

# Functional Analysis of Bacterial Genes

## A Practical Manual



*Edited by*

**Wolfgang Schumann**  
**S. Dusko Ehrlich**  
**Naotake Ogasawara**

# Contents

---

List of Contributors ix

Foreword xix

Preface xxi

Safety xxv

1 Aims of the Functional Analysis Project 1  
*S. Dusko Ehrlich*

2 *In silico* Genome Analysis 5

*Eduardo Rocha, Ivan Moszer, Maude Klaerr-Blanchard, Agnieszka Sekowska,  
Claudine Médigue, Alain Viari and Antoine Danchin*

3 Inactivation of *Bacillus subtilis* Genes Without Known Functions 21  
*Valérie Vagner, Étienne Dervyn and S. Dusko Ehrlich*

4 Analysis of Essential Genes 25

*Étienne Dervyn and S. Dusko Ehrlich*

5 Transcriptional Analysis of Large Regions of the Bacterial Genome 33  
*Rafael P. Mellado*

5A Useful Methods for Transcriptional Analysis 37  
*Rafael P. Mellado*

5B Northern Blot Analysis with non-RI Detection Systems 41  
*Hiroki Yamamoto, Kei Asai and Kazuo Kobayashi*

6 Micado, an Integrative Database Dedicated to the Functional Analysis of  
*Bacillus subtilis* and Microbial Genomics 45  
*Franck Samson, Véronique Biaudet-Brunaud, Shahinaz Gas, Étienne Dervyn,  
Gabriel Gallezot, Sandrine Duchet, S. Dusko Ehrlich and Philippe Bessières*

6A Phenotype Responses and Reporter Gene Activity from the Systematic  
Functional Analysis of *Bacillus subtilis* Unknown Genes 53

*Véronique Biaudet-Brunaud, Franck Samson, Shahinaz Gas, Étienne Dervyn,  
Gabriel Gallezot, Sandrine Duchet, S. Dusko Ehrlich and Philippe Bessières*

7 The Contribution of the EC Consortium to the Two-dimensional Protein  
Index of *Bacillus subtilis* 63

*J. Bernhardt, K. Büttner, J. Y. Coppée, C. Lelong, N. Ogasawara, C. Scharf,  
V. Vagner, R. Schmid, U. Völker and M. Hecker*

vi	
8	<b>Whole-Cell Fingerprinting – Pyrolysis Mass Spectrometry</b> 75 <i>Zoltán Prágai, Alan C. Ward and Colin R. Harwood</i>
9	<b>Metabolism of Small Molecules and Inorganics</b> 77 <i>Hans Henrik Saxild and Per Nygaard</i>
9A	<b>Screening for Genes Involved in Nucleotide and Nitrogen Metabolism</b> 79 <i>Hans Henrik Saxild, Bo Greve and Per Nygaard</i>
9B	<b>Screening for Growth and lacZ Reporter Gene Induction with Metal Ions and Chelators, Amino Acid Analogs, Antibiotics, Inhibitors of Oxidative Phosphorylation and Miscellaneous Stress Factors</b> 87 <i>Marie-Françoise Hullo, Agnieszka Sekowska, Elena Presecan-Siedel, Jean-Yves Coppée, Isabelle Martin-Verstraete, Philippe Glaser and Antoine Danchin</i>
10	<b>Nucleotide Metabolism</b> 95 <i>Hans Henrik Saxild and Per Nygaard</i>
11	<b>Utilization of Small Carbon-containing Molecules by <i>Bacillus subtilis</i></b> 101 <i>Sabine Fillinger, Dominique Le Coq and Stéphane Aymerich</i>
11A	<b>Screening for Genes Involved in the Metabolism of Small Carbon-containing Molecules</b> 105 <i>Sabine Fillinger, Dominique Le Coq and Stéphane Aymerich</i>
12	<b>Nitrogen Sources</b> 111 <i>Michel Débarbouillé, Rozenn Gardan, Maryvonne Arnaud and Georges Rapoport</i>
12A	<b>Induction or Repression by Nitrogen Sources</b> 115 <i>Rozenn Gardan, Maryvonne Arnaud, Denis Robichon and Michel Débarbouillé</i>
13	<b>Glucose Regulation in <i>Bacillus subtilis</i></b> 117 <i>Isabelle Martin-Verstraete, Maryvonne Arnaud and Georges Rapoport</i>
13A	<b>Catabolite Repression by Glucose</b> 121 <i>Maryvonne Arnaud, Rozenn Gardan and Denis Robichon</i>
14	<b>Cell Processes and Macromolecule Metabolism: a Quest for Genes Involved in Genetic Competence, and DNA Recombination and Repair</b> 123 <i>Rob Meima, Caroline Eschevins and Sierd Brön</i>
14A	<b>Cell Processes and Macromolecule Metabolism: Methods for the Selection of Mutants in Genetic Competence, DNA Recombination and Repair and Protein Secretion</b> 131 <i>Rob Meima, Caroline Eschevins and Sierd Bron</i>
15	<b>Molecular Tools for the Identification of Mutations Affecting Protein Secretion</b> 137 <i>Rob Meima, Pertti Koski, Caroline Eschevins, Matti Sarvas and Sierd Bron</i>

## CONTENTS

- 15A Screening for Mutants Defective in Secretion 143**  
*Pertti Koski and Matti Sarvas*
- 16 Genes Affecting Motility 149**  
*Alessandra M. Albertini and Alessandro Galizzi*
- 16A Screening for Motility 155**  
*Alessandra M. Albertini and Alessandro Galizzi*
- 17 Differentiation of *Bacillus subtilis* 157**  
*Anne Moir, Fiona Boland, Michele O'Rourke and Simon Foster*
- 17A Methods for Rapid Screening of Properties: Spore Resistance and Germination 163**  
*Anne Moir, Fiona Boland, Michele O'Rourke and Simon Foster*
- 18 Genes Affecting the Cell Envelope 167**  
*Zoltán Prágai, Maryam Lahooti and Colin R. Harwood*
- 18A Analysis of Cell Envelope Components 175**  
*Zoltán Prágai, Maryam Lahooti and Colin R. Harwood*
- 19 Peptidoglycan Structure and Structural Determination in Vegetative Cells of *Bacillus subtilis* 181**  
*Abdelmadjid Atrih, Gavin J. Horsburgh and Simon J. Foster*
- 20 Cell Cycle Gene Analysis 189**  
*Simone J. Séror and Jeffery Errington*
- 20A Identification and Analysis of Cell Cycle Genes 197**  
*Anne Masson, Françoise Vannier, Josef Seegers and Simone J. Séror*
- 21 Endospore Formation 201**  
*Jeffery Errington*
- 21A Isolation and Characterization of Genes Affecting Sporulation or the Cell Cycle 207**  
*Joy P. Rawlins, Helena B. Thomaides, Rut Carballido-Lopez and Jeffery Errington*
- 22 Selection and Characterization of Genes Affecting Germination 211**  
*Junichi Sekiguchi, Tatsuya Fukushima and Shu Ishikawa*
- 23 The Oxidative Stress Response 215**  
*Elizabeth Scanlan, Michael Hecker and Kevin M. Devine*
- 23A Screening for Increased Sensitivity or Resistance and Level of *lacZ* Reporter Gene Expression in Response to the Oxidizing Agents Hydrogen Peroxide and Methyl Viologen 227**  
*Elizabeth Scanlan, Mary O'Reilly, Kasper Krogh Andersen, David Noone and Kevin M. Devine*
- 24 The Osmotic Shock Response 231**  
*Ulrich Zuber*

24A	Salt Stress	235
	<i>Maryvonne Arnaud and Michel Débarbouillé</i>	
25	Phosphate Regulation	237
	<i>Maryam Lahooti, Zoltán Prágai and Colin R. Harwood</i>	
25a	Screening for Mutants Affected in their Response to Phosphate	245
	<i>Zoltán Prágai and Colin R. Harwood</i>	
26	The Heat Shock Response	251
	<i>Wolfgang Schumann and Michael Hecker</i>	
27	The Cold Shock Response	263
	<i>Wolfgang Schumann</i>	
28	pH-Inducible Genes	271
	<i>Wolfgang Schumann</i>	
28A	Analysis of the Mutant Collection for Defects in their Temperature and pH Shock Response	277
	<i>Tanja Ohanjan and Wolfgang Schumann</i>	
28B	Screening for Growth and <i>lacZ</i> Reporter Gene Induction or Repression under Glucose and Phosphate Starvation and under Heat, Oxidative and Osmotic Stress	281
	<i>Ulrich Zuber and Michael Hecker</i>	
29	List of <i>Bacillus subtilis</i> Unknown Genes with a Phenotype Determined by the Functional Analysis Project	283
	<i>Véronique Biaudet-Brunaud, Franck Samson, Shahinaz Gas, Étienne Dervyn, Gabriel Gallezot, Sandrine Duchet, S. Dusko Ehrlich and Philippe Bessières</i>	
30	List of <i>Bacillus subtilis</i> Genes of Known Function	293
	<i>Ivan Moszer and Antoine Danchin</i>	
Appendix 1	List of <i>Bacillus subtilis</i> Mutants Constructed by the European Functional Analysis Project	327
Appendix 2	List of <i>Bacillus subtilis</i> Mutants Constructed by the Japanese Functional Analysis Project	343
Appendix 3	List of Selected Suppliers	355
Appendix 4	Glossary	359
Index		367