DNA Prep from Yeast (quick protocol)

From plate culture

Break up the yeast
-Add 200uL Yeast DNA Extraction Buffer to 2ml screw cap tube
-Scrape culture off plate with a toothpick and swirl in the tube to remove yeast.
-Add 0.3g acid washed glass beads (0.4 scoop, Sigma G-8772)
-Add 200uL Phenol Chloroform (Sigma P-3803)
phenol chloroform is the bottom layer

Separate the DNA from the proteins and cell wall crap
-Close lids well and Fast Prep Set 5, 60secx2
-Quick spin to get liquid off the lid
-Add 200uL 1x TE pH 8.0
-Invert a few times
-Centrifuge at 10,000rpm for 5 min. This will cause layers to form. The top layer is the aqueous phase, followed by a white layer of proteins and the bottom is the beads and phenol chloroform.

Ethanol precipitation
-Transfer aqueous phase to fresh tubes. About 400uL. (It is okay to leave some liquid, but do not take white cell debris)
-Add 1mL 100% EtOH
-Invert a few times
-Store them at -20 for 10 minutes or longer.
-Centrifuge at 10,000rpm for 5 min.

Cleaning step
-Pour off EtOH carefully with vacuum.
if RNaseA treatment is necessary, start that procedure now
-Add 1mL 70% EtOH
-Carefully invert to wash all phenol off
-Centrifuge at 10,000rpm for 5 min.
-Use vacuum to remove EtOH completely *Carefully remove all traces of EtOH including in the lid. (IMPORTANT)

Resuspend
-Add 50uL TE pH 8.0
-Vortex.

Yeast DNA Extraction Buffer

2%	Triton
1%	SDS
100mM	NaCl
10mM	Tris pH 8.0
1mM	EDTA

Make 1L of Yeast DNA Extraction Buffer

20mL	Triton-X-100
10mL	SDS (10% stock)
20mL	NaCl (5M stock)
10mL	Tris-Base (1M stock, pH 8.0)
2mL	EDTA (0.5M stock, pH 8.0)
938mL	dI H2O
--1L Total	

Add all ingredients to dI H2O and mix well. Filter sterilize into 100mL bottles.