Preparing NGS libraries using the xGen™ DNA Library Prep Kit EZ and MGIEasy™ UDB Primers Adapter Kit for sequencing with the DNBSEQ-G400 system

The method presented here is provided by IDT. This method uses the xGen DNA Library Prep Kit EZ with the MGIEasy UDB Primers Adapter Kit A&B and the MGIEasy™ Dual Barcode Circularization Kit. This demonstrated protocol can be used as a starting point for creating next generation sequencing (NGS) libraries intended for downstream DNBSEQ-G400 sequencing. This method can be used in similar experiments but may not be fully optimized for your application. IDT does not guarantee these methods, and application scientists at IDT can only provide general guidance with limited troubleshooting support. For more information and support for MGI products and sequencing on the DNBSEQ-G400 System, see the MGI website.

Overview

The xGen DNA Library Prep Kit EZ from IDT is a fast and flexible solution designed to produce NGS libraries from a broad range of double-stranded DNA inputs. This demonstrated protocol describes the workflow for whole genome sequencing (WGS) using the xGen DNA Library Prep Kit EZ with the MGIEasy UDB Primers Adapter Kit and optional hybridization capture following library preparation. For sequencing, the DNBSEQ-G400 System requires circularized libraries containing DNBSEQ-G400-specific sequences. This protocol includes the MGIEasy UDB Primers Adapter Kit workflow followed by the MGIEasy Dual Barcode Circularization Kit workflow to convert the DNA library fragments into circular templates for sequencing.

There are four major activities outlined in this protocol:

- **Enzymatic preparation.** Performs fragmentation, end-repair, and dA-tailing of dsDNA.
- **Adapter ligation.** Performs ligation of MGIEasy UDB adapters to the enzymatically prepped samples.
- **PCR amplification.** Amplify and index libraries using MGIEasy UDB PCR primers.
- **MGIEasy circularization.** Prepare for DNBSEQ-G400 sequencing with denaturation, circularization, and digestion steps.

1	Prepare reagents	• Thaw reagents on ice • Prepare fresh 80% ethanol	Total time: varies (~20 min)
2	Perform enzymatic preparation	• Set up dsDNA fragmentation, end-repair, and A-tailing • Run Enzymatic Prep program	Total time: 40–60 min
3	Perform ligation	• Add MGIEasy UDB adapter	Total time: 20 min
4	Clean up ligation reaction	• Purify ligation product	Total time: 20 min
	Safe stopping point (store @ –20°C)		
5	Perform PCR & cleanup	• Add MGIEasy UDB PCR primers • Increase available library for sequencing	Total time: PCR, 10–30 min Cleanup, 20 min
	Safe stopping point (store @ –20°C)	Optional hybrid capture	
6	Denaturation	• Creates single-stranded library	Total time: 5 min
7	Single strand circularization	• Splint oligo used to generate circle	Total time: 40 min
8	Digestion	• Remove carryover linear DNA	Total time: 30 min
9	Perform cleanup	• Purify circularization reaction	Total time: 30 min

Consumables and equipment

Consumables—IDT

Item	Catalog #
xGen DNA Library Prep Kit EZ, 16 rxn	10009863
xGen DNA Library Prep Kit EZ, 96 rxn	10009821

Consumables—Other suppliers

Item	Supplier	Catalog #
MGIEasy UDB Primers Adapter Kit A&B	MGI	1000022801 or 1000022802
MGIEasy Dual Barcode Circularization Kit		1000020570
Absolute ethanol (200 proof)	Varies	Varies
Magnetic purification beads (choose one):		
SPRIselect™ purification beads, or equivalent	Beckman Coulter	B23317/B23318/B2331
AMPure® XP-PCR purification beads, or equivalent		A63880 or A63881
Digital electrophoresis chips and associated reagents (choose one):		
Experion™ DNA 1K Analysis Kit, or equivalent	Bio-Rad™	700-7107
High Sensitivity DNA Kit	Agilent	5067-4626
High Sensitivity D1000 ScreenTape®, or equivalent	Agilent	5067-5584
Fluorometric DNA quantification assay kit		
Qubit™ dsDNA HS Assay Kit, or equivalent	Thermo Fisher Scientific	Q32851 or Q32854
Qubit dsDNA BR Assay Kit, or equivalent		Q32850 or Q32853
Qubit ssDNA Assay Kit, or equivalent		Q10212
PCR tubes, 0.2 mL	Varies	Varies
Low-bind DNA Tubes, 1.5 mL		
Aerosol-resistant tips and pipettes ranging from 2–1000 µL		

Equipment

Item	Supplier	Catalog #
Digital electrophoresis		
Experion Electrophoresis Station, or equivalent	Bio-Rad	700-7010
2100 Electrophoresis Bioanalyzer™, or equivalent	Agilent	G2939BA
2200 TapeStation™ System/4200 TapeStation System, or equivalent	Agilent	G2965AA or G2991AA
Qubit 4 Fluorometer, or equivalent	Thermo Fisher Scientific	Q33226
Magnet options (choose one):		
Magnetic Separator Plate	Permagen	MSP750
Magnetic PCR Strip Magnetic Separator Rack		MSR812
Magnet for 1.5 mL tubes	Varies	Varies
Microcentrifuge		
Vortex		
Thermal cycler		

Reagent handling

Important: Always store kit reagents at –20°C, except for the Low EDTA TE buffer which can be stored at room temperature.

Note: The enzymes provided are temperature sensitive. Appropriate care should be taken during storage and handling. To maximize use of enzyme reagents, remove enzyme tubes from –20°C storage and place on ice for at least 10 minutes prior to pipetting. Attempting to pipette enzymes at –20°C may result in reagent loss.

Except for Buffer W1 and enzymes, briefly vortex the reagents after thawing them on ice. Spin all tubes in a microcentrifuge to collect contents before opening.

Thaw Buffer W1 (for Ligation Master Mix) at room temperature. Buffer W1 is viscous and requires special handling during pipetting. When ready for use, pipette slowly to draw the accurate quantity.

To create master mixes, scale reagent volumes as appropriate, using 5% excess volume to compensate for pipetting loss. Add reagents to the master mix in the specified order as stated throughout the protocol. Once prepared, master mixes should be stored on ice until used.

DNA input considerations

The xGen DNA Library Prep Kit EZ works with a broad range of DNA inputs, ranging from 100 pg to 1 µg. This kit can use high-quality gDNA, amplicons, and formalin-fixed paraffin-embedded (FFPE) DNA. To quantify the concentration of low-quality human DNA samples, qPCR can be performed using xGen Input DNA Quantification Primers (catalog # 10009856).

Important: For specific input quantities recommended in this protocol, refer to the total DNA quantified after fragmentation.

Fragmentation parameters

When using a new lot of the fragmentation enzyme, you may experience variation in the required fragmentation times. Refer to your certificate of analysis (CoA) for specific fragmentation time recommendations for the lot number that you received. Fragmentation times provided in this protocol are for high-quality samples. You may need to determine shorter fragmentation time for samples of compromised quality (e.g., FFPE).

EDTA in elution buffers

The enzymatic preparation reaction is sensitive to high concentrations of EDTA. A high concentration of EDTA, such as 1 mM in standard 1X TE buffer, will slow the reaction, resulting in larger insert sizes. Alternatively, no EDTA (i.e., if eluted in Tris buffer only) will result in faster fragmentation and smaller insert sizes. Our standard enzymatic prep conditions are determined using 0.1 mM EDTA TE (as provided in this kit [Low EDTA TE]).

If DNA is eluted in standard TE with 1 mM EDTA, perform a buffer exchange using a column or bead-based purification protocol. 3x SPRIselect (Beckman Coulter) is recommended for minimum loss of sample gDNA, although you may need to optimize your buffer exchange method depending on sample source. Alternatively, you can adjust the amount of Reagent K2 used in the Enzymatic Prep step to no more than 3X to achieve the desired fragment length (up to 4.5 µL of Reagent K2 per reaction).

If DNA is resuspended in 10 mM Tris (e.g., Buffer EB from Qiagen, 10 mM Tris-HCl, pH 8.5) or water without EDTA, Reagent K2 is not needed during Enzymatic Prep.

Note: For more information on automation, size-selection, and avoiding contamination, see the full xGen DNA Library Prep Kit EZ protocol.

Protocol

Enzymatic prep

Important: Keep the Enzymatic Prep Master Mix and the DNA samples on ice until they are loaded in the thermal cycler to safeguard against fragmentation. Enzymes are active at room temperature and may fragment DNA to undesired sizes.

1. Transfer the DNA sample to a sterile 0.2 mL PCR tube. Adjust sample volume to a total of 19.5 µL using Low EDTA TE, then place the tube on ice.

Components	Volume per sample (µL)
Low EDTA TE	(19.5 – x)
DNA	x
Total volume	19.5

2. Set up the thermal cycler with the Enzymatic Prep program, as described below, with the lid set to 70°C (heated lid required).

Step	Temperature* (°C)	Time
Hold	4	∞
Fragmentation	32	Variable (see note)
Inactivation	65	30 minutes
Hold	4	Less than 1 hour

* Lid temperature needs to be set to 70°C.

Important: Fragmented samples should not be stored at 4°C for longer than 1 hour.

Note: See your Certificate of Analysis (CoA) for fragmentation time recommendations for the individual lot number that you received. Reaction times may be optimized for individual samples. Specifically, for sample inputs <25 ng, longer fragmentation times may be required.

3. Begin the Enzymatic Prep program by chilling the thermal cycler to 4°C.
4. Prepare the Enzymatic Prep Master Mix by adding the components in the order shown:

Enzymatic Prep Master Mix	
Components	Volume per reaction (µL)
• Buffer K1	3.0
• Reagent K2	1.5
• Enzyme K3	6.0
Total volume	10.5

5. Vortex the Enzymatic Prep Master Mix for 5 seconds, then briefly centrifuge. Keep mix on ice until ready to use.

   Important: Ensure that the Enzymatic Prep Master Mix is mixed thoroughly before and after the addition of DNA samples to prevent incomplete fragmentation.

6. Add 10.5 µL of the premixed Enzymatic Prep Master Mix to each tube containing DNA samples and enough Low EDTA TE to reach a final volume of 30 µL.
7. Thoroughly vortex the sample tubes for 5 seconds.
8. Briefly centrifuge the sample tubes, then immediately place in the chilled thermal cycler and advance the Enzymatic Prep program to the 32°C fragmentation step.
9. While the Enzymatic Prep program runs, prepare the Ligation Master Mix.

MGI-specific adapter ligation

1. Before starting adapter ligation, preset a thermal cycler according to the program listed below with lid heating **OFF.**

Ligation program		
Step	Temperature* (°C)	Time
Ligation	20	20 minutes
Hold	4	Hold

* Lid temperature should be OFF.

2. For DNA input <25 ng, dilute adapters (MGI UDB adapter) as shown:

Important: Adapter dilution is necessary to achieve low levels of adapter dimer. For certain applications, adapter dilution may be adjusted to achieve best results.

DNA input	Adapter*
≥25 ng	No dilution
10 ng	10-fold (1:10)
1 ng	20-fold (1:20)
100 pg	30-fold (1:30)

3. Prepare the Ligation Master Mix. Add components in the order shown.

Enzymatic Prep Master Mix	
Components	Volume per sample (µL)
• Buffer W1	12
• Enzyme W3	4
• MGI UDB adapter*	5
Low EDTA TE	9
Total Master Mix	30

* If preparing the Ligation Master Mix ahead of time, add the adapter to the Master Mix just prior to use.

Important: Slowly pipette the viscous Buffer W1 to avoid bubbles.

4. When the Enzymatic Prep program is complete, add 30 µL pre-mixed Adapter Ligation Master Mix to each tube containing 30 µL fragmented DNA.
5. Thoroughly mix samples by moderate vortexing for 5 seconds and briefly centrifuge.
6. Place samples in the pre-programmed thermal cycler and run the Ligation program from step 1 of this section.
7. After the ligation program is complete, proceed immediately to Post-ligation cleanup.

Post-ligation cleanup

Important: Make sure magnetic purification beads are equilibrated to room temperature before starting this section.

1. Prepare fresh 80% ethanol solution.
2. Vortex the beads until the solution is homogenous.
3. Add 48 µL of beads to each sample at room temperature (ratio of bead to sample is 0.8).
4. Thoroughly mix samples by moderate vortexing for 5 seconds, then briefly centrifuge.
5. Incubate the samples for 5 minutes at room temperature.
6. Place the samples on a magnetic rack until the solution clears and a pellet has formed (~2 minutes).
7. Remove and discard the supernatant without disturbing the pellet (less than 5 µL may be left behind).
8. Add 180 µL of freshly prepared 80% ethanol solution to the sample while it is still on the magnetic rack. Be careful to not disturb the pellet.
9. Incubate for 30 seconds, then carefully remove the ethanol solution using a pipette.
10. Repeat steps 8 and 9 for a second ethanol wash.
11. Quick spin the samples in a microcentrifuge, then place them back on the magnetic rack. Remove any residual ethanol solution from the bottom of the tube with a clean pipette tip.
12. Remove the samples from the magnetic rack.
13. Add 20 µL Low EDTA TE to the sample tubes.
14. Incubate the samples at room temperature for 2 minutes.
15. Place the samples on a magnetic rack until the solution clears and a pellet has formed (~2 minutes).
16. Carefully transfer the clear solution into a clean tube, being careful to avoid any bead carryover.

Safe Stop: DNA libraries can be stored overnight at –20°C.

PCR amplification using MGIEasy PCR primers

1. Set up the thermal cycler with the PCR program as shown below, with a heated lid set to 105°C. Adjust the number of cycles based on input amount and workflow. Note that a yield >1 pmol is needed for direct sequencing and >500 ng is needed for hybridization capture.

PCR amplification program			
Step	Cycles	Temperature (°C)	Time
Initial denaturation	1	98	45 seconds
Denaturation	Varies (see table Cycling recommendation for PCR-amplified workflows)	98	15 seconds
Annealing		60	30 seconds
Extension		72	30 seconds
Final extension	1	72	1 minute
Hold	1	4	∞

* Lid should be heated to 105°C.

Cycling recommendations*	
DNA input	Minimum recommended cycles for >500 ng
100 ng	8†
25 ng	10
10 ng	11
1 ng	14

* Optimization may be needed to determine correct cycling conditions for different sample types.

† When indexing by PCR, a minimum of 3 cycles is required to attach adapter sequences, irrespective of whether a sufficient library amount is available following ligation.

2. Add the following components to the eluted library sample:

Components	Volume per reaction (µL)
• PCR Master Mix	25
MGIEasy PCR primers	5
Sample	20
Total volume	50

3. Gently vortex for 5 seconds and briefly centrifuge.
4. Place samples into pre-programmed thermal cycler and run the PCR Amplification program.
5. When the PCR program is complete, vortex the room temperature beads until the solution is homogenous.
6. Proceed to Post-PCR cleanup.

For the master list of sequences, see the DNBSEQ Dual Barcode Adapter & Barcode Sequences guide on the MGI website.

Post-PCR cleanup

1. Add the specified bead volume to each sample as shown:

Average insert size (bp)	Sample volume (µL)	Bead volume (µL)
350	50	32.5 (ratio: 0.65)
200	50	90 (ratio: 1.8)

2. Vortex sample tubes, then briefly centrifuge.
3. Incubate the samples for 5 minutes at room temperature.
4. Place the samples on a magnetic rack until the solution clears and a pellet has formed (~2 minutes).
5. Remove and discard the supernatant, using a clean pipette tip, without disturbing the pellet (less than 5 µL may be left behind).
6. Add 180 µL of freshly prepared 80% ethanol solution to the sample while it is still on the magnetic rack. Be careful to not disturb the pellet.
7. Incubate for 30 seconds, then carefully remove the ethanol solution.
8. Repeat steps 6 and 7 for a second ethanol wash.
9. Quick spin the samples in a microcentrifuge, then place them back on the magnetic rack. Remove any residual ethanol solution from the bottom of the tube with a clean pipette tip.
10. Add 21 µL of Low EDTA TE to the sample tubes and mix well until homogenous.
11. Incubate sample tubes at room temperature for 2 minutes.
12. Place the samples on a magnetic rack until the solution clears and a pellet is formed (~2 minutes).
13. Carefully transfer 20 µL clear solution (eluted DNA) into a clean tube, being careful to avoid any bead carryover.

    Safe Stop: Libraries can be stored overnight at –20°C.

14. The library is now ready for quantification, which can be performed using fluorometric methods (e.g., Qubit Fluorometer).

    Note: If performing direct sequencing, continue with the MGIEasy Dual Barcode Circularization protocol below. If performing hybridization capture, see Appendix A.

MGIEasy Dual Barcode Circularization protocol for DNBSEQ-G400 sequencing

For the remainder of the protocol, use reagents from the MGIEasy Dual Barcode Circularization Kit, which is necessary for sequencing using the DNBSEQ-G400 System. The circularization protocol allows library inputs of 1.0 pmol in 48 µL. Use the following equation to calculate the mass in ng that corresponds to 1 pmol of dsDNA sample: the mass (ng) corresponding to 1 pmol PCR products = (DNA library size [bp]/1000 bp) x 660 ng. Input amounts as low as 0.5 pmol can be used if needed but may negatively affect results. Uniquely indexed libraries can be pooled for a single circularization reaction (do not pool libraries with same index pair). After quantification of linear libraries, normalize libraries to 1.0 pmol and pool based on the sequencing throughput requirements for each sample.

Note: For more information or troubleshooting, visit the MGI website.

Important: Remove MGIEasy DNA Clean Beads from refrigerator and bring to room temperature for at least 30 minutes prior to Enzymatic Digestion Cleanup.

Denaturation

1. Ensure all reagents are thawed completely, invert to mix thoroughly, and store on ice during the remainder of protocol.
2. Transfer 1 pmol of pooled library to a new PCR tube. Add TE Buffer to bring the total volume to 48 µL if needed.
3. Set up the thermal cycler with the Denaturation program, as described below, with the lid set to 105°C.

Step	Temperature * (°C)	Time
1	95	3 minutes

* Lid temperature needs to be set to 105°C.

4. Immediately place the reaction on ice for 2 minutes and centrifuge briefly.

Single strand circularization

1. Set up the thermal cycler with the Single Strand Circularization program, as described below, with the lid set to 105°C.

Step	Temperature * (°C)	Time
1	37	30 minutes
Hold	4	Hold

* Lid temperature needs to be set to 105°C.

2. Prepare the Single Strand Circularization Master Mix by adding the components in the order shown:

Circularization Master Mix	
Components	Volume per sample (µL)
• Dual Barcode Splint Buffer	11.6
• DNA Rapid Ligase	0.5
Total volume	12.1

3. Place on ice and mix by pipetting 10x.
4. Add 12.1 µL of Single Strand Circularization Mix to each reaction, vortex 3 times (3 seconds each), and briefly centrifuge.
5. Place samples into pre-programmed thermal cycler and run the **Single Strand Circularization Program**.
6. Once completed, immediately remove the tube from the thermal cycler, place on ice, and proceed to the next step.

Enzymatic digestion

1. Set up the thermal cycler with the Enzymatic Digestion program, as described below, with the lid set to 105°C.

Step	Temperature * (°C)	Time
1	37	30 minutes
Hold	4	Hold

* Lid temperature needs to be set to 105°C.

2. Prepare the Enzymatic Digestion Master Mix by adding the components in the order shown:

Circularization Master Mix	
Components	Volume per sample (µL)
• Digestion Buffer	1.4
• Digestive Enzyme	2.6
Total volume	4.0

3. Place on ice and mix by pipetting 10x.
4. Add 4 µL of Enzymatic Digestion Mix to each reaction, vortex 3 times (3 sec each), and briefly centrifuge.
5. Place samples into pre-programmed thermal cycler and run the Enzymatic Digestion Program.
6. Once completed, remove the tube from the thermal cycler and centrifuge briefly.
7. Add 7.5 µL of Digestion Stop Buffer to the reaction. Vortex 3 times (3 sec each) and centrifuge briefly.
8. Transfer the total reaction to a new 1.5 mL tube.

Enzymatic digestion cleanup

1. Ensure DNA Clean Beads are at room temperature. Vortex and mix thoroughly.
2. Prepare fresh 80% ethanol solution.
3. Add 170 µL of DNA Clean Beads to the Enzymatic Digestion product. Gently pipette at least 10 times to mix thoroughly.
4. Incubate the samples for 10 minutes at room temperature.
5. Centrifuge briefly and place the samples on a magnetic rack until the solution clears and a pellet has formed (~2–5 minutes).
6. Remove and discard the supernatant using a clean pipette tip, without disturbing the pellet.
7. Add 500 µL of freshly prepared 80% ethanol solution to the sample while it is still on the magnetic rack. Be careful to not disturb the pellet.

8. Incubate for 30 seconds, then carefully remove the ethanol solution.
9. Repeat steps 7 and 8 for a second ethanol wash.
10. Remove tube from the magnet and centrifuge briefly. Place tube back on the magnet and allow beads to separate.
11. Remove all remaining ethanol using a small volume pipet tip.
12. Air-dry the beads uncapped for 2–5 minutes until the pellet loses shine. Do not over dry beads.
13. Once dry, remove the samples from the magnet.
14. Add 22 µL of TE Buffer to the sample tubes and mix by pipetting 10x or until beads are fully resuspended.
15. Incubate sample tubes at room temperature for 10 minutes.
16. Centrifuge briefly and place the samples on a magnetic rack until the solution clears and a pellet has formed (~2–5 minutes).
17. Carefully transfer 20 µL supernatant (eluted circularized library) into a clean 1.5 mL tube, being careful to avoid any bead carryover.

    Safe Stop: Libraries can be stored at –20°C for up to 1 month.
18. The circularized library is now ready for quantification using Qubit ssDNA Assay Kit.

Example of data output

Example libraries were prepared from 50 ng input of Coriell DNA (Coriell Institute) using the xGen DNA Library Prep Kit EZ and MGIEasy UDB Primers Adapter workflow. The sample DNA was enzymatically fragmented to create 350 bp inserts. After library preparation, hybridization capture was performed as single or 12-plex using the xGen Hybridization and Wash v2 Kit and xGen Exome Hyb Panel v2 with xGen Custom Blocking Oligos and custom amplification primers that were designed for use with libraries containing the MGI-specific adapter and index sequences. In Figure 1, a TapeStation High-Sensitivity DNA (Agilent) trace of an example library shows final captured library fragments prior to MGIEasy Dual Barcode circularization. Table 1 shows the sequencing metrics obtained from sequencing 28 hybridization captured libraries on the DNBSEQ-G400; the sequencing run met all manufacturer run specifications.

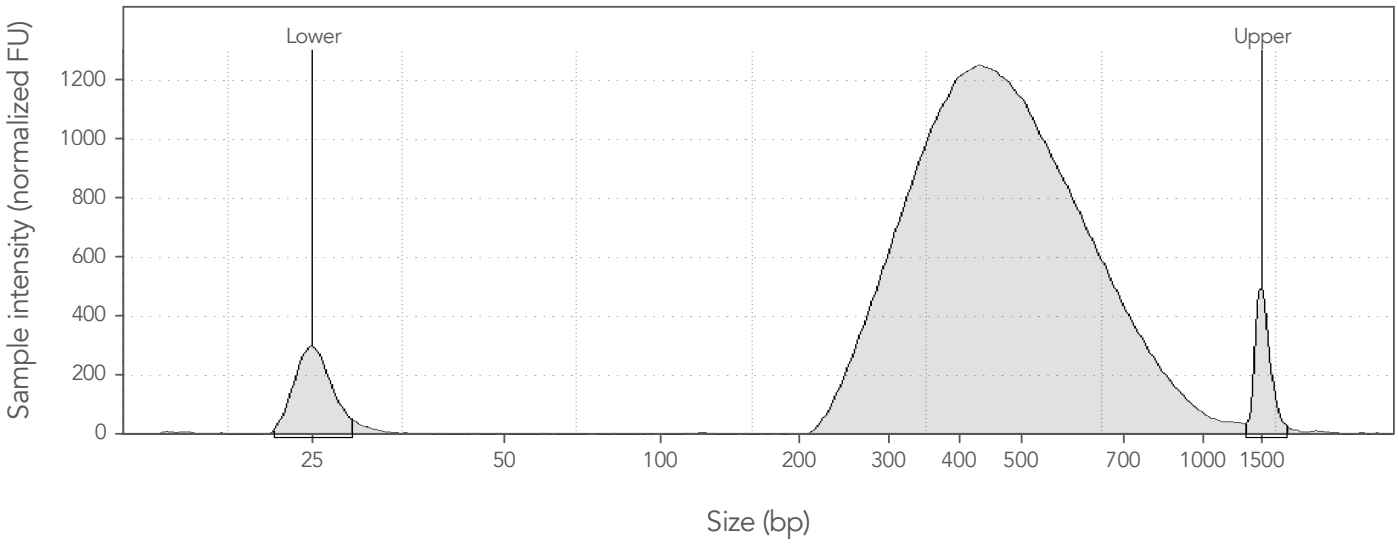

Figure 1. Representative TapeStation (Agilent) trace for 350 bp DNA fragments subjected to library preparation and hybridization capture. The library was generated using the xGen DNA Library Prep Kit EZ and MGIEasy UDB Primers Adapter Workflow with IDT synthesized adapter and

PCR Primer sequences that are compatible with the DNBSEQ-G400.

Table 1. DNBSEQ-G400 sequencing run metrics.*

Lane	Cycle number	Total reads (M)	Split rate (%)	Total Q30 (%)
L01	320	496.57	97.09	94.07
L02	320	497.77	97.14	94.71
L03	320	503	97.23	95.06
L04	320	494.58	97.17	94.62

* Table 1 shows the sequencing metrics obtained on the DNBSEQ-G400 system. The sequencing run included 28 hybridization captured libraries generated using the xGen DNA Library Prep Kit EZ and MGIEasy UDB Primers Adapter workflow with IDT synthesized adapter and PCR primer sequences that are compatible with the DNBSEQ-G400 and circularized using the MGIEasy Dual Barcode Circularization Kit.

Appendix A: Hybridization capture

To perform hybridization capture on the libraries, follow the **xGen hybridization capture of DNA libraries protocol** with the following adjustments. Because the libraries contain MGI-specific adapter and index sequences, custom blocking oligos and amplification primers are necessary. Do not use xGen Universal Blockers or the xGen Library Amplification Primer Mix as the sequences are not compatible with libraries generated with MGI adapters and index primers. **Contact us** to order the custom blockers and amplification primers.

Additionally, the post-capture PCR cycles may need to be adjusted to achieve the necessary yield for circularization. Review the first paragraph in the **circularization section** for yield information required post-capture and adjust PCR cycling accordingly. After the hybridization capture protocol is complete, perform the circularization protocol as described above.

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