A-24: Transfection of malaria parasites and transient expression of firefly Luciferase

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The objective of this study was to develop a method for the transfection of malaria parasites i.e. introduction and expression of foreign genes within the parasites. Although transfection has been achieved with prokaryotic cells such as bacteria and even mammalian cells, the only parasite organisms to have been successfully transfected were *Leishmania* and *Toxoplasma* species.

The malaria parasite presents a unique challenge for transfection because it is intracellular for most of its life cycle: therefore introduced DNA must cross multiple membranes before reaching the parasite nucleus. Since these multiple barriers would be likely to reduce the efficiency of introducing DNA into the parasite a parasite stage which was extracellular, the female gametes and fertilized zygotes was chosen. The pgs28 gene of *Plasmodium gallinaceum* is expressed at high levels in the gametes (sexual stage) and fertilized gametes (zygotes). This gene has been cloned with sufficient flanking DNA to ensure that the necessary 5' and 3' controlling elements for the expression of the gene was available for developing a transfection vector. The transient transfection vector was developed by constructing a chimeric gene in which the firefly luciferase gene was inserted in frame into the coding region of the pgs28 gene of *P. gallinaceum* (pgs28.1 luc). 100μg of the plasmid DNA (pgs28.1 luc) was introduced into *P. gallinaceum gametes* and fertilized zygotes ($1x10^7$ /200μl) by electroporation 25uF & 0.5KV), and luciferase expression assayed in 24 to 48 hours. Luciferase activity was detected only in the gametes/zygotes transfected with the pgs28.1 luc and not in parasites electroporated with control plasmids. This study demonstrate the first successful introduction and expression of a foreign gene in malaria parasites and demonstrate the feasibility of this approach to the development of methods for the functional analysis of parasite genes.