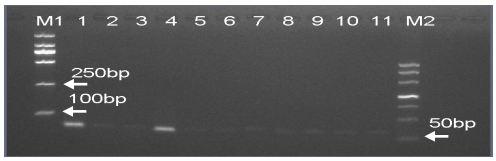
Fig. S1. Primer specific test by individual PCR. A: Primers for detection of *Nosema bombycis*. B: Primers for detection of BmNPV. C: Primers for detection of BmDNV. From left to right, each lane is M1： DL2000 marker (TaKaRa, Dalian, China); 1: *N. bombycis*; 2: BmNPV; 3: BmDNV; 4: mixture of *N. bombycis*, BmNPV, and BmDNV; 5: BmCPV-1; 6: BmIFV; 7: *Bacillus thuringiensis*; 8: *Beauveria bassiana*; 9: *Nomuraea rileyi*; 10: normal (uninfected) silkworm; 11: Mulberry leaves; M2： DL500 marker (TaKaRa, Dalian, China).

A



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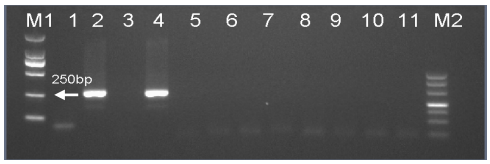

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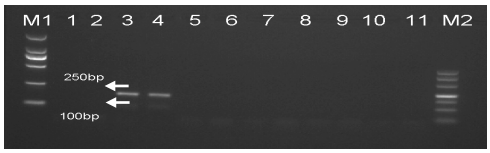
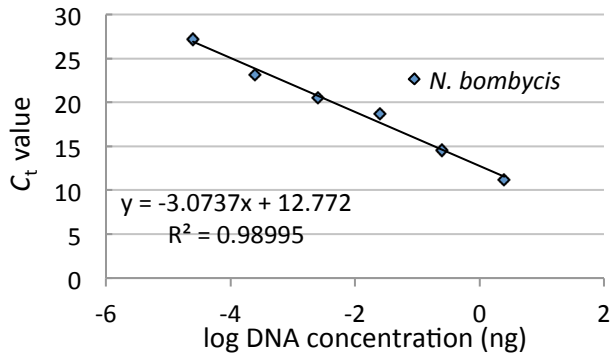


Fig. S2. C_t values obtained from serial dilution of *Nosema bombycis*, BmNPV, and BmDNV plasmid DNA in the simplex PCR systems, plotted versus the logarithm of the DNA concentrations (8.5×10^8 copies/uL ≈ 2.5 ng/uL). A: Simplex PCR system for detection of *N. bombycis*; B: Simplex PCR system for detection of BmNPV; C: Simplex PCR system for detection of BmDNV.

A



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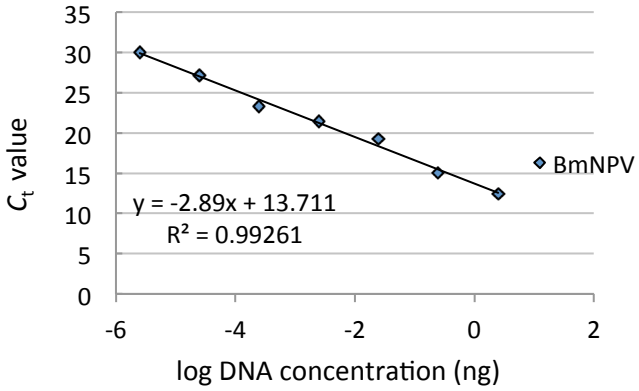

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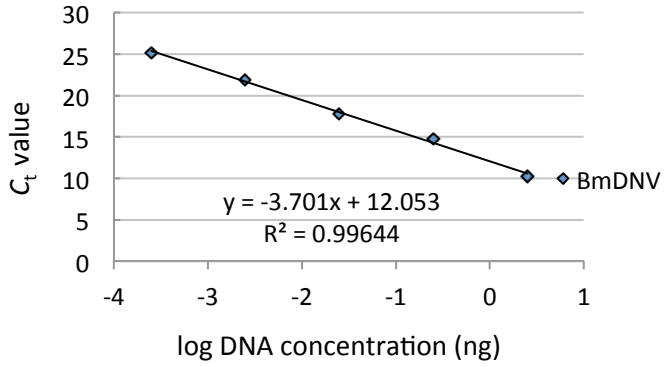
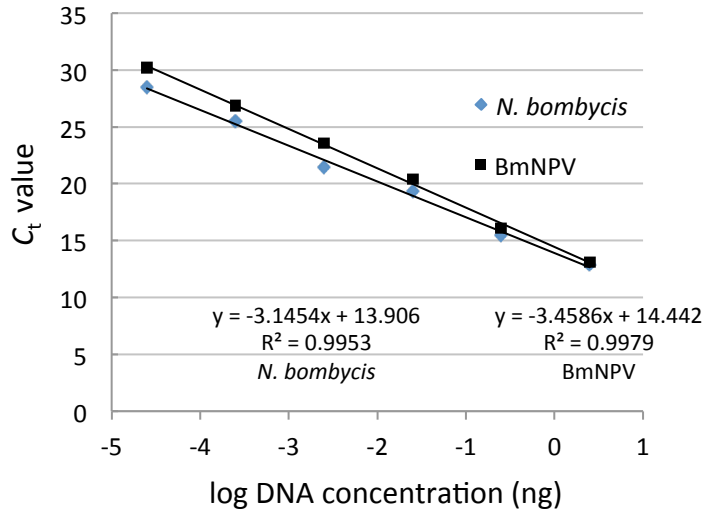


Fig. S3. C_t values obtained from serial dilution of *Nosema bombycis*, BmNPV, and BmDNV plasmid DNA in the duplex PCR systems, plotted versus the logarithm of the DNA concentrations (8.5×10^8 copies/uL ≈ 2.5 ng/uL). A: Duplex PCR system for detection of *N. bombycis* and BmNPV; B: Duplex PCR system for detection of *N. bombycis* and BmDNV; C: Duplex PCR system for detection of BmNPV and BmDNV.

A



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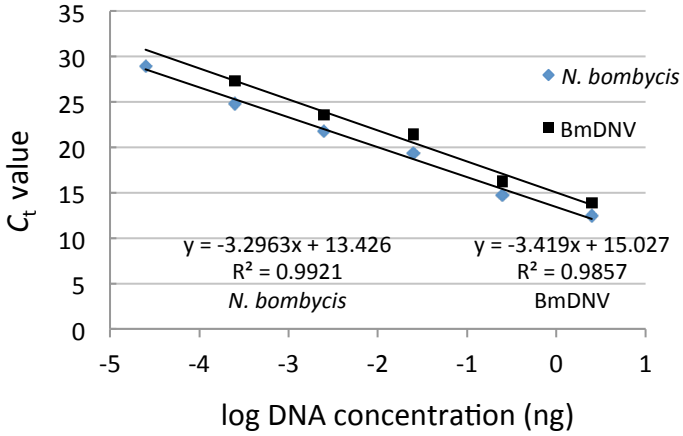

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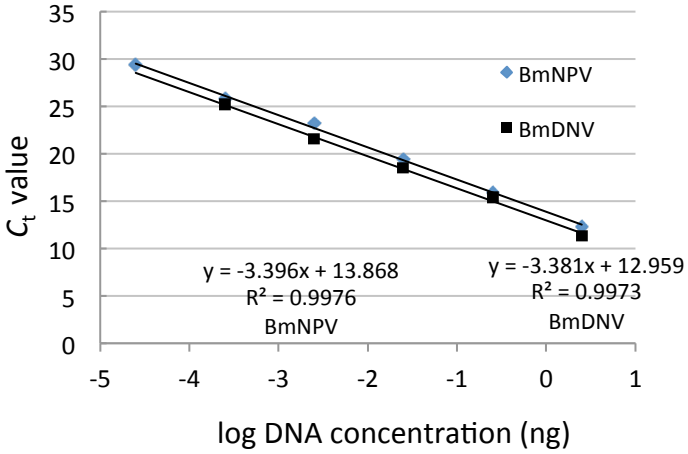
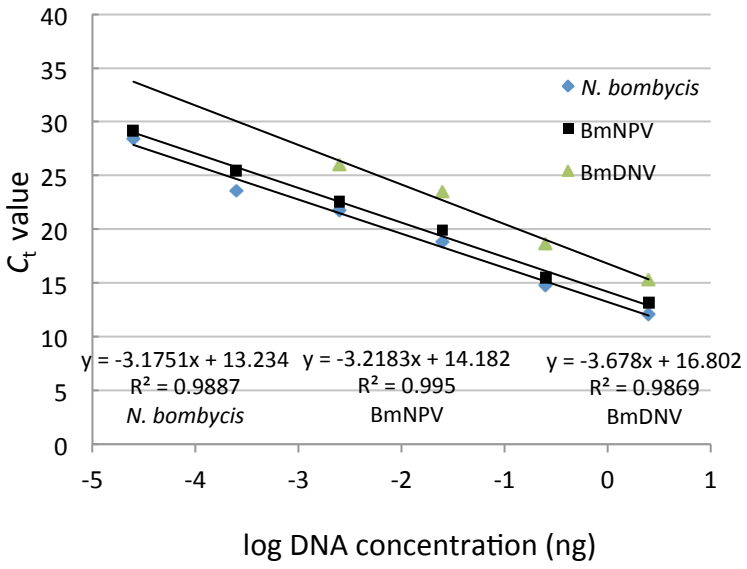


Fig. S4. C_t values obtained from serial dilution of *Nosema bombycis*, BmNPV, and BmDNV plasmid DNA in the multiplex PCR systems, plotted versus the logarithm of the DNA concentrations (8.5×10^8 copies/uL ≈ 2.5 ng/uL). A: The primer/probe sets between different pathogens were mixed in the ratio of 1:1:1 (*N. bombycis*:BmNPV:BmDNV); B: The primer/probe sets between different pathogens were mixed in the ratio of 2:2:3 (*N. bombycis*:BmNPV:BmDNV)

A



B