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## Introduction

Traditional next generation sequencing library preparation is time-consuming, and requires specialized shearing instrumentation as well as several enzymatic reactions and cleanup steps.

Transposase-based methods present well-known advantages:

- Decreased sample input
- Decreased turnaround time
- Increased throughput capacity.

However, transposase-based library construction methods have been known to increase duplication as well as bias in difficult genome regions, which has limited this approach for whole genome libraries.

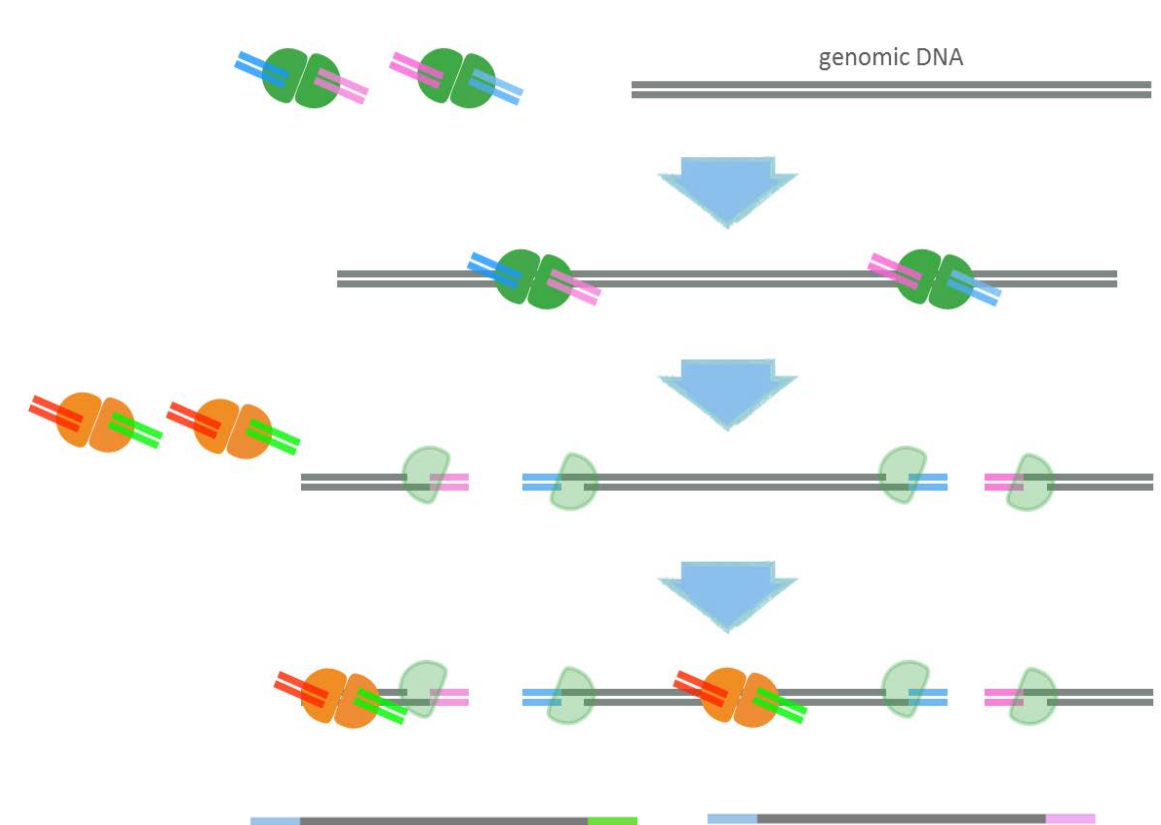
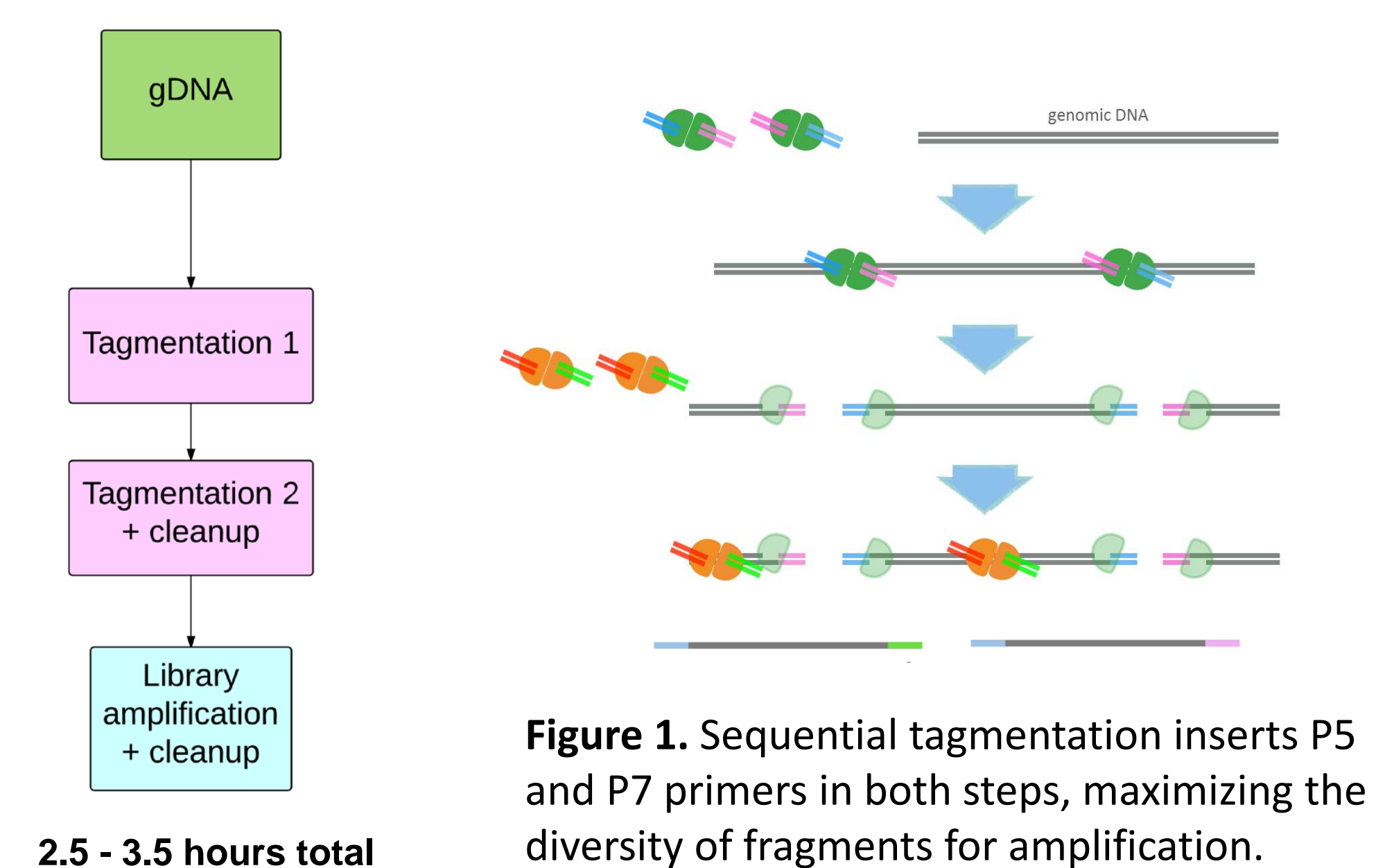
Here we present a novel method for library preparation from human gDNA using sequential tagmentation by two transposases. Initial data shows improved quality over current transposase-based methods:

- More balanced representation across the GC spectrum
- Increased library diversity and decreased duplication

## Workflow Detail

Simple overall workflow enables:

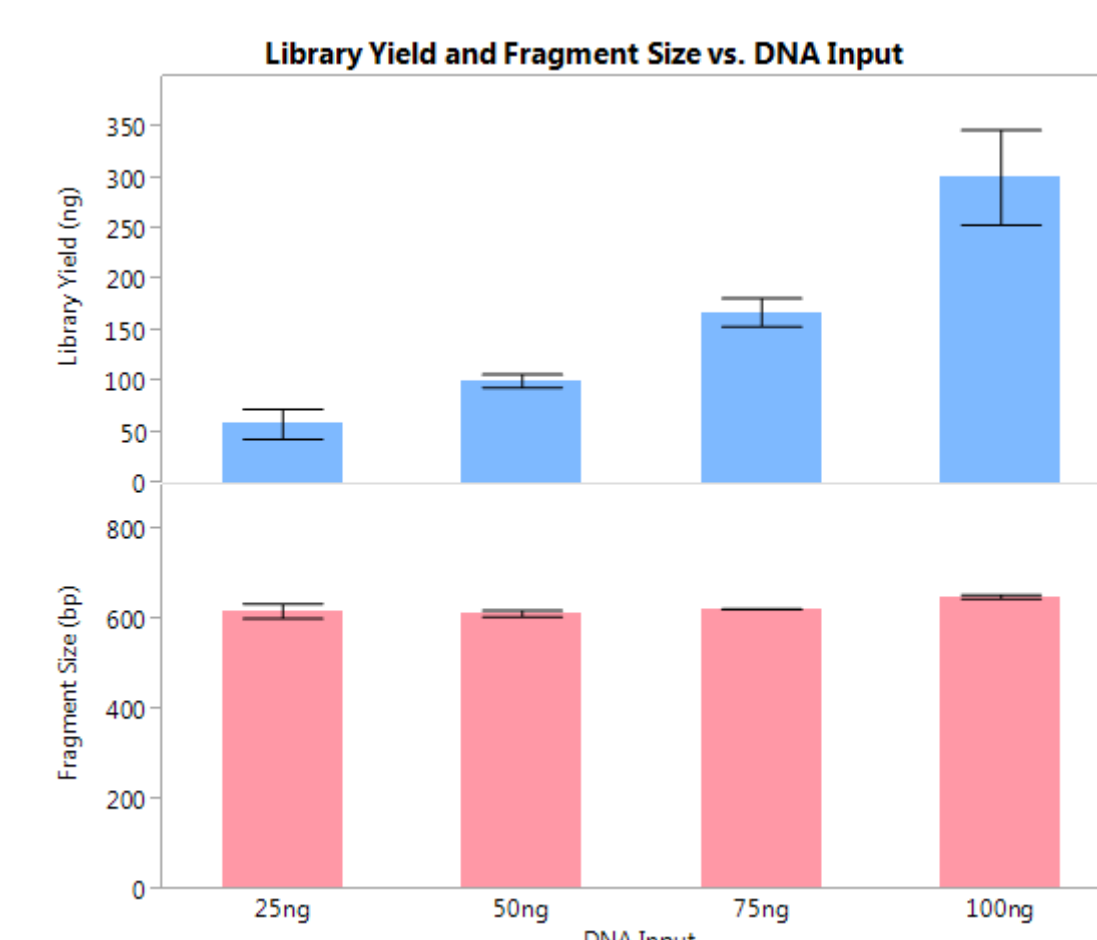
- rapid overall processing time
- no requirement for shearing instrumentation
- high throughput using automation for every step



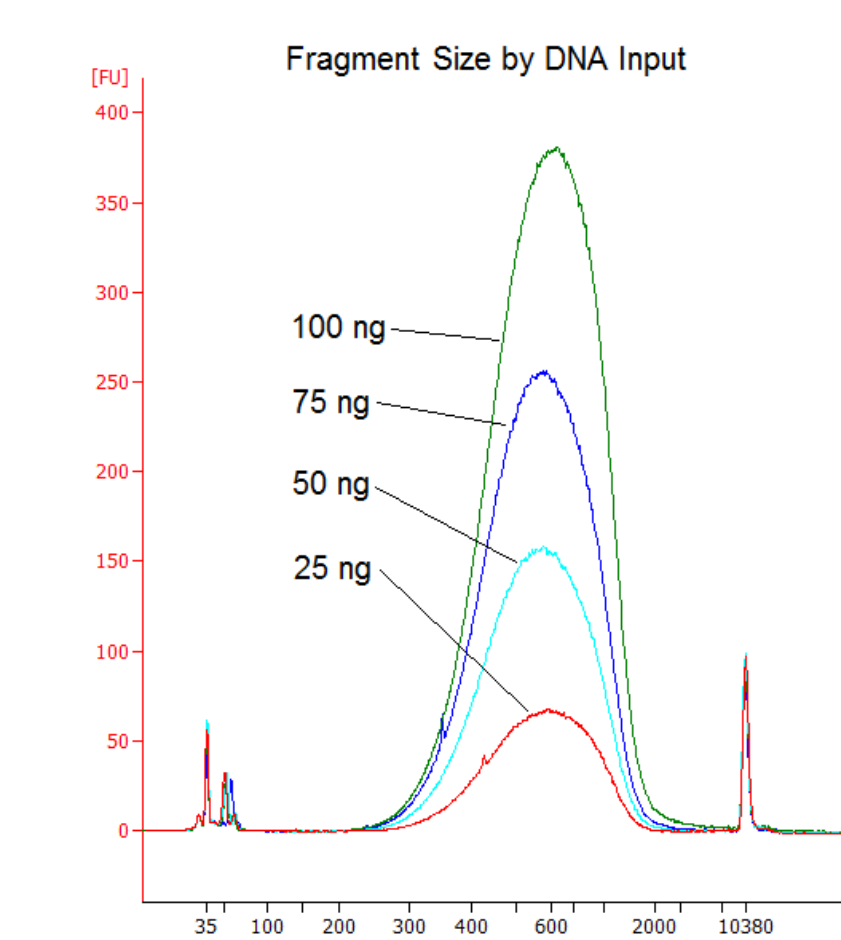
**Figure 1.** Sequential tagmentation inserts P5 and P7 primers in both steps, maximizing the diversity of fragments for amplification.

## Library Construction QC Metrics

Whole genome libraries can be prepared from as little as 25 ng of input DNA



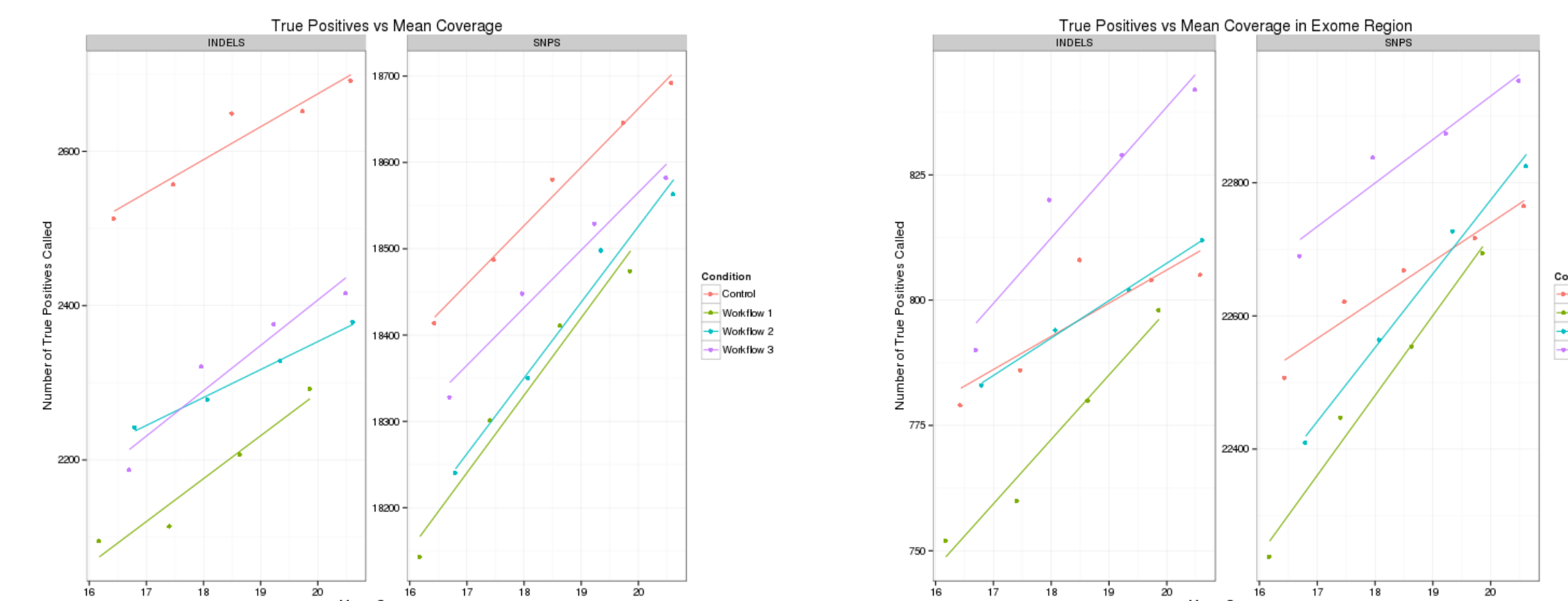
**Figure 2.** Library yield is dependent on gDNA input, but input amount does not significantly affect library fragment size



**Figure 3.** Post-PCR fragment size as measured by Bionanalyzer is independent of DNA input amount

## Sensitivity and Accuracy

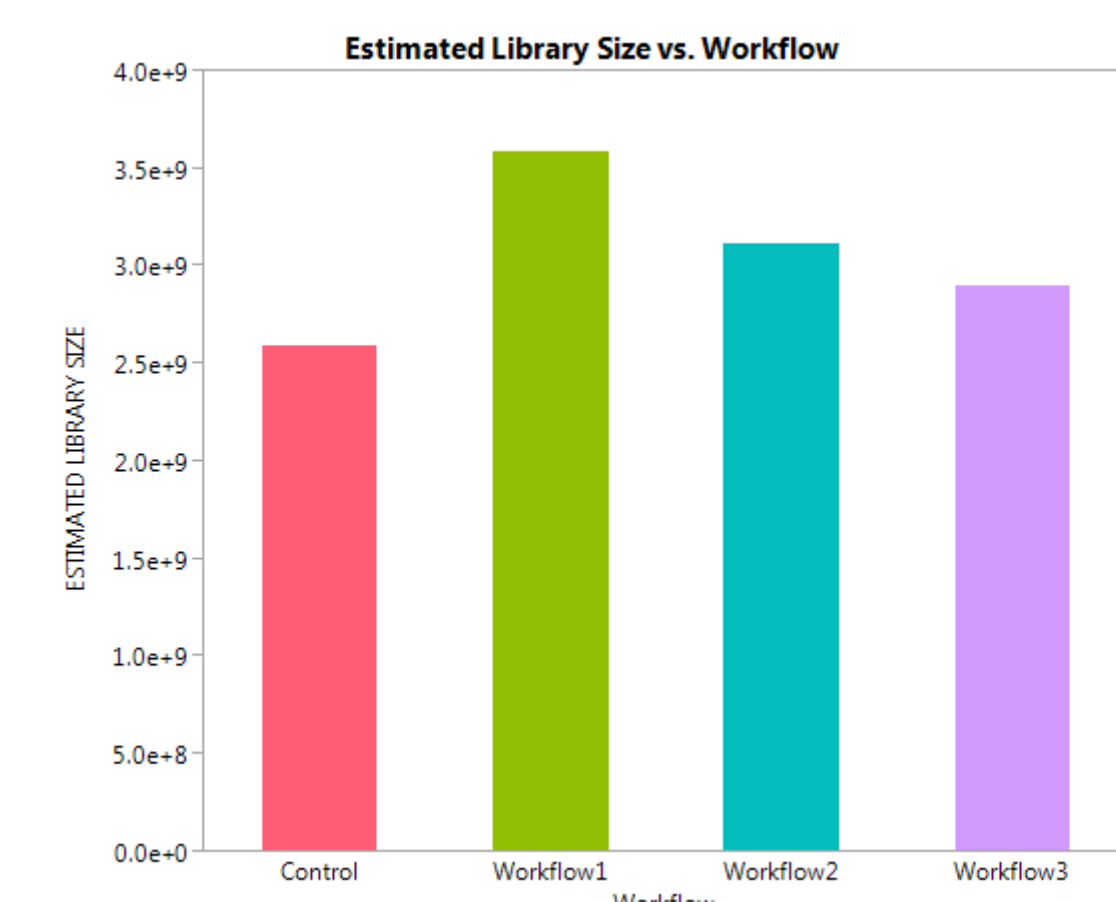
Precision and heterozygous concordance are similar between these workflows and traditional Nextera, with >99% for SNPs and >98% for Indels on Chromosome 20.



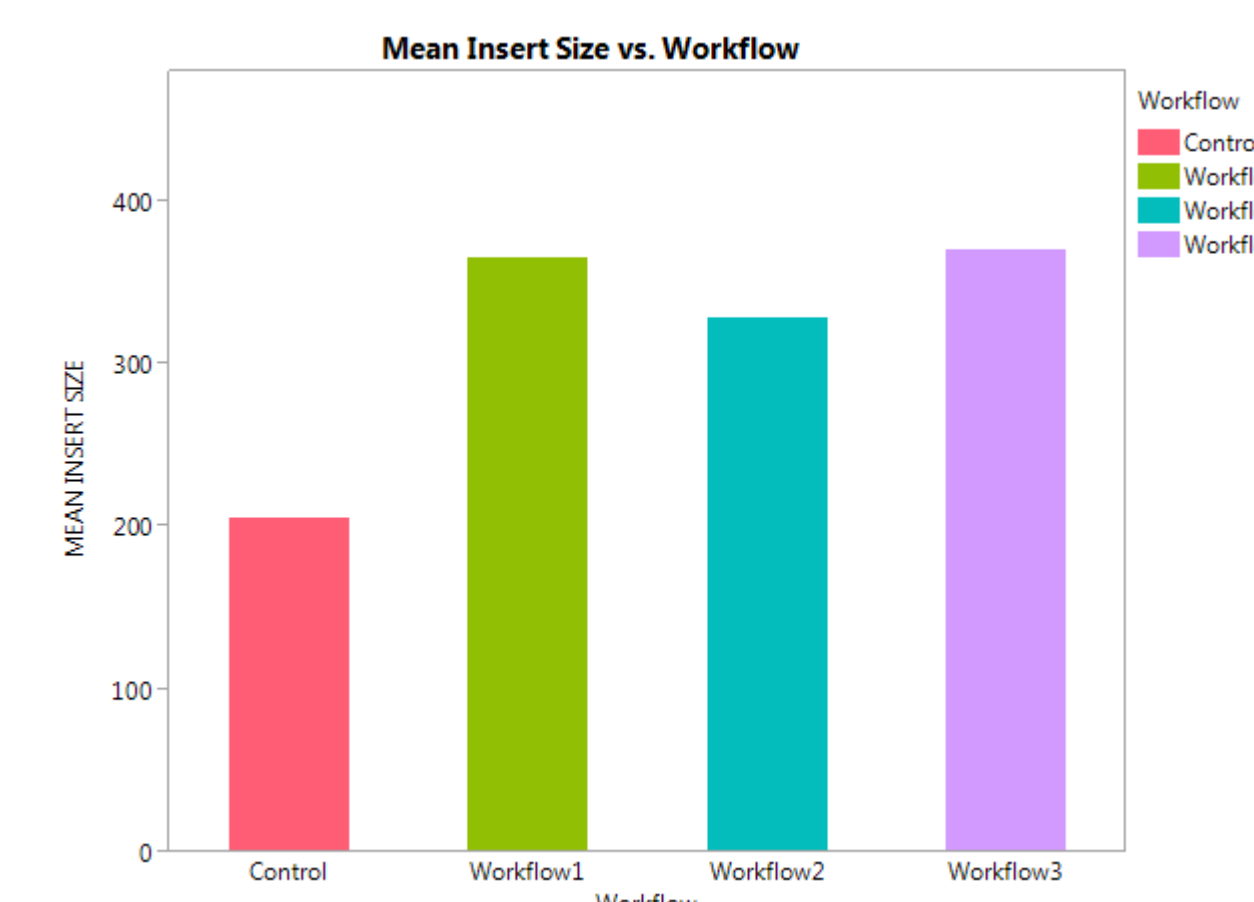
**Figures 8 and 9.** Sensitivity for SNPs and Indels on Chromosome 20 is higher for the control workflow at ~20x coverage, however in the exome region one of the sequential workflows appears to be more sensitive than the control. Down-sampling was used to examine sensitivity at comparable coverage between all methods.

## Sequencing QC Metrics

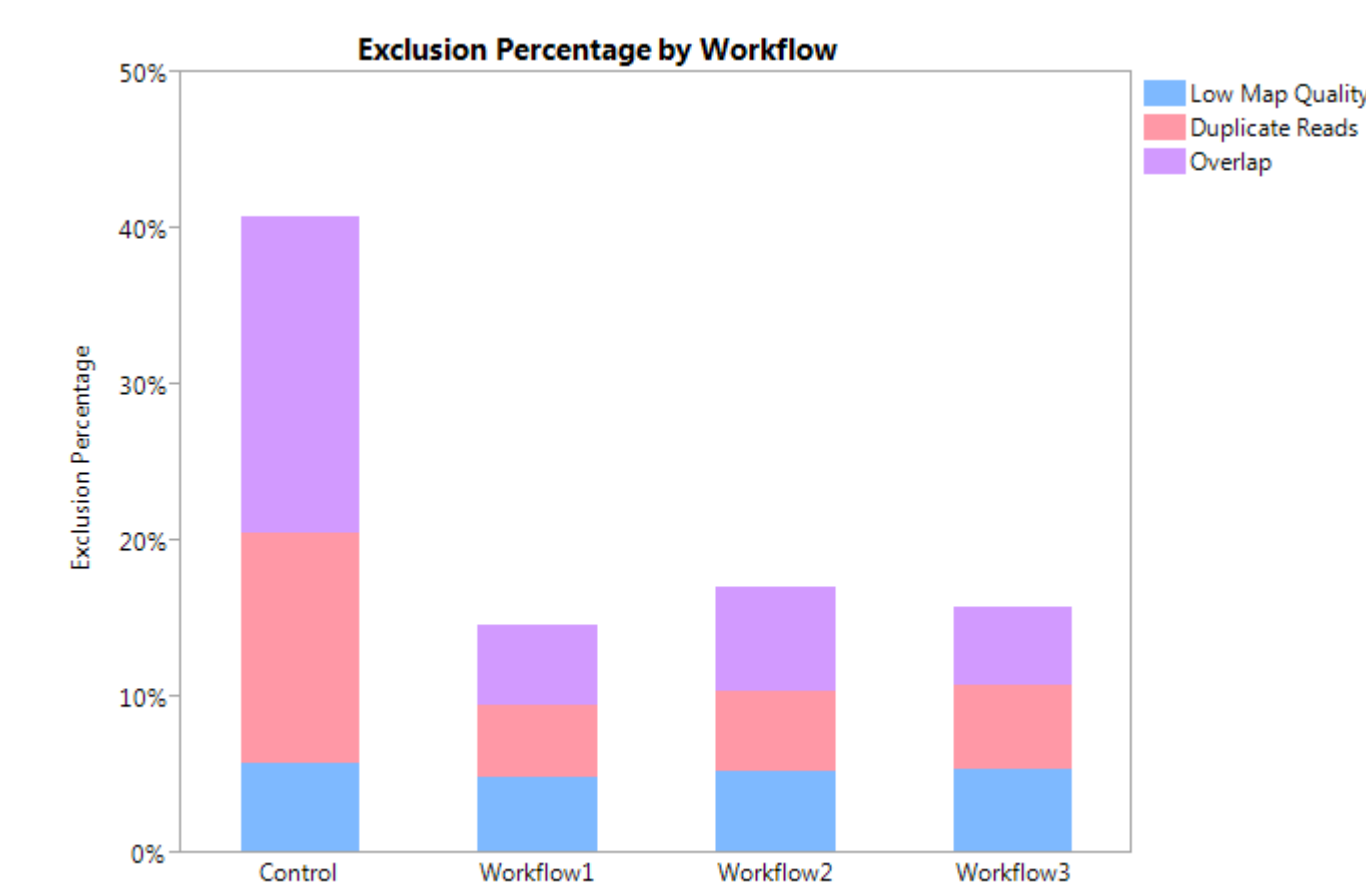
Analysis of 3 sequential tagmentation workflows compared here to traditional Nextera tagmentation as a control method. The 3 workflows differ in concentration and order of transposases added.



**Figure 4.** Sequential tagmentation libraries have higher diversity (estimated library size) than control Nextera libraries.

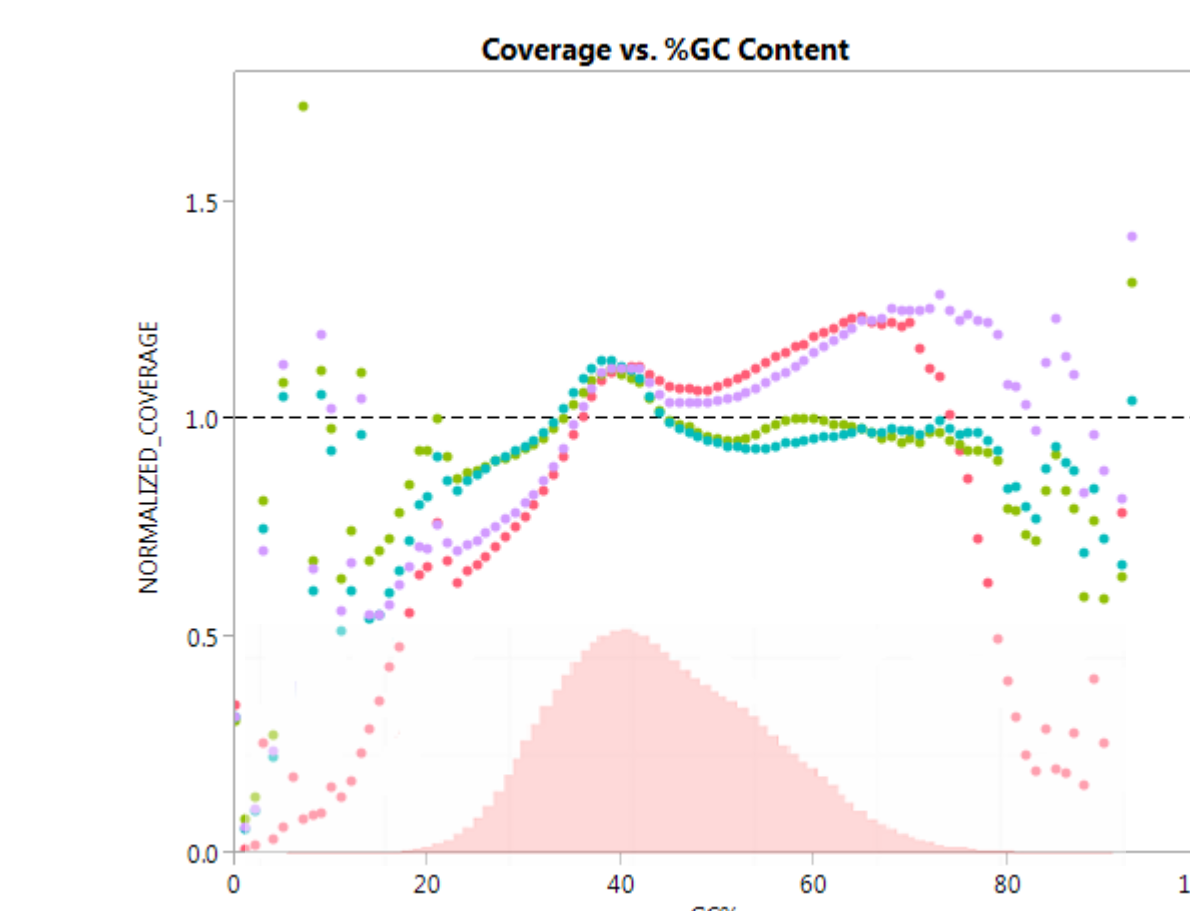


**Figure 5.** Sequential tagmentation libraries have larger mean insert size than control Nextera libraries.

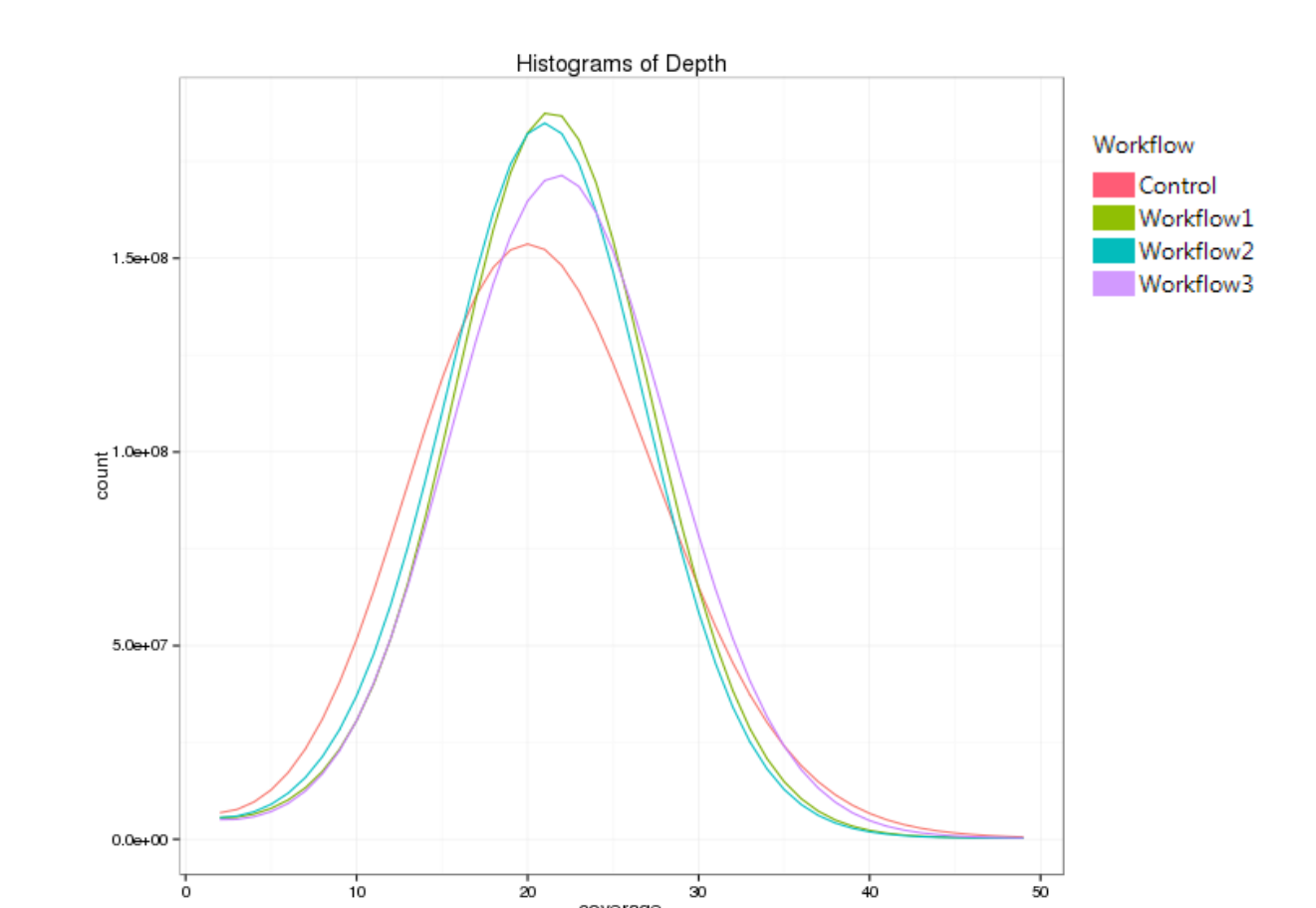


**Figure 6.** Sequential tagmentation libraries have significantly lower exclusion due to insert overlap and duplication rates

- Low map quality – bases filtered due to mapping quality <20
- Duplicate reads – bases filtered out due to PCR duplication or optical duplication
- Overlap - bases filtered as a second observation from an insert with overlapping reads.



**Figure 10.** GC bias varies between workflows. Two of the workflows show improved balance of across the GC spectrum as compared to Nextera.



**Figure 11.** All three workflows show improved evenness of coverage as compared to Nextera, enabling greater sequencing depth.

## Conclusion

- The initial data review suggests that sequential tagmentation using two novel transposases can be used to generate high-quality libraries from human gDNA, enabling benefits such as reduced input and sample preparation time.
- Whole genome analysis of these libraries indicates low duplication and high diversity, with improved evenness of coverage as compared to Nextera.
- While the sensitivity is superior in the exome regions, further development will aim to improve sensitivity overall.