Yeast genomic preparation for sequencing (08/30/17)

This protocol starts with cell pellets from 5 ml of culture.

Resuspend cells in 300 μl of Lysis buffer.

Add ~0.3 g Glass beads

Add 300 μl of phenol:chloroform:isoamyl alcohol

Vortex for 10 min.

Spin 5 min at top speed.

Remove aqueous layer (~250 μl) and transfer to a new tube.

Add 5 μl RNase (100 mg/ml). Incubate 37°C for at least 1 hour.

Add 300 μL of phenol:chloroform:isoamyl alcohol. Vortex for 15 seconds.

Transfer contents to a phase-lock gel (light) tube.

Spin 5 min at top speed.

Dump aqueous layer into a tube with 500 μl of ethanol. Precipitate DNA in –20° C freezer for at least 30 min (overnight).

Pellet DNA by spinning for 10 minutes at top speed. Decant or aspirate ethanol.

Add 1 ml of 70% ethanol, vortex, and spin for 2 min at top speed. Decant or aspirate ethanol.

Dry DNA pellet in speed-vac for ~10 min. Resuspend pellet in 100 ul of ddH_20.

Check and record DNA concentration using Qubit fluorometer

Lysis Buffer
2% Triton X-100
1% SDS
100 mM NaCl
10 mM Tris-Cl, pH 8.0
1 mM EDTA

To make 50 ml of Lysis Buffer
40.9 ml H_2O
5 ml of 20% Triton X-100
2.5 ml of 20% SDS
1 ml of 5 M NaCl
500 ul of 1 M Tris-Cl, pH 8.0
100 ul of 0.5 M EDTA