

## Set of Sequencing Primers (SeqGN16 & SeqGP16)

For sequencing analysis of PCR amplicons produced with Mastermix16S Complete, Mastermix 16S Primer.  
**For research use only**

Cat. No. S-775-100

100 reactions each

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### Product overview

#### Kit/Component

| Set of Sequencing Primers, non DNA-free: |                |
|--|----------------|
|  | <b>100 rxn</b> |
| SeqGN16 (10 pmol/μl)                     | 2 x 0.1 ml     |
| SeqGP16 (10 pmol/μl)                     | 2 x 0.1 ml     |

### Product description

**SeqGN16-Sequencing Primer** is an oligonucleotide for sequencing analysis of PCR amplicons produced with Mastermix16S Complete or Mastermix 16S Primer. The oligonucleotide is homologous to conserved regions of the 16S rRNA gene of mostly Gram-negative bacteria, including *Acinetobacter* spp., *Escherichia* spp., *Enterobacter* spp., *Serratia* spp., *Klebsiella* spp., *Neisseria* spp., *Porphyromonas* spp., *Proteus* spp., *Pseudomonas* spp., *Stenotrophomonas* spp. (including some Gram-positive bacteria: e.g. *Propionibacterineae*). 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 bacteria detected by Mastermix16S Complete or Mastermix16S Primer. Sequencing and sequence analysis, however, are not part of this certified product, but have been included in the validation of Mastermix16S Complete and Mastermix16S Primer.

**SeqGP16-Sequencing Primer** is an oligonucleotide for sequencing analysis of PCR amplicons produced with Mastermix16S Complete or Mastermix 16S Primer. The oligonucleotide is homologous to conserved regions of the 16S rRNA gene of mostly Gram-positive bacteria, including *Enterococcus* spp., *Lactococcus* spp., *Lactobacillus* spp., *Listeria* spp., *Staphylococcus* spp. and *Streptococcus* spp. (excluding: *Clostridiales*; *Actinomycetales* and *Propionibacterineae*). 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 bacteria detected by Mastermix16S Complete or Mastermix16S Primer. Sequencing and sequence analysis, however, are not part of this certified product, but have been included in the validation of Mastermix16S Complete and Mastermix16S Primer.

### **Purification of Amplicons**

For sequencing of amplicons, the PCR reactions need to be purified by a commercial PCR purification kit. Qiagen's QIAquick® PCR Purification Kit (cat. no. 28104) has shown satisfactory results. For this purpose, use the aliquot remaining after analysis of the PCR reaction mixture (approximately 17 µl) and follow the instructions of the manufacturer of the kit. Elute the purified amplicon from the column using 30 µl sterile deionised water. The procedure may not take more than 15 to 20 min.

### **Sequencing**

Apply the purified eluted amplicon DNA to a sequencing reaction as advised by the manufacturer of the sequencing system. Mastermix16S 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). The primers bind to regions within the amplicon for the DNA synthesis of sequences from Gram-positive and Gram-negative bacteria. For sequencing, follow the instructions of the manual (for download see <http://www3.appliedbiosystems.com/index.htm>).

### **BLASTN Analysis for Strain Identification**

In cases of clear reads, identification of detected bacteria can be performed by an online search with the nucleotide sequence obtained. For guidance, see <http://www.ncbi.nlm.nih.gov/>. 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  $\geq 97\%$  are the level of a bacterial species, less sequence identities indicate higher taxonomic levels (genus, family). This may be the result of a reading error of the sequencing reaction or an unknown strain detected. In such a case it is recommended to confirm the result by repeating the sequencing and performing another homology search.

### **Stability**

Stable at -15 to -25°C for 24 months.

Please address any questions relating the Sequencing Primer to the support hotline:

**Email: [support@molzym.com](mailto:support@molzym.com) / Tel.: +49(0)421-696162-0**