EasySeq™ *Microbiology and Infectious Disease*

Full-length 16S Library Prep Kit

NGS Library Prep by Reverse Complement PCR for Oxford Nanopore Technologies®

Safest and Simplest NGS Library Prep Workflow Available

- A complete analysis of the bacterial 16S rRNA gene with up to species level identification and sample-to-result within 8 hours
- Safe and cost-efficient workflow with confidence in highly sensitive, robust test results

NimaGen.

Innovators in DNA Sequencing Technologies

EasySeq™ NGS Library Prep by RC-PCR

The Next Revolution in Microbial NGS

EasySeq™ Full-length 16S Library Prep Kit utilizes proprietary Reverse Complement PCR (RC-PCR) technology to create a simple and safe one-tube, single reaction next-generation sequencing (NGS) library prep workflow compatible with Oxford Nanopore Technology (ONT) platforms (Figure 1).

In this reaction, target amplification and sample-specific barcoding 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 for individual clean-up (Figure 1). After clean-up, sequence adaptors are simply attached in a single ligation reaction. 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 barcode plates containing pre-spotted and dehydrated barcoded primers.

Figure 2 | RC-PCR Kinetics

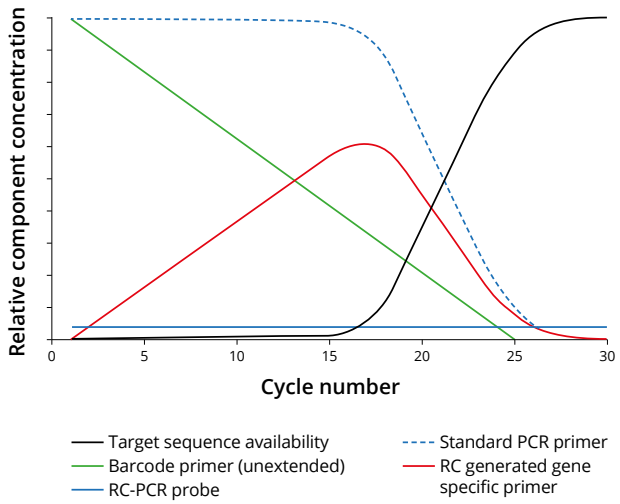


Figure 1 | EasySeq™ RC-PCR workflow

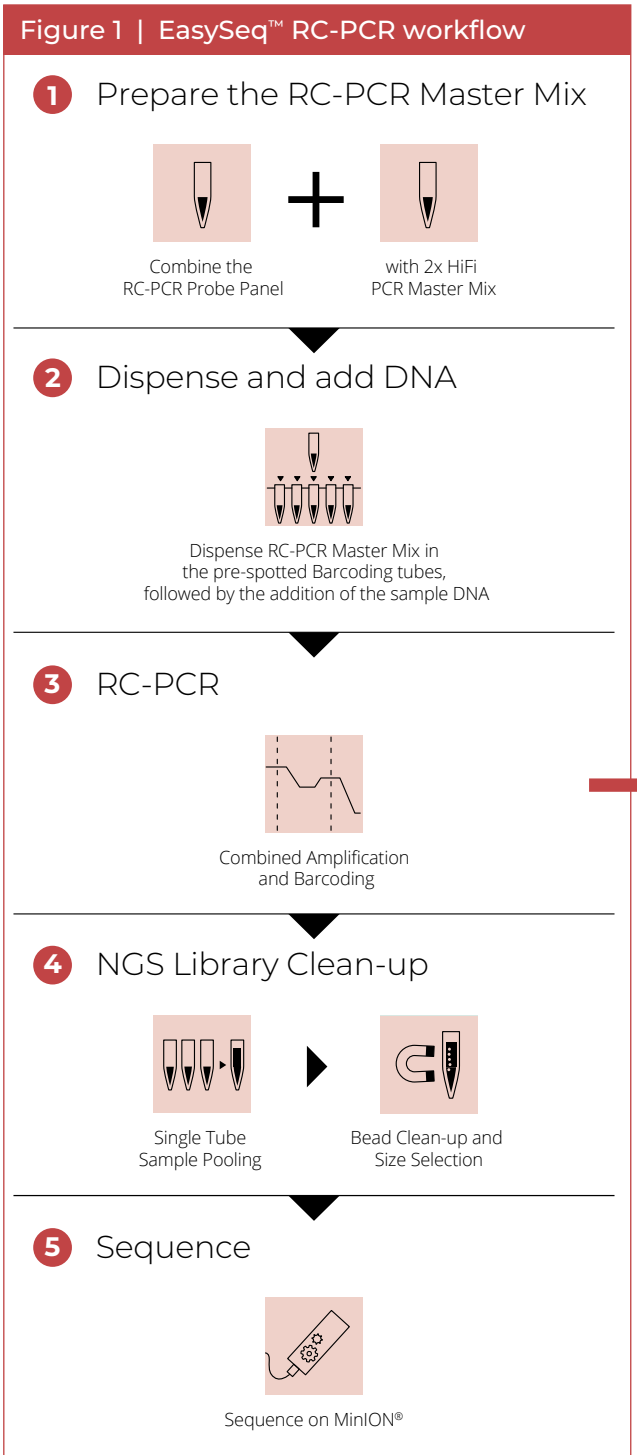
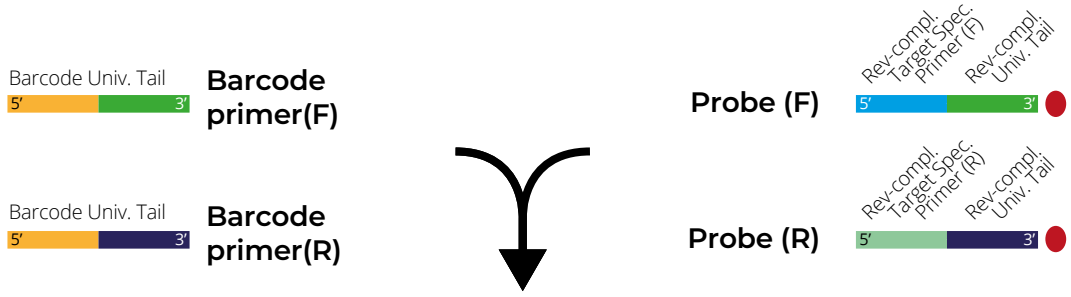
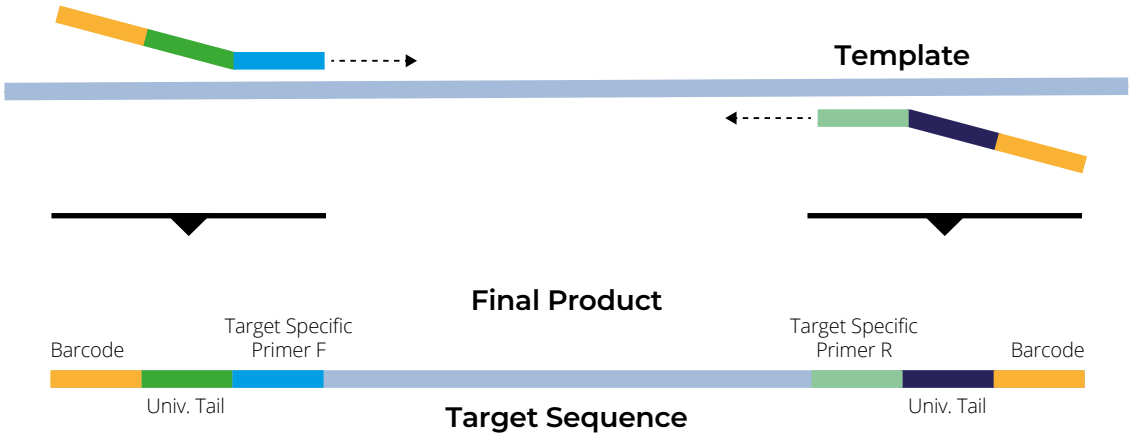


Figure 3 | RC-PCR principle ONT



Combine the Probe Panel with the Master Mix,
dispense in the barcoded plate, add sample DNA and start the RC-PCR program.

At the first annealing step, RC-PCR probes tails hybridize to the barcoded primer tails,
followed by extension of the barcoded primers with the gene-specific sequences.
This step synthesizes functional barcoded primers. In the following cycles,
targeted regions are amplified, while also creating more primers.



After bead clean-up, sequence adaptors are simply attached in a single ligation reaction.
This results in a ready-to-sequence, ONT compatible library.

Cost-efficient workflow

- Sample-to-result within 8 hours
- Breakable barcode plate ensures optimal usage, minimizing waste
- One closed-tube, single reaction workflow with simultaneous barcoding and target amplification reduces labor time
- Sample pooling for library clean-up significantly reduces usage of required magnetic beads and consumables
- 96 barcodes available allows for high multiplexing, thereby reducing cost

Confidence in test results

- Closed-tube RC-PCR workflow significantly reduces pipetting errors, minimizing risk of sample contamination
- Excellent composition representation with low input DNA samples as little as 0.1 pg of microbial DNA
- Uniform barcode performance ensures robust and sensitive test results
- Unique RC-PCR kinetics promote high target specificity and coverage uniformity from low DNA input samples
- Sample tracking dye in pre-spotted barcode plate ensures accuracy

Choice and flexibility

- Automation compatibility for high-throughput workflows
- Compatible with all ligation sequencing kits (LSK) of Oxford Nanopore Technologies®

Introduction

All species of archaea and bacteria have a 16S gene that encodes for the small subunit of the ribosomes. This 16S rRNA gene has highly variable regions (V1-V9) that are used as the gold standard for taxonomical classifications. Furthermore, this 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 used in a wide variety of applications from bacterial identification to deconvolution of complex microbial communities.

Traditionally, capillary sequencing or methods utilizing PCR have been used to sequence bacteria; however, these methods are labor intensive, cannot detect multiple bacteria within a sample and are limited by the fact that some bacteria are difficult to culture. NGS is the best method for quickly identifying bacteria as it a highly sensitive and culture-free technique, even allowing for detection of extremely low-abundance bacteria in mixed samples.

NimaGen's EasySeq™ Full-length 16S Library Prep Kit, powered by Reverse Complement (RC-PCR), provides a high-sensitive, cost-efficient workflow to sequence the entire 16S gene to characterize the bacteria up to species level and obtain a sample-to-result within 8 hours from DNA isolated from environmental, feed and food, human or animal derived samples (e.g. gut, skin, feces) without the need for culture. This kit is compatible with Oxford Nanopore Technologies®.

With a plethora of information available through NGS, determining specific bacterial species or bacterial composition in (complex) samples is made possible in a single run. This provides the versality that allows us to unravel the role and connection of microbes across a wide range of hosts and environments like never before.

EasySeq™ Full-length 16S Library Prep Kit

This kit is designed to sequence all hypervariable regions of the 16S rRNA gene in one working day to deconvolute complex bacterial communities and allow taxonomical assignment up to species level. This enables microbiologists fast evaluation of presence of bacteria and their respective abundance in various types of sample material. As the Oxford Nanopore Technologies® platform allows real-time sequencing, results can be obtained while sequencing is still ongoing.

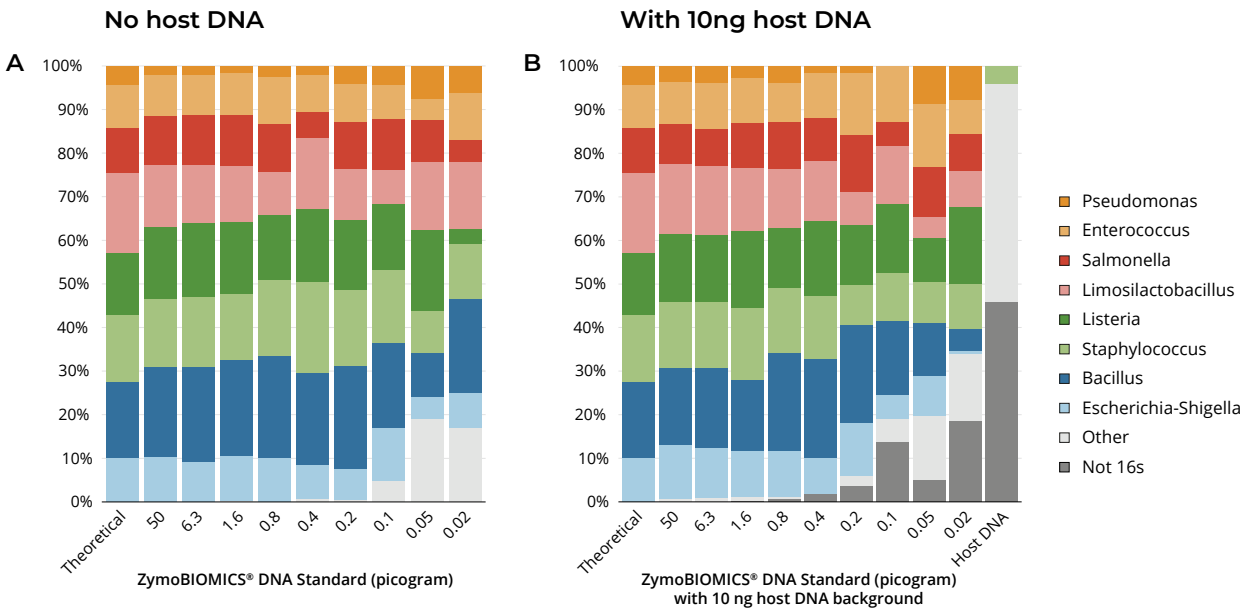
The singleplex Reverse Complement PCR generates one amplicon, which can then be sequenced on any Oxford Nanopore Technologies® platform using any ligation sequence kit (LSK). Depending on the required read-depth, up to 96 samples can be multiplexed.

The kit delivers high-quality NGS data with an excellent representation of the relative abundance of bacteria (Figure 4). Furthermore, the kit is very sensitive and robust in samples without (Figure 4A) or with host background DNA (Figures 4B). It also demonstrates a uniform performance across all 96 barcodes, including composition representation (Figure 5).

Table 1 | EasySeq™ Full-length 16S Library Prep Kit Specifications

Parameter	Specification
Library Prep Method	Singleplex Reverse Complement PCR
Targets	V1-V9 (Full-length)
Number of Amplicons	1
Number of Probe Panels	1
Input DNA Requirement	≥ 1 pg

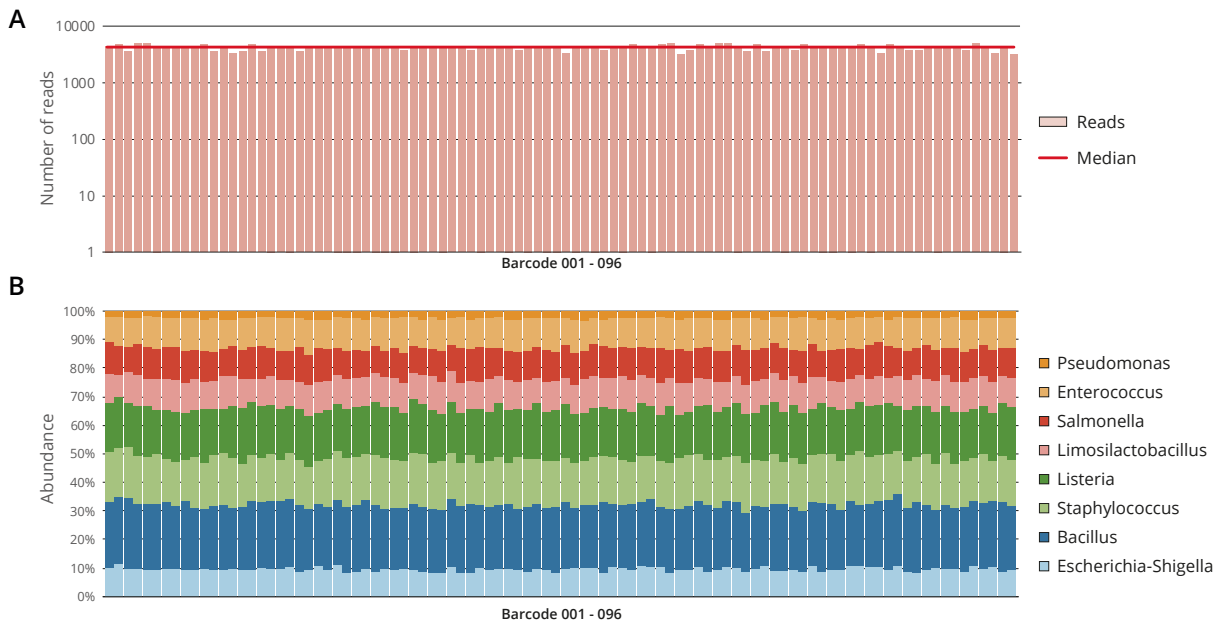
Figure 4 | Influence of microbial DNA input on bacterial identification and abundance in a sample



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 sequencing data shown in Figures 4 and 5 were preprocessed with open-source software NanoFilt (trimming and filtering), and analyzed with open-source software EMU with the default 16S database.

Figure 5 | Barcode uniformity



Ordering Information

EasySeq™ NGS Library Prep Kit for Full-length 16S

Part Number	Description
RC-ONT-16SFL096	EasySeq™ Full-length 16S Library Prep Kit *1 panel/sample, includes PCR Master Mix, 96 rxn*

Barcode Plates for use with EasySeq™ Full-length 16S Library Prep Kit

Part Number	Description
BC096-P01	1 x 96 Dehydrated, Colored Barcodes *Pre-spotted in 96-well plate - Barcode #0001-0096*

Note: Barcode 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 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™ Full-length 16S Library Prep Kit

Product Use
For Research Use Only

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