Precautions: Zymolase^{Ø}-100T has a low solubility. Use as a suspension. If it is necessary to make a sterile enzyme solution of more than 0.05%, make a 2% enzyme stock solution by dissolving Zymolase^{Ø} -100T in a buffer solution (pH 7.5) which contains 5% glucose. Pipette suspension leaving any material that has sedimented in the container. Nitrocellulose filters are not recommended. Dilute sterile enzyme suspension on to desired concentration.

Spheroplasting Protocol:

1. Centrifuge yeast culture at 5000 rpm (3000 xg) for 5 min at room temperature.
2. Harvest and record wet weight of cell pellet.
3. Suspend cells in 1.4 ml/wet g cells of TE Buffer (100 mM Tris [MP 8196231, pH 8.0 containing 100 mM EDTA [MP195173]).
4. QS to a final volume of 3.5 ml/wet g cells with DI Water.
5. Add 17.5 ul (1/200th of vol.)/wet g cells beta-mercaptoethanol (MP 806445) to remove the outer cell mannan layer.
6. Incubate at 30^{o}C with gentle shaking. Time required is 15 min for a log phase culture and 45 min for a stationary phase culture.
7. Centrifuge at 5000 rpm for 5 min at room temperature.
8. Resuspend in 4.0 ml S Buffer/wet g cells (1.0 M Sorbitol [MP 102938], 10 mM PIPES (MP 190257), pH 6.5).
9. Centrifuge at 5000 rpm for 5 min.
10. Resuspend in 4.0 ml S Buffer/wet g cells and add - 50 U Zymolyases /g wet weight yeast cells (250 ul if protocol in ADDENDUM used). It is best to initially optimize the amount of enzyme required for your system.
11. Incubate at 30^{o}C with gentle shaking for 30 min. Monitor the extent of spheroplasting as follows:

    Add 1 ul sample to 20 ul S Buffer. Spheroplasts should remain intact.
    Add 1 ul sample to 20 ul DI H_20. Spheroplasts should burst.
    Compare the two samples under a microscope.

12. When the cells appear to be $\geq$90% spheroplasts, usually 45 to 60 min, harvest by centrifuging at 5000 rpm for 5 min at 4^{o}C.
13. Resuspend spheroplasts in 2 ml/wet g cells S Buffer and centrifuge at 5000 rpm for 5 min. Repeat this step for 2 washes.
14. Spheroplast pellet may be stored frozen at -70^{o}C.

Lysis of Spheroplasts for Nuclei:

"Gentle" lysis of spheroplasts may be performed by suspending the pellet in 3 volumes of 18% Ficoll (MP 160003) in 10 mM PIPES Buffer, pH 6.5, with 0.5 mM $CaCl_2$ (MP 195088). The lower pH (6.5) buffer is used to slow down endogenous proteolytic activity.

Preparation of Yeast Genomic DNA

The following procedure is fast and suitable for the preparation of genomic DNA from small Yeast cultures.

1. To an overnight 10 ml yeast culture cell pellet add 280 ul TE Buffer (See Step 3, Spheroplasting Procedure), 300 ul DI water and 3 ul beta-mercaptoethanol (MP 806445).
2. Incubate at 30^{o}C for 45 min.
3. Centrifuge 2-3 sec at top speed in a microfuge, discard supernatant fluid and suspend in 500 ul S Buffer (See Step 8, Spheroplasting Procedure). Repeat spin and discard supernatant fluid.
4. Suspend cell pellet in 500 ul S Buffer containing 1 mg/ml Zymolyase 20T (MP 32-092-1) (or the enzyme concentration you have found optimal).
5. Incubate for 1 hr at 30^{o}C.
6. Repeat Step 3.
7. Suspend in 200 ul TE Buffer containing 0. 1 % SDS (MP 190522) and 2 ug Proteinase K (MP 809252).
8. Incubate 3 hr at 37^{o}C with occasional mixing.
9. Change to 65^{o}C incubator and incubate for 20 min.
10. Remove from incubator and cool to room temperature.
11. Extract with 200 ul of a 1 part: 1 part mixture Tris saturated phenol:chloroform. Vortex and spin down in a microfuge. Remove and save upper (aqueous) layer.
12. Extract supernatant fluid with 200 ul chloroform and repeat vortexing and microfuging step.
13. Add 500 ul 95% ethanol to the supernatant fluid. Precipitate 10 min at 20^{o}C.
14. Centrifuge at 15,000 xg for 20 min at 4^{o}C.
15. Air dry or dry in a Speed Vac and suspend in 200 ul TE Buffer containing 150 mM NaCl and 1 ug Ribonuclease A (MP 101076).
16. Incubate for 1 hr at 37^{o}C.
17. Repeat extraction Steps 11 and 12.
18. Add 2.5 volumes 95% ethanol. Precipitate for 10 min at 20^{o}C.
19. Resuspend in 30 ul DI water. Measure A_{260} nm of a 1:500 dilution. Calculate yield using Extinction Coefficient
20. Yield will be approximately 40-50 ug.

ADDENDUM

Preparation of Zymolyase 100T Solutions

The following may be used for the procedures described herein (this does not preclude other modes of preparation which may be equally adequate):

Prepare a solution of 200 units/ml lyophilized material dissolved in autoclaved S buffer (See Step 8, Spheroplasting Procedure).

This will be good for up to 2 weeks at 4^{o}C if kept free from microbial contamination.

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Note: This product may contain a preservative such as sodium azide, thimerosal or proclin. Please see lot specific chemical credential for preservative information.