I. Introduction

The Unique Dual Index Kits contain unique, dual-indexed PCR primers for amplification of Illumina®-compatible NGS libraries. When used as a set, these kits offer unique dual indexes (UDIs) for multiplexing up to 384 samples. The primers are supplied predispensed in 96-well plates and are available in two formats:

- 96 unique dual indexes (Cat. Nos. 634752, 634753, 634754, and 634755)
- 24 unique dual indexes (Cat. No. 634756, a subset of Cat. No. 634752)

Refer to the product page for the full list of Takara Bio RNA-seq and DNA-seq kits compatible with these products.

All indexes have been functionally validated to work with Illumina sequencing systems (e.g., MiSeq®, NovaSeq™, MiniSeq™, NextSeq®, and HiSeq® platforms) using two- or four-channel chemistry for base calling. They have not been validated with systems using one-channel chemistry.

II. List of Components

Name	Cat. No.	UDIs	Volume/well	Concentration
Unique Dual Index Kit (1–96)	634752	U001–U096	5 µl	6.25 µM/oligo 12.5 µM/oligo pair
Unique Dual Index Kit (97–192)	634753	U097–U192	5 µl	6.25 µM/oligo 12.5 µM/oligo pair
Unique Dual Index Kit (193–288)	634754	U193–U288	5 µl	6.25 µM/oligo 12.5 µM/oligo pair
Unique Dual Index Kit (289–384)	634755	U289–U384	5 µl	6.25 µM/oligo 12.5 µM/oligo pair
Unique Dual Index Kit (1–24)*	634756	U001–U024	5 µl	6.25 µM/oligo 12.5 µM/oligo pair

*The indexes in Unique Dual Index Kit (1–24) are a subset of Unique Dual Index Kit (1–96) (Cat. No. 634752)

III. Index Sequences

The UDIs are 8-nt long i5 and i7 dual index sequences. UDIs 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).

UDIs U097–U384 have been developed and validated by Takara Bio USA, Inc. and use the same adapter as IDT for Illumina-TruSeq DNA and RNA UD Indexes.

MS-Excel files (XLSX) containing a full list of these indexes and corresponding plate maps can be downloaded from takarabio.com.

- Unique Dual Index Kit (1-96) Indexes and Plate Map
- Unique Dual Index Kit (97-192) Indexes and Plate Map
- Unique Dual Index Kit (193-288) Indexes and Plate Map
- Unique Dual Index Kit (289-384) Indexes and Plate Map
- Unique Dual Index Kit (1-24) Indexes and Plate Map

For Illumina sequencing, enter indexes from the downloaded CSV file. For UDIs U001–U096 you may use 'IDT-ILMN TruSeq DNA UD Indexes (96 indexes)' from the dropdown menu of Illumina Experiment Sheet. UDIs U097–U384 may only be entered from the downloaded CSV file.

NOTE: Do NOT select 'IDT-ILMN TruSeq DNA UD Indexes v2 - 96 Indexes' from the dropdown.

IV. General Considerations

A. Best Practices

- We do not recommend subjecting the UDI kits to more than four freeze/thaw cycles.
- Prior to use, remove PCR plates containing the desired UDIs from the freezer and bring to the benchtop. Let the plates thaw at room temperature for 10 min.
- **IMPORTANT:** After thawing, spin the UDI plate(s) in a tabletop centrifuge to bring the contents to the bottom of the wells prior to dispensing.

B. Multiplexing and Index Pooling

It is important to select appropriate indexes that are unique and meet the Illumina-recommended compatibility and color balance requirements. UDIs should be chosen from a minimum number of columns to achieve greater color balance.

1. Low plexity (2–8 samples)

For low-plex pooling involving between two and eight samples per sequencing run, follow the guidelines in Illumina's Index Adapters Pooling Guide (Illumina, Document #1000000041074-v8).

- As shown in Figure 1, below, for Unique Dual Index Kit (1–96) or (1–24) (Cat. Nos. 634752 and 634756), pool libraries of plexity 2–8 down a column. Do NOT pool libraries across a row. For example: for a plexity of two, use U001–U002 (green box); for a plexity of three, use U001–U003 (blue box); and so on.
- Any combination of UDIs from the same column is acceptable. For example, for a plexity of two, U001–U002 or U007–U008 is valid. For a plexity of 4, U001–U004 or U005–U008 is valid.

	1	2	3	4	5	6	7	8	9	10	11	12
A	U001	U009	U017	U025	U033	U041	U049	U057	U065	U073	U081	U089
B	U002	U010	U018	U026	U034	U042	U050	U058	U066	U074	U082	U090
C	U003	U011	U019	U027	U035	U043	U051	U059	U067	U075	U083	U091
D	U004	U012	U020	U028	U036	U044	U052	U060	U068	U076	U084	U092
E	U005	U013	U021	U029	U037	U045	U053	U061	U069	U077	U085	U093
F	U006	U014	U022	U030	U038	U046	U054	U062	U070	U078	U086	U094
G	U007	U015	U023	U031	U039	U047	U055	U063	U071	U079	U087	U095
H	U008	U016	U024	U032	U040	U048	U056	U064	U072	U080	U088	U096

Figure 1. Index map and multiplexing strategy for Unique Dual Index Kit (1–96). (Cat. No. 634752) For multiplexing libraries of plexity ≥2, we recommend pooling indexes down a column (as shown in the example given in the colored boxes). Do **not** pool libraries across rows if processing less than eight samples per sequencing run.

2. Higher plexity (>8 samples)

If pooling more than eight samples, use UDIs from multiple columns. For example, for a plexity of 9, U001–U009 or U001–U004 + U009–U013 is valid.

- When using Unique Dual Index Kits (97–192), (193–288), and (289–384) (Cat. Nos. 634753, 634754, and 634755), pool at least two full columns per sequencing lane, as shown in Figure 2.

  NOTE: A minimum of 16 libraries should be pooled with these kits.

	1	2	3	4	5	6	7	8	9	10	11	12
A	U097	U105	U113	U121	U129	U137	U145	U153	U161	U169	U177	U185
B	U098	U106	U114	U122	U130	U138	U146	U154	U162	U170	U178	U186
C	U099	U107	U115	U123	U131	U139	U147	U155	U163	U171	U179	U187
D	U100	U108	U116	U124	U132	U140	U148	U156	U164	U172	U180	U188
E	U101	U109	U117	U125	U133	U141	U149	U157	U165	U173	U181	U189
F	U102	U110	U118	U126	U134	U142	U150	U158	U166	U174	U182	U190
G	U103	U111	U119	U127	U135	U143	U151	U159	U167	U175	U183	U191
H	U104	U112	U120	U128	U136	U144	U152	U160	U168	U176	U184	U192

Figure 2. Multiplexing strategy for Unique Dual Index with UDIs 97–384. (Cat. Nos. 634753, 634754, and 634755) When multiplexing libraries using these kits, any two columns may be pooled together (example given using Unique Dual Index Kit (97–192) with shaded red indexes). Do not pool <16 libraries using these index sets.

C. Product Compatibility

The UDI kits are designed for use with the Takara Bio RNA-seq and DNA-seq kits listed below. Please use the recommended volume as indicated in the table (Volume of UDI primers) for the kit of interest.

- If the protocol calls for 1 µl *each* of forward and reverse indexing primer, use 2 µl (total) of the UDI primers
- If the protocol calls for 2 µl of forward and reverse indexing primer, use 4 µl of the UDI primers

Table 1. UDI volumes for Takara Bio RNA-seq kits (Total RNA-seq on next page)

Immune profiling kit	Cat. Nos.	Volume of UDI primers
SMART-Seq® Human BCR (with UMIs)	634777, 634778, 634776	2 µl
SMART-Seq Human TCR (with UMIs)	634780, 634781, 634779	2 µl
SMART-Seq Mouse TCR (with UMIs)	634814, 634815, 634816	2 µl
SMART-Seq Mouse BCR (with UMIs)	634351, 634352, 634353	2 µl
SMARTer® Human TCR a/b Profiling Kit v2	634478, 634479	2 µl

mRNA-seq kit	Cat. Nos.	Volume of UDI primers
SMART-Seq mRNA LP (with UMIs)	634762, 634765, 634766	2 µl
SMART-Seq mRNA LP	634768, 634769, 634771	2 µl
SMART-Seq mRNA Single Cell LP	634786, 634787, 634788	2 µl
SMART-Seq mRNA HT LP	634792, 634793, 634794	2 µl
SMART-Seq Single Cell PLUS Kit	R400750, R400751	2 µl
SMART-Seq v4 PLUS Kit	R400752, R400753	2 µl
SMART-Seq HT PLUS Kit	R400748, R400749	2 µl

Total RNA-seq	Cat. Nos.	Volume of UDI primers
SMART-Seq Total RNA Pico Input with UMIs (ZapR Mammalian)	634354, 634355, 634356	2 µl
SMART-Seq Total RNA Pico Input (ZapR Mammalian)	634357, 634358, 634359	2 µl
SMART-Seq Total RNA Single Cell (ZapR Mammalian)	634360, 634361, 634362	2 µl
SMART-Seq Total RNA High Input (RiboGone Mammalian)	635045, 635046, 635047	2 µl
SMART-Seq Total RNA Mid Input	635048, 635049, 635050	2 µl
SMART-Seq Stranded Kit	634442, 634443, 634444	2 µl
SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian	634485, 634486, 634487, 634488	2 µl
SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian	634411, 634412, 634113, 634414	2 µl
SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian Components	634417, 634418, 634419	2 µl
SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian	635005, 635006, 635007	2 µl
SMARTer Stranded Total RNA Sample Prep Kit - Low Input Mammalian	634861	2 µl
SMARTer Stranded RNA-Seq Kit	634836, 634837, 634838, 634839	2 µl
SMARTer Stranded RNA-Seq Kit HT	634862	2 µl
SMARTer Stranded Total RNA Sample Prep Kit - HI Mammalian	634873, 634874, 634875, 634876, 634877, 634878	2 µl
SMARTer smRNA-Seq Kit for Illumina	635029, 635030, 635031	4 µl

Table 2. UDI volumes for Takara Bio DNA-seq kits

DNA-seq kit	Cat. Nos.	Volume of UDI primers
PicoPLEX® Gold Single Cell DNA-seq Kit	R300669, R300670, R300698	2 µl
ThruPLEX® DNA-Seq Kit	R400674, R400675, R400676, R400677	2 µl
ThruPLEX Plasma-Seq Kit	R400679, R400680, R400681, R400682	2 µl
DNA SMART™ ChIP-Seq kits	634865, 634866, 634867	4 µl
EpiXplore™ Meth-Seq DNA Enrichment Kit	635023	4 µl

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