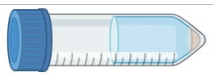
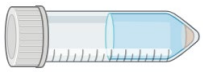
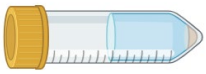
Cell collection

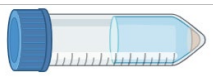
Materials Required

Cold PBS	
2 pre-chilled 50ml Falcon tubes	
2 re-usable 50ml Falcon tubes labeled BALANCE	

1. Label your 2 sample 50ml Falcon tubes (white top) with the sample IDs
2. Add _____mLs (from above) of your cell culture to a pre-chilled 50-ml (**white** top) Falcon tube
3. Add _____mLs (from above) of water to a 50-ml Falcon Tube (yellow) labeled BALANCE
4. Centrifuge at 3,000g for 5 minutes at 4°C.
5. Remove as much supernatant as possible and dispose of it into a container with 10% bleach
6. Resuspend the pellet in 10 mL ice-cold PBS (Blue 50ml Falcon Tube)
7. Centrifuge at 3,000g for 5 minutes at 4°C.
8. Remove as much supernatant as possible and dispose of it into a container with 10% bleach

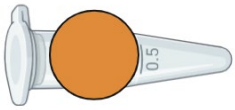
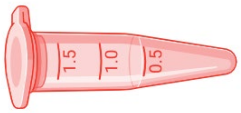
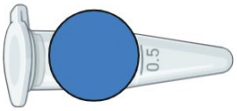
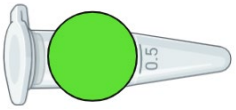
STEP 3 - Spheroplast (Cells without a cell wall) collection

Materials Required

Cold Spheroplast Buffer: 1M sorbitol, 1X PBS pH 7.4, 0.1 M EDTA	
Zymolyase (5U/µl)	
Cold PBS	
30°C incubator	
1.5 ml microcentrifuge tube	

1. Label your 1.5 ml tubes with your sample IDs
2. Add 500 µL of Spheroplast Buffer (Red 15ml Falcon Tube) to your yeast pellet
3. Gently resuspend the pellet
4. Transfer the resuspended pellet to 1.5ml microcentrifuge tube
5. Add **10 µl Zymolyase (5U/µ)] (White** dot 1.5ml tube)
6. Pipette up and down to mix with a wide-bore tip to mix.
7. Incubate at 30°C for 20 minutes.
8. Invert and gently flick tubes every 5 minutes to keep cells suspended in solution.
9. Gently pellet spheroplasts by centrifugation for 3 minutes at 300 x g
10. Remove the supernatant.
11. Using a 1000 µl wide-bore pipette tip, gently resuspend the spheroplast pellet in 200 µl of cold PBS (Blue 50ml Falcon Tube)

STEP 4 - Quick-DNA™ Miniprep Plus Kit

Resuspended Proteinase K	
BioFluid & Cell Buffer (Red)	
Genomic Binding Buffer	
DNA Pre-Wash Buffer	
g-DNA Wash Buffer	
DNA Elution Buffer	
1x Zymo-Spin column per sample	
2 Collection Tubes column per sample	
1 microcentrifuge column per sample	
55°C incubator	

1. Add 200 µl BioFluid & Cell Buffer (Red) (red tube) to the sample
2. Add 20 µl Proteinase K (orange dot tube) to the sample
3. Vortex for 10-15 seconds
4. Incubate at 55°C for 10 minutes
5. Add 400 µl of Genomic Binding Buffer (blue dot tube)

6. Place a Zymo-Spin Column in a collection tube
7. Transfer 800 µl of your mixture to the column
8. Centrifuge at 12,000 x g for 1 minute
9. Discard the flow-through and collection tube
10. Place spin column in a new collection tube

11. Add 400 µl of DNA Pre-Wash Buffer (green dot tube) to the spin column
12. Centrifuge at 12,000 x g for 1 minute
13. Empty the collection tube

14. Add 700 µl g-DNA Wash Buffer (orange cap 15ml tube) to the spin column.
15. Centrifuge at 12,000 x g for 1 minute
16. Empty the collection tube

17. Add 200 µl g-DNA Wash Buffer (orange cap 15ml tube) to the spin column
18. Centrifuge at 12,000 x g for 1 minute
19. Discard the flow-through and collection tube

20. Transfer the spin column to a clean microcentrifuge tube
21. Add 50 µl DNA Elution Buffer (yellow tube)
22. Incubate for 5 minutes at room temperature
23. Centrifuge at 12,000 x g for 1 minute
24. Your DNA will now be at the bottom of your microcentrifuge tube