Methods in Molecular Biology • 8

Practical Molecular Virology

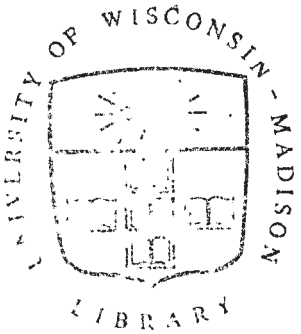
Viral Vectors for Gene Expression

Edited by

Mary K. L. Collins

Chester Beatty Laboratories, Institute of Cancer Research, London, UK

Humana Press • Clifton, New Jersey



Crescent Manor
PO Box 2148
Clifton, New Jersey 07015

Printed in the United States of America

Library of Congress Cataloging in Publication Data

Practical molecular biology: viral vectors for gene expression/
edited by Mary Collins.
p. cm. – (Methods in molecular biology : 8)
Includes index.
ISBN 0-89603-191-8
1. Genetic vectors. 2. Viruses. I. Collins, Mary II. Series:
Methods in molecular biology : v. 8.
[DNLM: 1. Gene Expression Regulation, Viral. 2. Genetic Vectors.
3. Viruses. W1 ME9616F v. 8 / QW 160 P895]
QH442.2.P73 1991
574.87'322–dc20
DNLM/DLC
for Library of Congress 91-7049
CIP

CHAPTER 5

Selectable Markers for Eukaryotic Cells

Richard Vile

1. Introduction

The transfer of DNA sequences into a population of cells can rarely, if ever, be achieved with 100% efficiency. Typically, transfection of cells with the calcium phosphate method will transduce only between 0.1 and 1% of the cells with the sequences of interest *(1)*, although some workers have achieved higher efficiencies *(2)*. If the transferred sequences do not confer a selective growth advantage, it is essential to use selection for transduced cells. The marker gene itself may be the gene of interest, e.g., to label a certain cellular population; alternatively, expression of the marker may merely be a convenient selection for the cellular population expressing another gene that has been cotransfected with the marker. Critically, selectable markers permit *positive* selection (i.e., the cells of interest are not killed); this is in contrast to systems in which demonstration of infection with a virus leads to death of the recipient cell (such as VSV pseudotypes, *see* Chapter 9).

1.1. Types of Selectable Markers

1.1.1. Dominant Selectable Markers

Many dominant selectable markers are bacterial genes that can be expressed in eukaryotic cells when placed under the control of suitable promoter elements. The recipient cells do not have to posses specific genotypes.

From: *Methods in Molecular Biology, Vol. 8:*
Practical Molecular Virology: Viral Vectors for Gene Expression
Edited by: M. Collins © 1991 The Humana Press Inc., Clifton, NJ

Frequently, two or more of these selection systems can be used together to introduce sequentially different sequences into the same cells. In such cases, it is necessary to titrate the selective conditions in tandem, rather than relying on the optimal conditions worked out for each selection separately.

1.1.1.1. Neomycin Phosphotransferase *(neo)*

One of the most frequently used dominant selectable markers has been the gene that confers resistance selectively to a group of antibiotics, including Geneticin® (G418-sulfate, or G418). Geneticin® is an aminoglycoside that is toxic to both prokaryotic and eukaryotic cells (obtained from GIBCO). However, certain bacterial strains contain mobile genetic elements (transposons) that encode dominantly acting genes with protein products that convert the toxic drug into tolerable byproducts. Two separate resistance genes are located on bacterial transposons Tn601 and Tn5. These genes are called aminoglycoside phosphotransferase 3' I and II [APH(3')I and APH(3')II], respectively, or neomycin phosphotransferase, *neo,* for short. Their functional transfer into eukaryotic cells permits those cells to grow in media containing G418.

1.1.1.2. Histidinol Dehydrogenase

The prokaryotic protein histidinol dehydrogenase catalyzes the NAD-linked oxidation of L-histidinol to histidine. L-Histidinol is normally toxic to eukaryotic cells, but cells stably expressing the histidinol dehydrogenase *(His D)* gene can survive in the presence of otherwise lethal doses of this drug *(3)*. Therefore, in medium lacking the essential amino acid histidine, but containing histidinol, expression of *His D* has the dual effect of removing the inhibitor and providing a required nutrient from its breakdown. Cells expressing the *His D* gene (following transfection or infection) can be grown in DMEM medium *lacking histidine* with added L-histidinol (Sigma Chemical Company), which has the advantage of being cheaper than G418 over long periods of selection.

1.1.1.3. Hygromycin Phosphotransferase

Hygromycin B is an aminocyclitol antibiotic produced by *Streptomyces hygroscopius (4)*. It is toxic to prokaryotic and eukaryotic cells, since it inhibits protein synthesis at the level of ribosomal translocation and aminoacyl-tRNA recognition. A plasmid-coded gene, hygromycin B phosphotransferase, has been isolated from *Escherichia coli* and has been shown to permit direct selection for hygromycin B resistance following transfection of eukaryotic cell lines *(5)*.

1.1.1.4. Xanthine-Guanine Phosphoribosyltransferase (GPT)

The bacterial enzyme xanthine-guanine phosphoribosyltransferase (GPT) *(6)* can provide, when expressed in mammalian cells, a purine salvage

function that is normally provided by expression of the mammalian enzyme hypoxanthine phosphoribosyltransferase (HPRT). However, unlike the mammalian enzyme, which cannot use xanthine for purine nucleotide synthesis, expression of *gpt* permits the synthesis of GMP from xanthine, via XMP. Therefore, it is possible to select for cells that are expressing *gpt* by supplying xanthine as the sole source of precursor for guanine nucleotide synthesis. Cells must also be grown in the presence of aminopterin, to prevent synthesis of inosine monophosphate (IMP) and, simultaneously, mycophenolic acid is added to inhibit IMP dehydrogenase, thus preventing the formation of guanosine monophosphate. Therefore, if cells are grown in hypoxanthine and xanthine, only cells transformed with the *gpt* gene will be capable of generating the guanine that they require to grow.

1.1.1.5. Other Dominant Selectable Markers

Several other dominant-selectable-marker systems exist that rely on the expression of prokaryotic genes in eukaryotic cells. The *neo* marker is very versatile, since it allows selection in both prokaryotes and eukaryotes, and is widely used. However, it is very expensive to use in the relatively large quantities required to keep cell lines under continual selection. As well as those discussed above, other marker genes are available, including the dihydrofolate reductase gene (DHFR) *(7,8)*, with selection using methotrexate in the range of 0.1–10 μ*M*; the *E. coli* tryptophan synthetase B gene (*trp B*), with selection in medium containing indole in place of tryptophan *(3)*, and, more recently, the puromycin resistance gene *(9)*, with subsequent selection in puromycin in the range of 1.0–10 μg/mL. The particular choice of system depends on the availability of the respective antibiotics/marker plasmids, the preexisting phenotype of the target cell types, and, increasingly, on budgetary constraints.

1.1.2. Selection by Exploitation of Cellular Mutations

1.1.2.1. Thymidine Kinase Selection

This selection relies on the existence of cells deficient in the expression of the *TK* gene *(10)*. Such cells are unable to make IMP from thymidine and can be grown in aminopterin to prevent other pathways being used for IMP synthesis. Such conditions will kill tk^- cells. If the *TK* gene is now delivered by infection or transfection, along with thymidine and hypoxanthine added to the medium, transfected cells will survive. The only recipient cells that can be used for this type of selection are, necessarily, tk^- (e.g., mouse L tk^- cells), so such a selection system is not as broadly useful as the dominantly acting ones described earlier.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.