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# Design of vectors for transgene expression: The use of genomic comparative approaches

Lluís Montoliu *, Rosa Roy, Lucía Regales, Ángel García-Díaz

*Department of Molecular and Cellular Biology, Centro Nacional de Biotecnologia (CNB-CSIC), Campus de Cantoblanco, C/ Darwin 3, 28049 Madrid, Spain*

**Abstract**

The design of transgenes has always been limited by the extent of available information on the endogenous locus whose expression pattern had to be replicated. Those genes whose expression domain had not been entirely documented resulted, usually, in transgenes with an unpredictable expression patterns and suboptimal performance in transgenic animals. The use of genomic comparative approaches, highlighting evolutionary conserved homologous DNA sequences, helps to identify crucial regulatory elements that are associated to a given expression domain. The inclusion of these conserved regulatory sequences in transgenic constructs would normally result in optimal expression levels of transgenes in recipient animals. The use of artificial chromosome-type transgenes usually ensures the inclusion of these preserved regulatory elements that are required for the faithful expression of the gene. These constructs could also contain insulators, a subset of regulatory sequences whose application is being addressed in transgenesis. Therefore, the generation of transgenic animals with genomic-type constructs is the recommended approach to achieve optimal transgene expression, according to the expected pattern of the corresponding endogenous locus.
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*Keywords:* Transgene; Expression; Insulator; Genomic DNA

**Résumé**

La conception des transgènes a toujours été limitée par le manque d'information sur les locus endogènes dont on souhaite répliquer le patron d'expression. Les gènes dont le domaine d'expression n'a pas été entièrement documenté ont, à l'état de transgène, un patron d'expression souvent

* Corresponding author. Tel.: +34 915854844; fax: +34 915854506.
*E-mail address:* montoliu@cnb.uam.es (L. Montoliu).

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imprévisible et moins satisfaisant que ce que l'on attend chez les animaux. L'approche comparative de la structure des génomes permet de mettre en évidence des séquences hautement conservées au cours de l'évolution, ce qui contribue à l'identification d'éléments régulateurs cruciaux associés à des domaines donnés caractérisés par une expression bien définie des gènes qu'ils contiennent. L'utilisation de vecteurs de type chromosomes artificiels assure la présence de ces éléments régulateurs indispensables pour une expression fiable des gènes. Ces constructions peuvent aussi contenir des isolateurs, une sous catégorie de séquences régulatrices dont l'utilisation est abordée via la transgenèse. L'obtention d'animaux trangéniques à l'aide de constructions contenant de longs fragments d'ADN génomique est donc une approche recommandée si l'on souhaite une expression optimale des trangènes respectant le patron d'expression attendu des gènes correspondant du locus.
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*Mots clés :* Transgène ; Expression ; Isolateur ; ADN génomique

The ability of genetically modify animals has been instrumental to generate additional and useful knowledge for the adequate interpretation of the genetic information included in the first available mammalian genomes. Functional genomic approaches have enabled the correlation between structural, and hence descriptive information (primary sequence of DNA), and putative function associated with a given piece of a genome. Both the over-expression of genomic loci via standard transgenesis procedures and their precise inactivation through homologous recombination techniques in embryonic stem cells or, more recently, in somatic cells along with nuclear transfer techniques, have served for advancing our current understanding of how genes actually work and execute their function [1,2].

With regard to traditional transgenic animals, those produced by standard pronuclear microinjection of DNA of unfertilised oocytes, the use of large genomic-type constructs, such as bacterial (BAC) or yeast (YAC) artificial chromosomes, normally ensures optimal transgene expression, recapitulating the expression pattern of the endogenous gene [3]. This is currently explained because the large size of these genomic transgenes usually allows the inclusion of most, if not all, regulatory elements that are required for the faithful expression of a gene. Therefore, selecting the most appropriate regulatory sequences that surround a gene of interest is fundamental for a successful project involving the use of transgenic animals.

Classically, several approaches have been devised to look for the existence of potential regulatory elements. The first type of analyses involved studying the structural properties of the chromatin fragment that included and surrounded our gene of interest. Among other techniques, the controlled digestion of chromatin with DNAseI and other nucleases indicated, on one hand, sites that were prone to digestion (interpreted as pieces of ''open'' or ''accessible'' chromatin). These sites are known as hypersensitive sites (HSS) to DNAseI digestion. On the other hand, sequences that were essentially refractory to DNAseI digestion were currently interpreted as pieces of ''closed'' or ''inaccessible'' chromatin [4,5]. Normally, HSS coincided with the presence of binding sites for powerful nuclear proteins, functioning as enhancer elements, which were crucial for the correct expression of the gene [6]. In addition, several databases were compiled with information of consensus

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