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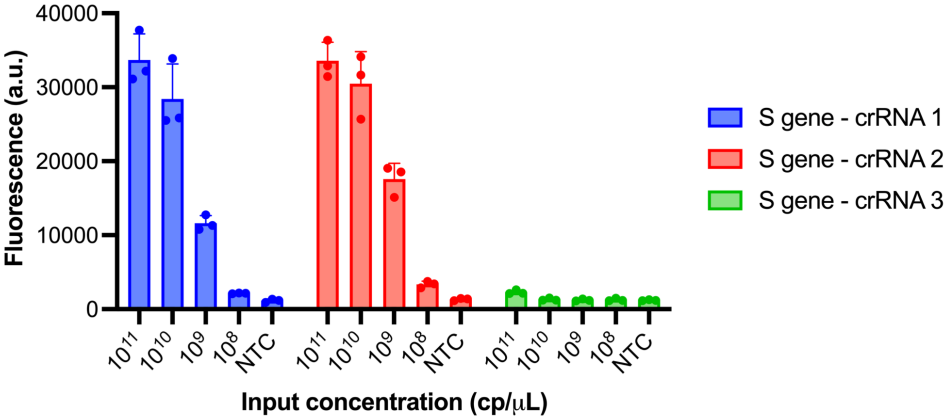
Supplementary information

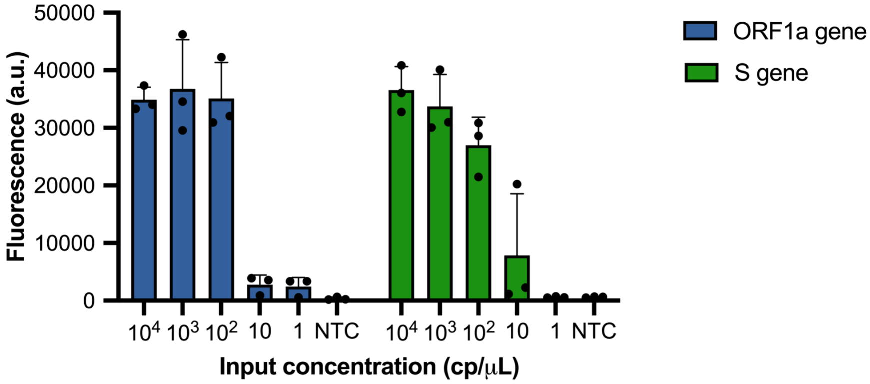
Simplified Cas13-based assays for the fast identification of SARS-CoV-2 and its variants

In the format provided by the authors and unedited

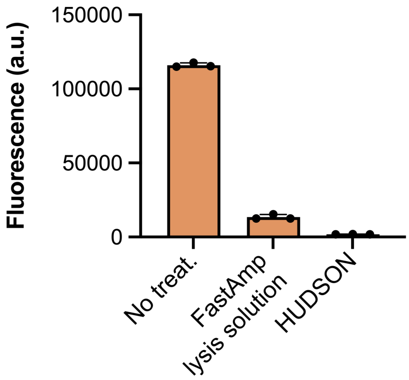
Contents



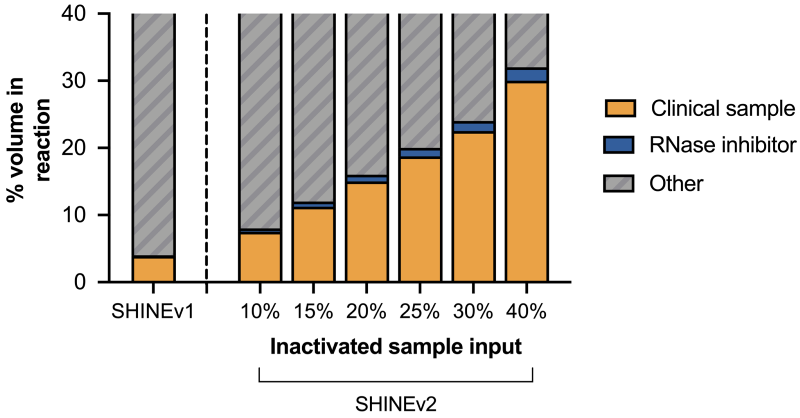
Supplementary Fig. 1 l Selection of most active S gene crRNA. Cas13a detection of synthetic RNA target (S gene) using different crRNAs. NTC, no target control. Centre = mean and error bar = standard deviation (s.d.) for 3 technical replicates.



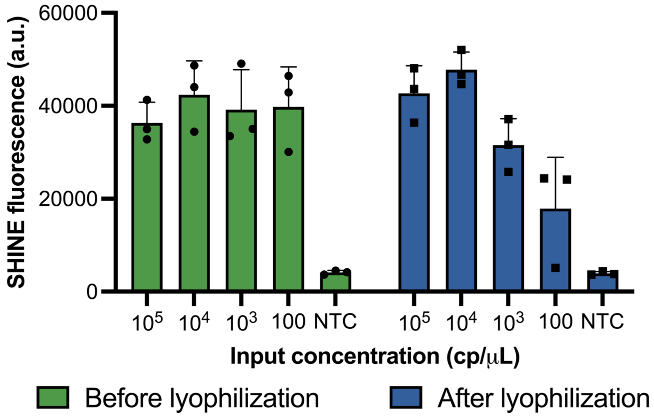
Supplementary Fig. 2 | SHINE assay comparison. Background-subtracted fluorescence of the ORF1a and S gene SHINE assays using synthetic SARS-CoV-2 RNA after 90 minutes. NTC, no target control. Centre = mean and error bars = s.d. for 3 technical replicates.



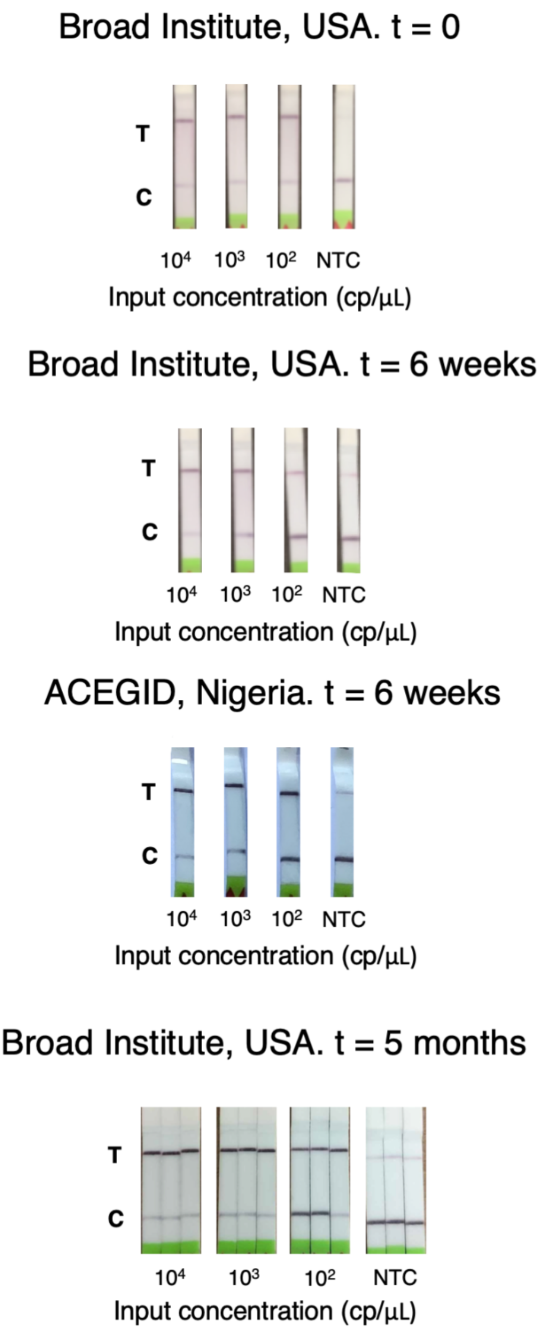
Supplementary Fig. 3 | RNase inactivation in saliva by FastAmp lysis solution. RNase activity in pooled saliva samples untreated or treated with FastAmp Lysis reagent supplemented with 5% RNase inhibitor or treated with HUDSON (a heat- and chemical- treatment). Activity measured using RNaseAlert at room temperature for 30 minutes. Centre = mean and error bars = s.d. for 3 technical replicates.



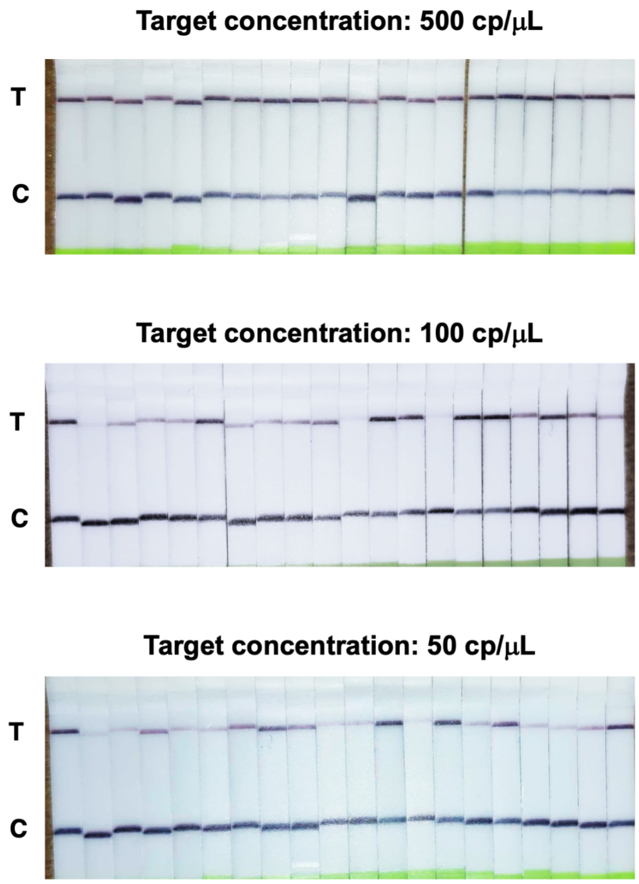
Supplementary Fig. 4 | Inactivated sample input is increased in SHINEv.2. Volume (%) of clinical sample and RNase inhibitor in the final reaction for SHINEv.1 and SHINEv.2 as a function of inactivated sample input.



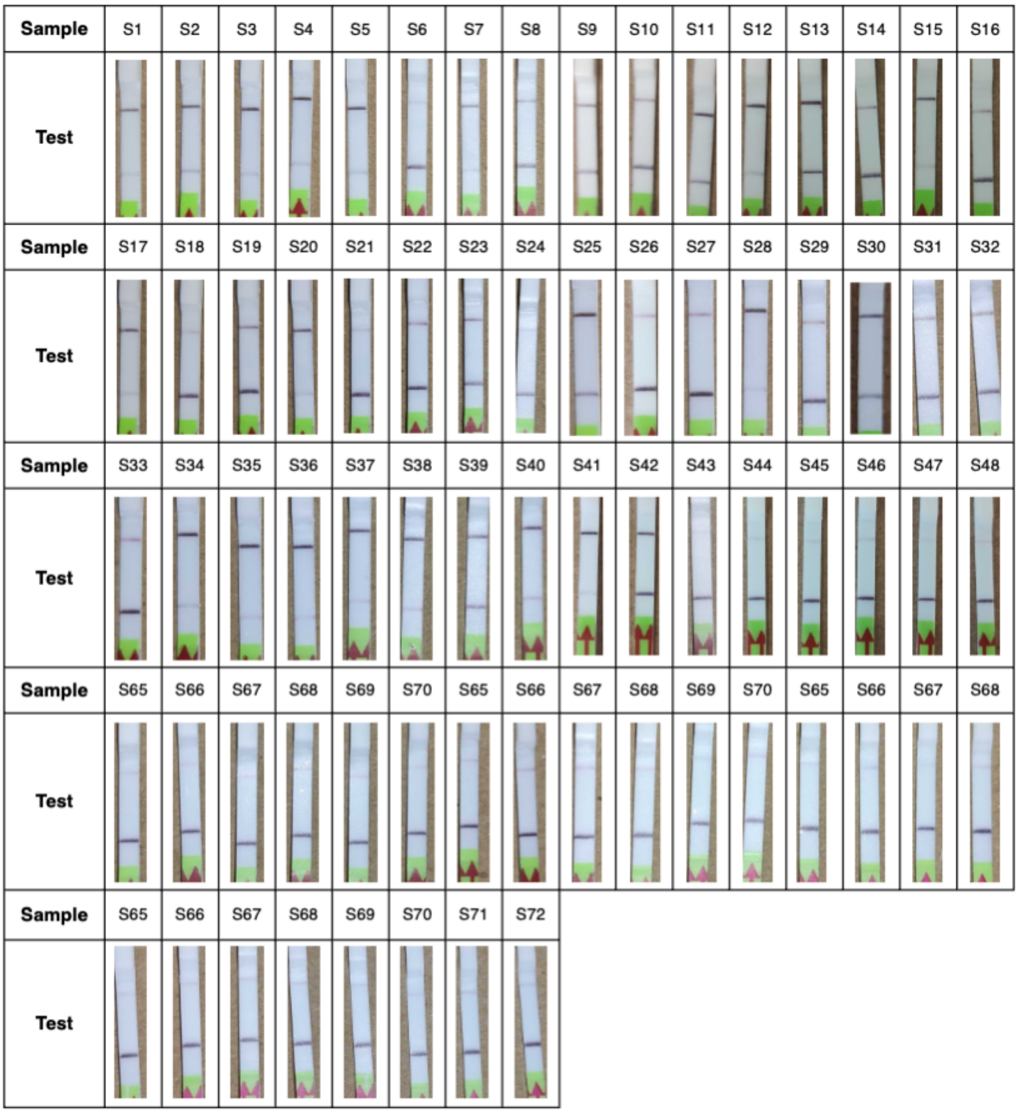
Supplementary Fig. 5 I The activity of SHINE is preserved after lyophilization. SHINE fluorescence on synthetic RNA target before and after lyophilization. Fluorescence measured after 90 minutes. NTC, no target control. Centre = mean and error bars = s.d. for 3 technical replicates.



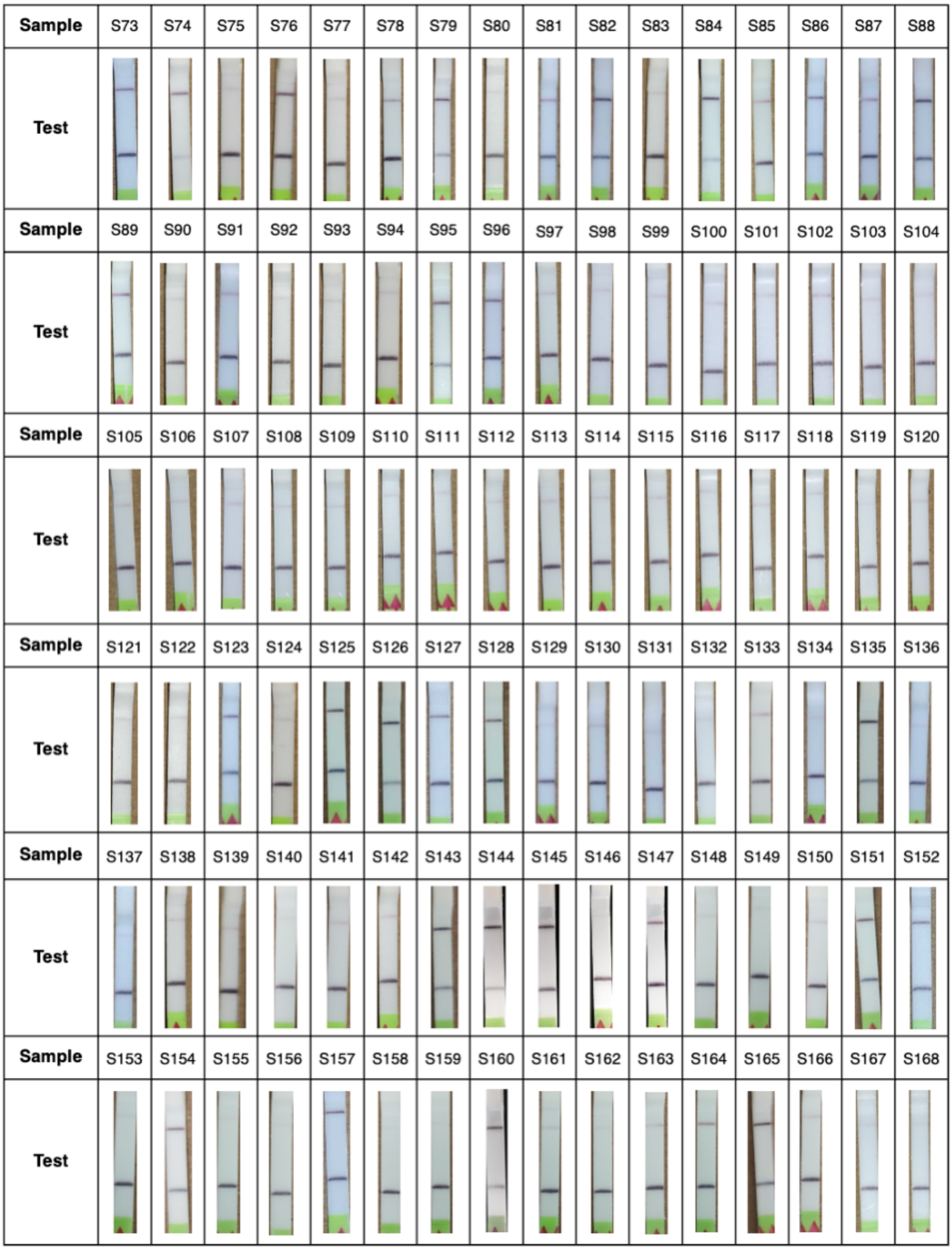
Supplementary Fig. 6 | SHINEv.2 can be transported overseas and is stable for at least 5 months. Lateral flow detection of full-genome synthetic RNA standards in lysis solution-treated UTM using SHINEv.2, before or after transportation, and after various lengths in storage. SHINEv.2 tests after 5-month storage tested in triplicate. Incubated for 90 minutes. C = control band; T = test band; NTC = no target control.



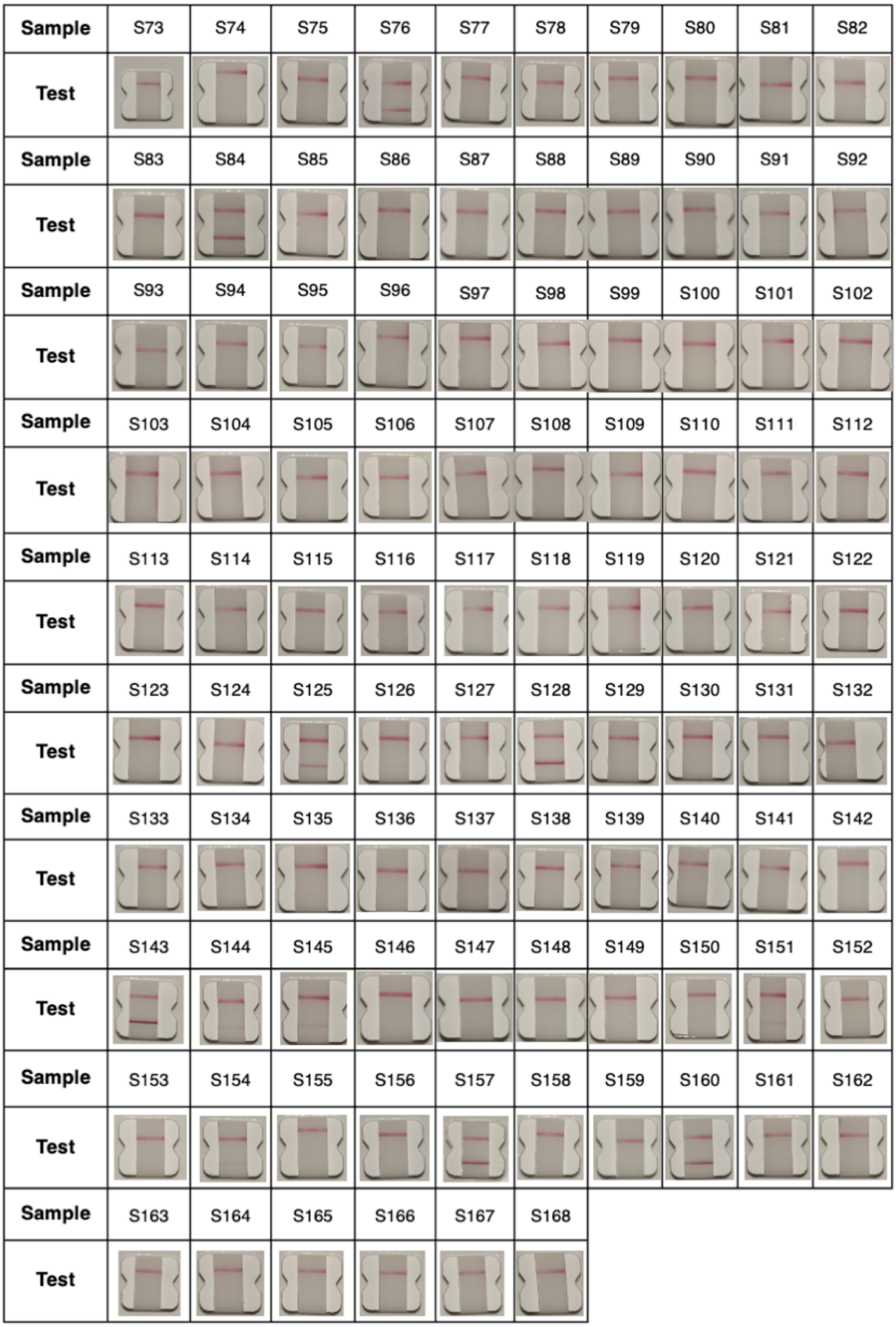
Supplementary Fig. 7 | SHINEv.2 has an analytical sensitivity of 200cp/μL. Determination of analytical limit of detection with 20 replicates of SHINEv.2 at different concentrations of SARS-CoV-2 RNA from lysis solution-treated contrived samples. Incubated for 90 minutes. Results for 200cp/μL sample input shown in Fig. 1i.



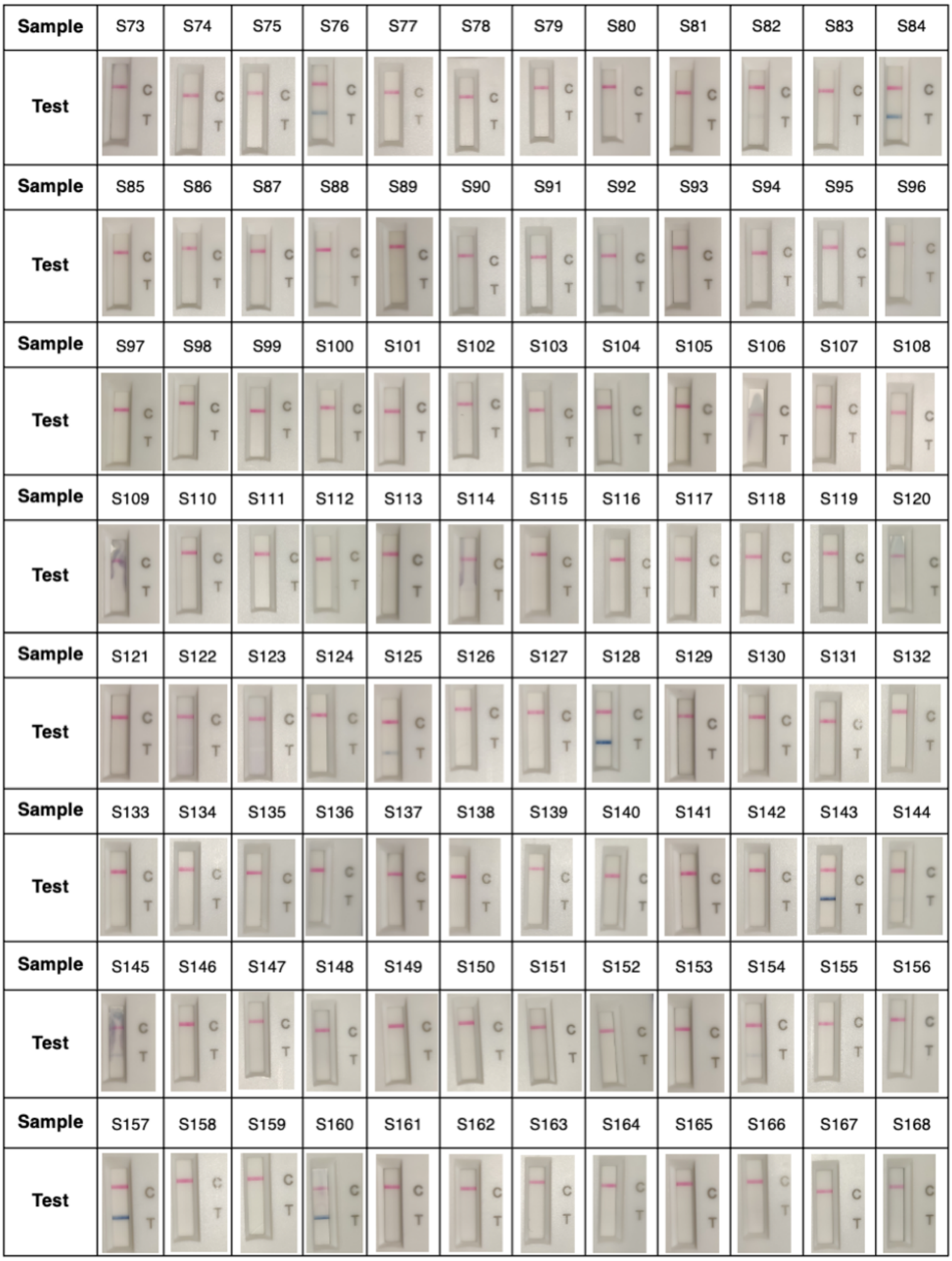
Supplementary Fig. 8 | SHINEv.2 results on population-representative clinical samples. Images of positive and negative test results with SHINEv.2 on unextracted nasopharyngeal swab samples. Samples were incubated for 90 minutes at 37°C.



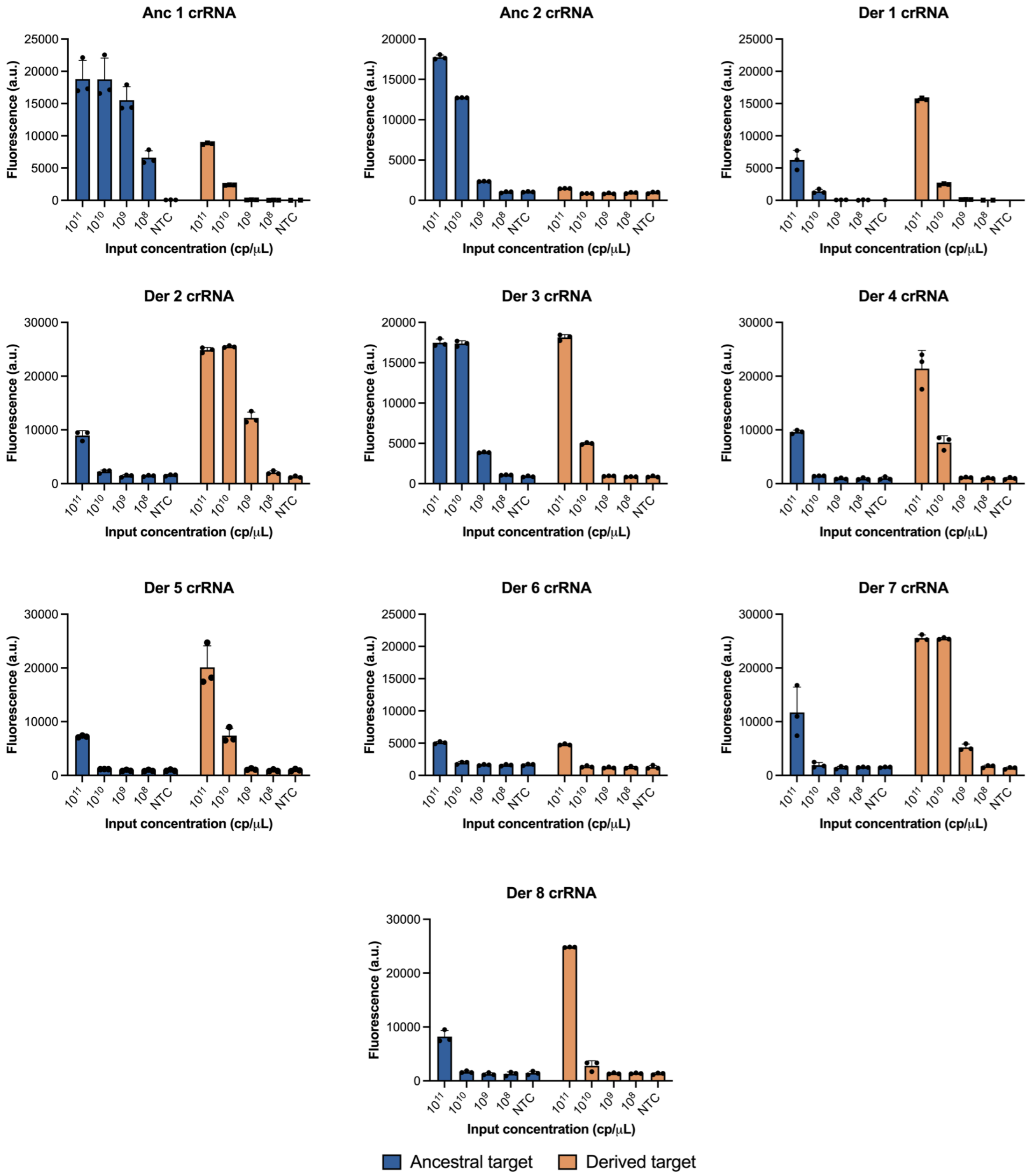
Supplementary Fig. 9 | SHINEv.2 results on clinical samples. Images of positive and negative test results with SHINEv.2 on unextracted nasopharyngeal swab samples. Samples were incubated for 90 minutes at 37°C.



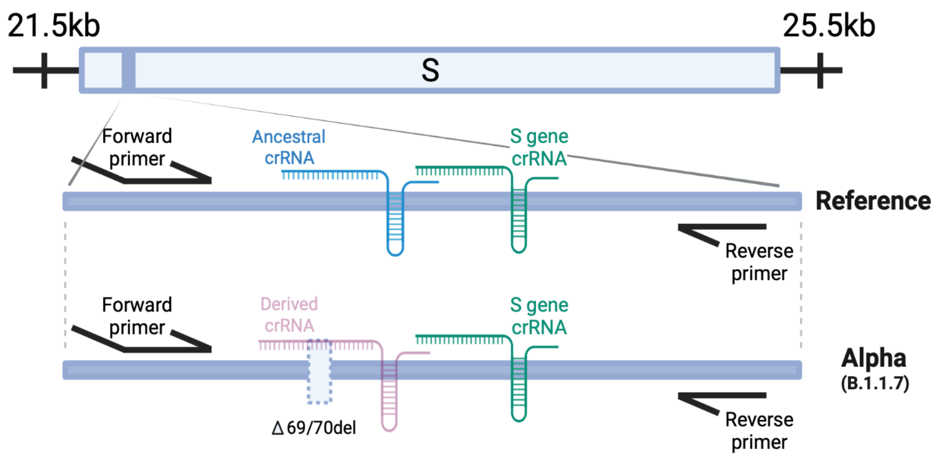
Supplementary Fig. 10 | BinaxNow results on clinical samples. Images of positive and negative test results with BinaxNow on unextracted NP swab samples.



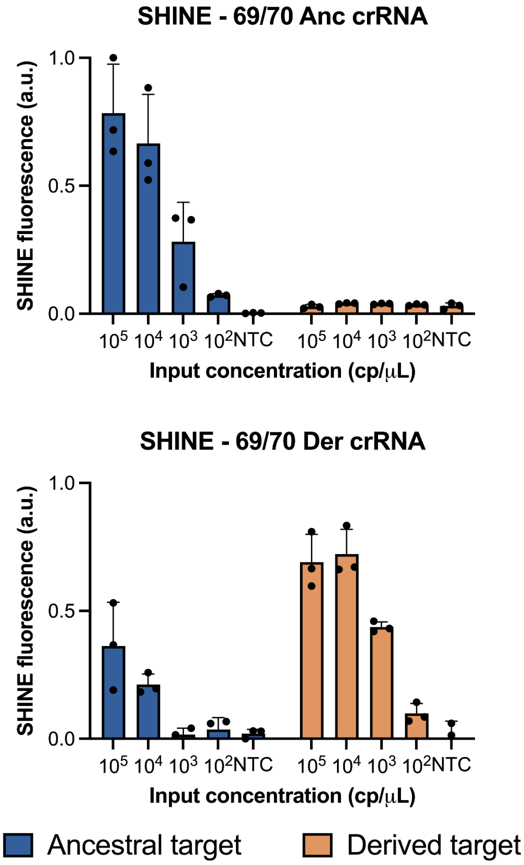
Supplementary Fig. 11 | CareStart results on clinical samples. Images of positive and negative test results with CareStart on unextracted nasopharyngeal NP swab samples.



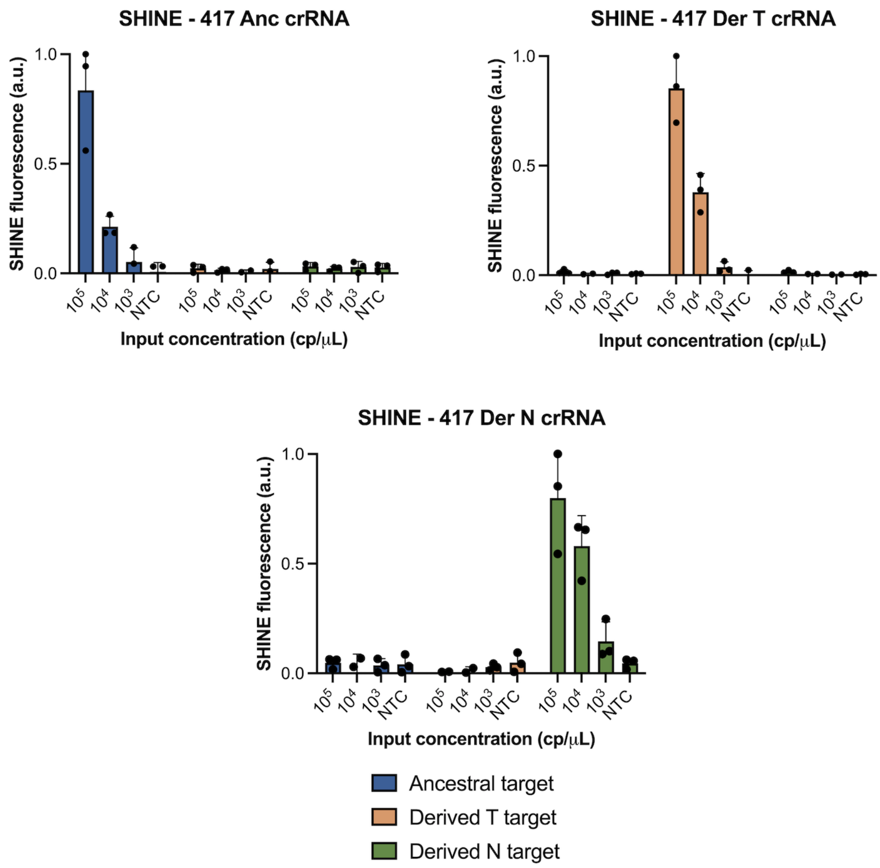
Supplementary Fig. 12 l Selection of ancestral (anc) and derived (der) crRNAs for the S gene 69/70 deletion assay. Cas13a detection of ancestral and derived synthetic RNA targets with different anc and der crRNAs. NTC, no target control. Centre = mean and error bar = standard deviation (s.d.) for 3 technical replicates.



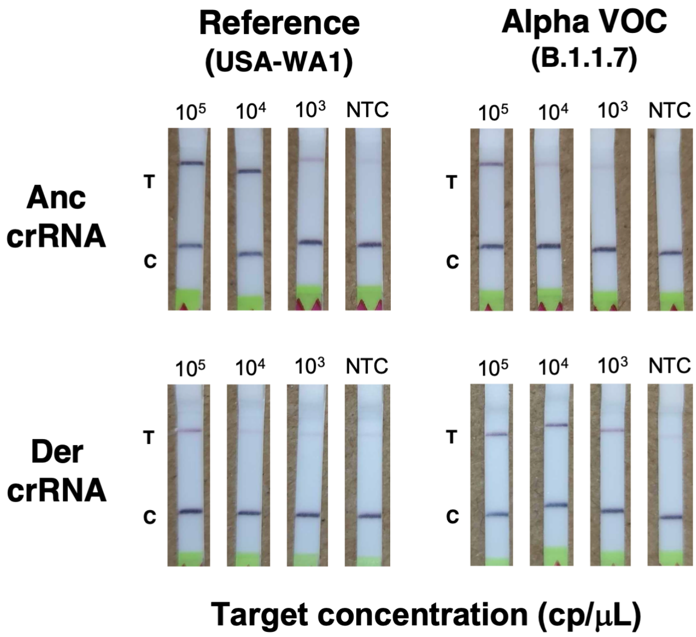
Supplementary Fig. 13 | Location of the SHINEv.2 assay for the 69/70 deletion detection. Schematic of location of the S gene and 69/70 deletion SHINEv.2 assays within the S gene of SARS-CoV-2 for the reference strain and Alpha VOC. Dashed rectangle, deletion at amino acid positions 69 and 70 in the S gene (Δ69/70del).



Supplementary Fig. 14 | Performance of 69/70 deletion assay on synthetic RNA targets. SHINE fluorescence of the 69/70 ancestral (anc) and derived (der) assays on synthetic SARS-CoV-2 RNA targets after 90 minutes. NTC, no target control. Centre = mean and error bars = s.d. for 3 technical replicates.

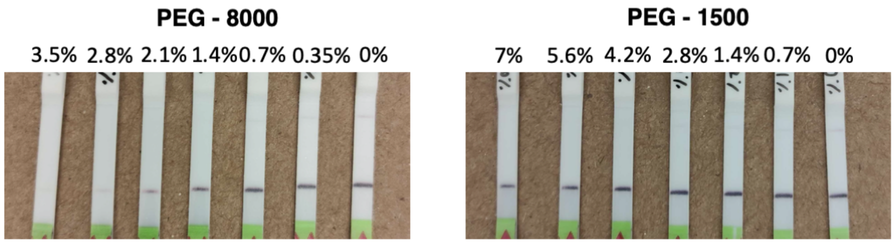


Supplementary Fig. 15 | Performance of 417 SNP detection assay on synthetic RNA targets. SHINE fluorescence of the 417 ancestral (anc), derived T (der T) and derived N (der N) assays on synthetic SARS-CoV-2 RNA targets after 90 minutes. NTC, no target control. Centre = mean and error bars = s.d. for 3 technical replicates.

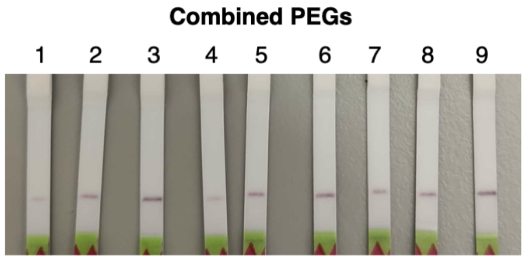


Supplementary Fig. 16 | Lateral flow detection of Alpha VOC in contrived clinical samples. Colorimetric lateral-flow-based detection of SARS-CoV-2 RNA in contrived clinical samples using the 69/70 SHINEv.2 assay. SHINEv.2 incubation time: 90 minutes. NTC, no-target control. T, test line; C, control line.

a

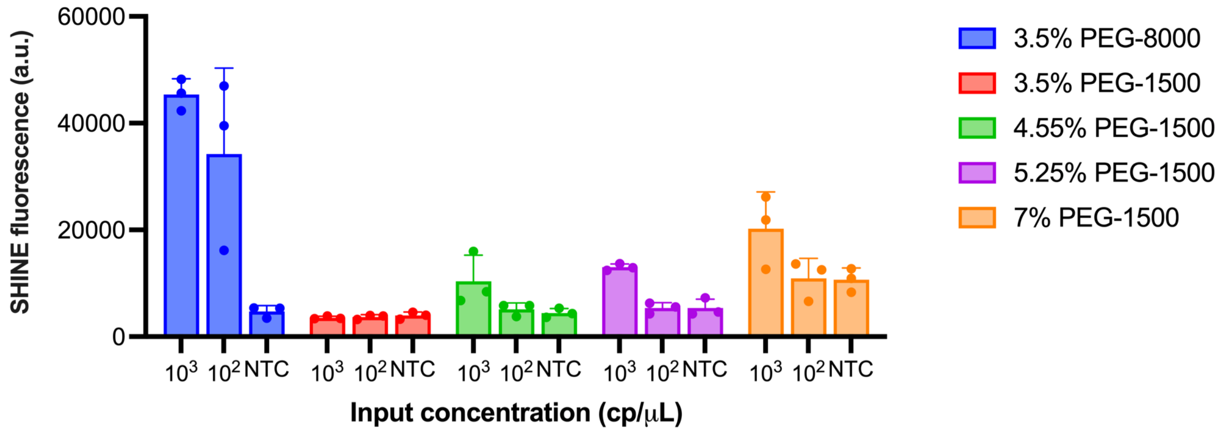


b

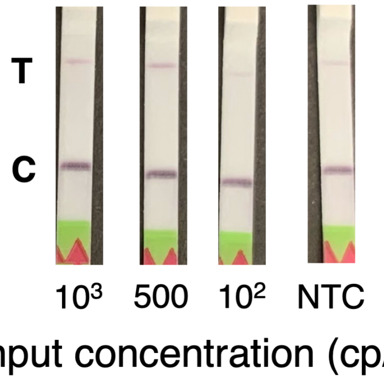


	1	2	3	4	5	6	7	8	9
PEG-1500	4.2%	4.2%	4.2%	3.5%	3.5%	3.5%	2.8%	2.8%	2.8%
PEG-8000	1.4%	0/7%	0.35%	1.4%	0.7%	0.35%	1.4%	0.7%	0.35%

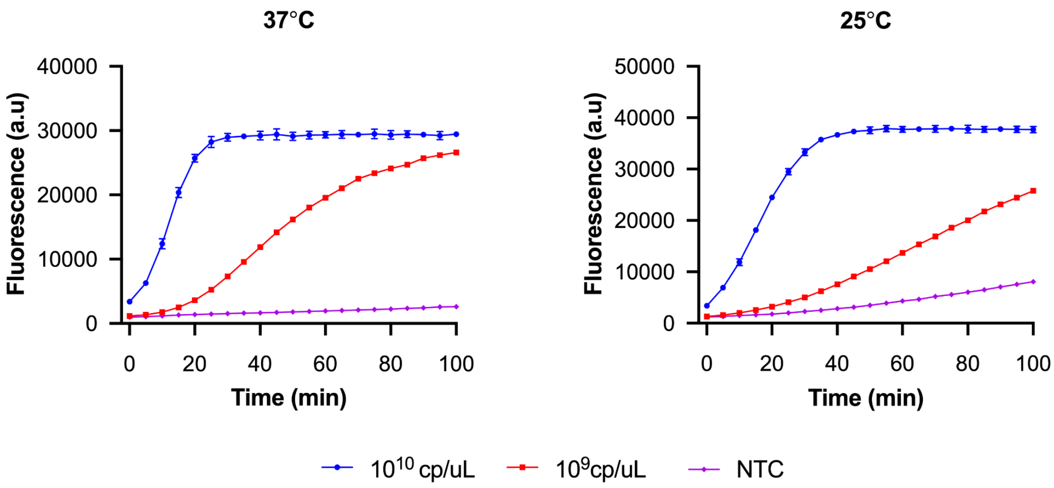
Supplementary Fig. 17 I Effect of PEG on flow through the strip. (a,b), images of lateral flow strips from SHINEv.2 reactions using PEGs with different molecular weights and concentrations **(a)** separately and **(b)** in combination, in the absence of an RNA target.



Supplementary Fig. 18 | Effect of PEG concentration on SHINE performance. SHINE fluorescence on synthetic SARS-CoV-2 RNA targets (S gene) relative to PEG molecular weight and concentration. Reactions incubated for 90 minutes. NTC, no target control. Centre = mean and error bars = s.d. for 3 technical replicates.



Supplementary Fig. 19 | Performance of equipment-free SHINEv.2 at room temperature. Lateral flow detection of full genome synthetic RNA standards in lysis solution-treated UTM using SHINEv.2, after a 90 minute incubation at 25°C. C = control band; T = test band; NTC = no target control.



Supplementary Fig. 20 l Temperature has a limited effect on Cas13-based detection. Cas13-detection of synthetic RNA target (S gene) at 37°C and 25°C. NTC, no target control. Centre = mean and error bar = standard deviation (s.d.) for 3 technical replicates.

Supplementary Table 1 | Patient sample information.

Samples S1 - S168 and VOC01 - VOC12 were used without extraction, whereas RNA was extracted from samples E1 - E20 before use. See *methods* for details. UTM, universal viral transport medium; VTM, viral transport medium; RP, RNase P; n.d., not determined.

SHINEv.2 sample ID	Sample matrix	N1 viral titer (copies/μL)	N1 Ct	N2 Ct	RP Ct	Figures
S1	VTM	486092	19.3	23.7	32.4	Supplementary Fig. 8
S2	VTM	37355	23.2	25	36.9	Supplementary Fig. 8
S3	VTM	33470	23.3	24.5	32.9	Supplementary Fig. 8
S4	VTM	209	30.8	32.2	33.8	Supplementary Fig. 8
S5	VTM	128108	21.3	22.9	32.5	Supplementary Fig. 8
S6	VTM	312	30.1	32.1	33.1	Supplementary Fig. 8
S7	VTM	482212	19.3	21.5	34.1	Supplementary Fig. 8
S8	VTM	1110	28.5	30.5	34.1	Supplementary Fig. 8
S9	VTM	24475	23.8	25.5	31.2	Supplementary Fig. 8
S10	VTM	3256	26.8	28.7	32.4	Supplementary Fig. 8
S11	VTM	1520	28	30.1	29.7	Supplementary Fig. 8
S12	VTM	6051	25.9	27.8	28.5	Supplementary Fig. 8
S13	VTM	780	29	31.1	30.7	Supplementary Fig. 8
S14	VTM	2013	27.6	29.7	31.7	Supplementary Fig. 8
S15	VTM	597551	19	20.5	34.5	Supplementary Fig. 8
S16	VTM	981	28.6	31	34	Supplementary Fig. 8
S17	VTM	324715	19.9	21.1	35.2	Supplementary Fig. 8
S18	VTM	133	32	32.6	28.8	Supplementary Fig. 8
S19	VTM	156186	21	22.5	31.7	Supplementary Fig. 8
S20	VTM	1010050	18.2	19.2	28.7	Supplementary Fig. 8
S21	VTM	102	32.9	33.2	32.7	Supplementary Fig. 8
S22	VTM	9731	25.2	26.3	33.7	Supplementary Fig. 8
S23	VTM	7591	25.6	27.1	30.9	Supplementary Fig. 8
S24	VTM	81	33.4	34	26.7	Supplementary Fig. 8
S25	VTM	5332	25.8	26.6	36.2	Supplementary Fig. 8
S26	VTM	727	29	29.9	31.3	Supplementary Fig. 8
S27	VTM	15654	24	24.6	26.5	Supplementary Fig. 8
S28	VTM	25046	23.3	23.5	34.6	Supplementary Fig. 8
S29	VTM	21779	23.5	23.4	30.3	Supplementary Fig. 8
S30	VTM	7215	25.3	25.1	30.3	Supplementary Fig. 8
S31	VTM	2455	27	27.8	34.4	Supplementary Fig. 8
S32	VTM	3162	26.6	27	29.8	Supplementary Fig. 8

S33	VTM	3179	26.6	26.8	33.6	Supplementary Fig. 8
S34	VTM	55277	22	22.2	34	Supplementary Fig. 8
S35	VTM	291593	19.3	19	33.8	Supplementary Fig. 8
S36	VTM	37000	22.7	22.9	33.3	Supplementary Fig. 8
S37	VTM	484666	18.5	18.6	31.7	Supplementary Fig. 8
S38	VTM	59845	21.9	22.5	29.9	Supplementary Fig. 8
S39	VTM	268003	19.4	19.4	27.2	Supplementary Fig. 8
S40	VTM	15899	24	24.6	33.4	Supplementary Fig. 8
S41	VTM	43486	22.4	23	28.3	Supplementary Fig. 8
S42	VTM	16193	24	24.2	31	Supplementary Fig. 8
S43	VTM	-	-	-	31.4	Supplementary Fig. 8
S44	VTM	-	-	-	31.3	Supplementary Fig. 8
S45	VTM	-	-	-	31.8	Supplementary Fig. 8
S46	VTM	-	-	-	33.8	Supplementary Fig. 8
S47	VTM	-	-	-	33.4	Supplementary Fig. 8
S48	VTM	-	-	-	36	Supplementary Fig. 8
S49	VTM	-	-	-	34.4	Supplementary Fig. 8
S50	VTM	-	-	-	32.7	Supplementary Fig. 8
S51	VTM	-	-	-	35.5	Supplementary Fig. 8
S52	VTM	-	-	-	33	Supplementary Fig. 8
S53	VTM	-	-	-	33.1	Supplementary Fig. 8
S54	VTM	-	-	-	32.6	Supplementary Fig. 8
S55	VTM	-	-	-	33.8	Supplementary Fig. 8
S56	VTM	-	-	-	31.6	Supplementary Fig. 8
S57	VTM	-	-	-	28.3	Supplementary Fig. 8
S58	VTM	-	-	-	30.7	Supplementary Fig. 8
S59	VTM	-	-	-	30	Supplementary Fig. 8
S60	VTM	-	-	-	31.2	Supplementary Fig. 8
S61	VTM	-	-	-	29.9	Supplementary Fig. 8
S62	VTM	-	-	-	31.9	Supplementary Fig. 8
S63	VTM	-	-	-	31.3	Supplementary Fig. 8
S64	VTM	-	-	-	31	Supplementary Fig. 8
S65	VTM	-	-	-	31.8	Supplementary Fig. 8
S66	VTM	-	-	-	33.3	Supplementary Fig. 8
S67	VTM	-	-	-	32.9	Supplementary Fig. 8
S68	VTM	-	-	-	31.1	Supplementary Fig. 8
S69	VTM	-	-	-	32.2	Supplementary Fig. 8

S70	VTM	-	-	-	31.7	Supplementary Fig. 8
S71	VTM	-	-	-	31.8	Supplementary Fig. 8
S72	VTM	-	-	-	31.3	Supplementary Fig. 8
S73	VTM	883	27.8	29.3	29.7	Supplementary Fig. 9-11
S74	VTM	1078	27.5	29	26.9	Supplementary Fig. 9-11
S75	VTM	7	35.6	37.8	31.5	Supplementary Fig. 9-11
S76	VTM	194575	19.4	20.3	28.9	Supplementary Fig. 9-11
S77	VTM	107	31	32.7	31.9	Supplementary Fig. 9-11
S78	VTM	109	31	33	30	Supplementary Fig. 9-11
S79	VTM	328	29	31.1	24.6	Supplementary Fig. 9-11
S80	VTM	34	33	35.1	29.5	Supplementary Fig. 9-11
S81	VTM	778	28	29.8	30	Supplementary Fig. 9-11
S82	VTM	4739	25.2	26.9	29.3	Supplementary Fig. 9-11
S83	VTM	33	33	35.6	29.4	Supplementary Fig. 9-11
S84	VTM	599754	17.7	18.6	29.5	Supplementary Fig. 9-11
S85	VTM	271	29.4	31.6	29.5	Supplementary Fig. 9-11
S86	VTM	931	27.7	29.2	29.4	Supplementary Fig. 9-11
S87	VTM	540	28.6	30.8	29.4	Supplementary Fig. 9-11
S88	VTM	3281	25.7	27.9	29.1	Supplementary Fig. 9-11
S89	VTM	739	28.1	29.8	24.6	Supplementary Fig. 9-11
S90	Saline	-	-	-	29.7	Supplementary Fig. 9-11
S91	Saline	232	29.6	32.2	28.2	Supplementary Fig. 9-11
S92	Saline	-	-	-	29	Supplementary Fig. 9-11
S93	Saline	1	37.7	-	26.4	Supplementary Fig. 9-11
S94	Saline	3	37	38.5	27.3	Supplementary Fig. 9-11
S95	VTM	188	29.9	32.1	30.3	Supplementary Fig. 9-11
S96	UTM	3739	25.5	27.8	28.9	Supplementary Fig. 9-11
S97	UTM	-	-	-	32.3	Supplementary Fig. 9-11
S98	UTM	-	-	-	29.8	Supplementary Fig. 9-11
S99	UTM	-	-	-	30.6	Supplementary Fig. 9-11
S100	UTM	-	-	-	28.8	Supplementary Fig. 9-11
S101	UTM	-	-	-	30.2	Supplementary Fig. 9-11
S102	UTM	-	-	-	29.8	Supplementary Fig. 9-11
S103	UTM	-	-	-	31.1	Supplementary Fig. 9-11
S104	UTM	-	-	-	34.1	Supplementary Fig. 9-11
S105	UTM	-	-	-	30.5	Supplementary Fig. 9-11

S106	UTM	-	-	-	31.7	Supplementary Fig. 9-11
S107	UTM	-	-	-	28.4	Supplementary Fig. 9-11
S108	UTM	-	-	-	30.1	Supplementary Fig. 9-11
S109	UTM	-	-	-	27.4	Supplementary Fig. 9-11
S110	UTM	-	-	-	28.8	Supplementary Fig. 9-11
S111	UTM	-	-	-	29.6	Supplementary Fig. 9-11
S112	UTM	-	-	-	28	Supplementary Fig. 9-11
S113	UTM	-	-	-	30.6	Supplementary Fig. 9-11
S114	UTM	-	-	-	29.7	Supplementary Fig. 9-11
S115	UTM	-	-	-	29.2	Supplementary Fig. 9-11
S116	UTM	-	-	-	31	Supplementary Fig. 9-11
S117	UTM	-	-	-	30	Supplementary Fig. 9-11
S118	UTM	-	-	-	30.8	Supplementary Fig. 9-11
S119	UTM	-	-	-	29.1	Supplementary Fig. 9-11
S120	UTM	-	-	-	27.7	Supplementary Fig. 9-11
S121	UTM	3	36.8	38.7	30	Supplementary Fig. 9-11
S122	VTM	-	-	-	27.7	Supplementary Fig. 9-11
S123	VTM	653	28	30	26.1	Supplementary Fig. 9-11
S124	VTM	193	29.8	31.8	31.3	Supplementary Fig. 9-11
S125	VTM	31094	22	23.4	31.9	Supplementary Fig. 9-11
S126	VTM	1130	27.1	29.4	29.6	Supplementary Fig. 9-11
S127	VTM	527	28.3	30.7	31.8	Supplementary Fig. 9-11
S128	VTM	2323088	15.4	16.6	28.6	Supplementary Fig. 9-11
S129	VTM	-	-	37.7	32.2	Supplementary Fig. 9-11
S130	VTM	129	30.2	33.1	31.6	Supplementary Fig. 9-11
S131	VTM	-	-	38.6	30.6	Supplementary Fig. 9-11
S132	VTM	34	32.6	35	30.3	Supplementary Fig. 9-11
S133	VTM	82	31.2	33.4	31.3	Supplementary Fig. 9-11
S134	VTM	10	34.5	36.7	32	Supplementary Fig. 9-11
S135	VTM	1224	27	29	29.1	Supplementary Fig. 9-11
S136	VTM	4	36	38.3	28.2	Supplementary Fig. 9-11
S137	UTM	-	-	-	29.9	Supplementary Fig. 9-11
S138	UTM	-	-	-	30.2	Supplementary Fig. 9-11
S139	UTM	-	-	-	27.8	Supplementary Fig. 9-11
S140	UTM	2	37.2	39.3	31.4	Supplementary Fig. 9-11
S141	UTM	4	35.9	38.5	26.4	Supplementary Fig. 9-11
S142	UTM	3	36	38.6	29.6	Supplementary Fig. 9-11

S143	VTM	3706009	14.7	15.8	27.3	Supplementary Fig. 9-11
S144	VTM	3118	25.6	27.5	29.7	Supplementary Fig. 9-11
S145	VTM	13588	23.1	24.4	28.8	Supplementary Fig. 9-11
S146	VTM	7	34.9	37	25.8	Supplementary Fig. 9-11
S147	VTM	1471	26.5	27.9	27.8	Supplementary Fig. 9-11
S148	VTM	3	36.5	38.2	30.3	Supplementary Fig. 9-11
S149	VTM	12	34.4	37.3	25.8	Supplementary Fig. 9-11
S150	VTM	9	34.6	38	29.5	Supplementary Fig. 9-11
S151	VTM	2925	25.5	27.2	28.1	Supplementary Fig. 9-11
S152	VTM	345	28.8	30.2	32.1	Supplementary Fig. 9-11
S153	VTM	5	35.6	37.5	28.3	Supplementary Fig. 9-11
S154	VTM	20694	22.4	23.4	29.5	Supplementary Fig. 9-11
S155	VTM	46	32	33.8	30.1	Supplementary Fig. 9-11
S156	VTM	14	33.8	36.6	29.1	Supplementary Fig. 9-11
S157	VTM	187051	18.9	19.9	26.1	Supplementary Fig. 9-11
S158	VTM	21	33.3	35.1	28.8	Supplementary Fig. 9-11
S159	VTM	13	34.2	36.6	30.8	Supplementary Fig. 9-11
S160	VTM	412032	17.7	18.8	27.4	Supplementary Fig. 9-11
S161	VTM	10	34.4	37.7	29.4	Supplementary Fig. 9-11
S162	VTM	172	30	32.3	28.1	Supplementary Fig. 9-11
S163	VTM	23	33.4	35.8	26.4	Supplementary Fig. 9-11
S164	VTM	386	28.7	29.7	30.9	Supplementary Fig. 9-11
S165	VTM	3907	25	26.3	28.9	Supplementary Fig. 9-11
S166	VTM	16	33.8	35	31.8	Supplementary Fig. 9-11
S167	VTM	-	-	-	30.3	Supplementary Fig. 9-11
S168	VTM	3	36.9	38.2	32.6	Supplementary Fig. 9-11
E1	VTM	65806	20.9	20.0	28.2	Fig. 3e
E2	UTM	23588	22.1	23.2	27.9	Fig. 3e
E3	VTM	1213	26.8	25.8	30.6	Fig. 3e
E4	VTM	3237	25.5	26.8	31.2	Fig. 3e
E5	VTM	753	27.3	29.3	32.1	Fig. 3e
E6	VTM	738	27.6	27.2	29.8	Fig. 3e
E7	Unknown	7804	24.1	24.2	31.2	Fig. 3e
E8	Unknown	4495	24.9	24.2	29.1	Fig. 3e
E9	Unknown	291	29.0	28.6	30.0	Fig. 3e
E10	Unknown	12954	23.3	23.3	33.5	Fig. 3e
E11	UTM	-	-	-	32.0	Fig. 3e

E12	UTM	-	-	-	30.2	Fig. 3e
E13	UTM	-	-	-	30.7	Fig. 3e
E14	UTM	-	-	-	29.9	Fig. 3e
E15	UTM	-	-	-	30.4	Fig. 3e
E16	UTM	-	-	-	29.5	Fig. 3e
E17	UTM	-	-	-	30.2	Fig. 3e
E18	UTM	-	-	-	33.8	Fig. 3e
E19	UTM	-	-	-	30.6	Fig. 3e
E20	UTM	-	-	-	31.1	Fig. 3e
VOC01	VTM	52016	22.2	n.d	n.d	Fig. 3f
VOC02	VTM	1957	28.0	n.d	n.d	Fig. 3f
VOC03	VTM	14210	24.5	n.d	n.d	Fig. 3f
VOC04	VTM	9888	25.1	n.d	n.d	Fig. 3f
VOC05	VTM	5212	25.9	n.d	n.d	Fig. 3f
VOC06	VTM	36147	23.0	n.d	n.d	Fig. 3f
VOC07	VTM	33457	23.1	n.d	n.d	Fig. 3f
VOC08	VTM	17855	24.0	n.d	n.d	Fig. 3f
VOC09	VTM	-	-	n.d	n.d	Fig. 3f
VOC10	VTM	-	-	n.d	n.d	Fig. 3f
VOC11	VTM	-	-	n.d	n.d	Fig. 3f
VOC12	VTM	-	-	n.d	n.d	Fig. 3f

Supplementary Table 2 | Oligonucleotides used in this study.

Name	Oligo type	Sequence	Gene - Assay
ORF1a crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACCUCUUCUUCAGGUUGAAGAGCAGCAGAA	ORF1ab
S gene crRNA - 1	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUGGUAGGACAGGGUUAUCAAACCUCUUA	S
S gene crRNA - 2	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACACAGGGUUAUCAAACCUCUUAGUACCAU	S
S gene crRNA - 3	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACACCAUUUAAUGAUGGUGUUUAUUUUGCU	S
S - 69/70 anc crRNA 1	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACACCAUUGGUCCCAGAGACAUGUAUAGCA	S - 69/70
S - 69/70 anc crRNA 2	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUCCCAGAGACAUGUAUAGCAUGGAACCA	S - 69/70
S - 69/70 der crRNA 1	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACCUUAGUACCAUUGGUCCCAGAUAUAGCA	S - 69/70
S - 69/70 der crRNA 2	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUCCCAGAUAUAGCAUGGAACCAAGUAAC	S - 69/70
S - 69/70 der crRNA 3	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACCAGAUAUAGCAUGGAACCAAGUAACAUU	S - 69/70
S - 69/70 der crRNA 4	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGGUCCCAGAUAUAGCAUGGAACCAAGUA	S - 69/70
S - 69/70 der crRNA 5	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUUGGUCCCAGAUAUAGCAUGGAACCAAG	S - 69/70
S - 69/70 der crRNA 6	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACCAUUGGUCCCAGAUAUAGCAUGGAACCA	S - 69/70
S - 69/70 der crRNA 7	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACACCAUUGGUCCCAGAUAUAGCAUGGAAC	S - 69/70
S - 69/70 der crRNA 8	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACAGTACCATTGGTCCCAGATATAGCATGG	S - 69/70

S - 417 anc crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGCATTCTCTCCAGTTTGCCCTGGAGCGA	S - 417
S - 417 derN crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUAUAAUUAUAAUCAGCAACAUCUCCGCA	S - 417
S - 417 derT crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUAUAAUCAGCAGUCGUACCAGUUUGCUA	S - 417
S - 452 anc crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGUUCCUAAACAAUCUAUACAGGUAAUCA	S - 452
S - 452 der crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACCUAUAGCUGUAAUUAUAAUUACCACCAA	S - 452
S - 156-158 anc crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUAAACUCUGAACUCACUUUCCAUCCAAC	S - 156-158
S - 156-158 der crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUAAACUCCACUUUCCAUCCAACUUUUGU	S - 156-158
S - 142-145 anc crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUGUUGUUUUUGUGGUAAUAAACACCCAA	S - 142-145
S - 142-145 der crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUGUUGUUUUUGUGGUCCAAAAAUGGAUC	S - 142-145
RNase P - crRNA	Cas12a crRNA	UAAUUUCUACUAAGUGUAGAUAGGCCCAGCUGGCCCGCUGC	RNase P
ORF1a fwd primer	RPA primer	GAAATTAATACGACTCACTATAGGGCCAAGGTAAACCTTTGGAATTTGGTGCCAC	ORF1ab
ORF1a rev primer	RPA primer	ACTATCATCATCTAACCAATCTTCTTCTTG	ORF1ab
S/S - 69/70 fwd primer	RPA primer	GAAATTAATACGACTCACTATAGGGCAACTCAGGACTTGTTCTTACCTTTCTTTTCC	S & S - 69/70
S/S - 69/70 rev primer	RPA primer	AAGCAAAATAAACACCATCATTAAAT	S & S - 69/70
S - 417 fwd primer	RPA primer	GAAATTAATACGACTCACTATAGGGTCTATGCAGATTCATTTGTAATTAGAGGTG	S - 417
S - 417 rev primer	RPA primer	ATAACGCAGCCTGTAAAATCATCTGGTAAT	S - 417
S - 452 fwd primer	RPA primer	GAAATTAATACGACTCACTATAGGGAGCTTGGAATTCTAACAATCTTGATTCT	S - 452
S - 452 rev primer	RPA primer	AGTTGAAATATCTCTCTCAAAAGGTTTGA	S - 452

S - 156-158 fwd primer	RPA primer	GAAATTAATACGACTCACTATAGGGTTTTTGGG TGTTTATTACCACAAAAACA	S - 156-158
S - 156-158 rev primer	RPA primer	AGAAAAGGCTGAGAGACATATTCAAAAG	S - 156-158
S - 156-158 fwd primer	RPA primer	GAAATTAATACGACTCACTATAGGGGCTACTAA TGTTGTTATTAAAGTCTGTGAA	S - 142-145
S - 156-158 rev primer	RPA primer	CTAGAATAAACTCTGAACTCACTTTCCATC	S - 142-145
RNase P Fwd primer	RPA primer	GAAATTAATACGACTCACTATAGGGGTGGAAT ACACCCTTAGGAAAAGGCTTC	RNase P
RNase P Rev primer	RPA primer	CAAGCCGTGAATGTAGATCTCAGAGCAC	RNase P
ORF1a synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGGGTGAGTTT AAATTGGCTTCACATATGTATTGTTCTTTCTAC CCTCCAGATGAGGATGAAGAAGAAGGTGATTG TGAAGAAGAAGAGTTTGAGCCATCAACTCAAT ATGAGTATGGTACTGAAGATGATTACCAAGGT AAACCTTTGGAATTTGGTGCCACTTCTGCTGCT CTTCAACCTGAAGAAGAGCAAGAAGAAGATTG GTTAGATGATGATAGTCAACAAACTGTTGGTCA ACAAGACGGCAGTGAGGACAATCAGACAACTA CTATTCAAACAATTGTTGAGGTTCAACCTCAAT TAGAGATGGAACTTACACCAGTTGTTCAGACT ATTGAAGTGAATAGTTTTAGTGGTTATTTAAAA CTTACTGACAATGTATACATTAAAAATGCAGAC ATTGTGGAAGAAGCTAAAAAGGTAAAACCAAC AGTGGTTGTTAATGCAGCCAATGTTTACCTTAA ACATGGAGGAGG	ORF1ab
S - 69/70 anc synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGTGACAAAGT TTTCAGATCCTCAGTTTTACATTCAACTCAGGA CTTGTTCTTACCTTTCTTTTCCAATGTTACTTGG TTCCATGCTATACATGTCTCTGGGACCAATGG TACTAAGAGGTTTGATAACCCTGTCCTACCATT TAATGATGGTGTTTATTTTGCTTCCACTGAGAA GTCTAACATAATAAGAGGCTGGATTTTTGGTAC TACTTTAGATTCGAAGACCCAGTCCCTACTTAT TGTTAATAACGCTACTAATGTTGTTATTAAAGT CTGTGAATTTCAATTTTGTAATGATCC	S - 69/70
S - 69/70 der synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGTGACAAAGT TTTCAGATCCTCAGTTTTACATTCAACTCAGGA CTTGTTCTTACCTTTCTTTTCCAATGTTACTTGG TTCCATGCTATATCTGGGACCAATGGTACTAA GAGGTTTGATAACCCTGTCCTACCATTTAATGA TGGTGTTTATTTTGCTTCCACTGAGAAGTCTAA CATAATAAGAGGCTGGATTTTTGGTACTACTTT AGATTCGAAGACCCAGTCCCTACTTATTGTTAA TAACGCTACTAATGTTGTTATTAAAGTCTGTGA	S - 69/70

		ATTTCAATTTTGTAATGATCC	
S - 417 anc synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGTCTATGCAG ATTCATTTGTAATTAGAGGTGATGAAGTCAGAC AAATCGCTCCAGGGCAAACTGGAAAGATTGCT GATTATAATTATAAATTACCAGATGATTTTACAG GCTGCGTTATAGCTTGGAATTCTAACAATCTTG ATTCTAAGGTTGGTGGTAATTATAATTACCTGT ATAGATTGTTTAGGAAGTCTAATCTCAAACCTT TTGAGAGAGATATTTCAACTGAAATCTATCAGG CCGGTAGCACACCTTGTAATGGTGTTGAAGGT TTTAATTGTTACTTTCCTTTACAATCATATGGTT TCCAACCCACTAATGGTGTTGGTTACCAACCA TACAGAGTAGTAGTACTTTCTTTTGAACTTCTA CATGCACCAGCAA	S - 417
S - 417 derN synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGTCTATGCAG ATTCATTTGTAATTAGAGGTGATGAAGTCAGAC AAATCGCTCCAGGGCAAACTGGAAATATTGCT GATTATAATTATAAATTACCAGATGATTTTACAG GCTGCGTTATAGCTTGGAATTCTAACAATCTTG ATTCTAAGGTTGGTGGTAATTATAATTACCTGT ATAGATTGTTTAGGAAGTCTAATCTCAAACCTT TTGAGAGAGATATTTCAACTGAAATCTATCAGG CCGGTAGCACACCTTGTAATGGTGTTGAAGGT TTTAATTGTTACTTTCCTTTACAATCATATGGTT TCCAACCCACTAATGGTGTTGGTTACCAACCA TACAGAGTAGTAGTACTTTCTTTTGAACTTCTA CATGCACCAGCAA	S - 417
S - 417 derT synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGTCTATGCAG ATTCATTTGTAATTAGAGGTGATGAAGTCAGAC AAATCGCTCCAGGGCAAACTGGAACGATTGCT GATTATAATTATAAATTACCAGATGATTTTACAG GCTGCGTTATAGCTTGGAATTCTAACAATCTTG ATTCTAAGGTTGGTGGTAATTATAATTACCTGT ATAGATTGTTTAGGAAGTCTAATCTCAAACCTT TTGAGAGAGATATTTCAACTGAAATCTATCAGG CCGGTAGCACACCTTGTAATGGTGTTGAAGGT TTTAATTGTTACTTTCCTTTACAATCATATGGTT TCCAACCCACTAATGGTGTTGGTTACCAACCA TACAGAGTAGTAGTACTTTCTTTTGAACTTCTA CATGCACCAGCAA	S - 417
S - 452 anc synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGGTCTATGCA GATTCATTTGTAATTAGAGGTGATGAAGTCAGA CAAATCGCTCCAGGGCAAACTGGAAAGATTGC TGATTATAATTATAAATTACCAGATGATTTTACA GGCTGCGTTATAGCTTGGAATTCTAACAATCTT GATTCTAAGGTTGGTGGTAATTATAATTACCTG TATAGATTGTTTAGGAAGTCTAATCTCAAACCT TTTGAGAGAGATATTTCAACTGAAATCTATCAG GCCGGTAGCACACCTTGTAATGGTGTTGAAGG	S - 452

		TTTTAATTGTTACTTTCCTTTACAATCATATGGTTTCCAACCCACTAATGGTGTTGGTTACCAACCATACAGAGTAGTAGTACTTTCTTTTGAACTTCTACATGCACCAGCAA	
S - 452 der synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGGTCTATGCAGATTCATTTGTAATTAGAGGTGATGAAGTCAGACAAATCGCTCCAGGGCAAACTGGAAAGATTGCTGATTATAATTATAAATTACCAGATGATTTTACAGGCTGCGTTATAGCTTGGAATTCTAACAATCTTGATTCTAAGGTTGGTGGTAATTATAATTACCGGTATAGATTGTTTAGGAAGTCTAATCTCAAACCTTTTGAGAGAGATATTTCAACTGAAATCTATCAGGCCGGTAGCAAACCTTGTAATGGTGTTGAAGGTTTTAATTGTTACTTTCCTTTACAATCATATGGTTTCCAACCCACTAATGGTGTTGGTTACCAACCATACAGAGTAGTAGTACTTTCTTTTGAACTTCTACATGCACCAGCAA	S - 452
S - 156-158 anc synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGGCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGAAAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCCTTTTCTTATGGACCTTGAAGGAAAACAGGGTAATTTCAAAAATCTTAGGGAATTTGTGTTTAAGAAT	S - 156-158
S - 156-158 der synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGGCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGAAAGTGGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCCTTTTCTTATGGACCTTGAAGGAAAACAGGGTAATTTCAAAAATCTTAGGGAATTTGTGTTTAAGAAT	S - 156-158
S - 142-145 anc synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGGGCTGGATTTTTGGTACTACTTTAGATTCGAAGACCCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGAAAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCCTTTTCTTATGGACCTTGAAGGAAAACAGGGTAATTT	S - 142-145
S - 142-145 der synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGGGCTGGATTTTTGGTACTACTTTAGATTCGAAGACCCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAATTTTGTAATGATCCATTTTTGGACCACAAAAACAACAAAAGTTGGATGGAAAGTGAGTTCAGAGTTTATTCTAGTGC	S - 142-145

		GAATAATTGCACTTTTGAATATGTCTCTCAGCC TTTTCTTATGGACCTTGAAGGAAAACAGGGTAA TTT	
RNase P synthetic DNA target	gBlock	GAAATTAATACGACTCACTATAGGGGTGCTGT GGAGGCTGAACTGGATCCAGTGGAATACACC CTTAGGAAAAGGCTTCCCAGCCGCCTGCCCC GGAGACCCAATGACATTTATGTCAACATGAAG ACGGACTTTAAGGCCCAGCTGGCCCGCTGCC AGAAGCTGCTGGACGGAGGGGCCCGGGGTCA GAACGCGTGCTCTGAGATCTACATTCACGGCT TGGGCCTGGCCATCAACCGCGCCATCAACATC GCGCTGCAGCTGCAGGCGGGCAGCTTCGGGT CCTTGCAGGTGGCTGCCAATACCTCCACCGTG GAGCTTGTTGATGAGCTGGAGCCAGAGACCG ACACACGGGAGCCACTGACTCGGATCCGCAA CAACTCAGCCATCCACATCCGAGTCTTCAGGG TCACACCCAAGTAATTGAAAAGACACTCCTCC ACTTATCCCCTCCGTGATATGGCTCTTCGCAT GCTGAGTA	RNase P
5C-HEX-reporter	Fluorescence DNA reporter	5' - /5HEX/CCCCC/3IABkFQ/-3'	-
6U-FAM-reporter	Fluorescence RNA reporter	5'- /56-FAM/rUrUrUrUrUrU/3IABkFQ/-3'	-
FAMBio 5C LFA reporter	LFA DNA reporter	/56-FAM/CCCCCCCCCCCCCC/3Bio/	-
FAMBio 14U LFA reporter	LFA RNA reporter	/5BiosG/rUrUrUrUrUrUrUrUrUrUrUrUrUrU/36-FAM/	-