Tyler's Transformation and Fluorescence

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

›

›

Procedure

Cotransformation:

1. Add 1-5µL of each plasmid to comp cells, standard transformation protocol, plate on plates that have the selection markers for both plasmids.

Measuring fluorescence:

2. Day 1: Streak necessary plates/perform necessary co-transformations

3. Day 2:

    1. Pick 3 colonies for each case you are testing (I would use Cas9+mrfp-grna (targeting guide), Cas9+mrfp-untargeting_grna (no guide inserted into the vector, control for how much fluorescence you would get if Cas9 did not cleave any mRFP, Cas9 (control that shows the level of fluorescence without rfp being expressed whatsoever)
    2. Autoclave a flask of 25mL of LB for each pre-culture you are creating (if you used my suggestion, it would take 9 flasks

4. Day 3:

    1. Inoculate with pre-culture into the 25mL of LB at ~1:50 dilution, grow up for 5hrs (ensure the culture is in stationary phase).
    2. Pipette 200µL of each culture into a well in a 96 well plate. You then want to measure absorbance at 600 (gives you OD600) and fluorescence of mRFP (555,584). When presenting data, you want to divide the measured mRFP fluorescence by the OD600.