

EasySeq™

# Microbiology & Infectious Disease 16S Microbiome Library Prep Kit

for illumina®

NGS Library Prep by Reverse Complement PCR



Safest and Simplest  
NGS Library Prep  
Workflow Available

- A singleplex assay for variable regions V3-V4 for deconvolution of bacterial communities like the microbiome
- Safe and cost-efficient workflow for confidence in test results, allowing informed decision making towards microbe management control



**NimaGen.**

Innovators in  
DNA Sequencing  
Technologies

# EasySeq™ NGS Library Prep by RC-PCR

## The Next Revolution in Microbial NGS

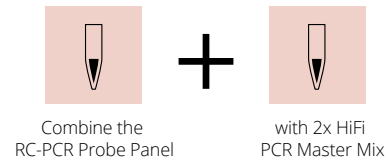
EasySeq™ 16S Microbiome Library Prep Kit utilizes unique Reverse Complement PCR (RC-PCR) technology to create a simple and safe one-tube, single reaction next-generation sequencing (NGS) library prep workflow (Figure 1). In this reaction, target amplification, sequencing adapter addition, and sample specific unique dual indexing all occur simultaneously in a closed-tube workflow, as simple as any normal PCR reaction (Figure 3). All samples can be pooled after the PCR for clean-up in a single tube using magnetic beads (AMPure XP or AmpliClean™ recommended), thereby eliminating the need to clean-up reactions separately (Figure 1). Therefore, RC-PCR greatly reduces the amount of hands-on steps and the associated risks of pipetting errors, as well as sample swaps and cross-contamination.

The unique kinetics of RC-PCR result in high sensitivity and specificity because target-specific primers are synthesized during the reaction. Therefore, concentrations of primers and amplicons are more in line, which reduces potential primer dimerization and off-target primer binding (Figure 2).

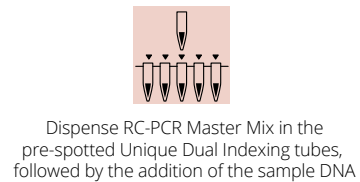
Every EasySeq™ NGS Library Prep Kit includes a target specific Probe Panel and the RC-PCR Master Mix, compatible with universal index (IDX) plates containing pre-spotted and dehydrated Unique Dual Index (UDI) primers.

Figure 1 | EasySeq™ RC-PCR workflow

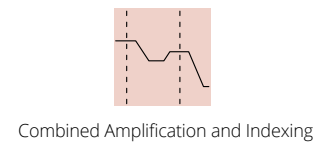
### 1 Prepare the RC-PCR Master Mix



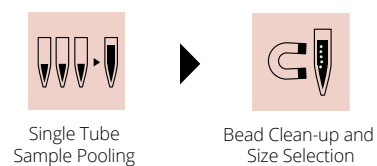
### 2 Dispense and add DNA



### 3 RC-PCR



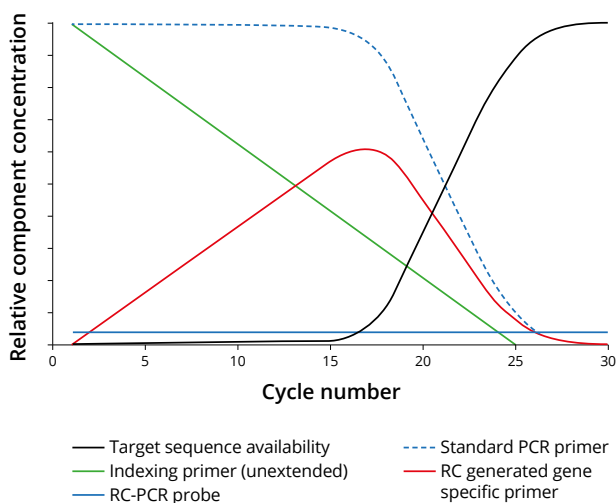
### 4 NGS Library Clean-up



### 5 Sequence

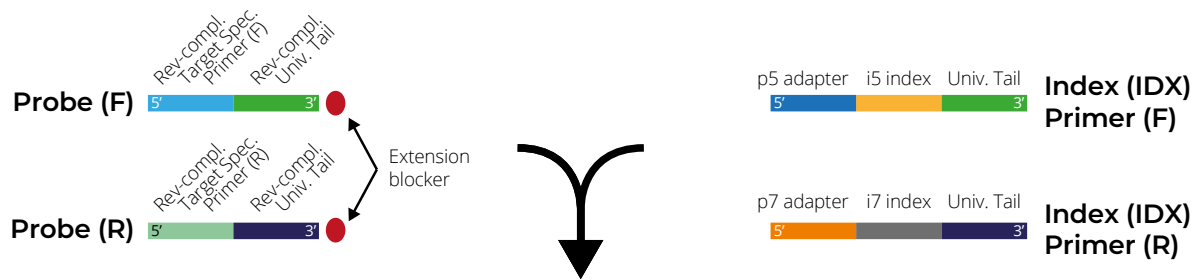


Figure 2 | RC-PCR Kinetics



# EasySeq™ NGS Library Prep by RC-PCR

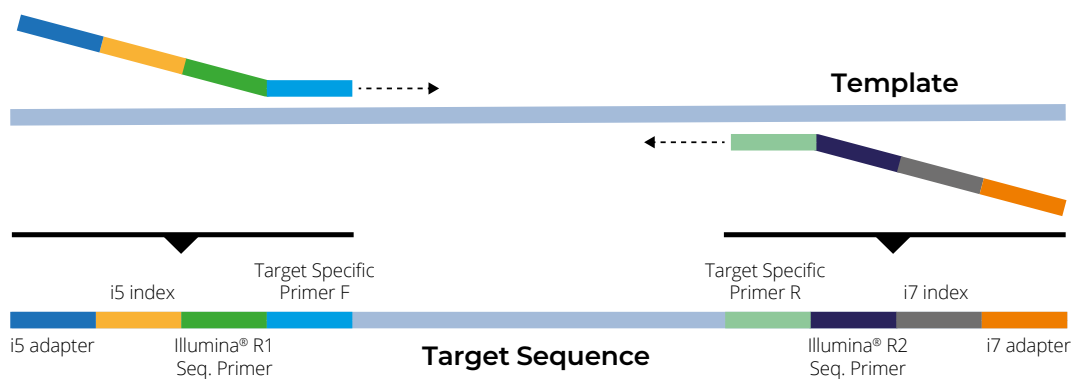
Figure 3 | Reverse Complement PCR Principle



Combine the probe panel with the mastermix, dispense in the IDX plate, add sample DNA and start the RC-PCR program.



At the first annealing step, RC-PCR probe tails hybridize to the IDX primer tails, followed by extension of the IDX primers with gene specific primer sequences. This step synthesizes functional indexed primers, including Illumina® adapters. In the following cycles, target regions are amplified, while also creating more primers.



This results in a ready-to-sequence, Illumina® compatible library, in a single step.

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### Cost-efficient workflow

- Breakable universal index (IDX) plates ensure optimal usage, minimizing waste
- One closed-tube, single reaction workflow with simultaneous indexing and target amplification reduces labor time
- Single tube sample pooling for library clean-up significantly reduces usage of required magnetic beads and consumables
- Well-balanced read distributions maximizes sequencing instrument flow cell capacity
- The use of phase-shifted primers reduces PhiX spike-in to increase flow cell throughput

### Confidence in test results

- Closed-tube RC-PCR workflow significantly reduces hands-on time and pipetting error, minimizing risk of sample contamination
- Sample tracking dye in pre-spotted UDI plates ensures accuracy
- Unique RC-PCR kinetics promote high-target specificity and coverage uniformity (optimized read depth balance) from low DNA input
- UDI of 10 bp increases discriminatory power and help to prevent index hopping

### Choice and flexibility

- A variety of 96-well breakable UDI plates available for matching your sample workload
- Automation compatibility for high-throughput workflows
- Compatible with various Illumina® platforms

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## Introduction

All species of archaea and bacteria have a 16S gene that codes for the small subunit of the ribosomes. This gene has highly variable regions (V1 till V9) used for taxonomical classifications. The gene also has conserved regions that can be used as targets for primers to amplify the highly variable regions. Through introduction of Next-generation Sequencing (NGS), the 16S rRNA method is now also widely used to deconvolute complex microbial communities. NGS is the best technique to study any microbiome as Sanger sequencing fails to detect multiple bacteria in one sample and culture is labor intensive and many bacterial species are extremely difficult to culture.

NimaGen's EasySeq™ 16S Microbiome Library Prep Kit, powered by Reverse Complement PCR (RC-PCR), provides a highly sensitive, cost-effective way to characterize the

microbiome of various sample types like environmental, fecal, or food samples through analysis of the hypervariable regions V3-V4. It can be used to decipher complex bacterial communities that are poorly described or difficult-to-culture. As NGS is a highly-sensitive method even extremely low-abundant bacteria can be detected. This kit can be used with DNA extracted from many different sample types and is compatible with the Illumina® platform.

The microbiome plays a role in human health like the gut microbiome in Crohn's disease and allergies. 16S rRNA sequencing is also a useful tool to study the role of any microbiome in animal health and the environment. Integration of human, animal, and environment areas, allows studying microbiomes from a One Health perspective for example the food chain and food safety testing.



# EasySeq™ 16S Microbiome Library Prep Kit

This kit is designed to sequence the hypervariable regions V3-V4 of the 16S rRNA gene to deconvolute complex bacterial communities, enabling microbiologists to evaluate bacterial diversity and detect the abundance of microbes in various environments.

The single-plex Reverse Complement PCR generates one amplicon, which can be sequenced on the Illumina® MiSeq and NextSeq 1000/2000 platforms (Table 1), both suitable for 16S sequencing. Depending on the platform, up to 768 samples can be multiplexed (Table 2).

The kit delivers high-quality NGS data with very few chimeric reads (depending on the number of PCR cycles), resulting in a stable calling of bacterial genera from as little as 5 pg input DNA (Figures 4 and 5).

**Table 1 | EasySeq™ 16S Microbiome Library Prep Kit Specifications**

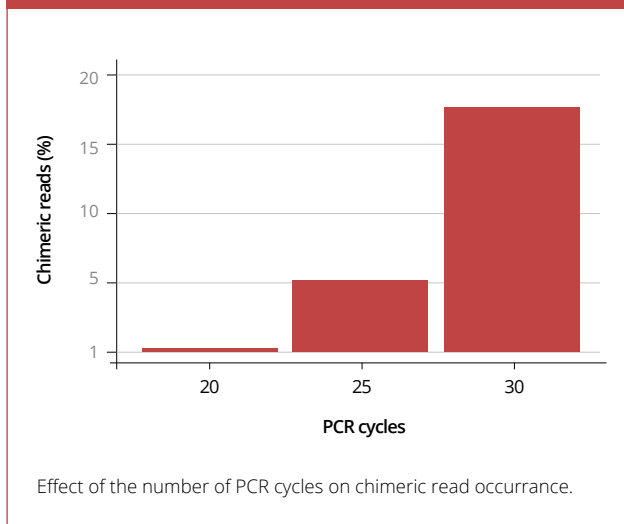
Parameter	Specification
Library Prep Method	Singleplex Reverse Complement PCR
Compatible with Illumina® sequencers and reagent kits	MiSeq: V3 (600), V2 (500), V2 Nano (500), NextSeq 1000/2000: P1 (600), P2 (600)
Targets	V3 - V4
Number of Amplicons	1
Number of Probe Panels	1
Input DNA Requirement	≥ 5 pg

**Table 2 | Sample Multiplexing**

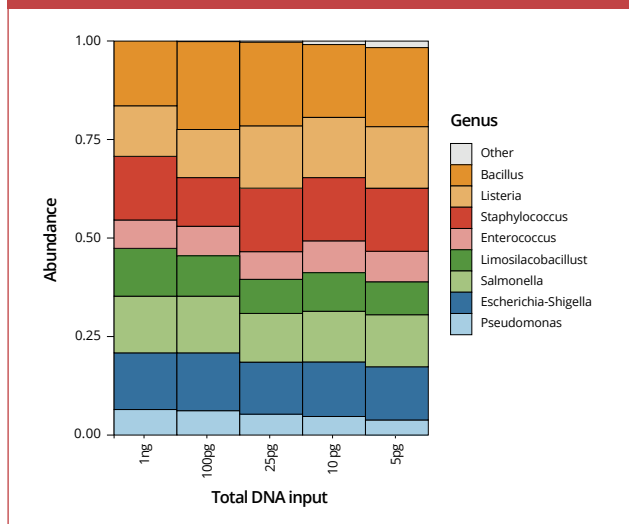
Sequencer	Reagent kit	Number of samples*
MiSeq	V3 (600 cycles)	250
MiSeq	V2 (500 cycles)	150
MiSeq	V2 Nano (500 cycles)	10
MiSeq	V2 Nano (300 cycles)	768
NextSeq 1000/2000	P1 (600 cycles)	768
NextSeq 1000/2000	P2 (600 cycles)	768

\* Theoretical maximum with 100,000 paired-end reads per sample or maximum number of indices available (n=768) if less than 100,000 reads are needed.

**Figure 4 | Effect of the number of PCR cycles on chimeric read occurrence**



**Figure 5 | Influence of DNA input amounts on abundance of bacterial genera in a sample**



# Analysis

After sequencing, basecalling and demultiplexing is performed, the FASTQ files per sample can be processed in an analysis pipeline of choice but primer sequences should be removed prior to analysis. The microbiome sequencing data shown were analyzed with open-source software DADA2

and visualized with the R-package microViz. In the DADA2 analysis, forward and reverse reads were quality filtered and trimmed prior to merging. After the merge step, chimeric reads were removed following taxonomical assignment using the SILVA 16S database.

# Ordering Information

## EasySeq™ NGS Library Prep Kit for 16S Microbiome

Part Number	Description
RC-16SMB096	EasySeq™ 16S Microbiome Library Prep Kit 1 pool/sample, includes PCR Master Mix, 96 rxn

## Unique Dual Index Plates for use with EasySeq™ 16S Microbiome Library Prep Kit

Part Number	Description
IDX96-U01	1 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0001-0096
IDX96-U02	1 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0097-0192
IDX96-U03	1 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0193-0289
IDX96-U04	1 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0290-0386
IDX96-U05	1 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0387-0483
IDX96-U06	1 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0484-0580
IDX96-U07	1 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0581-0677
IDX96-U08	1 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0678-0774

**Note:** Index (IDX) plates to be ordered separately.

## Magnetic Beads for NGS Library Clean-up

Part Number	Description
AP-005	AmpliClean™ Cleanup Kit, Magnetic Beads (AMPure XP alternative), 5 mL

**Note:** AmpliClean™ Magnetic Beads are ordered separately to complete the workflow from input DNA to sequencing-ready NGS libraries. AmpliClean™ magnetic beads are identical to AMPure XP beads for manual or automated purification and cleanup

## Recommended Alpaqua Magnet Kit Plates

Part Number	Description
A001322	96S Super Magnet
A000400	Magnum FLX™ Enhanced Universal Magnet Plate

**Note:** Alpaqua 96S Super Magnet is identical to Beckman Coulter P/N A32782. Magnum FLX™ facilitates up to 4.5x faster separation than 96S Super Magnet, from large volume and viscous samples, with low volume elution. Both magnet plates provide integrated Spring Cushion Technology, enabling maximized sample aspiration and protecting instruments and consumables.

# NimaGen.

## Product and Company Information

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### Product Name

EasySeq™ 16S Microbiome Library  
Prep Kit

### Product Use

For Research Use Only

Version 1.1 - October 2023

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