Phage Display Library

Amplification of the VCSM13 Helper Phage

N.B. The infective titer of the final supernatant should be between 10^{11} and 10^{12} plaque-forming units per milliliter (pfu/mL).

1. Inoculate a single colony of *Escherichia coli* XL1-Blue (or TG1) into 3mL LB + appropriate antibiotic and grow overnight at 37°C, 200rpm.
2. Inoculate the freshly grown culture into 10mL LB (ratio=1:100)+ antibiotic and grow at 37°C until an OD_{600} of 0.3 has been reached.
3. Add VCSM13 helper phage at a multiplicity of infection (MOI) of 20:1 (phage-to-cell ratio) and incubate at 37°C for 30 minutes.
4. Add kanamycin to the solution until a final concentration of 70 µg/mL has been reached and continue growth for 8 hours in 37°C.
5. Incubate at 65°C for 15 minutes and centrifuge the culture at 4,600x g for 15 min.
6. Aliquot the supernatant into new tubes, titer and store at 4°C.
7. Add DMSO to a final concentration of 7% (v/v) for storage at -80°C.

Titration of the VCSM13 Helper Phage

1. Inoculate a single colony of *Escherichia coli* strain XL1-Blue into 10mL LB + appropriate antibiotic in a 100mL flask and grow the culture at 37°C for approx. 6 hours (OD_{600} may not exceed 1.0).
2. Prepare dilutions of the VCSM13 helper phage (...) in 20mM TRIS-HCl pH8 and combine 1µL of each dilution with 200µL XL1-Blue cells.
3. Incubate the helper phage-XL1-Blue cells for 15 minutes at 37°C.
4. Add 3mL of NZY top agar, melted and cooled down to ~48°C and plate this liquid mixture upon a pre-existing, pre-warmed NZY agar plates. Incubate these for 10 minutes at room temperature.
5. Subsequently invert the plates and move to the 37°C stove. Incubate overnight.
6. Determine the titer, using the following formula:

$$\left(\frac{Number\ of\ plaques\ (pfu) \times Dilution\ factor}{Volume\ plated\ (\ L)} \right) \times 1{,}000\,\mu L\ /\ mL$$

Production of Phages Displaying the POI

1. Inoculate a single colony of *Escherichia coli* strain XL1-Blue harbouring the phagemid of interest into 3mL 2xYT, supplemented with 1% (w/v) glucose and appropriate antibiotic. Grow overnight at 37°C.
2. Inoculate the fresh culture (1:200) into 5mL 2xYT, supplemented with 1% (w/v) glucose and antibiotics in a 50mL flask. Incubate this flask in 37°C until an OD_{600} of 0.5-0.6 has been reached.
3. Add the VCSM13 helper phage at an MOI of 20. Incubate at 37°C for 30 minutes without shaking and subsequently at the same temperature, with shaking.
4. Spin the culture down at 3,300x g for 20 minutes, at room temperature. Discard the supernatant and resuspend the cell pellet in 50mL 2xYT, supplemented with the appropriate antibiotics and 0.25mM isopropyl-β-d-thiogalactoside (IPTG).
5. Transfer the cell suspension to a 250mL, conical flask and grow overnight at 30°C.

PEG/NaCl Precipitation of Phages

1. From the *'Production of Phages Displaying the POI'* end culture, centrifuge the culture at 4,600x g at 4°C for 10 minutes.
2. Transfer the supernatant to a 50mL tube, containing 2gr of PEG6000 and 1.5gr of NaCl. Dissolve the components in the supernatant and incubate on ice for 2 hours.
3. Precipitate the phages by centrifugation at 4,600x g at 4°C for 20 minutes again and resuspend the phage pellet in 1.5mL 20mM TRIS-HCl pH8.
4. Remove residual debris by centrifugation at 16,000x g, 4°C for 10 minutes. Transfer the supernatant to a clean Eppendorf.
5. Add 400µL 20% (w/v) PEG6000 and 2.5M NaCl solution to the dissolved phages, mix well and incubate on ice for 2 hours.
6. Precipitate the phages by centrifugation again (16,000x g, 4°C, 10 minutes), discard the supernatant and resuspend the pellet in 250µL 20mM TRIS-HCl pH8. Store phages directly at 4°C, or add DMSO to a final concentration of 7% (v/v) for storage at -80°C.

Infective Titer of Phage Particles

1. Inoculate a single colony of *Escherichia coli* XL1-Blue into 10mL LB + appropriate antibiotics in a 100mL flask and grow the culture at 37°C for approx. 6 hours (OD_{600} may not exceed 1.0).
2. Prepare the serial dilutions of the produced phages in 20mM TRIS-HCl pH8 and combine 10µL of the 109-1012 dilutions with 100µL of XL1-Blue cells.
3. Incubate the phages and the XL1-Blue cells for 15 minutes at 37°C.
4. Plate each dilution on LB agar (with antibiotics) plates and incubate overnight at 37°C.
5. To determine the infective titer (CFU/mL), use the following formula:

$$Colony\ forming\ units\ per\ mL = \left(\frac{Number\ of\ colonies\ (cfu) \times Dilution\ factor}{Volume\ plated\ (\ \ L)} \right)$$

Detection of POI Display by Western Blot Analysis

1. Run a 10% SDS-PAGE gel with the prepared phage samples (1012 phage particles/lane) according to standard protocol.
2. Soak the SDS-PAGE gel for a minimum of 5 minutes in Transfer buffer.
3. Soak a PVDF membrane for 5 minutes in 100% methanol and then for 5 minutes in transfer buffer.
4. Soak the transfer pads and Whatmann paper in transfer buffer.
5. Assemble the cassette, starting on the black side:

transfer pad>Whatmann paper>gel>pre-wetted PVDF membrane>2x Whatmann papers>transfer pad

6. Transfer to the PVDF membrane by electro-blotting overnight in 10mM CAPS, pH11 and 10% (v/v) methanol at constant 10V using a tank transfer system.
7. Block the membrane for 1 hour in TBSTG.
8. Incubate the membrane for 1 hour with anti-POI antiserum in TBSTG (1:1,000 as a starting point, exact dilution value must be determined for each antiserum).
9. Wash three times with TBST, 15 minutes for each step.
10. Wash once with alkaline phosphatase buffer, for 5 minutes.
11. Detect proteins with substrates BCIP and NBT, in alkaline phosphatase buffer.
12. Further develop the blot until the desired intensity has been achieved.

Amplification of Eluted Phages

1. Add the eluted phages to 5mL XL-1Blue cells with an OD_{600} of 0.6.
2. Incubate 30 minutes at 37°C, without shaking.
3. Transfer this mix to 40mL 2YT-AG with 10E10 Helper phage.
4. Incubate 2 hours for 37°C, with shaking at 200rpm.
5. Harvest cells at 3,300x g for 10 minutes, discard the supernatant and add 40mL 2YT-AK.
6. Incubate overnight at 30°C.

Phage Display

1. Add 5mL Blocking Buffer, TBS-T 0.1% + 0.1% gelatin for 1 hour.
2. Wash 0.5mL beads with 5 mL TBS-T 0.1%. Repeat two more times.
3. Add 500µL phage stock in 5mL TBS-T 0.1% to the beads and incubate for 1 hour at room temperature on a roller bank.
4. Spin down the beads for 10 minutes at 3,300x g.
5. Wash with TBS-T 0.1-0.4%. Repeat two more times.
6. Elute phages by adding 500µL of 50mM glycine-HCl and incubate for 10 minutes.
7. Neutralize the reaction by adding 50µL of 1M TRIS-HCl, pH8.0 and 450µL PBS.