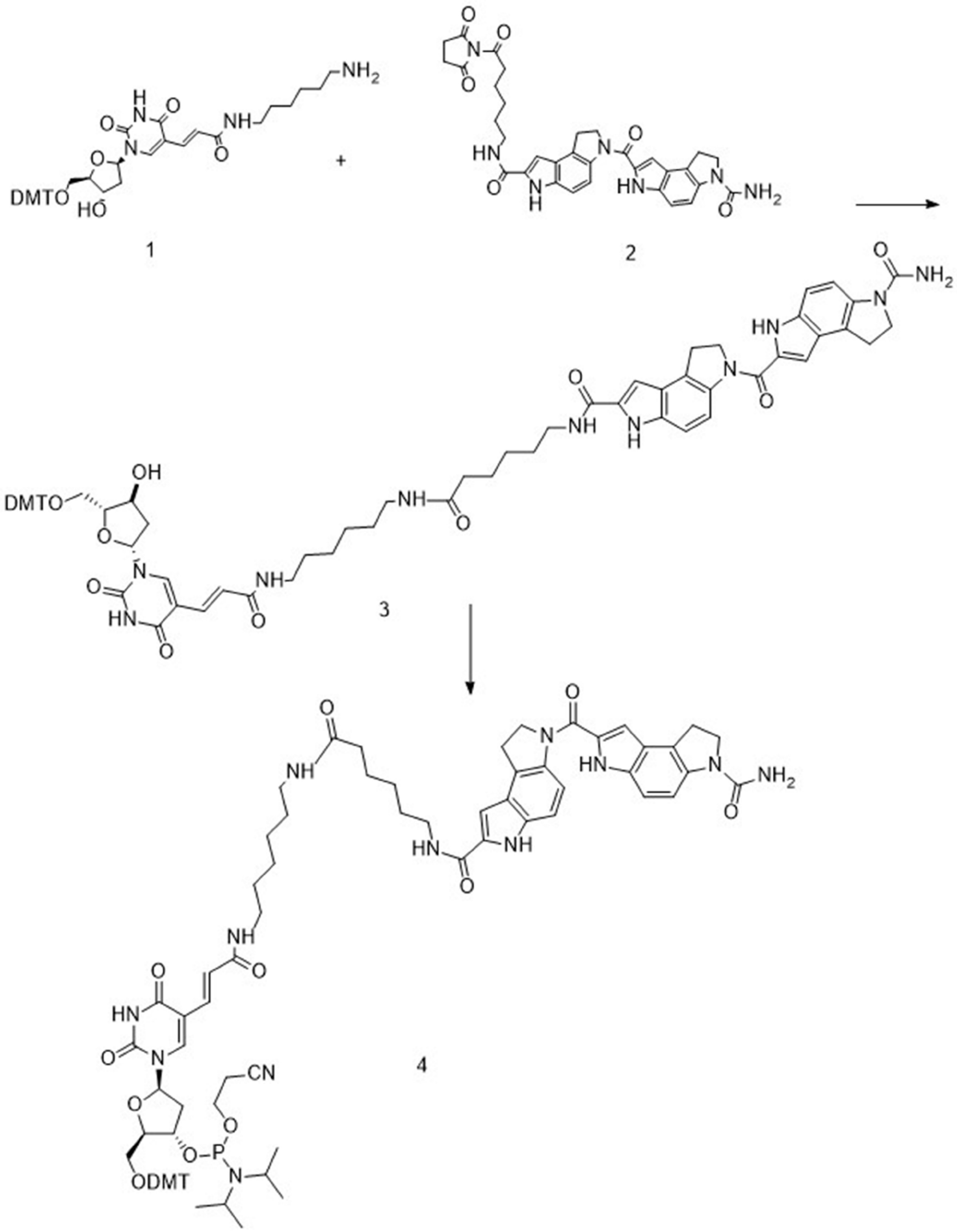

**Figure S1** Structure and advantages of the DQ Probe. DQ is a modified probe used in molecular biology and PCR. TFA Amino 5-DMT-2-deoxyuridine was deprotected with ammonium hydroxide to generate free amine, which was then coupled with DPI 3 NHS Ester to generate DPI3-DMT deoxyuridine, which was then phosphorylated to produce a fully protected Nucleoside phosphoramidite named DQ, which can be used for Oligo synthesis. It integrates a molecule capable of interacting with DNA in the minor groove, effectively increasing the melting temperature (Tm) of the probe. DQ binds to DNA less strongly than MGB, but this feature has the advantage of minimizing inhibition during the PCR process. DQ is a promising alternative to the conventional TaqMan probe because it can attain a higher Tm while maintaining a lower PCR inhibition. DMTO/ODMT: Dimethyl tin oxide; NH: An amino group; $NH_2$: An amino group consists of one nitrogen atom and two hydrogen atoms; OH: The Hydroxyl group (Hydroxyl group) with the chemical formula -OH, composed of hydrogen and oxygen atoms.

**Table S1** Detection of ORF/N genes of wild type for 125 copies/mL by 20 detections

| Reactions | ORF CT value | N gene CT value |
|---|---|---|
| 1 | NT | NT |
| 2 | NT | NT |
| 3 | NT | NT |
| 4 | NT | NT |
| 5 | NT | NT |
| 6 | NT | NT |
| 7 | NT | NT |
| 8 | NT | NT |
| 9 | NT | NT |
| 10 | NT | NT |
| 11 | NT | NT |
| 12 | NT | NT |
| 13 | NT | NT |
| 14 | NT | NT |
| 15 | NT | NT |
| 16 | NT | NT |
| 17 | NT | NT |
| 18 | NT | NT |
| 19 | NT | NT |
| 20 | NT | NT |

The CT value of ORF and N genes of wild type for 125 copies/mL by 20 replications. In the 20 times of 125 copies/mL repeated tests, neither O gene nor N gene could be detected and the detection rate was 0%.

**Table S2** Detection of ORF/N genes of wild type for 250 copies/mL by 20 detections

| Reactions | ORF CT value | N gene CT value |
|---|---|---|
| 1 | 37.76 | 39.76 |
| 2 | 37.45 | 37.8 |
| 3 | 37.09 | 37.37 |
| 4 | 37.96 | 37.51 |
| 5 | 37.80 | 36.77 |
| 6 | 37.81 | 37.23 |
| 7 | NT | 36.95 |
| 8 | 37.98 | 37.39 |
| 9 | 37.77 | 37.91 |
| 10 | 37.37 | 36.54 |
| 11 | 37.25 | 37.09 |
| 12 | 37.91 | 37.76 |
| 13 | 37.06 | 37.46 |
| 14 | 37.81 | 37.45 |
| 15 | 37.51 | 36.96 |
| 16 | 37.61 | 37.59 |
| 17 | 37.88 | 37.81 |
| 18 | 37.34 | 37.71 |
| 19 | 36.65 | 37.79 |
| 20 | 37.93 | 37.98 |

The CT value of ORF and N genes of wild type for 250 copies/mL by 20 replications. In the 20 times reactions, both O and N genes were detected 19 times which the CT value <38 cycles and with a detection rate of 95%.

**Table S3** Sensitivities of the developed qRT-PCR assays

| Copies/mL | subtype | Assay | Gene | CT ± SD | $R^2$ (250–4000 Copies/mL) | Subtype | CT ± SD | $R^2$ (250–4000 Copies/mL) |
|---|---|---|---|---|---|---|---|---|
| 4000 | Wild type | Assay 1 | ORF | 31.82± 0.02 | 0.9936 | BA.1 | 31.23±0.16 | 0.9817 |
| | | | N | 31.15±0.14 | 0.9888 | | 28.79±0.15 | 0.9987 |
| | | Assay 2 | 6970del | NT | | | 32.79±0.16 | 0.9974 |
| | | | T547K | NT | | | 33.13±0.21 | 0.9895 |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| | | | P681H | NT | | | 34.02±0.27 | 0.9636 |
| | | | T842I | NT | | | NT | |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 2000 | | Assay 1 | ORF | 33.23±0.06 | | | 32.45±0.19 | |
| | | | N | 32.3±0.19 | | | 29.80±0.09 | |
| | | Assay 2 | 6970del | NT | | | 33.93±0.08 | |
| | | | T547K | NT | | | 34.48±0.03 | |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| | | | P681H | NT | | | 35.38±0.06 | |
| | | | T842I | NT | | | NT | |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 1000 | | Assay 1 | ORF | 34.00±0.64 | | | 33.16±0.49 | |
| | | | N | 33.74±0.43 | | | 30.74±0.08 | |
| | | Assay 2 | 6970del | NT | | | 34.99±0.28 | |
| | | | T547K | NT | | | 35.54±0.34 | |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| | | | P681H | NT | | | 35.89±0.32 | |
| | | | T842I | NT | | | NT | |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 500 | | Assay 1 | ORF | 35.43±0.57 | | | 34.61±0.31 | |
| | | | N | 34.29±0.13 | | | 31.93±0.14 | |
| | | Assay 2 | 6970del | NT | | | 36.22±0.47 | |
| | | | T547K | NT | | | 36.81±0.86 | |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| | | | P681H | NT | | | 36.83±0.62 | |
| | | | T842I | NT | | | NT | |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 250 | | Assay 1 | ORF | 37.01±0.35 | | | 36.26±0.72 | |
| | | | N | 35.78±0.03 | | | 32.95±0.19 | |
| | | Assay 2 | 6970del | NT | | | 37.46±0.88 | |
| | | | T547K | NT | | | 37.48±0.99 | |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| | | | P681H | NT | | | 38.64±0.88 | |
| | | | T842I | NT | | | NT | |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 4000 | BA.2 | Assay 1 | ORF | 31.92±0.06 | 0.9758 | BA.3 | 32.05±0.25 | 0.9626 |
| | | | N | 31.23±0.06 | 0.9572 | | 30.64±0.23 | 0.9791 |
| | | Assay 2 | 6970del | NT | | | 31.22±0.01 | 0.9924 |
| | | | T547K | NT | | | NT | |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| | | | P681H | 34.21±0.25 | 0.9832 | | 31.81±0.18 | 0.9965 |
| | | | T842I | 34.16±0.65 | 0.9906 | | NT | |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 2000 | | Assay 1 | ORF | 32.71±0.22 | | | 33.05±0.1 | |
| | | | N | 32.01±0.09 | | | 31.77±0.21 | |
| | | Assay 2 | 6970del | NT | | | 31.97±0.14 | |
| | | | T547K | NT | | | NT | |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| | | | P681H | 34.84±0.11 | | | 32.71±0.22 | |
| | | | T842I | 35.10±0.69 | | | NT | |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 1000 | | Assay 1 | ORF | 33.57±0.12 | | | 34.41±0.15 | |
| | | | N | 33.22±0.19 | | | 32.91±0.06 | |
| | | Assay 2 | 6970del | NT | | | 33.23±0.23 | |
| | | | T547K | NT | | | NT | |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| | | | P681H | 36.33±0.51 | | | 34.07±0.29 | |
| | | | T842I | 36.87±1.04 | | | NT | |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 500 | | Assay 1 | ORF | 34.66±0.11 | | | 34.88±0.36 | |
| | | | N | 34.47±0.48 | | | 33.51±0.25 | |
| | | Assay 2 | 6970del | NT | | | 33.88±0.17 | |
| | | | T547K | NT | | | NT | |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| | | | P681H | 37.05±0.25 | | | 35.01±0.03 | |
| | | | T842I | 37.79±0.61 | | | NT | |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 250 | | Assay 1 | ORF | 36.29±0.51 | | | 37.06±0.8 | |
| | | | N | 36.86±0.18 | | | 35.31±0.69 | |
| | | Assay 2 | 6970del | NT | | | 34.89±0.7 | |
| | | | T547K | NT | | | NT | |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| | | | P681H | 37.88±0.47 | | | 36.1±0.78 | |
| | | | T842I | 39.03±0.30 | | | NT | |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 4000 | BA.4 | Assay 1 | ORF | 32.01±0.23 | 0.9991 | BA.5 | 31.70±0.24 | 0.9914 |
| | | | N | 31.03±0.11 | 0.9989 | | 31.07±0.17 | 0.999 |
| | | Assay 2 | 6970del | 33.66±0.17 | 0.9946 | | 34.32±0.27 | 0.9994 |
| | | | T547K | NT | | | NT | |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | 33.14±0.18 | 0.9676 | | 33.65±0.26 | 0.998 |
| | | | P681H | 32.64±0.17 | 0.983 | | 33.65±0.34 | 0.975 |
| | | | T842I | 34.20±0.14 | 0.9976 | | 34.79±0.13 | 0.9847 |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | 32.21±0.21 | 0.9914 | | NT | |
| | | | P681R | NT | | | NT | |
| 2000 | | Assay 1 | ORF | 33.02±0.19 | | | 32.54±0.22 | |
| | | | N | 32.06±0.30 | | | 32.18±0.27 | |
| | | Assay 2 | 6970del | 34.74±0.22 | | | 35.24±0.26 | |
| | | | T547K | NT | | | NT | |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | 34.21±0.26 | | | 34.67±0.24 | |
| | | | P681H | 33.51±0.32 | | | 34.64±0.32 | |
| | | | T842I | 35.53±0.09 | | | 36.30±0.12 | |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | 33.25±0.06 | | | NT | |
| | | | P681R | NT | | | NT | |
| 1000 | | Assay 1 | ORF | 34.01±0.04 | | | 33.89±0.24 | |
| | | | N | 33.24±0.17 | | | 33.19±0.15 | |
| | | Assay 2 | 6970del | 35.67±0.29 | | | 36.29±0.09 | |
| | | | T547K | NT | | | NT | |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | 34.88±0.46 | | | 35.65±0.43 | |
| | | | P681H | 34.48±0.12 | | | 35.56±0.66 | |
| | | | T842I | 36.46±0.46 | | | 37.57±0.46 | |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | 34.14±0.06 | | | NT | |
| | | | P681R | NT | | | NT | |
| 500 | | Assay 1 | ORF | 35.19±0.70 | | | 34.89±0.56 | |
| | | | N | 34.34±0.12 | | | 34.12±0.29 | |
| | | Assay 2 | 6970del | 37.14±0.12 | | | 37.19±0.14 | |
| | | | T547K | NT | | | NT | |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | 36.53±0.39 | | | 36.58±0.20 | |
| | | | P681H | 36.06±0.58 | | | 36.98±0.65 | |
| | | | T842I | 37.78±0.62 | | | 38.24±0.63 | |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | 35.32±0.29 | | | NT | |
| | | | P681R | NT | | | NT | |
| 250 | | Assay 1 | ORF | 36.22±0.19 | | | 36.38±0.54 | |
| | | | N | 35.61±0.18 | | | 35.11±0.64 | |
| | | Assay 2 | 6970del | 38.03±0.76 | | | 38.11±0.31 | |
| | | | T547K | NT | | | NT | |
| | | | R346K | NT | | | NT | |
| | | Assay 3 | L452R | 36.82±0.45 | | | 37.38±0.76 | |
| | | | P681H | 36.62±0.63 | | | 38.88±0.18 | |
| | | | T842I | 38.99±0.36 | | | NT | |
| | | Assay 4 | L452Q | NT | | | NT | |
| | | | KSF141-143 | 36.77±0.39 | | | NT | |
| | | | P681R | NT | | | NT | |
| 4000 | BA.1.1 | Assay 1 | ORF | 32.54±0.36 | 0.9836 | BA.2.12.1 | 32.26±0.20 | 0.9964 |
| | | | N | 31.53±0.18 | 0.9915 | | 31.39±0.15 | 0.9983 |
| | | Assay 2 | 6970del | 33.04±0.07 | 0.9896 | | NT | |
| | | | T547K | 33.42±0.12 | 0.9826 | | NT | |
| | | | R346K | 34.78±0.27 | 0.9699 | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| 0 | | | P681H | 34.63±0.09 | 0.9246 | | 33.86±0.11 | 0.9839 |
| | | | T842I | NT | | | 33.80±0.21 | 0.9855 |
| | | Assay 4 | L452Q | NT | | | 34.86±0.24 | 0.9141 |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 2000 | | Assay 1 | ORF | 33.39±0.21 | | | 33.32±0.75 | |
| | | | N | 32.32±0.13 | | | 32.58±0.11 | |
| | | Assay 2 | 6970del | 33.73±0.02 | | | NT | |
| | | | T547K | 34.08±0.20 | | | NT | |
| | | | R346K | 35.86±0.53 | | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| | | | P681H | 35.62±0.16 | | | 34.51±0.16 | |
| | | | T842I | NT | | | 34.85±0.20 | |
| | | Assay 4 | L452Q | NT | | | 35.66±0.36 | |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 1000 | | Assay 1 | ORF | 34.35±0.06 | | | 34.66±0.25 | |
| | | | N | 33.65±0.32 | | | 33.83±0.23 | |
| | | Assay 2 | 6970del | 34.89±0.40 | | | NT | |
| | | | T547K | 35.09±0.17 | | | NT | |
| | | | R346K | 37.07±0.22 | | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| | | | P681H | 36.29±0.33 | | | 35.55±0.41 | |
| | | | T842I | NT | | | 36.25±0.40 | |
| | | Assay 4 | L452Q | NT | | | 37.73±0.74 | |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 500 | | Assay 1 | ORF | 35.35±0.45 | | | 35.80±0.23 | |
| | | | N | 35.04±0.15 | | | 35.04±0.31 | |
| | | Assay 2 | 6970del | 35.55±0.39 | | | NT | |
| | | | T547K | 36.04±0.30 | | | NT | |
| | | | R346K | 37.40±0.51 | | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| | | | P681H | 37.51±0.36 | | | 36.60±0.39 | |
| | | | T842I | NT | | | 37.89±0.49 | |
| | | Assay 4 | L452Q | NT | | | 37.98±1.55 | |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 250 | | Assay 1 | ORF | 36.94±0.25 | | | 36.71±0.12 | |
| | | | N | 36.38±1.37 | | | 36.02±0.52 | |
| | | Assay 2 | 6970del | 36.81±0.31 | | | NT | |
| | | | T547K | 37.49±0.53 | | | NT | |
| | | | R346K | 39.12±0.62 | | | NT | |
| | | Assay 3 | L452R | NT | | | NT | |
| | | | P681H | 38.74±0.23 | | | 37.03±1.73 | |
| | | | T842I | NT | | | 38.52±0.27 | |
| | | Assay 4 | L452Q | NT | | | - | |
| | | | KSF141-143 | NT | | | NT | |
| | | | P681R | NT | | | NT | |
| 4000 | Delta | Assay 1 | ORF | 32.97±0.06 | 0.9753 | | | |
| | | | N | 31.61±0.11 | 0.9633 | | | |
| | | Assay 2 | 6970del | NT | | | | |
| | | | T547K | NT | | | | |
| | | | R346K | NT | | | | |
| | | Assay 3 | L452R | 34.51±0.12 | 0.9522 | | | |
| | | | P681H | NT | | | | |
| | | | T842I | NT | | | | |
| | | Assay 4 | L452Q | NT | | | | |
| | | | KSF141-143 | NT | | | | |
| | | | P681R | 33.95±0.20 | 0.9897 | | | |
| 2000 | | Assay 1 | ORF | 33.79±0.22 | | | | |
| | | | N | 32.77±0.28 | | | | |
| | | Assay 2 | 6970del | NT | | | | |
| | | | T547K | NT | | | | |
| | | | R346K | NT | | | | |
| | | Assay 3 | L452R | 35.57±0.37 | | | | |
| | | | P681H | NT | | | | |
| | | | T842I | NT | | | | |
| | | Assay 4 | L452Q | NT | | | | |
| | | | KSF141-143 | NT | | | | |
| | | | P681R | 34.99±0.18 | | | | |
| 1000 | | Assay 1 | ORF | 35.50±0.35 | | | | |
| | | | N | 34.12±0.30 | | | | |
| | | Assay 2 | 6970del | NT | | | | |
| | | | T547K | NT | | | | |
| | | | R346K | NT | | | | |
| | | Assay 3 | L452R | 36.37±0.59 | | | | |
| | | | P681H | NT | | | | |
| | | | T842I | NT | | | | |
| | | Assay 4 | L452Q | NT | | | | |
| | | | KSF141-143 | NT | | | | |
| | | | P681R | 36.91±1.40 | | | | |
| 500 | | Assay 1 | ORF | 36.22±0.27 | | | | |
| | | | N | 35.31±0.42 | | | | |
| | | Assay 2 | 6970del | NT | | | | |
| | | | T547K | NT | | | | |
| | | | R346K | NT | | | | |
| | | Assay 3 | L452R | 38.86±0.69 | | | | |
| | | | P681H | NT | | | | |
| | | | T842I | NT | | | | |
| | | Assay 4 | L452Q | NT | | | | |
| | | | KSF141-143 | NT | | | | |
| | | | P681R | 37.8±0.34 | | | | |
| 250 | | Assay 1 | ORF | 38.28±0.58 | | | | |
| | | | N | 36.45±0.47 | | | | |
| | | Assay 2 | 6970del | NT | | | | |
| | | | T547K | NT | | | | |
| | | | R346K | NT | | | | |
| | | Assay 3 | L452R | 39.25±0.24 | | | | |
| | | | P681H | NT | | | | |
| | | | T842I | NT | | | | |
| | | Assay 4 | L452Q | NT | | | | |
| | | | KSF141-143 | NT | | | | |
| | | | P681R | 39.26±0.36 | | | | |

**Table S4** Variation in CT values (ΔCT) of the wild type at 100,000, 10,000, and 5,000 copies/mL

| Gene | | 1 reaction | 2 reaction | 3 reaction | ΔCT average | | 1 reaction | 2 reaction | 3 reaction | | 1 reaction | 2 reaction | 3 reaction |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ORF | | 27.04 | 27.1 | 27.08 | | | 30.52 | 30.4 | 30.36 | | 31.63 | 31.54 | 31.4 |
| N | | 28.03 | 27.88 | 28 | | | 31.43 | 31.93 | 31.13 | | 32.92 | 32.66 | 32.78 |
| 6970del | | NT | NT | NT | | | NT | NT | NT | | NT | NT | NT |
| T547K | | NT | NT | NT | | | NT | NT | NT | | NT | NT | NT |
| R346K | | NT | NT | 35.22 ΔCT=8.14 | 8.14 | | NT | NT | NT | | NT | NT | NT |
| L452R | 100000 copes/mL | 37.5 ΔCT=9.7 | 37.84 ΔCT=10.74 | 37.77 ΔCT=10.69 | 10.37 | 10000 copes/mL | NT | NT | NT | 5000 copes/mL | NT | NT | NT |
| P681H | | NT | NT | NT | | | NT | NT | NT | | NT | NT | NT |
| T842I | | NT | NT | NT | | | NT | NT | NT | | NT | NT | NT |
| L452Q | | NT | NT | NT | | | NT | NT | NT | | NT | NT | NT |
| KSF141-143 | | NT | NT | NT | | | NT | NT | NT | | NT | NT | NT |
| P681R | | NT | NT | NT | | | NT | NT | NT | | NT | NT | NT |

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**Table S5** The variation of CT values (ΔCT) determined for BA.1, BA.2, BA.5, Delta variants by 3 clinical samples

| BA.1 | Sample 1 | | Sample 2 | | Sample 3 | | ΔCT average |
|---|---|---|---|---|---|---|---|
| Gene | CT Value | ΔCT | CT Value | ΔCT | CT Value | ΔCT | |
| ORF | 27.72 | \ | 24.51 | \ | 28.24 | \ | \ |
| 6970del | 28.12 | 0.4 | 24.55 | 0.04 | 28.89 | 0.65 | 0.36 |
| T547K | 28.5 | 0.78 | 25.58 | 1.07 | 28.23 | -0.01 | 0.61 |
| R346K | NT | NT | NT | NT | NT | NT | \ |
| L452R | 35.92 | 8.2 | 33.15 | 8.64 | 36.89 | 8.65 | 8.5 |
| P681H | 27.83 | 0.11 | 26.14 | 1.63 | 29.29 | 1.05 | 0.93 |
| T842I | 37.82 | 10.1 | 34.25 | 8.99 | 36.91 | 8.67 | 9.25 |
| L452Q | NT | NT | NT | NT | NT | NT | \ |
| KSF141-143 | NT | NT | NT | NT | NT | NT | \ |
| P681R | 36.61 | 8.89 | 35.73 | 11.22 | 37.12 | 8.88 | 9.66 |
| | | | | | | | |
| BA.2 | Sample 1 | | Sample 2 | | Sample 3 | | |
| Gene | CT Value | ΔCT | CT Value | ΔCT | CT Value | ΔCT | ΔCT average |
| ORF | 26.02 | \ | 22.8 | \ | 27.16 | \ | \ |
| 6970del | NT | NT | NT | NT | NT | NT | \ |
| T547K | 35.11 | 9.09 | 34.64 | 11.84 | 38.27 | 11.11 | 10.68 |
| R346K | NT | NT | NT | NT | NT | NT | \ |
| L452R | 34.66 | 8.64 | 31.2 | 8.4 | 36.76 | 9.51 | 8.85 |
| P681H | 26.03 | 0.01 | 23.01 | 0.21 | 28.27 | 1.11 | 0.44 |
| T842I | 26.59 | 0.57 | 23.83 | 1.03 | 28.77 | 1.61 | 1.07 |
| L452Q | NT | NT | NT | NT | NT | NT | \ |
| KSF141-143 | NT | NT | NT | NT | NT | NT | \ |
| P681R | 34.66 | 8.64 | 35.15 | 12.35 | 37.64 | 10.48 | 10.49 |
| | | | | | | | |
| BA.5 | Sample 1 | | Sample 2 | | Sample 3 | | |
| Gene | CT Value | ΔCT | CT Value | ΔCT | CT Value | ΔCT | ΔCT average |
| ORF | 21.96 | \ | 33.13 | \ | 28.68 | \ | \ |
| 6970del | 23.59 | 1.63 | 35.17 | 2.04 | 30.64 | 1.96 | 1.87 |
| T547K | 32.19 | 10.23 | NT | NT | NT | NT | 10.23 |
| R346K | NT | NT | NT | NT | NT | NT | \ |
| L452R | 22.20 | 0.24 | 34.06 | 0.93 | 31.45 | 2.77 | 1.31 |
| P681H | 21.86 | -0.1 | 34.23 | 1.1 | 29.16 | 0.48 | 0.49 |
| T842I | 22.61 | 0.65 | 34.53 | 1.4 | 30.66 | 1.98 | 1.34 |
| L452Q | NT | NT | NT | NT | NT | NT | \ |
| KSF141-143 | NT | NT | NT | NT | NT | NT | \ |
| P681R | 34.69 | 12.73 | NT | NT | NT | NT | \ |
| | | | | | | | |
| Delta | Sample 1 | | Sample 2 | | Sample 3 | | |
| Gene | CT Value | ΔCT | CT Value | ΔCT | CT Value | ΔCT | ΔCT average |
| ORF | 25.91 | \ | 23.27 | \ | 31.33 | \ | \ |
| 6970del | NT | NT | NT | NT | NT | NT | \ |
| T547K | 35.63 | 9.72 | 37.28 | 14.01 | NO CT | NO CT | 11.86 |
| R346K | NT | NT | NT | NT | NT | NT | \ |
| L452R | 26.3 | 0.39 | 23.53 | 0.26 | 34.42 | 3.09 | 1.25 |
| P681H | 35.53 | 9.62 | 35.5 | 12.23 | NT | NT | 10.93 |
| T842I | 34.83 | 8.92 | 34.04 | 10.77 | NT | NT | 9.85 |
| L452Q | NT | NT | NT | NT | NT | NT | \ |
| KSF141-143 | NT | NT | NT | NT | NT | NT | \ |
| P681R | 26.76 | 0.85 | 24.51 | 1.24 | 31.45 | 0.12 | 0.74 |

ΔCT average: average value of ΔCT of three specimens. The positive result is determined by that the mutation of BA.1, BA.2 and BA.5 and Delta were all < 38 and the ΔCT of the corresponding mutation sites were all <8.

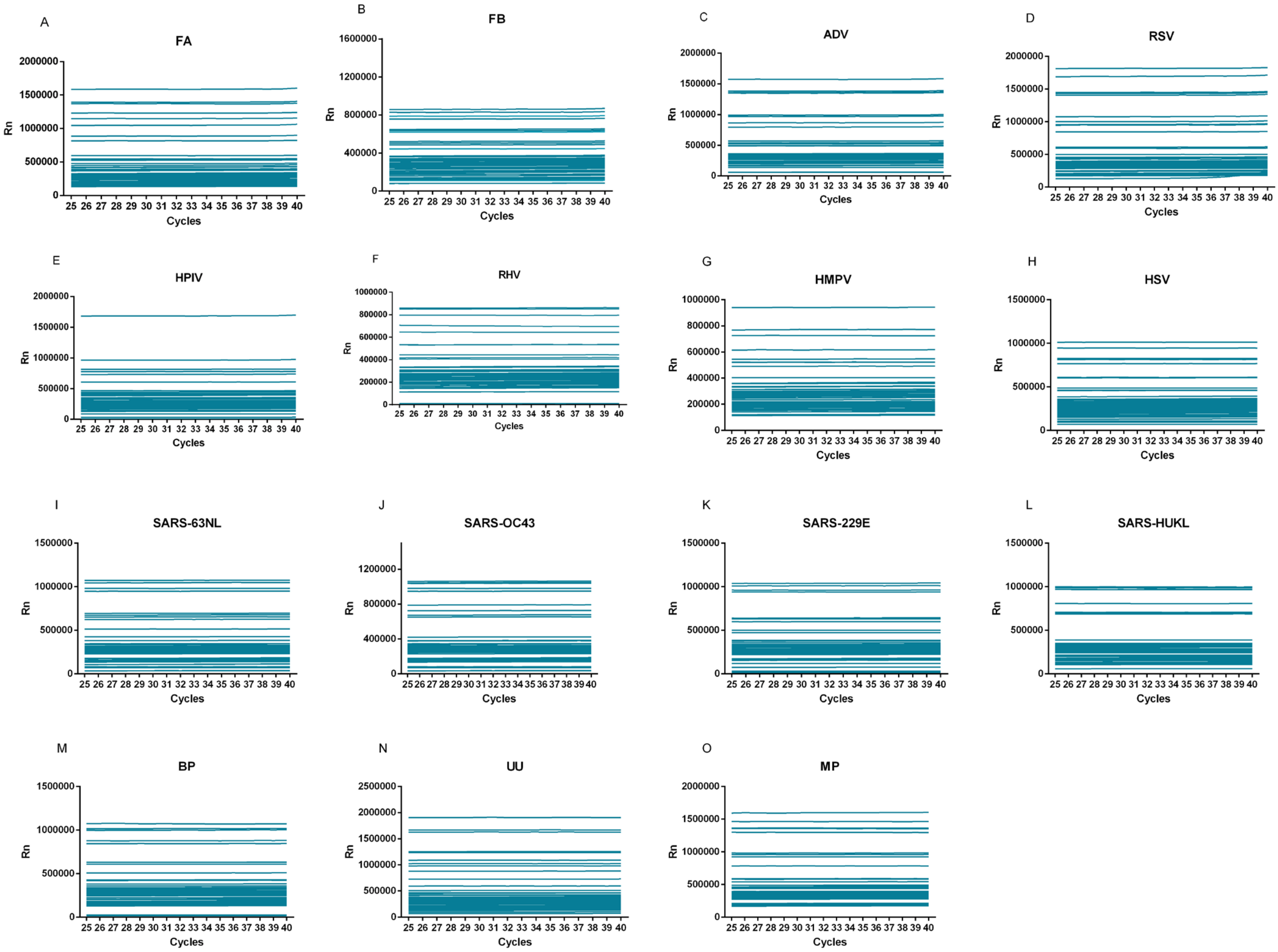

**Figure S2** Amplification curve of cross reaction. Amplification curve of cross reaction of Nucleic acids from other pathogens. (A) Influenza A virus (FA); (B) Influenza B virus (FB). (C) Adenovirus (ADV); (D) Respiratory syncytial virus (RSV); (E) Human parainfluenza virus (HPIV);(F) rhinovirus (RV); (G)human metapneumovirus (HMPV); (H) herpes simplex virus type (HSV); (I) SARS-HKU1; (J) SARS-NL63; (K) SARS-OC43; (L) SARS-229E; (M) Bacillus pertussis (BP); (N) Ureaplasma Urealyticum (UU); (O) Mycoplasma pneumoniae (MP). The pathogens were used as templates to check for cross-reactivity of the developed assay with common respiratory pathogens. The amplification curves for each mutation site (ORF, N, Spike: 6970del, L452R, P681H, T547K, P681R, L452Q, and R346K; NSP: KSF-141-143; NSP3: T842I) are shown. "Rn" : Normalized reporter, is the ratio of the fluorescence emission intensity of the fluorescence reporter group to the fluorescence emission intensity of the reference dye. The exponential and linear phases of the amplification curve can be observed.

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