Resolution at Scale: plexWell Rapid Single Cell for Highly Multiplexed Plate-Based scRNA-seq

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Introduction

The information gain from plate-based scRNA sequencing methods is potentially significant, characterized by more transcripts detected per cell and full-length transcript information.

The challenge with these workflows is the lack of ready-to-use, cost-effective, and scalable solutions to support them. The plexWell Rapid Single Cell Kit couples simple, yet sensitive, ready-to-go cDNA amplification chemistry with the unique multiplexed normalizing plexWell library preparation technology to allow researchers to quickly and efficiently profile single-cell whole transcripts from a wide range of cell types.

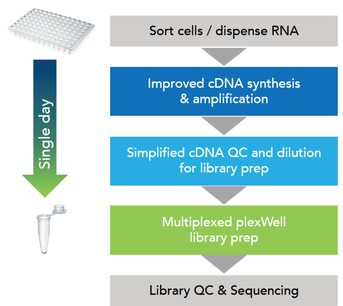
Key benefits

- Sorted cells to sequencing-read full-length cDNA libraries in one day
- Scalable, plate-based workflow
- Auto-normalization reduces sample QC
- Sensitive and robust transcript identification and expression analysis
- More information from every cell
- No specialized equipment required

Sequencing applications

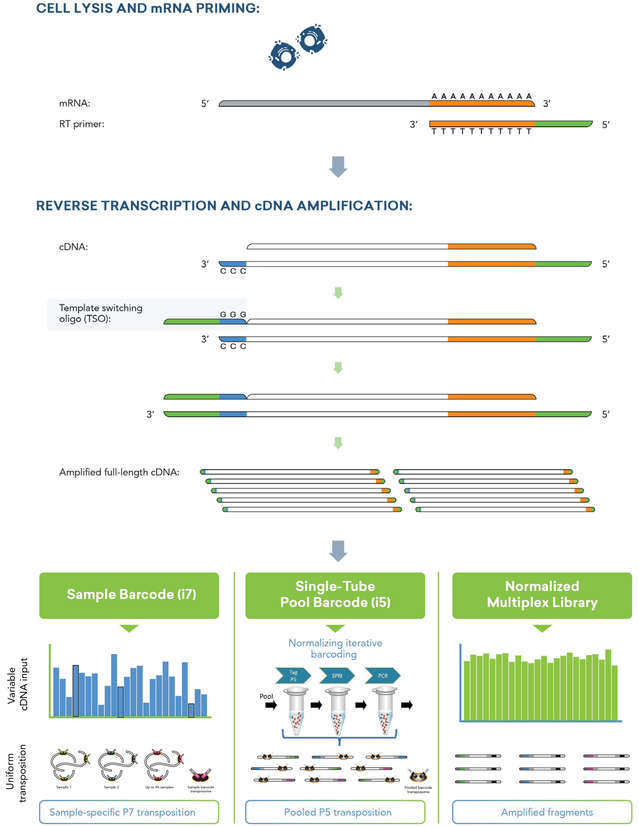
- Single cell transcriptome sequencing
- Allele-specific expression profiling
- Isoform detection
- SNP and variant information across full-length transcripts

Methods



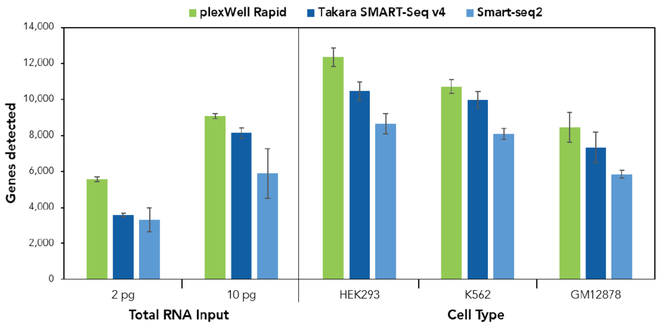
plexWell Single Cell Rapid Workflow Overview:

- Cells dispensed into lysis reagents
- Reverse transcription, first-strand synthesis
- Optimized Template-Switching for second-strand synthesis
- cDNA Amplification
- plexWell sample-barcoding
- plexWell pool-barcoding (single tube) and library amplification
- Final libary QC and sequencing



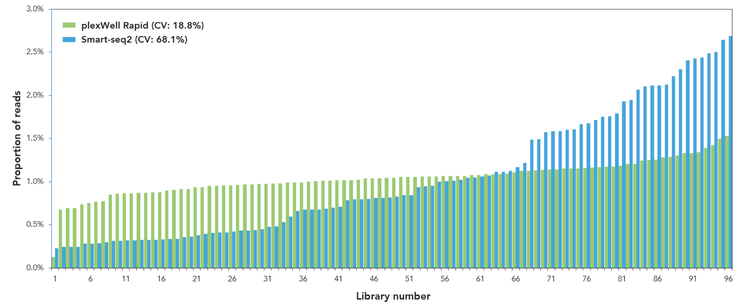
Results

Transcript sensitivity



plexWell Rapid Single Cell RNA Library Prep Kits enable high-sensitivity gene expression analysis and highly uniform transcript coverage. Performance with libraries prepared from 2 pg or 10 pg of total HEK293 RNA or single cells, using the plexWell Rapid Single Cell RNA-Seq Library Prep Kit, SMART-Seq v4 Ultra Low Input RNA Kit (Takara Bio), or the Smart-seq2 method (Picelli S. (2019).

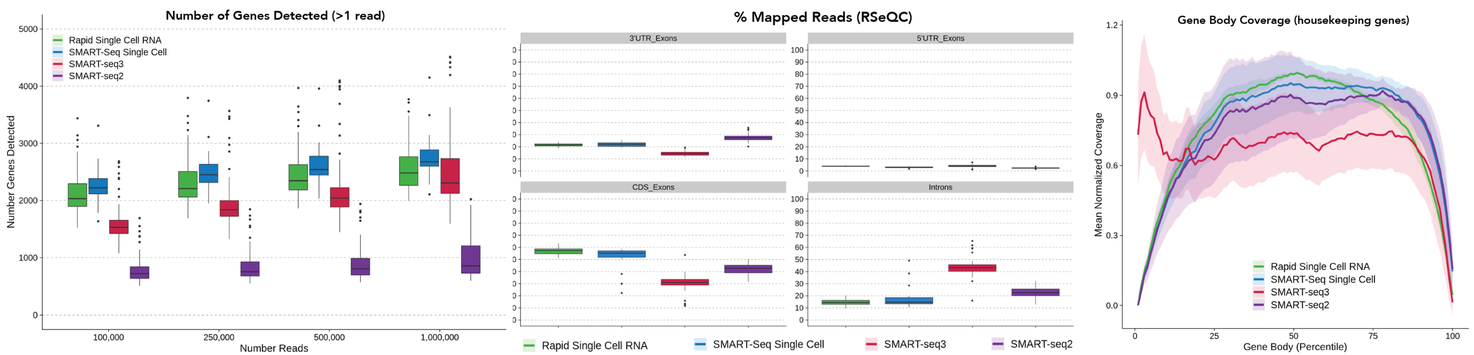
Multiplexing uniformity



plexWell auto-normalization ensures a highly even read distribution across single cells. Libraries were prepared from individual HEK293 cells and sequenced on a NextSeq® 500 instrument. All 96 cDNA samples were quantified and normalized for the Smart-seq2 workflow. For the plexWell workflow, cDNAs were diluted, by applying a global dilution factor derived from QC data for only 24 of the samples.

Application highlight: High-performance scRNA-Seq of PBMCs sensitive and efficient full-length transcriptome profiling of challenging samples

Single cell transcriptome profiling is now routinely employed in immunological studies, to better understand heterogeneity in cell populations with overlapping phenotypic markers; cell development and differentiation; hematopoietic pathways and regulatory networks that predict immune function. Single cell RNA-Seq of peripheral blood mononuclear cells (PBMCs) is particularly challenging because of ultra-low cellular RNA content (estimated at ≤1 pg per cell). In addition to a more streamlined workflow, the plexWell™ Rapid Single Cell RNA Library Prep Kit offers improved sensitivity (more genes detected; irrespective of sequencing depth); higher efficiency (less reads mapping to undesired content) and/or more uniform full-length transcript coverage as compared to homebrewed and commercially available reagents and protocols.



plexWell Rapid Single Cell RNA Library Prep Kits offer improved sensitivity, higher efficiency, and/or more uniform transcript coverage in full-length transcriptome profiling of PBMCs. CD45+ human PBMCs were sorted using a BD FACSAria™ Fusion cell sorter (BD Biosciences). Libraries were prepared according to standard or published protocols, using the plexWell Rapid Kit (green, 96 cells), Smart-seq3 method (red, 96 cells; Hagemann-Jensen M, et al. Protocols.io v3 (Feb 2020) and Nat. Biotechnol. (2020). 38: 708–714); Smart-seq2 method (purple, 48 cells; Picelli S. (2019), as cited previously), the or SMART-Seq Single Cell Kit (blue, 24 cells; Takara Biosciences). Sequencing (2 x 75 bp) was performed on an Illumina® NextSeq® instrument.