تقيم كلية طب الكندي

دورة بعنوان

Statistical analysis of medical data

تقديم

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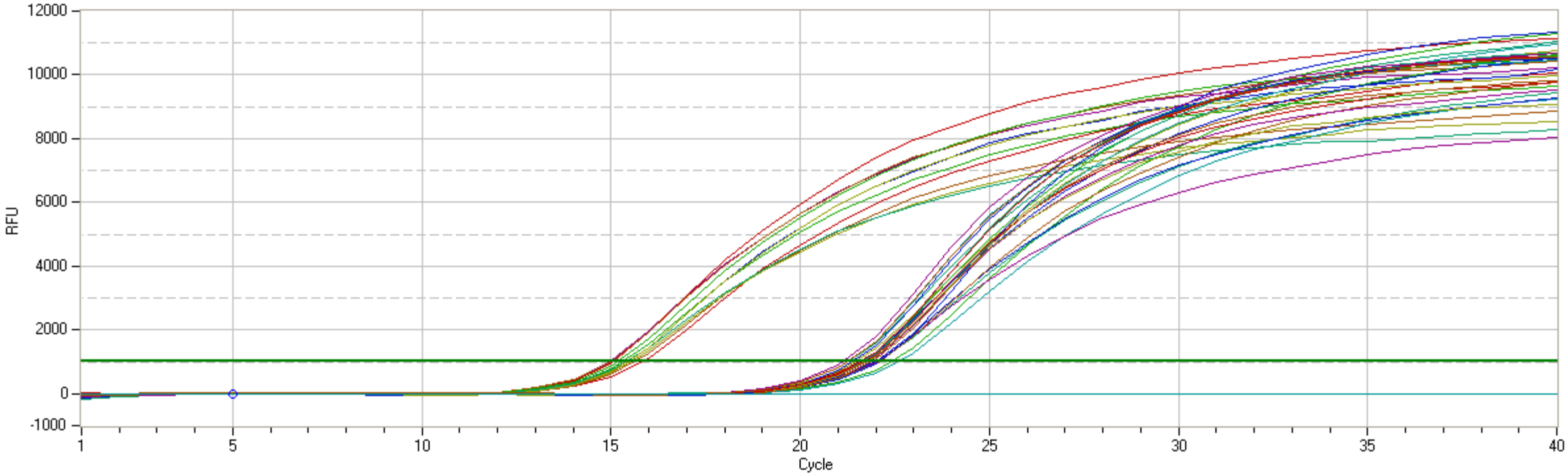
يوم٢٦/٢/٢٠٢٣

المكان: قاعة المؤتمرات

Data analysis in RT-PCR experiments with using Δ Δ method

Question: In a study of the impact of cholesterol on the gene expression of *hmcs1* gene, an RT-PCR experiment was conducted to quantify this impact. *Actin beta* was used as housekeeping gene, the control represent the gene expression without cholesterol while the sample represent the gene expression with using the cholesterol. Does the difference in gene expression between control and sample is due to the impact of cholesterol or by chance? data quantified using delta delta method.



Sample type	Gene	Cq		Sample type	Gene	Cq
control	actin beta	15.06		Control	hmcs1	19.5
control	actin beta	14.51		Control	hmcs1	19.06
control	actin beta	14.77		Control	hmcs1	19.25
control	actin beta	14.42		Control	hmcs1	19.17
control	actin beta	14.7		Control	hmcs1	19.1

ΔCt control = Cq control (gene) – Cq control (actin beta)

= 19.5 – 15.06 = 4.44

= 19.06 – 14.51 = 4.55

= 19.25 – 14.77 = 4.48

= 19.17 – 14.42 = 4.75

= 19.1 – 14.7 = 4.4

Mean = 4.524

Sample type	Gene	Cq		Sample type	Gene	Cq
Sample	actin beta	14.72		Sample	hmcs1	20.22
Sample	actin beta	14.53		Sample	hmcs1	22.01
Sample	actin beta	14.49		Sample	hmcs1	21.42
Sample	actin beta	14.39		Sample	hmcs1	21.11
Sample	actin beta	14.24		Sample	hmcs1	22.14

ΔCt sample = Cq sample (gene) – Cq sample (actin beta)

= 20.22 – 14.72 = 5.5

= 22.01 – 14.53 = 7.48

= 21.42 – 14.49 = 6.93

= 21.11 – 14.39 = 6.72

= 22.14 – 14.24 = 7.9

ΔΔCt control = ΔCt control - Avg. ΔCt control

Gene expression, Control = 2^- ΔΔCt Control

= 4.44 – 4.524 = -0.084	= 2^- (-0.084) = 1.06
= 4.55 – 4.524 = 0.026	= 2^- (0.026) = 0.98
= 4.48 – 4.524 = -0.044	= 2^- (-0.044) = 1.03
= 4.75 – 4.524 = 0.226	= 2^- (0.226) = 0.86
= 4.40 – 4.524 = -0.124	= 2^- (-0.124) = 1.09

ΔΔCt Sample = ΔCt sample - Avg. ΔCt control

Gene expression, Sample = 2^- ΔΔCt Sample

= 5.50 – 4.524 = 0.976	= 2^- (0.976) = 0.51
= 7.48 – 4.524 = 2.956	= 2^- (2.956) = 0.13
= 6.93 – 4.524 = 2.406	= 2^- (2.406) = 0.19
= 6.72 – 4.524 = 2.196	= 2^- (2.196) = 0.22
= 7.90 – 4.524 = 3.376	= 2^- (3.376) = 0.10

Control	Sample	
1.06	0.51	
0.98	0.13	
1.03	0.19	
0.86	0.22	
1.09	0.10	

Mean

$$s_x^2 = \frac{\sum (X - \overline{X})^2}{n} =$$

0.006504

0.0214

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_{x_1}^2}{n_1 - 1} + \frac{S_{x_2}^2}{n_2 - 1}}}$$

	P						
one-tail	0.1	0.05	0.025	0.01	0.005	0.001	0.0005
two-tails	0.2	0.1	0.05	0.02	0.01	0.002	0.001
DF							
1	3.078	6.314	12.706	31.821	63.656	318.289	636.578
2	1.886	2.92	4.303	6.965	9.925	22.328	31.6
3	1.638	2.353	3.182	4.541	5.841	10.214	12.924
4	1.533	2.132	2.776	3.747	4.604	7.173	8.61
5	1.476	2.015	2.571	3.365	4.032	5.894	6.869
6	1.44	1.943	2.447	3.143	3.707	5.208	5.959
7	1.415	1.895	2.365	2.998	3.499	4.785	5.408
8	1.397	1.86	2.306	2.896	3.355	4.501	5.041
9	1.383	1.833	2.262	2.821	3.25	4.297	4.781
10	1.372	1.812	2.228	2.764	3.169	4.144	4.587

, $df = (n_1-1) + (n_2-1) = 8$

t = (1.004 – 0.23)/ sqrt((0.006/4) + (0.021/4))

t = 9.27

9.27 > 2.306 , Reject null hypothesis

9.27 > 5.041 , highly significant difference

THANK YOU