ZAP Express *Eco*R I Library

INSTRUCTION MANUAL

Revision A

BN #939201-12

For In Vitro Use Only

LIMITED PRODUCT WARRANTY

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

ORDERING INFORMATION AND TECHNICAL SERVICES

United States and Canada

Agilent Technologies
Stratagene Products Division
11011 North Torrey Pines Road
La Jolla, CA 92037

Telephone	(858) 373-6300
Order Toll Free	(800) 424-5444
Technical Services	(800) 894-1304
Internet	techservices@agilent.com
World Wide Web	www.stratagene.com

Europe

Location	Telephone	Fax	Technical Services
Austria	0800 292 499	0800 292 496	0800 292 498
Belgium	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 15775	0800 15740	0800 15720
France	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 919 288	0800 919 287	0800 919 289
Germany	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 182 8232	0800 182 8231	0800 182 8234
Netherlands	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 023 0446	+31 (0)20 312 5700	0800 023 0448
Switzerland	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 563 080	0800 563 082	0800 563 081
United Kingdom	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 917 3282	0800 917 3283	0800 917 3281

All Other Countries

Please contact your local distributor. A complete list of distributors is available at www.stratagene.com.

ZAP Express *Eco*R I Library

CONTENTS

ZAP Express *Eco*R I Library

MATERIALS PROVIDED

Materials provided	Quantity
Amplified premade library constructed in the ZAP Express vector*,[a]	1 ml
XL1-Blue MRF′ strain[b]	0.5-ml bacterial glycerol stock
XLOLR strain[b]	0.5-ml bacterial glycerol stock
ExAssist interference-resistant helper phage[c,d]	1 ml
R408 interference-resistant helper phage[d,e]	1 ml

[a] Premade libraries have been amplified one time and frozen in the presence of 7% DMSO. Upon arrival, store at –80°C. Do not pass through more than two freeze–thaw cycles.

[b] Use the XLOLR strain for plating excised phagemids and the XL1-Blue MRF′ strain for all other manipulations. For host strain shipping and storage conditions, see *Bacterial Host Strains*.

[c] The titer of the ExAssist interference-resistant helper phage is ~1.0×10^{10} pfu/ml. This supercoiled single-stranded DNA migrates at ~5 kb on an agarose gel. The ExAssist helper phage is recommended for excision of the pBK-CMV phagemid vector from the ZAP Express vector. It should not be used for single-stranded rescue in general, because this f1 helper phage possesses 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] Retiter after 1 month. (Take care not to contaminate the ZAP Express vector with this high-titer filamentous helper phage.) Store at –80°C.

[e] The titer of the R408 Interference-Resistant Helper Phage is ~7.5×10^{10} pfu/ml. This supercoiled single-stranded DNA migrates at ~4 kb on an agarose gel. The R408 Interference-Resistant Helper Phage is recommended for single-stranded rescue (see *Appendix: Recovery of Single-Stranded DNA from Cells Containing the pBK-CMV Phagemid Vector*).

ADDITIONAL MATERIALS REQUIRED

Isopropyl-1-thio-β-D-galactopyranoside (IPTG)
5-Bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-gal)
Nitrocellulose membrane

STORAGE CONDITIONS

Premade Library: –80°C

Bacterial Glycerol Stocks: –80°C

Helper Phage: –80°C

* U.S. Patent Nos. 5,286,636 and 5,128,256; European Patent No. 286200 and an equivalent foreign patent.
Revision A

NOTICES TO PURCHASER

The use of the CMV Promoter is covered under U.S. Patent Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation and licensed FOR RESEARCH USE ONLY. For further information, please contact UIRF at 319-335-4546.

The ZAP Express vector is covered by United States Patent Nos. 5,128,256 and 5,286,636. The purchase of this vector includes a limited, nonexclusive license under such patent rights to use the vector for the cloning, expression, and characterization of genes. This license does not grant rights to (1) use the ZAP Express vector for the reproduction, amplification, or modification of the vector; (2) offer the ZAP Express vector or any derivative thereof for resale; (3) distribute or transfer the ZAP Express vector or any derivative thereof to any third party; or (4) incorporate the ZAP Express vector or any derivative thereof in any genomic or cDNA library for resale, distribution, or transfer to any third party. No other license, express, implied, or by estoppel, is granted. For information concerning the availability of licenses to reproduce and/or modify the ZAP Express vector, please contact Stratagene products division Technical Services Department at 1-800-894-1304.

This product is for research purposes only and must be used in accordance with NIH guidelines for recombinant DNA.

Overview of the ZAP Express Vector System

The ZAP Express vector (see Figure 1) allows both eukaryotic and prokaryotic expression while also increasing both cloning capacity and the number of unique lambda cloning sites.[1] The ZAP Express vector has 12 unique cloning sites which will accommodate DNA inserts 0–12 kb long. The 12 unique cloning sites are *Apa* I, *Bam*H I, *Eco*R I, *Hin*d III, *Kpn* I, *Not* I, *Sac* I, *Sal* I, *Sma* I, *Spe* I, *Xba* I, and *Xho* I. Inserts cloned into the ZAP Express vector can be excised out of the phage in the form of the kanamycin-resistant pBK-CMV phagemid vector (see Figure 2) by the same excision mechanism found in Lambda ZAP vectors.[2,3]

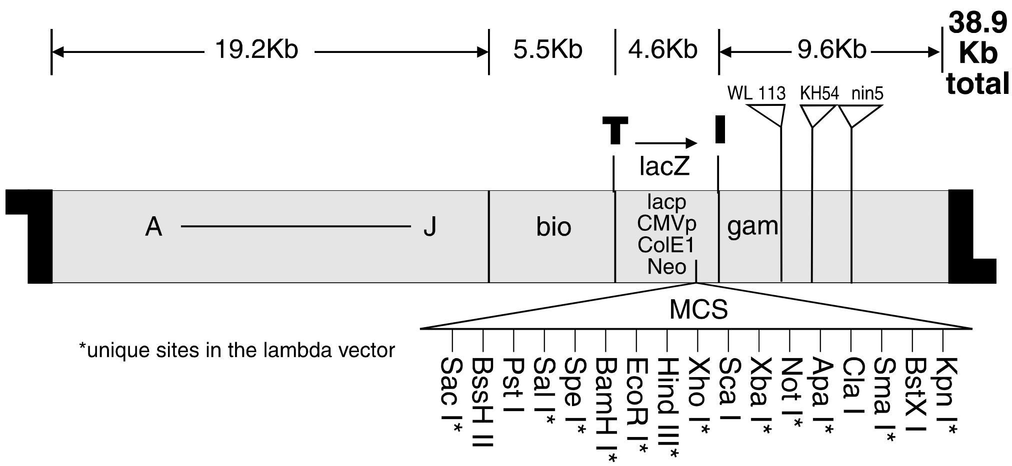


FIGURE 1 Map of the ZAP Express vector. Phage Arms Genotype: *Δcl*, *Δatt*, *Δint*, *Δxis*, *ΔWL113*, *ΔKH54*, *Δnin5*, *red⁻ gam⁺*, *bio*.

Clones in the ZAP Express vector can be screened with either DNA probes or antibody probes, and in vivo rapid excision of the pBK-CMV phagemid allows insert characterization in a plasmid system. The polylinker of pBK-CMV has 17 unique cloning sites flanked by T3 and T7 promoters, and it has 3 standardized primer sites for DNA sequencing. The plasmid has the bacteriophage f1 origin of replication allowing rescue of single-stranded DNA, which can be used for DNA sequencing or site-directed mutagenesis. Unidirectional deletions can be made using exonuclease III and mung-bean nuclease by taking advantage of the unique positioning of 5´ and 3´ restriction sites. Transcripts made from the T3 and T7 promoters generate riboprobes useful in Southern and Northern blotting, and the *lacZ* promoter may be used to drive expression of fusion proteins suitable for Western blot analysis or protein purification.

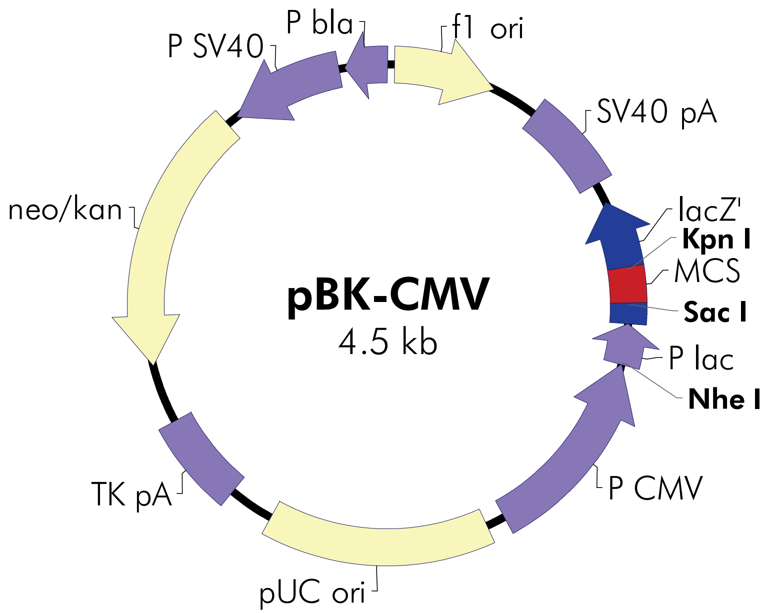
Eukaryotic expression of inserts is driven by the cytomegalovirus (CMV) immediate early (IE) promoter with the SV40 transcription terminator and polyadenylation signal.

Stable selection of clones in eukaryotic cells is made possible by the presence of the neomycin/kanamycin-resistance gene, which is driven by the SV40 early promoter with TK transcription termination and polyadenylation signals to render transfectants resistant to G418 (geneticin).

Premade Zap Express *Eco*R I libraries are primed with an oligo poly(dt) linker-primer, digested to create blunt ends, and ligated with *Eco*R I adaptors. The adaptors are comprised of 9- and 13-mer oligonucleotides, which are complementary to each other and have an *Eco*R I cohesive end. The adaptors have the following sequence:

```
5´ AATTCGGCACGAG 3´
3´     GCCGTGCTC 5´
```

The pBK-CMV Vector



pBK-CMV Multiple Cloning Site Region
(sequence shown 952–1196)

T7 Promoter → ; Kpn I ; BstX I ; Sma I

GTAAAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTGGGTACACTTACCTGGTACCCCACCCGGGTGGAAA...

M13 –20 primer binding site ; T7 primer binding site

Apa I ; Not I ; Xba I ; Sca I ; Xho I ; Hind III ; EcoR I ; BamH I ; Spe I ; Acc I Sal I ; Pst I

...ATCGATGGGCCCGCGGCCGCTCTAGAAGTACTCTCGAGAAGCTTTTTGAATTCTTTGGATCCACTAGTGTCGACCTGCAG...

BssH II ; Sac I ; ← T3 Promoter ; ← β-gal α-fragment

...GCGCGCGAGCTCCAGCTTTTGTTCCCTTTAGTGAGGGTTAATTTCGAGCTTGGCGTAATCAAGGTCATAGCTGTTTCCTGT

T3 primer binding site ; BK reverse primer binding site

Feature	Nucleotide Position
f1 origin of ss-DNA replication	24–330
SV40 polyA signal	469–750
β-galactosidase α-fragment coding sequence (lacZ′)	812–1183
multiple cloning site	1015–1122
lac promoter	1184–1305
CMV promoter	1306–1895
pUC origin of replication	1954–2621
HSV-thymidine kinase (TK) polyA signal	2760–3031
neomycin/kanamycin resistance ORF	3209–4000
SV40 promoter	4035–4373
bla promoter	4392–4518

Figure 2 The pBK-CMV phagemid vector. The complete sequence and list of restriction sites are available at *www.stratagene.com*.

BACTERIAL HOST STRAINS

Host Strain Genotypes

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

[a] Use the XLOLR strain for excision only.

XL1-Blue MRF´ Bacterial Strain Description

The RecA$^-$ *E. coli* host strain XL1-Blue MRF´ is supplied with the ZAP Express library.[3] Because the pBK-CMV phagemid vector does not require a *supF* genotype, the amplified library grows very efficiently on the XL1-Blue MRF´ strain. In addition, use of the correct host strain is important when working with the pBK-CMV phagemid vector as the F´ episome present in the XL1-Blue MRF´ strain serves three purposes.

First, the Δ*M15 lac*Z 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 *lac*Z 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 ZAP Express clone to a pBK-CMV phagemid vector requires superinfection with a filamentous helper phage, the F´ episome is required for in vivo excision (see *In Vivo Excision of the pBK-CMV Phagemid Vector from the ZAP Express 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, 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 ZAP Express 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 ZAP Express vector. The SURE strain and the SOLR strain are not compatible with the ZAP Express system, since these strains contain the kanamycin-resistance gene found in the pBK-CMV phagemid vector. Using these strains with the ZAP Express 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
XLOLR strain	LB-tetracycline[a]	LB broth with supplements[a-c]	—	LB-kanamycin[a]
XL1-Blue MRF´ strain	LB-tetracycline[a]	LB broth with supplements[a-c]	NZY[a]	—

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

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

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

Establishing an Agar Plate Bacterial Stock

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

Note *The host strains may thaw during shipment. The vials should be stored immediately at –20° or –80°C, but most strains remain viable longer if stored at –80°C. It is best to avoid repeated thawing of the host strains in order to maintain extended viability.*

1. Revive the stored cells by scraping off splinters of solid ice with a sterile wire loop.

2. Streak the splinters onto an LB agar plate containing the appropriate antibiotic (see *Recommended Media*), if one is necessary.

3. Incubate the plate overnight at 37°C.

4. Seal the plate with Parafilm® laboratory film and store the plate at 4°C for up to 1 week.

5. Restreak the cells onto a fresh plate every week.

Preparing a –80°C Bacterial Glycerol Stock

1. In a sterile 50-ml conical tube, inoculate 10 ml of LB broth with the appropriate antibiotic (see *Recommended Media*) with one colony from the plate. Grow the cells to late log phase.

2. Add 4.5 ml of a sterile glycerol-liquid medium solution (prepared by mixing 5 ml of glycerol + 5 ml of the appropriate medium) to the bacterial culture from step 1. Mix well.

3. Aliquot into sterile centrifuge tubes (1 ml/tube).

This preparation may be stored at –20°C for 1–2 years or at –80°C for more than 2 years.

Color Selection by IPTG and X-gal

The color selection by α-complementation with the ZAP Express vector requires a high amount of IPTG and X-gal for generation of the blue color (see *Titering Procedure*). Transcription and translation of the fusion protein are normal, but the large polylinker present within the pBK-CMV phagemid vector, which is present in the ZAP Express 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 ZAP Express vector is much less per cell than the copy number of pBK-CMV phagemid vector derivatives. 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.

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 $1000 \times g$ for 10 minutes then gently resuspended in 10 ml of 10 mM $MgSO_4$. Before use, dilute cells to an OD_{600} of 0.5 with 10 mM $MgSO_4$. Bacterial cells prepared in this manner can be used for all phage manipulations described within the manual. Highest efficiencies are obtained from freshly prepared cells.

HELPER PHAGE

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

Storing the Helper Phage

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

Titering the Helper Phage

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

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

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

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

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

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

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

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

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

Amplifying the Helper Phage

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

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

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

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

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

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

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

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

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

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

9. For further details about helper phage titering or amplification, please see *Titering the Helper Phage* or Reference 4.

PREPARING PLATING CULTURES

1. Streak the XL1-Blue MRF´ cells onto an LB–tetracycline agar plate. Incubate the plate overnight at 37°C.

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

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

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

Note *The lower temperature keeps the bacteria from overgrowing, thus reducing the number of nonviable cells. Phage can adhere to nonviable cells resulting in a decreased titer.*

4. Pellet the bacteria at 1000 × *g* for 10 minutes.

5. Carefully decant the media off the cell pellet and gently resuspend the pellet in 25 ml of sterile 10 mM $MgSO_4$.

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

6. Dilute the cells to $OD_{600} = 0.5$ with 10 mM $MgSO_4$. Approximately 600 µl of $OD_{600} = 0.5$ cells are needed for each 150-mm plate and 200 µl of $OD_{600} = 0.5$ cells for each 100-mm plate.

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

TITERING PROCEDURE

A background test can be completed by plating several hundred plaques on a plate (see *Color Selection by 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. Nonrecombinant (background) plaques are blue, while recombinant plaques are white.

1. Prepare the host bacteria as outlined in *Preparing Plating Cultures*.

2. 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.

3. Incubate the phage and bacteria for 15 minutes at 37°C 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

AMPLIFYING THE ZAP EXPRESS 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 ZAP Express library.

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

Day 1

1. Prepare the host bacteria as outlined in *Preparing Plating Cultures.*

    Note *In order to obtain the highest amplification efficiency, use freshly prepared host strains.*

Day 2

2. Combine aliquots of the library suspension containing ~5 × 10^4 pfu of bacteriophage with 600 μl of host cells at an OD_{600} of 0.5 in Falcon® 2059 polypropylene tubes. To amplify 1 × 10^6 plaques, use a total of 20 aliquots (each aliquot contains 5 × 10^4 plaques/150-mm plate).

    Note *Do not add more than 300 μl of phage volume/600 μl of cells.*

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

4. 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.

5. After the NZY top agar solidifies, incubate the plates at 37°C for 6–8 hours. Do not allow the plaques to grow larger than 1–2 mm. On completion, the plaques should be touching.

6. 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

7. 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.

8. Remove the cell debris by centrifugation for 10 minutes at 500 × *g*.

9. Recover and transfer the supernatant 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.

10. Check the titer of the amplified library using host cells and serial dilutions of the library. (Assume ~10^9–10^{11} pfu/ml.)

Performing Plaque Lifts

1. Titer the 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.

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

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] When using the ZAP Express vector, perform in vivo excision on the isolates to obtain the insert-containing pBK-CMV phagemid vector (see *In Vivo Excision of the pBK-CMV from the ZAP Express Vector* and *In Vivo Excision Protocols Using ExAssist Helper Phage with XLOLR Strain*).

ANTIBODY SCREENING IN *Escherichia coli*

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 [*pico*Blue immunoscreening kit, Stratagene Catalog #200371 (goat anti-rabbit) and #200372 (goat anti-mouse)].

EUKARYOTIC SCREENING WITH THE ZAP EXPRESS LIBRARY

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

Selective Assay

Devising a selective assay for eukaryotic library screening requires a cell line that can grow in nonselective media and where expression of a transfected gene permits growth in selective media. An example of this method is screening for a thymidine kinase (TK) gene in L-TK$^-$ cells. If TK$^-$ cells are grown in HAT media, only those cells transfected with a clone coding for a protein capable of replacing TK will grow.

Panning Assay

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

Functional Assay

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

After a clone has been identified within the eukaryotic cells, the clone can be retrieved by several methods. Plasmid DNA within the tissue culture cells can be collected using the Hirt, Birnboin, and Doly procedures,[6,7] then transferred into *E. coli* cells for amplification and plasmid DNA preparations. Simmons *et al.*[8] were able to screen libraries in COS cells, where the presence of the SV40 T antigen increases the copy number of phagemids containing the SV40 origin of replication. This results in a higher episomal copy number, which may help in the retrieval of the plasmids. Inserts can also be isolated by polymerase chain reaction (PCR) amplification of the tissue culture cells using T3/T7 primer sets. The resulting PCR fragment can be digested using restriction sites flanking the insert, then recloned into pBK-CMV phagemid DNA for further analysis.

Note *Screening libraries in eukaryotic cells can be extremely laborious. Many functional assays are not sensitive enough to detect a clone from pools of nonrelated clones. Therefore, it is worth considering the use of techniques, such as differential PCR,[9] selective hybridization,[10] and degenerate oligonucleotides, to develop DNA probes for initial screening using prokaryotic plaques. Positive clones can then be screened by eukaryotic transfection and expression.*

Protocol

Eukaryotic screening can be performed with cesium-banded, double-stranded phagemid DNA prepared from the excised library (see *In Vivo Excision of the pBK-CMV Phagemid Vector from the ZAP Express Vector*). The library can be introduced into the eukaryotic cells as separate pools or as an entire library, depending on the assay system.

EUKARYOTIC EXPRESSION

The CMV promoter is considered to be a strong promoter and to function in many different cell lines.[11] However, expression in eukaryotic cells is sensitive to many factors. If little or no expression is observed in eukaryotic cells, several factors can be considered.

1. For library screening where the insert may not be full length, the *lacZ* ATG allows the expression and detection of fusion protein. However, the 5´-untranslated sequences and amino-terminal fusion can affect expression levels of some inserts. If the clone is identified by prokaryotic screening techniques and is known to be full length (i.e., containing its own ATG and Kozak sequence[12]), the prokaryotic 5´ UT sequences may be removed by digesting the excised phagemid with *Nhe* I and *Spe* I, by religating, and by screening for clones which lost the 200-bp fragment. Other cloning strategies may be used if these sites exist within the insert. Removal of this region has been shown to increase expression levels in some inserts. This effect may be due to increased specific activity of the expressed protein by eliminating the expression of fusion proteins, increased RNA stability, or increased translation efficiency by removing the competing upstream *lacZ* ATG.

2. Methylation of some insert DNA can prevent expression in some cell lines.[13]

3. The promoter may not be functional in some cell lines and should be tested before screening a library.

IN VIVO EXCISION OF THE PBK-CMV PHAGEMID VECTOR FROM THE ZAP EXPRESS VECTOR

The ZAP Express 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.[2,14] This in vivo excision depends on the placement of the DNA sequences within the lambda phage genome and on the presence of a variety of proteins, including filamentous (e.g., M13) bacteriophage-derived proteins. The M13 phage proteins recognize a region of DNA normally serving as the f1 bacteriophage "origin of replication." This origin of replication can be divided into two overlying parts: (1) the site of initiation and (2) the site of termination for DNA synthesis.[15] These two regions are subcloned separately into the ZAP Express vector. The lambda phage (target) is made accessible to the M13-derived proteins by simultaneously infecting a strain of *E. coli* with both the lambda vector and the M13 helper phage.

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

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

IN VIVO EXCISION PROTOCOLS USING EXASSIST HELPER PHAGE WITH XLOLR STRAIN

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

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

Mass excision can be used to generate subtraction libraries and subtracted DNA probes. Converting the library to the phagemid form also allows screening of the phagemid library in eukaryotic cells by transformation of eukaryotic cells with supercoiled plasmid DNA.[1,16]

Single-Clone Excision Protocol

Day 1

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

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

Day 2

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

4. Combine the following components in a Falcon 2059 polypropylene tube:

   200 μl of XL1-Blue MRF´ cells at an OD_{600} of 1.0
   250 μl of phage stock (containing >1 × 10^5 phage particles)
   1 μl of the ExAssist helper phage (>1 × 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 Falcon 2059 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 Falcon 2059 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 Falcon 2059 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 Falcon 2059 polypropylene tube. This stock contains the excised pBK-CMV phagemid packaged as filamentous phage particles and may be stored at 4°C for 1–2 months.

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

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

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

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

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

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

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

R408 helper phage is recommended for the single-stranded rescue procedure. The single-stranded rescue procedure can be found in *Appendix: Recovery of Single-Stranded DNA from Cells Containing the pBK-CMV Phagemid Vector*.

Mass Excision Protocol

Day 1

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

Day 2

2. Gently spin down the XL1-Blue MRF´ and XLOLR cells (1000 × *g*). Resuspend each of the cell pellets in 25 ml of 10 mM $MgSO_4$. Measure the OD_{600} of the cell suspensions, then adjust the concentration of the cells to an OD_{600} of 1.0 (8×10^8 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 8×10^8 cells/ml)
   10^9 pfu of ExAssist helper phage (10:1 helper phage-to-cells ratio)

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

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

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

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

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

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

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

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

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

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

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

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

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

APPENDIX: RECOVERY OF SINGLE-STRANDED DNA FROM CELLS CONTAINING THE PBK-CMV PHAGEMID VECTOR

The pBK-CMV vector is a phagemid that can be secreted as single-stranded DNA (ssDNA) in the presence of M13 helper phage. These phagemids contain the intergenic (IG) region of a filamentous f1 phage. This region encodes all of the *cis*-acting functions of the phage required for packaging and replication. In *E. coli* with the F^+ phenotype (containing an F´ episome), pBK-CMV phagemid vectors will be secreted as single-stranded f1 "packaged" phage when the bacteria have been infected by a helper phage. Because these filamentous helper phages (M13, f1) will not infect *E. coli* without an F´ episome coding for pili, **it is essential to use the XL1-Blue MRF´ strain or a similar strain containing the F´ episome.**[17,18]

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

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

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

Single-Stranded Rescue Protocol

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

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

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

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

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

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

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

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

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

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

10. Remove ethanol and dry the DNA pellet.

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

12. Analyze 1–2 μl on an agarose gel.

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

Troubleshooting

Plating

Observations	Suggestions
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 ZAP Express phage titer
	Ensure that the platings are performed using agar plates containing kanamycin
	The lambda phage stock aliquot used for in vivo excision cannot contain chloroform, as chloroform lyses the *E. coli* cells. Briefly spin the lambda phage stock to ensure that the chloroform is separated completely before removing the aliquot

PREPARATION OF MEDIA AND REAGENTS

LB Agar (per Liter) 10 g of NaCl 10 g of tryptone 5 g of yeast extract 20 g of agar Add deionized H_2O to a final volume of 1 liter Adjust pH to 7.0 with 5 N NaOH Autoclave Pour into petri dishes (~25 ml/100-mm plate)	**LB Broth (per Liter)** 10 g of NaCl 10 g of tryptone 5 g of yeast extract Add deionized H_2O to a final volume of 1 liter Adjust to pH 7.0 with 5 N NaOH Autoclave
LB Broth with Supplements Prepare 1 liter of LB broth Autoclave Add the following filter-sterilized supplements prior to use 10 ml of 1 M $MgSO_4$ 3 ml of a 2 M maltose solution or 10 ml of 20% (w/v) maltose	**LB–Kanamycin Agar (per Liter)** 1 liter of LB agar Autoclave Cool to 55°C Add 7.5 ml of 10 mg/ml kanamycin (filter-sterilized) Pour into petri dishes (~25 ml/100-mm plate)
LB–Tetracycline Agar (per Liter) 1 liter of LB agar Autoclave Cool to 55°C Add 1.5 ml of 10 mg/ml tetracycline (filter-sterilized) Pour into petri dishes (~25 ml/100-mm plate) Store plates in a dark, cool place or cover plates with foil if left out at room temperature for extended time periods as tetracycline is light-sensitive	**LB–Tetracycline Broth (per Liter)** Prepare 1 liter of LB broth Autoclave Cool to 55°C Add 1.25 ml of 10 mg/ml-filter-sterilized tetracycline Store broth in a dark, cool place as tetracycline is light-sensitive
NZY Agar (per Liter) 5 g of NaCl 2 g of $MgSO_4 \cdot 7H_2O$ 5 g of yeast extract 10 g of NZ amine (casein hydrolysate) 15 g of agar Add deionized H_2O to a final volume of 1 liter Adjust the pH to 7.5 with NaOH Autoclave Pour into petri dishes (~80 ml/150-mm plate)	**NZY Broth (per Liter)** 5 g of NaCl 2 g of $MgSO_4 \cdot 7H_2O$ 5 g of yeast extract 10 g of NZ amine (casein hydrolysate) Add deionized H_2O to a final volume of 1 liter Adjust the pH to 7.5 with NaOH Autoclave

NZY Top Agar (per Liter)	SM Buffer (per Liter)
1 liter of NZY broth Add 0.7% (w/v) agarose Autoclave	5.8 g of NaCl 2.0 g of $MgSO_4 \cdot 7H_2O$ 50.0 ml of 1 M Tris-HCl (pH 7.5) 5.0 ml of 2% (w/v) gelatin Add deionized H_2O to a final volume of 1 liter
20× SSC Buffer (per Liter) 175.3 g of NaCl 88.2 g of sodium citrate 800.0 ml of deionized H_2O 10.0 N NaOH Adjust to pH 7.0 with a few drops of 10.0 N NaOH Add deionized H_2O to a final volume of 1 liter	**2× YT Broth (per Liter)** 10 g of NaCl 10 g of yeast extract 16 g of tryptone Add deionized H_2O to a final volume of 1 liter Adjust to pH 7.5 with NaOH Autoclave
TE Buffer 10 mM Tris-HCl (pH 7.5) 1 mM EDTA	

REFERENCES

1. Alting-Mees, M., Hoener, P., Ardourel, D., Sorge, J. and Short, J. M. (1992) *Strategies* 5(3):58-61.
2. Short, J. M., Fernandez, J. M., Sorge, J. A. and Huse, W. D. (1988) *Nucleic Acids Res* 16(15):7583-600.
3. Short, J. M. and Sorge, J. A. (1992) *Methods Enzymol* 216:495-508.
4. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
5. 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.
6. Hirt, B. (1967) *J Mol Biol* 26(2):365-9.
7. Birnboim, H. C. and Doly, J. (1979) *Nucleic Acids Res* 7(6):1513-23.
8. Simmons, D. L., Satterthwaite, A. B., Tenen, D. G. and Seed, B. (1992) *J Immunol* 148(1):267-71.
9. Liang, P. and Pardee, A. B. (1992) *Science* 257(5072):967-71.
10. Lee, M. G. and Nurse, P. (1987) *Nature* 327(6117):31-5.
11. Foecking, M. K. and Hofstetter, H. (1986) *Gene* 45(1):101-5.
12. Kozak, M. (1989) *J Cell Biol* 108(2):229-41.
13. MacGregor, G. R., Mogg, A. E., Burke, J. F. and Caskey, C. T. (1987) *Somat Cell Mol Genet* 13(3):253-65.
14. Jerpseth, B., Greener, A., Short, J. M., Viola, J. and Kretz, P. L. (1992) *Strategies* 5(3):81–83.
15. Dotto, G. P., Horiuchi, K. and Zinder, N. D. (1984) *J Mol Biol* 172(4):507-21.
16. Schweinfest, C. W., Henderson, K. W., Gu, J. R., Kottaridis, S. D., Besbeas, S. *et al.* (1990) *Genet Anal Tech Appl* 7(3):64-70.
17. Dente, L., Cesareni, G. and Cortese, R. (1983) *Nucleic Acids Res* 11(6):1645-55.
18. Mead, D. A., Skorupa, E. S. and Kemper, B. (1985) *Nucleic Acids Res* 13(4):1103-18.

ENDNOTES

Falcon® is a registered trademark of Becton Dickinson and Company.
Parafilm® is a registered trademark of American National Can.
Pyrex® is a registered trademark of Corning Glass Works.
Whatman® is a registered trademark of Whatman Ltd.

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.