plexWell
Library Preparation Kits

Scalable true multiplexing through optimized workflows and built-in normalization

The *true multiplexed* solution for preparing NGS libraries from hundreds to thousands of samples. The simple and scalable plexWell™ workflow provides far greater consistency across samples and fewer handling steps downstream.

Library preparation. Simplified.
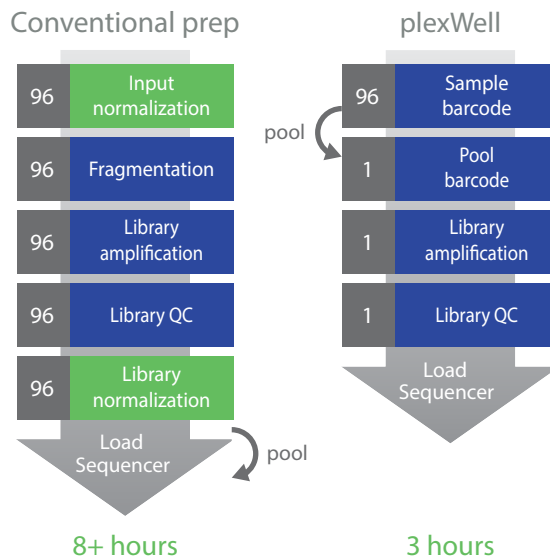
plexWell technology

Simple and scalable workflows

Conventional prep

96	Input normalization
96	Fragmentation
96	Library amplification
96	Library QC
96	Library normalization

Load Sequencer

8+ hours

plexWell

96	Sample barcode
1	Pool barcode
1	Library amplification
1	Library QC

Load Sequencer

3 hours

pool

pool

plexWell workflow saves hours over conventional library prep by eliminating the need for time-intensive DNA normalization and library quantitation.

Sample barcode

96 wells

1 tube

60 minutes
(25 minutes hands-on)

- - - - - - safe stopping point - - - - - -

Pool barcode

1 tube

45 minutes
(15 minutes hands-on)

- - - - - - safe stopping point - - - - - -

Library amplification

1 tube

75 minutes
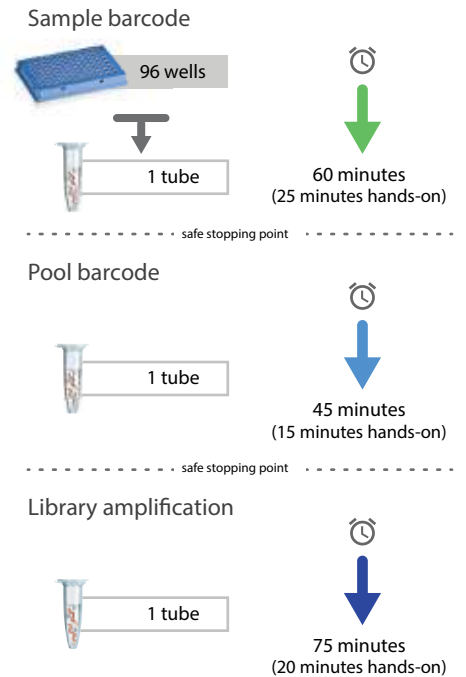(20 minutes hands-on)

Key benefits

- Multiplex thousands of samples with easy to use assay-ready plates

- Eliminate library normalization

- Uniform insert sizes and sample read counts

- Improved sequencing performance

- Exceptionally low index hopping on patterned flowcells

Sequencing applications

- Low-pass whole genome

- scRNA-seq

- Whole human genome

- Amplicons/plasmids, synthetic constructs

- Whole microbial genome

- Metagenomics/ microbe screening

Normalization without extra work

A primary technical benefit of plexWell™ is the ability to generate normalized libraries from large numbers of samples with a wide range of input concentrations through a proprietary reagent-based solution—no additional steps or equipment needed.

plexWell technology solves normalization

Multiplexing challenges

- Different amounts of input DNA

- Differences in GC or AT composition

- Pooling large numbers of samples
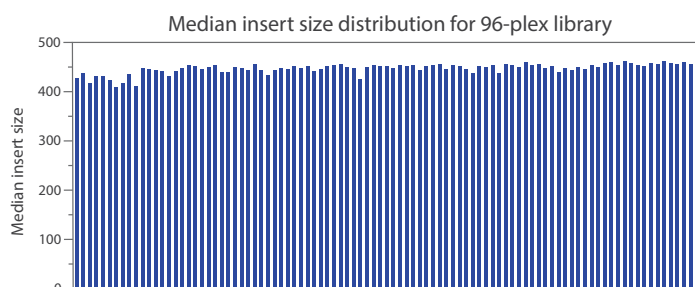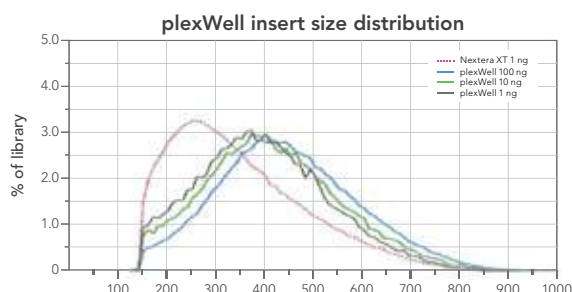
- Requires unique dual indexes for pooling

Desired results

- Objective is to achieve fewer drop outs or jackpots

- More even distribution results in better use of sequencers

- Less rework of failed samples

plexWell features

- A primary benefit of plexWell libraries is the improvement of multiplexing uniformity, and fewer dropouts per sequencing run.

- plexWell™ libraries achieve a high level of multiplexing uniformity without sacrificing other performance features such as uniformity across different GC levels.

- The combination of multiplexing power and raw sequencing performance means that more samples can be confidently sequenced on the same large sequencing run together.

More uniform insert sizes

plexWell insert size distribution

Nextera XT 1 ng
plexWell 100 ng
plexWell 10 ng
plexWell 1 ng

Median insert size distribution for 96-plex library

Superior tolerance with variable input range

plexWell technology yields balanced multiplexed libraries containing highly uniform insert size distributions and sample read counts.

Read count normalization
96-plex read output % across variable input

Compression of input DNA range
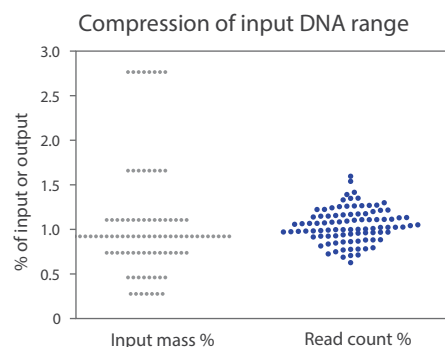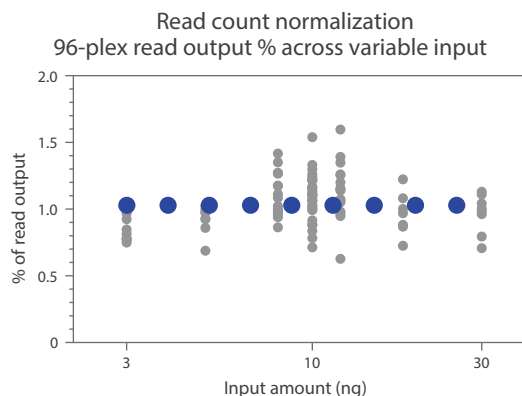
Thousands of indexes available with minimal index hopping

- With conventional library prep, free adapter (or primer) carry-over can contribute to index hopping on patterned flowcells.

- plexWell libraries are indexed in the first steps of the library prep process and have less carry-over.

- The low rate of index hopping with plexWell libraries (0.01%) eliminates the need for unique dual-indices for most applications.

Performance analysis on patterned flowcell

plexWell

	i5 index 1	i5 index 2
i7 index A	43.413%	0.009%
i7 index B	0.010%	56.568%

Conventional

|	i5 index 502	i5 index 505
i7 index 702	53.069%	0.304%
i7 index 704	0.223%	46.405%

plexWell libraries deliver an order of magnitude less index hopping than conventional libraries

Deliver multiplexing performance

Competitive comparison

Compared to conventional library preps, plexWell technology enables the library generation of hundreds to thousands of samples with balanced multiplexed libraries containing highly uniform insert size distributions and sample read counts.
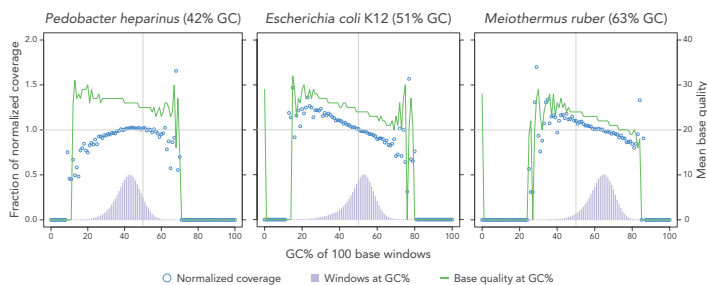
Nextera vs. plexWell
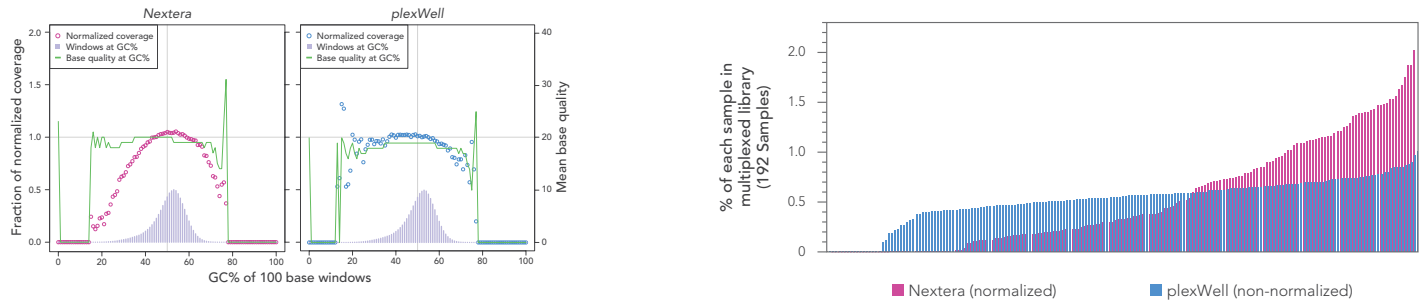
plexWell for multiple applications

Below illustrates the robustness of plexWell to solve inherent issues with many applications as well as its superiority in delivering better sequencing performance and scalable cost-effective workflows.
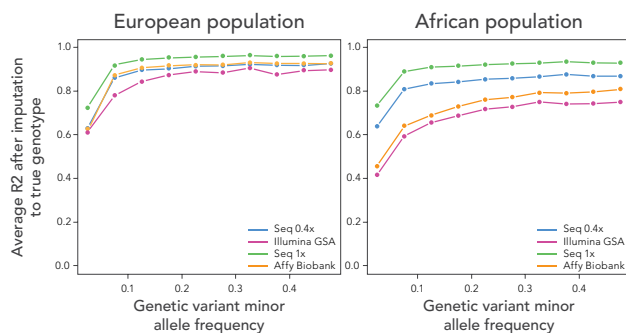
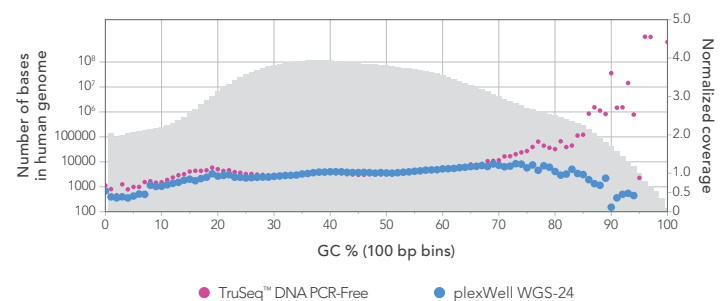### Microbial genomes: Uniformity across different GC content

scRNA-seq: High-throughput gene detection

Number of genes detected for different read counts
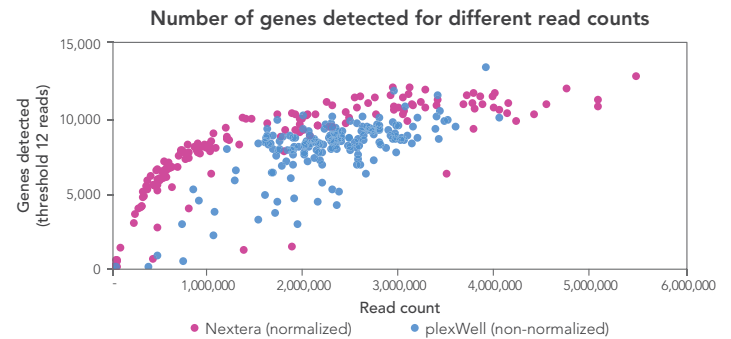
Low-pass whole genome: High-throughput variant detection


Whole genome: High uniformity of coverage

plexWell technology: How does it work?

plexWell™ utilizes a transposase to selectively tag DNA samples in a unique sequential manner. Samples are sequentially tagged with Illumina P7 and P5 primers resulting in normalized libraries with unique barcode combinations. The sequential tagging process provides more control of each step versus other transposon methods and less prone to bias and input DNA variations.

Sample barcoding – *Unfragmented DNA is tagged with sample-specific i7-barcoded adapters*

P7 primers and unique i7 indexes are inserted randomly into each DNA sample by a transposon. The same amount of P7 is added to each sample regardless of DNA input amount. This limiting reagent normalizes the samples, which are now pooled into a single tube.
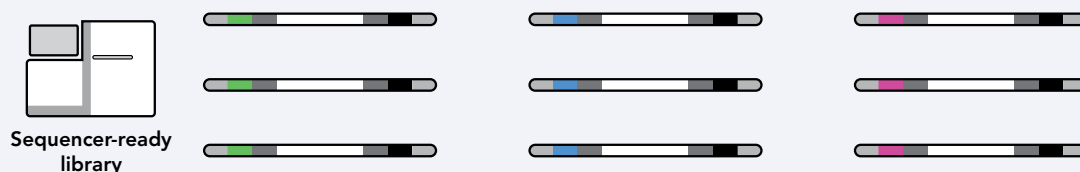

| Dispense DNA into PlexWell™ plate | Sample 1 | Sample 2 | Up to 96 samples | Sample barcode transposon |
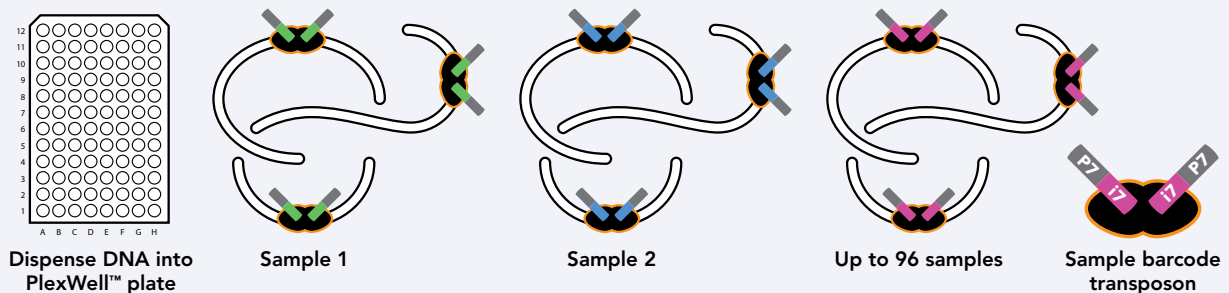
Pool barcoding – *Pooled sample-barcoded DNA is tagged with pool-specific i5-barcoded adapters*

The pool-specific i5 barcoded adapters containing P5-adapters are now added in the second transposition reaction. Excess pool barcoding reagent inserts the same average distance from every sample barcode.
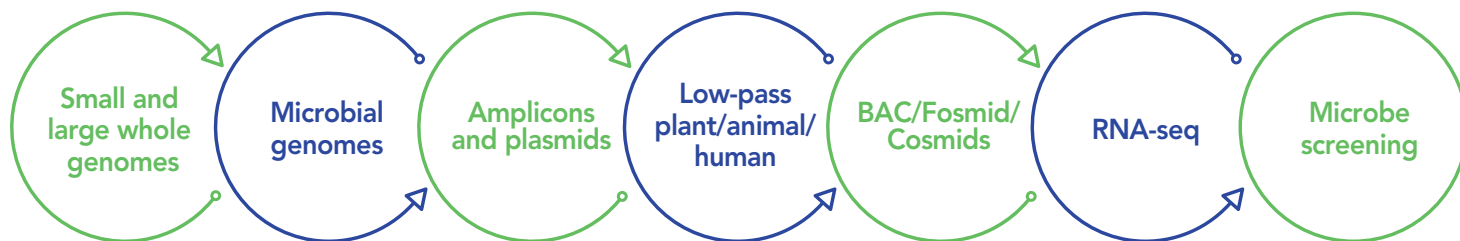

Pool into single tube

Pooled barcode transposon
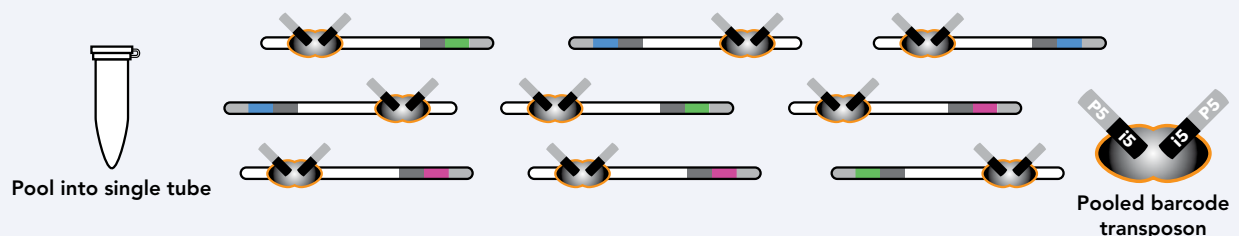
Amplified and final library – *Barcoded library fragments are amplified*

The final step is a library amplification with universal primers and final SPRI purification. The pooled library is now ready to be loaded in an Illumina® sequencer to produce normalized or balanced distribution of sequencing reads.

Sequencer-ready library

Multiplexed applications of plexWell technology

| Small and large whole genomes | Microbial genomes | Amplicons and plasmids | Low-pass plant/animal/ human | BAC/Fosmid/ Cosmids | RNA-seq | Microbe screening |

plexWell kit configurations for every scale

Flexible plexWell™ kits 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 with a nearly identical workflow and all of the same performance benefits regardless of sample number or batch size.

Please visit www.cellink.com/seqwell to find the kit that is right for your DNA sequencing applications.

Ordering information

Product No.	Description	Plex level
PW096	plexWell 96 Library Preparation Kit	96 samples
PW384	plexWell 384 Library Preparation Kit	96 to 384 samples
PWP24	plexWell Plus 24 Library Preparation Kit	8 to 96 samples
PWGS24	plexWell WGS 24 Library Preparation Kit	24 samples

Custom product configurations for larger numbers of samples available. Volume pricing available for all products.
Inquire about custom configurations, volume pricing, and trial offers at sales@cellink.com.

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