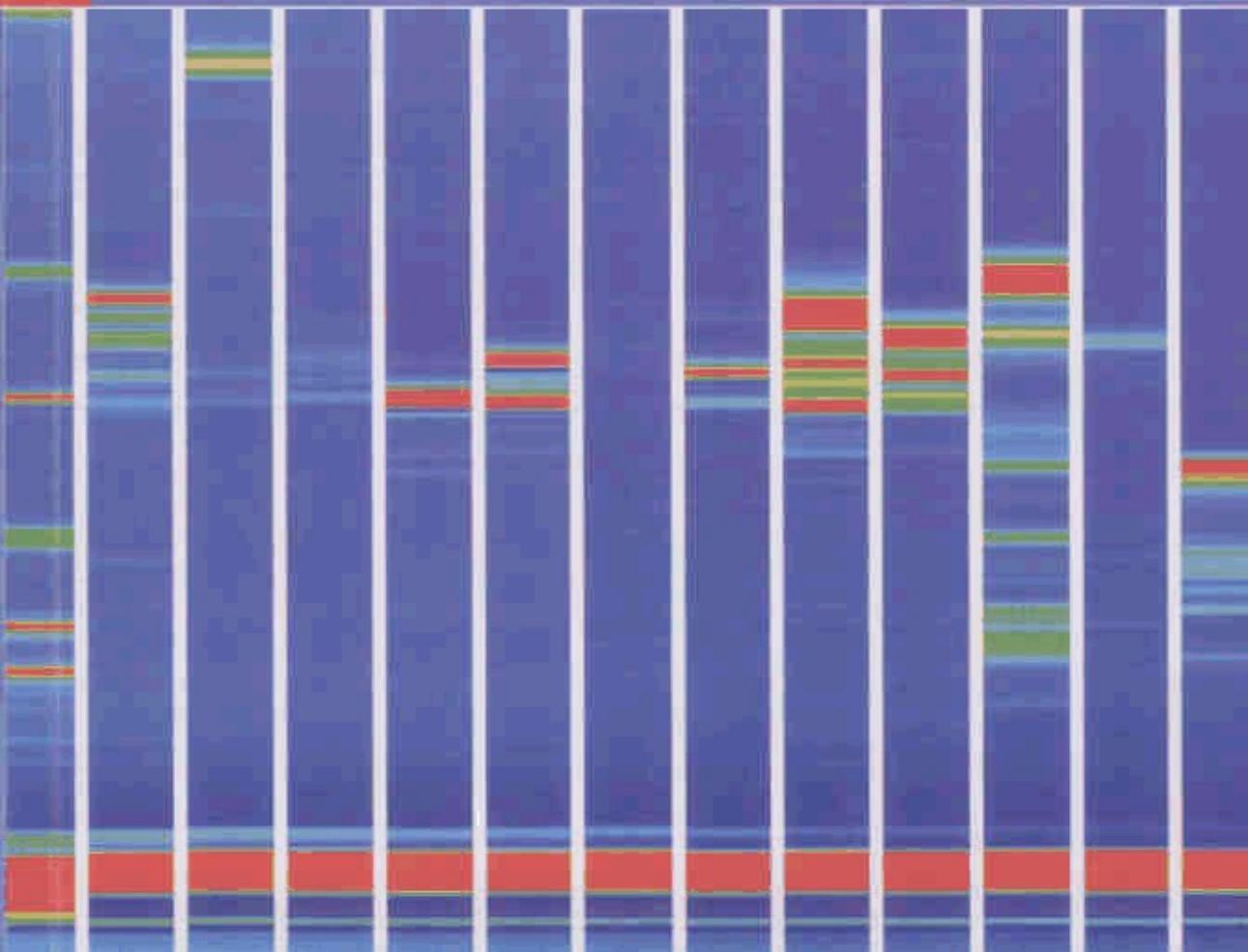


# Expression Systems

edited by Michael R. Dyson  
and Yves Durocher



# Contents

Contributors	xi
Preface	xiv
Abbreviations	xv
<b>Color section</b>	xix

## **Chapter 1. Expression strategy**

*Michael R. Dyson*

1. Introduction	1
2. Methods and approaches	3
2.1 Case study 1: mouse Rab3a	4
2.2 Case study 2: human epidermal growth factor receptor	8
2.3 Expression of previously unexpressed proteins	9
3. Troubleshooting	10
4. References	10

## **Chapter 2. Protein expression in *Escherichia coli***

*Rosalind Kim*

1. Introduction	13
2. Methods and approaches	14
2.1 Ligation-independent cloning	14
2.2 Small-scale expression	19
2.3 Cell paste growth	22
2.4 Clear lysate preparation	23
2.5 Purification of His <sub>6</sub> -tagged protein	24
2.6 Tobacco etch virus protease digestion of fusion protein	25
3. Troubleshooting	27
4. References	28

**Chapter 3. Expression engineering of synthetic antibodies using ribosome display***Lydia M. Contreras Martínez and Matthew P. DeLis*

1. Introduction	29
2. Methods and approaches	31
2.1 Principles of ribosome display	31
2.2 Experimental overview of ribosome display	32
2.3 ScFv gene sequence and control elements	33
2.4 Construction of an scFv ribosome display vector (pscFvDisplay)	34
2.5 Amplification and purification of the DNA target	37
2.6 <i>In vitro</i> transcription of a purified DNA library	39
2.7 <i>In vitro</i> translation of a mRNA library	40
2.8 Affinity selection of ribosome complexes and mRNA isolation	44
2.9 Reverse transcriptase PCR	44
2.10 <i>In vivo</i> analysis of selected scFvs	49
3. Troubleshooting	49
4. References	50

**Chapter 4. Refolding proteins from inclusion bodies***Renaud Vincentelli*

1. Introduction	53
2. Methods and approaches	54
2.1 Preparation and purification of inclusion bodies	57
2.2 Production	59
2.3 Solubilization of inclusion bodies	60
2.4 Refolding of solubilized proteins	60
2.5 Analysis of refolded protein	63
2.6 Conclusion	64
3. References	64

**Chapter 5. Selection of protein variants with improved expression using green fluorescent protein-derived folding and solubility reporters***Stéphanie Cabantous and Geoffrey S. Waldo*

1. Introduction	67
2. Methods and approaches	68
2.1 GFP insertion technology	68
2.2 Principles of GFP insertion	69
2.3 Methodology	71
2.4 Selecting optima with improved solubility	72
3. Troubleshooting	85
4. References	86

**Chapter 6. Protein expression in the wheat-germ cell-free system***Tatsuya Sawasaki and Yaeta Endo*

1. Introduction	87
2. Methods and approaches	88
2.1 Principles of the new wheat-germ cell-free system	88
2.2 Preparation of the extract from wheat embryos	89
2.3 Development of the 5'UTR of mRNA to enhance translation	92
2.4 Expression vector pEU	92
2.5 PCR-directed generation of DNA template	93
2.6 Preparation of mRNA	95
2.7 Translation	97
2.8 Adapting the CFCF reaction for transcription and translation in one tube	100
2.9 Applications based on the cell-free system	102
3. Troubleshooting	106
4. References	107

**Chapter 7. *Saccharomyces cerevisiae*: a microbial eukaryotic expression system***Christine Lang*

1. Introduction	109
2. Methods and approaches	110
2.1 Vectors and promoters	110
2.2 Strains	112
2.3 Analysis of expression	113
2.4 Recommended protocols	114
3. Troubleshooting	120
4. References	121

**Chapter 8. Expression of proteins in *Pichia pastoris****Geoff P. Lin-Cereghino, Wilson Leung, and Joan Lin-Cereghino*

1. Introduction	123
1.1 Background	124
1.2 Choosing a plasmid	124
1.3 Choosing a host strain	127
2. Methods and approaches	130
2.1 Transformation	130
2.2 Screening of transformants	133
2.3 Small-scale expression	135
2.4 Optimization	141
2.5 Small-scale purification	141
2.6 Considerations for scaling up expression and purification	142
3. Troubleshooting	143
4. References	144

**Chapter 9. Improved baculovirus expression vectors***Richard B. Hitchman, Robert D. Possee, and Linda A. King*

1. Introduction	147
2. Methods and approaches	147
2.1 Principles of <i>flashBAC</i>	147
2.2 Insect cell culture requirements	149
2.3 Maintaining insect cells in monolayer culture	153
2.4 Construction of recombinant baculoviruses using <i>flashBAC</i>	155
2.5 Automated production of recombinant baculoviruses	162
3. Troubleshooting	167
4. References	167

**Chapter 10. Transient transfection of insect cells for rapid expression screening and protein production***Kathryn H. Loomis, Courtney R. Rockwell, Heather D. Sternard, Keith W. Yaeger, and Robert E. Novy Jr*

1. Introduction	169
2. Methods and approaches	170
2.1 Media and insect cells	170
2.2 Transient expression vectors	171
2.3 Transfection reagent	171
2.4 Insect cell lysis	172
2.5 IMAC purification of His•Tag fusion proteins	172
2.6 Recommended protocols	172
2.7 Examples of results	177
3. Troubleshooting	180
4. References	182

**Chapter 11. Generation of stable CHO cell lines for protein expression***Zhijian Lu, Haley Laken, Jimin Zhang, Xiaotian Zhong, and Richard Zollner*

1. Introduction	183
1.1 Cell lineage	184
1.2 Selection markers	185
2. Methods and approaches	185
2.1 Cell line maintenance	185
2.2 Cryopreservation of CHO cells	188
2.3 Cell line construction	190
2.4 Scaling up of CHO cells for recombinant protein production	196
2.5 Specialized application: host engineering	198
3. References	200

**Chapter 12. Transient expression in HEK293-EBNA1 cells***Roseanne Tom, Louis Bisson, and Yves Durocher*

1. Introduction	203
2. Methods and approaches	206
2.1 Cell culture	206
2.2 Plasmid DNA preparation	209
2.3 Preparation of PEI	210
2.4 Transfection of 293E and 293-6E cells	212
2.5 Purification of His-tagged r-proteins	218
2.6. Results	220
3. Troubleshooting	221
4. References	223

**Chapter 13. Nisin- and subtilin-controlled gene expression systems for****Gram-positive bacteria***Oscar P. Kuipers and Jan Kok*

1. Introduction	225
2. Methods and approaches	228
2.1 The NICE system	228
2.2 The SURE system	235
2.3 Future applications	238
3. Troubleshooting	238
4. References	239

**Chapter 14. Protein production using lentiviral vectors***Rénald Gilbert, Sophie Broussau, and Bernard Massie*

1. Introduction	241
1.1 Properties of lentiviral vectors	241
1.2 Essential components of LVs	241
1.3 Strategies to control protein expression from LVs	243
2. Methods and approaches	244
2.1 Production of LVs	244
2.2 Concentration of LVs	250
2.3 LV titration	252
2.4 Cell marking and protein production using LVs	255
3. Troubleshooting	258
4. References	259

**Chapter 15. Expression in mammalian cells using BacMam viruses***Hsiao-Ping Lee and Yu-Chen Hu*

1. Introduction	261
2. Methods and approaches	262
2.1 Construction and production of BacMam virus	262

x ■ CONTENTS

2.2	Transduction of mammalian cells using culture medium as the surrounding solution	262
2.3	Improved protocol for BacMam transduction	265
2.4	Protein production in a BelloCell-500 bioreactor	267
2.5	Determination of baculovirus transducing ability in mammalian cells	271
3.	Troubleshooting	274
4.	References	274
 <b>Appendix 1</b>		
List of suppliers		277
 <b>Index</b>		281