

## Establishing malaria parasite transfection technology in South Africa

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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 such 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.

In order to establish malaria parasite transfection technology in South Africa, firefly luciferase and green fluorescent protein (GFP) reporter constructs were prepared. In attempt to simplify these constructs, a *var* intron (PFC0005w), previously reported to have bidirectional promoter activity [1], was utilized to drive expression through two genes (i.e. the antibiotic-resistance gene, human *dhfr* and the reporter gene) in a head-to-head orientation. In addition, protocols were adjusted by including DNA packaging reagents to improve uptake into the parasite and by using shaking instead of stationary parasite cultures to improve the parasite proliferation and selection rate. Successfully transfected parasites were selected by the antifolate WR99210.

The first transfected parasites appeared after 33 days indicating that the *dhfr* gene was expressed at sufficient levels to produce drug resistant parasites. Luciferase and GFP expression was also confirmed. The established technology will be applied in the future to validate potential targets as identified by CSIR functional genomics investigations.

[1] Epp D., Raskolnikov D. *et al.* ***Malaria Journal*** 7:86 (BioMed Central, 2008).