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Title: Transformation of Bb-Trigger Plasmid (R0010, B0034, PsB1C3)

Purpose: To transform the 5 – Alpha E. Coli Strain (NEB) with the ligated BbParts R0010, B0034, PsB1C3.

Materials & Methods:

Take the 3 groups of Ligations (Rxn, No Insert [Psb1C3 only], No Enzyme) that Burnadette completed on last Thursday 7/22/10, and 2 Controls: positive (w/PET 17b) and Negative (w/o DNA added) and perform the Transformation protocol upon them.

Transformation Protocol (C2988) (From NEB)

Protocol

1. Thaw a tube of NEB 5-alpha Competent *E. coli* cells on ice until the last ice crystals disappear. Mix gently and carefully pipette 50 µl of cells into a transformation tube on ice.
2. Add 1-5 µl containing 100 pg-1 µg of plasmid DNA to the cell mixture. Carefully flick the tube 4-5 times to mix cells and DNA. **Do not vortex.**
3. Place the mixture on ice for 30 minutes. Do not mix.
4. Heat shock at exactly 42°C for exactly 30 seconds. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950 µl of room temperature SOC into the mixture.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate. [Changed to 90 Minutes]
8. Warm selection plates to 37°C.
9. Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in SOC.
10. Spread 50-100 µl of each dilution onto a selection plate and incubate overnight at 37°C. Alternatively, incubate at 30°C for 24-36 hours or 25°C for 48 hours. [Rxn plated @ 700 & 300 ul, All others plated @ 700 ul… No serial Dilutions performed 7.26.10 – 6:15pm]

Calculations: None

Conclusion: Awaiting culture results to follow with Gel-electrophoresis analysis, (Thursday 7/28/10?).

Discussion: A lot of time was lost this afternoon, searching for the key for parts ligated last Thursday (7/22/10). Eventually a box in the deli-fridge was discovered which I recognized from Bernadette's original restrictions on Tuesday (7/20/10), which had a key on it which referenced the parts which were left in the PCR machine for incubation. Another reminder to keep good notes logged.