# Strip Blots

*The strip blot is a way to probe the same antigen with multiple antibodies at once.*

1. Obtain a 2-D SDS PAGE gel (or remove the wells from a standard gel to create one big well).

2. Load your antigen in the big well, run and transfer to nitrocellulose as usual. Amido black stain blot, and allow it to dry completely. Once blot is dry, use a waterproof marker to mark the top of the blot. This will be used to maintain proper orientation later. The dry blot can then be wrapped in a paper towel and saran wrap, and stored in the -20°C freezer until ready to use.

3. Obtain plastic trays with 8 long slots (we have S&S Accutran™ Incubation trays), a metal ruler (important – metal, not plastic – you need a straight edge), a scalpel, and a glass plate.

4. Using the metal ruler and the scalpel, cut the blot into thin slices, such that each slice contains some of the antigen. Make sure the strips are thin enough to fit inside the holder slots.

5. Insert strips inside holder.

6. Block each strip with 1-2mL of 5% Milk in TBST, 1 hour at room temperature on rocker. Ensure strips are completely covered.

7. After one hour, use aspirator to remove blocking solution – cover aspirator with gel loading tip to prevent sucking up the strip.

8. Probe with first antibodies in 1-2mL of 2.5% Milk/TBST
   If testing purification of antibodies from serum, test the serum (1/500), wash (1/500), elutions (1/1000)

9. Incubate overnight in the cold room on a rocker. Cover tray with parafilm.

10. Next day, wash strips 3x quick 3x 5 minutes with TBST (when aspirating off washes, again be careful not to suck up strips)

11. Add secondary antibody at appropriate dilution in 1-2mL of 2.5% milk in TBST. Incubate on rocker 1 hour at room temperature.

12. Wash 3x quick 3x 5 minutes with TBST.

13. Cover strips with freshly made ECL solution, and expose strips to film in the dark room to develop.