Transfection of bloodstream *T. brucei* Lister 427 using the AMAXA Nucleofector® apparatus

Plan your transfection either in early morning and add selective medium at least 6 h later, or transfect in late afternoon and add selective medium next morning. It's good to always mock transfect one cuvette of parental cells without DNA. This will confirm that your selective drug can kill the untransfected cells. If you need to check transfection efficiency, use one of the standard pHD309 tubulin-targeting constructs.

Use the AMAXA Nucleofector® II with Program X-001 and Human T-cell Nucleofector solution (Cat No. VPA-1002).

Grow cells to mid log phase: for BF 427 lines in HMI-9 medium, a concentration of 0.8–1.0 x 10^6 cells/ml is usually optimum.

In most cases, plan to do 1 nucleofection with 30 million cells for each construct. 50 million cells will yield more transfectants (100-150). With 5 million cells, you obtain 10x less clones (10-15). Even if this number of clones is enough, it is not recommended to nucleofect such a low number of cells, because the apparatus detects an error (probably a low resistance). After nucleofection, cells are routinely plated in three 24-well plates in 10-fold serial dilutions. Once you obtain reproducible results, you might be able to reduce the number of dilutions plated.

Make sure the bench-top centrifuge is at RT. For each cuvette —

1. Prepare 90 ml HMI-9 medium with appropriate drugs for parental cell growth. Distribute among three Falcon tubes as follows: 30 ml in Tube A, 27 ml in Tubes B and C.
2. Harvest 30 million cells by centrifugation at 1,500 rpm for 10 min at RT.
3. Resuspend in 100 µl of Amaxa Human T-cell solution at 4°C.
4. Add 10 μg of DNA (in 5 µl) to the cuvette and IMMEDIATELY add 100 µl cells (or add the cells before the DNA): replace the cap of the cuvette and transfect.
5. Transfer the entire cell-DNA mixture to Tube A, containing 30 ml of the prepared medium (cell density = 10 million cells/ml). Invert several times to mix well.
6. Transfer 3 ml of cells from Tube A to Tube B. Invert several times to mix well. (cell density = 1million cells/ml). Repeat procedure for Tube C (cell density = 0.1million cells/ml)
7. Distribute 1 ml aliquots of each dilution to three 24-well plates and incubate at 37°C.
8. After at least 6 h or overnight, prepare 75 ml HMI-9 medium with the selective drug at double the normal concentration. Aliquot 1 ml to each well of the three 24-well plates.
9. Transformed cells are easily seen on the 5^{th} or the 6^{th} day after transfection.