

## I. Introduction

SMARTer DNA single index kits are designed for use with SMARTer ThruPLEX® and SMARTer PicoPLEX® library preparation kits to construct libraries for multiplexed sequencing on Illumina® sequencers. These kits contain indexed PCR primers carrying the TruSeq® DNA Single Index sequences and offer a total of 24 indexes for the multiplexing of up to 24 samples. The indexed PCR primers are supplied predispensed in two different sets of 12 individual tubes, for 96 total reactions (Cat. Nos. R400695 and R400697). Each single index tube (12S Set A and 12S Set B) contains sufficient volume for eight uses.

## II. List of Components

Store all components at -20°C.

| Product Name                              | Cat. No. | Single index tube set | Number of reactions | Volume per well or tube |
|---|----------|-----------------------|---------------------|-------------------------|
| SMARTer DNA Single Index Kit - 12S Set A* | R400695  | 12S Set A             | 96                  | 40 µl                   |
| SMARTer DNA Single Index Kit - 12S Set B* | R400697  | 12S Set B             | 96                  | 40 µl                   |

\*Contains 12 different single index primer combinations in individual tubes labeled as shown below.

| SMARTer DNA Single Index Kit - 12S Set A (Cat. No. R400695; 96 rxns) |                                |
|--|--------------------------------|
| S1 - SMARTer DNA Single Index  | S7 - SMARTer DNA Single Index  |
| S2 - SMARTer DNA Single Index  | S8 - SMARTer DNA Single Index  |
| S3 - SMARTer DNA Single Index  | S9 - SMARTer DNA Single Index  |
| S4 - SMARTer DNA Single Index  | S10 - SMARTer DNA Single Index |
| S5 - SMARTer DNA Single Index  | S11 - SMARTer DNA Single Index |
| S6 - SMARTer DNA Single Index  | S12 - SMARTer DNA Single Index |

| SMARTer DNA Single Index Kit - 12S Set B (Cat. No. R400697; 96 rxns) |                                |
|--|--------------------------------|
| S13 - SMARTer DNA Single Index                                       | S20 - SMARTer DNA Single Index |
| S14 - SMARTer DNA Single Index                                       | S21 - SMARTer DNA Single Index |
| S15 - SMARTer DNA Single Index                                       | S22 - SMARTer DNA Single Index |
| S16 - SMARTer DNA Single Index                                       | S23 - SMARTer DNA Single Index |
| S18 - SMARTer DNA Single Index                                       | S25 - SMARTer DNA Single Index |
| S19 - SMARTer DNA Single Index                                       | S27 - SMARTer DNA Single Index |

## III. General Considerations

### A. Best Practices

- Prior to use, remove the kit from the freezer and thaw for 10 minutes on the bench top. Spin in a tabletop centrifuge to collect contents at the bottom of the tubes. Thoroughly wipe the tubes with 70% ethanol and allow them to dry.

**B. Product Compatibility**

The SMARTer DNA single index kits are designed for use with the SMARTer NGS library preparation kits listed below. Please refer to the kit-specific user manual for instructions on using the indexed PCR primers provided in the SMARTer DNA single index kits.

- SMARTer PicoPLEX Gold Single Cell DNA-Seq Kit (Cat. Nos. R300669, R300670, and R300698)
- SMARTer ThruPLEX DNA-Seq Kit (Cat. Nos. R400674, R400675, R400676, and R400677)
- SMARTer ThruPLEX Plasma-Seq Kit (Cat. Nos. R400679, R400680, R400681, and R400682)
- SMARTer ThruPLEX Tag-seq 96D Kit (Cat. Nos. R400586)
- ThruPLEX Tag-seq kits (Cat. Nos. R400584 and R400585)

**C. Multiplexing and Index Pooling**

It is very important to select appropriate single indexes that are unique and meet the Illumina-recommended compatibility requirements. For lowplex pooling guidelines, please refer to Illumina's Index Adapters Pooling Guide (Illumina, [Publication Document #1000000041074 v02](#)).

**IV. SMARTer DNA Single Index Sequences**

The SMARTer DNA single indexes are 6-nt long and employ the TruSeq DNA Single Index format (Tables I and II). Enter the sequences below directly on the sample sheet.

**Table I. SMARTer DNA single index sequences for Set A**

| <b>SMARTer DNA Single Index Kit - 12S Set A<br/>(Cat. Nos. R400694 and R400695)</b> |                               |
|---|-------------------------------|
| <b>Index name</b>   | <b>Bases for sample sheet</b> |
| S1  | ATCACG                        |
| S2  | CGATGT                        |
| S3  | TTAGGC                        |
| S4  | TGACCA                        |
| S5  | ACAGTG                        |
| S6  | GCCAAT                        |
| S7  | CAGATC                        |
| S8  | ACTTGA                        |
| S9  | GATCAG                        |
| S10   | TAGCTT                        |
| S11   | GGCTAC                        |
| S12   | CTTGTA                        |

# SMARTer® DNA Single Index Kit Protocol-At-A-Glance

Table III. SMARTer DNA single index sequences for Set B

| SMARTer DNA Single Index Kit - 12S Set B<br>(Cat. Nos. R400696 and R400697) |                        |
|---|------------------------|
| Index name  | Bases for sample sheet |
| S13   | AGTCAA                 |
| S14   | AGTTCC                 |
| S15   | ATGTCA                 |
| S16   | CCGTCC                 |
| S18   | GTCCGC                 |
| S19   | GTGAAA                 |
| S20   | GTGGCC                 |
| S21   | GTTTCG                 |
| S22   | CGTACG                 |
| S23   | GAGTGG                 |
| S25   | ACTGAT                 |
| S27   | ATTCCT                 |

**IMPORTANT:** When using the indexed PCR primers provided in the SMARTer DNA single index kits, make sure to follow the instructions in the user manual that is specific for the kit you are using them with ([Section III.B](#)).

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|--|--|
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This document has been reviewed and approved by the Quality Department.