**SUPPLEMENTARY MATERIAL**

**Table SM1.** Preparation of PCR mixture\*.

|  |  |
| --- | --- |
| Reagent | Volume/reaction |
| 2019-nCoV reaction solutionRTase MixROX\*\*Internal control A (amplification directly) | N x 14 µLN x 6 µLN x 0.5 µLN x 0.5 µL |
| Total volume/well | 20.5 µL (IC from NA extraction step)21 µL (IC from amplification step) |

 \*PCR reaction mixture can be stored below 8°C for 3 hours.

\*\*ROX is used as a reference dye.

**Table SM2.** Cycle condition setting.

|  |  |  |  |
| --- | --- | --- | --- |
| Reaction | Temperature (°C) | Time | Cycle |
| Reverse transcription | 50 | 15 minutes | 1 |
| Initial denaturation | 95 | 3 minutes | 1 |
| Pre-amplification | 95 | 5 seconds | 5 |
| 60 | 40 seconds |
| Amplification | 95 | 5 seconds | 40 |
| 60 | 40 seconds |
| Collect the signals (FAM/JOE/Cy5) |



**Fig. SM1.** Amplification plot of E gene from COVID-19-negative samples.



**Fig. SM2.** Amplification plot of E gene from COVID-19-positive samples.



**Fig. SM3.** Amplification plot of ORF1ab from COVID-19-negative samples.



**Fig. SM4.** Amplification plot of ORF1ab from COVID-19-positive samples.

**Table SM3.** Pre-scan for COVID-19-negative samples, and COVID-19-positive samples.

|  |  |  |
| --- | --- | --- |
| Sample code | Sample group | Wavelengths (nm) |
| Excitation peak | Average | Emission peak | Average |
| N1N2N3N4N5N6N7N8N9N10N11N12N13N14N15N16N17N18N19N20 | Negative | 343±0.89342±0.49342±0.75343±0.98342±1.36342±1.20343±0.00342±0.49343±0.40343±0.49343±0.98343±0.49343±1.36343±0.75342±0.75343±1.36343±0.49342±0.49343±0.63343±0.80 | 342.71±0.91 | 350±0.89349±1.62349±0.40349±0.98350±1.36351±0.49349±1.36349±1.36349±0.75349±0.49349±0.49350±0.49349±0.80350±0.49349±0.49349±0.80350±0.40350±1.49349±0.49349±1.01 | 349.57±0.98 |
| P1P2P3P4P5P6P7P8P9P10P11P12P13P14P15P16P17P18P19P20 | Positive | 351±0.40337±4.53350±0.40348±0.80339±3.14339±5.15362±0.00343±1.17338±1.47341±1.33338±5.43362±5.60342±1.47341±1.60342±1.55332±1.36339±4.38340±5.18343±1.20342±3.25 | 343.41±8.21 | 359±0.40340±10.35359±0.40348±0.80346±3.16363±5.02369±0.40350±1.26345±1.02366±1.41362±5.43365±1.20366±1.55365±1.26366±4.03357±1.17360±4.00362±4.83367±1.20364±2.80 | 358.82±9.04 |

**Table SM4.** Emission wavelengths for COVID-19-negative samples and COVID-19-positive samples.

|  |  |  |
| --- | --- | --- |
| Sample code | Sample group | Wavelengths (nm) |
| First emission peak | Average | Second emission peak | Average |
| N1N2N3N4N5N6N7N8N9N10N11N12N13N14N15N16N17N18N19N20 | Negative | 517±0.40503±0.40512±0.40507±0.49518±0.80516±0.80505±0.40502±0.40502±0.49503±0.49517±0.40505±0.40508±0.40518±0.49506±0.49506±0.49510±0.40504±0.40503±0.49503±0.49 | 508.37±5.68 | 685±0.49685±0.49685±0.49685±0.49685±0.00685±0.00685±0.49685±0.49685±0.49685±0.40685±0.40685±0.49685±0.49685±0.00685±0.00685±0.00685±0.49685±0.49685±0.40685±0.49 | 685.27±0.44 |
| P1P2P3P4P5P6P7P8P9P10P11P12P13P14P15P16P17P18P19P20 | Positive | 502±0.40505±0.40496±0.40502±0.40524±0.40531±0.40516±0.40520±0.40518±0.40532±0.40526±0.40532±0.40533±0.40527±0.40531±0.40524±0.40530±0.40533±0.40534±0.49532±0.40 | 522.47±11.78 | 700±0.40679±0.40699±0.40691±0.40678±0.40694±0.40722±0.40681±0.40674±0.40692±0.40695±0.40700±0.40695±0.40696±0.40697±0.40674±0.40686±0.00692±0.40696±0.40696±0.40 | 692.18±10.89 |

**Table SM5.** ANOVA testing for the first emission data.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Single Factor |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| SUMMARY |  |  |  |  |  |  |
| *Groups* | *Counta* | *Sumb* | *Averagec* | *Varianced* |  |  |
| Negative | 100 | 50837 | 508.37 | 32.63949 |  |  |
| Positive | 100 | 52247 | 522.47 | 140.191 |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SSe* | *dff* | *MSg* | *Fh* | *P-valuei* | *F critj* |
| Between Groups | 9940.5 | 1 | 9940.5 | 115.0318 | 1.88E-21 | 3.888853 |
| Within Groups | 17110.22 | 198 | 86.41525 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 27050.72 | 199 |   |   |   |   |

a*Count* = amount of data

b*Sum* = total sum of data

c*Average* = means of data

d*Variance* = the expectation of the squared deviation of a random variable from its mean

e*SS* = sum of squares

f*df* = degrees of freedom

g*MS* = mean square

h*F* = ratio of variation between sample means toward to variation within the samples

i*P-value* = the probability of obtaining results

j*F-crit* = a specific value compared to F value

**Table SM6.** LSD testing for the first emission data.

|  |  |  |
| --- | --- | --- |
| $$Ӯ=|Ӯ\_{i}-Ӯ\_{j}|$$ | LSD value | Conclusion |
| $$|Ӯ\_{1}-Ӯ\_{2}|$$ | 14.1 | 2.59251 | Ӯ > LSD |

Note:

$Ӯ$ **=** absolute mean difference

$Ӯ\_{i}$ = mean of variety i

$Ӯ\_{j}$ = mean of variety j

**Table SM7.** ANOVA testing for the second emission data.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Single Factor |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| SUMMARY |  |  |  |  |  |  |
| *Groups* | *Counta* | *Sumb* | *Averagec* | *Varianced* |  |  |
| Negative | 100 | 68527 | 685.27 | 0.199091 |  |  |
| Positive | 100 | 69218 | 692.18 | 119.9673 |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SSe* | *Dff* | *MSg* | *Fh* | *P-valuei* | *F critj* |
| Between Groups | 2387.405 | 1 | 2387.405 | 39.735 | 1.85E-09 | 3.888853 |
| Within Groups | 11896.47 | 198 | 60.08318 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 14283.88 | 199 |   |   |   |   |

a*Count* = amount of data

b*Sum* = total sum of data

c*Average* = means of data

d*Variance* = the expectation of the squared deviation of a random variable from its mean

e*SS* = sum of squares

f*df* = degrees of freedom

g*MS* = mean square

h*F* = ratio of variation between sample means toward to variation within the samples

i*P-value* = the probability of obtaining results

j*F-crit* = a specific value compared to F value

**Table SM8.** LSD testing for the second emission data.

|  |  |  |
| --- | --- | --- |
| $$Ӯ=|Ӯ\_{i}-Ӯ\_{j}|$$ | LSD value | Conclusion |
| $$|Ӯ\_{1}-Ӯ\_{2}|$$ | 6.91 | 2.16173 | Ӯ > LSD |

Note:

$Ӯ$ **=** absolute mean difference

$Ӯ\_{i}$ = mean of variety i

$Ӯ\_{j}$ = mean of variety j

**Table SM9.** RT-qPCR data for COVID-19-positive samples with various Ct value.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| No. | Ct value | Conclusion | Sample code for fluorescence analysis | Group of Ct value |
| E gene | Number of copies | ORF1ab | Number of copies |
| 1. | 22.82 | 6.87x104 | 22.58 | 1.01x105 | Positive | P21 | <25 |
| 2. | 23.17 | 5.41x104 | 23.02 | 6.97x104 | Positive | P22 |
| 3. | 24.69 | 1.74x104 | 23.03 | 1.56x104 | Positive | P23 |
| 4. | 26.91 | 4.23x103 | 26.29 | 4.48x103 | Positive | P24 | <30 |
| 5. | 28.48 | 1.44x103 | 27.76 | 1.31x103 | Positive | P25 |
| 6. | 27.46 | 2.89x103 | 26.58 | 3.53x103 | Positive | P26 |
| 7. | 32.71 | 80.20 | 31.18 | 74.30 | Positive | P27 | <35 |
| 8. | 33.65 | 42.20 | 31.36 | 63.90 | Positive | P28 |
| 9. | 31.91 | 1.38x102 | 30.66 | 1.15x102 | Positive | P29 |

**Table SM10.** Pre-scan for COVID-19-positive samples with various Ct value.

|  |  |  |
| --- | --- | --- |
| Sample code | Ct value | Wavelengths (nm) |
| Excitation peak | Average | Emission peak | Average |
| P21 | <25 | 352±0.40 | 353.53±10.51 | 360±0.40 | 358.60±14.80 |
| P22 | 341±0.80 | 341±7.86 |
| P23 | 367±0.00 | 375±0.00 |
| P24 | <30 | 354±3.56 | 353.13±8.61 | 362±3.43 | 360.60±9.16 |
| P25 | 363±0.49 | 371±0.49 |
| P26 | 342±1.74 | 349±1.55 |
| P27 | <35 | 366±2.06 | 357.60±10.76 | 374±1.62 | 364.00±13.87 |
| P28 | 343±1.36 | 345±7.28 |
| P29 | 364±0.75 | 372±0.40 |

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**Fig. SM5.** Excitation (A) and emission (B) peaks of COVID-19-positive samples with code P21-P29.



**Fig. SM6.** Principal component analysis for COVID-19-positive samples with various Ct value.