3

Escherichia coli Host Strains

Nicola Casali

1. Introduction

To successfully perform molecular genetic techniques it is essential to have a full understanding of the properties of the various *Escherichia coli* host strains commonly used for the propagation and manipulation of recombinant DNA. *E. coli* is an enteric rod-shaped Gram-negative bacterium with a circular genome of 4.6 Mb ***(1)***. It was originally chosen as a model system because of its ability to grow on chemically defined media and its rapid growth rate. In rich media, during the exponential phase of its growth, *E. coli* doubles every 20–30 min; thus, during an overnight incubation period, a single selected organism will double enough times to yield a colony on an agar plate, or 1–2 billion cells per milliliter of liquid media. The ease of its transformability and genetic manipulation has subsequently solidified the role of *E. coli* as the host of choice for the propagation, manipulation, and characterization of recombinant DNA. In the past 60 yr *E. coli* has been the subject of intensive research and more is now known about these bacilli than any other organisms on earth.

A wide variety of *E. coli* mutants have been isolated and characterized. Almost all strains currently used in recombinant DNA experiments are derived from a single strain: *E. coli* K-12, isolated from the feces of a diphtheria patient in 1922 ***(2)***. This chapter will discuss characteristics of *E. coli* host strains that are important for recombinant DNA experiments in order to aid in the choice of a suitable host and circumvent possible problems that may be encountered. Common mutations and genotypes that are relevant to recombinant DNA experiments are summarized in **Table 1**. A complete listing of genetically defined genes has been compiled by Berlyn et al. ***(3)***.

1.1. Genotype Nomenclature

A genotype indicates the genetic state of the DNA in an organism. It is associated with an observed behavior called the phenotype. Genotypes of *E. coli* strains are described in accordance with a standard nomenclature proposed by Demerec et al. ***(4)***. Genes are given three-letter, lowercase, italicized names that are often mnemonics

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Table 1
Properties of Common Genotypes of *E. coli* Host Strains

Mutation	Description	Significance
Amy	Expresses amylase	Allows amylose utilization
ara	Mutation in arabinose metabolism	Blocks arabinose utilization
dam	Blocks adenine methylation at GATC sequences	Makes DNA susceptible to cleavage by some restriction enzymes
dcm	Blocks cytosine methylation at CC(A/T)GG sequences	Makes DNA susceptible to cleavage by some restriction enzymes
(DE3)	λ lysogen carrying the gene for T7 RNA polymerase	Used for T7 promoter-based expression systems
deoR	Regulatory gene mutation allowing constitutive expression of genes for deoxyribose synthesis	Allows replication of large plasmids
dnaJ	Inactivation of a specific chaperonin	Stabilizes expression of certain recombinant proteins
dut	dUTPase activity abolished	In combination with *ung*, allows incorporation of uracil into DNA; required for Kunkel mutagenesis
e14$^-$	A prophagelike element carrying *mcrA*	*See mcrA*
endA1	Activity of nonspecific endonuclease I abolished	Improves yield and quality of isolated plasmid DNA
F'	Host contains an F' episome with the stated features	Required for infection by M13 vectors
gal	Mutation in galactose metabolism	Blocks galactose utilization
gor	Mutation in glutathione reductase	Facilitates cytoplasmic disulfide bond formation
gyrA	DNA gyrase mutation	Confers resistance to nalidixic acid
hflA	Inactivation of a specific protease	Results in high-frequency lysogenization by lambda
hsdR	Inactivation of *Eco* endonuclease activity	Abolishes *Eco* restriction but not methylation ($r^- m^+$)
hsdS	Inactivation of *Eco* site-recognition activity	Abolishes *Eco* restriction and methylation ($r^- m^-$)
Hte	Unknown	Enhances uptake of large plasmids
lacI^q	Constitutive expression of the *lac* repressor	Inhibits transcription from the *lac* promoter
lacY	Lactose permease activity abolished	Blocks lactose uptake; improves IPTG-induced control of *lac* promoters
lacZ	β-Galactosidase activity abolished	Blocks lactose utilization

(continued)

Table 1 *(continued)*

Mutation	Description	Significance
*lacZ*ΔM15	Partial deletion of β-galactosidase gene	Allows α-complementation for blue/white selection of recombinant colonies in *lacZ* mutant hosts
leu	Mutation in leucine biosynthesis	Requires leucine for growth on minimal media
lon	Inactivation of Lon protease	Increases yield of some recombinant proteins
Δ(*malB*)	Mutation in maltose metabolism; deletes most of the region encompassing *malEFG* and *malK lamB malM*	Blocks maltose utilization; eliminates expression of maltose-binding protein (MalE)
mcrA, *mcrBC*	Mutation in methylcytosine-specific restriction systems	Allows more efficient cloning of DNA containing methylcytosines
metB	Mutation in methionine biosynthesis	Requires methionine for growth on minimal media; promotes high specific activity labeling with ^{35}S-methionine
mrr	Mutation in methyladenosine-specific restriction system	Allows more efficient cloning of DNA containing methyladenines
mtl	Mutation in mannitol metabolism	Blocks mannitol utilization
mutD	Inactivates DNA polymerase III subunit	Increases frequency of spontaneous mutation
mutS	Deficient in mismatch repair	Stabilizes DNA heteroduplexes during site-directed mutagenesis
nupG	Mutation in nucleoside transport	Increases plasmid uptake
ompT	Mutation in outer-membrane protease	Improves yield of some recombinant proteins
ϕ80	Carries the prophage ϕ80	Often expresses *lacZ*ΔM15
P1	Carries the prophage P1	Expresses the P1 restriction system
P2	Carries the prophage P2	Inhibits growth of *red*$^+$ *gam*$^+$ λ vectors
phoA	Mutation in alkaline phosphatase	Blocks phosphate utilization; used for PhoA-based reporter systems
phoR	Regulatory gene mutation	Used for *pho* promoter-based expression systems
pnp	Inactivates polynucleotide phosphorylase	Increases stability of some mRNAs resulting in increased protein expression

(continued)

Table 1 ***(continued)***

Mutation	Description	Significance
proAB	Mutations in proline biosynthesis	Requires proline for growth in minimal media
recA	Homologous recombination abolished	Prevents recombination of introduced DNA with host DNA, increasing stability of inserts
recBC	Exonuclease and recombination activity of ExoV abolished	Reduces general recombination; enhances stability of palindromes in λ vectors
recD	Exonuclease activity of ExoV abolished	Enhances stability of palindromes in λ vectors
recE	Recombination deficiency	Reduces recombination between plasmids
recF	Recombination deficiency	Reduces recombination between plasmids
recJ	Recombination deficiency	Reduces recombination between plasmids
relA	Eliminates stringent factor resulting in relaxed phenotype	Allows RNA synthesis in the absence of protein synthesis
rne	Inactivates RNase E	Increases stability of some mRNAs resulting in increased protein expression
rpoH (or *htpR*)	Inactivates a heat-shock sigma factor	Abolishes expression of some proteases; improves yield of certain recombinant proteins at high temperature
rpsL	Mutation in small ribosomal protein S12	Confers resistance to streptomycin
sbcA	Mutation in RecE pathway	Improves growth of *recB* mutant hosts
sbcB	ExoI activity abolished	Allows general recombination in *recBC* mutant strains
sbcC	Mutation in RecF pathway	Enhances stability of long palindromes in λ and plasmid vectors
srl	Mutation in sorbitol metabolism	Blocks sorbitol utilization
sup	Suppressor mutation	Suppresses ochre (UAA) and amber (UAG) mutations (*see* **Table 3**)
thi	Mutation in thiamine biosynthesis	Thiamine required for growth in minimal media
thr	Mutation in threonine biosynthesis	Threonine required for growth in minimal media
Tn*10*	Transposon	Encodes resistance to tetracycline

(continued)

Table 1 *(continued)*

Mutation	Description	Significance
Tn*5*	Transposon	Encodes resistance to kanamycin
tonA	Mutation in outer-membrane protein	Confers resistance to bacteriophage T1
traD	Mutation in transfer factor	Prevents conjugal transfer of F' episome
trp	Mutation in tryptophan biosynthesis	Tryptophan required for growth in minimal media
trxB	Mutation in thioredoxin reductase	Facilitates cytoplasmic disulfide bond formation
tsp	Mutation in a periplasmic protease	Improves yield of secreted proteins and proteins isolated from cell lysates
tsx	Mutation in outer-membrane protein	Confers resistance to bacteriophage T6
umuC	Mutation in SOS repair pathway	Enhances stability of palindromes
ung	Uracil *N*-glycosylase activity abolished	Prevents removal of uracil incorporated into DNA; *see dut*
uvrC	Mutation in UV repair pathway	Enhances stability of palindromes
xylA	Mutation in xylose metabolism	Blocks xylose utilization

Source: Compiled from **refs. *3*** and ***5*** and information supplied by Invitrogen, New England Biolabs, Novagen, and Stratagene.

suggesting the function of the gene. If the same function is affected by several genes, the different genes are distinguished with uppercase italic letters, for example *recA*, *recB*, *recC*, and *recD* all affect recombination. By convention, *E. coli* genotypes list only genes that are defective, but the superscript symbols "–" and "+" are occasionally used redundantly for clarity or to emphasize a wild-type locus. Phenotypes are capitalized and the letters are followed by either superscript "+" or "–," or sometimes "r" for resistant or "s" for sensitive. Although convention dictates that phenotypes are not specified in the genotype designation, they are sometimes included, when not easily inferred. For example, *rpsL*(Str^r) indicates that a mutation in the gene for ribosomal protein small subunit S12 confers resistance to streptomycin.

Specific mutations are given allele numbers that are usually italic arabic numerals such as *hsdR17*. If the exact locus is not known, then the capital letter is replaced by a hyphen, as in *arg-3*. An amber mutation (*see* **Subheading 2.1.1.**) is denoted by *am* following the gene designation and a temperature-sensitive mutation that renders the gene inactive at high temperature, is denoted by *ts*. A constitutive mutation is denoted by superscript q; thus *lacI*^{q} indicates constitutive expression of the gene for the *lac* repressor.

Deletions are denoted by Δ. If Δ is followed by the names of deleted genes in parentheses, as in Δ(*lac-pro*), then all of the genes between the named genes are also deleted. An insertion is indicated by "::" preceded by the position of the insertion and followed by the inserted DNA; for example, *trpC22*::Tn*10* denotes an insertion of Tn*10* into *trpC*. Alternatively, the map position of an insertion can be denoted by a three-letter code. The first letter is always *z*, the second and third letters indicate 10-min and 1-min intervals, respectively, and are designated by the letters *a–i*. Thus, *zhg*::Tn*10* indicates an insertion of Tn*10* at 87 min. A fusion is denoted by the symbol ϕ followed by the fused genes in parentheses. A prime denotes that a fused gene is incomplete and can be used before or after the gene designation to denote deletions in the 5' or 3' regions, respectively. A superscript "+" indicates that the fusion involves an operon rather than a single gene. For example, ϕ(*ompC*'-*lacZ*$^+$) indicates a fusion between *ompC*, deleted in the 3' region, and the *lac* operon.

F$^+$ and Hfr (*see* **Subheading 2.2.**) strains are denoted by the relevant symbol at the start of the genotype and strains are assumed to be F$^-$ unless indicated. If the strain is F', then this is indicated at the end of the genotype with the genes carried by the F plasmid listed in square brackets. Plasmids and lysogenic phage, carried by the strain, are listed in parentheses at the end of the genotype and may include relevant genetic information.

2. General Properties of Cloning Hosts

The genotypes and features of a representative selection of popular host strains used for general recombinant DNA cloning procedures are listed in **Table 2**. An extended listing of available strain genotypes can be found in **ref. *5***. Many useful strains are available through the American Type Culture Collection (www.atcc.org) and the *E. coli* Genetic Stock Center at Yale (cgsc.biology.yale.edu), as well as from commercial suppliers such as Stratagene, Promega, Novagen, Invitrogen, and New England Biolabs.

2.1. Disablement

Many laboratory *E. coli* strains carry mutations that reduce their viability in the wild and preclude survival in the intestinal tract ***(6)***. These often confer auxtrophy, that is, they disable the cell's ability to synthesize a critical metabolite, which, therefore, must be supplied in the medium. Such mutations can also serve as genetic markers and may be useful for correct strain confirmation.

2.1.1. Suppressor Mutations

Some vectors contain nonsense mutations in essential genes as a means of preventing spread to natural bacterial populations. Nonsense mutations are chain-termination codons; they are termed amber (UAG) or ochre (UAA) mutations ***(5)***. Vectors containing these mutations can only be propagated in strains of *E. coli* that contain the appropriate nonsense suppressors. Amber and ochre suppressors are usually found in tRNA genes, and alter the codon-recognition loop so that a specific amino acid is occasionally inserted at the site of the nonsense mutation. Nonsense suppressors commonly used in cloning strains are given in **Table 3**.

Table 2
Properties of Representative *E. coli* Strains Used for Vector Propagation and Cloning Procedures

Strain[a]	Genotype[b]	Blue-white screening	Cloning methylated DNA	Generation of unmethylated DNA	Reduced recombination	Production of ssDNA	Transformation of large plasmids	Supression of amber mutations	Suppliers[c]
DH10B	Δ(*araABC-leu*)*7697 araD139 deoR endA1 galK galU* Δ(*lac*)*X74 mcrA* Δ(*mcrCB-hsdSMR-mrr*) *nupG recA1 rpsL*(Str^r) (ϕ80 *lacZ*ΔM15)	•	•		•				I
DH5α	*deoR endA1 gyrA96 hsdR17* Δ(*lac*)*U169 recA1 relA1 supE44 thi-1* (ϕ80 *lacZ*ΔM15)	•			•			•	AI
DM1	*ara dam dcm gal1 gal2 hsdR lac leu mcrB thr tonA tsx zac*::Tn*9*(Cam^r)			•					I
GeneHogs	*araD139* Δ(*ara-leu*)*7697 deoR endA1 galU galK* Δ(*lac*)*X74 mcrA* Δ(*mrr-hsdRMS-mrcBC*) *nupG recA1 rpsL*(Str^r) (ϕ80 *lacZ*ΔM15) λ	•	•		•		•		I
HB101[d]	*ara-14 galK2 proA2 lacY1 hsdS20 mtl-1 recA13 rpsL20*(Str^r) *supE44 xyl*-5				•			•	BIP
INV110	*ara dam dcm dupE44 endA galK galT* Δ(*lac-proAB*) *lacY leu* Δ(*mcrCB-hsdSMR-mrr*)*102*::Tn*10*(Tet^r) *rpsL*(Str^r) *thi-1 thr tonA tsx* F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$ *traD36*]	•	•	•		•			I
JM109	*endA1 gyrA96 hsdR17* Δ(*lac-proAB*) *recA1 relA1 supE44 thi-1* F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$ *traD36*]	•			•	•		•	AP
JS5	Δ(*araABC-leu*)*7697 araD139 galU galK hsdR2* Δ(*lac*)*X74 mcrA mcrBC recA1 rpsL*(Str^r) *thi* F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$ Tn*10*(Tet^r)]	•			•	•			B
LE392	*galK2 galT22 hsdR514 lacY1 mcrA metB1 supE44 supF28 trpR55*							•	A

(continued)

Table 2 *(continued)*

Strain[a]	Genotype[b]	Blue-white screening	Cloning methylated DNA	Generation of unmethylated DNA	Reduced recombination	Production of ssDNA	Transformation of large plasmids	Supression of amber mutations	Suppliers[c]
NM522	Δ(*hsdMS-mcrB*)*5* Δ(*lac-proAB*) *supE thi-1* F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$]	•				•		•	AS
SCS110	*ara dam dcm endA galK galT* Δ(*lac-proAB*) *lacY leu rpsL*(Str^r) *supE44 thi-1 thr tonA tsx* F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$ *traD36*]	•		•		•		•	S
STBL4	*endA1 gal gyrA96 Δ(lac-proAB) mcrA Δ*(*mcrCB-hsdSMR-mrr*) *recA1 relA1 supE44 thi-1* F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$ Tn*10*(Tet^r)]	•	•		•	•	•	•	I
SURE	e*ndA1 gyrA96 lac mcrA* Δ(*mcrCB-hsdSMR-mrr*)*171 recB recJ relA1 sbcC supE44 thi-1 umuC::*Tn*5*(Kan^r) *uvrC* F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$]	•	•		•	•	•	•	AS
TG1	Δ(*hsdMS-mcrB*)*5* Δ(*lac-proAB*) *supE thi-1* F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$ *traD36*]	•				•		•	S
XL10-Gold	*endA1 gyrA96 lac* Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 recA1 thi-1 relA1 supE44* Hte F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$ Tn*10*(Tet^r) Amy Cam^r]	•	•		•	•	•	•	S
XL1-Blue MRF'	*endA1 gyrA96 lac* Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 recA1 relA1 supE44 thi-1* F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$ Tn*10*(Tet^r) Amy Cam^r]	•	•		•	•		•	S

Note: Data compiled from suppliers' catalogs.

[a] All strains are derived from *E. coli* K-12 unless otherwise stated.

[b] Cam is chloramphenicol; Kan is kanamycin; Str is streptomycin; Tet is tetracycline.

[c] A is ATCC; B is Bio-Rad; I is Invitrogen; P is Promega; S is Stratagene.

[d] This strain is a hybrid of *E. coli* K-12 and *E. coli* B.

Table 3
Properties of Common *E. coli* Suppressor Mutations

Mutation	Codons suppressed	Amino acid inserted	tRNA gene supplied
supB	Amber, ochre	Glutamine	*glnU*
supC	Amber, ochre	Tyrosine	*tyrT*
supD	Amber	Serine	*serU*
supE	Amber	Glutamine	*glnV*
supF	Amber	Tyrosine	*tyrT*

2.2. Fertility Status

Some *E. coli* strains carry an F episome or fertility factor, which can be found in several different forms ***(7)***. It may be carried as a double-stranded single-copy circular extrachromosomal plasmid, designated F^+, or if it harbors additional genes, F'. These extrachromosomal forms can transfer themselves to recipient cells, which are F^-, and occasionally cause the mobilization of other plasmids (*see* Chapter 6). In Hfr cells (high-frequency chromosome donation), the F factor is integrated into the bacterial chromosome and can cause chromosomal transfer. Mutations in the locus *tra* inhibit transfer and mobilization.

Strains containing the F factor produce surface pili, which are required for infection by vectors based on filamentous phage. The F factor also permits the production and rescue of single-stranded DNA from M13 vectors when coinfected with a helper phage (*see* Chapter 13).

2.3. Restriction and Modification Systems

Restriction–modification systems play a role in preventing genetic exchange between groups of bacteria by enabling the host to recognize and destroy foreign DNA. An archetypal system consists of a DNA methylase and its cognate restriction endonuclease. The methylase covalently modifies host DNA, by transfer of a methyl group from *S*-adenosylmethionine to a cytosine or adenine residue, within the recognition sequence of its cognate restriction enzyme. Methylation prevents digestion at this site, limiting digestion to incoming foreign DNA ***(8)***. The restriction–modification systems present in an *E. coli* host will affect the pattern and extent of recombinant DNA methylation and can significantly affect the success of restriction digestions and bacterial transformations. Many common laboratory strains of *E. coli* that are deficient in one or more restriction–modification systems are available to counteract this problem.

2.3.1. Dam and Dcm Methylation

Derivatives of *E. coli* K-12 normally contain three site-specific DNA methylases: Dam, Dcm and *Eco*K. DNA adenine methylase, encoded by *dam*, methylates adenine residues in the sequence GATC ***(9,10)***. This sequence will occur approximately once every 256 bp in a theoretical piece of DNA of random sequence. DNA cytosine methylase, encoded by *dcm*, methylates the internal cytosine residue in the sequence CC(A/T)GG, which occurs on average once every 512 bp ***(9,11)***. Almost

all commonly used cloning strains are Dam$^+$ Dcm$^+$. Strains that are *recA*$^-$ (*see* **Subheading 2.4.**) are always *dam*$^+$, because the combination *recA*$^-$ *dam*$^-$ results in a lethal phenotype.

Methylation may interfere with cleavage of DNA cloned and propagated in *dam*$^+$ and *dcm*$^+$ *E. coli* strains. Not all restriction endonucleases are sensitive to methylation. For example, Dam-modified DNA is not cut by *Bcl*I (TGATCA); however, it is cut by *Bam*HI (GGATCC) ***(12)***. The restriction enzyme database, REBASE (rebase.neb.com), contains comprehensive information on the methylation sensitivity of restriction endonucleases ***(13)***. Not all DNA isolated from *E. coli* is completely methylated. For example, only about 50% of λ DNA sites are Dam methylated, presumably because λ DNA is rapidly packaged into phage heads. Thus, restriction of such DNA with a Dam-sensitive restriction endonuclease will yield a partial digestion pattern.

The presence of Dam or Dcm methylation can also affect the efficiency of plasmid transformation. For example, Dam-modified DNA cannot be efficiently introduced into a *dam*$^-$ strain, because replication initiation is inhibited when DNA is hemimethylated. Thus, a transformed plasmid is able to replicate once but not again ***(14)***.

Dam$^-$ Dcm$^-$ strains have the disadvantage that these mutations are mutagenic. This is because in wild-type strains, newly synthesized DNA is hemimethylated and any errors introduced by the polymerase are corrected by mismatch repair systems to the original methylated strand. However, in Dam$^-$ Dcm$^-$ strains, neither strand is methylated and the mismatch is equally likely to be resolved to the newly synthesized strand as to the correct one ***(15)***.

2.3.2. Eco*K* System

The *E. coli* K-12 *Eco*K methylase modifies the indicated adenine residues of the target sequence A(^{m}A)CN$_6$GTGC, and its complement GC(^{m}A)CN$_6$GTT ***(8,16)***. The cognate endonuclease will cleave DNA that is unmodified at this sequence. The *Eco*K system is encoded by the *hsdRMS* locus, where *hsdR* encodes the endonuclease, *hsdM* the methylase, and *hsdS* the site-recognition subunit. *E. coli* strains used for cloning are generally either *hsdR*$^-$, resulting in a restriction minus phenotype (r_K^- m_K^+), or *hsdS*$^-$, resulting in a restriction and methylation deficiency (r_K^- m_K^-). Strains derived from *E. coli* B are (r_B^+ m_B^+) and carry the equivalent *Eco*B endonuclease and methylase, which modify the adenosine in the sequence TGAN$_8$TGCT ***(17)***.

Because *Eco*K sites are rare, occurring approximately once every 8 kb, this type of methylation does not generally interfere with restriction digestion. However, transformation of unmodified plasmid DNA into *hsdR*$^+$ strains results in more than a 1000-fold reduction in efficiency and can lead to underrepresentation of fragments containing *Eco*K sites in libraries. Thus, if transferring DNA between strains with different *Eco*K genotypes, a plasmid should be passed through an *hsdM*$^+$ strain before introduction into an *hsdR*$^+$ *strain.*

2.3.3. McrA, McrBC, and Mrr Restriction

E. coli K-12 also contains several methylation-dependent restriction systems, namely McrA, McrBC, and Mrr. The methylcytosine restricting endonucleases, McrA

and McrBC, cleave methylcytosines in the sequences CG and (A/C)G, respectively ***(18–21)***. Mrr (methyladenine recognition and restriction) cleaves methyladenines, but the precise recognition sequence is unknown ***(22,23)***. None of these three systems cleave Dcm- or Dam-modified DNA and are, thus, generally of little concern when subcloning DNA from *dam*$^+$ *dcm*$^+$ *E. coli*, but using strains mutant in these systems may be desirable if cloning highly methylated DNA from other sources. In addition, when cytosine methylases are used in cloning procedures, such as adding linkers, the recombinant DNA should be transformed into an *mcrA*$^-$ *mcrBC*$^-$ strain to avoid Mcr restriction ***(8)***.

Most of these restriction determinants are clustered in a single "immigration control" locus allowing the removal of *hsdRMS*, *mcrBC*, and *mrr* by a single deletion: Δ(*mcrCB-hsdSMR-mrr*) ***(19)***.

2.4. Recombination

Following successful transformation of a plasmid vector into *E. coli*, host recombination systems can catalyze rearrangement of the recombinant molecule. This is a particular problem when the cloned DNA contains direct or inverted repeats and can result in duplications, inversions, or deletions. If the resulting product is smaller than the original molecule, it will replicate faster and quickly dominate the population. Mutations in the host that suppress recombination can help maintain the integrity of cloned DNA. Recombination properties are especially relevant to the choice of hosts for library propagation in order to avoid misrepresentation because of the unequal growth of specific clones. However, recombination-deficient strains are generally unfit and suffer from enhanced sensitivity to DNA-damaging agents, deficiency in repairing double-strand breaks in DNA, slow growth rate, and the rapid accumulation of nonviable cells ***(24)***; thus, depending on the application, Rec$^+$ strains may still be preferable.

E. coli contains three main recombination pathways encoded by *recBCD*, *recE*, and *recF* ***(25,26)***. All three pathways depend on the product of *recA*, with the notable exception of recombination of certain plasmids and phage promoted by the RecE pathway. Hence, *recA*$^-$ is the most stringent Rec$^-$ condition and mutations in *recA* reduce recombination 10,000-fold compared to wild type, almost completely blocking recombination.

The RecBCD, or exonuclease V (ExoV), pathway is predominant in wild-type *E. coli* K-12. Strains with single mutations in *recB* or *recC*, and *recBC* double mutants are defective in this pathway and have indistinguishable phenotypes exhibiting recombination rates 100- to 1000-fold lower than wild type ***(27)***. These strains are unfit and tend to accumulate extragenic suppressor mutations in both *sbcB* (suppressor of RecBC$^-$), encoding ExoI, and *sbcC* ***(28–30)***. The secondary mutations enable efficient recombination to be catalyzed by the RecF pathway and restore viability ***(25)***. In *recBC*$^-$ strains, the RecE (ExoVIII) pathway is activated by mutations in *sbcA* ***(31)***. Both *recE* and *sbcA* map to the cryptic lambdoid prophage *rac* that is present in most *E. coli* K-12 strains ***(32)***. In contrast, mutation in *recD*, which encodes the nuclease activity of ExoV, results in a healthy Rec$^+$ phenotype that does not acquire secondary mutations ***(33)***.

Cloned palindromes or interrupted palindromes are highly unstable in wild-type *E. coli*. Both *recBC*$^-$ ***(34)*** and *recD*$^-$ ***(35,36)*** strains are good hosts for palindrome stabilization in λ-derived vectors. However, most cloning plasmids are unstable in

recBC$^-$ and *recD*$^-$ strains and are difficult to maintain, even with selection ***(33,37)***. The problem is especially severe with high-copy-number ColE1 derivatives; this is probably the result of recombination-initiated rolling-circle replication, which results in long linear multimers that do not segregate properly at cell division ***(38)***. Mutation in *recA* or *recF* is able to suppress this effect ***(37,39)***. Mutations in *sbcBC* also independently stabilize cloned palindromes and *sbcC*$^-$ strains are permissive for palindromes in plasmids as well as phage ***(35,36,40)***.

2.4.1. Recombination Systems in λ-Infected Hosts

Bacteriophage λ is injected into the *E. coli* host as a linear molecule that rapidly circularizes and, during the early phase of infection, replicates by a bidirectional θ-type mechanism, yielding monomeric circles. Subsequently, replication converts to a rolling-circle σ-type mechanism, generating linear concatemers that are suitable substrates for packaging into phage heads ***(41)***.

Rolling-circle replication is inhibited by host RecBCD, which degrades the linear concatameric DNA. Thus, efficient propagation by rolling-circle replication requires a *recBC*$^-$ *sbcB*$^-$ or *recD*$^-$ host. Alternatively, the exonucleolytic activity RecBCD can be inhibited by the product of the λ *gam* gene, which may be carried on the λ vector itself or on a separate plasmid ***(42,43)***.

Infection of *recBCD*$^+$ strains with *gam*$^-$ λ will result in the production of the progeny phage only if a suitable recombination pathway exists to convert monomeric circles, produced by θ-replication, to multimeric circles that are acceptable substrates for packaging. Either λ-encoded Red recombinase or host RecA are able to catalyze this reaction ***(42)***. Most λ are *gam*$^-$ *red*$^-$ and, therefore, require a RecA$^+$ host for propagation.

The presence of the octameric sequence GCTGGTGG, termed a χ (chi) site ***(44)***, in the *gam*$^-$ λ genome can overcome inefficient multiplication in a *recBC*$^+$ background ***(45)***. The χ site in the λ recombinant causes increased recombination, by a RecBCD-dependent pathway, requiring RecA, resulting in more efficient conversion from monomeric to multimeric circular forms. It should be noted that cloned sequences containing a χ site will be overrepresented in libraries constructed in *gam*$^-$ χ$^-$ vectors if propagated in a *recBC*$^+$ host.

2.5. α-Complementation

Many current molecular biology techniques rely on the pioneering studies of the *lac* operon by Jacob and Monod in the 1960s ***(46)***. The *lac* operon consists of three genes: *lacZYA*, encoding β-galactosidase, which cleaves lactose to glucose and galactose, a permease, and a transacetylase. The *lac* repressor, encoded by the neighboring *lacI* gene, derepresses transcription of the *lac* operon in the presence of lactose ***(47)***.

Cells bearing 5' deletions in *lacZ* produce an inactive C-terminal fragment of β-galactosidase termed the ω-fragment; similarly, cells with a 3' deletion in *lacZ* (*lacZ'*) synthesize an inactive N-terminal α-fragment. However, if both fragments are produced in the same cell then β-galactosidase activity is restored ***(48)***. This phenomenon, known as α-complementation, is the basis for the visual selection of clones containing recombinant vectors by "blue-white screening" (*see* Chapter 19). The vector expresses the α-fragment and requires a host that expresses the ω-fragment. Gen-

erally, the host is engineered to carry the chromosomal deletion Δ*(lac-proAB)*; this mutation is partially complemented by *lacZΔ*M15, which consists of the lac operon minus the *lacZ*' segment and is often carried, along with *lacI*^{q} ***(49)***, on the lambdoid prophage ϕ80 or the F' plasmid. The F' episome is also usually *proAB*$^+$ to rescue proline auxotrophy and allow maintenance of the plasmid on proline-deficient minimal media.

To select for recombinant *E. coli*, bacilli are grown on media containing the nonfermentable lactose analog isopropyl-β-D-thiogalactoside (IPTG), which inactivates the *lac* repressor and derepresses ω-fragment synthesis. In the presence of IPTG, the chromogenic lactose analog 5-bromo-4-chloro-3-indoxyl-β-D-galactopyranoside (X-Gal) is cleaved by β-galactosidase to a blue-colored product. Cloning vectors that allow blue-white screening contain a multiple cloning site embedded within the α-fragment. Insertion of a DNA fragment within this region abolishes production of the α-fragment, and colonies grown on IPTG and X-Gal appear white.

3. Hosts for Mutagenesis

The frequency of spontaneous mutation in *E. coli* may be increased by three to four orders of magnitude by mutations in *mutD*, which encodes the 3'→5' exonuclease subunit of the DNA polymerase III holoenzyme ***(50,51)***. Thus, random mutagenesis can be achieved by maintaining plasmids in a *mutD*$^-$ strain for a number of generations and subsequently transforming the mutated plasmid into a *mutD*$^+$ "tester" strain. This method provides a useful alternative to chemical mutagenesis.

Site-directed mutagenesis methods frequently involve intermediates that contain wild-type/mutant heteroduplexes. Such heteroduplexes are stabilized in *mutS* mutants, which are deficient in mismatch repair, leading to high mutation efficiencies.

Kunkel mutagenesis requires a specialized *dut*$^-$ *ung*$^-$ host strain, which does not express dUTPase or uracil-*N*-glycosylase, resulting in the occasional substitution of uracil for thymine in newly synthesized DNA ***(52)***. In this procedure, single-stranded template DNA is prepared from a *dut*$^-$ *ung*$^-$ host; next, a mutant primer is annealed to the template and the second strand is synthesized. Subsequent transformation of the heteroduplex into an *ung*$^+$ strain will result in digestion of the uracil-containing parental strand, enriching for the mutant strand.

Various hosts that are useful for mutagenesis procedures are listed in **Table 4**.

4. Specialized Strains for Protein Expression

E. coli is a popular host for the overexpression of recombinant proteins (*see* Chapters 28 and 29). There are a number of factors that can influence protein yields and careful strain choice can greatly improve the chance of successful expression. Recent innovations have resulted in the availability of many new host strains, a selection of which are given in **Table 5**.

4.1. Repressors

E. coli expression vectors utilize highly active inducible promoters and the correct host strain must be used to ensure proper tight regulation ***(53)***. Many common vectors

Table 4
Properties of *E. coli* Strains Used as Hosts for Mutagenesis

Strain[a]	Genotype[b]	Application	Supplier[c]
BMH 71-18 *mutS*	Δ(*lac-proAB*) *mutS*::Tn*10*(Tet^r) *supE thi-1* F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$]	Used for site-directed mutagenesis	P
CJ236	*dut1 mcrA relA1 spoT1 thi-1 ung1* (pCJ105 F$^+$ Cam^r)	Used for generation of uracil-substituted DNA for Kunkel mutagenesis	B
MV1190	Δ(*lac-proAB*) Δ(*srl-recA*)*306*::Tn*10*(Tet^r) *supE thi* F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$ *traD36*]	Used for enrichment of mutant DNA	B
XL1-Red	*endA1 gyrA96 hsdR17 lac mutD5 mutS mutT relA1 supE44 thi-1* Tn*10*(Tet^r)	Used for random mutagenesis	S
XL-*mutS*	*endA1 gyrA96 lac* Δ*mcrA183* Δ(*mcrCB-hsdSMR-mrr*)*173 mutS*::Tn*10*(Tet^r) *relA1 supE44 thi-1* F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$ Tn*5*(Kan^r)]	Used for site-directed mutagenesis	S

Note: Date compiled from suppliers' catalogs.

[a] All strains are derived from *E. coli* K-12.

[b] Cam is chloramphenicol; Kan is kanamycin; Tet is tetracycline.

[c] B is Bio-Rad; P is Promega; S is Stratagene.

use the *lac* promoter, the related *lacUV5* promoter, or the *tac* promoter, which is a synthetic hybrid of the *lac* and *trp* promoters (*see* Chapter 29). These promoters are repressed in the presence of the chromosomal *lacI*^{q} allele; however, high-copy-number plasmids require *lacI* or *lacI*^{q} to be supplied *in trans*, on a compatible plasmid, to prevent leakiness. The *lac* promoters can be regulated by the lactose analog IPTG. Improved control can be achieved by using *lacY* mutants that prevent Lac permease-mediated active transport of IPTG. IPTG thus enters the cell in a concentration-dependent manner and the recombinant protein is uniformly expressed in all cells.

Another popular system is based on the bacteriophage T7 RNA polymerase (RNAP) and puts the recombinant protein under the control of the T7 late promoter. The T7 RNAP is regulated by the IPTG-inducible *lacUV5* promoter and is usually supplied *in trans* from the λ(DE3) lysogen. For the expression of toxic proteins, tighter control can be achieved in hosts that express T7 lysozyme, a natural inhibitor of T7 RNAP; by inhibiting basal levels of RNAP, expression of the target gene is reduced prior to induction. The plasmids pLysS or pLysE express T7 lysozyme at low and high levels, respectively, enabling variable levels of expression control (*see* Chapter 28).

4.2. Stability

Host proteases can interfere with the isolation of intact recombinant proteins; degradation may be avoided by the use of protease-deficient hosts. In *E. coli*, *lon* encodes a major ATP-dependent protease and strains that contain deletions of this gene greatly improve the yield of many recombinant proteins ***(54,55)***. An *rpoH* mutation represses Lon expression and also independently decreases the rate of protein degradation ***(56)***. Mutations in the gene for the outer-membrane protease OmpT also improve the recovery of intact recombinant proteins, especially if purified from whole-cell lysates ***(57)***.

Rapid degradation of mRNA may be the limiting factor in the expression of certain genes, particularly when using T7 RNAP-based systems in which transcription is not coupled to translation. An *rne* mutation, abolishing RNaseE activity, eliminates a major source of RNA degradation increasing the availability of mRNA for translation ***(58,59)***.

ABLE C and ABLE K strains express a heterogenous DNA polymerase I and reduce the copy number of ColE1-derived plasmids by 4-fold and 10-fold, respectively. The resulting reduction in the basal expression level of toxic recombinant proteins improves cell viability. The availability of both strains allows the choice of the highest plasmid copy number that is still permissive for growth ***(60)***.

4.3. Codon Bias

The frequency with which amino acid codons are utilized varies between organisms and is reflected by the abundance of the cognate tRNA species. This codon bias can have a significant impact on heterologous protein expression, so that genes that contain a high proportion of rare codons are poorly expressed ***(61,62)***. A subset of the codons for arginine, isoleucine, glycine, leucine, and proline are rarely used in *E. coli*. The forced high-level expression of genes containing these codons results in a depletion of internal tRNA pools and can lead to translational stalling, frame shifting, premature termination, or amino acid misincorporation ***(63)***. Recombinant protein expression can

Table 5
Properties of *E. coli* Strains Commonly Used for Recombinant Protein Expression

Strain	Genotype[a]	Derivation[b]	Key features	Supplier[c]
ABLE C, ABLE K	*hsdS lac mcrA mcrBC mcrF mrr* (Kan^r) F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$ Tn*10*(Tet^r)]	C strain	Reduces plasmid copy number; useful for expression of toxic proteins	S
AD494[d,e]	Δ(*araABC-leu*)*7697* Δ*lacX74* Δ*malF3* Δ*phoAPvuII phoR trxB*::Kan^r F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$]	K-12	Enhances cytoplasmic disulfide bond formation	N
B834[d,e]	*gal hsdS*$_B$ *met ompT*	B strain	Protease deficient; used for labeling with ^{35}S-methionine	N
BL21[d–f]	*gal hsdS*$_B$ *ompT*	B834	Protease deficient	INS
BL21 Star[d,e]	*gal hsdS*$_B$ *ompT rne131*	BL21	Improves stability of mRNA	I
BL21 CodonPlus-RIL[d]	*endA gal ompT hsdS*$_B$ Dcm$^+$ Hte Tet^r (pACYC-RIL *argU ileY leuW* Cam^r)	BL21	Expresses rare tRNAs; useful for AT-rich genomes	S
BL21 CodonPlus-RP[d]	*endA gal ompT hsdS*$_B$ Dcm$^+$ Hte Tet^r (pACYC-RP *argU proL* Cam^r)	BL21	Expresses rare tRNAs, useful for GC-rich genomes	S
BL21 *trxB*[d,e]	*gal hsdS*$_B$ *ompT trxB15*::Kan^r	BL21	Enhances cytoplasmic disulfide bond formation	N
BLR[d,e]	*gal hsdS*$_B$ *ompT* Δ(*srl-recA*)*306*::Tn*10*(Tet^r)	BL21	Stabilizes repetitive sequences and prevents loss of l prophage	N

Origami[d,e]	*araD139* Δ(*araABC-leu*)*7697 galE galK gor522*::Tn*10*(Tet^r) Δ*lacX74* Δ*phoAPvuII phoR rpsL*(Str^r) *trxB*::Kan^r F'[*lacI*^{q} *lacZ*ΔM15 *pro*$^+$]	K-12	Greatly enhances cytoplasmic disulfide bond formation	N
Rosetta[d,e]	*gal hsdS*$_B$ *lacY1 ompT* (pRARE *araW argU glyT ileX leuW proL metT thrT tyrU thrU* Cam^r)	Tuner	Expresses rare tRNAs; improves IPTG-mediated expression control	N
TKB1	*gal hsdSB ompT* (DE3) (pTK Tet^r)[g]	B strain	Generates phosphorylated proteins	S
TKX1	*endA1 gyrA96 lac* Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 recA1 relA1 supE44 thi-1* F'[*lacI*^{q} *lacZ*ΔM15 *proAB*$^+$ Tn*5*(Kan^r)] (pTK Tet^r)[g]	K-12	Generates phosphorylated proteins	S
Tuner[d,e]	*gal hsdS*$_B$ *lacY1 ompT*	BL21	Improves IPTG-mediated expression control	N

Note: Data compiled from suppliers' catalogs.

[a] Cam is chloramphenicol; Kan is kanamycin; Str is streptomycin; Tet is tetracycline.

[b] All B strain derivatives are naturally *lon* and *dcm*.

[c] I is Invitrogen; N is Novagen; S is Stratagene.

[d] Available as a lysogen of λ(DE3).

[e] Available as (DE3)pLysS.

[f] Available as (DE3)pLysE.

[g] ColE1-compatible plasmid harboring *elk* controlled by the *trp* promoter.

be rescued by using hosts that express tRNAs for rare codons and thus provide "universal" translation ***(64,65)***. *E. coli* hosts are available that supply genes for rare tRNAs, particularly *argU* (AGA/AGG), *ileY* (AUA), *glyT* (GGA), *leuW* (CUA), and *proL* (CCC), in combinations optimized for the expression of genes from AT- or GC-rich genomes. The laborious classical method of altering individual codons in the target gene, by site-directed mutagenesis, is obviated by the availability of these useful hosts.

4.4. Solubility and Posttranslational Processing

Overproduction of heterologous proteins in *E. coli* often results in misfolding and segregation into insoluble inclusion bodies. The cytoplasmic chaperones, DnaK-DnaJ and GroES-GroEL, assist proper folding in wild-type *E. coli* and there is evidence that co-overproduction of either complex increases the yield of soluble proteins from recombinant *E. coli* ***(66)***.

The *E. coli* cytoplasm is a reducing environment that strongly disfavors the formation of stable disulfide bonds. Mutations in *trxB* and *gor*, which encode thioredoxin and glutathione reductases, facilitate cytoplasmic disulfide bond formation and increase the efficiency of oxidized recombinant protein accumulation. Thus gor^{-} $trxB^{-}$ mutants are useful for the production of proteins whose solubility depends on proper oxidation ***(67–69)***.

Wild-type *E. coli* lack the ability to phosphorylate tyrosine residues. However, specialized host strains that carry the *elk* tyrosine kinase gene are able to produce tyrosine-phosphorylated proteins that may be required for affinity screening of expression libraries or for the purification of SH2 domain-containing proteins ***(70,71)***.

5. Conclusion

Since the first mutants of *E. coli* K-12 were isolated in the 1940s, laboratory strains have been heavily mutagenized by treatment with X-rays, ultraviolet irradiation, and nitrogen mustard. Thus, they may carry unidentified mutations and it can be useful to try more than one strain background if experiments are unsuccessful.

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