SINGULAR GENOMICS

Fluent BioSciences

APPLICATION NOTE

# PIPseq Compatibility with the Singular Genomics G4 Sequencing Platform

## Introduction

In the ever-growing field of genomic analysis, researchers have increasing needs for accessible, scalable, and cost-effective solutions to accelerate and amplify their research goals. Single cell transcriptomic analysis exemplifies these needs, requiring tens of thousands of individual sequencing reads per cell across hundreds to millions of cells. Fluent BioSciences and Singular Genomics are proud to demonstrate compatibility of PIPseq single cell RNA sequencing in Particle-templated Instant Partitions with the Singular Genomics G4 Sequencing Platform.

PIPseq is a novel, simple, and highly scalable single cell RNA sequencing approach that provides cost-effective library preparation at any application scale. Researchers can select PIPseq kits scaled to 2000, 20,000, or 100,000 cells captured per single tube reaction. PIPseq reactions can be processed individually or in multi-sample batches, and PIPseq's convenient workflow easily accommodates sporadic sample availability, complex time course studies, or remote sample processing in unimproved laboratory spaces.

The Singular Genomics G4 Sequencing Platform is an innovative benchtop sequencer combining novel 4-color rapid sequencing by synthesis (SBS) chemistry with advanced engineering to provide single-day turnaround times for a broad range of applications. The G4's ability to deliver fast results and run 1–4 flow cells in parallel, each with 4 independently addressable lanes, enables laboratories with highly efficient operations. More information about G4 specifications, such as run time, accuracy, and quality metrics can be found on the Singular Genomics website.

In combination, integrating PIPseq sample preparation with the G4 Platform offers new and experienced researchers the tools for efficient, cost-effective, and scalable single cell analysis in any laboratory. In this application note, human PBMCs were processed in PIPseq T20 V4.0 and prepared as Singular Genomics and Illumina compatible sequencing libraries, and single cell analysis of both libraries through PIPseeker analysis is presented. Protocol modifications to convert PIPseq libraries for compatibility with the G4 Platform are also detailed.

## Methods

Standard PIPseq cDNA may be readily adapted for the G4 Platform with minimal modification from existing protocols. Users will require standard PIPseq protocols and reagents (V4.05) and must purchase some additional PCR and sequencing primers from their preferred vendor (IDT, Sigma-Aldrich, etc).

## G4 Compatible PCR Primers

| PRIMER NAME | SEQUENCE | INDEX SEQUENCE |
|---|---|---|
| Fluent-S1-Index-1 | ACAAAGGCAGCCACGCACTCCTTCCCTGT**TAAGACCCTACTT**ACACTCTTTCCCTACACGAC*G*C | TAAGACCCTACT T |
| Fluent-S1-Index-2 | ACAAAGGCAGCCACGCACTCCTTCCCTGT**CGAAGTACATCCC**ACACTCTTTCCCTACACGAC*G*C | CGAAGTACATCC C |
| Fluent-S2-Index-1 | CTCCAGCGAGATGACCCTCACCAACCACT**GGGACATATTGAC**GTCTCGTGGGCTCGGAGATGTG TATAAGAGAC*A*G | GGGACATATTGA C |
| Fluent-S2-Index-2 | CTCCAGCGAGATGACCCTCACCAACCACTT**AGGACGTAACGG**GTCTCGTGGGCTCGGAGATGTG TATAAGAGAC*A*G | TAGGACGTAACG G |

Upon receipt, primers must be reconstituted to 200 uM, and then further diluted to 40 uM for aliquoting and storage at -20C.

Singular Genomics libraries were prepared per standard PIPseq protocol (PIPseq V4.0 T20 Userguide Rev 8.3), with the exception that indexing PCR primers were replaced with the recommended G4 compatible PCR primers noted above. The PCR primers add on necessary S1 and S2 flow cell binding regions as well as 12 bp unique dual indices (UDI's) with a single nucleotide spacer. Fluent Biosciences protocols and user guides are available at www.fluentbio.com.

## G4 Custom Read 2 Sequencing Primer

| PRIMER NAME | SEQUENCE |
|---|---|
| PIPseq R2 sequencing primer | GTCTCGTGGGCTCGGAG ATGTGTATAAGAGACAGT |

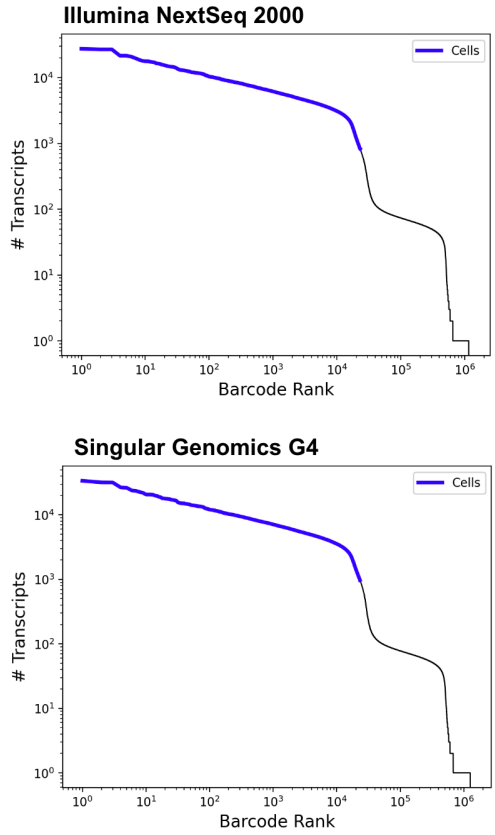
Singular Genomics libraries were sequenced on the G4 using an F3 100 cycle sequencing kit (Cat.# 700,124) and a custom read 2 sequencing primer (Table above, IDT). Sequencing primer was resuspended to 300 uM in 100uL volume in IDTE. The sequencing format was Read 1 = 54 and Read 2 = 67 cycles.

## Bioinformatics Methods

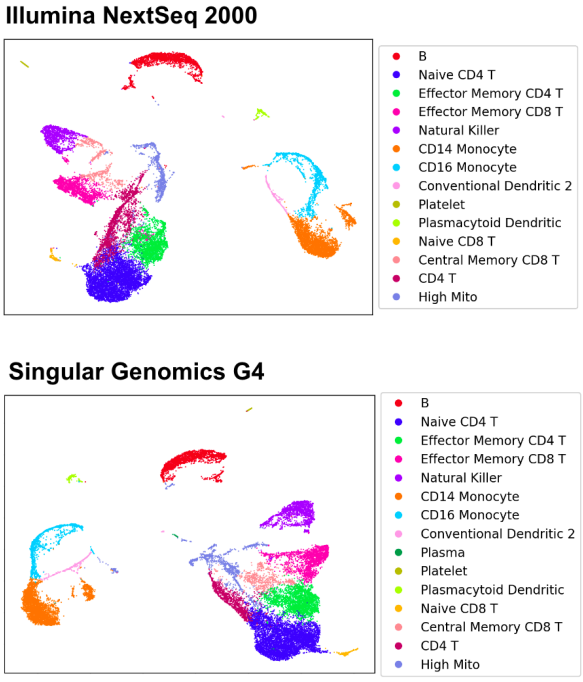
Demultiplexed FASTQs from Singular Genomics G4 or Illumina NextSeq 2000 were processed with PIPseeker v2.01.04, using the human PIPseeker mapping reference (GRCh38.p13, GENCODE v40 2022.04, Ensembl 106). Because the PBMC sample was derived from the same PIPseq T20 library, the data were normalized to the same read depth. The G4 sequencing data (with N-masking feature turned off) was processed at full depth and the matched sample generated on the Illumina NextSeq 2000 was normalized ~403.4 million reads using the `--downsample-to` option in PIPseeker. Cell type annotation was also performed for PBMCs and liver nuclei samples using the respective human PBMC and mouse liver PIPseeker annotation references. The resulting matrix files were merged and processed using Seurat v5 without batch-correction. UMAP plots were generated using the first 30 principal components. Correlation plots of normalized, aggregated gene expression values were also generated using the above samples.

# Results

## Figure 1: Barcode-Rank Plots




## Figure 2: UMAP Plot With Cell Type Annotations



## Table 1: Comparison of Key Performance Metrics between G4 and NextSeq 2000

| | SINGULAR GENOMICS G4 | ILLUMINA NEXTSEQ 2000 | % DIFFERENCE |
|---|---|---|---|
| MAPPING RATE | 91.52% | 89.49% | 2.03% |
| MEDIAN TRANSCRIPTS / CELL | 3304 | 2884 | 14.56% |
| MEDIAN GENES / CELL | 1265 | 1148 | 10.19% |

## Results + Discussion

The G4 Sequencing Platform generated similar metrics and output as the NextSeq 2000. UMAP (uniform manifold approximation and projection) dimensional reduction was performed on the joint G4 and NextSeq datasets, and nearly identical embeddings were observed with the same number of clusters identified between the two platforms. Furthermore, strong correlations of the pseudo-bulk profiles were observed between the G4 and NextSeq 2000. These results confirm that the G4 Platform maintains all the expected biology as the Illumina NextSeq 2000.

Combining the power of the G4 Sequencing Platform with PIPseq enables researchers to achieve high-quality, cost-efficient results. PIPseq library construction for the Singular Genomics G4 Platform is simple and requires only the purchase of a few indexing primers from your desired DNA primer vendor.

The G4 Sequencing Platform provides high sequencing quality and is fully compatible with PIPseeker analysis of PIPseq data. Sequencing quality is comparable to the NextSeq 2000 in matched sample analysis, with small, noted differences in barcode QC, transcript, and gene sensitivity. The high similarity of clustering and cell type annotation results signify high concordance of gene expression between platforms. In conclusion, the Singular Genomics G4 Sequencing Platform is a high-quality, accessible option for routine PIPseq analysis.