MosaiX™: High-Performance DNA Library Prep with Directional Tagmentation Powered by TnX™

David Bays, Zac Zwirko, Christianto Putra, Yanyan Liu, Maura Costello, Joe Mellor

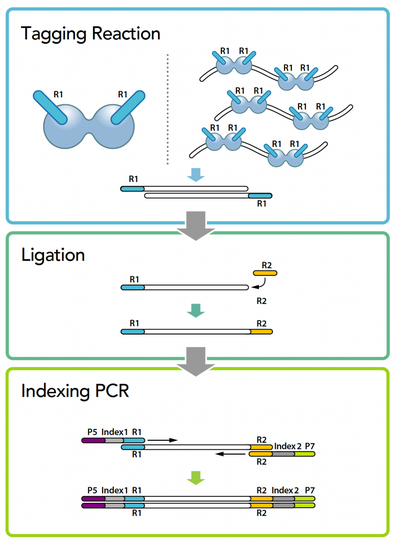
seqWell, Inc. Beverly, MA USA

Introduction

The MosaiX DNA Library Prep Kit uses a novel Directional Tagmentation chemistry with seqWell's engineered TnX transposase to generate high-performance libraries in a streamlined workflow. The chemistry is designed to support a wide range of applications including whole genome, whole exome, and metagenomics delivering superior metrics compared with standard Tn5-based tagmentation. MosaiX Directional Tagmentation also performs well with FFPE DNA, producing higher yields and lower percentages of chimeric reads than other library prep chemistries. In addition, we present a normalization method (currently in development) that can be used with MosaiX to auto-normalize both fragment size and read counts across a 10× range of DNA inputs, significantly reducing the time and cost of individual library QC.

MosaiX Library Preparation Kit Overview

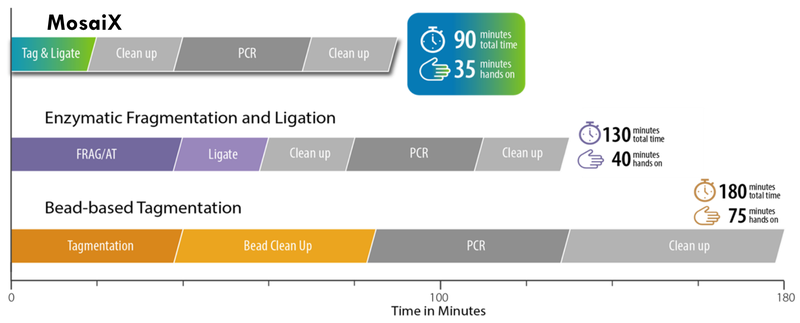
Direction Tagmentation with TnX Engineered Transposase



- seqWell's Directional Tagmentation chemistry combines TnX transposase tagging with adapter ligation into a single high-performance workflow.
- DNA is fragmented and Read 1 (R1) adapter is added to the 5' ends via TnX tagmentation followed by ligation of Read 2 (R2) adapters to the 3' ends.
- Directional Tagmentation generates complex libraries with all fragments having correct R1 + R2 configuration.
- Unique dual indexes (UDIs) are added via PCR primers, allowing for flexibility.

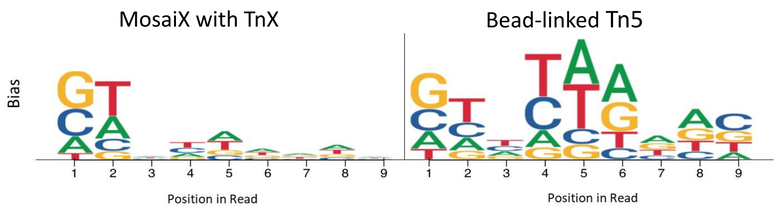
MosaiX Fast Workflow

- MosaiX takes ≤90 minutes from start to finish with only 35 minutes of hands-on time.
- The workflow is up to 3x shorter than competitors.



TnX – Engineered for Reduced Insert Site Bias Compared to Tn5

- MosaiX with TnX (left) has lower insertion site bias compared to Tn5 methods (right), leading to more even coverage and less biased libraries.



Robust Performance in a Variety of Applications

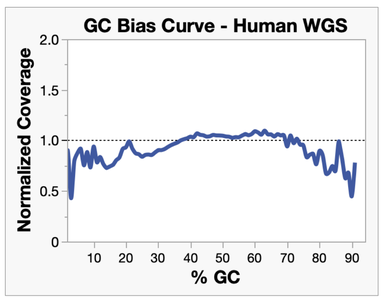
Higher Complexity in WGS Libraries

- WGS libraries were prepared from 50 ng of human DNA (NA12878) using both MosaiX and a bead-linked Tn5 kit.
- **MosaiX WGS libraries achieved higher coverage** at 105 Gb due to lower duplication (see below) which enables sequencing cost savings.

Library Preparation Method	PF Gb	Mean Coverage (X)	% Bases ≥20X	% Duplication	Estimated Library Size
MosaiX with TnX	105	27.4	84%	10%	3,374,667,137
Bead-linked Tn5 tagmentation	105	23.9	80%	14%	2,871,092,622

Even Coverage Across Entire GC Spectrum

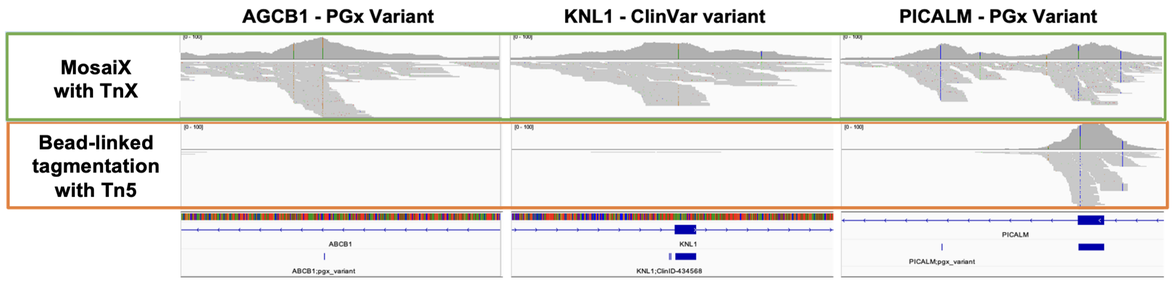
- At left, normalized coverage is plotted by %GC content to generate a GC bias curve for a human MosaiX library.
- The bias curve is flat and near the 1.0 line, indicating even coverage across the entire 10 – 90% GC spectrum.



High Quality Exome Data with Fewer Target Dropouts

- Bot MosaiX and bead-linked Tn5 libraries were also captured using Twist's Exome 2.0.
- Exome data generated using MosaiX libraries has lower % duplication and higher HS Library Size indicating **more complex libraries** vs bead linked Tn5 (see table below).
- MosaiX libraries also had **lower % of zero coverage targets** (lower rate of target dropouts), **including some medically relevant targets** vs Tn5 data (IGV plots below).

Library Preparation Method	PF Gb	% Duplication	HS Library Size (complexity)	% Zero Covg Targets
MosaiX with TnX	6 Gb	4.2%	285,255,870	0.69%
Bead-linked Tn5 tagmentation	6 Gb	7.9%	167,531,020	0.82%



Lower Rate of Chimeric Reads in FFPE

Competitive Benchmarking Using Horizon FFPE References

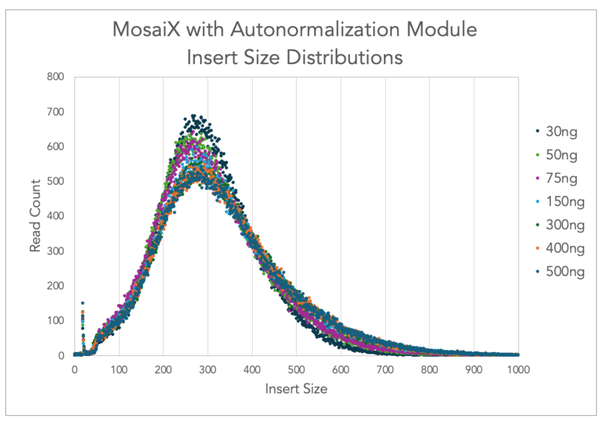
- MosaiX was benchmarked against bead-linked Tn5 and fragmentase plus ligation library methods using 10 ng Horizon's FFPE DNA standards (mild, moderate, and severe degradation).
- While all 3 methods struggled with the severely degraded sample (not sequenced), **MosaiX's outperformed the competitor kits** with higher yields, lower % duplication, and most notably **lower % chimeric read formation on the mild and moderately degraded DNAs.**

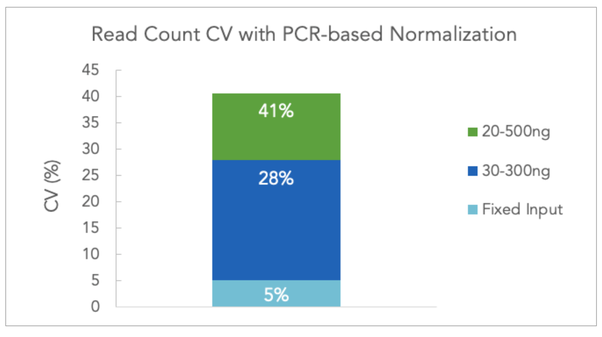
FFPE Sample	DIN	Kit	Library Yield (ng)	Mean Seq Insert (bp)	% Duplication	% Chimeric Reads
Horizon Mildly Degraded	6.8	MosaiX	2849	261	3.3%	4.5%
		Fragmentase + Ligation	761	418	7.8%	8.6%
		Bead-linked Tn5	276	230	4.1%	10.7%
Horizon Moderately Degraded	3.7	MosaiX	804	205	3.9%	6.2%
		Fragmentase + Ligation	194	277	9.7%	18.5%
		Bead-linked Tn5	98	155	5.6%	20.3%
Horizon Severly Degraded	1.6	MosaiX	171	*Not Sequenced*		
		Fragmentase + Ligation	190			
		Bead-linked Tn5	37			

In Development: MosaiX Normalization Module

Auto-Normalization over 10x DNA Input Range

- A novel PCR-based normalization method optimized for MosaiX is in development that eliminating the need to normalize input DNA.
- This normalizing method yields **consistent insert size** and **read counts across a 10x DNA input range** (30 – 300 ng) enabling pooling of libraries before QC (graphs at right).
- Auto-normalized libraries show only a mild reduction in complexity and evenness (see exome data below).





	MosaiX Standard	MosaiX Plus Norm Module
PF Gb	4.2	4.2
% Duplication	12.2%	13.3%
Fold 80 Penalty	1.38	1.47
Mean Target Covg	34.5X	33.7X
% Target Bases ≥20X	91.58%	88.31%