Quick and Easy Assembly of a One-step qRT-PCR Kit for COVID-19 Diagnostics Using In-House Enzymes

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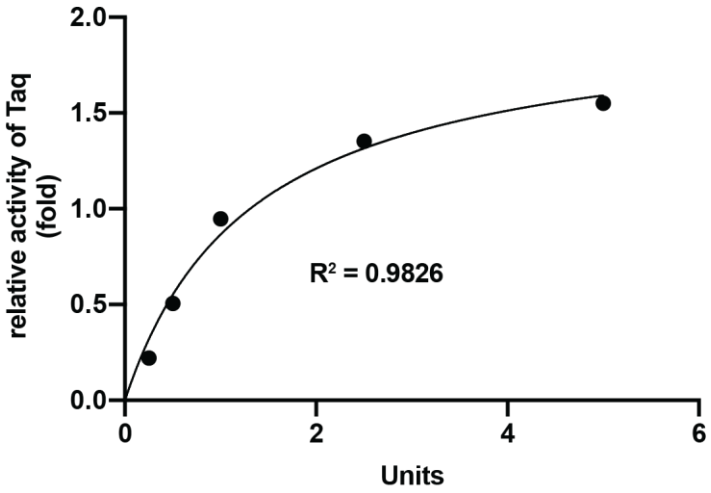
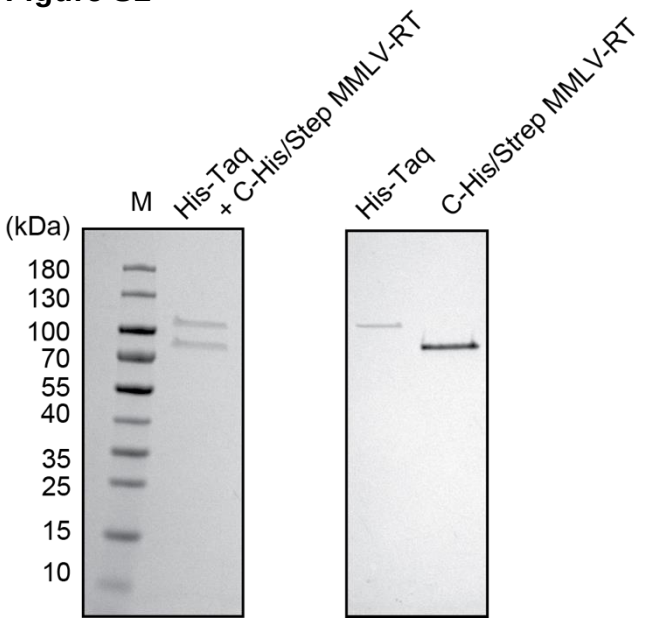
Figure S1



Standard titration curve of native Taq Pol's activity.
The PCR assays were conducted with serially diluted N-Taq Pol (Thermofisher) (see Materials and Methods section), and the standard titration curve of N-Taq Pol activity was plotted.

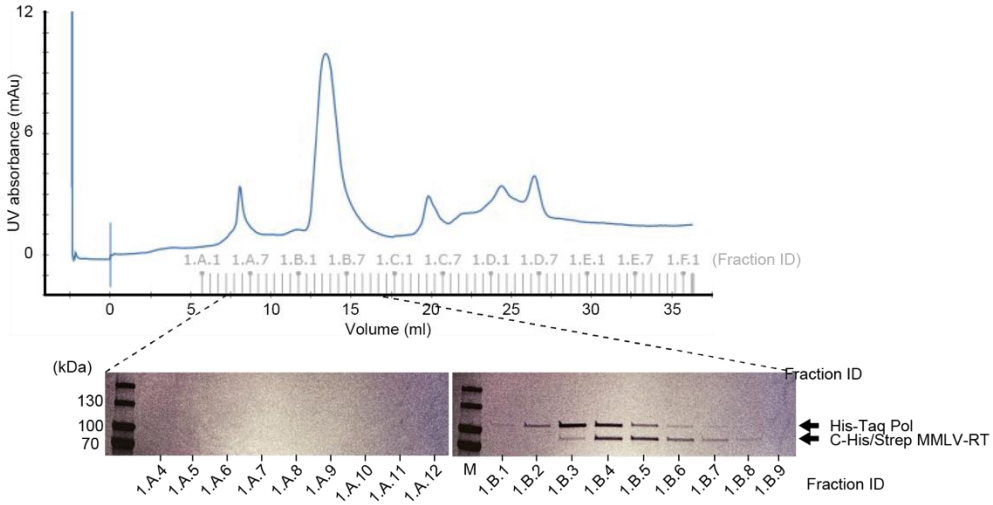
Figure S2



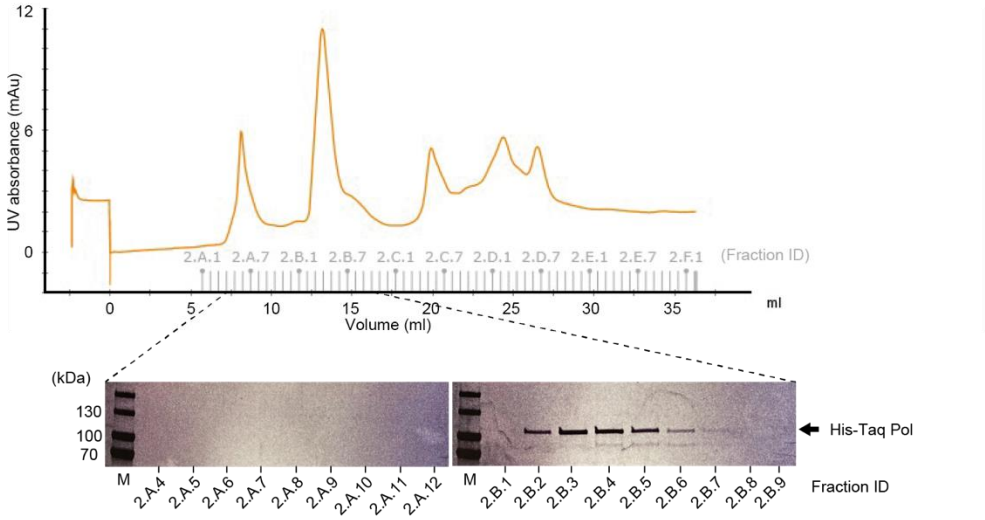
SDS-PAGE analysis of His-Taq and C-His/Strep MMLV-RT after the PCR.
After the PCR products were analyzed in Figure 3C, the reaction mixture leftover (500 ng of C-His/Strep MMLV-RT vs. 20 units of His-Taq Pol) was subjected to the SDS-PAGE analysis (left panel). Fresh purified His-Taq Pol and C-His/Strep MMLV-RT were loaded as a size control (right panel).

Figure S3

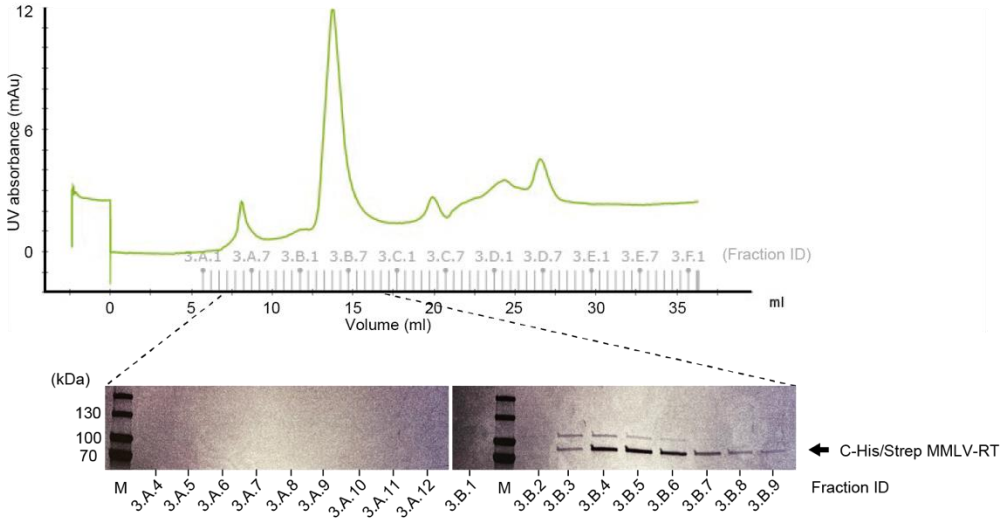
A C-His/Strep MMLV-RT (78.5 kDa) + His-Taq (93.4 kDa)



B His-Taq (93.4 kDa)



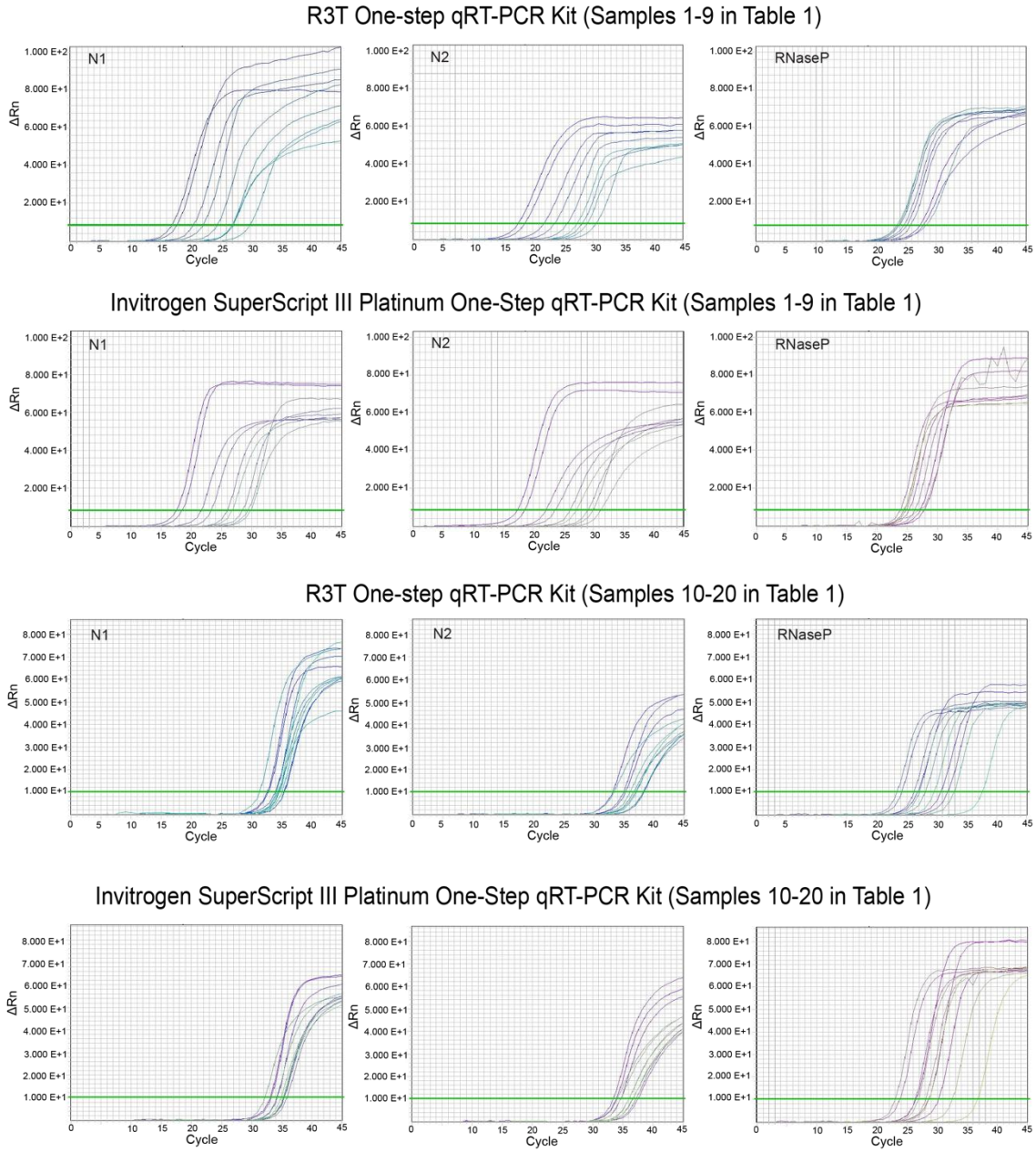
C C-His/MMLV-RT (78.5 kDa)



Elution profiles and SDS-PAGE gels of C-His/Strep MMLV-RT and His-Taq Pol from SEC.

The elution profiles from size-exclusion chromatography (SEC) and SDS-PAGE gels are shown for the mixture of C-His/Strep MMLV-RT and His-Taq (A), His-Taq only (B), and C-His/Strep MMLV-RT only (C). The protein samples were mixed with the PCR buffer and incubated on ice for 10 minutes. And the incubated samples were loaded onto the Superdex 200 10/30 GL column (GE Healthcare).

Figure S4



Comparison of amplification curves from the different positive patient samples.

The amplification curves of Ct values in Table 1 are shown. N1, N2, and RNaseP represent the primer sets used with the R3T One-step qRT-PCR kit or the Invitrogen SuperScript III Platinum One-Step qRT-PCR kit.

Table S1

Comparision of R3T One-step qRT-PCR System with TaqPath 1-Step RT-qPCR System.

R3T One-step qRT-PCR System							Thermofisher TaqPath 1-Step RT-qPCR System							
		Ct value in patient samples							Ct value in patient samples					
Sr. No.	Sample	N1	N2	RP	Expected	Result	Sr. No.	Sample	N	S	ORF 1ab	MS2	Expected	Result
1	RC-26	19.4	15.9	27.7	+	Positive SARS CoV-2	1	RC-26	18.8	19.8	20.1	30.3	+	Positive SARS CoV-2
2	RC-3	13.3	17.1	28.5	+	Positive SARS CoV-2	2	RC-3	16.9	17.7	17.2	37.1	+	Positive SARS CoV-2
3	RC-7	18	18.7	26.6	+	Positive SARS CoV-2	3	RC-7	16.4	16	15.7	32.8	+	Positive SARS CoV-2
4	RC-4	24.6	25.4	29.8	+	Positive SARS CoV-2	4	RC-4	25	25.4	24.8	26.5	+	Positive SARS CoV-2
5	RC-5	12.3	18.9	29.9	+	Positive SARS CoV-2	5	RC-5	17.5	18.6	18	36.6	+	Positive SARS CoV-2
6	RC-13	17.7	18.5	24.8	+	Positive SARS CoV-2	6	RC-13	16.8	16.1	16.1	37	+	Positive SARS CoV-2
7	RC-30	26.5	24.1	28.2	+	Positive SARS CoV-2	7	RC-30	27.1	26.8	27.4	28.6	+	Positive SARS CoV-2
8	RC-31	16.6	14.3	26.6	+	Positive SARS CoV-2	8	RC-31	15.9	16	16.3	34.6	+	Positive SARS CoV-2
9	RC-38	33	28.9	24.9	+	Positive SARS CoV-2	9	RC-38	31.9	30.2	30.9	26.5	+	Positive SARS CoV-2
10	RC-39	25.2	21.9	22.6	+	Positive SARS CoV-2	10	RC-39	24.6	23.9	24.2	26.7	+	Positive SARS CoV-2
11	RC-40	16.6	14.1	22.9	+	Positive SARS CoV-2	11	RC-40	16.6	15.1	15.3	36.3	+	Positive SARS CoV-2
12	RC-44	24.9	22.4	25.8	+	Positive SARS CoV-2	12	RC-44	24	22.4	22.6	29	+	Positive SARS CoV-2
13	RC-45	24.5	22.8	24.5	+	Positive SARS CoV-2	13	RC-45	23.4	21.7	21.3	29.9	+	Positive SARS CoV-2
14	RC-46	19.7	18.9	27.9	+	Positive SARS CoV-2	14	RC-46	18.7	17.2	17.3	33	+	Positive SARS CoV-2
15	RC-47	22	21.2	26	+	Positive SARS CoV-2	15	RC-47	21	19.7	20.1	30	+	Positive SARS CoV-2
16	RC-9	28.6	29.5	26.7	+	Positive SARS CoV-2	16	RC-9	27.5	25.5	24.7	27	+	Positive SARS CoV-2
17	RC-10	27.4	28.2	32.1	+	Positive SARS CoV-2	17	RC-10	21.5	24.4	24	24.1	+	Positive SARS CoV-2
18	RC-11	35.6	34.7	29.2	+	Positive SARS CoV-2	18	RC-11	32.9	33.8	34.9	23.5	+	Positive SARS CoV-2
19	RC-12	33.7	32.2	24.6	+	Positive SARS CoV-2	19	RC-12	31.3	31.8	31.5	27	+	Positive SARS CoV-2
20	RC-6	33.5	34	29.8	+	Positive SARS CoV-2	20	RC-6	32.6	34.6	34.9	27	+	Positive SARS CoV-2
21	RC-36	–	–	27.7	–	Negative SARS CoV-2	21	RC-36	–	–	–	27.9	–	Negative SARS CoV-2
22	RC-37	–	–	28.3	–	Negative SARS CoV-2	22	RC-37	–	–	–	27.7	–	Negative SARS CoV-2
23	RC-49	–	–	26.4	–	Negative SARS CoV-2	23	RC-49	–	–	–	26,6	–	Negative SARS CoV-2
24	RC-64	–	–	25.5	–	Negative SARS CoV-2	24	RC-64	–	–	–	26.8	–	Negative SARS CoV-2
25	RC-65	–	–	27.1	–	Negative SARS CoV-2	25	RC-65	–	–	–	26.5	–	Negative SARS CoV-2

N1 and N2: N-gene; RP: RNaseP; NTC: No template

N: N-gene; S: Spike Protein gene; MS2: Phage Control