Benchmarks

Mammalian Expression Vector with Two Multiple Cloning Sites for Expression of Two Foreign Genes

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Biological processes generally involve interactions between more than one gene product. Therefore, certain studies may require expression of multiple genes in a given cell. To make such experiments easier, we have created a new mammalian expression vector, pRSC, that has three independent transcriptional units (Figure 1). The first transcriptional unit contains the Rous sarcoma virus (RSV) promoter followed by a multiple cloning site and the Simian virus 40 (SV40) polyadenylation site. The second contains the cytomegalovirus (CMV) promoter followed by another multiple cloning site and the bovine growth hormone (BGH) polyadenylation site. The third contains the neomycin-resistance gene expressed under the control of the SV40 promoter to allow for the selection of transfected cells in G418. This plasmid has the PUC 19 backbone so that it can be grown to

high-copy numbers in the presence of ampicillin in *E. coli*.

When designing new double-gene expression vectors based on pRSC, one should take into consideration that the CMV promoter is generally stronger than the RSV promoter (1). The purpose of using three different promoters instead of using one promoter three times is to avoid the formation of DNA secondary structures and DNA recombination caused by the presence of repeating sequences within a plasmid. It may also reduce the potential competition between the promoters for transcription factors. Another expression vector, pHook™-2 (Invitrogen, San Diego, CA, USA), also uses the CMV, RSV and SV40 promoters in three separate transcription units to express three genes simultaneously. However, pHook-2 is developed for different kinds of applications as pRSC, and it only has one multiple cloning site for the insertion of genes.

Since DNA fragments and polymerase chain reaction (PCR) products can be easily adapted for any of the unique sites in pRSC's two multiple cloning sites using the KiSS cloning system (2,3,6), constructing vectors to express two foreign genes in the same

cells is now technically much easier. Such improvements could potentially contribute to studies of interaction, complementation and synergistic events among various gene products.

We have successfully used pRSC to express two pairs of genes.

REFERENCES

1. Boshart, M., F. Weber, G. Jahn, K. Dorsch-Hasler, B. Fleckenstein and W. Schaffner. 1985. A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* 41:521-530.
2. Hadjeb, N. and G.A. Berkowitz. 1996. Preparation of T-overhang vectors with high PCR product cloning efficiency. *BioTechniques* 20:20-22.
3. Marchuk, D., M. Drumm, A. Saulino and F.S. Collins. 1991. Construction of T-vectors, a rapid and general system for direct cloning of unmodified PCR products. *Nucleic Acids Res.* 19:1154.
4. Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
5. Tsang, T.C., V. Copeland and G.T. Bowden. 1991. A set of cassette cloning vectors for rapid and versatile adaptation of restriction fragments. *BioTechniques* 10:330.
6. Tsang, T.C., D.T. Harris, E.T. Akporiaye, S.F. Schluter, G.T. Bowden and E.M. Hersh. 1996. Simple method for adapting DNA fragments and PCR products to all of the commonly used restriction sites. *BioTechniques* 20:51-52.

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Figure 1. Map of pRSC. pRSC was constructed by inserting the RSV transcriptional unit from pREP4 (Invitrogen) into pcDNA3 (Invitrogen). The multiple cloning sites of both pREP4 and pcDNA3 were first modified to give pRSC the unique multiple cloning sites. pREP4 was digested with *Bam*HI and *Not*I, and pcDNA3 was digested with *Eco*RI and *Hind*III. Both were then treated with DNA polymerase followed by re-ligation of the blunt ends (4). The 1.1-kb *Sall* piece of the modified pREP4, which contains the entire RSV transcriptional unit, was then adapted for the *Bam*HI site using p34H (5). The resulting *Bam*HI fragment, which contains compatible overhangs with the *Bgl*II site, was cloned into the *Bgl*II site of the modified pcDNA3 to yield pRSC.