Lambda EMBL3/BamHI Vector Kit

INSTRUCTION MANUAL
Catalog #241211 (Lambda EMBL3/BamHI Vector Kit) and
#241612 (Lambda EMBL3/BamHI Gigapack III Gold Cloning Kit)
Revision A

For In Vitro Use Only
241211-12
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# Lambda EMBL3/BamH I Vector Kit

## MATERIALS PROVIDED

<table>
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<tr>
<td><strong>Quantity</strong></td>
<td><strong>Catalog #241211</strong></td>
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<tr>
<td>Lambda EMBL3 vector double-digested with EcoR I and BamH I, CIAP-treated&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10 μg</td>
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<td>pME/BamH I test insert (−12 kb)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5 μg</td>
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<tr>
<td>XL1-Blue MRA</td>
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<td>XL1-Blue MRA (P2)</td>
<td>0.5-ml bacterial glycerol stock</td>
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<td>Gigapack III Gold-11 packaging extract&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>λcl857 Sam7 wild-type lambda control DNA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>VCS257 host strain&lt;sup&gt;g&lt;/sup&gt;</td>
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<sup>a</sup> Shipped as a liquid at 1 μg/μl in 5 mM Tris-HCl (pH 7.5) and 0.1 mM EDTA. On arrival, store the EMBL3 vector at −20°C. After thawing, aliquot and store at −20°C. Do not pass through more than two freeze–thaw cycles. For short-term storage, store at 4°C for 1 month.

<sup>b</sup> Shipped as a liquid at 0.25 μg/μl in 5 mM Tris-HCl (pH 7.5) and 0.1 mM EDTA. On arrival, store the pME/BamH I test insert at −20°C. After thawing, aliquot and store at −20°C. Do not pass through more than two freeze–thaw cycles. For short-term storage, store at 4°C for 1 month.

<sup>c</sup> For host strain shipping and storage conditions, please see *Preparing the Host Strains*.

<sup>d</sup> The XL1-Blue MRA and XL1-Blue MRA (P2) strains have been modified to enhance the stability of clones containing methylated DNA, as well as nonstandard DNA structures.

<sup>e</sup> Gigapack III packaging extract is very sensitive to slight variations in temperature. Storing the packaging extracts at the bottom of a −80°C freezer directly from the dry ice shipping container is required in order to prevent a loss of packaging efficiency. Transferring tubes from one freezer to another may also result in a loss of efficiency. **Do not allow the packaging extracts to thaw!** Do not store the packaging extracts in liquid nitrogen as the tubes may explode.

<sup>f</sup> The λcl857 Sam7 wild-type lambda control DNA is shipped frozen and should be stored at −80°C immediately on receipt.

<sup>g</sup> The VCS257 host strain, included for plating the λcl857 Sam7 positive control, is shipped as a frozen bacterial glycerol stock (see *Preparing the Host Strains* for additional storage instructions) and should also be stored at −80°C immediately on receipt. This control host strain is a derivative of DP50 supF and should be used only when plating the packaged test DNA. The control DNA used with Gigapack III Gold packaging extract requires a supF mutation in the bacterial host to plate efficiently.

## STORAGE CONDITIONS

- **Lambda EMBL3 Vector:** −20°C
- **Test Insert:** −20°C
- **Bacterial Glycerol Stocks:** −80°C
- **Packaging Extracts:** −80°C
INTRODUCTION

Overview of the Lambda EMBL3 Vector System

The Lambda EMBL3 vector is a genomic replacement lambda phage vector capable of accepting BamHI-compatible fragments (Sau3A I, Mbo I, Bgl II, or BamHI) ranging in size from 9 to 23 kb (Figure 1). The arms are prepared by double digestion with BamHI and EcoRI followed by a selective precipitation which removes the small BamHI/EcoRI linker that separates the arms from the stuffer fragment. Because this treatment leaves the arms with BamHI ends and the stuffer fragment with EcoRI ends, there is no need to physically separate them. Target DNA cloned into the BamHI sites of EMBL3 may be removed by digestion with SalI.

The Lambda EMBL3 system takes advantage of spi (sensitive to P2 inhibition) selection. Lambda phages containing active red and gam genes are unable to grow on host strains that contain P2 phage lysogens. Lambda phages without these genes are able to grow on strains lysogenic for P2 such as XL1-Blue MRA (P2), a P2 lysogen of XL1-Blue MRA. The red and gam genes in Lambda EMBL3 DNA are located on the stuffer fragment; therefore, wild-type Lambda EMBL3 phage cannot grow on XL1-Blue MRA (P2). When the stuffer fragment is replaced by an insert, the recombinant Lambda EMBL3 becomes red–/gam–, and the phage is able to grow on the P2 lysogenic strain. Therefore, by plating the library on the XL1-Blue MRA (P2) strain, only recombinant phages are allowed to grow. The strain XL1-Blue MRA is also provided as a control strain and later for growth of the recombinant when the selection is no longer necessary.

Lambda EMBL3 Vector Map

Figure 1 Map of the Lambda EMBL3 replacement vector.
## PREPARING THE HOST STRAINS

### Host Strain Genotypes

<table>
<thead>
<tr>
<th>Host strain</th>
<th>Genotype</th>
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<tr>
<td>XL1-Blue MRA strain</td>
<td>Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 gyrA96 relA1 lac</td>
</tr>
<tr>
<td>XL1-Blue MRA (P2) strain</td>
<td>XL1-Blue MRA (P2 lysogen)</td>
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### Growing and Maintaining the Host Strains

The bacterial host strains are shipped as bacterial glycerol stocks. For the appropriate media, please refer to the following table:

<table>
<thead>
<tr>
<th>Host strain</th>
<th>Agar plates for bacterial streak§</th>
<th>Medium for bacterial glycerol stock§</th>
<th>Medium for bacterial cultures for titering phage (final concentration)</th>
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<td>XL1-Blue MRA strain</td>
<td>LB</td>
<td>LB</td>
<td>LB with 0.2% (w/v) maltose–10 mM MgSO₄</td>
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<tr>
<td>XL1-Blue MRA (P2) strain</td>
<td>LB</td>
<td>LB</td>
<td>LB with 0.2% (w/v) maltose–10 mM MgSO₄</td>
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<tr>
<td>VCS257 strainb</td>
<td>LB</td>
<td>LB</td>
<td>LB with 0.2% (w/v) maltose–10 mM MgSO₄</td>
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</tbody>
</table>

* The XL1-Blue MRA and XL1-Blue MRA (P2) host strains are modified to enhance the stability of clones containing methylated DNA; in addition, these strains enhance the stability of nonstandard DNA structures.

b For use with Gigapack III packaging extract and wild-type control only. Supplied with Gigapack III packaging extract.

On arrival, prepare the following from the bacterial glycerol stock using the appropriate media as indicated in the previous table:

**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 an LB agar plate (see Preparation of Media and Reagents).
3. Incubate the plate overnight at 37°C.
4. Seal the plate with Parafilm® laboratory film and store the plate at 4°C for up to 1 week.
5. Restreak the cells onto a fresh plate every week.

*See Preparation of Media and Reagents.*
Preparing a –80°C Bacterial Glycerol Stock

1. In a sterile 50-ml conical tube, inoculate 10 ml of appropriate liquid medium with one colony from the plate. Grow the cells to late log phase.

2. Add 4.5 ml of a sterile glycerol–liquid medium solution (5 ml of glycerol + 5 ml of appropriate medium) to the bacterial culture from step 1. Mix well.

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.
LIGATING THE INSERT

**Note** *In all ligations, the final glycerol content should be less than 5% (v/v). Do not exceed 5% (v/v) glycerol. Due to the high molecular weight of the lambda vector, the contents may be very viscous. It is important to microcentrifuge the contents of the lambda vector tube briefly at 11,000 × g and then to mix the solution gently by stirring with a yellow pipet tip prior to pipetting.*

Prepare a ligation reaction mixture containing the following components:
- 1.0 μl of predigested Lambda EMBL3/BamH I (1 μg)
- 1.2 μl of pME/BamH I insert (0.3 μg)
- 0.5 μl of 10× ligase buffer§
- 0.5 μl of 10 mM rATP (pH 7.5)
- 2 U of T4 DNA ligase
- Water up to a final volume of 5 μl

Incubate the ligation at 4°C overnight.

When ligating your own insert, you may use up to 2.5 μl in volume. Use an equal molar ratio of your BamH I-compatible insert DNA (*Sau*3A I, *Mbo* I, *Bgl* II, or *Bam*H I) with the Lambda EMBL3 arms. The Lambda EMBL3 vector can accommodate inserts ranging from 9 to 23 kb. If ligating a 20,000-bp insert to the arms, use 0.4 μg of insert for every 1 μg of arms. If the insert used is free from contaminants and contains a high percentage of ligatable ends, expect about $1 \times 10^6$–$1.5 \times 10^7$ recombinant plaques when using high-efficiency packaging extract, such as Gigapack III Plus or Gigapack III Gold packaging extracts.*

**Note** *The Lambda EMBL3 vector arms provided have been pre-treated with calf intestine alkaline phosphatase (CIAP). Do not CIAP-treat the insert DNA. We recommend size fractionation of the insert DNA to minimize cloning of multiple inserts.*

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§See *Preparation of Media and Reagents.*

*Gigapack III Gold packaging extract [Catalog #200201 (Gold-4), #200202 (Gold-7), and #200203 (Gold-11)]. Gigapack III Plus packaging extract [Catalog #200204 (Plus-4), #200205 (Plus-7), and #200206 (Plus-11)].
**Packaging**

**General Information**

Packaging extracts are used to package recombinant lambda phage with high efficiency. The single-tube format of Gigapack III packaging extract simplifies the packaging procedure and increases the efficiency and representation of libraries constructed from highly methylated DNA. Each packaging extract is restriction minus (HsdR⁻ McrA⁺ McrBC− McrF⁻ Mrr⁻) to optimize packaging efficiency and library representation. When used in conjunction with restriction-deficient plating cultures, Gigapack III packaging extract improves the quality of DNA libraries constructed from methylated DNA.²⁻⁵

Optimal packaging efficiencies are obtained with lambda DNAs that are concatemeric. Ligations should be carried out at DNA concentrations of 0.2 µg/µl or greater, which favors concatemers and not circular DNA molecules that only contain one cos site. DNA to be packaged should be relatively free from contaminants. Polyethylene glycol (PEG), which is contained in some ligase buffers, can inhibit packaging. The volume of DNA added to each extract should be between 1 and 4 µl. To obtain the highest packaging efficiency [i.e., the number of plaque-forming units per microgram (pfu/µg) of DNA], package 1 µl of the ligation reaction and never more than 4 µl. Increased volume (i.e., >4 µl) yields more plaque-forming units per packaging reaction, but fewer plaque-forming units per microgram of DNA.

DNA that is digested with restriction enzymes and religated packages less efficiently (by a factor of 10–100) than uncut lambda DNA. For example, uncut wild-type lambda DNA packages with efficiencies exceeding 1 × 10⁹ pfu/µg of vector when using a Gigapack III packaging extract. However, predigested vector, when ligated to a test insert, yields ~5 × 10⁶ – 1 × 10⁷ recombinant plaques/µg of vector.

**Gigapack III XL Packaging Extract**

Gigapack III XL packaging extract (Gigapack III XL packaging extract [Catalog #200207 (XL-4), #200208 (XL-7), and #200209 (XL-11)]) is an in vitro packaging extract, which preferentially size selects for extra large inserts, while maintaining the highest packaging efficiencies commercially available. This extract is specifically designed for use in generating genomic libraries. For example, a 20-kb insert will be packaged with a 95% higher efficiency than a 14-kb insert when using replacement vectors such as the Lambda EMBL3 vector.
Packaging Instructions

For optimal packaging efficiency, package 1 μl of the ligation and never more than 4 μl. For further selection of large inserts, we recommend using Gigapack III XL packaging extract, a size-selective packaging extract.

Preparing the Host Bacteria

Note Prepare an overnight culture of the VCS257 strain (see the table in Preparing the Host Strains) prior to performing the protocol for the positive wild-type lambda DNA control (see Positive Wild-Type Lambda DNA Control for the Gigapack III Packaging Extract).

1. Streak the bacterial glycerol stock onto the appropriate agar plates (see the table in Preparing the Host Strains). Incubate the plates overnight at 37°C.

2. Inoculate an appropriate medium, supplemented with 10 mM MgSO₄ and 0.2% (w/v) maltose, with a single colony.


Note The lower temperature keeps the bacteria from overgrowing, thus reducing the number of nonviable cells. Phage can adhere to nonviable cells resulting in a decreased titer.

4. Pellet the bacteria at 500 × g for 10 minutes.

5. Gently resuspend the cells in half the original volume with sterile 10 mM MgSO₄.

6. Dilute the cells to an OD₆₀₀ of 0.5 with sterile 10 mM MgSO₄.

Note The bacteria should be used immediately following dilution.

Packaging Protocol

Note Polyethylene glycol, which is contained in some ligase buffers, can inhibit packaging.

1. Remove the appropriate number of packaging extracts from a –80°C freezer and place the extracts on dry ice.

2. Quickly thaw the packaging extract by holding the tube between your fingers until the contents of the tube just begins to thaw.

3. Add the experimental DNA immediately (1–4 μl containing 0.1–1.0 μg of ligated DNA) to the packaging extract.
4. Stir the tube with a pipet tip to mix well. **Gentle** pipetting is allowable provided that air bubbles are not introduced.

5. Spin the tube quickly (for 3–5 seconds), if desired, to ensure that all contents are at the bottom of the tube.

6. Incubate the tube at room temperature (22°C) for 2 hours. **Do not exceed 2 hours.**

   **Note**  
   The highest efficiency occurs between 90 minutes and 2 hours. Efficiency may drop dramatically during extended packaging times.

7. Add 500 μl of SM buffer to the tube.

8. Add 20 μl of chloroform and mix the contents of the tube gently. Spin the tube briefly to sediment the debris.

9. The supernatant containing the phage is ready for titering. The supernatant may be stored at 4°C for up to 1 month.

**Testing the Efficiency of Gigapack III Packaging Extract with the Wild-Type Lambda Control DNA (Optional)**

Use the following procedure to test the efficiency of the Gigapack III packaging extract with the \( \lambda cI857 \) Sam7 wild-type lambda control DNA:

1. Thaw the frozen wild-type lambda control DNA on ice and gently mix after thawing.

2. Using 1 μl of the wild-type lambda control DNA (~0.2 μg), proceed with steps 1–10 in the **Packaging Protocol**.

   **Note**  
   Because of the high titer achieved with the wild-type lambda control DNA, Stop the control packaging reaction with 1 ml of SM buffer. This should make the plaques easier to count.

3. Prepare two consecutive 10\(^{-2}\) dilutions of the packaging reaction from step 10 in the **Packaging Protocol** in SM buffer. (The final dilution is 10\(^{-4}\)).

4. Add 10 μl of the 10\(^{-4}\) dilution to 200 μl of the VCS257 host strain. (This strain is recommended for plating the wild-type lambda control DNA only.) Incubate at 37°C for 15 minutes. Add 3 ml of NZY top agar\(^8\), melted and cooled to ~48°C, and quickly pour the dilution onto dry, prewarmed NZY agar plates.

5. Incubate the plates for at least 12 hours at 37°C. Count the plaques. Approximately 400 plaques should be obtained on the 10\(^{-4}\) dilution plate when the reaction is stopped with 1 ml of SM buffer.

\(^8\)See *Preparation of Media and Reagents*. 

Lambda EMBL3/BamH I Vector Kit
TITERING PROCEDURE

1. Streak the bacterial glycerol stock onto the appropriate agar plates (see the table in Preparing the Host Strains). Incubate the plates overnight at 37°C.

2. Inoculate an appropriate medium, supplemented with 10 mM MgSO₄ and 0.2% (w/v) maltose, with a single colony.


   Note The lower temperature keeps the bacteria from overgrowing, thus reducing the number of nonviable cells. Phage can adhere to nonviable cells resulting in a decreased titer.

4. Pellet the bacteria at 500 × g for 10 minutes.

5. Gently resuspend the cells in half the original volume with sterile 10 mM MgSO₄.

6. Dilute the cells to an OD₆₀₀ of 0.5 with sterile 10 mM MgSO₄.

   Note The bacteria should be used immediately following dilution.

7. Prepare dilutions of the final packaged reaction in SM buffer. Add 1 μl of the final packaged reaction to 200 μl of host cells diluted in 10 mM MgSO₄ to an OD₆₀₀ of 0.5. If desired, also add 1 μl of a 1:10 dilution of the packaged reaction in SM buffer to 200 μl of host cells.

8. Incubate the phage and the bacteria at 37°C for 15 minutes to allow the phage to attach to the cells.

9. Add 3 ml of NZY top agar, melted and cooled to ~48°C, and plate immediately on prewarmed NZY agar plates.
10. Count the plaques and determine the titer in plaque-forming units per milliliter (pfu/ml).

Both recombinant and nonrecombinant phage will grow on XL1-Blue MRA, but only recombinant phage will grow on XL1-Blue MRA (P2). Plaques should be visible after 8–12 hours of incubation at 37°C.

**Note** Historically, the host strain LE392 has been used with this vector; however, E. coli restriction systems in this strain have a significant negative effect on the efficiency of DNA cloning and the ability to generate libraries representative of the gene population. The strains provided, XL1-Blue MRA and XL1-Blue MRA (P2), are mcrA⁻, mcrB⁻ and mrr⁻; these modifications have been demonstrated to cause up to a 10-fold increase in the yield of recombinant phage containing methylated DNA. In addition, these strains have been further modified to enhance the stability of nonstandard DNA structures. Due to the removal of the red/gam genes during the preparation of the replacement vector phage with insert DNA is unable to plate on a recA⁻ host strain.

**AMPLIFYING THE LIBRARY**

It is usually desirable to amplify libraries prepared in lambda vectors to make a large, stable quantity of a high-titer stock of the library. However, more than one round of amplification is not recommended, since slower growing clones may be significantly underrepresented.

**Day 1**

1. Prepare the host strains as outlined in *Preparing the Host Strains*.

**Day 2**

2. Dilute the cells to an OD₆₀₀ of 0.5 in 10 mM MgSO₄. Use 600 μl of cells at an OD₆₀₀ of 0.5/150-mm plate.

3. Combine aliquots of the packaged mixture or library suspension containing ~5 × 10⁴ pfu of bacteriophage with 600 μl of host cells at an OD₆₀₀ of 0.5 in 14-ml BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059). To amplify 1 × 10⁶ plaques, use a total of 20 aliquots (each aliquot contains 5 × 10⁴ plaques/150-mm plate).

**Note** Do not add more than 300 μl of phage/600 μl of cells.

4. Incubate the tubes containing the phage and host cells for 15 minutes at 37°C.

5. Mix 6.5 ml of NZY top agar, melted and cooled to ~48°C, with each aliquot of infected bacteria and spread evenly onto a freshly poured 150-mm NZY bottom agar plate.
6. Incubate the plates at 37°C for 6–8 hours. Do not allow the plaques to get larger than 1–2 mm. On completion, the plaques should be touching.

7. Overlay the plates with ~8–10 ml of SM buffer. Store the plates at 4°C overnight (with gentle rocking if possible). This allows the phage to diffuse into the SM buffer.

**Day 3**

8. Recover the bacteriophage suspension from each plate and pool it into a sterile polypropylene container. Rinse the plates with an additional 2 ml of SM buffer and pool. Add chloroform to a 5% (v/v) final concentration. Mix well and incubate for 15 minutes at room temperature.

9. Remove the cell debris by centrifugation for 10 minutes at 500 × g.

10. Recover the supernatant and transfer it to a sterile polypropylene container. If the supernatant appears cloudy or has a high amount of cell debris, repeat steps 8 and 9. If the supernatant is clear, add chloroform to a 0.3% (v/v) final concentration and store at 4°C. Store aliquots of the amplified library in 7% (v/v) DMSO at −80°C.

11. Check the titer of the amplified library using host cells and serial dilutions of the library. (Assume ~10⁹–10¹¹ pfu/ml.)

**PERFORMING PLAQUE LiftS**

1. Titer the library to determine the concentration (prepare fresh host cells to use in titering and in screening).

2. Plate on large 150-mm NZY agar plates (≥2-day-old) to 50,000 pfu/plate with 600 μl of host cells at an OD₆₀₀ of 0.5/plate and 6.5 ml of NZY top agar/plate. (Use 20 plates to screen 1 × 10⁶.)

3. Incubate the plates at 37°C for ~8 hours.

4. Chill the plates for 2 hours at 4°C to prevent the top agar from sticking to the nitrocellulose membrane.

**Note** Use forceps and wear gloves for the following steps.

5. Transfer the plaques onto a nitrocellulose membrane (Stratagene Catalog #420106–#420108) for 2 minutes. Use a needle to prick through the agar for orientation. (If desired, waterproof ink in a syringe needle may be used.)

If making duplicate nitrocellulose membranes, allow the second membrane to transfer for ~4 minutes.
Note  
Pyrex® dishes are convenient for the following steps. All solutions should be at room temperature.

a. Denature the nitrocellulose membrane after lifting by submerging the membrane in a 1.5 M NaCl and 0.5 M NaOH denaturation solution for 2 minutes.

Note  
If using charged nylon, wash with gloved fingertips to remove the excess top agar.

b. Neutralize the nitrocellulose membrane for 5 minutes by submerging the membrane in a 1.5 M NaCl and 0.5 M Tris-HCl (pH 8.0) neutralization solution.

c. Rinse the nitrocellulose membrane for no more than 30 seconds by submerging the membrane in a 0.2 M Tris-HCl (pH 7.5) and 2× SSC buffer solution.§

6. Blot briefly on a Whatman® 3MM paper.

7. Crosslink the DNA to the membranes using the autocrosslink setting on the Stratalinker UV crosslinker* (120,000 μJ of UV energy) for ~30 seconds. Alternatively, oven bake at 80°C for ~1.5–2 hours.

8. Store the stock agar plates of the transfers at 4°C to use after screening.

HYBRIDIZING AND SCREENING

Following the preparation of the membranes for hybridization, perform prehybridization, probe preparation, hybridization, and washes for either oligonucleotide probes or double-stranded probes and then expose the membranes to film as outlined in standard methodology texts.6,7 Following these procedures, perform secondary and tertiary screenings also as outlined in the standard methodology texts. 6,7 After an isolate is obtained, refer to Sambrook et al.7 for suggested phage miniprep and maxiprep procedures.

§See Preparation of Media and Reagents.

* Stratagene Catalog #400071 (1800) and #400075 (2400).
## Troubleshooting

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<th>Suggestions</th>
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<tr>
<td>Packaging efficiency is too low</td>
<td>Ensure that the packaging extracts are properly stored. Gigapack III packaging extract is very sensitive to slight variations in temperature; therefore, store the packaging extracts at the bottom of a –80°C freezer and avoid transferring tubes from one freezer to another. <strong>Do not allow the packaging extracts to thaw</strong></td>
</tr>
<tr>
<td></td>
<td>Avoid the use of ligase buffers containing PEG, which can inhibit packaging</td>
</tr>
<tr>
<td></td>
<td>The DNA concentration in the packaging extract may be too low. Ligate at DNA concentrations of 0.2 μg/μl or greater and package between 1 and 4 μl of the ligation reaction</td>
</tr>
<tr>
<td></td>
<td>Packaging extract protein concentration may be too low. Never package &gt;4 μl of the ligation reaction to prevent dilution of the proteins contained within the packaging extract</td>
</tr>
<tr>
<td>During titering, neither a bacterial lawn nor plaques is observed on the plate</td>
<td>Chloroform, added after packaging to prevent bacterial contamination, may be present while titering. Be sure to spin down the chloroform completely prior to removing an aliquot of the viral stock for titering</td>
</tr>
</tbody>
</table>
# PREPARATION OF MEDIA AND REAGENTS

<table>
<thead>
<tr>
<th>LB Agar (per Liter)</th>
<th>LB Broth (per Liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 g of NaCl</td>
<td>10 g of NaCl</td>
</tr>
<tr>
<td>10 g of tryptone</td>
<td>10 g of tryptone</td>
</tr>
<tr>
<td>5 g of yeast extract</td>
<td>5 g of yeast extract</td>
</tr>
<tr>
<td>20 g of agar</td>
<td>Add deionized H₂O to a final volume of</td>
</tr>
<tr>
<td>Add deionized H₂O to a final volume of 1 liter</td>
<td>1 liter</td>
</tr>
<tr>
<td>Adjust pH to 7.0 with 5 N NaOH</td>
<td>Adjust to pH 7.0 with 5 N NaOH</td>
</tr>
<tr>
<td>Autoclave</td>
<td>Autoclave</td>
</tr>
<tr>
<td>Pour into petri dishes (~25 ml/100-mm plate)</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LB Broth (per Liter)</th>
<th>NZY Agar (per Liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 g of NaCl</td>
<td>5 g of NaCl</td>
</tr>
<tr>
<td>10 g of tryptone</td>
<td>2 g of MgSO₄ · 7H₂O</td>
</tr>
<tr>
<td>5 g of yeast extract</td>
<td>5 g of yeast extract</td>
</tr>
<tr>
<td>Add deionized H₂O to a final volume of 1 liter</td>
<td>10 g of NZ amine (casein hydrolysate)</td>
</tr>
<tr>
<td>Adjust the pH to 7.5 with NaOH</td>
<td>15 g of agar</td>
</tr>
<tr>
<td>Autoclave</td>
<td>Add deionized H₂O to a final volume of 1 liter</td>
</tr>
<tr>
<td></td>
<td>Adjust the pH to 7.5 with NaOH</td>
</tr>
<tr>
<td></td>
<td>Autoclave</td>
</tr>
<tr>
<td></td>
<td>Pour into petri dishes (~80 ml/150-mm plate)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NZY Broth (per Liter)</th>
<th>NZY Agar (per Liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 g of NaCl</td>
<td>5 g of NaCl</td>
</tr>
<tr>
<td>2 g of MgSO₄ · 7H₂O</td>
<td>2 g of MgSO₄ · 7H₂O</td>
</tr>
<tr>
<td>5 g of yeast extract</td>
<td>5 g of yeast extract</td>
</tr>
<tr>
<td>Add deionized H₂O to a final volume of 1 liter</td>
<td>10 g of NZ amine (casein hydrolysate)</td>
</tr>
<tr>
<td>Adjust the pH to 7.5 with NaOH</td>
<td>15 g of agar</td>
</tr>
<tr>
<td>Autoclave</td>
<td>Add deionized H₂O to a final volume of 1 liter</td>
</tr>
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<td></td>
<td>Adjust the pH to 7.5 with NaOH</td>
</tr>
<tr>
<td></td>
<td>Autoclave</td>
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<td></td>
<td>Pour into petri dishes (~80 ml/150-mm plate)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>10× Ligase Buffer</th>
<th>NZY Top Agar (per Liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 mM Tris-HCl (pH 7.5)</td>
<td>Prepare 1 liter of NZY broth</td>
</tr>
<tr>
<td>70 mM MgCl₂</td>
<td>Add 0.7% (w/v) agarose</td>
</tr>
<tr>
<td>10 mM dithiothreitol (DTT)</td>
<td>Autoclave</td>
</tr>
</tbody>
</table>

**Note**: rATP is added separately in the ligation reaction.

<table>
<thead>
<tr>
<th>SM Buffer (per Liter)</th>
<th>20× SSC Buffer (per Liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.8 g of NaCl</td>
<td>175.3 g of NaCl</td>
</tr>
<tr>
<td>2.0 g of MgSO₄ · 7H₂O</td>
<td>88.2 g of sodium citrate</td>
</tr>
<tr>
<td>50.0 ml of 1 M Tris-HCl (pH 7.5)</td>
<td>800.0 ml of deionized H₂O</td>
</tr>
<tr>
<td>5.0 ml of 2% (w/v) gelatin</td>
<td>Adjust to pH 7.0 with a few drops of 10 N NaOH</td>
</tr>
<tr>
<td>Add deionized H₂O to a final volume of 1 liter</td>
<td>Add deionized H₂O to a final volume of 1 liter</td>
</tr>
<tr>
<td>Autoclave</td>
<td>Autoclave</td>
</tr>
</tbody>
</table>

Lambda EMBL3/BamH I Vector Kit
REFERENCES


ENDNOTES

Parafilm® is a registered trademark of American Can Company.
Pyrex® is a registered trademark of Corning Glass Works.
Whatman® is a registered trademark of Whatman Ltd.

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at http://www.stratagene.com/MSDS/. Simply enter the catalog number to retrieve any associated MSDS’s in a print-ready format. MSDS documents are not included with product shipments.