**T. brucei PCF transfection**

Date :

Cell line :

DNA to transfect :

**Day1 : Everything on ice otherwise stated**

Cell count :

Required volume for 1 tranfection (3.107 cells) :

Spin the cells of culture 800 g (1800rpm in Jouan) for 10 minutes

Washed the pelleted cell **twice** with 10 mL of PBS

Spin @800g for 10 minutes

Resuspend the pellet with 150 µL per transfection of 1x Roditi buffer freshly made.

Add 10 µL of the **DNA** into 150µL of resuspended cells and mix gently

Transfer the mix into a 2 mm electroporation cuvette

Zap in the AMAXA, use program X-001.

Put the cells in 10 mL of pre-warmed medium (10% FCS) with parental only selective drug(s) in a 25-cm2 flask.

Incubate @ 27°C.

**Day2 :**

Add the selective drug into the 10mL flask

- Take 5 ml of the flask and add it to 45 mL of cloning medium with selective drugs

Aliquot 2 mL per well into a 24 wells plate => 1 :10 dilution

- Take 2 mL of the flask and add it to 48 mL of cloning medium selective drugs

Aliquot 2 mL per well into a 24 wells plate => 1 :25 dilution**\*\***

Incubate at 27°C until selection.

**\*\***for neomycin resistance, maybe add another dilution (1:100): 0.5 mL of flask into 48.5 mL cloning medium

**For 100 mL of Cloning medium (make 150 mL if you do 3 dilutions):**

FCS (10% stock, 20% final) 10 mL

Conditioned medium 40 mL

SDM-79 50 mL

**Roditi buffer (950** µl):

600 µl water

350 µl 3xRoditi buffer

100 µl 1.5 mM CaCl2