Twist PCR-Free WGS Library Preparation Kit Protocol

For use with the Twist NGS Workflow

This Twist Whole Genome Sequencing Workflow details all the necessary steps for generating PCR-free indexed libraries for sequencing on next-generation sequencing (NGS) systems with the Twist PCR-Free WGS Library Preparation Kit. The workflow uses reagents to prepare genomic DNA (gDNA) libraries with enzymatic fragmentation and full-length Y-shaped adapters. This library preparation workflow has been optimized for use with the reagents specified and should only be performed with them or their equivalents.

Twist NGS workflow. *The complete NGS workflow takes you from sample preparation to NGS sequencing and data analysis.*

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Get in touch at **sales@twistbioscience.com** or learn more at **twistbioscience.com/products/ngs**

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 PCR-FREE WGS LIBRARY PREPARATION KIT (REAGENTS FOR LIBRARY PREPARATION)			
128388: 16 rxn 128389: 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 ADAPTER SYSTEM			
107381, 107469, 107470, 107471: 384 samples, 96-well plate	Twist Full Length UDI Adapters, Plate 1-16	Full Length Adapter Set, 1 reaction per index	–20°C

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MATERIALS SUPPLIED BY USER

The following materials or their equivalent are required to generate libraries using the Twist PCR-Free WGS Library Preparation Kit.

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 High Sensitivity DNA Kit (Optional)	Agilent Technologies
qPCR Library Quantification Kit	—
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
Real-Time PCR Instrument	—

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 PCR-Free WGS Library Preparation Kit 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 75 ng of high-quality gDNA.
- Reagents are compatible with mass input range of 40 to 300 ng, but may require optimization.
- When using degraded DNA or low mass inputs, modify adapter input following Appendix A: Obtaining Larger Insert Sizes and Handling Alternative Mass Inputs.

FOR TECHNICAL SUPPORT, CONTACT CUSTOMERSUPPORT@TWISTBIOSCIENCE.COM.

PROTOCOL OVERVIEW

This protocol begins with genomic DNA (gDNA) and generates indexed libraries for whole genome sequencing applications. It features enzymatic fragmentation and full-length unique dual index (UDI) adapters. This protocol allows you to perform gDNA library preparation (Steps 1–2) in under 3 hours.

ENZYMATIC FRAGMENTATION WITH FULL LENGTH UDI ADAPTERS (GENOMIC DNA 75 NG STARTING MATERIAL)	**TIME**
STEP 1 **Perform DNA Fragmentation, End Repair, and dA-Tailing** dA-Tailed gDNA Fragments	**1 hour 45 minutes**
STEP 2 **Ligate Full-Length Twist UDI Adapters, Purify, and Perform QC** Indexed gDNA 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

- gDNA: 75 ng per sample recommended
- 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	25°C	15 minutes
3	65°C	30 minutes
4	4°C	HOLD

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 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.

1.4 Add 35 µl of each diluted gDNA sample 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 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 Indexed Adapters, Purify, and Perform QC (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 INDEXED ADAPTERS, PURIFY, AND PERFORM QC

STEP 2 LIGATE INDEXED ADAPTERS, PURIFY, AND PERFORM QC

Ligate adapters to the dA-tailed DNA fragments from Step 1 and purify them to generate indexed gDNA libraries. Perform QC to complete the protocol.

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 Library Preparation Kit 2:
  - DNA Purification Beads
- From the Twist Full Length UDI Adapter Set:
  - Twist Full Length UDI Adapters (plate)

⚠ IMPORTANT: Use of Amplification Primers, ILMN tubes 100220 and 100583 contained in the Twist Library Preparation Kit 1 are not required.

Before You Begin

- Thaw or place on ice:
  - Twist Full Length UDI Adapters (plate)
  - 4X Twist DNA Ligation Buffer
  - 20X Twist DNA Ligation Mix
- Prepare 1 ml of 80% ethanol for each sample.
- Equilibrate DNA Purification Beads to room temperature for at least 30 minutes.
- 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 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	14.5 µl
Total	**44.5 µl**

**Prepare a master mix for multiple reactions.*

2.2 Add 5.5 µl diluted Twist Full Length UDI Adapters (see table below and Appendix A for dilution recommendations) into each sample well or tube containing the dA-tailed DNA fragments from Step 1. Mix gently by pipetting and keep on ice.

DNA INPUT (ng)	VOLUME OF FULL LENGTH ADAPTER (µl)	VOLUME OF 10MM TRIS-HCL PH 8.0 (µl)
≤100	1	4.5
200	2	3.5
300	3	2.5

2.3 Add 44.5 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 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 benchtop. Proceed to the Purify step.

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

PURIFY

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

2.6 Add 60 µl of homogenized (0.6X) DNA Purification Beads to each ligation sample from Step 2.4. Mix well by vortexing (refer to Appendix A for optimization of library size).

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 52 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 50 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.

2ND PURIFY

2.18 Vortex the pre-equilibrated DNA Purification Beads until mixed.

2.19 Add 35 µl (0.7X) of homogenized DNA Purification Beads to each ligation sample from Step 2.17. Mix well by vortexing.

2.20 Incubate the samples for 5 minutes at room temperature.

2.21 Place the samples on a magnetic plate for 1 minute.

2.22 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.

2.23 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.

2.24 Repeat this wash once, for a total of two washes, while keeping the samples on the magnetic plate.

2.25 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.

2.26 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.

2.27 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.

2.28 Incubate at room temperature for 2 minutes.

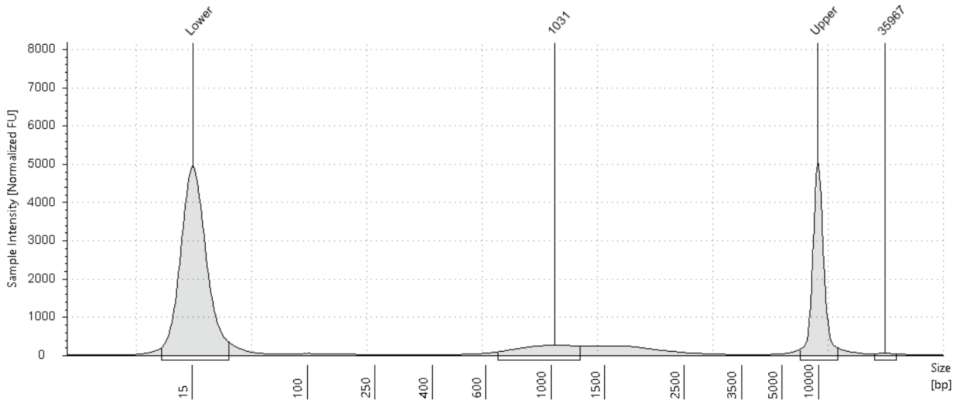
2.29 Place the plate or tubes on a magnetic plate and let stand for 3 minutes or until the beads form a pellet.

2.30 Transfer 20 µl of the clear supernatant containing the 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

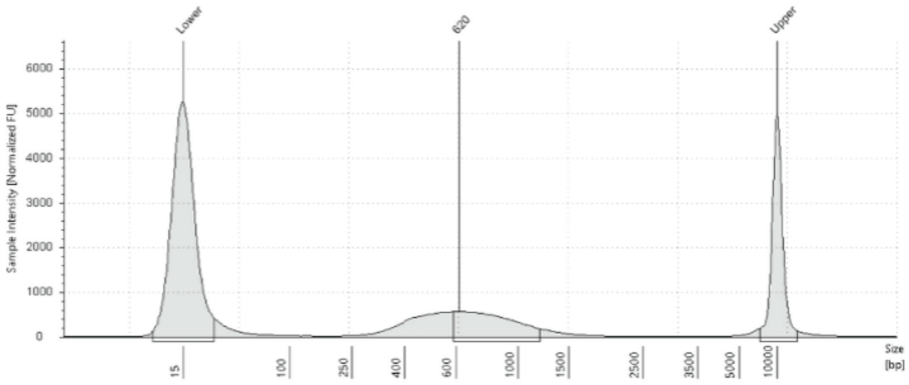
2.31 Quantify each library using an appropriate qPCR-based method. A capillary electrophoresis assay can be used to assess the quality of the libraries.

Running these PCR-free libraries on a fragment analyzer will yield inaccurate sizing results. This is because the Y-adapters added during ligation are partially single stranded and cause slower migration through the gel matrix. Directly below is an example of a library generated with input of 75 ng of high-quality gDNA into a 15-minute fragmentation at 25°C and directly run on an Agilent Tapestation. Notice the library has an average tapestation size above 1 kb, but the sizing is unreliable due to no PCR having been performed.



Representative electropherogram of a purified library generated with input of 75 ng of high-quality gDNA into a 15-minute fragmentation at 25°C without PCR.

If you are running a new sample type and accurate sizing is required, take a test sample and run 3 PCR cycles. Shown below is the trace of a 3 PCR cycle amplified library made from 75 ng of high-quality gDNA into a 15-minute fragmentation at 25°C. Notice the average tapestation size is 620 bp. This sizing is reliable since the sample has been PCR amplified.



Representative electropherogram of a purified library generated with input of 75 ng of high-quality gDNA into a 15-minute fragmentation at 25°C and 3 cycles of PCR.

STOPPING POINT: If not proceeding immediately to sequencing, store the indexed libraries at –20°C.

END OF WORKFLOW

APPENDIX A: OBTAINING LARGER INSERT SIZES AND HANDLING ALTERNATIVE MASS INPUTS

OPTIMIZING LIBRARY SIZE

For applications that require longer insert sizes, the library length can be increased by adjusting fragmentation conditions and the post-ligation SPRI bead ratio. Reducing fragmentation increases overall library size, but can generate broad libraries where shorter fragments preferentially cluster on flow cells during sequencing. Adjusting the post-ligation SPRI bead ratio can remove shorter fragments prior to sequencing. A sequential 0.6X and 0.7X bead ratio is a recommended starting point for generating average library insert sizes >300 bp. To optimize for other insert sizes, test a range of bead ratios between 0.4X and 0.8X. Increase the ratio to retain smaller fragments and reduce the ratio to remove smaller fragments. Note that reducing the bead ratio will reduce library yield.

ADAPTER LOADING

In PCR-free applications, a small amount of full-length adapters can carry over into sequencing and cluster on the flow cell, which reduces the desired library reads. To minimize residual adapters in the final libraries, the volume of full-length adapters can be adjusted. Refer to the table below for guidance, add additional buffer as needed to reach a total volume of 5.5 µl. In addition, performing an optional second 1X SPRI ratio purification after ligation on low mass input or degraded samples can remove residual adapter dimers.

DNA INPUT (ng)	VOLUME OF FULL LENGTH ADAPTER (µl)	VOLUME OF 10MM TRIS-HCL PH 8.0 (µl)
≤100	1	4.5
200	2	3.5
300	3	2.5

APPENDIX B: FULL LENGTH UDI ADAPTER SEQUENCES AND PLATE MAP

The Twist Full Length UDI Adapter set contains indexes of 10 nucleotides. To avoid potential index clashes, do not sequence in combination with other adapter sets.

For a complete guide to the Twist Full Length UDI Adapter sequences, please refer to the Full Length UDI Adapter Sequences Reference Spreadsheet and Full Length UDI Adapter Sample Sheet Templates. These files are available for download here: https://www.twistbioscience.com/resources/data-files/full-length-unique-dual-index-sequences-reference-spreadsheets-and-sample

Representative Plate Layouts.
Twist Full Length UDI Adapter, Plates 1 - 16 (107381, 107469, 107470, 107471)

Plate 1.

	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 2.

	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 3.

	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 4.

	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 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)

**IMPORTANT: Use of Amplification Primers, ILMN tubes 100220 and 100583 contained in the Twist Library Preparation Kit 1 are not required.*

END OF APPENDIX

LAST REVISED: JUNE 9, 2026

REVISION	DATE	DESCRIPTION
4.0	June 9, 2026	· Fixed wording for Step 2 in Protocol Overview section · In Step 2 Reagents Required and Before You Begin sections, added wording and fixed typo to properly reference Twist Full Length UDI Adapters · In Step 2.19, wording now properly refers to Step 2.17 · In Step 2.31, added details regarding obtaining accurate sizing results · Fixed typos throughout the document referring to amplification
3.0	Feb 24, 2026	· Updated mass input compatibility range in Guidelines for gDNA Samples and Protocol Overview sections · Fixed typo in Step 1.1 for thermal cycler program step 2 to 25°C · Fixed other stylistic errors throughout
2.0	Feb 4, 2026	· In Step 1.1, fixed temperature of thermal cycler step 2 to 25°C