Bacterial Colony Hybridization (Colony lift)

AIM: Identify which bacterial colonies on a petri dish have integrated the desired insert.

Materials:

- Freshly grown and well separated bacterial colonies on petri dishes
- Whatman Filter #1, trimmed to fit in the petri dish, does not need to be sterile
- Coomassie brilliant blue R-250 powder
- Radiolabelled probe specific to the desired sequence. Note: The easiest way to prepare the probe is by random-primed probe (see appropriate protocol) using the insert PCR fragment as a template.
- Large Pyrex tray
- Clean petri dishes
- Parafilm
- Hybridization oven set at 42°C

Solutions:

Solution A	200ml
50mM NaOH	1ml of 10N
0.5% SDS	5ml of 20%
ddH2O	194ml
Make fresh.	

SSC 20X	100ml
3M NaCl	17,5g
300mM Na2citrate•2H2O	8,8g
ddH2O	up to 100ml
Adjust to pH7.0 with HCl	
Store at RT.	

Hybridization mix	10ml
5X SSC	2.5ml of 20X
0.5% SDS	250ul of 20%
50% Formamide	5ml of 100%
50mM Tris pH7.5	500ul of 1M
5mM EDTA pH7.5	100ul of 0.5M
5x Denhardt's solution	0.5ml of 100x
ddH2O	1.15ml
Make fresh. Warm to 42°C.	

Wash solution 1 100ml

5x SSC	25ml of 20X
0.5% SDS	2.5ml of 20%
ddH2O	72.5ml

Wash solutions 2,3 & 4

Prepare dilutions:

Wash solution 2 (2x SSC, 0,2% SDS)	→ 100ml of Wash soln 1 + 150ml ddH2O
Wash solution 3 (0.2x SSC, 0.02% SDS)	→ 10ml of Wash soln 1 + 240ml ddH2O
Wash solution 4 (0.1x SSC, 0.01% SDS)	→ 2ml of Wash soln 1 + 98ml ddH2O

Method:

Lift colonies

1. Grow the bacterial colonies to be screened O/N (or for necessary time). Do not forget to include a negative control (eg. empty plasmid).
2. Number each filter with a pencil and place some marks (3), not evenly →
3. Overlay the filter on top of the colonies, without allowing the filter to slide. Gently press the filter to the surface of the plate, and ensure that it is evenly wetted.
4. For later alignment, replicate the three marks of the filter on the back of the plate.
5. Lift the filter off the agar using clean forceps, without allowing the filter to slide. Repeat for all your dishes.
6. Near the side of one of the filters (ideally in an area clear of colonies), drop about 1ng of the PCR fragment (insert) used for ligation. This will serve as a positive control for hybridization. Circle the drop with a pencil and label it P.C.
7. Lay the filter, colony side up, for about 2 min. on a doubled sheet of blotting paper soaked in a dish with solution A. (Add enough solution A to soak the paper but drain any excess. Don't let solution A flow over the lifted filters).
8. Put the blotting paper (with the filters) and the dish in a microwave oven and bake it at full power for 5 min.
9. Meanwhile, return the agar plates to 37 °C for several hours (or leave at room T°C O/N) to allow the colonies to regrow.

The filters may be wrapped in saranwrap and stored dry indefinitely, in which case the plates should be stored at 4 °C after regrowing.

Hybridization

10. Soak the filters in ≥10 ml wash solution 1.
11. Place 10 ml hybridization mix in a clean petri dish, then transfer each filter individually to the hybridization mix, colony side up, ensuring even wetting and avoiding air bubbles between the filters. Place a blank pre-wetted filter on the top to reduce the risk of the top colony-filter drying out.
12. Incubate at 42°C for ≥1h.
13. Denature the probe at 100°C for 2min and chill on ice.
14. Remove the filters from the hybridization solution, add the probe to the solution, then transfer the filters back to the solution individually, avoiding air bubbles, again placing

the blank filter in the top. From now on, you must use protective shields as the solution is highly radioactive.
15. Seal the lid of the petri dish with parafilm and incubate O/N (or longer) at 42°C.

Washes

16. Using forceps, gently transfer the filters from the petri dish to larger container (eg. Pyrex tray or plastic dish. Discard the hybridization solution in the petri dish.
17. Quickly rinse the filters twice with ≥10ml of wash solution 1.
18. Wash the filters twice with ≥100ml of wash solution 2 at room temp for 20min each. Agitate using a rocking platform, but do so very gently or you'll end up with paper pulp!
19. Wash twice with ≥100ml of wash solution 3 at room temp for 20min each.
20. Quickly evaluate the background radioactivity using the InstantImager (or Geiger) and keep washing with wash solution 3 until background is acceptable.

Colony staining and positive colony identification

21. Dissolve a pinch (very grains) of Coomasie blue in an eppendorf of 95% ethanol. Add this to 10ml of wash solution 4.
22. Transfer the filters to the stain solution and incubate at room temp for about 20min.
23. Remove the stain solution (which can be reused if necessary) and wash the filters with wash solution 4 until the colonies are clearly visible from the background stain.
24. Blot off any excess liquid and wrap the filters on a saran wrap. Stick phosphorescent dots for alignement and expose the filters on film (O/N in a cassette at room temp).
25. Identify positive insert-containing colonies by aligning the autoradiography with the filters and the plates. The positive control (step 6) should also be clearly visible.

Reference:

Protocol adapted fromk method used in Ares's lab and from Buluwela L. et al. (1989) NAR 17.452.