|  |  |  |  |
| --- | --- | --- | --- |
| **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 | 35x60 | 9560 | 5060 | 72 | 4 | 72 | 323 |
| \**Nosema apis*Na& *N. ceranae*Nc(Gisder & Genersch 2013) | 0.2 | 1.5 | 2 | 2.5 | 0.2Na0.2Nc | 0.2Na0.2Nc | 1 | 10 | 4| 95 | 35x60 | 9460 | 5860 | 72 | 5 | 72 | 297Na662Nc |
| *Apicystis bombi*(Meeus et al. 2010) | 0.4 | 1.5 | 2 | 1.25 | 0.5 | 0.5 | 1 | 10 | 2 | 94 | 35x30 | 9430 | 6045 | 72 | 3 | 72 | 260 |
| *Apidae*A(internal) and *Crithida bombi*Cb(Meeus et al. 2010) | 0.4 | 1.5 | 3 | 1.25 | 0.1A0.5Cb | 0.2A0.5Cb | 2 | 15 | 2 | 94 | 35x30 | 9430 | 5745 | 72 | 3 | 72 | 130A420Cb |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| **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 | 40x3 s | 953 min | 60 | 702 |