Lambda FIX II Library

INSTRUCTION MANUAL

BN #936001-11

Revision A

For In Vitro Use Only

936001-11

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Lambda FIX II Library

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Lambda FIX II Library

MATERIALS PROVIDED

Materials provided	Quantity
Amplified premade library constructed in the Lambda FIX II vector[a]	1 ml
XL1-Blue MRA host strain[b]	0.5-ml bacterial glycerol stock
XL1-Blue MRA (P2) host strain[b]	0.5-ml bacterial glycerol stock

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

[b] For host strain shipping and storage conditions, please see *Preparing the Host Strains*.

STORAGE CONDITIONS

Premade Library: –80°C
Bacterial Glycerol Stocks: –80°C

Revision A

INTRODUCTION

The Lambda FIX II vector is a replacement vector used for cloning large fragments of genomic DNA (see Figures 1 and 2). The Lambda FIX II system takes advantage of spi (sensitive to P2 inhibition) selection. Lambda phages containing active *red* and *gam* genes are unable to grow on host strains that contain P2 phage lysogens. Lambda phages without these genes are able to grow on strains lysogenic for P2 such as XL1-Blue MRA (P2), a P2 lysogen of XL1-Blue MRA. The *red* and *gam* genes in the Lambda FIX II DNA are located on the stuffer fragment; therefore, the wild-type Lambda FIX II phage cannot grow on XL1-Blue MRA (P2). When the stuffer fragment is replaced by an insert, the recombinant Lambda FIX II vector becomes Red–/Gam–, and the phage is able to grow on the P2 lysogenic strain. Therefore, by plating the library on the XL1-Blue MRA (P2) strain, only recombinant phages are allowed to grow. The strain XL1-Blue MRA is also provided as a control strain and later for growth of the recombinant when the selection is no longer necessary. The unique arrangement of the polylinker for the Lambda FIX II vector permits the isolation of the insert and flanking T3 and T7 bacteriophage promoters as an intact cassette by digestion with *Not* I. T3 and T7 promoters flanking the insertion sites can be used to generate end-specific RNA probes for use in chromosomal walking and restriction mapping.

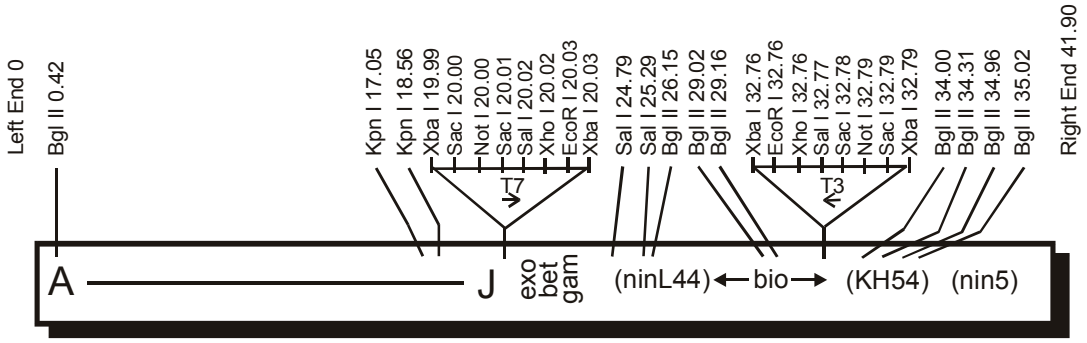

Figure 1 Map of the Lambda FIX II replacement vector.

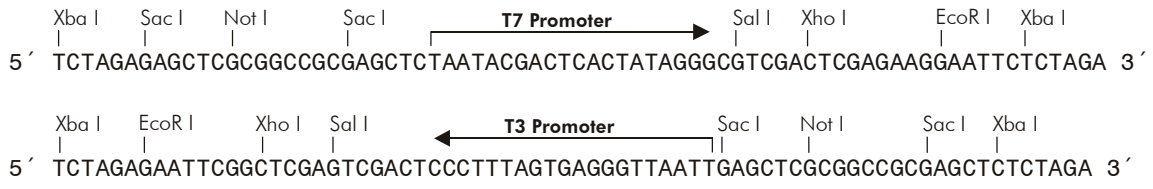

Figure 2 Multiple cloning site sequence of the Lambda FIX II replacement vector.

PREPARING THE HOST STRAINS

Host Strain Genotypes

Host strain	Genotype
XL1-Blue MRA strain	*Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 gyrA96 relA1 lac*
XL1-Blue MRA (P2) strain	XL1-Blue MRA (P2 lysogen)

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:

Host strain	Agar plates for bacterial streak[§]	Medium for bacterial glycerol stock[§]	Medium for bacterial cultures for titering phage (final concentration)
XL1-Blue MRA strain[a]	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
XL1-Blue MRA (P2) strain[a]	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
VCS257 strain[b]	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$

[a] The XL1-Blue MRA and XL1-Blue MRA (P2) host strains are modified to enhance the stability of clones containing methylated DNA; in addition, these strains enhance the stability of nonstandard DNA structures.

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

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[§]. 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.

[§]See *Preparation of Media and Reagents*

Preparing a –80°C Bacterial Glycerol Stock

1. In a sterile 50-ml conical tube, inoculate 10 ml of appropriate liquid medium 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. 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.

PREPARING THE PLATING CULTURES

Day 1

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

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. The cells may be stored for 2–3 days at 4°C.

[§]See *Preparation of Media and Reagents*

TITERING PROCEDURE

1. Prepare the host bacteria as outlined in *Preparing the 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. (Best results are obtained with gentle shaking.)
5. Add 2.5–3 ml of NZY[§] top agar (48°C) and plate on NZY plates.
6. Invert the plates and incubate overnight at 37°C.
7. Count the plaques and determine the plaque-forming units per milliliter (pfu/ml) concentration of the library.

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.*

Day 1

1. Prepare the host strains as outlined in *Preparing the 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 14-ml BD Falcon polypropylene round bottom tubes. To amplify 1×10^6 plaques, use a total of 20 aliquots (each aliquot contains 5×10^4 plaques/150-mm plate).

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

[§]See *Preparation of Media and Reagents*

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 bottom 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. We recommend 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

1. Titer the library to determine the concentration (prepare fresh host cells to use in titering and in screening).

2. Plate on large 150-mm NZY agar plates (≥2-day-old) to 50,000 pfu/plate with 600 µl of host cells at an OD_{600} of 0.5/plate and 6.5 ml of NZY top agar/plate. (Use 20 plates to screen 1×10^6.)

3. Incubate the plates at 37°C for ~8 hours.

4. 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.*

5. Transfer the plaques onto a nitrocellulose membrane for 2 minutes. Use a needle to prick through the 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 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*

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

7. 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.

8. Store the stock agar plates of the transfers at 4°C to use after screening.

HYBRIDIZING AND SCREENING

Following the preparation of the membranes for hybridization, perform prehybridization, probe preparation, hybridization, and washes for either oligonucleotide probes or double-stranded probes and then expose the membranes to film as outlined in standard methodology texts. Following these procedures, perform secondary and tertiary screenings also as outlined in the standard methodology texts.[1,2] After an isolate is obtained, refer to Sambrook *et al.*[1] for suggested phage miniprep and maxiprep procedures.

RAPID RESTRICTION MAPPING

The insertion sites of the Lambda FIX II vector are flanked by T3 and T7 promoters, which permit the generation of end-specific hybridization probes. End-specific probes can be made once a recombinant clone containing an insert is isolated. In addition, the Lambda FIX II vector has unique *Not* I sites flanking the RNA promoters, which permits the excision from the lambda vector of insert DNA with the T3 and T7 promoter sequences as an intact fragment.

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 (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

NZY Agar (per Liter)

5 g of NaCl
2 g of $MgSO_4 \cdot 7H_2O$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
15 g of agar
Add deionized H_2O to a final volume of 1 liter
Adjust the pH to 7.5 with NaOH
Autoclave
Pour into petri dishes (~80 ml/150-mm plate)

NZY Broth (per Liter)

5 g of NaCl
2 g of $MgSO_4 \cdot 7H_2O$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
Add deionized H_2O to a final volume of 1 liter
Adjust the pH to 7.5 with NaOH
Autoclave

NZY Top Agar (per Liter)

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

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

TE Buffer

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

REFERENCES

1. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
2. 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.

ENDNOTES

MSDS INFORMATION

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