Directional Tagmentation for Sequencing of Challenging DNA Samples

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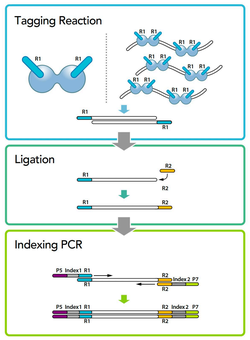
Introduction

The MosaiX™ DNA Library Prep Kit generates high-quality libraries in a rapid, automatable workflow using directional tagmentation with seqWell's engineered TnX™ transposase. Compared with Tn5, TnX has lower insertion site bias and reduced dependence on GC content, allowing greater coverage uniformity in whole-genome and exome sequencing. Directional tagmentation maximizes library diversity by preventing the redundant adapter tagging events inherent to traditional tagmentation, an advantage that is especially critical for low-input or degraded samples containing enzymatic inhibitors. To enable directionality, read 1 adapters are attached to the 5′ end of insert DNA via tagmentation, while read 2 adapters are attached to the 3′ end using a proprietary ligation strategy. Here we compare MosaiX DNA Library Prep to bead-linked tagmentation as well as enzymatic fragmentation with ligation using mock metagenomic communities, FFPE DNA of varying degradation, and contrived samples containing common inhibitors such as sodium humate, EDTA, and hematin.

MosaiX Library Preparation Kit Overview

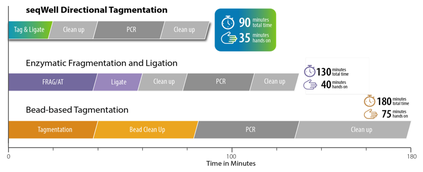
Directional Tagmentation with TnX Engineered Transposase

- Compatible with common elution buffers: DNA input can be suspended in Tris, TE or low TE without buffer exchange or adjustment of fragmentation time.
- Traditional tagmentation is non-directional, leaving 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 uses Directional Tagmentation, which attaches Read 1 adapters to 5′ ends and ligates Read 2 adapters to 3′ ends without the need for end-repair, A-tailing, or phosphorylation.
- Unique dual indexes (UDIs) are added via PCR.



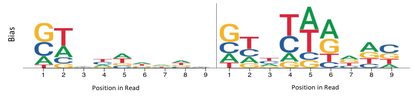
MosaiX Fast and Automatable Workflow

- Minimal hands-on time.
- MosaiX chemistry is compatible with common liquid-handling platforms for scalable, high-throughput processing.
- Streamlined workflow enables faster turnaround time than traditional tagmentation or ligation.



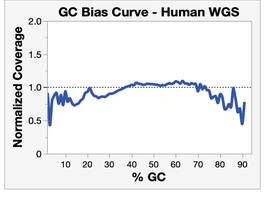
TnX: Reduced Insertion Site Bias Compared to Tn5

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



MosaiX with Even Coverage Across Wide GC Range

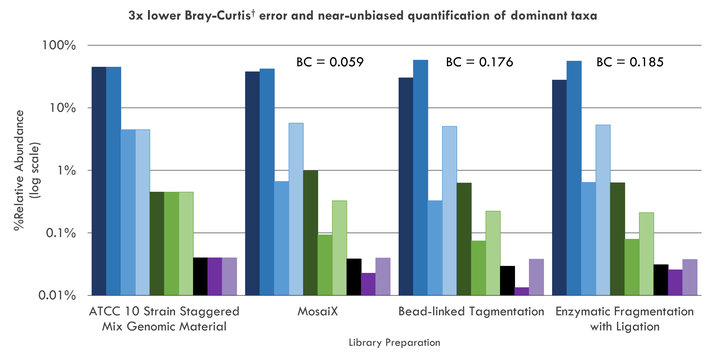
- Normalized coverage is plotted by %GC content to generate a GC bias curve for a human MosaiX library.
- The bias curve is flat and near the 1.0 line, indicating even coverage across the entire 10 – 90% GC spectrum.



Metagenomic Samples

MosaiX More Accurately Recovers Community Composition and Exhibits Comparable Assembly Performance

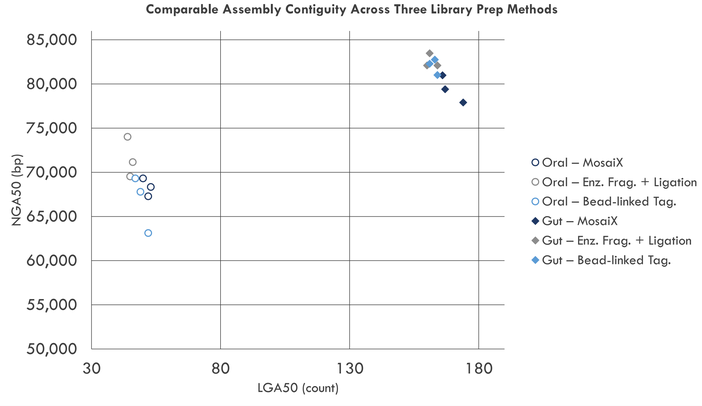
- ATCC 10 Strain Staggered Mix (MSA-1001) community prepared with various library prep chemistries



†Bray-Curtis (BC): a dissimilarity metric comparing observed to expected composition; 0 means identical, 1 means completely different.

Staphylococcus epidermidis (33% GC)
Rhodobacter sphaeroides (69% GC)
Bacillus cereus (35% GC)
Escherichia coli (51% GC)
Clostridium beijerinckii (28% GC)
Lactobacillus gasseri (35% GC)
Streptococcus mutans (37% GC)
Enterococcus faecalis (37% GC)
Bifidobacterium adolescentis (59% GC)
Deinococcus radiodurans (67% GC)

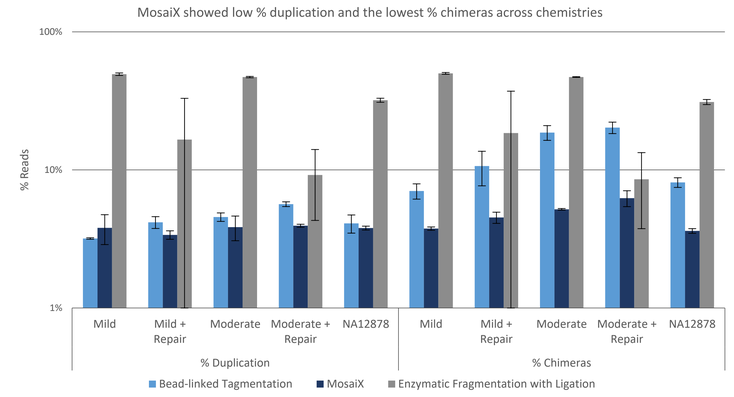
- Assembly performance on ATCC Oral (MSA-1004) and Gut (MSA-1006) Microbiome Genomic Mixes



FFPE DNA and Tolerance to Inhibitors

Directional Tagmentation Reduces Chimera Formation in FFPE DNA

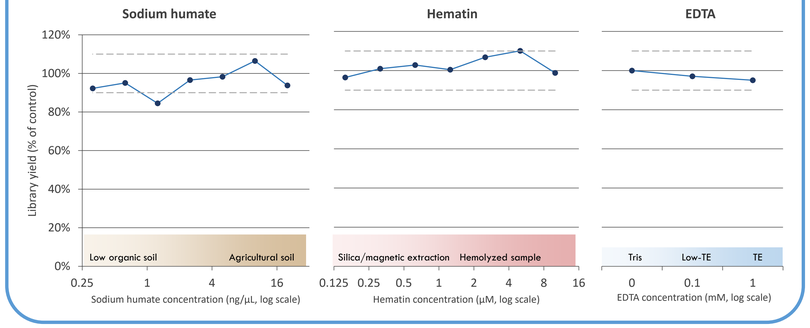
- Libraries from 10 ng of Horizon FFPE DNA (Mild, Moderate and Severe), with or without FFPE DNA repair (NEB E7360), and intact NA12878 were sequenced on a MiSeq i100 and downsampled to 400,000 reads.
- DIN values for Horizon FFPE Mild, Moderate and Severe are 6.8, 3.7 and 1.6, respectively. None of the 3 chemistries produced robust libraries from Severe FFPE DNA.*
- All methods followed the manufacturers recommended starting conditions without optimization: MosaiX (13 cycles), bead-linked tagmentation (8 cycles), and enzymatic fragmentation with ligation (11 cycles).
- MosaiX produced low duplication and a higher estimated library size despite more PCR cycles.



*Severe FFPE DNA is not shown because no chemistry produced a robust library.

MosaiX is Resistant to Common Inhibitors

- MosaiX library yield was unaffected by sodium humate and hematin.



Conclusions

Library prep: MosaiX with TnX has lower insertion site bias than Tn5 and gives even coverage across 10–90% GC in a fast, automatable workflow.

Metagenomics: MosaiX more accurately recovers community composition and comparable assembly contiguity in mock microbial community.

FFPE and inhibitors: MosaiX produced the lowest duplication rate and fewest chimeras in FFPE DNA. Directional tagmentation also proved the most robust against EDTA, sodium humate, and hematin.

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