

Contents

Part I Cloning of the Antigen-binding Site from Hybridoma

1 Cloning of Variable Domains from Mouse Hybridoma by PCR	3
Nina Strebe, Frank Breitling, Dieter Moosmayer, Bodo Brocks, and Stefan Dübel	
2 Coning Hybridoma cDNA by RACE	15
Andrew Bradbury	
3 Construction of scFv Fragments from Hybridoma or Spleen Cells by PCR Assembly	21
Jonas V. Schaefer, Annemarie Honegger, and Andreas Plückthun	

Part II Generation of Antibody Repertoires

4 Mouse Immune Libraries for the Generation of ScFv Fragments Directed Against Human Cell Surface Antigens	47
Christian Kellner, Sahar Mohseni Nodehi, and Matthias Peipp	
5 Human Antibody Gene Libraries	65
Michael Hust and Stefan Dübel	
6 Synthetic Antibody Libraries	85
Pierre Martineau	

7 Immune Libraries from Nonhuman Primates (NHP)	99
Thibaut Pelat, Michael Hust, and Philippe Thullier	
8 Generation of Rabbit Immune Libraries	115
Rüdiger Ridder and Hermann Gram	
 Part III Selection of Antibody Fragments from Combinatorial Libraries	
9 Immunosorbent Selections	127
Roland E. Kontermann	
10 Phage Display and Selection in Microtitre Plates	139
Michael Hust and Michael Mersmann	
11 Phage Display and Selections on Biotinylated Antigens	151
Patrick Chames and Daniel Baty	
12 Phage Display and Subtractive Selection on Cells	165
Steffen U. Eisenhardt and Karlheinz Peter	
13 Selection of Phage Antibody Libraries for Binding and Internalization into Mammalian Cells	183
Yu Zhou and James D. Marks	
14 Improving Phage Display Throughput by Using Hyperphage, Miniaturized Titration and pVIII (g8p) ELISA	197
Frank Breitling, Olaf Broders, Saskia Helmsing, Michael Hust, and Stefan Dübel	
15 Yeast Display and Selections	207
Kelly Davis Orcutt and K. Dane Wittrup	
16 The Generation of Transgenic Mice Expressing Human Antibody Repertoires	235
Xiangang Zou, Guocheng Lan, Michael J. Osborn, and Marianne Brüggemann	
17 Selection of Antibody Fragments by Means of the Filter-Sandwich Colony Screening Assay	255
Sabine Rauth, Martin Schlapschy, and Arne Skerra	
18 Semi-automated Magnetic Bead-Based Antibody Selection from Phage Display Libraries	267
Zoltán Konthur, Jeannine Wilde, and Theam Soon Lim	

Part IV Engineering and Production of Immunoglobulins

19 Aspects of Isotype Selection	291
Zehra Kaymakcalan, Alexander Ibraghimov, Andrew G. Goodearl, and Jochen G. Salfeld	
20 Generation of Heavy and Light Chains (Chimeric Antibodies)	307
Kirstin A. Zettlitz	
21 Humanising Antibodies by CDR Grafting	319
David Gareth Williams, David J. Matthews, and Tarran Jones	
22 Humanization by Resurfacing	341
Johan Desmet, Karen Vanhoorelbeke, and Hans Deckmyn	
23 Human Antibodies by Guided Selection	355
Sang Jick Kim, Insoo Park, and Hyo Jeong Hong	
24 In Silico De-Immunization	369
Philippe Alard, Johan Desmet, and Ignace Lasters	
25 Affinity Maturation by Chain Shuffling and Site Directed Mutagenesis	377
Jianlong Lou and James D. Marks	
26 Affinity Maturation by Random Mutagenesis and Phage Display	397
Holger Thie	
27 Engineering of the Fc Region for Improved PK (FcRn Interaction)	411
Vania E. Kenanova, Tove Olafsen, Jan T. Andersen, Inger Sandlie, and Anna M. Wu	
28 Antibody-Dependent Enzyme Prodrug Therapy (ADEPT)	431
Richard Begent, Surinder Sharma, and Kerry Chester	
29 Production of Recombinant Human IgG Antibodies in the Baculovirus Expression System	453
Mifang Liang and Stefan Dübel	
30 Expression of IgA Molecules in Mammalian Cells	471
Thomas Beyer, Stefan Lohse, Michael Dechant, and Thomas Valerius	

Part V Antibody Characterization

31	Expression of Complete Antibodies in Transgenic Plants	489
	Doreen M. Floss and Udo Conrad	
32	Expression of Full Length Monoclonal Antibodies (mAb) in Algal Chloroplast	503
	Miller Tran and Stephen P. Mayfield	
33	Expression of IgG Antibodies in Mammalian Cells	517
	Thomas Jostock and Jiandong Li	
34	Protein A/G Chromatography	531
	Kirstin A. Zettlitz	
35	Epitope Analysis Using Synthetic Peptide Repertoires Prepared by SPOT Synthesis Technology	537
	U. Beutling and R. Frank	
36	Epitope Mapping by Printed Peptide Libraries	573
	Frank Breitling, Christopher Schirwitz, Thomas Felgenhauer, Ines Block, Volker Stadler, and Ralf Bischoff	
37	Antibody Epitope Mapping Using Yeast Display	591
	Consuelo Garcia-Rodriguez, Yu Zhou, and James D. Marks	
38	Size Exclusion Chromatography	607
	Bernd Voedisch and Holger Thie	
39	Structural Characterization of Antibodies by Mass Spectrometry	613
	Marie-Claire Janin-Bussat, Jean-Marc Strub, Elsa Wagner-Rousset, Olivier Colas, Christine Klinguer-Hamour, Nathalie Corvaia, Alain van Dorselaer, and Alain Beck	
40	Antibody Glycans Characterization	635
	Marie-Claire Janin-Bussat, Elsa Wagner-Rousset, Christine Klinguer-Hamour, Nathalie Corvaia, Alain van Dorselaer, and Alain Beck	
41	Affinity Measurements by Competition ELISA	657
	Pierre Martineau	

42 Anti-Histidine Antibodies as Tools for Reversible Capturing of His-Tagged Fusion Proteins for Subsequent Binding Analysis 667
H.-M. Zenn, S. Hutschenreiter, and F.W. Herberg

43 Affinity Measurements Using Quartz Crystal Microbalance (QCM) 683
Thomas Johansson

44 Affinity Measurements with Radiolabeled Antibodies 695
Verena Boschert and Peter Scheurich

45 Neutralization Tests 705
Philippe Thullier and Dorothea (Thea) Sesardic

46 Functional Characterization of Antibodies Neutralizing Soluble Factors *In Vitro* and *In Vivo* 723
Geertruida M. Veldman, Zehra Kaymakalan, Renee Miller, Leena Kalghatgi, and Jochen G. Salfeld

47 Competitive ELISA 739
Torsten Meyer

48 Quantification of Human IgG and Related Fc Fusion Proteins by a Human IgG/Fc Capture ELISA 743
Torsten Rülker, Doris Meier, and Thomas Schirrmann

49 Determination of Fc-Mediated Antibody-Effector Functions by Chromium Release Assay 749
Tina Otz

50 Binding Studies with Flow Cytometry 765
Thomas Schirrmann

Index 781