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Yeast gDNA isolation

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Yeast Protocols, Tools, an...

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We use this protocol and it's working

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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 Na₂EDTA




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  0.9 % w/v NaCl solution

II

- 5 Centrifuge  3000 x g, 00:02:00 , discard supernatant

2m

- 6 Resuspend in  500 μ L **sorbitol buffer**

Sorbitol buffer:

1M Sorbitol

0.1M Na₂EDTA

- 7 Add  20 μ L 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  3000 x g, 00:05:00 , discard supernatant



5m

- 10 Resuspend the cells in  500 μ L **yeast resuspension buffer**

**Yeast resuspension buffer:**


50 mM Tris-HCl pH 7.5

20 mM Na₂EDTA

11 Add  50 μ L  10 % w/v SDS and shake vigorously



12 Add  5 μ L of  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 μ L 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 500-700 μ L 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 Na₂EDTA

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 μ L **TE solution**

TE solution:

10mM Tris pH 7.4

1mM Na₂EDTA

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 μ g 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)