

Sample Preparation

Samples were air-dried on filter paper (MilliporeSigma, Burlington, MA, USA) for 1 hr and added to 150 μ L chilled (4°C) Nuclei Lysis (NL) solution (Promega, WI, USA) in a 1.7 mL microcentrifuge tube (Thermo Fisher Scientific, Waltham, MA). Samples were pulverized in NL solution with a sterilized pestle, combined with 17 μ L of 20 mg/mL water suspended solution of Proteinase K (New England Biolabs), and incubated for 2 hr at 65°C in a dry bath incubator (Thermo Fisher Scientific, Waltham, MA). TE buffer suspended RNase A solution (5 μ L of 4 mg/mL) (Promega, WI) was added to the mixture and incubated for 30 mins at 37°C. Protein Precipitation solution (200 μ L; Promega, WI, USA) was added to the mixture, chilled at 4°C for 5 mins, and centrifuged for 4 mins at a maximum speed of 12,400 RPM (Thermo Fisher Scientific, Waltham, MA). The supernatant (700 μ L) was removed and added to a new 1.7 mL microcentrifuge tube. Molecular grade isopropanol (600 μ L) (Thermo Fisher Scientific, Waltham, MA) was added to the 1.7 mL tube and centrifuged at maximum speed. The resulting supernatant was removed (1100 μ L), and a second precipitation was performed with 600 μ L of 70% ethanol (Decon Laboratories Inc, King of Prussia, PA) with a 20 min centrifuge cycle at maximum speed. The ethanol supernatant was removed, and the pellet was air-dried for 1 hr and resuspended in 40 μ L of DNA rehydration solution (Promega, WI) for an hour at 65°C.