Isolation of YAC DNA for microinjection to produce transgenic animals

Annette Hammes
Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Strasse 10, 13092 Berlin-Buch, Germany.

Andreas Schedl
Institute of Human Genetics, International Centre for Life, Central Parkway, Newcastle upon Tyne, NE1 3BZ, U.K.

Equipment and reagents

- Agarose plugs containing YAC (yeast artificial chromosome) DNA at a high concentration (see protocols described elsewhere, e.g. in Schedl, A., Larin, Z., Montoliu, L., Thies, E., Kelsey, G., Lehrach, H., and Schütz, G. (1993b). A method for the generation of YAC transgenic mice by pronuclear microinjection. *Nucleic. Acids. Res.* ***21***, 4783-4787)
- Pulsed field gel electrophoresis system (e.g. from Bio-Rad)
- Low melting point (LMP) agarose, e.g. NuSieve (FMC)
- High quality agarose, e.g. SeaKem (FMC)
- TENPA buffer: 10 mM Tris pH 7.4, 1 mM EDTA, 100 mM NaCl, 70 μM spermidine, 30 μM spermine[a]
- Injection buffer: 10 mM Tris pH 7.4, 0.1 mM EDTA, 100 mM NaCl, 70 μM spermidine, 30 μM spermine[a]
- Agarase, e.g. GELase (Epicentre)
- Dialysis membrane (VMWP 02500, 0.025 μm pore size, from Millipore)

Method

1. Cast a 0.25 × TAE gel using high-quality agarose, e.g. Seakem (FMC) at a concentration of 1% with a preparative lane of about 5 cm (tape together several teeth of the comb).
2. Wash the agarose plugs containing the yeast DNA extensively (4 × 15 min in TE, and 2 × 15 min in water).
3. Load the plugs next to one another in the preparative lane and seal with 1% LMP agarose in 0.25× TAE.
4. Run the gel in cooled 0.25× TAE under conditions optimized for the separation of the YAC from the endogenous chromosomes.
5. Cut off each side of the gel including 0.5 cm of the preparative lane and stain for 30 min in 0.25× TAE buffer containing ethidium bromide (0.5 μg/ml).
6. Mark the position of the YAC lane under UV light using a scalpel.

© Oxford University Press 5 October, 2001 All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, without the prior permission in writing of Oxford University Press, or as expressly permitted by law, or under terms agreed with the appropriate reprographics rights organization.

7 Reposition the stained parts of the gel next to the preparative lane and excise the gel slice containing the YAC DNA using the marked parts as guidance.

8 Cut out a slice containing endogenous yeast chromosomes above and below the YAC to serve as controls for the second gel run.

9 Place the excised gel slices on a second gel tray at an angle of exactly 90 ° to the PFGE run, with the YAC slice in the middle flanked by the two marker slices. Cast a 4% Nusieve (FMC) gel in 0.25× TAE around it.

10 Run overnight at a voltage of 6 V/cm with circulating buffer at 4 °C.

11 Cut off the two marker slices and stain with ethidium bromide solution to locate the DNA. All of the YAC DNA should be concentrated within the LMP gel. If electrophoresis has not proceeded far enough, continue the gel run.

12 Excise the equivalent position of the YAC DNA.

13 Equilibrate the gel slice in 20 ml of TENPA buffer for at least 1.5 h.

14 Transfer the slice to a 1.5 ml microcentrifuge tube, remove any additional buffer that has been carried over and incubate for 3 min at 68 °C.

15 Spin for 5 sec in a microcentrifuge to bring down all pieces of agarose.

16 Incubate for further 5 min at 68 °C.

17 Spin in a microcentrifuge for 5 sec, place the tube containing the molten agarose at 42 °C and incubate for 5 min.

18 Meanwhile, load 4 U of Gelase (Epicentre) per 100 mg melted agarose into a Gilson pipette and keep at room temperature to allow the temperature to adjust. Do not add the Gelase straight from the –20 °C freezer, otherwise parts of the agarose may set.

19 Slowly release the Gelase while stirring the solution gently with the pipette. Incubate at 42 °C for 2–3 h.

20 Transfer the resulting DNA solution onto a dialysis membrane (Millipore, 0.025 µm pore size, VMWP 02500) and dialyse for 30 min against injection buffer.

21 Check the DNA concentration as described below *(Testing the exact concentration of DNA constructs used for microinjection to produce transgenic animals)* and dilute the DNA solution with injection buffer to a final concentration of 2 ng/µl for injection.

22 Store the DNA at 4 °C. Do not use the same batch for microinjection for longer than 2 weeks. Storage in solution for longer than this may result in fragmentation of the DNA.

© Oxford University Press 5 October, 2001.

Notes

a When isolating YACs <200 kb, spermidine and spermine can be omitted from both the TENPA and injection buffer.

© Oxford University Press 5 October, 2001.