Normalizing UDI Library Construction for Sensitive Genomic Applications

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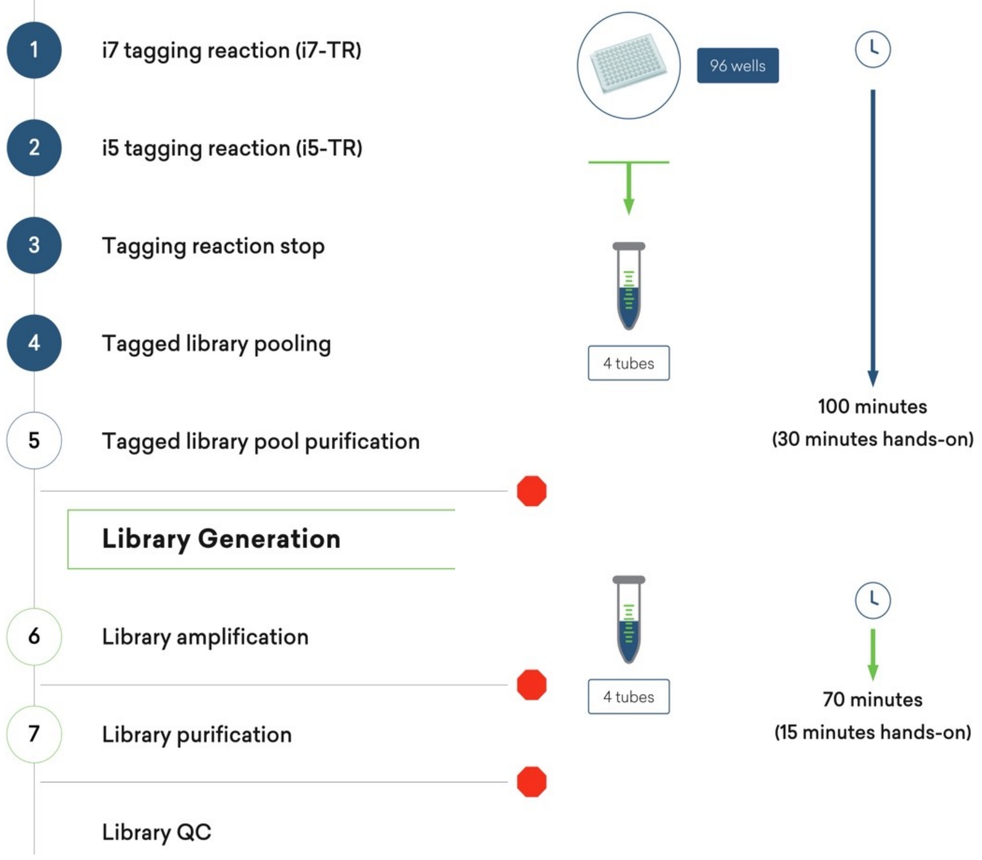
Introduction

Most available unique dual indexing (UDI) methods incorporate indexing into the library PCR step, which can be labor- and cost-intensive as libraries must be amplified, purified, quantified, and normalized individually prior to pooling for sequencing. In the purePlex™ DNA Library Preparation workflow, auto-normalization is achieved through sequential transposition events of full-length indexed adapters in the presence of a novel normalization reagent. This approach allows incorporation of unique dual indexing and permits pooling of samples immediately following the tagging steps such that purification and amplification of fragments occur after pooling, reducing the QC and labor costs over traditional UDI workflows.

A distinct advantage of the purePlex DNA library preparation workflow is the elimination of individual sample and library normalization. Herein we demonstrate the auto-normalization of the purePlex DNA library preparation method in terms of reads per sample, median insert, and performance across bacterial genomes with GC content ranging from 29-69%. In contrast to other transposase mediated UDI library prep kits, such as Nextera XT DNA Library Preparation kit, the insert size is preserved across all samples without the need for careful normalization of the starting DNA and regardless of GC content.

With a 10-fold input range and robust performance across a variety of genomes and sample types, we anticipate this method to have a wide applicability to NGS workflows that require both a streamlined protocol as well as the sensitivity and performance of UDIs.

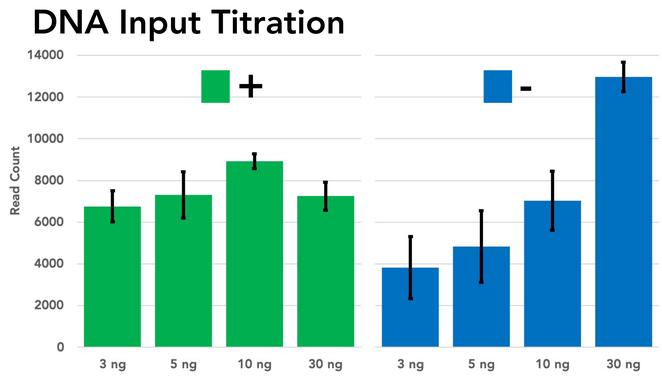
purePlex™ Workflow



Results

Auto-Normalization

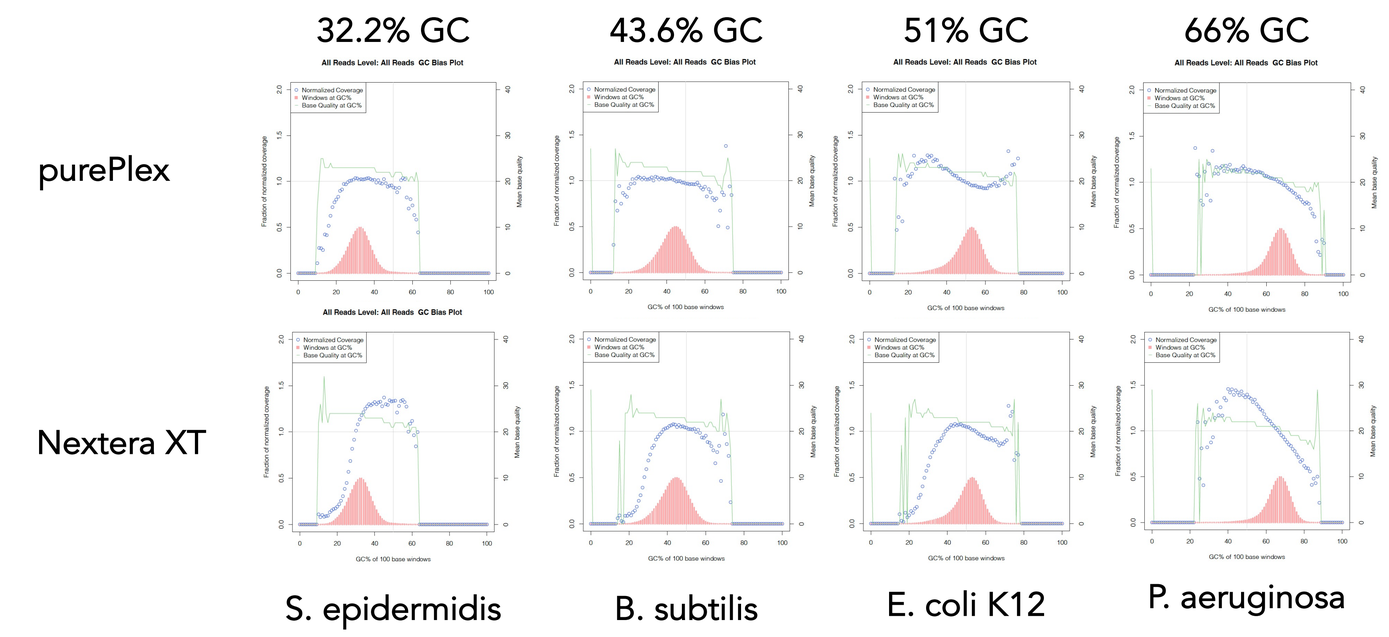
Samples were normalized to inputs of 3, 5, 10, and 30 ng then underwent purePlex library prep with (+) and without (-) normalization reagent. Read counts for each sample are equal, regardless of input, when normalization reagent is used. In contrast, without normalization reagent, sample read count scales with input.



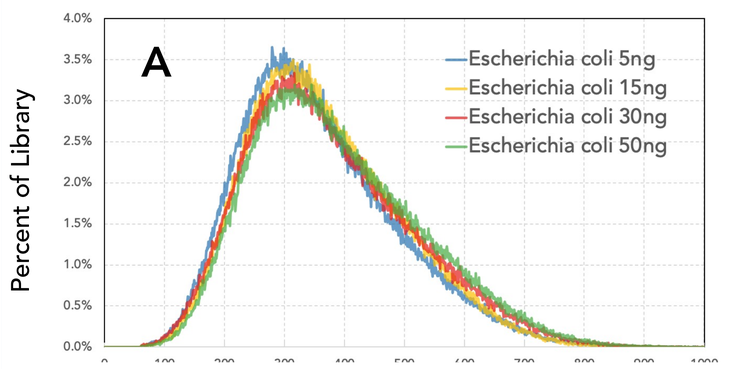
Robust performance for all GC contents

Libraries were prepared from bacterial genomic DNA (ATCC) ranging in GC content from 29-69%. purePlex Library preparation utilized each DNA at multiple inputs from 5-50 ng, whereas Nextera XT library preps required normalization to 1 ng input. Sequencing was performed on a NextSeq 550 300 cycle kit.

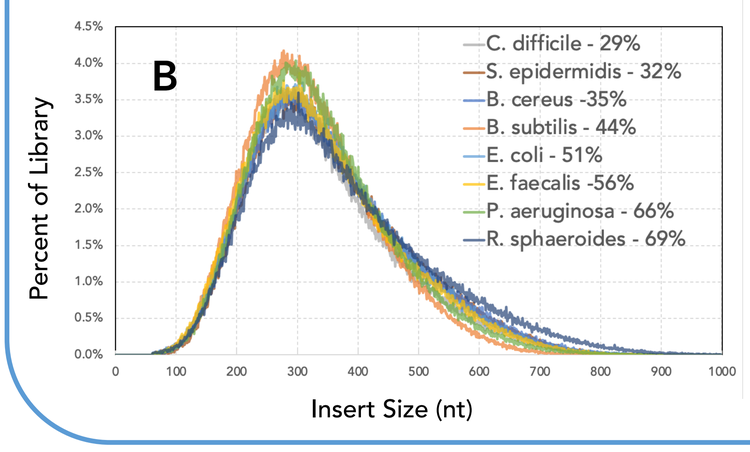
GC Bias plots, below, demonstrate purePlex library prep performs as well as or better than Nextera XT for low and high GC content samples.

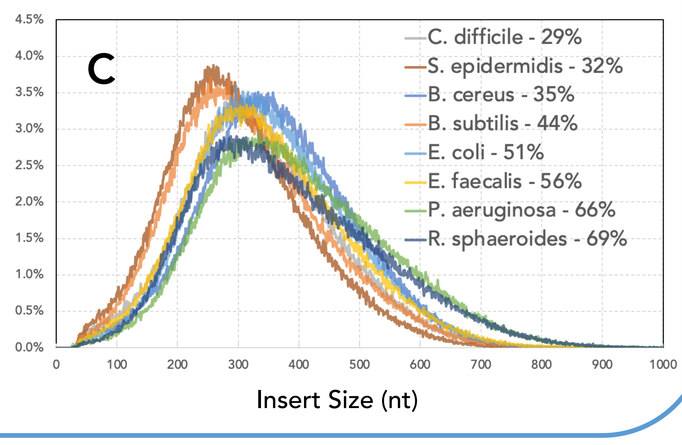


Insert Size



The insert size within a pool of samples is consistent regardless of input (panel A, left) or GC content (panel B, lower left). In contrast, Nextera XT libraries have varied fragment distributions from GC content even after normalizing the sample input.





Conclusions

- The purePlex DNA Library Preparation kit normalizes read count and insert size over a 10-fold input range, alleviating the burden of individual sample normalization and library pooling prior to sequencing.
- The purePlex DNA Library Preparation kit outperforms Nextera XT in terms of GC bias
- The purePlex DNA Library Preparation Kit generates consistent and tunable insert size regardless of sample input and GC content.