I. Introduction

SMARTer RNA unique dual index kits contain unique dual-indexed PCR primers for amplification of indexed Illumina®-compatible NGS libraries. These primers can be used in SMARTer NGS kits that typically use any of the Indexing Primer Set for Illumina, Indexing Primer Set HT for Illumina or the Indexing Primer Set HT for Illumina v2. Consult our website for a full list of compatible kits. For example, these primers are compatible with the SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian, the SMART-Seq® Stranded Kit, and the SMARTer smRNA-Seq Kit for Illumina.

These kits contain indexed PCR primers and together offer up to 192 unique dual indexes for multiplexing up to 192 samples. The indexed PCR primers are supplied pre-dispensed in 8-tube PCR strips (individually labeled U1 to U192) and are available in two formats: two sets of 96 unique dual indexes (Cat. Nos. 634452 and 634457) and a set of 24 unique dual indexes (Cat. No. 634451) that represents a subset of Cat. No. 634452. Each dual index is provided in sufficient amounts for four uses, with the exception of some SMARTer kits that require a larger amount due to a larger PCR reaction volume (which results in only two uses).

All indexes have been functionally validated to work with Illumina sequencing systems using two- or four-channel chemistry for base calling. They have not been validated with systems using one-channel chemistry.

II. List of Components

Store all components at –20°C.

Product name	Cat. No.	Concentration	Volume per tube
SMARTer RNA Unique Dual Index Kit - 24U*	634451	12.5 µM	8 µl
SMARTer RNA Unique Dual Index Kit - 96U Set A	634452	12.5 µM	8 µl
SMARTer RNA Unique Dual Index Kit - 96U Set B	634457	12.5 µM	8 µl

* The indexes in the SMARTer RNA Unique Dual Index Kit - 24U are a subset of the SMARTer RNA Unique Dual Index Kit - 96U Set A.

SMARTer RNA Unique Dual Index Kit - 24U (Cat. No. 634451; 96 rxns)
U25 - SMARTer RNA Unique Dual Index to U48 - SMARTer RNA Unique Dual Index

SMARTer RNA Unique Dual Index Kit - 96U Set A (Cat. No. 634452; 384 rxns)
U1 - SMARTer RNA Unique Dual Index to U96 - SMARTer RNA Unique Dual Index

SMARTer RNA Unique Dual Index Kit - 96U Set B (Cat. No. 634457; 384 rxns)
U97 - SMARTer RNA Unique Dual Index to U192 - SMARTer RNA Unique Dual Index

III. General Considerations

A. Best Practices

- It is not recommended to subject SMARTer RNA unique dual index kits to more than four freeze/thaw cycles.
- Prior to use, remove PCR strips containing the desired unique dual indexes from the freezer and bring to the benchtop. Thaw for 10 minutes at room temperature, then spin in a tabletop centrifuge to pellet contents at the bottom of the tubes. Ensure the caps show no visible condensation prior to opening tubes.

B. Product Compatibility

The SMARTer RNA unique dual 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 RNA unique dual index kits. As a rule, if the protocol calls for 1 µl of each forward and reverse (or 5′ and 3′) PCR primer, use 2 µl of the SMARTer RNA unique dual index. If the protocol calls for 2 µl of each forward and reverse (or 5′ and 3′) PCR primer, use 4 µl of the SMARTer RNA unique dual index.

- SMARTer Stranded RNA-Seq Kit (Cat. Nos. 634836, 634837, 634838, 634839)
- SMARTer Stranded RNA-Seq Kit HT (Cat. No. 634862)
- SMARTer Stranded Total RNA Sample Prep Kit - Low Input Mammalian (Cat. No. 634861)
- SMARTer Stranded Total RNA Sample Prep Kit - HI Mammalian (Cat. Nos. 634873, 634874, 634875, 634876, 634877, 634878)
- SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian (Cat. Nos. 635005, 635006, 635007)
- SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian (Cat. Nos. 634411, 634412, 634113, 634414)
- SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian Components (Cat. Nos. 634417, 634418, 634419)
- SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian (Cat. Nos. 634485, 634486, 634487, 634488)
- SMART-Seq Stranded Kit (Cat. Nos. 634442, 634443, 634444)
- SMARTer smRNA-Seq Kit for Illumina (Cat. Nos. 635029, 635030, 635031)
- SMARTer Human TCR a/b Profiling Kit v2 (Cat. Nos. 634478, 634479)
- DNA SMART™ ChIP-Seq kits (Cat. Nos. 634865, 634866, 634867)

C. Multiplexing and Index Pooling

It is important to select appropriate single indexes that are unique and meet the Illumina-recommended compatibility requirements. For low-plex pooling involving less than 8 samples per sequencing lane, use only Cat No 634451 or 634452, which carry IDT for Illumina TruSeq® DNA and RNA UD Indexes. Follow the guidelines in Illumina's Index Adapters Pooling Guide (Illumina, Publication Document

#1000000041074) and as shown in Figure 1, below: pool libraries of any plexity > 2 down a column (2-plex, 3-plex, etc.). Do **not** pool libraries across a row.

When using Cat. No. 634457 (SMARTer RNA Unique Dual Index Kit - 96U Set B), pool at least two full columns per sequencing lane. Any two or more columns can be pooled together (Figure 2, below). The SMARTer RNA Unique Dual Index Kit - 96U Set B was designed to increase the multiplexing capability of the SMARTer RNA Unique Dual Index Kit - 96U Set A set and should not be used pooling less than 16 libraries. Please note that the indexes in the SMARTer RNA Unique Dual Index Kit - 96U Set B do not overlap with indexes in Cat. No. 634452 (SMARTer RNA Unique Dual Index Kit - 96U Set A), enabling up to 192 samples to be multiplexed.

Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6	Strip 7	Strip 8	Strip 9	Strip 10	Strip 11	Strip 12
U1–U8	U9–U16	U17–U24	U25–U32	U33–U40	U41–U48	U49–U56	U57–U64	U65–U72	U73–U80	U81–U88	U89-U96
1	9	17	25	33	41	49	57	65	73	81	89
2	10	18	26	34	42	50	58	66	74	82	90
3	11	19	27	35	43	51	59	67	75	83	91
4	12	20	28	36	44	52	60	68	76	84	92
5	13	21	29	37	45	53	61	69	77	85	93
6	14	22	30	38	46	54	62	70	78	86	94
7	15	23	31	39	47	55	63	71	79	87	95
8	16	24	32	40	48	56	64	72	80	88	96

Figure 1. Index map and multiplexing strategy for the SMARTer RNA Unique Dual Index Kit - 96U Set A (Cat. No. 634452). For multiplexing libraries of plexity >2, we recommend pooling indexes down a column (example given in colored boxes). Do not pool libraries between rows.

Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6	Strip 7	Strip 8	Strip 9	Strip 10	Strip 11	Strip 12
U97–U104	U105–U112	U113–U120	U121–U128	U129–U136	U137–144	U145–U152	U153–U160	U161–168	U169–176	U177–U184	U185-U192
97	105	113	121	129	137	145	153	161	169	177	185
98	106	114	122	130	138	146	154	162	170	178	186
99	107	115	123	131	139	147	155	163	171	179	187
100	108	116	124	132	140	148	156	164	172	180	188
101	109	117	125	133	141	149	157	165	173	181	189
102	110	118	126	134	142	150	158	166	174	182	190
103	111	119	127	135	143	151	159	167	175	183	191
104	112	120	128	136	144	152	160	168	176	184	192

Figure 2. Index map and multiplexing strategy for the SMARTer RNA Unique Dual Index Kit - 96U Set B (Cat. No. 634457). When multiplexing libraries using this kit, any two columns may be pooled together (example given with shaded red indexes). Please note that this kit should not be used for pooling <16 libraries.

IV. SMARTer RNA Unique Dual Index Sequences

The SMARTer RNA unique dual indexes are 8-nt long i5 and i7 dual index sequences.

- SMARTer RNA Unique Dual Indexes U001–U096 correspond to the IDT for Illumina-TruSeq DNA and RNA UD Indexes - UDI0001–UDI0096 according to the Illumina Adapter Sequences Document (1000000002694 v10)
- SMARTer RNA Unique Dual Indexes U097–U192 have been designed at Takara Bio USA, Inc. and carry the same TruSeq adapters as IDT for Illumina-TruSeq DNA and RNA UD Indexes

Table 1 (continues over six pages) documents the complete list of the SMARTer RNA unique dual indexes. A CSV file containing a full list of these indexes can also be downloaded from our website in the SMARTer RNA Unique Dual Index Kits - Index Sequences file.

For Illumina sequencing, enter indexes from the downloaded CSV file*, or, for SMARTer RNA Unique Dual Indexes U001–U096 , using the Illumina Experiment Manager software:

1. "Library Prep Workflow" dropdown menu—select a 'TruSeq Stranded' option
2. "Index Adapters" dropdown menu—select 'IDT-ILMN TruSeq RNA UD Indexes (96 indexes)'

   NOTE: Do NOT select 'IDT-ILMN TruSeq RNA UD Indexes v2 - 96 Indexes'

*SMARTer RNA Unique Dual Indexes U097–U192 may only be entered from the downloaded CSV file.

IMPORTANT: When using the indexed PCR primers provided in the SMARTer RNA unique dual-index kits, make sure to follow the instructions in the user manual specific to the compatible product (see Section III.B) you are using with the indexing kits.

Table 1. SMARTer RNA Unique Dual Index Sequences

i7 index name	i7 bases for sample sheet	i5 bases for sample sheet (MiSeq®, NovaSeq™, HiSeq® 2000/2500)	i5 bases for sample sheet (MiniSeq™, NextSeq®, HiSeq 3000/4000)
U1	CCGCGGTT	AGCGCTAG	CTAGCGCT
U2	TTATAACC	GATATCGA	TCGATATC
U3	GGACTTGG	CGCAGACG	CGTCTGCG
U4	AAGTCCAA	TATGAGTA	TACTCATA
U5	ATCCACTG	AGGTGCGT	ACGCACCT
U6	GCTTGTCA	GAACATAC	GTATGTTC
U7	CAAGCTAG	ACATAGCG	CGCTATGT
U8	TGGATCGA	GTGCGATA	TATCGCAC
U9	AGTTCAGG	CCAACAGA	TCTGTTGG
U10	GACCTGAA	TTGGTGAG	CTCACCAA
U11	TCTCTACT	CGCGGTTC	GAACCGCG
U12	CTCTCGTC	TATAACCT	AGGTTATA
U13	CCAAGTCT	AAGGATGA	TCATCCTT
U14	TTGGACTC	GGAAGCAG	CTGCTTCC
U15	GGCTTAAG	TCGTGACC	GGTCACGA

i7 index name	i7 bases for sample sheet	i5 bases for sample sheet (MiSeq®, NovaSeq™, HiSeq® 2000/2500)	i5 bases for sample sheet (MiniSeq™, NextSeq®, HiSeq 3000/4000)
U16	AATCCGGA	CTACAGTT	AACTGTAG
U17	TAATACAG	ATATTCAC	GTGAATAT
U18	CGGCGTGA	GCGCCTGT	ACAGGCGC
U19	ATGTAAGT	ACTCTATG	CATAGAGT
U20	GCACGGAC	GTCTCGCA	TGCGAGAC
U21	GGTACCTT	AAGACGTC	GACGTCTT
U22	AACGTTCC	GGAGTACT	AGTACTCC
U23	GCAGAATT	ACCGGCCA	TGGCCGGT
U24	ATGAGGCC	GTTAATTG	CAATTAAC
U25	ACTAAGAT	AACCGCGG	CCGCGGTT
U26	GTCGGAGC	GGTTATAA	TTATAACC
U27	CTTGGTAT	CCAAGTCC	GGACTTGG
U28	TCCAACGC	TTGGACTT	AAGTCCAA
U29	CCGTGAAG	CAGTGGAT	ATCCACTG
U30	TTACAGGA	TGACAAGC	GCTTGTCA
U31	GGCATTCT	CTAGCTTG	CAAGCTAG
U32	AATGCCTC	TCGATCCA	TGGATCGA
U33	TACCGAGG	CCTGAACT	AGTTCAGG
U34	CGTTAGAA	TTCAGGTC	GACCTGAA
U35	AGCCTCAT	AGTAGAGA	TCTCTACT
U36	GATTCTGC	GACGAGAG	CTCTCGTC
U37	TCGTAGTG	AGACTTGG	CCAAGTCT
U38	CTACGACA	GAGTCCAA	TTGGACTC
U39	TAAGTGGT	CTTAAGCC	GGCTTAAG
U40	CGGACAAC	TCCGGATT	AATCCGGA
U41	ATATGGAT	CTGTATTA	TAATACAG
U42	GCGCAAGC	TCACGCCG	CGGCGTGA
U43	AAGATACT	ACTTACAT	ATGTAAGT
U44	GGAGCGTC	GTCCGTGC	GCACGGAC
U45	ATGGCATG	AAGGTACC	GGTACCTT
U46	GCAATGCA	GGAACGTT	AACGTTCC
U47	GTTCCAAT	AATTCTGC	GCAGAATT
U48	ACCTTGGC	GGCCTCAT	ATGAGGCC
U49	ATATCTCG	ATCTTAGT	ACTAAGAT
U50	GCGCTCTA	GCTCCGAC	GTCGGAGC
U51	AACAGGTT	ATACCAAG	CTTGGTAT
U52	GGTGAACC	GCGTTGGA	TCCAACGC
U53	CAACAATG	CTTCACGG	CCGTGAAG

i7 index name	i7 bases for sample sheet	i5 bases for sample sheet (MiSeq®, NovaSeq™, HiSeq® 2000/2500)	i5 bases for sample sheet (MiniSeq™, NextSeq®, HiSeq 3000/4000)
U54	TGGTGGCA	TCCTGTAA	TTACAGGA
U55	AGGCAGAG	AGAATGCC	GGCATTCT
U56	GAATGAGA	GAGGCATT	AATGCCTC
U57	TGCGGCGT	CCTCGGTA	TACCGAGG
U58	CATAATAC	TTCTAACG	CGTTAGAA
U59	GATCTATC	ATGAGGCT	AGCCTCAT
U60	AGCTCGCT	GCAGAATC	GATTCTGC
U61	CGGAACTG	CACTACGA	TCGTAGTG
U62	TAAGGTCA	TGTCGTAG	CTACGACA
U63	TTGCCTAG	ACCACTTA	TAAGTGGT
U64	CCATTCGA	GTTGTCCG	CGGACAAC
U65	ACACTAAG	ATCCATAT	ATATGGAT
U66	GTGTCGGA	GCTTGCGC	GCGCAAGC
U67	TTCCTGTT	AGTATCTT	AAGATACT
U68	CCTTCACC	GACGCTCC	GGAGCGTC
U69	GCCACAGG	CATGCCAT	ATGGCATG
U70	ATTGTGAA	TGCATTGC	GCAATGCA
U71	ACTCGTGT	ATTGGAAC	GTTCCAAT
U72	GTCTACAC	GCCAAGGT	ACCTTGGC
U73	CAATTAAC	CGAGATAT	ATATCTCG
U74	TGGCCGGT	TAGAGCGC	GCGCTCTA
U75	AGTACTCC	AACCTGTT	AACAGGTT
U76	GACGTCTT	GGTTCACC	GGTGAACC
U77	TGCGAGAC	CATTGTTG	CAACAATG
U78	CATAGAGT	TGCCACCA	TGGTGGCA
U79	ACAGGCGC	CTCTGCCT	AGGCAGAG
U80	GTGAATAT	TCTCATTC	GAATGAGA
U81	AACTGTAG	ACGCCGCA	TGCGGCGT
U82	GGTCACGA	GTATTATG	CATAATAC
U83	CTGCTTCC	GATAGATC	GATCTATC
U84	TCATCCTT	AGCGAGCT	AGCTCGCT
U85	AGGTTATA	CAGTTCCG	CGGAACTG
U86	GAACCGCG	TGACCTTA	TAAGGTCA
U87	CTCACCAA	CTAGGCAA	TTGCCTAG
U88	TCTGTTGG	TCGAATGG	CCATTCGA
U89	TATCGCAC	CTTAGTGT	ACACTAAG
U90	CGCTATGT	TCCGACAC	GTGTCGGA
U91	GTATGTTC	AACAGGAA	TTCCTGTT

i7 index name	i7 bases for sample sheet	i5 bases for sample sheet (MiSeq®, NovaSeq™, HiSeq® 2000/2500)	i5 bases for sample sheet (MiniSeq™, NextSeq®, HiSeq 3000/4000)
U92	ACGCACCT	GGTGAAGG	CCTTCACC
U93	TACTCATA	CCTGTGGC	GCCACAGG
U94	CGTCTGCG	TTCACAAT	ATTGTGAA
U95	TCGATATC	ACACGAGT	ACTCGTGT
U96	CTAGCGCT	GTGTAGAC	GTCTACAC
U97	AAGGAGCG	GACTATTG	CAATAGTC
U98	GTCATCTA	GCGGTTCT	AGAACCGC
U99	CTTCATGG	CATTGCCA	TGGCAATG
U100	TTAAGCGT	GCATGCAA	TTGCATGC
U101	GGTAGTTG	TGGACCAT	ATGGTCCA
U102	ACTATATA	TTGGCTCC	GGAGCCAA
U103	ACGGAGGA	GGTTAGTC	GACTAACC
U104	GACTGCGG	ATCCTTCA	TGAAGGAT
U105	GGCAGGAC	CCGCCTAA	TTAGGCGG
U106	AACCGCCA	TCAACTTG	CAAGTTGA
U107	TATCGGCT	ACGAGATG	CATCTCGT
U108	TCAGATTC	ACTTAGTA	TACTAAGT
U109	ACTGCTCT	CAACTGCA	TGCAGTTG
U110	ATATATAC	TCTAGAGG	CCTCTAGA
U111	CAGTCGAT	CTGCTGAA	TTCAGCAG
U112	GAGCATAC	AACCTAGA	TCTAGGTT
U113	TGAATGGC	AAGCGTCC	GGACGCTT
U114	TATATTGA	TAGCATTG	CAATGCTA
U115	ACCGCTAC	CTTGCGAG	CTCGCAAG
U116	GTCAAGAG	AAGTACGC	GCGTACTT
U117	ACGCGAGA	GCAACTAA	TTAGTTGC
U118	CCTCTCAT	ACTAGTTC	GAACTAGT
U119	GAGTCGTC	CGTAGGCA	TGCCTACG
U120	ATACTATA	GGCGGTAT	ATACCGCC
U121	CCTGCCAA	CAGCAACT	AGTTGCTG
U122	AGTTATGC	GATTACTT	AAGTAATC
U123	GCGTACGG	TCCTTGCA	TGCAAGGA
U124	TTCATACG	AACCAACG	CGTTGGTT
U125	AAGAAGAC	GCCGGCAG	CTGCCGGC
U126	GCAAGATC	CGAATCCA	TGGATTCG
U127	AACGCCAT	TGGAGTAC	GTACTCCA
U128	CAAGTACT	GCTTGATG	CATCAAGC
U129	AGCGGCAA	CCAACCAG	CTGGTTGG

i7 index name	i7 bases for sample sheet	i5 bases for sample sheet (MiSeq®, NovaSeq™, HiSeq® 2000/2500)	i5 bases for sample sheet (MiniSeq™, NextSeq®, HiSeq 3000/4000)
U130	TTCCAAGC	ACCGTTGA	TCAACGGT
U131	CTATTCAT	AACTAGTC	GACTAGTT
U132	AAGCAATA	GAGTAACG	CGTTACTC
U133	GTTCTACG	CTAGTTGC	GCAACTAG
U134	AGAATCTC	GGTCAGAT	ATCTGACC
U135	CGGTCCGT	AATAGACG	CGTCTATT
U136	GGAACGGT	GTACTCGA	TCGAGTAC
U137	CTCTACTT	GTTAGCGG	CCGCTAAC
U138	ACGCTGCA	CGTTAATG	CATTAACG
U139	AAGGATTC	ACCGTCTC	GAGACGGT
U140	TGATCTCA	CAGACCTT	AAGGTCTG
U141	GGAGTATG	CTTGCCGC	GCGGCAAG
U142	CAGCGGAC	TAGTATCT	AGATACTA
U143	GCGCCTTC	TGACTCAA	TTGAGTCA
U144	ATATCCGC	TGGTTGAC	GTCAACCA
U145	GAGGAGTA	CGAATATT	AATATTCG
U146	TGACGAAC	AATATGAG	CTCATATT
U147	AACTTGAT	GGATATCG	CGATATCC
U148	CTTGAGTC	CGTTGCGG	CCGCAACG
U149	TCCTAGGT	CAACTCTT	AAGAGTTG
U150	CCATCTTA	TAGTTAGG	CCTAACTA
U151	AAGTTGGA	ACCGCGTT	AACGCGGT
U152	GGCCGTTC	TGAGCAGG	CCTGCTCA
U153	TAACTAGC	AAGCAGGT	ACCTGCTT
U154	CAACGCAA	AGGATGAT	ATCATCCT
U155	AGGCTTCT	CTAATAGG	CCTATTAG
U156	GAGGACCT	CCTAGCGA	TCGCTAGG
U157	ACCTTATT	TGCCTCTC	GAGAGGCA
U158	GGCGTTGC	AGAACCGG	CCGGTTCT
U159	TGCTAACG	TTACTTCT	AGAAGTAA
U160	CCAACGTT	CCGGTTAG	CTAACCGG
U161	GGCAGCCG	GTATACCA	TGGTATAC
U162	TAGGCCGT	CGACGGTC	GACCGTCG
U163	CGTATTGC	GGTAGAAT	ATTCTACC
U164	GATGATAA	AGCAAGGC	GCCTTGCT
U165	AAGTCATT	GTTCAGGC	GCCTGAAC
U166	CAGTTGCC	AACCGAAC	GTTCGGTT
U167	AAGGTAAG	TTCAGCAG	CTGCTGAA

i7 index name	i7 bases for sample sheet	i5 bases for sample sheet (MiSeq®, NovaSeq™, HiSeq® 2000/2500)	i5 bases for sample sheet (MiniSeq™, NextSeq®, HiSeq 3000/4000)
U168	TCTTATAT	TGGTATGA	TCATACCA
U169	CTCTAAGA	CGCCGGAT	ATCCGGCG
U170	AAGACTTA	TTCGATGA	TCATCGAA
U171	TCTCCGTC	AACGAAGT	ACTTCGTT
U172	CCGATCCT	TATATGCC	GGCATATA
U173	GTTCAATA	CTTATCAG	CTGATAAG
U174	GATTCCAG	TGGTAATT	AATTACCA
U175	ATCGGTTC	GTACGGCT	AGCCGTAC
U176	TCCTCAAT	ATTGATTA	TAATCAAT
U177	ATTATCGA	TTAGGATG	CATCCTAA
U178	GTCGCTAG	ATCAAGCA	TGCTTGAT
U179	CGCAGAAG	ACGATTAC	GTAATCGT
U180	TCAGGACC	TTACCATT	AATGGTAA
U181	ATCAGAGA	TAGCAGCC	GGCTGCTA
U182	CTGGATAA	GTAGCCGG	CCGGCTAC
U183	GTCTCGCG	CCATACCT	AGGTATGG
U184	TCGAATAA	CTGAGTTC	GAACTCAG
U185	CAACCTCT	TGGCCATC	GATGGCCA
U186	AGTTGAAC	AACGCGCA	TGCGCGTT
U187	CTTCGTTA	GGCCAGTA	TACTGGCC
U188	GCTGCGGC	GAGCTAGT	ACTAGCTC
U189	AGTAACCT	TACGGTAC	GTACCGTA
U190	TAAGATAT	AACTACCT	AGGTAGTT
U191	TGGTCCTG	GAATAGGC	GCCTATTC
U192	GTCCTAAC	TCGGTCAT	ATGACCGA

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