Singulator™ 100 Nuclei Protocol Submission

Please fill out form as thoroughly as possible. For additional questions and support, email community@s2genomics.com. For more information on the Singulator™ 100 and single-cell processing, head over to www.s2genomics.com.

General Information | Study Identification

Protocol Name: Nuclei Isolation of Immature Embryos of wild Sorghum purpureosericeu

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Tissue Species: Sorghum purpureosericeum

Tissue Type: immature embryo

Tissue State: Frozen

Mass (mg): 30mg

Pre-Singulator™ 100 Processing| Run Summary:

Prior to the experiment, 60 embryos at the stage of development approx. 20 DAP were collected and deep frozen. The experiment was performed using SingleShot Low Volume Nuclei Isolation in with S2 Genomic's NIR/NSR supplemented with 1,500 U of Protector RNase inhibitor. The run details and parameters are specified in the following section. The total run duration was 19m 07s.

Singulator™ 100 Nuclei Protocol Parameters

Reagents: S2 NSR & NIR

Custom Formulation:

Protocol Type: Low Volume Nuclei

Auto Mince: (•) Yes ( ) No

Incubation Time: 10 minutes

Incubation Temperature: Cold

Mixing Type: Top

Mixing Speed: Fast

Disruption Type: Default

Disruption Speed: Fast

Post-Singulator™ 100 Processing

Centrifuge Time & Speed: 5 min/500g/cold

Additional Cleanups/Notes:

After the centrifugation, the supernatant was decanted and the pellet was resuspended gently in 1mL of NSR with 1,000 U of RNase inhibitor added. The nuclei suspension was further processed by flow cytometry. The sample was filtered through 20um mesh and the nuclei were stained with DAPI. In total, we aimed at sorting of 100,000 nuclei for the subsequent one run of single-cell RNA-seq using the 10X gemonics platform.
The resulting data were compared to the data set obtained from previously performed single-cell RNA-seq experiment, in which the nuclei were isolated by chopping the freshly collected embryos. In both replicas, the condition of flow sorting, single-cell RNA-seq, as weel as the parameters of the transcriptome analysis were identical, the method of nuclei isolation was the only step different.