**Salting-out Protocol for Extracting Genomic DNA**

**Materials**

Proteinase K (20 mg/ml)

RNase A (100 mg/ml)

1 % SDS cell lysis buffer (100mM Tris-Cl; 50 mM EDTA; 1 % SDS)

7.5 M ammonium acetate

0.5 M EDTA

Nuclease-free water

100 % cold isopropanol

70 % ethanol

**Equipment**

1.5 ml microcentrifuge tubes

Blue roll

Microcentrifuge

Vortexer

Homogeniser (e.g. TissueLyser, MP FastPrep 24) (optional)

Microbeads (optional)

**Protocol**

**Sample preparation**

1. Remove sample from preservative and dap on blue roll to remove excess ethanol (if required).
2. Add samples (up to 30 mg) to 1.5 ml microcentrifuge tubes.
3. Add one or more microbeads to each tube and homogenise samples using a homogeniser (optional).

**Digestion**

1. Add the following to each tube:
   1. 350 µl 1 % SDS cell lysis buffer.
   2. 42 µl 0.5 M EDTA.
   3. 20 µl proteinase K.
2. Mix by vortexing. Spin down. Incubate at 65oC for 4 hours.
3. Add 2 µl RNase A. Mix by vortexing. Incubate at 37oC for 30 minutes.

**Remove proteins and cellular debris**

1. Add 140 µl 7.5 M ammonium acetate to each tube. Mix by vortexing. Incubate at 4oC for 10 minutes.
2. Centrifuge at 12,000 xg for 10 minutes.
3. Transfer supernatant to a new 1.5 ml microcentrifuge.
4. Repeat steps 7-9.

**Precipitation of DNA**

1. Add 680 µl cold isopropanol (volume ratio 1:1). Mix by inverting gently 50 times. Centrifuge at 8000 xg for 5 minutes.
2. Carefully discard the supernatant, avoiding contact with the pellet. If a lot of supernatant remains, pulse centrifuge the tubes and discard the supernatant using a smaller pipette, again avoiding contact with the pellet. Drain the tube by placing on sterile blue roll, taking care that the pellet remains in the tube (~30 minutes).

**Washing of DNA**

1. Add 400 µl 70 % ethanol. Invert the tube several times to wash the DNA pellet. Centrifuge at 8000 xg for 1 minute.
2. Carefully discard the supernatant, avoiding contact with the pellet. If a lot of supernatant remains, pulse centrifuge the tubes and discard the supernatant using a smaller pipette, again avoiding contact with the pellet.
3. Allow to air dry to 10-20 minutes. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.

**Rehydration of DNA**

1. Re-suspend dried pellets with 100 µl nuclease-free water.
2. Incubate at room temperature for at least 60 minutes or incubate in the fridge overnight.
3. Pulse centrifuge the tubes and store DNA at -20oC.