|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Primers & source** | **Assay mix** | | | | | | | | **Thermal cycling** | | | **Amplicon size (bp)** |
| **dNTP (nM)** | **MgCl2 (nM)** | **5xbuffer (μl)** | **Taq (U)** | **Primer F (μM)** | **Primer R (μM)** | **Template (μl)** | **Total volume (μl)** | **1**  **Denaturing**  **Min | Temp** | **2 Replication Sec | Temp** | **3**  **Elongation Min | Temp** |
| *Nosema bombi*  (Klee et al. 2006) | 0.3 | 3.75 | 2 | 0.25 | 0.2 | 0.2 | 2 | 10 | 4 | 95 | 35x  60 | 95  60 | 50  60 | 72 | 4 | 72 | 323 |
| \**Nosema apis*Na& *N. ceranae*Nc  (Gisder & Genersch 2013) | 0.2 | 1.5 | 2 | 2.5 | 0.2Na  0.2Nc | 0.2Na  0.2Nc | 1 | 10 | 4| 95 | 35x  60 | 94  60 | 58  60 | 72 | 5 | 72 | 297Na  662Nc |
| *Apicystis bombi*  (Meeus et al. 2010) | 0.4 | 1.5 | 2 | 1.25 | 0.5 | 0.5 | 1 | 10 | 2 | 94 | 35x  30 | 94  30 | 60  45 | 72 | 3 | 72 | 260 |
| *Apidae*A(internal) and *Crithida bombi*Cb  (Meeus et al. 2010) | 0.4 | 1.5 | 3 | 1.25 | 0.1A  0.5Cb | 0.2A  0.5Cb | 2 | 15 | 2 | 94 | 35x  30 | 94  30 | 57  45 | 72 | 3 | 72 | 130A  420Cb |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| **RT-PCR** | **Probe (nM)** | **Taqman Fast Virus 1-step Master mix(μl)** | | | **Primer F (μM)** | **Primer R (μM)** | **Template (μl)** | **Total volume (μl)** | **1**  **Reverse transcription**  **Min | Temp** | **2**  **Denaturing**  **Sec | Temp** | **3**  **Annealing and elongation Time | Temp** | **Amplicon size (bp)** |
| Deformed wing virus  (Chen et al. 2005) | 200 | 5 | | | 0.65 | 0.65 | 2 | 10 | 5 | 50 | 20 | 95 | 40x  3 s | 95  3 min | 60 | 702 |