

Appendix 1 Methods and results to confirm BTV in *Culicoides*

1) One step RT-PCR

One step RT-qPCR method exclusive probe was used to amplify the BTV NS3 fragments from the two RNA samples of *Culicoides* (A46 and B3) positive for BTV. The reaction solution (25 µl) was confected using the Quant One Step PrimeScript RT-PCR Kit (Takara) according to the manufacturer's instructions, containing 2.5 µl of RNA and 20 µM of specific primers. The RT-PCR cycling program consisted of: 50°C, 10 min; 94°C, 2 min; 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, 33 cycles (for A46) or 37 cycles (for B3).

2) Sequencing

The two PCR products were sent to Kunming Shuoqing Biological Technology Company (China) for Sanger sequencing with an ABI3739XL machine (Applied Biosystems). The overlap regions of the forward sequences and reverse sequences were employed.

3) Sequences

>A46 BTV-NS3

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ACCTCGGGGCGCCACTCTACCTACTGATCTTAGGTTAATGGTAGTTCGAAACCATCTAG
CGGAACTGACGAGAACTCAGTGAAACTCATCCTCACCGCATCATTGTATGATTGCTTCT
TCATAACTTCCTTCTTGATCATGTCAATCTGCTGATTTAACCTCTTTCGCTCTTCGCGC
AAACCATCATTATGAAAGTCGCACCTAGATTTACCACTCCGAGCATTGGATTTAGACTC
TTAAACCACTGTGGTATTTCCGCTGATGTACCATTAAGTTTAAAAGCCCACTCATATCA
CTTGAAAGCGTGCATACTGACGTCAGCAATGCTACGACAGCGGCAACTAACAACGTCA
TGTGTATGATAGCTCTCTTTTCTTTAAACCACCTAGATCACTCTTTAACTTTGGGAGAA
TCTGTTCAATTAACATGACGTTTGATCTGTCTTAGCCTCACATCATCACGAAACGTTCTG
CGTACGATGCGAATGCAGCTTCTCCGCCTTCTGTGTTTGC GTTGACACCAGTTGTGTTT
GACATCGCTTTATCCAATATTTCAAGGGCAACGTTGGCATTGATGATGGCATTGGGGC
ACTCGGAGCATATCTCGGTGGTTGCGAAATTGTATCATCCACACGAACCAAACTTAGCT
CTTCGACTCTCTCCTGATTATGTTTCATTTTTTCTTCCTCGAACCTTTGGATCAGCCCGG
ATAGCATGGCAGCG
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>B3 BTV-NS3

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AAGAGCTAAGTTTGGTTCGTGTGGATGATACAATTTGCAACCACCGAGATATGCTCCG
AGTGCCCCAATGCCATCATCAATGCCAACCGTTGCCCTTGAAATATTGGATAAAGCGAT
GTCAAACACAACCTGGTGCAACGCAAACACAGAAGGCGGAGAAAGCTGCATTTCGCATC
GTACGCAGAAGCGTTTCGTGATGATGTGAGGCTAAGACAGATCAAACGTCATGTTAAT
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GAACAGATTCTCCCAAAGTTAAAGAGTGATCTAGGTGGTTTAAAGAAAAAGAGAGCTA
TCATACACATGACGTTGTTAGTTGCCGCTGTCGTAGCATTGCTGACGTCAGTATGCACG
CTTCAAGTGATATGAGTGTGGCTTTTAAACTTAATGGTACATCAGCGGAAATACCACA
GTGGTTTAAAGAGTCTAAATCCAATGCTCGGAGTGGTAAATCTAGGTGCGACTTTCATAA
TGATGGTTTGCGCGAAGAGCGAAAGAGGGTTAAATCAGCAGATTGACATGATCAAGAA
GGAAGTTATGAAGAAGCAATCATACAATGATGCGGTGAGGATGAGTTTCACTGAGTTC
TCGTCAGTCCGCTAGATGGTTTCGAACTACCATTAACCTAAGATCAGTAGGTAGAGTG
GCGCCCCGAGGT