Serological Identification of Recombinantly Expressed Clones (SEREX)

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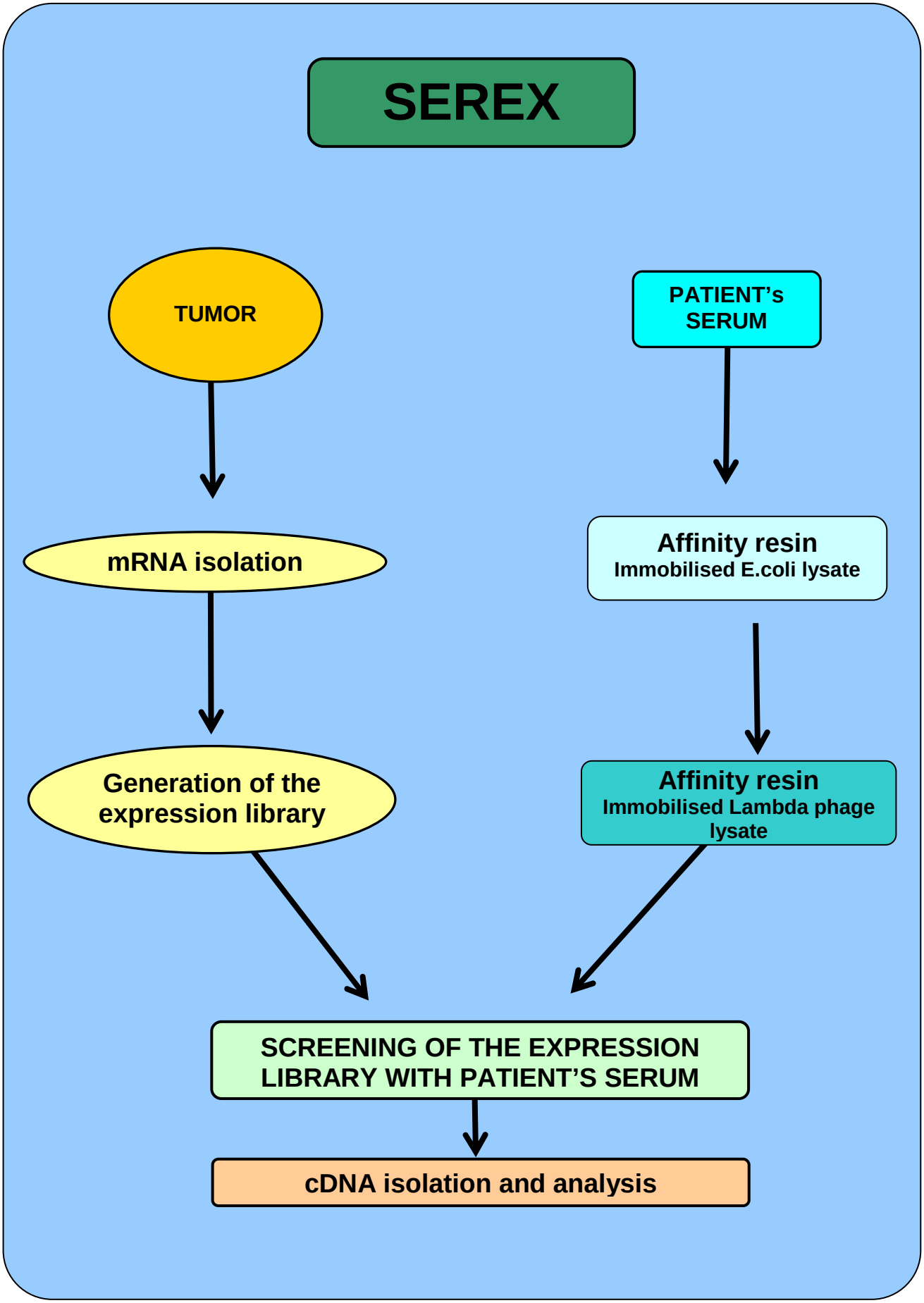
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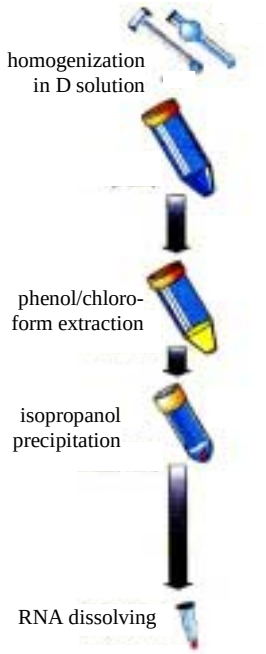
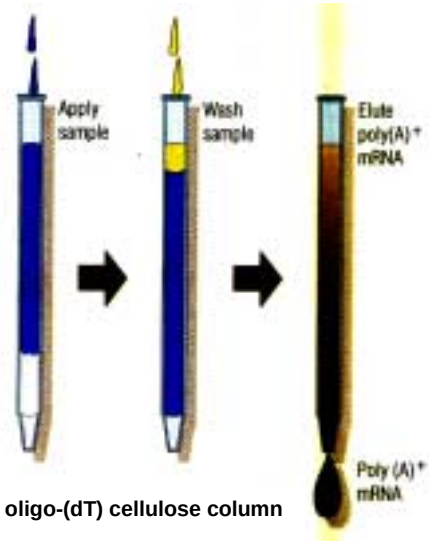
CHAPTER 1

mRNA isolation

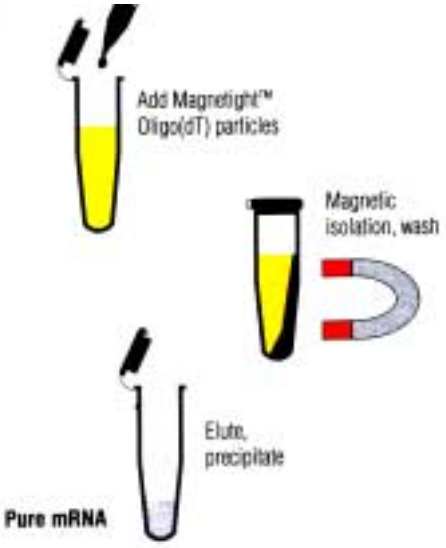
Total RNA extraction



mRNA isolation



oligo-(dT) cellulose column



Overview of the procedure

The quality and quantity of the mRNA used is of fundamental importance to the construction of a large, representative cDNA library. The guanidinium thiocyanate (GTC)-phenol-chloroform extraction method to isolate total RNA is rapid, yet it produces large amounts of high-quality, undegraded RNA. Although MMLV-RT is not inhibited by ribosomal RNA (rRNA) and transfer RNA (tRNA) contamination, it is advisable to select the poly(A) fraction. The amounts of rRNA and tRNA vastly outnumber the mRNA and will decrease the efficiency of the system. Poly(A) RNA is selected on oligo(dT) cellulose columns or oligo(dT) magnetic beads.

Ribonucleases A and TI are widely used in almost all molecular biology labs and are nearly indestructible. Ribonucleases are produced by microbes and have also been found in the oils of the skin. Make an effort to use tubes and micropipet tips which have been handled only with gloves. Use freshly autoclaved and baked tips and tubes. Usually these precautions are sufficient, but to be absolutely certain that microcentrifuge tubes and other components intended for use with RNA are not contaminated, the components can be treated with DEPC. Diethylpyrocarbonate is extremely toxic and should be handled with care.

Submerge the microcentrifuge tubes in a 0.1% (v/v) DEPC-treated water solution. Leave the beaker of submerged tubes in a fume hood overnight and then dispose of the DEPC-treated water. Autoclave the microcentrifuge tubes for at least 30 minutes. Even though the tubes may still have a sweet DEPC odor, the DEPC is completely inactivated by this procedure. Place the tubes in a drying oven overnight.

Remember, once the RNA is converted to first-strand cDNA, RNases are no longer a concern. Caution should still be exercised in maintaining a sterile, DNase-free environment.

1.1. Isolation of total RNA*

1. Homogenize frozen tissue (0.5g) with 5 ml of solution D in a glass-Teflon homogenizer and subsequently transfer to a 50 ml polypropylene tube.

2. Add sequentially 0.5 ml of 2M sodium acetate (pH4.0), 5 ml of phenol (water saturated), and 1 ml of chloroform to the homogenate with thorough mixing after the addition of each reagent. Shake the final suspension vigorously for 30 sec and cool on ice for 10-15 min.

3. Centrifuge the sample at 10000g for 20 min at 4°C.

4. After centrifugation, transfer the upper aqueous phase to a fresh tube, mix with 5 ml of isopropanol, and then place at -20°C for at least 1 hr to precipitate RNA.

5. Sediment again at 10000g for 20 min at 4°C and dissolve the resulting RNA pellet in 1.5-2 ml of solution D, and precipitate with 1 volume of isopropanol at -20°C for 1 hr.

6. After centrifugation for 10 min at 4°C wash the RNA pellet in 70% ethanol, centrifuge for 5 min. Remove supernatant, air dry the pellet and then dissolve it in 0.3ml of DEPC treated water at 65°C for 10-15 min. This RNA solution may kept at -20°C for short term or at -70°C for long term storage.

<u>D solution</u>
4 M guanidinium thiocyanate
25 mM sodium citrate, pH7.0
0.5% sarcosyl
0.1 M 2-mercaptoethanol

**. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162: 156–159.*

1.2. Quantifying of total RNA*

RNA can be quantified by measuring the optical density of a diluted RNA solution (1:500 with DEPC treated water). The conversion factor for RNA at the wavelength of 260 nm is 40 ug/ml for 1OD unit.

**. Maniatis, T., et al., eds. (1989) "Molecular Cloning: A Laboratory Manual. Second Edition," pp. 7.11–7.12. Cold Spring Harbor Laboratory Press.*

1.3. RNA electrophoresis*

After electrophoresis, intact total RNA will appear as smear with clear visible major bands for 28S and 18S rRNA. Formaldehyde gel should be used for RNA electrophoresis to disrupt possible secondary structure in RNA sample.

1. Formaldehyde gel preparation:
0.8 g agarose
5ml 20x RNA borate buffer
87 ml of distilled water.
Boil solution, put it into 60°C water bath and add 8ml formaldehyde. Put gel onto gel-casting tray.

2. RNA sample preparation:
1µl of isolated total RNA solution
1 µl EtBr (1mg/ml)
2µl RNA loading buffer.
Mix; heat sample at 65°C for 5 min and immediately put it on ice for 5 min. Load RNA onto gel.

3. Electrophoresis: run RNA sample in 1x RNA borate buffer at 5 V/1 cm of gel (running time of approx. 0.5 hr.).

20x RNA borate buffer	RNA loading buffer
24.73 g Sodium tetraborate (Borax)	197 µl formamide
38.14g Boric acid	64 µl formaldehyde
8 ml 0.5M EDTA (pH8)	39 µl 20x RNA borate buffer
Add distilled water to 1L.	

**. Maniatis, T., et al., eds. (1989) "Molecular Cloning: A Laboratory Manual. Second Edition," pp. 7.43–7.46. Cold Spring Harbor Laboratory Press.*

1.4. Isolation of mRNA

a) Purifying with Dynabeads Oligo(dT) beads (*"Dynal" protocol*).

1. Adjust the volume of 75 ug isolated RNA to 100 µl with DEPC treated water.

2. Heat to 65°C for 2 min to disrupt secondary structures.

3. In the meantime, transfer 200 µl resuspended Dynabeads Oligo(dT) from the stock tube suspension to an RNase-free Eppendorf tube placed in a Dynal MPC. After 30 sec (or when suspension is clear) remove the supernatant and wash the Dynabeads once by resuspending in 100µl 2x Binding buffer (solution B).

4. Remove the 2x Binding buffer from the Dynabeads while the vial is placed in the Dynal MPC.

Transfer the vial to another rack and resuspend the Dynabeads in 100µl 2x Binding buffer (solution B).

5. Add the total RNA to the Dynabeads suspension. Mix gently but thoroughly and anneal by rotating on a roller for 3-5 min at room temperature.

6. Place the vial in the Dynal MPC for 30 sec and remove the supernatant. Transfer the vial to another rack.

7. Wash twice with 200 µl Washing buffer (solution E) using the Dynal MPC. The Dynabeads must be mixed thoroughly in the washing buffer. Make sure to remove the supernatant completely between each washing step.

8. Add 10-20 µl Elution solution (solution F) and keep at 65°C for 2 min. Immediately place the tube in the Dynal MPC and transfer the supernatant containing the mRNA to a new RNase-free tube.

The used Dynabeads oligo(dT) may be regenerated as follows:

- resuspend the used Dynabeads Oligo(dT) (250 µl) in 200 µl reconditioning solution (solution G). Transfer the suspension to a new RNase-free tube and mix by shaking;

- incubate at 65°C for 2 min;

- place the tube in the Dynal MPC for at least 30 sec, and remove the supernatant. Repeat step 1 and 3 twice;

- resuspend the Dynabeads in 200 µl storage buffer Oligo(dT) (solution H). Place the tube in the Dynal MPC for at least 30 sec, remove and discard the supernatant. Repeat this washing step 3 times or until the pH of the supernatant is below 8.0;

- resuspend the Dynabeads in the desired volume of storage buffer Oligo(dT) (solution H). The Dynabeads are now reconditioned and ready for another mRNA isolation. Store the Dynabeads Oligo(dT) at 4°C.

2x Binding buffer (solution B)
20 mM Tris-HCl (pH 7.5)
1.0 M LiCl
2 mM EDTA

Washing buffer (solution E)
10 mM Tris-HCl (pH 8.0)
0.15 M LiCl
1 mM EDTA

Elution solution (solution F)
10 mM Tris (pH 7.5)

Recondition solution (solution G)
0.1 M NaOH

Storage buffer Oligo(dT) (solution H)
250 mM Tris-HCl (pH 8.0)
20 mM EDTA
0.1% Tween-20
0.02% Sodium Azide

b) Purifying with oligo-(dT)cellulose Poly(A) quick mRNA isolation kit *("Stratagene" protocol)**.

1. Prepare the RNA sample by denaturing at 65°C for 5 min and by adding the appropriate amount of 10× sample buffer on ice.

2. Preheat the elution buffer to 65°C.

3. Remove the caps from both ends of the push column.

4. Slowly push the storage buffer through the column at a rate of ~1 drop every 2 sec.

5. Apply 200 µl of high-salt buffer to the push column. Slowly push the high-salt buffer through the column at the same rate of 1 drop every 2 sec.

6. Repeat the previous step with another 200-µl aliquot of high-salt buffer.

7. Apply the prepared RNA sample (from the first step above) to the push column and push the RNA sample through the column at a rate of ~1 drop every 2 sec.

8. Reapply the RNA sample eluent to the push column and push the sample through the column at a rate of ~1 drop every 2 sec.

9. Apply 200 µl of high-salt buffer to the push column and push the high-salt buffer through the column at a rate of 1 drop/sec.

10. Repeat the high-salt wash with one additional 200-µl aliquot of high-salt buffer.

11. Wash the push column three times using 200-µl aliquots of low-salt buffer and pushing the low-salt buffer through the column at a rate of 1 drop/sec.

12. Elute the mRNA using four 200-µl aliquots of the preheated elution buffer (see the second step above), push the elution buffer through the column at a rate of 1 drop/sec and save the eluent.

13. If the RNA concentration is too low for subsequent use, precipitation is required. Add 10x sample buffer to a final concentration of 1. Mix well, add 2.5 volumes of ice cold 100% (v/v) ethanol and mix well again. Store overnight at –20°C. Centrifugation in a microcentrifuge at high speed for 20–30 min or at 12,000 *g* is sufficient to precipitate RNA in most cases. Wash the pellet with 70% (v/v) ethanol prepared with DEPC-treated water.

14. Dry the pellet for 1–2 min in a lyophilizer. Resuspend the pellet in a desired buffer that is free of ribonuclease contamination. Treatment with DEPC is recommended for the buffer. For long-term storage, store the mRNA in 100% (v/v) ethanol at –20°C or in DEPC-treated water at –80°C.

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

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

Low-salt buffer
10 mM Tris-HCl (pH 7.5)
1 mM EDTA
0.1 M NaCl

10x sample buffer
10 mM Tris-HCl (pH 7.5)
1 mM EDTA
5 M NaCl

High-salt buffer
10 mM Tris-HCl (pH 7.5)
1 mM EDTA
0.5 M NaCl

***** *Maniatis, T., et al., eds. (1989) "Molecular Cloning: A Laboratory Manual. Second Edition," pp. 7.26–7.30. Cold Spring Harbor Laboratory Press.*

1.5. Quantifying of mRNA*

If the amount of poly(A) RNA is below 1.5 ug/synthesis reaction, the RT may synthesize unclonable hairpin structures. If the amount of poly(A) RNA is above 7 ug, the percentage of cDNAs which are full length may decrease. The ZAP-cDNA synthesis kit is optimized for 5 ug of poly(A) RNA, but successful libraries have been generated using the minimums and maximums described here.

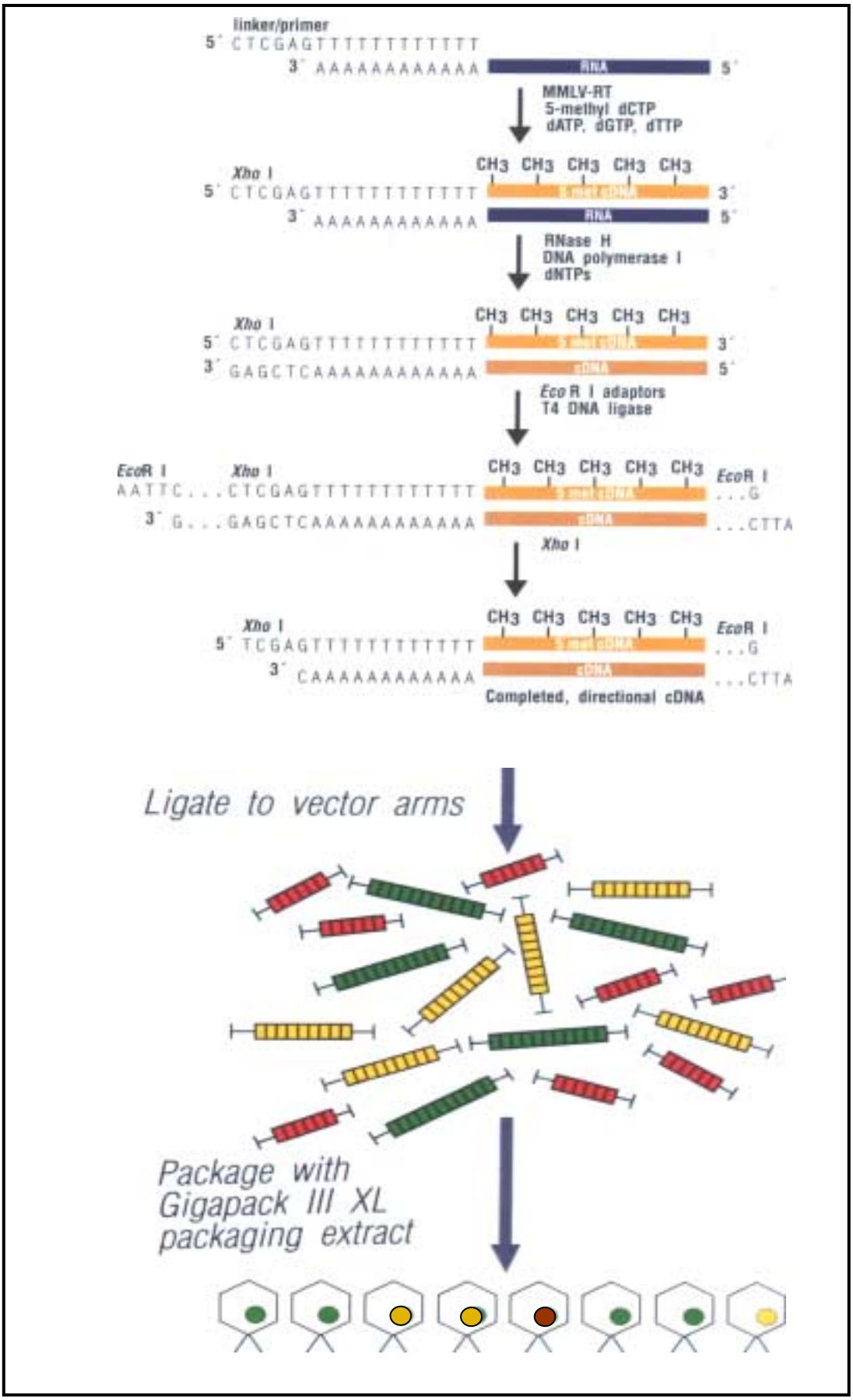
RNA can be quantified by measuring the optical density of a diluted RNA solution. The conversion factor for RNA at the wavelength of 260 nm is 40 ug/ml/OD unit as shown in the example below.

Two microliters of a poly(A) RNA sample are added to 498 µl of water (e.g., OD = 0.1). Therefore, 0.1 OD unit x dilution factor x 40 ug of RNA/ml = 1000 ug of RNA/ml.
If a sample has significant rRNA contamination, the actual amount of mRNA available for cDNA conversion will be overestimated by this procedure.

**. Maniatis, T., et al., eds. (1989) "Molecular Cloning: A Laboratory Manual. Second Edition," pp. 7.26–7.30. Cold Spring Harbor Laboratory Press.*

CHAPTER 2

cDNA library construction


linker/primer
5' CTCGAGTTTTTTTTTTTT
3' AAAAAAAAAAAA
RNA
5'
MMLV-RT
5-methyl dCTP
dATP, dGTP, dTTP
CH3 CH3 CH3 CH3 CH3
Xho I
5' CTCGAGTTTTTTTTTTTT
3' AAAAAAAAAAAA
3'
RNA
5'
RNase H
DNA polymerase I
dNTPs
3' GAGCTCAAAAAAAAAAAA
cDNA
EcoR I adaptors
T4 DNA ligase
EcoR I
AATTC...
...G
...CTTA
Xho I
Completed, directional cDNA
TCGAGTTTTTTTTTTTT
CAAAAAAAAAAAA
Ligate to vector arms
Package with
Gigapack III XL
packaging extract

Overview of the procedure

All steps below except 2.7 are according to "Stratagene" Uni-ZAP XR cDNA library synthesis protocol. When working with any enzyme required throughout the ZAP-cDNA synthesis protocol, flick the bottom of the tube to thoroughly mix the enzymes. Do not vortex.

2.1. Synthesizing first-strand cDNA

1. Preheat a 37°C water bath.

2. Thaw, vortex and spin down the contents of the non-enzymatic tubes. Place the tubes on ice *(moloney murine leukemia virus reverse transcriptase is temperature sensitive and should remain at -20°C until needed).*

3. The final volume of the first-strand synthesis reaction is 50 µl: the volume of added reagents and enzymes is 12.5 µl, which allows the mRNA and DEPC-treated water to be added in a volume up to 37.5 µl. As a control, use the following annealing reaction with 25 µl (5 ug) of test RNA and 12.5 µl of DEPC-treated water.

4. In an RNase-free microcentrifuge tube, add the following reagents in order:
5 µl of 10x first-strand buffer
3 µl of first-strand methyl nucleotide mixture
2 µl of linker-primer (1.4 ug/µl)
X µl of DEPC-treated water
1 µl of RNase Block Ribonuclease Inhibitor (40 U/µl).

5. Mix the reaction and then add X µl of poly (A) RNA (5 ug). Mix gently.

6. Allow the primer to anneal to the template for 10 minutes at room temperature.

7. Add 1.5 µl of MMLV-RT (50 U/µl) to the first-strand synthesis reaction. The final volume of the first-strand synthesis reaction should now be 50 µl.

8. Mix the sample gently and spin down the contents in a microcentrifuge.

9. Incubate the first-strand synthesis reactions, including the control reaction, at 37°C for 1 hr.

10. Prepare a 16°C water bath for second-strand synthesis. If a water bath with a cooling unit is not available, use a PCR machine.

11. After 1 hr, remove the first-strand synthesis reactions from the 37°C water bath and place them on ice.

2.2. Synthesizing second-strand cDNA

1. Thaw all nonenzymatic second-strand components. Briefly vortex and spin in a micro-centrifuge before placing the tubes on ice (*it is important that all reagents be <16°C when the DNA polymerase I is added*).

2. Add the following components in this order to the first-strand synthesis reaction on ice:
20 µl of 10x second-strand buffer
6 µl of second-strand dNTP mixture
14 µl of sterile distilled water (DEPC-treated water is not required).

3. Add the following enzymes to the second-strand synthesis reaction:
2 µl of RNase H (1.5 U/µl)
11 µl of DNA polymerase I (9.0 U/µl).

4. Quickly vortex the contents of the tube, spin the reaction in a microcentrifuge, and incubate for 2.5 hrs at 16°C. Temperatures above 16°C can cause the formation of hairpin structures, which are unclonable and interfere with the efficient insertion of correctly synthesized cDNA into the prepared vector.

5. After second-strand synthesis for 2.5 hrs at 16°C immediately place the tube on ice*.*

2.3. Blunting the cDNA termini

1. Add the following to the second-strand synthesis reaction:
23 µl of blunting dNTP mix
2 µl of cloned Pfu DNA polymerase (2.5 U/).

2. Quickly vortex the reaction and spin in a microcentrifuge. Incubate the reaction at 72°C for 30 min. Do not exceed 30 min.

3. Thaw the 3 M sodium acetate.

4. Remove the reaction and add 200 µl of phenol-chloroform [1:1 (v/v)] and vortex (*the phenol must be equilibrated to pH 7-8 because acidic phenol may denature the DNA)*.

5. Spin the reaction in a microcentrifuge at maximum speed for 2 min at room temperature and transfer the upper aqueous layer, containing the cDNA, to a new tube. Be careful to avoid removing any interface that may be present.

6. Add an equal volume of chloroform and vortex.

7. Spin the reaction in a microcentrifuge at maximum speed for 2 min at room temperature and transfer the upper aqueous layer, containing the cDNA, to a new tube.

8. Precipitate the cDNA by adding the following to the saved aqueous layer:
20 µl of 3 M sodium acetate
400 µl of 100% (v/v) ethanol
Vortex the reaction.

9. Precipitate overnight at -20°C.

10. In order to orientate the direction of precipitate accumulation, place a mark on the microcentrifuge tube or point the tube hinge away from the center of the microcentrifuge as an indicator of where the pellet will form.

11. Spin in a microcentrifuge at maximum speed for 60 min at 4°C.

12. Avoid disturbing the pellet and carefully remove and discard supernatant (t*he conditions of synthesis and precipitation produce a large -white pellet. The pellet accumulates near the bottom of the microcentrifuge tube and may taper up along the marked side of the tube)*.

13. Gently wash the pellet by adding 500 ul of 70% (v/v) ethanol to the side of the tube away from the precipitate. Do not mix or vortex.

14. Spin in a microcentrifuge at maximum speed for 2 min at room temperature with the orientation marked as in step 10.

15. Aspirate the ethanol wash and lyophilize the pellet until dry.

16. Resuspend the pellet in 9 µl of EcoR I adapters and incubate at 4°C for at least 30 min to allow the cDNA to resuspend.

2.4. Ligating the EcoR I adapters

1. Add the following components to the tube containing the blunted cDNA and the EcoR I adapters:
1 µl of 10x ligase buffer
1 µl of 10 mM rATP
1 µl of T4 DNA ligase (4 U/µl).

2. Spin down the volume in a microcentrifuge and incubate overnight at 8°C. Alternatively, the ligations can be incubated at 4°C for 2 days.

3. In the morning, heat inactivate the ligase by placing the tubes in a 70°C water bath for 30 min.

2.5. Phosphorylating the EcoR I ends

1. Once the ligase is heat inactivated, spin the reaction in a microcentrifuge for 2 sec. Cool the reaction at room temperature for 5 min.

2. Phosphorylate the adapter ends by adding the following components:
1 µl of 10x ligase buffer
2 µl of 10 mM rATP
6 µl of sterile water
1 µl of T4 polynucleotide kinase (10 U/µl).

3. Incubate the reaction for 30 min at 37°C.

4. Heat inactivate the kinase for 30 min at 70°C.

5. Spin down the condensation in a microcentrifuge for 2 sec and allow the reaction to equilibrate to room temperature for 5 min.

2.6. Digesting with Xho I

1. Add the following components to the reaction:
28 µl of Xho I buffer supplement
3 µl of Xho I (40U/µl).

2. Incubate the reaction for 1.5 hrs at 37°C.

3. Add 5 µl of 10x STE buffer and 125 µl of 100% (v/v) ethanol to the microcentrifuge tube.

4. Precipitate the reaction overnight at -20°C.

5. Following precipitation, spin the reaction in a microcentrifuge at maximum speed for 60 min at 4°C.

6. Discard the supernatant, dry the pellet completely, and resuspend the pellet in 10 µl of sterile water for cDNA size fractionating.

2.7. cDNA size fractionation*

1. Prepare 50 ml 1% low-melting point agarose gel without EtBr in 1xTAE buffer.

2. Load the cDNA sample and DNA size marker (1 kb ladder or lambda/HindIII) in 1x sample buffer onto gel and run them at 5-10 V/cm gel until distance between xylencyanol and bromphenol blue reaches 1-1.5 cm.

3. Cut the slice of gel with DNA size marker and put it into EtBr solution (weak red color) with gentle shaking for 10-15 min.

4. Visualize under UV the slice of gel with DNA size marker and mark the 0.5 kb and 6-8 kb fragments. Put them on thin peaces of paper.

5. Carefully join the slices of gel with DNA size marker and cDNA sample and excise the cDNA bands between 0.5 and 6-8 kb with a clean, sharp scalpel blade.

6. Extract the cDNA from the gel *("Qiagen" QIAEXII agarose gel extraction protocol):*
- add 3 volumes of buffer QX1 to 1 volume of gel;
- resuspend QIAEX II by vortexing for 30 sec. Add 30 µl QIAEXII to the sample;
- incubate at 50°C for 10 min to solubilize the agarose and bind the DNA. Mix by vortexing every 2 min to keep QIAEXII in suspension. Check that the color of the mixture is yellow;
- centrifuge the sample for 30 sec and carefully remove supernatant with a pipet;
- wash the pellet with 500 µl of buffer QX1;
- wash the pellet twice with 500 µl of buffer PE;
- air-dry the pellet for 10-15 min or until the pellet becomes white;
- to elute DNA, add 20 µl of 10 mM Tris-HCl, pH 8.5 and resuspend the pellet by vortexing. Incubate at 50°C for 5 min;
- centrifuge for 30 sec. Carefully pipet the supernatant into a clean tube;
- add 2 µl of 3M NaOAc and 50 µl of 100% (v/v) ethanol to the microcentrifuge tube;
- precipitate the reaction overnight at -20°C;
- following precipitation, spin the reaction in a microcentrifuge at maximum speed for 60 min at 4°C;
- carefully wash the pellet in 500 µl of 70% ethanol;
- air-dry the pellet and resuspend it in 2.5 µl of sterile water.

<u>50x TAE buffer</u>
242g Tris base
57.1 ml glacial acetic acid
100 ml 0.5 M EDTA (pH 8.0)
Add distilled water to 1 L

<u>6x Sample buffer</u>
0.25% bromphenol blue
0.25% xylene cyanol FF
30% glycerol in water

**. Maniatis, T., et al., eds. (1989) "Molecular Cloning: A Laboratory Manual. Second Edition," pp. 6.30–6.34. Cold Spring Harbor Laboratory Press.*

2.8. Ligating cDNA into the Uni-ZAP XR vector

1. Set up a control ligation to ligate the test insert into the Uni-ZAP XR vector as follows:
1.0 µl of the Uni-ZAP XR vector (1 ug)
1.6 µl of test insert (0.4 ug)
0.5 µl of 10x ligase buffer
0.5 µl of 10 mM rATP (pH 7.5)
1 µl of water
Then add 0.5 µl of T4 DNA ligase (4 U/µl).

2. To prepare the sample ligation, add the following components:
2.5 µl of resuspended cDNA (~100 ng)
0.5 µl of 10x ligase buffer
0.5 µl of 10 mM rATP (pH 7.5)
1 µl of the Uni-ZAP XR vector (1ug/1µl)
Then add 0.5 µl of T4 DNA ligase (4 U/µl).

3. Incubate the reaction tubes overnight at 12°C or for up to 2 days at 4°C.

2.9. Packaging procedure

1. Remove the appropriate number of packaging extracts from the -80°C freezer and place the extracts on dry ice.

2. Quickly thaw the packaging extract by holding the tube between your fingers until the contents of the tube just begins to thaw.

3. Add the ligated DNA (4 µl) to the packaging extract immediately.

4. Stir the tube with a pipet tip to mix well. Gentle pipetting is possible provided that air bubbles are not introduced.

5. Spin the tube quickly (for 3-5 sec), if desired, to ensure that all contents are at the bottom of the tube.

6. Incubate the tube at room temperature (22°C) for 2 hrs. Do not exceed 2 hrs (*the highest efficiency occurs between 90 min and 2 hrs. Efficiency may drop dramatically during extended packaging times*).

7. Add 500 µl of SM buffer to the tube.

8. Add 20 µl of chloroform and mix the contents of the tube gently.

9. Spin the tube briefly to sediment the debris.

10. The supernatant containing the phage is ready for titering and may be stored at 4°C.

2.10. Preparing the host bacteria

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

1. Revive the stored cells by scraping off splinters of solid ice with a sterile wire loop.

2. Streak the splinters onto an LB agar plate containing the appropriate antibiotic, if one is necessary.

3. Incubate the plate overnight at 37°C.

4. Seal the plate with Parafilm® laboratory film and store the plate at 4°C for up to 1 week *(restreak the cells onto a fresh plate every week).*

5. Inoculate an appropriate medium, supplemented with 10 mM $MgSO_4$ and 0.2% (w/v) maltose, with a single colony.

6. Grow at 37°C, shaking for 4-6 hrs (do not grow past an OD of 1). Alternatively, grow overnight at 30°C, shaking at 200 rpm (*the lower temperature keeps the bacteria from overgrowing, which reduces the number of non viable cells. Phages can adhere to non viable cells resulting in a decreased titer)*.

7. Spin the cells at 500 x *g* for 10 min and discard the supernatant.

8. Gently resuspend the cells in half the original volume with sterile 10 mM $MgS0_4$.

9. Dilute the cells to an OD of 0.5 with sterile 10 mM $MgSO_4$.

2.11. Plating and tittering

The color selection by a complementation with the Uni-ZAP XR vector requires higher amounts of IPTG and X-gal for the generation of the blue color. Transcription and translation of the fusion protein are normal, but the large polylinker present within the pBluescript phagemid, which is present in the Uni-ZAP XR vector, is partly responsible for the reduced activity of the 3-galactosidase protein - not the promoter. As would be expected, the copy number of the Uni-ZAP XR vector is much less per cell than the copy number of pBluescript phagemids. However, it is important to note that the color assay is used only for determining the ratio of recombinants to nonrecombinants within a newly constructed library and is not used for any other manipulations.

1. To plate the packaged ligation product, mix the following components:
- 1 µl of the final packaged reaction and 200 µl of XLI-Blue MRF' cells at an OD of 0.5 in 10 mM $MgSO_4$;
- 1 µl of a 1:10 dilution of packaged reaction and 200 µl of XLI-Blue MRF'cells at an OD of 0.5 in 10 mM $MgSO_4$.

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

3. Add the following components:
2-3 ml of NZY top agar (melted and cooled to ~48°C)
15 µl of 0.5 M IPTG (in water)
50 µl of X-gal [250 mg/ml (in DMF)].

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

5. Plaques should be visible after 6-8 hrs, although color detection requires overnight incubation. Background plaques are blue and should be $<1 \times 10^5$ pfu/1ug of arms, while recombinant plaques will be white (clear) and should be 10- to 100-fold above the background.

6. Incubate the plates for at least 12 hrs at 37°C.

7. Count the plaques. Calculate the efficiency using the following equation:

$$\frac{\text{(Number of plaques) X (dilution factor) X (total packaging volume)}}{\text{(Total number of micrograms packaged) X (number of microliters plated)}}$$

LB Broth
10 g NaCl
10 g of tryptone
5 g of yeast extract
Add deionized H_2O to a final volume of 1L, autoclave

LB Agar
10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized H_20 to a final volume of 1L, autoclave, cool to 55°C, pour into petri dishes (~15 ml/100-mm plate)

LB-Tetracycline Agar
Prepare 1 liter of LB agar
Autoclave, cool to 55°C, add 12.5 mg of filter-sterilized tetracycline, pour into petri dishes (~15 ml 10-mm plate)

LB-Kanamycin Agar
Prepare 1 liter of LB agar
Autoclave, cool to 55°C, add 50 mg of filter-sterilised kanamycin, pour into petri dishes (~15 ml/100-mm plate)

LB Top Agar (per Liter)
Prepare 1L of LB broth
Add 0.7% (w/v) agarose
Autoclave, cool to 55°C, pour into petri dishes (~15 ml/100-mm plate)

NZY Broth
5 g of NaCl
2 g of $MgS0_4$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
Add deionized H_2O to a final volume of 1L, autoclave

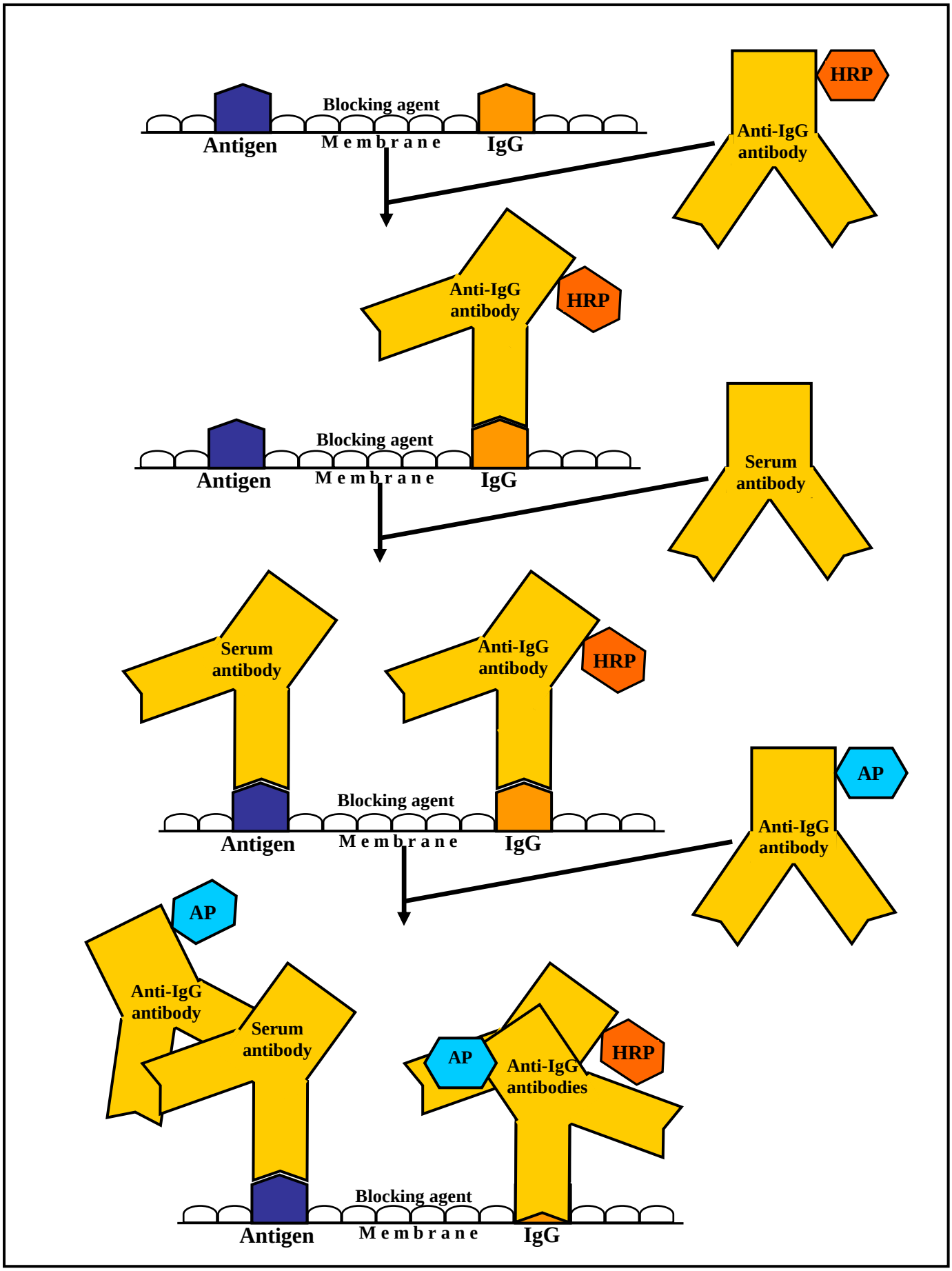
NZY Agar
5 g of NaCl
2 g of $MgS0_4$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
15 g of agar
Add deionized H_2O to a final volume of 1L, autoclave, cool to 55℃, pour into petri dishes (~25 ml/150-mm plate)

NZY Top Agar
Prepare 1L of NZY broth
Add 0.7% (w/v) agarose
Autoclave

SM Buffer
5.8 g of NaCl
2.0 g of $MgS0_4$
50.0 ml of 1 M Tris-HCl (pH 7.5)
5.0 ml of 2% (w/v) gelatin
Add deionized H_2O to a final volume of 1L, autoclave

CHAPTER 3

Immunoscreening of Uni-ZAP XR library


Blocking agent
Antigen
Membrane
IgG
HRP
Anti-IgG antibody
Anti-IgG antibody
HRP
Blocking agent
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Serum antibody
Serum antibody
Anti-IgG antibody
HRP
Blocking agent
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Membrane
IgG
AP
Anti-IgG antibody
AP
Anti-IgG antibody
Serum antibody
AP
Anti-IgG antibodies
HRP
Blocking agent
Antigen
Membrane
IgG

Overview of the procedure

Components normally present in many antisera also bind to antigens normally produced by E. coli and the bacteriophage lambda. Use of immobilized E. coli lysate and pseudo screening prior to the immunological screening of phage plaques and bacterial colonies will aid in reducing the high background signals produced by these binding activities.

3.1. Prepare the patient`s serum for immunoscreening*

a).Synthesis of Anti-E.coli Sepharose 4B beads (for 5 ml wet-volume beads).

1. Overgrow 400 ml XL1-blue MRF` culture in LB with 0.2% maltose and 10 mM $MgSO_4$ (use fresh plated bacteria).

2. Precool the culture at 4°C and pellet bacteria by centrifugation at 1000 g for 10 min at 4°C.

3. Resuspend the bacterial pellet in 40 ml of 0.1 M Sodium Borate (Borax) pH 8 with 1M NaCl. Transfer the resuspended pellet to 50 ml falcon tube and mix thoroughly on shaker for 5-10 sec.

4. Add to suspension 80 mg of lysozyme and mix thoroughly on shaker for 5-10 sec.

5. Incubate suspension on a rotor-wheel at room temperature for 20 min.

6. Precool the suspension on ice-water bath and add 80 µl of Triton X-100. Mix suspension on shaker throughly for 10 sec. It is turbidity should decrease and viscosity increase.

7. Incubate the suspension at 4°C on a rotor-wheel for 30-60 min.

8. Pass the suspension through a 30-50 ml syringe needle by 8-10 times. Viscosity of suspension should decrease.

9. Centrifuge the suspension at 5000 g at 4°C for 20 min.

10. At this time start to prepare CnBr activated sepharose 4B for coupling:
- suspend 2g of CnBr activated Sepharose 4B in 40 ml of 1mM HCl on rotor-wheel at room temperature for 15 min;
- wait until Sepharose 4B beads sediment, carefully decant the supernatant and wash pellet of beads with 60-80 ml of 0.1 M $NaHCO_3$;
- wait until beads sediment (approx 15-20 min), carefully decant supernatant. Leave tube with wet beads at room temperature for coupling.

11. Transfer the supernatant from step 9 into 50 ml falcon tube and placed it on ice water bath.

12. Adjust the pH of the supernatant to 9.0 with conc. NaOH.

13. Incubate the suspension of E. coli lysate with sepharose beads on a rotor–wheel at 4°C overnight.

14. Next morning, remove samples from the wheel and wait until sepharose beads sediment and carefully decant the supernatant. Incubate the sepharose beads with 40-50 ml of 0.1M Tris pH 8 for 2 hrs at room temperature on a rotor-wheel.

15. Wait until sepharose beads sediment and wash them with 40-50 ml TBS.

16. Wait until sepharose beads sediment, carefully decant the supernatant and resuspend the pellet of beads in 10 ml of TBS with 0.02% NaN_3 as preservative. Store suspension of beads at 4°C.

b).Synthesis of Anti-E.coli/Lambda Sepharose 4B beads (for 5 ml wet-volume beads).
1. Overgrow 400 ml XL1-blue MRF` culture in LB with 0.2% maltose and 10 mM $MgSO_4$ (use freshly plated bacteria).

2. Precool the culture to 4°C and pellet bacteria by centrifugation at 1000 g for 10 min at 4°C.

3. Suspend pellet of bacteria in 16 ml of 10mM $MgSO_4$.

4. Mix 1.6 ml of suspension with 4 ml of nonrecombinant lambda (make stock of nonrecombinant lambda in 5 ml SM from blue plaques after amplification on 15 cm dish to full lysis). Leave the other suspension of bacteria at 4**°**C.

5. Incubate suspension of bacteria with nonrecombinant lambda for 4 hrs in 40 ml of sterile LB with 10 mM $MgSO_4$ and 0.2% maltose at 37**°**C. Clear lysate should be visible.

6. Add to the lysate the bacterial suspension from step 5 and incubate for 2 hrs at 37**°**C. No lyses should be visible.

7. Centrifugate the suspension at 1000 g, 4**°**C for 20 min.

The others steps as 3-16 for Synthesis of Anti-E.coli Sepharose 4B beads (see above).

c). Immunoadsorption of patient`s serum against anti-E.coli/lambda immobilized lysates.
1. Pre-equilibrate 10 ml wet-volume synthesized beads (5 ml of Anti-E. coli beads + 5 ml of Anti-E. coli/lambda beads) with 50 ml of TBS.

2. Dilute 5 ml of patient's serum with TBS to 50 ml.

3. Add diluted serum to the pre-equilibrated beads and incubate the suspension on rotor-wheel at 4°C overnight.

4. Next morning, wait until beads sediment and carefully transfer serum supernatant to a new tube. Add 0.02% NaN_3 to serum and store it at 4°C.

5. The immunoadsorption of the sera may be successfully performed with the use of FPLC system by circular passes of the sera (5 to 10 times) through the column with immunoadsorbent prepared as described earlier.

The used beads may be regenerated by washing them with 200 ml of 1M propionic acid (13.4 M stock) followed by washing with 200 ml of TBS. Store regenerated beads in 10 ml TBS with 0.02% NaN_3 at 4°C.

d). Immunoadsorption of patient's serum against lytic membranes (pseudo screening).
1. Inoculate an 5 ml of LB, supplemented with 10 mM $MgSO_4$ and 0.2% (w/v) maltose, with a single colony of XL1-blue MRF` and grow overnight at 30°C, shaking at 200 rpm *(the lower temperature keeps the bacteria from overgrowing, which reduces the number of nonaviable cells. Phage can adhere to nonviable cells resulting in a decreased titer)*.

2. Spin the cells at 5000 *g* for 30 sec and discard the supernatant. Dilute the cells to an OD of 0.5 with sterile 10 mM $MgSO_4$.

3. Mix the 1 µl of nonrecombinant lambda (from stock obtained as above) and 600 µl of diluted XL1-blue MRF`. Incubate the phage and the bacteria at 37°C for 15 min to allow the phage to attach to the cells.
4. Add 8-10 ml of NZY top agar (melted and cooled to ~48°C) to the suspension of bacteria and phage, mix gently.

5. Plate immediately onto the 15 cm plates with NZY agar (totally use 10 plates) and allow the plates to set for 10 min at room temperature. Invert the plates and incubate at 37°C until plaques will be visible (approx. 4-6 hours).

6. If plaques are visible, carefully place 142 mm circle nitrocellulose membranes (avoid bulbs) on plates and incubate them overnight at 37°C.

7. Next morning, take the membranes off and wash them in TBST for 30 min at room temperature under vigorous shaking.

8. Rub the side of transfer of each membrane (use gloves) to remove residual agar. Place in fresh TBST for 15 min under vigorous shaking at room temperature.

9. Block each membrane in 30 ml of 5% dry milk powder in TBS for 1 hr at room temperature.

10. Wash membranes twice in TBS.

11. Fill up the 50 ml of immunoabsorbed on immobilized E.coli/lambda lysates serum (see above) to 100 ml with TBS containing 0.02% NaN_3 and 0.4% dry milk powder.

12. Pre-absorb 100 ml of diluted serum (1:20 dilution) with 10 prepared membranes on a shaker overnight at room temperature.

13. Next morning, pre-absorbed serum is diluted again with TBS containing 0.02% NaN_3 and 0.2% dry milk powder to 500 ml (1:100 dilution) and is ready for use in immunoscreening.

LB Broth
10 g NaCl
10 g of tryptone
5 g of yeast extract
Add deionized H_2O to a final volume of 1L, autoclave

10xTBS
0.1 M Tris-HCl (pH 8.0)
1.5 M NaCl
Adjust to 1L with distilled water

10xTBST
0.1 M Tris-HCl (pH 8.0)
1.5 M NaCl
0.05% Tween 20
Adjust to 1L with distilled water

NZY Agar
5 g of NaCl
2 g of $MgS0_4$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
15 g of agar
Add deionized H_2O to a final volume of 1 L, autoclave, pour into petri dishes (~25 ml/150-mm plate)

NZY Top Agar
5 g of NaCl
2 g of $MgS0_4$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
0.7% (w/v) agarose
Add deionized H_2O to a final volume of 1L, autoclave

**. Maniatis, T., et al., eds. (1989) "Molecular Cloning: A Laboratory Manual. Second Edition," pp. 12.13, 12.25-12.26, 12.27-12.28. Cold Spring Harbor Laboratory Press.*

3.2. cDNA library serum immunoscreening*

Do not allow the filters to dry out after transferring the plaques at any time during the immunoscreening.

1. Inoculate a single colony from the freshly plated XL1-Blue MRF`cells into 10 ml LB medium, containing 0.2% maltose and 10 mM $MgS0_4$ and grow overnight at 30°C with shaking. Do not overgrow cells (up to OD_{600} <1.0).

2. In the morning, spin down cells in eppendorf tubes at max rpm for 30 sec. Remove the supernatant and resuspend cells in 10 mM $MgS0_4$ to OD_{600} **=** 0.5.

3. Make appropriate dilutions of the titered primary library (see above) and add 10^4 pfu to 0.6 ml of prepared plating cells. Mix well and incubate at 37°C for 15 min (without shaking) to allow the phage to absorb on cells.

4. Add 15 µl of 1 M IPTG and 9-10 ml of melted NZY top agar (keep melted top agar at 48°C in a water bath), mix well and pour the mixture onto a prewarmed, dry NZY agar 15 cm plate (optional - one day old prepared) avoiding bubbles and solidifying of the agar in the tube. Cool the plates at room temperature for 10 min to allow the top agar to harden.

5. Invert the plates and incubate at 37°C until plaques become visible (approx. 4.5-5 hrs). Check if the titer is correct (on completion, the plaques should be touching).

7. Following this incubation period, treat the nitrocellulose membranes (use 132-mm circular nitrocellulose membranes for 150 cm plates):
- dilute IPTG in sterile distilled water to 10 mM;
- wet the nitrocellulose membranes by submersing them in the IPTG solution until the membranes are completely wet;
- after wetting the nitrocellulose membranes place them on Whatman 3MM paper to air dry.

8. Take plates out of the incubator and place premarked nitrocellulose filters onto the top agar surface very carefully, avoiding folding. Incubate plates at 37°C overnight.

9. Next morning, chill plates at 4°C for at least 1 hr.

10. Mark the filters by stabbing with an 18-gauge needle through the filter and the agar.

11. Remove filters carefully from plates and immerse them in the 1xTBST. Incubate filters in the 1xTBST on a platform shaker for 30-60 min (use 15 cm Petri dish with 150 ml of solution for 8-10 of 132 mm filters for washing).

12. Using gloves, rub off the side of transfer of each filter very thoroughly (quite harshly to remove bacteria and agar).

13. Transfer filters into fresh TBST buffer and wash them for 30 min.

14. Add blocking buffer (1xTBST containing 5% dry milk without fat) to filters and incubate for 1 hr at room temperature.

15. Wash filters twice in 1xTBS.

16. Add Goat anti-Human IgG labelled with HRP (1:2000 dilution) in 1xTBS, 1% dry milk. Incubate for 1.5 hrs on the shaker at room temperature (use for incubation 15 cm Petri dish with 100 ml of solution for 8-10 of 132 mm filters).

17. Wash filters 3 times in 1xTBST, 20 min per wash.

18. After the last wash add 50 mM Tris HCI, pH 7.4 containing DAB (0.3 mg/ml, should be filtered after dissolving) and 1 µl/ml of 30 % H_2O_2 and incubate for approx. 30 min.

19. When IgG plaques are clearly visible stop the reaction by washing filters in TBST buffer. Identify all IgG positive plaques on the light box and mark them with a pen (be careful because some plaques are very weakly stained). Do not allow filters to dry.

20. Rinse filters twice in 1xTBST and twice in 1xTBS.

21. Add preabsorbed serum and incubate overnight at room temperature (use a 15 cm Petri dish with 150 ml of diluted 1:100 serum for 8-10 of 132 mm filters for incubations).

22. Next day, wash filters 3 times in TBST, 20 min each time. After last wash rinse filters in TBS.

23. Add Goat anti-Human IgG labeled with AP (1:3000 dilution) in 1xTBS, 1% dry milk. Incubate at room temperature for 1.5 hrs (use a 15 cm Petri dish with 100 ml of solution for 8-10 of 132 mm filters for incubations).

24. Wash filters three times in 1xTBST, 20 min per wash and twice in TBS. Rinse filters with the AP-buffer.

25. Add AP-developing solution to filters and incubate on the shaker till plaques are clearly visible (use a 15 cm Petri dish with 100 ml of solution for 8-10 of 132 mm filters for incubations).

26. Stop reaction at an appropriate time by washing with a large excess of distilled water.

27. Identify and mark positives on filters. Overlap markings on filters and corresponding plates.

28. Pick positive plaques using a cut blue tip and place each agar plug in a separate tube containing 1 ml of SM and 20 µl of chloroform. Vigorously vortex and incubate at 4°C overnight to release the phage. It is safe to store primary phage for a long time at 4°C.

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

- overlay the immunoscreened plates with -8-10 ml of SM buffer. Store the plates at 4°C overnight (with gentle rocking if possible). This allows the phage to diffuse into the SM buffer;

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

- remove the cell debris by centrifugation for 10 minutes at 500 x g;

- recover and transfer the supernatant to a sterile polypropylene container. Add chloroform to a 0.3% (v/v) final concentration and store at 4°C.

29. For a secondary round of immunoscreening, dilute primary stock of positive plaque at 1:500 in SM and plate 2 µl from this dilution on 10 cm Petri dish.

30. Repeat all steps as above for primary immunoscreening (incubation period of 82 mm filters on Petri dish should be reduced to 3-3.5 hrs).

31. Pick up individual secondary positive plaques into 0.5 ml SM with 20 µl chloroform for in vivo excision procedure.

10xTBS
0.1 M Tris-HCl (pH 8.0)
1.5 M NaCl
Adjust to 1L with distilled water

10xTBST
0.1 M Tris-HCl (pH 8.0)
1.5 M NaCl
0.05% Tween 20
Adjust to 1L with distilled water

SM Buffer
5.8 g of NaCl
2.0 g of $MgS0_4$
50.0 ml of 1 M Tris-HCl (pH 7.5)
5.0 ml of 2% (w/v) gelatin
Add deionized H_2O to a final volume of 1L, autoclave

AP-buffer
100 mM Tris HCI (pH 9.5)
100 mM NaCl
5 mM $MgCl_2$

AP-development buffer
200 ml of AP buffer
360 µl of NBT (stock: 100 mg/ml in 70% DMF; always add first)
360 µl of BCIP (stock: 50 mg/ml in 100% DMF)

LB Broth
10 g NaCl
10 g of tryptone
5 g of yeast extract
Add deionized H_2O to a final volume of 1L, autoclave

NZY Agar
5 g of NaCl
2 g of $MgS0_4$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
15 g of agar
Add deionized H_2O to a final volume of 1L, autoclave, pour into petri dishes (~25 ml/150-mm plate)

NZY Top Agar
5 g of NaCl
2 g of $MgS0_4$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
0.7% (w/v) agarose
Add deionized H_2O to a final volume of 1L, autoclave

** Stratagene `s picoBlue immunoscreening protocol.*

3.3. *In vivo* excision of the pBluescript phagemid from the Uni-ZAP XR vector*

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

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

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

1. Grow separate overnight cultures of XL 1-Blue MRF' and SOLR cells in LB broth, supplemented with 0.2% (w/v) maltose and 10 mM $MgS0_4$, at 30°C.

2. Spin down the XLl-Blue MRF' and SOLR cells at max speed in eppendorf tube for 30 sec. Resuspend the XL 1-Blue MRF' and SOLR cells at an OD_{600} of 1.0 in 10 mM $MgS0_4$.

3. Combine the following components in a Falcon 2059 polypropylene tube:
200 µl of XL 1 -Blue MRF' cells at an OD_{600} of 1.0
250 µl of secondary positive phage stock (containing >1 x 10^5 phage particles)
1 µl of the ExAssist helper phage (>1 x 10^6 pfu/µl).

4. Incubate the Falcon 2059 polypropylene tube at 37°C for 15 min.

5. Add 3 ml of LB broth and incubate the Falcon 2059 polypropylene tube for 2.5-3 hrs at 37°C with shaking. Because clonal representation is not relevant, single-clone excision reactions can be safely performed overnight.

6. Heat the Falcon 2059 polypropylene tube at 65-70°C for 20 minutes and then spin the tube at 1000 x *g* for 15 min.

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

8. To plate the excised phagemids, add 200 µl of freshly grown SOLR cells from step 2 (OD_{600} = 1.0) to 1.5-ml microcentrifuge tube. Add 100 µl of the phage supernatant from step 7

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

10. Streak 10-20 µl of the cell mixture from microcentrifuge tube on LB-ampicillin agar plates (50 ug/ml) and incubate the plates overnight at 37°C.

Colonies appearing on the plate contain the pBluescript double-stranded phagemid with the cloned DNA insert. Helper phage will not grow, since helper phage is unable to replicate in the Su" (nonsuppressing) SOLR strain and does not contain ampicillin-resistance genes. SOLR cells are also resistant to lambda phage infection, thus preventing lambda phage contamination after excision. To maintain the pBluescript phagemid, streak the colony on a new LB-ampicillin agar plate. For long-term storage, prepare a bacterial glycerol stock and store at -80°C. At this stage, colonies may be selected for plasmid preps, or the cell mixture may be plated directly onto filters for colony screening.

LB Broth
10 g NaCl
10 g of tryptone
5 g of yeast extract
Add deionized H_2O to a final volume of 1L, autoclave

LB-Ampicillin Agar
Prepare 1L of LB agar, autoclave, cool to 55°C, add 50 mg of filter-sterilized ampicillin, pour into petri dishes (~15 ml 10-mm plate)

** Stratagene `s Uni-ZAP XR cDNA library synthesis protocol*