Transfection of constructs in diplonemids to enhance the HR pathway, using RS-1 (3-(benzylamino) sulfonyl)-4-bromo-N-(4-bromophenyl) benzamide).

Version 1

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ABSTRACT

RS-1 (RAD51-stimulatory compound 1) is a stimulator of homologous recombination (HR) protein RAD51

PROTOCOL STATUS

In development

We are still developing and optimizing this protocol

1 Step 1: Countthe cells and plan to 1 nucleofection with 5x 10^7 cells for each construct.

2 Step 2: Harvestthe cells by centrifugation at 1300xg for 5 min at room temperature in Swing Bucket Rotor.

3 Step 3: 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).

4 Step 4: Add 5-10ug of (PCR) (linearized DNA) along with 0.3 uM RS-1 enhancer into the cuvette (resuspend in 10ul of H2O).

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 to electroporate and repeat the same as mentioned in tranformation protocol.

7 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.