xGen™ ssDNA & Low-Input DNA Library Prep Kit

REVISION HISTORY

Version	Release date	Description of changes
5	June 2023	Corrected amount of Enzyme W4 provided in kit
4	January 2023	Update Buffer W3 naming and contact information
3	June 2022	Adjusted product size offerings
2	April 2022	Adaptase adapter name update
1	December 2021	Initial release

Table of contents

OVERVIEW

The **xGen™ ssDNA & Low-Input DNA Library Prep Kit** prepares comprehensive NGS libraries from ssDNA and dsDNA for sequencing on Illumina® platforms. Libraries can be prepared from 10 pg to 250 ng of input DNA. The Adaptase™ technology is a proprietary process that makes the xGen ssDNA & Low-Input DNA Library Prep Kit compatible with ssDNA, making it an ideal choice for NGS library prep from samples containing damaged, denatured, or otherwise single-stranded DNA. This kit does not require intact dsDNA, allowing users to investigate heavily damaged as well as ssDNA and dsDNA mixed samples.

SUPPORTED APPLICATIONS AND SAMPLE TYPES

The xGen ssDNA & Low-Input DNA Library Prep Kit is suitable for the following sample types:

- Heavily damaged samples
- Samples with a mixture of ssDNA and dsDNA
- Low-input DNA enriched by chromatin immunoprecipitation (ChIP) or other methods
- Viromes, metagenomes, and relatively less abundant ssDNA and dsDNA phage/virus
- Microbial samples that have undergone denaturing extraction to lyse difficult-to-extract strains
- Ancient DNA samples when retention of fragments containing uracil because of damage is not desired

For samples that require hybridization capture for targeted sequencing, **contact us** for more information.

xGEN ssDNA & LOW-INPUT DNA LIBRARY WORKFLOW

The xGen ssDNA & Low-Input DNA Library Prep Kit workflow (**Figure 1**) takes about 1.5 hours before Indexing PCR. The major steps to perform this protocol are:

- **Adaptase™ technology:** The Adaptase reaction simultaneously performs tailing and ligation of R2 Stubby Adapter to the 3′ end of the double-stranded or single-stranded DNA in a template-independent manner.
- **Extension:** The Extension reaction generates a second strand complementary to the template fragments.
- **Ligation**: The Ligation reaction adds R1 Stubby Adapter to the original strand.
- **Indexing PCR:** PCR is used to incorporate sample indexes and sequences needed for Illumina® sequencing. Indexing primers are sold separately.

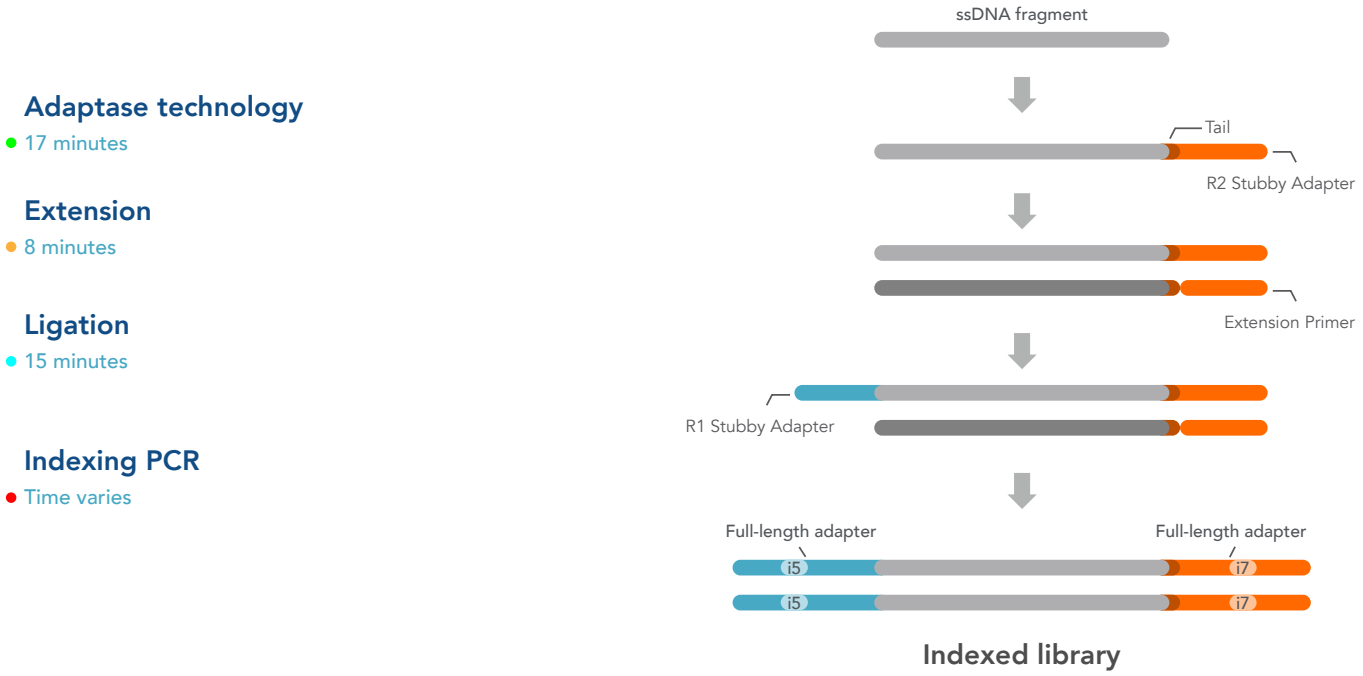

Figure 1. Workflow for the xGen ssDNA & Low-Input DNA Library Prep Kit. Four steps are performed and explained in detail in this protocol.

CONSUMABLES AND EQUIPMENT

These DNA library prep kits contain sufficient reagents for the preparation of 16 or 96 libraries (10% excess volume provided).

Consumables from IDT—Kit contents*

Workflow stage	Component	16 rxn (µL)	96 rxn (µL)	Storage
xGen Adaptase Module	• Buffer G1	71	423	–20°C
	• Reagent G2	71	423	
	• Reagent G3	44	264	
	• Enzyme G4	18	106	
	• Enzyme G5	18	106	
	• Enzyme G6	18	106	
Extension	• Reagent Y1	35	212	
Ligation	• Buffer B1	71	423	
	• Reagent B2	176	1056	
	• Enzyme B3	35	212	
Extension & Indexing PCR	• Reagent W2	194	1162	
	• Buffer W3	484	2904	
	• Enzyme W4	52.8	317	
Other reagents	Low EDTA TE	6000	24000	Room temperature

* Customizable kit contents are available from IDT.

Customizable kit—IDT

Workflow component	Product name	Index number	Reaction size (rxn)	Catalog number
xGen Core Reagents	xGen ssDNA & Low-Input DNA Library Prep Kit	N/A	16	10009859
	xGen ssDNA & Low-Input DNA Library Prep Kit	N/A	96	10009817
xGen Normalase™ Module (optional)	xGen Normalase Module	N/A	96	10009793
xGen CDI Primers*	xGen CDI Primers	D501-D508/ D701-D712	96	10009815
xGen Normalase CDI Primers*	xGen Normalase CDI Primers	D501N-D508N/ D701N-D712N	96	10009794
xGen Normalase UDI Primer Plates*	xGen Normalase UDI Primer Plate 1	SU001-SU096	96	10009796
	xGen Normalase UDI Primer Plate 2	SU097-SU192	96	10009797
	xGen Normalase UDI Primer Plate 3	SU193-SU288	96	10009798
	xGen Normalase UDI Primer Plate 4	SU289-SU384	96	10009799
	xGen Normalase UDI Primer Set 1	SU001-SU384	4x96	10009795
	xGen Normalase UDI Primer Set 2	SU385-SU768	4x96	10009800
	xGen Normalase UDI Primer Set 3	SU769-SU1152	4x96	10009811
	xGen Normalase UDI Primer Set 4	SU1153-SU1536	4x96	10009812

* For index sequences, see the Index Sequence Master List. For adapter sequences, See Appendix B. For custom indexing options, contact us.

Note: xGen Normalase CDI and UDI primers are compatible with Normalase workflows and non-Normalase workflows.

Consumables—Other suppliers

Item	Supplier	Catalog number
SPRISelect® or AMPure® XP beads	Beckman Coulter	B23317/B23318/B23319 A63880/A63881/A63882
Digital electrophoresis chips and associated reagents (choose one): Experion® DNA 1K Analysis Kit High Sensitivity DNA Kit High Sensitivity D1000 ScreenTape® or equivalent	 Bio-Rad Agilent Agilent	 700-7107 5067-4626 5067-5584
KAPA Biosystems® Library Quantification Kit–Illumina/Universal	Roche	KK4824
KAPA HiFi ReadyMix (only required if performing targeted capture)	Roche	KK2602
Qubit™ dsDNA HS Assay Kit	Thermo Fisher Scientific	Q32851 or Q32584
96-well PCR plates	Various suppliers	Varies
DNA LoBind® Tubes, 1.5 mL	Various suppliers	Varies
PCR tubes, 0.2 mL	Various suppliers	Varies
Serological pipettes (5–25 mL)	Various suppliers	Varies
50 mL conical tubes	Various suppliers	Varies
Aerosol-resistant, low-retention pipettes and tips, 2–1000 µL	Various suppliers	Varies
Absolute ethanol (200 proof)	Various suppliers	Varies
Nuclease-free water	Various suppliers	Varies

Equipment

Item	Supplier	Catalog number
Covaris® ultrasonicator	Covaris	Varies
0.2 mL magnets for individual tubes and plates	Permagen	MSR812 (tubes) or MSP750 (plates)
Fluorometer (Qubit™) or similar input DNA quantification instrument	Various suppliers	Varies
Bioanalyzer® or equivalent for library size determination	Agilent	Varies
Quantitative PCR instrument	Various suppliers	Varies
Microcentrifuge	Various suppliers	Varies
Programmable thermal cycler	Various suppliers	Varies
Vortex	Various suppliers	Varies

GUIDELINES

Reagent handling

- Store the xGen ssDNA & Low-Input DNA Library Prep Kit reagents at –20˚C, except for the xGen Low EDTA TE Buffer, which is stored at room temperature.

  Note: The enzymes provided in this kit are temperature sensitive and appropriate care should be taken during storage and handling.

- For all non-enzyme reagents, thaw on ice, then briefly vortex to mix well. Remove enzyme tubes from –20°C storage and place on ice for 10 minutes before use. Attempting to pipette enzymes at –20°C may result in reagent shortage. Spin all tubes in a microcentrifuge to collect contents before opening.
- To create master mixes, scale reagent volumes as appropriate, using 5% excess volume to compensate for pipetting loss. Add reagents in the order listed ON ICE, then pulse vortex to mix and briefly centrifuge.
- Prepare a fresh 80% ethanol solution using 200 proof/absolute ethanol and nuclease-free water. Approximately 2.0 mL of 80% ethanol solution will be used per sample.

Avoid cross-contamination

To reduce the risk of DNA and library contamination, physically separate the laboratory space, equipment, and supplies where pre-PCR and post-PCR processes are performed.

Follow these instructions to avoid cross-contamination:

- Clean lab areas using 0.5% sodium hypochlorite (10% bleach).
- Use barrier pipette tips to avoid exposure to potential contaminants.
- Always change tips between each sample.

Size selection during cleanup steps

This protocol has been performed with SPRISelect beads (Beckman Coulter) but can also be used with AMPure XP beads (Beckman Coulter) or equivalent. However, if other beads are used, solutions and conditions for DNA binding may differ.

- The size selections utilized in this protocol perform a Left Side Size Selection to remove small fragments and unused adapter. For customizing size selection, use Beckman Coulter's SPRISelect User Guide for desired conditions not included in this protocol.

DNA input considerations

For direct sequencing applications, the xGen ssDNA & Low-Input DNA Library Kit has been tested for an input range of 10 pg to 250 ng input DNA per library preparation.

DNA concentration may be assessed using a NanoDrop® instrument (Thermo Fisher Scientific) with A260/A280 ratio or another absorbance-based method; or, if samples are dsDNA, by using Qubit™ or another fluorometric-based method. Accurate determination of DNA is important for determining the number of PCR cycles required at the final step of the workflow. For low-quality human DNA samples, we suggest quantification by qPCR using xGen Input DNA Quantification Primers to accurately assess the usable amount of human DNA in the samples and their integrity.

For ultra-low input or unquantifiable samples, proceed with library prep and apply the number of PCR cycles for the lowest input supported.

Tip: Please consider genome complexity and sample quality when choosing input DNA quantity. Although libraries may be successfully prepared from ultra-low inputs, reduced representation of genome complexity may be observed.

DNA fragmentation

When working with high molecular weight DNA, the DNA must be fragmented prior to library preparation. Fragmentation to either 200 or 350 bp is supported in this protocol. This kit has been tested using Covaris® fragmented DNA. If DNA is single-stranded, Covaris shearing can be performed but may produce smaller fragments, so adjustments may be required to obtain the correct length of fragments.

Optional concentration step

If the DNA concentration is too low to provide sufficient input quantity in an aliquot of 15 µL DNA starting volume as specified in the Perform Adaptase reaction section, concentrate material using the Zymo Research® DNA Clean & Concentrator or other similar method and elute in 15 µL of xGen Low EDTA TE Buffer. Otherwise, proceed directly to the Adaptase section.

Automation

- This protocol is readily automatable. A 10% overage volume of reagents is supplied to accommodate automation. Please contact us if you require additional reagent overage volume or would like to learn about our custom packaging options.
- While IDT does not supply automated liquid handling instruments or consumables, our automation team collaborates with automation solution providers and customers to develop automated scripts for use of our kits with liquid handling platforms routinely used in NGS library preparation. Contact us to discuss automating your xGen ssDNA & Low-Input DNA Library Kit with your automated liquid handling system.

PROTOCOL

Perform Adaptase reaction

1. Add 15 µL of each fragmented sample into a low nucleic acid binding PCR plate or 0.2 mL PCR tube.

   Note: If sample volume < 15 µL, use Low EDTA TE to bring the volume up to 15 µL.

2. For each sample, make the following Adaptase Master Mix.

Component	Volume per sample (µL)
Low EDTA TE	11.5
• Buffer G1	4
• Reagent G2	4
• Reagent G3	2.5
• Enzyme G4	1
• Enzyme G5	1
• Enzyme G6	1
Total volume	25

3. Pulse-spin the Master Mix for 10 seconds, then briefly centrifuge. Keep the Master Mix on ice.
4. Place the samples in the pre-heated thermal cycler and run the Denaturation program.

Denaturation program*

Step	Temperature (°C)	Time
1	95	2 min

* Set the lid temperature to 105°C.

5. After the Denaturation Program ends, immediately place samples on ice for 2 minutes. Proceed directly to the Adaptase Master Mix addition step to preserve the maximum amount of ssDNA substrate.
6. Add 25 µL of Adaptase Master Mix to each sample and mix by pipetting or gentle vortexing. Briefly centrifuge.
7. Place the samples in the pre-heated thermal cycler and run the Adaptase program.

Adaptase program*

Step	Temperature (°C)	Time
1	37	15 min
2	95	2 min
3	4	Hold

* Set the lid temperature to 105°C.

8. While the Adaptase Program runs, make the Extension Master Mix in preparation for the Extension reaction. Enzyme W4 should be added to the Master Mix just before use.

Component	Volume per sample (µL)
Low EDTA TE	18.5
• Reagent Y1	2
• Reagent W2	7
• Buffer W3	17.5
• Enzyme W4	2
Total Volume	47

9. Pulse-spin the Master Mix for 10 seconds, then briefly centrifuge. Keep the Master Mix on ice.
10. After the Adaptase Program reaches 4°C, proceed immediately to Perform extension.

Perform extension

1. Add 47 µL of Extension Master Mix to each sample. Mix by pipetting or gentle vortexing. Briefly centrifuge.
2. Place the samples in the pre-heated thermal cycler and run the Extension program.

Extension program*

Step	Temperature (°C)	Time
1	98	30 sec
2	63	15 sec
3	68	5 min
4	4	Hold

* Set the lid temperature to 105°C.

Perform post-extension cleanup

Input	Number of cleanups	Sample volume (µL)	Bead volume (µL)	Elution volume (µL)
≥1 ng, 200 bp	Single cleanup	87	104 (ratio: 1.2X)	20
≥1 ng, 350 bp	Single cleanup	87	70 (ratio: 0.8X)	20
<1 ng, 200 bp	1st cleanup	87	104 (ratio: 1.2X)	50
	2nd cleanup	50	60 (ratio: 1.2X)	20
<1 ng, 350 bp	1st cleanup	87	70 (ratio: 0.8X)	50
	2nd cleanup	50	40 (ratio: 0.8X)	20

1. Add the specified Bead Volume of SPRISelect beads to each well. Mix by vortexing. Briefly centrifuge.
2. Incubate the samples at room temperature for 5 minutes off-magnet.
3. Place the samples on a magnetic rack until a pellet is formed and the solution clears (about 2 minutes).

   Important: If solution is not clear after 2 minutes, keep the plate on the magnet until solution clears. If beads are discarded, sequencing library yield and quality may be adversely affected.

4. Remove and discard the cleared supernatant; make sure not to remove any beads.

5. Keeping the samples on the magnet, add 200 µL of 80% ethanol, then incubate for 30 seconds. Remove and discard the supernatant.
6. Repeat previous step for a second wash with 80% ethanol.
7. Briefly centrifuge samples and place back on magnet.
8. Use a P20 pipette tip to remove any residual ethanol.
9. Remove samples from magnet, then add specified Elution Volume of Low EDTA TE buffer and gently vortex to resuspend the pellet.
10. Allow the samples to incubate at room temperature for 5 minutes to elute DNA off beads.
11. Place the samples on a magnet and wait for the liquid to clear completely for 1–2 minutes.

    Note: Depending on the strength of your magnet, you may need to wait longer.

12. Carefully transfer specified Elution Volume of eluted DNA into a new well/tube. If necessary, perform 2nd cleanup.
13. Proceed to Perform ligation, or pause here.

    Safe Stop: Samples can be briefly stored at 4°C until ready to proceed, or at –20°C if stored overnight.

Perform ligation

1. For each sample, make the following Ligation Master Mix. Enzyme B3 should be added to the Master Mix just before use.

Component	Volume per sample (µL)
Low EDTA TE	4
• Buffer B1	4
• Reagent B2	10
• Enzyme B3	2
Total volume	20

2. Pulse-vortex the Master Mix for 10 seconds, then briefly centrifuge. Keep the Master Mix on ice.
3. Add 20 µL of Ligation Master Mix to each sample. Mix by pipetting or gentle vortexing. Briefly centrifuge.
4. Place the samples in the pre-heated thermal cycler and run the Ligation Program.

Ligation program*

Step	Temperature (°C)	Time
1	25	15 min
2	4	Hold

* Set the lid temperature to OFF.

Perform post-ligation cleanup

Input	Sample volume (µL)	Bead volume (µL)	Elution volume (µL)
All inputs, 200 bp	40	40 (ratio: 1.0X)	20
All inputs, 350 bp	40	32 (ratio: 0.8X)	20

1. Add the specified bead volume of SPRISelect beads to each well. Mix by vortexing. Briefly centrifuge.
2. Incubate the samples at room temperature for 5 minutes off-magnet.
3. Place the samples on a magnetic rack until a pellet is formed and the solution clears (about 2 minutes).

   Important: If solution is **not** clear after 2 minutes, keep the plate on the magnet until solution clears. If beads are discarded, sequencing library yield and quality may be adversely affected.
4. Remove and discard the cleared supernatant, making sure not to remove any beads.
5. Keeping the samples on the magnet, add 200 µL of 80% ethanol, then incubate for 30 seconds. Remove and discard the supernatant.
6. Repeat previous step for a second wash with 80% ethanol.
7. Briefly centrifuge samples and place back on magnet.
8. Use a P20 pipette tip to remove any residual ethanol.
9. Remove samples from magnet, then add specified elution volume of Low EDTA TE buffer and gently vortex to resuspend the pellet.
10. Allow the samples to incubate at room temperature for 5 minutes to elute DNA off beads.
11. Place the samples on a magnet and wait for the liquid to clear completely for 1–2 minutes.

    Note: Depending on the strength of your magnet, you may need to wait longer.
12. Carefully transfer 20 µL of eluted DNA into a new well/tube. Proceed to Perform indexing PCR, or pause here.

    Safe Stop: Samples can be briefly stored at 4°C until ready to proceed, or at –20°C if stored overnight.

Perform indexing PCR

1. Add indexing primers directly to each sample. If using Normalase Indexing Primers, see Appendix A and the Normalase Kit protocol for complete instructions.

Indexing options	Reagents	Volume per sample (µL)
xGen UDI Primer Pairs	Pre-mixed primer pair	5.0
xGen CDI Primer Pairs	i5 primer i7 primer	2.5 2.5

Note: The indexing primers are provided separately as part of the Indexing Kit. If using alternative indexing primers, contact us to make sure that they are compatible with this protocol. See the Index Sequence Master List for index sequences.

2. For each sample, make the following Indexing PCR Master Mix. Enzyme W4 should be added to the Master Mix just before use.

Component	Volume per sample (µL)
Low EDTA TE	10
• Reagent W2	4
• Buffer W3	10
• Enzyme W4	1
Total volume	25

Note: For targeted hybridization capture applications, substitute KAPA HiFi HotStart ReadyMix in place of kit reagents.

3. Pulse-vortex the Master Mix for 10 seconds, then briefly centrifuge.
4. Add 25 µL of Indexing PCR Master Mix to each sample. Mix by pipetting or gentle vortexing. Briefly centrifuge.
5. Place the samples in the pre-heated thermal cycler and run the Indexing PCR program.

Indexing PCR program

Step	Cycles	Temperature (°C)	Time
1	1	98	30 sec
2	Based on sample input (see table below)	98	10 sec
		60	30 sec
		68	60 sec
3	1	4	Hold

* Set the lid temperature to 105°C.

Input (ng)	PCR cycles
250	3–5
100	4–6
10	7–9
1	10–12
0.1	14–16
0.01	17–19

Note: The number of cycles required to produce enough library for direct sequencing will depend on input quantity and quality. In the case of low-quality samples, the number of cycles required may vary based on the quality of the sample and amount of usable DNA present. Approximate guidelines for high-quality DNA are indicated above, but the exact number of cycles will be determined by you.

6. After the program completes, proceed to Post-Indexing PCR cleanup.

Perform post-indexing PCR cleanup

Input (bp)	Sample volume (µL)	Bead volume (µL)	Elution volume (µL)
200	50	40.0 (ratio: 0.8X)	20
350	50	42.5 (ratio: 0.85X)	20

1. Add the specified bead volume of SPRISelect beads to each well. Mix by vortexing. Briefly centrifuge.
2. Incubate the samples at room temperature for 5 minutes off-magnet.
3. Place the samples on a magnetic rack until a pellet is formed and the solution clears (about 2 minutes).

   Important: If solution is not clear after 2 minutes, keep the plate on the magnet until solution clears. If beads are discarded, sequencing library yield and quality may suffer.
4. Remove and discard the cleared supernatant, making sure not to remove any beads.
5. Keeping the plate on the magnet, add 200 µL of 80% ethanol, then incubate for 30 seconds. Remove and discard the supernatant.
6. Repeat previous step for a second wash with 80% ethanol.
7. Briefly centrifuge samples and place back on magnet.
8. Use a P20 pipette tip to remove any residual ethanol.
9. Remove samples from magnet, then add specified elution volume of Low EDTA TE buffer and gently vortex to resuspend the pellet.
10. Allow the samples to incubate at room temperature for 5 minutes to elute DNA off beads.
11. Place the samples on a magnet and wait for the liquid to clear completely, ~2 minutes.

    Note: Depending on the strength of your magnet, you may need to wait longer.
12. Carefully transfer entire eluate of sample into a new well/tube. If necessary, perform 2nd cleanup.

    Important: If you are direct sequencing on patterned flow cells, perform the following second cleanup to ensure the removal of any unincorporated primers which can increase index hopping on patterned flow cells. A second purification step is not necessary if you are using xGen Unique Dual Indexing Primer Pairs and Adapters, xGen Normalase Module, or xGen Hybridization Capture.

Input (bp)	Number of cleanups	Sample volume (µL)	Bead volume (µL)	Elution volume (µL)
200	1st cleanup	50	40.0 (ratio: 0.8X)	50
	2nd cleanup	50	40.0 (ratio: 0.8X)	20
350	1st cleanup	50	42.5 (ratio: 0.85X)	50
	2nd cleanup	50	42.5 (ratio: 0.85X)	20

The library is now ready for quantification.

Library quantification

Accurate library quantification is essential to properly load the sequencing instrument. Libraries can be quantified using fluorometric methods (e.g., Qubit™ Fluorometer) or qPCR. There are many commercially available qPCR kits available for library quantification. Digital electrophoresis (e.g., Agilent TapeStation™ or Agilent Bioanalyzer®) can also be used to assess the library size.

Data analysis and bioinformatics

Using the xGen Adaptase technology in this kit (xGen ssDNA & Low-Input DNA Library Prep Kit), adds a low-complexity dinucleotide tail with an average length of 8 bases to the 3' end of each fragment during the addition of the first NGS adapter molecule. If these tails are not trimmed bioinformatically from the sequencing data, it is normal and expected to observe them at the beginning of Read 2 (R2). When read length is close to fragment size, the tail may also be observed toward the end of Read 1 (R1) data.

Note: Bioinformatic trimming of the low complexity Adaptase tail from these libraries may be necessary to ensure the tails do not interfere with correct sequence alignment, depending on the aligner being used. For specific tail trimming recommendations, contact us.

APPENDIX A: PERFORM NORMALASE™ MODULE

Review this section and the Normalase Kit protocol before setting up your Normalase PCR. To achieve expected results, amplify each library using Normalase primers with the appropriate number of cycles and thermal cycler conditions (see below) to obtain a library yield of ≥12 nM in a 20 µL eluate.

Normalase indexing primers complete the adapter sequences, amplify, and condition libraries for downstream Normalase Module steps. Assemble using standard PCR reagents , except you will substitute standard indexing primers with i5 and i7 Normalase Indexing Primers.

1. For CDI prep, add 2 µL of both Normalase combinatorial dual index (CDI) primers to each sample; for UDI prep, add 4 µL of the Normalase unique index primer (UDI) pair. The total volume will be 24 µL.

Indexing options	Reagents	Volume per sample (µL)
Normalase UDI	Premixed primer pair	4
Normalase CDI	i5 primer i7 primer	2 2

2. Assemble the PCR Master Mix on ice. Mix thoroughly and pulse-spin to collect contents. Add 26 µL of the mix to each sample tube, again mix thoroughly, then pulse-spin to collect contents (50 µL total reaction volume), and place in the thermal cycler.

Normalase UDI

Component	Volume per reaction (µL)
PCR Master Mix	25
Reagent R7	1
Total Master Mix	**26**
Eluted sample + primers	24
Total volume	**50**

Normalase CDI

Component	Volume per reaction (µL)
PCR Master Mix	25
Reagent R6	1
Total Master Mix	**26**
Eluted sample + primers	24
Total volume	**50**

3. Run the following thermal cycler program, adjusting the number of cycles depending on the input amount and sample quality. Lid heating set to 105°C.

PCR program

Step	Cycles	Temperature (°C)	Time
Activate Enzyme	1	98	30 sec
Deanture	Perform X cycles*	98	10 sec
Anneal		60	30 sec
Extend		68	60 sec
Final extension	1	68	5 min
Hold	1	4	Hold

* The recommended minimum number of cycles for each input to provide ≥12 nM yield for the Normalase workflow; see below.

gDNA input (ng)	Minimum number of cycles for ≥12 nM
250	6
100	8
10	11
1	14
0.1	18
0.01	21

4. The number of cycles required may vary based on the input amount, as detailed above. Recommendations shown are for high-quality input DNA.
5. Proceed to Post-indexing PCR cleanup.
6. Proceed to Normalase step I, Pooling, and Normalase step II in the Normalase Module protocol.

APPENDIX B: INDEXED ADAPTER SEQUENCES

The full-length adapter sequences are below. The underlined text indicates the location of the index sequences which are 8 bp for CDI and 10 bp for UDI. These sequences represent the adapter sequences following completion of the indexing PCR step.

Index 1 (i7) Adapters

5´–GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXX(XX)ATCTCGTATGCCGTCTTCTGCTTG–3´

Index 2 (i5) Adapters

5´–AATGATACGGCGACCACCGAGATCTACACYYYYYYYY(YY)ACACTCTTTCCCTACACGACGCTCTTCCGATCT–3´

Refer to the accompanying Index Sequences Master List for index sequences when preparing your Illumina® sequencing sample sheet on your instrument of choice.

APPENDIX C: TROUBLESHOOTING

Problem	Possible cause	Suggested remedy
Incomplete resuspension of beads after ethanol wash during SPRISelect steps	Over-drying of beads	Continue pipetting the liquid over the beads to break up clumps for complete resuspension.
Shortage of enzyme reagents	Pipetting enzymes at –20°C	Allow enzyme reagents to equilibrate on-ice for 10 minutes before pipetting.
Low library yields	Low quality sample	Use the Alu Primers (sold separately) to determine the integrity of your human DNA sample and adjust input quantity accordingly. Increase the number of PCR cycles.
	Over-drying of beads	Continue pipetting the liquid over the beads to break up clumps for complete resuspension.

xGen™ ssDNA & Low-Input DNA Library Prep Kit

For more information, go to: **www.idtdna.com/ContactUs**

> For more than 30 years, IDT's innovative tools and solutions for genomics applications have been driving advances that inspire scientists to dream big and achieve their next breakthroughs. IDT develops, manufactures, and markets nucleic acid products that support the life sciences industry in the areas of academic and commercial research, agriculture, medical diagnostics, and pharmaceutical development. We have a global reach with personalized customer service.

> SEE WHAT MORE WE CAN DO FOR **YOU** AT **WWW.IDTDNA.COM**.

Doc ID: RUO21-0369_005 06/23

IDT
INTEGRATED DNA TECHNOLOGIES