Fast and highly sensitive full-length single-cell RNA-sequencing using FLASH-seq

Vincent Hahaut, Dinko Pavlinic, Walter Carbone, Pierre Balmer, Mathieu Quinodoz, Magdalena Renner, Maguelone Roma, David Rawling, Joseph Mellor, Cameron Cowan, Simone Picelli

Institute of Molecular and Clinical Ophthalmology Basel (IOB), Basel, Switzerland; Department of Ophthalmology, University of Basel, Basel, Switzerland; Novartis Institutes for Biomedical Research, Basel, Switzerland; seqWell, Beverly, Massachusets, USA

Plate-based single-cell RNA-sequencing (scRNA-seq) methods provide high transcriptome coverage and full-length mRNA information but have limited throughput. We present FLASH-seq (FS), a full-length scRNA-seq protocol with increased sensitivity and reduced hands-on time. FLASH-seq can be performed in ~4.5 hours, is simple to automate and miniaturize, inexpensive (~$1 / cell) and shows broad compatibility with downstream library preparation methods, including "home-brewed" Tn5 transposase (Picelli et al., 2014) and the entire range of plexWell™ kits (seqWell). A modified version of FLASH-seq includes Unique Molecular Identifiers (UMIs) for molecule counting, making use of a modified Template Switching Oligonucleotide (TSO) to minimize strand-invasion artifacts.

FLASH-seq

SMART-seq protocols, such as Smart-seq2 (SS2, Picelli et al., 2013) and Smart-seq3 (SS3, Hagemann-Jensen et al., 2020), rely on the template-switching activity of retrorviral reverse transcriptases to study full-length transcriptomes at single-cell resolution, thus enabling the characterization of splice isoforms, allelic variants, single nucleotide polymorphisms and transcriptional start sites.

Our new Smart-seq protocol, called FLASH-seq (FS), combines RT and PCR in a single reaction and includes several modifications and improvements which are the result of >300 tests. FS is 2-3.5h shorter than SS2 and SS3 (Fig. 1a) and displays greater gene detection and cell-to-cell correlation (Fig. 1b-c).

A modified version, called FS Low-Amplification, eliminates the need of intermediate cleanups and generates sequencing-ready libraries in ~4.5 h. Another variation, FS-UMI, makes use of UMIs for accurate molecule counting and isoform reconstruction. FS and FS-UMI showed compatibility with the commercially available plexWell™ library preparation kit (seqWell), which further simplifies the workflow and improves normalization across samples with variable cDNA yields.

FLASH-seq low amplification

Minute amounts of cDNA are required to generate sequencing libraries. We reduced the number of PCR amplification cycles in HEK293T cells (19 vs 12, Fig. 2a) and PBMCs (21 vs 16) and performed a direct tagmentation of the resulting cDNA, thus skipping all intermediate steps. The resulting protocol is ~4.5 h long (Fig. 1a) and shows comparable performance to the standard FLASH-seq (Fig. 2a). A fully automated version of FLASH-seq low amplification guarantees a throughput 2.3-times higher than standard FLASH-seq.

Performing too few PCR cycles leads to lower data quality (intergenic, intronic, multi-mapped reads, etc.) (Fig. 2a-b). A titration is therefore recommended.

FLASH-seq vs 10x Genomics

Smart-seq2 remained for many years the gold standard among scRNA-seq methods thanks to its affordability, sensitivity, robustness and simplicity. However, the current 3' Next CEM 10x Genomics Kit displays similar gene detection and greater throughput (Mereu et al., 2020).

In human retinal organoid cells (Fig. 4a-b), FLASH-seq unique genes outperformed Smart-seq3 in terms of gene detection and number of unique molecules detected (Fig. 3c-d). Moreover, Flash-seq displayed better performance compared to Smart-seq2 in terms of gene detection, gene diversity (Fig. 4c) and gene coverage.