Title: qPCR method for the Detection of *Salmonella* in Papaya –General Laboratory Procedure

Overview

A real-time PCR method has been developed by FDA Pacific Regional Laboratory Southwest (PRL-SW) with custom designed primers and TaqMan probe to detect the presence of a 262-bp fragment of the *Salmonella*-specific *invA* gene. The method supports the utility of real-time PCR as a diagnostic tool in testing food samples for *Salmonella* contamination and, in turn, enhancing consumer protection and the public health. This assignment is special procedure for detecting *Salmonella* in papayas.

Procedure

A. Equipment & Materials

1. Micro centrifuge
2. Sterile micro centrifuge tubes
3. Micro pipettes (2-20 µL, 20-200 µL, 100-1000 µL, or equivalent)
4. Aerosol barrier pipette tips to fit pipettes
5. Gloves
6. 100°C heater block
7. Vortex mixer
8. qPCR equipment

Option 1: SmartCycler® II (Cepheid, Sunnyvale, CA)

- SmartCycler® reaction tubes, 25 µL, Cat. No. 900-0085 (Fisher Scientific)
- SmartCycler® cold blocks and racks (Cepheid, Sunnyvale, CA)
- SmartCycler® mini-centrifuge (Model C-1200, Labnet International Inc., Woodbridge, N.J.)

Option 2: ABI 7500 Fast system (Foster City, CA)

- ABI Fast 96-well plate, Cat no. 4346906 (Life Technologeis)
- ABI Optical adhensive film, Cat no. 4360954 (Life technologies)
- 96-well plate centrifuge

B. Media & Reagents

1. Modified Buffer Peptone Water (mBPW), see Appendix 1.
2. *Salmonella* qPCR Master Mix (Dehydrated form),
   See instruction in Appendix 2 & 8. The Master Mix may be obtained by contacting FDA, Division of Field Science at 301-796-6586
3. qPCR reagents
   (A) Smart Cycler II:
   Ready-To-Go PCR Beads Cat. No. 27-9558-01, GE Healthcare, Inc. Piscataway, NJ)
   (B) ABI 7500 Fast system
   VeriQuest Probe qPCR Master Mix (2X) (Cat#:75650 Affymetrix, Inc.)
4. DNase-free, RNase-free water
5. Tetrathionate (TT) broth (Difco)
6. Rappaport-Vassiliadis (RV) medium (see BAM for preparation)
7. Tryptic Soy Agar (TSA) (Difco)
8. 0.85% Saline
9. Positive control (*Salmonella* Gaminara ATCC 8324, or other available strain)

10. Negative control (*Klebsiella pneumoniae* ATCC 13883, or other available strain)

C. Sample preparation

1. Aseptically place the whole papaya sample into a Whir-Pak bag.
   Add the volume that allow the papayas to float (1-1.5 W/V) of mBPW to each papaya sample.
2. Close bag and shake gently.
3. Let the sample soak in enrichment broth for 2 hours at room temperature.
4. Aseptically remove papaya from the bag.
5. Incubate individual enrichment broth at 42 ^{o}C incubator for 24 hours. Positive and negative controls are prepared concurrently by inoculating the appropriate cultures into 10mL of mBPW and incubating using the same conditions as for the samples.
6. After incubation, the sub sample pre-enrichments can then be "wet composited" as described below or sub samples can be analyzed individually.

Wet compositing procedure:

(a) For qPCR procedure

From each of 5 incubated sub samples, remove **1** ml of each pre-enriched mBPW broth into one sterilized tube (total 5 mL of one composite).

(b) For cultural BAM procedure

- From each of 5 incubated sub samples, remove **0.1** ml from pre-enrichment mBPW broth and place into a tube or flask containing 50 ml Rappaport-Vassiliadis (RV) medium. For the other 5 incubated sub samples, remove **0.1** ml mBPW broth and place into a tube or flask containing 50 ml RV medium. Incubate the 2 RV medium composites at 42 ± .2º C in a circulating, thermostatically controlled water bath for 18-24 h.
- Additionally, from each of 5 incubated sub samples, remove **1.0** ml mBPW broth pre-enrichment and place into a tube containing 50 ml tetrathionate (TET) broth. For the other 5 incubated sub samples, remove **1.0** ml mBPW broth and place into a tube containing 50 ml TET broth. Incubate the 2 TET broth composites at 42 ± .2º C in a circulating, thermostatically controlled water bath for 18-24 h.
- Analysis of RV and TET composites proceeds according BAM method.

7. Perform qPCR procedure from 24 h enriched mBPW broth (individual or composite).
8. Perform the qPCR from each individual sub-sample if the qPCR result is positive from composite samples.
9. Continue the BAM procedure from each qPCR positive sub samples.

D. Cultue cofirmation

For qPCR positive sample, refer to the procedure described in the BAM (Bacteriological Analytical Manual, the BAM On-line, Chapter 5, *Salmonella* procedure "http://www.cfsan.fda.gov/~ebam/bam0toc.html"). Transfer 1 ml and 0.1 ml of incubated mBPW broth into 10 mL of TT broth (Tetrathionate), and 10 ml of RV (Rappaport-Vassiliadis) broth, respectively. Incubate at 42 ^{o}C for 24 ± 2 h. All three selective media, Hektoen Enteric Agar (HE), Xylose Lysine Desoxycholate Agar (XLD), and Bismuth Sulfite Agar (BS) must be used according to the BAM procedure.

E. qPCR Procedure

24 h-qPCR

After enrichment, transfer 250 µL of each individual (or composite) incubated mBPW broth into separate 1.5 mL eppendorf tubes and prepare DNA templates (see Section H). Run qPCR on
1. SmartCycler® -see Sections E through J, Appendices 2-7 and see Section K to interpret results.
Or
2. ABI 7500 FAST system-see Sections E through J, Appendices 8-17 and see Section K to interpret results

48 h-qPCR –positive samples from 24 h-qPCR if Ct > 35
Combine 250 µL of each incubated TT and RV broth into one 1.5 ml eppendorf tube and prepare DNA template as in Section H. Run qPCR on
1. SmartCycler® see Sections E through J, Appendices 2-7 and see Section K to interpret results.
Or
2. ABI 7500 FAST system--see Sections E through J, Appendices 8-17 and see Section K to interpret results

F. PCR work flow

Note: *Laboratories must have in place a facility specific PCR work flow SOP to properly separate the preparation of the PCR master mix, the addition of test sample and the SmartCycler® operation to eliminate the possibility of contamination*.

Designated work areas are required for performing PCR It is recommended that pre-PCR manipulations be performed in two separated laminar flow hoods. The "clean" hood is used for Master Mix, qPCR reaction mix and reagent preparation. The "dirty" hood is for preparation of sample DNA template preparation and addition of templates to the SmartCycler® tubes. The use of aerosol resistant pipette tips is required to minimize the chance of crossover contamination associated with using micropipetters. Good laboratory practices and aseptic technique should also be followed to minimize sample or reagent contamination. Once the master mix has been prepared and distributed into Smart-Cycler tubes in the "clean" hood, the tubes are transported to the "dirty" hood for addition of template DNA. For regulatory samples, positive-, negative-, and reagent controls shall be included with each amplification experiment. Open tubes one at a time to aliquot samples. Close promptly after each sample has been added. Distribute negative controls first. Next, distribute test samples with positive controls distributed last. Verify that all tubes are securely capped before removing from the hood for transport to a SmartCycler® which is located in a designated post-PCR area.

G. qPCR Reaction Mix preparation and set up

(a) qPCR Reaction preparation

Smart Cycler II

1. Follow the guideline in Appendix 2 to prepare the qPCR Reaction Mix containing qPCR *Salmonella* Master Mix and Ready-to-Go PCR beads. *Note: This solution should be prepared and distributed into SmartCycler® tubes in the "clean" hood or similarly designated area.*
2. Add 22.5 µL PCR qPCR Reaction Mix to each reaction tube held in loading racks or the SmartCycler® cold blocks. Close all the tubes securely.
3. Remove the racks or cold blocks containing the tubes with the qPCR Reaction Mix to the sample addition area (see Section H).

ABI 7500 Fast

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See Appendices 8

(b) qPCR set up

Smart Cycler II- See Appendices 3-7

ABI 7500 Fast- See Appendices 9-17

H. DNA template preparation

Note: *Before beginning this procedure, preheat heating block to 100°C or prepare a boiling water bath and verify that this temperature has been reached and is maintained during the heating. The templates are prepared in the "dirty" hood or similarly designated area.*

1. Positive DNA template preparation from the *Salmonella* control strain
   Transfer one colony of *Salmonella* Gaminara ATCC 8324 reference strain (or other available strain) from TSA plate (overnight fresh culture) into 1.5 mL eppendorf tube containing 250µL DNAase free pure water. Heat the suspended culture at 100 °C for 20 minutes then cool in ice for 5 minutes. Centrifuge at 8000 rpm for 5 minutes then transfer the supernatant into a new eppendorf tube. Use as stock DNA template and store at -70 °C when not in use. The concentration of positive control DNA template should be adjusted by dilution (if it is too high) to generate Ct values in the range of 30-35.

2. Sample DNA template preparation
   (a) Aliquot 250µL of mBPW sample broth enrichment or combine 250 µL from each incubated (42°C for 24 hr) TT and RV broth directly to a micro-centrifuge tube .
   (b) Centrifuge at 10,000 x g (~8,000 rpm) for 5 min. Remove the supernatant without disturbing the pellet.
   (c) Add 250 µL DNase-free, RNase-free water and re-suspend the pellet. Repeat step 2b one more time if necessary.
   (d) Heat the micro centrifuge tube containing the re-suspended pellet at 100°C for 20 min then cool in ice for 5 min.
   (e) Centrifuge at 10,000 x g (~8,000 rpm) for 5 min.
   (f) The supernatant can be used directly as template for the real-time PCR assay (keep at 4°C), or at -70°C for long term storage.

I. Addition of Samples to SmartCycler® tubes

1. Ensure the prepared SmartCycler® tubes containing the qPCR Reaction Mix are properly labeled and have been placed in the sample addition area ("dirty" hood or similarly designated area).
2. Opening one reaction tube at a time, add 2.5 µL template (prepared as described above in section G) to the appropriate reaction tube. **Note: Check the pipette tip before and after releasing 2.5 µL template into each tube to make sure the template has been added.**
3. Completely close the reaction tube.
4. Spin briefly to bring the PCR mix with sample down to the bottom of the tube.

J. Running Samples in the SmartCycler®

1. Configure the qPCR instrument defining the instrument "Protocol" and "Graphs" on the SmartCycler® following the procedures outlined in Appendices 3 & 4.

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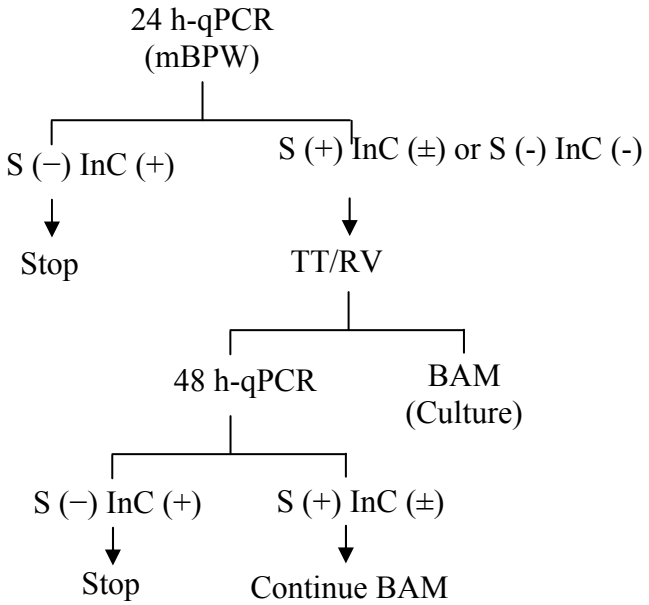
1. Add tubes containing samples and controls to the SmartCycler® and start run.

K. Interpretation and of results and actions to be taken (See Appendix 5)

The variety and variable nature of food matrices and the presence of inhibitory factors can impact qPCR amplification. The inclusion of the Internal Control (IC) monitors the efficiency of the qPCR reaction. In cases where the IC fails to perform as expected (see below) whether or not the target amplifies, the enrichment is transferred to RV/TT and incubated for 24hrs. This procedure serves both to dilute out inhibitors as well as enriching for the target *Salmonella*. Both 48hr qPCR and BAM culture confirmation are performed. Follow the guidelines in the following table.

	Sample (FAM)	Internal Control (Cy5)	Action
1	–	+	Stop, report as negative
2	+	+ or –	Report as can not rule out and continue to perform 48-h qPCR (if Ct>35) and BAM
3	–	- or Ct ≥ 3 units above the Ct value of the IC for the negative control sample	Re-run. If same result is obtained, perform 48-h qPCR and BAM. Report results of BAM analysis.



S = sample
InC = Internal Control

L. Confirmation

Confirmation of *Salmonella* culture isolates must be made in conjunction with presumptive qPCR results.

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Reference

(1) Cheng, C.-M., W. Lin , K. T.Van, L. Phan, N. T. Tran and D. Farmers. 2008. Rapid Detection of *Salmonella* in Foods using Real-Time PCR. J. Food. Prot. 71:2436-2441.

(2) Cheng, C.-M., K. T.Van, W. Lin, R. M. Ruby. 2009. Inter-Laboratory Validation of a Real-Time PCR 24-Hour Rapid Method for Detection of *Salmonella* in Foods. J. Food. Prot. 72:945-951.

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Appendix 1

MODIFIED BUFFERED PEPTONE WATER

mBPW can be prepared using either one of the following formulations:

Recipe 1.

Bio-Gel Peptone (Pancreatic Digest of Gelatin)	10g
Sodium Chloride	5g
Disodium Phosphate	7g
Monopotassium Phosphate	3g
Distilled Water	1.0 liter

Heat with gentle agitation to dissolve. Autoclave 15 min at 121°C. Final pH, 7.2 ± 0.2.

Recipe 2
(1) Buffered Peptone Water (BD cat# 218105)

20 g per liter contains:

Bio-Gel Peptone (Pancreatic Digest of Gelatin)	10g
Sodium Chloride	5g
Disodium Phosphate	3.5g
Monopotassium Phosphate	1.5g

(2)

Disodium Phosphate	3.5g
Monopotassium Phosphate	1.5g

Take (1) 20 g then add (2) which is equivalent to Recipe 1

Distilled Water	1.0 liter

Heat with gentle agitation to dissolve. Autoclave 15 min at 121°C. Final pH, 7.2 ± 0.2.

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Appendix 2 SmartCycler II-

Instructions for Rehydrating *Salmonella* Master Mix and Preparing qPCR Reaction Mix

(a) Storage & Preparation

1. The dehydrated Master Mix from PRL-SW must be kept in the sealed aluminum foil pouch at ambient temperature until used. On the day of PCR run, take out sufficient master mix tubes (40 reactions per tube) and re-hydrate with **900 µL** of DNase-free, RNase-free water in one tube. Wait for 10 minutes then **vortex** and spin briefly and keep the tube in ice. Calculate and take sufficient re-hydrated Master Mix (22.5 µL per reaction) into one clean 1.5 mL eppendorf tube, add **Ready-To-Go PCR bead** (**1.5 beads per reaction**, i.e. 3 beads per 2 reactions). The FDA PRL-SW *Salmonella* Master Mix **DOES NOT** contain Taq® polymerase, dNTPs, $MgCl_2$ and buffer. **The addition of the Ready-To-Go PCR bead is essential.**
2. If the sample is odd number, round it up to an even number. For example, if you have 9 samples, then prepare 10 samples, i.e. take 225 µL (22.5 µL X 10) of re-hydrated Master Mix solution and add 15 Ready-To-Go PCR beads.
3. Wait for 5 minutes until the liquid is clear then spin briefly. Aliquot 22.5 µL of the qPCR Reaction Mix into each SmartCycler® tube. Vortex , then spin down the tube briefly to be sure the qPCR Reaction Mix is entirely in the tube..

   Note: These operations should be performed in the "clean" hood or similarly designated area.
4. The remaining re-hydrated Master Mix should be stored at refrigerator (4 °C) and used within 2 months. Do not freeze and thaw.
5. Remove SmartCycler® tubes containing the qPCR Reaction Mix to the "dirty" hood (or similarly designated area). Add 2.5 µL of sample or control DNA template into each SmartCycler® tube. Take care to inspect the pipette tip to ensure all the sample has been delivered into the tube. It is recommended to use the prepared qPCR Reaction Mix within an hour of preparation.
6. The dehydrated Master Mix is stable for 1 year at ambient temperature in the pouch. (The shelf-life/stability of the dehydrated Master Mix is still in progress.)

(b) Quality Check

Each lab needs to prepare DNA template from the *Salmonella* positive control strain*. The Ct value of *Salmonella* positive control should be in the range between **30 and 35**. The "Internal Control" is included in the Master Mix and the Ct value is around **35**. Freshly re-hydrated Master Mix is used to determine the Ct value and the maximum fluorescence response unit. The same DNA template will be used as the positive control. The positive control and the DNase-free, RNase-free water used as negative control should be included for each PCR run. The known Ct value and the maximum fluorescence response unit of positive control are the two indices for evaluating if the Master Mix performs as it supposed to be. Any significant increase in the Ct value (> 10%) or decrease in the maximum fluorescence response unit (> 50%) is considered a sign of degraded quality of Master Mix and the sample should be retested using a freshly re-hydrated Master Mix.

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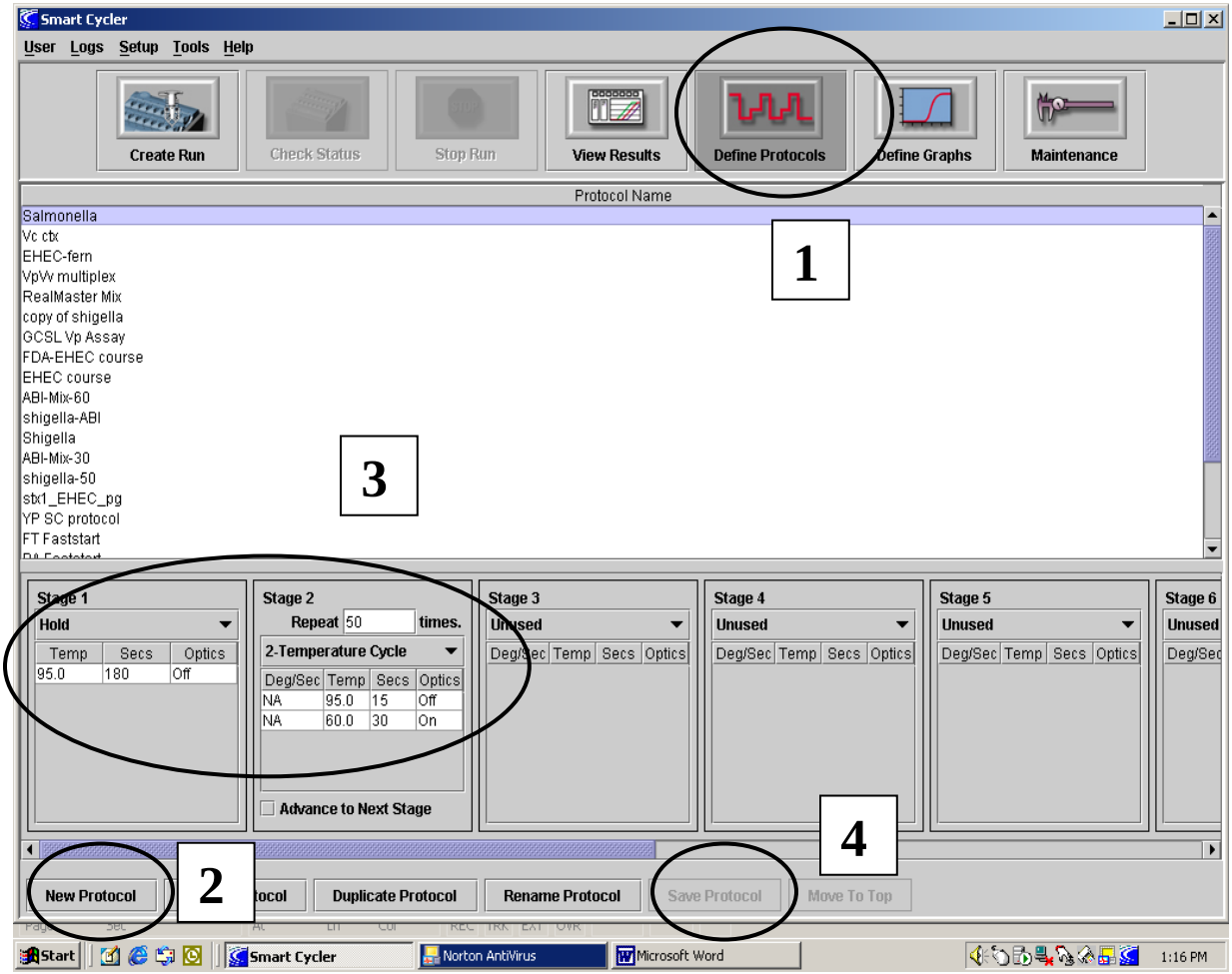
Appendix 3: SmartCycler II- Defined Protocols and Graphs (Page 1 of 2)

Define Protocols

1. *Click on "Define Protocols"*
2. *Click on "New Protocol" and type "Salmonella" and "OK".*
3. Key in the following parameters:
   Stage 1: Hold
   95 °C; 180 sec; optics off
   Stage 2: 2-Temperature Cycle
   Repeat 50 times
   95 °C; 15 sec; optics off
   60 °C; 30 sec; optics on
4. Save Protocol



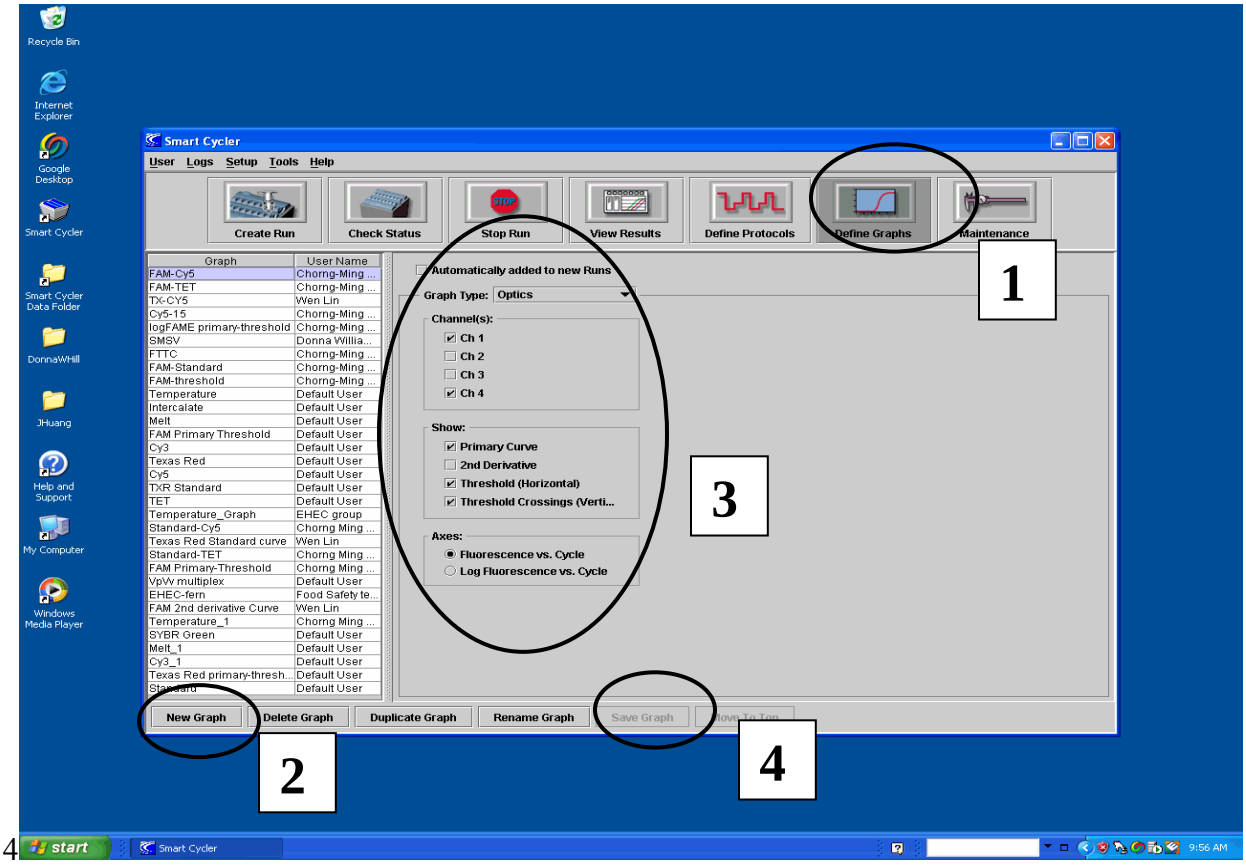
Title: qPCR method for the Detection of *Salmonella* in Papaya –General Laboratory Procedure

Appendix 4: SmartCycler II- Defined Protocols and Graphs (Page 2 of 2)

Define Graphs

1. Click on "Define Graphs"
2. Click on "New Graph" and key in "FAM-Cy5" and "OK".
3. Mark the following parameters:
   Graph Type: Optics
   Channel(s): 1 and 4
   Show: Primary curve
   Threshold (Horizontal)
   Threshold Crossings (Vertical)
   Axes: Fluorescence vs. Cycle
4. Save Graph

Note: Salmonella = FAM = SmartCycler® I and II, Channel 1
Internal Control=Cy5= SmartCycler® I and II, Channel 4

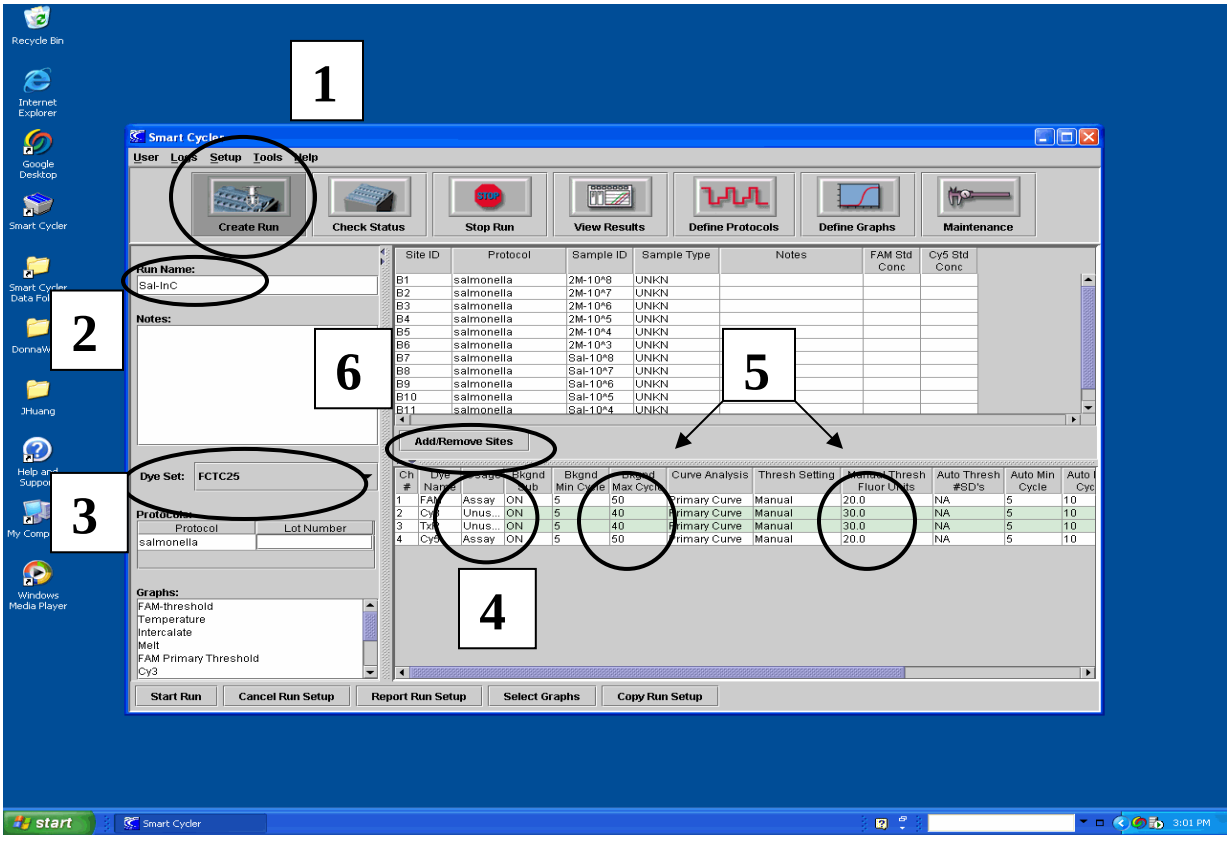


Title: qPCR method for the Detection of *Salmonella* in Papaya –General Laboratory Procedure

Appendix 5: SmartCycler II- Creating and Starting a Run (Page 1 of 2)

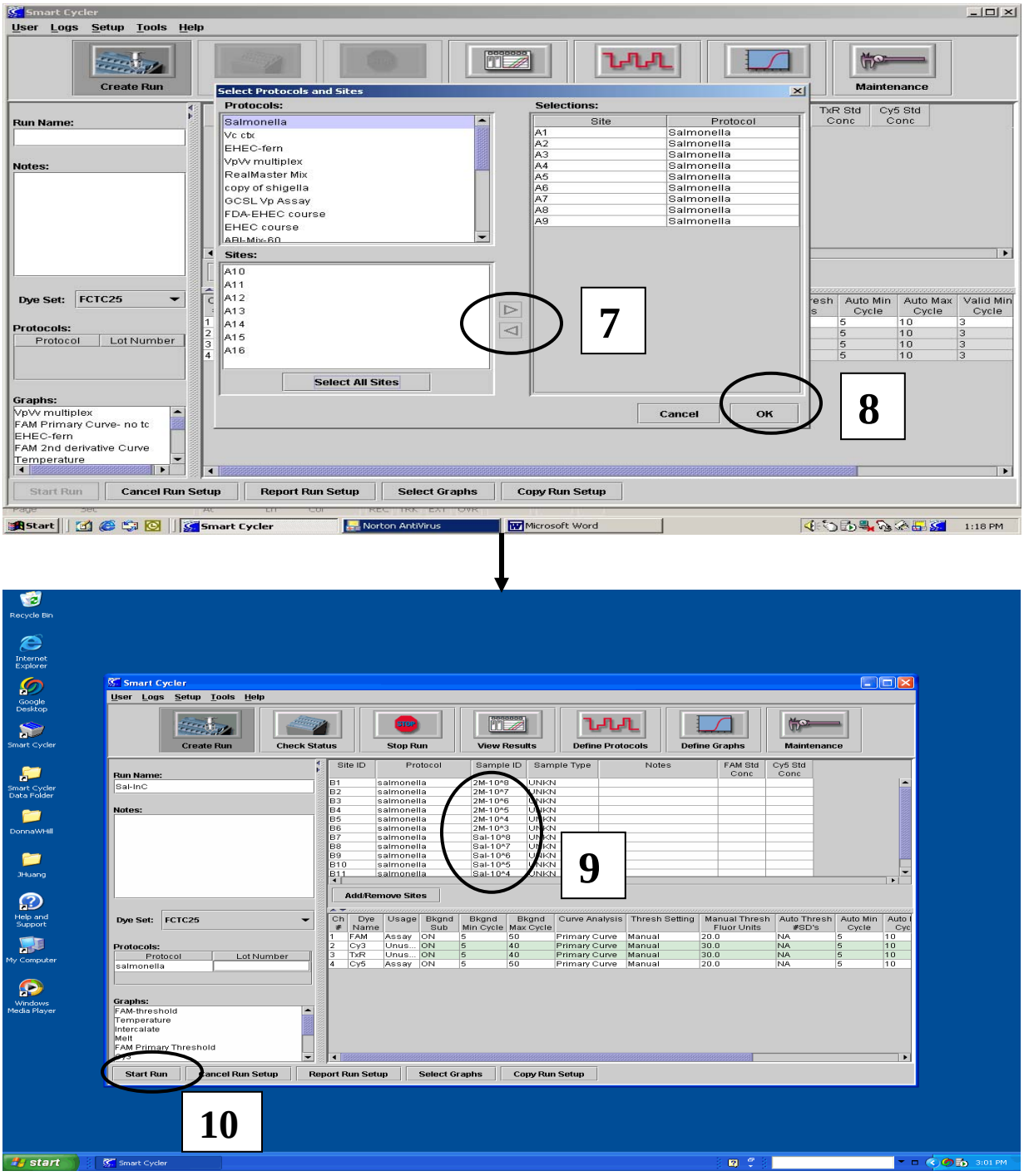
Create Run

1. Click on “Create Run”
2. Run Name: Name Experiment-e.g. ML-JH-pepper-BPW, ML-JH-pepper-TT.
3. Dye Set: Select “FCTC25”.
4. From “Usage”, change Cy3, and TxR channel on SmartCycler® II to “unused”.
5. Change “Bkgnd Max Cycle” to 50 and “Manual Thresh Fluor Units”=20 for both “FAM” and “Cy5”, and retain all others use default.
6. Click on Add/Remove Sites.
7. Select appropriate protocol-“Salmonella” and associate with Block/sites (A1, A2, ... etc.).
8. Click on “►”, then “OK”.
9. Enter sample identification information.
10. When all SmartCycler® tubes are in the associate sites, click on “Start Run



Title: qPCR method for the Detection of *Salmonella* in Papaya –General Laboratory Procedure

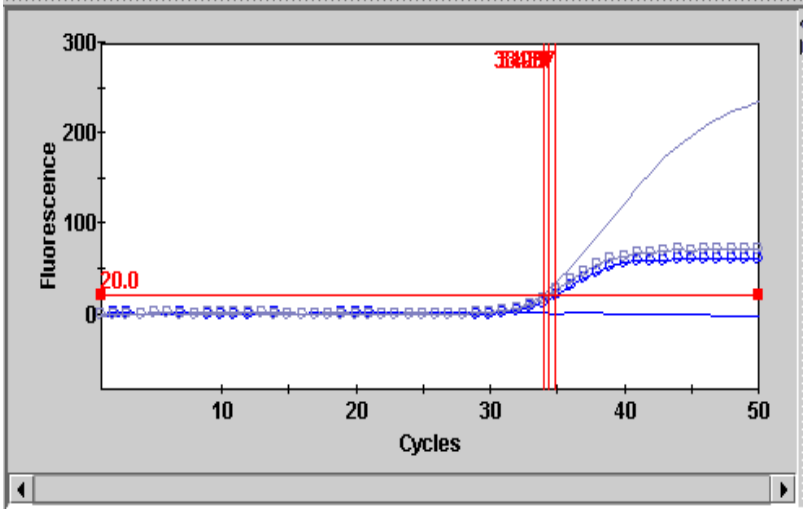
Appendix 6: SmartCycler II- Creating and Starting a Run (Page 2 of 2)



Title: qPCR method for the Detection of *Salmonella* in Papaya –General Laboratory Procedure

Appendix 7: SmartCycler II- qPCR *Salmonella* report

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	Salmo... Std/Res	Salmonella Ct	InC Std/Res	InC Ct
B9	salmonella	NEG-H2O	UNKN		OK	NEG	0.00	POS	34.87
B10	salmonella	POS-S. Gaminera ATCC 8324	UNKN		OK	POS	33.95	POS	34.39



Sit...	Protocol	Sample ID
B9	salmonella	NEG-H2O
B10	salmonella	POS-S. Gaminera ...

Note: FAM-*Salmonella,* Cy5- Internal Control

Title: qPCR method for the Detection of *Salmonella* in Papaya –General Laboratory Procedure

Appendix 8: ABI 7500 Fast System- Instructions for Rehydrating *Salmonella* Master Mix and Preparing qPCR Reaction Mix

(1) Rehydrating qPCR *Salmonella* Master Mix

The dehydrated Master Mix from FDA must be kept in the sealed aluminum foil pouch at ambient temperature until used. On the day of PCR run, take out sufficient master mix tubes (50 reactions per tube) and re-hydrate with **90 µL** (10X) of DNase-free, RNase-free water in one tube for ABI 7500 FAST. Vortex and wait for 10 minutes then **vortex** again, spin briefly and keep the tube in ice. The remaining re-hydrated Master Mix should be stored at refrigerator (4 °C) and used within 2 months. Do not freeze and thaw. The FDA PRL-SW *Salmonella* Master Mix **DOES NOT** contain Taq® polymerase, dNTPs, $MgCl_2$ and buffer. **The addition of the VeriQuest Probe qPCR Master Mix for ABI 7500 FAST and Ready-To-Go PCR bead for SmartCycler is essential.**

(2) qPCR Reaction Mix for ABI 7500 FAST

Reagents	I rx (µL)	10 rxs (µL)
VeriQuest Probe qPCR Master Mix (2X)	10	100
qPCR *Salmonella* Master Mix (10X)	1.8	18
Water	6.2	62
Total (µL)	18	180

- Add a 18 µl aliquot of PCR reaction mix into ABI 96-well plate
- Add a **2 µl** aliquot of sample and/or control (negative and positive) DNA template into each assigned reaction (well).
- Seal the plate with ABI 96-well adhesive film by film applicator.
- Remove the bubble by tapping then spin at 3000 rpm for 2 min.
- Create run on ABI 7500 FAST machine.

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Appendix 9: ABI 7500 Fast System- Define protocol

Turn on the computer and ABI 7500 FAST Teal-Time PCR system. It shows "Quick start" →click "cancel". From "File"→ New→ New Document Wizard (Define Document) → "Next" → "Select Detectors" → "Next" → "Set Sample Plate" → " Finish" → Click on "Instrument"

1. Key in the following parameters:
   Stage 1: Reps:1
   50 °C; 2:00
   Stage 2 : Reps:1
   95 °C; 10:00
   Stage 3:
   Reps 50 times
   95 °C; 0:15;
   60 °C; 0:30
2. Sample Volume (µL): 20
   Run Mode: Standard 7500
   Data collection:
   Stage 3, step 2 (60.0@ 0:30)

Save Protocol as a template in Drive D: →Applied Biosystems →7500 system → templates→ File Name: *Salmonella*, save as a type:of SDS Template [*sdt]

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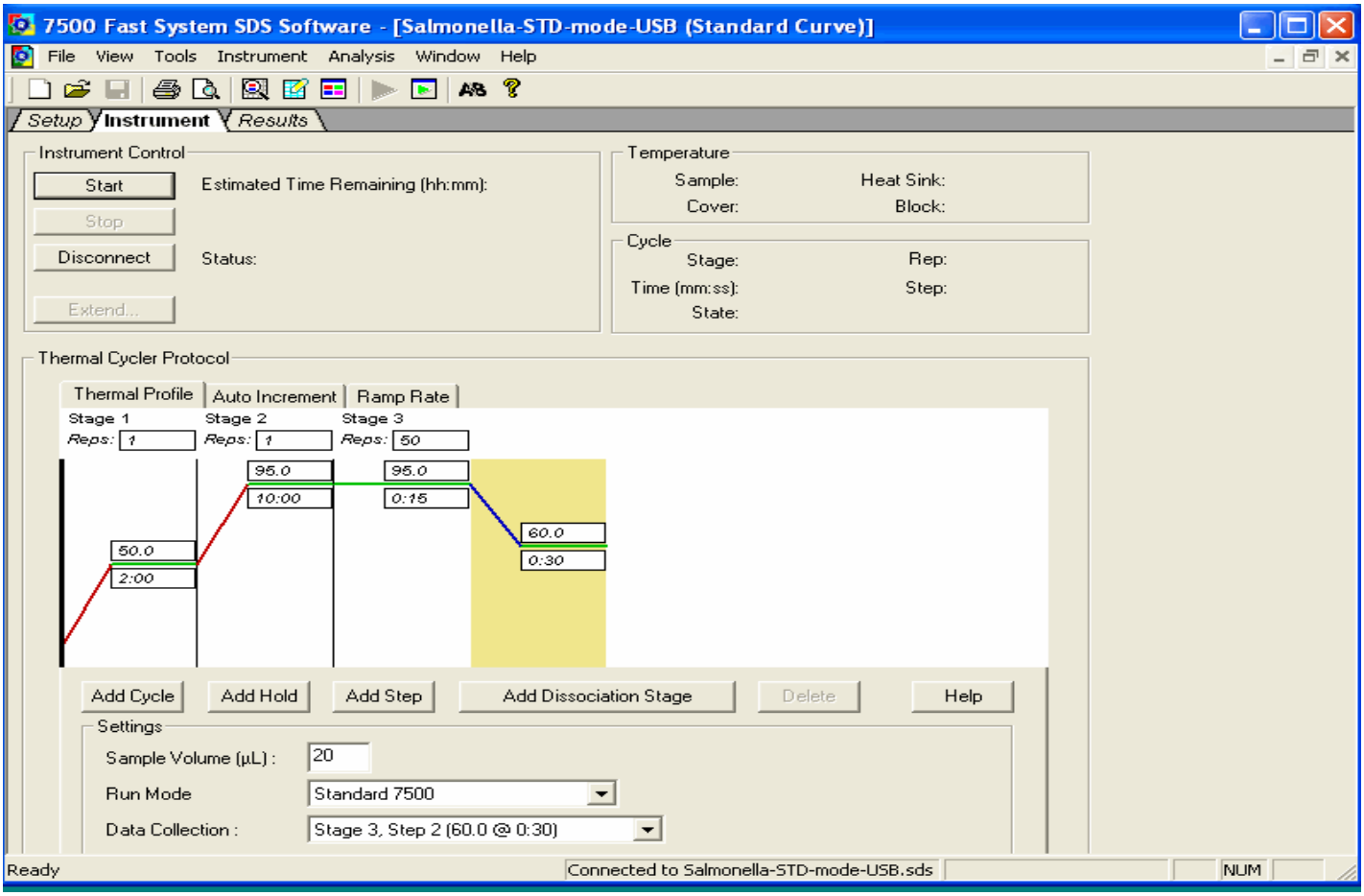

Appendix 10: ABI 7500 Fast System- Create Detectors: *Salmonella* & InC

Select "Tools" → "Detector Manager" → File → "New" → "Name": Salmonella, "Reporter Dye: FAM, "Quencher Dye": None, "Color": Green → Click OK. Create another one, →File → New→ "Name": InC, Reporter Dye: Cy5, "Quencher Dye": None, "Color": Purple → Click "Done"

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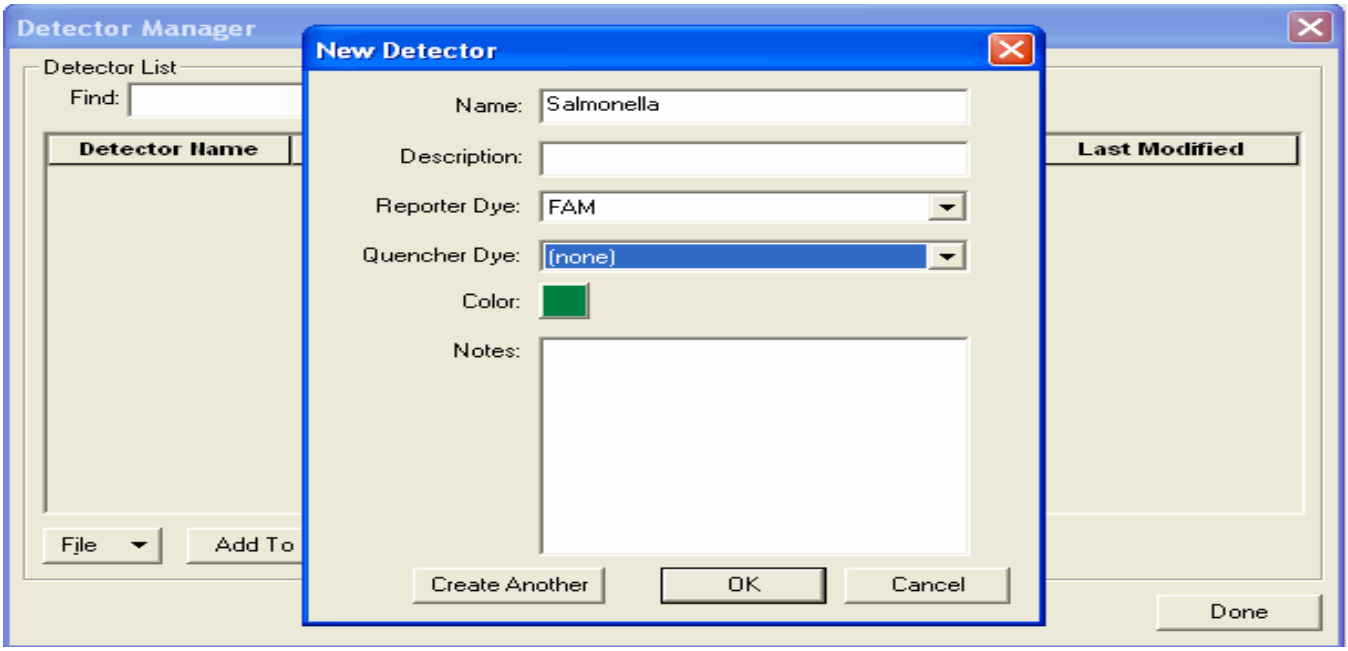
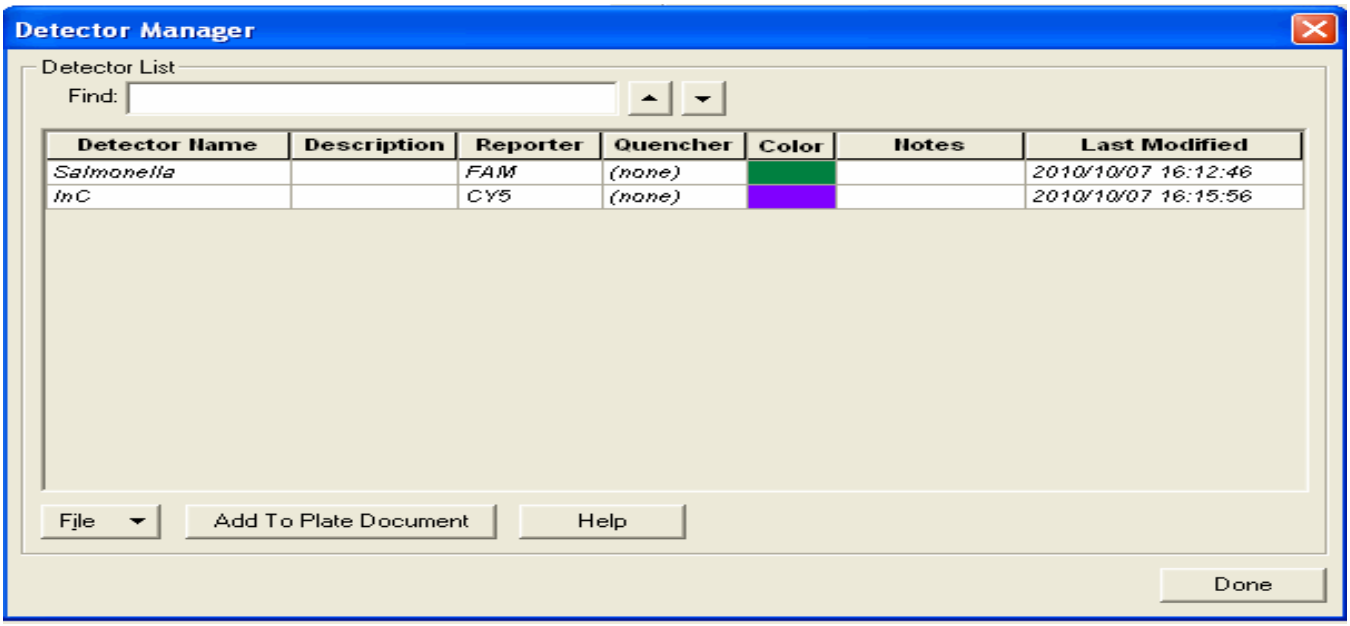


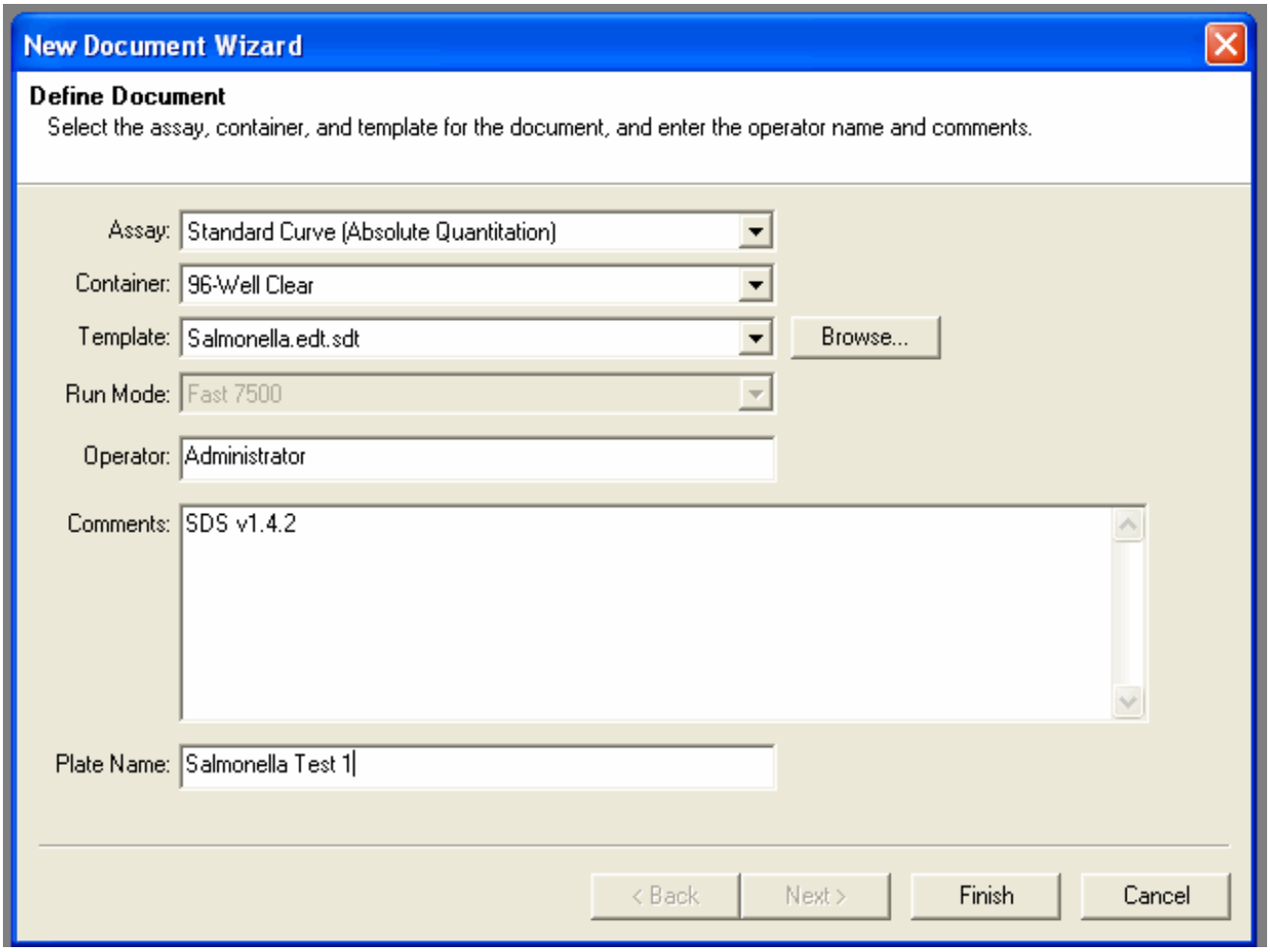


Appendix 11: ABI 7500 Fast System- Start a new Run

Turn on the computer and ABI 7500 FAST Real-Time PCR system.
From "File" → Click "New" → "New Document Wizard"-"Define Document"
Assay: Standard Curve (Absolute Quantitation)

Title: qPCR method for the Detection of *Salmonella* in Papaya –General Laboratory Procedure

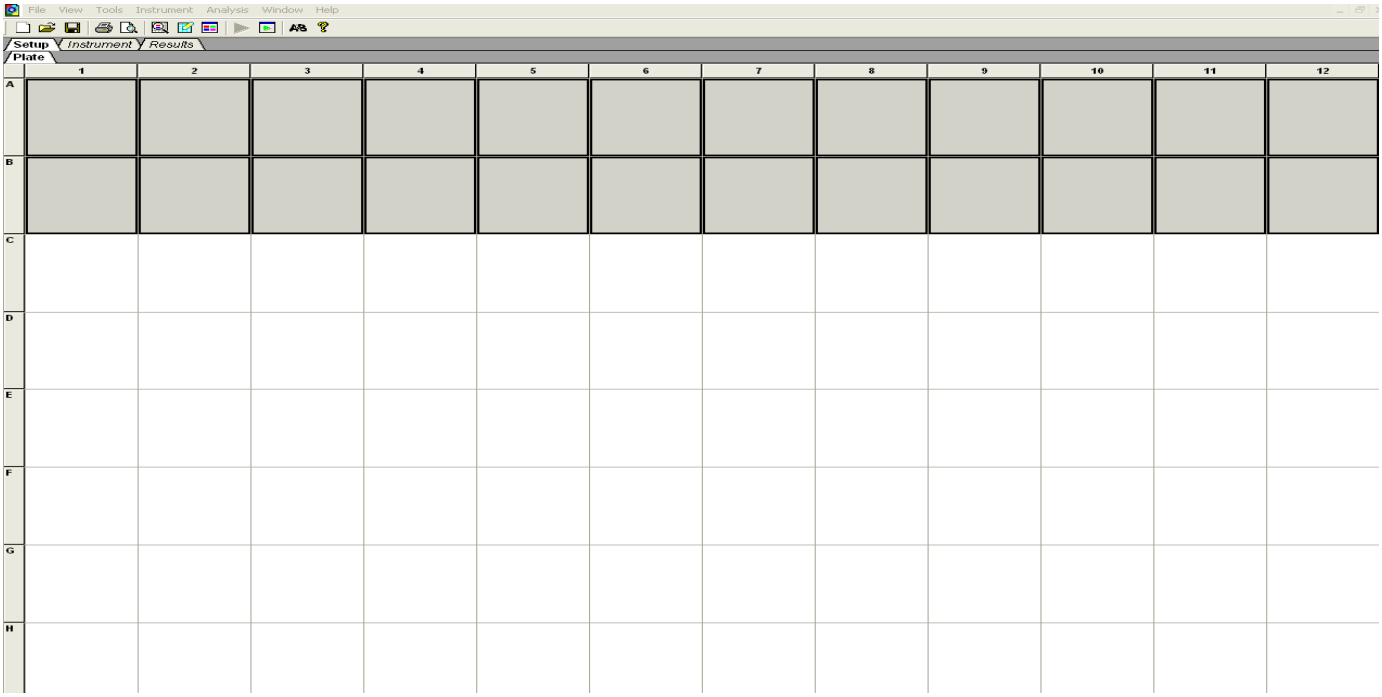
Template: Click on "Browse" → "template" → Select "Salmonella. sdt"
Plate Name: "Salmonella Test 1" → "Finish"



Appendix 12: ABI 7500 Fast System- Plate setting-1

→ Highlight and drag all wells you need

Title: qPCR method for the Detection of *Salmonella* in Papaya –General Laboratory Procedure



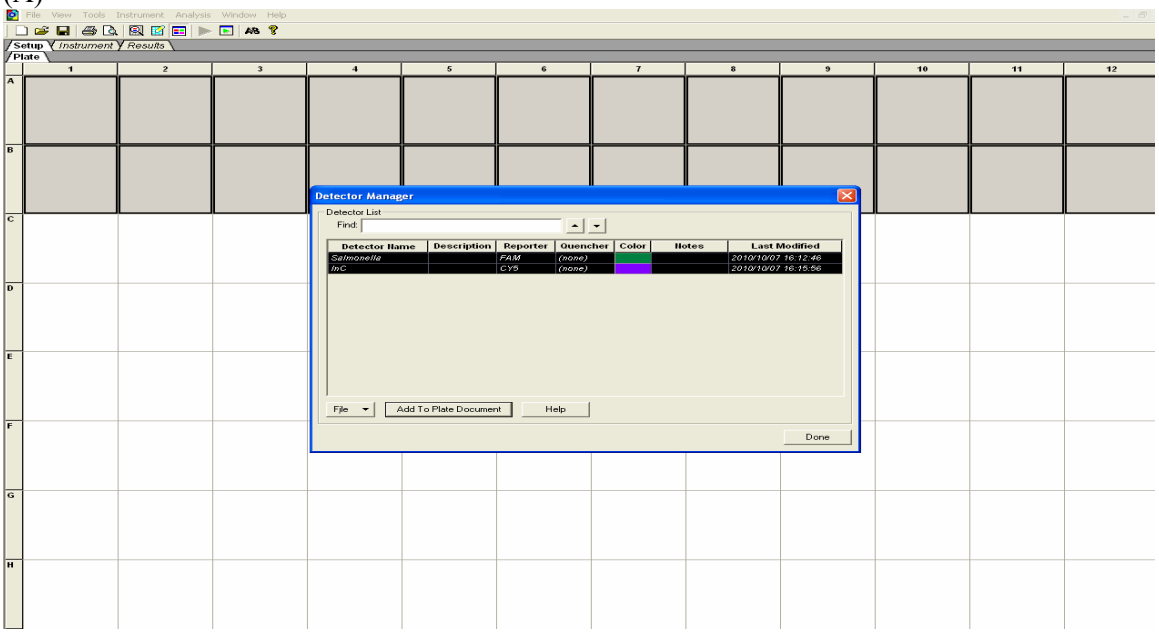
Title: qPCR method for the Detection of *Salmonella* in Papaya –General Laboratory Procedure

Appendix 13: ABI 7500 Fast System- Plate setting-2

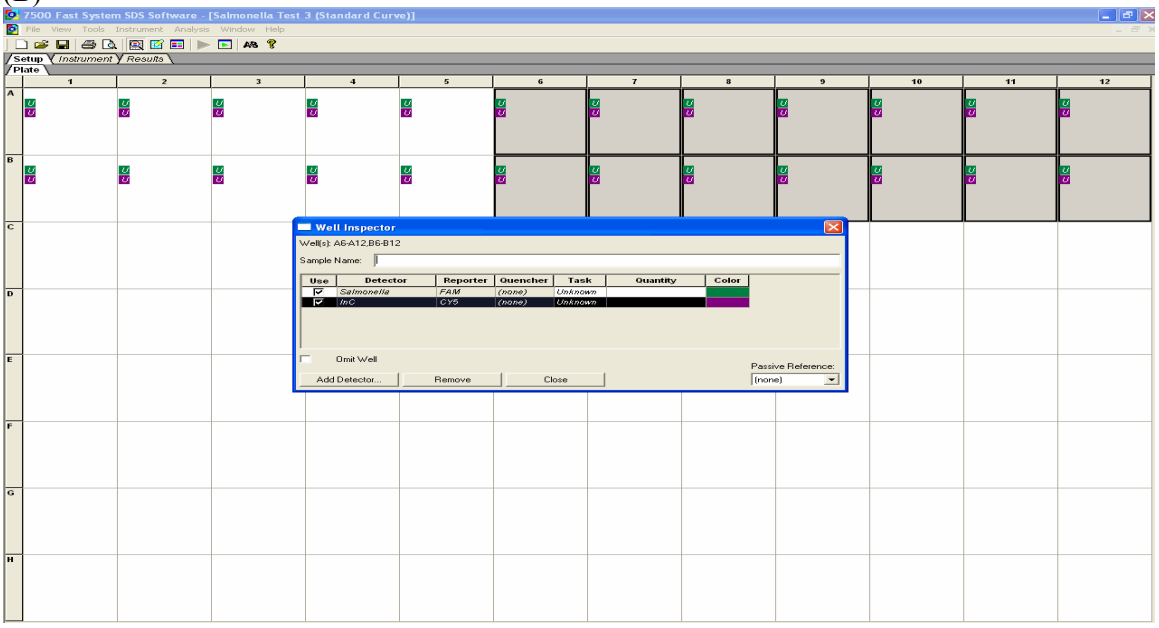
(A) .From "Tool" → Select "Detector Manager" → Shift select "Salmonella" and "InC" → Click on "Add to plate Document" → OK→OK→Done

(B) From "View" → Select "Well Inspector", mark both "Salmonella" and "InC" For "Passive Reference", select "ROX→ Close

(A)

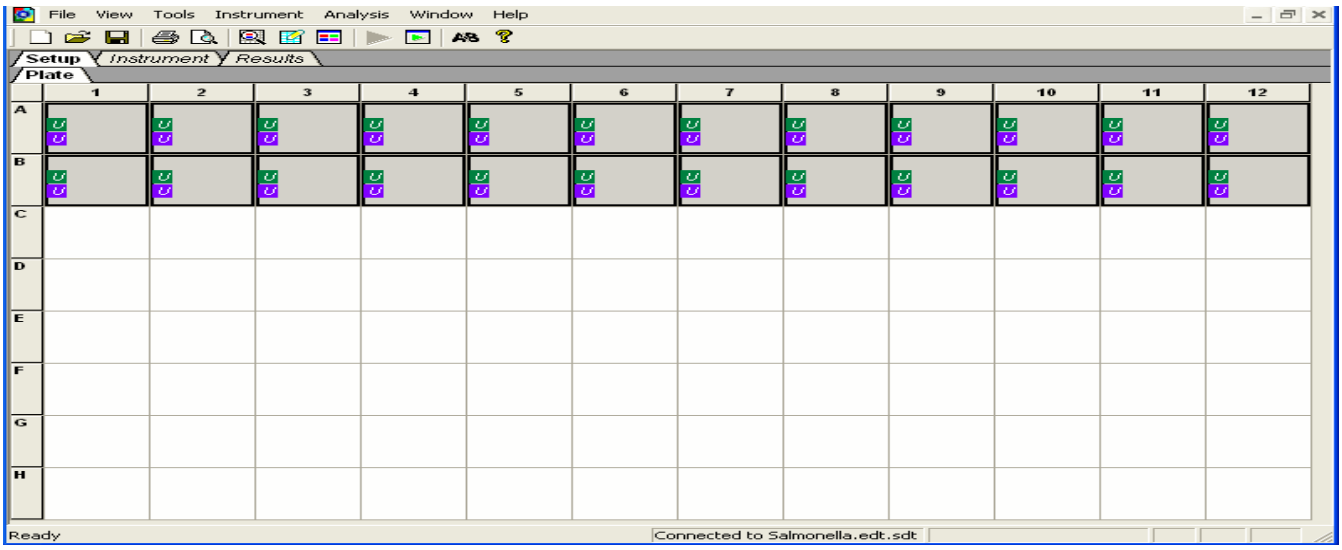


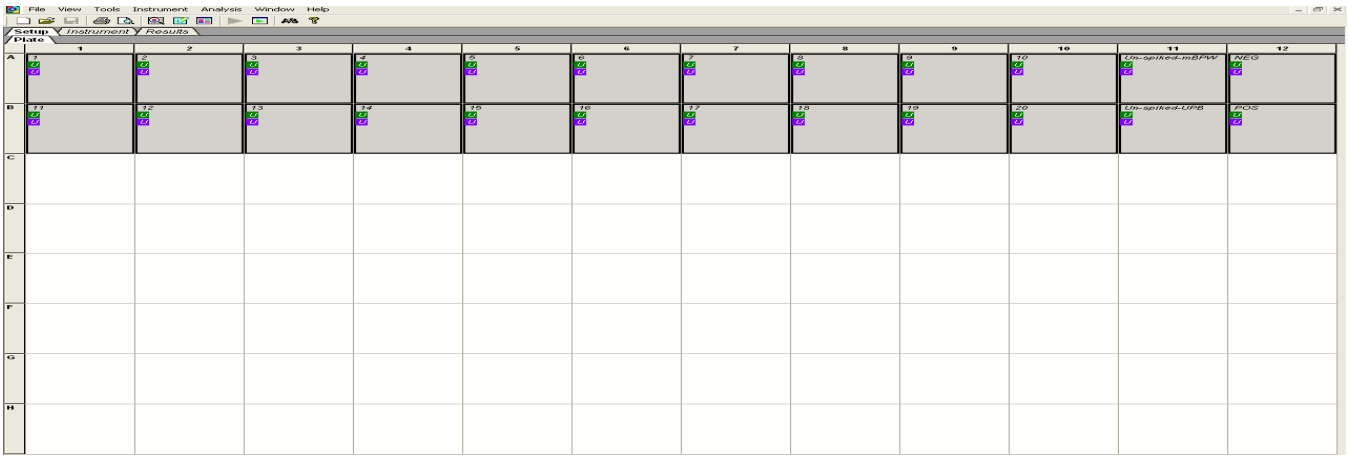
(B)



Title: qPCR method for the Detection of *Salmonella* in Papaya –General Laboratory Procedure
Appendix 14: ABI 7500 Fast System- Plate setting-3

Double click on each well → Type in "Sample Name" → Close

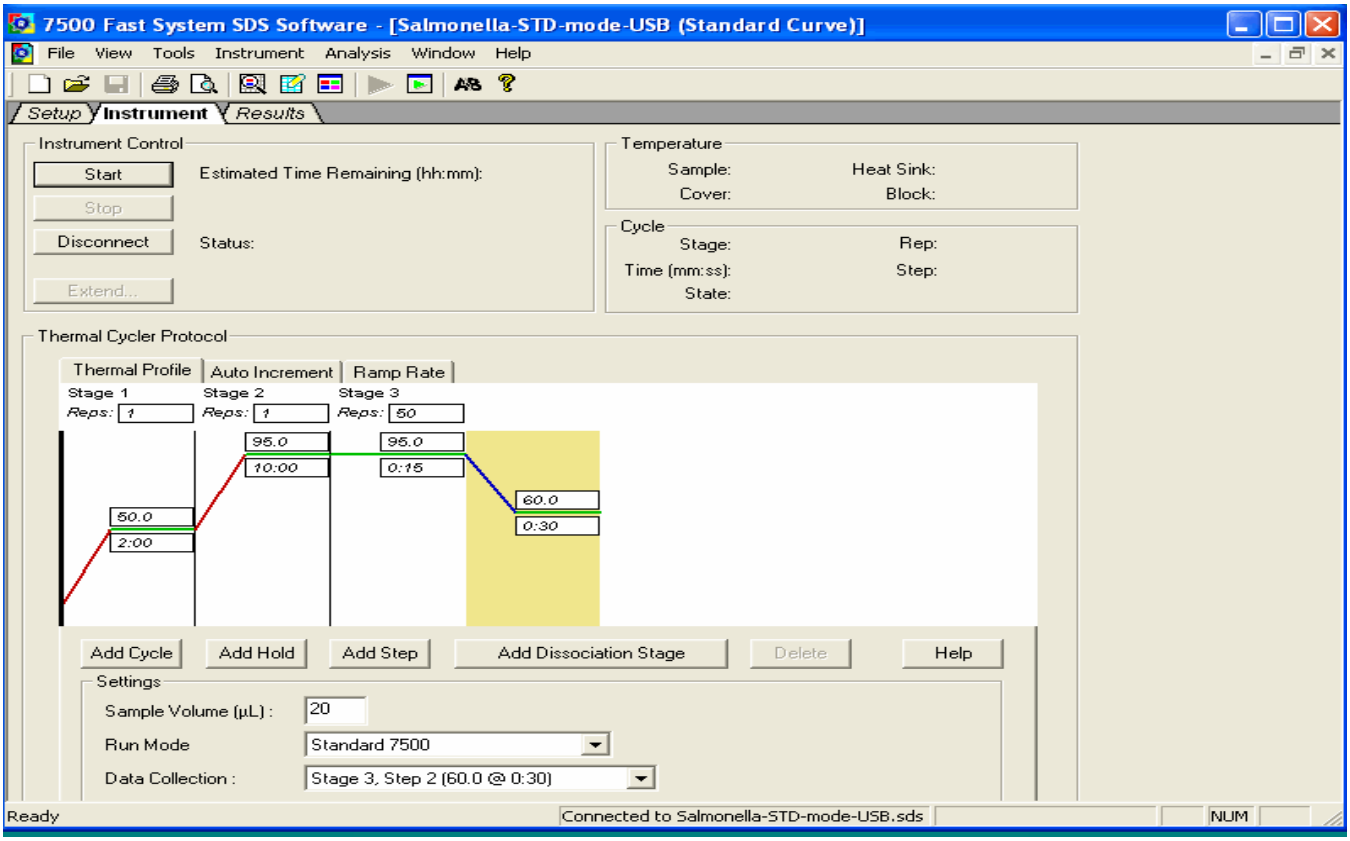




Title: qPCR method for the Detection of *Salmonella* in Papaya –General Laboratory Procedure

Appendix 15: ABI 7500 Fast System- Start Run

Click on “Instrument” then click on “Start



Title: qPCR method for the Detection of *Salmonella* in Papaya –General Laboratory Procedure

Appendix 16: ABI 7500 Fast System- Results-Amplification Plot

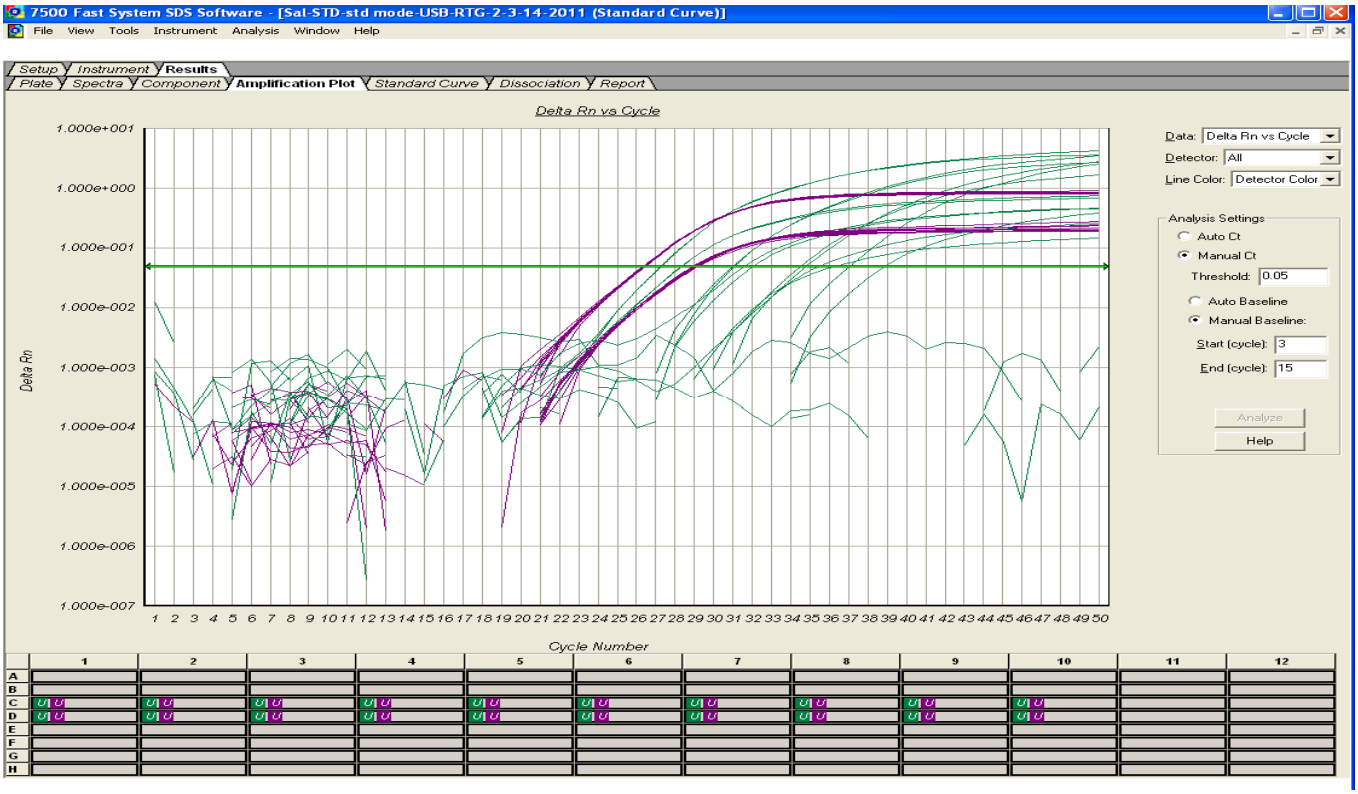
Results

Click on "Results" → Select "Amplification Plot" → Data: "Delta Rn vs. Cycle", Detector: All, Line Color: Select "Well color" or "Detector Color".

Analysis Settings

Select Manual Ct, Threshold: **0.05** (w/ ROX)

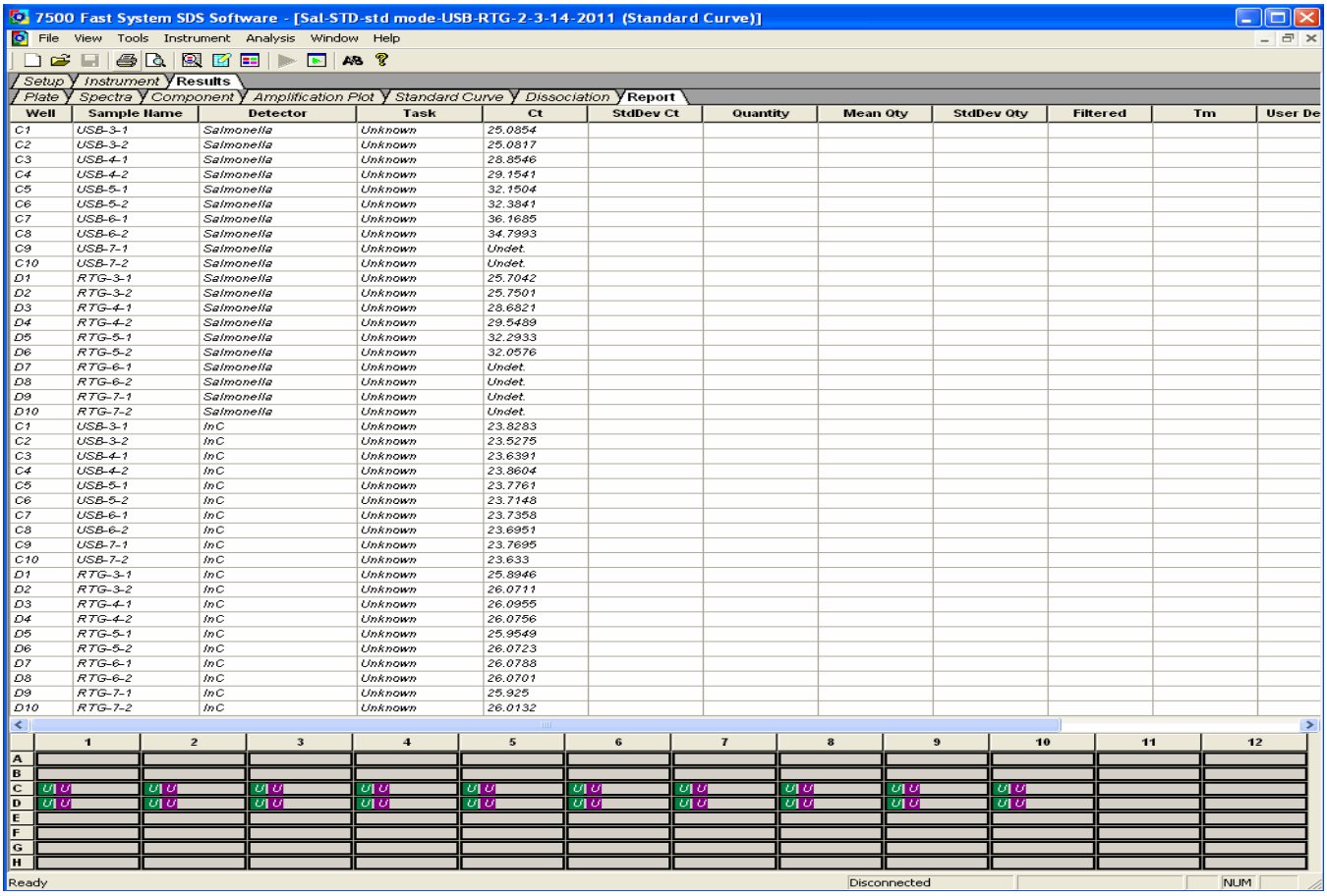
Select Manual Baseline, Start (cycle): 3, End (cycle): 15



Title: qPCR method for the Detection of *Salmonella* in Papaya –General Laboratory Procedure

Appendix 17: ABI 7500 Fast System- Results-Report

Click on “Report”, then click on the well or click on the left upper corner for all wells
Positive: w/ Ct value, Negative: “Undet”



Well	Sample Name	Detector	Task	Ct	StdDev Ct	Quantity	Mean Qty	StdDev Qty	Filtered	Tm	User De
C1	USB-3-1	Salmonella	Unknown	25.0854							
C2	USB-3-2	Salmonella	Unknown	25.0817							
C3	USB-4-1	Salmonella	Unknown	28.8546							
C4	USB-4-2	Salmonella	Unknown	29.1541							
C5	USB-5-1	Salmonella	Unknown	32.1504							
C6	USB-5-2	Salmonella	Unknown	32.3841							
C7	USB-6-1	Salmonella	Unknown	36.1685							
C8	USB-6-2	Salmonella	Unknown	34.7993							
C9	USB-7-1	Salmonella	Unknown	Undet.							
C10	USB-7-2	Salmonella	Unknown	Undet.							
D1	RTG-3-1	Salmonella	Unknown	25.7042							
D2	RTG-3-2	Salmonella	Unknown	25.7501							
D3	RTG-4-1	Salmonella	Unknown	28.6821							
D4	RTG-4-2	Salmonella	Unknown	29.5489							
D5	RTG-5-1	Salmonella	Unknown	32.2933							
D6	RTG-5-2	Salmonella	Unknown	32.0576							
D7	RTG-6-1	Salmonella	Unknown	Undet.							
D8	RTG-6-2	Salmonella	Unknown	Undet.							
D9	RTG-7-1	Salmonella	Unknown	Undet.							
D10	RTG-7-2	Salmonella	Unknown	Undet.							
C1	USB-3-1	InC	Unknown	23.8283							
C2	USB-3-2	InC	Unknown	23.5275							
C3	USB-4-1	InC	Unknown	23.6391							
C4	USB-4-2	InC	Unknown	23.8604							
C5	USB-5-1	InC	Unknown	23.7761							
C6	USB-5-2	InC	Unknown	23.7148							
C7	USB-6-1	InC	Unknown	23.7358							
C8	USB-6-2	InC	Unknown	23.6951							
C9	USB-7-1	InC	Unknown	23.7695							
C10	USB-7-2	InC	Unknown	23.633							
D1	RTG-3-1	InC	Unknown	25.8946							
D2	RTG-3-2	InC	Unknown	26.0711							
D3	RTG-4-1	InC	Unknown	26.0955							
D4	RTG-4-2	InC	Unknown	26.0756							
D5	RTG-5-1	InC	Unknown	25.9549							
D6	RTG-5-2	InC	Unknown	26.0723							
D7	RTG-6-1	InC	Unknown	26.0788							
D8	RTG-6-2	InC	Unknown	26.0701							
D9	RTG-7-1	InC	Unknown	25.925							
D10	RTG-7-2	InC	Unknown	26.0132							