



Lambda gt11/*EcoR* I/CIAP-Treated Vector Kit

Instruction Manual

**Catalog #234211 (Lambda gt11/*EcoR* I/CIAP-Treated Vector Kit) and
#234612 (Lambda gt11/*EcoR* I/CIAP-Treated/Gigapack III Cloning Kit)**

Revision B

Research Use Only. Not for Use in Diagnostic Procedures.

234211-12



Agilent Technologies

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Agilent. Agilent shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

Email

techservices@agilent.com

World Wide Web

www.genomics.agilent.com

Telephone

Location	Telephone
United States and Canada	800 227 9770
Austria	01 25125 6800
Benelux	02 404 92 22
Denmark	45 70 13 00 30
Finland	010 802 220
France	0810 446 446
Germany	0800 603 1000
Italy	800 012575
Netherlands	020 547 2600
Spain	901 11 68 90
Sweden	08 506 4 8960
Switzerland	0848 8035 60
UK/Ireland	0845 712 5292
All Other Countries	Please visit www.genomics.agilent.com and click Contact Us

Lambda gt11/EcoR I/CIAP-Treated Vector Kit

CONTENTS

Materials Provided	1
Storage Conditions	1
Introduction	2
Preparing the Host Strains	3
Host Strain Genotypes.....	3
Growing and Maintaining the Host Strains.....	3
Preparing a -80°C Bacterial Glycerol Stock.....	4
Color Selection by IPTG-X-gal.....	4
Ligating the Insert	5
Packaging	6
General Information.....	6
Packaging Instructions.....	6
Titering Procedure	9
Amplifying the Library	10
Day 1.....	10
Day 2.....	10
Day 3.....	11
Performing Plaque Lifts	11
Hybridizing and Screening	12
Antibody Screening Protocol	12
Troubleshooting	13
Preparation of Media and Reagents	14
References	15
Endnotes	15
MSDS Information	15

Lambda gt11/EcoR I/CIAP-Treated Vector Kit

MATERIALS PROVIDED

Material provided	Quantity	
	Catalog #234211	Catalog #234612
Lambda gt11 vector digested with EcoR I and dephosphorylated ^a	10 µg	10 µg
pRheo/EcoR I test insert (~2.8 kb) ^b	1.25 µg	1.25 µg
Host strains ^{c,d}		
Y1088	0.5-ml bacterial glycerol stock	0.5-ml bacterial glycerol stock
Y1089r-	0.5-ml bacterial glycerol stock	0.5-ml bacterial glycerol stock
Y1090r-	0.5-ml bacterial glycerol stock	0.5-ml bacterial glycerol stock
Gigapack III Gold-11 packaging extract ^e	—	11 × 25 µl
λcl857 Sam7 wild-type lambda control DNA ^f	—	1.05 µg
VCS257 host strain ^g	—	1 ml

^a Shipped as a liquid at 1 µg/µl in TE buffer (see *Preparation of Media and Reagents*). On arrival, store the gt11 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 0.25 µg/µl in TE buffer. On arrival, store the pRheo/EcoR 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 The Y1088 host strain gives a high lambda phage yield and is ideal for amplifying the gt11 library; Y1090r- produces stable fusion proteins and should be used for screening the library with antibody probes; and the Y1089r- host is recommended for inducing expression for large protein preparations.

^e 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.

^f The λcl857 Sam7 wild-type lambda control DNA is shipped frozen and should be stored at –80°C immediately on receipt.

^g 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 gt11 Vector: –20°C

Bacterial Glycerol Stocks: –20 or –80°C

INTRODUCTION

The Lambda gt11 expression vector (Figure 1) is used for the construction of cDNA libraries. It can accept inserts up to 7.2 kilobases in length at a unique *EcoR* I site near the C-terminal end of the coding region of the *lacZ* gene. The insertion site is 53 base pairs upstream from the β -galactosidase termination codon. Recombinant phage may be recognized with blue–white (clear) color identification when plated on *lac* hosts in the presence of IPTG (isopropyl- β -D-thio-galactopyranoside) and X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside). DNA sequences cloned into the *EcoR* I site of the vector may be expressed as fusion proteins under the control of the *lac* promoter. Libraries constructed in this vector can be screened with antibodies as well as nucleic acid probes.^{1,2}

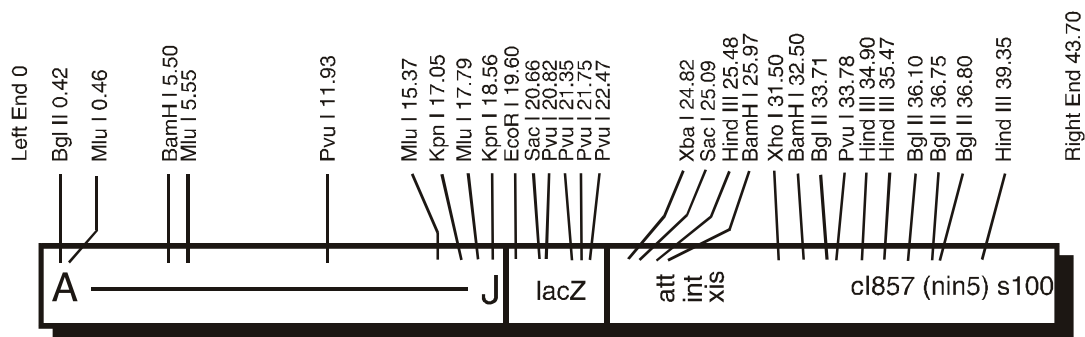


FIGURE 1 Map of the Lambda gt11 insertion vector.

PREPARING THE HOST STRAINS

Host Strain Genotypes

Host strain	Genotype
Y1088 strain	<i>e14-(McrA-) Δ(lac)U169 supE supF hsdR metB trpR tonA21 proC::Tn5 (Kanr) [pMC9 Amp^r Tetr]</i> (Note: pMC9 is pBR322 with <i>lacI^q</i> inserted.)
Y1089r- strain	<i>Δ(lac)U169 Δ(lon)[?] araD139 strA mcrB hflA150::Tn10 (Tet^r) [pMC9 Amp^r Tet^r]</i> (Note: pMC9 is pBR322 with <i>lacI^q</i> inserted.)
Y1090r- strain	<i>Δ(lac)U169 Δ(lon)[?] AraD139 strA supF mcrA mcrB hsdR trpC22::Tn10(Tetr) [pMC9 Amp^r Tetr]</i> (Note: pMC9 is pBR322 with <i>lacI^q</i> inserted.)

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)
Y1088 strain	LB-ampicillin ^a	LB-ampicillin ^a	LB with 0.2% (w/v) maltose–10 mM MgSO ₄
Y1089r- strain	LB-ampicillin ^a	LB-ampicillin ^a	LB with 0.2% (w/v) maltose–10 mM MgSO ₄
Y1090r- strain	LB-ampicillin ^a	LB-ampicillin ^a	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 50 μg/ml

^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.

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.

Color Selection by IPTG–X-gal

The color selection with the Lambda gt11 vector is a background test that can be completed by plating several hundred plaques on a plate. Add 15 µl of 0.5M IPTG (in water) and 25 µl of 250 mg/ml X-gal (in DMF) to 2–3 ml of NZY top agar[§] (48°C). Plate immediately on NZY agar plates.[§] Plaques are visible after incubation for 6–8 hours at 37°C. Background plaques are blue, while recombinant plaques will be white (clear).

[§]See *Preparation of Media and Reagents*

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.*

1.0 µl of the Lambda gt11 predigested with *EcoR* I (1 µg)
0.8 µl of pRheo/*EcoR* I test insert (0.2 µg)
0.5 µl of 10× ligase buffer (see *Preparation of Media 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 12–14°C overnight or at 4°C for 2 days.

When ligating the insert, use a volume up to 2.5 µl. Use an equal molar ratio of the *EcoR* I-compatible insert DNA with the Lambda gt11 vector. The Lambda gt11 vector can accommodate inserts ranging from 0 to 7.2 kb, and is ~43,700 base pairs in length. If ligating a 4,000-bp insert to the vector, use 0.1 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 the Gigapack III Plus or Gigapack III Gold packaging extracts (see the following table for ordering information).

Packaging Extract	Amount	Catalog #
Gigapack III Gold packaging extracts	4 vials	200201
	7 vials	200202
	11 vials	200203
Gigapack III Plus packaging extracts	4 vials	200204
	7 vials	200205
	11 vials	200206

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.^{3,4,5,6}

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 1×10^9 pfu/µg of vector when using a Gigapack III packaging extract. However, predigested vector, when ligated to a test insert, yield $\sim 5 \times 10^6 - 1 \times 10^7$ recombinant plaques/µg of 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 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.
3. Grow at 37°C, shaking for 4–6 hours (do not grow past an OD₆₀₀ 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 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 (see *Preparation of Media and Reagents*) 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 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 μ l of the wild-type lambda control DNA (~0.2 μ g), proceed with steps 1–10 in the *Packaging Protocol*.

Note *In order to compensate for 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 μ l of the 10^{-4} dilution to 200 μ l 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.
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.

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.
3. Grow at 37°C, shaking for 4–6 hours (do not grow past an OD₆₀₀ 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 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).

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.

Note *It is recommended to amplify the Lambda gt11 library in the Y1088 host strain. This strain is restriction negative but methylation positive. All recombinant phage should be passed through Y1088 before plating on a restriction positive host.*

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 $\sim 5 \times 10^4$ pfu of bacteriophage with 600 µl of host cells at an OD₆₀₀ of 0.5 in BD 14-ml polypropylene 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 $\sim 48^\circ\text{C}$, with each aliquot of infected bacteria and spread evenly onto a freshly poured 150-mm NZY 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 $500 \times g$.
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 $\sim 10^9$ – 10^{11} pfu/ml.)

PERFORMING PLAQUE LIFTS

1. Titer the library to determine the concentration (prepare fresh host cells to use in titring 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 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 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 (see *Preparation of Media and Reagents*).
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.^{7,8} Following these procedures, perform secondary and tertiary screenings also as outlined in the standard methodology texts.^{7,8} After an isolate is obtained, refer to Sambrook *et al.*⁸ for suggested phage miniprep and maxiprep procedures.

ANTIBODY SCREENING PROTOCOL

A complete manual for immunoscreening is supplied with the *picoBlue* immunoscreening kit. This kit is available with goat anti-rabbit antibodies (Catalog #200371) and goat anti-mouse antibodies (Catalog #200372).

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

TROUBLESHOOTING

Observations	Suggestion(s)
Packaging efficiency is too low	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 use of ligase buffers containing PEG, which can inhibit packaging
	To ensure DNA concentration is sufficient, ligate at DNA concentrations of $0.2\ \mu\text{g}/\mu\text{l}$ or greater and package between 1 and $4\ \mu\text{l}$ of the ligation reaction
	Never package $>4\ \mu\text{l}$ of the ligation reaction, which causes dilution of the proteins contained within the packaging extract
Neither a bacterial lawn nor plaques is observed on the plate	Be sure to spin down the chloroform completely prior to removing an aliquot for titering as chloroform can inhibit the reaction

PREPARATION OF MEDIA AND REAGENTS

Note All media must be autoclaved before use.

<p>LB Agar (per Liter) 10 g of NaCl 10 g of tryptone 5 g of yeast extract 20 g of agar Add deionized H₂O 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)</p>	<p>LB Broth (per Liter) 10 g of NaCl 10 g of tryptone 5 g of yeast extract Add deionized H₂O to a final volume of 1 liter Adjust to pH 7.0 with 5 N NaOH Autoclave</p>
<p>10× Ligase Buffer 500 mM Tris-HCl (pH 7.5) 70 mM MgCl₂ 10 mM dithiothreitol (DTT)</p> <p>Note <i>rATP is added separately in the ligation reaction</i></p>	<p>NZY Agar (per Liter) 5 g of NaCl 2 g of MgSO₄ · 7H₂O 5 g of yeast extract 10 g of NZ amine (casein hydrolysate) 15 g of agar Add deionized H₂O 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)</p>
<p>TE Buffer 10 mM Tris-HCl (pH 7.5) 1 mM EDTA</p>	
<p>NZY Broth (per Liter) 5 g of NaCl 2 g of MgSO₄ · 7H₂O 5 g of yeast extract 10 g of NZ amine (casein hydrolysate) Add deionized H₂O to a final volume of 1 liter Adjust the pH to 7.5 with NaOH Autoclave</p>	<p>NZY Top Agar (per Liter) Prepare 1 liter of NZY broth Add 0.7% (w/v) agarose Autoclave</p>
<p>SM Buffer (per Liter) 5.8 g of NaCl 2.0 g of MgSO₄ · 7H₂O 50.0 ml of 1 M Tris-HCl (pH 7.5) 5.0 ml of 2% (w/v) gelatin Add deionized H₂O to a final volume of 1 liter Autoclave</p>	<p>20× SSC Buffer (per Liter) 175.3 g of NaCl 88.2 g of sodium citrate 800.0 ml of deionized H₂O Adjust to pH 7.0 with a few drops of 10 N NaOH Add deionized H₂O to a final volume of 1 liter</p>

REFERENCES

1. Young, R. A. and Davis, R. W. (1983) *Proc Natl Acad Sci U S A* 80(5):1194-8.
2. Young, R. A. and Davis, R. W. (1983) *Science* 222(4625):778-82.
3. Kohler, S. W., Provost, G. S., Kretz, P. L., Dycaico, M. J., Sorge, J. A. *et al.* (1990) *Nucleic Acids Res* 18(10):3007-13.
4. Kretz, P. L., Kohler, S. W. and Short, J. M. (1991) *J Bacteriol* 173(15):4707-16.
5. Kretz, P. L., Reid, C. H., Greener, A. and Short, J. M. (1989) *Nucleic Acids Res* 17(13):5409.
6. Kretz, P. L. and Short, J. M. (1989) *Strategies in Molecular Biology* 2(2):25-26.
7. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G. *et al.* (1987). *Current Protocols in Molecular Biology*. John Wiley and Sons, New York.
8. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

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

Material Safety Data Sheets (MSDSs) are provided online at <http://www.genomics.agilent.com>. MSDS documents are not included with product shipments.