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Working

Transfection of construct containing kinetoplastid Blastocrithidia sp. p57 UTR's in Diplonema papillatum using AMAXA Nucleofactor apparatus.

Version 2

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ABSTRACT

Transfection of construct containing kinetoplastid *Blastocrithidia* sp. p57 UTR's in *Diplonema papillatum* using AMAXA Nucleofactor apparatus.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

SAFETY WARNINGS

BEFORE STARTING

Construct Design: A construct containing kinetoplastid Blastocrithidia sp. p57 containing V5-tagged aminoglycoside 3'-phosphotransferase gene (conferring resistance to neomycin) surrounded by regulatory sequences derived from the hexokinase gene was linearized with SwaI and named as p57-V5+G418 plasmid.

1 Count the cells, cells should be in log phase and for one nucleofection we used 5x 10^7 cells.

2 Harvest the cells by centrifugation at 1300xg for 5 min at room temperature in Swing Bucket Rotor.

3 Resuspend the cell pellet in 100ul of AMAXA Human T- cell solution at 4°C (from refrigerator combine 81.8ul of Human T-cell nucleofector solution + 18.2ul Supplement).

4 Add 3ug of linearized as p57-V5+G418 PLASMID into the cuvette(resuspend in 10ul of H2O).Note: Do not add anything in case of Negative control except Amaxa Buffer

5 Put everything into the cuvette, close the cap and place in the electroporator, cuvette should only fit in one direction, but metal sides should face towards you.

6 Press for the Program X-001 of amaxa - Nucleofector II_to electroporate.

Transfer the entire cell- DNA transfectant into 10ml of Diplonema media (chloramphenicol) with the supplied disposable micropipettes.Note:

7 Make sure labeled flasks with media should be ready before electroporation.

8 Immediately observe the flasks containing transfectants under a microscope and place them in the incubator at 27°C and let them grow for 8h.

9 After 8 hours took out the flasks and make controls which should be without antibiotics - put 1ml of the electroporated cells in the first well of 24 well plates.

10 In the rest - 9ml -transfectants add G418 for the selection.

11 Add 1.5ml of electroporated cells in the first row (6 wells) of 24 well plates. Put 900ul of the media (+ selectable drug) in the rest of the wells. Take 100ul from first well and transfer to the next to make 10x serial dilution and so on...up to the lowest concentration 5 x 102 cells/ml.

12 Do the same with the negative control plating.