Supplemental Figure S1. Microscopic blood smear of *P. falciparum* cultures. Examples of iRBCs containing (A) a single ring, (B) multiple rings, (C) early trophozoites, and (D) late trophozoites.
Supplemental Figure S2. Flow sort single-ring iRBC of *P. falciparum* culture. Distribution of uninfected RBCs and iRBCs in synchronous (A) and asynchronous (B) *P. falciparum* cultures stained by Hoechst 33342 and thiazole orange, which emitted fluorescence filtered by DAPI and FITC, respectively. Infected RBCs and single-ring iRBCs were gated and marked with “++” and Rings, respectively.

A.

![Flow cytometry plot](image1)

B.

![Flow cytometry plot](image2)
Supplemental Figure S3. Subpatent parasitemia measured by the Gen3.0 (A) and Gen3.5 (B) assays.

Synchronous *P. falciparum* cultures were used to prepare High Pos, Mid Pos and Low Pos controls of different densities. Nominal densities of them were $4 \times 10^5, 2 \times 10^3, 1.0 \times 10^2$ parasites/mL blood, respectively, used for evaluation of the Gen3.0 assay and they were $9 \times 10^5, 4 \times 10^3, 2 \times 10^2$ parasites/mL blood, respectively, for the Gen3.5 assay. Multiple replicates were run by *P. falciparum* qRT-PCR (Pf) and pan *Plasmodium* qRT-PCR (Pan) of each assay.