High-Throughput Single Cell Sequencing on the G4™ Using the Novel Max Read™ Kit

Timothy Looney (timl@singulargenomics.com), Singular Genomics, **Eli Glezer**, Singular Genomics, **Daan Witters**, Singular Genomics, **Martin Fabani**, Singular Genomics, **Ryan Shultzaberger**, Singular Genomics, **Liz Lamarca**, Singular Genomics, **Bubba Brooks**, Singular Genomics, **Kenneth Gouin III**, Singular Genomics, **Niek van Wietmarschen**, Singular Genomics

Background

There remains a need for cost-effective, high throughput sequencing solutions to reduce the cost of scRNA-Seq studies. We previously introduced the Max Read™ Kit, which enables up to 800M paired reads per flow cell on the G4 Sequencing Platform without compromising read quality. Here, we evaluate the performance of the Max Read Kit for scRNA-seq by sequencing a 10x Genomics 3' RNA-Seq library using the G4, comparing results to those from the Illumina® NextSeq 2000.

Methods

A 10x Genomics 3' RNA-Seq library was prepared from ~7000 total cells comprising human healthy donor peripheral blood mononuclear cells spiked with 10% Jurkat and 10% Ramos cells following the standard 10x Genomics protocol, then split into two aliquots. One aliquot was converted to Max Read format for sequencing in replicate on the G4 Platform with the F3 flow cell, while the other aliquot was sequenced on the NextSeq 2000. 200M read pairs were used for downstream analysis following processing via Cell Ranger, scanpy and scVI tools.

Results

Analysis of library size normalized, log transformed UMI counts per gene revealed high technical and cross-platform correlation (Pearson's cor =0.9952 and 0.9907, respectively). Celltypist and scVI leiden clustering labels for G4 and NextSeq 2000 datasets were nearly identical (Adjusted Rand Index = 0.9938) as were the estimated abundances of key immune cell types and expression of canonical immune cell markers.

Conclusions

We demonstrate excellent accuracy, reproducibility, and throughput of the Max Read format when applied to scRNA-seq. Using Max Read Kits for single cell analysis (28x91bp paired read format) customers may expect ~800M reads per F3 flow cell, sufficient for one typical ~10,000 cell scRNA-seq library per each of the four flow cell lanes. With a cost of ~$1 per million read pairs, the Max Read Kit enables cost-effective scRNA-Seq without compromising data quality.