
بسم الله الرحمن الرحيم

Gene Expression and Genotype

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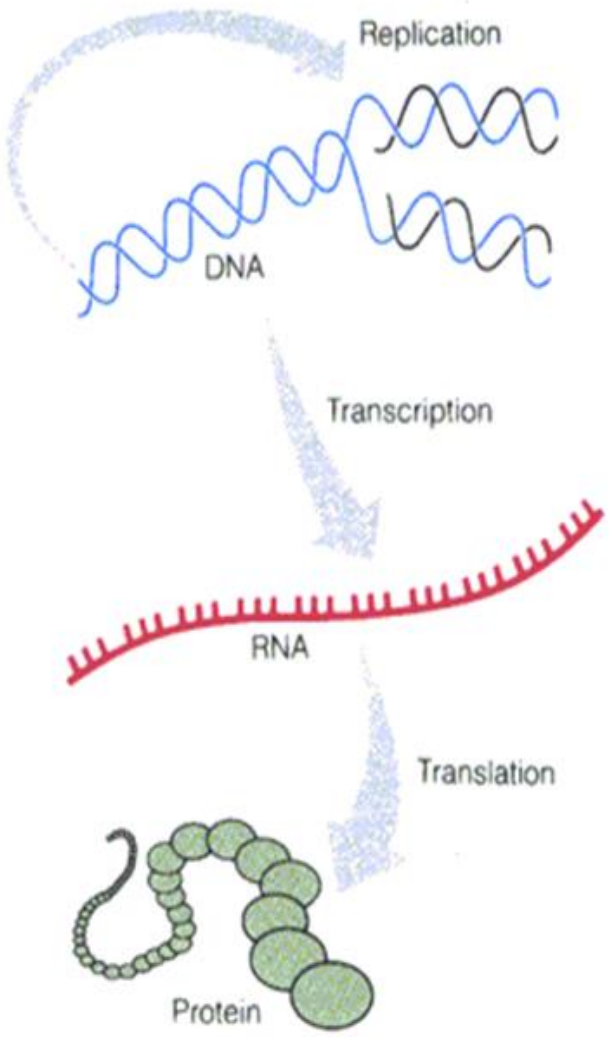
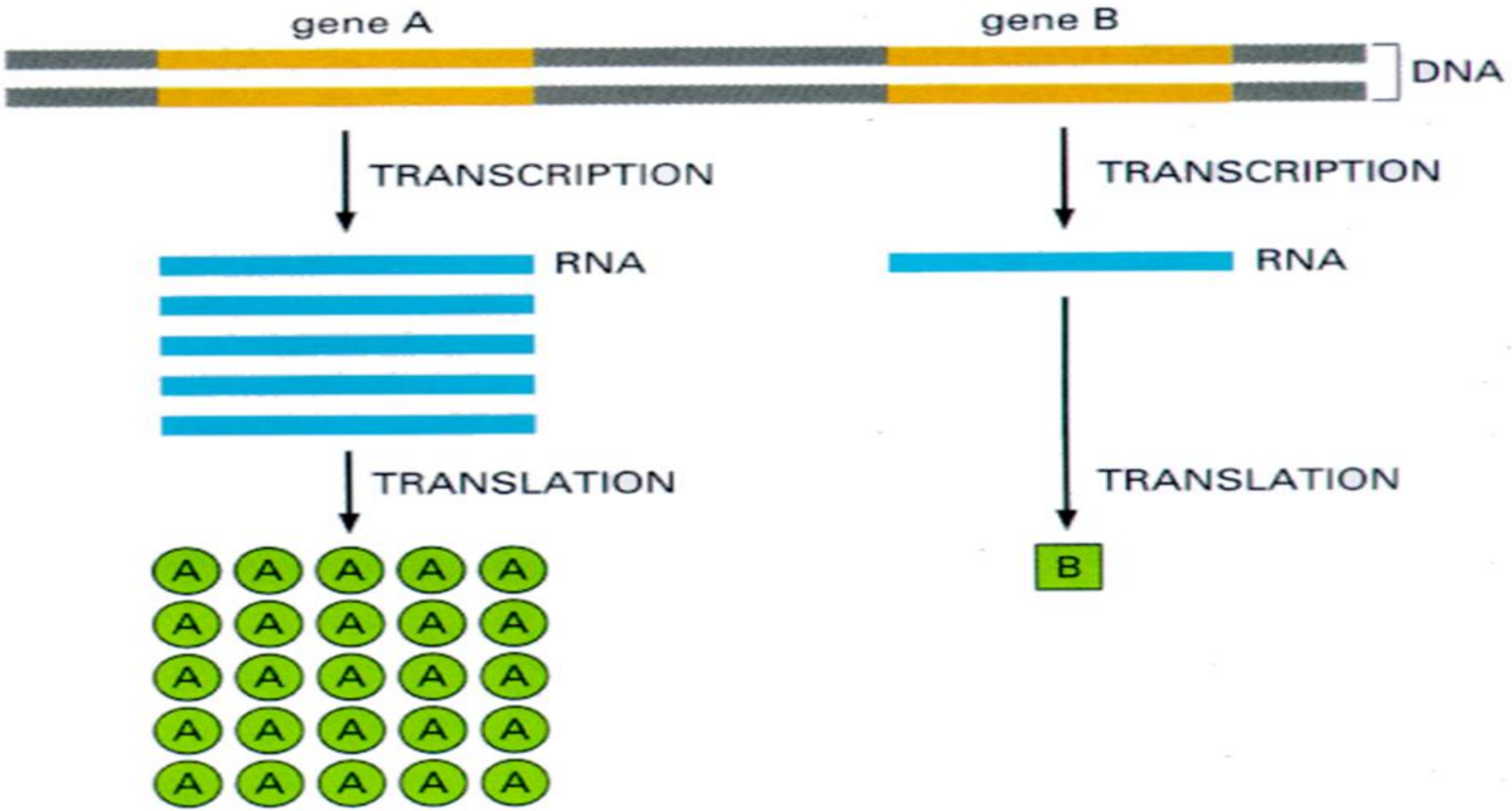
The Central Dogma



The central dogma states that information in nucleic acid can be perpetuated or transferred, but the transfer of information form into protein is irreversible.

Gene Expression

- Genes Can Be Expressed with Different Efficiencies at Different Times and Environments



Tissue

extract RNA

copy into cDNA (reverse transcriptase)

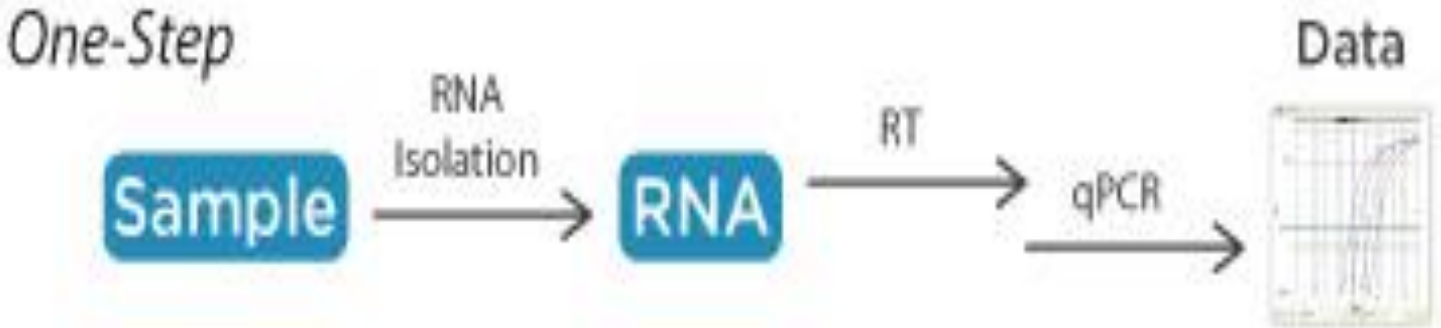
do real-time PCR

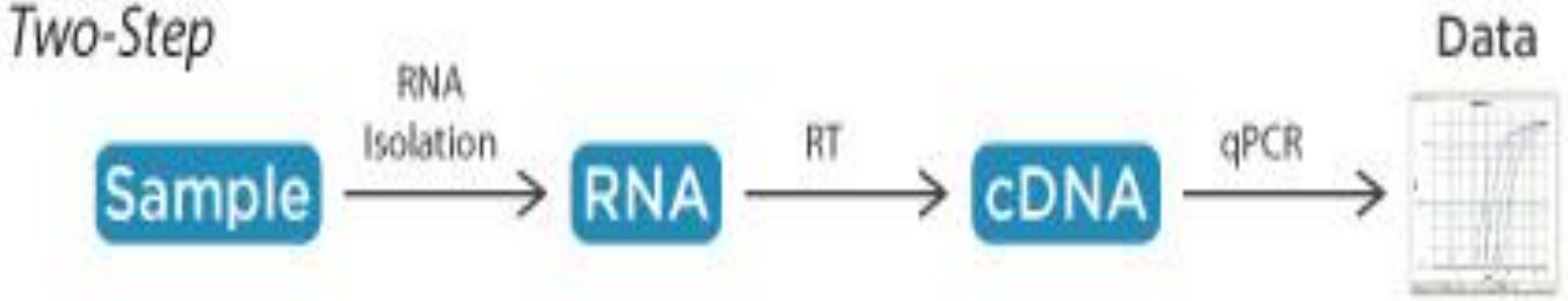
analyze results

Importance of reverse transcriptase primers

- **Oligo (dt)**
- **Random hexamer (NNNNNN)**
- **Specific**

- **One-Step RT-PCR**
- **Two-Step PCR**





Choosing between one-step and two-step RT-qPCR

Two-Step Protocol	One-Step Protocol	
Primers used in RT	•Oligo(dT) primers •Random hexamers •Gene-specific primers •A mix of these	•Gene-specific primers
Advantages	•Choice of primers •Optimize reactions for maximum yield •Modulate amount of RT that goes into PCR—controlling for target abundance •Perform multiple PCR reactions on the same cDNA sample •Adjust for challenging PCR (e.g., GC-rich sequences) •Experiment with different RT and Taq enzymes	•Quick setup and limited handling •Easy processing of multiple samples for repetitive tests, or high-throughput screening •Fewer pipetting steps, reducing potential errors •Eliminates possibility of contamination between the RT and qPCR steps
Considerations	•Requires more setup, hands-on, and machine time •Additional pipetting increases the chances for experimental errors and contamination •Uses more reagents	•Must "start over," or save RNA aliquot and perform new RT to analyze new target or repeat amplifications •Reaction conditions are not optimal—efficiency & thus quantification are affected •Primer dimers a bigger potential problem
Best for:	•amplifying multiple targets from a single RNA source •when you plan to reuse cDNA for additional amplifications	•working with multiple RNA samples to amplify only a few targets •assays performed repeatedly

Housekeeping gene

A gene involved in basic functions needed for the sustenance of the cell. Housekeeping genes are constitutively expressed

(they are always turned ON).

housekeeping genes

- same copy number in all cells
- expressed in all cells
- medium copy number advantageous
- no pseudogene
- no alternate splicing in region you want to PCR

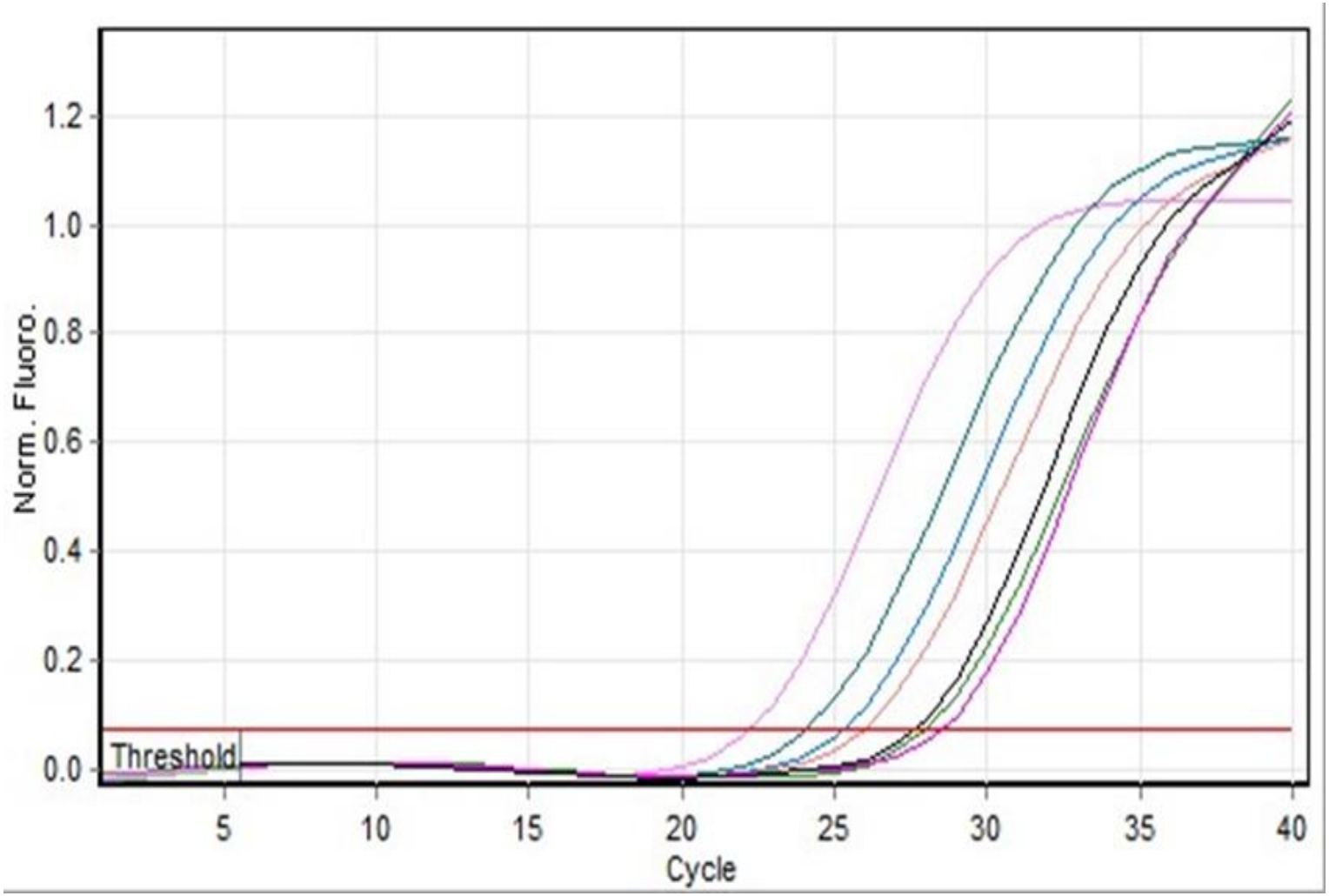
housekeeping genes

Commonly used housekeeping genes

- Glyceraldehyde-3-phosphate dehydrogenase mRNA
- Beta-actin mRNA
- MHC I (major histocompatibility complex I) mRNA
- Cyclophilin mRNA
- mRNAs for certain ribosomal proteins

E.g. RPLP0 (ribosomal protein, large, P0; also known as 36B4, P0, L10E, RPPO, PRLP0, 60S acidic ribosomal protein P0, ribosomal protein L10, Arbp or acidic ribosomal phosphoprotein P0)

- 28S or 18S rRNA


Norm. Fluoro.
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Threshold
5
10
15
20
25
30
35
40
Cycle

Comparison of GAPDH Fold expression between study groups.

Group	Means Ct of *GAPDH*	2^{-Ct}	experimental group/ Control group	Fold of gene expression
Healthy nonsmoker	24.42	4.45 E-8	4.45 E-8/4.45 E-8	1.00
Healthy smoker	24.53	4.12 E-8	4.12 E-8/4.45 E-8	0.93
Lung cancer nonsmoker	24.42	4.45 E-8	4.45 E-8/4.45 E-8	1.00
Lung cancer smoker	24.34	4.70 E-8	4.70 E-8/4.45 E-8	1.05

1. ΔCT

The expression ratio was calculated without a calibrator sample $2^{-\Delta Ct}$ according to the following equation:

ΔCT (test) = CT gene of interest (target, test) – CT internal control

Finally, the expression ratio was calculated according to the formula

$$\mathbf{2^{-\Delta Ct} = Normalized\ expression\ ratio}$$

Fold of APEX1 expression Depending on $2^{-\Delta Ct}$ Method

groups	Means Ct of *APEX1*	Means Ct of *GAPDH*	ΔCt (Means Ct of *APEX1* - Means Ct of *GAPDH*)	$2^{-\Delta Ct}$	experimental group/ Control group	Fold of gene expression
Lung cancer smoker	27.44	24.34	3.1	0.116	0.116/0.007	16.57
Lung cancer non smoker	27.99	24.42	3.57	0.084	0.084/0.007	12.00
Healthy smoker	29.68	24.53	5.15	0.028	0.028/0.007	4.00
Healthy nonsmoker	31.88	24.42	7.1	0.014	0.007/0.007	1.00

2- ΔΔ CT

ΔCT (test) = CT gene of interest (target, test) – CT internal control

ΔCT (calibrator) = CT gene of interest (target, calibrator) – CT The ΔCT of the test samples was normalized to the ΔCT of the calibrator:

ΔΔ CT was calculated according to the following equation:

ΔΔ CT=ΔCT (test)- ΔCT(calibrator)

Finally, the expression ratio was calculated according to the formula

$2^{-\Delta\Delta Ct}$ = Normalized expression ratio.

Fold of APEX1 expression Depending on $2^{-\Delta\Delta Ct}$ Method

groups	Means Ct of *Apex1*	Means Ct of *GAPDH*	Mean ΔCt Target (ct *Apex1* - ct *GAPDH)*	Mean ΔCt Calibrator (ct *Apex1*- ct *GAPDH*	ΔΔCt	$2^{-\Delta\Delta Ct}$	experimental group/ Control group	Fold of gene expression
Lung cancer smoker	27.44	24.34	3.1	8.78	-5.68	51.26	51.26/3.20	16.01
Lung cancer non smoker	27.99	24.42	3.57	8.78	-5.21	37.01	37.01/3.20	11.6
Healthy smoker	29.68	24.53	5.15	8.78	-3.63	12.38	12.38/3.20	3.9
Healthy non smoker	31.88	24.42	7.1	8.78	-1.68	3.20	3.20/ 3.20	1.00

qPCRsoft 3.0 - [Real time pcr project - Muhanad 1.rtp]

File Edit View Genotyping Extras Window Help

Settings | Monitoring | Analysis | Documentation

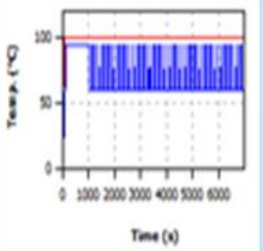
Window 0 - Muhanad 1.rtp

ddCt quantification | Melting curve | Genotyping

General

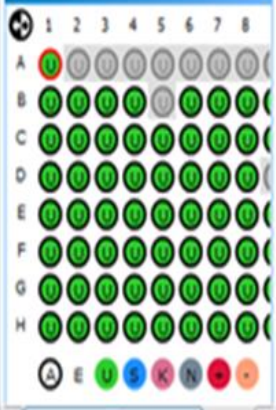
Title: Genotype
Operator: Muhanad
Day: 15/11/2015
Time: 11:14:59
Device: T-Opt2011 1306

Thermal Cycler



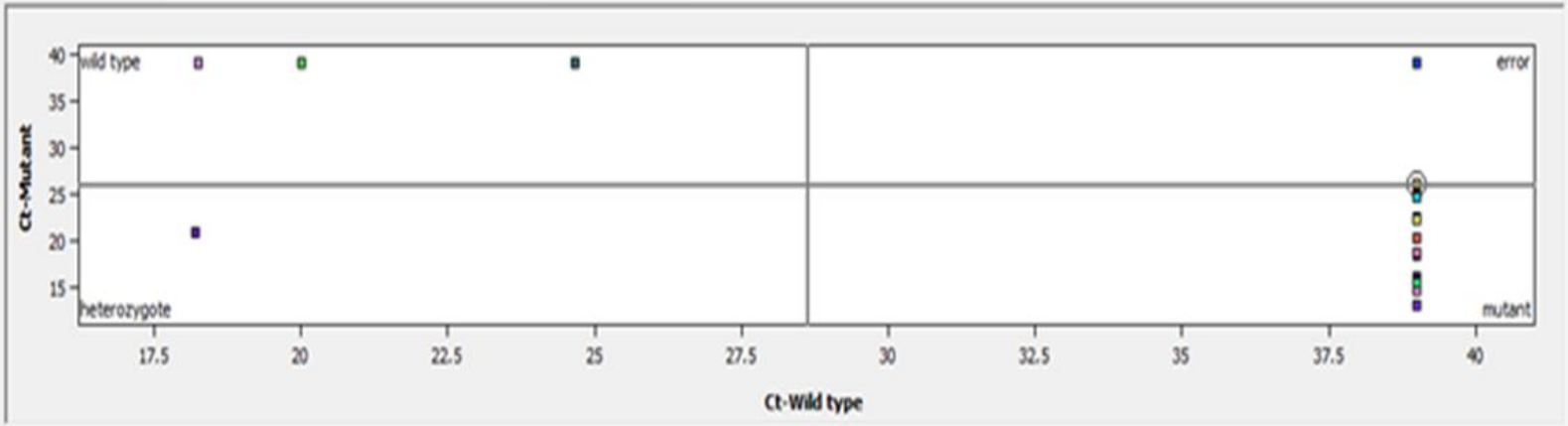
Scan

Samples



Genotyping: 0

Wild type: FAM
Include passive reference
Group: Group 1
CutOff Wild type: 28.61
Mutant: VIC
CutOff Mutant: 26.047



Wild type-FAM | Mutant-VIC | Scatter plot | End point

Well	Sample name	Sample type	Genotyp	Reaction ...	Reaction ...	Genotyp R...	Ct Wild type	Mean Ct ...	Std.Dev. ...	Ct Mutant	Mean Ct M...	Std.Dev. ...
A1	P-1	Unknown	error	no	no	error	No Ct	39	0	26.09	26.09	0
A10		Unknown	error	no	no	error	No Ct	39	0	No Ct	39	0
A11		Unknown	mutant	no	yes	mutant	No Ct	39	0	22.37	22.37	0
A12		Unknown	error	no	no	error	No Ct	39	0	No Ct	39	0
B1		Unknown	error	no	no	error	No Ct	39	0	No Ct	39	0
B2		Unknown	error	no	no	error	No Ct	39	0	No Ct	39	0
B3		Unknown	error	no	no	error	No Ct	39	0	No Ct	39	0
B4		Unknown	error	no	no	error	No Ct	39	0	No Ct	39	0

Connected with "TOptical" Administrator

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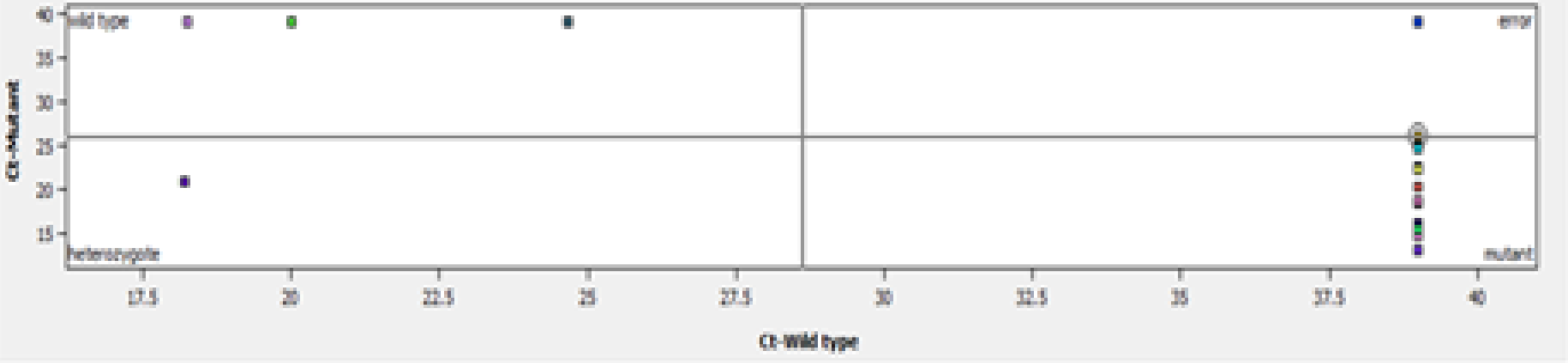
wild type
error
heterozygote
mutant
Ct-Mutant
Ct-Wild type
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27.5
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32.5
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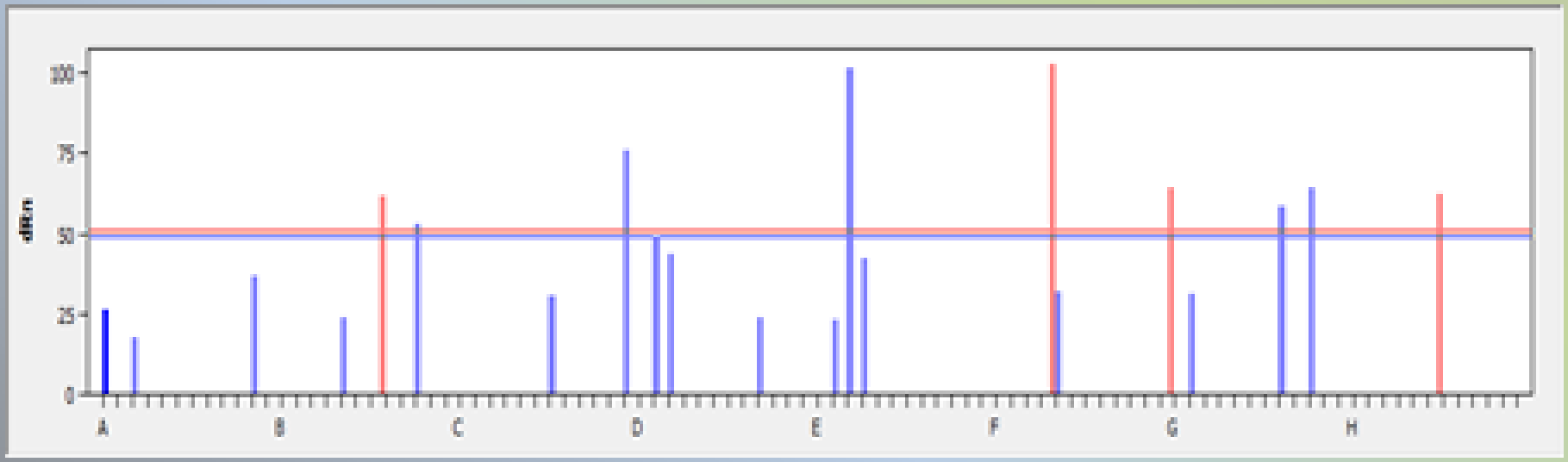
37.5
40


dRn
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75
50
25
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A
B
C
D
E
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G
H

Comparison of the Genotype and Allele Frequencies of *APEX1* gene polymorphism Asp148Glu between Lung cancer smoker group and Healthy group

APEX1 polymorphsim Asp148Glu	Frequencies (%)		P value	Odd ratio (95% CI)
	Healthy non-smoker (n=30)	Lung cancer smoker (n=40)		
TT	70.00 (n=21)	35.00 (n=14)	---	1.00 (Reference)
TG	23.33 (n=7)	37.50 (n=15)	0.041	3.26 (1.04-9.88)
GG	6.66 (n=2)	27.50 (n=11)	0.012	8.25 (1.58-23.02)
T	81.67 (49)	53.75 (43)	---	1.00 (Reference)
G	18.33 (11)	46.25 (37)	0.0001	5.17 (2.35-11.38)

Comparison of the Genotype and Allele of *APEX1* gene polymorphsim Asp148Glu between Lung cancer nonsmoker group and Healthy non smoker group

APEX1 polymorphsim Asp148Glu	Frequencies (%)		P value	Odd ratio (95% CI)
	Healthy non-smoker (n=30)	Lung cancer non-smoker (n=40)		
TT	70.00 (n=21)	47.50 (n=19)	---	1.00 (Reference)
TG	23.33 (n=7)	37.50 (n=15)	0.121	2.36 (0.79-7.05)
GG	6.66 (n=2)	15.00 (n=6)	0.171	3.3 (0.59-18.45)
T	81.67 (49)	66.25 (53)	---	1.00 (Reference)
G	18.33 (11)	33.75 (27)	0.045	2.26 (1.01-5.05)

Comparison of the Genotype and Allele of *APEX1* gene polymorphism Asp148Glu between Healthy smoker group and Healthy group

APEX1 polymorphsim Asp148Glu	Frequencies (%) Asp148Glu		P value	Odd ratio (95% CI)
	Healthy non-smoker (n=30)	Healthy smoker (n=30)		
TT	70.00 (n=21)	43.33 (n=13)	---	1.00 (Reference)
TG	23.33 (n=7)	33.33 (n=10)	0.167	2.30 (0.70-7.57)
GG	6.66 (n=2)	23.33 (n=7)	0.048	5.65 (1.01-31.47)
T	81.67 (49)	60.00 (36)		1.00 (Reference)
G	18.33 (11)	40.00 (24)	0.010	2.96 (1.29-6.83)


Thank You!