Amplification of single phage clones

Material

- PEG/2,5 M NaCl
- LB media
- Cuvettes
- Mikrocentrifug
- TE buffer
- Iodine buffer
- 95% ethanol
- 70% ethanol stored in - 20 degrees

1. Do overnight culture. Dilute 1:100, and add 1 ml in a cultivating tube, one for each plaque.
2. Add one plaque to each tube from a plate with <100 plaques. Use for example a pipette tip to stab the plaques with.
3. Incubate for 4-4,5 h (not more) in 37 degrees celsius.
4. Transfer each culture to a microcentrifgetube and spinn for 30 s at 14 000 rpm.
5. Transfer supernatant to new tube, respin.
6. Transfer 80 % of supernatant to new tube (use pipette here). **THIS IS AMPLIFIED PHAGE STOCK!**(can be stored for weeks in 4 degrees).

Phage DNA purification

Materials:

- Centrifuge
- Minimum of 14 000 rpm:s
- 1000 and 200 µL pipette (filter tips)
- DNA LoBind centrifuge tubes (one per each plaque)
- Iodine buffer (aliquot stored in dark)
- 99,7 % EtOH (Falcon tube)
- Freshly prepared 70% EtOH (falcon tube)
- stored at -20°C
- Deionized water

Procedure

1. Transfer 500 µL from the amplified phage stock (extracted from the first centrifugation) to separate DNA LoBind centrifuge tubes.
2. Add 200 µL of PEG and invert several times. Leave for 10 - 20 min in room temperature.
3. During waiting time store aliquot of the 99,7% EtOH in freezer. Aliquot the Iodine solution and cover with aluminium foil.

4. Timer: 7 m
5. Centrifuge at 14 000 rpm for 10 minutes in 4°C.
6. Pipette out supernatant and discard. Re-spin briefly and pipette out the remaining supernatant carefully and discard.
7. Resuspend the "pellet" in 100 µL Iodine buffer, by vigorously tapping the tubes.
8. Add 250 µL of 99,7% EtOH and incubate for 10-20 min at room temperature. After incubation time centrifuge at 14 000 rpm for 10 minutes in 4°C.
9. During the waiting time prepare the 70% EtOH and place it in the -20 freezer.
10. Pipette out supernatant and discard. Add 500 µL 70% EtOH and respin at same time and speed.
11. Discard the supernatant, and briefly dry the pellet.
12. Suspend the pellet in 30 µL of deionized H_2O.