A simple, ultra-rapid full-length single-cell RNA-sequencing method with streamlined cDNA synthesis and novel one-step tagmentation chemistry

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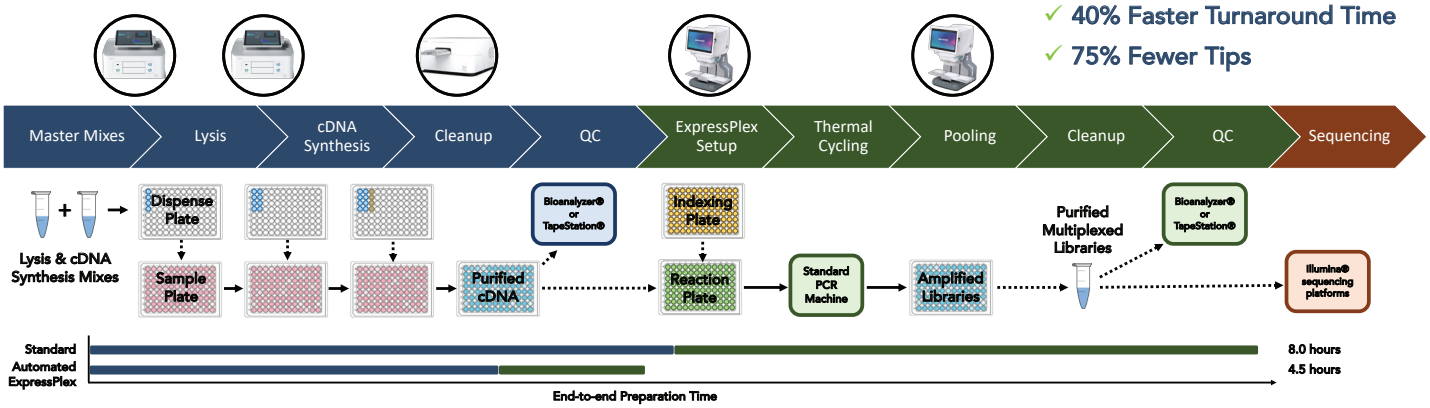
Introduction

Single-cell RNA-sequencing (scRNA-seq) is a powerful tool for understanding transcriptomic differences across cell types; however, most high-throughput scRNA-seq technologies only provide information on 3′ or 5′ portions of mRNA targets. Methods using template switching cDNA synthesis followed by tagmentation can generate sequencing data for the entire mRNA transcript but have historically been complex and time consuming. We report a novel reagent system called ExpressPlex™ that greatly simplifies and expedites library preparation, requiring only two pipetting actions and a single thermocycling procedure to prepare amplified libraries from cDNA. We implement this procedure downstream of a modified FLASH-seq cDNA synthesis protocol to enable faster, easier and automation-friendly full-transcript scRNA-seq. As a proof of concept, we automated and miniaturized this workflow using I.DOT™ and C.WASH™ from Bico® alongside the SPT Labtech Apricot®.

Results

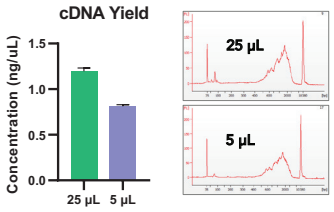
Automated scRNA-seq Workflow with ExpressPlex Library Prep Module

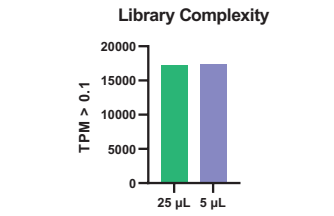
- ✓ 80% cDNA Reagent Savings
- ✓ 40% Faster Turnaround Time
- ✓ 75% Fewer Tips



Automated scRNA-seq library preparation workflow with ExpressPlex module saves time, consumables and reagents: Lysis and cDNA synthesis reagent master mixes are prepared manually. Lysis mix is dispensed by I.DOT. Cells or total RNA are deposited in wells containing Lysis reagent and briefly heated. cDNA Synthesis master mix is distributed in sample wells by the I.DOT then synthesis and amplification are executed on a thermal cycler. Magnetic SPRI beads dispensed in sample wells using the I.DOT. Cleanup is carried out automatically by the C.WASH instrument. Purified cDNA is quantified and QC'd by Agilent Bioanalyzer® or similar. Purified cDNA and Indexing Reagents are transferred to an ExpressPlex Reaction Plate using the Apricot. Library tagmentation and amplification occur in a single thermal cycling protocol. Libraries are pooled using the Apricot and purified. Libraries are quantified and QC'd. The multiplexed library can be sequenced on Illumina® platforms.

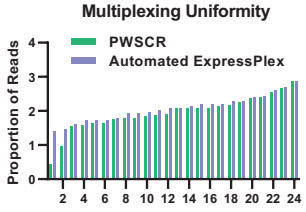
Miniaturized cDNA Synthesis

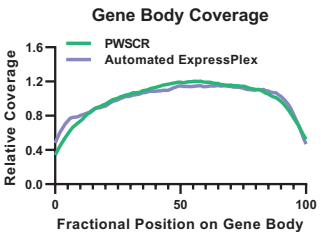


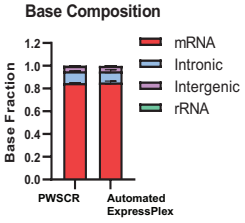


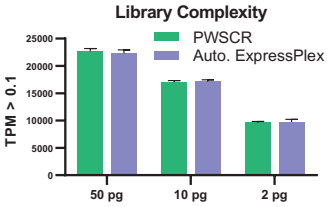
cDNA synthesis reaction can be miniaturized using liquid handling automation: Automated lysis and cDNA synthesis was carried out in 5 or 25 µL reaction volumes with 2 or 10 pg K562 total RNA used as input. cDNA yield was determined using a PicoGreen™ kit (Thermo). Purity and size distribution was assessed using a Bioanalyzer HSD kit (Agilent).

Automated ExpressPlex RNA-seq Workflow Performance



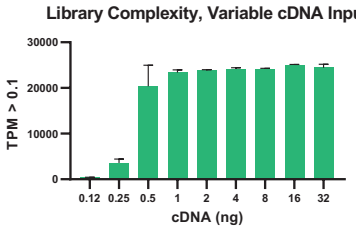


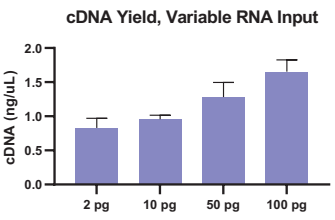




Automated scRNA-seq with ExpressPlex yields high quality sequencing data with exceptional library complexity, consistent with established methods: cDNA synthesis was carried out using the standard plexWell Single Cell Rapid (PWSCR) workflow or the miniaturized, automated workflow at 2-50 pg RNA input. Library preparation was carried out using the plexWell Low Input Module (part of the PWSCR kit) or the ExpressPlex Module. Each library was prepared as a 24-plex comprising n = 8 samples at 3 input levels. Libraries were pooled and 2x76 paired end sequencing was performed on an Illumina NextSeq2000®. Data were down-sampled to 500,000 reads for comparative analysis.

Wide Dynamic Range of Inputs





Automated scRNA-seq with ExpressPlex reagents accommodates a wide range of inputs: Varying amounts of pooled cDNA were used as input for ExpressPlex library prep. Libraries were pooled and 2x76 paired end sequencing was carried out to determine library complexity. Optimized PCR cycle counts were used to generate cDNA from varying K562 total RNA inputs.

Conclusions

- The plexWell Single Cell Rapid kit enables miniaturization of cDNA synthesis to a 5 µL reaction volume with no information loss
- Pairing the plexWell Single Cell Rapid cDNA synthesis module with the ExpressPlex library prep module enables easier, faster library preparation over a wide range of RNA and cDNA inputs without sacrificing sequencing data quality or content
- Both cDNA synthesis and library preparation modules can be automated using common liquid handling capabilities, saving both hands-on and total turnaround time and limiting the number of consumables needed