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Ultrasensitive CRISPR-based diagnostic for field-applicable detection of Plasmodium species in symptomatic and asymptomatic malaria

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Supplementary Information for

Ultrasensitive CRISPR-based Diagnostic for Field-applicable Detection of *Plasmodium* Species in Symptomatic and Asymptomatic Malaria

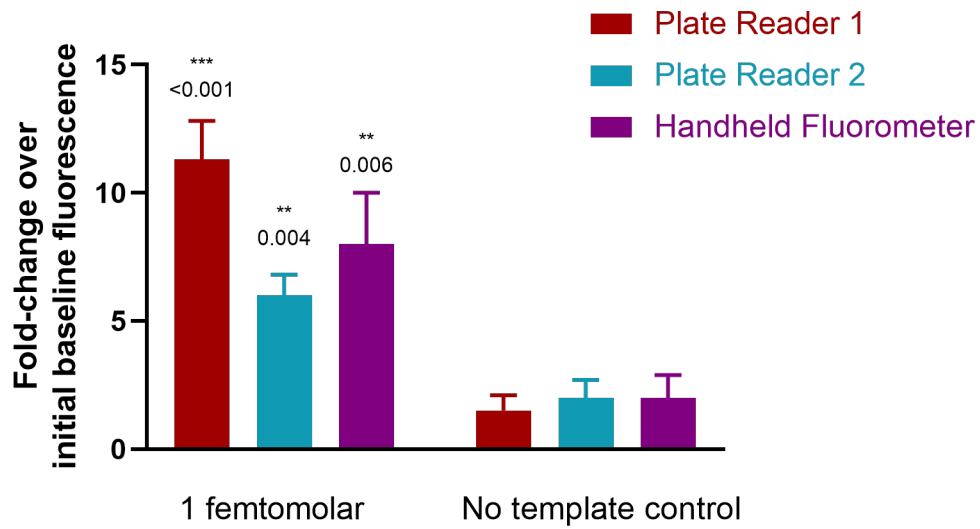
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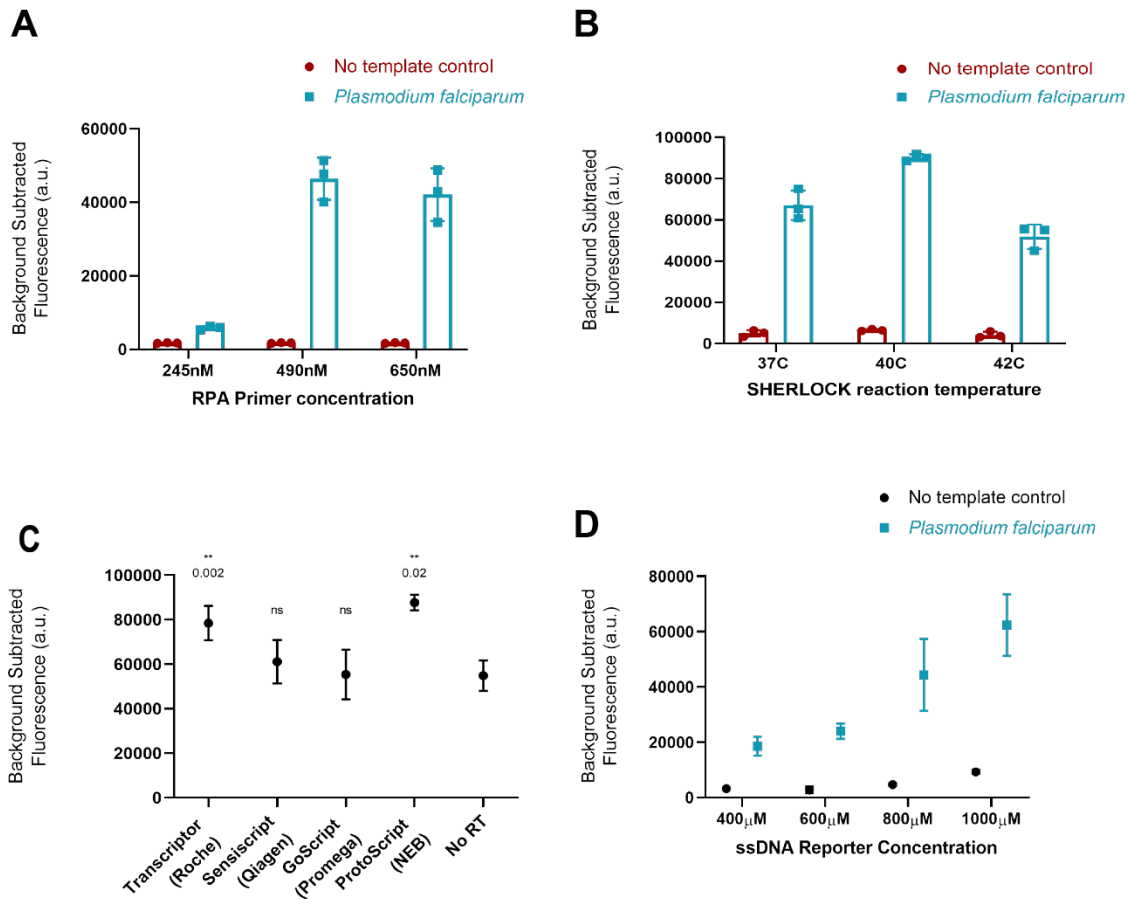
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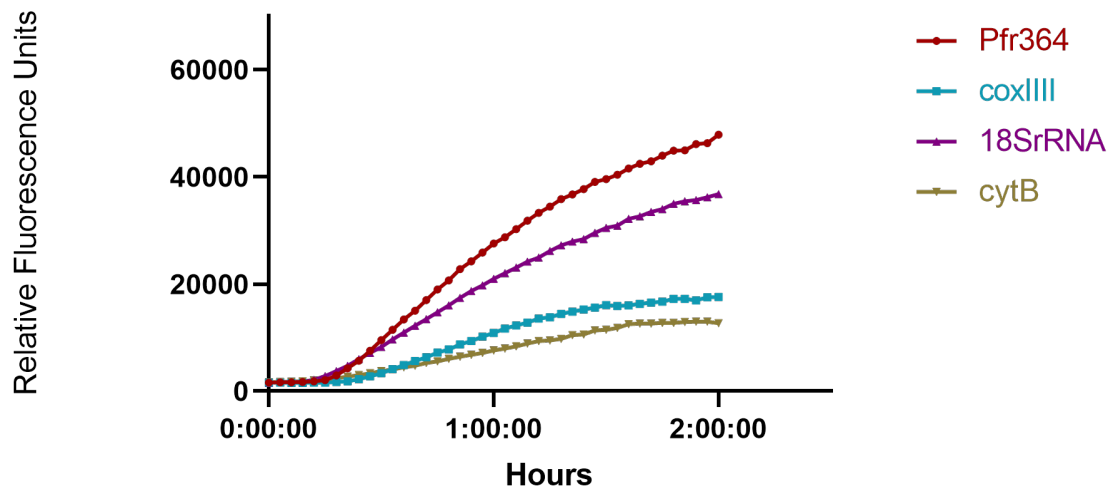
Figures S1 to S8



Supp. Fig. 1: Mean fold-change over baseline initial fluorescence of 1 fM (602 parasites/ μ L) *P. falciparum* whole genomic DNA spiked into whole blood compared with a no-template control in our final assay workflow (10 minutes S-PREP followed by rehydration of *P. falciparum* lyophilized SHERLOCK assay). Asterisks indicate significant differences in fluorescence between simulated sample and no-template control. Error bars indicate standard deviation of three replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



Supp. Fig. 2: Optimization of SHERLOCK parameters, including: A) RPA primer concentration, B) reaction temperature, C) reverse-transcriptase (RT) brand, and D) ssDNA reporter concentration. All optimizations were performed using 1 fM (602 parasites/ μ L) concentrations of whole genomic *P. falciparum* DNA in water, except for RT brand selection, for which a 1 fM concentration of live intraerythrocytic *P. falciparum* spiked into whole blood was used. Asterisks indicate significant differences in fluorescence of different RT brands from a 1 fM *P. falciparum* reaction where no RT was added assessed by a Student's two-tailed t-test. Bars and lines: mean \pm S.D. of three technical replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



Supp. Fig. 3: Optimization of *P. falciparum* target sequences during RPA screen, comparing best performing primer pairs of each target: Pfr364, cytochrome oxidase III (cox III), 18S rRNA, and cytochrome B (cytB).

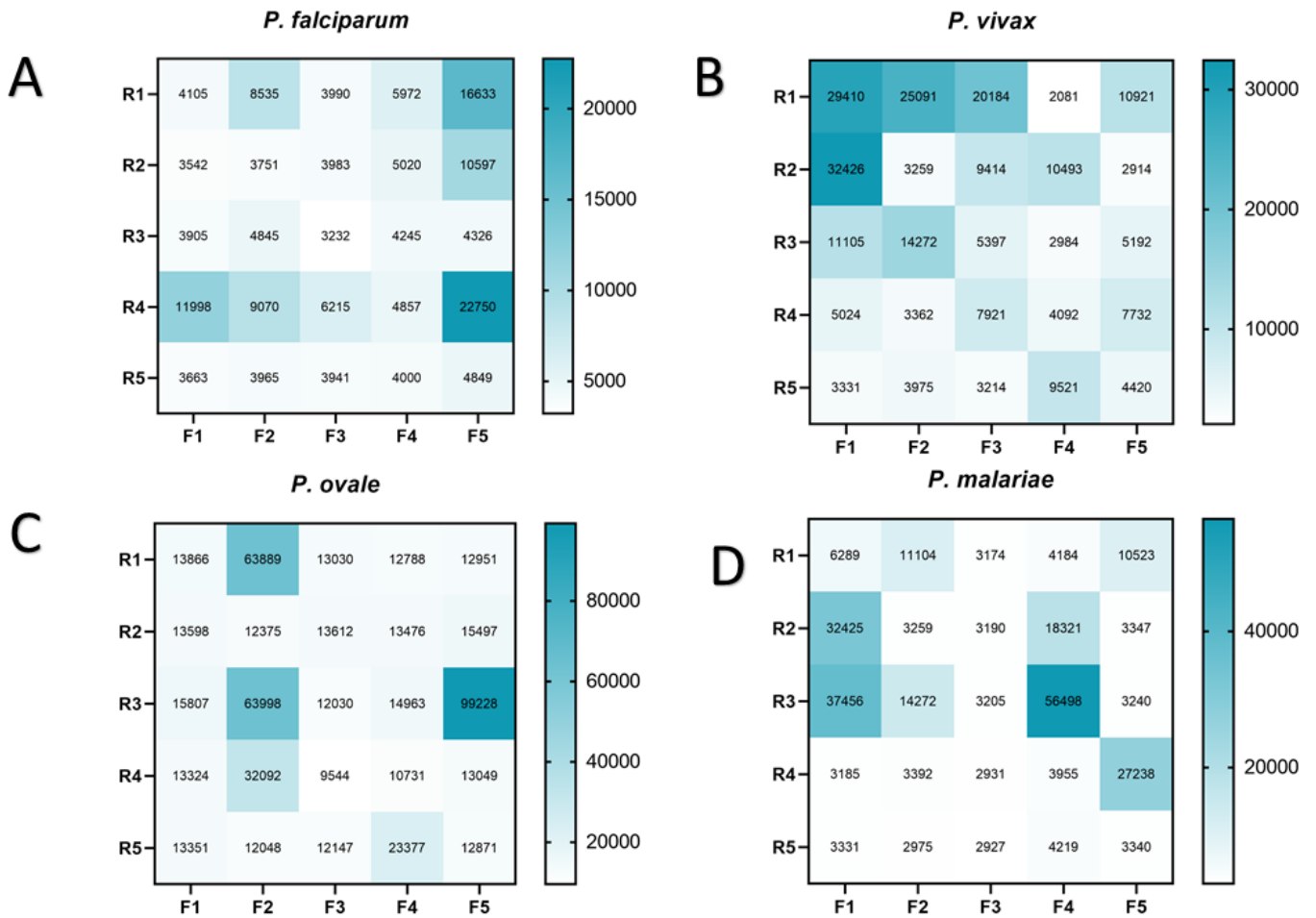
	RPA Forward Primer	gRNA match	RPA Reverse Primer
<i>P. falciparum</i> sub-telomeric region	AACGCTGCATTTTGGTCCATTTTGGACATTACG	-----GGTTTCTACGCTTATGTTTT-----	-----GGGCAGGGAAACATGGGGCAATTGTTCCTTTA
<i>P. vivax</i> LT635620	ATGTGTTTATTTTGGTCCATTTTGGCATTFTT	-----GCGCCTATTTTTTC-----	-----CATTTCCTACGTTTCGCACAATTCCGCATTTG
<i>P. ovale</i> LT594505.1	TCTTTTCAACGACATGTCCTTCGTAATCTTACG	-----AAATAGAAGGGATAG-----	-----AGGGAGAACATGGGGCAATTGTTGTGAC
<i>P. malariae</i> LT594635.1	TTTTTTTACATTTGTTCCATTTTGGACATTTA	-----GGGTGCTACAAATCCGTTGC-----	-----ATAACACCAAGTTTGCACGAATTTT

	RPA Forward Primer	gRNA match	RPA Reverse Primer
<i>P. vivax</i> mitochondrial region	CCTTACGTACTCTAGCTTTTAAACACAATATTATTGTC	-----TATATTCATTATTCTGAATA-----	-----ACCATATAATTTCAACAAAATGCCAGTATAAATTTGT
<i>P. falciparum</i> M76611	CCTTACGTACTCTAGCTATGAACACAATGTCCTATTC	-----CATGTCATTATTCTGAATA-----	-----ACCAGATTATTTCAACAAAATGCCAATATAAATTTGT
<i>P. ovale</i> HQ712052	CCTTACGTACTCTAGCTATTTACACAAAATATATTAT	-----ATGTTACATTATTCAGAATA-----	-----ACCATATTATTCATATAAATGCCAATATAAATTTGT
<i>P. malariae</i> LT594637.1	CCTTACGTACTCTAGCTTTGTACACAAAATTAATTCGT	-----TATATGTTCAATTATTCTGAATA-----	-----ACCATACTATTTCTATAAATGCCAATATAAATTTGT

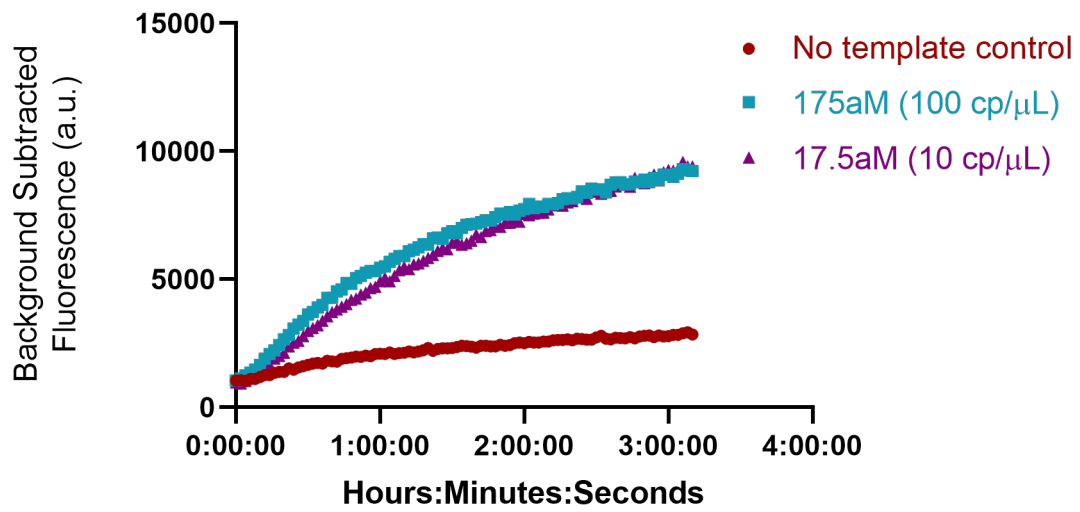
	RPA Forward Primer	gRNA match	RPA Reverse Primer
<i>P. ovale</i> 18S rRNA gene	AAGTTAAGGGAGTGAAGACGATCAGATACCGTCG	-----AATAAGAAAATTCCTTTCCGG-----	-----CCTTATGAGAAATCAAAGTCTTTGGGTTCTGGGGCGAGTA
<i>P. falciparum</i> 18S rRNA MN852864.1	AAGTTAAGGGAGTGAAGACGATCAGATACCGTCG	-----AAAAATAAAGTCACTCTTC-----	-----CCTTATGAGAAATCAAAGTCTTTGGGTTCTGGGGCGAGTA
<i>P. vivax</i> DQ660817	AAGTTAAGGGAGTGAAGACGATCAGATACCGTCG	-----AATAAGGATAGTCTCTCCGG-----	-----CCTTATGAGAAATCAAAGTCTTTGGGTTCTGGGGCGAGTA
<i>P. malariae</i> AF145336	AAGTTAAGGGAGTGAAGACGATCAGATACCGTCG	-----TAAAAGAGACATTCATATAT-----	-----CCTTATGAGAAATCAAAGTCTTTGGGTTCTGGGGCGAGTA

	RPA Forward Primer	gRNA match	RPA Reverse Primer
<i>P. malariae</i> 18S rRNA gene	AACGAAAGTTAAGGGAGTGAAGACGATCAGATACCG	-----TTTTAGATAGCTTCCTTCAG-----	-----CCTTATGAGAAATCAAAGTCTTTGGGTTCTGGGGCGAGTA
<i>P. falciparum</i> 18S rRNA MN852864.1	AACGAAAGTTAAGGGAGTGAAGACGATCAGATACCG	-----TTTCAGATTGCTTCCCTCAG-----	-----CCTTATGAGAAATCAAAGTCTTTGGGTTCTGGGGCGAGTA
<i>P. vivax</i> DQ660817	AACGAAAGTTAAGGGAGTGAAGACGATCAGATACCG	-----CCTTAGATTCTTCCTTCAG-----	-----CCTTATGAGAAATCAAAGTCTTTGGGTTCTGGGGCGAGTA
<i>P. ovale</i> AB182489.1	AACGAAAGTTAAGGGAGTGAAGACGATCAGATACCG	-----TCTTAGATTGCTTCCCTTCAG-----	-----CCTTATGAGAAATCAAAGTCTTTGGGTTCTGGGGCGAGTA

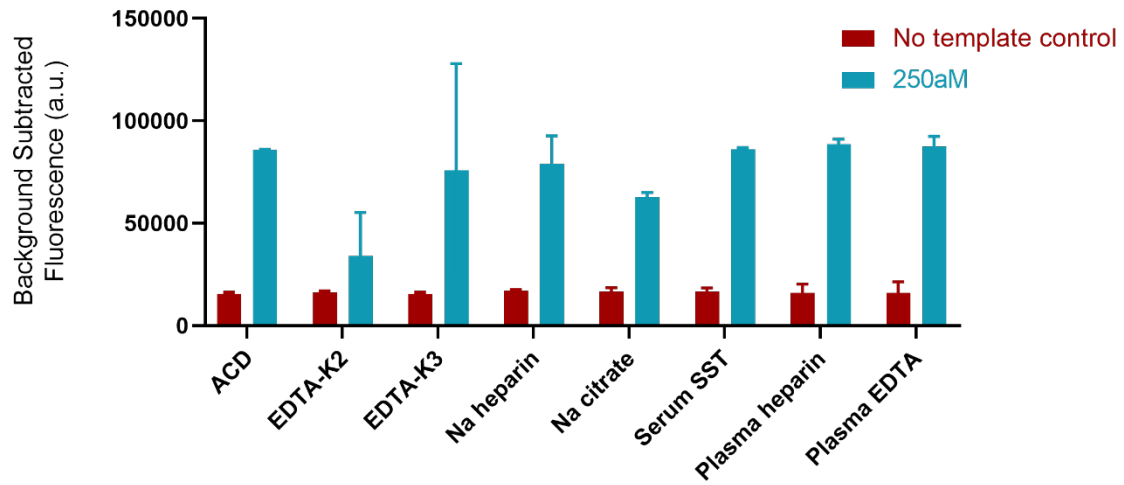
Supp. Fig. 4: Best performing RPA primers and gRNA sequences for development of *Plasmodium* SHERLOCK assays aligned with the closest homologous genes of other *Plasmodium* species or sequences with lowest matching E-values in NCBI database. First bolded line of each block demonstrates RPA primer and gRNA locations of target sequence in the 5' to 3' direction on the same strand. Alignments below are listed with GenBank accession number; blue color denotes nucleotide matches of RPA forward and reverse primers as well as gRNA sequences.



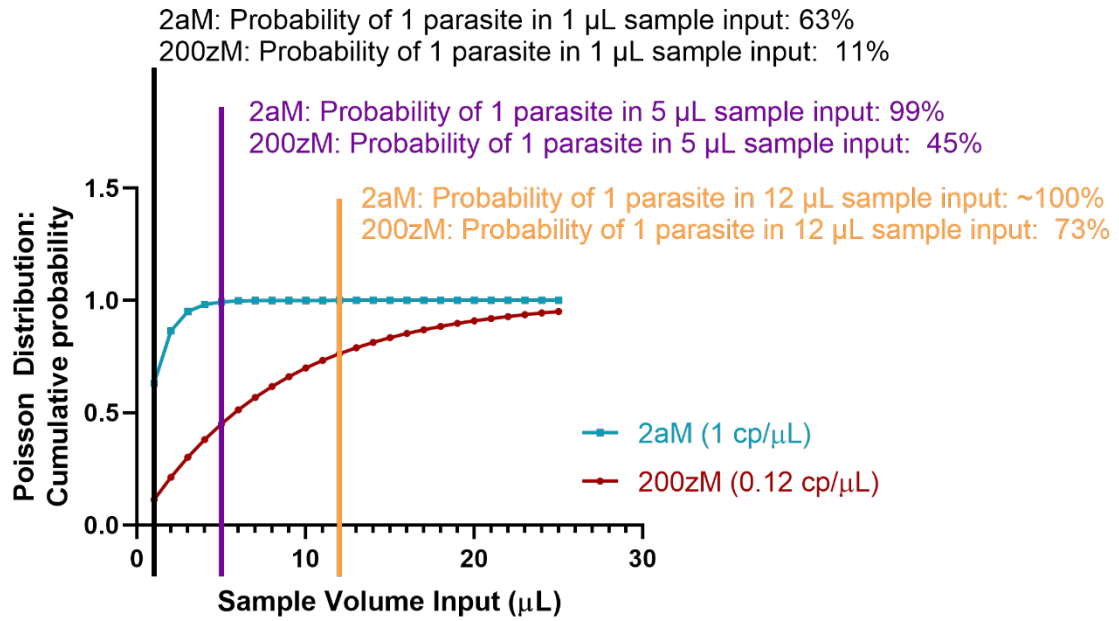
Supp. Fig. 5: Relative fluorescence unit (RFU) measurements of RPA screens comprised of 25-set combination of five forward (F1-F5) and five reverse primers (R1-5) for best-performing sequence targets for *Plasmodium* species targets.



Supp. Fig. 6: Detection of simulated RNA sample (synthetic SARS-CoV-2 synthetic RNA spiked in water) prepared with S-PREP and tested in SARS-CoV-2 SHERLOCK assay



Supp. Fig. 7: Specimen collection types compatible with SHERLOCK tested by spiking live intraerythrocytic *P. falciparum* into whole blood collected in various sample collection tubes to a final concentration of 250 aM (150 parasites/ μ L) and then prepared with S-PREP (1:3 dilution) for rehydration of lyophilized *P. falciparum* SHERLOCK reaction.



Supp. Fig. 8: Theoretical limits of detection in a perfect assay (one pathogen copy per reaction will be detected 100% of the time) where at attomolar and zeptomolar concentrations of pathogen, a Gaussian distribution is no longer an appropriate approximation of pathogen distribution.