Isolation of Yeast DNA
Grow 10 ml culture in YPD overnight
Spin at 3000 g for 5 min
Decant
Resuspend in 0.5 ml ddH2O
Transfer to 1.5 ml tube
Spin for 5 sec and pour off supernatant
Resuspend cells in 200 ul of breaking buffer
Add 0.3 g glass beads (200 ul)
Add 2 00 ul phenol/chloroform/isoamyl
Vortex at high speed for 3 min
Add 200 ul TE buffer and vortex briefly
Spin 5 min at high speed
Transfer aqueous layer to new 1.5 ml tube
Add 1 ml 100% ETOH
Mix by inversion
Spin 3 min at high speed
Remove supernatant
Resuspend pellet in 400 ul TE buffer
Add 30 ul of 1 mg/ml Dnase free Rnase A
Mix by inversion
Incubate 5 min at 37C
Add 10 ul 4 M Ammonium acetate
Add 1 ml 100% ETOH
Mix by inversion
Spin 3 min at high speed
Decant
Dry
Resuspend DNA in 100 ul TE buffer
Anticipate 20 ul DNA

Breaking Buffer
2% (v/v) Triton X-100
1% (v/v) SDS
100 mM NaCl
10 mM Tris-Cl, pH 8
1 mM EDTA, pH 8

<u>100 ml</u>
2 g Triton X
1 g SDS or 10 ml 10% SDS
0.585 g NaCl
1 ml Tris-HCl
0.2 ml of 0.5 M EDTA
H2O to 100 ml