*Online Supplemental Material for*

**Pradel et al. http://www.jem.org/cgi/doi/10.1084/jem.20031274**

## Supplemental Materials and Methods

*RNA Isolation and Transcript Level Analysis.* RNA from mixed asexual stages (rings, trophozoites, and schizonts) or from mature, transformation-competent gametocytes was isolated from pellets of intact (i.e., not saponin-lysed) intraerythrocytic parasites using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. RNA preparations were treated with RNase-free DNase (Invitrogen) to remove genomic DNA contamination, followed by phenol/chloroform extraction and ethanol precipitation. Synthesis of cDNA was accomplished using a Superscript II cDNA synthesis kit (Invitrogen). Real time PCR assays were performed using gene-specific primers by conventional thermocycling or via the SYBR Green incorporation method using a LightCycler system PCR machine (7900HT Sequence Detection System; Applied Biosystems).

*Southern Blot Analysis.* Genomic DNA was isolated from mature gametocyte cultures of NF54 wild-type parasites, *PfCCp2*-KO clones D-11H and F-1D, and *PfCCp3*-KO clones H-3D and I-9C by lysis of erythrocytes with 0.2% saponin and subsequent incubation of parasites in TSE buffer containing 1.5% SDS and 0.5 M $NaClO_4$ overnight at room temperature. DNA was purified by phenol/chloroform extraction and ethanol precipitation. Restriction digest patterns diagnostic of wild-type versus disruptant loci having an integrated a KO construct were guided by genome sequence analysis of the extended *PfCCp2* or *PfCCp3* locus (see Fig. 8 A). 1 μg genomic DNA was digested with three different combinations of restriction enzymes (see Fig. 8, B and C) and restriction digest products were separated by agarose gel electrophoresis and transferred to Hybond N membrane (Amersham Biosciences). A 418 bp hybridization probe corresponding to *PfCCp2* was amplified by PCR using a gene-specific 5′ primer, 5′-TTAGATGGATGTATTACTGC-3′ and 3′ primer, 5′-GCACTGAATCTGGAGCAGTATCC-3′, and a 454-bp probe corresponding to *PfCCp3* was amplified using 5′ primer, 5′-GGTGTAAAGCCACGTTTG-3′ and 3′ primer, 5′-AGTACCTGTTGTAGCTGC-3′. The gel-purified hybridization probe was labeled with digoxigenin and Southern hybridization was performed according to the manufacturer's instructions using the DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Diagnostics).


J. Exp. Med. © The Rockefeller University Press • 0022-1007
Volume 199 June 7, 2004
http://www.jem.org/cgi/doi/10.1084/jem.20031274