Electronic Supplementary Material (ESI) for Natural Product Reports.

SUPPLEMENTARY MATERIAL

Identifying the cellular targets of natural products using T7 phage display

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Equipment Required

- Freezers (–80 °C, –20 °C) and refrigerator (4 °C)
- Incubator with orbital shaker (37 °C)
- Spectrophotometer (OD 600 nm)
- Refrigerated laboratory centrifuge (4 °C; tubes and plates)
- Thermocycler (PCR)
- pH meter
- Microwave oven
- Ice maker
- Temperature-controlled water bath (50 °C)
- DNA electrophoresis power supply
- DNA gel tank (7 cm and 15 cm wide)
- DNA gel trays (7 × 10 cm and 15 × 10 cm) and combs
- UV transilluminator with camera
- Single-channel micropipettors (0.1 μL to 1 mL)
- Multi-channel micropipettors (1 μL to 200 μL)
- Electronic serological pipette filler
- Access to DNA sequencing facility

Disposable Plasticware

- Sterile PS Petri plates (100 × 15 mm) with lids
- 50 / 250 mL sterile PP centrifuge tubes with screw caps
- 10 mL PS culture tubes with press-seal lids
- 1.5 mL and 0.5 mL sterile PP microcentrifuge tubes
- 10 μL, 200 μL and 1 mL sterile micropipette barrier tips
- 96-well sterile untreated PS microtitre plates with lids
- 96-well flexible vinyl (PVC) microtitre plates
- 96-well thin-walled PCR tube plates with caps or mats
- 8-well thin-walled PCR tube strips with caps
- 1 mL to 100 mL sterile PS serological pipettes
- 20 mL sterile PP Luer-tip syringes
- 0.22 μm sterile syringe filter units

Reagents and Kits

- T7Select10-3b phage display library (Novagen)
- *Escherichia coli* strain BLT5615 (Novagen)
- NeutrAvidin-coated 8-well strip plates (Pierce)
- Tryptone, Yeast Extract, Agar (Bacto)
- Agarose
- Carbenicillin disodium salt
- Isopropyl β-D-1-thiogalactopyranoside (IPTG)
- Taq DNA polymerase (Qiagen)
- T7-FWD primer (5′-TCTTCGCCCAGAAGCTGCAG-3′)
- T7-REV primer (5′-CCTCCTTTCAGCAAAAAACCCC-3′)
- Deoxynucleotide triphosphates (dATP, dTTP, dGTP, dCTP)
- *Hin*fI restriction endonuclease (New England Biolabs)
- Nucleic acid stain (e.g. ethidium bromide, GelRed, SYBRsafe etc.)
- Super-fine agarose for DNA electrophoresis
- DNA ladder (50 bp to 2000 bp)
- QIAquick PCR purification kit (Qiagen)
- Glucose, sucrose
- Bromophenol blue
- Tween 20
- Sodium dodecyl sulfate (SDS)
- Tris, acetic acid, EDTA disodium, glycerol
- KH_2PO_4, $Na_2HPO_4.12H_2O$, NH_4Cl, KCl, NaCl, $MgCl_2$
- Ultrapure water
- Nuclease-free water

Table S1. Reagent preparation

Reagent	Ingredients	Instructions
20×M9 salts	20 g NH_4Cl 60 g KH_2PO_4 120 g $Na_2HPO_4.12H_2O$ Water to make 1 L of solution	Adjust pH to 7.4 Autoclave 20 min / 121 °C
20% Glucose	200 g glucose Water to make 1 L of solution	Sterile filter (0.22 μm) into sterile bottle
5% Carbenicillin	1 g carbenicillin disodium Water to make 20 mL of solution	Sterile filter (0.22 μm) Store at –20 °C in 1 mL aliquots
M9TB	9.28 g tryptone 4.64 g NaCl 50 mL 20×M9 salts 1 mL $MgCl_2$ (1 M) Water to make 1 L of solution	Autoclave 20 min / 121 °C Cool to room temperature Add 20 mL sterile 20% glucose Add 1 mL sterile 5% carbenicillin
2×YT	17 g tryptone 10 g yeast extract 5 g NaCl Water to make 1 L of solution	Adjust pH to 7.4 Autoclave 20 min / 121 °C
24% IPTG	2.4 g isopropyl β-D-1-thiogalactopyranoside (IPTG) Water to make 10 mL of solution	Sterile filter (0.22 μm) Store at –20 °C in 1 mL aliquots
80% Glycerol	80 mL glycerol Water to make 100 mL of solution	Autoclave 20 min / 121 °C
1% SDS	1 g sodium dodecyl sulfate Water to make 100 mL of solution	Sterile filter (0.22 μm)
1% Tween 20	1 mL Tween 20 Water to make 100 mL of solution	Sterile filter (0.22 μm)
Phosphate Buffered Saline (PBS)	8 g NaCl 0.2 g KCl 1.44 g Na_2HPO_4 0.24 g KH_2PO_4 Water to make 1 L of solution	Adjust pH to 7.4 Autoclave 20 min / 121 °C
Phage Wash Buffer (PWB)	0.5 mL Tween 20 999.5 mL PBS	Adjust pH to 7.4 Autoclave 20 min / 121 °C
LB Agar	10 g tryptone 5 g yeast extract 10 g NaCl 15 g agar Water to make 1 L of solution	Adjust pH to 7.4 Autoclave 20 min / 121 °C Cool to 50 °C in a water bath Add 1 mL sterile 5% carbenicillin Pour plates (15 mL) and store at 4 °C
LB Agarose	10 g tryptone 5.2 g yeast extract 5.2 g NaCl 6 g agarose Water to make 1 L of solution	Adjust pH to 7.4 Autoclave 20 min / 121 °C Cool to 50 °C in a water bath Add 1 mL sterile 5% carbenicillin Store at 4 °C in 30 mL aliquots
50×TAE	242 g Tris 57.1 mL acetic acid 18.6 g EDTA disodium salt Water to make 1 L of solution	Adjust pH to 8.0 Autoclave 20 min / 121 °C
1×TAE	20 mL 50×TAE Water to make 1 L of solution	Use immediately
0.5% Ethidium bromide (10000×)	5 mg ethidium bromide 1 mL water	Caution: suspected mutagen – avoid contact Store in unbreakable opaque plastic bottle
PCR Master Mix	10 μL T7-UP Primer (10 μM) 10 μL T7-DOWN Primer (10 μM) 20 μL dNTPs (10 mM each dNTP) 100 μL Taq buffer (10×) 835 μL nuclease-free water	Store frozen at –20 °C Defrost and add 5 μL Taq DNA polymerase before use Add 1 μL phage lysate to 49 μL PCR master mix
DNA Fingerprinting Mix	10 μL *Hin*fI enzyme (1000 U/mL) 50 μL NEB Buffer 2 (10×) 240 μL nuclease free water	Store at 4 °C for up to 1 week
DNA Loading Buffer (6×)	4 g sucrose 10 mg bromophenol blue Water to make 10 mL	Sterile filter (0.22 μm) Store at 4 °C

TIPS: Uninoculated agar Petri plates can be stored lid-side down at 4 °C for at least one month. While 10 mL of agar is sufficient for plates being used immediately, slightly thicker plates (15–20 mL) will not dry out as quickly and will last longer at 4 °C. Bubbles formed when pouring agar plates can be eliminated with a quick blast from the flame of a Bunsen burner while the agar is still molten.

Preparation of Microtitre Strip Plates

1. Prepare a stock solution (1 μmol/mL in DMSO) of both the biotinylated control probe and the biotinylated affinity probe (natural product). Perform a 1/100 dilution of each stock solution with PBS to give 10 nmol/mL working concentrations (1% DMSO).

2. Add 250 μL PBS to each well of two NeutrAvidin-coated 8-well strips and incubate at room temperature for 1 h. Discard PBS from all wells.

3. Add 200 μL of the 10 nmol/mL biotinylated control compound solution to each well of the first 8-well strip and 200 μL of the 10 nmol/mL biotinylated affinity probe (natural product) solution to each well of the second 8-well strip. Incubate at room temperature for 1 h.

4. Discard solutions and wash every well with PBS (5 × 250 μL). Store strips at 4 °C until required.

TIPS: NeutrAvidin-coated 8-well strips can be easily snapped into individual wells to provide spatial separation of different experiments, although finding a suitable rack to hold the tiny wells can be challenging. Experiment with different tip boxes and tube racks until a suitable holder is found.

5. *E. coli* strain BLT5615 is provided as a 10% glycerol stock that must be stored at –80 °C. Prepare an initial culture of BLT5615 by streaking a small quantity of the frozen stock across the entire surface of an LB agar plate with a sterile loop. Seal the plate with Parafilm and incubate lid-side down at 37 °C overnight or until bacterial colonies are clearly visible.

6. Store the plate at 4 °C for up to 3 weeks.

TIPS: It is not necessary to defrost the stock solution of BLT5615 before use – just dip the sterile loop directly into the surface of the frozen stock to avoid repeated freeze-thaw cycles. Streaking the agar plate should yield well-isolated individual colonies. If the initial culture is overgrown with bacteria, repeat the process using a streak plate dilution method to achieve well-separated individual colonies.

Growth of T7 Lysates

TIPS: T7 phages are hardy and can remain infectious on surfaces for many months. Always use disposable plasticware for all steps and avoid generating aerosols. Autoclaving is not effective for inactivating phages and contaminated solutions should be treated with sodium hypochlorite (1% final concentration) for at least 10 min prior to disposal. Decontaminate benchtops and micropipettors regularly with 1% sodium hypochlorite and biosafety cabinets with UV light. Dilute solutions of sodium hypochlorite decompose slowly, so fresh solutions should be prepared every 4 weeks.

7. Inoculate an aliquot of M9TB medium (20 mL in a 50-mL centrifuge tube) with a single colony of *E. coli* BLT5615 from the initial culture plate. Replace the tube lid loosely and use adhesive tape to stop the lid from falling off or tightening during swirling. Incubate at 37 °C overnight with gentle swirling (120 rpm).

8. The next morning, inoculate two fresh aliquots of M9TB medium (100 mL in 250-mL centrifuge tubes) each with 5 mL of the saturated overnight culture of *E. coli* BLT5615. Replace the lids loosely and incubate at 37 °C with vigorous swirling (200 rpm). Monitor the optical density of the cultures (600 nm) at regular intervals using a spectrophotometer and continue incubation until the OD_{600} reaches 0.4 (approx. 2–3 h).

9. Add 100 μL of 24% IPTG to each culture to induce production of the T7 phage coat protein (gp10A) and continue incubation for exactly 30 min.

10. Place one culture on slushy ice for later use. IPTG-treated cells can be stored on ice for 12 h.

11. Inoculate the second culture with 1 μL of a T7Select library. Continue incubation at 37 °C with vigorous swirling (200 rpm) until bacterial cell lysis occurs (approx. 2 h). Cell lysis results in the culture transitioning from cloudy to almost completely clear over a 3–5 min period.

12. Once lysis is complete, immediately cool the culture on slushy ice. Centrifuge at full speed for 10 min at 4 °C to precipitate cellular debris. Carefully decant the supernatant into a clean 50-mL centrifuge tube and add 100 μL of 1% Tween 20. Store on slushy ice until required (use as soon as possible).

13. For long-term archival storage, combine 90 μL of clarified phage lysate and 10 μL of 80% glycerol in a sterile 0.5-mL microcentrifuge tube. Cap the tube and flick gently to mix. Label the tube as “Round 0” and store at –80 °C.

TIPS: While T7 phages are robust, the proteins displayed on their surfaces are not. It is important to stop incubation immediately after bacterial cell lysis occurs to minimise degradation of displayed proteins, so inspect the cultures regularly. The addition of a protease inhibitor cocktail after lysis may be worth considering, although this may cause problems if the cellular target of the natural product turns out to be a protease. T7 phage lysate for biopanning experiments should be used as soon as possible after centrifugation.

Biopanning: Round 1

TIPS: Biopanning multiple T7 phage libraries at the same time can become quite hectic and it is important to be well organised to avoid mistakes. Physically separating different experiments to different parts of the bench or incubator can help to minimise human errors and prevent accidental cross-contamination of phage lysates. Using different coloured labels or bottle caps for each different reagent can also reduce the chance of grabbing the wrong bottle in the heat of the moment.

14. Add 200 μL of clarified phage lysate to one well of the biotinylated control compound strip plate. Cover with polyethylene film and incubate at room temperature for 1 h to eliminate non-specific binders.

15. Transfer the phage lysate from the control well to one well of the biotinylated affinity probe (natural product) strip plate. Cover with polyethylene film and incubate at room temperature for 2 h.

16. Discard phage lysate into an appropriate biological waste container and quickly wash the well with PWB (1 × 250 μL for 5 s).

17. Add 100 μL of 1% SDS to the well. Cover with polyethylene film and incubate at room temperature for 30 min to elute bound phages.

18. Transfer the entire SDS eluate to a clean 1.5-mL microcentrifuge tube and dilute with 900 μL 2×YT. Label this tube "Round 1 – 0.1×". Store at 4 °C until required (short periods only).

TIPS: This is an appropriate place to pause the biopanning experiments if required. Two rounds of biopanning can be comfortably performed in one day with good planning and organisation. Remember to inoculate a fresh aliquot of M9TB with *E. coli* BLT5615 for the following day (see Step 7). SDS will usually crystallise as lustrous needles from the 0.1× solutions when left overnight at 4 °C. These crystals do not cause any problems and need not be removed. Simply pipette off the supernatant carefully without disturbing the crystals.

Biopanning: Subsequent Rounds

19. Add 200 μL of the 0.1× phage solution from the previous round to 20 mL of IPTG-treated *E. coli* BLT5615 cells (either freshly prepared from a saturated overnight culture, or using the previously prepared IPTG-treated cells stored on slushy ice).

20. Incubate the culture at 37 °C with vigorous swirling (200 rpm) until bacterial cell lysis occurs.

21. Repeat Steps 12–20 for each round of biopanning. Generally, 5 or 6 rounds are sufficient for the library to converge to a manageable number of clones.

TIPS: As the phage titre increases with each round of biopanning, the volume of 0.1× 2×YT solution used to inoculate the BLT5615 cells should be progressively reduced to keep lysis times above 1 h. Likewise, the stringency of the washing step (number of washes and total washing time) should be progressively increased with each round of biopanning. In the initial T7 library, there will only be a relatively small number of clones displaying proteins capable of binding to the probe, so washing too aggressively at the start may result in all target phages being lost.

Titring T7 Lysates

22. Pre-warm an LB agar plate at 37 °C for 30 min.

23. Completely loosen the lid of a 50-mL centrifuge tube containing 30 mL of LB agarose and place tube in a Pyrex beaker. Heat the tube in a microwave oven on low power until completely molten.

24. Tighten lid and invert tube several times to mix thoroughly (Caution – Hot!).

25. Place tube in a 50 °C water bath and leave for 30 min to equilibrate.

26. Decant 5 mL of the 50 °C LB agarose into a clean 10-mL culture tube. Working quickly, add 250 μL of IPTG-treated *E. coli* BLT5615 cells and 5 μL of 24% IPTG. Invert the tube several times and immediately pour onto the surface of the pre-warmed LB agar plate. Rotate the plate to ensure the agarose forms an even layer on the surface of the agar.

27. Leave the plate in a biosafety cabinet or laminar flow hood with the lid removed until the agarose has set and the surface is dry (approx. 45 min).

28. While the agarose plate is drying, add 100 μL of the 0.1× phage solution from each round of biopanning to the first column of a flexible 96-well PVC assay plate. Using a multichannel micropipette, transfer 10 μL of each solution from the first column to the second column and add 90 μL of 2×YT to each well to generate a 10^{-2} dilution. Continue performing 10-fold dilutions with 2×YT from 10^{-2} to 10^{-8} using clean pipette tips for each dilution.

TIPS: To minimise micropipette tip usage, the same tips can be used to very gently add 2×YT to each serial dilution from 1 cm above the plate wells. However, care must be taken not to contaminate these tips or splash phage lysate from the wells.

29. Using a multichannel pipettor, carefully transfer 1 μL of each phage dilution to the surface of the solidified agarose plate to form an 8 × 6 array, being careful not to overlap spots.

TIPS: To prevent smearing, it is essential that the surface of the top agarose is completely dry before applying the phage lysate. For best results, avoid touching the surface of the agarose with the pipette tips – allow the surface tension of the drops to pull them onto the agarose surface

30. Leave the plate in a biosafety cabinet or laminar flow hood with the lid removed until the phage lysate has absorbed into the agarose (approx. 10 min).

TIPS: Make a mark on the side of the plate to indicate orientation of the array and label the lid with the experiment details. Do not mark the bottom of the plate as this will obscure the plaques.

31. Replace the lid and incubate plate at 37 °C until small clear areas (plaques) are clearly visible against the background lawn of bacteria (approx. 3 h; Manuscript Figure 2). Do not incubate the plate too long or the plaques will grow too large and begin overlapping each other.

32. Identify the dilution for each round of biopanning that contains a countable number of plaques (usually 5–50). Count the number of plaques visible in this dilution, then back-calculate the phage titre in plaque-forming units per millilitre (pfu/mL), taking into account the volume of lysate used (1 μL) and the dilution factor.

TIPS: For the more senior members of the laboratory, a hand lens can be useful to help count the plaques.

33. Plot the titre vs selection round. Generally, a sigmoidal plot can be fitted to the data, with a large increase in phage titre indicative of a successful selection. A titre that gradually decreases is indicative of a failed experiment.

Picking Plaques

34. Pre-warm an LB agar plate and melt some LB agarose as described in Steps 23–27.

35. Identify the 2×YT dilution from the previous titring experiment that yielded a countable number of plaques (5–50 in 1 μL) and calculate the volume required to give ~250 plaques (5–50 μL).

36. Decant 5 mL of the 50 °C LB agarose into a clean 10-mL culture tube. Working quickly, add 250 μL of IPTG-treated *E. coli* BLT5615 cells, 5 μL of 24% IPTG and the volume of 2×YT phage dilution identified above. Invert the tube several times and immediately pour onto surface of the pre-warmed LB agar plate. Rotate the plate to ensure agarose forms an even layer on the surface of the agar.

37. Leave the plate in a biosafety cabinet or laminar flow hood with the lid removed until the agarose has set (approx. 15 min). Replace the lid and incubate plate at 37 °C until plaques are clearly visible against the background lawn of bacteria (approx. 3 h).

TIPS: Plates containing T7 plaques can be stored at 4 °C for short periods of time. However, bacterial cell lysis will still continue (albeit more slowly) and eventually the plaques will begin overlapping.

38. Add 100 μL of IPTG-treated *E. coli* BLT5615 cells to each well of a sterile 96-well PS microtitre plate.

39. Using a hand-held 10-μL micropipette tip, stab the centre of one plaque from the agarose plate and transfer the tip to the first well of the 96-well plate containing BLT5616. Swirl gently to mix. Continue transferring plaques from the agarose plate to the 96-well plate until 95 plaques have been transferred. Leave one well uninfected as a control.

TIPS: T7 phages displaying larger proteins tend to grow more slowly than those displaying smaller proteins, or no proteins at all ("überphage"). When picking plaques, aim for the smaller ones as these are more likely to display intact proteins. When transferring plaques to the 96-well plate, leave the previous 10-μL micropipette tip in the plate until the next plaque is ready to be transferred as a marker to indicate the next empty well to infect.

40. Place a lid on plate, seal with Parafilm, and incubate at 37 °C until all bacterial cells have lysed (approx. 2 h).

41. Centrifuge plate at full speed for 10 min at 4 °C to precipitate cellular debris.

42. Using a multichannel pipettor, carefully transfer 45 μL of the supernatant from each well to a clean sterile 96-well PS microtitre plate containing 5 μL of 80% glycerol per well.

43. Store plate at –80 °C (long-term storage).

TIPS: Certain 96-well plates can crack or completely fail when centrifuged. A rubber mat placed under the plates can help prevent cracking, but be careful to balance these correctly. A slower centrifuge speed may also be required.

Amplifying DNA

44. Combine 1 μL of phage lysate (either from T7 phage sublibrary or from single T7 phage plaque) with 49 μL of PCR Master Mix in a PCR tube, PCR strip or PCR plate.

45. Perform 35 rounds of PCR using the thermocycler program indicated in Table S2.

46. Store the amplified DNA at 4 °C until required.

Table S2. Standard thermocycler program for PCR of T7 phage DNA inserts

Cycles	Temperature	Time
1	94 °C	150 s
35	94 °C	45 s
	55 °C	60 s
	72 °C	30 s
1	72 °C	10 min
1	4 °C	∞

TIPS: PCR amplification of DNA inserts from T7Select libraries using Qiagen Taq DNA polymerase is very reproducible and rarely fails. There is no need to use additional $MgCl_2$/Q-solution or any other additives.

Gel Electrophoresis of T7 Sublibraries

47. In a Pyrex conical flask, suspend 0.7 g of high resolution super-fine agarose in 40 mL of 1× TAE buffer. Microwave the suspension on high until boiling. Remove from oven (Caution: Hot!) and swirl until fully dissolved, ensuring no solid remains on the side of the flask. Stand the 1.75% agarose solution in a 50 °C water bath until required.

48. In a 50-mL centrifuge tube, carefully add 4 μL of 10000× ethidium bromide solution (Caution: suspected mutagen) or other DNA stain. Working quickly, add 40 mL of 50 °C agarose solution, cap the tube and invert several times to mix thoroughly, making sure there are no lumps in the solution. Immediately pour the agarose into a 7×10 cm casting tray containing two rows of well combs (15 wells per comb) and leave to set (approx. 45 min). Transfer the gel to an electrophoresis gel tank and flood with 1× TAE. Carefully remove the well combs.

TIPS: If using a casting dam, gently rock the gel tray from side to side to break the seal at each end before retracting the movable dam wall to prevent the gel from tearing along the perforated line of wells. Make sure the top of the gel is flooded with buffer before removing the combs to prevent damage to the wells. Thin combs (0.75 mm) are easier to remove from the agarose and tend to produce sharper bands than thick combs (1.5 mm). Always use the same bottle/batch of buffer for both the gel and the gel tank.

49. Add 5 μL of DNA Ladder to the leftmost and rightmost wells of both rows of the DNA gel.

50. On a strip of Parafilm, place a well separated array of 1-μL dots of 6× DNA Loading Buffer. Mix each dot with 5 μL of amplified DNA and pipette up and down to mix. Transfer the entire solution to the DNA gel.

51. Once all wells have been loaded, run the gel at 150 V for 1 min to move the DNA quickly into the gel, then turn voltage down to 80 V and run until the loading dye runs almost to the end of the gel (approx. 30 min).

52. Remove the gel and visualise DNA using a transilluminator (Manuscript Figure 3).

TIPS: Adding gel stain to the molten agarose is the most convenient method of visualising the DNA, although the stain will migrate in the opposite direction to the DNA and may yield uneven illumination. For publication-quality images, the gel should be stained and destained after performing the separation.

Gel Electrophoresis of T7 Plaques

53. Repeat Steps 47-48 using 1.6 g of high resolution super-fine agarose, 80 mL of 1× TAE buffer and 8 μL of 10000× DNA stain to yield a 2% agarose gel in a 15 × 10 cm casting tray containing two rows of well combs (26 or 30 wells per comb).

TIPS: Digesting an aliquot of the DNA from each individual phage plaque with a frequent cutting restriction endonuclease allows a unique DNA fingerprint to be generated for each clone. This provides a rapid and cost-effective method of determining whether two clones contain the same DNA insert, closely related inserts or completely different inserts, thereby minimising the number of DNA sequencing reactions required. The restriction endonuclease *Hin*fI, which cuts DNA at the sequence G|ANTC, is robust, relatively inexpensive and produces a suitable number of fragments from cDNA inserts in the 100–1000 bp size range.

54. In a 96-well PCR plate, combine 2 μL of each amplified DNA solution with 3 μL of DNA Fingerprinting Solution. Incubate plate in a water bath at 37 °C for 1 h. Add 1 μL of 6× DNA Loading Buffer to each well of the plate.

55. In a flexible PVC 96-well assay plate, combine 1 μL of 6× DNA Loading Buffer, 3 μL of nuclease-free water and 2 μL of undigested amplified DNA from each T7 plaque.

56. Add 5 μL of DNA Ladder to the leftmost and rightmost wells of both rows of the DNA gel.

57. Add each undigested DNA solution to the top row of wells on the DNA gel and directly underneath add the corresponding *Hin*fI-digested DNA solutions to the bottom row.

58. Run the gel and visualise the DNA as described in Steps 51–52. (Manuscript Figure 4).

TIPS: To expedite loading the gels, well-combs are available that are compatible with the tip spacing of standard multichannel pipette. These are generally configured with two wells between each pipette tip, requiring samples to be loaded in a staggered arrangement. This can lead to confusion if care is not taken. Aligning the multichannel pipette can also be challenging when using the thin (0.75 mm) combs, and practise is recommended using non-critical samples.

DNA Sequencing

59. Purify 10 μL of DNA amplified from individual plaques using a QIAquick PCR purification kit, following the manufacturer's instructions. This will yield 30 μL of purified DNA from each plaque.

TIPS: For best DNA sequencing results, centrifuge the purified DNA for 5 min at maximum speed to precipitate any stray resin particles from the DNA purification column and carefully transfer the supernatant to a clean tube.

60. Combine 8 μL of purified DNA and 4 μL of T7-FWD primer (1 μM) and submit for DNA sequencing. For large DNA inserts, repeat using the T7-REV primer to ensure the entire insert is sequenced.

TIPS: The T7SelectUP and T7SelectDOWN primers supplied by Novagen anneal too close to the restriction sites used for cloning the cDNA libraries. Consequently, these parts of the DNA sequence can become obscured by the early-eluting DNA sequencing dye peaks, making it difficult or impossible to determine the reading frame of the inserted fragments. The T7-FWD and T7-REV primers we use anneal further back from the cloning sites and do not suffer from this problem.

61. Perform a BLAST nucleotide search on the DNA sequence to identify the DNA insert (http://blast.ncbi.nlm.nih.gov/)

62. Locate the restriction site near the beginning of the DNA sequence (**GAATTC** for *Eco*R1). The first codon in the same reading frame as the gp10A coat protein is **AAT**. Ensure the foreign DNA insert is in the same reading frame as the coat protein by counting in groups of three to the start codon (**ATG**). If the BLAST search indicates "Plus/Minus" in the strand column, this indicates that the sequence has been inserted into the T7 phage vector backwards. Also look for the stop codon(s) (**TAA**/**TGA**/**TAG**) in the correct reading frame to determine whether a full-length protein or a peptide fragment is being displayed and whether a leader sequence is being translated before the encoded protein (Manuscript Figure 5).