Yeast Plasmid Prep

[Note, this is for extracting created plasmids out of yeast after in vivo ligation/assembly has been done—see IVL protocol]

1. After transformation of the DNA fragments into yeast and recovery on selective plates for 2 days, scrape yeast off the plate (avoid agar) and use roughly ¼ the surface area of the plate assuming equal and full coverage (if single colonies are spread across, then troubleshoot. The procedure should yield close to an entire lawn of yeast; many thousands of colonies).
2. Resuspend the yeast into 500 uL of “Genomic Solution #1” (1M sorbitol, 0.1 M Na₂EDTA pH 7.5); slightly higher pH also works. Mix by vortexing 10 seconds.
3. Add 5 uL of stock Zymolyase solution (100T, stored at 4°C; 25 mg/mL in 50% glycerol).
4. Place on rotator for 1-1.5 hours at 37°C to activate the Zymolyase enzyme.
5. Spin samples at 11,000 rpm for 1 minute.
6. Pipette off the solution and keep the pellet.
[Note: From here on is a “modified” plasmid miniprep protocol]
7. Add 250 uL of “Resuspension Buffer #1” and mix by pipetting up and down.
8. Add 1 “scoop” of glass beads (0.5mm diameter glass beads) roughly 100-200 uL volume to each tube.
9. Vortex for 4.5-5 minutes at maximum.
10. Add Lysis buffer (from miniprep kit) 250 uL and mix by inverting tube gently 6-10 times.
11. Incubate at room temperature for 4.5-5 minutes.
12. Add Neutralization buffer (from miniprep kit) 350 uL and mix by inversion 10 times.
13. Immediately spin at 13,200 rpms (max speed) for 5 minutes.
14. Pour solution into a fresh Eppendorf tube.
15. Spin for 1 minute at max (to gather any additional precipitate).
16. Pour solution into miniprep spin column.
17. Spin for 1 minute at max speed.
18. Pour off flow-through.
19. Add 500 uL of Ethanol wash buffer (from miniprep kit).
20. Spin for 1 minute at max speed.
21. Pour off flow-through.
22. Add 500 uL of Ethanol wash buffer a second time.
23. Spin for 1 minute at max speed.
24. Pour off flow-through.
25. Spin the empty spin column at max speed for 1 minute.
26. Remove the bottom portion of the tube and keep the column. Place the spin column inside a fresh Eppendorf tube (labeled).
27. Add 30 uL of elution buffer (miniprep kit) directly to the column filter (do not touch the filter) and incubate for 1 minute at room temperature.
28. Spin at max speed for 2 minutes.
29. Discard the spin column and keep the Eppendorf tube with the eluted DNA. Ensure the cap was not damaged or broken.
30. Can store 1-2 days at 4°C fridge. Or, immediately transform into competent E. coli. Use 7-10 uL of this yeast plasmid DNA.
[Note: Do NOT use this DNA for PCR or digest. This DNA is a mixture of many possible IVL products, uncut vector, other assemblies, etc. Immediately transformation into bacterial separates this mixture into single clonal isolates in each bacterial colony.]