Transfection of *P. falciparum*

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Equipment:

- Gene Pulser electroporator with Capacitance Extender (Bio-Rad)
- Electroporation cuvettes (0.2 cm gap) (Bio-Rad)
- 37ºC incubator chamber with proper gas mixture
- Microscope

Materials and Reagents:

- *P. falciparum* culture
- Washed human RBCs (50:50)
- Complete media (with drug(s) if appropriate)
- RPMI 1640 for washing RBCs
- Sterile 6-well tissue culture plates
- WR99210, blasticidin S HCl, G418, pyrimethamine, etc. (as needed)
- QIAGEN Plasmid Maxi kit
- Amicon Microcon columns (Millipore)

Buffers:

- Cytomix (120 mM KCl (anhydr); 0.15 mM $CaCl_2$; 10 mM KH_2PO_4 (anhydr); 25 mM HEPES; 2 mM EGTA; 5 mM $MgCl_2$; Adjust pH to 7.6 with KOH)

Preparation of parasites:

- You will need 80-100 µl of at least 5% ring infected RBCs per transfection.
- Ensure the cells are as healthy as possible, at the correct stage, and around the ideal parasitemia for transfection.

Preparation of DNA:

- You will need 50-100 µg of each plasmid per transfection.
- Plasmid isolation using Llinás Lab plasmid mini preparation protocol or QIAGEN Plasmid Maxi Kit (o/n cultures of 500 ml is recommended for the Maxi kit).
- Resuspend isolated plasmid DNA in filtered sterile cytomix (350-500 µl).
- Make a 1:10 dilution of plasmid DNA and spec on Nanodrop. Determine DNA concentration and volume of sample needed per transfection. If needed, concentrate DNA using Amicon Microcons (YM-30). Follow the manual provided, remembering to rinse the reservoir with sterile water prior to applying your sample, never let the membrane dry out, and practice sterile technique (this DNA and buffer are going into your cultures).
- Store plasmids in cytomix at 4ºC, so that whenever the parasites are ready, transfection may be performed.

Transfection of parasites:

- Smear and change the media of the parasites early in the morning (usually about 3 hours before transfection).
- Determine the parasitemia and decide whether the culture is adequate for transfection (look for large, healthy rings).

- Have everything ready to go, then start! Be nice to your parasites!! Try and finish the whole transfection procedure in about 30 min from the time the parasites are harvested to when the transformed parasites are returned to the incubator.
- Remember sterile technique. Do as much as possible in the hood.
- Label all electroporation cuvettes, tubes, and TC plates prior to transfection.
- Prepare labeled microcentrifuge tubes with 50-100 µg of plasmid(s) per transfection.
- Aliquot into a labeled 6-well TC plate 2.5 ml of media, and 300 µl of fresh 50:50 washed RBCs per well/transfection. These volumes should ensure a final culture volume of 5 ml, with 4% hematocrit.

1. For any standard 2% HCT culture at 5% parasitemia, remove 5 ml of resuspended culture per transfection. Spin 1300 rpm, 4 min RT, low brake. Aspirate S/N, being careful not to suction cells.
2. Resuspend cells in same volume of cytomix, and gently mix. Spin 1300 rpm, 3 mins RT, low brake.
3. Aspirate S/N as above and resuspend cells with ~325 µl of cytomix per transfection. Add 300 µl to appropriate DNA containing microcentrifuge tube. If needed add cytomix to bring the final volume to 400-500 µl (~425µl). Mix and add to appropriate cuvette. Avoid bubbles!
4. Take the following with you to the electroporator: cuvettes, sterile complete media (1 ml per transfection), P1000 and tips, pen and paper to write time constants.
5. **Electroporate cells**:
   - Settings—2 mm cuvette setting, 0.31 kV, 950 µF, max capacitance. **Make sure the red/black leads are plugged into the Gene Pulser II apparatus, NOT the Pulse Controller II/PLUS**. NO ADDITIONAL resistance is needed.

6. Insert cuvette into shocking chamber and slide into place. Press both Pulse buttons and release when beep is heard. Record time constant. Time constants between 7 and 11 msec are good. You will always hear a slight "pop." If you chill the cuvettes BRIEFLY (<30 secs) immediately before zapping, they will not pop as much into the top of the cuvette.

7. Immediately after electroporating, add 1 ml CM to each cuvette (aseptically on the bench). BE GENTLE, add the media slowly down the side of the cuvette.
8. When all electroporations are finished, transfer them back to the hood.
9. GENTLY, resuspend the cells by pipetting up/down in the cuvette to remove all/most of the cells and transfer to your pre-prepared wells, but DO NOT take any crusty cell debris. Wash cuvettes with another 1 ml of CM and add to well.
10. Place plates back in incubator. This is Day 0.
11. One to two hours post-transfection, aspirate the media and any cell debris on well walls. GENTLY resuspend the cells with 5 ml of fresh media. The next day change media with standard RPMI. On day two add media with appropriate drugged RPMI to select for your plasmid(s). NOTE: Make sure to use the proper drug containing media! One or more drugs may be needed depending on your plasmid(s). Adding drugged media at this time will ensure that your parasitemia will not get too high causing the parasites to become sick. (Some common concentrations used for 3D7 are: 2.5 nM WR99210, 1.5 μg/ml Blasticidin, and/or 125 μg/ml G418).

Maintenance of transfected parasite lines

- Change the media every day for the first 6 days using drugged media.
- Monitor the parasitemia with 2 μl smears. You will need to cut your culture or split into 2 wells if the parasitemia is over 5-6%, because the drug will not kill the parasites quickly enough (2.5 nM WR99210 kills by day 4, and 2.5 μg/ml Blasticidin kills by day 6).
- After day 6, change media every other day and smear (2 μl) every four to seven days to check for parasites.
- On day 6 post-transfection add an extra 100 μl of 50% washed RBCs to each 5 ml culture. On day 10 there should be minimal parasite debris left, with few gametocytes and RBCs should be in good condition.
- If there are a lot of gametocytes (>1%) on days 6-10, your culture is very stressed, and it may take longer for parasites to appear.
- Between days 10-14 cut cultures between 3:5 or 1:2 to add fresh RBCs and remove old lysing cells, and then again every 7-8 days.
- Fresh RBCs need to be added to the culture each week. A culture with a high % of old cells (14 days +) will lyse. Your cultures are about to lyse if they are very dark despite having a low parasitemia, >50% of the RBCs on your smear is blebby, or if you can see a layer of reddish color immediately over your cells from the side view. Alternatively, you can add 50-80 μl of fresh RBCs to culture to provide new cells or if the hematocrit is low, but this does not get rid of old, lysing cells!!!

When parasites come up...

- For episomal, attB, or piggyBac systems, you should see parasites between day 14-28, but transfections may come up as early as day 10, or as late as day 45, depending on the parasite strain, health of starting culture, stress during transfection, parasitemia on day 2, care during the first 10 days, etc.
- Hopefully, between days 45-90 (as earlier as day 24, and as late as, day 150) you might get stable recombinant integrants of the plasmid into the genome (these integrants will grow quicker than episomally transformed). Extract gDNA and perform PCRs to confirm integration. NOTE: Episomally transformed cultures grow slower than normal cells.
- As soon as parasitemia is at least ~2% TP (greater than 50% rings), freeze down cultures. You should freeze down two aliquots on 2 separate days. Remaining culture should be expanded

and harvested for genomic DNA extraction (for microsatellite analysis, plasmid rescue, and/or PCR screening for integration).

- Once genomic DNA is extracted, culture can be sized-down to 2 ml and should be kept at low parasitemia, such that they will need cutting twice a week (Monday and Friday), and feeding every other day.
- Transfected cultures should be frozen down once a month (2 aliquots).

Freezing Protocol

- From a 5 ml culture 4-5% hematocrit of at least 2% TP (greater than 50% rings), spin down 2.5 ml, 1300 rpm, 4 min RT, low brake. Aspirate media. Gently flick the pellet loose and add 1 ml of Glycerolyte 57 solution DROPWISE. Aliquot 0.5 ml per cryovile labeled with %Rings, Date, Strain, Gene Integrated, and your initials. Use these proportions if you want to freeze a larger or smaller volume (5 ml culture to 2 ml of Glycerolyte 57 or 1.25 ml culture to 0.5 ml of Glycerolyte 57).