Sussex Research Online

[Protocol] Colony polymerase chain reaction with Schizosaccharomyces pombe

Article (Accepted Version)

Murray, Johanne M, Watson, Adam T and Carr, Antony M (2016) [Protocol] Colony polymerase chain reaction with Schizosaccharomyces pombe. Cold Spring Harbor Protocols, 2016 (5). ISSN 1559-6095

This version is available from Sussex Research Online: http://sro.sussex.ac.uk/id/eprint/72034/

This document is made available in accordance with publisher policies and may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the URL above for details on accessing the published version.

Copyright and reuse:
Sussex Research Online is a digital repository of the research output of the University.

Copyright and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable, the material made available in SRO has been checked for eligibility before being made available.

Copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

http://sro.sussex.ac.uk

Protocol 3 : DNA extraction from S. pombe

Short title: DNA extraction

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

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

Tel: +44 1273 678122

j.m.murray@sussex.ac.uk

a.t.watson@sussex.ac.uk

a.m.carr@sussex.ac.uk

Abstract:

DNA is extracted from *S. pombe* cells for various uses including: templating PCRs, Southern blotting, library construction, 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 SDS (Moreno et al. 1991). Cell debris, SDS and SDS-protein complexes are subsequently removed by adding potassium acetete followed 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. When screening large number of individual strains by PCR the more rapid approach of 'colony PCR' may be used. There are numerous colony PCR protocols available and fundamental to them all is that the colony must be fresh (overnight) and as few cells as possible are used.

Extraction of chromosomal DNA from S. pombe.

Reagents:

CSE (50mM citrate/phosphate , pH5.6 (71g/litre Na2HPO4, 11.5g/litre citric acid), 40mM EDTA, pH8.0, 1.2M sorbitol) <R>

Zymolyase 20T or Lyticase

5 x TE (50mM Tris-HCl, pH 7.5, 5mM EDTA) <R>

10% SDS

5M potassium acetate

3M sodium acetate pH5.2

isopropanol

75% ethanol

RNAse A (10mg/ml)

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

Proteinase K (10mg/ml)

Materials:

Phase contrast microscope with 40X objective
Bench top centrifuge cooled to 4^{O}C
55^{O}C waterbath
Microfuge

Protocol:

Number of cells and reagent volumes represent a 'mini-prep', which is ample for PCR or one lane on a Southern blot. Cell numbers and reagent volumes are scaled for larger preparations.

1. Pellet 5x10^{7} cells by centrifugation, resuspend in 1ml ddH_2O and transfer to an Microfuge tube.
2. Centrifuge at 17,000 x g, 1 min and resuspend pellet in 1ml CSE containing 1mg/ml zymolyase 20T or 1mg/ml lyticase
3. Incubate at 37^{O}C for 15 minutes
4. Check digestion of cell walls using phase-contrast microscope on a 5ul sample to which 5ul of 10%SDS has been added (the cells lose their characteristic refringence and become black).
5. If digestion incomplete, incubate further at 37^{O}C and repeat step 4.
6. Centrifuge at 17,000 x g, 1 min
7. Resuspend in 450ul 5XTE
8. Add 50ul 10%SDS, mix by inversion and incubate at room temperature 5 min.
9. Add 150ul 5M potassium acetate, mix thoroughly by inversion and incubate on ice 10 min.
10. Centrifuge 17,000 x g, 10 min at maximum speed and transfer 600ul of supernatant to 600ul of isopropanol and mix.
11. Centrifuge 17,000 x g, 10 min and maximum speed and wash DNA pellet with 75% ethanol
12. Dry pellet

<u>for PCR:</u>

13. Resuspend in 200ul ddH2O and 5ul RNAse (10mg/ml) and incubate 15 min at 37^{O}C

14. Use 1ul per PCR reaction.

<u>for Southern blot:</u>

13. Resuspend pellet in 500ul 5XTBE and 5ul RNAse (10mg/ml) and incubate 15 min at 37^{O}C

14. Add 4ul 10% SDS and 20ul proteinase K (10mg/ml) and incubate either 60 min at 55^{O}C or overnight 30^{O}C.

15. Add 500ul phenol/chloroform/isoamyalcohol (50:49:1) and mix gently. Centrifuge 17,000 x g, 5 min.

16. Remove upper aqueous phase to fresh tube and add a further 500ul of phenol/chloroform/isoamyalcohol (50:49:1) and mix gently. Centrifuge 17,000 x g, 5 min.

17. Remove upper aqueous phase to fresh microfuge tube and add 500ul isopropanol and 25ul of 3M sodium acetate pH5.2 and incubate on ice for 10 min.

18. Centrifuge 17,000 x g, 10 min and wash DNA pellet with 75% ethanol.

19. Dry pellet and resuspend in appropriate volume for restriction enzyme digestion.

Colony PCR.

There are many variant colony PCR protocols. We provide three examples that are used successfully in our labs.

Protocol 1:

Reagents:

dd$H_2$0

Materials:

99^{O}C heat block, waterbath or PCR machine

0.2ml PCR tubes

Picofuge

Protocol

1. Transfer a *very small amount* of fresh colony to 5ul of ddH_2O in a 0.2ml PCR tube
2. Heat to 99^0C for 10 minutes in a heat block, boiling waterbath or PCR machine
3. Centrifuge maximum speed in a 0.2ml tube picofuge and add 20ul of PCR reaction mix

Protocol 2:

Reagents:

0.02N NaOH

sterile ddH_20

Materials:

99^0C heat block, waterbath or PCR machine.

0.2ml PCR tubes

Picofuge

Protocol

1. Resuspend a *very small amount* of fresh colony in 10µl 0.02N NaOH in a 0.2ml PCR tube
2. Heat to 99^0C for 10 minutes in a heat block, waterbath or PCR machine
3. Increase volume to 100µl with TE
4. Centrifuge maximum speed in a 0.2ml tube picofuge and dilute 5µl 10-fold with ddH_2O and use 5µl in a 25ul PCR reaction

Protocol 3:

Reagents:

Spheroplast buffer (0.6 M sorbitol, 50 mM KH_2PO_4/K_2HPO_4, pH 7.5. (It is important the buffer does not contain either EDTA or citrate)

10mg/ml Zymolyase stock dissolved in ddH_2O (may be kept at -20^0C for 3 months)

20mg/ml lysing enzyme stock dissolved in ddH_2O (may be kept at -20^{0}C for 3 months)

Materials:

96^{0}C heat block, waterbath or PCR machine

37^{0}C waterbath

Microfuge

Microfuge tubes

Protocol

1. Prepare digesting solution. Add 5µl of 10mg/ml Zymolyase (Nacalai) and 5µl of 20mg/ml lysing enzyme (Sigma) into 100µl of spheroplasting buffer (100µl of digesting solution is sufficient for 4 colony PCR reactions).
2. Resuspend small amount of fresh colony in the 25µl digesting solution (cell suspension needs to be a little cloudy) in an Eppendorf tube.
3. Incubate at 37^{0}C for at least 40 min.
4. Incubate at 96^{0}C for 5min
5. Snap cool on ice.
6. Centrifuge at 17,000 x g, 5 min
7. Use 1µl of the supernatant as DNA template in a 25µl PCR reaction

References

Moreno S, Klar A, Nurse P. 1991. Molecular genetic analysis of fission yeast Schizosaccharomyces pombe. *Methods Enzymol* **194**: 795-823.