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Plasmid Extract from Budding Yeast (*Saccharomyces cerevisiae*)

Gal Haimovich*

Dept. of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel
*For correspondence: gal.haimovich@weizmann.ac.il

[Abstract] Plasmids are widely used tools in yeast research. In many cases, plasmid libraries are used in genetic screens or in yeast two hybrid screens. In such cases, it is necessary to extract plasmids carrying unknown genetic elements from positive clones that were isolated in the screen.

This is a simple protocol to extract plasmid DNA from budding yeast cultures (Robzyk and Kassir, 1992). The amount produced is small, but it is sufficient for PCR or for transformation into bacteria, where the plasmid can be amplified to provide sufficient amounts for downstream uses (*e.g.*, restriction enzyme analysis, sequencing).

Keywords: Budding yeast, Plasmid extraction, Miniprep

Materials and Reagents

1. Pipette tips (1,000 µl, 200 µl, 20 µl)
2. 15 ml test-tube or equivalent
3. 1.7 ml tubes
4. 0.5 ml PCR tubes
5. Kimwipes (*e.g.*, KCWW, Kimberly-Clark, catalog number: 34120)
6. Isolated yeast clone carrying the required plasmid
7. Difco Yeast nitrogen base without amino acid (*e.g.*, BD, catalog number: 291940)
8. Yeast Synthetic Drop-out medium supplements (Sigma-Aldrich)
   Note: Choose drop-out supplement based on the required plasmid selection in yeast (e.g., Sigma-Aldrich, catalog number: Y1501 for drop-out without Uracil).
9. D-Glucose (*e.g.*, Sigma-Aldrich)
10. Sucrose (*e.g.*, Sigma-Aldrich)
11. Tris base (*e.g.*, Sigma-Aldrich)
12. EDTA (*e.g.*, Sigma-Aldrich)
13. Triton X-100
14. 100% ethanol
15. 70% ethanol
16. Ammonium acetate
17. HCl 1 N
18. NaOH 10 N
19. Nuclease-free water

20. Double distilled water
21. Ice
22. 0.5 mm glass beads (*e.g.*, MP Biomedicals, catalog number: 116540449-1kg)
23. Synthetic selective medium (see Recipes)
24. STET buffer (see Recipes)
25. 7.5 M ammonium acetate (see Recipes)

Equipment

1. 1,000 µl, 200 µl, 20 µl pipettes
2. Yeast incubator/shaker suitable for 15 ml tubes or equivalent
3. Refrigerated centrifuge suitable for 1.7 ml tubes
4. Vacuum aspirator (optional)
5. Heating block set to 100 °C
6. Vortex mixer (optional: with multi-tube head) (*e.g.,* VWR, catalog number: 10153-836)
7. Oven (> 160 °C)
8. -20 °C freezer
9. Glass beaker (use appropriate size to amount of glass beads; a 1 L beaker is sufficient for 1 kg beads)

Procedure

1. Inoculate the yeast clone into 5 ml selective media in a 15 ml tube.
2. Grow the culture overnight (14-18 h) in a suitable incubator/shaker.
   Note: Most budding yeast strains are cultured at 30 °C. However, some strains are temperature sensitive and require lower temperatures (e.g., 24-26 °C).
3. Fill a 1.7 ml tube with 1.4 ml of the yeast culture, centrifuge for 30 sec at top speed; discard supernatant by pipette (optional: use vacuum aspirator). Repeat twice to harvest 4.2 ml in total.
4. Resuspend the cell pellet in 100 µl STET buffer (vortex well).
5. Add ~200 µl of acid-washed 0.5 mm glass beads.
   Note: The glass beads (0.5 mm) should be acid-washed as follows: Put glass beads in a glass beaker and move to a chemical hood. Add 1 N HCl at a volume that will cover the beads. Let stand for 1 h. Pour the HCl into an appropriate chemical waste container according to institutional regulations. Wash multiple times with a large volume of double-distilled sterile water (for 1 kg of glass beads use at least 7-8 x 1 L of water). Use a pipette to remove as much excess liquid as possible. Cover the glass beaker with aluminum foil and bake the glass beads in an oven (> 160 °C) overnight. Use 0.5 ml PCR tube (or similar) to scoop glass beads (to about half-full) and pour into the 1.7 ml tubes with the yeast (see Video 1).

Video 1. Scooping acid-washed glass beads with 0.5 ml PCR tube

6. Vortex at max speed for 5 min.
   Optional: For multiple samples, use vortex with multi-tube head.
7. Add 100 µl STET. Briefly vortex again.
8. Boil (100 °C) for 3 min in a heating block.
9. Place on ice for 1 min.
10. Spin in a centrifuge for 10 min at 20,000 *x g* (4 °C).
11. Transfer 100 µl of supernatant to a new 1.7 ml tube.
    Note: Do not take more, to reduce the chance of picking up debris that will affect yield.
12. Add 50 µl of 7.5 M ammonium acetate (to a final conc. of 2.5 M) to the tube. Vortex well.
13. Incubate in a -20 °C freezer for 1 h.
14. Centrifuge for 10 min at 20,000 *x g* (4 °C). A pellet of debris is sometimes visible.
15. Transfer 100 µl of supernatant to a new 1.7 ml tube
    Note: Do not take more, to reduce the chance of picking up debris that will affect yield.
16. Add 200 µl of ice-cold ethanol 100% and vortex well!
17. Centrifuge for 10 min at 20,000 *x g* (4 °C).
18. Aspirate supernatant by vacuum. For extra care, use the 200 µl pipette instead of vacuum aspirator. Make sure you see the pellet clearly while aspirating (it is quite small and visible as a white dot)–this is the plasmid DNA.
19. Carefully add 800 µl of ice-cold 70% ethanol. If the pellet is still visible, aspirate supernatant as before. Otherwise, centrifuge for 5 min and then aspirate.
20. Place the tube upside-down on kimwipe paper to drain. Then leave the tube open (right side up) on the bench to dry (5-10 min).
21. Resuspend the pellet in 20 µl of nuclease-free water. Vortex gently for 10 min.
22. The expected concentration is typically below the detection level of common lab equipment (*e.g.*, Nanodrop). Hence it is not necessary to measure the concentration.

23. The plasmid DNA can be used directly for PCR or be transformed into competent bacteria (the plasmid needs to contain a bacterial origin of replication and antibiotic resistance gene for selection).
24. Use 10 µl of eluted plasmid to transform competent bacteria. For negative control, use 10 µl water for transformation.
25. If successful, single colonies should appear based on the antibiotic selection of the plasmid, and no colonies should grow with the negative control.

Recipes

1. Synthetic selective medium (1 L)
   6.67 g Yeast nitrogen base w/o amino acids
   Amino acid drop-out mix (use appropriate amount according to instructions–each dropout is different)
   20 g glucose
   Add double distilled water to 1 L
   Adjust pH to 5.8 with 10 N NaOH
   Autoclave
2. STET buffer
   Prepare stock solutions:
   25% sucrose (w/v)
   1 M Tris-HCl pH 8
   0.5 M EDTA pH 8
   10% Triton X-100 (v/v)

   For 50 ml STET buffer:

Final concentration	**vol. of stock solution**
8% Sucrose	16 ml of 25%
50 mM Tris pH 8	2.5 ml of 1 M Tris pH 8
50 mM EDTA pH 8	5 ml of 0.5 ml EDTA pH 8
5% Triton X-100	25 ml of 10%
Nuclease-free water	1.5 ml

3. 7.5 M ammonium acetate
   Dissolve 5.78 g of ammonium acetate into nuclease-free water to get 10 ml final volume

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interests. This protocol was adapted from Robzyk and Kassir (1992).

References

1. Robzyk, K. and Kassir, Y. (1992). A simple and highly efficient procedure for rescuing autonomous plasmids from yeast. *Nucleic Acids Res* 20(14): 3790.

