An Auto-normalizing Directional Tagmentation Method for Robust NGS Library Preparation

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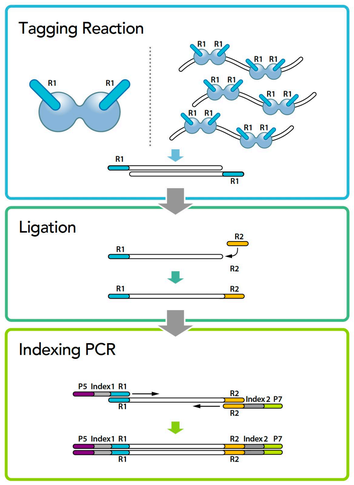
Introduction

The original MosaiX™ DNA Library Prep Kit was designed to maximize library complexity but lacked the auto-normalization capability featured across seqWell's product portfolio. **MosaiX 2.0 introduces auto-normalization** through a combination of large-fragment suppression and excess amplification blocker, eliminating the need for pre- and post-library preparation quantification. Retaining the speed and performance of the original workflow, MosaiX 2.0 generates ready-to-sequence libraries in approximately 90 minutes, streamlining library preparation for applications including whole-genome sequencing (WGS) and human target capture sequencing.

MosaiX 2.0 Library Preparation Kit Overview

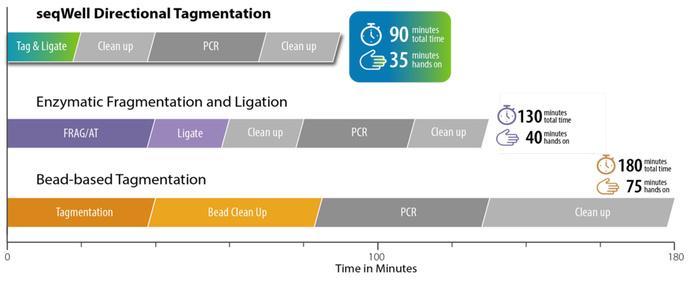
Directional Tagmentation with engineered TnX™ transposase

- seqWell's Directional Tagmentation chemistry combines TnX transposase tagging with adapter ligation into a single high-performance workflow.
- To maximize conversion of input DNA, MosaiX 2.0 uses Directional Tagmentation, which attaches Read 1 adapters to 5′ ends and ligates Read 2 adapters to 3′ ends without end repair.
- Unique dual indexes (UDIs) are added via PCR.



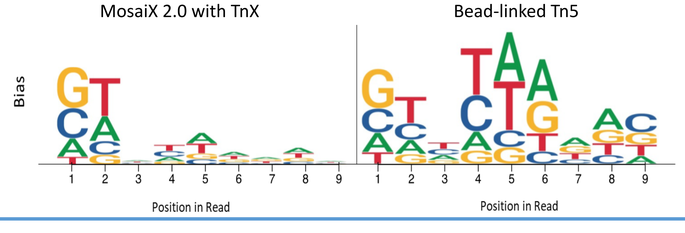
Fast, automatable workflow

- MosaiX 2.0 takes ≤ 90 minutes from start to finish with only 35 minutes of hands-on time.
- Master-mix-based chemistry is compatible with common liquid-handling platforms for scalable, high-throughput processing.



TnX: reduced insertion site bias compared with Tn5

- MosaiX 2.0 with TnX (left) has lower insertion site bias than Tn5-based methods (right), giving more even coverage and less biased libraries.

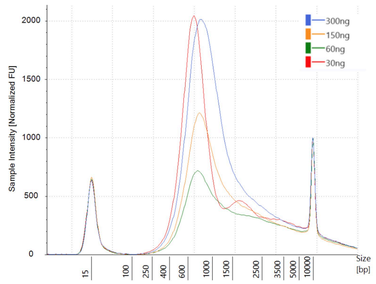


Auto-normalization & Competitive Performance in Human WGS and WES

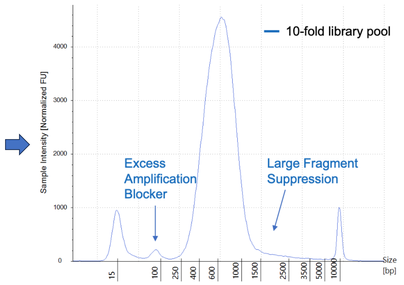
Auto-normalization

Our new proprietary technology produces libraries with uniform insert size and normalized total PCR product yield. It does this by combining **large fragment suppression** with an **excess amplification blocker,** reducing the need for quantification and QC before and after library prep.

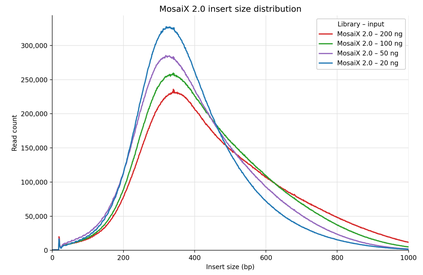
Standard library prep: 10-fold DNA input range, fixed tagmentation strength and PCR cycle number



With auto-normalization: 10-fold DNA input range, fixed tagmentation strength and PCR cycle number



10-fold input range sequenced on a NovaSeq X



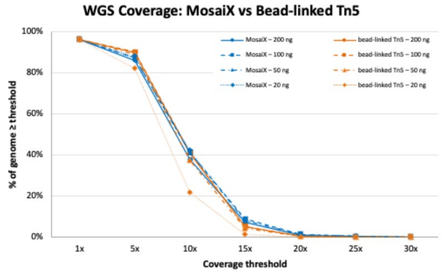
High performance in WGS

Compared with bead-linked Tn5, MosaiX 2.0 gave more consistent coverage across a 10-fold range of DNA input (right), with no protocol changes needed for different input amounts.

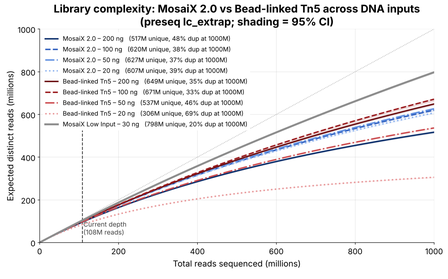
Library complexity of MosaiX 2.0 libraries made from 20–100 ng (right) are tightly grouped and tracks closely with bead-linked Tn5 libraries made from 100–200 ng. Curves were extrapolated from 108M to 1,000M reads with preseq lc_extrap. MosaiX Low Input (formerly MosaiX) shows the highest complexity but requires a fixed input amount.

GC bias of the 200 ng libraries (right) shows similar uniformity across the human genome for both workflows.

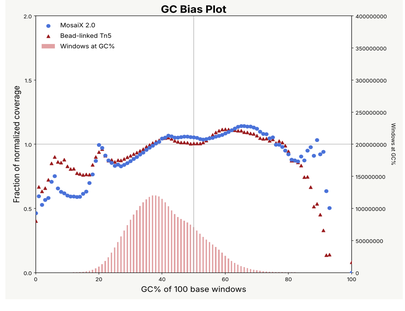
WGS Coverage: MosaiX vs Bead-linked Tn5



Library complexity: MosaiX 2.0 vs Bead-linked Tn5 across DNA inputs (preseq lc_extrap; shading = 95% CI)



GC Bias Plot



High performance in WES

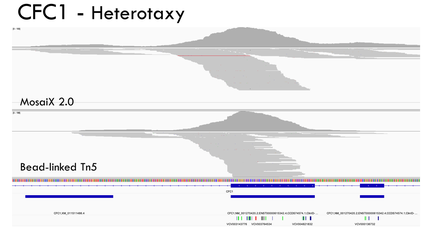
Library Prep Method	PF Gb	% Bases ≥ 20X	Insert Size (bp)	% Selected	% Dup.	HS Library Size	Fold 80	% Zero Coverage
MosaiX 2.0	9 Gb	98.4%	370	0.87	9.6%	101,795,965	1.38	**0.65%**
Bead-linked Tn5 tagmentation	9 Gb	98.2%	183	0.917	8.9%	128,895,138	1.29	0.82%

In a head-to-head comparison with bead-linked Tn5, MosaiX 2.0 had ~20% fewer target bases with zero coverage (table above). Its longer inserts likely explain the smaller HS library size.

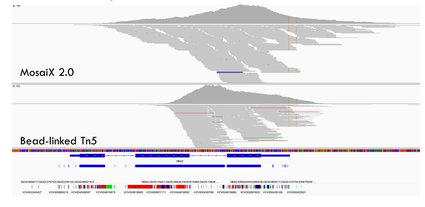
IGV views (right) show clinically relevant target exons that are covered in MosaiX 2.0 libraries but missing in bead-linked Tn5 libraries.

Libraries were made from 50 ng of NA12878, captured with the Twist Exome 2.0 panel, and sequenced on a NovaSeq X.

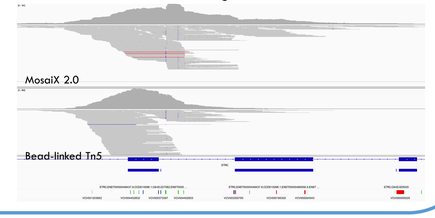
CFC1 - Heterotaxy



HBA2 - Alpha-thalassemia



STRC - DFNB16 hearing loss



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