



# PATENT SPECIFICATION

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(54) Title:                            Ace2 as a target gene for the molecular identification of yeast and fungal species.

(72) Inventor:                    THOMAS GERARD BARRY  
   TERRY JAMES SMITH  
   MAJELLA MAHER  
   MARCIN JANKIEWICZ  
   LOUISE O'CONNOR  
   NINA TUIITE  
   SINEAD LAHIFI

(73) Patent Granted to:        NATIONAL UNIVERSITY OF IRELAND, GALWAY, a body incorporated by statute, University Road Galway, Ireland

**Title**

Ace2 as a target gene for the molecular identification of yeast and fungal species.

**Field of the Invention**

5 The present invention relates to nucleic acid primers and probes for use in the identification of one or more yeast species. More specifically the invention relates to the Ace2 gene, the corresponding RNA, specific probes, primers and oligonucleotides related thereto and their use in diagnostic assays to detect and / or discriminate between yeast species.

**Background to the Invention**

10 Yeast and fungal infections represent a major cause of morbidity and mortality among immunocompromised patients. The number of immunocompromised patients at risk of yeast and fungal infection continues to increase each year, as does the spectrum of fungal and yeast agents causing disease. Mortality from fungal infections, particularly invasive fungal infections, is 30% or greater in certain risk groups. The array of  
15 available anti-fungal agents is growing; however, so too is the recognition of both intrinsic and emerging resistance to antifungal drugs. These factors are contributing to the increased need for cost containment in laboratory testing and have led to laboratory consolidation in testing procedures.

Invasive fungal infections are on the increase. In 2003, it was estimated that there  
20 were 9 million at risk patients of which 1.2 million developed infection. *Candida* species now rank as the most prominent pathogens infecting immunosuppressed patients. In particular, infections are common in the urinary tract, the respiratory system and the bloodstream, at the site of insertion of stents, catheters and orthopaedic joints. Approximately 10% of the known *Candida* spp. have been implicated in human  
25 infection. Invasive candidiasis occurs when candida enters the bloodstream and it is estimated to occur at a frequency of 8/100,000 population in the US with a mortality rate of 40%. *Candida albicans* is the 4<sup>th</sup> most common cause of bloodstream infection. Emerging mycoses agents include *Fusarium*, *Scedosporium*, *Zygomycetes* and *Trichosporon* spp. ("*Stakeholder Insight: Invasive fungal infections*", Datamonitor, Jan  
30 2004).

Immunocompromised patients including transplant and surgical patients, neonates, cancer patients, diabetics and those with HIV/AIDs are at high risk of developing invasive fungal infections (Datamonitor report: Stakeholder opinion -Invasive

fungal infections, options outweigh replacements 2004). A large number of severe cases of sepsis are reported each year. Despite improvements in its medical management, sepsis still constitutes one of the greatest challenges in intensive care medicine. Microorganisms (bacteria, fungi and yeast) responsible for causing sepsis are traditionally detected in hospital laboratories with the aid of microbiological culture methods with poor sensitivity (25-82%), which are very time-consuming, generally taking from two to five days to complete, and up to eight days for the diagnosis of fungal infections.

Definitive diagnosis of infection caused by yeast or fungus is usually based on either, the recovery and identification of a specific agent from clinical specimens or microscopic demonstration of fungi or yeasts with distinct morphological features. However, there are numerous cases where these methods fail to provide conclusive proof as to the infecting agent. In these instances, the detection of specific host antibody responses can be used, although again this can be affected by the immune status of the patient. Time is critical in the detection and identification of bloodstream infections typically caused by bacteria, yeast or fungi. Effective treatment depends on finding the source of infection and making appropriate decisions about antibiotics or antifungals quickly and efficiently.

Fungal and yeast nucleic acid based diagnostics have focused heavily on the ribosomal RNA (rRNA) genes, RNA transcripts, and their associated DNA / RNA regions. The rRNA genes are highly conserved in all fungal species and they also contain divergent and distinctive intergenic transcribed spacer regions. Ribosomal rRNA comprises three genes: the large sub-unit gene (28S), the small sub-unit gene (18S) and the 5.8S gene. The 28S and 18S rRNA genes are separated by the 5.8S rRNA and two internal transcribed spacers (ITS1 and ITS2). Because the ITS region contains a high number of sequence polymorphisms, numerous researchers have concentrated their efforts on these as targets (Atkins and Clark, 2004). rRNA genes are also multicopy genes with >10 copies within the fungal genome.

A number of groups are working on developing new assays for fungal and yeast infections. US2004044193 relates to, amongst a number of other aspects, the transcription factor CaTEC1 of *Candida albicans*; inhibitors thereof, and methods for the diagnosis and therapy of diseases which are connected with a *Candida* infection; and also diagnostic and pharmaceutical compositions which contain the nucleotide sequences, proteins, host cells and/or antibodies. WO0183824 relates to hybridization

assay probes and accessory oligonucleotides for detecting ribosomal nucleic acids from *Candida albicans* and/or *Candida dubliniensis*. US6017699 and US5426026 relate to a set of DNA primers, which can be used to amplify and speciate DNA from five medically important *Candida* species. US 6747137 discloses sequences useful for diagnosis of *Candida* infections. EP 0422872 and US 5658726 disclose probes based on 18S rRNA genes, and US 5958693 discloses probes based on 28S rRNA, for diagnosis of a range of yeast and fungal species. US 6017366 describes sequences based on chitin synthase gene for use in nucleic acid based diagnostics for a range of *Candida* species.

It is clear though, that development of faster, more accurate diagnostic methods are required, particularly in light of the selection pressure caused by modern antimicrobial treatments which give rise to increased populations of resistant virulent strains with mutated genome sequences. Methods that enable early diagnosis of microbial causes of infection enable the selection of a specific narrow spectrum antibiotic or antifungal to treat the infection (Datamonitor report: Stakeholder opinion -Invasive fungal infections, options outweigh replacements 2004; Datamonitor report: Stakeholder Opinion-Sepsis, under reaction to an overreaction, 2006).

Only after pathogens are correctly identified can targeted therapy using a specific antibiotic or antifungal begin. Many physicians would like to see the development of better *in vitro* amplification and direct detection diagnostic techniques for the early diagnosis of yeast and fungi ("*Stakeholder Insight: Invasive fungal infections*", Datamonitor, Jan 2004). The present invention provides novel fungal and yeast nucleic acid targets for application in Nucleic Acid Diagnostics (NAD) tests. These are rapid, accurate diagnostic tests for clinically significant bacterial and fungal pathogens for bioanalysis applications in the clinical sector.

Ace2 is a DNA-binding cell cycle regulated transcription factor. Ace2 functions similarly to the transcription factor SW15, yet they activate distinct genes. The translated Ace2 protein is present in the nucleus in early G1 phase of the cell cycle and thus, specifically activates the expression of genes in the G1 phase. In particular, it activates the CTS1 gene. CTS1 is a chitinase-encoding gene required to degrade the cell wall between parent and daughter cells during cytokinesis. There are 7 Ace2 sequences publicly available in the NCBI GenBank database including 5 *Candida* spp. sequences. There are no published Ace2 sequences available for *Aspergillus* spp. The current inventors have designed PCR primers to amplify the region of Ace2 in *Candida*

*spp.* equivalent to base pair position 1736 to 2197 in *C. albicans*. This region of the *Ace2* gene has an application for the molecular identification and / or discrimination of yeast species.

### **Definitions**

5                    "Synthetic oligonucleotide" refers to molecules of nucleic acid polymers of 2 or more nucleotide bases that are not derived directly from genomic DNA or live organisms. The term synthetic oligonucleotide is intended to encompass DNA, RNA, and DNA/RNA hybrid molecules that have been manufactured chemically, or synthesized enzymatically *in vitro*.

10                    An "oligonucleotide" is a nucleotide polymer having two or more nucleotide subunits covalently joined together. Oligonucleotides are generally about 10 to about 100 nucleotides. The sugar groups of the nucleotide subunits may be ribose, deoxyribose, or modified derivatives thereof such as OMe. The nucleotide subunits may be joined by linkages such as phosphodiester linkages, modified linkages or by non-  
15 nucleotide moieties that do not prevent hybridization of the oligonucleotide to its complementary target nucleotide sequence. Modified linkages include those in which a standard phosphodiester linkage is replaced with a different linkage, such as a phosphorothioate linkage, a methylphosphonate linkage, or a neutral peptide linkage. Nitrogenous base analogs also may be components of oligonucleotides in accordance  
20 with the invention.

                    A "target nucleic acid" is a nucleic acid comprising a target nucleic acid sequence. A "target nucleic acid sequence," "target nucleotide sequence" or "target sequence" is a specific deoxyribonucleotide or ribonucleotide sequence that can be hybridized to a complementary oligonucleotide.

25                    An "oligonucleotide probe" is an oligonucleotide having a nucleotide sequence sufficiently complementary to its target nucleic acid sequence to be able to form a detectable hybrid probe:target duplex under high stringency hybridization conditions. An oligonucleotide probe is an isolated chemical species and may include additional nucleotides outside of the targeted region as long as such nucleotides do not  
30 prevent hybridization under high stringency hybridization conditions. Non-complementary sequences, such as promoter sequences, restriction endonuclease recognition sites, or sequences that confer a desired secondary or tertiary structure such as a catalytic active site can be used to facilitate detection using the invented probes. An

oligonucleotide probe optionally may be labelled with a detectable moiety such as a radioisotope, a fluorescent moiety, a chemiluminescent, a nanoparticle moiety, an enzyme or a ligand, which can be used to detect or confirm probe hybridization to its target sequence. Oligonucleotide probes are preferred to be in the size range of from about 10 to about 100 nucleotides in length, although it is possible for probes to be as much as and above about 500 nucleotides in length, or below 10 nucleotides in length.

A "hybrid" or a "duplex" is a complex formed between two single-stranded nucleic acid sequences by Watson-Crick base pairings or non-canonical base pairings between the complementary bases. "Hybridization" is the process by which two complementary strands of nucleic acid combine to form a double-stranded structure ("hybrid" or "duplex"). A "fungus" or "yeast" is meant any organism of the kingdom Fungi, and preferably, is directed towards any organism of the phylum Ascomycota.

"Complementarity" is a property conferred by the base sequence of a single strand of DNA or RNA which may form a hybrid or double-stranded DNA:DNA, RNA:RNA or DNA:RNA through hydrogen bonding between Watson-Crick base pairs on the respective strands. Adenine (A) ordinarily complements thymine (T) or uracil (U), while guanine (G) ordinarily complements cytosine (C).

The term "stringency" is used to describe the temperature, ionic strength and solvent composition existing during hybridization and the subsequent processing steps. Those skilled in the art will recognize that "stringency" conditions may be altered by varying those parameters either individually or together. Under high stringency conditions only highly complementary nucleic acid hybrids will form; hybrids without a sufficient degree of complementarity will not form. Accordingly, the stringency of the assay conditions determines the amount of complementarity needed between two nucleic acid strands forming a hybrid. Stringency conditions are chosen to maximize the difference in stability between the hybrid formed with the target and the non-target nucleic acid.

With "high stringency" conditions, nucleic acid base pairing will occur only between nucleic acid fragments that have a high frequency of complementary base sequences (for example, hybridization under "high stringency" conditions, may occur between homologs with about 85-100% identity, preferably about 70-100% identity). With medium stringency conditions, nucleic acid base pairing will occur between nucleic acids with an intermediate frequency of complementary base sequences (for

example, hybridization under “medium stringency” conditions may occur between homologs with about 50-70% identity). Thus, conditions of “weak” or “low” stringency are often required with nucleic acids that are derived from organisms that are genetically diverse, as the frequency of complementary sequences is usually less.

5           ‘High stringency’ conditions are those equivalent to binding or hybridization at 42° C. in a solution consisting of 5xSSPE (43.8g/l NaCl, 6.9 g/l NaH<sub>2</sub>PO<sub>4</sub>H<sub>2</sub>O and 1.85 g/l EDTA, ph adjusted to 7.4 with NaOH), 0.5% SDS, 5xDenhardt’s reagent and 100µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 0.1xSSPE, 1.0%SDS at 42° C. when a probe of about 500 nucleotides in length is used.

10           “Medium stringency’ conditions are those equivalent to binding or hybridization at 42° C. in a solution consisting of 5XSSPE (43.8 g/l NaCl, 6.9 g/l NaH<sub>2</sub>PO<sub>4</sub>H<sub>2</sub>O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5xDenhardt’s reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 1.0xSSPE, 1.0% SDS at 42° C, when a probe of about  
15 500 nucleotides in length is used.

          ‘Low stringency’ conditions are those equivalent to binding or hybridization at 42° C. in a solution consisting of 5xSSPE (43.8 g/l NaCl, 6.9 g/l NaH<sub>2</sub>PO<sub>4</sub>H<sub>2</sub>O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.1% SDS, 5xDenhardt’s reagent [50xDenhardt’s contains per 500ml: 5g Ficoll (Type 400, Pharamcia), 5 g BSA  
20 (Fraction V; Sigma)] and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 5xSSPE, 0.1% SDS at 42° C, when a probe of about 500 nucleotides in length is used.

          In the context of nucleic acid *in-vitro* amplification based technologies, “stringency” is achieved by applying temperature conditions and ionic buffer conditions  
25 that are particular to that *in-vitro* amplification technology. For example, in the context of PCR and real-time PCR, “stringency” is achieved by applying specific temperatures and ionic buffer strength for hybridisation of the oligonucleotide primers and, with regards to real-time PCR hybridisation of the probe/s, to the target nucleic acid for *in-vitro* amplification of the target nucleic acid.

30           One skilled in the art will understand that substantially corresponding probes of the invention can vary from the referred-to sequence and still hybridize to the same target nucleic acid sequence. This variation from the nucleic acid may be stated in terms of a percentage of identical bases within the sequence or the percentage of perfectly

complementary bases between the probe and its target sequence. Probes of the present invention substantially correspond to a nucleic acid sequence if these percentages are from about 100% to about 80% or from 0 base mismatches in about 10 nucleotide target sequence to about 2 bases mismatched in an about 10 nucleotide target sequence. In preferred embodiments, the percentage is from about 85% to about 100%. In more preferred embodiments, this percentage is from about 90% to about 100%; in other preferred embodiments, this percentage is from about 95% to about 100%, *e.g.* 95%, 96%, 97%, 98%, 99%, or 100%.

By "sufficiently complementary" or "substantially complementary" is meant nucleic acids having a sufficient amount of contiguous complementary nucleotides to form, under high stringency hybridization conditions, a hybrid that is stable for detection. Substantially complementary to can also refer to sequences with at least 90% identity to, *e.g.*, 95, 96, 97, 98, 99, or 100% identity to, a given reference sequence.

The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, about 60% identity, preferably 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (*see, e.g.*, NCBI web site at [ncbi.nlm.nih.gov/BLAST/](http://ncbi.nlm.nih.gov/BLAST/) or the like). Such sequences are then said to be "substantially identical." This definition also refers to, or may be applied to, the complement of a test sequence. The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. As described below, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is 50-100 amino acids or nucleotides in length.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are



designated, if necessary, and sequence algorithm program parameters are designated. Preferably, default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program  
5 parameters.

A "comparison window," as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of  
10 contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for  
15 similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (*see, e.g., Current Protocols in Molecular Biology* (Ausubel *et al.*, eds. 1987-2005, Wiley  
20 Interscience)).

A preferred example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, *Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990), respectively. BLAST and BLAST 2.0  
25 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either  
30 match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for

initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

“Nucleic acid” refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form, and complements thereof. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs)

By "nucleic acid hybrid" or "probe:target duplex" is meant a structure that is a double-stranded, hydrogen-bonded structure, preferably about 10 to about 100 nucleotides in length, more preferably 14 to 50 nucleotides in length, although this will depend to an extent on the overall length of the oligonucleotide probe. The structure is sufficiently stable to be detected by means such as chemiluminescent or fluorescent light detection, autoradiography, electrochemical analysis or gel electrophoresis. Such hybrids include RNA:RNA, RNA:DNA, or DNA:DNA duplex molecules.

"RNA and DNA equivalents" refer to RNA and DNA molecules having the same complementary base pair hybridization properties. RNA and DNA equivalents have different sugar groups (i.e., ribose versus deoxyribose), and may differ by the presence of uracil in RNA and thymine in DNA. The difference between RNA and DNA equivalents do not contribute to differences in substantially corresponding nucleic acid sequences because the equivalents have the same degree of complementarity to a particular sequence.

By "preferentially hybridize" is meant that under high stringency hybridization conditions oligonucleotide probes can hybridize their target nucleic acids to form stable probe:target hybrids (thereby indicating the presence of the target nucleic acids) without forming stable probe:non-target hybrids (that would indicate the presence of non-target nucleic acids from other organisms). Thus, the probe hybridizes to target nucleic acid to a sufficiently greater extent than to non-target nucleic acid to enable one skilled in the art to accurately detect the presence of (for example *Candida*) and distinguish these species from other organisms. Preferential hybridization can be measured using techniques known in the art and described herein.

By "theranostics" is meant the use of diagnostic testing to diagnose the disease, choose the correct treatment regime and monitor the patient response to therapy. The theranostics of the invention may be based on the use of an NAD assay of this invention on samples, swabs or specimens collected from the patient.

### **Object of the Invention**

It is an object of the current invention to provide sequences and/or diagnostic assays to detect and identify one or more yeast species. The current inventors have used the *Ace2* gene sequence to design primers and probes that are specific to *Candida Ace2* genes. Such primers not only allow the detection of yeast and fungal species but also allow distinction between *Candida* species. The current invention further provides for primers and probes that allow discrimination between different *Candida* species.

### **Summary of the Invention**

The present invention provides for a diagnostic kit for detection and identification of yeast species, comprising an oligonucleotide probe capable of binding to at least a portion of the *Ace2* gene or its corresponding mRNA. The oligonucleotide

probe may have a sequence substantially homologous to or substantially complementary to a portion of the Ace2 gene or its corresponding mRNA. It will thus be capable of binding or hybridizing with a complementary DNA or RNA molecule. The Ace2 gene may be yeast Ace2 gene. The nucleic acid molecule may be synthetic. The kit may  
5 comprise more than one such probe. In particular, the kit may comprise a plurality of such probes. In addition the kit may comprise additional probes for other organisms, such as, for example, bacterial species or viruses.

The identified sequences are suitable not only for *in vitro* DNA/RNA amplification based detection systems but also for signal amplification based detection  
10 systems. Furthermore, the sequences of the invention identified as suitable targets provide the advantages of having significant intragenic sequence heterogeneity in some regions, which is advantageous and enables aspects of the invention to be directed towards group or species-specific targets, and also having significant sequence  
15 homogeneity in some regions, which enables aspects of the invention to be directed towards genus-specific yeast and fungal primers and probes for use in direct nucleic acid detection technologies, signal amplification nucleic acid detection technologies, and nucleic acid *in vitro* amplification technologies for yeast and fungal diagnostics. The Ace2 sequences allows for multi-test capability and automation in diagnostic assays.

20 One of the advantages of the sequences of the present invention is that the intragenic Ace2 nucleotide sequence diversity between closely related yeast species enables specific primers and probes for use in diagnostics assays for the detection of yeast to be designed. The Ace2 nucleotide sequences, both DNA and RNA can be used with direct detection, signal amplification detection and *in vitro* amplification  
25 technologies in diagnostics assays. The Ace2 sequences allow for multi-test capability and automation in diagnostic assays.

The kit may further comprise a primer for amplification of at least a portion of the Ace2 gene. Suitably the kit comprises a forward and a reverse primer for a portion of the Ace2 gene.

30 The portion of the Ace2 gene may be equivalent to a portion of the region of the gene from base pair position 1736 to base pair position 2197 in *C. albicans*. Particularly preferred, are kits comprising a probe for a portion of the Ace2 *C. albicans* gene and / or a probe for a portion of the region of the gene equivalent to base pair position 1736 to

base pair position 2197 in *C. albicans*. Equivalent regions to base pair position 1736 to base pair position 2197 can be found in other organisms, but not necessarily in the same position.

5 The probe may preferentially hybridize to a portion of the Ace2 gene sequence selected from the group consisting of SEQ ID NOs: 4-7 and 32-39 or their corresponding mRNA.

The kit may also comprise additional probes. The probe may have a sequence selected from the group consisting of SEQ ID Nos: 3, 30, 31, and a sequence substantially homologous to or substantially complementary to those sequences, which  
10 can also act as a probe for the Ace2 gene.

It is desirable that the probe has a sequence as defined by SEQ ID NO: 30.

The kit may comprise at least one forward *in vitro* amplification primer and at least one reverse *in vitro* amplification primer, the forward amplification primer having a sequence selected from the group consisting of SEQ ID Nos: 1, 20-24, and sequences  
15 being substantially homologous or complementary thereto which can also act as a forward amplification primer, and the reverse amplification primer having a sequence selected from the group consisting of SEQ ID Nos: 2, 25-29, and a sequence being substantially homologous or complementary thereto which can also act as a reverse  
20 amplification primer. It is desirable that said forward primer sequence is selected from the group consisting of SEQ ID NOs: 21, 22, and 23, and said reverse primer sequence is selected from the group consisting of SEQ ID NOs: 27, 28, and 29. The diagnostic kit may be based on direct nucleic acid detection technologies, signal amplification nucleic acid detection technologies, and nucleic acid *in vitro* amplification technologies is  
25 selected from one or more of Polymerase Chain Reaction (PCR), Ligase Chain Reaction (LCR), Nucleic Acids Sequence Based Amplification (NASBA), Strand Displacement Amplification (SDA), Transcription Mediated Amplification (TMA), Branched DNA technology (bDNA) and Rolling Circle Amplification Technology (RCAT) ), or other *in vitro* enzymatic amplification technologies.

The invention also provides a nucleic acid molecule selected from the group  
30 consisting of SEQ ID NO.1 to SEQ ID NO. 40 and sequences substantially homologous thereto, or substantially complementary to a portion thereof and having a function in diagnostics based on the Ace2 gene.

The nucleic acid molecule may comprise an oligonucleotide having a sequence substantially homologous to or substantially complementary to a portion of a nucleic acid molecule of SEQ ID NO.1 to SEQ ID NO. 40. The invention also provides a method of detecting a target organism in a test sample comprising the steps of:

- 5 (i) Mixing the test sample with at least one oligonucleotide probe as defined above under appropriate conditions; and
- (ii) Hybridizing under high stringency conditions any nucleic acid that may be present in the test sample with the oligonucleotide to form a probe:target duplex; and
- 10 (iii) Determining whether a probe:target duplex is present; the presence of the duplex positively identifying the presence of the target organism in the test sample.

The nucleic acid molecule and kits of the present invention may be used in a diagnostic assay to detect the presence of one or more yeast and/or fungal species, to measure yeast and/or fungal titres in a patient or in a method of assessing the efficacy of a treatment regime designed to reduce yeast and/or fungal titre in a patient or to measure yeast and/or fungal contamination in an environment. The environment may be a hospital, or it may be a food sample, an environmental sample e.g. water, an industrial sample such as an in-process sample or an end product requiring bioburden or quality assessment.

20 The kits and the nucleic acid molecule of the invention may be used in the identification and/or characterization of one or more disruptive agents that can be used to disrupt the Ace2 gene function. The disruptive agent may be selected from the group consisting of antisense RNA, PNA, and siRNA.

In some embodiments of the invention, a nucleic acid molecule comprising a species-specific probe can be used to discriminate between species of the same genus.

25 The oligonucleotides of the invention may be provided in a composition for detecting the nucleic acids of yeast and fungal target organisms. Such a composition may also comprise buffers, enzymes, detergents, salts and so on, as appropriate to the intended use of the compositions. It is also envisioned that the compositions, kits and methods of the invention, while described herein as comprising at least one synthetic oligonucleotide, may also comprise natural oligonucleotides with substantially the same sequences as the synthetic nucleotide fragments in place of, or alongside synthetic oligonucleotides.

The invention also provides for an *in vitro* amplification diagnostic kit for a target yeast and/or fungal organism comprising at least one forward *in vitro* amplification primer and at least one reverse *in vitro* amplification primer, the forward amplification primer being selected from the group consisting of one or more of or a sequence being substantially homologous or complementary thereto which can also act as a forward amplification primer, and the reverse amplification primer being selected from the group consisting of one or more of or a sequence being substantially homologous or complementary thereto which can also act as a reverse amplification primer.

The invention also provides for a diagnostic kit for detecting the presence of candidate yeast and/or fungal species, comprising one or more DNA probes comprising a sequence substantially complementary to, or substantially homologous to the sequence of the Ace2 gene of the candidate yeast and/or fungal species. The present invention also provides for one or more synthetic oligonucleotides having a nucleotide sequence substantially homologous to or substantially complementary to one or more of the group consisting of the Ace2 gene or mRNA transcript thereof, the yeast Ace2 gene or mRNA transcript thereof, the yeast Ace2 gene or mRNA transcript thereof, one or more of SEQ ID NO 1-SEQ ID NO 40.

The nucleotide may comprise DNA. The nucleotide may comprise RNA. The nucleotide may comprise a mixture of DNA, RNA and PNA. The nucleotide may comprise synthetic nucleotides. The sequences of the invention (and the sequences relating to the methods, kits compositions and assays of the invention) may be selected to be substantially homologous to a portion of the coding region of the Ace2 gene. The gene may be a gene from a target yeast or fungal organism. The sequences of the invention are preferably sufficient so as to be able form a probe:target duplex to the portion of the sequence.

The invention also provides for a diagnostic kit for a target yeast or fungal organism comprising an oligonucleotide probe substantially homologous to or substantially complementary to an oligonucleotide of the invention (which may be synthetic). It will be appreciated that sequences suitable for use as *in vitro* amplification primers may also be suitable for use as oligonucleotide probes: while it is preferable that amplification primers may have a complementary portion of between about 15 nucleotides and about 30 nucleotides (more preferably about 15-about 23, most

preferably about 20 to about 23), oligonucleotide probes of the invention may be any suitable length. The skilled person will appreciate that different hybridization and or annealing conditions will be required depending on the length, nature & structure (eg. Hybridization probe pairs for LightCycler, Taqman 5' exonuclease probes, hairpin loop structures etc. and sequence of the oligonucleotide probe selected.

Kits and assays of the invention may also be provided wherein the oligonucleotide probe is immobilized on a surface. Such a surface may be a bead, a membrane, a column, dipstick, a nanoparticle, the interior surface of a reaction chamber such as the well of a diagnostic plate or inside of a reaction tube, capillary or vessel or the like.

The target yeast or fungal organism may be selected from the group consisting of *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. dubliniensis*, *C. guilliermondii*, *C. norvegiensis*, *C. famata*, *C. haemuloni*, *C. kefir*, *C. utilis*, *C. viswanathii* and *Aspergillus species*.

The target yeast organisms may be a *Candida* species for the given set of primers already experimentally demonstrated, and more preferably, selected from the group consisting of *C. albicans*, *C. tropicalis*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. guilliermondii*, *C. norvegiensis*, *C. famata*, *C. haemuloni*, *C. kefir*, *C. utilis*, *C. viswanathii*.

Under these circumstances, the amplification primers and oligonucleotide probes of the invention may be designed to a gene specific or genus specific region so as to be able to identify one or more, or most, or substantially all of the desired organisms of the target yeast organism grouping.

The test sample may comprise cells of the target yeast and/or fungal organism. The method may also comprise a step for releasing nucleic acid from any cells of the target yeast or fungal organism that may be present in said test sample. Ideally, the test sample is a lysate of an obtained sample from a patient (such as a swab, or blood, urine, saliva, a bronchial lavage, dental specimen, skin specimen, scalp specimen, transplant organ biopsy, stool, mucus, or discharge sample). The test samples may be a food sample, a water sample an environmental sample, an end product, end product or in-process industrial sample.

The invention also provides for the use of any one of SEQ ID NO.1 to SEQ ID NO. 40 in a diagnostic assay for the presence of one or more yeast species. The species



may be selected from the group consisting of *C. albicans*, *C. tropicalis*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. guilliermondii*, *C. norvegiensis*, *C. famata*, *C. haemuloni*, *C. kefir*, *C. utilis*, *C. viswanathii*.

The invention also provides for kits for use in clinical diagnostics, theranostics, food safety diagnostics, industrial microbiology diagnostics, environmental monitoring, veterinary diagnostics, bio-terrorism diagnostics comprising one or more of the synthetic oligonucleotides of the invention. The kits may also comprise one or more articles selected from the group consisting of appropriate sample collecting instruments, reagent containers, buffers, labelling moieties, solutions, detergents and supplementary solutions. The invention also provides for use of the sequences, compositions, nucleotide fragments, assays, and kits of the invention in theranostics, Food safety diagnostics, Industrial microbiology diagnostics, Environmental monitoring, Veterinary diagnostics, Bio-terrorism diagnostics.

The nucleic acid molecules, composition, kits or methods may be used in a diagnostic nucleic acid based assay for the detection of yeast species.

The nucleic acid molecules, composition, kits or methods may be used in a diagnostic assay to measure yeast or fungal titres in a patient. The titres may be measured *in vitro*.

The nucleic acid molecules, composition, kits or methods may be used in a method of assessing the efficacy of a treatment regime designed to reduce yeast and/or fungal titre in a patient comprising assessing the yeast and/or fungal titre in the patient (by *in vivo* methods or *in vitro* methods) at one or more key stages of the treatment regime. Suitable key stages may include before treatment, during treatment and after treatment. The treatment regime may comprise an antifungal agent, such as a pharmaceutical drug.

The nucleic acid molecules, composition, kits or methods may be used in a diagnostic assay to measure potential yeast and/or fungal contamination, for example, in a hospital.

The nucleic acid molecules, composition, kits or methods may be used in the identification and/or characterization of one or more disruptive agents that can be used to disrupt the Ace2 gene function. Suitable disruptive agents may be selected from the group consisting of antisense RNA, PNA, siRNA.

### **Brief description of the Figures**

**Figure 1:** Primers binding sites in (grey highlights) *Ace2* gene in *Candida albicans* (XM\_707274.1). The amplified region of interest is underlined (Position 1736 to 2197). Product size= 462 base pairs

5 **Figure 2:** Location of *C. albicans* probe P1-CanAce2 binding site (underlined and bolded in the amplified fragment of *Ace2* gene. PCR primers CanAce2-F/CanAce2-R are highlighted.

**Figure 3:** Real-time PCR assay for *C. albicans* based on the *Ace2* gene with TaqMan probe P1-CanAce2. Specificity of the assay was tested using a panel of DNA from four  
10 other *Candida* species and *Aspergillus fumigatus*. The three *C. albicans* strains tested were detected in the real-time PCR and there was no cross-reaction with DNA for other species.

**Figure 4:** Location of indicated *C. albicans* primers and probes. Figure 4(a) primer ACF1 is marked in bold, while primer ACR1 is marked in bold underline; Figure 4(b) primer ACF2 is marked in bold and primer ACR2 is marked in bold underline; Figure 4(c) primer  
15 ACF3 is marked in bold and primer ACR3 is marked in bold underline; and Figure 4(d) probe is marked in bold, ACALB1 on - strand, ACALB2 in + strand.

**Figure 5:** Primer (ACF2b/ACR3b) binding sites (grey highlights) and ACALB1 probe (bold text) in *Ace2* of *Candida albicans* (XM\_707274.1). The amplified region of interest is underlined. (Position of the region of interest: 1879-2004).

20 **Figure 6:** Inclusivity panel of *C. albicans* strains. Amplification plot from Real-time PCR assay for *C. albicans* based on the *Ace2* gene with TaqMan probe ACALB1 and primer set ACF2b/ACR3b. Specificity of the assay was tested using a panel of DNA from 20 *C. albicans* strains and 84 strains covering 19 spp. of clinically relevant and related *Candida*. (a) The 20 *C. albicans* strains tested were detected. (b) No cross-  
25 reaction was seen with DNA from 84 strains covering 19 other *Candida* species. Signal obtained only from (+) controls.

**Figure 7:** Exclusivity vaginitis/ vaginosis panel. Amplification plot from Real-time PCR assay for *C. albicans* based on the *Ace2* gene with TaqMan probe ACALB1 and primer set ACF2b/ACR3b. A signal was obtained only for (+) control (*C. albicans*)  
30 samples. No signal was obtained for the organisms in the exclusivity panel.

**Figure 8:** Amplification plot from Real-time PCR assay for *C. albicans* based on the *Ace2* gene with TaqMan probe ACALB1. Sensitivity of the assay was tested using

various inputs of template DNA from *C. albicans*. The LOD of the assay was found to be ~1 cell equivalent.

### **Detailed Description of the Drawings**

#### 5 **EXAMPLE 1**

##### **Materials and Methods**

##### **Cell Culture**

*Candida* species were cultured in Sabouraud broth (4% wt/vol glucose, 1% wt/vol peptone, 1.5 % agar) for 48 hours at 37°C in a shaking incubator.

#### 10 **DNA Extraction**

Cells from *Candida* spp. were pre-treated with lyticase or zymolase enzymes prior to DNA isolation. DNA was isolated from *Candida* spp. using either the MagNA Pure System (Roche Molecular Systems) in combination with the MagNA pure Yeast and Bacterial isolation kit III according to the manufacturers protocol, or the Qiagen

15 DNeasy Plant Mini kit (silica-based DNA purification in spin column format).

##### **DNA sequencing of Ace2 gene regions in *Candida* species**

The publicly available sequences of the Ace2 genes of *Candida* species were acquired from the NCBI database and aligned using ClustalW. The PCR Primer set, namely CanAce2-F/CanAce2-R (Figure 1, Table 1) was designed and used to amplify  
20 the Ace2 gene region in *Candida* spp. equivalent to base pair position 1736 to base pair position 2197 in *C. albicans* with primer set CanAce2-F/CanAce2-R. The Ace2 gene regions were amplified in *C. albicans*, *C. tropicalis* and *C. dubliniensis* by conventional PCR on the iCycler BioRad PCR machine or the PTC200 Peltier thermocycler (MJ Research) using the reagents outlined in Table 2 and the thermocycling conditions  
25 described in Table 3.

The PCR reaction products were purified with Roche High Pure PCR Product Purification kit or with the ExoSAP-IT kit (USB) according to the manufacturers' protocol and subsequently sent for sequencing to Sequiserve, Germany using the forward amplification primer CanAce2-F. DNA sequence information was generated for  
30 three *Candida* species. (*C. albicans*, *C. tropicalis* and *C. dubliniensis*).

**Table 1: PCR primers designed to amplify the Ace2 gene regions in *Candida* spp.**

Primer Name	Primer Sequence
CanAce2-F	TATCACCTTTGAAAAACAATTACC
CanAce2-R	CACCAACACAAATATTTTCGATC

5 **Table 2: Conventional PCR reagents used to amplify the Ace2 gene regions in *Candida* spp.**

PCR Reaction Mix	SAMPLE
	x 1
10 x Buffer(100 mM Tris HCl, 15 mM MgCl <sub>2</sub> , 500 mM KCl pH 8.3)	5 µl
dNTP's Mix, Roche (10mM dNTP)	1 µl
Primer Forward CanAce2-F (10µM)	1 µl
Primer Reverse CanAce2-R (10µM)	1 µl
Polymerase TaqPol, Roche 1U/µl	1 µl
H <sub>2</sub> O Amgen/Accugene	36-39 µl
Genomic DNA Template	2-5 µl
TOTAL VOLUME	50 µl

10 **Table 3: Conventional PCR reaction conditions applied to amplify the Ace2 gene regions in *Candida* spp.**

PCR Thermal profile			Lid preheating was ON
Step	Temp	Time	X 35
1	94°C	1 min	
2	53 or 50°C	1 min	
3	72°C	1 min	
4	72°C	7 min	
5	8°C	Hold	

**Table 4: TaqMan probe for *C. albicans* based on Ace2 gene region**

P1-CanAce2	CTGTCACCATTGAATGGAGTCCAG
------------	--------------------------

**Table 5: Real-time PCR reagents**

5

Preparation of PCR Reaction Mix	SAMPLE
LightCycler®FastStartDNA Master HybProbe, Roche Cat. 03 003 248 001	x 1
HybProb mix 10 x conc. (Red cap)	2 µl
MgCl <sub>2</sub> stock solution (Blue cap) (Final conc. in reaction is 3 mM)	1.6 µl
Probe P1-CanAce2	2 µl
Primer Forward CanAce2-F	1 µl
Primer Reverse CanAce2-R	1 µl
H <sub>2</sub> O PCR-grade	10.4 µl
Template	2 µl
<b>TOTAL VOLUME</b>	<b>20 µl</b>

**Table 6: Real-time PCR thermocycling conditions**

PCR Thermal profile				
Cycle	Step	Temp	Time	<b>X 50</b>
Activation	1	95°C	10 min	
Amplification	1	95°C	10 sec	
	2	62- 65°C	20 sec	
	3	70°C	10 sec	
Cooling	1	40°C	Hold	

## RESULTS

### Primer and Probe Design

The sequence information available for the Ace2 gene in *Candida* spp. was aligned with the newly generated sequence information for the Ace2 gene in *Candida* spp. and analysed using bioinformatics tools. Species-specific probes were designed based on the compiled Ace2 sequence information for *Candida albicans* (Table 4). Figures 2 shows the relative positions of the PCR primers and TaqMan DNA probe for the amplification and detection of *C. albicans*. The specificity of the TaqMan probes for the identification of *C. albicans* was demonstrated in a real-time PCR assay on the LightCycler using the reagents and thermocycling conditions outlined in Tables 5. For the *C. albicans* assay based on the Ace2 gene, PCR primers CanAce2-F/CanAce2-R were combined with TaqMan probe, P1-CanAce2. The specificity of the assay for the detection of *C. albicans* was confirmed by including DNA from a range of closely related *Candida* species and *A. fumigatus* species in the *C. albicans* real-time PCR assay. The assay detected *C. albicans* but did not detect or cross-react with DNA from any other *Candida* species tested or with *A. fumigatus* DNA. Figure 3 shows the *C. albicans* real-time PCR assay and the specificity of the assay for *C. albicans*.

### 20 EXAMPLE 2

Primers and probes for specific detection and identification were designed following *in silico* analysis of generated sequences. Three forward and three reverse primers were generated and two probes were designed as follows. Figures 4(a) to 4(d) discloses the location of these sequences in the Ace2 subsequence.

25 ACF1: SEQ ID NO:20: ATCAAAGAATCATCACCA

ACF2: SEQ ID NO:21: AGACTTCATTGTTACCAC

ACF3: SEQ ID NO:24: CACCAGGTGAATTGG

ACR1: SEQ ID NO:25: CATTGTATCGACGAGTG

ACR2: SEQ ID NO:26: TGTATCGACGAGTGAAT

30 ACR3: SEQ ID NO:27: TTCGCACATTGTATCG

ACALB1: SEQ ID NO:30: 6FAM-ATATCTTATCCTCATCCGGTCCT--BHQ1

ACALB2: SEQ ID NO:31: 6FAM-AGGACCGGATGAGGATAAGATAT--BHQ1

The primer sets were evaluated using the following assay conditions: UNG treatment: 50°C 2min followed by 95°C 1min. The amplification included 50 cycles, 95°C 10sec, 60°C 30 sec, followed by a 2 min cooling at 40°C.

Based on initial assay performance (e.g. fluorescence and efficiency), primer set ACF3/ ACR3 was chosen for further evaluation using ACALB1 probe. Initial inclusivity and exclusivity experiments were performed to evaluate the potential of the assay using the chosen assay oligonucleotides. Specificity of the assay was tested using a panel of DNA from 14 *C. albicans* strains and 23 strains representing 19 other *Candida* species. All 14 *C. albicans* strains tested were detected. No cross-reaction was seen with DNA from 19 the other *Candida* species, *i.e.*, signal was obtained only from the positive *C. albicans* control.

The Ace2 assay was shown to be specific to *C. albicans*, and initial performance was good under conditions tested.

Next, eight different combinations of primers were tested in order to reduce cycling times. The following conditions were tested: UNG treatment for 2min at 50°C, 1min at 95°C, followed by amplification of 50 cycles, 95°C 5sec, 60°C 10sec, followed by a cooling step of 2min at 40°C.

The combination of primer set ACF2/ACR3 and the ACALB1 probe performed the best under these conditions. The LOD of the assay is ~1-10 cell equivalents. This primer combination demonstrated higher fluorescence at lower template inputs, and 3 out of 3 one-cell equivalents were detected with earlier C<sub>p</sub> values. Slight modifications were made to the primer sequences in order to further shorten cycle times, while retaining sensitivity and specificity (Table.7).

**Table 7: Modifications to primer sequences**

Primer name	SEQ ID NO	Tm
ACF2	21	53°C
ACF2b	22	57.1°C
ACF2c	23	59.5°C
ACR3	27	52.7°C
ACR3b	28	56.8°C
ACR3c	29	60.2°C

Further reduced cycling conditions were tested, *i.e.*, amplification step of 95°C for 1 second and 60°C for 10 seconds. 10-fold dilutions of *C. albicans* DNA were prepared and inputs of  $1 \times 10^5$  to 0.1 cell equivalents were used as template. The combination of ACF2b and ACR3b primers were selected for inclusivity and exclusivity testing due to higher fluorescence at lower template inputs and 3 out of 3 one-cell equivalents were detected with earlier Cp values than other primer sets, as indicated in Table 8.

**Table 8: CP values obtained for different Tm primer sets.**

Average CP values for samples					
Template input	ACF2 / ACR3	ACF2b / ACR3b	ACF2c / ACR3c	ACF2b / ACR3b	ACF2c / ACR3c
100000	(3/3) 21.92	(3/3) 22.08	(3/3) 22.52		
1000	(3/3) 25.55	(3/3) 25.65	(3/3) 25.95		
100	(3/3) 29.05	(3/3) 29.19	(3/3) 29.60		
10	(3/3) 32.56	(3/3) 32.67	(3/3) 33.01		
1	(3/3) 35.88	(3/3) 36.05	(3/3) 36.58		
0.1	(2/3) 40	(3/3) 39.34	(3/3) 40	0	0

Twenty strains of *Candida albicans* were selected to demonstrate inclusivity of detection with this primer set (see Table 9, Figure 5 and Figure 6). Real-time PCR assay for *C. albicans* was carried out as described with TaqMan probe ACALB1 and primer set ACF2b/ACR3b. All twenty *C. albicans* strains tested were detected, and no signal obtained from (-) controls (see Figure 6(a)).



**Table 9: *C. albicans* strains used in inclusivity experiments**

No.	Strain	Species	Source	Origin	Notes
1	3156	<i>C. albicans</i>	PHLS/NCPF	1965 Serotype B	UK ✓
2	3345	<i>C. albicans</i>	PHLS	Arm abscess	UK ✓
3	3328	<i>C. albicans</i>	PHLS	Renal transplant patient	UK ✓
4	178	<i>C. albicans</i>	UCHG	Clinical isolate	Galway Ireland ✓
5	180	<i>C. albicans</i>	UCHG	Clinical isolate	Galway Ireland ✓
6	320	<i>C. albicans</i>	UCHG	Clinical isolate	Galway Ireland ✓
7	369	<i>C. albicans</i>	UCHG	Clinical isolate	Galway Ireland ✓
8	765	<i>C. albicans</i>	UCHG	Clinical isolate	Galway Ireland ✓
9	562	<i>C. albicans</i>	CBS	patient with interdigital mycosis	Uruguay ✓
10	15640	<i>C. albicans</i>	HEM	Blood, tuberculosis	Belgium ✓
11	9659	<i>C. albicans</i>	HEM	Blood	Iowa USA ✓
12	16733	<i>C. albicans</i>	HEM	Blood, Candidemia AIDS	Belgium ✓
13	1893	<i>C. albicans</i>	CBS	Soil	New Zealand ✓
14	1899	<i>C. albicans</i>	CBS	man, infected skin of child	Republic of Congo ✓
15	1912	<i>C. albicans</i>	CBS	man, sputum, asthma patient	Norway ✓
16	2688	<i>C. albicans</i>	CBS	infection of urino-genital tract	Switzerland, Bern ✓
17	2701	<i>C. albicans</i>	CBS	man, pus from lung, dead mall	Italy, Paris ✓
18	8642	<i>C. albicans</i>	CBS	sputum, from 19-year-old man	Germany, Dresden ✓
19	2736	<i>C. albicans</i>	CBS	man, mouth of child	Portugal, Oporto ✓
20	2700	<i>C. albicans</i>	CBS	macroglossia mycetica	Brazil ✓

5                    In order to demonstrate exclusivity of the assay, the Ace2 assay was tested for performance against 84 strains covering 19 spp. of clinically relevant and related *Candida* (see Table 10 below). In order to ensure that all DNA tested is PCR amplifiable, the *Candida* DNA samples were amplified with universal fungal primers U1 and U2. All DNA tested was shown to be PCR amplifiable. A template input of 10<sup>5</sup> CE/rxn was used, which is higher than normally tested. A higher input was tested because the organisms tested are related *Candida* and Ace2 sequence information was not confirmed for 17 species prior to testing. Each input was tested in triplicate. No cross-reactions were observed, demonstrating the specificity of the Ace2 assay for *C. albicans* detection (see Figure 6(b)).

**Table 10: Candida Specificity panel – Clinically relevant and related *Candida* spp.**

<b><i>C. albicans</i> Ace2 specificity panel - Tested in triplicate</b>					
1	3605	<i>C. glabrata</i>	PHLS NCPF	Diabetes	UK
2	8018	<i>C. glabrata</i>	PHLS NCPF	Blood	UK
3	3863	<i>C. glabrata</i>	PHLS NCPF	Blood	UK
4	90876	<i>C. glabrata</i>	ATCC	Blood	Germany
5	3959	<i>C. glabrata</i>	IHEM	Blood	Belgium, Leuven
6	9556	<i>C. glabrata</i>	IHEM	Blood	Brussels, Belgium
7	138	<i>C. glabrata</i>	CBS		?
8	860	<i>C. glabrata</i>	CBS	man, urine, of diabe	Netherlands
9	861	<i>C. glabrata</i>	CBS	mouth	Netherlands, Groningen
10	7307	<i>C. glabrata</i>	CBS	sputum, of 63-year	New Zealand, Masterton
1	3873	<i>C. tropicalis</i>	PLHS NCPF	Blood, neutropenic	UK
2	3870	<i>C. tropicalis</i>	PLHS NCPF	Blood	UK
3	4225	<i>C. tropicalis</i>	IHEM	Blood	Brussels, Belgium
4	5557	<i>C. tropicalis</i>	IHEM	Blood	Grenoble, France
5	94 Type	<i>C. tropicalis</i>	CBS		?
6	2313	<i>C. tropicalis</i>	CBS	superficial interdigit	Egypt, Cairo
7	5701	<i>C. tropicalis</i>	CBS	infected hand	Hungary
8	6361	<i>C. tropicalis</i>	CBS	blood, of 53-year-old	USA
9	8072	<i>C. tropicalis</i>	CBS	man, blood, at auto	USA, New York
10	10256	<i>C. tropicalis</i>	CBS	blood sample	France, Paris
1	5579	<i>C. krusei</i>	IHEM	Blood	France
2	9560	<i>C. krusei</i>	IHEM	Sputum, bronchom	Sri Lanka
3	2052	<i>C. krusei</i>	CBS	sputum from case d	Italy, Milano
4	2046	<i>C. krusei</i>	CBS	pus from infected fir	Argentina, Buenos Aires
5	Type 573	<i>C. krusei</i>	CBS	Sputum	Sri Lanka
6	3165	<i>C. krusei</i>	PHLS NCPF	Sputum	UK
7	3922	<i>C. krusei</i>	PHLS NCPF	Human blood	UK
8	3847	<i>C. krusei</i>	PHLS NCPF	Abdominal fistula	UK
9	3433	<i>C. krusei</i>	DSMZ	fruit juice or berries	n/a
10	70075	<i>C. krusei</i>	DSMZ	human faeces	n/a
1	96137	<i>C. parapsilosis</i>	ATCC	Leg wound	Texas
2	1716	<i>C. parapsilosis</i>	IHEM	Human nail, onycho	Gosselies, Belgium
3	604	<i>C. parapsilosis</i>	CBS		Puerto Rico
4	2194	<i>C. parapsilosis</i>	CBS		Austria
5	2196	<i>C. parapsilosis</i>	CBS		Dominican Republic
6	2197	<i>C. parapsilosis</i>	CBS	bladder	Denmark, Copenhagen
7	4024	<i>C. parapsilosis</i>	IHEM	human ear canal	Belgium
8	2916	<i>C. parapsilosis</i> group III <i>metapsilosis</i>	CBS	sputum	Norway
9	96143	<i>C. parapsilosis</i> group III <i>metapsilosis</i>	ATCC	unknown	California
10	96141	<i>C. parapsilosis</i> group II <i>orthopsilosis</i>	ATCC	Blood	Texas

1	*3949 Type	<i>C. dubliniensis</i>	PHLS NCPF	Oral cavity HIV + pa	Ireland
2	*14280 Type	<i>C. dubliniensis</i>	IHEM		*Same strain as 3949
1	16625	<i>C. dubliniensis</i>	IHEM	human mouth	Besançon, France
1	16971	<i>C. dubliniensis</i>	IHEM	human sputum	Brussels, Belgium
1	7988	<i>C. dubliniensis</i>	CBS	man, oral cavity, HI	Australia, Victoria, Melbourne
1	7987	<i>C. dubliniensis</i>	CBS	oral cavity of HIV-inf	Ireland, Dublin
1	8500	<i>C. dubliniensis</i>	CBS	blood, of 38-year-old	Netherlands, Nijmegen
1	8501	<i>C. dubliniensis</i>	CBS	child, with neutrope	Netherlands, Nijmegen
1	8013	<i>C. lusitaniae</i>	PHLS NCPF	Sputum, hydronephr	UK
1	19472	<i>C. lusitaniae</i>	IHEM	citrus fruit	Israel
1	7270	<i>C. lusitaniae</i>	CBS	sputum, from pneum	New Zealand
1	10294	<i>C. lusitaniae</i>	IHEM		Maryland USA
1	4600	<i>C. lusitaniae</i>	IHEM	human blood candid	Italy, Milano
1	8167	<i>C. guilliermondii</i>	PHLS NCPF	Blood, patient with	UK
2	2672	<i>C. guilliermondii</i>	CBS	case of cystitis, man	
3	6021	<i>C. guilliermondii</i>	CBS	soil	Japan, Niigata Province
4	2682	<i>C. guilliermondii</i>	CBS	semen of bull	UK
1	2070	<i>C. lipolytica</i>	CBS	corneal lesion	Italy, Siena
2	2071	<i>C. lipolytica</i>	CBS	man, finger nail, 49	Austria
3	8218	<i>C. lipolytica</i>	DSMZ	Diesel fuel storage	USA
1	5312	<i>C. rugosa</i>	CBS	sake starter yeast	Japan
2	613	<i>C. rugosa</i>	CBS	faeces of Man	?
3	1948	<i>C. rugosa</i>	CBS	sputum	Norway
4	6703	<i>C. rugosa</i>	IHEM	human urine	Belgium, Brussels
1	1922	<i>C. norvegensis</i>	CBS	sputum, of patient v	Norway
2	2144	<i>C. norvegensis</i>	CBS	sputum	Netherlands, Gouda
3	2145	<i>C. norvegensis</i>	CBS	abdominal infiltrate	Netherlands, Dordrecht
1	5295	<i>Stephanosascus cifarii</i>	CBS	pig, with infected th	Netherlands
2	70749	<i>Stephanosascus cifarii</i>	DSMZ	dried yeast	N/A
1	70040	<i>C. catenulata</i>	DSMZ	?	Frankfurt
2	70136	<i>C. catenulata</i>	DSMZ	hyperkeratinic foot	N/A
1	3428	<i>C. famata</i>	DSMZ	spoilt sake	n/a aka <i>Debaryomyces hansenii</i>
2	70590	<i>C. famata</i>	DSMZ	harzer cheese	n/a aka <i>Debaryomyces hansenii</i>
1	7802	<i>C. haemulonii</i>	CBS	ulcer on toe	USA
2	70624	<i>C. haemulonii</i>	DSMZ	intestinal tract of H	Florida
1	3898	<i>C. keyfr</i>	PHLS NCPF	Blood, neutropenic	UK
2	70073	<i>C. keyfr</i>	DSMZ		aka <i>Kluyveromyces marxianus</i>
1	70336	<i>C. pulcherrima</i>	DSMZ	Red dates	Egypt
2	70879	<i>C. pulcherrima</i>	DSMZ	flower of <i>Trifolium p</i>	n/a
1	70163	<i>C. utilis</i>	DSMZ	fodder yeast from w	Germany
2	70167	<i>C. utilis</i>	DSMZ	protein from rye gra	n/a
1	4024	<i>C. viswanathii</i>	CBS	cerebro-spinal fluid,	India
2	5362	<i>C. viswanathii</i>	CBS	sputum	India
1	2000	<i>C. zeylanoides</i>	CBS	sputum, of bronchit	Norway
2	70185	<i>C. zeylanoides</i>	DSMZ	Butter	Germany

Exclusivity was further demonstrated using a vaginitis/vaginosis panel with 45 species of clinically relevant organisms in triplicate (see Table 11 below) using the shorter cycle conditions. A template input of  $\sim 10^4$  CE/rxn was used. No cross-reactions were observed, further demonstrating the specificity of the Ace2 assay (see Figure 7).

**Table 11: Vaginosis/Vaginitis specificity panel organisms tested against Ace2 assay.**

#	Organism	#Vagin number
1	<i>Acinetobacter lwoffii</i>	2403 <sup>1</sup>
2	<i>Alcaligenes faecalis</i>	13644
3	<i>Alcaligenes faecalis</i> subsp. <i>faecalis</i>	30030 <sup>1</sup>
4	<i>Anaerococcus vaginalis</i>	7457 <sup>1</sup>
5	<i>Atopobium vaginae</i>	15829 <sup>1</sup>
6	<i>Chlamydia trachomatis</i>	VR-348B
7	<i>Citrobacter freundii</i>	8090
8	<i>Cryptococcus neoformans</i>	11959
9	<i>Enterobacter cloacae</i> subsp. <i>cloacae</i>	13047
10	<i>Enterococcus faecalis</i>	20371
11	<i>Enterococcus faecium</i>	20477 <sup>1</sup>
12	<i>Escherichia coli</i>	30083 <sup>1</sup>
13	<i>Gardnerella vaginalis</i>	4944 <sup>1</sup>
14	<i>Haemophilus influenzae</i>	9999
15	Herpes simplex virus 1	1A 08-921-000
16	Herpes simplex virus 2	1A 08-922-000
17	HIV-1	1A 08-707-000
18	Human papilloma virus type 16	1A 08-754-100
19	Human papilloma virus type 18	1A 08-755-100
20	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	30104 <sup>1</sup>
21	<i>Lactobacillus acidophilus</i>	20079 <sup>1</sup>
22	<i>Lactobacillus fermentum</i>	20055
23	<i>Lactobacillus oris</i>	4864 <sup>1</sup>
24	<i>Lactobacillus parabuchneri</i>	5707 <sup>1</sup>
25	<i>Moraxella catarrhalis</i>	11994
26	<i>Moraxella (Moraxella) osloensis</i>	6998 <sup>1</sup>
27	<i>Morganella morganii</i> subsp. <i>morganii</i>	30164
28	<i>Neisseria gonorrhoeae</i>	9188 <sup>1</sup>
29	<i>Peptococcus niger</i>	20475 <sup>1</sup>
30	<i>Rhophomonas asaccharolytica</i>	20707 <sup>1</sup>
31	<i>Prevotella bivia</i>	20514 <sup>1</sup>
32	<i>Proteus mirabilis</i>	4479 <sup>1</sup>
33	<i>Proteus vulgaris</i>	2140
34	<i>Providencia stuartii</i>	4539 <sup>1</sup>
35	<i>Pseudomonas aeruginosa</i>	50071 <sup>1</sup>
36	<i>Pseudomonas putida</i>	291 <sup>1</sup>
37	<i>Saccharomyces cerevisiae</i>	70449 <sup>1</sup>
38	<i>Serratia marcescens</i>	1608
39	<i>Staphylococcus aureus</i>	346
40	<i>Staphylococcus epidermidis</i>	20044 <sup>1</sup>
41	<i>Staphylococcus intermedius</i>	20373 <sup>1</sup>
42	<i>Streptococcus agalactiae</i>	15081
43	<i>Streptococcus salivarius</i>	20560 <sup>1</sup>
44	<i>Trichomonas vaginalis</i>	30001
45	<i>Veillonella gluyckii</i> Serotype V	20739 <sup>1</sup>

Probit analysis was performed in order to statistically determine the LOD (limit of detection) of the Ace2 assay using ACF2b/ ACR3b/ ACALB1 with shorter cycle times. The LOD of the Ace2 assay was determined by testing 8 template inputs and 12 replicates. *C. albicans* strain CBS 562 was selected for LOD determination. The results are shown below in Table 12 and Figure 8. An LOD of 0.839, with a probability of greater than or equal to 95% was found by Probit analysis.

**Table 12: Input levels (cell equivalents) and results of Probit analysis**

Number of replicates detected	Input level	Number of replicates tested
12	10	12
12	5	12
12	4	12
12	3	12
12	2	12
11	1	12
11	0.5	12
1	0.1	12

**Discussion**

The number of yeast and fungal infections among immuno-compromised patients is escalating. Contributing to this increase is the growing resistance of many yeast and fungal species to antifungal drugs. There is, therefore, a need to develop a fast, accurate diagnostic method to enable early diagnosis of yeast and fungal species. Early diagnosis will enable the selection of a specific narrow spectrum antibiotic or antifungal to treat the infection. The current invention provides for sequences and/or diagnostic assays to detect and identify one or more yeast and fungal species. The current inventors have exploited the sequence of the *Ace2* gene in *Candida* species to design primers and probes specific for regions of this gene. *Ace2* is an ideal candidate for the design of primers and probes directed towards the detection of yeast and fungal species-specific targets and for the detection of genus specific diagnostic targets respectively. The current invention allows the detection of yeast and fungal species but also allows distinction between *Candida* species.

**Summary**

Using newly generated and publicly available sequences, the inventors have designed new primers and probes that are surprisingly specific to *Candida Ace2* polynucleotide sequences under short cycle times identified here. Such primers and probes not only allow the detection of yeast and fungal species but also allow surprisingly effective distinction between *Candida* species and specific detection of *C. albicans*, even under short cycle times. The current invention further provides for

primers and probes that allow excellent discrimination between different *Candida* species.

The words “comprises/comprising” and the words “having/including” when  
 5 used herein with reference to the present invention are used to specify the presence of  
 stated features, integers, steps or components but does not preclude the presence or  
 addition of one or more other features, integers, steps, components or groups thereof.

It is appreciated that certain features of the invention, which are, for clarity,  
 described in the context of separate embodiments, may also be provided in combination  
 10 in a single embodiment. Conversely, various features of the invention, which are, for  
 brevity, described in the context of a single embodiment, may also be provided  
 separately or in any suitable sub-combination.

In so far as any sequence disclosed herein differs from its counterpart in the  
 attached sequence listing in PatentIn3.3 software, the sequences within this body of text  
 15 are to be considered as the correct version.

#### SEQ IDs

N or x= any nucleotide; w=a/t, m=a/c, r=a/g, k=g/t, s=c/g, y=c/t, h=a/t/c, v=a/g/c,  
 d=a/g/t, b=g/t/c. In some cases, specific degeneracy options are indicated in parenthesis:  
 20 e.g.: (a/g) is either A or G.

#### SEQ ID NO 1: CanAce2-F

TATCACCTTTGAAAAACAATTACC

#### 25 SEQ ID NO 2: CanAce2-R

CACCAACACAAATATTTTCGATC

#### SEQ ID NO 3: P1-CanAce2

CTGTCACCATTGAATGGAGTCCAG

30

#### SEQ ID NO 4:

>CA3165-Ace2/ACE2F *C. albicans*

AAACACACCAACGAAGCAACCCCCTGTCACCATTGAATGGAGTCCAGTTATATCACCA  
 AACTCAAAACAACCATTACACAAACAAATCAAAGAATCATCACCACGACGCCGAATCA  
 AAAAGACTTCATTGTTACCACCAGGTCAATTGGACAATTACTGGACAGGACCCGGATGA  
 GGATAAGATATATACTTGTACTTACAAAACTGTGGGAAAAAATTCACTCGTCGATAC  
 5 AATGTGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTCAATTCT  
 GTCCGAAPAGATTTGTCAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGA  
 AGCTAGATATAGCAAATGTCCTTGTGGTAAAGAGTTTGCTAGATTAGATGCATTAAGA  
 AAACATCAGGATCGAAATATTTGTGTTGGTG

10 **SEQ ID NO 5:**

>CA765-Ace2/ACE2F *C. albicans*

AAACACTCCCACAAAGCAACCCCCTGTCACCATTGAATGGAGTCCAGTTATATCACCC  
 AACTCAAAGCAACCATTACACAAACAAATCAAAGAATCATCACCACGACGCCGAAT  
 CAAAAGACTTCATTGTTACCACCAGGTGAATTGGACAATTACTGGACAGGACCCGGA  
 15 TGAGGATAAGATATATACTTGTACTTACAAAACTGTGGGAAAAAATTCACTCGTCG  
 ATACAATGTGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTCA  
 ATTCTGTCCGAAAAGATTTGTCAGACAACATGATTTGAATCGTCACGTGAAAGGGCA  
 CATTGAAGCTAGATATAGCAAATGTCCTTGTGGTAAAGAGTTTGCTAGATTAGATGC  
 ATTAAGAAAACATCAGGATCGAAATATTTGTGTTGGTG

20

**SEQ ID NO 6:**

>CA562-Ace2/ACE2F *C. albicans*

AAACACT000ACAAAGCAACCCCCTGTCACCATTGAATGGAGTCCAGTTATATCACCA  
 AACTCAAAGCAACCATTACACAAACAJATCAAAGAATCATCACCACGACGCCGAATCA  
 25 AAAAGACTTCATTGTTACCACCAGGTGAATTGGACAATTACTGGACAGGACCCGGATGA  
 GGATAAGATATATACTTGTACTTACAAAACTGTGGCAAAAAAATTCACTCGTCGATAC  
 AATGTGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTCAATTCT  
 GTCCGAAPAGATTTGTCAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGA  
 AGCTAGATATAGCAAATGTCCTTGTGGTAAAGAGTTTGCTAGATTAGATGCCTTTGAGA  
 30 AAGCACCAGGATCGAAATATTTGTGTTGGTG

**SEQ ID NO 7:**>CA2700-Ace2/ACE2F *C. albicans*

AAACACTCCCACAAAGCAACCCCTGTCACCATTGAATGGAGTCCAGTTATATCACCA  
 AACTCAAAGCAACCATTACACAAACAAATCAAAGAATCATCACCACGACGCCGAATCA  
 5 AAAAGACTTCATTGTTACCACCAGGTGAATTCGACAATTA CTGGACAGGACCGGATGA  
 GGATAAGATATATACTTGTACTTACAAAACTGTGGGAAAAAATTC ACTCGTCGATAC  
 AATGTGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTCAATTCT  
 GTCCGAAPAGATTTGTCAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGA  
 AGCTAGATATAGCAAATGTCCTTGTGGTAAAGAGTTTGCTAGATTAGATGCATTAAGA  
 10 AAACATCAGGATCGAAATATTTGTGTTGGTG

**SEQ ID NO 8:**>CD3494 /ACE2F *C. dubliniensis*

AAACACTCCCACAAAGCAACCCCTGTCACCATTGAATGGAGTCCAGTTATATCACCA  
 15 AACTCAAAGCAACCATTACACAAACAAATCAAAGAATCGTCACCACGACGCCGAATCA  
 AAAAGACCTCATTGTTGCCACCAGGTGAATTTGGATAATTA CTGGACAGGACCGGATGA  
 GGATAAGATATATACTTGTACTTACAAAACTGTGGGAAAAAATTC ACTCGTCGATAT  
 AATGTGCGAAGTCATATTCAAACACATTTAAGTGATCGTCCATTTGGTTGTCAAGTTCT  
 GTCCCAAAGATTTGTTAGACAACATGATTTGAATCGTCACGTGAAAGGACACATTGA  
 20 AGCCAGATATAGTAAATGTCCTTGTGGTAAAGAGTTTGCTAGATTAGATGCTTTGAGA  
 AAGCACCAGGATCGAAATATTTGTGTTGGTG

**SEQ ID NO 9:**>CD7987 /ACE2F *C. dubliniensis*

AAACACACCAACGAAGCAACCCCTGTCACCATTGAATGGAGTCCAGTTATATCACCA  
 25 AACTCAAACAACCATTACACAAACAAATCAAAGAATCGTCACCACGACGCCGAATCA  
 AAAAGACCTCATTGTTGCCACCAGCTGAATTTGGATAACTATTGGACAGGACCAGATAA  
 AGACAAAATATATACCTGTACCTACAAAACTGTGGGAAAAAATTC ACTCGTCGATAT  
 AATGTGCGAAGTCATATTCAAACACATTTAAGTGATCGTCCATTTGGTTGTCAAGTTCT  
 30 GTCCCAAAGATTTGTTAGACAACATGATTTGAATCGTCACGTGAAAGGACACATTGA  
 AGCCAGATATAGTAAATGTCCTTGTGGTAAAGAGTTTGCTAGATTAGATGCTTTGAGA  
 AAGCACCAGGATCGAAATATTTGTGTTGGTG



**SEQ ID NO 10:**>CD8501S23 /ACE2F *C. dubliniensis*

AACACACCAACGAAGCAACCCCCTGTCACCATTGAATGGAGTCCAGTTATATCACCAA  
 5 ACTCAAAACAACCATTACACAAACAAATCAAAGAATCGTCACCACGACGCCGAATCAA  
 AAAGACCTCATTGTTGCCACCAGGTGAATTGGATAATTACTGGACAGGACCCGGATGAG  
 GATAAGATATATACTTGTACTTACAAAACACTGTGGGAAAAAATTCCTCGTCGATATA  
 ATGTGCGAAGTCATATTCAAACACATTTAAGTGATCGTCCATTTGGTTGTCAGTTCTG  
 TCCCAAAGATTTGTTAGACAACATGATTTGAATCGTCACGTGAAAGGACACATTGAA  
 10 GCCAGATATAGTAAATGTCCTTGTGCTAAAGAGTTTGCTAGATTAGATGCTTTGAGAA  
 AGCACCAGGATCGAAATATTTGTGTTGGTG

**SEQ ID NO 11:**>94-Ace2/ACE2F *C. tropicalis*

TATCACCTTTGAAAPCCAATTACCAAGTACACCAACTAAACAACAAAATTCCCAAGTT  
 15 ACTATTGAATGGAGCCCTGTTATTTACCAAATTCCAAAGCATCATTATCTAAACAAC  
 TCAAAGAATCATCATTACAAACGTCGCCACGTAGAAGAATTA AAAAGACTTCATTATT  
 GCCACCTGGAGA ACTTGATAATTATTGGATTGGACCCGATGAAAATAAAATTTATACA  
 TGTA CTTATAAAA ACTGTTTTAAAAAATTTACAAGAAGATATAATGTTAGAAAGTCATA  
 20 TTCAA ACTCATT TTTGTCCTAAAAGATTTGTTTCGACAACATGATTTAAATCGTCATGTT  
 AAAGGACATATTGAAGCAAGATATAGTAAATGGACATATTGAAGCAAGATATAGTAAA  
 TGTCCATGTGGTAAAGAATTTGCAAGATTAGATGCTTTAAGAAAACATC  
 AAGATCGAAATATTTGTATTGGTG

**25 SEQ ID NO 12:**>CT8157-Ace2/ACE2F *C. tropicalis*

TACACCAACTAAACAACAAAATTCCCAAGTTACTATTGAATGGAGCCCTGTTATTTCA  
 CCAAATTCCAAAGCATCATTATCTAAACA ACTCAAAGAATCATCATTACAAACGTCGC  
 CACGTAGAAGAATTA AAAAGACTTCATTATTGCCACCTGGAGAACTTGATAATTATTG  
 30 GATTGGACCCGATGAAAATAAAATTTATACATGTA CTTATAAAA ACTGTTTTAAAAA  
 TTTACAAGAAGATATAATGTTAGAAAGTCATATTCAA ACTCATTTAPGTGATAGACCAT  
 TTGGATGTCAATTTTGTCTAAAAGATTTGTTTCGACAACATGATTTAAATCGTCATGT

TAAAGGACATATTGAAGCAAGATATAGTAAATGTCCATGTGGTAAAGAATTTGCAAGA  
TTAGATGCTTTAAGAAAACATCAAGATCGAAATATTTGTGTTGGTG

**SEQ ID NO 13:**

5 >C12311-Ace2/ACE2F *C. tropicalis*

TACACCAACTAAACAACAAAATTCCTAAGTTACTATTGAATGGAGCCCTGTTATTTCA  
CCAAATTCCAAAGCATCATTATCTAAACAACCTCAAAGAATCATCATTACAAACGTCGC  
CACGTAGAAGAATTAAAAAGACTTCATTATTGCCACCTGGAGAACTTGATAATTATTG  
GATTGGACCCGATGAAAATAAAATTTATACATGTACTTATAAAAACCTGTTTTAAAAA  
10 TTTACAAGAAGATATAATGTTAGAAGTCATATTCAAACCTCATTTAAGTGATAGACCAT  
TTGGATGTCAATTTTGTCTTAAAGATTTGTTTCGACAACATGATTTAAATCGTCATGT  
TAAAGGACATATTGAAGCAAGATATAGTAAATGTCCATGTGGTAAAGAATTTGCAAGA  
TTAGATGCTTTAAGAAAACATCAAGATCGAAATATTTGTGTTGGTG

15 **SEQ ID NO 14:**

>CT2424-Ace2/ACE2F *C. tropicalis*

TACACCAACTAAACAACAAAATTCCTAAGTTACTATTGAATGGAGCCCTGTTATTTTC  
ACCAAATTCCAAAGCATCATTATCTAAACAACCTCAAAGAATCATCATTACAAACGTCG  
CCACGTAGAAGAATTAAAAAGACTTCATTATTGCCACCTGGAGAACTTGATAATTAT  
20 TGGATTGGACCCGATGAAAATAAAATTTATACATGTACTTATAAAAACCTGTTTTAA  
AAAATTTACAAGAAGATATAATGTTAGAAGTCATATTCAAACCTCATTTAAGTGATAG  
ACCATTTGGATGTCAATTTTGTCTTAAAGATTTGTTTCGACAACATGATTTAAATCG  
TCATGTTAAAGGACATATTGAAGCAAGATATAGTAAATGTCCATGTGGTAAAGAATT  
TGCAAGATTAGATGCTTTAAGAAAACATCAAGATCGAAATATTTGTGTTGGTG

25

**SEQ ID NO 15:**

>gi168487491|reflXM\_707274.11 *Candida albicans* SC5314 putative transcription  
factor (Ca019.13543), mRNA GENE ID: 3645995 ace2 1 putative zinc finger protein  
similar to *S. cerevisiae* ACE2 (YLR131C) cell cycle-specific transcriptional  
30 regulator of CUP1 [*Candida albicans* SC5314]

ATGCATTGGAAATTTCTGAACTTTCGAAAGTACCATCTTTCTTTCCATTTAAATTTA

TTTGATCTTTCTCTCTTTTTTATTTCCCTTTTACTGCTTTCCCATCCTATATATTTGT  
TTCTTTAATCAAGTCCATTCAATTCAGAAGCACACAACCAAGTCTTATAATGAATAAA  
TTGACTTGTTTGATGATTACTCGACCAAAGGGTCAACCATCCCTCTTCTAATGAA  
AATTTTGACCAATTATTTTAAAGTTCGGAAGCCAATGATATGGAATTTCTCTTTTTT  
5 ATTTCTTTTACTGCTTTCCCATCCTATATATTTGTTTCTTTAATCAAGTCCATTCA  
TTCACCATTAATAACGATTTCCAACACACACCAAATAAGTCAAAATCTCACAGTAGA  
CAATACAGTGGAACAGCAATTTTGGTTTTGCTGACCATAATAAGATTTGTCCATT  
AACGGTGTCAACAATGACTTGTGCAACAATCCAATAAAGCCATCAATACACAATCG  
GTTTCACCTGGTGAGTTGTTGAAACGTTCAAGAGGTTCTCAAACACCAACACCAACA  
10 TCAGCACTACCAGACACTGCCCAAGATATTTTAGATTTTAATTTTGAGGAAAAACCA  
ATATTGTTGCTTGAGGAGGATGAGCTTGAGGAAGAAAAACATAAACAACAACAAGA  
ATGATGACACAGCTGTACCTTTGAAGAGAGTAAACCACTCCTAGTCAATCACCATTT  
GTTCAACAGCCACAAACCATGAAACAAGAAAGCCACACAAGAAGACAAACGAGTAC  
ATTGTTGCCAATGAAAATCCCAATTCGTACAAATTTCCACCATCTCCATCTCCGACT  
15 GCCAAACGCCAGCAGTATCCACCATCATCTCCAATACCATAACAATCCAAAATCAGAT  
TCAGTTGGTGGCAATTCTTACTCTGAAAATACTTGCAAGTCTTTAAACAAAACCCAG  
CAAATTGAATATGTGGATGATATTGAGCCACTTTTACAGGAAGATAATAACAATATG  
AAATATATTCCAATTCCTCGTACAAGAGCCAATGCTGTATCAAAAACAGAAACAGTG  
ACACCTCCATTACAGTCAAAAATGATTCTCAACAATTAGAACCATTGAAGACACCA  
20 CAACCACAACCAAAAACAACAACAGCAACAGCAACCAAATAATGAACAGGATAAAA  
GAATTTACTGCTAATATCAATTTCAACACCTTCTTACCTCCACCTACTCCGCCTAAT  
TTGATTAATGGATCTCCCGATTGGAATTCATCGCCAGAACCACATTCTCCTTCCCA  
GGAAGATTGCAACCACCGCAACAGATATCCCAATTCACCAGAATTTAGGTGCCATG  
GGTAATAATATCAACTTTTATACACCCATGTATTACGAGCTTCCAGTACAAGCTGAA  
25 CAACCGCAACCACAACCACAACCACATCAACAGCAACATCAACAGCAACAGCATCAA  
CCTGAATTACAAAACACTTACCAACAAATTAACACATTCAACAGCAACAGCAAATG  
CTACAACATCAGTTTCACAACCAAAAACAATCAACTCCGCCAACAAACCCCAAATCAA  
TTCCAGAACCAGAACCAGAACCAGAAACCAAATAAAGACTCCTTACTCACAA  
CAAAATTTTAATTTGTCTCCTGCAAAAACAATTAATTCAAATGTAGGTTCCATGCAT  
30 TTATCACCTTTGAAAATCCATGCATTTATCACCTTTGAAAAACAATTACCAAPCAC  
TCCCACAAAGCAACCCCTGTACCATTCAACAGCATCAACCTGAATTACAAAACAC  
TTACCAACAAATTAACACATTCAACAGCAACAGCAAATGCCGACGCCGAATCAAAA

AGACTTCATTGTTACCACCAGGTGAATTGGACAATTACTGGACAGGACCGGATGAGG  
 ATAAGATATATACTTGTACTTACAAAACTGTGGGAAAAAATTCACTCGTTCGATACA  
 ATGTGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTCAATTCT  
 GTCCGAAAAGATTTGTTCAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTG  
 5 AAGCTAGATATAGCAAATGTCCTTGTGGTAAAGAGTTTGCTAGATTAGATGCATTAA  
 GAAAACATCAGGATCGAAATATTTGTGTTGGTGGGAATAAAAAATGTTATTAGTAAAC  
 CCACAAAGAAAAAGGGAACATAAACAACACTCAACAACAATTGCTTAAAACAGATACAG  
 TGGTTGAGAGGATAGAAAAACAGTTGCTACAGGAAGATAAGAGTGTTACTGA  
 GGAGTTTTTAATGTTGCAATAG

10

**SEQ ID NO 16:**

>gi|168487564|ref|XM\_707238.1| *Candida albicans* SC5314  
 hypothetical transcription factor (Ca019.6124) partial mRNA  
 ATGCATTGGAAATTTCTGAACTTTTCGAAAGTACCATCTTTCTTTCCATTTAAATTTAT  
 15 TTGATCTTTCTCTCTTTTTTATTTCCTTTTACTGCTTTCCCATCCTATATATTTGTTT  
 CTTTAATCAAGTCCATTCATTCAGAAGCACACAACCAAGTCTTATAATGAATAAATTT  
 GACTTGTTTGATGATTACTCGACCAAAGGGTCAACCATCCCTCTTCCCTAATGAAAATT  
 TTGACCAATTATTTTTAAGTTCCGAAGCCAATGATATGGAATTTTTGTTAATGAAAC  
 ATTGATGGGATTACAAGATTTAGATGTTCTTCTGGTTATGGAATTTCCCAAATACC  
 20 ATTAATAACGATTTCCAACACACACCAAATAAGTCAAAATCTCACAGTAGACAATACA  
 GTGGAACAGCAATTTTTGGTTTTGCTGACCATAATAAAGATTTGTCCATTAACGGTGT  
 CAACAATGACTTGTGCAAACAATCCAATAAAGCCATCAATACACAATCGGTTTCACCT  
 GGTGAGTTGTTGAAACGTTCAAGAGGTTCTCAAACACCAACACCAACATCAGCACTAC  
 CAGACACTGCCCAAGATATTTTAGATTTTAATTTTGAGGAAAAACCAATATTGTTGCT  
 25 TGAGGAGGATGAGCTTGAGGAAGAAAAACATAAACAACAACAAGAATGATGACACAG  
 CTGTCACCTTTGAAGAGAGTAACCACTCCTAGTCAATCACCATTTGTTCAACAGCCAC  
 AAACCATGAAACAAAGAAAGCCACACAAGAAGACAAACGAGTACATTGTTGCCAATGA  
 AAATCCCAATTCGTACAAATTTCCACCATCTCCATCTCCGACTGCCAAACGCCAGCAG  
 TATCCACCATCATCTCCAATACCATACAATCCAAAATCAGATTCAGTTGGTGGCAATT  
 30 CTTACTCTGCAAATACTTGCAGTCTTTAAACAAAACCCAGCAAATGAAATATGTGGA  
 TGATATTGAGCCACTTTTACAGGAAGATAATAACAATATGAAATATATTTCCAATCCC

GTACAAGAGCCAATGCTGTATCAAAAACAGAAACCAGTGACACCTCCATTACAGTCAC  
 AAAATGATTCTCAACAATTAGAACCATTGAAGACACCACAACCACAACCAAAACAACA  
 ACAACAGCAACAGCAACCAAATAATGAACAGGATAAAGAATTTACTGCTAATATCAAT  
 TTCAACACCTTCTTACCTCCACCTACTCCGCCTAATTTGATTAATGGATCTCCCGATT  
 5 GGAATTCATCGCCAGAACCACATTCTCCTTCCCCAGGAAGATTGCAACCACCGCAACA  
 GATATCCCCAATTCACCAGAATTTAGGTGCCATGGGTAATAATATCAACTTTTATACA  
 CCCATGTATTACGAGCTTCCAGTACAAGCTGAACAACCGCAACCACAACCACAACCAC  
 ATCAACAGCAACATCAACAGCAACAGCATCAACCTGAATTACAAAACACTTACCAACA  
 AATTAACACATTCAACAGCAACAGCAAATGCTACAACATCAGTTTCACAACCAAAAC  
 10 AATCAACTCCGCCAACACACCCCAAATCAATTCAGAACCAGAACCAGAACCAGAACC  
 AAAATCAAACCTAAGACTCCTTACTCACAACAAAGTCAATTCTCACCTACACATTCCAA  
 TTTTAATTTGTCTCCTGCAAAACAACCTAATTCAAATGTAGGTTCCATGCATTTATCA  
 CCTTTGAAAAACAATTACCAAACACTCCCAAAAGCAACCCCTGTACCATTGAAT  
 GGAGTCCAGTTATATCACCAAACCTCAAAGCAACCATTACACAAACAAATCAAAGAATC  
 15 ATCACCACGACGCCGAATCAAAAAGACTTCATTGTTACCACCAGGTGAATTGGACAAT  
 TACTGGACAGGACCGGATGAGGATAAGATATATACTTGTACTTACAAAACCTGTGGGA  
 AAAAATTCACTCGTCGATACAATGTGCGAAGTCATATTCAGACACATTTAAGTGATCG  
 TCCATTTGGTTGTCAATTCTGTCCGAAAAGATTTGTCAGACAACATGATTTGAATCGT  
 CACGTGAAAGGGCACATTGAAGCTAGATATAGCAAATGTCCTTGTGGTAAAGAGTTTG  
 20 CTAGATTAGATGCATTAAGAAAACATCAGGATCGAAATATTTGTGTTGGTGGGAATAA  
 AAATGTTATTAGTAAACCCACAAAGAAAAGGGAACCTAATAACACTCAACAACAATTG  
 CTTAAAACAGATACAGTGGTTGAGAGGATAGAAAACAGTTGCTACAGGAAGATAAGA  
 GTGTTACTGAGGAGTTTTTAATGTTGCAATAG

25 **SEQ ID NO 17:**

>*C. tropicalis* Ace2 from whole genomic DNA sequence. Assembled manually (17146-19101)

ATCATGGATAAGTTTGATTTATTTAGTGACTTTTCAAGTAGACCTTCAACTATACCT  
 TTACCTAGTGATAATTTGATCAATTTTTATTAACCTCAGAACCAAATGATATTGAT  
 30 TTCTTATTTAACGAACTTTAAATGGTTTACAAGATTTAGATGTTCTTCTGGTTAC  
 GGGTTTAAACCAACAACAAGCACCATCACAACAACAACAGCCATATCATTACCTCAT  
 AGATCTAAAACACATAGTCGACAATATAGTGGTACTGCAATTTTTGGTTTCACAGAA

CATACAAGAGACTTATCTATAAACGGAATTAATAATGACTTGTATAAAACAATATGTG  
 AATAAAGGACCTGAAAATAATTCTATTTACCAAACGATTTATTAAGAGAAACCAAT  
 AATCATCAACAAAATCCAATACCAACACAAGATATTTTAAATTTCAATTTTGATGAA  
 AAACCAATACTTTTATTAGAACAAGATGAGTTGGAAGATGAGAAACATTATTCGGCT  
 5 AATATTTACAATCCTTACAAAATCACAACCACAGGAATATGTTGATGATATTGGT  
 CCATTATTACAAGGAGATGAATTGTCTGATATTAAGTATATCCCCATTCCCGTTCAG  
 GAACCAGTTGTGTTTGATAAACAAGAAAAAGGAATTCAGCCCCATTGGATCAATCA  
 GAAGAATCAAAACAACCATCAAGAAAAGAACAATCTGACCAACAACAGGTACCACAA  
 CCAGTACCACAACCAGCAGCAGCACCACCACAACAGAAACAACAACATTTCCAGGA  
 10 AATTTCAATTTCAATACATATTTACCACCTCCTTCTTCACCACCACCTAATTTGAGT  
 AATGGGTCTCCTGACTGGAATAGCTCTCCAGAACCACAATCTCCTTCGCCAAGTAGA  
 TTACAACCAAATCAACAAATTTGCCAGTTCATCAAATTTACGTGCCATGGGTAGC  
 AATGTGAATTTTTATAATCCTGTGTATTATGAACAACATCCTCAACAACCTCCACCT  
 TCACTACCACCTCATTCTCAACCTTCTCAGCAACTGCAACTGCCTGGAATGCGTCAG  
 15 ACTTACGAACAAATCAAACAAATTCACAACAACAACAATGTTGCAACAACAATTT  
 CACTCAACAACAACCACTCAACAACAACCTCAATCATCACCAATTTATTCCACTTTG  
 AATTCATCACCAATTAACAATTTAATCCTGATACAGTGAATAACTTACAAATATTT  
 CACCTTTGAAAACCAATTACCAAGTACACCAACTAAACAACAACAAATTTCCAAGTTA  
 CTATTGAATGGAGCCCTGTTATTTACCAAATTCCAAAGCATCATTATCTAAACAAC  
 20 TCAAAGAATCATCATTACAAACGTCGCCACGTAGAAGAATFAAAAAGACTTCATTAT  
 TGCCACCTGGAGAACTTGATAATTATTGGATTGGACCCGATGAAAATAAAATTTATA  
 CATGTACTTATAAAAACCTGTTTTAAAAAATTTACAAGAAGATATAATGTTAGAAGTC  
 ATATTCAAACCTCATTTAAGTGATAGACCATTTGGATGTCAATTTTGTCTAAAAGAT  
 TTGTTGACAACATGATTTAAATCGTCATGTTAAAGGACATATTGAAGCAAGATATA  
 25 GTAAATGTCCATGTGGTAAAGAATTTGCAAGATTAGATGCTTTAAGAAAAACA  
 TCAAGATCGAAATATTTGTATTGGTGGTAACAAA

**SEQ ID NO 18:**

> gi1460199391emblAJ630371.11 *Candida glabrata*  
 30 Acc2 gene for transcription factor  
 ATGAATACTTTCCAGGCGGATTGGGGAGAGATTCCTCAAATGCCAAAGGACCAAGTG  
 TTCACGCCCCAGGACCAACTAACGAATTACAACGATAACATGATGGACAACCTTGCTT

GACTTCAATTATAACGATGTTGATGCTCTCCTGTCGGAGGAGTTGAAGGACTTAGAT  
ATACCATTGGCGCCTTCGCCTAGAGACTTGAATATGAATGCCGAACAAAGTCTCAAT  
TGGATGCAGGATATACAGGGACACAGGTCCAATAAACCTTCGATGTTCGCATAAGAGA  
GGAATGAGTGGTACCGCAATATTTGGCTTCAAAAATCATAACAAAACCTCAGCATT  
5 GCTAGTTTTAGCAAGAATACAGACATTATCAATGAAGCTGAGAACGAGAATGTAAAA  
GGAAACACAGATAACCAGAATGGCTTTGTATTAAGCCAAGTTCTTCTGAAACAGCAG  
GAAGAGCTTCGATTAGCTTTAGAAAAACAGAAAGAAGTAAATAGAACTTAGAGCGG  
CAACTGCGAGAAAATAGATTACAACAAGAACATATACAGCGTGTGCTTCACGATCAG  
GAAGCTGTAACAAGTCAATTAACAGCTCAAAACGTAACCTGAATCGCCATCAAAACAA  
10 AGAAGTCCAACCAAATATCAAGGAGATGACGCCATCATAGTTACAAAAACTCATCT  
TCTGGCGGCTATGTTTTCCACCTCCACCTCGAGTTACATTAATAACGAAGCTGTT  
ACTCCACCGCTATCATTCTCAAGGTTTAGCAATATTGATCAAATGGAATCTTCAGAT  
CCACTCAATTACCTGCAACCAAATGCTGATTTACAGAGGCTTATGCATCACACAAG  
ACACCCGAATCATCATTTGGAAAGGAACATGCTTCAGTGCTTCAACATCGGAATTT  
15 TTGAGACCTACCAATGCTGCTAGAGAATCATCATCAAAGGCAATGTATAGCTCACCA  
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ATTTTACAACCCCAAGATGACTATCAGAACACTGCAAGTCCGCCTTCGCAAATGTTG  
AACTTAGAAGAAGCCAATTTAGAGAATGAACAAAAGAACAATGGGAAAATGCTACGT  
GCACCAGTAGAAATTATGCCAACTATACCGGGATCAAAAAATAATACTCCCATGACT  
20 GCTAATAAGTCTGGATTCATGCCTCAGAAACATACTTTCCAGCAT  
ACTCCTGTGAAAGCGAAAAATAATGTGGACATGAACGAGAGATCATTGGTACGGCCG  
GAGCTATCAGGAACACCACTCAATAAGAGCGTACAAAATGGAATGCATTTTAGAGAA  
GAGGACGACAGTAATATATTGCACCATATATCAGAAATACCGCAAGGATCAACATCA  
CATAATAATACCACCGCTGGCGATGATTCAAATGTGGACAATAGGTTACAATTTTCT  
25 AATACCGAAAGTAGTCCAAGCCGGCAAAGGAAAAACCTACTACATTACCACCAGGT  
TATATTGATCGATTTGTAAAGAACTACCTGACAAGAATTTTGAATGTTTATTTCT  
AACTGTGGTAAATTTTTTAGGAGAAGGTATAATATAAAGTCCCATATTCAAACACAT  
CTAGAAGATAAACCATATAAGTGTGACTTTGAGGGATGCACTAAAGCGTTTGTGAGG  
AATCATGACCTTGCTAGACACAAGAAGACTCATGATAAGCATTTTTCTGTCCCTGC  
30 GGGAAAAAATTCAGCTCAGAACAATCAATGATGAAGCACAAGAATAGGCATAACTGC  
ACTGGCCCTGCCAGAGTTCCTGATTCAAAATGGTATCCAAGTCTCCTAGAAAACAA  
TCTTCTCCCACTAAACTTTCTTCAGCGATAATGAATAGCCCCATAAAGGAAAATTAT

CTGAAAGAAAATACCAACCTACATATCGATCAATTAAGAATGGATCCTAAAATGAGA  
 AATGCACTAGAGGATGGGGGGCTATTGAAGCCAGTTGAGAGAACTGAAGCAATGGCT  
 TTTCCATCTCCACTATCGGGATATAGTGATTTGGGTCCCCTTTTAGAGATTTAGGG  
 ACGATTGAGGAGTAG

5

**SEQ ID NO 19:**

>gi|50294246|ref|XM\_449535.1| *Candida glabrata* CBS138 hypothetical protein  
 (CAGL0M0323g) partial mRNA

ATGAATACTTTCCAGGCGGATTGGGGAGAGATTCCTCAAATGCCAAAGGACCAAGTGT  
 10 TCACACCCCAGGACCAACTAACGAATTACAACGATAACATGATGGACAACTTGCTTGA  
 CTTCAATTATAACGATGTTGATGCTCTCCTGTCCGAGGAGTTGAAGGACTTAGATATA  
 CCATTGGCGCCTTCGCCTAGAGATCTGAATATGAATGCCGAACAAAGTCTCAATTGGA  
 TGCAGGATATACAGGGACACAGGTCAAATAAACCTTCGATGTTCGCATAAGAGAGGAAT  
 GAGTGGTACCGCAATATTTGGCTTCAAAAATCATAACAAAACCTCAGCATTGCTAGT  
 15 TTTAGCAAGAATACAGACATTATCAATGAAGCTGAGAACGAGAATGTAAAAGGAAACA  
 CAGATAACCAGAATGGCTTTGTATTAAGCCAAGTTCTTCTGAAACAGCAGGAAGAGCT  
 TCGATTAGCTTTAGAAAAACAGAAAGAAGTAAATAGAACTTAGAGCGGCAACTGCGA  
 GAAAATAGATTACAACAAGAACATATACAGCGTGTGCTTCAAGATCAGGAAGCTGTAA  
 CAAGTCAATTAACAGCTCAAAACGTAAGTGAATCGCCATCAAAACAAAGAAGTCCAAC  
 20 CAAATATCAAGGAGATGACGCCATCATAGTTACAAAAACTCATCTTCTGGCGGCTAT  
 GTTTTCCACCTCCACCTCGAGTTACATTAAATAACGAAGCTGTTACTCCACCGCTAT  
 CATTCTCAAGGTTTAGCAATATTGATCAAATGGAATCTTCAGATCCACTCAATTACCT  
 GCAACCAAATGCTGATTTACAGAGGCTTATGCATCACACAAGACACCCGAATCATCA  
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 25 CTGCTAGAGAATCATCATCAAAGGCAATGTATAGCTCACCAAACAGTATGATFTCACC  
 TCATAGGAAAAAAGATTCTGTTCTCTCAACAGTTTCTACTATTTTACAACCCCAAGAT  
 GACTATCAGAACAAGTCCGCCTTCGCAAATGTTGAACTTAGAAGAAGCCAATT  
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 CTATACCGGGATCAAAAAATAATACTCCCATGACTGCTAATAAGTCTGGATTCATGCC  
 30 TCAGAAACATACTTTCCAGCATACTCCTGTGAAAGCGAAAAATAATGTGGACATGAAC



GAGAGATCATTTGGTACGGCCGGAGCTATCAGGAACACCACTCAATAAGAGCGTACAAA  
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ACCGCAAGGATCAACATCACATAATAATACCACCGCTGGCGATGATTCAAATGTGAGC  
AATAGGTTACAATTTTCTAATACCGAAAGTAGTCCAAGCCGGCAAAGGAAAAAGCCTA  
5 CTACATTACCACCAGGTTATATTGATCGATATGTTAAAGAACTACCTGACAAGAATTT  
TGAATGTTTATTTCTAACTGTGGTAAATTTTTTAGGAGAAGGTATAATATAAAGTCC  
CATATTCAAACACATCTAGAAGATAAACCATATAAGTGTGACTTTGAGGGATGCACTA  
AAGCGTTTGTGAGGAATCATGACCTTGCTAGACACAAGAAGACTCATGATAAGCATT  
TTCTTGTCCCTGCGGGAAAAAATTCAGCTCAGAACAATCAATGATGAAGCACAAGAAT  
10 AGGCATAACTGCACTGGCCCTGCCAGAGTTCCTGATTCCAAAATGGTATCCAAGTCTC  
CTAGAAAACAATCTTCTCCCACTACTTTCTTCAGCGATAATGAATAGCCCCATAAAGG  
AAAATTATCTGAAAGAAAATACCAACCTACATATCGATCAATTAAGAATGGATCCTAA  
AATGAGAAATGCACTAGAGGATGGGGGGCTATTGAAGCCAGTTGAGAGAACTGAAGCA  
ATGGCTTTTCCATCTCCAATATCGGGATATAGTGATTTGGGTTCCTTTTAG  
15 AGATTTAGGGACGATTGAGGAGTAG

**SEQ ID NO 20:** > ACF1 primer

ATCAAAGAATCATCACCA

20

**SEQ ID NO 21:** > ACF2 primer

AGACTTCATTGTTACCAC

25 **SEQ ID NO 22:** > ACF2b primer

AAGACTTCATTGTTACCACC

**SEQ ID NO 23:** > ACF2c primer

30 AGACTTCATTGTTACCACCAG

**SEQ ID NO 24:** > ACF3 primer

CACCAGGTGAATTGG

**SEQ ID NO 25:** > ACR1 primer

5 CATTGTATCGACGAGTG

**SEQ ID NO 26:** > ACR2 primer

10 TGTATCGACGAGTGAAT

**SEQ ID NO 27:** > ACR3 primer

15 TTCGCACATTGTATCG

**SEQ ID NO 28:** > ACR3b primer

ACTTCGCACATTGTATCG

20 **SEQ ID NO 29:** > ACR3c primer

TGACTTCGCACATTGTATCG

**SEQ ID NO 30:** > ACALB1 probe

25 ATATCTTATCCTCATCCGGTCCT

**SEQ ID NO 31:** > ACALB2 probe

30 AGGACCGGATGAGGATAAGATAT

**SEQ ID NO 32:**

>CA3345/ACE2-Ace2 sequence generated for *C. albicans*

AAACACTCCCACAAAGCAACCCCTGTCACCATTGAATGGAGTCCAGTTATATCACCAA  
ACTCAAAGCAACCATTACACAAACAAATCAAAGAATCATCACCACGACGCCGAATCAAA  
35 AAGACTTCATTGTTACCACCAGGTGAATTGGACAATTACTGGACAGGACCGGATGAGGA  
TAAGATATATACTTGTACTTACAAAACTGTGGGAAAAAATTCACTCGTTCGATACAATG

TGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTCAATTCTGTCCG  
 AAAAGATTTGTCAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGAAGCTAG  
 ATATAGCAAATGTCCTTGTGGTAAAGAG

5 **SEQ ID NO 33:**

>CA16733/ACE2-Ace2 sequence generated for *C. albicans*

AAACACTCCCACAAAGCAACCCCTGTCAACCATTTGAATGGAGTCCAGTTATATCACCAA  
 ACTCAAAGCAACCATTACACAAACAAATCAAAGAATCATCACCACGACGCCGAATCAAA  
 AAGACTTCATTGTTACCACCAGGTGAATTGGACAATTA CTGGACAGGACCGGATGAGGA  
 10 TAAGATATATACTTGTACTTACAAAACTGTGGGAAAAAATTC ACTCGTCGATACAATG  
 TGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTCAATTCTGTCCG  
 AAAAGATTTGTCAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGAAGCTAG  
 ATATAGCAAATGTCCTTGTGGTAAAGA

15 **SEQ ID NO 34:**

>CA1899/ACE2-Ace2 sequence generated for *C. albicans*

AAACACTCCCACAAAGCAACCCCTGTCAACCATTTGAATGGAGTCCAGTTATATCACCAA  
 ACTCAAAGCAACCATTACACAAACAAATCAAAGAATCATCACCACGACGCCGAATCAAA  
 AAGACTTCATTGTTACCACCAGGTGAATTGGACAATTA CTGGACAGGACCGGATGAGGA  
 20 TAAGATATATACTTGTACTTACAAAACTGTGGGAAAAAATTC ACTCGTCGATACAATG  
 TGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTCAATTCTGTCCG  
 AAAAGATTTGTCAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGAAGCTAG  
 ATATAGCAAATGTCCTTGTGGTAAAGAGTT

25 **SEQ ID NO 35:**

>CA1912/ACE2-Ace2 sequence generated for *C. albicans*

AAACACTCCCACAAAGCAACCCCTGTCAACCATTTGAATGGAGTCCAGTTATATCACCAA  
 ACTCAAAGCAACCATTACACAAACAAATCAAAGAATCATCACCACGACGCCGAATCAAA  
 AAGACTTCATTGTTACCACCAGGTGAATTGGACAATTA CTGGACAGGACCGGATGAGGA  
 30 TAAGATATATACTTGTACTTACAAAACTGTGGGAAAAAATTC ACTCGTCGATACAATG  
 TGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTCAATTCTGTCCG  
 AAAAGATTTGTCAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGAAGCTAG  
 ATATAGCAAATGTCCTTGTGGTAAAGAG

**SEQ ID NO 36:**

>CA2312/ACE2-Ace2 sequence generated for *C. albicans*

AAACACTCCCACAAAGCAACCCCTGTCACCATTGAATGGAGTCCAGTTATATCACCAA  
 5 ACTCAAAGCAACCATTACACAAACAAATCAAAGAATCATCACCACGACGCCGAATCAAA  
 AAGACTTCATTGTTACCACCAGGTGAATTGGACAATTA CTGGACAGGACCGGATGAGGA  
 TAAGATATATACTTGTACTTACAAAACTGTGGGAAAAAATTCACTCGTCGATAACAATG  
 TGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTCAATTCTGTCCG  
 AAAAGATTTGTCAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGAAGCTAG  
 10 ATATAGCAAATGTCCTTGTGGTAAAGAGT

**SEQ ID NO 37:**

>CA2688/ACE2-Ace2 sequence generated for *C. albicans*

AAACACTCCCACAAAGCAACCCCTGTCACCATTGAATGGAGTCCAGTTATATCACCAA  
 15 ACTCAAAGCAACCATTACACAAACAAATCAAAGAATCATCACCACGACGCCGAATCAAA  
 AAGACTTCATTGTTACCACCAGGTGAATTGGACAATTA CTGGACAGGACCGGATGAGGA  
 TAAGATATATACTTGTACTTACAAAACTGTGGGAAAAAATTCACTCGTCGATAACAATG  
 TGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTCAATTCTGTCCG  
 AAAAGATTTGTCAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGAAGCTAG  
 20 ATATAGCAAATGTCCTTGTGGTAAAGAGTT

**SEQ ID NO 38:**

>CA2701/ACE2-Ace2 sequence generated for *C. albicans*

AAACACTCCCACAAAGCAACCCCTGTCACCATTGAATGGAGTCCAGTTATATCACCAA  
 25 ACTCAAAGCAACCATTACACAAACAAATCAAAGAATCATCACCACGACGCCGAATCAAA  
 AAGACTTCATTGTTACCACCAGGTGAATTGGACAATTA CTGGACAGGACCGGATGAGGA  
 TAAGATATATACTTGTACTTACAAAACTGTGGGAAAAAATTCACTCGTCGATAACAATG  
 TGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTCAATTCTGTCCG  
 AAAAGATTTGTCAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGAAGCTAG  
 30 ATATAGCAAATGTCCTTGTGGTAAAGA

**SEQ ID NO 39:**

>CA15640/ACE2-Ace2 sequence generated for *C. albicans*

AAACACTCCCACAAAGCAACCCCTGTCACCATTGAATGGAGTCCAGTTATATCACCAA  
ACTCAAAGCAACCATTACACAAACAAATCAAAGAATCATCACCACGACGCCGAATCAA  
AAGACTTCATTGTTACCACCAGGTGAATTGGACAATTACTGGACAGGACCGGATGAGGA  
TAAGATATATACTTGTACTTACAAAACTGTGGGAAAAAATTCACTCGTCGATAACAATG  
5 TGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTCAATTCTGTCCG  
AAAAGATTTGTGACACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGAAGCTAG  
ATATAGCAAATGTCCTTGTGGTAAAG

**SEQ ID NO 40:**

10 >T94/ACE2 - Ace2sequence generated for *C. tropicalis*  
AAGATACACCAACTAAACAACAAAATTCCCAAGTTACTATTGAATGGAGCCCTGTTATT  
TCACCAAATTCCAAAGCATCATTATCTAAACAACCTCAAAGAATCATCATTACAAACGTC  
GCCACGTAGAAGAATTA AAAAGACTTCATTATTGCCACCTGGAGAACTTGATAATTATT  
GGATTGGACCCGATGAAAATAAAATTTATACATGTACTTATAAAAAC TTTTTAAAAA  
15 TTTACAAGAAGATATAATGTTAGAAGTCATATTCAAACTCATTTAAGTGATAGACCATT  
TGGATGTCAATTTTGTCTAAAAGATTTGTTTCGACAACATGATTTAAATCGTCATGTTA  
AAGGACATATTGAAGCAAGATATAGTAA

**Claims**

1. A diagnostic kit for a yeast or fungal species comprising an oligonucleotide probe capable of binding to at least a portion of the Ace2 gene or its corresponding mRNA.
- 5 2. A kit as claimed in claim 1, wherein the portion of the Ace2 gene is a portion of the region of the gene from base pair position 1736 to base pair position 2197 *C. albicans* Ace2 gene.
3. A kit as claimed in any of claim 1 or claim 2, wherein the probe is selected from the group consisting of SEQ ID NOs: 3, 30, 31 or sequences substantially similar or  
10 complementary thereto which can also act as a probe, and/or wherein the kit further comprises a forward amplification primer being selected from the group consisting of SEQ ID Nos: 1, 20-24, and sequences substantially similar or complementary thereto which can also act as a forward amplification primer and a reverse amplification  
15 primer being selected from the group consisting of SEQ ID NOs: 2, 25-29, and sequences substantially similar or complementary thereto which can also act as a reverse amplification primer..
4. A nucleic acid molecule selected from the group consisting of: SEQ ID NO 1 through SEQ ID NO 40 and sequences substantially homologous or substantially  
20 complementary thereto or to a portion thereof and having a function in diagnostics based on the Ace2 gene.
5. A kit substantially as described herein with reference to the accompanying figures.



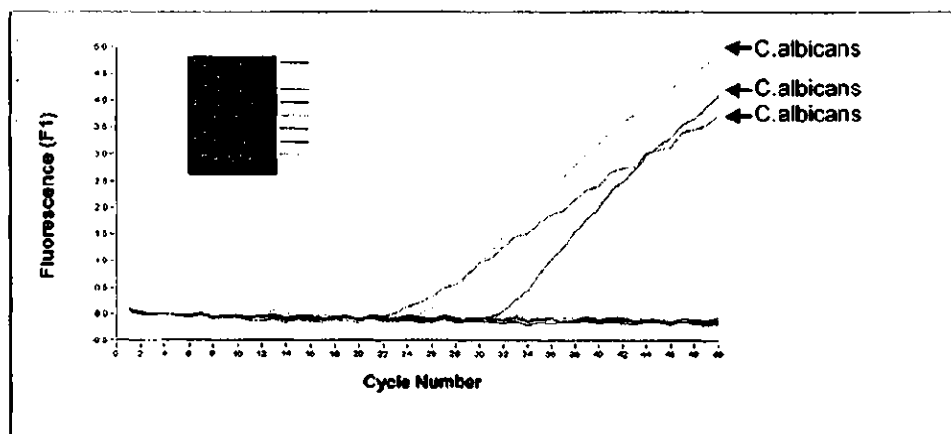


Figure 3



(a)

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GAATTGGACAATTACTGGACAGGACCGGATGAGGATAAGATATATACTTGTACTTACAAAAACTGTGGGAA  
AAAATT**CCTCGTCGATACAATGTGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTC**  
AATTCTGTCCGAAAAGATTTGTGAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGAAGCTAGA  
TATAGCAAATGTCCTTGTGGTAAAGAGTTTGCTAGATTAGATGCATTAAGAAAACATCAGGATCGAAATAT  
TTGTGTTGGTG

(b)

AAACACTCCCACAAAGCAACCCCTGTCACCATTGAATGGAGTCCAGTTATATCACCAAACCTCAAAGCAAC  
CATTACACAAACAAATCAAAGAATCATCACCACGACGCCGAATCAAAAAGACTTCATTGTTACCACCAGGT  
GAATTGGACAATTACTGGACAGGACCGGATGAGGATAAGATATATACTTGTACTTACAAAAACTGTGGGAA  
AAAATT**CCTCGTCGATACAATGTGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTC**  
AATTCTGTCCGAAAAGATTTGTGAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGAAGCTAGA  
TATAGCAAATGTCCTTGTGGTAAAGAGTTTGCTAGATTAGATGCATTAAGAAAACATCAGGATCGAAATAT  
TTGTGTTGGTG

(c)

AAACACTCCCACAAAGCAACCCCTGTCACCATTGAATGGAGTCCAGTTATATCACCAAACCTCAAAGCAAC  
CATTACACAAACAAATCAAAGAATCATCACCACGACGCCGAATCAAAAAGACTTCATTGTTACCACCAGGT  
GAATTGGACAATTACTGGACAGGACCGGATGAGGATAAGATATATACTTGTACTTACAAAAACTGTGGGAA  
AAAATT**CCTCGTCGATACAATGTGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTC**  
AATTCTGTCCGAAAAGATTTGTGAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGAAGCTAGA  
TATAGCAAATGTCCTTGTGGTAAAGAGTTTGCTAGATTAGATGCATTAAGAAAACATCAGGATCGAAATAT  
TTGTGTTGGTG

(d)

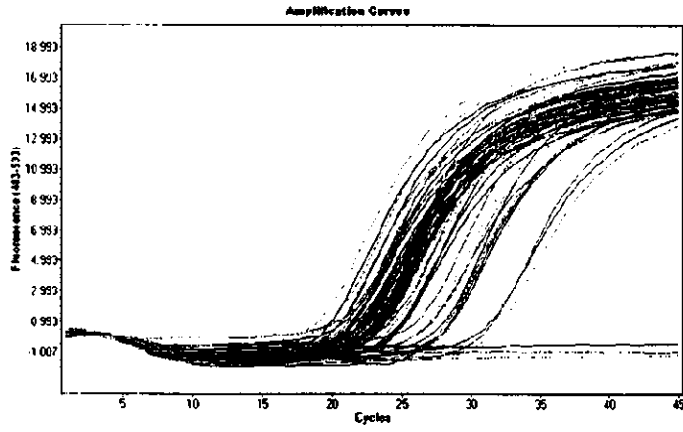
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GAATTGGACAATTACTGGACAGGACCGGATGAGGATAAGATATATACTTGTACTTACAAAAACTGTGGGAA  
AAAATT**CCTCGTCGATACAATGTGCGAAGTCATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTC**  
AATTCTGTCCGAAAAGATTTGTGAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATTGAAGCTAGA  
TATAGCAAATGTCCTTGTGGTAAAGAGTTTGCTAGATTAGATGCATTAAGAAAACATCAGGATCGAAATAT  
TTGTGTTGGTG

**Figure 4.**

ATGCATTGGAAATTTCTGAACTTTCGAAAGTACCATCTTCTTTCCATTTAAATTTATTTGATCTTTCTCT  
 CTTTTTTATTTTCTTTTACTGCTTTCCCATCCTATATATTTGTTTCTTTAATCAAGTCCATTCATTCAGAA  
 GCACACAACCAAGTCTTATAATGAATAAAATTTGACTTGTTTGATGATTACTCGACCAAAGGGTCAACCATC  
 CCTCTTCTAATGAAAATTTTGACCAATTATTTTTAAGTTCGGAAGCCAATGATATGGAATTTTTGTTTAA  
 TGAAACATTGATGGGATTACAAGATTTAGATGTTCTTCTGTTTATGGAATTCGCCAAAATACCATTAATA  
 ACGATTTCCAACACACACCAAATAAGTCAAAATCTCACAGTAGACAATACAGTGGAACAGCAATTTTTGGT  
 TTTGCTGACCATAATAAAGATTTGTCCATTAACGGTGTCAACAATGACTTGTGCAAACAATCCAATAAAGC  
 CATCAATACACAATCGGTTTTACCTGGTGAGTTGTTGAAACGTTCAAGAGGTTCTCAAACACCAACACCAA  
 CATCAGCACTACCAGACACTGCCAAAGATATTTTAGATTTTAAATTTTGAGGAAAAACCAATATGTTGCTT  
 GAGGAGGATGAGCTTGAGGAAGAAAAACATAAAACAACAACAAGAATGATGACACAGCTGTCCCTTTGAA  
 GAGAGTAACCACTCCTAGTCAATCACCATTTGTTCAACAGCCACAAACCATGAAACAAGAAAGCCACACA  
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 CAATCTTACTCTGCAAAATACTTGCACTCTTTAAACAAAACCCAGCAAATGAATATGTGGATGATATTG  
 AGCCACTTTTACAGGAAGATAATAACAATATGAAATATATTCCAATTCCTGTACAAGAGCCAAATGCTGTAT  
 CAAAAACAGAAACCAGTGACACCTCCATTACAGTCACAAAATGATTCTCAACAATTAGAACCATTGAAGAC  
 ACCACAACCACAACCAAAAACAACAACAGCAACAGCAACCAAATAATGAACAGGATAAAGAATTTACTG  
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 CCAGAATTTAGGTGCCATGGGTAATAATATCAACTTTTATACACCCATGTATTACGAGCTTCCAGTACAAG  
 CTGAACAACCGCAACCACAACCACAACCACATCAACAGCAACATCAACAGCAACAGCATCAACCTGAATTA  
 CAAAACACTTACCAACAAATTAACACATTTCAACAGCAACAGCAAATGCTACAACATCAGTTTCAACAACA  
 AAACAATCAACTCCGCCAACAACCCCAAATCAATTCCAGAACCAGAACCAGAACCAGAAATCAAAA  
 CTAAGACTCCTTACTCACAACAAAGTCAATTTCTCACCTACACATTTCAATTTTAAATTTGTCTCTGCAAAA  
 CAACTTAATTCAAATGTAGGTTCCATGCATTTATCACCTTTGAAAAACAATTACCAAACACTCCACAAA  
 GCAACCCCTGTCAACCATTGAATGGAGTCCAGTTATATCACCAACTCAAAGCAACCATTACACAACAACA  
 TCAAGAATCATCACCACGACGCCGAATCAAAATGATGAGGTTGAGGTTGAGGTTGAGGTTGAGGTTGAGGTT  
TGGACAGGACCGGATGAGGATAAGATATATACTTGTAATACAAAACTGTGGGAAAAAATTCACTCGTCC  
ATACATCTGCGGAG CATATTCAGACACATTTAAGTGATCGTCCATTTGGTTGTCAATTCGTCCGAAAA  
 GATTTGTCAGACAACATGATTTGAATCGTCACGTGAAAGGGCACATGAAAGCTAGATATAGCAAATGTCTT  
 TGTGGTAAAGAGTTTGCTAGATTAGATGCATTAAGAAAACATCAGGATCGAAATATTTGTGTTGGTGGGAA  
 TAAAAATGTTATTAGTAAACCCACAAGAAAAAGGGAACATAAACAATCAACAACAATGCTTAAAAACAG  
 ATACAGTGGTTGAGAGGATAGAAAAACAGTTGCTACAGGAAGATAAGAGTGTACTGAGGAGTTTTTAATG  
 TTGCAATAG      2352bp

**Figure 5.**

(a)



(b)

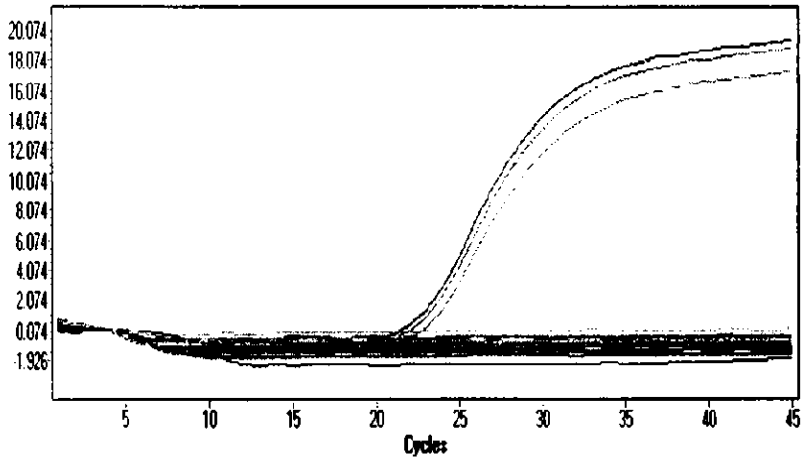


Figure 6.

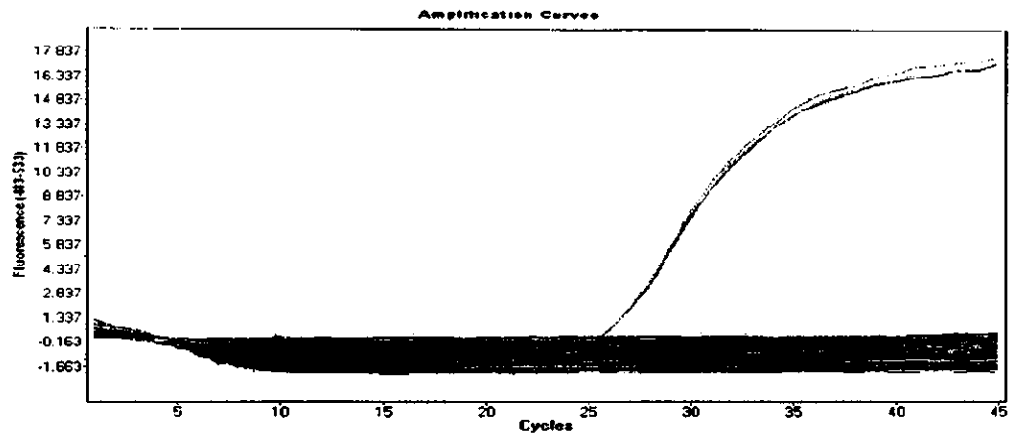
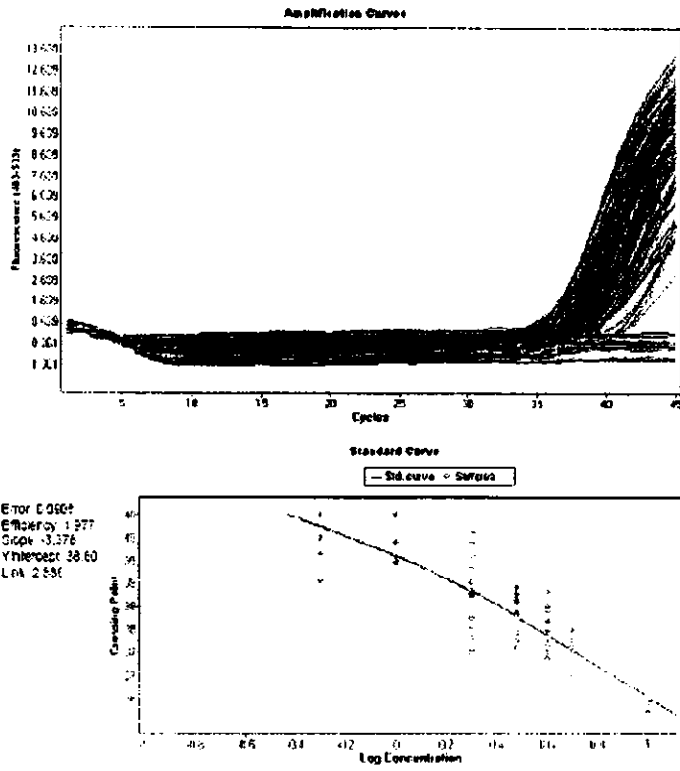


Figure 7.



**Figure 8.**

MC0370