

ThruPLEX® DNA-seq Kit Single Index Plate Guide

For Use With: ThruPLEX DNA-seq 48S Kit, CAT. NO. R400427

ThruPLEX® DNA-seq Single Index Plate (SIP) contains 48 unique Indexing Reagents pre-dispensed and sealed in a microplate. The Indexing Reagents consist of amplification primers containing Illumina®-compatible single indexes, each with a unique 8nt Sanger index sequence developed by the Wellcome Trust Sanger Institute in Cambridge, UK (see next page). Each well of the SIP contains sufficient volume of the Indexing Reagent for a single use. No more than 4 freeze/thaw cycles are recommended for the SIP. 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.

- **Note:** Information about the Sanger index sequences can be found in *Nature Methods* **7**, 111-118 (2010).

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

ThruPLEX DNA-seq Single-Indexed Library Structure

5' AATGATACGGCGACCACCGAGATCTACACAGGCGAAGACACTCTTCCCTACACGACGCTCTCCGATCT-----Insert-----
-----Insert-----AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC **NNNNNNNN**ATCTCGTATGCCGTCTTCTGCTTG 3'
Sanger index

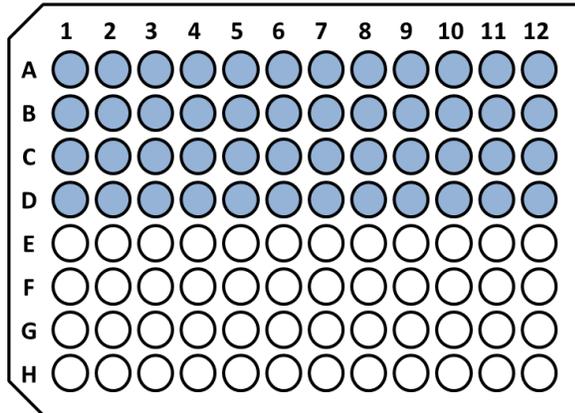
SIP Handling Instructions: The SIP is sealed with pierceable sealing foil and can be frozen and thawed no more than 4 times. Follow the instructions given below to avoid potential index cross contamination. Before removing Indexing Reagents from the SIP, make sure the two corner notches of the plate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

- Thaw the SIP 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 (MiSeq only), 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 SIP 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: ThruPLEX DNA-seq 48S Kit is designed for medium to high throughput applications; therefore, your experiment should be designed to use the entire plate of Indexing Reagents. Multiplexing less than the full set of 48 libraries is possible on the MiSeq only because MiSeq RTA v1.17.28 and later can process low-plexity index reads. Select index combinations that meet the Illumina recommended compatibility requirements. For more information on multiplexing and index pooling, please refer to the ThruPLEX DNA-seq Kit Instruction Manual at www.rubicongenomics.com/resources/manuals/.

Index Plate Map: The 48S Single Index Plate contains Illumina-compatible indexes with 8nt Sanger sequences. The colored wells indicate well positions containing Indexing Reagents.

Single Index late (48S)



Well	Sequence	Well	Sequence	Well	Sequence	Well	Sequence
A1	ATCACGTT	B1	TGGTTGTT	C1	TGCGATCT	D1	GGCACAAAC
A2	CGATGTTT	B2	TCTCGGTT	C2	TTCCTGCT	D2	TCTCACGG
A3	TTAGGCAT	B3	TAAGCGTT	C3	TAGTGACT	D3	TCAGGAGG
A4	TGACCACT	B4	TCCGTCTT	C4	TACAGGAT	D4	TAAGTTTCG
A5	ACAGTGGT	B5	TGTACCTT	C5	TCCTCAAT	D5	TCCAGTCG
A6	GCCAATGT	B6	TTCTGTGT	C6	TGTGGTTG	D6	TGTATGCG
A7	CAGATCTG	B7	TCTGCTGT	C7	TAGTCTTG	D7	TCATTGAG
A8	ACTTGATG	B8	TTGGAGGT	C8	TTCCATTG	D8	TGGCTCAG
A9	GATCAGCG	B9	TCGAGCGT	C9	TCGAAGTG	D9	TATGCCAG
A10	TAGCTTGT	B10	TGATACGT	C10	TAACGCTG	D10	TCAGATTC
A11	GGCTACAG	B11	GTGCTACC	C11	TTGGTATG	D11	TACTAGTC
A12	CTTGTACT	B12	GGTTGGAC	C12	TGAACTGG	D12	TTCAGCTC

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 8nt index sequences were developed by the Wellcome Trust Sanger Institute in Cambridge, UK; additional information can be found in *Nature Methods* 7, 111 - 118 (2010).

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