MosaiX™: A Directional Tagmentation Approach with Optional Auto-normalization for Rapid, High-Throughput NGS Library Preparation

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

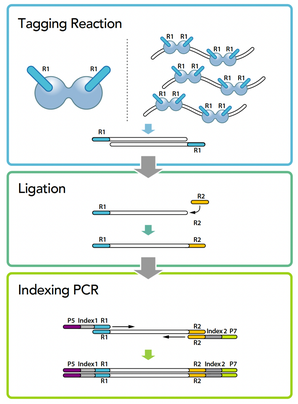
seqWell, Inc. Beverly, MA USA

Introduction

The MosaiX DNA Library Prep Kit uses a novel Directional Tagmentation chemistry with seqWell's engineered TnX transposase to generate high quality libraries in a rapid high-throughput workflow. The chemistry is designed to support a wide range of applications including whole-genome, whole-exome, and metagenomics delivering superior performance compared to standard Tn5-based tagmentation. Here we present a normalization method (Alpha Testing) that can be used with MosaiX to auto-normalize both fragment size and read counts across a 10-fold range of DNA inputs, significantly reducing the time and cost of individual library QC.

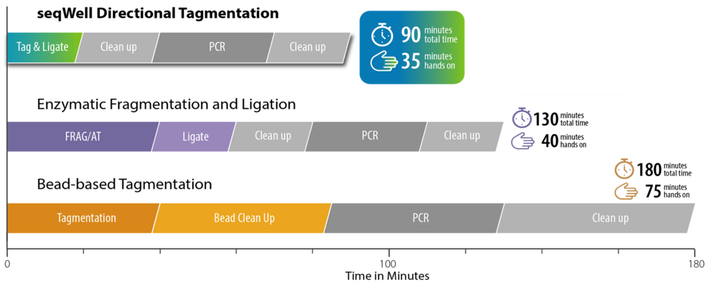
MosaiX Library Preparation Kit Overview

Directional Tagmentation with TnX Engineered Transposase



- **Robust against common inhibitors:** DNA input can be suspended in Tris, TE, or low TE without the need for buffer exchange or adjustment of fragmentation time.
- Traditional tagmentation is non-directional rendering 50% of input DNA unusable due to redundant tagging events. This raises input requirements, reduces sensitivity, and increases duplication rate.
- To maximize conversion of input DNA, MosaiX relies on Directional Tagmentation, which attaches read1 adapters to 5' ends and ligates read2 to 3' ends without the need for end-repair.
- Unique dual indexes (UDIs) are added via PCR.

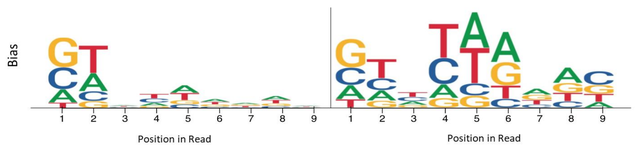
MosaiX Fast, Easily Automated Workflow



- Minimal hands-on time.
- Master-mix based chemistry enable compatibility with various liquid-handling platforms for scalable, high-throughput processing.
- Streamlined workflow enable faster turnaround time.

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 Various Applications

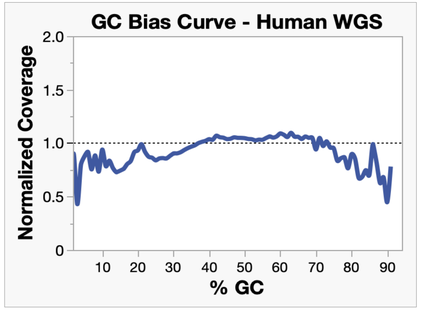
Higher Complexity in WGS Libraries Compared to Bead-linked Tagmentation

- **Reduced duplication leads to higher coverage with equivalent sequencing output.**

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

- 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.

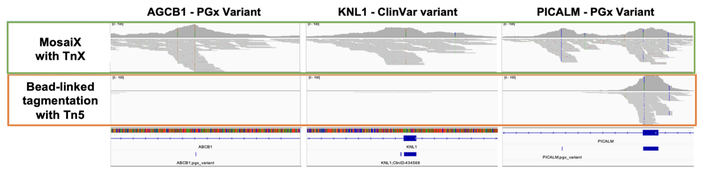


High Quality Exome Data with Fewer Target Dropouts

- Both MosaiX and bead-linked Tn5 libraries were captured using Twist Exome 2.0
- MosaiX libraries have lower % duplication and higher HS Library Size indicating **more complex libraries** vs bead linked Tn5 (see table 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%

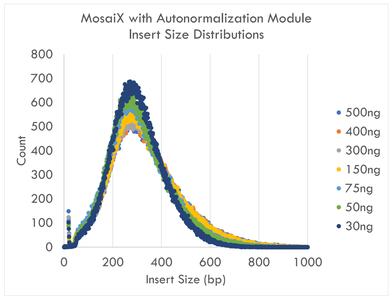
- **Lower % of zero coverage targets** (lower rate of target dropouts), **including some medically relevant targets** vs Tn5 data (IGV plots below).

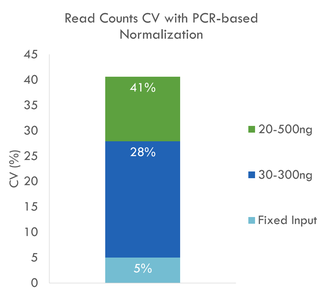


Auto-Normalization Performance (Alpha Testing)

Auto-Normalization Over 10-fold DNA Input Range

- A novel PCR-based normalization method optimized for MosaiX eliminates the need to normalize input DNA.
- This normalizing method yields **consistent insert size** and **read counts across a 10-fold DNA input range** (30 – 300 ng) enabling pooling of libraries before QC (see graphs below).





Minimal Impact on Complexity and Evenness of Coverage

- Auto-normalized libraries show only a mild reduction in complexity and evenness of coverage (see table below).

Sequencing Metrics	Exome		Whole Genome	
	MosaiX	Autonorm Module	MosaiX	Autonorm Module
PF Gb	4.1	4.1	132	132
Fold 80 Penalty	1.35	1.43	1.13	1.19
% Duplication	14.6%	16.9%	19.0%	19.0%
% Target Bases ≥50X	79.4%	70.4%	77.3%	72.4%
Mean Target Covg (X)	65.9	63.3	57.9	57.6

Retained Auto-Normalization Feature Across Various DNA Quality

- Fragmentase was used to generate samples with varying DNA Integrity Numbers (DIN).
- Libraries from 30 ng input DNA across the DIN range generated robust library yield and size (graphs below).