Panfungal Sequencing Primer (non DNA-free)

Revision Date: 10/2015 **Last Update:** 10/2020 **Version:** 03

Product Information

Content

Panfungal Sequencing Primer, non DNA-free	
	100 rxn
SeqYeast18 (10 pmol/µl)	2x 0.1ml

Catalog Number (Cat. No.)**:** **S-785-100** **100** reactions

Application of the Product: For sequencing analysis of PCR amplicons produced with *Mastermix 18S Complete.*

For research use only

Product Description

The binding site of the primer is located at the 3'-terminus of the lagging strand of amplicon. Sequencing of amplicons together with online homology search is an option for the identification of yeasts and other fungi detected by *Mastermix 18S Complete*. Sequencing and sequence analysis, however, are not part of this certified product, but have been included in the validation of *Mastermix 18S Complete*.

SeqYeast18: Sequencing Primer is an oligonucleotide for sequencing analysis of PCR amplicons produced with *Mastermix 18S Complete*.

The oligonucleotide is homologous to conserved regions of the 18S rRNA gene of mostly yeast and fungi.

Storage and Stability

Store at –15 to –25°C upon delivery.

Stable for 24 months from the date of manufacturing under proper storage condition. Guarantee for full performance of reagents and buffers is given through the expiration date printed on the label at the outer box, if the packed material is undamaged upon arrival and the reagents are unopened.

Material Safety Data Sheet (MSDS)

Please contact Molzym for an actual MSDS (according to Regulation (EC) No. 1907/2006) of this product:
Tel.: +49(0)421-69 61 62 0 • **E-Mail:** info@molzym.com

Usage of the Sequencing Primer

Purification of Amplicons

For sequencing of amplicons, the PCR reactions need to be purified by a commercial PCR purification kit. QIAquick® PCR Purification Kit (Qiagen, cat. no. 28104) has shown satisfactory results. For this purpose, use the aliquot remaining after analysis of the PCR reaction mixture and follow the instructions of the manufacturer of the kit. Elute the purified amplicon from the column using 30µl sterile deionised water. The procedure may not take more than 15 to 20 minutes.

Sequencing

Apply the purified eluted amplicon DNA to a sequencing reaction as adviced by the manufacturer of the sequencing system. For example Mastermix18S has been validated using Applied Biosystems DNA Analyzer ABI 3730XL apparatus and BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA).

For the identification of the *Mastermix 18S Complete* use *SeqYeast18*.

SeqYeast18 is a primer binding to region within the amplicon for the DNA synthesis of sequences from yeasts and other fungi. For sequencing, follow the instructions of the manuals of the sequencer and the used sequencing kit.

Example:

As an example, the following protocol for QIAquick®-purified amplicons using the ABI Prism310® may give satisfying results. Use 2µl of purified DNA for cycle sequencing. Add 4µl Big-Dye® Reaction mix (containing polymerase und nucleotides), 0.5µl sequencing primer SeqYeast18 (10pmol/µl) and PCR-grade water to fill up to a final volume of 20µl.

Incubate in the Eppendorf Mastercycler under the following conditions:

Initial denaturation at 95°C for 1min; 26 cycles at 95°C for 30s, 55°C for 30s and 60°C for 4min. Apply the sequencing reaction to a CentriSep® column (Princeton Separations) and spin the column at 750x*g* for 2min. Combine 5µl of the eluate containing the products of the sequencing reaction with 20µl formamide (or TSR reagent containing formamide) and incubate at 95°C for 4min. Apply the reaction mix to the capillary of the ABI Prism310®.

Validate the performance of the used sequencing system. For this, analyse the purified amplicons of the positive PCR controls (high and low concentrated standard DNA; e.g., Molzym´s Positive Control DNA (P1); cat. no. S-200-050, 50 reactions). Both controls should give readable results.

Alternatively use an overnight sequencing service (e.g., GATC Biotech AG, Konstanz, Germany).

BLASTN Analysis for Strain Identification

In cases of clear reads, identification of detected fungi can be performed by an online search with the nucleotide sequence obtained. For guidance, see NCBI Blast http://www.ncbi.nlm.nih.gov/ or SepsiTest-BLAST (http://www.sepsitest-blast.de/). Hits with retrieved unidentified isolates should be ignored. Rather, search the list of hits for a species designation with the highest score index. **Note:** Sequence identities ≥97 to <99% should be interpreted as on the genus level, ≥99% as on the species level. This may be the result of reading errors of the sequencing reaction. In such a case it is recommended to inspect the densitogram read-out for overlying sequences indicating the presence of more than one strain in the sample.

Please address any questions relating the mastermix and the sequencing primers to the support hotline:

E-Mail: support@molzym.com • **Tel.:** +49(0)421 69 61 62 0

Contact

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