A novel, fully integrated scRNA-seq workflow enables streamlined library preparation with sensitive and robust transcript detection

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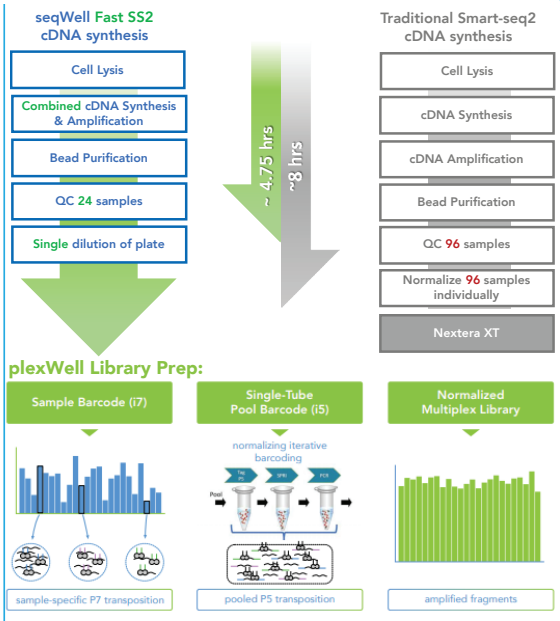
Introduction

Single cell RNA sequencing (scRNA-seq) has become an important tool for understanding gene expression of heterogeneous tissues and cell populations at individual cell resolution. While significant advances in scRNA-seq technology have been made in recent years, the gold standard for assay sensitivity remains plate-based workflows with full length transcript resolution, most notable of which is Smart-seq2 (1). Despite the sensitivity and compatibility of these workflows with RNA-seq applications beyond standard gene expression, they remain cumbersome, often taking multiple days to produce sequencing-ready libraries.

Here, we introduce seqWell Fast SS2. This streamlined workflow reduces the number of reagent additions, shortens incubations, reduces cDNA QC burden, and collapses 96 wells into a single tube for cDNA library preparation while maintaining the high-quality performance associated with Smart-seq2.

Methods



Single HEK293 cell assessment workflows:
- seqWell Fast SS2, which includes plexWell for multiplexed cDNA library prep
  - 24 single K562 cells (cDNA processed in duplicate) were also included for this workflow
- Home-brew Smart-seq2, which uses Nextera XT for cDNA library prep
- A commercially-available kit similar in workflow to Smart-seq2 (Competitor T)

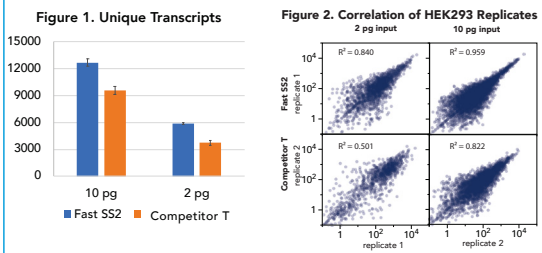
Bulk HEK293 RNA assessment workflows:
- seqWell Fast SS2 cDNA synthesis paired with Nextera XT
- Competitor T cDNA synthesis paired with Nextera XT

cDNA QC Details:
- Nextera XT required QC and normalization to 125 pg for each cDNA sample
- seqWell Fast SS2 reduced the QC burden to ¼ of cDNA samples and required a single dilution factor be applied to each sample type on the plate.
- cDNA was quantified via picogreen and quality was assessed using TapeStation traces.

Sequencing & Analysis Details:
- All libraries were downsampled to 500K paired reads (2 x 75)
- TPM >0.1 used to call transcripts.

Bulk HEK293 RNA Results

Achieve robust results with as little as 2 pg of RNA.



Figures 1 and 2. Transcript identification and expression data for bulk RNA. 1) Using bulk RNA extracted from HEK293 cells, Fast SS2 detected a greater number of transcripts at both 10 pg and 2 pg inputs in comparison to Competitor T. 2) Transcript expression results show improved correlation between technical replicates using Fast SS2.

Single-Cell RNA-seq Sequencing Performance

Fast SS2 workflow enables reduced cDNA QC and normalization without sacrificing data quality.

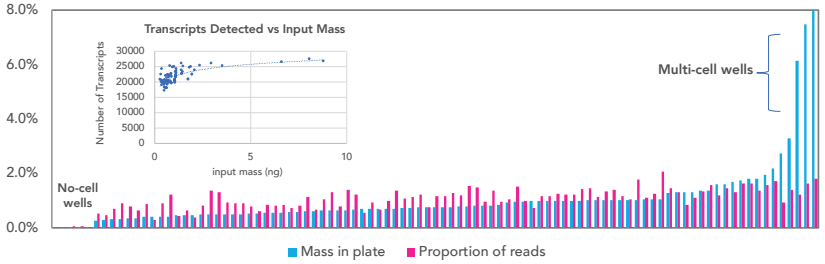
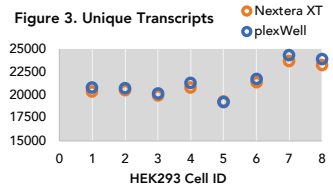
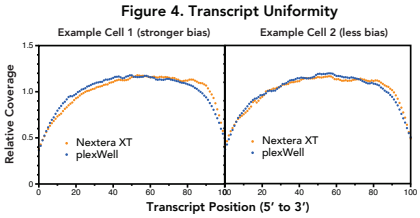
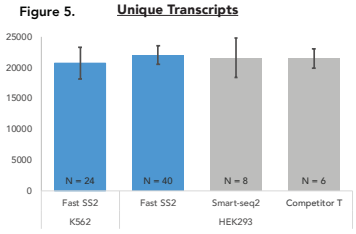


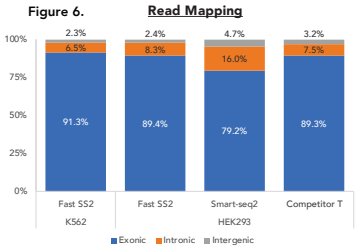
Figure 2 (above). cDNA mass impact on sequencing read distribution across cells with Fast SS2. plexWell provides even read distribution despite large variation in cDNA mass inputs across a plate of 96 cells. No-cell wells were identified by TapeStation traces and verified via normalized coverage uniformity plots (data not shown). Multi-cell wells were identified using picogreen yields (data not shown). A single outlier K562 well was removed due to suspected pipetting error, corroborated by the performance of its technical replicate.





Figures 3 and 4. DNA library prep workflow impact on transcript identification and coverage uniformity. cDNA generated from 8 single HEK293 cells was taken through both Nextera XT (125 pg) and plexWell (variable mass) DNA library prep workflows. 3) Transcript identification results were equivalent between the two downstream workflows. 4) plexWell samples exhibited more even transcript coverage in comparison to Nextera XT, indicating that that coverage uniformity can be significantly impacted by DNA library prep workflow.





Figures 5 and 6. Transcript identification and read mapping performance across workflows. 5) Using single HEK293 cells, Fast SS2 detected a similar number of unique transcripts in comparison to Smart-seq2 and Competitor T, indicating equivalent sensitivity for transcript identification. 6) Read mapping results indicate a similar performance to Competitor T, and improved specificity for exonic regions in comparison to Smart-seq2. K562 single cell results are comparable to HEK293 for both sets of metrics.

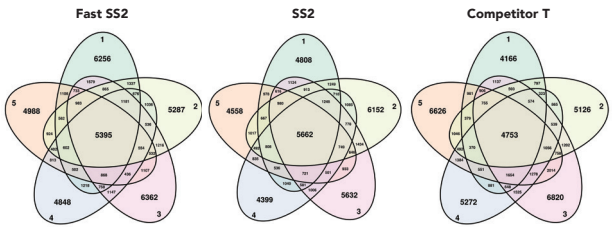


Figure 7 (left). Transcript overlap between individual cells. Fast SS2 shows a similar consistency in transcript detection from single cells in comparison to Smart-seq2, and improved consistency in comparison to Competitor T. Workflow consistency aids in the reduction of cell-to-cell experimental variation.

Conclusions

seqWell Fast SS2 offers a streamlined and fully integrated solution for scRNA-seq library preparation, enabling sample preparation from sorted cells to sequencing-ready libraries in a single day. Additionally, the inclusion of seqWell's plexWell technology for downstream cDNA library preparation reduces the cDNA QC and sample normalization burden typically associated with plate-based, full-length transcript sequencing workflows.

In comparison to both home-brewed and commercially-available gold-standard Smart-seq2-like workflows, Fast SS2 provides both sensitive and reproducible transcript identification and expression data.

References

(1) Picelli, et al Full-length RNA-seq from single cells using Smart-seq2; Nature Protocols volume 9, pages 171–181 (2014)