**Table S1:** Primers used in the study and thermocycling conditions

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Endosymbiont target** | **Target gene** | **Primer name** | **Primer sequence** | **Concentration in mastermix** | **Estimated size (bp)** | **Multiplex experiment**  | **Thermocycling conditions** |
| *B. aphidicola* | 16S rRNA | 16SA1 | AGA GTT TGA TCM TGG CTC AG  | 0.2 µM | 270 | 1  | Denaturation at 94°C for 3 min35 cycles of: 94°C for 30 s, 58°C for 30 s and 72°C for 60 sFinal step at 72°C for 10 min |
| Buch\_R\_CV2 | CCC CCA CTT TRG TTT TTC AAC  | 0.2 µM |
| *Arsenophonus* spp*..* | 16S rRNA | 16SA1 | AGA GTT TGA TCM TGG CTC AG  | 0.2 µM | 456 |
| Ars16S\_R2 | CCT TAA CAC CTT CCT CAC GAC  | 0.2 µM |
| Spiroplasma | 16S rRNA | 16SA1 | AGA GTT TGA TCM TGG CTC AG  | 0.2 µM | 1500 | 2 |
| Spi500R | ATC ATC AAC CCT GCC TTT GG  | 0.2 µM |
| *R. insecticola* | 16S rRNA | 16SA1 | AGA GTT TGA TCM TGG CTC AG  | 0.1 µM | 840 |
| PAUS16SR | TCG GAC GCC ATA ACA CTA GG  | 0.2 µM |
| *H. defensa* | 16S rRNA | 16SA1 | AGA GTT TGA TCM TGG CTC AG  | 0.2 µM | 480 |
| PABS480R | GGT ATT CGC ATT TAT CGC TTC  | 0.2 µM |
| *Rickettsiella* spp*.* | 16S rRNA | P136F | GGG CCT TGC GCT CTA GGT  | 0.2 µM | 300 |
| P136Ric-470R | TGG GTA CCG TCA CAG TAA TCG A  | 0.2 µM |
| *F. symbiotica* | 16S rRNA | PAXS\_F | AGT TTG ATC ATG GCT CAG ATT G  | 0.2 µM | 500 | 3 |
| PAXS\_R | GCA ACA CTC TTT GCA TTG CT  | 0.2 µM |
| *S. symbiotica* | 16S rRNA | 16SA1 | AGA GTT TGA TCM TGG CTC AG  | 0.2 µM | 1140 |
| PASS1140R | TTT GAG TTC CCG ACT TTA TCG  | 0.2 µM |
| *Rickettsia* spp*.* | 16S rRNA | 16SA1 | AGA GTT TGA TCM TGG CTC AG  | 0.2 µM | 600 |
| Ric600R | TTT GAA AGC AAT TCC GAG GT  | 0.2 µM |
| ParG-A435 | CAT GGT AGG CRY AGA ACC TAC CA | 0.5 µM |

Positive controls were included in each assay.