### Cloning Strain Properties

There are many properties to consider when choosing a strain for your cloning experiments. Requirements such as high-quality plasmid preparations, blue/white screening and fast colony growth necessitate specific strain choices. The following selection chart highlights the characteristics of NEB's cloning strains to help select the optimal strain for a particular experiment.

#### Cloning Strain Properties

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>TRANSFORMATION EFFICIENCY</th>
<th>AVAILABLE FORMATS</th>
<th>STRAIN BACKGROUND</th>
<th>LIBRARY CONSTRUCTION</th>
<th>BLUE/WHITE SCREENING</th>
<th>TRANSFORMATION EFFICIENCY</th>
<th>METHYLATION PHENOTYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEB 5-alpha</td>
<td>1-3 x 10^9</td>
<td>n/a</td>
<td>K12</td>
<td>–</td>
<td>–</td>
<td>&gt; 1 x 10^9</td>
<td>Dam –/–</td>
</tr>
<tr>
<td>NEB Turbo</td>
<td>1-3 x 10^9</td>
<td>50, 200</td>
<td>K12</td>
<td>–</td>
<td>–</td>
<td>&gt; 2 x 10^10</td>
<td>Dam +/–</td>
</tr>
<tr>
<td>NEB Stable</td>
<td>1-3 x 10^9</td>
<td>50, 200, 96, 384, Strips</td>
<td>K12</td>
<td>–</td>
<td>–</td>
<td>&gt; 1 x 10^9</td>
<td>Dam –/–</td>
</tr>
<tr>
<td>NEB 5-alpha F’</td>
<td>1-3 x 10^9</td>
<td>50, 200</td>
<td>K12</td>
<td>–</td>
<td>–</td>
<td>&gt; 1 x 10^9</td>
<td>Dam –/–</td>
</tr>
<tr>
<td>NEB Turbo</td>
<td>1-3 x 10^9</td>
<td>50, 200</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>&gt; 1 x 10^9</td>
<td>Dam –/–</td>
</tr>
</tbody>
</table>

- **Important for high-quality plasmid preparation.**
- **Subcloning**
- **Fast growth (< 8 hours)**
- **T1 phage resistance (fhuA2)**
- **Free of animal products**
- **Strains for cloning toxic genes**
- **Bulk sales available for custom packaging**
- **Compatible with NEBuilder® HiFi DNA Assembly and Gibson Assembly® reactions, as well as ligation reactions**
- **Strains for cloning toxic genes**
- **Free of animal products**
- **T1 phage resistance (fhuA2)**
- **Media and control plasmid included**
- **A variety of convenient formats**
- **Bulk sales available for custom packaging**

### Cloning Strain Features

NEB’s growing line of competent cells includes several popular strains for cloning. Our cloning strains include derivatives of the industry standards, DH5α® and DH10B®. NEB Turbo is unique to NEB and allows colony growth after 6.5 hours. NEB’s dam/dcm strain enables isolation of plasmids free of Dam and Dcm methylation. NEB Stable is recommended in all difficult cloning experiments. Our cells are all extensively tested for phage resistance, antibiotic resistance and sensitivity, blue/white screening and transformation efficiency. High efficiency, 5 minute transformation and electroporation protocols are provided, when applicable.

#### Featured Online Tools

The Tools & Resources tab, accessible on our homepage, contains a selection of interactive technical tools. These tools can also be accessed directly in the footer of every web page.

- **NEBcloner®**
  - Use this tool to select another company's competent cell product and find out which NEB strain is compatible. Choose either the product name or catalog number from the available selection, and this tool will identify the recommended NEB product, highlight its advantages, and provide a link for ordering the product.

- **Competitor Cross-Reference Tool**
  - Use this tool to find the right products and protocols for each step (digestion, end modification, ligation and transformation) of your next traditional cloning experiment. Also, find other relevant tools and resources to enable protocol optimization.

- **NEBioCalculator**
  - NEBioCalculator is a collection of calculators and converters that are useful in planning bench experiments in molecular biology laboratories.
Protein Expression Strain Properties

There are many properties to consider when choosing a strain for your protein expression experiments. Requirements such as cytoplasmic disulfide bond formation and regulated protein expression necessitate specific strain choices. The following selection chart highlights the characteristics of NEB’s protein expression strains to help select the optimal strain for a particular experiment.

**Protein Expression Strain Features**

NEB offers a wide variety of competent cell strains ideal for many protein expression applications. These strains address the needs of protein expression control, toxic protein expression, cytoplasmic disulfide bond formation and difficult targets. NEB Express, T7 Express and SHuffle strains are available with varying levels of expression control. Only NEB offers the exceptional control of expression from the *lacI* gene that reduces basal expression from T7 strains without inhibiting IPTG-induced expression. Lemo21(DE3) features tunable T7 expression for difficult targets such as membrane proteins and proteins prone to insoluble expression. Our NiCo21(DE3) strain is designed for the expression and purification of His-tagged proteins. Each strain is provided with a detailed protocol for optimal expression.

**Protein Expression Strain Properties**

- **NEB Express**: 0.6-1 x 10⁷ 50 – K12
- **BL21(DE3)**: 1 x 10⁷ 50 – B
- **T7 Expression**: 0.6-1 x 10⁷ 200 – B
- **T7 Express lysF**: 0.6-1 x 10⁷ 200 – B
- **SHuffle T7**: 1 x 10⁷ 50 – K12
- **SHuffle Expression**: 1 x 10⁷ 50 – B
- **SHuffle T7**: 1 x 10⁷ 50 – B
- **SHuffle T7 Express lysF**: 1 x 10⁷ 50 – B
- **NEB Express +**: 0.6-1 x 10⁷ 200 – B

**Strain Properties**

- **CYTOPLASMIC DISULFIDE BOND FORMATION**
  - **cyto**
  - **disul**

- **POLYMERASE**
  - **T7 RNA**

- **TRANSMISSION**
  - **EF**
  - **EF**

- **OUTGROWTH MEDIUM & CONTROL**
  - **B**
  - **B**

- **RESISTANCE**
  - **Dam**
  - **M. EcoKI**

- **METHYLATION PHENOTYPE**
  - **Dcm**
  - **Dcm**

- **CONSTRUCTION**
  - **lysY**
  - **I q**

- **TOOL**
  - **Competitor Cross Reference Tool**
  - **NEBcloner®**

- **AVANTAGES**
  - **Deficient in proteins Lmr and DmpT**
  - **Free of animal products**
  - **T1 plaque resistance (fhuA8)**
  - **Media and control plasmid included with some strains**
  - **A variety of convenient formats**
  - **Bulk sales available for custom packaging**
Difficult to Express Proteins
Lemo21(DE3) is a notable T7 expression strain designed for the expression of challenging proteins. A derivative of BL21(DE3), Lemo21(DE3) offers the host features of this popular expression strain, with the added benefit of being able to control expression levels by varying the level of T7 lysozyme (lysY), the natural inhibitor of T7 RNA Polymerase. The fine control of expression makes Lemo21(DE3) ideal for membrane proteins, toxic proteins, secreted proteins and proteins prone to insoluble expression.

Enhancing Transformation Efficiency
Transformation efficiency is defined as the number of colony forming units (cfu) that would be produced by transforming 1 µg of plasmid into a given volume of competent cells. However, 1 µg of plasmid is rarely transformed. Instead, efficiency is routinely calculated by being produced by transforming 1 µg of plasmid into a given volume of competent cells. Efficiency calculations can be used to compare cells or ligations. Our recommended protocols and tips are presented here to help you achieve maximum results.

Transformation Tips
Thawing
- Cells are best thawed on ice
- DNA should be added as soon as the last trace of ice in the tube disappears
- Cells can be thawed by hand, but warming above 0°C decreases efficiency

Incubation of DNA with Cells on Ice
- Incubate on ice for 30 minutes
- Expect a 2-fold loss in TE for every 10 minutes this step is shortened

Heat Shock
- Both temperature and time are specific to the transformation volume and vesel. Typically, 30 seconds at 42°C is recommended

Outgrowth
- Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in TE for every 15 minutes this step is shortened
- Use NEB 10-beta/Stable Outgrowth Medium with NEB 10-beta and NEB Stable Competent E. coli. Use SOC for all other strains
- Outgrowth Medium gives 2-fold higher TE than LB medium
- Incubation with shaking or rotation results in 2-fold higher TE

Plating
- Selection plates can be used warm or cold, wet or dry with no significant effects on TE
- Warm, dry plates are easier to spread and allow for the most rapid colony formation

DNA
- DNA should be purified and resuspended in water or TE Buffer
- Up to 10 µl of DNA from a ligation mix can be used with only a 2-fold efficiency loss
- Purification by either a spin column or phenol/chloroform extraction and ethanol precipitation is ideal

- The optimal amount of DNA is lower than commonly recognized. Using clean, supercoiled pUC19, the efficiency of transformation is highest in the 100 pg–1 ng range. However, the total yield of disulfide bonded proteins when compared to periplasmic expression. SHuffle yields of disulfide bonded proteins when compared to periplasmic expression. SHuffle yields of disulfide bonded proteins when compared to periplasmic expression. SHuffle yields of disulfide bonded proteins when compared to periplasmic expression. SHuffle yields of disulfide bonded proteins when compared to periplasmic expression.

- Disulfide Bonds
- SELENIUM and REFRIGERATION greatly enhance the fidelity of disulfide bond formation. Cytoplasmic expression also results in significantly higher protein yields of disulfide bonded proteins when compared to periplasmic expression. SHuffle strains are sensitive to kan, amp, tet and in most cases, cam which makes them able to express proteins from a wide variety of expression vectors offering greater versatility in experimental design.

Proteins with Multiple Disulfide Bonds
SELENIUM and REFRIGERATION greatly enhance the fidelity of disulfide bond formation. Cytoplasmic expression also results in significantly higher protein yields of disulfide bonded proteins when compared to periplasmic expression. SHuffle strains are sensitive to kan, amp, tet and in most cases, cam which makes them able to express proteins from a wide variety of expression vectors offering greater versatility in experimental design.
## Ordering Information

### Cloning Strains

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>NEB #</th>
<th>SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEB Turbo Competent E. coli</td>
<td>C2984H/I</td>
<td>20 x 0.05 ml / 6 x 0.2 ml</td>
</tr>
<tr>
<td>NEB 10-beta Competent E. coli (High Efficiency)</td>
<td>C3019H/I/P</td>
<td>20 x 0.05 ml / 6 x 0.2 ml / 1 x 96 well plate</td>
</tr>
<tr>
<td>NEB 10-beta Electrocompetent E. coli</td>
<td>C3020K</td>
<td>6 x 0.1 ml</td>
</tr>
<tr>
<td>NEB 5-alpha Competent E. coli (High Efficiency)</td>
<td>C2987H/I/P/R/U</td>
<td>20 x 0.05 ml / 6 x 0.2 ml / 1 x 96 well plate / 1 x 384 well plate / 96 x 50 µl/tube</td>
</tr>
<tr>
<td>NEB 5-alpha Competent E. coli (Subcloning Efficiency)</td>
<td>C2988J</td>
<td>6 x 0.4 ml</td>
</tr>
<tr>
<td>NEB 5-alpha F′/ICOMPETENT E. coli (High Efficiency)</td>
<td>C2992H/I</td>
<td>20 x 0.05 ml / 6 x 0.2 ml</td>
</tr>
<tr>
<td>dam/dcm Competent E. coli</td>
<td>C29925H/I</td>
<td>20 x 0.05 ml / 6 x 0.2 ml</td>
</tr>
<tr>
<td>NEB Stable Competent E. coli (High Efficiency)</td>
<td>C3040H/I</td>
<td>20 x 0.05 ml / 6 x 0.2 ml</td>
</tr>
<tr>
<td>NEB Cloning Competent E. coli Sampler</td>
<td>C1010S</td>
<td>8 x 0.05 ml (2 vials of 4 strains)</td>
</tr>
<tr>
<td>Component Sold Separately: SOC Outgrowth Medium</td>
<td>B9020S</td>
<td>4 x 25 ml</td>
</tr>
<tr>
<td>Component Sold Separately: NEB 10-beta/Stable Outgrowth Medium</td>
<td>B9035S</td>
<td>4 x 25 ml</td>
</tr>
</tbody>
</table>

**Note:** Store Competent Cells at –80°C. Once thawed, do not refreeze. Storage at –20°C will result in a significant decrease in transformation efficiency. Cells lose efficiency whenever they are warmed above –80°C, even if they do not thaw.

### Protein Expression Strains

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>NEB #</th>
<th>SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEB Express Competent E. coli (High Efficiency)</td>
<td>C2523H/I</td>
<td>20 x 0.05 ml / 6 x 0.2 ml</td>
</tr>
<tr>
<td>NEB Express F′/I COMPETENT E. coli (High Efficiency)</td>
<td>C3037I</td>
<td>6 x 0.2 ml</td>
</tr>
<tr>
<td>T7 Express Competent E. coli (High Efficiency)</td>
<td>C2566H/I</td>
<td>20 x 0.05 ml / 6 x 0.2 ml</td>
</tr>
<tr>
<td>T7 Express lysY COMPETENT E. coli (High Efficiency)</td>
<td>C3010I</td>
<td>6 x 0.2 ml</td>
</tr>
<tr>
<td>T7 Express lysY/I′ COMPETENT E. coli (High Efficiency)</td>
<td>C3013J</td>
<td>6 x 0.2 ml</td>
</tr>
<tr>
<td>SHuffle T7 Competent E. coli</td>
<td>C3026J</td>
<td>12 x 0.05 ml</td>
</tr>
<tr>
<td>SHuffle Express Competent E. coli</td>
<td>C3028J</td>
<td>12 x 0.05 ml</td>
</tr>
<tr>
<td>SHuffle T7 Express Competent E. coli</td>
<td>C3029J</td>
<td>12 x 0.05 ml</td>
</tr>
<tr>
<td>SHuffle T7 Express lysY COMPETENT E. coli</td>
<td>C3030J</td>
<td>12 x 0.05 ml</td>
</tr>
<tr>
<td>BL21 Competent E. coli</td>
<td>C2530H</td>
<td>20 x 0.05 ml</td>
</tr>
<tr>
<td>BL21(DE3) Competent E. coli</td>
<td>C2527H/I</td>
<td>20 x 0.05 ml / 6 x 0.2 ml</td>
</tr>
<tr>
<td>Lemo21(DE3) Competent E. coli</td>
<td>C2528J</td>
<td>12 x 0.05 ml</td>
</tr>
<tr>
<td>NiCo21(DE3) Competent E. coli</td>
<td>C2529H</td>
<td>20 x 0.05 ml</td>
</tr>
<tr>
<td>Component Sold Separately: SOC Outgrowth Medium</td>
<td>B9020S</td>
<td>4 x 25 ml</td>
</tr>
</tbody>
</table>

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