PROTOCOL: TOPO cloning

Material and chemicals:

competent cells of *Escherichia coli* DH5α
2 µl PCR product (diluted according to the amount of DNA)
0.5 µl NaCl solution
0.5 µl Topoisomerase I-activated pCR™4-TOPO® vector(keep on ice to avoid ligation of the vector itself)
Xgal solution (final concentration in medium 40 µg/ml)
SOC (super optimal broth) medium
ampicillin (final concentration in medium 50 µg/ml)

Workflow:

1. Mix the reagents and spin them down.
2. Incubate the mixture for 30 min at room temperature.
3. Add 2 µl of prepared mixture to 50 µl naturally competent *Escherichia coli* DH5α
4. Perform heat shock as you can see below.

Variant 1

1. Place the mixture on ice for 15 minutes.
2. Heat shock the mixture at 42 °C for 30 seconds.
3. Place on ice for 2 minutes.
4. Pipette 250 µl of medium (SOC - super optimal broth, or LB medium).
5. Keep at 37 °C and 300-500 rpm for 60 minutes.
6. Take 50 ul of the mixture and plant it on agar plate with ampicillin and Xgal (40 µg/ml). Then take 200 µl of remaining mixture and plant it on another agar plate with ampicillin and Xgal (do not centrifuge).

Variant 2

1. Place the mixture on ice for 15 minutes.
2. Heat shock the mixture at 42 °C for 45 seconds.
3. Place on ice for 2 minutes.
4. Add 900 µl of medium (SOC - super optimal broth, or LB medium).
5. Keep at 37 °C and 300-500 rpm for 60 minutes.
6. Centrifuge the mixture at 6000 rpm for 2-3 minutes.
7. Take 1 µl of the supernatant and plant it on agar plate with ampicillin (50 mg/l) and Xgal (40 µg/ml). Then take 90 µl of remaining mixture and plant it on another agar plate with ampicillin and Xgal.

Notes:

- Keep the agar plates in dark and leave them to grow overnight at 37 °C.
- Next day evaluate blue and white colonies - the white colonies indicate the successfully transformed cells containing plasmid with insert.
- To prove transformation sequencing using M13 primers can be performed.