Nucleus Purification

Sample (1.5 g) was ground with 5 mL 1xNuclei Isolation buffer using a mortar and pestle. Nuclei were isolated according to Plant Nuclei Isolation/Extraction Kit (Sigma, St. Louis, MO, USA) with some modifications. The procedures were as follows: The homogenates were filtered through a double layer of Filter Mesh 100 and transferred to a 15 mL tube, and then centrifuged at 1,300 x g for 10 min at 4°C. The resulting pellet was resuspended in Nuclei Isolation buffer containing protease inhibitor mixture (Roche, Werk Penzberg, Germany), and phosphatase inhibitor mixture (Sigma), and then layered on top of a 1.5 M sucrose cushion prepared in 1xNuclei Isolation buffer. After centrifugation at 12,000 x g for 10 min at 4°C, the resulting supernatant was removed and the pellet was resuspended in Nuclei Isolation buffer containing protease inhibitor mixture. The suspension was again layered on top of a 1.5 M sucrose cushion and then centrifuged at 12,000 x g for 10 min at 4°C to pellet the nuclei-enriched fraction. The purified nuclei was washed 2 times using Nuclei Isolation buffer containing protease inhibitor mixture. Nucleus was vortexed at 4°C for 20 min with extraction buffer containing protease inhibitor mixture and phosphatase inhibitor mixture, and then sonicated in ice water for 20 min. Vortex and sonication were repeated once. After sonication, the homogenates was centrifuged at 12,000 x g for 30 min at 4°C and the supernatant was collected as nuclear protein.