Electroporation of *Leishmania tarentolae* with Multiporator

- Inoculate *L. tarentolae* preculture 1:20 in 10 ml LEXSY BHI* medium (Jena Bioscience, Cat. No. ML-411) on Friday afternoon and incubate in ventilated tissue culture (TC) flask upright @ 26°C until Monday
- On Monday afternoon dilute preculture 1:10 in 10 ml LEXSY BHI* medium and incubate in TC flask flat @ 26°C o/n
- Grow culture to ca. 6 x 10^7 cells/ml (OD 1.4); ensure by microscopy that the cells are vital and of droplike shape; per transfection 4 x 10^7 cells are needed
- Have ready on wet ice electroporation cuvettes (d=2 mm, long electrodes) and DNA dissolved in IsoOsmolaric Buffer (IOB, Eppendorf)
- Have ready per transfection 10 ml of LEXSY BHI Medium in ventilated TC flasks at 26°C
- Spin *Leishmania* culture 3-5 min at 2000 x g (ca. 20°C)
- Resuspend cells in same volume of HypoOsmolaric Buffer (HOB, Eppendorf)
- Spin *Leishmania* cells again 3-5 min at 2000 x g (ca. 20°C)
- Resuspend cells at 10^8 cells/ml in HOB and keep on ice for 10 min (0.4 ml per transfection)
- Add 5-10 µg DNA in 10-20 µl IOB to 0.4 ml cells, mix well and transfer to electroporation cuvette kept on ice (avoid air bubbles!)
- Pulse with Multiporator (Eppendorf) for d=2 mm cuvettes at 1000 V and 160 µsec
- Return cuvettes with electroporated cells to wet ice for exactly 10 min
- Transfer cells with a Pasteur pipette carefully to 10 ml preincubated at 26°C LEXSY BHI Medium in ventilated TC flasks and incubate o/n at 26°C without selection
- Add selection antibiotic after ca. 20 h and continue incubation at 26°C in suspension culture or plate cells on LEXSY BHI Agar (Jena Bioscience, Cat.No. ML-451) with selection antibiotic(s).

* contains Hemin and PenStrep

R. Breitling, Jena Bioscience 20.01.2010