MosaiX™ DNA Library Prep Kit

Catalog numbers:
301458; MosaiX DNA Library Kit, 24 Reactions
301464; MosaiX DNA Library Kit, 96 Reactions
301443: MosaiX Compatible UDI Primers - Set 1

User Guide

v20260331

Table of Contents

Introduction 3

Molecular Diagram 4

Workflow Diagram 5

MosaiX Kit Components 6

Considerations Before You Begin

- User-Supplied Reagents, Equipment, Consumables, and Thermal Cycler Programs 7
- Reagent Handling 9

Procedure: MosaiX DNA Library Preparation

- Tagmentation Reaction 11
- Ligation Reaction 14
- Post-Ligation Bead Purification 15
- PCR Amplification 17
- Post-PCR Bead Purification 19

Appendix A: Library QC 21

Appendix B: Indexing Primer Information 22

Appendix C: Using MosaiX Libraries in Twist Target Enrichment 23

MosaiX™ DNA Library Prep Kit – Quick User Guide 24

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 limitation by combining tagmentation and ligation into a single streamlined workflow, ensuring that all tagging events produce functional PCR products with P5 on the 5' end and P7 on the 3' end.

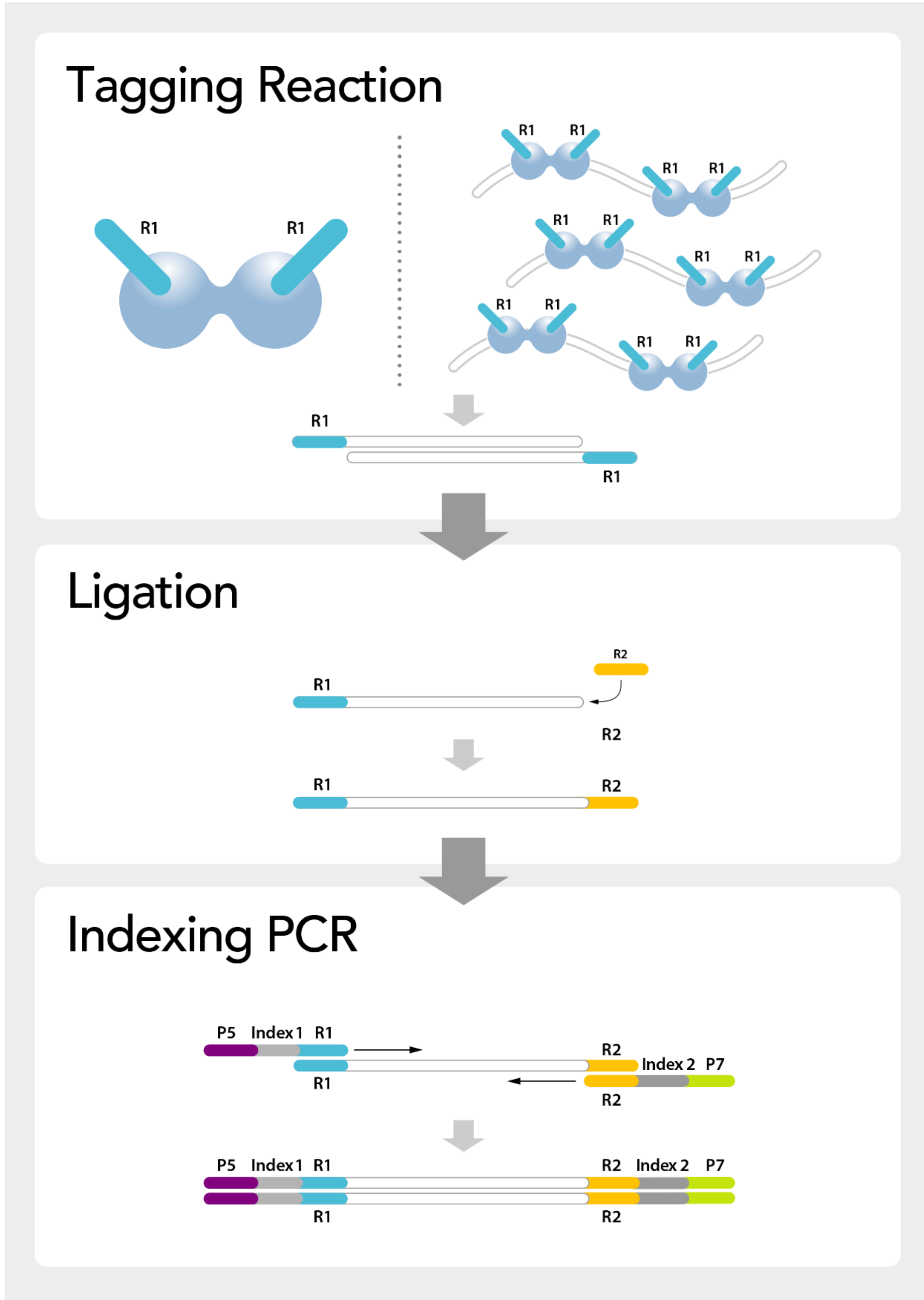
First, TnX fragments genomic DNA to the desired size range while simultaneously attaching the read 1 adapter sequence to the 5' end of each fragment. Next, a proprietary ligation step adds the read 2 adapter to the 3' end. The resulting intermediate libraries are then purified and amplified with primers containing 10 bp unique dual indexes (UDIs), enabling cost-effective and highly accurate demultiplexing. A workflow diagram is provided on the following page.

The MosaiX Kit is ideal for preparing high quality complex libraries from genomic DNA that generate sequencing mean insert sizes of 300 - 500 bp for whole-genome sequencing (WGS) or 200 - 350 bp for target enrichment using hybrid capture. These insert sizes are optimized for 2 x 150 bp sequencing on Illumina platforms.

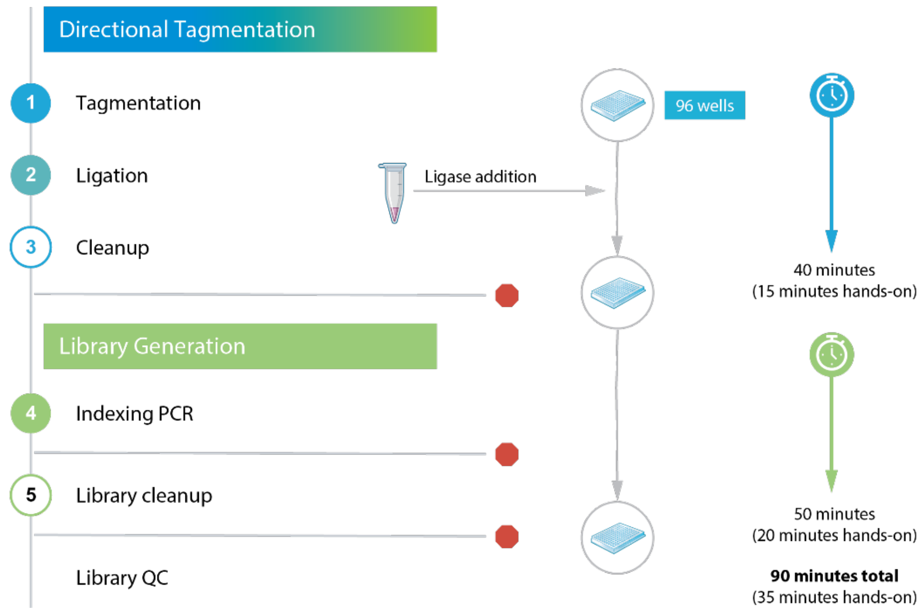
This protocol was optimized with a DNA input range of 1- 40 ng of high to moderate (DIN ≥ 6) genomic DNA quality. The protocol is compatible with lower DNA quality, but PCR cycle optimization may be required. Please see https://seqwell.com/resource-category/faqs/ for more details. Suggested modifications 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 cleanup ratios, adapter dilutions, etc) may need to be further optimized based on customer DNA source and quality. Although the kit is robust in the presence of common PCR inhibitors, the use of purified DNA is recommended for best results.

MosaiX kits contain all enzymes, reaction buffers, adapters, and magnetic cleanup beads (MAGwise) required. Compatible indexing primers are sold separately, or 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 DNA Library Prep 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 DNA Library Prep 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 UDI Primers (available for purchase - see Appendix B for additional info):

Item	Component	REF	Description	Storage	Qty
Box	MosaiX Compatible UDI Primers - Set 1	301443	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:

- Primers – 10 µM Unique dual index (UDI) PCR primers are required for library amplification and must be obtained separately. seqWell offers a set of 96 UDI primers for purchase, or customers may supply their own compatible primers using index sequences of their choice. Please refer to Appendix B of this User Guide for more information.
- 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 target enrichment by 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

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

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

45	sec	98°C	Initial denaturation
15	sec	98°C	***n*** cycles (See Step 4a 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:

Review FAQs. FAQs can be found here: https://seqwell.com/resource-category/faqs/. Review these prior to your first run.

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 0.1 – 4 ng/µl.

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)
40	4 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 seqWell's UDI Index Primers (sold separately), 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 cleanup 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 300 - 500 bp; or
- For target enrichment by hybrid capture style libraries - to generate libraries that sequence with a mean insert size of 200 - 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 1 – 40 ng of DNA. Before beginning the procedure, ensure that all genomic DNA samples have been adjusted to a fixed concentration from **0.1 ng/µl to 4 ng/µl** in 1X TE buffer **(total DNA input of 1 - 40 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 any library prep method, library complexity is linked to DNA input. For demanding applications such as downstream hybrid capture or high coverage WGS, it is recommended to start with ≥10 ng of input DNA to ensure ample library yield and complexity.

1. Tagmentation Reaction

Reagents used in this step: TnX Read 1 Tagging Reagent (white), Diluent (red), 5X Reaction Buffer (orange), and Tagmentation Enhancer (yellow)

Briefly vortex and pulse-spin all reagents.

a. Determine volumes of TnX Read 1 Tagging Reagent (white) and Diluent (red) based on the normalized starting DNA input being used for a given batch of samples (see table on next page).

Tagging Strength Recommendation per Reaction:

Normalized DNA Concentration	Total DNA Input	TnX Read 1 Tagging Reagent (µl)	Diluent (µl)
4.0 ng/µl	40 ng	8	0
2.5 ng/µl	25 ng	6.5	1.5
1.0 ng/µl	10 ng	3	5
0.5 ng/µl	5 ng	2	6
0.1 ng/µl	1 ng	0.4	7.6

Important Note: *It is critical to normalize the genomic DNA to a consistent input amount to ensure consistent fragmentation size.*

b. Prepare TAGMENTATION MASTER MIX as follows:

i. Based on the normalized DNA input concentration, 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 above by the total number of samples being processed including an extra 12.5% - 15% overage for pipetting dead volume.

For example, for 8 samples using a 10 ng total DNA input:

- TnX Tagging Reagent (white) : 3 µl x 8 samples x 12.5% extra = 27 µl
- Diluent (red) : 5 µl x 8 samples x 12.5% extra = 45 µl

Note: *For DNA input of 40 ng, only TnX Read 1 Tagging Reagent is added to the tube (with 12.5% overage); no Diluent is required.*

ii. 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).

iii. Mix thoroughly and pulse-spin briefly.

iv. To that same tube, add 5X Reaction Buffer (orange) and Tagmentation Enhancer (yellow) according to the table below, multiplying the volume of each reagent by the number of samples being processed and also including the 12.5% - 15% overage for pipetting dead volume (keep overage % consistent for all calculations).

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

Reagent	Volume per sample
5X Reaction Buffer (orange)	5 µl
Tagmentation Enhancer (yellow)	2 µl
TnX Read 1 Tagging Reagent ***added to tube in previous step***	8 µl
Total Master Mix Volume	**15 µl**

v. Mix thoroughly and pulse-spin briefly. Keep the TAGMENTATION MASTER MIX on ice until use.

c. Transfer 10 µl of each normalized DNA to a new 96-well REACTION PLATE or set of REACTION TUBE(S).

d. Dispense 15 µl of TAGMENTATION MASTER MIX to each DNA sample in the REACTION PLATE or TUBE(S).

e. Mix thoroughly by pipetting up and down ≥10x with pipette set to 15 µl.

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

f. 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

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 "c." 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**. Avoid leaving the tagged plate on the thermal cycler for more than 5 minutes.

2. Ligation Reaction

Reagents used in this step: Ligase (green), 5X Reaction Buffer (orange), Read 2 Adapter (blue), and Ultrapure Water

Briefly vortex and pulse-spin all reagents.

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

b. 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.

   Multiply the per sample volumes in the table below by the number of samples being processed and include 12.5% overage.

   Note: *If 1 ng of starting DNA input was used in Tagmentation, make a 4-fold dilution of the Read 2 adapter (blue) in 10 mM Tris-HCl to prevent adapter dimer formation. Make enough diluted adapter to allow for 4 µl per well plus extra for dead volume.*

Reagents	Volumes per sample
Read 2 Adapter (blue)	4 µl
5X Reaction Buffer (orange)	3 µl
Ultrapure Water	7 µl
Ligase (green)	1 µl
Total Master Mix Volume	**15 µl**

c. Mix thoroughly and pulse spin briefly. Place the LIGATION MASTER MIX on ice until use.

d. Unseal the REACTION PLATE or TUBE(S) carefully and dispense 15 µl of LIGATION MASTER MIX to each well or tube.

e. Mix by thoroughly pipetting up and down ≥10x with pipette set to 15 µl. It is crucial that the reactions are well mixed.

   Note: *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 **MSX_LIGATE** program, with lid-heating OFF:

25°C for 5 minutes
4°C hold

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

3. Post-Ligation MAGwise Bead Purification

Reagents used in this step: MAGwise beads, freshly prepared 80% Ethanol, 10mM Tris-HCl

a. Pulse-spin the REACTION PLATE or TUBE(S). Open carefully.

b. Resuspend room temperature MAGwise beads by vortexing until fully homogeneous.

c. Add 40 µl of MAGwise beads (1X volumetric equivalent) to each sample and mix thoroughly by pipetting up and down at least 10-15 times with pipette set to 40 µl. (Optional: vortex and pulse spin for tubes/strips).

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

e. Place the tube(s) or plate on a compatible magnetic stand until supernatant is completely clear (~5 minutes).

f. Remove and discard the supernatant. Do not disturb the beads.

g. Keeping samples on the magnet, wash beads with 80% ethanol:

   i. Add 180 µl 80% ethanol.

   ii. Incubate ≥30 seconds.

   iii. Remove and discard the supernatant.

   iv. Repeat wash once more (total of 2 washes).

h. Perform the next steps quickly to avoid overdrying the bead pellets:

   i. Pulse-spin and return to magnet (<30 seconds).

ii. Remove residual ethanol using a small pipet tip (≤20 µl).

__Important Note:__ DO NOT air-dry bead pellets prior to elution as DNA recovery may be compromised.

iii. Remove from the magnet and add 22 µl of 10mM Tris-HCl to each sample and mix thoroughly.

i. Incubate for 5 minutes to elute the purified DNA.

j. Return to magnet for ~2 minutes or until the supernatant is completely clear.

k. Transfer 20 µl of purified DNA to a new 96-well plate or PCR tube(s).

SAFE STOPPING POINT
Proceed immediately to the PCR Amplification step
or store the purified DNA at -20°C

4. PCR Amplification

Reagents used in this step: 2X Amplification Master Mix (clear) and 10 µM UDI Primers

Briefly vortex and pulse-spin all reagents.

Note: *MosaiX UDI Primer plates containing 96 UDI primers (10 µM each) are available for purchase. Index sequences for these primers are available to download from the MosaiX product page under the Resources section. For information on using alternative primers, please see Appendix B.*

a. Determine the number of cycles needed (see table below), and program the thermal cycler (see step 4d). 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 target enrichment by hybrid capture workflows 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 (Suggested for WGS)**	**≥500 ng of library (Suitable for Hybrid Capture)**
40	6	8
25	6	9
10	8	11
5	10	12
1	12	14

b. To set up the PCR reaction:

i. Unseal the 96-well plate or 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. Transfer 5 µl of 10 µM UDI primers to the reaction plate or tube(s). Record well positions for index tracking.

iv. Add 25 µl of 2X Amplification Master Mix (clear) to each sample and pipette-mix thoroughly. Final volume: 50 µl.

c. Seal or cap and pulse-spin the 96-well plate or tube(s).

d. Transfer to a thermal cycler and run the **MSX_PCR** cycling program below, with lid-heating set to 105˚C:

45	sec	98°C	Initial denaturation
15	sec	98°C	***n*** cycles (See Step 4a 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,
or store the amplified PCR products for up to 24 hours at -20°C

5. Post-PCR MAGwise Bead Purification

Reagents used in this step: MAGwise beads, freshly prepared 80% Ethanol, 10mM Tris-HCl

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

b. Add 50 µl of Ultrapure water to bring the volume of the reaction up to 100 µl. Mix thoroughly.

c. Resuspend room temperature MAGwise beads by vortexing until fully homogeneous.

d. Add the recommended volume of MAGwise below and mix thoroughly:

   The following bead volumes are suggested based on downstream application:

   - For WGS (300-500 bp sequencing mean insert size): add 75 µl (0.75X).
   - For target enrichment by hybrid capture (200 - 350 bp sequencing mean insert size): add 80 µl (0.8X). Using a lower bead ratio may result in yields slightly lower than 500 ng.

   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. Do not go below 0.5X MAGwise bead ratio to avoid sample loss.*

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

f. Place the plate or tube(s) on a magnetic stand until the supernatant is completely clear (~5 minutes).

g. Remove and discard the supernatant. Do not disturb beads.

h. Keeping samples on the magnet, wash beads with 80% ethanol:

   i. Add 180 µl 80% ethanol.

   ii. Incubate ≥30 seconds.

   iii. Remove and discard the supernatant.

   iv. Repeat wash once more (total of 2 washes).

i. Perform the next steps quickly to avoid overdrying the bead pellets:

    i. Pulse-spin and return to magnet (<30 seconds).

    ii. Remove residual ethanol using a small pipet tip (≤20 µl).

    Important Note: *DO NOT air-dry bead pellets prior to elution as DNA recovery may be compromised.*

    iii. Remove from the magnet. Add 26 µl 10mM Tris-HCl to each sample and mix thoroughly.

j. Incubate for 5 minutes to elute the purified DNA.

k. Return to magnet for ~2 minutes or until the supernatant is completely clear.

l. Transfer 24 µl of purified DNA to a new 96-well plate or PCR tube(s). The transferred supernatant contains the purified final library.

m. 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 (0.75X bead cleanup), library concentrations should be >5 ng/µl (>100 ng total).
- For the hybrid capture method (0.8X bead cleanup), library concentrations should be >22 ng/µl (>500 ng total).

Library Sizing:

Assess library sizing by electrophoretic analysis. We suggest running a 1:10 (WGS) or 1:25 (hybrid capture) 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 ~550-750 bp for 0.8X bead ratio and ~600-800 bp for 0.75X 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 larger 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:

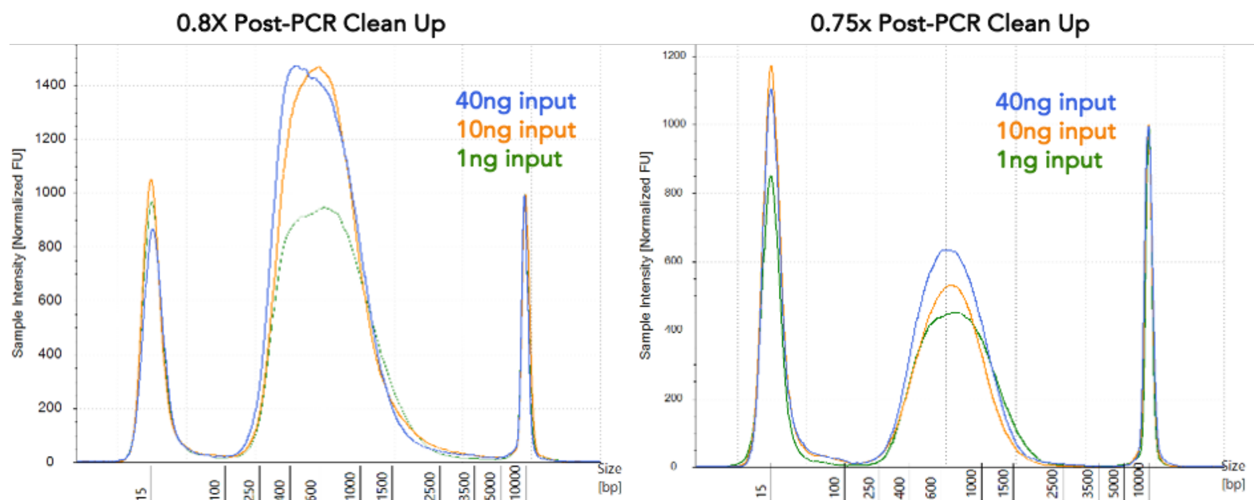


Figure 1. Representative MosaiX library size distributions generated using a TapeStation 2200 with High Sensitivity D5000 DNA reagents and ScreenTapes. The libraries were prepared from human genomic DNA (inputs of 40 ng, 10 ng, and 1 ng) and were size selected by either a 0.8X (left– hybrid capture) or a 0.75X (right - WGS) post-PCR bead cleanup ratio. Prior to loading on a ScreenTape, the libraries were diluted 1:25.

Appendix B: Information on MosaiX Indexing Primers

seqWell has 96 unique dual indexed primers (10 bp indexes) available for purchase. 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 ease of use, we recommend ordering or aliquoting primers in plate format*

For additional guidance on indexing primers, please contact support@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 support@seqwell.com.

MosaiX™ DNA Library Prep Kit – Quick User Guide

This quick protocol is provided as a streamlined reference for users who have previously completed the workflow. If you are new to the protocol or need additional guidance, we recommend using the full version for step-by-step instructions and best practices.

1. Before You Start

- Quantify and normalize 10 µl of Input DNA using Qubit / PicoGreen to 4, 2.5, 1, 0.5 or 0.1 ng/µl.
- Prepare fresh 80% ethanol.
- Equilibrate MAGwise beads to room temperature.

Thermal Cycler Programs:

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

MSX_LIGATE: 25°C for 5 minutes (lid heating OFF)
4°C hold

MSX_AMP: 98°C for 45 seconds (lid heating 105°C)
n cycles of: (see table 5)
98°C for 15 seconds
60°C for 30 seconds
72°C for 30 seconds
72°C for 1 minute
4°C hold

2. Tagmentation

- Prepare the TnX Read 1 Reagent strength as below.

Table 1. Recommended Tagging Strength.

DNA Input	TnX Read 1 (white)/Rxn	Diluent (red) /Rxn
40 ng	8 µl	0 µl
25 ng	6.5 µl	1.5 µl
10 ng	3 µl	5 µl
5 ng	2 µl	6 µl
1 ng	0.4 µl	7.6 µl

- Prepare Tagging Master Mix.

Table 2. Tagging Master Mix.

Reagents	Vol / Reaction
5X Reaction Buffer (orange)	5 µl
Tagmentation Enhancer (yellow)	2 µl
TnX Read 1 (from table 1)	8 µl

- Combine 10 µl of input DNA with 15uL of Tagging Master Mix and mix thoroughly.
- Run **XTAG** program.

3. Ligation

- Prepare Ligation Master Mix.

Table 3. Ligation Master Mix.

Reagents	Vol / Reaction
Read 2 Adapter (blue)	4 µl
5X Reaction Buffer (orange)	3 µl
Ultrapure Water	7 µl
Ligase (green)	1 µl

Note: For 1 ng of starting DNA input, make a 4-fold dilution of the Read 2 adapter (blue) in 10 mM Tris-HCl to prevent adapter dimer formation.

- Add 15 µL Ligation Master Mix per sample and mix thoroughly.
- Run **MSX_LIGATE** program.

4. Post-Ligation Cleanup

- Add 40 µL MAGwise beads (1X) and mix thoroughly.
- Incubate for 5 min.
- Magnetize until clear and remove supernatant.
- Wash twice with 180 µL 80% ethanol.
- Elute with 22 µL 10 mM Tris and incubate for 5 min.
- Recover 20 µL eluate.

SAFE STOPPING POINT
Store the purified DNA at -20°C or continue to PCR

5. PCR Amplification

- Set up the PCR by adding reagents as listed below.

Table 4. PCR Setup.

Reagents	Vol / Reaction
Purified DNA	20 µl
UDI Primers	5 µl
2X Amplification Mix (clear)	25 µl

Note: Record the UDI Primers well positions for index tracking.

Table 5. Recommended PCR cycles.

DNA Input	PCR cycles for WGS Library (*≥10 nM)*	PCR cycles for Hyb Cap Library (*≥500 ng)*
40 ng	6	8
25 ng	6	9
10 ng	8	11
5 ng	10	12
1 ng	12	14

- Run **MSX_AMP** program.

SAFE STOPPING POINT
Store the amplified PCR products for up to 24 hours at -20°C or continue to Post-PCR Cleanup

6. Post-PCR Cleanup

- Add 50 µL of water to bring the volume to 100 µL.
- Add MAGwise based on your application:
  Bead ratios:
  WGS → 75 µL beads (0.75X)
  Hyb Cap → 80 µL beads (0.8X)
- Mix thoroughly and incubate for 5 min.
- Magnetize until clear and remove supernatant.
- Wash twice with 180 µl 80% ethanol.
- Elute with 26 µL Tris / TE and incubate for 5 min.
- Recover 24 µL library.

7. Library QC

- Run a 1:10 (WGS) or 1:25 (Hyb Cap) diluted aliquot of purified library on the TapeStation using the High Sensitivity D5000 kit.
- Quantification: Qubit / PicoGreen.
  Expected yield: WGS: >5 ng/µL or Hyb Cap: >22 ng/µL
- Size distribution: 250–1500 bp (TapeStation).
  Expected avg frag size: WGS: 600 – 800 bp Hyb Cap: 550 – 750 bp

Technical Assistance

Please review FAQs at https://seqwell.com/resource-category/faqs/.

For additional technical assistance with the MosaiX DNA Library Prep Kit, contact seqWell Technical Support.

E-mail: support@seqwell.com

Protocol revision history:

Version	Release Date	Prior Version	Description
V20260331	**31 March 2026**	**N/A**	**First version**

seqWell, Inc.
66 Cherry Hill Drive
Beverly, MA 01915
USA
+1-855-737-9355
https://seqwell.com/

© 2026 seqWell. All rights reserved.
For Research Use Only – Not for Diagnostic Use
UserGuide_MX20260331

seqWell, the seqWell logo, TnX, and MosaiX are trademarks of seqWell, Inc.
All other trademarks are property of their respective owners.