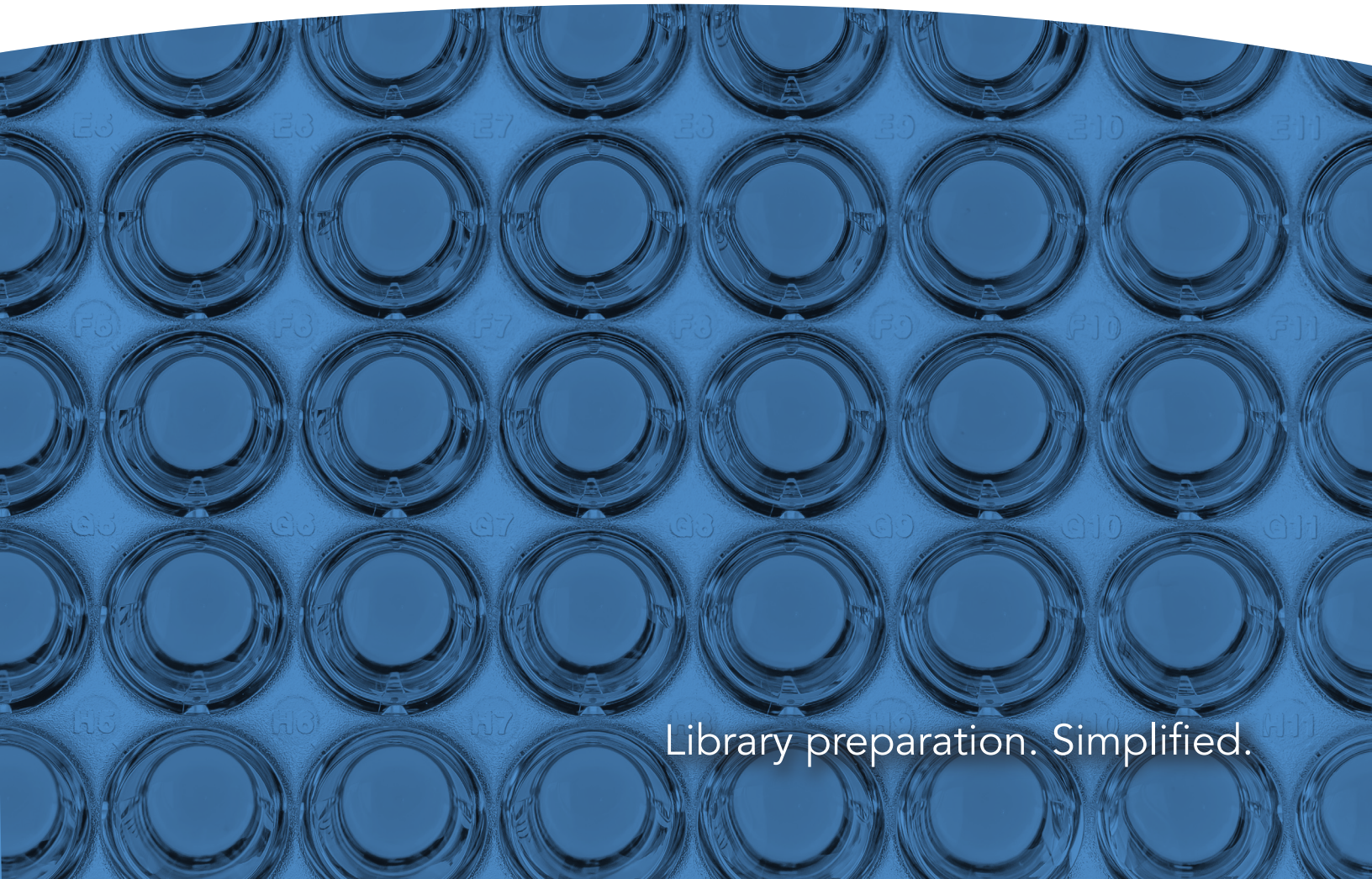
plexWell Rapid Single Cell
RNA Library Prep Kits

End-to-end library preparation for scalable, cost-effective single-cell transcriptome analysis

Novel improvements to the Smart-seq2 method for cDNA generation, combined with truly multiplexed library preparation, enables sensitive and robust detection and analysis of full-length transcripts at single-cell resolution.



Key benefits

- Sorted cells to sequencing-ready, 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

Streamlined, fully integrated workflow

Single-day protocol, scalable to your project needs

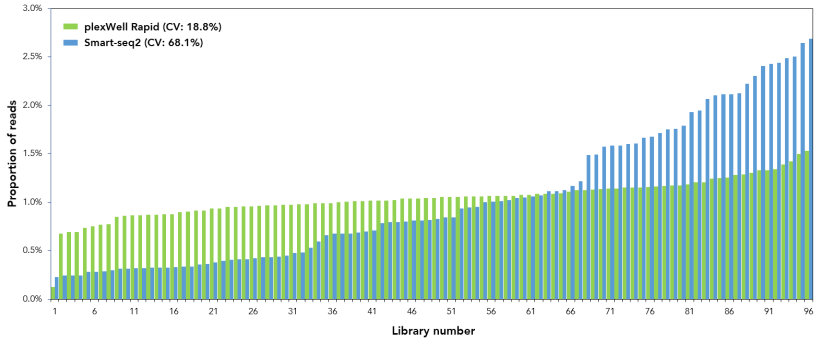
- Compatible with sorted cells or low RNA inputs (2 pg – 10 ng), arrayed in plates or strip tubes
- Novel improvements to Smart-seq2 method*
- Combined cDNA synthesis and amplification reduces hands-on and overall workflow time
- Unique features of plexWell™ library prep eliminates QC and dilution of every individual cDNA sample
- Yields normalized, amplified libraries
- Truly multiplexed library prep is highly scalable and reduces library prep and sequencing cost

*Picelli S, et al. Nat. Protoc. (2014). 9: 171–181.

The streamlined, fully integrated plexWell Rapid Single Cell RNA Library Prep protocol enables the production of 96 or 384 sequencing-ready, full-length transcriptome libraries in a single day, without the need for specialized equipment.

Novel improvements to the Smart-seq2 method allow for combined cDNA synthesis and amplification, without an impact on performance. Compared to the original Smart-seq 2 and other commercially available protocols, the plexWell Rapid cDNA generation module requires fewer reagent additions and shorter incubation times. This reduces sample and reagent handling, hands-on time, and overall library preparation time.

Unique features of the plexWell technology, including auto-normalization, further simplifies the process by reducing the effort required to QC and dilute cDNA samples prior to library prep, and collapses 96 cDNAs into a single, multiplexed library. This eliminates major bottlenecks in single-cell transcriptome projects, whilst improving data quality and reducing overall project costs.

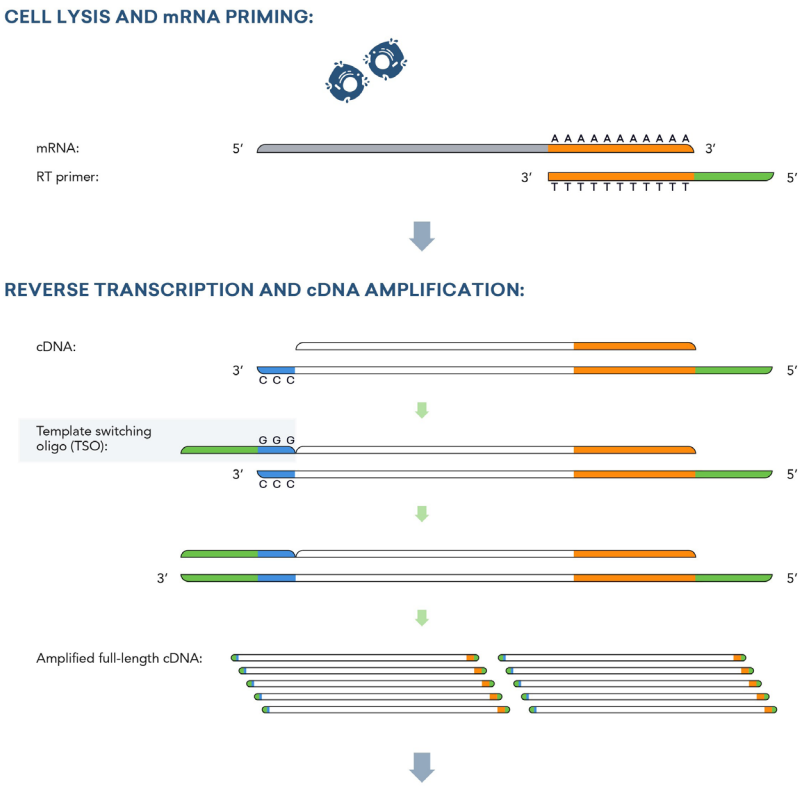


plexWell auto-normalization ensures a highly even read distribution across single cells

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.

Unique features enable true multiplexing

Streamlined cDNA synthesis, iterative barcoding and auto-normalization



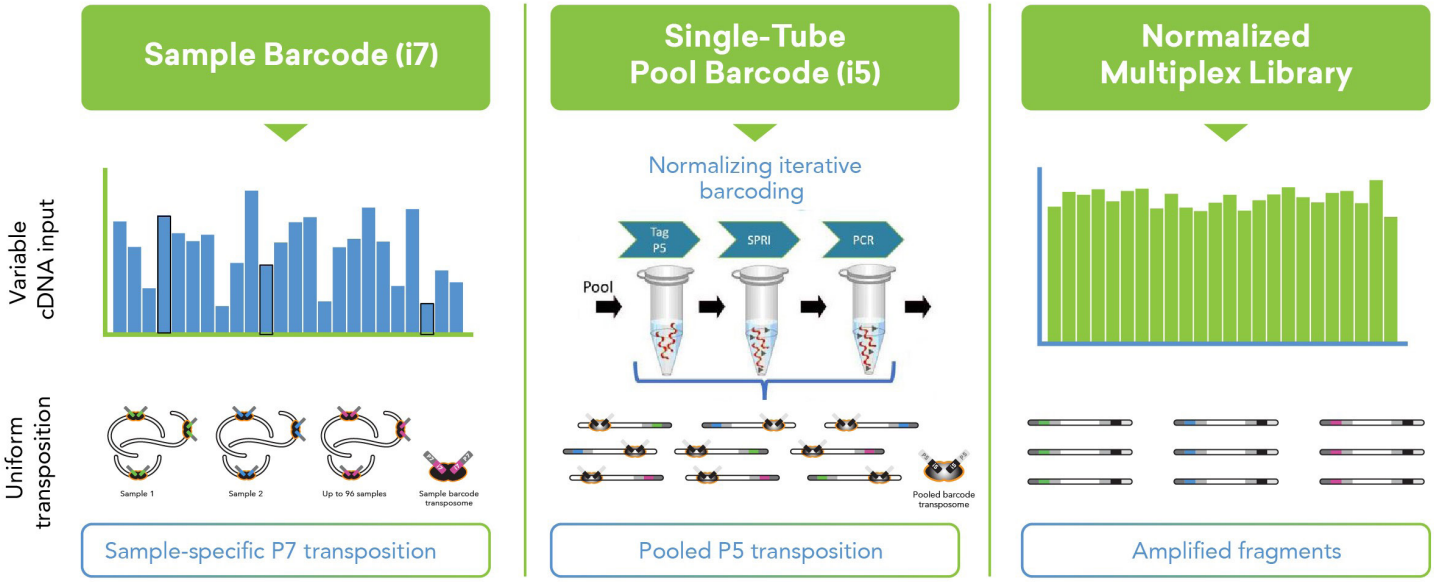
Streamlined cDNA synthesis, iterative barcoding and auto-normalization reduce sample QC, hands-on and overall workflow time

In the novel plexWell™ Rapid workflow, cells or total RNA are dispensed into wells containing lysis reagents. Poly-T priming during the reverse transcription reaction enriches for mRNA transcripts. Untemplated nucleotide addition at the 3′-end of the first cDNA strand enables hybridization of the template switching oligo (TSO). As reverse transcription continues, common priming sites are created at both ends of all cDNA molecules. Subsequent amplification generates a sufficient amount of double-stranded material for library preparation.

The plexWell library preparation technology (illustrated in the bottom half of the figure) employs an innovative, sequential transposition strategy to tag cDNA with P7 and P5 sequences. This results in normalized libraries with unique barcode combinations for multiplexed Illumina® sequencing.

In the first transposition (Sample Barcoding) reaction, each full-length cDNA is tagged with a sample-specific i7-barcoded sequence. The reagent-limiting nature of this step normalizes variable cDNA inputs. This eliminates the need to individually QC and manually normalize every sample. Since every sample receives a unique barcode in the first step of the process, up to 96 samples can be pooled into a single tube for downstream processing. This greatly simplifies and streamlines subsequent enzymatic reactions and bead-based purification steps.

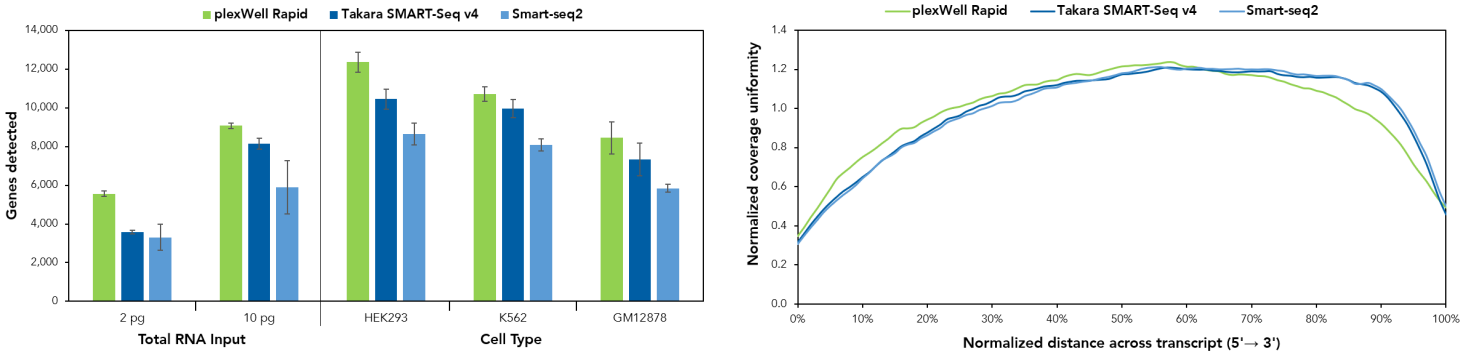
In the second (Pool Barcoding) transposition reaction, a pool-specific i5-barcoded sequence is added to each of the pooled samples. Excess pool barcoding reagent enables a high level of control over final library fragment length. Library amplification completes the Illumina adapter sequence to yield a normalized, sequencing-ready library pool



Robust single-cell RNA-Seq ...

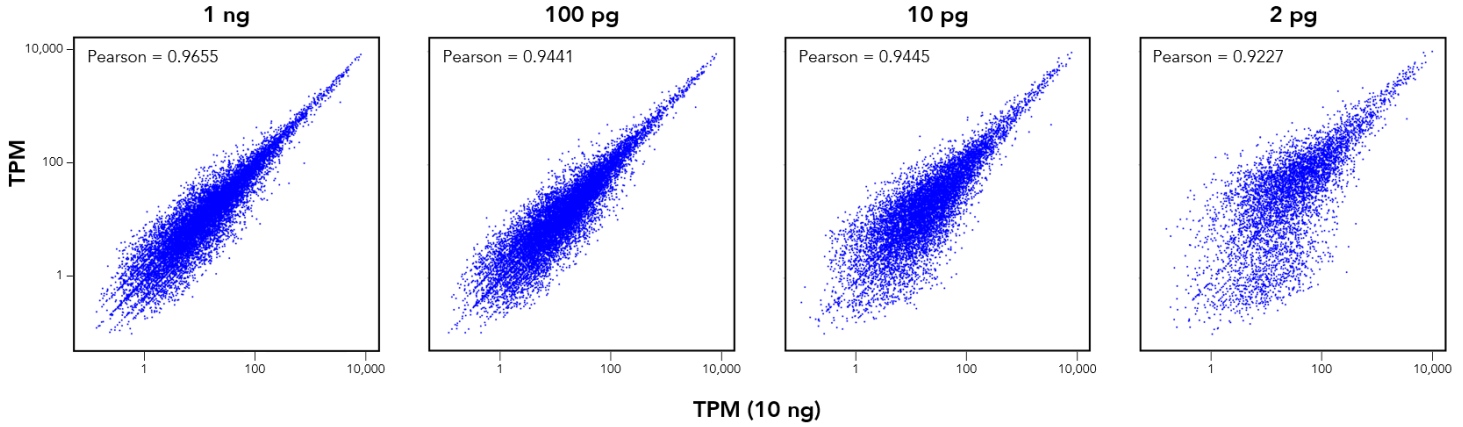
Maximize the information obtained from every cell

The plexWell™ Rapid Single Cell RNA Library Prep Kit is designed for high-sensitivity gene expression profiling, and provides uniform coverage across the entire length of transcripts. The workflow has been optimized to maintain library complexity, even with ultra-low inputs. This improves the detection of low-abundance genes and rare cell populations, preserves whole-gene SNP and indel variant information, and allows for isoform and allele-specific expression analysis from single cells and low picogram cDNA inputs.



plexWell Rapid Single Cell RNA Library Prep Kits enable high-sensitivity gene expression analysis and highly uniform transcript coverage

Libraries were prepared from 2 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). In: Proserpio V. (ed) Single Cell Methods. Methods in Molecular Biology, vol. 1979). Standard protocols were followed in all cases. For single-cell analysis, cells from the same suspension were sorted directly into each workflow's lysis master mix, using a BD FACSAria™ Fusion cell sorter (BD Biosciences). Libraries were sequenced (2 x 75 bp) on an Illumina® NextSeq® 500 instrument. Data were randomly downsampled to 0.5 million read pairs per sample. Read mapping and transcript/gene quantification was performed with Salmon v0.14.1. For the graph on the left, bars represent the mean of three replicates (for extracted RNA) or three to six replicates (for single cells), whereas error bars designate the standard deviation. Coverage uniformity plots on the right were generated from single HEK293 cells only. Lines represent the average, normalized transcript coverage uniformity for the top 1,000 expressed transcripts across all cells evaluated (three to six cells per workflow).



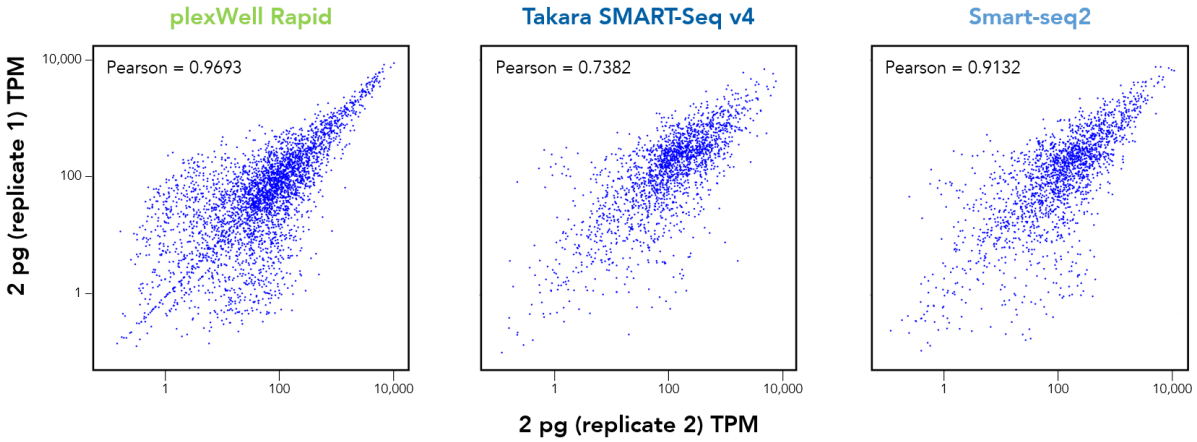
plexWell Rapid Single Cell RNA Library Prep Kits produce high-complexity libraries from ultra-low inputs

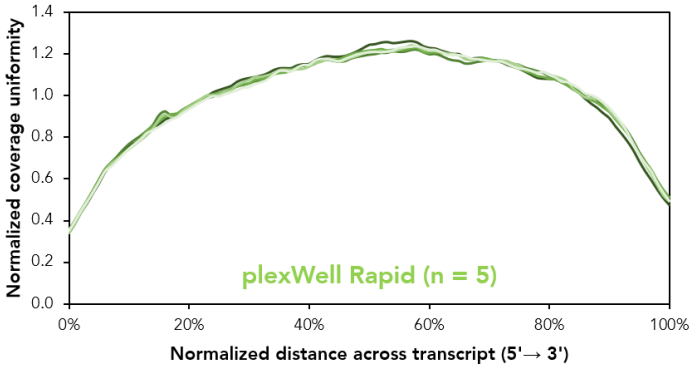
Libraries were prepared (from 2 pg, 10 pg, 100 pg, 1 ng or 10 ng HEK293 total RNA (RIN >9.0) using the plexWell Rapid Single Cell RNA-Seq Library Prep Kit and recommended protocol. Sequencing and data analysis were performed as described above. Correlation plots were generated from transcripts wit TPM >0.1. The Pearson correlation for each comparison is given in the top left of the plot.

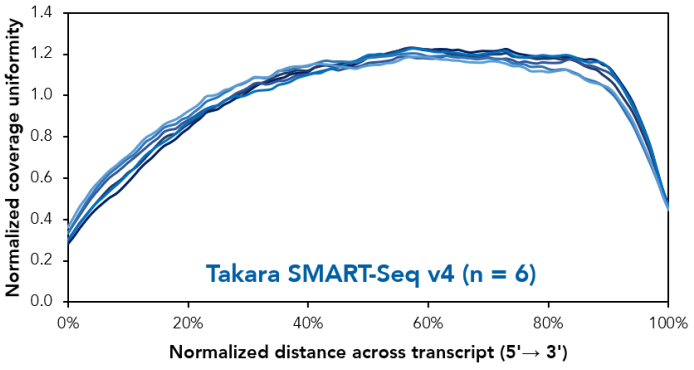
… ensures reliable results

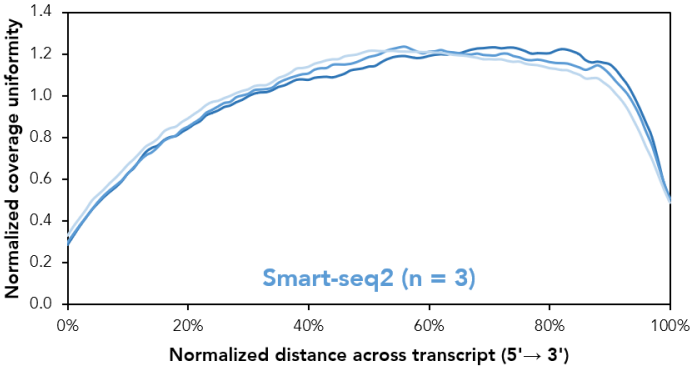
Achieve reproducibility across transcript expression levels and lengths

Reliable library preparation methods are needed to distinguish experimental variation from measured variation that is biologically significant. This is particularly important for low-input analyses. The plexWell™ Rapid Single Cell RNA Library Prep Kit delivers reproducible results across technical and biological replicates. It outperforms with respect to detection of both low- and high-abundance transcripts, as well as uniformity of coverage across transcript length.









plexWell Rapid Single Cell RNA Library Prep Kits enable reproducible detection of low-and high-abundance transcripts and uniform transcript coverage for ultra-low inputs and single cells

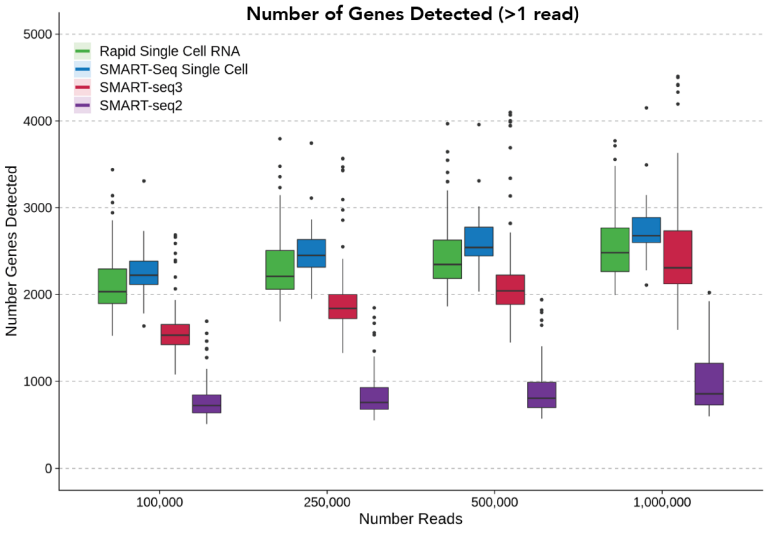
Libraries were prepared using the plexWell Rapid Single Cell RNA Library Prep Kit, Takara SMART-Seq v4 Ultra Low Input RNA Kit, or published Smart-seq2 method (Picelli S. (2019), as cited previously). For correlation plots, inputs were 2 pg single-use aliquots of total HEK293 RNA (RIN > 9.0). Coverage uniformity plots were generated from the analysis of three to six sorted single HEK293 cells. Standard protocols were followed in all cases. Sequencing and data analysis were performed as described previously.

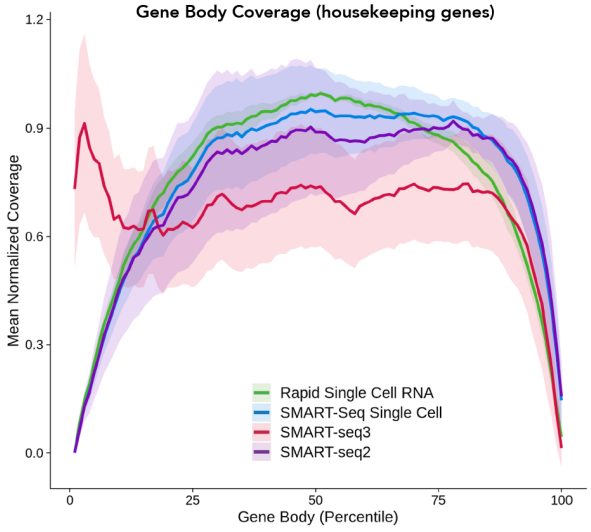
In correlation plots, all transcripts with TPM > 0.1 that were identified in both replicates are shown. R values are Pearson correlation coefficients. In coverage uniformity plots, lines represent the average, normalized transcript coverage uniformity for the top 1,000 expressed transcripts across all cells evaluated.

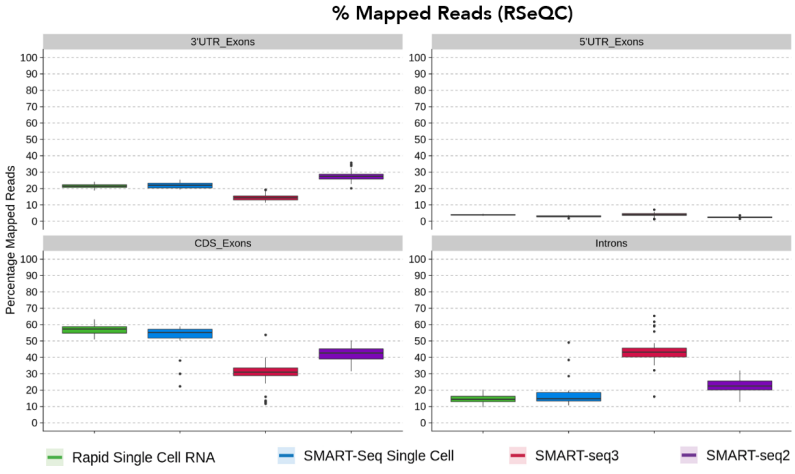
Application Highlight: High-performance scRNA-Seq of PBMCs

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 (i) improved sensitivity (more genes detected; irrespective of sequencing depth), (ii) higher efficiency (less reads mapping to undesired content), and/or (iii) 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.

Data courtesy of Simone Picelli, Institute of Molecular and Clinical Opthalmology; Basel, Switzerland.

plexWell™ Rapid vs. droplet-based 5′- and/or 3′-single cell RNA-Seq methods

Single-cell RNA sequencing is a fast-growing field, offering a variety of commercial and "homebrew" library prep methods. Appropriate method selection depends on research objectives, the expected level of cellular heterogeneity and subpopulation frequency. The plexWell Rapid Single Cell RNA Library Prep Kit and droplet-based 5′- and/or 3′-methods (e.g. 10X Genomics Chromium System) represent solutions at different ends of the spectrum and are compared below. Plate-based approaches (such as plexWell Rapid) complement droplet-based methods by providing more data on the identified cell subpopulations of interest.

Parameter	plexWell Rapid Single Cell	Droplet-based 5′- and/or 3′-methods
Throughput (required cells per run)	Low to medium (no limitation)	High (≥20,000)
Single cell isolation	FACS or index sorting (into 96- or 384-well plates)	Droplet-based
Long term cell storage	Yes	No
Transcript coverage	Full-length	5'- or 3'-ends only
cDNA generation / library prep	Individual wells (no UMIs) / single-tube	Single tube (with UMIs) / single tube
Key Applications	• Gene expression analysis (SNP-based, allele-specific) • Detection of isoforms, splice variants, alternative transcripts and fusions • Cell-type discovery, tissue composition analysis	• Gene expression analysis (gene-level/digital quantification) • Time-course or differential treatment studies • Cell-type discovery, tissue composition analysis
Key benefits	• More genes detected per cell, with improved detection of low-abundance genes • Profiling of rare cell populations • Flexible experimental design, overall project cost • No specialized library prep equipment required	• Fast turnaround time for large projects • Screening of large cell populations • Compatible with certain multi-omics approaches
Key drawbacks	• Lower throughput (numbers of cells) • More labor intensive	• Lower cell capture efficiency, which may skew biological conclusions • Large cell numbers required to be economical • Drop-out of low-abundance genes • Detects fewer genes per cell

Ordering information

Product No.	Description	Pack size	Plex level
PWSCR96	plexWell Rapid Single Cell RNA Library Prep Kit	96 samples	Up to 96-plex
PWSCR-A, -B or -C*	plexWell Rapid Single Cell RNA Library Prep Kit	384 samples	96- to 1,152-plex

*Contains different sets of barcodes. Custom product configurations available for large projects. Inquire at sales@seqwell.com.

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