Identifying fundamental mechanisms underlying apical branching morphogenesis in a model fungus *Aspergillus nidulans*.

By

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Abstract

Aspergillus nidulans is a filamentous fungus that for over 60 years has served as a robust model for studies of gene regulation and cell biology in complex eukaryotes. The most conspicuous feature of this species is the formation of hyphal branches through either lateral or apical branching to enable efficient colonization of its niche. Hyphal branching in filamentous fungi is regulated through a wide range of signaling pathways including extracellular and intracellular mechanisms. Notably, the role of protein kinases (PKs) in controlling apical branching morphogenesis remains an open question. Here, I have identified presumptive signaling pathways involved in hyphal branching by performing a bioinformatics screen for *A. nidulans* homologues of the human genes involved in neuronal migration, morphogenesis and branching (Chapter 2). I then employed two different assays to systematically investigate the roles of 100 PKs in apical branching (Chapters 2 and 3). My approach includes use of a quantitative microscopy assay (QMA) coupled with statistical analysis, which enabled me to identify PKs involved in regulation of apical branching morphogenesis in *A. nidulans.* Identifying PKs involved in mediating apical branching will encourage and support further investigations to characterize the main regulatory pathways implicated in branching morphogenesis in filamentous fungi.

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Dedication

For my mother and my brother, who always support and motivate me to follow my goals. Without their support I would not be where I am today.

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Chapter 1:

Introduction

1.1 Filamentous Fungi

Fungi are multicellular eukaryotic organisms that are classified in a distinct kingdom (Coudert et al. 2019). The presence of chitin in their cell wall is the main feature distinguishing them from other kingdoms such as Animalia, Plantae and Chromista (Tang et al. 2015). The vast majority of fungi colonizing terrestrial ecosystems are filamentous fungi (Li et al. 2017; Bielčik et al. 2019; Garrigues et al. 2020). They grow rapidly on plants, algal debris and biological wastes, and have long been recognized as critical sources of enzyme production especially for decomposing and recycling of plant debris and animal tissues (Li et al. 2017; Bielčik et al. 2019; Garrigues et al. 2020). Filamentous fungi also play an important role in medicine. For example, they can produce penicillin as an antibiotic, cyclosprine as an immunosuppressant medication, or lovastatin for treating cardiovascular diseases. In agriculture, filamentous fungi are also prominent as pathogens that cause numerous diseases, such as root rot and seed rot diseases in mung bean and sunflower respectively (Gawai 2018; Li et al. 2017; Nützmann et al. 2012; Pfliegler et al 2019). Economically, filamentous fungi are of great interest due to their fundamental role in manufacturing various enzymes, proteins and secondary metabolites (Hofmann et al. 2008). Some filamentous fungi can be life-threatening opportunistic pathogens that jeopardize human welfare (Bielčik et al. 2019; Mousavi et al. 2016). For example, serious diseases caused by *Aspergillus spp.* include invasive pulmonary aspergillosis, aspergilloma, and various forms of hypersensitivity such as allergic asthma, pneumonitis, and allergic bronchopulmonary aspergillosis (ABPA) (Bielčik et al. 2019; Mousavi et al. 2016). Therefore, a better understanding of fungal morphology and the mechanisms involved in its development will accelerate the future development of therapeutic interventions designed to improve medical and agricultural fields (Klei & Veenhuis 2006).

Filamentous fungi are widely used as model organisms for research because they grow very fast on different environments (Klei & Veenhuis 2006). In addition, the availability of complete genome sequences coupled with their close phylogenetic placement to animals facilitates their application as models for human cells (Klei & Veenhuis 2006).

Aspergillus nidulans belongs to the phylum Ascomycota and family Trichocomaceae. This fungus is also known as *Emericella nidulans* when in its sexual form (Tarawneh et al. 2013). Self-fertilization allows this fungus to form specialized spore-producing structures called fruiting bodies in the absence of a mating partner, which is why it is called a homothallic fungus (Tarawneh et al. 2013). *Aspergillus nidulans* is widely used as an experimental model to study gene regulation and cellular processes that occur in more complex systems such as humans, mice, worms and fruit flies (Oakley 2017). It's well-characterized genetics combined with its simple structure have made this fungus an excellent model to characterize conserved processes in multicellular organisms (Adams and Yu 1998; Brown et al. 1996; Timberlake 1990; Todd et al. 2007; Pontecorvo et al. 1952). Due to its relation to other medically important *Aspergillus* species such as *A. niger, A. oryzae, A. flavus,* and *A. fumigatus*, *A. nidulans* has paved the way for mycologists to find new genetic pathways involved in fungal development (Adams and Yu 1998; Brown et al. 1996; Timberlake 1990; Todd et al. 2007; Pontecorvo et al. 1952). *Aspergillus nidulans* adapts and grows rapidly in different substrates such as soil, plant and animal debris. It can also grow under various media and nutritional conditions (Harris 2010; Krijgsheld et al. 2012). This fungus produces spores through three lifecycles; sexual, asexual and para-sexual which are controlled by different environmental factors such as light, oxygen, and nutrient accessibility (Adams et al. 1998; Todd et al. 2007). Asexual sporulation, which leads to germination of asexual spores (conidia)

(Figure 1-1), is considered the most common mode of reproduction in filamentous fungi particularly *in A. nidulans* (Adams et al. 1998).

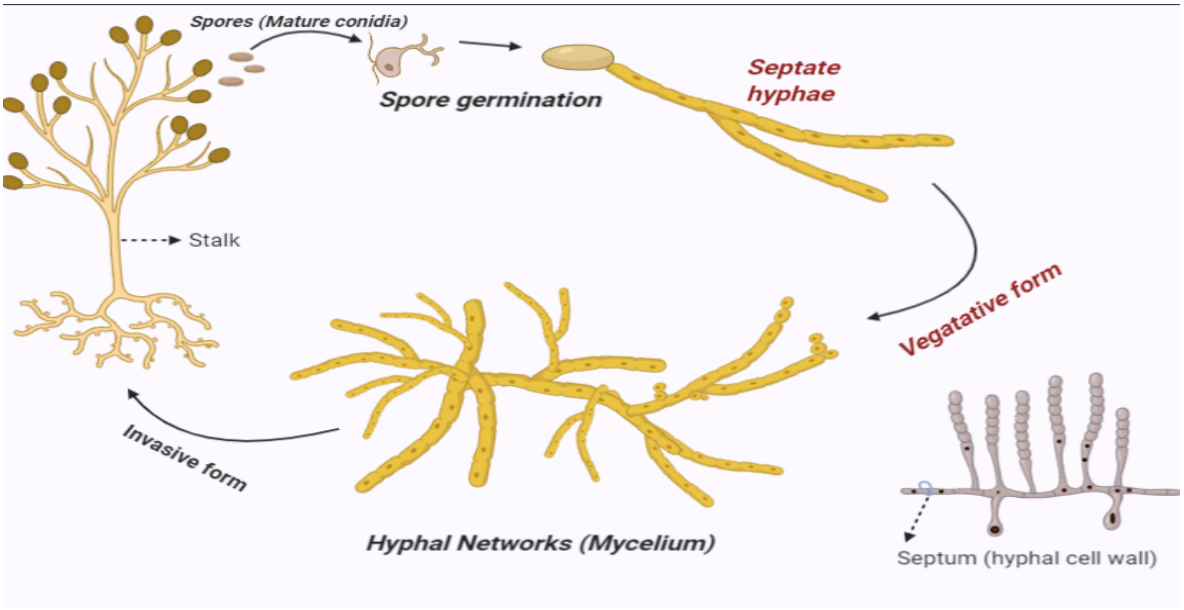

Figure 1-1. An illustration of asexual life cycle of *Aspergillus nidulans,* which forms an interconnected hyphal network called mycelium forming the invasive type of this fungus.

Aspergillus nidulans is normally haploid, containing 8 well-characterized chromosomes which range in size from 2.8 Mb to 4.4 Mb, and include an estimated 11,000–12,000 genes (Oakley 2001). They have been seen as diploid cells as well, which form from heterokaryons and generate diverse haploid mitotic segregants (Oakley 2001; Osmani 2006). Wild type colonies of this fungus are green, while mutant colonies exhibit diverse colors including yellow or white (Hofmann et al. 2008).

1.2 Hyphal growth

Asexual reproduction of vegetative growth in filamentous fungi starts with germinating conidia and is followed by the formation of tubular hyphae to form a specialized polarity axis (Adams et al. 1998; Steinberg et al. 2017) (Figure 1-1). In this manner, vegetative growth helps

filamentous fungi to generate highly polarized cells (hyphae) that are considered as the fundamental property of these fungi (Coudert et al. 2019; Dorter and Momany 2016; Harris 2010; Riquelme et al. 2018; Steinberg et al. 2017). Polarity means that the required biochemical elements for growth such as protein complexes, lipids or even organelles have been distributed asymmetrically in a cell as mediated by both microtubule and actin cytoskeleton (Diepeveen et al. 2017; Drubin 2000; Takeshita 2015; Xiang & Oakley 2010). Hyphae in many filamentous fungi are divided into different compartments by internal cross-walls called septa (Figure. 1-2A and C). Each compartment contains one or more nuclei (Harris 2019; Harris 2008; Stajich et al, 2009; Trinci 1978) (Figure 1-2C). Cross-walls (septa) in filamentous fungi play an important role in compartmentalizing cytoplasm, organelles or nuclei (Harris 2019; Takeshita 2015).

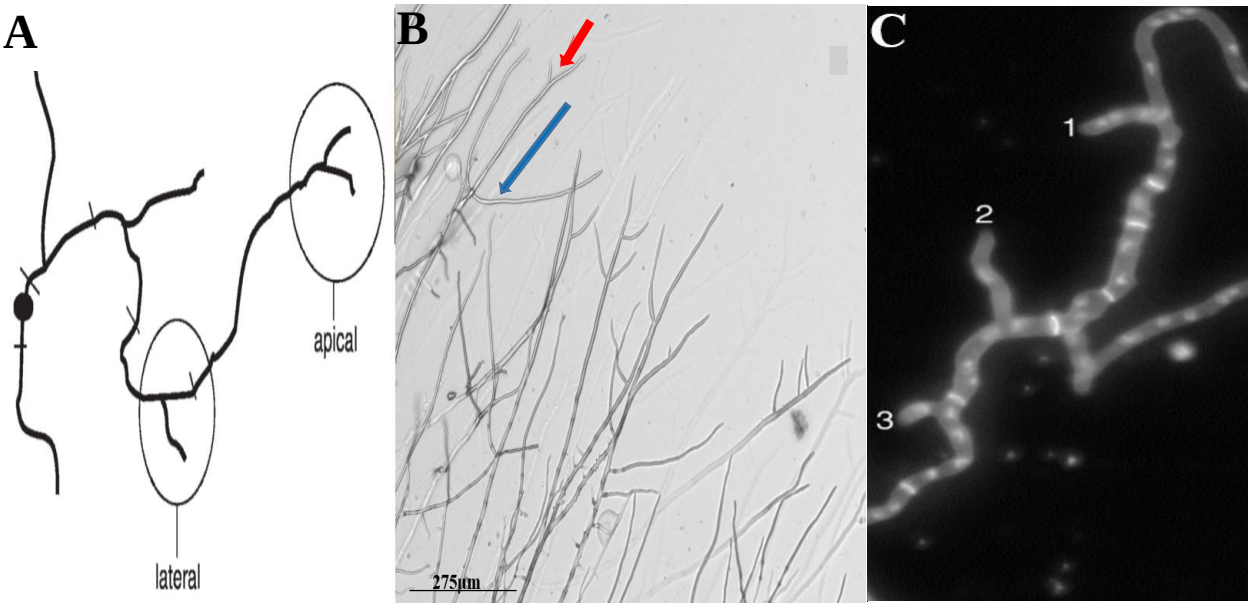

Figure. 1-2. A) Illustrates a schematic drawing of a fungal hypha that shows both apical and lateral branching patterns (taken from Harris 2008). The filled black oval represents the spore. Tick marks on the primary hyphae represent septa. B) Illustrates *Aspergillus nidulans* wild type strain (strain FGSC CDS1008). The red arrow shows an apical branch and blue arrow shows a lateral branch (10X magnification). C) A segment of *Aspergillus nidulans* (strain FGSC28) taken from Harris

2008. Hypha stained with Calcofluor White to show septa and nuclei. Three lateral branches are shown in this image.

Hyphae in filamentous fungi helps to absorb nutrients essential for their growth (Harris 2010; Harris 2019; Takeshita 2015). In fact, multiple hyphae radiate outwards from the initial spore and by increasing the number of hyphal tips, they are capable of assimilating sufficient nutrients from different environments (Harris 2010; Harris 2019; Takeshita 2015). Transferring essential nutrients into interior regions helps to develop and maintain the next generation of spores (Harris 2010; Harris 2019; Takeshita 2015). Hyphae develop a network of interconnected cells known as mycelium (plural mycelia) (Harris 2010; Harris 2019; Takeshita 2015). Therefore, the mycelium is the main mode of vegetative growth and plays a pivotal role in branching morphogenesis (Harris 2010; Van Peer1 et al. 2009). Most importantly, maintenance of cell polarity allows fungi to extend their hyphal tips continuously (Harris 2006). This occurs within the cell by the transport of cell wall precursors to the hyphal tip and placing multiple nuclei as well as other organelles in the right position within the elongating hyphae (Harris 2006; Harris 2010). In fact, hyphal networks or mycelia help fungi to colonize various substrates by hyphal branching (Rayner et al. 1995; Seiler &Plamann 2003).

1.3 Hyphal branching

Filamentous fungi produce tree-like structures known as hyphae. Development of a fungal colony is highly dependent on the growth rate, orientation, and the position of newly formed branches (Harris 2008; Moore et al. 2005; Trinci 1978). Branching is the main mechanism by which the new polarity axis is generated from the parental hypha (Harris 2008; Harris 2019; Riquelme & Bartnicki-Garcia 2004). The new hypha can branch and form other hyphae as well,

thereby increasing the density and the numbers of new hyphal tips in a mycelium (Harris 2008; Harris 2019; Riquelme & Bartnicki-Garcia 2004) (Figure 1-3).

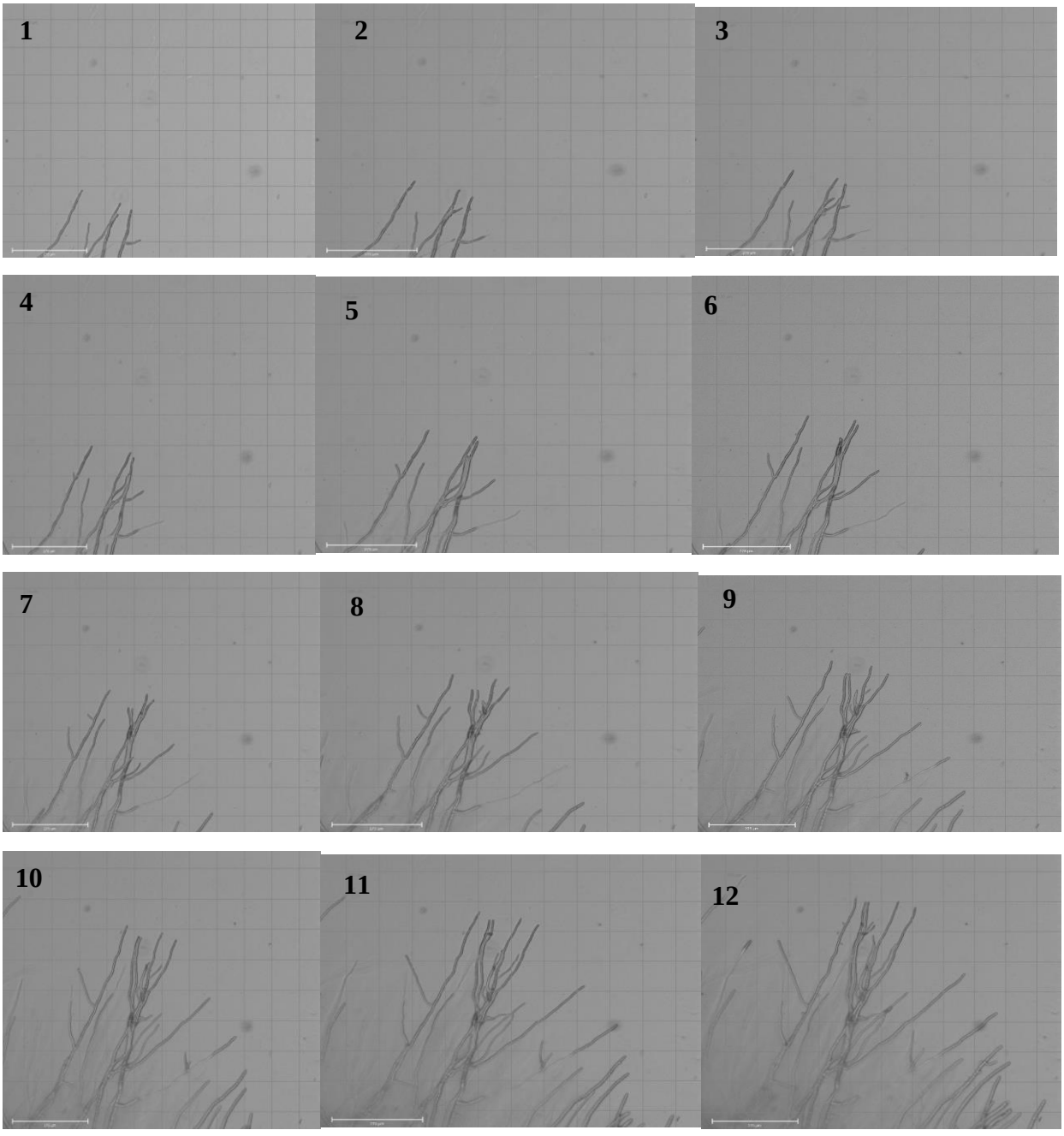

Figure 1-3. 12 images of hyphal growth and branching patterns in real time associated with *Aspergillus nidulans* wild type strain, 2E1 (strain FGSC CDS1008). The images were captured every 30 minutes over the course of 6 hours at 10X by EVOS microscope.

Hyphal growth and branching morphogenesis are not confined to filamentous fungi (Hallé 2001; McGowan et al. 2020; Pepper et al. 2015). There are other organisms including higher plants, bacterial actinomycetes and alga-related oomycetes that develop and grow through branching morphogenesis (Hallé 2001; McGowan et al. 2020; Pepper et al. 2015). Branching systems are also developed in highly polarized structures of animals such as the nervous system, respiratory system, internal vasculature, and many internal glands that are involved in transferring information, exchanging gases and fluids (Coudert et al. 2019; Spurlin & Nelson 2016). A good example of the importance of branching in animals is the transfer of information in dendritic cells that is vital for different functions including perception, learning and intellectual ability. Therefore, any defects in dendritic branching might lead to various neurological disorders specifically in humans (Coudert et al. 2019; Spurlin & Nelson 2016). While fungi and plants are able to expand their branches to absorb essential nutrients from the environment, the process of branching in animal organs is limited to embryogenesis and includes two patterns called "clefting" and "budding" which are the equivalent of "apical" and "lateral" branching patterns in fungi, respectively (Coudert et al. 2019; Harris 2008). The main reason why branching has been so well-developed in fungi and plants is due to their sessile conditions compared to animals which are mobile organisms (Hallé 2001).

1.4 Patterns of hyphal branching

Hyphal branching in filamentous fungi can be defined via two distinct patterns: apical and lateral branching (Coudert et al. 2019; Harris 2008; Riquelme and Bartnicki-Garcia, 2004).

Apical branching occurs by extending the apexes of parental hyphae and splitting them into two distinct tips (Figure 1-2A&B) (Coudert et al. 2019; Harris 2008; Riquelme and Bartnicki-Garcia, 2004) (Figure 1-3). Studies by Katz et al. (1972) and Trinci (1974) showed that excessive

propagation of the exocytic vesicles from the normal rate at the parental apex might trigger apical branching phenotype in filamentous fungi. Since the parental tip lacks enough capacity to incorporate all of the exocytic vesicles, a new branch or branches arise at the hyphal tip (Katz et al. 1972; Trinci 1974). In a study conducted on *Saccharomyces cerevisiae*, it was shown that spontaneous polarization model might be involved in regulating an apical branching phenotype (Irazoqui et al 2005; Marco et al. 2007). During this process, the numbers of polarity elements such as GTPases and Cdc42 exceed a normal rate at the hyphal tip, this activates other key elements like exocytic vesicles, endocytic vesicles and actin filaments (required to maintain cell polarity) that leads to emerging a new polarity axis from the parental hyphal tip (apical dominance). This results to form apical branches (Figure 1-4) (Irazoquiet al 2005; Marco et al. 2007).

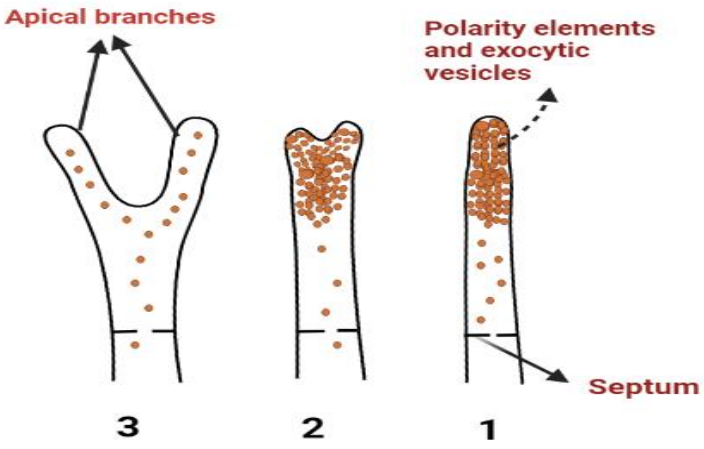

Figure 1-4. A schematic image of forming apical branches through "spontaneous polarization" model.

Apical branching is also regulated through the Spitzenkorper (Spk) (Riquelme and Bartnicki-Garcia 2004). The Spitzenkorper is a dark-phase material containing various vesicles, ribosomes and microfilaments and it is an organizing center to direct these elements to the plasma membrane (Harris et al. 2005; Riquelme & Bartnicki-Garcia 2004). Riquelme & Bartnicki-Garcia (2004) showed that in *Neurospora crassa*, cytoplasmic reservoir changes, induce displacement mediated by changes in morphological features of Spk and result in the disappearance of Spk temporarily at apical compartments. This results in a temporary disruption in vesicle flow throughout the cytoplasm. Following that, after reappearance of primary Spk at parental hyphal tip and formation of new Spk within the new hyphal axis (or deformation of primary hyphal tip), the elongation process associated with both parental and new hyphae resumes. This process results in two or more apical branches forming from the deformed hyphal apex (Riquelme & Bartnicki-Garcia 2004). By disrupting hyphal tip integrity or compromising the apical dominance, apical branching can be considered as a general response that maintains growth in response to various challenges or stress (Harris 2008; Katz et al. 1972; Trinici 1974). Whereas apical branching is common in fungal hyphae, lateral branching is the most prevalent pattern of branching in moss and algal filaments (Coudert et al. 2019). It is worth noting that, while apical cells are typically involved in absorbing nutrients and sensing the local environment, the sub apical cells play fundamental role in generating new branches through lateral branching (Coudert et al. 2019; Harris 2008).

Lateral branching occurs when new branches emerge from subapical hyphal compartments (Figure 1-2B and C) (Coudert et al. 2019; Harris 2008; Katz et al. 1972; Robertson 1959; Trinci 1969, 1970 and 1987).

Due to suppression of lateral branch formation in the vicinity of apical dominance, lateral branching only occurs at a considerable distance from the hyphal tip (Harris 2008). Therefore, sub apical compartments or septated segments that are far enough from the apex of parental hypha are the main origin for the formation of lateral branches (Harris 2008; Bartnicki-Garcia & Riquelme 2004). In *N. crassa* lateral branches are formed in a range of 17 to 30μm from the hyphal tip (Bartnicki-Garcia & Riquelme 2004; Trinci 1970). This separation suggests that factors inducing the generation of lateral branching might be independent of those underlying apical branch formation (Bartnicki-Garcia & Riquelme 2004). This view also has been supported by Trinci (1970) who suggested, unlike apical branching, neither the elongation rate of hyphal growth nor the shape of its apex are affected by lateral branching in filamentous fungi. Accordingly, the existence of a large cytoplasmic reservoir containing Spk vesicles in *N. crossa* allows this fungus to expand its lateral branches without perturbing the growth rate of parental hypha since both parental axis and the nascent lateral branch use their own source of Spk to expend their hyphal growth rate (Bartnicki-Garcia & Riquelme 2004).

While Spk vesicles are lost temporarily at the hyphal tip to form an apical branch in *N. crossa*, the substantial role of incipient Spk vesicles in initiating and extending lateral branching phenotype in this fungus is undeniable (Bartnicki-Garcia & Riquelme 2004; Harris 2008). Consistent with this view, a dark-phase material containing large amounts of cell wall precursors and vesicles in the vicinity of the site where a new lateral branch will be formed, generates a new Spk which is independent from the parental Spk. Therefore, the new formed Spk plays a key role in extending lateral branches at a considerable rate (Bartnicki-Garcia & Riquelme 2004; Harris 2008).

Trinci (1987) showed that the lateral branching process can be triggered by a model called "The septum as a barrier". This model explains that the location of septa can be an important factor in establishing the pattern of branching (Figure 1-2C). After forming a new complete septum, the flow of exocytic vesicles towards the hyphal apex is interrupted. This results in aggregation of those vesicles just behind the newly formed septum. Following that, excessive vesicle aggregation facilitates their fusion to the hyphal wall and changes the direction of branching (Figure 1-5). This process triggers the formation of lateral branches.

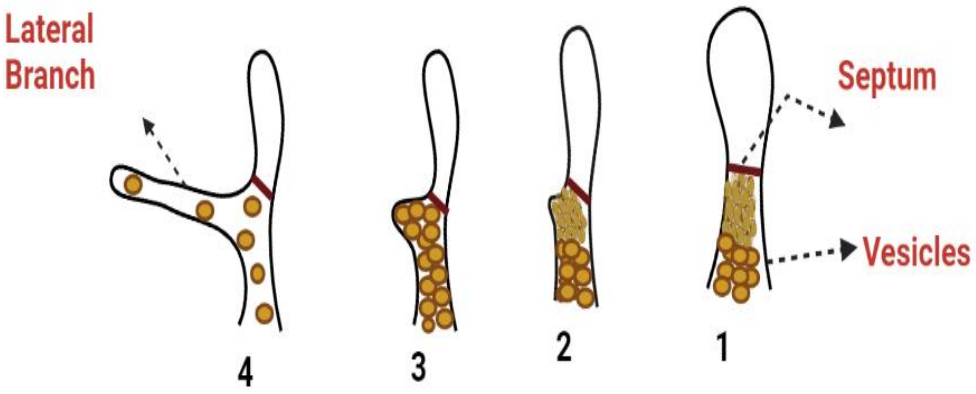

Figure 1-5. A schematic image of forming lateral branches through "The septum as a barrier" model.

Therefore, lateral branching might be considered as an efficient way to accommodate the numbers of secretory vesicles required for mycelium development (Trinci 1987). Interestingly, septa might not be considered as a vital process to form a branch in fungi based on an evidence from Zygomycetes fungi, which have aseptate hyphae, and are able to form hyphal branches (Harris 2008).

Lateral branching not only helps to increase interhyphal communication but also facilitates signaling between various hyphae in the same colony (Bartnicki-Garcia & Riquelme 2004; Harris

2008). Consequently, this pattern of branching results in generating a broad network of hyphae called mycelium (Bartnicki-Garcia & Riquelme 2004; Harris 2008). In accordance with a previous study, it has been shown that the colony development is highly dependent on maintaining apical dominance (Harris 2008). Therefore, loss of apical dominance could lead to chaotic growth and formation of lateral branches (Harris 2008).

There are a variety of other extracellular and intracellular signals including calcium ions, reactive oxygen species (ROS), actin, Cdc42.Rac GTPases, choline, cAMP and cGMP that could have impacts on branching dynamics (Coudert et al. 2019). However, to date little is known about the main regulatory pathways involved in controlling apical branching in filamentous fungi, particularly *A. nidulans* (Niu et al. 2020; Ribeiro et al. 2019). Finding those regulatory pathways and showing how they might underlie apical branching is a topic of great interest.

1.5 Mechanisms that underlie branch formation

A genetic screen related to 950 morphological mutants in *N. crassa* conducted by Seiler and Plamann (2003) revealed meaningful information about the process of hyphal branch formation. This information includes the process of selection of branch positions, the development of those positions in growth region, the generation of a small nascent branch and maturation of a new hyphal apex.

In addition to finding some of the mechanisms that might underlie branch formation through applying large-scale genetic screen (Seiler and Plamann 2003), functional characterization of particular genes involved in branch formation also has contributed to elucidate regulatory pathways of branching not only in *A. nidulans* but also in other filamentous fungi (Ganem et al. 2004; Harris, 2008; Yu et al. 1996). Deletion mutants associated with components of G-protein

coupled receptors (GPCRs) might abolish the formation of lateral branches (Ganem et al. 2004; Harris, 2008; Yu et al. 1996). This finding highlights the important role of GPCRs in regulating branch formation and is supported by another study by Niu *et al.* (2020). The study discussed an endogenous factor called oxylipin 5,8-diHODE that helps to trigger the formation of lateral branching mediated by G-protein coupled receptors in pathogenic fungi such as *Aspergillus fumigatus* and *Aspergillus flavusan*. While other functions in filamentous fungi such as protein kinases or circadian clock proteins are thought to be involved in regulating lateral branch formation and fungal development (Watters et al. 2011, 2013), it is still vital to identify the pathways by which those proteins are regulated to elucidate how branching morphology is controlled and conserved within the filamentous fungi.

It is believed that signals controlled by reactive oxygen species (ROS) and calcium (Ca^{++}) are able to reinforce apical dominance integrity (Takemoto et al. 2009). For example, in *A. nidulans* and *Epichloe festucae*, ROS is responsible in maintaining apical dominance integrity and it could be generated by NADPH oxidase (NOX) (Takemoto et al. 2009). Therefore, some mutations that disrupt the function of NOX might cause to some defects and form abnormal branching phenotypes (Takemoto et al. 2009). It is worth noting that, NOX can be regulated by other factors including GTPaseRac1 (Tanaka et al. 2008), Cdc24 and the scaffold protein Bem1 (Takemoto et al. 2011). Cdc24 and Bem1 are well-conserved in *S. cerevisiae* (Bi and Park 2012). In *N. crassa* the presence of Ca^{++} at the hyphal tip is the main suppressor factor to prevent the formation of any branches at apical dominance (Silverman-Gavrila & Lew 2003).

The morphogenetic machinery including cytoskeletal elements and microfilaments are not only involved in regulating the expansion of cell surface in fungi, but they also trigger the generation of germ tubes from spores (Steinberg et al. 2017). It has been shown that the positions

where new lateral branches will form are rich in cytoplasmic microtubules and microfilaments (Berepiki et al. 2010; Mourino-Perez et al. 2006; Sampson and Heath 2005; Torralba et al. 1998). However, it is not clear yet whether these elements in filamentous fungi are capable of initiating these lateral branching process (Harris et al. 1997). In *A. nidulans*, the emergence of lateral branches is closely associated with the presence of microfilaments at those sites, since the deletion of *SepA* gene in this fungus (responsible for polymerization of actin) abolishes the generation of lateral branches (Harris et al. 1997).

Amongst the key factors that might underlie branch formation in filamentous fungi, signaling pathways such as protein kinase A (PKA) and target-of-rapamycin (TOR) pathways are well conserved (Broach 2012). Another key regulator of branch formation in fungi is cyclin-dependent-kinase or CDK (Fiddy and Trinci 1976; Lin and Momany 2004). CDK is involved in monitoring nuclear division, suggesting that branch formation is controlled by nuclear division as well (Fiddy and Trinci 1976; Lin and Momany 2004). To support the significant role of CDK in branch formation and its conservation from Fungi to Animalia Kingdom, there is some strong evidence that in mammalian neuronal cells, a protein kinase called CDK5 helps to regulate dendritic arborization (branching structure) (Menon &Gupton 2018; Ploostera et al. 2017).

1.6 How branching morphogenesis is regulated in other organisms

The evolution of multicellularity has been one of the major reasons for the formation of various Kingdoms including Animalia, Fungi, Plantae and Chromista (Coudert et al. 2019). One of the most conspicuous features shared between these lineages is branching (Coudert et al. 2019). While there are some obvious biological differences between filamentous organisms and animals, the principles controlling branch formation might be shared between them (Coudert et al. 2019). To acquire a deep knowledge about how branching morphogenesis is controlled in filamentous

fungi, it is important to examine branching system in other organisms as well (Coudert et al. 2019). Branching organs in land plants can be distinguished by parenchymatous bodies, and in animals can be observed through kidney, nervous system and respiratory system (Ochoa-Espinosa et al. 2012; Wang et al. 2017). Although filamentous organisms are capable of extending their branches to assimilate nutrients from various environments, branching in animal organs is confined to a determined position and consists of a broad network of tubes called ducts (Coudert et al. 2019). Another obvious difference is, while in filamentous organisms the presence of filamentous cells helps to initiate branching, in Animalia kingdom epithelium cells are responsible for this feature (Affolter et al. 2009; Iber et al. 2013; Lang et al. 2018; Ochoa-Espinosa et al. 2012; Wang et al. 2017). Fritsch et al. (1945) and Fiddy et al. (1976) found that in *Ectocarpus, Sphacelaria* (a brown alga) and *P. patens* (a kind of moss) new branches arise from sites which are far enough from the apical dominance and the position of that branch has no interference in extending the apical tip. This view is consistent with what was found by Trinci (1970) in filamentous fungi that showed the growth rate of lateral branches is independent from the elongation rate of parental hyphal tip. These points of views suggest the main mechanisms and cues that specify branch position, can be shared between these organisms (Bartnicki-Garcia & Riquelme 2004; Trinci 1970).

Treating fungal mycelium with an ionophore enhances cytoplasmic Ca^{++} in filamentous organisms and increases the apical branching phenotype (Reiss et al. 1983). Reiss et al. (1983) also confirmed that treatment of moss protonemata with a calcium channel blocker (papaverine) would cause a chaotic growth pattern and enhance lateral branch formation at both sides of a newly formed septum. On the other hand, mutations in genes that control formation of actin patches or use of actin depolymerizing drugs in the brown alga *Sphacelaria* and the giant kelp *Macrocystis* lead to defects in branching pattern since actin patches determine the sites where a nascent branch

is formed (Katsaros 2003; Varvarigos et al. 2019). These studies highlight the pivotal role of calcium ions and the actin cytoskeleton in determining the branch position in various filamentous organisms including brown algae, moss as well as *P. patens* and *N. crassa* (Reissig et al. 1983; Reiss et al. 1983).

Ashbya gossypii is a filamentous fungus that contains septate hyphae and shows an intrinsic attitude to emerge lateral branches at sites adjacent the newly formed septum (Philippsen et al. 2005). Mutations in genes encoding the formation of septa cause this fungus to lose the ability to form lateral branches (DeMay et al. 2009; Yamazaki et al. 2013). This confirms that septa (inter-region rings) are a kind of a marking system to specify the sites of branch formation particularly lateral branches (DeMay et al. 2009; Yamazaki et al. 2013). However, the specific role of this conserved feature in other filamentous fungi such as *N. crassa* remains mysterious (Lindsey et al. 2010).

Furthermore, external cues such as local inhibitory factors might determine branch positions not only in filamentous organisms but also in animal organs so that the formation of a new branch at a specific area prevents the formation of other branches at that site by inducing some local inhibitory factors (Berepiki et al. 2013; Hernández-Rodríguez et al. 2012; Lindsey et al. 2010). This view is not only accurate for mosses and brown algae, but also confirms the mechanism that was found in animals by which generating lateral branches is inhibited to prevent excessive branch formation in those organs.

Consistent with previous findings, mutations in polarization factors in *A. nidulans* such as GTPases including Cdc42 or RacA results in abnormal branching phenotype (Katsaros et al. 2003; Si et al. 2016). For example, deletion of *cdc42* and Cdc42 GTPase activating protein (GAP) RgaA in *A. nidulans* will cause the loss of lateral branches and an abnormal increase in the branching

phenotype respectively (Katsaros et al. 2003; Si et al. 2016). Moreover, septins AspA, AspB, and AspC which are placed at the apex of a newly formed lateral branch are responsible for determining the suitable position of a branch (Hernández-Rodríguez et al. 2012; Lindsey et al. 2010). Therefore, mutations in each of these genes might lead to an increase in the amount of branching and causes an abnormal branch phenotype in which multiple branches emerge from one compartment in a hypha (Hernández-Rodríguez et al. 2012; Lindsey et al. 2010). This view is supported by hyper branching phenotype due to septin mutants in both *N. crassa* and *Candida albicans* (Berepiki et al. 2013; Li et al. 2012). Tong et al. (2007) believed that in *S. cerevisiae*, Cdc42 GAPRga1 (a component of GTPases), coordinating with septins played a vital role in regulating the time and pattern of bud emergence.

Receptor kinase–peptide ligand molecules regulate branch formation in animals (Wang et al 2017). First, the receptors in epithelium sense the kinase–peptide ligand molecules, that are located close to the mesenchyme (developing organ) and then produce the cells that are responsible for branch formation (Ochoa-Espinosa et al. 2012; Wang et al. 2017). The viscosity feature of both mesenchymal and epithelial tissues contributes to branch formation in animals as well (Nagatsu et al. 2007). In another study done by Flanagan et al. (2009), it was found that the mechanical features of the environment have direct impact on branching morphology. For example, the neuronal cells that were grown on different substrates of varying rigidity showed different branch densities. Similarly, rigid environments have impacts on determining the branching patterns of mosses and fungi (Flanagan et al. 2009) While there are other factors that have been shown to induce branch formation in filamentous organisms and animals including Auxin inhibitory gradients, cell wall thickness, cell wall poro-elasticity, CLE peptide–CLAVATA receptor-like kinase, and organelles (Coudert et al. 2019), explaining those pathways and examining each in different organisms is

beyond the scope of this review. Most importantly, although there are many factors which might impact the branch formation in filamentous fungi (Coudert et al. 2019), the pathways underpinning apical branch formation in *A. nidulans* remain largely unknown.

1.7 Signal transduction and protein kinases in filamentous fungi

There is strong evidence that fungi are highly susceptible to any changes in the environment indicating that they have complex mechanisms of sensing activated by various signal transduction pathways which result in orchestrated changes in fungal morphology, cell physiology and adherence (Biswas et al. 2007). For example, the pathogenic fungus *C. albicans* that is well-known for its adaptation to various physiological extremes is capable of switching between different morphological forms (e.g. single-celled, budding yeast and filamentous form), and adjusts its growth based upon distinct environmental stresses (Berman 2006; Garaizar 2006). Consistent with this view, it has been found that amino acids signaling pathways including the Csy1 sensor, which is homologous to Ssy1 in yeast, plays a key role in forming hyphal filaments in *C. albicans* and causes the formation of hyphal branches (Brega et al. 2004; Forsberg et al. 2001). Hyphal branching morphogenesis mediated by signal transduction pathways allows fungi to adjust themselves to a new environment (Brega et al. 2004; Forsberg et al. 2001).

Previous studies showed that protein kinases (PKs) play a fundamental role in various aspects of regulating and signaling transmission in filamentous fungi (Dickman &Yarden 1999; Souza et al. 2013). Therefore, they might be important in regulating branching morphogenesis as well (Kosti1et al. 2010). PKs add a phosphate group to a substrate protein and modify the function of that protein (Cohen 2000; Turra et al. 2014). The coordination of multiple PK pathways is necessary for inducing polar growth, nutrient or stress responses and asexual or sexual development (Turra et al. 2014; Cohen 2000).

PKs are potential factors to enhance the pathogenicity of fungi (Dickman &Yarden 1999). Indeed, the existence of cAMP-dependent protein kinase (PKA) in *Aspergillus* and *Neurospora* is well known (Dickman &Yarden 1999). Following that, it has been shown that the overexpression of PKA-C subunit genes in *Aspergillus niger* has a direct impact on changing either the growth or development of this fungus. It has been revealed that the mutants in R subunit of PKA (or *mcb* gene) in *N. crassa* cause the complete loss of growth (Dickman &Yarden 1999). These findings suggest that the genes encoding fungal protein kinases are conserved well thorough the kingdom. Overall, based upon what fungi sense in the environment, they extend their branches in response to various environmental stimuli (Biswas et al. 2007).

1.8 Objectives for this thesis:

1. To identify fungal homologues of human genes implicated in signaling pathways (including PKs, G proteins and their respective regulators) and regulation of branching morphogenesis. This will be accomplished through the use of a bioinformatics screen that queries a large set of genes that function in dendritic branching in humans to identify homologous in *A. nidulans* (Chapter 2).
2. To identify candidate protein kinases implicated in regulation of apical branching morphogenesis in *A. nidulans*. This experiment was performed by using a set of 100 PK deletion mutants using two different screens:
3. A quantitative microscopy assay designed to detect strains showing abnormal apical branching phenotypes (hypo or hyper apical branching phenotype) (Chapter 2) and a media-shift assay designed to detect strains that fail to adjust branching patterns upon a dramatic shift in growth conditions (Chapter 3).

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Chapter 2:

Identification of protein kinases that potentially regulate apical branching morphogenesis in *Aspergillus nidulans.*

Abstract

Fungal species penetrate various substrates to acquire necessary nutrients or turn into invasive life-threatening pathogens. These features are due to the formation of highly polarized cells called hyphae that are the main basis for the formation of branching morphology. Similar to hyphal branching, dendritic branching in neuronal cells is the vital step in regulating brain functions in some animals. Although branching morphogenesis has been studied extensively in other eukaryotic kingdoms such as Animalia, Plantae and Chromista from the simplest to the most complex organisms, in kingdom Fungi the pathways and genes that underlie branching morphogenesis remain poorly understood. Since there are structural similarities in branching morphology between the most complex eukaryotic systems like humans and the simplest ones like filamentous fungi, it was very interesting to identify whether basic mechanisms underlie branching morphogenesis have been shared and conserved from humans to fungi. The primary focus of this study was to use genes known to be involved in the branching morphogenesis of human neuronal cells as queries for a bioinformatics screen designed to determine whether they were conserved in *Aspergillus nidulans*. By predicting some protein kinases (PKs) that play important role in hyphal branching in *A. nidulans*, this research gave us a clue to examine the role of a specific set of 100 kinase deletion strains in regulation of apical branching in *A. nidulans*. Therefore, by developing a quantitative microscopy assay (QMA) along with a novel approach to define a standard reference interval (RI) used for detecting the abnormal strains in fungal population, I quantified the number of apical branches associated with 100 kinase deletion mutants to find strains demonstrating hypo- or hyper apical branching morphogenesis. Significant correlations were found between the absence of the 6 protein kinases and aberrant apical branching phenotype in *A. nidulans*, under two environmental conditions MAG and MN media. Among the 6 kinases, I suggest that cyclic AMP

(cAMP)-dependent protein kinase (protein kinase A [PKA]) plays an important role in regulation of apical branching in *A. nidulans.* Collectively, this research not only shows the signaling pathways might be involved in regulation of hyphal branching in *A. nidulans*, but also provides great insight into identifying other regulatory pathways that underlie apical branching morphogenesis in this fungus.

2.1 Introduction

Branching is one of the most obvious features of hyphal morphogenesis in filamentous fungi (Coudert et al. 2019; Dorter and Momany 2016; Harris 2010; Riquelme et al. 2018; Steinberg et al. 2017). Hyphal branching plays a fundamental role in organizing various cellular tasks in fungi, including sensing environmental cues and responding to them (Biswas et al. 2007), assimilating necessary nutrients to develop the fungal colony (Coudert et al. 2019; Harris 2008; Spurlin & Nelson 2016), or even inducing the invasive forms of these organisms that leads to fungal pathogenicity (Mayer et al. 2013; Naglik 2011). Each of these functions is likely to be monitored by diverse signaling pathways (reviewed by Coudert et al. 2019; Niu et al. 2020; Ribeiro et al. 2019). By extending hyphal growth primarily through apical and lateral branching, filamentous fungi are able to colonize the terrestrial ecosystem (Coudert et al. 2019; Harris 2008; Spurlin & Nelson 2016). In these organisms, compromising apical dominance integrity leads to apical branch formation that is a general response to continued growth (Harris 2008; Katz et al. 1972; Trinici 1974). Apical branching suggests de novo formation of a branch mainly through splitting the apex of parental hypha into two distinct tips (Harris 2008; Harris 2019; Riquelme and Bartnicki-Garcia, 2004) (Figures 2-1).

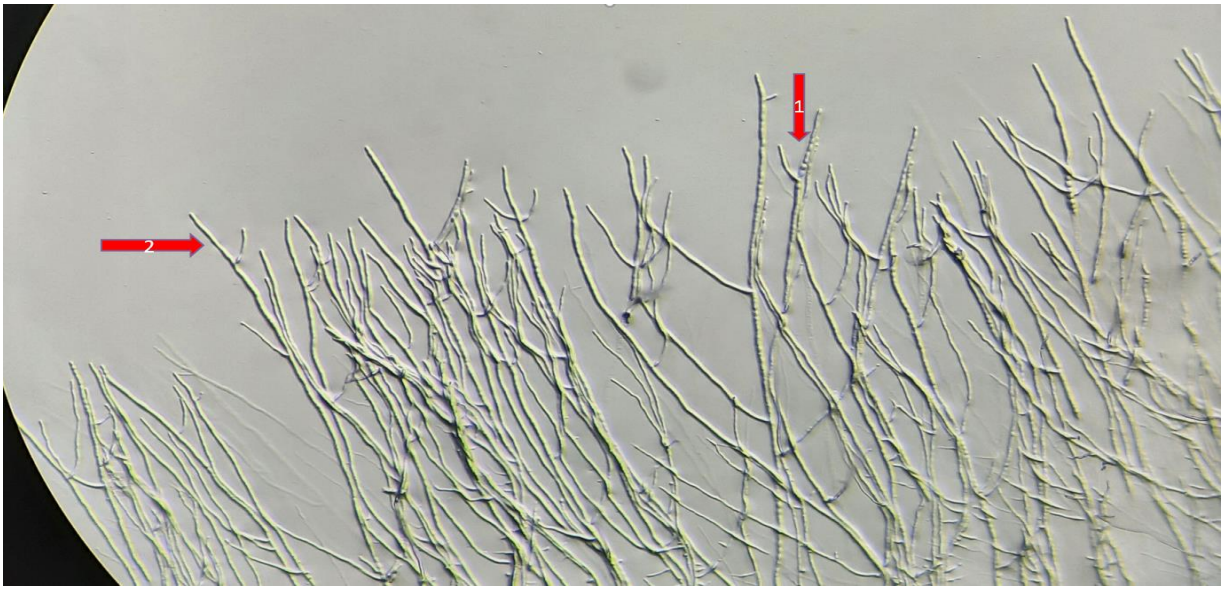

Figure 2-1. Branching patterns in fungal mycelia. Branched hyphae from *Aspergillus nidulans* (strain FGSC CDS1008) colony with 6X magnification by dissecting microscope. Arrow 1 shows lateral branching pattern in which after forming a new branch the hypha has been septated completely, while arrow 2 is associated with forming apical branch showing that the apex of parental hypha has been split into two tips in which there is no septation after forming a new branch.

It has been shown that a wide range of internal and external factors induce fungal development (Coudert et al. 2019; Nakamura et al. 2018; Niu et al. 2020; Philippsen et al. 2005; Reissig et al. 1983; Waters et al. 2017). For example, recently it was illustrated that in two pathogenic *Aspergillus* species, *A. fumigatus* and *A. flavus*, a class of fungal developmental signals called oxylipins, activated through G-protein-coupled receptors (GPCRs) can induce lateral branch formation in these species (Niu et al. 2020). Other pathways that trigger branch formation in filamentous fungi include calcium ions (Ca^{++}) (Reissig et al. 1983), reactive oxygen species (ROS) (Takeda et al. 2008), actin cytoskeleton (Katsaros et al. 2003), Cdc42/Rho GTPase (Si et al. 2016), strigolactone (Waters et al. 2017), Choline, cAMP and cGMP (Markham et al.

1993), nuclear division (Philippsen et al.2005), septum formation, and cell wall integrity (Gladfelter et al. 2001; Gladfelter et al. 2006; Pan et al. 2007). Any mutations that suppress these mechanisms might induce abnormal morphology that could be associated with either decreased (hypo-) or increased (hyper-) branching phenotype.

2.1.1 Characteristic features of branching morphology shared among animals and fungi

Unlike fungi, the mechanisms that underlie branching morphogenesis in land plants and animal organs (e.g. lung, kidney, neuronal cells, blood vessels, respiratory system, mammary glands, prostate and liver) have been characterized at both cellular and molecular levels (Affolter 2012; Affolter et al. 2009; Lang et al. 2018; Ochoa-Espinosa & Iber et al. 2013; Wang et al. 2017). However, the main principles involved in branching morphogenesis in filamentous fungi still remain mysterious.

Similar to filamentous fungi, branching morphogenesis has evolved in other multicellular organisms to achieve the goal of importing, transporting or secreting required materials to other compartments which allows those organisms to facilitate differentiation and developmental processes (Menon & Gupton 2018; Spurlin & Nelson 2016). Like hyphal branching which is fundamental to the formation of mycelia (hyphal network) in filamentous fungi (Harris 2010;Takeshita 2015; Harris 2019), previous studies indicate that extensive formation of branched polarized cells such as dendrites and axons, is the main basis of originating complex neuronal wiring which monitors all vital functions in animals including sensory perception, learning, intellectual ability and memory (Etxebeste & Espeso 2016; Menon & Gupton 2018; Spurlin and Nelson 2016).

Another obvious characteristic of branching morphogenesis shared between animals and fungi is that it primarily occurs through two general patterns (Coudert et al. 2019; Harris 2008; Riquelme and Bartnicki-Garcia, 2004). Apical branching (in fungi) or clefting (in animals) refer to bifurcation of the extending tip (either hyphal or dendritic axis) (Coudert et al. 2019; Harris 2008). In contrast, lateral branching (in fungal hyphae) or budding (in animals' cells) occur through the formation of nascent branches from sites distal to the extending hyphal tip (Coudert et al. 2019; Harris 2008; Riquelme and Bartnicki-Garcia, 2004). Branching has evolved to expand the surface area used for self-propagation, but also to enhance hyphal fusion in fungi or synaptic connections (formed between various neuronal cells) in animals. Further, branching allows these organisms to exchange necessary nutrients or transfer important information to other cells respectively (Menon & Gupton 2018). Consistent with this view, various neurodevelopmental disorders in humans and defects in fungal development might be caused by failure in providing proper branching pattern in these organisms.

2.1.2 PKA pathway in filamentous fungi

Signaling pathways have been evolved in eukaryotic systems, specifically fungi, that regulate a broad range of functions and enable them to sense and respond to the environmental stresses including low nutrient levels, ultraviolet (UV) radiation, low or high temperature, oxygen limitation, dryness and oxidative stress to survive and adapt themselves to the environmental changes (Hagiwara et al. 2016; Landry et al. 2013; Vidal et al. 2016). PKA pathway involved in signaling pathway, has been well conserved and studied in eukaryotic systems specifically fungi that mediates a wide range of intrinsic cellular processes including sensing nutrients in the environment (Holsbeeks et al. 2005), responding to environmental cues (Casado et al. 2011) as well as regulating fungal development (Freitas et al. 2010) and pathogenicity (Fuller et al. 2011;

Liebmann et al. 2004). The activation of PKA is highly dependent on binding cyclic adenosine 3′,5′ monophosphate (cAMP) as a second messenger to regulatory subunits that triggers structural changes and results to activation of catalytic subunits in PKA pathway (Taylor et al. 1990) (Figure.2-2).

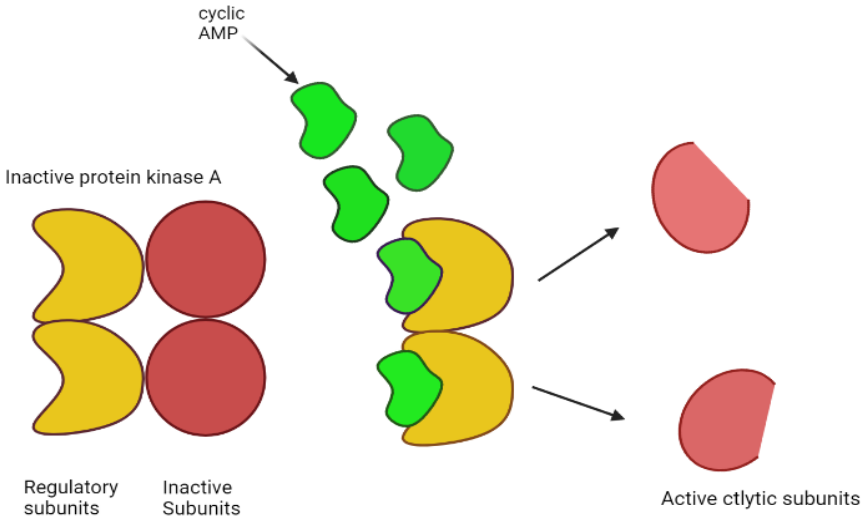

Figure 2-2. The activation of PKA pathway. Binding cAMP to the regulatory subunits of PKA pathway leads to conformational change and activation of inactive catalytic subunits (such as pkaA and pkaB).

PkaA and PkaB are the subunits of PKA pathway in *A. nidulans* (Ni et al. 2005). According to the previous studies, lacking PkaA (AN6305) leads to strong growth defects, while this defect can be compensated by up-regulation of PkaB (AN4717) under conditions of osmotic stress (de Souza et al. 2013). In addition, in most of the fungal species such as *Fusarium solani* and *A. fumigatus*, PkaC another subunit of PKA, regulates conidial germination (Banno et al. 2005; Liebmann et al. 2004).

Major studies over the past few years have shown that in *Saccharomyces cerevisiae*, the cAMP-PKA pathway is mainly responsible for sensing glucose in the environment and turning it to ethanol upon its activation (Oliver et al. 2002; Rolland et al. 2002). This view supports the major role of PKA pathway in the fermentation process in *S. cerevisiae* (Oliver et al. 2002; Rolland et al. 2002). Also, other studies indicate the vital role of PKA in inducing filamentous growth (filamentation) in both *S. cerevisiae* and *Candida albicans* (Cao et al. 2006; Cullen and Sprague 2012; Gimeno and Fink 1994). Inducing filamentation in *C. albicans* enhances the pathogenicity of this fungus (Cao et al. 2006; Cullen and Sprague 2012; Gimeno and Fink 1994). Consequently, the PKA pathway is directly associated with fungal pathogenicity (Borges-Walmsley & Walmsley 2000). In yeasts, PKA is the main mediator to modulate various stress responses through its opposite influence on stress response elements (STREs) (Smith et al. 1998). For example, an increase in the expression of Msn2 and Msn4 transcription factors would trigger STREs resulting to an excessive sensitivity to oxidative stress. Blocking Mns2/4 by PKA pathway therefore reduces the expression of STRE genes (Smith et al. 1998).

In *Aspergillus niger* the formation of conidiophore and hyphal growth are due to the activation of PKA pathway. This notion is supported completely by Benčina & Legiša (2000), who illustrated that by initiating the process of conidia germination, the level of *PkaC* (a subunit of PKA) gradually decreased showing that hyphal morphogenesis in *A. niger* is dependent on the function of PKA pathway. In *Schizosaccharomyces pombe*, PKA mediates the sexual life cycle and gluconeogenesis (Higuchi *et al*., 2002).

Ribeiro et al. (2019) showed a global perspective of proteins and functional pathways regulated either directly or indirectly by PKA through a comprehensive phosphorylation analysis of PKA pathway. One of the proteins that is activated indirectly by PkaA (subunit of cAMP) is

CreA (Ribeiro et al. 2019). Consistent with this view, the transcription factor CreA in *A. nidulans* is the main factor to repress the expression of genes coding lignocellulolytic enzymes when glucose is present. Therefore, CreA activates the expression of CCR (Carbon Catabolite Repression) to inhibit the usage of alternative carbon sources in this fungus. While PKA deletion strain leads to decrease the expression of CreA, there is no evidence showing that there is a physical interaction between CreA and PkaA (Ribeiro et al. 2019). Therefore, this result strongly suggests that CreA is regulated by another kinase regulated by PKA (Ribeiro et al. 2019). While the important role of some protein kinases like PKA or mitogen-activated protein kinase A (MPKA) pathway in regulating fungal development has been well-studied (Chen et al. 2007; Soberg et al. 2017), the role of these kinases as well as other PKs in regulating apical branching morphogenesis in filamentous fungi remain mysterious.

2.1.3 Standard reference interval (RI)

To date no one has established a standard reference interval in filamentous fungi to identify precisely the strains showing abnormal branching pattern in a fungal population. However, in this study the RI was established through finding the upper and lower limits for each dataset that enabled me to decide which deletion strain showed either hypo- or hyper- apical branching compared to the wild type strain (2E1). The method by which RI was established in this protocol, was defined by the International Federation of Clinical Chemistry (IFCC) in 1970 through a non-parametric approach to find the reference range for biochemical analytes like glucose in blood test (equation 1). In equation 1 R stands for rank number, α is either 2.5^{th} or 97.5^{th} percentiles and n is sample size (Figure 2-3). In this research the data for each round of QMA (there were 4 rounds) were distributed normally meaning that the mean and the median associated with each round were the same. It is worth noting that, the recommended sample size by (IFCC) to find RI through non-

parametric approach is $40 \leq n < 120$, where n is sample size (Friedrichs et al. 2012). The non-parametric method to measure RI is also included in several clinical laboratory software programs, including CBstat, Reference Value Advisor freeware and MedCalc (Friedrichs et al. 2012). By establishing an appropriate RI for each dataset not only the abnormal strains can be found precisely, but also it prevents to select abnormal strains arbitrarily.

Equation 1: Rank (R) = α percentile $(n+1)/100$

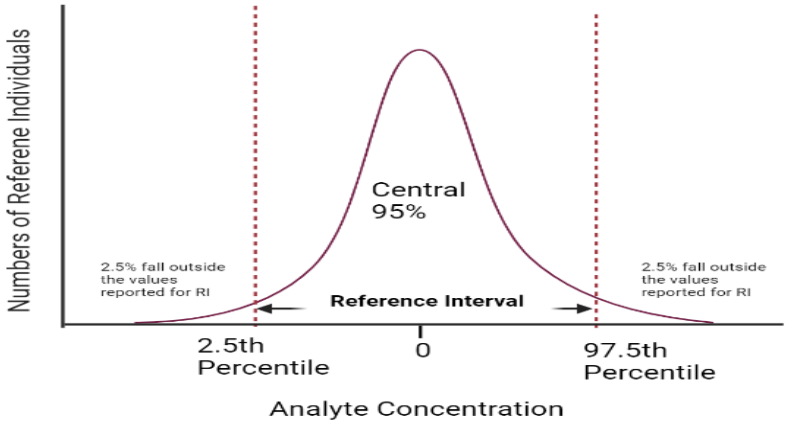

Figure 2-3. The reference interval when the data is distributed normally, contains 95% of the healthy individuals meaning that 5% of the results are abnormal. This means that 2.5% of the time a value will be less than the lower limit of this interval, and 2.5% of the time a value will be larger than the upper limit of this interval. The values located at 2.5^{th} and 97.5^{th} percentiles show the lower and upper reference limits respectively.

Overall, in this chapter according to the architectural similarities associated with branching in dendritic cells and fungal hyphae (Coudert et al. 2019; Wang et al. 2017) (Figure 2-4), our approach is based on the hypothesis that there must be some conserved molecular mechanisms including protein kinases (PKs), GTPases, G proteins or other regulators among humans and fungi that are involved in branching morphology.

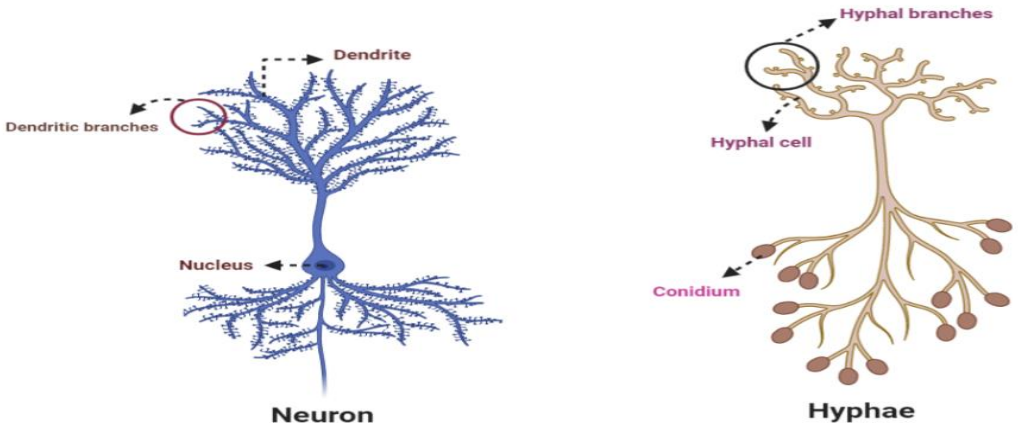

Figure 2-4. A schematic drawing of fungal hyphae and neuronal cell that shows structural similarities among fungi and animals.

Since PKs are involved in various aspects of signaling transduction pathways (Kosti1et al. 2010), through a bioinformatics screen this study potentially identified the PKs involved in branching morphogenesis that were highly conserved in *A. nidulans*. This gave us a good entry way to hypothesize that whether PKs are involved in regulation of apical branching morphogenesis in *A. nidulans.* Since apical branching plays a key role in sensing the environmental cues (Coudert et al. 2019; Harris 2008) and responses can be activated by signal transduction pathways in filamentous fungi (Biswas et al. 2007). Therefore, to address this hypothesis, a pre-existing library of 100

kinase deletion strains (Table 2-1) was screened through a quantitative microscopy assay (QMA) leading to identifying the abnormal strains that showed either hypo- or hyper- apical branching phenotype under two environmental conditions. Through this assay abnormal strains were identified by establishing the reference interval (RI) as a novel approach that has been defined in this paper, in filamentous fungi for the first time. Most importantly, this study not only provides a novel approach to find the kinase deletion strains showing defects in apical branching morphogenesis in filamentous fungi, but also it uncovers hitherto unknown signaling pathways inducing apical branching phenotype in a model fungus *A. nidulans* and paves the way to investigate largely unknown principles involved in branching morphogenesis in filamentous fungi.

Table 2-1. The list of 100 kinase deletion library.

Del#	FGSC#	ANID#	Cell	Name (Sc likely orthologue)
1	A1282	ANID_00038.1	1A1	AtmA (Sc Tel1)
2	A1284	ANID_00144.1	1A2	PkcA (Sc Pkc1)
3	A1287	ANID_00699.1	1A3	Uncharacterized
4	A1288	ANID_00822.1	1A4	KfsA (Sc Kin4)
5	A1289	ANID_00931.1	1A5	PbsA (Sc Pbs2)
6	A1290	ANID_00988.1	1A6	Uncharacterized
7	A1291	ANID_10019.1	1A7	Uncharacterized
8	A1292	ANID_10082.1	1A8	Uncharacterized
9	A1293	ANID_10153.1	1A9	SskB (Sc Ssk2)
10	A1294	ANID_01017.1	1A10	HogA (Sc Hog1)
11	A1295	ANID_10193.1	1A11	Uncharacterized
12	A1296	ANID_10325.1	1A12	Uncharacterized
13	A1297	ANID_10462.1	1B1	Uncharacterized
14	A1298	ANID_10485.1	1B2	Uncharacterized
15	A1299	ANID_10515.1	1B3	Uncharacterized
16	A1300	ANID_10800.1	1B4	Uncharacterized
17	A1301	ANID_10819.1	1B5	Uncharacterized
18	A1302	ANID_10869.1	1B6	Uncharacterized
19	A1303	ANID_10895.1	1B7	Uncharacterized
20	A1304	ANID_10937.1	1B8	Uncharacterized
21	A1305	ANID_01097.1	1B9	Uncharacterized
22	A1306	ANID_11032.1	1B10	Uncharacterized
23	A1307	ANID_01171.1	1B11	Uncharacterized

24	A1309	ANID_01560.1	1B12	PlkA (Sc Cdc5)
25	A1310	ANID_01632.1	1C1	Uncharacterized
26	A1311	ANID_01665.1	1C2	Uncharacterized
27	A1312	ANID_01789.1	1C3	Uncharacterized
28	A1313	ANID_01867.1	1C4	PhoB (Sc Pho85)
29	A1314	ANID_02067.1	1C5	Uncharacterized
30	A1315	ANID_02246.1	1C6	Uncharacterized
31	A1316	ANID_02265.1	1C7	Uncharacterized
32	A1317	ANID_02269.1	1C8	SteC (Sc Ste11)
33	A1318	ANID_02373.1	1C9	Uncharacterized
34	A1319	ANID_02412.1	1C10	CmkA (Sc Cmk2)
35	A1320	ANID_02489.1	1C11	Uncharacterized
36	A1322	ANID_02943.1	1C12	RfeA
37	A1323	ANID_03001.1	1D1	Uncharacterized
38	A1324	ANID_03065.1	1D2	CmkB
39	A1326	ANID_03422.1	1D3	Uncharacterized
40	A1328	ANID_03719.1	1D4	Uncharacterized
41	A1331	ANID_04196.1	1D5	Uncharacterized
42	A1332	ANID_04238.1	1D6	SchA (Sc Sch9)
43	A1333	ANID_04279.1	1D7	ChkB (Sc Dun1)
44	A1334	ANID_04385.1	1D8	SepH
45	A1335	ANID_04483.1	1D9	Uncharacterized
46	A1336	ANID_04536.1	1D10	Uncharacterized
47	A1338	ANID_04668.1	1D11	MpkC
48	A1339	ANID_04717.1	1D12	PkaB

49	A1341	ANID_04935.1	1E1	Uncharacterized
50	A1343	ANID_04980.1	1E2	Uncharacterized
51	A1344	ANID_05494.1	1E3	ChkA (Sc Chk1)
52	A1345	ANID_05511.1	2A1	Uncharacterized
53	A1347	ANID_05674.1	2A2	Uncharacterized
54	A1348	ANID_05728.1	2A3	Uncharacterized
55	A1349	ANID_05757.1	2A4	Uncharacterized
56	A1350	ANID_05759.1	2A5	Uncharacterized
57	A1354	ANID_06044.1	2A6	NpkA
58	A1355	ANID_06192.1	2A7	Uncharacterized
59	A1356	ANID_06207.1	2A8	Uncharacterized
60	A1357	ANID_06243.1	2A9	ImeB
61	A1358	ANID_06305.1	2A10	PkaA (Sc Tpk2)
62	A1360	ANID_06347.1	2A11	Uncharacterized
63	A1362	ANID_06508.1	2A12	Uncharacterized
64	A1363	ANID_06768.1	2B1	Uncharacterized
65	A1364	ANID_06975.1	2B2	UvsB (Sc Mec1)
66	A1365	ANID_07104.1	2B3	Uncharacterized
67	A1366	ANID_07185.1	2B4	Uncharacterized
68	A1367	ANID_07321.1	2B5	Uncharacterized
69	A1368	ANID_07537.1	2B6	Uncharacterized
70	A1369	ANID_07563.1	2B7	Uncharacterized
71	A1370	ANID_07572.1	2B8	SsrB (Sc Rim15)
72	A1371	ANID_07678.1	2B9	Uncharacterized
73	A1372	ANID_07695.1	2B10	Uncharacterized
74	A1373	ANID_07737.1	2B11	Uncharacterized
75	A1374	ANID_07986.1	2B12	Uncharacterized
76	A1375	ANID_08190.1	2C1	Uncharacterized
77	A1377	ANID_01315.1	2C2	Uncharacterized

78	A1378	ANID_02363.1	2C3	Uncharacterized
79	A1379	ANID_02581.1	2C4	Uncharacterized
80	A1380	ANID_03101.1	2C5	PhkB
81	A1381	ANID_03214.1	2C6	Uncharacterized
82	A1382	ANID_03946.1	2C7	SldA (Bub1/BubR1)
83	A1383	ANID_04113.1	2C8	Uncharacterized
84	A1385	ANID_04322.1	2C9	Uncharacterized
85	A1386	ANID_04447.1	2C10	Uncharacterized
86	A1387	ANID_04479.1	2C11	NikA
87	A1389	ANID_04818.1	2C12	Uncharacterized
88	A1391	ANID_08827.1	2D1	CmkC
89	A1392	ANID_08830.1	2D2	HalA (Sc Sat4)
90	A1393	ANID_08865.1	2D3	Uncharacterized
91	A1395	ANID_11101.1	2D4	Uncharacterized
92	A1396	ANID_01800.1	2D5	TcsB (Sc Sln1)
93	A1397	ANID_05296.1	2D6	TcsA
94	A1398	ANID_06820.1	2D7	Uncharacterized
95	A1399	ANID_07945.1	2D8	Uncharacterized
96	A1400	ANID_08261.1	2D9	PHOA (Sc Pho85)
97	A1401	ANID_09008.1	2D10	FphA
98	A1402	ANID_09048.1	2D11	Uncharacterized
99	A1404	ANID_11786.1	2D12	MPKA An5666
100	A1405	Control strain	2E1	CDS1008
101	A4	Parental strain		

2.2 Materials and Methods

2.2.1 Bioinformatics screen

3,520 genes including protein kinases, G proteins, GTPases and their respective regulators (involved in signaling pathways) associated with neuronal migration defects, abnormal neuron morphology and abnormal peripheral nervous system implicated in branching in humans, were selected from Human Phenotype Ontology (HPO) database (https://hpo.jax.org/app/) and used as queries for this work to find the conserved regulatory genes involved in signaling pathways that control branching morphogenesis in *A. nidulans*. To identify fungal homologues of the human genes, NCBI BLASTP analyses (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) were performed with using each *Homo sapiens* protein sequence as the query against *A. nidulans* as the optional model organism. The BLASTP showed the page containing best hits in *A. nidulans* for each *Homo sapiens* query protein as well as details about the alignments and links to the relevant database page for the proteins obtained for *A. nidulans*. Since the lower E-value ($E < 1e - 50$) reduces the chance of random matches among the query and the organism's sequence (or shows the significant match among them) (Lipman et al. 1984), best hits in this experiment were selected based upon $E < 1e - 50$. Among the best hits, finding the highest alignment score (equal or higher that 200) for each query was the basis for selecting the genes that were highly conserved among *A. nidulans* and *Homo sapiens*. Taken together, this information enabled us to decide which gene showed the strongest homology among others. Also, those genes in *A. nidulans* were identified through ASpGD database (http://www.aspergillusgenome.org) to study about their functions in more detail.

2.2.2 Strains and Media composition

Among the128 deletion strains related to *A. nidulans* kinases included expanded groups of 15 histidine kinases, 7 SRPK (serine-arginine protein kinases) kinases and an interesting group of 11 filamentous fungal specific kinases constructed previously by de Souza et al. 2013, library of 100 kinase viable deletion mutants (containing 99 deletion strains+ 1 wild type strain) (Table 2-1) (available from Fungal Genetic Stock Centre FGSC; http://www.fgsc.net/Aspergillus/KO_Cassettes.htm), was used, for this work. In this library, the control strain FGSC CDS1008 (2E1) (*wA3; argB2; ΔnkuA^{Ku70}::argB pyroA4; sE15 nirA14 chaA1 fwA1*) was considered as wild type (WT) strain (http://www.fgsc.net/Aspergillus/KO_Cassettes.htm). The strain FGSC A4 Glasgow wild type (*veA*$^{+}$) was considered as reference and parental strain in this study since it lacks any mutations (http://www.fgsc.net/). The deletions in table 2-1, are a precise gene replacement that removes the full gene (open reading frame from start to stop) and no other flanking region are affected. So, the only thing affected by that deletion is the PK coding gene. The process of how these deletions were constructed and rescued are explained by de Souza et al. 2013.

Media used for this research include MAG (malt extract 20g, agar 20g, dextrose 10g, peptone 2g, vitamin mix 2ml, trace elements 1ml, 1L ddH_2O) and Minimal-Nitrate or MN (1L ddH_2O, agar 18 g, 20x salts no nitrate 50 ml (ddH2O 1000ml, KCl 10.4g, MgSO4-7H2O 10.4g, KH2PO4 30.4g), dextrose 10g and ammonium tartrate 2g/L or 10ml (as nitrogen source).

2.2.3 Cell culture and Quantitative assay

A library of 100 *A. nidulans* kinase deletion mutants plus A4 as reference strain stored at -80°C were used and cultured on both MAG (supplemented with vitamin mix) and MN medium. While there were 128 mutant strains for this work, just 100 strains were selected since about 20% of the deletion strains were lethal and I could not study about their functions.

***Cell culture on MAG medium*—**Briefly, by using freezer stocks (including 100 kinase deletion strains plus A4 strain), 2µl of each individual strain was inoculated separately at the center of a 100mm solid MAG plate. The samples were then incubated at 28°C for 48 h. This experiment included 2 technical replicates for each sample used from -80 stocks. After evaluating those plates under dissecting microscope (explained in the following sections), the plates were stored as master plates (MPs) at 10°C room for further experiments. Inoculating the set of 100 kinase deletion strains on MAG media from -80 stocks was repeated 2 times.

The above experiment was repeated another time by using freshly harvested spores found in master plates. In other words, the spores of each strain were inoculated into 2µl of autoclaved sdH2O, then they were grown at the center of a large solid MAG plate (supplemented with vitamin mix). The samples were incubated at 28°C for 48 h. This experiment contained 2 technical replicates for each individual strain from MPs.

Overall, for culturing strains on MAG medium, there were 100 isogenic strains (the 100 strains were identical genetically except one difference which was kinase deletion) as well as 4 technical replicates (for each individual strains) meaning that the experiment was repeated 4 times (4 rounds) for the library of 100 kinase deletion strains.

***Cell culture on MN medium*—**Mutant strains (including 99 kinase deletion strains), wild type (2E1) plus A4 strains were grown on Minimal-Nitrate or MN medium and the same procedures described above for culturing them on MAG media, were followed for MN as well.

However, those plates were incubated at 28°C for 72 h. In this step like the previous one, spores from both -80 stocks and MPs were used. Therefore, this experiment contained 100 isogenic strains as well as 4 technical replicates (for each individual strains). Then after hyphal growth on each media, each individual strain was assessed in terms of the number of apical branches through a quantitative microscopy assay (QMA) (described in the following section).

Quantitative microscopy assay (QMA)—Through this assay, each individual plate (associated with either MAG or MN medium) was assessed in terms of the number of apical branches. Since in some colonies the density of hyphae on one side of the colony was higher than the other side and in other words the branches were not distributed equally on both sides of the colony, each individual colony, was divided into two sides with 200 hyphae counted on each side (400 hyphae totally) (Figure 2-5).

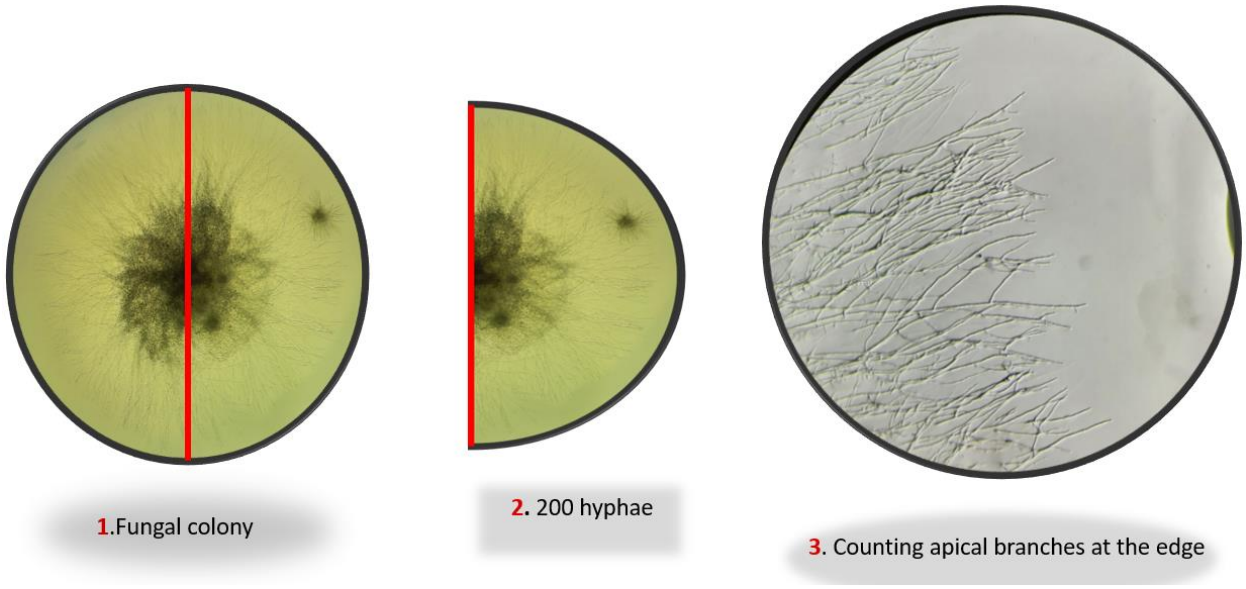

Figure 2-5. Quantitative microscopy assay (QMA) performed for branched hyphae from *Aspergillus nidulans* (strain FGSC CDS1008) colony with 4X magnification. The colony was divided in to two sides with 200 hyphae counted on each side. Then the number of apical branches

at the edge of each hypha were quantified. This quantitative microscopy assay was conducted under dissecting microscope with either 4x or 10x magnifications.

Then the numbers of apical branches at the edge of hyphae on both sides were counted and an average was taken among the numbers counted for both sides of the colony. This experiment was conducted for each round on each medium and the data was recorded in an Excel spreadsheet and used for further analysis (Appendix III) (Figure 2-6).

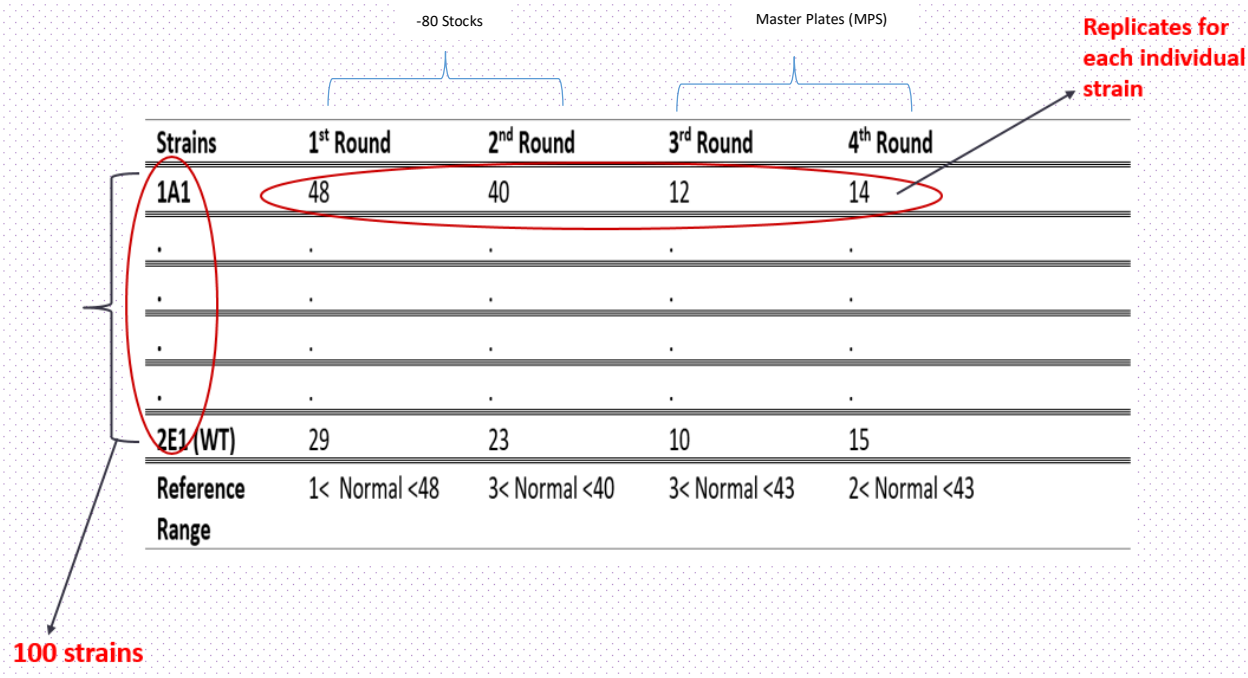

Figure 2-6. Illustrates how numerical results associated with QMA for each individual strain were sorted and organized in Excel spreadsheet for each round/replicate on each media (MAG or MN).

The whole procedures associated with cell culturing and QMA on both MAG and MN media have been illustrated by figure 2-7.

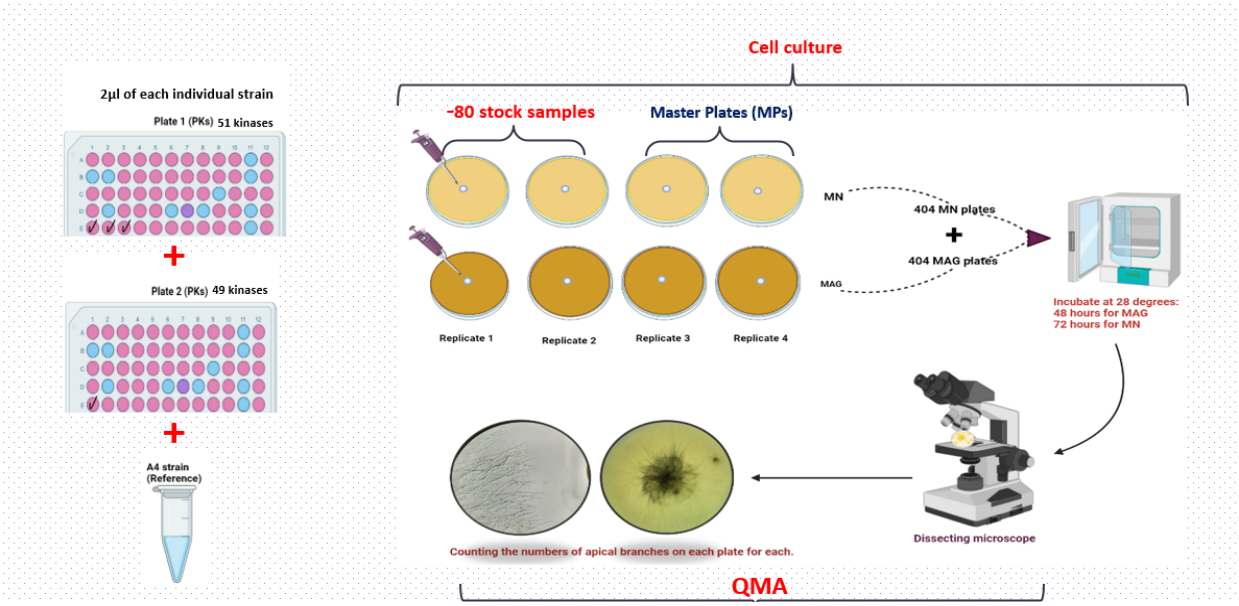

Figure 2-7. The process of inoculating mutant strains on both MAG and MN media as well as QMA. There are 4 technical replicates for each mutant strain on each media (either MAG or MN). 2 replicates are associated with -80 stocks and 2 replicates are related to MPs.

2.2.4 Establishing standard reference interval (RI) in filamentous fungi

To find the RI for each round of quantitative assay (2 rounds for -80 stocks and 2 rounds for MPs) on each medium (MN & MAG), following procedures were followed:1) the values for each round were sorted by Excel from the smallest amount to the largest amount, 2) the rank numbers were assigned to each value in the dataset and finally, the rank number of the α percentile (which could be either 2.5^{th} or 97.5^{th} percentiles) as $\alpha\ (n+1)/100$, where n is the sample size, was obtained. As the rank number is not always an integer, then it is required to round the rank number to the closest integer to define exact values at the percentile ranking of either 2.5% or 97.5% (Figure 2-8).

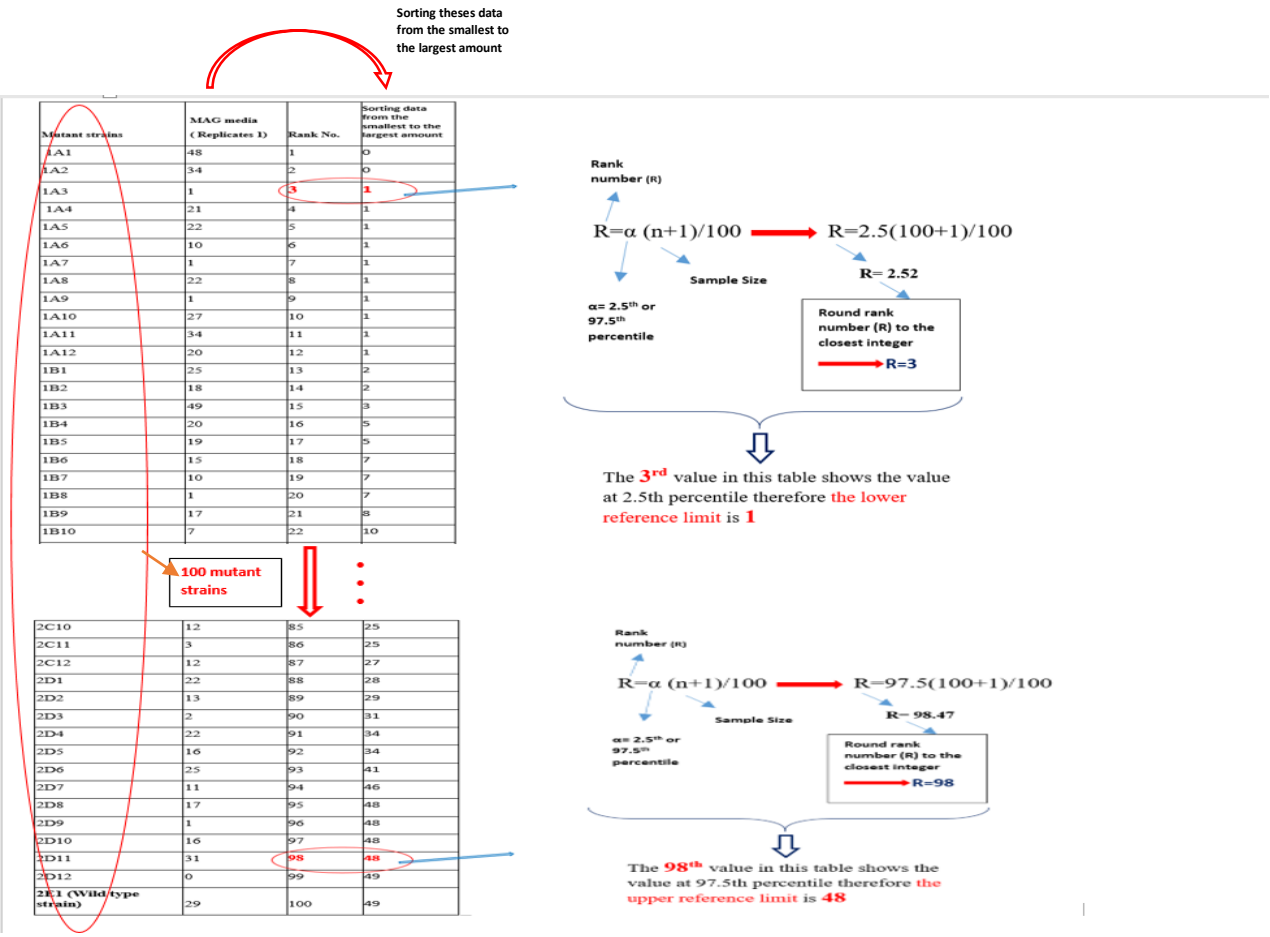

Figure 2-8. The process of finding the upper and the lower reference limits for the 1st round of QMA on MAG media. So, any strains in this round showing values lower than 1 or higher than 48 should be regarded as the strain illustrating abnormal apical branching morphology.

For example, in this work for the first round of -80 stocks on MAG medium, I considered n=100 and α=97.5th percentile to obtain the upper limit. Consequently, 98.4 was obtained as the rank number which fell between 98 and 99 and corresponded to the data values of 48 and 49 respectively. Since 98.4 is closer to 98 rather than 99, 98 should be considered as the rank number showing the value existed at 97.5th percentile which is 48. Then 48 was considered as the upper limit for the first round of -80 stocks on MAG media (Figure 2-8). The same procedure also was performed to obtain the lower limit by substituting 2.5th percentile in equation 1 for each dataset. The RI for each data set on either MAG or MN media can be found in Appendix III.

2.2.5 Confirmation test by EVOS microscope

To observe colonies by EVOS microscope, a thin layer of either MAG or MN media is required. To make thin layers, the media was melted into 2 separate small flasks (one for MN & one for MAG) for about 45 seconds in microwave and poured into the large petri dish to cover 1/3 plates. When plates were solidified, 2µl of each deletion strain marked as abnormal strains in previous step (*ΔpkaA* (2A10), *ΔnikA*(2C11), *ΔImeB*(2A9), *AN7695* (2B10), *AN7537* (2B6), *AN8865* (2D3) as well as WT strain (2E1), were inoculated on both MAG & MN separately and maintained at 28°C (2 days for MAG and 3 days for MN). After incubation time, the colony on each plate was separated carefully from the rest of MAG or MN medium by using a pincer and spatula (without harming the entire colony or hyphae) and transferred to a slide to observe under EVOS. Each slide was assessed with 10x and 20x magnifications to confirm the number of apical branches obtained previously through quantitative assay by dissecting microscope (Figure 2-9)

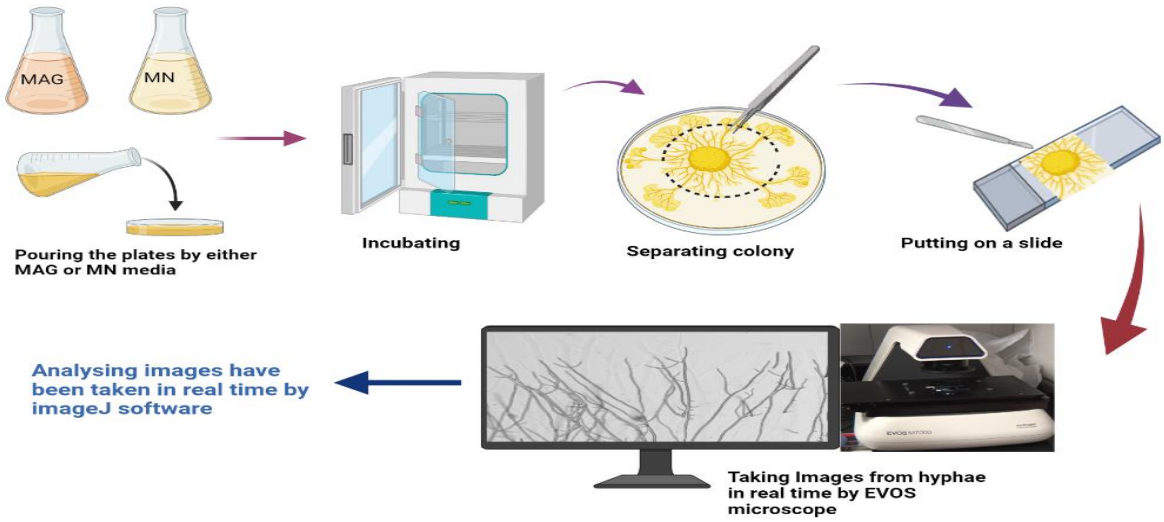

Figure 2-9. Performing QMA for the top 6 mutant strains under EVOS microscope.

Also, there were 4 technical replicates for each individual deletion strain in this part of experiment on each media (2times from -80 stocks and 2 times from MPs) meaning that this

experiment was repeated 4 times (4 rounds). In addition, the entire steps that were done for quantifying strains by dissecting microscope were repeated for EVOS as well. Those results were recorded as an Excel spreadsheet (Appendix IV). After capturing live images from 2A10 and 2E1 strains, the distance of newly formed apical branches from the hyphal tip as well as their angles were measured by ImageJ software.

2.2.6 Statistical analysis

The student t-test for two samples assuming unequal variances using Excel was performed for determining the statistical significance between the strains marked as abnormal through RI analysis, and the wild type strain (2E1) (statistical significance was a *P* value less than 0.05). Student t-tests were performed for all 99 kinase deletion strains plus the wild type strain (2E1). In this step each mutant strain with its replicates was organized in one group and compared to the second group including the wild type strain (2E1) with its replicates. This analysis was performed for the entire mutant strains grown on each media, separately. All data for this analysis are available in an Excel spreadsheet (Appendix V). In addition, to confirm that there is no significant difference between the samples obtained from -80 stocks and MPs, they were compared together using t-test for paired two samples for mean (Appendix VI).

2.3 Results

2.3.1 Results of bioinformatics screen

The results of blast search suggest that, among 3,520 genes involved in neuronal defects in humans only 275 genes were selected that showed the highest alignment score in blast search (=<200) as well as the E-value lower than 1e-50. This means that these 275 genes were highly conserved in *A. nidulans* which among them 28, 8 and 4 genes were assigned to PKs, GTPases

and G-proteins respectively, while other remaining genes (235 genes) were involved in other stress response pathways including high-osmolarity glycerol (HOG) pathway, target of rapamycin (TOR) pathway and reactive oxygen species (ROS). The results show that there are conserved PKs involved in signaling pathway. Therefore, deletion of those predicted PKs in *A. nidulans* might lead to some defects in branching morphogenesis (Appendixes I &II) (Figure 2-10).

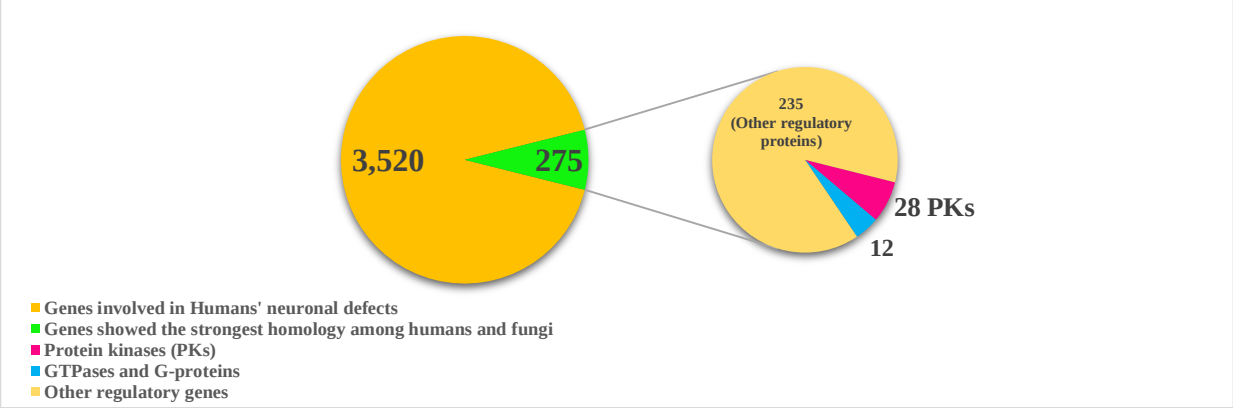

Figure 2-10. This pie chart shows among 3,520 genes associated with humans' neuronal defects, 275 genes showed the strongest homology in *A. nidulans* which among those just 28 Pks were found that were highly conserved in this fungus.

A genetic screen through QMA identifies protein kinases mediating apical branching morphogenesis.

In total there were 101 mutant strains including 99 kinase deletion strains plus one wild type strain (2E1) as well as A4 strain used for this experiment. The 100 deletion strains and A4 strain were screened for four rounds (2 rounds for -80 stocks and 2 rounds for MPs) on either MAG or MN medium (supplemented with vitamin mix) with applying QMA combined with establishing RI for each round.

2.3.2 Results of QMA on MAG medium— After performing QMA for each individual strain and defining the standard reference interval (RI), the strains that showed a value less than the lower reference limit or larger than the upper reference limit were detected as abnormal strains. The results of screening on MAG medium illustrated 24 kinase deletion strains that showed abnormality in terms of the number of apical branches (Appendix VII). Based upon that 2A10, 2A9, 2B6, 2C11, 2B9, 2B4, 1A3, 1B8, 2D12, 2D3,1A9, 2C1,1D5, 1A7, 2D9 and 1B12 deletion strains revealed hypo-apical branching phenotype. Also, 2B10, 1D11, 2C3, 1D6, 1A1, 1B3, 2B7 and 1D8 deletion strains showed hyper-apical branching phenotype. A total of 9 kinase deletion strains (2A10, 2A9, 2D3, 2B6, 2C11, 2B10, 2C1, 2D12 and 1A1) were found among the 24 mutant strains, that revealed abnormal branching phenotype either hypo- or hyper- apical branching more than 2 times during the four rounds of screening and quantifying, while the other remaining 15 strains showed abnormal phenotype once or twice on MAG media (Appendix VII).

2.3.3 Results of QMA on MN medium— To confirm the results of QMA on MAG media, the growth condition was changed to MN media and the role of 100 kinase deletion strains as well as A4 strain in regulating apical branching morphogenesis was assessed additional time in new environment. As a result, of the 100 deletion strains, 25 deletion strains were identified showing aberrant apical branching morphology, which 6 of them showed either decreased or increased apical branching phenotype more than 2 times during the four rounds of screening including 2A10, 2A9, 2D3, 2B6, 2C11, 2B10 (Appendix VII). In other words, the top 6 mutant strains flagged as abnormal strains in MAG experiments were confirmed again through MN experiment. The other strains that revealed abnormal phenotype once during the QMA on MN medium, revealed normal phenotype on MAG media (Figure 2-11).

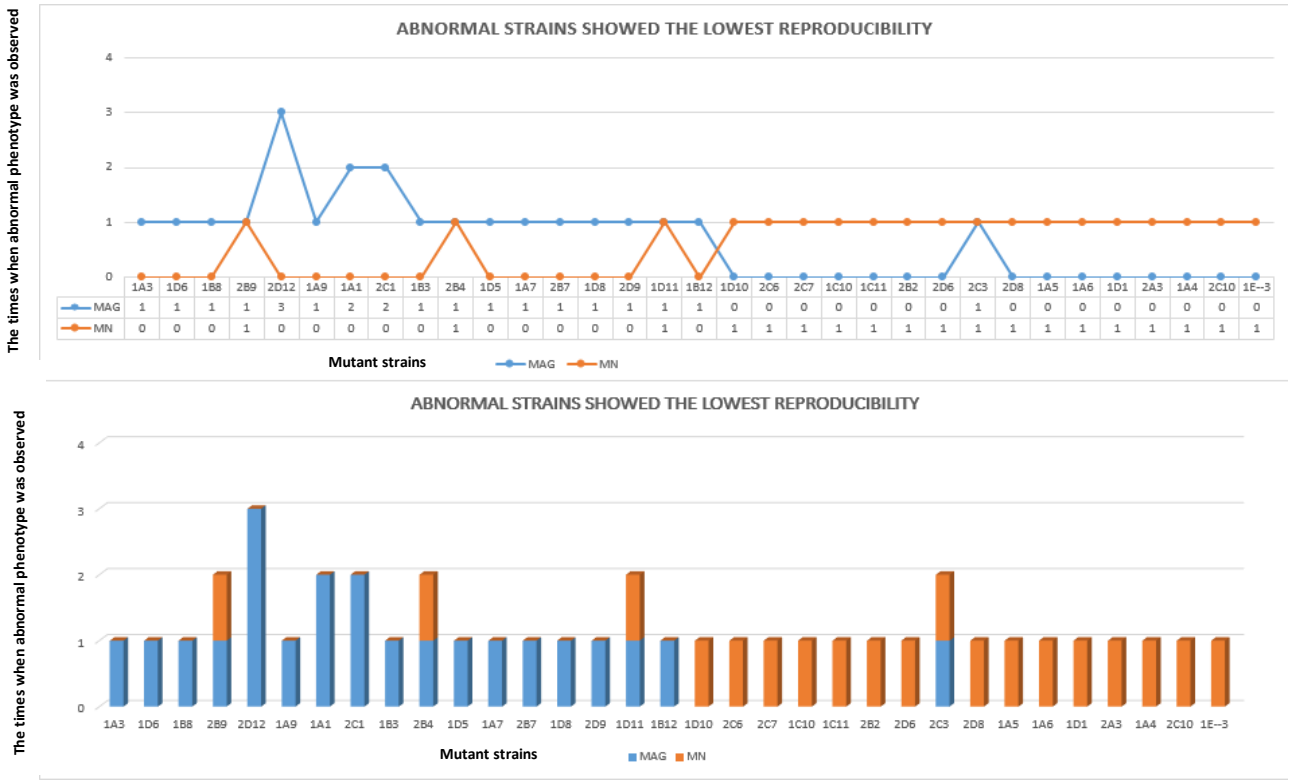

Figure 2-11. The repetitiveness of abnormal strains revealed hypo- or hyper apical branching phenotype once or twice during the four rounds of quantitative microscopy assay.

***2.3.4 Results of QMA on both MAG and MN medium*—**Taken together, when the results of MAG medium were compared with MN medium, 10 mutant strains including 2A10, 2A9, 2D3, 2B6, 2C11, 2B10, 2B9, 2B4, 1D11, 2C3 were found to be common among the two media types, of which the top 6 strains were selected showing the highest reproducibility of abnormal apical branching phenotypes on both media types (Table 2-2) (Appendix VII).

Table 2-2. The top 6 mutant strains as the result of QMA and RI analysis on MAG and MN media.

#Rank	Cell	Gene Name	The times when abnormal apical branching pattern was observed on MAG medium	Abnormal Phenotype on MAG medium	The times when abnormal apical branching pattern was observed on MN medium	Abnormal phenotype on MN medium
1	**2A10**	**pkaA**	**4**	**Hypo**	**4**	**Hypo**
2	2A9	ImeB	3	Hypo	3	Hypo
3	2D3	ptkA	3	Hypo	2	Hypo
4	2B6	Uncha	3	Hypo	2	Hypo
5	2C11	nikA	3	Hypo	2	Hypo
6	2B10	Uncha	2	Hyper	2	Hyper

2A10 and 2A9 showed hypo- apical branching phenotype for 4 and 3 times respectively on both media, while 2B6 and 2C11 revealed hypo-apical branching phenotype 3 times on MAG and 2 times on MN media respectively. Hypo-apical branching phenotype which was observed 3 times on MAG medium and 2 times on MN media is associated with 2D3 strain. By contrast, for 2B10 strain the number of apical branches were increased more than the defined normal range for 2 times during the four rounds of screening and quantifying (Table 2-2) (Appendix VII). 2B6 and 2B10 are uncharacterized kinases.

Figure 2-12 illustrates the mutant strains that revealed aberrant apical branching phenotype more than 2 times on either MAG or MN medium, while figure 2-11 shows the mutant strains revealed abnormal apical branching phenotype once or twice on either MAG or MN. The ones showed abnormal phenotype on one media, revealed normal phenotype on the other medium (Figure 2-11).

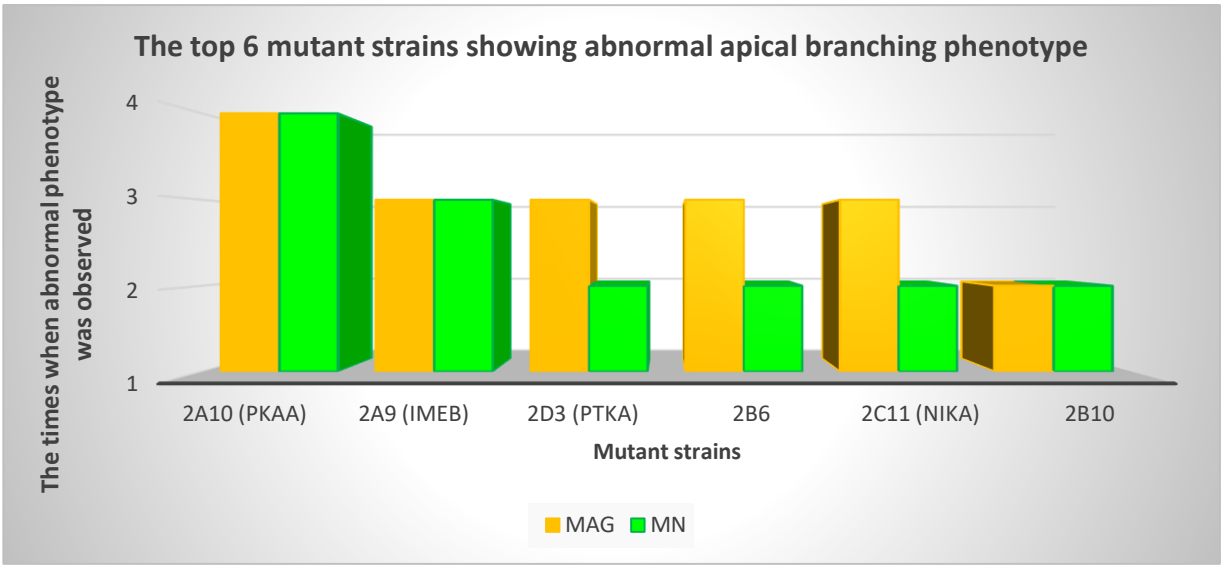

Figure 2-12. The reproducibility of either hypo- or hyper- apical branching phenotype by the top 6 abnormal strains on MAG and MN media. 2A10 (*pkaA*) deletion strain shows hypo apical branching phenotype 4 times that is more than other strains.

***2.3.5 Results of student t-test for both MAG and MN medium*—**To validate the results of the observed differences between the strains marked as abnormal through RI analysis (the top 6 mutant strains) and the wild type strain (2E1) statistically, all replicates related to each individual strain were organized in different categories and compared with wild type strain (2E1) using student t-test for two samples assuming unequal variances. As a result, among 99 kinase deletion strains, 6 common strains were found on both media that revealed significant difference statistically compared to the wild type strain including 2D3, 2B6, 2A9, 2B10, 2A10 and 2C11 (Table 2-3) (Appendix VII).

Table 2-3. The student t-test results of MAG and MN media.

#Rank	Cell	Gene Name	Results of student t-test on MAG medium (P<0.05)	Results of student t-test on MN medium (P<0.05)
1	2A10	pkaA	0.007	0.004
2	2A9	ImeB	0.008	0.004
3	2D3	ptkA	0.007	0.006
4	2B6	Uncha	0.018	0.006
5	2C11	nikA	0.007	0.005
6	2B10	Uncha	0.008	0.030

Therefore, through statistical analysis I was able to select the top 6 strains as interesting candidates that showed significant difference with the WT strain in terms of showing abnormal apical branching phenotype. This suggests these strains may play a significant role in regulating apical branching morphogenesis. Before comparing mutant strains with the wild type strain, A4 as a reference strain was compared with 2E1 (WT) and no significant difference was found among them. Also, the replicates associated with -80 stocks were compared with the replicates related to MPs and there was no significant difference between them (Appendix VI).

***2.3.6 Results of confirmation test by EVOS microscope*—**To validate the results of the statistical analysis, those 6 strains as well as 2E1 strain were assessed additional time under the EVOS microscope (Appendix IV). Using EVOS as a fluorescence microscope provided the maximum magnification of 20X (compared with a maximum magnification of 11.5X for the dissecting microscope) allowing more precise quantification of apical branches. Strains 2D3 (ptkA), 2B6 (AN7537), 2A9 (ImeB), 2B10 (AN7695), 2A10 (pkaA) and 2C11 (nikA) showed an abnormal apical branching pattern on both MAG and MN medium during the four rounds of screening and quantification by EVOS microscope (Table 2-4).

Table 2-4. The results of QMA and student t-test by EVOS microscope on MAG and MN media.

#Rank	Cell	Gene Name	The times when abnormal apical branching pattern was observed on MAG medium	Abnormal phenotype on MAG medium	Results of student t-test on MAG medium (P<0.05)	The times when abnormal apical branching pattern was observed on MN medium	Abnormal phenotype on MN medium	Results of student t-test on MN medium (P<0.05)
1	2A10	pkaA	4	Hypo	0.007	4	Hypo	0.004
2	2A9	ImeB	3	Hypo	0.008	3	Hypo	0.004
3	2D3	ptkA	3	Hypo	0.007	2	Hypo	0.006
4	2B6	Uncharacterized (AN7537)	3	Hypo	0.061	2	Hypo	0.006
5	2C11	nikA	3	Hypo	0.007	2	Hypo	0.005
6	2B10	Uncharacterized (AN7695)	2	Hyper	0.008	2	Hyper	0.030

These results confirm the results of statistical analysis in previous step (Table 2-3) (Appendix IV) that shows there is a significant difference between wild type and the top 6 mutant strains. To better understand this significant difference and monitor apical branching pattern in real time, EVOS microscope was used to capture images in real time. For this experiment, among the top 6 kinases just one of the mutant strains, 2A10 (pkaA) was selected and compared with WT strain. There are some reasons why 2A10 strain was selected for capturing live images among the top 5 kinases. First, this strain showed abnormal apical branching phenotype more than other strains with a strong growth defect on both MAG and MN media as well as showing a significant difference with WT strain (Table 2-3). Secondly, we know all the phosphoproteomics data and targets for 2A10 (pkaA) strain (Ribeiro et al. 2019), whereas the phosphoproteomics data for other top 5 PKs is not available. The other point is, the experiment was time consuming if I wanted to compare all the top 6 kinase with the WT under EVOS microscope.

While new apical branches in the 2A10 strain emerged at an average distance of 92.86 μm from the hyphal tip (Figure 2-13) (Table 2-5), in the 2E1 strain (WT) the new apical branches emerged with a mean distance of 80.82 μm from the hyphal tip (Figure 2-14) (Table 2-6). I could not obtain standard deviation for the length because there was a lot of variation among the data.

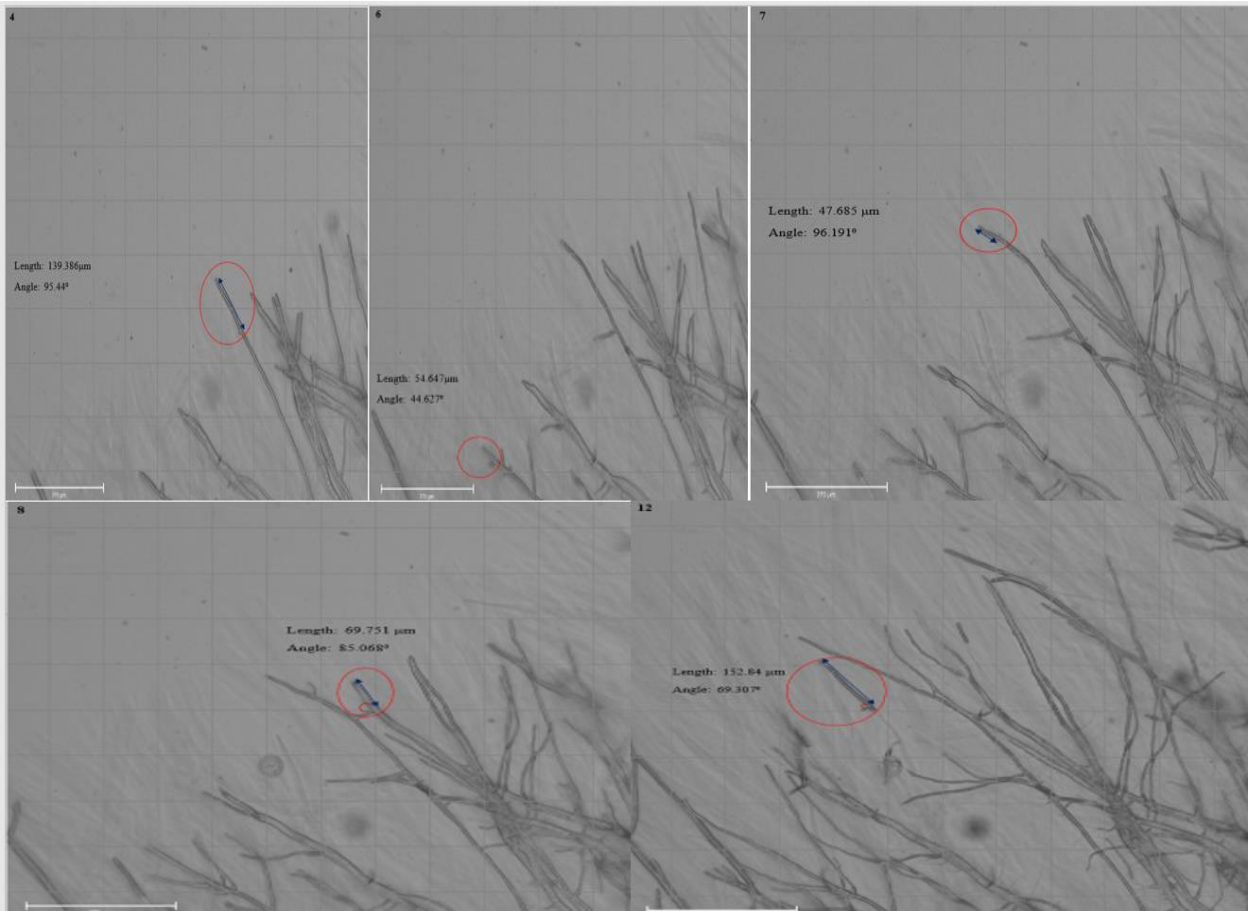

Figure 2-13. Shows images of hyphal growth and branching patterns associated with 2A10 or *pkaA* deletion strain in real time. These images have been taken every 30 minutes by EVOS microscope and 10X magnifications during 6 hours of hyphal growth (among the 12 images, here only images 4, 6, 7 & 12 have been shown).

Table 2-5. The length and angle of apical branches in 2A10 strain.

Image No.	Length (µm)	Angle
4	139.386	95.44
6	54.647	44.627
7	47.685	96.191
8	69.751	85.068
12	152.84	69.307
Average:	**92.86**	**78.12**

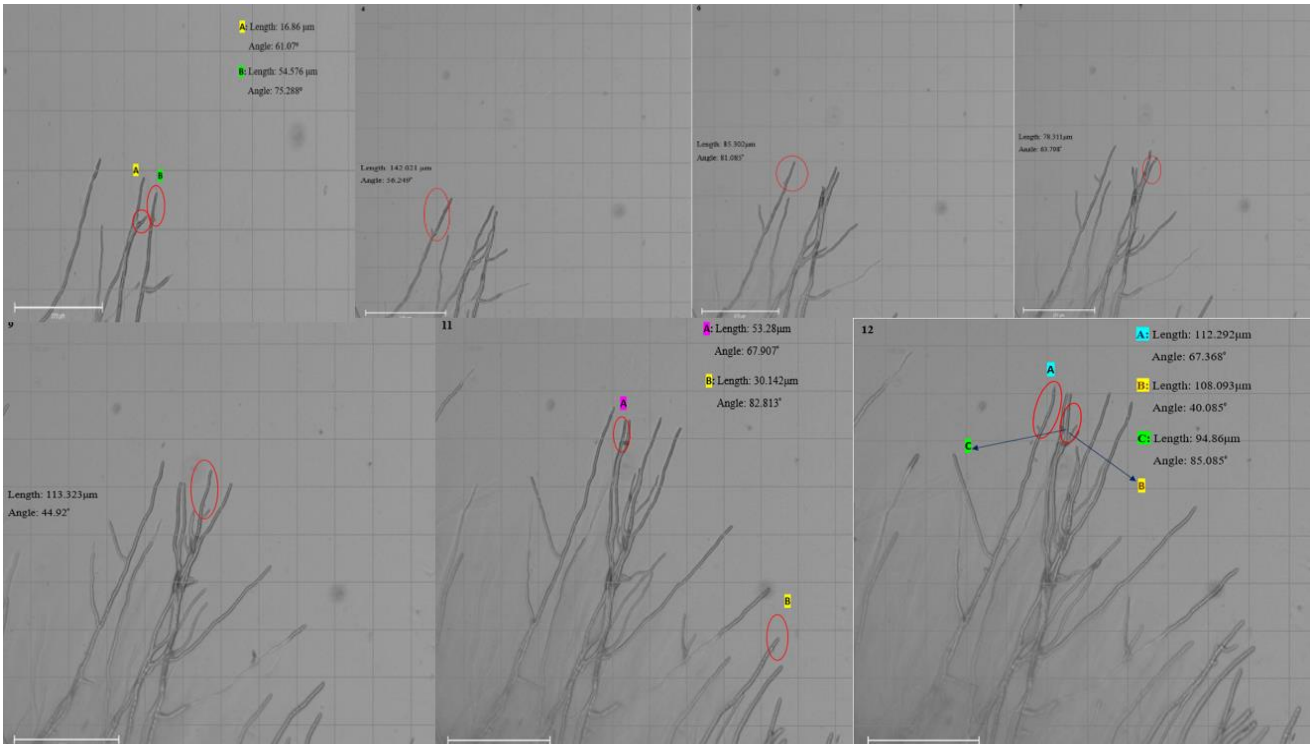

Figure 2-14. Illustrates images of hyphal growth and branching patterns in real time associated with *Aspergillus nidulans* wild type strain, 2E1 (strain FGSC CDS1008). The images have been captured during 6 hours by EVOS microscope and 10X magnifications every 30 minutes (among the 12 images here only images 2, 4, 6, 7, 9, 11 & 12 have been shown).

Table 2-6. This table shows the length and angle of new apical branches associated with 2E1 strain.

Image No.	Positions	Length (µm)	Angle
	A	16.86	61.07
2	B	54.576	75.288
4	…	142.021	56.249
6	…	85.302	81.085
7	…	78.311	63.798
9	…	113.323	44.92
	A	53.28	67.907
11	B	30.142	82.813
12	A	112.292	67.368
	B	108.093	40.085
	C	94.86	85.085
Average		**80.82**	**65.96**

The second obvious difference that could be observed through these images is, 2A10 strain showed hypo-apical branching pattern (5apical branches during 6 hours of hyphal growth) (Figure 2-13) compared to 2E1 which showed 11 apical branches during 6 hours of hyphal growth (Figure 2-14). The third clear difference in 2E1 strain, was the average angle between the new branches and parental hyphae. So, the average angle in 2E1 (65.96°) was narrower than the average angle in 2A10 strain (78.12°) (Figures 2-13 & 2-14, Tables 2-5 & 2-6). These results suggest that while the data associated with tables 2-5 & 2-6 are not statistically significant and there is a lot of variation in the process, using EVOS microscope helped us to follow apical branching process in real time which was not achievable if we wanted to use dissecting microscope. This showed that apical branching process might be monitored by different regulations and environmental factors

that we need to understand first how those factors regulate apical branching. So, we need to be careful about the conclusions we make about branching since there is a lot of variations.

2.4 Discussion

Bioinformatics screen identified highly conserved PKs involved in branching morphology and signaling pathway in *A. nidulans.*

Among the list of 28 PKs that showed strongest homology in *A. nidulans*, 6 PKs were selected that play role in hyphal branching in other fungal species. Among those pkaR kinase was identified that is the target of PKA pathway (Ribeiro et al. 2019). Another kinase in this list (Table 2-7) named torA is activated through MPKA pathway (Chelius et al. 2019).

Table 2-7. This table shows the list of PKs might be involved in hyphal branching in *A.nidulans*.

GENE_ENTREZ_ID	GENE_SYMBOL (*Homo sapiens*)	DISEASE_IDS	AN IDs (*Aspergillus nidulans*)	Feature type	Function	Accession Number
207	AKT1	OMIM:615109,OMIM:114480,OMIM:176920,OMIM:167000,OMIM:114500	pkcB/AN5973	ORF, Verified,	Protein with similarity to protein kinase C; involved in polar axis establishment and germling growth; mutant is inviable and forms microcolonies	XP_663577.1
10000	AKT3	OMIM:615937	pkcA/AN0106	ORF, Verified	Protein kinase C; essential gene; required for wild-type level of growth, cell wall integrity and penicillin biosynthesis	BAD02338.1
2475	MTOR	OMIM:616638,OMIM:607341	torA/AN5982	ORF, Verified,	Tor kinase homolog; essential; heterozygous diploids show increased sensitivity to DL-aspartic acid beta-hydroxamate in ammonium medium and enhanced growth on threonine; null mutants have early septation defect and arrest as short germlings	CAG30554.1
5297	PI4KA	OMIM:616531	stt4/AN4278	ORF, Uncharacterized,	Essential 1-phosphatidylinositol 4-kinase with a predicted role in phospholipid metabolism; mutants arrest in the cell cycle	XP_661882.1
1760	DMPK	OMIM:160900	cotA /AN5529	ORF, Verified,	Essential NDR ser/thr protein kinase; role in cell polarity; RAM-signaling pathway component; MobB/CotA kinase complex thought to regulate cell polarity growth by maintaining cellular calcium homeostasis; mutant forms brown microcolonies	XP_663133.1
5573	PRKAR1A	OMIM:610489,OMIM:255960,OMIM:160980,OMIM:101800	pkaR /AN4987	ORF, Uncharacterized,	Putative protein kinase A (PKA) regulatory subunit	XP_662591.1

Both MPKA and PKA pathways are involved in various functions such as sensing the environmental cues, responding to cellular stresses, hyphal growth, inducing lateral branch

formation in *A. nidulans* and other necessary functions in this fungus (Casado et al. 2011; Freitas et al. 2010; Fuller et al. 2011; Liebmann et al. 2004; Thevelein et al.2005). For example, in *N. crassa* mutants in the R subunit of PKA (or *mcb* gene) had complete loss of growth (Dickman & Yarden 1999). In table 2-7 pkcB, pkcA, cotA (Shi et al. 2008), Cka1(Rethinaswamy et al. 1998, Snell et al. 1994) and stt4 kinases were identified. These specific kinases may play fundamental roles in hyphal branching, hyphal growth, conidia formation as well as polarized growth (de Souza et al. 2013). Deletion of those genes may result in the induction of abnormal branching patterns (de Souza et al. 2013). Knowing this gives us a confidence to validate that PKs are not only the major part of the signaling pathway but also they might play a significant role in regulation of branching morphogenesis in *A. nidulans.* This gives us the confidence to investigate the role of a specific set of 100 PKs (Table 2-1) experimentally, in governing apical branching pattern in *A. nidulans* in the next steps. However, none of the 28 PKs that showed the strongest homology in bioinformatics screen was presented among the 100 kinase deletion library. Because those 28 PKs were completely lethal and we were not able to consider them and study their functions (Appendix II). The results of blast search can be found in Appendixes I&II.

2.4.1 Interpretation of results associated with MAG and MN media—Since fungi show various behaviors in different environmental conditions by sensing different environmental stimuli (Biswas et al. 2007), two media used for this study to precisely indicate the main regulators of apical branching morphogenesis among 100 kinase deletion strains. Through a genetic screen of 100 kinase deletion strains on both MAG and MN medium, 39 mutant strains were flagged as abnormal which mediate apical branching morphology by indicating either hypo- or hyper- apical branching phenotype (Appendix VII). Of the 39 mutant strains 2D3, 2B6, 2B10, 2C11, 2A10 and 2A9 showed abnormal phenotype more than 2 times during the four rounds of QMA on both MAG

and MN media (Figure 2-12). Therefore, these mutant strains can be regarded as interesting candidates which control apical branching morphogenesis.

While 2D12 (mpkA) showed hypo- apical branching phenotype 3 times on MAG medium (with the average of 0 apical branches), the average of apical branches related to this strain during the four rounds of quantifying on MN media was 13. This indicates the normal phenotype based upon the defined RI (Appendix III). Although several studies have shown that mitogen-activated protein kinase [MPKA] (or 2D12 strain) plays an integral role in growth and differentiation (Bussink and Osmani 1999; Martínez-Soto and Ruiz-Herrera 2017; Valiante et al. 2008), since this strain showed a very different behavior and found on MN media as a strain showing normal phenotype after 4 trials, it may not be considered as a potential candidate regulating apical branching morphogenesis in this experiment.

Another interesting candidate nominated to regulate apical branching morphogenesis in *A. nidulans* in this study is 2D3 or ptkA kinase (AN8865). The results of this study indicates that the *Δptka* strain shows hypo- apical branching (average of 3 apical branches) 3 times and 2 times (average of 4 apical branches) on MAG and MN media respectively. This suggests that this mutant shows abnormal hyphal growth with forming dense, needle like shape hyphae and indicates strong growth defects on both media. This finding can be further supported by Shim et al. (2002) and Garriga et al. (2010) illustrating that in mammalian cells like *Caenorhabditis elegans,* ptkA homologue, Cdk9 is essential for the expression of genes during early development and deletion of this gene results in up and down- regulation of some specific genes involved in RNA synthesis. These finding are also supported by a study conducted by de Souza et al. 2013 who determined ptkA kinase deletion strain shows strong growth defects in the environments containing NaCl. Suggesting that the strains lacking ptkA function might be sensitive to nutritional changes in the

environment. One of the ingredients used for MN media in this research is 20X salts. This is probably one of the reasons for why this deletion strain showed strong growth defects, very dense and needle like-shape hyphae on this medium. Consistent with these views, while defects in ptkA impair hyphal growth and it leads to hypo apical branching phenotype depends on the growth conditions, the amount of studies conducted in this area to fully characterize the function of this gene in regulating hyphal branching is not enough (de Souza et al. 2013; Kempf et al. 2013).

The other candidate is ppk33(AN7537) or the 2B6 strain as an uncharacterized kinase in *A. nidulans* whose deletion showed hypo-apical branching phenotype 3 times on MAG with 1 apical branch on average and the same phenotype 2 times on MN with 4 apical branches on average. This indicates that this kinase may be one of the regulators of apical branching phenotype as deletion of this kinase leads to abnormal apical branching phenotype on two different growth conditions. However, it cannot be considered as the main regulator of apical branching since it revealed normal phenotype 2 times on MN media. Therefore, to regulate apical branching the function of this kinase may be dependent on the function of other PKs or it may have some interactions with other kinases. Following that, Chen et al. (1993), illustrated *Saccharomyces cerevisiae* ppk33 homologuesYPK1 is associated with defects in vegetative growth, while de Souza et al. 2013 suggested in the presence of NaCl, deletion of ppk33 leads to inhibit the growth rate in *A. nidulans*. The other uncharacterized kinase in this study that revealed hyper apical branching phenotype 2 times on both media was 2B10 strain (AN7695) whose yeast homologue is snf1. While this strain showed normal phenotype 2 times on both growth conditions, it may not be regarded as the main regulators of apical branching morphogenesis. Also, Sanz (2003) indicated *S. cerevisiae* AN7695 homologue Snf1 plays a key role in regulating a wide range of functions in yeasts including cellular response to several forms of stress, such as nutrient limitation, salt stress

and heat shock, while there is no evidence suggesting that this kinase plays role in underlying hyphal branching morphogenesis. Therefore, its function in *A. nidulans* remains mysterious.

Deletion of 2C11 strain (AN4479) or nikA kinase in *A. nidulans* results in strong growth defects (Hagiwara et al. in 2013). The observed colonies lacking *nikA* in *A. nidulans* in this study exhibited a hypo-apical branching phenotype 3 times on MAG and 2 times on MN media during the four rounds of QMA with the average of 1 and 4 apical branches respectively. This indicates that this kinase is involved in regulating hyphal growth and apical branching morphogenesis. This is an agreement with Hagiwara et al. (2013), who illustrate that in *Aspergillus fumigatus* deletion mutants associated with nikA caused low conidia production, abnormal hyphae, sensitivity to high osmolarity stresses, resistance to cell wall perturbing reagents such as congo red and calcofluor white, as well as to fungicides such as fludioxonil, iprodione, and pyrrolnitrin. Therefore, nikA plays a fundamental role in regulating several functions in *A. fumigatus* (Hagiwara et al. in 2013). Deletion of nikA in *S. cerevisiae* impairs responses to oxidative stress (Raitt et al. 2000). While nikA can be considered as the candidate regulating apical branching, there are still two fundamental candidates such as pkaA and ImeB kinases that should be compared to them.

In this study hypo-apical branching phenotype was found 3 and 4 times as a result of deleting ImeB (AN6243) (2A9 strain) and pkaA (AN6305) (2A10 strain) kinases respectively on both growth conditions in *A. nidulans.* Previous studies revealed a genetic coordination between ImeB and PkaA genes in regulating hyphal morphogenesis (Bayram et al. 2009; Mitchell et al. 1990). For example, in yeasts Ime2 that is a subunit of ImeB act in concert with the cyclin dependent kinase Cdk1 which triggers meiosis (Mitchell et al. 1990; Yue et al.2017). Interestingly, Cdk1 located at the downstream of the cyclic AMP-dependent protein kinase shows that ImeB gene is activated indirectly through PKA pathway so that the growth rate is impaired severely by

defects in ImeB gene (Yue et al.2017). Another study shows that in *A. nidulans* the strains which lack the function of ImeB result in abnormal differentiation of sexual cells as well as a strong growth defect (Bayram et al. 2009; Bayram & Braus 2012) suggesting this kinase might play role in generating abnormal hyphal branch formation in *A. nidulans*. These findings are consistent with our finding as we observed reduction in the number of apical branches 3 times during the four rounds of counting for the strain lacking ImeB gene function.

The previous findings suggest that PKA is involved in a broad range of processes in eukaryotic systems and mutations in PKA lead to many defects in cellular regulations. In addition, in a research by Ribeiro et al. (2019), all of the phosphorylation targets of PKA pathway were determined in *A. nidulans*, while no research has been done yet to characterize the phosphorylation targets of other top 5 kinases obtained in this research (ptkA, ImeB, nikA, AN7537, AN7695). In this chapter also the results showed that deletion of *PkaA* in *A. nidulans* leads to hypo-apical branching phenotype 4 times on 2 media types constantly during the four rounds of screening assay compared to other top 5 deletion strains in this experiment (Table 2-2) (Appendix VII). Student t-test in this experiment confirmed that there is a significant difference between the mutant (2A10) and the wild type strains statistically (Table 2-3). The significant differences that were obtained on both environmental conditions, MAG and MN media, between the *2A10*(*pkaA*) deletion strain and wild type strain were 0.007 and 0.004 respectively. Also the result of statistical analysis was confirmed by observing the 2A10 strain under EVOS microscope with capturing images in real time that made it possible to detect the differences between 2A10 and the WT (2E1) strain (Figures 2-13 & 2-14). These finding can be supported by the study done by de Souza et al. 2013 which indicated that the *pkaA* deletion strain shows defects in growth rate with needle-like shape hyphae. Taken together the results of this study suggest that *pkaA* kinase might be the most potential

regulator of apical branching morphology in *A. nidulans* compared to other top 5 kinases nominated as the potential kinases that regulate apical branching morphogenesis in this research.

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Chapter 3:

Identification of protein kinases that potentially modulate apical branching morphogenesis in *Aspergillus nidulans* following an environmental shift.

Abstract

The ability of fungal hyphae to sense their local environment have a significant impact on growth and morphogenesis. Filamentous fungi differentiate and develop by various mechanisms directed by intracellular and extracellular factors. However, the important protein kinases required for adjustment of apical branching pattern when hyphae experience a sudden environmental change, remain poorly understood. mpkA, an important kinase involved in the MAPK (mitogen-activated protein kinase) pathway, mediates a wide range of intrinsic cellular processes including sensing nutrients in the environment, responding to environmental cues as well as regulating fungal development and pathogenicity. This study suggests that this kinase plays a fundamental role in monitoring apical branching in *A. nidulans* when the growth condition underlies dramatic change. Using *A. nidulans* as a model organism in this study, I developed a quantitative microscopy assay (QMA) along with a proper statistical approach (identifying standard reference interval (RI), for detecting the abnormal strains in fungal population. This method allowed the screening of the number of apical branches associated with 100 kinase deletion mutants to find hypo- or hyper apical branching morphogenesis that each strain showed on two different media including MAG+proline hole and proline+MAG hole. Significant correlation was found between the absence of the top 6 potential kinases and aberrant apical branching phenotype in *A. nidulans* under two different environmental conditions. Collectively, this research not only reveals the specific role of MAPK pathway as the regulator of apical branching in *A. nidulans* when environmental conditions change, but also provides insights into other regulatory pathways that underlie apical branching morphogenesis.

3.1 Introduction

Filamentous fungi are micro-organisms with a sessile lifestyle that can be found in a wide variety of substrates including plant debris, soil, decaying woods and even living animal and human tissues (Hagiwara et al. 2016). One of the most conspicuous features of filamentous fungi is that they are known as saprophytic organisms, playing a fundamental role in recycling carbon or nitrogen in nature (NAR & GM 1995). While the beneficial role of a variety of fungal species has been indicated in medicine, agricultural and fermentation industry, there are some fungi that can endanger the lives of humans or animals' life by forming an invasive vegetative growths called mycelia (hyphal networks) (Harris 2010; Van Peer1 et al. 2009). Therefore, development of a fungal colony is highly dependent on generating a broad network of hyphal branches to obtain necessary nutrients from their surroundings (Harris 2010; Van Peer1 et al. 2009). Sensing and absorbing sufficient nutrients are regulated through signaling mechanisms that allow fungi to sense and respond to subtle fluctuations caused by several abiotic and biotic stressors including temperature changes, dryness, nutrient levels, oxygen availability, ultraviolet (UV) radiation and oxidative stress (Biswas et al. 2007; Harris 2008; Moore et al. 2005; Trinci 1978). Consistent with this view, based upon the genetic analysis and genome sequencing of *Aspergillus oryzae, A. nidulans, and A. fumigatus*, it has been shown that the stress response molecules in fungi are targets of rapamycin (TOR) signaling, protein kinase A (PKA) signaling pathways, mitogen-activated protein kinase cascades (MAPK), and calcineurin signaling as well as upstream signaling machinery (G-protein coupled receptors, G-protein subunits, and two-component signaling (TCS) system). These mechanisms have been studied extensively in *Saccharomyces cerevisiae* (Ende et al. 2019; Moriya and Johnston 2003). For example, the MAPK pathway, that has been highly conserved in fungi, animals and plants, consists of 3 subunits including MAPK, MAPK kinase

(MAPKK), and MAPK kinase kinase (MAPKKK) (Karunanithi and Cullen 2012). Phosphorylation of these MAPK cascades simultaneously transfer required environmental signals from cytosol to the nucleus leading to appropriate cellular responses (Chen &Thorner 2007; Karunanithi and Cullen 2012). In *S. cerevisiae* it has been showed that MAPK is involved in regulating various functions such as the mating-pheromone response pathway, the pseudohyphal development pathway, the high osmolarity glycerol (HOG) pathway, the protein kinase C or the cell wall integrity pathway and the spore wall assembly pathway (Assis et al. 2020; Chelius et al. 2019). Also, the PKA signaling pathway plays a critical role in regulating cellular physiology and morphological switches in response to nutrient availability (Kayikc & Magwene 2004).

Nutrient sensing is of great importance not only in filamentous fungi but also in other eukaryotic systems because it is critical for adaptation of micro-organisms to environmental conditions and for establishing appropriate developmental programmatic changes in response to nutrient stresses (Caza &Kronstad 2019). Filamentous fungi are well adapted to maintain high rates of growth in environments containing sufficient amount of glucose which suggest that glucose, is a preferred carbon source in these organisms (Sabina & Brown 2020). Genetic studies revealed that organisms ranging from bacteria to humans have sophisticated sensing and signaling machinery specialized to detect, uptake and utilize the glucose in the environment (Hoffman 2015; Sabina & Brown 2020). The first studies to understand the mechanisms of sugar sensing were conducted on the yeast *S. cerevisiae* (Conrad et al. 2013). When glucose is available in the environment, genes required for the growth on alternative carbon sources are repressed by Carbon Catabolite Repression (CCR) mechanism (explained in chapter 2) (Kim et al. 2013; Ronne 1995). The repression regulatory pathway plays a fundamental role in fungi to regulate responses to nutrient availability (Kruckeberg et al. 1998). The Snf3/Rtg2, the transmembrane glucose sensor

has been evolved in *S. cerevisiae* to allow it to sense glucose (Kruckeberg et al. 1998). This mechanism leads not only to activate yeast casein kinase but also induces hexose transporter genes coding for glucose transporters (Kruckeberg et al. 1998). Snf3 in *S. cerevisiae* is expressed to sense glucose, fructose and mannose (Dietvorst et al., 2010). In *Candida albicans*, which is one of the most important pathogens of humans, it has been shown that sensing nutrients like glucose plays an essential role in inducing the pathogenicity trait in this fungus through affecting on its morphology and switching its morphogenesis from yeast-to-hypha (Rutherford et al. 2019).

While a variety of studies have been conducted to elucidate the pathways by which glucose is sensed in filamentous fungi (Assis et al. 2020; Chelius et al. 2019; Chen & Thorner 2007; Karunanithi and Cullen 2012), the main signaling pathway underling this mechanism is mysterious. However, one of the mechanisms has been explored thoroughly in filamentous fungi to sense nutrients in the environment is the cyclic AMP/protein kinase A (cAMP/PKA) signal transduction pathway (Caza &Kronstad 2019). The PKA pathway and its function in sensing environmental cues have been well characterized in *S. cerevisiae* (Caza &Kronstad 2019). For example, through this pathway in *S. cerevisiae* the component required for sensing machinery such as adenylyl cyclase Cyr1 is activated through GTPases such as Ras1 and Ras2 which in turn promotes the production of cAMP, which results to activate PKA (Caza & Kronstad 2019; Reis et al. 2019). PKA is composed of two catalytic and two regulatory subunits (Figure 2-2, chapter 2) (Reis et al. 2019; Caza & Kronstad 2019).

Another mechanism involved in nutrient-sensing is G-protein coupled receptors (GPCRs), which are a family of transmembrane receptors (Reis et al. 2019). Since these mechanisms for sensing of environmental cues and responding to them have been conserved evolutionary, it is not surprising to observe them not only in many fungi but also in plants and humans (Liu et al. 2013;

Xue et al. 2008). GPCRs consists of 3 heterotrimeric G proteins subunits including α, β, and γ (Reis et al. 2019). Xue et al. (1998) revealed that Gpr1, a glucose-sensing G-protein coupled receptor (GPCR) is involved in promoting cAMP production through the activation of Cyr1 resulting to sense sufficient amount of glucose in the environment. In addition, MAPKs are involved in nutrient-sensing by forming a complex containing multiple protein kinases including glycogen synthase kinase (GSK) that plays a critical role in glucose metabolism in environments containing glucose (Assis et al. 2020). This results in potentially phosphorylating the MAPK PbsA of the high osmolarity glycerol (HOG) pathway that leads to glucose-sensing in filamentous fungi (Assis et al. 2020).

Since carbon-sensing is regulated by various signaling transduction pathways (Assis et al. 2020) and protein kinases are involved in regulation of apical branching morphogenesis (Assis et al. 2020; chapter 2), we hypothesized that a shift from glucose to proline as carbon sources or vice versa is mediated by some protein kinases. This will predict the protein kinases that play a fundamental role in regulating apical branching phenotype in model organism *A. nidulans* when the growth condition undergoes a dramatic change.

I conducted this research by inoculating the pre-existing 100 kinase deletion library (used in chapter 2, Table 3-1), on three different media including proline+MAG holes, MAG+proline hole and proline+proline holes (control experiment). It does not matter if we use proline+proline holes or MAG+MAG holes as control experiments. The main goal was to ensure that the "edge" surrounding the holes does not itself lead to a change in morphology. I also performed a quantitative microscopy assay (QMA) and defined a standard reference interval (RI) for the resulting data, that I was able to detect the potential protein kinases that regulate apical branching when the environment undergoes dramatic changes. By performing this research, I identified 2D3

(ptkA), 2D12 (mpkA) and 2A4 (AN5757) as the most essential kinases playing role in regulating apical branching when the fungal habitat experiences changes. Most importantly, this study uncovers hitherto unknown signaling pathways that are critical to mediate apical branching morphogenesis when the growth condition undergoes a dramatic change in a model fungus *A. nidulans*.

3.2 Materials and Methods

3.2.1 Strains and Media composition

Of the128 deletion strains related to *A. nidulans* kinases including expanded groups of 15 histidine kinases, 7 SRPK (serine-arginine protein kinases) kinases and an interesting group of 11 filamentous fungal specific kinases constructed previously by de Souza et al. 2013, library of 100 kinase viable deletion mutants (containing 99 deletion strains+ 1 wild type strain) (Table 3-1) (available from Fungal Genetic Stock Centre FGSC; http://www.fgsc.net/Aspergillus/KO_Cassettes.htm), was used in this work. In this library, the control strain FGSC CDS1008 (2E1) (*wA3; argB2; ΔnkuA^{Ku70}::argB pyroA4; sE15 nirA14 chaA1 fwA1*) was considered as a wild type (WT) strain (http://www.fgsc.net/Aspergillus/KO_Cassettes.htm). Also, strain FGSC A4 Glasgow wild type (*veA*$^{+}$) was considered as reference and parental strain in this study since it lacks any mutations (http://www.fgsc.net/).

The media include MAG (malt extract 20g, agar 20g, dextrose 10g, peptone 2g, vitamin mix 2ml, trace elements 1ml, 1L ddH_2O), MAG media with the same recipe described above but without the vitamin mix, MNV–ethanol (ddH2O (1L), 20X nitrate salts (ddH2O 800ml, NaNO3 120g, KCl 10.4g, MgSO4-7H2O 10.4g KH2PO4 30.4g), agar 18gr,vitamin mix 1ml, dextrose was

replaced by 1% ethanol by volume), MNV–glycerol (MNV with 1% ethanol was replaced by 1% glycerol by volume) (***Note***: for the media containing 1% ethanol, just after autoclaving ethanol was added to the medium) and the last medium was proline (20x salts no nitrate 25ml, agar 9g, proline 5ml) served as both carbon and nitrogen source.

Cell culture on MAG + proline hole—Briefly, by using freezer stocks (including 100 kinase deletion strains plus A4 strain), 2µl of each individual strain was inoculated separately on MAG medium containing a large proline hole. The samples were then incubated at 28°C for 4 days. This experiment included 4 technical replicates for each strain used from -80 stocks meaning that the experiment was repeated 4 times (4 rounds) for the set of 100 kinase deletion library. Then those plates were evaluated under a dissecting microscope (explained in the following sections), to observe the transition or changes in the number of apical branches when the growth condition changed (Figure 3-1)

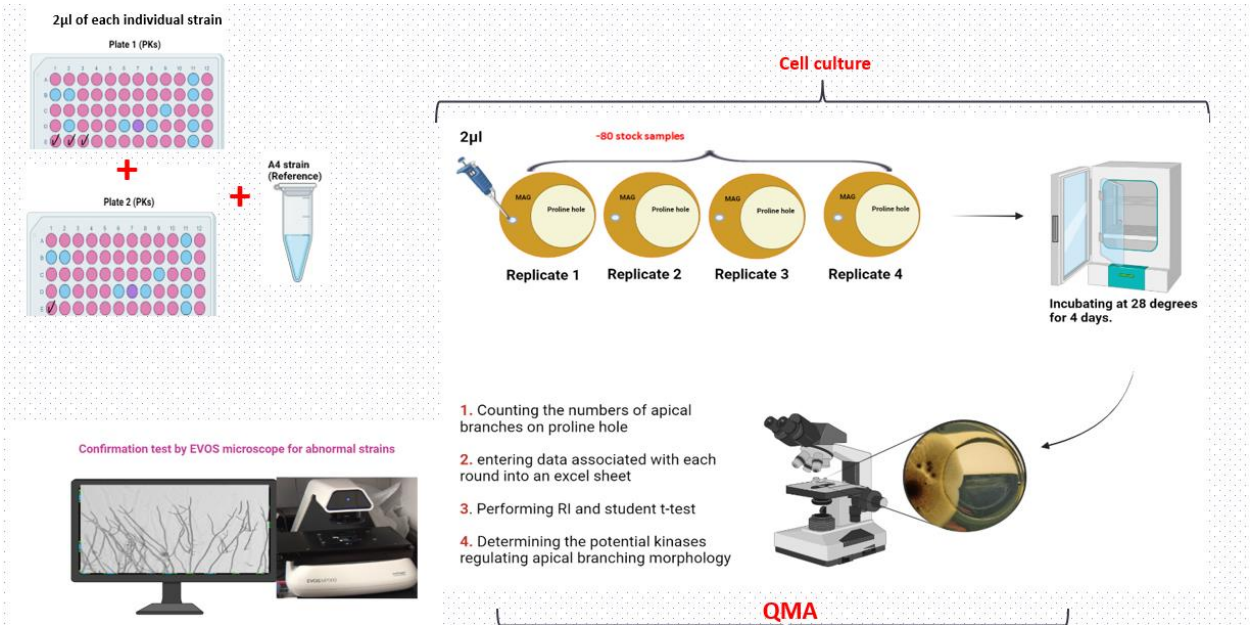

Figure 3-1. Cell culture and QMA on MAG+proline hole. The hole is filled by proline media.

Cell culture on proline + proline holes and proline + MAG holes—99 mutants, a wild type strains (2E1) plus A4 as reference strain stored at -80°C were grown on proline medium containing either four proline or MAG holes. The holes were considered as technical replicates in this experiment, therefore there were 4 technical replicates for each individual strain. Following that, each strain was inoculated in the center of the 60mm petri dish surrounded by either four proline or MAG holes. Those holes were punched by 200µl pipette tips, discharged from the medium and filled by either MAG or proline medium. Proline + proline holes plates were used as control group. There were 101 Proline + proline holes plates and 101 proline + MAG hole plates for this experiment each contained 4 technical replicates. The samples were then incubated at 28°C 14 days for proline + proline holes and 8 days for proline + MAG holes (Figure 3-2).

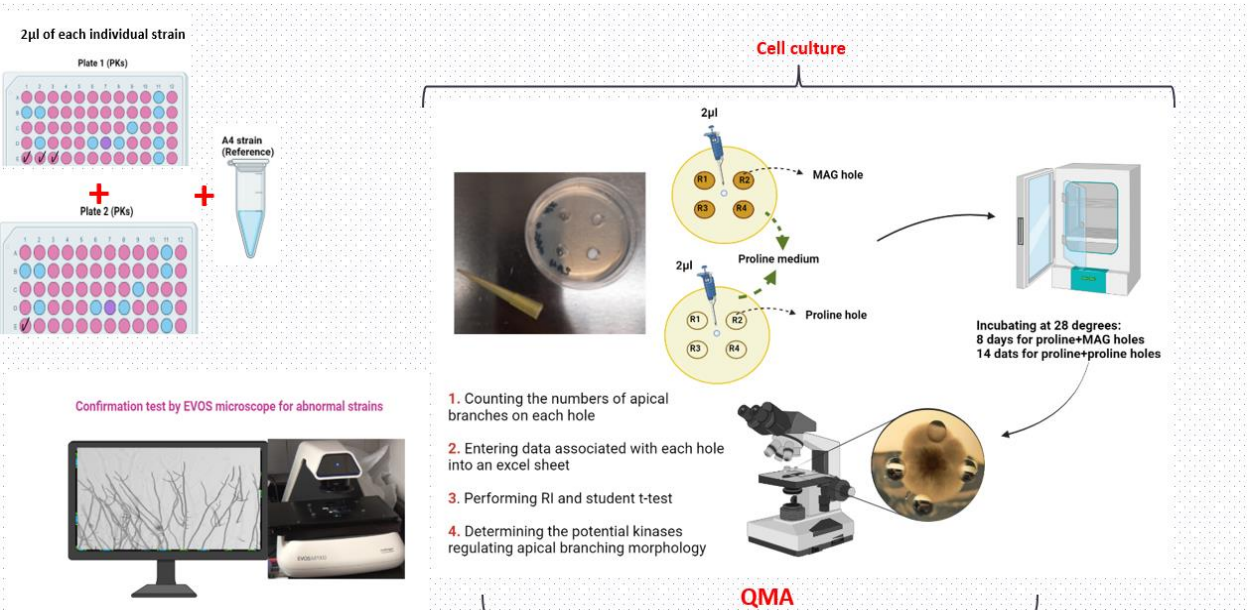

Figure 3-2. Cell culture and QMA on proline+proline holes & proline+MAG holes. The holes were filled with either MAG or MN media. The R1, R2, R3 & R4 on each media are the replicates for each individual mutant strain.

Cell culture on MNV-glycerol, MNV-ethanol, MAG without vitamin and proline—2µl of 2A10 strain that showed the lowest number of apical branches in chapter 2 as well as 2E1 (WT) and A4 (as reference strain) were used and inoculated in the center of large petri dishes separately on 4 different media types which were differ in terms of the source of carbon including MNV-glycerol, MNV-ethanol, MAG without vitamin and proline. 2A10, 2E1 and A4 strains grown on MNV-glycerol, MNV-ethanol, MAG (without vitamin) were assessed, double checked and compared with the same strains grown on proline medium in terms of the number of apical branches each strain showed (each strain on one medium was compared with itself on another medium in terms of the number of apical branches). There were 4 technical replicates for each strain in this experiment. The strains plated on MNV-glycerol or MNV-ethanol were incubated at 28°C for 3 days and those whose plated on MAG (without vitamin) and proline were maintained at 28°C for 2 and 6 days respectively (Figure 3-3).

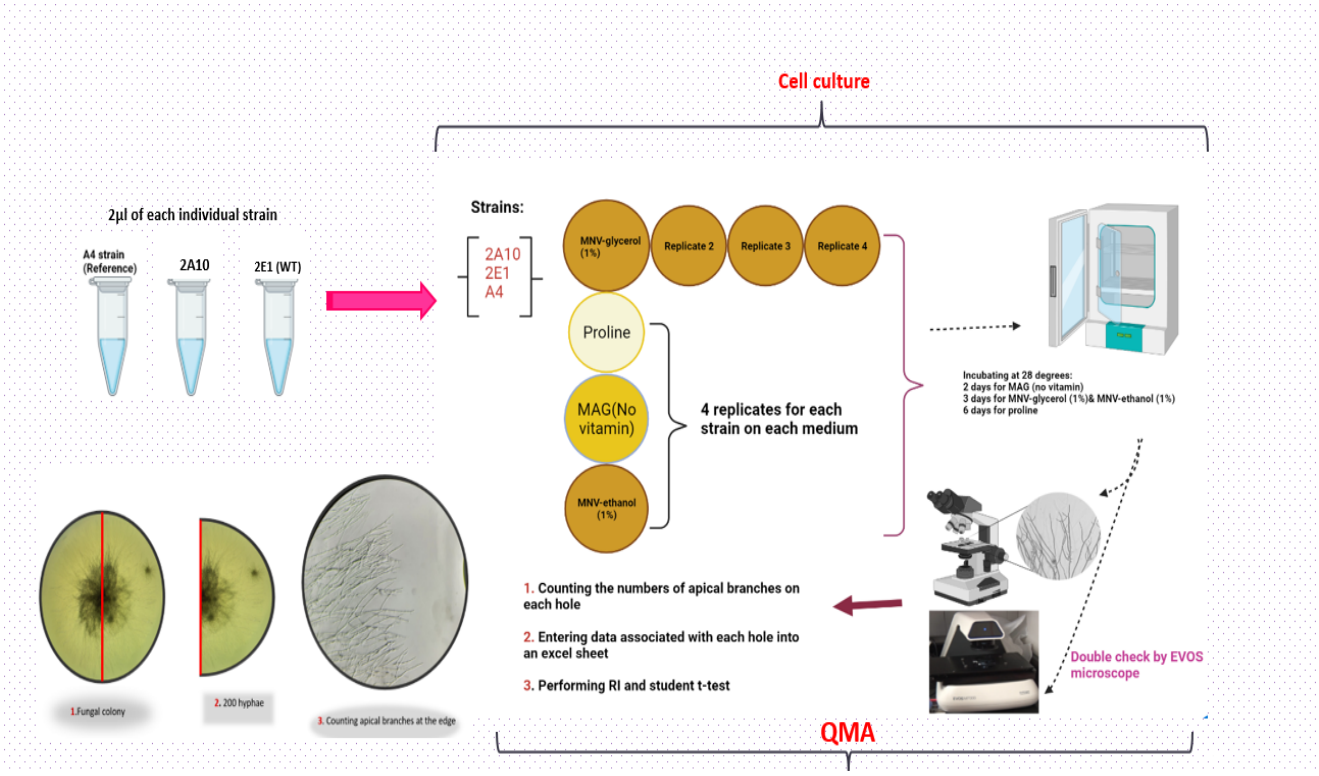

Figure 3-3. Cell culture on MNV-glycerol, MNV-ethanol, MAG without vitamin and proline.

3.2.2 Quantitative microscopy assay (QMA)

QMA for MAG + proline hole and proline + MAG holes **—**Through this assay, each individual plate (associated with MAG + proline hole and proline + MAG holes) was assessed in terms of the number of apical branches they revealed in each hole. Then, the number of apical branches at the edge of hyphae in each hole were counted. This experiment was conducted for each of the 4 replicates (4 rounds) on each medium and the data was recorded as an Excel spreadsheet for each experiment, then used for further analysis (Appendixes VIII&IX). This quantitative microscopy assay was conducted under dissecting microscope with 4x and10x magnifications for the objectives.

QMA for MNV-glycerol, MNV-ethanol, MAG without vitamin and proline**—** each individual plate (associated with MNV-glycerol, MNV-ethanol, MAG without vitamin and proline) was assessed in terms of the number of apical branches they revealed. The procedures were followed according to QMA in chapter 2 (Figure 2-5). This experiment was conducted for each replicate on each medium and the data was recorded as an excel spreadsheet and used for further analysis (Appendix X).

3.2.3 Establishing standard reference interval (RI) in filamentous fungi

To find the RI for each round of quantitative assay (4 rounds performed for -80 stocks) on each medium (MAG + proline hole and proline + MAG holes), the procedures explained for calculating RI in chapter 2 were followed. The RI for each data set on each media can be found in Appendixes VIII&IX.

3.2.4 Confirmation test by EVOS microscope

Proline+ proline holes, Proline + MAG holes and MAG + proline hole**—**To observe colonies by EVOS microscope, making thin layer of each media (either MAG or proline) on a slide is required. Those slides then were used to punch some holes on them. Briefly, to make a thin layer

of each medium on slides, the media was melted for about 45 seconds in microwave and used to cover the surface of the slide that was placed in the large petri dish. When slides were solidified, holes were punched on them, discharged from the medium and filled with new media (either MAG or proline). Following that 2µl of each deletion strain marked as abnormal strains in previous step such as 2A4, 2D3 and 2D12 (Appendix XII) as well as WT strain (2E1), were inoculated in the center of each slide surrounded by holes and maintained at 28°C (6 days for MAG+ proline hole, 8 days for proline+ MAG holes, 14 days for proline+ proline holes). This timeline was reduced to 2 and 4 days for wild type and reference strains on either MAG+proline or proline+MAG respectively. After this timeline the colony on each plate was observed by EVOS microscope with 10x and 20x magnifications to confirm the number of apical branches obtained previously through quantitative assay by dissecting microscope. Also, there were 4 technical replicates for each individual deletion strain in this part of the experiment on each medium meaning that this experiment was repeated 4 times (4 rounds). In addition, the entire steps that were done for quantifying strains by dissecting microscope were repeated for EVOS as well. Those results were recorded as an Excel spreadsheet (Appendix XI).

MNV-glycerol, MNV-ethanol, MAG without vitamin and proline— All the procedures explained in chapter 2, figure 2-9 for plating colonies and observing them under EVOS microscope were followed. The strains that were plated in this step were *ΔpkaA* (2A10), WT strain (2E1) & A4 and they maintained at 28°C (2 days for MAG, 3 days for MNV-glycerol& MNV-ethanol and 6 days for proline). Also, there were 4 technical replicates. In addition, the entire steps that were done for quantifying strains by dissecting microscope were repeated for EVOS as well. Those results were recorded as an excel spreadsheet (Appendix XIII).

3.2.5 Statistical analysis

The student t-test for two samples assuming unequal variances using Excel was performed for determining the statistical significance of observed differences between the strains marked as abnormal through RI analysis, and the wild type strain (2E1) (*P* value less than 0.05). Student t-test was performed for all 99 kinase deletion strains plus the wild type strain (2E1) for the results of each experiment (MNV-glycerol, MNV-ethanol, MAG without vitamin, proline, MAG + proline hole and proline + MAG holes). In this step each mutant strain with its replicates was organized in one group and compared to the second group including the wild type strain (2E1) with its replicates. This analysis was performed for the entire mutant strains grown on each media, separately, while for MNV-glycerol, MNV-ethanol, MAG (without vitamin) and proline experiments just each mutant strain was compared with itself in different medium. All data for this analysis are available in Excel spreadsheets (Appendix XIV). In addition, by using this test it was confirmed there is no significant difference between A4 and wild type strain.

3.3 Results

3.3.1 Results of MAG + proline hole—Of the 100 mutant strains, 13 strains were flagged as abnormal strains in this experiment which among those just 6 strains including 2D3, 2D12, 2A4, 1A1, 1C1 and 2A6 showed the highest repetitiveness more than 2 times in terms of illustrating abnormal apical branching phenotype in each round of QMA, when the growth condition changes dramatically from glucose to proline (Appendix XII). While 1A1, 1C1 and 2A6 showed hyper-(increased) apical branching phenotype 3, 2 and 2 times respectively, 2D3, 2D12 and 2A4 revealed hypo-apical branching phenotype constantly during the four rounds of QMA (Appendix XII). Interestingly, while 2D3, 2D12, 2A4, 1A1 and 1D1 strains showed significant difference when they compared to the wild type (2E1), the other remaining strains did not reveal any significant

difference during QMA. Most importantly, 2D3, 2D12 and 2A4 strains experienced a reduction in the number of apical branching with showing 1, 0 and 1 apical branches on average, while this number for wild type and reference strain is 25 and 26 respectively (Appendix IX). The other remaining 7 strains (Appendix XII) revealed hyper apical branching once except 1D8 that showed a reduction in the number of apical branches (Appendix IX). In terms of phenotype, the growth rate of the aforementioned strains (2D3, 2D12 and 2A4) (Figures 3-4, 3-5, 3-6) were lower than other mutant strains and WT strain (Figure 3-7) showing that they experienced a very strong growth defects, while they maintained more than other strains at 28 degrees.

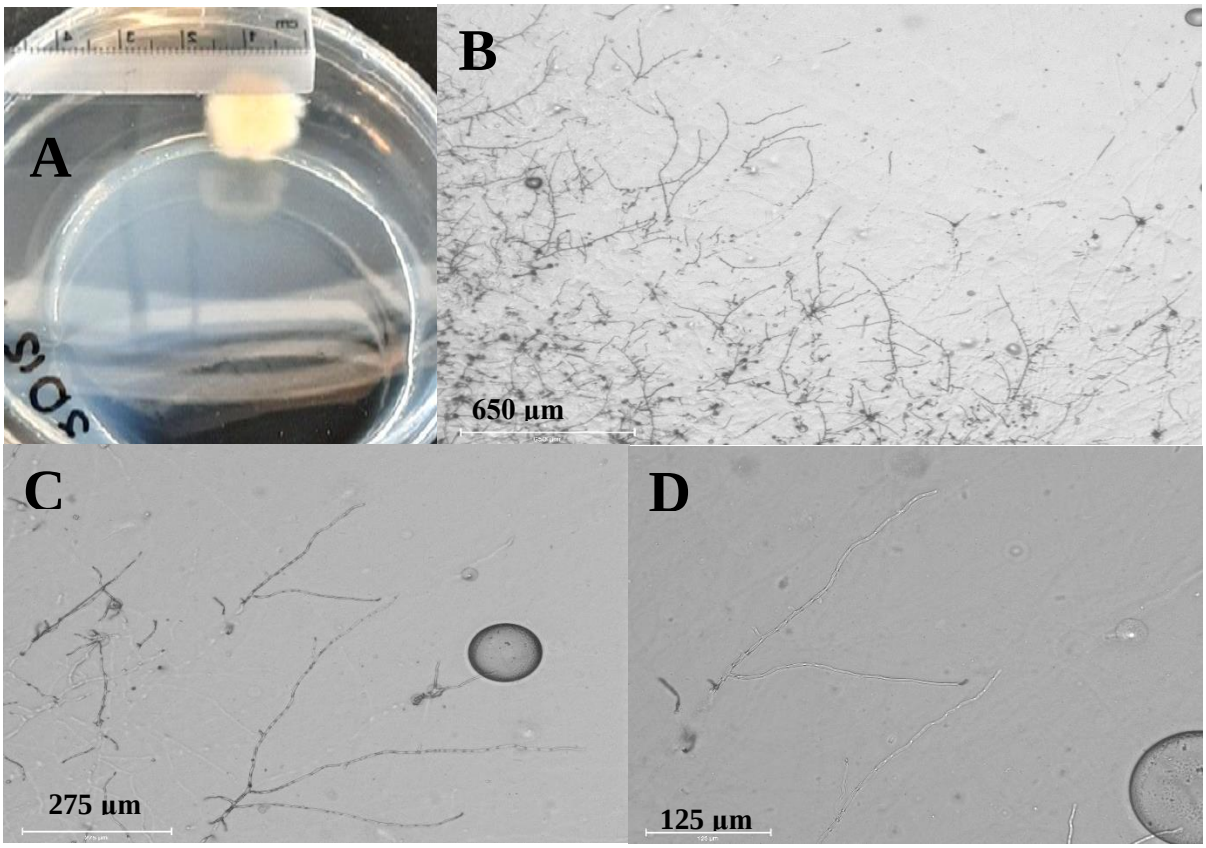

Figure 3-4. An illustration of 2D12 (mpkA) strain on MAG+ proline hole. Image A shows the size of colony that is 1.6cm after 6 days of growing. Images B, C &D have been captured by EVOS microscope with 4X, 10X and 20X magnifications respectively from the proline holes. Images C&D show needle like shape hyphae with no apical branches.

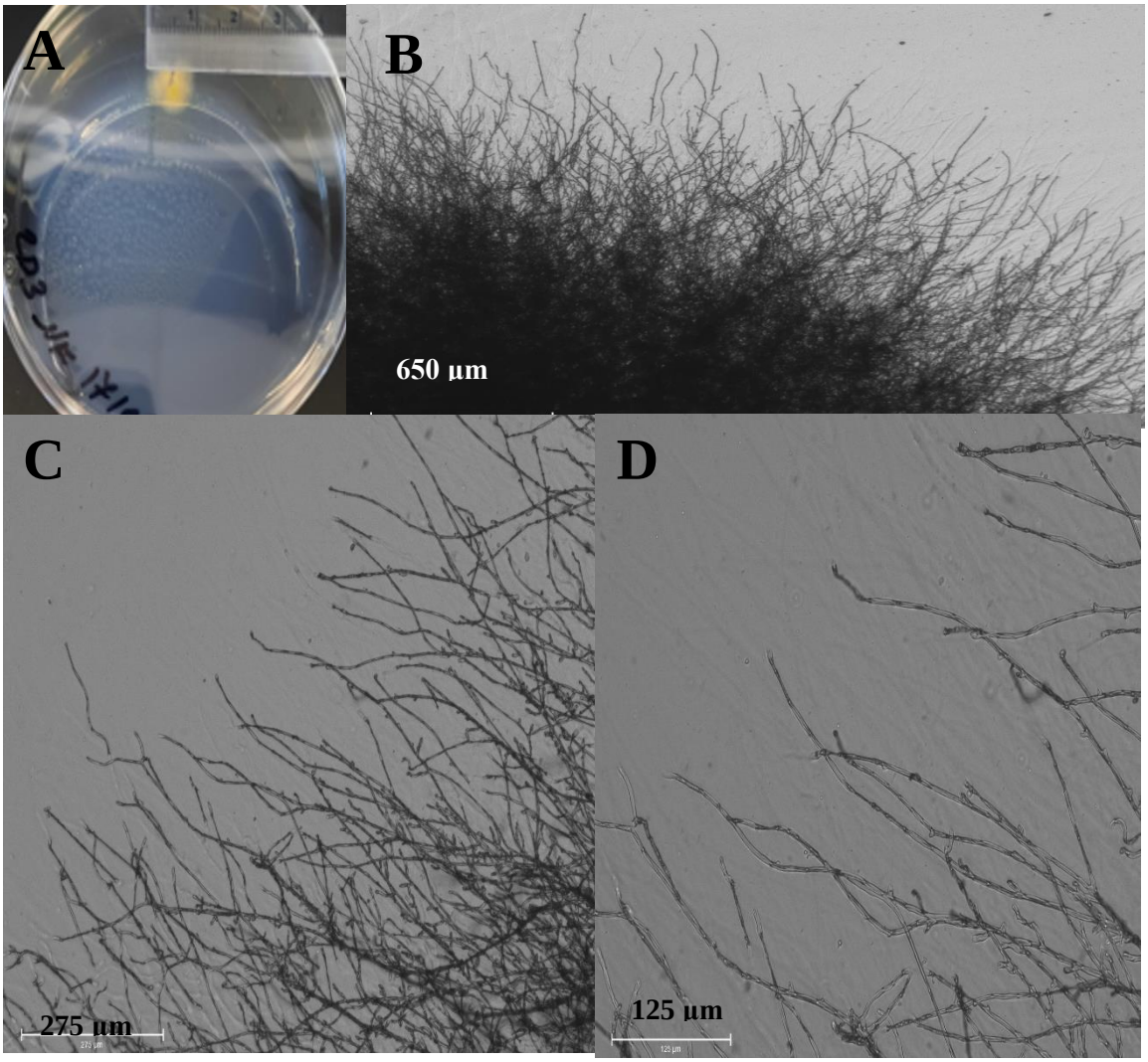

Figure 3-5. An illustration of 2D3 (ptkA) strain on MAG+ proline hole. Image A shows the size of colony that is 1CM after 6 days of growing. Images B, C &D have been captured by EVOS microscope with 4X, 10X and 20X magnifications respectively when hyphae reached to a proline hole. Very dense colony has been formed and Image D shows no apical branches for this strain.

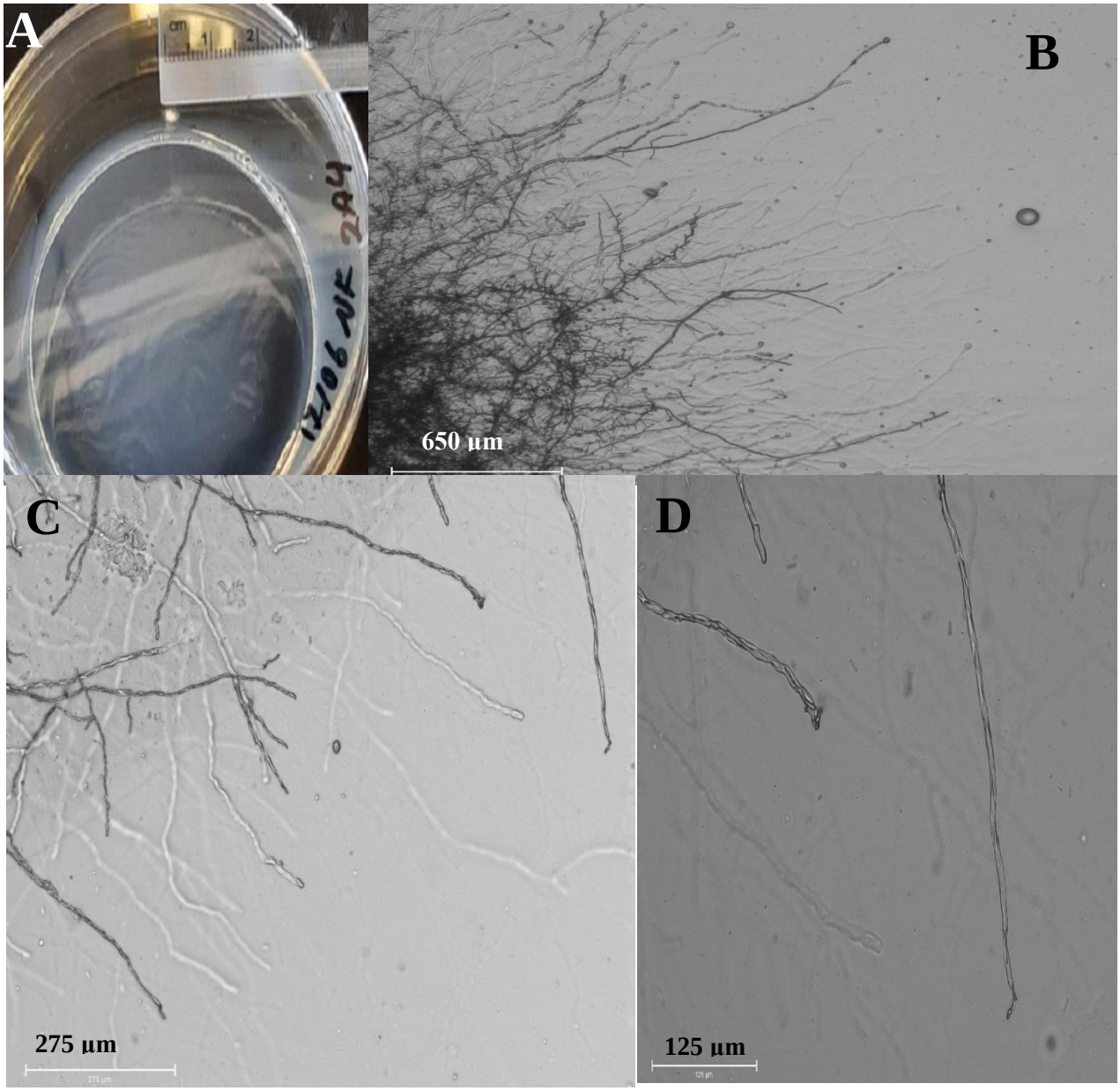

Figure 3-6. An illustration of 2A4 (AN5757) strain on MAG+ proline hole. This deletion strain revealed a very sever growth defect with the colony size of 3mm after 6 days of growing. Images B, C & D have been captured by EVOS microscope with 4X, 10X and 20X magnifications respectively after reaching the hyphal branches to the proline hole. Image D shows no apical branches.

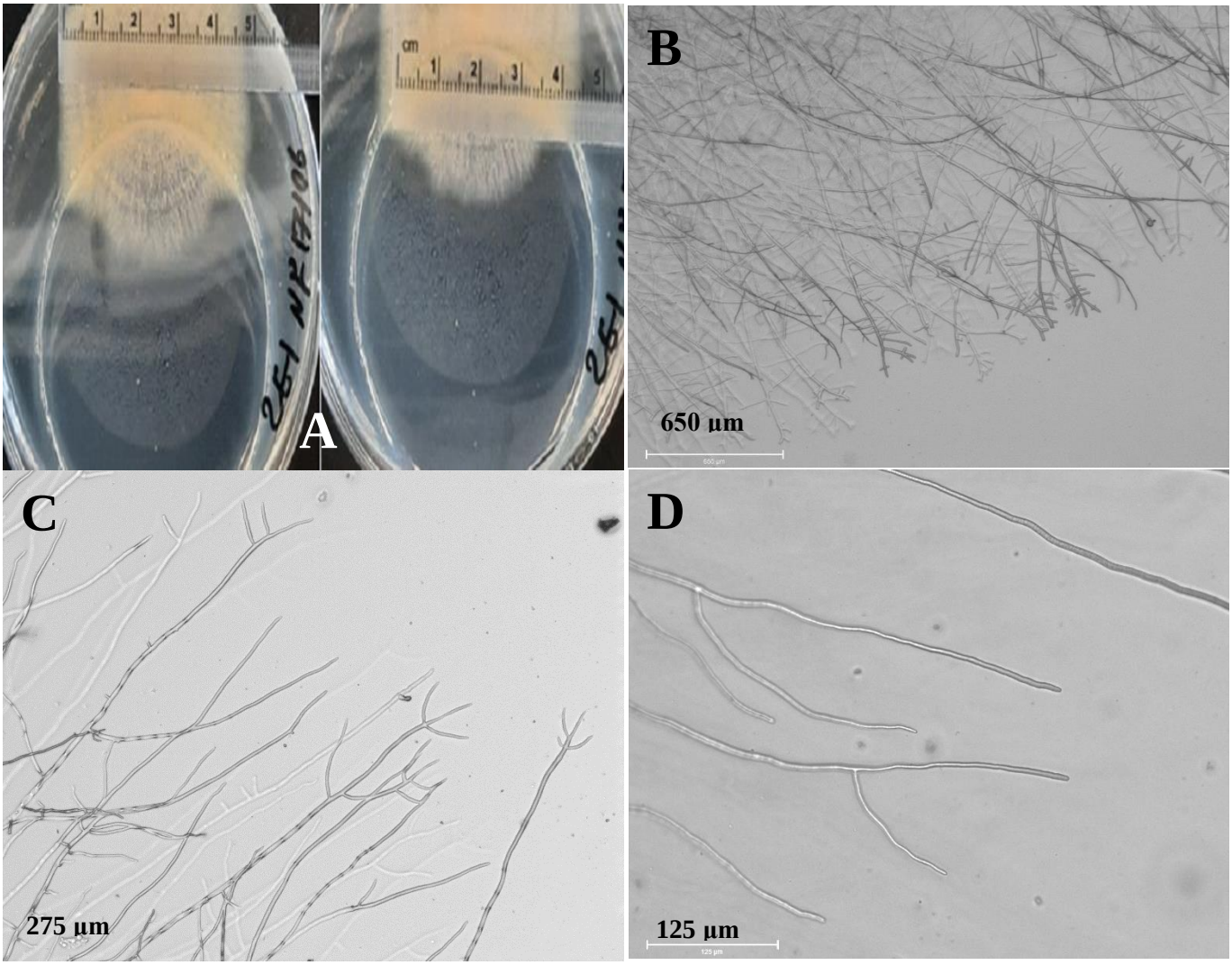

Figure 3-7. An illustration of 2E1, wild type strain. 2E1 showed a great growth rate compared to other mutant strains (2D12, 2D3 &2A4). Image A shows a colony size of 4.5 cm and 3.5cm associated with MAG and its transition to proline hole respectively. While this strain grew for 2 days, it showed apical branches equal to the number of apical branches assigned to A4 as reference strain. Images B (4X), C (10X) &D (20X) have been captured by EVOS microscope showing some apical branches generated by this strain.

3.3.2 Results of proline + MAG holes— Of the 100 mutant strains, 17 strains were marked as abnormal strains when they were grown on proline medium containing MAG holes. Among those strains 6 strains including 2C5, 2B5, 1D8, 2A4, 2D3 and 2D12 were found that showed hypo-apical branching phenotype more than 2 times, while 1D9 showed hyper apical branching 2 times through this experiment. Interestingly, the strains showed the highest repetitiveness in terms of illustrating abnormal apical branching morphogenesis, indicated the significant difference when they compared to the wild type strain. Most importantly, 2D3, 2D12 and 2A4 strains not only showed the most significant difference which were 0.003, 0.003 and 0.006 respectively but they also showed 1, 1 and 0 apical branches respectively on average. This suggests that these strains illustrated abnormal phenotype constantly during the four rounds of QMA. In terms of the growth rate, I found 2D3, 2D12 and 2A4 (Figures 3-8, 3-9 & 3-10) experienced a very strong growth defect compared to wild type (Figure 3-11) and other mutant strains, therefore they reached to the MAG holes after 8 days.

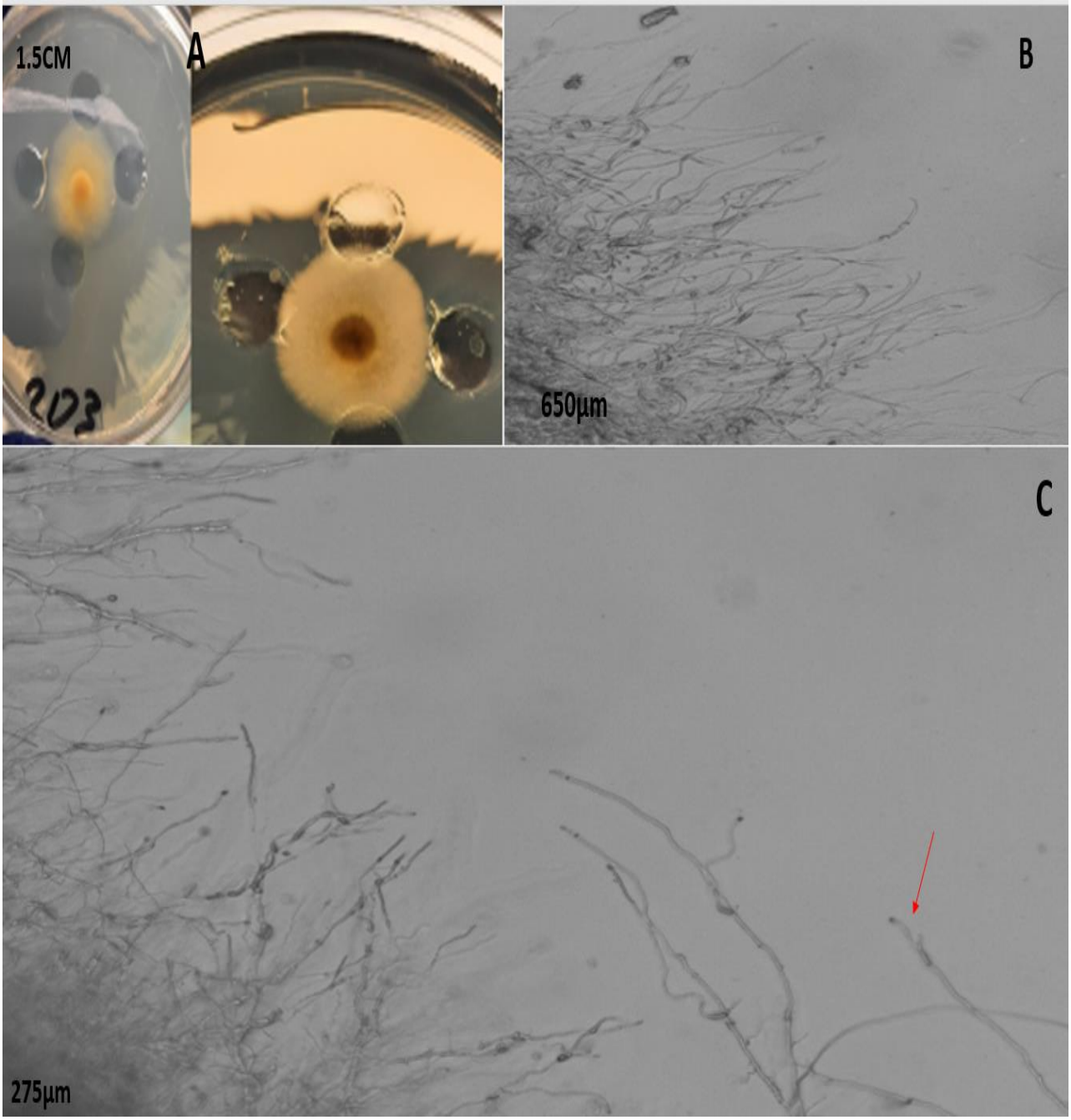

Figure 3-8. An illustration of 2D3 (ptkA) strain on proline+ MAG holes. Image A shows the size Of a colony which is 1.5cm after 8 days of growing. While a very dense colony has been formed, Images B & C reveal one apical branch for this strain. Images B & C have been captured by EVOS microscope with 4X and 10x magnifications respectively on MAG hole.

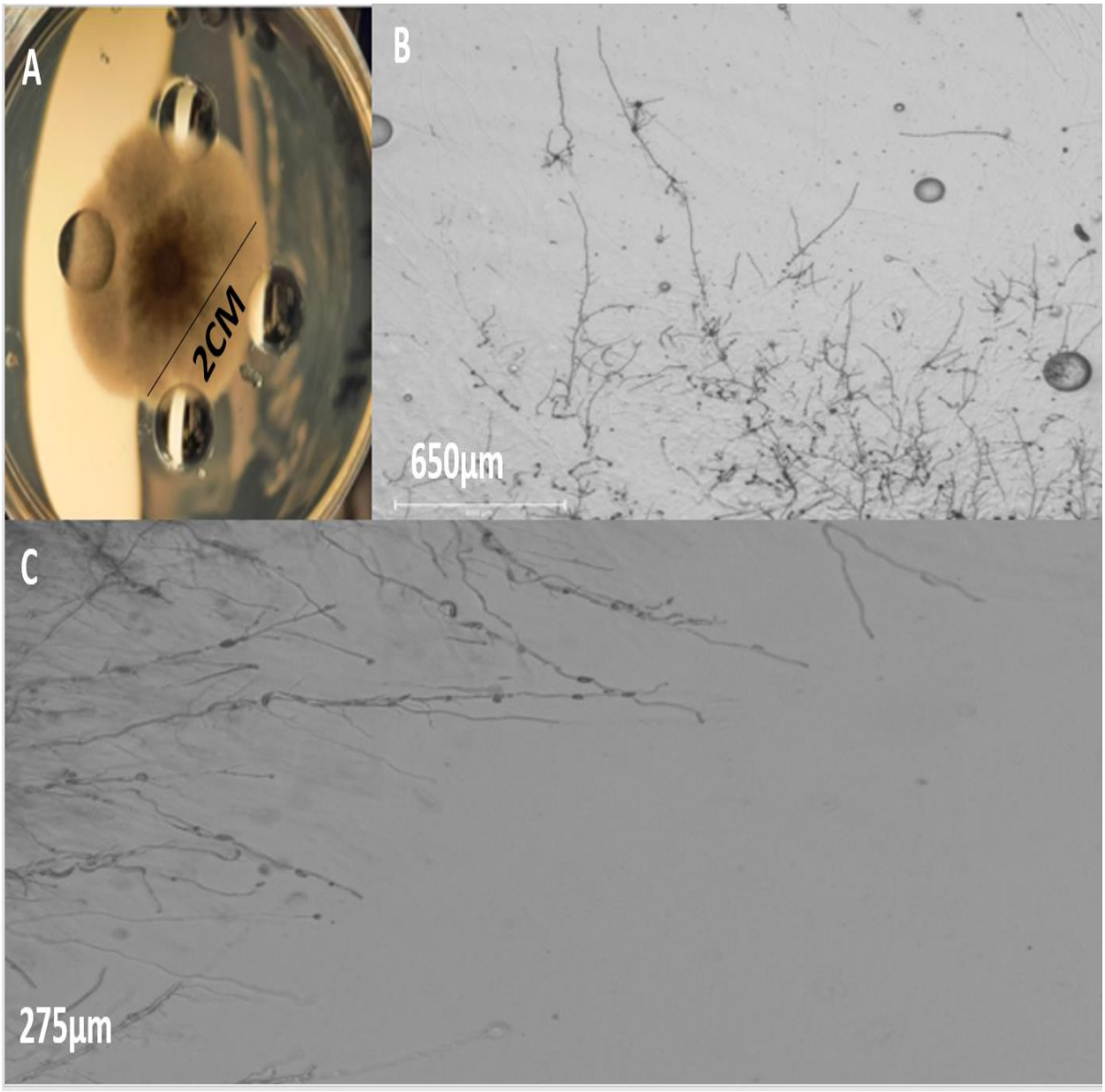

Figure 3-9. An illustration of 2D12 (mpkA) strain on Proline+ MAG holes. Image A shows the size of a colony that is 2cm after 8 days of growing. Images B &C have been taken under the EVOS microscope with 4X &10X magnifications respectively from the MAG hole. Image C shows needle like shape hyphae with no apical branches.

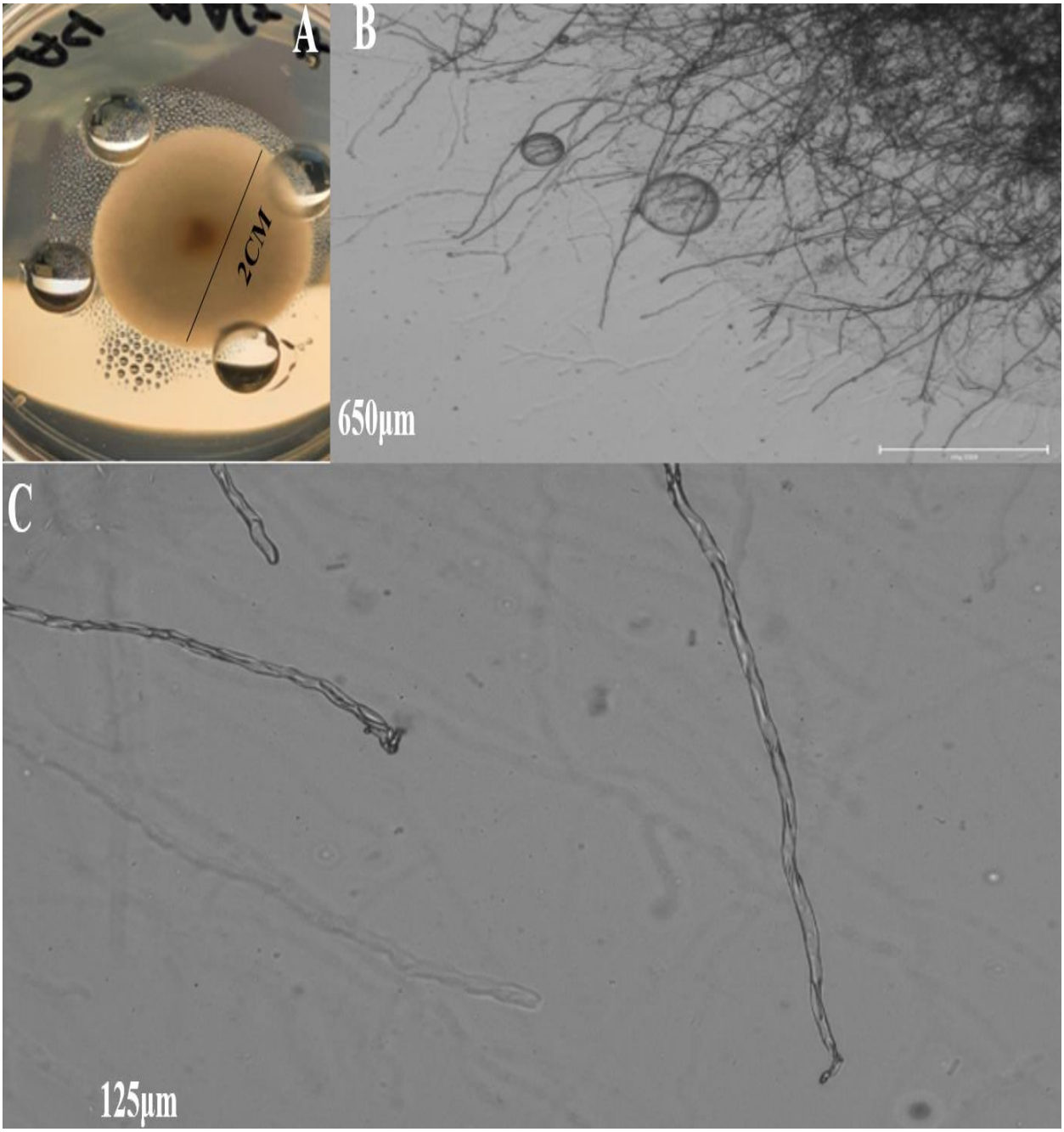

Figure 3-10. An illustration of 2A4 (AN5757) strain on proline + MAG holes. This deletion strain revealed a great growth rate with the colony size of 2cm after 8 days of growing, while the colony formed very dense hyphae. Images B &C have been captured by EVOS microscope with 4X and 10X magnifications respectively after reaching the hyphal branches to the MAG hole.

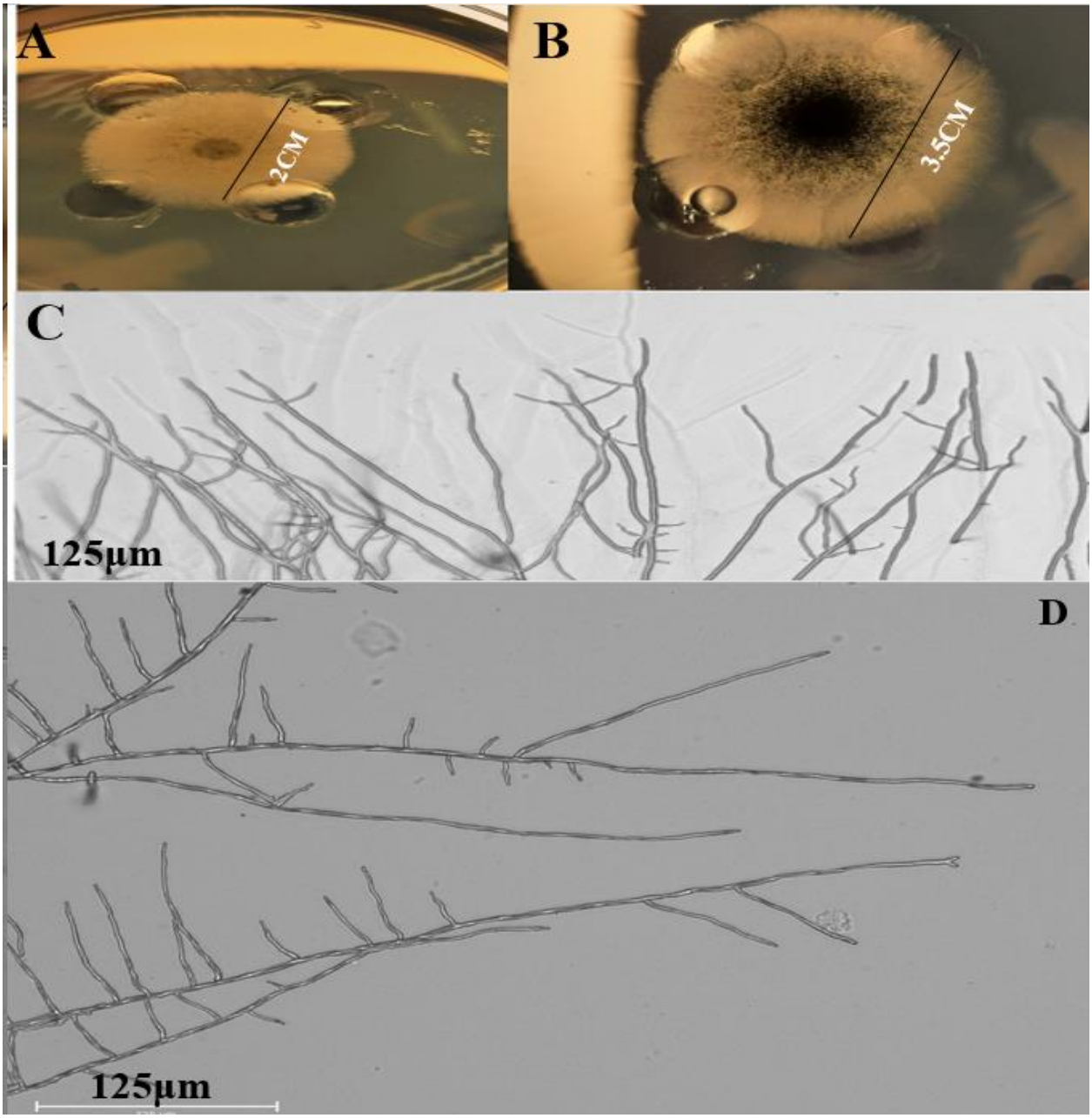

Figure 3-11. Images A&B show 2E1 (wild type, 2cm) and A4 (reference strain, 3.5cm) on proline +MAG holes after 4 days of growing. Images C & D indicate apical branches generated by 2E1 & A4 strains respectively on MAG holes with 20X magnifications.

Using the EVOS microscope allowed to confirm the results of this experiment additional time. The strains that showed the highest repetitiveness more than 2 times (2C5, 2B5, 1D8, 2A4, 2D3 and 2D12) were inoculated on some slides and after letting them to grow, 2D3, 2D12 and

2A4 were the strains showed the highest repetitiveness with the lowest number of apical branches confirming the results obtained by previous step (using dissecting microscope) (Appendix VIII)

3.3.3 Comparing the results of proline + MAG holes and MAG+ proline hole— Comparing the results of proline + MAG holes and MAG+proline hole experiments reveal the top 6 mutant strains that were found to be common among these two experiments including 2D3, 2D12, 2A4, 1C8, 1D8 and 1D9 (Table 3-1).

Table 3-1. Results of proline + MAG holes and MAG+ proline hole

#Rank	Cell	Gene Name	The times when abnormal apical branching pattern was observed on **MAG+proline hole** medium	Abnormal phenotype on **MAG+proline hole** medium	Results of student t-test on **MAG+proline hole** medium (P<0.05)	The times when abnormal apical branching pattern was observed on **Proline+MAG holes** medium	Abnormal phenotype on **Proline+ MAG holes** medium	Results of student t-test on **Proline+MAG holes** medium (P<0.05)
1	2D12	mpkA	4	Hypo	0.004	4	Hypo	0.003
2	2D3	ptkA	4	Hypo	0.005	4	Hypo	0.003
3	2A4	ckiB	4	Hypo	0.005	4	Hypo	0.006
4	1D8	SepH	1	Hypo	0.031	3	Hypo	0.005
5	1D9	cmkD	1	Hyper	0.432 (Not significant)	2	Hyper	0.007
6	1C8	steC	1	Hyper	0.334 (Not significant)	1	Hyper	0.312(Not significant)

These mutant strains not only indicated significant difference through both experiments when they compared to the wild type, but also were marked as strains showed the constant behavior in terms of generating the lowest number of apical branches thorough this study. While 1C8, 1D8 and 1D9 showed hyper-, hypo- and hyper apical branching phenotype respectively once on MAG + proline hole experiments, these strains showed the same phenotype 1, 3 and 2 times respectively on proline+ MAG hole experiment. While 1D8 and 1D9 revealed significant difference on proline+MAG hole, no significant difference was found among these strains and the wild type

strains on MAG+ proline hole experiment. 1C8 neither showed any significant difference on MAG+ proline hole nor on proline + MAG hole (Table 3-1).

3.3.4 Results of proline + proline holes— After inoculating 100 mutant kinases in proline plates containing proline holes and maintaining them for about 14 days at 28 degrees, no results were obtained meaning that no branches reached to the proline holes. While some colonies grew better than others, they never reached the proline holes (Figures 3-12 & 3-13).

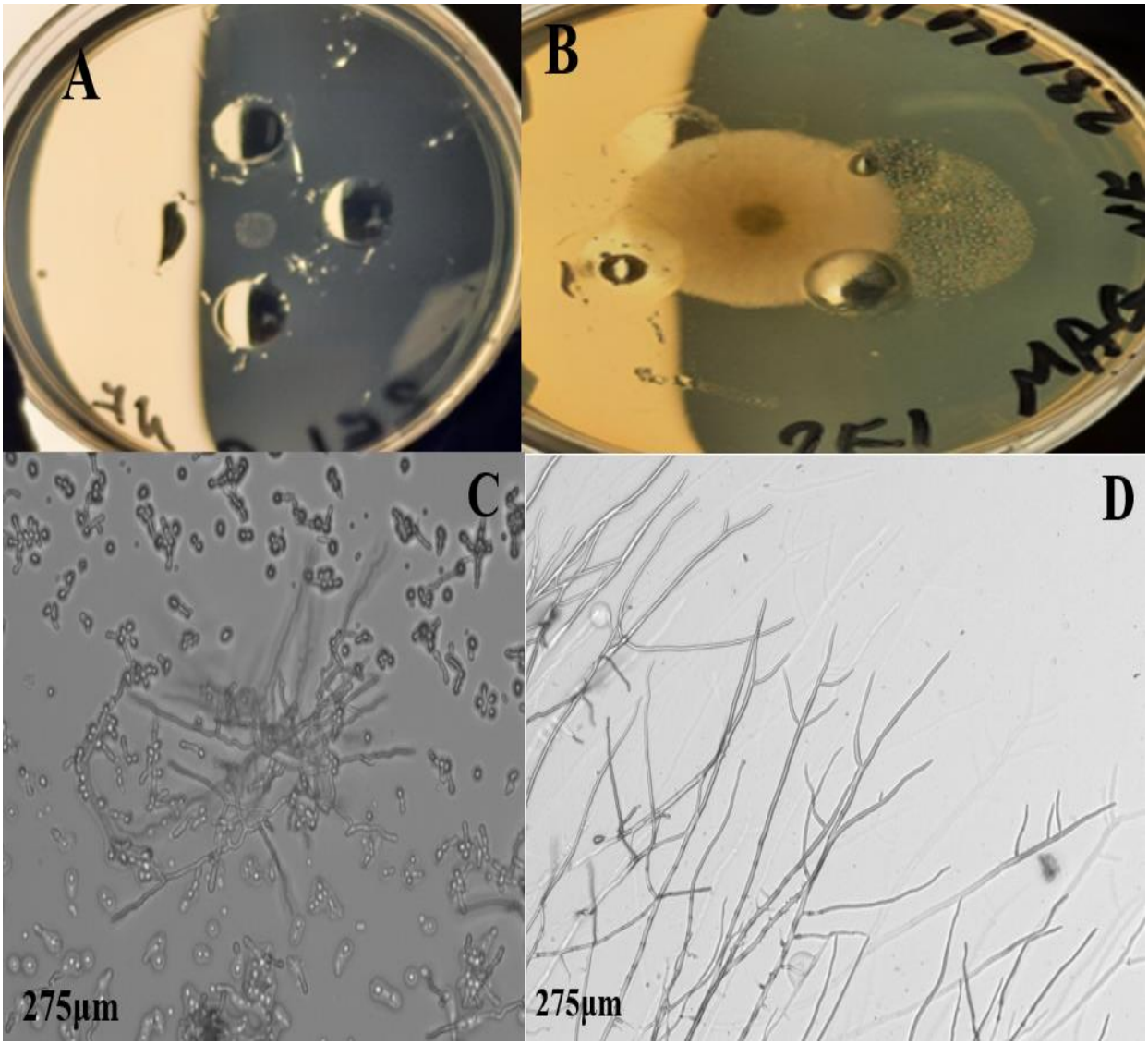

Figure 3-12. Images A-C & B-D reveal 2E1 strain on proline+proline holes and proline+MAG holes respectively. While it grew on proline+proline holes medium (after 14 days of growing), it was arrested at the initial phase of growing and did not reach peoline holes. This strain however on images B-D illustrated a very well growth rate on proline+MAG holes and reached the MAG holes. These images have been taken under EVOS microscope with 10X magnification.

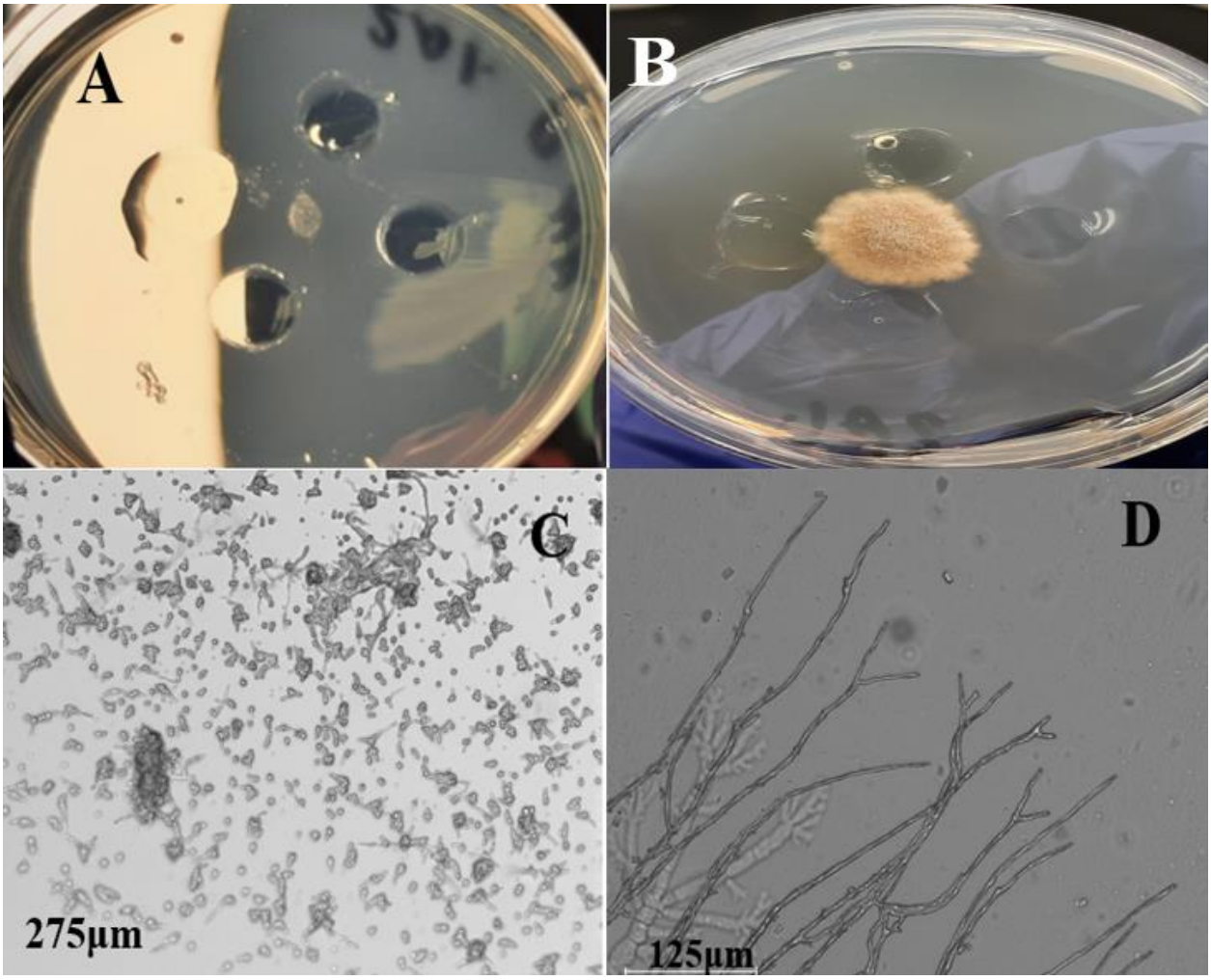

Figure 3-13. Images A-C & B-D reveal 2A10 strain on proline+proline holes and proline+MAG holes respectively. While it grew on proline+proline holes medium (after 14 days of growing), it was arrested at the initial phase of growing. 2A10 however on images B-D illustrated a very well growth rate on proline+MAG holes and reached the MAG hole. These images have been taken under EVOS microscope with 10X magnification.

*3.3.5 **Results of MNV-glycerol, MNV-ethanol, MAG without vitamin and proline—*** Through this experiment after plating 2E1(WT), A4 (reference strain) and 2A10 and comparing the number of apical branches associated with each strain with itself on different medium, we determined that there is no significant difference in the number of apical branches between the strains grown on MNV-glycerol, MNV-ethanol, MAG without vitamin, while there was significant difference in the number of apical branches between the strains grown on proline and other media types (MNV-glycerol, MNV-ethanol, MAG without vitamin) (Table 3-2) (Appendix XIV). These results were confirmed through EVOS microscope as well (Appendix XIII).

Table 3-2. Results of student t-test assuming unequal variances associated with each strain on different media ($P<0.05$).

Media type	2A10 (mutant strain)	2E1 (WT)	A4 (Reference)
MAG vs. Proline	**0.0007** **(significant difference)**	**0.004** **(significant difference)**	**1.8098E-05** **(significant difference)**
MN vs. MNV-1% glycerol	0.7	0.2	0.6
MN vs. MNV-1% ethanol	0.3	0.2	0.3
MN vs. MAG	0.4	0.6	0.4
MAG vs. MAG (without vitamin)	0.4	0.2	0.8

Taken together, these results showed 2D3, 2D12 and 2A4 are the only mutant strains repeated constantly in terms of showing hypo apical branching phenotype compared to others through two different assays including proline+MAG holes and MAG+proline hole (Figure 3-14).

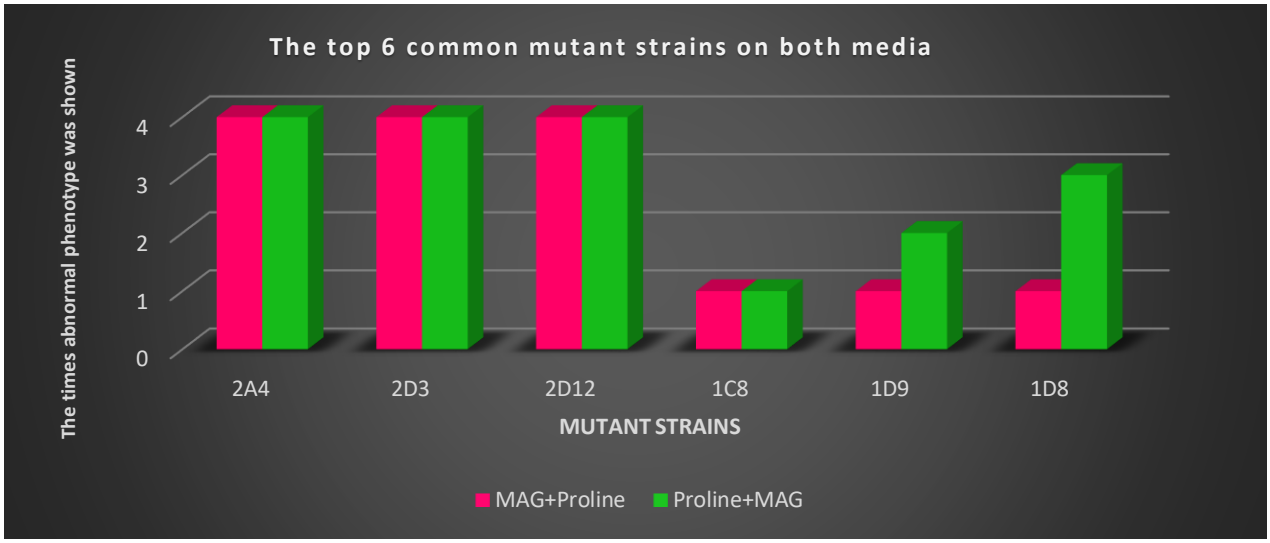

Figure 3-14. Illustrates the top 6 PKs showed abnormal apical branching phenotype through a media shift assay.

3.4 Discussion

Although there are a wide range of mechanisms not only in filamentous fungi but also in yeasts including the PKA pathway, the MAPK pathway and the HOG pathway that are involved in regulating hyphal growth in filamentous fungi and mediating environmental sensing in these organisms (Assis et al. 2020; Chelius et al. 2019; Chen & Thorner 2007; Karunanithi and Cullen 2012), the main pathways or protein kinases by which apical branching morphogenesis is regulated when the growth condition changes dramatically remain largely unknown in *A. nidulans*. Furthermore, apical branching has been shown to play a key role in sensing environmental cues (Dickman & Yarden in 1999). Dickman & Yarden in 1999 illustrated that protein kinases are the major factors to manage a wide range of signaling transmissions in filamentous fungi. Consequently, this study was able to screen the significant role of 100 kinase deletion strains (Table 2-1) in monitoring apical branching morphogenesis when the growth condition shifts from glucose to proline as another carbon source and vice versa to measure the number of apical branches. This study was conducted through defining a systematic methodology for measuring the number of apical branches which consists of a combination of quantitative microscopy assay (QMA) along with identifying reference interval (RI) (explained in chapter 2) for each dataset to detect abnormal strains. Although it has not been determined yet which regulatory pathway involved in regulating apical branching morphogenesis in *A. nidulans* when the environment undergoes changes, this study provides evidences suggesting that 2D12 (mpkA), 2D3 (ptkA) and 2A4 (AN5757) might be the main regulators of apical branching phenotype when the environment is changed. However, among those 2D12 is the potential kinase since there is a wide range of studies showing that this kinase regulates various tasks not only in filamentous fungi but also in other eukaryotic systems (Assis et al. 2020; Chelius et al. 2019). Two different media used in this

study to assess the number of apical branches when the growth condition changes dramatically from glucose to proline (MAG+proline hole) and vice versa (proline+MAG holes).

3.4.1 Interpretation of MAG + proline hole results—Through a genetic screen of 100 kinase deletion strains on MAG+ proline hole medium, 13 deletion strains (Appendix XII) were found which among those 2D3 (ptkA), 2D12 (MAPK) and 2A4 (ckiB/AN5757) strains not only showed the significant difference but also revealed decreased apical branching phenotype constantly during the four rounds of quantitative microscopy assay (QMA). These results suggest that, these mutant strains can be regarded as interesting candidates mediating apical branching morphogenesis when the environment undergoes sudden fluctuations. Furthermore, the lowest number of apical branches has been assigned to 2D12, 2D3 and 2A4 whose indicated 0, 1 and 1 apical branch respectively on average. These results suggest that among these strains 2D12 might be the potential kinase mediating apical branching phenotype when the growth condition changes dramatically from MAG to proline. The other reason why this strain has been considered as the potential candidate in this research is, MAPK has been characterized and studied very well not only in filamentous fungi but also in other eukaryotic systems (Assis et al. 2020; Chelius et al. 2019) compared to ptkA and AN5757 kinases.

Bathe et al. (2010) and Kempf et al. (2013) showed that in *A. nidulans,* ptkA kinase is involved in conidiophore development and interacts with 2 cyclins named as pclA and pchA. Therefore, deletion of pchA leads to severe growth defects in this fungus suggesting that the function of ptkA is highly dependent on pchA activity. Consistent with this view I found the lowest number of apical branches when ptkA was deleted in *A. nidulans* as well as a very strong growth defects which as shown in figure 3-5. This supports the role of ptkA in development and

morphogenesis in *A. nidulans*. Also, it has been indicated deletion of ptkA yeast homologue cdK9, results to impair the viability of *Saccharomyces pombe* (Pei et al. 2006).

Through the results of QMA obtained in this experiment AN5757 or 2A4 strain was shown as a strain which not only revealed a very strong growth defects compared to other strains but also did not indicate any apical branches when it reached to the proline hole (Figure 3-6). While this strain has not been characterized yet in *A. nidulans* and there is lack of evidence to support the role of AN5757 in this fungus, a few studies have been performed indicating the important role of AN5757 yeast homologue, YCK2 which plays a key role in yeast morphogenesis, development and glucose sensing (Reddi & Culotta 2013; Robinson et al. 1993; Robinson et al. 1999; Snowdon and Johnston 2016). Also, YCK2 plays a vital role in phosphorylation and regulation of glucose sensor Rgt2p in *S. cerevisiae* (Reddi & Culotta 2013). These evidences fully support our results indicating AN5757 might be involved in sensing environmental cues as well as regulating apical branching morphogenesis in *A. nidulans* when the environmental condition changes suddenly.

Multiple studies have indicated that MAPK has been involved in various functions including sensing environmental cues, up-taking glucose and signaling transduction pathways (Assis et al. 2020; Chelius et al. 2019). For example, in *A. nidulans* deletion of some MAPK associated protein kinases such as *Δste7, ΔmpkB, and ΔmpkC* in environments containing sufficient amount of glucose led to delay in glucose transport and resulted in reduced growth rate in this fungus (Assis et al. 2020). Bussink and Osmoni (1999) suggested that *mpkA* deletion strains in *A. nidulans* result in forming colonies with delayed conidial germination and abnormal hyphal tips. This research confirms our observation indicating that *mpkA* deletion strain or 2D12 strain showed a very strong growth defect with needle like shape hyphae (Figure 3-4) when this strain was grown on MAG and reached to proline hole. In *A. fumigatus* it has been shown *mpkC* deletion

strains are not able to grow on environments containing sorbitol or mannitol as the sole carbon source indicating the function of this kinase in utilizing major source of carbon like glucose (Reyes et al. 2006). Furthermore, MAPK genes tmk1 and tmk2 in *Trichoderma reesei* have been shown to detect, transport, and metabolize different source of carbon during degradation of plant cell wall (Graciano de Paula et al. 2018). In *S. cerevisiae* it has been revealed two genes involved in MPKA pathway including Mig1 and Mig2 that induce filamentous growth in response to sensing glucose (Karunanithi & Cullen 2012). In addition, MAPK related genes are implicated to form invasive growth by inducing hyphal growth and filametation in pathogenic fungi like *Candida albicans* (Rutherford et al. 2019). These documents provide strong evidence suggesting the essential role of MPKA pathway in sensing environmental cues and inducing filamentous growth in fungi.

In addition, 1A1 (atmA), 1C1 (atg1) and 2A6 (npkA) revealed abnormal apical branching phenotype more than 2 times in this study suggesting that these strains may mediate apical branching morphogenesis in response to environmental changes as well. It has been indicated that atmA plays a key role in regulating and maintaining polarized hyphal growth as well as the formation of polarity axis in *A. nidulans* suggesting that deletion strains lacking this function impair hyphal growth (Malavazi et al. 2007). This leads to form aberrant hyphal branching morphogenesis (Malavazi et al. 2007). In *A. nidulans* atmA plays role in glucose uptake by interacting with target of rapamycin (TOR) pathway (Krohn et al. 2014). These findings support our observation since the strain lacking atmA function led to generate increased apical branching phenotype when the colony expanded its hyphal branches from MAG and reached to the proline hole. In another study, Fagundes et al. (2004) suggested deletion mutants associated with npkA kinase have no impacts on hyphal branching, growth rate or conidial formation in *A. nidulans*. This confirms our results since in this research while slight increase was observed 2 times in the

number of apical branches formed on proline medium, this strain showed no significant difference with the wild type strain. Also, it grew properly without showing any dense hyphae or defects in forming colony. In fact, the significant role of 2D12 or mpkA kinase, compared to all aforementioned strains is considerable based upon its major role in regulating various functions in various organisms.

3.4.2 Interpretation of Proline + MAG hole results— Through this experiment by screening the changes in the pattern of apical branching morphogenesis particularly the number of apical branches when the growth condition changed from proline to MAG holes, of the 100 mutant kinase deletion strains we found 17 strains showed abnormal phenotype. Among those 7 (Appendix XII) strains indicated either hypo-or hyper-apical branching phenotype more than 2 times. The most interesting candidates in terms of showing constant hypo-apical branching phenotype during the four rounds of QMA were 2D3, 2D12 and 2A4 strains same as the previous experiment (MAG+proline hole). In addition to these strains there were other strains that showed the highest repetitiveness in terms of showing abnormal phenotype when they reached to the MAG hole including 2C5, 1D9, 1D8 and 2B5. Among these mutant strains 1D9 (cmkD) and 1D8 (sepH) have been characterized, while the other 2 remaining strains have not been studied well in filamentous fungi. cmkD kinase has been revealed as part of HogA MAPK pathway therefore it plays role in stress response and developmental processes (Arroyo et al. 2015). However, deletion mutants associated with this kinase revealed no defects in hyphal growth in *A. nidulans* in this study. By contrast, some studies indicated that 1D8 or sepH has been involved in septation, hyphal growth and it is required for actin ring formation during cytokinesis suggesting sepH plays role in mediating branching morphogenesis (Bruno et al. 2001; de Souza et al. 2013; Harris et al. 1994). Therefore, strains lacking this function experienced a sever defect in growth in this research. This

indicates that this kinase may also play role in monitoring apical branching when environment undergoes dramatic changes. Studying about the orthologous of 2C5 and 2B5 elucidate that in *C. albicans* 2C5 is involved in inducing fungal virulence, while 2B5 *S. cerevisiae* homologue, GCN2 plays an essential role in stress response pathways (Barrio et al. 2000; Selitrennikoff et al. 2001). However, there is lack of evidence to know what functions they have in *A. nidulans*.

3.4.3 Common protein kinases found among proline + MAG holes and MAG+ proline hole—Comparing the result of MAG+proline hole and proline +MAG holes led to find the top 6 kinases including 2D3 (ptkA), 2D12 (mpkA), 2A4 (AN5757), 1D8 (sepH), 1D9 (cmkD) and 1C8 (setC) that were found to be common on these type of media which showed abnormal apical branching phenotype upon reaching to either proline hole or MAG hole (Table 3-1) (Appendix XII). Among those based upon the strong evidences existed to confirm the important role of MAPK pathway in various functions in filamentous fungi and since *mpkA* deletion strain showed hypo-apical branching phenotype constantly on both MAG+proline hole and proline +MAG holes, this research suggests this kinase might be regarded as the most potential kinase regulating apical branching morphogenesis upon environmental changes.

Other potential strains are 2D3 and 2A4 since they showed decreased apical branching phenotype 4 times during the four rounds of QMA. Some studies showed the key role of these kinases in fungal development, while 2A4 strain has been not fully characterized in *A. nidulans* to know what its function. However, in this research based upon the phenotype of this strain and the numbers of apical branches we realized deletion of this strain will result in strong growth defects and delay in growth time (Figures 3-6 & 3-10) that prohibits hyphae to sense the environmental cues properly when the environment change from MAG to proline and vice versa. Then these fluctuations as well as lacking 2A4 strain would impair apical branching morphogenesis. 1C8 has

been shown to cooperate with MAPK pathway to regulate signaling and sensing as well as mediating fungal growth therefore it is part of MAPK pathway which means it can be regulated by MAPK pathway. As a result, 2D12 could be considered as an interesting candidate which mediates apical branching morphogenesis when the growth condition changes dramatically.

3.4.4 Interpretation of Proline + proline hole &MNV-glycerol, MNV-ethanol, MAG without vitamin and proline results— After plating 100 kinase deletion strains on proline medium containing proline holes no results were observed. While some of the strains formed colony on the medium, they were not able to expand their hyphal growth to reach the proline hole (Figures 3-12 & 3-13). To find whether this caused by proline or other factors have led them to stop forming colony, I plated the wild type strain (2E1), A4 as reference strain and one of the mutant strains 2A10 (which in chapter 2 was shown as the potential candidate in regulating apical branching morphogenesis) on different media types which were rich in glucose including MNV-glycerol, MNV-ethanol and MAG without vitamin. These strains were plated on proline medium as well. Consequently, the results suggest a significant difference among the strains plated on proline and the strains plated on other media types in terms of generating apical branches, while there was no significant difference among the strains inoculated on MNV-glycerol, MNV-ethanol and MAG without vitamin (Table 3-2) (Appendix XIV). This suggests that proline may not be a great source of carbon for filamentous fungi, since the number of apical branches formed on proline medium by those strains, showed significant reduction compared to those strains plated on other media types. Therefore, these observations can explain why strains did not grow properly on proline+ proline holes experiment and no outcomes were obtained. There are some evidences showing that when there is sufficient amount of glucose in the environment a mechanism named as Carbon Catabolite Repression (CCR) is activated in fungi which prevents them to use alternative carbon

sources (Adnan et al. 2018). However, when the environment lacks enough amount of glucose this mechanism is repressed leading fungi to use the alternative source of carbon (Adnan et al. 2018; Marggraf 1747; Sabina & Brown 2020). While this alternative source of carbon induces them to grow, they are not able to expand their hyphal branches to reach the maximum growth rate (Adnan et al. 2018; Marggraf 1747; Sabina & Brown 2020). This evidence might be another reason to indicate why some of the fungal strains did not grow well on proline medium containing proline holes (neither in the medium nor on the holes). Since they were not able to sense enough nutrients like glucose.

Overall, based upon the results of proline+MAG holes and MAG+proline hole experiments the most interesting candidates that might be involved in modulating apical branching morphogenesis through environmental fluctuations in *A. nidulans* are 2D3 (ptkA), 2D12(mpkA) and 2A4(AN5757) which among those according to the strong evidences exist for MPKA pathway, in this research it is suggested that 2D12 (mpkA) kinase might be the most potential candidate regulating apical branching morphogenesis in filamentous fungi through environmental fluctuations.

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Chapter 4:
Discussion & Future Directions

Discussion

Finding protein kinases involved in signaling pathway and regulation of hyphal branching in filamentous fungi is fundamental in particular for groups of organisms that are considered as the most important pathogens of humans, animals and plants. It is crucial to know what regulatory pathways induce filamentous fungi to expand their hyphal branches (Coudert et al. 2019; Niu et al. 2020; Ribeiro et al. 2019) or what kind of genes help pathogenic yeasts like *Candida albicans* to trigger yeast to hyphal transition (Cullen and Sprague 2012; Cao et al. 2006; Gimeno and Fink 1994). This would lead to generate some fundamental information for scientists who are working in the field of medicine and agriculture to find proper treatments for fungal diseases involving humans, animals and plants.

Since there are some morphological similarities among branched structures in humans and fungi (Wang et al. 2017), finding fungal homologues of the human genes involved in branching morphology, results to predict signaling pathways shared among humans and *A. nidulans* that control branching morphology (chapter 2). Most of the genes associated with human neuronal defects that were blasted against *A. nidulans* in chapter 2 were involved in signaling transduction pathways. It was suggested that some of them which showed the strongest homology are protein kinases (PKs) in *A. nidualns* regulating fundamental tasks including conidial formation, hyphal growth and signaling (Chapter 2). This allowed me to hypothesize that PKs might be involved in signaling transduction pathways and therefore regulating branching morphogenesis in fungi. Consequently, to test this hypothesis two separate assays were performed to examine the role of a specific set of 100 PKs in regulating apical branching morphogenesis in *A. nidulans* (Chapters 2&3).

In this study (Chapter 2), applying quantitative microscopy assay (QMA) to measure apical branches as well as appropriate statistical tests, led to find the top six mutant strains including 2A10 (pkaA), 2A9 (ImeB), 2D3 (ptkA), 2B6 (AN 7537), 2C11 (nikA) and 2B10 (AN7695) as the most potential kinases in regulating apical branching morphogenesis. Among those kinases it is suggested that 2A10 is the most potential candidate regulating apical branching morphogenesis. Since *pkaA* deletion strain not only showed a very strong growth defect compared to other strains, but also through a research by Ribeiro et al. 2019 we know all of the phosphorylation targets of PKA pathway, while information about the targets of other top 5 kinases is not available. Therefore, these strong evidences could support our results. For example, in *S. cerevisiae* and *Candida albicans* PKA induces filamentous growth (filamentation) (Cao et al. 2006; Cullen and Sprague 2012; Gimeno and Fink 1994). So, it would not surprise if pkaA strain plays role in apical branching morphology.

Another research performed in this project was to elucidate the role of the 100 pre-existing protein kinases in mediating apical branching morphogenesis in *A. nidulans* when the growth condition changes dramatically. This research was conducted under two different environmental conditions in terms of having different source of carbon. By applying QMA and statistical tests to detect abnormal strains showing either hypo-or hyper-apical branching phenotype when the condition changed from glucose to proline and vice versa, It was identified that the top 6 deletion strains showed abnormal apical branching phenotype. Those strains were 2D12 (mpkA), 2D3 (ptkA), 2A4 (AN5757), 1D8, 1D9 and 1C8. Among those kinases 2D12 was suggested as an interesting candidate regulating apical branching morphogenesis when the growth condition undergoes a dramatic change. Since not only *mpkA* deletion strain showed a very strong growth defect and the lowest number of apical branches constantly under two different environmental

conditions (Appendix XII), but also we know all the phosphorylation targets of this kinase (Chelius et al. 2019) in *A. nidulans* and it has been very well characterized compared to other top 5 kinases. For example, mpkA kinase regulates cell wall stress pathway and so many types of environmental stress weather directly or indirectly cause of some types of cell wall stress (Chelius et al. 2019). Another point is based upon the analysis of phosphoproteomics data associated with mpkA it has been elucidated this kinase plays role in lateral branching (Chelius et al. 2019). Therefore, this supports what I found in this research showing that mpkA plays role in apical branching in *A. nidulans.* So, it would not surprise if mpkA plays a prominent role in regulating apical branching among other kinases in this research (chapter 3).

Future Directions

In the future it will be very interesting to find all of the phosphoproteomics data associated with the top 5 kinases in this study (chapters 2&3) because to date no one has yet identified the targets for each of these kinases. While it will be very expensive and time consuming, I would think it would really help to elucidate the main regulatory pathways controlling apical branching morphology in filamentous fungi. Also, I would think in the future it will be fascinating if we try to investigate the role of GPCRs (G-protein coupled receptors) in monitoring apical branching in filamentous fungi. Since this signaling pathway has been very well conserved in eukaryotic organisms and it has been shown that it plays fundamental role in induction of lateral branch formation in filamentous fungi (Niu et al. 2020).

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Appendix I. Conserved homologous genes among *Aspergillus nidulans* and *Homo sapiens*.

GENE_ENTREZ_ID	GENE_SYMBOL (*Homo sapiens*)	GO/function in regulating neuronal development and branching (Homo sapiens)	DISEASE_IDS	AN IDs (*Aspergillus nidulans*)	Feature type	WD 40 domains	Function	Accession Number
60	ACTB	0001738/morphogenesis of a polarized epithelium	OMIM:607371,OMIM:243310	actA/AN6542	ORF, Verified		Gamma-actin	P20359.2
207	AKT1	0030030: cell projection organization/0060644mammary gland epithelial cell differentiation/0010975regulation of neuron projection development	OMIM:615109,OMIM:114480,OMIM:176920,OMIM:167000,OMIM:114500	pkcB/AN5973	ORF, Verified,		Protein with similarity to protein kinase C; involved in polar axis establishment and germling growth; mutant is inviable and forms microcolonies	XP_663577.1
10000	AKT3	0048854 brain morphogenesis/0007165signal transduction	OMIM:615937	pkcA/AN0106	ORF, Verified		Protein kinase C; essential gene; required for wild-type level of growth, cell wall integrity and penicillin biosynthesis	BAD02338.1
440138	ALG11		OMIM:613661	AN5725	ORF, Uncharacterized,		Protein with a predicted role in asparagine-linked glycosylation; S. cerevisiae ortholog Alg11p has alpha-1,2-mannosyltransferase activity	XP_663329.1
375	ARF1	0007015 actin filament organization	OMIM:618185	arlA/AN5912	ORF, Uncharacterized,		Predicted ADP ribosylation factor GTPase	XP_658730.1
10564	ARFGEF2	0035556 intracellular signal transduction	OMIM:608097	hypB/AN6709	ORF, Verified,		Putative ADP ribosylation factor guanine nucleotide exchange factor; Sec7-domain protein; role in hyphal morphogenesis; mutants display abnormal hyphae, hyphal tip cell death and excessive septum formation	XP_664313.1
440	ASNS		OMIM:615574	AN4401	ORF, Uncharacterized		Putative asparagine synthase with a predicted role in asparagine metabolism	XP_662005.1
23545	ATP6V0A2		OMIM:219200,OMIM:278250	AN5606	ORF, Uncharacterized,		Ortholog(s) have proton-transporting ATPase activity, rotational mechanism activity and role in cellular protein-containing complex assembly, endocytosis, polyphosphate metabolic process, vacuolar acidification, vacuole organization	XP_663210.1
523	ATP6V1A		OMIM:618012,OMIM:617403	vmaA/AN8021	ORF, Verified,		Putative vacuolar ATPase (V-ATPase), subunit A; required for normal growth and conidiation; mutation confers resistance to concanamycin; mutant fails to grow at alkaline pH; protein induced by farnesol	CAD54042.1
529	ATP6V1E1		OMIM:617402	AN8674	ORF, Uncharacterized,		Ortholog(s) have fungal-type vacuole membrane, vacuolar proton-transporting V-type ATPase, V1 domain localization	XP_681943.1
545	ATR		OMIM:614564,OMIM:210600	uvsB/AN6975	ORF, Verified,		Putative 1-phosphatidylinositol-3-kinase; role in checkpoint regulation during entry into mitosis and the DNA damage response; mutants have moderate growth defect, are sensitive to camptothecin, hydroxyurea and DEO, abnormal colony color	AAD54313.1
1020	CDK5	0048813 dendrite morphogenesis/0021954 central nervous system neuron development/0030866 cortical actin cytoskeleton organization/0030182neuron differentiation/0031175neuron projection development	OMIM:616342	phoA/AN8261	ORF, Verified,		Cyclin-dependent protein kinase; role in regulation of development in response to phosphate availability; interacts with cyclin Pho80; lethal in combination with mutations in phoB; mutant is sensitive to NaCl	AAC42260.1
1021	CDK6	0048699 generation of neurons	OMIM:616080	nimX/AN4182	ORF, Verified,		Cyclin-dependent protein kinase involved in cell cycle control; required for cell cycle progression through G1 and G2; regulated by NimE cyclin and NimT phosphatase; homolog of S. pombe cdc2; transcript camptothecin upregulated	XP_661786.1
1062	CENPE		OMIM:616051	kinA/AN5343	ORF, Verified,		Kinesin-family protein; involved in microtubule destabilization; required for normal growth and nuclear positioning	XP_662947.1
11113	CIT		OMIM:617090	cotA/AN5529	ORF, Verified,		Essential NDR ser/thr protein kinase; role in cell polarity; RAM-signaling pathway component; MobB/CotA kinase complex thought to regulate cell polarity growth by maintaining cellular calcium homeostasis; mutant forms brown microcolonies	XP_663133.1
9276	COPB2		OMIM:617800	AN5972	ORF, Uncharacterized,	YES	Ortholog(s) have ubiquitin binding activity	CBF70445.1
1376	CPT2		OMIM:600649,OMIM:255110,OMIM:614212,OMIM:608836	facC/AN1059	ORF, Verified,		Carnitine acetyltransferase, required for utilization of acetate as carbon source; transcriptional induction by acetate is mediated by FacB; carbon catabolite repression is mediated by CreA; predicted role in the carnitine shuttle	AAC82487.1
1457	CSNK2A1	0007165 signal transduction	OMIM:617062	cka1/AN1485	ORF, Uncharacterized,		Protein kinase; mutants are lethal forming only microcolonies	XP_659089.1
8450	CUL4B		OMIM:300354	culD/AN10008	ORF, Uncharacterized,		Predicted ubiquitin protein ligase; role in ubiquitin-dependent protein catabolism and cullin-RING ubiquitin ligase complex localization; NeddH-associated protein	CBF90329.1
1778	DYNC1H1		OMIM:158600,OMIM:614563,OMIM:614228	nudA/AN0118	ORF, Verified,		Cytoplasmic dynein heavy chain, component of the microtubule motor protein involved in nuclear migration; locus contains the conserved upstream open reading frame (uORF) AN0118-uORF	XP_657722.1
1781	DYNC1I2		OMIM:618492	nudI/AN1454	ORF, Verified,	Yes	Cytoplasmic dynein intermediate chain, component of the microtubule motor protein involved in nuclear migration	XP_659058.1
2108	ETFA		OMIM:231680	AN6699	ORF, Uncharacterized,		Ortholog(s) have mitochondrion localization	CBF71275.1
2109	ETFB		OMIM:231680	AN10278	ORF, Uncharacterized,		Ortholog(s) have mitochondrion localization	CBF86442.1

2271	FH		OMIM:150800,OMIM:606812	AN8707	ORF, Uncharacterized,		Putative fumarate dehydratase with a predicted role in the TCA cycle	XP_681976.1
9896	FIG4		OMIM:612691,OMIM:612577,OMIM:611228,OMIM:216340	AN7314	ORF, Uncharacterized,		Ortholog(s) have phosphatidylinositol-3,5-bisphosphate 5-phosphatase activity, role in phosphatidylinositol dephosphorylation and PAS complex, extrinsic component of membrane, fungal-type vacuole membrane, nuclear periphery localization	XP_680583.1
84340	GFM2		OMIM:618397	AN2223	ORF, Uncharacterized,		Ortholog(s) have role in mitochondrial genome maintenance, regulation of mitochondrial membrane potential	XP_659827.1
29925	GMPPB		OMIM:615352,OMIM:615350,OMIM:615351	AN5586	ORF, Uncharacterized,		Putative mannose-1-phosphate guanylyltransferase with a predicted role in mannose/mannitol, fructose, and sorbose/sorbitol metabolism	Q5B1J4.2
2775	GNAO1	0031175 neuron projection development	OMIM:615473,OMIM:617493	fadA/N0651	ORF, Verified,		Alpha subunit of a heterotrimeric G protein composed of FadA, SfaD, GpgA and involved in regulation of proliferation and conidiophore development; mutant produces increased amounts of extracellular proteinase during carbon starvation	XP_658255.1
2782	GNB1	0007165 signal transduction	OMIM:613065,OMIM:616973	sfaD/AN0081	ORF, Verified,	Yes	Beta subunit of a heterotrimeric G protein composed of FadA, SfaD, GpgA and involved in regulation of proliferation and conidiophore development; mutant produces increased amounts of extracellular proteinase during carbon starvation	XP_657685.1
10243	GPHN		OMIM:149400,OMIM:615501	cnxE/AN3778	ORF, Verified,		Putative Gephyrin-related protein involved in molybdenum cofactor biosynthesis; molybdopterin cofactor required for the activity of nitrate reductase	XP_661382.1
3295	HSD17B4		OMIM:261515,OMIM:233400	foxA/AN7111	ORF, Verified,		Peroxisomal multifunctional enzyme involved in fatty acid beta-oxidation; required for growth on very long-chain fatty acids; transcription is induced by fatty acids	CBF79061.1
3614	IMPDH1		OMIM:180105,OMIM:613837	AN10476	ORF, Verified,		IMP dehydrogenase/GMP reductase; expression reduced after exposure to farnesol	CBF75305.1
23522	KAT6B		OMIM:603736,OMIM:606170	nmy1/AN5640	ORF, Uncharacterized,		Putative MYST-type acetyltransferase	XP_663244.1
10765	KDM5B	0060444 branching involved in mammary gland duct morphogenesis/0060763 mammary duct terminal end bud growth	OMIM:618109	AN8211	ORF, Uncharacterized,		Ortholog(s) have histone demethylase activity (H3-trimethyl-K4 specific) activity	XP_681480.1
3832	KIF11		OMIM:152950	bimC/AN3363	ORF, Verified,		Kinesin-family protein required for the normal completion of mitosis; required for separation of mitotic spindle pole bodies	XP_660967.1
9928	KIF14		OMIM:616258,OMIM:617914	uncA/AN7547	ORF, Verified,		Member of kinesin-3 motor protein family; binds detyrosinated cytoplasmic microtubules, not mitotic spindle; mutation causes defects in vesicle transport and slow growth	XP_680816.1
3800	KIF5C		OMIM:615282	kinA/AN5343	ORF, Verified,		Kinesin-family protein; involved in microtubule destabilization; required for normal growth and nuclear positioning	CAC19836.1
374654	KIF7		OMIM:614120,OMIM:607131,OMIM:200990	kipB/AN4513	ORF, Verified,		Microtubule (MT) depolymerase; Kip3 family kinesin; travels toward MT plus end; roles in mitotic spindle positioning, MT cytoskeletal morphology, mitotic progression, chromosome maintenance; mitotic, astral, cytoplasmic MT localization	XP_662117.1
11253	MAN1B1		OMIM:614202	AN5748	ORF, Uncharacterized,		Putative mannosyl-oligosaccharide 1,2-alpha-mannosidase with a predicted role in mannose polymer metabolism	XP_663352.1
4292	MLH1		OMIM:609310,OMIM:276300,OMIM:158320x	AN0126	ORF, Uncharacterized,		Ortholog(s) have ATP binding, ATPase activity, DNA insertion or deletion binding, bubble DNA binding, dinucleotide insertion or deletion binding, double-stranded DNA binding and heteroduplex DNA loop binding,	XP_657730.1
4436	MSH2		OMIM:120435,OMIM:276300,OMIM:158320	AN10621	ORF, Uncharacterized,		Ortholog(s) have ATP binding, ATPase activity, DNA insertion or deletion binding, Y-form DNA binding, double-strand/single-strand DNA junction binding and four-way junction DNA binding	CBF76294.1
2956	MSH6		OMIM:614350,OMIM:608089,OMIM:276300	mshA/AN1708	ORF, Uncharacterized,		Putative MutS homolog with a predicted role in DNA mismatch repair; transcript upregulated in response to camptothecin	XP_659312.1
2475	MTOR	0045182translation regulator activity/0007420brain development/0030838positive regulation of actin filament polymerization/0060999positive regulation of dendritic spine development/0014042 positive regulation of neuron maturation/0010976positive regulation of neuron projection development/0032956regulation of actin cytoskeleton organization	OMIM:616638,OMIM:607341	torA/AN5982	ORF, Verified,		Tor kinase homolog; essential; heterozygous diploids show increased sensitivity to DL-aspartic acid beta-hydroxamate in ammonium medium and enhanced growth on threonine; null mutants have early septation defect and arrest as short germlings	CAG30554.1
23327	NEDD4L		OMIM:617201	hulA/AN1339	ORF, Verified,		Putative HECT ubiquitin ligase	XP_658943.1
54888	NSUN2		OMIM:611091	AN0757	ORF, Uncharacterized,		Ortholog(s) have tRNA (cytosine-5-)-methyltransferase activity, tRNA binding activity	CBF88845.1
55644	OSGEP		OMIM:617729	kaeA/AN6569	ORF, Uncharacterized,		Putative component of the EKC/KEOPS complex, involved in regulation of arginine metabolism	XP_664173.1
5048	PAFAH1B1	0048854brain morphogenesis/0001764neuron migration	OMIM:607432	nudF/AN6197	ORF, Verified,	Yes	Nuclear migration protein; mutants defective in asexual and sexual sporulation; similar to human LIS-1; interacts directly with NudC; spindle-pole body-localized; may facilitate minus-end-directed dynein movement	XP_663801.1
5130	PCYT1A		OMIM:608940	AN1357	ORF, Uncharacterized,		Putative cholinephosphate cytidylyltransferase with a predicted role in phospholipid metabolism	CBF87654.1

5189	PEX1		OMIM:214100,OMIM:234580,OMIM:601539	pexA/AN5991	ORF, Verified,		Putative peroxisomal protein (peroxin) with a role in fatty acid utilization; required for growth on long chain fatty acids	XP_663595.1
5830	PEX5		OMIM:214110,OMIM:202370,OMIM:616716	pexE/AN10215	ORF, Verified,		Putative peroxisomal targeting signal 1 (PTS1) receptor protein; required for growth on long chain fatty acids; inactivation impairs triacetylfusarinine C (TAFC) siderophore biosynthesis; mutant is growth impaired in iron-depleted medium	CBF85028.1
5190	PEX6		OMIM:614862,OMIM:616617,OMIM:614863	pexF/AN2925	ORF, Verified,		Putative peroxisomal import protein (peroxin) with a role in fatty acid utilization; required for growth on long chain fatty acids	CBF83738.1
5297	PI4KA	0007165 signal transduction	OMIM:616531	stt4/AN4278	ORF, Uncharacterized,		Essential 1-phosphatidylinositol 4-kinase with a predicted role in phospholipid metabolism; mutants arrest in the cell cycle	XP_661882.1
9373	PLAA	0007399 nervous system development/1903861positive regulation of dendrite extension/2001224 positive regulation of neuron migration/0007165signal transduction	OMIM:617527	AN7704	ORF, Uncharacterized,	**Yes**	Ortholog(s) have role in cellular protein catabolic process, proteasome-mediated ubiquitin-dependent protein catabolic process and cytoplasm, nucleus localization	CBF79941.1
5395	PMS2		OMIM:614337,OMIM:276300	AN6316	ORF, Deleted, Uncharacterized,		Ortholog(s) have ATP binding, ATPase activity, DNA insertion or deletion binding, dinucleotide insertion or deletion binding, double-stranded DNA binding, heteroduplex DNA loop binding, single-stranded DNA binding activity	XP_663920.1
11284	PNKP		OMIM:613402,OMIM:616267	AN5751	ORF, Uncharacterized,		Ortholog(s) have ATP-dependent polydeoxyribonucleotide 5'-hydroxyl-kinase activity, polynucleotide 3'-phosphatase activity and role in DNA 3' dephosphorylation involved in DNA repair, base-excision repair, single strand break repair	XP_663355.1
				AN10316	ORF, Uncharacterized,		Putative RNA polymerase III, large subunit; ortholog of S. cerevisiae Rpo31p; expression reduced after exposure to farnesol	CBF87003.1
				AN0809	ORF, Uncharacterized,		Ortholog(s) have DNA binding activity and RNA polymerase II, core complex, nuclear chromatin localization	XP_658413.1
10585	POMT1		OMIM:236670,OMIM:613155,OMIM:609308	pmtC/AN1459	ORF, Verified,		Subfamily 4 protein O-mannosyltransferase; involved in hyphal growth and conidia formation	XP_659063.1
29954	POMT2		OMIM:613156,OMIM:236670,OMIM:613150,OMIM:613158	pmtA/AN5105	ORF, Verified,		Pmt 2 subfamily protein O-mannosyltransferase; required for normal germination, hyphal growth and conidiophore development; mutants are sensitive to growth at high temperature and to cell wall perturbing agents	XP_662709.1
29968	PSAT1		OMIM:610992,OMIM:616038	AN10298	ORF, Verified,		3-phosphoserine aminotransferase; intracellular; protein abundance decreased by menadione stress	CBF86787.1
5859	QARS1	0007420 brain development	OMIM:615760	AN9157	ORF, Uncharacterized,		Has domain(s) with predicted ATP binding, aminoacyl-tRNA ligase activity, glutamine-tRNA ligase activity, role in glutaminyl-tRNA aminoacylation, tRNA aminoacylation, tRNA aminoacylation for protein translation and cytoplasm localization	XP_682426.1
5879	RAC1	0030036actin cytoskeleton organization/0007015 actin filament organization/0030041 actin filament polymerization/0007163 establishment or maintenance of cell polarity/0035556 intracellular signal transduction/0032956regulation of actin cytoskeleton organization	OMIM:617751	racA/AN4743	ORF, Uncharacterized,		Rho GTPase protein required for normal colony growth and conidiophore development; synthetically lethal with modA null	XP_662347.1
9401	RECQL4		OMIM:268400,OMIM:266280	musN/AN2087	ORF, Verified,		DNA helicase of the RecQ family, involved in response to DNA damage; interacts with 1-phosphatidylinositol-3-kinase UvsB	XP_659691.1
6197	RPS6KA3	0035556intracellular signal transduction/0007165 signal transduction	OMIM:300844,OMIM:303600	pkcB/AN5973	ORF, Verified,		Protein with similarity to protein kinase C; involved in polar axis establishment and germling growth; mutant is inviable and forms microcolonies	XP_663577.1
6301	SARS1		OMIM:617709	AN8867	ORF, Uncharacterized,		Has domain(s) with predicted ATP binding, aminoacyl-tRNA ligase activity, serine-tRNA ligase activity, role in seryl-tRNA aminoacylation, tRNA aminoacylation for protein translation and cytoplasm localization	XP_682136.1
10262	SF3B4		OMIM:154400	AN6501	ORF, Uncharacterized,		Ortholog(s) have U2 snRNP localization	XP_664105.1
150094	SIK1	0035556intracellular signal transduction	OMIM:616341	snf1/AN7695	ORF, Uncharacterized,		Putative protein kinase; reduced growth on AVICEL medium; required for CreA derepression and endocellulase production	CBF79923.1
79751	SLC25A22		OMIM:609304	AN8785	ORF, Uncharacterized,		Ortholog(s) have L-aspartate transmembrane transporter activity, L-glutamate transmembrane transporter activity, antiporter activity, uniporter activity	XP_682054.1
6812	STXBP1		OMIM:612164	AN4724	ORF, Uncharacterized,		Ortholog(s) have role in vesicle-mediated transport and prospore membrane localization	CBF76920.1
23043	TNIK	0007010cytoskeleton organization/0035556intracellular signal transduction/0048812neuron projection morphogenesis	OMIM:617028	mst1/AN5674	ORF, Uncharacterized,		MAP kinase, kinase, kinase, kinase (MAPKKKK); mutants undergo premature but incomplete sexual development	XP_663278.1
8295	TRRAP		OMIM:618454	AN8000	pseudogene, Verified,		Subunit of the SAGA transcriptional regulatory complex, possible pseudogene	XP_681269.1
7846	TUBA1A		OMIM:611603	tubA/AN0316	ORF, Verified,		Alpha-tubulin, forms a heterodimer with beta-tubulin that promotes microtubule assembly	XP_657920.1
				tubB/AN7570	ORF, Verified,		Alpha-tubulin, forms a heterodimer with beta-tubulin that promotes microtubule	XP_680839.1

							assembly; non-essential subunit required for meiosis and formation of ascospores	
203068	TUBB		OMIM:615771,OMIM:156610	benA/AN1182	ORF, Verified,		Beta-tubulin, highly conserved component of microtubules; A. nidulans has two beta-tubulin genes, benA and tubC; temperature sensitive mutants are blocked in mitosis and in nuclear division	XP_658786.1
				tubC/AN6838	ORF, Uncharacterized,		Beta-tubulin, highly conserved component of microtubules; A. nidulans has two beta-tubulin genes, tubC and benA; expression of tubC increases during conidiation	XP_664442.1
7283	TUBG1		OMIM:615412	mipA/AN0676	ORF, Verified,		Gamma-tubulin, essential component of the spindle pole body, required for microtubule function	P18695.3
7284	TUFM		OMIM:610678	AN1084	ORF, Verified,		Putative elongation factor EF-Tu; intracellular, menadione stress-induced protein	CBF88179.1
55697	VAC14	0007165signal transduction	OMIM:617054	AN10682	ORF, Uncharacterized,		Ortholog(s) have molecular adaptor activity, protein binding, bridging activity and role in phosphatidylinositol biosynthetic process, positive regulation of lipid kinase activity, protein localization to vacuolar membrane	CBF81734.1
26276	VPS33B		OMIM:208085	hbrA/AN2418	ORF, Uncharacterized,		Putative protein with a predicted role in vacuolar sorting	XP_660022.1
10352	WARS2		OMIM:617710	AN6488	ORF, Uncharacterized,		Putative tryptophanyl-tRNA synthetase with a predicted role in tRNA charging for translation	CBF70835.1
80232	WDR26		OMIM:617616	AN8017	ORF, Uncharacterized,	**YES**	Ortholog of A. fumigatus Af293 : Afu5g02400, A. niger CBS 513.88 : An02g10400, A. oryzae RIB40 : AO090102000352, Aspergillus wentii : Aspwe1_0052944 and Aspergillus sydowii : Aspsy1_0059906	XP_681286.1
9213	XPR1		OMIM:616413	AN4733	ORF, Uncharacterized,		Ortholog(s) have role in signal transduction and plasma membrane localization	CBF76903.1
16	AARS1		OMIM:616339,OMIM:613287	AN9419	ORF, Uncharacterized,		Ortholog(s) have alanine-tRNA ligase activity, role in alanyl-tRNA aminoacylation and cytoplasm, mitochondrion localization	Q5AQL1.2
19	ABCA1		OMIM:604091,OMIM:205400	AN8812	ORF, Uncharacterized,		Has domain(s) with predicted ATP binding, ATPase activity, nucleoside-triphosphatase activity, nucleotide binding activity	XP_682081.1
	ABCC8		OMIM:240800,OMIM:610374,OMIM:256450,OMIM:125853,OMIM:606176	AN7729	ORF, Uncharacterized,		Ortholog(s) have ATPase-coupled glutathione S-conjugate transmembrane transporter activity, ATPase-coupled phytochelatin transmembrane transporter activity, bilirubin transmembrane transporter activity	XP_680998.1
				AN0225	ORF, Uncharacterized,		Ortholog(s) have ATPase-coupled monocarboxylic acid transmembrane transporter activity, role in monocarboxylic acid transport and fungal-type vacuole localization	XP_657829.1
215	ABCD1	1990535neuron projection maintenance	OMIM:300100	amyD/AN3308	ORF, Uncharacterized,		Putative alpha-amylase with a predicted role in starch metabolism; predicted glycosyl phosphatidylinositol (GPI)-anchor	XP_658618.1
35	ACADS		OMIM:201470	scdA/AN0824	ORF, Verified,		Putative mitochondrial acyl-coA dehydrogenase involved in short-chain fatty acid beta-oxidation; required for growth on short-chain fatty acids	XP_658428.1
50	ACO2		OMIM:616289,OMIM:614559	acoA/AN5525	ORF, Verified,		Putative aconitate hydratase with a predicted role in the TCA cycle; intracellular; protein abundance decreased by menadione stress; protein expressed at increased levels in a hapX mutant versus wild-type	XP_663129.1
				AN5300	ORF, Uncharacterized,		Putative aconitate hydratase with a predicted role in the TCA cycle	XP_662904.1
				AN3894	ORF, Uncharacterized,		Putative aconitate hydratase with a predicted role in the TCA cycle	XP_661498.1
2182	ACSL4	0060996dendritic spine development/0030182neuron differentiation	OMIM:300387	faaA/AN6014	ORF, Verified,		Putative long-chain-fatty-acid-CoA ligase with a predicted role in fatty acid metabolism	CBF70362.1
122622	ADSS1		OMIM:617030	adB/AN0893	ORF, Uncharacterized,		Putative adenylosuccinate synthase with a predicted role in purine metabolism; induced by ammonium and adenosine	XP_658497.1
10939	AFG3L2		OMIM:610246,OMIM:614487	AN4557	ORF, Uncharacterized,		Ortholog(s) have ATP binding, ATPase activity, metallopeptidase activity and role in cellular protein-containing complex assembly, proteolysis, signal peptide processing	XP_662161.1
191	AHCY		OMIM:613752	AN1263	ORF, Uncharacterized,		Putative adenosylhomocysteinase with a predicted role in methionine metabolism; expression reduced after exposure to farnesol	XP_658867.1
210	ALAD		OMIM:612740	AN1403	ORF, Uncharacterized,		Ortholog(s) have porphobilinogen synthase activity, zinc ion binding activity and role in heme biosynthetic process	XP_659007.1
5832	ALDH18A1		OMIM:616586,OMIM:219150,OMIM:601162,OMIM:616603	AN5799	ORF, Uncharacterized,		Putative gamma-glutamyl phosphate reductase with a predicted role in proline metabolism	XP_663403.1
85365	ALG2		OMIM:616228,OMIM:607906	AN6874	ORF, Uncharacterized,		Putative mannosyltransferase; transcript levels increase during the unfolded-protein response (UPR); S. cerevisiae ortholog Alg2p has glycolipid 6-alpha-mannosyltransferase activity	XP_664478.1
23600	AMACR		OMIM:614307,OMIM:214950	AN9522	ORF, Uncharacterized,		Has domain(s) with predicted CoA-transferase activity and role in metabolic process	XP_868904.1
271	AMPD2		OMIM:615809,OMIM:615686	AN8872	ORF, Uncharacterized,		Putative AMP deaminase with a predicted role in nucleotide salvage pathways	XP_682141.1
162	AP1B1	0001822kidney development	OMIM:242150	AN3029	ORF, Uncharacterized,		Ortholog(s) have role in cellular response to drug	XP_660633.1
				AN5950	ORF, Uncharacterized,		Ortholog(s) have role in filamentous growth and cell cortex of cell tip, cell division site, site of polarized growth localization	XP_663554.1

23400	ATP13A2		OMIM:606693,OMIM:617225	AN8864	ORF, Uncharacterized,		Has domain(s) with predicted ATPase activity, ATPase-coupled cation transmembrane transporter activity, metal ion binding, nucleotide binding activity, role in cation transport and integral component of membrane localization	CBF77849.1
476	ATP1A1		OMIM:618314,OMIM:618036	enaB/AN1628	ORF, Verified,		Putative membrane ATPase with a predicted role in energy metabolism	CBF85251.1
538	ATP7A	0021954central nervous system neuron development/0048812neuron projection morphogenesis/0021860 pyramidal neuron development	OMIM:304150,OMIM:300489,OMIM:309400	AN3624/ygA	ORF, Verified,		Protein required for conidia pigmentation, predicted to act as a copper transporter; mutants produce yellow conidia at high pH and green conidia at low pH	XP_661228.1
546	ATRX		OMIM:309580,OMIM:301040,OMIM:300448	AN2255	ORF, Uncharacterized,		Has domain(s) with predicted ATP binding, DNA binding, helicase activity, nucleic acid binding activity	XP_659859.1
9790	BMS1		OMIM:107600,	AN6334	ORF, Uncharacterized,		Has domain(s) with predicted GTP binding, GTPase activity, role in SRP-dependent cotranslational protein targeting to membrane, ribosome biogenesis and nucleus localization	XP_663938.1
811	CALR	2000510positive regulation of dendritic cell chemotaxis	OMIM:187950,OMIM:254450,	clxA/AN3592	ORF, Uncharacterized,		Putative calnexin with a predicted role in protein folding and protein quality control on the endoplasmic reticulum (ER) membrane	CBF75819.1
22948	CCT5		OMIM:256840	AN1904	ORF, Uncharacterized,		Ortholog(s) have chaperonin-containing T-complex localization	CBF85795.1
1103	CHAT		OMIM:254210	AN6279/acuJ	ORF, Verified,		Carnitine acetyltransferase, required for utilization of acetate and fatty acids; transcription induction in response to acetate mediated by FacB; transcription induction in response to long-chain fatty acids mediated by FarA	XP_663883.1
55636	CHD7		OMIM:214800,OMIM:612370	AN1255	ORF, Uncharacterized,		Ortholog(s) have nucleosome-dependent ATPase activity	XP_658859.1
1183	CLCN4		OMIM:300114,	AN2308	ORF, Uncharacterized,		Has domain(s) with predicted ion channel activity, voltage-gated chloride channel activity, role in chloride transport, transmembrane transport and membrane localization	XP_659912.1
4512	COX1		OMIM:540000	oxiA/AN20014	ORF, Uncharacterized,		Subunit I of cytochrome c oxidase, which is the terminal member of the mitochondrial inner membrane electron transport chain; one of three mitochondrially-encoded subunits; 2nd and 3rd introns contain maturase-related open reading frames	P00402.2
				ndhE/AN20001	ORF, Uncharacterized,		Mitochondrially-encoded subunit 5 of NADH dehydrogenase	YP_006303580.1
1355	COX15		OMIM:615119,OMIM:256000	AN1915	ORF, Uncharacterized,		Mitochondrially-encoded subunit 5 of NADH dehydrogenase	XP_659519.1
4514	COX3		OMIM:535000,OMIM:540000	oxiC/AN20012	ORF, Uncharacterized,		Subunit III of cytochrome c oxidase, which is the terminal member of the mitochondrial inner membrane electron transport chain; one of three mitochondrially-encoded subunits	YP_006303578.1
1371	CPOX		OMIM:121300,	AN5130	ORF, Verified,		Ortholog(s) have coproporphyrinogen oxidase activity, role in heme biosynthetic process and cell surface, cytosol, fungal biofilm matrix, mitochondrion, yeast-form cell wall localization	CBF80916.1
1509	CTSD		OMIM:610127	AN2903/pepE	ORF, Uncharacterized,		Aspartic protease; protein expressed at decreased levels in a hapX mutant versus wild-type	XP_660507.1
55157	DARS2		OMIM:611105	AN1710	ORF, Uncharacterized,		Ortholog(s) have aspartate-tRNA ligase activity, role in mitochondrial aspartyl-tRNA aminoacylation and mitochondrion localization	XP_659314.1
1639	DCTN1	0007399 nervous system development	OMIM:607641,OMIM:168605,OMIM:105400	AN6323/nudM	ORF, Verified,		p150 subunit of dynactin; forms comet-like structures at microtubule plus ends	XP_663927.1
8560	DEGS1		OMIM:618404	AN4405	ORF, Uncharacterized,		Ortholog(s) have sphingolipid delta-4 desaturase activity and role in cellular response to glucose starvation, hyphal growth, response to heat, sphingolipid biosynthetic process, sphingosine biosynthetic process	XP_662009.1
55526	DHTKD1		OMIM:615025,OMIM:204750	AN5571/kgdA	ORF, Uncharacterized,		Putative oxoglutarate dehydrogenase (lipoamide) with a predicted role in the TCA cycle	XP_663175.1
1743	DLST		OMIM:618475,	AN3466/kgdB	ORF, Uncharacterized,		Putative dihydrolipoamide S-succinyl transferase with a predicted role in the TCA cycle	XP_661070.1
1760	DMPK	0035556intracellular signal transduction	OMIM:160900	AN5529/cotA	ORF, Verified,		Essential NDR ser/thr protein kinase; role in cell polarity; RAM-signaling pathway component; MobB/CotA kinase complex thought to regulate cell polarity growth by maintaining cellular calcium homeostasis; mutant forms brown microcolonies	XP_663133.1
1763	DNA2		OMIM:615807,OMIM:615156	AN3653	**The summary information has been deleted for this AN ID**		Ortholog(s) have 5' overhang single-stranded DNA endodeoxyribonuclease activity, 5'-flap endonuclease activity, DNA helicase activity, chromatin binding and nuclease activity,	XP_661257.1
5611	DNAJC3		OMIM:616192	AN3463	ORF, Uncharacterized,		Ortholog(s) have sequence-specific DNA binding activity	XP_661067.1
10059	DNM1L		OMIM:614388,OMIM:610708	AN8874	ORF, Uncharacterized,		Ortholog(s) have GTPase activity, identical protein binding, protein homodimerization activity	XP_682143.1
				AN8023/vpsA	ORF, Verified,		Protein required for vacuole biogenesis	XP_681292.1
1798	DPAGT1		OMIM:608093,OMIM:614750,	AN5888	ORF, Uncharacterized,		Ortholog(s) have UDP-N-acetylglucosamine-dolichyl-phosphate N-acetylglucosaminephosphotransferase activity	XP_663492.1
1778	DYNC1H1		OMIM:614228,OMIM:158600,OMIM:614563	nudA/AN0118	ORF, Verified,		Cytoplasmic dynein heavy chain, component of the microtubule motor protein involved in nuclear migration; locus	XP_657722.1

							contains the conserved upstream open reading frame (uORF) AN0118-uORF	
1892	ECHS1		OMIM:616277	echA/AN5916	ORF, Verified,		Mitochondrial enoyl-CoA hydratase, involved in fatty acid beta-oxidation; required for growth on short-chain fatty acids and for catabolism of isoleucine and valine; transcription is induced by fatty acids	CBF70577.1
8518	ELP1		OMIM:223900	AN10551	ORF, Uncharacterized,		Ortholog(s) have tRNA binding activity	CBF77576.1
2068	ERCC2		OMIM:610756,OMIM:278730,OMIM:601675	AN9436	ORF, Uncharacterized,		Ortholog(s) have 5'-3' DNA helicase activity, DNA helicase activity, damaged DNA binding activity	CBF88902.1
2071	ERCC3		OMIM:616390,OMIM:610651	AN8201	ORF, Uncharacterized,		Putative DNA helicase; transcript upregulated in response to camptothecin	CBF74098.1
2072	ERCC4		OMIM:610965,OMIM:278760,OMIM:615272,	AN8713	ORF, Uncharacterized,		Ortholog(s) have 3' overhang single-stranded DNA endodeoxyribonuclease activity	CBF78182.1
2073	ERCC5		OMIM:278780,OMIM:616570,	AN5216	ORF, Uncharacterized,		Ortholog(s) have role in interstrand cross-link repair, nucleotide-excision repair, nucleotide-excision repair involved in interstrand cross-link repair	XP_662820.1
2184	FAH		OMIM:276700,	fahA/AN1896	ORF, Verified,		Fumarylacetoacetate hydrolase, catalyzes the last step in the phenylalanine catabolic pathway; intracellular; protein abundance decreased by menadione stress; mutation in human ortholog causes type I hereditary tyrosinaemia	AAA85778.1
22909	FAN1		OMIM:614817,	AN6678	ORF, Uncharacterized,		Ortholog(s) have role in interstrand cross-link repair	XP_664282.1
2271	FH		OMIM:606812,OMIM:150800	AN8707	ORF, Uncharacterized,		Putative fumarate dehydratase with a predicted role in the TCA cycle	XP_681976.1
9896	FIG4		OMIM:611228,OMIM:612577,OMIM:216340,OMIM:612691	AN7314	ORF, Uncharacterized,		Ortholog(s) have phosphatidylinositol-3,5-bisphosphate 5-phosphatase activity, role in phosphatidylinositol dephosphorylation and PAS complex, extrinsic component of membrane, fungal-type vacuole membrane, nuclear periphery localization	XP_680583.1
24140	FTSJ1		OMIM:309549	AN1751	ORF, Uncharacterized,		Ortholog(s) have tRNA (cytosine-2'-O-)-methyltransferase activity, tRNA (guanosine-2'-O-)-methyltransferase activity and role in tRNA methylation	XP_659355.1
				AN11125	ORF, Uncharacterized,		Ortholog(s) have glycine-tRNA ligase activity, role in DNA-templated transcription, termination, glycyl-tRNA aminoacylation, mitochondrial glycyl-tRNA aminoacylation and cytoplasm, mitochondrion localization	CBF77921.1
2632	GBE1		OMIM:263570,OMIM:232500	AN2314	ORF, Uncharacterized,		Putative 1,4-alpha-glucan branching enzyme with a predicted role in starch metabolism	Q9Y8H3.3
2639	GCDH		OMIM:231670	AN2762	ORF, Uncharacterized,		Putative acyl-coA dehydrogenase	XP_660366.1
2643	GCH1		,OMIM:128230,OMIM:233910	AN8188	ORF, Uncharacterized,		Putative GTP cyclohydrolase I with a predicted role in folate biosynthesis	CBF74068.1
2645	GCK		OMIM:125853,OMIM:125851,OMIM:606176,OMIM:602485	AN7459/hxkA	ORF, Verified,		Putative hexokinase with a predicted role in carbohydrate metabolism	XP_680728.1
2729	GCLC		OMIM:230450	AN3150	ORF, Uncharacterized,		Putative gamma-glutamylcysteine synthetase with a predicted role in glutathione biosynthesis	CBF83306.1
2664	GDI1		OMIM:300849	AN5895	ORF, Uncharacterized,		Putative Rab GDP-dissociation inhibitor; ortholog of S. cerevisiae Gdi1p	XP_663499.1
2673	GFPT1			gfaA/AN10709	ORF, Uncharacterized,		Putative glutamine-fructose-6-phosphate transaminase	AAW49003.1
29926	GMPPA		OMIM:615510	AN1911	ORF, Uncharacterized,		Putative mannose-1-phosphate guanyltransferase	XP_659515.1
29925	GMPPB		OMIM:615350,OMIM:615351,OMIM:615352,	AN5586	ORF, Uncharacterized,		Putative mannose-1-phosphate guanylyltransferase with a predicted role in mannose/mannitol, fructose, and sorbose/sorbitol metabolism	Q5B1J4.2
2767	GNA11		OMIM:145981,OMIM:615361	fadA/AN0651	ORF, Verified,		lpha subunit of a heterotrimeric G protein composed of FadA, SfaD, GpgA and involved in regulation of proliferation and conidiophore development; mutant produces increased amounts of extracellular proteinase during carbon starvation	XP_658255.1
59345	GNB4		OMIM:615185	sfaD/AN0081	ORF, Verified,	**Beyond WD 40 domain**	Beta subunit of a heterotrimeric G protein composed of FadA, SfaD, GpgA and involved in regulation of proliferation and conidiophore development; mutant produces increased amounts of extracellular proteinase during carbon starvation	AAC33436.1
2821	GPI		OMIM:613470	AN6037/swoM	ORF, Verified,		Putative glucose-6-phosphate isomerase with a predicted role in gluconeogenesis and glycolysis; mutant defective in hyphal polarity and conidiation	XP_663641.1
57531	HACE1		OMIM:616756	AN1966/hulE	ORF, Uncharacterized,		Putative HECT ubiquitin ligase	XP_659570.1
3035	HARS1		OMIM:616625,OMIM:614504	AN0046	ORF, Verified,		Putative histidyl-tRNA synthetase with a predicted role in tRNA aminoacylation; intracellular, menadione stress-induced protein; expression reduced after exposure to farnesol	CBF90310.1
55869	HDAC8		OMIM:300882	AN4493/rpdA	ORF, Verified,		Histone deacetylase; expression of rpdA is induced by trichostatin A	XP_662097.1
3098	HK1		OMIM:605285,OMIM:235700,OMIM:617460	AN7459/hxkA	ORF, Verified,		Putative hexokinase with a predicted role in carbohydrate metabolism	XP_680728.1
3145	HMBS		OMIM:176000	hemC/AN0121	ORF, Uncharacterized,		porphobilinogen deaminase; heme biosynthesis enzyme that facilitates growth in reactive nitrogen species conditions; transcriptionally induced by reactive nitrogen species	CBF90157.1
3265	HRAS	0007165 signal transduction	OMIM:162900,OMIM:188470,OMIM:163200,OMIM:109800,OMIM:137550,OMIM:218040	AN0182/rasA	ORF, Verified,		Small monomeric GTPase of the Ras superfamily involved in regulation of development; involved in conidiophore formation and conidial germination	XP_657786.1

3295	HSD17B4		OMIM:261515,OMIM:233400	foxA/AN7111	ORF, Verified,		Peroxisomal multifunctional enzyme involved in fatty acid beta-oxidation; required for growth on very long-chain fatty acids; transcription is induced by fatty acids	CBF79061.1
3329	HSPD1		OMIM:612233,OMIM:605280	AN6089	ORF, Verified,		Putative 60 kilodalton heat shock protein	XP_663693.1
55699	IARS3		OMIM:616008	AN10393	ORF, Uncharacterized,		Has domain(s) with predicted ATP binding, aminoacyl-tRNA editing activity, aminoacyl-tRNA ligase activity, isoleucine-tRNA ligase activity and role in isoleucyl-tRNA aminoacylation, tRNA aminoacylation for protein translation	CBF82864.1
3508	IGHMBP2		OMIM:604320,OMIM:616155	AN7652	ORF, Uncharacterized,		Has domain(s) with predicted ATP binding, DNA binding, nucleoside-triphosphatase activity, nucleotide binding activity	XP_680921.1
3735	KARS1		OMIM:613916,OMIM:613641	AN1913	ORF, Uncharacterized,		Putative lysyl-tRNA synthetase with a predicted role in lysine metabolism	XP_659517.1
547	KIF1A		OMIM:610357,OMIM:614255,OMIM:201300,OMIM:614213	AN7547/uncA	ORF, Verified,		Member of kinesin-3 motor protein family; binds detyrosinated cytoplasmic microtubules, not mitotic spindle; mutation causes defects in vesicle transport and slow growth	XP_680816.1
3798	KIF5A		OMIM:617235,OMIM:617921,OMIM:604187	AN5343/kinA	ORF, Verified,		Kinesin-family protein; involved in microtubule destabilization; required for normal growth and nuclear positioning	XP_662947.1
3845	KRAS		OMIM:211980,OMIM:609942,OMIM:163200,OMIM:108010,OMIM:601626,OMIM:114480,OMIM:600268,OMIM:137215,OMIM:109800,ORPHA:1333,OMIM:615278,OMIM:260350,ORPHA:1340,OMIM:614470	AN0182/rasA	ORF, Verified,		Small monomeric GTPase of the Ras superfamily involved in regulation of development; involved in conidiophore formation and conidial germination	XP_657786.1
1130	LYST		OMIM:214500	AN0239	ORF, Uncharacterized,	Whithin and Beyond WD40	Ortholog(s) have role in regulation of vacuole organization	CBF89900.1
4126	MANBA		,OMIM:248510	AN1742/mndA	ORF, Uncharacterized,		Putative beta-1,4-mannosidase with a predicted role in polysaccharide degradation	XP_659346.1
4141	MARS1		OMIM:616280,OMIM:615486	AN1380	ORF, Uncharacterized,		Ortholog(s) have methionyl glutamyl tRNA synthetase complex localization	XP_658984.1
4143	MAT1A		OMIM:250850	sasA/AN1222	ORF, Verified,		Putative S-adenosylmethionine synthetase; predicted role in methionine metabolism; expression reduced after exposure to farnesol; strongly expressed during vegetative growth, downregulated during development in asexual or sexual cultures	XP_658826.1
4191	MDH2		OMIM:617339	mdhA/AN6717	ORF, Verified,		Putative mitochondrial malate dehydrogenase with a predicted role in the TCA cycle; intracellular; protein abundance decreased by menadione stress	CBF71313.1
4292	MLH1		OMIM:609310,OMIM:158320,OMIM:276300	AN0126	ORF, Uncharacterized,		Ortholog(s) have ATP binding, ATPase activity, DNA insertion or deletion binding, bubble DNA binding, dinucleotide insertion or deletion binding, double-stranded DNA binding and heteroduplex DNA loop binding,	XP_657730.1
4311	MME	0007611 learning or memory/0030324lung development/0019233sensory perception of pain/0001822 kidney development	OMIM:617017,OMIM:617018	AN3091	ORF, Verified,		Has domain(s) with predicted metalloendopeptidase activity, metallopeptidase activity and role in proteolysis	XP_660695.1
4337	MOCS1		OMIM:252150	cnxABC/AN0947	ORF, Verified,		Protein required for the synthesis of precursor Z, an intermediate in the molybdopterin cofactor biosynthesis pathway; molybdopterin cofactor is required for the activity of nitrate reductase	XP_658551.1
4436	MSH2		OMIM:158320,OMIM:276300,OMIM:120435	AN10621	ORF, Uncharacterized,		Ortholog(s) have ATP binding, ATPase activity, DNA insertion or deletion binding, Y-form DNA binding, double-strand/single-strand DNA junction binding and four-way junction DNA binding,	CBF76294.1
4436	MSH2		OMIM:158320,OMIM:276300,OMIM:120436	AN5006	ORF, Uncharacterized,		Ortholog(s) have ATP binding, ATPase activity, DNA insertion or deletion binding, Y-form DNA binding, double-strand/single-strand DNA junction binding and four-way junction DNA binding,	XP_662610.1
2956	MSH6		OMIM:614350,OMIM:608089,OMIM:276300	AN1708/mshA	ORF, Uncharacterized,		Putative MutS homolog with a predicted role in DNA mismatch repair; transcript upregulated in response to camptothecin	XP_659312.1
4524	MTHFR		OMIM:181500,OMIM:601634,OMIM:236250,OMIM:188050	metF/AN5883	ORF, Verified,		Putative methylenetetrahydrofolate reductase (NADPH) with predicted role in one-carbon metabolism; mutation causes methionine auxotrophy and decreased mycelial pigment production; expression induced by homocysteine and decreased by farnesol	XP_663487.1
79784	MYH14		OMIM:614369,OMIM:600652	AN4706/myoB	ORF, Verified,		Myosin II; required for normal conidiation, septum formation and for correct chitin deposition	XP_662310.1
4649	MYO9A		OMIM:618198	AN8862/myoV	ORF, Verified,		Myosin V; involved in the movement of vesicles to the hyphal tip; essential for polarized growth in the absence of microtubules	XP_682131.1
259232	NALCN		OMIM:615419,ORPHA:1146,OMIM:616266	AN1168/cch1	ORF, Verified,		Putative voltage-gated calcium channel; locus contains the conserved upstream open reading frame (uORF) AN1168-uORF	XP_658772.1
79731	NARS2		OMIM:616239,OMIM:618434	AN3073	ORF, Uncharacterized,		Putative asparaginyl-tRNA synthetase with a predicted role in tRNA charging for translation	CBF83457.1
4540	ND5		OMIM:535000,OMIM:540000	ndhE/AN20001	ORF, Uncharacterized,		Mitochondrially-encoded subunit 5 of NADH dehydrogenase	YP_006303567.1
4704	NDUFA9		OMIM:618247	AN10440	ORF, Uncharacterized,		Ortholog(s) have membrane, mitochondrion localization	CBF75541.1
79133	NDUFAF5		OMIM:618238	AN4918	ORF, Uncharacterized,		Ortholog(s) have intracellular localization	XP_662522.1

4719	NDUFS1		OMIM:618226	AN4288	ORF, Uncharacterized,		Protein expressed at decreased levels in a hapX mutant versus wild-type; AN4288 and AN9411 in version 4 of the A. nidulans annotation were identical, AN9411 has been merged with AN4288 such that AN4288 remains and AN9411 was deleted	XP_661892.1
4720	NDUFS2		OMIM:618228	AN2414	ORF, Uncharacterized,		Putative NADH dehydrogenase (ubiquinone) with a predicted role in energy metabolism	CBF86800.1
4722	NDUFS3		OMIM:618230	AN10229	ORF, Uncharacterized,		Ortholog(s) have role in ascospore formation, mitochondrial respiratory chain complex I assembly, programmed cell death and membrane, mitochondrion, plasma membrane localization	CBF85447.1
374291	NDUFS7		OMIM:618224	AN4297	ORF, Uncharacterized,		Ortholog(s) have role in iron-sulfur cluster assembly, mitochondrial respiratory chain complex I assembly and mitochondrial respiratory chain complex I, peripheral segment, mitochondrion, plasma membrane localization	CBF77819.1
4728	NDUFS8		,OMIM:618222	AN5703	ORF, Uncharacterized,		Putative electron-transferring-flavoprotein dehydrogenase with a predicted role in energy metabolism	CBF81370.1
4723	NDUFV1		OMIM:618225	AN5629	ORF, Uncharacterized,		Putative NADH dehydrogenase (ubiquinone) with a predicted role in energy metabolism	XP_663233.1
4729	NDUFV2	0007399 nervous system development	OMIM:618229	AN6077	ORF, Uncharacterized,		Putative NADH dehydrogenase (ubiquinone) with a predicted role in energy metabolism	XP_663681.1
55768	NGLY1		OMIM:615273,	AN3787	ORF, Uncharacterized,		Ortholog(s) have metal ion binding, misfolded protein binding, peptide-N4-(N-acetyl-beta-glucosaminyl)asparagine amidase activity, structural constituent of cell wall activity	XP_661391.1
25836	NIPBL	2001224 positive regulation of neuron migration	OMIM:122470,	AN7499	ORF, Uncharacterized,		Ortholog(s) have double-stranded DNA binding activity	XP_680768.1
5063	PAK3	0016358dendrite development/0030833regulation of actin filament polymerization	OMIM:300558	AN2067/ste20	ORF, Uncharacterized,		Predicted PAK (p21-activated kinase) family protein; similar to Saccharomyces cerevisiae Ste20p; mutants have increased pigment production	XP_659671.1
5160	PDHA1		OMIM:312170	AN5162/pdhB	ORF, Verified,		Putative pyruvate dehydrogenase (lipoamide) with a predicted role in pyruvate metabolism	XP_662766.1
5165	PDK3		OMIM:300905	AN6207/pkpC	ORF, Uncharacterized,		Putative protein kinase; reduced growth on AVICEL medium	XP_663811.1
23590	PDSS1		OMIM:614651	AN10340	ORF, Uncharacterized,		Ortholog(s) have di-trans,poly-cis-decaprenylcistransferase activity, trans-hexaprenyltranstransferase activity and role in farnesyl diphosphate biosynthetic process, mevalonate pathway, ubiquinone biosynthetic process	CBF84055.1
5189	PEX1		OMIM:214100,OMIM:234580,ORPHA:44,OMIM:601539	AN5991/pexA	ORF, Verified,		Putative peroxisomal protein (peroxin) with a role in fatty acid utilization; required for growth on long chain fatty acids	XP_663595.1
5830	PEX5		OMIM:202370OMIM:214110,OMIM:616716	AN10215/pexE	ORF, Verified,		Putative peroxisomal targeting signal 1 (PTS1) receptor protein; required for growth on long chain fatty acids; inactivation impairs triacetylfusarinine C (TAFC) siderophore biosynthesis; mutant is growth impaired in iron-depleted medium	CBF85028.1
5190	PEX6		OMIM:614863,OMIM:616617,OMIM:614862	AN2925/pexF	ORF, Verified,		Putative peroxisomal import protein (peroxin) with a role in fatty acid utilization; required for growth on long chain fatty acids	CBF83738.1
5238	PGM3		OMIM:615816	AN4234/pcmA/	ORF, Uncharacterized,		Putative phosphoacetylglucosamine mutase with a predicted role in chitin biosynthesis	CBF74428.1
5373	PMM2		OMIM:212065	AN10710	ORF, Uncharacterized,		Ortholog(s) have phosphomannomutase activity, role in establishment or maintenance of cell polarity, protein targeting to ER and cytoplasmic stress granule, cytosol, fungal biofilm matrix localization	CBF81230.1
11284	PNKP		OMIM:613402,OMIM:616267	AN5751	ORF, Uncharacterized,		Ortholog(s) have ATP-dependent polydeoxyribonucleotide 5'-hydroxyl-kinase activity, polynucleotide 3'-phosphatase activity and role in DNA 3' dephosphorylation involved in DNA repair, base-excision repair, single strand break repair	XP_663355.1
10908	PNPLA6		OMIM:215470,OMIM:612020,OMIM:245800,OMIM:275400	AN2481	ORF, Uncharacterized,		Ortholog(s) have lysophospholipase activity, role in phosphatidylcholine catabolic process, regulation of phospholipid biosynthetic process and endoplasmic reticulum localization	XP_660085.1
5428	POLG		OMIM:258450,OMIM:613662,OMIM:203700,OMIM:607459,OMIM:157640,OMIM:603041	AN0040	ORF, Uncharacterized,		Ortholog(s) have 3'-5' exonuclease activity, DNA-directed DNA polymerase activity, role in mitochondrial DNA catabolic process, mitochondrial DNA replication, mitochondrial genome maintenance and mitochondrion localization	XP_657644.1
5428	POLG		OMIM:258450,OMIM:613662,OMIM:203700,OMIM:607459,OMIM:157640,OMIM:603041	AN10316	ORF, Uncharacterized,		Putative RNA polymerase III, large subunit; ortholog of S. cerevisiae Rpo31p; expression reduced after exposure to farnesol	CBF87003.1
5428	POLG		OMIM:258450,OMIM:613662,OMIM:203700,OMIM:607459,OMIM:157640,OMIM:603041	AN0809	ORF, Uncharacterized,		Ortholog(s) have DNA binding activity and RNA polymerase II, core complex, nuclear chromatin localization	XP_658413.1
55703	POLR3B		OMIM:146110,OMIM:607694,OMIM:614381	AN0321	ORF, Uncharacterized,		Ortholog(s) have nuclear chromatin localization	XP_657925.1
55703	POLR3B		OMIM:146110,OMIM:607694,OMIM:614381	AN9120	ORF, Uncharacterized,		Ortholog(s) have DNA binding, DNA-directed 5'-3' RNA polymerase activity, RNA binding activity and role in chromatin silencing by small RNA, transcription by RNA polymerase II	CBF82508.1

10585	POMT1		OMIM:613155,OMIM:609308,OMIM:236670	AN1459/pmtC	ORF, Verified,		Subfamily 4 protein O-mannosyltransferase; involved in hyphal growth and conidia formation	XP_659063.1
29954	POMT2		OMIM:613156,OMIM:613158,OMIM:613150,OMIM:236670	AN5105/pmtA	ORF, Verified,		Pmt 2 subfamily protein O-mannosyltransferase; required for normal germination, hyphal growth and conidiophore development; mutants are sensitive to growth at high temperature and to cell wall perturbing agents	XP_662709.1
5521	PPP2R2B		OMIM:604326	pabA/AN1545	ORF, Verified,	**YES**	Putative regulatory subunit of protein phosphatase 2A (PP2A)	CBF85082.1
5573	PRKAR1A		OMIM:610489,OMIM:255960,OMIM:160980,OMIM:101800	AN4987/pkaR	ORF, Uncharacterized,		Putative protein kinase A (PKA) regulatory subunit	XP_662591.1
5631	PRPS1	0007399nervous system development	OMIM:301835,OMIM:300661,OMIM:311070,OMIM:304500	AN1965/prs2	ORF, Verified,		Putative ribose-phosphate pyrophosphokinase with a predicted role in histidine metabolism; interacts with prs1 and prs3	XP_659569.1
7879	RAB7A		OMIM:600882	rabS/AN0089	ORF, Verified,		Putative small GTPase involved in endosomal maturation and vacuolar biogenesis	BAB88682.1
5981	RFC1		OMIM:614575	uvsF/AN6303	ORF, Verified,		Protein with similarity to DNA replication factor C; mutations cause hypersensitivity to UV; transcript upregulated in response to camptothecin	AAB63523.2
6197	RPS6KA3	0007417central nervous system development/0035556 intracellular signal transduction	OMIM:300844,OMIM:303600	AN5973/pkcB	ORF, Verified,		Protein with similarity to protein kinase C; involved in polar axis establishment and germling growth; mutant is inviable and forms microcolonies	XP_663577.1
50484	RRM2B		OMIM:612075,OMIM:613077,	AN0067	ORF, Uncharacterized,		Putative ribonucleotide reductase small subunit; transcript upregulated in response to camptothecin	XP_657671.1
51128	SAR1B		,OMIM:246700	AN0411	ORF, Verified,		Putative GTPase with a predicted role in ER to Golgi transport	XP_658015.1
6390	SDHB		OMIM:171300,OMIM:115310,OMIM:606864,OMIM:606764	AN2332	ORF, Uncharacterized,		Putative succinate dehydrogenase with a predicted role in the TCA cycle	XP_659936.1
10801	SEPTIN9		OMIM:162100	AN1394/aspD	ORF, Uncharacterized,		Putative septin	XP_658998.1
23064	SETX		OMIM:606002,OMIM:602433	AN8671	ORF, Uncharacterized,		Ortholog(s) have 5'-3' DNA helicase activity, 5'-3' DNA/RNA helicase activity, protein domain specific binding activity	XP_681940.1
8879	SGPL1		OMIM:617575	AN1989	ORF, Uncharacterized,		Putative dihydrosphingosine-1-phosphate lyase with a predicted role in sphingoglycolipid metabolism	XP_659593.1
6557	SLC12A1		OMIM:601678	AN4478	ORF, Uncharacterized,		Ortholog(s) have L-arginine transmembrane transporter activity, potassium:chloride symporter activity	XP_662082.1
8402	SLC25A11		OMIM:618464	AN6254/ dicA	ORF, Uncharacterized,		Putative mitochondrial dicarboxylate:inorganic phosphate antiporter	XP_663858.1
291	SLC25A4		OMIM:609283,OMIM:615418,OMIM:617184	AN4064	ORF, Verified,		Putative ADP/ATP carrier protein with a predicted role in energy metabolism; palA-dependent expression independent of pH	XP_661668.1
9197	SLC33A1		OMIM:614482,OMIM:612539	AN4836	ORF, Uncharacterized,		Has domain(s) with predicted acetyl-CoA transmembrane transporter activity and integral component of membrane localization	XP_662440.1
84679	SLC9A7		OMIM:301024	AN2288/nhxA	ORF, Uncharacterized,		Ortholog(s) have potassium:proton antiporter activity, sodium:proton antiporter activity	XP_659892.1
8243	SMC1A		,OMIM:300590	AN2963	ORF, Uncharacterized,		Ortholog(s) have cohesin ATPase activity, double-stranded DNA binding, topological DNA entrapment activity and role in mitotic cohesin loading, mitotic cohesin ssDNA (lagging strand) loading, mitotic sister chromatid cohesion	XP_660567.1
9126	SMC3		OMIM:610759	sudA/AN6364	ORF, Verified,		Protein of the chromosome scaffold, involved with BimD in chromosome segregation; member of the DA-box protein family; transcript upregulated in response to camptothecin	XP_663968.1
6683	SPAST		OMIM:182601	AN3691	ORF, Uncharacterized,		Ortholog(s) have role in endocytic recycling, filamentous growth and hyphal tip localization	CBF75598.1
6687	SPG7		OMIM:607259	AN4557	ORF, Uncharacterized,		Ortholog(s) have ATP binding, ATPase activity, metallopeptidase activity and role in cellular protein-containing complex assembly, proteolysis, signal peptide processing	XP_662161.1
6712	SPTBN2		OMIM:600224,OMIM:615386	AN7707	ORF, Uncharacterized,		Protein with similarity to alpha-actinin; predicted role in actin filament bundling	XP_680976.1
10558	SPTLC1		OMIM:162400	lcbA/AN3728	ORF, Verified,		Serine palmitoyltransferase, catalyzes the first step in biosynthesis of sphinganine, a long-chain base component of sphingolipids; target of an antifungal drug, myriocin	XP_661332.1
9517	SPTLC2		OMIM:613640	AN1102	ORF, Uncharacterized,		Putative serine C-palmitoyltransferase with a predicted role in sphingoglycolipid metabolism	XP_658706.1
8803	SUCLA2		OMIM:612073	AN7000	ORF, Uncharacterized,		Putative succinate-CoA ligase (ADP-forming) with a predicted role in the TCA cycle	CBF79284.1
6897	TARS1		OMIM:618546	AN5662	ORF, Uncharacterized,		Has domain(s) with predicted ATP binding, aminoacyl-tRNA ligase activity, threonine-tRNA ligase activity, role in tRNA aminoacylation, tRNA aminoacylation for protein translation, threonyl-tRNA aminoacylation and cytoplasm localization	XP_663266.1
6904	TBCD	0048667 cell morphogenesis involved in neuron differentiation	OMIM:617193	AN0108	ORF, Uncharacterized,		Ortholog of A. fumigatus Af293 : Afu5g11940, A. niger CBS 513.88 : An18g02440, Aspergillus wentii : Aspwe1_0131348 and Aspergillus sydowii : Aspsy1_0195151	XP_657712.1
6908	TBP		OMIM:607136,OMIM:168600	AN4976	ORF, Uncharacterized,		Putative TATA-binding protein; contains a uORF in the upstream leader sequence	Q12731.1
10312	TCIRG1		,OMIM:259700	AN5606	ORF, Uncharacterized,		Ortholog(s) have proton-transporting ATPase activity, rotational mechanism activity and role in cellular protein-	XP_663210.1

							containing complex assembly, endocytosis, polyphosphate metabolic process, vacuolar acidification, vacuole organization	
55775	TDP1		OMIM:607250	AN5903	ORF, Uncharacterized,		Has domain(s) with predicted phosphoric diester hydrolase activity, role in DNA repair and nucleus localization	XP_663507.1
7156	TOP3A		OMIM:618097,OMIM:618098	AN4555	ORF, Uncharacterized,		Ortholog(s) have role in maintenance of rDNA, mitotic DNA replication, postreplication repair and RecQ family helicase-topoisomerase III complex, nucleus, site of double-strand break localization (	XP_662159.1
7167	TPI1		OMIM:615512	tpiA/AN6900	ORF, Verified,		Putative triose-phosphate isomerase with a predicted role in gluconeogenesis and glycolysis; protein induced by farnesol	XP_664504.1
1200	TPP1		OMIM:204500,OMIM:609270	AN7159	ORF, Uncharacterized,		Ortholog(s) have exopeptidase activity, tripeptidyl-peptidase activity	XP_664763.1
10381	TUBB3		,OMIM:614039,OMIM:600638	benA/AN1182	ORF, Verified,		Beta-tubulin, highly conserved component of microtubules; A. nidulans has two beta-tubulin genes, benA and tubC; temperature sensitive mutants are blocked in mitosis and in nuclear division	XP_658786.1
10381	TUBB3		,OMIM:614039,OMIM:600638	tubC/AN6838	ORF, Uncharacterized,		Beta-tubulin, highly conserved component of microtubules; A. nidulans has two beta-tubulin genes, tubC and benA; expression of tubC increases during conidiation	XP_664442.1
7317	UBA1		OMIM:301830	AN10266	ORF, Verified,		Putative ubiquitin-activating enzyme; ortholog of S. cerevisiae Uba1p; transcript upregulated in response to camptothecin; protein induced by farnesol	CBF86317.1
7317	UBA1		OMIM:301830	AN2174	ORF, Verified,		Putative ubiquitin-activating enzyme; ortholog of S. cerevisiae Uba1p; transcript upregulated in response to camptothecin; protein induced by farnesol	XP_659778.1
7415	VCP		OMIM:616687,OMIM:167320,OMIM:613954	AN7254	ORF, Verified,		Protein with a conserved CDC48, cell division protein N-terminal domain and two ATPase domains of the AAA-superfamily; protein expressed at increased levels during osmoadaptation; protein induced by farnesol	CBF78760.1
23230	VPS13A		ORPHA:2388,OMIM:200150	AN5579	ORF, Uncharacterized,		Ortholog(s) have role in ascospore-type prospore membrane assembly, late endosome to vacuole transport, mitochondrion organization, protein retention in Golgi apparatus, protein targeting to vacuole, regulation of inclusion body assembly	XP_663183.1
7453	WARS1		OMIM:617721	AN10475	ORF, Uncharacterized,		Has domain(s) with predicted ATP binding, aminoacyl-tRNA ligase activity, tryptophan-tRNA ligase activity, role in tRNA aminoacylation for protein translation, tryptophanyl-tRNA aminoacylation and cytoplasm localization	CBF75186.1
7508	XPC		OMIM:278720	AN3890	ORF, Uncharacterized,		Ortholog(s) have role in mismatch repair, nucleotide-excision repair	XP_661494.1
8565	YARS1		OMIM:608323	AN0057	ORF, Uncharacterized,		Has domain(s) with predicted ATP binding, aminoacyl-tRNA ligase activity, tyrosine-tRNA ligase activity, role in tRNA aminoacylation for protein translation, tyrosyl-tRNA aminoacylation and cytoplasm localization	XP_657661.1
10730	YME1L1		OMIM:617302	AN5588	ORF, Uncharacterized,		Ortholog(s) have ATP-dependent peptidase activity	CBF81608.1
5832	ALDH18A1		OMIM:616586,OMIM:616603,OMIM:601162,OMIM:219150	AN5799	ORF, Uncharacterized,		Putative gamma-glutamyl phosphate reductase with a predicted role in proline metabolism	XP_663403.1
1639	DCTN1	0007399nervous system development/1990535neuron projection maintenance	OMIM:168605,OMIM:105400,OMIM:607641	nudM/AN6323	ORF, Verified,		p150 subunit of dynactin; forms comet-like structures at microtubule plus ends	XP_663927.1
9896	FIG4		OMIM:611228,OMIM:216340,OMIM:612577,OMIM:612691	AN7314	ORF, Uncharacterized,		Ortholog(s) have phosphatidylinositol-3,5-bisphosphate 5-phosphatase activity, role in phosphatidylinositol dephosphorylation and PAS complex, extrinsic component of membrane, fungal-type vacuole membrane, nuclear periphery localization	XP_680583.1
3508	IGHMBP2		OMIM:604320,OMIM:616155	AN7652	ORF, Uncharacterized,		Has domain(s) with predicted ATP binding, DNA binding, nucleoside-triphosphatase activity, nucleotide binding activity	XP_680921.1
3798	KIF5A		OMIM:617921,OMIM:604187,OMIM:617235	kinA/AN5343	ORF, Verified,		Kinesin-family protein; involved in microtubule destabilization; required for normal growth and nuclear positioning	XP_662947.1
10908	PNPLA6		OMIM:612020,OMIM:275400,OMIM:245800,OMIM:215470	AN2481	ORF, Uncharacterized,		Ortholog(s) have lysophospholipase activity, role in phosphatidylcholine catabolic process, regulation of phospholipid biosynthetic process and endoplasmic reticulum localization	XP_660085.1
11128	POLR3A		OMIM:264090,OMIM:607694	AN10316	ORF, Uncharacterized,		Putative RNA polymerase III, large subunit; ortholog of S. cerevisiae Rpo31p; expression reduced after exposure to farnesol	CBF87003.1
11128	POLR3A		OMIM:264090,OMIM:607694	AN0809	ORF, Uncharacterized,		Ortholog(s) have DNA binding activity and RNA polymerase II, core complex, nuclear chromatin localization	XP_658413.1
55703	POLR3B		OMIM:146110,OMIM:607694,OMIM:614381	AN0321	ORF, Uncharacterized,		Ortholog(s) have nuclear chromatin localization	XP_657925.1
55703	POLR3B		OMIM:146110,OMIM:607694,OMIM:614381	AN9120	ORF, Uncharacterized,		Ortholog(s) have DNA binding, DNA-directed 5'-3' RNA polymerase activity, RNA binding activity and role in chromatin silencing by small RNA, transcription by RNA polymerase II	CBF82508.1
6342	SCP2		OMIM:613724	AN4353	ORF, Uncharacterized,		Ortholog(s) have glyoxysome localization	XP_661957.1
23064	SETX	0007399 nervous system development	OMIM:606002,OMIM:602433	AN8671	ORF, Uncharacterized,		Ortholog(s) have 5'-3' DNA helicase activity, 5'-3' DNA/RNA helicase activity, protein domain specific binding activity	XP_681940.1
6687	SPG7	0007399 nervous system development	,OMIM:607259	AN4557	ORF, Uncharacterized,		Ortholog(s) have ATP binding, ATPase activity, metallopeptidase activity and role in cellular protein-containing complex	XP_662161.1

							assembly, proteolysis, signal peptide processing	
7277	TUBA4A		OMIM:616208	tubA/AN0316	ORF, Verified,		Alpha-tubulin, forms a heterodimer with beta-tubulin that promotes microtubule assembly	XP_657920.1
7277	TUBA4A		OMIM:616208	tubB/AN7570	ORF, Verified,		Alpha-tubulin, forms a heterodimer with beta-tubulin that promotes microtubule assembly; non-essential subunit required for meiosis and formation of ascospores	XP_680839.1
7317	UBA1		OMIM:301830,	AN10266	ORF, Verified,		Putative ubiquitin-activating enzyme; ortholog of S. cerevisiae Uba1p; transcript upregulated in response to camptothecin; protein induced by farnesol	CBF86317.1
7317	UBA1		OMIM:301830,	AN2174=AN10266	ORF, Verified,		Putative ubiquitin-activating enzyme; ortholog of S. cerevisiae Uba1p; transcript upregulated in response to camptothecin; protein induced by farnesol	XP_659778.1
7415	VCP		OMIM:613954,OMIM:616687,OMIM:167320	AN7254	ORF, Verified,		Protein with a conserved CDC48, cell division protein N-terminal domain and two ATPase domains of the AAA-superfamily; protein expressed at increased levels during osmoadaptation; protein induced by farnesol	CBF78760.1

Guide

Numbers of genes associated with these proteins:

PKs (protein kinases)	**GTPases**	**G proteins**	Proteins involved in hyphal growth
25	8	4	10

Genes highlighted with blue are those involved in regulating branching morphogenesis in organs like neuronal cells and kidney

Appendix II. Conserved homologous genes among *Aspergillus nidulans* and *Homo sapiens.*

GENE_ENTREZ_ID	GENE_SYMBOL (*Homo sapiens*)	GO/function in regulating neuronal development and branching (Homo sapiens)	GENE_ENTREZ_ID	AN IDs (*Aspergillus nidulans*)	Feature type	WD 40 domains	Function	Accession Number
207	AKT1	0030030: cell projection organization/0060644mammary gland epithelial cell differentiation/0010975regulation of neuron projection development	207	pkcB/AN5973	ORF, Verified,		Protein with similarity to protein kinase C; involved in polar axis establishment and germling growth; mutant is inviable and forms microcolonies	XP_663577.1
10000	AKT3	0048854 brain morphogenesis/0007165signal transduction	10000	pkcA/AN0106	ORF, Verified		Protein kinase C; essential gene; required for wild-type level of growth, cell wall integrity and penicillin biosynthesis	BAD02338.1
375	ARF1	0007015 actin filament organization	375	arlA/AN5912	ORF, Uncharacterized,		Predicted ADP ribosylation factor GTPase	XP_658730.1
10564	ARFGEF2	0035556 intracellular signal transduction	10564	hypB/AN6709	ORF, Verified,		Putative ADP ribosylation factor guanine nucleotide exchange factor; Sec7-domain protein; role in hyphal morphogenesis; mutants display abnormal hyphae, hyphal tip cell death and excessive septum formation	XP_664313.1
1020	CDK5	0048813 dendrite morphogenesis/0021954 central nervous system neuron development/0030866 cortical actin cytoskeleton organization/ 0030182neuron differentiation/0031175neuron projection development	1020	phoA/AN8261	ORF, Verified,		Cyclin-dependent protein kinase; role in regulation of development in response to phosphate availability; interacts with cyclin Pho80; lethal in combination with mutations in phoB; mutant is sensitive to NaCl	AAC42260.1
1021	CDK6	0048699 generation of neurons	1021	nimX/AN4182	ORF, Verified,		Cyclin-dependent protein kinase involved in cell cycle control; required for cell cycle progression through G1 and G2; regulated by NimE cyclin and NimT phosphatase; homolog of S. pombe cdc2; transcript camptothecin upregulated	XP_661786.1
1457	CSNK2A1	0007165 signal transduction	1457	cka1/AN1485	ORF, Uncharacterized,		Protein kinase; mutants are lethal forming only microcolonies	XP_659089.1
2775	GNAO1	0031175 neuron projection development	2775	fadA/N0651	ORF, Verified,		Alpha subunit of a heterotrimeric G protein composed of FadA, SfaD, GpgA and involved in regulation of proliferation and conidiophore development; mutant produces increased amounts of extracellular proteinase during carbon starvation	XP_658255.1
2782	GNB1	0007165 signal transduction	2782	sfaD/AN0081	ORF, Verified,	Yes	Beta subunit of a heterotrimeric G protein composed of FadA, SfaD, GpgA and involved in regulation of proliferation and conidiophore development; mutant produces increased amounts of extracellular proteinase during carbon starvation	XP_657685.1
10765	KDM5B	0060444 branching involved in mammary gland duct morphogenesis/0060763 mammary duct terminal end bud growth	10765	AN8211	ORF, Uncharacterized,		Ortholog(s) have histone demethylase activity (H3-trimethyl-K4 specific) activity	XP_681480.1
2475	MTOR	0045182translation regulator activity/0007420brain development/0030838positive regulation of actin filament polymerization/0060999positive regulation of dendritic spine development/0014042 positive regulation of neuron maturation/0010976positive regulation of neuron projection development/0032956regulation of actin cytoskeleton organization	2475	torA/AN5982	ORF, Verified,		Tor kinase homolog; essential; heterozygous diploids show increased sensitivity to DL-aspartic acid beta-hydroxamate in ammonium medium and enhanced growth on threonine; null mutants have early septation defect and arrest as short germlings	CAG30554.1
5048	PAFAH1B1	0048854brain morphogenesis/0001764neuron migration	5048	nudF/AN6197	ORF, Verified,	Yes	Nuclear migration protein; mutants defective in asexual and sexual sporulation; similar to human LIS-1; interacts directly with NudC; spindle-pole body-localized; may facilitate minus-end-directed dynein movement	XP_663801.1
5297	PI4KA	0007165 signal transduction	5297	stt4/AN4278	ORF, Uncharacterized,		Essential 1-phosphatidylinositol 4-kinase with a predicted role in phospholipid metabolism; mutants arrest in the cell cycle	XP_661882.1
9373	PLAA	0007399 nervous system development/1903861positive regulation of dendrite extension/2001224 positive regulation of neuron migration/0007165signal transduction	9373	AN7704	ORF, Uncharacterized,	**Yes**	Ortholog(s) have role in cellular protein catabolic process, proteasome-mediated ubiquitin-dependent protein catabolic	CBF79941.1

							process and cytoplasm, nucleus localization	
5859	QARS1	0007420 brain development	5859	AN9157	ORF, Uncharacterized,		Has domain(s) with predicted ATP binding, aminoacyl-tRNA ligase activity, glutamine-tRNA ligase activity, role in glutaminyl-tRNA aminoacylation, tRNA aminoacylation, tRNA aminoacylation for protein translation and cytoplasm localization	XP_682426.1
5879	RAC1	0030036actin cytoskeleton organization/0007015 actin filament organization/0030041 actin filament polymerization/0007163 establishment or maintenance of cell polarity/0035556 intracellular signal transduction/0032956regulation of actin cytoskeleton organization	5879	racA/AN4743	ORF, Uncharacterized,		Rho GTPase protein required for normal colony growth and conidiophore development; synthetically lethal with modA null	XP_662347.1
6197	RPS6KA3	0035556intracellular signal transduction/0007165 signal transduction	6197	pkcB/AN5973	ORF, Verified,		Protein with similarity to protein kinase C; involved in polar axis establishment and germling growth; mutant is inviable and forms microcolonies	XP_663577.1
150094	SIK1	0035556intracellular signal transduction	150094	snf1/AN7695	ORF, Uncharacterized,		Putative protein kinase; reduced growth on AVICEL medium; required for CreA derepression and endocellulase production	CBF79923.1
23043	TNIK	0007010cytoskeleton organization/0035556intracellular signal transduction/0048812neuron projection morphogenesis	23043	mst1/AN5674	ORF, Uncharacterized,		MAP kinase, kinase, kinase, kinase (MAPKKKK); mutants undergo premature but incomplete sexual development	XP_663278.1
55697	VAC14	0007165signal transduction	55697	AN10682	ORF, Uncharacterized,		Ortholog(s) have molecular adaptor activity, protein binding, bridging activity and role in phosphatidylinositol biosynthetic process, positive regulation of lipid kinase activity, protein localization to vacuolar membrane	CBF81734.1
215	ABCD1	1990535neuron projection maintenance	215	amyD/AN3308	ORF, Uncharacterized,		Putative alpha-amylase with a predicted role in starch metabolism; predicted glycosyl phosphatidylinositol (GPI)-anchor	XP_658618.1
2182	ACSL4	0060996dendritic spine development/0030182neuron differentiation	2182	faaA/AN6014	ORF, Verified,		Putative long-chain-fatty-acid-CoA ligase with a predicted role in fatty acid metabolism	CBF70362.1
162	AP1B1	0001822kidney development	162	AN3029	ORF, Uncharacterized,		Ortholog(s) have role in cellular response to drug	XP_660633.1
538	ATP7A	0021954central nervous system neuron development/0048812neuron projection morphogenesis/0021860 pyramidal neuron development	538	AN3624/ygA	ORF, Verified,		Protein required for conidia pigmentation, predicted to act as a copper transporter; mutants produce yellow conidia at high pH and green conidia at low pH	XP_661228.1
811	CALR	2000510positive regulation of dendritic cell chemotaxis	811	clxA/AN3592	ORF, Uncharacterized,		Putative calnexin with a predicted role in protein folding and protein quality control on the endoplasmic reticulum (ER) membrane	CBF75819.1
1639	DCTN1	0007399 nervous system development	1639	AN6323/nudM	ORF, Verified,		p150 subunit of dynactin; forms comet-like structures at microtubule plus ends	XP_663927.1
1760	DMPK	0035556intracellular signal transduction	1760	AN5529/cotA	ORF, Verified,		Essential NDR ser/thr protein kinase; role in cell polarity; RAM-signaling pathway component; MobB/CotA kinase complex thought to regulate cell polarity growth by maintaining cellular calcium homeostasis; mutant forms brown microcolonies	XP_663133.1
3265	HRAS	0007165 signal transduction	3265	AN0182/rasA	ORF, Verified,		Small monomeric GTPase of the Ras superfamily involved in regulation of development; involved in conidiophore formation and conidial germination	XP_657786.1
4311	MME	0007611 learning or memory/0030324lung development/0019233sensory perception of pain/0001822 kidney development	4311	AN3091	ORF, Verified,		Has domain(s) with predicted metalloendopeptidase activity, metallopeptidase activity and role in proteolysis	XP_660695.1
4729	NDUFV2	0007399 nervous system development	4729	AN6077	ORF, Uncharacterized,		Putative NADH dehydrogenase (ubiquinone) with a predicted role in energy metabolism	XP_663681.1
25836	NIPBL	2001224 positive regulation of neuron migration	25836	AN7499	ORF, Uncharacterized,		Ortholog(s) have double-stranded DNA binding activity	XP_680768.1
5063	PAK3	0016358dendrite development/0030833regulation of actin filament polymerization	5063	AN2067/ste20	ORF, Uncharacterized,		Predicted PAK (p21-activated kinase) family protein; similar to Saccharomyces cerevisiae Ste20p; mutants have	XP_659671.1

5631	PRPS1	0007399nervous system development	5631	AN1965/prs2	ORF, Verified,		increased pigment production Putative ribose-phosphate pyrophosphokinase with a predicted role in histidine metabolism; interacts with prs1 and prs3	XP_659569.1
6197	RPS6KA3	0007417central nervous system development/0035556 intracellular signal transduction	6197	AN5973/pkcB	ORF, Verified,		Protein with similarity to protein kinase C; involved in polar axis establishment and germling growth; mutant is inviable and forms microcolonies	XP_663577.1
6904	TBCD	0048667 cell morphogenesis involved in neuron differentiation	6904	AN0108	ORF, Uncharacterized,		Ortholog of A. fumigatus Af293 : Afu5g11940, A. niger CBS 513.88 : An18g02440, Aspergillus wentii : Aspwe1_0131348 and Aspergillus sydowii : Aspsy1_0195151	XP_657712.1
1639	DCTN1	0007399nervous system development/1990535neuron projection maintenance	1639	nudM/AN6323	ORF, Verified,		p150 subunit of dynactin; forms comet-like structures at microtubule plus ends	XP_663927.1
23064	SETX	0007399 nervous system development	23064	AN8671	ORF, Uncharacterized,		Ortholog(s) have 5'-3' DNA helicase activity, 5'-3' DNA/RNA helicase activity, protein domain specific binding activity	XP_681940.1
6687	SPG7	0007399 nervous system development	6687	AN4557	ORF, Uncharacterized,		Ortholog(s) have ATP binding, ATPase activity, metallopeptidase activity and role in cellular protein-containing complex assembly, proteolysis, signal peptide processing	XP_662161.1

Guide

Numbers of genes associated with these proteins:

PKs (protein kinases)	**GTPases**	**G proteins**	Proteins involved in hyphal growth
25	8	4	10

Genes highlighted with blue are those involved in regulating branching morphogenesis in organs like neuronal cells and kidney

Appendix III. Results of QMA on MAG and MN media.

Guide:

Gray color shows the strains revealed once or twice either hypo or hyper apical branching during the four rounds of counting

Other colors show the strains showed either hypo or hyper apical branching more than 2 times during the four rounds of counting

MAG media

		Source: Minus 80 stocks		Source: Master plates (MPs)	
No.	**Mutant strains**	**Numbers of apical branches in the 1st round of inoculating colonies on MAG media (Replicates 1)**	**Numbers of apical branches in the 2nd round of inoculating colonies on MAG media (Replicates2)**	**Numbers of apical branches in the 3rd round of inoculating colonies on MAG media (Replicates 3)**	**Numbers of apical branches in the 4th round of inoculating colonies on MAG media (Replicates 4)**
1	1A1	48	40	12	14
2	1A2	34	5	12	4
3	1A3	1	18	14	13
4	1A4	21	12	7	5
5	1A5	22	21	8	14
6	1A6	10	19	8	15
7	1A7	1	15	12	10
8	1A8	22	26	7	3
9	1A9	1	30	12	11
10	1A10	27	22	7	16
11	1A11	34	18	15	16
12	1A12	20	28	8	8
13	1B1	25	23	8	10
14	1B2	18	16	6	21
15	1B3	49	8	10	12
16	1B4	20	34	5	12
17	1B5	19	11	12	10
18	1B6	15	14	10	12
19	1B7	10	21	6	20
20	1B8	1	10	13	13
21	1B9	17	9	8	15
22	1B10	7	23	8	12
23	1B11	11	27	18	10
24	1B12	1	10	35	15
25	1C1	19	22	25	10
26	1C2	14	21	11	13
27	1C3	17	21	27	24
28	1C4	14	21	17	14
29	1C5	18	18	18	15
30	1C6	11	21	14	16
31	1C7	18	16	19	21
32	1C8	14	18	11	18
33	1C9	16	13	13	9
34	1C10	17	30	14	15
35	1C11	20	10	8	9
36	1C12	20	21	11	17
37	1D1	11	20	24	12
38	1D2	17	26	11	6
39	1D3	8	13	8	7
40	1D4	13	9	23	8
41	1D5	0	20	25	14
42	1D6	48	14	26	11
43	1D7	15	18	11	8
44	1D8	48	20	14	9
45	1D9	25	18	13	11
46	1D10	11	24	17	20
47	1D11	48	35	9	13
48	1D12	41	23	10	8
49	1E--1	7	25	25	15
50	1E--2	13	25	34	17
51	1E--3	7	28	11	8
52	2A1	11	13	15	20
53	2A2	16	10	18	8

54	2A3	19	28	13	5
55	2A4	10	15	9	8
56	2A5	19	14	15	4
57	2A6	11	19	18	17
58	2A7	22	25	10	8
59	2A8	17	19	20	18
60	2A9	5	3	1	2
61	2A10	1	3	3	2
62	2A11	16	10	18	17
63	2A12	14	17	20	13
64	2B1	12	14	20	15
65	2B2	11	12	9	13
66	2B3	10	12	10	7
67	2B4	1	17	19	10
68	2B5	13	18	14	18
69	2B6	2	0	3	0
70	2B7	49	18	18	18
71	2B8	20	18	14	14
72	2B9	1	16	10	12
73	2B10	46	45	30	50
74	2B11	20	23	28	25
75	2B12	17	12	8	15
76	2C1	1	20	12	43
77	2C2	25	24	26	19
78	2C3	28	30	43	13
79	2C4	22	11	11	19
80	2C5	5	17	8	9
81	2C6	19	7	10	13
82	2C7	14	4	15	18
83	2C8	19	22	25	20
84	2C9	10	16	14	8
85	2C10	12	17	9	15
86	2C11	3	3	3	2
87	2C12	12	14	14	6
88	2D1	22	23	13	4
89	2D2	13	20	7	20
90	2D3	2	3	3	2
91	2D4	22	12	9	13
92	2D5	16	19	15	7
93	2D6	25	17	10	15
94	2D7	11	26	10	12
95	2D8	17	9	8	10
96	2D9	1	31	13	10
97	2D10	16	15	17	8
98	2D11	31	20	6	7
99	2D12	0	0	0	3
100	2E1 (Wild type strain)	29	23	10	15
Reference Interval (RI) to determine abnormal apical branching:		**1<Normal phenotype <48**	**3< Normal phenotype <40**	**3< Normal phenotype <43**	**2< Normal phenotype <43**

MN media

Source: Minus 80 stocks	Source: Master plates (MPs)

No.	Mutant strains	Numbers of apical branches in the 1st round of inoculating colonies on MN media (Replicates 1)	Numbers of apical branches in the 2nd round of inoculating colonies on MN media (Replicates 2)	Numbers of apical branches in the 3rd round of inoculating colonies on MN media (Replicates 3)	Numbers of apical branches in the 4th round of inoculating colonies on MN media (Replicates 4)
1	1A1	10	21	16	16
2	1A2	6	15	12	8
3	1A3	11	15	20	18
4	1A4	6	15	11	37
5	1A5	26	16	18	25
6	1A6	2	12	14	30
7	1A7	21	17	16	18
8	1A8	11	11	12	14
9	1A9	21	16	14	25
10	1A10	14	15	25	19

11	1A11	7	14	13	13
12	1A12	11	15	20	18
13	1B1	11	15	20	18
14	1B2	15	17	13	20
15	1B3	17	14	12	22
16	1B4	11	24	18	15
17	1B5	12	22	14	8
18	1B6	18	10	10	12
19	1B7	13	15	20	12
20	1B8	14	19	10	11
21	1B9	15	20	17	14
22	1B10	13	15	13	16
23	1B11	11	10	14	17
24	1B12	18	14	15	16
25	1C1	13	15	8	15
26	1C2	14	21	11	13
27	1C3	13	10	16	15
28	1C4	14	21	17	14
29	1C5	18	18	18	15
30	1C6	11	21	14	16
31	1C7	18	16	19	21
32	1C8	14	18	11	18
33	1C9	16	13	18	14
34	1C10	26	20	15	20
35	1C11	26	10	8	9
36	1C12	20	21	11	17
37	1D1	26	14	17	14
38	1D2	12	15	14	20
39	1D3	10	15	15	17
40	1D4	16	14	13	17
41	1D5	11	15	10	23
42	1D6	13	18	20	20
43	1D7	16	17	15	18
44	1D8	20	15	23	8
45	1D9	11	15	15	17
46	1D10	26	7	15	17
47	1D11	18	15	34	16
48	1D12	17	11	8	9
49	1E--1	15	22	10	11
50	1E--2	17	24	19	20
51	1E--3	10	28	11	18
52	2A1	12	16	13	18
53	2A2	17	11	15	15
54	2A3	26	20	14	17
55	2A4	10	9	8	12
56	2A5	11	20	11	16
57	2A6	11	15	15	16
58	2A7	7	11	15	15
59	2A8	9	12	19	16
60	2A9	3	5	3	4
61	2A10	2	5	3	4
62	2A11	13	11	17	13
63	2A12	15	20	11	23
64	2B1	17	16	15	10
65	2B2	2	15	13	11
66	2B3	12	16	12	18
67	2B4	13	25	20	18
68	2B5	18	20	12	17
69	2B6	4	6	3	4
70	2B7	8	20	15	7
71	2B8	17	15	18	15
72	2B9	15	11	33	10
73	2B10	25	24	40	37
74	2B11	11	12	19	13
75	2B12	6	12	21	9

76	2C1	14	22	17	15
77	2C2	16	14	22	15
78	2C3	2	11	20	13
79	2C4	15	12	12	20
80	2C5	20	12	20	9
81	2C6	26	14	21	12
82	2C7	26	15	16	21
83	2C8	12	15	13	18
84	2C9	18	12	21	18
85	2C10	11	16	21	40
86	2C11	4	5	4	4
87	2C12	14	18	25	10
88	2D1	13	15	11	20
89	2D2	7	13	18	15
90	2D3	4	6	3	4
91	2D4	13	14	20	11
92	2D5	10	13	14	14
93	2D6	2	13	18	12
94	2D7	9	23	17	9
95	2D8	2	15	20	13
96	2D9	9	13	13	10
97	2D10	10	10	19	9
98	2D11	13	13	20	18
99	2D12	12	15	10	12
100	2E1 (Wild type strain)	15	14	25	12
Reference Interval (RI) to determine abnormal apical branching:		**2<Normal phenotype<26**	**5<Normal phenotype<25**	**3<Normal phenotype<33**	**4<Normal phenotype<37**

Appendix IV. Results of QMA and student t-test associated with both MAG and MN media through EVOS for the top 6 mutant strains.

	Results of QMA (quantitative microscopy assay) through EVOS microscope on MN media				
	Abnormal strains	Replicate 1	Replicate 2	Replicate 3	Replicate 4
1	2A10	2	5	3	4
2	2A9	3	5	3	4
3	2D3	4	6	3	4
4	2B6	4	6	3	4
5	2C11	4	5	4	4
6	2B10	25	24	40	37

	Results of QMA (quantitative microscopy assay) through EVOS microscope on MAG media				
	Abnormal strains	Replicate 1	Replicate 2	Replicate 3	Replicate 4
1	2A10	1	3	3	2
2	2A9	5	3	1	2
3	2D3	2	3	3	2
4	2B6	2	0	3	0
5	2C11	3	3	3	2
6	2B10	46	45	30	50

Student t-test results assuming unequal variances						
MN Medium	**2A10**	**2E.1(WT)**	**2A9**	**2E.1(WT)**	**2D3**	**2E.1(WT)**
Replicate1	2	15	3	15	4	15
Replicate2	5	14	5	14	6	14
Replicate3	3	25	3	25	3	25
Replicate4	4	12	4	12	4	12
Mean	3.5	16.5	3.75	16.5	4.25	16.5
Variance	1.6666667	33.66666667	0.916666667	33.66666667	1.583333333	33.66666667
Observations	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0	
df	6		6		6	
t Stat	-4.3740228		-4.33617454		-4.126544413	
P(T<=t) one-tail	0.0023493		0.002447823		0.003085415	
t Critical one-tail	1.9431803		1.943180281		1.943180281	
P(T<=t) two-tail	0.0046985		0.004895647		0.00617083	
t Critical two-tail	2.4469119		2.446911851		2.446911851	
MAG Medium	**2A10**	**2E.1 (WT)**	**2A9**	**2E.1 (WT)**	**2D3**	**2E.1 (WT)**
Replicate1	1	29	5	29	2	29
Replicate2	3	23	3	23	3	23
Replicate3	3	10	1	10	3	10
Replicate4	2	15	2	15	2	15
Mean	2.25	19.25	2.75	19.25	2.5	19.25
Variance	0.9166667	70.91666667	2.916666667	70.91666667	0.333333333	70.91666667
Observations	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0	
df	6		6		6	
t Stat	-4.0115842		-3.840499403		-3.968737481	
P(T<=t) one-tail	0.0035129		0.004276975		0.003688804	
t Critical one-tail	1.9431803		1.943180281		1.943180281	
P(T<=t) two-tail	0.0070257		0.00855395		0.007377608	
t Critical two-tail	2.4469119		2.446911851		2.446911851	

Final results of the times strains showed either hypo or hyper apical branching phenotype							
		MAG results			MN results		
Abnormal strains	**Gene name**	**Numbers of repeatability among 4 replicates**	**Apical branhcing Phenotype**	**t-test results**	**Numbers of repeatability among 4 replicates**	**Apical branhcing Phenotype**	**t-test results**
2A10	**PkaA (Sc Tpk2)**	**4**	**Hypo**	0.007(Significant)	**4**	**Hypo**	0.004(Significant)
2A9	**ImeB**	**3**	**Hypo**	0.008(Significant)	**3**	**Hypo**	0.004(Significant)
2D3	**Uncharacterized (Sc Sgv1)**	**3**	**hypo**	0.007(Significant)	**2**	**Hypo**	0.006(Significant)
2B6	**Uncharacterized**	**3**	**Hypo**	0.061 (Significant)	**2**	**Hypo**	0.006(Significant)
2C11	**NikA**	**3**	**Hypo**	0.007(Significant)	**2**	**Hypo**	0.005(Significant)
2B10	**Uncharacterized (Sc Snf1)**	**2**	**Hyper**	0.008(Significant)	**2**	**Hyper**	0.030(Not Significant)

Appendix V. Statistical analysis related to MAG and MN media for 100 kinase deletion strains.

Guide
Null hypothesis=*H0: P>0.05* (There is no significant difference)
Alternative hypothesis=*Ha:P<0.05* (There is a significant difference)

Abnormally High apical branching

Abnormally Low apical branching

MAG Media

1A1-12	1A1	2E.1 (WT)	1A2	2E.1 (WT)	1A3	2E.1 (WT)	1A4	2E.1 (WT)	1A5	2E.1 (WT)	1A6	2E.1 (WT)	1A7	2E.1 (WT)	1A8	2E.1 (WT)	1A9	2E.1 (WT)	1A10	2E.1 (WT)	1A11	2E.1 (WT)	1A12	2E.1 (WT)
Replicate1	48	29	34	29	1	29	21	29	22	29	10	29	1	29	22	29	1	29	27	29	34	29	20	29
Replicate2	40	23	5	23	18	23	12	23	21	23	19	23	15	23	26	23	30	23	22	23	18	23	28	23
Replicate3	12	10	12	10	14	10	7	10	8	10	8	10	12	10	7	10	12	10	7	10	15	10	8	10
Replicate4	14	15	4	15	13	15	5	15	14	15	15	15	10	15	3	15	11	15	16	15	16	15	8	15
Mean	22	16	13.75	19.25	11.5	19.25	11.25	19.25	16.25	19.25	13	19.25	9.5	19.25	14.5	19.25	13.5	19.25	18	19.25	20.75	19.25	16	19.25
Variance	244	43	194.9167	70.91667	53.66667	70.916667	50.91667	70.91667	42.91667	70.91667	24.66667	70.91667	36.33333	70.91667	125.6667	70.91667	145.6667	70.91667	74	70.91667	79.58333	70.91667	96	70.916667
Observations	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	3		5		6		6		6		5		5		6		5		6		6		6	
t Stat	0.613438		0.674665		-1.38868		1.449562		0.562363		1.278554		-1.88294		0.677564		-0.78142		0.207673		0.244542		0.503111	
P(T<=t) one-tail	0.291482		0.26491		0.107143		0.09868		0.29712		0.128592		0.059215		0.261651		0.23496		0.421177		0.40748		0.316409	
t Critical one-tail	2.353363		2.015048		1.94318		1.94318		1.94318		2.015048		2.015048		1.94318		2.015048		1.94318		1.94318		1.94318	
P(T<=t) two-tail	0.582964		0.52982		0.214286		0.197359		0.59424		0.257184		0.118429		0.523302		0.469919		0.842353		0.814959		0.632818	
t Critical two-tail	3.182446		2.570582		2.446912		2.446912		2.446912		2.570582		2.570582		2.446912		2.570582		2.446912		2.446912		2.446912	

1B1-12	1B1	2E.1 (WT)	1B2	2E.1 (WT)	1B3	2E.1 (WT)	1B4	2E.1 (WT)	1B5	2E.1 (WT)	1B6	2E.1 (WT)	1B7	2E.1 (WT)	1B8	2E.1 (WT)	1B9	2E.1 (WT)	1B10	2E.1 (WT)	1B11	2E.1 (WT)	1B12	2E.1 (WT)
Replicate1	25	29	18	29	49	29	20	29	19	29	15	29	10	29	1	29	17	29	7	29	11	29	1	29
Replicate2	23	23	16	23	8	23	34	23	11	23	14	23	21	23	10	23	9	23	23	23	27	23	10	23
Replicate3	8	10	6	10	10	10	5	10	12	10	10	10	6	10	13	10	8	10	8	10	18	10	35	10
Replicate4	10	15	21	15	12	15	12	15	10	15	12	15	20	15	13	15	15	15	12	15	10	15	15	15
Mean	16.5	19.25	15.25	19.25	19.75	19.25	17.75	19.25	13	19.25	12.75	19.25	14.25	19.25	9.25	19.25	12.25	19.25	12.5	19.25	16.5	19.25	20	16
Variance	76.33333	70.91667	42.25	70.91667	382.9167	70.916667	154.9167	70.91667	16.66667	70.91667	4.916667	70.91667	54.91667	70.91667	32.25	70.91667	19.58333	70.91667	53.66667	70.91667	61.66667	70.91667	175	43
Observations	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	6		6		4		5		4		3		6		5		5		6		6		3	
t Stat	0.453247		0.752022		0.046941		0.199631		1.33567		1.49284		0.891461		-1.96907		1.471647		1.209494		0.477659		0.469237	
P(T<=t) one-tail	0.33315		0.240244		0.482405		0.424817		0.126295		0.116155		0.203504		0.053025		0.100545		0.135981		0.324898		0.335448	
t Critical one-tail	1.94318		1.94318		2.131847		2.015048		2.131847		2.353363		1.94318		2.015048		2.015048		1.94318		1.94318		2.353363	
P(T<=t) two-tail	0.666301		0.480487		0.964481		0.849634		0.25259		0.23231		0.407008		0.106051		0.201109		0.271962		0.649796		0.670897	
t Critical two-tail	2.446912		2.446912		2.776445		2.570582		2.776445		3.182446		2.446912		2.570582		2.570582		2.446912		2.446912		3.182446	

1C1-12	1C1	2E.1 (WT)	1C2	2E.1 (WT)	1C3	2E.1 (WT)	1C4	2E.1 (WT)	1C5	2E.1 (WT)	1C6	2E.1 (WT)	1C7	2E.1 (WT)	1C8	2E.1 (WT)	1C9	2E.1 (WT)	1C10	2E.1 (WT)	1C11	2E.1 (WT)	1C12	2E.1 (WT)
Replicate1	19	29	14	29	17	29	14	29	18	29	11	29	18	29	14	29	16	29	17	29	20	29	20	29
Replicate2	22	23	21	23	21	23	21	23	18	23	21	23	16	23	18	23	13	23	30	23	10	23	21	23
Replicate3	25	10	11	10	27	10	17	10	18	10	14	10	19	10	11	10	13	10	14	10	8	10	11	10
Replicate4	10	15	13	15	24	15	14	15	15	15	16	15	21	15	18	15	9	15	15	15	9	15	17	15
Mean	19	19.25	14.75	19.25	22.25	19.25	16.5	19.25	17.25	19.25	15.5	19.25	18.5	19.25	15.25	19.25	12.75	19.25	19	19.25	11.75	19.25	17.25	19.25
Variance	42	70.91667	18.91667	70.91667	18.25	70.916667	11	70.91667	2.25	70.91667	17.66667	70.91667	4.333333	70.91667	11.58333	70.91667	8.25	70.91667	55.33333	70.91667	30.91667	70.91667	20.25	70.916667
Observations	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	6		4		4		4		3		4		3		4		4		6		5		5	
t Stat	-0.04705		0.949563		0.635404		0.607682		0.467631		0.796866		0.172917		0.880771		1.461074		0.044499		1.486436		0.41893	
P(T<=t) one-tail	0.481999		0.198052		0.279849		0.288104		0.335961		0.235076		0.436862		0.214091		0.108896		0.482975		0.098655		0.346328	
t Critical one-tail	1.94318		2.131847		2.131847		2.131847		2.353363		2.131847		2.353363		2.131847		2.131847		1.94318		2.015048		2.015048	
P(T<=t) two-tail	0.963998		0.396104		0.559697		0.576208		0.671922		0.470151		0.873725		0.428182		0.217793		0.96595		0.197311		0.692655	

t Critical two-tail	2.44691		2.776445		2.776445		2.776445		3.182446		2.776445		3.182446		2.776445		2.776445		2.446912		2.570582		2.570582	
1D1-12	**1D1**	**2E.1 (WT)**	**1D2**	**2E.1 (WT)**	**1D3**	**2E.1 (WT)**	**1D4**	**2E.1 (WT)**	**1D5**	**2E.1 (WT)**	**1D6**	**2E.1 (WT)**	**1D7**	**2E.1 (WT)**	**1D8**	**2E.1 (WT)**	**1D9**	**2E.1 (WT)**	**1D10**	**2E.1 (WT)**	**1D11**	**2E.1 (WT)**	**1D12**	**2E.1 (WT)**
Replicate1	11	29	17	29	8	29	13	29	0	29	48	29	15	29	48	29	25	29	11	29	48	29	41	29
Replicate2	20	23	26	23	13	23	9	23	20	23	14	23	18	23	20	23	18	23	24	23	35	23	23	23
Replicate3	24	10	11	10	8	10	23	10	25	10	26	10	11	10	14	10	13	10	17	10	9	10	10	10
Replicate4	12	15	6	15	7	15	8	15	14	15	11	15	8	15	9	15	11	15	20	15	13	15	8	15
Mean	16.75	19.25	15	19.25	9	19.25	13.25	19.25	14.75	19.25	24.75	19.25	13	19.25	22.75	19.25	16.75	19.25	18	19.25	26.25	19.25	20.5	19.25
Variance	39.58333	70.91667	74	70.91667	7.333333	70.91666667	46.91667	70.91667	116.9167	70.91667	282.25	70.91667	19.33333	70.91667	303.5833	70.91667	38.91667	70.91667	30	70.91667	340.9167	70.91667	231	70.91666667
Observations	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	6		6		4		6		6		4		5		4		6		5		4		5	
t Stat	0.475651		0.70609		2.317457		1.105471		-0.65668		0.585333		1.315789		0.36172		0.477093		-0.24886		0.68987		0.143879	
P(T<=t) one-tail	0.325573		0.253305		0.040682		0.155651		0.267872		0.294877		0.122671		0.36793		0.325088		0.406683		0.264107		0.445608	
t Critical one-tail	1.94318		1.94318		2.131847		1.94318		1.94318		2.131847		2.015048		2.131847		1.94318		2.015048		2.131847		2.015048	
P(T<=t) two-tail	0.651145		0.50661		0.081363		0.311301		0.535744		0.589754		0.245342		0.73586		0.650177		0.813367		0.528214		0.891215	
t Critical two-tail	2.446912		2.446912		2.776445		2.446912		2.446912		2.776445		2.570582		2.776445		2.446912		2.570582		2.776445		2.570582	
1E1-3	**1E1.1**	**2E.1 (WT)**	**1E1.2**	**2E.1 (WT)**	**1E1.3**	**2E.1 (WT)**																		
Replicate1	7	29	13	29	7	29																		
Replicate2	25	23	25	23	28	23																		
Replicate3	25	10	34	10	11	10																		
Replicate4	15	15	17	15	8	15																		
Mean	18	19.25	22.25	19.25	13.5	19.25																		
Variance	76	70.91667	86.25	70.91667	96.33333	70.91666667																		
Observations	4	4	4	4	4	4																		
Hypothesized Mean Difference	0		0		0																			
df	6		6		6																			
t Stat	0.206255		0.478598		0.889231																			
P(T<=t) one-tail	0.421706		0.324583		0.204057																			
t Critical one-tail	1.943118		1.94318		1.94318																			
P(T<=t) two-tail	0.843412		0.649166		0.408113																			
t Critical two-tail	2.446912		2.446912		2.446912																			
2A1-12	**2A1**	**2E.1 (WT)**	**2A2**	**2E.1 (WT)**	**2A3**	**2E.1 (WT)**	**2A4**	**2E.1 (WT)**	**2A5**	**2E.1 (WT)**	**2A6**	**2E.1 (WT)**	**2A7**	**2E.1 (WT)**	**2A8**	**2E.1 (WT)**	**2A9**	**2E.1 (WT)**	**2A10**	**2E.1 (WT)**	**2A11**	**2E.1 (WT)**	**2A12**	**2E.1 (WT)**
Replicate1	11	29	16	29	19	29	10	29	19	29	11	29	22	29	17	29	5	29	1	29	16	29	**14**	29
Replicate2	13	23	10	23	28	23	15	23	14	23	19	23	25	23	19	23	3	23	3	23	10	23	**17**	23
Replicate3	15	10	18	10	13	10	9	10	15	10	18	10	10	10	20	10	1	10	3	10	18	10	**20**	10
Replicate4	20	15	8	15	5	15	8	15	4	15	17	15	8	15	18	15	2	15	2	15	17	15	**13**	15
Mean	14.75	19.25	13	19.25	16.25	19.25	10.5	19.25	13	19.25	18	16	16.25	19.25	18.5	19.25	2.75	19.25	2.25	19.25	15.25	19.25	16	19.25
Variance	14.91667	70.91667	22.66667	70.91667	94.25	70.91666667	9.666667	70.91667	40.66667	70.91667	1	43	72.25	70.91667	1.666667	70.91667	2.916667	70.91667	0.916667	70.91667	12.91667	70.91667	10	70.91666667
Observations	4	4	4	4	4	4	4	4	4	4	3	3	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	4		5		6		4		6		2		6		3		6		6		4		4	
t Stat	0.971437		-1.29214		0.466864		-1.94946		1.183342		0.522233		0.501453		-0.17606		-3.84005		-4.01158		0.873739		-0.72259	
P(T<=t) one-tail	0.19317		0.126401		0.328534		0.061516		0.140715		0.326795		0.316958		0.435729		0.004277		0.003513		0.21579		0.254959	
t Critical one-tail	2.131847		2.015048		1.94318		2.131847		1.94318		2.919986		1.94318		2.353363		1.94318		1.94318		2.131847		2.131847	
P(T<=t) two-tail	0.386334		0.252802		0.657069		0.123033		0.28143		0.65359		0.633916		0.871457		0.008554		0.007026		0.43158		0.509918	
t Critical two-tail	2.776445		2.570582		2.446912		2.776445		2.446912		4.302653		2.446912		3.182446		2.446912		2.446912		2.776445		2.776445	
2B1-12	**2B1**	**2E.1 (WT)**	**2B2**	**2E.1 (WT)**	**2B3**	**2E.1 (WT)**	**2B4**	**2E.1 (WT)**	**2B5**	**2E.1 (WT)**	**2B6**	**2E.1 (WT)**	**2B7**	**2E.1 (WT)**	**2B8**	**2E.1 (WT)**	**2B9**	**2E.1 (WT)**	**2B10**	**2E.1 (WT)**	**2B11**	**2E.1 (WT)**	**2B12**	**2E.1 (WT)**
Replicate1	12	29	11	29	10	29	1	29	13	29	2	29	49	29	20	29	1	29	46	29	20	29	17	29
Replicate2	14	23	12	23	12	23	17	23	18	23	0	23	18	23	18	23	16	23	45	23	23	23	12	23

Replicate3	20	10	9	10	10	10	19	10	14	10	3	10	18	10	14	10	10	10	30	10	28	10	8	10
Replicate4	15	15	13	15	7	15	10	15	18	15	0	15	18	15	14	15	12	15	50	15	25	15	15	15
Mean	15.25	19.25	11.25	19.25	9.75	19.25	11.75	19.25	15.75	19.25	1	16	25.75	19.25	16.5	19.25	9.75	19.25	42.75	19.25	19	19.25	13	19.25
Variance	11.58333	70.91667	2.916667	70.91667	4.25	70.91666667	66.25	70.91667	6.916667	70.91667	3	43	240.25	70.91667	9	70.91667	40.25	70.91667	76.91667	70.91667	54.66667	70.91667	15.33333	70.91666667
Observations	4	4	4	4	4	4	4	4	4	4	3	3	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	4		3		3		6		4		4		5		4		6		6		6		4	
t Stat	0.880771		1.86206		2.191497		-1.28076		0.793442		-3.83065		0.736965		0.615239		-1.80205		3.865553		0.044617		1.345955	
P(T<=t) one-tail	0.214091		0.079752		0.058046		0.123778		0.235965		0.009304		0.247125		0.285838		0.060804		0.004154		0.48293		0.12477	
t Critical one-tail	2.131847		2.353363		2.353363		1.94318		2.131847		2.131847		2.015048		2.131847		1.94318		1.94318		1.94318		2.131847	
P(T<=t) two-tail	0.428182		0.159504		0.116092		0.247556		0.471931		0.018608		0.49425		0.571675		0.121607		0.008309		0.96586		0.249541	
t Critical two-tail	2.776445		3.182446		3.182446		2.446912		2.776445		2.776445		2.570582		2.776445		2.446912		2.446912		2.446912		2.776445	
2C1-12	**2C1**	**2E.1 (WT)**	**2C2**	**2E.1 (WT)**	**2C3**	**2E.1 (WT)**	**2C4**	**2E.1 (WT)**	**2C5**	**2E.1 (WT)**	**2C6**	**2E.1 (WT)**	**2C7**	**2E.1 (WT)**	**2C8**	**2E.1 (WT)**	**2C9**	**2E.1 (WT)**	**2C10**	**2E.1 (WT)**	**2C11**	**2E.1 (WT)**	**2C12**	**2E.1 (WT)**
Replicate1	1	29	25	29	28	29	22	29	5	29	19	29	14	29	19	29	10	29	12	29	3	29	12	29
Replicate2	20	23	24	23	30	23	11	23	17	23	7	23	4	23	22	23	16	23	17	23	3	23	14	23
Replicate3	12	10	26	10	43	10	11	10	8	10	10	10	15	10	25	10	14	10	9	10	3	10	14	10
Replicate4	43	15	19	15	13	15	19	15	9	15	13	15	18	15	20	15	8	15	15	15	2	15	6	15
Mean	19	19.25	23.5	19.25	28.5	19.25	15.75	19.25	9.75	19.25	12.25	19.25	12.75	19.25	21.5	19.25	12	19.25	13.25	19.25	2.75	19.25	11.5	19.25
Variance	316.6667	70.91667	9.666667	70.91667	151	70.91666667	31.58333	70.91667	26.25	70.91667	26.25	70.91667	36.91667	70.91667	7	70.91667	13.33333	70.91667	12.25	70.91667	0.25	70.91667	14.33333	70.91666667
Observations	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	4		4		5		5		5		5		5		4		4		4		6		4	
t Stat	-0.0254		0.946883		1.241872		0.691411		1.927502		1.420265		1.251892		0.509797		1.579731		1.315851		-3.91179		1.678744	
P(T<=t) one-tail	0.490477		0.198658		0.134679		0.260046		0.055924		0.107385		0.132991		0.318517		0.094659		0.129285		0.003938		0.084252	
t Critical one-tail	2.131847		2.131847		2.015048		2.015048		2.015048		2.015048		2.015048		2.131847		2.131847		2.131847		1.94318		2.131847	
P(T<=t) two-tail	0.980955		0.397315		0.269358		0.520091		0.111848		0.214769		0.265982		0.637034		0.189318		0.25857		0.007876		0.168503	
t Critical two-tail	2.776445		2.776445		2.570582		2.570582		2.570582		2.570582		2.570582		2.776445		2.776445		2.776445		2.446912		2.776445	
2D1-12	**2D1**	**2E.1 (WT)**	**2D2**	**2E.1 (WT)**	**2D3**	**2E.1 (WT)**	**2D4**	**2E.1 (WT)**	**2D5**	**2E.1 (WT)**	**2D6**	**2E.1 (WT)**	**2D7**	**2E.1 (WT)**	**2D8**	**2E.1 (WT)**	**2D9**	**2E.1 (WT)**	**2D10**	**2E.1 (WT)**	**2D11**	**2E.1 (WT)**	**2D12**	**2E.1 (WT)**
Replicate1	22	29	13	29	2	29	22	29	16	29	25	29	11	29	17	29	1	29	16	29	31	29	0	29
Replicate2	23	23	20	23	3	23	12	23	19	23	17	23	26	23	9	23	31	23	15	23	20	23	0	23
Replicate3	13	10	7	10	3	10	9	10	15	10	10	10	10	10	8	10	13	10	17	10	6	10	0	10
Replicate4	4	15	20	15	2	15	13	15	7	15	15	15	12	15	10	15	10	15	8	15	7	15	3	15
Mean	15.5	19.25	15	19.25	2.5	19.25	14	19.25	14.25	19.25	16.75	19.25	14.75	19.25	11	19.25	13.75	19.25	14	19.25	16	19.25	0.75	19.25
Variance	79	70.91667	39.33333	70.91667	0.333333	70.91666667	31.33333	70.91667	26.25	70.91667	38.91667	70.91667	56.91667	70.91667	16.66667	70.91667	158.25	70.91667	16.66667	70.91667	140.6667	70.91667	2.25	70.91666667
Observations	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	6		6		6		5		5		6		6		4		5		4		5		6	
t Stat	0.612543		0.809524		-3.96874		1.038383		1.014475		0.477093		0.796014		1.763085		-0.72664		1.121963		0.446861		-4.32559	
P(T<=t) one-tail	0.28133		0.224559		0.003689		0.173338		0.178452		0.325088		0.228177		0.076333		0.250014		0.162337		0.336833		0.002476	
t Critical one-tail	1.943178		1.94318		1.94318		2.015048		2.015048		1.94318		1.94318		2.131847		2.015048		2.131847		2.015048		1.94318	
P(T<=t) two-tail	0.562659		0.449118		0.007378		0.346676		0.356904		0.650177		0.456354		0.152666		0.500029		0.324674		0.673666		0.004952	
t Critical two-tail	2.446912		2.446912		2.446912		2.570582		2.570582		2.446912		2.446912		2.776445		2.570582		2.776445		2.570582		2.446912	

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1A1-12	1A1	2E.1 (WT)	1A2	2E.1 (WT)	1A3	2E.1(WT)	1A4	2E.1 (WT)	1A5	2E.1 (WT)	1A6	2E.1 (WT)	1A7	2E.1 (WT)	1A8	2E.1 (WT)	1A9	2E.1 (WT)	1A10	2E.1 (WT)	1A11	2E.1 (WT)	1A12	2E.1(WT)
Replicate1	10	15	6	15	11	15	6	15	26	15	2	15	21	15	11	15	21	15	14	15	7	15	11	15
Replicate2	21	14	15	14	15	14	15	14	16	14	12	14	17	14	11	14	16	14	15	14	14	14	15	14
Replicate3	16	25	12	25	20	25	11	25	18	25	14	25	16	25	12	25	14	25	25	25	13	25	20	25
Replicate4	16	12	8	12	18	12	37	12	25	12	30	12	18	12	14	12	25	12	19	12	13	12	18	12
Mean	15.75	16.5	10.25	16.5	16	16.5	21	17	21.25	16.5	14.5	16.5	18	16.5	12	16.5	19	16.5	18.25	16.5	11.75	16.5	16	16.5
Variance	20.25	33.66667	16.25	33.66667	15.3333	33.66666667	196	49	24.9167	33.6667	134.333	33.6667	4.666667	33.6667	2	33.6667	24.6667	33.6667	24.9167	33.6667	10.25	33.6667	15.3333	33.66666667
Observations	4	4	4	4	4	4	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	6		5		5		3		6		4		4		3		6		6		5		5	
t Stat	0.204282		1.769242		-0.14286		0.440262		1.241185		-0.30861		0.484544		1.506993		0.654654		0.457279		1.433537		-0.14286	
P(T<=t) one-tail	0.422443		0.068541		0.445991		0.340002		0.13043		0.386513		0.326668		0.114456		0.262848		0.33178		0.105577		0.445991	
t Critical one-tail	1.94318		2.015048		2.015048		2.353363		1.94318		2.131847		2.131847		2.353363		1.94318		1.94318		2.015048		2.015048	
P(T<=t) two-tail	0.844886		0.137081		0.891981		0.680005		0.260861		0.773025		0.653337		0.228912		0.536963		0.663561		0.211153		0.891981	
t Critical two-tail	2.446912		2.570582		2.570582		3.182446		2.446912		2.776445		2.776445		3.182446		2.446912		2.446912		2.570582		2.570582	

1B1-12	1B1	2E.1 (WT)	1B2	2E.1 (WT)	1B3	2E.1(WT)	1B4	2E.1 (WT)	1B5	2E.1 (WT)	1B6	2E.1 (WT)	1B7	2E.1 (WT)	1B8	2E.1 (WT)	1B9	2E.1 (WT)	1B10	2E.1 (WT)	1B11	2E.1 (WT)	1B12	2E.1(WT)
Replicate1	11	15	15	15	17	15	11	15	12	15	18	15	13	15	14	15	15	15	13	15	11	15	18	15
Replicate2	15	14	17	14	14	14	24	14	22	14	10	14	15	14	19	14	20	14	15	14	10	14	14	14
Replicate3	20	25	13	25	12	25	18	25	14	25	10	25	20	25	10	25	17	25	13	25	14	25	15	25
Replicate4	18	12	20	12	22	12	15	12	8	12	12	12	12	12	11	12	14	12	16	12	17	12	16	12
Mean	16	16.5	16.25	16.5	16.25	16.5	17	16.5	14	16.5	12.5	16.5	15	16.5	13.5	16.5	16.5	16.5	14.25	16.5	13	16.5	15.75	16.5
Variance	15.3333	33.66667	8.916667	33.6667	18.9167	33.66666667	30	33.6667	34.6667	33.6667	14.3333	33.6667	12.6667	33.6667	16.3333	33.6667	7	33.6667	2.25	33.6667	10	33.6667	2.916667	33.66666667
Observations	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	5		4		6		6		6		5		5		5		4		3		5		4	
t Stat	-0.14286		-0.07662		0.068952		0.125327		0.604858		1.154701		0.440732		-0.84853		0		0.750871		-1.05931		0.247999	
P(T<=t) one-tail	0.445991		0.471303		0.473634		0.452179		0.281714		0.151019		0.332905		0.217437		0.5		0.253631		0.168963		0.408173	
t Critical one-tail	2.015048		2.131847		1.94318		1.94318		1.94318		2.015048		2.015048		2.015048		2.131847		2.353363		2.015048		2.131847	
P(T<=t) two-tail	0.891981		0.942604		0.947268		0.904359		0.563742		0.300398		0.67781		0.434875		1		0.507261		0.337926		0.816346	
t Critical two-tail	2.570582		2.776445		2.446912		2.446912		2.446912		2.570582		2.570582		2.570582		2.776445		3.182446		2.570582		2.776445	

1C1-12	1C1	2E.1 (WT)	1C2	2E.1 (WT)	1C3	2E.1(WT)	1C4	2E.1 (WT)	1C5	2E.1 (WT)	1C6	2E.1 (WT)	1C7	2E.1 (WT)	1C8	2E.1 (WT)	1C9	2E.1 (WT)	1C10	2E.1 (WT)	1C11	2E.1 (WT)	1C12	2E.1(WT)
Replicate1	13	15	14	15	13	15	14	15	18	15	11	15	18	15	14	15	16	15	26	15	26	15	20	15
Replicate2	15	14	21	14	10	14	21	14	18	14	21	14	16	14	18	14	13	14	20	14	10	14	21	14
Replicate3	8	25	11	25	16	25	17	25	18	25	14	25	19	25	11	25	18	25	15	25	8	25	11	25
Replicate4	15	12	13	12	15	12	14	12	15	12	16	12	21	12	18	12	14	12	20	12	9	12	17	12
Mean	12.75	16.5	14.75	16.5	13.5	16.5	16.5	16.5	17.25	16.5	15.5	16.5	18.5	16.5	15.25	16.5	15.25	16.5	20.25	16.5	13.25	16.5	17.25	16.5
Variance	10.9167	33.66667	18.9167	33.6667	7	33.66666667	11	33.6667	2.25	33.6667	17.6667	33.6667	4.333333	33.6667	11.58333	33.6667	4.916667	33.6667	20.25	33.6667	72.9167	33.6667	20.25	33.66666667
Observations	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	5		6		4		5		3		5		4		5		4		6		5		6	
t Stat	1.122324		0.482663		-0.94088		0		0.25029		0.279145		0.648886		0.371647		-0.40248		1.021409		-0.62961		0.204282	
P(T<=t) one-tail	0.156176		0.32322		0.200021		0.5		0.409262		0.395656		0.275893		0.362692		0.35395		0.173233		0.278299		0.422443	
t Critical one-tail	2.015048		1.94318		2.131847		2.015048		2.353363		2.015048		2.131847		2.015048		2.131847		1.94318		2.015048		1.94318	
P(T<=t) two-tail	0.312352		0.646439		0.400041		1		0.818525		0.791311		0.551786		0.725385		0.707915		0.346465		0.556598		0.844886	
t Critical two-tail	2.570582		2.446912		2.776445		2.570582		3.182446		2.570582		2.570582		2.570582		2.776445		2.446912		2.570582		2.446912	

t Critical two-tail	2.57058 2		2.44691 2		2.77644 5		2.57058 2		3.18244 6		2.57058 2		2.77644 5		2.57058 2		2.77644 5		2.44691 2		2.57058 2		2.44691 2	
1D1-12	**1D1**	**2E.1 (WT)**	**1D2**	**2E.1 (WT)**	**1D3**	**2E.1(WT)**	**1D4**	**2E.1 (WT)**	**1D5**	**2E.1 (WT)**	**1D6**	**2E.1 (WT)**	**1D7**	**2E.1 (WT)**	**1D8**	**2E.1 (WT)**	**1D9**	**2E.1 (WT)**	**1D10**	**2E.1 (WT)**	**1D11**	**2E.1 (WT)**	**1D12**	**2E.1(WT)**
Replicate1	26	15	12	15	10	15	16	15	11	15	13	15	16	15	20	15	11	15	26	15	18	15	17	15
Replicate2	14	14	15	14	15	14	14	14	15	14	18	14	17	14	15	14	15	14	7	14	15	14	11	14
Replicate3	17	25	14	25	15	25	13	25	10	25	20	25	15	25	23	25	15	25	15	25	34	25	8	25
Replicate4	14	12	20	12	17	12	17	12	23	12	20	12	18	12	8	12	17	12	17	12	16	12	9	12
Mean	17.75	16.5	15.25	16.5	14.25	16.5	15	16.5	14.75	16.5	17.75	16.5	16.5	16.5	16.5	16.5	14.5	16.5	16.25	16.5	20.75	16.5	11.25	16.5
Variance	32.25	33.66667	11.58333	33.66667	8.916667	33.66666667	3.333333	33.66667	34.91667	33.66667	10.91667	33.66667	1.666667	33.66667	43	33.66667	6.333333	33.66667	60.91667	33.66667	79.58333	33.66667	16.25	33.66666667
Observations	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	6		5		4		4		6		5		3		6		4		6		5		5	
t Stat	0.30792 3		0.37164 7		0.68959 3		0.49319 7		0.42262 8		0.37441 5		0		0		0.63245 6		-0.05141		0.79873		1.48616 3	
P(T<=t) one-tail	0.38427 5		0.36269 2		0.26418 5		0.32386 2		0.34364 7		0.36172 5		0.5		0.5		0.28071 9		0.48033 3		0.23034 3		0.09869	
t Critical one-tail	1.94318		2.01504 8		2.13184 7		2.13184 7		1.94318		2.01504 8		2.35336 3		1.94318		2.13184 7		1.94318		2.01504 8		2.01504 8	
P(T<=t) two-tail	0.76855		0.72538 5		0.52837 1		0.64772 3		0.68729 4		0.72344 9		1		1		0.56143 8		0.96066 6		0.46068 6		0.19737 9	
t Critical two-tail	2.44691 2		2.57058 2		2.77644 5		2.77644 5		2.44691 2		2.57058 2		3.18244 6		2.44691 2		2.77644 5		2.44691 2		2.57058 2		2.57058 2	
1E1-3	**1E1.1**	**2E.1 (WT)**	**1E1.2**	**2E.1 (WT)**	**1E1.3**	**2E.1(WT)**																		
Replicate1	15	15	17	15	10	15																		
Replicate2	22	14	24	14	28	14																		
Replicate3	10	25	19	25	11	25																		
Replicate4	11	12	20	12	18	12																		
Mean	14.5	16.5	20	16.5	16.75	16.5																		
Variance	29.66667	33.66667	8.666667	33.66667	68.91667	33.66666667																		
Observations	4	4	4	4	4	4																		
Hypothesized Mean Difference	0		0		0																			
df	6		4		5																			
t Stat	0.50262 5		1.07586 3		0.04936 6																			
P(T<=t) one-tail	0.31657		0.17127 7		0.48126 9																			
t Critical one-tail	1.94318		2.13184 7		2.01504 8																			
P(T<=t) two-tail	0.63314		0.34255 3		0.96253 9																			
t Critical two-tail	2.44691 2		2.77644 5		2.57058 2																			
2A1-12	**2A1**	**2E.1 (WT)**	**2A2**	**2E.1 (WT)**	**2A3**	**2E.1(WT)**	**2A4**	**2E.1 (WT)**	**2A5**	**2E.1 (WT)**	**2A6**	**2E.1 (WT)**	**2A7**	**2E.1 (WT)**	**2A8**	**2E.1 (WT)**	**2A9**	**2E.1 (WT)**	**2A10**	**2E.1 (WT)**	**2A11**	**2E.1 (WT)**	**2A12**	**2E.1(WT)**
Replicate1	12	15	17	15	26	15	**10**	15	11	15	11	15	7	15	9	15	3	15	2	15	13	15	**15**	15
Replicate2	16	14	11	14	20	14	**9**	14	20	14	15	14	11	14	12	14	5	14	5	14	11	14	**20**	14
Replicate3	13	25	15	25	14	25	**8**	25	11	25	15	25	15	25	19	25	3	25	3	25	17	25	**11**	25
Replicate4	18	12	15	12	17	12	**12**	12	16	12	16	12	15	12	16	12	4	12	4	12	13	12	**23**	12
Mean	14.75	16.5	14.5	16.5	19.25	16.5	9.75	16.5	14.5	16.5	14.25	16.5	12	16.5	14	16.5	3.75	16.5	3.5	16.5	13.5	16.5	17.25	16.5
Variance	7.583333	33.66667	6.333333	33.66667	26.25	33.66666667	2.916667	33.66667	19	33.66667	4.916667	33.66667	14.66667	33.66667	19.33333	33.66667	0.916667	33.66667	1.666667	33.66667	6.333333	33.66667	28.25	33.66666667
Observations	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	4		4		6		3		6		4		5		6		6		6		4		5	
t Stat	0.54494 9		0.63245 6		0.71054 1		-2.2112		0.55117 8		0.72445 7		1.29455 1		0.68680 3		-4.33617		-4.37402		0.94868 3		-0.40168	
P(T<=t) one-tail	0.30737 4		0.28071 9		0.25201 9		0.05698 7		0.30070 9		0.25444 6		0.12601 7		0.25892 8		0.00244 8		0.00234 9		0.19825 1		0.35225 8	
t Critical one-tail	2.13184 7		2.13184 7		1.94318		2.35336 3		1.94318		2.13184 7		2.01504 8		1.94318		1.94318		1.94318		2.13184 7		2.01504 8	
P(T<=t) two-tail	0.61474 9		0.56143 8		0.50403 8		0.11397 4		0.60141 7		0.50889 1		0.25203 3		0.51785 7		0.00489 6		0.00469 9		0.39650 1		0.70451 5	

t Critical two-tail	2.77644		2.77644		2.44691		3.18244		2.44691		2.77644		2.57058		2.44691		2.44691		2.44691		2.77644		2.57058	
2B1-12	**2B1**	**2E.1 (WT)**	**2B2**	**2E.1 (WT)**	**2B3**	**2E.1(WT)**	**2B4**	**2E.1 (WT)**	**2B5**	**2E.1 (WT)**	**2B6**	**2E.1 (WT)**	**2B7**	**2E.1 (WT)**	**2B8**	**2E.1 (WT)**	**2B9**	**2E.1 (WT)**	**2B10**	**2E.1 (WT)**	**2B11**	**2E.1 (WT)**	**2B12**	**2E.1(WT)**
Replicate1	17	15	2	15	12	15	13	15	18	15	4	15	8	15	17	15	15	15	25	15	11	15	6	15
Replicate2	16	14	15	14	16	14	25	14	20	14	6	14	20	14	15	14	11	14	24	14	12	14	12	14
Replicate3	15	25	13	25	12	25	20	25	12	25	3	25	15	25	18	25	33	25	40	25	19	25	21	25
Replicate4	10	12	11	12	18	12	18	12	17	12	4	12	7	12	15	12	10	12	37	12	13	12	9	12
Mean	14.5	16.5	10.25	16.5	14.5	16.5	19	16.5	16.75	16.5	4.25	16.5	12.5	16.5	16.25	16.5	17.25	16.5	31.5	16.5	13.75	16.5	12	16.5
Variance	9.666667	33.66667	32.9166 7	33.66667	9	33.66666667	24.66667	33.66667	11.58333	33.66667	1.583333	33.66667	37.66667	33.66667	2.25	33.66667	114.9167	33.66667	67	33.66667	12.91667	33.66667	42	33.66666667
Observations	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	5		6		4		6		5		6		6		3		5		5		5		6	
t Stat	-0.60764		-1.53189		0.612372		0.654654		0.074329		-4.12654		0.947204		-0.08343		0.123057		2.99005		0.805837		1.034642	
P(T<=t) one-tail	0.284981		0.088217		0.286696		0.268482		0.471815		0.003085		0.192 0052		0.469382		0.453428		0.015223		0.226846		0.170362	
t Critical one-tail	2.015048		1.94318		2.131847		1.94318		2.015048		1.94318		1.94318		2.353363		2.015048		2.015048		2.015048		1.94318	
P(T<=t) two-tail	0.569962		0.176434		0.573392		0.536963		0.94363		0.006171		0.380104		0.938765		0.907 685		0.030446		0.453693		0.340724	
t Critical two-tail	2.570582		2.44691 2		2.776445		2.44691 2		2.570582		2.44691 2		2.44691 2		3.182446		2.570582		2.570582		2.570582		2.44691 2	
2C1-12	**2C1**	**2E.1 (WT)**	**2C2**	**2E.1 (WT)**	**2C3**	**2E.1(WT)**	**2C4**	**2E.1 (WT)**	**2C5**	**2E.1 (WT)**	**2C6**	**2E.1 (WT)**	**2C7**	**2E.1 (WT)**	**2C8**	**2E.1 (WT)**	**2C9**	**2E.1 (WT)**	**2C10**	**2E.1 (WT)**	**2C11**	**2E.1 (WT)**	**2C12**	**2E.1(WT)**
Replicate1	14	15	16	15	2	15	15	15	20	15	26	15	26	15	12	15	18	15	11	15	4	15	14	15
Replicate2	22	14	14	14	11	14	12	14	12	14	14	14	15	14	15	14	12	14	16	14	5	14	18	14
Replicate3	17	25	22	25	20	25	12	25	20	25	21	25	16	25	13	25	21	25	21	25	4	25	25	25
Replicate4	15	12	15	12	13	12	20	12	9	12	12	12	21	12	18	12	18	12	40	12	4	12	10	12
Mean	17	16.5	16.75	16.5	11.5	16.5	14.75	16.5	15.25	16.5	18.25	16.5	19.5	16.5	14.5	16.5	17.25	16.5	25.66667	17	4.25	16.5	16.75	16.5
Variance	12.66667	33.66667	12.9166 7	33.66667	55	33.66666667	14.25	33.66667	31.58333	33.66667	41.58333	33.66667	25.66667	33.66667	7	33.66667	14.25	33.66667	160.3333	49	0.25	33.66667	40.9166 7	33.66666667
Observations	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	5		5		6		5		6		6		6		4		5		3		6		6	
t Stat	0.140691 1		0.073258		-1.06199		0.505621		0.309492		0.403473		0.778936		0.62725		0.216695		1.037513		-4.20687		0.057896	
P(T<=t) one-tail	0.444471		0.472221		0.164553		0.317309		0.383707		0.350293		0.23281		0.28226		0.418505		0.18789		0.000282 1		0.478566	
t Critical one-tail	2.015048		2.015048		1.94318		2.015048		1.94318		1.94318		1.94318		2.132847		2.015048		2.353363		1.94318		1.94318	
P(T<=t) two-tail	0.889442		0.944441		0.329105		0.634619		0.767414		0.700585		0.465619		0.56452		0.837 01		0.376578		0.005643		0.955711	
t Critical two-tail	2.570582		2.570582		2.776445		2.570582		2.44691 2		2.44691 2		2.44691 2		2.776445		2.570582		3.182446		2.44691 2		2.44691 2	
2D1-12	**2D1**	**2E.1 (WT)**	**2D2**	**2E.1 (WT)**	**2D3**	**2E.1(WT)**	**2D4**	**2E.1 (WT)**	**2D5**	**2E.1 (WT)**	**2D6**	**2E.1 (WT)**	**2D7**	**2E.1 (WT)**	**2D8**	**2E.1 (WT)**	**2D9**	**2E.1 (WT)**	**2D10**	**2E.1 (WT)**	**2D11**	**2E.1 (WT)**	**2D12**	**2E.1(WT)**
Replicate1	13	15	7	15	4	15	13	15	10	15	2	15	9	15	2	15	9	15	10	15	13	15	12	15
Replicate2	15	14	13	14	6	14	14	14	13	14	13	14	23	14	15	14	13	14	10	14	13	14	15	14
Replicate3	11	25	18	25	3	25	20	25	14	25	18	25	17	25	20	25	13	25	19	25	20	25	10	25
Replicate4	20	12	15	12	4	12	11	12	14	12	12	12	9	12	13	12	10	12	9	12	18	12	12	12
Mean	14.75	16.5	13.25	16.5	4.25	16.5	14.5	16.5	12.75	16.5	11.25	16.5	14.5	16.5	12.5	16.5	11.25	16.5	12	16.5	16	16.5	12.25	16.5
Variance	14.91667	33.66667	21.58333	33.66667	1.583333	33.66666667	15	33.66667	3.583333	33.66667	44.91667	33.66667	46.33333	33.66667	57.66667	33.66667	4.25	33.66667	22	33.66667	12.66667	33.66667	4.25	33.66666667
Observations	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	5		6		6		5		4		6		6		6		4		6		5		4	
t Stat	0.502 14		0.874475		-4.12654		0.573382		1.228848		-1.18447		0.447214		-0.8371		1.705196		1.206271		0.146911		-1.38 04	
P(T<=t) one-tail	0.318448		0.207743		0.003085		0.296605		0.140324		0.140508		0.336206		0.217303		0.083 167 9		0.136557		0.444471		0.119794	
t Critical one-tail	2.015048		1.94318		1.94318		2.015048		2.132847		1.94318		1.94318		1.94318		2.132847		1.94318		2.015048		2.132847	

P(T<=t) two-tail	0.63689		0.41548		0.00617		0.59121		0.28647		0.28101		0.67041		0.43460		0.16335		0.27311		0.88894		0.23958	
	7		7		1				9		6		2		7		7		4		2		9	
t Critical two-tail	2.57058		2.44691		2.44691		2.57058		2.77644		2.44691		2.44691		2.44691		2.77644		2.44691		2.57058		2.77644	
	2		2		2		2		5		2		2		2		5		2		2		5	

Appendix VI. T-Test for paired two samples for mean to find significant difference between -80 stocks and Master Plates (MPs).

Guide
Gray color: shows strains revealed one or two times abnormal apical branching phenotype
Green color shows strains revealed more than two times abnormal apical branching phenotype

MAG media

1A1-12	1A1		1A2		1A3		1A4		1A5		1A6		1A7		1A8		1A9		1A10		1A11		1A12	
	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs
Replicate1	48	12	34	12	1	14	21	7	22	8	10	8	1	12	22	7	1	12	27	7	34	15	20	8
Replicate2	40	14	5	4	18	13	12	5	21	14	19	15	15	10	26	3	30	11	22	16	18	16	28	8
Mean	44	13	19.5	8	9.5	13.5	16.5	6	21.5	11	14.5	11.5	8	11	24	5	15.5	11.5	24.5	11.5	26	15.5	24	8
Variance	32	2	420.5	32	144.5	0.5	40.5	2	0.5	18	40.5	24.5	98	2	8	8	420.5	0.5	12.5	40.5	128	0.5	32	0
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	6.2		1.0952381		-0.444444444		3		3		3		-0.375		4.75		0.2666667		1.857142857		1.235294		4	
P(T<=t) one-tail	0.05090192		0.2355413		0.3668750		0.1024163		0.1024163		0.1024163		0.3857997		0.066048		0.4170476		0.1572264		0.216616		0.0779791	
t Critical one-tail	6.31375151		6.3137515		6.31375151		6.3137515		6.31375151		6.3137515		6.31375151		6.31375151		6.3137515		6.31375151		6.313751		6.313751	
P(T<=t) two-tail	0.10180385		0.4710826		0.7337501		0.2048327		0.2048327		0.2048327		0.7715995		0.1320962		0.8340953		0.3145284		0.433233		0.1559583	
t Critical two-tail	12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.7062047		12.706204		12.7062047		12.70620		12.70620	
1B1-12	1B1		1B2		1B3		1B4		1B5		1B6		1B7		1B8		1B9		1B10		1B11		1B12	
	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs
Replicate1	25	8	18	6	49	10	20	5	19	12	15	10	10	6	1	13	17	8	7	8	11	18	1	35
Replicate2	23	10	16	21	8	12	34	12	11	10	14	12	21	20	10	13	9	15	23	12	27	10	10	15
Mean	24	9	17	13.5	28.5	11	27	8.5	15	11	14.5	11	15.5	13	5.5	13	13	11.5	15	10	19	14	5.5	25
Variance	2	2	2	112.5	840.5	2	98	24.5	32	2	0.5	2	60.5	98	40.5	0	32	24.5	128	8	128	32	40.5	200
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	7.5		0.4117647		0.813953488		5.2857142		1.333333333		2.333333333		1.666666667		-1.666666667		0.2		0.833333333		0.416667		-1.34483	
P(T<=t) one-tail	0.04219246		0.3756659		0.2825334		0.0595173		0.2048327		0.1288810		0.1720209		0.1720209		0.4371670		0.2788579		0.374334		0.2035229	
t Critical one-tail	6.31375151		6.3137515		6.31375151		6.3137515		6.31375151		6.3137515		6.31375151		6.31375151		6.3137515		6.31375151		6.313751		6.313751	
P(T<=t) two-tail	0.08438492		0.7513318		0.5650668		0.119034		0.4096655		0.2577621		0.3440417		0.3440417		0.8743340		0.5571587		0.748668		0.4070457	
t Critical two-tail	12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.7062047		12.706204		12.7062047		12.70620		12.70620	
1C1-12	1C1		1C2		1C3		1C4		1C5		1C6		1C7		1C8		1C9		1C10		1C11		1C12	
	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs
Replicate1	19	25	14	11	17	27	14	17	18	18	11	14	18	19	14	11	16	13	17	14	20	8	20	11
Replicate2	22	10	21	13	21	24	21	14	18	15	21	16	16	21	18	18	13	9	30	15	10	9	21	17

Mean	20.5	17.5	17.5	12	19	25.5	17.5	15.5	18	16.5	16	15	17	20	16	14.5	14.5	11	23.5	14.5	15	8.5	20.5	14
Variance	4.5	112.5	24.5	2	8	4.5	24.5	4.5	0	4.5	50	2	2	2	8	24.5	4.5	8	84.5	0.5	50	0.5	0.5	18
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	0.333333333		2.2		-1.85714285		0.4		1		0.25		-1.5		1		7		1.5		1.181818182		2.6	
P(T<=t) one-tail	0.397583618		0.135799755		0.157226421		0.378881086		0.25		0.422020887		0.187167042		0.25		0.045167244		0.187167042		0.223535353		0.116875151	
t Critical one-tail	6.313751515		6.313751511		6.313751515		6.313751511		6.313751515		6.313751511		6.313751515		6.313751515		6.313751511		6.313751515		6.313751511		6.313751515	
P(T<=t) two-tail	0.795167235		0.271599551		0.314452842		0.757762122		0.5		0.844041744		0.374334084		0.5		0.090334437		0.374334084		0.447070676		0.233750302	
t Critical two-tail	12.70620474		12.70620473		12.70620474		12.70620473		12.70620474		12.70620473		12.70620474		12.70620474		12.70620473		12.70620474		12.70620473		12.70620474	
1D1-12	**1D1**		**1D2**		**1D3**		**1D4**		**1D5**		**1D6**		**1D7**		**1D8**		**1D9**		**1D10**		**1D11**		**1D12**	
	Minus 80	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**
Replicate1	11	24	17	11	8	8	13	23	0	25	48	26	15	11	48	14	25	13	11	17	48	9	41	10
Replicate2	20	12	26	6	13	7	9	8	20	14	14	11	18	8	20	9	18	11	24	20	35	13	23	8
Mean	15.5	18	21.5	8.5	21.5	8.5	11	15.5	10	19.5	31	18.5	16.5	9.5	34	11.5	21.5	12	17.5	18.5	41.5	11	32	9
Variance	40.5	72	40.5	12.5	40.5	12.5	8	112.5	200	60.5	578	112.5	4.5	4.5	392	12.5	24.5	2	84.5	4.5	84.5	8	162	2
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	-0.23809523		1.857142856		1.857142857		-0.818181812		-0.612903226		1.3157894		2.333333333		1.956521739		3.8		-0.2		3.588235333		2.875	
P(T<=t) one-tail	0.425597235		0.157226422		0.157226421		0.281725522		0.324976296		0.206860119		0.128881058		0.150400446		0.081908688		0.437167042		0.086514141		0.10655	
t Critical one-tail	6.313751515		6.313751511		6.313751515		6.313751511		6.313751515		6.313751511		6.313751515		6.313751515		6.313751511		6.313751515		6.313751511		6.313751515	
P(T<=t) two-tail	0.851194469		0.314452844		0.314452842		0.563451033		0.649952592		0.413720388		0.257762117		0.300800892		0.163817366		0.874334084		0.173028333		0.213100101	
t Critical two-tail	12.70620474		12.70620473		12.70620474		12.70620473		12.70620474		12.70620473		12.70620474		12.70620474		12.70620473		12.70620474		12.70620473		12.70620474	
1E1-3	1E1.1		1E1.2		**1E1.3**																			
	Minus 80	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**																		
Replicate1	7	25	13	34	7	11																		
Replicate2	25	15	25	17	28	8																		
Mean	16	20	19	25.5	17.5	9.5																		
Variance	162	50	72	144.5	220.5	4.5																		
Observations	2	2	2	2	2	2																		
Hypothesized Mean Difference	0		0		0																			
df	1		1		1																			
t Stat	-0.285714286		-0.4482759		0.666666667																			
P(T<=t) one-tail	0.411414467		0.3658581		0.312832958																			
t Critical one-tail	6.313751515		6.313751511		6.313751515																			
P(T<=t) two-tail	0.822828934		0.7317162		0.625665916																			

t Critical two-tail	12.7062047 4		12.7062047		12.7062047 4																			
2A1-12	2A1		2A2		**2A3**		**2A4**		**2A5**		**2A6**		**2A7**		**2A8**		**2A9**		**2A10**		**2A11**		**2A12**	
	Minus 80	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**
Replicate1	11	15	16	18	19	13	10	9	19	15	11	19	22	10	17	20	5	1	1	3	16	18	**14**	**20**
Replicate2	13	20	10	8	28	5	15	8	14	4	18	17	25	8	19	18	3	2	3	2	10	17	**17**	**13**
Mean	12	17.5	13	13	23.5	9	12.5	8.5	16.5	9.5	14.5	18	23.5	9	18	19	4	1.5	2	2.5	13	17.5	15.5	16.5
Variance	2	12.5	18	50	40.5	32	12.5	0.5	12.5	60.5	24.5	2	4.5	2	2	2	2	0.5	2	0.5	18	0.5	4.5	24.5
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	-3.66666667		0		1.70588235 3		1.33333333		2.33333333		-0.7777778		5.8		-0.5		1.66666667		-0.33333333		-1.8		-0.2	
P(T<=t) one-tail	0.08475065 9		0.5		0.16877292 2		0.2048327 6		0.1288105 8		0.2895834 2		0.0543467 06		0.3524163 82		0.1720208 7		0.3975836 18		0.161414 5		0.437167	
t Critical one-tail	6.31375151 5		6.3137515 1		6.31375151 5		6.3137515 1		6.31375151 5		6.3137515 1		6.31375151 5		6.31375151 5		6.3137515 1		6.31375151 5		6.31375 15		6.31375 15	
P(T<=t) two-tail	0.16950131 9		1		0.33754584 5		0.4096655 3		0.2576211 7		0.5791668 5		0.1086934 11		0.7048327 65		0.3440417 4		0.7951672 35		0.322828 9		0.87433 41	
t Critical two-tail	12.7062047 4		12.7062047		12.7062047 4		12.7062047		12.7062047 4		12.7062047		12.7062047 4		12.7062047 4		12.7062047		12.7062047 4		12.7062 05		12.7062 05	
2B1-12	2B1		**2B2**		**2B3**		**2B4**		**2B5**		**2B6**		**2B7**		**2B8**		**2B9**		**2B10**		**2B11**		**2B12**	
	Minus 80	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**
Replicate1	12	20	11	9	10	10	1	19	13	14	2	3	49	18	20	14	1	10	46	30	20	28	17	8
Replicate2	14	15	12	13	12	7	17	10	18	18	0	0	18	18	18	14	16	12	45	50	23	25	12	15
Mean	13	17.5	11.5	11	11	8.5	9	14.5	15.5	16	1	1.5	33.5	18	19	14	8.5	11	45.5	40	21.5	26.5	14.5	11.5
Variance	2	12.5	0.5	8	2	4.5	128	40.5	12.5	8	2	4.5	480.5	0	2	0	112.5	2	0.5	200	4.5	4.5	12.5	24.5
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	-1.28571428 6		0.3333333 3		1		-0.44		-1		-1		1		5		-0.3846154		0.52380952 4		-1.66667		0.5	
P(T<=t) one-tail	0.21041657 6		0.3975836 2		0.25		0.3680583 6		0.25		0.25		0.25		0.0628329 58		0.3831249 4		0.3464112 48		0.172020 9		0.352416 4	
t Critical one-tail	6.31375151 5		6.3137515 1		6.31375151 5		6.3137515 1		6.31375151 5		6.3137515 1		6.31375151 5		6.31375151 5		6.3137515 1		6.31375151 5		6.31375 15		6.31375 15	
P(T<=t) two-tail	0.42083315 2		0.7951672 4		0.5		0.7361167 3		0.5		0.5		0.5		0.1256659 16		0.7662498 8		0.6928224 96		0.344041 7		0.70483 28	
t Critical two-tail	12.7062047 4		12.7062047		12.7062047 4		12.7062047		12.7062047 4		12.7062047		12.7062047 4		12.7062047 4		12.7062047		12.7062047 4		12.7062 05		12.7062 05	
2C1-12	2C1		2C2		**2C3**		**2C4**		**2C5**		**2C6**		**2C7**		**2C8**		**2C9**		**2C10**		**2C11**		**2C12**	
	Minus 80	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**
Replicate1	1	12	25	26	28	43	22	11	5	8	19	10	14	15	19	25	10	14	12	9	3	3	12	14
Replicate2	20	43	24	19	30	13	11	19	17	9	7	13	4	18	22	20	16	8	17	15	3	2	14	6
Mean	10.5	27.5	24.5	22.5	29	28	16.5	15	11	8.5	13	11.5	9	16.5	20.5	22.5	13	11	14.5	12	3	2.5	13	10
Variance	180.5	480.5	0.5	24.5	2	450	60.5	32	72	0.5	72	4.5	50	4.5	4.5	12.5	18	18	12.5	18	0	0.5	2	32
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		1		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	

t Stat	-2.83333333		0.66666667		0.0625		0.15789474		0.45454545		0.2		-1.15384615		-0.5		0.33333333		5		1		0.6	
P(T<=t) one-tail	0.10800019		0.31283296		0.48013147		0.45015207		0.36420025		0.43716704		0.22730212		0.35241638		0.39758362		0.06283295		0.25		0.32797971	
t Critical one-tail	6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151	
P(T<=t) two-tail	0.21600038		0.62566592		0.96026295		0.90030415		0.72840050		0.87433408		0.45460425		0.70483276		0.79516724		0.12566591		0.5		0.65595583	
t Critical two-tail	12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.7062047		12.706204		12.7062047		12.706205		12.706205	
2D1-12	2D1		2D2		2D3		**2D4**		**2D5**		**2D6**		**2D7**		**2D8**		**2D9**		**2D10**		**2D11**		**2D12**	
	Minus 80	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**
Replicate1	22	13	13	7	2	3	22	9	16	15	25	10	11	10	17	8	1	13	16	17	31	6	0	0
Replicate2	23	4	20	20	3	2	12	13	19	7	17	15	26	12	9	10	31	10	15	8	20	7	0	3
Mean	22.5	8.5	16.5	13.5	2.5	2.5	17	11	17.5	11	21	12.5	18.5	11	13	9	16	11.5	15.5	12.5	25.5	6.5	0	1.5
Variance	0.5	40.5	24.5	84.5	0.5	0.5	50	8	4.5	32	32	12.5	112.5	2	32	2	450	4.5	0.5	40.5	60.5	0.5	0	4.5
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	2.8		1		0		0.85714286		1.18181818		1.30769231		1.15384615		0.8		0.27272727		0.75		3.16666667		-1	
P(T<=t) one-tail	0.10918791		0.25		0.5		0.27443725		0.22353534		0.20780754		0.22730212		0.28522328		0.41524934		0.29516723		0.09736433		0.25	
t Critical one-tail	6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151	
P(T<=t) two-tail	0.21837582		0.5		1		0.54887455		0.44707064		0.41561507		0.45460425		0.57044657		0.83049868		0.59033447		0.19472855		0.5	
t Critical two-tail	12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.7062047		12.706204		12.7062047		12.706205		12.706205	

MN Media

1A1-12	**1A1**		**1A2**		**1A3**		**1A4**		**1A5**		**1A6**		**1A7**		**1A8**		**1A9**		**1A10**		**1A11**		**1A12**	
	Minus 80	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**
Replicate1	10	16	6	12	11	20	6	11	26	18	2	14	21	16	11	12	21	14	14	25	7	13	11	20
Replicate2	21	16	15	8	15	18	15	37	16	25	12	30	17	18	11	14	16	25	15	19	14	13	15	18
Mean	15.5	16	10.5	10	13	19	10.5	24	21	21.5	7	22	19	17	11	13	18.5	19.5	14.5	22	10.5	13	13	19
Variance	60.5	0	40.5	8	8	2	40.5	338	50	24.5	50	128	8	2	0	2	12.5	60.5	0.5	18	24.5	0	8	2
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	-0.09090909		0.07692308		-2		-1.58823529		-0.05882353		-5		0.66666667		-2		-0.125		-2.14285714		-0.714286		-2	
P(T<=t) one-tail	0.47114206		0.47556275		0.14758361		0.17886519		0.48129744		0.06283296		0.31283295		0.14758361		0.46041658		0.13898274		0.30256855		0.14758361	
t Critical one-tail	6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151		6.31375151	
P(T<=t) two-tail	0.94228413		0.95112555		0.29516723		0.35773038		0.96259488		0.12566592		0.62566591		0.29516723		0.92083315		0.27796548		0.60513569		0.29516722	
t Critical two-tail	12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.7062047		12.706204		12.7062047		12.706205		12.706205	
1B1-12	**1B1**		**1B2**		**1B3**		**1B4**		**1B5**		**1B6**		**1B7**		**1B8**		**1B9**		**1B10**		**1B11**		**1B12**	

	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs	Minus 80	MPs
Replicate1	11	20	15	13	17	12	11	18	12	14	18	10	13	20	14	10	15	17	13	13	11	14	18	15
Replicate2	15	18	17	20	14	22	24	15	22	8	10	12	15	12	19	11	20	14	15	16	10	17	14	16
Mean	13	19	16	16.5	15.5	17	17.5	16.5	17	11	14	11	14	16	16.5	10.5	17.5	15.5	14	14.5	10.5	15.5	16	15.5
Variance	8	2	2	24.5	4.5	50	84.5	4.5	50	18	32	2	2	32	12.5	0.5	12.5	4.5	2	4.5	0.5	4.5	8	0.5
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	-2		-0.2		-0.230769231		0.125		0.75		0.6		-0.4		3		0.5		-1		-2.5		0.2	
P(T<=t) one-tail	0.14758361		0.4371670		0.4278076		0.4604165		0.2951672		0.3279793		0.3788810		0.1024163		0.3524163		0.25		0.1211189		0.437167	
t Critical one-tail	6.31375151		6.3137515		6.31375151		6.3137515		6.31375151		6.3137515		6.31375151		6.31375151		6.3137515		6.31375151		6.3137515		6.313751	
P(T<=t) two-tail	0.29516723		0.8743340		0.8556153		0.9208331		0.5903344		0.6559582		0.7576211		0.2048327		0.7048327		0.5		0.2422379		0.874334	
t Critical two-tail	12.706204		12.706204		12.706204		12.706204		12.706204		12.706204		12.706204		12.706204		12.706204		12.706204		12.706205		12.70620	
1C1-12	**1C1**		**1C2**		**1C3**		**1C4**		**1C5**		**1C6**		**1C7**		**1C8**		**1C9**		**1C10**		**1C11**		**1C12**	
	Minus 80	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**
Replicate1	13	8	14	11	13	16	14	17	18	18	11	14	18	19	14	11	16	18	26	15	26	8	20	11
Replicate2	15	15	21	13	10	15	21	14	18	15	21	16	16	21	18	18	13	14	20	20	10	9	21	17
Mean	14	11.5	17.5	12	11.5	15.5	17.5	15.5	18	16.5	16	15	17	20	16	14.5	14.5	16	23	17.5	18	8.5	20.5	14
Variance	2	24.5	24.5	2	4.5	0.5	24.5	4.5	0	4.5	50	2	2	2	8	24.5	4.5	8	18	12.5	128	0.5	0.5	18
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	1		2.2		-4		0.4		1		0.25		-1.5		1		-3		1		1.117647		2.6	
P(T<=t) one-tail	0.25		0.1357997		0.0779791		0.3788810		0.25		0.4220208		0.1871670		0.25		0.1024163		0.25		0.2323343		0.1168751	
t Critical one-tail	6.31375151		6.3137515		6.31375151		6.3137515		6.31375151		6.3137515		6.31375151		6.31375151		6.3137515		6.31375151		6.3137515		6.313751	
P(T<=t) two-tail	0.5		0.2715995		0.1559582		0.7577621		0.5		0.8440417		0.3743340		0.5		0.2048327		0.5		0.4646686		0.2337501	
t Critical two-tail	12.706204		12.706204		12.706204		12.706204		12.706204		12.706204		12.706204		12.706204		12.706204		12.706204		12.706205		12.70620	
1D1-12	**1D1**		**1D2**		**1D3**		**1D4**		**1D5**		**1D6**		**1D7**		**1D8**		**1D9**		**1D10**		**1D11**		**1D12**	
	Minus 80	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**
Replicate1	26	17	12	14	10	15	16	13	11	10	13	20	16	15	20	23	11	15	26	15	18	34	17	8
Replicate2	14	14	15	20	15	17	14	17	15	23	18	20	17	18	15	8	15	17	7	17	15	16	11	9
Mean	20	15.5	13.5	17	12.5	16	15	15	13	16.5	15.5	20	16.5	16.5	17.5	15.5	13	16	16.5	16	16.5	25	14	8.5
Variance	72	4.5	4.5	18	12.5	2	2	8	8	84.5	12.5	0	0.5	4.5	12.5	112.5	8	2	180.5	2	4.5	162	18	0.5
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	1		-2.333333333		-2.333333333		0		-0.777777778		-1.8		0		0.4		-3		0.047619048		-1.133333		1.571428	
P(T<=t) one-tail	0.25		0.1288810		0.1288810		0.5		0.2895834		0.1614147		0.5		0.3788810		0.1024163		0.4848538		0.2301315		0.180395	

t Critical one-tail	6.313751515		6.313751515		6.313751515		6.313751515		6.313751515		6.313751515		6.313751515		6.313751515		6.313751515		6.313751515		6.313751515		6.313751515	
P(T<=t) two-tail	0.5		0.2577621		0.25762117		1		0.57916684		0.32282893		1		0.75776211		0.20483276		0.96970765		0.460263		0.360791	
t Critical two-tail	12.706204		12.70620		12.706204		12.70620		12.706204		12.70620		12.706204		12.706204		12.70620		12.706204		12.70620		12.70620	
1E1-3	**1E1.1**		**1E1.2**		**1E1.3**																			
	Minus 80	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**																		
Replicate1	15	10	17	19	10	11																		
Replicate2	22	11	24	20	28	18																		
Mean	18.5	10.5	20.5	19.5	19	14.5																		
Variance	24.5	0.5	24.5	0.5	162	24.5																		
Observations	2	2	2	2	2	2																		
Hypothesized Mean Difference	0		0		0																			
df	1		1		1																			
t Stat	2.666666667		0.333333		0.818181818																			
P(T<=t) one-tail	0.114200251		0.397583		0.28172551																			
t Critical one-tail	6.313751515		6.313751515		6.313751515																			
P(T<=t) two-tail	0.228400502		0.795167		0.56345103																			
t Critical two-tail	12.706204		12.70620		12.706204																			
2A1-12	**2A1**		**2A2**		**2A3**		**2A4**		**2A5**		**2A6**		**2A7**		**2A8**		**2A9**		**2A10**		**2A11**		**2A12**	
	Minus 80	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**
Replicate1	12	13	17	15	26	14	**10**	**8**	11	11	11	15	7	15	9	19	3	3	2	3	13	17	**15**	**11**
Replicate2	16	18	11	15	20	17	**9**	**12**	20	16	15	16	11	15	12	16	5	4	5	4	11	13	**20**	**23**
Mean	14	15.5	14	15	23	15.5	9.5	10	15.5	13.5	13	15.5	9	15	10.5	17.5	4	3.5	3.5	3.5	12	15	17.5	17
Variance	8	12.5	18	0	18	4.5	0.5	8	40.5	12.5	8	0.5	8	0	4.5	4.5	2	0.5	4.5	0.5	2	8	12.5	72
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	-3		-0.333333		1.666666667		-0.2		1		-1.666666667		-3		-2.333333333		1		0		-3		0.142857	
P(T<=t) one-tail	0.102416382		0.397583		0.17202087		0.43716704		0.25		0.17202087		0.102416382		0.12888105		0.25		0.5		0.102416		0.454832	
t Critical one-tail	6.313751515		6.313751515		6.313751515		6.313751515		6.313751515		6.313751515		6.313751515		6.313751515		6.313751515		6.313751515		6.313751515		6.313751515	
P(T<=t) two-tail	0.204832765		0.795167		0.34404173		0.87433408		0.5		0.34404174		0.204832765		0.25762117		0.5		1		0.204832		0.909665	
t Critical two-tail	12.706204		12.70620		12.706204		12.70620		12.706204		12.70620		12.706204		12.706204		12.70620		12.706204		12.70620		12.70620	
2B1-12	**2B1**		**2B2**		**2B3**		**2B4**		**2B5**		**2B6**		**2B7**		**2B8**		**2B9**		**2B10**		**2B11**		**2B12**	
	Minus 80	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**
Replicate1	17	15	2	13	12	12	13	20	18	12	4	3	8	15	17	18	15	33	25	40	11	19	6	21
Replicate2	16	10	15	11	16	18	25	18	20	17	6	4	20	7	15	15	11	10	24	37	12	13	12	9
Mean	16.5	12.5	8.5	12	14	15	19	19	19	14.5	5	3.5	14	11	16	16.5	13	21.5	24.5	38.5	11.5	16	9	15
Variance	0.5	12.5	84.5	2	8	18	72	2	2	12.5	2	0.5	72	32	2	4.5	8	264.5	0.5	4.5	0.5	18	18	72
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	2		-0.4666667		-1		0		3		3		0.3		-1		-0.8947368		-14		-1.285714		-0.666667	
P(T<=t) one-tail	0.14758361		0.3610172		0.25		0.5		0.10241638		0.1024163		0.4072264		0.25		0.2676657		0.0226978		0.2104166		0.312833	
t Critical one-tail	6.31375151		6.3137515		6.31375151		6.3137515		6.31375151		6.3137515		6.31375151		6.31375151		6.3137515		6.31375151		6.313751		6.313751	
P(T<=t) two-tail	0.29516723		0.7220345		0.5		1		0.20483276		0.2048327		0.8145284		0.5		0.5353314		0.0453957		0.4208332		0.6256659	
t Critical two-tail	12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.7062047		12.706204		12.7062047		12.70620		12.70620	
2C1-12	**2C1**		**2C2**		**2C3**		**2C4**		**2C5**		**2C6**		**2C7**		**2C8**		**2C9**		**2C10**		**2C11**		**2C12**	
	Minus 80	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**
Replicate1	14	17	16	22	2	20	15	12	20	20	26	21	26	16	12	13	18	21	11	21	4	4	14	25
Replicate2	22	15	14	15	11	13	12	20	12	9	14	12	15	21	15	18	12	18	16	40	5	4	18	10
Mean	18	16	15	18.5	6.5	16.5	13.5	16	16	14.5	20	16.5	20.5	18.5	13.5	15.5	15	19.5	13.5	30.5	4.5	4	16	17.5
Variance	32	2	2	24.5	40.5	24.5	4.5	32	32	60.5	72	40.5	60.5	12.5	4.5	12.5	18	4.5	12.5	180.5	0.5	0	8	112.5
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	0.4		-1.4		-1.25		-0.454545		1		2.333333		0.25		-2		-3		-2.428571		1		-0.157895	
P(T<=t) one-tail	0.37888105		0.1974315		0.2147767		0.3642002		0.25		0.1288810		0.4220208		0.1475836		0.1024163		0.1243340		0.25		0.4501521	
t Critical one-tail	6.31375151		6.3137515		6.31375151		6.3137515		6.31375151		6.3137515		6.31375151		6.31375151		6.3137515		6.31375151		6.313751		6.313751	
P(T<=t) two-tail	0.75776211		0.3948630		0.4295534		0.7284005		0.5		0.2577621		0.8440417		0.2951672		0.2048327		0.2486681		0.5		0.9003041	
t Critical two-tail	12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.7062047		12.706204		12.7062047		12.70620		12.70620	
2D1-12	**2D1**		**2D2**		**2D3**		**2D4**		**2D5**		**2D6**		**2D7**		**2D8**		**2D9**		**2D10**		**2D11**		**2D12**	
	Minus 80	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**	**Minus 80**	**MPs**
Replicate1	13	11	7	18	4	3	13	20	10	14	2	18	9	17	2	20	9	13	10	19	13	20	12	10
Replicate2	15	20	13	15	6	4	14	11	13	14	13	12	23	9	15	13	13	10	10	9	13	18	15	12
Mean	14	15.5	10	16.5	5	3.5	13.5	15.5	11.5	14	7.5	15	16	13	8.5	16.5	11	11.5	10	14	13	19	13.5	11
Variance	2	40.5	18	4.5	2	0.5	0.5	40.5	4.5	0	60.5	18	98	32	84.5	24.5	8	4.5	0	50	0	2	4.5	2
Observations	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	1		1		1		1		1		1		1		1		1		1		1		1	
t Stat	-0.428571		-1.444444		3		-0.4		-1.666667		-0.882353		0.272727		-0.8		-0.142857		-0.8		-6		5	
P(T<=t) one-tail	0.37111894		0.1927508		0.1024163		0.3788810		0.1720208		0.2698685		0.4152493		0.2852232		0.4548327		0.2852232		0.0525685		0.0628333	
t Critical one-tail	6.31375151		6.3137515		6.31375151		6.3137515		6.31375151		6.3137515		6.31375151		6.31375151		6.3137515		6.31375151		6.313751		6.313751	
P(T<=t) two-tail	0.74223788		0.3855017		0.2048327		0.7577621		0.3440417		0.5397370		0.8304986		0.5704465		0.9096655		0.5704465		0.1051369		0.1256659	
t Critical two-tail	12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.706204		12.7062047		12.7062047		12.706204		12.7062047		12.70620		12.70620	

Appendix VII. Final results of QMA associated with 100 kinase deletion library through dissecting microscope.

					MAG Medium			MN Medium		
Del #	**Cell**	**Org Strn #**	**ANID_#.1**	**Gene name**	**Repetitiveness of abnormal apical branching phenotype for each mutant strain during 4 rounds of counting**	**Abnormal apical branching phenotype during 4 rounds of counting**	**Student t-test results (P<0.05)**	**Repetitiveness of abnormal apical branching phenotype for each mutant strain during 4 rounds of counting**	**Abnormal apical branching phenotype during 4 rounds of counting**	**Student t-test results (P<0.05)**
1	**2A10**	KID79	ANID_06305.1	**PkaA (Sc Tpk2)**	**4**	**Hypo**	0.007(Significant)	**4**	**Hypo**	0.004(Significant)
2	**2A9**	KID78	ANID_06243.1	**ImeB**	**3**	**Hypo**	0.008(Significant)	**3**	**Hypo**	0.004(Significant)
3	**2D3**	KID117	ANID_08865.1	**ptkA (Sc Sgv1)**	**3**	**hypo**	0.007(Significant)	**2**	**Hypo**	0.006(Significant)
4	**2B6**	KID89	ANID_07537.1	**Uncharacterized**	**3**	**Hypo**	0.018 (Significant)	**2**	**Hypo**	0.006(Significant)
5	**2C11**	KID109	ANID_04479.1	**NikA**	**3**	**Hypo**	0.007(Significant)	**2**	**Hypo**	0.005(Significant)
6	**2B10**	KID93	ANID_07695.1	**Uncharacterized (Sc Snf1)**	**2**	**Hyper**	0.008(Significant)	**2**	**Hyper**	0.030(Significant)
7	**2B9**	KID92	ANID_07678.1	**Uncharacterized**	**1**	Hypo	0.1216(Not Significant)	**1**	hypo	0.906(Not Significant)
8	**2B4**	KID87	ANID_07185.1	**Uncharacterized (Sc Sky1, Sp Dsk1))**	**1**	Hypo	0.247(Not Significant)	**1**	hypo	0.536(Not Significant)
9	**1D11**	KID57	ANID_04668.1	**MpkC**	**1**	Hyper	0.528(Not Significant)	**1**	hyper	0.46(Not Significant)
10	**2C3**	KID99	ANID_02363.1	**Uncharacterized**	**1**	hyper	0.269(Not Significant)	**1**	Hypo	0.329(Not Significant)
11	**2D12**	KID129	ANID_11786.1	**MPKA (Sc Slt2) An5666**	**3**	**Hypo**	0.004 (Significant)	**No result**	Normal	0.239(Not Significant)
12	**1A3**	KID6	ANID_00699.1	**cak1**	**1**	Hypo	0.2142(Not Significant)	**No result**	Normal	0.891(Not Significant)
13	**1D6**	KID51	ANID_04238.1	**SchA (Sc Sch9)**	**1**	Hyper	0.5897(Not Significant)	**No result**	Normal	0.723(Not Significant)
14	**1B8**	KID23	ANID_10937.1	**Uncharacterized**	**1**	Hypo	0.106(Not Significant)	**No result**	Normal	0.434(Not Significant)
15	**1A9**	KID12	ANID_10153.1	**SskB (Sc Ssk2)**	**1**	Hypo	0.469(Not Significant)	**No result**	Normal	0.536(Not Significant)
16	**1A1**	KID1	ANID_00038.1	**AtmA (Sc Tel1)**	**2**	Hyper	0.582(Not Significant)	**No result**	Normal	0.844(Not Significant)
17	**2C1**	KID96	ANID_08190.1	**Uncharacterized**	**2**	Hypo	0.980(Not Significant)	**No result**	Normal	0.888(Not Significant)
18	**1B3**	KID18	ANID_10515.1	**Uncharacterized (Sc Prk1)**	**1**	Hyper	0.964(Not Significant)	**No result**	Normal	0.947(Not Significant)
19	**1D5**	KID50	ANID_04196.1	**Uncharacterized**	**1**	Hypo	0.535(Not Significant)	**No result**	Normal	0.687(Not Significant)
20	**1A7**	KID10	ANID_10019.1	**Uncharacterized (Sc Hrk1)**	**1**	Hypo	0.118(Not Significant)	**No result**	Normal	0.653(Not Significant)
21	**2B7**	KID90	ANID_07563.1	**chkC**	**1**	Hyper	0.494(Not Significant)	**No result**	Normal	0.38(Not Significant)
22	**1D8**	KID53	ANID_04385.1	**SepH**	**1**	Hyper	0.735(Not Significant)	**No result**	Normal	1(Not Significant)
23	**2D9**	KID125	ANID_08261.1	**PHOA (Sc Pho85)**	**1**	Hypo	0.5(Not Significant)	**No result**	Normal	0.163(Not Significant)
24	**1B12**	KID28	ANID_01560.1	**PlkA (Sc Cdc5)**	**1**	Hypo	0.670(Not Significant)	**No result**	Normal	0.816(Not Significant)
25	**2C10**	KID108	ANID_04447.1	**Uncharacterized**	**No result**	Normal	0.258 (Not significant)	**1**	hyper	0.375 (Not significant)
26	**1E--3**	KID63	ANID_05494.1	**ChkA (Sc Chk1)**	**No result**	Normal	0.408 (Not significant)	**1**	hyper	0.962 (Not significant)
27	**1D10**	KID55	ANID_04536.1	**Uncharacterized (Sc Psk1)**	**No result**	Normal	0.813(Not Significant)	**1**	Hyper	0.960(Not Significant)
28	**2C6**	KID103	ANID_03214.1	**Uncharacterized**	**No result**	Normal	0.214(Not Significant)	**1**	Hyper	0.700(Not Significant)
29	**2C7**	KID104	ANID_03946.1	**SldA (Bub1/BubR1)**	**No result**	Normal	0.265(Not Significant)	**1**	Hyper	0.465(Not Significant)
30	**1C10**	KID38	ANID_02412.1	**CmkA (Sc Cmk2)**	**No result**	Normal	0.965(Not Significant)	**1**	Hyper	0.346(Not Significant)
31	**1C11**	KID39	ANID_02489.1	**Uncharacterized (Sc Ssn3)**	**No result**	Normal	0.197(Not Significant)	**1**	Hyper	0.556(Not Significant)
32	**2B2**	KID85	ANID_06975.1	**UvsB (Sc Mec1)**	**No result**	Normal	0.159(Not Significant)	**1**	Hypo	0.176(Not Significant)
33	**2D6**	KID121	ANID_05296.1	**TcsA**	**No result**	Normal	0.65(Not Significant)	**1**	Hypo	0.281(Not Significant)
34	**1A4**	KID7	ANID_00822.1	**KfsA (Sc Kin4)**	**No result**	Normal	0.197 (Not significant)	**1**	hyper	0.688 (Not significant)
35	**2D8**	KID123	ANID_07945.1	**Uncharacterized**	**No result**	Normal	0.152(Not Significant)	**1**	Hypo	0.434(Not Significant)
36	**1A5**	KID8	ANID_00931.1	**PbsA (Sc Pbs2)**	**No result**	Normal	0.594(Not Significant)	**1**	Hyper	0.26(Not Significant)
37	**1A6**	KID9	ANID_00988.1	**Ikh1 (Sc Kns1)**	**No result**	Normal	0.257(Not Significant)	**1**	Hypo	0.773(Not Significant)
38	**1D1**	KID42	ANID_03001.1	**Uncharacterized**	**No result**	Normal	0.651(Not Significant)	**1**	Hyper	0.768(Not Significant)
39	**2A3**	KID68	ANID_05728.1	**Uncharacterized**	**No result**	Normal	0.657(Not Significant)	**1**	Hyper	0.504(Not Significant)

Appendix VIII. Results of quantitative assay on proline medium with MAG hole.

Guide:

This color shows abnormal strains found through this experiment (Proline medium with MAG hole)

No.	Mutant strains	Numbers of apical branches associated with 1st round of counting(Replicates 1)	Numbers of apical branches associated with 2nd round of counting (Replicates2)	Numbers of apical branches associated with 3rd round of counting (Replicates 3)	Numbers of apical branches associated with 4th round of counting (Replicates 4)
1	1A1	8	8	5	4
2	1A2	5	5	4	7
3	1A3	6	0	7	8
4	1A4	6	13	9	11
5	1A5	10	8	5	8
6	1A6	2	7	6	9
7	1A7	12	11	14	11
8	1A8	9	13	8	12
9	1A9	12	12	7	9
10	1A10	6	12	8	9
11	1A11	6	7	8	10
12	1A12	12	9	6	9
13	1B1	10	10	7	8
14	1B2	8	13	10	9
15	1B3	10	8	9	0
16	1B4	9	9	8	13
17	1B5	9	8	12	10
18	1B6	10	11	9	9
19	1B7	10	13	12	5
20	1B8	8	13	11	10
21	1B9	7	12	7	7
22	1B10	6	7	4	5
23	1B11	5	8	10	9
24	1B12	4	12	3	4
25	1C1	11	9	9	5
26	1C2	7	5	12	7
27	1C3	8	9	10	10
28	1C4	6	5	10	4
29	1C5	3	8	9	4
30	1C6	4	10	8	8
31	1C7	7	6	7	8
32	1C8	8	6	13	14
33	1C9	6	14	9	9
34	1C10	3	6	8	13
35	1C11	5	10	4	7
36	1C12	9	15	9	9
37	1D1	10	13	15	7
38	1D2	6	12	10	10
39	1D3	10	8	7	12
40	1D4	10	9	5	13
41	1D5	8	10	7	9
42	1D6	13	10	10	8
43	1D7	10	11	14	10
44	1D8	0	1	2	0
45	1D9	11	14	15	13
46	1D10	10	10	7	8
47	1D11	11	12	14	10
48	1D12	8	9	10	9
49	1E--1	6	6	6	8
50	1E--2	8	11	5	11
51	1E--3	10	9	11	11
52	2A1	6	7	6	8
53	2A2	8	4	4	5
54	2A3	5	5	6	10
55	2A4	0	0	0	0
56	2A5	9	11	12	8
57	2A6	2	10	9	8
58	2A7	5	5	5	6
59	2A8	2	8	6	4
60	2A9	10	4	4	5
61	2A10	4	8	6	5
62	2A11	4	9	9	8
63	2A12	5	9	9	7
64	2B1	5	4	9	10

65	2B2	7	5	10	6
66	2B3	6	5	7	8
67	2B4	8	6	6	10
68	2B5	0	1	0	1
69	2B6	6	9	5	7
70	2B7	2	8	5	7
71	2B8	1	6	6	1
72	2B9	8	6	9	4
73	2B10	6	13	14	15
74	2B11	11	8	10	13
75	2B12	10	5	4	9
76	2C1	6	9	9	8
77	2C2	6	8	4	5
78	2C3	9	7	2	9
79	2C4	6	5	9	9
80	2C5	0	1	2	3
81	2C6	8	5	6	7
82	2C7	4	6	6	5
83	2C8	4	7	7	6
84	2C9	5	7	10	4
85	2C10	7	6	8	7
86	2C11	9	6	8	8
87	2C12	5	6	10	6
88	2D1	6	6	8	6
89	2D2	6	7	8	0
90	2D3	0	1	0	0
91	2D4	6	7	8	5
92	2D5	10	8	9	5
93	2D6	5	8	14	6
94	2D7	7	5	9	7
95	2D8	6	9	6	6
96	2D9	3	6	7	6
97	2D10	7	8	7	0
98	2D11	7	8	6	10
99	2D12	0	1	0	0
100	**2E1 (Wild type strain)**	5	10	9	7
101	**A4 (Reference strain)**	6	7	14	10
Reference Interval (RI) to determine abnormal apical branching:		**0<normal phenotype<13**	**1<normal phenotype<14**	**0<normal phenotype<14**	**0<normal phenotype<15**

Gene name, function and AN_ID	**Abnormal strains from this experiment**	**Repetitiveness of abnormal apical branching phenotype**	**Abnormal branching phenotype**	Not significant difference
halA/ ORF verified / An 8830	2D2	1	**decreased apical branching (Hypo)**	Not significant difference
hk-8-7/ uncharacterized/ AN9048	2D10	1	Hypo-apical branching	Not significant difference
Scha overlaping with pkaA/ ORF verified/AN4238	1D6	1	**Increased apical branching (Hyper)**	Not significant difference
isr1/uncharacterized/ AN3001	1D1	1	Hyper-apical branching	Not significant difference
rfeA/uncharacterized/AN2943	1C12	1	Hyper-apical branching	Not significant difference
ffkC/uncharacterized/AN2373	1C9	1	Hyper-apical branching	Not significant difference
steC/ORF verified/AN2269	1C8	1	Hyper-apical branching	Not significant difference
caK1/ ORF verified/AN0699	1A3	1	Hypo-apical branching	Not significant difference
sepl/uncharacterized/AN11032	2B10	1	Hyper-apical branching	Not significant difference
prk1/uncharachterized/AN10515	1B3	**1**	Hypo-apical branching	Not significant difference
phkB/uncharacterized/AN3101	2C5	**2**	Hypo-apical branching	**Significant difference**
CmkD/ involved in mpkA pathway/ ORF verified/AN4483	1D9	**2**	Hyper-apical branching	**Significant difference**
hriA/uncharachterized/AN7321	2B5	**3**	Hypo-apical branching	**Significant difference**
	1D8	**3**	Hypo-apical branching	**Significant difference**
ckiB/uncharacterized/AN5757	2A4	**4**	Hypo-apical branching	**Significant difference**
ptkA/ORF verified/ AN8865	2D3	**4**	Hypo-apical branching	**Significant difference**
MpkA/ AN5666/ Verified	2D12	**4**	Hypo-apical branching	**Significant difference**

Appendix IX. Results of quantitative assay on MAG+ proline hole.

Guide:

This color shows abnormal strains found through this experiment (MAG+proline hole experiment)

No.	Mutant strains	Numbers of apical branches associated with 1st round of counting (Replicates 1)	Numbers of apical branches associated with 2nd round of counting (Replicates2)	Numbers of apical branches associated with 3rd round of counting (Replicates 3)	Numbers of apical branches associated with 4th round of counting (Replicates 4)
1	1A1	43	34	44	44
2	1A2	16	17	25	17
3	1A3	16	24	20	14
4	1A4	22	15	25	20
5	1A5	31	33	25	30
6	1A6	20	24	20	21
7	1A7	10	11	18	24
8	1A8	30	21	23	25
9	1A9	33	30	25	25
10	1A10	35	25	21	24
11	1A11	44	22	21	25
12	1A12	37	20	39	30
13	1B1	17	17	25	20
14	1B2	41	21	29	24
15	1B3	25	22	19	8
16	1B4	24	25	29	25
17	1B5	20	40	25	30
18	1B6	10	17	25	20
19	1B7	20	31	26	30
20	1B8	16	22	18	25
21	1B9	20	31	30	26
22	1B10	20	16	23	17
23	1B11	16	20	24	15
24	1B12	26	20	24	20
25	1C1	44	30	76	33
26	1C2	23	23	25	30
27	1C3	26	37	40	26
28	1C4	18	23	19	20
29	1C5	17	44	38	27
30	1C6	33	19	27	30
31	1C7	25	20	24	16
32	1C8	34	34	22	34
33	1C9	28	22	24	20
34	1C10	23	23	16	23
35	1C11	40	17	3	30
36	1C12	28	28	29	28
37	1D1	22	23	23	23
38	1D2	21	20	23	23
39	1D3	22	20	28	22
40	1D4	17	15	25	25
41	1D5	8	59	12	15
42	1D6	20	42	14	18
43	1D7	25	40	22	22
44	1D8	15	0	6	15
45	1D9	13	48	25	25
46	1D10	24	31	22	22
47	1D11	18	19	20	25
48	1D12	23	14	25	13
49	1E--1	27	20	18	24
50	1E--2	20	24	16	25
51	1E--3	22	27	24	19
52	2A1	30	25	15	20
53	2A2	30	16	25	25
54	2A3	27	13	25	23
55	2A4	1	0	2	1
56	2A5	35	24	17	25
57	2A6	30	25	40	40
58	2A7	21	30	10	27
59	2A8	23	15	18	28
60	2A9	28	23	19	18

61	2A10	23	32	25	23
62	2A11	15	24	28	26
63	2A12	15	16	25	19
64	2B1	25	34	23	33
65	2B2	24	25	15	15
66	2B3	16	14	15	28
67	2B4	30	27	22	30
68	2B5	25	23	25	27
69	2B6	10	20	18	23
70	2B7	31	20	26	26
71	2B8	20	35	32	25
72	2B9	36	22	20	20
73	2B10	24	30	25	30
74	2B11	20	32	25	25
75	2B12	32	18	32	32
76	2C1	16	17	28	19
77	2C2	28	23	33	30
78	2C3	15	25	19	20
79	2C4	20	15	28	25
80	2C5	18	21	32	28
81	2C6	25	30	23	30
82	2C7	17	20	17	25
83	2C8	30	18	28	18
84	2C9	30	16	28	14
85	2C10	32	20	22	20
86	2C11	15	26	27	14
87	2C12	25	30	26	26
88	2D1	35	22	20	20
89	2D2	24	28	16	20
90	2D3	2	0	1	1
91	2D4	17	30	25	25
92	2D5	30	24	23	23
93	2D6	22	23	20	20
94	2D7	22	23	21	21
95	2D8	34	20	27	27
96	2D9	34	20	21	21
97	2D10	26	30	22	22
98	2D11	26	13	28	15
99	2D12	1	0	0	0
100	**2E1 (Wild type strain)**	23	25	22	28
101	**A4 (Reference strain)**	24	30	22	26
Reference Interval (RI) to determine abnormal apical branching:		**1<normal phenotype<43**	**0<normal phenotype<44**	**2<normal phenotype<40**	**1<normal phenotype<34**

Gene name, function and AN_ID	**Abnormal strains from this experiment**	**Repetitiveness of abnormal apical branching phenotype**	**Abnormal branching phenotype**	**Results of student t-test**
ckiB/uncharacterized/AN5757	2A4	**4**	hypo	**0.005 Significant**
ptkA/ORF verified/ AN8865	2D3	**4**	hypo	**0.005 Significant**
MpkA/ AN5666/ Verified	2D12	**4**	hypo	**0.004 Significant**
steC/ORF verified/AN2269	1C8	1	**hyper**	0.334 Not significant
CmkD/ involved in mpkA pathway/ ORF verified/AN4483	1D9	1	**hyper**	0.432 Not significant
atmA/AN0038/ ORF verified	1A1	3	**hyper**	**0.025 significant**
atg1/AN1632/uncharacterized	1C1	2	**hyper**	0.289 Not significant
npkA/AN6044/ ORF verified	2A6	2	**hyper**	0.199 Not significant
env7/AN10193/uncharacterized	1A11	1	**hyper**	0.33 Not significant
ffkD/AN1789/uncharacterized	1C3	1	**hyper**	0.135 Not significant
ste20/AN2067/uncharacterized	1C5	1	**hyper**	0.164 Not significant
ffkF/AN4196/uncharacterized	1D5	1	**hyper**	0.832 Not significant
sepH/AN4385/ORF Verified	1D8	1	hypo	**0.031 significant**

Appendix X. QMA associated with wild type strains on 1% ethanol and 1% glycerol.

Dissecting Microscope 10X	EVOS microscope 10X

2E1 (CDS1008) Wild type			
MN (1% glycerol, 72h)		**2E1 (CDS1008) Wild type**	
Replicates	**Numbers of apical branches**	**MN (1% glycerol, 72h)**	
1	**10**	**Replicates**	**Numbers of apical branches**
2	**11**	1	**11**
3	**10**	2	**15**
4	**8**	3	**12**
MN (1% Ethanol, 72h)		4	**13**
Replicates	**Numbers of apical branches**	**MN (1% Ethanol, 72h)**	
1	10	**Replicates**	**Numbers of apical branches**
2	11	1	**12**
3	13	2	**10**
4	8	3	**15**
		4	**10**
A4 (Wild type)			
MN (1% glycerol, 72h)		**A4 (Wild type)**	
Replicates	**Numbers of apical branches**	**MN (1% glycerol, 72h)**	
1	**37**	**Replicates**	**Numbers of apical branches**
2	**40**	1	**38**
3	**32**	2	**42**
4	**44**	3	**40**
MN (1% Ethanol, 72h)		4	**43**
Replicates	**Numbers of apical branches**	**MN (1% Ethanol, 72h)**	
1	**36**	**Replicates**	**Numbers of apical branches**
2	**34**	1	**39**
3	**45**	2	**40**
4	**36**	3	**41**
		4	**40**
A4 (Wild type)			
MAG (No vitamins, 48h)		**A4 (Wild type)**	
Replicates	**Numbers of apical branches**	**MAG (No vitamins, 48h)**	
1	**28**	**Replicates**	**Numbers of apical branches**
2	**40**	1	**42**
3	**43**	2	**45**
4	**43**	3	**44**
		4	**40**
A4 (Wild type)			
MAG (vitamins added to MAG medium, 48h)		**A4 (Wild type)**	
Replicates	**Numbers of apical branches**	**MAG (vitamins added to MAG medium, 48h)**	
1	**45**	**Replicates**	**Numbers of apical branches**
2	**44**	1	**45**
3	**40**	2	**44**
4	**43**	3	**40**
		4	**43**
MN Media (Without glycerol or ethanol, this media was made by ammonium tartrate)			
Replicates	**2E 1 (Wild type strain)**		

#	Numbers of apical branches
1	15
2	14
3	25
4	12
MAG Media	
Replicates	2E 1
#	Numbers of apical branches
1	29
2	23
3	10
4	15
MAG Media	
Replicates	A4 (Reference strain)
#	Numbers of apical branches
1	45
2	44
3	40
4	43
MN Media	
Replicates	A4 (Reference strain)
#	Numbers of apical branches
1	40
2	45
3	39
4	42

Appendix XI. EVOS results associated with proline+ MAG hole

	Results of QMA (quantitative microscopy assay) through EVOS microscope on MN media				
	Abnormal strains	Replicate 1	Replicate 2	Replicate 3	Replicate 4
1	2A4	0	0	0	1
2	2D3	1	1	0	0
3	2D12	1	0	0	0

	2A4	2E1(WT)	2D3	2E1(WT)	2D12	2E1(WT)
Replicate1	0	5	1	5	0	5
Replicate2	0	10	1	10	0	10
Replicate3	0	9	0	9	0	9
Replicate4	1	7	0	7	0	7
Mean	0.333333333	8.666666667	0.333333333	8.666666667	0	8.666667
Variance	0.333333333	2.333333333	0.333333333	2.333333333	0	2.333333
Observations	3	3	3	3	3	3
Hypothesized Mean Difference	0		0		0	
df	3		3		4	
t Stat	-8.838834765		-8.83883476		-9.827076298	
P(T<=t) one-tail	0.001526149		0.001526149		0.000300623	
t Critical one-tail	2.353363435		2.353363435		2.131846786	
P(T<=t) two-tail	0.003052298		0.003052298		0.000601246	
t Critical two-tail	3.182446305		3.182446305		2.776445105	

Results: the results of Quantitative microscopy assay were confirmed as the same results were obtained/ strains showed the significant difference with the wild type strain

Appendix XII. Final results of QMA and t-test for proline+MAG hole and MAG+proline hole.

		MAG+Proline hole media				**Proline+MAG hole media**		
Del #	**Gene name, function and AN_ID**	**Abnormal strains from this experiment**	**Repeatability of abnormal strains among 4 replicates**	**Abnormal branching phenotype**	**Results of student t-test**	**Repeatability of abnormal strains among 4 replicates**	**Abnormal branching phenotype**	**Results of student t-test**
1	ckiB/uncharacterized/AN 5757	2A4	**4**	hypo	**0.005 Significant**	**4**	Hypo-apical branching	**Significant difference**
2	ptkA/ORF verified/ AN8865	2D3	**4**	hypo	**0.005 Significant**	**4**	Hypo-apical branching	**Significant difference**
3	MpkA/ AN5666/ Verified	2D12	**4**	hypo	**0.004 Significant**	**4**	Hypo-apical branching	**Significant difference**
4	steC/ORF verified/AN2269	1C8	1	**hyper**	0.334 Not significant	1	Hyper-apical branching	Not significant difference
5	CmkD/ involved in mpkA pathway/ ORF verified/AN4483	1D9	1	**hyper**	0.432 Not significant	**2**	Hyper-apical branching	**Significant difference**
6	sepH/AN4385/ORF Verified	1D8	1	hypo	**0.031 significant**	**3**	Hypo-apical branching	**Significant difference**
7	atmA/AN0038/ ORF verified	1A1	3	**hyper**	**0.025 significant**	No result	**Normal**	Not significant
8	atg1/AN1632/uncharacterized	1C1	2	**hyper**	0.289 Not significant	No result	**Normal**	Not significant
9	npkA/AN6044/ ORF verified	2A6	2	**hyper**	0.199 Not significant	No result	**Normal**	Not significant
10	env7/AN10193/uncharacterized	1A11	1	**hyper**	0.33 Not significant	No result	**Normal**	Not significant
11	ffkD/AN1789/uncharacterized	1C3	1	**hyper**	0.135 Not significant	No result	**Normal**	Not significant
12	ste20/AN2067/uncharacterized	1C5	1	**hyper**	0.164 Not significant	No result	**Normal**	Not significant
13	ffkF/AN4196/uncharacterized	1D5	1	**hyper**	0.832 Not significant	No result	**Normal**	Not significant
14	halA/ ORF verified / An 8830	2D2	No result	**Normal**	Not significant	1	**decreased apical branching (Hypo)**	Not significant difference
15	hk-8-7/ uncharacterized/ AN9048	2D10	No result	**Normal**	Not significant	1	Hypo-apical branching	Not significant difference
16	Scha overlaping with pkaA/ ORF verified/AN4238	1D6	No result	**Normal**	Not significant	1	**Increased apical branching (Hyper)**	Not significant difference
17	isr1/uncharacterized/ AN3001	1D1	No result	**Normal**	Not significant	1	Hyper-apical branching	Not significant difference
18	rfeA/uncharacterized/AN2943	1C12	No result	**Normal**	Not significant	1	Hyper-apical branching	Not significant difference
19	ffkC/uncharacterized/AN2373	1C9	No result	**Normal**	Not significant	1	Hyper-apical branching	Not significant difference
20	caK1/ ORF verified/AN0699	1A3	No result	**Normal**	Not significant	1	Hypo-apical branching	Not significant difference
21	sepl/uncharacterized/AN11032	2B10	No result	**Normal**	Not significant	1	Hyper-apical branching	Not significant difference
22	prk1/uncharachterized/AN10515	1B3	No result	**Normal**	Not significant	**1**	Hypo-apical branching	Not significant difference
23	phkB/uncharacterized/AN3101	2C5	No result	**Normal**	Not significant	**2**	Hypo-apical branching	**Significant difference**
24	hriA/uncharachterized/AN7321	2B5	No result	**Normal**	Not significant	**3**	Hypo-apical branching	**Significant difference**

Appendix XIII. Student t-test for strains associated with 1% glycerol or 1% ethanol associated with EVOS microscope.

t-Test: Two-Sample Assuming Unequal Variances

Replicates	2E 1 , MN media	2E.1 MN (1% glycerol) media	Replicates	2E 1 , MN media	2E.1 MN (1% Ethanol) media
1	15	11	1	15	12
2	14	15	2	14	10
3	25	12	3	25	15
4	12	13	4	12	10
Mean	16.5	12.75	Mean	16.5	11.75
Variance	33.66666667	2.916666667	Variance	33.66666667	5.583333333
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	4		df	4	
t Stat	1.239994122		t Stat	1.51636508	
P(T<=t) one-tail	0.141374456		P(T<=t) one-tail	0.102008695	
t Critical one-tail	2.131846786		t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.282748913		P(T<=t) two-tail	0.204017391	
t Critical two-tail	2.776445105		t Critical two-tail	2.776445105	
Replicates	A4 (Reference strain), MN media	A4 MN (1% glycerol) media	Replicates	A4 (Reference strain), MN media	A4 MN (1% Ethanol) media
1	40	38	1	40	39
2	45	42	2	45	40
3	39	40	3	39	41
4	42	43	4	42	40
Mean	41.5	40.75	Mean	41.5	40
Variance	7	4.916666667	Variance	7	0.666666667
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	6		df	4	
t Stat	0.434524095		t Stat	1.083472678	
P(T<=t) one-tail	0.339549947		P(T<=t) one-tail	0.169770864	
t Critical one-tail	1.943180281		t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.679099893		P(T<=t) two-tail	0.339541728	
t Critical two-tail	2.446911851		t Critical two-tail	2.776445105	
Replicates	A4 Reference strain, MAG media	A4 , MAG media (No vitamine added)	Replicates	2A10 (Mutant strain), MN media	2A10 (Mutant strain), MAG media
1	45	42	1	3	4
2	44	45	2	5	3
3	40	44	3	3	2
4	43	40	4	2	1
Mean	43	42.75	Mean	3.25	2.5
Variance	4.666666667	4.916666667	Variance	1.583333333	1.666666667
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	6		df	6	
t Stat	0.161514571		t Stat	0.832050294	
P(T<=t) one-tail	0.438494815		P(T<=t) one-tail	0.218618265	
t Critical one-tail	1.943180281		t Critical one-tail	1.943180281	
P(T<=t) two-tail	0.876989629		P(T<=t) two-tail	0.43723653	
t Critical two-tail	2.446911851		t Critical two-tail	2.446911851	
Replicates	2A10 (Mutant strain), MN media	2A10 MN (1% glycerol) media	Replicates	2A10 (Mutant strain), MN media	2A10 MN (1% Ethanol) media
1	3	6	1	3	4
2	5	5	2	5	4
3	3	3	3	3	5
4	2	1	4	2	3
Mean	3.25	3.75	Mean	3.25	4
Variance	1.583333333	4.916666667	Variance	1.583333333	0.666666667
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5	
t Stat	-0.39223227		t Stat	-1	
P(T<=t) one-tail	0.355524184		P(T<=t) one-tail	0.181608734	
t Critical one-tail	2.015048373		t Critical one-tail	2.015048373	
P(T<=t) two-tail	0.711048367		P(T<=t) two-tail	0.363217468	
t Critical two-tail	2.570581836		t Critical two-tail	2.570581836	

Results: There is no significant difference between the strains grown on different media

Appendix XIV. Comparing the number of apical branches on MAG and Proline medium associated with Wild type (2E1), A4(Reference strain) and 2A10 as mutant strain with using t-Test for two sample assuming unequal variances.

Replicates	2E1 Wild type strain, MAG media	2E1 Wild type strain, proline media	Replicates	A4 Reference strain, MAG media	A4 Reference strain, proline media
1	29	4	1	45	6
2	23	5	2	44	7
3	10	5	3	40	5
4	15	4	4	43	8
Mean	16	4.666666667	Mean	42.3333333	6.666666667
Variance	43	0.333333333	Variance	4.33333333	2.333333333
Observations	3	3	Observations	3	3
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	4		df	4	
t Stat	2.981997266		t Stat	23.9259274	
P(T<=t) one-tail	0.020329232		P(T<=t) one-tail	9.0491E-06	
t Critical one-tail	2.131846786		t Critical one-tail	2.13184679	
P(T<=t) two-tail	0.040658463		P(T<=t) two-tail	1.8098E-05	
t Critical two-tail	2.776445105		t Critical two-tail	2.77644511	

Replicates	2A10 mutant strain, MAG media	2A10 mutant strain, proline media
1	4	0
2	5	0
3	4	0
4	5	1
Mean	4.666666667	0.333333333
Variance	0.333333333	0.333333333
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	9.192388155	
P(T<=t) one-tail	0.000388954	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.000777907	
t Critical two-tail	2.776445105	

Result: **There is a significant difference between the results obtained by MAG and the ones obtained by proline medium**

Appendix XIV. Results of student t-test for 1%glycerol, Ethanol, Dissecting microscope

t-Test: Two-Sample Assuming Unequal Variances			Dissecting Microscope		
Replicates	2E 1 (Wild type strain), MN media	2E1 Wild type strain, MAG media	Replicates	A4 (Reference strain), MN media	A4 Reference strain, MAG media
1	15	29	1	40	45
2	14	23	2	45	44
3	25	10	3	39	40
4	12	15	4	42	43
Mean	16.5	19.25	Mean	41.5	43
Variance	33.66666667	70.91666667	Variance	7	4.666666667
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	6	
t Stat	-0.537813191		t Stat	-0.878310066	
P(T<=t) one-tail	0.306885782		P(T<=t) one-tail	0.206780388	
t Critical one-tail	2.015048373		t Critical one-tail	1.943180281	
P(T<=t) two-tail	0.613771564		P(T<=t) two-tail	0.413560776	
t Critical two-tail	2.570581836		t Critical two-tail	2.446911851	

Replicates	2E 1 , MN media	2E1 MN (1% glycerol) media	Replicates	2E 1 , MN media	2E1 MN (1% Ethanol) media
1	15	10	1	15	10
2	14	11	2	14	11
3	25	10	3	25	13
4	12	8	4	12	8
Mean	16.5	9.75	Mean	16.5	10.5

Variance	33.66666667	1.583333333	**Variance**	33.66666667	4.333333333
Observations	4	4	**Observations**	4	4
Hypothesized Mean Difference	0		**Hypothesized Mean Difference**	0	
df	3		**df**	4	
t Stat	2.273810187		**t Stat**	1.946657054	
P(T<=t) one-tail	0.053773322		**P(T<=t) one-tail**	0.061714984	
t Critical one-tail	2.353363435		**t Critical one-tail**	2.131846786	
P(T<=t) two-tail	0.107546644		**P(T<=t) two-tail**	0.123429967	
t Critical two-tail	3.182446305		**t Critical two-tail**	2.776445105	
Replicates	**A4 (Reference strain), MN media**	**A4 MN (1% glycerol) media**	**Replicates**	**A4 (Reference strain), MN media**	**A4 MN (1% Ethanol) media**
1	40	37	**1**	40	36
2	45	40	**2**	45	34
3	39	32	**3**	39	45
4	42	44	**4**	42	36
Mean	41.5	38.25	**Mean**	41.5	37.75
Variance	7	25.58333333	**Variance**	7	24.25
Observations	4	4	**Observations**	4	4
Hypothesized Mean Difference	0		**Hypothesized Mean Difference**	0	
df	5		**df**	5	
t Stat	1.138716467		**t Stat**	1.341640786	
P(T<=t) one-tail	0.153210951		**P(T<=t) one-tail**	0.118709655	
t Critical one-tail	2.015048373		**t Critical one-tail**	2.015048373	
P(T<=t) two-tail	0.306421902		**P(T<=t) two-tail**	0.23741931	
t Critical two-tail	2.570581836		**t Critical two-tail**	2.570581836	
Replicates	**A4 Reference strain, MAG media**	**A4 , MAG media (No vitamine added)**	**Replicates**	**2A10 (Mutant strain), MN media**	**2A10 (Mutant strain), MAG media**
1	45	28	**1**	3	1
2	44	40	**2**	5	3
3	40	43	**3**	3	3
4	43	43	**4**	2	2
Mean	43	38.5	**Mean**	3.25	2.25
Variance	4.666666667	51	**Variance**	1.583333333	0.916666667
Observations	4	4	**Observations**	4	4
Hypothesized Mean Difference	0		**Hypothesized Mean Difference**	0	
df	4		**df**	6	
t Stat	1.206271039		**t Stat**	1.264911064	
P(T<=t) one-tail	0.147089218		**P(T<=t) one-tail**	0.126405057	
t Critical one-tail	2.131846786		**t Critical one-tail**	1.943180281	
P(T<=t) two-tail	0.294178436		**P(T<=t) two-tail**	0.252810113	
t Critical two-tail	2.776445105		**t Critical two-tail**	2.446911851	
Replicates	**2A10 (Mutant strain), MN media**	**2A10 MN (1% glycerol) media**	**Replicates**	**2A10 (Mutant strain), MN media**	**2A10 MN (1% Ethanol) media**
1	3	2	**1**	3	5
2	5	5	**2**	5	3
3	3	1	**3**	3	1
4	2	3	**4**	2	1
Mean	3.25	2.75	**Mean**	3.25	2.5
Variance	1.583333333	2.916666667	**Variance**	1.583333333	3.666666667
Observations	4	4	**Observations**	4	4
Hypothesized Mean Difference	0		**Hypothesized Mean Difference**	0	
df	6		**df**	5	
t Stat	0.471404521		**t Stat**	0.654653671	
P(T<=t) one-tail	0.32700231		**P(T<=t) one-tail**	0.27080228	
t Critical one-tail	1.943180281		**t Critical one-tail**	2.015048373	
P(T<=t) two-tail	0.65400462		**P(T<=t) two-tail**	0.541604561	
t Critical two-tail	2.446911851		**t Critical two-tail**	2.570581836	

Results: There is no significant difference between the strains grown on different media

Appendix XIV. Student t-test assuming unequal variances for proline plus MAG hole experiment.

1A1-12	1A1	2E.1 (WT)	1A2	2E.1 (WT)	1A3	2E.1 (WT)	1A4	2E.1 (WT)	1A5	2E.1 (WT)	1A6	2E.1 (WT)	1A7	2E.1 (WT)	1A8	2E.1 (WT)	1A9	2E.1 (WT)	1A10	2E.1 (WT)	1A11	2E.1 (WT)	1A12	2E.1 (WT)
Replicate1	8	5	5	5	6	5	6	5	10	5	2	5	12	5	9	5	12	5	6	5	6	5	12	5
Replicate2	8	10	5	10	0	10	13	10	8	10	7	10	11	10	13	10	12	10	12	10	7	10	9	10
Replicate3	5	9	4	9	7	9	9	9	5	9	6	9	14	9	8	9	7	9	8	9	8	9	6	9
Replicate4	4	7	7	7	8	7	11	7	8	7	9	7	11	7	12	7	9	7	9	7	10	7	9	7
Mean	6.25	7.75	5.25	7.75	5.25	7.75	9.75	7.75	7.75	7.75	6	7.75	12	8.6666667	10.5	7.75	10	7.75	8.75	7.75	7.75	7.75	9	7.75
Variances	4.25	4.916667	1.583333	4.916667	12.91667	4.916667	8.916667	4.916667	4.25	4.916667	8.666667	4.916667	3	2.333333	5.666667	4.916667	6	4.916667	6.25	4.916667	2.916667	4.916667	6	4.916667
Observations	4	4	4	4	4	4	4	4	4	4	4	4	3	3	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	6		5		5		6		6		6		4		6		6		6		6		6	
t Stat	-0.99087		-1.96116		-1.18401		1.075466		0		-0.94965		2.5		1.691061		1.36197		0.598506		0		0.75665	
P(T<=t) one-tail	0.180006		0.053564		0.144813		0.16175		0.5		0.189477		0.03338 3		0.07093		0.11061		0.28569 5		0.5		0.23895 4	
t Critical one-tail	1.94318		2.015048		2.015048		1.94318		1.94318		1.94318		2.13184 7		1.94318		1.94318		1.94318		1.94318		1.94318	
P(T<=t) two-tail	0.360012		0.107129		0.289625		0.323499		1		0.378955		0.06676 7		0.14186		0.22212 2		0.57139		1		0.47790 8	
t Critical two-tail	2.446912		2.570582		2.570582		2.446912		2.446912		2.446912		2.77644 5		2.446912		2.446912		2.446912		2.446912		2.446912	
1B1-12	1B1	2E.1 (WT)	1B2	2E.1 (WT)	1B3	2E.1 (WT)	1B4	2E.1 (WT)	1B5	2E.1 (WT)	1B6	2E.1 (WT)	1B7	2E.1 (WT)	1B8	2E.1 (WT)	1B9	2E.1 (WT)	1B10	2E.1 (WT)	1B11	2E.1 (WT)	1B12	2E.1 (WT)
Replicate1	10	5	8	5	10	5	9	5	9	5	10	5	10	5	8	5	7	5	6	5	5	5	4	5
Replicate2	10	10	13	10	8	10	9	10	8	10	11	10	13	10	13	10	12	10	7	10	8	10	12	10
Replicate3	7	9	10	9	9	9	8	9	12	9	9	9	12	9	11	9	7	9	4	9	10	9	3	9
Replicate4	8	7	9	7	0	7	13	7	10	7	9	7	5	7	10	7	7	7	5	7	9	7	4	7
Mean	8.75	7.75	10	7.75	6.75	7.75	9.75	7.75	9.75	7.75	9.75	7.75	10	7.75	10.5	7.75	8.25	7.75	5.5	7.75	8	7.75	5.75	7.75
Variances	2.25	4.916667	4.666667	4.916667	20.91667	4.916667	4.916667	4.916667	2.916667	4.916667	0.916667	4.916667	12.66667	4.916667	4.333333	4.916667	6.25	4.916667	1.666667	4.916667	4.666667	4.916667	17.58333	4.916667
Observations	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	5		6		4		6		6		4		5		6		6		5		6		5	
t Stat	0.747087		1.453631		-0.3935		1.275586		1.429179		1.656157		1.073154		1.808389		0.299253		-1.75384		0.161515		-0.84327	
P(T<=t) one-tail	0.244316		0.098137		0.357013		0.12463		0.101442		0.086516		0.166121		0.060272		0.387421		0.069915		0.438495		0.218773	
t Critical one-tail	2.015048		1.94318		2.131847		1.94318		1.94318		2.131847		2.015048		1.94318		1.94318		2.015048		1.94318		2.015048	
P(T<=t) two-tail	0.488632		0.196273		0.714027		0.24926		0.202884		0.173031		0.332242		0.120544		0.774842		0.139829		0.877699		0.437545	
t Critical two-tail	2.570582		2.446912		2.776445		2.446912		2.446912		2.776445		2.570582		2.446912		2.446912		2.570582		2.446912		2.570582	
1C1-12	1C1	2E.1 (WT)	1C2	2E.1 (WT)	1C3	2E.1 (WT)	1C4	2E.1 (WT)	1C5	2E.1 (WT)	1C6	2E.1 (WT)	1C7	2E.1 (WT)	1C8	2E.1 (WT)	1C9	2E.1 (WT)	1C10	2E.1 (WT)	1C11	2E.1 (WT)	1C12	2E.1 (WT)
Replicate1	11	5	7	5	8	5	6	5	3	5	4	5	7	5	8	5	6	5	3	5	5	5	9	5
Replicate2	9	10	5	10	9	10	5	10	8	10	10	10	6	10	6	10	14	10	6	10	10	10	15	10
Replicate3	9	9	12	9	10	9	10	9	9	9	8	9	7	9	13	9	9	9	8	9	4	9	9	9
Replicate4	5	7	7	7	10	7	4	7	4	7	8	7	8	7	14	7	9	7	13	7	7	7	9	7
Mean	8.5	7.75	7.75	7.75	9.25	7.75	6.25	7.75	6	7.75	7.5	7.75	7	7.75	10.25	7.75	9.5	7.75	7.5	7.75	7	8.666667	10.5	7.75
Variances	6.333333	4.916667	8.916667	4.916667	0.916667	4.916667	6.916667	4.916667	8.666667	4.916667	6.333333	4.916667	0.666667	4.916667	14.91667	4.916667	11	4.916667	17.66667	4.916667	9	2.333333	9	4.916667
Observations	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	6		6		4		6		6		6		4		5		5		5		3		6	
t Stat	0.447214		0		1.242118		-0.8721		-0.94965		-0.14907		-0.63481		1.122722		0.877288		-0.10521		-0.85749		1.474331	
P(T<=t) one-tail	0.335206		0.5		0.141022		0.208341		0.189477		0.443191		0.280024		0.156277		0.210239		0.461488		0.227107		0.095416	

t Critical one-tail	1.943 18		1.943 18		2.131 847		1.943 18		1.94 318		1.943 18		2.13 184 7		2.01 504 8		2.01 504 8		2.01 504 8		2.35 336 3		1.94 318	
P(T<=t) two-tail	0.670 412		1		0.282 043		0.416 681		0.37 8955		0.886 382		0.56 004 7		0.31 255 5		0.42 047 8		0.92 029 6		0.45 421 5		0.19 083 3	
t Critical two-tail	2.446 912		2.446 912		2.776 445		2.446 912		2.44 6912		2.446 912		2.77 644 5		2.57 058 2		2.57 058 2		2.57 058 2		3.18 244 6		2.44 691 2	
1D1-12	**1D1**	**2E.1 (WT)**	**1D2**	**2E.1 (WT)**	**1D3**	**2E.1 (WT)**	**1D4**	**2E .1 (WT)**	**1D5**	**2E.1 (WT)**	**1D6**	**2E.1 (WT)**	**1D7**	**2E.1 (WT)**	**1D8**	**2E.1 (WT)**	**1D9**	**2E.1 (WT)**	**1D10**	**2E.1 (WT)**	**1D11**	**2E.1 (WT)**	**1D12**	**2E.1 (WT)**
Replicate1	10	5	6	5	10	5	10	5	8	5	13	5	10	5	0	5	11	5	10	5	11	5	8	5
Replicate2	13	10	12	10	8	10	9	10	10	10	10	10	11	10	1	10	14	10	10	10	12	10	9	10
Replicate3	15	9	10	9	7	9	5	9	7	9	10	9	14	9	2	9	15	9	7	9	14	9	10	9
Replicate4	7	7	10	7	12	7	13	7	9	7	8	7	10	7	0	7	13	7	8	7	10	7	9	7
Mean	10.66 667	8.6 666 67	9.5	7.7 5	9.25	7.7 5	9.25	7.7 5	8.5	7.75	10.25	7.7 5	11.2 5	7.75	1	8.66 6667	13.2 5	7.75	8.75	7.75	12	8.66 6667	9	7.75
Variances	10.33 333	2.3 333 33	6.333 333	4.9 166 67	4.916 667	4.9 16 66 7	10.91 667	4.9 16 66 7	1.66 6667	4.91 666 7	4.25	4.9 166 67	3.58 333 3	4.91 6667	1	2.33 3333	2.91 666 7	4.91 6667	2.25	4.91 6667	4	2.33 3333	0.66 666 7	4.91 6667
Observations	3	3	4	4	4	4	4	4	4	4	4	4	4	4	3	3	4	4	4	4	3	3	4	4
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	3		6		6		5		5		6		6		3		6		5		4		4	
t Stat	0.973 329		1.043 498		0.956 689		0.753 937		0.58 4613		1.651 446		2.40 098		-7.27 324		3.93 024 3		0.74 708 7		2.29 415 7		1.05 801 8	
P(T<=t) one-tail	0.201 089		0.168 463		0.187 833		0.242 428		0.29 2096		0.074 869		0.02 661 2		0.00 268 2		0.00 385 5		0.24 431 6		0.04 173 7		0.17 485 4	
t Critical one-tail	2.353 363		1.943 18		1.943 18		2.015 048		2.01 5048		1.943 18		1.94 318		2.35 336 3		1.94 318		2.01 504 8		2.13 184 7		2.13 184 7	
P(T<=t) two-tail	0.402 178		0.336 925		0.375 667		0.484 857		0.58 4192		0.149 737		0.05 322 4		0.00 536 4		0.00 771 1		0.48 863 2		0.08 347 4		0.34 970 9	
t Critical two-tail	3.182 446		2.446 912		2.446 912		2.570 582		2.57 0582		2.446 912		2.44 691 2		3.18 244 6		2.44 691 2		2.57 058 2		2.77 644 5		2.77 644 5	

1E1-3	**1E1.1**	**2E.1 (WT)**	**1E1.2**	**2E.1 (WT)**	**1E1.3**	**2E.1 (WT)**
Replicate1	6	5	8	5	10	5
Replicate2	6	10	11	10	9	10
Replicate3	6	9	5	9	11	9
Replicate4	8	7	11	7	11	7
Mean	6.5	7.7 5	8.75	7.7 5	10.25	7.7 5
Variances	1	4.9 166 67	8.25	4.9 166 67	0.916 667	4.9 16 66 7
Observations	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0	
df	4		6		4	
t Stat	-1.027 78		0.551 178		2.070 197	
P(T<=t) one-tail	0.181 069		0.300 709		0.053 603	
t Critical one-tail	2.131 847		1.943 18		2.131 847	
P(T<=t) two-tail	0.362 138		0.601 417		0.107 206	
t Critical two-tail	2.776 445		2.446 912		2.776 445	

2A1-12	**2A1**	**2E.1 (WT)**	**2A2**	**2E.1 (WT)**	**2A3**	**2E.1 (WT)**	**2A4**	**2E .1 (WT)**	**2A5**	**2E.1 (WT)**	**2A6**	**2E.1 (WT)**	**2A7**	**2E.1 (WT)**	**2A8**	**2E.1 (WT)**	**2A9**	**2E.1 (WT)**	**2A10**	**2E.1 (WT)**	**2A11**	**2E.1 (WT)**	**2A12**	**2E.1 (WT)**
	6	5	8	5	5	5	0	5	9	5	2	5	5	5	2	5	10	5	4	5	4	5	5	5
Replicate1	7	10	4	10	5	10	0	10	11	10	10	10	5	10	8	10	4	10	8	10	9	10	9	10
Replicate2	6	9	4	9	6	9	0	9	12	9	9	9	5	9	6	9	4	9	6	9	9	9	9	9
Replicate3	8	7	5	7	10	7	0	7	8	7	8	7	6	7	4	7	5	7	5	7	8	7	7	7
Replicate4	6.75	7.7 5	5.25	7.7 5	6.5	7.7 5	0	7.7 5	10	7.75	7.25	7.7 5	5.25	7.75	5	7.75	5.75	7.75	5.75	7.75	7.5	7.75	8.33 333 3	8.66 6667
Mean	0.916 667	4.9 166 67	3.583 333	4.9 166 67	5.666 667	4.9 16 66 7	0	4.9 16 66 7	3.33 3333	4.91 666 7	12.91 667	4.9 166 67	0.25	4.91 6667	6.66 666 7	4.91 6667	8.25	4.91 6667	2.91 666 7	4.91 6667	5.66 666 7	4.91 6667	1.33 333 3	2.33 3333
Variances	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3
Observations	0		0		0		0		0		0		0		0		0		0		0		0	
Hypothesized Mean Difference	4		6		6		3		6		5		3		6		6		6		6		4	
df	-0.828 08		-1.714 99		-0.768 47		-6.990 31		1.56 6699		-0.236 8		-2.19 971		-1.61 602		-1.10 236		-1.42 918		-0.15 369		-0.30 151	

t Stat	0.227086		0.06858		0.23568		0.003005		0.084114		0.411103		0.057602		0.078609		0.156275		0.101442		0.441445		0.389025	
P(T<=t) one-tail	2.131847		1.94318		1.94318		2.353363		1.94318		2.015048		2.353363		1.94318		1.94318		1.94318		1.94318		2.131847	
t Critical one-tail	0.454172		0.137171		0.471361		0.00601		0.168227		0.822207		0.115204		0.157218		0.312549		0.202884		0.88289		0.77805	
P(T<=t) two-tail	2.776445		2.446912		2.446912		3.182446		2.446912		2.570582		3.182446		2.446912		2.446912		2.446912		2.446912		2.776445	
t Critical two-tail																								
2B1-12	**2B1**	**2E.1 (WT)**	**2B2**	**2E.1 (WT)**	**2B3**	**2E.1 (WT)**	**2B4**	**2E.1 (WT)**	**2B5**	**2E.1 (WT)**	**2B6**	**2E.1 (WT)**	**2B7**	**2E.1 (WT)**	**2B8**	**2E.1 (WT)**	**2B9**	**2E.1 (WT)**	**2B10**	**2E.1 (WT)**	**2B11**	**2E.1 (WT)**	**2B12**	**2E.1 (WT)**
	5	5	7	5	6	5	8	5	0	5	6	5	2	5	1	5	8	5	6	5	11	5	10	5
Replicate1	4	10	5	10	5	10	6	10	1	10	9	10	8	10	6	10	6	10	13	10	8	10	5	10
Replicate2	9	9	10	9	7	9	6	9	0	9	5	9	5	9	6	9	9	9	14	9	10	9	4	9
Replicate3	10	7	6	7	8	7	10	7	1	7	7	7	7	7	1	7	4	7	15	7	13	7	9	7
Replicate4	7.666667	8.666667	7	8.666667	6.5	7.75	7.5	7.75	0.5	7.75	6.75	7.75	5.5	7.75	3.5	7.75	6.333333	8.666667	12	7.75	10.5	7.75	6	8.666667
Mean	10.33333	2.333333	7	2.333333	1.666667	4.916667	3.666667	4.916667	0.333333	4.916667	2.916667	4.916667	7	4.916667	8.333333	4.916667	6.333333	2.333333	16.66667	4.916667	4.333333	4.916667	7	2.333333
Variances	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	3	3	4	4	4	4	3	3
Observations	0		0		0		0		0		0		0		0		0		0		0		0	
Hypothesized Mean Difference	3		3		5		6		3		6		6		6		3		5		6		3	
df	-0.48666		-0.94491		-0.97435		-0.17066		-6.32832		-0.71459		-1.30357		-2.33513		-1.37281		1.829615		1.808389		-1.51186	
t Stat	0.329918		0.207207		0.187315		0.435049		0.003989		0.250853		0.12008		0.029115		0.131716		0.063415		0.060272		0.113878	
P(T<=t) one-tail	2.353363		2.353363		2.015048		1.943318		2.353363		1.943318		1.94318		1.94318		2.353363		2.015048		1.94318		2.353363	
t Critical one-tail	0.659835		0.414414		0.37463		0.870098		0.007978		0.501705		0.24016		0.058231		0.263432		0.12683		0.120544		0.227757	
P(T<=t) two-tail	3.182446		3.182446		2.570582		2.446912		3.182446		2.446912		2.447691		2.447691		3.182446		2.570582		2.447691		3.182446	
t Critical two-tail																								
2C1-12	**2C1**	**2E.1 (WT)**	**2C2**	**2E.1 (WT)**	**2C3**	**2E.1 (WT)**	**2C4**	**2E.1 (WT)**	**2C5**	**2E.1 (WT)**	**2C6**	**2E.1 (WT)**	**2C7**	**2E.1 (WT)**	**2C8**	**2E.1 (WT)**	**2C9**	**2E.1 (WT)**	**2C10**	**2E.1 (WT)**	**2C11**	**2E.1 (WT)**	**2C12**	**2E.1 (WT)**
	6	5	6	5	9	5	6	5	0	5	8	5	4	5	4	5	5	5	7	5	9	5	5	5
Replicate1	9	10	8	10	7	10	5	10	1	10	5	10	6	10	7	10	7	10	6	10	6	10	6	10
Replicate2	9	9	4	9	2	9	9	9	2	9	6	9	6	9	7	9	10	9	8	9	8	9	10	9
Replicate3	8	7	5	7	9	7	9	7	3	7	7	7	5	7	6	7	4	7	7	7	8	7	6	7
Replicate4	8	7.75	5.75	7.75	6.75	7.75	7.25	7.75	1.5	7.75	6.5	7.75	5.25	7.75	6	7.75	7	8.666667	7	8.666667	7.75	7.75	6.75	7.75
Mean	2	4.916667	2.916667	4.916667	10.91667	4.916667	4.25	4.916667	1.666667	4.916667	1.666667	4.916667	0.916667	4.916667	2	4.916667	9	2.333333	1	2.333333	1.583333	4.916667	4.916667	4.916667
Variances	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3	3	3	4	4	4	4
Observations	0		0		0		0		0		0		0		0		0		0		0		0	
Hypothesized Mean Difference	5		6		5		6		5		5		4		5		3		3		5		6	
df	0.190117		-1.42918		-0.50262		-0.33029		-4.87177		-0.97435		-2.0702		-1.33082		-0.85749		-1.58114		0		-0.63779	
t Stat	0.428347		0.101442		0.318289		0.376205		0.002293		0.187315		0.053603		0.120353		0.227107		0.105993		0.5		0.272581	
P(T<=t) one-tail	2.015048		1.94318		2.015048		1.943318		2.015048		2.015048		2.131847		2.015048		2.353363		2.353363		2.015048		1.94318	
t Critical one-tail	0.856695		0.202884		0.636579		0.752409		0.004586		0.37463		0.107206		0.240706		0.450421		0.211985		1		0.541762	
P(T<=t) two-tail	2.570582		2.446912		2.570582		2.446912		2.570582		2.570582		2.776445		2.570582		3.182446		3.182446		2.570582		2.446912	
t Critical two-tail																								
2D1-12	**2D1**	**2E.1 (WT)**	**2D2**	**2E.1 (WT)**	**2D3**	**2E.1 (WT)**	**2D4**	**2E.1 (WT)**	**2D5**	**2E.1 (WT)**	**2D6**	**2E.1 (WT)**	**2D7**	**2E.1 (WT)**	**2D8**	**2E.1 (WT)**	**2D9**	**2E.1 (WT)**	**2D10**	**2E.1 (WT)**	**2D11**	**2E.1 (WT)**	**2D12**	**2E.1 (WT)**
Replicate1	6	5	6	5	0	5	6	5	10	5	5	5	7	5	6	5	3	5	7	5	7	5	0	5
Replicate2	6	10	7	10	1	10	7	10	8	10	8	10	5	10	9	10	6	10	8	10	8	10	1	10
Replicate3	8	9	8	9	0	9	8	9	9	9	14	9	9	9	6	9	7	9	7	9	6	9	0	9
Replicate4	6	7	0	7	0	7	5	7	5	7	6	7	7	7	6	7	6	7	0	7	10	7	0	7
Mean	6.5	7.75	5.25	7.75	0.333333	8.666667	6.5	7.75	8	7.75	8.25	7.75	7	7.75	6.75	7.75	5.5	7.75	5.5	7.75	8	8.666667	0.333333	8.666667
Variance	1	4.916667	12.91667	4.916667	0.333333	2.333	1.666667	4.916	4.666667	4.916667	16.25	4.916667	2.666667	4.916667	2.25	4.916667	3	4.916667	13.66667	4.916667	4	2.333333	0.333333	2.333333

						333		667																
Observations	4	4	4	4	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3	3	3
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	4		5		3		5		6		5		6		5		6		5		4		3	
t Stat	-1.02778		-1.18401		-8.83883		-0.97435		0.161515		0.217357		-0.5447		-0.74709		-1.59934		-1.04388		-0.45883		-8.83883	
P(T<=t) one-tail	0.181069		0.144813		0.001526		0.187315		0.438495		0.418261		0.302797		0.24316		0.080431		0.17218		0.33509		0.001526	
t Critical one-tail	2.131847		2.015048		2.353363		2.015048		1.94318		2.015048		1.94318		2.015048		1.94318		2.015048		2.131847		2.353363	
P(T<=t) two-tail	0.362138		0.289625		0.003052		0.37463		0.87699		0.836521		0.605594		0.48632		0.160861		0.34359		0.67018		0.003052	
t Critical two-tail	2.776445		2.570582		3.182446		2.570582				2.570582		2.446912		2.570582		2.446912		2.570582		2.776445		3.182446	

A4 (Reference strain)	**A4**	**2E.1 (WT)**
Replicate1	6	5
Replicate2	7	10
Replicate3	14	9
Replicate4	10	7
Mean	9.25	7.75
Variance	12.91667	4.916667
Observations	4	4
Hypothesized Mean Difference	0	
df	5	
t Stat	0.710403	
P(T<=t) one-tail	0.254603	
t Critical one-tail	2.015048	
P(T<=t) two-tail	0.509206	
t Critical two-tail	2.570582	

Appendix XIV. T-test assuming unequal variances for MAG+proline hole experiment.

Guide:

Strains showing abnormal apical branching phenotype

1A1-12	1A1	2E.1 (WT)	1A2	2E.1 (WT)	1A3	2E.1 (WT)	1A4	2E.1 (WT)	1A5	2E.1 (WT)	1A6	2E.1 (WT)	1A7	2E.1 (WT)	1A8	2E.1 (WT)	1A9	2E.1 (WT)	1A10	2E.1 (WT)	1A11	2E.1 (WT)	1A12	2E.1 (WT)
Replicate1	43	23	16	23	16	23	22	23	31	23	20	23	10	23	30	23	33	23	35	23	44	23	37	23
Replicate2	34	25	17	25	24	25	15	25	33	25	24	25	11	25	21	25	30	25	25	25	22	25	20	25
Replicate3	44	22	25	22	20	22	25	22	25	22	20	22	18	22	23	22	25	22	21	22	21	22	39	22
Replicate4	44	28	17	28	14	28	20	28	30	28	21	28	24	28	25	28	25	28	24	28	25	28	30	28
Mean	40.6667	25	19.6667	25	19.3333	25	20	25	29.3333	25	21.6667	25	17.6667	25	23	25	26.6667	25	23.3333	25	22.6667	25	29.6667	25
Variances	33.3333	9	21.3333	9	25.3333	9	25	9	16.3333	9	4.33333	9	42.3333	9	4	9	8.33333	9	4.33333	9	4.33333	9	90.3333	9
Observations	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	3		3		3		3		4		4		3		3		4		4		4		2	
t Stat	4.17057		-1.67726		-1.67506		-1.48522		1.491202		-1.58114		-1.77281		-0.96077		0.693375		-0.79057		-1.10068		0.810998	
P(T<=t) one-tail	0.012547		0.096043		0.096258		0.117081		0.105087		0.094502		0.087186		0.203772		0.263116		0.236714		0.165231		0.251266	
t Critical one-tail	2.353363		2.353363		2.353363		2.353363		2.131847		2.131847		2.353363		2.353363		2.131847		2.131847		2.131847		2.919986	
P(T<=t) two-tail	0.025095		0.192086		0.192516		0.234161		0.210174		0.189004		0.174373		0.407544		0.526232		0.473427		0.330046		0.502532	
t Critical two-tail	3.182446		3.182446		3.182446		3.182446		2.776445		2.776445		3.182446		3.182446		2.776445		2.776445		2.776445		4.302653	

1B1-12	1B1	2E.1 (WT)	1B2	2E.1 (WT)	1B3	2E.1 (WT)	1B4	2E.1 (WT)	1B5	2E.1 (WT)	1B6	2E.1 (WT)	1B7	2E.1 (WT)	1B8	2E.1 (WT)	1B9	2E.1 (WT)	1B10	2E.1 (WT)	1B11	2E.1 (WT)	1B12	2E.1 (WT)
Replicate1	17	23	41	23	25	23	24	23	20	23	10	23	20	23	16	23	20	23	20	23	16	23	26	23
Replicate2	17	25	21	25	22	25	25	25	40	25	17	25	31	25	22	25	31	25	16	25	20	25	20	25
Replicate3	25	22	29	22	19	22	29	22	25	22	25	22	26	22	18	22	30	22	23	22	24	22	24	22
Replicate4	20	28	24	28	8	28	25	28	30	28	20	28	30	28	25	28	26	28	17	28	15	28	20	28
Mean	20.6667	25	24.6667	25	16.3333	25	26.3333	25	31.6667	25	20.6667	25	29	25	21.6667	25	29	25	18.6667	25	19.6667	25	21.3333	25
Variances	16.3333	9	16.3333	9	54.3333	9	5.33333	9	58.3333	9	16.3333	9	7	9	12.3333	9	7	9	14.3333	9	20.3333	9	5.33333	9
Observations	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	4		4		3		4		3		4		4		4		4		4		3		4	
t Stat	-1.49 12		-0.11471		-1.88624		0.609994		1.401719		-1.49 12		1.732051		-1.25		1.732051		-2.27093		-1.70561		-1.67748	
P(T<=t) one-tail	0.105087		0.457102		0.078666		0.287741		0.127704		0.105087		0.079151		0.139972		0.079151		0.042282		0.093313		0.084376	
t Critical one-tail	2.131847		2.131847		2.353363		2.131847		2.353363		2.131847		2.131847		2.131847		2.131847		2.131847		2.353363		2.131847	
P(T<=t) two-tail	0.210174		0.914204		0.157332		0.574819		0.254408		0.210174		0.158302		0.279944		0.158302		0.085639		0.186625		0.168752	
t Critical two-tail	2.776445		2.776445		3.182446		2.776445		3.182446		2.776445		2.776445		2.776445		2.776445		2.776445		3.182446		2.776445	

1C1-12	1C1	2E.1 (WT)	1C2	2E.1 (WT)	1C3	2E.1 (WT)	1C4	2E.1 (WT)	1C5	2E.1 (WT)	1C6	2E.1 (WT)	1C7	2E.1 (WT)	1C8	2E.1 (WT)	1C9	2E.1 (WT)	1C10	2E.1 (WT)	1C11	2E.1 (WT)	1C12	2E.1 (WT)
Replicate1	44	23	23	23	26	23	18	23	17	23	33	23	25	23	34	23	28	23	23	23	40	23	28	23
Replicate2	30	25	23	25	37	25	23	25	44	25	19	25	20	25	34	25	22	25	23	25	17	25	28	25
Replicate3	76	22	25	22	40	22	19	22	38	22	27	22	24	22	22	22	24	22	16	22	3	22	29	22
Replicate4	33	28	30	28	26	28	20	28	27	28	30	28	16	28	34	28	20	28	23	28	30	28	28	28
Mean	46.3333	25	26	25	34.3333	25	20.6667	25	36.3333	25	25.3333	25	20	25	30	25	22	25	20.6667	25	16.6667	25	28.3333	25
Variances	662.333 3	9	13	9	54.3333	9	4.33333	9	74.3333	9	32.3333	9	16	9	48	9	4	9	16.3333	9	182.333 3	9	0.33333	9
Observations	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	2		4		3		4		2		3		4		3		3		4		2		2	
t Stat	1.42610 1		0.36927 4		2.03133 4		-2.05548		2.150349		0.08980 3		-1.73205		1.14707 9		-1.44115		-1.49 12		-1.04348		1.88982 2	
P(T<=t) one-tail	0.14497		0.36532		0.06758 5		0.05450 5		0.08224 8		0.46705 2		0.07915 1		0.16726 9		0.12259 7		0.10508 7		0.20313 7		0.09968	

t Critical one-tail	2.91998 6		2.13184 7		2.35336 3		2.13184 7		2.91998 6		2.35336 3		2.13184 7		2.35336 3		2.35336 3		2.13184 7		2.91998 6		2.91998 6	
P(T<=t) two-tail	0.28994		0.73064		0.13517		0.10901		0.16449 6		0.93410 4		0.15830 2		0.33453 8		0.24519 4		0.21017 4		0.40627 5		0.19935 9	
t Critical two-tail	4.30265 3		2.77644 5		3.18244 6		2.77644 5		4.30265 3		3.18244 6		2.77644 5		3.18244 6		3.18244 6		2.77644 5		4.30265 3		4.30265 3	
1D1-12	**1D1**	**2E.1 (WT)**	**1D2**	**2E.1 (WT)**	**1D3**	**2E.1 (WT)**	**1D4**	**2E.1 (WT)**	**1D5**	**2E.1 (WT)**	**1D6**	**2E.1 (WT)**	**1D7**	**2E.1 (WT)**	**1D8**	**2E.1 (WT)**	**1D9**	**2E.1 (WT)**	**1D10**	**2E.1 (WT)**	**1D11**	**2E.1 (WT)**	**1D12**	**2E.1 (WT)**
Replicate1	22	23	21	23	22	23	17	23	8	23	20	23	25	23	15	23	13	23	24	23	18	23	23	23
Replicate2	23	25	20	25	20	25	15	25	59	25	42	25	40	25	0	25	48	25	31	25	19	25	14	25
Replicate3	23	22	23	22	28	22	25	22	12	22	14	22	22	22	6	22	25	22	22	22	20	22	25	22
Replicate4	23	28	23	28	22	28	25	28	15	28	18	28	22	28	15	28	25	28	22	28	25	28	13	28
Mean	23	25	22	25	23.3333 3	25	21.6666 7	25	28.6666 7	25	24.6666 7	25	28	25	7	25	32.6666 7	25	25	25	21.3333 3	25	17.3333 3	25
Variances	0	9	3	9	17.3333 3	9	33.3333 3	9	692.3333 3	9	229.3333 3	9	108	9	57	9	176.3333 3	9	27	9	10.3333 3	9	44.3333 3	9
Observations	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	
df	2		3		4		3		2		2		2		3		2		3		4		3	
t Stat	-1.15547		-1.5		-0.56254		-0.88736		0.23981 1		-0.0374		0.48038 4		-3.83761		0.97541 7		0		-1.44437		-1.81831	
P(T<=t) one-tail	0.18377 2		0.11529 2		0.30188 9		0.22012 4		0.41640 7		0.48678 2		0.33918 3		0.0156		0.21611 4		0.5		0.11106 8		0.08330 1	
t Critical one-tail	2.91998 6		2.35336 3		2.13184 7		2.35336 3		2.91998 6		2.91998 6		2.91998 6		2.35336 3		2.91998 6		2.35336 3		2.13184 7		2.35336 3	
P(T<=t) two-tail	0.36754 4		0.23058 4		0.60377 8		0.44024 8		0.83281 4		0.97356 5		0.67836 6		0.0312		0.43222 9		1		0.22213 6		0.16660 2	
t Critical two-tail	4.30265 3		3.18244 6		2.77644 5		3.18244 6		4.30265 3		4.30265 3		4.30265 3		3.18244 6		4.30265 3		3.18244 6		2.77644 5		3.18244 6	

1E1-3	**1E1.1**	**2E.1 (WT)**	**1E1.2**	**2E.1 (WT)**	**1E1.3**	**2E.1 (WT)**
Replicate1	27	23	20	23	22	23
Replicate2	20	25	24	25	27	25
Replicate3	18	22	16	22	24	22
Replicate4	24	28	25	28	19	28
Mean	20.6666 7	25	21.6666 7	25	23.3333 3	25
Variances	9.33333 3	9	24.3333 3	9	16.3333 3	9
Observations	3	3	3	3	3	3
Hypothesized Mean Difference	0		0		0	
df	4		3		4	
t Stat	-1.75292		-1		-0.57354	
P(T<=t) one-tail	0.07724 4		0.19550 1		0.29849 2	
t Critical one-tail	2.13184 7		2.35336 3		2.13184 7	
P(T<=t) two-tail	0.15448 9		0.39100 2		0.59698 5	
t Critical two-tail	2.77644 5		3.18244 6		2.77644 5	

2A1-12	**2A1**	**2E.1 (WT)**	**2A2**	**2E.1 (WT)**	**2A3**	**2E.1 (WT)**	**2A4**	**2E.1 (WT)**	**2A5**	**2E.1 (WT)**	**2A6**	**2E.1 (WT)**	**2A7**	**2E.1 (WT)**	**2A8**	**2E.1 (WT)**	**2A9**	**2E.1 (WT)**	**2A10**	**2E.1 (WT)**	**2A11**	**2E.1 (WT)**	**2A12**	**2E.1 (WT)**
	30	23	30	23	27	23	1	23	35	23	30	23	21	23	23	23	28	23	23	23	15	23	15	23
Replicate1	25	25	16	25	13	25	0	25	24	25	25	25	30	25	15	25	23	25	32	25	24	25	16	25
Replicate2	15	22	25	22	25	22	2	22	17	22	40	22	10	22	18	22	19	22	25	22	28	22	25	22
Replicate3	20	28	25	28	23	28	1	28	25	28	40	28	27	28	28	28	18	28	23	28	26	28	19	28
Replicate4	20	25	22	25	20.3333 3	25	1	25	22	25	35	25	22.3333 3	25	20.3333 3	25	20	25	26.6666 7	25	26	25	20	25
Mean	25	9	27	9	41.3333 3	9	1	9	19	9	75	9	116.3333 3	9	46.3333 3	9	7	9	22.3333 3	9	4	9	21	9
Variances	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Observations	0		0		0		0		0		0		0		0		0		0		0		0	
Hypothesized Mean Difference	3		3		3		2		4		2		2		3		4		3		3		3	
df	-1.48522		-0.86603		-1.1393		-13.1453		-0.98198		1.88982 2		-0.41257		-1.08661		-2.16506		0.51571 1		0.48038 4		-1.58114	

t Stat	0.11708 1		0.22509 2		0.16865		0.00286 9		0.19085 4		0.09968		0.35997 2		0.17835 8		0.04817 1		0.32082 8		0.33190 4		0.10599 3	
P(T<=t) one-tail	2.35336 3		2.35336 3		2.35336 3		2.91998 6		2.13184 7		2.91998 6		2.91998 6		2.35336 3		2.13184 7		2.35336 3		2.35336 3		2.35336 3	
t Critical one-tail	0.23416 1		0.45018 5		0.33731 1		0.00573 7		0.38170 7		0.19935 9		0.71994 4		0.35671 6		0.09634 1		0.64165 5		0.66380 8		0.21198 5	
P(T<=t) two-tail	3.18244 6		3.18244 6		3.18244 6		4.30265 3		2.77644 5		4.30265 3		4.30265 3		3.18244 6		2.77644 5		3.18244 6		3.18244 6		3.18244 6	
t Critical two-tail																								
2B1-12	**2B1**	**2E.1 (WT)**	**2B2**	**2E.1 (WT)**	**2B3**	**2E.1 (WT)**	**2B4**	**2E.1 (WT)**	**2B5**	**2E.1 (WT)**	**2B6**	**2E.1 (WT)**	**2B7**	**2E.1 (WT)**	**2B8**	**2E.1 (WT)**	**2B9**	**2E.1 (WT)**	**2B10**	**2E.1 (WT)**	**2B11**	**2E.1 (WT)**	**2B12**	**2E.1 (WT)**
	25	23	24	23	16	23	30	23	25	23	10	23	31	23	20	23	36	23	24	23	20	23	32	23
Replicate1	34	25	25	25	14	25	27	25	23	25	20	25	20	25	35	25	22	25	30	25	32	25	18	25
Replicate2	23	22	15	22	15	22	22	22	25	22	18	22	26	22	32	22	20	22	25	22	25	22	32	22
Replicate3	33	28	15	28	28	28	30	28	27	28	23	28	26	28	25	28	20	28	30	28	25	28	32	28
Replicate4	30	25	18.3333 3	25	19	25	26.3333 3	25	25	25	20.3333 3	25	24	25	30.6666 7	25	20.6666 7	25	28.3333 3	25	27.3333 3	25	27.3333 3	25
Mean	37	9	33.3333 3	9	61	9	16.3333 3	9	4	9	6.33333 3	9	12	9	26.3333 3	9	1.33333 3	9	8.33333 3	9	16.3333 3	9	65.3333 3	9
Variances	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Observations	0		0		0		0		0		0		0		0		0		0		0		0	
Hypothesized Mean Difference	3		3		3		4		3		4		4		3		3		4		4		3	
df	1.27688 5		-1.77471		-1.24212		0.45883 1		0		-2.06419		-0.37796		1.65118 6		-2.33487		1.38675		0.80295 5		0.46875 5	
t Stat	0.14575 5		0.08702		0.15122 3		0.33509		0.5		0.05396 9		0.36232 9		0.09863 3		0.05084 8		0.11889 8		0.2335		0.33560 2	
P(T<=t) one-tail	2.35336 3		2.35336 3		2.35336 3		2.13184 7		2.35336 3		2.13184 7		2.13184 7		2.35336 3		2.35336 3		2.13184 7		2.13184 7		2.35336 3	
t Critical one-tail	0.29151		0.17404		0.30244 5		0.67018		1		0.10793 9		0.72465 9		0.19726 7		0.10169 6		0.23779 6		0.467		0.67120 5	
P(T<=t) two-tail	3.18244 6		3.18244 6		3.18244 6		2.77644 5		3.18244 6		2.77644 5		2.77644 5		3.18244 6		3.18244 6		2.77644 5		2.77644 5		3.18244 6	
t Critical two-tail																								
2C1-12	**2C1**	**2E.1 (WT)**	**2C2**	**2E.1 (WT)**	**2C3**	**2E.1 (WT)**	**2C4**	**2E.1 (WT)**	**2C5**	**2E.1 (WT)**	**2C6**	**2E.1 (WT)**	**2C7**	**2E.1 (WT)**	**2C8**	**2E.1 (WT)**	**2C9**	**2E.1 (WT)**	**2C10**	**2E.1 (WT)**	**2C11**	**2E.1 (WT)**	**2C12**	**2E.1 (WT)**
	16	23	28	23	15	23	20	23	18	23	25	23	17	23	30	23	30	23	32	23	15	23	25	23
Replicate1	17	25	23	25	25	25	15	25	21	25	30	25	20	25	18	25	16	25	20	25	26	25	30	25
Replicate2	28	22	33	22	19	22	28	22	32	22	23	22	17	22	28	22	28	22	22	22	27	22	26	22
Replicate3	19	28	30	28	20	28	25	28	28	28	30	28	25	28	18	28	14	28	20	28	14	28	26	28
Replicate4	21.3333 3	25	28.6666 7	25	21.3333 3	25	22.6666 7	25	27	25	27.6666 7	25	20.6666 7	25	21.3333 3	25	19.3333 3	25	20.6666 7	25	22.3333 3	25	27.3333 3	25
Mean	34.3333 3	9	26.3333 3	9	10.3333 3	9	46.3333 3	9	31	9	16.3333 3	9	16.3333 3	9	33.3333 3	9	57.3333 3	9	1.33333 3	9	52.3333 3	9	5.33333 3	9
Variances	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Observations	0		0		0		0		0		0		0		0		0		0		0		0	
Hypothesized Mean Difference	3		3		4		3		3		4		4		3		3		3		3		4	
df	-0.96476		1.06841 4		-1.44437		-0.54331		0.54772 3		0.91766 3		-1.49 12		-0.97609		-1.20 51		-2.33487		-0.58977		1.06749	
t Stat	0.20291 5		0.18183 5		0.11106 8		0.31234 5		0.31100 1		0.20536		0.10508 7		0.20050 3		0.15727 8		0.05084 8		0.29841		0.17294 7	
P(T<=t) one-tail	2.35336 3		2.35336 3		2.13184 7		2.35336 3		2.35336 3		2.13184 7		2.13184 7		2.35336 3		2.35336 3		2.35336 3		2.35336 3		2.13184 7	
t Critical one-tail	0.40583		0.36366 9		0.22213 6		0.62469		0.62200 2		0.41072		0.21017 4		0.40100 7		0.31455 6		0.10169 6		0.59681 9		0.34589 4	
P(T<=t) two-tail	3.18244 6		3.18244 6		2.77644 5		3.18244 6		3.18244 6		2.77644 5		2.77644 5		3.18244 6		3.18244 6		3.18244 6		3.18244 6		2.77644 5	
t Critical two-tail																								
2D1-12	**2D1**	**2E.1 (WT)**	**2D2**	**2E.1 (WT)**	**2D3**	**2E.1 (WT)**	**2D4**	**2E.1 (WT)**	**2D5**	**2E.1 (WT)**	**2D6**	**2E.1 (WT)**	**2D7**	**2E.1 (WT)**	**2D8**	**2E.1 (WT)**	**2D9**	**2E.1 (WT)**	**2D10**	**2E.1 (WT)**	**2D11**	**2E.1 (WT)**	**2D12**	**2E.1 (WT)**
Replicate1	35	23	24	23	2	23	17	23	30	23	22	23	22	23	34	23	34	23	26	23	26	23	1	23
Replicate2	22	25	28	25	0	25	30	25	24	25	23	25	23	25	20	25	20	25	30	25	13	25	0	25
Replicate3	20	22	16	22	1	22	25	22	23	22	20	22	21	22	27	22	21	22	22	22	28	22	0	22
Replicate4	20	28	20	28	1	28	25	28	23	28	20	28	21	28	27	28	21	28	22	28	15	28	0	28
Mean	20.6666 7	25	21.3333 3	25	0.66666 7	25	26.6666 7	25	23.3333 3	25	21	25	21.6666 7	25	24.6666 7	25	20.6666 7	25	24.6666 7	25	18.6666 7	25	0	25
Variance	1.33333 3	9	37.3333 3	9	0.33333 3	9	8.33333 3	9	0.33333 3	9	3	9	1.33333 3	9	16.3333 3	9	0.33333 3	9	21.3333 3	9	66.3333 3	9	0	9
Observations	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Hypothesized Mean Difference	0		0		0		0		0		0		0		0		0		0		0		0	

df	3		3		2		4		2		3		3		4		2		3		3		2	
t Stat	-2.33487		-0.93301		-13.7957		0.693375		-0.94491		-2		-1.79605		-0.11471		-2.45677		-0.10483		-1.26386		-14.4338	
P(T<=t) one-tail	0.050848		0.20982		0.002607		0.263116		0.222222		0.069663		0.085176		0.457102		0.066667		0.461564		0.147779		0.002383	
t Critical one-tail	2.353363		2.353363		2.919986		2.131847		2.919986		2.353363		2.353363		2.131847		2.919986		2.353363		2.353363		2.919986	
P(T<=t) two-tail	0.101696		0.419641		0.005213		0.526232		0.444444		0.139326		0.170352		0.914204		0.133333		0.923128		0.295557		0.004766	
t Critical two-tail	3.182446		3.182446		4.302653		2.776445		4.302653		3.182446		3.182446		2.776445		4.302653		3.182446		3.182446		4.302653	

A4 (Reference strain)	**A4**	**2E.1 (WT)**
Replicate1	24	23
Replicate2	30	25
Replicate3	22	22
Replicate4	26	28
Mean	26	25
Variance	16	9
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	0.34641	
P(T<=t) one-tail	0.373245	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.746489	
t Critical two-tail	2.776445	