

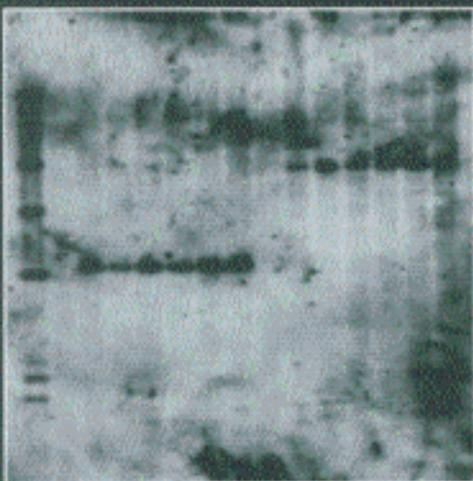
Molecular Methods of Plant Analysis

Volume 22

Testing for Genetic Manipulation in Plants

Edited by

J. F. Jackson and H. F. Linskens



Springer

Contents

1 Selectable and Screenable Markers for Rice Transformation

R.M. TWYMAN, E. STÖGER, A. KOHLI, T. CAPELL, and P. CHRISTOU

1.1	Introduction	1
1.2	Dominant Selectable Markers for Rice	3
1.2.1	Aminoglycoside 3'-Phosphotransferase (Neomycin Phosphotransferase)	3
1.2.2	Hygromycin Phosphotransferase	4
1.2.3	Phosphinothricin Acetyltransferase	4
1.2.4	Other Dominant Selectable Markers	5
1.3	Novel Selectable Markers	7
1.3.1	Innocuous Markers	7
1.3.2	Counterselectable Markers	7
1.4	Screenable Marker Genes	8
1.4.1	β -Glucuronidase (<i>gusA</i>)	9
1.4.2	Firefly Luciferase (<i>luc</i>)	11
1.4.3	Green Fluorescent Protein (<i>gfp</i>)	11
1.5	Strategies for Marker-Gene Delivery	13
	References	14

2 Use of Green Fluorescent Protein to Detect Transformed Shoots

J. MOLINIER and G. HAHNE

2.1	Introduction	19
2.2	GFP: Suitable as a Visually Selectable Marker <i>In Planta</i> ?	20
2.2.1	Important Properties of the Protein	20
2.2.2	Properties of a Useful Selectable Marker in Plant Transformation Technology	21
2.3	GFP Expression and Detection in Primary Transformed Tissues	22
2.3.1	Transient Expression and GFP Detection	22
2.3.2	Detection Equipment and Troubleshooting	22
2.3.3	Stable Expression and GFP Detection in Primary Transformed Tissues	23
2.4	GFP for Screening of Segregating Populations	28
2.5	Conclusion	29
	References	29

3 Luciferase Gene Expressed in Plants, Not in *Agrobacterium*

S.L. MANKIN

3.1	Introduction	31
3.2	Preventing Bacterial Expression	31
3.3	Imaging Luciferase Activity In Planta	32
3.4	Measuring Luciferase Activity in Plant Extracts	34
	References	35

4 Use of β -Glucuronidase (GUS) To Show Dehydration and High-Salt Gene Expression

K. NAKASHIMA and K. YAMAGUCHI-SHINOZAKI

4.1	Introduction	37
4.2	What Is GUS?	39
4.3	Trasgenic Plants Carrying Promoter-GUS Constructs	41
4.3.1	Construction of Promoter-GUS Fusion Genes	44
4.3.2	Introduction of Promoter-GUS Constructs into <i>Agrobacterium</i>	45
4.3.3	Transformation of Plants with <i>Agrobacterium</i>	46
4.3.3.1	Transformation of <i>Arabidopsis</i> Plants	46
4.4	Fluorometric Assay	49
4.4.1	Introduction	49
4.4.2	Stress Conditions	49
4.4.2.1	Plant Preparation	49
4.4.2.2	Dehydration	49
4.4.2.3	High Salinity	49
4.4.2.4	ABA Treatment	50
4.4.2.5	Other Treatments	50
4.4.3	Protein Assay	50
4.4.4	Sample Preparation	50
4.4.5	Fluorometric Assay	51
4.5	Histochemistry	51
4.5.1	Introduction	51
4.5.2	Histochemistry	52
4.6	Northern Analysis of GUS	52
4.6.1	Introduction	52
4.6.2	RNA Extraction	53
4.6.3	RNA Blotting	54
4.6.4	Northern Hybridization	55
4.7	Application of the GUS System	56
4.7.1	Transient Assay	56
4.7.2	Transactivation Experiment	57
4.7.3	Promoter Tagging (Enhancer Trap)	57
4.8	Conclusion	57
	References	59

5 Methods for Detecting Genetic Manipulation in Grain Legumes

H.-J. JACOBSEN and R. GREINER

5.1	Introduction	63
5.2	Detection at the DNA Level	64
5.3	PCR Analysis	65
5.4	Control PCR and Specific PCR Systems	66
5.5	Quantitative Approach	69
5.6	Competitive PCR	69
5.7	Real-Time PCR Systems	70
5.8	Concluding Remarks	70
	References	71

6 Elimination of Selectable Marker Genes from Transgenic Crops

A.P. GLEAVE

6.1	Introduction	73
6.2	Co-transformation	74
6.3	Transposon-Mediated Approaches	78
6.3.1	Transposon-Mediated Repositioning	78
6.3.2	Transposon-Mediated Elimination	80
6.4	Site-Specific Recombination	81
6.4.1	The Cre/loxP System	82
6.4.2	The FLP/frt System	86
6.4.3	The R/RS System	88
6.5	Intrachromosomal Homologous Recombination	89
6.6	Conclusions and Future Prospects	90
	References	91

7 GST-MAT Vector for the Efficient and Practical Removal**of Marker Genes from Transgenic Plants**

H. EBINUMA, K. SUGITA, E. MATSUNAGA, S. ENDO, and K. YAMADA

7.1	Introduction	95
7.2	<i>ipt</i> -Type MAT Vectors	96
7.2.1	Transposable Element	96
7.2.2	Site-Specific Recombination System	97
7.2.3	Advantages of the <i>ipt</i> Gene	101
7.3	Two-Step Transformation	103
7.3.1	Promoter of the <i>R</i> Gene	103
7.3.2	Promoter of the <i>ipt</i> Gene	105
7.3.3	Combination of the <i>ipt</i> and <i>iaaM/H</i> Genes	108
7.3.4	Transgene Stacking	110
7.4	Single-Step Transformation	112

7.5 Cloning Vector for Desired Genes	114
7.6 Concluding Remarks	115
References	116

8 Safety Assessment of Insect Protected Crops: Testing the Feeding Value of *Bt* Corn and Cotton Varieties in Poultry, Swine and Cattle

B. HAMMOND, E. STANISIEWSKI, R. FUCHS, J. ASTWOOD, and G. HARTNELL

8.1 Introduction	119
8.1.1 Food Safety Standards	119
8.1.2 Testing for Food and Feed Safety	120
8.2 Insect Protection Traits	122
8.3 Benefits	123
8.4 Safety Assessment of the Cry Insect-Control Proteins	124
8.5 Mode of Action	125
8.6 Substantial Equivalence Based on Compositional Analysis	126
8.7 Current Products	127
8.8 Grower Acceptance	127
8.9 Future Products	127
8.10 Farm-Animal Studies	128
8.10.1 <i>Bt</i> Corn	128
8.10.1.1 Poultry	128
8.10.1.2 Lactating Cows	131
8.10.1.3 Beef and Sheep	131
8.10.1.4 Swine	132
8.11 Cottonseed	133
8.12 Conclusions	134
References	135

9 Safety Assessment of Genetically Modified Rice and Potatoes with Soybean Glycinin

K. MOMMA, W. HASHIMOTO, S. UTSUMI, and K. MURATA

9.1 Introduction	139
9.2 Safety Assessment of Genetically Modified Crops	140
9.2.1 Genetically Modified Rice	141
9.2.2 Genetically Modified Potatoes	144
9.3 Concluding Remarks	146
References	149

10 Chromosomal and Genetic Aberrations in Transgenic Soybean

R.J. SINGH

10.1 Introduction	153
10.2 Times in Culture with 2,4-D Prior to Transformation	154
10.3 Genetic Background of the Explants	159

Contents	XIII
10.4 Seed Fertility in Transgenic Soybean	160
10.5 Cytological Basis of Gene Silencing	163
10.6 Conclusions	164
References	165
11 Transgenic Barley (<i>Hordeum vulgare</i> L.) and Chromosomal Variation	
M.-J. CHO, H.W. CHOI, P. BREGITZER, S. ZHANG, and P.G. LEMAUX	
11.1 Introduction	169
11.2 Chromosomal Variation in Nontransgenic Barley Plants	170
11.3 Chromosomal Variation in Transgenic Barley Plants	172
11.4 Fidelity and Quality of Transgenic Barley Plants	177
11.4.1 Comparative Analysis of Genomic Stability in Plants Derived from Tissues Generated Using Different in Vitro Proliferation Processes	177
11.4.2 Somaclonal Variation and Field Performance of Transgenic Plants Derived from Embryogenic Callus	179
11.4.3 Stability of Transgenes and Transgene Expression	180
11.5 Conclusions and Future Perspectives	184
References	185
 Subject Index	 189