MosaiX™ Library Preparation Kit

Catalog numbers:
301436: MosaiX Early Access Kit, 24 Reactions
301430: MosaiX Early Access Kit, 96 Reactions

Early Access User Guide

v20250404

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Introduction

The MosaiX DNA Library Prep Kit offers an innovative approach to transposase-based library construction. The kit utilizes a highly evolved hyperactive transposase, TnX™, engineered to improve library complexity and minimize insertion-site bias. Traditional transposase methods lose 50% of library diversity due to redundant tagging events, where identical adapter sequences are inserted at both ends of a molecule (P5-P5 or P7-P7). MosaiX with TnX overcomes this issue by combining tagmentation and ligation into a single workflow to ensure all tagging events produce functional PCR products with P5 on the 5’ end and P7 on the 3’ end.

First, TnX fragments genomic DNA to the desired size range and attaches the read 1 adapter sequence to the 5’ end of fragmented material. Next, the read 2 adapter is added to the 3' end using a proprietary ligation method. The resulting samples are then purified and amplified with primers containing 10 bp unique dual indexes (UDIs) enabling cost-effective and highly accurate demultiplexing. See workflow diagram on next page.

The MosaiX Kit is ideal for preparing high quality complex libraries from genomic DNA that generate sequencing mean insert sizes of 400 - 500 bp for whole-genome sequencing (WGS) or 250 - 350 bp for hybrid capture applications.

The DNA input range for this Early Access protocol is 50 - 100 ng of mid to high quality genomic DNA.

Additional Notes

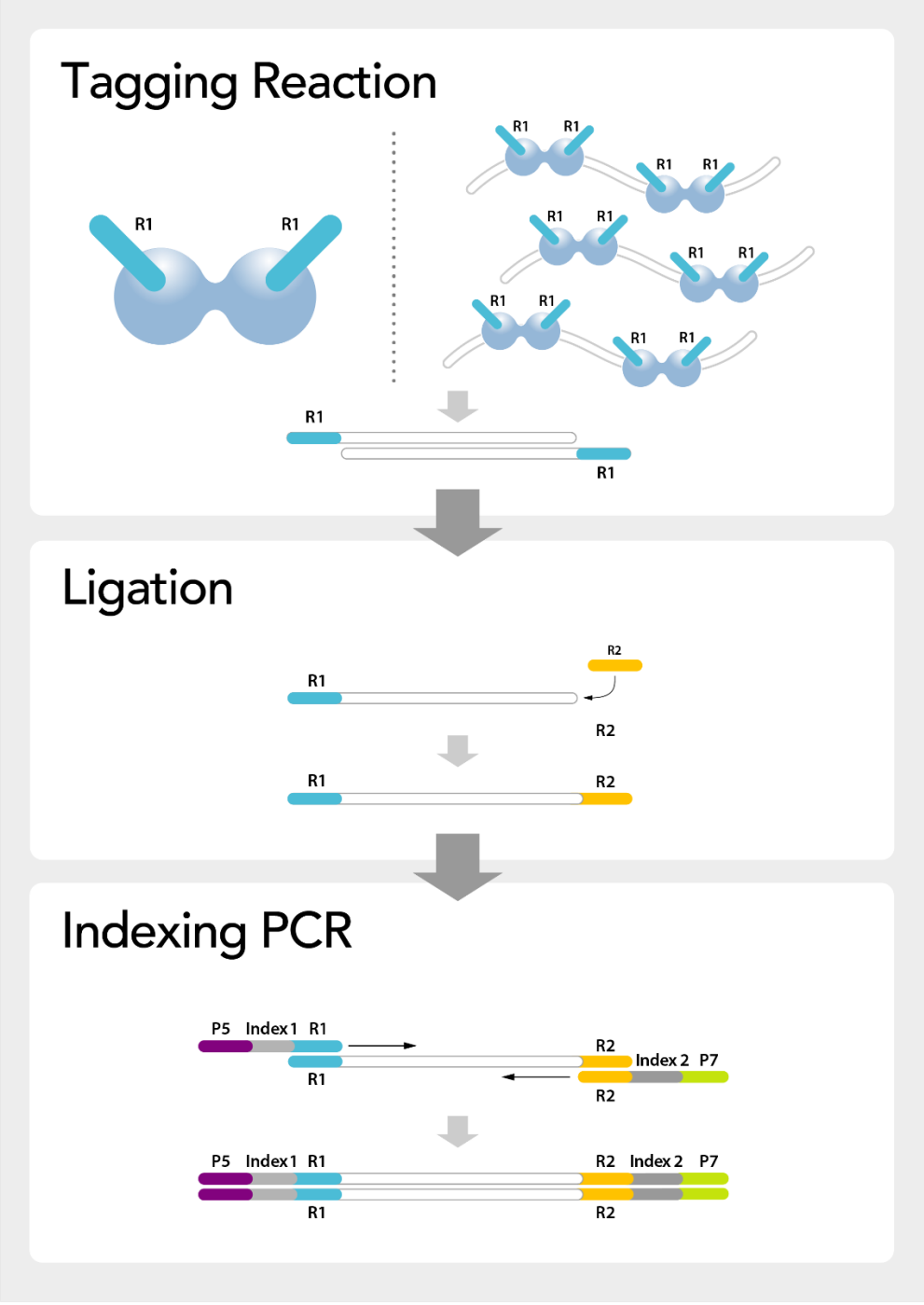
The MosaiX Early Access kits come with all enzymes, buffers, and adapters needed for fragmentation and ligation, as well as PCR master mix and purification beads (seqWell's MAGwise).

For Early Access, seqWell is also providing 24 or 96 unique dual indexed (UDI) primers free of charge to facilitate testing. However, for customers requiring larger numbers of UDIs, any transposase adapter compatible indexing primer sets at 10 µM of each primer can be used. These are readily available from other NGS reagent providers as ready-to-use primer plates, or these primers can be ordered custom from oligo providers. Please see page 12 for more details on the primer sequences or contact support@seqwell.com for recommendations on sources of indexing primers.

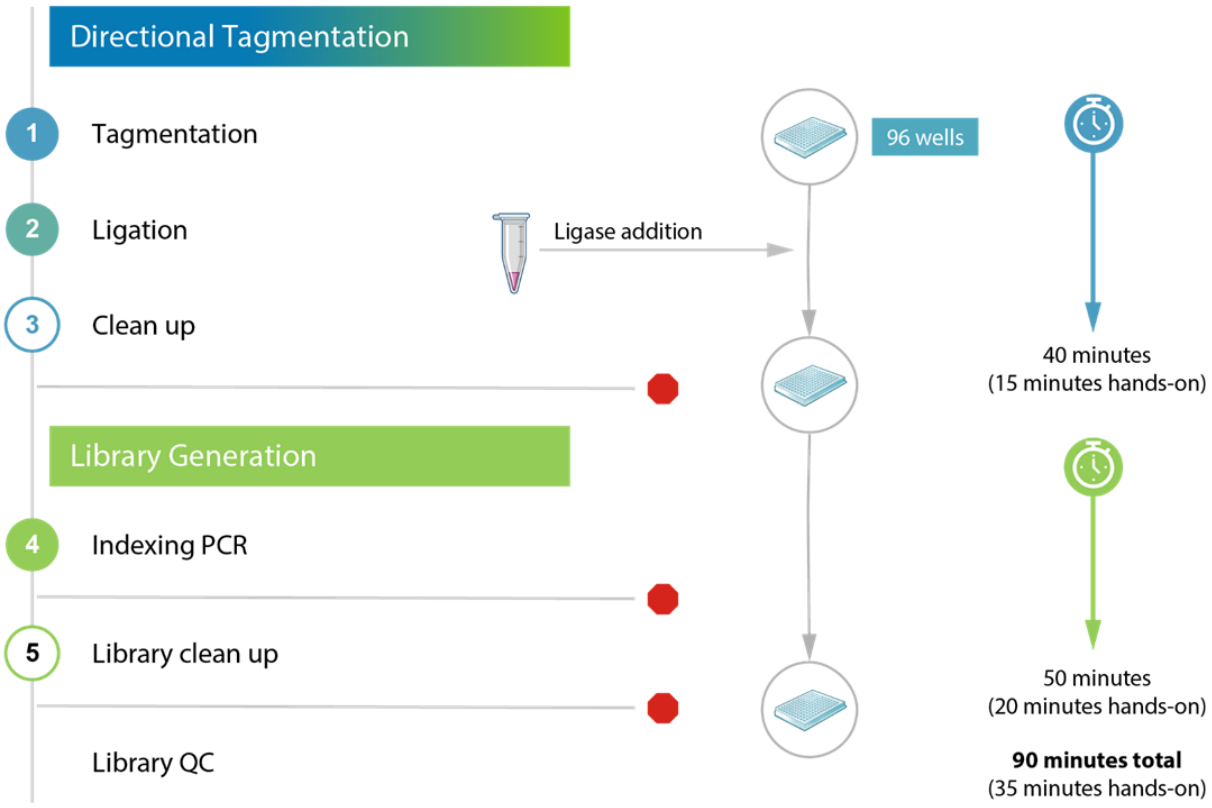
Hybrid capture, sequencing, and library QC reagents must be purchased separately.

Please refer to Appendix B for important information on required substitutions to blocking reagents for downstream hybrid capture workflows.

MosaiX Molecular Diagram



MosaiX Workflow Diagram



Total process time: ≤ 90 minutes
Total hands-on time: ≤ 35 minutes

Kit Components

Table 1. MosaiX Early Access Kit Components

MosaiX Early Access Kit, 24 Reactions

Catalog No.: 301436

Item	Component	REF	Description	Storage	Qty
Box	TnX Read 1 Tagging Reagent	301461	0.5 ml tube (white cap) – 225 µl	-20° C	1
	Ligation Enhancer	301465	0.5 ml tube (yellow cap) – 60 µl	-20° C	1
	5x Reaction Buffer	301471	0.5 ml tube (orange cap) – 240 µl	-20° C	1
	Read 2 Adapter	301463	0.5 ml tube (blue cap) – 60 µl	-20° C	1
	Ligase	301469	0.5 ml tube (green cap) – 30 µl	-20° C	1
	2x Amplification Master Mix	301473	2 ml tube (clear cap) – 750 µl	-20° C	1
	Diluent	301467	0.5 ml tube (red cap) – 135 µl	-20° C	1
Bottle	MAGwise paramagnetic beads	101003	10 ml bottle – 5 ml	4° C	1

MosaiX Early Access Kit, 96 Reactions

Catalog No.: 301430

Item	Component	REF	Description	Storage	Qty
Box	TnX Read 1 Tagging Reagent	301440	2 ml tube (white cap) – 900 µl	-20° C	1
	Ligation Enhancer	301444	0.5 ml tube (yellow cap) – 240 µl	-20° C	1
	5x Reaction Buffer	301450	2 ml tube (orange cap) – 960 µl	-20° C	1
	Read 2 Adapter	301442	0.5 ml tube (blue cap) – 240 µl	-20° C	1
	Ligase	301448	0.5 ml tube (green cap) – 120 µl	-20° C	1
	2x Amplification Master Mix	301452	2 ml tube (clear cap) – 1.5 ml each	-20° C	2
	Diluent	301446	2 ml tube (red cap) – 540 µl	-20° C	1
Bottle	MAGwise paramagnetic beads	101002	15 ml bottle – 15 ml	4° C	1

UDI primers provided for Early Access (see MosaiX Index List for UDI info):

UDI Index Primers, 24 reactions

Catalog No.: 301435

Item	Component	REF	Description	Storage	Qty
Box	UDI Index Primers	301435	96-well plate - 24 wells with 8 µl of primer mix per well	-20° C	1

UDI Index Primers, 96 reactions

Catalog No.: 301429

Item	Component	REF	Description	Storage	Qty
Box	UDI Index Primers	301429	96-well plate - 96 wells with 8 µl of primer mix per well	-20° C	1

User-Supplied Reagents, Equipment, Consumables, and Thermal Cycler Program

Reagents

- 100% Ethanol (molecular biology grade)
- 10 mM Tris-HCl, pH 8.0
- TE Buffer (10 mM Tris-HCl + 1mM EDTA, pH 8.0)
- Ultrapure Water (molecular biology grade)
- Qubit™ 1X dsDNA High Sensitivity (HS) Assay Kit (ThermoFisher P/N: Q33230), Quant-iT™ PicoGreen™ dsDNA Assay Kits (ThermoFisher P/N: P7589), or other validated dsDNA quantification assay
- High Sensitivity D5000 ScreenTape Assay for the TapeStation (Agilent P/N: 5067-5592, 5067-5593)

Equipment & Consumables

- 96-well thermal cycler
- Benchtop centrifuge to pulse-spin tubes and 8-tube PCR strips
- Plate centrifuge
- Vortex mixer
- Qubit or plate-based fluorometer
- TapeStation
- Magnetic stand for 8-tube PCR strip and/or 96-well plate
- Single-channel pipettors (1-20 µl, 20-200 µl, 100-1,000 µl)
- Multi-channel pipettors (1-10 µl, 10-200 µl)
- Pipette tips (low-retention barrier tips preferred)
- Eppendorf Tubes® (1.5 ml and 2.0 ml DNA LoBind Tubes)
- 96-well PCR plates or 8-tube PCR strip tubes with caps
- Evaporation resistant seals for PCR plates

Thermal Cycler Programs:

- **XTAG:** 37°C for 7 minutes (lid heating set to 100°C)
  95°C for 3 minutes
  4°C hold

- **LIGATE:** 25°C for 5 minutes (lid heating OFF)
  4°C hold

- **PCR Amplification:**

  98˚C for 45 seconds (lid heating set to 105°C)
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  8 cycles of:
  98˚C for 15 seconds
  60˚C for 30 seconds
  72˚C for 30 seconds

  72˚C for 1 minute
  4˚C HOLD

Before Starting the Procedure:

Measure and adjust input DNA concentration. Assay the DNA concentration of each sample using Qubit, PicoGreen or other validated dsDNA assay. Adjust input DNA concentration to 5 - 10 ng/µl using TE buffer (total input of 50 - 100 ng).

> **If available sample DNA quantity is below this recommended range, please contact earlyaccess@seqwell.com before proceeding for recommendations on adjusting tagging conditions to accommodate lower DNA input amounts.**

Program thermal cycler. For convenience, set up all applicable thermal cycler programs described in the protocol before starting.

Mix and pulse-spin kit components. Liquids can condense or shift locations inside containers during shipment and/or storage. Before dispensing from reagent tubes, always mix and pulse-spin before use.

Handling of MAGwise bads. MAGwise beads can be stored for up to 2 weeks at room temperature or for longer periods at 2° - 8°C. Equilibrate to room temperature for at least 30 minutes before use. Vortex well to thoroughly resuspend beads prior to use. When working with MAGwise, aspirate and dispense carefully to ensure that extra beads are not clinging to the outside of the tip. Always pipette mix thoroughly ≥10X or vortex immediately after beads have been added to ensure that the beads and samples are mixed homogeneously.

Prepare 80% ethanol fresh daily. The volume required will depend on the number of samples processed. Dilute ethanol in molecular biology grade ultrapure water.

If needed, prepare 10 mM Tris-HCl, pH 8.0. If you do not have access to pre-diluted 10 mM Tris-HCl pH 8.0, please prepare 10 mM Tris-HCl, pH 8.0 from a concentrated stock solution diluted with ultrapure water (molecular-biology grade).

If needed, prepare 1X TE buffer. If you do not have access to pre-diluted 1X TE, please prepare 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) from a concentrated stock solution diluted with ultrapure water (molecular-biology grade).

Safe stopping points are indicated in the protocol. For optimal results, proceed directly to the next step unless a safe stopping point is indicated.

For hybrid capture workflows, please see Appendix B for important information on required blocker substitutions to accommodate transposase libraries.

Information on MosaiX Indexing Primers

seqWell is providing 24 or 96 unique dual indexed primers (10 bp indexes) during Early Access to facilitate testing. However, you may also supply your own custom primers or use commercially available transposase-compatible primers from other library prep solution providers. See MosaiX Early Access Index List for index information.

Because transposases require a specific 19-base sequence in order to bind the adapters (known as the mosaic end of ME sequence), the primers used **must be compatible with transposase-specific adapters**. Primers that are compatible with traditional ligation-based library prep kits are not compatible with transposase-based adapters. If not using seqWell provided primers, verify that the sequences of your primers match the following:

i7 Indexing Primer:

5’ CAAGCAGAAGACGGCATACGAGAT[i7 index]GTCTCGTGGGCTCGG 3’

i5 Indexing Primer:

5’ AATGATACGGCGACCACCGAGATCTACAC[i5 index]TCGTCGGCAGCGTC 3’

Additional note: Primers i7 and i5 should be mixed at a concentration of 10 µM each.

For questions or recommendations for where to obtain additional indexing primers, please contact earlyaccess@seqwell.com.

Procedure – MosaiX DNA Library Prep

This user guide provides two protocol variations:

- Libraries sized for whole genome sequencing (WGS) to generate sequencing mean insert sizes of 400 - 500 bp: Starting on Page 14.
- Libraries sized for targeted hybrid capture to generate sequencing mean insert sizes of 250 - 350 bp: Starting on Page 24.

Important Note

The library fragment size distribution for both protocols has been optimized for the NovaSeq X Plus, which has a substantial size bias towards smaller fragments. To compensate for this, the libraries generated here are intentionally larger when assayed on a TapeStation (see Appendix A).

However, if smaller average fragment sizes are desired, this can be achieved by increasing the MAGwise bead ratios of the post-PCR clean up step, for example increasing from a 0.75X bead volume ratio to 1.0X ratio.

See Appendix B for expected fragment distributions when using different MAGwise ratios. For more guidance, please contact support@seqwell.com.

Before beginning either method:

- Ensure that each genomic DNA sample has been adjusted to 5 - 10 ng/µl **(total DNA input of 50 - 100 ng in 10 µl)** using 1X TE Buffer (10mM Tris-HCl, 1mM EDTA).
- For both methods, thaw 5X Reaction Buffer (orange), Ligation Enhancer (yellow), Read 2 Adapter (blue), and UDI primer plate at room temperature. For WGS method only, also thaw Diluent (red) at room temperature. Note: Diluent is not needed in the hybrid capture library workflow.
- Place TnX Read 1 Tagging Reagent (white), Ligase (green), and 2X Amplification Master Mix on ice.

Safe stopping points are noted throughout both protocols. If a safe stopping point is not indicated, please proceed directly to the next step.

MosaiX DNA Library Prep - Protocol for Libraries Sized Optimally for Whole Genome Sequencing (WGS)

Before you begin:

- Ensure that each genomic DNA sample has been adjusted to 5 - 10 ng/µl **(total DNA input of 50 - 100 ng in 10 µl)** using 1X TE Buffer (10mM Tris-HCl, 1mM EDTA).
- Thaw 5X Reaction Buffer (orange), Ligation Enhancer (yellow), Read 2 Adapter (blue), and Diluent (red) and UDI primer plate at room temperature at room temperature.
- Remove MAGwise beads from 4°C and equilibrate to room temperature for at least 30 minutes.
- Place TnX Read 1 Tagging Reagent (white), Ligase (green), and 2X Amplification Master Mix (clear) on ice.

1. Tagmentation Reaction

a. Vortex 5X Reaction Buffer (orange), Diluent (red) and Ligation Enhancer (yellow) at 90% speed for 2-3 seconds and pulse-spin to collect liquid at bottom of tube.

b. Vortex TnX Read 1 Tagging Reagent (white) at 60% speed for 2-3 seconds and pulse-spin to collect liquid at bottom of tube.

NOTE: If excessive bubbling is observed, continue to pulse-spin until solution is homogeneous.

c. To clean a 1.5 or 2.0 ml tube, prepare a TAGMENTATION MASTER MIX by adding the following components in the order listed below.

The table provides master mix volumes for 8, 24, and 96 samples. For other batch sizes, simply multiply the per sample volumes by the number of samples being processed and add 12.5% extra to allow for overage.

Reagents	Volume per sample	Master mix 8 samples *w/ 12.5% overage*	Master mix 24 samples *w/ 12.5% overage*	Master mix 96 samples *w/ 12.5% overage*
5X Reaction Buffer (orange)	5 µl	45 µl	135 µl	540 µl
Ligation Enhancer (yellow)	2 µl	18 µl	54 µl	216 µl
TnX Read 1 Tagging Reagent (white)	6 µl	54 µl	162 µl	648 µl
Diluent (red)	2 µl	18 µl	54 µl	216 µl
Total Volume	**15 µl**	**135 µl**	**405 µl**	**1620 µl**

d. After all reagents have been added to the TAGMENTATION MASTER MIX tube, cap and vortex at 70% speed for 2-3 seconds to mix and pulse spin down briefly. Place master mix on ice if not immediately proceeding to reaction set up.

e. Vortex genomic DNA samples and pulse-spin.

f. Add 10 µl of DNA (50-100 ng total input) to each well of a new 96-well REACTION PLATE or to REACTION TUBE(S).

g. Dispense 15 µl of TAGMENTATION MASTER MIX to each sample in the REACTION PLATE or TUBES(S) and mix thoroughly by pipetting up and down ≥10x at with pipette set to 15 µl. **It is crucial that the reactions are well mixed.** Note: a small amount of bubbles/foaming will not affect results.

h. Seal REACTION PLATE or TUBE(S) and transfer to a thermal cycler and run the XTAG program, with lid-heating set to 100°C:

    37°C for 7 minutes
    95°C for 3 minutes
    4°C hold

i. Return the Ligation Enhancer (yellow), TnX Read 1 Tagging Reagent (white), and Diluent (red) to the freezer.

    Useful tip: The LIGATION MASTER MIX used in the next step can be prepared while the XTAG program is running. Please refer to Section 2 below, steps “b.” though “e.” and once made, keep the mix on ice until ready to use.

j. Once the program is complete, proceed immediately to the next ligation step.

2. Ligation Reaction

a. Remove the REACTION PLATE or TUBE(S) from the thermal cycler and pulse spin briefly.

b. Vortex Ligase (green) at 60% speed for 2-3 seconds and pulse-spin to collect liquid at the bottom of the tube.

c. Vortex 5X Reaction Buffer (orange) and Read 2 Adapter (blue) at 90% speed for 2-3 seconds and pulse-spin to collect liquid at the bottom of the tube.

d. To clean a 1.5 ml or 2.0 ml tube, prepare a LIGATION MASTER MIX by adding the following components in the order listed below. The table provides master mix volumes for 8, 24, and 96 samples. For other batch sizes, simply multiply the per sample volumes by the number of samples being processed and add 12.5% extra to allow for overage.

Reagents	**Volume per sample**	**Master mix 8 samples** ***w/ 12.5% overage***	**Master mix 24 samples** ***w/ 12.5% overage***	**Master mix 96 samples** ***w/ 12.5% overage***
Read 2 Adapter (blue)	2 µl	18 µl	54 µl	216 µl
5X Reaction Buffer (orange)	3 µl	27 µl	81 µl	324 µl
Ultrapure Water	9 µl	81 µl	243 µl	972 µl
Ligase (green)	1 µl	9 µl	27 µl	108 µl
Total Volume	**15 µl**	**135 µl**	**405 µl**	**1620 µl**

e. After all reagents have been added to the LIGATION MASTER MIX tube, cap and vortex at 70% speed for 2-3 seconds to mix and pulse spin down briefly.

f. Unseal the REACTION PLATE or TUBE(S) and dispense 15 µl of LIGATION MASTER MIX to each sample and mix thoroughly by pipetting up and down ≥10x at with pipette set to 15 µl. **It is crucial that the reactions are well mixed.** Note: a small amount of bubbles/foaming will not affect results.

g. Seal REACTION PLATE or TUBE(S) and pulse-spin to collect liquid at the bottom of reaction vessels.

h. Transfer the REACTION PLATE or TUBE(S) to a thermal cycler and run the LIGATION program, with lid-heating OFF:

    25°C for 5 minutes
    4°C hold

i. Return the Read 2 Adapter (blue), 5X Reaction Buffer (orange), and Ligase (green) to the freezer.

j. Once the program is complete, proceed directly to the next step.

3. Bead Purification for WGS-Sized Libraries

The MAGwise bead volume ratio used here (0.65X) will produce an average library fragment size (with adapters) of 850 – 1050 bp, which should generate 400 - 500 bp sequencing mean insert sizes on the NovaSeq X Plus.

To generate smaller sized libraries, the ratio of MAGwise beads can be increased. See Appendix B for example fragment sizes using different bead ratios.

a. Pulse-spin the REACTION PLATE or TUBE(S) in a centrifuge then carefully open the tube or remove the seal.

b. Vortex room temperature MAGwise beads to ensure that the beads are fully resuspended. The bead mixture should be completely homogenous before use.

c. Working one column at a time, add 40 µl of MAGwise beads (1X volumetric equivalent) to each well and mix thoroughly by pipetting up and down at least 10-15 times with pipette set to 40 µl (if in a tube or strip tube, you may vortex briefly and pulse spin down). Ensure that the beads and sample mixture is fully homogenous.

d. Incubate on the bench for 5 minutes to allow the DNA to bind to the beads.

e. Place the tube(s) or plate on a magnetic stand and allow the bead pellet to form and the supernatant appears completely clear (~5 minutes).

f. Remove and discard the supernatant with pipette. Be careful not to disturb the bead pellet.

g. Wash beads with 80% ethanol.
    i. With the tube still in the magnetic stand, add 150 µl 80% ethanol.
    ii. After ≥30 seconds, remove and discard the supernatant being careful not to dislodge the bead pellet.
    iii. Repeat the previous step for a total of 2 washes with 80% ethanol. Remove and discard the supernatant.

h. Perform the next steps quickly to avoid overdrying the bead pellets:
    i. Pulse-spin, and return to magnet, letting beads settle (<30 seconds). Use a small pipet tip (≤20 µl) to remove any residual ethanol at the bottom of the tube. DO NOT air-dry bead pellet prior to elution as DNA recovery may be compromised.

ii. Add 22 µl of 10mM Tris-HCl to each tube and mix thoroughly by pipetting the solution along the inner wall of the tube multiple times to thoroughly resuspend the bead pellet.

i. Incubate the resuspended beads on the bench for 5 minutes to elute the purified DNA from the beads.

j. Return tube to magnetic stand and allow a bead pellet to reform on the inner wall of the tube (~2 minutes) until supernatant is completely clear.

k. When the supernatant has completely cleared, carefully transfer 20 µl of DNA eluate from each tube to a 96-well plate or 8-tube PCR strip(s) for library amplification. The transferred supernatant contains the purified DNA product.

SAFE STOPPING POINT

Proceed immediately to the next step
or store the pooled purified library at -20°C.

4. PCR Amplification

a. *Before beginning*:

   i. Remove 2X Amplification Master Mix (clear) from the freezer and thaw on ice. Once thawed, mix the 2X Amplification Master Mix by vortexing at 70% speed for at least 15 seconds and briefly spin down. **It is crucial to mix the Amplification Master Mix before use.**

   ii. Remove the UDI indexing primers from the freezer and thaw at room temperature.

   ***NOTE:** seqWell will supply 24 or 96 UDI indexing primers during Early Access. However, any transposase adapter compatible indexing primers at 10 µM each primer can be used. See page 12 for more details or contact us for additional recommendations for primer sources.

b. To set up the PCR reaction:

   i. Unseal the 96-well plate or tube(s) containing the purified unamplified libraries.

   ii. Spin down the primer plate* and remove the seal. (Note: if you are processing only a partial plate, you may carefully use a razor blade to cut the seal for only the wells of the primer plate you will be using. After use, re-seal the used wells).

   iii. With a pipette set to 5 µl, gently mix the primers in the primer plate by pipetting up and down 5x and then transfer 5 µl of primer to the reaction plate or tube(s). Note what wells you used for downstream index info.

   iv. Add 25 µl of well mixed 2X Amplification Master Mix (clear) to each well or tube and pipette mix ≥10X with pipette set to 25 µl. (The total PCR reaction volume is 50 µl).

c. Seal or cap and pulse-spin the 96-well plate or tube(s) then transfer to a thermal cycler and run the PCR Amplification cycling program below, with lid-heating set to 105˚C:

   98˚C for 45 seconds (lid heating 105°C)

   8 cycles of:

      98˚C for 15 seconds

      60˚C for 30 seconds

      72˚C for 30 seconds

   72˚C for 1 minute

   4˚C HOLD

NOTE: *This program takes ~20 minutes.*

d. Return primers and the 2X Amplification Master Mix (clear) to the freezer.

5. Post-PCR Bead Purification

a. Pulse-spin the 96-well plate or PCR tubes(s) in a centrifuge and carefully open.

b. To each well or tube, add 50 µl of Ultrapure water to bring the volume of the reaction up to 100 µl. Vortex or mix thoroughly by pipetting up and down ≥10x with pipette set to 50 µl.

c. Vortex room temperature MAGwise beads to ensure that the beads are fully resuspended. The bead mixture should be completely homogenous before use.

d. Working one column at a time, add 65 µl of MAGwise beads (0.65X volumetric equivalent) to each well and mix thoroughly by pipetting up and down at least 10-15 times with pipette set to 40 µl (if in a tube or strip tube, you may vortex briefly and pulse spin down). Ensure that the beads and sample mixture is fully homogenous.

e. Incubate on the bench for 5 minutes to allow the DNA to bind.

f. Place the tube(s) on a magnetic stand and let the bead pellet form on one side of the tube and wait until the supernatant appears completely clear (~5 minutes).

g. Remove and discard the supernatant with pipette. Be careful not to disturb the bead pellet.

h. Wash beads with 80% ethanol:
    i. With the tube still in the magnetic stand, add 150 µl 80% ethanol to cover the bead pellet.
    ii. After ≥30 seconds, remove and discard the supernatant being careful not to dislodge the bead pellet.
    iii. Repeat the previous step for a total of 2 washes with 80% ethanol. Remove and discard the supernatant.

i. Perform the next steps quickly to avoid over drying the bead pellets:
    i. Cap tubes or seal plate, pulse-spin, and return to magnet, letting beads settle (<30 seconds). Use a small pipet tip (≤20 µl) to remove any residual ethanol at the bottom of the tube. DO NOT air-dry bead pellet prior to Tris or Low TE addition. Overdrying bead pellet will compromise library recovery.
    ii. Add 24 µl 10mM Tris-HCl (or Low TE buffer) to each tube and mix by pipetting the solution along the inner wall of the tube multiple times to thoroughly resuspend the bead pellet. Incubate the resuspended beads on the bench for 5 minutes to elute the purified library from the beads.

j. Return tube to magnetic stand and allow a bead pellet to reform on the inner wall of the tube (~2 minutes).

k. When the supernatant has completely cleared, carefully transfer 22 µl of DNA eluate from each tube to a fresh tube. The transferred supernatant contains the purified library.

l. Proceed to downstream QC (Appendix A).

MosaiX DNA Library Prep - Protocol for Libraries Sized Optimally for Hybrid Capture

Before you begin:

- Ensure that each genomic DNA sample has been adjusted to 5 - 10 ng/µl **(total DNA input of 50 - 100 ng in 10 µl)** using 1X TE Buffer (10mM Tris-HCl, 1mM EDTA).
- Thaw 5X Reaction Buffer (orange), Ligation Enhancer (yellow), Read 2 Adapter (blue), and UDI primer plate at room temperature. Also remove MAGwise beads from 4°C to equilibrate to room temperature.
- Place TnX Read 1 Tagging Reagent (white), Ligase (green), and 2X Amplification Master Mix (clear) on ice.
  Note: Diluent (red) is *not* needed for the hybrid capture library workflow.

1. Tagmentation Reaction

a. Vortex 5X Reaction Buffer (orange) and Ligation Enhancer (yellow) at 90% speed for 2-3 seconds and pulse-spin to collect liquid at bottom of tube.

b. Vortex TnX Read 1 Tagging Reagent (white) at 60% speed for 2-3 seconds and pulse-spin to collect liquid at bottom of tube.

   NOTE: If excessive bubbling is observed, continue to pulse-spin until solution is homogeneous.

c. To clean a 1.5 or 2.0 ml tube, prepare a TAGMENTATION MASTER MIX by adding the following components in the order listed below.

   The table provides master mix volumes for 8, 24, and 96 samples. For other batch sizes, simply multiply the per sample volumes by the number of samples being processed and add 12.5% extra to allow for overage.

Reagents	Volume per sample	Master mix 8 samples *w/ 12.5% overage*	Master mix 24 samples *w/ 12.5% overage*	Master mix 96 samples *w/ 12.5% overage*
5X Reaction Buffer (orange)	5 µl	45 µl	135 µl	540 µl
Ligation Enhancer (yellow)	2 µl	18 µl	54 µl	216 µl
TnX Read 1 Tagging Reagent (white)	8 µl	72 µl	216 µl	864 µl
Total Volume	**15 µl**	**135 µl**	**405 µl**	**1620 µl**

d. After all reagents have been added to the TAGMENTATION MASTER MIX tube, cap and vortex at 70% speed for 2-3 seconds to mix and pulse spin down briefly. Place master mix on ice if not immediately proceeding to reaction set up.

e. Vortex genomic DNA samples and pulse-spin.

f. Add 10 µl of DNA (50-100 ng total input) to each well of a new 96-well REACTION PLATE or to REACTION TUBE(S).

g. Dispense 15 µl of TAGMENTATION MASTER MIX to each sample in the REACTION PLATE or TUBES(S) and mix thoroughly by pipetting up and down ≥10x at with pipette set to 15 µl. **It is crucial that the reactions are well mixed.** Note: a small amount of bubbles/foaming will not affect results.

h. Seal REACTION PLATE or TUBE(S) and transfer to a thermal cycler and run the XTAG program, with lid-heating set to 100°C:

    37°C for 7 minutes
    95°C for 3 minutes
    4°C hold

i. Return the Ligation Enhancer (yellow) and TnX Read 1 Tagging Reagent (white) to the freezer.

    Useful tip: The LIGATION MASTER MIX used in the next step can be prepared while the XTAG program is running. Please refer to Section 2 below, steps “b.” though “e.” and once made, keep the mix on ice until ready to use.

j. Once the program is complete, proceed immediately to the next ligation step.

2. Ligation Reaction

a. Remove the REACTION PLATE or TUBE(S) from the thermal cycler and pulse spin briefly.

b. Vortex Ligase (green) at 60% speed for 2-3 seconds and pulse-spin to collect liquid at the bottom of the tube.

c. Vortex 5X Reaction Buffer (orange) and Read 2 Adapter (blue) at 90% speed for 2-3 seconds and pulse-spin to collect liquid at the bottom of the tube.

d. To clean a 1.5 ml or 2.0 ml tube, prepare a LIGATION MASTER MIX by adding the following components in the order listed below. The table provides master mix volumes for 8, 24, and 96 samples. For other batch sizes, simply multiply the per sample volumes by the number of samples being processed and add 12.5% extra to allow for overage.

Reagents	**Volume per sample**	**Master mix 8 samples** ***w/ 12.5% overage***	**Master mix 24 samples** ***w/ 12.5% overage***	**Master mix 96 samples** ***w/ 12.5% overage***
Read 2 Adapter (blue)	2 µl	18 µl	54 µl	216 µl
5X Reaction Buffer (orange)	3 µl	27 µl	81 µl	324 µl
Ultrapure Water	9 µl	81 µl	243 µl	972 µl
Ligase (green)	1 µl	9 µl	27 µl	108 µl
Total Volume	**15 µl**	**135 µl**	**405 µl**	**1620 µl**

e. After all reagents have been added to the LIGATION MASTER MIX tube, cap and vortex at 70% speed for 2-3 seconds to mix and pulse spin down briefly.

f. Unseal the REACTION PLATE or TUBE(S) and dispense 15 µl of LIGATION MASTER MIX to each sample and mix thoroughly by pipetting up and down ≥10x at with pipette set to 15 µl. **It is crucial that the reactions are well mixed.** Note: a small amount of bubbles/foaming will not affect results.

g. Seal REACTION PLATE or TUBE(S) and pulse-spin to collect liquid at the bottom of reaction vessels.

h. Transfer the REACTION PLATE or TUBE(S) to a thermal cycler and run the LIGATION program, with lid-heating OFF:

    25°C for 5 minutes
    4°C hold

i. Return the Read 2 Adapter (blue), 5X Reaction Buffer (orange), and Ligase (green) to the freezer.

j. Once the program is complete, proceed directly to the next step.

3. Bead Purification

a. Pulse-spin the REACTION PLATE or TUBE(S) in a centrifuge then carefully open the tube or remove the seal.

b. Vortex room temperature MAGwise beads to ensure that the beads are fully resuspended. The bead mixture should be completely homogenous before use.

c. Working one column at a time, add 50 µl of MAGwise beads (1.25X volumetric equivalent) to each well and mix thoroughly by pipetting up and down at least 10-15 times with pipette set to 40 µl (if in a tube or strip tube, you may vortex briefly and pulse spin down). Ensure that the beads and sample mixture is fully homogenous.

d. Incubate on the bench for 5 minutes to allow the DNA to bind to the beads.

e. Place the tube(s) or plate on a magnetic stand and allow the bead pellet to form and the supernatant appears completely clear (~5 minutes).

f. Remove and discard the supernatant with pipette. Be careful not to disturb the bead pellet.

g. Wash beads with 80% ethanol.
    i. With the tube still in the magnetic stand, add 150 µl 80% ethanol.
    ii. After ≥30 seconds, remove and discard the supernatant being careful not to dislodge the bead pellet.
    iii. Repeat the previous step for a total of 2 washes with 80% ethanol. Remove and discard the supernatant.

h. Perform the next steps quickly to avoid overdrying the bead pellets:
    i. Pulse-spin, and return to magnet, letting beads settle (<30 seconds). Use a small pipet tip (≤20 µl) to remove any residual ethanol at the bottom of the tube. DO NOT air-dry bead pellet prior to elution as DNA recovery may be compromised.
    ii. Add 22 µl of 10mM Tris-HCl to each tube and mix thoroughly by pipetting the solution along the inner wall of the tube multiple times to thoroughly resuspend the bead pellet.

i. Incubate the resuspended beads on the bench for 5 minutes to elute the purified DNA from the beads.

j. Return tube to magnetic stand and allow a bead pellet to reform on the inner wall of the tube (~2 minutes) until supernatant is completely clear.

k. When the supernatant has completely cleared, carefully transfer 20 µl of DNA eluate from each tube to a 96-well plate or 8-tube PCR strip(s) for library amplification. The transferred supernatant contains the purified DNA product.

SAFE STOPPING POINT

Proceed immediately to the next step
or store the pooled purified library at -20°C.

4. PCR Amplification

a. *Before beginning*:
   i. Remove 2X Amplification Master Mix (clear) from the freezer and thaw on ice. Once thawed, mix the 2X Amplification Master Mix by vortexing at 70% speed for at least 15 seconds and briefly spin down. It is crucial to mix the Amplification Master Mix before use.
   ii. Remove the UDI indexing primers from the freezer and thaw at room temperature.

b. To set up the PCR reaction:
   i. Unseal the 96-well plate or tube(s) containing the purified unamplified libraries.
   ii. Spin down the primer plate* and remove the seal. (Note: if you are processing only a partial plate, you may carefully use a razor blade to cut the seal for only the wells of the primer plate you will be using. After use, re-seal the used wells).
   iii. With a pipette set to 5 µl, gently mix the primers in the primer plate by pipetting up and down 5x and then transfer 5 µl of primer to the reaction plate or tube(s). Note what wells you used for downstream index info.
   iv. Add 25 µl of well mixed 2X Amplification Master Mix (clear) to each well or tube and pipette mix ≥10X with pipette set to 25 µl. (The total PCR reaction volume is 50 µl).

   ***NOTE:** seqWell will supply 24 or 96 UDI indexing primers during Early Access. However, any transposase adapter compatible indexing primers at 10 µM each primer can be used. See page 12 for more details or contact us for additional recommendations for primer sources.

c. Seal or cap and pulse-spin the 96-well plate or tube(s) then transfer to a thermal cycler and run the PCR Amplification cycling program below, with lid-heating set to 105˚C:

98˚C for 45 seconds (lid heating 105°C)
8 cycles of:
  98˚C for 15 seconds
  60˚C for 30 seconds
  72˚C for 30 seconds
72˚C for 1 minute
4˚C HOLD

NOTE: *This program takes ~20 minutes.*

d. Return primers and the 2X Amplification Master Mix (clear) to the freezer.

5. Post-PCR Bead Purification

> The MAGwise bead volume ratio used here (0.75X) will produce an average library fragment size (with adapters) of 700 - 850 bp which should generate 250 - 350 bp sequencing mean insert sizes on the NovaSeq X Plus.
>
> To generate smaller sized libraries, the ratio of MAGwise beads can be increased. See Appendix B for example fragment sizes using different bead ratios.

a. Pulse-spin the 96-well plate or PCR tubes(s) in a centrifuge and carefully open.

b. Vortex room temperature MAGwise beads to ensure that the beads are fully resuspended. The bead mixture should be completely homogenous before use.

c. Working one column at a time, add 33.75 µl of MAGwise beads (0.75X volumetric equivalent) to each well and mix thoroughly by pipetting up and down at least 10-15 times with pipette set to 40 µl (if in a tube or strip tube, you may vortex briefly and pulse spin down). Ensure that the beads and sample mixture is fully homogenous.

d. Incubate on the bench for 5 minutes to allow the DNA to bind.

e. Place the tube(s) on a magnetic stand and let the bead pellet form on one side of the tube and wait until the supernatant appears completely clear (~5 minutes).

f. Remove and discard the supernatant with pipette. Be careful not to disturb the bead pellet.

g. Wash beads with 80% ethanol:
    i. With the tube still in the magnetic stand, add 150 µl 80% ethanol to cover the bead pellet.
    ii. After ≥30 seconds, remove and discard the supernatant being careful not to dislodge the bead pellet.
    iii. Repeat the previous step for a total of 2 washes with 80% ethanol. Remove and discard the supernatant.

h. Perform the next steps quickly to avoid over drying the bead pellets:
    i. Cap tubes or seal plate, pulse-spin, and return to magnet, letting beads settle (<30 seconds). Use a small pipet tip (≤20 µl) to remove any residual ethanol at the bottom of the tube. DO NOT air-dry bead pellet prior to Tris or Low TE addition. Overdrying bead pellet will compromise library recovery.
    ii. Add 24 µl 10mM Tris-HCl (or Low TE buffer) to each tube and mix by pipetting the solution along the inner wall of the tube multiple times to thoroughly resuspend the bead pellet. Incubate the resuspended beads on the bench for 5 minutes to elute the purified library from the beads.

i. Return tube to magnetic stand and allow a bead pellet to reform on the inner wall of the tube (~2 minutes).

j. When the supernatant has completely cleared, carefully transfer 22 µl of DNA eluate from each tube to a fresh tube. The transferred supernatant contains the purified library.

k. Proceed to downstream QC (Appendix A).

Appendix A: Library QC

Library quantification: Assess library concentration via Qubit™ 1X dsDNA High Sensitivity (HS) Assay, Quant-iT™ PicoGreen™ dsDNA Assay Kits or other validated dsDNA quantification assay.

- For the WGS method, library concentrations should be >5 ng/µl (>100 ng total).
- For the Hybrid Capture method, library concentrations should be >22 ng/µl (>500 ng total).

Library sizing: Assess library sizing by electrophoretic analysis. We suggest running a 1:10 diluted aliquot of purified library on the TapeStation using the High Sensitivity D5000 kit. The majority of library fragments should be between 250 and 1,500 bp in length. To determine the average clusterable fragment size, use a region analysis for 250-1500 bp, and use this value for size adjustment with fluorometric based assays or qPCR assays for determining library concentration.

Typical results on the TapeStation High Sensitivity D5000 assay shown in Figure 1, below.

> ***Note:*** The MosaiX standard protocols have been tuned for NovaSeq X Plus which has a more pronounced size bias than previous Illumina sequencers. To compensate, the library sizes generated here are intentionally larger, but these will sequence on the NovaSeq X Plus with appropriate mean insert sizes for capture and WGS, respectively.

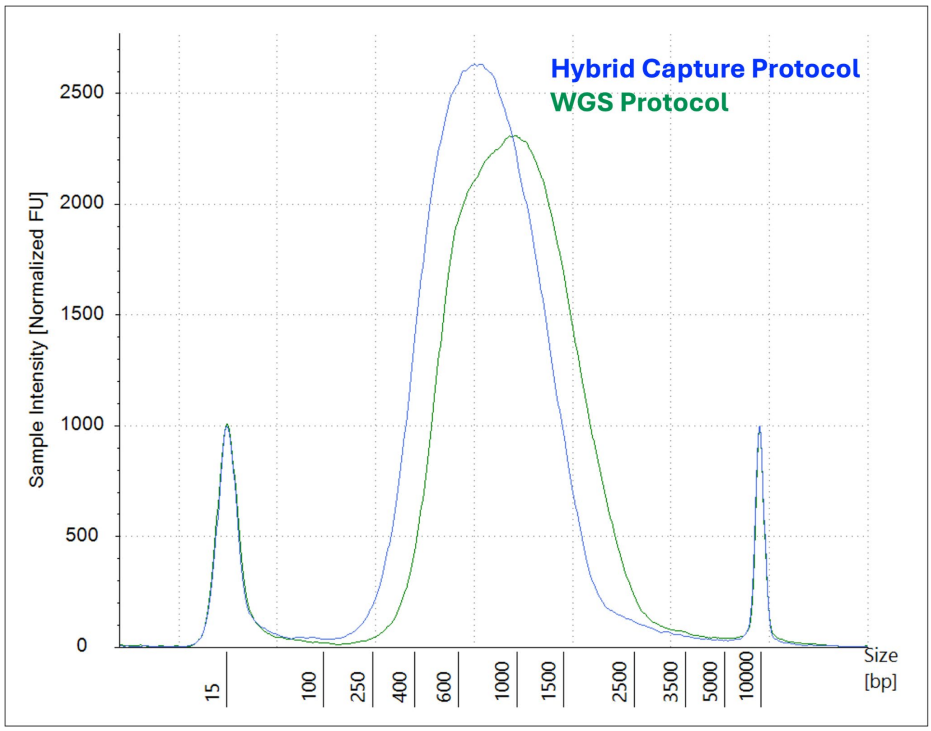
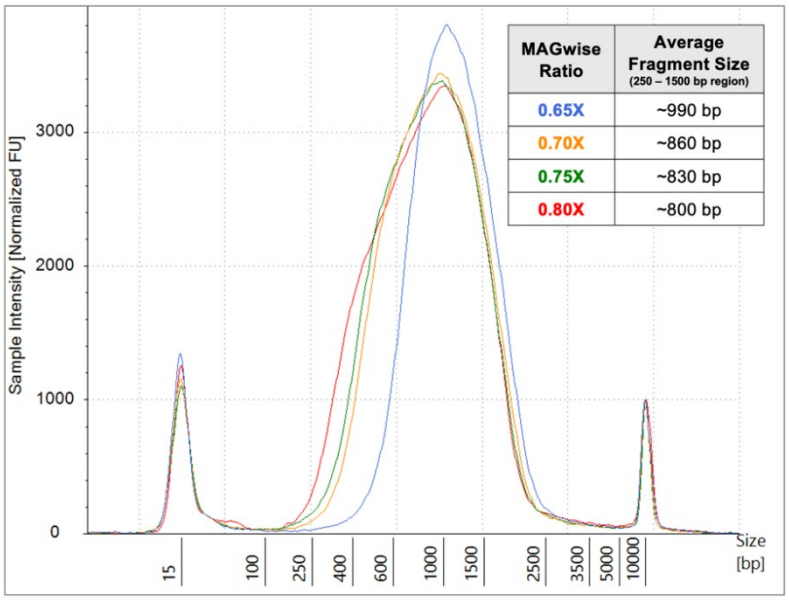


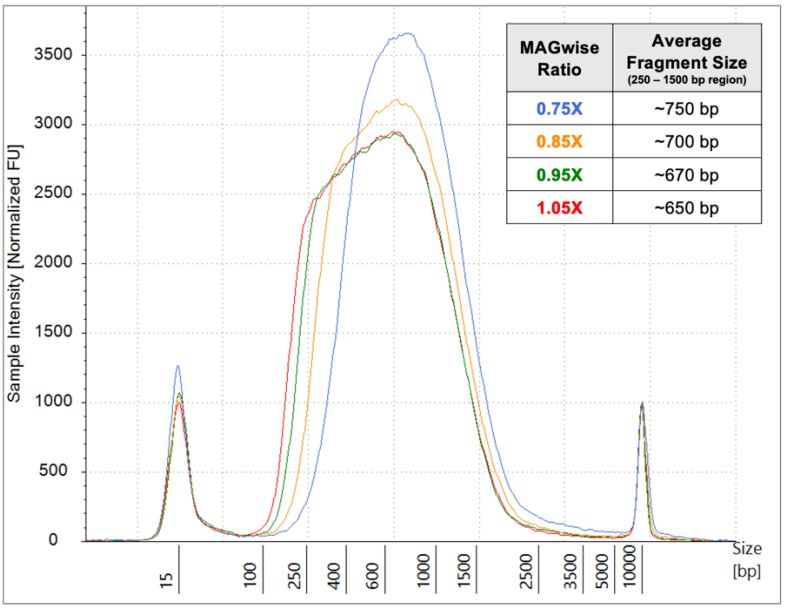
Figure 1. Representative MosaiX library trace generated using a TapeStation 2200 with HSD5000 DNA reagents and ScreenTapes. The libraries were prepared from human genomic DNA (input of 50 ng). Prior to loading on a High Sensitivity D5000 ScreenTape, the libraries were diluted 1:10. Average fragment size is ~850-1050 bp (WGS) and ~700-850 bp (Exome) using a region analysis of 250-1500 bp.

Appendix B: Adjusting Post-PCR MAGwise Bead Ratios to Generate Smaller Fragment Sizes

The library fragment size distributions generated for both methods have been optimized to compensate for the small fragment size bias observed on the NovaSeq X Plus. However, if a smaller fragment size is preferred, this can be achieved by increasing the MAGwise bead volume ratio in the post-PCR clean up step. Below are some examples for both workflows. For more guidance, please contact support@seqwell.com.



WGS workflow:
Post-PCR MAGwise ratio titration is shown at right. Average fragment sizes shown are on a TapeStation HS D5000 tape with a region analysis of 250 – 1500 bp.



Hybrid Capture workflow:
Post-PCR MAGwise ratio titration is shown at right. Average fragment sizes shown are on a TapeStation HS D5000 tape with a region analysis of 250 – 1500 bp.

Appendix C: Modifications to Twist Target Enrichment Protocol to Accommodate MosaiX Libraries

MosaiX adapters contain transposase specific adapter sequences that are not fully blocked by using Twist Bioscience's standard "Universal Blockers". Here, we provide information on modifications to the hybridization reaction set up and alternative blockers to improve % on target.

Reagents supplied by user from Twist:

- Twist Capture Panels - Exome 2.0, other off-the-shelf panels, or custom panels
- Twist Standard Hybridization V2 Reagent Kit – hybrid capture and wash buffers

NOTE: You DO NOT need to order Twist Bioscience's Universal Blocking module if using MosaiX libraries (see below).

Reagents supplied by user from other source:

- IDT xGen™ Universal Blockers, NXT (Part no. 1079584 for 16 rxn)
- IDT xGen™ Human Cot DNA (Part no. 1080768 for 150 µl)

Using MosaiX libraries in Twist Hybrid Capture:

1. Follow all steps as outlined by Twist for pooling and concentrating libraries.
2. For the hybridization reaction set up, under the "Prepare the Probe Solution" section, use the following substitutions to improve % on target when using seqWell TnX libraries:
    a. Substitute the 5 µl of Twist Blocking Solution listed in the table with 5 µl of Human Cot DNA.
    b. Substitute the 7 µl of Twist Universal Blockers listed in the table with 2 µl of IDT xGen Universal NXT blockers + 5 µl of water.
3. Once these substitutions have been made, continue with the standard Twist protocol.
    NOTE**:** Refer to Twist's user guides for all subsequent target capture and post processing instructions. For any capture specific troubleshooting, please reach out to Twist's customer support.

MosaiX libraries are also compatible with hybrid capture workflows from other technology providers. For guidance on other targeted capture workflows from these other vendors, please reach out to earlyaccess@seqwell.com.

Version	Release Date	Prior Version	Description of changes
V20250404	**April 4, 2025**	**N/A**	**Early Access V1 Protocol**

Technical Assistance

For technical assistance with MosaiX Library Preparation Kit (Early Access), contact seqWell Technical Support.

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