Protocol

DNA Preparation from *Schizosaccharomyces pombe*

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This protocol includes two methods for genomic DNA preparation from *Schizosaccharomyces pombe*. The first is a quick method for preparation of DNA suitable for polymerase chain reaction (PCR) genotyping. The second, longer method yields high-quality DNA that can be used for amplification of targeting cassettes.

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.

RECIPE: 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

Reagents for long DNA preparation

Buffer EB (QIAGEN miniprep kit)

Ethanol (96% and 70%)

Phenol:chloroform:isoamyl alcohol (P/C/I) (25:24:1 [pH 8.0])

Sodium acetate

Prepare 600 mM sodium acetate (NaOAc) in Buffer P1 (with RNase A) (QIAGEN miniprep kit).

YDEB <R>

Reagents for quick DNA preparation

NaOH (25 mM)

Schizosaccharomyces pombe cells

For long DNA preparation, we recommend starting with 1.5 mL of mid-log phase culture per sample.

Equipment

Equipment for long DNA preparation

Glass beads (0.5-mm [Biospec or similar])

Incubator at 37°C

Microcentrifuge

SpeedVac

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Tubes (screw-cap)

It is important to use screw-cap tubes for samples containing P/C/I.

Equipment for quick DNA preparation

Tabletop centrifuge with microplate plate adaptors

Thermocycler

Thin-wall polymerase chain reaction (PCR) tubes or plates

Vortex

METHOD

Quick DNA Preparation

1. In a thin-wall PCR tube or plate, resuspend a small amount of cells in 50 μ L of 25 mM NaOH. Incubate for 25 min at 100°C in a thermocycler.
2. Vortex the sample to mix and then centrifuge to pellet the cell debris.
3. Use 1–1.5 μ L of the supernatant per 25 μ L PCR for genotyping.

Long DNA Preparation

4. In a screw-cap tube, centrifuge 1.5 mL of mid-log phase culture at 12,000g for 10 sec to pellet the cells.
5. Add 200 μ L of YDEB and ~400 μ L of glass beads to each sample.
6. Add 200 μ L of P/C/I to each sample. Vortex at the maximum setting for 2–3 min to mix.
7. Centrifuge for 3 min at 12,000g.
8. Transfer 150 μ L of the upper phase to a new screw-cap tube.
9. Add 150 μ L of 600 mM NaOAc in Buffer P1 (with RNase A) to each sample.
10. Incubate for 10 min at 37°C.
11. Add 800 μ L of 96% ethanol to each sample. Vortex to mix.
12. Centrifuge for 3 min at 12,000g.
13. Discard the supernatant and wash the pellet with 500 μ L of 70% ethanol.
14. Centrifuge for 1 min at 12,000g.
15. Discard the supernatant and dry the pellet in a SpeedVac.
16. Dissolve the pellet in 50 μ L of Buffer EB.

RELATED INFORMATION

For colony PCR procedures, see Protocol: Colony Polymerase Chain Reaction with *Schizosaccharomyces pombe* (Murray et al. 2016).

RECIPE

YDEB

10 mM Tris (pH 8)

100 mM NaCl

1 mM EDTA

2% Triton X-100

1% sodium dodecyl sulfate (SDS)

Store at room temperature (it is stable for at least 6 mo).

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REFERENCES

Murray JM, Watson AT, Carr AM. 2016. Colony polymerase chain reaction with *Schizosaccharomyces pombe*. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot090993.

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