Whole-Genome Sequencing with the plexWell™ WGS-24 Library Preparation Kit

Introducing the first WGS library preparation kit with a true multiplexed workflow, delivering outstanding sequencing performance across 24 input samples.

Highlights

- **Efficient Multiplexed Workflow**

  plexWell WGS libraries (24-plex) are conveniently prepared in a single tube, which reduces associated labor, consumable and library QC costs by more than 10-fold.

- **More Samples per Sequencing Run**

  plexWell WGS-24 library prep kits automatically normalize read count and deliver uniform insert size distribution across all 24 DNA samples.

- **Outstanding Sequencing Results**

  Delivers best-in-class genome coverage and excellent SNV detection/callability for WGS.

Introduction

The plexWell WGS-24 Library Preparation Kit from seqWell Inc. is optimized for whole-genome sequencing of complex genomes on the Illumina® NovaSeq™ 6000 and other high throughput sequencing systems. Using advanced plexWell library prep technology, well-balanced multiplexed libraries (for 24 DNA samples) are produced in a single tube. Consequently, higher levels of sample multiplexing on sequencers are efficiently and reliably achieved.

Evolution of NGS Library Prep

Sequencing system output has increased at an astounding rate over the last several years, providing users with an opportunity to significantly reduce their sequencing costs per sample by simply loading more libraries per sequencing run. Until now, however, the methods for preparing and accurately pooling large numbers of libraries upstream of sequencing have been hampered by outdated NGS library prep kits that were originally designed to convert a single sample into a single sequence-ready NGS library. So when these older style library prep kits were modified by kit developers to make multiple libraries for running on the same sequencing run (*e.g.*, by supplementing kits with unique indexed primers), those kits were still constrained by a "one sample = one library" type workflow in which each sample was processed separately into a fully-purified library before mixing together with other libraries. One unfortunate consequence of the "one sample = one library" library prep paradigm has been that the associated complexity and cost (*i.e.*, the cost for labor, automation, kits, consumables, reagents, indexed primers, SPRI beads, electrophoresis, and qPCR) essentially scale-up in a linear fashion with the number of samples that are converted into NGS libraries.

plexWell: A Truly Multiplexed Library Prep Workflow

Growing demand for scaling-up library prep batch size for whole genome sequencing was the inspiration for developing plexWell WGS-24, featuring the world's first truly multiplexed library prep workflow (Figure 1). The sample-barcoding reagents used in the first step of the workflow are pre-dispensed in a convenient PCR plate format. After adding approximately 300 ng of genomic DNA per well and individually tagging the 24 DNA samples with unique barcodes, the tagged samples are all pooled together and processed to completion as a multiplexed library in a single tube format in about 3 hours (1 hour total hands-on time).
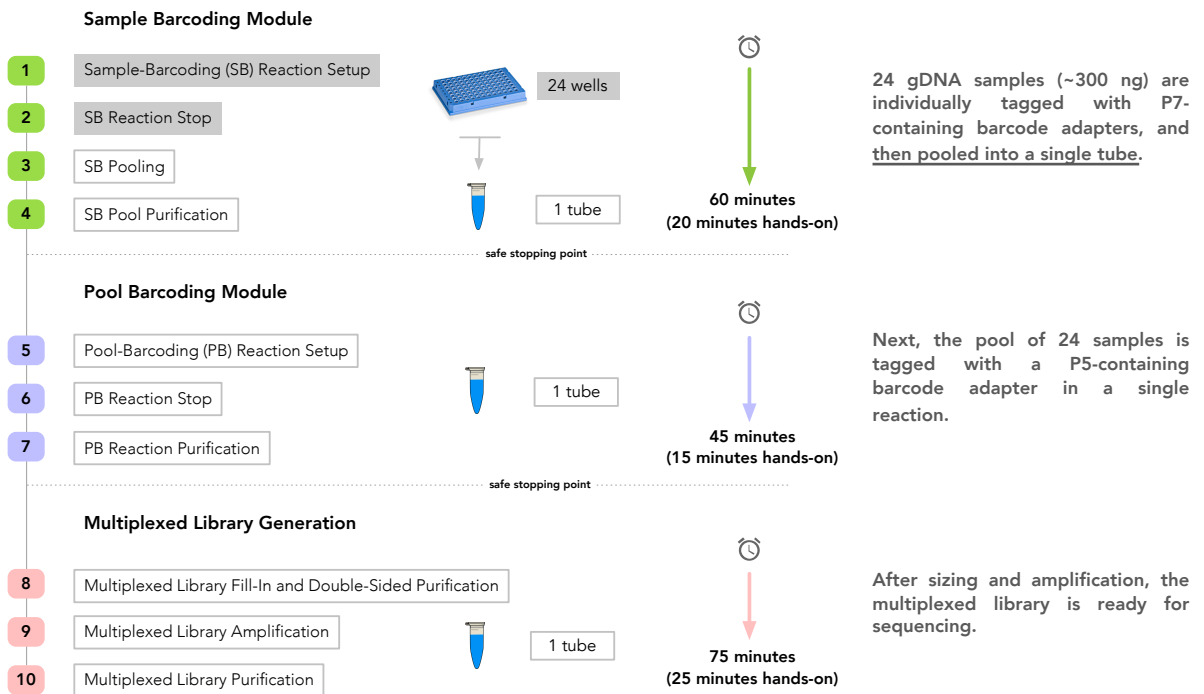

Sample Barcoding Module

1. Sample-Barcoding (SB) Reaction Setup
2. SB Reaction Stop
3. SB Pooling
4. SB Pool Purification

24 wells → 1 tube

60 minutes (20 minutes hands-on)

safe stopping point

24 gDNA samples (~300 ng) are individually tagged with P7-containing barcode adapters, and then pooled into a single tube.

Pool Barcoding Module

5. Pool-Barcoding (PB) Reaction Setup
6. PB Reaction Stop
7. PB Reaction Purification

1 tube

45 minutes (15 minutes hands-on)

safe stopping point

Next, the pool of 24 samples is tagged with a P5-containing barcode adapter in a single reaction.

Multiplexed Library Generation

8. Multiplexed Library Fill-In and Double-Sided Purification
9. Multiplexed Library Amplification
10. Multiplexed Library Purification

1 tube

75 minutes (25 minutes hands-on)

After sizing and amplification, the multiplexed library is ready for sequencing.

Figure 1. The Speed and Power of a Truly Multiplexed Library Prep Workflow: After sample-barcoding, all 24 DNA samples are pooled and processed to completion in a single tube. With plexWell's integrated normalization feature, it only takes 3 hours to produce a balanced, multiplexed library comprised of 24 samples. Quantification of only one multiplexed library (24-plex) is required. The multiplexed library prep workflow dramatically reduces (>10-fold) the consumable and labor costs associated with conventional NGS library preps.

Helping End an Era of Wasteful Sequencing

Another obstacle to scaling-up a conventional "one sample = one library" type workflow is that after each library is individually prepared and quantified, a mixture of libraries must then be formulated in a complex procedure known as library pool creation. The end-goal of creating a library pool is to generate adequate depth of sequencing coverage for every sample represented in the pool. In practice, it is difficult even for NGS experts to accurately control sample-to-sample variation in read balance when library pools are created from dozens of libraries. Poorly balanced library pools cause a higher rate of sample-dropout and lead to expensive rework. To minimize accidental under-sequencing, many labs pool and load fewer libraries per sequencing run (i.e., deliberate over-sequencing). Truth be told, as many terabases of worldwide sequencing capacity have been wasted due to deliberate over-sequencing as due to accidental under-sequencing. The wasteful practice of routine over-sequencing should no longer be accepted as a necessary cost of running a sequencer. The financial risk to users is greater now than ever before because a single sequencing run for WGS costs tens of thousands of dollars.

Precision Multiplexing is the Next Advance in Library Prep

Using the plexWell WGS-24 Library Preparation Kit, twenty-four WGS libraries can be prepared in a single tube and loaded onto a single sequencing run (NovaSeq 6000 with S4 flow cell). Due to plexWell's integrated normalization feature, manual library pool creation is not required. Typical read balance and insert size uniformity across 24 samples for a plexWell multiplexed library are shown below (Figure 2).
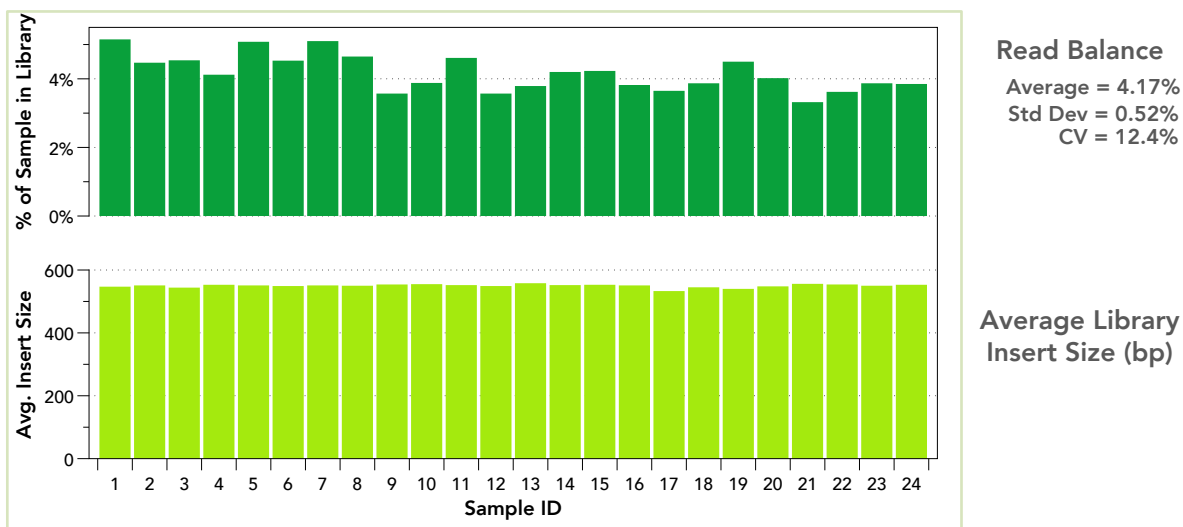

Read Balance

Average = 4.17%
Std Dev = 0.52%
CV = 12.4%

Average Library Insert Size (bp)

Figure 2. Uniform read counts and library insert sizes across 24 input samples <u>without</u> **manual pool correction or rebalancing.** A multiplexed library of 24 human gDNA samples (replicates of NA12878) was prepared using the plexWell WGS-24 Library Preparation Kit. After sequencing, reads were demultiplexed and mapped to the hg38 reference to calculate insert sizes.

Sequencing Results

plexWell WGS - Depth of Coverage Distribution @30X
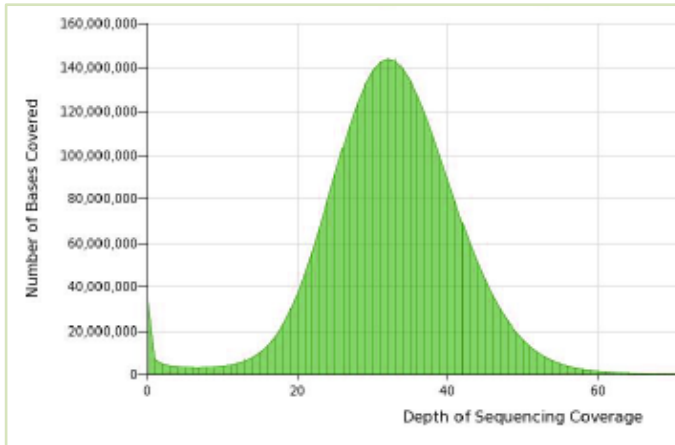
Coverage and Uniformity Metrics

% Q30 bases	85.59%
% aligned reads	94.35%
autosome mean coverage	30.40x
coverage @ 1x	99.50%
coverage @10x	98.25%
% duplicate aligned reads	5.69%

SNP Analysis Metrics

autosome callability	94.70%
% recall dbSNP SNVs	95.04%

Index-Hopping Rate

24-plex	< 0.05% sample-to-sample

Figure 3. Excellent uniformity of coverage and detection of SNVs for complex genomes. Typical data generated from a human (NA12878) plexWell WGS library sequenced on the Illumina NovaSeq 6000. In contrast to conventional NGS library pools, high-purity plexWell libraries exhibit extremely low read misassignment rates (<0.05% sample-to-sample) for patterned and non-patterned flow cells alike, and bypass the need for UDIs.
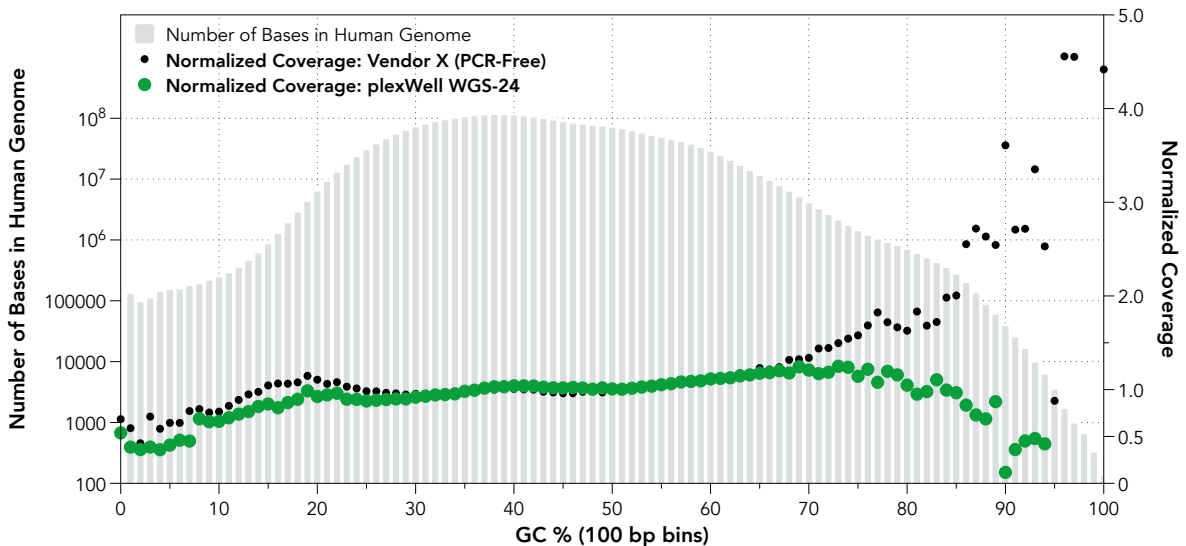

Figure 4. plexWell WGS libraries exhibit high uniformity of coverage, comparable to gold-standard PCR-free libraries. Multiplexed plexWell WGS-24 libraries (replicates of NA12878) were sequenced on the NovaSeq 6000 platform and a typical replicate was selected and compared to a typical sample prepared with the leading conventional PCR-free ligation-based library prep (Vendor X). GC bias was calculated in 100 bp bins on the hg38 reference genome on the basis of 1 million randomly selected reads analyzed with Picard.

Conclusions

plexWell is a breakthrough library prep technology from seqWell which scales-up better than other library prep kits in terms of reliability, plex-level, and sequencing performance. Featuring a transformational multiplexed library prep workflow, the new plexWell WGS-24 Library Preparation Kit enables researchers to effectively leverage the impressive increases in sequencing output represented by the launch of the Illumina NovaSeq 6000 sequencing system and higher output flow cells.

Ordering Information

Product	Part No.
plexWell WGS-24 Library Preparation Kit (24 samples)	WGS24

Please email product orders and order inquiries to:
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Learn More
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