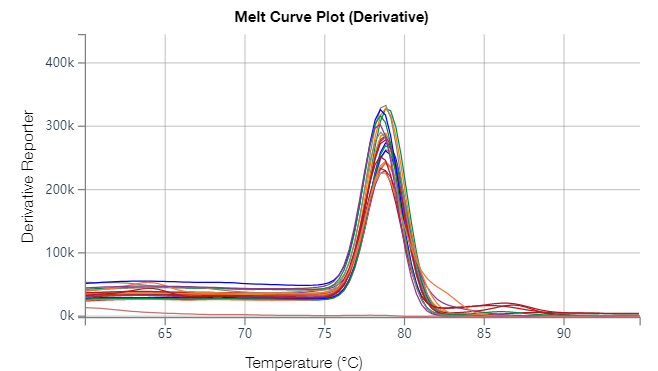
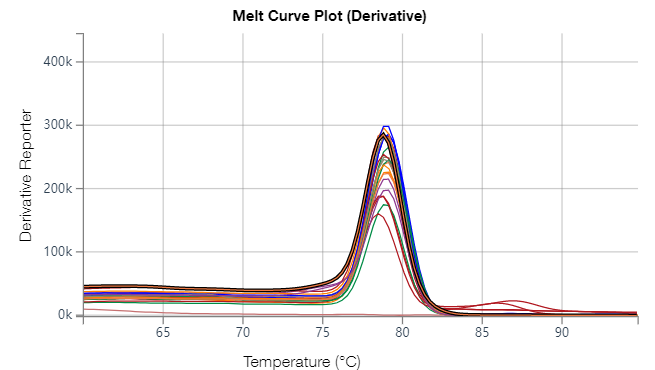
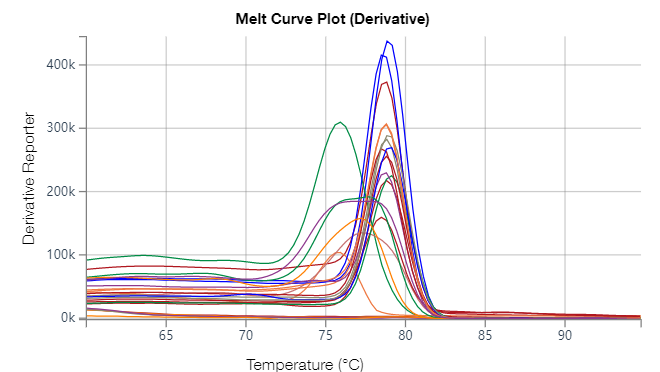
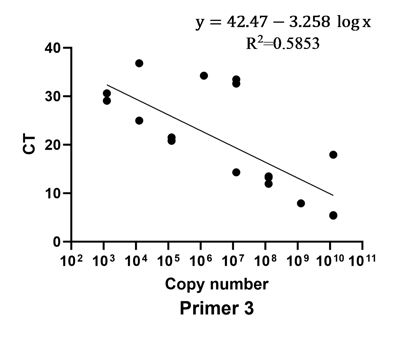
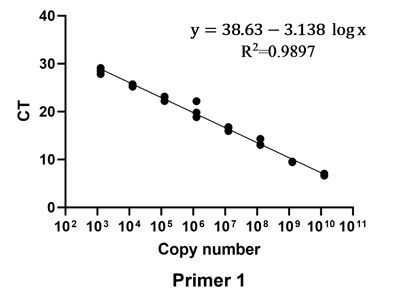
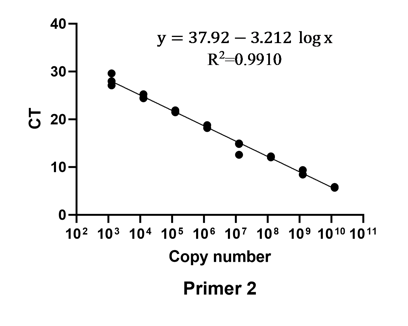
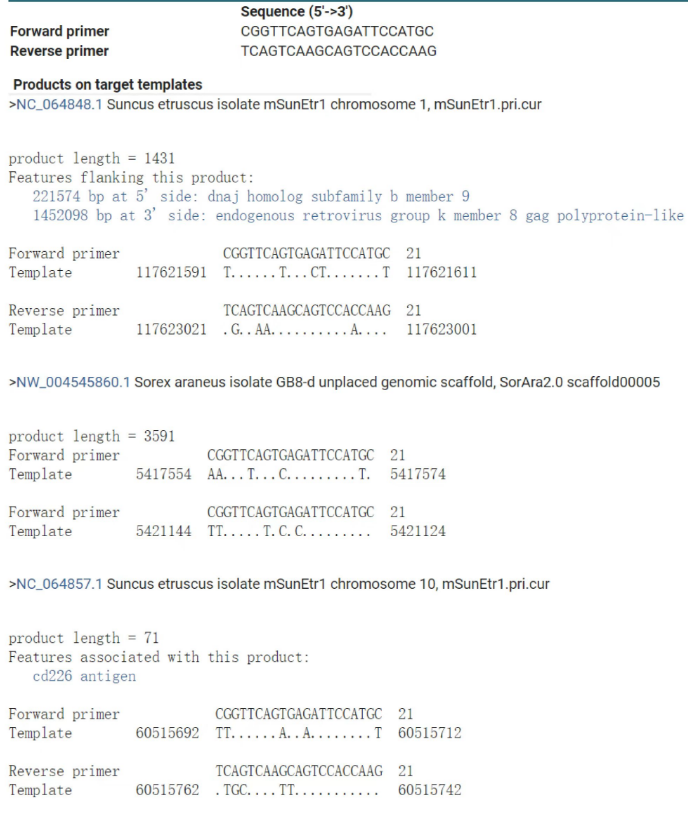
Supplementary Materials:

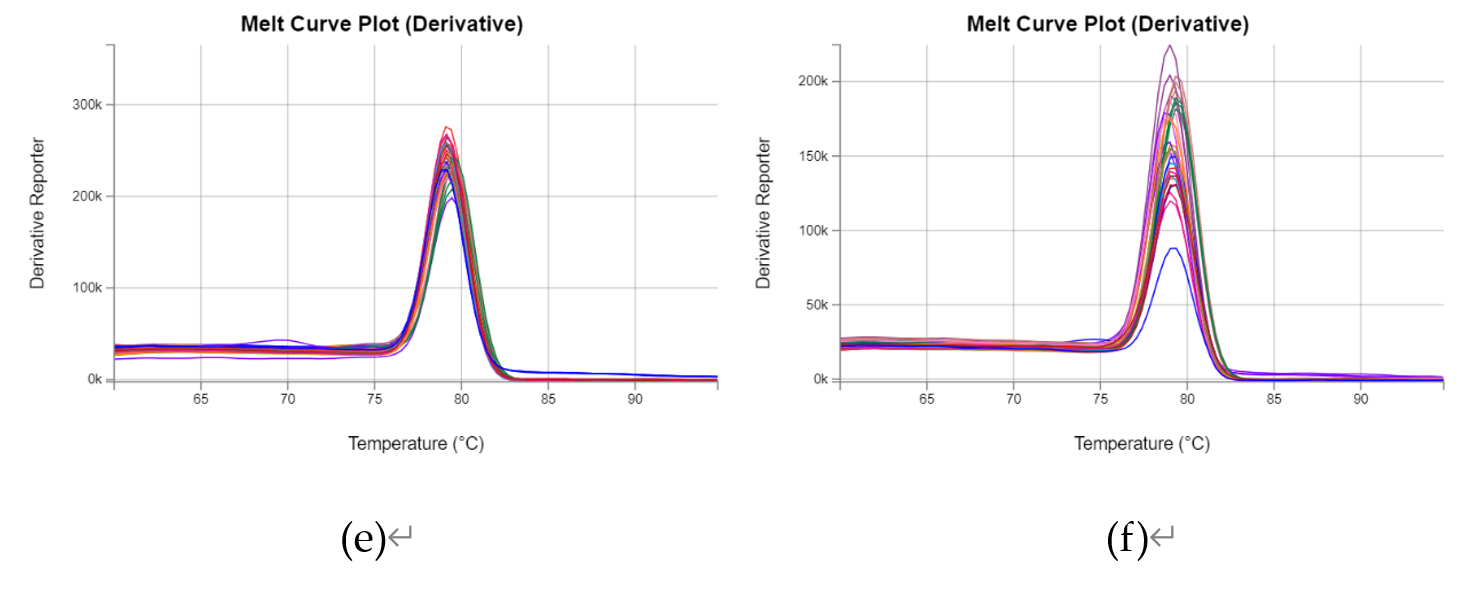
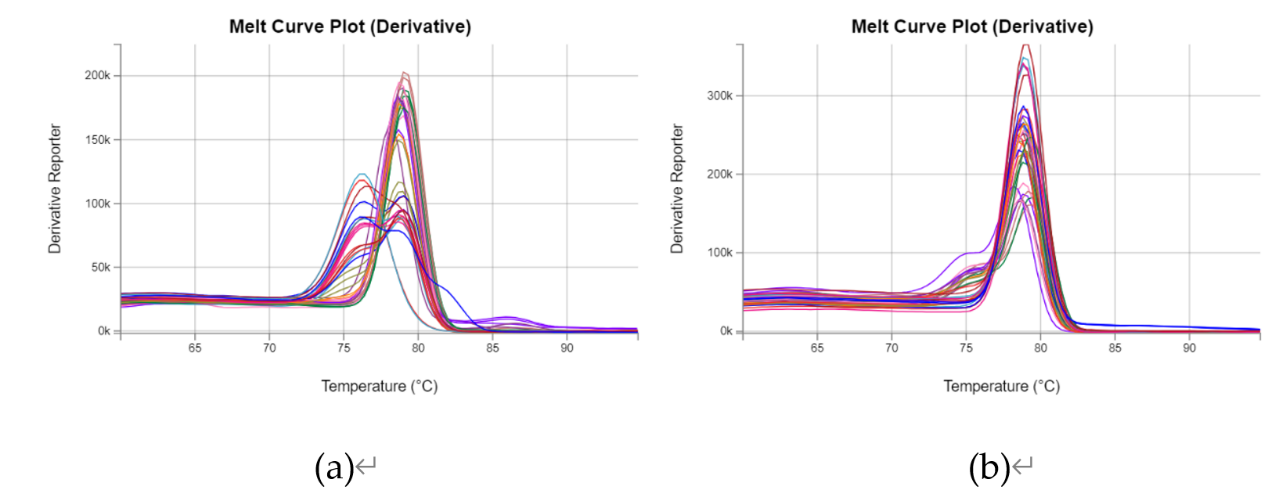
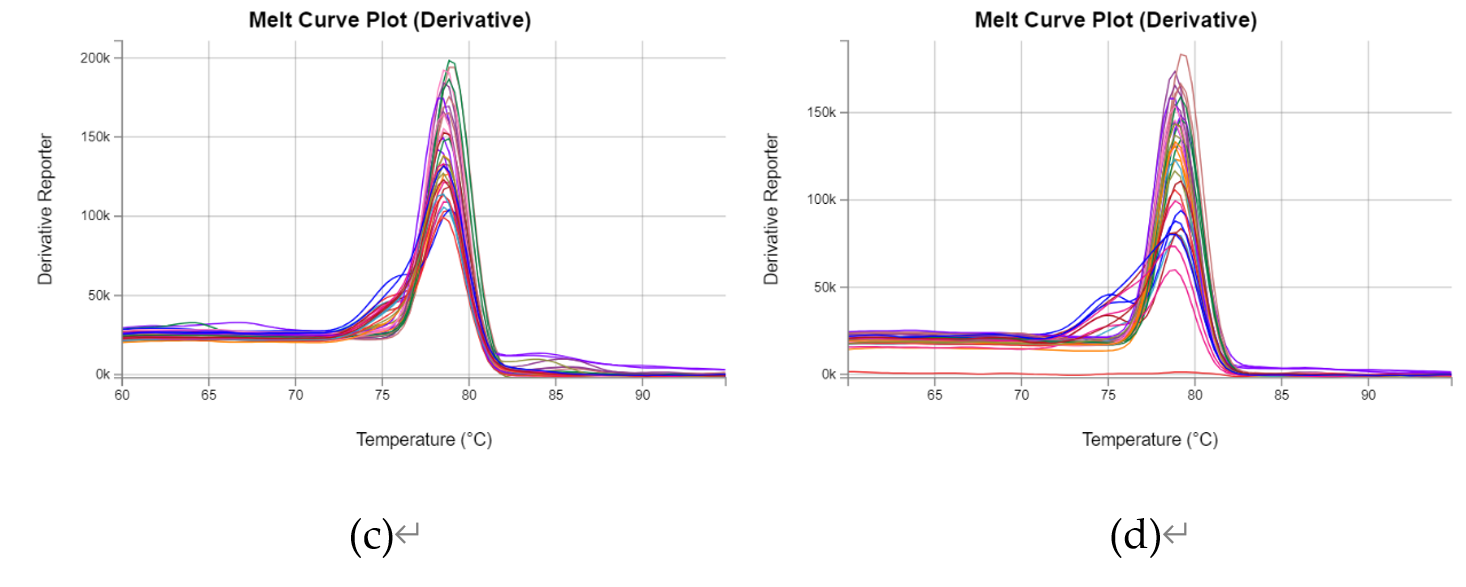
S1:

**Supplementary Figure S1.** Melt curves and standard curves for the preliminary experiments using three primers against the LayV L gene. Figures S1(a) and (b) present melt results and standard curve outcomes for Primer Set 1. Figures S1(c) and (d) depict melt results and standard curve outcomes for Primer Set 2. Figures S1(e) and (f) showcase the same for Primer Set 3. Both primer sets 1 and 2 exhibit a single sharp peak in the melt curve, indicative of specific amplification, whereas Primer Set 3 does not show a distinct peak. The standard curves for primer sets 1 and 2 display high R2 values, signifying a high fit of the data to the model. In contrast, Primer Set 3 exhibits a lower R2 value, suggesting a less optimal fit. The figures presented are representative of two replicate experiments within a single experimental group.

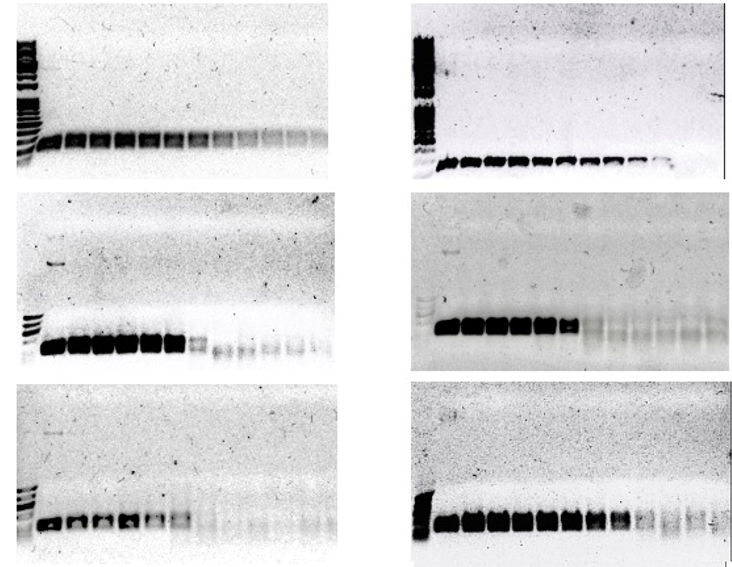
S2:



**Supplementary Figure S2.** Primer-BLAST result of the Langya virus L gene primer design when ignoring targets with six or more mismatches. When the Primer-BLAST program was further adjusted to ignore targets with six or more mismatches to the primers, three target templates were identified, as depicted in the figure. Consequently, Primer Set 2 has the potential to match gene templates of both *Suncus etruscus* and *Sorex araneus,* both belonging to the *Soricidae* family.

**S3:**

**Supplementary Figure S3.** Melt curves for three replication experiments using Primer set 1 and Primer set 2 against the LayV L gene. Figures S3(a), (b), and (c) present the results of Primer Set 1, while Figures S3(d), (e), and (f) display the results of Primer Set 2 from three replication experiments. Notably, some melt curves exhibit two peaks, observed exclusively in samples with lower copy numbers (<1000 DNA copies). The smaller peaks preceding the main expected peak may suggest the presence of primer dimers. A total of three replicates were conducted, with each replicate involving both primer sets, each comprising three experimental groups. The figure presented is representative of the melting curves for the three experimental groups in each of the three replicate experiments.

S4:

(a)

((c)

(e)

(f)

(b)

(d)

**Supplementary Figure S4.** Gel electrophoresis of qPCR products of three replication experiments using Primer set 1 and Primer set 2 against the LayV L gene. Figures S4(a), (c), and (e) depict the gel electrophoresis results for three groups of qPCR products using Primer Set 1, while Figures S4(b), (d), and (f) correspond to Primer Set 2. In Figures S4(a) and (b), the molecular weight standard RealBand 1 KB Puls (0.1-10kb) DNA Ladder (BBI-B600032-0500) was employed, whereas Figures S4(c), (d), (e), and (f) utilized the molecular weight standard StarMarker 50 bp Ladder (GenStar- M018-01). To confirm the specificity of the designed primers in generating a single PCR product, gel electrophoresis was employed for qPCR product detection. Consistently across all results, amplification by both primers is evident, displaying a product of approximately 200 bp with no other visible products. Given that the experiment was conducted with a total of three replicates, each repeated three times, random sets of qPCR results were selected for testing.