Despite a lot of efforts to control malaria, it still remains a major health problem. With the new development of transfection technology, it is now possible to determine the structure-function relationship of vaccine candidates. The aim of this study was to develop a baboon (Papio anubis) model for transfection and analysis of host-parasite interface of transfected P. knowlesi parasites. One baboon was infected with wild-type P. knowlesi parasites for generation of parasites to be transfected. At peak parasitaemia, the baboon was anaesthetized bled and blood stream parasites were harvested, transfected with DNA plasmid constructs containing pyrimethamine resistant form of dihydrofolate reductase thymydylate synthase (dhfr-ts) gene from Toxoplasma gondii as selectable marker and monkey interferon gamma (IFN-γ) gene as the transgene. Both the selectable marker and the transgene were engineered for expression under control of P. berghei DNA regulatory sequences. Equal volumes of electroporated parasites were injected into two baboons, followed by a daily oral administration of pyrimethamine. Transfected parasites were detected in peripheral blood at day 10 post-transfection. At day 15 post-transfection, blood was collected from the baboons, subjected to Plasmodipur filtration to remove leucocytes and used for DNA isolation. Analysis of isolated DNA by PCR showed presence of T. gondii dhfr-ts and IFN-γ genes in transfected parasites. Enzyme Linked Immunosorbent Assay for IFN-γ showed release of significant levels of IFN-γ by transfected parasites. These studies have developed a P. knowlesi transfection protocol, which involves in vitro gene insertion and subsequent selection of transfected parasites in a baboon system. This opens new possibilities for using the P. knowlesi-baboon model in vaccine development using cutting edge technology.