Lambda ZAP Premade Library

INSTRUCTION MANUAL

Revision A

BN #935202-12

For In Vitro Use Only

935202-12

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Lambda ZAP Premade Library

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Lambda ZAP Premade Library

MATERIALS PROVIDED

Material provided	Quantity
Amplified premade library constructed in the Lambda ZAP vector[a]	1 ml
Host strains[b]	
XL1-Blue MRF´ strain	0.5-ml bacterial glycerol stock
BB4 strain	0.5-ml bacterial glycerol stock
f1 helper phage[c]	
R408 interference-resistant helper phage	1 ml
VCSM13 interference-resistant helper phage	1 ml

[a] Shipped as a liquid in 7% (v/v) DMSO. On arrival, store the library at –80°C. Do not pass through more than two freeze–thaw cycles.

[b] Use the XL1-Blue MRF´ strain for plating excised phagemids and the BB4 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*).

STORAGE CONDITIONS

All Components: –80°C

ADDITIONAL MATERIALS REQUIRED

14-ml BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059)

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Revision A

INTRODUCTION

Overview of the Lambda ZAP Vector System

The Lambda ZAP vector[1, 2] system combines the high efficiency of lambda library construction and the convenience of a plasmid system (see Figure 1).

The Lambda ZAP vector has six unique cloning sites that will accommodate DNA inserts from <1 to 10 kb in length. Clones in the Lambda ZAP vector can be screened with either DNA probes or antibody probes. The Lambda ZAP vector allows rapid in vivo excision of the pBluescript phagemid, permitting your insert to be characterized in a plasmid system (see Figure 2). The polylinker of the pBluescript phagemid has 21 unique cloning sites flanked by T3 and T7 promoters and a choice of six 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.

The pBluescript phagemid in the Lambda ZAP vector contains the N-terminus of the *lacZ* gene, which can be α-complemented by the specific host strain used. There are 36 amino acids between the MET sequence and the *Eco*R I site. A total of 131 amino acids are coded for, but this is interrupted by the large polylinker.

Lambda ZAP Vector Map

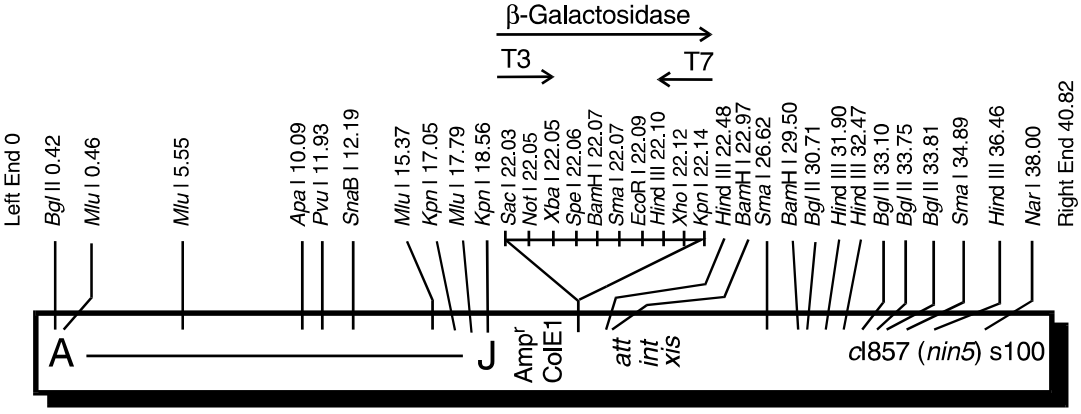
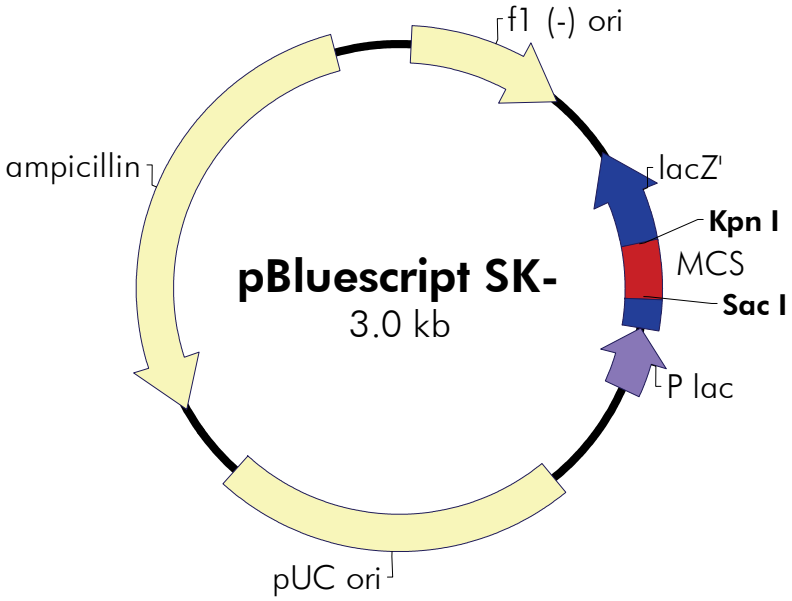
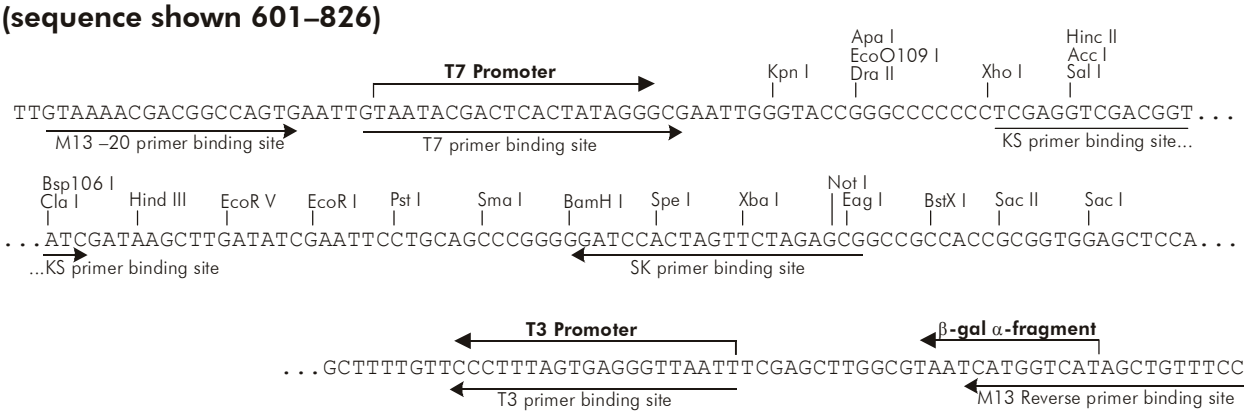


FIGURE 1 Map of the Lambda ZAP insertion vector.

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.stratagene.com* or from the GenBank® database (#X52324).

BACTERIAL HOST STRAINS

Host Strain Genotypes

Host strain	Genotype
BB4 strain	LE392.23 [F´*lacI*^{q}ZΔ*M15 proAB* Tn*10* (Tet^r)
XL1-Blue MRF´ strain	Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F´ *proAB lacI*^{q}ZΔ*M15* Tn*10* (Tet^r)]

BB4 and XL1-Blue MRF′ Bacterial Strain Descriptions

Two *Escherichia coli* host strains are supplied with the Lambda ZAP library: the XL1-Blue[3] MRF´, and BB4 strains. The BB4 strain is RecA$^+$, which supports vigorous growth of the Lambda ZAP phage. The RecA$^-$ *E. coli* host strain XL1-Blue MRF´ is also supplied for those hosts requiring a host recombination minus strain. Twelve hours of growth are usually required before plaques are visible on XL1-Blue MRF´ cells since XL1-Blue MRF´ supplies the *supE* tRNA suppresser, which only weakly suppresses the *s100* mutation in the Lambda ZAP vector. We recommend plating and screening the library on the BB4 strain. In addition, use of the correct host strain is important when working with the Lambda ZAP vector as the F´ episome present 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 Lambda ZAP 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 Lambda ZAP Vector*).

Third, the F´ episome contains the *lac* repressor (*lacI*^{q} gene), which blocks transcription from the *lac*Z 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, BB4 strain 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 Lambda ZAP 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 Lambda ZAP vector. Using these strains with the Lambda ZAP 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
BB4 strain	LB–tetracycline[a]	LB broth with supplements[a-c]	NZY[a]	—
XL1-Blue MRF´ strain	LB–tetracycline[a]	LB broth with supplements[a-c]	—	LB–ampicillin[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 bacterial 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.

Determining Background by Color Selection with IPTG and X-gal

The color selection by α-complementation with the Lambda ZAP 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 Lambda ZAP 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 Lambda ZAP 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 Lambda ZAP library: (1) the R408 interference-resistant helper phage and (2) the VCSM13 helper phage. The R408 helper phage is designed to allow efficient in vivo excision of the pBluescript phagemid from the Lambda ZAP vector. The 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 R408 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 R408 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 *Helper phage 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 R408 helper phage or between 1.0 × 10^{11} and 1.0 × 10^{12} pfu/ml for VCSM13 helper phage.

   Note *Helper phage 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 4.

TITERING THE LIBRARY

Preparing the Host Bacteria

1. Streak the BB4 cells onto an LB–tetracycline agar plate. Incubate the plate overnight at 37°C.

2. Inoculate 50 ml of LB broth with supplements in a sterile flask with a single colony of the BB4 host.

   Note *Do not add antibiotic to the overnight culture or to the titering plates. The antibiotic will bind to the bacterial cell wall and will inhibit the ability of the phage to infect the cell.*

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

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. The IPTG and X-gal should be added separately, with mixing in between additions, to the NZY top agar 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. Dilute the BB4 cells (from step 5 of *Preparing the Host Bacteria* in *Titering the Library*) to an OD_{600} of 0.5 with sterile 10 mM $MgSO_4$.

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

2. For amplified library titering, first dilute the amplified phage stock in SM buffer by the following amounts: 1:10,000, 1:100,000, 1:1,000,000. Add 1 µl of each dilution to 200 µl of host cells at an OD_{600} of 0.5.

   Note *The premade library has been through one round of amplification.*

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

4. Add the following components:

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

5. 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.

6. 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 10–100-fold above the background.

PERFORMING PLAQUE LIFTS

1. Titer the library suspension to determine the concentration using BB4 cells.

2. Combine the equivalent of 5×10^4 pfu/plate and 600 µl of freshly prepared BB4 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.[4, 5] Following these procedures, perform secondary and tertiary screenings also as outlined in the standard methodology texts.[4, 5] After an isolate is obtained, refer to Sambrook *et al.*[4] for suggested phage miniprep and maxiprep procedures.

ANTIBODY SCREENING PROTOCOL

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

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

IN VIVO EXCISION OF THE PBLUESCRIPT PHAGEMID FROM THE LAMBDA ZAP VECTOR

The Lambda ZAP 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.[6] These two regions are subcloned separately into the Lambda ZAP 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´ to 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 Lambda ZAP 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.

Signals for “packaging” the newly created phagemid are contained within the f1 terminator origin DNA sequence. They permit the circularized DNA to be “packaged” and secreted from the *E. coli*. Once the phagemid is secreted, the *E. coli* cells used for in vivo excision of the cloned DNA can be removed from the supernatant by heating at 70°C. The heat treatment kills all the *E. coli* cells while the phagemid remains resistant to the heat treatment. For production of double-stranded DNA, the “packaged” pBluescript DNA is mixed with fresh *E. coli* cells and spread on LB–ampicillin plates to produce colonies. DNA from minipreps of these colonies can be used for analysis of insert DNA including DNA sequencing, subcloning, mapping, and expression.

Miniprep DNA is also used for subsequent production of single-stranded DNA suitable for dideoxy-sequencing and site-specific mutagenesis.

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 BB4 and XL1-Blue MRF' cells in LB broth with supplements at 30°C.

Day 2

3. Gently spin down the BB4 and XL1-Blue MRF' 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 (8×10^8 cells/ml) in 10 mM $MgSO_4$.

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

   200 µl of BB4 cells at an OD_{600} of 1.0
   250 µl of phage stock (containing $>1 \times 10^5$ phage particles)
   1 µl of the R408 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 14-ml BD Falcon polypropylene round-bottom 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 XL1-Blue MRF' 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 dilute the supernatant to achieve single-colony isolation.

Colonies appearing on the plate contain the pBluescript double-stranded phagemid with the cloned DNA insert.

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 BB4 and XL1-Blue MRF' cells in LB broth with supplements at 30°C.

Day 2

2. Gently spin down the BB4 and XL1-Blue MRF' 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 (8×10^8 cells/ml) in 10 mM $MgSO_4$.

3. In a 50-ml conical tube, combine a portion of the amplified lambda bacteriophage library with BB4 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 R408 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 BB4 cells (1:10 lambda phage-to-cell ratio, noting that an OD_{600} of 1.0 corresponds to 8×10^8 cells/ml)
   - 10^9 pfu of R408 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 XL1-Blue MRF' 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.**[7, 8]

The Stratagene Products Division offers helper phages that *preferentially* package pBluescript phagemids. Typically, 30–50 pBluescript molecules are packaged/helper phage DNA molecule. 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.

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 ~4 kb for R408 or 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.

VCSM13 and R408 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), is more efficient at single-stranded DNA rescue and 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. We suggest R408 for excision of pBluescript from the Lambda ZAP vector and VCSM13 for single-stranded rescue.

Single-Stranded Rescue Protocol

1. Inoculate a single colony into 5 ml of 2× YT broth[§] containing 100 µg/ml ampicillin and VCSM13 or 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.

   Note *If using VCSM13, after 1–2 hours, add kanamycin to 70 µg/ml to select for infected cells.*

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

Observation	Suggestion
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 ZAP 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 (Stratagene Catalog #200170–200172) and ABLE electroporation competent cells (Stratagene Catalog #200160–200162) are available separately.

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–Ampicillin Agar (per Liter)

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

LB–Tetracycline Agar (per Liter)

Prepare 1 liter of LB agar
Autoclave
Cool to 55°C
Add 1.25 ml of 10 mg/ml-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

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

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)

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

TE Buffer

5 mM Tris-HCl (pH 7.5)
0.1 mM EDTA

SM Buffer (per Liter)

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

20× SSC Buffer (per Liter)

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

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

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ENDNOTES

MSDS INFORMATION

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