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Transfectionof constructs in diplonemids to block NHEJ pathway using the inhibitors of KU70/80 proteins. V.1

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We are still developing and optimizing this protocol

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Abstract

The process of NHEJ is completed when the DNA ligase complex ligates the broken ends of the DNA. Ku70-Ku80 are the DNA-dependent protein kinase heterodimeric regulatory factor that forms a link between two broken DNA ends and structurally support, align and protect them from further degradation. W7 inhibitor (W7(N-(6-aminohexyl)-5-chloro1-naphthalenesulfonamide) inhibits the cofactor of inositol hexakisphophate which effects the regulatory factor of Ku heterodimers.

Materials

Nucleofector^{TM} 2b Device , Human T Cell Nucleofector^{TM} Kit, Cell counter, Normal growth medium,Tissue culture plates, Microcentrifuge tubes

1 Step 0: Pre-incubation of Diplonema cells for 4 hours with W7 (5 mg/ml) beforeelectroporation. EC value of W7 was calculated by Alamar blue assay

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

3 Harvest thecells by centrifugation at 1300xg for 5 min at room temperature in Swing BucketRotor

4 Resuspendthe cell pellet in 100ul of AMAXA Human T- cell solution at 4C (from refrigerator combine 81.8ul of Human T-cell nucleofectorsolution + 18.2ul Supplement).

5 Add 5-10ug of (PCR) (linearized DNA) into the cuvette(resuspend in 10ul of H2O).

6 : 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.

7 Press for the Program X-001 to electroporate.

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

9 Immediately observe the flasks containing transfectants under a microscopeand place them in the incubator at 27C + shaker and let them grow for 6-8h.

10 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.

11 In the rest - 9ml -transfectants add Puromycin antibiotics in the flasks.

12 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 × 10^2 cells/ml and let them grow untill selection of clones is done.

13 Result: Unfortunately, targeting to the planned position (N-terminal tagging of alpha-tubulin with mCherry under puromycin^{R} selection) did not work in any of the obtained clones.