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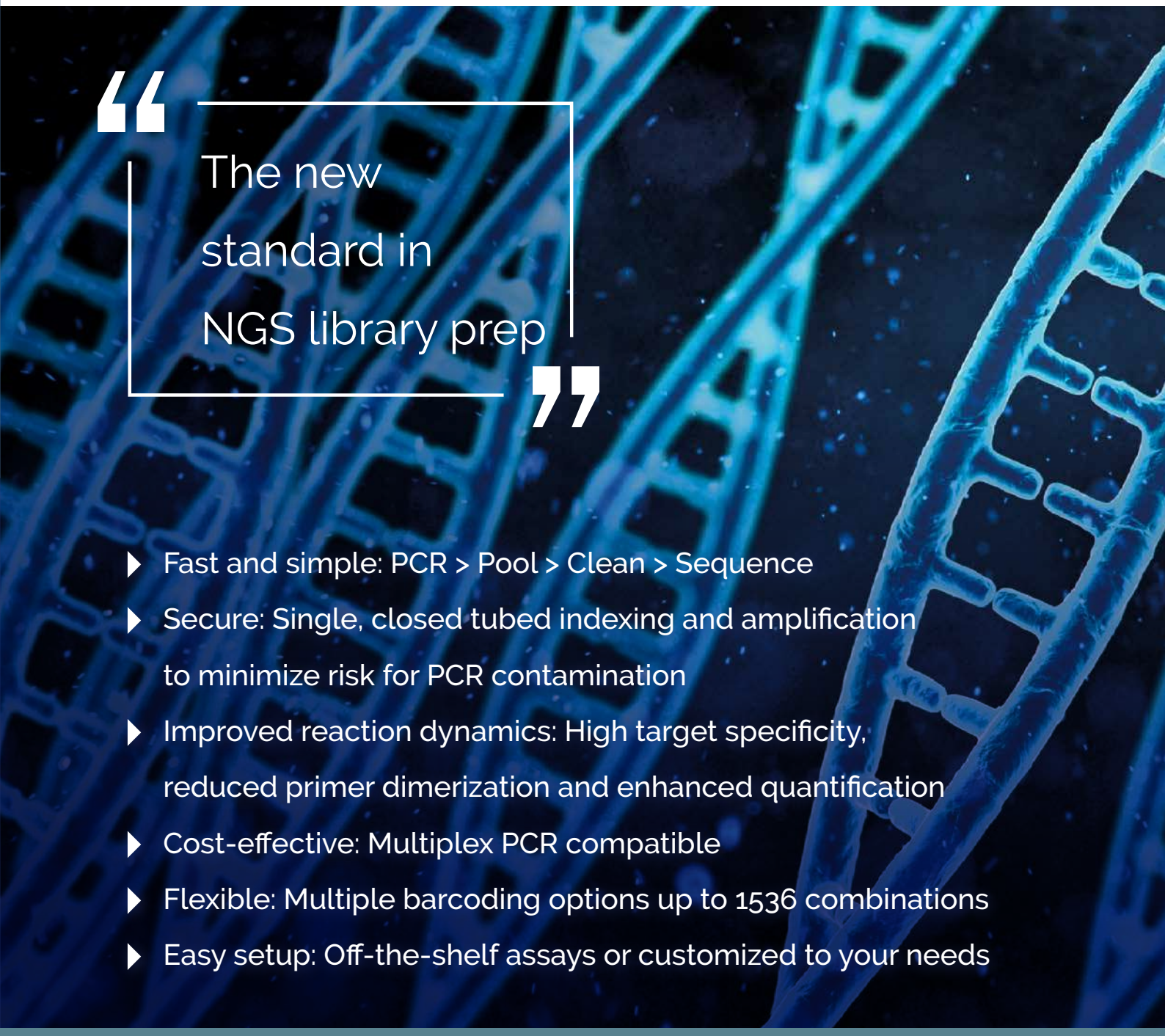
EasySeq™ NGS Reverse Complement PCR

Simultaneous Target Amplification and Barcoding



> The new standard in NGS library prep

- Fast and simple: PCR > Pool > Clean > Sequence
- Secure: Single, closed tubed indexing and amplification to minimize risk for PCR contamination
- Improved reaction dynamics: High target specificity, reduced primer dimerization and enhanced quantification
- Cost-effective: Multiplex PCR compatible
- Flexible: Multiple barcoding options up to 1536 combinations
- Easy setup: Off-the-shelf assays or customized to your needs

A single PCR reaction for Multiplex NGS library prep including flexible barcoding? It's now possible. RC-PCR, the most straight forward, secure and robust method for NGS Library generation.

Introduction

Current PCR based methods for Amplicon library preparation for Next Generation Sequencing (NGS) typically require a two-step PCR reaction:
A primary PCR is performed to amplify the ROI and add 5' universal tags while the secondary PCR step, using the primary PCR as the template, add the required functional NGS sequences and indexes.
This approach allows complex combinations of indexes to be added to PCR amplicons using a relatively small set of primers, however it requires multiple steps adding time and cost, particularly if large number of samples need to be analyzed.
In addition, as the primary and secondary PCR steps are performed separately, this requires opening tubes and/or tube transfers . Accordingly, there is a high risk of cross-contamination or carry-over. Since PCR involves exponential amplification, even low levels of contamination can have very significant impact that is difficult to detect, particularly when the process is used as a preparation step for sensitive analysis like NGS.
RC-PCR from NimaGen is the ideal solution to these problems, providing a new, patent protected method for generating amplicon constructs, with a single closed tube amplification and indexing reaction.
Furthermore, the method is fast, simple to use, increases targeting specificity and quantification, has the potential for multiplexing and significantly reduces the chances of amplifying incorrect sequences.

Kit Content (96 indexes)
1 tube with 10x concentrated RC-PCR probe Mix (20 µL)
12 x 8 PCR wells containing 4 µL Barcoding primer mix (96 unique indexes total)

RC PCR is patent protected (PCT/GB2016/050558, WO2016146968A1) and exclusively licenced to NimaGen B.V. Nijmegen

EasySeq™ RC-PCR workflow

1 Prepare the RC PCR Mastermix

Combine the RC Probe Mix + with 2x HiFi PCR MasterMix

2 Dispense and add DNA

Dispense this mix in the PCR wells, containing the predispensed universal primers with barcoding, followed by the addition of the sample DNA

3 PCR amplification

Perform thermal cycling

4 Pool samples

Pool all samples in one tube

5 Purify

Purify by single-tube AmpliClean → QC* (tapestation). Quantify (Qubit) and dilute > Illumina Run

** QC is optional*

6 Data analysis

Data analysis → Ready-to-go

Principle of RC-PCR

The reaction mix contains 2 probes (per target) and 2 primers:

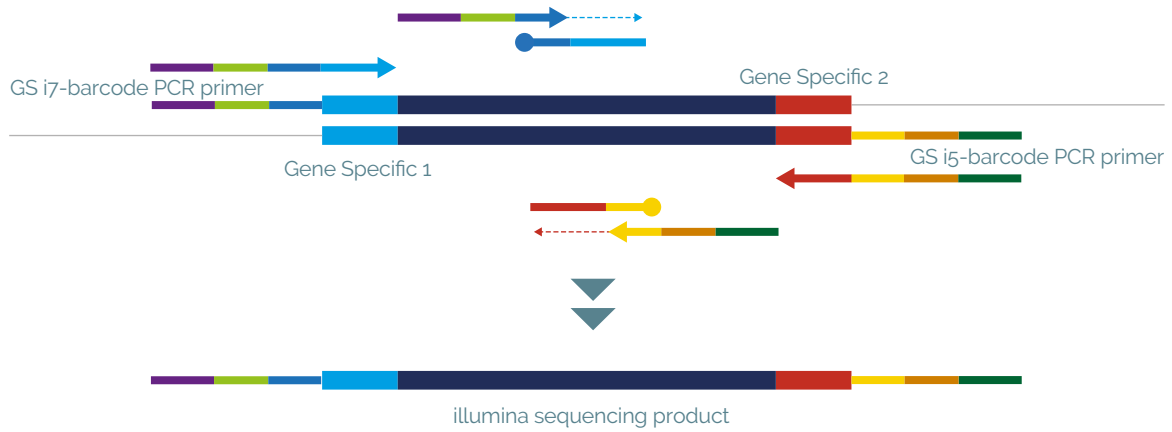
- 2 RC-PCR probes containing the reverse complement of the target specific primer, a 3' universal tail and a 3' amplification blocker
- 2 Universal indexing primers containing Illumina tags, sample specific barcodes, and the complement of the univeral tail
- HiFi PCR Mastermix

1 PCR Cycle 1: Tailed Gene Specifc PCR primer generation:

In this step, gene-specific PCR primers, including a sample specific barcode, illumina tags will be generated using the RC-PCR probes as a template,

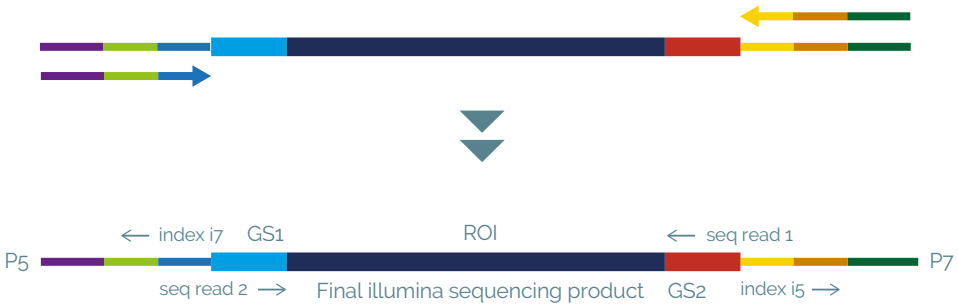
2 PCR Cycle 2 en 3: Generation of first copy of tailed target sequence

By implementing extended hybridization times, a highly efficient capturing and synthesis of the final complete construct will be generated.



3 PCR cycles 4+: Amplification of the library

Further amplification using universal barcoding PCR primers.



- For research use only -

Product and Company Information

Product name	EasySeq™ NGS Reverse Complement PCR Simultaneous Target Amplification and Barcoding
Product use	For Research Use Only
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