

Lambda DASH II/*Eco*R I Vector Kit

Instruction Manual

Catalog #247212 (*Eco*R I-Treated)
#247614 (*Eco*R I/Gigapack III Gold Packaging Extract)
#247714 (*Eco*R I/Gigapack III XL Packaging Extract)

Revision C.0

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Lambda DASH II/*Eco*R I Vector Kit

CONTENTS

Materials Provided **1**

Storage Conditions **1**

Introduction **2**

Lambda DASH II Vector Map **3**

Preparing the Host Strains **4**

Host Strain Genotypes 4

Growing and Maintaining the Host Strains 4

Preparing a –80°C Bacterial Glycerol Stock 4

Ligating the Insert **5**

Packaging **6**

General Information 6

Gigapack III Packaging Extract 6

Packaging Instructions 7

Titering the Packaging Reaction **9**

Amplifying the Library **10**

Day 1 10

Day 2 10

Day 3 10

Performing Plaque Lifts **11**

Hybridizing and Screening **12**

Rapid Restriction Mapping **12**

Troubleshooting **12**

Preparation of Media and Reagents **13**

References **14**

Endnotes **14**

MSDS Information **14**

Lambda DASH II/*Eco*R I Vector Kit

MATERIALS PROVIDED

Materials provided	Quantity		
	Catalog #247212	**Catalog #247614**	**Catalog #247714**
Lambda DASH II vector double digested with *Eco*R I and *Xho* I, CIAP treated[a]	10 µg	10 µg	10 µg
pDT-RI/*Eco*R I test insert (11.1 kb)[b]	2 µg	2 µg	2 µg
Host strains[c]			
XL1-Blue MRA strain	0.5-ml bacterial glycerol stock	0.5-ml bacterial glycerol stock	0.5-ml bacterial glycerol stock
XL1-Blue MRA (P2) strain	0.5-ml bacterial glycerol stock	0.5-ml bacterial glycerol stock	0.5-ml bacterial glycerol stock
Gigapack III Gold-11 packaging extract[d]	—	11 × 25 µl	—
Gigapack III XL-11 packaging extract[d]	—	—	11 × 25 µl
λcI857 *Sam*7 wild-type lambda control DNA[e]	—	1.05 µg	1.05 µg
VCS257 host strain[f]	—	1 ml	1 ml

[a] 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 Lambda DASH II 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.

[b] Shipped as a liquid at 0.4 µg/µl in 5 mM Tris-HCl (pH 7.5) and 0.1 mM EDTA. On arrival, store the pDT-RI/*Eco*R 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.

[c] For host strain shipping and storage conditions, please see *Preparing the Host Strains*.

[d] 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.

[e] The λcI857 *Sam*7 wild-type lambda control DNA is shipped frozen and should be stored at –80°C immediately on receipt.

[f] The VCS257 host strain, included for plating the λcI857 *Sam*7 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

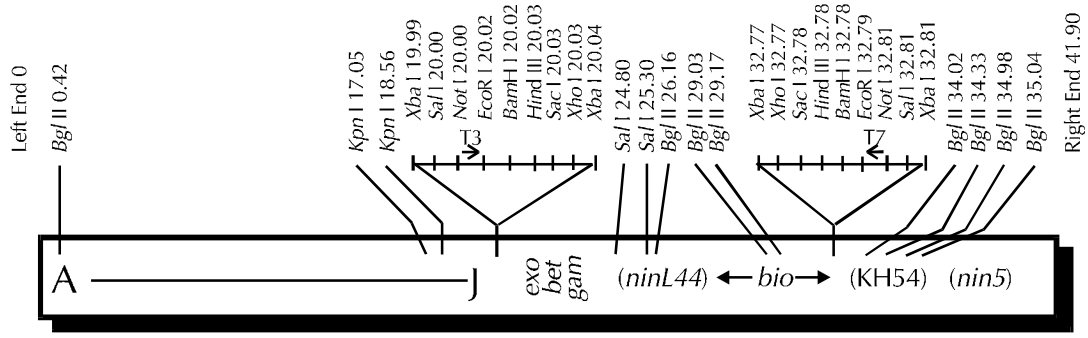
Lambda DASH II Vector: **–20°C**
Bacterial Glycerol Stocks: **–80°C**
Packaging Extracts: **–80°C**

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INTRODUCTION

Lambda DASH II is a replacement vector used for cloning large fragments of genomic DNA (see Figure 1). The Lambda DASH II 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 the Lambda DASH II DNA are located on the stuffer fragment; therefore, the wild-type Lambda DASH II phage cannot grow on XL1-Blue MRA (P2). When the stuffer fragment is replaced by an insert, the recombinant Lambda DASH II 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 XL1-Blue MRA strain is also provided as a control strain and later for growth of the recombinant when the selection is no longer necessary. Target DNA cloned into the *Eco*R I sites of the Lambda DASH II vector may be removed by digestion with *Not* I. The unique arrangement of the Lambda DASH II polylinker permits the isolation of the insert and flanking T3 and T7 bacteriophage promoters as an intact cassette by digestion with *Not* I. The T3 and T7 promoters flanking the insertion sites can be used to generate end-specific RNA probes for use in chromosomal walking and restriction mapping.

LAMBDA DASH II VECTOR MAP



Lambda DASH II Multiple Cloning Site Regions

[sequences shown: 19.99–20.04kb (top sequence) and 32.77–32.81kb (bottom sequence)]

Xba I Sal I Not I **T3 Promoter** EcoR I BamH I Hind III Sac I Xho I Xba I

```
TCTAGAGAGCTGTCGACGCGGCCGCGGAATTAACCCTCACTAAAGGGAACGAATTCGGATCCAAGCTTGAGCTCCTCGAGAAATTCTCTAGA
```

Xba I Xho I Sac I Hind III BamH I EcoR I **T7 Promoter** Not I Sal I Xba I

```
TCTAGAGAATTTCTCGAGGAGCTCAAGCTTGGATCCGAATTCTTCGCCCTATAGTGAGTCGTATTACGCGGCCGCGTCGACAGCTCTCTAGA
```

FIGURE 1 Linear map and multiple cloning site sequences for the Lambda DASH II replacement vector.

PREPARING THE HOST STRAINS

Host Strain Genotypes

Host strain	Genotype
XL1-Blue MRA strain	*Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 gyrA96 relA1 lac*
XL1-Blue MRA (P2) strain	XL1-Blue MRA (P2 lysogen)

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:

Host strain	Agar plates for bacterial streak	Medium for bacterial glycerol stock	Medium for bacterial cultures for titering phage (final concentration)
XL1-Blue MRA strain[a]	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
XL1-Blue MRA (P2) strain[a]	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
VCS257 strain[b]	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$

[a] 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. 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.

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 (prepared by mixing 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, do not exceed a 5% (v/v) glycerol content.*** *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, then gently mix the solution by stirring with a yellow pipet tip prior to pipetting.*

Prepare a ligation reaction mixture containing the following components:
1.0 µl of the Lambda DASH II vector predigested with *Eco*R I (1 µg)
1.0 µl of the pDT-RI/*Eco*R I insert (0.4 µg)
0.5 µl of 10× ligase buffer (see *Preparation of Media and Reagents*)
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 the insert, use a volume up to 2.5 µl. Use an equimolar ratio of the *Eco*R I-digested insert with the Lambda DASH II vector. The Lambda DASH II vector can accommodate inserts ranging from 9 to 23 kb. If ligating a 20,000-bp insert to the vector, use 0.4 µg of insert for every 1 µg of vector. If the insert used is free from contaminants and contains a high percentage of ligatable ends, expect about 1×10^6–1.5×10^7 recombinant plaques when using high-efficiency packaging extracts, such as Gigapack III Plus or Gigapack III Gold packaging extracts.

Note *The Lambda DASH II vector arms provided have been pre-treated with calf intestine alkaline phosphatase (CIAP). Do not CIAP-treat the insert DNA. Size fractionate the insert DNA to minimize cloning of multiple inserts.*

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.[1, 2, 3]

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 then 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 1×10^9 pfu/µg of vector when using a Gigapack III packaging extract. However, predigested vector, when ligated to a test insert, yields ~$5 \times 10^6 - 1 \times 10^7$ recombinant plaques/µg of vector.

Gigapack III Packaging Extract

Gigapack III XL packaging extract 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 DASH II 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, use 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* Testing the Efficiency of the Gigapack III Packaging Extract with the Wild-Type Lambda Control DNA*).*

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 $MgSO_4$ and 0.2% (w/v) maltose, with a single colony.

3. Grow at 37°C, shaking for 4–6 hours (do not grow past an OD_{600} of 1.0). Alternatively, grow overnight at 30°C, shaking at 200 rpm.

   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 and discard the supernatant.

5. *Gently* resuspend the cells in half the original volume with sterile 10 mM $MgSO_4$.

6. Dilute the cells to an OD_{600} of 0.5 with sterile 10 mM $MgSO_4$.

   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 reaction mixture 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.

9. Spin the tube briefly to sediment the debris.

10. 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 λ*c*I857 *Sam*7 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,[§] melted and cooled to ~48°C, and quickly pour the dilution onto dry, prewarmed NZY agar plates.

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

5. Incubate the plates for at least 12 hours at 37°C.

6. 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.

TITERING THE PACKAGING REACTION

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 $MgSO_4$ and 0.2% (w/v) maltose, with a single colony.

3. Grow at 37°C, shaking for 4–6 hours (do not grow past an OD_{600} of 1.0). Alternatively, grow overnight at 30°C, shaking at 200 rpm.

   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 $MgSO_4$.

6. Dilute the cells to an OD_{600} of 0.5 with sterile 10 mM $MgSO_4$.

   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 $MgSO_4$ to an OD_{600} 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 (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).

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 OD_{600} of 0.5 in 10 mM $MgSO_4$. Use 600 µl of cells at an OD_{600} of 0.5 for each 150-mm plate.

3. Combine aliquots of the packaged mixture or library suspension containing ~5×10^4 pfu of bacteriophage with 600 µl of host cells at an OD_{600} of 0.5 in 14-ml BD Falcon® polypropylene round-bottom tubes. To amplify 1×10^6 plaques, use a total of 20 aliquots (each aliquot contains 5×10^4 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 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 ~10^9–10^{11} 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 agar plates (≥2-day-old) to 50,000 pfu/plate with 600 µl of host cells at an OD_{600} of 0.5/plate and 6.5 ml of NZY top agar/plate. (Use 20 plates to screen 1×10^6.)

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 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-bound DNA 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 (see *Preparation of Media and Reagents*).

6. Blot the nitrocellulose membrane briefly on Whatman® 3MM paper.

7. Crosslink the DNA to the membrane 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.[4, 5] Following these procedures, perform secondary and tertiary screens also as outlined in the standard methodology texts.[4, 5] After an isolate is obtained, refer to Sambrook *et al.* for suggested phage miniprep and maxiprep procedures.

RAPID RESTRICTION MAPPING

The insertion sites of the Lambda DASH II vector are flanked by T3 and T7 promoters, which permit the generation of end-specific hybridization probes. End-specific probes can be made once a recombinant clone containing an insert is isolated. In addition, the Lambda DASH II vector has unique *Not* I sites flanking the RNA promoters, which permits the excision from the lambda vector of insert DNA plus the T3 and T7 promoter sequences as an intact fragment.

TROUBLESHOOTING

Observation	Suggestion(s)
Packaging efficiency is too low	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. **Do not allow the packaging extracts to thaw.**
	Avoid the use of ligase buffers containing PEG, which can inhibit packaging.
	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.
	Packaging extract protein concentration may be too low. Never package >4 µl of the ligation reaction to prevent dilution of the proteins contained within the packaging extract.
During titering, neither a bacterial lawn nor plaques is observed on the plate	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.

PREPARATION OF MEDIA AND REAGENTS

Note *All media must be autoclaved before use.*

LB Agar (per Liter)

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

LB Broth (per Liter)

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

NZY Broth (per Liter)

5 g of NaCl
2 g of $MgSO_4 \cdot 7H_2O$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
Add deionized H_2O to a final volume of 1 liter
Adjust the pH to 7.5 with NaOH
Autoclave

NZY Top Agar (per Liter)

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

NZY Agar (per Liter)

5 g of NaCl
2 g of $MgSO_4 \cdot 7H_2O$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
15 g of agar
Add deionized H_2O to a final volume of 1 liter
Adjust the pH to 7.5 with NaOH
Autoclave
Pour into petri dishes (~80 ml/150-mm plate)

SM Buffer (per Liter)

5.8 g of NaCl
2.0 g of $MgSO_4 \cdot 7H_2O$
50.0 ml of 1 M Tris-HCl (pH 7.5)
5.0 ml of 2% (w/v) gelatin
Add deionized H_2O to a final volume of 1 liter
Autoclave

20× SSC Buffer (per Liter)

175.3 g of NaCl
88.2 g of sodium citrate
800.0 ml of deionized H_2O
Adjust to pH 7.0 with a few drops of 10 N NaOH
Add deionized H_2O to a final volume of 1 liter

10× Ligase Buffer

500 mM Tris-HCl (pH 7.5)
70 mM $MgCl_2$
10 mM dithiothreitol (DTT)

Note *rATP is added separately in the ligation reaction*

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