LAB PROTOCOL: pGREEN BACTERIAL TRANSFORMATION

PREP

WATER BATH at 42°C
Foam tube holder for water bath

CONTAINER OF CRUSHED ICE
pGREEN plasmid
CaCl
1 Eppendorf tube: Label “pGREEN”

ROOM TEMPERATURE
SOC (Luria Broth may be substituted)
E. coli MM294 Culture on LB plate (cultured ~8-16 hours ago)

INCUBATOR AT 37°C
1 LB+AMP Agar Plate (Label “LB + Amp + pGREEN”)

SUPPLIES

Trash container for pipettor tips and loops
Pipettors for 100µL, 250µL, 10µL
Sterile Transfer Pipets (100µL, 250µL, 10µL)
5 Glass Beads for spreading on plate
1 Sterile Transfer Loop for E. coli colony
Sharpie
parafilm for sealing plates

RESOURCES

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1. **Pipette 250µL of CaCl into pGREEN tube**
2. **Submerge pGREEN tube in ice**

3. **Use sterile loop to transfer an isolated colony of E. coli from LB plate to pGREEN tube. Try not to transfer agar.**
    - Immerse loop and vigorously spin in tube to dislodge E. coli cells.
    - Hold tube to light to observe that cells have fallen off loop.
    - Suspend cells. (Flick tube with middle finger to unclump cells.)
    - Avoid creating bubbles or splashing sides.
    - Examine against light. There should not be visible clumps of cells. Solutions should appear milky white.
4. **Return pGREEN tube to ice**

5. **Pipette 10µL of plasmid to pGREEN tube**
    - Flick to mix in and suspend cells
6. **Return pGREEN tube to ice.**
    - Insert tube into foam tube holder with solution hanging out bottom entirely and re-submerge on ice while in foam holder.
7. **Incubate pGREEN tube while in foam holder on ice for 15min.**
    - Carefully carry ice container over to water bath so you are ready for heat shock step.

8. **Heat shock cells in 42°C water bath.**
    - Place foam holder with pGREEN tube into water bath for 45 seconds.

9. **Return pGREEN tube to ice for 1 or more minutes.**

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10. **Pipette 250µL SOC to pGREEN tube. (1:1 ratio for CaCl:SOC).**
    - Flick tube gently to mix the SOC with the cell suspension solution.
    - Place pGREEN tube on rocker in incubator for 30 minute recovery.

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11. **Pipette 100µL from pGREEN tube to LB+AMP plate. Divvy it out as you pipette by releasing drops around the plate.**
12. **Spread solution on plate with glass beads**
    - Slightly open plate lid and shake out 5 glass bead onto plate
    - Use back & forth shaking motion to move beads across surface to evenly spread. (not swirling round & round) for 30-60 seconds.
    - Remove beads: To Remove glass beads, hold plate vertically over trash container. Clam shell lower part of plate and tap out beads into container.

13. **Seal plates with parafilm.**
14. **Place upside down in incubator at 37°C (98.6°F) for 24-36 hours.**

15. **Observe florescence with UV Flashlight.**