Preparing clones for sequencing

Overnight cultures and archiving of clones

1. Prepare an archive LB + ampicillin + X-gal + IPTG plate by making a grid on the back of the Petri dish with a felt pen. Grid cells should be approximately 1 x 1 cm; number each cell.
2. For each colony you intend to test, fill a 0.2 mL PCR tube with 50 µl sterile water. Number the tubes.
3. Select single white colonies from plates with a pipette tip. Gently streak the colony across a grid on the other plate. Do not discard the tip.
4. Take the same tip and place it in the appropriately numbered PCR tube.
5. Repeat for each colony you intend to test.
6. Cover the plate and incubate at 37° C overnight. Remove the pipette tips from the tubes.
7. Place the tubes in a thermocycler. Incubate at 99° C for 10 minutes.
8. Cool the tubes to room temperature and proceed with colony PCR (link)

Growing cells for Plasmid Isolation

1. For each colony to be sequenced, prepare a 15 mL tube with 5 mL of LB broth with 100 µg/mL of ampicillin.
2. Select colonies with known inserts and touch with a pipette tip.
3. Place the tip in LB culture and grow at 37° C shaking incubator overnight. Use this culture in the plasmid isolation protocols (1,2).

Materials necessary

15 ml culture tubes
LB broth
100 mg/ml ampicillin stock
LB + amp + X-gal + IPTG
0.2 ml PCR tubes
Sterile DI water
10, 20, and 1000 µl pipette tips

Equipment necessary

37° C incubator
37° C shaking incubator
1000 µL micropipette
10 µl micropipette