© 1997 Oxford University Press

Nucleic Acids Research, 1997, Vol. 25, No. 7 **1467–1468**

Growth and storage of YAC clones in Hogness Freezing Medium

Ekkehard Werner[1,2], Anthony A. Holder[2] and Jörg D. Hoheisel[1,*]

[1]Molecular-Genetic Genome Analysis, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 506, D-69120 Heidelberg, Germany and [2]Division of Parasitology, National Institute of Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

Received November 7, 1996; Revised and Accepted February 19, 1997

ABSTRACT

To date, frozen storage of YAC libraries have relied on the administration of glycerol to the medium subsequent to cell growth. By adding Hogness Freezing Medium prior to inoculation, cultures can be frozen directly after cell growth, with no adverse effect on the stability of the YAC DNA or on the viability of the cells even after repeated freezing and defrosting. Although a relatively simple modification, the procedure notably improves the handling of YAC libraries and significantly reduces the risk of contamination, especially when dealing with large numbers of clones.

Compared to the handling of bacterial cloning systems, such as in *Escherichia coli*, the manipulation of YAC clones requires a considerably higher degree of sophistication with regard to sterility. Due to both the lack of positive selection and the slowness of cell division and thus the length of incubation periods, the danger of contamination is much more profound for yeast cultures. More surprisingly, the frozen storage of YAC libraries that consist of large numbers of individual clones in microtitre dish cultures still relies on the administration of glycerol to the medium subsequent to cell growth to the stationary phase (1–9 and references therein). Not only does this procedure involve an extra manipulation, but also the additional step increases the risk of contamination.

Having had good experience with the addition of Hogness Freezing Medium [final concentration: 36 mM K_2HPO_4, 13.2 mM KH_2PO_4, 0.4 mM $MgSO_4$, 1.7 mM Na_3-citrate, 6.8 mM $(NH_4)_2SO_4$, 4.4% (v/v) glycerol] to the growth media of bacterial cultures *prior* to inoculation, we wondered whether the same could be done to yeast cultures. In contrast to the mere addition of glycerol, the medium buffers the solution and has a positive effect on the crystallisation process. The low salt concentration, however, is very unlikely to trigger any stress reaction in the yeast cells which may result in an increase in recombination frequency. The applicability of the protocol was tested on YAC clones of *Plasmodium falciparum* (4), *Arabidopsis thaliana* (9) and human (10). The *Plasmodium* DNA has a very low GC-content of $<20\%$ that should act as a sensitive indicator for reduced stability of the cloned DNA; in human clones on the other hand there are areas of high GC-content as well as a high percentage of repetitive sequences.

Clones were replicated from normal glycerol culture into 384-well microtitre dishes containing YPD medium. To one copy of a library, glycerol was added to 20% (v/v) after the 2 day incubation period, and a parallel copy was grown in medium supplemented with Hogness Freezing Medium. Both copies were stored frozen at –80°C. For inoculation of subsequent cultures, the dishes were defrosted at room temperature and stirred. DNA plugs were prepared in parallel from identical 5 ml cultures in YPD that had been inoculated from the respective copies. Figure 1 shows typical results of such preparations. No significant difference in quality or amount of YAC or yeast DNA could be observed after the two storage methods. Also, when spotted onto Nylon filters identical colony growth rates and, subsequent to an *in situ* attachment of the DNA (5), identical hybridisation results were detected (not shown).

Library microtitre dishes containing the Hogness Freezing Medium were repeatedly frozen solid at –80°C and completely defrosted for clone replication. After 20 such cycles, still no apparent loss of viability could be observed by counting the number of colonies growing after replicating the clones onto YPD agar plates, irrespective of the origin of the cloned DNA. In the laboratory, libraries of the above organisms are being kept in Hogness Freezing Medium and subjected to various types of analysis, such as physical and transcriptional mapping, with no apparent effect on the experimental results by the mode of storage.

REFERENCES

1 Sherman,F., Fink,G.R. and Hicks,J.B. (1986) *Methods in Yeast Genetics.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
2 Albertsen,H.M., Abderrahim,H., Cann,H.M., Dausset,J., Le Paslier,D. and Cohen,D. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4256–4260.
3 Riley,J.H., Ogilvie,D. and Anand,R. (1992) In Anand,R. (ed.), *Techniques for the Analysis of Complex Genomes.* Academic Press, London, pp. 59–80.
4 Rubio,J.P., Triglia,T., Kemp,D.J., de Bruin,D., Ravetch,J.V. and Cowman,A.F. (1995) *Genomics* **26**, 192–198.
5 Cox,R.D., Meier-Ewert,S., Ross,M., Larin,Z., Monaco,A.P. and Lehrach,H. (1993) *Methods Enzymol.* **255**, 637–653.
6 Foote,S., Vollrath,D., Hilton,A. and Page,D.C. (1992) *Science* **258**, 60–66.

*To whom correspondence should be addressed. Tel: +49 6221 424680; Fax: +49 6221 424682; Email: j.hoheisel@dkfz-heidelberg.de

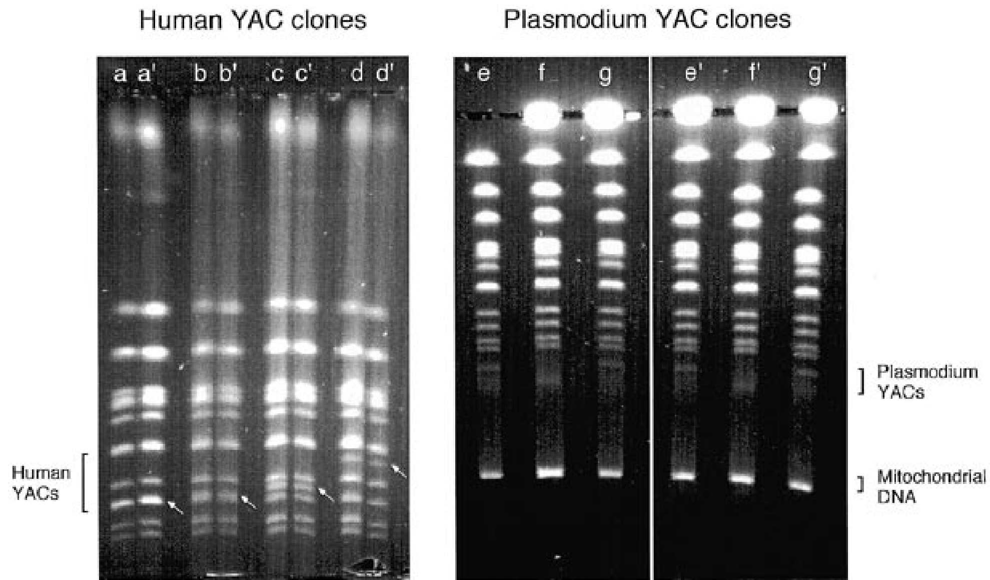

Figure 1. Comparison of YAC DNA isolated from different inoculates. Identical YAC clones stored frozen in glycerol culture (a–g) or growth medium supplemented with Hogness Freezing Medium (a′–g′) were used to inoculate 5 ml YPD cultures. Intact yeast DNA was prepared and separated on pulsed-field gels under standard conditions. There is no significant difference in yield or quality. In the preparations of *Plasmodium* YACs a smeared background of sheared DNA ran in the same size range as the YAC clones and is visible due to the high DNA concentration that was loaded to the gel. The agarose blocks that had been in slots (a–e) and (a′–d′) were displaced during staining and destaining of the gel.

7 Qin,S., Zhang,J., Isaacs,C.M., Nagafuchi,S., Jani Sait,S.N., Abel,K.J., Higgins,M.J., Nowak,N.J. and Shows,T.B. (1993) *Genomics* **16**, 580–585.
8 Libert,F., Lefort,A., Okimoto,R., Womack,J. and Georges,M. (1993) *Genomics* **18**, 270–276.
9 Schmidt,R., West,J., Love,K., Lenehan,Z., Lister,C., Thompson,H., Bouchez,D. and Dean,C. (1995) *Science* **270**, 480–483.
10 Zehetner,G. and Lehrach,H. (1994) *Nature* **367**, 489–491.