

## Phage Screen

### Titerting Phage

1. Make serial dilutions of phage in SM buffer (store O/N at 4oC)
2. Mix 1ul  $10^{-2}$  thru  $10^{-7}$  dilutions with 200ul BB4 cells
3. Incubate at 37oC for 15 minutes with shaking
4. Add 3.5mL top agar
5. Pour on NZCYM plates
6. Incubate at 42oC (~5 hours) or 37oC (~8 hours) [titerting plates can grow O/N at 37oC if necessary. Just make sure one dilution has plaques that can be counted.]
7. Count plaques and average to find number of plaques per a particular dilution
8. Average # of plaques x dilution factor = pfu/ul original stock

### Primary Screen:

1. Calculate the amount of phage needed to plate 70,000 pfu's per each of 10 to 12 large plates
2. Plate on large NZCYM plates
3. Incubate at 42oC (~5 hours) or 37oC (~8 hours)
4. Chill at 4oC for 2 hours to overnight
5. Lift plaques on nitrocellulose (leave filters on plates for two minutes; mark plates asymmetrically)
6. Incubate filters in denaturing buffer for 2 minutes
7. Incubate filters in neutralizing buffer for 5 minutes
8. Wash filters in rinse buffer no more than 30 seconds
9. Crosslink on autocrosslinking setting
10. Hybridize
11. Pick positive colonies off plates
12. Resuspend in 500ul SM buffer
13. Vortex several times
14. Store at 4oC

### Secondary Screen

1. Plate  $10^{-3}$  and  $10^{-4}$  dilutions on small NZCYM plates
2. Follow rest of protocol for primary screen

### Tertiary Screen

1. Plate  $10^{-2}$  and  $10^{-3}$  dilutions on small NZCYM plates
2. Follow rest of protocol for 1? screen

## **Plating Protocol**

Small plates :

1. Mix 1 ul phage and 200ul BB4 cells
2. Incubate at 37oC for 15 min
3. Add 3.5mL top agar
4. Pour on NZCYM plates

Large plates :

1. Mix phage and 600ul BB4 cells
2. Incubate at 37oC for 15 min
3. Add 7.5mL top agar
4. Pour on NZCYM plates

## **Hybridization**

1. Pre-hybe at 60oC for 15 minutes
2. Boil 100 ul salmon sperm DNA and probe for 5 minutes
3. Hybridize at 60oC for 4 hours to O/N
4. Wash with 2xSSC/0.1% SDS 2x15 minutes at 58oC
5. Wash with 0.1xSSC/0.1%SDS 1x30 minutes at 58oC
6. Expose to film

## **BB4 and XL-1 Blue Cell Cultures**

1. Inoculate LB with 10mM MgSO<sub>4</sub> and 10% maltose with cells (late in the day)
2. Grow O/N at 27oC
3. Spin down at 1000xg
4. Resuspend in 10mM MgSO<sub>4</sub> to OD<sub>600</sub>=0.6
5. Store at 4oC
6. Good for 1-2 weeks

## **Zap-Out**

1. Mix:           250 ul BB4  
                  200 ul phage in SM buffer  
                  1.0 ul RO48 Helper Phage
2. Incubate at 37oC for 15 minutes
3. Add 3mL LB
4. Incubate at 37oC 3 hours to O/N
5. Heat to 65oC for 20 minutes
6. Spin at 1000x g for 15 minutes

7. Pour phage sup into new 15mL tube
8. Mix 10ul phage sup and 200ul XL-1 Blue cells
9. Incubate at 37oC for 15 minutes
10. Plate 50 and 200ul cell mixture on LB/Amp plates
11. Grow at 37oC O/N