

PROTEIN EXPRESSION

Customised vectors by gene synthesis

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▶ The heterologous expression of foreign proteins in organisms like *Escherichia coli*, *Saccharomyces cerevisiae* or different mammalian cell lines is a daily procedure in many laboratories. In addition to the adaptation of the coding sequence, the choice and decision on the best expression vector is a critical step in the process.

It is well known that the strength of the promoter – and in some cases the copy number, promoted by the origin of replication – have a significant influence

on the expression level of a foreign protein^[1]. In terms of expression, optimising the coding sequence itself has attracted major interest. The open reading frame

(ORF) contains several factors that can be adapted to significantly enhance protein expression. Parameters like codon-usage and the implementation of beneficial sequences (Kozak sequence, etc.) or the avoidance of detrimental sequences (internal splice sites, internal polyadenylation sites etc.) represent common optimisation qualities^[2].

In some instances, maximising protein expression is a secondary goal. In gene therapy, a modulated expression is of major interest. The ability to activate and silence genes will be an essential feature of constructs aiming at the alleviation of recurrent symptoms in neurodegenerative disorders. Cell-specific activation or silencing of the therapeutic gene helps to avoid side effects due to gene overexpression^[3].

Role of application

Sometimes the application itself dictates the nature of the DNA construct. Plasmid DNA vaccination procedures rely on a sufficient expression of the foreign gene after introduction of the genetic information into the host's cells. In addition to the foreign protein, adjuvants are used to stimulate the immune system of the host organism. The specific immunity of the gene of interest can also be raised by the existence of CpG motifs (PuPuCpG-PyPy) within the plasmid DNA^[4]. The respective CpG motif will function as an intrinsic adjuvant to trigger a strong cell-mediated immune (CMI) response, allowing one to dispense with conventional adjuvants. If the plasmid DNA will be used for gene therapeutic purposes, the inflammatory response is rather harmful, and CpG-motifs are therefore to be avoided.

Besides this immune modulating activity, CpGs play an essential part in gene regulation. Activating and silencing gene expression involves the methylation status of CpG dinucleotides. It has been demonstrated that the usage of expression vectors free of any CpG dinucleotides render a sustained in vivo transgene expression, simultaneously totally abrogating inflammation^[5],

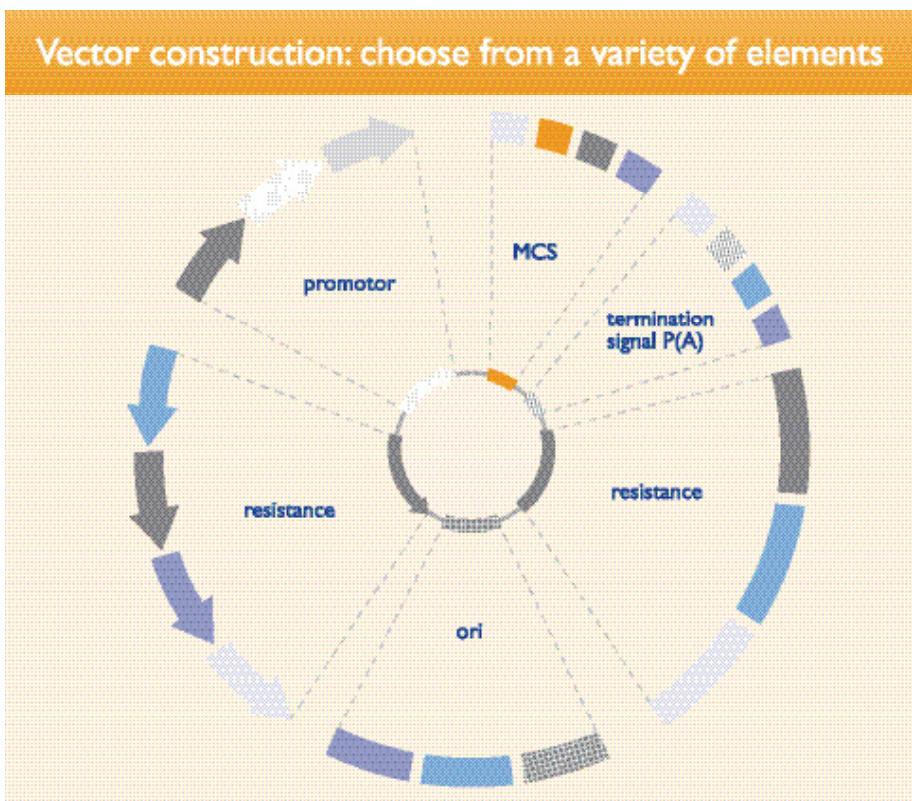


Fig. 1: Vector construction: Individual elements can be combined to construct the optimal vector for every application.

which vividly illustrates that both CpG attributes have to be taken into account when designing expression constructs.

Vector selection

As these examples show, many factors have to be considered when designing expression experiments. The choice of the right vector system is certainly one of the crucial parameters to the success of the above-mentioned strategies. GENEART's vector platform allows fast and specific generation of application-specialised plasmids with distinct characteristics. GENEART's technology platform contributes to individual vector design through modular, easily exchangeable fragments comprising an assortment of regulatory and functional elements.

An individual vector can be constructed to meet specific customer requirements by assembling standardised modules. Individual elements can be derived from PCR amplification (where applicable) or generated synthetically to provide custom-made features. It is possible to avoid or introduce immunologically relevant sequences like CpG motifs to adapt the vector to the specific needs of the customer. While avoiding "junk" sequences, the designed plasmids also exhibit the minimal size to guarantee best transformation/transfection efficiencies. Significantly reduced homology to wild-type sequences, counteracting the risk of homologous recombination events and optimised cell or tissue specific promoters, also enhance the expression and improve the outcome of experiments.

In addition to the customised vector synthesis, experienced GENEART scientists will assist customers during the design of the specific plasmids. The combination of GENEART's state-of-the-art synthesis technology and the individualised scientific advice will guarantee the best possible outcome for any kind of expression experiment. ▼

References

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