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Dr. Tom Beneke

Cell and Developmental Biology
Biocenter, University of Wuerzburg
Am Hubland • D-97074 Würzburg
tom.beneke@uni-wuerzburg.de

Transfecting base editor guide plasmids into *Leishmania*
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Step-by-step protocol

Base editor plasmids that contain guide target sequences can be transfected into *Leishmania* species following conditions outlined in Schumann Burkard et al., Mol Biochem Parasitol 2011; 175, 91-94.

Transfection Buffer

1. Prepare the following buffers:

3x Tb-BSF buffer stock
200 mM Na_2HPO_4
70 mM NaH_2PO_4
15 mM KCl
150 mM HEPES pH 7.4

$CaCl_2$ stock
1.5 mM $CaCl_2$ in water

Cell-Plasmid-Transfection-Mix

1. Dilute 5-10 µg of plasmid DNA into 50 µl ddH_2O and heat sterilize for 5 minutes at 95°C
2. For each transfection (see transfection protocol below) a Cell-Transfection-Mix is prepared by mixing:
    - 25 µl $CaCl_2$
    - 83 µl 3x Tb-BSF
    - 92 µl ddH_2O and

Transfection protocol

1. Prepare an exponentially growing culture of *Leishmania*
2. Prepare the required volume of transfection buffer according to instructions above (ensure all solutions are sterile)
3. Pre-warm medium and collect the required number of cells (at least 5E6 cells per transfection) at 800g for 5 minutes
4. Take off supernatant and wash in 1 – 5 ml of Cell-Transfection-Mix (as specified above)
5. Resuspend pellet in desired final volume of Cell-Transfection-Mix
6. Mix 200 µl Cell-Transfection-Mix with 50 µl heat sterilized plasmid DNA and transfer into electroporation cuvette. (Note: Leave the cells in the cuvettes for the shortest possible time. When doing a big batch of transfections, do no more than five cuvettes at a time and keep the remaining cells in the Eppendorf tube until use)
7. Transfection: one pulse with X-001 (Amaxa Nucleofector 2b)
8. Quickly and carefully transfer cells into 5 ml pre-warmed medium (cell concentration should not be higher than 1E6 cells/ml) and rinse cuvette once with medium.
9. Leave cells to incubate for 8-16 h, then add the required selection drug and incubate until drug resistant populations emerge