Nuclei Isolation from Immature Embryos of Wild *Sorghum purpureosericeum* with the Singulator™ 100 system

Karafiátová M[1], Bartoš J[1], Doležel J[1], Khan N[2], Pereira N[2], Chear K[2], Meyer D[2], Jovanovich S[2], Bashkin J[2*], (1) Inst. of Experimental Botany of the Czech Academy of Sciences, Centre of the Region Hana for Biotechnological and Agricultural Research, Šlechtitelů 31, Olomouc, 779 00, Czech Republic., (2) S2 Genomics, Livermore, CA, *johnb@s2genomics.com

Single-cell sequencing is revealing the next level of complexity in biological systems. snSEQ workflows for multiple applications require reproducible generation of high-quality single-cell or nuclei suspensions. To facilitate these workflows, S2 Genomics has developed the patented Singulator™ 100 and 200 Systems to automate the dissociation of solid tissues into single cell or nuclei suspensions in single-use cartridges using coupled enzymatic or chemical dissociation and mechanical disruption.

Here we demonstrate the utility of the Singulator platform for the isolation of nuclei from plant embryos, and the impact of the improved nuclei quality over manually prepared nuclei on single nuclei sequencing, enabling the identification of a candidate marker gene associated with the B Chromosome in a specific cell type that may be related to the regulation of the B Chromosome elimination during embryonic development.

Singulator Operation and Sample Preparation

The Singulator 100 and 200 Systems (**Figure 1**) can automatically process fresh tissue samples into single cell suspensions, while nuclei can be isolated from fresh, frozen, or OCT preserved tissue. Isolating nuclei from plant material using manual methods can be difficult, so we sought to expand the application of the Singulator to include plants. In this experiment, the Singulator 100 was used to isolate nuclei from immature embryos of wild sorghum, which were then subjected to single nuclei sequencing. In parallel, nuclei were isolated manually *via* chopping fresh embryos. Nuclei from both preparation methods were further purified by flow cytometry with DAPI staining, until ~100,000 of each type were collected (**Figure 2**).

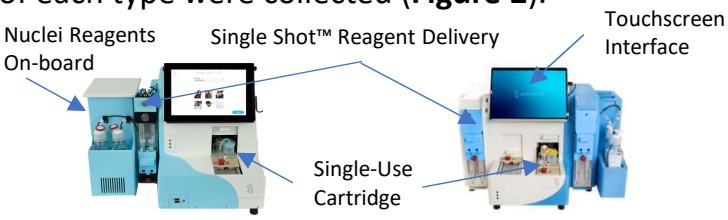

Figure 1. Singulator™ 100 (left) and 200 (right) Systems.

snSEQ libraries were prepared and sequenced, targeting 5,000 nuclei from each preparation. **Table 1** summarizes the sequencing results. The data from the Singulator-derived nuclei shows consistent improvement over the manually prepared sample for most metrics, with substantially more Reads, UMIs per Cell, and Genes per Cell detected.

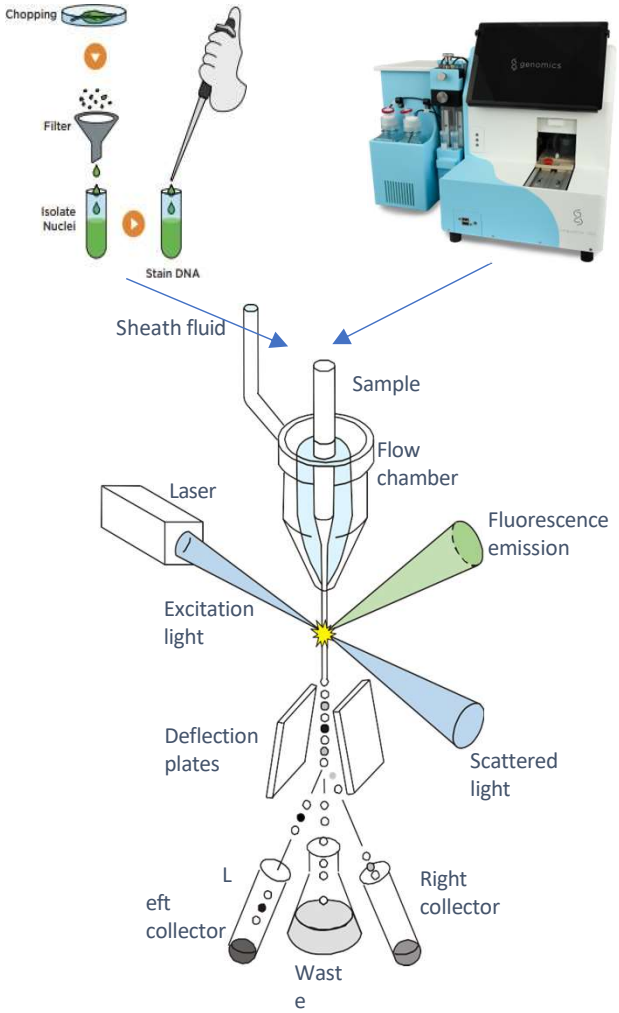

Figure 2. Preparation of nuclei either manually or on the Singulator 100. Singulator isolations were performed using S2 Nuclei Isolation and Nuclei Storage Reagents, and the Low Volume Nuclei Isolation Protocol, with Incubation Time set to 10 minutes. Nuclei from both preparation methods were centrifuged for 5 minutes at 500 g and 4°C, then resuspended and purified by FACS with DAPI staining. RNase Inhibitor was present throughout.

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snSEQ Results and B Chromosome

B Chromosomes are a non-Mendelian inherited component of genomes across many eukaryotic organisms, with largely unknown function. One feature of B chromosome is its structural instability and elimination from specific tissues during plant development. The mechanism of this highly regulated process is poorly understood. The wild grass *S. purpureosericeum* is a useful model organism, for which B chromosome has been partially sequenced (https://doi.org/10.1093/jxb/eraa548). Single nuclei sequencing could provide insights into cell-type specific, B chromosome associated transcriptional networks and, in turn, reveal underlying processes associated with B chromosome regulation. Here, we evaluated the Singulator 100 for nuclei isolation and subsequent snSEQ analysis as part of our research into Chromosome B biology. **Figure 3** shows UMAP clustering for snSEQ data derived from manually and Singulator-prepared samples from monocot embryos. The cluster resolution is significantly improved for nuclei isolated with the Singulator, consistent with the improved quality metrics (**Table 1**). The improved data quality results in the emergence of one B chromosome-specific gene, PGSB-gene-167089, as uniquely expressed in one cell type.

	Manual	Singulator
Reads	430,374,095	593,349,709
Valid Barcodes	97.16%	96.63%
Reads Mapped to Genome	91.44%	92.99%
Estimated Number of Cells	4,689	4,772
Median Reads per Cell	16,343	15,119
Median UMI per Cell	1332	**1628**
Median GeneFull per Cell	978	**1218**

Table 1. Summary sequencing quality metrics for libraries from nuclei isolated manually vs. on the Singulator 100. The data show consistently improved results from the Singulator-derived nuclei, with substantially higher UMI's and Genes per cell detected.

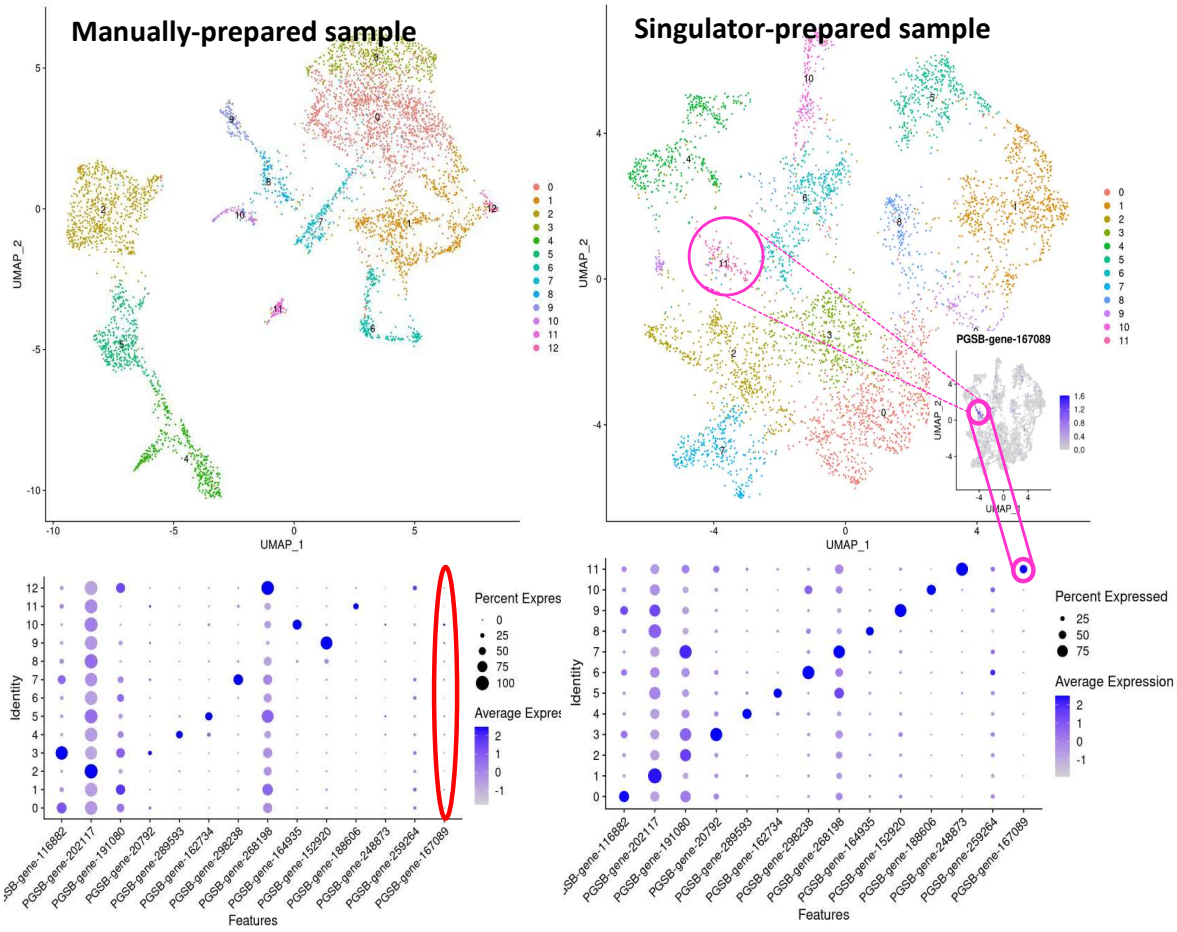

Figure 3. UMAP cluster and heat plots for snSEQ data of manually isolated nuclei (left) and Singulator isolated nuclei (right). Singulator data show significantly improved cluster separation and improved specificity of canonical marker genes. Of potential importance is the unique expression of PSGB-gene-167089, a B chromosome specific gene, in cluster 11.