

Customer guidelines for Next-Generation Sequencing



Requirements for Amplicon sequencing

This document provides information about the primer sequences used for amplicon sequencing, how to design a custom amplicon and the bioinformatic processing.

We perform amplicon sequencing with two different PCRs. The first PCR amplifies the target region and adds Illumina Adapters to the sequence. We standardly use Nextera Adapters. The second PCR adds the Indices and the P5- and P7-Tag to the sequences.

We accept non-amplified DNA, PCR-clean amplicons from the first PCR and PCR-clean amplicons from the second PCR. Please keep in mind to use Unique Dual Indices if you plan to send 2nd-PCR-Amplicons that will be run on the NovaSeq 6000 platform.

The design of the final amplicon:

P5tag-i5-P5NexteraAdapter-AMPLICON-P7NexteraAdapter-i7-P7tag

The standard 16S primer sequences we use for the first PCR:

515F(Parada): TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-GTGCCAGCMGCCGCGGTAA

806R(Apprill): GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-GGACTACHVGGGTWTCTAAT

The primers we use for the second PCR:

P5-PCR index primer: AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC

P7-PCR index primer: CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG

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Custom Primer Design

If you plan to design your own custom amplicons, use the sequences from the first PCR and replace the non-coloured sequences with your sequences of choice.

Bioinformatic analysis

We standardly use nf-core and ampliseq pipelines to analyse 16S amplicons.

Further information

Please contact us if you need more information and also find more information in the chapters “Illumina 16S Metagenomic Sequencing Library Preparation” and “Illumina Adapter Sequences” on the Illumina webpage.