**Supplementary table 1.** PCR settings and primers used for the detection of tick-borne pathogens in tick nucleic acids extracts.

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| --- | --- | --- | --- | --- | --- |
| **Target organism** | **Target gene (amplicon size, bp)** | **Method** | **Primer and probe names and 5´–3’ sequences** | **Cycling conditions** | **Reference** |
| TBEV | 3’ non-coding region  (67 bp) | qRT-PCR | F-TBE1: GGG CGG TTC TTG TTC TCC  R-TBE1: ACA CAT CAC CTC CTT GTC AGA CT  TBE-probe-W: TGA GCC ACC ATC ACC CAG ACA CA | 48 °C – 5 min, 95 °C – 30 sec, (95 °C – 3 sec, 60 °C – 30 sec) × 45 | [18] |
| E gene  (465 bp) | RT-PCR | E frw1: GTT GTG TGG YTG ACY STG GA  E rev1: TCK GAK ACY TCY CTC CAC AC | 65 °C – 5 min, on ice – 1 min, 25 °C – 5 min, 50 °C – 45 min, 70 °C – 15 min | [19] |
| nested PCR | 283F1: GAG AYC AGA GTG AYC GAG GCT GG  827R1: AGG TGG TAC TTG GTT CCM TCA AGT | (94 °C – 1 min, **57 °C** – 1 min, 72 °C – **2 min**) × 35 | [13] |
| 349F2: GTC AAG GCG KCT TGT GAG GCA A  814R2: TTC CMT CAA TGT GYG CCA CAG G | (94 °C – 1 min, 60 °C – 1 min, 72 °C – 120 sec) × 30 |
| *B. burgdorferi* s.l. | *5S-23S intergenic spacer*  (245-256 bp) | nested PCR | NC1: CCT GTT ATC ATT CCG AAC ACA G  NC2: TAC TCC ATT CGG TAA TCT TGG G | (94 °C – **30 sec**; 58 °C – **30 sec**; 72 °C – **1 min**) × 35 | [21] |
| NC3: CTG CGA GTT CGC GGG AGA  NC4: TCC TAG GCA TTC ACC ATA | (94 °C – **30 sec**; 52 °C – **30 sec**; 72 °C – **1 min**) × 30 |
| *B. miyamotoi* | *p66*  (532 bp) | nested PCR | M1F: TTC TAT ATT TGG ACA CAT GTC  M2R: CAG ATT GTT TAG TTC TAA TCC G | (94 °C – **30 sec**, **52** °**C**– **30 se**c, 72 °C – **1 min**) × 35 | [20] |
| M3F: CTA AAT TAT TAA ATC CAA AAT CG  M4R: GGA AAT GAG TAC CTA CAT ATG | (94 °C – **30 sec, 49** °C– **30 sec**, 72 °C – **1 min**) × 35 |
| *glpQ*  (379 bp) | nested PCR | glpQMiy-Q1F: CAC CAT TGA TCA TAG CTC ACA G  glpQMiy-Q2R: CTG TTG GTG CTT CAT TCC AGT C | (94 °C – **30 sec**, **57** °C – **30 sec**, 72 °C – **1 min**) × 35 |
| glpQMiy-Q3F: GCT AGT GGG TAT CTT CCA GAA C  glpQMiy-Q4R: CTT GTT GTT TAT GCC AGA AGG GT | (94 °C – **30 sec**, **59** °C – **30 sec**, 72 °C – **1 min**) × 35 |
| Anaplasmataceae | *16S rRNA*  (524 bp) | nested PCR | Ehr1: GAA CGA ACG CTG GCG GCA AGC  Ehr2: AGT AYC GRA CCA GAT AGC CGC | (94 °C – **30 sec**, 57 °C – **30 sec**, 72 °C – **1 min**) ×35 | [10] |
| Ehr3: TGC ATA GGA ATC TAC CTA GTA G  Ehr4: CTA GGA ATT CCG CTA TCC TCT | 94 °C – **30 sec**, **48 °C** – **30 sec**, 72 °C – **1 min**) × 35 |
| *16S rRNA* (1350 bp) | nested PCR | Ehr1: GAA CGA ACG CTG GCG GCA AGC  Ehr6: GAC CCA ACC TTA AAT GGC TGC | (94 °C – **30 sec**, **58 °C** – **30 sec**, 72 °C – **1 min**) × 35 |
| Ehr7: TAA CAC ATG CAA GTC GAA CG  Ehr8: CTT CGA GTT AAG CCA ATT CC | (94 °C – **30 sec**, **50 °C** – **30 sec**, 72 °C – **1 min**) × 35 |
| *groESL*  (1300 bp) | nested PCR | HS1: TGG GCT GGT AMT GAA AT  HS6: CCI CCI GGI ACI AYA CCT TC | (95 °C – 60 sec, 48 °C – 120 sec, 72 °C – 90 sec) × 3; (88 °C – 60 sec, 50 °C – 2 min, 72 °C – 1.5 min) × 27 |
| HS43: ATW GCW AAR GAA GCA TAG TC  HSVR: CTC AAC AGC AGC TCT AGT AGC | (95 °C – 60 sec, 52 °C – 2 min, 72 °C – 1.5 min) × 30 |
| *Rickettsia* | *gltA*  (74 bp) | qPCR | CS-F: TCG CAA ATG TTC ACG GTA CTT T  CS-R: TCG TGC ATT TCT TTC CAT TGT G  CS-P (probe): TGC AAT AGC AAG AAC CGT AGG CTG GAT G | 95 °C – 3min, (95 °C – 10 sec, 60 °C – 60 sec) × 40 | [11] |
| *gltA*  (667 bp) | nested PCR | glt1: GAT TGC TTT ACT TAC GAC CC  glt2: TGC ATT TCT TTC CAT TGT GC | (94°C – **30 sec, 48°C** – **30 sec**, 72°C – **60 sec**) × 35 | [23] |
| glt3: TAT AGA CGG TGA TAA AGG AAT C  glt4: CAG AAC TAC CGA TTT CTT TAA GC | (94°C – **30 sec, 45.5°C** – **30 sec**, 72°C – **60 sec**) × 30 |
| *sca4*  (843 bp) | nested PCR | Sc4-1: ATG TCT CTG AAT TAA GCA ATG C  Rj2837r: CCT GAT ACT ACC CTT ACA TC | (94°C – 60 sec, 52°C – 60 sec, 72°C – 2 min) × 35; |
| sc4-3: AAT TAT TAG GCT CTG TAT TAA AGA  sc4-4: GAA AGG ATA GCA CGA AAA GTA | (94°C – 60 sec, 50°C – 60 sec, 72°C – 2 min) × 30 |
| *ompB*  (769 bp) | PCR | 120-2788F: AAA CRA TAA TCA AGG TAC TGT  120-3599R: ACY STG GAR AGT GTG GTG AC | (95°C – 30 sec, 51°C – 30 sec, 72°C – 60 sec) × 35 | [20][20] |

Cycling conditions modifications are indicated bold.

**reaction mix contents:**

**qRT-PCR**:final volume of 20 uL, containing Quanta qScript One-Step Fast qRT-PCR kit with low ROX (Quantabio, Beverly, MA, USA), forward and reverse primers at final concentration of 900 nM each, 200 nM of probe and 5 uL of RNA/positive/negative control.

**RT-PCR:** SuperScript III Reverse Transcriptase kit (ThermoFisher Scientific, USA)

**qPCR:** final volume of 20 uL, containing Takyon Low ROX Probe qPCR Mastermix (Kaneka Eurogentec, Belgium, EU) with each primer concentration at 900 nM and probe at 200 nM

**PCR and nested PCR:** final volume of 25 µl, containing 10X DreamTaq PCR buffer (ThermoFisher Scientific, USA), per 0.8 mM of each dNTPs, 0.5 mM of each forward and reverse primers, per 1.5 mM MgCl2 for outer and 1 mM MgCl2 for inner reaction and 1 U of DreamTaq DNA Polymerase (ThermoFisher Scientific, USA) and 5 µl of cDNA, DNA or PCR reaction product