

Answers to questions about

Selecting *E. coli* cloning hosts

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Why are some *E. coli* strains designated cloning hosts, while others are designated expression hosts?

DNA cloning requires a bacterial host strain with high transformation efficiency, that does not cleave the recombinant DNA, provides good yields of high quality plasmid or phage DNA, and supplies components for various vector screening systems.

An expression strain, on the other hand, must have the appropriate regulatory proteins for expression of cloned genes—a requirement that may not be compatible with attributes of cloning strains. To improve yields of expressed proteins from the cloned genes, expression strains are often deficient in some proteases. For the pET system, expression hosts are λ DE3 lysogens, which have the inducible gene for T7 RNA polymerase.

What genetic changes are important for DNA cloning host strains?

A number of features have been incorporated into *E. coli* DNA cloning strains to facilitate transformation of the initial ligation reactions, screening for recombinants, stability of the recombinant plasmid, and high quality plasmid DNA preparations. Genetic features to look for are:

lacZ Δ *M15* encodes an enzymatically inactive β -galactosidase lacking amino acids 11-41. Some cloning vectors encode the deleted β -galactosidase amino acids (*lacZ* α or *lacZ'*), which, when expressed, can associate with the inactive protein in the host to generate active enzyme. The combination of host strain and vector allow blue/white screening for recombinant plasmids by α -complementation.

recA encodes a protein essential for homologous recombination in *E. coli*. Plasmids in host strains deficient in *recA* are less likely to incur deletions or other rearrangements. Plasmid DNA prepared from these strains contains monomer circles and lack the multimers common in preparations from *RecA*⁺ strains.

endA encodes a thermostable DNase that can degrade plasmid DNA during purification. Host strains deficient for *endA* allow higher yields of plasmid DNA.

hsdRMS encodes the enzymes responsible for the *Eco* restriction-modification system. For transformation with unmodified recombinant plasmid DNA, strains must be restriction minus.

mcrAB,
mcrB,
mmr encode enzymes that restrict foreign DNA. Strains that are deleted for these genes are preferred for the construction of genomic DNA libraries.

What is a restriction system?

Prokaryotic restriction systems use site-specific endonucleases to recognize and cleave foreign DNA. Self DNA is distinguished from foreign DNA by the methylation state of the DNA. *E. coli* has one restriction-modification system and three restriction systems.

What should I know about the host's restriction-modification system?

A restriction-modification system consists of a matched pair of enzymes that recognize the same site: the site-specific restriction endonuclease cleaves unmethylated foreign DNA and the site-specific methylase modifies the bacterial DNA soon after it is replicated, to protect it from restriction. The *E. coli* restriction-modification system, *hsdRMS*, restricts only unmethylated DNA. The restriction enzyme in the system consists of two subunits, the restriction subunit (HsdR) and the specificity subunit (HsdS); the modification enzyme consists of two subunits, the modification subunit (HsdM) and HsdS. Strains with mutations in *hsdR* are restriction minus, strains with mutations in *hsdS* are restriction minus, modification minus. Strains are never restriction plus, modification minus as this is a lethal phenotype. Cloning host strains are typically restriction minus and frequently modification plus, like the NovaBlue, NovaBlue T1^R, and NovaF⁻ strains.

When should I select a host that is also negative for the other restriction systems?

E. coli laboratory strains have three other restriction systems: *mcrAB*, *mcrB*, *mmr*, which restrict DNA that is methylated in a pattern different from the *E. coli* host. DNA isolated from mammals, plants, lower eukaryotes, and many other bacteria is methylated in a variety of ways and susceptible to restriction unless the host is negative for these restriction systems. Strains deleted for these genes, such as NovaXG and NovaXGF' Zappers™ Electrocompetent Cells, and ER1647 (in the λBlueSTAR™ Cloning Kit), are preferred for the initial construction of genomic DNA libraries.

Should I be concerned about Dam and Dcm methylation?

E. coli has two other methylases that modify DNA at the Dam site and the Dcm site. For example, in genomic DNA isolated from wild type *E. coli* K12, about 1.5% of the A residues and about 0.8% of the C residues are methylated (6-methyladenine, 5-methylcytosine). *E. coli* does not restrict DNA based on the methylation state at either Dam or Dcm sites, which have functions related to recombination, repair, and replication. However, Dam/Dcm methylation does block the activity of some restriction enzymes (e.g., *Cla* I, *Alw*N I, *Msc* I, *Stu* I), so the methylation state of the DNA should be considered when devising a cloning strategy based on these enzymes. Dam methylated DNA transforms Dam⁻ strains inefficiently due to difficulties replicating hemimethylated DNA. Because most laboratory *E. coli* K12 strains, including all Novagen cloning strains, are *dam*⁺ *dcm*⁺, DNA grown in these cells is methylated and should not be used to transform *dam*⁻ strains.

How does the genotype help me select a cloning host?

Based on standard *E. coli* nomenclature, only the variant genes are listed in the genotype. The exception is when a strain contains an F', for which the wildtype genes on the F' are listed, unless otherwise noted.

NovaBlue *endA1 hsdR17*(*r*_{K12}⁻ *m*_{K12}⁺) *supE44 thi-1 recA1 gyrA96 relA1 lac* F'[*proA*⁺*B*⁺ *lacI*^q*ZΔM15*::Tn 10]

NovaBlue T1^R *endA1 hsdR17*(*r*_{K12}⁻ *m*_{K12}⁺) *supE44 thi-1 recA1 gyrA96 relA1 lac tonA* F'[*proA*⁺*B*⁺ *lacI*^q*ZΔM15*::Tn 10]

NovaF⁻ F⁻ *endA1 hsdR17*(*r*_{K12}⁻ *m*_{K12}⁺) *supE44 thi-1 recA1 gyrA96 relA1 lac*

NovaXG F⁻ *mcrA Δ(mcrC-mrr) endA1 recA1 φ80dlacZΔM15 ΔlacX74 araD139 Δ(ara-leu)7697 galU galK rpsL nupG λ⁻ tonA*

NovaXGF' *mcrA Δ(mcrC-mrr) endA1 recA1 φ80dlacZΔM15 ΔlacX74 araD139 Δ(ara-leu)7697 galU galK rpsL nupG λ⁻ tonA* F'[*lacI*^q Tn 10]

Product	Size	Cat. No.	Price
NovaBlue Singles™ Competent Cells	11 rxn 22 rxn	70181-3 70181-4	
NovaBlue T1 ^R Singles™ Competent Cells	11 rxn 22 rxn	71318-3 71318-4	
NovaF ⁻ Competent Cells	0.4 ml 1 ml	71133-3 71133-4	
NovaXG Zappers™ Electrocompetent Cells	10 rxn 20 rxn	71315-3 71315-4	
NovaXGF' Zappers™ Electrocompetent Cells	10 rxn 20 rxn	71317-3 71317-4	
NovaBlue GigaSingles™ Competent Cells	11 rxn 22 rxn	71227-3 71227-4	
Veggie™ NovaBlue Singles™ Competent Cells	11 rxn 22 rxn	71251-3 71251-4	
HT96™ NovaBlue Competent Cells	1 plate 4 plates	71011-3 71011-4	

Components

Cat. No. 70181	
• 11 × 50 µl or 22 × 50 µl	Singles Competent Cells
• 2 × 2 ml or 4 × 2 ml	SOC Medium
• 10 µl	Test Plasmid
Cat. No. 71318	
• 11 × 50 µl or 22 × 50 µl	NovaBlue T1 ^R Singles Competent Cells
• 2 × 2 ml or 4 × 2 ml	SOC Medium
• 10 µl	Test Plasmid
Cat. No. 71133	
• 2 × 0.2 ml or 5 × 0.2 ml	NovaF ⁻ Competent Cells
• 2 × 2 ml or 4 × 2 ml	SOC Medium
• 10 µl	Test Plasmid
Cat. Nos. 71315, 71317	
• 5 × 50 µl or 10 × 50 µl	NovaXG or XGF' Zappers Electrocompetent Cells
• 10 µl	Test Plasmid
Cat. No. 71227	
• 11 × 50 µl or 22 × 50 µl	Competent Cells
• 2 × 2 ml or 4 × 2 ml	SOC Medium
• 10 µl	Test Plasmid
Cat. No. 71251	
• 11 × 50 µl or 22 × 50 µl	Veggie NovaBlue Singles Competent Cells
• 2 × 2 ml or 4 × 2 ml	Veggie SOC Medium
• 10 µl	Test Plasmid
Cat. No. 71011	
• 1 or 4 plates	HT96 NovaBlue Competent Cells
• 1 × 14 ml or 4 × 14 ml	SOC Medium
• 1 × 10 µl or 2 × 10 µl	Test Plasmid
• 1 or 4 pkg	8-Cap Strips
• 1 or 4	Reagent Reservoirs