LIBRARY SCREENING

Overview.

Screening a λ library involves the following steps: titering the library to determine the PFU (plaque forming units per mL of library stock), primary plating of the library, preparing filters of phage, hybridization screening of filters, secondary screening, tertiary screening, preparation of λDNA, and subcloning of insert into plasmid vector. It's not too difficult, just time consuming....

For background reading, read Maniatis or Current Protocols.

Titering the Library.

1. Grow an overnight culture of the appropriate E. coli strain for your phage library in λ broth with 0.2% maltose and 10mM $MgSO_4$.

2. Microwave λ top agarose to boiling. Place in a water bath (ie. beaker with water) on a hot plate. Maintain the temperature at 45˚ to 50˚C. Pre-warm LB plates to 37˚C; this will prevent the agarose from solidifying too soon in step 7, ie. before you've had a chance to evenly spread it.

3. Prepare 200 µL 100-fold serial dilutions of the phage library in SM, ie. 10^{-2}, 10^{-4}, 10^{-6}, etc. to 10^{-14}. Note: 10^{-2} means 2 µL phage in 198 µL SM.

4. Aliquot 300 µL of the overnight culture for each dilution.

5. Add 100 µL of the phage dilution to the E. coli.

6. Incubate at room temperature, 20 min, then at 37˚C for 10 min.

7. Add 2.5 mL of λ top agarose to the E. coli. Quickly vortex and pour onto an LB plate (no antibiotic). Rock the plate back and forth to evenly spread agarose/E. coli mix.

8. Incubate at 37˚C for approximately 12 hours. You will see little holes in the lawn of bacteria. Find an appropriate dilution with a reasonable number of plaques (ie. "holes") to count. Titer = #plaques x dilution x 10. For example, 20 plaques on the 10^{-8} dilution plate indicates a λ phage library titer of $2x10^{10}$ PFU/mL.

Plating a Library.

- First, you must decide how many plaques you will need. For a genomic library, 5 genomes worth of plaques should be plated. For a cDNA library, it depends on the abundance of the transcript; generally, 10^6 plaques is sufficient.
- For a primary plating, use the 16 cm plates, which can handle 100,000-150,000 plaques. For secondary and tertiary platings, use 10 cm plates.
- Depending on which plates you're using, follow the table below for the appropriate amounts of bacteria, phage dilution, and top agarose to use.

	Bacteria	Phage Dilution	Top Agarose
10cm Plate	300µL	100µL	2.5mL
16cm Plate	500µL	300µL	7.0mL

1. Grow an overnight culture of the appropriate E. coli strain for your phage library in broth with 0.2% maltose and 10mM $MgSO_4$.

2. Microwave top agarose to boiling. Place in a water bath (ie. beaker with water) on a hot plate. Maintain the temperature at 45˚ to 50˚C. Pre-warm LB plates to 37˚C; this will prevent the agarose from solidifying too soon in step 7, ie. before you've had a chance to evenly spread it.

3. Aliquot the appropriate amount of overnight culture (see table).

4. Add the appropriate amount of phage diluted in SM (see table). Incubate at room temperature, 20 min, then at 37˚C for 10 min.

5. Add the appropriate amount of top agarose to the E. coli (see table). Quickly vortex and pour onto a pre-warmed LB plate (no antibiotic). Rock the plate back and forth to evenly spread agarose/E. coli mix.

8. Incubate at 37˚C for approximately 9-12 hours. DO NOT OVERGROW! I recommend checking the plates hourly after 8 hours of growth. If you cannot do this, you may place the plates at 4˚C and then return them to 37˚C when you can monitor them more closely. For the primary screening, the plaques should be about 1 mm in diameter with minimal overlap with adjacent plaques (ideally, no overlap). For the secondary and tertiary screenings, the plaques can be larger, but they should be distinct, ie. far enough away from neighboring plaques to be easily identified.

Preparing Nitrocellulose Filters.

- By capillary action, phage are transferred onto the nitrocellulose. The filters are then washed to denature the phage proteins and DNA.

1. Place the plates at 4˚C for >1 hour to solidify the top agarose.

2. Label the nitrocellulose filters with a ball-point pen.

3. Remove plate from 4˚C and place nitrocellulose filter with the labeled side facing up onto the plate, center first, and allow the edges to fall slowly (see figure 1.). Leave filter on plate for 30 sec. Meanwhile, poke asymmetric holes through agarose/agar (see figure 2.). Note: If duplicate filters are being done, for the second filter, poke through the filter at the same holes as the first filter.

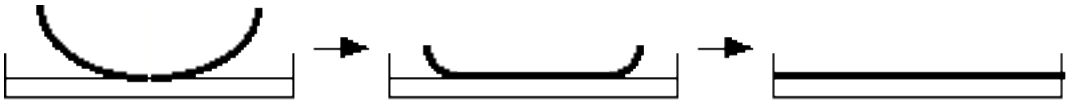
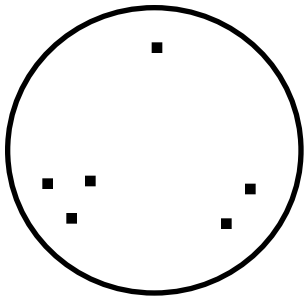


Figure 1.



My favorite asymmetric pattern

Figure 2.

Note: I usually remove the plates from 4˚C one at a time to ensure that the top agarose is just as solidified for the last plate as the first. This is particularly important when doing duplicate filters because the top agarose tends to stick to the nitrocellulose when it is warmer.

4. Air dry filters for > 10 min on Whatman paper with the phage side up. Note: I usually get all the filters to this stage before proceeding, thus some of filters may be air dried for 30min or more.

5. Wash filters for 2 min in each of the following in succession:

   A. 0.2M NaOH
      1.5M NaCl

   B. 0.4M Tris-Cl, pH7.6
      2X SSC

C. 2X SSC

Note: I generally prepare 500mL of each solution and wash 4 or 6 filters at a time depending on the size of the tray.

6. Place filters on Whatman paper to dry with the phage side up.

7. Vacuum bake the filters between two pieces of Whatman paper for 90 min at 80˚C.

Hybridization.

1. Prehybridize the filters at 65˚C for > 1 hour in

   0.5 M Na_2HPO_4, pH7.2
   1 mM EDTA
   1 % BSA
   7 % SDS

   Note: Prepare a little extra (~10 mL for every 20 filters) of the prehybridization solution since it is also the hybridization solution.

2. Transfer filters into hybridization bags with a maximum of 20 in one bag.

3. Add 10 mL (pre)hybridization solution.

4. Boil labeled probe (0.5 to 1.0 x 10^6 cpm per mL of hybridization solution) and 2 mg salmon sperm DNA for 3 min.

5. Add probe/salmon sperm DNA to hybridization solution and close bag.

6. Hybridize at 65˚C overnight.

7. Wash filters at RT in Low Stringency Wash buffer twice.

   40 mM Na_2HPO_4, pH7.2
   1 mM EDTA
   5% SDS
   0.5% BSA

8. Wash filters at RT in High Stringency Wash buffer.

   40 mM Na_2HPO_4, pH7.2
   1 mM EDTA
   1% SDS

9. Repeat high stringency washes at 3˚C increments until filters appear to have background radioactivity.

10. Sandwich the filters in saran wrap while they are still wet/damp and expose to x-ray film for 24 hours.

11. Line up the developed autorad with the sandwiched filters and mark the locations of the poked holes onto the autorad.

12. Line up a phage plate with the autorad (Note: the autorad will have to be flipped over.).

13. Using a pasteur pipette, remove a plug of top agarose/agar from the phage plate aligned over a positive autorad signal and transfer it to an eppendorf containing SM with a drop of chloroform. You may want to take a few plugs to be certain of getting the positive plaque.

    Note: the "positives" will sometimes appear as streaks. In this case, target removing a plug corresponding to the head not the tail of the streak.

14. Place the samples at 4˚C for > 1 hour. The samples can then be used for secondary/tertiary plating or to prepare λ DNA.

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Preparation of λ DNA

1. Grow an overnight culture of the appropriate E. coli strain for your phage library in λ broth with 0.2% maltose and 10mM $MgSO_4$.

2. Incubate the following at 37˚C for 15 min:

    100 µL E. coli culture
    100 µL 10 mM $MgCl_2$ / 10 mM $CaCl_2$
    100 µL phage in SM

3. Transfer sample to 25 mL NZC media.

4. Incubate in 37˚C shaker until lysis occurs (~8 hours).

5. Add a few drops of chloroform and mix gently.

6. Carefully decant into 30 mL Corex tube leaving chloroform behind.

7. Centrifuge at 10000 RPM, 10 min, 4˚C.

8. Transfer supernatant to 50 mL Falcon tube.

9. Add 12.5 µL 10 mg/mL RNase
   2.5 µL 10 mg/mL DNase

10. Incubate at 37˚C for 1 hour.

11. Transfer to SW-28 ultracentrifuge tube.

12. Add NZC media to fill tube to prevent collapse during spin.

13. Centrifuge at 25000 RPM, 90 min, 4˚C.

14. Discard supernatant. Resuspend pellet in 100 µL 50 mM Tris-Cl, pH8.0.

15. Transfer sample to eppendorf tube.

16. Phenol extract well with 100 µL buffered phenol, twice. Note: for effective extraction, tape eppendorf tube to bottom of bacterial shaker for 20 min each extraction.

17. Chloroform extraction with 100 µL 24:1 chloroform:isoamyl alcohol.

18. Transfer aqueous layer to fresh eppendorf.

19. Add 10 µL 3M NaOAC
    200 µL cold ethanol

20. Microfuge for 10 min, 4˚C.

21. Wash pellet with 70% ethanol

22. Resuspend pellet in 100 µL TE.

23. Use 3 µL for restriction digests.