untitled protocol

IGEM-EGYPT 2017

Abstract

Flow Cytometry

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Protocol

Step 1.
 Inoculate single colony of freshly transformed DH5Pro or TOP10 cells in 4 ml LB medium containing

Step 2.
50µg/ml chloramphenicol (or appropriate antibiotic).

Step 3.
 Grow the culture overnight at 37 C with shaking (250 rpm).

Step 4.
 Next day re-inoculate the cultures into 4 ml fresh LB medium having antibiotics and varying inducer

Step 5.
concentrations. Inducer concentrations can be varied from 0-1mM of IPTG or 0-200 ng/ml aTc.

Step 6.
 Collect the samples at different time intervals of 3, 6 and 9 hours.

Step 7.
 Monitor the growth rate by measuring optical density at 600 nm.

Step 8.
 Measure the fluorescence in a Becton Dickinson FACS Calibur flow cytometer equipped with a 488 nm argon

Step 9.
laser and a 515-545 nm emission filter (FL-1) and a 585-610 nm emission filter (FL-2).

Step 10.
 Make sure that machine has settings for E. coli.

Step 11.
 To measure the fluorescence, add 3-5 µl of the growing culture in 1 ml PBS (phosphate buffer saline, pH-7.5).

Step 12.
Measurement should be done at low flow rate (1000 events/second).

Step 13.
 For each sample, collect 50,000 events.

Step 14.

 Analyze the fluorescence in both FL-1 and FL-2 channel using FlowJo software (BD Biosciences).

Step 15.

 Determine the background fluorescence by using controls (cells having empty plasmid vector).