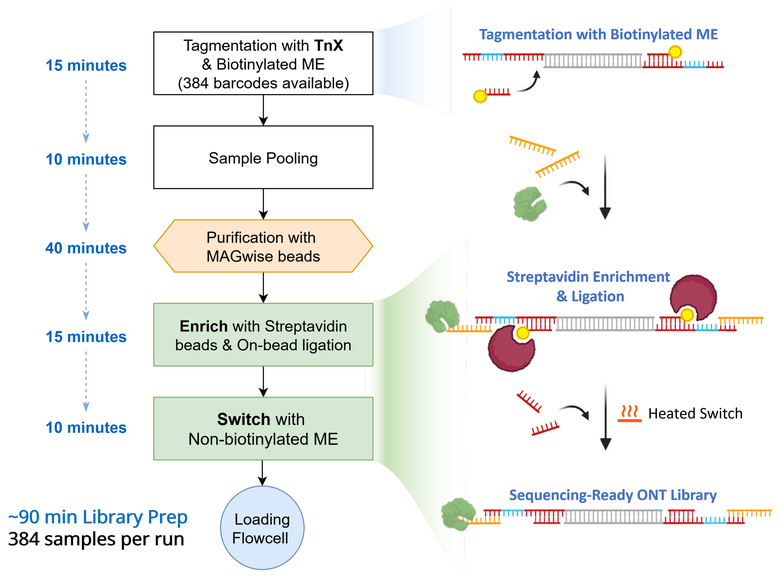
Highly Multiplexed Auto-Normalized Library Prep on the Oxford Nanopore Platform using TnX™ Transposase

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Introduction

Oxford Nanopore Technologies (ONT) long-read sequencing enables rapid clinical diagnostics, though current transposase-based library preparation is limited to 96 barcodes and can produce substantial variation in read counts across transposomes. Here, we introduce Enrich & Switch Library Preparation workflow, a new ONT library preparation using TnX, a novel engineered transposase designed to reduce insertion bias and improve coverage uniformity and barcode balance. Our TnX-based workflow supports 384 barcodes, automatically normalizes libraries across an 8-fold input range, and enriches tagged molecules to improve pore occupancy and recovery from low-input samples. Across plasmids, amplicons, and microbial organisms spanning diverse GC contents, TnX delivers more uniform read counts, reduced species bias, and improved N50.

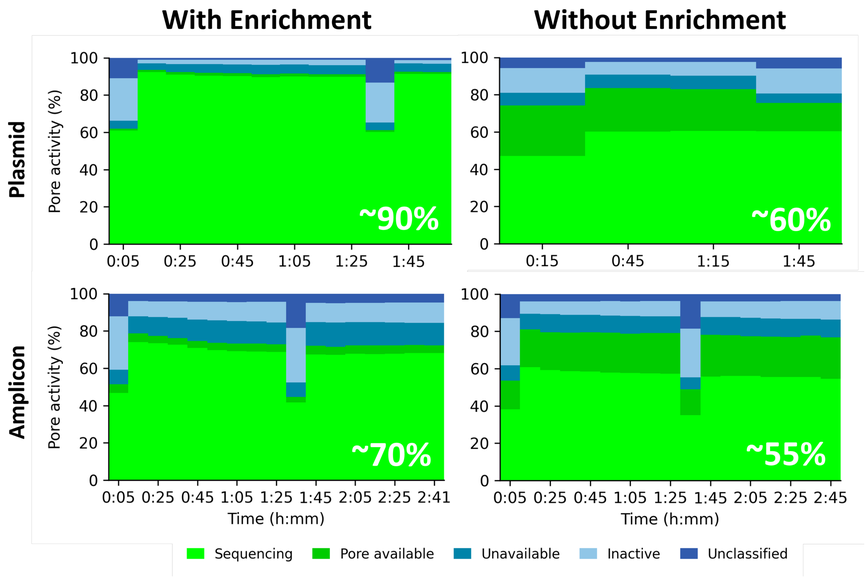
Enrich & Switch Library Preparation Workflow Overview



- Input DNA is tagmented and biotin-labeled with 384 uniquely barcoded TnX transposomes.
- After MAGwise cleanup, streptavidin beads capture tagged molecules to normalize input, and ONT Native adapters are ligated on-bead.
- A heated switch to non-biotinylated ME oligos releases sequencing-ready libraries.

Figure 1.
Enrich & Switch Library Preparation Kit workflow for high-throughput 384-plex ONT library preparation with molecular schematic.

Streptavidin Enrichment Boosts Pore Occupancy



- Streptavidin enrichment captures only tagged molecules, so more of the loaded library can be sequenced.
- Compared with workflows without enrichment, pore occupancy rose from ~60% to ~90% for plasmids and from ~55% to ~70% for amplicons, with fewer idle pores.

Figure 2. Pore activity with and without streptavidin enrichment.
Upper: plasmids prepared with Enrich & Switch (left) or a similar workflow without enrichment (right).
Lower: amplicons prepared with Enrich & Switch (left) or the ONT Rapid Barcoding Kit (right) which has no enrichment step. Percentages show estimated pore occupancy.

Enrich & Switch Performance Across Different Samples

Methods

Library preparation

Plasmid: 384 pUC19 plasmids (2.7 kb, NEB) at 25, 50, 100 and 200 ng input (8-fold range) across four 96-well plates.
Amplicon: 96 amplicons (~400 bp) at 25, 50, 100 and 200 ng input.
Microbial: genomic DNA from 3 bacteria (Table 1) at 50 ng input.

Table 1. Summary of bacterial genomic DNA samples used in the study.

Organism	Strain	Genome size	GC content
Pseudomonas aeruginosa	ATCC 27853	6.84 Mb	66.1%
Escherichia coli	B str. REL606	4.63 Mb	50.8%
Bacillus cereus	ATCC 14579	5.41 Mb	35.3%

All libraries were prepared with the Enrich & Switch workflow.

Sequencing & Analysis

Each pool was sequenced on a MinION R10.4.1 flow cell (>1,200 active pores) to 1 Gb of estimated data. Reads were demultiplexed, filtered, trimmed and QC'd using Dorado in a custom Nextflow pipeline (seqWell-ONT-dorado-demux), now publicly available on GitHub.

Plasmid

Auto-normalized, full-length plasmids · 1 Gb in < 2 h

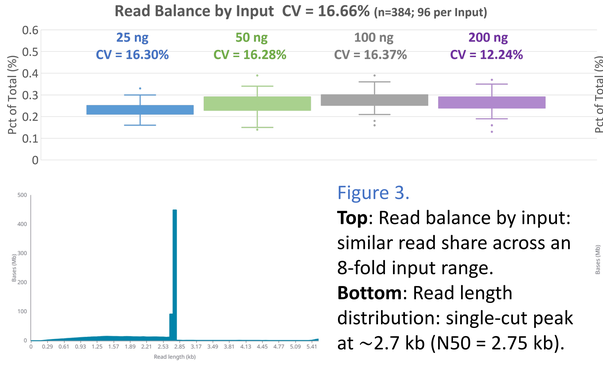


Figure 3.
Top: Read balance by input: similar read share across an 8-fold input range.
Bottom: Read length distribution: single-cut peak at ~2.7 kb (N50 = 2.75 kb).

Amplicon

Even read balance across 8-fold input · 1 Gb in ~2.5 h

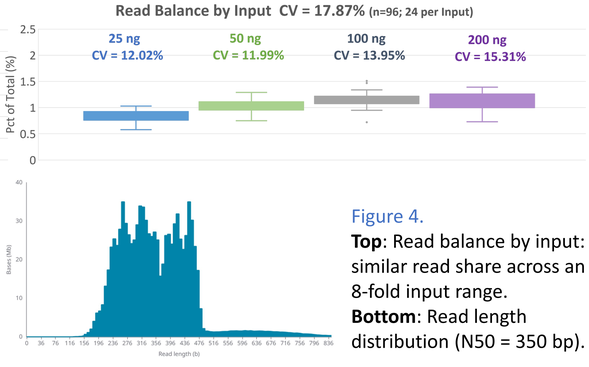


Figure 4.
Top: Read balance by input: similar read share across an 8-fold input range.
Bottom: Read length distribution (N50 = 350 bp).

Microbial

Balanced genomes across 35–66% GC · 1 Gb in < 2 h

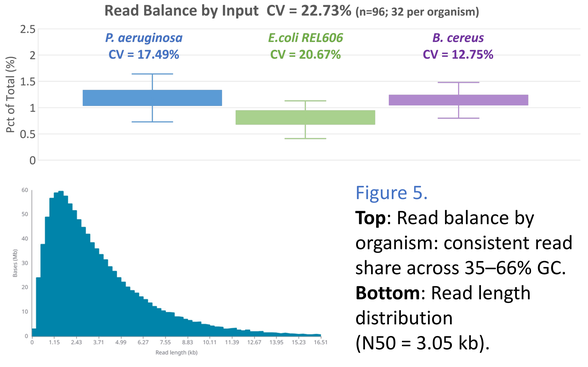


Figure 5.
Top: Read balance by organism: consistent read share across 35–66% GC.
Bottom: Read length distribution (N50 = 3.05 kb).

Conclusion

Novel Enrich & Switch workflow delivers 384-plex ONT libraries in ~90 minutes, with higher pore occupancy, auto-normalized read counts and consistent performance across plasmids, amplicons and microbial genomes.

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