Colony PCR

Protocol for Colony PCR

Materials:

- Nuclease free water
- Bacterial Colonies
- LB plates (ampicillin)
- 10X Buffer Promega
- DNA RedTaq polymerase (5U/µl)
- dNTPs
- PCR tubes
- Primers
- Agarose
- GelRed
- Buffer TAE 0,5X
- Buffer TAE 1.5X
- Molecular Marker
- Loading buffer
- Electrophoresis chamber

Procedure:

1. Prepare a master mix in an 0,5 ml tube using the following volumes. Multiply the volume per the number of reactions. Remember to prepare one or two reaction volumes extra to prevent any loss in tube reaction volumes.

Reagent	Volume/mass
Colony	X
5X Buffer Green GoTaq	2 µl
DNA GoTaq polymerase	1 µl
dNTPs	0,2 µl
Forward primer	0,6 µl

Reverse primer	0,6 µl
NFW	Fill up to 20 l (16,6 µl)
Total	20 µl

2. Place 20 µl of the master mix in one tube for every colony that is going to be checked.
3. Take the colony with the bacteriological loop and streak a small line in a specific location in the agar plate, label in the back of the plate the corresponding colony streaked. Then after streaking the colony, resuspend the bacterial loop in one reaction tube with the colony PCR mix.
4. Repeat for every colony.
5. After every colony is streaked and resuspended, place the agar plate in incubation at 37°C for 24h.
6. Run the PCR reaction with the corresponding PCR program for each pair of primers and PCR product.

Confirmation:

1. Solve 1,5g of Agarose in 100 mL of buffer TAE 1X by heating the mix in the microwave. Be careful to prevent bubbles.
2. Add 1 µl of GelRed
3. Pour in the electrophoresis chamber and let the gel solidify.
4. Draw your run map.
5. When the gel is ready remove the well comb and charge the samples according to the run map.
    a. 5 µl of sample
    b. 2 µl of loading buffer
6. Add to the first well 5 µl of the molecular marker.
7. Run the electrophoresis for 45 mins at 90 V.