Genomic DNA extraction (via bead beater) (From Yeates and Gillings, with Neil Wilson mods)

Notes: This protocol is versatile and can be used for any kind of cells/samples (bacteria, soil, faeces etc). The DNA obtained is lower quality than from chemical/enzymatic lysis, and is sheared to usually < 10 kb in size. This is fine for PCR, but may not be ok for other purposes.

Hazards: Binding matrix contains guanidine which is an irritant. Wear gloves and safety glasses.

1. Prepare the beadbeater tubes by adding two scoops* of tiny beads, two scoops of small beads, and one large bead to each 2 ml screw cap tube. Autoclave with lids off, loosely covered in foil. Autoclave lids separately, also loosely wrapped in foil. Put caps on tubes aseptically once cooled.

   * one 'scoop' is approx 100 ul volume. tiny beads are ~100-200 micron, small beads are ~500 micron, and large bead is 5 mm. All 'beads' are acid washed glass beads. eg. Sigma G1145-100G

2. Add 300 µl or 300 mg of sample (soil, cells etc.) per tube, then 150 µl of **SH solution**. Mix by vortexing until the sample is evenly mixed with the solution.

3. Add 750 µl of **Lysis Buffer** per tube, vortex again, then freeze at -80C for at least 20 min (prep can be indefinitely stored at this point for later processing).

4. Thaw samples. Ensure fully thawed and cap is on tightly. Bead beat 30 seconds in beadbeater.

5. Incubate samples at 50°C for 1 hr with occasional mixing, then freeze again at -80°C for >20 min.

6. Add 120 µl of **K-acetate** solution, incubate on ice for 10 min, centrifuge at ~10,000 g for 5 min.

7. Transfer 700 µl of supernatant to a new (labelled) 1.5 ml tube. Find the **'binding matrix'** and vortex the bottle/tube to resuspend the silica particles, then immediately add 500 µl of matrix to DNA sample. Incubate 5 minutes at room temp, mixing occasionally to keep the matrix suspended.

8. Centrifuge at ~10,000 g for 30 seconds to pellet the matrix-bound DNA.

9. Pour off the supernatant into the discard bottle. Be careful not to back-contaminate your DNA sample from the discard bottle ! Tap gently on paper towel to remove last drops of supernatant.

10. Add 750 µl of **SEWS** and resuspend the pellet by vigorous vortexing/flicking (approx 30 sec). Allow matrix to sit in SEWS buffer ~1 minute, with occasional mixing.

11. Centrifuge at ~10,000 g for 30 seconds, then discard supernatant. Leave the tubes open for 15 min in heating block at 60°C to allow residual ethanol (in SEWS buffer) to evaporate. Its very important to remove all the ethanol or DNA will misbehave later ! When fully dry, the matrix pellet should be a uniform bright white colour, with no darker patches.

12. Add 200 µl **EB** to the pellet, vortex/flick vigorously, heat 60°C for 5 min (lid closed this time!).

13. Centrifuge at ~10,000 g for 1 min, then transfer the DNA-containing supernatant to a new labelled tube. Note that you will only recover ~100 µl liquid. Be careful to avoid the matrix material.

SH solution: Sodium hexametaphosphate (200 mM). Sterilised by autoclaving.

Lysis buffer: Tris base (50 mM), EDTA (50 mM), guanidine-HCl (1 M), Triton X-100 (0.5% w/v), adjust to pH 10 with NaOH. Sterilised by autoclaving.

K-acetate: 7 M potassium acetate. Sterilised by autoclaving.

Binding matrix: MP Biomedicals™ Binding Matrix. This can be diluted 1:2 with 6 M guanidine-HCl to make it go further. The raw product has more binding capacity than is needed.

SEWS (salt/ethanol wash solution): 100 mM sodium acetate in 70% ethanol. Make 30 ml of 333 mM sodium acetate, autoclave this, then after cooling, add 70 ml of 100% ethanol.

EB: 10 mM Tris HCl, pH 8