***Delftia* qPCR + Probe Assay**

**Purpose:** To confirm the presence of *Delftia acidovorans* in collected samples using a sequence-specific probe for the DACI\_4753 gold cluster gene

**Methods:**

1. Prepare a 96-well qPCR plate
2. Prepare master mix according to:

|  |  |
| --- | --- |
| Mix for 1 reaction |  |
| 5x Qiagen QuantiFast Pathogen master mix | 5μL |
| 10μM Forward Primer\* | 1μL |
| 10μM Reverse Primer\*\* | 1µL |
| 10μM DACI\_4753 probe\*\*\* | 0.5μL |
| 10x Internal Control Assay | 2.5μL |
| 10x Internal Control DNA | 2.5μL |
| RNase-free water | 11.5μL |
| Template DNA | 1μL |

Total volume is 25μL

1. Run in thermal cycler according to:

|  |  |  |
| --- | --- | --- |
| Initial Denaturation | 95 oC | 5 min |
| Denaturation | 95 oC | 15 sec |
| Annealing | 60 oC | 30 sec |
| Melt curve | 65 oC – 95 oC |  |

Steps 2-3 repeated 44x

Be sure to read FAM, not HEX for the probe, and VIC for the internal control

1. Store samples at -20 oC

\*Forward primer sequence : 5’ – AGG CCG AAG GTG TTT GAT T – 3’

\*\*reverse primer sequence : 5’ – TCT CGG TCT GGG AGA TCT TT – 3’

\*\*\*Probe sequence : 5’ - /56-FAM/CA CGC AGC A/ZEN/A AGC CAG GAA GTC /3IABkFQ/ - 3’