**DNeasy modified column-less protocol**

This protocol combines the lysis and protein precipitation steps of the Qiagen DNeasy kit with a standard alcohol precipitation step. DNA from this protocol will be of higher (30-50kb) molecular weight than the standard Qiagen column based method.

**1)Add 400 μl Buffer AP1 and 1 μl RNase A stock solution (100 mg/ml) to a maximum of 100 mg (wet weight) or 20 mg (dried) ground plant tissue.** Optional: add 1x PVP and 10mM DTT.

**2) Incubate the mixture for up to 20 min at 65°C. Mix 2 or 3 times during incubation by inverting tube.** This step lysis the cells.

**3) Add 200 μl Buffer AP2 to the lysate, mix, and incubate for 20 min on ice.** This step precipitates detergent, proteins, and polysaccharides.

**4) Centrifuge the lysate for 5 min at 20,000 x g.**

**5) Transfer supernatant to a fresh tube, avoiding the cellular debris pellet; add 600 μl of isopropanol mix by inversion and incubate on ice for 15-30 minutes.**

**6) Centrifuge for 20 minutes at >14,000 x g. Discard supernatant being careful not to disturb DNA pellet.**

**7) Resuspend and wash pellet in 500 μl of 70% ethanol, mix by inversion and spin for 10 minutes at >14,000 x g. Discard supernatant and leave pellet to air dry.**

**8) Resuspend DNA pellet in buffer of choice. If pellet is slow to resuspend, heat sample to 50°C for 10-15 minutes.**

**Buffer AP1 – aka Lysis Buffer**

10 mM Tris-HCl pH8.0

1 mM EDTA pH 8.0

0.1% SDS

0.1M NaCl

+1X PVP (optional, recommended for tissue with many secondary compounds)

+10mM DTT (optional, recommended for tissue with many secondary compounds)

+ RNase (optional)

**Buffer AP2 – aka Protein Precipitation buffer**

3M potassium / 5M acetate

made by adding:

 60 ml of 5M potassium acetate (98.14 g in 200 ml dH20)

11.5 ml of glacial acetic acid

28.5 ml dH20