Protocol for SINEUP activity in transfected mammalian cells

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Background

SINEUP technology was recently discovered in our research group (Carrieri et al., Nature 2012). It is based on the activity of long non-coding RNA molecules that regulate the production of target proteins (figure 1). The advantage of SINEUPs is that are composed by two independent modules: the first module (SINEUP binding domain) is responsible for the specificity of action and the second module (SINEUP effector domain) increments the production of the selected protein. By molecular design, it is possible to generate SINEUPs to virtually any protein of interest.

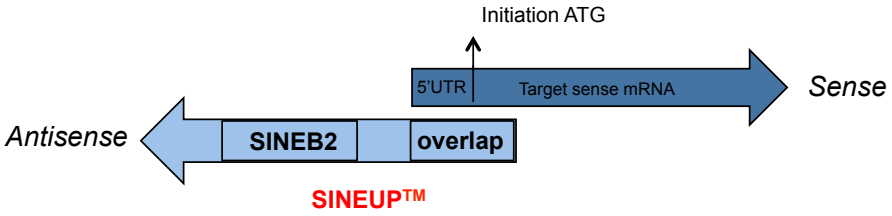

Figure 1. Schematic representation of SINEUP domains.

This protocol will provide experimental details on how to test SINEUP activity targeting a specific mRNA of interest to increase production of translated protein.

Layout of the experimental plan

- Sub-clone target-specific overlap to the 5' of SINEUP sequence
- Obtain a full-length clone (with 5'UTR) of gene of interest into a mammalian expression vector (sense mRNA)
- Proceed with testing in mammalian cells (HEK 293 T cells are suggested)

Protocol

- 6 well-multiwell (or 35mm tissue culture dishes)
- Plate HEK 293T cells at 0.5x 10^6 cells/well about 16 hours before transfection
- Transfection reagent:

Protocol A) FuGENE® HD (Promega, Cat. No. E2311)
Mix for transfection:
Medium to a final volume: 100µl
Volume of FuGENE® HD: 6µl

Total DNA amount: 2µg (0.3µg target gene plasmid + 1.8µg SINEUP plasmid)

Protocol B) Lipofectamine (Invitrogen)
Mix for transfection:
Volume of Lipofectamine:
Total DNA amount: 4µg (0.6µg target gene plasmid + 3.4µg SINEUP plasmid)

[Note. Suggested sense/antisense ratio for transfection is 1:6. Additional trouble-shooting may be required. When available, include cells transfected with SINEUP-scramble or empty expression plasmid in place of SINEUP-gene of interest as control.]

- Harvest cells 24-48h hours after transfection
- Lysate cell pellets in 500µl SDS-PAGE loading buffer
- Briefly sonicate samples
- Boil 5' at 95°C
- Quickly cool on ice
- Load 5µl of sample on a 12% SDS-PAGE
- Visualize with standard western blotting techniques using anti-GFP monoclonal antibody (Clontech, Cat. No. 632380).

Data analysis

To measure SINEUP activity:
a) Test protein production (WB, luminometer, FACS, etc)
b) Test expression of transfected SINEUP (qRT-PCR)
c) Test stability of mRNA of interest (qRT-PCR).

SINEUP activity is the increment of protein levels in SINEUP-transfected cells as compared to control cells with stable levels of protein-coding mRNA (figure 2).

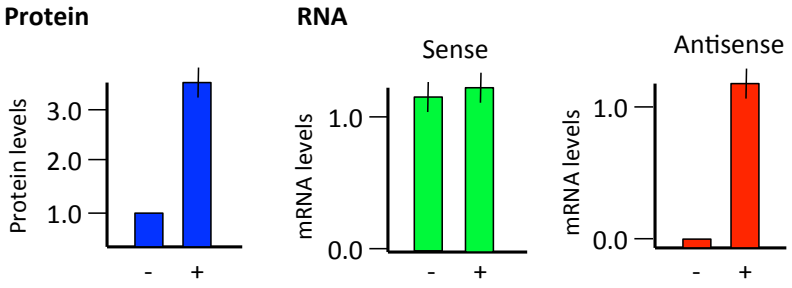

Figure 2. Analysis of SINEUP activity in transfected cells.