© 1994 Oxford University Press
Nucleic Acids Research, 1994, Vol. 22, No. 6 1117–1118

Production of high titre *helper-free* recombinant retroviral vectors by lipofection

Ariberto Fassati, Yoshiyuki Takahara[1], Frank S.Walsh and George Dickson*
Department of Experimental Pathology, U.M.D.S. Guy's Hospital, London SE1 9RT, UK and [1]Fine Chemical Development Laboratory, Central Research Laboratories, Ajinomoto Co. Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki 210, Japan

Received January 18, 1994; Accepted February 7, 1994

High titre stocks of replication-defective retroviral vectors are generally prepared by stable introduction of cloned proviral DNA into packaging cell lines that supply in-trans all the proteins necessary for viral assembly (1). However, different vectors are packaged with different efficiencies and the expression of transgenes and activity of heterologous internal promoter elements may vary widely, dependent upon poorly understood and often ill-defined features of recombinant proviral structure (2). Optimization and comparative screening of proviral structures by isolation and selection of stable producer cell clones is thus an extremely time consuming and labour intensive procedure. A more rapid and simpler alternative for the preparation of high titre retroviral stocks is the short-term production of virus via transient transfection of packaging cells. This approach often yields low viral titres due to the inefficiency of standard transfection procedure such as the use of $CaPO_4$-DNA precipitates (3,4). However, methods have been recently described for the transient production of high titre retroviral stocks using $CaPO_4$–DNA precipitates and Cos cells or other SV40 large T antigen expressing cells (5,6). Here we describe a simple method using high efficiency Lipofectamine-mediated gene transfer to transiently and reproducably prepare recombinant retroviral vectors at titres in excess of 10^5 cfu/ml. Cultures of the ecotropic packaging cell line Ampli-GPE (7) were seeded in 60 mm dishes (1.3×10^4, cells/cm^2) and 24 hours later cells were transfected by exposure to Lipofectamine–DNA complexes prepared according to the manufacturer (Gibco BRL, Paisley, Scotland).

Cultures were incubated at 37°C for 6 hours in the presence of 3 ml of Dulbecco's modified Eagle medium containing 10 μg/ml of Lipofectamine and 2 μg/ml of supercoiled plasmid DNA. Under these conditions 20–30% of cells were stained positively for β-galactosidase activity (8) following transfection with BAG plasmid containing the Lac-Z gene (3). Supernatants from cells transfected with BAG or pBABE Neo retroviral vectors (9) were collected after 72 hours, filtered through a 0.45 μm filter and stored at −70°C. Viral titres were determined by infecting NIH 3T3 fibroblasts, that were seeded 18–22 hours before at a concentration of 10^4/cm^2, with various dilutions of the viral stocks in the presence of 8 μg/ml polybrene. The number of cells expressing β-galactosidase was counted 48 hours later in the case of the BAG vector, while the number of G418 resistant colonies was counted 12 days later for pBABE Neo. As shown in table 1, titres of greater than 10^5 were reproducibly obtained by Lipofectamine-mediated gene transfer for both the BAG and pBABE Neo vectors, which compares favourably with titres obtained from stably-transfected clonal producer cells. To achieve high and consistent titres of virus using the Lipofectamine procedure we found it to be essential that the 5:1 (w/w) ratio of lipid to DNA was precisely maintained as well as the concentration of the cells (1.3×10^4/cm^2) that had to be accurately dispersed. The absence of helper virus in the transiently produced stocks was confirmed by mobilization assay (1) using 3T3 fibroblasts that stably express an indicator previrus. Cultures were passaged at high density for 7 days, supernatants were then used to infect NIH 3T3 fibroblasts as described. In conclusion the present method can be used to rapidly and conveniently prepare large numbers of high titre, helper-free stocks of recombinant retroviral vectors, thus allowing efficient screening and characterisation of variant proviral structures and packaging cell lines.

Table 1. Retroviral production from Ampli-GPE cells after Lipofectamine-mediated transfection

Expr No	Plasmid Construct	Recombinant Retrovirus Titre[a] (Mean ± SD)	Helper virus titre[a]
1	BAG	$1.6\times10^5 \pm 0.3$	<1
2	BAG	$2.2\times10^5 \pm 0.5$	<1
3	BAG	$2.0\times10^5 \pm 0.5$	<1
4	pBABE Neo	$1.0\times10^5 \pm 0.3$	<1
5	pBABE Neo	$1.2\times10^5 \pm 0.6$	<1

[a]Infectious titres (LacZ-positive cells or G418 resistant colonies/ml) were determined as previously described (6). For helper virus determination, 300 μl of undiluted viral stocks were tested.

ACKNOWLEDGEMENTS

We thank Drs Jack Price, Hartmut Land and Mary Collins for providing plasmids and indicator cells. This work was funded by the Muscular Dystrophy Group of Great Britain and the Italian Telethon.

* To whom correspondence should be addressed

Downloaded from http://nar.oxfordjournals.org/ at Royal Holloway, University of London on May 10, 2012

REFERENCES

1. Morgenstern,J.P. and Land,H. (1991) In Murray,E.J. (ed.), *Methods in Molecular Biology*. Humana Press Inc., Clifton, NJ, Vol. 7, pp. 181–206.
2. Boris-Lawrie,K.A. and Temin,H.M. (1993) *Curr. Opin. Genet. Develop.* **3**, pp. 102–109.
3. Price,J., Turner,D. and Cepko,C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 156–160.
4. Miller,A.D., Trauber,D.R. and Buttimore,C. (1986) *Somat. Cell. Mol. Genet.* **12**, 175–183.
5. Landau,N.R. and Littman,D.R. (1992) *J. Virol.* **66**, 5110–5113.
6. Pear,W.S., Nolan,G.P., Scott,M.L. and Baltimore,D. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 8392–8396.
7. Takahara,Y., Hamada,K. and Housman,D.E. (1992) *J. Virol.* **66**, 3725–3732.
8. Lim,K. and Chae,C.-B. (1989) *BioTechniques* **7**, 576–579.
9. Morgenstern,J.P. and Land,H. (1990) *Nucleic Acids Res.* **18**, 3587–3596.

Downloaded from http://nar.oxfordjournals.org/ at Royal Holloway, University of London on May 10, 2012