# Transfection of *T. brucei* Lister 427 and its derivatives

PLEASE NOTE: The Amaxa Nucleofector® transfection technology, which we have been using since August 2006, gives nearly 100-fold increase in stable transfection of BLOODSTREAM forms. PLEASE SEE THE SEPARATE PROTOCOL (transfection_amaxa.pdf) to use this machine. We have not tested it for PROCYCLIC forms, as we do little work with these, and the efficiency with the BTX Electroporator is sufficient for our purposes.

The following is our previously published protocol, for using the BTX Electroporator.
Procedures for bloodstream and procyclic forms differ. Solutions and electroporator parameters are the same. 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 include one cuvette of electroporation with just water and the parental cells. This will make sure your selective drug can kill the cells that are not transfected. If you need to check transfection efficiency, use one of the standard pHD309 constructs.

## *Cytomix*

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|---|---|
| EGTA pH 7.6 | 2 mM |
| KCl | 120 mM |
| $CaCl_2$ | 0.15 mM |
| $K_2HPO_4/KH_2PO_4$ pH 7.6 | 10 mM |
| HEPES pH 7.6 | 25 mM |
| $MgCl_2.6H_2O$ | 5 mM |
| Glucose (Dextrose) | 0.5% |
| BSA | 100 µg/ml |
| Hypoxanthine | 1 mM |

Check pH is 7.6: adjustment should not be necessary. Filter-sterilize and store at 4°C.

## *Electroporator parameters*

BTX Electro Square Porator, ECM 830
1,700 v
pulse length time: 100 µs
pulse interval (time): 200 ms
number of pulses: 3

## *Bloodstream forms*

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 5 electroporations with each construct, and use 20 million cells for each electroporation. However, if you know your construct always has higher or lower transfection efficiencies as expected, you can adjust the number of electroporations accordingly, but always use 20 million cells per electroporation. Also, the 20 million cells come from each electroporation will be evenly distributed to each well of a 24-well plate. If you need to check transfection efficiency, use one of the standard pHD309 constructs.

1. Make sure the bench-top centrifuge is at RT.
2. Prepare 120 ml HMI-9 medium with appropriate drugs for parental cell growth in a bottle.
3. Harvest cells by centrifugation at 1,500 rpm for 10 min at RT.
4. Resuspend at 20 million cells/0.5 ml in Cytomix at RT.
5. Prepare one cuvette for each transfection. The same cuvette can be used for all 5 electroporations (as long as both the cell and DNA are the same).
6. Add 5–10 $\mu$g of DNA fragment to the cuvette and IMMEDIATELY add 0.5 ml cells (in Cytomix) (or add the cells before the DNA): replace the cap of the cuvette and zap.
7. Transfer the entire cell-DNA mixture to 120 ml of the prepared medium.
8. Repeat step 6, transferring the DNA-cell mixture to the same 120 ml of medium after each repeat.
9. Distribute 1 ml aliquots of cells to five 24-well dishes and incubate at 37°C.
10. After at least 6 h or overnight, prepare 125 ml HMI-9 medium with the selective drug at double the normal concentration. Aliquot 1 ml to each well of the five 24-well dishes.
11. Transfected cells usually grow up the 5$^{th}$ or the 6$^{th}$ day after transfection.

## *Procyclic forms*

Grow cells to mid log phase: for PF 427 lines in SDM-79 medium, a concentration of 6–10 x $10^6$ cells/ml is usually optimum.

In most cases, plan to do 2 electroporations with each construct, one for getting the pool and one for getting clones of transfected cells. Use 20 million cells for each electroporation.

1. Make sure the bench-top centrifuge is at 4ºC.
2. Prepare 10 ml SDM-79 medium with appropriate drugs for parental cell growth in a small flask for each electroporation.
3. Harvest cells by centrifugation at 1,5000 rpm for 10 min at 4ºC
4. Wash cells with 10 ml Cytomix at 4°C (this step seems optional. It's certainly possible to get many clones without this wash, though whether this affects transfection efficiency hasn't been rigorously tested.)
5. Resuspend at 20 million cells/0.5 ml in Cytomix at 4°C.
6. Prepare one cuvette for each transfection. The same cuvette can be used for both electroporations (as long as both the cell and DNA are the same).
7. Add 5–10 $\mu$g of DNA fragment to the cuvette, IMMEDIATELY add 0.5 ml cells (in Cytomix) (or add the cells before the DNA): replace the cap of the cuvette and zap. Keep the cuvette on ice if not zapped at once.
8. Transfer the entire cell-DNA mixture from one electroporation to 10 ml of the prepared medium and incubate at 27°C. Repeat the electroporation and transfer the cell-DNA mixture to another flask with 10 ml prepared medium.
9. After at least 6 h or overnight, add selective drug to the cells. Keep one flask growing at 27ºC overnight. Next day, take 2 ml of cells add 4 ml of medium and transfer to a new flask, then take 3 ml of cell and add 3 ml of medium and transfer to yet another flask. Keep cells growing at 27ºC until the pool of transfected cells grow up.
10. To the second flask, add selective medium to total ~19.5 ml and distribute 200 $\mu$l aliquots into each well of a 96-well plate. Keep cells at 27ºC until clones grow up (aim for no more than 20 wells per plate, to ensure each is a clone, but not necessarily independent).