# Supplemental Data

## IDENTIFICATION OF THE COMPETITOR RNA SEQUENCE

To identify a competitor sequence, 100 randomized versions of the dual hybridization acceptor probe sequence were generated (http://www.molbiol.ru/eng/scripts/01_16.html). Each sequence was compared against the genomes of all *Plasmodium* spp. by nBLAST (www.plasmodb.org) and *Homo sapiens* (NCBI nBLAST). Estimates for melting temperature ($T_m$), hairpin formation, self-dimer formation, and heterodimer formation (with forward PL1473 and reverse PL1679 *Plasmodium* genus-specific primers and *P. falciparum*-specific donor PFALF and acceptor PFALR dual hybridization probes) were made using OligoAnalyzer 3.1 (http://www.idtdna.com/ANALYZER/Applications/OligoAnalyzer/). Candidate sequences were sequentially eliminated if they had 1) homology to any *Plasmodium* sequences (e-value < 1.0); 2) homology to any *Homo sapiens* sequences (e-value < 1.0); 3) predicted hairpin formation with Gibbs free energy ($\Delta G$) < –3.0 kcal/mol or formation at > 45.0°C; 4) predicted self-dimer formation at $\Delta G$ < –7.0 kcal/mol; 5) predicted heterodimer formation with any of the RT-PCR reagents at $\Delta G$ < –7.0 kcal/mol; 6) dinucleotide runs of 4 pairs or more; 7) single nucleotide runs of 4 bp of more; or 8) calculated $T_m \leq 45.0$°C.

Most candidate sequences were eliminated based on homology to *Plasmodium* spp. (86 sequences). Fourteen candidates lacked homology to *Plasmodium* spp. or *H. sapiens.* Nine of these had unfavorable predicted hairpin characteristics, and four had unfavorable predicted self-dimer or heterodimer formation or long di- or single nucleotide repeats. One sequence with no significant homology to *Plasmodium* spp. or *H. sapiens* had generally favorable predicted chemical self- and component-binding behavior (ATGTGTAATTATTATAAGAACTCAT). To increase the $T_m$ of this sequence and reduce the likelihood of self-dimerization, the sequence was modified by nucleotide substitution. On this basis, a competitor RNA acceptor probe binding sequence was selected; this sequence contains two more G/C bases compared with the native A-type *P. falciparum* 18S rRNA sequence (TAGTGTTATCAGTATAAGAACTCAT).

## PLASMID INSERT SEQUENCES

Plasmid insert (+) sense sequences are listed from the upstream transcriptional start site in the T7 promoter ("g") to the downstream EcoRI restriction digest position ("g"); the actual RT-PCR amplicon sequences are underlined.

**18S rRNA standard.** gCGAATTGGGTACGATCGATGCGGCCTCCGTGAATCCGATTTGTCTGGTTCATTCCGATAACGAACGAGATCTTAACCTGCTAATTAGCGGCGAGTACACTATATTCTTATTTGAAATTGAACATAGGTAACTATACATTTATTCAGTAATCAAATTAGGATATTTTTATTAAAATATCCTTTTCCCTGTTCTACTAATAATTTGTTTTTTACTCTATTTCTCTCTTCTTTTAAGAATGTACTTGCTTGATTGAAAAGCTTCTTAGAGGAACATTGTGTGTCTAGCACAAGGAAGTTGAAGGCAGCAACAGGTCTGTGATGTCCTCAGATGAACTAGGCTGCACGCGTGCTACACTGATATATGGAACGAGTTCCTAAGACTATGCTTATATTCGTATCTTTGCTTATTg

**Competitor RNA standard.** gCGAATTGGGTACGATCGATGCGGCCTCCGTGAATCCGATTTGTCTGGTTCATTCCGATAACGAACGAGATCTTAACCTGCTAATTAGCGGCGAGTACACTATATTCTTATTTGAAATTGAACATAGGTAACTATACTAGTGTTATCAGTATAAGAACTCATATTTTTATTAAAATATCCTTTTCCCTGTTCTACTAATAATTTGTTTTTTACTCTATTTCTCTCTTCTTTTAAGAATGTACTTGCTTGATTGAAAAGCTTCTTAGAGGAACATTGTGTGTCTAGCACAAGGAAGTTGAAGGCAGCAACAGGTCTGTGATGTCCTCAGATGAACTAGGCTGCACGCGTGCTACACTGATATATGGAACGAGTTCCTAAGACTATGCTTATATTCGTATCTTTGCTTATTg