TaqMan qPCR protocol for Detection of the Bacterial Blight Pathogen, *Xanthomonas citri* pv. *malvacearum*, from Cotton Seed

(Wang et al. 2018: https://doi.org/10.1094/PDIS-07-18-1150-RE)

Negative Control and Required Replications

Cotton seed that is unlikely to be infected, such as from a known resistant variety, grown in a field where the disease has not been observed, *Xanthomonas citri* pv. *malvacearum* (*Xcm*) cells and sterile water should serve as positive and negative controls, respectively. At least three independent replications are needed for each seed sample.

DNA extraction and qPCR assay:

1. Grind 10 grams of acid-delinted seed using a sterilized blender, such as one purchased from Waring, specifically the Blender 7011S, Torrington, CT, USA for 20 s.
2. Add 30 ml sterile distilled water to a 50-ml sterile Falcon tube containing the ground seed and vortex for 5 s (Setting no. 9, Vortex Genie; Scientific Industries, Bohemia, NY).
3. Place the seed samples on the lab bench for 20 min and collect the supernatant after centrifugation (300 *g* for 5 min) at 25ºC to remove large seed tissue pieces.
4. Centrifuge the supernatant (≈ 5 to 10 ml) at 4,750 *g* for 10 min to collect bacterial cells and extract genomic DNA from the resulting pellet using Powersoil DNA Isolation kit (Mo Bio Laboratories Inc., Carlsbad, CA) as described by the manual with the following modification as outlined below.
5. Break cells using a bead beater homogenizer (Mini Beadbeater-8, BioSpec Products, Inc. Bartlesville, OK, USA) (2 min at the maximum speed).
6. Measure DNA concentrations using a NanoDrop spectrophotometer (ThermoFisher Scientific, USA) and Genomic DNA of *Xcm* is used as a positive control and two negative controls (sterile distilled water and the genomic DNA from the non-*Xcm* infested seed) are included.
7. Conduct qPCR reaction on the StepOnePlus Real-Time PCR System with the Maxima Probe qPCR Master Mix (Thermo Fisher Scientific, Inc.). The qPCR is carried out in a 25 µl reaction volume containing 12.5 µl Maxima Probe qPCR Master Mix (2X), 0.75 µl of each forward and reverse primers (10 µM each), 0.5 µl of the probe (10 µM), ROX (300 nM), 100 ng DNA and nuclease-free water to make up to 25µl reaction volume.
8. The qPCR program (50ºC 2 min and then 95ºC 10 min followed by 40 cycles of 95ºC for 15 s and 60ºC for 1 min) is used based on the manufacturer's recommendations.
9. Record Ct values. Conduct at least three independent replications for each seed sample screened. And calculate the Ct means and their standard deviations.
10. The samples with significantly lower Ct values than that of the healthy seeds are considered *Xcm* positive.

Validation of qPCR results: An aliquot (0.5 ml for each sample) of the supernatant (collected from Step 4 above) is serially diluted on nutrient yeast broth (NBY) agar plates to isolate bacteria. Bacterial colonies same or similar to *Xcm* (colony morphology and color) are subsequently purified on fresh NBY agar plates and genomic DNA extracted using Wizard Genomic DNA Purification Kit for 16S rDNA sequence confirmation.

Notes: The parameters of centrifugation and the qPCR program are subject to change if different instruments are used in place of those outlined above. If so, further optimization may be needed. For the specific details of the protocol and primer and probe sequences, please refer to the full article in the journal Plant Disease (Wang et al. 2018: available on First Look at https://doi.org/10.1094/PDIS-07-18-1150-RE).