Optimized protocol for bacterial uptake into yeast spheroplasts and regeneration handling

1. Grow cells in liquid culture to a mid-logarithmic stage (oD 0.5 - 0.6), aliquot cells into 50mL falcons
2. Centrifuge the cells at 5000g for 5 min at RT
3. Resuspend each cell pellet in sterile water at a concentration of OD 10 (=2.5mL)
4. Centrifuge at 5000g for 5 min at RT
5. Resuspend each cell pellet in "softening medium" at a concentration of OD 10 (=2.5mL) softening medium: 100 mM Hepes-KOH, pH 9.4, 10 mM dithiothreitol (DTT) (*always prepare fresh softening medium*Hepes-KOH may be substituted with another buffer, e.g., Tris pH 9.4 or Pipes-KOH pH 9.4)
6. Incubate for 15 min at RT
7. Centrifuge at 5000g for 5 min at RT
8. Resuspend each cell pellet in "spheroplasting medium" at a concentration of OD 5 (=5mL) spheroplasting medium: 1X YNB, 2% glucose, 1X amino acids, 50 mM Hepes-KOH, pH 7.2, 1 M sorbitol (spheroplasting medium can be stored at RT indefinitely)
9. Add 5U of Zymolyase (if you are using another chassis then *S. cerevisiae*, you might have to increase the concentration or switch to Novozyme)
10. Incubate for 60 min at 30°C (or an otherwise appropriate temperature; for instance, if the strain has a temperature-sensitive growth defect, incubate at the permissive temperature)
11. Centrifuge at 2500g for 3-4 min
12. Wash spheroplasts in spheroplasting medium at concentration of oD 10 (=2.5mL)
13. Repeat washing step
14. Prepare samples containing 500µL of each spheroplasts and bacteria (OD of approximately 20 for *E. coli* stock. In general, a ratio of 1:100 between yeast and bacteria should be used)
15. Let the mix incubate for 15 min at 30°C and 110rpm
16. Spin down cells at 2500g for 5 min
17. Discard supernatant and resuspend pellet directly in 500µL 25% PEG with 50mM $CaCl_2$ (molecular weight of PEG should be corresponding to the bacterial size)
18. Spin down cells at 2500g for 5 min
19. Discard supernatant and resuspend pellet directly in 250µL 25% PEG with 50mM $CaCl_2$ (molecular weight of PEG should be corresponding to the bacterial size) and 250µL regeneration medium (1M sorbitol in YPD, pH adjusted to 4.1 to make environment *E. coli* unfriendly)
20. Spin down cells at 2500g for 5 min
21. Discard supernatant and resuspend pellet directly in 100µl 25% PEG with 50mM $CaCl_2$ (molecular weight of PEG should be corresponding to the bacterial size) and 400µL

regeneration medium (1M sorbitol in YPD, pH adjusted to 4.1 to make environment *E. coli* unfriendly)

22. Spin down cells at 2500g for 5 min
23. Resuspend in 2 mL of regeneration medium
24. Regeneration of the cells for at least 2h in 30°C with 110rpm to ensure cell wall regeneration. Microscopy can be done earlier when cells are fixated on a 1.5% agar pad containing 1M sorbitol

Based on the work of:

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Yoshida, Naoto, and Misa Sato. "Plasmid uptake by bacteria: a comparison of methods and efficiencies." *Applied microbiology and biotechnology* 83.5 (2009): 791-798.

Guerra-Tschuschke, I., I. Martin, and M. T. Gonzalez. "Polyethylene glycol-induced internalization of bacteria into fungal protoplasts: electron microscopic study and optimization of experimental conditions." *Applied and environmental microbiology* 57.5 (1991): 1516-1522.