***T. brucei* PCF nucleofection with Nucleofector 2b**

1. Count cells. Spin down 1E8 cells per transformation. Include one sample as “mock” (no DNA) control.
2. Resuspend in residual liquid (~0.5 ml). Transfer to microcentrifuge tube. Spin again. Remove supernatant (all but 20 µl). Resuspend cells in residual liquid.
3. Add 100 µl supplemented human T-cell nucleofection solution.
4. Add 10 µg linearized plasmid DNA (add nothing to mock).
5. Add solution to cuvette. Place in Nucleofector 2b device.
6. Nucleofect using program X-014.
7. Remove cells from cuvette using pipette provided and place into 10 ml media without selecting drug.
8. 18-24 h later spin down cells and resuspend in fresh media containing selecting drug.