A novel multiplexed library construction method that streamlines sample preparation prior to targeted hybrid capture

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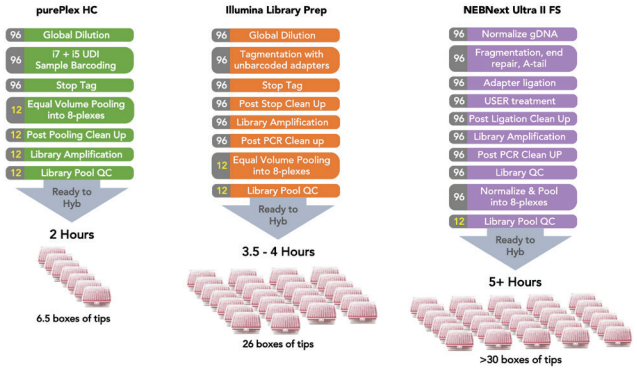
Introduction

Here, we describe "purePlex HC", a novel multiplexed library preparation chemistry optimized for hybrid capture. Building from seqWell's purePlex™ chemistry, this method utilizes unique dual indexes (UDIs) in a streamlined workflow that permits pooling of samples immediately following transposase (Tn5) mediated tagging. We demonstrate the suitability of this method in multiple human exome capture technologies and compare ease of use and performance to other commonly used library preparation kits.

- Streamlined workflow dramatically reduces labor, time, and consumables required to generate libraries for capture
- Generates multiplexed libraries ready for hybridization in around 2 hours across a DNA input range of 50-200 ng
- Libraries have high molecular complexity and are compatible with multiple commercially available hybrid capture technologies

"purePlex HC" workflow

Fig 1:



- Simultaneously fragments & adds full length UDI adapters
- Samples can then be multiplexed prior to further downstream steps, reducing labor, time, and consumables vs other methods.

Compatibility in exome capture – Twist and IDT

- 8-plex "purePlex HC" libraries were prepared from NA12878 (GIAB) with a starting DNA input range of 50-200 ng in replicate.
- Pooled libraries were then captured with both Twist Exome 2.0 and IDT xGen Exome v2 using standard manufacturer SOPs.
- Post capture, libraries were sequenced on NextSeq 2000 with 2x100 reads and downsampled to 6 Gb prior to exome analysis.
- Metrics at 6 Gb for the 100 ng input libraries are shown in Table 1 (below) and are on par with Twist and IDT published specs:

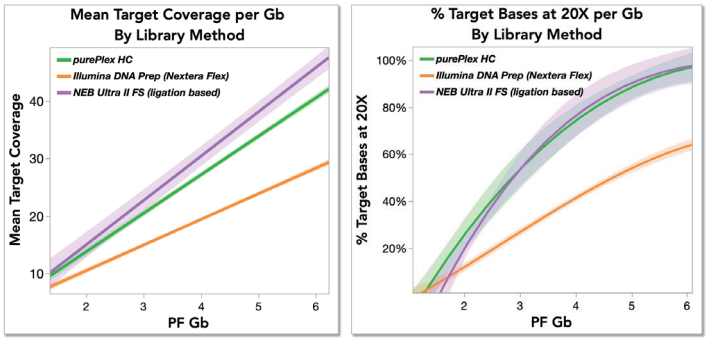
Table 1:

Key Metrics	Twist Exome 2.0	IDT xGen Exome V2
% Selected	88.7%	89.3%
Mean Target Cvg	43.9X	49.5X
% Bases ≥ 20X	95.0%	89.8%
% Zero Target Cvg	0.7%	1.0%
Fold 80 Penalty	1.36	2.11

Performance in Twist exome compared to other library prep methods

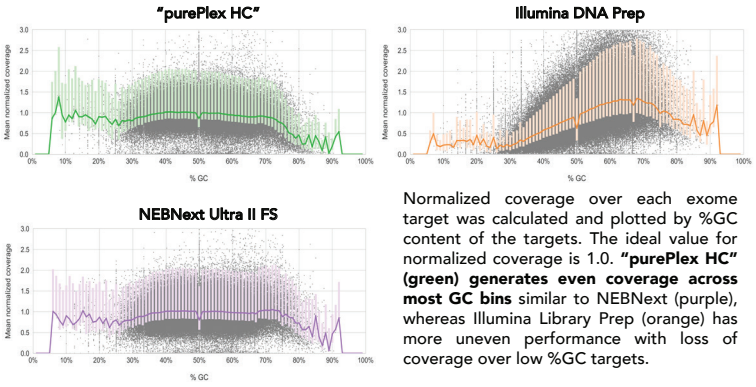
- 8-plex pooled libraries were prepared from NA12878 with starting DNA input of 50-200 ng using the following methods.
  - "purePlex HC" (Tn5-based)
  - Illumina DNA Prep Kit (Tn5-based) - *standard kit SOP*
  - NEBNext Ultra II FS (ligation-based) – *standard kit SOP*
- All were captured using Twist Exome 2.0, sequenced on NextSeq 2000 2x100, and analyzed with Picard HS Metrics.

Fig 2: "purePlex HC" reaches coverage targets with less sequencing compared to Illumina DNA prep and similar sequencing to NEBNext ligation-based library prep



Replicate 8-plexes were made using the 3 different library prep methods, captured using Twist Exome 2.0, and sequenced with at varying depths between 2-6 Gb. The Mean Target Coverage and % Bases at 20X for all samples are plotted by Gb of total sequencing, demonstrating that **"purePlex HC" reaches coverage targets with less sequencing than Illumina DNA Prep and similar sequencing to NEBNext.**

Fig 3: GC Bias by library prep method - Normalized coverage across exome targets of varying GC content



Normalized coverage over each exome target was calculated and plotted by %GC content of the targets. The ideal value for normalized coverage is 1.0. **"purePlex HC" (green) generates even coverage across most GC bins** similar to NEBNext (purple), whereas Illumina Library Prep (orange) has more uneven performance with loss of coverage over low %GC targets.

Table 2: Comparison of key hybrid capture metrics (downsampled to 6 Gb)

Key Metrics at 6 Gb (2x100)	purePlex HC	Illumina DNA Prep	NEBNext Ultra II FS
% Selected	88.7%	89.5%	92.0%
% Duplication	25.5%	53.8%	26.6%
Mean Insert Size	318 bp	129 bp	172 bp
Fold 80 Penalty	1.36	2.20	1.37
% Bases at 20X	95.0%	59.2%	97.0%
% Zero Target Cvg	0.73%	1.41%	0.93%

Summary and Conclusions

- The "purePlex HC" workflow is faster, less labor intensive, and uses less consumables than either ligation-based NEBNext Ultra II or Tn5-based Illumina DNA Prep. Performance in exome is nearly equivalent to NEBNext and superior to Illumina.
- This product is still in development. Contact info@seqwell.com for more information and alpha testing opportunities.