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Add Coelenterazine-h

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igemnthuth[1]
[1]iGEM_NTHU_Taiwan

iGEM NTHU

igemnthuth

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We use this protocol and it's working

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Abstract

Add **Coelenterazine-h** as the substrate (light-sensitive; prepare fresh on the day of use) to activate the full-length RenG luciferase and measure its maximum luminescence value. This serves as the relative standard for subsequent SLCA interaction signals. Since three configurations will be tested, record the strains and culture conditions to ensure consistency.

Next, measure the luminescence intensity of the three SLCA constructs to compare their complementation efficiency and background signals, thereby establishing the optimal pairing of the SpyTag/SpyCatcher system and the positioning of the RenG fragments.

- **SLCA assay 1:** Full-length RenG fusion protein, serving as the maximum luminescence reference.
- **SLCA assay 2:** SpyTag + RenG (N-terminal) with SpyCatcher + RenG (C-terminal).
- **SLCA assay 3:** SpyTag + RenG (C-terminal) with SpyCatcher + RenG (N-terminal).

Materials

- Strains harboring expression plasmids with the following constructs (designed plasmids already introduced by electroporation):

  Full-length RenG

  SpyTag–RenG (NTD, aa 1–155) and **SpyCatcher–RenG (CTD, aa 156–314)**

  SpyTag–RenG (CTD) and **SpyCatcher–RenG (NTD)**

- **THYE liquid medium** (with appropriate antibiotic)
- **Coelenterazine-h** (working range **2–10 µM**, pre-dissolved in DMSO; optimize experimentally to identify an appropriate concentration for downstream use)
- **Sterile white 96-well luminometer plate** (luminescence-compatible)
- **ddH_2O** or **PBS**

Strain Pre-culture and Preparation

1 For each strain, pick a single colony and inoculate into THYE medium containing the appropriate antibiotic. Incubate overnight at **37 °C, 220 rpm**.

2 The next day, dilute **1:50** into fresh THYE medium with antibiotic and continue incubation until **OD_{600} ≈ 0.4–0.6** (mid-log phase).

Dispensing into a 96-well Plate

3 Dispense **100 µL** of log-phase culture into each well of a **white 96-well luminometer plate**, preparing **triplicates** per condition.

4 In a separate row, dispense **blank controls** (medium only, no cells).

Adding Coelenterazine-h Substrate

5 Prepare the **Coelenterazine-h working solution** at **[ ] µM** in DMSO (light-protected).

6 Quickly add **20 µL** of Coelenterazine-h to each well (final concentration **[ ] µM**) and mix immediately (gentle pipetting or plate tapping).

Luminescence Measurement

7 Within **3–5 minutes** after substrate addition, measure luminescence for each well using a plate reader.

8 Record the **raw RLU (Relative Light Units)** for each well and subtract the **blank** (medium-only) background.

9 In **SLCA assay 1**, the luminescence of **full-length RenG** is defined as the **maximum (Max RLU)**; report other assay signals as **Relative Activity = (sample RLU) / (Max RLU)**.