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RNAi induction

In 1 collection

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Abstract

Back-dilution, induction, and creating frozen *E. coli* feeding stocks for downstream RNAi

Preparing stocks

15m

1 In a sterile hood, streak out HT115 *E. coli* frozen stock onto LB Agar plate (containing [M] 0.05 mg/mL Ampicillin and [M] 0.0125 mg/mL Tetracycline)

Ampicillin selects for the L4440 plasmid containing the knock-down construct
In a sterile hood, treak out HT115 *E. coli* strain (deficient in RNAse III)

2 Incubate overnight at 37 °C

Set up

15m

3 In a sterile hood, pick a single colony to inoculate 10 mL LB containing 10 µL Ampicillin and 25 µL Tetracycline.

Final concentrations Ampicillin ([M] 0.05 mg/mL), and Tetracycline ([M] 0.0125 mg/mL)

3.1 Streak used inoculation loop onto a fresh LB Agar plate (containing [M] 0.05 mg/mL Ampicillin and [M] 0.0125 mg/mL Tetracycline) to create a back-up stock plate

Incubate LB Agar plate overnight at 37 °C

4 Incubate LB overnight with shaking at 180 rpm, 37°C

Induction

5h 30m

5 In a sterile hood, add 600 µL of overnight *E. coli* pre-culture to 15 mL LB containing 15 µL Ampicillin and 37.5 µL Tetracycline.

Final concentrations Ampicillin ([M] 0.05 mg/mL), and Tetracycline ([M] 0.0125 mg/mL)
Conduct remainder of experiment under sterile conditions

6 Incubate with shaking at 180 rpm, 37°C, 02:30:00

2h 30m

7 Add 60 µL ([M] 0.4 millimolar (mM)) IPTG

This induces template expression within the L4440 plasmid construct

8 Incubate with shaking at 180 rpm, 37°C, 03:00:00 3h

9 In a sterile hood, prepare 150 mL NCL containing 300 µL Ampicillin, 600 µL IPTG, and 30 µL β-sitosterol

Ampcillin ([M] 0.1 mg/mL), IPTG ([M] 0.4 millimolar (mM)), and β-sitosterol ([M] 0.0008 mg/mL)

Match OD and freeze 11m

10 Spin down *E. coli* at 3200 x g, 00:02:00 2m

11 Remove supernatant, and add 20 mL MQ

12 Spin down *E. coli* at 3200 x g, 00:02:00 2m

13 Resuspend with 3 mL NCL

NCL (containing Ampicillin, IPTG, and β-sitosterol) prepared earlier

14 Add 800 µL of NCL and 200 µL resuspended *E. coli* to a cuvette, and measure OD_{600}

Use OD dilution calculator:
https://docs.google.com/spreadsheets/d/1hJUsVOjwcuhKSeFQaAbNhn1ZXleTpNXN8RAR4g7zWDE/edit?usp=sharing

Make sure to pipette *E. coli* up and down 3 times before removing from falcon, and again after adding to cuvette for OD measurement

15 In a sterile hood, add required volumes of resuspended *E. coli* and NCL to give 1.5 mL of *E. coli* at OD 3

Use OD dilution calculator as above

Larger experiments may require greater volumes, scale up your stock accordingly
Conduct remainder of the protocol under sterile conditions

16 Add 37.5 µL of 80% glycerol per 1.5mL *E. coli* (to give 2%), and vortex

17 Add 60 µL of *E. coli* per well to a 96-well plate

Ensure *E. coli* are in a consistent layout for every 96-well plate
This is enough for 20 aliquots per *E. coli* stock
Do not use the outside wells

18 Add lids to each stock plate, and store at -20 °C until required