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λTriplEx™ & λTriplEx2™ Libraries User Manual

PT3003-1 (PR09529)

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See List of Components for storage conditions

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I. Introduction

Unlike other λ expression vectors, which can generate a polypeptide sequence from one reading frame in each recombinant λ clone, λTriplEx™ expresses cloned sequences in all three reading frames (Figure 1). This feature triples the number of productive clones in a λTriplEx library, giving a three-fold greater chance of finding positive plaques. λTriplEx2™ has all the features of λTriplEx, and also contains asymmetrical *Sfi* I A & B sites in its MCS. These sites eliminate adaptor ligation and facilitate directional cloning.

λTriplEx is a phagemid vector with *cre-lox*-mediated subcloning

Very high-titer libraries (i.e., > 1 x 10^6 independent clones) can be constructed in λ vectors because of the high efficiency of packaging and transduction of recombinant λ phage into *E. coli* (Sambrook *et al*., 1989). In λTriplEx phagemid vectors, the cloning sites are located within a plasmid that is embedded in a λ phage genome and flanked by *loxP* sites at the λ junctions (Figure 2). Transducing a λTriplEx lysate into an appropriate *E. coli* strain such as BM25.8 promotes *Cre* recombinase-mediated release and circularization of pTriplEx at the *loxP* sites (Elledge *et al*., 1991). Because helper phage is not required, *Cre-lox*-mediated phagemid conversion is an easy and reliable way to obtain a plasmid vector containing a desired cDNA clone. pTriplEx contains elements that facilitate its selection and autonomous replication in *E. coli* (Appendix A, Figure 8).

λTriplEx expresses each cloned protein in all three reading frames

Double-stranded cDNAs have heterogeneous 5' ends and, when cloned into conventional λ expression vectors, are under the control of only one translational start site. Consequently, in conventional λ expression libraries, only about one-third of the cDNA clones will have the coding sequence in the same frame as the single translation initiation site on the vector. Even when using a directional cloning strategy, approximately two-thirds of the clones in the library are unproductive. This problem is avoided in λTriplEx because each insert is translated in all three reading frames. Furthermore, the expression cassette in λTriplEx incorporates the 5' untranslated region (UTR) of the *ompA* gene from *E. coli*. This UTR stabilizes the mRNA and leads to an increased level of expression (Emory *et al*., 1992). These features significantly increase the likelihood that a recombinant vector containing the target cDNA will be detected in expression screening.

λTriplEx cDNA libraries are ideal for immunoscreening

Triple frame expression makes λTriplEx cDNA libraries especially well-suited to screening methods designed for detecting proteins expressed in *E. coli*. Such methods include screening with antibodies, with DNA or RNA probes (for nucleic acid binding proteins), or with labeled proteins (for interacting proteins). λTriplEx cDNA libraries can also be screened using standard nucleic acid hybridization methods. λTriplEx LD-Insert Screening Amplimers (#9107-1; available separately) can be used to sort and characterize candidate clones using long-distance PCR. Sequencing primers are provided with every λTriplEx order.

I. Introduction *continued*

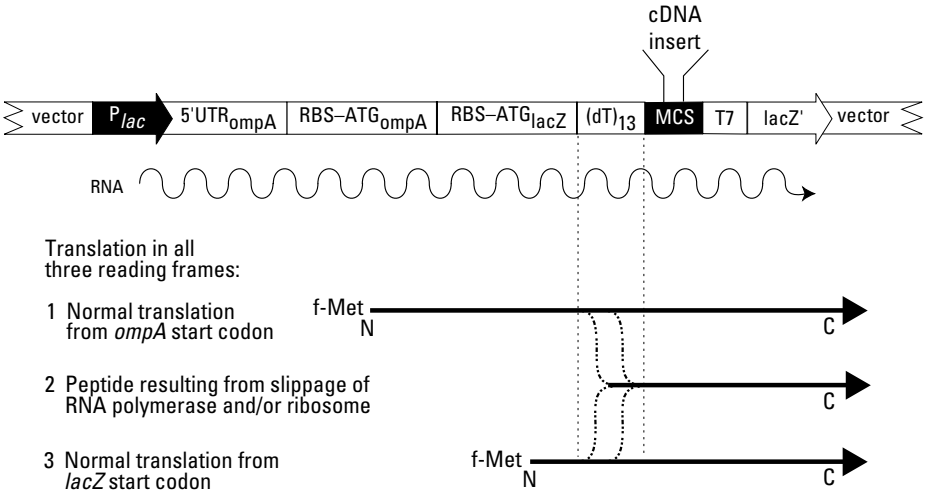

Figure 1. Generation of polypeptides from all three reading frames in a single recombinant λTriplEx clone. λTriplEx contains two translation start sites (i.e., two sets of ribosome-binding sites and ATG start codons) in different reading frames and a slip site (a stretch of dTs) that can cause ribosomes to shift frames between the regulated *lac* promoter and the MCS. RNA polymerase may also slip during transcription of the $(dT)_{13}$ region. By the time the ribosomes begin translating the insert, roughly one-third will be in each of the three reading frames.

Novel triple reading frame design

λTriplEx is similar to other λ expression vectors in that its multiple cloning site (MCS) is embedded in the coding sequence for the α-polypeptide of β-galactosidase (*lacZ'*). Also, as in other λ vectors, proteins are expressed as fusions with a short peptide encoded by the vector. However, unlike other expression vectors, λTriplEx has two sets of translation initiation signals, including two ATG start codons in different reading frames (Figure 1). In addition, a stretch of dTs between the second start codon and the MCS provides a slip site where RNA polymerase can skip nucleotides during transcription (Wagner *et al.*, 1990), and where ribosomes can easily slip into another reading frame during translation (Atkins, *et al.*, 1990). Consequently, within each cell, approximately one-third of the ribosomes will be in each reading frame when they begin to translate the cDNA insert. Thus, every recombinant λTriplEx vector containing a coding insert will express some protein (or peptide fragments) from the correct reading frame.

I. Introduction *continued*

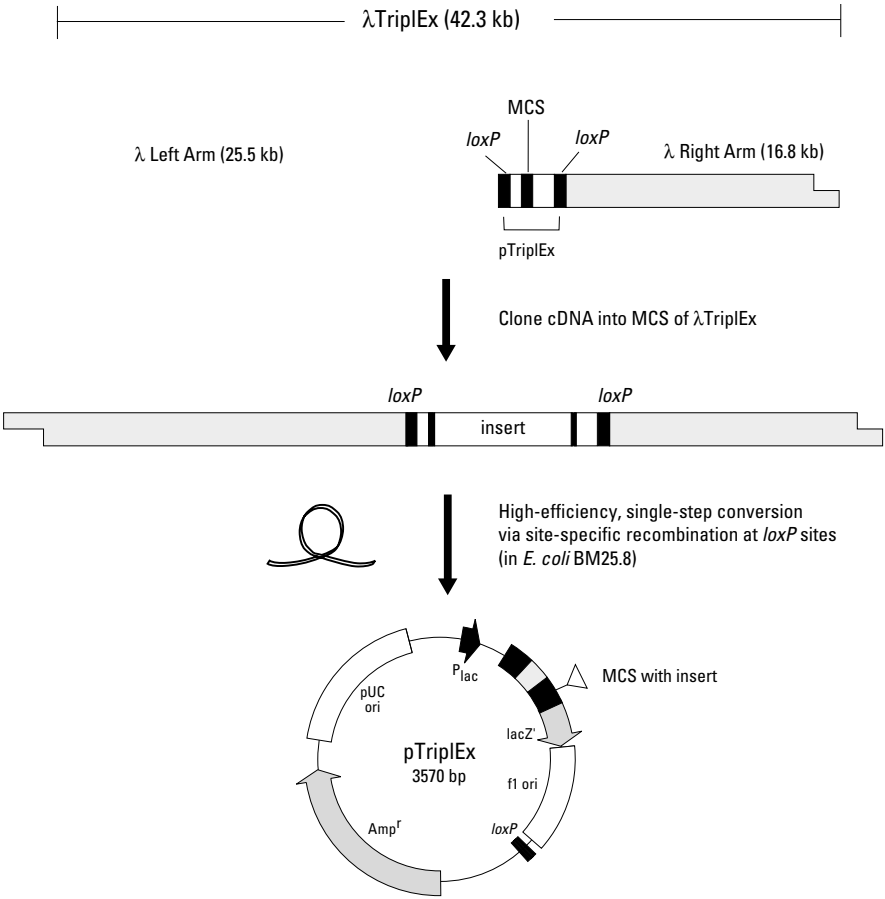

Figure 2. Conversion of a recombinant λTriplEx to pTriplEx. The λTriplEx MCS is located within an embedded plasmid, which is flanked by *loxP* sites at the λ junctions. Transduction of a λTriplEx lysate into *E. coli* strain BM25.8 promotes *Cre* recombinase-mediated release and circularization of pTriplEx at the *loxP* sites. pTriplEx carries the *bla* gene for ampicillin resistance, and the pUC ori for autonomous replication, in *E. coli*. The MCS provides 20 unique restriction sites including *Eco*R I, *Xba* I, *Xho* I, and *Not* I to facilitate the subcloning and analysis of inserts. See Appendix A for a more detailed pTriplEx vector map and the MCS sequence.

I. Introduction *continued*

Blue/white screening, regulated expression

In λTriplEx nonrecombinants, translation in-frame with the *lacZ'* peptide results in sufficient protein for *lacZ* α-complementation (Sambrook *et al*., 1989). This feature allows easy identification of recombinant phage by transducing an appropriate host such as *E. coli* XL1-Blue and screening for blue plaques on medium containing IPTG and X-gal. The ratio of white to blue plaques gives a quick estimate of recombination efficiency. The regulated *lac* promoter, which controls expression of cloned inserts, is repressed by the absence of the inducer IPTG until the library is screened (Young & Davis, 1983).

New library construction technology enriches for full-length cDNAs

CLONTECH's Large-Insert cDNA Libraries represent the ultimate resource for obtaining full-length clones. Our new library construction process combines our SMART™ technology with size fractionation to ensure an average insert size that's twice that of other libraries. These libraries are constructed in λTriplEx2, a novel phagemid cDNA cloning vector that results in higher library complexity. Together, these features greatly increase the chance of finding your full–length clones.

II. List of Components

A. λTriplEx DNA

Storage conditions:

- λTriplEx DNA can be stored at 4°C for up to 2 weeks, –20°C for up to 3 months, and –70°C for up to one year. Avoid repeated freeze-thaw cycles.
- *E. coli* host strains: Store at –20°C for up to 2 weeks; store at –70°C (in 25% glycerol) for up to 1 year.
- Control insert and sequencing primers: Store at –20°C.

Cat. #6160-1

- 20 µg **λTriplEx, uncut**
  (check Product Analysis Certificate for concentration)

Cat. #6161-1

- 10 µg **λTriplEx arms, *Eco*R I-digested, dephosphorylated** (0.5 µg/µl)
- 10.0 µl **Control insert, *Eco*R I-digested**
  (0.25 µg/µl. Insert size is 2.9-kb long)

Cat. #6162-1

- 10 µg **λTriplEx arms, *Eco*R I/*Xba* I-digested, dephosphorylated** (0.5 µg/µl)
- 10.0 µl **Control insert, *Eco*R I/*Xba* I-digested**
  (0.25 µg/µl. Insert size is 2.9-kb long)

Components included with all λTriplEx DNAs:

- 0.5 ml ***E. coli* XL-1 Blue** (in 25% glycerol; genotype in Section II.C**)**
- 0.5 ml ***E. coli* BM25.8** (in 25% glycerol; genotype in Section II.C)
- 50 µl **5' Sequencing primer** (20 µM)
  5'- TCCGAGATCTGGACGAGC -3'
- 50 µl **3' Sequencing primer** (20 µM)
  5'- TAATACGACTCACTATAGGG -3'

B. λTriplEx Libraries

Storage conditions:

Note: All premade and custom λTriplEx libraries are shipped in 7% DMSO. Removing the DMSO prior to using the library is not necessary.

- **Premade λTriplEx libraries, λTriplEx2 Large-Insert libraries, and amplified λTriplEx2 custom libraries**

  Short-term (<2 months) Store working aliquots of the library at 4°C. The titer of these aliquots should be stable for up to 2 months.

II. List of Components *continued*

Long-term (≥2 months) Divide the library into 50-µl aliquots and store them at –70°C. Storage at –70°C should keep the library titer constant for years. Avoid repeated freeze/thaw cycles of the stock sample.

- **Unamplified custom libraries** can be stored at 4°C for **up to 2 weeks only**. If you plan to keep an unamplified library longer than 2 weeks, you have two options: (1) Divide the unamplified library into 50-µl aliquots and store them at –70°C. (2) Amplify the library (Section V.E), and store it as described for premade and amplified custom libraries. If you amplify your library, remember to add 7% DMSO before storing at –70°C. Avoid repeated freeze/thaw cycles.
- ***E. coli* host strains:** Store at –20°C for up to 2 weeks; store at –70°C (in 25% glycerol) for up to 1 year.

Components included with all λTriplEx cDNA Libraries:

- 0.2 ml **Library lysate** (in 1X lambda dilution buffer + 7% DMSO)
- 0.5 ml ***E. coli* XL1-Blue** (in 25% glycerol; genotype in Section II.C)
- 0.5 ml ***E. coli* BM25.8** (in 25% glycerol; genotype in Section II.C)
- 50 µl **Purified human β_2-microglobulin clone in λTriplEx** (>10^8 pfu/ml in 1X lambda dilution buffer and 7% DMSO) Store at 4°C for up to 2 months; store at –70°C for several years. Avoid repeated freeze/thaw cycles.
- 20 µl **Polyclonal rabbit anti-human β_2-microglobulin serum** (250 µg/ml) Store at 4°C for up to 1 year.
- 50 µl **5' Sequencing primer** (20 µM) Store at –20°C.
- 50 µl **3' Sequencing primer** (20 µM) Store at –20°C.

C. Host Strain Information

TABLE I: BACTERIAL HOST STRAIN GENOTYPES

Strain	Genotype
XL1-Blue	*endA*1, *gyrA*96, *hsdR*17, *lac*$^-$, *recA*1, *relA*1, *supE*44, *thi*-1, [F' *lacI*^{q}*Z ΔM15, proAB*, Tn*10*]
	Note: Tn*10* confers resistance to tetracycline.
	Reference: Wood *et al.*, 1985
BM25.8	*supE*44, *thi Δ(lac–proAB) [F' traD*36, *proAB*$^+$, *lacI*^{q}*Z ΔM15] λimm434 (kan*^{R}*)P1 (cam*^{R}*) hsdR (*r_{k12}*–* m_{k12}*–)*
	Note: BM25.8 is lysogenic for phages λ and P1 and is used for plasmid excision.
	Reference: Palazzolo *et al.*, 1990

II. List of Components *continued*

TABLE II: HOST STRAIN APPLICATIONS & MEDIA ADDITIVES

Host strain	Stock plate	Application(s)
XL1-Blue	LB/tet (15 μg/ml)	• Library plating & screening • Blue/white (β-galactosidase) screening • Regulated expression of cloned genes
BM25.8	LB/kan (50 μg/ml)/ cam (34 μg/ml)	• *cre-lox*-mediated excision of pTriplEx or pTriplEx2 from λTriplEx or λTriplEx2

Note: For λ phage transductions including plaque titering, and library plating and screening, use the following media additives for optimal adsorption of phage to bacteria:

- 10 mM $MgSO_4$ in LB agar and LB top agar
- 10 mM $MgSO_4$ and 0.2% maltose in LB broth when growing overnight bacterial cultures for λ phage transductions.
- Before using **XL1-Blue** overnight cultures in phage transductions, centrifuge the cells, pour off the supernatant, and resuspend the pellet in 10 mM $MgSO_4$ in H_2O. For details, see pages 21 & 22.
- Before using **BM25.8** overnight cultures in phage transductions, add $MgCl_2$ to a final concentration of 10 mM. For details, see pages 21 & 42.
- If the bacterial strains are always maintained on stock plates containing the appropriate antibiotic, there is no need to add antibiotics to the LB broth when growing overnight cultures.

III. Additional Materials Required

- Store at room temperature (20–22°C) unless specified otherwise.

A. Long-term library storage

- **100% Dimethylsulfoxide** (DMSO)

B. Routine culture and plating of *E. coli*

- **Kanamycin stock solution** (25 mg/ml in H_2O; 500X) Store at –20°C.
- **Tetracycline stock solution** (15 mg/ml in H_2O; 1000X) Store at –20°C.
- **Chloramphenicol stock solution** (34 mg/ml in 100% ethanol; 1000X) Store at –20°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.
- **LB agar plates**
  Prepare LB broth as described above. Add agar (15 g/L) and autoclave. Pour plates and store at 4°C.
- **LB/tet agar plates**

  Prepare LB broth as described above. Add agar (15 g/L) and autoclave. Cool to 50°C before adding tetracycline (final concentration 15 µg/ml). Pour plates and store at 4°C.
- **LB/kan/cam agar plates**

  Prepare LB broth as described above. Add agar (15 g/L) and autoclave. Cool to 50°C and add kanamycin (final concentration 50 µg/ml) and chloramphenicol (final concentration 34 µg/ml). Pour plates and store at 4°C.

C. Ligation of vector to insert

- **10X Ligation buffer** (Store at –20°C.)

  500 mM Tris-HCl (pH 7.8)
  100 mM $MgCl_2$
  100 mM DTT (dithiothreitol)
  0.5 mg/ml BSA
- **ATP** (10 mM) Store at –20°C.
- **T4 DNA ligase** Store at –20°C.

D. λ phage packaging extract

We recommend that you choose a commercially available packaging system that will give you at least 1 x 10^9 pfu/µg of DNA, such as Gigapack® III Gold-4; Stratagene, Cat. #200201.

III. Additional Materials Required *continued*

E. Transduction and titering of λ phage in *E. coli*

- **$MgSO_4$** (1 M stock solution)

  Dissolve 24.65 g of $MgSO_4 \cdot 7H_2O$ in 100 ml deionized H_2O. Autoclave.

- **20% Maltose stock solution**

  Dissolve 20 g maltose in 80 ml of deionized H_2O; bring volume to 100 ml. Filter sterilize and store at 4°C.

- **LB/$MgSO_4$ agar plates**

  To 1 L of LB broth (Section III.B), add 10 ml 1 M $MgSO_4$ (10 mM final concentration) and 15 g agar. Autoclave. Pour plates and allow agar to solidify. Invert and store at 4°C.

- **LB/$MgSO_4$ broth**

  To 1 L of LB broth (Section III.B), add 10 ml 1 M $MgSO_4$ (10 mM final concentration). Autoclave.

- **LB/$MgSO_4$/maltose broth**

  Prepare 1 L of LB/$MgSO_4$ broth as described above. After autoclaving, cool to 50°C and add maltose to a final concentration of 0.2% (10 ml of 20% maltose stock solution).

- **LB/$MgSO_4$ soft top agar**

  To 1 L of LB broth (Section III.B), add 10 ml 1 M $MgSO_4$ (10 mM final concentration) 7.2 g agar. Autoclave and store at 4°C.

- **10X Lambda dilution buffer**

	Final Conc.	To prepare 1 L of solution
NaCl	1.0 M	58.3 g
$MgSO_4 \cdot 7H_2O$	0.1 M	24.65 g
Tris-HCl (pH 7.5)	0.35 M	350.0 ml of 1 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 (0.01% final concentration)

  Add H_2O to a final volume of 1 L. Autoclave and store at 4°C.

  Note: The 0.01% gelatin in the 1X lambda dilution buffer stabilizes the library titer for long-term storage. Adding gelatin is optional when diluting the phage for immediate titering.

F. Blue/white (β-galactosidase) screening in *E. coli* (strain XL1-Blue only)

- **IPTG** (0.1 M in H_2O)
  Isopropyl β-D-thiogalactopyranoside. Filter-sterilize. Store at 4°C.

- **X-Gal** (0.1 M in dimethylformamide [DMF])
  5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside. Store at –20°C.

III. Additional Materials Required *continued*

G. Hybridization screening

- **DNA denaturing solution**
  1.5 M NaCl
  0.5 N NaOH
- **Filters** (positively charge-modified, supported nylon filters, such as Schleicher & Schuell's Nytran Plus™)
- **Hybridization probe** (oligonucleotide or DNA; radioactively labeled)
- **Neutralizing solution**
  1.5 M NaCl
  0.5 M Tris-HCl (pH 8.0)
- **Salmon sperm DNA** (for blocking non-specific hybridization)
  Shear by passing through an 18-gauge needle three times. Boil the sheared DNA for 10 min. Quickly chill in an ice bath. Store at –20°C.
- **ExpressHyb™ Hybridization Solution** (#8015-1, -2)
- **Radioactive ink**
  Mix a small amount of ^{32}P with waterproof black ink.
- **20% SDS**
- **20X SSC**

	20X Conc.	To prepare 1 L
NaCl	3.0 M	175.3 g
Sodium citrate•$2H_2O$	0.3 M	88.2 g

  Adjust to pH 7.0 with 10 N NaOH. Add H_2O to a final volume of 1 L.
- **20X SSPE**

	20X Conc.	To prepare 1 L
NaCl	3.0 M	175.3 g
NaH_2PO_4•H_2O	0.2 M	27.6 g
EDTA	0.02 M	40 ml of 0.5 M

  Adjust to pH 7.4 with 10 N NaOH. Add H_2O to a final volume of 1 L.
- **Wash buffer 1**
  2X SSC
  0.5% SDS
- **Wash buffer 2**
  1X SSC
  0.1% SDS
- **Wash buffer 3**
  0.2X SSC
- **Whatman 3MM filter paper**
- **Kodak XAR Film**

III. Additional Materials Required *continued*

H. Immunoscreening

- **Primary antibody** (preferably polyclonal, with strong, specific affinity for the target protein)
- **Secondary antibody** (enzyme-linked or biotin-labeled)
- **Substrates and buffers** (appropriate for the detection system you plan to use)

  This manual provides a protocol for detecting alkaline phosphatase (AP)-conjugated secondary antibodies using the substrates NBT and BCIP. Use the following components with AP-linked secondary antibodies:

  1. **AP Buffer**

	10X	1X
Tris-HCl (pH 9.5)	1.0 M	100 mM
NaCl	1.0 M	100 mM
$MgCl_2$	50 mM	5 mM

  2. **NBT** (Nitro blue tetrazolium chloride; 75 mg/ml)

     Caution: NBT is an irritant. Avoid skin contact; do not inhale dust.

  3. **BCIP** (5-Bromo-4-chloro-3-indolyl phosphate; 50 mg/ml)

     Caution: Possible carcinogen. Wear protective clothing; do not inhale dust.

  4. **Stop solution**

20 mM	Tris-HCl (pH 8.0)
5 mM	EDTA

- **10X Lambda dilution buffer** (Section III.E)
- **LB/$MgSO_4$ agar plates** (Section III.E)
- **LB/$MgSO_4$ soft top agarose**

  To 1 L of LB broth (Section III.B), add

  10 ml 1 M $MgSO_4$ (10 mM final concentration)

  7.2 g agarose

  Autoclave and store at 4°C.
- **Nitrocellulose filters** (Schleicher & Schuell's Protran® filters or equivalent)
- **13 x 100-mm sterile plastic test tubes**
- **12 x 75-mm (5 ml) sterile plastic or polypropylene test tubes**
- **Waterproof ink**
- **IPTG** (10 mM in H_2O)

  Isopropyl β-D-thiogalactopyranoside. Filter-sterilize. Store at 4°C.
- **TBS buffer**

	10X	1X
Tris-HCl (pH 8.0)	0.1 M	10 mM
NaCl	1.5 M	150 mM

III. Additional Materials Required *continued*

- **TBST** (1X TBS + 0.05% Tween-20)
- **Blocking solution** (prepare fresh on the day of use)
  1% gelatin in TBST, or 1% BSA (bovine serum albumin) in TBS

I. Converting λTriplEx/λTriplEx2 to pTriplEx/pTriplEx2

- **LB broth** (Section III.B)
- **LB/kan/cam agar plates** (Section III.B)
- **Carbenicillin stock solution** (50 mg/ml in H_2O)
  Store at –20°C.
- **LB/carb agar plates**
  To 1 L of LB broth (Section III.B), add
  15 g agar
  Autoclave. Cool to 50°C before adding carbenicillin (50 µg/ml).
  Pour plates and store at 4°C.
- **$MgCl_2$** (1 M stock solution)
  Dissolve 20.3 g of $MgCl_2 \cdot 6H_2O$ in 100 ml of deionized H_2O. Autoclave.
- **25% Glycerol**

IV. Premade and Custom λTriplEx Libraries

A. λTriplEx cDNA Library Construction

Like our other 5'-STRETCH PLUS™ cDNA Libraries, λTriplEx and λTriplEx2 cDNA Libraries are prepared using a modified Gubler & Hoffman procedure (1983). First-strand cDNA synthesis is primed by an *Xba* I-site-containing oligo-dT primer-adaptor (Figure 3A) for λTriplEx libraries. An *Sfi* IB-site-containing oligo-dT primer-adaptor (Figure 3B) or *Sfi* IB-site-containing random primer-adaptor (Figure 3C) is used for λTriplEx2 libraries. Adaptor and linker sequences are shown in Figure 4. The first- and second-strand cDNA synthesis procedures and post-synthesis cDNA handling are optimized to produce libraries with larger inserts, and therefore, a greater probability of containing full-length transcripts.

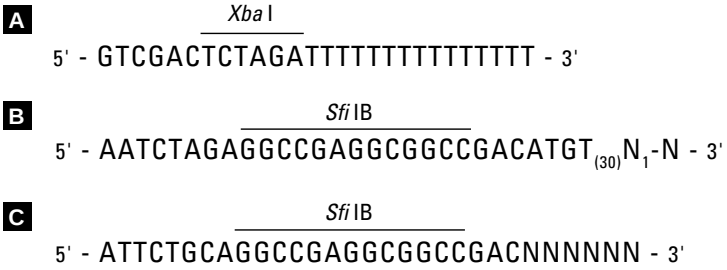

Figure 3. Primer-adaptor sequences: Panel A. *Xba* I-$(dT)_{15}$ primer-adaptor for λTriplEx libraries. **Panel B.** *Sfi* IB-$(dT)_{30}$ primer-adaptor for λTriplEx2 libraries. **Panel C.** *Sfi* IB random primer-adaptor for λTriplEx2 libraries.

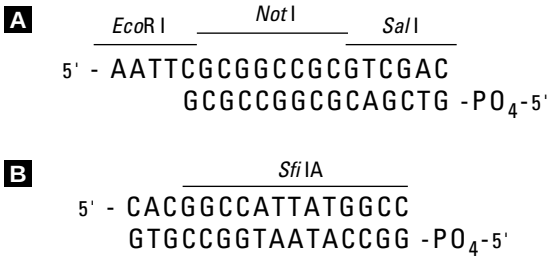

Figure 4. Adaptor and linker sequences. Panel A. Adaptor used for λTriplEx libraries. **Panel B.** Linker used for λTriplEx2 libraries.

IV. Premade and Custom λTriplEx Libraries *continued*

B. Large-Insert Library Construction

Figure 5 illustrates the key features in the construction of our Large-Insert cDNA Libraries. First, we use the SMART III™ Oligonucleotide and long-distance PCR (LD-PCR; 1, 2) to generate full-length, double-stranded cDNA. SMART, which stands for Switching Mechanism at the 5' end of the RNA Template, ensures high representation of full-length cDNAs (3). After synthesizing and digesting the cDNAs with Sfi I, we enrich for large, full-length clones by size fractionation on a low-melting-point agarose gel. Finally, we excise cDNAs greater than 3.0 kb and directionally clone them into our λTriplEx2 phagemid, which is specifically designed for efficient cloning of large inserts.

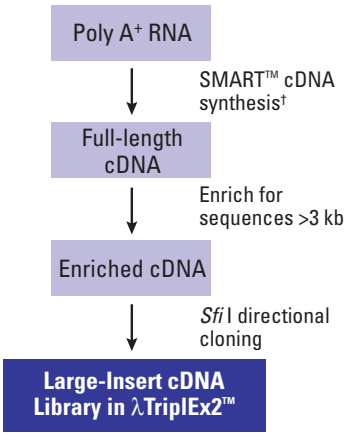

Figure 5. Large-Insert cDNA Library construction process.

C. Library amplification

All premade λTriplEx libraries, Large-Insert libraries, and amplified custom libraries have been amplified only once. Carefully amplified libraries are faithful copies of unamplified libraries. All phage libraries are amplified on solid medium to avoid the unequal growth rates of clones that may occur in liquid medium.

Custom libraries can be ordered amplified or unamplified. **Unamplified custom libraries** can be stored at 4°C for **up to two weeks only**. If you plan to keep an unamplified library longer than two weeks, see Section II for important storage information.

IV. Premade and Custom λTriplEx Libraries *continued*

D. Packaging extract

Mcr⁻ packaging extracts are used during library construction.

E. Titer

1. General Information

Library titer information is provided on the Product Analysis Certificate (PAC). The lysate titer is determined at the time the library was constructed. Retiter the library upon receipt to verify the titer before proceeding with screening. **For a titering protocol, see Section V.F.** If the lysate contains 10^8 pfu/ml or greater, the library is representative, and you may proceed with screening procedures. If library titer is below 10^8 pfu/ml, please 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 are 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.

2. Procedures

The protocols in this manual can be used 1) if you are constructing a library in λTriplEx; 2) if you have purchased a pre-made library in λTriplEx or λTriplEx2, or 3) if you have purchased a custom library in λTriplEx or λTriplEx2.

If you have purchased a library which has been amplified once at CLONTECH, you should determine the titer of the amplified library as stated in section VII.F of this manual. To determine the titer, you must first plate out the bacterial cultures as described in section VII.B.

F. 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 have more than one million independent clones and are representative of the complexity of the cDNA population or genomes involved.

G. Recombination Frequency

The recombination frequencies for λTriplEx libraries are determined after library amplification. Although the recombination frequency that we obtained is included on the PAC, you may wish to replate the library upon receipt to confirm this value. Use the protocol for blue/white screening provided in Section V.D.

V. Library Construction Protocols

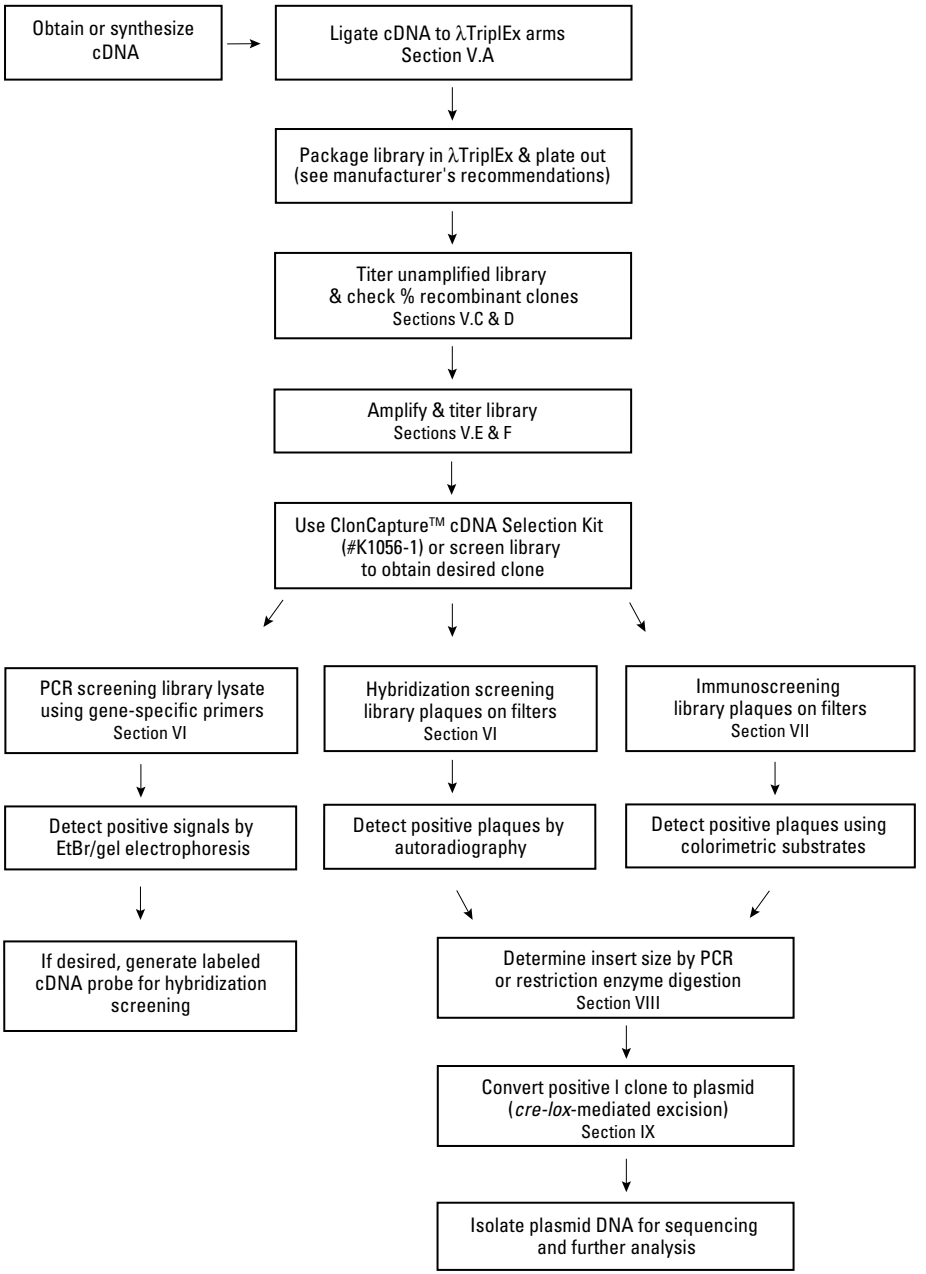

Figure 6. Guide to the λTriplEx library construction and screening protocols.

V. Library Construction Protocols *continued*

A. Ligating cDNA to λTriplEx Arms

In the ligation reaction, the cDNA:vector ratio is critical in determining transformation efficiency and, ultimately, the number of independent clones in the library. We recommend setting up three parallel ligations using three molar ratios of vector to cDNA (Table III). These ratios have been optimized for ligation of adaptor-ligated cDNA to restriction enzyme-digested, dephosphorylated λTriplEx DNA. The ligations in Table III will give you three different molar ratios of vector (~42 kb) to cDNA (~1.5 kb), within the range of 3.5:1 to 1.2:1. If you modify the molar ratio further, keep a constant concentration of vector DNA and vary the cDNA concentration.

Important : For λ packaging reactions, use a λ phage packaging system that gives at least 1×10^9 pfu/µg of DNA. Follow the supplier's protocol, and perform a parallel reaction with the control DNA provided with the kit.

1. Set up a test ligation to determine the efficiency of vector ligation to a control insert. Use 1 µl of vector, 1 µl of control insert, 1.5 µl of deionized H_2O, and the other reagents listed in Table III. Incubate at 16°C overnight. Perform a λ phage packaging reaction and titer the resulting phage (Section V.C). You should obtain ≥1×10^7 pfu/µg of input vector.

   Note: A control insert is provided with restriction enzyme-digested λTriplEx DNA.

2. Label three 0.5-ml tubes and add the reagents in Table III. Mix gently; avoid producing air bubbles. Spin tubes briefly to bring contents to the bottom of the tube. Incubate at 16°C overnight.

TABLE III: LIGATIONS USING THREE DIFFERENT RATIOS OF cDNA TO VECTOR

Component	1st ligation (µl)	2nd ligation (µl)	3rd ligation (µl)
cDNA (25–75 ng/µl)	0.5	1.0	1.5
Vector (500 ng/µl)	1.0	1.0	1.0
10X ligation buffer	0.5	0.5	0.5
ATP (10 mM)	0.5	0.5	0.5
T4 DNA ligase	0.5	0.5	0.5
Deionized H_2O	2.0	1.5	1.0
Total Volume (µl)	5.0	5.0	5.0

3. Perform a separate λ phage packaging reaction for each ligation.
4. Titer each of the resulting libraries (Section VI.C). From the three ligations combined, you should obtain 1–2×10^6 independent clones. The unamplified libraries can be stored at 4°C for 2 weeks.

V. Library Construction Protocols *continued*

5. [Optional] If you obtained <1–2 x 10^6 clones, you may wish to do another ligation with the remaining cDNA. Use the cDNA: vector ratio that gave the best results in the initial ligations. Scale-up the volumes of all reagents according to the amount of cDNA used, and package and titer this scaled-up ligation. If the titer is still low, see Section VII.G.
6. To increase your library's stability, combine the packaging reactions from Step A.4; you should have >1 x 10^6 independent clones. Amplify the library as described in Section V.E. You can store this library at 4°C for 2 months or at –70°C in 7% DMSO for at least one year.

B. Bacterial Culture Plating

E. coli strains XL1-Blue and BM25.8 are provided as stocks in LB medium with 25% glycerol. You can store them for at least 1 year at –70°C. See Section II.C for strain genotypes and recommended applications.

1. Make a *primary streak plate*: streak a small portion (~5 µl) of frozen stock onto an LB agar plate with antibiotic, and without $MgSO_4$.
   - Use **LB/tet for XL1-Blue** stock plates.
   - Use **LB/kan/cam for BM25.8** stock plates.
2. Incubate **XL1-Blue** plate at **37°C** overnight. Incubate **BM25.8** plate at **31°C** overnight. Wrap plates in Parafilm® and store at 4°C for up to 2 weeks.
3. To prepare a *working stock plate*, pick an isolated colony from the primary streak plate and streak it onto an LB/$MgSO_4$ agar plate with antibiotics.
4. Incubate **XL1-Blue** plate at **37°C** overnight. Incubate **BM25.8** plate at **31°C** overnight. Wrap plates in Parafilm and store at 4°C for up to 2 weeks. These plates are the source of fresh colonies for inoculating liquid cultures and for preparing the next working stock plate.
5. Prepare a new working stock plate from the previous working stock every two weeks maintain a source of fresh colonies.
6. If you suspect contamination on your current working stock plate, prepare a new primary streak plate from the frozen culture.

C. Titering the Unamplified Library

Titering the unamplified library will give an estimate of the number of independent clones in it. A library with at least 1 x 10^6 independent clones is generally representative of mRNA complexity. The following protocol can be used to determine (1) the efficiency of the ligation of vector to positive control insert and (2) the background titer of the vector alone.

V. Library Construction Protocols *continued*

Notes:

- When plating bacteria + phage mixtures using melted top agar, maintain the agar at 45°C; higher temperatures will kill the bacteria.
- Before plating with top agar, prewarm the agar plates to 37°C; make sure the agar surface is free of excess moisture droplets.
- When preparing media for phage transductions or plaque titering, use recipes containing 10 mM $MgSO_4$ for optimal adsorption of phage to bacteria. For the same reason, add 0.2% maltose to the LB broth when growing overnight bacterial cultures for transduction/titering.

1. Pick an isolated colony from the XL1-Blue working stock plate (Step B.4). Inoculate 15 ml of LB/$MgSO_4$/maltose broth in a 50-ml test tube. Incubate at 37°C overnight with shaking at 140 rpm until the OD_{600} reaches 2.0. Centrifuge the cells for 5 min at 5,000 rpm, pour off the supernatant, and resuspend the pellet in 7.5 ml of 10 mM $MgSO_4$.
2. Plan the number of 90-mm LB/$MgSO_4$ plates you will need. Warm and dry them as explained in the note above.
3. Make appropriate dilutions of each of the packaging extracts (Step A.4) in 1X lambda dilution buffer. As a general guideline, an appropriate dilution for an **unamplified** λ lysate is **1:5 to 1:20**. Perform the following steps for each dilution of each extract you wish to plate.
4. Add 1 µl of diluted phage to 200 µl of the XL1-Blue overnight culture. Allow the phage to adsorb at 37°C for 10–15 min.
5. Add 2 ml of melted LB/$MgSO_4$ top agar. Mix by quickly inverting and immediately pour onto 90-mm LB/$MgSO_4$ plates prewarmed to 37°C. Swirl the plates quickly to allow even distribution of the top agar.
6. Cool the plates at room temperature for 10 min to allow the top agar to harden. Invert them and incubate at 37°C for 6–18 hr. Check the plates periodically for plaques development.
7. Count the plaques and calculate the phage titer (pfu/ml):

$$\text{pfu/ml} = \frac{\text{number of plaques} \times \text{dilution factor} \times 10^3\ \mu\text{l/ml}}{\mu\text{l of diluted phage plated}}$$

8. [For test ligations from Step A.4] Compare the titers to determine the optimal ratio of vector arms:cDNA insert. If you have <1–2 x 10^6 plaques (clones) altogether, you may wish to repeat the ligation, using the optimal ratio of vector to insert. See Step A.5 and Section V.G.1.

D. Determining the Percentage of Recombinant Clones

For blue/white screening in *E. coli* XL1-Blue, follow the procedure for titering an unamplified library on LB/$MgSO_4$ plates (Section V.C), **except** add IPTG and X-gal to the melted top agar. For every 2 ml of melted top agar, use 50 µl each of the 0.1 M IPTG and X-gal stock solutions (Section III.F). Aim for 500–1,000 plaques per 90-mm plate. Incubate at 37°C for 6–18 hr, until plaques and blue color develop.

V. Library Construction Protocols *continued*

The ratio of white (recombinant) to blue (nonrecombinant) plaques will give you a quick estimate of recombination efficiency. A successful ligation using the control insert should give at least 75% recombinants. If your recombination efficiency for the control is lower, see Section V.G.2.

E. Library Amplification

The number of plates you will need depends on the number of independent clones in the library. With λTriplEx, aim for 1 x 10^5 clones (or plaques) per 150-mm plate. **λTriplEx plaques are small (~0.5 mm diameter).**

1. Pick an isolated colony from the primary working plate of XL1-Blue (Step B.4), and inoculate 15 ml of LB/$MgSO_4$/maltose broth. Incubate at 37°C overnight with shaking at 140 rpm until the OD_{600} reaches 2.0. Centrifuge the cells for 5 min at 5,000 rpm, pour off the supernatant, and resuspend the pellet in 7.5 ml of 10 mM $MgSO_4$.
2. Warm and dry the requisite number of LB/$MgSO_4$ agar plates.
3. Set up the required number of sterile 5-ml (12 x 75 mm) tubes with 500 µl of overnight XL1-Blue culture (Step E.1) and sufficient diluted lysate to yield 1 x 10^5 plaques per 150-mm plate.
4. Incubate in a 37°C water bath for 15 min.
5. Fill each tube to the top with melted LB/$MgSO_4$ soft top agar and cap.
6. Quickly mix and pour the bacteria + phage onto LB/$MgSO_4$ agar plates. Swirl plates quickly while pouring to promote even agar distribution.
7. Cool plates at room temperature for 10 min to allow the top agar to harden. Invert them and incubate at 37°C for 6–18 hr, until the plaques become nearly confluent.
8. Add 12 ml 1X lambda dilution buffer to each plate; put at 4°C overnight.
9. Shake plates at ~50 rpm for 1 hr at room temperature.
10. Pool library lysate by pouring the λ phage lysates into a sterile beaker.
11. To clear the lysate of cell debris and to lyse any intact cells:
    a. Mix the phage lysate well and pour it into a sterile, 50-ml polypropylene, screw-cap tube.
    b. Add 10 ml of chloroform. Replace the cap and vortex for 2 min.
    c. Centrifuge in a Beckman J2-21 centrifuge at 7,000 rpm (5,000 x g) for 10 min. Collect the supernatant in a new sterile 50-ml tube, fasten cap tightly, and place at 4°C.
12. Determine the titer of the amplified library (Section F).
13. You may store the amplified library at 4°C for 6 months. For storage up to one year, make 1-ml aliquots, add DMSO to a final concentration of 7%, and store at –70°C. Avoid repeated freeze/thaw cycles.

V. Library Construction Protocols *continued*

F. Titering the Amplified Library

1. Pick an isolated colony from the XL1-Blue working stock plate (Step B.4), and inoculate 15 ml of LB/$MgSO_4$/maltose broth without antibiotics. Incubate at 37°C overnight with shaking at 140 rpm until the OD_{600} reaches 2.0. Centrifuge the cells for 5 min at 5,000 rpm, pour off the supernatant, and resuspend the pellet in 7.5 ml of 10 mM $MgSO_4$.
2. Warm 4 90-mm-size LB/$MgSO_4$ agar plates to 37°C and dry them.
3. Prepare dilutions of phage lysate (library):
   a. Pipet 10 µl of the library lysate into 1 ml of 1X lambda dilution buffer (Dilution 1 = 1:100).
   b. Transfer 10 µl of Dilution 1 into a second tube containing 1 ml of 1X lambda dilution buffer (Dilution 2 = 1:10,000).
4. Prepare 4 tubes as described in Table IV, using the XL1-Blue overnight culture (Step F.1) and phage Dilution 2 from Step F.3.

TABLE IV: PLATING DILUTIONS FOR TITERING AN AMPLIFIED LIBRARY

Tube	1X Lambda Dilution Buffer	Bacterial Overnight Culture	Phage Dilution 2
1	100 µl	200 µl	5 µl
2	100 µl	200 µl	10 µl
3	100 µl	200 µl	20 µl
4 (Control)	100 µl	200 µl	0 µl

5. Incubate tubes in a 37°C water bath for 15 min.
6. Add 3 ml of melted (45°C) LB/$MgSO_4$ top agar to each of the 4 tubes.
7. Quickly mix and pour each tube's contents onto separate LB/$MgSO_4$ agar plates. Swirl plates to promote even agar distribution.
8. Harden soft agar by cooling plates at room temperature for 10 min.
9. Incubate plates (inverted position) at 37°C for at least 6–7 hr.
10. Count the plaques and calculate the titer (pfu/ml) as follows:

$$\text{pfu/ml} = \frac{\text{number of plaques} \times \text{dilution factor*} \times 10^3\ \mu\text{l/ml}}{\mu\text{l of diluted phage plated}}$$

* In this case, the dilution factor = 1×10^4

11. A successfully amplified library will have a very high titer (~10^9 pfu/ml).

V. Library Construction Protocols *continued*

G. Troubleshooting Library Construction

1. Low titer of unamplified library

Ensure that you are using a λ phage packaging system designed to yield at least 1 x 10^9 pfu/µg of control λ DNA.

If the titer of the unamplified library's combined ligations is <10^6 pfu/µg DNA, but the control packaging reaction yielded at least 5 x 10^8 pfu/µg, you may have a problem with vector ligation (Section V.A.). You cannot check the ligation reaction retroactively. However, before you repeat the ligations, check the concentration of your cDNA preparation. Electrophorese 1 µl of resuspended cDNA on an EtBr-agarose gel next to a defined amount of control DNA. Alternatively, spot 1 µl of the cDNA on an EtBr-containing agarose plate next to small spots of defined amounts (10–1,000 ng) of control DNA. The resuspended cDNA's concentration should be 100–200 ng per µl.

If the concentration is within this range, you may have a problem with adaptor or linker ligation, or with phosphorylation of the adaptor-ligated cDNA. These steps cannot be checked retroactively. However, you can check indirectly by ligating the adaptors to a purified, blunt-ended DNA fragment of known size, ligating it to the vector provided, and checking for titer and percentage of recombinants. If these results are as expected, repeat the ligation with your adaptor-ligated cDNA, adjusting the cDNA to vector ratio to the ratio in Table II that gave the best initial results. For example, if you obtained the greatest number of plaques using a ratio of 1.5:1.0, then use ratios of 1.5:1.0, 1.75:1.0, and 2.0:1.0.

2. Low (<75%) recombination efficiency

Our pre-digested λTriplEx Arms (#6161-1 and 6162-1) have been dephosphorylated. Consequently, background due to religation of nonrecombinant vector molecules should be minimal. If you have digested the uncut vector yourself, dephosphorylate it before you clone in the cDNA. Check background due to religated vector by performing a control ligation with the vector alone (Section V.A). A high titer with a low recombination efficiency may mean that your cDNA population contains small DNA fragments, such as adaptor-dimers, which are preferentially ligated into the vector. Adaptor-dimer inserts are generally too small to disrupt lacZ α-complementation, and plaques are blue. Repeat your cDNA synthesis and size-fractionate the product to remove fragments <400 bp. Check the size distribution of the cDNA before cloning it into the vector.

Low recombination efficiency may also result from a problem with ligation of adaptors to the cDNA and phosphorylation of adaptor-ligated cDNA. To check these steps indirectly, see Section V.G.1.

V. Library Construction Protocols *continued*

3. Small insert sizes

If > 50% of your clones appear to have insert sizes <0.5 kb, your cDNA preparation probably contained small cDNA fragments, unligated adaptors, adaptor-dimers, unincorporated primers, and primer-dimers. These small fragments are preferentially ligated to the vector, and should be completely removed before vector ligation. If you repeat the cDNA synthesis procedure, take extra steps to size-fractionate the product and check the size distribution before you ligate the cDNA to the vector.

VI. PCR and Hybridization Screening

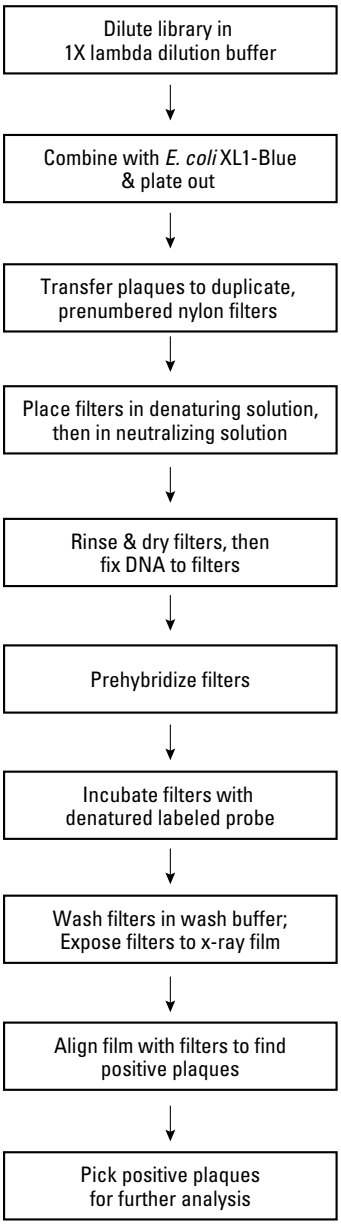

Figure 7. Hybridization screening a λTriplEx cDNA library using a DNA or oligonucleotide probe.

VI. PCR and Hybridization Screening *continued*

A. Screening a Library by PCR

You can use your gene-specific primers and PCR to screen λTriplEx libraries. Use 1 µl of amplified library lysate in a 50-µl reaction volume as a PCR template. Use an 8–10 µl sample of the PCR product on a 1.2% agarose/EtBr gel to determine if the fragment has been amplified. 22–30 cycles are usually required to obtain a visible product. Optimize PCR conditions for each set of primers. You may use PCR products of the expected size to generate DNA probes for hybridization screening.

B. Hybridization Screening—General Considerations

λTriplEx libraries can be screened by hybridization with DNA probes (Section VI.D) or oligonucleotide probes (Section VI.E). In either case, we recommend that you prepare at least two replicate filters of each plate to be screened (Section VI.C). Additionally, determine conditions giving lowest background by performing a hybridization experiment with a blank filter or, preferably, a control filter containing nonrecombinant vector plaques. The protocol below is for use with a radioactive probe.

The handbooks edited by Sambrook *et al.* (1989) and Ausubel *et al.* (1995) contain further information on hybridization using nucleic acid probes. Jacobs *et al.* (1988), Lathe (1985), Suggs *et al.* (1981), Wallace *et al.* (1981), and Wood *et al.* (1985) contain information on using oligonucleotide probes, and Woo (1979) has information about DNA probes.

Important notes:

- Use soft top **agarose** in the following procedures. Commercially supplied **agar** contains components that may interfere with hybridizations.
- Promote even spreading of melted agarose by swirling plates quickly after pouring.
- Use positively charge-modified, supported nylon filters (Schleicher & Schuell's Nytran Plus works well). Nitrocellulose filters are too brittle for hybridization screening.
- Your hybridization probe should contain at least 10^7 cpm. Its specific activity should be >8 x 10^8 cpm/µg, and the final concentration of hybridization solution should be 1–2 x 10^6 cpm/ml.
- We recommend ExpressHyb™ Hybridization Solution (#8015-1) in place of standard prehybridization and hybridization solutions. If you are using a rapid hybridization solution, follow the protocol accompanying it.

C. Transferring Plaques to Nylon Filters

1. Pick an isolated colony from the working stock plate of XL1-Blue host cells, and inoculate 15 ml of LB/$MgSO_4$/maltose broth in a 20-ml test tube. Incubate at 37°C overnight with shaking at 140 rpm, until its OD_{600} reaches 2.0. Centrifuge the cells for 5 min at 5,000 rpm, pour off the supernatant, and resuspend the pellet in 7.5 ml of 10 mM $MgSO_4$.

VI. PCR and Hybridization Screening *continued*

2. Dilute the titered amplified library in 1X lambda dilution buffer (Table V). Combine dilutions with the indicated volume of overnight cell culture.

TABLE V: LIBRARY PLATING VOLUMES FOR HYBRIDIZATION SCREENING

Component	for each 90-mm plate	for each 150-mm plate
Cell culture	200 µl	500 µl
Library phage	1–2 x 10^4 pfu	2–5 x 10^4 pfu
Melted top agarose	3 ml	5 ml

3. Incubate bacteria + phage mixture at 37°C for 15 min.
4. Add the indicated volume of melted LB top agarose/$MgSO_4$. Quickly cap the tube and invert once. Immediately pour mixture onto a prewarmed, dry LB/$MgSO_4$ plate.
5. Cool the plates at room temperature for 10 min to allow the top agar to harden. Invert plates and incubate at 37°C overnight. Alternatively, you may incubate the plates at 40–42°C until plaques become distinctly visible. For λTriplEx, this process takes 5–7 hr. Avoid confluent lysis.
6. Chill plates at 4°C for at least 1 hr to allow the LB top agarose to harden. You may store these plates at 4°C overnight.
7. Number a nylon filter with a soft pencil or ball point pen. Using forceps, place the filter onto the LB soft top agarose. Avoid trapping air bubbles. Mark the filter in three asymmetric locations. To do so, stab through the filter and into the agar with an 18-gauge needle attached to a syringe containing ink.
8. After **2 min**, peel off the filter carefully. Float it, plaque side up, for 30 sec in a petri dish containing DNA denaturing solution. Then immerse the filter for 5 min.
9. Remove the filter and immerse it in neutralizing solution for 5 min.
10. Briefly rinse the filter in 2X SSC, and blot-dry on Whatman 3MM paper.
11. Place a second filter onto the same plate, and mark it with ink at the same locations. Peel the filter off after **3 min**. Denature the DNA; repeat Steps 8–10. You may prepare four filters from a single plate. Successive filters should be left on the plate 1 min longer than previous filters.
12. Use a UV crosslinker to fix the DNA to the filter. Follow the manufacturer's recommended conditions, or simply bake filters at 80°C for 2 hr.

D. Hybridization Using DNA Probes >200 bp

1. Place ExpressHyb in a heat-sealable plastic bag. Use 10 ml for one filter or 15 ml for two. Use 2–3 extra volumes for each additional filter you will be treating in the same plastic bag.

VI. PCR and Hybridization Screening *continued*

2. Place filters in the plastic bag and seal it. Avoid trapping air bubbles in it. If you are placing multiple filters in a single bag, use enough solution to wet all filters. Do not allow filters to adhere to each other, and ensure that each filter is in direct contact with the solution.
3. Incubate filters in ExpressHyb at 42°C for 4 hr with agitation.
4. Denature the labeled DNA probe by heating at 100°C for 10 min. Immediately chill the probe on ice.
5. Cut one corner of the plastic bag and pour off the ExpressHyb. Make hybridization solution by mixing fresh ExpressHyb with the probe.
6. Incubate the filters in hybridization solution at 42°C for 16–20 hr.
7. Carefully remove the filters from the plastic bag, and place the hybridization solution in your radioactive liquid waste.
8. Wash the filters at room temperature in **wash buffer 1** for 15–20 min. Do not allow them to dry. Use a minimum of 15 ml of wash buffer for one filter, or 25 ml for two filters; use 5 ml extra for each additional filter.
9. Wash the filters in **wash buffer 2** at 65°C for 1 hr. Repeat once.

   Note: If background is too high, you may wish to add a 1-hr wash in wash buffer 3. Monitor background with a Geiger counter; if you detect more than 1000 cpm, wash further.
10. Blot-dry the filters on Whatman 3MM paper at room temperature.

    Note: At this point, you may seal the filters individually in plastic bags to keep them moist. If they remain moist, you may wash them again to reduce background noise. Also, keep them moist if you plan to strip them for rehybridizing to a different probe.
11. Proceed to Section F for signal detection by autoradiography.

E. Hybridization Using Oligonucleotide Probes <200 bp

1. Place the required volume of ExpressHyb in a heat-sealable plastic bag. Use 10 ml for one filter, 15 ml for two, and 2–3 ml for each additional filter.
2. Place filters in the plastic bag and seal it. See step D.2 for tips.
3. Determine the probe's estimated dissociation temperature (T_d). Incubate the filters at 20°C below this temperature for 4–6 hr on a platform shaker at ~60 rpm. For probes of length 14–27 nt, the T_d is:

   T_d (°C) = 4(G+C) + 2(A+T)

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

   For longer probes:

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

   n = the number of nucleotides

   You can decrease the T_d by approximately 13°C if you decrease the incubation solution's Na^+ concentration from 1 M (6X SSPE) to 0.2 M (1X SSPE). Use 1X SSPE in the prehybridization and hybridization solutions for oligonucleotides with a T_d higher than 80°C.

VI. PCR and Hybridization Screening *continued*

4. Denature the oligonucleotide probe: heat it at 100°C for 2 min to release any secondary structure. Chill the probe quickly on ice.
5. Cut one corner of the bag and pour off the solution. Make hybridization solution by mixing fresh ExpressHyb with the probe.
6. Incubate the filters in the hybridization solution at the appropriate temperature for 16–20 hr.
7. Carefully remove the filters from the plastic bag. Place the solution in your radioactive liquid waste container.
8. 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.

   Note: Use a minimum of 15 ml of wash buffer for one filter, 25 ml for two, and 5 ml extra for each additional filter.
9. Replace the wash solution with fresh solution 20°C below the T_d. Wash filters under these higher stringency conditions for 15 min. If background is too high (≥ 1000 cpm as measured with a Geiger counter), increase the high-stringency wash time to 30 min.
10. Blot-dry the filters on Whatman 3MM paper at room temperature. See step D.10 for filter storage information.
11. Proceed to Section F.

F. Signal Detection by Autoradiography

1. Cut a piece of Whatman 3MM filter paper to the size of the x-ray film you will use. Tape duplicate filters on a single piece of 3MM filter backing. Place them alongside one another and in the same orientation.
2. Mark the filter backing at three asymmetric locations with radioactive ink so that each filter can be aligned with its radioautographic print. Cover in plastic wrap.
3. Expose filters to Kodak XAR film at –70°C for 16–24 hr. Use an intensifying screen to enhance the signal.
4. After developing the film, align it with the filters to find positive plaques. If you cannot pick an isolated 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. Rescreen, pick an isolated plaque and proceed to Section VIII or IX.

VII. Immunoscreening

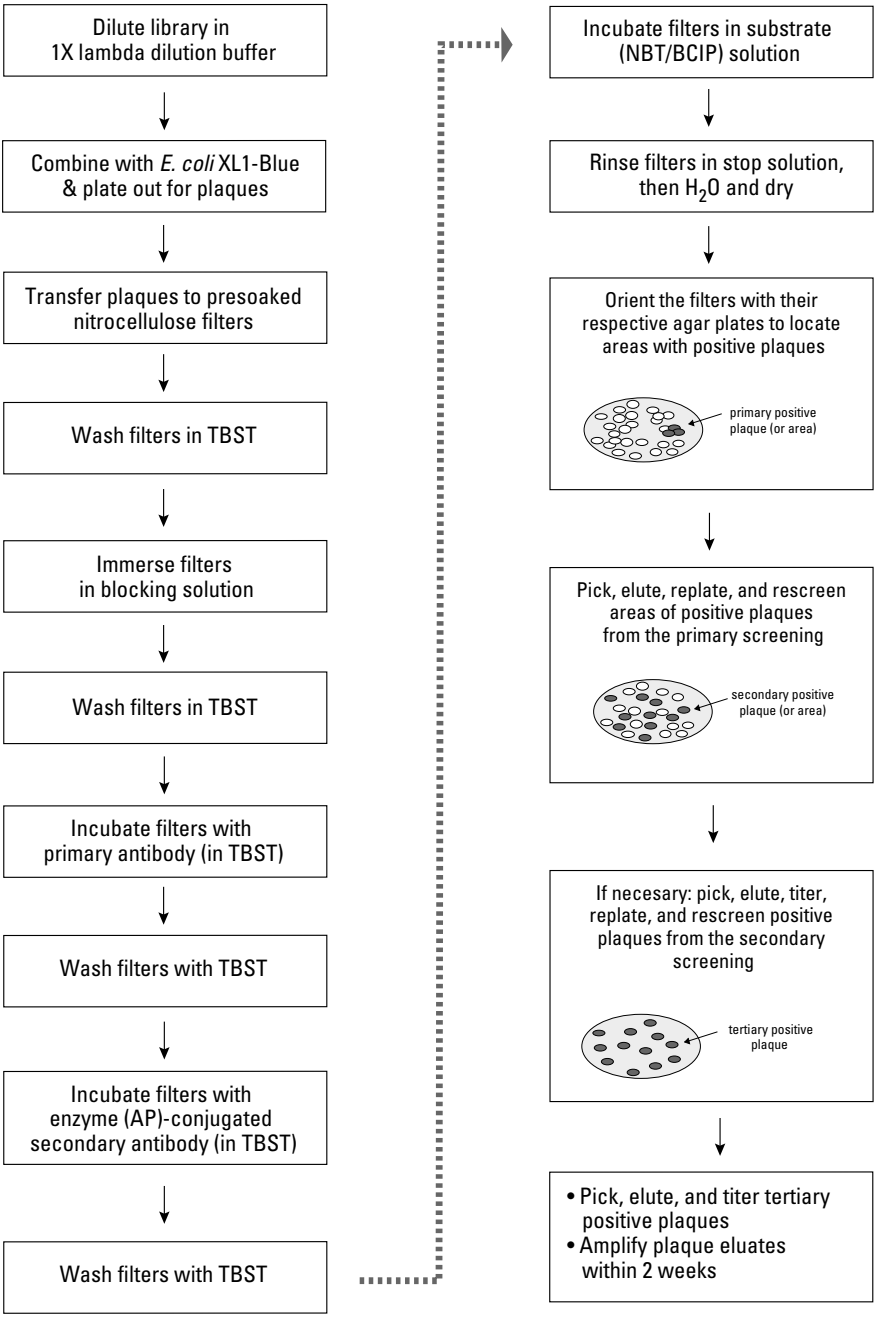

Figure 8. Overview of immunoscreening a λTriplEx library.

VII. Immunoscreening *continued*

cDNA that is cloned into λTriplEx and λgt11 ωεχτορσ is expressed as a fusion protein whose N-terminus is encoded by the vector sequence, and whose C-terminus is encoded by an open reading frame in the cDNA. After plating the λ expression library for plaques, the fusion proteins are immobilized on nitrocellulose filters. Antibodies are used to identify the specific target protein(s), and, ultimately, the cDNAs. Bound primary antibodies are detected with a species-specific secondary antibody conjugated to biotin or to enzymes such as alkaline phosphatase (AP) or horseradish peroxidase (HRP). Enzyme-conjugated and biotinylated secondary antibodies are commercially available and should be diluted according to the manufacturer's instructions.

This procedure describes detecting AP-conjugated secondary antibodies with the colorimetric substrates NBT and BCIP. If you choose another enzyme detection system, you will need different substrates. For further information on library immunoscreening, see Harlow & Lane (1988); Sambrook *et al.* (1989); Huynh *et al.* (1984); Young & Davis (1985; 1983a,b).

A purified human β_2-microglobulin clone in λTriplEx and rabbit anti-human β_2-microglobulin (primary antibody) are provided with all λTriplEx libraries as a positive control for immunoscreening. You must purchase an appropriate secondary antibody. If your experimental primary antibody was raised in rabbits, you can use the same AP-conjugated secondary antibody for library screening and control experiments.

A. Preparation and General Tips for Immunoscreening a Library

1. Selecting a primary antibody
   - We recommend polyclonal antibodies, as cDNA libraries typically encode a variety of protein fragments.
   - Use a primary antibody that can detect fusion proteins immobilized on a filter, such as for Western blots, ELISAs, or RIAs. These antibodies should also work for immunoscreening expression libraries in *E. coli*. If the target protein is available, we recommend performing a Western blot to confirm that the antibody has a strong and specific affinity for it.
   - Primary antibodies specific for a polysaccharide or other modifying groups on some eukaryotic proteins may not be suitable for detecting fusion proteins expressed in *E. coli*.
2. Before immunoscreening, optimize the concentrations of both antibodies by detecting positive clones on a filter or a portion of a filter. This test will provide data on your secondary antibody's affinity for your primary antibody.

   Note: Library customers should use the human β_2-microglobulin cloned insert and anti-human β_2-microglobulin antibody.

   In preliminary tests, include a control with only your secondary antibody, in order to determine if it binds nonspecifically. If it does, see Step F.5.d.

VII. Immunoscreening *continued*

3. Determine the number of 100- or 150-mm LB/$MgSO_4$ agar plates you will need, and warm and dry them.
4. Melt the requisite amount of LB/$MgSO_4$ top agarose. Use 2.5 ml when plating on 100-mm plates, and 5 ml when plating on 150-mm plates.
5. Determine the requisite number of nitrocellulose filters. **Do not** substitute nylon filters for nitrocellulose filters. For 150-mm plates, use 132-mm filters; for 100-mm plates, use 82-mm filters. Prenumber the filters with a soft lead pencil.
6. **Presoak the nitrocellulose filters in 10 mM IPTG (Step B.6) and blot-dry them.** The filters should be **damp** at the time of use.
7. **Do not allow the filters to dry after transferring the plaques or at any time during immunoscreening.** Antibodies can bind nonspecifically and *reversibly* to *wet* filters. They attach *permanently* if the filters become *dry*.
8. Use sterile forceps when handling filters.
9. You may reuse wash buffers, blocking solution, and stop solution 3–4 times in a day. If you reuse wash buffers, rotate the additional batches of filters through them in the same sequence as for the first batch.
10. Perform all filter washes, blocking reactions, and antibody incubations at room temperature with gentle agitation on a platform shaker (60–80 rpm) unless specified otherwise. Each filter wash lasts 5–10 min.
11. Buffer and solution volumes: use 15 ml for a filter in a 100-mm petri dish, or 25 ml for a filter in a 150-mm petri dish. If you process more than one filter at a time, use the larger petri dish or a large container, and enough solution to cover the filters. You may stack covered petri dishes or containers on the platform shaker.
12. **If you process multiple filters in a single container, ensure that they do not stick to each other.**

B. Transferring Plaques to Nitrocellulose Filters

1. Pick an isolated colony from the working stock plate of XL1-Blue host cells and use it to inoculate 15 ml of LB/$MgSO_4$/maltose broth in a 50-ml test tube. Incubate at 37°C overnight with shaking at 140 rpm, until the OD_{600} of the culture reaches 2.0. Centrifuge the cells for 5 min at 5,000 rpm, pour off the supernatant, and resuspend the pellet in 7.5 ml of 10 mM $MgSO_4$.
2. Dilute library in 1X lambda dilution buffer. Aim for 3×10^3 library plaques per 100-mm plate or 1.2×10^4 library plaques per 150-mm plate.

   Notes:

   - Always include one filter that is treated with secondary antibody alone to test the general background.
   - For a positive control filter, aim for 100 pfu of the positive control clone per 100-mm plate. Use the human β_2-microglobulin clone or any well-characterized cloned protein

VII. Immunoscreening *continued*

for which you have primary antiserum may be used. Plate several dilutions of the positive clone in the range of 1:1,000 and 1:10,000, or titer the clone before use.

3. Add 1 µl of diluted phage to 200 µl of the XL1-Blue overnight culture to each of 13 100-mm sterile tubes. Allow the phage to adsorb to the bacteria at 37°C for 15 min.
4. For each phage/bacteria mixture, add 2.5 ml (100-mm plate) or 5 ml (150-mm plate) of melted LB/$MgSO_4$ top agarose. Quickly cap the tube and invert it once. Pour immediately onto a prewarmed LB/$MgSO_4$ plate. Swirl the plates gently to allow even distribution of the agarose.
5. Cool the plates at room temperature for 10 min to allow the top agar to harden. Invert the plates and incubate at **40–42°C for 3–5 hr**. Check the plates periodically for plaque development.
6. While plates are incubating, presoak the prenumbered nitrocellulose filters in 10 mM IPTG (for ~20 min) and blot-dry.
7. When plaques are clearly visible, remove the plates from the incubator and use sterile forceps to place a **damp** nitrocellulose filter onto each plate. Avoid trapping air bubbles under the filters.

   Note: IPTG is a gratuitous inducer used to enhance the expression of β-galactosidase fusion proteins. To avoid smearing the plaques, be sure that the filter is damp only (not dripping wet) before placing it on the agar plate.
8. Use waterproof ink to spot orientation marks on the filter and its plate. See step VI.C.7 for details.
9. Incubate at 37°C for 3–5 hr. Maintain plate temperature at 37°C: bacteriophage growth is retarded at lower temperatures.
10. Remove the filter. If agarose sticks to the filter, replace it carefully, refrigerate the plate at 4°C for 30 min, and then attempt to remove the filter. Place filter immediately in TBST to begin washing.
11. Wash the filters in TBST 3–5 times. If necessary, you may leave them in TBST overnight at 4°C after the final wash.
12. You may wrap the agar plates in plastic wrap and store them 4°C until the immunoscreening results are available.

C. Blocking Nonspecific Binding Sites

1. Immerse the filters in room-temperature TBST containing 1% gelatin. See Step A.11 above for volume guidelines.

   Notes:

   - 1% gelatin solutions will form a gel if they are cooled below 20°C.
   - Other blocking solutions may also be used; see Step F.5.d.
2. Incubate the filters at room temperature for 30–45 min at 60 rpm on a platform shaker. Proceed directly to the next step.

VII. Immunoscreening *continued*

D. Binding the Primary Antibody

1. Remove the filters from the blocking solution and wash 3–5 times with TBST.
2. Dilute the primary antibody in TBST. See Step A.11 for volume guidelines. The same diluted antibody solution may be reused at least once, and may be stored at 4°C for up to 2 weeks between uses. See Step F.4.i for further information on reusing diluted primary antibody.

   Notes:

   - Primary polyclonal antibody dilutions are typically between 1:200 and 1:5,000, depending on the background levels and the signal strength of the bound antibody.
   - Dilute rabbit anti-human β_2-microglobulin control antibody at 1:1,000.
3. Incubate filters in the primary antibody solution at room temperature for 1 hr at 60 rpm on a platform shaker. **Do not allow the filters to become dry during the subsequent steps.**

   Note: You may incubate the filters at 4°C overnight in the primary antibody.
4. Wash the filters 3–5 times with TBST.

E. Detection Using an AP-Conjugated Secondary Antibody

1. Dilute the AP-conjugated goat anti-rabbit secondary antibody in TBST, according to the manufacturer's directions. Do not store or reuse secondary antibody.

   Note:

   - The secondary antibody is typically used at a much higher dilution (1:5,000–1:10,000) than the primary antibody.
2. Incubate each filter in the diluted secondary antibody at room temperature for 1–2 hr on a platform shaker (60 rpm). **Do not incubate >2 hr.**
3. Wash the filters 3–5 times with TBST.
4. Prepare the development solution. Use 100 µl of NBT and 75 µl of BCIP per 25 ml of 1X AP buffer.

   Note: Prepare development solution daily. Keep it in the dark until you use it.
5. Place filters in the substrate solution. Cover container and place on a platform shaker (60 rpm) at room temperature.
6. Inspect the filters at 10-min intervals until you see the desired signal-to-noise ratio. This process takes ~20–60 min. **Do not overdevelop.**
7. Rinse each filter in stop solution.
8. Rinse the filters in distilled H_2O and air-dry.
9. Identify positive areas by aligning the filter's orientation marks over those of the original plate. Pick plugs containing positive clones with a sterile, yellow plastic pipette tip (such as for a P100 or P200 pipettor). Place each plug in a separate 1.5-ml microcentrifuge tube containing 500 µl of 1X lambda dilution buffer. You may wish to store all of them for further study.

VII. Immunoscreening *continued*

10. Vortex the suspended agar plugs vigorously to release the phage. Place the plugs 4°C overnight to continue the elution process.
11. Titer the eluted primary positive areas (Section V.C).
12. The primary positive areas usually contain multiple clones, and you must purify and verify the desired clones. To do so, obtain isolated plaques. Replate the primary eluted plaques at a concentration of 200–1,000 pfu per 150-mm plate. Repeat the immunoscreening process.
13. Pick 2 or 3 well-isolated positive plaques and place each agar plug in a separate tube containing 500 µl of 1X lambda dilution buffer. Vortex and elute the phage as described in Step 10. These eluates are secondary phage eluates.
14. Titer the secondary phage eluates (Section V.C).
15. If the secondary phage eluate appears to contain a mixture of positive and negative clones, replate the eluate, repeat the immunoscreening process, and repeat step 13. These eluates are tertiary phage eluates.
16. Titer the eluted tertiary screened plaques (Section V.C).
17. Determine the insert sizes of positive screened plaques by PCR or restriction enzyme digestion (Section VIII). Target clones may be amplified or converted to plasmid for further analysis (Section IX).

F. Troubleshooting Library Immunoscreening

1. Plaques smeared on the filters

a. There may have been moisture droplets on the agar surface during bacteria + phage mixture plating. See the notes in Section V.C.

b. The presoaked nitrocellulose filters may have been too wet when they were used to transfer the plaques.

2. Plaques too small

a. The agar plates may have been too dry or the cell density too high at the time of library plating.

b. The plating temperature (Step VII.B.5) may have been too high. Do not let the incubation temperature go above 42°C.

3. Plaque size not uniform on the plate

The melted soft agar may have been unevenly distributed when plating. Swirl the plates gently when plating phage to promote even distribution, and place plates on a level bench while the agar solidifies.

4. Weak or no signal

a. Signal intensity decreases as plaque density increases. For best results, plate no more than 3×10^3 pfu per 100-mm plate, or 1.2×10^4 pfu per 150-mm plate.

VII. Immunoscreening *continued*

b. The nitrocellulose filters may have been too dry when used to transfer plaques, causing a decrease in IPTG's effectiveness to induce protein expression.

c. If you used a monoclonal antibody, switch to a polyclonal antibody.

d. Ensure that your primary antibody is suitable for immunoscreening (see Step VII.A.1 for more information).

e. Ensure that the secondary antibody is compatible with the primary antibody. For example, if you are using a primary antibody raised in rabbit, you will need a secondary antibody that is directed against rabbit IgGs. Perform a Western blot to confirm that the secondary antibody has a reasonable affinity for the primary antibody.

f. Serum-based blocking agents may interfere with the antibody–antigen interaction. We recommend 1% gelatin as the blocking agent because it is less likely to interfere in this way. For this reason, 5% nonfat dry milk in TBST is also better than serum products.

g. Antibody concentrations may be too low. See Step VII.A.2 for tips.

h. Incubation time with primary antibody may be too short. You may obtain a stronger reaction by incubating the filters with the primary antibody at 4°C overnight.

i. Improper storage may cause a primary antibody to lose binding activity. You can store diluted primary antibody at 4°C for up to 2 weeks. However, if it has been stored >2 weeks, do not use it or check its performance on a Western blot or a control filter before using it for screening. You can also purchase a new batch of primary antibody.

j. The enzymatic activity of the secondary antibody may decrease due to improper storage or handling. Test by adding 1 ml of the diluted antibody conjugate to 1 ml of color development solution. Intense purple color should appear within 5 min. **Do not store or reuse diluted secondary antibody.**

k. The activity of the enzyme linked to the secondary antibody may have decreased due to contaminants in the water or other reagents. Use reagents of the highest quality available and use only deionized water to prepare buffers and make dilutions.

5. High background

a. Incubation with the substrate may have been too long. After placing the filters in substrate solution, check them every 5–10 min. Stop the reaction immediately when the color reaches the desired intensity.

b. Make sure that you have used **agarose** —not agar—for the top layer during library plating. Agar contains components that can cause background problems.

VII. Immunoscreening *continued*

c. Filters may have been insufficiently washed. Perform all washes specified in the protocol; use longer wash times (10 min) and the maximum number (five) if necessary. Use at least 25 ml of wash buffer per filter per wash, and read the cautions about washing multiple filters a single container (Steps VII.A.11 & 12).

d. Insufficient blocking of nonspecific binding sites will contribute to background.

- Use sufficient blocking buffer to cover all filters in the container (Step VII.A.11). If you are treating multiple filters a single container, do not allow them to stick together.
- Increase blocking time. For 1% gelatin blocking buffers, you may block for 2 hr **maximum**. For serum-based blocking solutions, you may block overnight at room temperature.
- If you suspect that gelatin or nonfat dry milk does not work well with your system, try 20% fetal calf serum (FCS) or 3% bovine serum albumin (BSA) in TNT buffer (Sambrook *et al.*, 1989). Include controls without primary or secondary antibody, and wash the filters thoroughly.

e. Try diluting one or both antibodies.

f. If you did not use Protran filters (from Schleicher & Schuell), make sure that the filters you used were nitrocellulose, and that they had not been treated with Triton X-100 during manufacture.

g. Your primary or secondary antibody may bind nonspecifically to many proteins, even on blocked filters. Perform control incubations using one filter containing nonrecombinant plaques (formed by the vector alone) and another containing a sample of the library plaques. If nonspecific binding is the problem, decrease the concentration of the problem reagent, or take steps to ensure more complete blocking (5.d). Alternatively, obtain a primary antibody that is more specific for the desired protein, or a secondary antibody that is more specific for the primary antibody (6.d).

6. False positives

a. Replate the eluted primary positive area and repeat the immunoscreening. We recommend replating and rescreening the secondary eluted positive plaque. As a result, further analysis is performed on tertiary screened positive plaques.

b. Antibodies can bind nonspecifically to remnants of agarose on the filters. Ensure that top agarose does not adhere to the filters when you remove them from the plates. See Step VII.B.10 for tips.

c. Check the primary antibody dilution; false positives may result if antiserum is too concentrated.

VII. Immunoscreening *continued*

d. The primary antibody or the conjugate may contain components that cross-react with endogenous *E. coli* proteins.

- The antisera may require pretreatment with an *E.coli* cell extract to reduce interference caused by cross-reacting proteins (Ausubel *et al.*, 1995; Sambrook *et al.*, 1989).
- Alternatively, a serial dilution containing defined amounts of native and denatured target antigen can be spotted on separate filters and screened with different dilutions of the primary antibody . The aim is to find a dilution that displays a high level of specific reactivity with the antigen but does not react nonspecifically with prokaryotic proteins.

VIII. Analysis of Inserts

A. Amplifying Inserts by PCR

- You can use PCR to determine the insert sizes of positive clones. Use vector-specific insert screening primers. λTriplEx LD-Insert Screening Amplimers (#9107-1) are available from CLONTECH.
- PCR amplification can yield sufficient material for characterizing inserts by restriction enzyme digestion. You can also use PCR to generate cDNA hybridization probes from eluted phage plaques.

1. Pick well-isolated positive plaques from hybridization screening plates (Step VI.F.4), or immunoscreening plates (Step VII.E.17). Place each agar plug in 25 µl of H_2O. Vortex the plug.
2. Use 5 µl of the eluted plaque as the PCR template. Store the rest at 4°C.
3. Estimate insert sizes by electrophoresing an 8-µl sample of the PCR product on a 0.8% agarose gel with 1-kb ladder DNA size markers.

B. Excising Inserts by Restriction Enzyme Digestion

Restriction enzymes can be sensitive to the purity of the DNA preparation. For this reason, using soft top **agarose**–not agar–is critical when plating phage in preparation for DNA isolation (see Section VI. B for information).

Recombinant phage DNA can be isolated from purified phage particles using standard procedures (Sambrook *et al.*, 1989)

1. Digest 5–10 µl of purified recombinant λ DNA with the appropriate restriction enzyme at 37°C for 3–16 hr.
   - For directionally cloned libraries, use *Xba* I and *Eco*R I (double digest). For nondirectionally cloned libraries, use *Not* I. You may use *Eco*R I, but we do not recommended it as you may cut the insert at an internal *Eco*R I site. *Sal* I is also not recommended, because it is not unique within λTriplEx. For λTriplEx2 libraries, use *Sfi* I.
2. Heat samples at 70°C for 10 min to inactivate the enzyme.
3. Analyze the digest on a 0.8% agarose minigel.
4. If you see abundant undigested DNA, try the following steps:
   a. Reduce the DNA concentration.
   b. Increase the amount of restriction enzyme.
   c. Digest the DNA overnight.
   d. Combine the above suggestions.
   e. Further purifying of phage DNA on a CHROMA SPIN™-400 Column may help.
   f. Amplify the insert by PCR (see Section VIII.A).

IX. Converting λTriplEx to pTriplEx

A. Background

Converting a λTriplEx/λTriplEx2 clone to a pTriplEx/pTriplEx2 clone requires excising and circularizing a complete plasmid from the recombinant phage. The plasmid is released by *cre*-recombinase-mediated recombination at *loxP* sites (Figure 2). Release occurs automatically when recombinant phage is transduced into a bacterial host expressing *cre* recombinase. In this system, *E. coli* BM25.8 growing at 31°C provides *cre*-recombinase activity. Conversion may be performed on either individual positive plaques picked from the secondary or tertiary screening plates, or the entire library. Released plasmids differ from pTriplEx by a 100-bp *loxP* insert at the *Cla* I site (Figure 2). Excised plasmids are propagated stably in *E. coli*. Do not attempt blue/white screening with strain BM25.8.

B. Single Plaque Conversion Protocol

1. Pick an isolated colony from the working stock plate of BM25.8 host cells (Step V.B.4). Use it to inoculate 10 ml of LB broth in a 50-ml test tube. Incubate at **31°C** overnight with shaking at 150 rpm until the OD_{600} of the culture reaches 1.1–1.4.
2. Add 100 µl of 1 M $MgCl_2$ to the 10-ml overnight culture of BM25.8 (10 mM final concentration of $MgCl_2$).
3. Pick a well-isolated positive plaque from the secondary or tertiary screening plates (Step VI.F.4 or VII.E.17). Place it in 350 µl of 1X lambda dilution buffer. Vortex and incubate at 37°C for 3–4 hr with shaking (200–250 rpm). You may also elute phage at 4°C overnight.
4. In a 20-ml test tube, combine 200 µl of overnight cell culture with 150 µl of the eluted positive plaque. Reserve the remainder of the eluted plaque in case you need to repeat the conversion.
5. Incubate at **31°C** for 30 min without shaking.
6. Add 400 µl of LB broth.
7. Incubate at **31°C** for an additional 1 hr with shaking (225 rpm).
8. Using a sterile glass spreader, spread 1–10 µl of infected cell suspension on an LB/carbenicillin plate to obtain isolated colonies. Incubate plates at **37°C**.

   Note: You may use ampicillin instead of carbenicillin, but you may obtain more satellite colonies.
9. Pick several well-isolated colonies from each clone and prepare plasmid DNA separately from each. We recommend the NucleoSpin Miniprep Kit (#K3063-1, -2) for plasmid DNA preparations and for quick analysis of insert size. Plasmid DNA isolated with this kit is suitable for direct or automated fluorescent sequencing. You may also use Birnboim & Doly's method (1979) for quick analysis of insert size. Isolated plasmid DNA should be sufficiently pure for direct sequencing. You may use the pTriplEx sequencing primers with standard ds DNA sequencing protocols.

IX. Converting λTriplEx to pTriplEx *continued*

C. Library Conversion Protocol

Notes:

- This protocol was developed for use with standard λTriplEx and λTriplEx2 libraries. It is not recommended for use with the Large-Insert libraries.
- The day before conversion of the entire phage cDNA library, check the titer of the λTriplEx cDNA library as described in Section V. F.

1. Grow BM25.8 cells overnight from a single colony in 10 ml of LB medium at **31ºC** with shaking at 190 rpm.
2. Remove 1 ml of overnight culture and use it to inoculate 10 ml of LB medium (1:10 dilution). Continue growing at **31ºC** with shaking until the OD_{600} reaches 1.2 (2–2.5 hrs). Transfect cells on the same day.
3. Add 100 µl of 1 M $MgCl_2$ to the 10 ml freshly grown BM25.8 culture (10 mM final concentration).
4. Mix 200 µl of BM25.8 cells and λTriplEx cDNA containing 2 x 10^6 pfu (Section A) in a sterile 5-ml tube. Mix gently by pipetting.
5. Incubate this mixture of cells and phage for 1 hour at **31ºC** without shaking.
6. After incubation is complete, add 500 µl of LB medium and incubate for 1 hr at **31ºC** with shaking at 190 rpm. At this point, conversion of the entire library to plasmid form is complete.
7. Using a sterile spreader, spread 10 and 100 µl of converted cDNA library (diluted 1:100 in LB medium) on 150-mm LB agar plates containing 50 µg/ml carbenicillin.

   Note: You may use ampicillin instead of carbenicillin, but you may obtain more satellite colonies.
8. Grow the LB agar plates overnight at **37ºC**.
9. Count colonies and determine the total number of converted recombinant clones per µl. Count the total number of recombinant clones present in the whole volume of the converted plasmid library generated at Step 6.
10. Prepare 150–200 150-mm LB agar plates with 50 µg/ml carbenicillin.
11. Plate 20,000–30,000 converted clones on each 150-mm LB-carb plate (3–5 x 10^6 clones) and grow overnight at **37ºC** (not longer than 14 hr).

D. Plasmid DNA isolation from converted pTriplEx cDNA library

1. Add 10 ml of LB medium to each 150-mm plate to wash the grown cells from the surface of agar plate.
2. Gently remove the cells from the surfaces of the agar plates with a sterile spreader and transfer the cells in the medium from all the plates to sterile 500-ml centrifuge bottles.
3. Centrifuge at 10,000 rpm for 20 min at 4ºC.
4. At this point, follow a protocol for CsCl preparation of plasmid DNA, or use a NucleoBond Plasmid Kit (included; also see Related Products).

X. References

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XI. Related Products

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- λTriplEx™ (*Eco*R I-digested) 6161-1
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- Poly A^+ RNA many
- Biotin-21-dUTP 5021-1, -3
- Biotin-21-UTP 5024-1
- ExpressHyb™ Hybridization Solution 8015-1
- Oligonucleotide and DNA Probes many
- CSPD™ Chemiluminescent Substrate 8055-1, -2
- CHROMA SPIN™ Columns many
- Advantage® 2 cDNA PCR Kits K1910-1, -2
- Advantage® 2 cDNA Polymerase Mix 8430-1, -y
- Macherey-Nagel NucleoSpin® Plus Miniprep Kits K3063-1, -2
- SMART™ PCR cDNA Library Construction Kit K1051-1
- ClonCapture™ cDNA Selection Kit K1056-1

Appendix A: Restriction Maps of λTriplEx & λTriplEx2

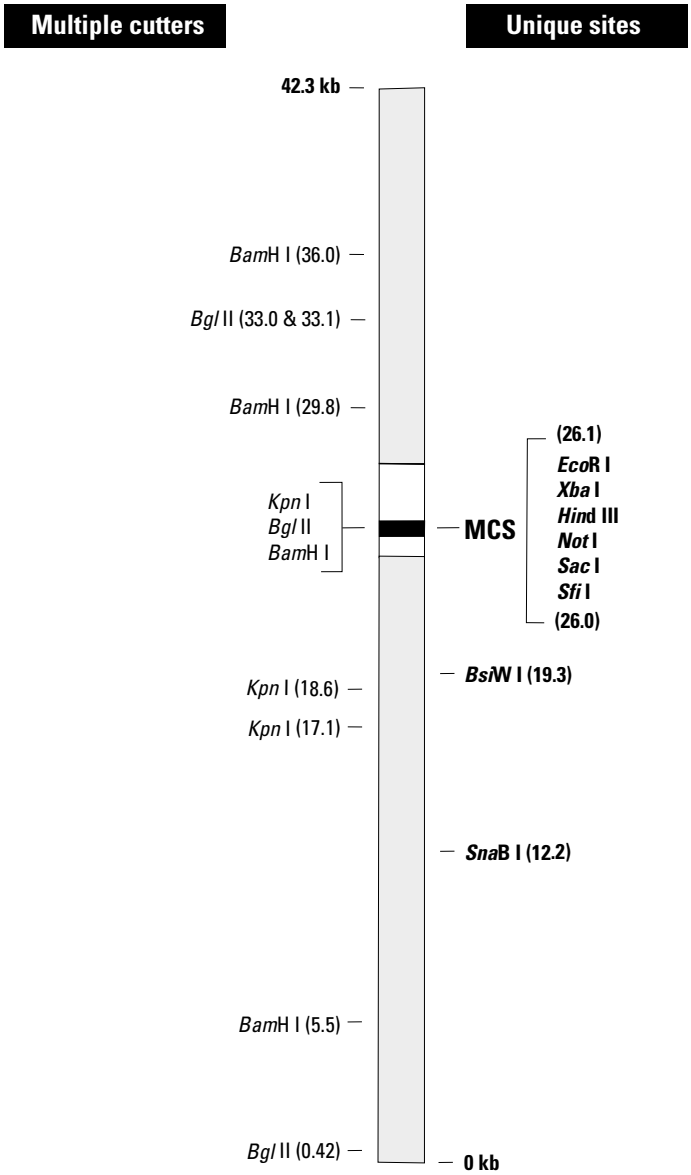

Figure 9. Restriction Map of λTriplEx.

Appendix A: Restriction Maps of λTriplEx & λTriplEx2, *cont'd*

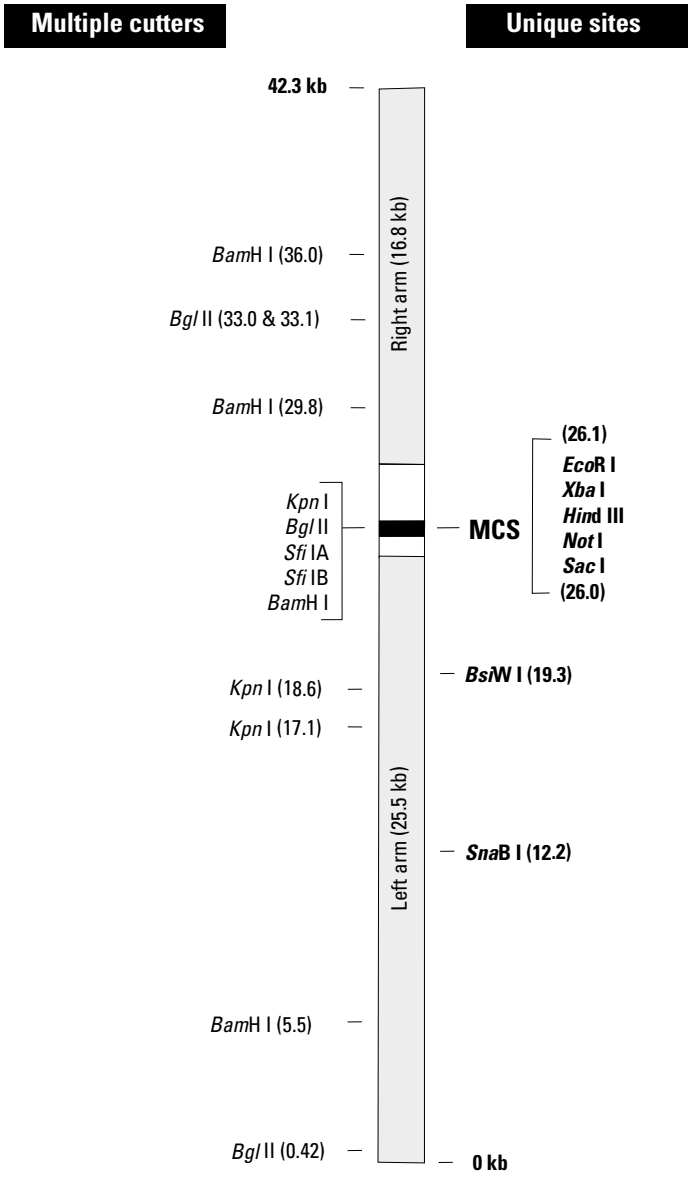

Figure 10. Restriction Map of λTriplEx2.

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