Advanced Cell Biology & Biotechnology

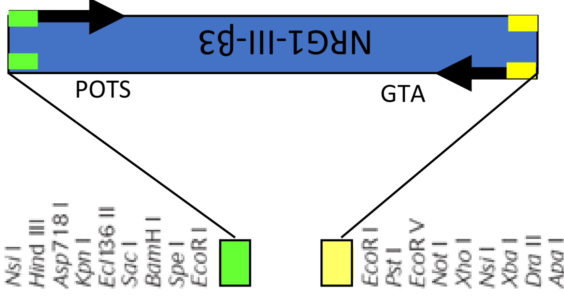
Biotechnology Project Lab

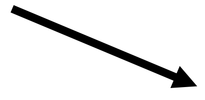
Giovanna Gambarotta
& Isabella Tarulli

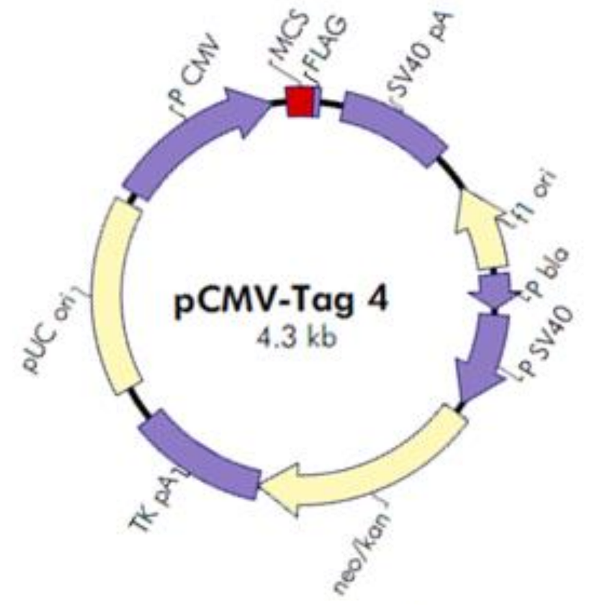
The lecture of November 28th 2021 is about to begin....

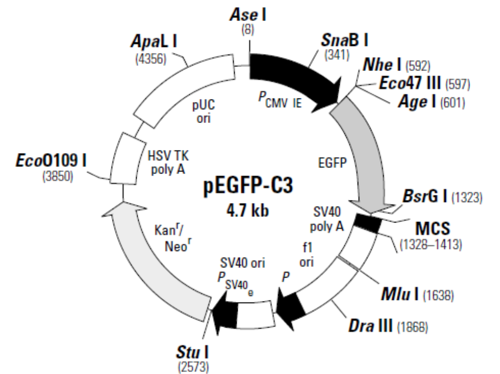
- summary of the previous lesson
- quantitative real time PCR (qRT-PCR-26-11-2021)
- example of qRT-PCR data analysis
- exercise with qRT-PCR data (Excel files)

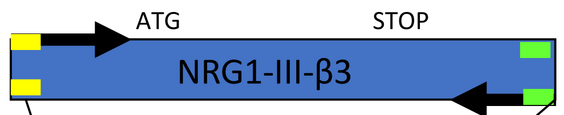
Subcloning NRG1IIIβ3 into the expression vector pCMV-Tag4 containing a FLAG

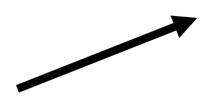


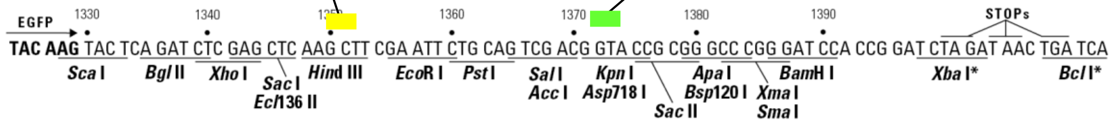












T3 promoter

A ATT AAC CCT CAC TAA AGG GAA CAA AAG CTG GAG CTC CAC CGC GGT GGC GGC CGC TCT A...

Sac I, BstX I, Sac II, Not I

...GC CCG GGC GGA TCC CCC GGG CTG CAG GAA TTC GAT ATC AAG CTT ATC GAT ACC GTC GAC*

Srf I, BamH I, Pst I, EcoR I, EcoR V, Hind III, Acc I/Sal I

Xho I — FLAG tag: D Y K D D D D K

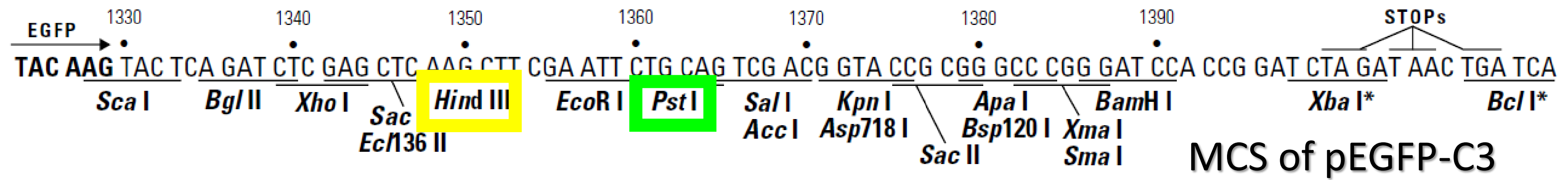
...CTC GAG GAT TAC AAG GAT GAC GAC GAT AAG TAG GGCCCGGTACCT...

STOP

T7 promoter

...TAATTAATTAAGGTACCAGGTAAGTGTACCCAATTCGCCCTATAGTGAGTCGTATTA

MULTIPLE STOP CODONS



Example of NRG1IIIβ3 cloned in pEGFP-C3

Sac I / Ecl136 — HindIII — sense primer — ATG

EGFP — Sca I — Bgl II — Xho I

TAC AAG TAC TCA GAT CTC GAG CTC aagcttggaggtgagccgatggagatttattccccagacatgtctgaggtagctggcgggaggtcctccagcccctccactcagctgagtgcag
ccccatctcttgatgggcttccggcagcggaggaacatataccagacacccacacagaagatgagagaagccctggactcctgggcctggcggtgccctgctgtgtgtgcctggaagctgagc
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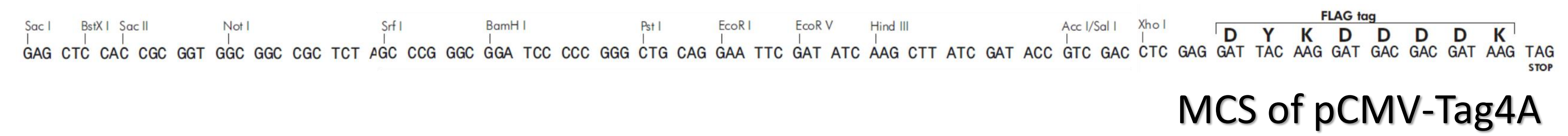
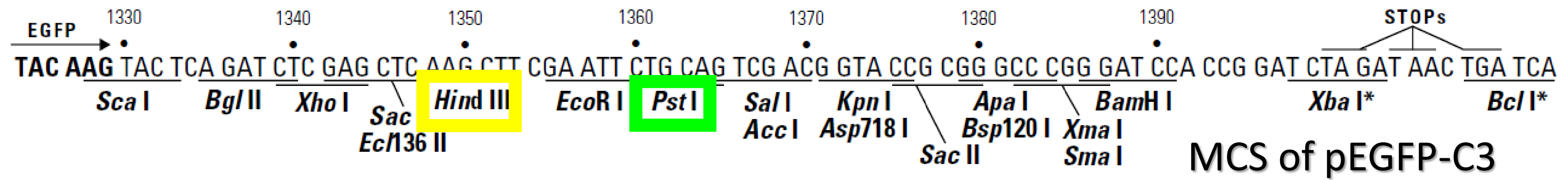
tcctacccaccttgaccctgggggttaggccaggaccctgtgatttctctggatccaactgctgccccagccattttggtatcatctgaggcatacacttcacctgtctctaaggctcagtctgaa
gctggggctcatgttacagtacaaggtgaccatgctgctgtggcctctgaaccttcagcagtaccgacccggaagaaccggctgtctgcttttcctccctttcactctactgcaccgcccttccctt
ctccagctcggacccctgaggtgagaacacccaagtcaggaactcagccacaaacaacagaaactaacctgcaaactgctcctaaactttccacatcgacatccacgactgggaccagccat
ctcataaagtgcgcggagaaggagaaaactttctgtgtgaatgggggcgagtgcttcacggtgaaggacctgtcaaacccgtcaagatacttgtgcaagtgcccaaatgagtttactggtgat
cgttgccaaaactacgtaatggccagcttctacagtacgtccactcctttctgtctctgcctgagtagctgcag TCG ACG GTA CCG CGG GCC CGG GAT CCA CCG GAT CTA GAT AAC TGA TCA

Sal I / Acc I — Kpn I / Asp718 I — Sac II — Apa I / Bsp120 I — Xma I / Sma I — BamH I — Xba I* — Bcl I*

STOP — antisense primer — PstI





MCS of pEGFP-C3

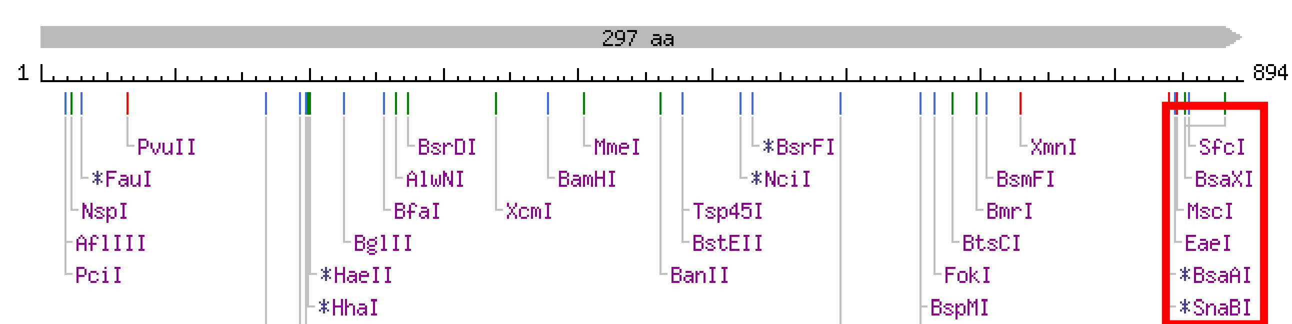
Example of NRG1IIIβ3 cloned in pEGFP-C3

EGFP Sca I Bgl II Xho I Sac I Ecl136 HindIII sense primer ATG

TAC AAG TAC TCA GAT CTC GAG CTC aagcttggaggtgagccgatggagatttattccccagacatgtctgaggtagctggcgggaggtcctccagcccctccactcagctgagtgcag
ccccatctcttgatgggcttccggcagcggaggaacatataccagacacccacacagaagatgagagaagccctggactcctgggcctggcggtgccctgctgtgtgtgcctggaagctgagc
gcctgagagggtgtctcaactccgagaagatctgcattgttcccattctggcttgcctagtcagcctctgcctctgcattgctggcctgaagtgggtatttgtggacaagatatttgaatacgactc
tcctacccaccttgaccctgggggttaggccaggaccctgtgatttctctggatccaactgctgccccagccattttggtatcatctgaggcatacacttcacctgtctctaaggctcagtctgaa
gctggggctcatgttacagtacaaggtgaccatgctgctgtggcctctgaaccttcagcagtaccgacccggaagaaccggctgtctgcttttcctccctttcactctactgcaccgcccttccctt
ctccagctcggacccctgaggtgagaacacccaagtcaggaactcagccacaaacaacagaaactaacctgcaaactgctcctaaactttccacatcgacatccacgactgggaccagccat
ctcataaagtgcgcggagaaggagaaaactttctgtgtgaatgggggcgagtgcttcacggtgaaggacctgtcaaacccgtcaagatacttgtgcaagtgcccaaatgagtttactggtgat
cgttgccaaaactacgtaatggccagcttctacagtacgtccactccctttctgtctctgcctgagtagctgcag TCG ACG GTA CCG CGG GCC CGG GAT CCA CCG GAT CTA GAT AAC TGA TCA

SnaBI MscI SfcI antisense primer STOP PstI

Sal I Acc I Kpn I Asp718 I Sac II Apa I Bsp120 I Xma I Sma I BamH I Xba I* Bcl I*



Single cutters of NRG1IIIβ3

Enzymes you can use for subcloning in pCMV-Tag4

pCMV-Tag 4A Multiple Cloning Site Region

ATT AAC CCT CAC TAA AGG GAA CAA AAG CTG GAG CTC CAC CGC GGT GGC
GGC CGC TCT AGC CCG GGC GGA TCC CCC GGG CTG CAG GAA TTC GAT ATC
AAG CTT ATC GAT ACC GTC GAC*CTC GAG **GAT TAC AAG GAT GAC GAC GAT**
AAG TAG GGC CCG GTA CCT TAA TTA ATT AAG GTA CCA GGT AAG TGT ACC
CAA TTC GCC CTA TAG TGA GTC GTA TTA

pCMV-Tag 4B Multiple Cloning Site Region

TTA ACC CTC ACT AAA GGG AAC AAA AGC TGG AGC TCC ACC GCG GTG GCG
GCC GCT CTA GCC CGG GCG GAT CCC CCG GGC TGC AGG AAT TCG ATA TCA
AGC TTA TCG ATA CCG TCG ACA CTC GAG **GAT TAC AAG GAT GAC GAC GAT**
AAG TAG GGC CCG GTA CCT TAA TTA ATT AAG GTA CCA GGT AAG TGT ACC
CAA TTC GCC CTA TAG TGA GTC GTA TTA

pCMV-Tag 4C Multiple Cloning Site Region

TAA CCC TCA CTA AAG GGA ACA AAA GCT GGA GCT CCA CCG CGG TGG CGG
CCG CTC TAG CCC GGG CGG ATC CCC CGG GCT GCA GGA ATT CGA TAT CAA
GCT TAT CGA TAC CGT CGA CAA CTC GAG **GAT TAC AAG GAT GAC GAC GAT**
AAG TAG GGC CCG GTA CCT TAA TTA ATT AAG GTA CCA GGT AAG TGT ACC
CAA TTC GCC CTA TAG TGA GTC GTA TTA

EcoRI	PstI	SacII
EcoRV	BamHI	
HindIII	SacI	**FLAG**

-> Translate the protein that you obtain after the subcloning: if you have the correct frame you will obtain the NRG1 followed by the **FLAG: DYKDDDDK**

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- summary of the previous lesson
- quantitative real time PCR (qRT-PCR-26-11-2021)
- example of qRT-PCR data analysis
- exercise with qRT-PCR data (Excel files)

- PCR reaction
- conventional versus real time PCR
- real time PCR principles
- threshold cycle C_T
- efficiency
- relative quantification
- reference genes
- primers
- **detection chemistry**
- GLP in real time PCR

1. DNA-binding (intercalating) agents (SYBR Green I, Eva Green, LC Green)
2. Hydrolysis probes (TaqMan)
3. Hybridization (Beacons, Scorpions, Pleiades)
4. Hybridization with FRET probes (Light Cycler)

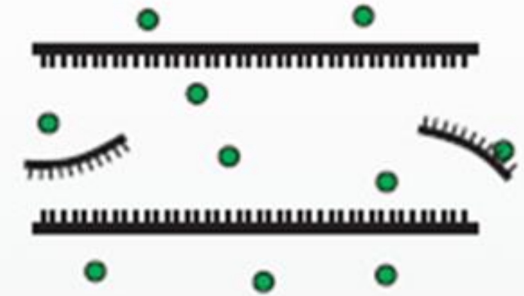
Principles of Real-Time Detection Chemistry

SYBR Green technique:

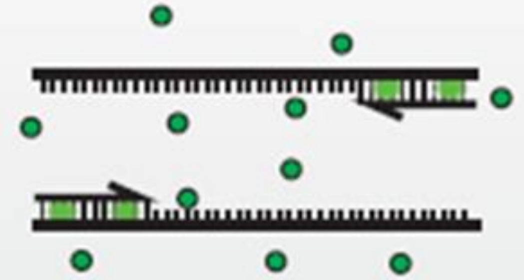
- **SYBR Green fluorescence** is enormously increased upon binding to double-stranded DNA.
- during the extension phase, more and more SYBR Green will bind to the PCR product, resulting in an increased fluorescence.
- consequently, during each subsequent PCR cycle, more fluorescence signal will be detected.

SYBR Green I fluoresces only when bound to dsDNA.

Denature



Anneal



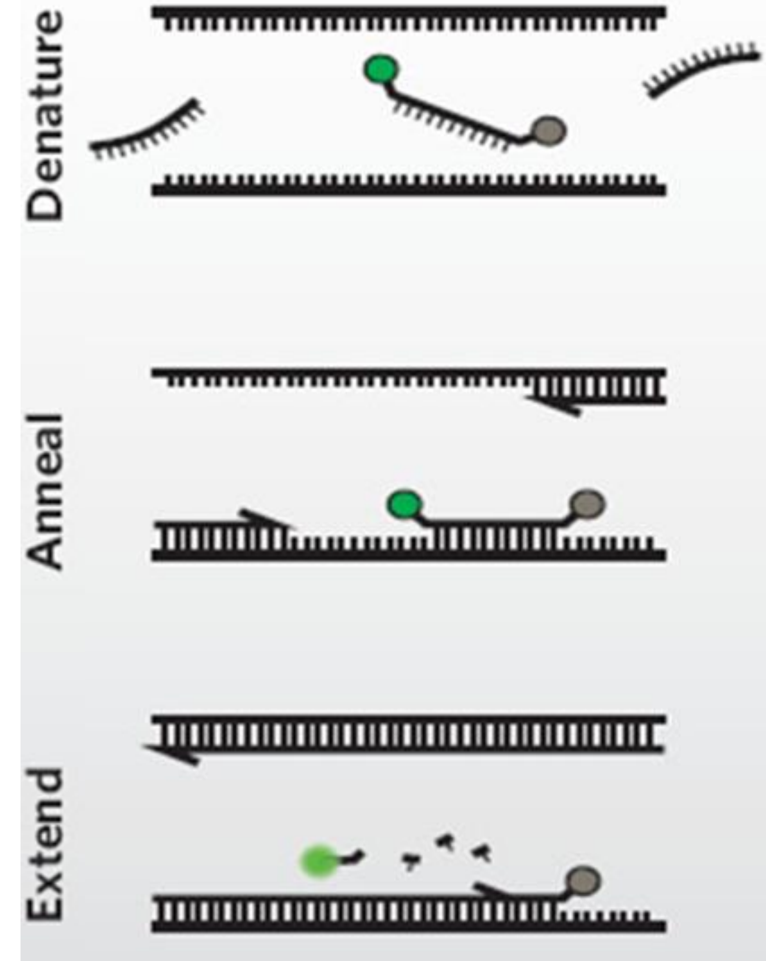
Extend

- Pros: relatively cheap, doesn't require probe design
- Cons: nonspecificity can lead to false positives, not attuned for complex protocols

Principles of Real-Time Detection Chemistry

Hydrolysis probe technique:

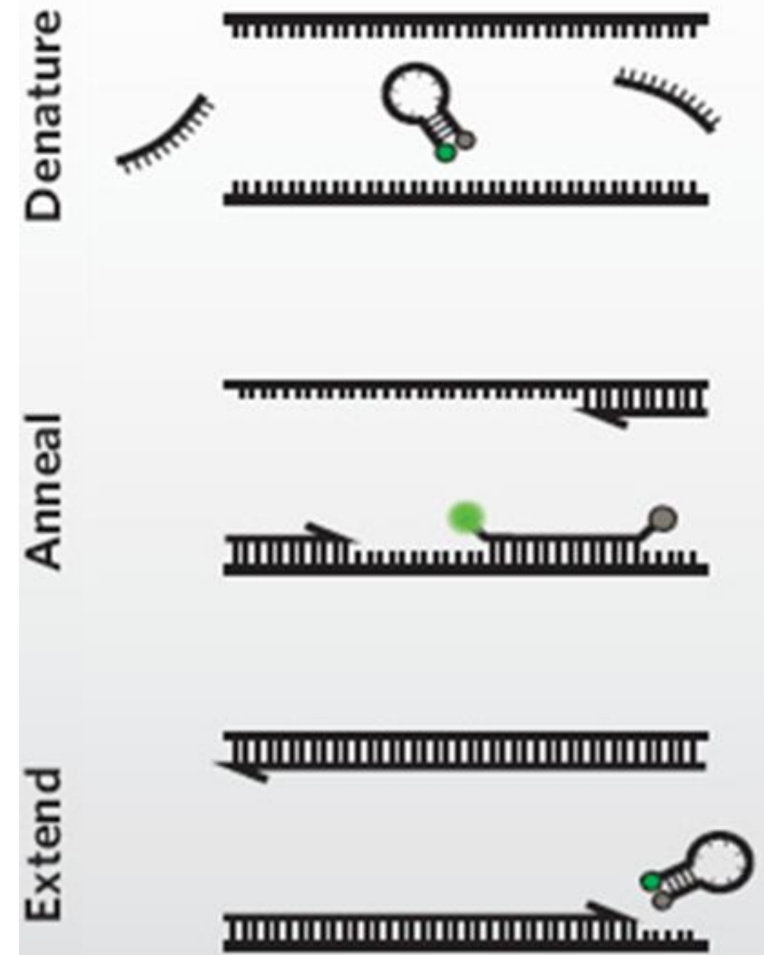
- the hydrolysis probe is conjugated with a **quencher fluorochrome**, which absorbs the fluorescence of the **reporter fluorochrome** as long as the probe is intact.
- upon amplification of the target sequence, the hydrolysis probe is displaced and subsequently hydrolyzed by the Taq polymerase.
- this results in the separation of the reporter and quencher fluorochrome and consequently the fluorescence of the reporter fluorochrome becomes detectable.
- during each consecutive PCR cycle this fluorescence will further increase because of the progressive and exponential accumulation of free reporter fluorochromes.



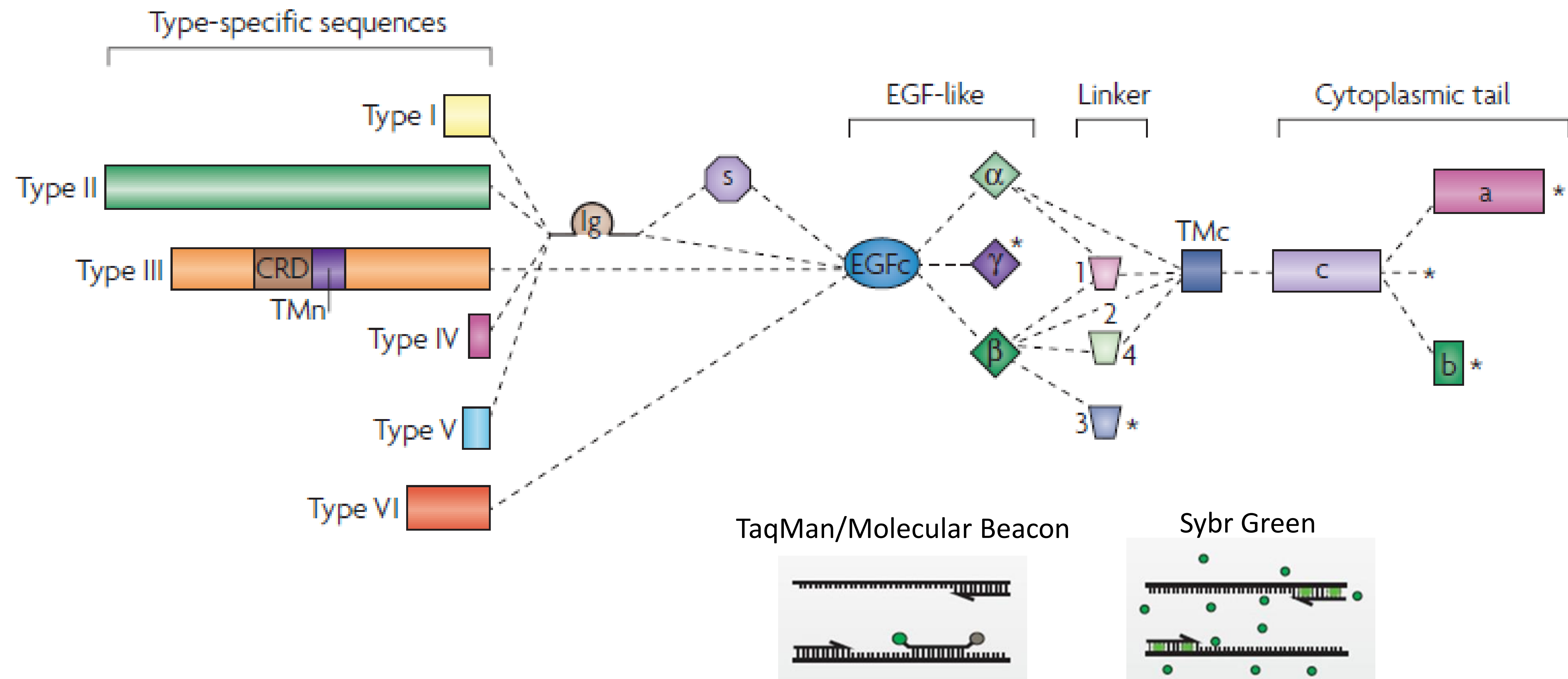
Principles of Real-Time Detection Chemistry

Hybridization probes technique:

- in this technique the probe is labelled with a **reporter fluorochrome** at the 3' end and a **quencher fluorochrome** at the 5' end.
- in the unbound conformation the quencher interacts with the reporter: the fluorescence is OFF
- when the probe anneals to the target sequence this results in the separation of the reporter and quencher fluorochrome and consequently the fluorescence of the reporter fluorochrome becomes ON
- upon amplification of the target sequence, the probe is displaced, and the fluorescence is OFF



Real-Time PCR for NRG1 isoforms



- emits a strong fluorescent signal upon binding to double-stranded DNA
- non-specific binding is a disadvantage
- requires extensive optimization
- requires melting curve analysis to ensure specificity
- longer amplicons create a stronger signal
- may be multiplexed when coupled with melting curve analysis

When to Choose SYBR Green

- detection of thousands of molecules
- general screening of transcripts prior to moving to probe based assays
- when the PCR system is fully optimized
- no primer dimers or non-specific amplicons

When Not to Choose SYBR Green

- allelic discrimination assays
- multiplex reactions
- amplification of rare transcripts

The highest risk in PCR is the contamination

- it is important to have two separate laboratory rooms for real time and DNA manipulation
- use pipettes and reagents dedicated only to PCR
- change gloves when you go to the PCR room
- check everytime for contamination by introducing negative controls in your reactions
- after the PCR reaction, discard the plate in a different room
- run the agarose gel in a different room (in the conventional PCR)
- never introduce plasmidic DNA in the PCR room!!!!

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RT (reverse transcriptase)

	stock	RT +	RT-	mix	final concentration
RNA		0,5-1 µg	0,5-1 µg		1 µg
Buffer	5x	5 µl	5 µl		1x
BSA	1 µg/µl	2,5 µl	2,5 µl		0.1 µg/µl
Triton	1%	1,25 µl	1,25 µl		0.05%
dNTPs	10mM	1,25 µl	1,25 µl		0,5mM
random primers	50 µM	3,75 µl	3,75 µl		7.5 µM
reverse transcriptase	200u/µl	1 µl	——		200u
RNAse inhibitor	33u/µl	1 µl			33u
water		.... µl	.... µl		
tot	25 µl	25 µl	25 µl		

- the cDNA can be diluted 10 folds or more for real time PCR analysis
- if you dilute 10 folds, you add 225 µl water to each sample
- for each gene, you will use 15 µl for the technical triplicate
- if you dilute 10 folds you can analyse 15 genes
- if you dilute 20 folds you can analyse 30 genes
- when you dilute 10 folds, the Ct shifts 3,3 cycles, when you dilute 2 folds Ct shifts 1 cycle

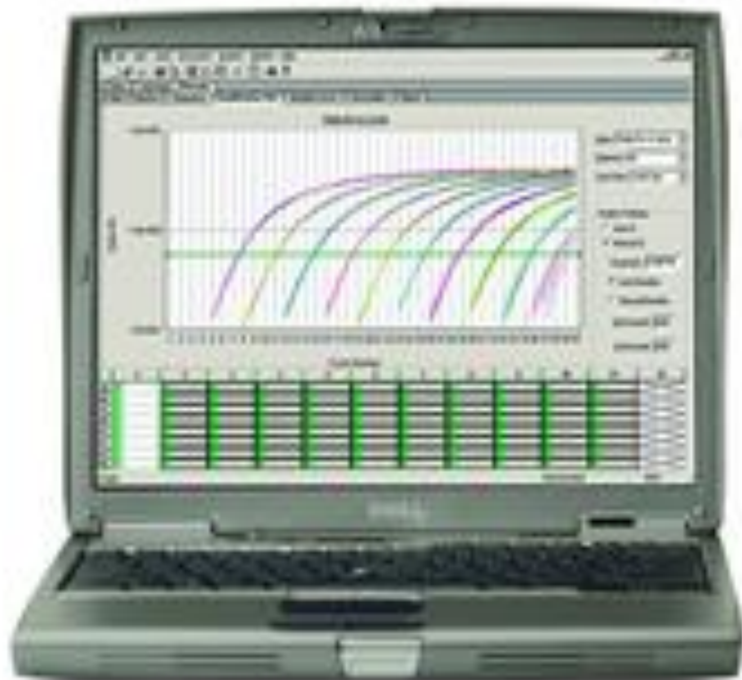
For each gene (target or reference) prepare a mix containing primers and syber mix:

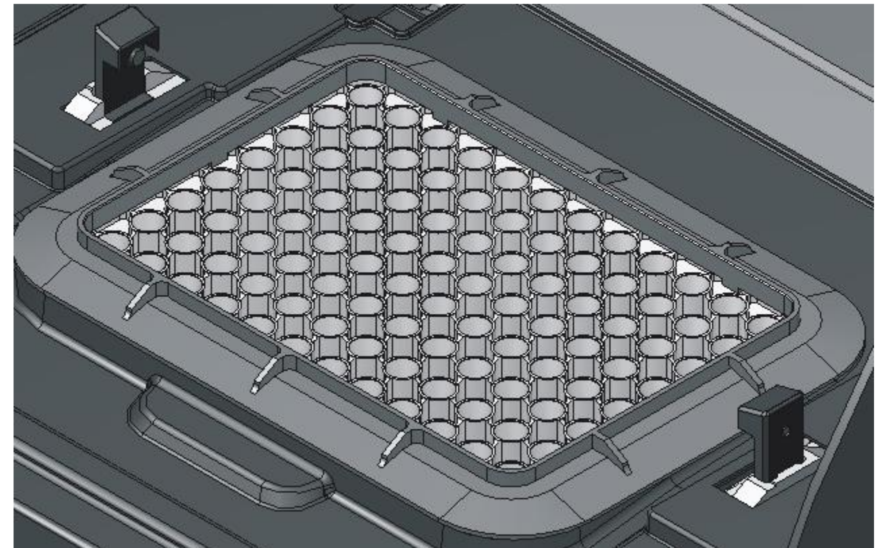
Mix for n samples x 3 (technical triplicate)

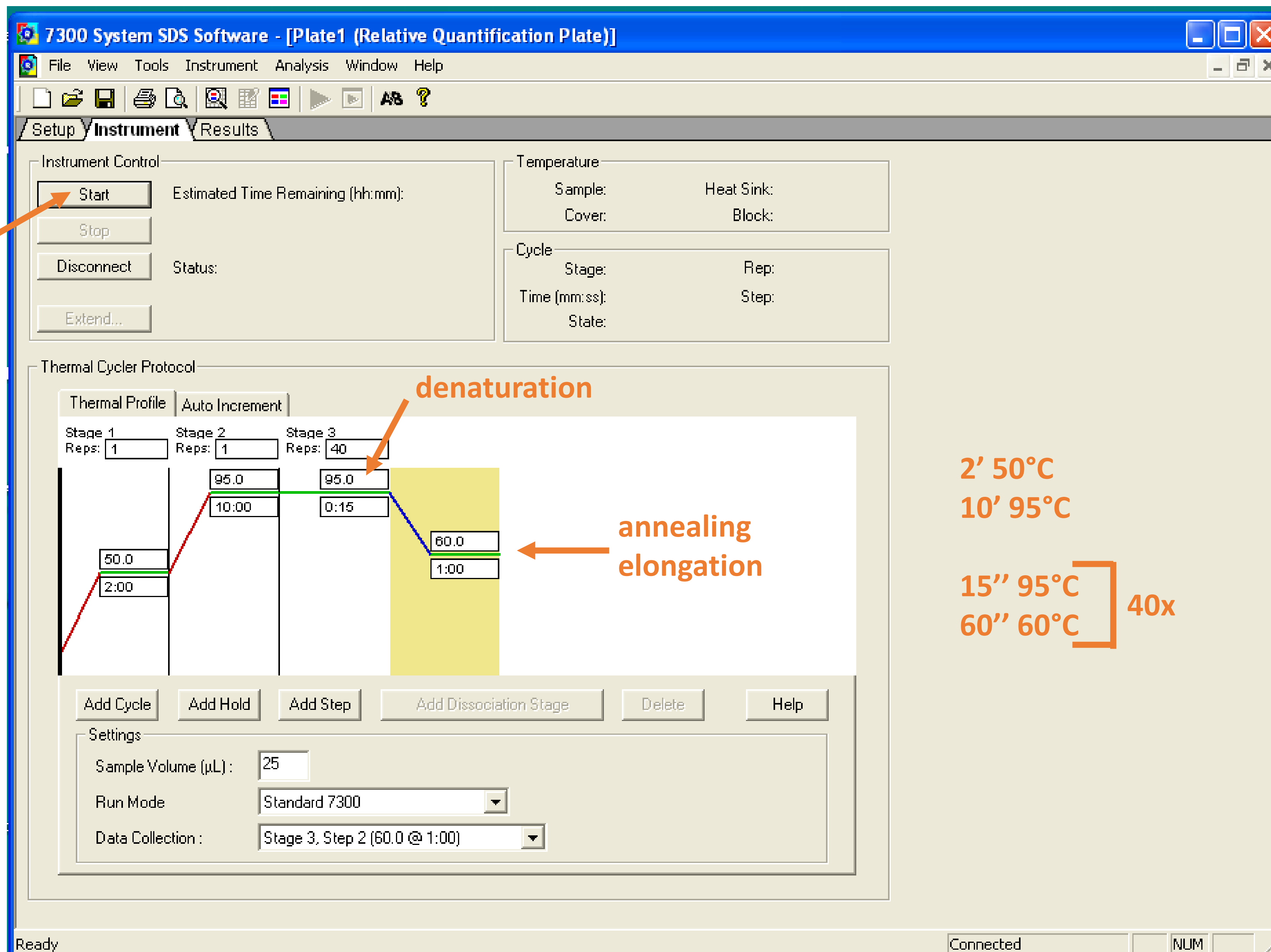
MIX/each gene		1 sample	MIX	final concentration
primer sense	7,5 µM			300 nM
primer antisense	7,5 µM			300 nM
SYBR green I	2 x			1x
water				
tot		20 µl		

For each sample (to be done in technical triplicate)
mix 5µl diluted cDNA and 20 µl mix

Solo per uso didattico - vietata la riproduzione o la vendita

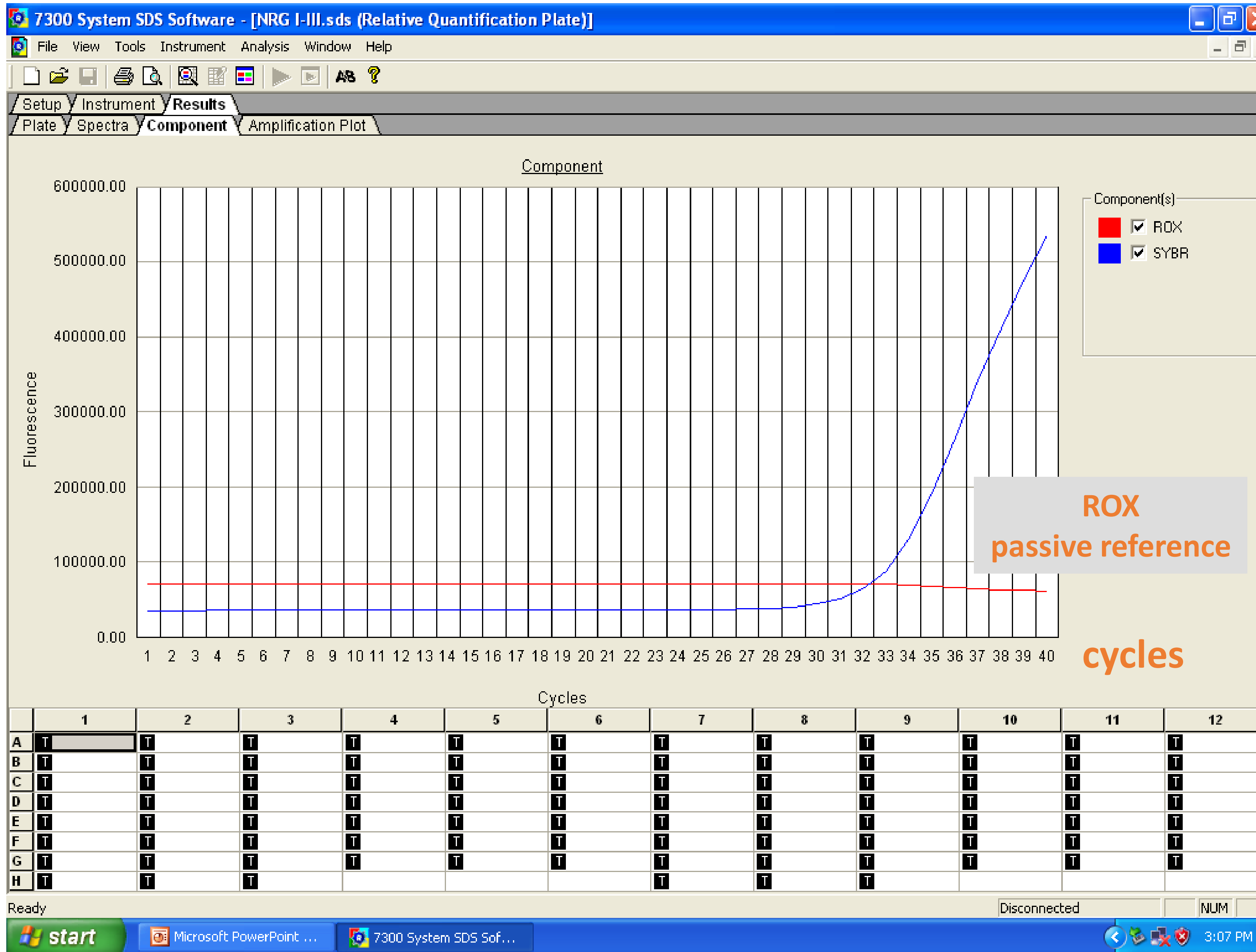






Solo per uso didattico - vietata la riproduzione o la vendita

Fluorescence



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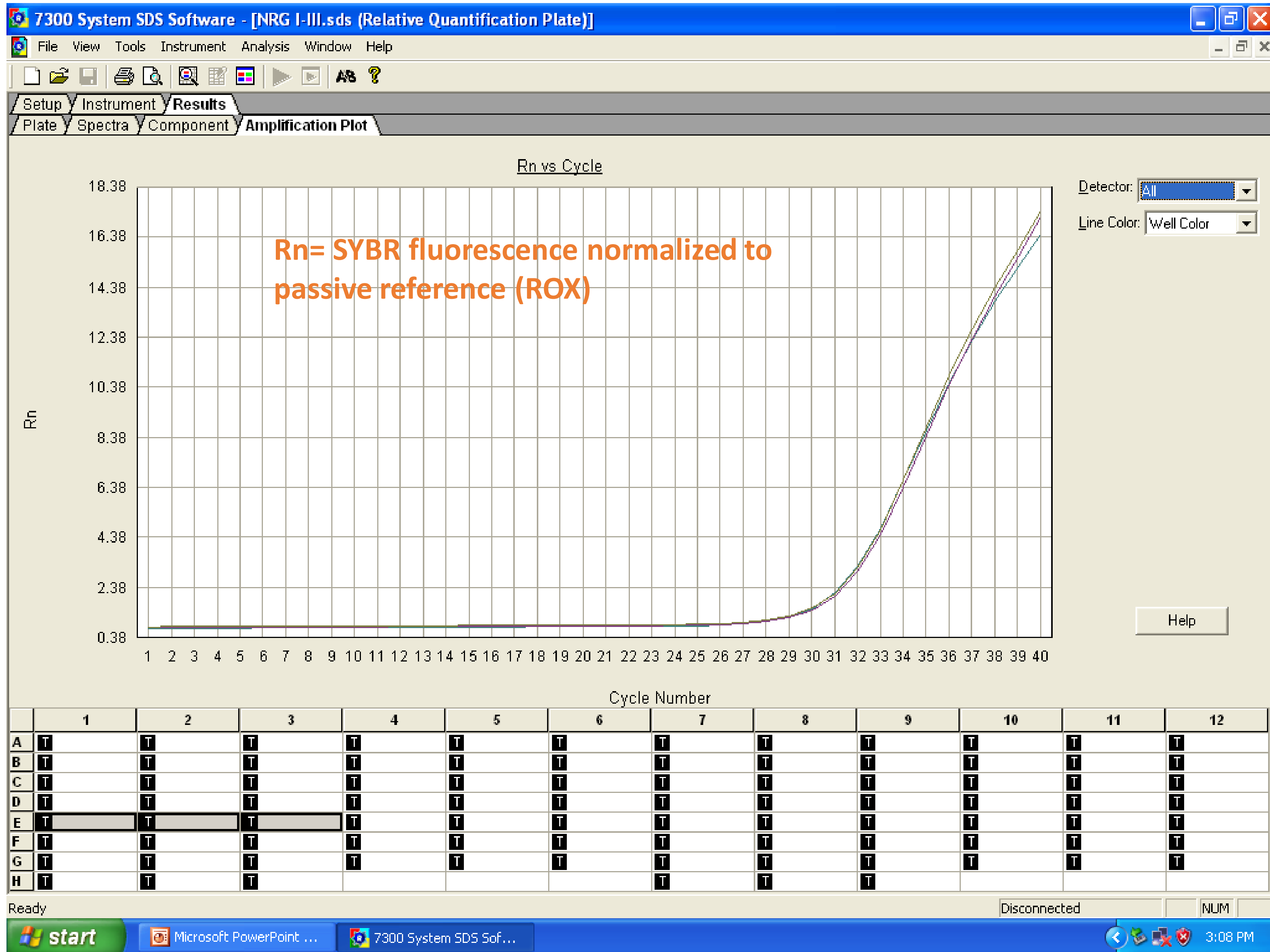

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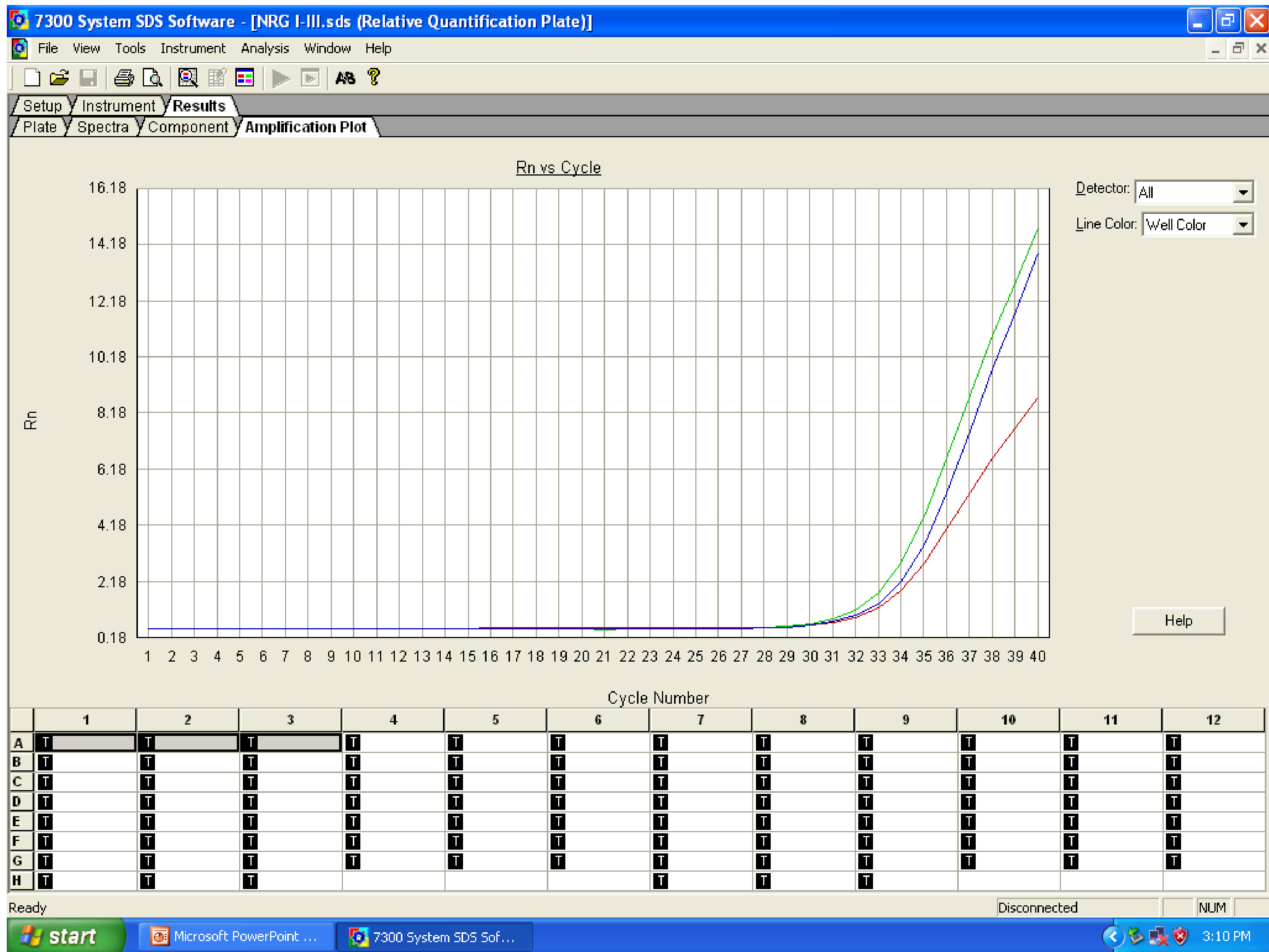

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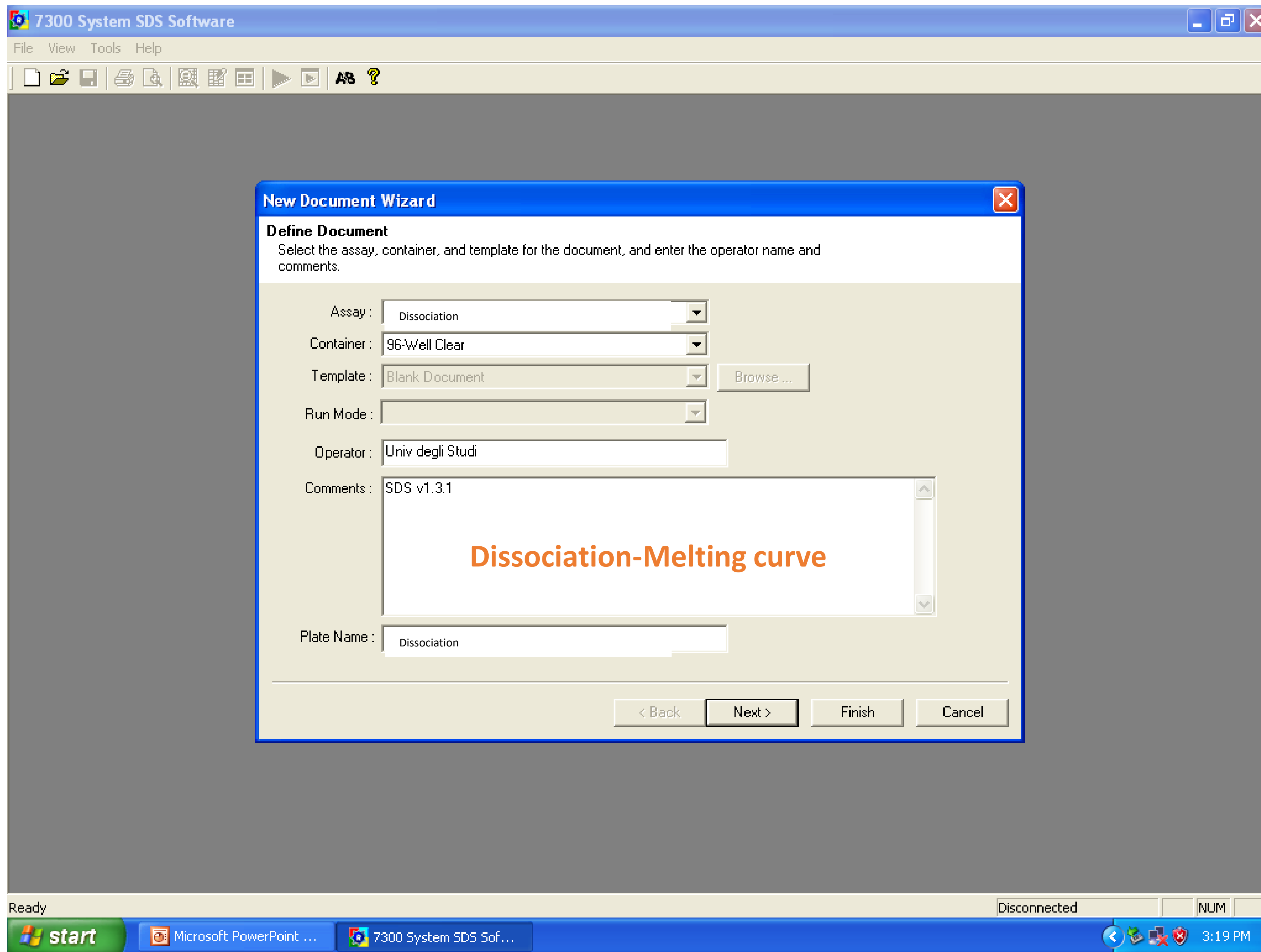

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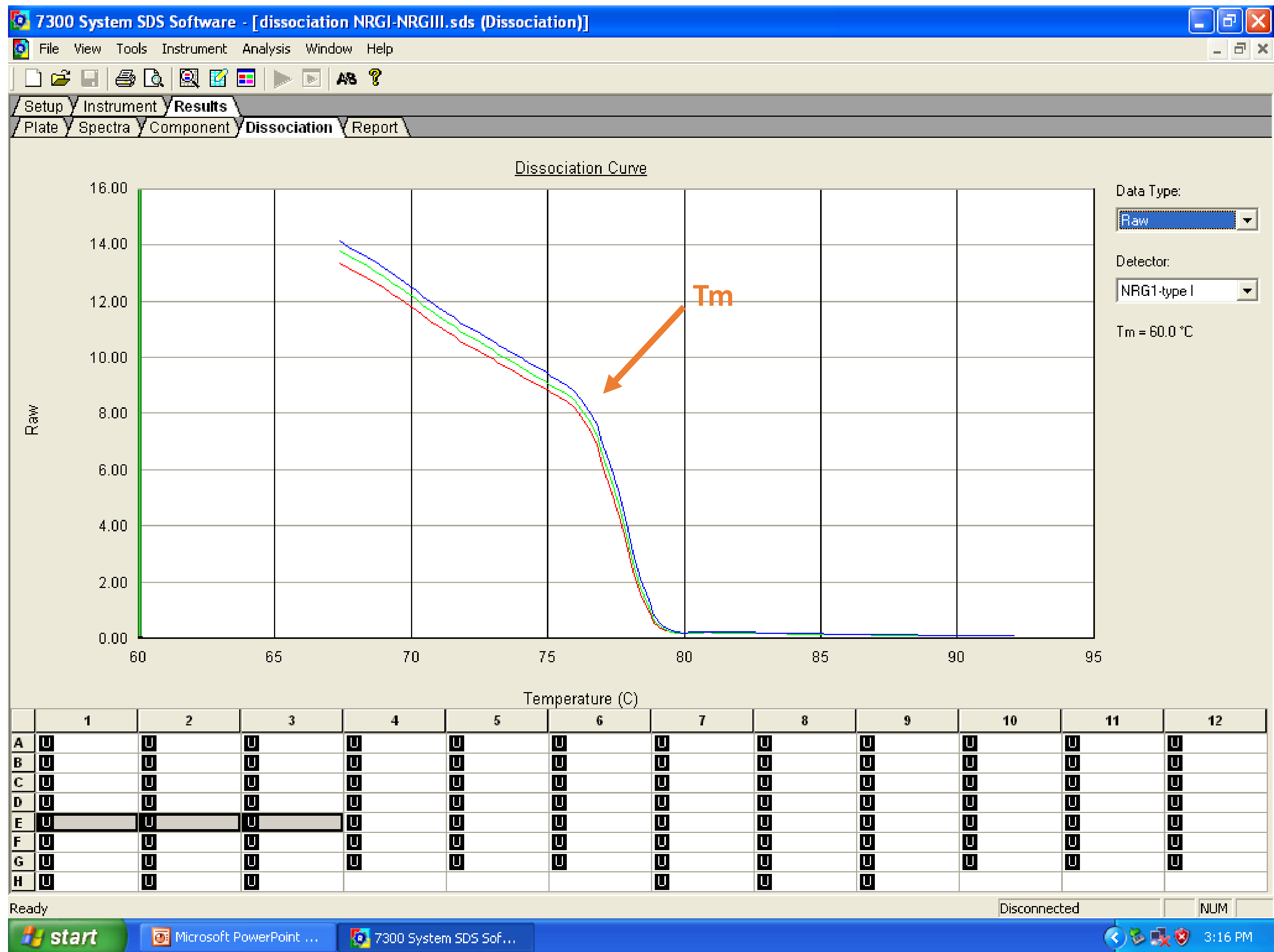

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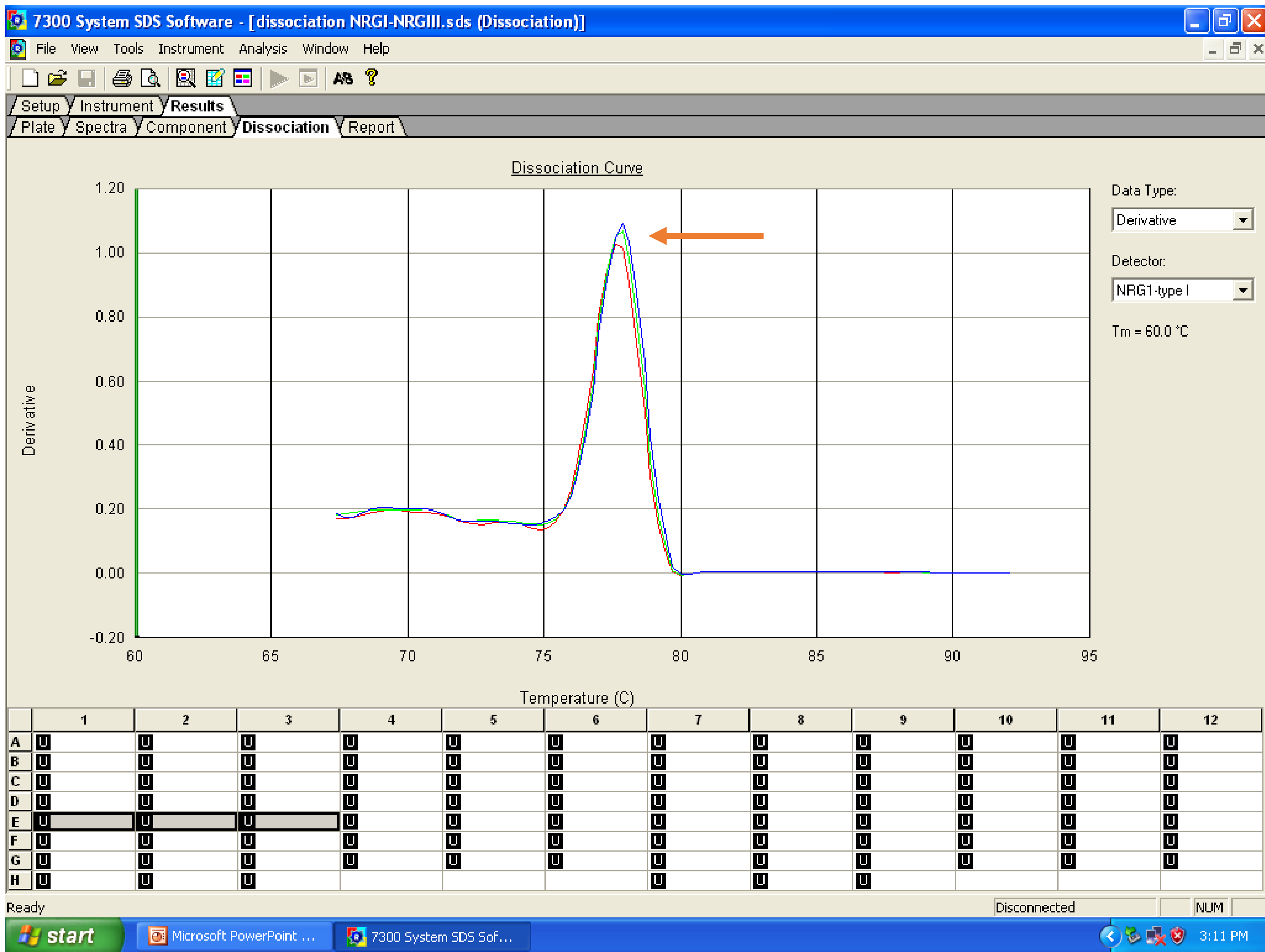

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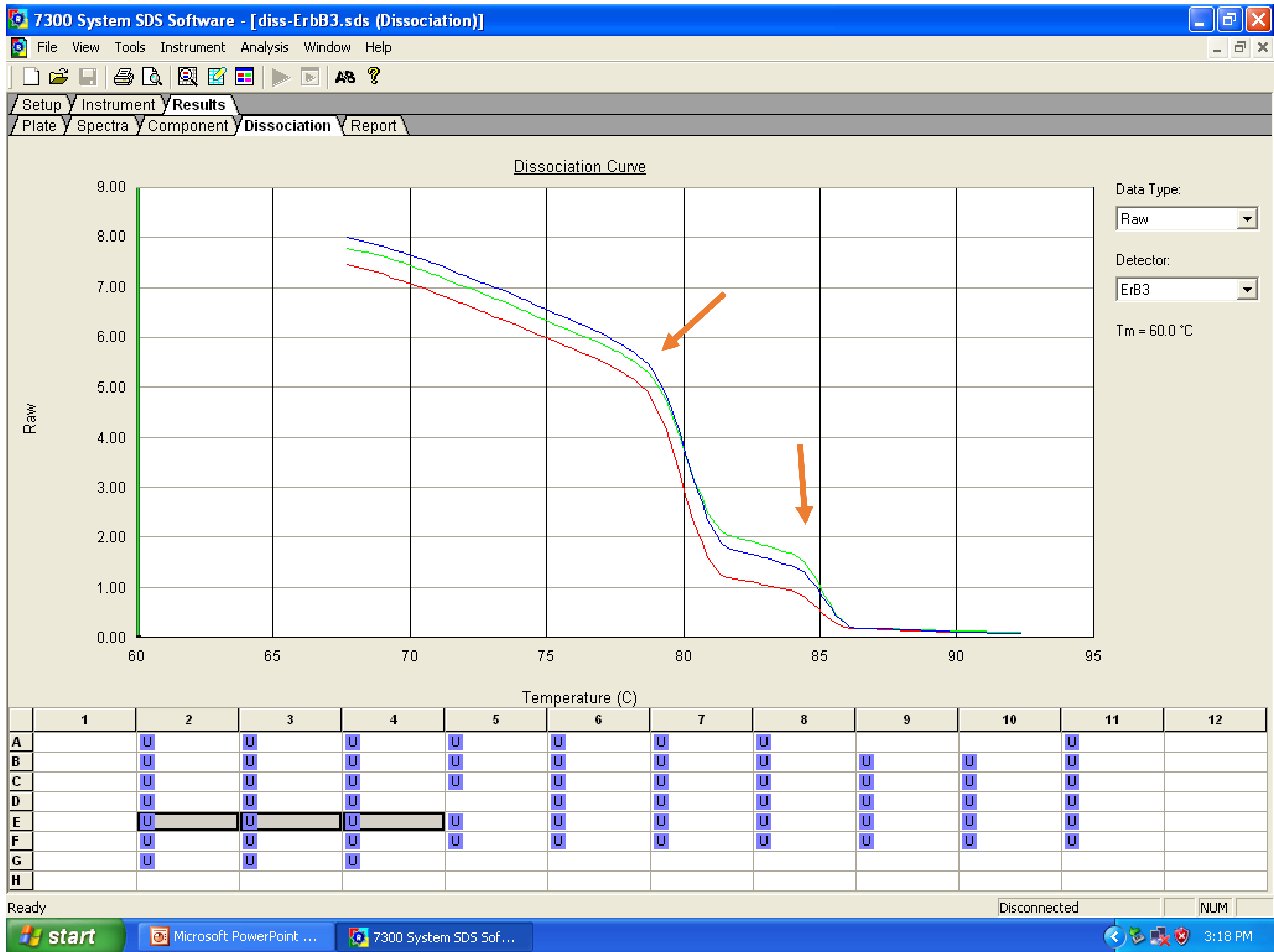

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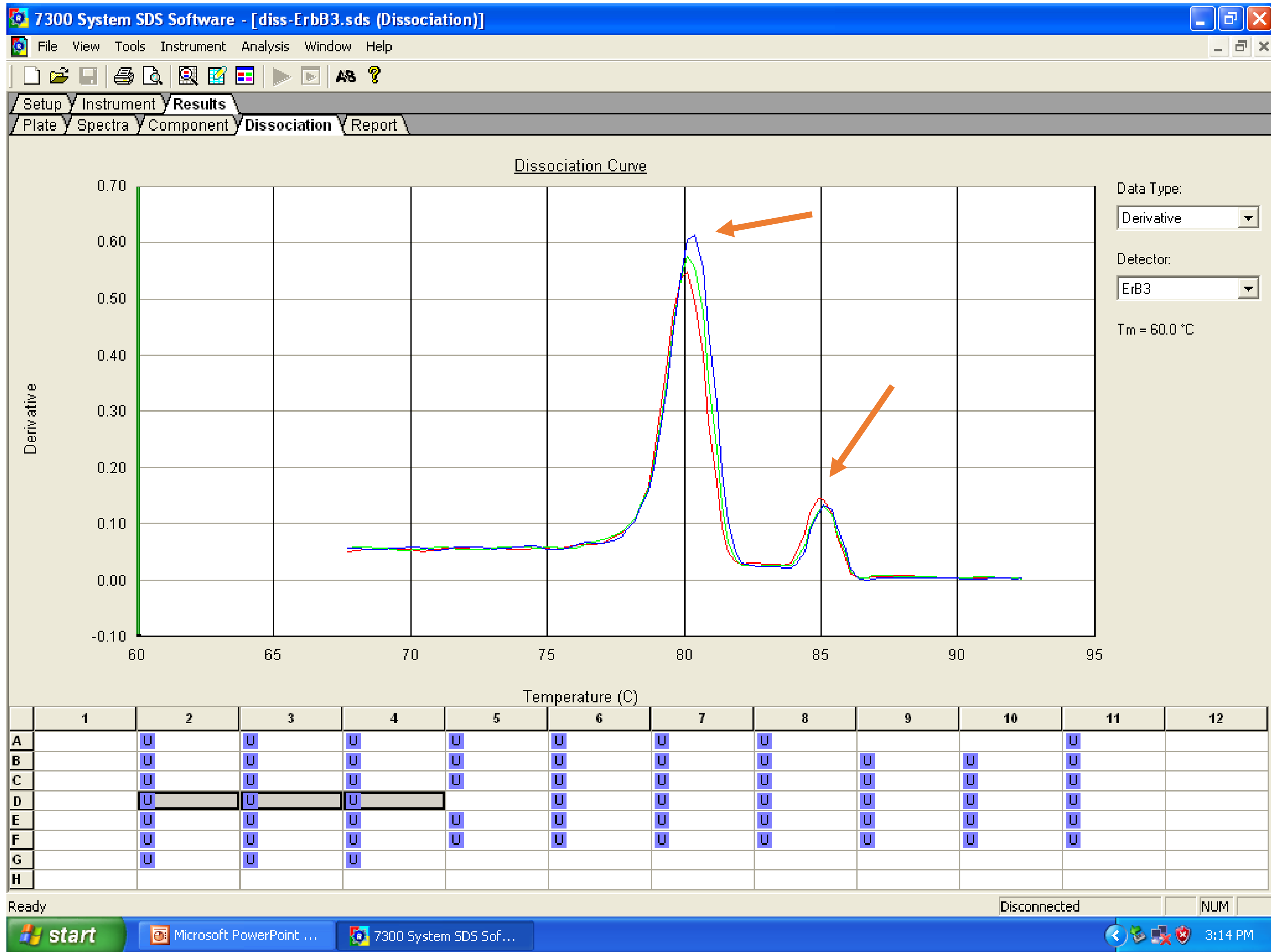

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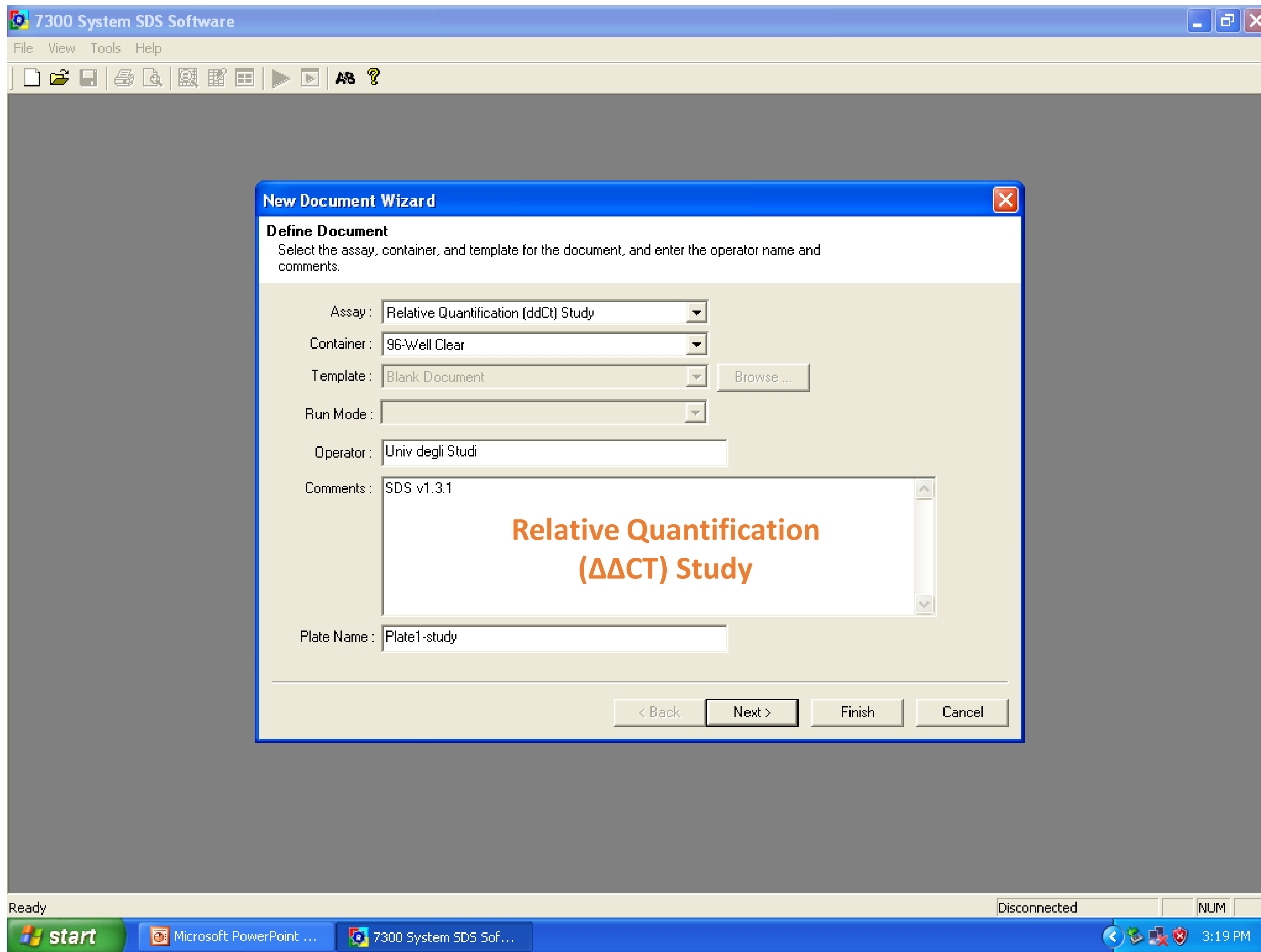

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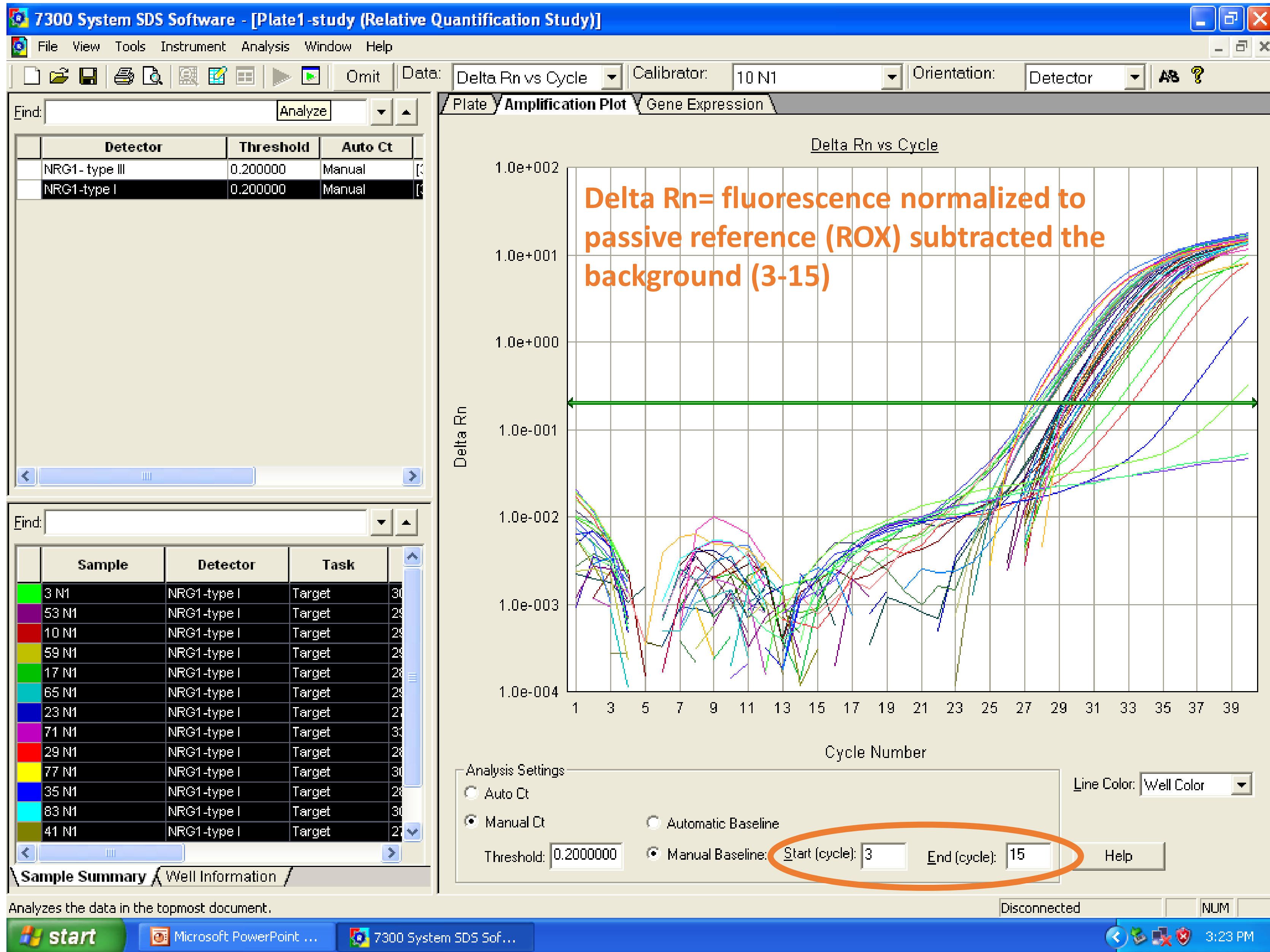

Detector	Threshold	Auto Ct
NRG1- type III	0.200000	Manual
NRG1-type I	0.200000	Manual

Sample	Detector	Task
3 N1	NRG1-type I	Target
53 N1	NRG1-type I	Target
10 N1	NRG1-type I	Target
59 N1	NRG1-type I	Target
17 N1	NRG1-type I	Target
65 N1	NRG1-type I	Target
23 N1	NRG1-type I	Target
71 N1	NRG1-type I	Target
29 N1	NRG1-type I	Target
77 N1	NRG1-type I	Target
35 N1	NRG1-type I	Target
83 N1	NRG1-type I	Target
41 N1	NRG1-type I	Target

Solo per uso didattico - vietata la riproduzione o la vendita

7300 System SDS Software - [Plate1-study (Relative Quantification Study)]

File View Tools Instrument Analysis Window Help

Omit Data: Delta Rn vs Cycle Calibrator: 10 N1 Orientation: Detector

Find:

	Detector	Threshold	Auto Ct	Baselin
	NRG1- type III	0.200000	Manual	[6,14]
	NRG1-type I	0.200000	Manual	[6,14]

CTR=water+mix= negative control

Undetectable or 39,045 Ok!!

Find:

Plate	Well	Sample	Detector	Ct	Ct std err	Omit
NRG I-III	F3	35 N1	NRG1-type I	27.930	0.060	
NRG I-III	F4	83 N1	NRG1-type I	30.235	0.103	
NRG I-III	F5	83 N1	NRG1-type I	30.555	0.103	
NRG I-III	F6	83 N1	NRG1-type I	30.258	0.103	
NRG I-III	G1	41 N1	NRG1-type I	27.331	0.078	
NRG I-III	G2	41 N1	NRG1-type I	27.428	0.078	
NRG I-III	G3	41 N1	NRG1-type I	27.162	0.078	
NRG I-III	G4	CTR N1	NRG1-type I	Undet.		
NRG I-III	G5	CTR N1	NRG1-type I	39.045		
NRG I-III	G6	CTR N1	NRG1-type I	Undet.		
NRG I-III	H1	47 N1	NRG1-type I	29.320	0.080	
NRG I-III	H2	47 N1	NRG1-type I	29.500	0.080	
NRG I-III	H3	47 N1	NRG1-type I	29.227	0.080	

Sample Summary | Well Information

Plate | Amplification Plot | Gene Expression

Delta Rn vs Cycle

Delta Rn: 1.0e+000, 1.0e-001, 1.0e-002, 1.0e-003, 1.0e-004

Cycle Number: 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39

Analysis Settings

Auto Ct

Manual Ct

Automatic Baseline

Threshold: 0.2000000

Manual Baseline: Start (cycle): 6 End (cycle): 14

Line Color: Well Color

Help

Ready Disconnected NUM

start Microsoft PowerPoint ... 7300 System SDS Sof... 3:30 PM

Solo per uso didattico - vietata la riproduzione o la vendita

Microsoft Excel - NRGI-NRGIII.xls

File Modifica Visualizza Inserisci Formato Strumenti Dati Finestra ?

W30

	A	B	C	D	E	F	G	H	I	R	S	T	U	V	W	X
28	Endogenou															
29																
30	Plate	Well ID	Well	Sample	Detector	Task	Ct	Ct std err	Avg Ct	Threshold	Auto Ct	Baseline	Start	End		
31																
32	NRG I-III	7	A7	3 NIII	NRG1- typ	Target	33.119	0.293	32.588	0.2	Manual	Manual	7	15		
33	NRG I-III	8	A8	3 NIII	NRG1- typ	Target	32.539	0.293	32.588	0.2	Manual	Manual	7	15		
34	NRG I-III	9	A9	3 NIII	NRG1- typ	Target	32.107	0.293	32.588	0.2	Manual	Manual	7	15		
35	NRG I-III	10	A10	53 NIII	NRG1- typ	Target										
36	NRG I-III	11	A11	53 NIII	NRG1- typ	Target	32.749	0.225	32.975	0.2	Manual	Manual	7	15		
37	NRG I-III	12	A12	53 NIII	NRG1- typ	Target	33.2	0.225	32.975	0.2	Manual	Manual	7	15		
38	NRG I-III	19	B7	10 NIII	NRG1- typ	Target										
39	NRG I-III	20	B8	10 NIII	NRG1- typ	Target	31.587	0.118	31.705	0.2	Manual	Manual	7	15		
40	NRG I-III	21	B9	10 NIII	NRG1- typ	Target	31.824	0.118	31.705	0.2	Manual	Manual	7	15		
41	NRG I-III	22	B10	59 NIII	NRG1- typ	Target	32.801	0.127	32.937	0.2	Manual	Manual	7	15		
42	NRG I-III	23	B11	59 NIII	NRG1- typ	Target	33.19	0.127	32.937	0.2	Manual	Manual	7	15		
43	NRG I-III	24	B12	59 NIII	NRG1- typ	Target	32.82	0.127	32.937	0.2	Manual	Manual	7	15		
44	NRG I-III	31	C7	17 NIII	NRG1- typ	Target	32.753	0.022	32.732	0.2	Manual	Manual	7	15		
45	NRG I-III	32	C8	17 NIII	NRG1- typ	Target	32.71	0.022	32.732	0.2	Manual	Manual	7	15		
46	NRG I-III	33	C9	17 NIII	NRG1- typ	Target										
47	NRG I-III	34	C10	65 NIII	NRG1- typ	Target										
48	NRG I-III	35	C11	65 NIII	NRG1- typ	Target	33.647	0.151	33.798	0.2	Manual	Manual	7	15		
49	NRG I-III	36	C12	65 NIII	NRG1- typ	Target	33.949	0.151	33.798	0.2	Manual	Manual	7	15		
50	NRG I-III	43	D7	23 NIII	NRG1- typ	Target	33.543	0.083	33.586	0.2	Manual	Manual	7	15		
51	NRG I-III	44	D8	23 NIII	NRG1- typ	Target	33.468	0.083	33.586	0.2	Manual	Manual	7	15		
52	NRG I-III	45	D9	23 NIII	NRG1- typ	Target	33.747	0.083	33.586	0.2	Manual	Manual	7	15		
53	NRG I-III	46	D10	CTR NIII	NRG1- typ	Target	Undet.			0.2	Manual	Manual	7	15		
54	NRG I-III	47	D11	CTR NIII	NRG1- typ	Target	Undet.			0.2	Manual	Manual	7	15		
55	NRG I-III	48	D12	CTR NIII	NRG1- typ	Target	Undet.			0.2	Manual	Manual	7	15		
56	NRG I-III	55	E7	29 NIII	NRG1- typ	Target										
57	NRG I-III	56	E8	29 NIII	NRG1- typ	Target	34.482	0.314	34.796	0.2	Manual	Manual	7	15		
58	NRG I-III	57	E9	29 NIII	NRG1- typ	Target	35.109	0.314	34.796	0.2	Manual	Manual	7	15		
59	NRG I-III	58	E10	71 NIII	NRG1- typ	Target	34.093	0.32	34.413	0.2	Manual	Manual	7	15		
60	NRG I-III	59	E11	71 NIII	NRG1- typ	Target										
61	NRG I-III	60	E12	71 NIII	NRG1- typ	Target	34.733	0.32	34.413	0.2	Manual	Manual	7	15		
62	NRG I-III	67	F7	35 NIII	NRG1- typ	Target	34.249	0.296	33.786	0.2	Manual	Manual	7	15		

NRGI-NRGIII

Pronto NUM

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Solo per uso didattico - vietata la riproduzione o la vendita

1 - copy the Ct of the target gene and of the reference gene for calibrator and samples

	Ct-target gene	Ct-Reference gene
Calibrator	$Ct_{(target)C}= 25$	$Ct_{(reference)C} = 24$
Sample 1	$Ct_{(target)1}= 22$	$Ct_{(reference)1} = 23$
Sample 2	$Ct_{(target)2}= 23$	$Ct_{(reference)2} = 24$
Sample 3	$Ct_{(target)3}= 27$	$Ct_{(reference)3} = 23$

2 - normalize the Ct of the target gene to that of the reference gene for calibrator and samples, calculating the ΔCt

	Ct-target gene	Ct-Reference gene	ΔCt
Calibrator	$Ct_{(target)C} = 25$	$Ct_{(reference)C} = 24$	$\Delta Ct_C=Ct_{(target)C}-Ct_{(reference)C} = 1$
Sample 1	$Ct_{(target)1} = 22$	$Ct_{(reference)1} = 23$	$\Delta Ct_1=Ct_{(target)1}-Ct_{(reference)1} = -1$
Sample 2	$Ct_{(target)2} = 23$	$Ct_{(reference)2} = 24$	$\Delta Ct_2=Ct_{(target)2}-Ct_{(reference)2} = -1$
Sample 3	$Ct_{(target)3} = 27$	$Ct_{(reference)3} = 23$	$\Delta Ct_3= Ct_{(target)3}-Ct_{(reference)3} = 4$

3 - normalize the ΔCt of the samples to the ΔCt of calibrator, calculating the ΔΔCt

	ΔΔCt	-ΔΔCt	$2^{-\Delta\Delta Ct}$
Calibrator	$\Delta\Delta Ct_C = \Delta Ct_C-\Delta Ct_C = 1-1 = 0$	0	$2^{-\Delta\Delta Ct_C} = 2^0=1$
Sample 1	$\Delta\Delta Ct_1 = \Delta Ct_1-\Delta Ct_C = -1-1 = -2$	2	$2^{-\Delta\Delta Ct_1} = 2^2=4$
Sample 2	$\Delta\Delta Ct_2 = \Delta Ct_2-\Delta Ct_C = -1-1 = -2$	2	$2^{-\Delta\Delta Ct_2} = 2^2=4$
Sample 3	$\Delta\Delta Ct_3 = \Delta Ct_3-\Delta Ct_C = 4-1 = 3$	-3	$2^{-\Delta\Delta Ct_3} = 2^{-3}=0,125$

4 - finally, calculate the normalized relative quantification= $2^{-\Delta\Delta CT}$

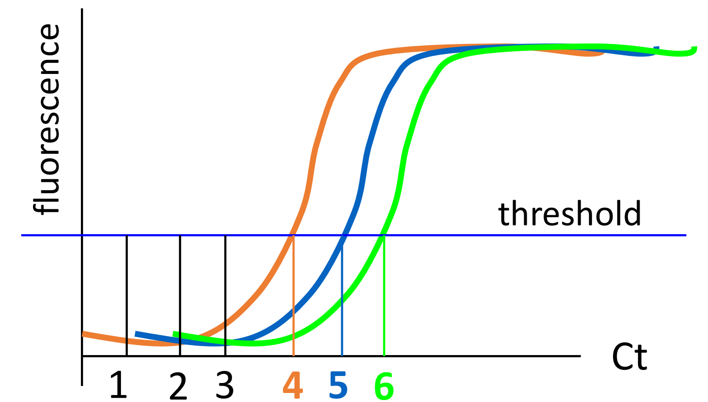
Biotechnology Project Lab

Giovanna Gambarotta
& Isabella Tarulli

The lecture of November 28th 2021 is about to begin….

- summary of the previous lesson
- quantitative real time PCR (qRT-PCR-26-11-2021)
- example of qRT-PCR data analysis
- exercize with qRT-PCR data (Excel files)

cycle	Sample A (calibrator)	Sample B	Sample C
0	**2**	**4**	**8**
1	4	8	16
2	8	16	32
3	16	32	64
4	32	64	**128**
5	64	**128**	256
6	**128**	256	512



- If the detectable amount of DNA is **128**

-the Ct value for sample A will be 6,

-the Ct value for sample B will be 5,

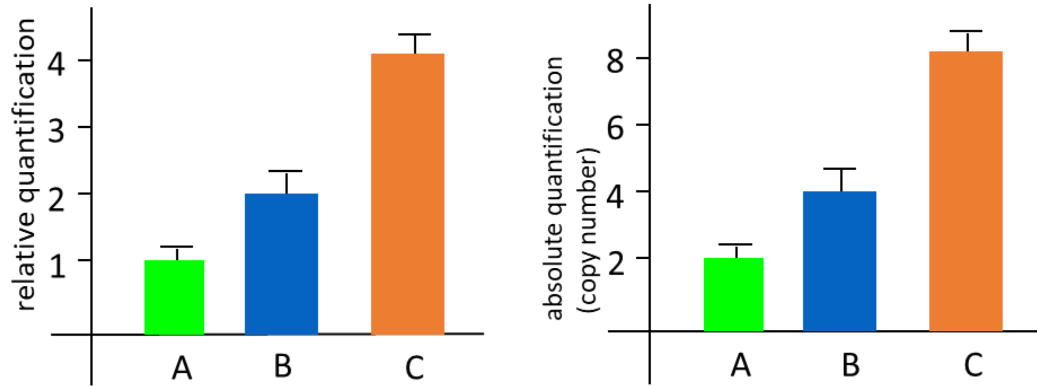
-the Ct value for sample C will be 4

The logarithm of a number is the exponent to which another number, the base, must be raised to produce that number.

At each cycle, during the exponential phase, the DNA doubles

Ct is the number of amplification cycles necessary to obtain a detectable amount of DNA -> **Ct are base 2 logarithms**.

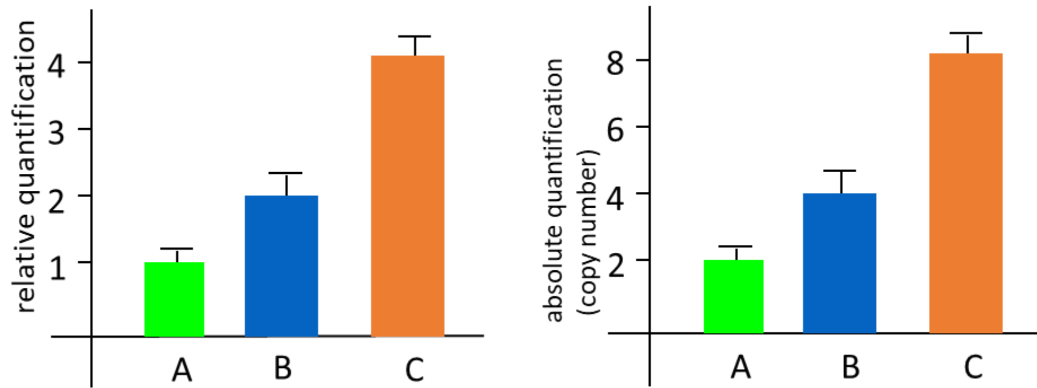
- if the starting material is low, you will need many amplification cycles (Ct high),
- if the starting material is high, you will need less amplification cycles (Ct low).



cycle	Sample A (calibrator)	Sample B	Sample C
0	**2**	**4**	**8**
1	4	8	16
2	8	16	32
3	16	32	64
4	32	64	**128**
5	64	**128**	256
6	**128**	256	512

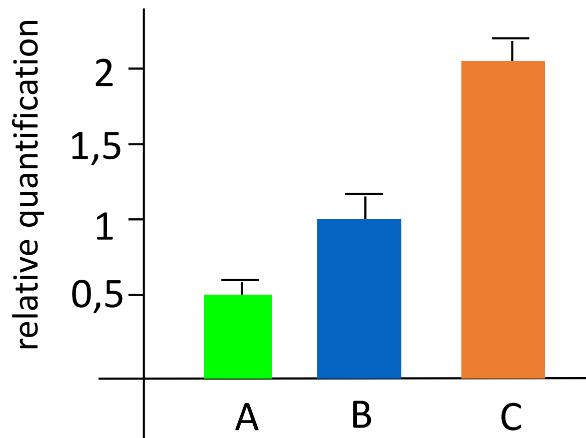
If calibrator is B, which will be relative expression of A and C?

If calibrator is C, which will be relative expression of A and B?

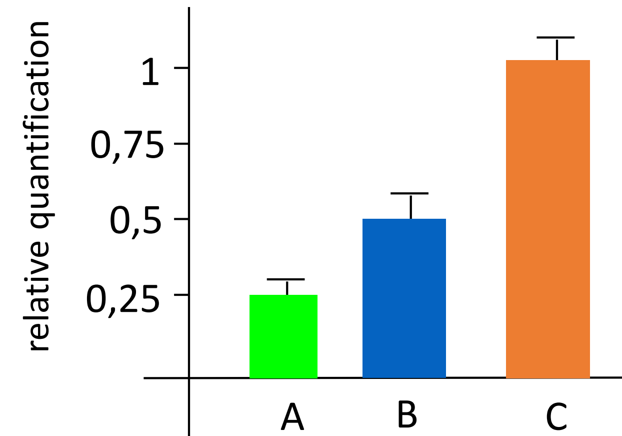


cycle	Sample A (calibrator)	Sample B	Sample C
0	**2**	**4**	**8**
1	4	8	16
2	8	16	32
3	16	32	64
4	32	64	**128**
5	64	**128**	256
6	**128**	256	512

If calibrator is B, which will be relative expression of A and C?



If calibrator is C, which will be relative expression of A and B?



- how does Ct change when you dilute your sample?
- how does Ct change when you have different copy numbers?

If the CT of my calibrator is 18,

Which will be the CT if

- I dilute it 2 fold?	10 fold?
- I dilute it 4 fold?	100 fold?
- I dilute it 8 fold?	1000 fold?
- I dilute it 16 fold?	10000 fold?

($\log_2 10=3.322$)

If the CT of my calibrator is 20

- Which will be the CT of a sample in which the gene expression is 2 fold?
- Which will be the CT of a sample in which the gene expression is 4 fold?
- Which will be the CT of a sample in which the gene expression is 8 fold?
- Which will be the CT of a sample in which the gene expression is 16 fold?

- Which will be the CT of a sample in which the gene expression is 1/2 ? (=0,5)?
- Which will be the CT of a sample in which the gene expression is 1/4 (=0,25)?
- Which will be the CT of a sample in which the gene expression is 1/8 (=0,125)?
- Which will be the CT of a sample in which the gene expression is 1/16 (=0,0625)?

If the CT of my calibrator is 18,

Which will be the CT if

- I dilute it 2 fold? 19
- I dilute it 4 fold? 20
- I dilute it 8 fold? 21
- I dilute it 16 fold? 22

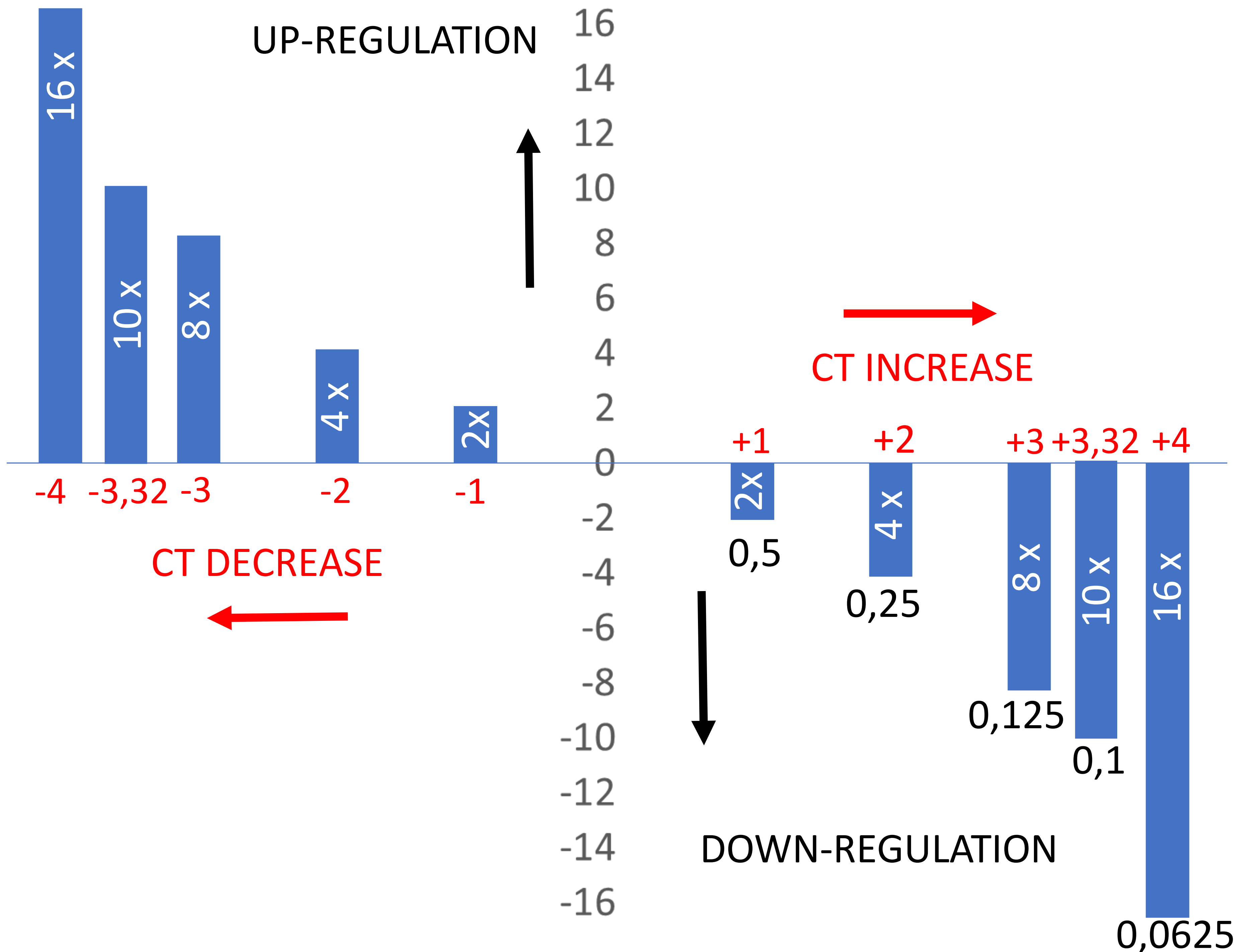
10 fold? 21,322
100 fold? 24,633
1000 fold? 27,966
10000 fold? 31,288

($\log_2 10=3.322$)

If the CT of my calibrator is 20

- Which will be the CT of a sample in which the gene expression is 2 fold? 19
- Which will be the CT of a sample in which the gene expression is 4 fold? 18
- Which will be the CT of a sample in which the gene expression is 8 fold? 17
- Which will be the CT of a sample in which the gene expression is 16 fold? 16

- Which will be the CT of a sample in which the gene expression is 1/2 ? (=0,5)? 21
- Which will be the CT of a sample in which the gene expression is 1/4 (=0,25)? 22
- Which will be the CT of a sample in which the gene expression is 1/8 (=0,125)? 23
- Which will be the CT of a sample in which the gene expression is 1/16 (=0,0625)? 24



UP-REGULATION
16
14
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
16 x
10 x
8 x
4 x
2x
-4
-3,32
-3
-2
-1
CT DECREASE
CT INCREASE
+1
+2
+3
+3,32
+4
2x
4 x
8 x
10 x
16 x
0,5
0,25
0,125
0,1
0,0625
DOWN-REGULATION

- Please, prepare an expression graphic for soluble NRG1 & ErbB2 expression, like in the following examples focused on ErbB3

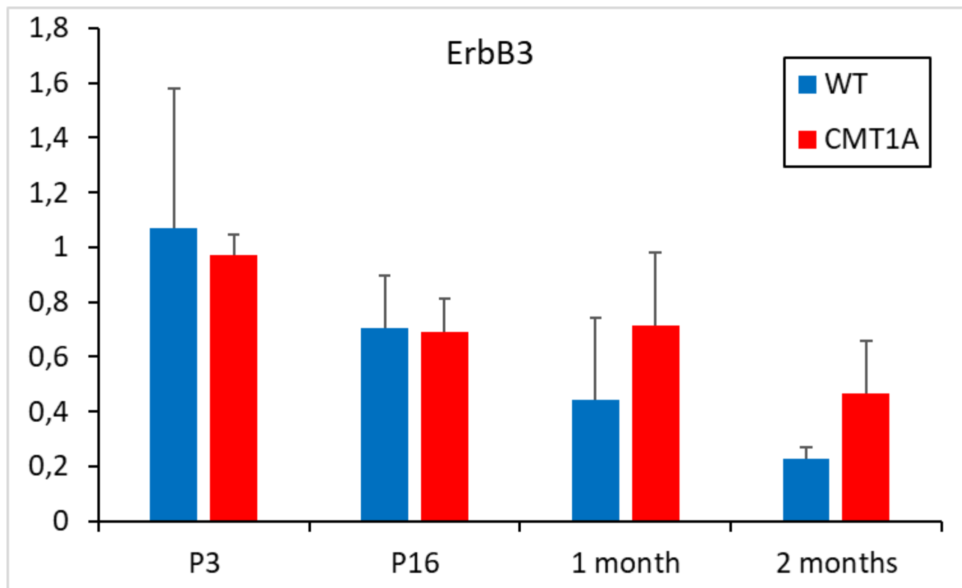
- If you need help, I will show you how to carry out CT analysis & graphic preparation

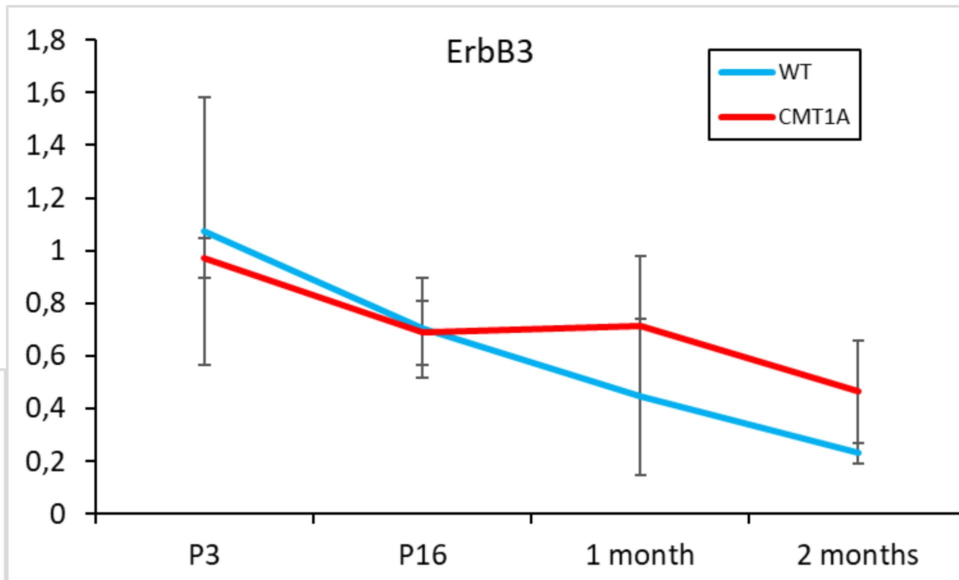
4	strain	age	sample number	CT soluble NRG1	CT ErbB2	CT HKG
5	WT	P3	59.55	31,393	22,62	24,33
6			59.57	30,725	22,33	24,23
7			59.59	30,416	22,33	24,09
8		P16	59.67	29,293	23,83	24,68
9			59.68	29,415	23,7	25,06
10			59.69	28,97	23,51	24,63
11		1 month	59.73	31,263	25,25	25,32
12			59.74	30,977	25,96	25,74
13			59.75	30,649	24,72	25,24
14		2 months	59.79	32,22	27,13	27,12
15			59.80	32,027	27,82	26,7
16			59.81	33,01	28,12	27,41
17	CMT1A +/-	P3	59.61	30,658	22,87	24,69
18			59.63	30,221	22,41	24,14
19			59.65	29,808	22,3	23,99
20		P16	59.70	25,753	22,6	24,65
21			59.71	25,477	23,75	24,59
22			59.72	25,852	23,02	24,79
23		1 month	59.76	25,096	23,86	24,65
24			59.77	25,851	24,08	24,74
25			59.78	25,588	24,83	25,05
26		2 months	59.82	26,295	25,57	26,2
27			59.83	27,057	26,04	26,19

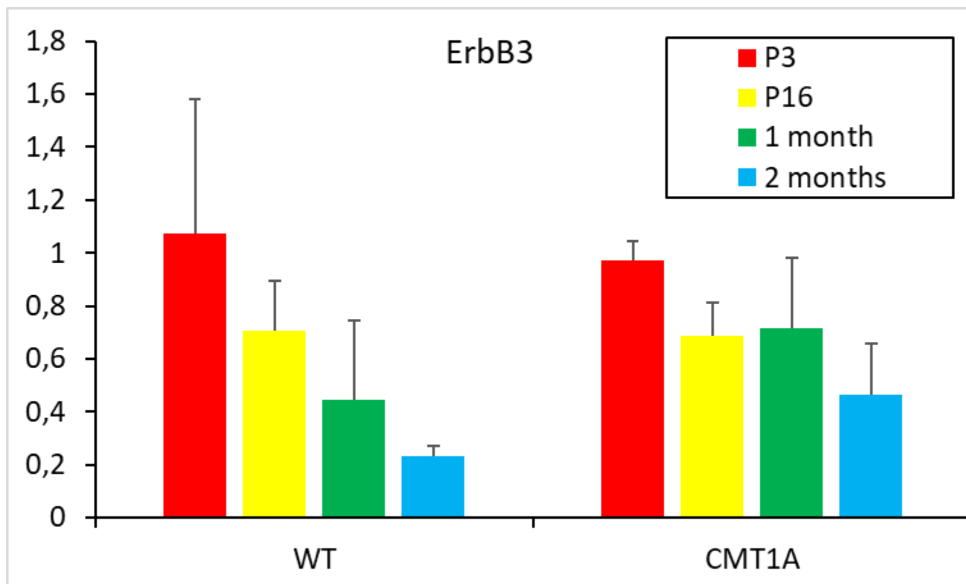
soluble NRG1 | ErbB2 | ErbB3 | HKG

	B	C	D	E	F	G	H	I	J	K	L
4	strain	age	sample number	CT ErbB3	CT HKG	Δ CT	ΔΔCT	−ΔΔCT	2^-ΔΔCt	average	standard deviation
5	WT	P3	59.55	20,115	24,33	-4,219	0,218	-0,218	0,85975649	1,07189	0,5081638
6			59.57	19,072	24,23	-5,161	-0,724	0,724	1,65175533		
7			59.59	20,155	24,09	-3,931	0,506	-0,506	0,70417211		
8		P16	59.67	20,933	24,68	-3,748	0,689	-0,689	0,62028365	0,70646	0,1885933
9			59.68	21,418	25,06	-3,642	0,795	-0,795	0,57634317		
10			59.69	20,309	24,63	-4,321	0,116	-0,116	0,92274249		
11		1 month	59.73	22,639	25,32	-2,678	1,759	-1,759	0,29545289	0,44461	0,2981065
12			59.74	23,296	25,74	-2,44	1,997	-1,997	0,2505204		
13			59.75	21,15	25,24	-4,093	0,344	-0,344	0,78785389		
14		2 months	59.79	24,729	27,12	-2,392	2,045	-2,045	0,24232245	0,23037	0,0399401
15			59.80	24,694	26,7	-2,009	2,428	-2,428	0,18582287		
16			59.81	24,899	27,41	-2,51	1,927	-1,927	0,26297544		
17	CMT1A	P3	59.61	20,242	24,69	-4,44567	-0,009	0,00867	1,00602768	0,97079	0,0756747
18			59.63	19,881	24,14	-4,259	0,178	-0,178	0,88392753		
19			59.65	19,524	23,99	-4,469	-0,032	0,032	1,02242853		
20		P16	59.70	20,628	24,65	-4,025	0,412	-0,412	0,75158074	0,68878	0,1222355
21			59.71	20,54	24,59	-4,054	0,383	-0,383	0,76684133		
22			59.72	21,218	24,79	-3,569	0,868	-0,868	0,54790588		
23		1 month	59.76	20,988	24,65	-3,664	0,773	-0,773	0,58519932	0,71455	0,2651889
24			59.77	20,279	24,74	-4,465	-0,028	0,028	1,01959768		
25			59.78	21,502	25,05	-3,545	0,892	-0,892	0,53886657		
26		2 months	59.82	22,956	26,2	-3,243	1,194	-1,194	0,43708931	0,46516	0,1945434
27			59.83	22,323	26,19	-3,864	0,573	-0,573	0,6722175		
28			59.84	23,591	26,22	-2,632	1,805	-1,805	0,28618104		

soluble NRG1 | ErbB2 | **ErbB3** | HKG


ErbB3
WT
CMT1A
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
P3
P16
1 month
2 months


ErbB3
WT
CMT1A
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
P3
P16
1 month
2 months


ErbB3
P3
P16
1 month
2 months
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
WT
CMT1A