MosaiX™ DNA Library Prep Kit

Catalog numbers:
301458; MosaiX Early Access v2 Kit, 24 Reactions
301464; MosaiX Early Access v2 Kit, 96 Reactions

Early Access (V2) User Guide

v20250910

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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.

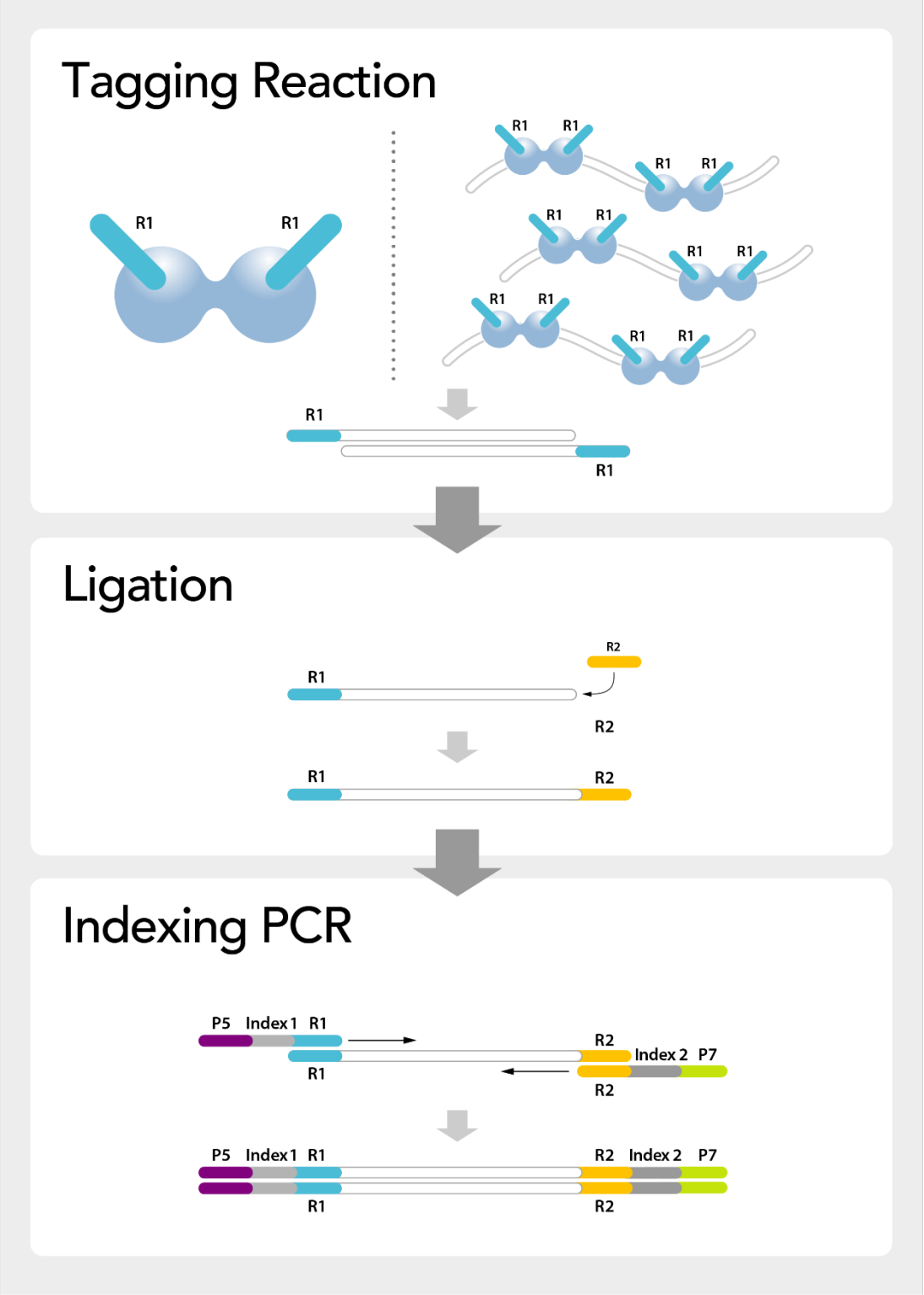
First, TnX fragments genomic DNA to the desired size range and attaches the read 1 adapter sequence to the 5' end of fragmented material. Next, the read 2 adapter is added to the 3' end using a proprietary ligation method. The resulting samples are then purified and amplified with primers containing 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 350 - 500 bp for whole-genome sequencing (WGS) or 250 - 350 bp for hybrid capture applications, optimal for 2 x 150 bp sequencing on Illumina platforms.

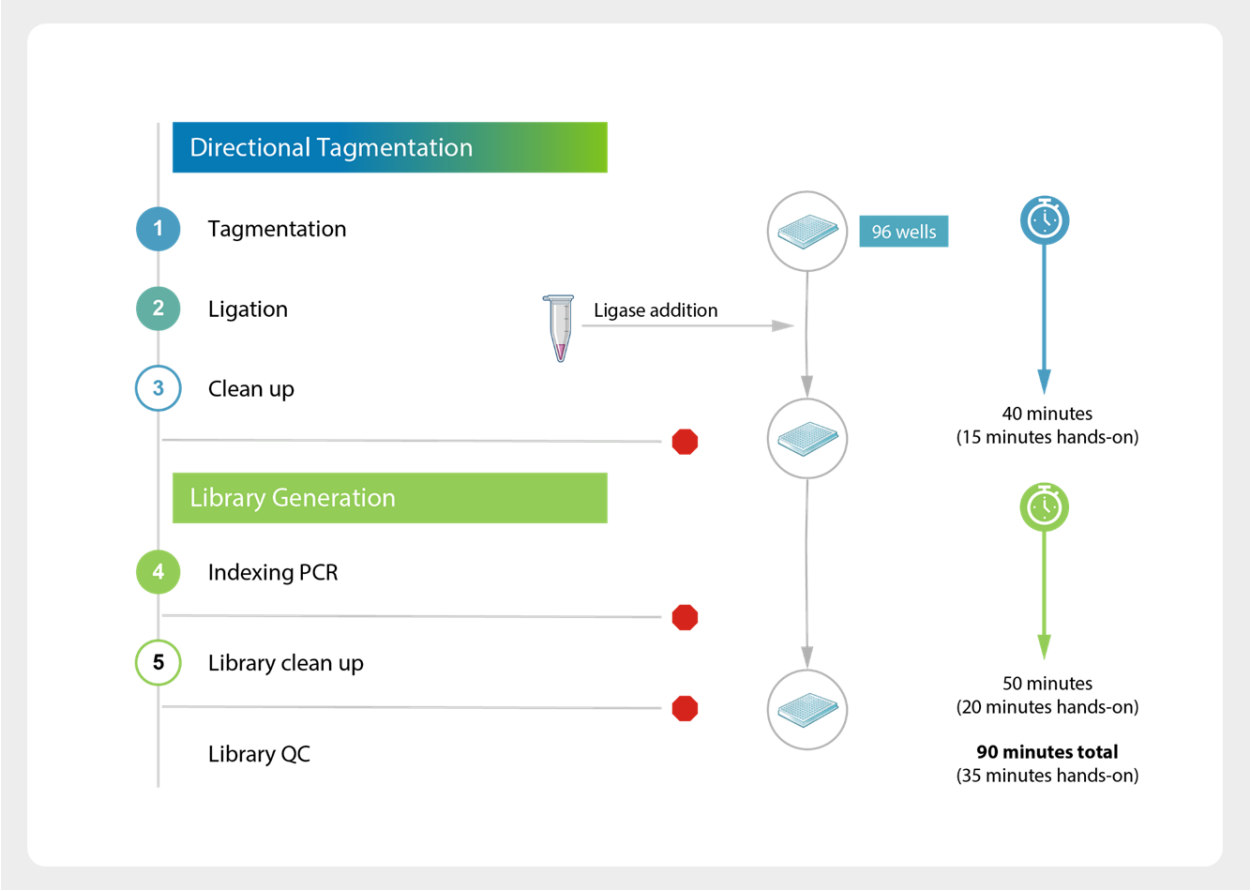
The optimal DNA input range for MosaiX is 5 - 50 ng of mid to high quality genomic DNA, however inputs as low as 1 ng can be accommodated with additional protocol modifications. Suggested modifications are provided throughout the user guide to help reduce over fragmentation and generation of adapter dimer. Nonetheless, for inputs <5 ng some conditions (tagging reagent volume, bead clean up ratios, adapter dilutions, etc) may need to be further optimized based on customer DNA source and quality.

MosaiX kits contain all enzymes, reaction buffers, adapters, and magnetic clean up beads (MAGwise) required. In addition, during the Early Access period, seqWell is providing 24 or 96 unique dual index (UDI) PCR primers, mixed at 10 µM each, at no additional cost. Index sequences for these primers are available to download from https://seqwell.com/resource-category/kit-user-guides/. Alternatively, customers may source their own compatible primers using indexes of their choice; please see Appendix B of this User Guide for more information.

MosaiX Molecular Diagram



MosaiX Workflow Diagram



Total process time: ≤ 90 minutes

Total hands-on time: ≤ 35 minutes

MosaiX Kit Components

MosaiX Early Access v2 Kit, 24 Reactions (301458):

Item	Component	REF	Description	Storage	Qty
Box	TnX™ Read 1 Tagging Reagent	301424	0.5 ml tube (white cap) - 225 µl	-20° C	1
	Tagmentation Enhancer	301477	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	301432	0.5 ml tube (blue cap) - 120 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	301431	0.5 ml tube (red cap) - 250 µl	-20° C	1
Bottle	MAGwise purification beads	101003	10 ml bottle - 5 ml	4° C	1

MosaiX Early Access v2 Kit, 96 Reactions (301464):

Item	Component	REF	Description	Storage	Qty
Box	TnX™ Read 1 Tagging Reagent	301433	2 ml tube (white cap) - 900 µl	-20° C	1
	Tagmentation Enhancer	301481	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	301455	0.5 ml tube (blue cap) - 480 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 tube	-20° C	2
	Diluent	301427	2 ml tube (red cap) - 1000 µl	-20° C	1
Bottle	MAGwise purification beads	101002	15 ml bottle - 15 ml	4° C	1

MosaiX Compatible UDI primers (included free of charge with Early Access kits):

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

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
- Electrophoretic analysis reagents - Agilent High Sensitivity D5000 ScreenTape Assay for TapeStation (Agilent P/N: 5067-5592, 5067-5593) or equivalent

Please note if MosaiX libraries will be used in hybrid capture workflows: All hybrid capture reagents must be purchased separately from their respective vendors. Please also refer to Appendix C for important information on using tagmentation-based libraries in hybrid capture workflows.

Equipment & Consumables

- 96-well thermal cycler with heated lid
- Benchtop centrifuge to pulse-spin tubes and 8-tube PCR strips
- Plate centrifuge (if using plates)
- Vortex mixer
- Magnetic stand for individual tubes and/or 8-tube PCR strip and/or 96-well plate
- Single-channel pipettors (0.5-10 µl, 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 recommended)
- 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 plate seals for PCR plates
- Electrophoretic analysis equipment - Agilent TapeStation
- Fluorometer for dsDNA quantification assay

Thermal Cycler Programs:

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

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

- **PCR Amplification:** (Lid heating set to 105°C)

45	sec	98°C	Initial denaturation
15	sec	98°C	***n*** cycles (See Step 4c on page 17 for cycle number recommendations)
30	sec	60°C	
30	sec	72°C	
1	min	72°C	Final extension
Hold		4°C	

Before starting the procedure:

Measure and adjust input DNA concentration. Assay the concentration of each genomic DNA sample using Qubit, PicoGreen or other validated dsDNA assay. The optimal DNA concentration is between 0.5 – 5 ng/µl, however DNA with concentrations as low as 0.1 ng/µl may be used with additional protocol modifications (see notes throughout).

Based on available DNA and application needs, **normalize all DNA samples within a batch to a set concentration** to ensure consistent fragment sizing, according to the table below:

Note: *If performing downstream hybrid capture on MosaiX libraries, it is recommended to start with ≥10 ng to ensure ample library yield and complexity.*

Total Input DNA (ng)	Target DNA Conc. (ng)
50	5 ng/µl
25	2.5 ng/µl
10	1.0 ng/µl
5	0.5 ng/µl
1	0.1 ng/µl

Program thermal cycler. For convenience, set up thermal cycler in use with all applicable programs described in the protocol on page 8 before starting.

Mix and pulse-spin kit components. Always mix and pulse-spin reagents before use. Please note any reagent-specific handling instructions throughout the procedure.

Handling of MAGwise beads. 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.

Thaw Reagents. Equilibrate 5X Reaction Buffer (orange), Tagmentation Enhancer (yellow), Diluent (red), Read 2 Adapter (blue), and UDI primer plate to room temperature.

Place Reagents on Ice. Place TnX Read 1 Tagging Reagent (white), Ligase (green), and 2X Amplification Master Mix (clear) on ice.

Prepare 80% ethanol fresh daily. Volume required will depend on the number of samples processed (~500 µl per sample). Dilute ethanol in molecular biology grade ultrapure water.

If needed, prepare 10 mM Tris-HCl, pH 8.0 and 1X TE buffers. If you do not have pre-diluted 10 mM Tris-HCl pH 8.0 and 1X TE, please prepare 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.

Obtain PCR Primers. If not using the primers included with the Early Access Kit, see Appendix B for information on sourcing compatible primers.

If using MosaiX libraries in downstream hybrid capture, please see Appendix C for important information on choosing the right blocking reagents

Procedure: MosaiX DNA Library Preparation

This User Guide provides two options for suggested post-PCR bead clean up procedures to generate libraries with different mean fragment size distributions:

- For whole genome sequencing (WGS) style libraries to generate libraries that sequence with a mean insert size of 350 - 500 bp; or
- For hybrid capture style libraries to generate libraries that sequence with a mean insert size of 250 - 350 bp.

Additionally, fragment sizes may be further customized by varying the volume of TnX Read 1 Tagging Reagent and/or by varying the MAGwise bead volume ratios. Please contact support@seqwell.com for recommendations on how best to customize library fragment sizes.

To ensure consistent results, the optimal input into MosaiX is 5 – 50 ng of DNA. However, inputs as low as 1 ng may be used with additional modifications (see notes throughout). Before beginning the procedure, ensure that all genomic DNA samples have been adjusted to a fixed concentration from **0.1 ng/µl to 5 ng/µl** in 1X TE buffer **(total DNA input of 1 - 50 ng in 10 µl**), depending on available DNA and application. Fixing the input amount across all samples will lead to a more consistent library size and yield.

For libraries to be used in downstream hybrid capture, starting with ≥10 ng total DNA input is recommended to ensure ample library yield and complexity for hybridization.

1. Tagmentation Reaction

a. The table below provides the per sample volumes of TnX Read 1 Tagging Reagent (white) and Diluent (red) based on the normalized genomic DNA input concentration. It is important to normalize the genomic DNA to a consistent input amount to ensure consistent fragment size.

b. Prepare TAGMENTATION MASTER MIX as follows:

   i. Calculate the volume of TnX Read 1 Tagging Reagent (white) and Diluent (red) required by multiplying the volume of each reagent needed for one sample from the table below by the total number of samples being processed (including an extra 12.5% overage for pipetting dead volume).

For example, for 8 samples using a 25 ng total DNA input:

- 4.5 µl TnX Tagging Reagent x 8 samples x 12.5% extra = 40.5 µl
- 3.5 µl Diluent x 8 samples x 12.5% extra = 31.5 µl

***Note**: For DNA input of 50 ng, only TnX Read 1 Tagging Reagent is added to the tube (with 12.5% overage); no Diluent is required.*

Tagging Strength Recommendation per Reaction:

Normalized DNA Concentration	Total DNA Input	TnX Read 1 Tagging Reagent (µl)	Diluent (µl)
5.0 ng/µl	50 ng	8	0
2.5 ng/µl	25 ng	4.5	3.5
1.0 ng/µl	10 ng	2.5	5.5
0.5 ng/µl	5 ng	1.5	6.5
0.1 ng/µl	1 ng	0.5	7.5

ii. Vortex Diluent (red), 5X Reaction Buffer (orange) and Tagmentation Enhancer (yellow) at 90% speed for 2-3 seconds and pulse-spin to collect liquid at the bottom of the tube. Vortex TnX Read 1 Tagging Reagent (white) at 60% speed for 2-3 seconds and pulse-spin to collect liquid at the bottom of the tube.

iii. To a clean 1.5 ml tube (or 2.0 ml tube if preparing >72 samples), add the volumes calculated above of both TnX Read 1 Tagging Reagent (white) and Diluent (red).

iv. Mix well by vortexing at 70% speed for 2-3 seconds and pulse-spin briefly to collect liquid at bottom of the tube. Proceed directly to the next step.

v. To that same tube, add 5X Reaction Buffer (orange) and Tagmentation Enhancer (yellow) according to the table below.

Volume calculations are provided for 8, 24, and 96 samples (with 12.5% overage included). For other batch sizes, multiply the per sample volumes in the 1st column by the number of samples being processed and include 12.5% overage.

***Note**: The 5X Reaction Buffer is viscous. To transfer volumes accurately, pipette slowly and do not pre-wet pipette.*

Reagents	Volumes per sample	Volumes for 8 samples *w/ 12.5% overage*	Volumes for 24 samples *w/ 12.5% overage*	Volumes for 96 samples *w/ 12.5% overage*
5X Reaction Buffer (orange)	5 µl	45 µl	135 µl	540 µl
Tagmentation Enhancer (yellow)	2 µl	18 µl	54 µl	216 µl
TnX Read 1 Tagging Reagent ***added to tube in previous step***	8 µl	72 µl	216 µl	864 µl
Total Master Mix Volume	**15 µl**	**135 µl**	**405 µl**	**1620 µl**

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

c. Vortex genomic DNA and pulse-spin. Add 10 µl of normalized DNA to each well of a new 96-well REACTION PLATE or to REACTION TUBE(S).

d. 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.

A small amount of bubbles/foaming after pipette mixing will not affect results.

e. Seal REACTION PLATE or TUBE(S) and pulse spin. 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

f. Return Tagmentation Enhancer (yellow), TnX Read 1 Tagging Reagent (white), and Diluent (red) to the freezer. Keep the 5X Reaction Buffer (orange) on ice as it will be used in the next step.

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.” through “e.” and once made, keep the mix on ice until ready to use.

g. Once the program is complete, remove plate from thermal cycler and **proceed immediately to the ligation reaction set up**. Try to avoid leaving the tagged plate on the thermal cycler for more than 5 minutes.

2. Ligation Reaction

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

b. Vortex Ligase (green), 5X Reaction Buffer (orange) and Read 2 Adapter (blue) at 70% speed for 2-3 seconds and pulse-spin to collect liquid at the bottom of the tube.

c. To a clean 1.5 ml tube (or 2.0 ml tube if >72 samples processed at a time), prepare a LIGATION MASTER MIX by adding the following components in the order listed in the table below.

   i. Volume calculations are provided below for 8, 24, and 96 samples (with 12.5% overage included). For other batch sizes, multiply the per sample volumes in the 1st column by the number of samples being processed and include 12.5% overage.

   Note: *For starting DNA input <5 ng, the Read 2 Adapter (blue) must be diluted to reduce the creation of adapter dimer. We suggest at least a 5-fold dilution of the Read 2 adapter in 10 mM Tris-HCl. However, the ideal dilution may need to be optimized based on DNA quality, source, and quantity. Make enough diluted adapter to allow for 4 µl per well plus extra 12.5% for dead volume.*

Reagents	Volumes per sample	Volumes for 8 samples *w/ 12.5% overage*	Volumes for 24 samples *w/ 12.5% overage*	Volumes for 96 samples *w/ 12.5% overage*
Read 2 Adapter (blue)	4 µl	36 µl	108 µl	432 µl
5X Reaction Buffer (orange)	3 µl	27 µl	81 µl	324 µl
Ultrapure Water	7 µl	63 µl	189 µl	756 µl
Ligase (green)	1 µl	9 µl	27 µl	108 µl
Total Master Mix Volume	**15 µl**	**135 µl**	**405 µl**	**1620 µl**

d. 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. Place on ice if not proceeding immediately to the next step.

e. Unseal the REACTION PLATE or TUBE(S) carefully and dispense 15 µl of LIGATION MASTER MIX to each sample and mix by thoroughly pipetting up and down ≥10x at with pipette set to 15 µl. It is crucial that the reactions are well mixed.

   A small amount of bubbles/foaming after pipette mixing will not affect results.

f. Seal REACTION PLATE or TUBE(S) and pulse-spin to collect liquid.

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

    25°C for 5 minutes
    4°C hold

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

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

3. Post Ligation MAGwise Bead Purification

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

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 tube or 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 alternatively vortex briefly and pulse spin down).

d. After beads have been added to all samples and mixed, 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 pellets 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 well or 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 PCR Amplification step
or store the purified DNA at -20°C

4. PCR Amplification

a. Remove the UDI indexing primers from the freezer and thaw at room temperature. Once thawed, spin down the primer plate.

b. Also remove 2X Amplification Master Mix (clear) from the freezer and thaw on ice. Once thawed, mix the 2X Amplification Master Mix (clear) by vortexing at 70% speed for at least 2-3 seconds and pulse-spin to collect liquid at the bottom of the tube.

c. Determine the number of cycles needed (see table below), and program the thermal cycler (see step 4f). The table below shows the recommended number of cycles, depending on starting input amount and application. A higher cycle number is shown for libraries to be used in downstream hybrid capture applications in order to generate 500 ng or more of library.

***Note**: These are suggested cycle numbers determined using high quality purified genomic DNA. Cycling parameters may need to be optimized depending on sample source, DNA quality, and downstream application.*

	Recommended cycles to generate:	
Starting gDNA input (ng)	**≥10 nM of library (Suitable for WGS)**	**≥500 ng of library (Suitable for Hybrid Capture)**
50	5	7
25	6	8
10	7	10
5	9	11
1	12 – 13	16 – 18

d. To set up the PCR reaction:

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

ii. Spin down the primer plate and remove the seal.

If processing <96 samples, carefully use a razor blade to cut the seal to uncover just the wells being used. After, re-seal the used wells to prevent cross contamination.

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 the wells 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).

e. Seal or cap and pulse-spin the 96-well plate or tube(s) and return primers and the 2X Amplification Master Mix (clear) to the freezer.

f. Transfer plate or tube(s) to a thermal cycler and run the **PCR Amplification** cycling program below, with lid-heating set to 105˚C:

45	sec	98°C	Initial denaturation
15	sec	98°C	***n*** cycles (See Step 4c for cycle number recommendations)
30	sec	60°C	
30	sec	72°C	
1	min	72°C	Final extension
Hold		4°C	

SAFE STOPPING POINT

Proceed immediately to the Post-PCR bead purification on the next page, or store the amplified PCR products for up to 24 hours at -20°C

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 tube or column at a time, add the appropriate volume of MAGwise beads to each well and mix thoroughly by pipetting up and down at least 10-15 times (if in a tube or strip tube, you may vortex briefly and pulse spin down).

   The following bead volumes are suggested based on downstream application:

   - For WGS (350 - 500 bp sequencing mean insert size): add 70 µl of MAGwise beads (0.7X volumetric equivalent) to each well.
   - For hybrid capture (250 - 350 bp sequencing mean insert size): add 80 µl of MAGwise beads (0.8X volumetric equivalent) to each well.

   Note: *The ratio of MAGwise to amplified library volume may be adjusted to suit your particular application and needs; increasing the volume of beads decreases the mean fragment size and decreasing the volume will increase the mean fragment size. It is not recommended to go below 0.5X MAGwise bead ratio as yield will be compromised.*

e. After beads have been added to all samples and well-mixed, incubate on the bench for 5 minutes to allow the DNA to bind to the beads.

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 200 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 pellets prior to Tris or Low TE addition. Over-drying bead pellets will compromise library recovery.

ii. Add 26 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 24 µl of DNA eluate from each tube to a fresh tube. The transferred supernatant contains the purified final library.

l. 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 WGS (0.7X bead clean up), library concentrations should be >5 ng/µl (>100 ng total).
- For hybrid capture (0.8X bead clean up), 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.

Average fragment size is ~650-750 bp for 0.8X bead ratio and ~800-900 bp for 0.7X bead ratio using a region analysis on the TapeStation software set to 250-1500 bp.

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

Typical results on the TapeStation High Sensitivity D5000 assay shown below:

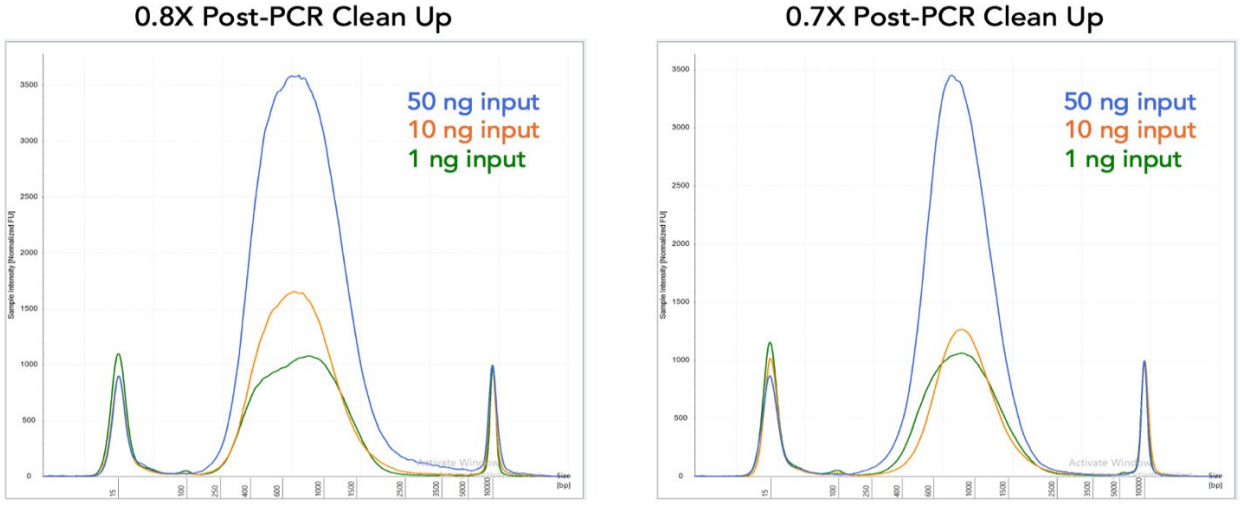


Figure 1. Representative MosaiX library size distributions on TapeStation 2200 with High Sensitivity D5000 DNA reagents and ScreenTapes. The libraries were prepared from human genomic DNA (inputs of 50 ng, 10 ng, and 1 ng) and were size selected by either a 0.8X (left– hybrid capture) or a 0.7X (right - WGS) post-PCR bead clean up ratio. Prior to loading on a ScreenTape, the libraries were diluted 1:10.

Appendix B: Information on MosaiX Indexing Primers

During the Early Access period, seqWell is providing customers with 24 or 96 unique dual indexed primers (10 bp indexes free of charge). The sequences of those indexes can be found at https://seqwell.com/resource-category/kit-user-guides/.

However, you may also source your own custom primers or use commercially available ***transposase-compatible*** primers from other library prep solution providers.

Because transposases require a specific 19-base sequence in order to bind adapters (known as the mosaic end or ME sequence), primers designed to be used with ligation adapters (i.e. "TruSeq" style adapters) are not compatible with transposase-based adapters as they do not contain this ME sequence.

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'`

***Note**: Primers i7 and i5 should be pre-mixed at a concentration of 10 µM each.*

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

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.
   a. 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.

Technical Assistance

For additional technical assistance with MosaiX Early Access, contact seqWell Technical Support.
Email: support@seqwell.com

Protocol revision history:

Version	Release Date	Prior Version	Description
V20250327	**27 March 2025**	**N/A**	**Early Access V1 User Guide release**
V20250910	**10 September 2025**	**V20250327**	1. Part numbers changed. 2. Tagmentation Reaction Set Up: • Guidance on tagging reagent volumes for a DNA input range of 1 - 50 ng. 3. Ligation Reaction Set Up & Bead Clean Up: • Guidance on suggested reduced adapter volumes for DNA inputs <5 ng • Adjustment to post-ligation MAGwise bead ratio 4. PCR Amplification & Bead Clean Up: • Suggested PCR cycle dependent on DNA input 5. Adjustment to post-PCR MAGwise bead ratio 6. Formulation changes for TnX Read 1 Tagging Reagent, Ligation Enhancer, and Read 2 Adaptor.

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