Genomic DNA extraction (via bead beater "FastPrep" method)

Notes: This protocol is versatile and can be used for any kind of cells/samples (animal, plant, microbe, soil, faeces etc). Note that the DNA obtained is of lower quality than that obtained via chemical/enzymatic lysis (e.g. above protocol), and it will be sheared to ~ 1-10 kb in size. This is fine for PCR, but may not be ok for making clone libraries. Reference: Yeates and Gillings (1998)

Hazards: Binding matrix contains conc. guanidine thiocyanate which is an irritant. Wear gloves and safety glasses for any steps involving binding matrix.

Protocol

1. Prepare bead-beater tubes ahead of time: Get glass beads plus the scoop and funnel from the chemicals cabinet (under 'G' for Glass) and put 2 large beads and two scoops each of the medium and small size beads into each tube. Sterilise by autoclaving.
2. Add 122 µl of MT buffer and 780 µl sodium phosphate buffer to the bead beater tube, then add your sample (200-500µl volume). Leave a little bit of headspace for good beating action. For pure cultures, the pellet from a 5 ml broth or a large loopful (~half of a streak plate) is good.
3. Place tubes in bead beater and process for 30 sec at speed 5.5
4. Centrifuge ~ 10000 g for 5 min to pellet beads and debris
5. Transfer supernatant to 1.5ml microfuge tube
6. Add 125 µl PPS and mix
7. Centrifuge ~ 10000 g for 5 min to pellet the protein precipitate
8. Transfer 700 µl of supernatant to a new 1.5 ml microfuge tube. If high yield is important, you can transfer the remaining liquid (~300-600 µl) to a second tube, and process in parallel.
9. Add 700 µl Binding Matrix Suspension and mix gently for 5 minutes
10. Centrifuge ~ 10000 g for 1 min to pellet matrix-bound DNA
11. Remove supernatant (avoid Binding matrix)
12. Add 500 µl of salt/ethanol wash solution and resuspend pellet by vortexing
13. Centrifuge ~ 10000 g for 1 min and discard supernatant
14. Repeat steps 12-13
15. Invert tube with lid open on paper towel and drain off excess salt/ethanol (~5 min), then transfer tubes to 60ºC incubator for ~20 mins to evaporate residual ethanol. When fully dry, the pellet should look white, not grey/brown.
16. Resuspend matrix by vortexing in 200 µl TE buffer. Incubate 60°C for 10 min to dissolve DNA.
17. Centrifuge ~ 10000 g for 1 min and transfer supernatant to new tube. Store at -20ºC

Solutions:

MT buffer*: 1% SDS, 0.5% Teepol, 5% PVP40, 10 mM Tris (pH 8), 10 mM EDTA (pH 8). Autoclaved.
Sodium phosphate: 0.1M Na_2HPO_4 (pH 7.0), sterilise by autoclaving
PPS: 7.5M potassium acetate, sterilise by autoclaving
Binding Matrix Suspension: (bought from MPBio = silica in guanidine solution). Can be diluted 2:1 in guanidine thiocyanate (6 M) solution to make stock go further.
Salt/ethanol wash solution: 70% ethanol, 100 mM sodium acetate
(add ethanol to autoclaved 100 mM sodium acetate after cooling)
TE buffer: 10 mM Tris-HCl, 1 mM EDTA. pH 8.0, sterilise by autoclaving

* This MT recipe is modified from original version. Has more PVP and more EDTA