***Delftia acidovorans* detection qPCR**

**Purpose:** To amplify the DACI\_4753 gene in collected genomic DNA samples to determine which samples may contain *Delftia acidovorans*

**Methods:**

1. Obtain a clean 96-well qPCR plate
2. Dilute all genomic DNA samples to 5 ng/μL, including *D. acidovorans* SPH-1
3. Dilute primers to a 10μM working stock
4. Create Master Mix according to:

|  |  |  |  |
| --- | --- | --- | --- |
| Mix for 1 reaction |  | Mix for 80 reactions |  |
| SYBR green 2x master mix | 10μL | SYBR green 2x master mix | 800μL |
| 10μM Forward Primer | 1μL | 10μM Forward Primer | 80μL |
| 10μM Reverse Primer | 1μL | 10μM Reverse Primer | 80μL |
| PCR Grade H2O | 7μL | PCR Grade H2O | 560μL |
| DNA | 1μL | DNA per well | 1μL |

\*Total of 20μL per reaction

1. Load 1μL of 5 ng/μL DNA sample to each well; load samples in duplicate
2. Add 19μL of Master Mix to each well
3. Cover plate with stick-on film and roll over top to seal
4. Run in qPCR thermal cycler according to:

|  |  |  |
| --- | --- | --- |
| Initial Denaturation | 94 oC | 5 min |
| Denaturation | 94 oC | 30 sec |
| Annealing | 55 oC | 15 sec |
| Extension | 72 oC | 30 sec |
| Melt Curve | 65 oC to 95 oC | 0.5o increments for 5 sec |

\*Steps 2-4 repeated 40x

1. Run samples on agarose gel (100V) to confirm amplification
2. Store plate at -20 oC