Protocol

Extraction of Chromosomal DNA from *Schizosaccharomyces pombe*

Johanne M. Murray,¹ Adam T. Watson,¹ and Antony M. Carr¹

Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Falmer, Brighton,
E. Sussex BN1 9RQ, United Kingdom

Extraction of DNA from *Schizosaccharomyces pombe* cells is required for various uses, including templating polymerase chain reactions (PCRs), Southern blotting, library construction, and high-throughput sequencing. To purify high-quality DNA, the cell wall is removed by digestion with Zymolyase or Lyticase and the resulting spheroplasts lysed using sodium dodecyl sulfate (SDS). Cell debris, SDS, and SDS–protein complexes are subsequently precipitated by the addition of potassium acetate and removed by centrifugation. Finally, DNA is precipitated using isopropanol. At this stage, purity is usually sufficient for PCR. However, for more sensitive procedures, such as restriction enzyme digestion, additional purification steps, including proteinase K digestion and phenol–chloroform extraction, are recommended. All of these steps are described in detail here.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.

Reagents

CSE buffer <R>

Ethanol (75%)

Isopropanol

Phenol:chloroform:isoamyl alcohol (50:49:1)

Potassium acetate (5 M)

Proteinase K (10 mg/mL)

RNase A (10 mg/mL)

Schizosaccharomyces pombe strain of interest and liquid growth medium appropriate for that strain

For general advice concerning fission yeast growth media and culture conditions, see Introduction: Growth and the Environment of Schizosaccharomyces pombe (Petersen and Russell 2016).

Sodium acetate (3 M, pH 5.2)

Sodium dodecyl sulfate (SDS) (10%)

TE (5×) <R>

¹Correspondence: a.m.carr@sussex.ac.uk; j.m.murray@sussex.ac.uk; a.t.watson@sussex.ac.uk

From the Fission Yeast collection, edited by Iain M. Hagan, Antony M. Carr, Agnes Grallert, and Paul Nurse.

© 2016 Cold Spring Harbor Laboratory Press

Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot090985

Water (double-distilled, sterile)
Zymolyase 20T or Lyticase

Equipment

Centrifuge for 50-mL glass tubes containing 10-mL cultures
Glass flasks (fluted, 1-L)
Glass tubes (50-mL, sterile) with loose caps
Hemocytometer
Ice bucket
Incubator (37°C)
Microcentrifuge
Microcentrifuge tubes
Phase-contrast microscope with 40× objective
Rotating wheel (for glass tubes)
Water bath (30°C–55°C)

METHOD

This protocol is based on the approach first described by Moreno et al. (1991) for digesting the cell wall with Zymolyase or Lyticase and lysing the resulting spheroplasts using SDS. It presents the number of cells and reagent volumes required to purify ~1 µg of DNA (commonly termed a “mini-prep”), which is ample for polymerase chain reaction (PCR) or one lane on a Southern blot. Cell numbers and reagent volumes should be scaled for larger preparations.

Extraction of DNA

1. Grow cells in 10 mL of appropriate growth medium in 50-mL glass tubes at 30°C, rotating to aerate, to a density of 1×10^7 cells/mL.

Determine cell density using a hemocytometer and microscope. If larger quantities of DNA are required, cells can be grown in bulk using large flasks and a shaking incubator and processed by scaling up the volumes given below and using larger tubes.

2. Pellet 5×10^7 cells by centrifugation, resuspend in 1 mL of ddH₂O, and transfer to a microcentrifuge tube.
3. Centrifuge at 17,000g for 1 min, and resuspend pellet in 1 mL of CSE buffer containing 1 mg/mL Zymolyase 20T or 1 mg/mL Lyticase.
4. Incubate for 15 min at 37°C.
5. Check digestion of cell walls by observation of a 5-µL sample containing 5 µL of 10% SDS under a phase-contrast microscope.

Cells lose their characteristic refringence and become black when the cell wall is digested. If digestion is incomplete, incubate for an additional 15 min at 37°C and repeat Step 5.

6. Centrifuge at 17,000g for 1 min.
7. Resuspend pellet in 450 µL of 5× TE.
8. Add 50 µL of 10% SDS, mix by inversion, and incubate for 5 min at room temperature.
9. Add 150 µL of 5 M potassium acetate, mix thoroughly by inversion, and incubate on ice for 10 min.
10. Centrifuge at 17,000g for 10 min. Transfer 600 µL of supernatant to 600 µL of isopropanol in a fresh microcentrifuge tube, and mix.
11. Centrifuge at 17,000g for 10 min, and wash DNA pellet with 75% ethanol.
12. Air-dry pellet.

Proceed to Step 13 if DNA is to be used for PCR. Proceed to Steps 14–20 if DNA is to be used for Southern blot analysis.

Preparation of DNA for PCR

13. Resuspend pellet in 200 μ L of ddH₂O containing 5 μ L of 10 mg/mL RNase, and incubate for 15 min at 37°C.

Use 1 μ L per PCR.

Purification of DNA for Southern Blotting

14. Resuspend pellet in 500 μ L of 5 $\times$ TE containing 5 μ L of 10 mg/mL RNase, and incubate for 15 min at 37°C.
15. Add 4 μ L of 10% SDS and 20 μ L of 10 mg/mL proteinase K, and incubate either for 60 min at 55°C or overnight at 30°C.
16. Add 500 μ L of phenol:chloroform:isoamyl alcohol (50:49:1) and mix gently. Centrifuge at 17,000g for 5 min.
17. Transfer the upper aqueous phase to a fresh microcentrifuge tube. Add an additional 500 μ L of phenol:chloroform:isoamyl alcohol (50:49:1), and mix gently. Centrifuge at 17,000g for 5 min.
18. Transfer the upper aqueous phase to a fresh microcentrifuge tube. Add 500 μ L of isopropanol and 25 μ L of 3 M sodium acetate (pH 5.2), and incubate on ice for 10 min.
19. Centrifuge at 17,000g for 10 min, and wash the DNA pellet with 75% ethanol.
20. Air-dry the pellet, and resuspend in an appropriate volume of 1 $\times$ TE for restriction enzyme digestion.

RELATED INFORMATION

For alternative approaches for rapid preparation of DNA from *S. pombe* for PCR, see Protocol: Colony Polymerase Chain Reaction with *Schizosaccharomyces pombe* (Murray et al. 2016).

RECIPES

Citrate-Phosphate Buffer (50 mM, pH 5.6)

Na ₂ HPO ₄ (anhydrous)	7.10 g
Citric acid	11.50 g

Dissolve in 1 L of distilled water. Sterilize by autoclaving. (The final solution contains 50 mM Na₂HPO₄)

CSE Buffer

Sorbitol	1.2 M
EDTA (pH 8.0)	40 mM

Prepare the sorbitol and EDTA in citrate-phosphate buffer (50 mM, pH 5.6) <R>. Filter-sterilize.

TE Buffer (5 $\times$)

Tris-Cl (pH 7.5)	50 mM
EDTA (pH 8.0)	5 mM

Sterilize the buffer by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle. Store at room temperature.

REFERENCES

- Moreno S, Klar A, Nurse P. 1991. Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol* **194**: 795–823.
- Murray JM, Watson AT, Carr AM. 2016. Colony polymerase chain reaction with *Schizosaccharomyces pombe*. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot090993.
- Petersen J, Russell P. 2016. Growth and the environment of *Schizosaccharomyces pombe*. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top079764.

Cold Spring Harbor Protocols

Extraction of Chromosomal DNA from *Schizosaccharomyces pombe*

Johanne M. Murray, Adam T. Watson and Antony M. Carr

Cold Spring Harb Protoc; doi: 10.1101/pdb.prot090985

Email Alerting Service

Receive free email alerts when new articles cite this article - [click here](#).

Subject Categories

Browse articles on similar topics from *Cold Spring Harbor Protocols*.

[DNA Purification](#) (160 articles)
[Molecular Biology, general](#) (1293 articles)
[Yeast](#) (288 articles)

To subscribe to *Cold Spring Harbor Protocols* go to:
<http://cshprotocols.cshlp.org/subscriptions>