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Yeast gDNA isolation

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is Sparrow[1]

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is Sparrow
UIUC

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Abstract

This protocol is based on https://cshprotocols.cshlp.org/content/2020/10/pdb.prot098152.full
doi:10.1101/pdb.prot098152

The protocol details how to perform a small scale yeast gDNA isolation including a proteinase K incubation step to visualize linear cytoplasmic protein primed plasmids.

This is used in combination with the system OrthoRep which relies on error prone replication of linear cytoplasmic plasmids.
Rix, G., Watkins-Dulaney, E.J., Almhjell, P.J. *et al.* Scalable continuous evolution for the generation of diverse enzyme variants encompassing promiscuous activities. *Nat Commun* **11**, 5644 (2020). https://doi.org/10.1038/s41467-020-19539-6

Materials

Sorbitol buffer:

1M Sorbitol
0.1M Na2EDTA

Zymolyase solution:
Zymolyase 20T (12.5 mg/mL) or Zymolyase 100T (2.5 mg/mL) in **Sorbitol buffer**

Yeast resuspension buffer:
50 mM Tris-HCl pH 7.5
20 mM Na2EDTA

TE solution:
10mM Tris pH 7.4
1mM Na_2EDTA

DNA extraction

2h 12m

1 Start a 10 mL yeast culture and grow to saturation overnight or over 2 days

Note: for p1_wt (but not p1_rec) containing strains, p1 can be lost if grown in YPD for prolonged periods of time (based on my estimations, 2 or 3 1:1000 passages). Be mindful of this.

2 Aliquot 5 mL of culture to a 15 mL falcon tube and centrifuge 3000 x g, 00:05:00 5m

3 Pour off supernatant and pipette out the rest.

4 Resuspend the cells in 1 mL [M] 0.9 % w/v NaCl solution

5 Centrifuge e 3000 x g, 00:02:00 , discard supernatant 2m

6 Resuspend in 500 µL **sorbitol buffer**

Sorbitol buffer:

1M Sorbitol
0.1M Na2EDTA

7 Add 20 µL of **zymolyase solution**

Zymolyase solution:
Zymolyase 20T (12.5 mg/mL) or Zymolyase 100T (2.5 mg/mL) in **Sorbitol buffer**

8 Incubate 37 °C 01:00:00 with gentle shaking 1h

9 Centrifuge e 3000 x g, 00:05:00 , discard supernatant 5m

10 Resuspend the cells in 500 µL **yeast resuspension buffer**

Yeast resuspension buffer:
50 mM Tris-HCl pH 7.5
20 mM Na2EDTA

11 Add 50 µL [M] 10 % w/v SDS and shake vigorously

12 Add 5 µL of [M] 20 mg/mL Proteinase K

Note: NEB proteinase K solution comes at this concentration

13 Incubate at 65 °C for 00:30:00 30m

14 Add 200 µL of 5M potassium acetate

15 Incubate On ice 00:30:00 30m

DNA isolation 1h 15m

16 Pellet by centrifugation at max speed for 00:05:00 5m

16.1 **Note:**

At this step, a "short" protocol can be done - it will not produce sufficient DNA for visualization of p1, but is sufficient for PCR amplification from p1.

16.2 **Recover** supernatant and place into a miniprep kit silica column. Wash as per manufacturer instructions or with homemade Wash 1 / Wash 2 buffers, and elute in water or EB.

Note: this short protocol is not suitable for electrophoresis visualization of gDNA and should only be used for PCR amplification from the eluted DNA.

17 **Recover supernatant in a fresh microcentrifuge tube. Aim to get 500<x<700 uL of sample, and avoid getting debris.**

18 Add pure isopropanol equal to the volume of the sample (~ 765 µL) and mix gently

19 Incubate for 00:05:00 Room temperature 5m

Note: do not allow more than 5 minutes to pass

20 Centrifuge at max speed for 00:10:00 10m

21 **Discard supernatant**

22 Air dry the pellet 00:10:00 10m

23 Add 300 µL of **TE solution** and add 0.75 µL of RNAse A at [M] 20 mg/mL

Note: NEB RNAse A comes in this concentration

TE solution:
10mM Tris pH 7.4
1mM Na_2EDTA

24 Incubate 37 °C 00:30:00 30m

25 Add 30 µL 3M sodium acetate and mix by inversion

26 Add 200 µL isopropanol and mix, then centrifuge at max speed for 00:00:20 20s

27 **Discard supernatant**

28 Allow pellet to air dry 00:10:00 10m

29 Resuspend in 50-150 µL **TE solution**

TE solution:
10mM Tris pH 7.4
1mM Na_2EDTA

Pellet might be hard to resuspend especially if there is a lot of DNA. Be gentle, but persistent.

30 Quantify using NanoDrop. Remember to blank using TE instead of water.

DNA is clean enough for PCR or sequencing.

Gel analysis

31 Optional step for p1 analysis

Cast a 0.75% agarose 1X TAE gel

Load ~ 1 µg gDNA

Run at 85 V for 100+ minutes

Look for the following bands and no others

p2_wt: 13.5 kb

p1_wt (size will be variable depending on landing pad used - for GA-Y319 = 8.9 kb; for GR-Y718 = 5.6 kb)

p1_rec (size will be variable = your construct size + 4.6 kb)