RT-PCR

|  |  |  |
| --- | --- | --- |
| Reagent | CHP (µl) | CDC (µl) |
| Water | 6.5 | 7.5 |
| 2x PCR buffer\* | 25 | 25 |
| MgSO4 (5 mM) | 2 | 2 |
| Primer MVN1 (5 uM) | 2 |  |
| Primer MVN2R (5 uM) | 2 |  |
| Primer MeV214 (10 uM) |  | 1.5 |
| Primer MeV216 (10 uM) |  | 1.5 |
| Enzyme mix\* | 2 | 2 |
| RNase Inhibitor (20 U/ul) | 0.5 | 0.5 |
| RNA | 10 | 10 |

\*Superscript III One-Step RT-PCR (Invitrogen)

Nested PCR

|  |  |  |
| --- | --- | --- |
| Reagent | CHP (µl) | CDC (µl) |
| Water | 28.75 | 34.7 |
| 10x PCR buffer\* | 5 | 5 |
| MgCl2 (25 mM) | 1 | 1 |
| dNTPs (2.5 mM) | 4 | 4 |
| Primer MVN3 (5 uM) | 5 |  |
| Primer MVN4R (5 uM) | 5 |  |
| Primer MeV210 (10 uM) |  | 1 |
| Primer MeV217 (10 uM) |  | 1 |
| Taq Polymerase (5 U/ul)\* | 0.25 | 0.3 |
| RT-PCR product | 1 | 3 |

\*Taq Polymerase (GE Healthcare)

Cycling conditions RT-PCR

|  |  |  |
| --- | --- | --- |
| Temperature (°C) | Time | Cycles |
| 55 | 30 min | 1 |
| 94 | 2 min | 1 |
| 94 | 15 sec | 50 CHP  40 CDC |
| 58 CHP, 55 CDC | 30 sec |
| 68 | 30 sec |
| 68 | 5 min | 1 |
| 10 | hold | 1 |

Cycling conditions Nested PCR

|  |  |  |
| --- | --- | --- |
| Temperature (°C) | Time | Cycles |
| 94 | 3 min | 1 |
| 94 | 30 sec | 30 CHP  40 CDC |
| 55 | 30 sec |
| 72 | 30 sec |
| 72 | 2 min CHP, 5 min CDC | 1 |
| 10 | hold | 1 |