Glass Bead Preparation of Total Yeast DNA Using the Fast Prep (MP Bio)

1. Grow 5 ml yeast cells to stationary phase (30°C overnight).

2. Spin desired volume of cells (usually 1.5-3 mL) in 1.5 mL tubes. Wash cells 1X in ddH_2O.

3. Resuspend in 300 µl lysis buffer. Add 300 µl of acid-washed glass beads. Seal the tube with a small piece of parafilm paper.

4. Place tubes in FastPrep. Run 2X 45 seconds at 4.5 m/s (Program 1).

5. Remove the tubes. In order to remove the glassbeads, pierce a small hole with a 23G1 needle (blue). Heating the needle will help. To avoid the liquid to go out of the hole, uncap the tube to release the pressure before piercing. Place the pierced tube on top of another 1.5 mL tube.

6. Spin at 1000 rpm for 10-15 seconds (the Eppendorf centrifuge works best). Leave one space between each tube (easier to remove them from the centrifuge). You should now have the glassbeads on top and the extract on the bottom.

7. To the 300 µl of extract, add 200 µl of lysis buffer.

8. Extract 2X with 500 µl phenol-chloroform, vortex and spin.

9. To the upper layer, add 1 ml cold ethanol 100% to supernatant. Vortex; place at -20°C overnight OR 20-30 minutes at -80°C.

10. Spin 20 min at 4°C. Wash in 70% Ethanol. Dry the pellet.

11. Resuspend in 50 µl TE, add 2.5 µl RNase (10mg/ml), incubate at 37 °C at least 30 min.

12. Add 150 µl TNE; add 5 µl Proteinase K (10mg/ml), incubate 37 °C at least 30 min.

13. Extract 1X with 200 µl phenol-chloroform, vortex and spin.

14. Extract 1X with 200 µl chloroform, vortex and spin.

15. Add 500 µl of cold ethanol 100% to supernatant. Chill at –80°C, 30 min (or >1h at -20°C).

16. Spin 20 min, 4 °C. Add 1 ml cold ethanol 70%. Spin 5 min, 4 °C. Dry the pellet.

17. Resuspend pellet in 30 - 50 µl TE. Quantify the DNA using your favourite method.

Lysis Buffer (for 50 ml)

5 ml Tris 1M (100mM)
5 ml EDTA 0.5M (50mM)
2.5 ml NaCl 5M (250 mM)
5 ml SDS 10% (1%)
Complete at 50 ml with H_2O

TNE (for 50 ml)

500 µl Tris 1M pH8,0 (10 mM)
1 ml NaCl 5M (0,1 M)
100 µl EDTA 0,5 M pH8,0 (1mM)
Complete at 50 ml with H_2O