

Supplementary data

Table S1: Primers used for Sanger sequencing to verify the sequences of the mutant A(H1N1)pmd09 and A(H3N2) NA-plasmids

pRF-CMV Fw	GATAGCGGTTGACTCACG
H3N2sNA-2f	GCAAAAGCAGGAGTAAAGATGAA
H3N2sNA-418f	CCTTGGACAGGGAACAAAC
N2-550f	ATGGTCCAGCTCAAGTTGTCA
N2-645R	CCATCGTAAATGAAGCTAGC
H3N2sNA-1436r	CGAAAGCTTATATAGGCATGAGA
pRF-polyA-Rv	CTCTAGCATTAGGTGACC

H3N2 MUTANT GGACATCTGGGTGACAAGAGTAACCTTATGTGTCAT
||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
H3N2 WT GGACATCTGGGTGACAAGAGAAACCTTATGTGTCAT

Figure S1: Results of sequence of mutant and wild-type H3N2 around the mutation E119V (GAA > GTA)

Table S2: Mean results for the RT-ddPCR and the phenotypic test

Mixes	Expected		RT-ddPCR		Phenotypic (IC ₅₀)
	WT	Mutant	WT	Mutant	
Mix1	0	100	0.21 ± 0.01%	99.79 ± 0.01%	1.0 ± 0.0
Mix2	10	90	-	-	0.9 ± 0.4
Mix3	20	80	-	-	1.1 ± 0.6
Mix4	30	70	-	-	1.2 ± 1.2
Mix5	40	60	-	-	1.1 ± 0.8
Mix6	50	50	41.90 ± 0.31%	58.10 ± 0.31%	0.7 ± 0.5
Mix7	80	20	60.02 ± 2.16%	39.98 ± 2.16%	0.9 ± 0.8
Mix8	90	10	75.69 ± 2.60%	24.33 ± 2.60%	4.8 ± 3.5
Mix9	95	5	85.86 ± 1.13%	14.14 ± 1.13%	11.6 ± 12.2
Mix10	99	1	97.03 ± 0.48%	2.97 ± 0.48%	64.1 ± 35.1
Mix11	99.5	0.5	98.40 ± 0.13%	1.60 ± 0.13%	117.7 ± 6.7
Mix12	99.9	0.1	99.60 ± 0.04%	0.40 ± 0.04%	158.8 ± 14.0
Mix13	100	0	99.88 ± 0.08%	0.12 ± 0.08%	177.6 ± 24.2

Table S3: Raw ddPCR data and phenotypic test data

Mix	Expected MT%	RT-ddPCR							Phenotypic test
		E119 (copies/µL)	V119 (copies/µL)	Accepted droplets	Negative droplets	E119+ droplets	V119+ droplets	E119+/V119+ droplets	
1	100	2.5	2980	9805	774	3	9010	18	167.5
1	100	1.8	2640	8693	916	3	7764	10	178.9
1	100	2.5	4650	7006	135	0	6856	15	186.5
2	90	-	-	-	-	-	-	-	165.5
2	90	-	-	-	-	-	-	-	168.2
2	90	-	-	-	-	-	-	-	142.8
3	80	-	-	-	-	-	-	-	123.1
3	80	-	-	-	-	-	-	-	117.5
3	80	-	-	-	-	-	-	-	112.4
4	70	-	-	-	-	-	-	-	97.0
4	70	-	-	-	-	-	-	-	68.2
4	70	-	-	-	-	-	-	-	27.2
5	60	-	-	-	-	-	-	-	25.0
5	60	-	-	-	-	-	-	-	8.8
5	60	-	-	-	-	-	-	-	1.0
6	50	1373	3890	8727	106	213	2611	5797	8.7
6	50	507.2	779.4	9653	2431	2318	3653	1251	4.0
6	50	247	724	9471	4203	916	3476	876	1.6
7	20	614	329	5973	2676	1839	868	590	0.0
7	20	232	156	8486	6120	1313	849	204	1.5
7	20	1177	699	6186	11239	2176	1036	1735	1.1
8	10	662	150	7756	3902	2925	518	411	0.2
8	10	555	151	9465	5222	3105	683	455	1.2
8	10	1031	286	9579	3140	4373	847	1219	0.6

9	5	874	99	5875	2558	2842	236	239	0.3
9	5	798	111	8963	4170	3987	377	429	1.2
9	5	1044	115	8769	3256	4699	355	459	1.9
10	1	1074	18.3	5310	2089	3139	42	40	2.4
10	1	1019	24.8	12080	4977	6851	104	148	1.2
10	1	1042	21	7859	3184	4536	58	81	0.1
11	0.5	1062	11.1	6618	2662	3894	21	41	1.7
11	0.5	746	9.9	9698	5102	4515	44	37	1.1
11	0.5	1153	11.3	12937	4814	7999	42	82	0.5
12	0.1	1169	2.8	9621	3553	6045	8	15	0.5
12	0.1	794	2.2	8507	4322	4169	9	7	1.0
12	0.1	1016	3.1	7257	3054	4184	7	12	1.2
13	0	730	1.2	9022	4842	4171	7	2	1.0
13	0	1210	0.59	12047	4304	7737	3	3	1.0
13	0	1088	0.5	7612	3018	4591	0	3	1.0

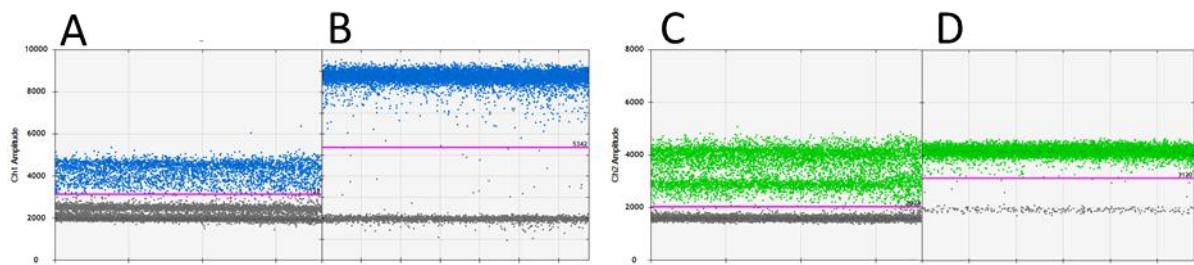


Figure S2: 1D view in QuantaSoft of results on optimization quencher. These figures show the difference in discriminating the viral populations before and after optimization of the quencher and replacing the BHQ-1 (A, C) quencher by a MGB-quencher (B, D). The negative droplets are shown in black, while droplets containing the FAM-labelled (A, B) E119-wild-type are blue and the HEX-labelled (C, D) V119-mutant are green

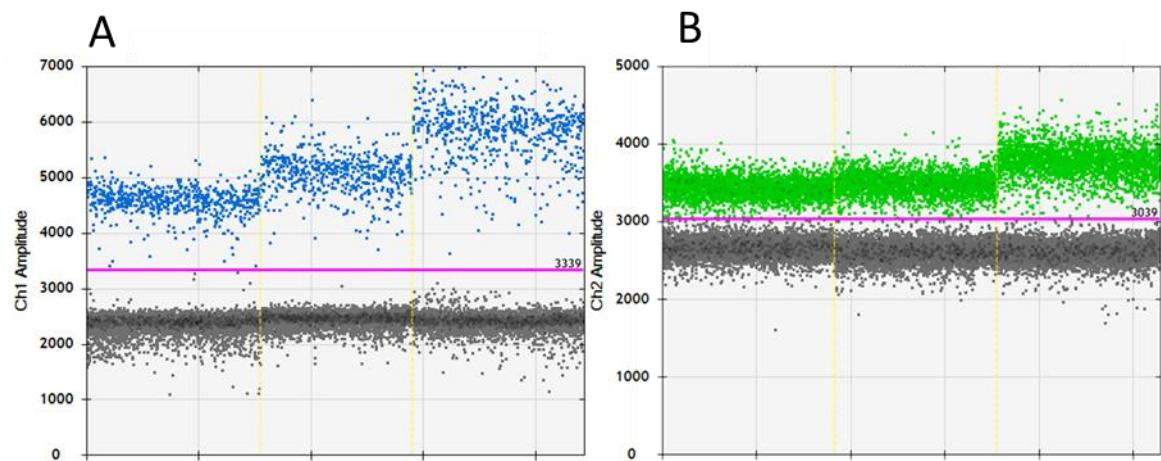


Figure S3: 1D view in QuantaSoft of results on optimization of the annealing temperature using a thermal gradient. These figures show the difference in discriminating the viral populations using a thermal gradient ranging from 58°C to 54.8°C (samples from left to right: 58°C, 56.5°C, 54.8°C). The negative droplets are shown in black, while droplets containing the FAM-labelled (A) E119-wild-type are blue and the HEX-labelled (B) V119-mutant are green.