Schizont Transfection using Amaxa Nucelofector – Treeck Lab (June 2026)

Preparation of DNA

The total amount of DNA used per transfection is a minimum of 10 ug of each plasmid (but ideally around 25 ug) in a maximum volume of 10 ul TE. If transfecting a Cas9-sgRNA plasmid with a repair template, make sure to linearise the repair template and use a molar ratio of 1:6 of gRNA plasmid to repair template. Use this website to calculate molarity of your DNA (https://www.promega.co.uk/resources/tools/biomath/)

- Precipitate DNA in 2 volumes of 100% EtOH and 0.1 volume 3M Sodium Acetate. You can also precipitate and use the DNA straight away or incubate overnight, but overnight incubation is not necessary.
- Pellet the DNA by spinning at max speed (~20,000 rcf) for 2-5 minutes at room temperature and wash with 700-1000 ul of 70% EtOH twice
- After final EtOH wash, add 700 ul of 70% EtOH, and invert the tube a few times to make sure that the entire internal surface is decontaminated with the EtOH.
- Transfer the tube to a biosafety cabinet and remove as much of the EtOH as possible, then dry the pellet for 15 minutes (residual EtOH will evaporate; make sure to dry completely – DNA will look almost transparent when fully dry)
- Resuspend the DNA in 10 µl TE (sterile) and proceed with the transfection or store in -20ºC

Preparation of erythrocytes for invasion after transfection

- Mix 300 ul of packed erythrocytes and 1.5 ml Complete Media in small tissue culture flask (T25) for each sample
- Preheat to 37ºC while preparing parasites

Preparation of parasites

*Note 1: Generally, each transfection requires ≥25 ul schizonts. However, using as little as 10 ul schizonts has been successful.

*Note 2: In our experience quantity is more important than quality (i.e., synchronisty).

- In the cycle before, purify schizonts on Percoll cushion and allow the schizonts to invade fresh erythrocytes while shaking. If possible, perform a second synchronisation ~4h later with Sorbitol.

- On the day of transfection, purify schizonts on Percoll cushion once most begin to rupture, then place in RPMI such that there are 25 µl schizonts/500 µl RPMI
- Transfer 500 µl aliquots of resuspended parasites to microcentrifuge tubes
- Pellet parasites briefly in microcentrifuge
- Try to keep parasites warm until you are ready to transfect, since they will slow down and not egress if left at room temperature for too long.

Transfection

- Label AMAXA cuvettes beforehand and pre/open AMAXA pastettes in the biosafety cabinet, since these can be a bit fiddly to open.

- Add 100 ul of supplemented P3 to your DNA that was previously resuspended in 10 ul TE (P3 buffer is supplied with the Lonza transfection kit; beware that this goes off 3 months after supplementation)
- Remove supernatant from pelleted parasites (from above).
- Resuspend parasites in DNA/P3 transfection solution mixture
- Transfer to the appropriately labelled AMAXA cuvette
- Transfect in AMAXA device using setting FP158 for P3 Primary cells
- After transfection, immediately remove cuvette and transfer the parasites with the special AMAXA pastette to a flask with erythrocytes and medium – it is crucial that this is done as quickly as possible.
- After all transfections are done, incubate the flasks with the blood and transfected parasites in the shaking incubator at 37˚C for at least thirty minutes
- Add 8 ml RPMI and place in 37˚C incubator.

Selection and transfection aftercare

The following day, replace medium with medium containing the appropriate drug

Selectable Marker Genes	**Drug**	**Working Concentration**	**How long for parasites to die fully? (Time kept on drug)**	**Selection procedure**
hDHFR	WR99210 OR Trimethoprim (TMP)	WR: 8x IC50 (= 0.59 nM) OR TMP: 8x IC50 (= 10 uM)	4-6 days	Can be added immediately or the day after transfection
BSDr	Blasticidin S deaminase	8x IC50 (=5 µg/ml)	7-10 days	Add the day after transfection
NeoR	Neomycin (G418)	225 µg/ml	7-10 days	Add the day after transfection
PKG_{T618Q}	ML10	50 nM	7-10 days	Can be added immediately or the day after transfection
yFCU	Ancoti (5-fluoroytosine)	1 mM	2 weeks	Negative selection

Feed transfections every 2-3 days. Make sure to add ~50 ul of fresh blood every week to prevent catastrophic lysis.

Start smearing cultures ~7-10 days post transfection and continue to monitor regularly from this point onwards.