6. Selection of positive clones

After ligation and transformation, there will be some colonies appearing on the agar plate. However, not all clones would be the correct one. In the past lab, you have included negative controls, which would most likely still give out colonies. Those are usually due to the unlikely even of plasmid self-ligation. While theoretically the same would also happen in your experimental plate, it is possible to screen among the clones to determine which contains the correctly ligated insert. Another possible mistake could be that the plasmid or the insert were wrongly propagated, which might cause mutations. All these false positives will be screened away by colony PCR, restriction mapping and DNA sequencing.

Materials

NEB standard Taq with buffer
Prefix primers and Suffix reverse complement primers
ddH_2O
dNTPs
Agarose
TAE buffer
200ml flask
DNA gel dye
Ampicillin
LB
Mini-prep
Restriction enzymes: *EcoR I, Pst I*
NEB Buffer 3
pRSFDuet empty plasmid

Procedures

Signal to noise ratio

1. Count colonies on each control plate.
2. Divide number of colonies of the ligation product plate by that of the no-pcr

plate.

3. Decide number of clones to pick.

Colony PCR and DNA sequencing

1. Setup 1X PCR reaction as follow:
   10X Standard Taq Reaction Buffer: 2.5ul
   10mM dNTPs : 0.5ul
   10uM Forward Primer : 0.5ul
   10uM Reverse Primer : 0.5ul
   Taq DNA Polymerase : 0.125ul
   Nuclease-free water : to 25ul
2. Divide an agar plate into 16 squares with markers.
3. Pick a single colony, streak into a square in the ampicillin agar plate and then in a 1X reaction, and repeat as needed, incubate at 37C overnight.
4. Setup PCR routine as follow:
   Initial Denaturation: 95 ℃ 30 s
   30 Cycles: 95 ℃ 30 s
   60 ℃ 60 s
   68 ℃ 60 s
   Final Extension: 68 ℃ 5 min
   Hold: 4 ℃
5. Run 1% agarose gel to confirm existence of correct band.
6. Pick 2 clones with correct band in agarose gel into 5 ml LB culture with ampicillin and shake it overnight at 37 ℃, 220 rpm.
7. Aliquot 1 ml of overnight culture to sequencing.
8. Mini-prep according to the manufacturer's protocol.
9. Take OD 260 and OD 280, 260/280 ratio and DNA concentration.

Restriction mapping

1. Digest the plasmid using *EcoR I* and *Pst I*, with uncut and single cut controls.
2. Run DNA gel with ladder, uncut, *EcoR I*, *Pst I*, Double cut, PCR product, single cut empty vector.

(This video shows the procedures of colony PCR)