Institute of Molecular and Clinical Ophthalmology Basel

www.iob.ch

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

Vincent Hahaut[1,2], Dinko Pavlinic[1,2], Walter Carbone[3], Pierre Balmer[1,2], Mathieu Quinodoz[1,2], Magdalena Renner[1,2], Guglielmo Roma[3], David Rawling[4], Joseph Mellor[4], Cameron Cowan[1,2], Simone Picelli[1,2]

[1]Institute of Molecular and Clinical Ophthalmology Basel (IOB), Basel, Switzerland; [2]Department of Ophthalmology, University of Basel, Basel, Switzerland; [3]Novartis Institutes for Biomedical Research, Basel, Switzerland; [4]seqWell, Beverly, Massachusetts, 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 retroviral 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 2-3.5 h shorter than SS2 and SS3 (Fig. 1 a) and displays greater gene detection and cell-to-cell correlation (Fig. 1 b-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.

Fig. 1a

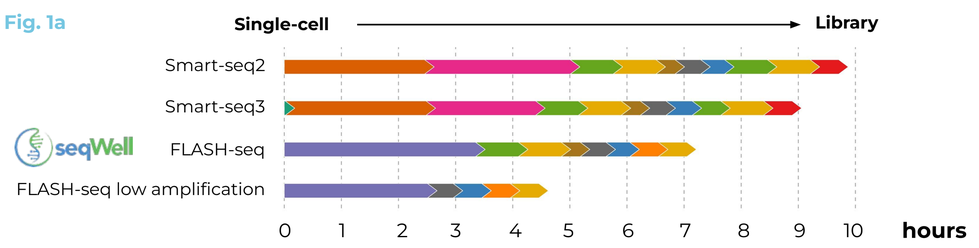


Fig. 1b

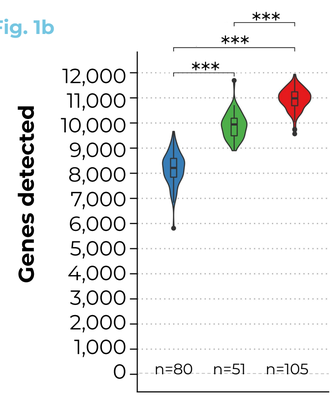
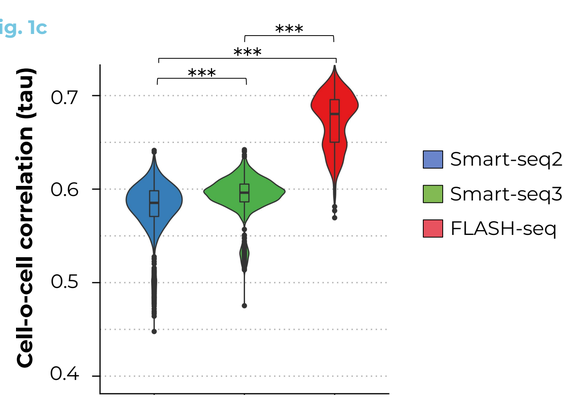


Fig. 1c



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⇒≤12, Fig. 2a) and PBMCs (21⇒≤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.

Fig. 2a

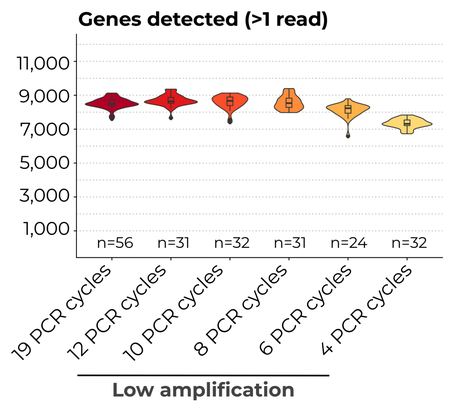
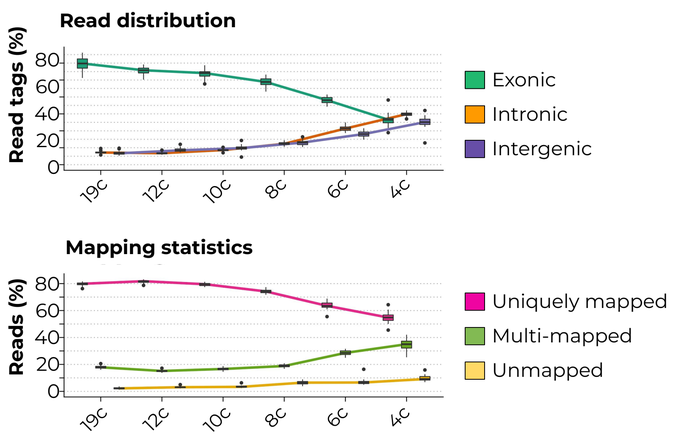


Fig. 2b



FLASH-seq UMI

To demonstrate the flexibility of FLASH-seq, we added UMIs to the FLASH-seq TSO, similarly to Smart-seq3. We observed that the proximity of the UMI and riboguanosines increased the risk of strand-invasion events (Fig. 3a-b). The addition of a spacer isolating the 2 sequences minimized these artifacts.

FLASH-seq UMI outperformed Smart-seq3 in terms of gene detection and number of unique molecules detected (Fig. 3c-d).

Fig. 3a

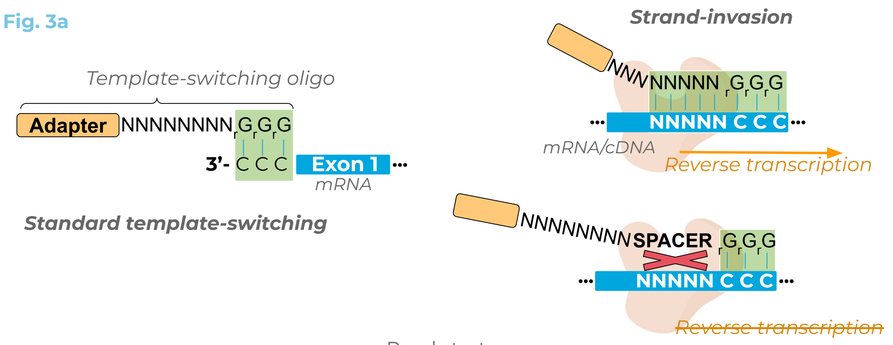
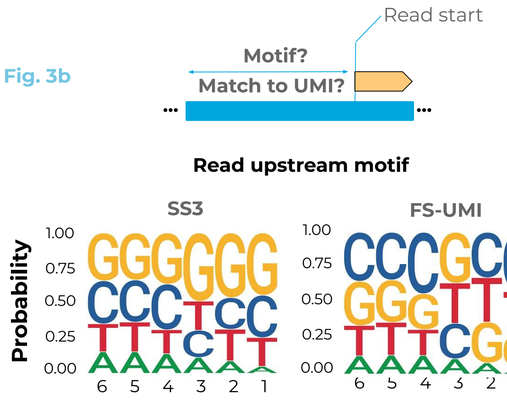


Fig. 3b



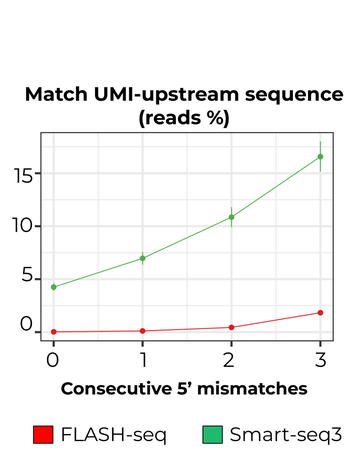


Fig. 3c

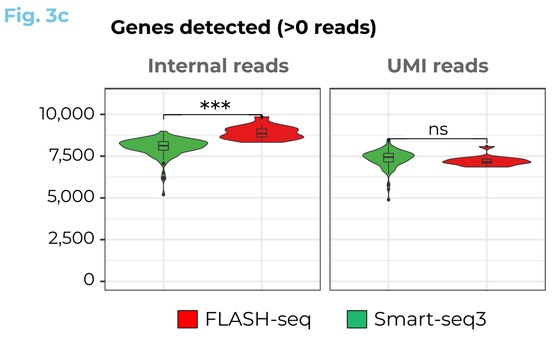
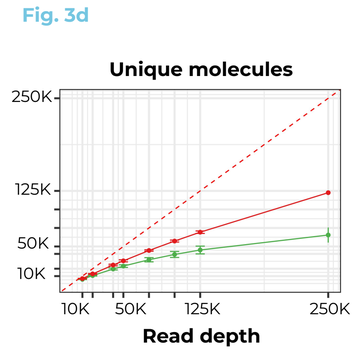


Fig. 3d



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 GEM 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 combination of reagents outperformed 10x in terms of gene detection, gene diversity (Fig. 4c) and gene coverage.

Fig. 4a

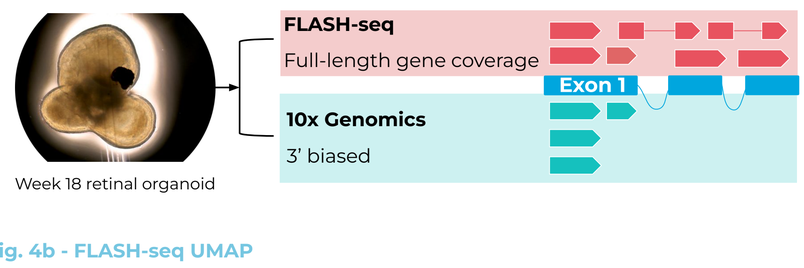


Fig. 4b - FLASH-seq UMAP

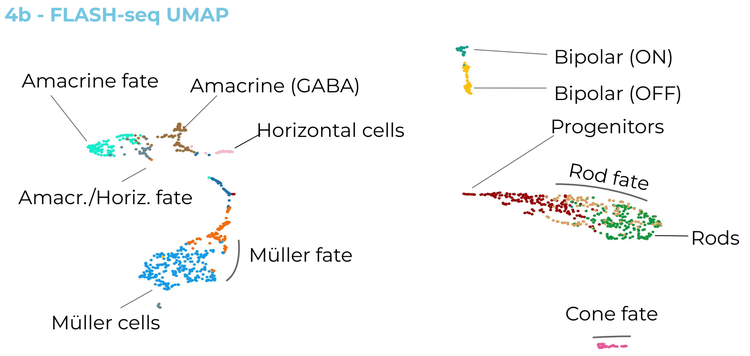


Fig. 4c