Screening Bacteriophage Lambda Libraries

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Preparing Host Cells

Grow an overnight culture of appropriate host strain (XL1-Blue MRF' for cDNA libraries) in LB plus antibiotic if relevant. The next morning, set up 50 ml NZY medium supplemented with 0.2% maltose (1:100 dilution of 20% maltose/dH20 stock; sterile filtered) and 10mM MgSO4 (1:100 dilution of 1M MgS04/dH20 stock; sterile filtered). Inoculate culture 1:100 with overnight bacterial culture and grow with 200 to 300 rpm agitation at 37 degrees C. Grow bacteria to an O.D. 600nm~0.5-1.0. This will take 2-5 hours-- check O.D. periodically. If cells grow past O.D. 600~ 1, they are not suitable for use and should be discarded. Pour cells into a conical centrifuge tube, centrifuge 10 minutes 2000 rpm. Remove supernatant and resuspend in 1/2 original cell volume in sterile 10 mM MgS04. Gently mix to fully resuspend cells. Store at 4 degrees C. Use within 7 days for screening libraries and within 14 days for titering or in vivo excision of lambdaZAP.

Preparing Plating Supplies

Melt top agarose (NZY broth plus 7% w/v agarose) [NZY broth per liter: 5g NaCl, 2 g MgSO4.7H2O, 5 g yeast extract 10 g NZ amine. Adjust pH to 7.5 with NaOH. Autoclave] in microwave until completely melted. You will need 8 ml for each 150mm plate and 3 ml for each 100mm plate. Cool top agarose to 48 degrees C by placing it in a water bath. For each plate, transfer 8 ml of top agarose to a sterile glass culture tube and place tube in a heating block set to 48 degrees. If top agarose is too hot, it will kill the phage and bacteria, if it is too cold, it will congeal too quickly and will not spread properly on the plates. Remove the appropriate number of NZY plates (NZY plates: NZY broth plus 15 g/liter Bacto Agar. Autoclave and pour. Dry plates overnight and store at 4 degrees C.) from the cold and prewarm to 37 degrees C. Prewarmed plates are easier to pour! It is impossible to pour even 48 degree C top agarose onto cold plates!

Titering libraries

Phage suspensions are stable at 4 degrees C in SM for several months, provided they are stored with chloroform. However, the titer does decrease with time, so you should check it every few months. Phage libraries are best stored in aliquots at -80 degrees C in 7% DMSO v/v. In preparation for titering, have 48 degree C top agarose, 37 degree C pre-warmed NZY plates, and a plating stock of bacteria in 10 mM MgSO4 available. Aliquot host cells into the appropriate number of sterile culture tubes. You will need 0.6 ml for 150mm plates and 0.2 ml for 100mm plates. Using SM Buffer (SM per liter: 5.8 g NaCl, 2 g MgSO4.H2O, 50 ml Tris-HCl pH 7.5, 5 ml 2% gelatin. Sterile filter or autoclave. N.B. Magnesium is critical to the viability of bacteriophage lambda.) make dilutions of main stock of library. For an amplified library [anticipated titer 10^8-10^{10} plaque forming units (pfu)/ml] make a 1:1000 dilution and add 1 ul of this to 600 ul plating cells (titer plate "A"). Dilute this 1:1000 dilution 1:100 and add 10 ul of this dilution to 600 ul plating cells (titer plate "B"), and 1 ul of this dilution to 600 ul plating cells (titer plate "C"). Incubate cells with phage in tube at 37 degrees for 10'. Remove plates from 37 degree C incubator. Working quickly, transfer 48 degree C top agarose from culture tube in heat block into culture tube containing cells and phage. Do this with enough force to ensure mixing of cells and top agarose, but not so quickly that you generate air bubbles. Pour cell and top agarose suspension into the center of the plate and swirl rapidly but gently so that the entire plate is coated. Avoid air bubbles, blank patches, ridges, and other problems and strive for a totally smooth coating. If you have problems, try to work in a warmer room, i.e. very air conditioned room may be problematic. Wait 1 minute for top agarose to harden. When you are done plating and top agarose has set, incubate upside down at 37 degrees C for 4-6 hours. Count plaques and calculate titer in terms of pfu/ml.

Plating Libraries for Screening

Have available: 48 degree C top agarose, prewarmed NZY plates (150mm plates will be used throughout this protocol), fresh host cells in 10mM MgSO4, and a cDNA library of known titer. Dilute library in SM; you will need 50,000 pfu/150mm plate for a primary screen. In some cases, less dense or more dense plating strategies are required. Mix 0.6 ml host cells with 50,000 pfu in a sterile culture tube. Incubate at 37 degrees 10'-15' to allow phage to adsorb to host cells. Remove plates from warm room in preparation for plating. Add 8 ml top agarose to 1st culture tube containing cells and phage and pour onto plate. Repeat for all tubes and plates. Let top agarose harden at room temperature for one minute, then transfer all plates to

37 degree incubator. Plates should be incubated with agar surface up, spread out and not stacked. This ensures even growth from plate to plate. Monitor growth of phage--plaques should be visible at 4 hours. Stop growth when plaques are the appropriate size. This will probably not exceed 5-6 hours and a plaque size of 1mm, but circumstances vary. Cool plates in refrigerator or cold room in preparation for doing lifts. You can do lifts after 30 minutes of cooling or the next day. However, do the lifts within 24 hours of stopping growth.

Lifts: Transferring Phage from Plate to Membranes

When plates are cool, prepare room to do the lifts. You will need:

Denaturing Solution [1.5M NaCl, 0.5N NaOH; per liter: 87.66 g NaCl and 20g NaOH]

Neutralizing Solution [1.5M NaCl, 0.5M Tris-HCl pH 7.5; per liter: 87.66g NaCl and 60.55 g Trizma base; adjust pH to 7.5 with HCl]

2XSSC [dilute 20XSSC with dH2O]

Hybond N+ from Amersham (Catalog # RPN137B); mark with pencil or Paper Mate medium black pen; prepare enough filters for a minimum of 2 and a maximum of 6 lifts per plate

India Ink to mark orientation, in a 1cc syringe with 22g needle

3mm or similar blotting paper

large plastic cafeteria trays for processing filters

Remove plates from cold. Remove protective wrapping from Hybond-N+ wearing gloves and center first filter over first plate. Hold it so the center of the filter touches the center of the plate. Gently lower the rest of the filter onto the plate until it is totally smoothed down and touching all parts of the plate, writing side facing up. Do not pick up and reposition! Do not allow puckers or air bubbles! Incubate filters on plates according to the following schedule:

1st lift	30 seconds
2nd lift	1 minute
3rd lift	2 minutes
4th lift	3 minutes
5th lift	4 minutes
6th lift	5 minutes

Mark filter and plate to orient using India Ink: puncture both filter and agar while gently injecting a small amount of ink. Make 3 asymmetric patterns. i.e. 1 dot, 3 dots arranged in a triangle and 3 dots arranged in a line. This will create an inky hole in the plate and the filter for later alignment purposes. Be

careful to not inject too much ink or you will obliterate surrounding marks. Using flat forceps, carefully pick up edge of filter without gouging top agarose and carefully lift filter off of plate. If top agarose sticks to filter, try to pick up the opposite corner of the filter and ease the top agarose back onto the plate. Do all the lifts first and place finished filters, DNA side up, on 3mm filter paper. Denature phage by placing filters [DNA side up !] on 3mm filters sitting in a large plastic tray and soaked with denaturing solution for 7 minutes. Transfer to a second tray with 3mm filter soaked with neutralizing solution for 7 minutes. Process them in batches, with 12 filters per tray. Rinse filters by transferring to a pan with 2XSSC and arrange on 3mm filter paper to dry. Bake in a vacuum oven at 80 degrees C for 2 hours to fix DNA to nitrocellulose filter or UV crosslink in Stratalinker (Autocrosslink) or Photodyne unit (2 minutes). If baking in oven, dry filters in warm room for 30 minutes first. Then separate filters with clean paper towels. If UV crosslinking, filters do not need to be dried first. Important: DNA side must face UV light or DNA will not be crosslinked!

Making Radioactive DNA Probes

Use Stratagene Prime-It II random primer kit to label 25-50 ng of purified DNA insert. Purify probe through G50 Sephadex and count 1 ul on A2 program. Should be at least 200,000 cpm/ul.

Screening Libraries with Radioactive Probes

Remove filters from oven, wet in 2XSSC, and prehybridize in Buck Buffer (2-4 ml/filter) 1 hour to 6 hours, 65 degrees C, with shaking or rotation. This can be in seal-a-meal bags, hybridization bottles, or a tupperware pan or petri dish sealed to prevent leaks.
Do not at any point allow filters to dry; this will cause non-specific sticking of the probe and significant background on the x-ray film exposure.

Buck Buffer
0.5M Sodium Phosphate pH 7.3
1% BSA
4% SDS
do not filter or autoclave store at 25 degrees C.
Add sheared salmon sperm DNA, boiled for 10 minutes, then ice chilled to a final concentration of 0.5 mg/ml

Denature probe by boiling for 10 minutes, then chilling on ice. Remove prehybridization solution, and add probe to more Buck Buffer + ssDNA (10-20 ml) to filters. Hybridize overnight at 65 degrees C. (~16 hours).

Remove probe and freeze -20 degrees C. Can reuse within one week. Wash filters with 0.2XSSC/0.5% SDS 3x30 minutes, 65 degrees C., with agitation. Remove filters from wash solution, dry on 3mm filter paper and tape to 37x43 mm filter paper (6 filters/piece of paper). Orient with fluorescent ink and expose to X-Ray film in cassette with intensifying screen, -80 degrees C, overnight. Develop film and put up for a longer exposure as necessary.