# Lambda ZAP-CMV RI Predigested Vector Kit

## Instruction Manual

**Catalog #239221**

Revision C.0

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239221-12

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# LAMBDA ZAP-CMV RI PREDIGESTED VECTOR KIT

## CONTENTS

# Lambda ZAP-CMV RI Predigested Vector Kit

## MATERIALS PROVIDED

| Materials provided | Quantity |
|---|---|
| Lambda ZAP-CMV RI vector digested with *Eco*R I and CIAP treated[a] | 10 µg |
| RHEO test insert digested with *Eco*R I | 1.25 µg |
| XL1-Blue MRF′ strain[b] | 0.5-ml bacterial glycerol stock |
| XLOLR strain[b] | 0.5-ml bacterial glycerol stock |
| ExAssist interference-resistant helper phage[c,d] | 1 ml |
| R408 Interference-Resistant Helper Phage[d,e] | 1 ml |

[a] On arrival, store the Lambda ZAP-CMV RI 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] Use the XLOLR strain for plating excised phagemids and the XL1-Blue MRF′ strain for all other manipulations. For host strain shipping and storage conditions, see *Bacterial Host Strains*.

[c] The titer of the ExAssist interference-resistant helper phage is ~$1.0 \times 10^{10}$ pfu/ml. This supercoiled single-stranded DNA migrates at ~5 kb on an agarose gel. ExAssist helper phage is recommended for excision of the pCMV-Script EX phagemid vector from the Lambda ZAP-CMV RI vector. It should not be used for single-stranded rescue.

[d] Retiter after 1 month. (Take care not to contaminate the Lambda ZAP-CMV RI vector with this high-titer filamentous helper phage.) Store at –80°C.

[e] The titer of the R408 interference-resistant helper phage is ~$7.5 \times 10^{10}$ pfu/ml. This supercoiled single-stranded DNA migrates at ~4 kb on an agarose gel. The R408 interference-resistant helper phage is recommended for single-stranded rescue (see *Appendix: Recovery of Single-Stranded DNA from Cells Containing the pCMV-Script EX Phagemid Vector*).

## STORAGE CONDITIONS

**Lambda ZAP-CMV RI Vector:** –20°C

**Test Insert:** –20°C

**Helper Phage:** –80°C

**Bacterial Glycerol Stocks:** –80°C

## ADDITIONAL MATERIALS REQUIRED

Gigapack III Plus or Gigapack III Gold packaging extract (Catalog #200204 and #200201, respectively)

## NOTICE TO PURCHASER

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Revision C.0

# INTRODUCTION

## Overview of the Lambda ZAP-CMV RI Vector System

The Lambda ZAP-CMV RI vector (predigested with *Eco*R I) allows eukaryotic expression.[1, 2] The five unique cloning sites of the Lambda ZAP-CMV RI vector, *Sac* I, *Not* I, *Srf* I, *Eco*R I, and *Xho* I, accommodate DNA inserts up to 6.5 kb in length (see Figure 1). Inserts cloned into the Lambda ZAP-CMV RI vector can be excised out of the phage in the form of the kanamycin-resistant pCMV-Script EX phagemid vector (see Figure 2) by the same excision mechanism used with the Lambda ZAP vectors.[1, 3, 4]

Clones in the Lambda ZAP-CMV RI vector can be screened with DNA probes and in vivo rapid excision of the pCMV-Script EX phagemid vector allows insert characterization in a plasmid system. Alternatively, the entire library can be mass excised for functional screening in mammalian cells. The polylinker of pCMV-Script EX phagemid has 15 unique cloning sites flanked by T3 and T7 promoters and has three primer sites for DNA sequencing. The plasmid has the bacteriophage f1 origin of replication allowing rescue of single-stranded DNA, which can be used for DNA sequencing or site-directed mutagenesis. Unidirectional deletions can be made using exonuclease III and mung bean nuclease by taking advantage of the unique positioning of 5´ and 3´ restriction sites. Transcripts made from the T3 and T7 promoters generate riboprobes useful in Southern and Northern blotting.

***Note*** *The pCMV-Script EX vector differs from the pCMV-Script vector by 29 bases located downstream of the f1 origin.*

Eukaryotic expression of inserts in pCMV-Script EX is driven by the cytomegalovirus (CMV) immediate early (IE) promoter with the SV40 transcription terminator and polyadenylation signal. Stable selection of clones in eukaryotic cells is made possible by the presence of the neomycin-and kanamycin-resistance gene, which is driven by the SV40 early promoter with TK transcription polyadenylation signals to render transfectants resistant to G418 (geneticin).

The pCMV-Script EX vector does not contain an ATG initiation codon. A translation initiation sequence must be incorporated if the DNA fragment to be cloned does not have an initiating ATG codon or an optimal sequence for initiating translation, such as the Kozak sequence [GCC(A/G)CCATGG].

## Lambda ZAP-CMV Vector Map

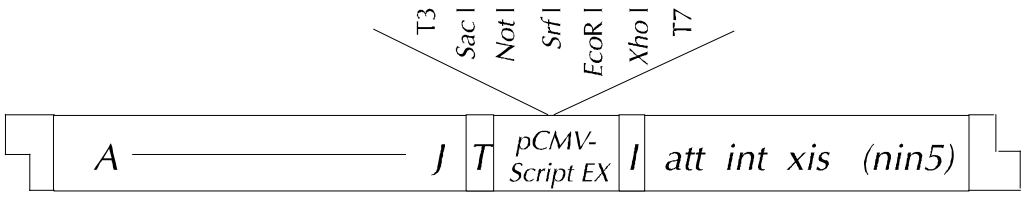
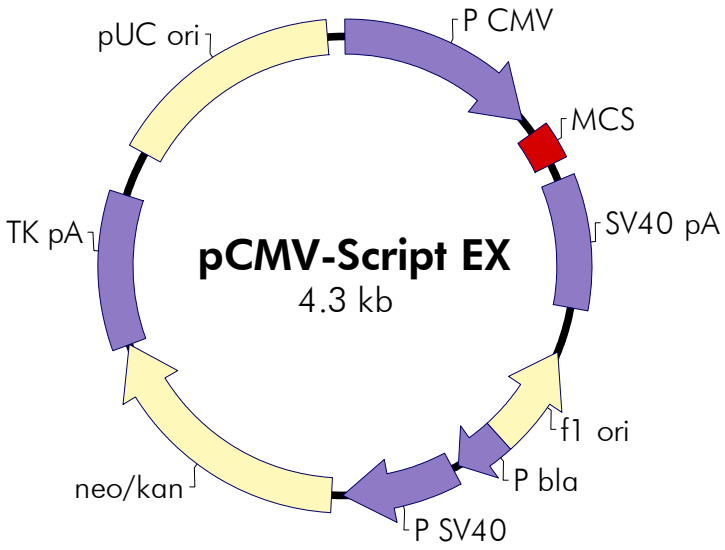



**FIGURE 1** Map of the Lambda ZAP-CMV vector.

## pCMV-Script EX Vector Map




## pCMV-Script EX Multiple Cloning Site Region
**(sequence shown 620–799)**

```
                                                Sac I    BstX I  Sac II          Not I
                T3 promoter                       |         |      |              |
AATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCGCTCTA...

     Srf I          BamH I              Pst I       EcoR I    EcoR V    Hind III                Acc I/Sal I
      |               |                  |           |         |          |                       |
...GCCCGGGCGGATCCCCCGGGCTGCAGGAATTCGATATCAAGCTTATCGATACCGTCGAC...

     Xho I          Apa I       Kpn I
      |              |           |                                                  T7 promoter
...CTCGAGGGGGGGCCCGGTACCAGGTAAGTGTACCCAATTCGCCCTATAGTGAGTCGTATTAC
```

| Feature | Nucleotide Position |
|---|---|
| CMV promoter | 1–602 |
| T3 promoter and T3 primer binding site | 620–639 |
| multiple cloning site | 651–758 |
| T7 promoter and T7 primer binding site | 778–799 |
| SV40 polyA signal | 811–1194 |
| f1 origin of ss-DNA replication | 1332–1638 |
| *bla* promoter | 1692–1816 |
| SV40 promoter | 1836–2174 |
| neomycin/kanamycin resistance ORF | 2209–3000 |
| HSV-thymidine kinase (TK) polyA signal | 3001–3450 |
| pUC origin | 3588–4255 |

**Figure 2** Circular map and polylinker sequence of the pCMV-Script EX vector.

# BACTERIAL HOST STRAINS

## Host Strain Genotypes

| Host strain | Genotype |
|---|---|
| XL1-Blue MRF′ strain | Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F′ *proAB lacI*$^{q}$*Z*Δ*M15* Tn*10* (Tet$^{r}$)] |
| XLOLR strain[a] | Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 thi-1 recA1 gyrA96 relA1 lac* [F′ *proAB lacI*$^{q}$*Z*Δ*M15* Tn*10* (Tet$^{r}$)] Su$^{-}$ (nonsuppressing) λ$^{r}$ (lambda resistant) |

[a] Use the XLOLR strain for excision only.

## XL1-Blue MRF′ Bacterial Strain Description

The RecA$^{-}$ *E. coli* host strain XL1-Blue MRF′ is supplied with the Lambda ZAP-CMV RI predigested vector kit.[2] Because the pCMV-Script EX phagemid vector does not require a *supF* genotype, the amplified library grows very efficiently on the XL1-Blue MRF′ strain. In addition, use of the correct host strain is important when working with the pCMV-Script EX phagemid vector due to the F′ episome present in the XL1-Blue MRF′ strain.

The F′ episome expresses the genes forming the F′ pili found on the surface of the bacteria. Without pili formation, filamentous phage (i.e., M13 or f1) infection could not occur. Because the conversion of a recombinant ZAP-CMV RI clone to a pCMV-Script EX phagemid vector requires superinfection with a filamentous helper phage, the F′ episome is required for in vivo excision (see *In Vivo Excision of the pCMV-Script EX Phagemid Vector from the Lambda ZAP-CMV RI Vector*).

***Note*** *The strains Y1088, Y1089, and Y1090 are not suitable for use with the Lambda ZAP-CMV RI vector as these strains contain the plasmid pMC9, a pBR322 derivative, which contains many of the same sequences as those found in the phagemid portion of the Lambda ZAP-CMV RI vector. Using these strains with the Lambda ZAP-CMV RI vector could result in recombination between the homologous sequences. The SURE and SOLR strains are not compatible with the Lambda ZAP-CMV RI system since these strains contain the kanamycin-resistance gene found in the pCMV-Script EX phagemid vector.*

## Recommended Media

| Host strain | Agar plates and liquid medium for bacterial streak and glycerol stock | Liquid medium for bacterial cultures prior to phage attachment | Agar plates and top agar for plaque formation | Agar plates for excision protocol |
|---|---|---|---|---|
| XLOLR strain | LB-tetracycline[a] | LB broth with supplements[a-c] | — | LB-kanamycin[a] |
| XL1-Blue MRF′ strain | LB-tetracycline[a] | LB broth with supplements[a-c] | NZY[a] | — |

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

[b] LB broth with 0.2% (w/v) maltose and 10 mM $MgSO_4$.

[c] Maltose and magnesium supplements are required for optimal lambda phage receptor expression on the surface of the XL1-Blue MRF′ host cell. The media supplements are not required for helper phage infection, but are included in both protocols for simplified media preparation.

## Establishing an Agar Plate Bacterial Stock

The bacterial host strains are shipped as bacterial glycerol stocks. On arrival, prepare the following plates from the bacterial glycerol stocks.

***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 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 containing the appropriate antibiotic (see *Recommended Media*), if one is necessary.

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 LB broth with the appropriate antibiotic (see *Recommended Media*) 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 the 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.

### Growth of Cells for Plating Phage

Bacterial cultures for plating phage should be started from a fresh plate using a single colony and should be grown overnight with vigorous shaking at 30°C in 50 ml of LB broth supplemented with 0.2% (w/v) maltose and 10 mM $MgSO_4$. (Do not use tetracycline in the presence of magnesium.) The lower temperature ensures that the cells will not overgrow. The cells should be spun at 1000 × *g* for 10 minutes then gently resuspended in 10 ml of 10 mM $MgSO_4$. Before use, dilute cells to an $OD_{600}$ of 0.5 with 10 mM $MgSO_4$. Bacterial cells prepared in this manner can be used for all phage manipulations described within the manual. Highest efficiencies are obtained from freshly prepared cells.

## HELPER PHAGE

Two different helper phages are provided with the Lambda ZAP-CMV RI predigested vector kit: (1) the ExAssist interference-resistant helper phage with XLOLR strain and (2) the R408 helper phage. The ExAssist interference-resistant helper phage with XLOLR strain is designed to allow efficient in vivo excision of the pCMV-Script EX phagemid vector from the Lambda ZAP-CMV RI vector while preventing problems associated with helper phage co-infection. The ExAssist helper phage contains an amber mutation that prevents replication of the phage genome in a nonsuppressing *E. coli* strain (e.g., XLOLR cells). Only the excised phagemid can replicate in the host, removing the possibility of co-infection from the ExAssist helper phage. Because ExAssist helper phage cannot replicate in the XLOLR strain, single-stranded rescue cannot be performed in this strain using ExAssist helper phage. XLOLR cells are also resistant to lambda infection, preventing lambda DNA contamination after excision.

### Storing the Helper Phage

The ExAssist helper phage and the R408 helper phage are supplied in 7% dimethylsulfoxide (DMSO) and should be stored at –80°C. The helper phage may be stored for short periods of time at –20°C or 4°C. It is important to titer the helper phage prior to each use. Expect titers of approximately $10^{10}$ pfu/ml for the ExAssist helper phage or $10^{10}$ pfu/ml for the R408 helper phage. If the titer drops over time, prepare a fresh high-titer stock of the helper phage as outlined in *Amplifying the Helper Phage*.

### Titering the Helper Phage

1. Transfer a colony of XL1-Blue MRF´ cells into 10 ml of LB broth with supplements in a 50-ml conical tube. Incubate the conical tube with shaking at 37°C until growth reaches an $OD_{600}$ of 1.0.

2. Dilute the phage ($10^{-4}$–$10^{-7}$) in SM buffer (See *Preparation of Media and Reagents*) and combine 1 µl of each dilution with 200 µl of XL1-Blue MRF´ cells ($OD_{600}$ = 1.0).

3. Incubate the helper phage and the XL1-Blue MRF´ cells for 15 minutes at 37°C to allow the phage to attach to the cells.

4. Add 3 ml of NZY top agar, melted and cooled to ~48°C, and plate immediately onto dry, prewarmed NZY agar plates. Allow the plates to set for 10 minutes.

5. Invert the plates and incubate overnight at 37°C.

   **Note** *ExAssist and R408 plaques will have a cloudier appearance than lambda phage plaques.*

6. To determine the titer [in plaque-forming units per milliliter (pfu/ml)], use the following formula:

$$\left[\frac{\text{Number of plaques (pfu)} \times \text{dilution factor}}{\text{Volume plated (µl)}}\right] \times 1000 \text{ µl / ml}$$

   where the volume plated (in microliters) refers to the volume of the helper phage solution added to the cells.

## Amplifying the Helper Phage

1. Transfer a colony of XL1-Blue MRF´ cells into 10 ml of LB broth with supplements in a 50-ml conical tube. Incubate the conical tube with shaking at 37°C until growth reaches an $OD_{600}$ of 0.3.

   **Note** *An $OD_{600}$ of 0.3 corresponds to 2.5 × $10^8$ cells/ml.*

2. Add the helper phage at a multiplicity of infection (MOI) of 20:1 (phage-to-cells ratio).

3. Incubate the conical tube at 37°C for 15 minutes to allow the phage to attach to the cells.

4. Incubate the conical tube with shaking at 37°C for 8 hours.

5. Heat the conical tube at 65°C for 15 minutes.

6. Spin down the cell debris and transfer the supernatant to a fresh conical tube.

7. The titer of the supernatant should be between 7.5 × $10^{10}$ and 1.0 × $10^{12}$ pfu/ml for ExAssist helper phage or between 1.0 × $10^{11}$ and 1.0 × $10^{12}$ pfu/ml for R408 helper phage.

   **Note** *ExAssist and R408 plaques will have a cloudier appearance than lambda phage plaques.*

8. Add dimethylsulfoxide (DMSO) to a final concentration of 7% (v/v) and store at –80°C.

9. For further details about helper phage titering or amplification, please see *Titering the Helper Phage* or Reference 5.

## LIGATING THE INSERT

**Notes** *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, then gently mix the solution by stirring with a yellow pipet tip prior to pipetting.*

*Polyethylene glycol (PEG), which is present in some ligase buffers, can inhibit packaging.*

The Lambda ZAP-CMV RI vector is shipped in 10 mM Tris-HCl (pH 7.0) and 0.1 mM EDTA and can be stored up to 1 month at 4°C or frozen in aliquots at –20°C for longer storage. The RHEO test insert should be stored at –20°C. However, do not put samples through multiple freeze–thaw cycles.

When ligating the sample insert, use a volume up to 2.5 µl. Use an equal molar ratio (or less to prevent multiple inserts) of the insert. The Lambda ZAP-CMV vector can accommodate inserts up to 6.5 kb. The Lambda ZAP-CMV vector is ~42 kb in length. If ligating a 4000-bp insert to the vector, use 0.1 µg of insert for every 1 µg of vector.

**Note** *A general rule when constructing cDNA libraries is to add between 50–150 ng of cDNA/µg of lambda arms.*

1. Set up a control ligation to ligate the test insert into the Lambda ZAP-CMV RI vector. Add the following components in order:

   1.0 µl of the predigested Lambda ZAP-CMV RI vector (1 µg)
   0.8 µl of RHEO test insert (0.2 µg)
   0.5 µl of 10× ligase buffer
   0.5 µl of 10 mM rATP (pH 7.5)
   *X* µl of water for a final volume of 5 µl
   *X* µl of T4 DNA ligase (2 Weiss U)

2. Prepare the sample ligation in a separate tube, using equal molar ratios of insert and vector. Add the following components in order:

   1.0 µl of the predigested Lambda ZAP-CMV RI vector (1 µg)
   *X* µl of the sample insert
   0.5 µl of 10× ligase buffer
   0.5 µl of 10 mM rATP (pH 7.5)
   *X* µl of water for a final volume of 5 µl
   *X* µl of T4 DNA ligase (2 Weiss U)

3. Incubate the reaction tubes overnight at 4°C.

After ligation is complete, package each ligation, including the control ligation. If the insert used is free from contaminants and contains a high percentage of ligatable ends, expect ~1 × $10^6$–1.5 × $10^7$ recombinant plaques/µg vector when using high-efficiency packaging extracts, such as Gigapack III Plus or Gigapack III Gold packaging extracts (Catalog #200204 and #200201, respectively). Protocols are provided below for packaging ligation reactions using these recommended extracts. See the appropriate Gigapack III packaging extract instruction manual for additional details and recommended packaging control reactions.

**Note** *We recommend using the high-efficiency Gigapack III Gold packaging extract[6] since this packaging extract is McrA⁻, McrB⁻, and Mrr⁻. Other commercially available packaging extracts can restrict hemimethylated DNA, therefore producing low-titer libraries.*

# PACKAGING REACTION

## General Information

Packaging extracts are used to package recombinant lambda phage with high efficiency, which increases the size of gene libraries.

Gigapack III Gold packaging extract increases the efficiency and representation of libraries constructed from highly methylated DNA. The packaging extracts are 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 Gold packaging extract should improve the quality of DNA libraries constructed from methylated DNA.[6, 7, 8, 9]

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. DNA may be used directly from ligation reactions in most cases; however, polyethylene glycol (PEG), which is contained in some ligase buffers, has been shown to inhibit packaging. The volume of DNA added to each extract should be <5 µl.

Undigested wild-type lambda DNA will be packaged with efficiencies exceeding 2 × $10^9$ plaques/µg of vector when using Gigapack III Gold packaging extract. Predigested arms, when ligated to a test insert, will yield ~1.0 × $10^7$ recombinant plaques/µg of vector.

## Packaging Instructions

### Packaging Protocol

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

1. Remove the appropriate number of packaging extracts from the –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. (Add 1 µl of the control ligation to a separate tube of packaging extract.)
4. Stir the 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 collect the liquid at the bottom of the tube.
6. Incubate the tube at room temperature (22°C) for 2 hours.
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.

## Titering the Packaging Reaction

### Preparing the Host Bacteria

1. Streak the XL1-Blue MRF´ cells onto LB agar plates containing the appropriate antibiotic (See *Recommended Media*). Incubate the plates overnight at 37°C.

   ***Note*** *Do not add antibiotic to the medium in the following step. The antibiotic will bind to the bacterial cell wall and will inhibit the ability of the phage to infect the cell.*

2. Prepare a separate 50-ml culture of XL1-Blue MRF´ cells in LB broth with supplements.

3. Incubate with shaking at 37°C 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 1000 × *g* for 10 minutes.

5. Gently resuspend the cell pellet in 25 ml sterile 10 mM $MgSO_4$.

   **Note** *For later use, store the cells at 4°C overnight in 10 mM $MgSO_4$.*

## Titering Protocol

1. Dilute the XL1-Blue MRF´ cells (from step 5 of *Preparing the Host Bacteria)* to an $OD_{600}$ of 0.5 with sterile 10 mM $MgSO_4$.

   **Note** *The bacteria should be used immediately following dilution.*

2. To determine the titer of the packaged ligation product, mix the following components:

   1 µl of the final packaged reaction
   200 µl of XL1-Blue MRF´ cells at an $OD_{600}$ of 0.5

   and

   1 µl of a 1:10 dilution of the final packaged reaction
   200 µl of XL1-Blue MRF´ cells at an $OD_{600}$ of 0.5

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

4. Add 3 ml of NZY top agar, melted and cooled to ~48°C, and plate immediately onto dry, prewarmed NZY agar plates. Allow the plates to set for 10 minutes. Invert the plates and incubate at 37°C.

5. Plaques should be visible after 6–8 hours. Count the plaques and determine the titer in plaque-forming units per milliliter (pfu/ml).

   **Note** *If insert size is crucial, one may excise a few clones at this step or a few clones prior to single-clone amplification (see* Single-Clone Excision Protocol *in* In Vivo Excision Protocols Using ExAssist Interference-Resistant Helper Phage with XLOLR Strain*).*

## AMPLIFYING THE LIBRARY

***Note*** *Primary libraries can be unstable; therefore, amplification of the libraries is recommended immediately.*

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. The following protocol is recommended for amplifying the Lambda ZAP-CMV RI library.

### Day 1

1. Grow a 50-ml overnight culture of XL1-Blue MRF´ cells in LB broth with supplements at 30°C with shaking.

### Day 2

2. Gently spin down the XL1-Blue MRF´ cells (1000 × *g*). Resuspend the cell pellet in 25 ml of 10 mM $MgSO_4$. Measure the $OD_{600}$ of the cell suspension, then dilute the cells to an $OD_{600}$ of 0.5 in 10 mM $MgSO_4$.

3. Combine aliquots of the packaged mixture or library suspension containing ~5 × $10^4$ pfu of bacteriophage with 600 µl of XL1-Blue MRF´ 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 to allow the phage to attach to the cells.

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 agar plate. Allow the plates to set for 10 minutes.

6. Invert the plates and incubate 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.)

    **Note** *Briefly spin the lambda phage stock to ensure that the chloroform is separated completely before removing the aliquot for titering.*

## PERFORMING PLAQUE LIFTS

1. Titer the amplified mixture or library suspension to determine the concentration using XL1-Blue MRF´ cells.

2. Combine the equivalent of 5 × $10^4$ pfu/plate and 600 µl of freshly prepared XL1-Blue MRF´ cells at an $OD_{600}$ of 0.5.

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

4. Add 6.5 ml of NZY top agar (~48°C) to the bacteria and phage mixture.

5. Quickly pour the plating culture onto a dry, prewarmed 150-mm NZY agar plate, which is at least 2 days old. Carefully swirl the plate to distribute the cells evenly. Allow the plates to set for 10 minutes. (Use 20 plates to screen 1 × $10^6$ pfu.)

6. Invert the plates and incubate at 37°C for ~8 hours.

7. 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.*

8. Place a nitrocellulose membrane onto each NZY agar plate for 2 minutes to allow the transfer of the phage particles to the membrane. Use a needle to prick through the membrane and agar for orientation. (If desired, waterproof ink in a syringe needle may be used.)

   **Notes** *If making duplicate nitrocellulose membranes, allow the second membrane to transfer for ~4 minutes.*

   *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*).

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

10. 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.

11. 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.[5, 10] Following these procedures, perform secondary and tertiary screenings also as outlined in the standard methodology texts.[5, 10] When using the Lambda ZAP-CMV RI vector, perform in vivo excision on the isolates to obtain the insert-containing pCMV-Script EX phagemid vector (see *In Vivo Excision of the pCMV-Script EX from the Lambda ZAP-CMV RI Vector* and *In Vivo Excision Protocols Using ExAssist Helper Phage with XLOLR Strain*).

* Catalog #400071 (1800 model) or #400075 (2400 model).

# IN VIVO EXCISION OF THE pCMV-SCRIPT EX PHAGEMID VECTOR FROM THE LAMBDA ZAP-CMV RI VECTOR

The Lambda ZAP-CMV RI vector is designed to allow simple, efficient in vivo excision and recircularization of any cloned insert contained within the lambda vector to form a phagemid containing the cloned insert.[3, 4] This in vivo excision depends on the placement of the DNA sequences within the lambda phage genome and on the presence of a variety of proteins, including filamentous (e.g., M13) bacteriophage-derived proteins. The M13 phage proteins recognize a region of DNA normally serving as the f1 bacteriophage "origin of replication." This origin of replication can be divided into two overlying parts: (1) the site of initiation and (2) the site of termination for DNA synthesis.[11] These two regions are subcloned separately into the Lambda ZAP-CMV RI vector. The lambda phage (target) is made accessible to the M13-derived proteins by simultaneously infecting a strain of *E. coli* with both the lambda vector and the M13 helper phage.

Inside *E. coli*, the "helper" proteins (i.e., proteins from M13 phage) recognize the initiator DNA that is within the lambda vector. One of these proteins then nicks one of the two DNA strands. At the site of this nick, new DNA synthesis begins and duplicates whatever DNA exists in the lambda vector "downstream" (3´) of the nicking site. DNA synthesis of a new single strand of DNA continues through the cloned insert until a termination signal, positioned 3´ of the initiator signal, is encountered within the constructed lambda vector. The ssDNA molecule is circularized by the gene II product from the M13 phage, forming a circular DNA molecule containing the DNA between the initiator and terminator. In the case of the Lambda ZAP-CMV RI vector, this includes all sequences of the pCMV-Script EX phagemid vector and the insert, if one is present. This conversion is the "subcloning" step, since all sequences associated with normal lambda vectors are positioned outside of the initiator and terminator signals and are not contained within the circularized DNA. In addition, the circularizing of the DNA automatically recreates a functional f1 origin as found in f1 bacteriophage or phagemids.

Signals for "packaging" the newly created phagemid are linked to the f1 origin sequence. The signals permit the circularized ssDNA to be "packaged" into phagemid particles and secreted from the *E. coli*. Following secretion of the phagemid particle, the *E. coli* cells used for in vivo excision of the cloned DNA are killed, and the lambda phage is lysed by heat treatment at 70°C. The phagemid is not affected by the heat treatment. *Escherichia coli* is infected with the phagemid and can be plated on selective media to form colonies. DNA from excised colonies can be used for analysis of insert DNA, including DNA sequencing, subcloning, and mapping. Colonies from the excised pCMV-Script EX phagemid vector can also be used for subsequent production of ssDNA suitable for dideoxy-sequencing and site-specific mutagenesis.

# In Vivo EXCISION PROTOCOLS USING EXASSIST HELPER PHAGE WITH XLOLR STRAIN

The ExAssist helper phage with XLOLR strain is designed to efficiently excise the pCMV-Script EX phagemid vector from the Lambda ZAP-CMV RI vector, while eliminating problems associated with helper phage co-infection. The ExAssist helper phage contains an amber mutation that prevents replication of the helper phage genome in a nonsuppressing *E. coli* strain such as XLOLR cells. This allows only the excised phagemid to replicate in the host, removing the possibility of co-infection from the ExAssist helper phage. Since the ExAssist helper phage cannot replicate in the XLOLR strain, single-stranded rescue cannot be performed in this strain using this helper phage.

**Note** *It is important not to contaminate the Lambda ZAP-CMV RI library with the filamentous helper phage, since small amounts of contaminating helper phage are sufficient to convert Lambda ZAP-CMV RI phage into pCMV-Script EX phagemids. If contamination should occur, contaminating filamentous phage can be removed without harming the library by adding a few microliters of chloroform to the Lambda ZAP-CMV RI phage stock.*

Mass excision can be used to generate subtraction libraries and subtracted DNA probes. Converting the library to the phagemid form also allows screening of the phagemid library in eukaryotic cells by transformation of eukaryotic cells with supercoiled plasmid DNA.

## Single-Clone Excision Protocol

### Day 1

1. Core the plaque of interest from the agar plate and transfer the plaque to a sterile microcentrifuge tube containing 500 µl of SM buffer and 20 µl of chloroform. Vortex the microcentrifuge tube to release the phage particles into the SM buffer. Incubate the microcentrifuge tube for 1–2 hours at room temperature or overnight at 4°C. (This phage stock is stable for up to 6 months at 4°C.)

2. Grow separate 50-ml overnight cultures of XL1-Blue MRF´ and XLOLR cells in LB broth with supplements at 30°C.

### Day 2

3. Gently spin down the XL1-Blue MRF´ and XLOLR cells (1000 × *g*). Resuspend each of the cell pellets in 25 ml of 10 mM $MgSO_4$. Measure the $OD_{600}$ of the cell suspensions, then adjust the concentration of the cells to an $OD_{600}$ of 1.0 ($8 \times 10^8$ cells/ml) in 10 mM $MgSO_4$.

4. Combine the following components in a 14-ml BD Falcon polypropylene tube:

   200 µl of XL1-Blue MRF´ cells at an $OD_{600}$ of 1.0
   250 µl of phage stock (containing $>1 \times 10^5$ phage particles)
   1 µl of the ExAssist helper phage ($>1 \times 10^6$ pfu/µl)

   **Note** *Briefly spin the lambda phage stock to ensure that the chloroform is separated completely before removing the aliquot used in the excision reaction.*

5. Incubate the BD Falcon polypropylene tube at 37°C for 15 minutes to allow the phage to attach to the cells.

6. Add 3 ml of LB broth with supplements and incubate the BD Falcon polypropylene tube for 2.5–3 hours at 37°C with shaking. Because clonal representation is not relevant, single-clone excision reactions can be safely performed overnight.

   **Note** *The turbidity of the media is not indicative of the success of the excision.*

7. Heat the BD Falcon polypropylene tube at 65–70°C for 20 minutes to lyse the lambda phage particles and the cells. Spin the tube at 1000 × *g* for 15 minutes to pellet the cell debris.

8. Decant the supernatant into a sterile BD Falcon polypropylene tube. This stock contains the excised pCMV-Script EX phagemid packaged as filamentous phage particles. (This stock may be stored at 4°C for 1–2 months.)

9. To plate the excised phagemids, add 200 µl of freshly grown XLOLR cells from step 3 ($OD_{600}$ = 1.0) to two 1.5-ml microcentrifuge tubes. Add 100 µl of the phage supernatant (from step 8 above) to one microcentrifuge tube and 10 µl of the phage supernatant to the other microcentrifuge tube.

10. Incubate the microcentrifuge tubes at 37°C for 15 minutes.

11. Add 300 µl of NZY broth and incubate the tubes at 37°C for 45 minutes to allow sufficient expression of the kanamycin-resistance gene product prior to plating on selective medium.

12. Plate 200 µl of the cell mixture from each microcentrifuge tube on LB-kanamycin agar plates (50 µg/ml) and incubate the plates overnight at 37°C.

Due to the high-efficiency of the excision process, it may be necessary to titrate the supernatant to achieve single-colony isolation.

Colonies appearing on the plate contain the pCMV-Script EX double-stranded phagemid vector with the cloned DNA insert. Helper phage will not grow, since helper phage is unable to replicate in $Su^-$ (nonsuppressing) XLOLR strain and does not contain kanamycin-resistance genes. XLOLR cells are also resistant to lambda phage infection, thus preventing lambda phage contamination after excision.

To maintain the pCMV-Script EX phagemid vector, streak the colony on a new LB–kanamycin agar plate. For long-term storage, prepare a bacterial glycerol stock and store at –80°C.

R408 helper phage is recommended for the single-stranded rescue procedure. The single-stranded rescue procedure can be found in *Appendix: Recovery Of Single-Stranded DNA From Cells Containing the pCMV-Script EX Phagemid Vector*.

## Mass Excision Protocol

### Day 1

1. Grow separate 50-ml overnight cultures of XL1-Blue MRF´ and XLOLR cells in LB broth with supplements at 30°C.

### Day 2

2. Gently spin down the XL1-Blue MRF´ and XLOLR cells (1000 × *g*). Resuspend each of the cell pellets in 25 ml of 10 mM $MgSO_4$. Measure the $OD_{600}$ of the cell suspensions, then adjust the concentration of the cells to an $OD_{600}$ of 1.0 ($8 \times 10^8$ cells/ml) in 10 mM $MgSO_4$.

3. In a 50-ml conical tube, combine a portion of the amplified lambda bacteriophage library with XL1-Blue MRF´ cells at a MOI of 1:10 lambda phage-to-cell ratio. Excise 10- to 100-fold more lambda phage than the size of the primary library to ensure statistical representation of the excised clones. Add ExAssist helper phage at a 10:1 helper phage-to-cells ratio to ensure that every cell is co-infected with lambda phage and helper phage.

   For example, use

   - $10^7$ pfu of the lambda phage (i.e., 10- to 100-fold above the primary library size)
   - $10^8$ XL1-Blue MRF´ cells (1:10 lambda phage-to-cell ratio, noting that an $OD_{600}$ of 1.0 corresponds to $8 \times 10^8$ cells/ml)
   - $10^9$ pfu of ExAssist helper phage (10:1 helper phage-to-cells ratio)

   **Note** *Briefly spin the lambda phage stock to ensure that the chloroform is separated completely before removing the aliquot used in the excision reaction.*

4. Incubate the conical tube at 37°C for 15 minutes to allow the phage to attach to the cells.

5. Add 20 ml of LB broth with supplements and incubate the conical tube for 2.5–3 hours at 37°C with shaking.

   **Notes** *Incubation times for mass excision in excess of 3 hours may alter the clonal representation.*

   *The turbidity of the media is not indicative of the success of the excision.*

6. Heat the conical tube at 65–70°C for 20 minutes to lyse the lambda phage particles and the cells.

7. Spin the conical tube at 1000 × *g* for 10 minutes to pellet the cell debris and then decant the supernatant into a sterile conical tube.

8. To titer the excised phagemids, combine 1 µl of this supernatant with 200 µl of XLOLR cells from step 2 in a 1.5-ml microcentrifuge tube.

9. Incubate the microcentrifuge tube at 37°C for 15 minutes.

10. Add 40 µl of 5× NZY broth (for a final concentration of 1×) and incubate the tube at 37°C for 45 minutes to allow sufficient expression of the kanamycin-resistance gene product prior to plating on selective medium.

11. Plate 100 µl of the cell mixture onto LB–kanamycin agar plates (50 µg/ml) and incubate the plates overnight at 37°C.

    **Note** *It may be necessary to further dilute the cell mixture to achieve single-colony isolation.*

Colonies may now be selected for plasmid preps, or the cell mixture may be plated directly onto filters for colony screening.

# EUKARYOTIC SCREENING WITH THE LAMBDA ZAP-CMV RI LIBRARY

Screening libraries in eukaryotic cells has proved to be an effective way of identifying clones otherwise nonidentifiable in prokaryotic screening systems. The screening technique used will depend on the clone of interest and on the type of assay available. An appropriate cell line for screening must be obtained, and an assay or reagent capable of identifying the cell or cells expressing the desired target protein must be developed. The panning assay and functional analysis of clone pools are two available techniques.

## Panning Assay

Clone identification by "panning" requires the transfection of a library into a cell line deficient in the desired surface protein. When the clone of interest is translated and expressed on the surface of eukaryotic cells, the translated protein product is made accessible to an antibody, ligand, or receptor coupled either directly or indirectly to a solid-phase matrix. Eukaryotic transfectant clones expressing the appropriate insert will bind to the affinity matrix, while cells not adhering are washed away. Either transient or stable transfection protocols can be used.[12]

## Functional Assay

Functional assay screening can also be performed on either transiently or stably transfected cells. Transient expression will likely require subdividing the amplified library into smaller pools of clones to prevent the dilution of a positive cell signal with an excess of negative clones. Each clone pool is amplified separately and transfected into the eukaryotic cells. The transfected cells are then tested for the expression of the desired clone. Once a pool is identified as containing the clone of interest, it is subdivided into smaller pools for a second round of prokaryotic amplification, eukaryotic transfection, and screening. After several rounds of enriching for the desired clone, a single clone can be isolated. The initial pool size is determined according to the sensitivity of the available assay so that a single clone within the pool is still theoretically detectable in the transfected cells. For example, if a positive assay signal is 1000-fold above background, pools containing 500–1000 members should still give a signal above background. The sensitivity of the assay dictates the initial size of the pools, as well as the number of pools required to screen. If stable transformants are created using G418 selection, pools of stable clones can be assayed. This simplifies the identification of isolated positive eukaryotic clones, because the eukaryotic colonies can be picked or diluted in microtiter tissue culture plates.

After a clone has been identified within the eukaryotic cells, the clone can be retrieved by several methods. Plasmid DNA within the tissue culture cells can be collected using the Hirt, Birnboim, and Doly procedures,[13, 14] then transferred into *E. coli* cells for amplification and plasmid DNA preparations. Simmons *et al.*[15] were able to screen libraries in COS cells, where the presence of the SV40 T antigen increases the copy number of phagemids containing the SV40 origin of replication. This results in a higher episomal copy number, which may help in the retrieval of the plasmids. Inserts can also be isolated by polymerase chain reaction (PCR) amplification of the tissue culture cells using T3/T7 primer sets. The resulting PCR fragment can be digested using restriction sites flanking the insert, then recloned into pCMV-Script EX phagemid DNA for further analysis.

**Note** *Screening libraries in eukaryotic cells can be extremely laborious. Many functional assays are not sensitive enough to detect a clone from pools of nonrelated clones. Therefore, it is worth considering the use of techniques, such as differential PCR,[16] selective hybridization[17]and degenerate oligonucleotides, to develop DNA probes for initial screening using prokaryotic plaques. Positive clones can then be screened by eukaryotic transfection and expression.*

## EUKARYOTIC EXPRESSION

The CMV promoter is considered to be a strong promoter and to function in many different cell lines.[18] However, expression in eukaryotic cells is sensitive to many factors. If little or no expression is observed in eukaryotic cells, several factors can be considered.

- Methylation of some insert DNA can prevent expression in some cell lines.[19]
- The promoter may not be functional in some cell lines and should be tested before screening a library.
- The pCMV-Script EX vector does not contain an ATG initiation codon. A translation initiation sequence must be incorporated if the DNA fragment to be cloned does not have an initiating ATG codon or an optimal sequence for initiating translation, such as the Kozak sequence [GCC(A/G)CCATGG].[20]

# APPENDIX: RECOVERY OF SINGLE-STRANDED DNA FROM CELLS CONTAINING THE pCMV-SCRIPT EX PHAGEMID VECTOR

The pCMV-Script EX vector is a phagemid that can be secreted as single-stranded DNA (ssDNA) in the presence of M13 helper phage. These phagemids contain the intergenic (IG) region of a filamentous f1 phage. This region encodes all of the *cis*-acting functions of the phage required for packaging and replication. In *E. coli* with the $F^+$ phenotype (containing an F´ episome), pCMV-Script EX phagemid vectors will be secreted as single-stranded f1 "packaged" phage when the bacteria have been infected by a helper phage. Because these filamentous helper phages (M13, f1) will not infect *E. coli* without an F´ episome coding for pili, **it is essential to use the XL1-Blue MRF´ strain or a similar strain containing the F´ episome.**[21, 22]

We offer helper phage that *preferentially* package the pCMV-Script EX phagemid vector. Typically, 30–50 pCMV-Script EX molecules are packaged per helper phage DNA molecule. The pCMV-Script EX phagemid vector is offered with the IG region in the minus orientation.

Yields of ssDNA can depend on the specific insert sequence, but for most inserts >1 µg of ssDNA can be obtained from a 1.5-ml miniprep if grown in XL1-Blue MRF´. A faint single-stranded helper phage band may appear on a gel at ~4 kb for R408 helper phage. This DNA mixture can be sequenced with primers that are specific for the pCMV-Script EX phagemid vectors and do not hybridize to the helper phage genome.

R408 helper phage can be used to produce a large amount of single-stranded pCMV-Script EX phagemid vector. Use the ExAssist interference-resistant helper phage with XLOLR strain for the excision of the pCMV-Script EX phagemid vector from the Lambda ZAP-CMV RI vector and use the R408 helper phage for single-stranded rescue.

## Single-Stranded Rescue Protocol

1. Inoculate a single colony into 5 ml of 2× YT broth[§] containing 50 µg/ml kanamycin and R408 helper phage at $10^7$–$10^8$ pfu/ml (MOI ~10).

2. Grow the culture at 37°C with vigorous aeration for 16–24 hours, or until growth has reached saturation.

3. Centrifuge 1.5 ml of the cell culture for 5 minutes in a microcentrifuge.

4. Remove 1 ml of the supernatant to a fresh tube, then add 150 µl of a solution containing 20% PEG8000 and 2.5 M NaCl. Allow phage particles to precipitate on ice for 15 minutes.

   **Note** *For increased yield, perform the PEG precipitation overnight at 4°C.*

5. Centrifuge for 5 minutes in a microcentrifuge. (A pellet should be obvious.)

6. Remove supernatant. Centrifuge the PEG pellets a few seconds more to collect residual liquid, then remove and discard the residual liquid.

7. Resuspend the pellet in 400 µl of 0.3 M NaOAc (pH 6.0) and 1 mM EDTA by vortexing vigorously.

8. Extract with 1 volume phenol–chloroform and centrifuge for 1–2 minutes to separate phases.

9. Transfer the aqueous phase to a fresh tube and add 1 ml of ethanol. Centrifuge for 5 minutes.

10. Remove ethanol and dry the DNA pellet.

11. Dissolve the pellet in 25 µl of TE buffer.[§]

12. Analyze 1–2 µl on an agarose gel.

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

# TROUBLESHOOTING

## Packaging

| Observations | Suggestions |
|---|---|
| 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 |
| | Ensure the DNA concentration is sufficient. Ligate at DNA concentrations of 0.2 µg/µl or greater and package between 1 and 4 µl of the ligation reaction |
| | Never package >4 µl 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 when titering or amplifying the library | The lambda phage stock aliquot used when determining titer and amplifying the library cannot contain chloroform, as chloroform lyses the *E. coli* cells. Briefly spin the lambda phage stock to ensure that the chloroform is separated completely before removing the aliquot |

## Excision

| Observations | Suggestions |
|---|---|
| The number of colonies is too low | Verify that the titer on the tubes is current and correct and use only calibrated pipettors. The molar ratios of lambda phage to cells to helper phage is critical |
| | If an excision is unsuccessful, prepare a high-titer stock of the phage and repeat the excision procedure, as excision efficiencies are directly related to the lambda phage titer |
| | Ensure that the platings are performed using agar plates containing kanamycin |
| | The lambda phage stock aliquot used for in vivo excision cannot contain chloroform, as chloroform lyses the *E. coli* cells. Briefly spin the lambda phage stock to ensure that the chloroform is separated completely before removing the aliquot |

## PREPARATION OF MEDIA AND REAGENTS

### 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

### LB Broth with Supplements

Prepare 1 liter of LB broth
Autoclave
Add the following filter-sterilized supplements prior to use
10 ml of 1 M $MgSO_4$
3 ml of a 2 M maltose solution or 10 ml of 20% (w/v) maltose

### LB–Kanamycin Agar (per Liter)

1 liter of LB agar
Autoclave
Cool to 55°C
Add 7.5 ml of 10 mg/ml kanamycin (filter-sterilized)
Pour into petri dishes (~25 ml/100-mm plate)

### LB–Tetracycline Agar (per Liter)

1 liter of LB agar
Autoclave
Cool to 55°C
Add 1.5 ml of 10 mg/ml tetracycline (filter-sterilized)
Pour into petri dishes (~25 ml/100-mm plate)
Store plates in a dark, cool place or cover plates with foil if left out at room temperature for extended time periods as tetracycline is light-sensitive

### LB–Tetracycline Broth (per Liter)

Prepare 1 liter of LB broth
Autoclave
Cool to 55°C
Add 1.25 ml of 10 mg/ml-filter-sterilized tetracycline
Store broth in a dark, cool place as tetracycline is light-sensitive

### 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)

### 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)

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

### 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

### 20× SSC Buffer (per Liter)

175.3 g of NaCl
88.2 g of sodium citrate
800.0 ml of deionized $H_2O$
10.0 N NaOH
Adjust to pH 7.0 with a few drops of 10.0 N NaOH
Add deionized $H_2O$ to a final volume of 1 liter

### 2× YT Broth (per Liter)

10 g of NaCl
10 g of yeast extract
16 g of tryptone
Add deionized $H_2O$ to a final volume of 1 liter
Adjust to pH 7.5 with NaOH
Autoclave

### TE Buffer

10 mM Tris-HCl (pH 7.5)
1 mM EDTA

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## ENDNOTES

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## MSDS INFORMATION

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