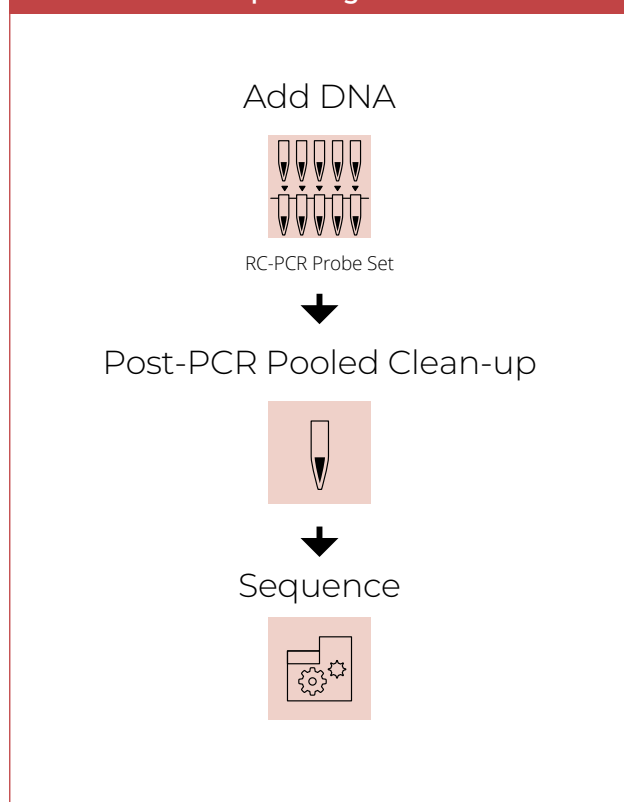


EasySeq™ 16S rRNA V3V4 Bacterial Identification Sequencing Kit

This kit is designed to sequence the hypervariable regions V3 and V4 in a singleplex reaction (Table 2), using a single amplicon that is aimed at deconvoluting complex bacterial communities. It is compatible with Illumina® MiSeq and NextSeq 1000/2000 (2x250bp) runs, both suitable for 16S metagenomic sequencing, enabling microbiologists to evaluate bacterial diversity and detect the abundance of microbes in various environments. The RC-PCR powered EasySeq™ 16S rRNA V3V4 Sequencing Kit delivers high-quality NGS data and optimum instrument throughput (Figure 4).

Parameter	Specification
Library Prep Method	Reverse Complement PCR
Compatible with	Illumina® MiSeq and NextSeq 1000/2000
Targets	V3 and V4
Number of Amplicons	1
Number of Probe Panels	1
Input DNA Requirement	0.1-10 ng

Figure 4 | EasySeq™ 16S rRNA V3V4 Bacterial ID Sequencing Kit Workflow



Ordering Information

EasySeq™ NGS Library Prep Kit for 16S rRNA V1-6 and V9 Bacterial ID

Part Number	Description
RC-16S096	EasySeq™ 16S rRNA V1-6 and V9 Bacterial ID Sequencing Kit 2 pools/sample, includes PCR Master Mix, 96 rxn

EasySeq™ NGS Library Prep Kit for 16S rRNA V3V4 Bacterial ID

Part Number	Description
RC-16SV3V4	EasySeq™ 16S rRNA V3V4 Bacterial ID Sequencing Kit 1 pool/sample, includes PCR Master Mix, 96 rxn

Unique Dual Index Plates for use with EasySeq™ 16S rRNA V1-6 and V9 Bacterial ID Sequencing Kit

Part Number	Description
IDX96-U01D	2 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0001-0096
IDX96-U02D	2 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0097-0192
IDX96-U03D	2 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0193-0289
IDX96-U04D	2 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0290-0386
IDX96-U05D	2 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0387-0483
IDX96-U06D	2 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0484-0580
IDX96-U07D	2 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0581-0677
IDX96-U08D	2 x 96 Dehydrated, Colored Unique Dual Indexes Pre-spotted in 96-well plate - UDI #0678-0774

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

Unique Dual Index Plates for use with EasySeq™ 16S rRNA V3V4 Bacterial ID Sequencing 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.

NimaGen.

Product and Company Information

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Product Name
EasySeq™ 16S rRNA V1-6 and V9
Bacterial ID Sequencing Kit
EasySeq™ 16S rRNA V3V4 Bacterial ID
Sequencing Kit

Product Use
For Research Use Only

Version 1.0 - March 2023

Legal Notice

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EasySeq™

Microbiology & Infectious Disease 16S rRNA Bacterial Identification

NGS Library Prep by Reverse Complement PCR



**Safest and Simplest
NGS Library Prep
Workflow Available**

- Available in two versions, matching your research needs:
 - V1-V6 and V9, multiplex assay
 - V3-V4, singleplex assay

- Detailed identification, classification and quantitation of microbes within complex biological mixtures

- 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 rRNA Sequencing Kit utilize unique RC-PCR technology to create a simple and safe one-tube, single reaction NGS library prep workflow. Multiplex 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. Multiplex reactions are then pooled and cleaned-up in a single tube using magnetic beads, 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.

RC-PCR kinetics results in high sensitivity and specificity, as target specific primers are synthesized during the reaction, so concentrations of primers and amplicons are more in line, thus reducing 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

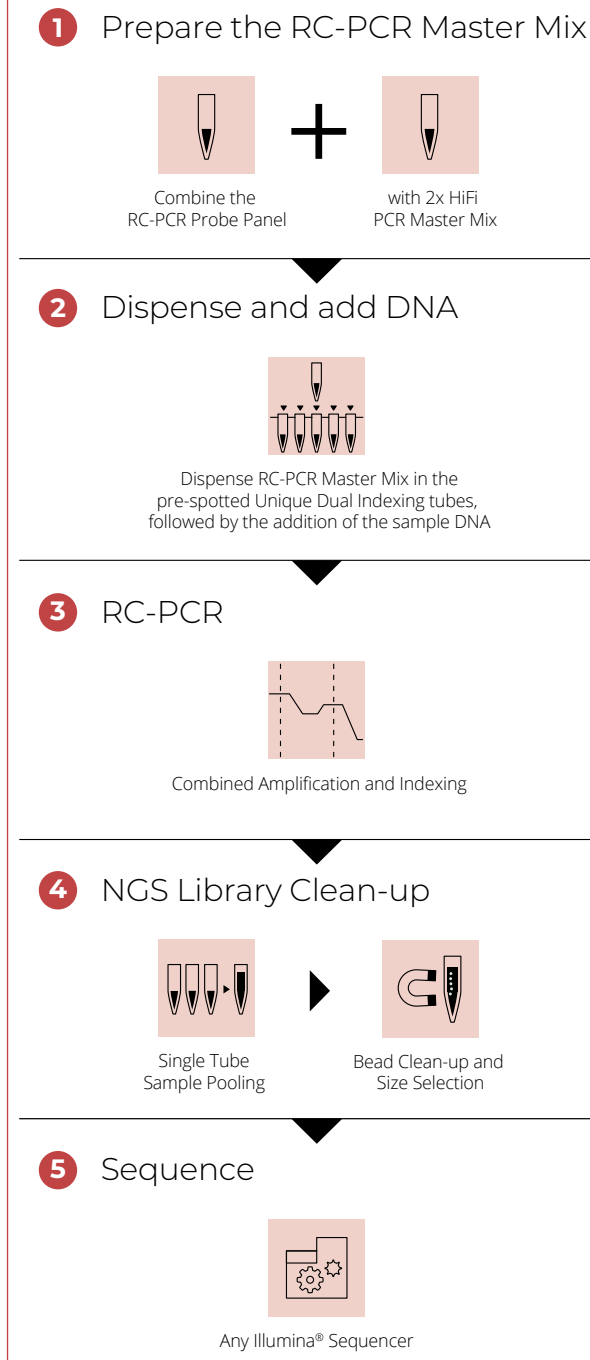
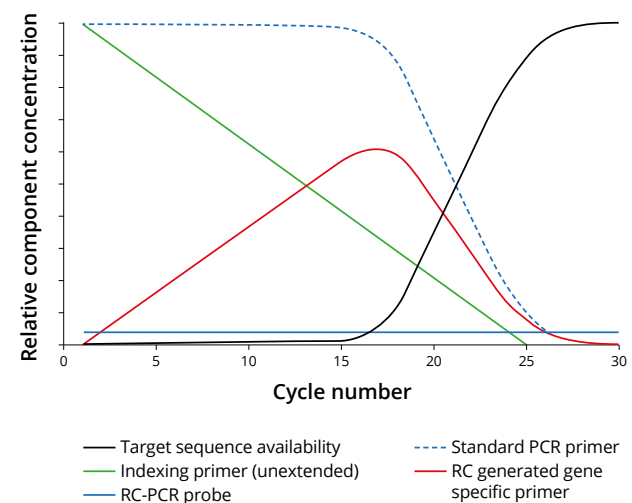


Figure 2 | RC-PCR Kinetics



Cost-efficient workflow

- Breakable index plates ensures 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

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

Introduction

All bacteria and archaea contain a ribosomal subunit known as 16S that is around 1,500 nucleotides long and contains nine hypervariable regions (V1-V9) spread out among highly conserved regions that are genus- or species-specific. Sequencing of the 16S ribosomal RNA (rRNA) gene, an established genetic marker, is the gold standard for the analysis of bacterial samples for identification and classification of both pure cultures and mixed sample analysis. Through introduction of Next-generation Sequencing (NGS), the 16S rRNA method is now also widely used to deconvolute complex microbial communities, such as soil samples and human gut microbiomes

NimaGen's EasySeq™ 16S rRNA Sequencing Kit for microbiome analysis, powered by Reverse Complement PCR (RC-PCR), provides a highly sensitive, cost-effective way to characterize the microbiota of clinical, animal-derived, environmental, or food samples. It can be used to identify even poorly described, difficult-to-culture, or phenotypically

aberrant bacterial strains, as well as determine novel pathogens or non-cultured bacteria. 16S rRNA sequencing performed on clinical samples contributes to bacterial identification in cases of negative culture due to an antibiotic therapy.

The EasySeq™ 16S rRNA Sequencing Kit is available in two versions:

- Analyzing hypervariable regions V1-V6 and V9 for mainly bacterial identification from clinical samples, for use with shorter amplicons in a two-pool multiplex reaction
- Analyzing hypervariable regions V3 and V4 in a singleplex reaction, mainly deciphering complex bacterial communities.

Both kits are used with DNA extracted from pure cultures and also allow direct amplification of DNA from clinical materials. Libraries are compatible with Illumina®.

EasySeq™ 16S rRNA V1-6 and V9 Bacterial Identification Sequencing Kit

This kit is designed for analysis of hypervariable regions V1-2-3-4-5-6 and V9 in two reactions (Table 1), compatible with short read (2x151bp) Illumina® runs. This kit, with shorter amplicons in a multiplex reaction, is commonly used for identifying bacteria in clinical samples. Thanks to improved reaction kinetics, intrinsic to the RC-PCR method, in combination with the well-designed and balanced probe panels, the EasySeq™ 16S rRNA V1-6 and V9 Sequencing Kit delivers high-quality NGS data and maximizes usage of sequencing instrument capacity (Figure 3).

Table 1 | EasySeq™ 16S rRNA V1-6 and V9 Bacterial ID Sequencing 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

Figure 3 | EasySeq™ 16S rRNA V1-6 and V9 Bacterial ID Sequencing Kit Workflow

