



Look What They've Done To My Shoes!

SCU_China Project 2016

Protocol
Lysis Team



Experimental principles: We constructed a device that can induce E.coli cell lysis when the temperature is raised up to 42°C. To test the device, we construct a plasmid which contains that device and also can express RFP. The E.coli containing our plasmid is supposed to lyse and release intracellular proteins including RFP when incubated at 42°C. So that there would be more RFP in the supernate after centrifugation if the device effect the suicide, compared with the cell incubated at 30°C at which the suicide gene won't be promoted.

Apparatus: Micro-plate reader

Method:

1. Transform the E.coli cell with the constructed plasmid.
2. Enlarge the transformed bacterium.
3. Culture 5ml LB liquid medium inoculated with 10 μ L bacteria solution from step 2 in a 50ml sterile tube at 30°C.
4. After being cultured for 16~20h, dilute the bacteria solution with equal-volume LB liquid medium. Distribute 2ml dilution in six 2ml tubes each.
5. Divide those tubes averagely into 2 groups. Incubate one at 30°C and the other at 42°C overnight (more than 10h)
6. Centrifuge the sample at maximum speed for 2 minutes. Take the supernatant and suspend the sedimentation with a same volume of LB medium.
7. Test the fluorescence intensity in the supernatant and in the resuspending by micro-plate reader.