



LIBRARY PREPARATION PROTOCOL

Enzymatic Fragmentation and Twist Universal Adapter System

For use with the Twist NGS Workflow

This Twist Library Preparation Kit provides the reagents needed to prepare genomic DNA (gDNA) libraries using enzymatic gDNA fragmentation and the Twist Universal Adapter System. The Twist Universal Adapter system consists of Twist Universal Adapters and Twist Unique Dual Indexed (UDI) Primers. This kit is also compatible with Twist HT Universal Adapter System, which consists of Twist Universal Adapters and Twist HT UDI Primers. This manual details the steps for generating the amplified, indexed libraries needed for downstream target enrichment and sequencing on Illumina next-generation sequencing (NGS) systems. This library preparation protocol is optimized for use with Twist Target Enrichment Kits and should only be performed with reagents specified or their equivalents.



Twist NGS workflow. The complete NGS workflow takes you from sample preparation to NGS sequencing and data analysis. A component of this workflow, the Twist Library Preparation Protocol works in conjunction with the other component protocols.

PROTOCOL COMPONENTS

Please read the product packaging and storage recommendations carefully for each kit and store components as recommended immediately upon arrival.

CATALOG #	NAME	DESCRIPTION	STORAGE
104174: 16 rxn 104175: 96 rxn	Twist Library Preparation EF Kit with Amp Mix	Reagents for library construction	—
	Twist Library Preparation EF Kit 1	· 5x Fragmentation Enzyme · 10x Fragmentation Buffer · DNA Ligation Mix · DNA Ligation Buffer · Amplification Primers, ILMN (Tubes 100220, 100583 are not required when used with universal adapters)	–20°C
	Twist Library Preparation Kit 2	DNA Purification Beads	2–8°C
	Equinox Library Amp Mix	Equinox Library Amp Mix (2x)	–20°C
101307: 16 rxn 101308, 101309, 101310, 101311: 96 rxn	Twist Universal Adapter System - TruSeq Compatible	Twist Universal Adapters and Twist UDI Primers, provides unique dual-indexed combinations with 1 reaction per index pair	–20°C
106390-106393, 106396, 106398-106400: 384 rxn, 96-well plate	Twist HT Universal Adapter System - TruSeq Compatible	Twist Universal Adapters and Twist HT UDI Primers, provide dual-indexed combinations with 1 reaction per index pair	—
106401-106408: 384 rxn, 384-well plate			

This product is for **research use only**.



DON'T SETTLE FOR LESS IN TARGETED SEQUENCING.

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INTENDED USE

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MATERIALS SUPPLIED BY USER

The following materials or their equivalent are required to generate libraries using the Twist Library Preparation Kit with Enzymatic Fragmentation and Twist Universal Adapter System or Twist HT Universal Adapter System.

PRODUCT	SUGGESTED SUPPLIER
REAGENTS AND CONSUMABLES	
Ethanol (200 proof)	—
Molecular biology grade water	—
10 mM Tris-HCl pH 8	—
Buffer EB	Qiagen
1.5-ml microcentrifuge tubes	VWR
Thin-walled PCR 0.2-ml strip-tubes	Eppendorf
96-well thermal cycling plates	VWR
1.5-ml compatible magnetic stand	Beckman Coulter
96-well compatible magnetic plate	Alpaqua, Permagen Labware
Qubit dsDNA Broad Range Quantitation Assay	Thermo Fisher Scientific
Agilent DNA 7500 Kit	Agilent Technologies
EQUIPMENT	
Pipettes and tips	—
Vortex mixer	—
Benchtop mini centrifuge for 0.2-ml tubes	—
Thermomixer for 1.5-ml tubes	Eppendorf
Thermal cycler (96 well) with heated lid	—
Fluorometer (Qubit 3.0)	Thermo Fisher Scientific
2100 Bioanalyzer	Agilent Technologies



GENERAL NOTES AND PRECAUTIONS

Wear appropriate protective equipment (lab coat, gloves, and protective glasses or goggles) at all times when performing this protocol.

For best results, read this document before performing the protocol, and follow the instructions provided. Twist cannot guarantee the performance of the Twist Library Preparation Kit using Enzymatic Fragmentation with either the Twist Universal Adapter System or the Twist HT Universal Adapter System if modifications are made to the protocol.

The Twist HT Universal Adapter System can be directly substituted for the Twist Universal Adapter System without any protocol modification. In all instances where UDI Primers are mentioned in this protocol, the HT UDI Primers can be used instead.

This library preparation method may yield more material than needed for target enrichment. Excess product can be stored at -20°C for later use.

Do NOT mix or combine the same reagents from the different lots.

Test the compatibility of your thermal cycler and PCR tubes by incubating at 95°C for up to 5 minutes to ensure the PCR tubes do not crack under heat and pressure. Adjust the tightness of the thermal cycler lid and/or use a spacer specific to the thermal cycler model.

GUIDELINES FOR gDNA SAMPLES

- Input DNA should be suspended in Molecular Biology Grade Water, 10 mM Tris-HCl pH 8.0, or Buffer EB.
- It is important to remove all cations and chelators from the starting gDNA sample. The presence of cations and chelators may affect the initial fragmentation reaction.
- For genomic (gDNA) samples, correct input quantity is critical for achieving optimal yield and library fragment length.
- The recommended DNA input is 50 ng of purified gDNA.
- Using higher or lower mass input may require optimization of the following steps in library preparation to achieve optimal performance.
 - Incubation time for fragmentation at 32°C (Step 1.1, page 7)
 - Amount of Twist Universal Adapter (Step 2.1, page 9)
 - Incubation time for ligation reaction (Step 2.5, page 10)
- Use the Thermo Fisher Scientific Qubit dsDNA Broad Range Quantitation Assay to accurately quantify input purified gDNA.
- Measuring DNA concentration by absorbance at 260 nm is not recommended.
- For technical support, contact customersupport@twistbioscience.com.



PROTOCOL OVERVIEW

This protocol begins with genomic DNA (gDNA) and generates amplified, indexed libraries for subsequent target enrichment. It features enzymatic fragmentation and Twist Universal Adapters with UDI or HT UDI Primers. This protocol allows you to perform gDNA library preparation (Steps 1–3) in 3 hours.

ENZYMATIC FRAGMENTATION WITH UNIVERSAL ADAPTERS AND UDI PRIMERS (GENOMIC DNA, 50 NG STARTING DNA MATERIAL)		TIME
STEP 1	Perform DNA Fragmentation, End Repair, and dA-tailing dA-tailed DNA fragments	1 hour
STEP 2	Ligate Twist Universal Adapters and Purify gDNA libraries ready for indexing	1 hour
STEP 3	PCR Amplify Using Twist UDI Primers, Purify, and Perform QC Amplified indexed libraries	1 hour



STEP 1 PERFORM DNA FRAGMENTATION, END REPAIR, AND dA-TAILING

Perform enzymatic fragmentation of input gDNA and subsequent end repair and dA-tailing to generate dA-tailed DNA fragments.

Reagents Required

- Genomic DNA (gDNA): 50 ng per sample
- Molecular biology grade water (chilled)
- Optional 10 mM Tris-HCl pH 8 or Buffer EB
- Qubit dsDNA Broad Range Quantitation Assay (or equivalent)
- From the Twist Library Preparation EF Kit 1:
 - 5x Fragmentation Enzyme
 - 10x Fragmentation Buffer

Before You Begin

- Thaw 5x Fragmentation Enzyme and gDNA samples on ice, then mix by flicking the tube with a finger.
- Thaw 10x Fragmentation Buffer on ice, then mix by pulse vortexing for 2 seconds.

PREPARE THE THERMAL CYCLER, SAMPLES, AND REAGENTS

- 1.1** Program the thermal cycler with the following conditions. Set the temperature of the heated lid to 70°C. Start the program to pre-chill the thermal cycler.

	TEMPERATURE	TIME
STEP 1	4°C	HOLD
STEP 2	32°C	22 minutes (adjustment may be required based upon DNA mass input and/or quality)
STEP 3	65°C	30 minutes
STEP 4	4°C	HOLD

- 1.2** Use the Qubit dsDNA Broad Range Quantitation Assay to determine the concentration of your gDNA samples.

- 1.3** Dilute the gDNA samples to 5 ng/μl with water, 10 mM Tris-HCl pH 8, or Buffer EB. Mix well with gentle pipetting.

- 1.4** Add 10 μl of each diluted gDNA sample (50 ng total gDNA) into a thin-walled PCR 0.2-ml strip-tube or well of a 96-well thermal cycling plate, and place on ice.

- 1.5** Pulse-spin to ensure all of the solution is at the bottom of the tube.

**PERFORM FRAGMENTATION, END REPAIR, AND dA-TAILING**

- 1.6** Prepare an enzymatic fragmentation master mix in a 1.5-ml microcentrifuge tube on ice. Use the volumes listed below. Mix thoroughly by gentle pipetting.

REAGENT	VOLUME PER REACTION*
Water (chilled)	25 µl
10x Fragmentation Buffer	5 µl
5x Fragmentation Enzyme	10 µl
Total	40 µl

**Prepare a master mix for multiple reactions.*

- 1.7** Add 40 µl enzymatic fragmentation master mix (from Step 1.6) to each 10 µl gDNA sample well or tube and mix well by gentle pipetting. Cap the tube and keep the reaction on ice.

- 1.8** Pulse-spin the sample plate or tubes and immediately transfer to the pre-chilled thermal cycler.

- 1.9** Proceed to steps 2–4 of the thermal cycler program (32°C step of the thermocycler program in Step 1.1 above).

NOTE: While the thermal cycler program is running, prepare the reagents for Step 2: Ligate Twist Universal Adapters and Purify (see Before You Begin).

- 1.10** When the thermal cycler program is complete and the sample block has returned to 4°C, remove the samples from the block and place on ice.

PROCEED IMMEDIATELY TO STEP 2: LIGATE TWIST UNIVERSAL ADAPTERS AND PURIFY



STEP 2

LIGATE TWIST UNIVERSAL ADAPTERS AND PURIFY

Ligate Twist Universal Adapters to the dA-tailed DNA fragments from Step 1 and purify to generate gDNA libraries ready for index introduction through amplification in Step 3.

Reagents Required

- dA-tailed DNA fragments (from Step 1.10)
- Ethanol
- Molecular biology grade water (chilled)
- Optional 10 mM Tris-HCl pH 8 or Buffer EB
- From the Twist Library Preparation EF Kit 1:
 - DNA Ligation Mix
 - DNA Ligation Buffer
- From the Twist (HT) Universal Adapter System:
 - Twist Universal Adapters
- From the Twist Library Preparation Kit 2:
 - DNA Purification Beads

Before You Begin

- Thaw on ice:
 - Twist Universal Adapters (tube; utilized for all samples)
 - DNA Ligation Mix
 - DNA Ligation Buffer
- Prepare 1 ml 80% ethanol for each sample (for use in Steps 2 and 3 of the protocol).
- Equilibrate DNA Purification Beads to room temperature for at least 30 minutes (for use in Steps 2 and 3 of the protocol).
- Program a thermal cycler to incubate the samples at 20°C with the heated lid set to minimum temperature or turned off. Start the program so that the cycler is at 20°C when the samples are prepared.

LIGATE TWIST UNIVERSAL ADAPTERS

2.1 Add 5 µl Twist Universal Adapters into each sample well or tube containing the dA-tailed DNA fragments for Step 1. Mix gently by pipetting and keep on ice.


2.2 Prepare the ligation master mix in a 1.5-ml microcentrifuge tube on ice as indicated below. Mix well by gentle pipetting.

REAGENT	VOLUME PER REACTION*
Water (chilled)	15 µl
DNA Ligation Buffer	20 µl
DNA Ligation Mix	10 µl
Total	45 µl

**Prepare a master mix for multiple reactions.*



- 2.3** Add 45 μ l of the ligation master mix to the sample from Step 2.1 and mix well by gentle pipetting.
- 2.4** Seal or cap the tubes and pulse-spin to ensure all solution is at the bottom of the tube.
- 2.5** Incubate the ligation reaction at 20°C for 15 minutes in the thermal cycler, then move the samples to the bench top. Proceed to the Purify step.

 **IMPORTANT:** Turn off the heated lid or set to minimum temperature.

NOTE: While the thermal cycler program is running, prepare the reagents for Step 3 (see Step 3: PCR Amplify Using Twist UDI Primers, Purify, and Perform QC).

PURIFY

- 2.6** Vortex the pre-equilibrated DNA Purification Beads until well mixed.
- 2.7** Add 80 μ l (0.8x) of homogenized DNA Purification Beads to each ligation sample from Step 2.5. Mix well by vortexing.
- 2.8** Incubate the samples for 5 minutes at room temperature.
- 2.9** Place the samples on a magnetic plate for 1 minute or until the supernatant is clear.
- 2.10** The DNA Purification Beads form a pellet, leaving a clear supernatant. Without removing plate or tubes from the magnetic plate, remove and discard the supernatant.
- 2.11** Wash the bead pellet by gently adding 200 μ l freshly prepared 80% ethanol (do not disturb the pellet). Incubate for 1 minute, then remove and discard the ethanol.
- 2.12** Repeat the wash once, for a total of two washes, while keeping the samples on the magnetic plate.
- 2.13** Carefully remove all remaining ethanol with a 10- μ l pipet, making sure not to disturb the bead pellet.
- NOTE:** Before pipetting, the bead pellet may be briefly spun to collect ethanol at the bottom of the plate or tube and returned to the magnetic plate.
- 2.14** Air-dry the bead pellet on the magnetic plate for 5 minutes or until the bead pellet is dry. Do not overdry the bead pellet.
- 2.15** Remove the plate or tubes from the magnetic plate and add 17 μ l water, 10 mM Tris-HCl pH 8, or Buffer EB to each sample. Mix by pipetting until homogenized.
- 2.16** Incubate at room temperature for 2 minutes.
- 2.17** Place the plate or tubes on a magnetic plate and let stand for 3 minutes or until the beads form a pellet.
- 2.18** Transfer 15 μ l of the clear supernatant containing the ligated and indexed libraries to a clean thin-walled PCR 0.2-ml strip-tube or well of a 96-well thermal cycling plate, making sure not to disturb the bead pellet.

STEP 3

PCR AMPLIFY USING TWIST UDI PRIMERS, PURIFY, AND PERFORM QC

Amplify the adapted gDNA libraries with Twist UDI or Twist HT UDI Primers, purify them, and perform quality control (QC) analysis to complete the protocol.

Reagents Required

- Ligated, adapted libraries (from Step 2.18)
- 80% Ethanol (from Step 2)
- Equilibrated DNA Purification Beads (from Step 2)
- Molecular biology grade water
- Optional 10 mM Tris-HCl pH 8 or Buffer EB
- Equinox Library Amp Mix (2x)
- If using the Twist Universal Adapter System:
 - Twist UDI Primers
- If using the Twist HT Universal Adapter System:
 - Twist HT UDI Primers

⚠ IMPORTANT: Use of Amplification Primers, ILMN tubes 100220, 100583 contained in the Twist Library Preparation EF Kit 1 are not required. Using these primers with the Twist (HT) Universal Adapter System will result in a failed PCR amplification.

Before You Begin

Thaw on ice:

- Twist UDI or Twist HT UDI Primers (plate with single use primers)
- Equinox Library Amp Mix (2x)

PREPARE THE THERMAL CYCLER AND PCR MIX

3.1

Program a thermal cycler with the following conditions. Set the temperature of the heated lid to 105°C.

STEP	TEMPERATURE	TIME	NUMBER CYCLES
1 Initialization	98°C	45 seconds	1
2 Denaturation Annealing Extension	98°C	15 seconds	6–8*
	60°C	30 seconds	
	72°C	30 seconds	
3 Final Extension	72°C	1 minute	1
4 Final Hold	4°C	HOLD	—

*6–8 cycles is recommended when starting with 50–100 ng of high quality gDNA.

**PERFORM PCR**

- 3.2** Add 10 µl of Twist UDI or Twist HT UDI Primer from the provided 96-well or 384-well plate to each of the gDNA libraries from Step 2.18 and mix well by gentle pipetting.
- NOTE:** For index selection and multiplexing refer to the pooling guidelines in Appendix A or B depending on the adapter system being used.
- 3.3** Add 25 µl of Equinox Library Amp Mix (2x) to the gDNA libraries from Step 3.2 and mix well by gentle pipetting.
- 3.4** Pulse-spin sample plate or tube and immediately transfer to the thermal cycler. Start the program.
- 3.5** Remove the sample(s) from the block when the thermal cycler program is complete. Proceed to purification.

PURIFY

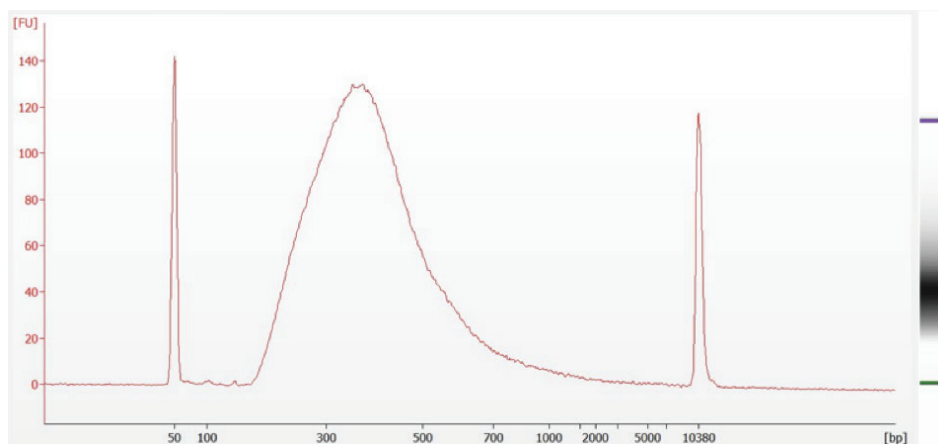
- 3.6** Vortex the pre-equilibrated DNA Purification Beads until mixed.
- 3.7** Add 50 µl (1x) of homogenized DNA Purification Beads to each ligation sample from Step 3.5. Mix well by vortexing.
- 3.8** Incubate the samples for 5 minutes at room temperature.
- 3.9** Place the samples on a magnetic plate for 1 minute.
- 3.10** The DNA Purification Beads form a pellet, leaving a clear supernatant. Without removing plate or tubes from the magnetic plate, remove and discard the supernatant.
- 3.11** Wash the bead pellet by gently adding 200 µl freshly prepared 80% ethanol (do not disturb the pellet), incubate for 1 minute, then remove and discard the ethanol.
- 3.12** Repeat this wash once, for a total of two washes, while keeping the samples on the magnetic plate.
- 3.13** Carefully remove all remaining ethanol with a 10-µl pipet, making sure not to disturb the bead pellet.
- NOTE:** Before pipetting, the bead pellet may be briefly spun to collect ethanol at the bottom of the plate or tube and returned to the magnetic plate.
- 3.14** Air-dry the bead pellet on the magnetic plate for 5 minutes or until the bead pellet is dry. Do not overdry the bead pellet.
- 3.15** Remove the plate or tubes from the magnetic plate and add 22 µl water, 10 mM Tris-HCl pH 8, or Buffer EB to each sample. Mix by pipetting until homogenized.
- 3.16** Incubate at room temperature for 2 minutes.

3.17 Place the plate or tubes on a magnetic plate and let stand for 3 minutes or until the beads form a pellet.

3.18 Transfer 20 μL of the clear supernatant containing the Amplified Indexed Libraries to a clean thin-walled PCR 0.2-ml strip-tube or well of a 96-well thermal cycling plate, making sure not to disturb the bead pellet.

PERFORM QC

3.19 Quantify and validate the size range of each library using the Thermo Fisher Scientific Qubit dsDNA Broad Range Quantitation Assay and Agilent DNA 7500 Assay. Final concentration values should be $\geq 80 \text{ ng}/\mu\text{L}$, and average fragment length should be 375–425 bp using a range setting of 150–1000 bp.



Electropherogram generated by an Agilent 7500 DNA analysis of gDNA library samples that were prepared as described. Note the single prominent peak at ~400 bp.

NOTES: If the average fragment length is not in the range of 375–425 bp:

- Input DNA concentration may be inaccurate. Typically, using too much DNA leads to shorter fragments, while not using enough DNA leads to larger fragments.
- The presence of cations and chelators may also affect the average fragment length.
- If neither of the above factors apply, optimize the 32°C fragmentation in Step 1.1 by changing the time in 3 minute increments—increase time to produce shorter fragments, and decrease time to produce longer fragments.

You can proceed with concentrations lower than 80 $\text{ng}/\mu\text{L}$, but low concentrations may reflect inefficient sample preparation and can result in low library diversity after hybridization.

STOPPING POINT: If not proceeding immediately to a Twist Target Enrichment System, store the amplified indexed libraries at -20°C .

END OF WORKFLOW



APPENDIX A: UDI ADAPTER SEQUENCES AND POOLING GUIDELINES

UDI SEQUENCES

For a complete guide of the Twist UDI sequences used in the Twist Universal Adapter System, please refer to the UDI Sequences Reference Spreadsheet and the Sample Sheet Template. All files are available for download at [twistbioscience.com/resources/protocol/unique-dual-index-sequences-protocol-reference-document-spreadsheet-and-sample](https://www.twistbioscience.com/resources/protocol/unique-dual-index-sequences-protocol-reference-document-spreadsheet-and-sample).

POOLING GUIDELINES

Twist UDI Primers are base balanced for next generation sequencing on a column basis. When pooling unique dual-indexed libraries for 8-plex hybridization, it is recommended that libraries be selected from a single column. Multiple columns may be selected in any desired combination across a single plate or multiple plates for sequencing.

Table 1. Twist UDI Primer plate layouts and pooling guidelines.

Twist Universal Adapter System: TruSeq Compatible, 16 Samples (101307)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9										
B	2	10										
C	3	11										
D	4	12										
E	5	13										
F	6	14										
G	7	15										
H	8	16										

*PLEASE NOTE: The indexes in the 16 sample plate are not the same in 96 samples, Plate A.



APPENDIX A: UDI ADAPTER SEQUENCES AND POOLING GUIDELINES

Twist Universal Adapter System: TruSeq Compatible, 96 Samples, Plates A to D (101308, 101309, 101310, 101311)

Plate A.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

Plate B.

	1	2	3	4	5	6	7	8	9	10	11	12
A	97	105	113	121	129	137	145	153	161	169	177	185
B	98	106	114	122	130	138	146	154	162	170	178	186
C	99	107	115	123	131	139	147	155	163	171	179	187
D	100	108	116	124	132	140	148	156	164	172	180	188
E	101	109	117	125	133	141	149	157	165	173	181	189
F	102	110	118	126	134	142	150	158	166	174	182	190
G	103	111	119	127	135	143	151	159	167	175	183	191
H	104	112	120	128	136	144	152	160	168	176	184	192

Plate C.

	1	2	3	4	5	6	7	8	9	10	11	12
A	193	201	209	217	225	233	241	249	257	265	273	281
B	194	202	210	218	226	234	242	250	258	266	274	282
C	195	203	211	219	227	235	243	251	259	267	275	283
D	196	204	212	220	228	236	244	252	260	268	276	284
E	197	205	213	221	229	237	245	253	261	269	277	285
F	198	206	214	222	230	238	246	254	262	270	278	286
G	199	207	215	223	231	239	247	255	263	271	279	287
H	200	208	216	224	232	240	248	256	264	272	280	288

Plate D.

	1	2	3	4	5	6	7	8	9	10	11	12
A	289	297	305	313	321	329	337	345	353	361	369	377
B	290	298	306	314	322	330	338	346	354	362	370	378
C	291	299	307	315	323	331	339	347	355	363	371	379
D	292	300	308	316	324	332	340	348	356	364	372	380
E	293	301	309	317	325	333	341	349	357	365	373	381
F	294	302	310	318	326	334	342	350	358	366	374	382
G	295	303	311	319	327	335	343	351	359	367	375	383
H	296	304	312	320	328	336	344	352	360	368	376	384



APPENDIX B: HT UDI ADAPTER SEQUENCES AND POOLING GUIDELINES

HT UDI SEQUENCES

The Twist HT UDI Primers in the Twist HT Universal Adapter System contain indexes of 12 nucleotides that are distinct from the Twist UDI Primers in the Twist Universal Adapter System. All 3,072 indexes are available in either 96-well or 384-well plate format. Representative plate layouts are below.

CAUTION: Sequence only one plate configuration at a time because the 96-well and 384-well plate formats both use the same 3,072 indexes.

For a complete guide of the Twist HT UDI sequences used in the Twist HT Universal Adapter System, please refer to the HT UDI Sequences Reference Spreadsheets and HT UDI Sample Sheet Templates. These files are available for download here: twistbioscience.com/resources/protocol/high-throughput-unique-dual-index-sequences-datafiles-reference-spreadsheet-and-sample

POOLING GUIDELINES

The Twist HT UDI Primers are base balanced for next-generation sequencing on a column basis. When pooling unique dual-indexed libraries for multiplex hybridization, it is recommended that libraries be selected from a single column. Multiple columns may be selected in any desired combination across a single plate or multiple plates for sequencing.

Table 2: Representative 96-Well Plate Layouts

Twist HT Universal Adapter System: TruSeq Compatible, 96-Well, Plates AAA01-AAA16 and BBB01-BBB16 (106390-106393, 106396, 106398-106400)

Plate 96W-AAA01

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
B	13	14	15	16	17	18	19	20	21	22	23	24
C	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
E	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
H	85	86	87	88	89	90	91	92	93	94	95	96

Plate 96W-AAA02

	1	2	3	4	5	6	7	8	9	10	11	12
A	97	98	99	100	101	102	103	104	105	106	107	108
B	109	110	111	112	113	114	115	116	117	118	119	120
C	121	122	123	124	125	126	127	128	129	130	131	132
D	133	134	135	136	137	138	139	140	141	142	143	144
E	145	146	147	148	149	150	151	152	153	154	155	156
F	157	158	159	160	161	162	163	164	165	166	167	168
G	169	170	171	172	173	174	175	176	177	178	179	180
H	181	182	183	184	185	186	187	188	189	190	191	192



Plate 96W-AAA03

	1	2	3	4	5	6	7	8	9	10	11	12
A	193	194	195	196	197	198	199	200	201	202	203	204
B	205	206	207	208	209	210	211	212	213	214	215	216
C	217	218	219	220	221	222	223	224	225	226	227	228
D	229	230	231	232	233	234	235	236	237	238	239	240
E	241	242	243	244	245	246	247	248	249	250	251	252
F	253	254	255	256	257	258	259	260	261	262	263	264
G	265	266	267	268	269	270	271	272	273	274	275	276
H	277	278	279	280	281	282	283	284	285	286	287	288

Plate 96W-AAA04

	1	2	3	4	5	6	7	8	9	10	11	12
A	289	290	291	292	293	294	295	296	297	298	299	300
B	301	302	303	304	305	306	307	308	309	310	311	312
C	313	314	315	316	317	318	319	320	321	322	323	324
D	325	326	327	328	329	330	331	332	333	334	335	336
E	337	338	339	340	341	342	343	344	345	346	347	348
F	349	350	351	352	353	354	355	356	357	358	359	360
G	361	362	363	364	365	366	367	368	369	370	371	372
H	373	374	375	376	377	378	379	380	381	382	383	384

Table 3: Representative 384-Well Plate Layout

Twist HT Universal Adapter System: TruSeq Compatible, 384-Well, Plates AAA01-AAA04 and BBB01-BBB04 (106401-106408)

Plate 384W-AAA01

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	97	2	98	3	99	4	100	5	101	6	102	7	103	8	104	9	105	10	106	11	107	12	108
B	193	289	194	290	195	291	196	292	197	293	198	294	199	295	200	296	201	297	202	298	203	299	204	300
C	13	109	14	110	15	111	16	112	17	113	18	114	19	115	20	116	21	117	22	118	23	119	24	120
D	205	301	206	302	207	303	208	304	209	305	210	306	211	307	212	308	213	309	214	310	215	311	216	312
E	25	121	26	122	27	123	28	124	29	125	30	126	31	127	32	128	33	129	34	130	35	131	36	132
F	217	313	218	314	219	315	220	316	221	317	222	318	223	319	224	320	225	321	226	322	227	323	228	324
G	37	133	38	134	39	135	40	136	41	137	42	138	43	139	44	140	45	141	46	142	47	143	48	144
H	229	325	230	326	231	327	232	328	233	329	234	330	235	331	236	332	237	333	238	334	239	335	240	336
I	49	145	50	146	51	147	52	148	53	149	54	150	55	151	56	152	57	153	58	154	59	155	60	156
J	241	337	242	338	243	339	244	340	245	341	246	342	247	343	248	344	249	345	250	346	251	347	252	348
K	61	157	62	158	63	159	64	160	65	161	66	162	67	163	68	164	69	165	70	166	71	167	72	168
L	253	349	254	350	255	351	256	352	257	353	258	354	259	355	260	356	261	357	262	358	263	359	264	360
M	73	169	74	170	75	171	76	172	77	173	78	174	79	175	80	176	81	177	82	178	83	179	84	180
N	265	361	266	362	267	363	268	364	269	365	270	366	271	367	272	368	273	369	274	370	275	371	276	372
O	85	181	86	182	87	183	88	184	89	185	90	186	91	187	92	188	93	189	94	190	95	191	96	192
P	277	373	278	374	279	375	280	376	281	377	282	378	283	379	284	380	285	381	286	382	287	383	288	384



END OF APPENDIX

LAST REVISED: February 21, 2023

REVISION	DATE	DESCRIPTION
5.0	Feb 21, 2023	• Updated for compatibility with Twist HT Universal Adapter System.
4.0	Mar 7, 2022	• Updated language for clarity in the Notes of step 3.19.
3.0	Sep 7, 2021	• General Notes and Precautions updated.
2.0	Apr 27, 2021	• Library Amplification Mix is now included with the Library Prep kit. • Kit catalog numbers, kit component list, and workflow steps are updated to include Library Amplification Mix. • Minor workflow steps updated for more clarity.