Idetification of microRNA-93-5P binding site on the BMP-2 mRNA 3'UTR using psicheck2.0 vector and luciferase system

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Abstract

This protocol describes the affinity activity of microRNA93 through luciferase activity in 293T cells that have been transformed with a firefly luciferase coding sequence downstream of the BMP2 3'UTR. Using this methods we were able to idetificated microRNA93-5P binding the downstream 3'UTR of BMP-3 and inhibite the BMP-2 protein formation or not.

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Materials

- luciferase assay reagent by Contributed by users
- psicheck2.0 by addgene

Protocol

Design psicheck2.0 plasmid which contain the BMP2 3'UTR prediction binding sequence

Step 1.

A: synthes the DNA sequence and subclone in to psicheck2.0

B: lysis bacterial and harvest plasmid

Grow low passage 293T cells from liquid nitrogen stock

Step 2.

1. Briefly thaw cells at 37 °C water bath.
2. Let the cell spin for 5 min at 1,500 *x g*. Aspirate media, re-suspend cells and plate in T75 culture flask and cultured at 37 °C with 5% CO_2.
3. Split the cells when they grow to 60% sub-confluency.
   The cell line should be subcultured for one or two time before performing the actual assay. Then plate the cells into 24-well plates as triplicate according to the experimental conditions

Transfection

Step 3.

1. Day 0: Seed cells (density as above-mentioned) in a total volume of 500 µl complete growth

media (DMEM/10%FBS).
2. Day 1: Transfection (use 3µg of psicheck2.0 and 50nm microRNA93 mimic, 2.5 µl lipo2000/well).
3. Day 2: Change media 12h after transfection.
4. Day 3: Change to serum deprived media.
5. Day 5: Lyse cells

Reading luminescence signal

Step 4.

1. Thaw dual-luciferase reporter reagents.
2. Flash and prime Berthold luminometer with firefly luciferase and renilla luciferase substrate reagents.Read firefly and renilla luciferase signals (firefly luciferase signal is detected at 560 nm and renilla luciferase signal is detected at 480 nm).