Preparation of target RNA for hybridization to arrays by *in vitro* transcription using T3, T7 or SP6 RNA polymerase

John K. Elder, Martin Johnson, Natalie Milner, Kalim U. Mir, Muhammad Sohail and Edwin M. Southern
Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK.

Reagents

- DNA template (at 0.5–1 μg/μl)
- T3, T7 or SP6 RNA polymerase (Promega)
- Transcription buffer (5 × transcription buffer from Promega): 200 mM Tris-HCl pH 7.9, 30 mM $MgCl_2$, 10 mM spermidine, 50 mM NaCl
- RNase inhibitor (recombinant RNasin®; Promega)
- 100 mM DTT (Promega)
- [α-^{32}P]UTP (3000 Ci/mmol) or [α-^{33}P]UTP (2500 Ci/mmol) (Amersham)
- rNTPs (Pharmacia): ATP, GTP, and CTP stored as 10 mM solutions, and UTP as 250 mM in nuclease-free distilled water (Promega)

Method

The reaction below is for 20 μl using labelled UTP.

1 Add the following components to a microcentrifuge tube at room temperature in this order:

• 5 × transcription buffer	4	μl
• 100 mM DTT	2	μl
• RNasin®	20	U
• 10 mM ATP, GTP, and CTP	1	μl each
• 250 μM UTP	1	μl
• Template DNA	2	μl
• [α-^{32}P]UTP or [α-^{33}P]UTP	2	μl
• T3, T7 or SP6 RNA polymerase	20	U
• Total volume	20	μl

© Oxford University Press 26 September, 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.

2 Mix and incubate at 37 °C for 1 h.

3 Remove 1 μl for quantitation (see Quantitation of transcripts).

4 Remove unincorporated label by Sephadex® G25 or G50 column chromatography.[a]

5 Save 1 μl of the purified transcript for quantitation in a microcentrifuge tube.

6 Check the integrity of the transcript by electrophoresis on a polyacrylamide or an agarose gel.

Notes

a Ready-to-use columns are available from several suppliers including Promega and Pharmacia.

© Oxford University Press 26 September, 2001.