

# A method for cDNA cloning in COS cells irrespective of subcellular site of expression

E.Horst, P.L.J.Wijngaard<sup>1</sup>, M.Metzelaar<sup>1</sup>, E.J.E.G.Bast<sup>1</sup> and H.C.Clevers<sup>1,\*</sup>

Department of Pathology, Free University Hospital, Amsterdam and <sup>1</sup>Department of Immunology, Academic Hospital Utrecht, Utrecht, The Netherlands

Submitted July 2, 1991

Several years ago, Seed and colleagues introduced a rapid and highly successful technique for the cloning of surface antigens defined by monoclonal antibodies. The technique is based upon transient transfection of a cDNA expression library in COS cells and the subsequent physical selection of cells expressing particular antigens by adhesion to monoclonal antibody-coated dishes (panning) (1, 2). Unfortunately, this method requires expression of the antigen at the plasma membrane. Here we report an adaptation of the original procedure which allows single-step cloning of antigens irrespective of their subcellular localization.

A CD8 cDNA inserted in the COS cell-expression vector pCDM8 (1) served to establish the procedure. The CD8 plasmid was diluted 1:10<sup>-5</sup> with DNA of an irrelevant (B cell) pCDM8 cDNA library. The DNA mixture was transfected into COS cells (1 µg of DNA/culture dish) as described (3). The cells were then grown for 48 hrs. COS cells expressing the CD8 antigen were visualized using immunoperoxidase staining (Bast, unpublished). In brief, the COS cells were rinsed with phosphate-buffered saline (PBS) and fixed in the culture dish for 10 minutes with methanol. All subsequent manipulations were performed at room temp. The monolayers were washed twice with PBS and preincubated with 5% Fetal Calf Serum (FCS)/PBS (10') followed by a 1 hr incubation with the CD8 antibody WT82 (1:1000 ascites in 5% FCS/PBS). The plates were washed twice with PBS followed by a 45' incubation with peroxidase-labeled Goat anti-Mouse Ig (DAKO), diluted 1:50 in 5% FCS/PBS. Peroxidase activity was subsequently visualized using a 5% dilution of a 4 mg/ml stock of 9-amino-3-ethylcarbazol in N,N'-dimethylformamide in 0.1 M NaAc (pH 4.8) containing 0.1% H<sub>2</sub>O<sub>2</sub> (30–60'). After washing with H<sub>2</sub>O, the plates were visually screened for CD8-positive cells with the use of an inverted microscope. We

observed 2–5 bright-red COS cells/dish. Positive cells were picked by scraping with a hand-held fine tip of a Gilson pipetman. Next, individual scraped cells were treated with proteinase K (0.1 µg/µl in 0.2 ml of 0.6% SDS/10 mM EDTA) for 30' at 37°C and a Hirt extract was performed (4). Plasmid cDNA was precipitated in the presence of 20 µg glycogen as carrier, dissolved in H<sub>2</sub>O, and transformed into *E.coli* strain MC 1061/P3 using high voltage electroporation, resulting in 30–200 colonies per extracted COS cell. Colony hybridization with a CD8 probe identified 2–10 CDS colonies per transformation. The reisolated CD8 plasmids were intact as determined by insert size and expression in COS cells. We have similarly visualized and isolated plasmids encoding the lysosomal membrane antigen CD63 (3). Furthermore, we have recently successfully employed the technique for the cloning of an intracellular antigen of the bovine intralymphocytic parasite *Theileria parva* (Wijngaard *et al.*, unpublished). The adaptation described here provides a valuable extension of the original COS cell cloning procedure.

## ACKNOWLEDGEMENTS

We thank Drs. B.Seed and A.Aruffo for providing us with the CD8 plasmid, pCDM8 and MC1061/P3, and Dr. W.Tax for the WT82 hybridoma.

## REFERENCES

1. Seed,B. and Aruffo,A. (1987) *Proc. Natl. Sci. USA* **84**, 3365–3369.
2. Wysocki,L.J. and Sato,V.L. (1978) *Proc. Natl. Sci. USA* **75**, 2844–2848.
3. Metzelaar,M., *et al.* (1991) *J. Biol. Chem.* **266**, 3239–3245.
4. Hirt,B. (1967) *J. Mol. Biol.* **26**, 365–369.

\* To whom correspondence should be addressed