Yeast DNA Miniprep Protocol

1. Grow 2-3 ml yeast culture in YPD or selective medium overnight to saturation.

2. Pour or pipet 1.5 mls of culture into microfuge tubes. Spin in microfuge for 10-20 seconds (longer spins will require more vortexing to resuspend the cells).

3. Aspirate or drain off as much of the supernatant as possible. Vortex tubes on the multi-tube vortexer for several minutes while preparing the zymolyase (lyticase) (20T) solution.

4. Add 250 µl zymolyase solution to the resuspended cells. Use a clean pipet tip to resuspend cells if necessary. Incubate 20-30 minutes at 37°. To check if zymolyase worked, place a 5 µl aliquot from several samples on a glass slide and add 2 µl of lysis solution to each spot. If the cell wall digestion is complete, the cells will lyse and the droplets will become clear and viscous. Lysis may observed under the microscope if desired.

5. Add 200 µl of lysis solution to each tube. Invert to mix.

6. Add 100 µl of KAc solution. Mix thoroughly by inverting. Do not allow samples to stand too long before mixing and do not vortex.

7. Spin tubes in microfuge for 10 minutes. The length of the spin may depend on the centrifuge. Prepare a new set of tubes.

8. Transfer supernatant to new tubes. If any of the precipitate is transferred, remove it with a pipet tip or spin again and transfer supernatant to a new tube.

9. Add 700 ul of isopropanol. Invert to mix and spin for 1-5 minutes.

10. Remove supernatant and add 300 ul of TE plus RNase at 30-60 µg/ml. Incubate at 37° for 30-60 mins or until the pellet is resuspended. RNase will aid in dissolving pellet.

11. Add 30 µl Na-Ac and 300 µl of isopropanol. Mix and then spin for 1-5 min. in microfuge.

12. Remove supernatant. Rinse with 95% ethanol (RT) and vacuum or air dry. Resuspend DNA in TE

Trouble shooting:

If the DNA yield is low, it may be that the zymolyase did not work. The spheroplasting step can be checked as described earlier. Multiple freezing and thawing may inactivate the enzyme; although, zymolyase may be frozen at least one time prior to use. Also, a lower DNA yield can be expected if the cells are grown to a lower cell density as when grown in selective medium instead of YPD.
If the DNA cannot be digested, or is being randomly digested by nucleases, it may be because some of the SDS/KAc precipitate was carried over into the new tube. It appears that this can be minimized by inverting the tubes to mix (no vortexing) and by extending the centrifugation if necessary.
Incomnplete enzyme reactions can be caused by salts carried over from the isopropanol precipitations. Try digesting in a larger volume or re-precipitating the DNA.

Solutions:

Zymolyase Solution	for 6 ml	per 1 ml
1.2 M Sorbitol	3.6 ml 2 M sorbitol	0.6 ml
10 mM tris pH 8	30 µl 2 M tris pH 8	5 µl
10 mM $CaCl_2$	30 µl 2 M $CaCl_2$	5 µl
1% BMe	60 µl BMe	10 µl
0.7 mg/ml Zymolyase	4 mg Zymolyase (20 units/mg)	0.7 mg
H_20	2.3 mls H_20	.38 ml
	6.0 mls total	

Lysis Solution	
50 mM tris pH8	1.25 ml 2 M tris pH8
50 mM EDTA	5 ml 0.5 M EDTA
1.2 % SDS	3 ml 20% SDS
	41 ml H_20
	50 mls total

KAc: 5 M potassium acetate neutralized to about pH 5.5 with glacial acetic acid. Mix 100 mls 5 M KAc (49 g CH_3COOK (anhydrous) in 100 mls total) and 172 mls 5 M glacial acetic acid diluted to 172 mls total.

Zymolyase stock solution: Make up a 10 mg/ml solution in zymolyase buffer and freeze at -20° in 350 ul aliquots. Melt in about 5.5 mls (depending on the number of samples you have) of zymolyase buffer prior to use. Caution: activity decreases over time.

The most recent version of this protocol may be found at the Haber Lab web page:
http://www.bio.brandeis.edu/haberlab/jehsite/protocol.html.