Mycotype® **Basidio**QS Microarray Detection Kit

Product description

Mycotype® **Basidio**QS Microarray Detection Kit contains optimised reagents for the detection of all relevant dry rot fungi. Employing the polymerase chain reaction (PCR), first, specific amplification of wood-rotting basidiomycetes' target DNA takes place. In the second step, the fungi are species- and genus-specifically identified using the DNA chip technology.

Applied DNA chip technology is based on *Arrayed Ligation Reaction* (ALR), where additionally to a hybridization reaction a specific enzymatic reaction takes place on a planar chip surface. This ligation reaction binds the ligation oligonucleotides to the immobilised DNA probes. Detection and analysis is achieved by measuring the fluorescence signals of the bound ligation oligos using a microarray scanner. The DNA chips are one-way products and cannot be reused after analysis. Please find a detailed description of the technology in section 5.

Mycotype® **Basidio**QS Microarray Detection Kit was specially designed to facilitate fast, simple-to-use, routine-fit and reliable diagnostics of dry rot fungi. Particular attention was paid to allow an identification and differentiation of simultaneously occurring fungi species in one sample (multiplex-analysis of mixed samples) and to achieve considerable reduction of analysis time, so far necessary for molecular biological methods. Thanks to the two-step process of specific amplification with basidiomycetes-specific primers and the differentiation of fungi by species- and genus-specific DNA probes a highly analytical specificity in diagnostics is guaranteed.

Mycotype® **Basidio**QS Microarray Detection Kit contains internal PCR and ALR controls (Quality Sensors "QS") which provide helpful information on the efficiency of the PCR and ALR and on the presence of reaction inhibitors.

The detection limit of the Mycotype® **Basidio**QS Microarray Detection Kit is 10 pg genomic DNA. The optimal range under standard conditions is **0.5-5 ng DNA**.

The test kit was validated and evaluated using PCR cycler BioRad PTC-200, Eppendorf Mastercycler ep-S and Techne TC-512, as well as scanner PerkinElmer ScanArray® Gx Plus and Ditabis Microarray Scanner MArS. The research and development, production, and distribution of Biotype® products are certified according to DIN EN ISO 9001 ff.

This product and its use are covered by one or more of the following patents owned by Oxford Gene Technology IP Limited (together "OGT"): European Patent Nos. 0 820 524 and 1 308 523, US Patents Nos. 6,150,095, 6,307,039, 6,770,751 and 7,192,707 and pending patents. The purchaser is licensed to practise methods and processes covered by these patents using this product for the purpose of botanical, medical, forensic, veterinary, environmental and food diagnosis, prognosis and monitoring only.

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1. Description of Mycotype® Basidio^{QS}

Table 1. Detectable dry rot fungi

Antrodia sinuosa
Antrodia vaillantii
Antrodia xantha
Bjerkandera adusta
Coniophora arida
Coniophora marmorata
Coniophora olivacea
Coniophora puteana
Daedalea quercina
Donkioporia expansa
Fomitopsis pinicola
Gloeophyllum abietinum
Gloeophyllum sepiarium
Gloeophyllum trabeum
Laetiporus spp.
Leucogyrophana mollusca
Leucogyrophana pinastri
Neo/ Lentinus lepideus
Oligoporus placenta
Phellinus ferruginosa
Pleurotus spp.
Schizophyllum commune
Serpula lacrymans
Serpula himantioides
Stereum spp.
Tapinella panuoides
Trametes (Coriolus) versicolor

Table 2. Quality Sensors of Mycotype® Basidio^{QS}

Quality Sensors	Description
PCR positive control	PCR process verification
ALR positive control	ALR process verification
ALR negative control	ALR specificity verification
Fluorescence marker	Production control and position marker

Content

Mycotype® **Basidio**^{QS} Microarray Detection Kit

Kit component	4 reactions	20 reactions	40 reactions
PCR mix	195 µl	955 µl	2x 955 µl
DNA polymerase	3 µl	8 µl	2x 8 µl
DNA glycosylase	5 µl	20 µl	2x 20 µl
ALR mix	30 µl	145 µl	2x 145 µl
DNA ligase	17 µl	83 µl	2x 83 µl
DNA chips (á 4 Arrays)	1 sld	5 slds	2x 5 slds
Test slide	1 sld	1 sld	1 sld

Ordering information

Mycotype® **Basidio**^{QS}	4	reactions	Cat. No.	51-27110-0004
Mycotype® **Basidio**^{QS}	20	reactions	Cat. No.	51-27110-0020
Mycotype® **Basidio**^{QS}	40	reactions	Cat. No.	51-27110-0040

Storage

Store all components at -20°C and avoid repeated thawing and freezing. The ALR mix and the DNA chips must be stored protected from light. The DNA chips should be kept unopened in the protective packaging at RT until first use. After opening the protective packaging we advise to store DNA chips together with desiccant at -20°C in the original packaging, or vacuum-packed at RT. The expiry date is indicated on the kit cover.

Additionally required reagents and materials

Additional reagents and materials are needed in order to use the Biotype® Microarray Detection Kit:

Reagent / Material

ALCONOX - Powdered Precision Cleaner (Alconox, Inc., US; 1125)
DNA-decontamination medium for cleaning of instruments and surfaces
Stainless steel slide holder (e.g. VWR International GmbH; 631-9811)
Fluorescence scanner for standard DNA chips (75 x 25 x 1 mm)
Horizontal shaker for reaction tubes (Vortex)
Micropipettes (10, 20, 200 µl)
PCR cycler
Reaction tubes (0.2 ml, 1.5 ml und 2.0 ml)
PCR purification kit (e.g. Macherey-Nagel GmbH & Co.KG; NucleoSpin® Extract II or Qiagen GmbH; MinElute PCR Purification Kit)
Nitrogen gas cylinder or centrifuge for DNA chips / microtiter plates (microplates)
Thermoblock for reaction tubes (37°C / 95°C)
Thermoblock with humidity chamber for hybridization (e.g. Eppendorf; exchangeable thermoblock for 1 to 4 slides; 5368 000.010)
Washing pot
Water heater
Centrifuge for reaction tubes

Warnings and safety instructions

The Microarray Detection Kit contains the following potentially hazardous chemicals:

Kit component	Chemical	Hazards
Primer mix	Sodium azide NaN_3	toxic if swallowed, develops toxic gases when it gets in contact with acids

Observe the Material Safety Data Sheets (MSDS) for all Biotype® products, which are available on request. Please contact the respective manufacturers for copies of the MSDS for any additionally needed reagents.

Quality assurance

All kit components undergo an intensive quality assurance process at Biotype Diagnostic GmbH. The quality of the test kits is permanently monitored in order to ensure unrestricted usability. If you have any questions regarding quality assurance, please do not hesitate to contact us.

Trademarks and patents

Mycotype® is a registered trademark of Biotype Diagnostic GmbH.
ScanArray® is a registered trademark of PerkinElmer, Inc.
The enzymatic detection of sequence variants on a planar surface is covered by patents. The patentee is Oxford Gene Technology (OGT) (Oxford, UK).

2. Workflow diagram

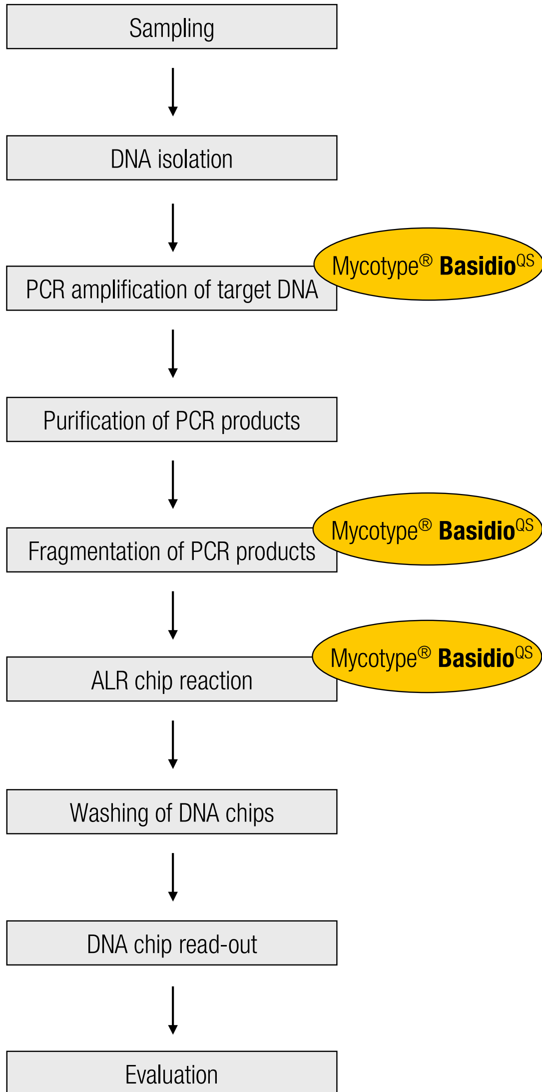

Fig. 1 From sample to report – identification of dry rot fungi with Mycotype® **Basidio**^{QS}

3. Brief instructions

Please read detailed instructions (section 4, page 10 et sqq.) prior starting experiment.

Sampling and DNA isolation

- For recommendations see section 4.1 (page 10)
- Depending on the grade of turbidity the DNA should be diluted 1:10 to 1:50 with 0.1x TE-buffer, if purified by precipitation
- The DNA may be used undiluted if purified using spin-columns

PCR amplification of target DNA

- All components should be mixed (vortex) and centrifuged for about 5 s before preparing the master mix

	Number of PCR approaches			
Component	**1**	**5**	**9**	**17**
PCR mix	23.8 µl	119.0 µl	214.2 µl	404.6 µl
DNA polymerase	0.2 µl	1.0 µl	1.8 µl	3.4 µl
Volume of the PCR master mix	*24.0 µl*	*120.0 µl*	*216.0 µl*	*408.0 µl*

- Mix shortly (vortex) and centrifuge (ca. 5 s) the master mix
- Disperse master mix and add 1 µl sample DNA per reaction tube
- Mix shortly (vortex) and centrifuge (ca. 5 s) the PCR mix

Amplification parameter

Temperature	Time	
94°C	4 min	
94°C	30 s	
60°C	60 s	**35 cycles**
72°C	60 s	
72°C	7 min	
10°C	∞	hold

Analysis of PCR products (optional)

- Maximum 5 µl per PCR approach can be analyzed using for example the agarose gel electrophoresis
- A weak ~650 bp long PCR product (5 ng / 5 µl) is sufficient for a successful ALR

Purification of PCR products

- Purify the PCR products using a PCR purification kit and elute in 22 µl elution buffer
- For recommendations see section 4.5 (page 14)

DNA fragmentation and denaturing

Component	Volume
purified PCR product	20.0 µl
DNA glycosylase	1.0 µl
- mix (vortex) and centrifuge shortly (ca. 5 s)	

- fragmentation for 15 min at 37°C
- denaturation for min. 10 min at 95°C

*** Attention:** The 95°C hot samples must be used directly in ALR.

Arrayed Ligation Reaction (ALR)

- Heat up the microarray-thermoblock* to 58°C and fill the humidity reservoirs with ddH_2O
- Place the DNA chips on the designated area
- All components should be mixed (vortex) and centrifuged for about 5 s

Note: Mark the DNA chips on the reverse side, outside the arrays using a touch-up pencil. Marks on the microfluidic would get lost after removal of the film in a later step!

	Number of ALR approaches			
Component	**1**	**5**	**9**	**17**
ALR mix	5.7 µl	28.5 µl	51.3 µl	96.9 µl
DNA ligase	3.3 µl	16.5 µl	29.7 µl	56.1 µl
Volume of the ALR master mix	*9.0 µl*	*45.0 µl*	*81.0 µl*	*153.0 µl*

- Mix shortly (vortex) and centrifuge (ca. 5 s) the master mix

Component	Volume
fragmented, denatured, 95°C hot PCR product	21.0 µl
ALR master mix	9.0 µl
- mix (vortex) and centrifuge shortly (ca. 5 s)	

- fill the mix directly into the fillers of the microfluidic film

*** Attention:** The fragmented and denatured PCR product must be tempered at 95°C until used. The ALR approach must be pipetted fast and with pressure into the fillers of the microfluidic film.

- As soon as all arrays are loaded close the microarray-thermoblock* and incubate for 20 min at 58°C

* As an alternative, the use of a hybridization chamber together with a tempered water bath is also possible.

Washing of DNA chips

- Remove the microfluidic film from DNA chip
- Place the DNA chips in a stainless steel slide holder and cover with 95°C ddH_2O for 1 min
- Slew the DNA chips gently for 2 min in a 50°C 0.3 % (w/v) Alconox solution (consisting of 1/3 Vol. 1 % (w/v) Alconox and 2/3 Vol. 95°C ddH_2O)
- Immerse the DNA chips three times consecutively for ca. 1 min in fresh 95°C ddH2O
- Clean and dry the DNA chips with gaseous nitrogen
- As an alternative, the DNA chips can be placed in the slide holder with a tissue paper underneath in a centrifuge for microplates and spun down for 1 min at 150 x g

Read-out and evaluation

- Calibrate scanner with calibration slide
- To this, select any area of the size of a Mycotype® **Basidio**QS array (~5.2 x 6.2 mm) and scan the calibration slide using adjustment for Cyanine 5 (Cy5, 633 nm)
- Adjust the PMT and laser strength of the scanner so that the averaged RFU-value equates to the indications accompanying the kit
- Scan the DNA chips using adjustment for Cyanine 5 (Cy5, 633 nm) and limit the area of the scan field to the size of an array
- Safe image in 16-Bit TIF format
- Evaluate signal intensities with MycoProof **Basidio** Software (for detailed instructions see section 4.10, page 14)

4. Detailed instructions

4.1 Sample preparation

In order to guarantee an optimal analysis of dry rot samples we recommend to follow the instructions of GLP (Good Laboratory Practice) at: United States Environmental Protection Agency (EPA): Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples, (www.epa.gov/nerlcwww/qa_qc_pcr10_04.pdf) EPA 815-B-04-001, October 2004

4.2 DNA isolation

Following methods and reagents for DNA isolation have been validated and can be recommended for the subsequent use with Mycotype® **Basidio**QS Microarray Detection Kit:

Starting material:	Fruiting body, mycelia or damaged wood
Required devices:	Mortar grinder, mills; e.g. Mixer Mill MM 400 (Retsch GmbH) or Homogenisator Precellys® 24 (PEQLAB Biotechnologie GmbH)
Reagents:	NucleoSpin® Plant II (Macherey-Nagel GmbH & Co.KG)

Attention: In order to avoid contamination the instruments must be DNA-free and cleaned accordingly (see below).

Cleaning of workbench

In order to avoid contamination, the DNA isolation should be performed, as far as possible, on a clean bench of the cabinet class II. For disinfecting the working area prior to or after DNA isolation we recommend to spray „Braun Meliseptol rapid“ (B. Braun Petzold GmbH) onto the surface and incubate for 10 min. Irradiate the surface with UV light for 15 min.

Cleaning of instruments

Immediately after DNA isolation immerse the used instruments (e.g. mortar grinder, pestle, stainless steel vessel, forceps, etc.) in the clean bench cabinet in a disinfection solution (e.g. 3 % Korsolex basic, BODE Chemie GmbH) for 15 min. Take out the instruments and wash thoroughly with water. Incubate subsequently the used instruments under a fume cupboard in 0.6 % Sodium hypochlorite solution for 15 min. Immerse the instruments for neutralisation in 0.1 % Sodium thiosulfate solution. Finally, wash the instruments thoroughly with water and dry with tissue paper.

As an alternative to the Sodium hypochlorite cleansing, the instruments and surfaces may be processed with DNA-ExitusPlus (AppliChem GmbH). To this end, immerse the instruments or spray onto the surfaces, incubate for 15 min, and wash subsequently with water.

Biotype Diagnostic GmbH assumes no liability for the chemical resistance or temperature stability of used instruments. This is to be enquired of the manufacturer.

4.3 PCR amplification

The in section 4.2 isolated DNA serves as a template. In case the DNA was purified following a precipitaion step, depending on the grade of turbidity the DNA should be diluted 1:10 to 1:50 with 0.1x TE-buffer. The DNA may be used undiluted if purified using spin-columns. The purification with spin-columns removes PCR inhibitors as e.g. humic acid, or polysaccharides as inulin, pectin or xylane.

The table below shows the volumes of all PCR reagents per 25 µL reaction volume, including a sample volume of 1 µL (template DNA). A master mix should be prepared when analysing more samples. Add one or two reactions to this number to compensate the pipetting error. The test kit contains for four reactions additional reagents for one more reaction. All components should be mixed (vortex) and centrifuged for about 5 s before preparing the PCR master mix.

	Number of PCR approaches			
Component	**1**	**5**	**9**	**17**
PCR mix	23.8 µl	119.0 µl	214.2 µl	404.6 µl
DNA polymerase	0.2 µl	1.0 µl	1.8 µl	3.4 µl
Volume of the PCR master mix	*24.0 µl*	*120.0 µl*	*216.0 µl*	*408.0 µl*

Mix shortly (vortex) and centrifuge (ca. 5 s) the PCR master mix, disperse to reaction tubes and add 1 µl of sample DNA each. Mix shortly (vortex) and centrifuge (ca. 5 s) prior to PCR.

Amplification parameter

Temperature	**Time**	
94°C	4 min	
94°C	30 s	
60°C	60 s	**35 cycles**
72°C	60 s	
72°C	7 min	
10°C	∞	hold

For a break in analysis store the PCR product at -20°C.

4.4 Analysis of PCR products (optional)

In order to control the PCR amplification it is possible to analyze maximum 5 µl per PCR approach using for example the agarose gel electrophoresis. The expected PCR product of the fungi DNA is ~650 bp long. If a weak product (5 ng / 5 µl) is detected there is sufficient for a successful ALR analysis.

It must be pointed out that even with undetectable PCR product a successful ALR analysis is possible.

Note: The test kit contains a 1.5-times amount on PCR reagents in order to provide for a repetition of PCR for half of the reactions.

4.5 Purification of PCR products

Following PCR the products are purified using a commercial PCR purification kit and eluted in 22 µl elution buffer. To keep the loss of PCR products of the fungi DNA, as well as of the PCR positive control as low as possible, it is recommended to use NucleoSpin® Extract II (Macherey-Nagel GmbH & Co.KG) or MinElute PCR Purification Kit (Qiagen GmbH).

For a break in analysis store the purified PCR product at -20°C.

4.6 Fragmentation and denaturing of PCR products

It is recommended to temper two thermoblocks for reaction tubes at 37°C and 95°C, respectively. If applicable, the incubation steps can also be performed in a PCR cycler.

Component	Volume
purified PCR product	20.0 µl
DNA glycosylase	1.0 µl
- mix (vortex) and centrifuge shortly (ca. 5 s)	
- fragmentation for15 min at 37°C	
- denaturation for min. 10 min at 95°C	

*** Attention:** The 95°C hot samples must be used directly in ALR.

4.7 Arrayed Ligation Reaction (ALR)

Preparation of ALR

The ALR should be prepared during the fragmentation and denaturing process. To this, heat up microarray-thermoblock* to 58°C and fill the humidity reservoirs with ddH_2O. Mark the DNA chips on the reverse side, outside the arrays using a touch-up pencil, since marks on the microfluidic would get lost after removal of the film in a later step. Place the DNA chips on the designated area of the microarray-thermoblock*.

* As an alternative, the use of a hybridization chamber together with a tempered water bath is also possible.

In a later step the ALR approach must be pipetted fast and with pressure into the fillers of the microfluidic film. To assure an accurate filling of the microfluidic chambers it is recommended to practice pipetting with the provided test slide using water.

The table below shows the volumes of all ALR reagents per 30 µL reaction volume, including a sample volume of 21 µL (target DNA). A master mix should be prepared when analysing more samples. Add one or two reactions to this number to compensate the pipetting error. The test kit contains for four reactions additional reagents for one more reaction. All components should be mixed (vortex) and centrifuged for about 5 s before preparing the ALR master mix.

Component	Number of ALR approaches 1	5	9	17
ALR mix	5.7 µl	28.5 µl	51.3 µl	96.9 µl
DNA ligase	3.3 µl	16.5 µl	29.7 µl	56.1 µl
Volume of the ALR master mix	*9.0 µl*	*45.0 µl*	*81.0 µl*	*153.0 µl*

Mix shortly (vortex) and centrifuge (ca. 5 s) the ALR master mix.

Performing ALR

Immediately after the 95°C incubation step, the fragmented and denatured PCR product is centrifuged (ca. 5 s) and complemented as follows:

Component	Volume
fragmented, denatured, 95°C hot PCR product	21.0 µl
ALR master mix	9.0 µl
- mix (vortex) and centrifuge shortly (ca. 5 s)	
- fill the mix directly into the fillers of the microfluidic film	

*** Attention:** The fragmented and denatured PCR product must be tempered at 95°C until used. The ALR approach must be pipetted fast and with pressure into the fillers of the microfluidic film.

- As soon as all arrays are loaded close the microarray-thermoblock* and incubate for 20 min at 58°C

* As an alternative, the use of a hybridization chamber together with a tempered water bath is also possible.

4.8 Washing of DNA chips

Remove the microfluidic film from the DNA chip by pulling on the free strap. The removal can be done for facilitation in a lukewarm water bath (~30°C). Immediately thereafter place the DNA chip in a stainless steel slide holder and immerse in 95°C ddH_2O for 1 min. The DNA chips are washed subsequently by gentle slewing for 2 min in a 50°C 0.3 % (w/v) Alconox solution (consisting of 1/3 Vol. 1 % (w/v) Alconox and 2/3 Vol. 95°C ddH_2O). Immerse the DNA chips three times consecutively for ca. 1 min in fresh 95°C ddH_2O. Finally, the DNA chips are taken out of the water bath and are directly cleaned and dried with gaseous nitrogen. This step is important to minimize background signals. As an alternative, the DNA chips can be placed in the slide holder with a tissue paper underneath in a centrifuge for microplates and spun down for 1 min at 150 x g.

Note: Tempering of ddH_2O at 95°C can be done by using a standard water boiler or a microwave oven.

4.9 Read-out of DNA chips

The read-out of the DNA chips is performed using a commercially available fluorescence scanner with the adjustment for Cyanine 5 (Cy5, 633 nm). Depending on the kit batch a fluctuation of performance and thus the signal intensity of the immobilized DNA probes may occur. In order to guarantee a stable quality of results, scanners PMT and laser strength must be calibrated for each DNA chip batch.

To this end, select in the scanners control software any area of the size of a Mycotype® **Basidio**QS array (~5.2 x 6.2 mm) and scan the calibration slide. Evaluate signal intensities of the calibration slide using the ALR software MycoProof **Basidio**. At this the averaged RFU value of all pixels is calculated. Adjust the PMT and laser strength of the scanner so that the averaged RFU-value equates to the indications accompanying the kit (yellow paper sheet).

Scan the DNA chips using adjustment for Cyanine 5 (Cy5, 633 nm) and limit the area of the scan field to the size of an array. Safe image in 16-Bit TIF format.

Positions of subarrays:
- Array 1: x = 9.75 mm; y = 16.75 mm
- Array 2: x = 9.75 mm; y = 32.75 mm
- Array 3: x = 9.75 mm; y = 48.75 mm
- Array 4: x = 9.75 mm; y = 64.75 mm

*** Attention:** The signal intensities of the PCR positive control should be in the range of 25 000 to 55 000 RFU. Otherwise, the PMT and laser strength of the scanner should be adjusted additionally.

4.10 Evaluation

When using the Biotype® ALR software MycoProof **Basidio** the TIF image will be opened in this program and an automated evaluation is performed, providing directly the results. For details please read the instruction manual of MycoProof **Basidio**.

In some cases a fixed signal percentage of an associated feature is subtracted from another feature signal to ensure a clear specificity. The following probes are offset:

- **AntVai1a** minus 1 % of the average value from SerLac2a and SerLac2b, if ConMar6a > 1000 RFU and SerLac > 2000 RFU
- **ConOli7a** minus 6 % of the average value from ConMar4a and ConMar6a
- **DaeQue2a** minus 6 % of the average value from AntXan1a and AntXan2a
- **GloSep1a** minus 10 % of the average value from AntSin1a and AntSin2a
- **LeuPin2a** minus 3 % of the average value from PleSpp1a and PleSpp2a
- **PleSpp1a** minus 2 % of the average value from AntXan1a and AntXan2a
- **SteSpp1a** minus 4 % of the average value from PleSpp1a and PleSpp2a
- **SteSpp1b** minus 5 % of the average value from FomPin4a and FomPin5a
  TapPan2b minus 4 % of the average value from LaeSpp1a and LaeSpp2a minus 2.5 % of the average value from PleSpp1a and PleSpp2a

The analysis results of both the feature and the single spots belonging to a certain quality sensor are shown as a deduced declaration (VALID/ invalid). A spot of a positive control is valid if the probe was found and the spot signal is above 1500 RFU. A spot of the negative control is valid, if the spot has not been found. A quality feature is valid, if all associated spots are valid. Otherwise, the feature is invalid.

Each fungi feature (e.g. *Antrodia sinuosa*) consists of two different DNA probes (e.g. AntSin1a, AntSin2a) existing as duplicates (spot / reference spot). The result of a feature is positive, if at least one DNA probe (e. g. AntSin1a) has been found completely (spot and reference spot) and the final fluorescence value is above 1500 RFU. Otherwise, the feature is negative. The final value of a DNA probe is the average fluorescence intensity of the two corresponding spots (signal/reference signal).

5. The principle of Mycotype® Basidio^{QS}

5.1 PCR amplification

The identification of fungi is based on the detection of an individual DNA region which is unique to each species. This ITS (*internal transcribed spacer*) region is located in the genomic rDNA area. The rDNA is made up of several gene sections which encode the ribosomal RNAs and are thus highly conserved (18S rDNA, 5.8S rDNA, 28S rDNA and 5S rDNA). These genes are separated by the *internal transcribed spacer* ITS I and ITS II, as well as the *intergenic spacer* IGS I and IGS II.

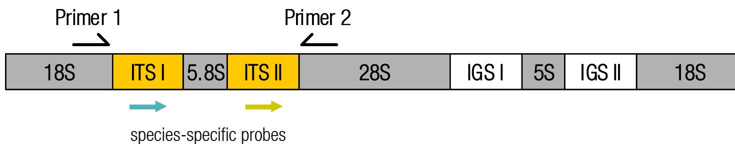

Fig. 2 Schematic diagram of the rDNA target region with the conserved gene sections for the ribosomal RNAs (grey) and the variable Spacer-regions ITS I and ITS II (yellow)

The PCR amplification of the DNA target region is done using Primer 1 and Primer 2, which bind within the conserved gene section. This primer pair facilitates specific amplification of the basidiomycetes' DNA. The identification and determination of fungi results by means of the ALR with species- and genus-specific DNA probes, which bind within the ITS I and ITS II regions.

5.2 DNA fragmentation

The PCR products have to be fragmented in order to ensure a uniform and sensitive ALR. To this end, Uracil-DNA Glycosylase hydrolyses N-glycosidic bonds of the in PCR integrated dUTPs releasing free uracil. In the subsequent incubation step at 95°C the PCR products are denatured resulting in short, single-stranded DNA fragments.

5.3 Arrayed Ligation Reaction (ALR)

To specifically detect the DNA of dry rot by ALR chip technology, first, fungi specific catcher molecules (DNA probes) are immobilised on a planar surface (Fig. 3, a). Complementary target sequences, amplified beforehand using PCR, attach themselves to these single-strand DNA probes (b). If both the DNA probe and the ligation oligonucleotide are complementary to the target sequence, the ligation oligonucleotide is attached to the DNA probe (c). Here, a thermophilic ligase catalyzes the formation of a phosphodiester bond between the 3' hydroxyl end of the DNA probe and the 5' phosphate end of the ligation oligonucleotide. During the subsequent high-temperature washing phase, the target sequences and the unbound ligation oligonucleotides are removed from the probe (d). However, the ligation oligonucleotides, which are attached covalently and specifically, remain on the DNA probes and thus also on the DNA chip, so can be detected using fluorescence measuring.

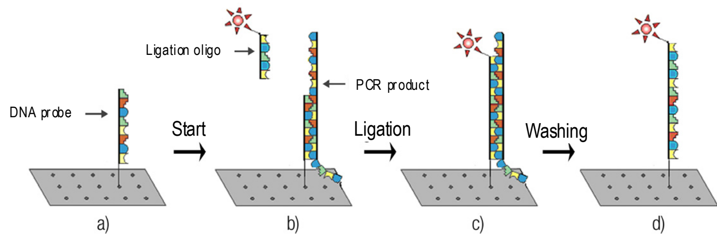

Fig. 3 ALR working pattern. a) Product-ready DNA chips b) hybridisation of PCR products with complementary DNA probe c) ligation of fluorescence labelled ligation oligonucleotide to the DNA probe d) washing of DNA chips.

6. Supplement

Table 3. Features and probes

Feature	Probe	Reference probe
Antrodia sinuosa	AntSin1a	AntSin2a
Antrodia vaillantii	AntVai1a	AntVai2a
Antrodia xantha	AntXan1a	AntXan2a
Bjerkandera adusta	BjeAdu1a	BjeAdu2a
Coniophora arida	ConAri1a	ConAri2a
Coniophora marmorata	ConMar4a	ConMar6a
Coniophora olivacea	ConOli5a	ConOli7a
Coniophora puteana	ConPut1a	ConPut2a
Daedalea quercina	DaeQue1a	DaeQue2a
Donkioporia expansa	DonExp1c	DonExp1f
Fomitopsis pinicola	FomPin4a	FomPin5a
Gloeophyllum abietinum	GloAbi1e2	GloAbi1e3
Gloeophyllum sepiarium	GloSep1a	GloSep2a
Gloeophyllum trabeum	GloTra1a	GloTra2a
Laetiporus spp.	LaeSpp1a	LaeSpp2a
Leucogyrophana mollusca	LeuMol1a	LeuMol2a
Leucogyrophana pinastri	LeuPin1a	LeuPin2a
Neo/Lentinus lepideus	LenLep1a	LenLep2a
Oligoporus placenta	OliPla1d	OliPla2a
Phellinus ferruginosa	PheFer1a	PheFer2a
Pleurotus spp.	PleSpp1a	PleSpp2a
Schizophyllum commune	SchCom1a	SchCom2a
Serpula lacrymans	SerLac2a	SerLac2b
Serpula himantioides	SerHim2a	SerHim2b
Stereum spp.	SteSpp1a	SteSpp1b
Tapinella panuoides	TapPan1b	TapPan2b
Trametes (Coriolus) versicolor	TraVer1a	TraVer2b
PCR positive control	PCR-PK	
ALR positive control	ALR-PK	
ALR negative control	ALR-NK	
Fluorescence marker	FLU-PK	

Table 4. Microarray layout

Position	1	2	3	4	5	6	7	8	9	10	11	12
A	ALR-PK	ALR-NK	AntSin1a	AntSin2a	AntVai1a	AntVai2a	AntXan1a	AntXan2a	BjeAdu1a	BjeAdu2a	-	FLU-PK
B	ALR-NK	-	ConAri1a	ConAri2a	ConMar4a	ConMar6a	ConOli5a	ConOli7a	ConPut1a	ConPut2a	DaeQue1a	DaeQue2a
C	DonExp1c	DonExp1f	FomPin4a	FomPin5a	GloAbi1e2	GloAbi1e3	GloSep1a	GloSep2a	GloTra1a	GloTra2a	LaeSpp1a	LaeSpp2a
D	LeuMol1a	LeuMol2a	LeuPin1a	LeuPin2a	LenLep1a	LenLep2a	OliPla1d	OliPla2a	PheFer1a	PheFer2a	PleSpp1a	PleSpp2a
E	SchCom1a	SchCom2a	SerLac2a	SerLac2b	SerHim2a	SerHim2b	SteSpp1a	SteSpp1b	TapPan1b	TapPan2b	TraVer1a	TraVer2b
F	AntSin1a	AntSin2a	AntVai1a	AntVai2a	AntXan1a	AntXan2a	BjeAdu1a	BjeAdu2a	ConAri1a	ConAri2a	ConMar4a	ConMar6a
G	ConOli5a	ConOli7a	ConPut1a	ConPut2a	DaeQue1a	DaeQue2a	DonExp1c	DonExp1f	FomPin4a	FomPin5a	GloAbi1e2	GloAbi1e3
H	GloSep1a	GloSep2a	GloTra1a	GloTra2a	LaeSpp1a	LaeSpp2a	LeuMol1a	LeuMol2a	LeuPin1a	LeuPin2a	LenLep1a	LenLep2a
I	PCR-PK	OliPla1d	OliPla2a	PheFer1a	PheFer2a	PleSpp1a	PleSpp2a	SchCom1a	SchCom2a	SerLac2a	SerLac2b	PCR-PK
J	FLU-PK	SerHim2a	SerHim2b	SteSpp1a	SteSpp1b	TapPan1b	TapPan2b	TraVer1a	TraVer2b	-	ALR-PK	ALR-PK

Exemplary image of a Mycotype® Basidio^{QS} microarray

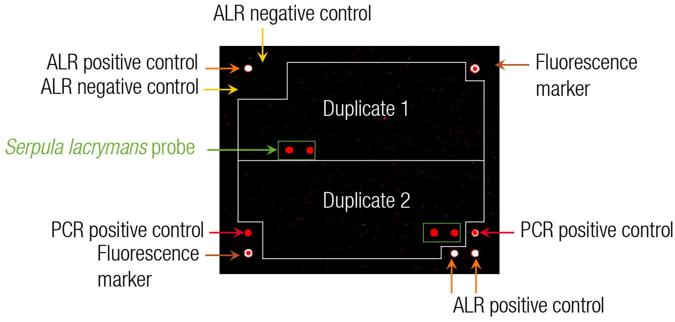

Fig. 4 Exemplary image of a Mycotype® **Basidio**^{QS} microarray following an analysis of a *Serpula lacrymans* sample. The positions of the control probes, as well as of the *Serpula lacrymans* probes are shown.