

EasySeq™

# Microbiology & Infectious Disease 16S Bacterial Identification Library Prep Kit

for illumina®

NGS Library Prep by Reverse Complement PCR



Safest and Simplest  
NGS Library Prep  
Workflow Available

- A multiplex assay that targets variable regions V1-V6 and V9 for bacterial identification
- 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 Bacterial Identification 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 2 | RC-PCR Kinetics

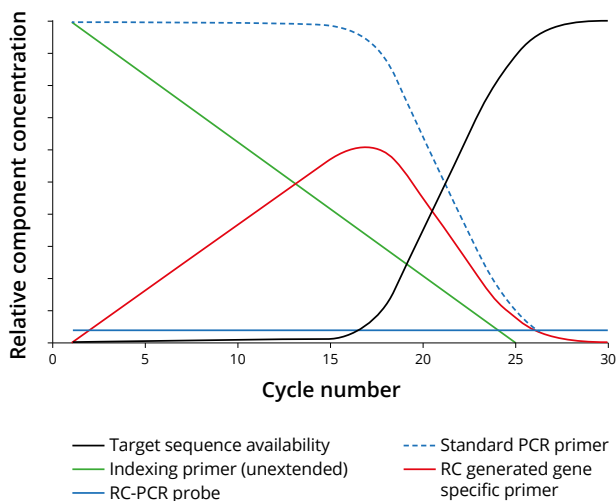
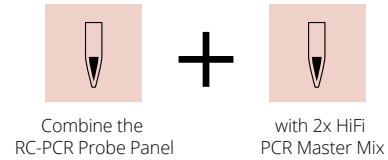
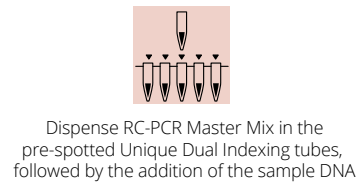


Figure 1 | EasySeq™ RC-PCR workflow

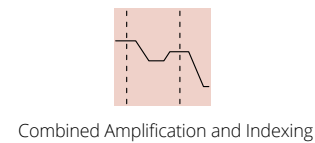
### 1 Prepare the RC-PCR Master Mix



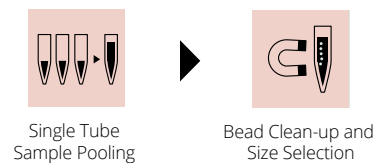
### 2 Dispense and add DNA



### 3 RC-PCR



### 4 NGS Library Clean-up

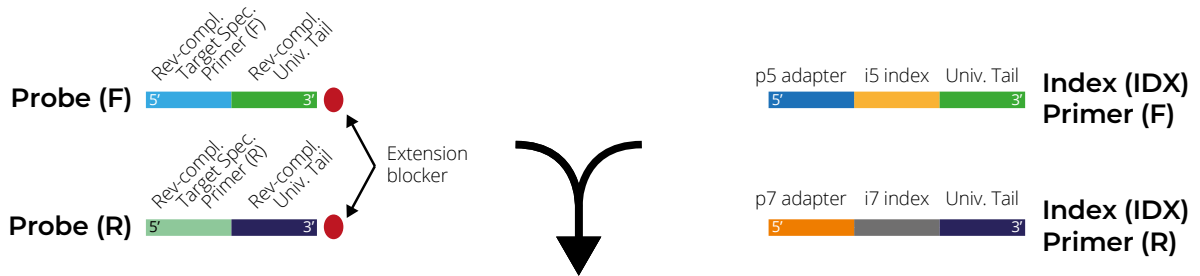


### 5 Sequence



# EasySeq™ NGS Library Prep by RC-PCR

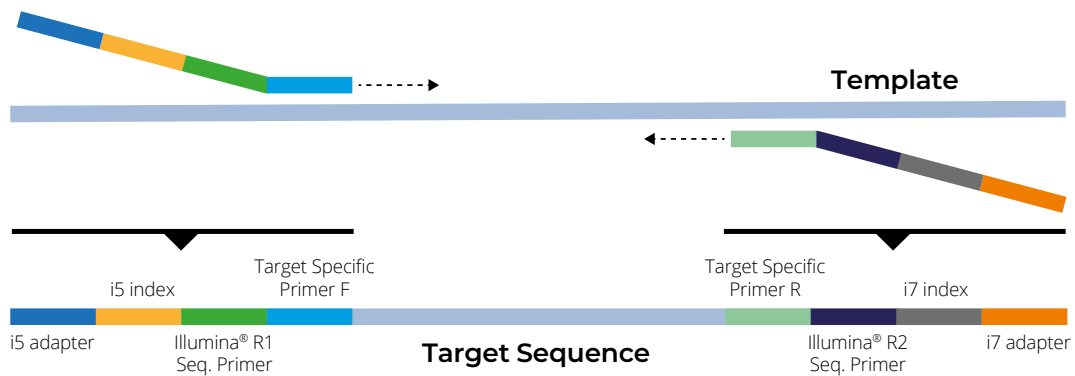
Figure 3 | Reverse Complement PCR Principle



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

### 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 identify one or multiple bacterial species in a single sample.

NimaGen's EasySeq™ 16S Bacterial Identification Library Prep Kit, powered by Reverse Complement PCR (RC-PCR), provides a highly sensitive, cost-effective way to identify bacterial species in culture positive and culture-negative samples through analysis of hypervariable regions V1-V6 and V9. It can be used to decipher polymicrobial samples where traditional Sanger sequencing fails. Furthermore, resources and time are saved because the kit does not require culture

and can be used with DNA extracted from many different sample types. The multiplex RC-PCR generates six amplicons that can be sequenced on various Illumina® platforms (Table 1). Depending on the platform, up to 768 samples can be multiplexed (Table 2).

One publication by Moorlag et al. (2023) summarizes the capabilities of this kit brilliantly, "Using 16S RC-PCR, we reveal a significant increase in the number of clinical samples in which a potentially clinically relevant pathogen is identified compared to the commonly used 16S Sanger method. Moreover, RCPCR allows automation and is well suited for implementation in a diagnostic laboratory<sup>1</sup>". The kit offers demonstrated high-quality data thanks to improved reaction kinetics, intrinsic to the RC-PCR method, in combination with the well-designed and balanced probe panels (figure 4) it maximizes usage of sequencing instrument capacity.

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1. Moorlag et al., Targeting the 16S rRNA Gene by Reverse Complement PCR Next-Generation Sequencing: Specific and Sensitive Detection and Identification of Microbes Directly in Clinical Samples, *Microbiology Spectrum*, doi: 10.1128/spectrum.04483-22



# EasySeq™ 16S Bacterial Identification Library Prep Kit

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

Parameter	Specification
Library Prep Method	Multiplex Reverse Complement PCR
Compatible with	Illumina® iSeq, MiSeq, MiniSeq, NextSeq
Targets	V1-2, V3, V4, V5, V6 and V9
Number of Amplicons	6
Number of Probe Panels	2
Input DNA Requirement	0.1 - 10 ng

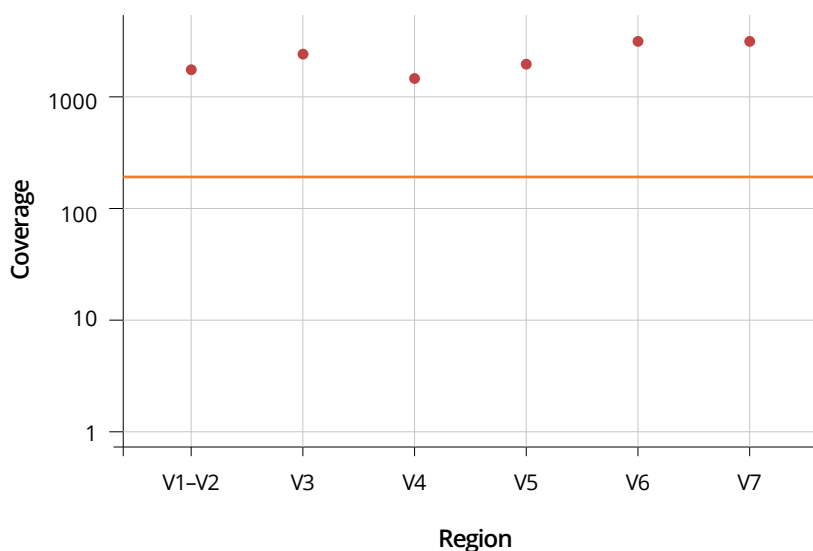
**Table 2 | Sample Multiplexing**

Sequencer	Reagent kit	Number of samples*	Number of polymicrobial samples**
iSeq 100	v2 (300 cycles)	768	333
MiniSeq	High output (300 cycles)	768	768
MiniSeq	Mid output (300 cycles)	768	666
MiSeq	V3 (600 cycles)	768	768
MiSeq	V2 (500 cycles)	768	768
MiSeq	V2 (300 cycles)	768	768
MiSeq	V2 Micro (300 cycles)	768	333
MiSeq	V2 Nano (500 cycles)	768	83
MiSeq	V2 Nano (300 cycles)	768	83
Nextseq 500/550	High output (300 cycles)	768	768
Nextseq 500/550	Mid output (300 cycles)	768	768
NextSeq 1000/2000	P1 (600 cycles)	768	768
NextSeq 1000/2000	P2 (600 cycles)	768	768

\* Theoretical maximum with 200 reads per probe or maximum number of indices available (n=768)

\*\* Theoretical maximum with 2000 reads per probe or maximum number of indices available (n=768)

**Figure 4 | EasySeq™ 16S Bacterial Identification Library Prep Kit target balance**



The orange line represents 200x coverage that is the advised minimum for cultures.

# Ordering Information

## EasySeq™ NGS Library Prep Kit for Bacterial Identification

Part Number	Description
RC-16S096	EasySeq™ 16S Bacterial Identification Library Prep Kit 2 pools/sample, includes PCR Master Mix, 96 rxn

## Unique Dual Index Plates for use with EasySeq™ 16S Bacterial Identification 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.

## Recommended Alpaqua Magnet 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 Bacterial Identification  
Library Prep Kit

### Product Use

For Research Use Only

Version 1.1 - October 2023

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