plexWell™ : Powerful Multiplexed Library Prep for Diverse NGS Applications

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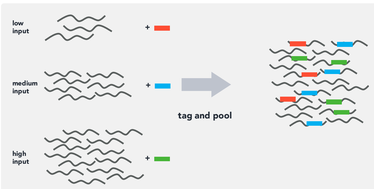
Introduction

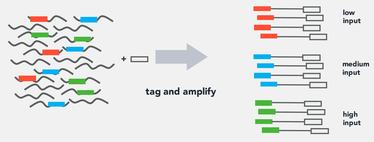
plexWell™ is streamlined, NGS library preparation technology that enables efficient preparation of libraries from thousands of distinct samples for sequencing in a single run. Principal features of plexWell technology include removal of the need for time-consuming measurement or adjustment of input DNA concentrations, and significant simplification of high-level multiplexing.

Methods

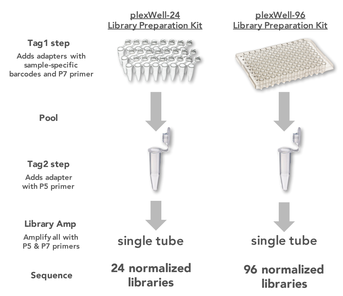
The technical foundation of the plexWell™ approach is a reagent-limited initial tagging step performed on many samples in parallel, coupled to a subsequent pooled library generation step; when applied in conjunction, these two steps yield an approximately equal number of sequenceable library fragments from each of a potentially large collection of samples. The two step process also allows a large combinatorial repertoire of sample barcode combinations



Step 1.
Input DNA is tagged with limiting quantities of sample-specific barcoded P7-containing adapters, and pooled together.



Step 2.
Pooled tagged material is tagged again with excess quantity of P5-containing adapters, yielding normalized multiplexed library.



- **Identical workflow can be scaled to different numbers of samples.**
- **Reagent can be tuned to produce multiplexed libraries of varying complexity.**
- **Simple two-step protocol eliminates separated normalization steps.**

Results

Multiplexing Performance: Plasmid Sequencing and Assembly

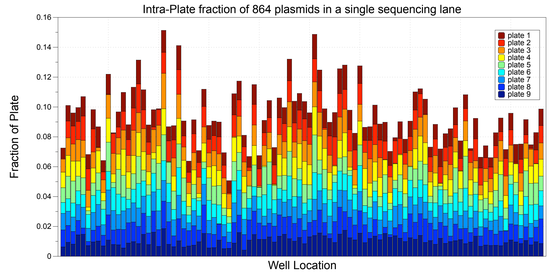


Figure 1. Sequencing of 9x96 (864) plasmids on a single MiSeq sequencing lane. Each column represents a single well (A01-12 to H01-H12, left to right). The dark red bar to the far left represents the fraction of well A01 on plate 1. Each plate of plasmids went through plexWell 96 library preparation without normalizing sample input. Each plate's library was QC'd by e-gel and qPCR then pooled for sequencing on the MiSeq (v3 600 cycle run as 2x251).

Bacterial Genome Sequencing: Normalized Library Yield

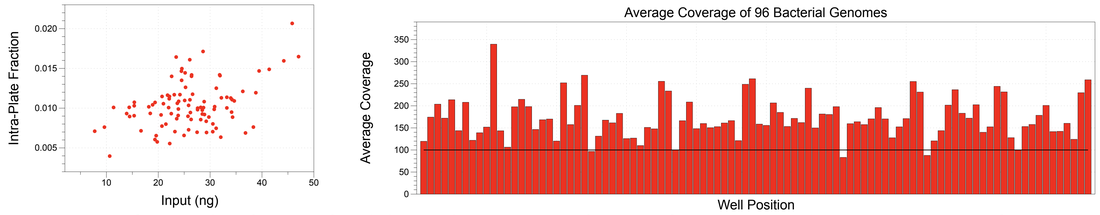


Figure 2. Left: Intra-plate fraction of each bacterial genome as a function of input DNA. Right: Average coverage for each bacterial genome. 92 of 96 bacterial genomes exhibited 100X or higher coverage. Un-normalized dsDNA samples were put through plexWell library preparation and sequenced on the NextSeq® using 300 bp chemistry to generate 230M paired end reads (70 Gbases). Data was used for de novo assembly of the bacterial genomes.

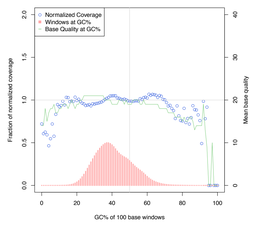
plexWell WGS for Multiplexed Human/Plant/Animal Genomes

plexWell human WGS performance: high uniformity and low sequence bias

uniformity of coverage in a 30X avg depth genome (NA12878)

Total PF Reads: 850,096,124
Reference genome: UCSC hg19
Total Aligned Reads: 723,877,464
Total Aligned Read Pairs: 347,263,644
Percent Q30 bases: 80.32%
Mean coverage (x) = 30.72
Fragment length median = 302 bp
Percent autosome coverage @ 1x = 99.89%
Percent autosome coverage @ 10x = 99.07%
Percent autosome coverage @ 15x = 96.93%

coverage of different human GC% regions (1M subsampled reads)



- **Efficient multiplexing: The superior multiplexing performance of plexWell WGS permits users to confidently load more samples per NGS run, and to better leverage the substantial reduction in sequencing costs made possible by the latest increases in NGS output (e.g., NovaSeq 6000 running the S4 flow cell).**
- **Excellent genome coverage: Sequencing metrics obtained with plexWell WGS libraries on the HiSeq 4000 were comparable to standard PCR-free libraries.**
- **Easy workflow: After a 15 min barcoding step, 24 human gDNA samples are conveniently compressed into a single tube, and then processed as a single library. Consequently, only one qPCR is required per 24 samples. Library prep for 24 samples takes about 2.5 hours with plexWell WGS, and the need for library pooling plans is completely eliminated.**
- **Robust performance: The integrated normalization properties of the plexWell WGS kit delivers consistent insert sizes and uniform read count across batches of 24 human samples.**

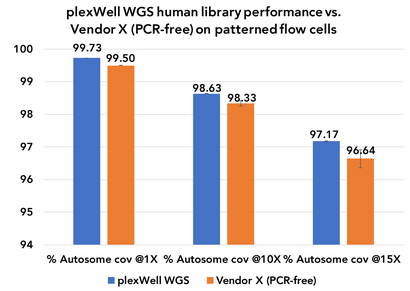


Figure 3. Human DNA libraries were made using the plexWell WGS library prep kit and a PCR-free library prep kit (Vendor X). After sequencing (HiSeq® 4000), the aligned reads from both libraries were downsampled to a mean autosome coverage of 33X, and percent autosome coverage was compared at 1X, 10X, 15X.

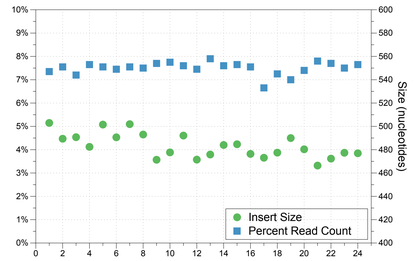


Figure 4. Uniform read counts and library insert sizes without manual pool correction. A human (NA12878 gDNA) library comprised of 24 samples (300 ng input) was prepared using the plexWell-HC library preparation kit.

plexWell™ Kits

Currently Available Kits

	PLEXWELL-96	PLEXWELL-384
Multiplexing Capability	96	96, 192, 188, 384
Sample Type	Best suited for low complexity samples such as amplicons, BACs, plasmids, COSMIDs, FOSMIDs, viral and bacterial genomes	
Input (ng)	1-100 ng	
Additional Verified Applications	cDNA (single-cell RNASeq), library preparation & sequencing from HLA amplicons, plant genomes, human DNA	

*Kits priced at $20/sample. Inquire at info@seqwell.com for volume discounts and custom configurations for multiplexing >384 samples

New plexWell Kit Configurations (Q1 2018*)

	PLEXWELL 4x24	PLEXWELL HC
Multiplexing Capability	24, 48, 72, 96	24
Sample Type	Low Complexity Amplicon–Bacterial Genomes	High Complexity
Input (ng)	1-100 ng	300 ng
Sequencer	MiSeq, NextSeq	NextSeq, HiSeq, NovaSeq

plexWell HC (WGS) kit available now: Please contact seqWell (info@seqwell.com) for inquiries.

Conclusions

plexWell has several advantages for numerous multiplexed library preparation applications:

Workflow

A key technical feature of the plexWell method is a "pooled library prep" step, which reduces the number of sample-handling steps.

Cost Reduction

Reduced sample QC cost - No need to quantify large numbers of input DNA samples, only a single QC step on entire pooled library

Performance

Robust ability to generate balanced multiplexed pools with controlled uniformity of abundance and size between samples translates into increased sequencing yields per sample

Acknowledgements

We thank Anoja Perera, Kate Hall, and Xin Gao at Stowers Institute (poster #71) for their demonstration of plexWell library prep for single-cell RNASeq. We are grateful for resources provided by the North Shore Genomics Center at Endicott College (Beverly, Mass.) as well as the intern sponsorship support from the Massachusetts Life Science Center.