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Author manuscript
Curr Protoc. Author manuscript; available in PMC 2022 January 01.

Published in final edited form as:
Curr Protoc. 2021 January ; 1(1): e20. doi:10.1002/cpz1.20.

Growth and Maintenance of *Escherichia coli* Laboratory Strains

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Abstract

Escherichia coli is a Gram-negative bacterium, commonly used in both teaching and research laboratories. This unit includes protocols for the growth and maintenance of *E. coli* in any teaching- or research-associated laboratory.

Keywords

Escherichia col; growth; maintenance

INTRODUCTION

Escherichia coli is a Gram-negative, rod-shaped bacterium that belongs to the *Enterobacteriaceae* family. Typical laboratory strains are considered to be nonpathogenic microorganisms and grow rapidly in and on a wide range of liquid or solid media, especially in the presence of oxygen (doubling time ~20 min), but can also grow under anaerobic conditions (facultative anaerobe). The phenotypes and genotypes of common laboratory *E. coli* strains have been well characterized, and have led to its use as a model teaching and research organism over many decades since the early 1940s.

This unit describes general techniques commonly used to grow and maintain the model organism *E. coli* in the laboratory setting. *E. coli* is a relatively robust microorganism that can be grown on or in a variety of different solid or liquid media, respectively, over a wide range of temperatures. Using the basic outlines described in this unit, the reader should be able to grow and maintain a culture(s) of *E. coli* for use in a wide array of assays, including, but not limited to, aseptic technique demonstrations, basic cloning, gene expression systems, and protein purification.

CAUTION:

E. coli is a Biosafety Level 2 (BSL-2) microorganism. Strict guidelines and regulations established by your institution regarding the safe handling and disposal of this bacterium should be followed. See *UNIT 1A.1* and other pertinent resources for more information.

Internet Resources
http://openwetware.org/index.php?title=E._coli_genotypes&oldid=577711
This Web site is for various E. coli strains and gene-specific information.

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STRATEGIC PLANNING

Strain Selection

E. coli has been well characterized and is typically subdivided into different serotypes based on its major surface antigens: the O-antigen, which is part of the lipopolysaccharide layer, and the H-antigen, which is the flagellin protein. The deciphering of the genetic code of *E. coli* and identification of different serotypes has led to the development of many commonly used strains, many of which are commercially available. It is important to identify the purpose of the experiment and downstream applications prior to choosing which *E. coli* strain should be used. A list of commonly used *E. coli* strains and their respective genotypes can be found in Table 5A.4.1, as well as the typical use for each strain.

Growth Conditions

E. coli, a member of the *Enterobacteriaceae* family, grows optimally at 37°C under aerobic conditions, although it is a facultative anaerobe and can therefore grow under anaerobic conditions. It has also been previously reported that some strains of *E. coli* have been known to grow at temperatures of up to 53°C (Fotadar et al., 2005), although this is not typical nor recommended for commonly used laboratory strains. *E. coli* is a relatively hearty bacterium and can survive at temperatures of 4°C for extended periods of time (up to 3 months) on solid media, although increased storage times at low temperatures may result in decreased viability. *E. coli* can also grow under a wide pH range; typical growth and maintenance is performed at a neutral pH of 7.0. Taking all optimal growth conditions into consideration (i.e., 37°C, aeration, pH of 7.0), a doubling time of ~20 min should occur when *E. coli* is grown in a rich liquid broth medium (such as Luria-Bertani broth) and will reach an overnight cell density of >10^9 cfu/ml (colony-forming units per milliliter) of culture.

Media

E. coli is able to grow on a wide range of carbon sources, but is typically grown in a rich medium, such as Luria-Bertani broth, or on rich media agar plates (see Reagents and Solutions for recipes or *APPENDIX 4A* for protocols describing the preparation of commonly used liquid and solid media). Each medium can be modified and/or adjusted to meet specific nutritional requirements for individual experiments. Supplements, such as specific chromogenic agents (Xgal or 5-bromo-4-chloro-indolyl-*β*-D-galactopyranoside) or antibiotics (see *APPENDIX 4A*) at desired concentrations can also be added to different media catering to the specific needs of the experiment. Also see Elbing and Brent (2002a) and *APPENDIX 4A* for additional details on media preparations.

BASIC PROTOCOL 1

GROWTH OF *E. COLI* FROM FROZEN STOCKS

E. coli, like many other laboratory strain stocks, are typically diluted in 30% glycerol (final concentration) and frozen at −80°C for long-term storage. Proper handling and growth initiated from these frozen stocks marks the beginning of any experiment, but also introduces the possibility of stock contamination and reduced stock viability. Careful aseptic technique is crucial when taking frozen culture sample from the stock vial for re-streaking.

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Re-streaking from these frozen stocks onto solid agar media is strongly recommended, rather than directly inoculating liquid broth, as it is good laboratory practice to initiate any experiment from a single colony. This re-streaking step should be performed quickly with very minimal thawing of the stock, as repeated freeze-thaw cycles will greatly decrease the viability of the entire stock culture.

Materials—*E. coli* frozen stock cultures

Luria-Bertani (LB) agar plates (standard size of 100 mm × 15 mm)

Wooden applicator sticks, sterile (Fisher, cat. no. 01–340)

37°C incubator

1. Using a sterile wooden applicator stick, remove a small ice chunk of bacteria from the frozen stock culture vial.

   It should be noted that repeated freeze-thaw cycles will decrease the viability of the stock culture; therefore, the frozen stock should NOT be defrosted when streaking onto an LB agar plate.

2. Streak the small ice chunk on an LB agar plate, so as to obtain single, well-isolated colonies.

   LB agar plates with appropriate antibiotics can be used, if necessary. For example, if the E. coli strain is carrying a plasmid with an antibiotic resistance marker, this strain can be revived on an LB plate with the antibiotic incorporated in the medium, so as to ensure that the plasmid is maintained as the bacteria grow overnight. See APPENDIX 4A for recommended antibiotic concentrations.

3. Incubate the plate overnight (typically 14 to 18 hr) at 37°C.

BASIC PROTOCOL 2

GROWTH OF *E. COLI* ON SOLID MEDIA

It is sometimes necessary to enumerate the number of bacteria in a liquid culture by performing serial dilutions and plating these serial dilutions on an agar plate in order to quantify the number of colony-forming units in a given volume of culture (cfu/ml). This protocol describes one technique that can be used to determine the cfu/ml of an overnight culture of *E. coli*. This simple technique can also be applied to spreading of transformation cultures, where competent *E. coli* cells have been electroporated or otherwise transformed (e.g., $CaCl_2$ treated) with an exogenous source of DNA (e.g., recombinant plasmids).

Materials—*E. coli* overnight liquid cultures (or frozen stock)

Luria-Bertani (LB) broth, sterile (see recipe)

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Luria-Bertani (LB) agar plates (standard size of 100 mm × 15 mm; see recipe), supplemented with antibiotics, if necessary (see *APPENDIX 4A*) 70% ethanol in a glass beaker (to sterilize glass spreader)

2-ml microcentrifuge tubes, sterile (USA Scientific, cat. no. 1620–2700)

Vortex mixer

Glass spreader

Bunsen burner (or alternative flame source to burn off the excess ethanol on the spreader)

37°C incubator

1. From an overnight culture, prepare 10-fold serial dilutions from 10^{-1} to 10^{-10} in 1.0 ml final volumes.

   For example, add 100 μl of the original overnight culture to 900 μl sterile LB broth. This is now a 10^{-1} dilution. Mix well by vortexing, and transfer a 100-μl aliquot of this dilution into the next 2-ml microcentrifuge tube containing 900 μl sterile LB broth. Continue this serial dilution until the final dilution is made.

   If growing E. coli from a frozen stock, please see Basic Protocol 1.

2. Apply the desired volume (e.g., 20 to 100 μl) of the different dilution series to individual agar media plates, labeled with the appropriate dilution. Apply the lowest concentration culture to the center of the appropriately labeled plate, and then repeat for the next increasing concentrations using the same tip. Be sure to work quickly as drier plates may absorb the liquid before you have the opportunity to spread it, resulting in an uneven distribution of the cells.

   Agar media plates should contain antibiotics, if appropriate. See APPENDIX 4A for antibiotic concentrations.

   Note that an overnight culture of E. coli grown in a rich medium, such as LB broth, will contain ~10^9 to 10^{10} colony-forming units (cfu) per ml of culture. Therefore, to observe single, well-isolated colonies, only dilutions of 10^{-7} to 10^{-9} need be plated.

3. Sterilize the glass spreader by soaking the spreader in a beaker containing 70%ethanol for about 1 min.

4. Remove the glass spreader and carefully pass the spreader through a flame to burn off the excess ethanol.

5. Allow the glass spreader to cool in the air. Do not place the glass spreader down, so as to keep it sterile.

   Cooling the spreader should take about 15 to 30 sec.

   Alternatively, to save time the flamed spreader can be touched to the agar plate where no culture has been deposited.

6. Using the glass spreader, carefully touch an open part of the agar medium surface where the bacterial culture is not applied to ensure it has completely cooled.

   If the glass spreader is not sufficiently cooled or applied directly to the bacterial culture, the heat from the spreader will heat up the liquid medium in the 20 μl drop effectively cooking the cells, killing them.

7. Spread the culture samples to evenly distribute the culture over the entire surface of the plate. Keep spreading until the surface of the agar plate is dry to ensure easily identifiable single, well-isolated colonies.

   If multiple dilutions are being spread on different plates, start with the highest dilution (lowest concentration), spread completely, and then move to the next highest dilution and spread to avoid re-sterilizing the spreader between dilutions.

8. Incubate the plate overnight (typically 14 to 18 hr) at 37°C.

BASIC PROTOCOL 3

GROWTH OF *E. COLI* IN LIQUID MEDIA

It is often necessary to inoculate *E. coli* into various liquid media. The type of liquid media and any additionally added supplements will depend on the purpose of the experiment, such as a specific liquid medium used for conducting growth curve experiments or using a rich liquid media for isolating chromosomal or plasmid DNA. For general purposes, growth in Luria-Bertani broth medium will usually suffice.

Materials—Luria-Bertani (LB) broth (see recipe)

E. coli single colonies on solid medium (with appropriate antibiotics, if necessary)

Antibiotics (if necessary; see *APPENDIX 4A*)

Capped glass culture tubes, sterile (Tubes—Fisherbrand, cat. no. 14–961-32; Caps—Fisherbrand, cat. no. 05–888) (or sterile Erlenmeyer flasks with caps)

Wooden applicator sticks, sterile (Fisher, cat. no. 01–340)

37°C incubator with a mechanism for rotating or shaking liquid cultures

1. Using aseptic techniques, carefully transfer a 5-ml aliquot of LB broth into a capped sterile glass culture tube.

   If a larger volume of culture is required, the LB broth can be divided into aliquots into sterile Erlenmeyer flasks and inoculated in a similar manner.

   Supplement the LB broth with antibiotics, if appropriate. See APPENDIX 4A for antibiotic concentrations.

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If more than one strain is to be inoculated and cultured, and all require the same antibiotic, it is recommended that a stock solution of LB broth containing the antibiotic be made.

2. With a sterile wooden applicator stick (or a flame-sterilized inoculating loop or stab), touch a single, well-isolated colony on the solid medium plate.

3. Inoculate the LB broth in the glass culture tube by resuspending the picked colony into the liquid medium.

4. Replace the cap on the glass culture tube and incubate the test tube (or flask) in a 37°C incubator with aeration for a desired amount of time.

BASIC PROTOCOL 4

STORAGE OF *E. COLI* FROZEN STOCKS IN GLYCEROL

For long-term storage, *E. coli* can be mixed with glycerol (final concentration of 30%) and stored at −80°C for an indefinite amount of time. These frozen stocks will be the source of all future experiments in which this particular strain will be used. Despite practicing aseptic technique and minimizing thawing time of the frozen stock, the samples will unavoidably be subjected to repeated freeze-thaw cycles. These freeze-thaw cycles will decrease the viability of the frozen stock; therefore, it is strongly recommended to freeze two identical vials of each *E. coli* strain, and only use one stock regularly while keeping the other as a back-up. Optimally, the two stocks should be kept in separate freezers, in case one freezer fails.

Materials—*E. coli* overnight liquid cultures

Luria-Bertani (LB) broth

Antibiotics (if necessary; see *APPENDIX 4A*)

37°C incubator with a mechanism for rotating or shaking liquid cultures

4-ml glass vials (Wheaton, cat. no. 224–882) containing 1.5 ml of sterile 50% (w/v) glycerol [alternatively, cryovials (Fisher, cat. no. 12–565-167N) containing half the volume can be used]

Vortex mixer

2-ml sterile microcentrifuge tubes (USA Scientific, cat. no. 1620–2700) Microcentrifuge

−80°C freezer

1. From a streak plate, pick a single, well-isolated colony and inoculate 3 to 5 ml LB broth (with appropriate antibiotics if necessary) as described in Basic Protocol 3.

2. Incubate the inoculated medium overnight (12 to 18 hr) at 37°C with aeration.

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a. Transfer 1.0 ml of the overnight culture into the glass freezer vial containing1.5 ml of 50% (w/v) glycerol and vortex to mix well. This will bring the final concentration of the glycerol to 30%.

b. If using the smaller volume cryovials, transfer 0.5 ml of the overnight culture into 0.75 ml of 50% glycerol and vortex to mix well.

c. Alternatively, to concentrate your stock culture, or for low-cell-density cultures, 2.0 ml of cell culture can be transferred into a 2-ml microcentrifuge tube. Centrifuge the sample for 2 min at maximum speed, room temperature, to pellet the cells. Remove the supernatant, and resuspend the cell pellet with 1.0 ml of fresh LB broth (with appropriate antibiotics). Freeze this resuspension as in step 3 (or 3a if using cryovials).

3. Label the vial and cap with appropriate strain names and other relevant information and freeze immediately in a −80°C freezer.

BASIC PROTOCOL 5

STORAGE OF *E. COLI* IN AGAR STABS

It is sometimes necessary to store *E. coli* strains in an alternative form from the frozen stock stored at −80°C, such as an agar stab. Although the agar stab provides some convenience for long-term storage especially when a −80°C freezer is not available, an agar stab is more commonly used when shipping a strain. Strain shipment requires adherence to strict federal, state and institutional guidelines, including primary and secondary containment. For a full list of federal, state, and/or institutional guidelines, please consult with your institutional Environmental Health and Safety Office. A brief description of how to prepare agar stabs from liquid cultures and from colonies grown on solid agar media is detailed here. Alternatively, see Elbing and Brent (2002b) for techniques and protocols for preparing and reviving bacteria from agar stabs.

Materials—*E. coli* overnight liquid cultures

E. coli streak plate with single, well-isolated colonies

Luria-Bertani (LB) agar stabs (see recipe)

Inoculating loop (Fisher, cat. no. 22–268-169)

Bunsen burner

Inoculating wire needle (Fisher, cat. no. 22–032-099) (or sterile double-pointed, round toothpicks)

37°C incubator

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Agar stabs from liquid cultures

1a. Sterilize the inoculating loop using the Bunsen burner and allow the loop to air-cool to room temperature.

2a. Carefully insert the sterile loop into the overnight culture of *E. coli* that is to be stored in the agar stab, and get a loopful of culture.

3a. With a loopful of culture on the inoculating loop, stab directly down into the agar stab all the way to the bottom of the vial and pull straight up.

4a. Incubate the vial at 37°C with the cap slightly loosened overnight (approximately 12 to 18 hr).

5a. Tightly screw the cap on so the stab will not dry out over time and store the vial in a cool (15°C to 22°C), dark place.

Agar stabs from solid media plates

1b. Sterilize the inoculating needle using the Bunsen burner and allow the stab to air-cool to room temperature or touch it to the agar plate where there is no growth.

Alternatively, sterile double-pointed, round toothpicks can be used if an inoculating stab is not available.

2b. Carefully touch the sterile stab (or toothpick) to a single, well-isolated colony.

3b. With the bacteria on the inoculating stab (or toothpick), stab directly down into the agar stab all the way to the bottom of the vial and pull straight up.

4b. Inoculate the vial at 37°C with the cap slightly loosened overnight (approximately 12 to 18 hr).

5b. Tightly screw the cap on and store the vial in a cool (15°C to 22°C), dark place.

REAGENTS AND SOLUTIONS

Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see **APPENDIX 2A**; for suppliers, see **SUPPLIERS APPENDIX**.

Glycerol, 50% (w/v)

50 ml of 100% glycerol [final concentration 50% (w/v); Fisher, cat. no. AC41098–5000]

Adjust volume to 100 ml with double-distilled water

Transfer 1.5 ml into 4-ml autoclavable glass vials with screw caps (Wheaton, cat. no. 224882) (or suitable cryovials; Fisher, cat. no. 12–565-167N)

Autoclave and allow to completely cool before tightening screw caps Store indefinitely at room temperature

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Luria-Bertani (LB) agar plates

10 g tryptone (BD, cat. no. 211705)

5 g yeast extract (BD, cat. no. 212750)

5 g NaCl (Fisher, cat. no. S217–500)

15 g agar (BD, cat. no. 214030)

Adjust volume to 1000 ml with double-distilled water

Autoclave, let cool to about 60°C (cool enough to handle without gloves) and then pour into sterile plastic petri dishes (Fisherbrand, cat. no. 08–757-12)

Allow the plates to cool and solidify at room temperature

Store the plates inverted in a plastic sleeve up to 6 months at 4°C

Luria-Bertani (LB) agar stabs

3 g agar (BD, cat. no. 214030)

5 g LB broth (see recipe)

5 g NaCl (Fisher, cat. no. S217–500)

Adjust volume to 500 ml with double-distilled water

Heat the solution with gentle mixing to dissolve all the agar and NaCl

Carefully transfer a 3-ml aliquot of the warm agar solution into 4-ml autoclavable glass vials (Wheaton, cat. no. 224882) and loosely tighten the caps

Autoclave and allow to cool at room temperature

Screw caps on tight to prevent drying of the agar during storage Store up to 1 year at room temperature

Luria-Bertani (LB) broth

10 g tryptone (BD, cat. no. 211705)

5 g yeast extract (BD, cat. no. 212750)

5 g NaCl (Fisher, cat. no. S217–500)

Adjust volume to 1000 ml with double-distilled water

Autoclave

Store indefinitely at room temperature

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COMMENTARY

Background Information

E. coli, first discovered and isolated by Theodor Escherich in 1885 from fecal samples of healthy individuals, is a Gram-negative, rod-shaped bacterium, which belongs to the *Enterobacteriaceae* family of bacteria. Although thousands of biotypes of *E. coli* can be found, most of the strains are relatively harmless, existing as commensal organisms that inhabit the lower intestine of warm-blooded animals. However, there are some strains of *E. coli* that can cause severe gastrointestinal disease, such as the enterohemorrhagic, enteropathogenic, and enterotoxigenic *E. coli* (EHEC, EPEC, and ETEC, respectively) strains. Today, most, if not all commonly used laboratory strains of *E. coli* can have their lineages traced back to either the *E. coli* K-12 or B strains.

Many attribute the nonpathogenic nature of the *E. coli* K-12 and B strains to its success as a model organism in research and teaching. It could also be equally argued that it is the genetic tractability of *E. coli*, which has promoted its use in research and teaching laboratories. Pioneering work by Max Delbruck and Salvador Luria in 1943 (Luria-Delbruck experiment; Nobel Prize 1969) using the *E. coli* B strain, perfectly illustrated the genetic tractability of *E. coli* by demonstrating that bacteria model to Darwin's theory of natural selection, and that mutations in bacteria occur in the absence of selection. However, it was not until the discovery of bacterial conjugation by Joshua Lederberg and Edward Tatum in 1946 that immortalized *E. coli* as a model organism to be used globally in research laboratories, and eventually teaching laboratories throughout the many decades to come. For its genetic tractability and ease of use for recombinant DNA research, in addition to the nonpathogenic nature of the *E. coli* K-12 and B strains making them safe for recombinant DNA work, *E. coli* has remained as a model organism, as clearly demonstrated by the work of Roy Curtiss in 1978 (Curtiss, 1978) and cloning vector transmissibility.

Since the original discovery and isolation of *E. coli* in 1885, hundreds of strains have been constructed that have allowed for a very comprehensive understanding of the enteric bacterium (Neidhardt, 1996), and many of these strains are commercially available. An abbreviated list of commonly used *E. coli* strains is given in Table 5A.4.1 with genotypes and typical uses for each strain, but a more comprehensive strain list, including gene-specific information, can be found online (see Internet References). Careful consideration must be taken when deciding which strain of *E. coli* will be used for one's own research or teaching purposes.

Critical Parameters and Troubleshooting

If no growth is observed after streaking from the frozen stock directly onto solid agar medium, it is possible that an insufficient amount of bacteria was initially taken from the stock vial. Try re-streaking and taking a larger "chip" of ice from the frozen stock. It is also imperative to ensure that the proper agar medium is used with all necessary supplements (e.g., correct antibiotics). As previously mentioned, it is also possible that if the stock vial has been frequently used and/or has undergone significant freeze-thaw cycles, the bacteria in

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the stock may no longer be viable. Consider thawing the entire stock vial, concentrating it, and plating the entire stock on a plate, or using the back-up stock.

When sub-culturing, or passing a growing culture repeatedly, the chance of mutation increases, and should therefore be avoided if possible. This is particularly a problem when dealing with serial liquid cultures, and may also result in unwanted and/or unknown genotypic, and therefore phenotypic, changes.

Anticipated Results

Basic Protocol 1 describes the streaking for single colonies from a frozen stock culture. After streaking and incubating the plate overnight at 37°C, single colonies should be observed away from the initial heavy re-streaking area. Colonies should be large and present typical *E. coli* colony morphology (e.g., semi-opaque, smooth, convex, round, and off-white in color). Basic Protocol 2 should yield the same results as observed in Basic Protocol 1, but will depend on the type of medium the strain is streaked out on. The growth of the *E. coli* culture in Basic Protocol 3 will be very obvious, as growth of the bacteria will result in increased turbidity of the culture medium. Finally, after incubation of the agar stabs in Basic Protocol 5, a column of growth should be observed where the needle was stabbed into the medium.

Time Considerations

All the Basic Protocols described in this unit should not take more than a few minutes each, aside from the time required to grow the bacteria overnight (e.g., 12 to 20 hr). Sometimes adjustments may be needed to allow for larger numbers of cultures to be processed in each Basic Protocol.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by NIAID grants AI025096 and AI039654.

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Table 5A.4.1

Genotypes of Commonly Used *E.coli* Strains and Typical Uses

Strain	Genotype	Typical use	Reference/source
BL21	B F- *dcm ompT hsdS*(r_B^- m_B^-) *gal* [*malB*$^+$]$_{K\text{-}12}$(λ^S)	Protein expression (T7 RNA polymerase based)	Stratagene
DH5α	F- *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR supE44* ϕ80 *lacZ* M15 (*lacZYA- argF*)-U169 *hsdR17*(r_K^- m_K^+) λ^-	Cloning (blue/white colony selection)	Grant et al. (1990); Meselson and Yuan (1968)
DH10B	F- *endA1 recA1 endA1 galU galK rpsL nupG rpsL lacX74 deoR* ϕ80 *lacZ* M15 *araD139* (*ara, leu*)7697 *mcrA* (*mrr-hsdRMS-mcrBC*) λ^-	Cloning (insertions of large DNA libraries)	Invitrogen; Casadaban and Cohen (1980); Grant et al. (1990)
ER2566	F- λ^- *fhuA2* [*lon*] *ompT lacZ*::T7 *gene1 gal sulA11* (*mcrC-mrr*)114::*IS10 R*(*mcr-73*:: *miniTn10*-Tet^S)2 *R*(*zgb*–210::*Tn10*)(Tet^S) *endA1* [*dcm*]	Protein expression (T7 RNA polymerase based)	New England Biolabs
HB101	F- *mcrB mrr hsdS20*(r_B^- m_B^-) *recA13 leuB6 ara-14 proA2 lacY1 galK2 xyl-5 mtl-1 rpsL20*(Sm^R) *glnV44* λ^-	General cloning	Boyer and Roulland-Dussoix (1969); Lacks and Greenberg (1977)
JM105	*endA1 glnV44 sbcB15 rpsL thi-1* (*lac-proAB*) [F' *traD36 proAB*$^+$ *lacI*^{q} *lacZ* M15] *hsdR4*(r_K^- m_K^+)	Cloning (blue/white colony selection)	Yanisch-Perron et al. (1985)
JM109	*endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB*$^+$ (*lac-proAB*) e14- [F' *traD36 proAB*$^+$ *lacI*^{q} *lacZ* M15] *hsdR17*(r_K^- m_K^+)	Cloning (blue/white colony selection)	New England Biolabs; Yanisch-Perron et al. (1985); Casadaban (1976)
MC4100	F- [*araD139*]$_{B/r}$ (*argF-lac*)169 λ^- *e14-flhD5301* (*fruK-yeiR*)725 (*fruA25*) *relA1 rpsL150*(Sm^R) *rbsR22* (*fimB-fimE*) 632(::*IS1*) *deoC1*	General cloning; *lac* gene fusions	Casadaban and Cohen (1979); Komeda and Iino (1979); Peters et al. (2003)

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