Panning Protocol for Proteins

by Reena Zutshi

Required solutions

TBST
Dissolve 10 mL of 1M Tris.HCl (pH 8.0)
8.7 g NaCl
0.5 mL Tween-20
in 1L of diH_20. Sterile filter.

2M Tris (Neutralizing Solution)
Dissolve 12.1 g Tris Base
in 40 mL diH_20.
Adjust pH to 9.2 with concentrated HCl.
Bring the total volume up to 50 mL with dH_20.

0.1 M Gly.HCl (Eluting Solution)
Dissolve 75 mg of glycine
10 mg of BSA
in 8 mL of diH_20.
Adjust pH to 2.2 with concentrated HCl.
Bring the total volume up to 10 mL with diH_20.

3% Milk / TBST (Blocking Solution)
Dissolve 300 mg fat free Carnation milk
in 10 mL of TBST.

Protocol

– Day 1 –

Make Starter Cultures:

§ For each clone, start a 10 mL 2xYT-AG growth in 50 mL orange cap centrifuge tubes from 1-2 mL of glycerol stocks. Grow at 37 °C overnight.

Plate XL1Blue cells on LB Tet plates:

§ Streak XL1Blue cells on LB Tet plate; incubate at 37 °C overnight.

– Day 2 –

Add Phage:

- Start a 10 mL 2xYT-AG growth for each clone in 50 mL orange cap centrifuge tubes from 1mL of overnight culture. Grow at 37 °C until log phase.
- Add 400 µL M13K07 helper phage to each.
- Shake at 37 °C for 1 hr.
- Spin cells down at 2500 rpm for 20 min in delicase centrifuge.
- Resuspend cells in 10 mL 2xYT-AK.
- Grow phage overnight (12 hours).

Immobilize GST-protein on microtiter plates:

- Wash wells of glutathione microtiter plate 3 x 2 min with 200 µL PBS.
- Dilute GST-protein in TBST (final concentration 5 µg/mL).
- Add 200 µL GST-protein to each well.
- Incubate at 4 °C overnight (12 hours).

Small growth of XL1-Blue cells:

- Pick an XL1Blue colony from the LB Tet plate. Add to 5 mL of 2xYT. Incubate at 37 °C overnight.

– Day 3 –

Phage precipitation:

- Spin cells down at 2500 rpm for 20 min in delicase centrifuge.
- Discard cells, filter broth through 0.45 µm filter into centrifuge tube.
- Add 2 mL 0.2 µm filtered PEG/NaCl to each tube.
- Incubate on ice for 45 min.
- Centrifuge cells for 35 min at 13000 rpm at 4 °C.
- Discard supernatant; dry pellets for ~ 2 min.
- Resuspend pellet in 1 mL TBST buffer.

Block microtiter plates:

- Wash wells 3 x 2 min with 200 µL TBST.
- Add 3% milk/TBST to each well.
- Incubate at 4 °C for 45 minutes.
- Wash wells 3 x 2 min with 200 µL TBST.

Grow XL1-Blue cells:

- Using the starter culture (dilute 1 Ù 25 from overnight growth), set up 10 mL (5 mL for input and 5 mL for output) 5 number of assays of 2xYT growths.
- Incubate at 37 °C until log phase growth (~8 hours).

Panning:

- Bind 200 µL phage to wells for 3 hours at 4 °C.
- Wash wells 5 times for 1 min at 4 °C with 200 µL TBST (in the first round).
- Elute phage with 200 µL 0.1 M glycine, pH 2.2 for 20 min.
- Transfer phage to eppys, neutralize with 4.5 µL 2 M Tris, pH 9.2.

Infection:

- Infect 5 mL XL1Blue culture with 100 µL input and 5 mL with 100 µL output phage.
- Incubate at 37 °C for 1 hr.

Titering:

- Make neat - 10^7 serial dilutions of output phage.
- Make 10^1 - 10^{10} serial dilutions of input phage.
- Plate 20 µL of dilutions of output phage and input phage on SOBAG plates.
- Incubate at 30 °C overnight (16 hours).

- Make glyercol stocks of necessary clones

– Day 4 –

- Pick 20 colonies from output titer plates and grow in 3 mL 2xYT overnight at 37˚C.

– Day 5 –

- Miniprep the cell culture to isolate DNA.
- Screen by digesting DNA with the appropriate restriction enzymes. Analyze on a 1% agarose gel.
- Send the positive clones (from screening) to Keck for sequencing.