SUPPORTING INFORMATION

A blueprint for academic labs to produce SARS-CoV-2 RT-qPCR test kits

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Running Head: George P. Burdell's RT-qPCR Formulation

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Supplemental Information:

Supplemental Tables S1-S5

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Supplemental Data Files S1-S3

Supplemental Tables

Supp Table S1. A functional[1] 1-step RT-qPCR mix using all commercial components

Component	Stock	Volume (µL)	Final concentration
Template	Quantitative Synthetic SARS-CoV-2 RNA: ORF, E, N (ATCC® VR-3276SD™)	5.0	50,000 copies
Primer/ probe:	IDT N1 primer/probe mix	1.5	500 nM primers
Commercial mix:	5x First Strand buffer (Thermo Fisher Scientific #18080093): 250 mM Tris pH 8.3, 375 mM KCl, 15 mM $MgCl_2$,	4.0	1X buffer: 50 mM Tris pH 8.3, 75 mM KCl, 3 mM $MgCl_2$,
	250 mM $(NH_4)_2SO_4$,	2.0	25 mM $(NH_4)_2SO_4$
	10 mM dNTP (Thermo Fisher Scientific #18427013),	0.8	400 µM
	100 mM DTT (Thermo Fisher Scientific #18080093),	1.0	5 mM
	Platinum II *Taq* Hot Start (Thermo Fisher Scientific #14966001),	0.4	
	Superscript III RT (Thermo Fisher Scientific #18080093),	0.5	
	RNAseOUT (Thermo Fisher Scientific #10777019),	1.0	
	25 µM ROX (Thermo Fisher Scientific #2223012)	0.4	500 nM
Water:	Molecular biology grade water	**xx**	
	Total volume	**20**	

[1]PCR efficiency untested; cryopreservation mechanism not identified

Supp Table S2. Lysis buffers.

	Buffer	NaCl (mM)	Imidazole (mM)	DTT (mM)	Glycerol (%)
A. GT-MMLV	10 mM Na/K phosphate pH 7.2	400	30	1.0	10
B. GT-RTX	10 mM Na/K phosphate pH 7.2	150	10		
C. GT-His-*Taq*	10 mM Na/K phosphate pH 7.2	300	10		
D. GT-rRI	10 mM Na/K phosphate pH 7.2	300	10	5.0	

Supp Table S3. Histrap purification buffers.

	Buffer	NaCl (mM)	Imidazole (mM)	DTT (mM)	Glycerol (%)
A. GT-MMLV					
Buffer A	20 mM HEPES pH 7.5	400	30	1.0	10
Buffer B	20 mM HEPES pH 7.5	400	300	1.0	10
B. GT-RTX					
Buffer A1	10 mM Na/K phosphate pH 7.2	150	20		
Buffer A2	10 mM Na/K phosphate pH 7.2	150	40		
Buffer B	10 mM Na/K phosphate pH 7.2	150	250		
C. GT-His-*Taq*					
Buffer A	10 mM Na/K phosphate pH 7.2	300	50		
Buffer B	10 mM Na/K phosphate pH 7.2	300	250		
D. GT-rRI					
Buffer A	10 mM Na/K phosphate pH 7.2	300	40	2.0	
Buffer B	10 mM Na/K phosphate pH 7.2	300	250	2.0	

Supp Table S4. Final storage buffers.

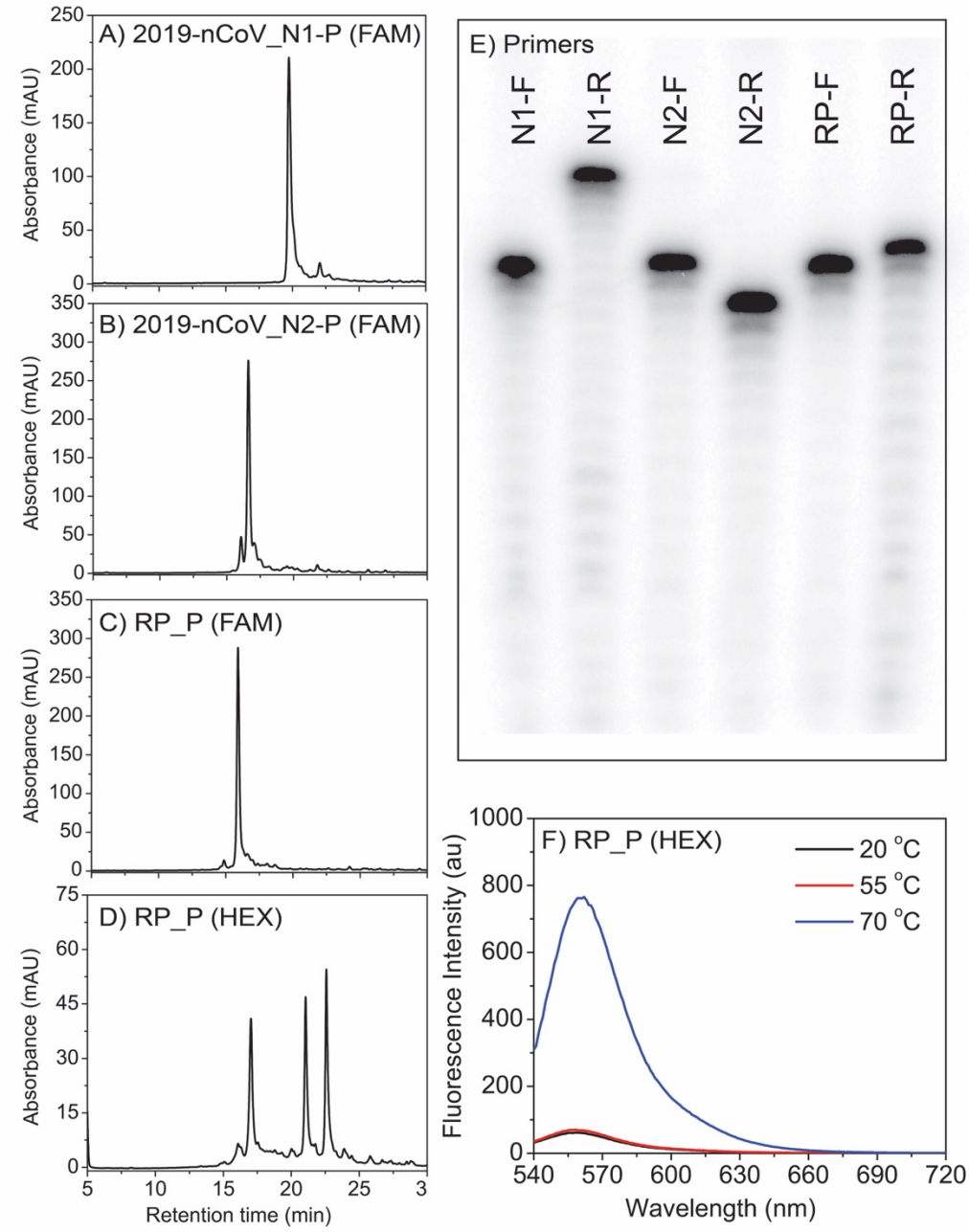
	Buffer	KCl (M)	NaCl (mM)	DTT (mM)	NP-40 (%)	TCEP (mM)	Glycerol (%)	Tween-20 (%)	EDTA (mM)
A. GT-MMLV									
Purified from BL21(DE3)	20 mM Tris-HCl pH 7.5		100		0.01	1.0	50		
Purified from ArcticExpress	20 mM Tris-HCl pH 8.0	0.1			0.01		50		
B. GT-RTX	50 mM Tris-HCl pH 8.0						50		
C. GT-*Taq* (no tags)	20 mM Tris-HCl pH 8.0	0.05		1.0*	0.5		50	0.5	0.1
D. GT-His-*Taq*									
Stringent protocol	20 mM Tris-HCl pH 8.0	0.05		1.0*	0.5		50	0.5	0.1
Final protocol	40 mM Tris-HCl pH 8.0						50		
E. GT-rRI	40 mM HEPES pH 7.5	0.1		8.0			50		

*make fresh

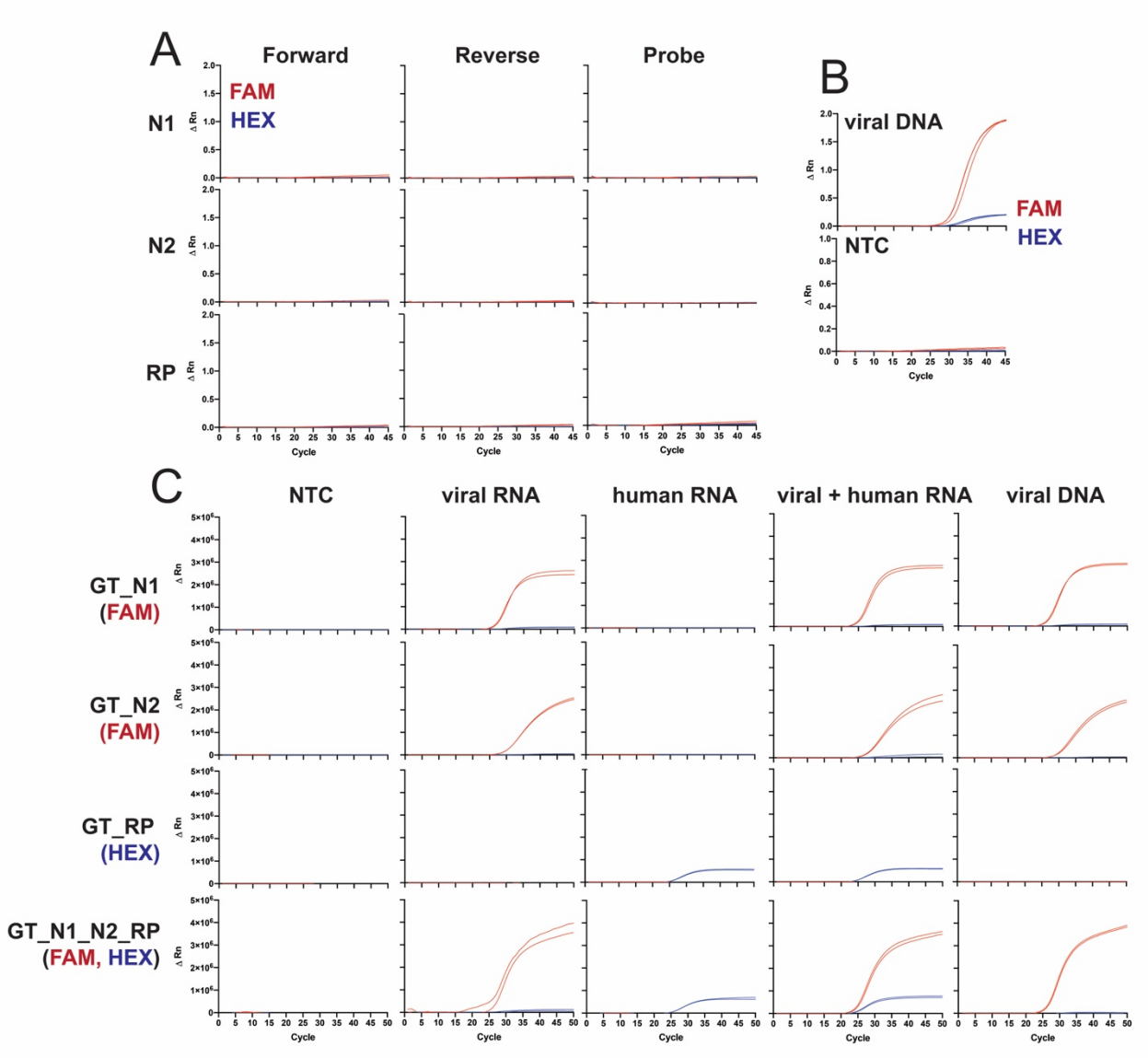
Supp Table S5. Anion exchange purification buffers.

	Buffer	KCl (M)	NaCl (mM)	DTT (mM)	Dextrose (mM)	EDTA (mM)
A. GT-MMLV						
Buffer A	20 mM Tris-HCl pH 8.9	0.05		1.0		
Buffer B	20 mM Tris-HCl pH 8.9	1.0		1.0		
B. GT-RTX						
Buffer A	100 mM Tris HCl pH 8.0					
Buffer B	100 mM Tris HCl pH 8.0		250			
C. GT-*Taq* (no tags)						
Buffer A	50 mM Tris HCl pH 7.9				50	1.0
Buffer B	50 mM Tris HCl pH 7.9		250		50	1.0
D. GT-His-*Taq*						
Buffer A	100 mM Tris HCl pH 8.0					
Buffer B	100 mM Tris HCl pH 8.0		1000			

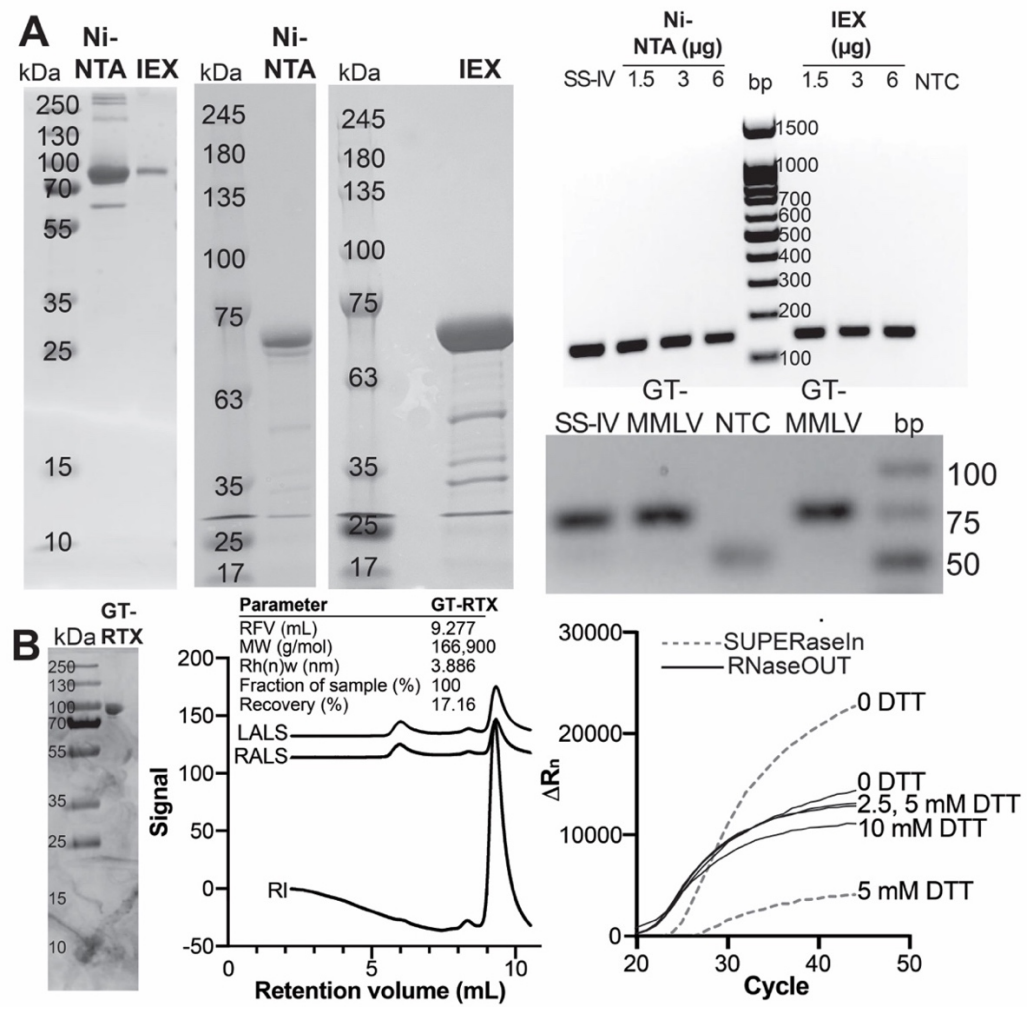
Supplemental Figures



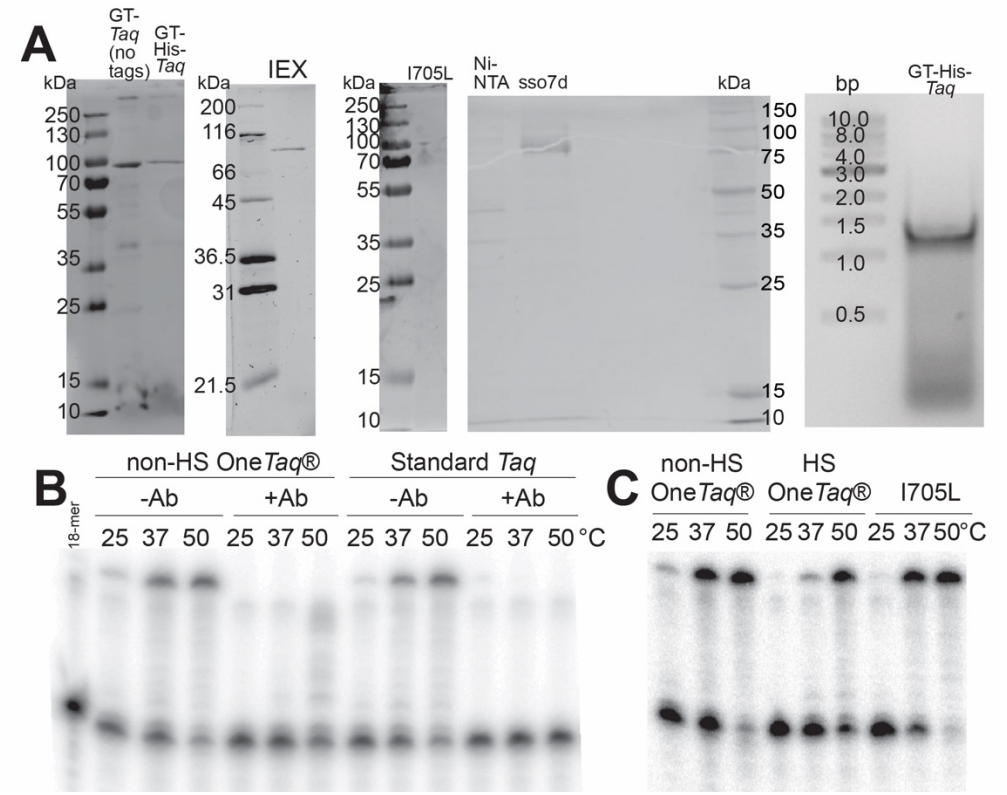
Supp Figure S1. Purity of GT-produced primers and probes temperature sensitivity of RP-P-HEX probe. Representative HPLC profiles of probes (monitored at 260 nm): **(A)** 2019-nCoV_N1-P, **(B)** 2019-nCoV_N2-P, **(C)** RP-P-FAM, and **(D)** RP-P-HEX. The largest peak is the desired product (in the case of HEX, the ~23 min peak is the desired product). **(E)** End-labeling of forward and reverse oligos of GT primers: 2019-nCoV_N1-F (20-mer), 2019-nCoV_N1-R (24-mer), 2019-nCoV_N2-F (20-mer), 2019-nCoV_N2-R (18-mer), 2019-nCoV_N2-R (18-mer), RP-F (19-mer), RP-R (20-mer). (**F**) Changes in the fluorescence intensity of RP-P-HEX probe (10 µM, in 100 µM NaCl) with temperature.



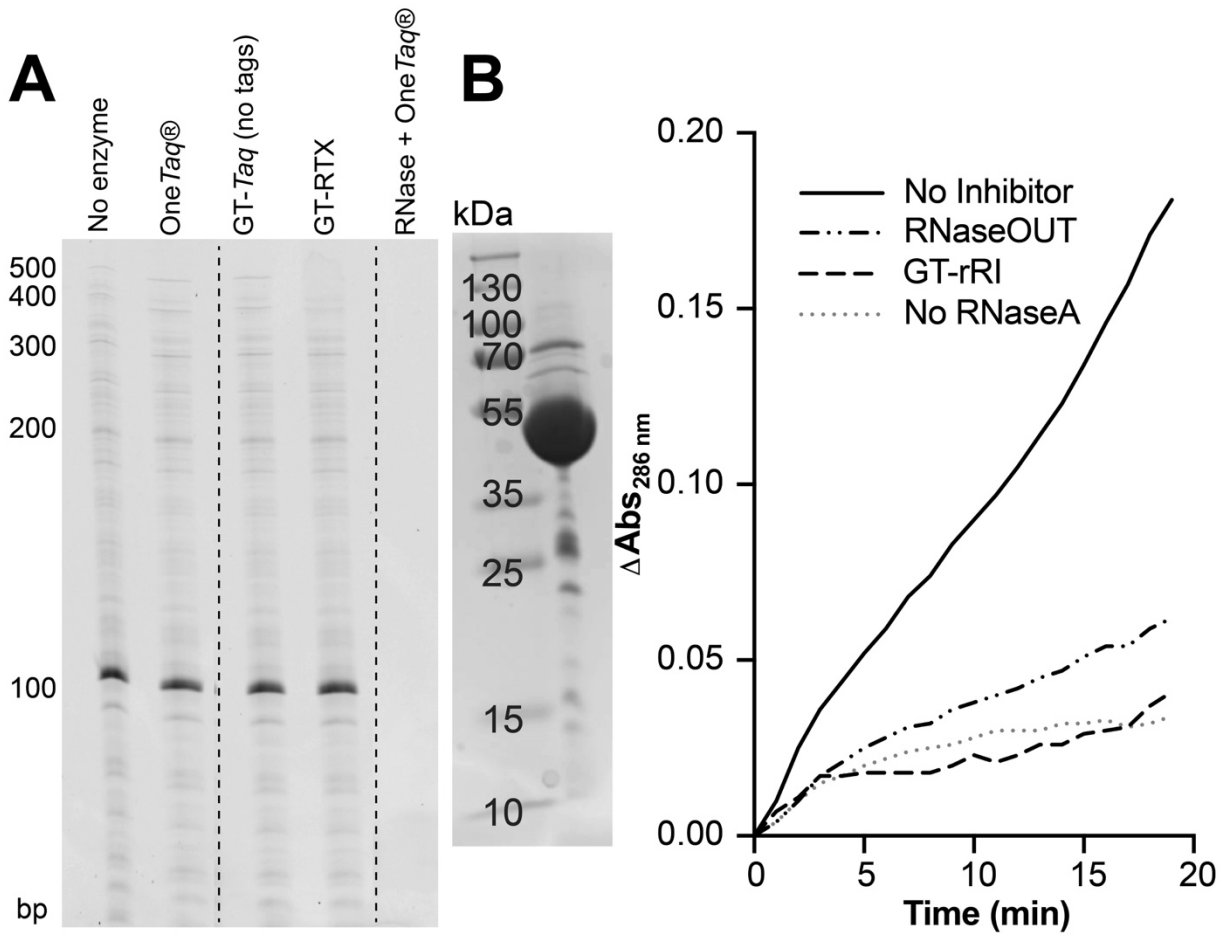
Supp. Figure S2. Quality control of GT primers and probes to ensure performance and absence of contamination with SARS-CoV-2 viral template. (A) GT primers and probes were individually tested for the presence of viral template contamination by adding them to TaqPath master mix that incorporated commercial IDT N1 and N2 primers and probes (FAM) and performing RT-qPCR. **(B)** Absence of amplification was indicative of uncontaminated material. Results were compared to a negative, no template control (NTC) and to 2019_nCoV_N_Positive Control (IDT) plasmid DNA. **(C)** Performance was validated by assaying the RT-qPCR results when GT probes and primers were combined with TaqPath and different SARS-CoV-2 and/or human templates. Results are plotted linearly.



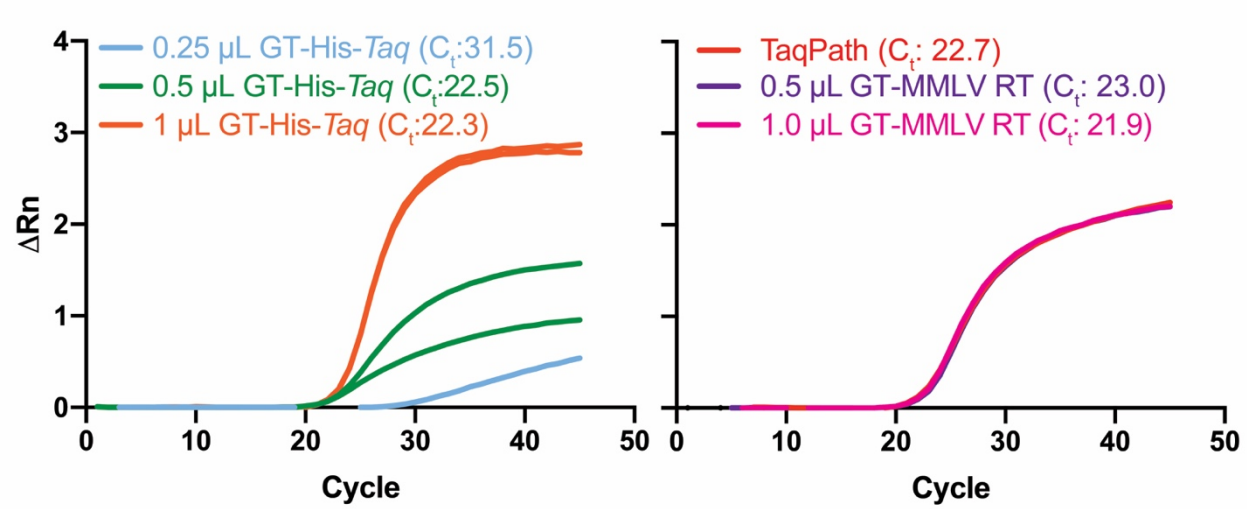
Supp Figure S3. Purification, activity, and characteristics of GT-MMLV M5 RT and GT-RTX reverse transcriptases. (A) *Top left:* Purified His-tagged GT-MMLV (12% SDS-PAGE, expected product: 77.8 kDa) from Ni-NTA column and ion exchange (IEX) chromatography. *Top gel:* 1.5% agarose gel showing reverse transcriptase activity of enzymes from both purification methods at a range of concentrations compared to SuperScript® (SS) IV RT (Thermo Fisher) and no template control (NTC). Only one product was observed at the expected product size of 150 bp. *Bottom gel:* A 2.0% agarose gel was used to separate RT-PCR products from reactions with SuperScript® (SS) IV RT (Thermo Fisher Scientific) and GT-MMLV, using the CDC N1 primer. The expected size of the N1 amplicon was 72 bp. N1 amplicons are similar in size to primer-dimer in NTC, complicating efforts to assess amplification efficiency by gel electrophoresis. **(B)** *Lower left:* Purified GT-RTX (12% SDS-PAGE, expected product: 95 kDa). *Middle:* OMNISEC results for RTX, calculated based on BSA as a standard, and triple detection using RI, RALS, and LALS detector. Molecular weight was determined using dn/dc based on BSA calculations. *Lower right:* Effect of DTT (0-10 mM) on RT-qPCR with GT-RTX and Platinum™ II *Taq* in i) RTX buffer (60 mM Tris-HCl pH 8.4, 25 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$) with SUPERase•In™ RNase inhibitor (Thermo Fisher Scientific #AM2694) or ii) SuperScript® III buffer with RNaseOUT Recombinant Ribonuclease Inhibitor (Thermo Fisher Scientific #10777019). ROX was not included in the RT-qPCR reaction. Cycling conditions were 60 °C for 20 min, 95 °C for 8 min, then 45 cycles of (95 °C for 15s followed by 55 °C for 30s).



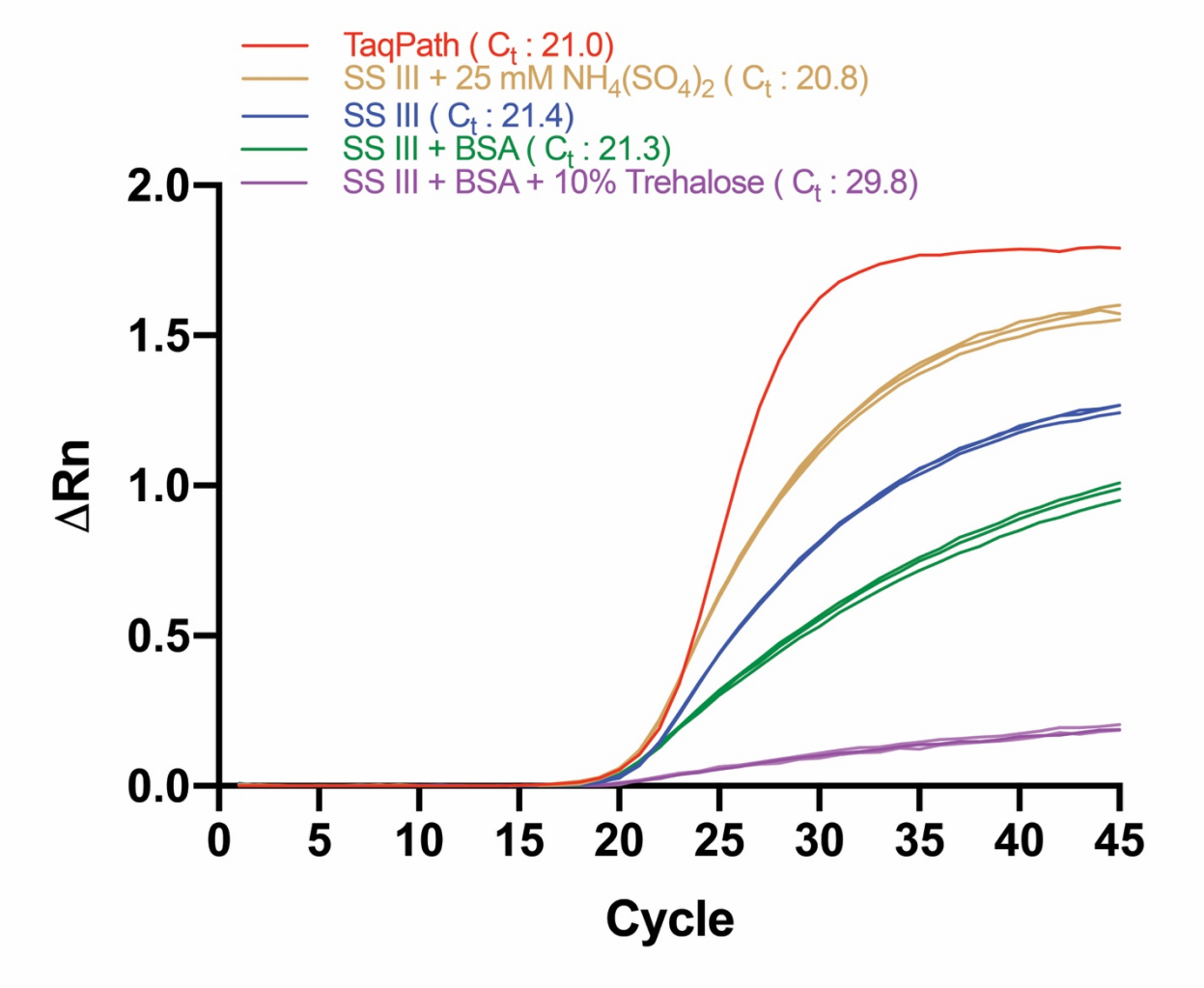
Supp Figure S4. Purification and activity of GT-*Taq*. **(A)** SDS-PAGE (12% polyacrylamide with Coomassie-staining or using Bio-Rad stain-free gel imaging) analysis of purified GT-His-*Taq* (94 kDa) after Ni-NTA column and ion exchange (IEX) chromatography, I705L *Taq* variant (94 kDa), and sso7d-*Taq* fusion after Ni-NTA purification (102 kDa). Right, 1.2% agarose gel showing DNA polymerase activity of GT-His-*Taq*. **(B)** Effect of Platinum® *Taq* monoclonal antibody (Thermo Fisher Scientific #10965-028) on two non-HS commercial DNA polymerases: One*Taq*® (NEB #M0480) and standard *Taq* (NEB #M0273). **(C)** Polymerization by different DNA polymerases as a function of incubation temperature: non-hot-start (HS) One*Taq*® (NEB #M0480); HS (aptamer-based) One*Taq*® (NEB #M0481); I705L (mutant *Taq*).



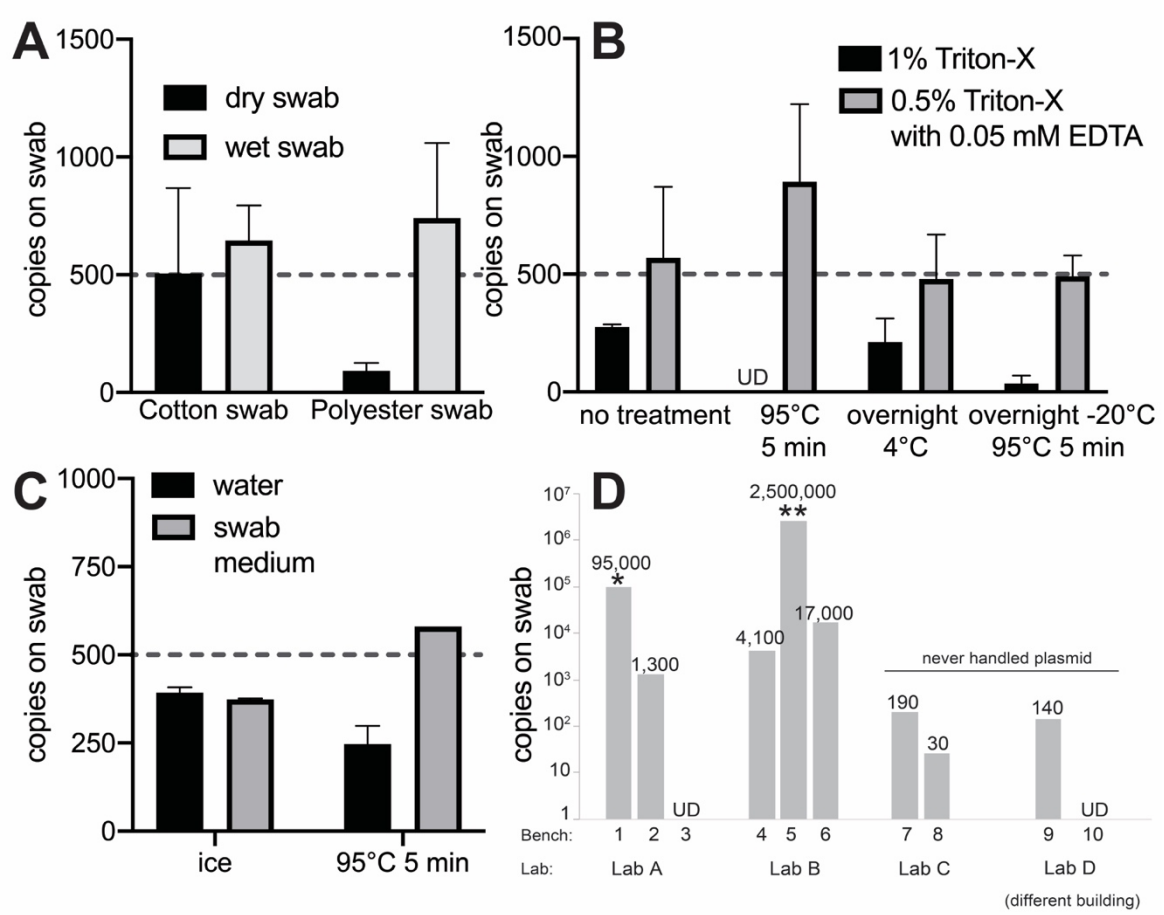
Supp. Figure S5. Purification and RNase inhibition by GT-rRI. (A) Quality control assay for testing RNase A activity. In the 'positive control' lane (far right), 1 µL of 1 pg/mL RNase A (Thermo Fisher Scientific #EN0531) stock solution was added to One*Taq*®. Complete degradation of the RNA ladder was observed, indicating RNase A activity. The other lanes show intact bands with the same intensity with no enzyme, One*Taq*®, GT-*Taq* polymerase, and GT-RTX enzymes, confirming the absence of RNase A in the enzymes. Dashed lines indicate boundaries between slices of the same gel that were cut and spliced together. **(B)** SDS-PAGE analysis of GT-rRI (expected product: 54 kDa) and assay for GT-rRI inhibition of RNaseA-catalyzed cCMP hydrolysis. A reduction in the rate of change in absorbance at 286 nm relative to 'no inhibitor' indicates GT-rRI activity.



Supp. Figure S6. Effect of increasing concentrations of GT-His-*Taq* (left) and GT-MMLV RT (right) on RT-qPCR with GT master mix and IDT N1 primers and probes. Template was 50,000 copies of synthetic SARS-CoV-2 RNA (ATCC).



Supp. Figure S7. Effect of additives on 1-step RT-qPCR with different commercial enzymes. RT-qPCR with SuperScript III Reverse Transcriptase (Thermo Fisher Scientific #18080093), Platinum™ II *Taq* DNA Polymerase (Thermo Fisher Scientific #14966001) and RNaseOUT Recombinant Ribonuclease Inhibitor (Thermo Fisher Scientific #10777019) in the buffer that accompanies Superscript III enzyme, amended with 0.5M betaine (and 25 mM $(NH_4)_2SO_4$, 1 mg/mL BSA and/or 10% trehalose, as shown). Cycling conditions were 60°C for 20 min, 95°C for 8 min, then 45 cycles of (95°C for 15s followed by 55°C for 30s).



Supp. Figure S8. Optimization of the sample collection method for environmental testing and laboratory plasmid contamination. (A) Wet swab is more effective at collecting DNA from a surface than a dry swab. The swab material (polyester (Puritan®) or cotton (Q-tips®)) and swabbing method (dry or pre-wet with 100 µL) were compared by swabbing 500 copies of plasmid DNA dropped on clean lab bench, then picking up by swabbing (see **Fig. 2A**). DNA was retrieved by adding 200 µL of 1% Triton-X for dry or 100 µL of 1% Triton-X for pre-wet swab, vortexing, and centrifugation. The DNA copies in 5 µL of the supernatant were quantified by RT-qPCR, then multiplied by 40 to estimate whole amount in swab. Error bar represents standard error of the mean from duplicated swab. **(B)** Swab medium (0.05 mM EDTA, 0.5% Triton-X) protects RNA during storage. 500 copies of full-length SARS-CoV-2 RNA was dropped on the clean surface, then picked up using a polyester swab moistened with swab medium or 1% Triton-X. The swab was processed by different treatment necessary for storage and processing, then compared for recovery. Error bar represents SEM of duplicated RT-qPCR reaction of single swab. **(C)** Swab medium (0.05 mM EDTA, 0.5% Triton-X) opens virion during simple heat treatment for efficient RT-qPCR without RNA extraction. Heat inactivated SARS-CoV-2 virion was suspended in water or swab medium (0.05 mM EDTA, 0.5% Triton-X) and RT-qPCR efficiency was compared without RNA extraction. Heat treatment was done on heat block at 95°C for 5 min. Error bar represents SEM of duplicated RT-qPCR reaction. Dashed lines indicate 500 copies (expected). UD: undetected. **(D)** Environmental testing results for GT laboratories involved in this study following contamination with a viral plasmid. Each bar represents a different lab bench. Lab A is where reagents are stored

and RT-qPCR reactions assembled. Bench 1 (*) is a high-traffic area across from the -20°C fridge where reagents, including the offending plasmid DNA, were stored. Lab B produced the positive control N-gene plasmid; most work was done at Bench 5 (**). Lab C and D contribute to enzyme production and never handled the positive control. While Labs A-C are in same building, Lab D is in completely different building. Bench 9's signal was confirmed to be DNA by no-RT reaction. UD: undetected.

Supplemental Data Files

S1. Protein Sequences

S2. MATLAB script

S3. Example script output Excel file