A New Gene Expression System Useful for the Analysis of ECM

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For the functional analysis of ECM molecules in vitro, we have been tried to carry out the exogenous expression of their cDNA in various cells. To this end, we have examined different types of plasmid vector such as pcDNA3.1 (Invitrogen) and pIRESpuro3 (Clontech). However, the results were not satisfactory in quality and quantity. Leaving aside a transient expression, it is not always easy to succeed in the stable expression at high-level in mammalian cells. On the other hand, we have known by experience that marker genes are stably expressed at high level in clonal cells obtained by gene trap experiments. Taking a cue from these observations, we have developed a new expression system combining gene trapping and site-specific gene integration methods.

Our system is composed of two parts. One is the establishment of target cell line that is stably expressing a reporter gene at high level. The other is the site-specific integration of cDNA into the position of reporter gene for its consistent expression. In this system, we use a retroviral promoter-trapping vector for expressing cDNA by an endogenous promoter. Our expression system is quite new and will have a broad range of applications, even though retrovirus infection for target cells is a minimum requirement.

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