# Laboratory Standard Operating Procedure for

# Avipoxvirus, Dr. Lisa Tell

1.) Primer Probe Mix

***Method***

1. Reconstitute primers with RNAase/DNAase-free water to 100pmol/µl concentration. Probes come reconstituted and can be used at stock concentration, also 100pmol/ul.
2. Combine primers and probes into yellow-capped 1.5mL tube with protocol below.
3. Primer/probe mix is good for about 1 year if kept in -20C freezer.

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| Avipoxvirus (Anna’s hummingbird specific):  (assay uses dual-labeled probe) |  |
| **vAAPV-124f (forward primer)** | 20.0µl |
| **vAAPV-246r (reverse primer)** | 20.0µl |
| **vAPV-159p (probe)** | 4.0 µl |
| **RNA/DNAase free water** | 196.0µl |
| **Total volume of PP mix (400 rxn):** | 240.0µl |
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|  |  |
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2.) Master Mix

-TaqMan Universal PCR Master Mix (cat # 4318157, AppliedBiosystems)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **AAPV** | n=1 | 10 | |  | 20 | |  | 30 | |  | 40 | |  | 50 | |  | 60 | |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Universal Master Mix | 6.00µl | 60.0 | |  | 120.0 | |  | 180.0 | |  | 240.0 | |  | 300.0 | |  | 360.0 | |
| Primer Probe mix | 0.58µl | 5.8 | |  | 11.6 | |  | 17.4 | |  | 23.2 | |  | 29.0 | |  | 34.8 | |
|  |  |  |  |  |
|  |  |  |  |  |
| Sample | 5.00µl | - | |  | - | |  | - | |  | - | |  | - | |  | - | |
| H2O | 0.42µl | 4.2 | |  | 8.4 | |  | 12.6 | |  | 16.8 | |  | 21.0 | |  | 25.2 | |
| Reaction volume | 12.00µl | 70.0 | |  | 140.0 | |  | 210.0 | |  | 280.0 | |  | 350.0 | |  | 420.0 | |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Master Mix per well: | 7.00µl |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **AAPV** | n=1 | 70 | |  | 80 | |  | 90 | |  | 100 | |  | 110 | |  | 120 | |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Universal Mastermix | 6.00µl | 420.0 | |  | 480.0 | |  | 540.0 | |  | 600.0 | |  | 660.0 | |  | 720.0 | |
| Primer Probe mix | 0.58µl | 40.6 | |  | 46.4 | |  | 52.2 | |  | 58.0 | |  | 63.8 | |  | 69.6 | |
|  |  |  |  |  |
|  |  |  |  |  |
| Sample | 5.00µl | - | |  | - | |  | - | |  | - | |  | - | |  | - | |
| H2O | 0.42µl | 29.4 | |  | 33.6 | |  | 37.8 | |  | 42.0 | |  | 46.2 | |  | 50.4 | |
| Reaction volume | 12.00µl | 490.0 | |  | 560.0 | |  | 630.0 | |  | 700.0 | |  | 770.0 | |  | 840.0 | |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Master Mix per well: | 7.00µl |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **AAPV** | n=1 | 130 | |  | 140 | |  | 150 | |  | 160 | |  | 170 | |  | 180 | |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Universal Master Mix | 6.00µl | 780.0 | |  | 840.0 | |  | 900.0 | |  | 960.0 | |  | 1020.0 | |  | 1080.0 | |
| Primer Probe mix | 0.58µl | 75.4 | |  | 81.2 | |  | 87.0 | |  | 92.8 | |  | 98.6 | |  | 104.4 | |
|  |  |  |  |  |
|  |  |  |  |  |
| Sample | 5.00µl | - | |  | - | |  | - | |  | - | |  | - | |  | - | |
| H2O | 0.42µl | 54.6 | |  | 58.8 | |  | 63.0 | |  | 67.2 | |  | 71.4 | |  | 75.6 | |
| Reaction volume | 12.00µl | 910.0 | |  | 980.0 | |  | 1050.0 | |  | 1120.0 | |  | 1190.0 | |  | 1260.0 | |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Master Mix per well: | 7.00µl |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

3.) Pipetting the qPCR plate

- 7µl master mix

- 5µl DNA

= 12µl total reaction volume

4.) qPCR cycling conditions

Stage 1: 2 min at 50oC

Stage 2: 10 min at 95oC

Stage 3: 40 cycles of:

15 sec at 95oC

1 min at 60oC

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| Prepared by | Samantha Mapes |  |  |
| Approved by | Samantha Mapes |  |  |

Signature Date