7 High Resolution Gene Expression Analysis in Mice Using Genetically Inserted Reporter Genes

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7.1 Introduction

Detailed analysis of the temporal and spatial patterns of expression of a gene in situ can provide a wealth of knowledge regarding the potential functional roles of a gene product. However, such detailed analysis of gene expression patterns represents a great challenge to biologists. Classical approaches such as northern blotting, RNAse protection, PCR assays and western blotting are highly reliable and sensitive means of detecting expression, but these methods completely lack spatial information indicating where the gene is expressed. Spatial information can be obtained by the use of similar approaches such as in situ hybridization and immunohistochemistry on tissue sections or whole tissue preparations. These methods, however, are limited by the availability of robust and reliable probes for the gene of interest as well as the availability of appropriately prepared tissues to probe. Even under optimal conditions, such approaches are not practical when the aim is to undertake an exhaustive screen to discover rare sites of expression in a range of ages.

The use of genetically inserted reporter genes with optimal detection characteristics, most notably the bacterial *LacZ* gene encoding β-galactosidase (β-Gal), in vivo by either targeted or transgenic insertion, allows one to screen a diverse array of tissues and developmental stages rapidly and easily, and to observe domains of expression with extremely high resolution. Coupling of reporter gene insertion with gene targeting and inactivation in mice represents an extremely powerful tool for elucidating gene function, allowing high resolution analysis of domains of expression coupled with the potential loss-of-function phenotypes of the gene of interest. This approach has been used with a great deal of success over the past 10 years in genetargeting, and considerably longer (but without the phenotypic component and with significant limitations) using

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randomly integrated constructs harboring the reporter gene downstream of the presumed promoter of a gene of interest. We favor the approach of utilizing a rational and standardized construct design that allows a chosen site of integration to be targeted. We will focus our discussion on the use of this approach.

The success of gene expression analysis using reporter genes in mice is highly dependent on a well designed targeting vector. Such designs rely greatly on the ability to place the reporter gene in a precise location within an endogenous locus. Until recently, the construction of such precisely designed vectors was hampered by the limitations of classical molecular biology techniques. However, the advent of the use of bacterial homologous recombination in generating targeting vectors has largely eliminated these difficulties and now allows the execution of practically any vector design with nucleotide precision. We have developed a technology we call "VelociGene" to achieve such goals with incredible success in a very large number of gene targeting projects. The reader is referred to our description of this method and Fig. 7.1 for further details (see (Valenzuela et al. 2003)).

Despite having the ability to minimize unwanted changes that may adversely affect reporter function by manipulating the genome with great precision, factors such as RNA stability and transcriptional and translational controls can also have significant effects on the normal expression of the gene of interest. Engineering the gene of interest by inserting a reporter and deleting and/or adding controlling elements (including the introduction of a non endogenous polyadenylation signal) might result in the reporter gene not being subject to all of the normal levels of control. Therefore, despite the clear advantages of using reporter genes in mice, it is important to bear in mind the parameters of the technology. Thus, it is appropriate to view a reporter gene in mice as most accurately reflecting promoter activity of the gene of interest rather than an ultimate indication of the precise levels of expression of the gene being targeted.

This chapter incorporates the extensive experience accrued from designing and analyzing hundreds of mouse gene-targeted models, in which a variety of reporter genes have been introduced in order to understand the normal or pathological pattern of expression of the gene of interest. This chapter should serve as a guide to users of transgenic and gene targeting technology in the selection of appropriate reporter genes for expression analysis; to provide some information regarding the design of optimal reporter gene constructs; and to provide detailed methods for the optimal detection of reporter genes in situ.

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