MAKING & SCREENING A PLASMID LIBRARY

1. Set up a ligation up with 100 ng of vector and a 3:1 molar insert to vector ratio. Incubate for 48 h in the fridge and transform via electroporation.

2. Let the cells recover for about an hour at 37°C and plate 10 μl on an LB + antibiotic plate. Keep the remaining cells in the fridge. Incubate the plate at 37°C overnight.

3. Calculate cell density from test plate and plan to plate out remaining transformation to a density of about 10,000 colonies per 20 cm^2 plate.

4. Either plate directly onto a plate and screen as for colony hybridizations or follow protocol below:

5. Place a sheet of Hybond-N directly on top of agar and plate cells on top of it. Incubate at 37°C.

6. After about 6-8 hours growth, you should see colonies forming on the membrane. When they are 1 to 2 mm in diameter, lift the membrane from the plate and place on saranwrap on top of a glass plate.

7. Carefully place another membrane on top and press down with another glass plate to make a replica. Return the replica (colony side up) to a fresh LB + antibiotic plate and let grow overnight at 30°C.

8. Denature, neutralize and then hybridize the original filter.

9. After overnight growth, seal the replica plate in parafilm and put in the cold room (it should be fine for up to a week). You can then recover your clones from the replica plate.