Supplementary Figures

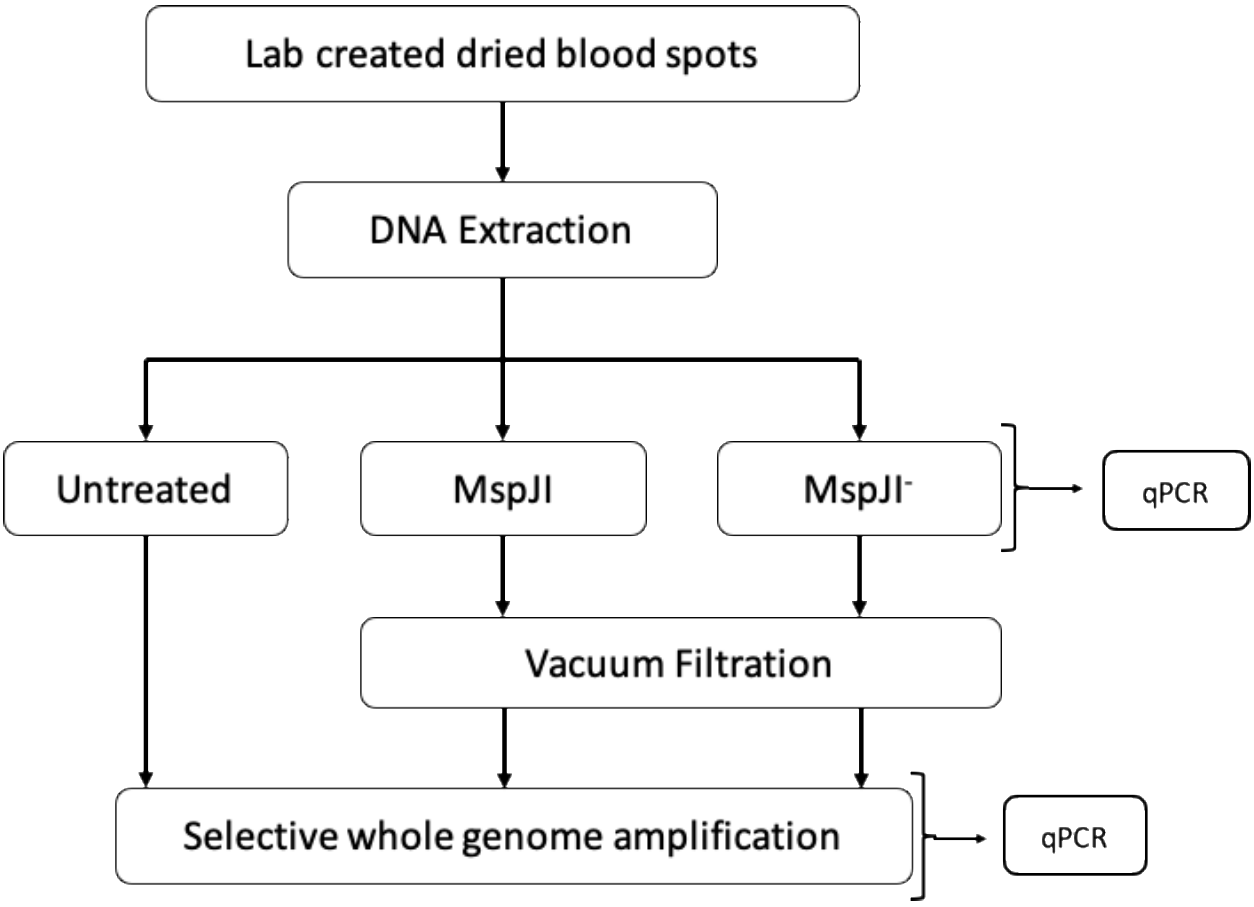

Figure S1. Flowchart describing the experimental design to optimize selective whole genome amplification. Vacuum filtration was done using the MultiScreen® Filter Plate (Millipore).

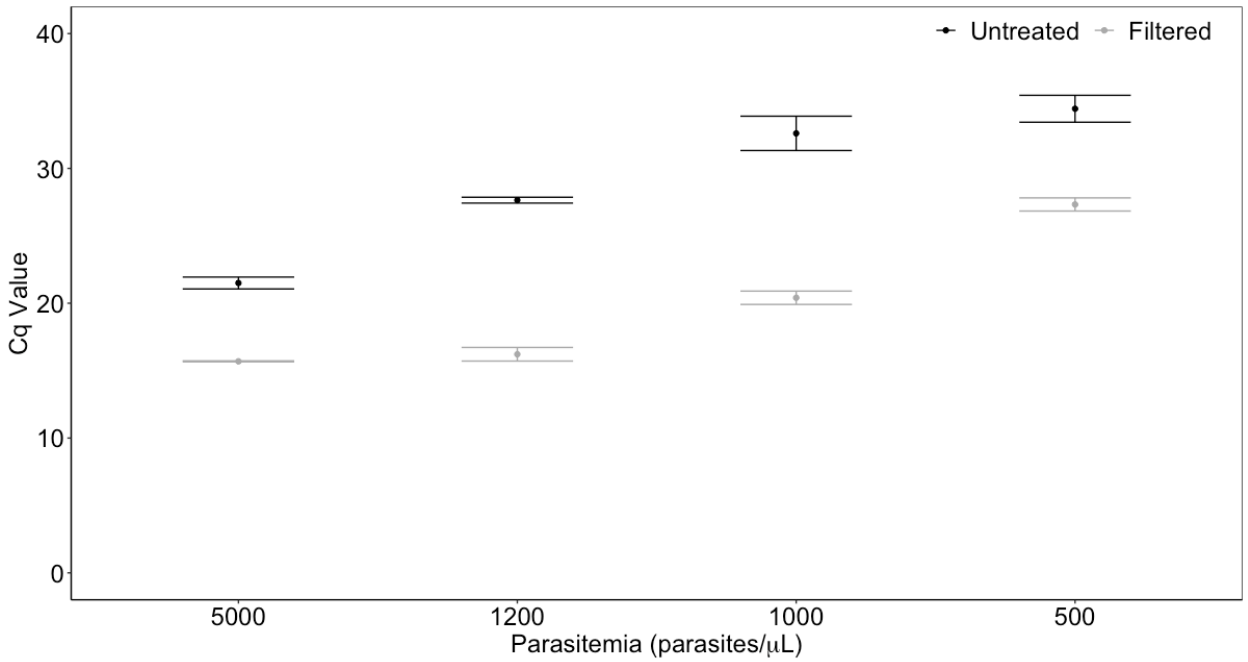

Figure S2. Effect of filtration on parasite DNA concentration in parasites with lower parasitemia (n = 3). *P. falciparum* Cq values for untreated-sWGA and filtered-sWGA samples with lower parasitemia. Error bars represent standard error.

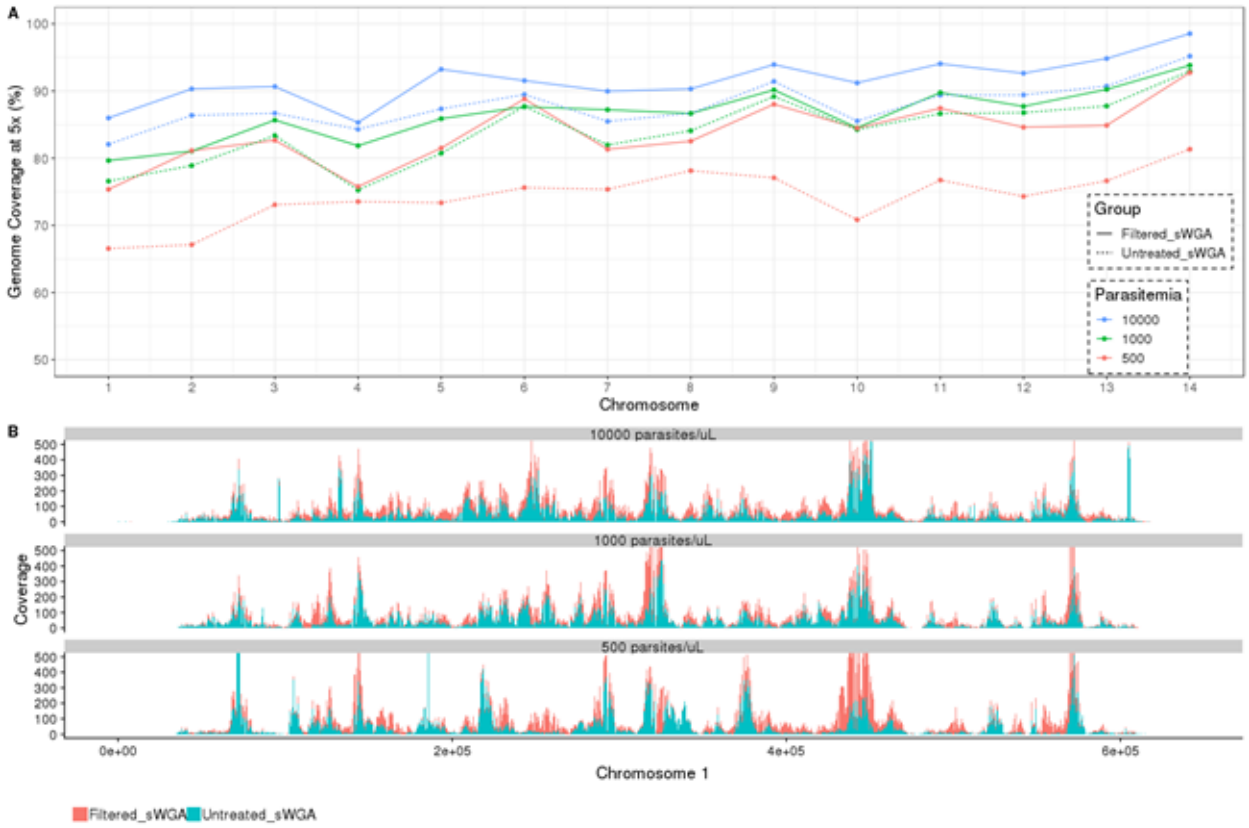

Figure S3. A) Average coverage per chromosome in untreated-sWGA *vs.* filtered-sWGA samples. B) Coverage over Chromosome 1.

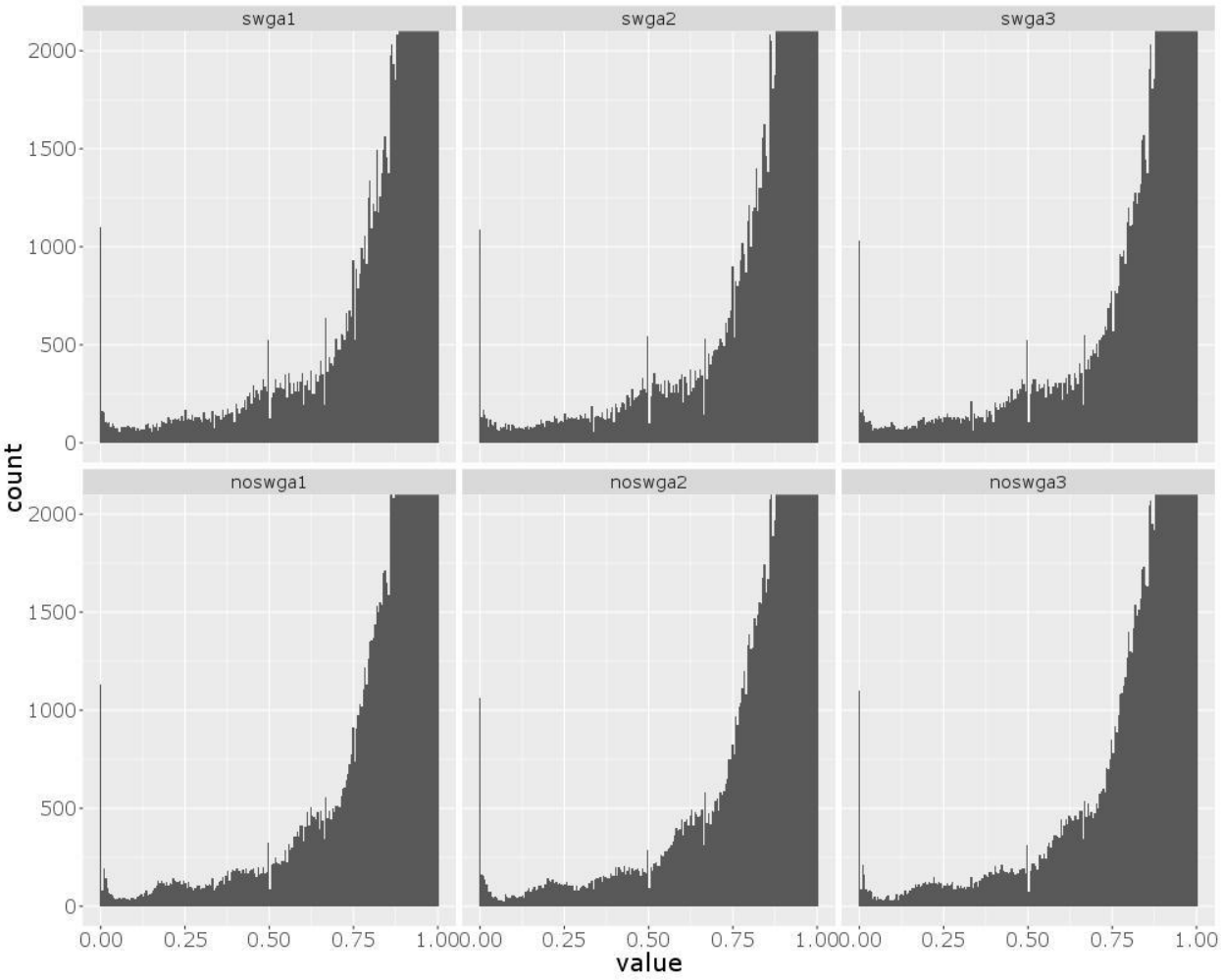

Figure S4. Distribution of reference allele frequencies for each site in the genome for lab-created mixtures of isolates that did (top row) or did not (bottom row) undergo sWGA prior to sequencing. The y-axis shows the number of variable positions for the proportion of reads carrying the 3D7 reference allele shown on the x-axis.

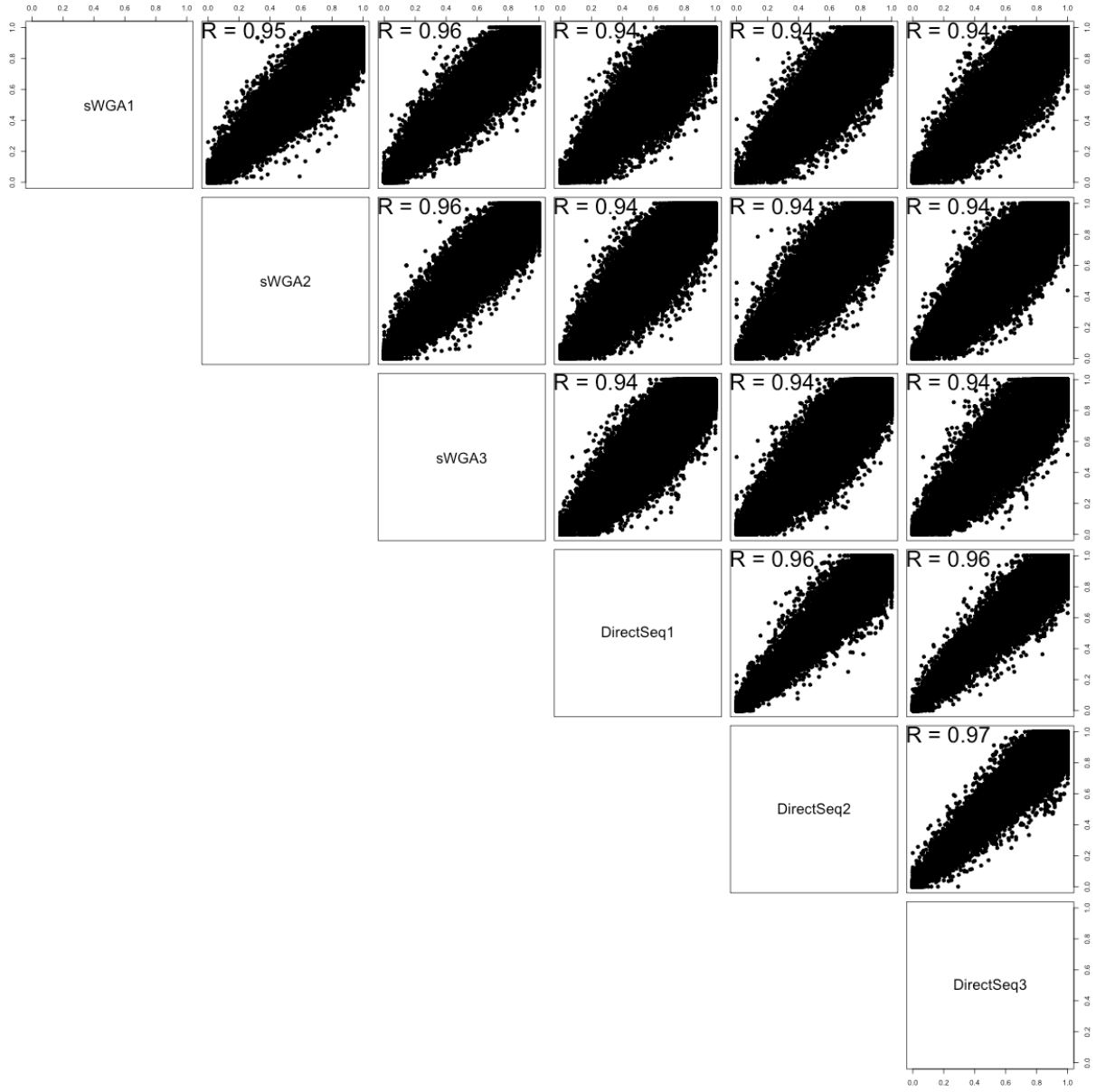

Figure S5. Correlation between reference allele frequency estimates for lab-created mixtures of isolates that either underwent sWGA prior to sequencing or that were directly s equenced. The plots show correlation between samples from within each group and also between groups (sWGA and no sWGA). The upper left side of the matrix shows the corresponding correlation coefficient r^2 for each comparison.

Supplementary Methods

DNA Extraction

DNA was extracted using the New Extraction Method [1] protocol (from blood) and elute with 50 μL TE buffer.

Filtration

25 μL DNA was diluted with 5 μL water. The diluted DNA was then filtered using Millipore vacuum filtration plate and elute with 30 μL water, followed by gentle agitation for 15 minutes.

sWGA

The sWGA reaction was performed in a 96 well plate. The fEach reaction mixture contained 0.50 μL 100x BSA (New England Biolabs), 2 μL 25 mM dNTPs, 1.25μL of each sWGA primer[1] (100 μM), 5 μL 10x Phi29 Reaction buffer (New England Biolabs), 3 μL 1250 U Phi29 Polymerase (New England Biolabs) and 17 μL filtered DNA. The reaction was then placed in a thermocycler programmed to run a stepdown protocol of 5 mins in 35 °C, 10 mins in 34 °C, 15 mins in 33 °C, 20 mins in 32 °C, 30 mins in 31 °C, 16 hours in 30 °C, 15 minutes in 65 °C to inactivate the enzymes and cooling to 4 °C. [2]

References

1. Zainabadi K, Adams M, Han ZY, Lwin HW, Han KT, Ouattara A, et al. A novel method for extracting nucleic acids from dried blood spots for ultrasensitive detection of low-density Plasmodium falciparum and Plasmodium vivax infections. Malar J. 2017 Sep 18;16(1):377.
2. Oyola SO, Ariani CV, Hamilton WL, Kekre M, Amenga-Etego LN, Ghansah A, et al. Whole genome sequencing of Plasmodium falciparum from dried blood spots using selective whole genome amplification. Malar J [Internet]. 2016 Dec 20 [cited 2018 Mar 28];15. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5175302/