Application Note:

Scaling Whole-Genome Sequencing to > 50,000 Single-cells using cellenONE®

Abstract

A breakthrough comparative study[1] of 50,000 single-cell genomes was recently made possible by cellenONE® unique capabilities and published by a Canadian consortium of researchers from the University of British Columbia, Memorial Sloan Kettering Cancer Center and BC Cancer Research Centre (BCCRC). This remarkable work combined cellenONE® technology, coupling high-resolution imaging and high-precision single cell isolation and dispensing, and amplification-free whole-genome sequencing of single human cells. The authors provide new insights into genome heterogeneity and clonal evolution in mammalian tissues, paving the way for ultra-high-throughput single-cell sequencing approaches using cellenONE®. DLP+ has since been implemented at BCCRC for sequencing more than 400,000 genomes from patient single-cells.

Materials and Methods

1. Isolation of single cells across various cell and tissue types, ranging 5 - 80 µm, thanks to **cellenONE® versatility and independence from Poisson's law;**
2. Use of **cellenONE® as a single-cell sorter & liquid dispenser for nanolitre-scale sequencing libraries**, drastically reducing reagent costs and hands-on time;
3. Development and open-source release of **scWGS data analysis pipeline** (url) and fully cured and annotated **cellmine genome database** (url);
4. Integration of **morphological measurements and images recorded by cellenONE® with genomic** features;
5. Visualize single nucleotide variants (SNVs), CNAs and breakpoints with **greater resolution by merging clonal data**, with shallow sequencing of a large number of cells.

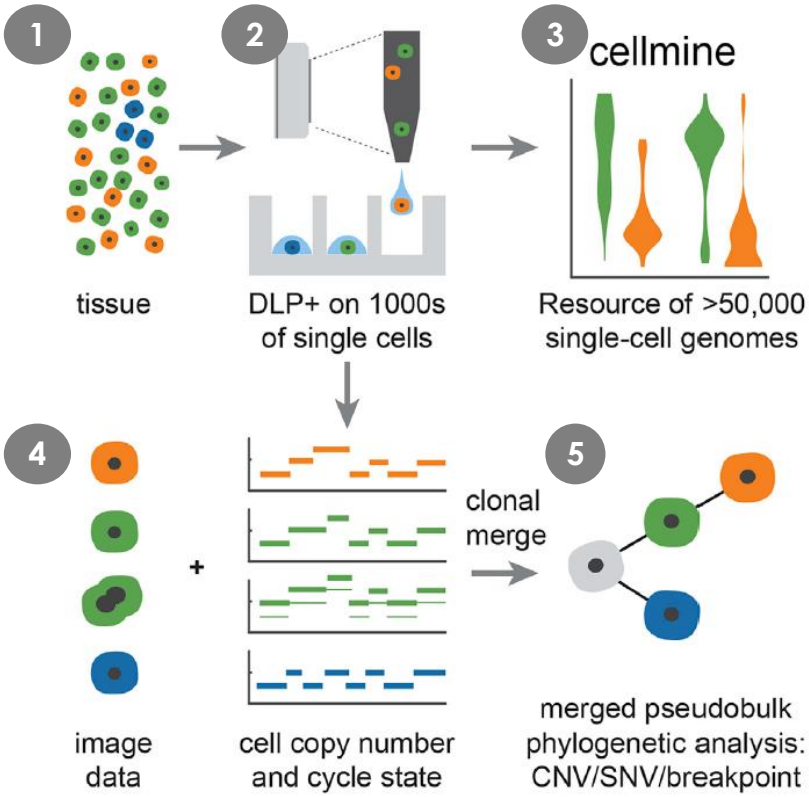


Figure 1. *DPL+ workflow beginning with patient tissue samples for processing on the cellenONE® through data analysis with the DLP+ pipeline.*

Results and Discussion

The authors previously described **Direct Library Preparation (DLP) method for single-cell whole-genome sequencing (scWGS) library preparation**[2]. Removing amplification biases, DLP yielded greater coverage uniformity and better detection of copy number alterations (CNAs) than other amplification-dependent methods with the same low-depth sequencing.

Development of a new **higher throughput and more affordable method, namely DLP+,** was made possible by using **cellenONE®** as it enabled dispensing of a single cell in every well of the microwell chip. Together with a complete open access bioinformatic platform. Although only **52,000 genomes have been released publicly, BCCRC has since sequenced over 10 times that number**. The DLP+ method is now being implemented at other highly renowned institutes around the world such as the Memorial Sloan Kettering Cancer Center in New York and the Francis Crick Institute in London.

[1]Laks, McPherson, Zahn et al. Clonal decomposition and DNA replication states defined by scaled single-cell genome sequencing. Cell, 2019, vol. 179, no 5, p. 1207-1221. e22. (url)

[2]Zahn, Steif, Laks, Eirew, VanInsberghe, Shah, Aparicio & Hansen, Scalable whole-genome single-cell library preparation without preamplification. Nature methods, 2017, vol. 14, no 2, p. 167. (url)

Results and Discussion

Features obtained through merging of clone genomes from fine needle aspirate (FNA) of a breast cancer:

- Reconstruction of copy number clonal architecture of malignant cells (Fig. 2B and 2C) and comparison with diploid reference germline (Fig. 2A).
- Identification of ancestral clonal and clone-specific amplifications
- Significant improvement of clonal inference through single cell demultiplexing and clone clustering.

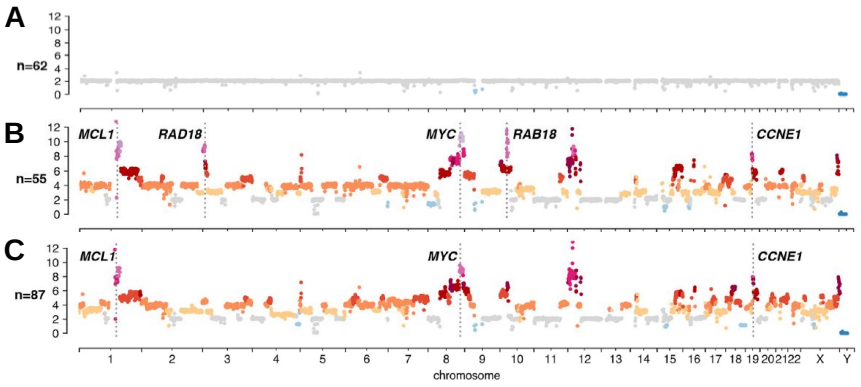


Figure 2. *Total clonal copy number of normal (A) and tumour clones (B and C) in a breast cancer FNA.*

Inference of clonal phylogenies avoiding the limitations of bulk deconvolution:

- Aggregation of cells sharing copy number profiles (Fig. 3A) for calculation of single-nucleotide resolution clonal genotypes. Inference of clone maximum likelihood phylogenetic tree, showing clade- and clone-specific SNVs (Fig. 3B).
- High congruence of phylogeny inferred from breakpoints, suggesting biological relevance of clonal decomposition inferred from copy number profiles.
- Greater efficiency of single-cell DLP+ in deconvolving copy number clones than simulated “bulk” method.

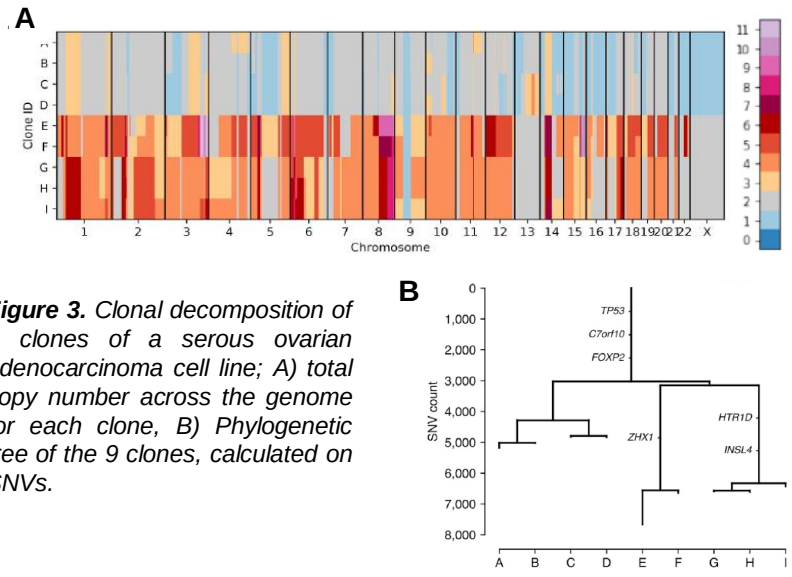
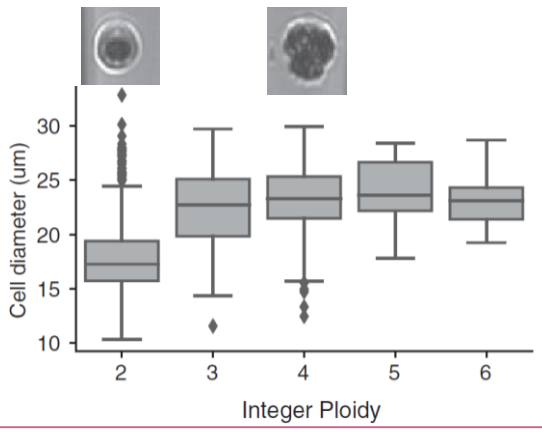


Figure 3. *Clonal decomposition of 9 clones of a serous ovarian adenocarcinoma cell line; A) total copy number across the genome for each clone, B) Phylogenetic tree of the 9 clones, calculated on SNVs.*

Correlation of cell morphology and genome features:

- Larger cell diameter was observed with ploidy > 2 in breast xenograft cancer cells (Fig. 4) and B lymphocyte cell line nuclei.
- Nucleus sizes were not always correlated with DNA replication states (i.e. cellular cycle phases).

Figure 4. *Cell diameter as a function of cell integer ploidy, with cellenONE® bright field images of typical diploid (left) and tetraploid (right) cells*



Conclusions

The combination of an efficient molecular biology method and cellenONE® technology led to the release of an unprecedented resource of tens of thousands of high-quality single-cell genomes. Such scaling of single-cell sequencing library preparation can be applied to most –omics methods, thanks to cellenONE® unique versatility. Miniaturisation of existing protocols together with increasing throughput will reduce single-cell technologies costs allowing these groundbreaking approaches to transition from research to clinical settings.

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