Chapter 1: *E. coli*, plasmids and bacteriophages
I. *Escherichia coli*:

- facultative aerobes
- grow rapidly at 37°C on minimal or rich medium.
- lag, log, stationary, and decline phases.
- Minimal medium: C-source, N-source, P-source, energy source, and trace metals.
- Rich medium: doubling time, 20-30 min,
- $1-2 \times 10^9$ cells/ml, when freshly saturated.
I. *Escherichia coli*:

- To get single colonies: streaking.
  - (gridding and checking for desired colonies)
- To grow:
  - use pre-warmed medium, and grow to saturation (overnight, 8-16 hrs).
  - For 5 ml culture:
    - inoculum, a single good colony, on a roller drum at 60 rpm.
  - For larger cultures:
    - inoculum, 1:100 of an overnight culture, Erlenmeyer of baffle flasks with > or = 5 times of culture volume, at 300 rpm (if less rpm, need flasks with >20 or = times of culture volume).
I. *Escherichia coli:*

- Monitoring growth:
  - hemacytometer:
    - look under 400x microscope,
    - 1 cell/square = 2 x 10^7 cells/ml.
  - spectrophotometer:
    - $\text{OD}_{600}$, OD needs to be >0.1 and < 0.9 for accurate reading,
    - $0.1\text{OD} = 10^8$ cells/ml.
  - serial 10 x dilutions and plating.

- CFU (colonies forming units) /ml.
Stocks:
in 15% glycerol at -700C. Do not thaw.

Bacterial strains frequently used:
DH5α, HB101, JH109, NovaBlue, AD494, BL21(DE3), etc.

- lacZΔM15: encode omega fragment of β-galactosidase
- IPTG, non-metabolizable inducer
- X-gal, non-inducing chromogenic substrate of β-galactosidase
- α complementation
• **sup**: wild type
• **supD, E, F, B, C**: nonsense suppressors.
• **lacI**: super LacI repressor.
• **DE3**: λ DE3, containing T7 RNA polymerase gene under *lacUV5* promoter control.
• **pLysS**: a pACYC184 - based plasmid with T7 lysozyme gene under weak promoter (*ptet*).

  T7 lysozyme is an inhibitor of T7 RNA polymerase.

  will reduce basal expression of target genes that under T7 promoter control.
• **lon**: a major protease gene
• **F’**: a defective F plasmid
II. Plasmids

- Detection
- Extraction
- Purification
- Transformation: CaCl$_2$ method and electroporation.

  Plasmids of $>15$kb are hard to transform by CaCl$_2$ method.
1. origin of replication
   copy number
   compatibility
   host range
2. selection marker
3. cloning sites: multiple cloning sites, MCS.
4. Selection/screening for inserts.
5. promoters for expression
   *lac*, *lacUV5*, *tac* promoter
   T3, T5, T7 and SP6 promoters: for *in vitro*
   transcription.
   T7 promoter: work in λ DE3 lysogen.
   T5 promoter: work in *E. coli* strains (with *E. coli*
   RNA polymerase).


7. Transcriptional terminator.

8. tag : for target protein detection and purification.
Cloning vectors
  eg. pBR322, pUC19
phagemid
  eg. pBluescript SK+ (pUC ori)
cosmid
  eg. pWE15
BAC (bacterial artificial chromosome)
  F-based, eg. pBeloBAc11
Expression vectors
  Transcription: need promoter and/or terminator.
  Be careful of antisense RNA.
  Translation: need RBS 4-11 bp upstream of start codon (ATG, GTG).

Promoter proving vector
  • eg. pEGFP-1
Shutter vectors
   eg. pRS303: replicate in *E. coli* and yeast,
   eg. pcDNA3.1: replicate in *E. coli* and
   mammalian cells expressing the
   SV40 large T antigen.

Broad-host-range mobilizable vector
   eg. pRR54
III. Bacteriophages

A. Lamda

- A temperate (both lysogenization and lysis) phage, infect *E. coli* and form turbid plaques.
- Linear ds DNA inside protein head, with cohesive ends (*cos* sites).
For lysogenization

• $\lambda$ integrates at $att$ sites ($attB$ and $attP$) by $\lambda$ Int and *E. coli* IHF and become a lysogen.
• Only $cI$ gene of $\lambda$ is expressed.
For lytic growth

- $\lambda$ enters *E. coli* and become ds ccc.
- $\Theta$ replication followed by rolling circle replication. (Ends are protected by $\lambda$ Gam).
- Transcription and translation of all $\lambda$ structural genes.
- Head assembly and packaged at 2 cos sites.
- Lysis, followed by infection of neighboring *E. coli* cells.

  *E. coli* cultures become clear.
  Form plaques on *E. coli* pre-seeded plates.
Why using lambda phages as vectors?

• The middle 1/3 of genome is dispensable.
• DNA can be inserted and packaged into phages \textit{in vitro}.
• Package efficiency is 10\%, infection efficiency is 1.

(Plasmid transformation is \(<0.1\%\) by the \text{CaCl}_2\) method.)
Why using lambda phages as vectors?

1. The middle 1/3 of genome is dispensable.
2. DNA can be inserted and packaged into phages \textit{in vitro}.
3. Package efficiency is 10%, infection efficiency is 1. (transformation is $< 0.1\%$ by the CaCl2 method.)

Q: If you got a transformation efficiency of $10^5/ \mu g$ pBR322, calculate how many plasmids are needed to be transformed in order to get one transformant.
4. Selection of insert DNA.

**Size selection:** <78% or >105% cannot be packed.

**spi** selection:
- $\text{red}^+\text{gam}^+ \lambda$ cannot form plaques on a P2 lysogen. (sensitive to $P2$ interference).
- DNA insertion results in $\lambda \text{red}^-\text{gam}^-$, forming plaques.

**hfl** selection:
- $\lambda$ cII protein is needed for making Int protein for lysogenization.
- *E. coli* HflA and HflB protein will inactivate cII protein.
- In hfl- *E. coli*, $\lambda$ become a lysogen.
- Cloning site is in $cl$ gene.
- $cl$ protein represses cII gene expression.
- DNA insertion will results in plaque formation.
• Use PEG to concentrate phage articles and then buffered phenol to extract phage DNA.

• Phage stocks are kept at 4\(^{0}\)C and chloroform was added to prevent bacterial growth.
B. M13
a Filamentous phage, with ss circular DNA.

• infect pili of *E. coli*
• after infection, become ds ccc and ss ccc.
• ss ccc is packaged into phage particles and secreted out of *E. coli* cells.
• infected *E. coli* cells are not lysed, but grow slower than un-infected *E. coli* cells.
• will still form turbid plaques.
• medium supernatants are full of phage particles, and good for preparation of ss ccc.

  Why ss ccc?
  very useful for mutagenesis study.
With plasmids of f1ori in an F$^+$ or Hfr E. coli strain, ss ccc can be prepared by infecting helper phage (M13K07, VCSM13 (Stratagene), or R408) at low MOI (multiplicity of infection).
Chapter 2: Preparation and analysis of DNA

Q: How to convert Da to bp, or vise versa?

Electrophoresis

• $V = IR$ ($V$: electric field, in volt; $I$: current, in milliamp; $R$: resistance, in ohms)
  Resistance is inversely proportional to cross-sectional area & the ionic strength of the buffer.

• $P = I^2R$ ($P$: power, in watts)
  Power is manifested as heat.
• Resistance of acrylamide gels increases during a run, as ions related to polymerization are electrophoresed out of the gel. Set a constant power.

• With two gels on one power supply, each gel receives 50% of total voltage and power indicated on the power supply.

• Be aware of lethal high voltages with some electrophoresis systems.
<table>
<thead>
<tr>
<th>Galactoside</th>
<th>Stock concentration&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Use</th>
<th>Characteristics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropyl-1-thio-β-D-galactoside (IPTG)</td>
<td>100 mM</td>
<td>Very effective inducer</td>
<td>Nonmetabolizable inducer</td>
<td>Barkley and Bourgeois, 1978 (pp. 177-220)</td>
</tr>
<tr>
<td>5-Bromo-4-chloro-3-indolyl-β-D-galactoside (Xgal)</td>
<td>20 mg/ml</td>
<td>Identification of lac&lt;sup&gt;2&lt;/sup&gt; bacteria, especially useful for detecting β-galactosidase made by recombinant vectors</td>
<td>Noninducing chromogenic substrate of β-galactosidase (cleavage of Xgal results in blue color); production of blue color independent of lac&lt;sup&gt;Y&lt;/sup&gt; gene product</td>
<td>Miller, 1972</td>
</tr>
<tr>
<td>Orthonitrophenyl-β-D-galactoside (ONPG)</td>
<td>10 mM</td>
<td>β-galactosidase assays</td>
<td>Chromogenic substrate of β-galactosidase (cleavage of ONPG results in yellow color)</td>
<td>Miller, 1972 (pp. 352-355)</td>
</tr>
<tr>
<td>6-O-β-D-Galactopyranosyl α-D-glucose (allo lactose)</td>
<td></td>
<td></td>
<td>Inducer of the lactose operon in vivo; lactose is converted into allolactose by β-galactosidase</td>
<td>Zabin and Fowler, 1978 (pp. 89-121)</td>
</tr>
<tr>
<td>Phenyl-β-D-galactoside (Pgal)</td>
<td>2 mg/ml</td>
<td>Selection for lac constitutive mutants</td>
<td>Noninducing substrate of β-galactosidase; uptake partly dependent on lac&lt;sup&gt;Y&lt;/sup&gt; gene product</td>
<td>Miller, 1978 (pp. 31-88)</td>
</tr>
<tr>
<td>Orthonitrophenyl-β-D-thiogalactoside (TONPG)</td>
<td>10 mM</td>
<td>Selection for lac&lt;sup&gt;−&lt;/sup&gt; mutants</td>
<td>Transported into cells by lac permease (the lac&lt;sup&gt;Y&lt;/sup&gt; gene product); inhibits cell growth at high concentration</td>
<td>Miller, 1978 (pp. 31-88)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Stock solutions should be dissolved in sterile water except for Xgal, which should be dissolved in N,N-dimethylformamide.
<table>
<thead>
<tr>
<th>Replicator</th>
<th>Prototype plasmid</th>
<th>Size (bp)</th>
<th>Markers on prototype</th>
<th>Copy number</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMB1</td>
<td>pBR322</td>
<td>4,362</td>
<td>Ap&lt;sup&gt;f&lt;/sup&gt;, Tet&lt;sup&gt;f&lt;/sup&gt;</td>
<td>High; 100-300</td>
<td>Bolivar et al., 1977</td>
</tr>
<tr>
<td>ColE1</td>
<td>pMK16</td>
<td>~4,500</td>
<td>Kan&lt;sup&gt;f&lt;/sup&gt;, Tet&lt;sup&gt;f&lt;/sup&gt;, ColE1&lt;sub&gt;imm&lt;/sub&gt;</td>
<td>High; &gt;15</td>
<td>Kahn et al., 1979</td>
</tr>
<tr>
<td>p15A</td>
<td>pACYC184</td>
<td>~4,000</td>
<td>Eml&lt;sup&gt;f&lt;/sup&gt;, Tet&lt;sup&gt;f&lt;/sup&gt;</td>
<td>High; ~15</td>
<td>Chang and Cohen, 1978</td>
</tr>
<tr>
<td>pSC101</td>
<td>pLG338</td>
<td>~7,300</td>
<td>Kan&lt;sup&gt;f&lt;/sup&gt;, Tet&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Low; ~6</td>
<td>Stoker et al., 1982</td>
</tr>
<tr>
<td>F</td>
<td>pDF41</td>
<td>~12,800</td>
<td>TrpE</td>
<td>Low; 1 to 2</td>
<td>Kahn et al., 1979</td>
</tr>
<tr>
<td>R6K</td>
<td>pRK353</td>
<td>~11,100</td>
<td>TrpE</td>
<td>Low; &lt;15</td>
<td>Kahn et al., 1979</td>
</tr>
<tr>
<td>R1 (R1&lt;sub&gt;drd-17&lt;/sub&gt;)</td>
<td>pBEU50</td>
<td>~10,000</td>
<td>Ap&lt;sup&gt;f&lt;/sup&gt;, Tet&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Low at 30°C; high above 35°C&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Uhlin et al., 1983</td>
</tr>
<tr>
<td>RK2</td>
<td>pRK2501</td>
<td>~11,100</td>
<td>Kan&lt;sup&gt;f&lt;/sup&gt;, Tet&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Low; 2 to 4</td>
<td>Kahn et al., 1979</td>
</tr>
<tr>
<td>λ&lt;sup&gt;dv&lt;/sup&gt;</td>
<td>λ&lt;sub&gt;dvgal&lt;/sub&gt;</td>
<td>—</td>
<td>Gal</td>
<td>—</td>
<td>Jackson et al., 1972</td>
</tr>
</tbody>
</table>

<sup>a</sup>Copy numbers are for the prototype plasmid. Plasmid vectors that contain replicators derived from these plasmids may have different copy numbers due to introduction of mutations into the replicator. For example, pUC vectors (pMB1-derived) have copy numbers of 1000-3000.

<sup>b</sup>Temperature sensitive.

<sup>c</sup>Not known.
<table>
<thead>
<tr>
<th>Strain&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Genotype</th>
<th>Reference&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC1061&lt;sup&gt;h&lt;/sup&gt;</td>
<td>hsdR&lt;sup&gt;2&lt;/sup&gt;, hsdM&lt;sup&gt;+&lt;/sup&gt;, hsdS&lt;sup&gt;+&lt;/sup&gt;, araD139Δ(ara-leu)754Δ(lac)x74 galE15 galK16 rpsL (Str&lt;sup&gt;+&lt;/sup&gt;) mcrA mcrB1</td>
<td>Casadaban and Cohen, 1980&lt;sup&gt;†&lt;/sup&gt;; M. Casadaban&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>MM294&lt;sup&gt;h&lt;/sup&gt;</td>
<td>endA thiA hsdR17 supE44</td>
<td>Backman et al., 1976&lt;sup&gt;†&lt;/sup&gt;; M. Meselson&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>NM539&lt;sup&gt;h&lt;/sup&gt;</td>
<td>supF hsdR (P2cox3)</td>
<td>Frischauer et al., 1983&lt;sup&gt;†&lt;/sup&gt;; Lindahl and Sunshine, 1972&lt;sup&gt;†&lt;/sup&gt;; N. Murray&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>P2392&lt;sup&gt;h&lt;/sup&gt;</td>
<td>hsdR514(rK&lt;sup&gt;+&lt;/sup&gt;mg&lt;sup&gt;+&lt;/sup&gt;) supE44 supF58 lacY galK2 galT22 metB1 trp55 mcrA (P2)</td>
<td>L. Klickstein, pers. comm.</td>
</tr>
<tr>
<td>PR722&lt;sup&gt;h&lt;/sup&gt;</td>
<td>F&lt;sup&gt;−&lt;/sup&gt;Δ(lacIZA&lt;sub&gt;65&lt;/sub&gt;) pro&lt;sup&gt;+&lt;/sup&gt;/proC::Tn5Δ(lacIYA&lt;sub&gt;169&lt;/sub&gt;) hsdS20 ara-14 galK2 rpsL20 (Str&lt;sup&gt;+&lt;/sup&gt;) xyl-5 mtl-1 supE44 leu</td>
<td>P. Riggs, pers. comm.</td>
</tr>
<tr>
<td>Q359&lt;sup&gt;h&lt;/sup&gt;</td>
<td>hsdR&lt;sup&gt;−&lt;/sup&gt; hsdM&lt;sup&gt;+&lt;/sup&gt; supE tonA (φ80&lt;sup&gt;+&lt;/sup&gt;) (P2)</td>
<td>Kam et al., 1980&lt;sup&gt;†&lt;/sup&gt;‡</td>
</tr>
<tr>
<td>RR1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Δ(gpt-proA)62 leuB6 thi-1 lacY1 hsdS20 rpsL20 (Str&lt;sup&gt;+&lt;/sup&gt;) ara-14 galK2 xyl-5 mtl-1 supE44 mcrB&lt;sub&gt;6&lt;/sub&gt;</td>
<td>Bolivar et al., 1977; see UNIT 16.5</td>
</tr>
<tr>
<td>Y1088&lt;sup&gt;fi&lt;/sup&gt;</td>
<td>supE supF metB trpR hsdR&lt;sup&gt;−&lt;/sup&gt; hsdM&lt;sup&gt;+&lt;/sup&gt; tonA21 strA ΔlacU169 mcrA proC::Tn5/pMC9</td>
<td>Huỳnh et al., 1984&lt;sup&gt;†&lt;/sup&gt;; Miller et al., 1984&lt;sup&gt;†&lt;/sup&gt;; R. Young&lt;sup&gt;†&lt;/sup&gt;; M. Calos (pMC9)&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>Y1089&lt;sup&gt;fi&lt;/sup&gt;</td>
<td>ΔlacU169 proA&lt;sup&gt;+&lt;/sup&gt; Δ(lon) araD139 strA hflA150 chr::Tn10/pMC9</td>
<td>See Y1088 references</td>
</tr>
<tr>
<td>Y1090&lt;sup&gt;fi&lt;/sup&gt;</td>
<td>ΔlacU169 proA&lt;sup&gt;+&lt;/sup&gt; Δ(lon) araD139 strA supF trpC22::Tn10 mcrA/pMC9</td>
<td>See Y1088 references</td>
</tr>
</tbody>
</table>

<sup>a</sup>The original E. coli K-12 strain was an F<sup>+</sup> λ lysogen, but most K-12 derivatives in common use have been cured of the F factor and phage and these are indicated only when present. All other genes in these strains are presumed to be wild-type except for the genotype markers noted in the second column.

<sup>b</sup>Reference for all mcr and mrr genotypes is Raleigh et al., 1988. Specific information for each strain can be found as indicated by: * reference for genotype of strain; † source of additional genotype information; ‡ thought to be responsible for original strain construction.

<sup>h</sup>AS1 is also known as MM294cI<sup>+</sup>. BNN102 is also known as C600 hflA.

<sup>fi</sup>Both CJ236 and BW313 are commonly used in oligonucleotide-directed mutagenesis. pC105, the plasmid CJ236 carries, is not relevant for this application.

<sup>†</sup>Three strains are in circulation. DH5<sup>Δ</sup> is a derivative of DH1 that transforms at slightly higher efficiency. DH5α<sup>Δ</sup> and DH5Δ<sup>φ</sup> are derivatives that carry a deletion of the lac operon and a φ<sup>B</sup> prophage that directs synthesis of the omega fragment of β-galactosidase. DH5<sup>Δ</sup> carries an F<sup>−</sup> factor as well. DH5α<sup>Δ</sup> and DH5Δ<sup>φ</sup> are proprietary strains and the cells are prepared in some way that allows them to be transformed with slightly higher efficiency than DH5.

<sup>†</sup>In this strain, the area of the chromosome that contains the hsd genes was derived from the related B strain of E. coli.

<sup>‡</sup>The continued presence of the F<sup>−</sup> factor in JM strains can be assured by starting cultures only from single colonies grown on minimal plates that do not contain proline. These strains encode the omega fragment of lacZ and are frequently used with vectors that direct the synthesis of the lacZ alpha fragment.

<sup>‡</sup>It is not known whether this strain has markers other than those listed.

<sup>p</sup>PMC9, the plasmid in the Y strains listed here, directs the synthesis of large amounts of lac repressor. It also confers resistance to tetracycline and ampicillin (Miller et al., 1984).
<table>
<thead>
<tr>
<th>Strain&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Genotype</th>
<th>Reference&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR58</td>
<td>sup&lt;sup&gt;0&lt;/sup&gt; galK2 galE::Tn10 (λc1857 ΔH1 bio&lt;sup&gt;-&lt;/sup&gt; uvrB kil&lt;sup&gt;+&lt;/sup&gt; cIII&lt;sup&gt;+&lt;/sup&gt;) Str&lt;sup&gt;f&lt;/sup&gt;</td>
<td>A. Shatzman, pers comm.&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>AR120</td>
<td>sup&lt;sup&gt;0&lt;/sup&gt; galK2 nad·::Tn10 (Tet&lt;sup&gt;+&lt;/sup&gt;) (λc1&lt;sup&gt;i&lt;/sup&gt; ind&lt;sup&gt;+&lt;/sup&gt; pl-lacZ fusion) Str&lt;sup&gt;f&lt;/sup&gt;</td>
<td>A. Shatzman, pers comm.&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>AS1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>endA1 thi-1 hsdR17(ry&lt;sup&gt;-&lt;/sup&gt;m&lt;sup&gt;k&lt;sup&gt;+&lt;/sup&gt;&lt;/sup&gt;) supE44 (λc1&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>A. Shatzman, pers comm.&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>BNN102&lt;sup&gt;c&lt;/sup&gt;</td>
<td>C600 hflA150 chr·::Tn10 mcrA1 mcrB</td>
<td>Young and Davis, 1983&lt;sup&gt;∗&lt;/sup&gt;</td>
</tr>
<tr>
<td>BW313&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Hfr lysA&lt;sup&gt;+&lt;/sup&gt; dut ung thi-1 recA spoT1</td>
<td>Kunkel et al., 1987&lt;sup&gt;‡†&lt;/sup&gt;</td>
</tr>
<tr>
<td>C600</td>
<td>thi-1 thr-1 leuB6 lacY1 tonA21 supE44 mcrA</td>
<td>Appleyard, 1954&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>CJ236&lt;sup&gt;d&lt;/sup&gt;</td>
<td>dut1 ung1 thi-1 relA1/pC1105 (Cm&lt;sup&gt;r&lt;/sup&gt;)</td>
<td>Kunkel et al., 1987&lt;sup&gt;‡†&lt;/sup&gt;; Joyce and Grindley, 1984&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>DH1</td>
<td>recA1 endA1 thi-1 hsdR17 supE44 gyrA96 (NaI&lt;sup&gt;r&lt;/sup&gt;) relA1</td>
<td>Hanahan, 1983&lt;sup&gt;‡‡&lt;/sup&gt;; D. Hanahan, pers. comm.&lt;sup&gt;††&lt;/sup&gt;</td>
</tr>
<tr>
<td>√ DH5αF&lt;sup&gt;e&lt;/sup&gt;</td>
<td>F'/endA1 hsdR17(ry&lt;sup&gt;-&lt;/sup&gt;m&lt;sup&gt;k&lt;sup&gt;+&lt;/sup&gt;&lt;/sup&gt;) supE44 thi-1 recA1 gyrA (NaI&lt;sup&gt;r&lt;/sup&gt;) relA1 Δ(lacZYA-argF)&lt;sub&gt;U169&lt;/sub&gt;(m80lacZΔM15)</td>
<td>See DH1 references</td>
</tr>
<tr>
<td>DK1</td>
<td>hsdR2 hsdM&lt;sup&gt;+&lt;/sup&gt; hsdS&lt;sup&gt;+&lt;/sup&gt; ara139 Δ ara-leu&lt;sub&gt;7697&lt;/sub&gt; Δ(lac)x74 galU galK rpsL (Str&lt;sup&gt;f&lt;/sup&gt;) mcrA mcrB1 Δ(srl-reca) &lt;sub&gt;396&lt;/sub&gt;</td>
<td>D. Kurnit and B. Seed, pers. comm.&lt;sup&gt;‡‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>ER1451</td>
<td>F' traD36 proA&lt;sup&gt;B&lt;/sup&gt; lacI&lt;sup&gt;+&lt;/sup&gt; Δ(lacZ)M15/endA gyrA96 thi-1 hsdR2 (or hsdR17) supE44 Δ(lac-proAB) mcrA mcrB</td>
<td>Raleigh et al., 1988&lt;sup&gt;‡‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>√ HB101&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Δ(gpt-proA)62 leuB6 thi-1 lacY1 hsdS&lt;sub&gt;20&lt;/sub&gt; recA rpsL20 (Str&lt;sup&gt;f&lt;/sup&gt;) ara-14 galK2 xyl-5 mit-1 supE44 mcrB&lt;sup&gt;B&lt;/sup&gt;</td>
<td>Boyer and Roulland-Dussoix, 1969&lt;sup&gt;‡‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>JM101&lt;sup&gt;g&lt;/sup&gt;</td>
<td>F' traD36 proA&lt;sup&gt;B&lt;/sup&gt; proB&lt;sup&gt;B&lt;/sup&gt; lacI&lt;sup&gt;+&lt;/sup&gt; lacZΔM15/supE thi Δ(lac-proAB)</td>
<td>Yanisch-Perron et al., 1985&lt;sup&gt;‡‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>JM102&lt;sup&gt;g&lt;/sup&gt;</td>
<td>F' traD36 proA&lt;sup&gt;B&lt;/sup&gt; proB&lt;sup&gt;B&lt;/sup&gt; lacI&lt;sup&gt;+&lt;/sup&gt; lacZΔM15/Δ(lac-pro)&lt;sub&gt;111&lt;/sub&gt; thi rpsL (Str&lt;sup&gt;f&lt;/sup&gt;) endA sbcB supE hsdR&lt;sup&gt;d&lt;/sup&gt;</td>
<td>See JM101 references</td>
</tr>
<tr>
<td>JM107&lt;sup&gt;g&lt;/sup&gt;</td>
<td>F' traD36 proA&lt;sup&gt;B&lt;/sup&gt; proB&lt;sup&gt;B&lt;/sup&gt; lacI&lt;sup&gt;+&lt;/sup&gt; lacZΔM15/relA1 gyrA96 (NaI&lt;sup&gt;r&lt;/sup&gt;) thi hsdR17 supE44 relA1 Δ(lac-proAB) mcrA</td>
<td>See JM101 references</td>
</tr>
<tr>
<td>√ JM109&lt;sup&gt;g&lt;/sup&gt;</td>
<td>F' traD36 proA&lt;sup&gt;B&lt;/sup&gt; proB&lt;sup&gt;B&lt;/sup&gt; lacI&lt;sup&gt;+&lt;/sup&gt; lacZΔM15/relA1 endA1 gyrA96 (NaI&lt;sup&gt;r&lt;/sup&gt;) thi hsdR17 supE44 relA1 Δ(lac-proAB) mcrA</td>
<td>See JM101 references</td>
</tr>
<tr>
<td>K38</td>
<td>HfrC (λ)</td>
<td>Russel and Model, 1984; see UNIT 16.2</td>
</tr>
<tr>
<td>KM392</td>
<td>hsdR514(ry&lt;sup&gt;-&lt;/sup&gt;m&lt;sup&gt;k&lt;sup&gt;+&lt;/sup&gt;&lt;/sup&gt;) supE44 supF58 lacY galK2 galT22 metB1 trp55 mcrA ΔlacUV69 proC::Tn5</td>
<td>T. St. John, pers. comm.&lt;sup&gt;†&lt;/sup&gt;; K. Moore&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>LE392</td>
<td>hsdR514(ry&lt;sup&gt;-&lt;/sup&gt;m&lt;sup&gt;k&lt;sup&gt;+&lt;/sup&gt;&lt;/sup&gt;) supE44 supF58 lacY galK2 galT22 metB1 trp55 mcrA</td>
<td>Borck, et al., 1976&lt;sup&gt;‡&lt;/sup&gt;; N. Murray, pers. comm.&lt;sup&gt;†&lt;/sup&gt;; L. Enquist&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
### pET Host Strain Genotypes

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Description/Application(s)</th>
<th>Antibiotic Resistance¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>NovaBlue</td>
<td>endA1 hisD17(λr12 m12) supE44 thi-1 recA1 gyrA96 relA1 lacF' proA8 B lacZΔM15::Tn10</td>
<td>non-expression host, general purpose cloning, plasmid props</td>
<td>Tetracycline (12.5 μg/ml)</td>
</tr>
<tr>
<td>BL21</td>
<td>F- ompT hsdS(rK mK) gal dcm</td>
<td>control non-expression host</td>
<td>none</td>
</tr>
<tr>
<td>BL21(DE3)</td>
<td>F- ompT hsdS(rK mK) gal dcm (DE3)</td>
<td>general purpose expression host</td>
<td>none</td>
</tr>
<tr>
<td>BL21(DE3)pLysS</td>
<td>F- ompT hsdS(rK mK) gal dcm (DE3) pLysS</td>
<td>high-stringency expression host</td>
<td>Chloramphenicol (34 μg/ml)</td>
</tr>
<tr>
<td>BLR</td>
<td>F- ompT hsdS(rK mK) gal dcm</td>
<td><em>recA</em> high-stringency expression host recommended for use with tandem repeats</td>
<td>Tetracycline (12.5 μg/ml)</td>
</tr>
<tr>
<td>BLR(DF3)</td>
<td>F- ompT hsdS(rK mK) gal dcm (DF3)</td>
<td><em>recA</em> high-stringency expression host recommended for use with tandem repeats</td>
<td>Chloramphenicol (34 μg/ml)</td>
</tr>
<tr>
<td>BLR(DF3)pLysS</td>
<td>F- ompT hsdS(rK mK) gal dcm (DF3) pLysS</td>
<td><em>recA</em> high-stringency expression host recommended for use with tandem repeats</td>
<td>Tetracycline (12.5 μg/ml)</td>
</tr>
<tr>
<td>NovaBlue(DF3)</td>
<td>endA1 hisD17(λr12 m12) supE44 thi-1 recA1 gyrA96 relA1 lacF' proA8 B lacZΔM15::Tn10 (DF3)</td>
<td><em>recA</em> K-12 expression host recommended for use with NovaTape® System</td>
<td>Tetracycline (12.5 μg/ml)</td>
</tr>
<tr>
<td>HMS174</td>
<td>F- recA1 hsdR(rK mK) Riff</td>
<td>control non-expression host</td>
<td>none</td>
</tr>
<tr>
<td>HMS174(DF3)</td>
<td>F- recA1 hsdR(rK mK) Riff (DF3)</td>
<td><em>recA</em> K-12 expression host</td>
<td>Chloramphenicol (34 μg/ml)</td>
</tr>
<tr>
<td>HMS174(DF3)pLysS</td>
<td>F- recA1 hsdR(rK mK) Riff (DF3) pLysS</td>
<td><em>recA</em> K-12 high-stringency expression host</td>
<td>Chloramphenicol (34 μg/ml)</td>
</tr>
<tr>
<td>AD494</td>
<td>Δara-leu7697 ΔlacX74 ΔphoAPVΔ phoR vonF3F′ lac(lacpro) · trxB · kan</td>
<td>trxB non-expression host; allows disulfide bond formation in E. coli cytoplasm</td>
<td>Kanamycin (15 μg/ml)</td>
</tr>
<tr>
<td>AD494(DF3)</td>
<td>Δara-leu7697 ΔlacX74 ΔphoAPVΔ phoR vonF3F′ lac(lacpro) · trxB · kan(DF3)</td>
<td>trxB expression host; allows disulfide bond formation in E. coli cytoplasm</td>
<td>Kanamycin (15 μg/ml)</td>
</tr>
<tr>
<td>AD494(DF3)pLysS</td>
<td>Δara-leu7697 ΔlacX74 ΔphoAPVΔ phoR vonF3F′ lac(lacpro) · trxB · kan(DF3) pLysS</td>
<td>trxB high-stringency expression host; allows disulfide bond formation in E. coli cytoplasm</td>
<td>Chloramphenicol (34 μg/ml)</td>
</tr>
<tr>
<td>B834</td>
<td>F- ompT hsdSφ(rK mK) gal dcm met</td>
<td>met auxotroph, parent of BL21 control non-expression host</td>
<td>none</td>
</tr>
<tr>
<td>B834(DF3)</td>
<td>F- ompT hsdSφ(rK mK) gal dcm met (DF3)</td>
<td>met auxotroph, parent of BL21 general expression host, 35S-met labeling</td>
<td>Chloramphenicol (34 μg/ml)</td>
</tr>
<tr>
<td>B834(DF3)pLysS</td>
<td>F- ompT hsdSφ(rK mK) gal dcm met (DF3) pLysS</td>
<td>met auxotroph, parent of BL21 high-stringency expression host, 35S-met labeling</td>
<td>Chloramphenicol (34 μg/ml)</td>
</tr>
</tbody>
</table>

---

Table notes:
1. The appropriate drug to select for the target plasmid must also be added.
2. Here, non-expression means that the strain does not contain the gene for T7 RNA polymerase and therefore will not express from a T7 promoter. These strains may be suited for expression from E. coli promoters such as lac, tac, and trp.
3. Expression means that the strain is a λDE3 lysogen, i.e., it carries the gene for T7 RNA polymerase under lacUV5 control. It is therefore suited to expression from T7 promoters.
4. High-stringency means that the strain carries pLysS, a pET-compatible plasmid that produces T7 lysozyme, thereby reducing basal expression of target genes. Even greater stringency is provided by pLysE hosts; these are available separately as glycerol stocks.

---

*Novagen*
<table>
<thead>
<tr>
<th>Suppressor</th>
<th>Map position&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Type of suppressor</th>
<th>Amino acid inserted</th>
<th>tRNA gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>supD (su1)</td>
<td>43</td>
<td>Amber</td>
<td>Serine</td>
<td>serU</td>
</tr>
<tr>
<td>supE (su2)</td>
<td>16</td>
<td>Amber</td>
<td>Glutamine</td>
<td>glnU</td>
</tr>
<tr>
<td>supF (su3)</td>
<td>27</td>
<td>Amber</td>
<td>Tyrosine</td>
<td>tyrT</td>
</tr>
<tr>
<td>supB (suB)</td>
<td>16</td>
<td>Ochre/amber</td>
<td>Glutamine</td>
<td>glnU</td>
</tr>
<tr>
<td>supC (suC)</td>
<td>27</td>
<td>Ochre/amber</td>
<td>Tyrosine</td>
<td>tyrT</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data compiled from Bachmann (1983) and Celis and Smith (1979).

<sup>b</sup>Given in minutes; see Bachmann (1983) for description.
**Figure 1.5.1** pBR322 is one of the classic cloning vectors from which many other vectors are derived. It contains an amplifiable pMB1 replicator and genes for ampicillin and tetracycline resistance. Insertion of DNA into either drug-resistance gene usually inactivates it, allowing selection of colonies bearing such plasmids (Bolivar et al., 1977; sequence in Sutcliffe, 1978).
**Figure 1.5.2** pUC19 belongs to a family of plasmid vectors that contain a polylinker in the alpha region of the lacZ gene. The polylinkers are the same as those used in the M13mp series (see Fig. 1.14.2). pUC18 has the same polylinker but in the opposite orientation. Under appropriate conditions, colonies that bear plasmids with an insert in the polylinker will be white instead of blue. These pMB1-derived plasmids maintain a very high-copy-number (1000 to 3000 per genome). Wild-type and recombinant plasmids confer ampicillin resistance and can be amplified with chloramphenicol (Norrander et al., 1983). In addition, wild-type plasmids confer a LacZ\(^+\) phenotype to appropriate cells (e.g., JM101 cells, Table 1.4.5).
Figure 1.5.3  pBluescript SK (+/−) is a commonly used phagemid vector with a polylinker in the alpha region of the **lacZ** gene and T3 and T7 promoter sequences flanking the cloning sites. pBluescript SK (+) has the f1 filamentous phage ori in the (+) orientation, allowing recovery of the sense strand of the **lacZ** gene as ssDNA. pBluescript SK (−) has the f1 ori in the opposite orientation, f1 (−), allowing recovery of the other strand. The position of the polylinker in the **lacZ** alpha region allows identification of inserts based on a blue/white color screen under the appropriate conditions. The T3 and T7 promoters are recognized by bacteriophage RNA polymerases, allowing transcription of inserted DNA to be initiated from either side of the polylinker.
Figure 1.5.4 pWE15 is an example of a cosmid vector used for cloning DNA fragments ~35 to 45 kb. The cos sites allow the DNA to be cut and packaged into phage heads by the appropriate lambda proteins. There is a single unique BamHI cloning site flanked by T3 and T7 promoter sequences. These promoters are particularly useful for production of labeled RNA probes corresponding to the ends of the insert DNA, and these can be used to identify overlapping cosmids for chromosomal walking and construction of cosmid contigs. NotI sites flanking the cloning site can be used to excise an intact insert fragment from the vector. The ColE1-derived ori and ampicillin resistance gene allow for replication and selection in bacteria. The SV40 promoter (included in SV40 ori) drives the neomycin phosphotransferase gene, which enables selection in eukaryotic cells. Map of pWE15 adapted from Wahl et al. (1987) with permission.
Figure 1.5.5 **pBelobAC11** is an example of a bacterial artificial chromosome vector based on the low-copy-number F factor replicator. BAC vectors are used for cloning large DNA fragments (100 to 500 kb) in *E. coli* and are used commonly in genome-mapping strategies. *oriS, repE, parA, parB,* and *parC* genes are the essential genes that compose the F factor replicator. *oriS* and *repE* genes are required for unidirectional replication of the plasmid, and *parABC* loci stably maintain the copy number at one to two per *E. coli* genome. There are two unique cloning sites (*HindIII* and *BamHI*) in the *lacZ* alpha region. The cloning region also contains T7 and SP6 promoter sequences flanking the cloning sites, *NotI* restriction sites flanking the cloning sites for potential excision of the insert, and *loxP* and *cosN* sites that can be cleaved by specific enzymes. The ends generated by cleavage at *loxP* or *cosN* can be used as fixed reference points in building an ordered restriction map by end labeling and partial restriction digestion. Map of **pBelobAC11** adapted from Shizuya et al. (1992) with permission.
pET Dsb Fusion Systems 39b and 40b

Vectors for export and periplasmic folding of target proteins

The pET Dsb Fusion Systems 39b and 40b are designed for cloning and high-level expression of peptide sequences fused with the 208 aa DsbA•Tag™ sequence [pET or the 236 aa DsbC•Tag™ sequence [pET-40b(+)].

DsbA and DsbC are periplasmic enzymes that catalyze the formation and isomerization of disulfide bonds, respectively (1–5). In contrast to the pET-32 Trx•Tag™ series, which is designed to increase solubility in the cytoplasm, the DsbA and DsbC•Tag vectors provide potential periplasmic localization and thus may enhance solubility and proper folding for non-reducing environments (6).

Advantages

- Fusion to DsbA or DsbC for export and increased protein solubility/folding in the periplasm
- Protease cleavage sites for complete fusion tag removal
- His•Tag™ sequence facilitates protein purification
- S•Tag™ sequence for sensitive protein quantification, non-radioactive detection, and efficient purification


NovaGen

pET-39-40b(+)

T7

DsbA-Tag
Spe I
His-Tag
Sac II
thrombin site
Sna I
S•Tag
Nsp V
Kpn I
Ek site
BstR I
Sfi I
Nco I
BarH I
EcoR I
Sac I
Sal I
Hind III
Not I
Eag I
Xho I
His-Tag
Avr II
T7 terminator

T7

DsbC-Tag
Spe I
His-Tag
Sac II
thrombin site
Sna I
Man I
S•Tag
Nsp V
Kpn I
Ek site
BstR I
Sfi I
Nco I
BarH I
EcoR I
Sac I
Sal I
Hind III
Not I
Eag I
Xho I
His-Tag
Avr II
T7 terminator
### pQE-30, pQE-31, and pQE-32 Vectors

**Positions of elements in bases**

<table>
<thead>
<tr>
<th>Element</th>
<th>pQE-30</th>
<th>pQE-31</th>
<th>pQE-32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector size (bp)</td>
<td>3461</td>
<td>3463</td>
<td>3462</td>
</tr>
<tr>
<td>Start of numbering at XhoI (CTCGAG)</td>
<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
</tr>
<tr>
<td>T5 promoter/lac operator element</td>
<td>7-87</td>
<td>7-87</td>
<td>7-87</td>
</tr>
<tr>
<td>T5 transcription start</td>
<td>61</td>
<td>61</td>
<td>61</td>
</tr>
<tr>
<td>6xHis-tag coding sequence</td>
<td>127-144</td>
<td>127-144</td>
<td>127-144</td>
</tr>
<tr>
<td>Multiple cloning site</td>
<td>145-192</td>
<td>147-194</td>
<td>146-193</td>
</tr>
<tr>
<td>Lambda t1 transcriptional termination region</td>
<td>208-302</td>
<td>210-304</td>
<td>209-303</td>
</tr>
<tr>
<td>r6B T1 transcriptional termination region</td>
<td>1064-1162</td>
<td>1066-1164</td>
<td>1065-1163</td>
</tr>
<tr>
<td>ColE1 origin of replication</td>
<td>1638</td>
<td>1640</td>
<td>1639</td>
</tr>
<tr>
<td>β-lactamase coding sequence</td>
<td>3256-2396</td>
<td>3258-2398</td>
<td>3257-2397</td>
</tr>
</tbody>
</table>

---

Qiagene
Figure 1.5.6  (Left) pTrc99A,B,C is a member of the pTrc series of plasmid expression vectors, which facilitate regulated expression of genes in *E. coli*. These vectors carry the strong hybrid trp/lac promoter, the lacZ ribosome-binding site (RBS), the MCS of pUC18 that allows insertion in three reading frames, and the rmB transcription terminators. These vectors are equally useful for expression of unfused proteins (resulting from insertion in the Ncol site) and fusion proteins (using one of the cloning sites in the correct translational frame). The presence of the lacZm allele ensures complete repression of the hybrid trp/lac promoter during cloning and growth in any host strain (see Amann et al., 1988, for further details).
Figure 1.5.7  pEGFP-1 (Clontech; Yang et al., 1996) is a selectable vector for monitoring promoter activity in mammalian cells via fluorescence of EGFP, a derivative of green fluorescent protein (GFP). Promoter sequences are introduced into a polylinker upstream of the EGFP gene, so that the function of the promoter can be assessed based on EGFP activity. A neomycin resistance gene downstream of the SV40 early promoter allows selection of stably transformed mammalian cells. The modifications in EGFP ensure expression in mammalian cells. EGFP has silent base mutations that correspond to human codon-usage preferences, and sequences flanking the coding region have been converted to a Kozak consensus translation initiation signal. The vector backbone contains an f1 ori for production of ssDNA, a pUC-derived ori for propagation in E. coli, and a kanamycin resistance gene for selection in bacteria.
**Figure 1.5.8** pRS303 is one of a series of shuttle vectors (also pRS305 and 306, not shown) created to facilitate manipulation of DNA in *S. cerevisiae*. The backbone is derived from pBluescript and contains features necessary for replication and maintenance in yeast. These plasmids contain an autonomously replicating sequence and a centromere sequence, CEN6, that ensures stable maintenance in yeast cells. They differ only in the selectable marker; pRS303 carries the HIS3 marker that complements a nonreverting his3 chromosomal mutation in specific yeast strains. Map of pRS303 adapted from Sikorski and Hieter (1989) with permission.
Figure 1.5.9  pcDNA3.1 is a selectable cloning and expression vector for use in mammalian cells. The features of this vector include a neomycin resistance gene driven by the SV40 early promoter (contained within the SV40 ori) and terminated by an SV40 polyadenylation signal for selection in mammalian cells. In addition, due to the inclusion of the SV40 ori, the vector can replicate as an episome in cells expressing the SV40 large T antigen. The polylinker cloning site is located downstream of strong cytomegalovirus enhancer-promoter sequences and upstream of the bovine growth hormone gene termination signals, allowing high-level expression of protein-coding sequences cloned into this vector. This vector also contains some of the more standard features of other plasmid vectors, including a ColE1 replicator for propagation in E. coli, the ampicillin resistance gene for selection in E. coli, the 11 ori for production of ssDNA, and the T7 promoter sequence for in vitro transcription of DNA inserted into the polylinker.
Figure 1.5.10  pRR54 is an example of a broad-host-range mobilizable plasmid vector. This vector contains replicator and stabilization sequences derived from the natural RK2 broad-host-range plasmid. oriV is the vegetative origin of replication, trfA encodes trans-acting functions necessary for replication, and par encodes a locus that enhances stability of the plasmid. This plasmid can be mated into diverse gram-negative species as long as the appropriate mobilization machinery, which contains the origin of conjugal transfer (oriT), is provided in trans. The plasmid carries the β-lactamase gene, allowing for ampicillin/carbenecillin selection. Map adapted from Roberts et al. (1990) with permission.
Figure 1.9.1  The $\lambda$ genome. Gray areas represent dispensable regions.

Figure 1.9.2  The $\lambda$ immunity region.
Figure 1.10.2  λZAP (Stratagene) carries pBluescript SK(−), which is excised in vivo upon infection with f1 or M13 helper phages. Inserts are cloned within a polylinker located in the lacZ gene within the Bluescript sequences. As with λgt11, a fusion protein may be expressed if the insert DNA is in frame with the lacZ sequence, so that libraries made in this vector can be screened with antibodies. In λZAP, T7 and T3 promoters flank the inserts, which allows RNA probes to be easily obtained. pBluescript M13(−), the excised plasmid (shown on the right), is normally propagated as a circular dsDNA, but infection with a helper phage enables the plasmid to be propagated as ssDNA. These properties facilitate sequencing of the insert, site-directed mutagenesis, and the construction of unidirectional deletions. DNA fragments up to 10 kb can be inserted. Unique XhoI, EcoRI, SphI, XbaI, NotI, and SstI cloning sites are available. λZAP/R is shown in the figure. λZAP/L has an inverted polylinker.
**Figure 1.14.1** M13mp18 is one of the M13mp vectors made by Messing and colleagues. Insertion of DNA into the polylinker inactivates the lacZ alpha fragment. Insert-containing phages form colorless plaques; vectors that do not contain inserts form blue plaques.