

ThruPLEX® DNA-seq Kit Dual Index Plate Guide

For Use With: ThruPLEX DNA-seq 48D Kit, CAT. NO. R400406
ThruPLEX DNA-seq 96D Kit, CAT. NO. R400407

ThruPLEX® DNA-seq Dual Index Plate (DIP) contains either 48 or 96 Indexing Reagents pre-dispensed and sealed in a microplate. The Indexing Reagents consist of amplification primers containing Illumina®-compatible dual indexes. Each well of the DIP contains a unique combination of Illumina's 8nt TruSeq® HT i5 and i7 index sequences (see next page) and has sufficient volume of the Indexing Reagent for a single use. No more than 4 freeze/thaw cycles are recommended for the DIP. The plate is sealed with foil that can be pierced with a multichannel pipet tip to collect the required amount of index to assemble the reactions.

Dual Indexed Library Structure: The prepared dual-indexed ThruPLEX DNA-seq library has the following structure.

ThruPLEX DNA-seq Dual-Indexed Library Structure

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5' AATGATACGGCGACCACCGAGATCTACACNNNNNNNNCACTCTTCCCTACACGACGCTCTCCGATCT----Insert----  
TruSeq HT i5 index  
----Insert----AGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNNNATCTCGTATGCCGTCTTCTGCTTG 3'  
TruSeq HT i7 index
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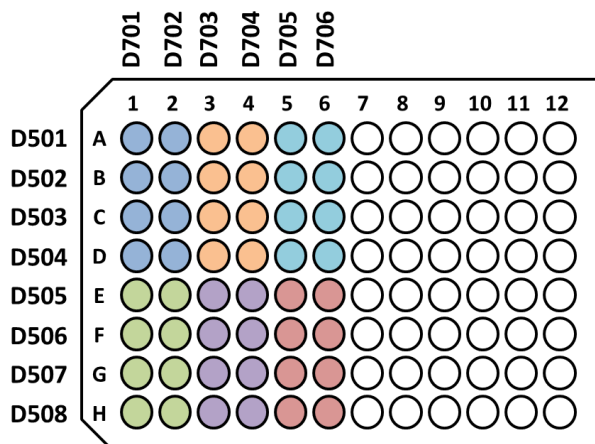
DIP Handling Instructions: The DIP is sealed with pierceable sealing foil and can be frozen and thawed no more than 4 times. Follow the instructions given below to avoid potential index cross contamination. Before removing Indexing Reagents from the DIP, make sure the two corner notches of the plate are on the left and the barcode label on the long side of the plate is facing you.

- Thaw the DIP for 10 min on the bench top prior to use.
- Once thawed, briefly centrifuge the plate to collect the contents to the bottom of each well.
- Thoroughly wipe the foil seal with 70% ethanol and allow it to dry completely.
- Pierce the seal above each well containing the specific index combination with a clean 200 µL filtered pipet tip; discard the tip.
- Use a new pipet tip to collect 5 µL of a specific index combination and add it to the reaction mixture at the Library Amplification Step. A multichannel pipette may be used if needed.
- If indexes from the entire plate are not used at the same time (low level multiplexing), cover any pierced index wells with scientific tape (e.g., VWR, CAT. NO. 89097-920, General-Purpose Laboratory Labeling Tape, 0.5") to mark the index as used.
- Once the DIP is used, wipe the seal with 70% ethanol and let it dry completely.
- Replace the plastic lid and return the plate to its sleeve and store at -20°C.

Low Level Multiplexing: Select appropriate dual index combinations that meet the Illumina recommended compatibility requirements. For more information on multiplexing and index pooling, please see index plate maps (next page) and refer to the ThruPLEX DNA-seq Kit Instruction Manual at www.rubicongenomics.com/resources/manuals/.

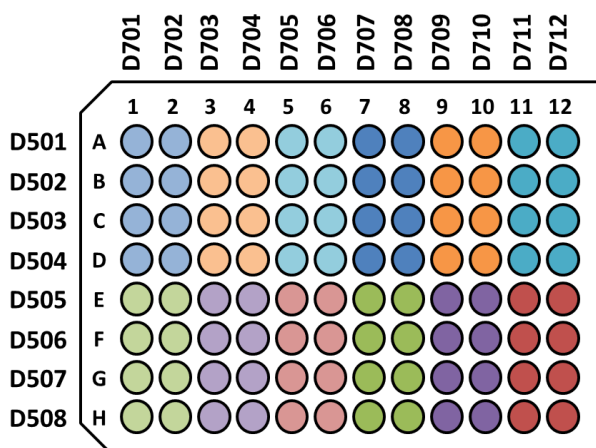
Index Plate Maps: The index combination at each well position is indicated by the column (i7) and row (i5) labels on the plate maps. The well colors illustrate one way to pool dual-index combinations for an 8-plex experiment; wells sharing the same color should be pooled together. For other ways to pool a low-plex (2- to 16-plex) experiment, please refer to Illumina's TruSeq Sample Preparation Pooling Guide (Illumina, Part# 15042173 Rev B, 2014).

Dual Index Plate (48B or 48D)



i7 Index	Sequence	i5 Index	Sequence
D701	ATTACTCG	D501	TATAGCCT
D702	TCCGGAGA	D502	ATAGAGGC
D703	CGCTCATT	D503	CCTATCCT
D704	GAGATTCC	D504	GGCTCTGA
D705	ATTCAGAA	D505	AGGCGAAG
D706	GAATTCGT	D506	TAATCTTA
		D507	CAGGACGT
		D508	GTACTGAC

Dual Index Plate (96A or 96D)



i7 Index	Sequence	i5 Index	Sequence
D701	ATTACTCG	D501	TATAGCCT
D702	TCCGGAGA	D502	ATAGAGGC
D703	CGCTCATT	D503	CCTATCCT
D704	GAGATTCC	D504	GGCTCTGA
D705	ATTCAGAA	D505	AGGCGAAG
D706	GAATTCGT	D506	TAATCTTA
D707	CTGAAGCT	D507	CAGGACGT
D708	TAATGCGC	D508	GTACTGAC
D709	CGGCTATG		
D710	TCCGCGAA		
D711	TCTCGCGC		
D712	AGCGATAG		

Technical Support: Call (734)-677-4845 (9AM-5:30PM EST) or contact support@rubicongenomics.com.

ThruPLEX® DNA-seq Kit is intended for **Research Use Only**. It may not be used for any other purposes including, but not limited to, use in diagnostics, forensics, therapeutics, or in humans. ThruPLEX DNA-seq may not be transferred to third parties, resold, modified for resale or used to manufacture commercial products without prior written approval of Rubicon Genomics, Inc.

ThruPLEX DNA-seq Kit is protected by U.S. Patents 7,803,550; 8,071,312; 8,399,199; 8,728,737 and corresponding foreign patents. Additional patents are pending.

The index sequences correspond to Illumina Index sequences for multiplexing and are copyrighted to Illumina, Inc.

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