NanoLuciferase Assay

afedoriouk[1], Morgan Farrell[1], Nicholas J Shikuma[1]

[1]San Diego State University

Shikuma Lab

Morgan Farrell

JAN 30, 2023

OPEN ACCESS

Protocol Citation: afedoriouk , Morgan Farrell, Nicholas J Shikuma 2023. NanoLuciferase Assay . **protocols.io** https://protocols.io/view/nanoluciferase-assay-cig2ubye

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

Created: Oct 27, 2022

Last Modified: Jan 30, 2023

PROTOCOL integer ID: 71930

ABSTRACT

Protocol for a luciferase assay to quantify promoter expression in marine bacteria.

MATERIALS

Nano-Glo live Cell Assay System **Promega Catalog #N2011**

Day 1: Streak out strains

30m

1 Streak out all strains from frozen stock onto the appropriate antibiotic plates. Including a positive and negative control. 30m

- *Pseudoalteromonas luteoviolacea* expressing kanamycin resistant backbone was struck onto NSWT with 300 µg/mL of kanamycin
- *Pseudoalteromonas* sp. PS5 expressing kanamycin resistant plamid was struck onto Marine Broth (MB) with 300 µg/mL of kanamycin

- Wild type marine bacteria were grown on MB only media

Day 2: Create Sub-Culture

2 Create sub-culture by inoculating 5 colonies from the plate into a 5 mL tube with appropriate media and antibiotics. Repeat for each strain into a separate 5 mL tube. 1h

- Biological replicates should be in separate sub-cultures

3 Incubate at 25ºC shaking at 200 rpm for 24 hours.

> **Note**
>
> Plan ahead! Start this step at a time that works best for you to perform the experiments on day 3.

Biofilm Only + 1 Day: Spot Cultures for Biofilm

4 Take the optical density (OD_{600}) measurement of the sub-cultures with a spectrophotometer to ensure they are in stationary phase. 10m

5 Take 1.5ml of sub-culture and transfer into a micro centrifuge tube. Spin down to concentrate bacteria. 15m

- Spin cultures at 4000g for 10 minutes
- Remove supernatant and avoid cell pellet

6 Resuspend bacterial pellet with 75 µL of liquid media. 10m

7 Spot the entire 75 µL onto a single agar plate of the appropriate media and antibiotics. 1d

- Inoculate plate for 24 hours at 25ºC

Day 3: Inoculate Experimental Cultures

8 Inoculate experimental culture from the sub-culture created in Day 2 with 1:100 dilution of media to sub-culture. 30m

9 In 125 mL flask add 25 mL of media and 250 µL of sub-culture. Repeat for each strain and biological replicate into its own flask. 5m

10 Take optical density OD_{600} measurement right after inoculation to have a time point 0 (T0). 5m

11 Continue to take optical density measurements to determine the major growth phases that will be tested in the NanoLuciferase assay. 8h

- Test 2-3 strains to determine where the OD_{600} is at. May need to retest every 30 minutes in the beginning to capture exponential phase.
- To measure OD_{600}: Fill cuvettes in with 1 mL of sample and flick each vial before taking OD_{600} measurement

Day 3: Performing NanoLuciferase Assay 2h 5m

12 Prepare mastermix for blanks reactions. 10m

Nano-Go Live Cell Assay System **Promega Catalog #N2011**

- 4 blanks
- 2.5 µL of Buffer x 4= 10 µL of Buffer + 0.5 µL of Substrate
- 17.5 µL of water x 4 = 70 µL
- 20 µL of blank media per well

Run the plate with the blanks making sure to label to results as blanks in the computer software.

> **Note**
>
> Always have the machine read the whole plate and not just selected cells that have samples.

13 Prepare luciferase plate by loading blank samples first to see if any of the wells including the blank are showing signal before the start of the assay. 20m

14 Prepare the sample only plate in a regular 96-well PCR plate. 20m

- 100 µL of each sample culture laid out the same as the luciferase plate.
- Keep the layout the same so you can use a multichannel pipette to transfer the samples from the PCR plate to the luciferase plate.
- Shake or mix each flask before pipetting out the sample so that it is evenly mixed

15 Dilute the samples when making the samples plate. 30m

- Past assays have been successful with the following dilution scheme.
- *Pseudoalteromonas luteoviolacea* was diluted in a range of 1:10 to 1:100.
- *Pseudoalteromonas* sp.PS5 was diluted in a range of 1:1,000 to 1:100,000.

Late stationary and biofilm growth phases showed higher expression and required larger dilutions. Additionally, constitutive promoters required larger dilutions than native promoters.

- Use serial dilution of 1:100 in a regular 96-well plate first then proceed to do 10-fold dilutions to get to the above dilution levels.

16 Prepare the luciferase experimental plate. Use the mastermix reaction template to calculate the amount for your number of samples. 5m

- i.e. For 72 reactions prep 80 reactions for mastermix
- 80 x 2.5 µL Buffer/Substrate = 200 µL Buffer + 10 µL Substrate
- 80 x 17.5 µL MilliQ Water = 1400µL MQ
- 20 µL of mastermix per well

17 Pipette 20 µL of mastermix in each well first. 20m

18 Then, pipette 20 µL of sample into luciferase plate with mastermix using a multichannel pipette. 5m

> **Note**
>
> Needs to be done as quickly as possible ~1 minute is ideal.

19 At each time point that you run the assay measure the optical density to normalize your luciferase data by OD_{600}. 15m

- Fill cuvettes in with 1 mL of sample.
- Flick each vial to mix before taking OD_{600} measurement.