S4 Generation of NS5B amplicons

Forward (positions 8250-8275) and reverse primers (8616-8638) were used as described previously (Murphy et al 2007). Final PCR reactions contained one times concentration HI-FI buffer, 0.02 unit/ μ L of Velocity Taq Polymerase, 250 μ M dNTP (Bioline), 200nM forward primer (Integrated DNA Technologies), 400nM reverse primer, one times concentration of Polymate (Bioline) and cDNA at 1/10 of the final reaction volume. PCRs were performed in a total volume of 50 μ L. Thermocycling conditions are described in the table below. PCR products were stored at -20°C until required.

Thermocycling conditions for generation of NS5B amplicons

| 1 cycle | 98°C 2 mins |
|-----------|--------------|
| | 98°C 30 secs |
| 40 cycles | 56°C 30 secs |
| | 72°C 1 min |
| 1 cycle | 72°C 10 mins |
| Hold | 4°C |