(19) DANMARK

(10) DK/EP 3382041 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *C 12 Q 1/6895 (2018.01)*

(45) Oversættelsen bekendtgjort den: **2021-10-25**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2021-09-01**

(86) Europæisk ansøgning nr.: **18163653.1**

(86) Europæisk indleveringsdag: **2018-03-23**

(87) Den europæiske ansøgnings publiceringsdag: **2018-10-03**

(30) Prioritet: **2017-03-30 EP 17000524**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

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(54) Benævnelse: **Assay til diagnosticering af dermatofytose**

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Description

[0001] The present invention relates to a primer pair comprising a forward primer and a reverse primer for amplifying a nucleic acid from a pathogen associated with a skin, hair and nail infection comprising SEQ ID NO22, a method comprising the step detecting in a sample a nucleic acid sequence comprising SEQ ID N022 from a pathogen associated with a skin, hair and nail infection, a use of the primer pair for the diagnosis of a disease and a kit comprising the primer pair for the diagnosis of a disease according to the appended claims.

[0002] Human pathogenic dermatophytes, which belong to the three genera Trichophyton, Microsporum and Epidermophyton, are fungi that infect human skin, nails and hair. While the genus Epidermophyton is represented only by a single species (E. floccosum), the genera Microsporum and Trichophyton comprised several different species. Recently, several species formerly assigned to Arthroderma have been reclassified and assigned to Trichophyton (Hoog et al., 2016), and this reassignment will be adhered to throughout this patent application.

[0003] Prevalence rates of dermatophyte skin, hair and nail infections in European countries vary between 3 and 22 %. Topical therapy is sufficient in most cases, but long term and often expensive systemic treatment is necessary if the infection is caused by specific strains of the genera Microsporum and Trichophyton such as T. verrucosum and T. mentagrophytes.

[0004] Systemic antifungals are associated with various side effects such as gastro-intestinal side effects, which occur in 3-5 % of the patients treated orally with terbinafine. In addition, bone marrow suppression and hepatic side effects may occur albeit less frequent. Therefore, the diagnosis of skin, hair and nail infection with a specific strain should be confirmed before a treatment regime is devised and the therapy initiated.

[0005] The current diagnosis of dermatophytes is based on microscopic identification of spores and hyphae in clinical specimens followed by in vitro culture and morphological identification of the fungus. Direct microscopic examination of skin, hair and nail material is often sufficient for the preemptive diagnosis of a fungal infection, but it does not lead to a specific species diagnosis. Although rapid and cheap, this technique has a relatively low sensitivity and shows false negative results in up to 15 % of all cases.

[0006] Application of culture enables specific species identification in 10-15 days in approximately 95 % of cases. However, for some slow growing or atypical isolates time of diagnosis is up to 3-6 weeks. Therefore, a simple rapid and specific method for the diagnosis of dermatophyte infections is required.

[0007] PCR-based methods have been introduced for the diagnosis of fungal infections. For example, US2010/0311041 discloses a method for extracting nucleic acids from fungi, a PCR method for detecting fungi in patient samples and a PCR kit for detecting dermatophytes and for diagnosing infections by the three genera Trichophyton, Microsporum and Epidermophyton.

[0008] WO 01/86003 A2, WO 2007/106407 A2 and Wang et al. (Wang, L. et al., 2006, BMC Genomics, Biomed Central Ltd, London, UK, vol. 7, no.1, 11, page 255) disclose nucleic acids capable of hybridizing to a nucleic acid sequence from T. tonsurans comprising SEQ ID NO:22 or the complementary strand of SEQ ID NO:22.

[0009] However, the methods disclosed in the state of the art have shortcomings. In particular, they do not allow the rapid and reliable distinction of closely related strains from the genus Trichophyton and Microsporum, which includes zoophilic and non-zoophilic species.

[0010] Therefore, a problem underlying the present invention is to provide methods and reagents for the diagnosis of a skin, hair and nail disease, namely a fungal skin, hair and nail infection.

[0011] Another problem underlying the present invention is to provide methods and reagents for identifying and distinguishing from closely related species a pathogen from the genus Trichophyton, in particular T. benhamiae (yellow) and T. concentricum.

[0012] The problem underlying the present invention is solved by the subject matter of the attached independent and dependent claims.

[0013] In a first aspect, the problem underlying the present invention is solved by a primer pair comprising a forward primer and a reverse primer capable of amplifying a nucleic acid from a pathogen associated with a skin, hair and nail infection comprising SEQ ID N022, preferably SEQ ID NO1 according to the appended claims.

[0014] Further described herein is a nucleic acid capable of hybridizing specifically to a nucleic acid sequence from a pathogen associated with a skin, hair and nail infection comprising SEQ ID N022, preferably SEQ ID NO1, or its complementary strand or a vector or cell comprising said nucleic acid sequence according to the appended claims.

[0015] In a preferred embodiment, the primer pair comprises a detectable label, preferably from the group comprising a fluorescent, radioactive, colloidal gold or enzymatically active label.

[0016] In a third aspect, the problem underlying the present invention is solved by a nucleic acid comprising the primer pair according to the present invention and, between the forward and the reverse primer, the nucleic acid sequence from a pathogen associated with a skin, hair and nail infection located in the pathogen's genome between the sequences of the forward and the reverse primer obtained by amplifying a sample comprising said pathogen using the primers according to the present invention as described in the appended claims.

[0017] Further described herein is a carrier comprising the nucleic acid according to the present invention.

[0018] The carrier may be a silane coated glass, plastic or silicon material microarray plate.

[0019] In a fifth aspect, the problem underlying the present invention is solved by a method comprising the step detecting in a sample a nucleic acid sequence comprising SEQ ID N022, preferably SEQ ID NO1, from a pathogen associated with a skin, hair and nail infection according to the appended claims. More preferably the nucleic acid comprises a sequence selected from the group comprising SEQ ID N02, SEQ ID N03, SEQ ID N04, SEQ ID N05, SEQ ID N06, SEQ ID N07, SEQ ID N08 and SEQ ID N09.

[0020] In a preferred embodiment, the method according to the present invention further comprises the steps:

a) providing a sample, preferably nail, hair or skin/nail material, from a patient
b) amplifying any nucleic acid comprising SEQ ID N022, preferably SEQ ID NO1, present in the sample using the primer pair, thus generating an amplicon if a nucleic acid sequence comprising SEQ ID N022 (or SEQ ID NO1) from a pathogen associated with a skin, hair and nail infection is present in the sample. More preferably the nucleic acid comprises a sequence selected from the group comprising SEQ ID N02, SEQ ID N03, SEQ ID N04, SEQ ID N05, SEQ ID N06, SEQ ID N07, SEQ ID N08 and SEQ ID N09 and a variant thereof.

[0021] In a preferred embodiment, the method according to the present invention further comprises the step:

c) detecting the amplicon.

[0022] In a preferred embodiment, the amplicon is detected by fluorescence, radioactivity, colloidal gold or chemiluminescence.

[0023] In a sixth aspect, the problem underlying the present invention is solved by a use of the primer pair, according to the present invention for the diagnosis of a disease, preferably a skin, hair and nail infection associated with a pathogen according to the appended claims.

[0024] In a seventh aspect, the problem underlying the present invention is solved by a kit comprising the primer pair according to the present invention, preferably for the diagnosis of a disease, more preferably a skin, hair and nail infection associated with a pathogen.

[0025] In an eighth aspect, the problem underlying the present invention is solved by a use of the primer pair, the nucleic acid or the carrier according to the present invention for the manufacture of a kit for the diagnosis of a disease, preferably a pathogen associated with a skin, hair and nail infection, more preferably with a skin, hair and nail infection having a nucleic acid comprising SEQ ID N022, preferably SEQ ID NO1 according to the appended claims.

[0026] Further described herein is the use of the primer pair, nucleic acid, carrier, use or kit for the identification of a fungus, preferably from the genus Trichophyton, more preferably from the group comprising T. tonsurans, T. equinum, T. interdigitale, T. benhamiae (african), T. benhamiae (yellow), T. concentricum, T. erinacei (african) and T. erinacei.

[0027] The pathogen is from the group comprising T. tonsurans, T. equinum, T. interdigitale, T. benhamiae (african), T. benhamiae (yellow), T. concentricum, T. erinacei (african) and T. erinacei.

[0028] The present invention is based on the inventors' surprising finding that various pathogens associated with a skin, hair and nail infection have in common a homologous metalloprotease related to the consensus sequence SEQ ID NO22 or SEQ ID NO1, with slight, but distinctive sequence differences between relevant strains, which differences may be used to distinguish in samples such as clinical samples from patients suffering from a skin, hair and nail infection various closely related strains of pathogens associated with a skin, hair and nail infection, from the genus Trichophyton, namely from the group comprising T. tonsurans, T. equinum, T. interdigitale, T. benhamiae (white), T. benhamiae (african), T. benhamiae (yellow), T. concentricum, T. erinacei (african) and T. erinacei.

[0029] The primer pair comprises a forward primer and a reverse primer capable of amplifying a nucleic acid from a pathogen associated with a skin, hair and nail infection, the pathogen is selected from the group comprising T. tonsurans, T. equinum, T. interdigitale, T. benhamiae (white), T. benhamiae (african), T. benhamiae (yellow), T. concentricum, T. erinacei (african) and T. erinacei.

[0030] Further herein described is that the nucleic acid capable of hybridizing specifically to a nucleic acid sequence from a pathogen associated with a skin, hair and nail infection is selected from the group comprising T. tonsurans, T. equinum, T. interdigitale, T. benhamiae (african), T. benhamiae (yellow), T. concentricum, T. erinacei (african) and T. erinacei.

[0031] According to the present invention, a nucleic acid comprising SEQ ID NO22 or 1 from a pathogen associated with a skin, hair and nail infection is detected. SEQ ID N022 or 1 is from a pathogen from the genus Trichophyton, namely from a pathogen from the group comprising T. tonsurans, T. equinum, T. interdigitale, T. benhamiae (white), T. benhamiae (african), T. benhamiae (yellow), T. concentricum, T. erinacei (african) and T. erinacei. In a preferred embodiment, the SEQ ID N022 or 1 is from T. tonsurans and is represented by SEQ ID N02. In another preferred embodiment, the SEQ ID N022 or 1 is from T. equinum and is represented by SEQ ID N03. In another preferred embodiment, the SEQ ID N022

or 1 is from T. interdigitale (anthrophilic + zoophilic), I, II, II, III*, IV, M and is represented by SEQ ID N04. In another preferred embodiment, the SEQ ID N022 or 1 is from T. benhamiae (yellow) and is represented by SEQ ID N05. In another preferred embodiment, the SEQ ID N022 or 1 is from T. benhamiae (white) and is represented by SEQ ID N06. In another preferred embodiment, the SEQ ID N022or 1 is from T. benhamiae (african) and is represented by SEQ ID N07. In another preferred embodiment, the SEQ ID N022 or 1 is from T. concentricum and is represented by SEQ ID N08. In another preferred embodiment, the SEQ ID N022 or 1 is from T. erinacei and is represented by SEQ ID N09.

[0032] The invention contemplates various reagents such as a primer pair comprising a forward primer and a reverse primer for amplifying a nucleic acid encoding said metalloprotease from a pathogen. Methods how to design a primer such that specific hybridization is ensured and primer dimerization or secondary structure formation is avoided are described in the state of the art, for example in Dennis, Y. M., Chius, R. W. K., and Allen Chan, K. C. (2006) Clinical applications of PCR, Humana Press, page 18. In a preferred embodiment, each primer has a length of 10 to 40, more preferably 12 to 35, more preferably 14 to 30 nucleotides. The term "forward primer", as used herein, relates to a primer hybridizing upstream of SEQ ID NO22 or 1 in the pathogen's genome such that the primer may be extended in a PCR reaction in the 5' to 3' direction, resulting in the synthesis of a nucleic acid comprising SEQ ID NO22 or 1.

[0033] The forward primer is a universal forward primer hybridizing specifically to a region of the pathogen's genome upstream of SEQ ID NO22 or 1 that is sufficiently conserved among the pathogens to be distinguished, preferably species from the genus Trichophyton, to the effect that it may be used to amplify SEQ ID NO22 or 1 of more than one species, even though variable parts of SEQ ID NO22 or 1 of said species differ. In a preferred embodiment, the forward primer hybridizes to a conserved region shared by T. tonsurans, T. equinum and T. interdigitale (anthrophilic + zoophilic), I, II, III, III*, IV, M, and comprises a sequence comprising GGGAGGGAGACTAGTTG or a variant thereof. In another preferred embodiment, the forward primer binds to a conserved region shared by T. benhamiae (yellow), T. benhamiae (white), T. benhamiae (african) T. concentricum and T. erinacei, and comprises a sequence comprising GCATTTCCCATGGCT or a variant thereof.

[0034] The term "reverse primer", as used herein, relates to a primer hybridizing specifically downstream of SEQ ID NO22 or 1 in the pathogen's genome such that the primer may be extended in a PCR reaction in the 5' to 3' direction, resulting in the synthesis of a nucleic acid comprising a sequence complementary to SEQ ID NO22 or 1.

[0035] The reverse primer is a universal reverse primer hybridizing specifically to a region of the pathogen's genome downstream of SEQ ID NO22 or 1 that is sufficiently conserved among the pathogens to be distinguished, preferably species from the genus Trichophyton, to the effect that it may be used to amplify the sequences complementary to SEQ ID NO22 or 1 of more than one species, even though the sequences comprising SEQ ID NO22 or 1 and the sequences complementary to them of said species differ. In a preferred embodiment, the reverse primer hybridizes to a conserved region shared by T. tonsurans, T. equinum and T. interdigitale (anthrophilic + zoophilic), I, II, III, III*, IV, M, and comprises a sequence comprising AATTTTTCGCCGCCAAG or a variant thereof. In another preferred embodiment, the reverse primer binds to a conserved region shared by T. benhamiae (yellow), T. benhamiae (white), T. benhamiae (african), T. concentricum and T. erinacei, and comprises a sequence comprising TGGCTCTGTTACGTG or a variant thereof.

[0036] In another preferred embodiment, the primer pair may be present in a composition comprising more than one primer pair, for example 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 24, 28, 32, 36, 40 or 48 or more primer pairs. In a preferred embodiment, the composition comprises a primer pair that may be used for amplifying the nucleic acids encoding SEQ ID NO22 or 1 from T. tonsurans, T. equinum and T. interdigitale (anthrophilic + zoophilic), I, II, III, III*, IV, M and in addition a primer pair that may be used for amplifying the nucleic acids encoding SEQ ID NO22 or 1 from T. benhamiae (yellow), T. benhamiae (white), T. benhamiae (african), T. concentricum and T. erinacei. In a preferred embodiment, the composition may comprise a primer pair that may be used to amplify or detect, by specifically hybridizing to it, one or more sequences that may be used to distinguish pathogens associated with a skin, hair and nail infection, preferably a sequence selected from the group comprising the nucleic acid sequences referred to as beta tubulin (Rezaei-Matehkolaei et al. (2014) Nucleotide sequence analysis of beta tubulin gene in a wide range of dermatophytes, Medical Mycology 42, 674), transcription elongation factor (Mirhendi et al. (2015), Translation elongation factor 1-alpha gene as a potential taxonomic and identification marker in dermatophytes, Medical Mycology 53, 215), internal transcribed spacer regions 1 and 2 (Gräser et al. (2008), The New Species Concept in Dermatophytes - a Polyphasic Approach, Mycopathologia 166, 239) and topoisomerase or a part thereof. Such primer composition may be used according to the present invention and analysed using a carrier comprising one or more nucleic acids capable of hybridizing specifically to a sequence selected from the group comprising the nucleic acid sequences referred to as beta tubulin, transcription elongation factor, internal transcribed spacer regions 1 and 2, and topoisomerase or a part thereof may be used to detect the presence of any such sequence.

[0037] The distance between the final base pair of the forward primer, in its 5'-3' orientation, and the first base pair of SEQ ID N022 or 1, in its 5'-3' orientation, is, in order of increasing preference, 200, 100 or less base pairs.

[0038] The distance between the final base pair of the reverse primer, in its 5'-3' orientation, and the final base pair of SEQ ID NO22 or 1, in its 5'-3' orientation, is, in order of increasing preference, 200, 100 or less base pairs.

[0039] The teachings of the present invention may not only be carried out using nucleic acids having the exact se-

quences referred to in this application explicitly, for example by function, name, sequence or accession number, or implicitly, but also using variants of such nucleic acids. In a preferred embodiment, the term "variant" of a nucleic acid comprises nucleic acids having at least 70, more preferably 75, 80, 85, 90, 95, 98, 99 or 99,5 % sequence identity with the reference or wild type nucleic acid, preferably with the ability to hybridize specifically to the same target as reference or wild type nucleic acid, as well as nucleic acids the complementary strand of which hybridizes, preferably under stringent conditions, to the reference or wild type nucleic acid. In a preferred embodiment, the term "hybridizes specifically", as used herein, means that a nucleic acid such as a primer or probe hybridizes under stringent conditions to the target nucleic acid. Stringency of hybridization reactions is readily determinable by one of ordinary skilled in the art, and in general is an empirical calculation dependent on primer or probe length, reaction temperature and salt concentration. In general longer primers or probes withstand higher temperatures for proper annealing, while shorter primers or probes less so. Hybridization generally depends on the ability of single or double stranded DNA to bind to complementary strands present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which may be used. As a result higher relative temperatures would tend to make the reaction conditions more stringent and beware unspecific bindings, while lower temperature less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel, F. M. (1995), Current Protocols in Molecular Biology. John Wiley & Sons, Inc. Moreover, the person skilled in the art may follow the instructions given in the manual Boehringer Mannheim GmbH (1993) The DIG System Users Guide for Filter Hybridization, Boehringer Mannheim GmbH, Mannheim, Germany and in Liebl, W., Ehrmann, M., Ludwig, W., and Schleifer, K. H. (1991) International Journal of Systematic Bacteriology 41: 255-260 on how to identify DNA sequences by means of hybridization. In a preferred embodiment, stringent conditions are applied for any hybridization, i.e. hybridization occurs only if the primers or probe is 70 %, preferably 75 %, 80 %, 85 %, 90 %, 95 % or 99 % or more identical to the target sequence. Nucleic acid having a lower degree of identity with respect to the target sequence may hybridize, but such hybrids are unstable and will be removed while the annealing step of a PCR or the washing steps after probe hybridization. In a washing step of a probe hybridization under stringent conditions, for example lowering the concentration of salt to 2 x SSC or, optionally and subsequently, to 0.25 x SSC, while the temperature is, in order of increasing preference, approximately 39 °C - 69 °C, approximately 41 °C - 67 °C, approximately 43 °C - 65 °C, approximately 45 °C - 63 °C, approximately 47 °C - 61 °C, approximately 49 °C - 59 °C, approximately 51 °C -57 °C, approximately 53 °C - 57 °C. In a particularly preferred embodiment, the temperature is approximately 51 °C - 57 °C or approximately 53 °C - 57 °C. In a preferred embodiment, the primer pair used in a PCR reaction comprises a detectable label, preferably from the group comprising a fluorescent, radioactive, colloidal gold or enzymatically active label. More preferably, the label is a fluorescent label preferably selected from the group comprising cy-3, cy-5, HEX, FAM, ROX and TAMRA. Suitable labels, ways to link them to nucleic acids such as primers and to detect such labels have been described in the state of the art.

[0040] In a preferred embodiment, the nucleotide sequence of each primer of the primer pair and/or the probe of the invention comprises or consists of a sequence that is capable of amplifying or hybridizing to a sequence set forth in SEQ ID NO22, 1 or the complement sequence thereof with the proviso that said primer pair and/or said probe is not capable to amply or hybridize to a sequence set forth in SEQ ID NO6, 29, 30 and/or the complement sequence thereof. Thus, in some embodiments the probe of the invention comprises or consists of a sequence that is capable of hybridizing to a sequence set forth in SEQ ID NO22 or the complement sequence thereof with the proviso that said probe is not capable to hybridize to a sequence set forth in SEQ ID NO6, 29, 30 and/or the complement sequence thereof. In alternative embodiments, the probe of the invention comprises or consists of a sequence that is capable of hybridizing to a sequence set forth in SEQ ID NO1 or the complement sequence thereof with the proviso that said probe is not capable to hybridize to a sequence set forth in SEQ ID N06, 29, 30 and/or the complement sequence thereof. Stringent conditions for hybridization are described above. Methods for the measurement of nucleotide hybridization are well-known in the art. In embodiments concerning the proviso that the primer pair and/or the probe of the invention is not capable to amply or hybridize to a sequence set forth in SEQ ID N06, 29, 30 and/or the complement sequence thereof, said sequence has a sequence identity of at least 85%, at least 90%, at least 95%, at least 96%, at least 97, at least 98%, at least 99% or 100% to a sequence set forth in SEQ ID N06, 29, 30 and/or the complement sequence thereof over its entire length.

[0041] In preferred embodiments, the nucleotide sequence of each primer of the primer pair of the invention comprises or consists of a sequence that is capable of amplifying or hybridizing to a sequence set forth in SEQ ID N022, 1 or the complement sequence thereof, wherein said nucleotide sequence of each primer of the primer pair of the invention comprises or consists of a sequence that is a fragment of the sequence set forth in SEQ ID N022, 1 or the complement sequence thereof, wherein said fragment has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97, at least 98%, at least 99% or 100% sequence identity to a sequence set forth in SEQ ID N022, 1 or the complement sequence thereof over a length of at least 5, at least 10, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25 or at least 26 consecutive nucleotides. Further herein described is that the nucleotide sequence of the probe comprises or consists of a sequence that is a fragment of the sequence set forth in SEQ ID N022 or the complement sequence thereof, wherein said fragment has at

least 90% sequence identity to the sequence set forth in SEQ ID N022 or the complement sequence thereof over a length of at least 5, at least 10, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25 or at least 26 consecutive nucleotides.

[0042] The probe has a length of not more than 250, not more than 200, not more than 150, not more than 100, not more than 90, not more than 80, not more than 70, not more than 65, not more than 60, not more than 55, not more than 50, not more than 45, not more than 40, not more than 35, not more than 30, not more than 25 or not more than 20 nucleotides fused to a detectable signal molecule.

[0043] Further herein described is that the nucleotide sequence of the probe may comprise or consists of a sequence that is a fragment of the sequence set forth in SEQ ID NO1 or the complement sequence thereof, wherein said fragment has at least 90% sequence identity to the sequence set forth in SEQ ID NO1 or the complement sequence thereof over a length of at least 5, at least 10, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25 or at least 26 consecutive nucleotides.

[0044] In other preferred embodiments of the invention, the above described properties of each primer of the primer pair and the probe are combined, namely (1) that said primer pair and/or said probe is not capable to amply or hybridize to a sequence set forth in SEQ ID N06, 29, 30 and/or the complement sequence thereof, (2) that the probe has a limited length and (3) that each primer of the primer pair and the probe comprise a fragment of SEQ ID N022 or the complement sequence thereof, wherein said fragment has at least 90% sequence identity to the sequence set forth in SEQ ID NO22 or the complement sequence thereof over a length of at least 5 consecutive nucleotides.

[0045] According to the present invention, a nucleic acid capable of hybridizing specifically to a nucleic acid sequence comprising SEQ ID NO22 or 1 from a pathogen associated with a skin, hair and nail infection or its complementary strand is provided. The nucleic acid may be an isolated nucleic acid. This nucleic acid may be used as a probe to detect the nucleic acid comprising SEQ ID NO22 or 1 from the pathogen and to distinguish it from other sequences, more specifically homologous sequences from other pathogens associated with such an infection also comprising SEQ ID NO22 or 1. Therefore, this nucleic acid comprises a strand capable of hybridizing specifically to the sequence comprising SEQ ID NO22 or 1 or a complementary sequence. The nucleic acid capable of hybridizing specifically to a nucleic acid sequence comprising SEQ ID NO22 or 1 from such a pathogen may be selected from the group comprising SEQ ID NO14, SEQ ID NO15, SEQ ID NO16, SEQ ID NO17, SEQ ID NO18, SEQ ID NO19, SEQ ID NO20, SEQ ID NO21, SEQ ID Nos. 24 to 28 and a variant thereof.

[0046] The nucleic acid capable of hybridizing specifically to a nucleic acid sequence from a pathogen associated with a skin, hair and nail infection comprising SEQ ID NO22 or 1 may be immobilized, preferably on a carrier. This way, it is more straightforward to separate said nucleic acid when it is annealed to a nucleic acid from a pathogen comprising SEQ ID NO22 or 1 from any other nucleic acids or other substances in a sample from a patient. The carrier may be made by coating a carrier with a nucleic acid capable of hybridizing specifically to a nucleic acid comprising SEQ ID NO22 or 1 or a variant thereof, more preferably a nucleic acid comprising a sequence selected from the group comprising SEQ ID N014, SEQ ID N015, SEQ ID N016, SEQ ID N017, SEQ ID N018, SEQ ID N019, SEQ ID N020, SEQ ID N021, SEQ ID Nos. 24 to 28 and a variant thereof. In a preferred embodiment, the carrier is a microarray plate. Suitable microarrays, ways how to prepare and how to use them are described in the state of the art, for example in Müller, H. J & Röder, T. (2004) Der Experimentator - Microarrays, Elsevier/Spektrum, Chapters 3 and 4.

[0047] According to the present invention, a method comprising the step detecting in a sample a nucleic acid comprising SEQ ID N022 or 1 from a pathogen associated with a skin, hair and nail infection is provided. The term "detecting", as used herein, means that the presence or absence of SEQ ID NO22 or 1 or a variant thereof is detected. The term means that it is determined whether or not the nucleic acid present comprises SEQ ID NO22 or 1 from one or more pathogens from the genus Trichophyton, namely from the group comprising T. tonsurans, T. equinum, T. interdigitale, T. benhamiae (white), T. benhamiae (african), T. benhamiae (yellow), T. concentricum, T. erinacei (african), T. erinacei and T. concentricum and optionally which of these pathogens' sequence it is. In a most preferred embodiment, the term means that it is determined whether or not a nucleic acid present in the sample is from T. benhamiae (yellow) or T. concentricum, thus distinguishing both organisms. In another preferred embodiment, the detection may be a semi-quantitive or quantitive detection. Methods that may be used to detect a specific sequence are described in the state of the art, for example in Lottspeich, F. & Engels, J. W. (2012), Bioanalytik, Springer Spektrum, 3rd edition. In a preferred embodiment, the method is selected from the group comprising microarray, nucleic acid sequencing, mass spectrometry and PCR, more preferably real-time PCR. In another preferred embodiment, the pathogen associated with a skin, hair and nail infection is detected by identifying the polypeptide partially encoded by SEQ ID NO22 or 1. The person skilled in the art is familiar with suitable methods, preferably selected from the group comprising immunoassays and mass spectrometry. Antibodies may be generated for distinguishing differences between nucleic acid sequences comprising SEQ ID NO22 or 1 at the protein level using standard methods described in the state of the art, for example Lottspeich, F. & Engels, J. W. (2012), Bioanalytik, Chapter 6.

[0048] The method according to the present invention may optionally comprise detecting in a sample one or more additional nucleic acid sequences in addition to the nucleic acid from a pathogen associated with a skin, hair and nail

infection. Such a sequence may be another SEQ ID NO22 or 1 from a pathogen associated with a skin, hair and nail infection. For example, the method may involve detecting a first nucleic acid comprising SEQ ID N022 or 1 from a pathogen from the group T. tonsurans, T. equinum and T. interdigitale (anthrophilic + zoophilic), I, II, III, III*, IV, M, and a second nucleic acid comprising another SEQ ID N022 or 1 from a pathogen from the group comprising T. benhamiae (yellow), T. benhamiae (white), T. benhamiae (african), T. concentricum and T. erinacei. Such additional sequences are preferably detected simultaneously together with the sequence encoding a metalloprotease from a pathogen associated with a skin, hair and nail disease. In a preferred embodiment, a multiplex PCR reaction is carried out to amplify the sequences to be detected, and this may optionally be followed by microarray analysis, preferably using fluorescence detection of the amplicons comprising SEQ ID NO22 or 1 of the sequence encoding a metalloprotease and any amplicons comprising one of the additional sequences.

[0049] In a preferred embodiment, the method may comprise step a) providing a sample, preferably from a patient. A sample examined using a method or reagent according to the present invention may be obtained from a patient suspected of suffering from a skin, hair and nail infection and is preferably a sample from a part of the patient's body which comprises keratin. In a more preferred embodiment, the sample is a nail, hair or skin sample, more preferably an isolated sample. The patient is preferably a mammalian patient, more preferably a human. Alternatively, the sample may be an environmental sample, for example from soil or the floor of potentially contaminated areas such as a swimming pool or a hospital. Prior to further analysis, the sample may be processed, for example by extracting any nucleic acids present. In a preferred embodiment, the term "extracting", as used herein, means that the nucleic acids are purified from the sample and/or concentrated, for example to remove any contaminants that may interfere with the amplification and/or detection. In a preferred embodiment, the nucleic acid is DNA or RNA, more preferably DNA. Methods and reagents for extracting nucleic acids, preferably for detecting pathogens, more preferably fungal pathogens, are commercially available. It is possible to use the sample directly or the extracted nucleic acid for the detection, but it is preferred that the method comprises step b) amplifying any nucleic acid comprising SEQ ID NO22 or 1 and optionally any additional sequence or sequences, thus generating one or more amplicons. Such amplification may be performed by PCR. Suitable methods and reagents are described in the state of the art, for example in Dennis, Y. M., Chius, R. W. K., and Allen Chan, K. C. (2006) Clinical applications of PCR, Humana Press, chapter 1. Essentially the sample or the nucleic acid extracted is contacted with at least one primer pair according to the present invention and optionally additional primer pairs followed by addition of a polymerase capable of amplifying the nucleic acid in the presence of any reagents required such as NTPs and bivalent cations in a PCR buffer. The resulting reaction mixture is subjected to several amplification cycles each comprising a denaturation step, which involves separating the two complementary strands of the nucleic acid, an annealing step, which sees the primers hybridize to the nucleic acid strands and an elongation reaction which involves the generation of complementary strands to both strands of the nucleic acid. As a result, a double-stranded amplicon is generated that comprises the primer pair and the sequence from the genomic nucleic acid of the pathogen located between the forward and the reverse primers of the primer pair. The amplicon is present at a concentration that exceeds the concentration of the nucleic acid in the sample, preferably, in order of increasing preference, more than 10, 10^2, 10^3, 10^4 or 10^5 times. Several reactions for the PCR amplification and generation of amplicons comprising additional sequences may be carried out. The forward and/or reverse primers may be labeled, resulting in a labeled amplicon.

[0050] In a preferred embodiment, the primer pair used in a PCR reaction comprises a detectable label, preferably from the group comprising a fluorescent, radioactive, colloidal gold or enzymatically active label. More preferably, the label is a fluorescent label preferably selected from the group comprising cy-3, cy-5, HEX, FAM, ROX and TAMRA. Suitable labels, ways to link them to nucleic acids such as primers and to detect such labels have been described in the state of the art.

[0051] Subsequently or concomitantly as step b) is carried out, any amplicon may be labeled, preferably by fluorescence, radioactivity, colloidal gold or chemiluminescence. In a preferred embodiment, the label may be linked to the primers prior to carrying out step b), for example if a fluorescent, radioactive, enzymatically active or chemiluminescent label is attached to one or both primers of the primer pair. In another preferred embodiment, the amplicon may be labeled as the amplification reaction progresses, for example by incorporation of labeled NTPs or a parallel labeling reaction. In another preferred embodiment, the amplicon may be labeled following step b), for example by attaching to the amplicon a fluorescent, radioactive, enzymatically active or chemiluminescent label or by adding to the amplicon a label binding to double-stranded DNA, for example a fluorescent intercalating agent such as ethidium or propidium bromide.

[0052] Subsequently or concomitantly as step b) is carried out, the amplicon or amplicons may be detected. For example, the PCR may be real-time PCR involving continuous fluorescence detection as the reaction progresses. If the amplicon is to be detected subsequently, the amplicon may be extracted from the PCR reaction mixture prior to step c). For a subsequent detection, the amplicon, extracted or not, may be contacted, under conditions allowing for a specific hybridization, with a nucleic acid capable of specifically hybridizing to a nucleic acid from a pathogen comprising SEQ ID NO22 or 1, preferably a nucleic acid comprising a sequence from the group comprising SEQ ID NO14, SEQ ID NO15, SEQ ID N016, SEQ ID NO17, SEQ ID NO18, SEQ ID NO19, SEQ ID NO20, SEQ ID NO21, SEQ ID Nos. 24 to 28 and a variant thereof. The nucleic acid comprising SEQ ID NO22 or 1 is preferably immobilized on a carrier. It may be

subjected to a washing step to remove contaminants prior to carrying out the detection. In such a setting, it is preferred that the amplicon is labeled and the label is detected in case a labeled amplicon present hybridized specifically to the nucleic acid comprising SEQ ID NO22 or 1.

[0053] Preferably, the detection is carried out such that an amplicon comprising SEQ ID NO22 or 1 from a pathogen associated with a skin, hair and nail disease may be distinguished from an amplicon comprising SEQ ID N022 or 1 from another pathogen associated with a skin, hair and nail disease or an amplicon comprising an additional or in fact any other sequence.

[0054] According to the present invention, a kit comprising the primer pair may be provided. The primer pair may be labeled. The inventive teachings provide a kit, preferably for diagnosing a disease. The kit may comprise instructions detailing how to use the kit and a means for contacting the nucleic acid capable of hybridizing specifically to SEQ ID NO22 or 1 from a pathogen with a sample from a subject, preferably a human subject, on a carrier, for example, a microarray. Furthermore, the kit may comprise a positive control, for example one or more nucleic acids comprising SEQ ID NO22 or 1 from a pathogen associated with a skin, hair and nail disease, and a negative control, for example a nucleic acid lacking SEQ ID NO22 or 1. Finally, such a kit may comprise a standard solution comprising one or more nucleic acids comprising SEQ ID NO22 or 1 from a pathogen associated with a skin, hair and nail disease for preparing a calibration curve. SEQ ID NO22 or 1 is from a pathogen from the genera Microsporum and Trichophyton, namely from a pathogen from the group comprising T. tonsurans, T. equinum, T. interdigitale, T. benhamiae (white), T. benhamiae (african), T. benhamiae (yellow), T. concentricum, T. erinacei (african) and T. erinacei.

[0055] According to the present invention, the primer pair, carrier, nucleic acid, cell, vector or kit may be used for the diagnosis of a disease or for the manufacture of a kit for the diagnosis of a disease, preferably a skin, hair and nail disease, more preferably a skin, hair and nail infection, more preferably a fungal skin, hair and nail infection, most preferably dermatophytosis. Said disease is an infection associated with a pathogen from the genus Trichophyton, namely from a pathogen from the group comprising T. tonsurans, T. equinum, T. interdigitale, T. benhamiae (white), T. benhamiae (african), T. benhamiae (yellow), T. concentricum T. erinacei (african) and T. erinacei.

[0056] In a preferred embodiment, the term "diagnosis", as used herein, refers to any kind of procedure aiming to obtain information supportive in the assessment whether a patient suffers or is likely or more likely than the average or a comparative subject, the latter preferably having similar symptoms, to suffer from a certain disease or disorder in the past, at the time of the diagnosis or in the future, to find out how the disease is progressing or is likely to progress in the future or to evaluate the responsiveness of a patient with regard to a certain treatment, for example the administration of suitable drugs such as drugs for the desensitization of allergic patients. In other words, the term "diagnosis" comprises not only diagnosing, but also prognosticating and/or monitoring the course of a disease or disorder.

[0057] Therefore, the term "diagnosis" does preferably not imply that the diagnostic methods or agents according to the present invention will be definitive and sufficient to finalize the diagnosis on the basis of a single test, let alone parameter, but may refer to a contribution to what is referred to as a "differential diagnosis", *i.e.* a systematic diagnostic procedure considering the likelihood of a range of possible conditions on the basis of a range of diagnostic parameters. This may include an indirect diagnosis, *i.e.* a negative result means that one disease may be ruled out but that, in turn, another disease is more likely to be present. The term "diagnosis" may also refer to a method or agent used to choose the most promising treatment regime for a patient. In other words, the method or agent may relate to selecting a treatment regimen for a subject. The term "diagnosis" may also refer to the identification of the causative pathogen of a disease or to distinguishing two closely related pathogens, preferably *T. benhamiae (yellow)* or *T. concentricum.*

[0058] The present invention is further illustrated by the following non-limiting figures, sequences and examples from which further features, embodiments, aspects and advantages of the present invention may be taken.

Fig. 1 shows a sequence alignment comprising the various sequences related to a metalloprotease with shared nucleic acid sequence motifs and the consensus sequence derived.

Fig. 2 shows captured microarrays with hybridized PCR products from *Trichophyton interdigitale (anthrophilic)* (A), *Trichophyton interdigitale (zoophilic)* (B), *Trichophyton tonsurans* (C) and *Trichophyton equinum* (D) templates. The specific probe for *Trichophyton tonsurans* is highlighted by a white circle and the dotted encircled spots are controls needed from the software to set the grid. Only the PCR products from the *Trichophyton tonsurans* template (C) hybridized to the specific probe (6a, 6b) and showed fluorescent signals.

Fig. 3 shows captured microarrays with hybridized PCR products *from Trichophyton benhamiae (yellow)* (A), *Trichophyton benhamiae (white)* (B), *Trichophyton benhamiae (african)* (C) and *Trichophyton concentricum* (D) templates. The specific probes for *Trichophyton benhamiae (yellow)* and *Trichopyhton concentricum* are highlighted by a white circle and the dotted encircled spots are controls needed from the software to set the grid. Only the PCR products from the *Trichophyton benhamiae (yellow)* (A) and *Trichophyton concentricum* (D) template hybridized to the specific probes and showed fluorescent signals.

Fig. 4 shows captured microarrays with hybridized PCR products from *Trichophyton benhamiae (white)* (A), *Trichophyton benhamiae (yellow)* (B) and *Trichophyton benhamiae (african)* (C) templates. The specific probes for *Trichophyton benhamiae (yellow)* and *Trichophyton benhamiae (white/african)* are highlighted by a white circle and the dotted encircled spots are controls needed from the software to set the grid. The PCR products from the *Trichophyton benhamiae (white)* (A), *Trichophyton benhamiae (yellow)* (B) and *Trichophyton benhamiae (yellow)* (C) template hybridized to the specific probes and showed fluorescent signals.

Fig. 5 shows captured microarrays with hybridized PCR products from *Trichophyton erinacei* (A), *Trichophyton benhamiae (white)* (B) and *Trichophyton benhamiae (african)* (C) templates. The specific probes for *Trichophyton erinacei* is highlighted by a white circle and the dotted encircled spots are controls needed from the software to set the grid. Only the PCR product from the *Trichophyton erinacei* (A) template hybridized to the specific probe and showed fluorescent signals.

Fig. 6 shows captured microarrays with hybridized PCR products from *Trichophyton benhamiae (white)* (A), *Trichophyton benhamiae (yellow)* (B) and *Trichophyton concentricum* (C) templates. The specific probes for *Trichophyton benhamiae (white/african),Trichophyton benhamiae (yellow)* and *Trichophyton concentricum* are highlighted by a white circle and the dotted encircled spots are controls needed from the software to set the grid. Only the PCR products from the *Trichophyton benhamiae (white),Trichophyton benhamiae (yellow)* and *Trichophyton concentricum* template hybridized to the specific probe and showed fluorescent signals.

[0059] The present invention relates to the following nucleic acid sequences (presented in 5'-3' orientation):

SEQ ID NO1:
Consensus sequence of metalloprotease-related nucleic acid to be amplified

CACNNNNNTAACCNTACCCnnnnTCCnnGnnGnnnGGnnnnnnAnCnnnTGGATTATGGnnTn
nTTCGTGGAnTAnGGTnnnnAnnCGATCnTGnnnATGGCACTnnTnGGTnnnnTGnGnCAnnTn
CCAAAAGnnGnnGCAGGGnnnnACCnnnTTnnnnnnTGGnAnGGTGTAGGCAATnnTTnTGnG
nCnnCATCGnnGAnGnnnATCGnnGnAGnA

SEQ ID N02:
Target sequence related to metalloprotease from *T. tonsurans*

CACGCTTATAACCGTACCCGA*GATCCTTGGCGTACGGATGCATA*ACGGCTGGATTATGG
GCTCCTTCGTGGATTATGGTCGAGACGCGATCTTGATGATGGCACTTATCGGTGAGATG
AGGCAGTTGCCAAAAGATGTTGCAGGGGAAGACCGAATTCATGGCTGGGAGGGTGTAG
GCAATAGTTCTGGGACGGCATCGTCGATGTTTATCGTAGCAGAACCACTGAACAGGGC
CACCCTCTGAAACGGATGCTTTGGAAATCGAGTAGAGATGCATGGAAACCTCCTTCCTG
GTTGCAGAATC

SEQ ID N03:
Target sequence related to metalloprotease from *T. equinum*

CACGCTCATAACCGTACCCG*AGATCCCTGGCGTGCG*GACGCATAACGGCTGGATTATG
GGCTCCTTCGTGGATTATGGTCGAGACGCGATCTTGATGATGGCACTTATCGGTGAGAT
GAGGCAGTTGCCAAAAGATGTAGCAGGGGAAGACCGAATTCATGGCTGGGAGGGTGTA
GGCAATAGTTCTGGGACGGCATCGTCGATGTTTATCGTAGCAGAACCACTGAACAGGG
CCACCCTCTGGAACGGATGCTTTGGAAATCGAGTAGAGATGCATGGAAACCTCCTTCCT
GGTTGCAGAATC

SEQ ID N04:
Target sequence related to metalloprotease from *T. interdigitale (anthrophilic + zoophilic), I, II, II, III*, IV,* M

CACGGTAATAACCGTACCCGAGGTCCCCGGCGTGCGGACTCATAACGGCTGGATTATG
GGCTCCTTCGTGGATTATGGTCGAGACGCGATCTTGACCATGGCACTTCTTGGTGAGAT
GGGGCAGTTGCCAAAAGATGTCGCAGGGAAAGACCGAATTCATGGCTGGGAGGGTGTA
GGCAATAGTTCTGCGACGGCATCGTCGATGTTTATCGTGGCAGAACCACTTAACAGGGC
CACCCTCTGAAACGGATGCTTTGGAAATCGAGTT*GAGATGCGCGGAAACCTC*CTTCCTG
GTTGCAGGATC

SEQ ID N05:
Target sequence related to metalloprotease from *T. benhamiae (yellow)*

CTCCGGTGAGAGAGTGCAATTGCACGATCGTAACCGTACCCCAAGTCCTTGCAGAACG
GTGTAACAACCCTTGGATTATGGAGTAATTCGTGGATTATGGTGTCGACACGATCCTGG
CCATGGCACTTATTGGTTTGGTGGGGCAGTTGCCAAAAGATGGAGCAGGGGAAGACCG
AGTTCTCGCCTGGAAGG*GTGTAGGCAATGATTTTGGGCCTACATC*GGTGACGTGCATC
GGAGCAGTAC

SEQ ID N06:
Target sequence related to metalloprotease from *T. benhamiae (white)*

CTCTGGTGAGAGAGTGCAGTTGCACGATTGTAACCATACCCATGGTCCT*TGCAGTGCG*
*GTGTGACAGCATTTGG*ATTATGGGCTCCTTCGTGGATTACGGTCCCGACACGATCCTGA
CGATGGCACTCATTGGTGCGGTGGGGCAGTTGCCAAAAGAGGGAGCAGGGGTGAACC
TCATTCCTAGCTGGAAAGGTGTAGGCAATGGTTCTGGGACTGCATCGGCGAGGACCAT
CGGAGCAGAAC

SEQ ID NO7:
Target sequence related to metalloprotease *T. benhamiae (African)*

CTCTTGTGAGAGAGTGCAGTTGCACGCTGATAACCGTACCCATGGTCCTTG*CAGTGCG*
*GCTTGACAACAC*TTGGATTATGGGCTCCTTCGTGGACTACGGTCCCGATACGATCCTGA
CGATGGCACTTATTGGTGCGGTGGGGCAGTTGCCAAAAGATGTCGCAGGGGTGAACCT
CATTCCTAGATGGAAAGGTGTAGGCAATGGTTCTGGGACCGCATCGGCGAGGACCATC
GGAGCAGAAC

SEQ ID N08:
Target sequence related to metalloprotease from *T. concentricum*

CTCCGGTGAGAGAGTGCAGTTGCACGATTGTAACCGTACCCCAAGTCCTTGCAGAACG
GTGTAACAACACTTGGATTATGGAGTAATTCGTGGATTATGGTGTCGACACGATCCTGG

CCATGGCACTTATTGGTTTGGTGGGGCAGTTGCCA*AAAGTTGGGGCAGGGGAAGAC*CG
AGTTCTCGCCTGGAAGGGTGTAGGCAATGGTTCTGGGCCTACATCGGTGACGTGCATC
GGAGTAGTAC

SEQ ID N09:
Target sequence related to metalloprotease from *T. erinacei*

CTCCCGTGAGAGAGTGCAGTTGCACTACTGTAACCGTACCCATGGTCC*TTGCAGTGTG GGTTAGCAACATTTG*GATTATGGAGTGCTTCGTGGATTACGGTCCCAACACGATCCTGA CCATGGCACTTCTTGGTTTTGTGCGCCATATTCCAAAAGATGGGGCAGGGGTGAACCTC ATTGTTAGATGGAAGGGTGTAGGCAATGGTTCTGGGACCGCATCGGCGAGGACCATCG GAGCAGTA

SEQ ID N010:
Universal forward primer (*Trichophyton tonsurans, Trichophyten equinum, Trichophyton interdigitale (antrophilic + zoophilic) I, II, III, III*, IV, M*):
GGGAGGGAGACTAGTTG

SEQ ID NO11:
Universal forward primer (*Trichophyton benhamiae (yellow), Trichophyton benhamiae (white), Trichophyton benhamiae (african), Trichophyton concentricum, Trichophyton erinacei*)
GCATTTCCCATGGCT

SEQ ID NO12:
Universal reverse primer (*Trichophyton tonsurans, Trichophyten equinum, Trichophyton interdigitale (antrophilic + zoophilic) I, II, III, III*, IV, M*)
AATTTTTCGCCGCCAAG

SEQ ID N013:
Universal reverse primer (*Trichophyton benhamiae (yellow), Trichophyton benhamiae (white), Trichophyton benhamiae (african), Trichophyton concentricum, Trichophyton erinacei*)
TGGCTCTGTTACGTG

SEQ ID NO14:
Trichophyton tonsurans probe
GATCCTTGGCGTACGGATGCATA

SEQ ID N015:
Trichophyten equinum probe
AGATCCCTGGCGTGCG

SEQ ID N016:
Trichophyton interdigitale (antrophilic + zoophilic) I, II, III, III, IV, M probe*
GAGATGCGCGGAAACCTC

SEQ ID NO17:
Trichophyton benhamiae (yellow) probe
GTGTAGGCAATGATTTTGGGCCTACAT

SEQ ID N018:
Trichophyton benhamiae (white) probe
TGCAGTGCGGTGTGACAGCATTTGG

SEQ ID NO19:
Trichophyton benhamiae (african) probe
CAGTGCGGCTTGACAACAC

SEQ ID N020:
Trichophyton concentricum probe
AAAGTTGGGGCAGGGGAAGA

SEQ ID NO21:
Trichophyton erinacei probe

TTGCAGTGTGGGTTAGCAACATTTG

SEQ ID N022:
Consensus sequence

cacnnnnntaaccntacccnnnntccnngnngnnnggnnnnnnancnnntggattatggnntnnttcgtggantanggtnn
nnanncgatcntgnnnatggcactnntnggtnnnntgnnncanntnccaaaagnngnngcagggnnnnaccnnnttnnnn
nntggnanggtgtaggcaatnnttntgnnncnncatcgnngangnnnatcgnngnagna

SEQ ID N023:
Primer
ctggccatggcacttattgg

SEQ ID N024:
Probe
gatgtaggcccaaaatcattgcctacac

SEQ ID N025:
Probe
ctgccccaacttttggcaactg

SEQ ID N026:
Probe
taggcaatgattttgggccta

SEQ ID N027:
Probe
catcggcgaggaccatcgga

SEQ ID N028:
Probe
gcaatggttctgggcctacatc

SEQ ID N029:
Sequence from T. rubrum

cttttgtgagagagtccagttgcacgcctgtaaccgtacccgaagtccttgcagtacggtttggccacatttggattatggagtgcttc
gtggactatagtggtgacacgatcctgaccatggcacttattggtccagtggggtagttgccaaaaggggccgcaggggaagac
cgcattctgaattggaagagtgtaggcaatggttctgggcctacatcggtgatgcatatcggagcagtgc

SEQ ID N030:
Sequence from T. verrucosum

ctattgggagggagtccagttgcactcctgtaaccgtacccatggtccttggcgtgcggggacataacatttggattatgggctcctt
cgtggattacggtcccaacacgatcctgacgatggcactccttgttgacgtggggcagttgccaaaaggggggcaggggaag
accgaattcatagatggaagggtgtaggcaatggttctgggactgcatcggcgatatttatcggagcagaac

[0060] **Examples:** The following examples demonstrate that different pathogen strains may be distinguished using the teachings according to the present invention.

Example 1

Design of primers and probes

[0061] For the identification of *T. tonsurans* in a sample, the metalloprotease gene was chosen as a useful target

region. Based on the comparison of oligonucleotide sequences, a set of primers (forward primer 5' cy3- GGGAGGGA-GACTAGTTG 3', reverse primer 5' cy3-AATTTTTCGCCGCCAAG 3') and a species-specific probe (5' C6-Amino-linker-GATCCTTGGCGTACGGATGCATA 3') for the detection of *Trichophyton tonsurans* were designed. The designed probes were checked for internal repeats, secondary structure, melting temperature and GC content.

DNA microarray

[0062] The designed probe for *T. tonsurans* and some controls were spotted with the sciFLEXARRAYER S11 (Scienion AG, Germany) on a solid carrier material as microscopically small spots located at defined positions.

DNA extraction from dermatophyte cultures

[0063] Cultures were performed on dermatophyte test medium agar (SIFIN, Germany, TN 2102). The DNA from cultured *T. tonsurans* (CBS 483.76), *T. interdigitale anthrophilic/zoophilic* (2235, Pelo1) and *T. equinum* (CBS 127.97) was extracted using the OmniPrep™ for Fungus kit (G-Biosciences, USA, no. 786-399) according to the manufacturer's instructions and identified at species level by internal transcribe spacer (ITS) sequencing.

PCR

[0064] Each PCR reaction was performed in a volume of 20 µl by the addition of 5 µl DNA extract (5 ng/µl) from *Trichophyton tonsurans, Trichophyton interdigitale (anthrophilic), Trichophyton interdigitale (zoophilic)* or *Trichophyton equinum.* Each reaction contained of 1 x Green GoTaq® Flexi Buffer (Promega, USA, X9801), 2.5 mM MgCl2 (Promega, USA, X9801), 0.4 mM each dNTPs (25 mM each) (Bioline, Germany, BIO-39029), 0.75 U GoTaq® Flexi DNA Polymerase (5 U/µl) (Promega, USA, M830), 0.5 µM forward primer and 0.5 µM reverse primer (Metabion, Germany). The amplification was performed in a ABI 2720 thermal cycler (Applied Biosystems, USA, no. 4359659) and consisted of a pre-melt step for 3 min at 96 °C and 35 cycles of 15 s at 96 °C (melt), 15 s at 52 °C (annealing), 40 s at 72 °C (extend) and finished with a 1 min hold at 72 °C.

Hybridization

[0065] The amplicons resulting from the PCR reaction comprised a fluorescent dye attached to the 5' end of the forward and/or reverse primers, which makes the amplicon detectable by a microarray scanner (EUROIMMUN AG, EUROArrayScanner, YG 0602-0101) if the PCR products bind to the complementary probe on the microarray. For the hybridization step, 25 µl PCR products were mixed with 65 µl hybridization buffer A (EUROIMMUN AG, hybridization buffer A, ZM0101-0108). 65 µl of this mixture was hybridized to the microarray using the EUROIMMUN titerplane technique (EUROIMMUN AG, titerplane + hybridization station, ZM 9999-0105 + YG 0615-0101). After one hour of incubation at 55 °C, the EUROArray slides were washed with special buffer solutions, according to the manufacturer's protocol, to remove non-specific bonding sequences (EUROIMMUN AG, wash reagent 1 + 2, ZM 0121-0050 + ZM0122-0012). After washing, the slides were dried with compressed air and only strongly paired strands remained hybridized. A hybridization with labeled PCR product generated a signal which was detected via microarray scanner.

Readout and evaluation

[0066] Final data readout and its evaluation were done using the EUROArrayScanner and EUROArrayScan software (EUROIMMUN AG, EUROArrayScan software, YG 0901-0101). Captured microarrays with hybridized PCR products from *Trichophyton tonsurans, Trichophyton interdigitale (anthrophilic), Trichophyton interdigitale (zoophilic)* and *Trichophyton equinum* templates are shown in **Fig. 2.**

[0067] The specific probe for *Trichophyton tonsurans* is highlighted by a white circle and the dotted encircled spots are controls needed from the software to set the grid. Only the PCR products from the *Trichophyton tonsurans* template (C) hybridized to the specific probe (6a, 6b) and showed fluorescent signals, which are absent when DNA from any of the other trains was used.

[0068] This demonstrates that the inventive method may be used to distinguish *Trichophyton* strains associated with a skin, hair and nail disease.

Example 2

Material and methods

[0069] DNA microarrays consist of DNA molecules (probes) that differ from one another by their DNA sequence. When the DNA of an organism contains segments that match to these defined probes at the microarray, the complementary DNA regions bind together (hybridize). Due to fluorescence labeled primers that are used in the polymerase chain reaction (PCR), a positive hybridization between probe and amplified target sequence can be detected via microarray scanner. An evaluated positive signal means that the target sequence could be detected. In this example the detection of the dermatophyte *Trichophyton benhamiae (yellow) and Trichophyton concentricum* via DNA microarray will be shown. For the verification of probe specificity the most closely related species *Trichophyton benhamiae (white)* and *Trichophyton benhamiae (african)* were also included in the analysis. The used method based on the EUROIMMUN DNA microarray platform.

Design of primers and probes

[0070] For the identification of *Trichophyton benhamiae (yellow) and Trichophyton concentricum* in a sample the metalloprotease gene was chosen as a useful target region. Based on the comparison of oligonucleotide sequences a set of primers (forward primer 5' cy3-CTGGCCATGGCACTTATTGG 3', reverse primer 5' cy3- TGGCTCTGTTACGTG 3') and species-specific probes for the detection of *Trichophyton benhamiae (yellow)* (5' C6-Amino-linker- GATGTAG-GCCCAAAATCATTGCCTACAC 3') *and Trichophyton concentricum* (5' C6-Amino-linker- CTGCCCCAACTTTT-GGCAACTG 3') were designed. The designed probes were checked for internal repeats, secondary structure, melting temperature (Tm) and GC content.

DNA microarray

[0071] The designed probe for *Trichophyton benhamiae (yellow), Trichophyton concentricum* and some controls were spotted with the sciFLEXARRAYER S11 (Scienion AG, Germany) to a solid carrier material as microscopically small spots located at defined positions.

DNA extraction from dermatophyte cultures

[0072] Cultures were performed on dermatophyte test medium agar (SIFIN, Germany, TN 2102). The DNA from cultured *Trichophyton benhamiae (yellow)* (CBS 623.66), *Trichophyton benhamiae* (white) (CBS 280.83), *Trichophyton benhamiae* (african) (CBS 808.72) and *Trichophyton concentricum* (CBS 563.83) was extracted using the OmniPrep™ for Fungus kit (G-Biosciences, USA, no. 786-399) according to the manufacturer's instructions and identified at species level by internal transcribe spacer (ITS) sequencing.

PCR

[0073] Each PCR reaction was performed in a volume of 20 µl by the addition of 5 µl DNA extract (5 ng/µl) from *Trichophyton benhamiae (yellow), Trichophyton benhamiae* (white), *Trichophyton benhamiae* (african) and *Trichophyton concentricum.* Each reaction contained of 1 x Green GoTaq® Flexi Buffer (Promega, USA, X9801), 2.5 mM MgCl2 (Promega, USA, X9801), 0.4 mM each dNTPs (25 mM each) (Bioline, Germany, BIO-39029), 0.75 U GoTaq® Flexi DNA Polymerase (5 U/µl) (Promega, USA, M830), 0,8 µM forward primer and 0,4 µM reverse primer (Metabion, Germany). The amplification was performed in a ABI 2720 thermal cycler (Applied Biosystems, USA, no. 4359659) and consisted of a pre-melt step for 3 min at 96 °C and 35 cycles of 15 s at 96 °C (melt), 15 s at 52°C (annealing), 40 s at 72 °C (extend) and finished with a 1 min hold at 72 °C.

Hybridization

[0074] The resulting PCR products were labelled with a fluorescent dye, which enables them to be detected by the microarray scanner (EUROIMMUN AG, EUROArrayScanner, YG 0602-0101) if the PCR products bind to the complementary probe on the microarray. For the hybridization step 25 µl PCR products were mixed with 65 µl hybridization buffer A (EUROIMMUN AG, hybridization buffer A, ZM0101-0108). 65 µl of this mixture was hybridized to the microarray using the EUROIMMUN titerplane technique (EUROIMMUN AG, titerplane + hybridization station, ZM 9999-0105 + YG 0615-0101). After one hour of incubation at 55 °C the EUROArray slides were washed with special buffer solutions, according to the manufacturer's protocol, to remove non-specific bonding sequences (EUROIMMUN AG, wash reagent

1 + 2, ZM 0121-0050 + ZM0122-0012). After washing the slides were dried with compressed air and only strongly paired strands remained hybridized. Hybridization with labeled PCR product generated a signal which was detected via microarray scanner.

Readout and evaluation

[0075] Final data readout and its evaluation were done using the EUROArrayScanner and EUROArrayScan software (EUROIMMUN AG, EUROArrayScan software, YG 0901-0101). Captured microarrays with hybridized PCR products from *Trichophyton benhamiae (yellow), Trichophyton benhamiae* (white), *Trichophyton benhamiae* (african) and *Trichophyton concentricum* templates are shown in Figure 3. Strains were identified as being a Trichophyton benhamiae (yellow) or Trichopyhton concentricum when the specific probes (white circle in Fig. 3) hybridized with cy3-labeled PCR products and the controls at the microarray also showed fluorescent signals due to a hybridization between labeled oligonucleotides in the hybridization buffer and probe sequences in the corner of the array.

Example 3

Material and methods

[0076] DNA microarrays consist of DNA molecules (probes) that differ from one another by their DNA sequence. When the DNA of an organism contains segments that match to these defined probes at the microarray, the complementary DNA regions bind together (hybridize). Due to fluorescence labeled primers that are used in the polymerase chain reaction (PCR), a positive hybridization between probe and amplified target sequence can be detected via microarray scanner. An evaluated positive signal means that the target sequence could be detected. In this example the detection of the dermatophyte *Trichophyton benhamiae (yellow) and Trichophyton benhamiae (white/african)* via DNA microarray will be shown. The used method based on the EUROIMMUN DNA microarray platform.

Design of primers and probes

[0077] For the identification of *Trichophyton benhamiae (yellow) or Trichophyton benhamiae (white/african)* in a sample the metalloprotease gene was chosen as a useful target region. Based on the comparison of oligonucleotide sequences a set of primers (forward primer 5' cy3-CTGGCCATGGCACTTATTGG 3', reverse primer 5' cy3- TGGCTCTGTTACGTG 3') and species-specific probes for the detection of *Trichophyton benhamiae (yellow)* (5' C6-Amino-linker- TAGGCAATGATTTTGGGCCTA 3') *and Trichophyton benhamiae (white/african)* (5' C6-Amino-linker- CATCGGCGAGGACCATCGGA 3') were designed. The designed probes were checked for internal repeats, secondary structure, melting temperature (Tm) and GC content.

DNA microarray

[0078] The designed probe for *Trichophyton benhamiae (yellow), Trichophyton benhamiae (white/african) and* some controls were spotted with the sciFLEXARRAYER S11 (Scienion AG, Germany) to a solid carrier material as microscopically small spots located at defined positions.

DNA extraction from dermatophyte cultures

[0079] Cultures were performed on dermatophyte test medium agar (SIFIN, Germany, TN 2102). The DNA from cultured *Trichophyton benhamiae (yellow)* (CBS 623.66), *Trichophyton benhamiae* (white) (CBS 280.83) and *Trichophyton benhamiae* (african) (CBS 808.72) was extracted using the OmniPrep™ for Fungus kit (G-Biosciences, USA, no. 786-399) according to the manufacturer's instructions and identified at species level by internal transcribe spacer (ITS) sequencing.

PCR

[0080] Each PCR reaction was performed in a volume of 20 µl by the addition of 5 µl DNA extract (5 ng/µl) from *Trichophyton benhamiae (yellow), Trichophyton benhamiae* (white) and *Trichophyton benhamiae* (african). Each reaction contained of 1 x Green GoTaq® Flexi Buffer (Promega, USA, X9801), 2.5 mM MgCl2 (Promega, USA, X9801), 0.4 mM each dNTPs (25 mM each) (Bioline, Germany, BIO-39029), 0.75 U GoTaq® Flexi DNA Polymerase (5 U/µl) (Promega, USA, M830), 0,4 µM forward primer and 1,6 µM reverse primer (Metabion, Germany). The amplification was performed in a ABI 2720 thermal cycler (Applied Biosystems, USA, no. 4359659) and consisted of a pre-melt step for 3 min at 96

°C and 35 cycles of 15 s at 96 °C (melt), 15 s at 52°C (annealing), 40 s at 72 °C (extend) and finished with a 1 min hold at 72 °C.

Hybridization

[0081] The resulting PCR products were labelled with a fluorescent dye, which enables them to be detected by the microarray scanner (EUROIMMUN AG, EUROArrayScanner, YG 0602-0101) if the PCR products bind to the complementary probe on the microarray. For the hybridization step 25 µl PCR products were mixed with 65 µl hybridization buffer A (EUROIMMUN AG, hybridization buffer A, ZM0101-0108). 65 µl of this mixture was hybridized to the microarray using the EUROIMMUN titerplane technique (EUROIMMUN AG, titerplane + hybridization station, ZM 9999-0105 + YG 0615-0101). After one hour of incubation at 55 °C the EUROArray slides were washed with special buffer solutions, according to the manufacturer's protocol, to remove non-specific bonding sequences (EUROIMMUN AG, wash reagent 1 + 2, ZM 0121-0050 + ZM0122-0012). After washing the slides were dried with compressed air and only strongly paired strands remained hybridized. Hybridization with labeled PCR product generated a signal which was detected via microarray scanner.

Readout and evaluation

[0082] Final data readout and its evaluation were done using the EUROArrayScanner and EUROArrayScan software (EUROIMMUN AG, EUROArrayScan software, YG 0901-0101). Captured microarrays with hybridized PCR products from *Trichophyton benhamiae (yellow), Trichophyton benhamiae* (white) and *Trichophyton benhamiae* (african) templates are shown in Figure 4. Strains were identified as being a Trichophyton benhamiae (yellow) or Trichophyton benhamiae (white/african) when the specific probes (white circle in Fig. 4) hybridized with cy3-labeled PCR products and the controls at the microarray also showed fluorescent signals due to a hybridization between labeled oligonucleotides in the hybridization buffer and probe sequences in the corner of the array.

Example 4

Material and methods

[0083] DNA microarrays consist of DNA molecules (probes) that differ from one another by their DNA sequence. When the DNA of an organism contains segments that match to these defined probes at the microarray, the complementary DNA regions bind together (hybridize). Due to fluorescence labeled primers that are used in the polymerase chain reaction (PCR), a positive hybridization between probe and amplified target sequence can be detected via microarray scanner. An evaluated positive signal means that the target sequence could be detected. In this example the detection of the dermatophyte *Trichophyton erinacei* via DNA microarray will be shown. For the verification of probe specificity the most closely related species *Trichophyton benhamiae (white)* and *Trichophyton benhamiae (african)* were also included in the analysis. The used method based on the EUROIMMUN DNA microarray platform.

Design of primers and probes

[0084] For the identification of *Trichophyton erinacei* in a sample the metalloprotease gene was chosen as a useful target region. Based on the comparison of oligonucleotide sequences a set of primers (forward primer 5' cy3- CTGGCCATGGCACTTATTGG 3', reverse primer 5' cy3-TGGCTCTGTTACGTG 3') and a species-specific probe for the detection of *Trichophyton erinacei* (5' C6-Amino-linker- GATGTAGGCCCAAAATCATTGCCTACAC 3') *was* designed. The designed probe was checked for internal repeats, secondary structure, melting temperature (Tm) and GC content.

DNA microarray

[0085] The designed probe for *Trichophyton erinacei* and some controls were spotted with the sciFLEXARRAYER S11 (Scienion AG, Germany) to a solid carrier material as microscopically small spots located at defined positions.

DNA extraction from dermatophyte cultures

[0086] Cultures were performed on dermatophyte test medium agar (SIFIN, Germany, TN 2102). The DNA from cultured *Trichophyton erinacei* (CBS 677.86), *Trichophyton benhamiae* (white) (CBS 280.83) and *Trichophyton benhamiae* (african) (CBS 808.72) was extracted using the OmniPrep™ for Fungus kit (G-Biosciences, USA, no. 786-399) according to the manufacturer's instructions and identified at species level by internal transcribe spacer (ITS) sequencing.

PCR

[0087] Each PCR reaction was performed in a volume of 20 µl by the addition of 5 µl DNA extract (5 ng/µl) from *Trichophyton erinacei, Trichophyton benhamiae* (white) and *Trichophyton benhamiae* (african). Each reaction contained of 1 x Green GoTaq® Flexi Buffer (Promega, USA, X9801), 2.5 mM MgCl2 (Promega, USA, X9801), 0.4 mM each dNTPs (25 mM each) (Bioline, Germany, BIO-39029), 0.75 U GoTaq® Flexi DNA Polymerase (5 U/µl) (Promega, USA, M830), 0,5 µM forward primer and 0,5 µM reverse primer (Metabion, Germany). The amplification was performed in a ABI 2720 thermal cycler (Applied Biosystems, USA, no. 4359659) and consisted of a pre-melt step for 3 min at 96 °C and 35 cycles of 15 s at 96 °C (melt), 15 s at 52°C (annealing), 40 s at 72 °C (extend) and finished with a 1 min hold at 72 °C.

Hybridization

[0088] The resulting PCR products were labelled with a fluorescent dye, which enables them to be detected by the microarray scanner (EUROIMMUN AG, EUROArrayScanner, YG 0602-0101) if the PCR products bind to the complementary probe on the microarray. For the hybridization step 25 µl PCR products were mixed with 65 µl hybridization buffer A (EUROIMMUN AG, hybridization buffer A, ZM0101-0108). 65 µl of this mixture was hybridized to the microarray using the EUROIMMUN titerplane technique (EUROIMMUN AG, titerplane + hybridization station, ZM 9999-0105 + YG 0615-0101). After one hour of incubation at 55 °C the EUROArray slides were washed with special buffer solutions, according to the manufacturer's protocol, to remove non-specific bonding sequences (EUROIMMUN AG, wash reagent 1 + 2, ZM 0121-0050 + ZM0122-0012). After washing the slides were dried with compressed air and only strongly paired strands remained hybridized. Hybridization with labeled PCR product generated a signal which was detected via microarray scanner.

Readout and evaluation

[0089] Final data readout and its evaluation were done using the EUROArrayScanner and EUROArrayScan software (EUROIMMUN AG, EUROArrayScan software, YG 0901-0101). Captured microarrays with hybridized PCR products from *Trichophyton erinacei, Trichophyton benhamiae* (white) and *Trichophyton benhamiae* (african) templates are shown in Figure 5. Strains were identified as being a *Trichophyton erinacei* when the specific probe (white circle in Fig. 5) hybridized with cy3-labeled PCR products and the controls at the microarray also showed fluorescent signals due to a hybridization between labeled oligonucleotides in the hybridization buffer and probe sequences in the corner of the array.

Example 5

Material and methods

[0090] DNA microarrays consist of DNA molecules (probes) that differ from one another by their DNA sequence. When the DNA of an organism contains segments that match to these defined probes at the microarray, the complementary DNA regions bind together (hybridize). Due to fluorescence labeled primers that are used in the polymerase chain reaction (PCR), a positive hybridization between probe and amplified target sequence can be detected via microarray scanner. An evaluated positive signal means that the target sequence could be detected. In this example the detection of the dermatophyte *Trichophyton benhamiae (white), Trichophyton benhamiae (yellow) and Trichophyton concentricum* via DNA microarray will be shown. The used method based on the EUROIMMUN DNA microarray platform.

Design of primers and probes

[0091] For the identification of *Trichophyton benhamiae (white), Trichophyton benhamiae (yellow) and Trichophyton concentricum* in a sample the metalloprotease gene was chosen as a useful target region. Based on the comparison of oligonucleotide sequences a set of primers (forward primer 5' cy3- GCATTTCCCATGGCT 3', reverse primer 5' cy3- TGGCTCTGTTACGTG 3') and species-specific probes for the detection of *Trichophyton benhamiae (white/african)* (5' C6-Amino-linker- CATCGGCGAGGACCATCGGA 3'), *Trichophyton benhamiae (yellow)* (5' C6-Amino-linker- TAG-GCAATGATTTTGGGCCTA 3') *and Trichophyton concentricum* (5' C6-Amino-linker- GCAATGGTTCTGGGCCTACATC 3') *were* designed. The designed probes were checked for internal repeats, secondary structure, melting temperature (Tm) and GC content.

DNA microarray

[0092] The designed probes for *Trichophyton benhamiae (white), Trichophyton benhamiae (yellow), Trichophyton*

concentricum and some controls were spotted with the sciFLEXARRAYER S11 (Scienion AG, Germany) to a solid carrier material as microscopically small spots located at defined positions.

DNA extraction from dermatophyte cultures

[0093] Cultures were performed on dermatophyte test medium agar (SIFIN, Germany, TN 2102). The DNA from cultured *Trichophyton benhamiae* (african) (CBS 808.72), *Trichophyton benhamiae* (yellow) (CBS 623.66) and *Trichophyton concentricum* (CBS 563.83) was extracted using the OmniPrep™ for Fungus kit (G-Biosciences, USA, no. 786-399) according to the manufacturer's instructions and identified at species level by internal transcribe spacer (ITS) sequencing.

PCR

[0094] Each PCR reaction was performed in a volume of 20 µl by the addition of 5 µl DNA extract (5 ng/µl) from *Trichophyton benhamiae (white), Trichophyton benhamiae (yellow), Trichophyton concentricum.* Each reaction contained of 1 x Green GoTaq® Flexi Buffer (Promega, USA, X9801), 2.5 mM MgCl2 (Promega, USA, X9801), 0.4 mM each dNTPs (25 mM each) (Bioline, Germany, BIO-39029), 0.75 U GoTaq® Flexi DNA Polymerase (5 U/µl) (Promega, USA, M830), 1,0 µM forward primer and 1,0 µM reverse primer (Metabion, Germany). The amplification was performed in a ABI 2720 thermal cycler (Applied Biosystems, USA, no. 4359659) and consisted of a pre-melt step for 3 min at 96 °C and 35 cycles of 15 s at 96 °C (melt), 15 s at 52°C (annealing), 40 s at 72 °C (extend) and finished with a 1 min hold at 72 °C.

Hybridization

[0095] The resulting PCR products were labelled with a fluorescent dye, which enables them to be detected by the microarray scanner (EUROIMMUN AG, EUROArrayScanner, YG 0602-0101) if the PCR products bind to the complementary probe on the microarray. For the hybridization step 25 µl PCR products were mixed with 65 µl hybridization buffer A (EUROIMMUN AG, hybridization buffer A, ZM0101-0108). 65 µl of this mixture was hybridized to the microarray using the EUROIMMUN titerplane technique (EUROIMMUN AG, titerplane + hybridization station, ZM 9999-0105 + YG 0615-0101). After one hour of incubation at 55 °C the EUROArray slides were washed with special buffer solutions, according to the manufacturer's protocol, to remove non-specific bonding sequences (EUROIMMUN AG, wash reagent 1 + 2, ZM 0121-0050 + ZM0122-0012). After washing the slides were dried with compressed air and only strongly paired strands remained hybridized. A hybridization with labeled PCR product generated a signal which was detected via microarray scanner.

Readout and evaluation

[0096] Final data readout and its evaluation were done using the EUROArrayScanner and EUROArrayScan software (EUROIMMUN AG, EUROArrayScan software, YG 0901-0101). Captured microarrays with hybridized PCR products from *Trichophyton benhamiae (white), Trichophyton benhamiae (yellow)* and *Trichophyton concentricum* templates are shown in Figure 6. Strains were identified as being a *Trichophyton benhamiae (whitelafrican), Trichophyton benhamiae (yellow)* and *Trichophyton concentricum* when the specific probe (white circle in Fig. 6) hybridized with cy3-labeled PCR products and the controls at the microarray also showed fluorescent signals due to a hybridization between labeled oligonucleotides in the hybridization buffer and probe sequences in the corner of the array.

SEQUENCE LISTING

[0097]

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<223> Probe

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<210> 27
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<223> Probe

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<210> 28
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atagatggaa gggtgtaggc aatggttctg ggactgcatc ggcgatattt atcggagcag 240

aac 243

REFERENCES CITED IN THE DESCRIPTION

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Patentkrav

1. Primerpar omfattende en forward-primer og en reverse-primer, der er i stand til at amplificere en nukleinsyre fra et patogen, der er associeret med en hud-, hår- og negleinfektion, hvor den amplificerede nukleinsyre omfatter SEQ ID NO22, hvor forward-primeren hybridiserer specifikt til en region af patogenets genom opstrøms for SEQ ID NO22, og reverse-primeren hybridiserer specifikt til en region af patogenets genom nedstrøms for SEQ ID NO22,
hvor afstanden mellem forward-primerens sidste basepar, i 5'-3'-orienteringen, og det første basepar af SEQ ID NO22, i 5'-3'-orienteringen, er 200, 100 eller færre basepar,
hvor afstanden mellem reverse-primerens sidste basepar, i 5'-3'-orienteringen, og det sidste basepar af SEQ ID NO22, i 5'-3'-orienteringen, er 200, 100 eller færre basepar,
hvor hver primer har en længde på 10 til 40, 12 til 35 eller 14 til 30 nukleotider, og
hvor patogenet er udvalgt fra gruppen omfattende *T. tonsurans, T. equinum, T. interdigitale, T. benhamiae (afrikansk), T. benhamiae (gul), T. concentricum* og *T. erinacei.*

2. Nukleinsyre omfattende (a) en forward-primer-sekvens og en reverse-primer-sekvens i overensstemmelse med primerparret ifølge krav 1 og, mellem forward- og reverse-primer-sekvensen, (b) nukleinsyresekvensen fra et patogen, der er associeret med en hud-, hår- og negleinfektion, omfattende SEQ ID NO22,
hvor patogenet er udvalgt fra gruppen omfattende *T. tonsurans, T. equinum, T. interdigitale, T. benhamiae (afrikansk), T. benhamiae (gul), T. concentricum* og *T. erinacei*, og
hvor nukleinsyren er opnået ved amplificering af en prøve omfattende patogenet og under anvendelse af primerparret ifølge krav 1.

3. Primerpar ifølge krav 1 eller nukleinsyre ifølge krav 2, omfattende et deteterbart mærke, fortrinsvis fra gruppen omfattende et fluorescerende, radioaktivt, kolloidt guld- eller enzymatisk aktivt mærke.

4. Fremgangsmåde omfattende trinnet detektering i en prøve af en nukleinsyresekvens omfattende SEQ ID NO22 fra et patogen, der er associeret med en hud-, hår- og negleinfektion, og hvor patogenet er udvalgt fra gruppen omfattende *T. tonsurans, T. equinum, T. interdigitale, T. benhamiae (afrikansk), T. benhamiae (gul), T. concentricum* og *T. erinacei.*

5. Fremgangsmåde ifølge krav 4, omfattende trinnene
a) amplificering af enhver nukleinsyre omfattende SEQ ID NO22, der er til stede i den tilvejebragte prøve, fortrinsvis negle-, hår- eller hudmateriale, under anvendelse af primerparret ifølge et hvilket som helst af kravene 1 eller 3, hvorved der genereres et amplikon, hvis en nukleinsyresekvens omfattende SEQ ID NO22 fra patogenet er til stede i prøven.

6. Fremgangsmåde ifølge krav 5, endvidere omfattende trinnet
b) detektering af amplikonet.

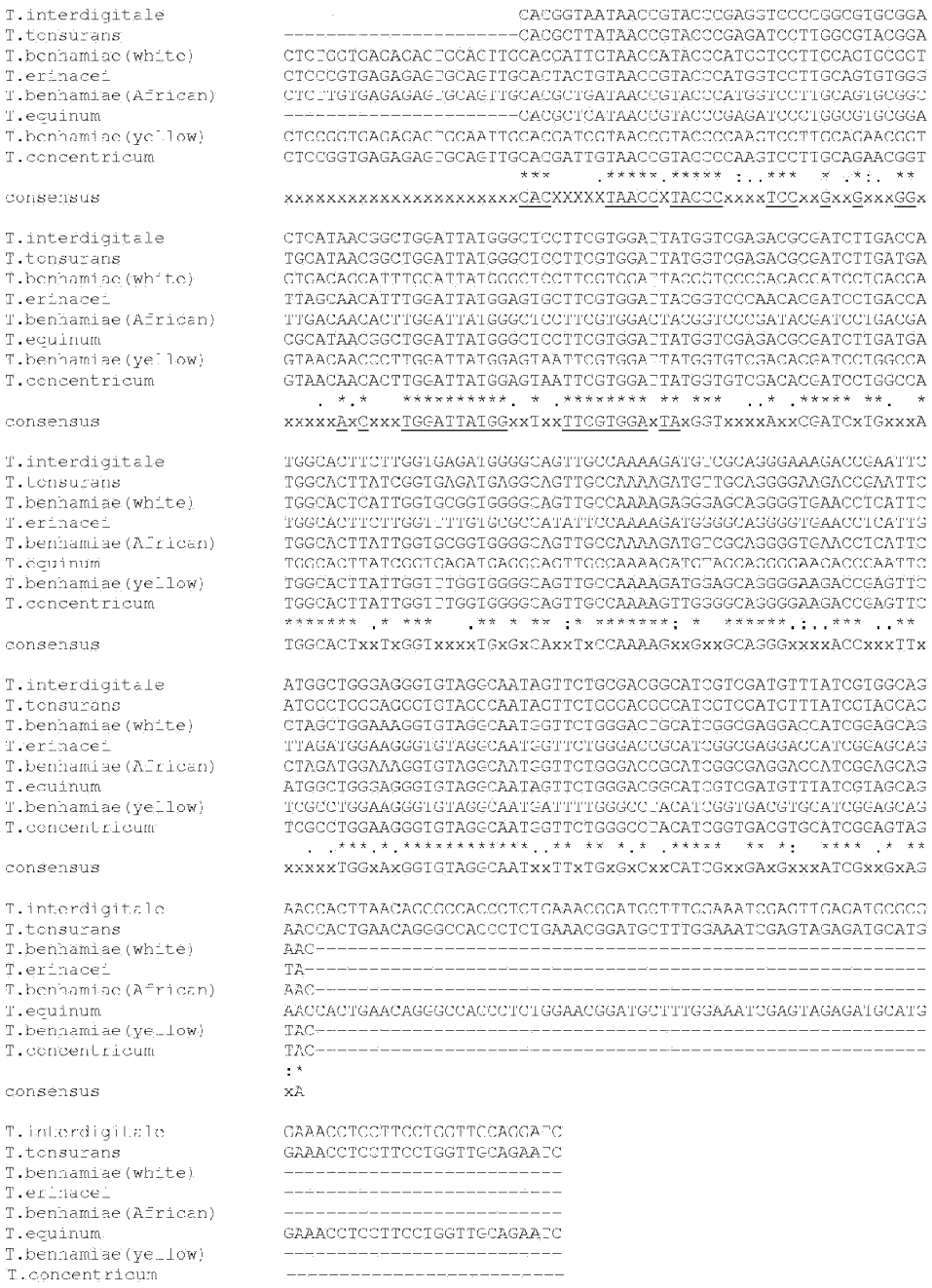
7. Fremgangsmåde ifølge krav 6, hvor amplikonet detekteres ved hjælp af fluorescens, radioaktivitet, kolloidt guld eller kemiluminescens.

8. Anvendelse af primerparret ifølge et hvilket som helst af kravene 1 eller 3 til diagnosticering af en hud-, hår- og negleinfektion, der er associeret med et patogen, og hvor patogenet er udvalgt fra gruppen omfattende *T. tonsurans, T. equinum, T. interdigitale, T. benhamiae (afrikansk), T. benhamiae (gul), T. concentricum* og *T. erinacei.*

9. Kit omfattende primerparret ifølge et hvilket som helst af kravene 1 eller 3, fortrinsvis til diagnosticering af en hud-, hår- og negleinfektion, der er associeret med et patogen, og
hvor patogenet er udvalgt fra gruppen omfattende *T. tonsurans, T. equinum,*

T. interdigitale, T. benhamiae (afrikansk), T. benhamiae (gul), T. concentricum og *T. erinacei.*

DRAWINGS



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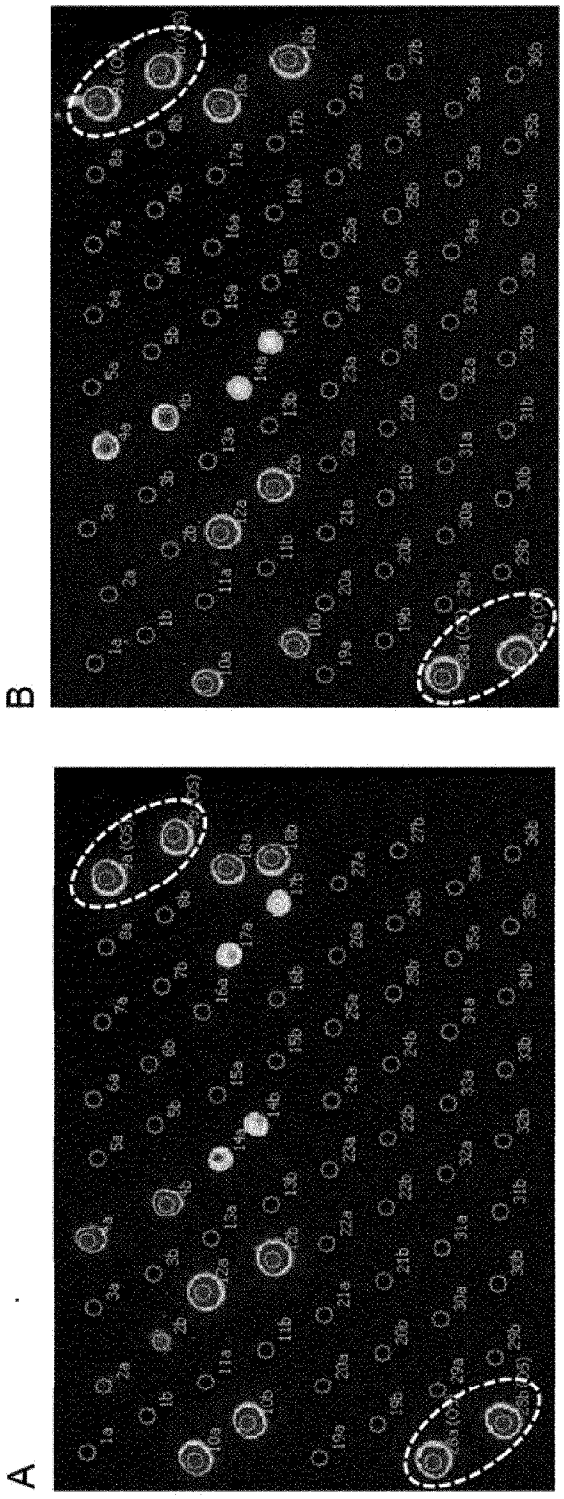

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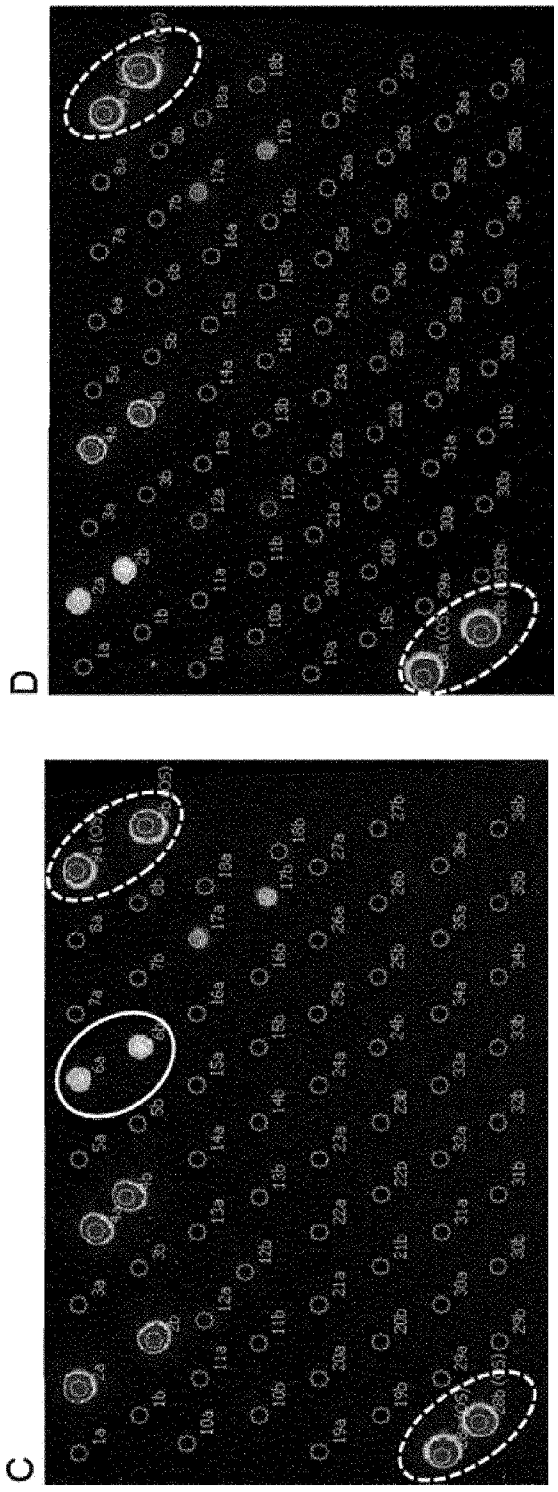

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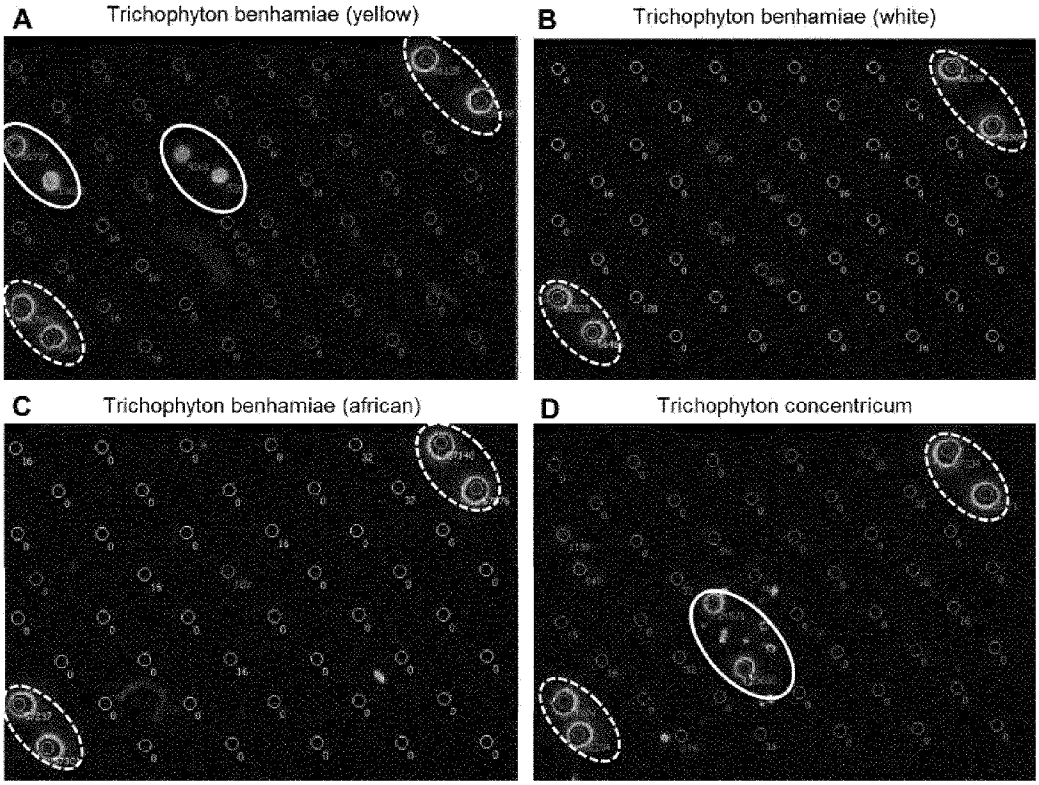
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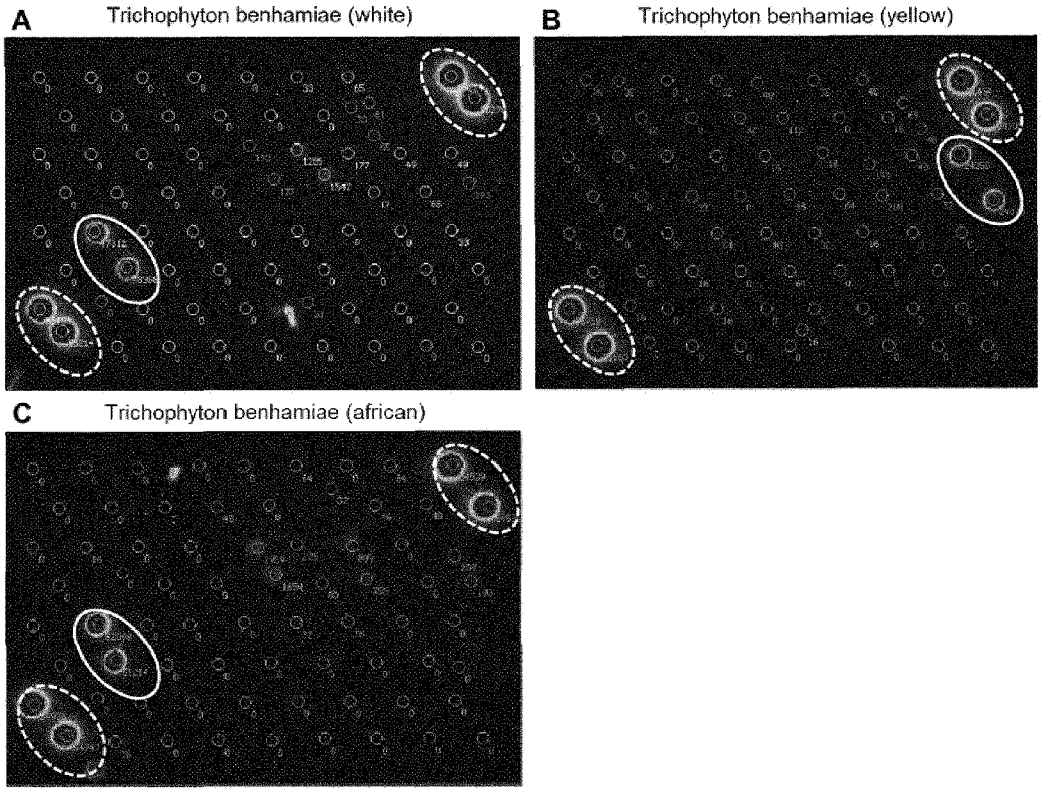
Trichophyton benhamiae (african)
D
Trichophyton concentricum

Fig. 3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Trichophyton benhamiae (white)
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Trichophyton benhamiae (yellow)
C
Trichophyton benhamiae (african)

Fig. 4

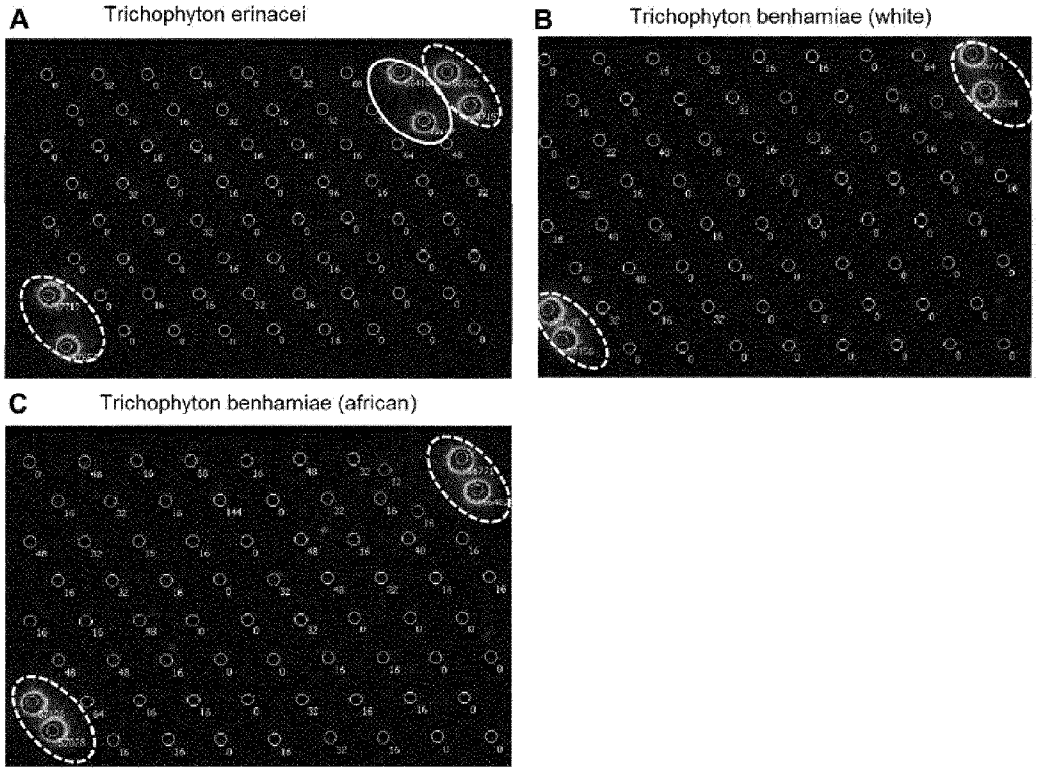
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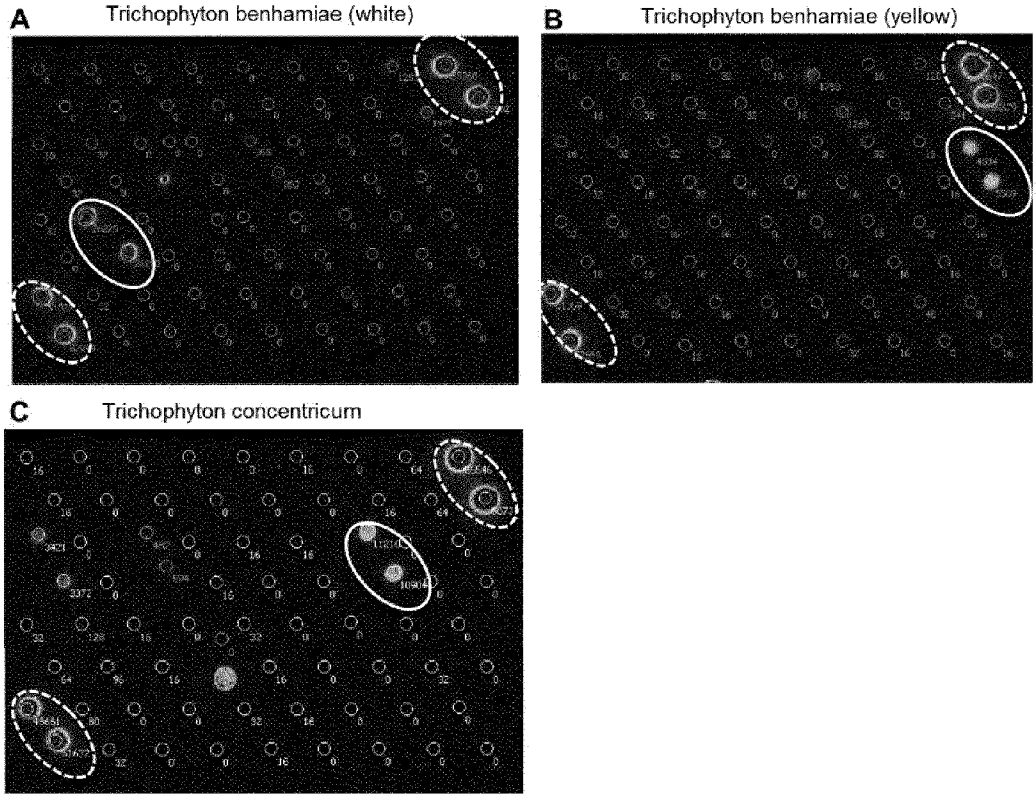
Fig. 5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Trichophyton benhamiae (white)
B
Trichophyton benhamiae (yellow)
C
Trichophyton concentricum

Fig. 6