Multiplexed NGS Library Prep Made Simple with plexWell™ Library Preparation Kits

Introducing a new and revolutionary library preparation technology with a true multiplexing workflow for accelerating precision high-throughput genomics.

Highlights

- **Multiplexed Library Preparation Made Simple**
  plexWell libraries are conveniently prepared in a single tube after a single tagging step, which reduces associated labor, consumable and library QC costs by more than 10-fold.
- **Eliminate Costly and Error-Prone Normalization Steps**
  plexWell library prep kits normalize for a wide range of DNA input amounts and deliver uniform read count and insert size distributions across multiple samples in a single library.
- **Maximize Your Research Output**
  by enabling more uniform representation of samples in a single library, plexWell library prep kits unlock the power of modern NGS instruments to deliver more data for more samples.

Introduction

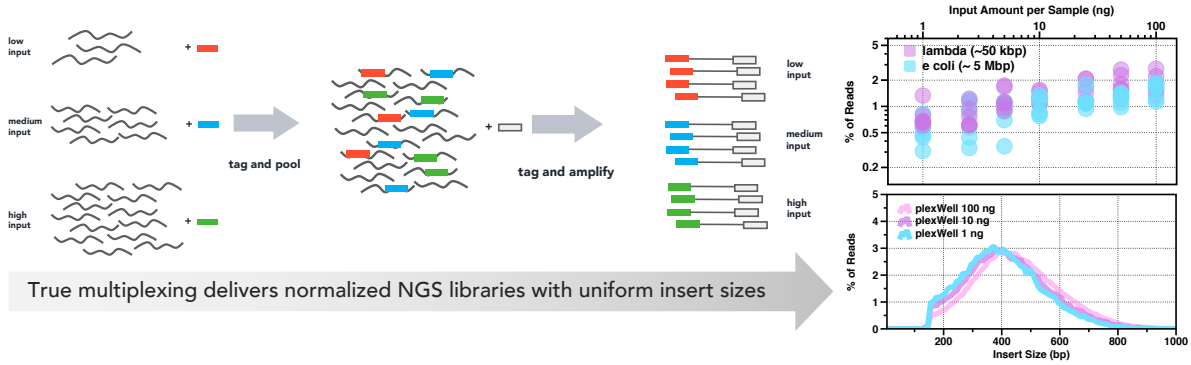
plexWell Library Preparation Kits from seqWell are designed to solve many of the difficult challenges that NGS users face when approaching medium- to large-scale multiplexed library preparation. With plexWell library prep technology, well-balanced libraries for up to 96 samples can be efficiently multiplexed in a single tube, and 100s of incoming samples can be readied for loading on to a sequencer in a single day with ease.

Addressing the Challenges of Multiplexed Library Prep

Researchers and NGS users have an ever-expanding array of powerful sequencing instruments allowing them to sequence larger numbers of samples at higher depth and lower cost. However, these phenomenal improvements in the speed and cost of sequencing have only intensified the need for library preparation solutions that keep pace. Traditional methods for preparing and accurately pooling large numbers of libraries upstream of sequencing are often hampered by outdated library prep technology that was originally designed to convert a single sample into a single sequence-ready NGS library. For many sequencing problems, especially where sequencing of larger numbers of samples is required, conventional library prep is a disproportionate and time-consuming bottleneck that acts as a barrier to achieving the potential workflow and economic benefits of scaling-up sequencing projects.

plexWell is a streamlined, NGS library preparation technology that enables efficient preparation of libraries from multiple samples for sequencing in a single run. The principal advantages of plexWell technology include reducing the need for time-consuming measurement and adjustment of input DNA concentrations, and significantly simplifying higher levels of multiplexing.

plexWell: A Simple Yet Powerful Library Preparation Chemistry



At the core of plexWell technology is a fundamentally new approach to multiplexed library preparation. Rather than making individual libraries out of multiple samples and then pooling them to make a multiplexed mixture ("prep, then pool"), with plexWell the process of making the libraries uses a simple tagging/barcoding step after which all samples are pooled and prepared as a single multiplexed library ("pool, then prep"). The plexWell process not only dramatically reduces the time and cost associated with making a sequencer-ready multiplexed library, it also accommodates and normalizes a wide range of DNA input amount while preserving the read-count and insert-size balance of all samples in the resulting multiplexed library.

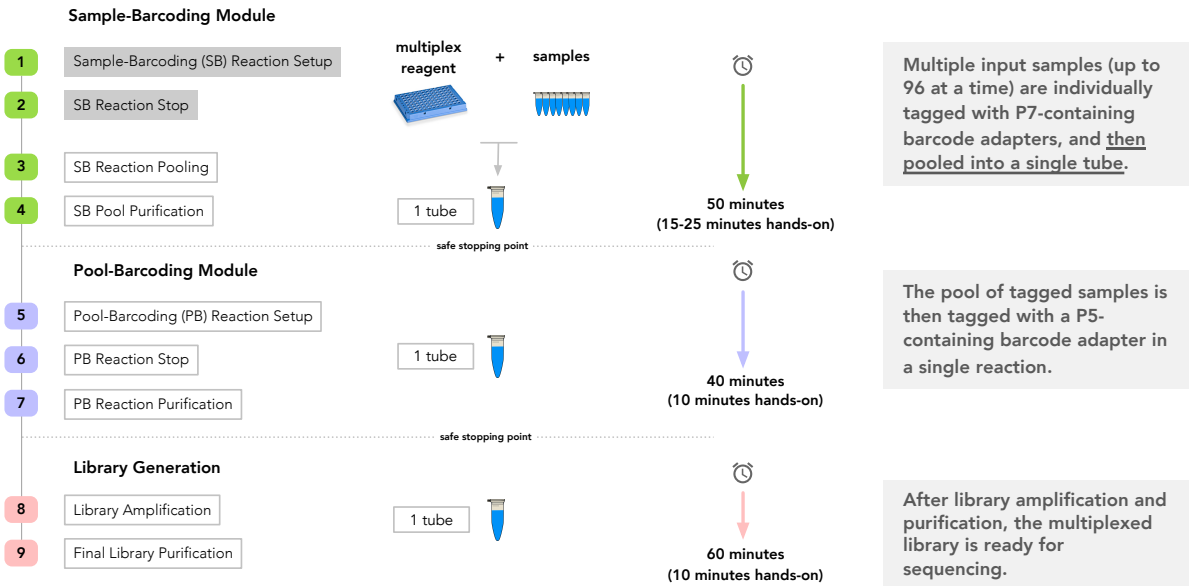


Figure 1. The Speed and Power of plexWell's True Multiplexing Library Prep Workflow. After sample-barcoding, from 8 up to 96 DNA samples are pooled and can be processed to library completion in a single tube. With plexWell's integrated normalization feature, it only takes 3 hours to produce a balanced, multiplexed library comprised of a single batch of samples. Quantification of only one multiplexed library is required for each batch. The plexWell library prep workflow dramatically reduces (>10-fold) the consumable and labor costs associated with conventional NGS library preps.

Solving the Normalization and Library Pooling Problem

In conventional ("prep, then pool") library prep workflows, each library is individually prepared and a mixture of libraries must then be accurately combined into a precisely controlled multiplexed library pool. 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). plexWell solves this challenge by integrating normalization directly into the library prep process itself. Balanced multiplexed libraries are created by auto-normalizing chemistry built into every plexWell kit, giving users confidence that a precise library mixture will be loaded on their sequencing run, not only saving the time and cost associated with complicated library pooling, but minimizing the rework cost and risk of library pooling errors on the sequencer.

A Single Library Prep Solution for Many Sample Types

Using plexWell Library Preparation Kits, Illumina-compatible libraries can be prepared in a single tube and loaded onto a single sequencing for efficient multiplexed sequencing. The robust auto-normalizing chemistry of the plexWell technology even allows for multiplexing of samples of different types (*e.g.*, varying GC content) as well as different target lengths.

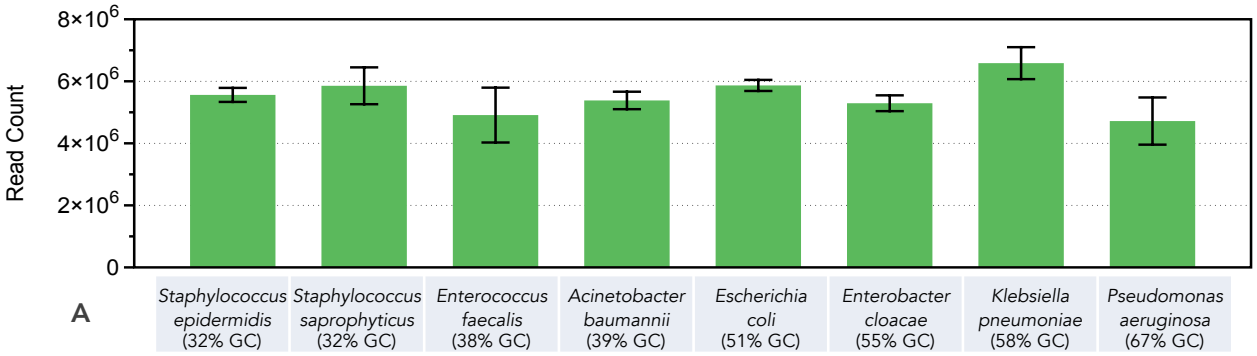
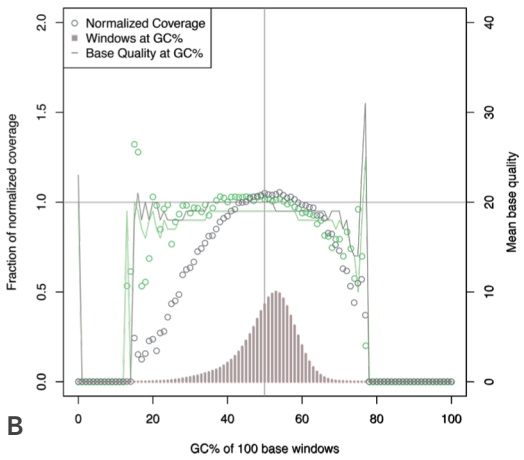


Figure 2. Summary of plexWell performance by sample type and across GC content. Figure 2A shows read count balance for eight different microbial gDNA sources (ranging in GC composition from 32% to 67%) shows that plexWell library uniformity is maintained across a wide range of input sample types. Samples were prepared in triplicate with the plexWell Plus 24 kit, sequenced and demultiplexed to obtain read count statistics (error bars represent standard deviation of mean read count). Figure 2B shows coverage uniformity for a single sample (*E. coli*) by GC% compared between a standard plexWell library (green) and Nextera XT (gray) library, demonstrating superior uniformity of coverage over a wider range of GC%.



Case Study: Multiplexed Microbial Genomic Sequencing

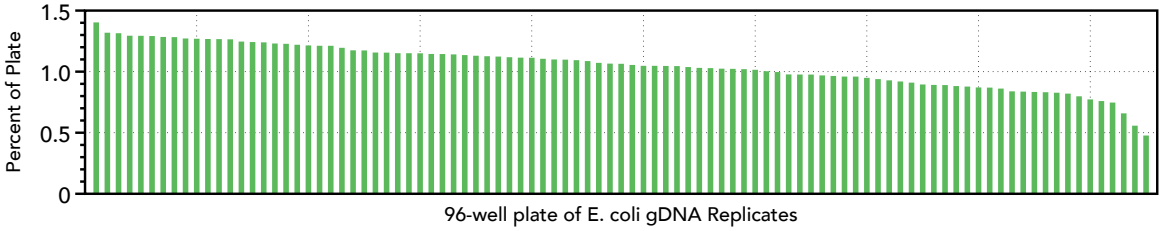


Figure 3. High uniformity of coverage and detection for multiplexed microbial gDNA sequencing. A full 96-well plate of E. coli gDNA (B strain) replicates were prepared as a plexWell multiplexed library for sequencing.

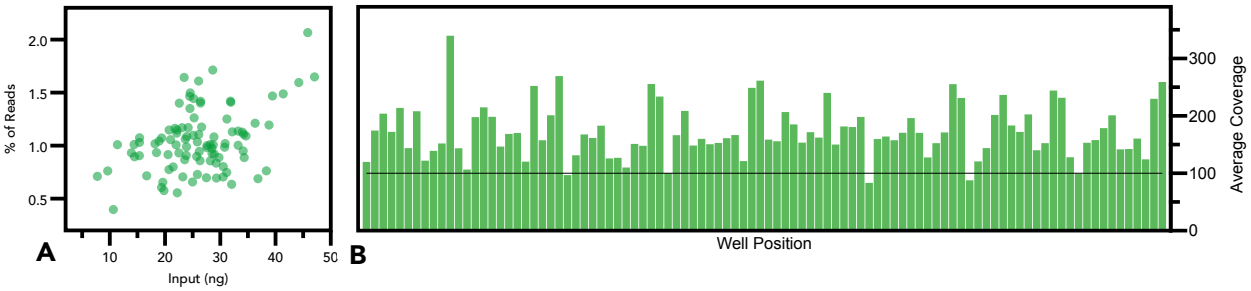
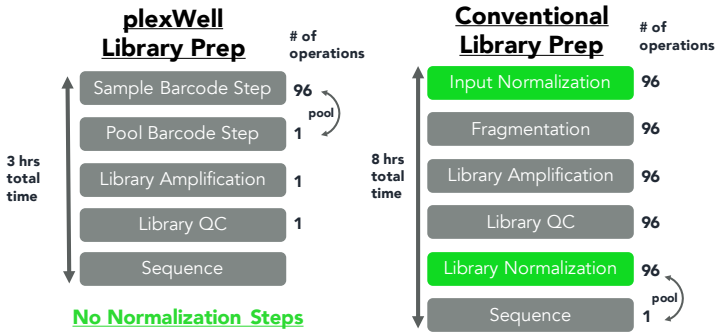


Figure 4. Sequencing of a typical 96-well microplate of bacterial DNA. Non-normalized dsDNA samples were prepared as a single 96-plex library with the plexWell 96 kit and sequenced on a NextSeq® instrument using a standard dual-indexed pair-end 151bp x 151bp read configuration to generate 230M paired end reads (70 Gbases). Data were subjected to *de novo* genome assembly of the bacterial genomes and coverage statistics were generated. Figure 4A (left) shows read count statistics for each bacterial genome as a function of input DNA. Figure 4B shows average coverage for each bacterial genome (92 of 96 bacterial genomes exhibited 100X or higher coverage).

For applications such as microbial genomics, plexWell offers significant time and material costs savings versus conventional "one sample = one library") library prep workflows by compressing the number of operations required to handle multiple samples during the library prep process, and by eliminating the expensive and time-consuming process of library normalization that typically is required.



plexWell Kit Configurations for Every Scale

seqWell has created a flexible series of plexWell kits that accommodate a range of potential multiplexed library preparation needs for different numbers of samples. These kits are designed to allow handling routine batch sizes encountered in the lab (e.g., 96-well plate format) with a nearly identical workflow and all of the same performance benefits regardless of sample number or batch size. For smaller batch sizes, the plexWell Plus 24 kit offers a flexible format for preparing 96 libraries in batches of 8 to 24 samples.

Library Prep Kit	plexWell Plus 24	plexWell 96	plexWell 384
Multiplexing Capacity (samples)	Batches of 8 - 24 (96 total)	96	96, 192, 288, 384
Typical Sample Types	Microbial gDNA, amplicons (multiplexed and clonal), plasmids, BAC, cosmid, fosmid, viral, low-pass human/plant/animal		
Input Range	3 - 30 ng		
Sequencer Compatibility	Illumina MiSeq®, NextSeq™, HiSeq®, NovaSeq™		

Ordering Information

Product	Part No.
plexWell Plus 24 Library Preparation Kit (8 to 96 samples)	PWP24
plexWell 96 Library Preparation Kit (96 samples)	PW096
plexWell 384 Library Preparation Kit (96 to 384 samples)	PW384

*Custom product configurations for larger numbers of samples are also available. Please inquire.

Please email product orders and order inquiries to orders@seqwell.com

Learn more at our website: www.seqwell.com/products/plexWell-kit

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