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Plasmids (2nd edition)
★ Platelets
Pollination Ecology
Postimplantation Mammalian Embryos
Preparative Centrifugation
Prostaglandins and Related Substances
Protein Blotting
Protein Engineering
Protein Function
Protein Phosphorylation
Protein Purification Applications
Protein Purification Methods
Protein Sequencing

Protein Structure
Protein Targeting
Proteolytic Enzymes
★ Pulsed Field Gel Electrophoresis
Radioisotopes in Biology
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Receptor-Ligand Interactions
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Antibody engineering

A Practical Approach

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Preface

Recombinant DNA methods have revolutionized the isolation and production of antibodies in recent years. Initially, recombinant antibodies were engineered and expressed in mammalian cells but by the late 1980s efficient expression of antibody fragments in bacteria had been demonstrated. More recently, the isolation of new antibody specificities has been transformed by the creation of large repertoires of antibody genes and the development of methods based on bacteriophage display to isolate specific antibodies. This has been paralleled by an increased understanding of the key residues affecting antibody structure and specificity together with more quantitative analysis of the binding properties of the antibodies. In addition, the mapping and sequencing of the repertoire of human antibody germline genes has put our understanding of the changes occurring during *in vivo* affinity maturation on a firmer footing.

We believe that now is the right time to assemble in one volume, protocols which allow the researcher to isolate a new antibody, analyse its properties, format the right antibody molecule or fragment, and produce sufficient quantities to be useful.

The book is structured in two parts: the first describes the generation and analysis of antibodies and the second covers engineering and production. Chapter 1 is designed to be the source of the standard 'repertoire' of phage-display protocols. In the following chapter the 'fine art' of the system, e.g. affinity maturation, is described. Methods for introducing antibody repertoires into transgenic mice are also described in the first section, as are methods for the analysis of the characteristics of antibodies with respect to affinity and sequence.

The second section describes the engineering of natural and man-made effector functions. The conversion of rodent antibodies to human antibodies by either CDR grafting or 'guided selection' using phage display is also covered. In addition, methods to manufacture significant quantities of various antibody-based molecules in both eukaryotic and prokaryotic systems are outlined.

Traditional monoclonal antibody technology has provided a wealth of reagents for research and diagnostic applications. The methods and technologies described here can help expand the versatility of antibody-based reagents in these areas. We have endeavoured in this book to provide researchers working with antibodies in research and diagnostics with a route into the 'new technologies', as practised by the leaders in the field.

This 'route' will be of equal value to workers in the field of antibody therapeutics. The ability to generate high-affinity, high-specificity human antibodies in appropriate formats will help circumvent some of the problems

Preface

associated with earlier work using mouse monoclonal antibodies and aid in their successful use *in vivo*. As we draw to the end of the millennium we have moved closer to the 'magic bullet' first hypothesized by Paul Ehrlich at the beginning of this century.

Cambridge
Maastricht
May 1996

J.McC.
H.R.H.
D.J.C.

Contents

List of Contributors

xvii

Abbreviations

xxi

1. Construction and use of antibody gene repertoires

1

A.R. Popr, M. J. Embleton, and R. Mernaugh

1. Introduction

1

2. Vectors for the display of proteins on the surface of bacteriophage fd

2

3. Preparation and cloning of antibody DNA

6

Preparation of mRNA

7

cDNA preparation

8

Primary PCR

9

Assembly of scFv fragments

17

Amplification and digestion

20

Ligation and transformation

22

4. Growth and expression of phage antibodies

23

Rescue of phage

23

Growth and soluble expression

25

Purification of soluble antibody

27

5. Selection of antibody variants displayed on the surface of bacteriophage

28

6. Analysis of phage-derived antibodies by ELISA

30

7. In-cell PCR assembly

32

Introduction

32

Application

37

8. Conclusions

38

References

39

2. Affinity maturation of antibodies using phage display

41

Kevin S. Johnson and Robert E. Hawkins

1. Introduction

41

General considerations

41

Contents

Mutagenesis	41
Selection	43
Screening	43
2. Mutagenesis	44
General considerations	44
Error-prone PCR	44
Site-directed mutagenesis	45
Mutagenesis using 'spiked' PCR primers	47
3. Selection of antibodies with altered properties	49
Selection of phage by panning	50
Solution capture on soluble antigen	51
Off-rate selection	53
4. Screening selected populations of antibodies	54
Affinity screen for phage antibody clones (K_d assay)	54
5. Sequence and fingerprint analysis	57
References	58
3. Human antibody repertoires in transgenic mice: manipulation and transfer of YACs	59
<i>Nicholas P. Davies, Andrei V. Popov, Xiangang Zou, and Mairianne Brüggemann</i>	
1. Introduction	59
2. Yeast artificial chromosomes	61
Library screening	62
Maintenance	62
3. Modification of YACs	63
Universal YAC vectors	64
Site-directed introduction	65
Mapping site-specific integration	67
Profile of single and multiple integrations	67
4. YAC transfer into embryonic stem cells	70
Spheroplast fusion	70
Picking and analysing clones	73
5. Conclusions	73
References	74
4. Measuring antibody affinity in solution	77
<i>Lisa Djavadi-Ohanian, Michel E. Goldberg, and Bertrand Friguet</i>	
1. General considerations	77
K_d does not directly reflect association or dissociation kinetics	77

x

Contents

True K_d cannot be determined when the mAb or the Ag is immobilized in a solid-phase assay	78
The determination of K_d must take into account the valency of the mAb and of the Ag molecule	78
2. Overview of methods to measure affinities in solution	78
Fluorescence	79
ELISA- and RIA-based methods	80
3. Affinity measurements in solution by competition ELISA	81
Theoretical aspects	81
Rationale	82
Requirements for the determination of K_d	82
Determination of K_d	86
Calculations	88
Determination of K_d with impure antibody	90
4. Affinity measurement in solution by an RIA-based method	91
Rationale	91
Requirements for the determination of K_d	91
Determination of K_d	94
5. Conclusions	95
References	96
5. Measuring antibody affinity using biosensors	99
<i>Laura J. Hefst, Anna M. Wu, Michael Neumaier, and John F. Shively</i>	
1. Introduction	99
2. Theoretical aspects	101
Measuring association and dissociation rate constants	101
Limitations on measuring affinity constants	102
3. Immobilization, binding, and regeneration of the BIAcore	103
Immobilization step	104
Regeneration step	108
4. Kinetic analysis of anti-CHA antibodies	108
Direct binding assays: comparison of murine and chimeric TR4.12	108
Indirect binding assays: comparison of murine and chimeric TR4.66	109
Assays for engineered antibody fragments	112
Assays for anti-idiotypic antibody	115
5. Conclusions	115

xi

Contents

Acknowledgements	116
References	116
6. Analysis of human antibody sequences	119
<i>Gerald Walter and Ian M. Tomlinson</i>	
1. Introduction	119
2. Amplification and cloning of antibody V genes	119
Germline V segments	123
Rearranged V genes	125
3. Sequencing of immunoglobulin genes	125
Sequencing primers	126
Template preparation	126
Sequencing techniques	129
4. Analysis of antibody sequences	137
Software packages for sequence analysis	137
Editing, translating, and comparing sequences	137
Multiple alignments	138
Databases	139
Statistical analyses	140
References	144
7. Rodent to human antibodies by CDR grafting	147
<i>Mary M. Bendig and S. Tarran Jones</i>	
1. Introduction	147
2. Cloning and sequencing mouse variable regions	147
3. Construction of a chimeric antibody	154
4. Design and construction of a reshaped human antibody	155
Analysis of the mouse variable regions	155
Design of the reshaped human antibody	155
Construction of the reshaped human antibody	157
5. Preliminary expression and analysis of the reshaped human antibodies	164
Acknowledgements	166
References	168

Contents

8. Converting rodent into human antibodies by guided selection	169
<i>Hennie R. Hoogenboom, Deborah J. Allen, and Andrew J. Roberts</i>	
1. Introduction	169
2. Cloning, expression, and characterization of rodent scFv fragments	173
3. Construction of large chain-shuffled repertoires in guided selection	174
The first DNA shuffle: combining murine V _H with a human V _L repertoire	175
Construction of fully human chain-shuffled repertoires by combining the selected human V _L genes with a human V _H repertoire	181
4. Selecting half-human or completely human antibodies by display and enrichment steps	182
References	185
9. Choosing and manipulating effector functions	187
<i>Inger Sandlie and Terje E. Michnalsen</i>	
1. Choosing effector functions	187
2. Modulation of effector functions	188
Complement activation and lysis	189
FcγR-mediated activities	189
3. Measuring complement activation and lysis <i>in vitro</i>	190
The structural requirements for complement activation	190
Comparing the IgG subclasses in complement activation and lysis	190
4. Measuring ADCC <i>in vitro</i>	195
The structural requirements for FcγR binding	195
Comparing the IgG subclasses in ADCC	195
5. Measuring phagocytosis and respiratory burst	197
6. Optimizing effector functions	200
Acknowledgements	201
References	201

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