Transfection of Diplonemids using AMAXA Nucleofactor apparatus

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Protist Research to Optimize Tools in Genetics (PROT-G) Julius Lukes

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

Program: X-001/ X-014

1 Count the cells and plan to 1 nucleofection with 5x 10^7 cells for each construct.

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 5-10ug of (PCR) (linearized DNA) into cuvette(resuspend in 10ul of H2O).
Do not add anything in case of Negative control

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/X-014 to electroporate.

7 Transfer the entire cell- DNA transfectant into 10ml of Diplonema media (chloramphenicol) with the supplied disposable micropipettes.
Note: 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 27C + shaker and let them grow for 6-8h

9 After 6-8 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 antibiotics (HYG or G418or Puro) on the basis of the selection marker in the flasks

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 10^2 cells/ml.

12 Do the same with the negative control plating.

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