

Differentially Expressed Genes in Plants: A Bench Manual

EDITED BY E. HANSEN AND G. HARPER



Taylor & Francis
Publishers since 1798

CONTENTS

| | |
|---|------------------|
| <i>Contributors</i> | <i>page</i> viii |
| <i>Preface</i> | ix |
| <i>Abbreviations</i> | x |
| 1 Differential Screening | 1 |
| Charles P. Scutt | |
| 1.1 Introduction | 1 |
| 1.2 Preparation of RNA from plant tissues | 4 |
| 1.3 cDNA synthesis and library construction | 8 |
| 1.4 Primary differential screening of cDNA libraries | 12 |
| 1.5 Secondary differential screening of cDNA libraries | 16 |
| 1.6 Dual-labelling with ³² P and ³⁵ S in differential screening | 19 |
| 1.7 Solutions | 20 |
| References | 20 |
| 2 Subtractive cDNA Cloning | 23 |
| Glyn Harper | |
| 2.1 Introduction | 23 |
| 2.2 Clone then subtract: subtraction of a phagemid cDNA library | 27 |
| 2.3 Subtract then clone: PCR-based subtractive cloning | 33 |
| 2.4 Library screening | 38 |
| 2.5 Troubleshooting | 40 |
| 2.6 Solutions | 42 |
| References | 43 |
| 3 Differential Display of mRNA | 45 |
| Gregory R. Heck and Donna E. Fernandez | |
| 3.1 Introduction | 45 |
| 3.2 Methods | 49 |

| | | |
|----------|---|------------|
| 3.3 | Troubleshooting | 57 |
| 3.4 | Solutions | 60 |
| | Acknowledgements | 60 |
| | References | 61 |
| 4 | The Yeast Two-Hybrid System | 63 |
| | Susanne E. Kohalmi, Jacek Nowak and William L. Crosby | |
| 4.1 | Introduction | 63 |
| 4.2 | 'Bait' and 'prey' vector constructs | 67 |
| 4.3 | Preparation of a two-hybrid cDNA expression library | 71 |
| 4.4 | Processing and characterization of 3-AT-resistant yeast colonies | 74 |
| 4.5 | Characterization of positive <i>E. coli</i> transformants | 77 |
| 4.6 | Potential problems | 77 |
| 4.7 | Outlook | 79 |
| 4.8 | Yeast strain genotypes | 79 |
| 4.9 | Growth media, solutions and supplies | 79 |
| | References | 81 |
| 5 | T-DNA Mediated Gene Tagging in <i>Arabidopsis</i> | 83 |
| | Jennifer F. Topping and Keith Lindsey | |
| 5.1 | Introduction | 83 |
| 5.2 | Gene tagging and the generation of mutants | 84 |
| 5.3 | T-DNA vectors as insertional mutagens | 84 |
| 5.4 | T-DNA promoter trap vectors | 86 |
| 5.5 | <i>Arabidopsis</i> as a model plant species | 87 |
| 5.6 | Protocols | 88 |
| 5.7 | Notes and comments | 98 |
| | Acknowledgements | 100 |
| | References | 100 |
| 6 | Two-Dimensional Polyacrylamide Gel Electrophoresis-Based Analysis for the Identification of Proteins and Corresponding Genes | 105 |
| | Guy Bauw and Marc Van Montagu | |
| 6.1 | Introduction | 105 |
| 6.2 | Two-dimensional electrophoresis of plant proteins | 106 |
| 6.3 | Protein extraction with phenol | 107 |
| 6.4 | Isofocusing, the first dimension | 108 |
| 6.5 | Second dimension: SDS-PAGE and protein staining | 110 |
| 6.6 | Protein blotting onto polyvinylidene difluoride membranes | 112 |
| 6.7 | Semi-preparative two-dimensional gel electrophoresis | 114 |
| 6.8 | Protein identification of 2D-PAGE-separated proteins by sequence analysis | 115 |
| | Acknowledgements | 117 |
| | References | 117 |

| | |
|---|------------|
| 7 Analysis of Isolated Genes | 119 |
| Ekkehard Hansen | |
| 7.1 Introduction | 119 |
| 7.2 Obtaining and analysing the complete gene | 119 |
| 7.3 Analysis of the expression of the isolated gene | 125 |
| Acknowledgement | 129 |
| References | 129 |
| | |
| <i>Index</i> | 135 |