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

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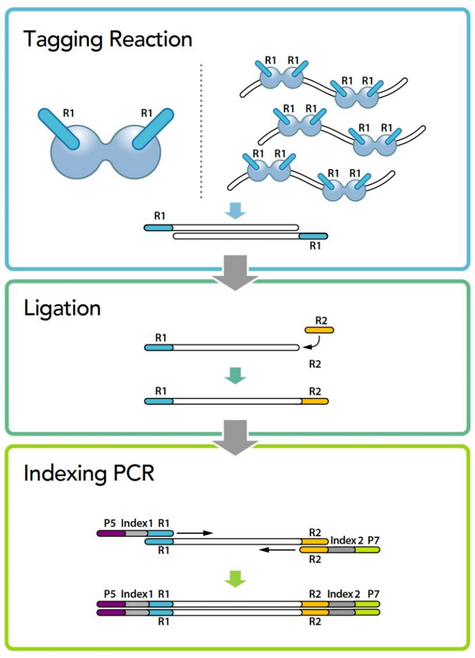
Introduction

The concentration and quialty of genomic DNA can vary widely, which can complicate choosing the right NGS library preparation method to address the broadest range of sample types. Standard Tn5 tagmentation-based library methods, while fast and efficient, are limited by strong insertion-site bias, lower complexity, and poor performance with compromised samples. The MosaiX DNA Library Prep kit overcomes these barriers via highly efficient **directional tagmentation featuring TnX**, an engineered transposase optimized through directed evolution for reduced bias, enhanced activity, and superior inhibitor tolerance. This combination of next generation transposase and novel workflow ensures **MosaiX has robust and class leading performance, even with challenging sample types such as formalin fixed paraffin embedded (FFPE) derived DNA.**

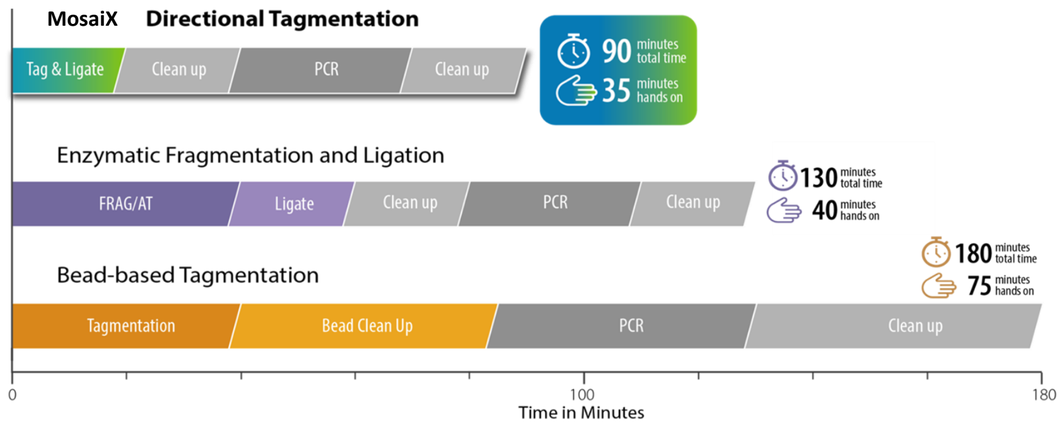
MosaiX Library Preparation Kit Overview

Direction Tagmentation with TnX Engineered Transposase



- seqWell's Directional Tagmentation chemistry combines the speed of transposase tagging with ligation.
- DNA is fragmented and Read 1 (R1) adapters are added to the 5' end of DNA via TnX tagmentation followed by ligation of Reads 2 (R2) adapters onto the 3' ends without a clean up between tagging and ligation.
- Because all library molecules have the proper R1/R2 ends, MosaiX generates more complex libraries compared to standard tagmentation where half of library molecules generated will be nonproductive with R1/R1 or R2/R2 ends.
- Unique dual indexes (UDIs) are added via PCR primers, allowing maximum flexibility.

MosaiX Simple 90 Minute Workflow



- MosaiX takes just ≤90 minutes from DNA in to sequencing ready library out, with only 35 minutes of hands-on time.
- The workflow is up to 3x shorter than competitor kits.

High Complexity and Reduced Insertion Site Bias

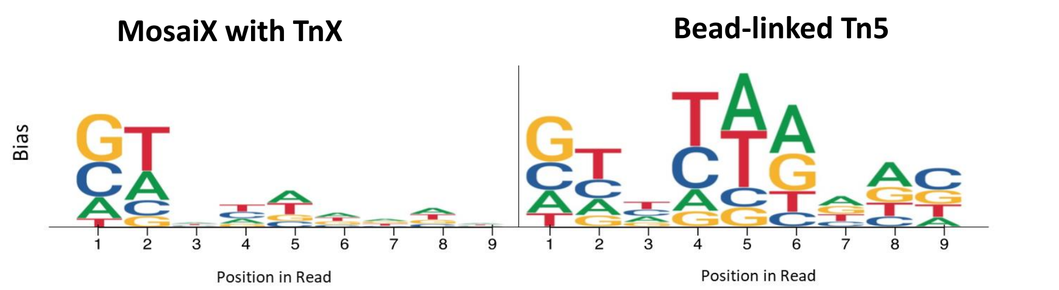
Higher Performing Libraries for Human WGS Applications

- Whole genome libraries were prepared using 50 ng of input DNA (NA12878) using both MosaiX and a bead-linked Tn5 kit and sequenced on NovaSeq X.
- When down sampled to the same 105 Gb, MosaiX libraries achieved higher coverage due to lower duplication, enabling sequencing cost savings (see below).

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

TnX – Engineered for Reduced Insert Site Bias Compared to Tn5

- MosaiX with TnX (left) has lower insertion site bias in the 1st nine bases of sequencing versus Tn5 data (right), leading to more evenly covered libraries.

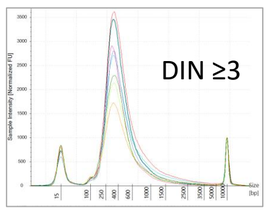


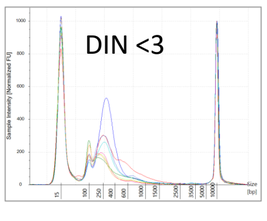
MosaiX Outperforms Other Methods with FFPE DNA

Robust Libraries from Mild to Moderately Degraded FFPE

- Commercially sourced FFPE samples with DNA Integrity Numbers (DIN) between 1.6 and 6.8 were prepared with MosaiX using 10 ng input.
- MosaiX generated robust libraries from both mildly and moderately degraded FFPE samples, though yields were poor and fragment sizes small for severely degraded samples with DIN scores <3.

Sample (Source)	DIN	Total Library Yield (ng)
FFPE Mildly Degraded (Horizon)	6.8	1095
FFPE Moderately Degraded (Horizon)	3.7	758
FFPE Liver (BioChain)	3.4	713
FFPE Colon (BioChain)	1.7	92
FFPE Severely Degraded (Horizon)	1.6	117
FFPE Pancreas (BioChain)	1.6	59
FFPE Lung (BioChain)	1.6	59





Competitive Benchmarking Using Horizon FFPE Reference Samples

- Using the Horizon FFPE reference samples (mildly, moderately, and severely degraded), MosaiX was then benchmarked against bead-linked Tn5 and fragmentase plus ligation library methods using 10 ng of DNA input.
- While all 3 methods struggled with the severely degraded sample, on moderate and mildly degraded samples **MosaiX's directional tagmentation outperformed the competitor kits** with higher yields, lower % duplication, and most notably **lower % chimeric read formation**.

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			

Summary and Conclusions

- MosaiX DNA Library Prep is fast, easy to use, and generates highly complex libraries suitable for demanding applications such as human WGS.
- The method is robust on a variety of sample types, including FFPE, and outperforms competitor kits.
- seqWell's directional tagmentation chemistry generates fewer chimeric reads in FFPE samples compared to both bead-linked Tn5 and fragmentase plus ligation competitor kits.