# Uni-ZAP XR Vector Kit and Uni-ZAP XR Gigapack Cloning Kits

***Eco*R I/*Xho* I/CIAP-Treated**

## Instruction Manual

**Catalog #237211 (Uni-ZAP XR Vector Kit) and**
**#237612 (Uni-ZAP XR Gigapack III Gold Cloning Kit)**
Revision C.0

**For Research Use Only. Not for use in diagnostic procedures.**

237211-12

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# Uni-ZAP XR Vector Kit and Uni-ZAP XR Gigapack Cloning Kits

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# Uni-ZAP XR Vector Kit and Uni-ZAP XR Gigapack Cloning Kits

Catalog #237211 (Uni-ZAP XR Vector Kit) and
#237612 (Uni-ZAP XR Gigapack III Gold Cloning Kit)

## MATERIALS PROVIDED

| Materials provided | Quantity | |
|---|---|---|
| | **Catalog #237211** | **Catalog #237612** |
| Uni-ZAP XR vector predigested with *Eco*R I and *Xho* I, CIAP treated[a] | 12 µg | 12 µg |
| pBR322 test insert digested with *Eco*R I and *Sal* I[a] | 2.5 µg | 2.5 µg |
| Host strains[b] | | |
| XL1-Blue MRF′ strain | 0.5-ml bacterial glycerol stock | 0.5-ml bacterial glycerol stock |
| SOLR strain | 0.5-ml bacterial glycerol stock | 0.5-ml bacterial glycerol stock |
| f1 helper phage[c] | | |
| ExAssist interference-resistant helper phage | 1 ml | 1 ml |
| VCSM13 Interference-Resistant Helper Phage | 1 ml | 1 ml |
| Gigapack III Gold packaging extract | | |
| Gigapack III Gold-11 packaging extract[d] | — | 11 × 25 µl |
| λcI857 *Sam*7 wild-type lambda control DNA[e] | — | 1.05 µg |
| VCS257 host strain[f] | — | 1 ml |

[a] Shipped as a liquid at 1 µg/µl (vector) and 0.25 µg/µl (test insert) in TE Buffer. *Sal* I is compatible with *Xho* I. On arrival, store the 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 SOLR strain for plating excised phagemids and the XL1-Blue MRF′ strain for all other manipulations. For host strain storage conditions, see *Bacterial Host Strains*.

[c] Retiter after 1 month. (Take care not to contaminate the Lambda ZAP vector with this high-titer filamentous helper phage.) Store at –80°C. We recommend VCSM13 interference-resistant helper phage for single stranded rescue (see *Appendix: Recovery of Single-Stranded DNA from Cells Containing pBluescript Phagemids*). ExAssist interference-resistant helper phage has α-complementing β-galactosidase sequences which may interfere with sequencing or site-directed mutagenesis where oligonucleotide primers hybridize to β-galactosidase sequences (e.g., M13–20 primer).

[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 immediately stored at –80°C.

[f] The VCS257 host strain, included for plating the λcI857 *Sam*7 positive control, is shipped as a frozen bacterial glycerol stock (see *Bacterial 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.

Revision C.0

## STORAGE CONDITIONS

**Vector:** –20°C
**Test Insert:** –20°C
**Helper Phage:** –80°C
**Bacterial Glycerol Stocks:** –80°C

# INTRODUCTION

## Overview of the Uni-ZAP XR Vector System

The Uni-ZAP XR vector system combines the high efficiency of lambda library construction and the convenience of a plasmid system with blue-white color selection. The Uni-ZAP XR vector (Figure 1) is double digested with *Eco*R I and *Xho* I and will accommodate DNA inserts from 0 to 10 kb in length. The Uni-ZAP XR vector can be screened with either DNA probes or antibody probes and allows in vivo excision of the pBluescript phagemid (Figure 2), allowing the insert to be characterized in a plasmid system. The polylinker of the pBluescript phagemid has 21 unique cloning sites flanked by T3 and T7 promoters and a choice of 6 different primer sites for DNA sequencing. The phagemid 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 with 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, and the *lac*Z promoter may be used to drive expression of fusion proteins suitable for Western blot analysis or protein purification.

## Uni-ZAP XR Vector Map

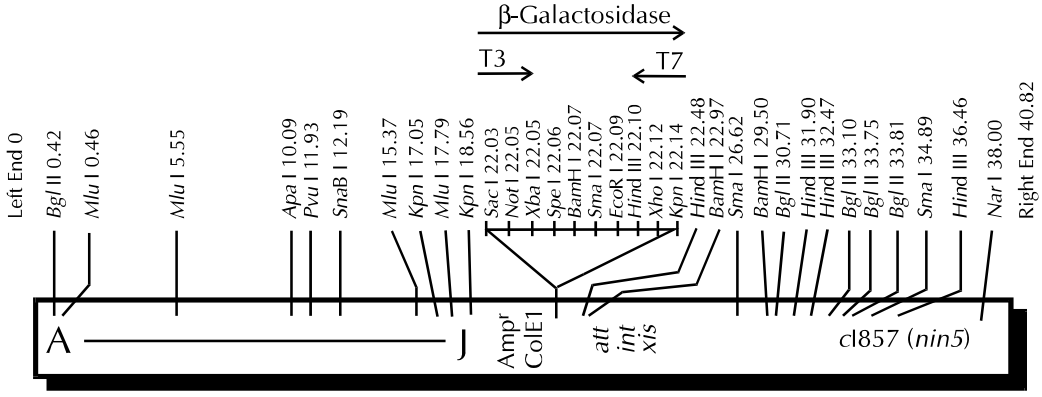
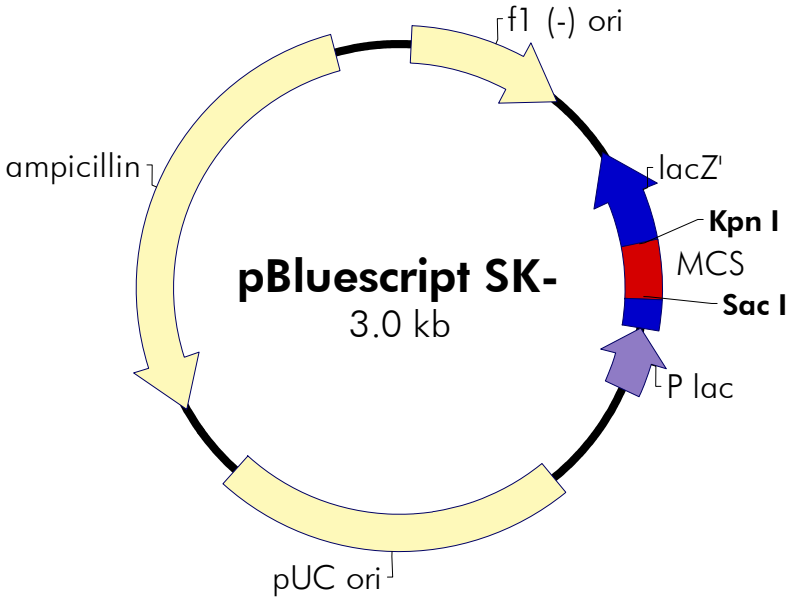
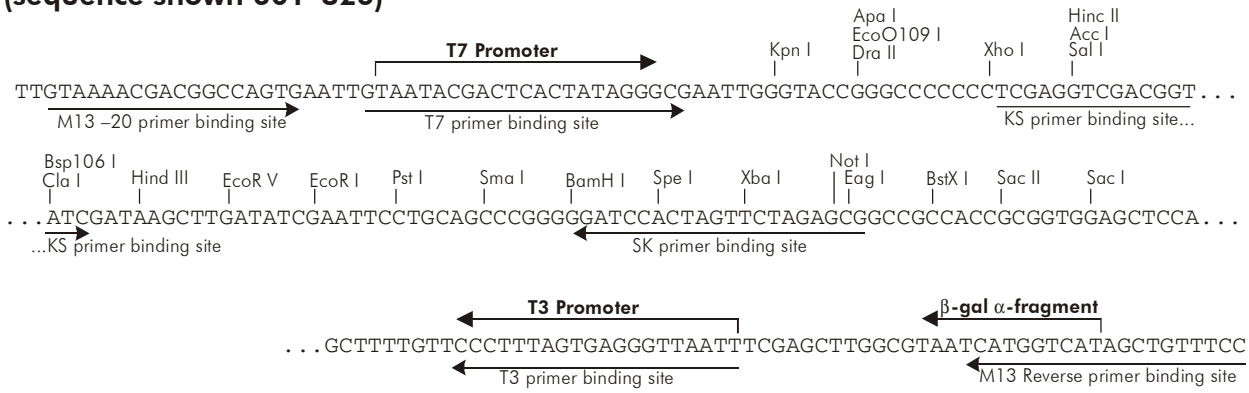



**FIGURE 1** Map of the Uni-ZAP XR insertion vector.

## pBluescript SK(–) Vector Map




## pBluescript SK (–) Multiple Cloning Site Region (sequence shown 601–826)




| Feature | Nucleotide Position |
|---|---|
| f1 (–) origin of ss-DNA replication | 24–330 |
| β-galactosidase α-fragment coding sequence (lacZ′) | 463–816 |
| T7 promoter transcription initiation site | 643 |
| multiple cloning site | 653–760 |
| T3 promoter transcription initiation site | 774 |
| *lac* promoter | 817–938 |
| pUC origin of replication | 1158–1825 |
| ampicillin resistance (*bla*) ORF | 1976–2833 |

**FIGURE 2** Circular map and polylinker sequence of the pBluescript SK(–) phagemid. The complete sequence and list of restriction sites are available from *www.genomics.agilent.com* or from the GenBank® database (#X52324).

## Special Considerations for Using the ZAP-cDNA Synthesis Kit

The ZAP-cDNA synthesis kit efficiently produces vector-ready directional cDNA that can be used in conjunction with the Uni-ZAP XR unidirectional vector. The ZAP-cDNA synthesis kit uses a hybrid oligo(dT) linker–primer that contains an *Xho* I site. First-strand synthesis is primed with the linker–primer and is transcribed using Moloney murine leukemia virus reverse transcriptase (MMLV-RT) and 5-methyl dCTP. The use of 5-methyl dCTP during first-strand synthesis hemimethylates the cDNA, protecting it from digestion from certain restriction endonucleases such as *Xho* I. Therefore, on *Xho* I digestion of the cDNA, only the unmethylated site within the linker–primer will be cleaved.

Hemimethylated DNA introduced into an McrA$^+$ McrB$^+$ strain would be subject to digestion by the *mcrA* and *mcrB* restriction systems. Therefore, it is necessary to initially infect an McrA$^-$ McrB$^-$ strain (e.g., XL1-Blue MRF´ strain supplied with the Uni-ZAP XR vector) when using the ZAP-cDNA synthesis kit. After passing the library through XL1-Blue MRF´ cells, the DNA is no longer hemimethylated and can be grown in McrA$^+$ McrB$^+$ strains (e.g., XL1-Blue strain).

**Note** *We recommend using high-efficiency Gigapack III Gold packaging extract, since this packaging extract is McrA$^-$, McrB$^-$, and Mrr$^-$. Other commercially available packaging extracts can destroy hemimethylated DNA, therefore producing low-titer libraries.*

Recombination frequency can be checked with blue–white color selection using XL1-Blue MRF´ cells or plating efficiencies of arms ligated to themselves versus arms ligated to cDNA can be compared.

# BACTERIAL HOST STRAINS

## Host Strain Genotypes

| Host strains | Genotype |
|---|---|
| SOLR strain[a] | e14$^-$(McrA$^-$) Δ(*mcrCB-hsdSMR-mrr*)*171 sbcC recB recJ uvrC umu*C::Tn*5* (Kan$^r$) *lac gyrA96 relA1 thi-1 endA1* $\lambda^R$ [F′ *proAB lacI*$^q$Z*ΔM15*] Su$^-$ (nonsuppressing) |
| 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*ΔM15* Tn*10* (Tet$^r$)] |

[a] Use the SOLR strain for excision only.

## XL1-Blue MRF′ Bacterial Strain Description

The RecA$^-$ *E. coli* host strain XL1-Blue MRF′ is supplied with the Uni-ZAP XR vector kit. Because the Uni-ZAP XR 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 Uni-ZAP XR vector as the F′ episome present in the XL1-Blue MRF′ strain serves three purposes.

First, the Δ*M15 lacZ* gene present on the F′ episome is required for the β-galactosidase-based nonrecombinant selection strategy. When cDNA is present in the polylinker, expression from the *lacZ* gene is disrupted and white plaques are produced. In contrast, without insert in the polylinker, the amino terminus of β-galactosidase is expressed and nonrecombinants can be scored visually by the presence of blue plaques. To produce an enzymatically active β-galactosidase protein, two domains are required: the α-region expressed by the vector and the Δ*M15 lacZ* domain expressed by the F′ episome. These two domains fold to form a functional protein, the α-region complementing the missing amino acids resulting from the Δ*M15* mutation. Therefore, in order to utilize the nonrecombinant selection strategy, the correct host strain must be used to produce a functional β-galactosidase protein.

Second, 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 Uni-ZAP XR clone to a pBluescript phagemid requires superinfection with a filamentous helper phage, the F′ episome is required for in vivo excision (see *In Vivo Excision of the pBluescript Phagemid from the Uni-ZAP XR Vector*).

Third, the F´ episome contains the *lac* repressor (*lacI*$^q$ gene), which blocks transcription from the *lacZ* promoter in the absence of the inducer isopropyl-1-thio-β-D-galactopyranoside (IPTG). This repressor is important for controlling expression of fusion proteins which may be toxic to the *E. coli*. Because the presence of the *lacI*$^q$ repressor in the *E. coli* host strain can potentially increase the representation or completeness of the library, XL1-Blue MRF´ is useful for screening the amplified library.

**Note** *The strains used for the Lambda gt11 vector (i.e., Y1088, Y1089, and Y1090) are not suitable for use with the Uni-ZAP XR vector because 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 Uni-ZAP XR vector. Using these strains with the Uni-ZAP XR vector could result in recombination between the homologous sequences.*

## 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 |
|---|---|---|---|---|
| SOLR strain | LB-kanamycin[a] | LB broth with supplements[a-c] | — | LB-ampicillin[a] |
| VCS257 strain[d] | LB[a] | LB broth with supplements[a-c] | NZY[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.

[d] For use with Gigapack III Gold packaging extract and wild-type control only. Supplied with Gigapack III Gold packaging extract.

## 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.

## Determining Background by Color Selection with IPTG and X-gal

The color selection by α-complementation with the Uni-ZAP XR vector requires higher amounts of IPTG and X-gal for generation of the blue color. Transcription and translation of the fusion protein are normal, but the large polylinker present within the pBluescript phagemid, which is present in the Uni-ZAP XR vector, is partly responsible for the reduced activity of the β-galactosidase protein—not the promoter. As would be expected, the copy number of the Uni-ZAP XR vector is much less per cell than the copy number of pBluescript phagemids. However, it is important to note that the color assay is used only for determining the ratio of recombinants to nonrecombinants within a newly constructed library and is not used for any other manipulations.

# HELPER PHAGE

Two different helper phages are provided with the Uni-ZAP XR vector kit: (1) the ExAssist interference-resistant helper phage with SOLR strain[1] and (2) the VCSM13 helper phage. The ExAssist interference-resistant helper phage with SOLR strain is designed to allow efficient in vivo excision of the pBluescript phagemid from the Uni-ZAP XR vector while preventing the problems that can be 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., SOLR cells). Only the excised phagemid can replicate in the host, removing the possibility of co-infection from the ExAssist helper phage. The ExAssist helper phage cannot be used for single-stranded rescue due to its inability to replicate in the SOLR strain. The other helper phage, VCSM13 helper phage, is recommended for single-stranded rescue procedures from the excised pBluescript phagemids (see *Appendix: Recovery of Single-Stranded DNA from Cells Containing pBluescript Phagemids*).

## Storing the Helper Phage

The ExAssist helper phage and the VCSM13 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 $10^{11}$ pfu/ml for the VCSM13 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 VCSM13 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.

   **Note** *When amplifying VCSM13 helper phage, add kanamycin to a final concentration of 25 µg/ml after 30 minutes of growth.*

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 VCSM13 helper phage.

   **Note** *ExAssist and VCSM13 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 2.

## 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 Uni-ZAP XR vector arms are 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 pBR322 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 Uni-ZAP XR vector can accommodate inserts ranging from 0 to 10 kb. The Uni-ZAP XR vector is ~41,000 bp in length. If ligating a 4,000-bp insert to the vector, use 0.1 µg of insert for every 1 µg of vector.

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

   1.0 µl of the predigested Uni-ZAP XR vector (1 µg)
   1.6 µl of the test insert (0.4 µg)
   0.5 µl of 10× ligase buffer
   0.5 µl of 10 mM ATP (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 Uni-ZAP XR vector (1 µg)
   *X* µl of the sample insert
   0.5 µl of 10× ligase buffer
   0.5 µl of 10 mM ATP (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 12–14°C or for 2 days 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 about $2 \times 10^6$–$1.5 \times 10^7$ recombinant plaques/µg of vector when using high-efficiency packaging extracts, such as Gigapack III Gold packaging extract [Agilent Catalog #200201 (Gold-4), #200202 (Gold-7) and #200203 (Gold-11)].

# PACKAGING REACTION

## 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.[3–6]

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 $1 \times 10^9$ pfu/µg of vector when using a Gigapack III packaging extract. However, predigested vector, when ligated to a test insert, yield ~$5 \times 10^6$–$1 \times 10^7$ 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.

### Packaging Protocol

***Note*** *Polyethylene glycol, which is present 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.
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 and transfer the supernatant to a fresh tube.
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

***Note*** *The VCS257 strain is for use with the Gigapack III Gold packaging extract and the positive wild-type lambda DNA control only.*

1. Streak the XL1-Blue MRF' and VCS257 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 separate 50-ml cultures of XL1-Blue MRF' and VCS257 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 each 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* in *Titering the Packaging Reaction*) 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).

## Testing the Efficiency of the 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 Instructions.*

   ***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 in SM buffer of the packaging reaction from step 10 of the *Packaging Protocol*. (The final dilution is $10^{-4}$.)

4. Dilute the VCS257 cells (from step 5 of *Preparing the Host Bacteria* in *Titering the Packaging Reaction*) to an $OD_{600}$ of 0.5 with sterile 10 mM $MgSO_4$.

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

5. Add 10 µl of the $10^{-4}$ packaging reaction dilution from step 3 to 200 µl of the VCS257 host strain from step 4. (The VCS257 strain is recommended for plating the wild-type lambda control DNA only.)

6. Incubate at 37°C for 15 minutes to allow the phage to attach to the cells.

7. 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.

8. Plaques should be visible after 12 hours. 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.

## DETERMINING BACKGROUND BY BLUE-WHITE COLOR SELECTION

A background test can be completed by plating several hundred plaques on a plate [see *Determining Background by Color Selection with IPTG and X-gal*]. Add 15 µl of 0.5 M IPTG (in water) and 50 µl of 250 mg/ml X-gal [in dimethylformamide (DMF)] to 2–3 ml of NZY top agar, melted and cooled to ~48°C. The higher concentrations of IPTG and X-gal used in the plating often result in the formation of a precipitate, which disappears after incubation. Add the IPTG and X-gal to the NZY top agar separately, with mixing in between additions, to minimize the formation of this precipitate. Plate immediately on NZY agar plates. Plaques are visible after incubation for 6–8 hours at 37°C, although color detection requires overnight incubation. Background plaques are blue, while recombinant plaques are white.

1. To plate 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

   **Note** *Use of any other cell line may result in a dramatically reduced titer. XL1-Blue MRF´ is a RecA$^-$ McrA$^-$ and McrCB$^-$ Mrr$^-$ strain and does not restrict methylated DNA.*

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

3. Add the following components:

   2–3 ml of NZY top agar (melted and cooled to ~48°C)
   15 µl of 0.5 M IPTG (in water)
   50 µl of X-gal [250 mg/ml (in DMF)]

4. Plate immediately onto dry, prewarmed NZY agar plates and 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, although color detection requires overnight incubation. Background plaques are blue and should be $<1 \times 10^5$ pfu/µg of arms, while recombinant plaques will be white (clear) and should be 10- to 100-fold above the background.

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

## 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.

The following protocol is recommended for amplifying the Uni-ZAP XR 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.[2, 7] Following these procedures, perform secondary and tertiary screenings also as outlined in the standard methodology texts.[2, 7] When using the Uni-ZAP XR vector, perform in vivo excision on the isolates to obtain the insert-containing pBluescript phagemid, or after an isolate is obtained, refer Reference 2 for suggested phage miniprep and maxiprep procedures.

## ANTIBODY SCREENING PROTOCOL

A complete instruction manual for immunoscreening is supplied with the *pico*Blue immunoscreening kit. This kit is available with goat anti-rabbit antibodies or goat anti-mouse antibodies [Agilent Catalog #200371 (goat anti-rabbit) and #200372 (goat anti-mouse)].

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

# In Vivo EXCISION OF THE pBLUESCRIPT PHAGEMID FROM THE UNI-ZAP XR VECTOR

The Uni-ZAP XR 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. 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 f1 bacteriophage-derived proteins. The f1 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.[8] These two regions are subcloned separately into the Uni-ZAP XR vector. The lambda phage (target) is made accessible to the f1-derived proteins by simultaneously infecting a strain of *E. coli* with both the lambda vector and the f1 bacteriophage.

Inside *E. coli*, the "helper" proteins (i.e., proteins from f1 or 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 single-stranded DNA molecule is circularized by the gene II product from the f1 phage, forming a circular DNA molecule containing the DNA between the initiator and terminator. In the case of the Uni-ZAP XR vector, this includes all sequences of the pBluescript SK(–) phagemid 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.

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

The ExAssist helper phage with SOLR strain is designed to allow efficient excision of the pBluescript phagemid from the Uni-ZAP XR vector, while eliminating 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 such as SOLR 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 SOLR strain, single-stranded rescue cannot be performed in this strain using this helper phage.

Mass excision can be used to generate subtraction libraries and subtracted DNA probes.

## 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 SOLR cells in LB broth with supplements at 30°C.

### Day 2

3. Gently spin down the XL1-Blue MRF´ and SOLR 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 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 pBluescript 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 SOLR 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. Plate 200 µl of the cell mixture from each microcentrifuge tube on LB-ampicillin agar plates (100 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 pBluescript double-stranded phagemid with the cloned DNA insert. Helper phage will not grow, since helper phage is unable to replicate in the $Su^-$ (nonsuppressing) SOLR strain and does not contain ampicillin-resistance genes. SOLR cells are also resistant to lambda phage infection, thus preventing lambda phage contamination after excision.

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

VCSM13 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 pBluescript Phagemids*.

## Mass Excision Protocol

### Day 1

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

### Day 2

2. Gently spin down the XL1-Blue MRF´ and SOLR 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 SOLR cells from step 2 in a 1.5-ml microcentrifuge tube.

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

10. Plate 100 µl of the cell mixture onto LB–ampicillin agar plates (100 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.*

At this stage, colonies may be selected for plasmid preps, or the cell mixture may be plated directly onto filters for colony screening.

# APPENDIX: RECOVERY OF SINGLE-STRANDED DNA FROM CELLS CONTAINING PBLUESCRIPT PHAGEMIDS

pBluescript is a phagemid that can be secreted as single-stranded DNA 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), pBluescript phagemids will be secreted as single-stranded f1 "packaged" phage when the bacteria has been infected by a helper phage. Since these filamentous helper phages (M13, f1) will not infect *E. coli* without an F´ episome coding for pili, **it is essential to use XL1-Blue MRF´ or a similar strain containing the F´ episome.**[11, 12]

pBluescript phagemids are offered with the IG region in either of two orientations: pBluescript (+) is replicated such that the sense strand of the β-galactosidase gene is secreted within the phage particles; pBluescript (–) is replicated such that the antisense strand of the β-galactosidase gene is secreted in the phage particles.

We offer helper phages that *preferentially* package pBluescript phagemids. Typically, 30–50 pBluescript molecules are packaged/helper phage DNA molecule. VCSM13 and R408 (Agilent Catalog #200251 and #200252, repectively) helper phage produce the largest amount of single-strand pBluescript. R408 (single-strand size ~4 kb) is more stable and can be grown more easily. VCSM13 (single-strand size ~6 kb), being more efficient, yields more single-stranded phagemid; however it is more unstable and reverts to wild-type more frequently. This difficulty can be addressed by periodically propagating VCSM13 in the presence of kanamycin. VCSM13 (a derivative of M13KO7) has a kanamycin gene inserted into the intergenic region, while R408 has a deletion in that region.

Yields of single-stranded (ss)DNA depend on the specific insert sequence. For most inserts, over 1 µg of ssDNA can be obtained from a 1.5-ml miniprep if grown in XL1-Blue MRF´. A faint single-strand helper phage band may appear on a gel at ~6 kb for VCSM13. This DNA mixture can be sequenced with primers that are specific for pBluescript and do not hybridize to the helper phage genome.

Site-specific mutagenesis is also possible using standard techniques. The advantages of using pBluescript phagemids for either purpose are as follows: (1) pBluescript phagemids do not replicate via the M13 cycle, lessening the tendency to delete DNA inserts, therefore it is unlikely that even 10-kb inserts will be deleted. (2) "Packaging" of pBluescript phagemids containing inserts is efficient since the pBluescript vector is significantly smaller than wild-type M13. (3) Oligonucleotide mutagenesis in pBluescript vectors is advantageous because the mutagenized insert is located between the T3 and T7 promoters. The resultant mutant transcripts can be synthesized in vitro without further subcloning.

## Single-Stranded Rescue Protocol

1. Inoculate a single colony into 5 ml of 2× YT broth[§] containing 100 µg/ml ampicillin and VCSM13 helper phage at $10^7$–$10^8$ pfu/ml (MOI ~10).

2. Grow the culture at 37°C with vigorous aeration for 1–2 hours.

3. Add kanamycin to 70 µg/ml to select for infected cells.

4. Continue growth at 37°C with vigorous aeration for 16–24 hours, or until growth has reached saturation.

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

6. 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.*

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

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

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

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

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

12. Remove ethanol and dry the DNA pellet.

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

14. 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 Uni-ZAP XR phage titer |
| | Poor rescue may be a result of toxic cDNA clones which can be isolated in lambda vectors but not in plasmid vectors. The ABLE C strain* and the ABLE K strain* reduce the copy number of common cloning vectors by ~4- and 10-fold, respectively, enhancing the probability that a toxic clone will be propagated. Positive clones observed on initial screening as lambda plaques can be excised and introduced into the ABLE strains. Excised phagemid libraries can also be screened directly in the ABLE strains |
| | 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 |

* ABLE competent cells (Agilent Catalog #200170–200172) and ABLE electroporation competent cells are available separately (Agilent Catalog #200160–200162).

## 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

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 pH to 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–Ampicillin Agar (per Liter)

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

### LB–Kanamycin Broth (per Liter)

Prepare 1 liter of LB broth
Autoclave
Cool to 55°C
Add 50 mg of filter-sterilized kanamycin

### LB–Kanamycin Agar (per Liter)

Prepare 1 liter of LB agar
Autoclave
Cool to 55°C
Add 5 ml of 10-mg/ml, filter-sterilized kanamycin
Pour into petri dishes (~25 ml/100-mm plate)

### LB–Tetracycline Broth (per Liter)

Prepare 1 liter of LB broth
Autoclave
Cool to 55°C
Add 12.5 mg of filter-sterilized tetracycline
Store broth in a dark, cool place as tetracycline is light-sensitive

### TE Buffer

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

### LB–Tetracycline Agar (per Liter)

Prepare 1 liter of LB agar
Autoclave
Cool to 55°C
Add 12.5 mg of filter-sterilized tetracycline
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

| **NZY Broth (per Liter)** | **NZY Agar (per Liter)** |
|---|---|
| 5 g of NaCl<br>2 g of $MgSO_4 \cdot 7H_2O$<br>5 g of yeast extract<br>10 g of NZ amine (casein hydrolysate)<br>Add deionized $H_2O$ to a final volume of 1 liter<br>Adjust the pH to 7.5 with NaOH<br>Autoclave | 5 g of NaCl<br>2 g of $MgSO_4 \cdot 7H_2O$<br>5 g of yeast extract<br>10 g of NZ amine (casein hydrolysate)<br>15 g of agar<br>Add deionized $H_2O$ to a final volume of 1 liter<br>Adjust the pH to 7.5 with NaOH<br>Autoclave<br>Pour into petri dishes (~25 ml/100-mm plate or ~80 ml/150-mm plate) |
| **NZY Top Agar (per Liter)** | **SM Buffer (per Liter)** |
| Prepare 1 liter of NZY broth<br>Add 0.7% (w/v) agarose<br>Autoclave | 5.8 g of NaCl<br>2.0 g of $MgSO_4 \cdot 7H_2O$<br>50.0 ml of 1 M Tris-HCl (pH 7.5)<br>5.0 ml of 2% (w/v) gelatin<br>Add deionized $H_2O$ to a final volume of 1 liter<br>Autoclave |
| **20× SSC Buffer (per Liter)** | **2× YT Broth (per Liter)** |
| 175.3 g of NaCl<br>88.2 g of sodium citrate<br>800.0 ml of deionized $H_2O$<br>Adjust to pH 7.0 with a few drops of 10 N NaOH<br>Add deionized $H_2O$ to a final volume of 1 liter | 10 g of NaCl<br>10 g of yeast extract<br>16 g of tryptone<br>Add deionized $H_2O$ to a final volume of 1 liter<br>Adjust to pH 7.5 with NaOH<br>Autoclave |

## REFERENCES


1. Hay, B. and Short, J. M. (1992) *Strategies* 5(1):16–18.
2. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
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. Dotto, G. P., Horiuchi, K. and Zinder, N. D. (1984) *J Mol Biol* 172(4):507-21.
9. Dente, L., Cesareni, G. and Cortese, R. (1983) *Nucleic Acids Res* 11(6):1645-55.
10. Mead, D. A., Skorupa, E. S. and Kemper, B. (1985) *Nucleic Acids Res* 13(4):1103-18.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