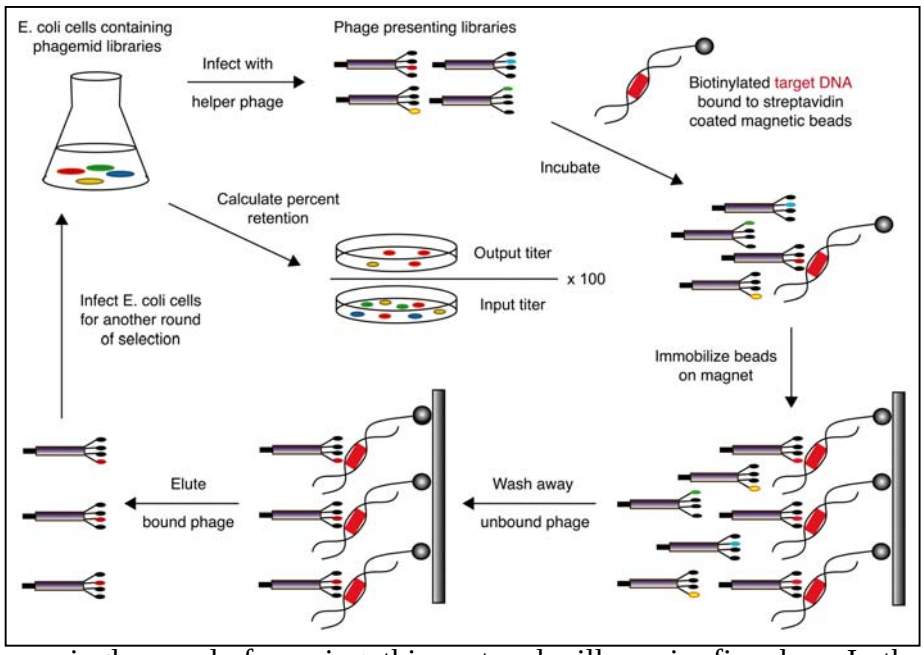
Phage Display: Panning Against DNA Targets

by Lori Yang

I. Experimental Strategy

The experimental strategy for panning of phage particles against DNA targets is outlined in the figure below. The phage particles are generated from the library in XL1-Blue cells upon infection with M13KO7 helper phage. The target DNA (for example: hsCRE, ATGAC) is biotinylated on one strand and can be immobilized using streptavidin-coated magnetic beads. The phage particles are then exposed to the DNA. Washing and elution of the beads isolates only the desired phage particles. These are then reinfected into XL1-Blue cells, serially diluted, and plated to determine the number of retained phage. For comparison, a sample of the phage particles without exposure to DNA are infected into XL1-Blue cells, serially diluted, and plated to determine the input titer. From the number of colonies, the percent retention of the phage particles on the DNA target site is determined. To isolate the phage particles with the highest specificity
and binding, multiple rounds of selection will be necessary.



For a single round of panning, this protocol will require five days. In the first and second days, all of the necessary solutions are prepared, the beads are washed and blocked, and the cell cultures are infected to produce phage particles. The following day, the biotinylated DNA is added to the beads, and the phage particles are isolated. The phage particles are mixed with the beads, then immobilized on a magnet. Washing and elution allows for the selection of high affinity binding phage particles. These selected phage particles are then reinfected into XL1-Blue cells and plated. On the final day, the plated cells are tallied to determine the retention percentages.The outlined procedure is effective when performing 4 assays. Typically, two of these can be positive and negative

controls. For example: pCANTAB-007 and pCANTAB-APP (or beads containing no DNA).

II. Solutions

1M NaOH

4.0 g NaOH (40 g/mol)
Add H_2O to 100 mL

2M $MgCl_2$

40.66 g $MgCl_2 \cdot H_20$ (203.30 g/mol)
Add H_2O to 100 mL and autoclave

LB-tet agar plates

1 g tryptone peptone
0.5 g yeast extract
1 g NaCl
1.5 g bacteriological grade agar
100 µL 1M NaOH
Add H_2O to 100 mL and autoclave
Cool to ~60˚C
Dissolve21.5 mg tet tablet
Pour in petri plates
Let solidify
Store upside down at 4˚C

40% glucose

200 g dextrose
Add H_2O to 500 mL
Heat to dissolve
0.2 µm filter
Store at 4˚C

1000X ampicillin (100 mg/mL)

1.0 g ampicillin
Add H_2O to 10 mL
0.2 µm filter
Store 1 mL and 100 µL aliquots at -20˚C

2X YT mix (in 1 L orange cap bottle)

255 g tryptone peptone
150 g yeast extract
75 g NaCl

2X YT (in 250 mL Erlenmeyer flask), 4

2.4 g 2X YT mix
Add H_2O to 75 mL and autoclave

2X YT (in 500 mL orange cap bottle), 2

16 g 2X YT mix
Add H_2O to 500 mL and autoclave

1000X kanamycin (50 mg/mL)

0.5 g kanamycin sulfate
Add H_2O to 10 mL
0.2 µm filter
Store 1 mL and 100 µL aliquots at -20˚C

1 µg/µL poly(dI-dC)•poly(dI-dC)

10 U poly(dI-dC)•poly(dI-dC)
Add H_2O to 500 µL
Store 100 µL aliquots at -20˚C

50% glycerol

100 mL glycerol
100 mL H_2O
Autoclave

1M Tris, pH 7.5

40.3 mL 1M Tris-HCl (157.60 g/mol)
9.7 mL 1M Tris base (121.14 g/mol)
or
6.06 g Tris base
Add H_2O
pH to 7.5 w/HCl
Add H_2O to 50 mL

0.5M EDTA, pH 8.0

186.12 g $Na_2EDTA \cdot 2H_2O$ (372.24 g/mol)
Add H_2O to 0.8 L
pH to 8.0 w/NaOH pellets
Add H_2O to 1 L
0.2 µm filter

2X B&W (binding and washing buffer)

5.84 g NaCl
0.5 mL 1M Tris, pH 7.5
0.1 mL 0.5M EDTA, pH 8.0
Add H_2O to 50 mL

SOB-AG agar plates

20 g tryptone peptone
5 g yeast extract
0.5 g NaCl
15 g bacteriological grade agar
Add H_2O to 1 L and autoclave
Cool to ~60˚C
Add 5 mL 2M $MgCl_2$
50 mL 40% glucose
1 mL 1000X ampicillin
Pour in petri plates
Let solidify
Store upside down at 4˚C

PEG/NaCl

40.0 g polyethylene glycol 8000
29.2 g NaCl (58.44 g/mol)
Add H_2O to 200 mL and autoclave

III. Protocol

– Day 1 –

2X YT-AG (in sterile flask)

200	mL	2X YT
200	µL	1000X ampicillin
10	mL	40% glucose

Store at 4˚C

Autoclave centrifuge tubes

Assays

pCANTAB-negative control
pCANTAB-positive control
pCANTAB-Library A
pCANTAB-Library B

Assay Starter Cultures

6:45pm - Add -control and +control glycerol stabs to 5 mL 2X YT-AG. Add Library A and Library B glycerol stocks (not pool 0) to 10 mL of 2X YT-AG in 50 mL orange cap tubes. Streak XL1-blue glycerol stock on LB-tet plate. Incubate at 37ºC.

– Day 2 –

6% nonfat milk in 1X B&W (in eppy)

60	mg	nonfat milk

Add 1X B&W to 1 mL

2X YT-AK

50	mL	2X YT
50	µL	1000X ampicillin
50	µL	1000X kanamycin

Buffer A

5.0	mL	10X PBS (-NaCl)
0.4	g	NaCl
0.25	mL	10% NP-40
0.1	mL	0.5M EDTA, pH 8.0
0.2	mL	100 mg/mL BSA

Add H_2O to 50 mL
0.2 µm filter; Store at 4˚C

3:35pm - Inoculate 13 mL of 2X YT-AG with 1.0 mL of starter culture (for pool 0, inoculate with glycerol stocks). Grow at 37°C to an OD_{600} of 0.8.

8:25pm - Add 400 µL of titered M13KO7 helper phage (~1 x 10^{11} pfu/mL) to each final **10 mL** growth.

8:30pm - Grow cell cultures for an additional hour at 37°C.

9:30pm - Spin cell cultures at 2500 rpm in the delicase centrifuge for 10 minutes. Decant the broth into bleach and resuspend the cells in 10 mL 2X YT-AK.

9:45pm - Incubate the now phage producing cells at 37°C for 12 hours.

Preparation of Beads (blocking)

For each assay, 0.5 mg of beads is necessary. Beads are provided in a 10 mg/mL solution from Dynal, therefore, for 4 assays the volume of beads would 200 µL. Minimum wash volume is 200 µL.

Wash the beads (1.0 vol. 200 µL) six times with 2X B&W (1.0 vol. 200 µL).

Each wash consists of 2 minutes rotating and 2 minutes on magnet.

8:15pm - Resuspend beads in 400 µL 6% nonfat milk in 1X B&W and rotate 14 hours.

XL1-blue Starter Culture

9:35pm - Pick XL1-blue colony from plate (Day 1). Add to 5 mL 2X YT. Incubate overnight at 37°C.

– Day 3 –

Isolation of Phage

9:45am - Spin the phage producing cell cultures (10 mL) at 2,500 rpm for 20

minutes. Get ice. Thaw dI-dC (1 µg/µL) and 1.0µM duplex DNA.

- Prepare a solution (Buffer A*) that contains 1 mL of Buffer A and 8 µL of dI-dC per assay (4 mL Buffer A + 32 µL 1 µg/µL dI-dC). Chill on ice.
- Prepare beads and bind DNA to beads (below).
    1. Filter the broth through a 0.45 µm filter using a 10 mL syringe into a sterile centrifuge tube. (The cells may be discarded.)
    2. To each centrifuge tube containing the 10 mL of broth, add 2 mL PEG/NaCl solution. *(These tubes should then be weighed and balanced to allow for centrifugation.)*

10:20am - Cool the solutions on ice for 45 minutes. Pre-cool either the Sorval centrifuge and rotor SA-600 or the Beckman centrifuge and rotor JA-20 to 4°C.

11:05am - Spin the broth/PEG solutions at 20,000 G, (SA-600-11,800 rpm, JA-20-13,000 rpm) for 30 minutes.

After centrifugation, decant the broth into bleach, then invert the tubes on paper towels to dry the phage particle pellet. *(Translucent white precipitate may not be visible.)*

A "phage solution" for each assay is prepared by adding 1 mL of Buffer A* to each centrifuge tube containing a phage pellet *(after drying). Chill on ice.*

Preparation of Beads - *continued from Day 2*

9:55am - Wash beads 5 times with 1X B&W (1.0 vol. 200 µL).

Each wash - 2 minutes rotating, 2 minutes on magnet.

Resuspend in (1.0 vol. 200 µL) 2X B&W.

Binding DNA to Beads	**Binding DNA to Beads (no DNA - control)**
• Add (1.0 vol. 200 µL) of 1.0 µM duplex DNA to the beads. • Rotate for 12 minutes. • Wash beads 3 times with Buffer A (1.0 vol. 200 µL). • Each wash - 2 minutes rotating, 2 minutes on magnet. • Resuspend (1.0 vol. 200 µL) Buffer A.	• Remove 50 µL of beads to eppy. • Add (0.75 vol. 150 µL) of 1.0 µM duplex DNA to remaining beads. • Rotate for 12 minutes. • Wash both sets of beads 3 times with Buffer A (1.0 vol. 200 µL). • Each wash - 2 minutes rotating, 2 minutes on magnet. • Resuspend (0.75 vol. 150 µL) Buffer A. • Resuspend - control in 50 µL Buffer A.

Incubation of Phage with DNA and Washing - *performed at 4°C or 25°C*

Aliquot 50 µL of beads per eppendorf tube.

Remove the buffer from the beads by pipetting. A "binding mix" solution for each assay is prepared by adding 0.4 mL of each "phage solution" to a tube of beads.

12:00pm - Rotate each "binding mix" for 2 hours.

Wash beads 5 times with 0.4 mL Buffer A.

Each wash - 2 minutes rotating, 2 minutes on magnet.

XL1-blue Culture From Starter Culture - *continued from Day 2*

12:05pm - Add 0.5 mL starter culture to 75 mL 2X YT in Erlenmeyer flask and

grow at 37°C to an OD_{600} of 0.8. *It may be beneficial to grow at 30°C and in 2% glucose.*

Elution of Phage

Add 0.4 mL 1X PBS w/4M NaCl to each "binding mix" to elute the phage particles.

2:40pm - Rotate at 25°C for 2 hours.

After 2 minutes on the magnet, the "elution phage" is collected by pipet. *The beads may now be discarded.*

XL1-blue Culture

4:30pm - *Don't forget about the XL1-blue cells from the starter culture!* After growing to an OD_{600} of 0.8, aliquot 7 mL into Falcon culture tubes (2 x number of assays 8).

Infection of XL1-blue with Phage

The elution titer is prepared by adding 0.2 mL of the "eluted phage" to a 7 mL aliquot of the XL1-blue culture.

The input titer is prepared by adding 0.1 mL of the "phage solution" to a 7 mL aliquot of the XL1-blue culture as well for use of a control.

Each new 7 mL culture is grown at 37°C for 1 hour.

Serial Dilutions

Make multiple serial dilutions for the elution titers by adding 100 µL of the 7 mL culture to 900 µL of 2X YT-AG (10^1, 10^2, 10^3, 10^4, 10^5, & 10^6 dilutions).

Likewise, make multiple serial dilutions for the input titers by adding 100 µL of culture to 900 µL of 2X YT-AG (10^1, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, & 10^9 dilutions).

Plate a 20 µL droplet from each of the above serial dilutions on SOB-AG agar plate (4 per plate) and incubate overnight at 37°C.

Glycerol Stocks

Glycerol stocks of XL1-blue cells containing selected Libraries A and B phagemids are prepared from elution titers by adding 0.8 mL to 0.4 mL 50% glycerol and freezing.

– Day 4 –

Identifying Selected Sequences

Pick colonies from the output titer plates, grow in 4 mL 2X YT-AG overnight at 37°C.

– Day 5 –

Identifying Selected Sequences - *continued from Day 4*

Miniprep 2 mL of culture.

Screen by digesting 7 µL of plasmid with the appropriate restriction enzymes in a 15 µL reaction. Run on a 1% agarose gel.

Submit premixed sequencing reactions to the Keck Facility: 15 µL miniprepped plasmid, 2 µL 4µM S1 primer, 7 µL H_2O.

Notes

Timepoints are approximate.

Discard into bleach anything that comes into contact with phage. Separate

pipetmen for phage may be used. Filter tips must be used.

The stringency of the selections can be varied by changing the temperature, number of washes, length of washes, or by adding competitor DNA.

Percent Retention Calculation

Tabulate the number of colonies for each SOB-AG agar plate. The following equations may be used to determine the retention percentages:

$$\text{Elution Titer} = \text{\# colonies} \times 10^{x} \times \frac{\text{7 mL culture}}{\text{0.02 mL plated}} \times \frac{\text{0.4 mL eluted}}{\text{0.2 mL infect}} \times \frac{\text{1 mL phage soln}}{\text{0.4 mL bind mix}} \times \frac{1}{\text{10 mL culture}}$$

$$\text{Input Titer} = \text{\# colonies} \times 10^{x} \times \frac{\text{7 mL culture}}{\text{0.02 mL plated}} \times \frac{\text{1 mL phage soln}}{\text{0.1 mL infect}} \times \frac{1}{\text{10 mL culture}}$$

$$\text{Percent retention} = \frac{\text{Elution titer}}{\text{Input titer}} \times 100$$

# colonies		**Fold dilution**								
assay	titer	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9
Negative control	Elution									
	Input									
Positive control	Elution									
	Input									
Library A	Elution									
	Input									
Library B	Elution									
	Input									