The purpose of this work instruction is to describe one-step reverse transcription conventional PCRs for confirmatory detection of pospiviroids (CEVd, CLVd, PCFVd, PSTVd, TASVd, TCDVd and TPMVd) and subsequent sequence analysis for identification of pospiviroids. This protocol is used for confirmation of the presence of pospiviroids following a tentative positive result in the real-time RT PCR assays.

I. Overall Pospiviroid Testing Workflow and LIMS information

This work instruction is to be used if the sample has a positive qPCR result using WI-B-T-1-63. If the sample failed to produce a valid Nad5 ABY result in Pospi C qPCR test, then the Internal Control Nad5 conventional PCR must be completed. If the sample produced a valid Nad5 ABY result in Pospi C qPCR test, the Internal Control Nad5 conventional PCR does not have to be completed. Use the outline below to determine which primer mix (pMix) to be used for the conventional PCR based off of the qPCR test results from WI-B-T-1-63.

If Pospi A qPCR tested positive in:	PSTVd FAM PCFVd VIC	Test using Generic Pospiviroid pMix
If Pospi B qPCR tested positive in:	CEVd_CLVd FAM	Test using **BOTH** Generic Pospiviroid and CLVd specific pMix
If Pospi C qPCR tested positive in:	TPMVd FAM	Test using Generic Pospiviroid pMix
If Pospi T qPCR tested positive in:	TASVd FAM	Test using Generic Pospiviroid pMix

In LIMS, assign the Pospi conventional RT-PCR test to the subsample of the lot that has the lowest Ct values. If there are mixed test results in the qPCR, assign the subsamples the Pospi Conventional RT-PCR. For instance; if one subsample tested positive for CEVd_CLVd FAM in Pospi B qPCR but all other subsamples tested negative for CEVd_CLVd FAM **AND** another subsample tested positive for another pospiviroid, both subsamples must be tested using the appropriate pMix(s) in accordance with this WI. The Pospi conventional RT-PCR test has 3 parameters assigned to it in LIMS: Pospi generic 195 bp, CLVd 370 bp, and Pospi Nad5 688 bp. Enter in the results (positive or negative) in the appropriate parameter for the pMix that is used for the conventional PCR testing. If the pMix was not used for testing, enter "N/A" in the appropriate result field. If the band is weak or faint, enter positive in results field and add a comment stating weak band.

II. Related Work Instructions

WI-B-T-2-16 Extraction of RNA from tomato seeds using Sbeadex Maxi Plant Kit
WI-B-T-1-63 Detection of Pospiviroids in Tomato Seeds using Multiplex RT-qPCR on Quant7.
WI-B-T-S-1 Gene Sequence Analysis of conventional PCR amplicons using SeqStudio

III. Equipment, Materials and Reagents

A. Equipment

1. PCR set-up workstation or dedicated PCR enclosure (any vendor)
2. Conventional PCR thermocycler (any vendor)
3. Micro centrifuge, bench-top, capable of ≥10,000 rpm (6,708 x g) (any vendor)
4. Vortex (any vendor)
5. Freezer, non-frost-free, capable of -20° C ± 2° C (any vendor)
6. Microwave (any vendor)
7. Analytical Balance, capable of weighing 0.5 mg to 20.5 g (any vendor)
8. Dedicated, annually-calibrated pipettes (P10, P50, P200, P1000, any vendor)
9. Gel Electrophoresis unit, capable of running a minimum of 14 samples (any vendor)
10. Horizontal orbital shaker (any vendor)
11. Power supply (any vendor)
12. Gel documentation system (any vendor)
13. 4200 TapeStation System (Agilent)
14. Vortex mixer IKA MS3 with adapter

B. Materials

1. Sterile filter (barrier) pipette tips (P10, P50, P200, P1000, any vendor)
2. Microcentrifuge tubes, 1.5-1.7 mL (pre-sterilized, certified DNase & RNase free, any vendor)
3. Thin-wall 0.2 mL PCR tubes (any vendor)
4. Ice
5. Gloves (any vendor)
6. Paper mat or towels, absorbent (any vendor)
7. Disposable, absorbent bench under pads (any vendor)
8. Loading tips (Agilent 5067- 5598, 1pk or 5067- 5599, 10pk)
9. Optical Tube 8x Strip (Agilent 401428) and Optical Cap 8x Strip (401425)
10. MicroAmp 8-Cap Strips/Adhesive Film for 96-Well Plate (C#: 4323032/C#: 4306311)
11. MicroAmp 8-Tube Strip/ 96-Well Reaction Plate (C#: 4316567/C#: 8010560)
12. 8-strip Septa/Plate Septa, 96 well (C#: A35643/C#: 4315933)

C. Reagents

1. SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase (100 Rx) (Invitrogen Cat. # 12574026)
2. D1000 ScreenTape (Agilent Cat. # 5067-5582)
3. D1000 Reagents (Agilent Cat. # 5067- 5583)
4. BigDye™ Terminator v3.1 Cycle Sequencing Kit (C#: 4337454)
5. BigDye XTerminator™ Purification Kit (C#: 4376486)
6. ExoSAP-IT™ Express PCR Product Cleanup (C#: 75001)
7. Genetic Analyzer Cartridge (SeqStudio C#: A32656)

8. Cathode Buffer Container (SeqStudio C#: A33401)
9. Integrated Capillary Protector (SeqStudio C#: A31923)
10. Molecular Grade (MG) Water (any vendor)\ 100 base pair DNA ladder (i.e.: BioMarker® Low or EXT Plus, BioVentures)
11. 10X TAE gel running buffer (or components for it)
12. Agarose (i.e.: UltraPure, Invitrogen # 15510-027)
13. 5 mg/mL Ethidium Bromide (any vendor)
14. PCR Purification kit (i.e.: Qiagen # 28704)
15. Gel Extraction Kit (i.e.: Qiagen # 28104)
16. Primers (see Tables 1, 2 & 3)

Order primers described in Tables 1 and 3 below from Integrated DNA Technologies, Inc.; Purification Standard Desalting:

Table 1: Generic Pospiviroid (PSTVd, TCDVd, CEVd, MPVd, TASVd, TPMVd and PCFVd) primer sequences (Verhoeven, et al., 2004)

Primer name *	Sequence 5'-3'	Working Concentration
Pospi1- FW	5' – GGG ATC CCC GGG GAA AC- 3'	10 µM
Pospi1-RE	5' – AGC TTC AGT TGT (T/A)TC CAC CGG GT- 3'	10 µM

Table 2. *Columnea latent viroid* (CLVd) specific primers (Olivier, et al., 2014)

Primer name *	Sequence 5'-3'	Working Concentration
Pospildeg-FW	5' – GGG AKC CCC GGG GMA AC- 3'	10 µM
pCLV4s	5' – GGG GCT CCT GAG ACC GCT C- 3'	10 µM

Table 3. Internal Control Nad5 primers (specific for plant genome) (Reference)

Primer name*	Sequence 5'-3'	Working Concentration
PNad5f	5' - GAT GCT TCT TGG GGC TTC TTK TT - 3'	5 µM
PNad5.656.F Complement	5' – GGT TGC CGC AAG GAA TGA - 3'	5 µM

IV. Primer preparation

Note: Primer stock and working solutions should be prepared after receipt of new reagents. Reagent solutions are stored in small aliquots in the freezer until needed. New reagents be tested using approved and validated positive and negative controls prior to testing samples.

When a PCR assay is being conducted, the analyst should use a 'working solution' tube of primers.

1. Tubes with lyophilized primers are centrifuged briefly (10-20 seconds at a minimum of 10,000 rpm (6,708 x g)) before opening to ensure that the lyophilized material is in the bottom of the tube.

The following steps must be done in a decontaminated PCR hood/enclosure:

2. Concentrated freezer stock solutions (100 µM) of primers:
   Primers are re-hydrated in a decontaminated PCR hood/enclosure to a **100 µM** stock solution with MG water. Mix well by vortexing at setting 7 for 30 seconds then centrifuge 10-20 seconds at a minimum of 10,000 rpm (6,708 x g) and place in ice for 30 minutes. Store at -20°C.

Note: To make 100 µM stock solution; use the lyophilized oligo concentration in nmol, multiply by 10. This is the total amount (µL) of nuclease free water should be added to the lyophilized primer.

3. Working concentration of primer mixes (pMix):
   a. **10** µM Generic Pospiviroid pMix: Add 100 µL of the 100 µM Pospi1- FW stock and 100 µL of the 100 µM Pospil1-RE stocks with 800 µL of MG water in a 1.5 mL micro centrifuge tube. Mix well by vortexing. Store in -20°C freezer in 100-200 µL aliquots.

   b. **10** µM CLVd-specific pMix: Add 100 µL of the 100 µM Pospildeg-FW stock and 100 µL of the 100 µM pCLV4s stock with 800 µL of MG water in a 1.5 mL micro centrifuge tube. Mix well by vortexing. Store in -20°C freezer in 100-200 µL aliquots.

   c. **5** µM Internal Control Nad5 pMix: Add 50 µL of the 100 µM PNad5f stock and 50 µL of the 100 µM PNad5mr stock with 900 µL of MG water in a 1.5 mL micro centrifuge tube. Mix well by vortexing. Store in -20°C freezer in 100-200 µL aliquots.

V. Master Mix preparation for Conventional RT-PCR One Step

Master Mix preparation must be done in a decontaminated PCR workstation/enclosure.

1. Remove all reagents from -20°C and thaw. Taq polymerase does not require thawing, place immediately on ice. Once frozen reagents are thawed, vortex briefly (5-10 seconds) and centrifuge briefly (10-20 seconds at a minimum of 10,000 rpm (6,708 x g)) to settle the liquid to the bottom of the tube. Place tubes in ice.

2. In a separate ice bucket, thaw prepared RNAs (if frozen) including all controls. Vortex the tubes briefly (3-5 seconds) at a speed setting of 7 and centrifuge 10-20 seconds at a minimum of 10,000 rpm (6,708 x g) to settle the liquid at the bottom of the tube. Place tubes in ice.

3. Prepare the Master Mix described in Table 4 and 5 for the desired number of samples, plus a positive, healthy, and non-template water control (NTC). To ensure adequate amount of master mix is made, prepare an additional 10% (multiply each reagent amount listed in tables 4 and 5 by 1.1) or for every 10 samples prepare 1 additional reaction. Lightly vortex the reaction mix and follow by a quick spin down in a centrifuge.

4. Pipette 48.0 µL into each 0.2 mL PCR tube. Lightly close the caps on the PCR tubes to prevent contamination during transportation to sample addition area. Ensure that the Master Mix is at the bottom of each tube by centrifuging the tubes for 10-20 seconds.

Do not add controls or samples RNA while working inside the PCR enclosure.

Table 4. RT-PCR Master Mix for Pospiviroid (generic primer) and CLVd (specific primer)

Reagents	Volume (µL) for each reaction	Final concentration
Molecular grade water	20	n/a
2X buffer	25	1X
Forward primer/Reverse primer mix **(10 µM mix)**	1	0.2 µM
SuperScript III RT/Platinum Taq Mix (2U/µL)	2	1u
Total	**48**	

Table 5. PCR Master Mix for Internal Control Nad5 (specific for plant genome)

Reagents	Volume (µL) for each reaction	Final concentration
Molecular grade water	20	n/a
2X buffer	25	1X
PNAD 5f & PNad5.656.F Complement Primer mix (5 µM mix)	1	0.1 µM
SuperScript III RT/Platinum Taq Mix (2U/µL)	2	1u
Total	**48**	

VI. Adding Test Samples and Controls

1. Take the PCR tubes containing the Master Mix to an assigned lab bench and place on a new disposable lab mat.
2. Add 2.0 µL of MG Water to the NTC PCR tube. Pipette up & down to mix.
3. Add 2.0 µL of the Healthy Seed Control that was prepared using WI-B-T-2-16. Pipette up & down to mix.
4. Add 2.0 µL of undiluted samples that was prepared using WI-B-T-2-16 to the appropriate PCR tube. Pipette up & down to mix.
5. Add 2.0 µL of the Positive Control(s) RNA to the appropriate tube. Pipette up and down to mix. The qPCR results will dictate which positive control(s) that must be run during the conventional PCR reaction. If the qPCR has multiple positive results, use ALL the controls as appropriate. See table 6 below for more information. The positive control may be extracted from a known positive sample that has been sequenced and matches the target Pospiviroid OR the control may be synthetic (G-block) containing the targeted sequence.

Table 6. cRT-PCR Positive Controls

If Pospi A qPCR tested positive in:	PSTVd FAM	PSTVd Positive Control
	PCFVd VIC	PCFVd Positive Control
If Pospi B qPCR tested positive in:	CEVd_CLVd FAM	CLVd using the CLVd pMix **AND** CLVd and CEVd using the generic pMix
If Pospi C qPCR tested positive in:	TPMVd FAM	TPMVd Positive Control
If Pospi T qPCR tested positive in:	TASVd FAM	TASVd Positive Control

6. After the addition of all samples and quality controls, close the caps and spin the PCR tubes in a micro centrifuge for 10 seconds.

Note: Do not use 50 µL of Master Mix as your NTC. The negative control indicates contamination of master mix reagents or contamination introduced by the analyst during sample addition, so it should be prepared in a similar manner including the addition of 2 µL of MG water instead of RNA.

VII. PCR Reaction

1. Turn on the thermocycler and allow the machine to run through its self-testing procedures. Place the PCR tubes into the thermocycler.
2. The PCR program is the same for all three reactions described in this WI. Enter the following reaction steps in the thermocycler or verify the saved protocol:
   - 50°C for 30 minutes
   - 94°C for 1.5 minutes
   - 15 cycles of the following:
     - 94°C for 30 seconds
     - 62°C for 90 seconds
     - 72°C for 45 seconds
   - 30 cycles of the following:
     - 94°C for 30 seconds
     - 59°C for 90 seconds
     - 72°C for 45 seconds
   - Extension 72°C for 7 minutes
   - Hold at 4°C
3. Start the Run

VIII. Electrophoresis

Note: Either the Agilent TapeStation or Gel Electrophoresis may be used to visualize the PCR reaction. It is not necessary to do both.

A. Agilent TapeStation Operating

1. Allow D1000 Reagents to equilibrate at room temperature for 30 minutes.
2. Launch the Agilent 4200 TapeStation Controller Software.
3. Flick the D1000 ScreenTape device and load it into the 4200 TapeStation instrument.
4. Place loading tips into the Agilent 4200 TapeStation instrument.
5. Vortex reagents and spin down before use.
6. Prepare ladder: For 1 – 15 samples: pipette 3 µL D1000 Sample Buffer and 1 µL D1000 Ladder at position A1 in a tube strip.
7. For each sample, pipette 3 µL D1000 Sample Buffer and 1 µL DNA sample in a well plate (5042- 8502) or a tube strip.
8. Apply foil seal to sample well plate and caps to tube strips with ladder or sample.
9. Mix liquids in sample and ladder vials using the IKA vortex at 2000 rpm for 1 min.
10. Spin down to position the sample and ladder at the bottom of the well plate and tube strip.
11. Load samples into the Agilent 4200 TapeStation instrument. Carefully remove caps of tube strips.
12. Place ladder in position A1 on tube strip holder in the 4200 TapeStation instrument.
13. Select required sample positions on the 4200 TapeStation Controller Software.

14. Click Start.
15. The Agilent TapeStation Analysis Software opens after the run is complete and displays results (Figure 1, 2 and 3).

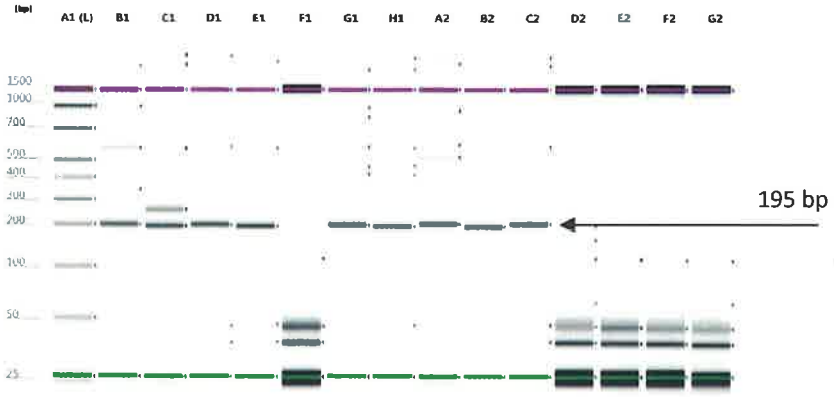

Figure 1. TapeStation Electrophoresis of PCR products for Generic detection of pospiviroids for One-Step RT-PCR . Lane A1(l): D1000 Ladder (Marker Sizes: 1500, 1000, 700, 500, 400, 300, 200, 100, 50, 25bp); Pospiviroid positive controls: Lanes B1 and C1 *Potato spindle tuber viroid* **(PSTVd)**, lane D1 *Tomato chlorotic dwarf viroid* **(TCDVd)**, lane E1 *Citrus exocortis viroid* **(CEVd),** Lane F1 *Columnea latent viroid* **(CLVd),** lane G1 *Mexican papita viroid* **(MPVd)**, lane H1 *Tomato apical stunt viroid* **(TASVd),** lane A2 *Tomato planta macho viroid* **(TPMVd)**, lane B2 *Pepper chat fruit viroid* **(PCFVd)** synthetic gene**,** lane C2 *Tomato chlorotic dwarf viroid* **(TCDVd)** seed extraction; lane D2 Healthy Tomato- RNA negative control 1, lane E2 Healthy Potato- RNA negative control 2, no target band present; Lanes F2 and G2: Buffer extraction and NTC (no bands expected).

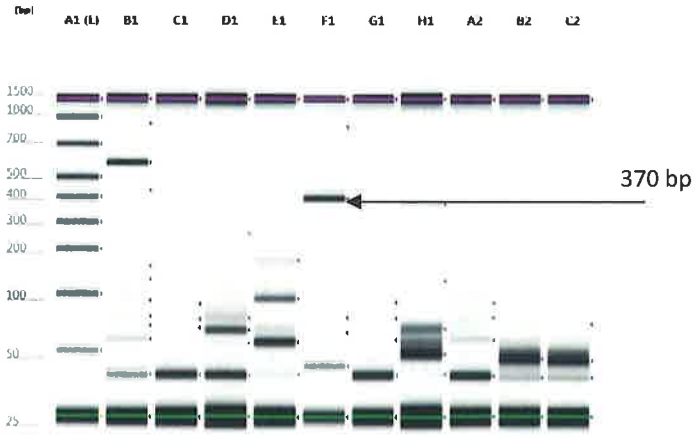

Figure 2. TapeStation Electrophoresis of PCR products for specific detection of CLVd for One-Step RT-PCR. Lane A1(l): D1000 Ladder (Marker Sizes: 1500, 1000, 700, 500, 400, 300, 200, 100, 50, 25bp); Pospiviroid positive controls: Lanes B1 (Non –specific band) and C1 *Potato spindle tuber viroid* **(PSTVd)**, lane D1 *Tomato chlorotic dwarf viroid* **(TCDVd)**, lane E1 *Citrus exocortis viroid* **(CEVd),** Lane F1 *Columnea latent viroid* **(CLVd),** lane G1 *Mexican papita viroid* **(MPVd)**, lane H1 *Tomato apical stunt viroid* **(TASVd),** lane A2 *Tomato planta macho viroid* **(TPMVd)**; lane B2 Healthy Tomato- RNA negative control 1, lane C2 Healthy Potato- RNA negative control 2, no target band present.

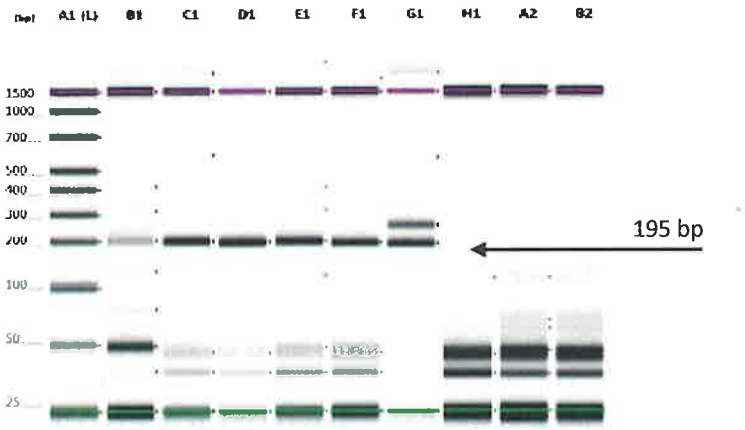

Figure 3. TapeStation Electrophoresis of PCR products for Generic detection of pospiviroids for One-Step RT-PCR. Lane A1(l): D1000 Ladder (Marker Sizes: 1500, 1000, 700, 500, 400, 300, 200, 100, 50, 25bp); Lanes B1, C1, D1 and E1 Pospiviroid positive samples; Lanes F1 and G1: Pospiviroid positive controls (1 and 2); H1: Negative sample, no target band present; Lanes A2 and B2: Buffer extraction and NTC (no bands expected).

B. Gel Electrophoresis

1. Prepare a 1.5% agarose gel(s) in 1X TAE buffer in accordance with JA-B-14 Agarose Gel Preparation. Gels should be sufficient size to accommodate the number of samples, plus controls and two lanes with DNA ladder flanking the PCR samples on each gel. Sample wells must be large enough to accommodate at least 15 µL.

2. Prepare DNA ladder, mix 4 µL of the 100 bp DNA ladder with 2 µL 6X loading buffer plus 6 µL water for each lane. Load 12 µL of ladder mixture on the left and right side of the sample PCR products.

3. Mix 8 µL of the PCR reaction with 2 µL of 6X loading buffer. Mix by pipetting up and down and load mixture into the gel. Load entire mixture into designated sample well.

4. Run the loaded gel in 1X TAE buffer for 1 hour at 100V (constant).

Note: The time to achieve good separation of the PCR products may vary depending on the equipment used, particularly the gel box and gel size. It is suggested to use the parameters specified above, if necessary adjust run time (not voltage) to achieve separation.

5. Stain the gels for 10-15 minutes in 0.58 µg/mL EtBr solution (35 µL of 5 mg/mL concentrated stock solution of EtBr in 300 mL of dH_2O) and destain 10-15 minutes in ddH2O, using a horizontal orbital shaker for each stage. Alternatively, GelRed may be used to stain the gel following the protocol outlined in JA-B-20.

6. Document the gel results using a digital imaging system.

7. Dispose of the gel, any contaminated gloves and paper toweling, into the EtBr hazardous waste receptacle.

Note: EtBr is a hazardous chemical. Please read MSDS before using.

IX. Assessment of the Generic Pospiviroid Conventional RT-PCR Results

1. Assess the Quality Controls. All controls must be valid in order to accept the sample results.
   a. **Ladders** must be distinct and well resolved in order to be valid. If the ladders are not distinct and/or well resolved, determine the cause and correct, then rerun the gel with the remainder of the PCR reactions for the samples and controls.

   b. **Healthy Plant Control** should **not** have a band of ~195 bp. If a band is present then the entire run is invalid and the conventional PCR reaction must be repeated. This result would indicate contamination with target pathogen, which may have occurred either during the cDNA preparation or the PCR run.

   c. **Non-template control** (NTC) should **not** contain a band of ~195 bp. If a band is present, then the entire run is invalid and all samples must be retested using this WI. This indicates contamination of the PCR run.

   d. **Positive control(s)**. All positive controls ran with the generic pMix **must** have one distinct band of ~195 bp. If no ~195 bp band is present, the run is invalid and all samples and controls must be retested using this WI. This indicates that the PCR reaction failed, typically a reagent was not added into the master mix or the control was not added to the reaction tube.

2. Assess the sample: If a ~195 bp is present, the sample tested positive for the presence of Pospiviroid. The sample should be sequenced to determine the particular Pospiviroid. If no band is present, the sample is considered negative for the presence of Pospiviroid.

X. Assessment of *Columnea latent viroid* (CLVd) Specific Conventional RT-PCR Results

1. Assess the Quality Controls. All controls must be valid in order to accept the sample results.
   a. **Ladders** must be distinct and well resolved in order to be valid. If the ladders are not distinct and/or well resolved, determine the cause and correct, then rerun the gel with the remainder of the PCR reactions for the samples and controls.

b. **Healthy Plant Control** should **not** have a band of ~370 bp. If a band is present then the entire run is invalid and the conventional PCR reaction must be repeated. This result would indicate contamination with target pathogen, which may have occurred either during the cDNA preparation or the PCR run.

c. **Non-template control** (NTC) should **not** contain a band of ~370 bp. If a band is present, then the entire run is invalid and all samples must be retested using this WI. This indicates contamination of the PCR run.

d. **CLVd Positive Control** must have one distinct band of ~370 bp. If no ~370 bp band is present, the run is invalid and all samples and controls must be retested using this WI. This indicates that the PCR reaction failed, typically a reagent was not added into the master mix or the control was not added to the reaction tube

2. Assess the sample: If a ~370 bp is present, the sample tested positive for the presence of CLVd. The sample should be sequenced to confirm the results. If no band is present, the sample is considered negative for the presence of CLVd.

XI. Assessment of Nad5 Internal Control RT-PCR Reaction

This test is performed to verify the quality of the DNA extracted from the tomato seed samples. It is only performed if the Nad5 ABY result in Pospi C qPCR is invalid. The Nad5 internal control of the test sample should produce clear band of ~688 bp. If the band does not exist or not clear, the sample should be retested using this WI. If after the second retest the sample does not produce a clear ~688 bp band, the sample should be re-extracted using WI-B-T-2-16 and retested with WI-B-T-1-63.

XII. Sequencing

Follow the protocol as outlined in WI-*B-T-S-1 Gene Sequence Analysis of conventional PCR amplicons using SeqStudio.*

XIII. Download and Assembling Sequences Using GENEIOUS

1. In Geneious, Select all ABI files then copy and paste into a folder for the samples to be evaluated by sequencing analysis.
2. Import the ABI files for the sequences amplified with forward and reverse primers.
3. Process for assembling sequences
4. Select the Forward and Reverse files for the sample to be processed.
5. Select Align/Assemble.
6. Select Novo Assemble.
7. Click OK.

8. Go to the beginning of the sequence and select the area that needs to be discarded (Cursor bottom to up, then right to left). Trim until have nice strong peaks. Right Click the mouse to "Delete Selected Bases".
9. Go to the end of the sequence and select the area that needs to be discarded (Cursor bottom to up, then left to right). "Right Click" on "Delete Selected Bases." This will produce a clean "Consensus Sequence."
10. Click on "Consensus."
11. Select "Extract".
12. The system will prompt the Extraction Name.
13. Manually add the information of the sample. Click OK and save. A prompt will appear "Do you wish to apply changes to the original sequences?" click "Yes."
14. Copy Name and Sequence and paste into a Notepad

XIV. Run General BLAST Analysis using NCBI:

1. Copy the sequence into BLAST https://blast.ncbi.nlm.nih.gov/Blast.cgi

2. Click "identity" to list the pospiviroids with the highest percent match at the top of the list. Observe that sequences have 100% alignments, and a long list of Pospiviroid and other species align with 99%. This indicates that the sequence corresponds to a Pospiviroid.

XV. References

1. Monger, W., Tomlinson, J., Boonham, N., Marn, M.V., Plesko, I.M., Molinero-Demilly,V., Tassus, X., Meekes, E., Toonen, M., Papayiannis, L., Perez-Egusquiza, Z., Mehle, N., Jansen, C., Nielsen, S.L., (2010) Development and inter-laboratory evaluation of real-time PCR assays for the detection of pospiviroids. J. Virol. Methods 169, 207–210.

2. Olivier, T., Demonty, E., Fauche, F., and Steyer, S. (2014). Generic detection and identification of pospiviroids. Arch Virol (2014) 159:2097–2102

3. Verhoeven, J. Th. J., Jansen, C. C. C., Willemen, T. M., Kox, L. F. F., Owens, R. A., Roenhorst, J. W., (2004). Natural infections of tomato by Citrus exocortis viroid, Columnea latent viroid, Potato spindle tuber viroid and Tomato chlorotic dwarf viroid. Eur. J. Plant Pathol. 110, 823–831

Document Revision History

Status	Revision Number	Effective Date	Description	Revised By
Original	Original	3/12/2019	To baseline the work instruction	Marco Galvez Avijit Roy
Revision	01	See electronic signature	Add footer	Ashlee Barth
These Revisions Require Staff Re-Training? Yes (No)				

Authors: Marco Galvez Gargurevich, NCSU Research Assistant, CPHST-Beltsville Laboratory
Avijit Roy PhD, Molecular Biologist, CPHST-Beltsville Laboratory

Approved By: Ashlee K. Barth CPHST-Beltsville Laboratory Quality Manager

ASHLEE BARTH Digitally signed by ASHLEE BARTH
Date: 2019.03.22 07:43:07 -04'00'

Approved By: Mark Nakhla, Ph.D. CPHST-Beltsville Laboratory Director

MARK NAKHLA Digitally signed by MARK NAKHLA
Date: 2019.03.27 09:01:47 -04'00'