User Manual

Lambda Library User Manual

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This protocol has been optimized for use with libraries constructed in the following vectors:

- λgt10
- λgt11
- other lambda-derived vectors
- EMBL3
- EMBL3 SP6/T7

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I. List of Components

Store reagents as described below.

Components included with all λ libraries

- **0.2 ml** **Liquid cDNA library lysate** (in 1X lambda dilution buffer and 7% DMSO)

  Notes:
  - For long-term storage (6 months or longer), store at –70°C. For short-term storage (less than 6 months), store at 4°C. For additional library storage information, please refer to Section IV.B.
  - All custom libraries are supplied without 7% DMSO. For long-term storage, store phage lysate at –70°C after adding DMSO to 7% or glycerol to 50%; for short-term storage, store at 4°C.

OR

- **0.3 ml** **Liquid genomic library lysate** (in 1X lambda dilution buffer and 7% DMSO)
- **0.5 ml** **Bacterial host strain** (in 25% glycerol)

  Notes:
  - All phage libraries are supplied with at least one bacterial host strain. Please refer to Section V for a list of the supplied host strains. For information about bacterial genotypes and references, see Appendix A.
  - Store bacterial cultures (in 25% glycerol) at –70°C. Bacterial cultures stored in this manner should remain viable for more than one year.

II. Additional Materials Required

- **Ampicillin** (50 µg/ml)
  Store at –20°C.
- **β-Mercaptoethanol** (14 M)
  Store at room temperature (22°C).
- **Carrier DNA**, sheared (10 µg/ml) (λ DNA or calf thymus DNA)
  Use of carrier DNA is optional; however, it will increase the transformation efficiency by 5- to 10-fold. Store at –20°C.
- **Cesium chloride** (CsCl)
  Store at room temperature (22°C).
- **Chloroform:isoamyl alcohol** (24:1)
  Store at room temperature (22°C).
- Biotinylated secondary antibody & avidin-horseradish peroxidase complex or secondary antibody conjugated with horseradish peroxidase & peroxidase substrate solution
- **50X Denhardt's solution**
  5.0 g Ficoll
  5.0 g Polyvinylpyrrolidone
  5.0 g BSA, Pentax Fraction V
  Add H_2O to a final volume of 500 ml. Store at –20°C.
- **100% Dimethylsulfoxide** (DMSO)
  Store at room temperature (22°C).
- **DNA denaturing solution**
  1.5 M NaCl
  0.5 N NaOH
  Store at room temperature (22°C).
- **DNase I**
  Store at –20°C.
- **EDTA** (0.5 M)
  Store at room temperature (22°C).
- **70% Ethanol**
  Store at 4°C.
- **95% Ethanol**
  Store at 4°C.
- **Ethidium bromide** (EtBr)
  10 mg/ml in H_2O. Store at room temperature (22°C).
- **Fetal calf serum**
  Store at 4°C.
- **100% Glycerol**
  Store at room temperature (22°C).

II. Additional Materials Required *continued*

- **IPTG** (10 mM & 100 mM)
  Isopropyl β-D-thiogalactopyranoside. Filter-sterilize. Store at 4°C.
- **10X Lambda dilution buffer**
  58.3 g 1.0 M NaCl
  24.65 g 0.1 M $MgSO_4 \cdot 7H_2O$
  350.0 ml 1.0 M Tris-HCl (pH 7.5) (final concentration of 0.35 M)
  Add H_2O to a final volume of 1 L. Autoclave and store at 4°C.
- **1X Lambda dilution buffer**
  100 ml 10X Lambda dilution buffer
  5 ml 2% Gelatin
  Add H_2O to a final volume of 1 L. Autoclave and store at 4°C.

  The 0.01% gelatin in the 1X lambda dilution buffer stabilizes the library titer for long-term storage. Gelatin is optional when diluting the phage for immediate titering.
- **LB agar plates**
  Prepare LB broth as described below. Add agar (15 g/L), and then autoclave. Pour plates, and store them at 4°C.
- **LB agar + 10 mM $MgSO_4$ plates**
  Prepare LB broth as described below. Add $MgSO_4$ (2.4 g/L) to a 10-mM final concentration and agar (7.5 g/L). Autoclave. Pour plates, and store at 4°C.
- **LB agar + amp + 10 mM $MgSO_4$ plates**
  Prepare LB agar plates as described above. Add $MgSO_4$ (2.4 g) to a 10-mM final concentration, and then autoclave. After autoclaving, cool LB broth to 50°C before adding ampicillin (50 µg/ml). Pour plates, and store them at 4°C.
- **LB agarose plates**
  Prepare LB broth as described below. Add agarose (15 g/L), and then autoclave. Pour plates, and store them at 4°C.
- **LB agarose + 10 mM $MgSO_4$ plates**
  Prepare LB agarose plates as described above. Add $MgSO_4$ (2.4 g/L) to a final concentration of 10 mM, and then autoclave. Pour plates, and store them at 4°C.
- **LB broth**
  10 g/L Bacto-tryptone
  5 g/L Bacto-yeast extract
  5 g/L NaCl
  Adjust pH to 7.0 with 5 N NaOH. Autoclave, and store broth at room temperature (22°C).
- **LB broth + 10 mM $MgSO_4$**
  Prepare LB broth as described above. Add $MgSO_4$ to a final concentration of 10 mM, and then autoclave. Store broth at room temperature (22°C).

II. Additional Materials Required *continued*

- **LB broth + 10 mM $MgSO_4$ + 0.2% maltose**
  Prepare LB broth + 10 mM $MgSO_4$ as described above. After autoclaving, cool LB broth to 50°C before adding maltose to a final concentration of 0.2%. Store at room temperature (22°C).
- **LB Soft top agar + 10 mM $MgSO_4$**
  Prepare LB broth as described above. Add $MgSO_4$ (2.4 g/L) to a 10-mM final concentration and agar (15 g/L), and then autoclave. Store at 4°C.
- **LB Soft top agarose + 10 mM $MgSO_4$**
  Prepare LB broth as described above. Add $MgSO_4$ to a 10-mM final concentration and agarose (7.2 g/L), and then autoclave. Store at 4°C.
- **$MgSO_4$** (1 M and 10 mM)
  Store at room temperature (22°C).
- **NaCl** (4 M)
  Store at room temperature (22°C).
- **Neutralizing solution**

1.5	M	NaCl
0.5	M	Tris-HCl (pH 8.0)

  Store at room temperature (22°C).
- **Hybridization filters**
  Use positively charge-modified, supported nylon filters such as Schleicher & Schnell's Nytran Plus.
- **Nonhomologous salmon sperm blocking DNA**
  Shear salmon sperm DNA by passing it 3 times through an 18-gauge needle. Boil the sheared DNA for 10 min. Quickly chill in ice H_2O. Store at –20°C.
- **NucleoBond®, NucleoSpin®, and NucleoTrap® Purification Products**
  Clontech offers a complete line of nucleic acid purification products that are useful for purifying library clones and inserts.
- **20%PEG**

20	g	PEG 8000 (Fisher Biotech)
90	ml	H_2O

  Stir gradually at room temperature to dissolve salt. Adjust volume to 100 ml with H_2O. Store at room temperature (22°C).
- **Peroxidase substrate solution**

2	ml	4-chloro-1-naphthol (3 mg/ml in methanol)
10	ml	TBS + 0.01 M imidazole
5	µl	30% hydrogen peroxide

  Store at 4°C.
- **Phenol:chloroform** (1:1)
  Equilibrate with TE buffer (pH 8.0). Store at 4°C, protected from light.

II. Additional Materials Required *continued*

- **Prehybridization solution 1** (for use with DNA probes)

50%	Formamide
5X	SSPE
5X	Denhardt's solution
0.1%	SDS
100 µg/ml	Denatured nonhomologous salmon sperm blocking DNA

  Store at –20°C.

- **Prehybridization solution 2** (for use with oligonucleotide probes)

6X	SSPE
5X	Denhardt's solution
0.25 %	SDS
100 µg/ml	Denatured nonhomologous salmon sperm blocking DNA

  Note: Make the prehybridization solutions on the day they will be used. Warm the solutions to dissolve the SDS, and filter through a 0.45–0.8-µm filter. Store at –20°C. You may also use Clontech's ExpressHyb™ hybridization solution (Cat. Nos. 636831, 636832) in place of either prehybridization solution.

- **Proteinase K**
  20 mg/ml in H_2O. Filter-sterilize. Store at –20°C.

- **Radioactive ink**
  Mix a small amount of ^{32}P with waterproof black ink.

- **RNase A** (DNase-free)
  Incubate a 10 mg/ml solution of RNase A in [(10 mM Tris-HCl, 15 mM NaCl), pH 7.5] at 90°C for 15 min, and then cool the solution slowly to room temperature. Store in small aliquots at –20°C.

- **20% SDS**
  Store at room temperature (22°C).

- **Sodium acetate** (3 M)
  Store at room temperature (22°C).

- **20X SSC**

175.3 g	NaCl (3.0 M)
88.2 g	Sodium citrate•$2H_2O$ (0.3 M)

  Adjust to pH 7.0 with 10 N NaOH. Add H_2O to a final volume of 1 L. Store at room temperature (22°C).

- **20X SSPE**

175.3 g	NaCl (3.0 M)
27.6 g	NaH_2PO_4•H_2O (0.2 M)
40 ml	0.5 M EDTA (final concentration of 0.02 M)

  Adjust to pH 7.4 with 10 N NaOH. Add H_2O to a final volume of 1 L. Store at room temperature (22°C).

II. Additional Materials Required *continued*

- **TBS buffer**
  50 mM Tris-HCl (pH 7.9)
  150 mM NaCl
  Store at room temperature (22°C).
- **TBST**
  TBS + 0.05% Tween-20
  Store at room temperature (22°C).
- **TE buffer**
  10 mM Tris-HCl (pH 7.5)
  1 mM EDTA
  Store at room temperature (22°C).
- **Tetracycline** (15 µg/ml)
  Store at –20°C.
- **Wash buffer 1**
  2X SSC
  0.5% SDS
  Store at room temperature (22°C).
- **Wash buffer 2**
  1X SSC
  0.1% SDS
  Store at room temperature (22°C).
- **Wash buffer 3**
  0.2X SSC
  Store at room temperature (22°C).
- **Whatman 3M paper**
- **X-Gal** (0.1 M)
  Dissolve in DMF. Store at –20°C.
- **Kodak XAR Film**

Additional materials required, by section:

Section IV.B.: Library Storage
- 100% DMSO
- 100% Glycerol

Section VI.A.: Bacterial Culture Plating
- LB broth + 10 mM $MgSO_4$ + 0.2% maltose
- LB agar plate (without $MgSO_4$)
- LB broth + 0.2% maltose
- Antibiotics

Section VI.B.: Library Plating/Titering
- LB soft top agar + 10 mM $MgSO_4$
- LB agar + 10 mM $MgSO_4$ plates
- 1X Lambda dilution buffer

II. Additional Materials Required *continued*

Section VI.D.: Library Screening Using Oligonucleotide or DNA Probes

- LB broth + 10 mM $MgSO_4$ + 0.2% maltose
- 1X Lambda dilution buffer
- LB soft top agarose + 10 mM $MgSO_4$
- Nitrocellulose filters (or nylon membranes)
- Radioactive ink
- DNA denaturing solution
- Neutralizing solution
- Whatman 3M paper
- Prehybridization solutions 1 and 2
- 3X SSC
- Wash buffers 1, 2, and 3
- 20% SDS
- 6X SSPE

Section VI.E.: Library Screening Using an Antibody Probe

- LB agar + 10 mM $MgSO_4$ plates
- LB agar + amp + 10 mM $MgSO_4$ plates
- LB broth + 10 mM $MgSO_4$ + 0.2% maltose
- 1X Lambda dilution buffer
- LB soft top agarose + 10 mM $MgSO_4$
- 10 mM IPTG
- Nitrocellulose filters
- Radioactive ink
- TBST
- TBST + 20% fetal calf serum
- Biotinylated secondary antibody
- Avidin-horseradish peroxidase complex
- Secondary antibody conjugated with horseradish peroxidase
- Peroxidase substrate solution

Section VI.F.: Isolation of DNA from Lambda Lysates

- LB broth + 10 mM $MgSO_4$ + 0.2% maltose
- 10 mM $MgSO_4$
- 1X Lambda dilution buffer
- Chloroform:isoamyl alcohol
- LB agarose + 10 mM $MgSO_4$ plates
- LB soft top agarose + 10 mM $MgSO_4$
- LB broth + 10 mM $MgSO_4$
- DNase I (RNase-free)
- RNase A (DNase-free)
- 20% PEG 8000
- 4 M NaCl
- Cesium chloride
- 0.5 M EDTA
- 20% SDS

II. Additional Materials Required *continued*

- Proteinase K
- Phenol:chloroform
- 3 M Sodium acetate
- 95% Ethanol
- 70% Ethanol
- TE buffer

Section VI.G.: Insert Excision

- NucleoBond®, NucleoSpin®, and NucleoTrap® Nucleic Acid Purification Products

III. Library Information

A. Library Construction

1. cDNA library preparation

All cDNA libraries are prepared according to a modified Gubler & Hoffman (1983) procedure. 5'-STRETCH PLUS cDNA Libraries are subjected to the most rigorous quality control tests of any commercially available library (see Product Analysis Certificate [PAC] for details).

First- and second-strand cDNA synthesis procedures and post-synthesis handling of the cDNA are optimized to produce libraries with larger inserts, and therefore, a greater probability of full-length transcripts.

5'-STRETCH and 5'-STRETCH PLUS cDNA Libraries were formerly prepared from mRNA that had been completely denatured by methylmercuric hydroxide, to release secondary structures. We have optimized first- and second-strand cDNA synthesis conditions in order to avoid this hazardous compound while maintaining larger insert sizes (1). The procedure uses unique oligo(dT)$_{25}$d(A/C/G) primers for first-strand cDNA synthesis; the 3'-end nucleotide (A/C/G) positions the primer at the junction of the poly-A tail and encoded transcript. Thus, only a relatively small fraction of the poly-A tail must be reverse transcribed to reach the transcript, and more mRNAs are fully reverse transcribed. Also, specially designed EcoR I hemiphosphorylated adaptors, rather than linkers, are used for cloning (see Section IV.A.4). 5'-STRETCH and 5'-STRETCH PLUS cDNA Libraries prepared using the new procedure are indicated by a catalog number in the 5000 range (e.g., HL5000a, ZL5000b).

After second-strand synthesis, cDNAs with molecular weights less than 400 bp are removed; remaining cDNAs are selected and cloned into lambda vectors.

2. Priming methods

cDNA libraries are oligo(dT)-, random-, or oligo(dT)+random-primed, (see PAC). For oligo(dT)+random-primed libraries, two priming reactions

III. Library Information *continued*

are performed for first-strand synthesis; each reaction is primed with either oligo(dT) or random primers. These reactions are pooled before second-strand synthesis, allowing approximately equal representation of oligo(dT)- and random-primed cDNAs in the same library.

Unidirectionally cloned libraries are primed with an oligo(dT) primer containing a vector-appropriate restriction enzyme site. An adaptor or linker containing the other appropriate enzyme site is ligated to the cDNA before cloning. If an adaptor was used, the cDNA is digested with the enzyme corresponding to the site present on the oligo(dT) primer so that the cDNA can be cloned unidirectionally. If a linker was used, the cDNA is digested with both enzymes before unidirectional cloning.

3. **EcoR I linker/adaptor sequences**
   We use an EcoR I linker or specially designed adaptor for libraries containing inserts cloned into the EcoR I site of the vector. If a linker was used, the cDNA is subjected to methylation to protect any internal EcoR I sites. The PAC will state whether or not an adaptor was used.

   CCGGAATTCCGG (linker sequence;12-mer)

   5'-AATTCGCGGCCGCGTCGAC-3'
   3'-GCGCCGGCGCAGCTG–P-5' (adaptor sequence)

   There are advantages to cloning cDNA using the specially designed EcoR I adaptor instead of a linker. No methylation or restriction enzyme digestion of the cDNA prior to cloning is required; therefore, internal EcoR I sequences in the cDNA will not be cut. The EcoR I adaptor also contains flanking Not I and Sal I sites so that the inserts can be removed from the phage using alternative restriction enzymes.

4. **Library amplification**
   Unless otherwise stated, all libraries are amplified once. Carefully amplified libraries are faithful copies of unamplified ones. All phage libraries are amplified on solid media in order to avoid unequal growth rates of clones that may occur with liquid media.

   If the titer of a library drops below a stable level, the library may be reamplified (to raise the titer) due to the rarity of the particular tissue from which the library was prepared. All reamplified libraries are subjected to the same rigorous quality control as are the once-amplified libraries. The PAC indicates if a library has been reamplified.

5. **Carrier**
   In the past, part of Clontech's cDNA library preparation protocol used yeast tRNA as a carrier. Some residual yeast genomic sequences may have been cloned with the cDNA when yeast tRNA was used . The PAC accompanying the library indicates if yeast tRNA was used.

6. **Packaging extract**

   McR $^{-}$ packaging extracts are used during library construction.

III. Library Information *continued*

B. Library Storage

1. **Short-term**
   Store working aliquots at 4°C; they should be stable for 6 months.
2. **Long-term—premade libraries**
   We recommend removing 2–3 aliquots (50 µl/aliquot) and storing them at –70°C. The titer of libraries stored at –70°C should remain stable for years. Work from a single tube stored at 4°C to avoid freeze/thaw cycles of the stock sample. You need not remove DMSO prior to use.
3. **Long-term—custom libraries**
   We recommend removing 2–3 aliquots (50 µl/aliquot), adding DMSO to each aliquot to a final concentration of 7%, and storing at –70°C. The titer of libraries stored at –70°C should remain stable for years. Use 1 tube at a time (stored at 4°C) to avoid repeated freeze/thaw cycles of the working stock sample. Alternatively, a sterile solution of glycerol can be added to a final concentration of 50% prior to storage at –70°C. DMSO or glycerol are equally good storage media; neither needs to be removed prior to library use.

C. Titer

The lysate titer is the value obtained when the library was constructed, and it is noted on the PAC. Retiter the library to determine the most accurate titer before screening. For additional information, please see Section VI.B.

Note: If the lysate contains at least 10^8 pfu/ml, the library is representative, and you may proceed with screening procedures. If the titer is below 10^8 pfu/ml, contact our Technical Service Department or your local distributor.

At 4°C, the titer may drop several-fold over a period of one year. Stability will vary between libraries, but low-titer or diluted libraries will be less stable over time. For example, under similar storage conditions, the titer of a library diluted to 10^6 pfu/ml will drop more drastically over time than an equivalent sample with a titer of 10^9 pfu/ml. In some cases, the titer can be stabilized with the addition of gelatin to a final concentration of 0.1 mg/ml.

D. Number of Independent Clones

This number is the number of independent recombinant colonies or independent clones that were in the library before amplification, which is performed to stabilize titer. Most libraries are have more than one million independent clones and are representative of the complexity of the cDNA population or genomes involved.

E. Recombination Frequency

Recombination frequencies for λgt11 libraries are determined after library amplification; for libraries in all other λ vectors, these values are determined

III. Library Information *continued*

before amplification. Although the recombination frequency value that we obtained is noted on the PAC, we recommend that you replate the library to determine this value most accurately. See Appendix D for recombination frequency determination for expression libraries—i.e., libraries with selection criteria such as blue/white selection in λgt11.

IV. Bacterial Host Strain Information

Each library is supplied with at least one bacterial host strain for library plating. Store host strains at –20°C when not in use. For information on bacterial host strain genotypes, please refer to Appendix A.

Notes: • Antibiotics are required for stock maintenance only and should **not** be used with immunoscreening protocols.

• Use LB agar plates that do not contain $MgSO_4$ for stock maintenance.

TABLE I: HOST STRAIN / ADDITIONAL INFORMATION

Vector	Bacterial Host	Additional Information
λgt10	C600 Hfl C600	3, 6 4
λgt11	Y1090r $^-$	1, 2, 5
EMBL3	K803	
EMBL3 SP6/T7	K802	
EMBL4	K803	

Additional Information:

1. K802 and LE392 may also be used for titering, but not for screening.
2. This vector will form tiny plaques in Y1090r $^-$.
3. This strain is recommended for recombinant screening.
4. This strain is recommended for vector propagation. It will also show nonrecombinants in unamplified libraries.
5. Add 50 µg/ml ampicillin.
6. Add 15 µg/ml tetracycline.

V. Library Protocols

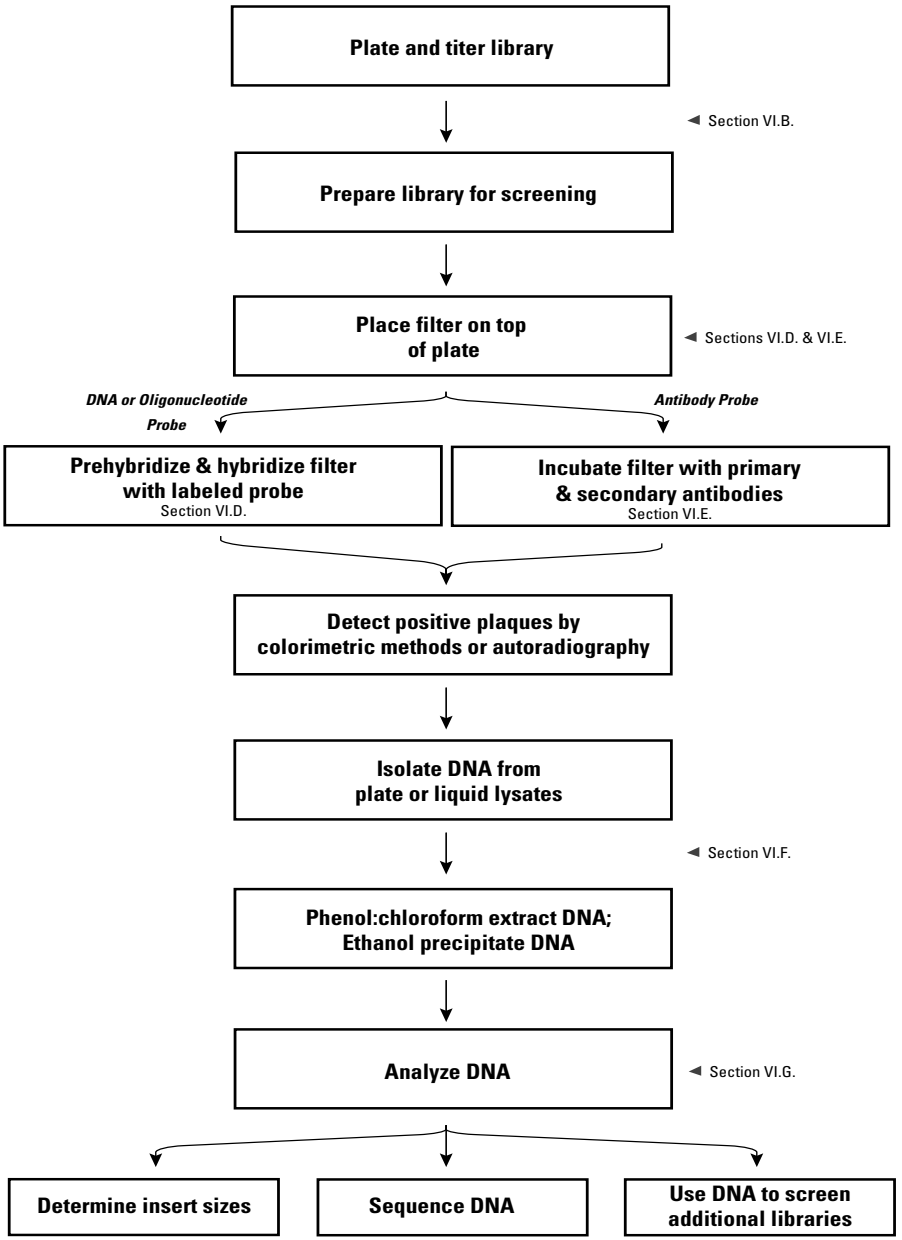

Figure 1. Overview of the Lambda Library Protocols.

V. Library Protocols *continued*

A. Bacterial Culture Plating

1. Streak 5 µl of the appropriate E. coli host strain (Table I) from the 25% glycerol stock culture provided onto a $MgSO_4$-free LB agar plate containing the appropriate media additives (Table I). Incubate at 37°C overnight. This plate is the master plate and should be stored at 4°C. Fresh streak plates should be prepared at 2-wk intervals.
2. Pick a single, isolated colony, and streak onto another $MgSO_4$-free LB agar plate containing the appropriate media additives. Incubate at 37°C overnight. This plate is the primary working plate and should be stored at 4°C.

B. Library Plating/Titering

1. Pick a single, isolated colony from the primary working plate (Step VI.A.2.), and inoculate LB broth + 10 mM $MgSO_4$ + 0.2% maltose (without antibiotics). Incubate on a shaker (200 rpm) at 37°C overnight until the OD_{600} of the culture reaches 2.0.
2. Preparation of titering dilutions of phage lysate (library)
   a. Pipet 2 µl of the library lysate into 1 ml of 1X lambda dilution buffer (Dilution 1 = 1:500).
   b. Transfer 2 µl of Dilution 1 into a second tube containing 1 ml of 1X lambda dilution buffer (Dilution 2 = 1:250,000).
   c. Prepare 4 tubes as described in Table II using the bacterial overnight culture obtained from Step B.1.

TABLE II: LIBRARY PLATING DILUTIONS

Tube	1X Lambda Dilution Buffer	Bacterial Overnight Culture	Phage Dilution 2
1	100 µl	200 µl	2 µl
2	100 µl	200 µl	5 µl
3	100 µl	200 µl	10 µl
4 (Control)	100 µl	200 µl	0 µl

3. Incubate tubes in a 37°C water bath for 15 min.
4. Add 3 ml of melted LB soft top agar + $MgSO_4$ (45°C) to each of the 4 tubes. Mix well.

V. Library Protocols *continued*

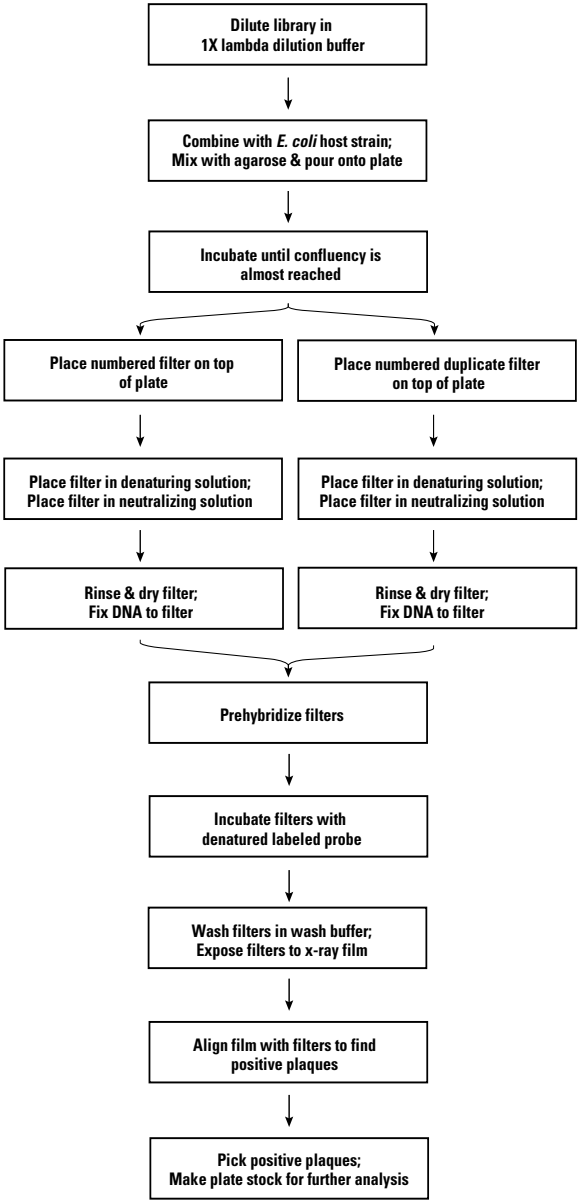

Figure 2. Library screening using a DNA or oligonucleotide probe.

V. Library Protocols *continued*

5. Pour the contents from each tube onto 4 separate 90-mm LB agar + $MgSO_4$ plates. Swirl the plates quickly after pouring to allow even spreading of the agar.

   Note: If the plates were prepared fresh and have been sitting at room temperature, prewarm plates at 37°C for 10–15 min. If plates have been stored at 4°C, prewarm plates at 37°C for 1 hr prior to use.
6. Cool plates at room temperature for 10 min to allow the inoculum to soak into the agar.
7. Incubate plates (inverted position) at 37°C for at least 6–7 hr.
8. Count the plaques to determine titer (pfu/ml). This plate is the secondary working plate and should be stored at 4°C.

$$\text{pfu/ml} = \frac{\text{\# of plaques}}{\mu\text{l used}} \times \text{dilution factor} \times 10^3\ \mu\text{l/ml}$$

C. Hybridization Screening—General Considerations

Lambda libraries can be screened by hybridization using either DNA probes or oligonucleotide probes (Section VI.D) . In either case, we recommend that you prepare at least two replicate filters of each plate to be screened (Section VI.C). Along with your library screening filters, perform a complete hybridization experiment with a blank filter (or preferably a control filter containing nonrecombinant vector plaques) to determine the conditions under which background noise is lowest. The protocol given below is for screening with a radioactively labeled probe and detecting positive signals by autoradiography.

Further information on hybridization using nucleic acid probes can be found in the laboratory handbooks edited by Sambrook et al. (2001) and Ausubel et al. (1987-1995). Also see Jacobs et al. (1988), Lathe (1985), Suggs et al. (1981), Wallace et al. (1981), and Wood et al. (1985) for further information on using oligonucleotide probes, and Woo (1979) for further information on using DNA probes.

Important notes:

- Use soft top **agarose** (rather than soft top agar) in the following procedures because commercially supplied agar contains components that may interfere with hybridizations.
- Always swirl plates quickly after pouring melted top agarose to promote even spreading of the agarose over the plate.
- Use positively charge-modified, supported nylon filters (Schleicher & Schuell's Nytran Plus works well). Nitrocellulose filters are too brittle for use in hybridization screening.
- The hybridization probe (DNA or oligonucleotide) should contain at least 10^7 cpm. The specific activity of the probe should be >8 x 10^8 cpm/µg, and the final concentration 1–2 x 10^6 cpm/ml of hybridization solution.

V. Library Protocols *continued*

- Commercially available rapid hybridization solutions (e.g., ExpressHyb Hybridization Solution, (Cat. No. 636831) may be substituted for standard prehybridization and hybridization solutions. If you are using a rapid hybridization solution, follow the protocol that is sent with that product.

  λgt11 libraries

  Y1090r $^-$, the host strain for λgt11 libraries, contains copies of pMC9, the parental plasmid of pBR322. In amplified λgt11 libraries, there is a 10^{-4} to 10^{-5} probability that the pMC9 sequence will recombine with the λgt11 vector. Therefore, when you are screening an amplified λgt11 library, we recommend that your probe contain no pBR322 or pBR322-like sequences.

  Studies have indicated that some λgt11 inserts may undergo homologous recombination with the vector arms (9). Therefore, false positives may result. We recommend that all positive clones be verified after identification.

D. Library Screening Using Oligonucleotide or DNA Probes

Note: It is essential to use agarose in the following procedures rather than agar as the top layer; agar may peel off the plate during the filter lift. Always swirl plates quickly after pouring to allow for even spreading of the agarose over the plate.

1. Filter replicas
   a. Pick a single, isolated bacterial colony from the primary working plate prepared in Step VI.A.2., and inoculate 15 ml of LB broth + 10 mM $MgSO_4$ + 0.2% maltose. Incubate with good aeration at 37°C overnight (or until stationary phase is reached).
   b. Based on the titer obtained in Section VI.B., prepare a sample of the phage library diluted in 0.1 ml of sterile 1X lambda dilution buffer so that the dilution will yield a maximum of approximately 10,000 pfu for each 90-mm plate (or approximately 30,000 pfu for each 150-mm plate). Combine the dilution with 200 µl of bacteria grown in Step a above (or 600 µl for a 150-mm plate). Incubate at 37°C for 15 min.
   c. Immediately add 3 ml of melted LB soft top agarose + 10 mM $MgSO_4$ to the cell suspension for each 90-mm plate (or 7 ml for each 150-mm plate). Mix and pour onto an LB agar + $MgSO_4$ plate prewarmed in a 37°C incubator.

      Note: The LB agar plates must be dry, otherwise the plaques may run together. Use 2-day-old plates that have been prewarmed to 37°C for at least 4–5 hr.

   d. Invert the plates and incubate at 37°C until the plaques reach a diameter not exceeding 1.5 mm or are just beginning to make contact with one another (3–8 hr). Avoid confluent lysis.

      Note: Plaque densities vary among λ phage and their plaque morphologies. Optimize the number of plaques per plate according to your specific phage.

V. Library Protocols *continued*

e. Chill the plates at 4°C for at least 1 hr to allow the LB soft top agarose to harden. Plates can now be stored at 4°C overnight.

f. Number a nylon membrane with a soft pencil or ball point pen. Using sterile forceps, place the filter onto the LB soft top agarose. Avoid trapping air bubbles. Mark the filter in 3 asymmetric locations by stabbing through the filter and into the agar with an 18-gauge needle attached to a syringe containing radioactive ink.

g. After 2 min, carefully peel off the filter. Float the filter on top of DNA denaturing solution, plaque-side up, for 30 sec. Then immerse the filter for 5 min.

h. Remove the filter and immerse in neutralizing solution for 5 min.

i. Briefly rinse the filter in 2X SSC, and place on a Whatman 3M paper to dry.

j. Place a second membrane onto the same plate, and mark it with ink at the same locations. Peel the filter off after 3 min. Denature the DNA; neutralize and rinse as described in Steps g–i. Additional membranes can be placed on the same plate and peeled off after 5 min.

k. Use a UV crosslinker to fix the DNA to the filter. Follow the manufacturer's recommended conditions, or simply bake membranes at 80°C for 2 hr.

l. Proceed to Step 2 for hybridization using DNA probes or to step 3 for hybridization with oligonucleotide probes.

2. Hybridization using DNA Probes (>200 bp)

a. Incubate the filters in prehybridization solution I in a heat-sealed plastic bag at 42°C for 4 hr with agitation. Avoid trapping air bubbles in the bag. The filters should come in direct contact with the solution.

Note: Commercially available rapid hybridization solutions (e.g., ExpressHyb™ Hybridization Solution, Cat. No. 636831) may be substituted for standard prehybridization and hybridization solutions.

b. Denature the labeled DNA probe by heating at 100°C for 10 min. Chill it quickly on ice, and then add it to the prehybridization solution in the plastic bag. Incubate the membranes in at least 10 ml of hybridization solution (prehybridization solution I containing probe) at 42°C for 16–20 hr (13). If using ExpressHyb™ solution, follow the recommendations provided for library screening.

Note: The probe should contain at least 10^7 cpm.

c. After hybridization, carefully and safely remove the filter from the plastic bag. Wash the filters at room temperature in wash buffer 1 for 15–20 min. Do not allow the filters to dry.

V. Library Protocols *continued*

d. Wash the filters in wash buffer 2 at 65°C for 1 hr. Repeat this wash once. If the background is still too high, you may wish to increase the washing stringency by incubating the filters for 1 hr in wash buffer 3.

Note: We recommend performing a complete experiment with a blank filter to determine the conditions under which background noise is lowest.

e. Wrap the filters in plastic wrap, and place them on Whatman 3M paper at room temperature. Pair duplicate filters, and place them onto a piece of filter paper backing cut to the size of the x-ray film to be used for autoradiography.

f. Mark the filter paper backing at 3 asymmetric locations with radioactive ink. Cover the filters in plastic wrap. Proceed with autoradiography at –70°C for 16–24 hr, using an intensifying screen to enhance the signal. We recommend using Kodak XAR Film.

Note: If the filters are kept slightly moist in the plastic wrap, they can be washed again to reduce background noise.

g. After developing, align film with the filters to find positive plaques. In cases where a single, isolated positive plaque cannot be picked, remove an agar plug containing several plaques into 1 ml of sterile 1X lambda dilution buffer. Replate to obtain 200–1,000 plaques on a 150-mm plate. Rescreen these plaques. Pick a single, well-isolated plaque for the plate stock. The plate stock is then used for further analysis. For information on the isolation of DNA from lambda lysates, please refer to Section VI.F.

3. Hybridization using oligonucleotide probes (6, 7, 11, 12, 14)

a. Incubate the filters in prehybridization solution 2 in a heat-sealed plastic bag at 20°C below the oligonucleotide probe's estimated dissociation temperature (T_d) for at least 4–6 hr. Avoid trapping air bubbles in the bag; the filters should come in direct contact with the prehybridization solution. For probes 14–27 nucleotides in length, the approximate T_d is:

T_d (°C) = 4(G+C) + 2(A+T)

under standard conditions of 1.0 M Na^+ (6X SSPE or 6X SSC)

For longer probes:

$T_d = 81.5°C + 16.6 \text{ Log}_{10} [Na^+] + 0.41(\%G/C \text{ content}) – 500/n$

n = the number of nucleotides

The T_d of the oligonucleotide can be decreased by approximately 13°C when the Na^+ concentration of the incubation solution is decreased from 1 M (6X SSPE) to 0.2 M (1X SSPE). 1X SSPE in the prehybridization and hybridization solutions should be used when employing oligonucleotides with a T_d higher than 80°C.

V. Library Protocols *continued*

b. Denature the oligonucleotide probe by heating at 100°C for 2 min (to release any secondary structure which may have formed). Chill probe quickly on ice. Add the denatured probe to the prehybridization solution in the plastic bag. Incubate the filters in hybridization solution (prehybridization solution containing probe) at the appropriate temperature for 16–20 hr.

c. After hybridization, carefully discard the solution, and wash the filters under low stringency conditions in a large volume of 2X SSC and 0.05% SDS for 1 hr. The wash buffer should be at room temperature or 30°C below the calculated T_d, whichever is higher. Replace the wash solution with fresh solution at a temperature 20°C below the calculated T_d. Wash the filters under these high stringency conditions for 15 min. If the background is still too high, increase the high stringency wash time to 30 min.

Note: We recommend performing a complete experiment with a blank filter to determine the conditions under which background noise is lowest.

d. Dry the filters by blotting on Whatman 3M paper at room temperature. Pair duplicate filters, and tape the dried filters onto a piece of filter paper backing, cut to the size of the x-ray film to be used for autoradiography. We recommend using Kodak XAR Film.

e. Mark the filter paper backing at 3 asymmetric locations with radioactive ink. Cover the filters in plastic wrap. Proceed with autoradiography at –70°C for 16–24 hr, using an intensifying screen to enhance the signal.

f. After developing, align film with the filters to select a positive plaque. In cases where a single, isolated positive plaque cannot be picked, remove an agar plug containing several plaques into 1 ml of sterile 1X lambda dilution buffer. Replate to obtain 200–1,000 plaques on a 150-mm plate. Rescreen these plaques. Pick a single, well-isolated plaque for the plate stock. The plate stock is then used for further analysis. For information on the isolation of DNA from lambda lysates, please refer to Section VI.F.

E. Library screening using an antibody probe

(Figure 2; Refs 2, 5, 16–18)

Cloning directly into the lacZ gene of λgt11 results in the expression of foreign DNA as part of a β-galactosidase fusion protein. λgt11 expresses the α-subunit of β-gal (96 kd). After plating, proteins released by the lysed cells are immobilized onto a nitrocellulose filter overlay. Your primary antibodies (rabbit, goat, or other) are used as probes to detect recombinants containing DNA sequences of interest by probing for specific antigens. Primary antibody binding is visualized by the use of a secondary antibody followed by colorimetric or other detection.

V. Library Protocols *continued*

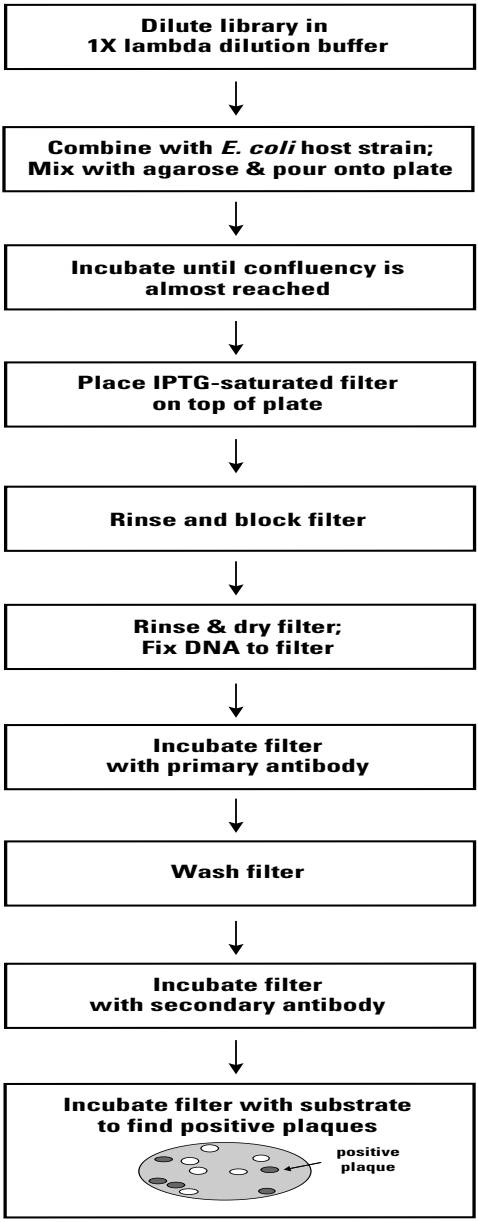

Figure 3. Library screening using an antibody probe.

V. Library Protocols *continued*

This protocol describes an avidin-horseradish peroxidase (avidin-HRP)-based system for colorimetric detection of the protein products expressed by the cloned cDNA. Antibody dilutions and other reagent must be optimized empirically or according to the manufacturer's recommendations.

1. Pick a single, isolated colony from the primary working plate (Step VI.A.2.), and grow the culture to saturation in 15 ml of LB broth + 10 mM $MgSO_4$ + 0.2% maltose at 37°C with good aeration.

   Note: Maltose addition is required for proper receptivity of cells to phage.

2. Mix 0.2 ml of the Y1090r $^-$ culture with 0.1 ml of sterile 1X lambda dilution buffer containing 10^4 pfu of λgt11 phage. Allow adsorption of phage to the cells at 37°C for 15 min.

   Note: Empirically determine the number of λgt11 clones to plate. 10^4 plaques/90-mm plate is generally acceptable. More than 10^4 plaques may cause a gradual decrease in the intensity of positive signals.

3. Add 3 ml of LB soft top agarose + 10 mM $MgSO_4$ for each 90-mm plate (or 7 ml for each 150-mm plate). Mix and pour onto an LB agar + 10 mM $MgSO_4$ plate. Swirl quickly while pouring to allow thorough mixing. Incubate plates at 42°C for 3.5 hr to allow lytic phage growth.

   Note: Do not use agar for the top layer of the plate.

4. Using forceps, place a dry nitrocellulose filter previously saturated in 10 mM IPTG on the top agarose. Avoid trapping air bubbles. Mark the filter in 3 asymmetric locations by stabbing through the filter and into the agarose with an 18-gauge needle attached to a syringe containing waterproof ink.

   Notes:
   - Do not substitute nylon filters for the nitrocellulose filters.
   - Filters must be dry so that moisture does not smear the plate. Air-drying the filters at room temperature is best.
   - IPTG, a gratuitous inducer, is used to enhance expression of the β-galactosidase fusion gene at 37°C. In the case of the chicken ovalbumin clone (cl), a 3-fold stimulation is detected.

5. Incubate the plates containing filters at 37°C for 3.5 hr.

   Notes:
   - Do not incubate overnight because increased background may result.
   - If a duplicate is required, simply overlay a second filter on top of the agarose after the first filter is removed. Incubate the second filter for an additional 3.5 hr.

6. Remove and rinse the filters in TBST.

   Note: If agarose sticks to the filter, cool the plates at 4°C for 15 min before lifting the filters.

V. Library Protocols *continued*

7. Incubate the filters in TBST + 20% fetal calf serum for 30 min. Use 5 ml for each 82-mm filter, or 10 ml for each 132-mm filter.

   Note: Fetal calf serum is a blocking agent used to saturate nonspecific protein binding sites on the nitrocellulose filter and reduce background.

8. Remove and rinse the filters in TBST.

NOTE: YOU MAY STOP HERE. STORE COMPONENTS AT 4°C.

9. Primary Antibody binding
   a. Incubate the filters in 5 ml for each 82-mm filter (or 10 ml for each 132-mm filter) of TBST + the appropriate dilution of your primary antibody at room temperature for 1 hr.
   b. Add 2 µl of your primary antibody/10 ml of solution used above.

   Note: The amount of primary antibody may have to be optimized.

10. Wash the filters in 3 changes of TBST; allow 3 min/change.
11. Detection of bound primary antibody with a biotinylated secondary antibody: An alternative detection procedure is described in Step 12. Using a biotinylated antibody will result in stronger signals, albeit with higher background.
    a. Add 20 µl of secondary antibody/10 ml of TBST. Incubate at room temperature for 30 min with gentle agitation.
    b. Place filters in the TBST containing biotinylated secondary antibody.
    c. Wash the filters in 3 changes of TBST; allow 3 min/change.
    d. Transfer the filters to TBST containing avidin-horseradish peroxidase complex. Incubate at room temperature for 30 min with gentle agitation.

       Note: Prepare avidin-horseradish peroxidase (HRP) complex by adding avidin and horseradish peroxidase conjugate to 10 ml of TBST. Consult the manufacturer's recomendations foramounts of these reagents to add. Incubate this mixture at room temperature for 30 min before use.

    e. Wash the filters 3 times in TBS (without Tween-20); allow 3 min/change.
    f. Incubate the nitrocellulose filters in peroxidase substrate solution. Allow the color to develop for approximately 30 min. Use 5 ml of peroxidase substrate solution for each 82-mm filter (or 10 ml for each 132-mm filter). Consult the manufacturer's recommendations to determine the optimal concentration of peroxidase substrate.

       Note: Positive plaques appear blue as a result of horseradish peroxidase staining. Once you locate them, remove them from the original plates, and purify recombinants by repeating the same screening procedure until all plaques are positive.

V. Library Protocols *continued*

g. Wash the filters in three changes of distilled H_2O, and allow the filters to air-dry.

Notes:
- The primary antibody is bound specifically by the biotinylated secondary antibody whose biotin moieties can be easily detected by avidin-conjugated horseradish peroxidase followed by immunostaining.
- The peroxidase stain on the filter is light sensitive. Store the filter in the dark to prevent fading.

12. Detection of bound primary antibody: Use of goat anti-rabbit antibody conjugated with horseradish peroxidase

Note: This detection procedure is alternative to that described in Step 11. You will obtain less background and slightly weaker positive signals when using goat anti-rabbit antibody conjugated with horseradish peroxidase for detection of primary antibody.

a. Add 8 µl of affinity-purified horseradish peroxidase-conjugated goat anti-rabbit IgG/10 ml of TBST.

b. Transfer the filters to the TBST containing IgG.

c. Incubate at room temperature for 1 hr with gentle agitation.

d. Wash filters 3 times in TBS (without Tween 20); allow 3 min/change.

e. Incubate the nitrocellulose filters in peroxidase substrate solution. Allow color development for approximately 30 min. Use 5 ml of peroxidase substrate solution for each 82-mm filter (or 10 ml for each 132-mm filter).

f. Wash with three changes of distilled H_2O, and allow the filters to air-dry.

13. Align the filters with the plates to select a positive plaque. If you cannot pick a single, isolated positive plaque, remove an agar plug containing several plaques, and place it in 1 ml of sterile 1X lambda dilution buffer. Replate to obtain 200–1,000 plaques on a 150-mm plate and rescreen. Pick a single, well-isolated plaque for the plate stock, and use this stock for further analysis. For information on the isolation of DNA from lambda lysates, please see Section VI.F.

V. Library Protocols *continued*

F. Isolation of DNA from Lambda Lysates

1. Preparation of bacterial host cells
   a. Incubate the appropriate bacterial host cells in 15 ml of LB broth (pH 7.5) + 10 mM $MgSO_4$ + 0.2 % maltose at 37°C overnight.
   b. Pellet the cells, and resuspend the pellet in 7.5 ml of 10 mM $MgSO_4$. Store the cells at 4°C for a maximum of 2–3 days following their preparation.
2. Phage stock preparation

   Note: The ratio of phage to bacterial host, which determines the ultimate success of any phage DNA preparation, must be determined empirically (9). A yield of 100–200 µg of phage DNA can be expected from 500 ml of phage lysate.

   a. Plate an appropriate number of lytic phage (usually 600–800 plaques per 90-mm plate) from the plate stock from Section VI.D.2.g., D.3.f., or E.13., so that a single plaque can be easily removed. Incubate at 37°C overnight.
   b. With the end of a pasteur pipette, transfer an agar plug containing a single plaque to a microcentrifuge tube containing 200 µl of 1X lambda dilution buffer.
   c. Add a drop of chloroform and vortex briefly.
   d. Let phage elute at 4°C overnight (or at 37°C with shaking [200 rpm] for 4–6 hr).

      Note: A low titer may result if phage is allowed to elute at 4°C for longer than 12 hr.

   e. Spin in a microcentrifuge at 10,000 rpm (8,000 x g) for 2 min to remove debris.
   f. Titer the supernatant as described in Section VI.B. Plate 10^5 pfu on a 150-mm LB agarose + 10 mM $MgSO_4$ plate.

      Note: Do not use agar for the plate as commercially available agar often contains strong inhibitors of restriction enzymes.

   g. Based on the titer obtained above, plate enough phage so that near confluency is obtained in 5–7 hr. Incubate at 37°C for 5–7 hr.
   h. Add 10 ml of 1X lambda dilution buffer to the plate, and incubate at 4°C overnight.
   i. Add a few drops of chloroform to the plate and swirl briefly. Pour the liquid (containing phage lysate) from the plate into a sterile 50-ml polypropylene tube.
   j. Add 2 ml of chloroform to the plate lysate, and vortex for 2 min.
   k. Centrifuge in a Beckman J2-21 centrifuge at 7,000 rpm (7,200 x g) for 10 min. Collect and save the supernatant.

      Note: This is a high-titer stock. Check the titer for future reference as described in Section VI.B. Titer should be approximately 10^{10} pfu/ml.

V. Library Protocols *continued*

3. Lysate preparation

Note: For preparation of large quantities of λ DNA, the liquid lysate method is more convenient than the plate lysate method; however λ DNA generally grows better in plate lysates.

a. Plate lysate

Note: Agarose is recommended for both the top and bottom layers of the plate since commercially available agar often contains strong inhibitors of restriction enzymes.

The number of plates required depends upon intended usage (i.e., preparations of 0.5–1 L of lysate requires 50–100 150-mm plates).

i. Based on the titer of the high-titer stock, prepare lysate dilutions such that approximately 10^5 pfu/ml are obtained on each 150-mm LB agarose + 10 mM $MgSO_4$ plate.

(a) Pick a single, isolated colony from the primary working plate (Step VI.A.2.), and inoculate LB broth + 10 $MgSO_4$ + 0.2% maltose. Incubate on a shaker (200 rpm) at 37°C overnight.

(b) Set up the appropriate number of tubes with 600 µl of bacterial culture and diluted lysate.

(c) Incubate in a 37°C water bath for 15 min.

(d) Add 5.5–6.5 ml of melted LB soft top agarose + 10 mM $MgSO_4$.

ii. Mix and pour inoculum onto 150-mm plates. Swirl the plates quickly while pouring to allow even spreading of the agarose.

iii. Incubate plates at 37°C for 5–6 hr.

Note: If the transfection is performed in the afternoon, incubate the plates overnight. However, daytime incubation is preferred so that the plates can be harvested at the first sign of confluency.

iv. Add 12 ml of 1X lambda dilution buffer to each plate. Store plates at 4°C overnight.

v. Incubate at room temperature for 1 hr with constant shaking.

vi. Remove and save the 1X lambda dilution buffer solution; rinse the plate surface with an additional 2 ml of 1X lambda dilution buffer.

vii. Pool the lambda dilution buffer solutions.This is the plate lysate.

viii. For lysate processing directions, proceed to Section VI.F.4.

b. Large-scale liquid lysate

Most liquid lysate protocols start by adding a small number of infected cells to a large volume of culture media (14). We have found that better results are achieved with a scaled-up version beginning with a higher quantity of infected cells.

i. Add 1–3 ml of phage stock (1–3 x 10^{10} pfu) to 1 L of host cells grown in LB broth to an OD_{600} of 0.6.

Note: Calibrate the spectrophotometer by taring with an LB broth blank.

V. Library Protocols *continued*

ii. Shake the culture at 37°C in a 4-L flask until lysis is apparent (6–10 hr, depending upon the vector).

Notes:
- When lysis occurs, the culture looks clear with some bacterial debris. If lysis does not occur, the culture appears turbid.
- Foaming during this period is a good indication of phage growth.

iii. After lysis, add 10 ml of chloroform, and shake for an additional 15 min.

Note: Chloroform will lyse cells which were not lysed during the previous incubation.

iv. Remove the flask. Centrifuge the lysate at 7,000 rpm (7,200 x g) for 10 min at 4°C in polypropylene bottles.

v. Combine the supernatants, and check the titer as in Section VI.B. This is the liquid lysate which is now ready for DNA extraction.

Note: If the titer is below 10^8 pfu/ml, repeat Steps i–v. For large quantities of DNA, the titer must be greater than 10^{13} pfu/L.

Standard yield = 440 µg of DNA/L:

(10^{13} pfu/L) x (40 kb/pfu) x (660,000 g/6 x 10^{23} kb) = 440 µg of DNA/L

If the titer is insufficient, increase the ratio of phage to bacteria. If lysis appears complete within 2–3 hr, the ratio of phage to bacteria is too high, and the bacterial population has been lysed prematurely. If lysis occurs too quickly, repeat Steps 3.b.i–v.

4. Lysate processing
   a. Centrifuge the bottled, pooled lambda dilution buffer solutions (obtained either from plate or liquid lysates) in a Beckman J2-21 at 8,500 rpm (10,000 x g) for 10 min in order to pellet debris.
   b. To the supernatant, add DNase I to 1 µg/ml and RNase A to 5 µg/ml. Incubate at room temperature for 30 min.
   c. Add 100% chloroform to a final concentration of 5%, and vortex for 30 sec.
   d. Centrifuge at 8,500 rpm (10,000 x g) for 10 min at 4°C to pellet the debris. Transfer the aqueous phase to a new centrifuge tube.
   e. Add an equal volume of 20% PEG/2.0 M NaCl to the aqueous phase. Incubate on ice for at least 1 hr.

      Note: The solution may be stored at 4°C overnight.

   f. Centrifuge the precipitated phage at 8,500 rpm (10,000 x g) for 15 min at 4°C. Discard the supernatant. A grayish smear should be evident on the side of each bottle.

      Notes:
      - Remove as much of the PEG solution as possible as PEG can inhibit restriction enzymes.
      - For rapid DNA preps, Steps g–l may be omitted. However, ultracentrifugation is recommended before preparative digestion of cDNA inserts for making probes or subcloning.

V. Library Protocols *continued*

g. Resuspend the pellets in 32 ml of 1X lambda dilution buffer.

h. Transfer the phage suspension into two 50-ml polypropylene tubes, and add an equal volume of chloroform. Vortex for 30 sec.

i. Centrifuge in a Beckman J2-21 centrifuge at 7,000 rpm (7,200 x g) for 10 min. Collect the supernatant, being careful to leave the PEG interface behind.

j. Add 0.5 g of CsCl per ml of phage suspension.

k. Pour the phage suspension into a 40-ml UltraClear™ tube, and centrifuge in a Beckman SW 28 rotor at 22,000 rpm (90,000 x g) for 2 hr at 20°C.

Note: The phage will pellet to the bottom of the tube.

l. Pour off the supernatant, and resuspend the clear, sticky phage pellet in 1 ml of 1X lambda dilution buffer.

m. Transfer to a 1-ml microcentrifuge tube, and spin down debris at 15,000 rpm for 10 min.

Note: Unless purity is critical (e.g., for making vector arms), the phage is now ready for DNA extraction (proceed to Step 5 below). If purity is essential, proceed to Step n.

5. DNA extraction

Note: You may wish to dialyze rather than precipitate the DNA because large DNAs are often difficult to resuspend once precipitated.

a. To the phage DNA, add EDTA to 20 mM, SDS to 0.5%, and proteinase K to 50 µg/ml final concentrations.

b. Incubate at 65°C for 1 hr.

c. Add an equal volume of phenol:chloroform, and mix by gentle inversion for 10 min.

d. Centrifuge in a Beckman J2-21 centrifuge at 7,000 rpm for 10 min at room temperature (or for 5 min in a microcentrifuge for smaller volumes).

e. Collect the supernatant, and repeat steps a–d until the interface is clean (usually one extraction is sufficient).

f. Repeat steps a–d with chloroform only to remove any residual phenol (the small amount of aqueous material left at the interface can be collected separately in 1.5-ml microcentrifuge tubes, centrifuged, and combined with the larger fraction).

g. Add $^1/_{10}$ volume of 3 M NaOAc and 2.5 volumes of 95% ethanol. Store at –20°C for at least 1 hr for DNA precipitation.

h. Centrifuge the DNA at 12,000–15,000 rpm for 15 min at room temperature.

i. Pour off the supernatant, and wash the pellet with 70% ethanol.

j. Centrifuge for 5 min, and pour off the supernatant.

V. Library Protocols *continued*

k. Spin down the residual ethanol. The last few drops can be removed with a micropipette.

l. Let the pellet dry until the edges of the pellet begin to turn clear.

Notes:
- If the DNA is not completely dry, it will resuspend well in TE buffer. If the DNA is dry, add 1 ml of TE buffer, and allow the pellet to resuspend at 4°C overnight.
- The DNA should form a sharp band on an agarose gel with some contaminating RNA. RNase A can be added along with restriction enzymes, thus eliminating an additional digestion. A spectrophotometer reading should give characteristic curves, with a maximum absorption at 260 nm. The $A_{260/280}$ ratio should be >1.8.

(Alternative Protocol)

1. To 20 ml of phage liquid stock, add 4 µl of DNase I (5 mg/ml) and 75 µl of RNase A (10 mg/ml)
2. Add 20 ml of 20% PEG 8000/2M NaCl in 1X lambda dilution buffer. Incubate for 1 h at 0°C.
3. Centrifuge at 4°C for 20 min at 10,000 x g.
4. Remove supernatant by aspiration. Stand tube in an inverted position on a paper towel.
5. Add 0.5 ml of cold 1X lambda dilution buffer and resuspend by pipeting/ scraping off the pellet. Transfer to an Eppendorf tube.
6. Spin at 4°C for 2 min at 10,000 rpm. Transfer supernatant to a new tube.
7. Add 2.5 µl of 20% SDS, 5 µl of 0.5 M EDTA (pH 8), and 1.25 µl of 20mg/ml proteinase K (50 µg/ml final concentration).
8. DNA Extraction
   a. Extract with 0.5 ml phenol. Vortex for 1 min, and centrifuge for 5 min at 12,500 rpm.
   b. Extract with 0.5 ml phenol/chloroform. Vortex for 1 min, and centrifuge for 5 min at 12,500 rpm.
   c. Extract with 0.5 ml chloroform. Vortex for 1 min, and centrifuge for 5 min at 12,500 rpm.
9. Precipitate
   a. Add 1 ml of EtOH and mix by gentle inversion. DO NOT VORTEX. You should see a precipitate.
   b. Spool out the DNA with a sealed capillary tube.
   c. Wash with 70% EtOH.
10. Resuspend in 50 µl of TE. Use 2–5 µl to digest, and analyse on an agarose gel.

V. Library Protocols *continued*

G. Insert Excision

1. Restriction enzyme digestion
   a. Digest 5–10 µl of purified DNA with the appropriate restriction enzyme at 37°C for 3–16 hr.
   b. Heat samples at 70°C for 10 min to inactivate the enzyme.
   c. Analyze the digest on a 0.8% agarose minigel.

      Note: Depending on lysate titer, digestion of the entire isolate may be necessary in order to view the digest pattern. Precipitation of more than the usual volume of lysate can be performed (i.e., >10 ml) if a low titer is anticipated. If an abundance of undigested DNA is seen, the following steps are recommended.

      i. Reduce the DNA concentration.
      ii. Increase the amount of restriction enzyme.
      iii. Digest the DNA overnight.
      iv. Combine the above suggestions.
      v. Purify the DNA using standard methods followed by a round of phage DNA purification on a CHROMA SPIN-400 Column.

2. Difficulty with insert excision

   Inserts in both λgt10 and λgt11 libraries can be excised by EcoR I digestion. However, restriction enzymes are often sensitive to the purity of the DNA preparation. If the insert cannot be excised using EcoR I, the following options are available.

   Note: Top agarose must be used during library plating, otherwise DNA purity is compromised and insert excision may prove difficult.

   a. Digest λgt10 clones with Hind III/Ava I.

      Digest λgt11 clones with BsiW I.

      Note: For additional restriction site information, please refer to Appendices B and C.

   b. 5'-STRETCH or 5'-STRETCH PLUS cDNA Libraries (5000 series)

      The 5000 series libraries were constructed with a specially designed EcoR I adaptor that contains flanking Not I and Sal I sites. Therefore, the inserts can be excised with a Not I or Sal I digestion.

   c. NucleoBond®, NucleoSpin®, and NucleoTrap® Purification Products

      Clontech offers a complete line of nucleic acid purification products that are useful for purifying library clones and inserts.

   d. Insert size determination by PCR†

      Following identification of positive clones in λgt10 or λgt11, insert sizes can be determined by the use of Clontech's Insert Screening Amplimers. A listing of Clontech's cDNA Insert Screening Amplimers is presented in Section VII. Each Amplimer Set contains a complete protocol for use in PCR assays.

VI. Related Products

For DNA or oligonucleotide probe labeling	Cat. No.	Size
• Biotin-21-dUTP (0.5 mM)	635701	100 µl
• Biotin-21-dUTP (10 mM)	635702	100 µl

Note: Clontech offers a wide range of products for nonisotopic labeling of probes. For additional information, please consult the current Clontech Catalog.

For library screening	Cat. No.	Size
• ExpressHyb™ Hybridization Solution	636831	250 ml

For phage DNA isolation and purification	Cat. No.	Size
• CHROMA SPIN™ Columns	many	
• NucleoBond®, NucleoSpin®, and NucleoTrap® Purification Products	many	

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Appendix A: Host Strain Information

BACTERIAL HOST STRAINS

Strain	Reference	Genotype
C600	9–11	lacY1, leuB6, mcrA$^-$, supE44, thi-1, thr-1, tonA21 For making lysates and for λgt10 propagation.
C600 hfl	10, 11	hflA150 [chr::Tn10], lacY1, leuB6, mcrA$^-$, supE44, thi-1, thr-1, tonA21 **Note**: Tn10 confers tet^r For selection of λgt10 recombinants; the high frequency lysogeny mutation suppresses plaque formation by cI$^+$ bacteriophages while allowing plaque formation by recombinant cI$^-$ bacteriophages.
K802	12	gal K2, gal T22, hsdR2 (r_k^-, m_k^+), lacY1, mcrA$^-$, mcrB$^-$, metB1, mrr$^+$, supE44 For propagation of λ vectors and their recombinants.

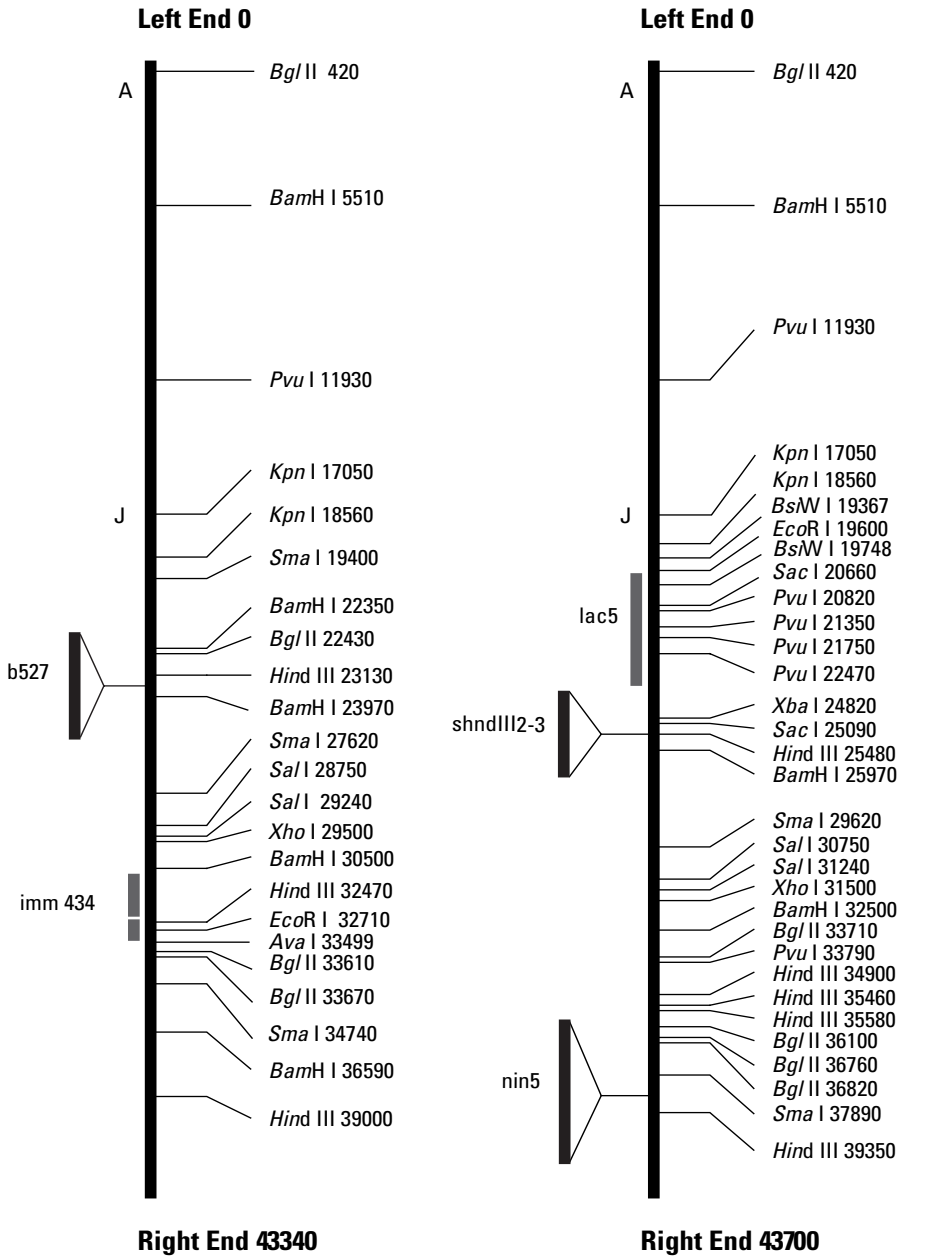
Appendix A: Host Strain Information *continued*

BACTERIAL HOST STRAINS

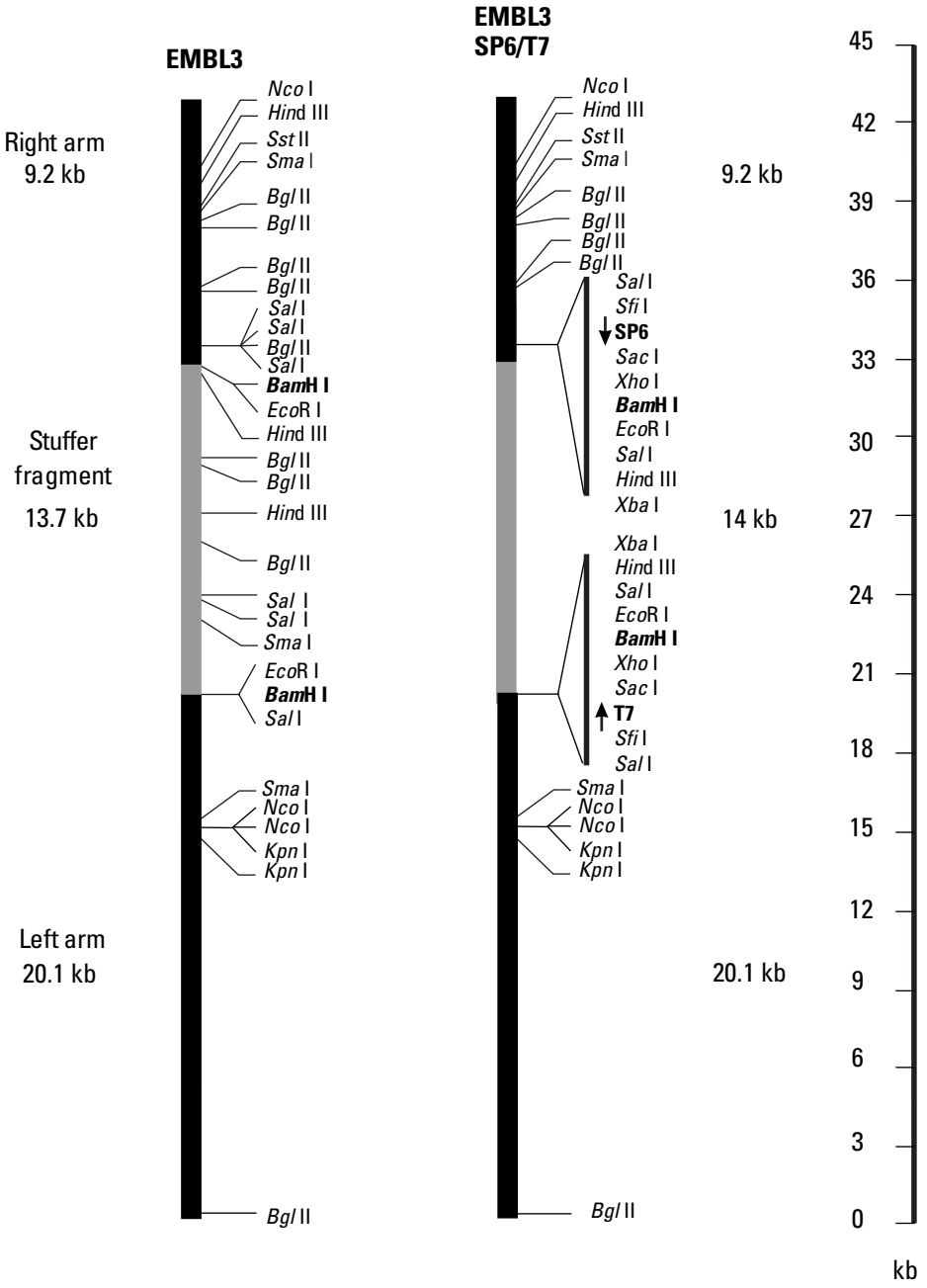
Strain	Reference	Genotype
K803	12	gal K2, gal T22, hsdS3 (r_k^-, m_k^-), lacY1, mcrA$^-$, mcrB$^-$, metB1, mrr$^+$, supE44 For propagation of λ vectors and their recombinants.
LE392	13, 14	gal K2, gal T22, hsdR514, lacY1, mcrA$^-$, metB1, supE44, supF58, trpR55 For propagation of λ vectors and their recombinants; a derivative of ED8654.
Y1090r$^-$	11	araD139, hsdR (r_k^-, m_k^+), mcrA$^-$, rpsL, supF, trpC22::Tn10, ΔlacU169, Δlon, (pMC9) **Note**: rpsL confers str^r, Tn10 confers tet^r, and pMC9 confers tet^r and amp^r Strain of choice for immunological screening of expression libraries and propagation of λgt11; expression of foreign proteins is controlled by high levels of lac repressor made by resident plasmid pMC9, which carries lacI^q; in lon$^-$ cells, β-galactosidase fusion proteins are often more stable than in wild-type cells; supF supresses normally defective lysis of λgt11 and λgt22, leading to a high frequency of lytic plaques and easier antibody screening.

Appendix B: Vector Maps

FIGURE 4. λgt10 λgt11



Appendix B: Vector Maps *continued*



Appendix C: Sequence Information

1. λgt10: Nucleic acid sequence around EcoR I Cloning site (15–17)

Note: EcoR I site is at position 251

```
1   ▼Hind III
AAGCTTCACA CCACGAACCA GCTCTAACCA TGCTAATCAA TGGATATTTC CCTTTGGGCT
TTCGAAGTGT GGTGCTTGGT CGAGATTGGT ACGATTAGTT ACCTATAAAG GGAAACCCGA

61                              ▼BstB I
CAACGTGCCC AACAAATCTA ACATTCGAAT CAGAGGTGCC ATTGAGCAGC CAGTCAACAC
GTTGCACGGG TTGTTTAGAT TGTAAGCTTA GTCTCCACGG TAACTCGTCG GTCAGTTGTG

121
TTACGCCAAG AGCTGACGCA AGTTCTGGTA AAAAGCGTGG TCGCTTAGTT TTACCGTTTT
AATGCGGTTC TCGACTGCGT TCAAGACCAT TTTTCGCACC AGCGAATCAA AATGGCAAAA

181                                                 5' insert screening amplimer
CGAGCTGCTC TATAGACTGC TGGGTAGTCC CCACCTTTTG AGCAAGTTCA GCCTGGTTAA
GCTCGACGAG ATATCTGACG ACCCATCAGG GGTGGAAAAC TCGTTCAAGT CGGACCAATT

241             ▼EcoR I
GTCCAAGCTG AATTCTTTTG CTTTTTACCC TGGAAGAAAT ACTCATAAGC CACCTCTGTT
CAGGTTCGAC TTAAGAAAAC GAAAAATGGG ACCTTCTTTA TGAGTATTCG GTGGAGACAA
                                  3' insert screening amplimer
301
ATTTACCCCC AATCTTCACA AGAAAAACTG TATTTGACAA ACAAGATACA TTGTATGAAA
TAAATGGGGG TTAGAAGTGT TCTTTTTGAC ATAAACTGTT TGTTCTATGT AACATACTTT

361                                                         ▼EarI
ATACAAGAAA GTTTGTTGAT GGAGGCGATA TGCAAACTCT TTCTGAACGC CTCAAGAAGA
TATGTTCTTT CAAACAACTA CCTCCGCTAT ACGTTTGAGA AAGACTTGCG GAGTTCTTCT

421
GGCGAATTGC GTTAAAAATG ACGCAAACCG AACTGGCAAC CAAAGCCGGT GTTAAACAGC
CCGCTTAACG CAATTTTTAC TGCGTTTGGC TTGACCGTTG GTTTCGGCCA CAATTTGTCG

461
AATCAATTCA ACTGATTGAA
TTAGTTAAGT TGACTAACTT
                                                    IMM 434 cI region
```

5' insert screening amplimer: 5'-AGC AAG TTC AGC CTG GTT AAG T-3'

3' insert screening amplimer: 3'-GGG ACC TTC TTT ATG AGT ATT-5'

2. λgt11: Nucleic acid sequence around Sac I–Kpn I region

Note: EcoR I site is at position 1070

```
1       ▼Sac I
GAGCTCCTGC ACTGGATGGT GGCGCTGGAT GGTAAGCCGC TGGCAAGCGG TGAAGTGCCT
CTCGAGGACG TGACCTACCA CCGCGACCTA CCATTCGGCG ACCGTTCGCC ACTTCACGGA
```

Appendix C: Sequence Information *continued*

```
 61
    CTGGATGTCG CTCCACAAGG TAAACAGTTG ATTGAACTGC CTGAACTACC GCAGCCGGAG
    GACCTACAGC GAGGTGTTCC ATTTGTCAAC TAACTTGACG GACTTGATGG CGTCGGCCTC

121                                 ▼Mlu I                      Drd I ▼
    AGCGCCGGGC AACTCTGGCT CACAGTACGC GTAGTGCAAC CGAACGCGAC CGCATGGTCA
    TCGCGGCCCG TTGAGACCGA GTGTCATGCG CATCACGTTG GCTTGCGCTG GCGTACCAGT

181                       ▼AlwN I
    GAAGCCGGGC ACATCAGCGC CTGGCAGCAG TGGCGTCTGG CGGAAAACCT CAGTGTGACG
    CTTCGGCCCG TGTAGTCGCG GACCGTCGTC ACCGCAGACC GCCTTTTGGA GTCACACTGC

241                                             Xcm I ▼   ▼BstX I
    CTCCCCGCCG CGTCCCACGC CATCCCGCAT CTGACCACCA GCGAAATGGA TTTTTGCATC
    GAGGGGCGGC GCAGGGTGCG GTAGGGCGTA GACTGGTGGT CGCTTTACCT AAAAACGTAG

301
    GAGCTGGGTA ATAAGCGTTG GCAATTTAAC CGCCAGTCAG GCTTTCTTTC ACAGATGTGG
    CTCGACCCAT TATTCGCAAC CGTTAAATTG GCGGTCAGTC CGAAAGAAAG TGTCTACACC

361                                 ▼Bgl I                      ▼ApaLI
    ATTGGCGATA AAAAACAACT GCTGACGCCG CTGCGCGATC AGTTCACCCG TGCACCGCTG
    TAACCGCTAT TTTTTGTTGA CGACTGCGGC GACGCGCTAG TCAAGTGGGC ACGTGGCGAC

421
    GATAACGACA TTGGCGTAAG TGAAGCGACC CGCATTGACC CTAACGCCTG GGTCGAACGC
    CTATTGCTGT AACCGCATTC ACTTCGCTGG GCGTAACTGG GATTGCGGAC CCAGCTTGCG

481                                                 ▼ApaLI
    TGGAAGGCGG CGGGCCATTA CCAGGCCGAA GCAGCGTTGT TGCAGTGCAC GGCAGATACA
    ACCTTCCGCC GCCCGGTAAT GGTCCGGCTT CGTCGCAACA ACGTCACGTG CCGTCTATGT

541 ▼                               ▼Mlu I
    CTTGCTGATG CGGTGCTGAT TACGACCGCT CACGCGTGGC AGCATCAGGG GAAAACCTTA
    GAACGACTAC GCCACGACTA ATGCTGGCGA GTGCGCACCG TCGTAGTCCC CTTTTGGAAT

601
    TTTATCAGCC GGAAAACCTA CCGGATTGAT GGTAGTGGTC AAATGGCGAT TACCGTTGAT
    AAATAGTCGG CCTTTTGGAT GGCCTAACTA CCATCACCAG TTTACCGCTA ATGGCAACTA

661                                                 ▼BspM I
    GTTGAAGTGG CGAGCGATAC ACCGCATCCG GCGCGGATTG GCCTGAACTG CCAGCTGGCG
    CAACTTCACC GCTCGCTATG TGGCGTAGGC CGCGCCTAAC CGGACTTGAC GGTCGACCGC

721
    CAGGTAGCAG AGCGGGTAAA CTGGCTCGGA TTAGGGCCGC AAGAAAACTA TCCCGACCGC
    GTCCATCGTC TCGCCCATTT GACCGAGCCT AATCCCGGCG TTCTTTTGAT AGGGCTGGCG

781                                                 Bbs I ▼   ▼ ▼BsiW I
    CTTACTGCCG CCTGTTTTGA CCGCTGGGAT CTGCCATTGT CAGACATGTA TACCCCGTAC
    GAATGACGGC GGACAAAACT GGCGACCCTA GACGGTAACA GTCTGTACAT ATGGGGCATG
```

Appendix C: Sequence Information *continued*

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 841
     GTCTTCCCGA GCGAAAACGG TCTGCGCTGC GGGACGCGCG AATTGAATTA TGGCCCACAC
     CAGAAGGGCT CGCTTTTGCC AGACGCGACG CCCTGCGCGC TTAACTTAAT ACCGGGTGTG

 901   vBstX I      vRleA                                        vAlwN I
     CAGTGGCGCG GCGACTTCCA GTTCAACATC AGCCGCTACA GTCAACAGCA ACTGATGGAA
     GTCACCGCGC CGCTGAAGGT CAAGTTGTAG TCGGCGATGT CAGTTGTCGT TGACTACCTT

 961     v Bsg I
     ACCAGCCATC GCCATCTGCT GCACGCGGAA GAAGGCACAT GGCTGAATAT CGACGGTTTC
     TGGTCGGTAG CGGTAGACGA CGTGCGCCTT CTTCCGTGTA CCGACTTATA GCTGCCAAAG

1021   vNde I v Xcm I              5' insert screening amplimer          Gsu I v, vEcoR I  Esp I v
     CATATGGGGA TTGGTGGCGA CGACTCCTGG AGCCCGTCAG TATCGGCGGA ATTCAGCTG
     GTATACCCCT AACCACCGCT GCTGAGGACC TCGGGCAGTC ATAGCCGCCT TAAGTCGAC

1081
     AGCGCCGGTC GCTACCATTA CCAGTTGGTC TGGTGTCAAA AATAATAATA ACCGGGCAGG
     TCGCGGCCAG CGATGGTAAT GGTCAACCAG ACCACAGTTT TTATTATTAT TGGCCCGTCC
       3' insert screening amplimer
1141
     CCATGTCTGC CCGTATTTCG CGTAAGGAAA TCCATTGTAC TGCCGGACCA CCGACTGTGA
     GGTACAGACG GGCATAAAGC GCATTCCTTT AGGTAACATG ACGGCCTGGT GGCTGACACT

1201          Xcm I
       Bgl I v  v    vNco I  v BsiW I                  vBspM I    Age I v     vRsr II
     GCCACTCCGG CCATGGCGTA CGCACTGACC TGCTTACTGA TTTGTAAAAC CGGTCCGGCC
     CGGTGAGGCC GGTACCGCAT GCGTGACTGG ACGAATGACT AAACATTTTG GCCAGGCCGG

1261
     ATCACGCTCA CATAACGTCC ACGCAGGCTC TCATAGTGAA ACGTATCCTC CCCGGTCATC
     TAGTGCGAGT GTATTGCAGG TGCGTCCGAG AGTATCACTT TGCATAGGAG GGGCCAGTAG

1321
     ACTGTGCTGC TCTTTTTCGA CGCGGCGAAC CCCAGGGAAG CCATCACCCC CACACTGTCC
     TGACACGACG AGAAAAAGCT GCGCCGCTTG GGGTCCCTTC GGTAGTGGGG GTGTGACAGG

1381        vRleA I                                          vAge I
     GTCAGCTCAT AACGGTACTT CACGTTAATC CCTTTCAGAT GACTCACACC GGTATCCCCG
     CAGTCGAGTA TTGCCATGAA GTGCAATTAG GGAAAGTCTA CTGAGTGTGG CCATAGGGGC

1441
     CCCGACAACG ACGGCAATGT ACCCGGTTTC ACTTGAAAAT AGCCCACCGT AAACGTACCA
     GGGCTGTTGC TGCCGTTACA TGGGCCAAAG TGAACTTTTA TCGGGTGGCA TTTGCATGGT

1501
     TGTCCACCTT CCGCACGGGC CGGAGTGACT GTCACCGCAA GTGCGGCAAA GACAGCAACG
     ACAGGTGGAA GGCGTGCCCG GCCTCACTGA CAGTGGCGTT CACGCCGTTT CTGTCGTTGC

1561
     GCAATACACA CATTACGCAT CGTTCACCTC TCACTGTTTT ATAATAAAAC GCCCGTTCCC
     CGTTATGTGT GTAATGCGTA GCAAGTGGAG AGTGACAAAA TATTATTTTG CGGGCAAGGG
```

Appendix C: Sequence Information *continued*

```
1621                                                    AlwN I v          v Bsg I
    GGACGAACCT CTGTAACACA CTCAGACCAC GCTGATGCCC AGCGCCTGTT TCTTAATCAC
    CCTGCTTGGA GACATTGTGT GAGTCTGGTG CGACTACGGG TCGCGGACAA AGAATTAGTG

1681                  v BspM I
    CATAACCTGC ACATCGCTGG CAAACGTATA CGGCGGAATA TCTGCCGAAT GCCGTGTGGA
    GTATTGGACG TGTAGCGACC GTTTGCATAT GCCGCCTTAT AGACGGCTTA CGGCACACCT

1741
    CGTAAGCGTG AACGTCAGGA TCACGTTTCC CCGACCCGCT GGCATGTCAA CAATACGGGA
    GCATTCGCAC TTGCAGTCCT AGTGCAAAGG GGCTGGGCGA CCGTACAGTT GTTATGCCCT

1801
    GAACACCTGT ACCGCCTCGT TCGCCGCGCC ATCATAAATC ACCGCACCGT TCATCAGTAC
    CTTGTGGACA TGGCGGAGCA AGCGGCGCGG TAGTATTTAG TGGCGTGGCA AGTAGTCATG

1861
    TTTCAGATAA CACATCGAAT ACGTTGTCCT GCCGCTGACA GTACGCTTAC TTCCGCGAAA
    AAAGTCTATT GTGTAGCTTA TGCAACAGGA CGGCGACTGT CATGCGAATG AAGGCGCTTT

1921                          v PflM I
    CGTCAGCGGA AGCACCACTA TCTGGCGATC AAAAGGATGG TCATCGGTCA CGGTGACAGT
    GCAGTCGCCT TCGTGGTGAT AGACCGCTAG TTTTCCTACC AGTAGCCAGT GCCACTGTCA

1981   Asp718
       v    v Kpn I
    ACGGGTACC
    TGCCCATGG
```

5' insert screening amplimer: 5'-GAC TCC TGG AGC CCG-3'

3' insert screening amplimer: 3'-CGC GGC CAG CGA TG G-5'

Enzyme	Position	Enzyme	Position	Enzyme	Position
Age I	1250 1429	BsiW 1	837 1218	Mlu I	148 573
AlwN I	201 952 1666	Bsm I	1734	Nco I	1212
		BspM I	712 1238 1695	Nde I	1023
ApaL I	411 526			PflM I	1942
		BstX I	286 903	RleA I	912 1387
Asp718	1985			Rsr II	1253
Bbs I	835	Drd I	175	Sac I	6
Bgl I	389 1208	EcoR I	1070	Xcm I	283 1028 1210
		Esp I	1079		
Bsg I	965 1673	Gsu I	1069		
		Kpn I	1989		

Appendix C: Sequence Information *continued*

There are no restriction sites in the previous sequence for the following enzymes:

Aat II	Afl II	ApaB I	Apa I
Asc I	Ase I	Avr II	BamH I
Bbe I	Bcg I	Bcl I	Bgl II
Bpu 10 I	Bsa I	Bsi I	Bsp120 I
BspE I	BspH I	BssH II	BstB I
BstE II	Bsu361	Cla I	Dra III
Eag I	Ear I	Eco571	EcoN I
Esp3 I	Fse I	HgiEI I	Hind III
Kas I	Mun I	Nar I	NgoM I
Nhe I	Not I	Nsi I	Pac I
PpuM I	Pst I	Pvu I	Sac II
Sal I	Sap I	Sce I	Sfi I
SgrA I	Spe I	Sph I	Sse I
TthIII I	Xba I	Xho I	Xma I

3. λgt11: Amino acid sequence around Sac I–Kpn I region

```
  1
GAG CTC CTG CAC TGG ATG GTG GCG CTG GAT GGT AAG CCG CTG GCA AGC GGT GAA
CTC GAG GAC GTG ACC TAC CAC CGC GAC CTA CCA TTC GGC GAC CGT TCG CCA CTT
Glu Leu Leu His Trp Met Val Ala Leu Asp Gly Lys Pro Leu Ala Ser Gly Glu

 55
GTG CCT CTG GAT GTC GCT CCA CAA GGT AAA CAG TTG ATT GAA CTG CCT GAA CTA
CAC GGA GAC CTA CAG CGA GGT GTT CCA TTT GTC AAC TAA CTT GAC GGA CTT GAT
Val Pro Leu Asp Val Ala Pro  Gln Gly Lys Gln Leu Ile  Glu Leu Pro Glu Leu

110
CCG CAG CCG GAG AGC GCC GGG CAA CTC TGG CTC ACA GTA CGC GTA GTG CAA CCG
GGC GTC GGC CTC TCG CGG CCC GTT GAG ACC GAG TGT CAT GCG CAT CAC GTT GGC
Pro Gln Pro Glu Ser  Ala Gly Gln Leu Trp Leu Thr  Val Arg Val  Val Gln Pro

164
AAC GCG ACC GCA TGG TCA GAA GCC GGG CAC ATC AGC GCC TGG CAG CAG TGG CGT
TTG CGC TGG CGT ACC AGT CTT CGG CCC GTG TAG TCG CGG ACC GTC GTC ACC GCA
Asn Ala Thr Ala Trp Ser Glu Ala  Gly His  Ile  Ser  Ala Trp Gln Gln Trp Arg

218
CTG GCG GAA AAC CTC AGT GTG ACG CTC CCC GCC GCG TCC CAC GCC ATC CCG CAT
GAC CGC CTT TTG GAG TCA CAC TGC GAG GGG CGG CGC AGG GTG CGG TAG GGC GTA
Leu Ala Glu Asn Leu Ser Val Thr Leu Pro Ala Ala  Ser His  Ala Ile  Pro  His

272
CTG ACC ACC AGC GAA ATG GAT TTT TGC ATC GAG CTG GGT AAT AAG CGT TGG CAA
GAC TGG TGG TCG CTT TAC CTA AAA ACG TAG CTC GAC CCA TTA TTC GCA ACC GTT
Leu Thr Thr Ser Glu Met Asp Phe Cys Ile  Glu Leu Gly Asn Lys Arg Trp Gln
```

Appendix C: Sequence Information *continued*

```
326
TTT AAC CGC CAG TCA GGC TTT CTT TCA CAG ATG TGG ATT GGC GAT AAA AAA CAA
AAA TTG GCG GTC AGT CCG AAA GAA AGT GTC TAC ACC TAA CCG CTA TTT TTT GTT
Phe Asn Arg Gln Ser Gly Phe Leu Ser Gln Met Trp Ile Gly Asp Lys Lys Gln

380
CTG CTG ACG CCG CTG CGC GAT CAG TTC ACC CGT GCA CCG CTG GAT AAC GAC ATT
GAC GAC TGC GGC GAC GCG CTA GTC AAG TGG GCA CGT GGC GAC CTA TTG CTG TAA
Leu Leu Thr Pro Leu Arg Asp Gln Phe Thr Arg Ala Pro Leu Asp Asn Asp Ile

434
GGC GTA AGT GAA GCG ACC CGC ATT GAC CCT AAC GCC TGG GTC GAA CGC TGG AAG
CCG CAT TCA CTT CGC TGG GCG TAA CTG GGA TTG CGG ACC CAG CTT GCG ACC TTC
Gly Val Ser Glu Ala Thr Arg Ile Asp Pro Asn Ala Trp Val Glu Arg Trp Lys

488
GCG GCG GGC CAT TAC CAG GCC GAA GCA GCG TTG TTG CAG TGC ACG GCA GAT ACA
CGC CGC CCG GTA ATG GTC CGG CTT CGT CGC AAC AAC GTC ACG TGC CGT CTA TGT
Ala Ala Gly His Tyr Gln Ala Glu Ala Ala Leu Leu Gln Cys Thr Ala Asp Thr

542
CTT GCT GAT GCG GTG CTG ATT ACG ACC GCT CAC GCG TGG CAG CAT CAG GGG AAA
GAA CGA CTA CGC CAC GAC TAA TGC TGG CGA GTG CGC ACC GTC GTA GTC CCC TTT
Leu Ala Asp Ala Val Leu Ile Thr Thr Ala His Ala Trp Gln His Gln Gly Lys

596
ACC TTA TTT ATC AGC CGG AAA ACC TAC CGG ATT GAT GGT AGT GGT CAA ATG GCG
TGG AAT AAA TAG TCG GCC TTT TGG ATG GCC TAA CTA CCA TCA CCA GTT TAC CGC
Thr Leu Phe Ile Ser Arg Lys Thr Tyr Arg Ile Asp Gly Ser Gly Gln Met Ala

650
ATT ACC GTT GAT GTT GAA GTG GCG AGC GAT ACA CCG CAT CCG GCG CGG ATT GGC
TAA TGG CAA CTA CAA CTT CAC CGC TCG CTA TGT GGC GTA GGC CGC GCC TAA CCG
Ile Thr Val Asp Val Glu Val Ala Ser Asp Thr Pro His Pro Ala Arg Ile Gly

704
CTG AAC TGC CAG CTG GCG CAG GTA GCA GAG CGG GTA AAC TGG CTC GGA TTA GGG
GAC TTG ACG GTC GAC CGC GTC CAT CGT CTC GCC CAT TTG ACC GAG CCT AAT CCC
Leu Asn Cys Gln Leu Ala Gln Val Ala Glu Arg Val Asn Trp Leu Gly Leu Gly

758
CCG CAA GAA AAC TAT CCC GAC CGC CTT ACT GCC GCC TGT TTT GAC CGC TGG GAT
GGC GTT CTT TTG ATA GGG CTG GCG GAA TGA CGG CGG ACA AAA CTG GCG ACC CTA
Pro Gln Glu Asn Tyr Pro Asp Arg Leu Thr Ala Ala Cys Phe Asp Arg Trp Asp

812
CTG CCA TTG TCA GAC ATG TAT ACC CCG TAC GTC TTC CCG AGC GAA AAC GGT CTG
GAC GGT AAC AGT CTG TAC ATA TGG GGC ATG CAG AAG GGC TCG CTT TTG CCA GAC
Leu Pro Leu Ser Asp Met Tyr Thr Pro Tyr Val Phe Pro Ser Glu Asn Gly Leu
```

Appendix C: Sequence Information *continued*

```
866
CGC TGC GGG ACG CGC GAA TTG AAT TAT GGC CCA CAC CAG TGG CGC GGC GAC TTC
GCG ACG CCC TGC GCG CTT AAC TTA ATA CCG GGT GTG GTC ACC GCG CCG CTG AAG
Arg Cys Gly Thr  Arg Glu Leu Asn Tyr  Gly Pro His Gln Trp  Arg Gly Asp Phe

920
CAG TTC AAC ATC AGC CGC TAC AGT CAA CAG CAA CTG ATG GAA ACC AGC CAT CGC
GTC AAG TTG TAG TCG GCG ATG TCA GTT GTC GTT GAC TAC CTT TGG TCG GTA GCG
Gln Phe Asn Ile  Ser  Arg Tyr Ser Gln Gln Gln Leu Met Glu Thr Ser His Arg

974
CAT CTG CTG CAC GCG GAA GAA GGC ACA TGG CTG AAT ATC GAC GGT TTC CAT ATG
GTA GAC GAC GTG CGC CTT CTT CCG TGT ACC GAC TTA TAG CTG CCA AAG GTA TAC
His Leu Leu His Ala Glu Glu Gly Thr Trp  Leu Asn Ile Asp Gly Phe His Met

1028 EcoR I
GGG ATT GGT GGC GAC GAC TCC TGG AGC CCG TCA GTA TCG GCG GAA TTC CAG CTG
CCC TAA CCA CCG CTG CTG AGG ACC TCG GGC AGT CAT AGC CGC CTT AAG GTC GAC
Gly  Ile  Gly Gly Asp Asp Ser Trp Ser Pro Ser Val Ser Ala  Glu Phe Gln Leu

1082
AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA TAA TAA TAA CCG
TCG CGG CCA GCG ATG GTA ATG GTC AAC CAG ACC ACA GTT TTT ATT ATT ATT GGC
Ser Ala Gly  Arg Tyr His  Tyr Gln Leu Val Trp Cys Gln Lys End End  End Pro

1136
GGC AGG CCA TGT CTG CCC GTA TTT CGC GTA AGG AAA TCC ATT GTA CTG CCG GAC
CCG TCC GGT ACA GAC GGG CAT AAA GCG CAT TCC TTT AGG TAA CAT GAC GGC CTG
Gly Arg Pro Cys Leu Pro Val Phe Arg Val Arg Lys Ser  Ile  Val Leu Pro Asp

1190
CAC CGA CTG TGA GCC ACT CCG GCC ATG GCG TAC GCA CTG ACC TGC TTA CTG ATT
GTG GCT GAC ACT CGG TGA GGC CGG TAC CGC ATG CGT GAC TGG ACG AAT GAC TAA
His Arg Leu End Ala Thr Pro Ala Met Ala Tyr  Ala Leu Thr Cys Leu Leu Ile

1244
TGT AAA ACC GGT CCG GCC ATC ACG CTC ACA TAA CGT CCA CGC AGG CTC TCA TAG
ACA TTT TGG CCA GGC CGG TAG TGC GAG TGT ATT GCA GGT GCG TCC GAG AGT ATC
Cys Lys Thr Gly Pro Ala  Ile  Thr Leu Thr End Arg Pro Arg Arg Leu Ser End

1298
TGA AAC GTA TCC TCC CCG GTC ATC ACT GTG CTG CTC TTT TTC GAC GCG GCG AAC
ACT TTG CAT AGG AGG GGC CAG TAG TGA CAC GAC GAG AAA AAG CTG CGC CGC TTG
End Asn Val Ser Ser Pro Val  Ile  Thr  Val Leu Leu Phe Phe Asp Ala Ala Asn

1352
CCC AGG GAA GCC ATC ACC CCC ACA CTG TCC GTC AGC TCA TAA CGG TAC TTC ACG
GGG TCC CTT CGG TAG TGG GGG TGT GAC AGG CAG TCG AGT ATT GCC ATG AAG TGC
Pro Arg Glu  Ala Ile  Thr Pro Thr Leu Ser Val Ser Ser End Arg Tyr Phe Thr
```

Appendix C: Sequence Information *continued*

1406

```
TTA ATC CCT TTC AGA TGA CTC ACA CCG GTA TCC CCG CCC GAC AAC GAC GGC AAT
AAT TAG GGA AAG TCT ACT GAG TGT GGC CAT AGG GGC GGG CTG TTG CTG CCG TTA
Leu Ile  Pro Phe Arg End Leu Thr Pro Val Ser Pro Pro Asp Asn Asp Gly Asn
```

1460

```
GTA CCC GGT TTC ACT TGA AAA TAG CCC ACC GTA AAC GTA CCA TGT CCA CCT TCC
CAT GGG CCA AAG TGA ACT TTT ATC GGG TGG CAT TTG CAT GGT ACA GGT GGA AGG
Val Pro Gly  Phe Thr End Lys End  Pro Thr  Val Asn Val Pro Cys Pro Pro Ser
```

1514

```
GCA CGG GCC GGA GTG ACT GTC ACC GCA AGT GCG GCA AAG ACA GCA ACG GCA ATA
CGT GCC CGG CCT CAC TGA CAG TGG CGT TCA CGC CGT TTC TGT CGT TGC CGT TAT
Ala  Arg Ala Gly Val Thr  Val Thr Ala Ser  Ala  Ala Lys Thr Ala  Thr Ala  Ile
```

1568

```
CAC ACA TTA CGC ATC GTT CAC CTC TCA CTG TTT TAT AAT AAA ACG CCC GTT CCC
GTG TGT AAT GCG TAG CAA GTG GAG AGT GAC AAA ATA TTA TTT TGC GGG CAA GGG
His Thr Leu Arg  Ile  Val His Leu Ser Leu Phe Tyr  Asn Lys Thr Pro Val Pro
```

1622

```
GGA CGA ACC TCT GTA ACA CAC TCA GAC CAC GCT GAT GCC CAG CGC CTG TTT CTT
CCT GCT TGG AGA CAT TGT GTG AGT CTG GTG CGA CTA CGG GTC GCG GAC AAA GAA
Gly Arg Thr Ser  Val Thr His Ser  Asp His Ala Asp Ala Gln Arg Leu Phe Leu
```

1676

```
AAT CAC CAT AAC CTG CAC ATC GCT GGC AAA CGT ATA CGG CGG AAT ATC TGC CGA
TTA GTG GTA TTG GAC GTG TAG CGA CCG TTT GCA TAT GCC GCC TTA TAG ACG GCT
Asn His His Asn Leu His  Ile  Ala Gly Lys Arg  Ile  Arg  Arg Asn Ile Cys Arg
```

1730

```
ATG CCG TGT GGA CGT AAG CGT GAA CGT CAG GAT CAC GTT TCC CCG ACC CGC TGG
TAC GGC ACA CCT GCA TTC GCA CTT GCA GTC CTA GTG CAA AGG GGC TGG GCG ACC
Met Pro Cys Gly Arg Lys Arg Glu Arg Gln Asp His Val Ser Pro Thr  Arg Trp
```

1784

```
CAT GTC AAC AAT ACG GGA GAA CAC CTG TAC CGC CTC GTT CGC CGC GCC ATC ATA
GTA CAG TTG TTA TGC CCT CTT GTG GAC ATG GCG GAG CAA GCG GCG CGG TAG TAT
His  Val Asn Asn Thr Gly Glu His Leu Tyr  Arg Leu Val  Arg Arg Ala  Ile  Ile
```

1838

```
AAT CAC CGC ACC GTT CAT CAG TAC TTT CAG ATA ACA CAT CGA ATA CGT TGT CCT
TTA GTG GCG TGG CAA GTA GTC ATG AAA GTC TAT TGT GTA GCT TAT GCA ACA GGA
Asn His Arg Thr Val  His Gln Tyr  Phe Gln  Ile Thr His Arg Ile  Arg Cys Pro
```

1892

```
GCC GCT GAC AGT ACG CTT ACT TCC GCG AAA CGT CAG CGG AAG CAC CAC TAT CTG
CGG CGA CTG TCA TGC GAA TGA AGG CGC TTT GCA GTC GCC TTC GTG GTG ATA GAC
Ala Ala Asp Ser Thr Leu Thr Ser Ala Lys Arg Gln Arg Lys His His  Tyr  Leu
```

Appendix C: Sequence Information *continued*

1946

```
GCG ATC AAA AGG ATG GTC ATC GGT CAC GGT GAC AGT ACG GGT ACC
CGC TAG TTT TCC TAC CAG TAG CCA GTG CCA CTG TCA TGC CCA TGG
Ala  Ile Lys Arg Met Val  Ile  Gly His Gly Asp Ser Thr Gly Thr
```

Appendix D: Recombination Frequency Determination

The following protocol is used to determine the recombination frequency for libraries cloned in λ expression vectors (e.g., λgt11).

Prepare recommended titering dilutions of phage lysate (library) as follows:

1. Pipet 2 µl of the library lysate into 1 ml of 1X lambda dilution buffer (Dilution 1 = 1:500).
2. Transfer 2 µl of Dilution 1 into a second tube containing 1 ml of 1X lambda dilution buffer (Dilution 2 = 1:250,000).
3. Prepare 4 tubes as described in Table IV using the bacterial overnight culture obtained in Step VI.B.1.

TABLE IV: LIBRARY PLATING DILUTIONS

Tube	1X Lambda Dilution Buffer	Bacterial Overnight Culture	Phage Dilution 2
1	100 µl	200 µl	2 µl
2	100 µl	200 µl	5 µl
3	100 µl	200 µl	10 µl
4 (Control)	100 µl	200 µl	0 µl

4. Incubate tubes in a 37°C water bath for 15 min.
5. Add 30 µl of 0.1 M X-Gal and 30 µl of 0.1 M IPTG to each of the 4 tubes.
6. Add 3 ml of melted LB soft top agar + $MgSO_4$ (45°C) to each of the 4 tubes. Mix well.
7. Pour the contents from each tube onto 4 separate 90-mm LB agar + $MgSO_4$ plates. Swirl the plates quickly after pouring to allow for even spreading of the agar.

   Note: If the plates were prepared fresh and have been sitting at room temperature, prewarm plates at 37°C for 10–15 min. If plates have been stored at 4°C, prewarm plates at 37°C for 1 hr prior to use.
8. Cool plates at room temperature for 10 min to allow the inoculum to soak into the agar.

Appendix D: Recombination Frequency *continued*

9. Incubate plates (inverted position) at 37°C for at least 6–7 hr.
10. Count the number of blue plaques and the number of white plaques.

Recombination frequency = $\frac{\text{number of blue plaques}}{\text{number of total plaques}} \times 100\%$

Appendix E: rpm Calculation

$$\text{rpm} = 1000 \times \sqrt{\text{RCF}/(1.12r)}$$

r = radius of rotor (in mm)
RCF = relative centrifugal field
g Force = RCF x g

Examples:

a. Swinging bucket rotor = Beckman Model TJ-6
   r = 125 mm
   RCF = 700
   therefore, rpm = 2236

b. Fixed-angle rotor = Eppendorf Model 5415C (microcentrifuge)
   r = 50 mm
   RCF = 700
   therefore, rpm = 3536