Protocol for Transformation - Chelsea (detailed)

Introduction

Protocol for Experiment 1 - Functional Analysis of Cas9 and DsbA-Cas9 to Cut mRFP sequence using specific gRNA

Materials

›

- Competent cells (10 uL/trial for Top10, 20 uL/trial for JC8031)
- Plasmid DNA (conc. 1 pg/uL- 10 ng/uL)
- 2 mL microcentrifuge tubes (chilled in -20 freezer)
- Agar plates (with right antibiotic resistance if needed)
- Ice (in bucket)
- Spreader beads/wand
- SOC Media for rescuing cells
- Water Bath (set to 42 C)
- Incubator/Shaker @ 37 C
- Floating test tube rack for water bath

Procedure

Procedue

1. Set water bath to 42 C
2. Thaw comp cell aliquots on ice for 15 minutes
3. Remove agar plates (containing the appropriate antibiotic) from storage at 4°C and let warm up to room temperature
4. Pipette 10 uL of competent cells in each 2 mL microcentrifuge tube (20 uL for JC8031). Keep tubes on ice at all times.
5. Pipette 1 uL of 1 pg/uL-10 ng/uL of plasmid DNA into each microcentrifuge tube, do not disturb in anyway (not even flick). For dual transformation, use 0.5 uL of each plasmid DNA to get to 1 uL total DNA per rxn
    a. e.g. If stocks of DNA are at 50 ng/ul, dilute 50 ng/uL to 10 ng/uL
6. Incubate tubes on ice for 20 mins
7. Heat shock tubes in water bath at 42 C for 60 secs. --- Timing must be exact or cells will die ---
8. Immediately move tubes to ice bucket, cover with ice, and incubate on ice for 5 mins
9. Add 10 uL (20 uL if using JC8031) of SOC (no antibiotics) and rescue for an hour and 15 minutes at 37 C in shaker

10. Plate on appropriate agar + antibiotic plates

11. Incubate overnight at 37