Establishing malaria parasite transfection technology in South Africa

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Introduction

- The most important contributing factor to the current malaria crisis is the **rapid spread of parasite resistance** to available anti-malarial drugs. Anti-malarial drug resistance is critical and the need for compounds with novel modes of action is beyond the point of urgency. A rational and a deliberate target-based drug discovery strategy as opposed to a “shotgun” screening-approach requires specific, well-characterized and **validated drug targets**.
- **Gene manipulation** is the only means by which the functions of individual proteins in living cells can confidently be confirmed and is thus **a fundamental requirement in target-based drug discovery**.

- Genetic manipulation of the malaria parasite, *Plasmodium falciparum*, is usually performed via **plasmid-based transfection** similar to those used in other eukaryotic organisms.
- However, a **major obstacle** faced by malaria research laboratories world-wide is the **poor transfection efficiencies** of current protocols due to the unique technical problems faced i.e. AT-richness and **intracellular location of the organism**.
- As a result successful transfection often requires **prolonged periods** (up to 2-3 months) of constant and patient culturing and selection. In addition, plasmids usually have a complicated composition and require lengthy cloning manipulations to prepare. **Improvements to the methodology would have wide impact and is constantly being sought**.

Objectives

- To successfully transfect malaria parasites with foreign genes
- To improve malaria parasite transfection efficiency in order to shorten the transfectant selection time
- To use the technology to validate promising drug targets

Methodology

- Parasite culture and transfection
  - *P. falciparum* 3D7 asexual cultures were maintained *in vitro* according to Trager and Jensen [1]
  - Parasites were transfected with 100 μg plasmid via electroporation (0.31 kV, 960 μF) [2]
  - Transfected parasites were selected for by 2.5 nM of the antifolate WR99210

- WR99210 culture media was changed daily for the first 6 days after which media was changed on alternative days until parasites appeared
- Parallel cultures were kept stationary and shaken at 50 rpm
- Cultures were visualized with microscopy

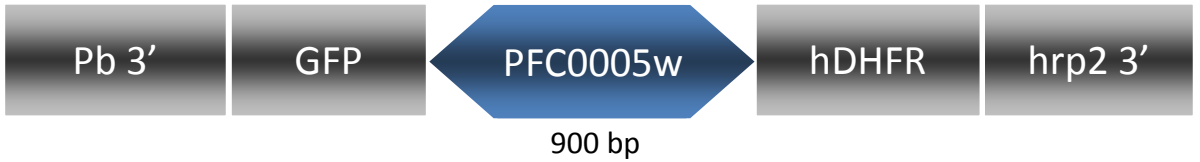
- Plasmid constructs
  - All constructs were prepared in NEB5α *Escherichia coli* cells using standard molecular biology techniques
  - The pHTK plasmid [3] was used for insertion of a foreign gene (GFP, luciferase) to replace thymidine kinase in the transgene cassette – control construct
  - The promoters of both cassettes of pHTK [3] were replaced with a single bidirectional promoter, the *var* intron PFC0005w [4]. A foreign gene (GFP, luciferase) replaced thymidine kinase in the transgene site – bidirectional construct

- Foreign gene expression
  - GFP expression was visualized by fluorescence microscopy
  - Luciferase expression was determined by RT-PCR
    - DNase-treated RNA was reverse transcribed and amplified with luciferase-specific primers
    - PCR products were visualized by agarose gel electrophoresis

Construct diagrams

Control construct

Bidirectional construct



Pb 3’- *Plasmodium berghei* 3’ termination region; GFP- *Renilla reniformis* humanized recombinant green fluorescent protein II; PFC0005w- *var* intron [4]; hDHFR- human dihydrofolate reductase; hrp2 3’- histidine-rich protein 2 termination region; h86 5’- heat shock protein 86 promoter; CAM 5’- calmodulin promoter

Results

- GFP-control construct

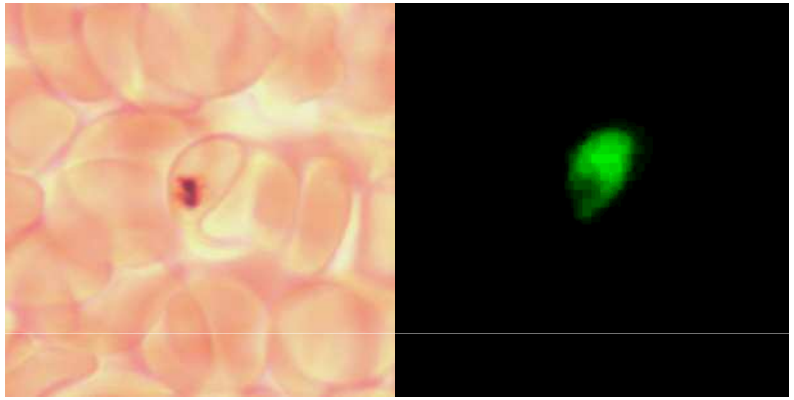


Fig 1: Bright field and fluorescence microscopy of GFP-control transfectants, only visible after 44 days, showing GFP expression.

- GFP-bidirectional construct

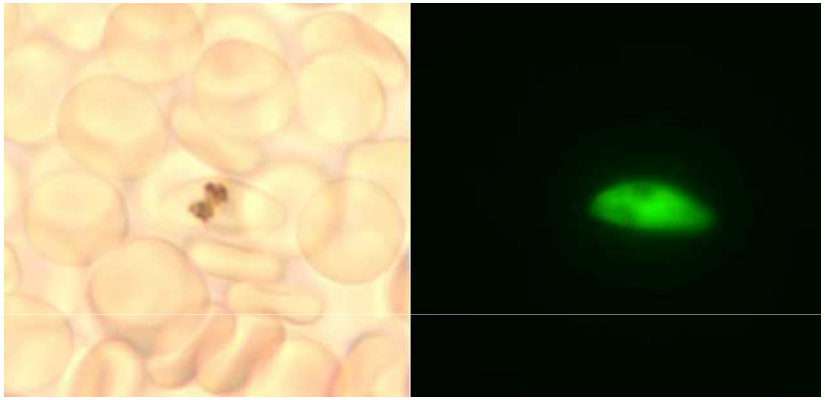


Fig 2: Bright field and fluorescence microscopy of GFP-bidirectional transfectants, already visible after 25 days, showing GFP expression

- Luciferase-bidirectional construct

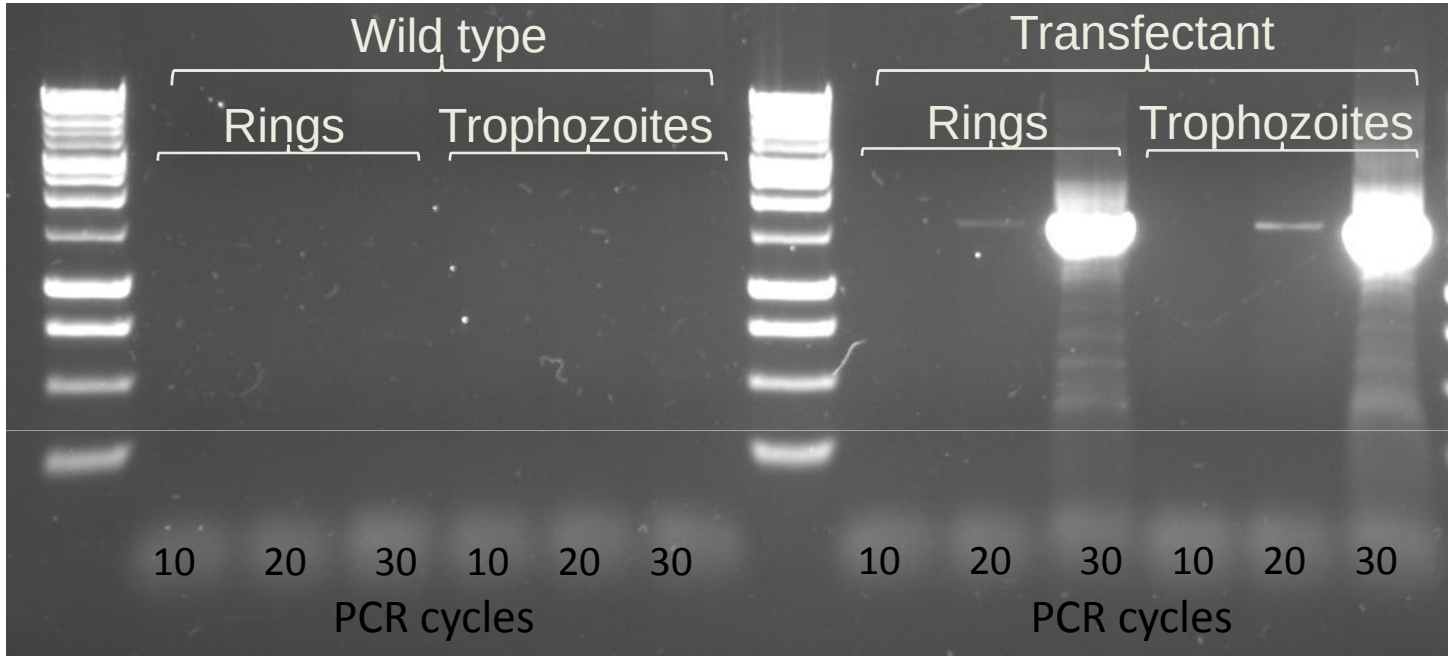


Fig 3: Gel electrophoresis of luciferase transcripts of wild type parasites vs. luciferase-bidirectional transfectants, produced via RT-PCR with luciferase-specific primers. The foreign luciferase transcripts are not present in the wild type parasite but are visible in the transfectant rings and trophozoites after 20 amplification cycles.

- Stationary vs. shaken transfectants

Table 1: Number of days before transfected parasites appeared in stationary vs. shaken cultures

	Stationary	Shaken
GFP-bidirectional transfectants	32 days n = 1	26.5 (±1.5) days n = 2
Luciferase-bidirectional transfectants	34 (±1) days n = 2	28 days n = 1

  - A similar result was recently reported [5]

Conclusion

- Both the control construct and bidirectional construct successfully transfected *P. falciparum* parasites
- However, transfectants from the larger control construct took almost 20 days longer to appear
- The smaller bidirectional construct therefore significantly improved transfection efficiency
- Shaking the transfected cultures also resulted in a slightly reduced selection time compared to stationary cultures
- Malaria parasite transfection technology is now established in South Africa

Future perspectives

- Applications:
  - The control construct (with separate promoters for the two cassettes) will be used to investigate transcriptional regulation (Promoter study)
  - The bidirectional construct will be used to determine the effect of overexpressing potential drug targets (Biomarker study)
  - Promising drug targets from functional genomics data are currently being validated
  - Two successful transfectants are under evaluation

References

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[5] Allen R. and Kirk K. (2010) *Mol. Biochem. Parasitol*. **169**, 63-65.

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