Preparation of genomic DNA for PCR

1. Centrifuge 1 mL of a saturated overnight culture of yeast cells at 10,000 x g for 5 min at RT.
2. Aspirate supernatant, then resuspend cell pellet in 1 mL of sterile water.
3. Transfer volume to screw-cap eppi tube and centrifuge at 10,000 x g for 5 min at RT.
4. Aspirate supernatant, then resuspend cell pellet in 400 µL of Rescue Buffer:

- 47.9 mL H_2O, 0.5 mL 1M Tris pH 8.0, 0.5 mL 10% SDS, 0.1 mL 0.5M EDTA pH 8.0, 1 mL 5M NaCl

5. Add 200 µL of sterile glass beads and 200 µL of phenol-chloroform-isoamyl alcohol.
6. Vortex 15 min at RT.
7. Centrifuge 5 min at full speed for 5 min.
8. Transfer 300 µL of aqueous phase (top) to new eppi tube, withdrawing liquid from the surface.
9. Add 300 µL of chloroform and vortex briefly, then centrifuge 5 min at full speed for 5 min.
10. Transfer 200 µL of aqueous (top) phase to new eppi tube.
11. Add 20 µL of 3 M NaOAc pH 5.2; vortex briefly, then add 200 µL of 100% isopropanol and vortex briefly.
12. Incubate on ice (or at -20˚C or at -80˚C) for ≥ 20 min.
13. Centrifuge 10 min at full speed at 4˚C.
14. Aspirate supernatant, then add 500 µL of 70% ethanol; vortex to dislodge pellet from bottom of tube.
15. Centrifuge 10 min at full speed at RT.
16. Aspirate supernatant., then dry pellet completely by speed-vac for 15 min (heat setting “high”).
17. Add 100 µL of sterile water, then allow DNA to resuspend for ≥30 min).
18. Vortex thoroughly, then pulse-spin.
19. Use 1 µL of gDNA as template for PCR.