Liquid Experiment, Plasmid Stability Assay

Introduction

Stability assays were performed with high-copy plasmids as described for “Plasmid stability, liquid experiment”. In ~ 10 replicates, 1 mL of LB medium was inoculated with a single CP colony for overnight incubation under chloramphenicol (30 µg/ml) selection. Then, to allow ~ 10 generations of growth in each cycle, a 1000-fold dilution with LB chloramphenicol was performed every 24 h. Assays continued for ~ 40 generations unless color was lost earlier in all replicates (Additional file 1: Table S1). Visualization was done in ambient light except for amilGFP where a UV lamp was used.

Materials

- overnight culture of DH5-alpha E.coli containing AmilCP + psb1c3 plasmid
    - Eppedorf tubes
    - liquid LB+chloramphenicol

Procedure

1. For ten replicates add 1 uL of liquid overnight culture to a labeled eppedorf tube (1, 2, 3, 4, 5..... 10)

2. Make a 1:1000 dilution in LB + chloramphenicol for each aliquote of culture in order to allow 10 generations grow in each cycle. (add 999 uL of Lb chloramphenicol to all tubes)

3. Put all tubes on incubation/growth in 37° C for 24 hours

4. Measure expression of AmilCP in each tube by comparing the colour intensity of the tubes. Rank by: ++ strong color; + weak color; -, colorless. Plate all cultures with high color intensity on LB+chloramphenicol agar plates.

5. take 1 uL of ech tube/culture and make a 1:1000 dilution in LB+ chloramphenicol again in a new eppedorf tube and put all tubes on incubation for 37° C in 24 hours. After incubation, measure the color intensity in each tube again and rank in the same way as before.

6. Repeat the procedure 4 times in order to get an assay spanning over 40 generation.