



Genomics

Essential Methods

Editors | Mike Starkey and Ramnath Elaswarapu

 WILEY-BLACKWELL

Contents

List of Contributors	xi
Preface	xv
1 High-Resolution Analysis of Genomic Copy Number Changes	1
<i>Mario Hermesen, Jordy Coffa, Bauke Ylstra, Gerrit Meijer, Hans Morreau, Ronald van Eijk, Jan Oosting and Tom van Wezel</i>	
1.1 Introduction	1
1.2 Methods and approaches	2
1.2.1 Oligonucleotide aCGH	2
1.2.2 SNP aCGH	15
1.2.3 Multiple ligation-dependent probe amplification (MLPA)	19
1.3 Troubleshooting	28
References	29
2 Identification of Polymorphic Markers for Genetic Mapping	33
<i>Daniel C. Koboldt and Raymond D. Miller</i>	
2.1 Introduction	33
2.2 Methods and approaches	34
2.2.1 Repositories of known genetic variants	34
2.2.2 Targeted resequencing for variant discovery	35
2.3 Troubleshooting	45
2.3.1 Primer design	45
2.3.2 PCR amplification	45
2.3.3 Working with binary trace files	46
2.3.4 Phred/Phrap	46
References	46
3 Genotyping and LOH Analysis on Archival Tissue Using SNP Arrays	49
<i>Ronald van Eijk, Anneke Middeldorp, Esther H. Lips, Marjo van Puijenbroek, Hans Morreau, Jan Oosting and Tom van Wezel</i>	
3.1 Introduction	49
3.2 Methods and approaches	50
3.2.1 Arrays	50
3.2.2 Genotyping	50

3.2.3	Linkage and association analysis	51
3.2.4	Formalin-fixed, paraffin-embedded tissue	51
3.2.5	Loss of heterozygosity	58
3.3	Troubleshooting	63
	References	64
4	Genetic Mapping of Complex Traits	67
	<i>Nancy L. Saccone</i>	
4.1	Introduction	67
4.2	Methods and approaches	68
4.2.1	Association methods: unrelated case-control samples	68
4.2.2	Association methods: family-based samples	81
4.2.3	Linkage methods: parametric LOD score analysis	82
4.2.4	Linkage methods: non-parametric methods	83
4.2.5	Summary and conclusions	84
4.3	Troubleshooting	84
4.3.1	Combining datasets	84
	References	85
5	RNA Amplification Strategies: Toward Single-Cell Sensitivity	91
	<i>Natalie Stickle, Norman N. Iscove, Carl Virtanen, Mary Barbara, Carolyn Modi, Toni Di Berardino, Ellen Greenblatt, Ted Brown and Neil Winegarden</i>	
5.1	Introduction	91
5.1.1	The need for amplification	91
5.1.2	Amplification approaches	93
5.2	Methods and approaches	100
5.2.1	T7 RNA polymerase-based <i>in vitro</i> transcription	100
5.2.2	Global-RT-PCR	107
5.3	Troubleshooting	115
	References	116
6	Real-Time Quantitative RT-PCR for mRNA Profiling	121
	<i>Stephen A. Bustin and Tania Nolan</i>	
6.1	Introduction	121
6.2	Methods and approaches	122
6.2.1	Sample selection	122
6.2.2	RNA extraction	123
6.2.3	Clinical and environmental samples	127
6.2.4	Reverse transcription	130
6.2.5	qPCR using SYBR green I dye detection	134
6.2.6	qPCR using labeled oligonucleotide probe detection	137
6.2.7	Quantification methods	140
6.2.8	RT-qPCR standardization	143
6.3	Troubleshooting	144
6.3.1	No/Poor/Late amplification	144
6.3.2	No-template, negative control yields an amplification product	147

6.3.3	No reverse transcriptase control yields an amplification product	148
6.3.4	Primer dimers formed	148
6.3.5	Multiple peaks in SYBR green I melt curve	148
6.3.6	Standard curve is unreliable (correlation coefficient <0.98 over at least 5 log dilution and with samples repeated in triplicate)	149
6.3.7	Erratic amplification plots/high well-to-well variation	149
	References	149
7	Gene Expression in Mammalian Cells	155
	<i>Félix Recillas-Targa, Georgina Guerrero, Martín Escamilla-del-Arenal and Héctor Rincón-Arano</i>	
7.1	Introduction	155
7.1.1	Artificial chromosomes and transgenesis	157
7.1.2	Gene transfer and expression problems	157
7.1.3	Position effects and chromatin	157
7.1.4	Tissue-specific regulatory elements	158
7.1.5	Sustained expression and chromatin insulators	158
7.2	Methods and approaches	159
7.2.1	Site-specific chromosomal integration in mammalian cells	159
7.2.2	Plasmid requirement	161
7.2.3	Chromosome transfer	163
7.3	Troubleshooting	169
	Acknowledgments	169
	References	170
8	Using Yeast Two-Hybrid Methods to Investigate Large Numbers of Binary Protein Interactions	173
	<i>Panagoula Charalabous, Jonathan Woodsmith and Christopher M. Sanderson</i>	
8.1	Introduction	173
8.2	Methods and approaches	174
8.2.1	Producing large numbers of bait or prey clones	174
8.2.2	Generating recombination-compatible inserts for gap repair cloning	177
8.2.3	Performing gap repair reactions	179
8.2.4	Identifying positive transformants	181
8.2.5	Yeast colony PCR	181
8.2.6	Bait and prey auto-activation tests	183
8.2.7	Targeted 'matrix'-style Y2H screens	184
8.3	Troubleshooting	188
	References	189
9	Prediction of Protein Function	191
	<i>Hon Nian Chua</i>	
9.1	Introduction	191
9.2	Methods and approaches	191
9.2.1	Annotation schemes	192
9.2.2	Working with multiple protein identifier systems	195

9.2.3	Sequence homology	196
9.2.4	Phylogenetic relationships	199
9.2.5	Sequence-derived functional and chemical properties	202
9.2.6	Protein–protein interaction maps	203
9.3	Troubleshooting	205
	References	205
10	Elucidating Gene Function through Use of Genetically Engineered Mice	211
	<i>Mary P. Heyer, Cátia Feliciano, João Peca and Guoping Feng</i>	
10.1	Introduction	211
10.2	Methods and approaches	212
10.2.1	Principles of targeted gene deletion in mice	212
10.2.2	Strategies for gene targeting in mice	215
10.2.3	Retrieval of DNA from BAC by recombineering	217
10.2.4	ES and MEF cell culture	222
10.2.5	Mating of chimeras and downstream applications	244
10.3	Troubleshooting	245
	References	246
11	Delivery Systems for Gene Transfer	249
	<i>Charlotte Lawson and Louise Collins</i>	
11.1	Introduction	249
11.2	Methods and approaches	250
11.2.1	The ideal gene therapy vector	250
11.2.2	Plasmid design	251
11.2.3	Viral vectors	252
11.2.4	Non-viral DNA vectors	263
11.2.5	Assessing the physical properties of a non-viral vector	267
11.2.6	Optimizing <i>in vitro</i> gene delivery	268
11.2.7	Optimization strategies	271
11.2.8	Reporter genes and assays	271
11.2.9	Cytotoxicity assays	272
11.2.10	Future steps for non-viral vector development	272
11.3	Troubleshooting	273
11.3.1	General points	273
	References	274
12	Gene Therapy Strategies: Constructing an AAV Trojan Horse	283
	<i>M. Ian Phillips, Edilamar M. de Oliveira, Leping Shen, Yao Liang Tang and Keping Qian</i>	
12.1	Introduction	283
12.1.1	General strategies for gene therapy: Basic methods	284
12.1.2	Gene therapy strategies: Delivering genes to cells	287
12.1.3	Viral delivery	288
12.1.4	Production, purification and titration of recombinant adeno-associated virus (rAAV)	291

12.2 Methods and approaches	292
12.3 Troubleshooting	303
References	304
13 An Introduction to Proteomics Technologies for the Genomics Scientist	307
<i>David B. Friedman</i>	
13.1 Introduction	307
13.2 Methods and approaches	309
13.2.1 Gel-based strategies	309
13.2.2 LC/MS strategies	312
13.2.3 MALDI imaging and profiling	314
13.3 Troubleshooting	316
13.3.1 Number of resolved features and modifications	316
13.3.2 Sample consumption, protein identification and depth of coverage	317
13.3.3 Statistical power	317
13.3.4 Conclusions	318
References	318
Index	325