Luminescence assay

Material

- Supernatant from HeLa cells expressing Gaussia luciferase
- Freshly prepared hGluc buffer (see below)
- Black, flat bottom 96 well plate
- Plate reader (must be able to detect luminescence)

Procedure

Preparation of luminescence buffer

Preparation of luminescence buffer (referred to as hGluc buffer) was done as described in [1]. The recipe is listed in the following table.

	Stock solution	Working solution	Volume [µL] of stock solutions to get 1 mL hGluc buffer
Tris-HCl, pH 8.0	1 M	90 mM	90
NaCl	100 mM	15 mM	150
NaI, pH 8.2	1.5 M	75 mM	50
Triton X-100	100 %	0.3 %	3
ddH_2O	-	-	705
Coelenterazine	5 mM in acidified methanol	10 µM	2

- Storage of stock solutions:
  - Coelenterazine stored at -20°C
  - Tris-HCl stored at 4°C
  - all other stock solutions stored at room temperature
- Always freshly prepare hGluc buffer on the day of use.
- Add coelenterazine only directly before performing luminescence assay.

Luminescence readout

For quantitative luminescence readout, Tecan Infinite® 200 PRO plate reader and Tecan i-control software were used.

1. Transfer 100 µL of cell culture supernatant to a black, flat bottom 96 well plate.
   Note: Remember to also examine supernatant from untreated cells. This will serve as negative control – resulting values represent noise/background signal.
2. Add 50 µL of freshly prepared hGluc buffer.
3. Mix by shaking.
4. Quantitatively measure luminescence via plate reader using the following parameters:
   a. Mode: Luminescence
   b. Integration time: 1000 ms
   c. Resting time: 10 ms
   d. Attenuations: none

   Note: Measurement has to be conducted right away as the luminescence signal intensity decreases by 80 % within 6 minutes.

Analysis

1. Calculate the mean of the negative controls. This value represents the background signal.

2. Subtract the background from every other value.
3. Perform normalization as needed.
   Note: For our promoter activity studies, we analyzed two different aspects.
   For comparison of the different promoters' basal activities, we performed normalization within the group of untreated controls (supernatant from transfected cells without LPS-stimulation, see protocol "LPS-stimulation of HeLa cells"). In this case, the CMV promoter served as reference and thus data was normalized on samples coming from cells carrying Gaussia luciferase under control of the CMV promoter.
   For studying the inducibility of promoter activity by LPS treatment, we performed normalization within each group of cells transfected with the same plasmid. In this case, untreated control served as reference to which data was normalized.

Notes

- Always wear a labcoat and gloves.

Citations

[1] Wider, D., & Picard, D. (2017). Secreted dual reporter assay with Gaussia luciferase and the red fluorescent protein mCherry. *PloS one*, *12*(12), e0189403. https://doi.org/10.1371/journal.pone.0189403