Promega

TECHNICAL REFERENCE

# *Genetic Markers in E. coli and EndA+ versus EndA- Strains*

| Symbol | Description | Effect of Mutation |
|---|---|---|
| *ara*-14 | Mutation in arabinose metabolism | Blocks arabinose catabolism. |
| *araD* | L-ribulose phosphate 4-epimerase mutation; part of an inducible operon araBAD repressed by L-arabinose | Blocks arabinose catabolism. |
| *argA* | N-Acetylglutamate synthase mutation; inhibited by the presence of arginine | Arginine required from growth in minimal media. |
| *cycA* | Mutation of a gene product involved in D-alanine, glycine, D-serine and D-cycloserine transport, and an L-alanine carrier | Mutants cannot use D-alanine as a carbon source. |
| *dam* | DNA adenine methylase mutation | Blocks methylation of adenine residues in the sequence 5′…G$^{m}$ATC…3′. |
| *dapD* | Succinyl-diaminopimelate aminotransferase mutation | Reflects impaired synthesis of succinyl CoA. Mutants need to be supplemented with succinate or lysine + methionine. |
| *dcm* | DNA cytosine methylase mutation | Blocks methylation of cytosine in the sequence 5′…C$^{m}$CAGG…3′ or 5′…C$^{m}$CTGG…3′. |
| *deoC* | Deoxyribose-phosphate aldolase mutation | |
| *deoR* | Regulatory gene mutation allowing constitutive expression of genes for deoxyribose synthesis | Allows efficient propagation of large plasmids. |
| *dut*1 | Mutation of deoxyuridine triphosphatase, which catalyzes the conversion of dUTP to dUMP and PPi | Mutants are impaired in conversion of dUTP to dUMP, leading to higher dUTP pools, which can lead to misincorporation of uracil instead of thymidine. Stable incorporation of dUTP needs mutation in *ung* gene. |
| *endA*1 | DNA-specific endonuclease I mutation | Improves quality of plasmid DNA isolations. |
| *galE* | Part of the galETK operon that encodes UDP galactose-4-epimerase | Mutant is more resistant to bacteriophage P1 infection. |
| *galK* | Galactokinase mutation | Blocks catabolism of galactose. |
| *galT* | Galactose-1-phosphate uridylyltransferase mutation | Blocks catabolism of galactose. |
| *gyrA*96 | DNA gyrase mutation | Confers resistance to nalidixic acid. |
| *hflA*150 | Protease mutation that leads to stabilization of cII gene products | Leads to high frequency of lysogeny by λ phages (1). |
| *hflB* | Gene encodes a possible protease component | Leads to high frequency of bacteriophage lambda lysogenization. |
| *hsdR* ($r_K^-$, $m_K$+) | Host DNA restriction and methylation system mutation. Restriction minus, modification positive for the *E. coli* K strain methylation system | Allows cloning without cleavage of transformed DNA by endogenous restriction endonucleases. DNA prepared from this strain can be used to transform $r_K$+ *E. coli* strains. |
| *hsdS*20 ($r_B^-$, $m_B^-$) | Mutation of specificity determinant for host DNA restriction and methylation system. Restriction minus, modification minus for the *E. coli* B strain methylation system | Allows cloning without cleavage of transformed DNA by endogenous restriction endonucleases. DNA prepared from this strain is unmethylated by the *hsdS*20 methylases. |
| *lacI*$^q$ | Overproduction of the *lac* repressor protein | Leads to high levels of the *lac* repressor protein, inhibiting transcription from the *lac* promoter. |
| *lacY* | Galactoside permease mutation | Blocks lactose utilization. |
| *lacZ*ΔM15 | Partial deletion of β-D-galactosidase gene | Allows complementation of β-galactosidase activity by α-complementation sequence in pGEM®-Z Vectors. Allows blue/white selection for recombinant colonies when plated on X-Gal. |
| *leuB* | β-isopropylmalate dehydrogenase mutation | Requires leucine for growth on minimal media. |
| Δ(*lon*) | Deletion of *lon* protease | Reduces proteolysis of expressed proteins. |
| *LysS* | pLysS plasmid is integrated into the host genome | Strains carrying this plasmid will be tet resistant and produce T7 lysozyme, a natural inhibitor of T7 RNA polymerase, thus lowering background transcription of sequences under the control of the T7 RNA polymerase promoter (2). |
| *mcrA* | Mutation in methylcytosine restriction system | Blocks restriction of DNA methylated at the sequence 5′…G$^{m}$CGC…3′. |
| *mcrB* | Mutation in methylcytosine restriction system | Blocks restriction of DNA methylated at the sequence 5′…AG$^{m}$CT…3′. |
| *metB* | Cystathionine γ-synthase mutation | Requires methionine for growth on minimal media. |
| *metC* | Cystathionine beta-lyase mutation; involved in methionine biosynthesis | Requires methionine for growth on minimal media. |
| *mtl* | Mutation in mannitol metabolism | Blocks catabolism of mannitol. |
| *mutS* | Methyl-directed mismatch repair mutation | Prevents repair of the newly synthesized, unmethylated strand. |
| *ompT* | Mutation of protease VII, an outer membrane protein | Reduces proteolysis of expressed proteins. |
| P2 | P2 bacteriophage lysogen present in host | λ phages containing the *red* and *gam* genes of λ are growth inhibited by P2 lysogens (3). |
| *proA* | γ-glutamyl phoshate reductase mutation | proA/argD mutant will not block proline synthesis but will be repressed by arginine. Mutants excrete proline on minimal media and are resistant to proline analogs. proA/argD/argR triple mutant grows slowly on minimal media + arginine. |
| *proAB* | Mutations in proline metabolism | Requires proline for growth in minimal media. |
| *recA*1, *recA*13 | Mutation in recombination | Minimizes recombination of introduced DNA with host DNA, increasing stability of inserts. Inserts are more stable in *recA*1 than *recA*13 hosts. |
| *recB*, *recC*, *recD* | Exonuclease V mutations. The Rec BCD trimer (exonuclease V) progressively degrades ssDNA and dsDNA in an ATP-dependent manner to form oligonucleotides; implicated in homologous recombination | Reduces general recombination and affects repair of radiation damage. Allows easier propagation of sequences with inverted repeats. |

# Genetic Markers in E. coli and EndA+ versus EndA- Strains

**Genetic Markers in *E. coli* (continued).**

| Symbol | Description | Effect of Mutation |
|---|---|---|
| *recF* | Recombination and repair mutation | Mutant cannot repair daughter strand gaps (post-replicational repair). |
| *relA* | ppGpp synthetase I mutation. | Allows RNA synthesis in the absence of protein synthesis. The ribosome-associated ppGpp synthetase I can sense ribosomes with an uncharged tRNA in the A site, a sign of starvation. |
| *rha* | Use L-rhamnose, a methylpentose | Blocks rhamnose catabolism. |
| *rpsL* | Mutation in subunit S12 of 30S ribosome | Confers resistance to streptomycin. |
| *sbcB* | Exonuclease I mutation | Allows general recombination in *rec*BC mutant strains. |
| *strA* | Mutant alters ribosome protein S12 | Confers resistance to streptomycin. |
| *supB*, *supC*, *supG*, *supL*, *supM*, *supN*, *supO* | Suppressor mutations | Suppresses ochre (UAA) and amber (UAG) mutations. |
| *supD*, *supE*, *supF* | Suppressor mutations | Suppresses amber (UAG) mutations. |
| *thi*-1 | Mutation in thiamine metabolism | Mutants require thiamine for growth in minimal media. |
| *thr* | Threonine biosynthesis mutation | Mutants are obligate threonine auxotrophs. |
| *thyA* | Thymidylate synthase; dTTP biosynthesis | Mutants are obligate thymidine auxotrophs. |
| Tn5 | Transposon | Encodes resistance to kanamycin. |
| Tn10 | Transposon | Encodes resistance to tetracycline. |
| *tonA* | Mutation in outer membrane protein | Confers resistance to bacteriophage T1. |
| *traD*36 | Transfer factor mutation | Prevents transfer of F′ episome. |
| *trpC* | Phosphoribosyl anthranilate isomerase mutation; part of tryptophan biosynthesis pathway | |
| *trpR* | trpR aporepressor; regulates the biosynthesis of tryptophan and its transport | |
| *tsx* | T6 and colicin K phage receptor; outer membrane protein involved in specific diffusion of nucleosides; transports the antibotic albicidin | Confers resistance to bacteriophage T6 and colicin K. |
| *ung*1 | Uracil-DNA N-glycosylase mutation | Allows uracil to exist in plasmid DNA. |
| *xyl*-5 | Mutation in xylose metabolism | Blocks catabolism of xylose. |

**References**

1. Hoyt, A. *et al.* (1982) *Cell* **31**, 565.
2. Studier, F.W. (1991) *J. Mol. Biol.* **219**, 37.
3. Kaiser, K. and Murray, N. (1985) In: *DNA Cloning*, Vol. 1, Glover, D., ed., IRL Press Ltd., Oxford, UK.

**Importance of EndA+ versus EndA– *E. coli* Strains.**

Endonuclease I is a 12kDa periplasmic protein encoded by the gene *end*A that degrades double-stranded DNA. The *E. coli* genotype *endA*1 refers to a mutation in the wildtype *endA* gene, which produces an inactive form of the nuclease. *E. coli* strains with this mutation are referred to as EndA negative (EndA–); the wildtype is indicated as EndA+. The table below contains a list of EndA– and EndA+ *E. coli* strains. High-quality DNA is easily obtained from both EndA+ and EndA– strains using Promega PureYield™ and Wizard® *Plus* SV Plasmid Purification Systems. However, the level of endonuclease I produced is strain-dependent, and these systems may not totally exclude endonuclease I from plasmid DNA prepared from very high endonuclease I-producing strains. In general, we recommend the use of EndA– strains whenever possible.

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| JM110 | |

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