SYBR Green qPCR Service

Customer Information

Customer Name:

Institute: College of Veterinary Science

Telephone: ……

Address:

Email:

1. Samples:

Item	Sample Name	qPCR Sample code
1	Control	HB211
2	Acute Quinalphos toxicity(ACT)	HB212
3	Chronic Qunalphos toxicity(CQT)	HB213
4	Acute Dimethoate Toxicity(ADT)	HB214
5	Chronoc Dimethoate Toxicity(CDT)	HB215
6		
7		
8		

Comparisons:

	Treatment	Control
……	……	……

2. A. Primers

This qPCR service request included 8 target genes and 10 samples.

Sample Name	Primer Name
Control	AvBD1
Acute Quinalphos toxicity(ACT)	AvBD6
Chronic Qunalphos toxicity(CQT)	AvBD7
Acute Dimethoate Toxicity(ADT)	GAPDH
Chronoc Dimethoate Toxicity(CDT)	ACTB

B. Procedure

Protocol for DNA Isolation

1. Cut up to 50-100 mg tissue into small pieces, and place in a 2.0 ml micro centrifuge tube. Add 350 µl Buffer TTL.
2. Add 20 µl Proteinase K and 5 µl RNAse A. Mix thoroughly by vortexing, and incubate at 65oC until the tissue is completely lysed. Briefly invert the tube during incubation.

3. Add 300 µl Buffer TBL to the sample, Vortex for 15-20 seconds and incubate at 65oC for 10 min and mix thoroughly by inverting. Then add 150 µl ethanol (96-100%), mixed by inverting a tube 15-20 times or Pulse vortexing. Incubate for 1 min at room temperature (18-25oC). Briefly centrifuge the MCT (1-2 sec at 2,000 x g) to remove drops from the lid (short spin only).

4. Transfer 650 µl lysate to the TransPureTM spin column, and centrifuge at 10,000rpm for 2 min. discard the flow through liquid.

5. Repeat above step, until the entire sample has been processed and retain column for further processing.

6. Place the column into same collection tube. Add 500 µlof Buffer TW. Centrifuge at 10,000rpm for 1 min. Discard the flow through.

7. Place the column into same collection tube. Add 650 µl of TW I. Centrifuge at 10,000rpm for 1 min. Discard the flow through.

8. Repeat the above “step-7” again.

9. Place the empty TransPureTM spin column, with the lid open into the same collection tube and centrifuge at 10,000 rpm for 2 min.

10. Place the column into a new sterile 1.5 ml Eppendorf tube, add 30 µl preheated (75oC-80oC) Elution buffer. Incubate at room temperature for 5 min. (perform this step twice 30 µl + 30 µl= 60 µl)

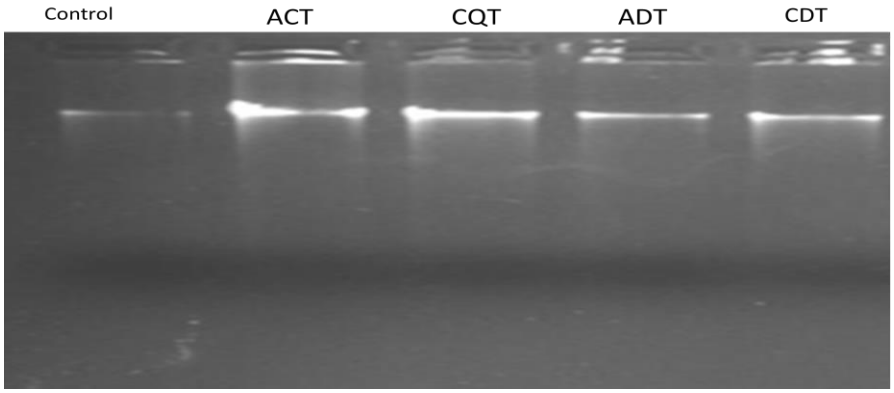
11. Centrifuge 10,000 rpm for 1 min to elute pure Tissue gDNA. The first elution normally yields 60-70% of DNA bound. A second elution with another 30 µl buffer will yield another 20 % of the DNA.

12. Discard the column, and save elute. Do not reuse binding columns or collection tubes.

Agarose gel electrophoresis

The amplified PCR products were separated by electrophoresis in 0.8% agarose gel run in 1× TAE buffer at 50V for 30 to 45 min. till DNA fragments are migrated well. The gel was photographed on UVP version gel documentation system, stored and analyzed using a gel documentation system with built in software.

Gel Photo:



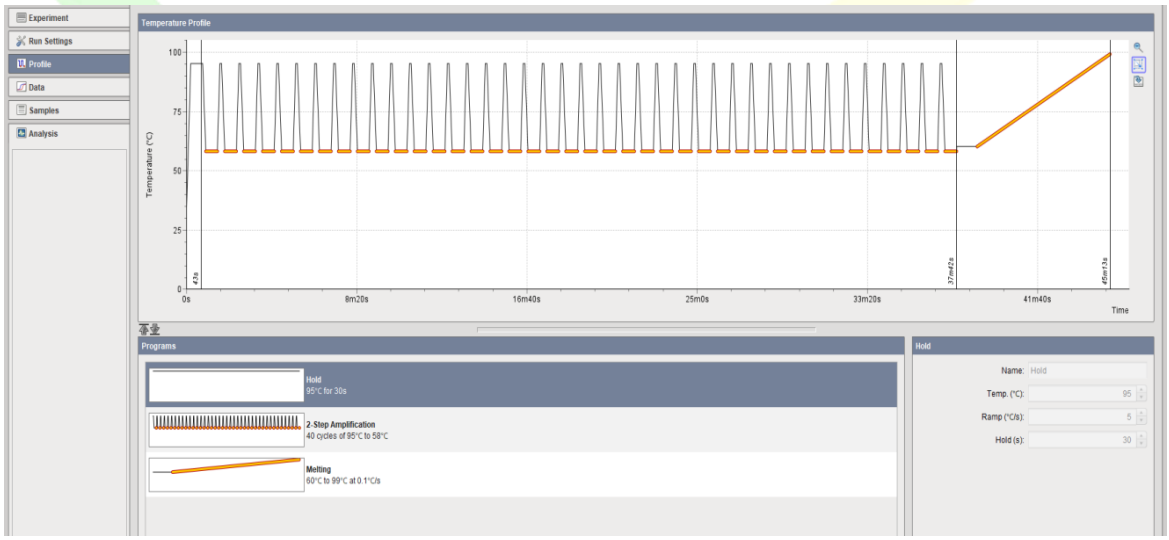
3. A. Quantitative PCR (q-PCR)

Each sample was tested in duplicate. Each reaction included 20 ng cDNA, 10pocomole of forward and reverse primers, and 2X Fast SYBR Green PCR Master Mix (TB Green Premix Ex Taq II , # RR820A).
10µl reaction volumes were used according to the table below:

Fast SYBR master mix(µl)	**nuclease-free water(µl)**	**RT product (20 ng/µl)**	**F+R primer (10pM)**	**total volume (µl)**
5	3	1	1	10

B. Temperature & Program file

A **Tata MD** CHECK **Express real time PCR** machine was used with the following program: (95°C → 30sec), and 40 cycles (95°C → 5sec, 58°C → 30sec) followed by Melting curve 60^{0}C to 99^{0}C.



4. Data Analysis

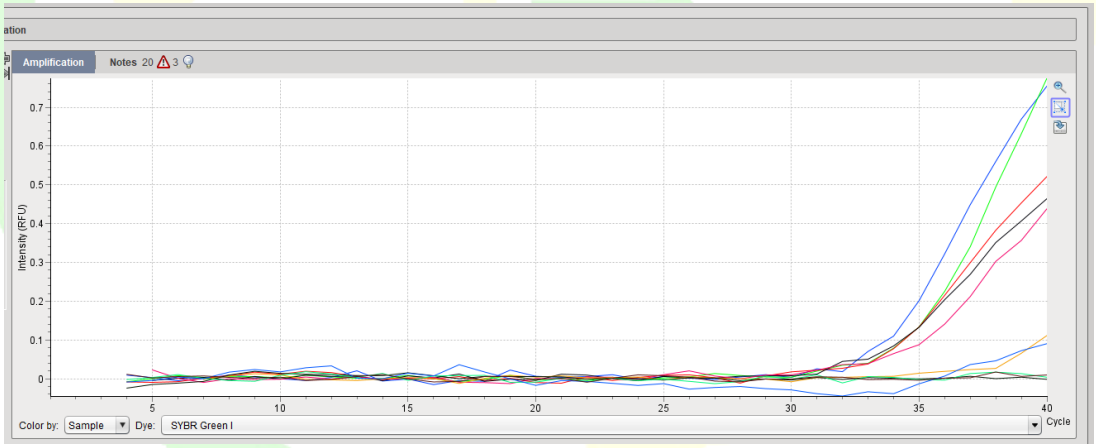
MyGo pro PCR software 3.6 was used for experimental setup and data analysis. Target gene qPCR data were determined by Cq Value.

A. AvBD1Gene

B. Plate Set up Real time PCR

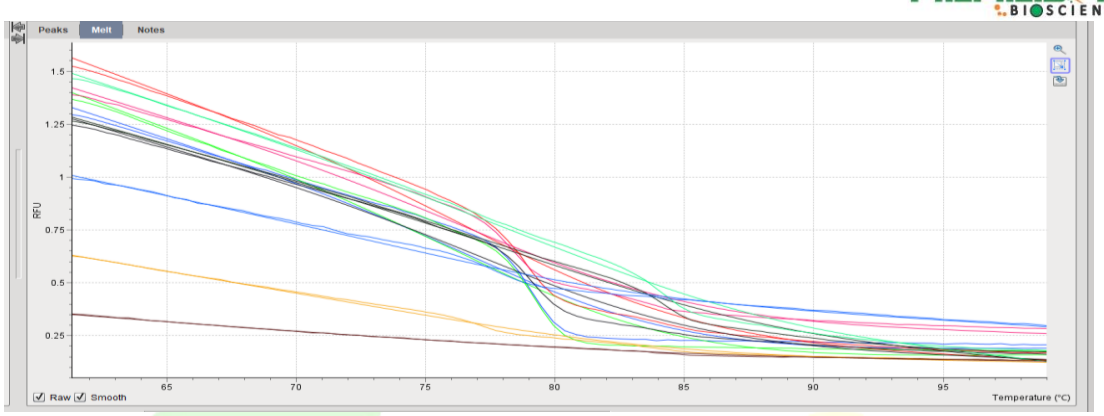
	1	2	3	4	5	6	7	8
A	Control	Control_1	AQT	AQT_1	CQT	CQT_1	ADT	ADT_1
B	CDT	CDT_1						
C								
D								

C. Amplification Curve



D. Melting curve

E.



F. CT VALUE

Sample Name	Primer	CT values
Control	**AvBD1**	32.63
Control_1	**AvBD1**	33.972
AQT	**AvBD1**	32.795
AQT_1	**AvBD1**	32.635
CQT	**AvBD1**	33.6
CQT_1	**AvBD1**	36.749
ADT	**AvBD1**	…..
ADT_1	**AvBD1**	……
CDT	**AvBD1**	……
CDT_1	**AvBD1**	……

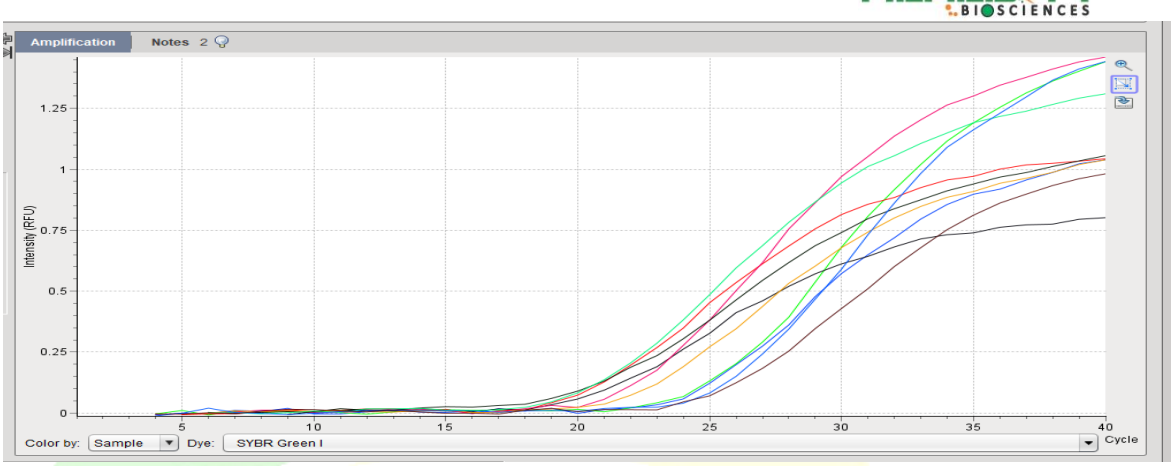
5.

A. AvBD6 Gene

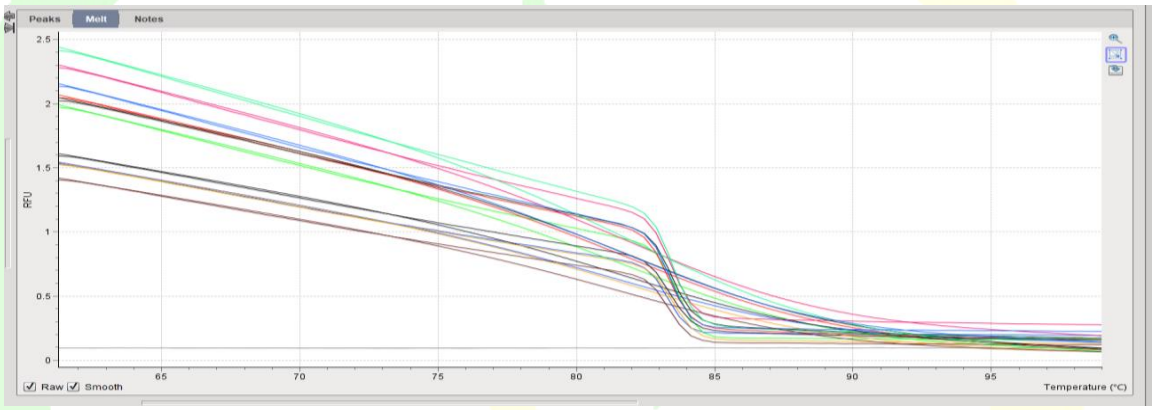
Plate Set up Real time PCR

	1	2	3	4	5	6	7	8
A	Control	Control_1	AQT	AQT_1	CQT	CQT_1	ADT	ADT_1
B	CDT	CDT_1						
C								
D								

B. Amplification Curve



C. Melting curve



D. CT value

Sample Name	Primer	CT values
Control	**AvBD6**	23.613
Control_1	**AvBD6**	24.511
AQT	**AvBD6**	19.427
AQT_1	**AvBD6**	19.775
CQT	**AvBD6**	21.358
CQT_1	**AvBD6**	21.407
ADT	**AvBD6**	20.336
ADT_1	**AvBD6**	19.801
CDT	**AvBD6**	24.897
CDT_1	**AvBD6**	24.535

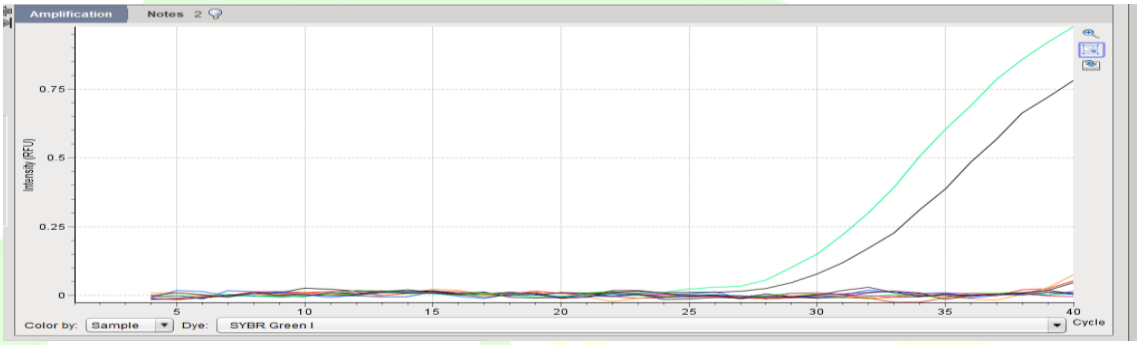
6A. AvBD7 Gene

Plate Set up Real time PCR

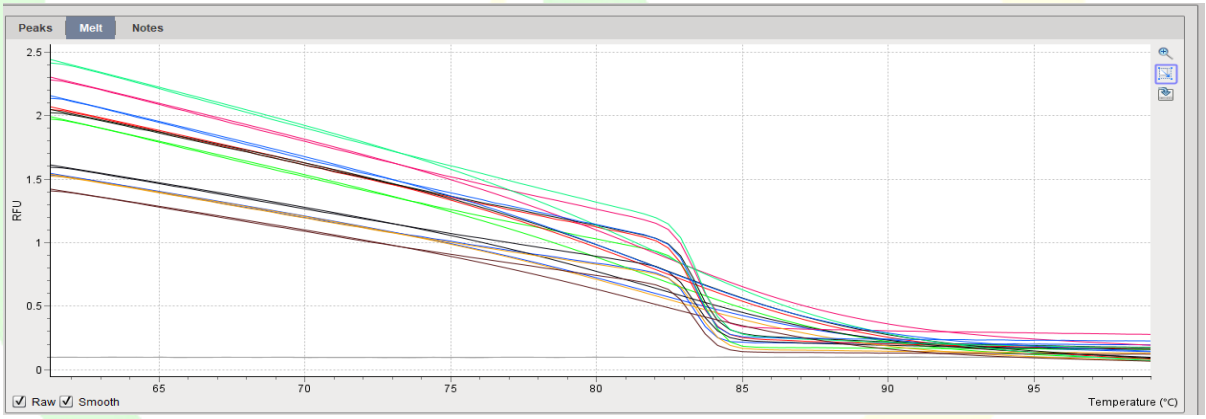
	1	2	3	4	5	6	7	8

A	Control	Control_1	AQT	AQT_1	CQT	CQT_1	ADT	ADT_1
B	CDT	CDT_1						
C								
D								

E. Amplification Curve



F. Melting curve



G. CT value

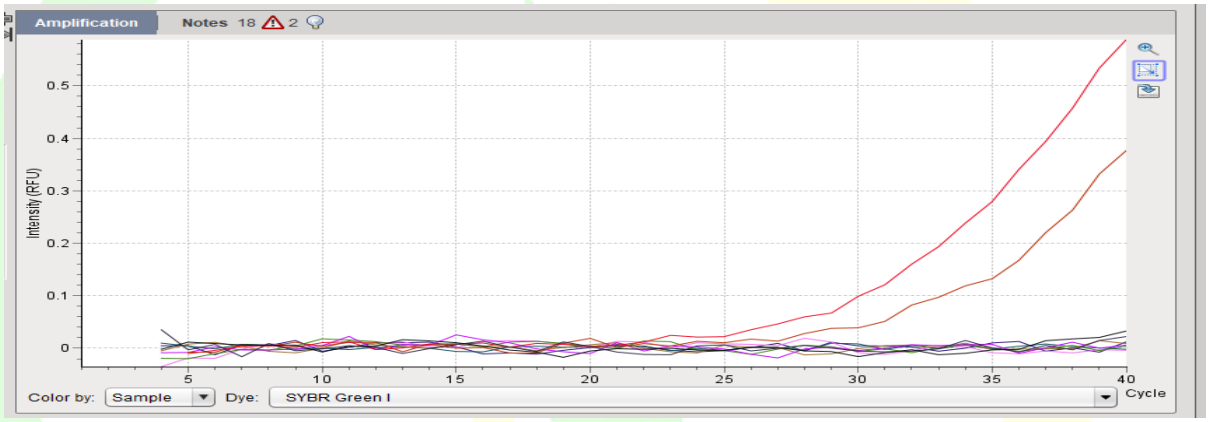
Sample Name	Primer	CT values
Control	**AvBD7**	…….
Control_1	**AvBD7**	…..
AQT	**AvBD7**	……
AQT_1	**AvBD7**	…….
CQT	**AvBD7**	……
CQT_1	**AvBD7**	…..
ADT	**AvBD7**	30.153
ADT_1	**AvBD7**	28.652
CDT	**AvBD7**	…….
CDT_1	**AvBD7**	……..

7A. GAPDH Gene

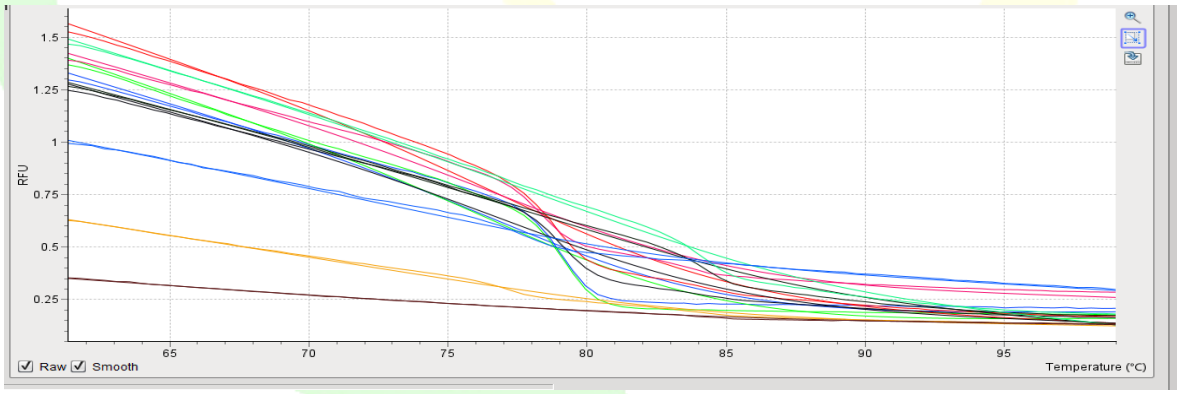
Plate Set up Real time PCR

	1	2	3	4	5	6	7	8
A	Control	Control_1	AQT	AQT_1	CQT	CQT_1	ADT	ADT_1
B	CDT	CDT_1						
C								
D								

H. Amplification Curve



I. Melting curve



J. CT value

Sample Name	Primer	CT values
Control	**GAPDH**	…….
Control_1	**GAPDH**	……..
AQT	**GAPDH**	……..
AQT_1	**GAPDH**	…….
CQT	**GAPDH**	…….

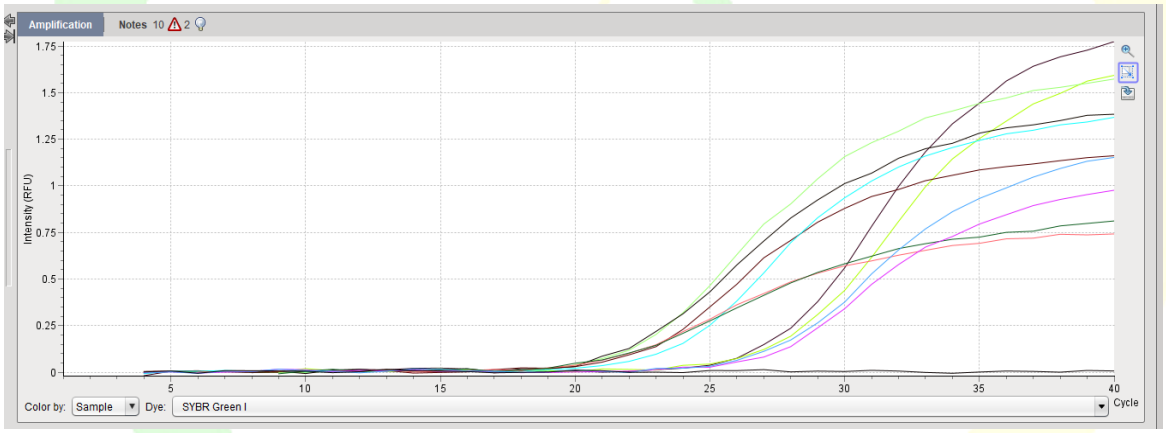
CQT_1	**GAPDH**	……..
ADT	**GAPDH**	34.748
ADT_1	**GAPDH**	33.327
CDT	**GAPDH**	……..
CDT_1	**GAPDH**	……..

8 A. ACTB Gene

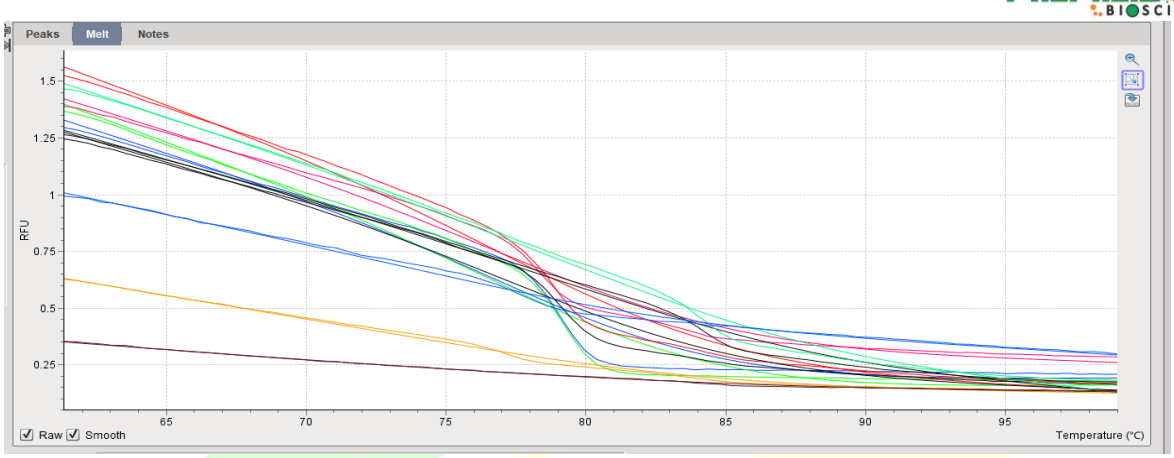
Plate Set up Real time PCR

	1	2	3	4	5	6	7	8
A	Control	Control_1	AQT	AQT_1	CQT	CQT_1	ADT	ADT_1
B	CDT	CDT_1						
C								
D								

K. Amplification Curve



L. Melting curve



M. CT value

Sample Name	Primer	CT values
Control	**ACTB**	26.636
Control_1	**ACTB**	27.112
AQT	**ACTB**	21.385
AQT_1	**ACTB**	21.063
CQT	**ACTB**	21.685
CQT_1	**ACTB**	22.875
ADT	**ACTB**	20.411
ADT_1	**ACTB**	20.566
CDT	**ACTB**	26.38
CDT_1	**ACTB**	26.512