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A Practical Guide

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A Practical Guide

Edited by

Donald S. Anson

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Adelaide, Australia*

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Preface

Reporter genes have played, and continue to play, a vital role in many areas of biological research by providing a ready means for qualitative and quantitative assessment of the activity of genes and location of gene products in different environments. For example, reporter genes have played a major role in defining the activity of different genetic elements that control transcription of genes, both *in vitro* and *in vivo* (1,2), in the study of cell lineages (2,3) and in determining the effectiveness of different gene transfer technologies (4,5). While early reporter genes required fixation of cells for visualization, or the preparation of cell extracts for quantitative assays, there has been a move towards reporter genes that can be assayed quantitatively and/or qualitatively in live cells and animals. The two widely used examples of such markers are the fluorescent proteins (6–8) and luciferase (9,10). However, despite the development of these new reporter genes, one of the earliest, *E. coli* β -galactosidase (LacZ), is still widely used as a marker and offers significant advantages, especially for histological analysis (11).

Most reporter genes originate from non-mammalian species, and most have subsequently been modified to enhance their expression in mammalian cells and/or to modify their characteristics, vastly increasing their usefulness. For example, codon optimisation for expression in mammalian cells has been applied to the fluorescent proteins (12), luciferase (13) and β -galactosidase (14). The characteristics of fluorescent proteins have been extensively modified, with variants of many different colours and characteristics now available (15,16 and see chapter by Patterson in this book). LacZ (14,17,18) and fluorescent proteins (18,19) can also be targeted to the nucleus of cells by addition of suitable trafficking signals. Fluorescent proteins are also widely used for making fusion proteins to allow analysis of sub-cellular trafficking and compartmentalisation of proteins of interest (2,20,21).

Reporter genes have provided powerful tools for analysis of gene expression, either by localisation and/or by quantitative analysis. Examples of the former include the use of LacZ, where staining of microscopic sections gives information on gene expression at cellular resolution, and fluorescent proteins, where direct visualisation of tissue can give similar information. The use of luciferase as a marker gene *in vivo* also results in information regarding the localisation of gene expression in the living animal, although at a much lower resolution. In this system *in vivo* gene expression can be quantified by photonic imaging (see chapter by Ray and Gambhir).

Most marker genes can be used to provide quantitative data regarding gene expression. However, in some cases this requires the preparation of cell lysates. For example, β -galactosidase (LacZ) can be quantified by ELISA or enzyme activity and kits for this are available from a number of manufacturers, as are kits for quantitative analysis of several other enzymatic reporter genes such as alkaline phosphatase and luciferase. Reporter genes that can be quantitatively assayed without the preparation of a cell lysate include luciferase (*via* photonic imaging), secreted alkaline phosphatase (enzyme assay) and fluorescent proteins (*via* FACS analysis or microscopy).

New marker/reporter gene systems are currently being developed. For example, systems based on the use of positron-emission tomography (PET) offer a means for non-invasive imaging of all tissues (22,23). While these are not covered in this book, the development of microPET machines suitable for imaging rodents means that this technology is likely to be rapidly developed over the next few years. As indicated above, molecular engineering is constantly being used to improve existing reporter systems.

This book will describe practical protocols for experimentation with the most useful reporter genes for mammalian systems that are available and will concentrate on those marker genes that are currently most commonly used.

Donald S. Anson

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