Twist TrueAmp Library Preparation Kit With Twist UMI Adapter System

For use with the Twist NGS Workflow

This Twist TrueAmp Library Preparation Kit provides the reagents needed to prepare genomic DNA (gDNA) libraries using enzymatic gDNA fragmentation and the Twist unique molecular identifiers (UMIs). The Twist UMI Adapter system consists of Twist UMI Adapters and Twist Unique Dual Indexed (UDI) Primers. This manual details the steps for generating the amplified, indexed libraries needed for downstream target enrichment and sequencing on Illumina's next-generation sequencing (NGS) systems. This library preparation protocol 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.*

For Research Use Only. *Not intended for use in diagnostic procedures.*

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
TWIST TRUEAMP LIBRARY PREPARATION KIT (REAGENTS FOR LIBRARY PREPARATION)			
116480: 16 rxn 116481: 96 rxn	Twist Library Preparation Kit 1	· 10X Twist Fragmentation Enzyme Mix · 5X Twist Fragmentation Buffer · 20X Twist DNA Ligation Mix · 4X Twist DNA Ligation Buffer	–20°C
	Twist Library Preparation Kit 2	DNA Purification Beads	2–8°C
	Twist TrueAmp Polymerase Mix	Twist TrueAmp Polymerase Mix	–20°C
TWIST ADAPTER SYSTEM			
105040: 16 rxn 105041, 105042, 105043, 105044: 96 rxn	Twist UMI Adapter System - TruSeq Compatible	Twist UMI Adapters and Twist UDI Primers provide UMI labeling and unique dual-indexed combinations with 1 reaction per index pair	–20°C

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MATERIALS SUPPLIED BY USER

The following materials or their equivalent are required to generate libraries using the Twist TrueAmp Library Preparation Kit and Twist UMI Adapter System.

PRODUCT	SUGGESTED SUPPLIER
REAGENTS AND CONSUMABLES	
Ethanol (200 proof)	—
Molecular biology grade water	—
10 mM Tris-HCl pH 8 (optional)	—
Buffer EB (optional)	Qiagen
1.5-ml microcentrifuge tubes	VWR
Thin-walled PCR 0.2-ml strip-tubes	Eppendorf
96-well thermal cycling plates	VWR
96-well compatible magnetic plate	Alpaqua, Permagen Labware
Qubit dsDNA Broad Range Quantification 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 TrueAmp Library Preparation Kit with the Twist UMI Adapter System if modifications are made to the protocol.

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

Test the compatibility of your thermal cycler and PCR tubes by incubating them 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

gDNA SAMPLES

- Use the Thermo Fisher Scientific Qubit dsDNA Broad Range Quantification Assay to accurately quantify input purified gDNA.
- Measuring DNA concentration by absorbance at 260 nm is not recommended.
- 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.
- The recommended DNA input is 50 ng of high-quality gDNA.
- Reagents are compatible with mass input of 1 ng to 500 ng, but may require optimization of the following step in library preparation to achieve optimal performance:
  - Incubation Time for Fragmentation (Step 1.1)
  - Amount of Twist UMI Adapter (Step 2.1)
  - Incubation Time for Ligation Reaction (Step 2.4)
  - PCR cycles for amplification (Step 3.1)

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 UMI Adapters with UDI Primers. This protocol allows you to perform gDNA library preparation in 3 hours.

ENZYMATIC FRAGMENTATION WITH UMI ADAPTERS AND UDI PRIMERS (GENOMIC DNA 50 NG STARTING MATERIAL)	**TIME**
STEP 1 **Perform DNA Fragmentation, End Repair, and dA-Tailing** dA-Tailed gDNA Fragments	**1 hour**
STEP 2 **Ligate Twist UMI Adapters and Purify** gDNA library pools 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
- Qubit dsDNA Broad Range Quantification Assay (or equivalent)
- From Twist Library Preparation Kit 1:
  - 10X Twist Fragmentation Enzyme Mix
  - 5X Twist Fragmentation Buffer

Before You Begin

- Thaw or place on ice:
  - Molecular biology grade water
  - gDNA
  - 10X Twist Fragmentation Enzyme Mix
  - 5X Twist Fragmentation Buffer

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 105°C.** Start the program to pre-chill the thermal cycler.

STEP	TEMPERATURE	TIME
1	4°C	HOLD
2	32°C	*Varies. Use table to the right and refer to Appendix B for fragmentation optimization.*
3	65°C	30 minutes
4	4°C	HOLD

INSERT SIZE	TIME
320-360 bp	5 minutes
280-320 bp	10 minutes
260-280 bp	15 minutes
220-260 bp	22 minutes
200-240 bp	30 minutes

1.2

Mix gDNA by flicking the tube with a finger. Use the Qubit dsDNA Broad Range Quantification Assay to determine the concentration of your gDNA samples.

NOTE: Measuring DNA concentration by absorbance at 260 nm is not recommended.

1.3 Bring 50 ng of each gDNA sample to a total volume of 35 µl with water, 10 mM Tris-HCl pH 8, or buffer EB. Mix well with gentle pipetting.
NOTE: If a mass input other than 50 ng is used, bring the target mass to a volume of 35 µl.

1.4 Add 35 µl of each diluted gDNA sample (50 ng total gDNA) into either a thin-walled PCR 0.2-ml strip-tube or a well of a 96-well thermal cycling plate.

1.5 Pulse-spin for 2 seconds to ensure all of the solution is at the bottom of the tube and place on ice.

PERFORM FRAGMENTATION, END REPAIR, AND DA-TAILING

1.6 Vortex the 5X Twist Fragmentation Buffer for 5 seconds. Pulse-spin for 2 seconds to collect all liquid at the bottom of the tube.

1.7 Invert 10X Twist Fragmentation Enzyme Mix a minimum of 10 times to homogenize or briefly vortex to ensure complete mixing. Pulse-spin for 2 seconds to collect all liquid at the bottom of the tube.

1.8 Prepare an enzymatic fragmentation mix in a 1.5-ml microcentrifuge tube on ice. Use the volumes listed below. Homogenize the master mix with moderate vortexing for 5 seconds or pipetting a minimum of half the total volume up and down 10 times (avoid formation of bubbles).

REAGENT	VOLUME PER REACTION*
5X Twist Fragmentation Buffer	10 µl
10X Twist Fragmentation Enzyme Mix	5 µl
Total	**15 µl**

**Prepare a master mix for multiple reactions.*

1.9 Add 15 µl enzymatic fragmentation mix (from Step 1.8) to each 35 µl gDNA sample tube or well. Homogenize with moderate vortexing for 5 seconds or by pipetting a minimum of half the total volume up and down 10 times (avoid formation of bubbles). Cap the tube(s) or seal the plate and keep the reaction on ice.
NOTE: Complete mixing is critical to achieve consistent fragment lengths.

1.10 Pulse-spin the sample plate or tube(s) for 2 seconds and immediately transfer it to the pre-chilled thermal cycler.

1.11 Initiate steps 2 to 4 of the thermal cycler program (refer to the table in Step 1.1).
NOTE: While the thermal cycler program is running, prepare the reagents for Step 2: Ligate Twist UMI Adapters and Purify (see Before You Begin).

1.12

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

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

STEP 2 LIGATE TWIST UMI ADAPTERS AND PURIFY

Ligate Twist UMI 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 1.12)
- Ethanol
- Molecular biology grade water
- 10 mM Tris-HCl pH 8 or Buffer EB (optional, for elution)
- From Twist Library Preparation Kit 1:
  - 4X Twist DNA Ligation Buffer
  - 20X Twist DNA Ligation Mix
- From Twist UMI Adapter System:
  - Twist UMI Adapters
- From Twist Library Preparation Kit 2:
  - DNA Purification Beads

Before You Begin

- Thaw or place on ice:
  - Twist UMI Adapters (tube; utilized for all samples)
  - 4X Twist DNA Ligation Buffer
  - 20X Twist DNA Ligation Mix
- Prepare 1 ml of 80% ethanol for each sample (for use in both Steps 2 and 3 of the protocol).
- Equilibrate DNA Purification Beads to room temperature for at least 30 minutes (for use in both Steps 2 and 3 of the protocol).
- Program a thermal cycler to incubate samples at 25°C with the heated lid set to a minimum temperature or turned off. Start the program so that the cycler has reached 25°C when the samples are done being prepared.

LIGATE TWIST UMI ADAPTERS

2.1

Prepare a ligation mix in a 1.5-ml microcentrifuge tube on ice. Use the volumes listed below. Homogenize the master mix by pipetting a minimum of half the total volume up and down 10 times (avoid formation of bubbles).

REAGENT	VOLUME PER REACTION*
4X Twist DNA Ligation Buffer	25 µl
20X Twist DNA Ligation Mix	5 µl
Water	17 µl
Total	**47 µl**

**Prepare a master mix for multiple reactions.*

2.2 Add 3 µl Twist UMI Adapters into each sample well or tube containing the dA-tailed DNA fragments from Step 1. Mix gently by pipetting and keep on ice.
NOTE: For mass inputs other than 50 ng, refer to Appendix B for guidance on adapter volume.

2.3 Add 47 µl of ligation mix from Step 2.1 to each sample. Pipette a minimum of half the total volume up and down 10 times to ensure complete mixing. Seal or cap the sample plate or tube(s) and pulse-spin for 2 seconds to ensure all solution is at the bottom of the tube.

2.4 Incubate the ligation reaction at 25°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 a minimum temperature.

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

PURIFY

2.5 Vortex the pre-equilibrated room-temperature DNA Purification Beads until well mixed.

2.6 Add 80 µl of homogenized (0.8X) DNA Purification Beads to each ligation sample from Step 2.4. Mix well by vortexing.

2.7 Incubate the samples for 5 minutes at room temperature.

2.8 Place the samples on a magnetic plate for 1 minute or until the supernatant is clear.

2.9 The DNA Purification Beads form a pellet, leaving a clear supernatant. Without removing the plate or tube(s) from the magnetic plate, remove and discard the supernatant.

2.10 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.

2.11 Repeat the wash once, for a total of two washes, while keeping the sample(s) on the magnetic plate.

2.12 Carefully remove all remaining ethanol with a 10-µl pipette, 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.

2.13 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.

2.14 Remove the plate or tube(s) from the magnetic plate and add 17 µl water to each sample. Mix by pipetting until homogenized.
NOTE: 10 mM Tris-HCl pH 8 or Buffer EB may also be utilized for elution.

2.15 Incubate at room temperature for 2 minutes.

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

2.17 Transfer 15 µl of the clear supernatant containing the ligated 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.

PROCEED TO STEP 3: PCR AMPLIFY USING TWIST UDI PRIMERS, PURIFY, AND PERFORM QC

STEP 3 PCR AMPLIFY USING TWIST UDI PRIMERS, PURIFY, AND PERFORM QC

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

Reagents Required

- Ligated libraries (from Step 2.17)
- 80% Ethanol (from Step 2)
- Equilibrated DNA Purification Beads (from Step 2)
- Molecular biology grade water
- 10 mM Tris-HCl pH 8 or Buffer EB (optional, for elution)
- From Twist TrueAmp Polymerase Mix:
  - Twist TrueAmp Polymerase Mix
- From Twist UMI Adapter System:
  - Twist UDI Primers

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

Before You Begin

- Thaw by placing on ice:
  - Twist TrueAmp Polymerase Mix
  - Twist UDI Primers (plate with single-use primers)

PREPARE THE THERMAL CYCLER

3.1

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

	STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
1	Initialization	98°C	45 seconds	1
2	Denaturation	98°C	15 seconds	*6–8**
	Annealing	60°C	30 seconds	
	Extension	68°C	30 seconds	
3	Final Extension	68°C	1 minute	1
4	Hold	4°C	HOLD	–

**6-8 cycles are recommended for Twist target enrichment workflows when starting with 50 ng high-quality gDNA. For mass inputs other than 50 ng, refer to Appendix B for guidance on PCR cycles.*

PERFORM PCR

3.2 Add 10 µl of Twist UDI Primer from the provided 96-well or 384-well plate to each of the gDNA libraries from Step 2.17 and mix well by gentle pipetting.
NOTE: For index selection and pooling guidelines for downstream target enrichment and sequencing, refer to Appendix A

3.3 Add 25 µl Twist TrueAmp Polymerase Mix to the gDNA libraries from Step 3.2 and mix well by gentle pipetting.

3.4 Pulse-spin the sample plate or tube(s) for 2 seconds 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 the 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 pipette, 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.

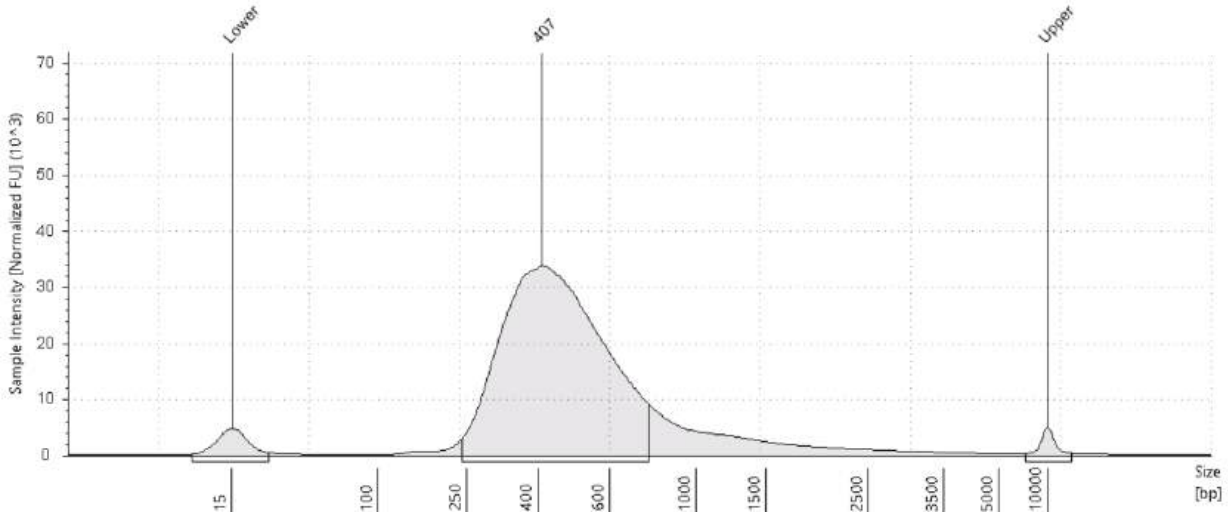
3.17 Place the plate or tubes on a magnetic plate and let stand for 3 minutes or until the beads form a pellet.

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 Quantification Assay and Tapestation D5000 Assay.

50 ng of high-quality gDNA into a 22-minute fragmentation at 32°C and 6 cycles of PCR should result in final concentration values of ≥60 ng/µl. Concentrations below 50 ng/µl may reflect inefficient sample preparation and can result in low library diversity after hybridization. Under these conditions, the average fragment length is typically observed between 400–500 bp using a range setting of 150–1000 bp.



Representative electropherogram of a purified library generated with input of 50 ng of high-quality gDNA into a 22-minute fragmentation at 32°C and 6 cycles of PCR.

STOPPING POINT: If not proceeding immediately to sequencing, 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 to the Twist UDI sequences used in the Twist UMI 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

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.

Twist UDI Primer Plate Layouts and Pooling Guidelines.
Twist UMI Adapter System: TruSeq Compatible, 16 Samples (105040)

	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										

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

APPENDIX A: UDI SEQUENCES AND POOLING GUIDELINES

Twist UMI Adapter System: TruSeq Compatible, 96 Samples, Plates A to D (105041, 105042, 105043, 105044)

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: OBTAINING LARGER INSERT SIZES AND HANDLING ALTERNATIVE MASS INPUTS

FRAGMENTATION OPTIMIZATION

Fragmentation rates may vary depending on the quality and type of starting material. The presence of contaminants like cations and chelators in DNA samples can also inhibit the fragmentation reaction. Buffers containing >0.1 mM EDTA will significantly slow the enzymatic fragmentation reaction. In order to achieve optimum performance of the fragmentation reaction, a DNA clean-up step may be used to remove contaminants before library preparation.

Begin optimization by selecting the desired size range condition from the tables in Step 1.1. Test a range of fragmentation times around this initial condition by adjusting the incubation time in 3-5 minute increments. Increase time to produce shorter fragments and decrease time to produce longer fragments. Greater control for long insert sizes can be achieved using the 30°C fragmentation temperature. For insert sizes >400 bp, fragmentation time can be reduced below 10 minutes. Additional finer optimizations for insert size may be carried out if necessary.

ADAPTER LOADING

For mass inputs other than 50 ng into fragmentation, the volume of Twist UMI Adapters added to ligation in Step 2.2 can be adjusted. Refer to the table on the right for guidance and, if required, add additional chilled water to reach a total volume of 3 µl.

MASS INPUT	VOLUME OF TWIST UMI ADAPTERS (10 µM)
>10 ng	3 µl
10 ng	2 µl
1 ng	1 µl

PCR CYCLE NUMBER

For mass inputs into fragmentation other than 50 ng, the number of PCR cycles in Step 3.1 can be adjusted. For FFPE samples, a minimum of 8 cycles is necessary.

Please contact customersupport@twistbioscience.com for additional guidance.

MASS INPUT	PCR CYCLE RECOMMENDATION*
500 ng	3 cycles
100 ng	4–6 cycles
50 ng	6–8 cycles
25 ng	7–9 cycles
10 ng	8–10 cycles
1 ng	11–12 cycles

**Cycle number recommendations are a starting point for Twist target enrichment workflows using high-quality gDNA and provide sufficient yield for use in 8-plex target enrichment. Cycle number should be modified for each sample type/application.*

APPENDIX C: KIT COMPONENTS

The following table details part numbers for each component provided in the kits required for this protocol.

BOX	COMPONENT	COMPONENT PART NUMBER
Twist Library Preparation Kit 1	10X Twist Fragmentation Enzyme Mix	126918 (16 rxn) 126919 (96 rxn)
	5X Twist Fragmentation Buffer	126920 (16 rxn) 126921 (96 rxn)
	20X Twist DNA Ligation Mix	126922 (16 rxn) 126923 (96 rxn)
	4X Twist DNA Ligation Buffer	126924 (16 rxn) 126925 (96 rxn)
	Amplification Primers, ILMN*	100220 (16 rxn)* 100583 (96 rxn)*
Twist Library Preparation Kit 2	DNA Purification Beads	100322 (16 rxn) 100584 (96 rxn)
Twist TrueAmp Polymerase Mix	Twist TrueAmp Polymerase Mix	116475 (16 rxn) 116476 (96 rxn)

**IMPORTANT: Use of Amplification Primers, ILMN tubes 100220 and 100583 contained in the Twist Library Preparation Kit 1 are not required. Using these primers with the Twist UMI Adapter System will result in a failed PCR amplification.*

END OF APPENDIX