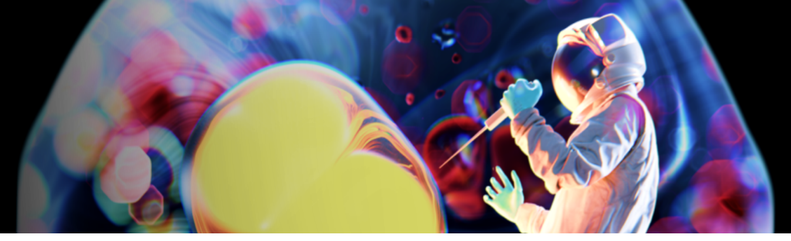
Droplet Genomics



BARCODING-FREE HIGH-THROUGHPUT SINGLE-CELL SEQUENCING ENABLED BY THE 1-READ-1-CELL PARADIGM

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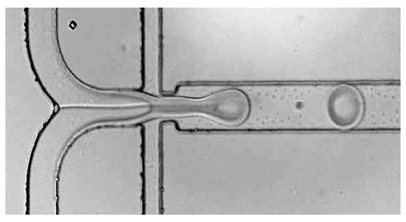
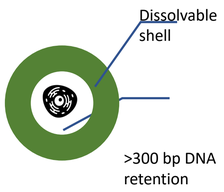
INTRODUCTION

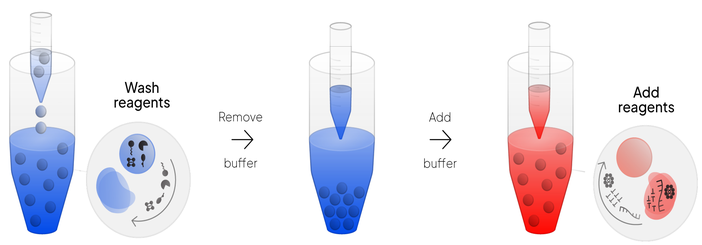
High-throughput single-cell sequencing is largely based on the use of cell barcodes. However, for lower target numbers (2-100s) studied in large numbers of cells (10^4-10^6), the use of cellular barcodes might be excessive, as every cell needs to be presented with a unique set of DNA oligonucleotides. This is expensive and technically challenging, even when performed in microfluidic droplets.
Here, we present the 1-read-1-cell principle for sequencing large numbers of cells in a barcoding-free fashion. Rather than being linked by barcode information, DNA or RNA targets originating from the same cell are concatenated into a single fragment addressable by long-read sequencing. To apply the 1-read-1-cell principle at high throughput, we have developed semi-permeable capsules (SPCs). SPCs take advantage of the single-cell compartmentalization rates characteristic of droplet microfluidics. However, in contrast to regular water-in-oil droplets SPCs enable straightforward multiple-step reactions without loss of compartmentalization.

Semi-Permeable Capsules (SPCs)[1]



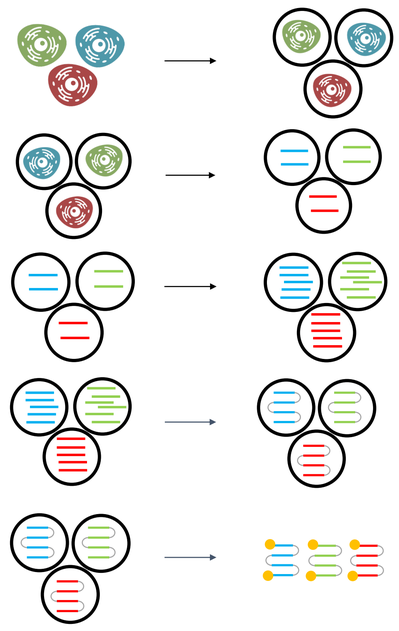
Microfluidic generation; 1mln. SPCs/hr



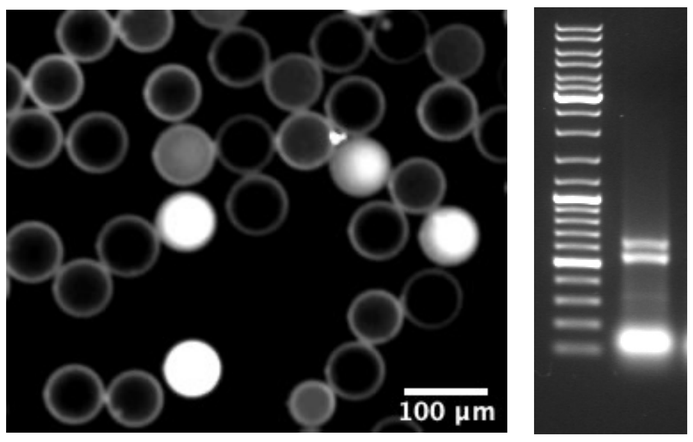


General SPC multistep reaction workflow

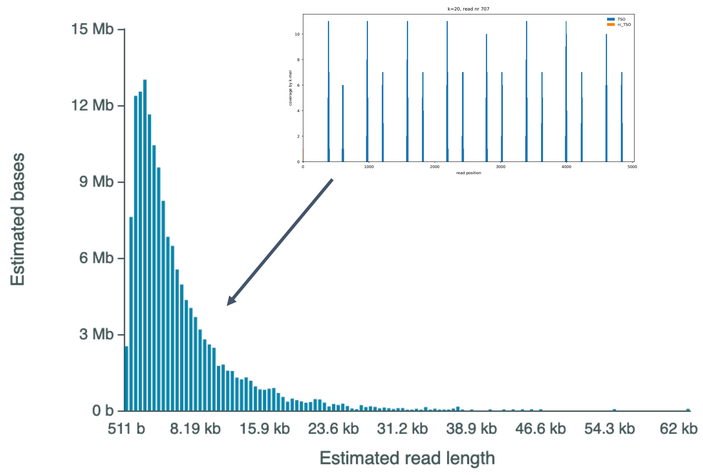
WORKFLOW

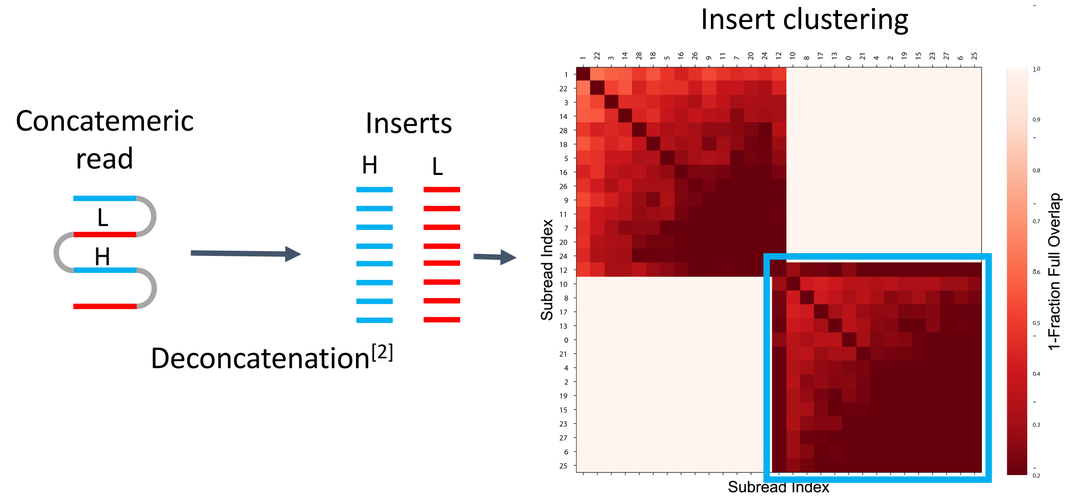


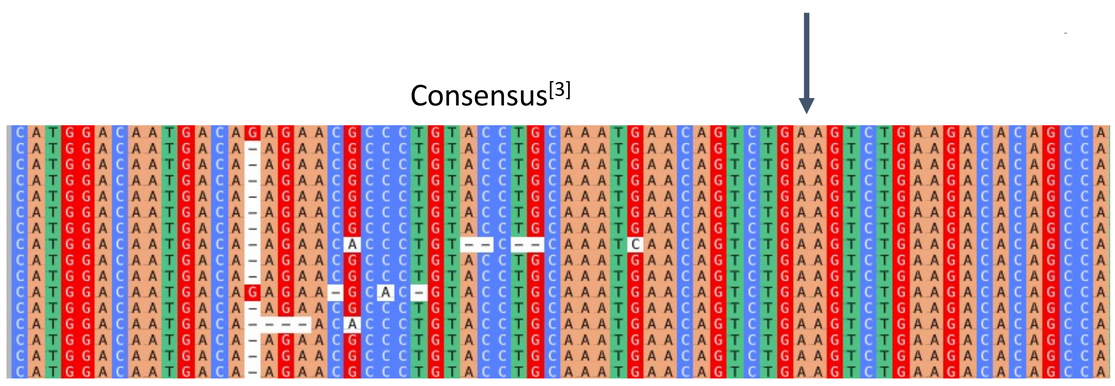
RESULTS



Amplified DNA inside SPC







We demonstrate our approach to recover native BCR heavy and light chain pairs in single cells, a critical step in monoclonal antibody discovery. Antibody-secreting mouse hybridoma cells were processed in SPCs to obtain single-cell-derived concatemeric Nanopore reads, encoding heavy and light chain sequences. Inserts obtained following deconcatenation in silico clustered into two groups by sequence similarity, consistently with the expectation. Insert redundancy in concatemers enabled sequencing error correction by consensus calling.

CONCLUSIONS

The 1-read-1-cell principle is enabled by the unique combination of the read lengths offered by Nanopore sequencing and the throughput and versatility of the SPC technology. We have applied the approach for BCR chain sequencing, an example of two-target analysis in mammalian cells. Notably, the principle can be extended beyond two targets, and can also be applied to hard-to-lyse microorganisms, inaccessible to the droplet format.

REFERENCES

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TRADEMARKS