Using GFP retrovirus to label tumor cells and vascular endothelia cells  

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Abstract Objective To prepare retrovirus which carry GFP gene and are able to label living cells simply and rapidly. Methods The recombinant retrovirus pLNCX-GFP was constructed by inserting 780 bp GFP cDNA fragment into the MCS site of retroviral plasmid pLNCX. Both ectopic packaging cell line Δ-Eco and amphotropic packaging cell line Δ-Ampo and PA317 were transfected by pLNCX-GFP with liposome. The supernate collected from transfected packaging cells was used to infect a variety of tumor cell lines and vascular endothelial cell lines. Results When packaging cells were transfected by retroviral vector pLNCX-GFP, the GFP expression could be observed in 25% ~ 40% of cells and GFP retrovirus then could be detected. However, G418 resistant clones showed more stable GFP expression and higher retrovirus titer. The GFP retrovirus from different packaging cell line showed variant ability to infect tumor cell lines and vascular endothelial cell lines and the tumor cells infected by GFP retrovirus showed stable GFP expression in vitro. GFP transduced tumor cells could grow in syngenic animal and continue expressing GFP. Conclusion Using GFP retrovirus to label target cells represent an important advantage over conventional plasmid because they can efficiently transfer GFP gene into target cells and GFP can be stably expressed in target cells in vitro or in vivo.

Key words: Green fluorescent protein, GFP, Retrovirus, Gene transduction

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**Fig 1**  FACS analysis of GFP retrovirus producing cells **φX-Ampho-GFP**
Fig 2  Amphophilic package cells APhoton-GFP which could express GFP protein and produce GFP retrovirus.

2.3 GFP APhotonA siRNA DENV E6771i, O N2 GFP APhoton APhot APhot PACP AAE7, apA, sO, Oqa, B3AQA, 2i 4é EEEU1X1, OUL APhoton-Ampo-GFP MAI-Os, O2EA1 MAI-OAEG C3O19 AYE cumpl AI -MAI O3P A2E61 E61 0/460% PA Lewis I, OUL 0% MA Lovo I, OUL 6% MA NCI H446 I, OUL 24 EEB1/’i GFP3 EMU1 1, EEB1/BEL-7402 I, O N2 24/’51 GFP N3O1/’i E

2.4 3ODE7 2ET I7 1i GFP MAOECEH 1AULAI, O N OCE GFP APhoton APhot 1/4B3MQAE7 A61 O I 2, OUL Boveri 133.

Fig 3 Stable GFP expression was observed in Lewis cells which was infected by GFP retrovirus and selected with G418

Fig 4 FACS analysis of GFP expressing Lewis cells
Fig 5 A C57 mouse bore a tumor that originated from injected GFP expressing Lewis cells.

Fig 6 A tumor cut from C57 mouse showed stable GFP expression.

1 Prasher DG, Eckenrode VK, Ward WC et al. Primary structure of the Ae-
侵犯胸壁肺癌的外科治疗

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