**Supplemental Figure Legends**

Figure S1. A schematic representation of the integration of the drug cassette into the genome of *P. berghei.* The PCR product (top) containing the *hdhfr* gene is integrated into the gene locus of PBANKA_103430 (middle) by a double crossover. The transcription of the *hdhfr* gene was controlled by the *P. berghei* elongation factor 1α promoter and terminated by the 3'-UTR of the dihydrofolate reductase-thymidine synthase (*dhfr-ts*) gene. The *hdhfr* gene conferred pyrimethamine resistance to the transgenic parasite.

Figure S2. (A) Three independent genomic libraries were made from the artificial drug-resistant parasites with PACv2, and the parasites that acquired resistance were then screened. CHEF electrophoreses of the resultant parasite populations were performed followed by Southern blot analyses using the *hdhfr* gene as the probe DNA. These analyses clearly show the presence of the drug resistance *hdhfr* gene in all parasite populations, demonstrating that it can be identified in each trial. (B) The parasites selected in trial 1 were further cloned using the limiting dilution procedure. Southern blot analysis using the *hdhfr* gene as the probe DNA shows that all of the cloned parasites included PACv2 that contained the *hdhfr* gene. The inserted DNA fragments of PACv2 in each clone were numbered as in Fig. S2C (below). (C) The sequence analyses of the DNA fragments inserted into PACv2 show that these parasites were derived from at least two independent clones: 15171-bp

and 12926-bp DNA fragments containing the *hdhfr* gene were introduced into PACv2. The upper section schematically represents the genomic sequence surrounding PBANKA_103430 with the *Hind*III recognition sites.