Table of Contents

Description 1
Systems and Components 2
Partial Digestion and Size Fractionation of Genomic DNA 3
Ligation to λBlueSTAR Arms 5
In Vitro Packaging and Plating 5
Amplification of the Phage Library 6
Preparation of Plaque Lifts for Screening 7
Automatic Subcloning by Cre-Mediated Excision 8
Recipes 8
References 9
Restriction Maps 10

Copyright © 1993, 1995-97 by Novagen, Inc. All rights reserved.

Description

λBlueSTAR is a λ replacement vector designed for the production of genomic DNA libraries with efficient cloning of DNA fragments 7–20 kbp in size. The vector contains the unique features of blue/clear visual screening of recombinants and cre-***loxP***-mediated autosubcloning. Dual polylinker regions contain nine cloning sites flanked by T7 and T3 phage promoters for convenient RNA probe synthesis. Novagen offers the uncut vector as well as prepared arms ready for high-efficiency cloning of insert DNA with ultra-low nonrecombinant background. Kits are available for cloning with either partially filled in *Xho* I arms or *Bam*H I arms. A further advantage of both λBlueSTAR systems is the protection of inserted genomic DNA from host restriction by the use of the *EcoK*, *mcr* PhageMaker® extracts and host strain ER1647 (Wyman et al., 1986; Wertman et al., 1986; Raleigh et al. 1989; Doherty et al., 1993).

Blue/clear screening is achieved by the presence of the complete *E. coli lacZ* gene and control region within the 13 kbp stuffer fragment. The native vector, as well as arms religated with the stuffer, yields phage that produce active β-galactosidase in infected cells. Nonrecombinants are easily identified as blue plaques on plates containing X-gal, whereas phage containing genomic DNA inserts produce clear plaques.

Plasmid subclones are easily obtained by plating λBlueSTAR phage on a strain expressing cre recombinase (BM25.8) in the presence of carbenicillin (or ampicillin). These conditions produce colonies that carry the genomic insert in a plasmid excised from the lambda vector.

Cloning with *Xho* I-digested, partially filled arms involves partial fill-in of *Sau*3A I (or *Mbo* I or other compatible enzyme) digested genomic DNA, followed by ligation to the vector arms (Zabarovsky and Allikmets, 1986; see Fig. 1). The partial fill-in strategy prevents tandem inserts and religation of arms to the stuffer fragment. Cloning with *Bam*H I-digested (non-filled) arms does not require partial fill-in of genomic DNA prior to ligation, since the *Bam*H I arms can be ligated directly to *Sau*3A I-digested genomic DNA. Both types of arms have been dephosphorylated to further reduce nonrecombinant background.

Either cloning method is well-suited for the preparation of genomic libraries and the choice between them is generally based on personal preference. The *Xho* I partially filled-in arms have the theoretical advantage of reducing the occurrence of tandem inserts, since fragment ends are rendered incompatible with each other by the fill-in reaction. In addition, this method should in theory allow library preparation from

unfractionated DNA. However, we highly recommend a size fractionation step to increase the efficiency of productive ligations and to eliminate the possibility of short fragments of genomic DNA ligating in tandem with the stuffer and arms to form packagable phage molecules. Unlike other vectors, λBlueSTAR has the advantage of detecting these undesired clones by simple blue/clear screening.

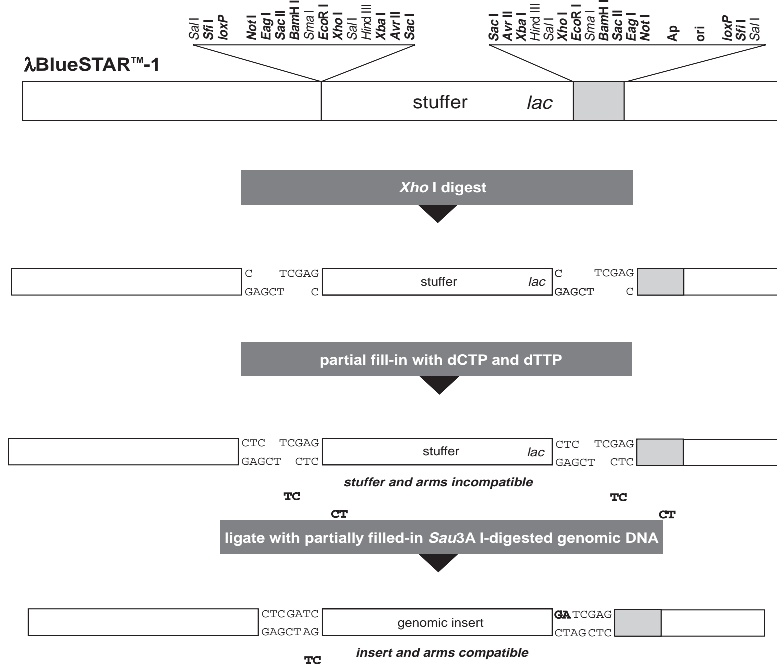

Fig. 1. *Xho* I partial fill-in cloning strategy.

Systems and Components

Kits contain enough components to make 2–3 genomic libraries.

λBlueSTAR *Xho* I Half-site Arms Kit **Cat. No. 69242-3**

- 6 µg λBlueSTAR *Xho* I partially filled-in, dephosphorylated arms
- 2 µg Half-site Control Insert
- 100 µl 10X Insert Fill-in Buffer
- 100 µl 100 mM DTT
- 0.2 ml Host strain ER1647, glycerol stock
- 0.2 ml Host strain BM25.8, glycerol stock
- Protocol

λBlueSTAR *Xho* I Half-site Arms Kit plus PhageMaker® **Cat. No. 69244-3**

- Same components as above plus 11 packaging extracts.

λBlueSTAR *Bam*H I Arms Kit **Cat. No. 69243-3**

- 6 µg λBlueSTAR *Bam*H I digested, dephosphorylated arms
- 2 µg *Bam*H I Control Insert
- 0.2 ml Host strain ER1647, glycerol stock
- 0.2 ml Host strain BM25.8, glycerol stock
- Protocol

λBlueSTAR *Bam*H I Arms Kit plus PhageMaker® **Cat. No. 69245-3**

- Same components as above plus 11 packaging extracts.

λBlueSTAR Uncut DNA **Cat. No. 69246-3**

- 10 µg λBlueSTAR uncut DNA
- 0.2 ml Host strain ER1647, glycerol stock
- 0.2 ml Host strain BM25.8, glycerol stock
- Protocol

Storage

Store packaging extracts and host strains at –70 °C. Store Control Inserts, 100 mM DTT and 10X Insert Fill-in Buffer –20 °C. Store uncut vector DNA and arms at 4 °C. Avoid multiple freeze-thaw cycles of Control Inserts.

Additional Reagents Needed for Library Construction

- Genomic DNA, 20–100 µg, 90% greater than 100 kbp in size
- *Sau*3A I or other compatible restriction enzyme and buffer
- Klenow DNA polymerase (*Xho* I Half-site Arms only)
- Agarose gel electrophoresis apparatus and reagents
- DNA fragment isolation reagents (e.g., Bio-Rad Prep-a-Gene® or equivalent)
- T4 DNA ligase and ligation reagents (Cat. No. 69838-3)
- PhageMaker lambda *in vitro* packaging system (Cat. No. 69307-3)
- SM buffer, LB media, LB agar plates, top agarose, X-gal (5-bromo-4-chloro-3-indolyl -β-D-galactoside)

Partial Digestion and Size Fractionation of Genomic DNA

To prepare for cloning, genomic DNA is partially digested with *Sau*3A I (or another enzyme that produces 5' GATC overhangs, such as *Mbo* I, *Bam*H I, *Bcl* I, or *Bgl* II) to obtain the desired size range of fragments. For λBlueSTAR *Xho* I Half-site Arms, a size range of 7–20 kbp is optimal and represents the range accepted by the vector for efficient packaging. In contrast, a range of 12–20 kbp is appropriate for the *Bam*H I cloning strategy. The larger minimum size prevents the occurrence of tandem inserts, since the unmodified *Bam*H I fragments can ligate with each other (a 24 kbp tandem insert would not form viable phage).

Small scale digestion

To optimize conditions that maximize the yield of fragments in the desired size range, a fixed amount of DNA is incubated with increasing dilutions of enzyme in a series of small-scale reactions. Once the reaction conditions are determined, the reaction is scaled up for library production.

1. Make fresh 1:15 and 1:150 dilutions of *Sau*3A I (usually supplied at 1–5U/µl) in 1X *Sau*3A I digest buffer + 100 µg/ml acetylated BSA. Keep the dilution tubes on ice and mix thoroughly by pipetting up and down. Further dilute the 1:150 dilution in the same buffer as follows: 1:2, 1:4, 1:6, 1:8, 1:10, 1:12, 1:20. Three microliters of each of these dilutions, including a no enzyme control, should be used with 1 µg of genomic DNA in eight 30 µl reactions. Assemble the following components:

   X µl (8 µg) genomic DNA
   25.5 µl 10X *Sau*3A I Buffer
   25.5 µl 1 mg/ml acetylated BSA
   Y µl Nuclease-free water
   229.5 µl total volume

   Mix well by gently stirring with the pipet tip and let sit on ice for 30 min to allow the solution to equilibrate. Dispense 27 µl of the mixture into 8 tubes. Add 3 µl of each *Sau*3A I dilution to seven of the tubes. Add 3 µl of the enzyme dilution buffer to the eighth for the no enzyme control. Mix gently, let equilibrate on ice for 10 min, and incubate at 37 °C for 30 min.

2. Stop the reaction by heating at 70 °C for 10 minutes.

3. Add 20 µl of each reaction to 5 µl of gel loading buffer, mix, and load onto a 0.5% agarose gel containing 0.5 µg/ml ethidium bromide. Include a lane of appropriate size markers (e.g., lambda *Hin*d III). Run at 5 volts/cm until bromphenol blue reaches the bottom of the gel. Photograph gel under a UV light source and determine the enzyme concentration that gives a maximum amount of DNA in the desired size range.

Large-Scale Digestion

1. Scale up the chosen reaction to digest 10–20 μg DNA. The best sequence representation in the library will be achieved by using 1/2 the amount of enzyme that gives the maximum amount of DNA in the desired size range (Seed et al., 1982). Remember to scale up exactly; i.e., to increase total reaction volume, keep ratios of components equivalent, and exactly duplicate conditions such as time and temperature.

2. Inactivate the enzyme by heating at 70 °C for 10 min. Check the digestion by running a sample (~0.3 μg DNA) on an agarose gel as discussed in Step A above. If there was not enough digestion, add more *Sau*3A I and repeat. If the size range is acceptable, proceed with partial fill-in (*Xho* I half-site strategy). For *Bam*H I arms, proceed to Size Fractionation (on the following page).

Partial Fill-In of *Sau*3A I ends (*Xho* I Half-site Arms only)

*Sau*3A I digestion leaves the 5' four base pair cohesive end d(GATC). For cloning into *Xho* I Half-site Arms, the internal two bases are filled-in with dATP and dGTP to yield a 5' d(GA) overhang that is complementary to the d(CT) overhangs created by partial fill-in of *Xho* I-digested vector.

1. Assemble the following components:

X μl	*Sau*3A I-digested genomic DNA (10–20 μg)
40 μl	10X Insert Fill-in Buffer (1X = 50 mM Tris-HCl pH 7.3, 10 mM $MgCl_2$, 1 mM dATP, 1 mM dGTP, 50 μg/ml BSA)
4 μl	100 mM DTT
Y μl	Klenow DNA polymerase (1 U/μg DNA)
Z μl	Nuclease-free water
400 μl	total volume

2. Incubate at 30 °C for 30 minutes.

3. Place at 70 °C for 10 minutes, then cool slowly by leaving the tube at room temperature for approximately 10 minutes.

4. Add 1/10 volume 3 M sodium acetate and 2 volumes 100% ethanol. Incubate at room temperature for 5 minutes, then centrifuge for 8 minutes at room temperature. Resuspend the DNA pellet in 20–30 μl TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA).

Size Fractionation

Prior to ligation with λBlueSTAR arms, a size fractionation step is recommended to remove fragments outside the desired size range that compete for vector arms during ligation. Add an appropriate volume of gel loading dye to the prepared genomic DNA and load onto a 0.5% agarose gel containing 0.5 μg/ml ethidium bromide. Adjust the width of the slot to accommodate the amount of DNA loaded. It is best to minimize the slot width as much as possible without overloading the lane, since higher recoveries are generally obtained with lower agarose:DNA ratios. Up to 10 μg of properly digested DNA can be loaded in a single 10 mm x 1.5 mm well. Include DNA size markers in an adjacent lane and run the gel at 5 V/cm or less. Visualize the DNA with a long wave UV light source and excise the gel piece containing DNA in the appropriate size range using a clean razor blade. Recover the DNA from the gel slice using a Prep-a-Gene® Kit or other suitable method and resuspend in a final volume of 20 μl (~0.5 μg DNA/μl). Determine the DNA concentration by reading the absorbance at 260 nm of a 2 μl sample diluted in 0.3 ml water (1 A_{260} unit corresponds to 50 μg DNA/ml). Expect a recovery of 50–70% using a successful recovery method. Store the genomic DNA at –20 °C until use.

Ligation to λBlueSTAR Arms

Test ligations are usually performed to optimize insert:vector ratios for maximum cloning efficiency. Highest efficiencies are normally obtained using insert:vector molar ratios between 0.5:1 and 3:1. The following set of reactions is an example of typical test ligations, in which 4 different amounts of insert DNA are ligated to 0.5 μg of vector arms.

1. Set up the following reactions:

	Sample 1	2	3	4
Insert:arms ratio	0.6	1.2	2.1	2.9
λBlueSTAR arms (0.5 μg = 0.017 pmol)	0.5 μg	0.5 μg	0.5 μg	0.5 μg
Genomic DNA (ave. size 15kbp) (0.1 μg = 0.01 pmol)	0.1 μg	0.2 μg	0.35 μg	0.5 μg
10X Ligase Buffer*	1 μl	1 μl	1 μl	1 μl
100 mM DTT	1 μl	1 μl	1 μl	1 μl
10 mM ATP	1 μl	1 μl	1 μl	1 μl
T4 DNA ligase (4 U/μl)	1 μl	1 μl	1 μl	1 μl
Nuclease-free water	to 10 μl total volume			

* (10X = 200 mM Tris-HCl pH 7.6, 100 mM $MgCl_2$, 500 μg/ml BSA)

If desired, two additional reactions can be performed for negative and positive controls. The negative control has no added DNA insert, and the positive control uses 0.5 μg (2 μl) of the appropriate Control Insert provided in the kit. All other components are the same. The negative control is usually unnecessary if plating on X-gal, since religated λBlueSTAR stuffer + arms (nonrecombinant background) will produce blue plaques that are easily distinguished from clear recombinants.

2. Mix gently and incubate 16 h (overnight) at 4 °C for *Xho* I Half-site Arms and 4–16 h at 16 °C for *Bam*H I Arms.

3. Proceed with packaging and plating as described below. Choose the ligation conditions that give the highest number of recombinants for a scaled-up ligation reaction. To ensure reproducibility, keep all reactants in the same proportions that were used in the test ligation. Remember to add no more than 10 μl of the ligation per 50 μl PhageMaker extract.

In Vitro Packaging and Plating

1. For genomic libraries we strongly recommended using packaging extracts that lack *EcoK*, *mcrA* and *mcrBC* restriction activities, such as Novagen's PhageMaker® System (Cat. No. 69307-3). Allow the extract to thaw on ice. Avoid leaving thawed extracts on ice for more than 10 min before adding the DNA. The volume of extract is 50 μl; tubes can be centrifuged briefly at 4 °C if necessary to collect the contents at the bottom. The extract can be subdivided into several pre-chilled tubes for testing several samples at once. If this is done, gently stir the extract with a pipet tip prior to removing an aliquot to a chilled tube. Scale down the amounts of ligation reaction and SM buffer added in the following steps in proportion to the amount of extract used.

2. Add up to 10 μl of ligation reaction per 50 μl extract. Mix by gently stirring with the pipet tip (do not vortex!). There is a linear relationship between amount of DNA added and number of plaque forming units (pfu) for amounts of DNA between 50 ng and 5 μg. A vial of ligated lambda control DNA is provided with the system. The volume of DNA added should not exceed 1/5 the volume of the packaging extract. To test the packaging efficiency independently, add 0.5 μg of the control DNA to the 50 μl extract.

3. Incubate the mixture at 22 °C for 2 h.

4. Stop the reaction by adding SM to 0.5 ml (e.g., 440 μl SM if 10 μl DNA was added to 50 μl of extract). If the packaging reaction is to be stored for more than 24 h, also add 25 μl chloroform and invert several times to mix.

5. The packaged phage may be stored for up to 1 week at 4 °C, although we recommend amplifying primary libraries within 48 h to avoid possible decreases in titer. If the library is not to be amplified, allow the chloroform to settle out (or spin briefly), remove the aqueous phase to a fresh tube, add fresh DMSO to a final concentration of 7% (for 0.5 ml this is 37.6 µl) and store the phage at –70 °C.

Plating Phage

The phage titer is determined by plating several dilutions of the packaging reaction on host strain ER1647. The proportion of phage containing inserts in libraries constructed in λBlueSTAR can be determined by including X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) in the top agarose.

1. Streak out the appropriate supplied host strain on an LB agar plate (add 12.5 µg/ml tetracycline to the cooled agar prior to pouring the plates for strain ER1647; use no antibiotic for LE392, which must be used for plating PhageMaker control DNA packaging reactions). The next morning, transfer a single colony to 50 ml LB medium supplemented with 0.2% maltose and 10 mM $MgSO_4$ in a 250 ml Erlenmeyer flask. It is not necessary to add tetracycline to liquid medium used for growing plating cells. Incubate at 37 °C with shaking at 250 rpm until the OD_{600} reaches 1. From a freshly streaked plate, the cells require approximately 5–7 hours to reach this density.
2. Store the host cells at 4 °C until needed (preferably not more than 48 h).
3. Make a series of dilutions of the packaged phage in SM buffer. As a general guideline, an appropriate dilution for recombinant phage in typical packaging reactions is 10^{-2} to 10^{-3}. When the positive control DNA is used, dilutions should be made to 10^{-5}. Make a 1/100 (10^{-2}) dilution first by adding 10 µl of the packaged phage to 990 µl SM and then make further dilutions from there.
4. In a sterile tube with at least a 4 ml capacity, mix 100 µl of the host cells with 100 µl of the appropriate phage dilution. Add the host cells first to avoid cross-contamination of the phage between samples.
5. Incubate the host/phage mixture at 37 °C for 30 min to allow the phage to adsorb to the host. Do not shake.
6. Add 3 ml of molten top agarose to the tube containing the host and phage. For blue/clear screening of λBlueSTAR recombinants, add 100 µl of a 50 mg/ml stock solution of X-gal in dimethylformamide or DMSO (stored at –20 °C) per 10 ml of top agarose prior to use. The top agarose must be no warmer than 47 °C. Immediately pour the contents of the tube onto an LB plate. Spread the top agarose evenly by gently swirling the plate.
7. Allow the plate to sit undisturbed on a level surface for a few minutes until the top agarose hardens, then incubate the plate inverted at 37 °C overnight.
8. Count the plaques and calculate the phage titer. The phage titer (pfu/ml) is the number of plaques on a plate times the dilution factor times ten (to account for the fact that 0.1 ml of dilution was plated). For example: if there were 200 plaques on a plate made from a 1/1,000 dilution, there are 200 x 1,000 x 10 = 2 x 10^6 pfu/ml of packaging reaction. If 0.5 µg of arms were packaged in a 50 µl reaction that was brought to 0.5 ml (1 µg arms/ml), the titer is 2 x 10^6 pfu/µg arms. The positive control DNA should produce more than 200 plaques when packaged as above and plated at 10^{-5}, which corresponds to a titer of 2 x 10^8 pfu/µg.

Amplification of the Phage Library

The following plate amplification protocol is based on that described by Ausubel et al. (1989) and is recommended for maintaining the most accurate clone representation in a library.

1. Inoculate 50 ml of LB supplemented with 0.2% maltose and 10 mM $MgSO_4$ by picking a single colony of ER1647 host cells from a freshly streaked plate. Shake at 37 °C until the cells reach an OD_{600} of 1. Store host cells at 4 °C until

needed (preferably less than 24 h).

2. If library was stored over chloroform, spin briefly in a microcentrifuge to separate out the chloroform. Use the packaged, titered phage (no chloroform) for the amplification.

3. In a sterile 15 ml tube (or 50 ml tube if necessary) combine phage and host cells at the ratio of 1 x 10^5 phage/0.25 ml cells (see note below). A library containing 1 x 10^6 primary recombinants requires 2.5 150 mm plates. Dispense phage/host mixture in 15 ml tubes such that each tube contains 4 x 10^5 phage. Allow the phage to adsorb by incubating for 30 minutes at 37 °C without shaking.

4. Add 10 ml molten top agarose (no warmer than 47 °C) to each tube containing the host and phage. Pour the contents of the tube onto a 150 mm H agar plate. Spread the top agarose evenly by gently swirling the plate.

6. Allow the plates to sit undisturbed on a level surface for a few minutes until the top agarose hardens, then incubate the plates inverted at 37 °C until plaques are visible, but not yet confluent (about 5–6 h).

7. To elute the phage, cover each plate with 10 ml SM and place upright on a level surface at 4 °C for 4–16 h.

8. Holding the plate at an angle, carefully remove the SM buffer using a sterile 10 ml pipet. Combine the SM from all of the plates in a single sterile capped tube. Add 100 µl chloroform per 10 ml and shake well. Centrifuge at 3,000 rpm for 5 minutes to remove debris and transfer the supernatant to a fresh sterile tube. Titer the phage as described in *In Vitro Packaging and Plating*. Expect a titer of about 10^9–10^{10} pfu/ml.

9. Add DMSO to a final concentration of 7% (0.075 ml DMSO/ml phage) and store library in aliquots at –70 °C.

Note: The volume of phage should not exceed the volume of host cells. If the titer of the library is low it may be necessary to add more host cells to the adsorption. A density of 4 x 10^5 pfu/150 mm plate should be maintained to obtain sufficient titers of the amplified library (final titers should be greateer than 10^9 pfu/ml).

Preparation of Plaque Lifts for Screening

1. For primary screening of λBlueSTAR libraries, make serial dilutions of the phage in SM (if necessary). Plan to plate about 3 x 10^4 phage per 82 mm plate (multiply quantities by 3.3 if using 150mm plates). Mix 100 µl cells with 100 µl of an appropriate phage dilution. Incubate at 37 °C for 20 min to allow the phage to adsorb to the host cells. Add 3 ml top agarose warmed to 47 °C, quickly pour on LB plates and spread evenly. Use slightly dry plates (~2 days old) so that the agarose will tend to stick to the plates when filters are lifted.

2. Invert plates and incubate at 37 °C until plaques are about 1–2 mm in size (approximately 5–7 hours). When plaques reach the desired size, cool the plates for 15–30 minutes at 4 °C.

3. Holding a dry nitrocellulose membrane by the edges (wear gloves!), slightly bend the filter in the center by holding it at the edges and allow the middle of the filter to make contact with the plate first. Allow the rest of the filter to be pulled down on the plate by capillary action. This procedure is meant to avoid trapping air bubbles. Leave the membrane on the plate for one minute once it is completely wet. Do not reposition the filter once it has made contact with the plate surface.

4. Mark the filter in at least three asymmetric locations around the edge by poking through it with a needle into the agar below. If desired, waterproof ink can be used to make the marks easier to locate.

5. Using a blunt forceps, carefully peel the membrane away from the top agarose surface. Float the membrane (DNA side up) in a shallow tray of 1.5 M NaCl,

0.5 M NaOH (denaturing solution) for 60 seconds. Duplicate filters can be made from the same plate by the same procedure. For each replicate, the incubation time on the plate should be increased by 30 seconds.

6. Transfer the membrane to another tray containing 1.5 M NaCl, 0.5 M Tris-HCl, pH 8.0 (neutralizing solution) for 60 seconds.
7. Place the membrane, DNA side up, on filter paper and let dry.
8. Put the membrane between sheets of filter paper, wrap in aluminum foil and bake in vacuum oven at 80 °C for 15–30 min.
9. Store membranes, wrapped in plastic, at 4 °C.

Automatic Subcloning by Cre-Mediated Excision of Plasmids from λBlueSTAR

1. Grow the host strain BM25.8 in LB supplemented with 10 mM $MgSO_4$ and 0.2% maltose to an OD_{600} of 1. (Maintain the strain on LB plates containing 34 µg/ml chloramphenicol and 50 µg/ml kanamycin. The strain is a double lysogen of P1 and λ*imm*^{434}kan, each of which is maintained by antibiotic selection.)
2. Mix 100 µl of an appropriate dilution of the phage with 100 µl of host cells.
3. Incubate the host/phage mixture at 37 °C for 30 min to allow the phage to adsorb to the host.
4. Spread 100 µl of the host/phage mixture directly on the surface of an LB agar plate containing 50 µg/ml carbenicillin with a bent glass rod. Ampicillin can also be used, but tends to result in more satellite colonies.
5. Let the mixture soak in for several minutes, and then incubate the plate inverted at 37 °C overnight.
6. Count the number of colonies. Expect an efficiency of excision of 10–20%. (For example, if the phage titer is 10^4 pfu/ml, there should be 1–2 x 10^3 drug-resistant cfu/ml).

Note: Colonies obtained in this manner contain plasmids which have been excised from the phage by site-specific recombination. The cre recombinase generates a mixture of plasmid multimers; furthermore, for unknown reasons plasmid replication in these cells can be inefficient. Therefore, plasmids prepared directly from colonies in BM25.8 by standard miniprep procedures are usually unsuitable for mapping by gel analysis or sequencing. Plasmids that are subcloned in BM25.8 should be isolated by a miniprep protocol and then transformed into another host for these purposes. Suitable hosts include HB101, JM109, NovaBlue, and DH5α (NovaBlue competent cells are available as Cat. No. 69825-3).

Recipes

LB
Per liter:
10 g tryptone
5 g yeast extract
10 g NaCl
adjust pH to 7.5 with 1 N NaOH
Autoclave
for plates, add 15 g/l agar

H Agar
Per liter:
10 g Bacto tryptone
8 g NaCl
15 g agar
autoclave

SM
Per liter:
5.8 g NaCl
2 g $MgSO_4$-$7H_2O$
50 ml 1 M Tris-HCl pH 7.5
5 ml 2% gelatin
Autoclave

Top agarose
Per 100 ml:
1 g bacto-tryptone
0.5 g NaCl
0.6 g agarose
Autoclave

References

Ausubel et al. (1989). "Current Protocols in Molecular Biology," John Wiley and Sons, New York.

Doherty, J.P., Lindeman, R., Trent, R.J., Graham, M.W. and Woodcock, D.M. (1993) *Gene* **124**, 29–35.

Raleigh, E.A., Murray, N.E., Revel, H., Blumenthal, R.M., Westaway, D., Reith, A.D., Rigby, P.W.J., Elhai, J and Hanahan, D. (1989) *Nuc. Acids Res.* **16**, 1563–1575.

Seed, B., Parker, R.C. and Davidson, N. (1982). *Gene* **19**, 201–209.

Wertman, K.F., Wyman, A.R. and Botstein, D. (1986) *Gene* **49**, 253–262.

Wyman, A.R., Wertman, K.F., Barker, D., Helms, C. and Petri, W.H. (1986) *Gene* **49**, 263–271.

Zabarovsky, E. R. and Allikmets, R.L. (1986) *Gene* **42**, 119.

Restriction Maps

λBlueSTAR™-1

Left polylinker region

Right polylinker region

Site	Position	Region
Left end	0	Left arm
Bgl II	420	
Apa I	10090	
Kpn I	17050	
Kpn I	18560	
Sma I	19400	
Sal I	19940	
Sfi I	19957	
loxP	19963-19996	Left polylinker
Not I	20025	
Eag I	20025	
Sac II	20031	
BamH I	20032	
Sma I	20038	
EcoR I	20041	
Xho I	20046	
Sal I	20051	
Hind III	20057	
Xba I	20062	
Avr II	20068	
Sac I	20078	
Avr II	20085	Stuffer
Xba I	20101	
Apa I	20384	
Sac I	20614	
Xba I	20614	
Apa I	21422	
Xba I	21482	
Xba I	22039	
Kpn I	22301	
Xba I	22339	
Kpn I	22994	
Kpn I	23395	
Kpn I	23646	
Hind III	23859	
Sac I	24247	
Xba I	24507	
Avr II	24619	
Avr II	24693	
Apa I	24970	
Xba I	25030	

Site	Position	Region
Xba I	25587	Stuffer
Kpn I	25849	
Xba I	25887	
Kpn I	26542	
Kpn I	26943	
Kpn I	27194	
Hind III	27407	
Sac I	27795	
Xba I	28055	
Avr II	28167	
Avr II	28241	
Apa I	29573	
Sac I	32171	
EcoR I	33235	
Sac I	33331	Right polylinker
Avr II	33333	
Xba I	33339	
Hind III	33344	
Sal I	33350	
Xho I	33355	
EcoR I	33360	
Sma I	33367	
BamH I	33369	
Sac II	33376	
Eag I	33376	
Not I	33376	
Ap gene	33544-34401	Plasmid Ap, ori
pUC origin	35128	
Kpn I	35408	
loxP	35411-35444	
Sfi I	35460	Right arm
Sal I	35510	
Hind III	35550	
Bgl II	36380	
Bgl II	36690	
Bgl II	37340	
Bgl II	37400	
Sma I	38480	
Hind III	39930	
Right end	44290	

Cloning sites shown in **bold**.

Genotype: sbhIλ1° <*loxP* polycloning site EMC *lacZ* polycloning site Ap ori *loxP*> KH54 chiD srIλ4° *nin5* srIλ5°

Not I (74)
Eag I (74)
Sac II (80)
BamH I (81)
Sma I (87)
EcoR I (90)
Xho I (95)
Sal I (100)
Hind III (106)
Xba I (111)
Avr II (117)
Sac I (127)

Not I (13425)
Eag I (13425)
Sac II (13425)
BamH I (13418)
Sma I (13416)
EcoR I (13409)
Xho I (13404)
Sal I (13399)
Hind III (13393)
Xba I (13388)
Avr II (13382)
Sac I (13380)

Avr II(134)
Xba I(150)
Xba I(663)
Sac I(663)
Xba I(1531)
Xba I(2088)
Xba I(2388)
Pfl1108 I(14361)
Sca I(13899)
ori
loxP
Ap
EMC
EcoR I(13284)
BsiW I(13051)
Bst1107 I(13045)
Sac I(12220)
Eco47 III(12115)
lacZ

pBlueSTAR™-1
(15,448bp)

Hind III(3908)
lambda
Sac I(4296)
Xba I(4556)
Avr II(4668)
Avr II(4742)
Xba I(5079)
Nar I(10051)
EMC
Xba I(5636)
Xba I(5936)
lambda
Avr II(8290)
Avr II(8216)
Xba I(8104)
Sac I(7844)
Hind III(7456)

pBlueSTAR-1 is a plasmid derived from the λBlueSTAR™-1 vector by autosubcloning (cre-mediated excision). If derived from the native vector, the plasmid carries the 13,253bp stuffer insert containing *lacZ*, and inert EMC viral and lambda sequences. The *lacZ* gene will give a blue colony phenotype when plated in the presence of X-gal (strains carrying *lacI* may also require IPTG to give a blue color). Enzymes that have unique sites and those in the polylinker that cut twice are shown in **bold** type. Note that *Sal* I, *Hin*d III and *Sma* I are not appropriate for cloning in λBlueSTAR-1. Enzymes that do not cut pBlueSTAR-1:

AflII	AgeI	AscI	BglII	Bpu1102I	Bsp1407I	Eco105I	EcoNI
FseI	MunI	NruI	PacI	PmeI	PshAI	Psp5II	PstI
RsrII	SfiI	SgrAI	SrfI	Sse8387I	SwaI		

TB #79 11/97