



Table of Contents

	Foreword	v
	Preface	vii
	Acknowledgments	ix
Introduction	Introduction to the <i>lac</i> System	3
	Format; Use of Materials	6
	Nomenclature	7
	Strain List	13
Unit I	Introductory Experiments	
Experiment 1	Determination of Viable Cell Counts: Bacterial Growth Curves	31
2	Preparation and Plaque Assay of a Phage Stock	37
3	Behavior of Mutants on Indicator Plates	47
4	Replica Plating	56
		xi

Unit II	Matings between Male and Female Cells	
Experiment 5	Episome Transfers: Direct Selection	82
6	Interrupted Matings and the Time of Marker Entry	86
7	Location of Markers on the <i>E. coli</i> Chromosome by the Gradient of Transmission	93
8	Determination of the Point of Origin of Hfr Strains	96
9	Test for the Sex Factor by Phage Sensitivity	98
10	Production of F ⁻ Phenocopies	101
11	Curing of Episomes from <i>E. coli</i> Strains with Acridine Orange	104
12	Recombination Deficient Strains: Influence on Episome Transfer and Recombination	107
 Unit III	 Mutagenesis and the Isolation of Mutants	
Experiment 13	Ultraviolet Light Mutagenesis	121
14	Nitrosoguanidine Mutagenesis	125
15	Mutagenesis with a Frameshift Mutagen: ICR 191	130
16	2-Aminopurine and Nitrous Acid Mutagenesis Other Methods: EMS, Hydroxylamine, 5-Bromouracil, Sodium Bisulfite, Mu-1 Phage	135
17	Reversion Tests	140
18	Isolation of Temperature-sensitive Mutants in the <i>lac</i> Operon	144
19	Isolation of Spontaneous Lac ⁻ and i ⁻ Mutants	146
20	Complementation Tests of Lac Mutants—Intracistronic Complementation	153
21	Fine Structure Mapping of Lac ⁻ Mutants	159
22	Isolation of Nonsense Mutants	167
23	Isolation of Nonsense Suppressor Strains	173
24	Detecting Polar Mutants in the <i>lac</i> Operon	180

Unit IV	Strain Construction	
	Episome Transfers	
Experiment 25	F' Factor Transfers—Inability to Select for Recipient	190
26	F' Factor Transfers—Inability to Select against Donor	193
27	Construction of a Temperature-sensitive Episome	196
28	Generalized Transduction; Use of P1 in Strain Construction	201
29	F' Factor-mediated Chromosome Transfer	206
	Manipulation of Genetic Markers	
30	Use of P2 Phage to Isolate His ⁻ Strains of <i>E. coli</i>	215
31	Selection of Thy ⁻ Strains with Trimethoprim	218
32	Isolation of Valine-resistant and Antibiotic-resistant Mutants	221
	A. Valine Resistance	
	B. Nalidixic Acid Resistance	
	C. Rifampicin Resistance	
	D. Ampicillin Resistance	
	Additional Markers Used for Selection	
	Isolation of Auxotrophic Mutants	
33	Penicillin and Ampicillin Treatment for the Isolation of Auxotrophic Mutants	230
	Other Enrichment Techniques	
34	Isolation and Mapping of Temperature-sensitive Lethal Mutants	235
	Construction of Hfr Strains	
35	Selection for Integration of F Factor	249
36	Conversion of an F ⁻ Strain to an Hfr Strain	253
	A. Conversion of an F ⁻ Strain to HfrH	
	B. Conversion of an F ⁻ Strain to Hfr Cavalli	
37	F' Factor Formation; Use of Rec ⁻ Strains to Isolate F' Factors Carrying Different Regions of the <i>E. coli</i> Chromosome	259
38	Use of Homogenotization in Strain Construction	265

Unit V	Transduction and the Isolation of Specialized Transducing Phage Lines	
Experiment 39	Construction of Specialized Transducing Phage Lines	274
	Positioning Genes Near Prophage Attachment Sites	
40	Transpositions of the <i>lac</i> Region	284
	A. Selection of F' <i>lac</i> Integrations	287
	B. Transpositions at the <i>tonB</i> Locus	290
41A	Insertion of λ DNA into New Sites on the Chromosome	293
41B	Bringing Two Genes into Close Proximity by Isolation of a Fused Episome	301
	Operon Fusion	
42A	Isolation of Fusion Strains for the <i>lac</i> and <i>trp</i> Operons	304
42B	Construction of <i>trpR</i> ⁻ Derivatives of <i>trp-lac</i> Fusion Strains	312
Unit VI	Transformation with λh80d<i>lac</i> DNA and Measurement of <i>lac</i> Messenger RNA	
	<i>Bill Haseltine and Benno Müller-Hill</i>	
Experiment 43	Preparation of λ h80d <i>lac</i> Phage and Transformation with λ h80d <i>lac</i> DNA	319
44	Isolation of RNA Labeled <i>in vivo</i> after IPTG Induction	328
45	Separation of the Strands of λ plac DNA	331
46	Hybridization with <i>lac</i> mRNA	338
47	Determination of the Sedimentation Constant of <i>lac</i> mRNA	344
Unit VII	Assays of the <i>lac</i> Operon Enzymes	
	<i>Terry Platt, Benno Müller-Hill, and Jeffrey H. Miller</i>	
Experiment 48	Assay of β -Galactosidase	352
49	Time Course of β -Galactosidase Induction	356
50	Assay of <i>lac</i> Permease	360

Table of Contents

xv

51	Assay of <i>lac</i> Repressor by Binding to Inducer	363
52	Assay of <i>lac</i> Repressor by Binding to Operator	367
53	Assay of Transacetylase	371
54	Assay for α Complementation	374

Unit VIII

Protein Purifications

Terry Platt

Experiment 55	Purification of the <i>lac</i> Repressor	385
56	Determination of the Binding Constant of <i>lac</i> Repressor for IPTG	394
57	Purification of β -Galactosidase	398

Unit IX

Cyclic AMP, Catabolite Repression, and Cell-free Enzyme Synthesis

Jack Greenblatt and Jeffrey H. Miller

Experiment 58	Cyclic AMP and Catabolite Repression	412
59	Isolation of Mutants Missing a Factor Necessary for the Expression of Catabolite-sensitive Operons	415
60	Cell-free Synthesis of β -Galactosidase	419
61	Catabolite Repression in a Cell-free System	425
62	Repression of β -Galactosidase Synthesis in a Cell-free System	427

Appendices

I	Formulas and Recipes	431
II	Operation of Mating Interrupter <i>Brooks Low</i>	436
III	Linkage Map of <i>E. coli</i>	439

IV	General Mapping Strategy: Mapping Strategy for Two Loci Concerned with Pyridoxine Biosynthesis <i>A. L. Taylor</i>	451
V	Lambda Genetic Elements	455
VI	Assay for <i>lac</i> Repressor by Millipore Filter <i>Terry Platt</i>	458
VII	Selected F' Factors from <i>E. coli</i> K12	460
VIII	Addresses for Laboratory Supplies	461