Protein Expression

A PRACTICAL APPROACH

Edited by
S. J. HIGGINS and B. D. HAMES

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Preface

Some years ago we edited a book for The Practical Approach series entitled *Transcription and translation: a practical approach*. When the time came to consider organizing a second edition, it rapidly became clear that no one book of the desired size could include in sufficient detail the myriad of important new techniques for investigating gene expression. As a result, a decision was taken to produce a collection of books to cover this important area. *Gene transcription: a practical approach* and two volumes of *RNA processing: a practical approach* have since been published. Now, this book, *Protein expression: a practical approach*, and its companion volume, *Post-translational processing: a practical approach*, complete the 'mini-series' by providing a comprehensive and up-to-date coverage of the synthesis and subsequent processing of proteins.

Protein expression: a practical approach describes in detail the expression of cloned DNA or RNA templates in all the major *in vitro* and *in vivo* systems, both prokaryotic and eukaryotic, as well as methods for monitoring expression. The *in vivo* systems include cultured mammalian cells (described comprehensively by Marlies Otter-Nilsson and Tommy Nilsson), yeast (by Mick Tuite and his colleagues), baculovirus (Bob Possee *et al.*), and *Xenopus* (Glenn Matthews). Expression *in vivo* in prokaryotes is covered by Ed Appelbaum and Allan Shatzman. On the *in vitro* side, the chapter by Mike Clemens and Ger Pruijn focuses on the purification of eukaryotic mRNA and its translation in cell-free extracts. The prokaryotic *in vitro* systems of note are those that offer coupled transcription–translation and hence these are the subject of the chapter by Boyd Hardesty's group. Finally, John Colyer's chapter provides essential techniques for monitoring protein expression.

Those researchers who wish to fully characterize the expressed protein product, and to follow its post-translational fate, are advised to also consult the companion volume, *Post-translational processing: a practical approach*, which covers protein sequence analysis, protein folding and import into organelles, protein modification (phosphorylation, glycosylation, lipid modification), and proteolytic processing.

The overriding goals of *Protein expression: a practical approach* are to describe, in precise detail, tried and tested versions of key protocols for the active researcher, and to provide all the support required to make the techniques work optimally, including hints and tips for success, advice on potential pitfalls, and guidance on data interpretation. We thank the authors for their diligence in writing such strong chapters and for accepting the editorial changes we suggested. The end-result is a comprehensive compendium of the best of current methodology in this subject area. It is a book designed both to be used at the laboratory bench and to be read at leisure to gain insight into future experimental approaches.

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Abbreviations

5-FOA	5-fluoro-orotic acid
A_{260}	absorbance at 260 nm
αAA	α-aminoadipate
AcMNPV	*Autographa californica* multiple nucleopolyhedrovirus
ATP	adenosine 5′-triphosphate
BCIP	5-bromo-4-chloro-3-indolyl phosphate
BFP	blue fluorescent protein
BSA	bovine serum albumin
cAMP	adenosine 3′,5′-cyclic monophosphate
CAT	chloramphenicol acetyltransferase
CIP	calf intestinal alkaline phosphatase
DAB	diaminobenzidine
DDAB	dimethyldioctadecyl ammonium bromide
DEAE	diethylaminoethyl
DEPC	diethyl pyrocarbonate
DHF	dihydrofolate
DHFR	dihydrofolate reductase
dia.	diameter
DMEM	Dulbecco's modified Eagle's medium
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
DOC	deoxycholate
DOTMA	*N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethyl ammonium chloride
dsRNA	double-stranded RNA
DTE	dithioerythritol
DTT	dithiothreitol
ε	molar extinction coefficient
ECL	enhanced chemiluminescence
EDTA	ethylenediaminetetraacetic acid
EGTA	ethyleneglycol-*O*,*O*′*bis*(2-aminoethyl)-*N*,*N*,*N*′,*N*′-tetraacetic acid
eIF	eukaryotic initiation factor
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
ER	endoplasmic reticulum
FA	folinic acid
FACS	fluorescence-activated cell sorting
FCS	fetal calf serum
FITC	fluorescein isothiocyanate

G-6-P	glucose 6-phosphate
GFP	green fluorescent protein
GST	glutathione *S*-transferase
GTP	guanosine 5′-triphosphate
GUS	β-glucuronidase
HBS	Hepes-buffered saline
h.p.i.	hours post-infection
hyg	hygromycin
HzSNVP	*Heliothis zea* single nucleopolyhedrovirus
IgG	immunoglobulin class G
IPTG	isopropyl-β-D-thiogalactoside
KLH	keyhole limpet haemocyanin
LB	Luria broth
LM	low molecular weight mixture
MBS	modified Barth's saline
MCS	multiple cloning site
MFα1	mating factor α1
MNPV	multiple nucleopolyhedrovirus
m.o.i.	multiplicity of infection
MOPS	3-(*N*-morpholino)propane sulfonic acid
M_r	relative molecular mass
mRNA	messenger RNA
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
NBT	nitroblue tetrazolium
NCYC	National Collection of Yeast Cultures
nd	not determined
neo	neomycin
Ni-NTA	nitrilo-triacetic acid chelated with Ni^{2+} ions
NMR	nuclear magnetic resonance
NPV	nucleopolyhedrovirus
OD_{650}	optical density (at 650 nm)
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
PEP	phosphoenolpyruvate
p.f.u.	plaque-forming units
pI	isoelectric point
P_i	inorganic phosphate
PMSF	phenylmethylsulfonyl fluoride
P-ser	phosphoserine
p.s.i.	pounds per square inch
P-thr	phosphothreonine

PTT	protein truncation test
P-tyr	phosphotyrosine
PVDF	polyvinylidene difluoride
RBS	ribosome binding site
rDNA	ribosomal DNA
RNase	ribonuclease
S30	supernatant from 30 000 *g* centrifugation
SDS	sodium dodecyl sulfate
Sf	*Spodoptera frugiperda*
SFV	Semliki forest virus
SNPV	single nucleopolyhedrovirus
ssRNA	single-stranded RNA
TBS	Tris-buffered saline
TBST	Tris-buffered saline containing Tween
TCA	trichloroacetic acid
TE	Tris-EDTA
TLC	thin-layer chromatography
tRNA	transfer RNA
UTP	uridine 5′-triphosphate
UTR	untranslated region
V	volts
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside
YGSC	Yeast Genetics Stock Center

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Protein expression in mammalian cells

MARLIES OTTER-NILSSON and TOMMY NILSSON

1. Introduction

Protein expression has become a major tool to analyse intracellular processes, both *in vitro* and *in vivo*. The choice of expression system depends entirely on the purpose of the study. For some cases, transient transfection is the most obvious choice because of its relatively short time investment. In other cases, homogeneous populations and large quantities of cells may be required, which involves making cell lines stably expressing the desired protein. It may also be advantageous to express proteins under inducible promotors; this is particularly true if the protein exerts pathological effects on the cell. There are several inducible systems available but none, so far, is easy and straightforward. Expression through virus infection is also possible. Here, large quantities of cells can be infected at the same time and the protein assayed for shortly after infection. The drawback, however, with this method is the need for special precautions when making and handling virus stocks and likely side-effects exerted by the virus on the cellular machinery upon infection. Protein expression can also be achieved directly via microinjection of plasmid DNA directly into the nucleus of the host cell. This allows for protein expression in single cells which may be desirable when performing video microscopy. Thus the choices of expression systems are multiple and one should carefully consider the range of these available before investing time and other resources in the experiments themselves. It is the goal of this chapter to describe the various expression systems so as to make this choice easier.

2. Viral and plasmid vectors

2.1 Semliki forest virus

A vector based on Semliki forest virus (SFV) has been developed by Liljeström and co-workers (1) and has turned out to be a very efficient expression system in mammalian cells. The virus has a genome of ssRNA which is

capped and polyadenylated and has a positive polarity, acting as a direct mRNA upon infection. It encodes its own RNA polymerase producing viral RNA transcripts. Vectors pSFV1, 2, and 3, which lack the structural protein genes of the virus, and helper vectors 1 and 2, encoding the structural viral proteins have all been described (1, 2). The pSFV1, 2, and 3 all have a polylinker with unique restriction sites, followed by stop codons in all three reading frames. The three vectors have minor differences with respect to their cloning sites. The pSFV3 vector has an additional ribosome binding site and initiation codon within the vector and is therefore the most convenient vector to use. The DNA encoding the protein of interest is cloned into one of the pSFV vectors under control of the viral promotor. The recombinant pSFV viral DNA and the helper vector are then linearized by *Spe*I (note: the insert should not contain a *Spe*I site!) and then used for *in vitro* transcription to obtain RNAs. Co-transfection of the helper RNA and the pSFV RNA into cells yields both protein and virions containing the recombinant RNA. Production of these virions is described in *Protocol 1*.

The recombinant pSFV virions can now be used to infect an appropriate mammalian cell line such as BHK, Vero, HeLa, or MDCK II (3), during which the gene of interest cloned into the recombinant virus is expressed (*Protocol 2*).

Protocol 1. Transfection of recombinant viral RNA into mammalian cells by electroporation

Equipment and reagents

- Electroporation equipment, e.g. Gene-pulser (Bio-Rad)
- Electroporation cuvettes
- Cell scraper (rubber policeman)
- Mammalian cells in tissue culture dishes in the logarithmic phase of growth
- Growth medium supplemented with 10% FCS
- PBS: 1.37 mM NaCl, 2.7 mM KCl, 8.2 mM Na_2HPO_4, 1.8 mM KH_2PO_4 pH 7.4
- Recombinant RNA and helper RNA encoding the structural viral proteins (1–5 μg)
- 0.1% crystal violet in 20% ethanol
- Trypsin/EDTA: 0.5 mg/ml trypsin, 0.2 mg/ml EDTA

A. *Infection of the cells*

1. Grow the cells to 80% confluency, then pour off the growth medium, and wash the cells with PBS.
2. Scrape the cells off the tissue culture dish using a rubber policeman, or detach the cells using trypsin/EDTA.
3. Centrifuge the cells at 400 *g* for 5 min.
4. Wash the cells with PBS by centrifugation (400 *g* for 5 min).
5. Resuspend the cells at 10^7 cells/ml in PBS, add 1–5 μg recombinant pSFV RNA, and 1 μg helper RNA in a total volume not exceeding 800 μl.