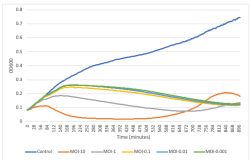
Oct 14, 2019

Growth curve analysis



DOI
dx.doi.org/10.17504/protocols.io.77jhrkn

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iGEM Wageningen 2019

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.77jhrkn

Protocol Citation: Sebastiaan Kuiper 2019. Growth curve analysis. **protocols.io https://dx.doi.org/10.17504/protocols.io.77jhrkn**

Protocol status: Working
We use this protocol and it's working

Created: October 14, 2019

Last Modified: October 14, 2019

Protocol Integer ID: 28619

Keywords: Growth curve analyses, Bacteriophages, Defense mechanisms, CRISPR, CRISPR-Cas, Cas9, Cpf1, Cas12a

Abstract

To observe the potential of defense mechanisms of either native or synthetic systems in *Escherichia coli* (and more) when incubated with a bacteriophage stock.

Materials

MATERIALS

96-well plate, flat bottom, tissue culture treated, black wall with clear bottom **Fisher Scientific Catalog #**3904

Microplate Reader Synergy Mx

STEP MATERIALS

96-well plate, flat bottom, tissue culture treated, black wall with clear bottom **Fisher Scientific Catalog #**3904

Protocol materials

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Preparations

1 Media and bacteriophage stock solutions :
- 1L Luria-Bertani (LB) media (with antibiotics)
- Desired Bacteriophage stock solution in LB media (with known Plaque Forming Units (PFU) ml^{-1})

2 **Fill in plate reader protocol as follows:**
- Set temperature: 37°C
  preheat before moving to next step
- Start kinetics:
  Runtime 15:00:00 (HH:MM:SS), Interval 0:04:00
- Shake:
  medium, 0:30 (MM:SS)
- Read:
  Absorbance Endpoint, Full Plate
  Wavelengths: 600
  Read Speed: Normal, Delay: 100 msec
- End kinetics

3 Prepare overnight cultures of desired samples (with associated antibiotics).

Plate reader

4 Measure OD600 of overnight cultures and dilute cultures to an OD600 of 0.02

5 Load 180 µL of diluted overnight culture into a

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Include a serie of LB (without bacteria) as a control and as zero point for the OD600 measurements!

6 Start plate reader protocol go to step #2 and let the bacteria grow to an OD600 of 0.11.

7 Prepare Bacteriophage PFU dilutions (with associated antibiotics) for;

MOI 10^{1} : 4.0 x 10^{10} PFU ml^{-1}
MOI 10^{0} : 4.0 x 10^{9} PFU ml^{-1}
MOI 10^{-1} : 8.0 x 10^{8} PFU ml^{-1}
MOI 10^{-2} : 8.0 x 10^{7} PFU ml^{-1}
MOI 10^{-3} : 8.0 x 10^{6} PFU ml^{-1}

An OD600 of 0.10 correlates to 8.0 x 10^{8} cells per ml.
The above concentrations are required when 20 µl of bacteriophage dilution is added into 180 µl of cell culture with an OD600 of 0.11 (1:10 dilution).

8 At the moment an OD600 of 0.11 is reached, the plate reader must be stopped and 20 µL of bacteriophage dilution* must be added to a final volume of 200 µL to both the samples and the LB controls.

* include as a control, a serie without bacteriophages and only LB media (with antibiotics)

9 Restart the plate reader protocol and measure over 15 hours the growth of the samples.