**BSF Transfection Protocol**

**(AMAXA)**

**Transfection:**

Count the BSF cells in 3.7% formaldehyde in vPBS.

Use between 2x107 and 4x107 cells per transfection from an exponential phase culture (about 3 x T75 flasks per transfection at 1x105 cells/mL the day before).

NB: for BSF 427 SmOx use the upper end of the range and for BSF 427 90-13 HN choose the lower end of the range.

Use 10ug of linearized plasmid per transfection.

For PCR transfections, use 200µl (4 x 50µL) of PCR that was ethanol precipitated, washed twice in 70% ethanol, sterile air dried under the culture hood and re-suspended in 10µL sterile H2O.

Wash the cells once in 5-10 mL of vPBS (spin 10mins, 1900 rpm Jouan centrifuge) then re-suspend in 150µL of transfection buffer (Roditi’s: see below).

Add the plasmid and pipette gently up and down.

Put the cells in the transfection cuvette (2 mm).

Zap in the AMAXA, use the X-001 programme.

Put the cells in 25 mL of pre-warmed medium (10% FCS) in a 75 cm2-aerated flask.

Dilute 1:5 and 1:25 (and 1:100 if using BSF 427 90-13 HN) and aliquot into 24-well plates adding 1 mL per well. Duplicate the 1:25 dilution plate:

7 mL transfection + 28 mL medium 1:5 x1 plate

5 mL dilution 1:5 + 20 mL medium 1:25 x2 plate

(1.25 mL dilution 1:5 + 23.75 mL medium 1:100 x1 plate)

Incubate in a humidified 37°C, 5% CO2 incubator.

24h later add 500µL of medium with 3x concentration of selection antibiotic.

Cells should come up in approximately one week.

**Antibiotics**

Neomycin 2.5µg/mL

Hygromycin 5 µg/mL

Phleomycin 2.5 µg/mL

Puromycin 0.1 µg/mL

Blasticidin 5µg/mL

**1x Electroporation Buffer (store at 4°C a few weeks only)**

(Refer to Gabriela Schumann Burkard, Pascal Jutzi, and Isabel Roditi, Molecular and Biochemical Parasitology 175, no. 1 (January 2011): 91–94, doi:10.1016/j.molbiopara.2010.09.002.)

90 mM NaPO4 pH7.3

5 mM KCL

0.15 mM CaCl2

50 mM HEPES pH 7.3

**Stock solutions:**

-0.5 M NaPO4 pH7.3

0.5 M Na2HPO4 77.4 mL

0.5 M NaH2PO4 22.6 mL 🡪 adjust to pH 7.3 with pH-meter

-0.5 M KCL

-50 mM CaCl2

-0.5 M HEPES pH 7.3 🡪 adjust with KOH

**Sam Dean’s tip :**

You can also prepare a 3x stock that lasts a long time, but should be “completed” with CaCl2 just prior to use.

**3 x Roditi buffer stock:**

200 mM Na2HPO4

70 mM NaH2PO4

15 mM KCl

150 mM HEPES pH 7.3

**Prepare 1X Roditi buffer just before each transfection:**

600 µl water,

350 µl 3xRoditi buffer,

100 µl 1.5 mM CaCl2

**Citation for the electroporation buffer:**

Gabriela Schumann Burkard, Pascal Jutzi, and Isabel Roditi, “Genome-Wide RNAi Screens in Bloodstream Form Trypanosomes Identify Drug Transporters,” Molecular and Biochemical Parasitology 175, no. 1 (January 2011): 91–94, doi:10.1016/j.molbiopara.2010.09.002.

**Subculture:**

**>> The cells concentration should not be above 1.5 to 2.106/mL <<**

**>> They might not recover <<**

* over 24h :

dilute the cells to **105/mL** in a 25cm2 flask with a final volume of 5 mL (or 15 mL in a 75 cm2 flask => keep the ratio 1mL / 5cm2 for flasks)

* over the week-end :

dilute the cells to **103/mL** in a 25cm2 flask with a final volume of 5 mL (or 15 mL in a 75 cm2 flask => keep the ratio 1mL / 5cm2 for flasks)

**Defrosting cells:**

Prepare a 24 well plate with 4 wells of adapted pre-warmed medium (1mL/well).

Defrost the vial and load 1 drop of the frozen stock in 1 well then 2 drops in the next well, then three drops in the following well and in the last well, load what is left in the tube.

**Freezing cells:**

Spin down 10 ml of **5.105 cells/mL** culture ( 800g, 10min), re-suspend the pellet of cells into 1 mL of IMDM complete + 7% glycerol. Aliquots in two cryotubes. Freeze them into a Mister Frosty box at -80°C. The day after put the tube into the liquid nitrogen container.

**BSF culture medium (IMDM incomplete), per 1 litre:**

* 3.024 g Sodium Bicarbonate (NaHCO3, Euromedex 6885-1)
* 0.136 g Hypoxanthine (Sigma H-9636)
* 0.110 g Sodium pyruvate (Sigma P-3662) Keep at 4°C.
* 0.039 g Thymidine (Sigma T-9250)
* 0.028 g Bathocuprone sulfate acide (Sigma B-1125).
* 1 bottle of IMDM powder for 1 litre (Iscove's Modified Dubecco's Medium, Gibco ref: 42200-014 #3011115). Kept in fridge at 4°C.
* 1 litre of Aguettant water.

Mix the powders and the IMDM into a 1 liter bottle with Aguettant water qsp 1 litre.

Filter sterilise (0.22 µm filter) (Nalgene) and aliquot 400 mL into bottles of 500 mL.

Store the media at + 4°C.

**IMDM complete**

Before use the IMDM incomplete, add in 400 mL of medium:

* 1 mL of 100 mM β mercaptoethanol (Sigma M-6250 stock: 14.3 M) in H20.
* 5 mL of 150 mM L-cysteine (Sigma C-1276, 0.2364 g in 10 mL water) in H20.
* 40 mL of FCS (Gibco) heated at 56°C 30 minutes.
* 2.5 mL 10 mg/mL Kanamycin (Sigma K-4000).

Filter sterilise, 0.22 µm.

Store the medium at + 4°C.

**10x vPBS(-Sucrose-Glucose) (Voorheis’s modified PBS):**

NaCl 80 g/l

KCl 2.2 g/l

Na2HPO4 22.7 g/l

KH2PO4 4.4 g/l

Adjust to pH7.4 and autoclave.

**For VPBS 1x:**

Dilute the 10x VPBS with 15.7 g/L sucrose and 1.8 g/l glucose final concentration.