# 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.

Here, we describe a novel approach for an auto-normalizing UDI library construction that permits pooling of samples immediately after sequential transposase mediated tagging steps. Purification and amplification of library fragments occurs after samples are pooled greatly reducing the cost and labor over traditional UDI workflows. This new method utilizes a novel normalization technology during tn5 transposition that generates uniform quantities of library molecules across a 10-fold DNA input range.

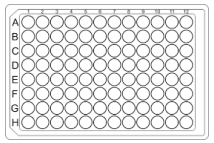
We anticipate the method will have wide applicability to NGS workflows requiring a streamlined workflow along with the sensitivity and performance of UDIs.

## Method

1. **i7 Tagging Reaction (i7-TR)**
   10 bp i7 indexes added
2. **i5 Tagging Reaction (i5-TR)**
   10 bp i5 indexes added
   Normalization reagent added
3. **Tagging reaction STOP**
4. **Equal volume pooling**
   24-plex pooling
5. **Pooled library purification**
6. **Pooled library amplification**
7. **Pooled library purification**
8. **Pooled library QC**

96-well plate

24-plex pooled libraries



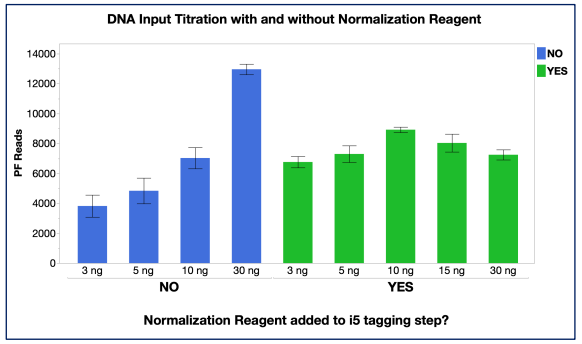

4 PCRs per 96

Only 4 pools to QC per 96

## Results

### Novel Normalization Reagent balances read counts across a 10-fold input range

- A core feature of seqWell's plexWell library preparation technology is integrated normalization, defined as uniform read count and insert size across a wide DNA input range. Until now, this has been achieved through sequential tagging including a pooled tagging step.
- Herein we achieved the same auto-normalization without a pooled tagging step by adding seqWell's proprietary **Normalization Reagent**, during the i5 tagging step.
- The results (graph at right) demonstrate **normalization over a 10-fold input range** versus a control sample set without Normalization Reagent added.




### Consistent read counts and insert sizes using UDI tagging with Normalization Reagent

- The UDI workflow normalizes read count and generates consistent insert sizes over 5-50 ng of DNA input. The insert size can be modified by altering the library purification conditions.
- Duplication rates for human DNA when down-sampled to 1 million reads per sample averaged <1%.
- 96 unique amplicons we prepped using the UDI reagents. Data was demultiplexed using UDIs or using the i7 index alone. Using UDIs effectively reduced unexpected alignments due to index misassignment/swapping.

| | Expected alignments | Unexpected alignments | % Misassigned |
|---|---|---|---|
| i7 only demultiplex | 10,585,107 | 207,191 | 1.92% |
| UDI demultiplex | 10,032,064 | 5,778 | 0.06% |