Bioluminescence Resonance Energy Transfer at various ratios of expressed proteins

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Abstract

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Protocol

Day 1 - plate cells

Step 1.

1. Plate HEK-293T cells in 384 plates with 3000 cells per well in 50 µL of medium

2. Centrifuge plate at 900 g for 1 min.

3. Incubate cells at 37°C, 5%CO2, for 3h.

Day 1 - transfection FuGENE6

Step 2.

1. Prepare a DNA mix with 25 ng of plasmid expressing Nluc-tagged and YFP-tagged protein in 1 µL of DMEM without FBS. Use a 1:1 ratio of plasmid or if specified, range the ratio of plasmids from 2:1 to 1:4 (Nluc:YFP).

2. Mix with 0.15 µL of DuGENE6 (Promega) in 4 µL of DMEM without FBS.

3. Incubate 30 min at room temperature and add gently 5µL to each well.

Day 3 - bioluminescence readout

Step 3.

Measure direct bioluminescence from the donor (Nluc) and the acceptor (YFP, noted NlucY) using a luminescence plate reader (ex: Wallac 1420 VICTOR 3V multilabel plate reader (PerkinElmer).

Analysis

Step 4.

1. The energy transfer between the Nluc and YFP (BRET) is calculated according to the following formulas (2 and 3) and normalized to a YFP-Nluc linked recombinant protein:

CF_{value} = $NlucY_{value}$ (prot-Nluc) / $Nluc_{value}$ (prot-Nluc) (2)

netBRET = [$NlucY_{value}$ - ($Nluc_{value}$ x CF_{value})] / $Nluc_{value}$ (3)

2. A threshold of specific interaction was determined using the mean+3SD of the negative controls within each experiment.