

Table S4. All threshold times generated in this study (5000 target copies)

Primer set	Primer sets name	Experiment	A-UG	B	C	D	F	G	Average
			T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)
1	B-CA	1.1		13.29	48.54	13.31	52.68		
		1.2		11.9	61	13.49	61		
		1.3		12.47		11.86	61		
		1.4		12.41		14.46	61		
		1.5		12.45		13.4	61		
		Average		12.5	54.77	13.3	59.34		34.98
		STD		0.5	8.81	0.93	5.89		4.03
2	B-PR	2.1		15.88	61	15.91	55.54		
		2.2		16.35	61	16.93	61		
		2.3		15.83	61	15.93	61		
		2.4		16.65	61	14.47	61		
		2.5		15.66	61	13.67	20.69		
		Average		16.07	61	15.38	51.85		36.08
		STD		0.41	0	1.3	3.86		1.39
3	B-RT	3.1		9.18	61	12.6	41.78		
		3.2		8.88	48.97	19.88	61		
		3.3		8.9	61	21.95	61		
		3.4		8.64	36.63	26.25	61		
		3.5		8.52	56.79	18.64	57.61		
		Average		8.82	52.88	19.86	56.48		34.51
		STD		0.25	10.33	4.99	13.59		7.29
4	B-CA + B-PR	4.1		13.4	16.58	13.77	61		
		4.2		12.93	31.71	14.5	61		

Primer set	Primer sets name	Experiment	A-UG	B	C	D	F	G	Average
			T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)
		Average		13.16	24.15	14.14	61		28.11
		STD		0.33	10.7	0.52	0		2.89
5	B-PR +B-RT	5.1		8.46	39.71	19.39	46.48		
		5.2		8.47	61	16.94	51.33		
		Average		8.46	50.36	18.17	48.91		31.47
		STD		0	15.05	1.73	3.43		5.05
6	ACeIN-1	6.1		10.98	35.57	56.75	50.4		
		6.2		10.84	13.33	46.51	57.59		
		Average		10.91	24.45	51.63	53.99		35.25
		STD		0.1	15.73	7.24	5.08		7.04
7	ACeIN-2	7.1		10.41	34.39	49.78	55.94		
		7.2		9.92	19.33	31.45	48.74		
		Average		10.16	26.86	40.61	52.34		32.49
		STD		0.34	10.65	12.96	5.09		7.26
8	ACeIN-3	8.1	16.51	9.28	33.58	27.5	54.62	15.58	
		8.2	12.22	9.67	61	41.48	61	13.27	
		8.3		9.97	13.4	12.57	61		
		8.4		8.66	14.36	18.94	61		
		8.5		9.26	12.97	13.88	18.83		
		8.6		9.36	15.34	12.58	61		
		8.7		8.3	14.29	61	25.69		
		8.8		8.48	14.53	13.37	61		
		Average	14.37	9.12	22.43	25.17	50.52	14.43	22.67
		STD	3.03	0.59	17.02	17.64	17.67	1.64	9.6

Primer set	Primer sets name	Experiment	A-UG	B	C	D	F	G	Average
			T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)
9	ACeCA	9.1		32.88	61	61	61		
		9.2		31.81	61	61	61		
		Average		32.34	61	61	61		53.84
		STD		0.75	0	0	0		0.19
10	ACeIN-4	10.1		61	61	61	61		
		10.2		61	61	61	61		
		Average		61	61	61	61		61
		STD		0	0	0	0		0
11	ACeIN-5	11.1		48.99	61	61	61		
		11.2		61	61	61	61		
		Average		55	61	61	61		59.5
		STD		8.49	0	0	0		2.12
12	ACeIN-6	12.1		54.75	61	61	61		
		12.2		51.48	61	61	61		
		Average		53.12	61	61	61		59.03
		STD		2.31	0	0	0		0.58
13	ACeIN-7	13.1	15.81	9.4	13.48	16.81	19.31	15.55	
		13.2	12.37	9.66	15.64	32.86	41.6	22.62	
		13.3		8.43	11.48	17.96	61		
		13.4		8.68	15.39	17.57	41.33		
		Average	14.09	9.04	14	21.3	40.81	19.08	19.72
		STD	2.44	0.5	1.68	6.69	14.75	5	5.18
14	ACeIN-8	14.1	20.37	10.54	12.47	16.8	21.8	12.96	
		14.2	12.95	9.94	10.71	14.68	61	21.83	

Primer set	Primer sets name	Experiment	A-UG	B	C	D	F	G	Average
			T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)
		14.3		9.49	12.38	30.68	61		
		14.4		9.46	11.99	22.59	51.48		
		Average	16.66	9.86	11.89	21.19	48.82	17.39	20.97
		STD	5.24	0.44	0.71	6.2	16.08	6.27	5.82
		15.1		35.85	61	61	61		
15	ACeIN-9	15.2		32.85	61	61	61		
		Average		34.35	61	61	61		54.34
		STD		2.12	0	0	0		0.53
		16.1		61	61	61	61		
16	ACeIN-10	16.2		46.75	61	61	61		
		Average		53.87	61	61	61		59.22
		STD		10.08	0	0	0		2.52
		17.1		16.43	26.34	61	41.93		
17	ACeIN-11	17.2		15.71	24.39	61	61		
		Average		16.07	25.36	61	51.47		38.47
		STD		0.51	1.38	0	13.48		3.84
		18.1		16.58	24.46	61	25.39		
18	ACeIN-12	18.2		15.29	24.98	32.46	40.64		
		Average		15.94	24.72	46.73	33.02		30.1
		STD		0.91	0.37	20.18	10.78		8.06
		19.1		33.65	48.68	61	61		
19	ACeIN-13	19.2		35.97	42.79	61	61		
		Average		34.81	45.74	61	61		50.64
		STD		1.64	4.16	0	0		1.45

Primer set	Primer sets name	Experiment	A-UG	B	C	D	F	G	Average
			T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)
20	ACeIN-14	20.1		37.59	61	61	61		
		20.2		41.49	61	61	61		
		Average		39.54	61	61	61		55.63
		STD		2.76	0	0	0		0.69
21	ACeIN-15	21.1		20.1	24.66	52.99	61		
		21.2		18.99	33.93	61	61		
		Average		19.54	29.3	56.99	61		41.71
		STD		0.79	6.56	5.67	0		3.25
22	ACeIN-16	22.1		25.97	30.71	30.99	61		
		22.2		26.94	31.53	51.53	61		
		Average		26.45	31.12	41.26	61		39.96
		STD		0.68	0.58	14.53	0		3.95
23	ACeIN-17	23.1	14.7	10.23	13.84	12.83	61	12.34	
		23.2	15.92	9.58	12.47	22.66	61	14.6	
		23.3		8.85	16.74	34.89	61		
		23.4		8.99	11.8	39.57	61		
		Average	15.31	9.41	13.71	27.49	61	13.47	23.4
		STD	0.87	0.55	1.9	10.48	0	1.6	2.56
24	ACeIN-18	24.1		14.35	14.49	30.99	26.83		
		24.2		12.43	19.51	19.52	61		
		Average		13.39	17	25.26	43.91		24.89
		STD		1.36	3.55	8.12	24.16		9.3
25	ACeIN-19	25.1		13.51	15.83	24.7	61		
		25.2		12.65	18.75	61	61		

Primer set	Primer sets name	Experiment	A-UG	B	C	D	F	G	Average
			T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)
		Average		13.08	17.29	42.85	61		33.55
		STD		0.61	2.06	25.67	0		7.09
26	ACeIN-20	26.1		16.96	20.65	61	61		
		26.2		13.8	20.45	61	61		
		Average		15.38	20.55	61	61		39.48
		STD		2.23	0.14	0	0		0.59
27	ACeIN-21	27.1		49.96	61	61	61		
		27.2		44.45	44.64	61	61		
		Average		47.2	52.82	61	61		55.51
		STD		3.9	11.57	0	0		3.87
28	ACeIN-22	28.1		42.81	57.68	61	61		
		28.2		50.66	61	61	61		
		Average		46.73	59.34	61	61		57.02
		STD		5.55	2.35	0	0		1.98
29	ACeIN-23	29.1		11.69	13.99	29.43	61		
		29.2		9.95	18.28	19.49	61		
		Average		10.82	16.13	24.46	61		28.1
		STD		1.23	3.04	7.03	0		2.83
30	AcelN-23+B-PR	30.1		10.93	17.5	18.4	29.55		
		30.2		9.97	16.78	16.84	38.22		
		Average		10.45	17.14	17.62	33.89		19.77
		STD		0.68	0.51	1.1	6.13		2.1
31	ACeIN-24	31.1		12.54	19.81	40.95	61		
		31.2		11.88	19.97	42.81	22.47		

Primer set	Primer sets name	Experiment	A-UG	B	C	D	F	G	Average
			T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)
		Average		12.21	19.89	41.88	41.74		28.93
		STD		0.46	0.12	1.32	27.24		7.29
32	ACeIN-25	32.1		9.16	12.53	13.92	31.99		
		32.2		8.65	15.51	14.91	61		
		Average		8.91	14.02	14.41	46.49		20.96
		STD		0.36	2.11	0.7	20.51		5.92
33	ACeIN-26	33.1	13.52	9.96	15.25	18.75	46.36	13.99	
		33.2	14.25	10.99	13.32	18.36	19.79	18.72	
		33.3	17.41	9.4	15.59	24.42	33.7	33.9	
		33.4	15.59	8.96	14.68	14.82	22.24	12.36	
		33.5	20.4	10.22	13.6	29.6	61	14.81	
		33.6	14.55	11.22	13.96	20.81	61	19.56	
		33.7		9.64	11.6	18.62	15.79		
		33.8		8.63	17.66	20.93	17.67		
		Average	15.95	9.88	14.46	20.79	34.69	18.89	19.11
		STD	2.56	0.91	1.8	4.49	19.07	7.86	6.12
34	ACeIN-27	34.1		12.51	61	35.82	61		
		34.2		12.59	14.4	37.14	61		
		Average		12.55	37.7	36.48	61		36.93
		STD		0.06	32.95	0.94	0		8.49
35	ACeIN-28	35.1	10.43	9.28	10.79	28.57	34.4	16.21	
		35.2	10.95	9.54	17.33	30.61	13.97	16.93	
		35.3		9.5	18.47	13.39	61		
		35.4		9.26	17.37	61	29.95		

Primer set	Primer sets name	Experiment	A-UG	B	C	D	F	G	Average
			T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)
		Average	10.69	9.4	15.99	33.39	34.83	16.57	20.15
		STD	0.37	0.13	3.04	17.27	16.91	0.51	6.37
36	ACeIN-29	36.1	12.72	8.96	17.67	20.4	61	61	
		36.2	15.32	10.92	15.61	21.74	24.46	10.89	
		36.3		12.28	12.37	34.77	21.7		
		36.4		10.55	13.82	27.55	18.42		
		Average	14.02	10.68	14.87	26.12	31.39	35.95	22.17
		STD	1.84	1.18	1.98	5.67	17.23	35.43	10.56
37	ACeIN-30	37.1	15.42	11.9	23.39	34.57	61	19.92	
		37.2	17.97	10.76	17.88	23.46	25.41	28.58	
		Average	16.69	11.33	20.64	29.01	43.2	24.25	24.19
		STD	1.8	0.81	3.89	7.85	25.17	6.12	7.61
38	ACeIN-31	38.1	20.93	14.54	19.5	61	31.74	26.55	
		38.2	25.56	15.5	23.84	61	61	22.7	
		Average	23.25	15.02	21.67	61	46.37	24.62	31.99
		STD	3.27	0.68	3.06	0	20.69	2.73	5.07
39	ACeIN-26+B-PR	39.1	13.68	8.45	15.84	14.68	61	11.77	
		39.2	18.52	9.65	13.61	14.93	41.52	16.33	
		Average	16.1	9.05	14.73	14.8	51.26	14.05	20
		STD	3.42	0.85	1.58	0.18	13.77	3.23	3.84
40	ACeIN-30+B-PR	40.1	43.96	10.27	44.57	20.69	61	26.6	
		40.2	15.94	10.5	12.4	19.97	23.93	19.58	
		Average	29.95	10.38	28.49	20.33	42.46	23.09	25.78
		STD	19.81	0.16	22.75	0.51	26.22	4.96	12.4

Primer set	Primer sets name	Experiment	A-UG	B	C	D	F	G	Average
			T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)
41	ACeIN-28+B-PR	41.1	12.49	10.39	54.62	18.65	61	12.93	
		41.2	10.4	9.46	12.63	24.85	32.44	13.88	
		Average	11.44	9.93	33.62	21.75	46.72	13.41	22.81
		STDE	1.48	0.66	29.7	4.39	20.19	0.68	9.51
42	ACeIN-32	42.1	12.8	9.94	16.35	19.75	61	15.8	
		42.2	15.38	10.68	14.89	46.73	61	18.6	
		42.3	17.86	10.51	17.86	24.72	22.38	17.96	
		42.4	18.61	10.45	14.84	25.47	61	18.47	
		Average	16.16	10.39	15.98	29.17	51.34	17.71	23.46
		STD	2.63	0.32	1.43	11.98	19.31	1.3	6.16
43	ACeIN-33	43.1	13.52	9.86	12.26	26.99	61	13.46	
		43.2	14.84	10.5	16.32	19.78	61	15.68	
		43.3	20.48	10.96	18.93	17.44	27.23	15.37	
		43.4	16.26	10.6	15.74	26.77	52.68	22.65	
		Average	16.27	10.48	15.81	22.75	50.48	16.79	22.1
		STD	3.02	0.46	2.74	4.87	15.99	4.03	5.18
44	ACeIN-34	44.1	15.88	10.33	13.68	16.45	24.95	19.51	
		44.2	15.82	9.47	14.57	31.59	61	14.7	
		44.3	15.47	10.6	13.44	15.6	61	61	
		44.4	16.43	10.47	15.7	26.88	61	13.4	
		Average	15.9	10.22	14.35	22.63	51.99	27.15	23.71
		STD	0.4	0.51	1.02	7.87	18.02	22.72	8.42
45	ACeIN-26+ACePR	45.1	21.81	12.8	14.53	46.97	39.66	17.29	
		45.2	21.52	11.53	32.41	31.29	61	34.88	

Primer set	Primer sets name	Experiment	A-UG	B	C	D	F	G	Average
			T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)	T _t (min)
		Average	21.66	12.16	23.47	39.13	50.33	26.09	28.81
		STD	0.2	0.9	12.64	11.08	15.09	12.44	8.72
46	ACeIN-26+F-IN	46.1	14.46	12.51	14.55	14.44	61	16.97	
		46.2	14.57	12.18	15.58	12.65	25.78	14.25	
		Average	14.51	12.35	15.06	13.54	43.39	15.61	19.08
		STD	0.08	0.23	0.73	1.26	24.91	1.93	4.86
47	ACeIN-26+F-IN+ACePR	47.1	22.62	14.35	19.98	15.94	44.97	13.74	
		47.2	16.76	11.81	16.92	13.89	25.29	18.44	
		Average	19.69	13.08	18.45	14.91	35.13	16.09	19.56
		STD	4.15	1.79	2.16	1.45	13.92	3.32	4.47

Samples consist of 5000 copies per reaction of HIV-1 templates. The threshold time (T_t) is defined as the reaction time that elapses until the fluorescent signal increases to 10% of maximum fluorescence intensity (I_{max}) above the baseline level. For cases where there was no amplification, we designate 61 min as T_t . Lack of entry indicates that no experiment was carried out.