A one-enzyme RT-qPCR assay for SARS-CoV-2, and procedures for reagent production

Sanchita Bhadra, Andre C. Maranhao, and Andrew D. Ellington¹

Center for Systems and Synthetic Biology
University of Texas at Austin
Austin, TX
78703

¹ Inquiries about manuscript to ellingtonlab@gmail.com

² Inquiries about vectors to <https://reclone.org/>

ABSTRACT

Given the scale of the ongoing COVID-19 pandemic, the need for reliable, scalable testing, and the likelihood of reagent shortages, especially in resource-poor settings, we have developed a RT-qPCR assay that relies on an alternative to conventional viral reverse transcriptases, a thermostable reverse transcriptase / DNA polymerase (RTX)¹. Here we show that RTX performs comparably to the other assays sanctioned by the CDC and validated in kit format. We demonstrate two modes of RTX use – (i) dye-based RT-qPCR assays that require only RTX polymerase, and (ii) TaqMan RT-qPCR assays that use a combination of RTX and Taq DNA polymerases (as the RTX exonuclease does not degrade a TaqMan probe). We also provide straightforward recipes for the purification of this alternative reagent. We anticipate that in low resource or point-of-need settings researchers could obtain the available constructs from Addgene or our lab and begin to develop their own assays, within whatever regulatory framework exists for them.

We lay out protocols for dye-based and TaqMan probe-based assays, in order to best compare with ‘gold standard’ reagents. These protocols should form the basis of further modifications that can simplify the assay to the use of overexpressing cells themselves as reagents.

Developing dye-based and TaqMan probe-based RT-qPCR assays with RTX

MATERIALS

- 10X RTX buffer: 600 mM Tris-HCl, 250 mM (NH₄)₂SO₄, 100 mM KCl, 20 mM MgSO₄, pH 8.4 @25°C
- 10X ThermoPol buffer (New England Biolabs): 200 mM Tris-HCl, 100 mM (NH₄)₂SO₄, 100 mM KCl, 20 mM MgSO₄, 1% Triton® X-100, pH 8.8 @25°C
- Forward PCR primer^a
- Reverse PCR primer^a
- TaqMan probe^a

- 20X EvaGreen solution
- 4 mM deoxyribonucleotides (dNTP)
- 5M Betaine
- 20 U/μL SUPERase.In RNase inhibitor
- 5 U/μL Taq DNA polymerase
- 0.2 mg/ml RTX polymerase^b
- Nuclease-free water
- qPCR tubes or plates^c
- Real-time PCR machine^c
- Cold-block or ice
- Barrier tips

^aIndividual primer and probe stocks or pre-made primer-probe mixes for CDC N1, N2 and N3 assays (**Table 1**) available from Integrated DNA Technologies may be used.

^bRTX polymerase with proofreading capability (RTX), and RTX polymerase without proofreading capability (RTX Exo-) have been compared.

^cLightCycler96 real-time PCR machine and 96-well plates with optical plastic film cover designed for use with LightCycler platform were used for these experiments.

Table 1. CDC TaqMan qRT-PCR primers and probes for SARS-CoV-2^a (adapted from <https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html>).

Name	Description	Sequence ^b
2019-nCoV_N1-F	2019-nCoV_N1 Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'
2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'
2019-nCoV_N1-P	2019-nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'
2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	5'-TTA CAA ACA TTG GCC GCA AA-3'
2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	5'-GCG CGA CAT TCC GAA GAA-3'
2019-nCoV_N2-P	2019-nCoV_N2 Probe	5'-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3'
2019-nCoV_N3-F	2019-nCoV_N3 Forward Primer	5'-GGG AGC CTT GAA TAC ACC AAA A-3'
2019-nCoV_N3-R	2019-nCoV_N3 Reverse Primer	5'-TGT AGC ACG ATT GCA GCA TTG-3'
2019-nCoV_N3-P	2019-nCoV_N3 Probe	5'-FAM-AYC ACA TTG GCA CCC GCA ATC CTG-BHQ1-3'

^aAccording to CDC, oligonucleotide sequences are subject to future changes as the 2019-Novel Coronavirus evolves. Refer to CDC website for latest updates.

^bFAM: 6-carboxyfluorescein; BHQ-1: Black Hole Quencher 1

RESULTS

Dye-based RT-qPCR reaction set up using only RTX DNA polymerase

EvaGreen dye-containing RT-qPCR reaction set up shown in **Table 2** was used for assembling CDC SARS-CoV-2 N1, N2, and N3 assays. The three assays target three different regions within the nucleocapsid phosphoprotein (N) gene near the 3'-end of the ~30 kb viral genome. In particular, N1 primers (**Table 1**) amplify a 72 nt long region from position 28287 to 28358 of the genome (GenBank Accession No. MN985325.1). The N2 primers produce a 67 bp amplicon from the genomic position 29164 to 29230. Meanwhile, the N3 primers amplify a 72 nt long region from genomic position 28681 to 28752. All reaction mixes were assembled on a cold-block and directly transferred to a real-time PCR machine programmed to cycle through the steps depicted in **Table 3**. Melt curve analysis was included after PCR amplification in order to distinguish target amplicons from spurious products.

Table 2. EvaGreen RT-qPCR reaction mix using only RTX DNA polymerase

	Volume (μL)	Final concentration
RNA sample	5	
Forward PCR primer	1.5	500 nM
Reverse PCR primer	1.5	500 nM
20X EvaGreen	1.25	1X
10X RTX buffer	2.5	1X
4 mM dNTP	2.5	400 μM
20 U/μL SUPERase.In RNase inhibitor	1	0.8 U/μL
RTX or RTX Exo- 0.2mg/ml	0.5	4 ng/μL
5M betaine	2.5	0.5M
Water	6.75	
Total reaction volume	25	

Table 3. RTX polymerase-based EvaGreen RT-qPCR cycling conditions

Step	Temperature	Duration	Signal acquisition
1 ^a	60 °C	30 min	None
2 ^a	95 °C	10 min	None
55 cycles of Steps 3 and 4			
3	95 °C	15 sec	None
4 ^b	55 °C	30 sec	Single
Melt curve analysis in Steps 5, 6, and 7			
5	95 °C	10 sec	None
6	65 °C	60 sec	None
7	97 °C	1 sec	Continuous ^c

^aAssay time may be reduced by shortening reverse transcription step (Step 1) to 15 min and the following denaturation step (Step 2) to 2 min.

^bSignal acquisition occurs in the FAM (fluorescein) channel during step 4.

^c20 measurements/°C temperature change

Representative results of SARS-CoV-2 RT-qPCR tests performed using synthetic RNA templates and only RTX or RTX Exo- DNA polymerases are depicted in **Figures 1 and 2**.

Figure 1. CDC SARS-CoV-2 N1, N2, and N3 RT-qPCR assays performed using indicated copies of synthetic RNA and RTX DNA polymerase. Panels A, B, and C depict N1, N2, and N3 assays measured in real-time using EvaGreen dye. Amplification curves from reactions containing 100,000 (black traces), 10,000 (blue traces), 1,000 (red traces), and 100 (pink traces) copies of SARS-CoV-2 synthetic N RNA are depicted. Negative control reactions either contained no templates (gray traces) or contained 100,000 copies of synthetic N RNA from MERS-CoV (green traces). Panels D, E, and F depict melting peaks of amplicons determined using the 'T_m calling' analysis in the LightCycler96 software. Average Cq values of all assays are tabulated.

Figure 2. CDC SARS-CoV-2 N1, N2, and N3 RT-qPCR assays performed using indicated copies of synthetic RNA and RTX Exo- DNA polymerase. Panels A, B, and C depict N1, N2, and N3 assays measured in real-time using EvaGreen dye. Amplification curves from reactions containing 100,000 (black traces), 10,000 (blue traces), 1,000 (red traces), and 100 (pink traces) copies of SARS-CoV-2 synthetic N RNA are depicted. Negative control reactions either contained no templates (gray traces) or contained 100,000 copies of synthetic N RNA from MERS-CoV (green traces). Panels D, E, and F depict melting peaks of amplicons determined using the 'Tm calling' analysis in the LightCycler96 software. Average Cq values of all assays are tabulated.

These results demonstrate that RTX DNA polymerase alone, with or without proofreading capability, can support dye-based RT-qPCR analyses. In our hands the full-length RTX DNA polymerase was slightly better than the Exo- version, especially in the N3 assay.

RTX polymerase-based TaqMan qRT-PCR reaction set up

In order to perform TaqMan RT-qPCR we developed a one-pot two enzyme system comprising RTX and Taq DNA polymerases. TaqMan qRT-PCR reaction set up shown in **Table 4** was used for assembling CDC SARS-CoV-2 N1, N2, and N3 assays using either RTX buffer or ThermoPol buffer. Control reactions lacking RTX polymerase were set up using the same recipe except RTX was

replaced with water. All reaction mixes were assembled on a cold-block and directly transferred to a real-time PCR machine programmed to cycle through the steps depicted in **Table 5**.

Table 4. RTX polymerase-based TaqMan qRT-PCR reaction mix

	Volume (μL)	Final concentration
RNA sample	5	
Forward PCR primer	1.5	402 nM
Reverse PCR primer	1.5	402 nM
TaqMan probe	1.5	102 nM
10X Assay buffer ^a	2.5	1X
4 mM dNTP	2.5	400 μM
20 U/μL SUPERase.In RNase inhibitor	1	0.8 U/μL
5 U/ μL Taq DNA polymerase	0.5	0.1 U/μL
RTX or RTX Exo- 0.2mg/ml or water	0.5	4 ng/μL
5M betaine	2.5	0.5M
Water	6	
Total reaction volume	25	

^a Assay buffer: Duplicate TaqMan RT-qPCR reactions were performed either in 1X ThermoPol buffer or in 1X RTX buffer as indicated.

Table 5. RTX polymerase-based TaqMan qRT-PCR cycling conditions

Step 1 ^a	60 °C	30 min
Step 2 ^a	95 °C	10 min
55 cycles of Steps 3 and 4		
Step 3	95 °C	15 sec
Step 4 ^b	55 °C	30 sec

^a Assay time may be reduced by shortening reverse transcription step (Step 1) to 15 min and the following denaturation step (Step 2) to 2 min.

^b Signal acquisition occurs in the FAM (fluorescein) channel during step 4.

Representative results of RTX polymerase-based SARS-CoV-2 TaqMan qRT-PCR tests performed using synthetic RNA templates are depicted in **Figures 3 and 4**. Both RTX and RTX Exo- when combined with Taq DNA polymerase were able to support one-pot TaqMan RT-qPCR assays for all three CDC SARS-CoV-2 assays. Under both buffer conditions tested, 1X ThermoPol versus 1X RTX buffer, RTX polymerase yielded more consistent amplification curves for all three CDC assays compared to RTX Exo- polymerase.

Figure 3. CDC SARS-CoV-2 N1, N2, and N3 TaqMan RT-qPCR assays performed in 1X ThermoPol buffer using indicated copies of synthetic RNA and RTX or RTX Exo- and Taq DNA polymerases. Panels A, B, and C depict TaqMan assays containing both RTX and Taq DNA polymerases. Panels D, E, and F depict TaqMan assays containing only Taq DNA polymerase. Panels G, H, and I depict TaqMan assays containing RTX Exo- and Taq DNA polymerases. Amplification curves from reactions containing 100,000 (black traces), 10,000 (blue traces), 1,000 (red traces), and 100 (pink traces) copies of SARS-CoV-2 synthetic N RNA are depicted. Negative control reactions either contained no templates (gray traces) or contained 100,000 copies of synthetic N RNA from MERS-CoV (green traces). Cq values of all assays are tabulated.

Figure 4. CDC SARS-CoV-2 N1, N2, and N3 TaqMan RT-qPCR assays performed in 1X RTX buffer using indicated copies of synthetic RNA and RTX or RTX Exo- and Taq DNA polymerases. Panels A, B, and C depict TaqMan assays containing RTX and Taq DNA polymerases. Panels D, E, and F depict TaqMan assays containing RTX Exo- and Taq DNA polymerases. Amplification curves from reactions containing 100,000 (black traces), 10,000 (blue traces), 1,000 (red traces), and 100 (pink traces) copies of SARS-CoV-2 synthetic N RNA are depicted. Negative control reactions either contained no templates (gray traces) or contained 100,000 copies of synthetic N RNA from MERS-CoV (green traces). Cq values of all assays are tabulated.

To compare the RTX-based TaqMan qRT-PCR with qRT-PCR performed using commercial master mix, duplicate reactions (**Table 6**) were set up using TaqPath™ 1-Step RT-qPCR Master Mix, CG (Thermo Fisher Scientific, Waltham, MA, USA). All reactions were assembled on cold block and then incubated at room temperature (25 °C benchtop) for 2 min prior to being loaded on a real-time PCR machine set to cycle through the steps indicated in **Table 7**.

Table 6. CDC N1, N2, N3 TaqMan qRT-PCR assay set up using commercial qRT-PCR master mix

	Volume (μL)
RNA sample	5
6.7 μM Forward PCR primer	1.5
6.7 μM Reverse PCR primer	1.5
1.7 μM TaqMan probe	1.5
4X TaqPath™ master mix	5
Water	5.5
Total reaction volume	20

Table 7. TaqPath™ TaqMan qRT-PCR cycling conditions

Step 1	50 °C	15 min
Step 2	95 °C	2 min
45 cycles of Steps 3 and 4		
Step 3	95 °C	3 sec
Step 4	55 °C	30 sec

Figure 5 depicts typical results from CDC N1, N2, and N3 TaqMan qRT-PCR reactions performed using synthetic RNA and TaqPath™ commercial qRT-PCR mastermix.

Figure 5. CDC SARS-CoV-2 N1, N2, and N3 TaqMan qRT-PCR assays performed using indicated copies of synthetic RNA and TaqPath™ commercial qRT-PCR mastermix. Amplification curves from reactions containing 100,000 (black traces), 10,000 (blue traces), 1,000 (red traces), and 100 (pink traces) copies of SARS-CoV-2 synthetic N RNA are depicted. Negative control reactions either contained no templates (gray traces) or contained 100,000 copies of synthetic N RNA from MERS-CoV (green traces). Cq values of all assays are tabulated.

The results with either a viral RT (in TaqPath™ commercial mastermix) or RTX are summarized in **Table 8**. As can be seen, RTX-based TaqMan assays are of comparable sensitivity and specificity to the gold standard TaqPath assay. While all three RTX-based TaqMan assays performed in ThermoPol buffer consistently yielded Cq values, these were somewhat delayed compared to Cq values obtained with the commercial TaqPath™ Master Mix. In contrast, RTX-based TaqMan assays when executed in RTX buffer yielded Cq values that were closer to Cq values obtained with the commercial mastermix.

Table 8. Comparison of Cq values of RTX-based and TaqPath-based SARS-CoV-2 TaqMan qRT-PCR assays

N1 qRT-PCR	RTX+Taq in ThermoPol	RTX+Taq in RTX buffer	TaqPath™
100,000 nCoV-2019 N RNA	26.19	22.18	22.02
10,000 nCoV-2019 N RNA	29.71	25.54	25.55
1,000 nCoV-2019 N RNA	33.21	28.88	28.79
100 nCoV-2019 N RNA	36.29	32.06	32.21
100,000 MERS N RNA	--		--
No template	--		--

N2 qRT-PCR	RTX+Taq in ThermoPol	RTX+Taq in RTX buffer	TaqPath™
100,000 nCoV-2019 N RNA	28.81	23.77	21.57
10,000 nCoV-2019 N RNA	32.47	27.46	25.16
1,000 nCoV-2019 N RNA	35.78	31.09	28.90
100 nCoV-2019 N RNA	39.53	34.97	32.58
100,000 MERS N RNA	--		--
No template	--		--

N3 qRT-PCR	RTX+Taq in ThermoPol	RTX+Taq in RTX buffer	TaqPath™
100,000 nCoV-2019 N RNA	28.03	23.28	21.20
10,000 nCoV-2019 N RNA	31.53	26.55	24.56
1,000 nCoV-2019 N RNA	35.16	29.77	27.98
100 nCoV-2019 N RNA	38.51	35.44	31.59
100,000 MERS N RNA	--		--
No template	--		--

Summary of assay performance

Overall, RTX performs well either as a single enzyme for RT-qPCR with intercalating dyes, such as EvaGreen, or as the RT component of a TaqMan based assay. At the lowest RNA concentrations examined (100 copies), the gold standard TaqPath assay general gave a signal at a Cq value of ca. 32. The more robust full-length RTX on its own actually performed slightly better, with Cq values typically from 29-30 (although the N3 primer set gives higher signal and higher background). When used as a substitute for a viral RT, RTX is slightly less sensitive overall, with Cq values typically from 32-35.

Purification of RTX

For sites wishing to produce their own RTX, the following protocol can be used.

Resuspension Buffer	<ul style="list-style-type: none"> • 50 mM Phosphate Buffer, pH 7.5 • 300 mM NaCl • 20 mM imidazole • 0.1% Igepal CO-630 (non-toxic Non-idet P40 equivalent) • 5 mM MgSO₄
Equilibration Buffer	<ul style="list-style-type: none"> • 50 mM Phosphate Buffer, pH 7.5 • 300 mM NaCl • 20 mM imidazole
Lysis Buffer	<ul style="list-style-type: none"> • 30 mL Resuspension Buffer + • 1x EDTA-free protease inhibitor tablet + • 30-60 mg HEW Lysozyme
Wash Buffer	<ul style="list-style-type: none"> • 50 mM Phosphate Buffer, pH 7.5 • 300 mM NaCl • 50 mM imidazole
Elution Buffer	<ul style="list-style-type: none"> • 50 mM Phosphate Buffer, pH 7.5 • 300 mM NaCl • 250 mM imidazole
Heparin Buffer A	<ul style="list-style-type: none"> • 40 mM Tris-HCl, pH 7.5 • 100 mM NaCl • 0.1% Igepal CO-630 (non-toxic Non-idet P40 equivalent)
Heparin Buffer B	<ul style="list-style-type: none"> • 40 mM Tris-HCl, pH 7.5 • 2 M NaCl • 0.1% Igepal CO-630 (non-toxic Non-idet P40 equivalent)

Table 7. Buffers for RTX purification

Ni-NTA Dialysis Buffer (2x 2 L)	<ul style="list-style-type: none"> • 40 mM Tris-HCl, pH 7.5 • 100 mM NaCl • 10 mM beta-mercapto ethanol (BME) or 1 mM DTT • 0.1% Igepal CO-630 (non-toxic Non-idet P40 equivalent)
Heparin Dialysis Buffer (2 L)	<ul style="list-style-type: none"> • 50 mM Tris-HCl, pH 8.0 • 50 mM KCl • 0.1% Tween-20 • 0.1% Igepal CO-630 (non-toxic Non-idet P40 equivalent)
Final Dialysis Buffer (1 L)	<ul style="list-style-type: none"> • 50% Glycerol • 50 mM Tris-HCl, pH 8.0

	<ul style="list-style-type: none"> • 50 mM KCl • 0.1% Tween-20 • 0.1% Igopal CO-630 (non-toxic Non-idet P40 equivalent)
--	--

Table 8. Buffers for dialysis of RTX

In brief, a T7-based *E. coli* expression strain such as BL21(DE3) was transformed with a RTX expression plasmid that contained an carboxyl terminal six histidine tag for purification. On the next day, an individual transformant colony was picked into desired media with appropriate antibiotics (e.g. ampicillin) and 1-2% glucose. This starter culture was grown overnight in shaking incubator at 37°C.

On day three, 1 L Superior Broth™ (Athena Enzyme Systems™) or other rich media with appropriate antibiotics in a 4 L Erlenmeyer flask was inoculated with 250-350 µL of the overnight starter culture. Incubate at 37°C, 250 rpm until the culture reaches OD₆₀₀ of 0.4-0.7 (0.5-0.6 is ideal). When desired OD₆₀₀ is reached, place flask(s) in 4°C cold room for 30-45 min and set shaking incubator to 18°C. Following cold incubation, induce expression of RTX by adding 1 mL of 1 M IPTG to each 1 L of culture. Place flask(s) in the 18°C shaking incubator for 16-18 hours.

Following overnight expression, cells were harvested by centrifuging cultures at 4°C, 5000 xg for 20 min. On ice, resuspend cell pellet in 30 mL cold **Lysis Buffer**. Transfer the resuspended cell pellet to a small 50 mL beaker with clean stir bar and place securely in an ice bath. With moderate stirring, sonicate the sample using 40% amplitude and 1 sec ON / 4 sec OFF for 4 min total sonication time. Centrifuge the resulting lysate at 4°C, 35,000 xg for 30 min. Carefully transfer the supernatant to a clean ultracentrifugation tube, shake in thermomixer at 400 rpm, 85°C for 10 min, and then place on ice for 10 min. Centrifuged the heat-treated lysate at 4°C, 35,000 xg for 30 min. Carefully transfer the supernatant to a clean tube and filter the clarified lysate using a 0.2 µm filter.

Perform IMAC column purification in a cold room or allow gravity flow to occur in a refrigerator. Prepare Ni-NTA agarose columns with final column volume (CV) of 1 mL. Apply the clarified lysate from 1 L of expressed culture to a previously equilibrated column and collect the flow-through. Wash column with 20 CV **Equilibration Buffer** and collect the flow-through. Wash column with 5 CV **Wash Buffer** and collect flow-through. Elute RTX from column with 5 mL **Elution Buffer** and transfer to a dialysis cassette with appropriate molecular weight cut-off. Dialyze eluate into 2 L of **Ni-NTA Dialysis Buffer** for 3-4 hours at 4°C. Then dialyze eluate into a second 2 L of **Ni-NTA Dialysis Buffer** overnight at 4°C.

Dialyzed eluate was then passed over an equilibrated 5 mL heparin column (HiTrap™ Heparin HP) and eluted along a sodium chloride gradient (100 mM to 2 M NaCl). The peak corresponding to RTX or its variants could be expected between 40-60% Buffer B. RTX fractions were collected, pooled, and dialyzed into 2 L **Heparin Dialysis Buffer** for 3-4 hours. Then dialysis cassettes were transferred into 2 L **Final Dialysis Buffer** overnight where it is expected that the protein sample

volume will decrease significantly. Following the final dialysis, protein sample was recovered and protein concentration was determined before storing at -20°C.

A similar protocol should work for Taq and other thermostable polymerases, but may require different dialysis and storage buffers.

Summary and opportunities for distribution

By obtaining a RTX expression vector and purifying the thermostable reverse transcriptase it should prove possible to carry out RT-qPCR reactions with a sensitivity similar to that already observed for approved kits, with few or no false positive results. The sequence information for plasmids for RTX [pET_RTX-6xHis] and its exonuclease deficient variant, RTX(exo-) [pET_RTX(exo-)-6xHis], bearing a C-terminal 6xHis tags can be found in the **Supplemental Information**. These plasmids will be uploaded to Addgene in short order, or can be obtained via <https://reclone.org/>. The Board of Regents of The University of Texas has licensed IP covering RTX to Promega Corporation.

Acknowledgements: We acknowledge the extraordinary contributions of the Schoggins lab in making synthetic RNA available on short notice for these assays.

Supporting Information

Figure S1. Plasmid map of pET_RTX-6xHis

LOCUS	pET_RTX-6xHis	8232 bp	DNA	circular	UNA	26-MAR-2020
DEFINITION	A new nucleotide sequence entered manually.					
ACCESSION	urn.local...f-bq4nr1q					
VERSION	urn.local...f-bq4nr1q					
KEYWORDS	.					
SOURCE	.					
ORGANISM	.					
FEATURES	Location/Qualifiers					
gene	140..1000					


```

        /created_by="User"
        /label="bla (AmpR)"
rep_origin    1042..1555
        /created_by="User"
        /modified_by="User"
        /label="M13 ori"
rep_origin    1666..2254
        /created_by="User"
        /modified_by="User"
        /label="pMB1 ori"
gene          complement(2623..2814)
        /created_by="User"
        /label="rop"
gene          complement(3371..4453)
        /created_by="User"
        /label="lacI"
promoter      5637..5654
        /created_by="User"
        /label="T7 Promoter"
operon        5656..5680
        /created_by="User"
        /label="lacO"
misc_feature  5722..5727
        /created_by="User"
        /modified_by="User"
        /label="NdeI"
CDS           5725..8085
        /created_by="User"
        /modified_by="User"
        /label="RTX"
misc_difference 5836..5838
        /created_by="User"
        /label="F38L"
misc_difference 6013..6015
        /created_by="User"
        /label="R97M"
misc_difference 6076..6078
        /created_by="User"
        /modified_by="User"
        /label="K118I"
misc_difference 6133..6135
        /created_by="User"
        /label="M137L"
misc_difference 6865..6867

```

```

        /created_by="User"
        /label="R381H"
misc_difference 6874..6876
        /created_by="User"
        /label="Y384H"
misc_difference 6889..6891
        /created_by="User"
        /label="V389I"
misc_difference 7120..7122
        /created_by="User"
        /label="K466R"
misc_difference 7201..7203
        /created_by="User"
        /label="Y493L"
misc_difference 7264..7266
        /created_by="User"
        /label="T514I"
misc_difference 7285..7287
        /created_by="User"
        /label="I521L"
misc_difference 7483..7485
        /created_by="User"
        /label="F587L"
misc_difference 7714..7716
        /created_by="User"
        /label="E664K"
misc_difference 7855..7857
        /created_by="User"
        /label="G711V"
misc_difference 7927..7929
        /created_by="User"
        /label="N735K"
misc_difference 8026..8028
        /created_by="User"
        /label="W768R"
misc_feature 8047..8052
        /created_by="User"
        /label="XhoI"
misc_feature 8053..8058
        /created_by="User"
        /label="BamHI"
terminator 8104..8226
        /created_by="User"
        /label="T7 Tphi transcription terminator"

```

ORIGIN

1 aactacgtca ggtggcactt ttcggggaaa tgtgcgcgga acccctattt gtttatttt
 61 cttaaatacat tcaaatatgt atccgctcat gagacaataa ccctgataaa tgcttcaata
 121 atattgaaaa aggaagagta tgagtattca acatttccgt gtcgccctta ttccctttt
 181 tgcggcattt tgccttctg tttttgctca cccagaaacg ctggtgaaag taaaagatgc
 241 tgaagatcag ttgggtgcac gagtgggtta catcgaactg gatctcaaca gcggtgaagat
 301 ccttgagagt tttcgccccg aagaacgttt cccaatgatg agcactttta agtttctgct
 361 atgtggcgcg gtattatccc gtgttgacgc cgggcaagag caactcggtc gccgcataca
 421 ctattctcag aatgacttgg ttgagtactc accagtcaca gaaaagcatc ttacggatgg
 481 catgacagta agagaattat gcagtgtctc cataacatg agtgataaca ctgcggccaa
 541 cttacttctg acaacgatcg gaggaccgaa ggagctaacc gcttttttgc acaacatggg
 601 ggatcatgta actcgcttg atcgttggga accggagctg aatgaagcca taccaaagca
 661 cgagcgtgac accacgatgc ctgtagcaat ggcaacaacg ttgcgaaac tattaactgg
 721 cgaactactt actctagctt cccggcaaca attaatagac tggatggagg cggataaagt
 781 tgcaggacca cttctgcgct cggcccttcc ggctggctgg tttattgctg ataatctgg
 841 agccggtgag cgtgggtctc gcggtatcat tgcagcactg gggccagatg gtaagccctc
 901 ccgtatcgta gttatctaca cgacggggag tcaggcaact atggatgaac gaaatagaca
 961 gatcgctgag ataggtgcct cactgattaa gcattggtaa ctgtcagacc aagtttactc
 1021 atatatactt tagattgatt taccccgggt gataatcaga aaagcccaa aaacaggaag
 1081 attgtataag caaatattta aattgtaaac gtaatatatt tgttaaaatt cgcgttaaatt
 1141 tttgtttaa tcagctcatt ttttaacaa taggccgaaa tcggcaaaat cccttataaa
 1201 tcaaaagaat agcccagat aggggtgagt gttgttcag ttggaacaa gagtccacta
 1261 ttaaagaacg tggactcaa cgtcaaaggg cgaaaaaccg tctatcaggg cgatggccca
 1321 ctacgtgaac catcaccaa atcaagtttt ttggggtcga ggtgccgtaa agcactaaat
 1381 cggaaacctt aaggagccc ccgatttaga gcttgacggg gaaagccggc gaacgtggcg
 1441 agaaaggaag ggaagaaagc gaaaggagcg ggcgctaggg cgctggcaag tgtagcggct
 1501 acgctgcgcg taaccaccac accgcccgcg cttaatgcgc cgctacaggg cgcgtaaaag
 1561 gatctaggtg aagatccttt ttgataatct catgacaaa atcccttaac gtgagttttc
 1621 gttccactga gcgtcagacc ccgtagaaaa gatcaaagga tcttcttgag atcctttttt
 1681 tctgcgcgta atctgctgct tgcaaacaaa aaaaccaccg ctaccagcgg tggtttggtt
 1741 gccgatcaa gagctaccaa ctctttttcc gaaggtaact ggcttcagca gagcgcagat
 1801 accaaatact gtccttctag tgtagccgta gtagggccac cacttcaaga actctgtagc
 1861 accgcctaca tacctcgctc tgctaactct gttaccagtg gctgctgcca gtggcgataa
 1921 gtcgtgtctt accgggttgg actcaagacg atagttaccg gataaggcgc agcggtcggg
 1981 ctgaacgggg gggtcgtgca cacagcccag cttggagcga acgacctaca ccgaactgag
 2041 atacctacag cgtgagctat gagaaagcgc cagcttccc gaaggagaa aggcggacag
 2101 gtatccggtg agcggcaggg tcggaacagg agagcgcacg agggagcttc cagggggaaa
 2161 cgcttggtat ctttatagtc ctgttgggtt tcgccacctc tgacttgagc gtcgattttt
 2221 gtgatgctcg tcaggggggc ggagcctatg gaaaaacgcc agcaacgcgg cctttttacg
 2281 gttcctggcc ttttgctggc cttttgctca catgttcttt cctgcgttat ccctgattc
 2341 tgttgataac cgtattaccg cttttgagt agctgatacc gctcgcgca gccgaacgac
 2401 cgagcgcagc gagtcagtga gcgaggaagc tatggtgcac tctcagtaca atctgctctg
 2461 atgccgcata gtaagccag tataactccc gctatcgcta cgtactggg tcatggctgc
 2521 gccccgacac ccgccaacac ccgctgacgc gccctgacgg gcttgtctgc tcccggcatc

2581 cgcttacaga caagctgtga ccgtctccgg gagctgcatg tgtcagaggt tttaccgctc
 2641 atcaccgaaa cgcgcgaggc agctgcggta aagctcatca gcgtggctgt gcagcgattc
 2701 acagatgtct gcctgttcat ccgcgtccag ctctgtgagt ttctccagaa gcgttaatgt
 2761 ctggcttctg ataaagcggg ccatgttaag ggcgggtttt tctgtttgg tcaactgatgc
 2821 ctccgtgtaa gggggatttc tgttcatggg ggtaatgata ccgatgaaac gagagaggat
 2881 gctcacgata cgggttactg atgatgaaca tgcccgggta ctggaacgtt gtgagggtaa
 2941 acaactggcg gtatggatgc ggcgggacca gagaaaaatc actcagggtc aatgccagcc
 3001 gaacgccagc aagacgtagc ccagcgcgtc ggcgccatg ccggcgataa tggcctgctt
 3061 ctgcgcgaaa cgtttgggtg cgggaccagt gacgaaggct tgagcgaggg cgtgcaagat
 3121 tccgaatacc gcaagcgaca ggccgatcat cgtcgcgctc cagcgaaagc ggtcctcgcc
 3181 gaaaaatgacc cagagcgtg cggcacctg tcctacgagt tgcatagata agaagacagt
 3241 cataagtgcg gcgacgatag tcattccccg cgcccaccgg aaggagctga ctgggttgaa
 3301 ggctctcaag ggcacggtc gagatcccgg tgcctaata gaagagtaac ttacattaat
 3361 tgcgttgcgc tcaactcccc ctttcagtc gggaaacctg tcgtgccagc tgcattaatg
 3421 aatcggccaa cgcgcgggga gaggcgggtt gcgtattggg cgccagggtg gttttctt
 3481 tcaccagtga gacgggcaac agctgattgc cttcaccgc ctggccctga gagagttgca
 3541 gcaagcggtc cagctgtggt tggccagca ggcgaaaatc ctgtttgatg gtggttaacg
 3601 gcgggatata acatgagctg tcttcggtat cgtcgtatcc cactaccgag atgtccgcac
 3661 caacgcgcag cccggactcg gtaatggcgc gcattgcgcc cagcgccatc tgatcgttgg
 3721 caaccagcat cgagtgaggc acgatgccct cattcagcat ttgcatggtt tgttgaaaac
 3781 cggacatggc actccagtcg ctttccgtt ccgctatcgg ctgaatttga ttgcgagtga
 3841 gatatttatg ccagccagcc agacgcggac gcgccgagac agaacttaat gggcccgcta
 3901 acagcgcgat ttgtgttgga ccaatgcga ccagatgctc cagccagct cgcgtaccgt
 3961 cttcatggga gaaaataata ctgttgatgg gtgtctggc agagacatca agaaataacg
 4021 ccggaacatt agtcaggca gttccacag caatggcatc ctggcatcc agcggatagt
 4081 taatgatcag cccactgacg cgttgcgcga gaagatttg caccgccgt ttacaggctt
 4141 cgacgccgt tcgttctacc atcgacacca ccacgtggc acccagttga tcggcgag
 4201 atttaatcgc cgcgacaatt tgcgacggcg cgtgcagggc cagactggag gtggcaacgc
 4261 caatcagcaa cgactgttg cccgccagt gttgtgccac gcggttgga atgtaattca
 4321 gctccgcat cgccgttcc acttttccc gcgttttcg agaaactgg ctggcctggt
 4381 tcaccacgcg gaaacggtc tgataagaga caccggcata ctctgcgaca tcgtataacg
 4441 ttactggtt cacattcacc accctgaatt gactctctc cgggcgctat catgccatac
 4501 cgcgaaagg tttgcgcat tcgatggtgt ccgggatctc gacgctctc cttatgcgac
 4561 tcctgcatta ggaagcagc cagtagtagg ttgaggcgt tgagcaccgc cgccgcaagg
 4621 aatggtgcat gccggcatgc gcgcccttcg tcttaagaa ttaattcca attccccagg
 4681 catcaataa aacgaaaggc tcagtcgaaa gactggcct ttcgtttat ctgttgtt
 4741 tcggtgaacg ctctctgag taggacaaat ccgcgggag cggatttgaa cgttgcgaag
 4801 caacggcccg gaggttgccg ggcaggacgc ccgcataaa ctgccaggaa ttaattcccc
 4861 aggcatacaa taaacgaaa ggctcagtcg aaagactggg ctttcgttt tatctgtt
 4921 ttgtcgtgta acgctctct gagtaggaca aatccgccg gagcggattt gaacgttgcg
 4981 aagcaacggc ccggagggtg gcgggcagga cggccgcat aaactgccag gaattaatc
 5041 cccaggcatc aaataaaacg aaaggctcag tcgaaagact gggccttctg tttatctgt
 5101 tgtttgtcgg tgaacgctc ctgagtagg acaaatccg cgggagcgga ttgaacgtt
 5161 gcgaagcaac ggcccggagg gtggcgggca ggacggccg cataaactgc caggaattaa

5221 ttccccaggc atcaataaaa acgaaaggct cagtcgaaag actgggcctt tcgttttatc
 5281 tgttgtttgt cggtagacgc tctctgagt aggacaaatc cgccgggagc ggatttgaac
 5341 gttgcgaagc aacggcccgg aggggtggcgg gcaggacgcc cgccataaac tgccaggaat
 5401 taattcccca ggcatcaaat aaaacgaaag gctcagtcga aagactgggc ctttcgtttt
 5461 atctgttgtt tgtcgggtgaa cgctctctg agtaggacaa atccgccggg agcggatttg
 5521 aacgttgcga agcaacggcc cggaggggtgg cgggcaggac gcccgccata aactgccagg
 5581 aattggggat cggaattaat tcccgttta aaccggggat ctgatcccg cgaaattaat
 5641 acgactcact ataggggaat tgtgagcgga taacaattcc cctctagaaa taattttgtt
 5701 taactttaag aaggagatat acatatgatt ctgacacgg attacatcac ggaagacggc
 5761 aagccgggta tccgtatttt caagaaagaa aacggcgaat tcaagattga atacgatcgg
 5821 acatttgaac cgtacctgta cgctctctc aaggatgata gcgcaatcga agaagtgaag
 5881 aaaatcaccg cagagcggca tggcacagtg gtaacagtta agcgggtcga gaaagtgcag
 5941 aagaagtct taggccggcc agtcgaagta tggaaattat acttcacaca tccacaggac
 6001 gttccggcga tcatggataa gattcgggag catccggcgg taatcgatat ctatgaatac
 6061 gatattccgt tcgctattcg ctaccttatt gacaaaggt tagttccaat ggagggtgat
 6121 gaggaactta aactgttagc attcgatatc gaaacacttt atcacgaagg tgaagagttt
 6181 gccgaaggtc cgattttaat gatctcgtac gccgatgaag aaggcgacg cgtaattacg
 6241 tggaaaaatg tggacctccc gtacgtagac gtagtgagca ctgagcgca gatgattaaa
 6301 cgtttccttc gggtagtaaa agaaaaagac ccagacgtgc tgattacgta taacggcgac
 6361 aactttgatt ttgctatct caagaagcgt tgcgaaaagt taggcattaa tttcgccctg
 6421 ggtcgggacg gttcagagcc gaaaattcag cggatgggag accgctttg tgtggaggta
 6481 aaaggtcgca tccatttcga tttatatccg gttatccggc gcacatcaa cttgccgact
 6541 tacacacttg aagcagttta cgaagcgggt ttcggccaac caaaagaaaa ggtttatgcc
 6601 gaggagatta ccaccgatg ggaaactggc gaaaacttgg agcgggtggc tcggtattcc
 6661 atggaagatg ccaagtgac ctacgaactg ggcaaagagt tttaccgat ggaagcaca
 6721 ttaagccgcc ttattggtca gtcctctgg gatgtgtcgc gttcttaac gggcaattta
 6781 gtcgaatgtt ttcttctcg gaaagcgtac gagcgtaacg agcttgctcc aaataagcca
 6841 gacgaaaaag aattggctcg gcgcatcag tcacatgagg gcggctacat taaggagcca
 6901 gaacggggct tgtgggagaa catcgtctac cttgatttc ggtctctta tccgtctatt
 6961 atcatcacac ataactctc gccagatacc ctgaaccgtg aaggctgtaa agaataatgat
 7021 gtggcaccac aggtcgcca tcgttttgt aaagacttc cgggcttcat tcatctctt
 7081 ctgggtgatt tgttagaaga gcgtcaaaag atcaagaaac gtatgaaagc gacaattgac
 7141 ccaattgaac gcaaattact tgattaccgt cagcgtgcaa tcaagatcct cgcaactct
 7201 ctgtacggtt attacggcta cgcacgcc cggtggtatt gcaaagaatg tgcagaatca
 7261 gtcattgctt ggggtcggga gtacctgacc atgacgatta aggaaattga ggagaaatac
 7321 ggtttcaagg tcatctatag tgacacggat ggtttcttg caacgattcc aggtgcggac
 7381 gcagaaactg taaagaaaaa ggcaatggag ttctgaagt atattaatgc gaagttgcc
 7441 ggcgccttg aattagagta cgaaggtttt tataagcgtg gcctgttcgt gacaaagaag
 7501 aaatacgcg taattgacga ggaaggcaag atcacaactc gtggcttga aattgttcgt
 7561 cgcgattgga gcgagatcgc aaaggagacc caagctcgtg tgttgaggc cctctgaag
 7621 gatggtgacg tcgaaaaagc cgtacgcacg gtaaggagg ttacagagaa gcttagcaag
 7681 tatgaggtcc caccagagaa acttgttatt cataaacaac tactcgca ccttaaagac
 7741 tataaggcca ctggtccaca cgtcgccgta gcaaagcggc ttgcggtcg gggcgtaag
 7801 attcggccag gcacggttat tagttacatc gtcctcaaag gctcaggccg gattgttgat

```

7861 cgcgcgattc catttgatga attgatccg acgaagcata aatatgatgc ggaatattac
7921 attgaaaaac aggttctgcc ggcgggtggag cgcattctac gtgcgttcgg ctatcgcaag
7981 gaggatttgc ggtaccagaa aactcgtcaa gtcggtttga gtgcccgtct gaagccgaaa
8041 ggtaccctcg agggatccag ccatcacat caccatcact gataagcggc cgcattgatc
8101 cggctgctaa caaagcccga aaggaagctg agttggctgc tgccaccgct gagcaataac
8161 tagcataacc cttgggggcc tctaaacggg tcttgagggg tttttgctg aaaggaggaa
8221 ctatatccgg at

```

//

Table S1. GenBank format of pET_RTX-6xHis

Figure S2. Plasmid map pET_RTX(exo-)-6xHis

LOCUS	pET_RTX(exo-)-6x	8232 bp	DNA	circular	UNA	26-MAR-2020
DEFINITION	A new nucleotide sequence entered manually.					


```

ACCESSION  urn.local...28-bq4yik3
VERSION    urn.local...28-bq4yik3
KEYWORDS   .
SOURCE
  ORGANISM
    .
FEATURES   Location/Qualifiers
  gene      140..1000
            /created_by="User"
            /label="bla (AmpR)"
  rep_origin 1042..1555
            /created_by="User"
            /modified_by="User"
            /label="M13 ori"
  rep_origin 1666..2254
            /created_by="User"
            /modified_by="User"
            /label="pMB1 ori"
  gene      complement(2623..2814)
            /created_by="User"
            /label="rop"
  gene      complement(3371..4453)
            /created_by="User"
            /label="lacI"
  promoter   5637..5654
            /created_by="User"
            /label="T7 Promoter"
  operon     5656..5680
            /created_by="User"
            /label="lacO"
  RBS        5708..5714
            /created_by="User"
            /label="RBS"
  misc_feature 5722..5727
            /created_by="User"
            /modified_by="User"
            /label="NdeI"
  CDS        5725..8085
            /created_by="User"
            /modified_by="User"
            /label="RTX"
  misc_difference 5836..5838
            /created_by="User"
            /label="F38L"

```

```

misc_difference 6013..6015
    /created_by="User"
    /label="R97M"
misc_difference 6076..6078
    /created_by="User"
    /modified_by="User"
    /label="K118I"
misc_difference 6133..6135
    /created_by="User"
    /label="M137L"
misc_difference 6352..6354
    /created_by="User"
    /label="N210D (exo- mutation)"
misc_difference 6865..6867
    /created_by="User"
    /label="R381H"
misc_difference 6874..6876
    /created_by="User"
    /label="Y384H"
misc_difference 6889..6891
    /created_by="User"
    /label="V389I"
misc_difference 7120..7122
    /created_by="User"
    /label="K466R"
misc_difference 7201..7203
    /created_by="User"
    /label="Y493L"
misc_difference 7264..7266
    /created_by="User"
    /label="T514I"
misc_difference 7285..7287
    /created_by="User"
    /label="I521L"
misc_difference 7483..7485
    /created_by="User"
    /label="F587L"
misc_difference 7714..7716
    /created_by="User"
    /label="E664K"
misc_difference 7855..7857
    /created_by="User"
    /label="G711V"
misc_difference 7927..7929

```

```

        /created_by="User"
        /label="N735K"
misc_difference 8026..8028
        /created_by="User"
        /label="W768R"
misc_feature 8047..8052
        /created_by="User"
        /label="XhoI"
misc_feature 8053..8058
        /created_by="User"
        /label="BamHI"
terminator 8104..8226
        /created_by="User"
        /label="T7 Tphi transcription terminator"

```

ORIGIN

```

1 aactacgtca ggtggcactt ttcggggaaa tgtgcgcgga acccctattt gtttatttt
61 cttaaatacat tcaaatatgt atccgctcat gagacaataa ccctgataaa tgcttcaata
121 atattgaaaa aggaagagta tgagtattca acatttccgt gtcgccctta ttccctttt
181 tgcggcattt tgccttctg ttttgctca cccagaaaacg ctggtgaaag taaaagatgc
241 tgaagatcag ttgggtgcac gagtgggtta catcgaactg gatctcaaca gcggtaatg
301 ccttgagagt tttcgccccg aagaacgttt cccaatgatg agcactttta aagtctgct
361 atgtggcgcg gtattatccc gtgtgacgc cgggcaagag caactcggtc gccgcataca
421 ctattctcag aatgacttgg ttgagtactc accagtcaca gaaaagcatc ttacggatgg
481 catgacagta agagaattat gcagtgtgc cataacatg agtgataaca ctgcggccaa
541 ctacttctg acaacgatcg gaggaccgaa ggagctaacc gctttttgc acaacatggg
601 ggatcatgta actcgcttg atcgttggga accggagctg aatgaagcca taccaaacga
661 cgagcgtgac accacgatgc ctgtagcaat ggcaacaacg ttgcgcaaac tattaactgg
721 cgaactactt actctagctt cccggcaaca attaatagac tggatggagg cggataaagt
781 tgcaggacca cttctgcgt cggcccttcc ggctggctgg tttattgctg ataaatctgg
841 agccggtgag cgtgggtctc gcggtatcat tgcagcactg gggccagatg gtaagccctc
901 ccgtatcgta gttatctaca cgacggggag tcaggcaact atggatgaac gaaatagaca
961 gatcgctgag ataggtgcct cactgattaa gcattggtaa ctgtcagacc aagtttactc
1021 atatatactt tagattgatt taccctgggt gataatcaga aaagcccaa aaacaggaag
1081 attgtataag caaatattta aattgtaaac gttaatattt tgttaaaatt cgcgttaa
1141 tttgttaaa tcagctcatt ttttaacaa taggccgaaa tcggcaaaat cccttataaa
1201 tcaaaagaat agcccagat aggggtgagt gttgttcag ttggaacaa gaggccacta
1261 ttaaagaacg tggactccaa cgtcaaaggg cgaaaaaccg tctatcaggg cgatggccca
1321 ctacgtgaac catcaccaa atcaagtttt ttggggtcga ggtgccgtaa agcactaaat
1381 cggaacccta aaggagccc ccgatttaga gcttgacggg gaaagccggc gaacgtggcg
1441 agaaaggaag ggaagaaagc gaaaggagcg ggcgctaggg cgctggcaag tgtagcggtc
1501 acgctgcgcg taaccaccac accgcccgcg cttaatgcgc cgctacaggg cgcgtaaaag
1561 gatctaggtg aagatccttt ttgataatct catgaccaa atccctaac gtgagttttc
1621 gttccactga gcgtcagacc ccgtagaaaa gatcaaagga tcttctgag atcctttttt
1681 tctgcgcgta atctgctgct tgcaaacaaa aaaaccaccg ctaccagcgg tggtttgtt

```

1741 gccggatcaa gagctacca cttttttcc gaaggtaact ggcttcagca gagcgagat
 1801 accaaatact gtccttctag tgtagccgta gtaggccac cacttcaaga actctgtagc
 1861 accgcctaca tacctcgctc tgctaactct gttaccagt gctgctgcca gtggcgataa
 1921 gtcgtgtctt accgggttgg actcaagacg atagttaccg gataaggcgc agcggtcggg
 1981 ctgaacgggg gggtcgtgca cacagcccag cttggagcga acgacctaca ccgaactgag
 2041 atacctacag cgtgagctat gaaaaagcgc cacgcttccc gaaggagaaa aggcgagacg
 2101 gtatccggtg agcggcaggg tcggaacagg agagcgacg agggagcttc cagggggaaa
 2161 cgcttggtat ctttatagtc ctgttggtt tcgccacctc tgacttgagc gtcgattttt
 2221 gtgatgctcg tcaggggggc ggagcctatg gaaaaacgc agcaacgcgg cttttttacg
 2281 gttcctggcc ttttgctggc cttttgctca catgttctt cctgcgttat cccctgattc
 2341 tgttgataac cgtattaccg ctttgagt gctgatacc gctcgccgca gccgaacgac
 2401 cgagcgacg gagtcagtga gcgaggaagc tatggtgcac tctcagtaca atctgctctg
 2461 atgccgcata gtaagccag tatacactcc gctatcgcta cgtgactggg tcatggctgc
 2521 gccccgacac ccgccaacac ccgtgacgc gccctgacgg gcttgctgc tcccgcatc
 2581 cgcttacaga caagctgtga ccgtctccg gagctgcatg tgcagaggt tttaccgtc
 2641 ataccgaaa cgcgcgaggc agctgcggtg aagctcatca gcgtggtcgt gcagcgattc
 2701 acagatgtct gcctgttcat ccgcgtccag ctcgttgagt ttctccagaa gcgttaatgt
 2761 ctggcttctg ataaagcggg ccatgttaag ggcgggtttt tctgtttgg tactgatgc
 2821 ctccgtgtaa gggggatttc tgttcattgg ggtaatgata ccgatgaaac gagagaggat
 2881 gctcacgata cgggttactg atgatgaaca tgcccggtta ctggaacgtt tgagggttaa
 2941 acaactggcg gtatggatgc ggcgggacca gagaaaaatc actcagggtc aatgccagcc
 3001 gaacgccagc aagacgtagc ccagcgcgtc ggccgccatg ccggcgataa tggcctgctt
 3061 ctcgccgaaa cgtttggtgg cgggaccagt gacgaaggct tgagcgaggc cgtgcaagat
 3121 tccgaatacc gcaagcgaca ggccgatcat cgtcgcgtc cagcgaaagc ggtcctcgcc
 3181 gaaaatgacc cagagcgtg ccggcacctg tcctacgagt tgcagtataa agaagacagt
 3241 cataagtgcg gcgacgatag tcatgccccg cgccaccgg aaggagctga ctgggttgaa
 3301 ggctctcaag ggcacggtc gagatcccg tgcctaatga gtgagctaac ttacattaat
 3361 tgcgttgccg tactgcccc ctttcagtc gggaaacctg tcgtgccagc tgcattaatg
 3421 aatcgcccaa cgcgcgggga gaggcggtt gcgtattggg cgccagggtg gttttctt
 3481 tcaccagtga gacgggcaac agctgattgc cttcaccgc ctggccctga gagagtga
 3541 gcaagcgtc cacgctggtt tgccccagca ggcgaaaatc ctgttgatg gtggttaacg
 3601 cggggatata acatgagctg tcttcggtat cgtcgtatcc cactaccgag atgtccgac
 3661 caacgcgac cccggactcg gtaatggcgc gattgcgcc cagcgccatc tgatcgttg
 3721 caaccagcat cgagtggga acgatgccct cattcagcat ttgatggtt tgtgaaaac
 3781 cggacatggc actccagtcg cttcccgtt ccgctatcgg ctgaatttga ttgcagtgta
 3841 gatatttatg ccagccagcc agacgcggac gcgccgagac agaactaat gggcccgcta
 3901 acagcgcat ttgctggtga ccaatgcga ccagatgctc cagccccagt cgcgtaccgt
 3961 cttcatggga gaaaataata ctgttgatgg gtgtctggc agagacatca agaaataacg
 4021 ccggaacatt agtcaggca gctccacag caatggcatc ctggctatcc agcgatagat
 4081 taatgatcag ccaactgacg cgttgccgga gaagatttg caccgccgt ttacaggctt
 4141 cgacgccgt tcgttctacc atcgacacca ccacgtggc acccagttga tcggcgag
 4201 attaatcgc cgcgacaatt tgcgacggc cgtgcaggc cagactggag gtggcaacgc
 4261 caatcagcaa cgactgttg cccgccagt gttgtgccac gcggttgga atgtaattca
 4321 gctccgcat cgccgttcc actttttcc gcgtttcgc agaaactgg ctggcctggt

4381 tcaccacgcg ggaaacggtc tgataagaga caccggcata ctctgcgaca tcgtataacg
 4441 ttactggttt cacattcacc accctgaatt gactctcttc cgggcgctat catgccatac
 4501 cgcgaaaggt ttgcgccat tcgatgggtg cgggatctc gacgctctcc cttatgcgac
 4561 tcctgcatta ggaagcagcc cagtagtagg ttgaggccgt ttagcaccgc cgccgcaagg
 4621 aatgggtcat gccggcatgc cgcccttcg tcttaagaa ttaattccca attccccagg
 4681 catcaataa aacgaaaggc tcagtcgaaa gactgggcct ttcgtttat ctgtgtttg
 4741 tcggtgaacg ctctctgag taggacaaat ccgccgggag cggattgaa cgttgcaag
 4801 caacggcccc gaggggtggc ggaggacgc ccgccataaa ctgccaggaa ttaattcccc
 4861 aggcataaaa taaaacgaaa ggctcagtcg aaagactggg ctttcgttt tatctgtgt
 4921 ttgtcggtag acgctctct gagtaggaca aatccgccgg gagcggattt gaacgttgcg
 4981 aagcaacggc ccggagggtg gcgggcagga cggcccat aaactgccag gaattaattc
 5041 cccaggcatc aaataaaacg aaaggctcag tcgaaagact gggccttcg ttttatctgt
 5101 tgtttgtcgg tgaacgtct cctgagtagg acaaatccgc cgggagcgga ttgaacgtt
 5161 gcgaagcaac ggccggagg gtggcgggca ggacggccgc cataaactgc caggaattaa
 5221 ttcccaggc atcaataaaa acgaaaggct cagtcgaaag actgggcctt tcgtttatc
 5281 tgttgtttgt cgtgaacgc tctctgagt aggacaaatc cgccgggagc ggatttgaac
 5341 gttgcgaagc aacggcccgg aggggtggcgg gcaggacgcc cgccataaac tgccaggaat
 5401 taattcccca ggcatacaat aaaacgaaag gctcagtcga aagactgggc ctttcgttt
 5461 atctgttgtt tgcggtgaa cgctctctg agtaggacaa atccgccggg agcggatttg
 5521 aacgttgca agcaacggcc cggagggtgg cgggcaggac gccgccata aactgccagg
 5581 aattggggat cggaattaat tcccgttta aaccggggat ctgatcccg cgaaattaat
 5641 acgactact ataggggaat tgtgagcgga taacaattcc ccttagaaa taattttgtt
 5701 taactttaag aaggagatat acatatgatt cttgacacgg attacatcac ggaagacggc
 5761 aagccgggta tccgtatttt caagaaagaa aacggcgaat tcaagattga atacgatcgg
 5821 acatttgaac cgtacctga cgctctctc aaggatgata gcgcaatcga agaagtga
 5881 aaaatcaccg cagagcggca tggcacagt gtaacagta agcgggtcga gaaagtgcag
 5941 aagaagtct taggccggcc agtcgaagta tggaaattat acttcacaca tccacaggac
 6001 gttccggcga tcatggataa gattcgggag catccggcgg taatcgatat ctatgaatac
 6061 gatattccgt tcgtattcg ctacctatt gacaaagggt tagttccaat ggagggtgat
 6121 gaggaactta aactgttagc attcgatac gaaacacttt atcacgaagg tgaagagttt
 6181 gccgaaggtc cgattttaat gatctctac gccgatgaag aaggcgcacg cgtaattacg
 6241 tggaaaaatg tggacctccc gtacgtagac gtagtgagca ctgagcgcga gatgattaaa
 6301 cgtttccttc gggtagtaaa agaaaaagac ccagacgtgc tgattacgta tgacggcgac
 6361 aactttgatt ttgcctatct caagaagcgt tgcgaaaagt taggcattaa ttgcacctg
 6421 ggtcgggacg gttcagagcc gaaaattcag cggatgggag accgctttg tgtggaggta
 6481 aaaggtcga tccatttcga tttatatccg gttatccgc gcacatcaa cttgccgact
 6541 tacacacttg aagcagttta cgaagcgggt ttcggccaac caaaagaaaa ggtttatgcc
 6601 gaggagatta ccaccgatg ggaaactgg gaaaacttgg agcgggtggc tcggtattcc
 6661 atggaagatg ccaaggtgac ctacgaactg ggcaaagagt tttaccgat ggaagcaca
 6721 ttaagccgcc ttattgttca gtccctctgg gatgtgtcgc gttcttaac gggcaattta
 6781 gtcgaatggt ttcttctcg gaaagcgtac gagcgtaac agcttgctcc aaataagcca
 6841 gacgaaaaag aattggctcg gcgcatcag tcacatgagg gcggctacat taaggagcca
 6901 gaacggggct tggggagaa catcgtctac cttgatttc ggtctctta tccgtctatt
 6961 atcatcacac ataactctc gccagatacc ctgaaccgtg aaggctgtaa agaataatgat

```

7021 gtggcaccac aggtcggcca tcgttttgt aaagacttcc cgggcttcat tccatctctt
7081 ctgggtgatt tgtagaaga gcgtcaaaag atcaagaaac gtatgaaagc gacaattgac
7141 ccaattgaac gcaaattact tgattaccgt cagcgtgcaa tcaagatcct cgcgaactct
7201 ctgtacggtt attacggcta cgcacgcgcc cgggtgtatt gcaaagaatg tgcagaatca
7261 gtcattgctt ggggtcggga gtacctgacc atgacgatta aggaaattga ggagaaatac
7321 ggtttcaagg tcatctatag tgacacggat ggtttcttg caacgattcc aggtgcggac
7381 gcagaaactg taaagaaaaa ggcaatggag ttctgaagt atattaatgc gaagttgcca
7441 ggcgccttg aattagagta cgaaggtttt tataagcgtg gcctgttcgt gacaaagaag
7501 aaatacgcg taattgacga ggaaggcaag atcacaactc gtggcttga aattgttcgt
7561 cgcgattgga gcgagatcgc aaaggagacc caagctcgtg tgttgaggc cctcctgaag
7621 gatggtgacg tcgaaaaagc cgtacgcacg gtaaggagg ttacagagaa gcttagcaag
7681 tatgaggtcc caccagagaa acttggtatt cataaacaac tcaactcgca ccttaaagac
7741 tataaggcca ctggtccaca cgtcgccgta gcaaagcggc ttgcggctcg gggcgtaag
7801 attcgccag gcacggttat tagttacatc gtcctcaaag gctcaggccg gattgtgat
7861 cgcgcgattc ctttgatga attgatccg acgaagcata aatatgatgc ggaatattac
7921 attgaaaaac aggttctgcc ggcggtggag cgcacttac gtgcgttcgg ctatcgcaag
7981 gaggatttgc ggtaccagaa aactcgtcaa gtcggttga gtgcccgtct gaagccgaaa
8041 ggtaccctcg agggatccag ccatcaccat caccatcact gataagcggc cgcattgatc
8101 cggctgctaa caaagcccga aaggaagctg agttggctgc tgccaccgct gagcaataac
8161 tagcataacc cttggggcc tctaaacggg tcttgagggg tttttgctg aaaggaggaa
8221 ctatatccgg at

```

//

Table S2. GenBank format of pET_RTX(exo-)-6xHis

REFERENCES

- 1 Ellefson, J. W. *et al.* Synthetic evolutionary origin of a proofreading reverse transcriptase. *Science* **352**, 1590-1593, doi:10.1126/science.aaf5409 (2016).