Transfection of Leishmania promastigotes by electroporation

1. Grow Leishmania to Late-log phase (5-10x10^7/ml cells) in 4ml culture.
2. Transfer 4ml culture to 15ml centrifuge tube and pellet cells at 4500rpm in 4°C for 5 mins.
3. Remove the supernatant and resuspend cells in 4 ml ice cold transfection buffer twice times (step2-3).
4. After wash steps, resuspend pellet in transfection buffer for cell count and adjust the final cell density to 10^8/ml (Keep on ice).
5. Pipet 300 µl cell suspension (3*10^7 cells) into 15-20 µg plasmid DNA (20-30 µl)
6. Chill cells and plasmid DNA on ice for 10 mins.
7. Transfer 320-330 µl mix into 0.2 cm Biorad E cuvette (pre-cold)
8. Electroporate using capacitance extender at 0.45kV, 500 µF and time constant range in 4-6 mllisec.
9. Immediately transfer electroporated cells in the flow hood to 3 ml M199+20% HIFBS for recovering (3-24hr).
10. Check for electroporation efficient (expected 50% cell death). Repeat it if no cell death.

Transfection buffer

1. Prepare autoclaved serum bottles before use.
2. Set up the Transfection buffer as follow:

21 mM	HEPES	(5 g/L)
150 uM	$CaCl_2$	(0.016 g/L)
5 mM	$MgCl_2$	(1.01g/L)
120 mM	KCl	(8.9 g/L)
0.7 mM	NaH_2PO4	(0.08 g/L)
6.0 mM	Glucose	(1.08 g/L)

3. Use 0.22 µm filter to sterilize transfection buffer
4. Keep at 4°C before electroporation .