

Supplementary Materials

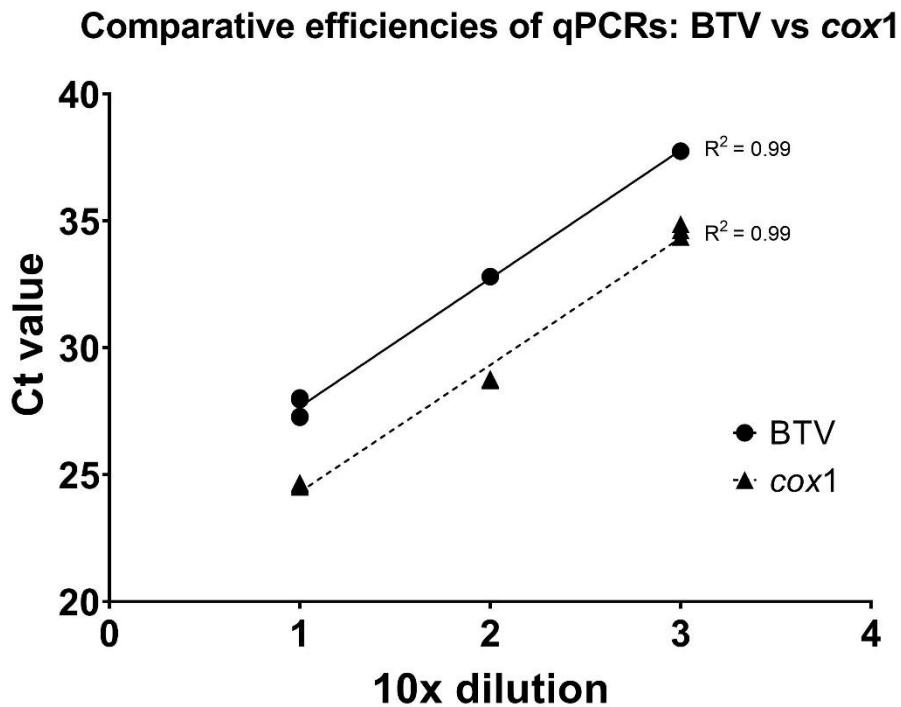


Figure S1. Comparative efficiencies of BTV (segment 10) and *Culicoides* mitochondrial cytochrome oxidase 1 gene (*cox1*) RT-PCR after DNase treatment. Ten-fold dilutions were prepared of a pool of BTV-infected midges, and each dilution was screened in triplicate for each target. Linear regressions and R² values were prepared using Graph Pad Prism 8.0.

Table S1. Amplicon Assay Primers. Round-one PCR of amplicon assay, BTV-specific primer sequences and concentrations. Final primer cocktail concentration (in μ M) is shown for each primer. D11 represents the concentration used for plaques screened from midges collected on day 11 post-blood meal; D23 represents the concentrations used for plaques screened from midges collected on day 23 post-blood meal.

| | Sequence | D11, μ M | D23, μ M |
|----------|---------------------------------------|--------------|--------------|
| S1_560F | TCC AGG GGA ATA GAG ATT TAT C | 0.13 | 0.12 |
| S1_1033R | TCG TGC GAG CCY AAW TTT TG | 0.13 | 0.12 |
| S2_1210F | TGG CGA TGT KTA CTT YAC MTT GCG | 0.10 | 0.11 |
| S2_1601R | GCA TCY TTY TCG AAA TCG ATT GTA AG | 0.10 | 0.11 |
| S3_2282F | TMC AGT TYC GAG CGG CTT TAA G | 0.08 | 0.10 |
| S3_2684R | GAG CGA TTG GGT GAT GTC CA | 0.08 | 0.10 |
| S4_1484F | TCG TGG GCG ATG AAT TTT GCT | 0.08 | 0.08 |
| S4_1961R | TCA CCT AGC AGT CAC GCA TTA TAA G | 0.08 | 0.08 |
| S5_177F | TCG ATG ATY GCA GCA ACT GAT G | 0.13 | 0.08 |
| S5_587R | TGT GCT GTC CAC GAA TGC CAA | 0.13 | 0.08 |
| S6_715F | TAG GCG GCR TCW GAA GAA GTG | 0.13 | 0.10 |
| S6_1099R | YGG GAT CTT AAA YYT CAT CAT YAC | 0.13 | 0.10 |
| S7_246F | TTT TGG ACC GAT ATC GCC AGA | 0.08 | 0.05 |
| S7_701R | TGT CCA TCC CAC GCT ATA ATG C | 0.08 | 0.05 |
| S8_594F | TTG GAT GAW GAG GCC AAA GAG AT | 0.08 | 0.09 |
| S8_1048R | CTT AGA GAC AAA AGC AAC ACG CT | 0.08 | 0.09 |
| S9_455F | TAC GGT ACG AAG ATT GAT GTT TAC AG | 0.10 | 0.09 |
| S9_902R | TTC CAA TGC GGA TCT CCA GTT G | 0.10 | 0.09 |
| S10_184F | TAA ATY CTG GAC AAA GCG ATG TC | 0.08 | 0.07 |

S10_549R ACT YTT TGC GCA AAC CAT CAT CA 0.08 0.07