Title	Nucleofection		
Protocol Number	MOLBIO19002	Written by	Duha A.
Adapted from	CFP lab (N. Douanne)	Version	1.0
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Materials:
Lonza nucleofector

Notes: Cuvette must be kept on ice if they're outside the fridge

Protocol:
1) Take out you're your DNA form -20°C. Measure the DNA concentration. For each sample, you'll need 1 -5 µg of plasmid DNA for successful transfection.
2) Count the parasites: a minimum of $5x10^7$ parasites is necessary for the nucleofection. Calculate the volume required to reach this number of parasites and then pour it into a 15 mL tube.
2) Centrifuge for 5 min at 2000 rpm at 20 ° C. Discard the supernatant
3) Resuspend the pellet in 100 µl of Human T-cell buffer and mix thoroughly
4) Take 100 µL of resuspended solution and place in a new eppendorf
e) Add 1-5 µg of the vector to be transfected (in 1-10uL)
5) For each sample (eppendorfs), transfer all the contents into the electroporation cuvettes (be careful to always identify each sample) and place them in the ice
6) Wipe the metal part of the cuvettes and proceed with nucleofection (program U-033)
7) Immediately add 1 mL of M199 medium (supplemented with FBS and Hemin) to the cuvettes
8) Prepare flasks for culture: 10 ml of M199 medium (supplemented with FBS and Hemin) + 10 µl of Biopterin [10 mM]
9) Transfer the entire contents of each electroporation cuvette into the corresponding flask
10) Homogenize and incubate the flask at 25 °C for 25 hrs.
11) Add the appropriate type and amount of antibiotic to the growing culture.