**BSF transformation solution**

**(AMAXA)**

**Transfection :**

Count the BSF cells in 3.7% formaldehyde in vPBS

Use 4.107 cells per transfection 427 90-13 single or double marker from an exponential phase culture (about 3xT75 flask per transfection at 1.105 the day before).

Use 10ug of linearized plasmid per transfection (resuspend the plasmid in 10ul H2O).

Wash the 1x in 5-10 mL of vPBS (spin 10 min, 1900 rpm Jouan) then resuspend in 100ul of transfection buffer.

Add the plasmid and pipette gently up and down

Put the cells in the cuvette (2 mm)

Zap in the AMAXA, use program Z001.

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

Dilute 1 :5, 1:25 and 1 :100 and aliquot in 24-well plates (1 mL)

7 mL transfection + 28 mL medium 1:5

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

1.25 mL dilution 1:5 + 23.75 mL medium 1:100

Incubate in humidified 37°C, 5% CO2 incubator

24h later add 500ul of medium with 3x antibiotics

Cells should come up in a 1-week.

**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

**Antibiotics**

Neomycin 2.5ug/mL

Hygromycin 5 ug/mL

Phleomycin 2.5 ug/mL

Puromycin 0.1 ug/mL

Blasticidin 5ug/mL

**Culture medium (IMDM complet)**

* 3.024 g Sodium Bicarbonate (NaHCO3, Sigma S-6014)
* 0.136 g Hypoxanthine (Sigma H-9636)
* 0.110 g Sodium pyruvate (Sigma P-3662)
* 0.039 g Thymidine (Sigma T-9250)
* 0.028 g Bathocuprone sulfate acide (Sigma B-1125).
* 1 bottle of IMDM powder for 1 liter (Iscove's Modified Dubecco's Medium , Gibco ref: 42200-014 #3011115).
* 1 liter of Aguettant water.

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

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

Store the media at + 4°C.

Before to use it :

In 400 mL of medium add :

* 1 mL of 100 mM β mercaptoethanol (Sigma M-6250 stock : 14.3 M)in water.
* 5 mL of 150 mM L-cysteine (Sigma C-1276 , 0.2364 g in 10 mL water) in water.
* 40 mL of FCS heated at 54°C 30 minutes.
* 2.5 mL 10 mg/mL Kanamycine (Sigma K-4000).

Filter sterilise (0.22 um).

Store the medium at + 4°C.

**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)

**Defreezee stabs :**

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

Defreeze 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.

**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 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.

**Freeze stabs :**

Spin down 10 ml of **5.105 cells/mL** culture( 800g, 10min), resuspend the pellet of cells into 1 mL of IMDM complet + 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.

**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.