EXPERIMENTS WITH GENE FUSIONS

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The cover displays a pencil drawing of the Chimera, by Nancy Trun. In Greek mythology Chimera is a fire-breathing she-monster, commonly represented with a lion's and goat's head, a goat's or lion's body, and a serpent's tail. In biology a chimera is a tissue composed of cells from at least two genetically distinct origins. In molecular genetics the term chimera has come to refer to: (1) a gene composed of two or more genetically distinct loci; specifically, a gene composed of the 5′ sequences from one gene fused with the 3′ sequences from a second gene (often the 3′ sequences from the *lacZ* gene of *Escherichia coli*); (2) a gene product having aminoterminal residues of one peptide and carboxyterminal residues from a second peptide.

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To our wives, Dalleen, Marta, and Kathy, for their encouragement, understanding, and continued support.

Preface

This manual is designed to demonstrate the use of gene fusions, transposable elements, and methods of recombinant DNA for genetic analysis in *Escherichia coli*. It consists of experiments and procedures used in the Advanced Bacterial Genetics course offered at Cold Spring Harbor Laboratory during the summers from 1981 to 1983. Through this manual, gene fusion technology becomes accessible to students and scientists who are familiar with the principles and basic manipulations of bacterial genetics as described in J.H. Miller's book, *Experiments in Molecular Genetics* (1972). Such a person can grasp the concepts described herein and perform all of the experiments in three weeks, provided he or she is willing to work in the lab 12–14 hours a day. This manual is not meant to be a compendium of recombinant DNA methods. Scientists, particularly those working with eukaryotic cells, who wish to learn this technology in more depth should consult *Molecular Cloning: A Laboratory Manual* by T. Maniatis, E.F. Fritsch, and J. Sambrook (1982).

Many people have contributed substantially to the production of this manual. Our teaching assistants—Scott Emr, Dolores Jackson, Ronald Taylor, Spencer Benson, Erhard Bremer, Stephen Garrett, and Susan Bear—gave many hours of their time, provided valuable advice, and helped us in ways too numerous to mention. The success of the course, and consequently of the manual, is a direct result of their enthusiasm and hard work. Special thanks must be given to Stephen Garrett, who provided insight into the genetics of *envZ* and constructed many of the strains used in this manual, and to Susan Bear, who by working with us for three years was able to give the course a special continuity and offer helpful suggestions for its improvement.

We thank the many colleagues who have generously provided us with unpublished experimental protocols. We have tried to reference the original sources for specific methods, and we apologize for any errors or omissions.

Over the past three years, Sylvia Lucas has given many hours to the production of this manual. She did all the typing and layout for the earlier versions, reminded us of our deadlines, and helped in the preparation of the manuscript for this edition. Without her, this book would still be in loose-leaf form. During the past year, as we were preparing the manual for publication, Lori Jenkins provided much-needed, additional secretarial support. Doug Owen deserves special thanks for his patience and editorial skills. He and Nancy Ford, Director of Cold Spring Harbor's Publications Department, shared their expertise to add coherence to the book and simplify the process

of publication. We are also grateful to Emily Harste for the typographic design of the book and cover.

We are particularly indebted to Jim Hicks and Jim Watson for their continued support of bacterial genetics and for their aid and encouragement to us during our tenure as instructors of the Advanced Bacterial Genetics course.

The success of any course is perhaps best judged by its students. Each of the former students listed below has helped to ensure that the techniques described in the manual actually work. We hope they have profited as much from this experience as we have.

One of these former students contributed her artistic talents, as well; we thank Nancy Trun for her rendering of Chimera, which appears on the cover of this manual.

Finally, we would like to acknowledge the magnanimous support given us by our respective institutions and employers. T.J.S. and M.L.B. were supported in part by the National Cancer Institute, DHHS, under contract NO1-CO-23909 with Litton Bionetics, Inc.

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Bacteriophages

Bacteriophage	*Genotype*[a]
B10	λ *tmm*21 cI
B17	λ *int*6 *red*3 *tmm*21 cI
B500	λ *h*80 *tmm*21 *c*
G6	λ *tmm*434 cI
G216	λ *b*2 *tmm*434 *cIts*
G244	λ *b*538 *tmm*434 cI Sam7
Y1	λ *cIts*857 cI *ind*
Y2	λ *b*2 *cIts*857
Y47	λ *cIts*857 Sam7
Y2223	λ *W*am403 *cIts*857
W14[b]	λ v_2 v_1 v_3
W30	λ *b*2 cI
W248	λ *h*80 Δ(*att-int*)9 cI
λNK561	λ *b*22I cI::Tn*10* *O*am29 *P*am80
λ*p* 1(209)[c]	
λ*plac*Mu1[d]	
λ*p*Mu507	λ *cIts*857 Sam7 Mu*A*$^+$*B*′
λ*p10-25*	λ Φ(*ompC*′-*lacZ*$^+$)*10-25*
λ*p16-13*	λ Φ(*ompF*′-*lacZ*$^+$)*16-13*
λTK10	λ Φ(*ompR*′-′*lacZ*)*hyb1*
λ*p*SG1	λ *p* 1(209)*lacY*::Tn*9*
λD69	λ *bam*λ 1^o Δ(*sr*Iλ 1-*sr*Iλ 2) *tmm*21 *nin*5 *shn*6^o
λ*p*RT2	λD69 *bam*λ3::(*envZ*$^+$)*ompR*$^+$
λ*p*SG10	λD69 *bam*λ3::*ompR*$^+$

Bacteriophage	***Genotype***[a]
λ*p*SG11	λpSG10 *h80*
λ*p*SG517[e]	λpRT2 Δ(*ompR*)*517*
λ*p*RT2-80	λ*p*RT2 *h80*
λ*p*RT2.3	λ*p*RT2 *envZ3*
λ*p*RT2.101	λ*p*RT2 *ompR101*
λ*D*am*sr*Iλ3	λ *D*am15 *b538 cIts857 sr*Iλ4[o] *nin5 sr*Iλ5[o]
λ*p*RT1*imm*434	λ *D*am *sr*Iλ3::(*ompR*$^+$) *imm*434
λNF1955[f]	
λ*p*1048[g]	λNF1955 Φ(*tryT'-lacY*$^+$)*1048*
λ*p*1081.1[h]	
λ*apmalB13*[i]	λ *malG*$^+$*F*$^+$*E*$^+$*K*$^+$*lamB' h80 cIts857* Sam7
hy2[j]	λ *hPA-2 imm*λ *vir*
K20[k]	
Mud1(*lac*, Ap)[l]	
P1*vir*	P1 *vir*
P1*cam*	P1::Tn*9clr*100
*ϕ*80*vir*	*ϕ*80 *vir*
*ϕ*80*p*SuIII	*ϕ*80 *psupF*$^+$

[a]Unless otherwise stated, all fusion transducing phages are derivatives of λ*p*1(209) (see Experiment 3, Fig. 7, p. 30).
[b]λ*vir*.
[c]See Experiment 3, Fig. 7 (p. 30).
[d]See Appendix L, Fig. 28 (p. 262).
[e]See Experiment 13, Fig. 15 (p. 80).
[f]See Appendix I, Fig. 24 (p. 252).
[g]See Experiment 2 and Berman and Jackson (1984).
[h]See Experiment 10, Fig. 13 (p. 65).
[i]See Marchal et al. (1978).
[j]The receptor for hy2 is OmpC.
[k]The receptor for K20 is OmpF.
[l]See Experiment 1, Fig. 1 (p. 8).

Plate 1
Selection of Lac$^+$ mutants on lactose MacConkey agar. This plate shows the result of streaking strain MBM7060 carrying pMLB952 on lactose MacConkey agar as described in Experiment 2, part I. The plate was incubated for 5 days at 37°C.

Plate 2
Mapping *ompR* mutants by using gene fusions and lactose MacConkey agar. A section of a lactose MacConkey plate is shown. A lawn of a strain carrying an *ompR101* mutation that abolishes expression of an *ompC-lac* fusion was spotted with ~100 pfu of four different phages. Clockwise from upper left: (1) λ*p*RT2.101, negative control; (2) λ*p*SG517, a phage that cannot complement *ompR101* but that can recombine with the chromosome to generate *ompR*$^+$; (3) λ*p*RT1*imm*434, which for reasons not fully understood exhibits weak complementation; (4) λ*p*RT2, complementation. See Experiment 13 for details.

Plate 3
Red-plaque test for λ site-specific excision (see Procedure 5). A *mutD*-mutagenized stock of λ *b*515 *b*519 *imm*21 (Enquist and Weisberg 1977) was plated on a lawn of LE292, using TB top agar and galactose TTC plates. The majority of the phage form plaques with red centers (excision proficient). White plaque mutants (excision defective) are seen at a frequency of about 1–2%. The *mutD*-mutagenesis protocol is described in Procedure 24.

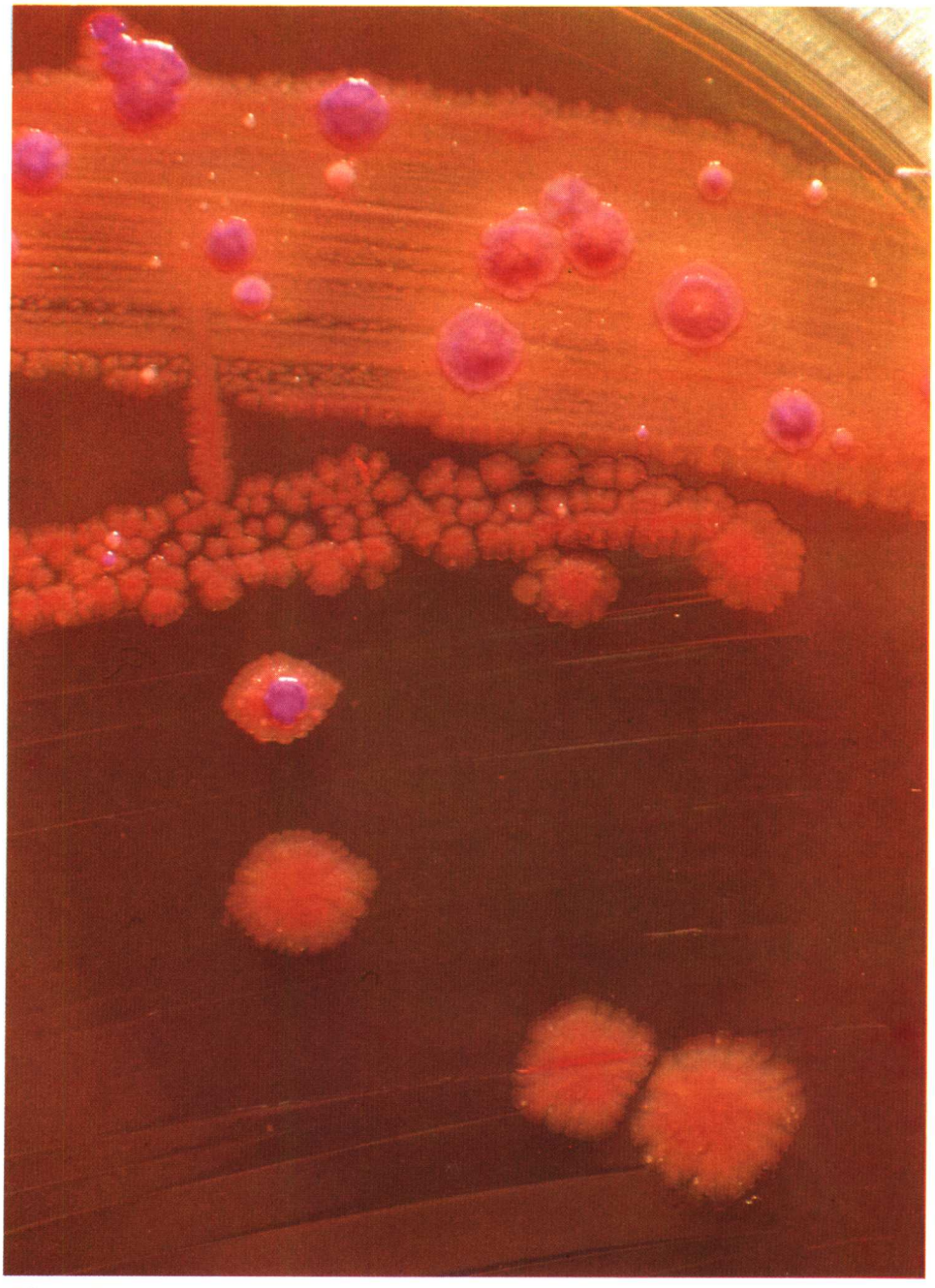

Plate 1

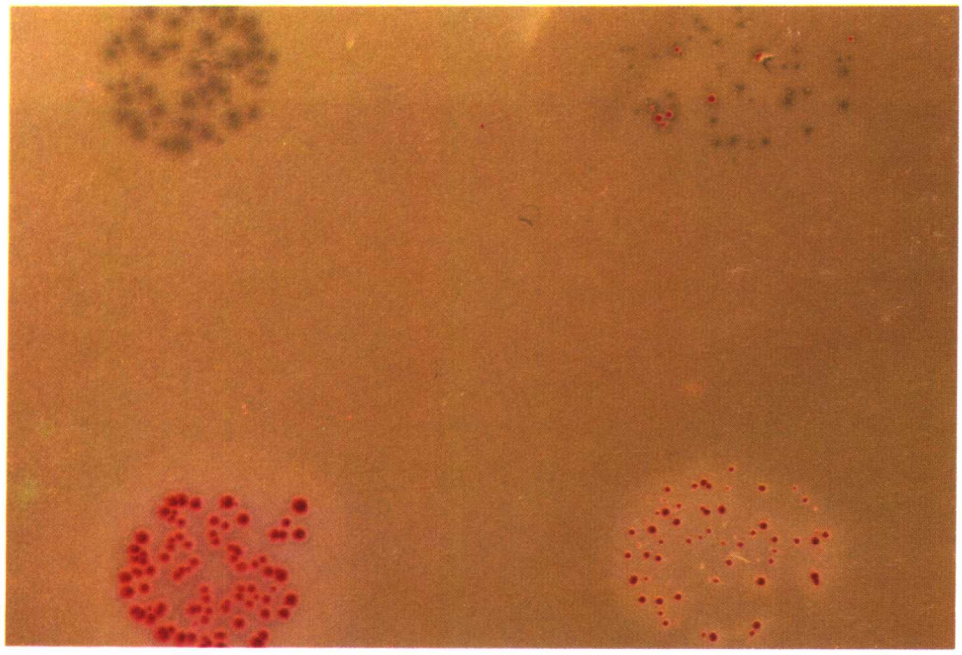

Plate 2

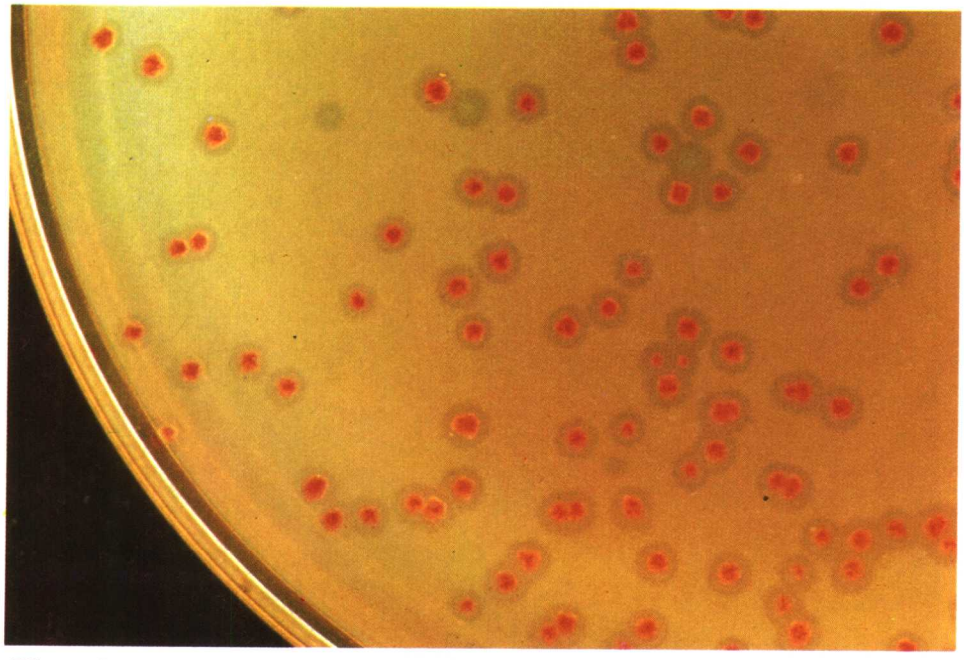

Plate 3

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Bacterial Strains

Strain[a]	Genotype[b]
BHB2688	F⁻ *recA* λ^r (λ *E*am4 *b*2 *red*3 *imm*434 *c*I*ts* S*a*m7)
BHB2690	F⁻ *recA* λ^r (λ *D*am15 *b*2 *red*3 *imm*434 *c*I*ts* S*a*m7)
KLF41	F′141/*leuB6 hisG1 recA1 argG6 metB1 lacY1 gal-6 xyl-7 mtl-2 malA1 rpsL104 tonA tsx supE44*
LE30	F⁻ *mutD5 rpsL azi galU95*
LE292	HfrH *argE*am *rpoB galT*:: (λΔ[*int-F*II])
LE392	F⁻ *supF supE hsdR galK trpR metB lacY tonA*
LE392.23	LE392 Δ(*argF-lac*)*U169*
MAL103	F⁻ Δ(*gpt-proAB-argF-lac*)XIII *rpsL* [MudI(*lac*, Ap)] (Mu*cts*62)
MB100	MC4100 *ara*$^+$ *leu ABCD*::Tn*10*
MB101	MBM7014 Φ(*araBA′-lacZ*$^+$)*101*[λ*p* 1(209)]
MBM7007	F⁻ *araC*am *araD* Δ(*argF-lac*)*U169 trp*am *malB*am *rpsL relA thi*
MBM7014	MBM7007 *supF*
MBM7060	MBM7014 (λ*p* 1048)
MBM7060(pMLB952)	
MC1000	F⁻ *araD139* Δ(*araABC-leu*)*7679 galU galK* Δ(*lac*)X74 *rpsL thi*
MC1000 (pMLB524)	
MC1000 (pMLB1034)	
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR*
MC4100 (pRT516)	
MH225	MC4100 Φ(*ompC′-lacZ*$^+$)*10-25*, [λ*p* 1(209)]
MH2101	MH225 *ompR101*
MH2472	MH225 *ompR472*

Strain[a]	Genotype[b]
MH513	MC4100 *ara*$^+$ Φ(*ompF'-lacZ*$^+$)*16-23*, [λ*p* 1(209)]
MH5101	MH513 *ompR101*
MH5473	MH513 *envZ473*
MH760	MC4100 *ompR472*
MH1160	MC4100 *ompR101*
MH1471	MC4100 *envZ473*
N3098	*lig7ts supF*
RT3	MC4100 *envZ3*
RT203	MH225 *envZ3*
SE3001	MC4100 Δ(*malK-lamB*)*1*
SE5000	MC4100 *recA56*
SG158(pRT516.101)[c]	MC4100 Φ(*malP'-lacZ*::*kan1081.1*)*1* [λ*p* 1(209)*c*I*ts*857]
SG263	MBM7014 *malPQ*::Tn*10*
SG265	F$^-$ Δ(*gpt-proAB-argF-lac*)XIII *ara argE*am *gyrA rpoB thi supP* (P1*cry*)
SG404	F'141/MC4100 *asd* (P1*cam*)
SG480[d]	MC4100 Δ(*malPQ-bioH-ompB*)*61*
SG608	MH225 (λ*p*RT2.3)
SG624	MH225 *envZ22*
SG626	MH225 *aroB*
SV101	MC4100 *malPQ*::Tn*10*
SW101	F$^-$ *araD139* Δ(*araABC-leu*)*7679 zab*::Tn*10* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR*
TK821	MC4100 *ompR331*::Tn*10*
TK827	MH513 *ompR331*::Tn*10*
594	*rpsL*

[a]All strains are *Escherichia coli* K-12.

[b]Fusions constructed as described in Experiment 1 (p. 7) or Appendix L (p. 261) of the manual contain a λ prophage that lies adjacent to the fusion in the chromosome. This is designated here as [λ*p* 1(209)]. Although the fusion phage is a derivative of λ*p* 1(209), it is not this phage per se. The event that generated the particular fusion, insertion or deletion, altered the bacterial DNA carried by λ*p* 1(209). As a consequence, the phage is "locked in" the chromosome (see Appendix H, p. 241).

[c]See Experiment 10, Fig. 14 (p. 67).

[d]See Experiment 8, Fig. 12 (p. 56).