Supplementary Table 1 Primer design and polymerase chain reaction protocols for each amplified region

PCR	Primer sequence (5'->3')	Amplified region	Protocol
First-round PCR	forward 5′-TGTATTCCCATCCCATCATC reverse 5′- AGWAGCTCCAAATTCTTTATAAGG	from nt 599 to 1936	95 °C for 5 min followed by 35 cycles of 95 °C for 20 s, 53°C for 20 s, and 72°C for 15 s, and finally 72 °C for 3 min
Second-round PCR	forward 5′- GTTGTAAAACGACGGCCAGTATGCGTGGAACCTTTGTGGCT reverse 5′- CACAGGAAACAGCTATGACCATGGGCGTTCACGGTGGTCT	from nt 1234 to 1631	95 ℃ for 2 min followed by 30 cycles of 95 ℃ for 15 s, 60°C for 20 s, and 72 ℃ for 15 s, and finally, 72 ℃ for 3 min
Third-round PCR	forward 5′-MID-GTTGTAAAACGACGGCCAGT reverse 5′-MID-CACAGGAAACAGCTATGACC	Complementary to universal M13 sequences	95°C for 2 min followed by 20 cycles of 95°C for 15 s, 60°C for 20 s, and 72°C for 15 s, and finally, 72°C for 3 min
qPCR	forward 5′–GATCCATACTGCGGAACTCCT reverse 5′–GHAGGATCCAGTTGGCAGYAC TaqMan probe 5′-LC610- CTTGTTTTGCTCGCAGCMGGTCTGG—BBQ	from nt 1263-1408	**HBV-RNA quantification:** 63°C for 3min and 95°C for 30 s followed by 45 cycles of 95°C for 10 s, 65°C for 30 s, and 72°C for 1 s, and finally 40°C for 10 s **Verification of DNAse I treatment:** 95°C for 10 min followed by 45 cycles of 95°C for 5 s, 65°C for 30 s, and 72°C for 15 s, and finally 37°C for 20 s

MID is a multiplex identifier or barcode sequence consisting of 10 specific nucleotides for each sample. BBQ is a non-fluorescent chromophore that absorbs fluorescence energy from a neighboring fluorophore, thereby preventing emission of fluorescent light.
Abbreviations: BBQ, BlackBerry Quencher.