

### GeneReleaser® YEAST Protocol

1. Estimate the number of organisms/ml from your culture (typically ~  $10^8$ /ml).
2. Prepare three (3) dilutions of the culture with isotonic saline so that the concentrations are  $10^3$ ,  $10^4$ ,  $10^5$  organisms/ml.
3. Place 20ul of resuspended GeneReleaser® in each of three standard 1.5ml centrifuge tubes.
4. Add 1ul of each dilution of the yeast cultures prepared in #2 above to one of the GeneReleaser® containing tubes. Label each tube.
5. Using a disposable pestle, grind the yeast cells in the presence of GeneReleaser® using 10 turns and thrusts of the pestle. Drain the pestle into the tube.
6. Discard the pestle.
7. Vortex the tubes and transfer the entire contents to a fresh 0.5ml amplification tube.
8. Overlay each tube with 35-50ul of mineral oil. Close the tubes.
9. Place tubes in a microwave safe rack.
10. Heat the tubes at maximum power for 7-10 minutes.
11. Transfer the tubes to a preheated thermocycler at 80°C for 5 minutes.
12. Add 80ul of 1.25 X master mix and begin amplification sequence immediately after adding master mix to the last tube.
13. It is very important that the first denaturation cycle be for 4 minutes at 94°C.
14. Following amplification analyze the products per standard protocols.
15. Evaluate the amplification results and for future amplifications employ the dilution of yeast cells giving the best results.
16. The preceding protocol is for 100ul reactions. Both GeneReleaser® and master mix volumes can be proportionately reduced for smaller reaction volume, i.e. 50ul and 25ul amplifications.

If you have further questions, please contact us at [support@bioventures.com](mailto:support@bioventures.com). You may also contact us via fax (1-877-286-0330) or through our toll-free phone number (1-877-852-7846).