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APLICACIONS BIOTECNOLOXICAS DE PCR EN TEMPO REAL

PCR EN TEMPO REAL

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PCR EN TEMPO REAL

CURVA ESTÁNDAR

Target	Slope	Y-Inter	R2	Eff%
Vaca	-2.939	12.817	0.894	118.915
Vaca correxido	-3.335	12.949	0.936	99.457
Cabalo	-6.253	18.645	0.577	44.52
Cabalo correxido	-3.494	18.465	0.9	93.295

SLOPE: PENDENTE, indica a eficiencia da reacción. Unha pendente de -3,32 correspóndese cunha eficiencia do 100%. Coincide cos ciclos necesarios para obter 10 veces máis copias na etapa exponencial:
$2^{Ct}=2^{3,32}=$ x9,987

Eff%: Tanto por cen de EFICIENCIA DA AMPLIFICACIÓN da reacción con respecto a dobrar a cantiade de adn en cada ciclo (2^{Ct})

$$Eff\%=10^{(-1/SLOPE)}-1=10^{(-1/-3.32)}-1=10^{(0,3)}-1=1,995-1=0,995=99,5\%$$

PCR EN TEMPO REAL

CURVA ESTÁNDAR

SLOPE	EFFICIENCY
-3,32	100%
-3,5	93%
-3,6	90%
-3,8	83%
-4,0	78%

A eficacia debería estar entre o 90 e o 100% = -3,6 $\geq$ slope $\geq$ -3,32

Y-INTER: INTERCEPCIÓN CO EXE Y, correpóndese co valor de Ct para unha mostra de cantitade 1

R^2: Coeficiente de regresión que indica a exactitude entre a recta de regresión e os puntos das dilucións das reaccións estandar. É 1 cando a coincidencia é total.

PCR EN TEMPO REAL

- Absolute quantitation
  - Standard curve
  - Standards must be accurately quantitated
  - Best used for viral load determination
- Relative quantitation
  - Standard curve
  - Standards are serial dilutions of a calibrator template
  - Best used for gene expression studies
- Comparative quantitation
  - Mathematical determination
  - Calibrator sample used as a 1x standard
  - Best used when particular ratios are expected or to verify trends

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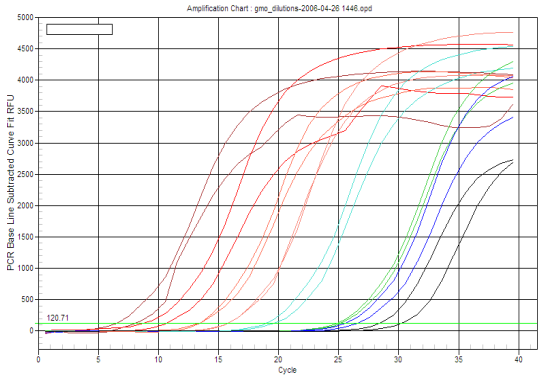
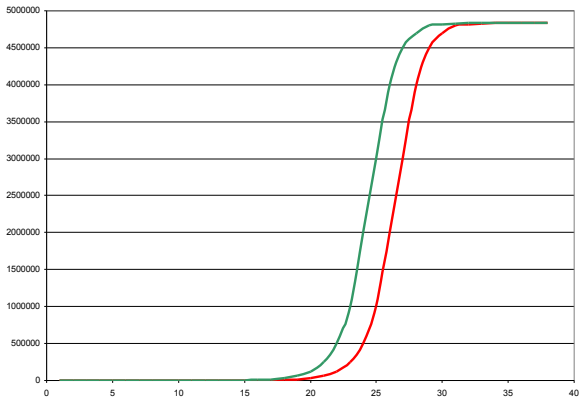


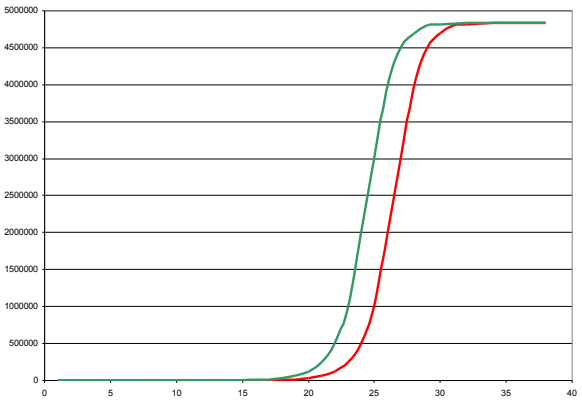
Quantification and Normalization

Quantification and Normalization

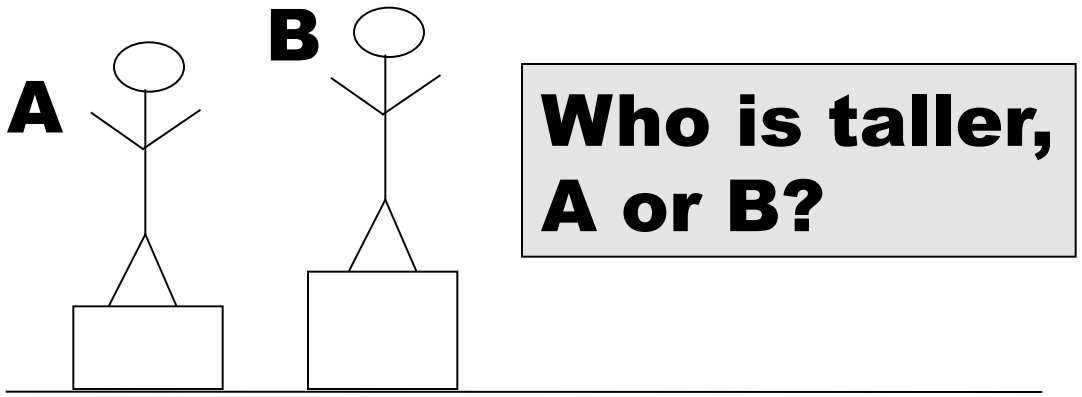


- How can we determine the GMO content of a food?
- We can do this simply by comparing the amount of the GMO “target gene” to the amount of the plant “reference gene”!
- By comparing the two amounts, we then have a basis to compare one food with another!
- This process is called “normalization”.

Normalization, what is it?



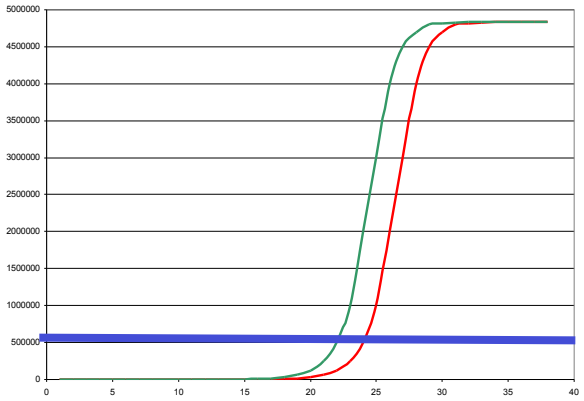
- An analogy to demonstrate the concept of “normalization”…



- Height of person A = (total height of A) – (height of box A)
- Height of person B = (total height of B) – (height of box B)

- In qPCR, the “box” may be the Ct value of a standard sample type (ie. 100% gmo food), or may be the Ct value of a reference gene compared to an unknown gene.

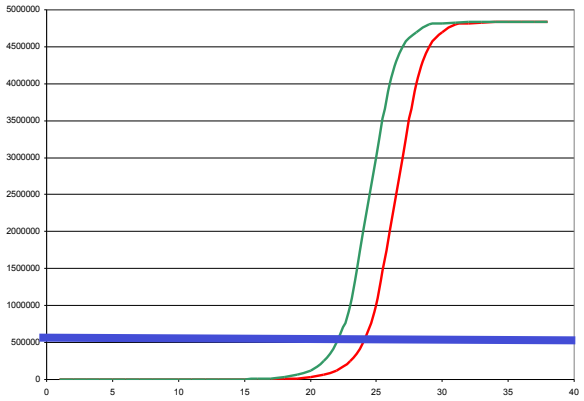
Quantification and Normalization



- Example: Ct values for the plant gene and GMO gene from different food samples.

Food	Plant Ct	GMO Ct
100% GMO	25	28
Non-GMO	26	36
Unknown 1	20	23
Unknown 2	27	31

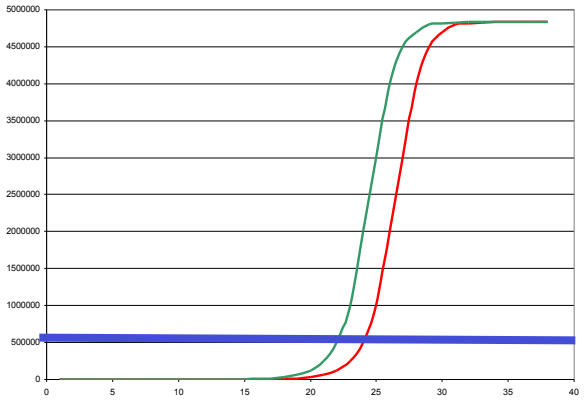
Quantification and Normalization



- First we'll compare relative amounts of plant DNA … relative to the 100% GMO control.
- Using the formula that relates relative quantity to 2^(Cta-Ctb), we can calculate amounts of DNA relative to a single sample.

Food	Ct Value	Delta Ct	Relative Quantity
100% GMO	25	0	1
Non-GMO	26	-1	0.5
Unknown 1	20	5	32
Unknown 2	27	-2	0.25

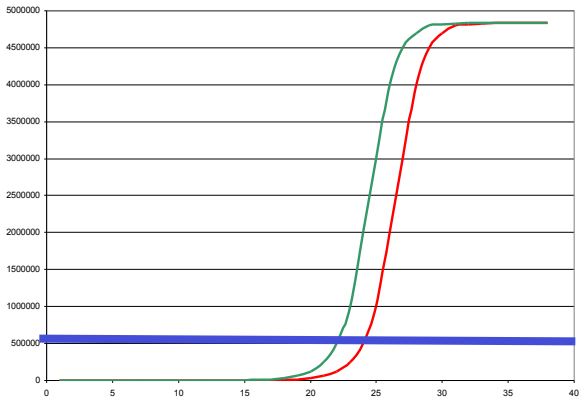
Quantification and Normalization



- Second we'll determine relative amounts of GMO DNA … again, relative to the 100% control.
- We can do the exact same calculations for the Ct values from the GMO genes of the same food samples.

Food	Ct Value	Delta Ct	Relative Quantity
100% GMO	28	0	1
Non-GMO	36	-8	0.004
Unknown 1	23	5	32
Unknown 2	31	-3	0.125

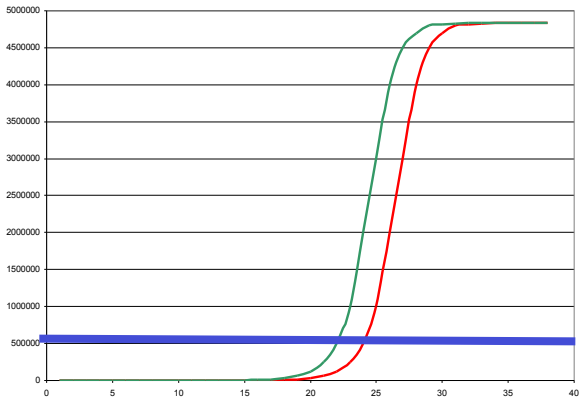
Quantification and Normalization



- Now we compare the relative amounts of plant to GMO DNA in each sample…
- …This gives us our relative GMO content.
- This is called delta-delta-Ct analysis, and is the basis of real-time quantification analysis.

Food	Plant DNA	GMO DNA	Relative GMO Content
100% GMO	1	1	1 (100%)
Non-GMO	0.5	0.004	0.008 (0.8%)
Unknown 1	32	32	1 (100%)
Unknown 2	0.25	0.125	0.5 (50%)

Quantification and Normalization



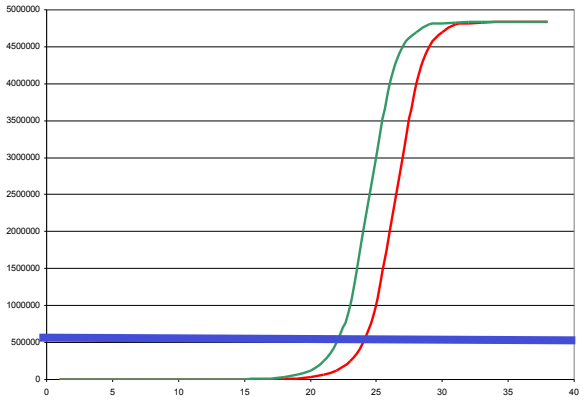
Summary

- First we determined how much plant DNA is in each sample,
- Second we determined how much GMO DNA is in each sample,
- Finally we corrected / normalized the GMO DNA against the plant DNA to determine relative amount of GMO for each sample.

Normalization

Food	Relative GMO Content
100% GMO	100%
Non-GMO	0.8%
Unknown 1	100%
Unknown 2	50%

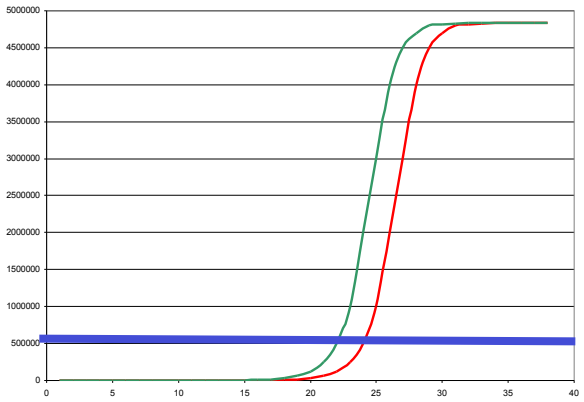
Quantification and Normalization



- Practice! Try this example.
- What is the GMO content of product A and product B??

Food	Plant Ct	GMO Ct
100% GMO	23	27
Non-GMO	24	39
Product A	22	30
Product B	25	29

Quantification and Normalization



- Results…
- What is the GMO content of product A and product B??

Food	Plant Ct	GMO Ct	Delta Ct Plant	Delta Ct GMO	GMO Plant ddCt	**Ratio 2^{ddCt}**	**Ratio %**
100% GMO	23	27	0	0	0	**1**	**100**
Non-GMO	24	39	-1	-12	-11	**0.0004**	**0.04**
Product A	22	30	1	-3	-4	**.0625**	**6.3**
Product B	25	29	-2	-2	0	**1**	**100**