Colony Lifts – for bacterial colony screening.

Prepared by Ms Alex Aitken

This allows a probe based method to screen numerous (100's) bacterial colonies for the desired insert DNA without the need to isolate colonies and analyse separately.

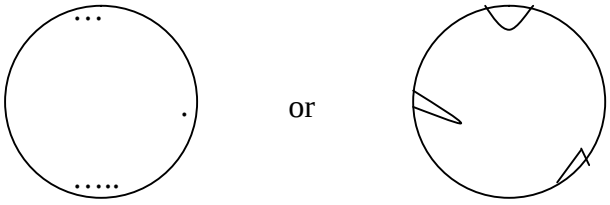
Denaturing Solution (500 ml)
- 1.5 M NaCl 44 g
- 0.1 M NaOH 2 g

Neutralization Solution (500 ml)
- 1.5 M NaCl 44 g
- 0.2 M Tris pH7.5 100 ml

Rinse Solution (500 ml)
- 2xSSC 50 ml

(20x SSC – 1litre: NaCl - 175.3 g + Sodium Citrate dihydrate 88.2 g -- pH 7.0)

1. Cool the agar plates containing the colonies to 4C (~30minutes).
2. Label the circular nitrocellulose filter papers with the plate number.
3. using forceps lay the filter discs onto the agar plate
    a. allow the middle (imagine noon-6 on a clock) to touch first
    b. the sides will then flatten on with no bubbles.
4. Allow capillary action to adhere the filter disc – DO NOT PAT DOWN.
5. Mark the filter discs and the plate uniquely to orientate the position of the disc eg
    a. use a needle to stab different numbers of holes in 3 different places on the perimeter
    b. use a scalpel blade to cut different shaped nicks from the perimeter.



6. Lift the filter and place colony side up onto a puddle (500ul on a piece of SaranWrap) of Denaturing Solution.
7. Leave for 1-2minutes – you will see the colonies diminish.
8. Blot colony side down dry onto a piece of towel.
9. Repeat 6-8.
10. Place colony side down onto a puddle of Neutralization Solution
11. Leave for 1minute.
12. Blot colony side down dry onto a piece of towel.
13. Repeat 10-12
14. Place colony side down onto a puddle of Rinse Solution.
15. Leave for 1minute.
16. Blot colony side down dry onto a piece of towel.
17. Repeat 14-16.
18. Leave to air dry on filter paper.
19. Bake filter at 80C for 1 hour.
20. Proceed to hybridisation; this method will depend on the probe used – follow manufacturers protocol.