Escherichia coli Host Strains

INSTRUCTION MANUAL

Part #200256-12
Revision A

For In Vitro Use Only
200256-12

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Escherichia coli Host Strains

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ESCHERICHIA COLI HOST STRAIN STORAGE CONDITIONS AND MEDIA

The host strains have been supplied as bacterial glycerol stocks. (Instructions for preparing host cells are in subsequent sections of this instruction manual.)

Storage Conditions

Store the vials at –80°C.

Host Strain Media

For the appropriate media, please refer to the following table:

Bacterial strain	Catalog #	Agar plate for bacterial streak[a,b]	Medium for bacterial glycerol stock[a,b]	Medium for bacterial cultures for titering phage (final concentration)[a,b]
ABLE C [c,d]	200306	LB–Tet–Kan	LB–Tet–Kan	—
ABLE K [c,d]	200307	LB–Tet–Kan	LB–Tet–Kan	—
AG1	200274	LB	LB	—
BB4	200269	LB–Tet	LB–Tet	LB with 0.2% maltose–10 mM $MgSO_4$
C600	200261	LB	LB	LB with 0.2% maltose–10 mM $MgSO_4$
JM101 [d]	200272	NZY	NZY	—
JM109 [d]	200271	NZY	NZY	—
JM110 [d]	200299	NZY	NZY	—
LE392	200266	LB	LB	LB with 0.2% maltose–10 mM $MgSO_4$
NM514	200297	LB	LB	LB with 0.2% maltose–10 mM $MgSO_4$
NM522 [d]	200270	NZY	NZY	—
NM554	200284	LB	LB	—
P2392	200267	LB	LB	LB with 0.2% maltose–10 mM $MgSO_4$
SCS-8	700288	LB–Tet	LB–Tet	NZY with 0.2% maltose–10 mM $MgSO_4$
SCS110 [d]	200275	NZY	NZY	—
SOLR	200298	LB–Kan	LB–Kan	LB without supplements
SURE [c,d,e,f]	200294	LB–Tet	LB–Tet	LB with 0.2% maltose–10 mM $MgSO_4$
VCS257	700256	LB	LB	LB with 0.2% maltose–10 mM $MgSO_4$

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(*table continued from the previous page*)

Bacterial strain	Catalog #	Agar plate for bacterial streak[a,b]	Medium for bacterial glycerol stock[a,b]	Medium for bacterial cultures for titering phage (final concentration)[a,b]
XL1-Blue[c,d]	200268	LB–Tet	LB–Tet	—
XL1-Blue MR[d]	200300	LB	LB	—
XL1-Blue MRA	200302	LB	LB	LB with 0.2% maltose–10 mM $MgSO_4$
XL1-Blue MRA (P2)	200303	LB	LB	LB with 0.2% maltose–10 mM $MgSO_4$
XL1-Blue MRF′[d]	200301	LB–Tet	LB–Tet	LB with 0.2% maltose–10 mM $MgSO_4$
XL1-Blue MRF′ Kan[d]	200309	LB–Kan	LB–Kan	LB with 0.2% maltose–10 mM $MgSO_4$
XLOLR	200304	LB–Tet	LB–Tet	LB without supplements
XPORT	200310	LB	LB	LB without supplements
Y1088	200263	LB–Amp	LB–Amp	LB with 0.2% maltose–10 mM $MgSO_4$
Y1089r$^-$	200260	LB–Amp	LB–Amp	LB with 0.2% maltose–10 mM $MgSO_4$
Y1090r$^-$	200281	LB–Amp	LB–Amp	LB with 0.2% maltose–10 mM $MgSO_4$

[a] See *Preparation of Media and Reagents*.

[b] NZY media may be substituted for LB in all cases.

[c] Stratagene electroporation-competent cells produce efficiencies greater than those achieved with the best chemical methods. These cells routinely produce high-efficiency transformations between 3.0×10^9 and 7.5×10^9 cfu/μg of pUC18 DNA.

[d] To transform any of these strains, using Stratagene competent cells is recommended. These cells offer extremely high efficiencies (up to 1×10^9 cfu/μg of pUC18), as well as convenience. Alternatively, the procedures described in Hanahan[1] may be used to obtain efficiencies of 10^7–10^8 cfu/μg of pUC18.

[e] When growing lambda phage for plaque formation, incubate plates at 39°C.

[f] We do not recommend the $CaCl_2$ procedure to make competent cells; instead we use a modified Hanahan protocol.[1]

PREPARATION OF HOST CELLS

On arrival, prepare the following from the bacterial glycerol stock:

Note *The host strains may thaw during shipment. The vials should be stored immediately at –20° or –80°C, but most strains remain viable longer if stored at –80°C. It is also best to avoid repeated thawing of the host strains in order to maintain extended viability.*

1. Revive the stored cells by scraping off splinters of solid ice with a sterile wire loop.

2. Streak the splinters onto the recommended plate containing the appropriate antibiotic.

3. Restreak the cells fresh each week.

Preparation of a –80°C Bacterial Glycerol Stock

1. In a sterile 50-ml conical tube, inoculate 10 ml of the appropriate liquid media (see the third column of the table in *Host Strain Media*) with one or two colonies from the plate. Grow the cells to late log phase (OD_{600} = ~1.0–2.0).

2. Add 4.5 ml of a sterile glycerol–liquid media solution (5 ml of glycerol + 5 ml of the appropriate media) to the bacterial culture from step 1. Mix well. (For the appropriate medium, see the third column of the table in *Host Strain Media*.)

3. Aliquot into sterile centrifuge tubes (1 ml/tube).

This preparation may be stored at –20°C for 1–2 years or at –80°C for more than 2 years.

HOST STRAIN GENOTYPES

For all *E. coli* strains, the genes listed signify that the bacterium carries a mutant allele. The genes present on the F′ episome, however, represent the wild-type alleles unless indicated. Strains should be considered λ^- and F^- unless otherwise designated.

Bacterial strain	Reference(s)	Genotype
ABLE C strain [a,b]	2	*E. coli* C *lac*(LacZω⁻) [Kan^r McrA⁻ McrCB⁻ McrF⁻ Mrr⁻ HsdR(r_K^- m_K^-)] [F′ *proAB lacI*^{q}Z*ΔM15* Tn*10* (Tet^r)]
ABLE K strain [a,b]	2	*E. coli* C *lac*(LacZω⁻) [Kan^r McrA⁻ McrCB⁻ McrF⁻ Mrr⁻ HsdR(r_K^- m_K^-)] [F′ *proAB lacI*^{q}Z*ΔM15* Tn*10* (Tet^r)]
AG1 strain[a]	1,3	*recA1 endA1 gyrA96 thi-1 hsdR17*(r_K^- m_K^+) *supE44 relA1* (uncharacterized mutation improves transformation efficiency)
BB4 strain	3,4	LE392.23 [F′ *lacI*^{q}Z*ΔM15 proAB* Tn*10* (Tet^r)]
C600 strain	5	e14⁻(McrA⁻) *supE44 thi-1 thr-1 leuB6 lacY1 tonA21*
JM101 strain[a]	6	*supE thi-1* Δ(*lac-proAB*) [F′ *traD36 proAB lacI*^{q}Z*ΔM15*]
JM109 strain[a]	6	e14⁻(McrA⁻) *recA1 endA1 gyrA96 thi-1 hsdR17*(r_K^- m_K^+) *supE44 relA1* Δ(*lac-proAB*) [F′ *traD36 proAB lacI*^{q}Z*ΔM15*]
JM110 strain[a]	6	*rpsL* (Str^r) *thr leu thi-1 lacY galK galT ara tonA tsx dam dcm supE44* Δ(*lac-proAB*) [F′ *traD36 proAB lacI*^{q}Z*ΔM15*]
LE392 strain	7	e14⁻(McrA⁻) *hsdR514 supE44 supF58 lacY1* or Δ(*lacIZY*)*6 galK2 galT22 metB1 trpR55*
NM514 strain	8	*hsdR514*(r_K^- m_K^-) *argH galE galX lycB7 strA* (Hfl$^+$)
NM522 strain[a]	9	*supE thi-1* Δ(*lac-proAB*) Δ(*mcrB–hsdSM*)*5* (r_K^- m_K^-) [F′ *proAB lacI*^{q}Z*ΔM15*]
NM554 strain	10	*recA13 araD139* Δ(*ara-leu*)*7696* Δ(*lac*)*I7A galU galK hsdR rpsL* (Str^r) *mcrA mcrB*

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Bacterial strain	Reference(s)	Genotype
P2392 strain	4	LE392 (P2 lysogen)
SCS-8 strain	11	*recA1 endA1 mcrA* Δ(*mcrBC-hsdRMS-mrr*) Δ(*argF-lac*)*U169* ϕ80*dlacZ*Δ*M15* Tn*10* (Tet^r)
SCS110 strain[a]		*rpsL* (Str^r) *thr leu endA thi-1 lacY galK galT ara tonA tsx dam dcm supE44* Δ(*lac-proAB*) [F′ *traD36 proAB lacI*^{q}ZΔ*M15*]
SOLR strain	12	e14$^-$(McrA$^-$) Δ(*mcrCB-hsdSMR-mrr*)*171 sbcC recB recJ uvrC umuC*::Tn*5* (Kan^r) *lac gyrA96 relA1 thi-1 endA1* λ^R [F′ *proAB lacI*^{q}ZΔ*M15*] Su$^-$ (nonsuppressing)
SURE strain[a,b]	12	e14$^-$(McrA$^-$) Δ(*mcrCB-hsdSMR-mrr*)*171 endA1 supE44 thi-1 gyrA96 relA1 lac recB recJ sbcC umuC*::Tn*5* (Kan^r) *uvrC* [F′ *proAB lacI*^{q}ZΔ*M15* Tn*10* (Tet^r)]
VCS257 strain		Derivative of DP50 *supF*[c]
XL1-Blue strain[a,b]	3	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*^{q}ZΔ*M15* Tn*10* (Tet^r)]
XL1-Blue MR strain[a]	13	Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac*
XL1-Blue MRA strain		Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 gyrA96 relA1 lac*
XL1-Blue MRA (P2) strain		XL1-Blue MRA (P2 lysogen)
XL1-Blue MRF′ strain[a]	13	Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F′ *proAB lacI*^{q}ZΔ*M15* Tn*10* (Tet^r)]
XL1-Blue MRF′ Kan strain[a]		Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F′ *proAB lacI*^{q}ZΔ*M15* Tn*5* (Kan^r)]
XLOLR strain		Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 thi-1 recA1 gyrA96 relA1 lac* [F′ *proAB lacI*^{q}ZΔ*M15* Tn*10* (Tet^r)] Su$^-$ (nonsuppressing) λ^R (lambda resistant)
XPORT		Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F′ *proAB lacI*^{q} *Z* Δ*M15*]
Y1088 strain	14	e14$^-$(McrA$^-$) Δ(*lac*)*U169 supE supF hsdR metB trpR tonA21 proC*::Tn*5* (Kan^r) [pMC9 Amp^r Tet^r] (Note: pMC9 is pBR322 with *lacI*^{q} inserted.)
Y1089r$^-$ strain	5	Δ(*lac*)*U169* Δ(*lon*)*? araD139 strA mcrB hflA150*::Tn*10* (Tet^r) [pMC9 Amp^r Tet^r] (Note: pMC9 is pBR322 with *lacI*^{q} inserted.)
Y1090r$^-$ strain	5	Δ(*lac*)*U169* Δ(*lon*)*? araD139 strA supF mcrA mcrB hsdR trpC22*::Tn*10* (Tet^r) [pMC9 Amp^r Tet^r] (Note: pMC9 is pBR322 with *lacI*^{q} inserted.)

[a] Strains are available as high-efficiency, chemically competent cells producing transformation efficiencies up to 1 × 10^9 cfu/μg of pUC18 DNA. Visit http://www.stratagene.com for details.

[b] Strains are available as higher efficiency, electroporation-competent cells producing transformation efficiencies up to 7.5 × 10^9 cfu/μg of pUC18 DNA. Visit http://www.stratagene.com for details.

[c] DP50 *supF* genotype: *supE44 supF58 hsdS3*(r_B^- m_B^-) *dapD8 lacY1 glnV44* Δ(*gal-uvrB*)*47 tyrT58 gyrA29 tonA53* Δ(*thyA57*).

PREPARATION OF MEDIA AND REAGENTS

Note *All media must be autoclaved prior to use.*

NZY Broth (per Liter)

5 g of NaCl
2 g of $MgSO_4 \cdot 7H_2O$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
Adjust the pH to 7.5 with NaOH

NZY–Kanamycin Broth (per Liter)

NZY broth
Autoclave
Cool to 55°C
Add 50 mg of filter-sterilized kanamycin

NZY Agar (per Liter)

5 g of NaCl
2 g of $MgSO_4 \cdot 7H_2O$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
15 g of agar
Adjust the pH to 7.5 with NaOH
Autoclave
Pour into petri dishes (~80 ml/150-mm plate)

NZY Top Agar (per Liter)

1 liter of NZY broth
Add 0.7% (w/v) agarose

NZY–Kanamycin Agar (per Liter)

NZY agar
Autoclave
Cool to 55°C
Add 50 mg of filter-sterilized kanamycin

LB Broth (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
Add deionized H_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave

LB Agar (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized H_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave
Pour into petri dishes (~25 ml/100-mm plate)

LB–Ampicillin Broth (per Liter)

1 liter of LB broth, autoclaved
Cool to 55°C
Add 10 ml of 10-mg/ml filter-sterilized ampicillin

LB–Ampicillin Agar (per Liter)

1 liter of LB agar, autoclaved
Cool to 55°C
Add 10 ml of 10-mg/ml filter-sterilized ampicillin
Pour into petri dishes (~25 ml/100-mm plate)

LB–Kanamycin Broth (per Liter)

1 liter of LB broth
Autoclave
Cool to 55°C
Add 50 mg of filter-sterilized kanamycin

LB–Kanamycin Agar (per Liter)

1 liter of LB agar
Autoclave
Cool to 55°C
Add 50 mg of filter-sterilized kanamycin
Pour into petri dishes (~25 ml/100-mm plate)

LB–Tetracycline Broth (per Liter)	LB–Tetracycline Agar (per Liter)
1 liter of LB broth Autoclave Cool to 55°C Add 12.5 mg of filter-sterilized tetracycline Store broth in a dark, cool place as tetracycline is light-sensitive	1 liter of LB agar Autoclave Cool to 55°C Add 12.5 mg of filter-sterilized tetracycline Pour into petri dishes (~25 ml/100-mm plate) Store plates in a dark, cool place or cover plates with foil if left out at room temperature for extended time periods as tetracycline is light-sensitive
LB–Tetracycline–Kanamycin Broth (per Liter)	**LB–Tetracycline–Kanamycin Agar (per Liter)**
1 liter of LB broth Autoclave Cool to 55°C Add 12.5 mg of filter-sterilized tetracycline Add 50 mg of filter-sterilized kanamycin Store broth in a dark, cool place as tetracycline is light-sensitive	1 liter of LB agar Autoclave Cool to 55°C Add 12.5 mg of filter-sterilized tetracycline Add 50 mg of filter-sterilized kanamycin Pour into petri dishes (~25 ml/100-mm plate) Store plates in a dark, cool place or cover plates with foil if left out at room temperature for extended time periods as tetracycline is light-sensitive

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