## **CONTENTS**

Contributors Preface	xvii
1. Comparative Biochemistry and In Vitro Pathway Reconstruction as Powerful Partners in Studies of Metabolic Diversity	1
P. Fan, G.D. Moghe, and R.L. Last	
<ol> <li>Introduction</li> <li>Characterization of Specialized Metabolite Diversity Using</li> </ol>	2
<ul><li>LC/MS and NMR</li><li>3. Phylogeny-Driven Analysis of the Biochemical Basis of Specialized Metabolite Diversity</li></ul>	4
4. Conclusions	15
Acknowledgments	16
References	16
2. De Novo Deep Transcriptome Analysis of Medicinal Plants for Gene Discovery in Biosynthesis of Plant Natural Products R. Han, A. Rai, M. Nakamura, H. Suzuki, H. Takahashi, M. Yamazaki, and K. Saito	19
<ol> <li>Methods for Transcriptomic Study Prior to High-Throughput Sequencing</li> <li>Deep Transcriptome Outline</li> </ol>	20 22
3. Preparation of the Plant Materials and RNA Extraction	24
4. cDNA Library Construction	27
5. High-Throughput Sequencing	30
6. Data Interpretation	31
7. Application of RNA-Seq on Medicinal Plants and Perspectives	38
Acknowledgments References	39 39
3. Genomics-Based Discovery of Plant Genes for Synthetic Biology of Terpenoid Fragrances: A Case Study in Sandalwood oil Biosynthesis  J.M. Celedon and J. Bohlmann	4 <b>7</b>
<ol> <li>Introduction</li> <li>Prior Knowledge of Sandalwood TPSs and P450s and Development</li> </ol>	48
of a Hypothesis	50

	3.	Replication, Sampling, and Statistical Design	51
	4.	Defining Temporal and Spatial Variables for Tissue Sampling	52
	5.	Tissue Sampling	53
	6.	Metabolite Profiling	53
	7.	Isolation of High-Quality RNA from Recalcitrant Tissues	55
	8.	Transcriptome Sequencing and De Novo Assembly	56
	9.	Transcriptome Mining and Annotation	57
	10.	Expression Analysis and Candidate Gene Selection	58
	11.	Functional Characterization of Candidate Genes	59
	12.	Product Identification	63
,	4ckı	nowledgments	65
1	Refe	erences	65
4.	ΑV	Vorkflow for Studying Specialized Metabolism	
		Nonmodel Eukaryotic Organisms	69
-	M.P	. Torrens-Spence, T.R. Fallon, and J.K. Weng	
	1.	Introduction	70
		"Omics"-Based Novel Specialized Metabolic Pathway Discovery	72
		Structure–Function Analysis of Specialized Metabolic Enzymes	80
		Reconstitution of Specialized Metabolic Pathways in Heterologous Systems	87
		Summary	90
		nowledgments	91
		erences	91
5	Ge	ne Discovery for Synthetic Biology: Exploring the Novel	
		tural Product Biosynthetic Capacity of Eukaryotic Microalgae	99
		O'Neill, G. Saalbach, and R.A. Field	
	1	Introduction	100
		Natural Product Synthases	102
		Genome Mining for the Identification of Natural Products	106
		Natural Product Discovery	113
		Conclusions	115
		nowledgments	116
	Ref	erences	116
6	ric.	Prenyltransferase and Polymer Analysis from a Natural	
		bber Perspective	121
		Kwon, EJ.G. Kwon, and D.K. Ro	
		Introduction	122
	2.	Rationale: Observation of Revertants from rer2 Mutant	125

Contents	vii

3.	Generation of rer2 and srt1 Double Knockout Yeast Strain	128
4.	Complementation of $rer2\Delta$ srt1 $\Delta$ with CPT and CBP	131
5.	CPT Biochemical Assay Using Yeast Microsomes	· 35
6.	General Discussion	142
Ac	knowledgments	143
Re	ferences	i 44
	eneration and Functional Evaluation of Designer	
	onoterpene Synthases	147
N.	Srividya, I. Lange, and B.M. Lange	
1.	Introduction	148
2.	Equipment	150
3.	Materials	150
4.	Step 1—Generation of Expression Constructs	153
5.	Step 2—Production of Purified, Recombinant	
	Target Enzyme	158
6.	Step 3—Functional Evaluation of Recombinant	
	Monoterpene Synthases	161
7.	Conclusions	164
Ac	knowledgments	164
Re	ferences	164
o D.	equels to Synthetic Biology: From Candidate Gene	
	entification and Validation to Enzyme Subcellular	
	ocalization in Plant and Yeast Cells	167
	Foureau, I. Carqueijeiro, T. Dugé de Bernonville, C. Melin, F. Lafontaine,	
	Besseau, A. Lanoue, N. Papon, A. Oudin, G. Glévarec, M. Clastre,	
В.	St-Pierre, N. Giglioli-Guivarc'h, and V. Courdavault	
1.	Introduction	168
2.	Identification of Candidate Genes Through Transcriptomic	
	Data Mining and Analysis	171
3.	Validation of Candidate Gene Function by Biolistic-Mediated VIGS	187
4.	Studying the Subcellular Localization of Biosynthetic Pathway	
	Enzymes in Plant and Yeast Cells to Alleviate Bottlenecks	
	in Bioengineering Approaches	191
5.	Concluding Remarks	202
A	cknowledgments	203
Re	eferences	203

9.	Functional Expression and Characterization of Plant ABC Transporters in <i>Xenopus laevis</i> Oocytes for Transport	
	Engineering Purposes	207
	D. Xu, D. Veres, Z.M. Belew, C.E. Olsen, H.H. Nour-Eldin, and B.A. Halkier	20,
	1. Introduction	208
	2. Preparation of cDNA of Plant ABC Transporter Genes by In Planta	
	"Exon Engineering"	211
	3. ABC Transporter Expression in <i>Xenopus</i> Oocytes	215
	4. Optimization of Transport Assay for Diffusible ABA in <i>Xenopus</i> Oocytes	217
	5. Case Study: Characterization of the ABA Exporter at ABCG25 in	
	Xenopus Oocytes	219
	6. Conclusions	220
	Acknowledgments	221
	References	221
10.	Quantifying the Metabolites of the Methylerythritol 4-Phosphate (MEP) Pathway in Plants and Bacteria by Liquid Chromatography–Triple Quadrupole Mass Spectrometry	225
	D. González-Cabanelas, A. Hammerbacher, B. Raguschke, J. Gershenzon,	
	and L.P. Wright	
	1. Introduction	226
	2. Preparation of Stable Isotope-Labeled Internal Standards	229
	3. Extraction of Methylerythritol Phosphate Pathway Intermediates from	
	Biological Sources	234
	4. Analysis of Methylerythritol Phosphate Pathway Metabolites by LC-MS/MS	236
	5. Discussion and Summary	244
	References	245
11	Establishing the Architecture of Plant Gene Regulatory	
	Networks	251
	F. Yang, W.Z. Ouma, W. Li, A.I. Doseff, and E. Grotewold	
	1. Introduction	252
	2. The cis-Regulatory Apparatus	254
	3. The <i>Trans</i> -Acting Factors	260
	4. Transcription Factor Centered Approaches	266
	5. Gene-Centered Approaches	274
	<b>6.</b> Resources for Studying Plant GRNs	281
	7. Conclusions	286
	Acknowledgment	287
	References	287

	ngineering of Tomato Glandular Trichomes for e Production of Specialized Metabolites	305		
R.V	R.W.J. Kortbeek, J. Xu, A. Ramirez, E. Spyropoulou, P. Diergaarde,			
1. 0	1. Otten-Bruggeman, M. de Both, R. Nagel, A. Schmidt, R.C. Schuurink, and P.M. Bleeker			
1.	Introduction	306		
2. 3.	Materials and Technology  Proof of Concept: Targeted Expression of a Terpene Precursor	310		
•	Gene in Tomato Glandular Trichomes	318		
4.	Summary	325		
Ac	knowledgments	326		
	ferences	327		
13. To	omato Fruits—A Platform for Metabolic Engineering			
	Terpenes	333		
M.	. Gutensohn and N. Dudareva			
1.	Introduction	334		
2.	Terpenoid Formation in Tomato Fruits	338		
3.	Transgene Expression in Ripening Tomato Fruits	340		
4.	Overexpression of Terpene Biosynthetic Genes in Tomato Fruits	343		
5.	Analysis of Terpenes in Tomato Fruits	349		
6.	Conclusions	350		
Ac	cknowledgments	352		
Re	eferences	352		
14. Li	braries of Synthetic TALE-Activated Promoters:			
М	lethods and Applications	361		
T.	Schreiber and A. Tissier			
	Introduction	361		
2.	Construction of Libraries of Synthetic Promoters Using Golden			
	Gate Cloning	366		
	Analyzing Promoter Activity in Transient Assays	371		
4.	Conclusion	373		
Re	eferences	375		
Autho	r Index	379		
Subjec	ct Index	419		