Normalizing NGS Library Preparation Improves Full-Length Single-Cell RNAseq Analysis

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Summary

Sequencing of transcripts from single cells has emerged as a powerful tool for understanding the heterogenous nature of cell function. Current approaches to single-cell RNAseq (scRNAseq) vary from single-cell encapsulation and 3'-end sequencing to partitioning of single cells and sequencing of full length cDNA.

A commonly used approach for generating full-length cDNA sequencing information from single cells involves miniaturized library prep performed from Smart-seq2 (1, 2) amplified cDNA. This approach has the shortcoming of requiring upstream normalization of amplified cDNA material, and sensitivity of miniaturized library prep methods to small variations in input DNA amount.

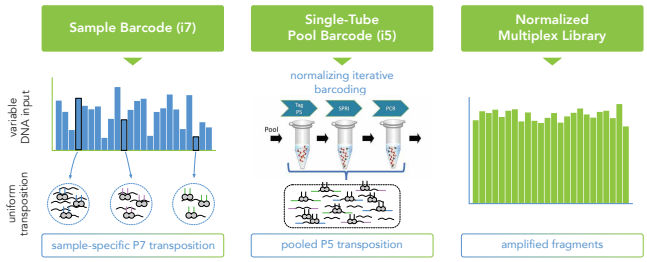
plexWell library preparation is a normalizing iterative barcoding method that allows for multiplexed libraries to be generated from input DNA having a wide range of concentrations. In this study we report the application of plexWell library prep technology to sequencing of single cell cDNA, and compare the results to miniaturized Nextera library prep.

Methods

Study Design Overview

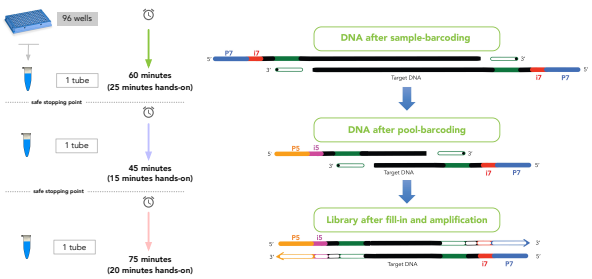
- 192 undiluted HEK cDNA samples were prepared as plexWell libraries (2 x 96)
- 288 diluted samples were prepared as miniaturized Nextera libraries
- Both sets of samples were sequenced via NextSeq500 (2 x 36bp)

plexWell Library Preparation Technology



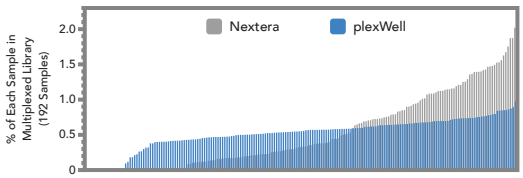
- plexWell libraries achieve normalization by using an iterative barcoding chemistry that selectively generates library molecules from a fixed number of sample-tagging events, followed by a pooled library prep that generates an even number of reads per sample

plexWell Workflow



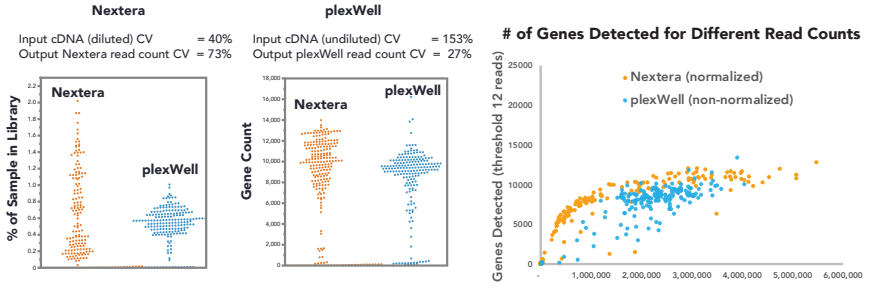
Analysis and Results

Normalization and Gene Count Analysis



plexWell achieves a significantly better level of multiplexing uniformity for highly multiplexed sequencing applications.
At left, sequencing results obtained for sequencing 192 samples of amplified single-cell cDNA with plexWell (blue) and For Nextera reagents (gray). Input DNA was pre-normalized, whereas plexWell library was made from un-normalized amplified cDNA. Read count variation for plexWell showed 27% CV versus 71% CV for Nextera.

Uniformity of Multiplexing scRNA-seq of 192 HEK cells



Reads per sample — values after read count threshold applied

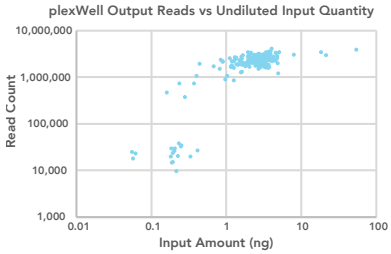
Prep method	Pretreatment	samples	samples over threshold	ave reads/sample	stdev	CV	median reads/sample
Nextera	Pre-normalized	241	188	1,802,834	1,285,852	71%	1,449,330
plexWell	Non-normalized	192	175	2,292,872	622,689	27%	2,299,380

Genes detected per sample — values after read count threshold applied

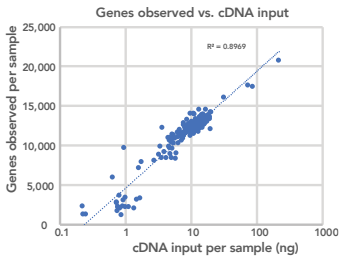
Prep method	Pretreatment	avg genes/sample	median genes/sample	ave genes/sample	stdev	CV	median genes/sample
Nextera	Pre-normalized	7932	9640	10,065	2,338	23%	10,303
plexWell	Non-normalized	8300	9372	9,084	2,081	23%	9,460

A larger percentage of plexWell samples achieve a baseline threshold of 7500 genes per sample.

	# of samples	% of nonzeros	% of all
Nextera	167	89%	69%
plexWell	150	86%	78%



The number of plexWell reads per sample is normalized across a wide range of input amounts.



The number of genes detected per sample is controlled primarily by the input mass, not the read count.

Conclusions

plexWell libraries achieve greater uniformity of coverage per sample, which translates in to a greater number of detected transcripts for a larger number of samples in a given sequencing run. Normalized libraries are difficult to achieve for conventional library prep workflows, especially when miniaturized chemistry is employed to improve the cost and scalability for highly multiplexed applications. plexWell libraries tolerate a wide range of input amounts per sample during multiplexed library prep, which results in a simpler workflow that eliminates the separate normalization steps that typically are required for conventional library prep.

References

Picelli, et al Full-length RNA-seq from single cells using Smart-seq2; Nature Protocols volume 9, pages 171–181 (2014)
Lafzi, et al Tutorial: guidelines for the experimental design of single-cell RNA sequencing studies; Nature Protocols volume 13, pages 2742–2757 (2018)