**Isolation of DNA from Filters using Hot Detergent and CTAB**

2X Lysis buffer is 100mM Tris pH 8.0, 40mM EDTA, 100mM NaCl, 1% SDS. Preheat to 70C.

Add zircon beads, about 50µL, to the filter along with 100µL lysis buffer. (You can adjust the amount of lysis buffer depending upon your sample volume – you want to cover your whole sample with buffer. When working with tissue pieces, you can also use a small eppendorf mortar and pestle to grind the tissues, or make your own by melting the tips of 1000micoL pipet tips.)

Vortex and then heat @ 70C for 5 minutes.

Vortex sample for 30 seconds on the homogenize setting.

Heat @ 70C for 5 minutes.

Vortex as before.

Transfer the solution to a new micorfuge tube and make the solution at least 0.7M with NaCl (if 100µL, add 35µL 3M NaCl). Add 10% CTAB to make a 1% solution (15µL) and incubate at 60 – 70C for 10 minutes.

Add an equal volume of phenol/chloroform (200µL), vortex, then spin @ 12,000 rpm for 5 minutes.

Remove the aqueous layer to a new eppendorf tube.

Precipitate supernatants using 100% isopropanol (0.6 volume isopropanol, no need to add extra salt.) Chill for at least 1 hour @ -80C, or overnight at –20C, before spinning @ 12,000 rpm for 10 minutes. Check bottom of tube carefully for very small whitish pellet. Air dry for 15 minutes.

Resuspend the pellet in 10 –20µL sterile milliQ water or 0.01M Tris pH 7.6, and determine concentration by A260 if desired.