Uni-ZAP® XR Library

Custom and Premade Libraries

INSTRUCTION MANUAL

Revision #078001d

BN #937111-12

STORAGE CONDITIONS

Custom Library: 4°C

Premade Library: –80°C

Bacterial Glycerol Stocks: –80°C

Helper Phage: –80°C

For in Vitro Use Only

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Uni-ZAP® XR Library

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Uni-ZAP® XR Library

MATERIALS PROVIDED

	Quantity	
Materials provided	**Premade Library**	**Custom Library**
Amplified premade library constructed in the Uni-ZAP® XR vector[a]	1 ml	—
Custom library constructed in the Uni-ZAP® XR vector[b]	—	
Host strains[c]		
XL1-Blue MRF´ strain	0.5-ml bacterial glycerol stock	0.5-ml bacterial glycerol stock
SOLR™ strain	0.5-ml bacterial glycerol stock	0.5-ml bacterial glycerol stock
f1 helper phage[d]		
ExAssist™ interference-resistant helper phage	1 ml	1 ml
VCSM13 Interference-Resistant Helper Phage	1 ml	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] Amplified custom libraries are shipped at 4°C without DMSO. Upon arrival, add DMSO to 7%, aliquot, and store at –80°C. Do not pass through more than two freeze–thaw cycles.

[c] Use the SOLR strain for plating excised phagemids and the XL1-Blue MRF´ strain for all other manipulations. For host strain storage conditions, see *Preparing the Host Strains*.

[d] Retiter after 1 month. (Take care not to contaminate the Uni-ZAP® XR vector with this high-titer filamentous helper phage.) Store at –20°C. Stratagene recommends VCSM13 interference-resistant helper phage for single stranded rescue. ExAssist™ interference-resistant helper phage has α-complementing β-galactosidase sequences which may interfere with sequencing or site-directed mutagenesis where oligonucleotide primers hybridize to β-galactosidase sequences (e.g., M13–20 primer).

ADDITIONAL MATERIALS REQUIRED

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

Revision #078001d

INTRODUCTION

The Uni-ZAP® XR vector system combines the high efficiency of lambda library construction and the convenience of a plasmid system with blue–white color selection. The Uni-ZAP XR vector (see Figure 1) is double digested with *Eco*R I and *Xho* I and will accommodate DNA inserts from 0 to 10 kb in length. The Uni-ZAP XR vector can be screened with either DNA probes or antibody probes and allows in vivo excision of the pBluescript® phagemid (see Figure 2), allowing the insert to be characterized in a plasmid system[1]. The polylinker of the pBluescript phagemid has 21 unique cloning sites flanked by T3 and T7 promoters and a choice of 6 different primer sites for DNA sequencing. The phagemid has the bacteriophage f1 origin of replication, allowing rescue of single-stranded DNA, which can be used for DNA sequencing or site-directed mutagenesis. Unidirectional deletions can be made with exonuclease III and mung bean nuclease by taking advantage of the unique positioning of 5´ and 3´ restriction sites. Transcripts made from the T3 and T7 promoters generate riboprobes useful in Southern and Northern blotting, and the *lac*Z promoter may be used to drive expression of fusion proteins suitable for Western blot analysis or protein purification.

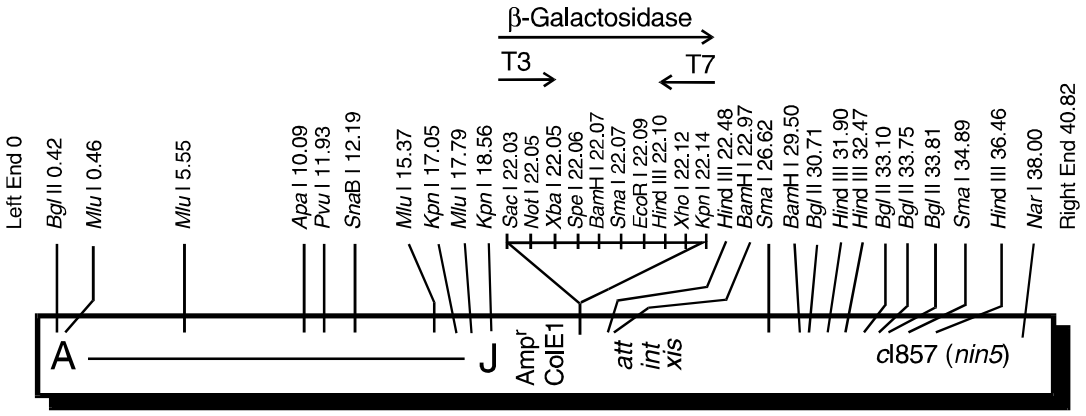


FIGURE 1. Map of the Uni-ZAP® XR insertion vector.

The pBluescript® SK (+/–) phagemid is a 2958-bp phagemid derived from pUC 19. The SK designation indicates the polylinker is oriented such that lacZ transcription proceeds from Sac I to Kpn I.

f1 (+) origin (6–462 bp) f1 filamentous phage origin of replication allowing recovery of the sense strand of the lacZ gene when a host strain containing the pBluescript phagemid is co-infected with helper phage.

f1 (–) origin (6–462 bp) f1 filamentous phage origin of replication allowing recovery of the antisense strand of the lacZ gene when a host strain containing the pBluescript phagemid is co-infected with helper phage.

ColE1 origin (1032–1912 bp) Plasmid origin of replication used in the absence of helper phage.

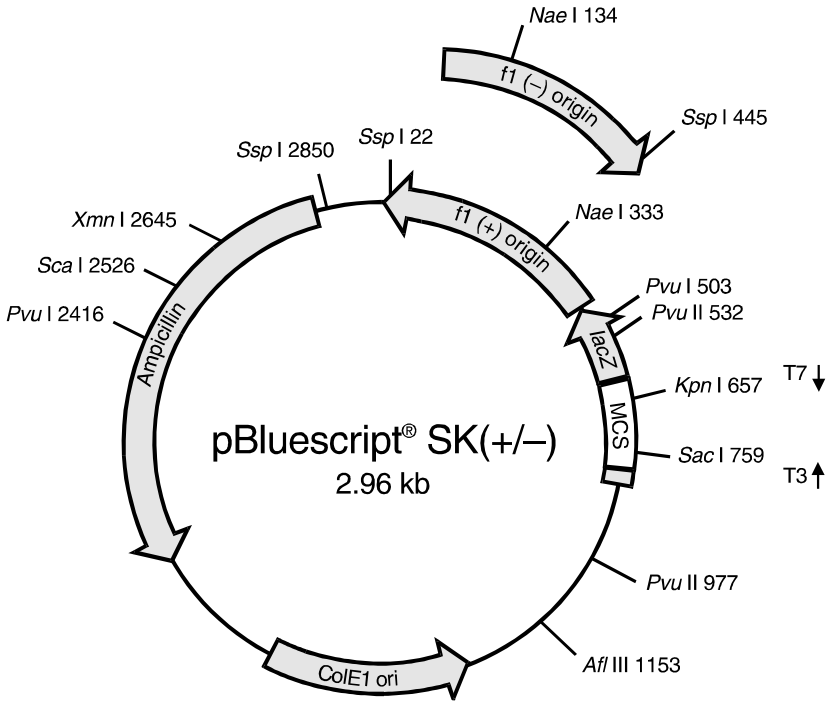
LacZ (Lac promotor: 816–938 bp) This portion of the lacZ gene provides α-complementation for blue–white color selection of recombinant phagemids. An inducible lac promotor upstream from the lacZ gene permits fusion protein expression with the b-galactosidase gene product.

MCS (657–759 bp) Multiple cloning site flanked by T3 and T7 promotors. (Please see the polylinker sequence below.)

Ampicillin (1975–2832 bp) Ampicillin-resistance gene for antibiotic selection of the phagemid vector.

Please note: The upper strand is designated the (+) strand, and the lower strand is designated the (–) strand.

GenBank® #52325 [SK(+)] and #52324 [SK(–)].



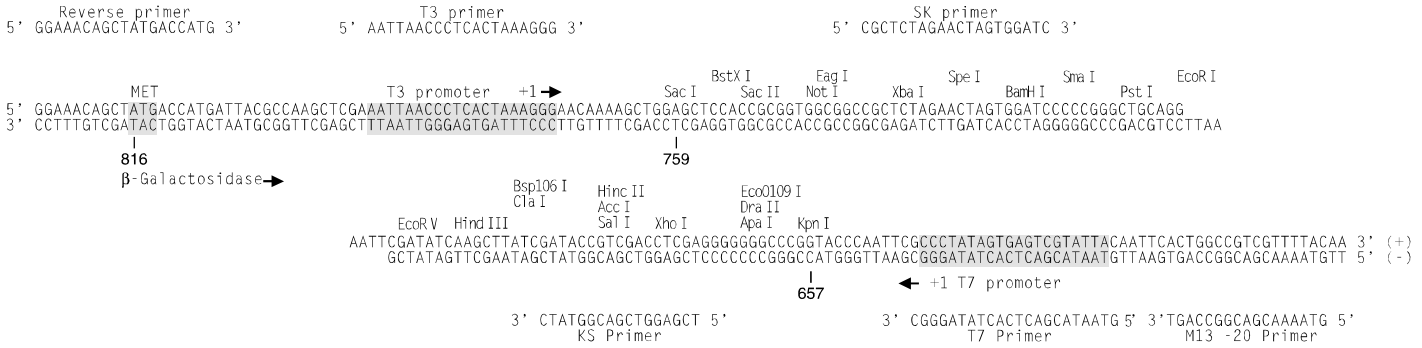


FIGURE 2 Circular map and polylinker of the pBluescript® SK(+/–) phagemid.

The library was synthesized using the ZAP-cDNA® synthesis method.[2] The linker–primer was designed with a GAGA sequence to protect the *Xho* I restriction enzyme recognition site and an 18-base poly(dT) sequence. The restriction site allows the finished cDNA to be inserted into the vector unidirectionally in the sense orientation with respect to the *lacZ* promotor. The linker–primer is a 50-base oligonucleotide with the following sequence:

5′ GAGAGAGAGAGAGAGAGAGAACTAGT**CTCGAG**$(T)_{18}$ 3′
GAGA sequence *Xho* I

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 pBluescript SK(–) plasmid in the Uni-ZAP XR 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 from the MET sequence to the *Eco*R I site. A total of 131 amino acids are coded for, but this is interrupted by the large polylinker.

Host Strains

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

First, the Δ*M15 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 Uni-ZAP XR clone to a pBluescript phagemid requires superinfection with a filamentous helper phage, the F′ episome is required for in vivo excision (see In Vivo *Excision of the pBluescript Phagemid from the Uni-ZAP XR Vector*).

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

Note *The strains used for the Lambda gt11 vector (i.e., Y1088, Y1089, and Y1090) are not suitable for use with the Uni-ZAP XR vector because these strains contain the plasmid pMC9, a pBR322 derivative, which contains many of the same sequences as those found in the phagemid portion of the Uni-ZAP XR vector. Using these strains with the Uni-ZAP XR vector could result in recombination between the homologous sequences.*

Helper Phage

Two different helper phages are provided with the Uni-Zap XR library: (1) the ExAssist interference-resistant helper phage with SOLR strain[4] and (2) the VCSM13 helper phage. The ExAssist interference-resistant helper phage with SOLR strain is designed to allow efficient in vivo excision of the pBluescript phagemid from the Uni-ZAP XR vector while preventing the problems that can be associated with helper phage co-infection. The ExAssist helper phage contains an amber mutation that prevents replication of the phage genome in a nonsuppressing *E. coli* strain (e.g., SOLR cells). Only the excised phagemid can replicate in the host, removing the possibility of co-infection from the ExAssist helper phage. The ExAssist helper phage cannot be used for single-stranded rescue due to its inability to replicate in the SOLR strain. The other helper phage, VCSM13 helper phage, is recommended for single-stranded rescue procedures from the excised pBluescript phagemids.

Storing the Helper Phage

The ExAssist helper phage and the VCSM13 helper phage may be stored at –80°, –20°, or 4°C. Some loss of titer may occur after 2 months of storage at 4°C. The stability will increase to 1 year at –80°C. If the titer drops over time, prepare a fresh high-titer stock of the helper phage as outlined in *Amplification*.

Titering the Helper Phage

If the ExAssist helper phage has been stored at 4°C for >1 month or passed through a freeze–thaw cycle, titer the helper phage with XL1-Blue MRF´ cells.

1. Dilute the phage (10^{-4}–10^{-7}) in TE buffer and combine 1 µl of each dilution with 200 µl of XL1-Blue MRF´ cells (OD_{600} = 1.0) as outlined in steps 2–5 below. Expect titers of $<10^{10}$ pfu/ml.

2. 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.

3. Add 3 ml of NZY top agar,§ melted and cooled to ~48°C, and then pour immediately onto prewarmed NZY agar plates.§

4. Incubate the plates overnight at 37°C.

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

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

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

   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 or XL1-Blue MRF´ cells from a fresh LB–tetracycline plate into 10 ml of 2× YT broth (see *Preparation of Media and Reagents*) in a 50-ml conical tube.

2. 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.*

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

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

4. Grow the culture 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 VCSM13 helper phage.

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

For further details about helper phage titering or amplification, please see *Titering Procedure* or reference 5.

PREPARING THE HOST STRAINS

Host Strain Genotypes

Host strains	Genotype
SOLR strain[a]	e14$^-$(McrA$^-$) Δ(*mcrCB-hsdSMR-mrr*)*171 sbcC recB recJ uvrC umuC::*Tn*5* (Kan^r) *lac gyrA96 relA1 thi-1 endA1* λ^R [F´ *proAB lacI*^{q}*Z*Δ*M15*] Su$^-$ (nonsuppressing)
XL1-Blue MRF´ strain	Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F´ *proAB lacI*^{q}*Z*Δ*M15* Tn*10* (Tet^r)]

[a] Use the SOLR strain for excision only.

Growing and Maintaining the Host Strains

The bacterial host strains are shipped as bacterial glycerol stocks. For the appropriate media, please refer to the following table:

Note *The mcrA, mcrCB and mrr mutations prevent restriction of methylated DNA, making XL1-Blue MRF´ compatible with cloning both genomic DNA and cDNA constructed using Stratagene's ZAP-cDNA® synthesis kit.*

Host strain	Agar plates for bacterial streak	Medium for bacterial glycerol stock	Medium for bacterial cultures for titering phage (final concentration)
SOLR strain[a]	LB–kanamycin[b]	LB–kanamycin[b]	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
XL1-Blue MRF´ strain	LB–tetracycline[b]	LB–tetracycline[b]	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$

[a] Use the SOLR strain for excision only.

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

On arrival, prepare the following from the bacterial glycerol stock using the appropriate media as indicated in the previous table:

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

1. Revive the stored cells by scraping off splinters of solid ice with a sterile wire loop.
2. Streak the splinters onto an LB agar plate (see *Preparation of Media and Reagents*) containing the appropriate antibiotic, 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 appropriate liquid medium containing antibiotic with one colony from the plate. Grow the cells to late log phase.

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

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

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

Color Selection by IPTG and X-gal

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

PREPARING PLATING CULTURES

Day 1

1. Inoculate 50 ml of LB broth supplemented with 0.2% (w/v) maltose and 10 mM $MgSO_4$ in a sterile flask with a single colony of the appropriate bacterial host.

   Note ***Do not*** *add antibiotic to the overnight culture or to the titering plates.*

2. Grow overnight with shaking at 30°C. This temperature ensures that the cells will not overgrow. Phage can adhere to dead cells as well as to live ones and can lower the titer.

Day 2

3. Spin the cells down in a sterile conical tube for 10 minutes at 2000 rpm.

4. Carefully decant the media off the cell pellet and **gently** resuspend the pellet in ~15 ml of 10 mM $MgSO_4$. (Do not vortex.)

5. 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.

6. Use freshly prepared cells for titering and amplifying the library.

‡ See *Realated Stratagene Products*

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. Stratagene suggests that the IPTG and X-gal should be added separately, with mixing in between additions, to the 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. Make dilutions of the lambda phage in SM buffer.

3. Add 1 µl of the lambda phage to 200 µl of host cells diluted in 10 mM $MgSO_4$ to OD_{600} = 0.5. If desired, also add 1 µl of a 1:10 dilution of the packaged material in SM buffer to 200 µl of host cells.

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

5. 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)]

6. Plate immediately onto NZY agar plates and allow the plates to set for 10 minutes. Invert the plates and incubate at 37°C.

7. 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 plaqures will be white (clear) and should be 10–100-fold above the background.

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

AMPLIFYING THE LIBRARY

It is usually desirable to amplify libraries prepared in lambda vectors to make a large, stable quantity of a high-titer stock of the library. However, more than one round of amplification is not recommended, since slower growing clones may be significantly underrepresented.

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

The following protocol is recommended for amplifying the Uni-ZAP XR library:

Day 1

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

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

Day 2

2. Dilute the cells to an OD_{600} of 0.5 in 10 mM $MgSO_4$. Use 600 µl of cells at an OD_{600} of 0.5/150-mm plate.

3. Combine aliquots of the packaged mixture or 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/600 µl of cells.*

4. Incubate the tubes containing the phage and host cells for 15 minutes at 37°C.

5. Mix 6.5 ml of NZY top agar, melted and cooled to ~48°C, with each aliquot of infected bacteria and spread evenly onto a freshly poured 150-mm NZY agar plate.

6. Incubate the plates at 37°C for 6–8 hours. Do not allow the plaques to get larger than 1–2 mm. On completion, the plaques should be touching.

7. Overlay the plates with ~8–10 ml of SM buffer. Store the plates at 4°C overnight (with *gentle* rocking if possible). This allows the phage to diffuse into the SM buffer.

Day 3

8. Recover the bacteriophage suspension from each plate and pool it into a sterile polypropylene container. Rinse the plates with an additional 2 ml of SM buffer and pool. Add chloroform to a 5% (v/v) final concentration. Mix well and incubate for 15 minutes at room temperature.

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

10. Recover the supernatant and transfer it to a sterile polypropylene container. If the supernatant appears cloudy or has a high amount of cell debris, repeat steps 8 and 9. If the supernatant is clear, add chloroform to a 0.3% (v/v) final concentration and store at 4°C. Stratagene recommends storing aliquots of the amplified library in 7% (v/v) DMSO at –80°C.

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

PERFORMING PLAQUE LIFTS

Titering

Titer the library using fresh host cells to determine the concentration as outlined in *Plating and Titering*.

Plating

Plate the library at 50,000 pfu/plate on a series of large 150-mm NZY agar plates, which are at least 2 days old, following the procedure outlined below.

1. Add the equivalent of 50,000 pfu/plate to 600 µl of host cells at an OD_{600} of 0.5.

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

3. Add 6.5 ml of NZY top agar to the bacteria and phage mixture.

4. Quickly pour the plating culture onto an NZY agar plate and carefully swirl the plate to distribute the cells evenly. (Use 20 plates to screen 1 × 10^6 pfu.)

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

6. Chill the plates for 2 hours at 4°C to prevent the NZY top agar from sticking to the nitrocellulose membrane.

Lifting

Note *Use forceps and wear gloves for the following steps.*

1. 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 membrane after lifting by submerging the membrane in a 1.5 M NaCl and 0.5 M NaOH denaturation solution for 2 minutes.

      Note *If using charged nylon, wash with gloved fingertips to remove the excess NZY 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 (see *Preparation of Media and Reagents*) solution.

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

3. 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.

4. Store the agar plates at 4°C.

HYBRIDIZING AND SCREENING

Following the preparation of the membranes for hybridization, perform prehybridization, probe preparation, hybridization, and washes for either oligonucleotide probes or double-stranded probes and then expose the membranes to film as outlined in standard methodology texts.[5,6] Following these procedures, perform secondary and tertiary screenings also as outlined in the standard methodology texts.[5,6] After an isolate is obtained, refer to Sambrook *et al.*[5] for suggested phage miniprep and majorprep procedures.

‡ See *Related Stratagene Products.*

ANTIBODY SCREENING PROTOCOL

A complete manual for immunoscreening is supplied with Stratagene's *pico*Blue™ immunoscreening kit. This kit is available with goat anti-rabbit antibodies and goat anti-mouse antibodies (see *Related Stratagene Products*).

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

The Uni-ZAP XR vector is designed to allow simple, efficient in vivo excision and recircularization of any cloned insert contained within the lambda vector to form a phagemid containing the cloned insert. This in vivo excision depends on the placement of the DNA sequences within the lambda phage genome and on the presence of a variety of proteins, including f1 bacteriophage-derived proteins. The f1 phage proteins recognize a region of DNA normally serving as the f1 bacteriophage origin of replication. This origin of replication can be divided into two overlying parts: (1) the site of initiation and (2) the site of termination for DNA synthesis.[7] These two regions are subcloned separately into the Uni-ZAP XR vector. The lambda phage (target) is made accessible to the f1-derived proteins by simultaneously infecting a strain of *E. coli* with both the lambda vector and the f1 bacteriophage.

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

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

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

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

Single-Clone Excision Protocol

Day 1

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

2. Grow separate overnight cultures of XL1-Blue MRF´ and SOLR cells in LB broth (see *Preparation of Media and Reagents*), supplemented with 0.2% (w/v) maltose and 10 mM $MgSO_4$, at 30°C.

Day 2

3. Gently spin down the XL1-Blue MRF´ and SOLR cells (1000 × *g*). Resuspend the XL1-Blue MRF´ and SOLR cells at an OD_{600} of 1.0 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 $>1 \times 10^5$ phage particles)
   1 µl of the ExAssist helper phage ($>1 \times 10^6$ pfu/µl)

5. Incubate the Falcon 2059 polypropylene tube at 37°C for 15 minutes.

6. Add 3 ml of LB broth 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 and then spin the tube at 1000 × *g* for 15 minutes.

8. Decant the supernatant into a sterile Falcon 2059 polypropylene tube. This stock contains the excised pBluescript phagemid packaged as filamentous phage particles. (This stock may be stored at 4°C for 1–2 months.)

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

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

11. Plate 200 µl of the cell mixture from each microcentrifuge tube on LB–ampicillin agar plates (50 µg/ml) (see *Preparation of Media and Reagents*) and incubate the plates overnight at 37°C.

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

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

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

VCSM13 helper phage is recommended for the the single-stranded rescue procedure. The single-stranded rescue procedure can be found in Stratagene's *pBluescript® Exo/Mung DNA Sequencing System Instruction Manual*.

Mass Excision Protocol

Day 1

1. Grow separate overnight cultures of XL1-Blue MRF´ and SOLR cells in LB broth, supplemented with 0.2% (w/v) maltose and 10 mM $MgSO_4$, at 30°C.

Day 2

2. Gently spin down the XL1-Blue-MRF´ and SOLR cells (1000 × *g*). Resuspend the XL1-Blue MRF´ and the SOLR cells in 10 mM $MgSO_4$ to an OD_{600} of 1.0 (8×10^8 cells/ml).

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 0.3 corresponds to 2.5×10^8 cells/ml)
   10^9 pfu of ExAssist helper phage (10:1 helper phage-to-cells ratio)

4. Incubate the conical tube at 37°C for 15 minutes to allow absorption.

5. Add 20 ml of LB broth 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.

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

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

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

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

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

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

TROUBLESHOOTING

Observations	Possible causes	Suggestions
The number of colonies is too low	The molar ratios of lambda phage to cells to helper phage is critical	Verify that the titer on the tubes is current and correct and use only calibrated pipettor
	Excision efficiencies are directly related to the Uni-ZAP XR phage titer	If an excision is unsuccessful, prepare a high-titer stock of the phage and repeat the excision procedure
	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[a] and the ABLE™ K strain[a] reduce the copy number of common cloning vectors by ~4- and 20-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
	Chloroform, which is added after packaging to prevent bacterial contamination, lyses the *E. coli* before the helper phage can infect and excise	Be sure to spin down the chloroform completely prior to removing an aliquot of the lambda phage for in vivo excision

[a] See *Related Stratagene Products*

PREPARATION OF MEDIA AND REAGENTS

Note *All media must be autoclaved before use.*

LB Agar (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized H_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave
Pour into petri dishes (~25 ml/100-mm plate)

LB Broth

10 g of NaCl
10 g of trytone
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–Tetracycline Broth (per Liter)

Prepare 1 liter of LB broth
Autoclave
Cool to 55°C
Add 1.5 ml of 10 mg/ml tetracycline (filter-sterilized)
Store broth in a dark, cool place as tetracycline is light-sensitive

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

LB–Ampicillin Agar (per Liter)

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

LB–Kanamycin Broth (per Liter)

Prepare 1 liter of LB broth
Autoclave
Cool to 55°C
Add 7.5 ml of 10 mg/ml kanamycin (filter-sterilized)

LB–Tetracycline Agar (per Liter)

Prepare 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

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)

LB Top Agar (per Liter)

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

NZY Top Agar (per Liter)

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

SM Buffer (per Liter)

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

RELATED STRATAGENE PRODUCTS

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ABLE™ C strain and ABLE™ K strain [Catalog #200305 (2 × 0.5-ml glycerol stock)]
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ABLE™ K strain [Catalog #200307 (0.5-ml glycerol stock)]
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ENDNOTES