

Supplementary Table 1. Primers, probes, cycling conditions

Species	Primer or probe	[μ M]	Sequence (5'- 3') ^b	Ref .	Cycling Conditions	
<i>P. falciparum</i>	Falciparum F	0.45	CCGACTAGGTGTTGGATGAAAGTGTTAA	(5)	<u>RT-PCR Program</u> 50°C for 20 minutes (RT) 95°C 15 minutes 94°C 45 seconds 60°C 75 seconds (Data) Repeat to 94oC 44 times	<u>Ramp speed</u> 1.7°C/s 1.7°C/s
	Plasmodium R	0.60	AACCCAAAGACTTTGATTTCTCATAA			
<i>P. vivax</i>	Falciparum P	0.10	Cy5-AGCAATCTAAAAGTCACCTCGAAAGATGACT-BHQ2	[2]		
	Vivax R	0.10	CCGACTAGGCTTTGGATGAAAGATTTTA FAM-AGCAATCTAAGAATAAACTCCGAAGAGAAAATTCT-			
Human	Vivax P	0.10	BHQ2			
	Actin F	0.10	ACCGAGCGCGGCTACAG			
	Actin R	0.10	CTTAATGTCACGCACGATTTC			
	Actin P	0.10	VIC-TTCACC ACCACGGCCGAGC-MGB			

^aProbe sequence is as previously published [6], with modified fluorophores.

^bCy5, cyanine; BHQ_2, black hole quencher 2; FAM, carboxyfluorescein; VIC (Life Technologies, Carlsbad, CA); MGB, minor-groove binder

Supplementary Table 2. Resulting Ct values and statistical information showing impact of storage time on Ct values

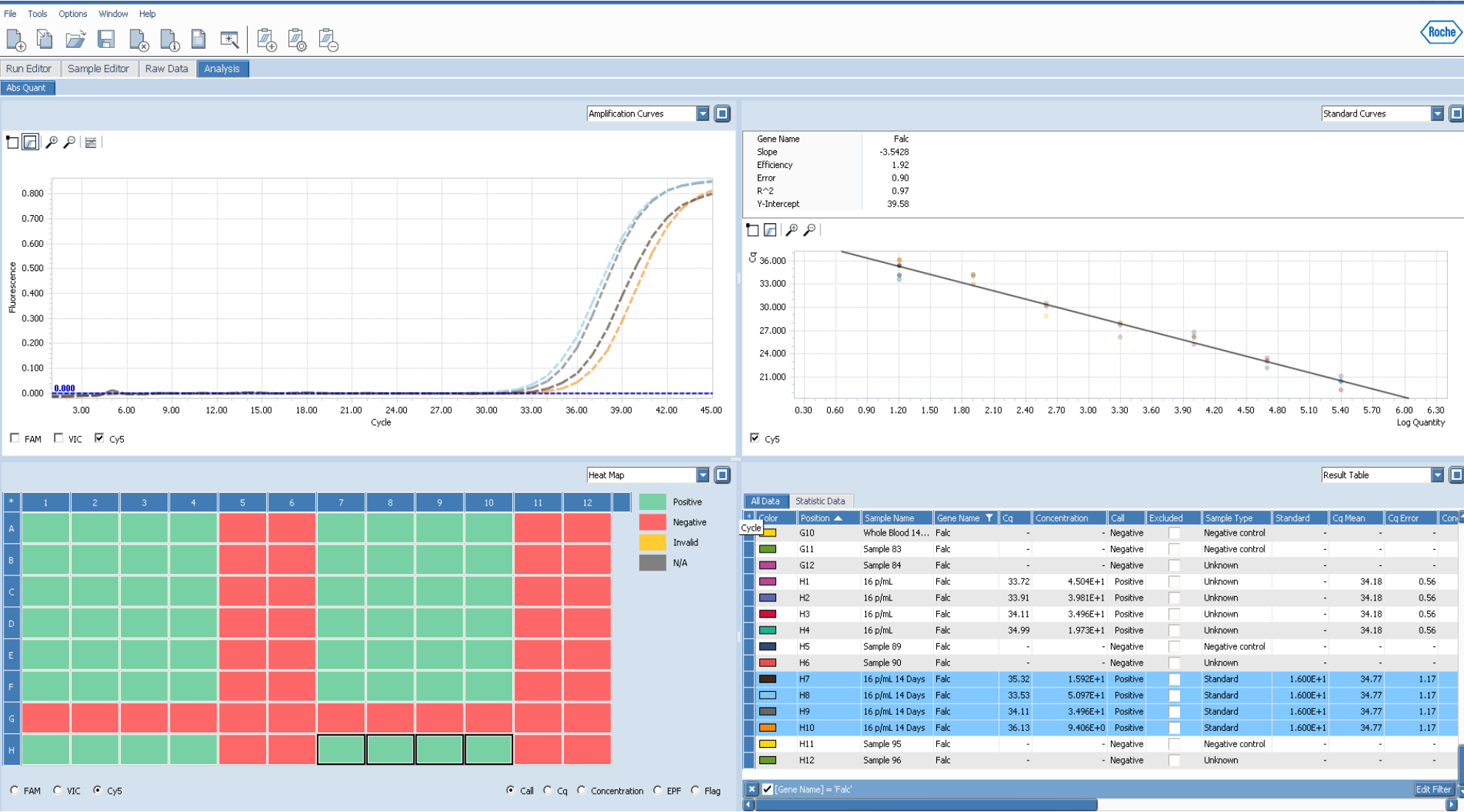
parasites/mL	14 Days @ 28 °C/80% RH			3 Days @ 28 °C/80% RH		
	Cq	Cq Mean	Cq Error	Cq	Cq Mean	Cq Error
250,000	18.69	20.68	1.40	20.47	20.98	0.47
250,000	20.95	20.68	1.40	20.72	20.98	0.47
250,000	21.96	20.68	1.40	21.19	20.98	0.47
250,000	21.11	20.68	1.40	21.52	20.98	0.47
50,000	24.70	24.27	0.45	22.39	23.17	0.57
50,000	23.80	24.27	0.45	23.47	23.17	0.57
50,000	24.59	24.27	0.45	23.13	23.17	0.57
50,000	23.97	24.27	0.45	23.68	23.17	0.57
10,000	26.55	26.44	0.33	24.53	25.45	0.67
10,000	26.27	26.44	0.33	25.40	25.45	0.67
10,000	26.85	26.44	0.33	25.84	25.45	0.67
10,000	26.10	26.44	0.33	26.03	25.45	0.67
2,000	29.34	28.79	0.45	29.34	28.79	0.45
2,000	28.58	28.79	0.45	28.58	28.79	0.45
2,000	28.94	28.79	0.45	28.94	28.79	0.45
2,000	28.29	28.79	0.45	28.29	28.79	0.45
400	32.90	31.14	1.54	32.90	31.14	1.54
400	31.10	31.14	1.54	31.10	31.14	1.54
400	31.41	31.14	1.54	31.41	31.14	1.54
400	29.16	31.14	1.54	29.16	31.14	1.54
80	34.94	32.84	1.43	31.94	32.33	0.28
80	32.30	32.84	1.43	32.53	32.33	0.28
80	32.42	32.84	1.43	32.33	32.33	0.28
80	31.70	32.84	1.43	32.52	32.33	0.28
16	36.25	35.17	0.83	33.71	34.24	0.63
16	34.68	35.17	0.83	34.07	34.24	0.63
16	35.34	35.17	0.83	34.94	34.24	0.63
16	34.39	35.17	0.83	-	-	-
0	-	-	-	-	-	-
0	-	-	-	-	-	-
0	-	-	-	-	-	-
0	-	-	-	-	-	-

Supplementary Table 3. Evaluation of inter-assay and intra-assay variability and removal of reverse transcriptase from RT PCR on 250,000

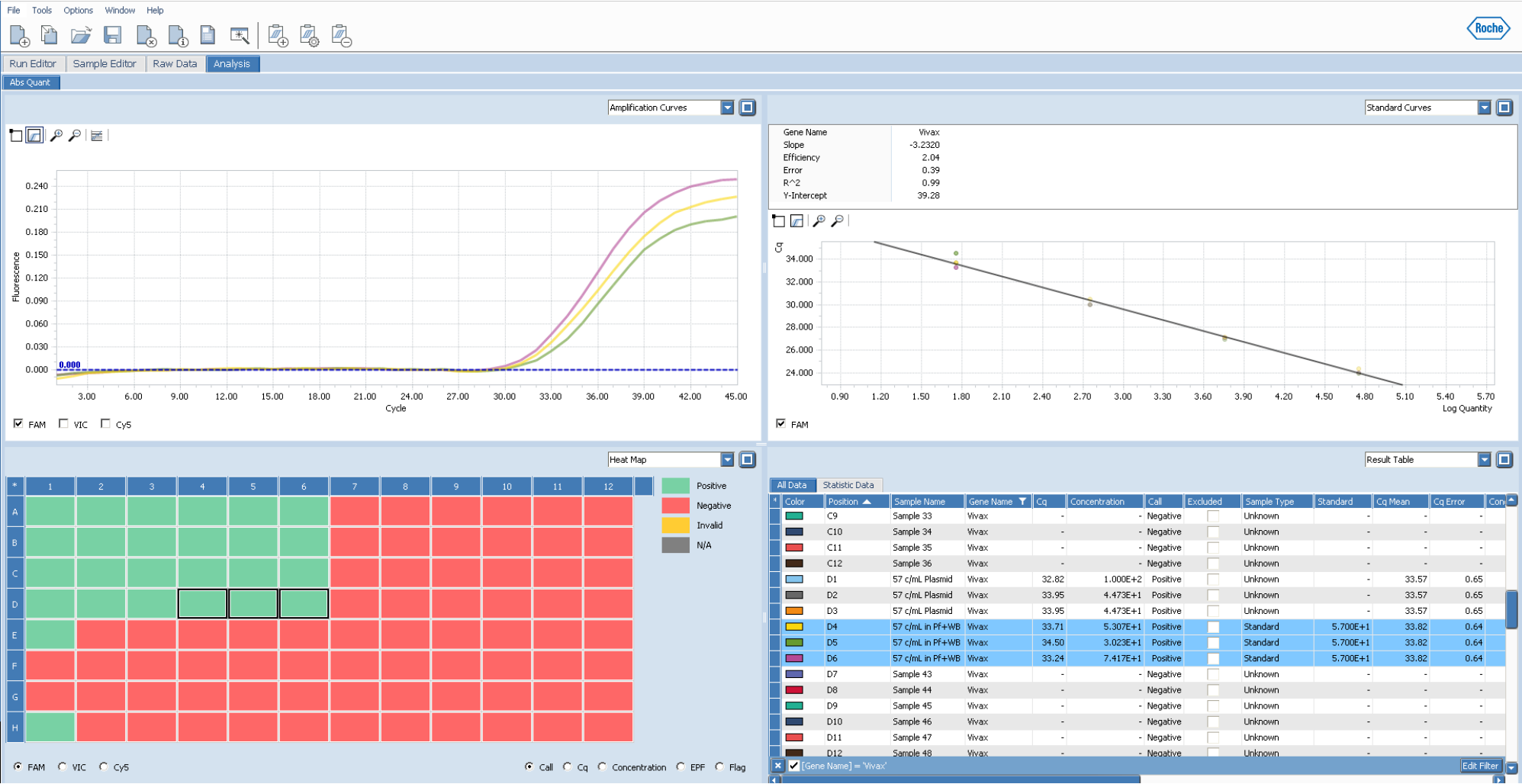
***Plasmodium falciparum* parasites per mL in whole blood**

	With Reverse Transcriptase			Without Reverse Transcriptase		
	Cq	Cq Mean	Cq Error	Cq	Cq Mean	Cq Error
Experiment 1	18.87	18.93	0.10	29.99	29.98	0.31
Experiment 1	19.02	18.93	0.10	29.53	29.98	0.31
Experiment 1	18.82	18.93	0.10	30.23	29.98	0.31
Experiment 1	19.02	18.93	0.10	30.15	29.98	0.31
Experiment 2	18.61	18.69	0.20			
Experiment 2	18.95	18.69	0.20			
Experiment 2	18.47	18.69	0.20			
Experiment 2	18.74	18.69	0.20			
Experiment 3	18.81	18.85	0.11			
Experiment 3	18.99	18.85	0.11			
Experiment 3	18.74	18.85	0.11			
Experiment 3	18.86	18.85	0.11			

Supplementary Figure 1. Example data output from standard curve of serially diluted *P. falciparum* parasites diluted in whole blood



Supplementary Figure 2. Example data output from standard curve of serially diluted *P. vivax* plasmid diluted in 10⁴ *P. falciparum* parasites and whole blood



Supplementary Figure 3. Example Probit Graph of standard curve samples stored in tropical conditions for 3 days

