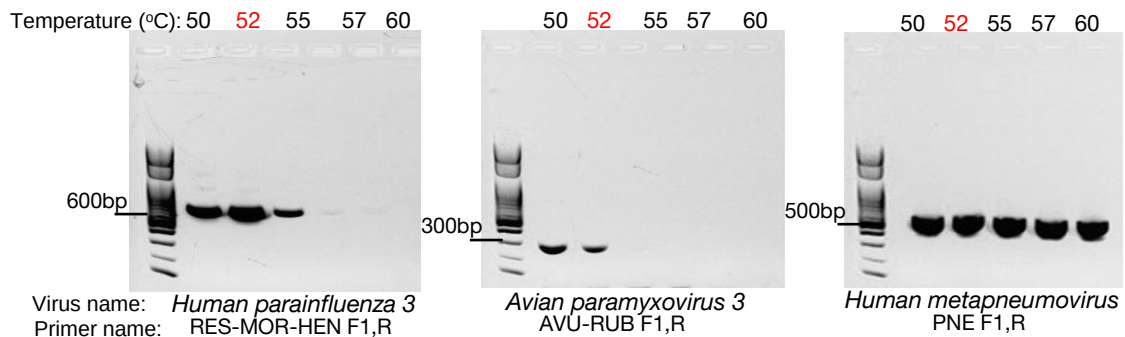
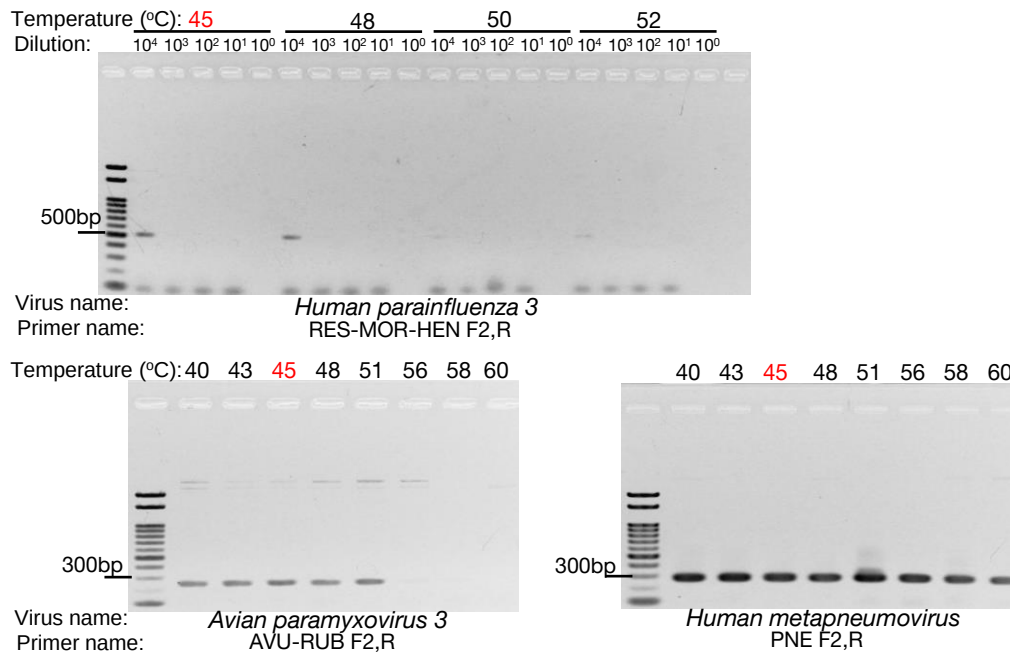


a. Optimization of annealing temperature from outer PCR

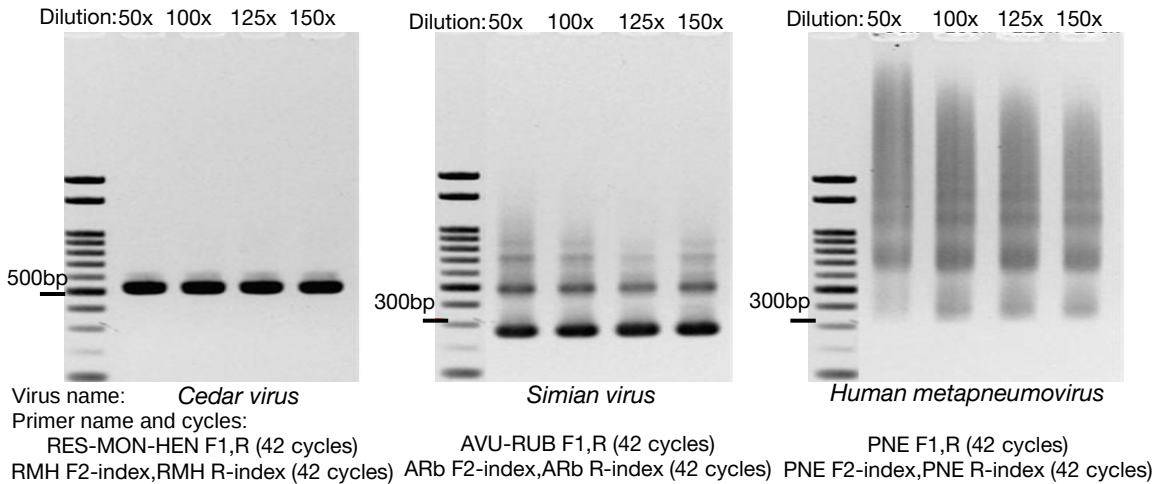


b. Optimization of annealing temperature from inner PCR

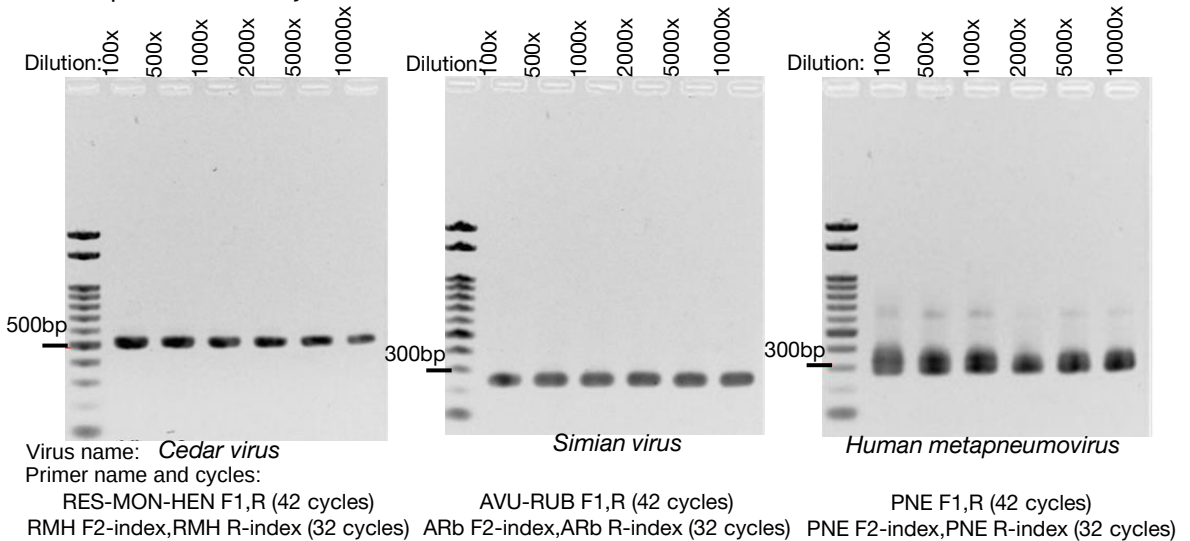


Supplementary Fig. S1. Optimization of annealing temperature from pan-paramyxovirus primers by semi-nested PCR. The electrophoresis with 1.5% agarose gel concentration were used to evaluated optimization results. In the outer PCR reaction, DNA recombinants from each genus group from paramyxoviruses were utilized as templates. The optimization was achieved in various steps: (a) Optimization of annealing temperature from outer PCR. The optimal annealing temperature was established by gradient PCR from 50 °C to 60 °C and plasmids with 10^8 cp/reaction were used for the PCR template. To facilitate all primer groups, 52 °C was chosen as the optimal annealing temperature for outer primers. (b) Optimization of annealing temperature from inner PCR. To set up the optimum annealing temperature for limited viral copy number, serial dilution of plasmid and gradient annealing temperature were used in the PCR reactions of RMH-F2-index and RMH-R2-index. The limit detection of those PCR reactions was 10^4 copy/reaction. Subsequently, the 10^4 copies/reaction were applied in other primer groups. The optimum annealing temperature for inner PCR was established at 45°C for all primer groups.

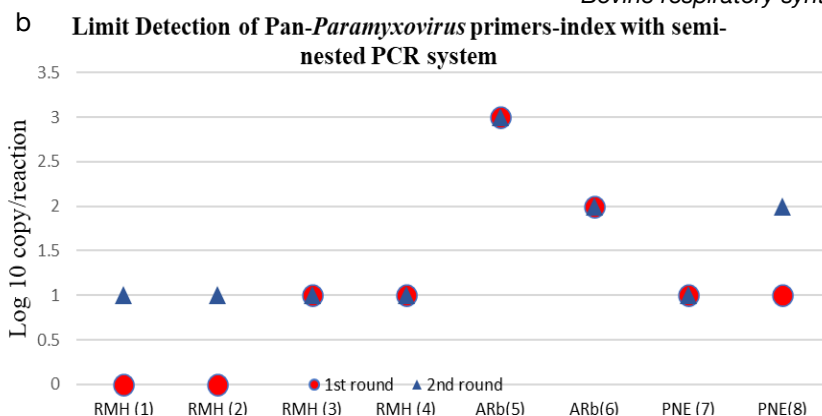
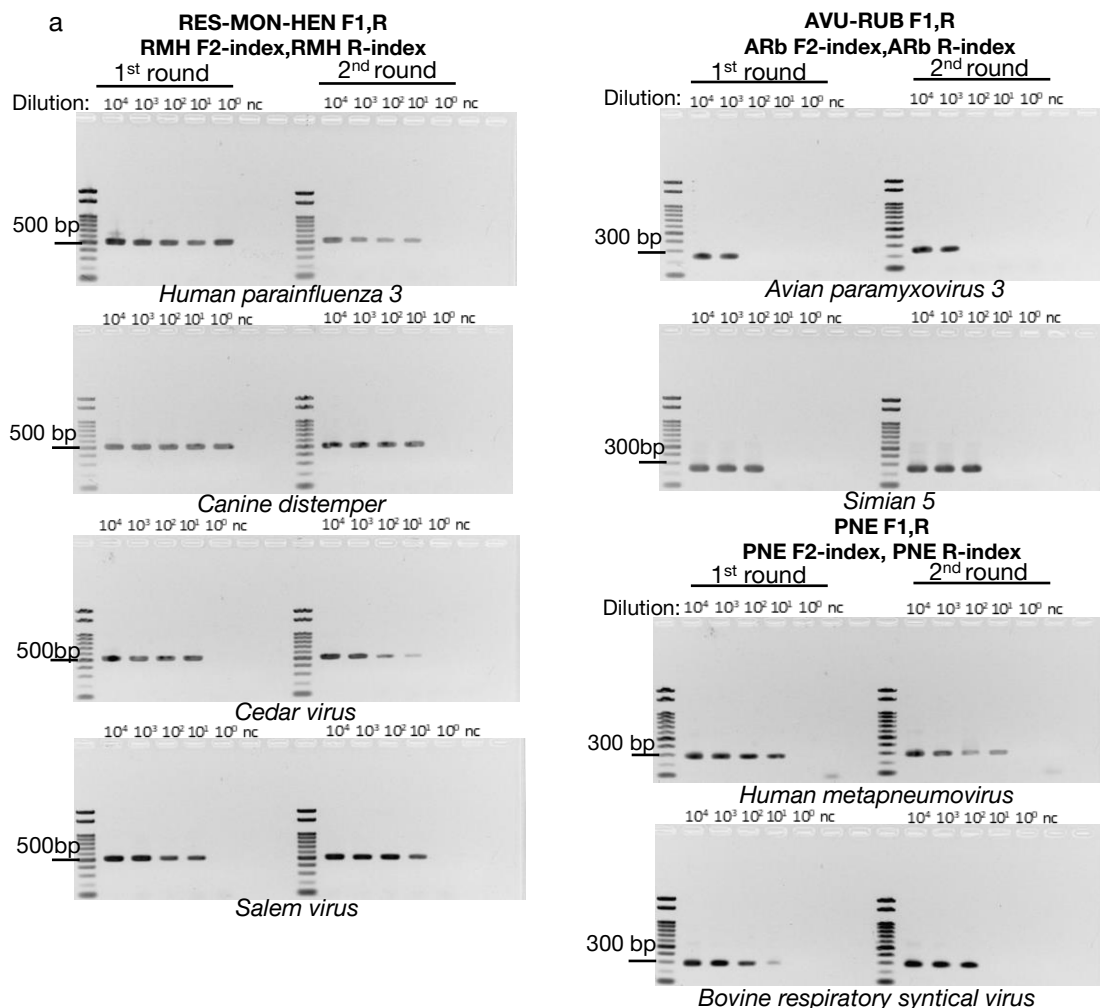
a. Optimization of dilution template for inner PCR



b. Optimization of cycles from outer and inner PCR in combination



Supplementary Fig. S2. Optimization of template dilution and cycles from pan-paramyxovirus primers by semi-nested PCR. The electrophoresis with 1.5% agarose gel concentration were used to evaluated optimization results. In the outer PCR reaction, DNA recombinants from each genus group from paramyxoviruses were utilized as templates. The optimization was achieved in various steps: **(a)** Optimization of dilution template for inner PCR. The amplicons of outer PCR from each primer group were diluted from 50 to 150 times and used for inner PCR's template. The amplification step was done in 42 cycles. There were no significant results between different dilutions of templates. However, unspecific band were occurred in ARb and PNE amplicons, suggested to reduced the cycles. **(b)** Optimization of cycles from outer and inner PCR in combination. To perform the clear band size, the outer primers were applied in 42 cycles, then diluted more up to 10^4 times. The cycles of inner PCR were reduced to 32 for all primer groups. Finally, to establish the inner PCR, we selected 100 times of the template's dilutions with 32 cycles. Additionally, PNE cycles was suggested to be shortened to 15 cycles.



Primers and templates:

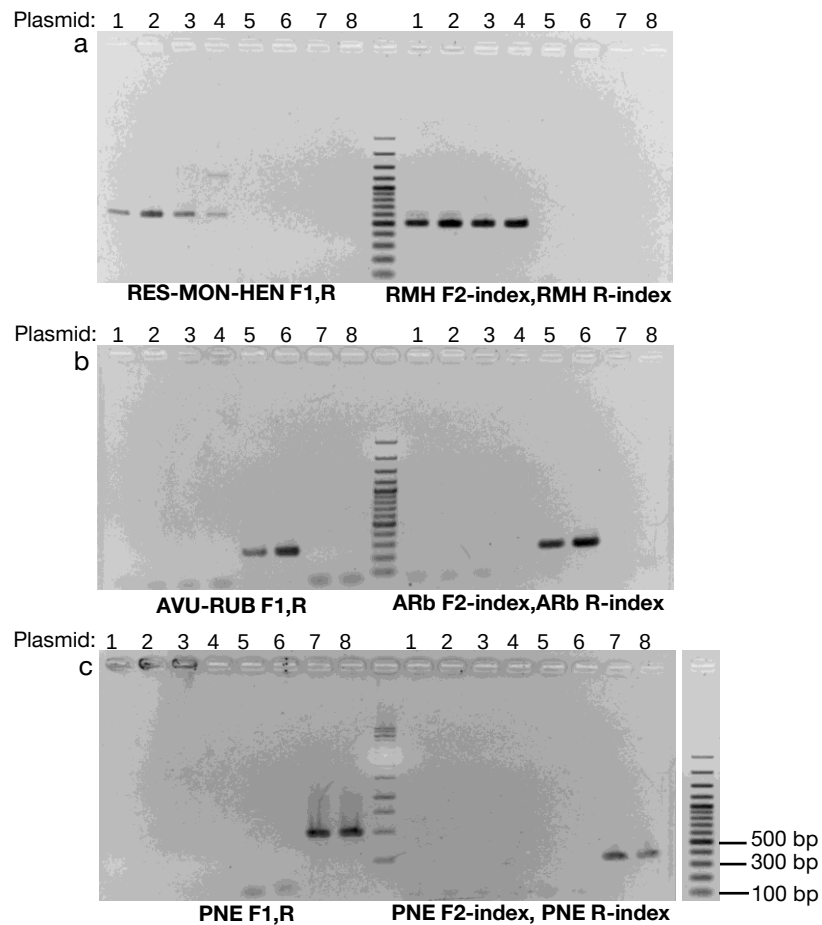
RMH (1): RMH-F2/R-index (*Human parainfluenza 3*) ARb (5): ARb-F2/R-index (*Avian paramyxovirus 3*)

RMH (2): RMH-F2/R-index (*Canine distemper*) ARb (6): ARb-F2/R-index (*Simian 5*)

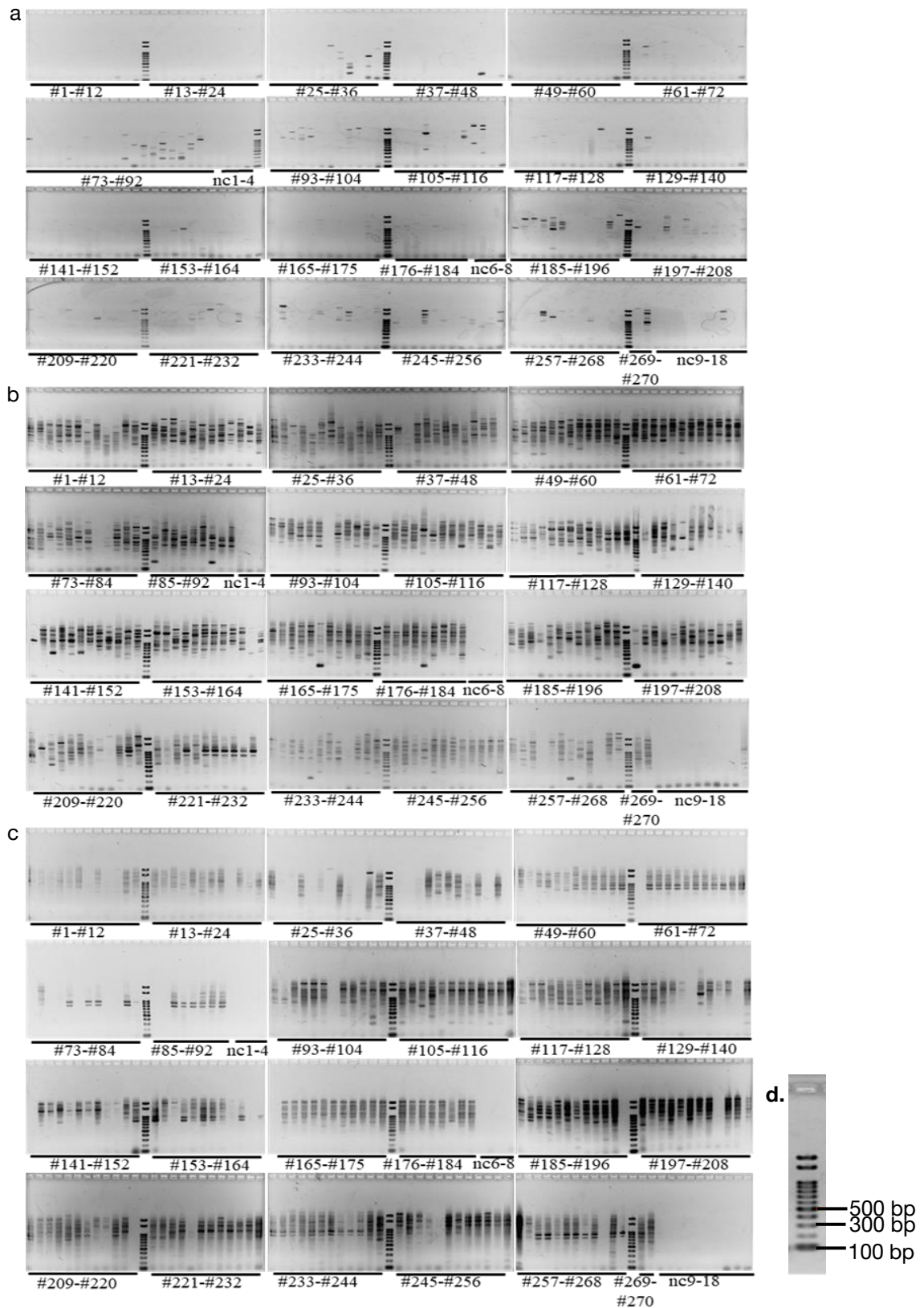
RMH (3): RMH-F2/R-index (*Cedar virus*) PNE (7): PNE-F2/R-index (*Human metapneumovirus*)

RMH (4): RMH-F2/R-index (*Salem virus*) PNE (8): PNE-F2/R-index (*Bovine respiratory syncytial virus*)

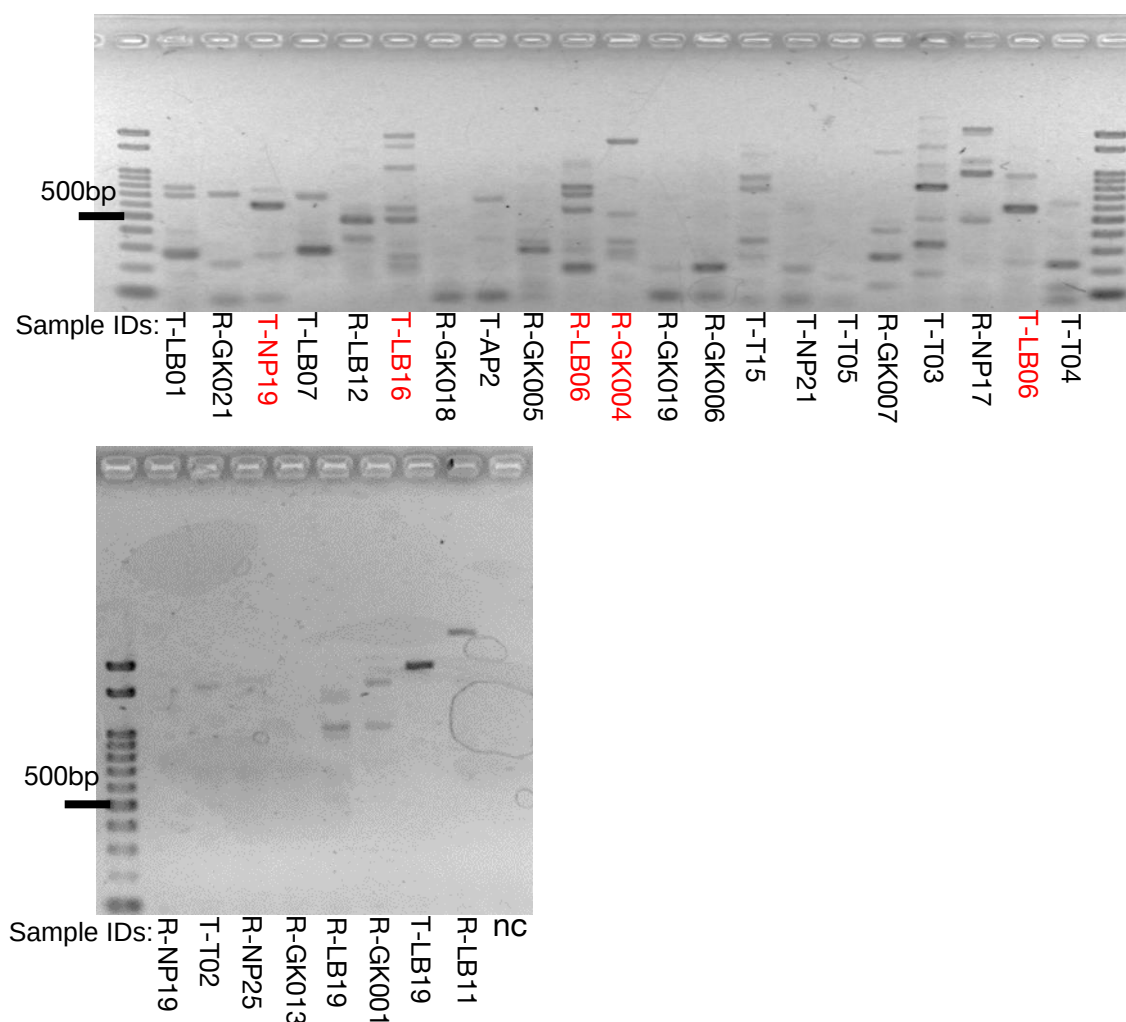
Supplementary Fig. S3. Limit Detection of pan-paramyxovirus primers-index with a semi-nested PCR system. (a) The validation were applied with serial dilution of plasmid from 10⁴ to 10⁰ copies/reaction. Gel images of inner PCR amplicons were showing the expected size from 200-500 bp. Two times replications (1st round and 2nd round) were performed to increase the accuracy of validation results. (b) The limit detection or RMH primer group is 1-10 copies/reaction, ARb 100-1000 copies/reaction, and PNE 10-100 copies/reaction.



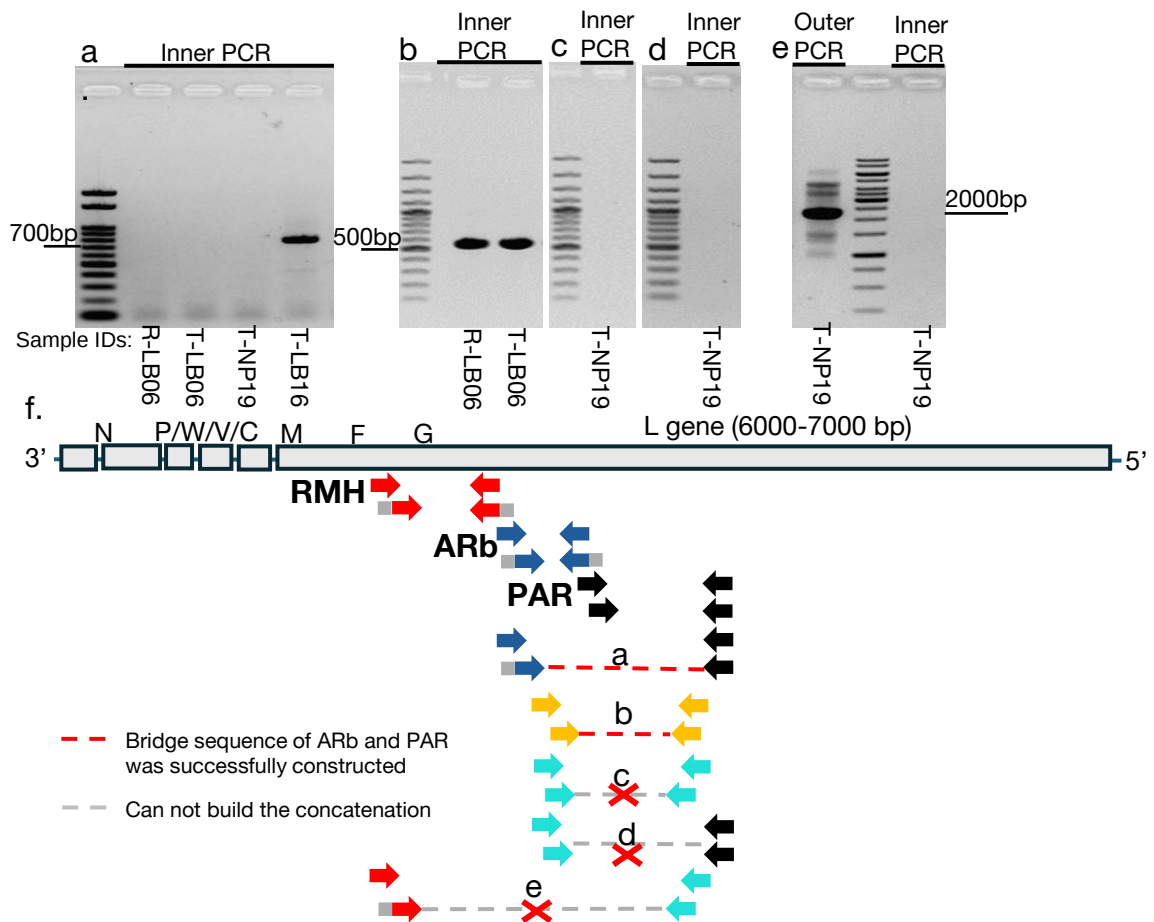
Supplementary Fig. S4. Specificity of pan-paramyxovirus PCR. (a) RMH PCR. (b) ARb PCR. (c) PNE PCR. Numbers above the gel images represent template plasmids used for the test. They include partial genome sequence of (1) *Human parainfluenza 3*, (2) *Canine distemper*, (3) *Cedar virus*, (4) *Salem virus*, (5) *Avian paramyxovirus 3*, (6) *Simian virus 5*, (7) *Human metapneumovirus*, and (8) *Bovine respiratory syncytial virus*, respectively. The viruses 1-4 belong to the RMH group. The viruses 5 and 6 belong to the ARb. The viruses 7 and 8 belong to the PNE. 10^4 copies were used for each reaction.



Supplementary Fig. S5. Agarose gel electrophoresis of PCR amplicon from semi-nested PCR using RMH, ARb and PNE primer in bat samples. (a) The amplicons from inner PCR using RMH primer with length target 487 bp. (b) The amplicons from inner PCR using ARb primer with length target 224 bp. (c) The amplicons from inner PCR using PNE primer with length target 461 bp. (d) The 100bp Ladder were used to determine expected length of amplicon.



Supplementary Fig. S6. Agarose gel electrophoresis of semi-nested PCR from PARs primers. The PAR PCR was applied to twenty-nine bat samples detected for paramyxovirus-like sequences by the ARb PCR system. The PAR amplicons were applied with electrophoresis using 1.5% agarose gel and showed the expected length of 540 bp. Hence, the expected band size was shown in five amplicons while others are non-specific. Sample IDs that perform the expected band are shown in red color.



Supplementary Fig. S7. The Bridge PCR of ARb and PAR regions. The initial bridge PCR for the ARb and PAR regions was conducted using a conventional semi-nested RT-PCR, which was performed in this study. Furthermore, a nested RT-PCR with expected amplicon size 600-700 bp was conducted using specific primers (yellow and light blue arrows) derived from the obtained ARb and PAR sequences to generate bridging amplicons. The bridge amplicons of ARb and PAR regions were successfully obtained from 3 samples : T-LB16, T-LB06, and R-LB06, and subsequently confirmed by sequencing.

- The semi-nested PCR was applied to amplify four samples with AVU-RUB-F1, ARb-F2-index, and PAR-R primers. One amplicon displayed the predicted band size of 778 bp.
- The nested PCR was used to amplify tracheal and rectal swab samples from LB16 bat using ARb-F1.C4, PAR-R1.C6, ARb-F2.C4, and PAR-R2.C6 primers. Both amplicons were shown expected amplicon size.
- One sample (T-NP19) was amplified with nested PCR using ARb-F1.C1, PAR-R1.C7, ARb-F2.C1, and PAR-R2.C7 primers. Visible band cannot be observed.
- The semi-nested PCR was used to amplify the same sample (T-NP19) using ARb-F1.C1, ARb-F2.C1, and PAR-R primers. Visible band cannot be observed.
- Sample T-NP19 was amplified with pan-paramyxovirus primers from the *Henipavirus* group, including RES-MOR-HEN-F1, PAR-R1.C7, RMH-F2-index, and PAR-R2.C7. The predicted band size was 1930 bp in the outer PCR, but the results of the inner PCR were negative. This finding indicates that the amplification was non-specific, as evidenced by the absence of viral sequences in nanopore sequencing.
- The positions of RMH, ARb, PAR, and new primers to created bridge amplicons.