# NAIL FUNGAL

## qPCR PANEL HANDBOOK

For Research Use Only. Not intended for diagnostic use.

## CAT NO: YSP-Nail-62B10

96 well plates

## VERSION 1.0

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# INTENDED USE

This Nail Fungal Panel offers an efficient and user-friendly solution for the qualitative detection of the pathogenic markers listed in the **Panel Targets** section of this document (see final pages). It is designed to be used by trained professionals in a suitable molecular biology laboratory.

YouSeq panels comprise a set of multiplex real-time qPCR assays utilising hydrolysis probe technology to identify target nucleic acids for various research purposes. The primer/probes are pre-dispensed into a qPCR plate to be used in conjunction with good quality nucleic acids extracted from a variety of sample types and include essential control assays to ensure dependable results.

# CONTENTS

| Component | Included |
|---|---|
| Primer/probe qPCR Plate | 10 x 96-well plates |
| PCR plate seals (to be used to seal the plate for PCR amplification) | 10 x cap seals |

# RECOMMENDED REAGENTS & MATERIALS

General laboratory equipment (pipettes, pipette tips, (micro)centrifuge tubes, vortex mixer, plate centrifuge etc.)

Total nucleic acid extraction kit

Tetra™ 2X qPCR MasterMix: YS-qP-TMM-10

qPCR Panel Reagents Pack (inc. Positive Control): YSPL-Rg-62B10

qPCR instrument with channels to detect FAM, VIC/HEX, ROX & CY5.

# SUPPLEMENTARY MATERIALS

The following documents are available on request, please contact our customer success team at support@youseq.com.

| Document | Description |
|---|---|
| YSP-Nail-62B10 - Plate Layout Template | Plate Layout Template files, built specifically for the panel and available for a range of qPCR instruments, to simplify your qPCR software setup. |
| YouSeq qPCR Data Analysis Pipeline IFU | User guide for YouSeq's real-time qPCR automated data analysis solution. |

# BEST PRACTICE

### Decontamination:

Before beginning laboratory work, thoroughly decontaminate any work surfaces and pipettes being used, to eliminate potential contamination.

### General use and set-up:

All components should be fully defrosted with contents at the bottom of the plate wells before opening. To ensure contents are at the bottom, centrifuge or gently tap the plate. After use, reagents should be returned to the freezer.

After any reagents are resuspended or combined, the tube should be pulse vortexed to ensure it is mixed well.

It is advised to set up the plate and reaction mix on ice to minimise artefact formation, which may reduce sensitivity.

When preparing the qPCR reaction mix, it is recommended to incorporate an overage into the calculations to compensate for potential losses incurred during pipetting.

### Set-up environments:

It is best practice to set up qPCR plates in two different environments - a clean (no template) lab and PCR (template) lab.

No Template Control(s) (NTC) and Positive Control(s) (PTC) for each multiplex on the plate should be included in every run. To reduce contamination, NTCs and samples can be set up and sealed in a clean lab before moving to the PCR lab.

# BREAK-AWAY PLATES

The supplied plates and seals can be cut at any junction point between wells using a pair of scissors. This allows for smaller plates to be run at the user's discretion.

### Considerations:

When cutting the qPCR plate, make sure each section retains visible orientation markers, such as column numbers or row letters. This ensures the wells in the remaining plate sections can be confidently identified after the cut.

The plate ID is typically located on only one side of the plate. If cutting separates the unused portion from the plate ID, re-label that unused section (using ink or adhesive labelling) before storing it. This helps maintain traceability.

Ink or adhesive labelling should only be applied to the exterior surface of the plate and only on sections being stored. Before using any labelled plate section in a future qPCR run, ensure all adhesive labels are removed to avoid interference with the instrument.

Only the included plate cap seals should be used to seal the plate for PCR amplification.

Figure 1: Example of a 96 well break-away plate before and after cutting. The cutting orientation shown may not be suitable for this specific panel. For guidance, refer to the Plate Layout section (pg. 4)

# PLATE LAYOUT

Multiplex primer/probe mixes are pre-dispensed into the plates provided according to the layout shown in Figure 2. For full target details, please refer to the Panel Targets section of this document (see final pages).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| B | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| C | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| D | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| E | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| F | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| G | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| H | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |

Figure 2: 96 well plate layout indicating the location of each unique multiplex primer/probe mix.

# SAMPLE LAYOUT

Addition of processed samples, PTCs and NTCs will follow the layout in Figure 3.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | PTC 1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | NTC 1 |
| B | PTC 2 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | NTC 2 |
| C | PTC 3 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | NTC 3 |
| D | PTC 4 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | NTC 4 |
| E | PTC 5 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | NTC 5 |
| F | PTC 6 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | NTC 6 |
| G | PTC 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | NTC 7 |
| H | PTC 8 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | NTC 8 |

Figure 3: Example 96 well plate to indicate the final dispense of nucleic acid samples and control location.

**Please note**: The product is associated with a specific Plate Layout Template file for the qPCR instrument of choice. This file comes with samples and targets pre-arranged in their correct positions and is designed to be imported directly into the software of various qPCR instruments.

To request the panel-specific Plate Layout Template file, please contact support@youseq.com.

It is strongly recommended to use these template files, as manually setting up the plate or editing the template may disrupt the automation of YouSeq's qPCR Data Analysis Pipeline.

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# RECOMMENDED BENCH SIDE PROTOCOL

## qPCR REACTION SET-UP

1. Remove the plate from the pouch and briefly spin the plate in a plate centrifuge. This will ensure the primer/probe mix is at the bottom of the wells before removing the plate seal.

**Please note:** If the plates needs to be cut, please do so at this stage before removing the seal, returning the unused portion of the plate to the freezer.

2. In an appropriately sized (micro)centrifuge tube, combine the following reagents to create a reaction mix that will cover all required wells (e.g. samples, NTCs and PTCs).

**Please note:** Include an overage to account for volume loss during pipetting.

| Component | Volume (for 1 well) |
|---|---|
| 2X qPCR MasterMix | 10 µL |
| DNase/RNase Free Water | 5 µL |
| Reaction mix volume | 15 µL |

3. Mix the combined reagents by briefly vortexing or inverting.

**Please note:** For best results reaction mixes should be prepared and kept on ice until the amplification protocol is ready to start.

4. Remove plate seal and dispense 15 µL of the reaction mix into all required wells.

5. For each NTC, add 5 µL of DNase/RNase Free water into required well(s).

6. For each sample, add 5 µL of the extracted DNA into required well(s).

**Please note**: It is best practice to seal the NTC and sample wells, using the supplied cap seals, before proceeding to the PTC steps.

7. For each PTC, add 5 µL of the prepared panel-specific PTC, into required well(s).

**Please note**: The PTC is a contamination risk and should be handled in a designated PCR (template) laboratory. Prepare the PTC reagent following the instructions supplied in the product handbook.

8. Seal the plate using the supplied cap seals.

9. Briefly vortex the sealed plate, then spin in a plate centrifuge to ensure all reagents are fully resuspended and at the bottom of the wells before running.

# RECOMMENDED qPCR AMPLIFICATION PROTOCOL

1. Open the relevant qPCR instrument software and import the provided Plate Layout Template into the software using the 'Import' option. This will load the layout for the panel into the software (e.g. Target & sample names).

2. Within the loaded Plate Layout Template replace the pre-allocated sample names (e.g. Sample 1, Sample 2, etc.) with the sample IDs prior to data analysis. This can be performed either before the run has commenced or after the run has completed.

**Please note**: Manual plate setup or editing the Channels, Targets or Controls within the Plate Layout Template will impact the automation of YouSeq's qPCR Data Analysis Pipeline.

4. Load the plate onto the qPCR instrument.

| Temperature | Time | Number of Cycles |
|---|---|---|
| 95°C | 3 minutes | - |
| 95°C | 15 seconds | x 5 |
| 60°C | 60 seconds | |
| 95°C | 15 seconds | x 35 |
| 60°C 📷 | 60 seconds | |

📷 Collect fluorogenic data through FAM, VIC/HEX, ROX & CY5 channels during this step.

5. Start the run.

# ANALYSIS OF RESULTS

For automated analysis, output run files can be uploaded to YouSeq's qPCR Data Analysis Pipeline if this is the preferred method. Access to a unique analysis environment will be provided by the YouSeq Customer Success team.

YouSeq's qPCR Data Analysis Pipeline will provide a summary of the result for all samples in a PDF or CSV output, depending on user preference. Further details can be found on how to use the pipeline in the YouSeq qPCR Data Analysis Pipeline IFU.

Some users may prefer to analyse their data manually or adapt the output to custom workflows. For those opting out of the automated pipeline, the high data volume from YouSeq panels can be challenging to manage. For assistance adapting the pipeline, troubleshooting issues, or exploring manual analysis options, the Customer Success Team can provide help with personalised guidance at support@youseq.com.

# PRODUCT SPECIFICATIONS

## Storage

Store at -20°C from arrival. The primer/probe qPCR plate shelf life is outlined as an expiry date on the box label.

## Suitable input material

The primer/probe qPCR plate will work well with any source of good quality nucleic acid. Good quality is defined as nucleic acid with high integrity (not degraded). Poor quality input nucleic acid is a leading cause of test failure.

## Commensal Organisms

Commensal organisms are present as part of a healthy human microbiome and are found on the skin and in the respiratory tract. Contamination of samples and primer/probe qPCR plate is therefore possible, often seen as re-occurring late amplification for any assay targeting a commensal organism.

## Regulatory Status

This product has been developed for Research Use Only (RUO) and is not intended for diagnostic use. It should not be used for diagnosis of disease or infection unless specifically approved by the regulatory authorities in the country of use.

## Quality Control

In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each lot of YouSeq Nail Fungal Panel is tested against predetermined specifications to ensure consistent product quality. The primers/probes typically demonstrate ≥95% in silico specificity to their intended targets and are periodically checked against newly available sequence information to maintain their detection profile.

## Technical Assistance

For customer support, please contact:

e-mail: support@youseq.com
phone: +44 (0)333 577 6697

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Not available in all countries.


Internal product identifier
V.1
JSU0199

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# PANEL TARGETS

| MPX | Target | Abbreviation | Target Gene | Potential cross-reactivity | Channel |
|---|---|---|---|---|---|
| 1 | **Alternaria SPP** | Alter.SPP | AP-2 (adaptor complex subunit alpha) gene | | VIC/HEX |
| | **Epidermophyton floccosum** | E.flocc | beta tubulin gene | | ROX |
| | **Aspergillus fumigatus** | A.fumig | cyp51A gene | | FAM |
| | **RNaseP Endogenous Control** | Endo Ctrl | RNaseP gene | | CY5 |
| 2 | **Trichophyton violaceum** | T.viola | DNA topoisomerase II gene | | CY5 |
| | **Trichophyton soudanense** | T.souda | hypothetical protein gene | T. rubrum, T. violaceum | VIC/HEX |
| | **Trichophyton tonsurans** | T.tonsu | 25S and 18S ribosomal RNA genes | | FAM |
| | **Trichophyton rubrum** | T.rubrum | squalene epoxidase gene | T. violaceum, T. soudanense | ROX |
| 3 | **Fusarium solani complex** | F.solan | RNA polymerase large subunit (RBP1) gene | F. ensiforme, F. azukiicola, C. rosea. | VIC/HEX |
| | **Fusarium oxysporum complex** | F.oxysp | calmodulin gene | F.acutatum, F.udum | ROX |
| | Fluoroquinolone (QnrA1&2) resistance | Qnr | qnrA gene | QnrA3, QnrA4, QnrA5, QnrA6, QnrA7 | CY5 |
| | Fluoroquinolone (QnrB) resistance | Qnr | QnrB gene | QnrE | CY5 |
| | Fluoroquinolone (QnrS) resistance | Qnr | QnrS gene | | CY5 |
| | Class A beta-lactamase (CTX-M-Grp 1) resistance | CTX-M | CTX-M gene | | FAM |
| | Class A beta-lactamase (CTX-M-Grp2) resistance | CTX-M | CTX-M gene | blaOXY | FAM |
| | Class A beta-lactamase (CTX-M-Grp8/25) resistance | CTX-M | CTX-M gene | blaOXY, blaCRH | FAM |
| | Class A beta-lactamase (CTX-M-Grp9) resistance | CTX-M | CTX-M gene | | FAM |
| 4 | **Candida albicans** | C.albic | RPR1 gene | | CY5 |
| | **Candida parapsilosis** | C.parap | TAC1 gene | | FAM |
| | **Candida tropicalis** | C.tropi | ALST2 gene | | ROX |
| | **Candida glabrata** | C.glabr | RPR1 gene | | VIC/HEX |
| 5 | **Pseudomonas aeruginosa** | P.aerug | regA gene | | ROX |
| | **Scopulariopsis brevicaulis** | S.brevi | RNA polymerase II largest subunit (RPB1) gene | | VIC/HEX |
| | **Trichophyton mentagrophytes** | T.menta | hsp60 gene | | CY5 |
| | **Microsporum SPP** | Micro.SPP | ITS1 gene | | FAM |
| 6 | **Candida krusei** | C.kruse | RPR1 gene | | FAM |
| | **Candida dubliniensis** | C.dubli | RPR1 gene | | VIC/HEX |
| | **Staphylococcus Methicillin (mecA) resistance** | MecA gene | MecA gene | M.fleurettii, M.lentus, M.sciuri, M.vitulinus | CY5 |
| | Class B beta-lactamase (BlaNDM) resistance | NDM.IMP.VIM | blaNDM gene | blaAFM | ROX |
| | Class B beta-lactamase (BlaIMP) resistance | NDM.IMP.VIM | blaIMP gene | | ROX |
| | Class B beta-lactamase (blaVIM) resistance | NDM.IMP.VIM | blaVIM gene | | ROX |
| 7 | **Malassezia SPP** | Mala.SPP | NAD4 gene | | ROX |
| | **Candida lusitaniae** | C.lusit | beta tubulin gene | | CY5 |

| | | | | | |
|---|---|---|---|---|---|
| | **Extended spectrum beta-lactamases (blaTEM) resistance** | blaTEM | blaTEM gene | | VIC/HEX |
| | Macrolide/Lincosamide/Streptogramin (ermA) resistance | MLSRes | ermA gene | | FAM |
| | Macrolide/Lincosamide/Streptogramin (ermA) resistance | MLSRes | ermA gene | | FAM |
| | Macrolide/Lincosamide/Streptogramin (ermB) resistance | MLSRes | ermB gene | | FAM |
| | Macrolide/Lincosamide/Streptogramin (ermC) resistance | MLSRes | ermC gene | | FAM |
| 8 | **Staphylococcus aureus** | S.aureus | femB gene | S.argenteus, S.roterodami , S.schweitzeri | CY5 |
| | **Scytalidium dimidiatum** | S.dimid | RBP2 gene | | FAM |
| | Vancomycin A resistance | Vanco | VanA gene | VanM | VIC/HEX |
| | Vancomycin B resistance | Vanco | VanB gene | | VIC/HEX |
| | Class A beta-lactamase (blaSHV) resistance | SHV.KPC | blaSHV gene | blaLEN | ROX |
| | Class A beta-lactamase (blaKPC) resistance | SHV.KPC | blaKPC gene | | ROX |

*Most target genes are detected using an individual assay within the specified channel. Some targets are strategically chosen to be detected in the same channel or have multiple assays to cover all species etc; a positive signal may be from one or more of the target genes.

## Potential Cross-Reactivity

Due to extreme homology, it is possible that some of the target sequences may be present and detected in other related species. These potential cross-reactivity scenarios have been outlined in the Panel Targets List found at the back of this handbook.