I. Introduction

DNA single index kits are designed for use with ThruPLEX® and 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
DNA Single Index Kit - 12S Set A*	R400695	12S Set A	96	40 µl
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.

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

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

DNA Single Index Kit Protocol-At-A-Glance

B. Product Compatibility

The DNA single index kits are designed for use with the 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 DNA single index kits.

- PicoPLEX Gold Single Cell DNA-Seq Kit (Cat. Nos. R300669, R300670, and R300698)
- ThruPLEX DNA-Seq Kit (Cat. Nos. R400674, R400675, R400676, and R400677)
- ThruPLEX Plasma-Seg Kit (Cat. Nos. R400679, R400680, R400681, and R400682)
- 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, <u>Publication Document #1000000041074 v02</u>).

IV. DNA Single Index Sequences

The DNA single indexes are 6-nt long and employ the TruSeq DNA Single Index format (Tables 1 and 2). Enter the sequences below directly on the sample sheet.

Table 1. DNA single index sequences for Set A

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

Table 2. DNA single index sequences for Set B

DNA Single Index Kit - 12S Set B (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 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).

Contact Us			
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This document has been reviewed and approved by the Quality Department.