

Tips and tricks of the trade

Fine Tuning Multicloning Sites

Cloning a gene into the multicloning site of an expression vector is so natural to molecular biologists that they waste few thoughts on it. To get optimal expression results, however, they should ponder this issue more.

Lab Hint

Multicloning sites (MCSs) also known as polylinkers are short sequences in plasmids or expression vectors that contain about 20 unique restriction sites. MCSs allow a wide choice of restriction enzymes to be used for cloning. Almost any commercial vector, and very likely the one you are currently using, is equipped with a MCS. Over the years, researchers have optimised vectors containing MCSs in several ways. They have re-engineered the promoter site, improved the transcription step, reduced background expression or manipulated codon bias to improve translation rate.

They have, however, paid little attention to the fact that promoters are usually placed upstream of the MCS and that some base pairs of the MCS appear in the 5'-untranslated region (UTR) of the transcribed mRNA. It was common belief amongst molecular biologists that MCSs are benign elements that have no influence on translational efficiency of the target gene. But is this really true?

Nathan C. Crook and his collaborators at the University of Austin, Texas, took a deeper look at this issue in the eukaryote *Saccharomyces cerevisiae* and came up with some interesting results (*Nucleic Acids Research*, 2011, Vol. 39, No. 14). Obviously, the bases stemming from the MCS may form secondary structures in the 5'-untranslated region that can hinder or even inhibit translation. Small stem loops may, for example, hamper the scanning of the UTR by the ribosomal 43S initiation complex, leading to a decrease in translation initiation.



Secondary structures in the untranslated region of mRNA, originating from base pairs of expression vectors' multicloning sites, may inhibit expression of recombinant proteins.

Using the RNA analysis programme Nupack (<http://nupack.org/>) that calculates RNA folding, Crooks *et al.* created a predictive model to design trouble-free MCSs. According to their results, it is not necessarily the length of the MCS-hangover that leads to problematic secondary structures but the actual content of the sequence. Besides that, it seems that the translational inhibition depends on the position of the secondary structure in the 5'-UTR.

Based on their model, the group generated a set of promoter-specific MCSs, predicted by the programme to form minimal or no 5'-UTR secondary structures and tested the expression of several genes cloned into the optimised MCSs. All of the redesigned MCSs showed reduced or even negli-

gible multicloning site inhibition. However, there is no such thing as a perfect multipurpose MCS. According to the Texas group, optimisation of 5'-UTR secondary structure is context-specific and depends on the upstream promoter. Nonetheless, it may be worth the effort of redesigning the multicloning sites of your vectors for better protein expression in eukaryotic cells.

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