# Supplemental Material

## Establishment of one-step duplex TaqMan real-time PCR for detection of feline coronavirus and panleukopenia virus

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**Table S1 Synthesized nucleotide sequences based on base mutations in the binding sites of FCoV strain specific primers**

| GenBank | Nucleotide |
|---|---|
| FJ938054.1 | cgtccctgtttggtaagtcgtctagtattagctgcggtggttccgcccgtcgtagttgggtagaccgggttccgt<br>cctgtgatctcccctcgccggccgccaggagaatgagttccaaacaatttaagatcctcgttaatgaggactatc<br>aagtcaacgtgcctagccttctttcc |
| HQ392471.1 | cgtccctgtttggtaagtcgtctagtattagcttcggcggttycgcccgtcgcagttgggtagaccgggttccgt<br>cctgtgatctccctcgccggccgccaggagaatgagttccaaacattttaagatcctcgttaatgaggactatc<br>aagtcaacgtgcctagccttttctttcc |
| FJ938056.1 | cgccccctgtttggtaagtcgtctagtattagctgcggcggttccgcccgtcgtagttgggtagaccgggttccg<br>tcctgtgatctccctcgccggccgccaggagaatgagttccaaacaatttaagatcctcgttaatgaggactac<br>caagtcaacgtgtctagccttctttcc |
| FJ938058.1 | cgccccctgtttggtaagtcgtctagtattagctgcggcggttccgcccgtcgtagttgggtagaccgggttccg<br>tcctgtgatctccctcgccggccgccaggagaatgagttccaaacaatttaagatcctcgttaatgaggactac<br>caaatcaacgtgtctagcttcctttcc |
| MN165107.1 | ccgccccctgtttggtaagtcgtctagtattagctgcggcggttccgcccgtcgtagttgggtagaccgggttcc<br>gtcctgtgatctccctcgccggccgccaggagaatgagttccaaacaatttaagatcctcgttaatgaggacta<br>tcaagtcaacgtgcctagccttcctttcc |
| FJ938051.1 | ccgtccctgtttggtaagtcgcctagtattagctgcggcggttccgcccgtcgtagttgggtagaccgggttcc<br>gtcctgtgatctccctcgccggccgccaggagaatgagttccaaacaatttaagatcctcgttaatgaggacta<br>ccaagtcaacgtgcctagccttcctttcc |
| HQ012367.1 | tcgtccttgtttggtaagtcgtctagtattagctgcggcggttctgcccgtcgtagttgggtagaccgggttccgt<br>cctgtgatctccctcgccggccgccaggagaatgagttccaaacaatttaagatcctcgttaatgaggactatc<br>aagtcaacgtgcctagcctacctttcc |
| HQ392470.1 | ccgtccttgtttggtaagtcgtctagtattagctgcggcggttccgcccgtcgtagttgggtagaccgggttccg<br>tcctgtgatctccctcgccggccgccaggagaatgagttccaaacaatttaagatcctcgttaatgaggactat<br>caagtcaacgtgcctagccttcctttcc |
| HQ012370.1 | ccgtccctgtttggtaagtcgtctagtattagctgcggcggttccgcccgtcgtagttgggtagaccgggttcc<br>gtcctgtgatctccctcgccggccgccaggagaatgagttccaaacaatttaagatcctcgttaatgaggacta<br>ccaagtcaacgtgcctagtcttcctttcc |
| DQ010921.1 | ccgtccctgtttggtaagtcgtctagtattagctgcggcggttccgcccgtcgtagttgggtagaccgggttcc<br>gtcctgtgatctccctcgccggccgccaggagaatgagttccaaacaatttaagatcctcgttaatgaggacta<br>ccaagtcaacgttcctagccttcctttcc |
| FJ938052.1 | ccgtccctgtttggtaagtcgtctagtattagctgcggcggttccgcccgtcgtagttgggtagaccgggttcc<br>gtcctgtgatctccctcgccggccgccaggagaatgagttccaaacaatttaagatcctcgttaatgaggacta<br>ccaagtcaacgtgcctagccgtccttcc |
| FJ938061.1 | ccgtccctgtttggtaagtcgtctagtattagctgcggcggttccgcccgtcgtagttgggtagaccgggttcc<br>gtcctgtgatctccctcgccggccgccacgagaatgagttccaaacaatttaagatcctcgttaatgaggacta<br>ccaagtcaacgtgcctagccaccctttcc |

Note: The nucleotides marked in red in the table indicate the positions of targeted mutations in the synthesized nucleotide sequences that correspond to the specific primers used in the cat coronavirus detection method established in this experiment. The underlines mark the targeting positions for the upstream primer, probe, and downstream primer, respectively.

**Table S2 The quantitative qPCR data of Fig. 2**

| Run order | Annealing temperature (℃) | Primer concentration (μM) | Probe concentration (μM) | Ct values |
|---|---|---|---|---|
| 1 | 62 | 0.1 | 0.25 | 20.2 |
| 2 | 59.5 | 0.3 | 0.25 | 19.1 |
| 3 | 62 | 0.3 | 0.1 | 19.2 |
| 4 | 62 | 0.5 | 0.25 | 19.4 |
| 5 | 59.5 | 0.5 | 0.4 | 19.3 |
| 6 | 59.5 | 0.3 | 0.25 | 19.1 |
| 7 | 59.5 | 0.3 | 0.25 | 19.1 |
| 8 | 57 | 0.5 | 0.25 | 19.6 |
| 9 | 59.5 | 0.1 | 0.4 | 19.3 |
| 10 | 59.5 | 0.5 | 0.1 | 19.8 |
| 11 | 59.5 | 0.1 | 0.1 | 19.4 |
| 12 | 57 | 0.3 | 0.4 | 19.8 |
| 13 | 57 | 0.1 | 0.25 | 20.8 |
| 14 | 59.5 | 0.3 | 0.25 | 19.1 |
| 15 | 59.5 | 0.3 | 0.25 | 19.1 |
| 16 | 57 | 0.3 | 0.1 | 19.9 |
| 17 | 62 | 0.3 | 0.4 | 20.4 |

**Table S3 The qPCR reaction system**

| Reagent | Dose | Final concentration |
|---|---|---|
| One Step Prime Script III RT-qPCR，with UNG (2 X) | 10 μl | 1 X |
| FCoV-F1（10 μM） | 0.6 μl | 0.3 μM |
| FCoV-R1（10 μM） | 0.6 μl | 0.3 μM |
| FCoV-Ra（10 μM） | 0.6 μl | 0.3 μM |
| FCoV-P1（10 μM） | 0.4 μl | 0.2 μM |
| FPLV-F3（10 μM） | 0.4 μl | 0.2 μM |
| FPLV-R3（10 μM） | 0.4 μl | 0.2 μM |
| FPLV-P3（10 μM） | 0.6 μl | 0.3 μM |
| Nucleic acid | 2 μl | |
| ROX Reference Dye II (50 X) | 0.4 μl | |

| | |
|---|---|
| $ddH_2O$ | 4 μl |
| Total | 20 μl |

**Table S4 Reaction conditions of the qPCR**

| Steps | Temperature | Time | Number of cycles |
|---|---|---|---|
| Reverse transcription reaction | 25 ℃ | 10 min | 1 |
| | 52 ℃ | 5 min | 1 |
| | 95 ℃ | 10 s | 1 |
| PCR reaction | 95 ℃ | 5 s | 40 |
| | 60 ℃ | 30 s | |