MosaiX™: Rapid Directional Tagmentation Utilizing TnX™ Engineered Transposase for High Performance in Diverse DNA Sequencing Applications

Maura Costello[1], Zac Zwirko[1], Christianto Putra[1], David Bays[1], Yanyan Liu[1], Filippo Lucchini[2], Matteo Orlandi[2], Marzia Rossato[2], Massimo Delledonne[2], Gavin Rush[1], Joe Mellor[1]

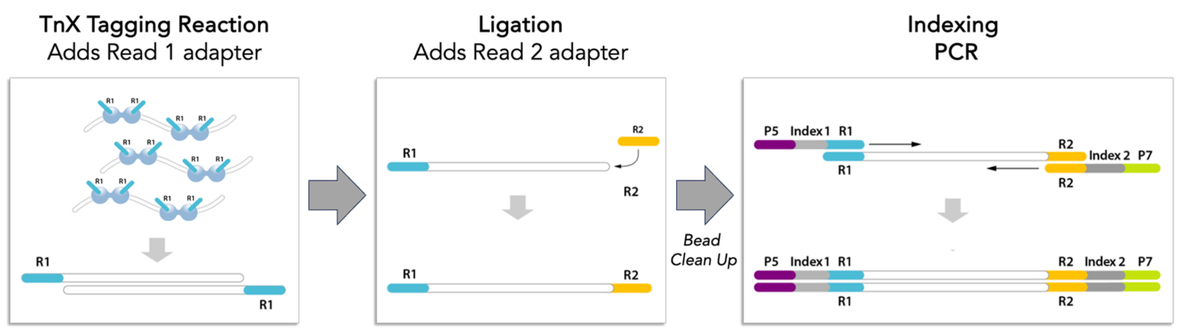
[1]seqWell, Inc. Beverly, MA USA; [2] University of Verona, Department of Biotechnology, Verona, Italy

Introduction

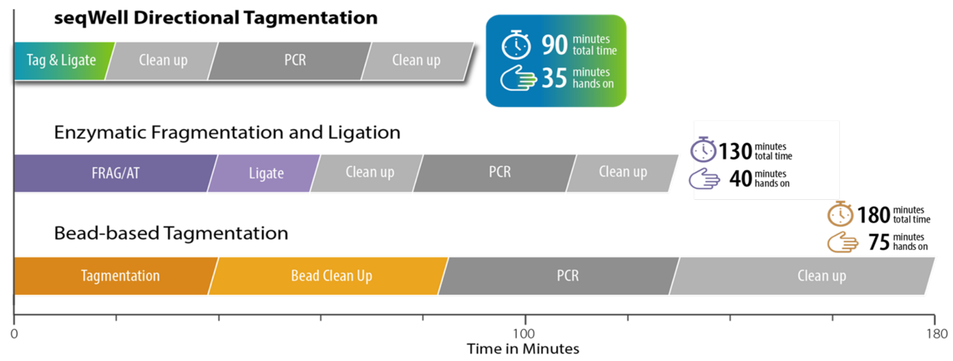
Transposase tagmentation is a highly streamlined approach to library preparation. However, using standard Tn5 tagging methods can pose quality challenges due to strong insertion-site bias and lowered complexity as half of all library fragments generated can share the same adapter on both ends, making them non-productive.

We present the **MosaiX™ Library prep kit** that employs a proprietary chemistry based on **directional tagmentation** and **incorporates TnX™, an engineered transposase.** TnX was developed through directed enzyme evolution for reduced insertion bias, improved activity, and enhanced inhibitor tolerance. Within the MosaiX workflow, DNA is fragmented and Read 1 adapters added to the 5' end via TnX followed by 3' ligation of Read 2 adapters, generating a high proportion of productive library molecules. The streamlined workflow produces high-quality ready-to-sequence libraries in just 90 minutes and is ideal for applications like human target capture and whole genome sequencing.

MosaiX: seqWell Directional Tagmentation Featuring Next-Generation TnX Engineered Transposase



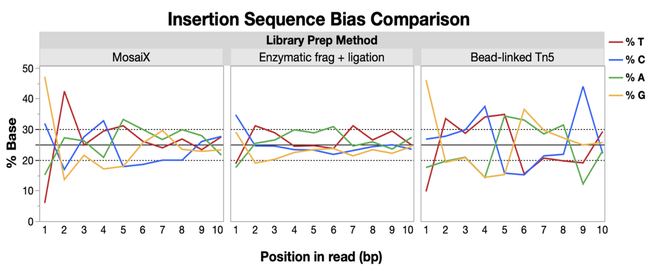
MosaiX library preparation employs proprietary directional tagmentation and ligation technology, combining the speed of transposase workflows with the high quality of ligation.

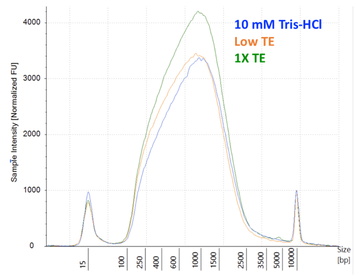


MosaiX is faster than both enzymatic fragmentation with ligation and bead-linked tagmentation methods, requiring ≤90 minutes from start to finish.

Competitive Performance in Human WES and WGS Applications

Reduced insertion bias & robust tagging chemistry





Read start base bias for MosaiX with TnX, enzymatic fragmentation, and bead-linked Tn5. TnX has reduced bias vs. Tn5 (left). MosaiX fragmentation profiles are not affected by EDTA and DNA can be diluted in a variety of buffers (right).

High performance in WGS

MosaiX delivers higher complexity and achieves a higher mean coverage compared to bead-linked Tn5 with the same amount of sequencing.

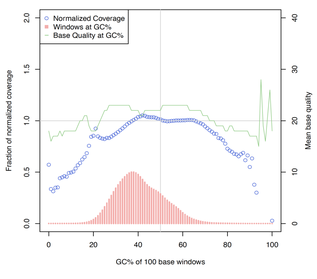
Library Preparation Method	PF Gb	Mean Coverage (X)	% Bases ≥20X	% Duplication	Estimated Library Size
MosaiX with TnX	105	27.4	84%	10%	3,374,667,137
Bead-linked Tn5 tagmentation	105	23.9	80%	14%	2,871,092,622

WGS metrics. Replicates of 50 ng of NA12878 were prepared using MosaiX or bead-linked Tn5 transposition, sequenced on NovaSeq™ X Plus at 2x150, and down-sampled to 105 Gb.

Prof. Massimo Delledonne's lab at University of Verona demonstrated robust MosaiX WGS data on par with other methods, including PCR-free.

Sample	Total Reads	PF Gb	Mean Insert Size (bp)	% Duplication	Mean Coverage
MosaiX WGS Rep 1	412M	124	400	7%	31.4X
MosaiX WGS Rep 2	478M	143	397	8%	35.8X

WGS metrics (*left*) and GC bias (*right*) for two replicates of 50 ng of NA12878 prepared using MosaiX and sequenced on NovaSeq X Plus at 2x150.



Library Prep Method	Mean Coverage	% Bases ≥1X	% Bases Genotyped	Fold 80
MosaiX (avg of both reps)	33.6	93.9	89.6	1.28
Bead-linked Tn5	30.6	93.8	89.8	1.21
Bead-linked Tn5 PCR-Free	38.3	94.1	90.0	1.26
Fragmentase + Ligation PCR-Free	39.7	94.3	90.2	1.21

Comparison of MosaiX WGS performance to three other library prep methods including PCR-free at similar read depths.

High performance in WES (Twist Exome 2.0)

In a three-way comparison of MosaiX, enzymatic fragmentation, and bead-linked Tn5, MosaiX has the highest molecular complexity (HS Library Size) and less target dropouts versus bead-linked Tn5.

Library Prep Method	PF Gb	% Bases ≥20X	Insert Size (bp)	% Selected	% Duplication	HS Library Size	Fold 80	% Zero Cvg
MosaiX with TnX	6 Gb	97.6%	269	85.0%	4.2%	285,255,870	1.35	0.69%
Enzymatic fragmentation + ligation	6 Gb	98.0%	298	85.5%	6.0%	201,223,608	1.33	0.68%
Bead-linked Tn5 tagmentation	6 Gb	98.1%	183	93.3%	7.9%	167,531,020	1.30	0.82%

Exome metrics and GC bias curves. All methods were prepared with 50 ng of NA12878 and captured using Twist Exome 2.0..

IGV plot showing clinically relevant targets present in MosaiX that are missing in libraries prepared using bead-linked Tn5.

Exome data generated from MosaiX libraries by Prof. Delledonne's lab at University of Verona had lower % duplication and achieved higher coverage at 30 million reads compared to fragmentase libraries.

Library Prep Method	Total Reads	Mean Coverage	% Duplication	% Bases at ≥10X	Fold 80	% Bases Genotyped	True Pos	False Pos	False Neg
MosaiX	30M	73X	12%	99.60%	1.33	96.70%	24,836	4,508	896
Fragmentase + Ligation	30M	67X	26%	99.40%	1.34	96.70%	24,865	4,442	867

Twist Exome 2.0 data for 50 ng NA12878 MosaiX libraries sequenced on NovaSeq X Plus and compared to previously sequenced fragmentase libraries at 30 million reads (*above*). MosaiX had lower duplication and more even GC coverage (*right*). Genotyping results were similar.