**SUPPLEMENTARY MATERIAL**

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

|  |  |
| --- | --- |
| Reagent | Volume/reaction |
| 2019-nCoV reaction solution  RTase Mix  ROX\*\*  Internal control A (amplification directly) | N x 14 µL  N x 6 µL  N x 0.5 µL  N 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 |
| N1  N2  N3  N4  N5  N6  N7  N8  N9  N10  N11  N12  N13  N14  N15  N16  N17  N18  N19  N20 | Negative | 343±0.89  342±0.49  342±0.75  343±0.98  342±1.36  342±1.20  343±0.00  342±0.49  343±0.40  343±0.49  343±0.98  343±0.49  343±1.36  343±0.75  342±0.75  343±1.36  343±0.49  342±0.49  343±0.63  343±0.80 | 342.71±0.91 | 350±0.89  349±1.62  349±0.40  349±0.98  350±1.36  351±0.49  349±1.36  349±1.36  349±0.75  349±0.49  349±0.49  350±0.49  349±0.80  350±0.49  349±0.49  349±0.80  350±0.40  350±1.49  349±0.49  349±1.01 | 349.57±0.98 |
| P1  P2  P3  P4  P5  P6  P7  P8  P9  P10  P11  P12  P13  P14  P15  P16  P17  P18  P19  P20 | Positive | 351±0.40  337±4.53  350±0.40  348±0.80  339±3.14  339±5.15  362±0.00  343±1.17  338±1.47  341±1.33  338±5.43  362±5.60  342±1.47  341±1.60  342±1.55  332±1.36  339±4.38  340±5.18  343±1.20  342±3.25 | 343.41±8.21 | 359±0.40  340±10.35  359±0.40  348±0.80  346±3.16  363±5.02  369±0.40  350±1.26  345±1.02  366±1.41  362±5.43  365±1.20  366±1.55  365±1.26  366±4.03  357±1.17  360±4.00  362±4.83  367±1.20  364±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 |
| N1  N2  N3  N4  N5  N6  N7  N8  N9  N10  N11  N12  N13  N14  N15  N16  N17  N18  N19  N20 | Negative | 517±0.40  503±0.40  512±0.40  507±0.49  518±0.80  516±0.80  505±0.40  502±0.40  502±0.49  503±0.49  517±0.40  505±0.40  508±0.40  518±0.49  506±0.49  506±0.49  510±0.40  504±0.40  503±0.49  503±0.49 | 508.37±5.68 | 685±0.49  685±0.49  685±0.49  685±0.49  685±0.00  685±0.00  685±0.49  685±0.49  685±0.49  685±0.40  685±0.40  685±0.49  685±0.49  685±0.00  685±0.00  685±0.00  685±0.49  685±0.49  685±0.40  685±0.49 | 685.27±0.44 |
| P1  P2  P3  P4  P5  P6  P7  P8  P9  P10  P11  P12  P13  P14  P15  P16  P17  P18  P19  P20 | Positive | 502±0.40  505±0.40  496±0.40  502±0.40  524±0.40  531±0.40  516±0.40  520±0.40  518±0.40  532±0.40  526±0.40  532±0.40  533±0.40  527±0.40  531±0.40  524±0.40  530±0.40  533±0.40  534±0.49  532±0.40 | 522.47±11.78 | 700±0.40  679±0.40  699±0.40  691±0.40  678±0.40  694±0.40  722±0.40  681±0.40  674±0.40  692±0.40  695±0.40  700±0.40  695±0.40  696±0.40  697±0.40  674±0.40  686±0.00  692±0.40  696±0.40  696±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.

|  |  |  |  |
| --- | --- | --- | --- |
|  | | LSD value | Conclusion |
|  | 14.1 | 2.59251 | Ӯ > LSD |

Note:

**=** absolute mean difference

= mean of variety i

= 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.

|  |  |  |  |
| --- | --- | --- | --- |
|  | | LSD value | Conclusion |
|  | 6.91 | 2.16173 | Ӯ > LSD |

Note:

**=** absolute mean difference

= mean of variety i

= 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 |

****

**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.