Real time PCR workshop

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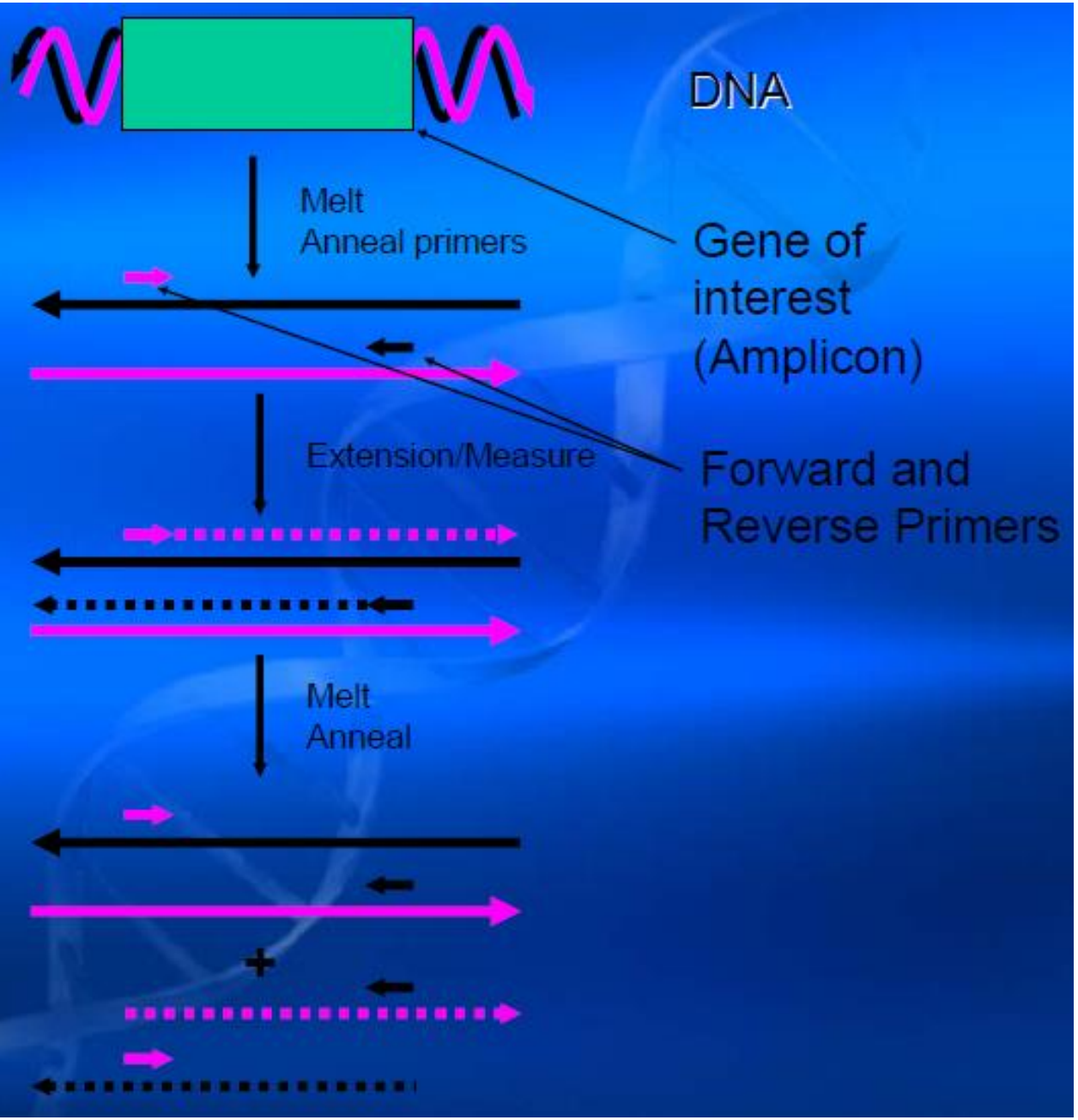
Important notes

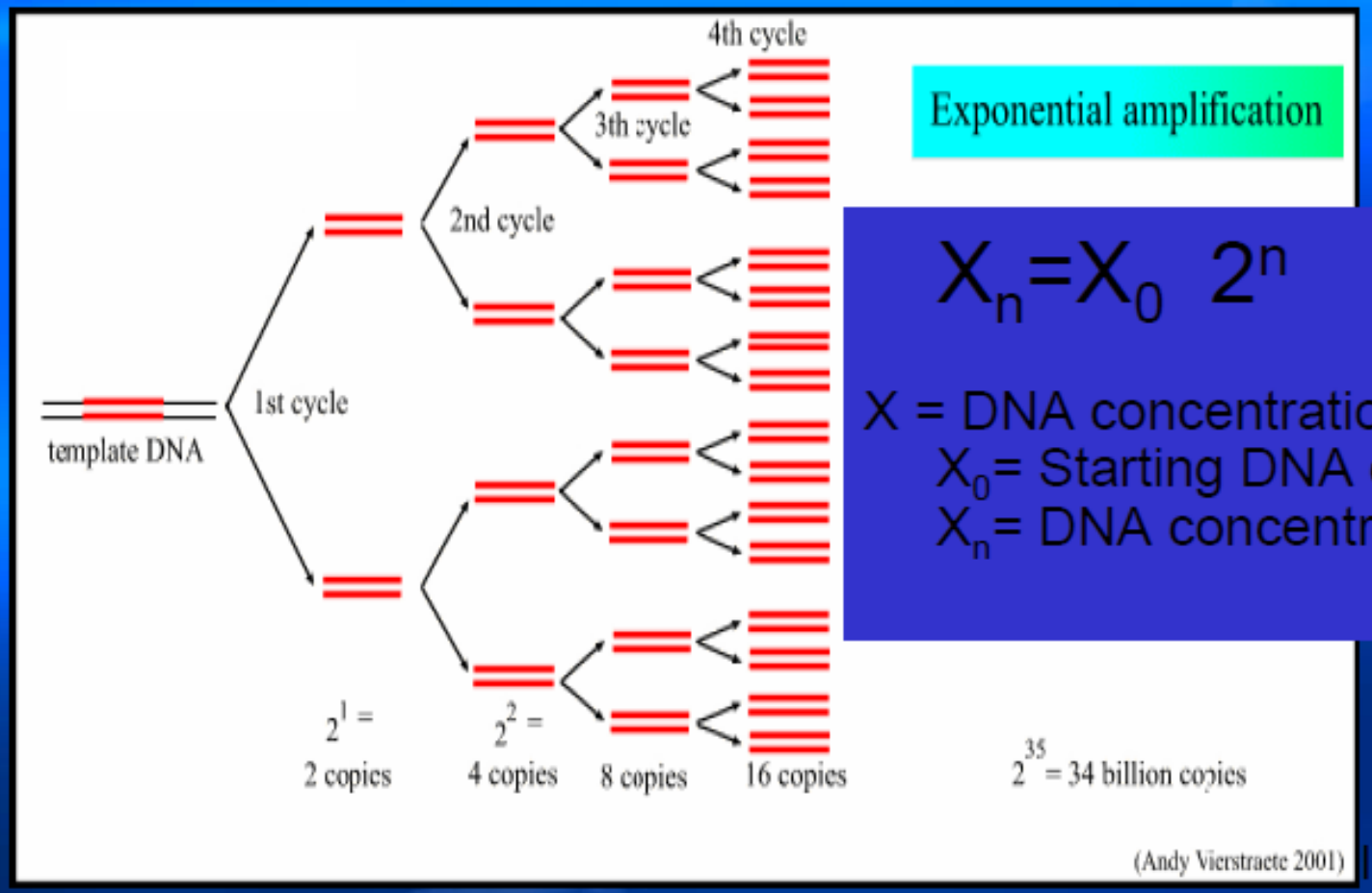
- ✓ principle
- ✓ machine design
- ✓ methods of detection
- ✓ amplification plot
- ✓ differences between qPCR and PCR
- ✓ primer design
- ✓ how to design your experiment
- ✓ controls
- ✓ comparative and quantitative types
- ✓ Applications

Polymerase Chain Reaction



QPCR Molecular Mechanism

Exponential amplification of the original DNA sequence (template) to create copies of part of the sequence (amplicon)



Exponential amplification

$$X_n = X_0 \ 2^n$$

X = DNA concentration

X_0 = Starting DNA concentration

X_n = DNA concentration at cycle n

Influence of Reaction Efficiency

CYCLE	AMOUNT OF DNA 100% EFFICIENCY	AMOUNT OF DNA 90% EFFICIENCY	AMOUNT OF DNA 80% EFFICIENCY	AMOUNT OF DNA 70% EFFICIENCY
0	1	1	1	1
1	2	2	2	2
2	4	4	3	3
3	8	7	6	5
4	16	13	10	8
5	32	25	19	14
6	64	47	34	24
7	128	89	61	41
8	256	170	110	70
9	512	323	198	119
10	1,024	613	357	202
11	2,048	1,165	643	343
12	4,096	2,213	1,157	583
13	8,192	4,205	2,082	990
14	16,384	7,990	3,748	1,684
15	32,768	15,181	6,747	2,862
16	65,536	28,844	12,144	4,866
17	131,072	54,804	21,859	8,272
18	262,144	104,127	39,346	14,063
19	524,288	197,842	70,824	23,907
20	1,048,576	375,900	127,482	40,642
21	2,097,152	714,209	229,468	69,092
22	4,194,304	1,356,998	413,043	117,456
23	8,388,608	2,578,296	743,477	199,676
24	16,777,216	4,898,763	1,338,259	339,449
25	33,554,432	9,307,650	2,408,866	577,063
26	67,108,864	17,684,534	4,335,959	981,007
27	134,217,728	33,600,615	7,804,726	1,667,711
28	268,435,456	63,841,168	14,048,506	2,835,109
29	536,870,912	121,298,220	25,287,311	4,819,686
30	1,073,741,824	230,466,618	45,517,160	8,193,466

AFTER 1 CYCLE

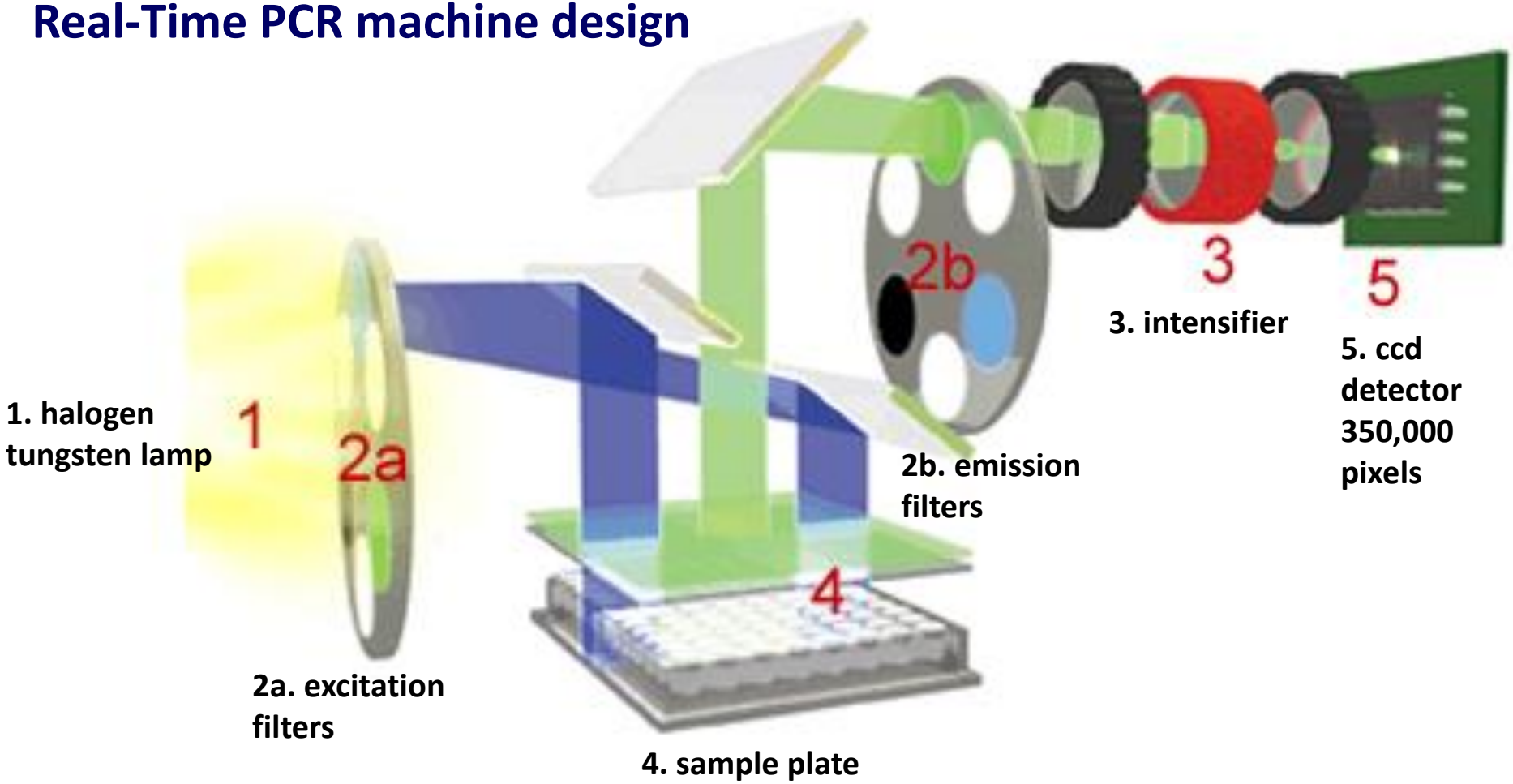
100% = 2.00x

90% = 1.90x

80% = 1.80x

70% = 1.70x

Real-Time PCR machine design



1. halogen tungsten lamp

2a. excitation filters

2b. emission filters

3. intensifier

4. sample plate

5. ccd detector 350,000 pixels

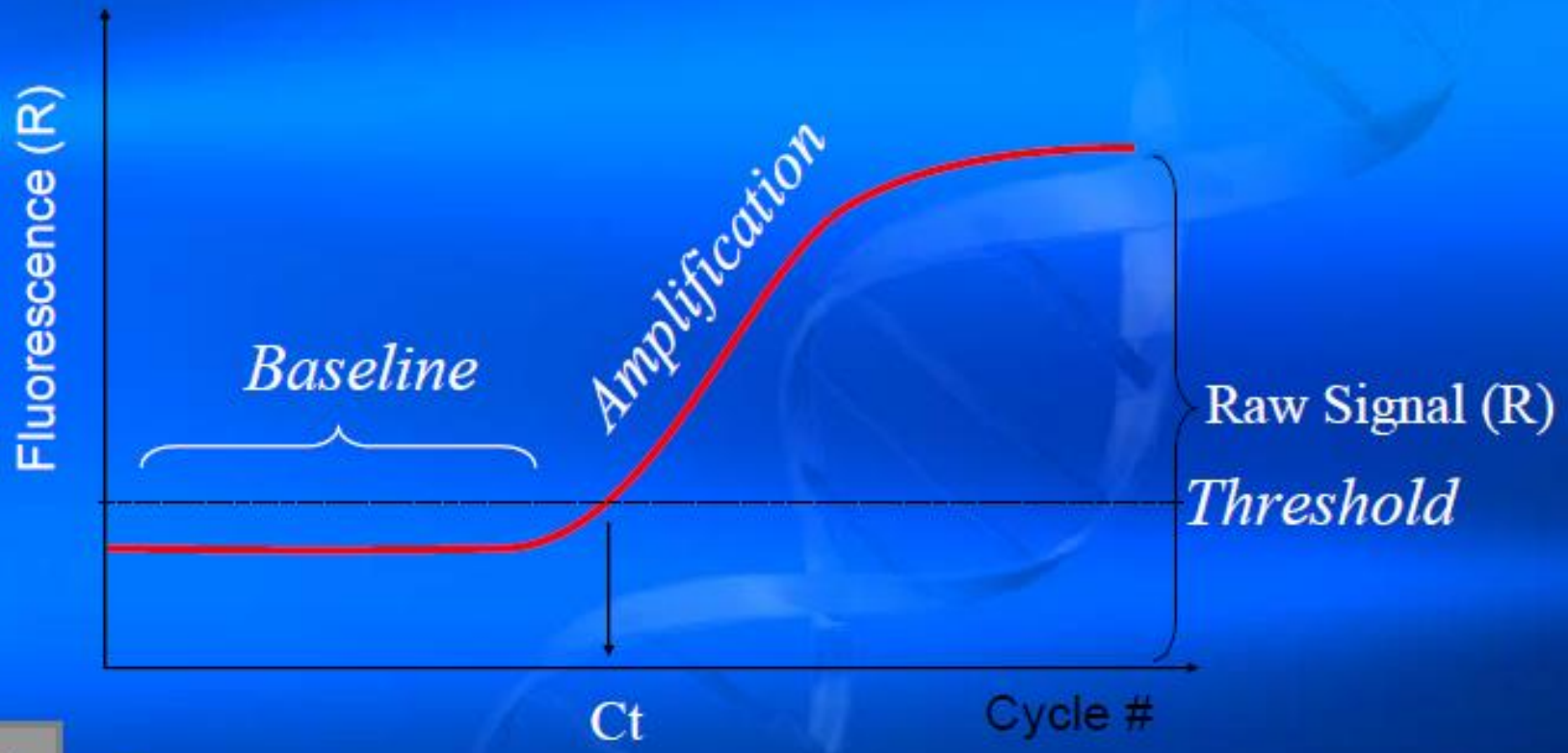
Fig. 1.2. Representation of Optical Detection System layout.

www.biorad.com

Real time PCR is the most accurate method to detect:

- Copy number of each gene
- Amount of gene expression
- Efficiency of drugs
- Virus infection
- Different type of Pathogensn(CMV, streptococcus, mycobacterium, HIV , ...)
- Methylation of DNA
- Different type of mutations

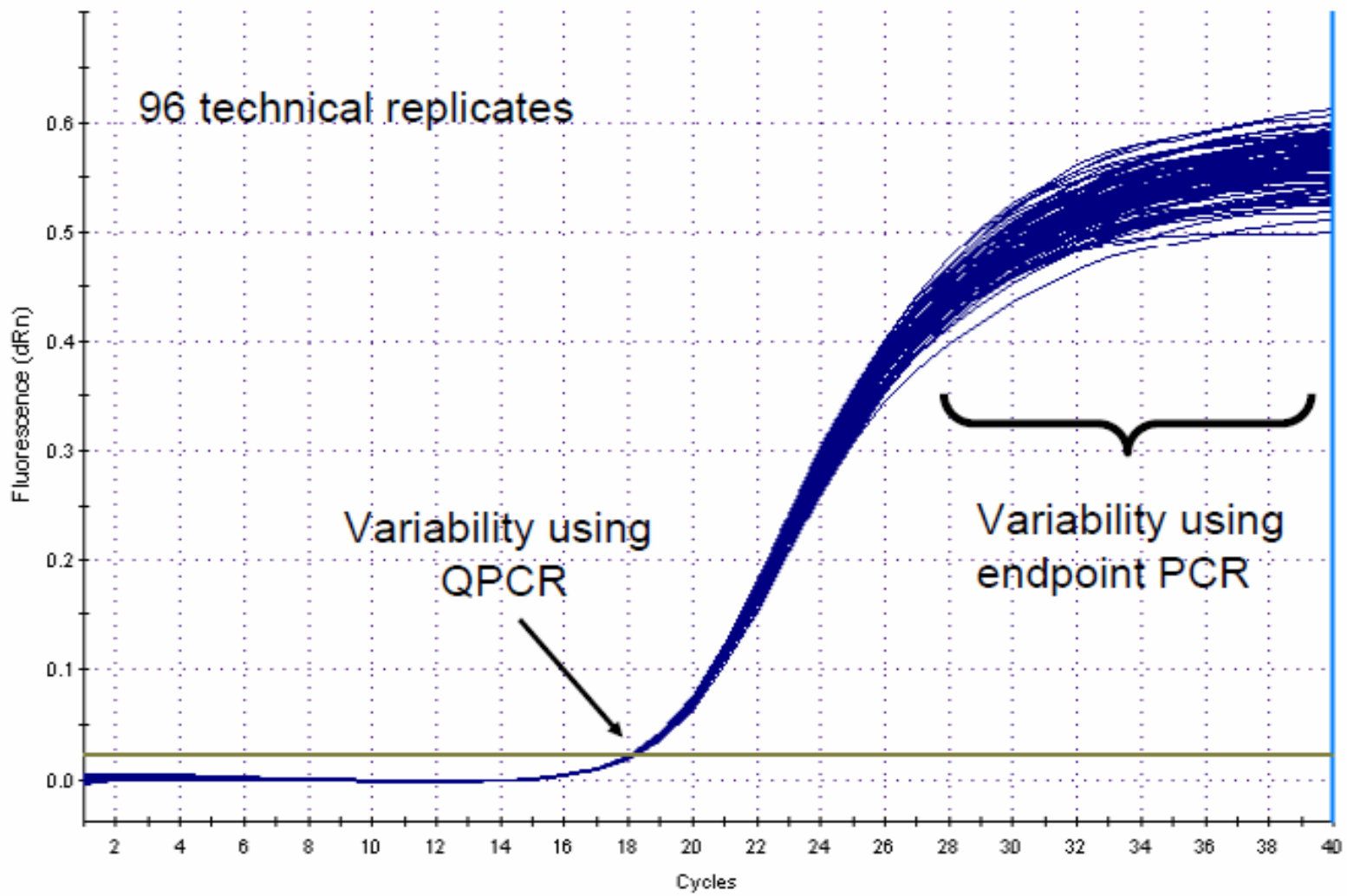
Typical PCR Amplification Plot



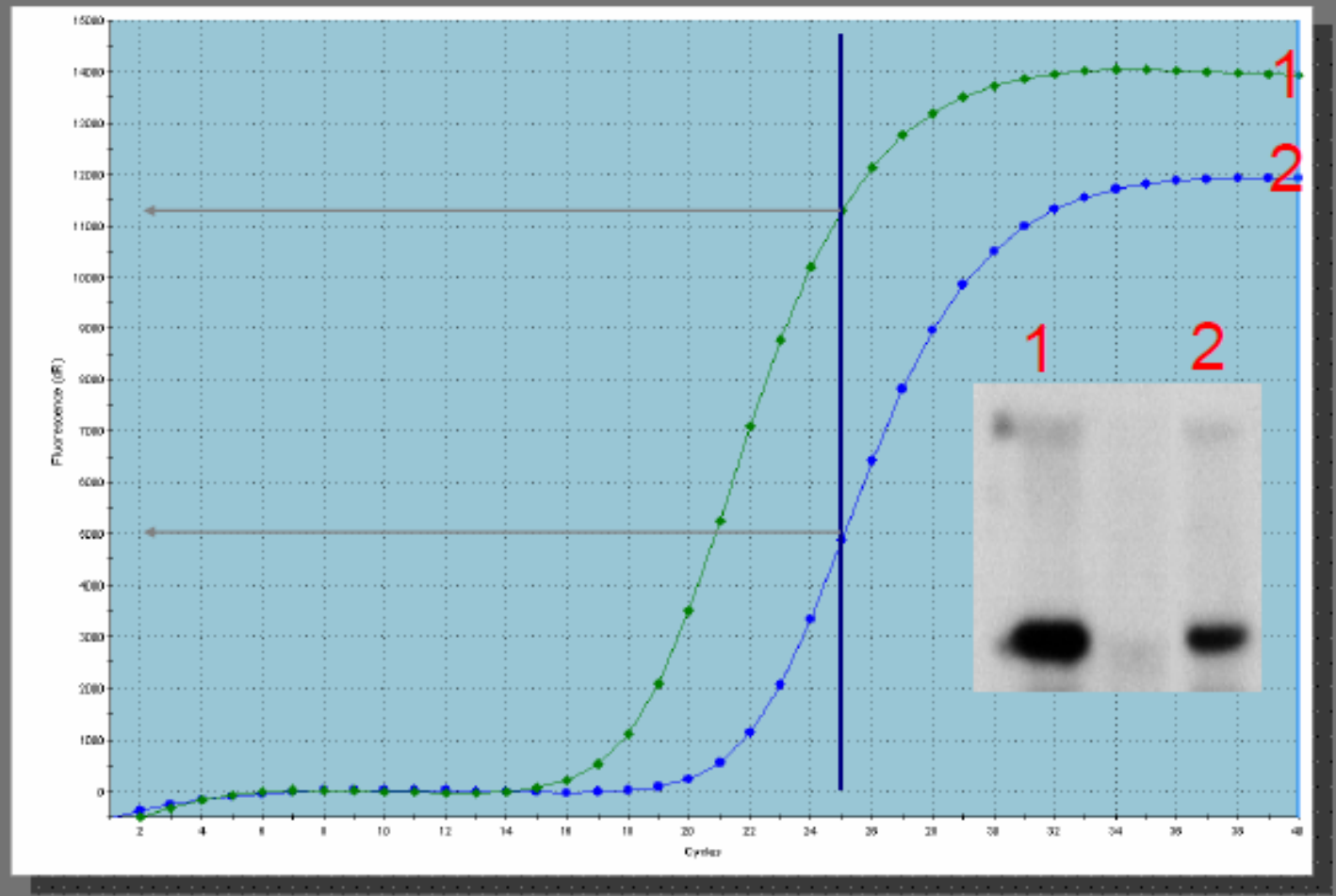
C_t = Fractional PCR cycle number at which the fluorescence intensity crosses the established threshold line.

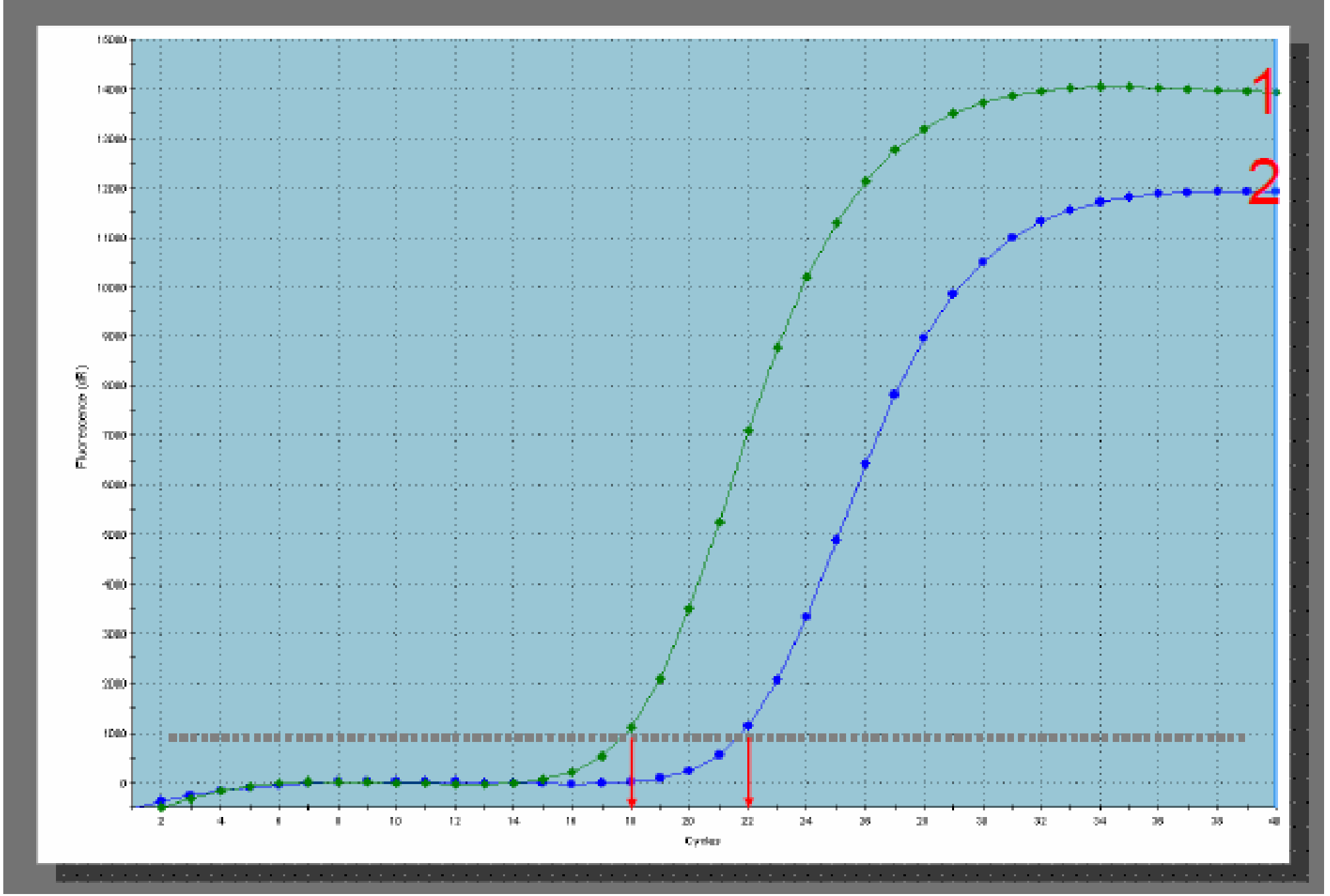
- A 1C_t difference between samples represents **2x** more transcript

Why is QPCR superior to PCR

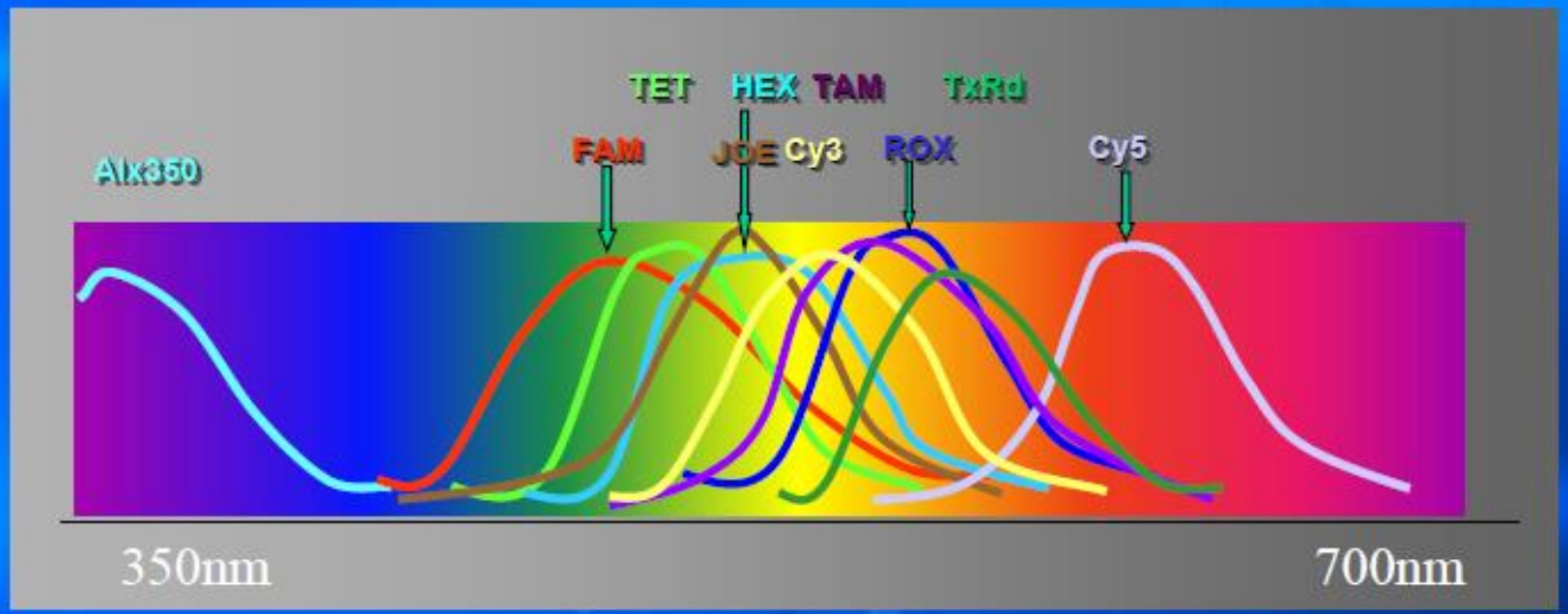


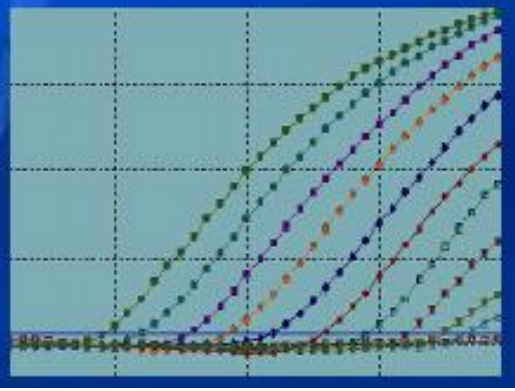
Gel-based quantification



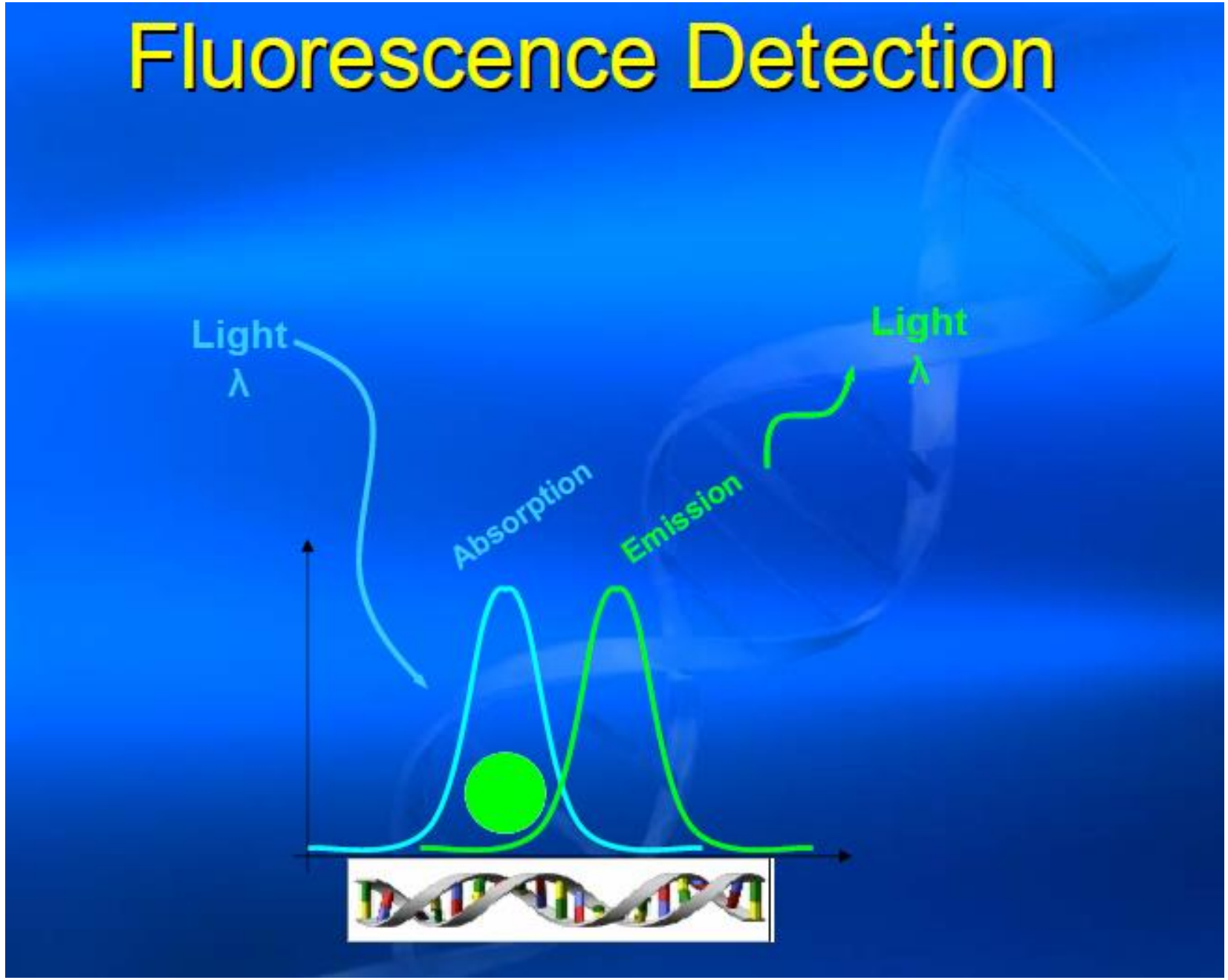

1
2
Fluorescence (dR)
Cycles

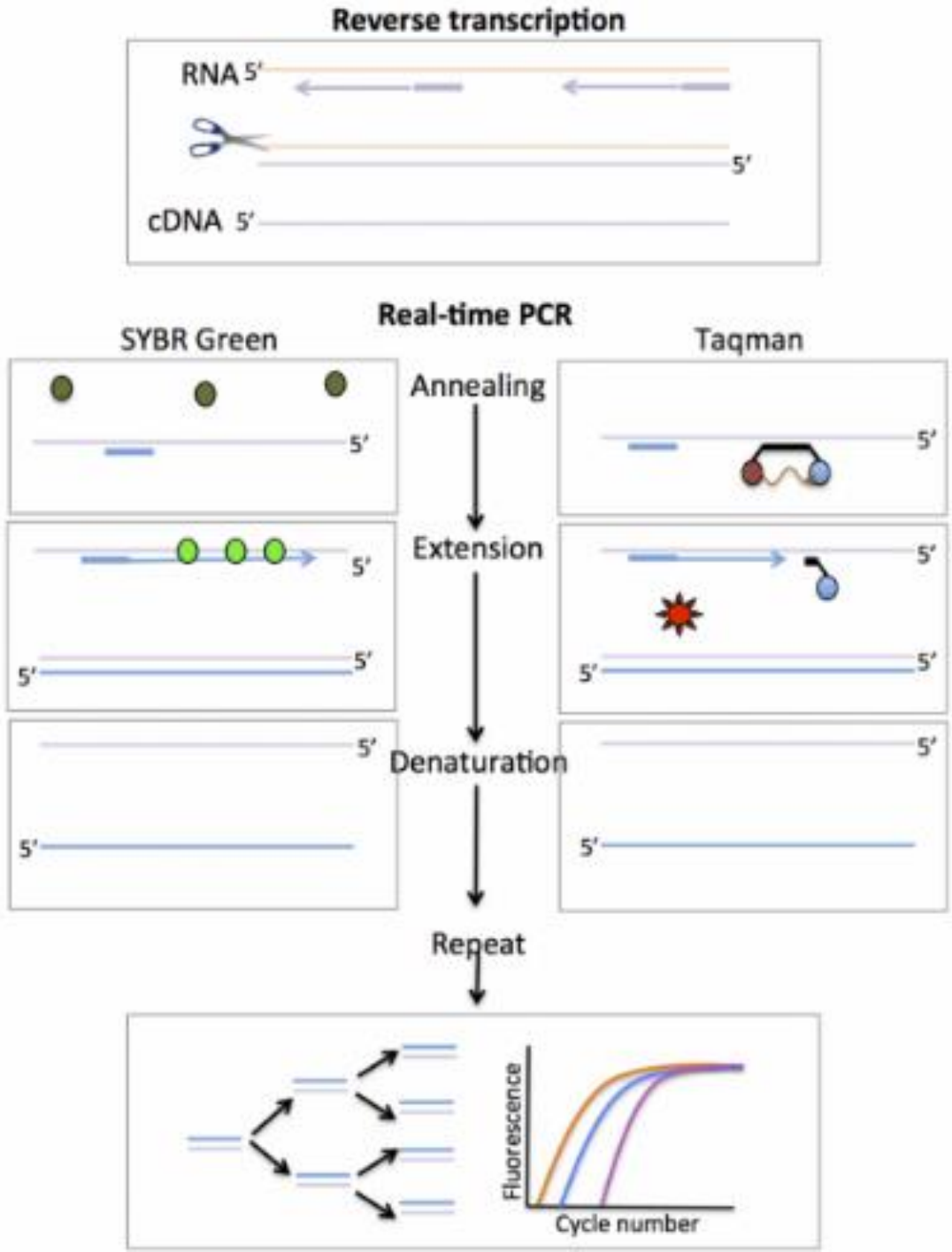
Chemistries used in QPCR





Fluorescence Detection



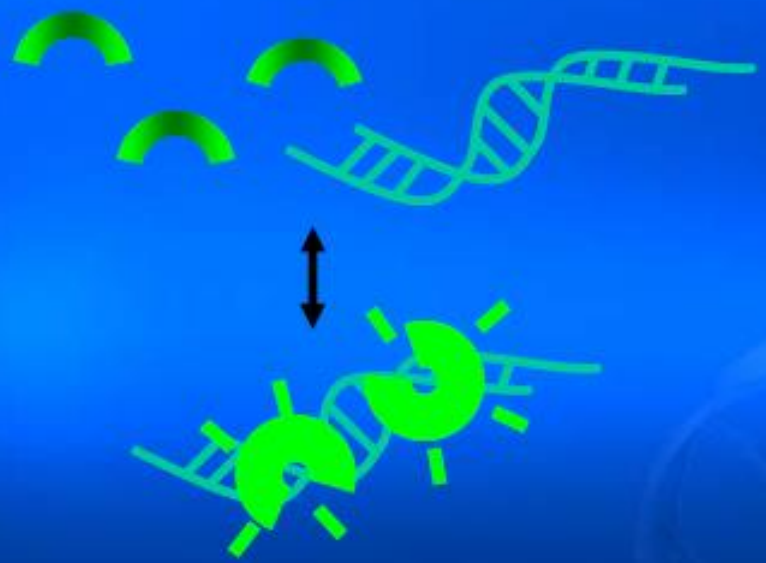

Reverse transcription
RNA 5'
5'
cDNA 5'
Real-time PCR
SYBR Green
Taqman
Annealing
Extension
Denaturation
Repeat
Fluorescence
Cycle number

Quantitative PCR Chemistries

dsDNA Binding	SYBR Green
Probe Based Detection	TaqMan®
	Molecular Beacons
	Lux® primers
	Hybridization probes
	ScorpionsTM
	Amplifluor® probes
	FRET

Chemistries

SYBR green
dsDNA binding dyes



1000x increase in
fluorescence

Pro:

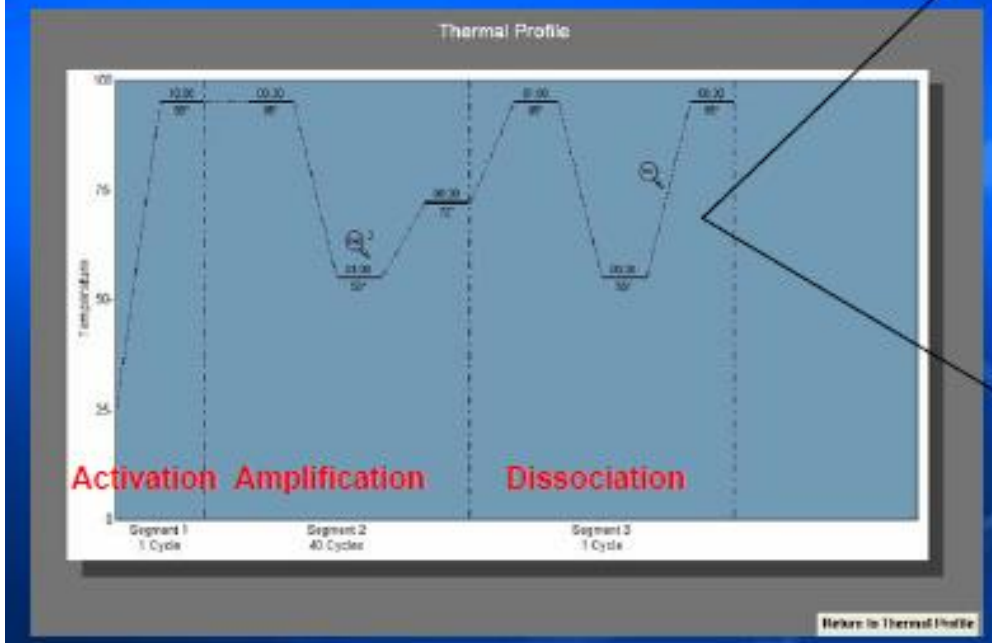
- Ease of use
- Inexpensive
- Good for high throughput screenings
  → lots of genes: this is your chemistry
- Great for first screens and optimization
- Can detect amplicon heterogenity

Con:

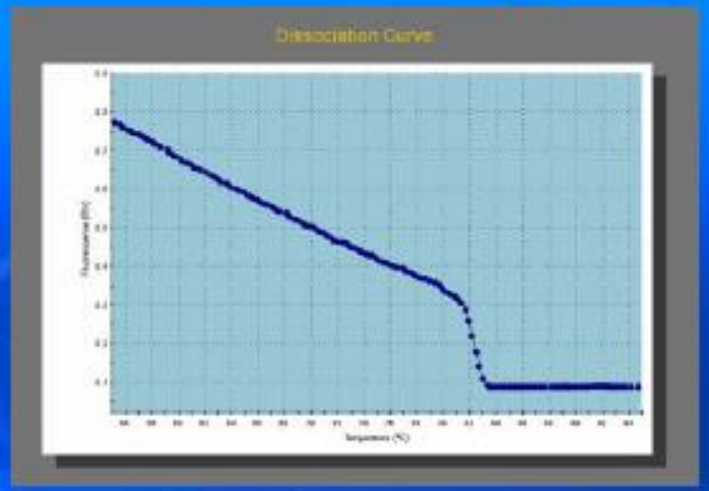
- Sequence unspecific – detects any double strand in your reaction
- Can not multiplex reactions

SYBR green

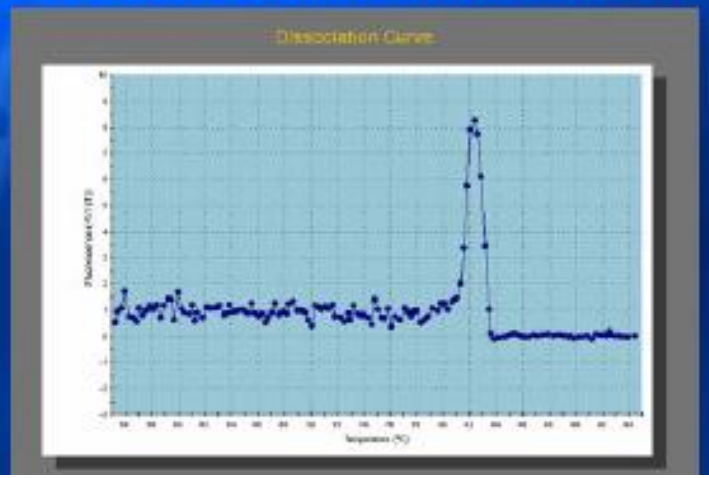
SYBR Green I™ Thermal Profile



Raw Fluorescence [R]

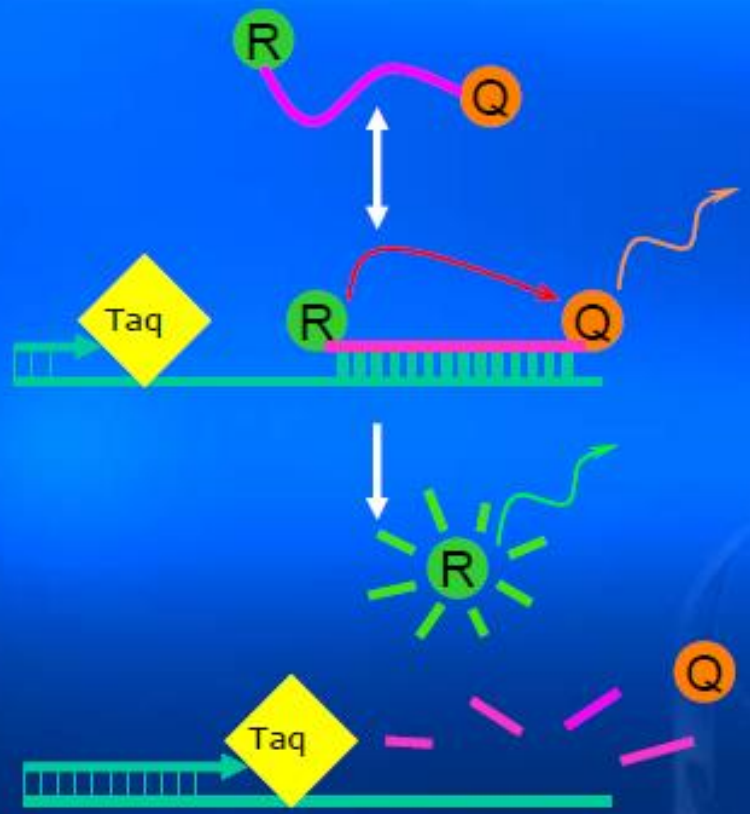


Negative First Derivative [-R'(T)]



Chemistries

Taqman probes



Pro:

- Sequence specific
- Possibility to do multiplex
  - have GOI and normalizer in the same well, doing comparative quantification

Con:

- More difficult to design
- Expensive

Linear Taqman Probe Design

- Probe T_m 5-10°C higher than primers
- ≤ 30 bp in length
- No G next to reporter fluorophore
- < 4 contiguous Gs
- PCR blocker at 3' end
- Compatible reporters and quenchers

Primer Selection

- Try to achieve similar Tm for all primers: Ideal ~60°C. (Future multiplexing or use of Taqman™ assays in mind)
- Forward and reverse primer should have ΔTm <2°C (SYBR: 75 – 400, 200bp ; Taqman 75-150, 125bp)
- 40-60% GC content to prevent G/C region self-hybridization
- ΔG of primer dimer/cross primer dimer formation > -4 kcal/mol to avoid stable primer dimers
- Design via software (Always use the same one):
- Always perform a BLAST search with your amplicon and primers
  (→ Specificity of the PCR)

Optimase ProtocolWriter™

This tool will generate PCR protocols for use with Optimase, the high-fidelity polymerase product from Transgenomic. To generate a PCR "Develop PCR protocol", this software will generate the appropriate PCR protocol.

Forward primer sequence: AGAAGAGCGACCCTCACATCA

Reverse primer sequence: TCCAGGTGGTACGTGTGATTG

PCR product length: 82 bp

Protocol type: Simple 3-step PCR

Develop PCR Protocol

Note: In the sequence fields above, include only the template-specific portion of the primer--do not include any GC clamps or tai

Optimase ProtocolWriter™

The following PCR protocol is designed for use with Optimase, the high-fidelity polymerase product from Transgenomic.

Forward primer sequence: AGAAGAGCGACCCTCACATCA (Tm = 56.6°C)
Reverse primer sequence: TCCAGGTGGTACGTGTGATTG (Tm = 56.6°C)
PCR product length: 82 bp
Protocol type: Simple 3-step PCR protocol

Step 1: 95°C, 2 min.
Step 2: 95°C, 30 sec.
Step 3: 59.6°C, 30 sec.
Step 4: 72°C, 10.0 sec.
Step 5: Repeat steps 2-4 29 more times
Step 6: 72°C, 5 min.
Step 7: 4°C, forever

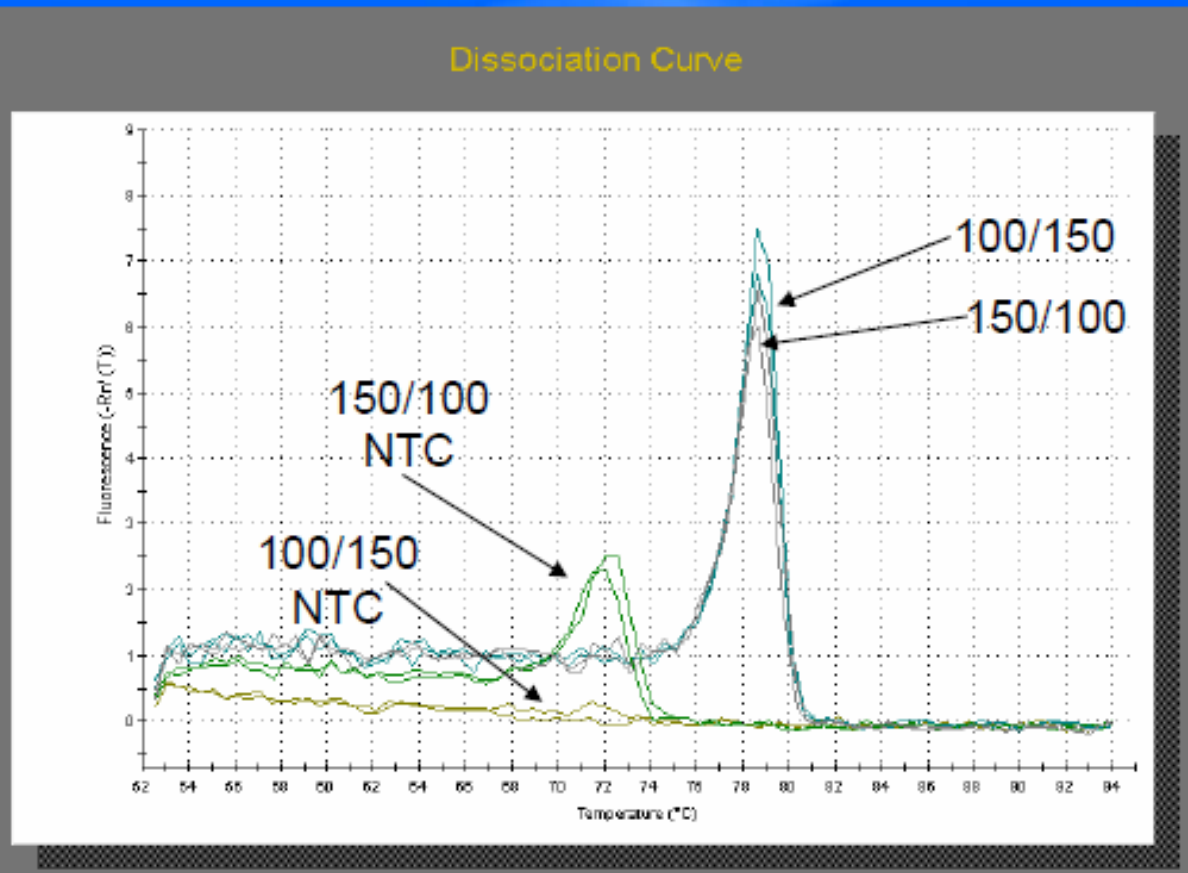
Purchase Optimase Polymerase kit

Assay Optimization
Primers

Primer titration 50 nM – 200 nM
duplicates for pos. Control & NTC

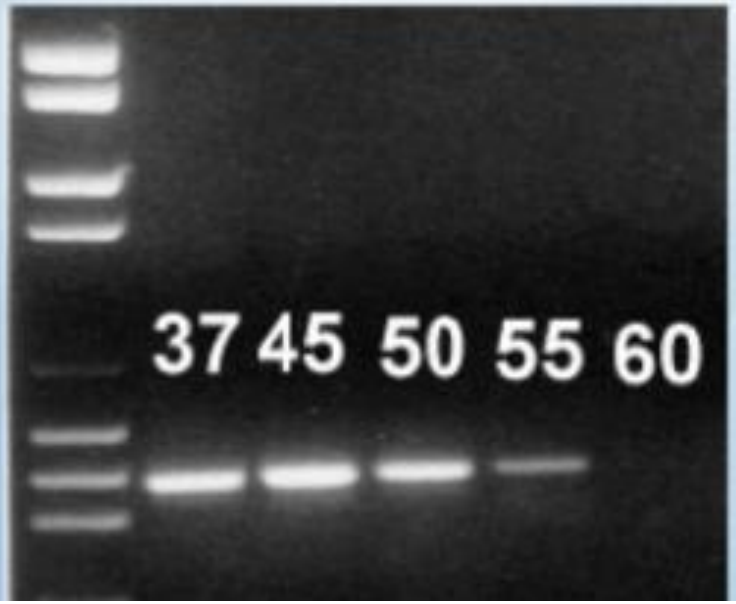
Aims:

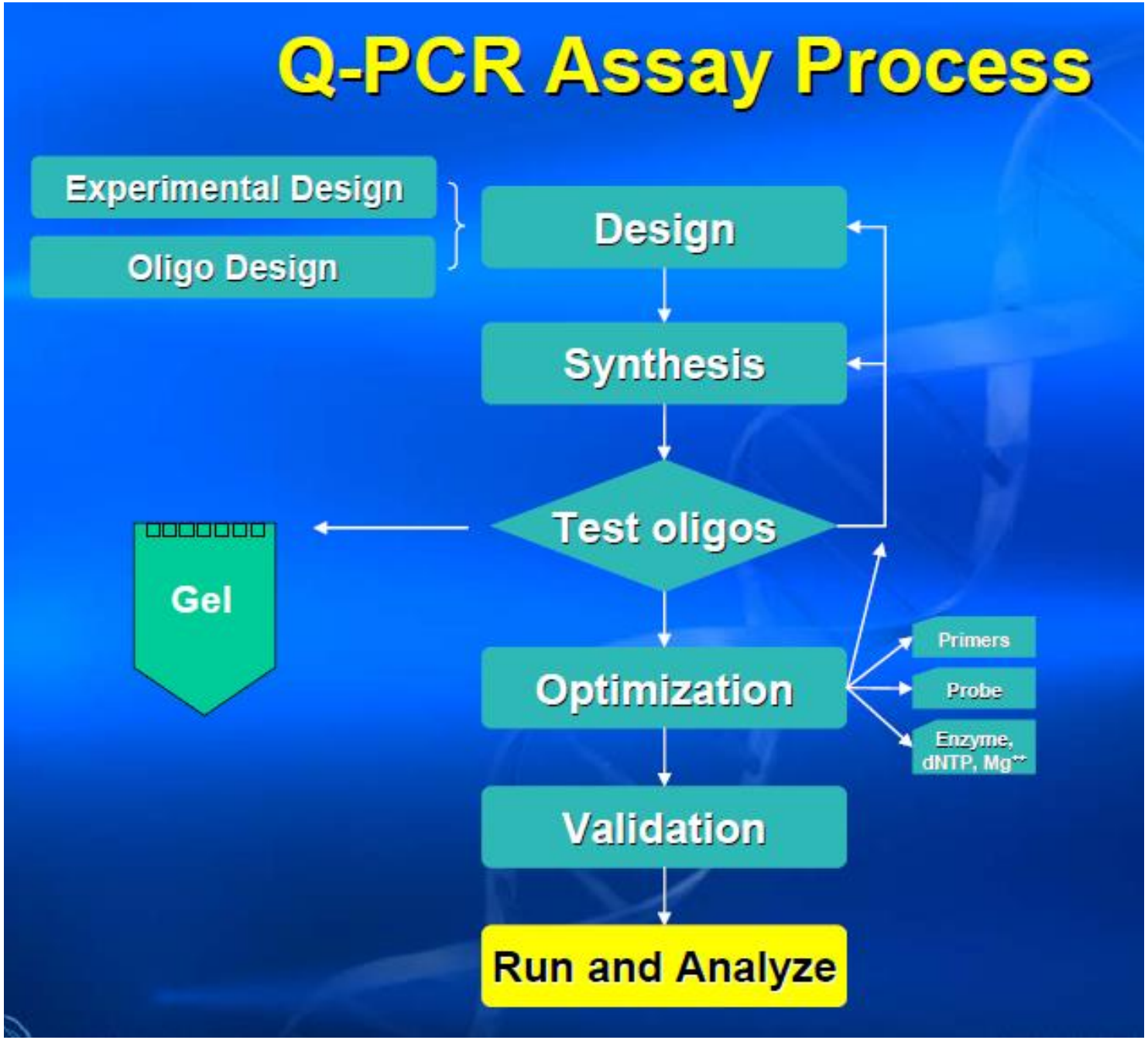
- low Ct values
  → sensitivity
- no unspecific amplification or primer dimers
  → specificity
- Low interreplicate variability
- high efficiency of amplification



Annealing temperatures

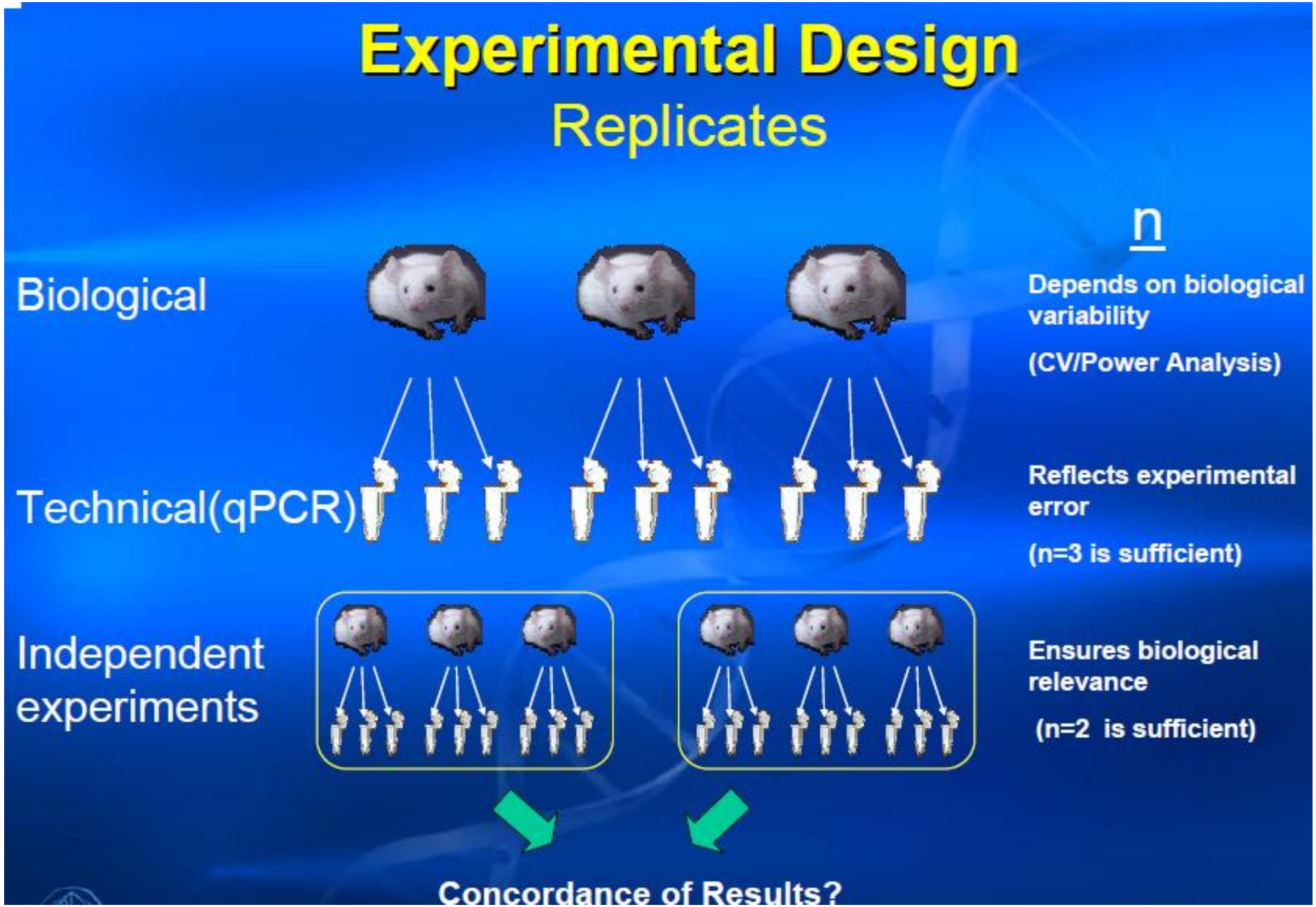
37 – 60°C
gradient




Q-PCR Assay Process
Experimental Design
Oligo Design
Design
Synthesis
Test oligos
Gel
Optimization
Primers
Probe
Enzyme, dNTP, Mg++
Validation
Run and Analyze

Experimental Design

Replicates



QPCR Assay Controls

Initial efforts should identify good control materials to run during assay setup and validation

- Establish a range of acceptable QPCR performance data
- Controls will dictate what data is good or bad and what should be included in down-stream analysis.
- Justification for omitting data or re-assay

QPCR ASSAY CONTROLS

Review the most common controls to include in any QPCR experiment

- Systematic Experimental Error Control
- Positive QPCR controls
- Negative QPCR controls

QPCR Assay Controls

Passive Reference Fluor

Passive Reference Fluor (ROX) spiked into QPCR master mix at outset of assay setup

- Rox fluor emission used to correct for artifacts in signal measurement from wells
  - Bubbles in sample volumes, plasticware inconsistency, variation in sample volume.
- Include Rox, measure signal, assign it as the Reference Dye in Mx software setup
- Will improve data uniformity and reduce correlation of variance (%CV) among technical replicates

QPCR Assay Controls
Positive QPCR Control

Positive Controls- Common Sources of material

- Pooled RNA/cDNA unknowns from experiments
- Linearized/nicked plasmid cDNA
- Purified PCR product
- Stratagene Reference RNAs

QPCR Assay Controls
Positive QPCR Control

Positive Controls- Some sample that contains your gene of interest (GOI) and should be detected by QPCR

- Ideal control should be similar to the unknowns you will be analyzing, ie RNA in same matrix as tissue or cell samples

QPCR Assay Controls

Negative QPCR Controls

- No Template controls (NTC)
  - No cDNA added to QPCR reaction
  - Detects primer dimer, contaminating template, or probe degradation across cycles
- No Reverse Transcription Control (NoRT)
  - RNA sample undergoing reaction w/o RT
  - Detects contaminating gDNA in RNA
- No Amplification Control (NAC)
  - No Taq DNA polymerase added to QPCR reaction
  - May indicate high background

Commonly used standards

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

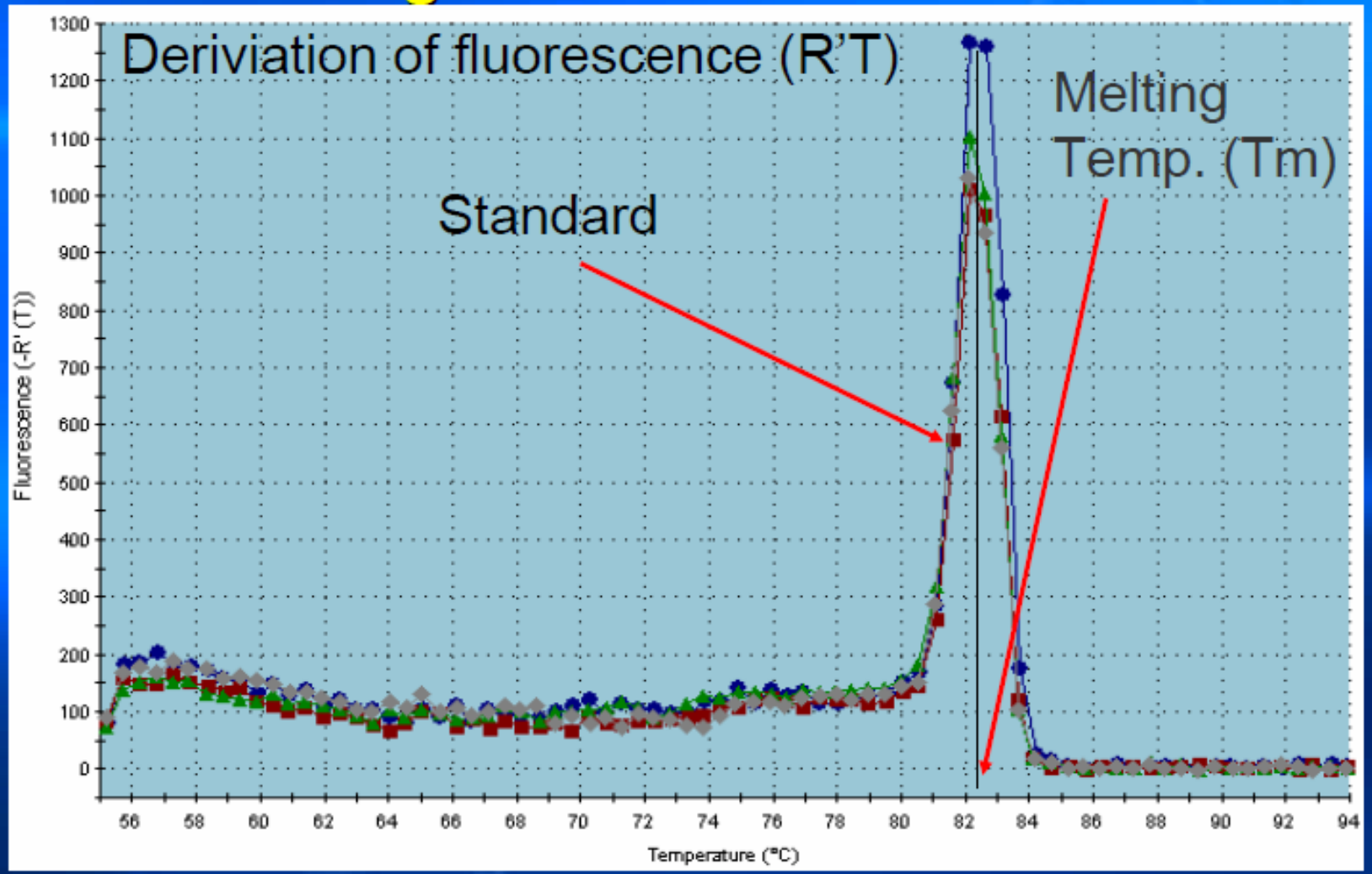
  E.g. RPLP0 (ribosomal protein, large, P0; also known as 36B4, P0, L10E, RPPO, PRLP0, 60S acidic ribosomal protein P0, ribosomal protein L10, Arbp or acidic ribosomal phosphoprotein P0)
- 28S or 18S rRNA

Table 7. Housekeeping genes commonly used as endogenous references

Gene	Gene symbol		Relative expression level*	
	Human	Mouse	Human	Mouse
18S ribosomal RNA	RRN18S	Rn18s	++++	++++
Actin, beta	ACTB	Actb	+++	+++
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	Gapdh	+++	+++
Phosphoglycerate kinase 1	PGK1	Pgk1	+++	++
Peptidylprolyl isomerase A	PPIA	Ppia	+++	+++
Ribosomal protein L13a	RPL13A	Rpl13a	+++	+++
Ribosomal protein, large, P0	RPLP0		+++	
Acidic ribosomal phosphoprotein PO		Arbp		+++
Beta-2-microglobulin	B2M	B2m	++ – +++	++ – +++
Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	YWHAZ	Ywhaz	++ – +++	+
Succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	SDHA	Sdha	++	+
Transferrin receptor	TFRC	Tfrc	++	+
Aminolevulinate, delta-, synthase 1	ALAS1	Alas1	+	+
Glucuronidase, beta	GUSB	Gusb	+	+
Hydroxymethylbilane synthase	HMBS	Hmbs	+	++ – +++
Hypoxanthine phosphoribosyltransferase 1	HPRT1	Hprt1	+	+
TATA box binding protein	TBP	Tbp	+	+
Tubulin, beta	TUBB		+	
Tubulin, beta 4		Tubb4		+

QPCR Assay Control Specificity

Negative QPCR Control



QPCR Assay Control Specificity

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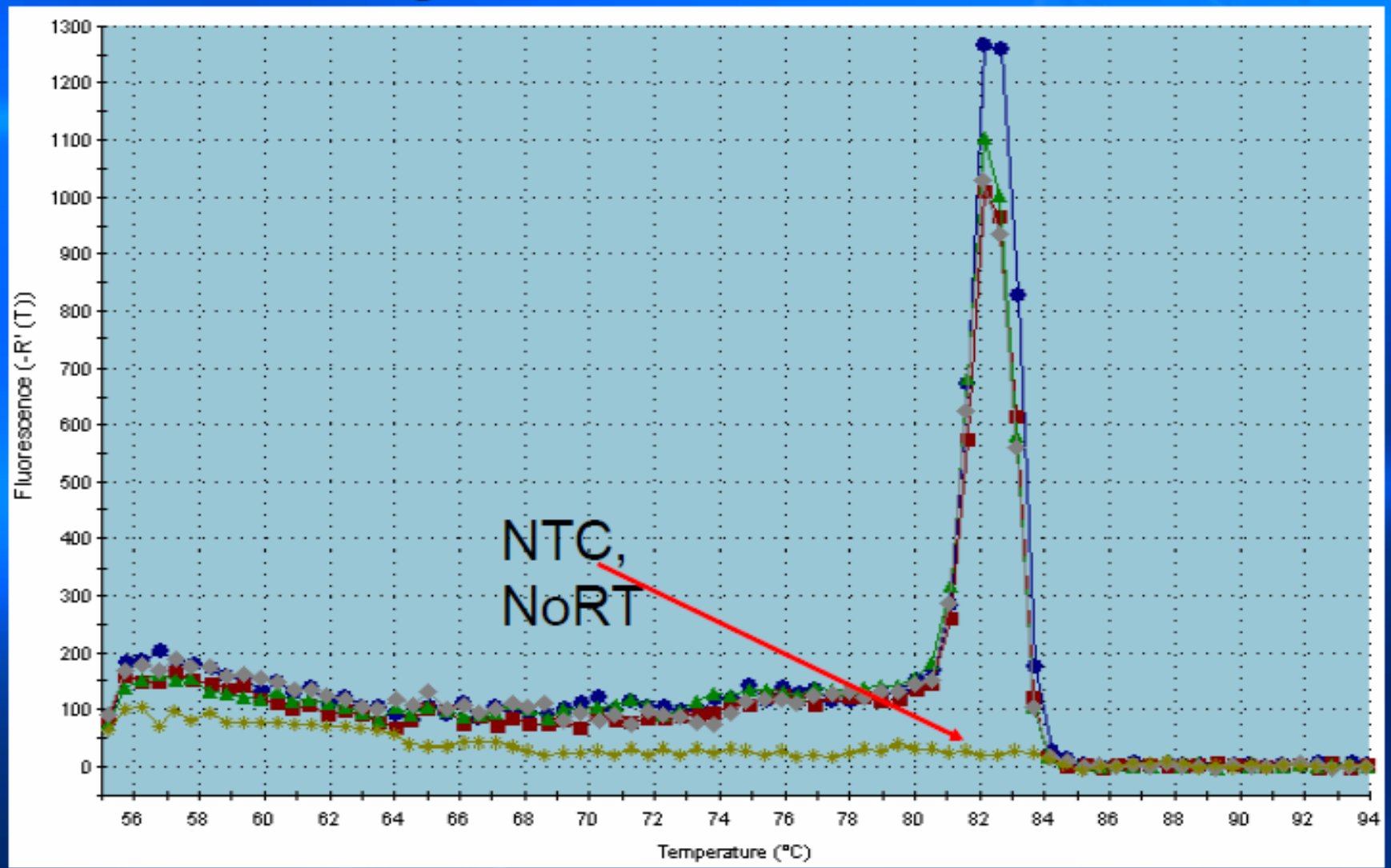

QPCR Assay Control Specificity

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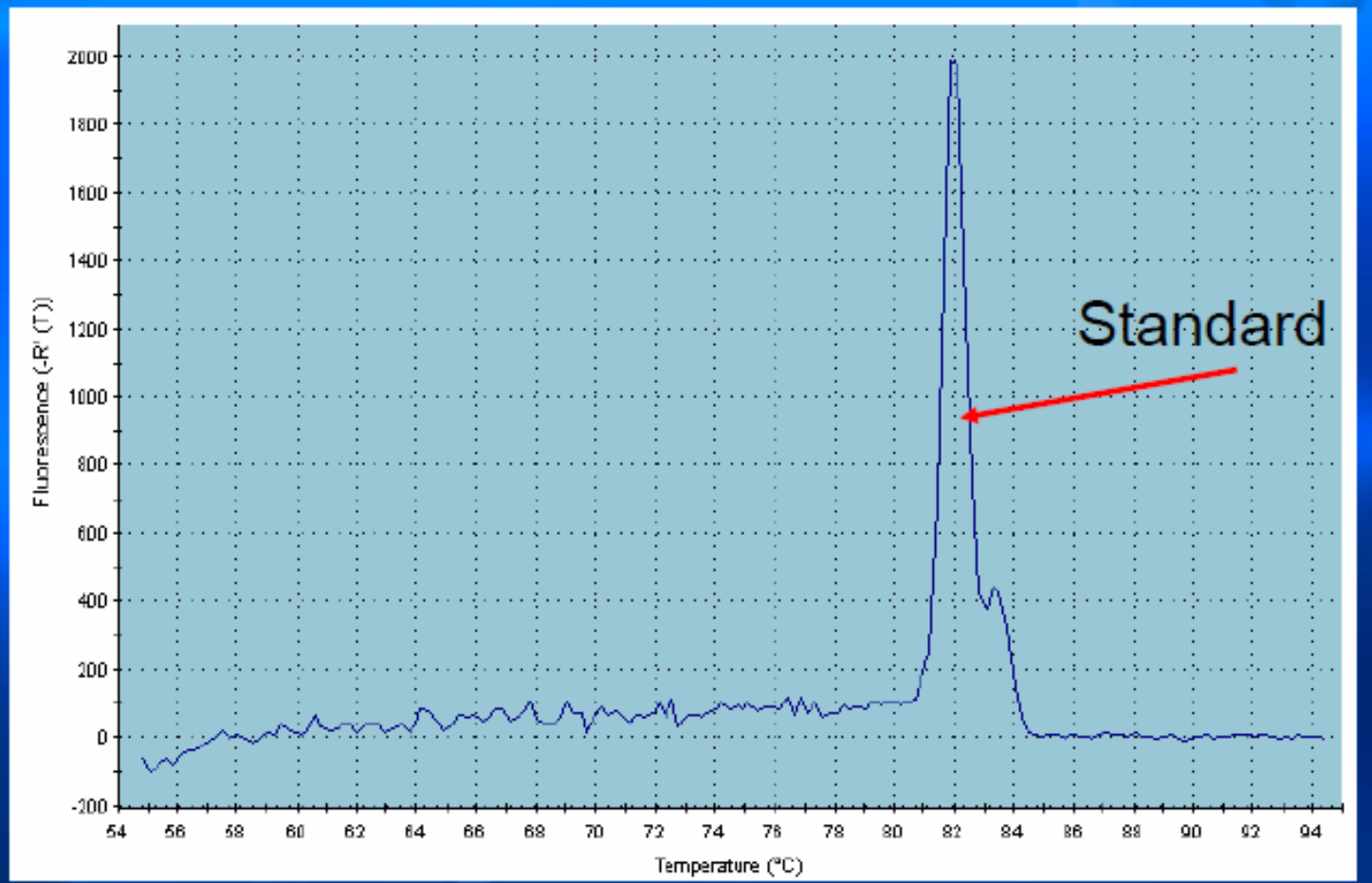

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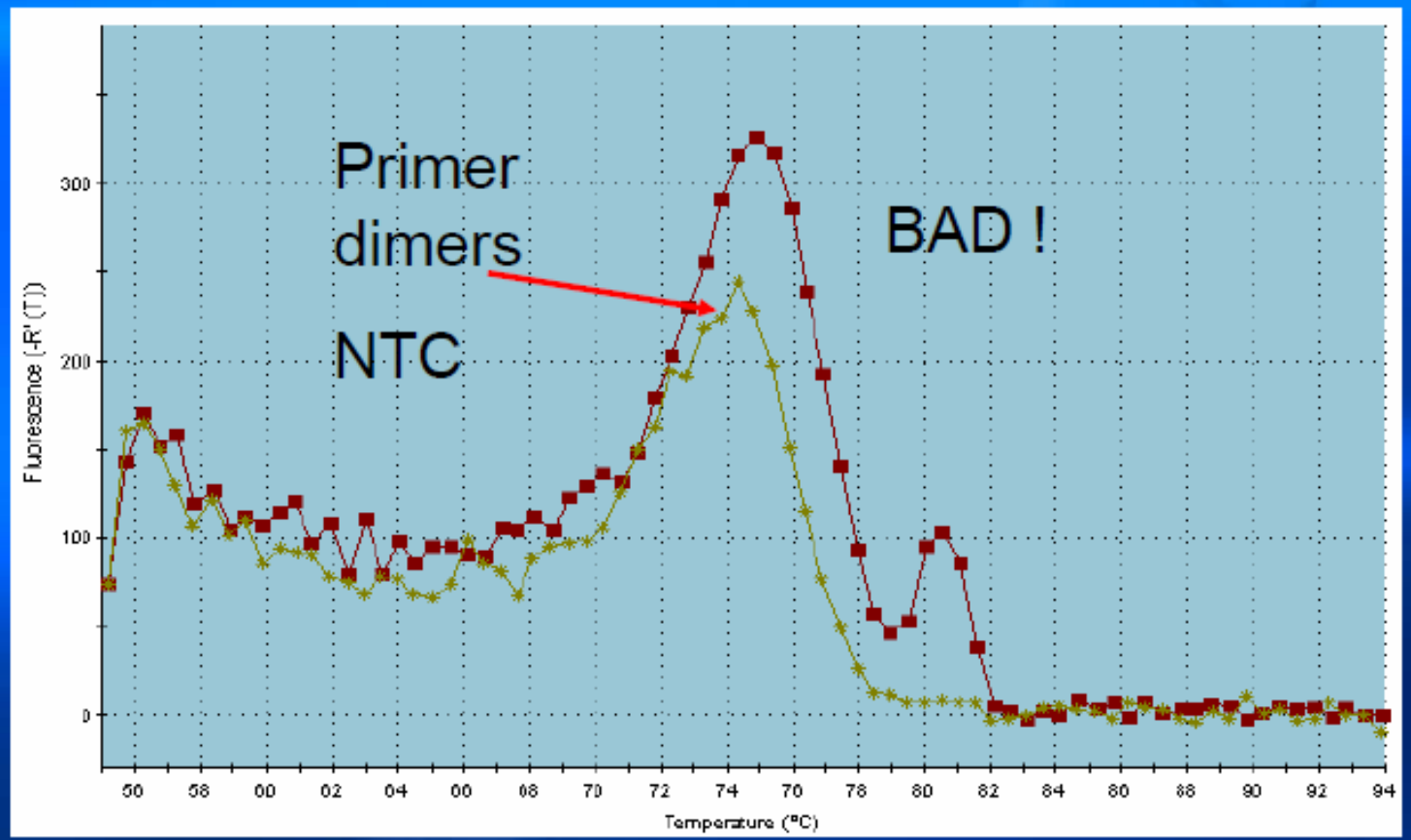
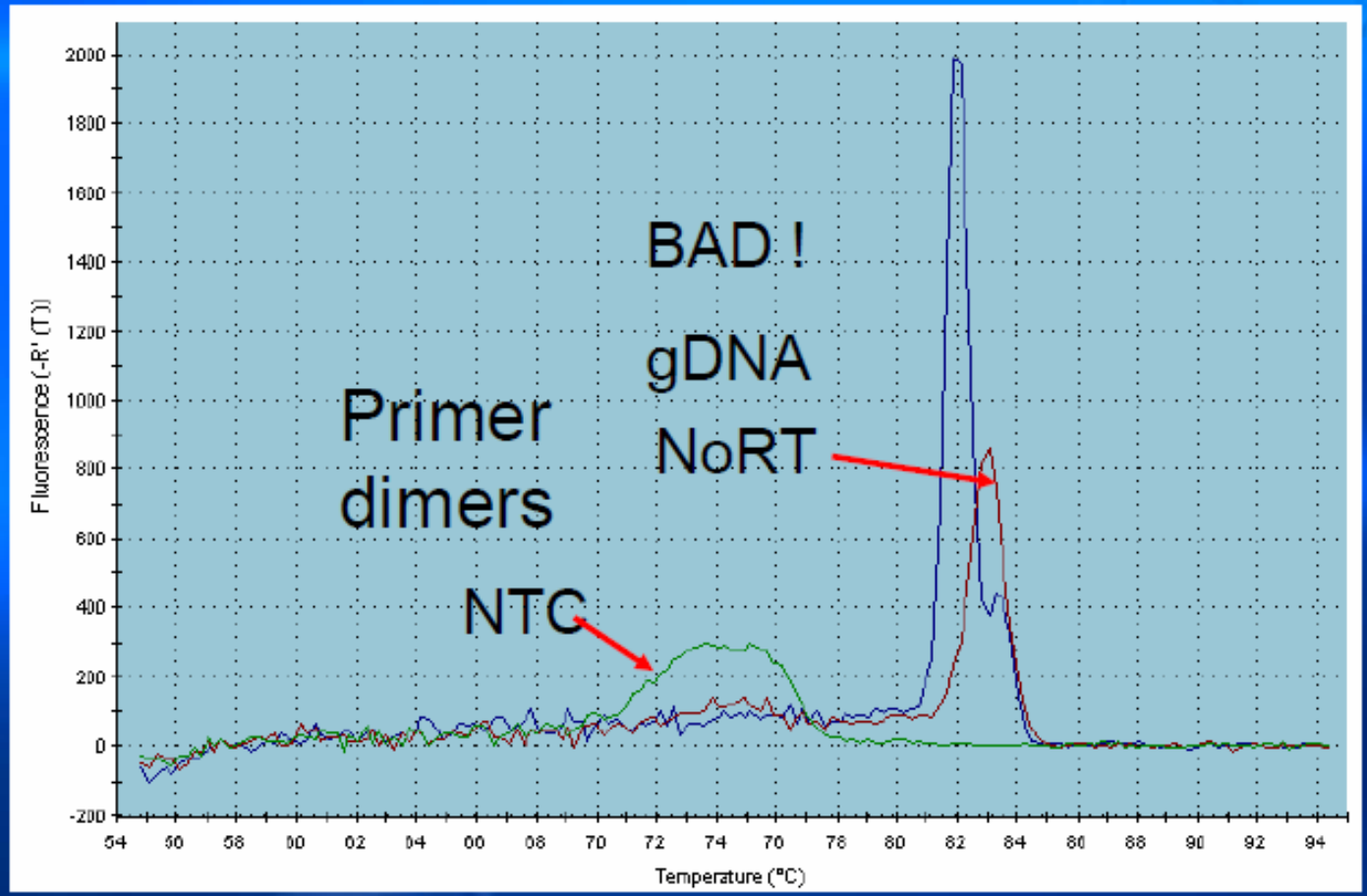
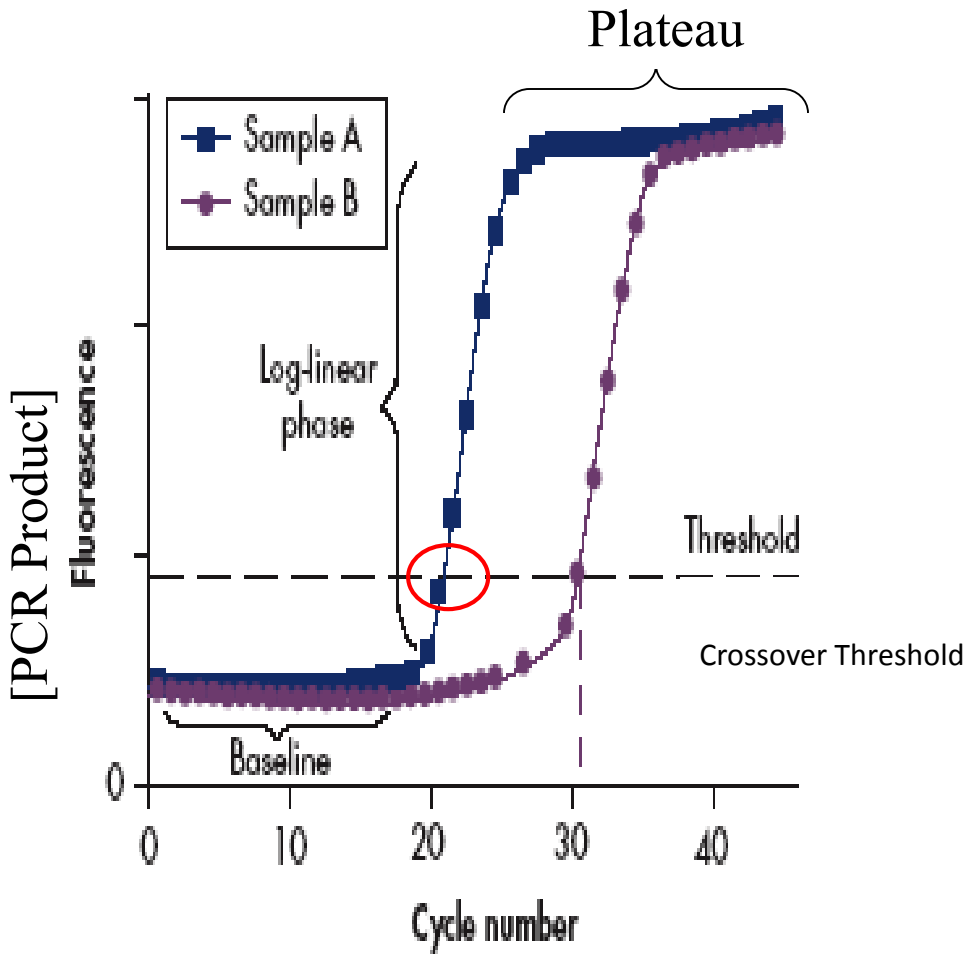


QPCR Assay Control Specificity

Negative QPCR Control



The kinetics of Real time PCR

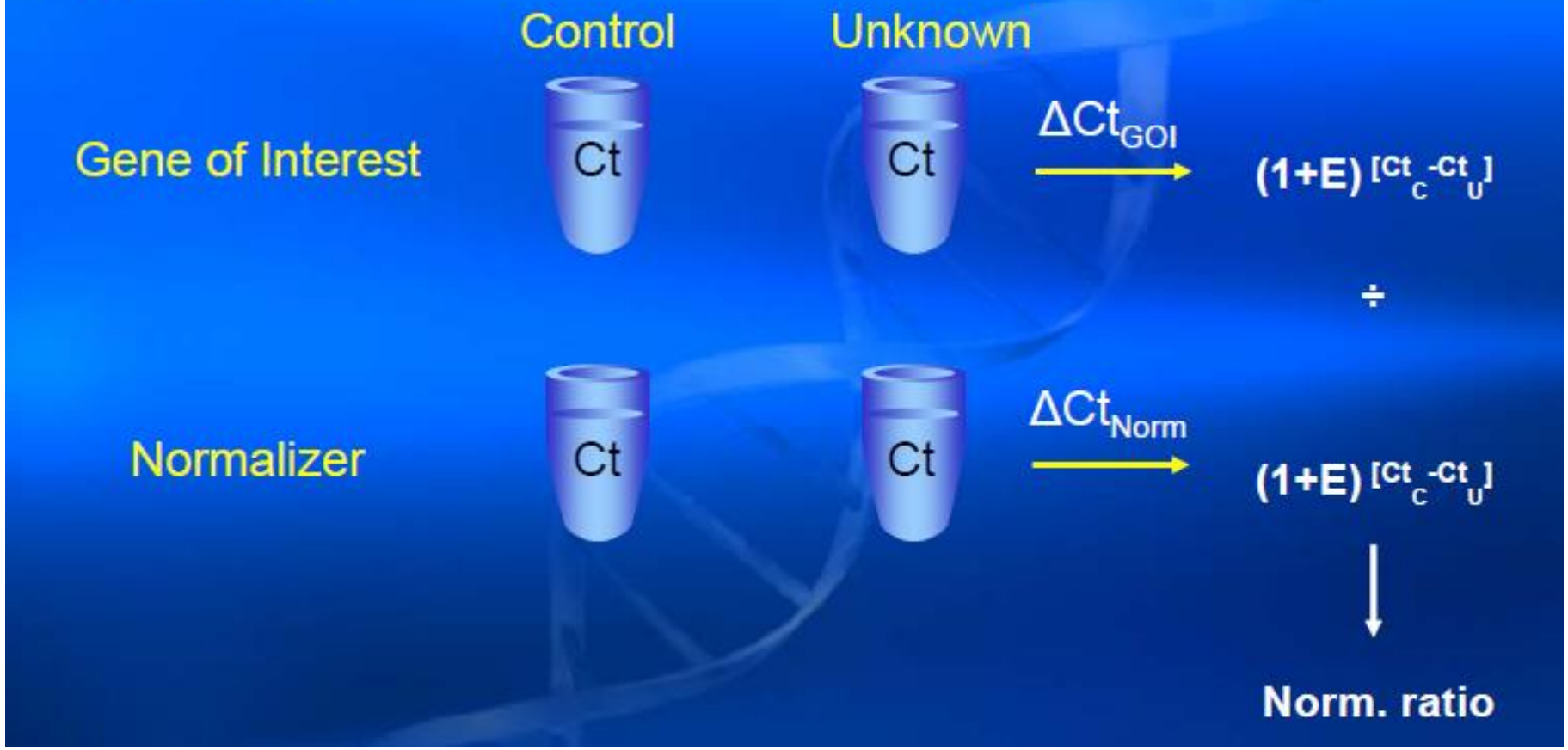


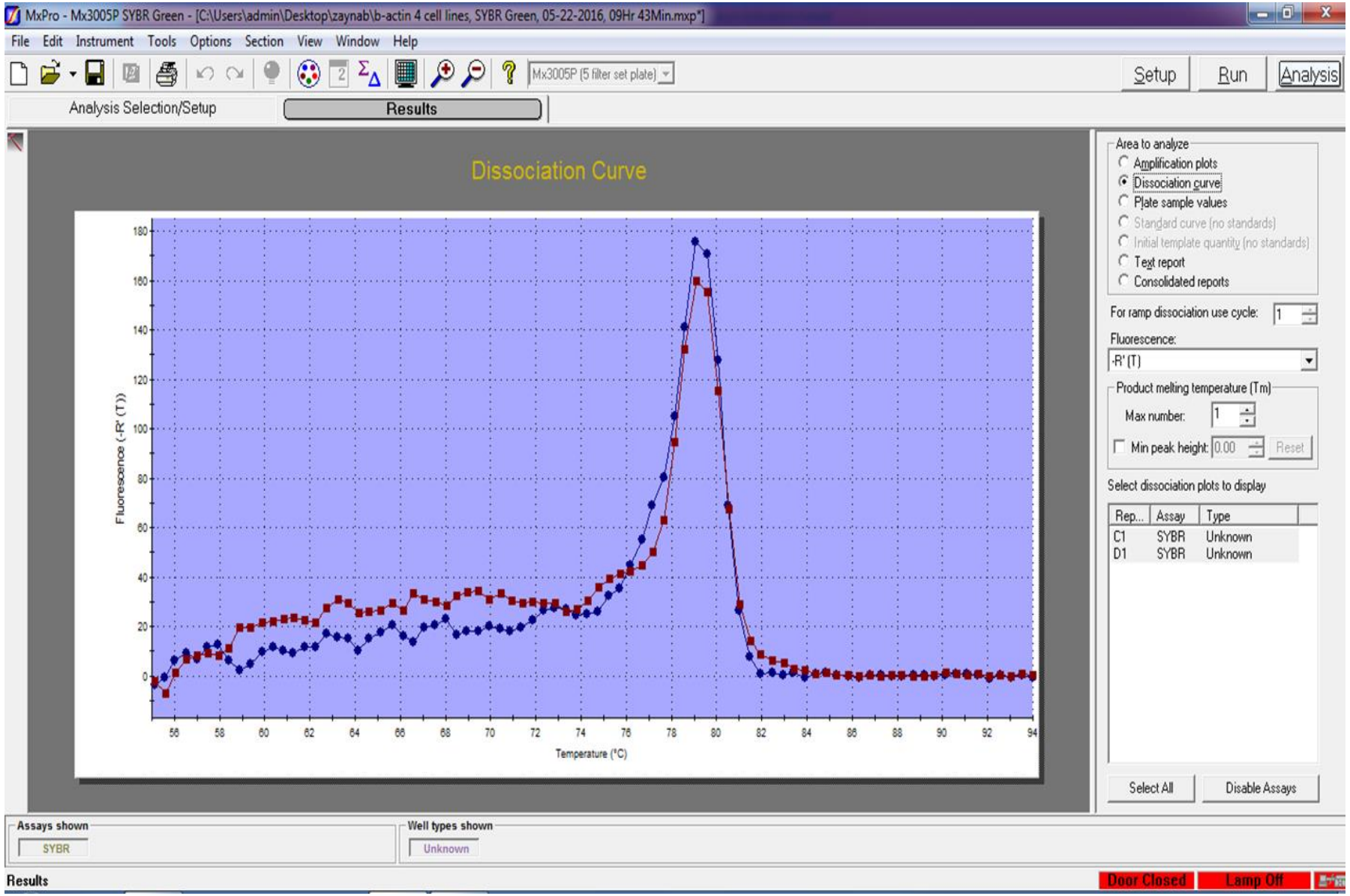
- Amplification is associated with fluorescence.
- No need for end point analysis by Gel electrophoresis
- CT- 10X baseline mean for cycles 2-10

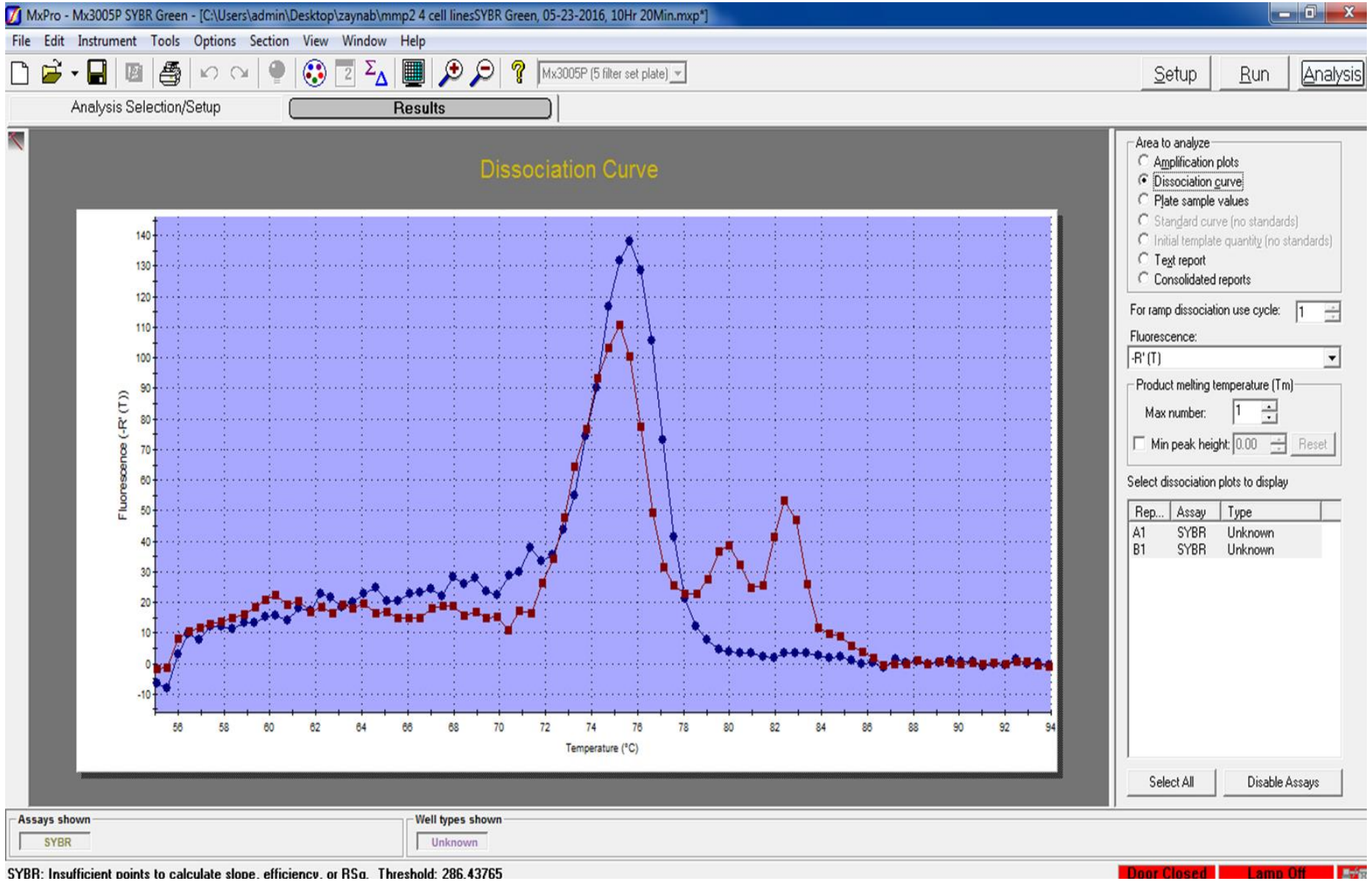
- **Always look at the Y axis**
  - Linear vs Log

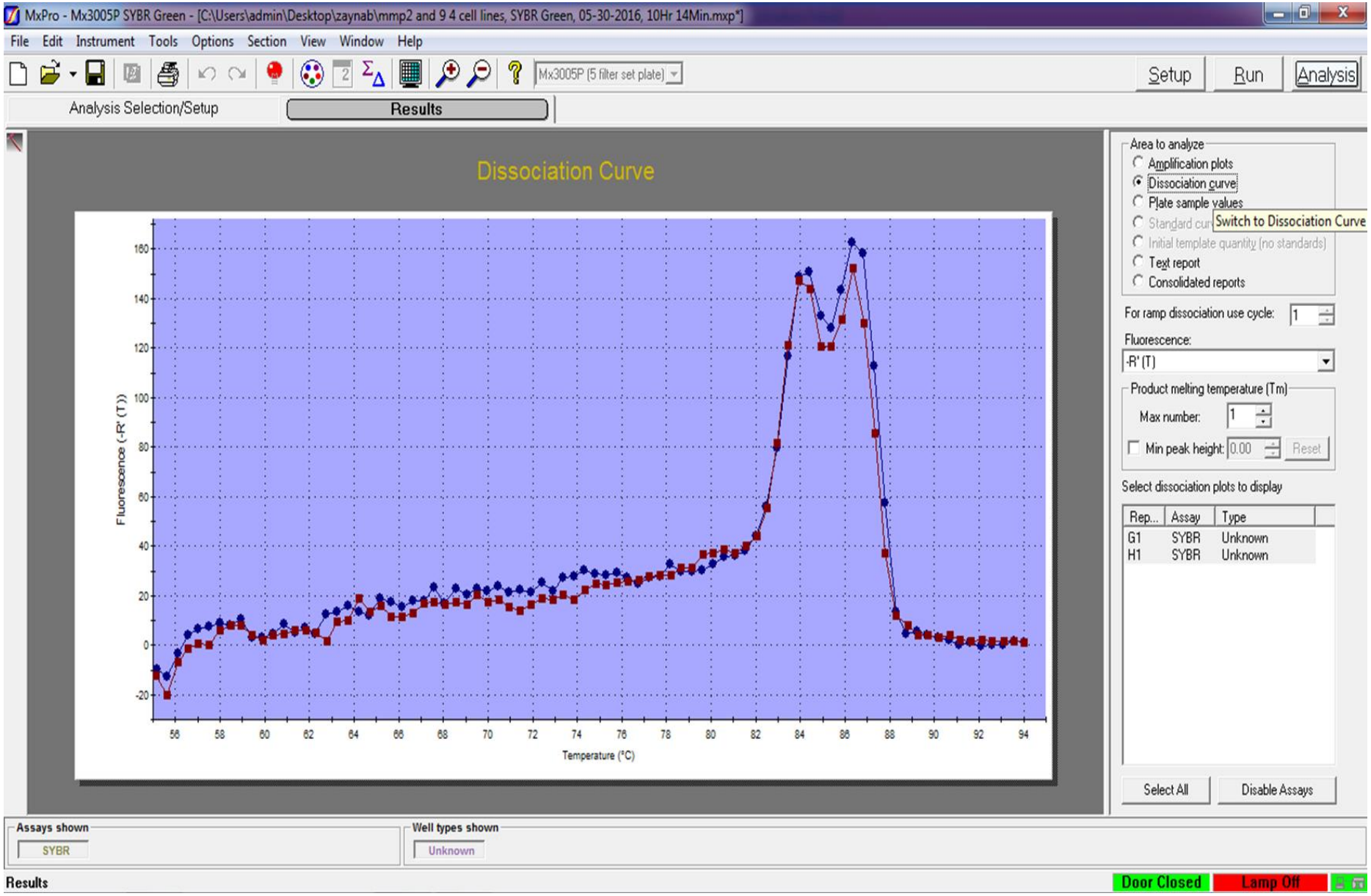
Comparative Quantification

Given two samples: What is the difference in gene expression?




MxPro - Mx3005P SYBR Green - [C:\Users\admin\Desktop\zaynab\b-actin 4 cell lines, SYBR Green, 05-22-2016, 09Hr 43Min.mxp*]
File Edit Instrument Tools Options Section View Window Help
Mx3005P (5 filter set plate)
Setup
Run
Analysis
Analysis Selection/Setup
Results
Dissociation Curve
Fluorescence (-R' (T))
Temperature (°C)
Area to analyze
Amplification plots
Dissociation curve
Plate sample values
Standard curve (no standards)
Initial template quantity (no standards)
Text report
Consolidated reports
For ramp dissociation use cycle:
Fluorescence:
-R' (T)
Product melting temperature (Tm)
Max number:
Min peak height:
Reset
Select dissociation plots to display
Rep... Assay Type
C1 SYBR Unknown
D1 SYBR Unknown
Select All
Disable Assays
Assays shown
SYBR
Well types shown
Unknown
Results
Door Closed
Lamp Off


MxPro - Mx3005P SYBR Green - [C:\Users\admin\Desktop\zaynab\mmp2 4 cell linesSYBR Green, 05-23-2016, 10Hr 20Min.mxp*]
File Edit Instrument Tools Options Section View Window Help
Mx3005P (5 filter set plate)
Setup
Run
Analysis
Analysis Selection/Setup
Results
Dissociation Curve
Fluorescence (-R' (T))
Temperature (°C)
Area to analyze
Amplification plots
Dissociation curve
Plate sample values
Standard curve (no standards)
Initial template quantity (no standards)
Text report
Consolidated reports
For ramp dissociation use cycle:
Fluorescence:
-R' (T)
Product melting temperature (Tm)
Max number:
Min peak height:
Reset
Select dissociation plots to display
Rep...
Assay
Type
A1 SYBR Unknown
B1 SYBR Unknown
Select All
Disable Assays
Assays shown
SYBR
Well types shown
Unknown
SYBR: Insufficient points to calculate slope, efficiency, or RSq. Threshold: 286.43765
Door Closed
Lamp Off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Amplification plots
Dissociation curve
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Text report
Consolidated reports
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Max number:
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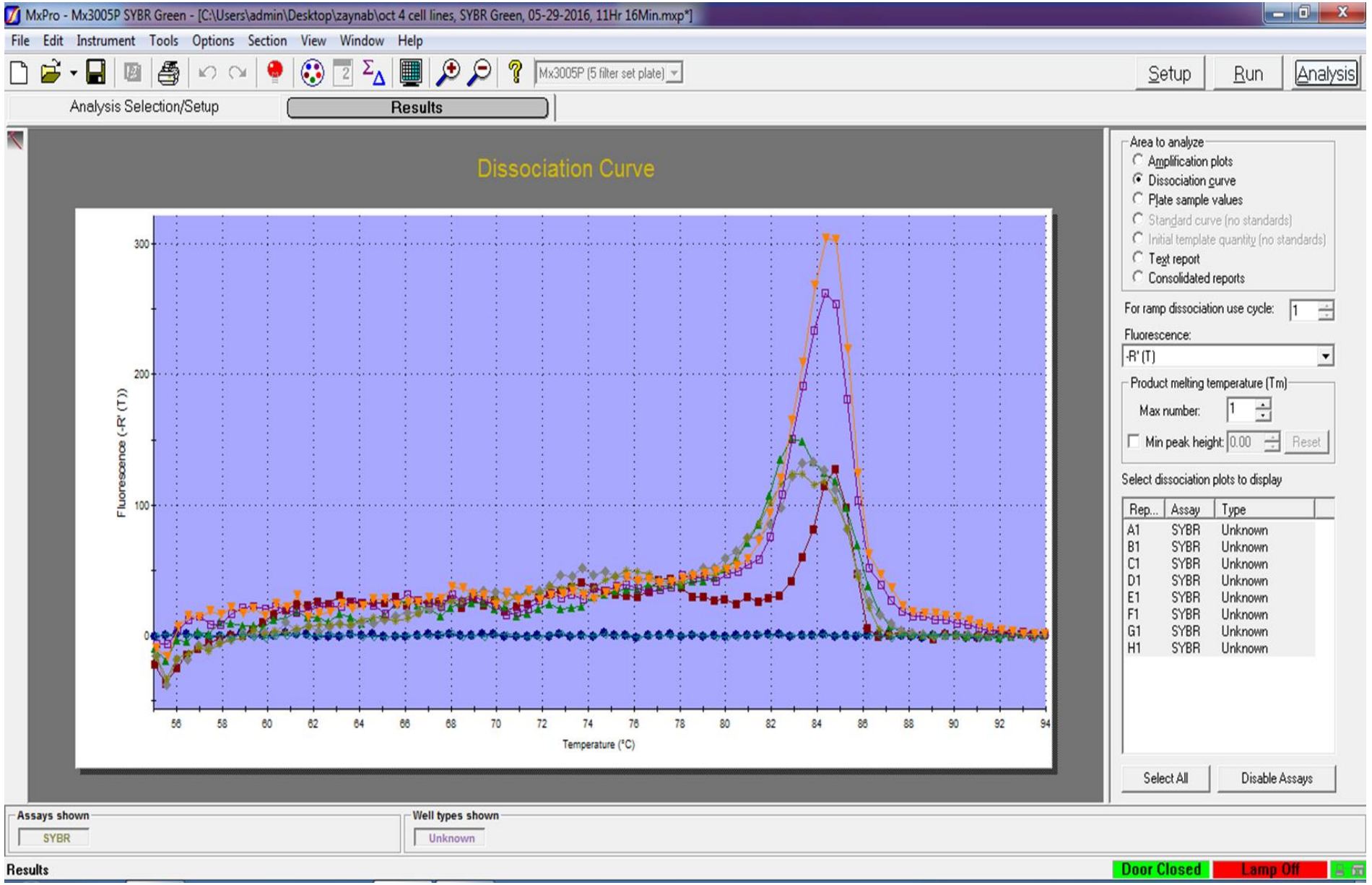
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A1 SYBR Unknown
B1 SYBR Unknown
C1 SYBR Unknown
D1 SYBR Unknown
E1 SYBR Unknown
F1 SYBR Unknown
G1 SYBR Unknown
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Select All
Disable Assays
Assays shown
SYBR
Well types shown
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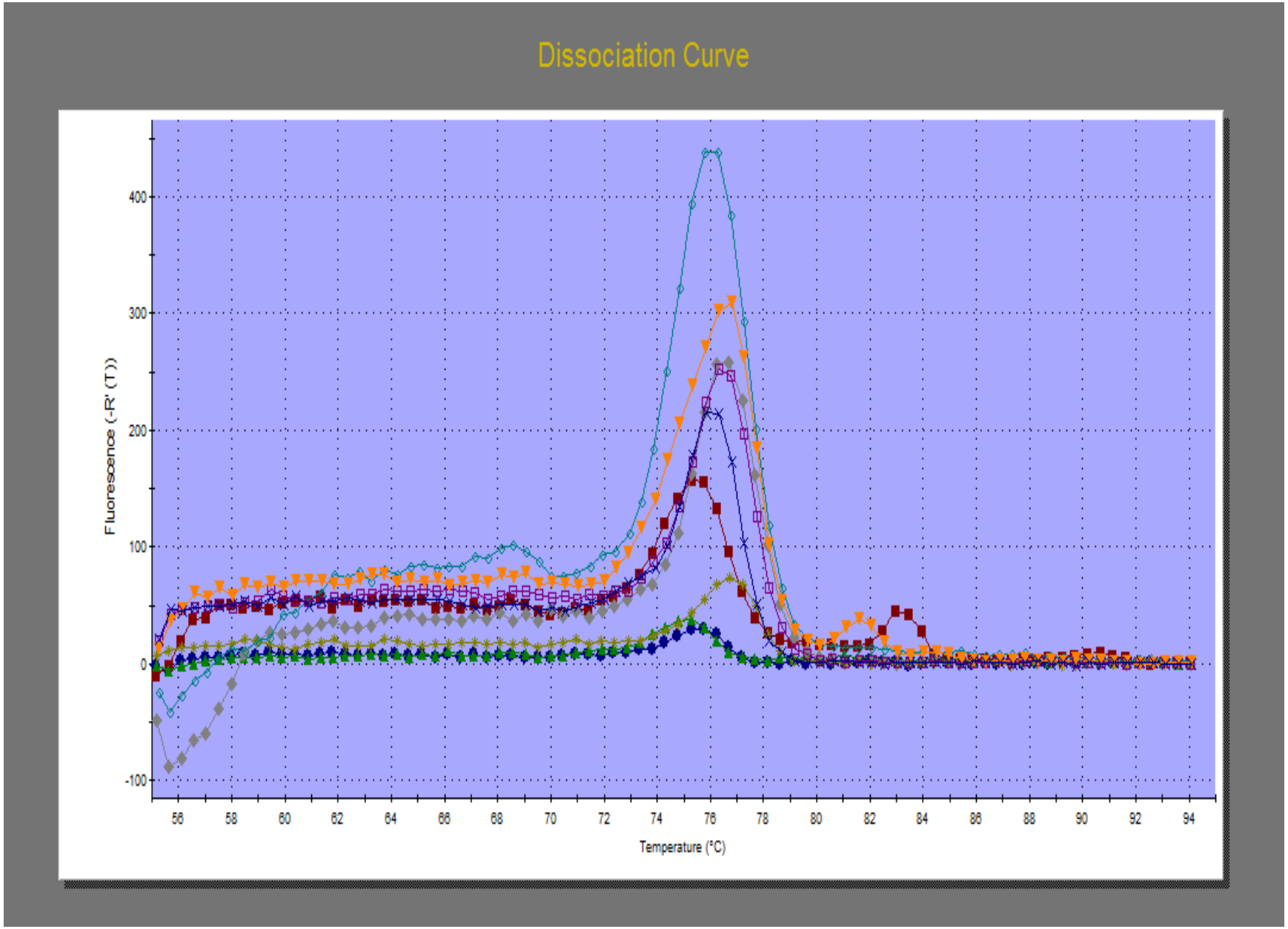
Results
Door Closed
Lamp Off


Dissociation Curve
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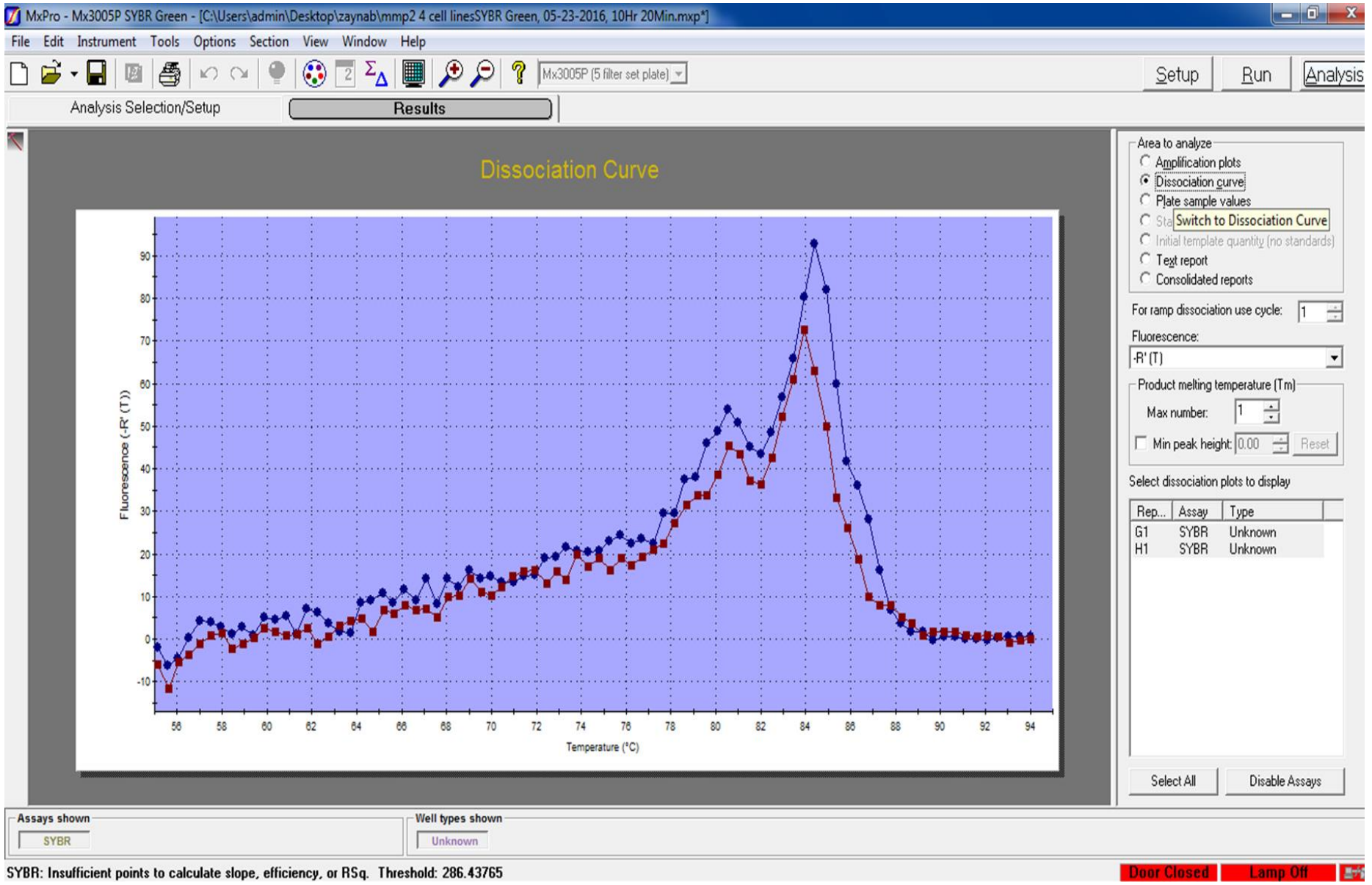
Temperature (°C)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Analysis Selection/Setup
Results
Dissociation Curve
Fluorescence (-R' (T))
Temperature (°C)
Area to analyze
Amplification plots
Dissociation curve
Plate sample values
Switch to Dissociation Curve
Initial template quantity (no standards)
Text report
Consolidated reports
For ramp dissociation use cycle:
Fluorescence:
-R' (T)
Product melting temperature (Tm)
Max number:
Min peak height:
Reset
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Rep... Assay Type
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H1 SYBR Unknown
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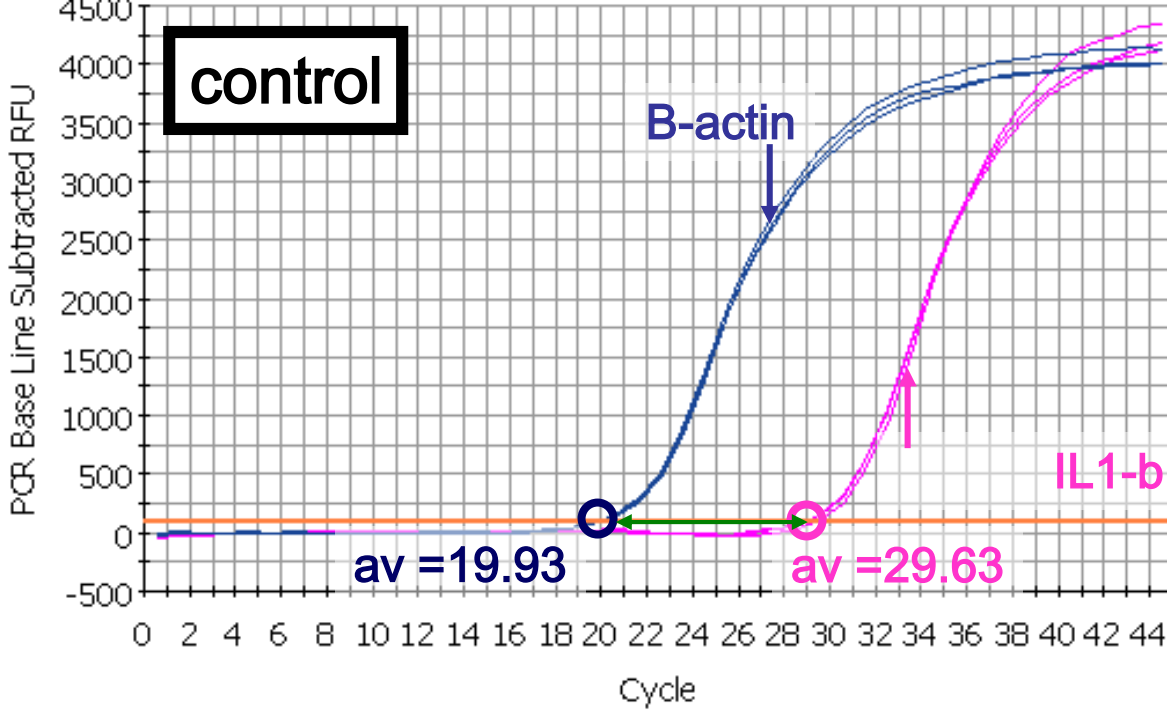
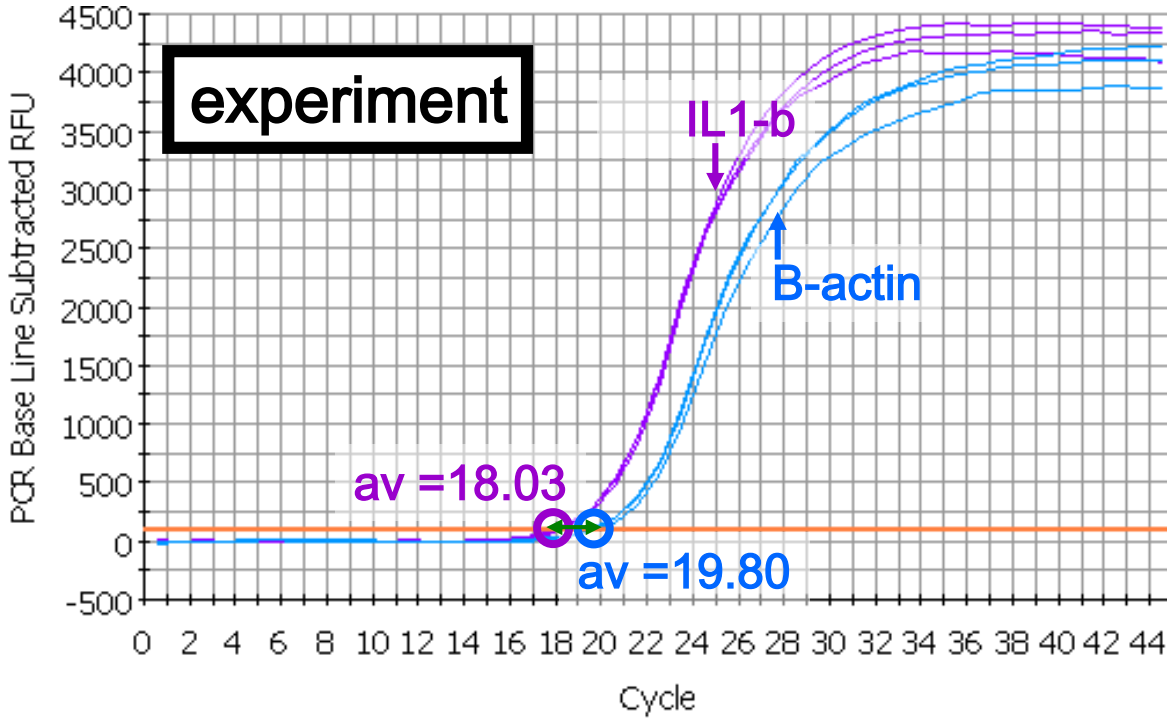
Lamp Off



Δ Ct = target - ref

Δ Ct = 9.70



Δ Ct = target - ref

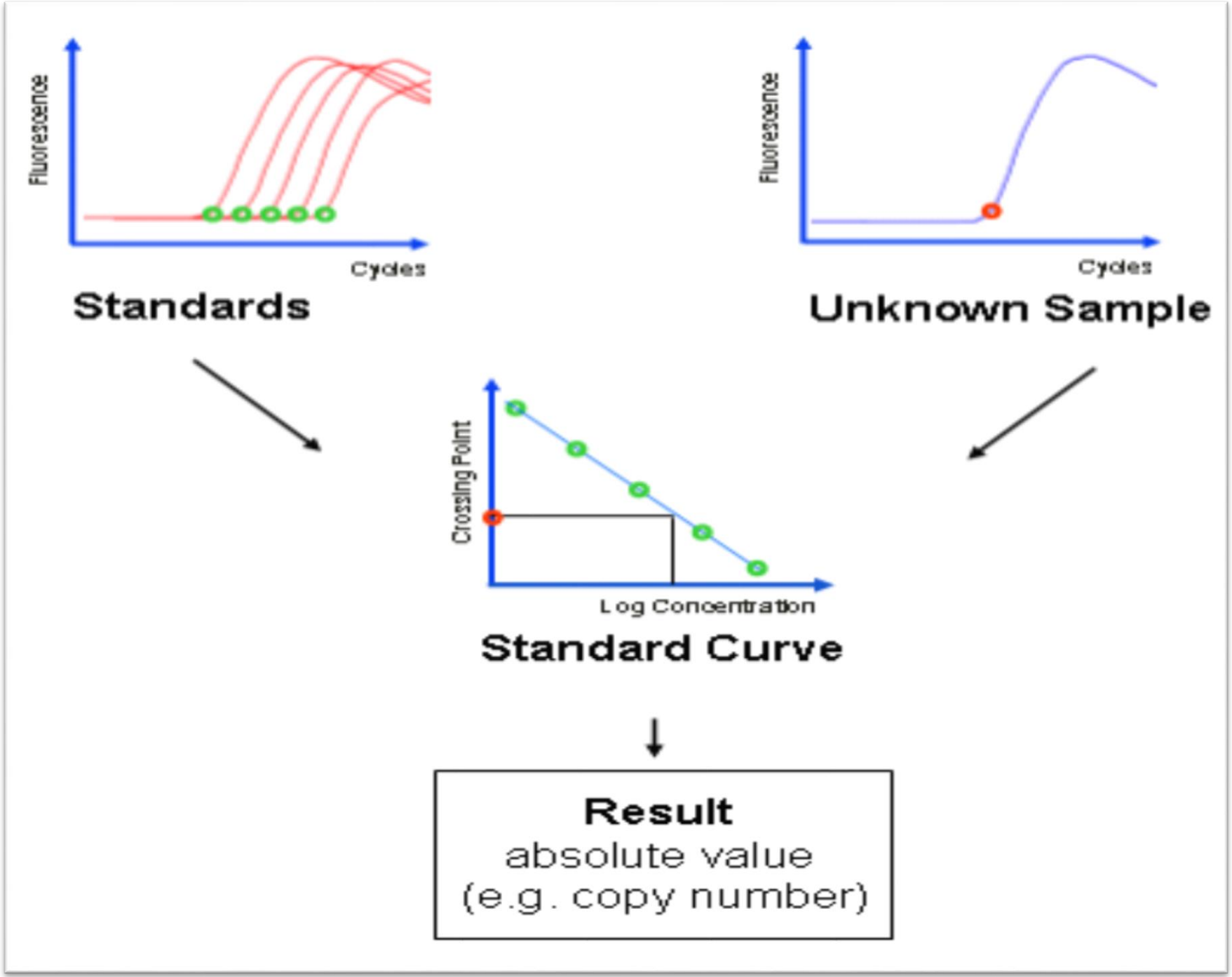
Δ Ct = -1.7

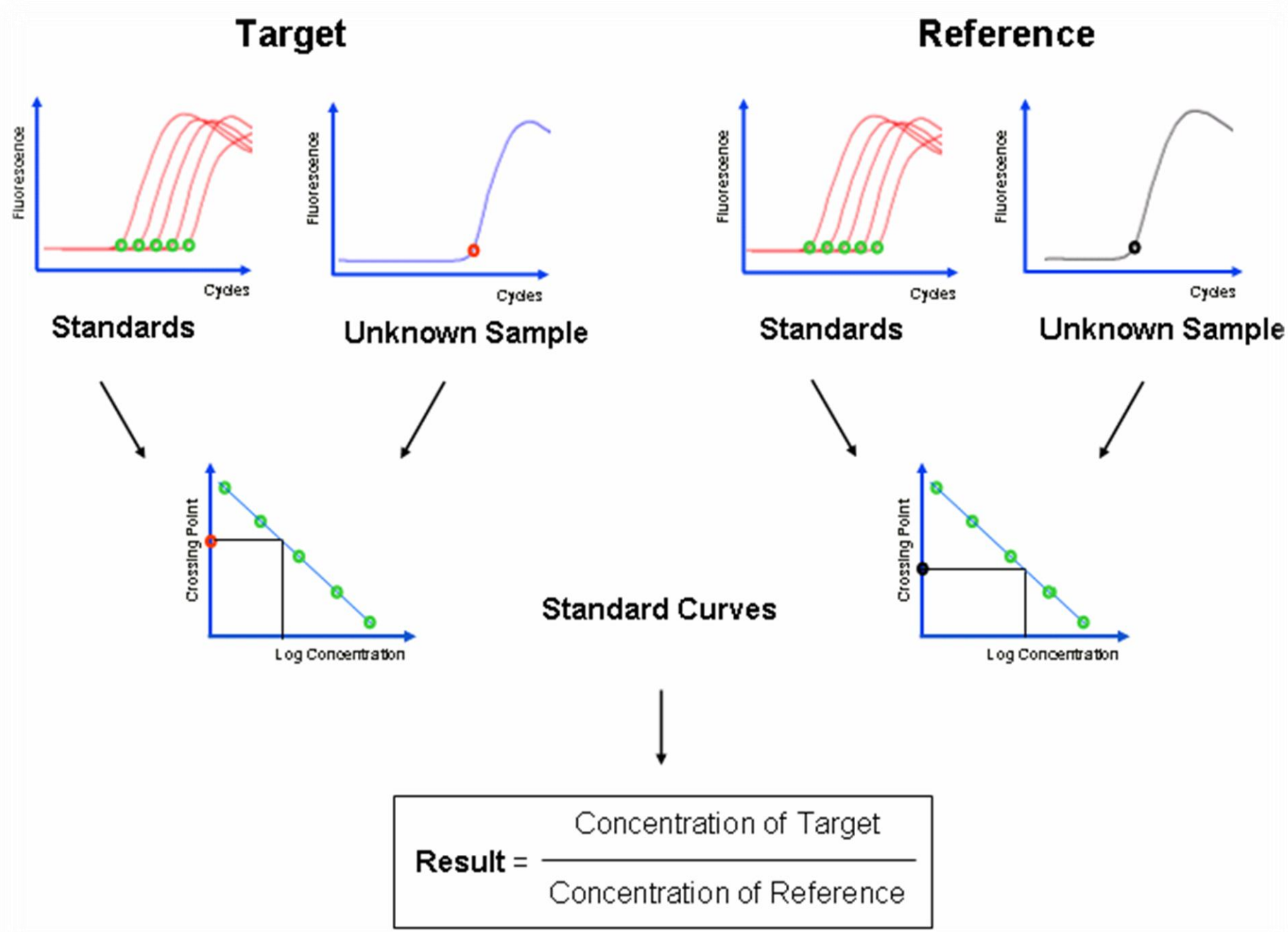
Difference = ΔCt-ΔCt

= $\Delta\Delta$Ct

= 9.70-(-1.7)

= 11.40


Fluorescence
Cycles
Standards
Fluorescence
Cycles
Unknown Sample
Crossing Point
Log Concentration
Standard Curve
Result
absolute value
(e.g. copy number)


Target
Reference
Fluorescence
Cycles
Standards
Unknown Sample
Crossing Point
Log Concentration
Standard Curves
Result = Concentration of Target / Concentration of Reference

Real Time PCR Applications Include:

- Quantitative mRNA expression studies.
- DNA copy number measurements in genomic or viral DNAs.
- Allelic discrimination assays or SNP genotyping.
- Verification of microarray results.
- Drug therapy efficacy.
- DNA damage measurement.

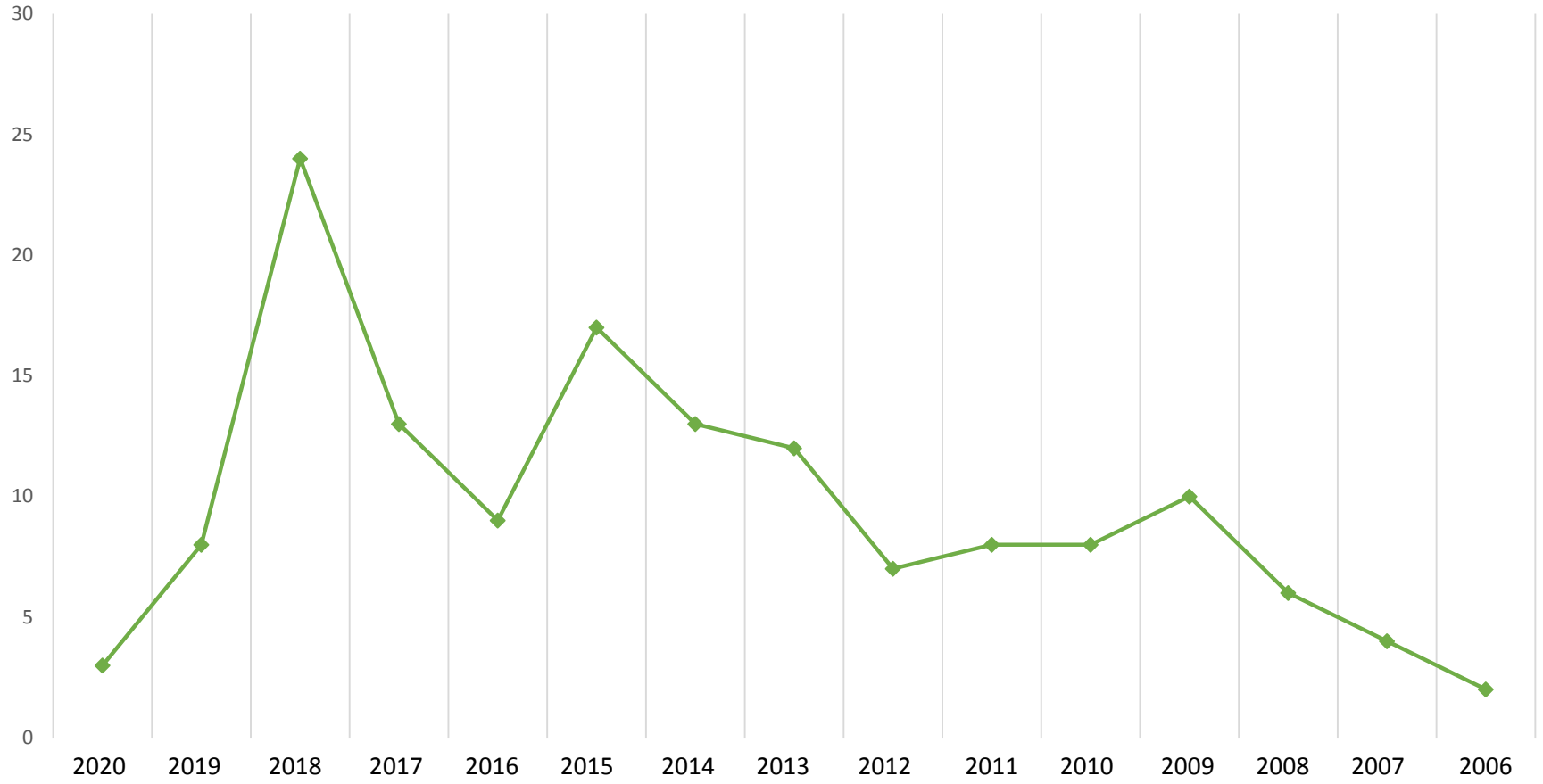
Hope it will be easy for you

You can fell free to contact me

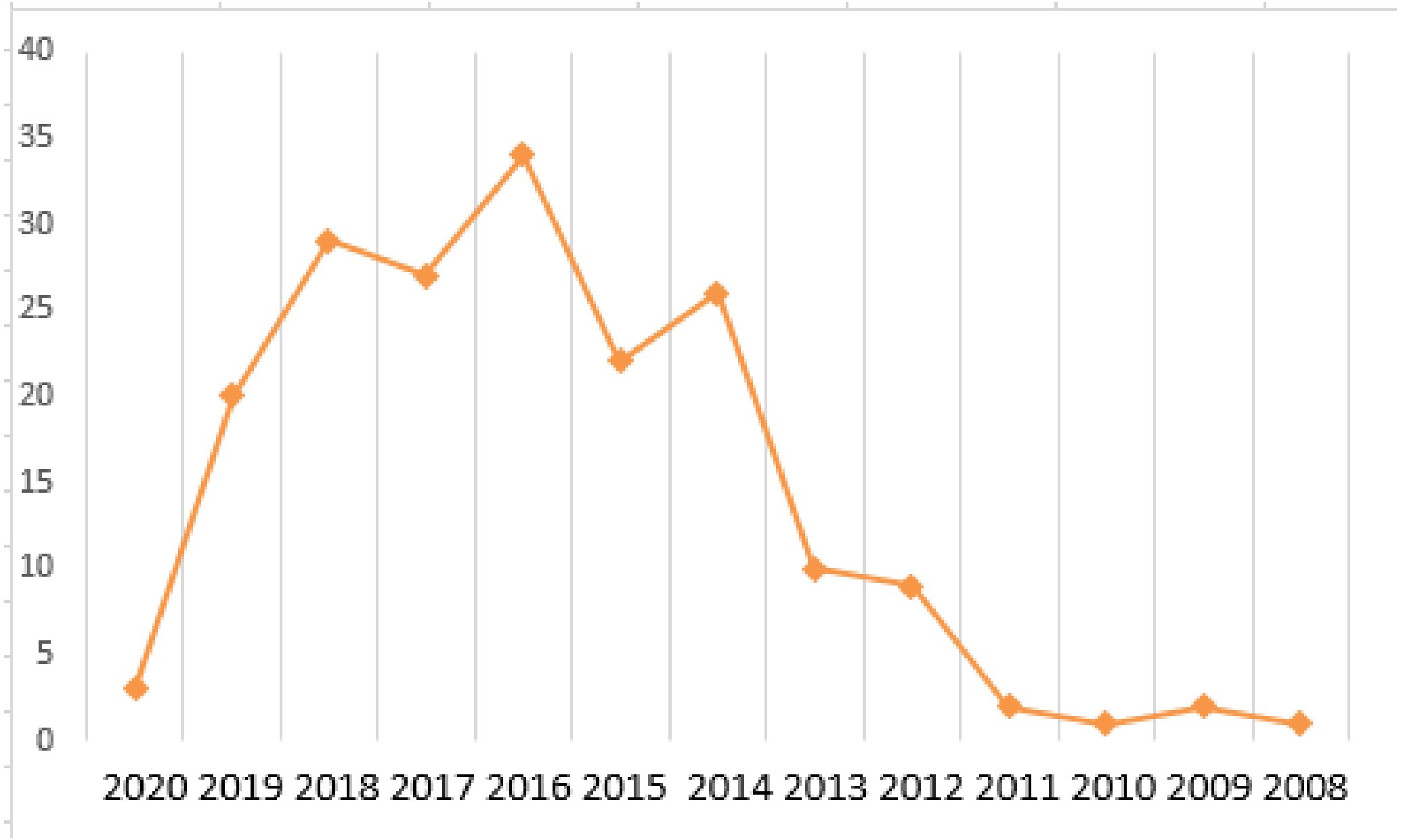
zaynab.saad@iccmgr.org

Thank you for attending this workshop

Molecular techniques according to Iraqi academic scientific journals



Molecular techniques



Real time pcr

Novel expression of microRNAs in serum samples of Iraqi breast cancer women

Saad, Zaynab, Arif, Muhammad, Yassen, Nahi, Jasim, Hameed, Jelawe, Majed and Brown, James (2014). Novel expression of microRNAs in serum samples of Iraqi breast cancer women. *American Journal of Biomedicine*, 2 (5), pp. 567-574.

Abstract

Although a lot of hard work against cancer to reduces its spread but it still continues to kill with abandon. The need for a biomarker for cancer early detection becomes the most mind concentrated scientists. MicroRNAs the tiny non coding RNA molecules opened new path for the scientists to determine the cancer in its early stages. Expression of microRNAs profiles has been investigated to be involved in cancer development. Here we determined the expression of microRNAs in serum of Iraqi healthy volunteers and other women diagnosed with breast cancer. MicroRNAs expression has been determined by using real time qPCR and delta method has been

EJSO EUROPEAN JOURNAL OF SURGICAL ONCOLOGY THE JOURNAL OF CANCER SURGERY

ABSTRACT ONLY | VOLUME 42, ISSUE 5, PS20, MAY 01, 2016

A differential expression of miRNA in plasma and breast tissue: A potential biomarker

M.Arif Nasir • Zaynab Abdul-Ghany • Zartasht Carmichael • James E.P. Brown • Amtul R. Carmichael

DOI: https://doi.org/10.1016/j.ejso.2016.02.083

Article Info

Related Articles

Introduction: Breast cancer is a complex disease and is the leading cause of cancer mortality in women after lung cancer. A non-coding class of RNA called micro RNA (miRNA) is implicated in many diseases including breast cancer where it has been suggested as a potential biomarker.

Biomedical Research
An International Journal of Medical Sciences

Research Article - Biomedical Research (2018) Volume 29, Issue 21

Starvation contributes to elevated levels of heat shock proteins and cancer stem cell markers in an esophageal cancer cell line.

Amer Talib Tawfeeq*, Noah Abd-Alkader Mahmood, Zaynab Saad Abd-Alghni

Department of Molecular Biology, Molecular Biology Lab, Iraqi Center for Cancer and Medical Genetics Research, Iraq

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Department of Molecular Biology
Molecular Biology Lab
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Iraq

Accepted date: November 26, 2018

DOI: 10.4066/biomedicalresearch.29-18-1138

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Abstract

The presence of cancer stem cells as a subpopulation residing in the apex of solid tumor cell hierarchy has been introduced as a new hypothesis capable of describing the ability of tumors to be more aggressive, highly metastasized, and chemo-radiotherapy resistant. The origin of these cells is still controversial: one hypothesis describes the stress conditions in the tumor microenvironment as one of the driving forces behind the existence of these cells. In this study, we test if nutrition depletion conditions (deprivation of serum and glucose) would be one of such forces. Esophagus adenocarcinoma cell line SKGT-4 was exposed to starvation by depleting glucose and fetal bovine serum from growth media at different times. The viability of the cells during this condition was determined by standard MTT assay and the cells' morphological changes were observed by crystal violet staining and trypan blue staining. The expression levels of stress-related proteins, heat shock protein 90 (HSP90) and heat shock protein 70 (HSP70), as well as some known cancer stem cell markers, CD44, ALDH1A1, and ABCG2, were determined using quantitative real-time PCR. Levels of necrosis and apoptosis were followed in cell populations under stress using a mixture of fluorescence staining and observation under a fluorescence microscope. The results indicated a loss of cell viability during the extended times of incubation in starved condition compared with the non-starved condition. Cells under starvation suffered from noticeable morphological changes combined with widespread necrosis and apoptosis. Levels of HSP90, HSP70, and cancer stem cell marker expression were significantly increased in starved condition compared with non-starved condition ($p \leq 0.01$). In conclusion, although starvation as a result of serum and glucose depletion leads to induced necrosis and/or apoptosis in most of the cells, it may induce stress-resistant mechanisms in cells that remain viable (stress-resistant cells).

Matrix Metalloproteinases MMP 2 and MMP 9 Expression in Stages II-III Breast Cancer in Iraqi Women

Noah Abd-Alkader Mahmood, Rajaa M. Fakhoury, +1 author Mohamed E Moustafa · Published 2015

Breast cancer is the most common invasive cancer in women worldwide. Metalloproteinases MMP2 and MMP9 participate in tumor invasion and metastasis by degrading extracellular matrix. In this study, we investigated the expression of MMP2 and MMP9 in breast tissues of Iraqi women with stage II and III breast cancer. The correlation between the expression levels of MMP2 and MMP9 in stage II-III breast cancer and clinicopathological features was also examined. The expression levels of MMP2 and MMP9 in the breast were determined by real-time PCR and immunohistochemistry in 64 patients with stages II-III samples and in 21 benign tumors from Iraqi women. The mRNA levels of MMP2 and MMP9 were significantly higher in breast cancer stages II-III than those in benign breast tumor tissues at P< 0.05. Immunohistochemistry also revealed that the protein levels of MMP2 and MMP9 were 72% and 64% in patients with stages II-III breast cancer as compared to 28% and 23% in benign breast tumor. The increased levels of MMP2 and MMP9 in stages II-III breast cancer were correlated to tumor grade (P=0.04 and 0.01, respectively), stage (P=0.03 and 0.05) and type (P=0.004 and 0.05) and lymph node metastasis (P=0.009 and 0.04), respectively.MMP2 and MMP9 expression levels were increased in stages II-III of breast cancer in Iraqi women and their levels were correlated with tumor grade, stage and type and lymph node metastasis.These metalloproteinases can be used as biomarkers for breast cancer progression and metastasis. LESS

Journal of Medical and Biological Science Research

Vol. 1 (2), pp.13-23, April, 2015

Research Paper

OCT3/4, ALDH-1 and CD44 Expression Levels in Iraqi Women with Stage II-III Breast Cancer

Noah A. Mahmood[1,2*], Rajaa M. Fakhoury[1], Nahi Y. Yaseen[2], Mohamed E. Moustafa[1]

Accepted 23 April, 2015

[1]Department of Biological and Environmental Sciences, Faculty of Science, Beirut Arab University, Beirut, Lebanon.
[2]Iraqi Center for Cancer and Medical Genetic Research, University of AL-Mustansiriya, Baghdad, Iraq.

ABSTRACT

Cancer stem cell (CSC) markers OCT3/4, ALDH-1 and CD44 play important roles in metastasis and resistance to conventional cytotoxic agents. In this study, we investigated the expression levels of CSC markers OCT3/4, ALDH-1 and CD44 in Iraqi women with stage II-III breast cancer or with benign breast tumor. We also investigated the association between the expression levels of these markers and some clinicopathological features. The expression of OCT 3/4, ALDH-1 and CD44 in breast tissues was determined using real-time PCR and immunohistochemistry in 64 patients with breast cancer stage II or III samples as well as in 21 corresponding benign tumors. OCT3/4, ALDH-1 and CD44 mRNA levels were highly expressed in stage III (p= 0.004, 0.015 and 0.008, respectively) and in stage II (p= 0.043, 0.045 and 0.028, respectively) as compared to those in benign tumors. There was no significant variation in the expression of these markers between stages II and III (p= 0.18, 0.30 and 0.49). In addition, immunohistochemistry showed that OCT3/4, ALDH-1 and CD44 expression levels were increased in 82.2%, 68.8% and 53.1%, respectively in stage II-III breast cancer as compared to 14%, 28%, and 33% in benign tumor. The expression levels of OCT3/4, ALDH-1 and CD44 were correlated to tumor grades, types and lymph node metastasis. These results indicate that the expression levels of CSC markers OCT3/4, ALDH-1 and CD44 were increased in stage II-III breast cancer but not in benign breast tumor in Iraqi women.

Iraqi Journal of Cancer and Medical Genetics

ABCG2 (BCRP) m RNA expression level by using real- time PCR and immunohistochemistry associated with clinicopathological features in Iraqi women with stage II-III breast cancer.

Noah A. Mahmood[1], Isra'a M. Ressan Al-Sudani[2], Hala Khalid Al-Sammarraie[3] and Tee-baa H. Jaffar[1]

1 Iraqi Center for Cancer and Medical Genetic Research/ Al-Mustansiriya University.
2 Department of pathology/ College of medicine/ Al-Mustansiriya University.
3 Forensic DNA Research and Training Center/ Al-Nahrain University.

Abstract:

Background: Breast cancer is the most frequent cancer and cause of death among women worldwide. ABCG2 (ATP–binding cassette sub-family G member 2) is an ABC transporter superfamily and endogenous expression of ABCG2 in different certain cancer reflect intrinsic drug resistance. It is also a molecular determinant of pharmacokinetic properties of many drugs in humans. In this study, we determined the expression levels of ABCG2 in breast tissues of Iraqi women with stage II and III breast cancer. The correlation between the expression levels of ABCG2 and clinicopathological features was analyzed.
Methods: The expression levels of ABCG2 in the breast was determined by using real- time PCR and immunohistochemistry in 64 patients with stage II and III samples and in 21 benign tumors from Iraqi women.
Results: We found that the expression level of ABCG2 mRNA were significantly increased in breast cancer stage II-III tissues than those in benign tumor tissues. There was a significant variation between the mRNA levels of ABCG2 in stage II and stage III at $P< 0.05$. Immunohistochemistry revealed that the protein expression levels of ABCG2, was also increased in 83% of patients with stage II and III breast cancer as compared to 17% in benign tumor. The increased expression levels of ABCG2, in stage II-III breast cancer were correlated to tumor stages ($P=0.03$), tumor grades ($P=0.01$), tumor types ($P=0.01$) and lymph node metastasis ($p=0.0001$), respectively.
Conclusion: ABCG2 expression level in Iraqi women with stage II and III breast cancer were highly correlated with tumor stages, grades, types and metastasis and they could be used a potential markers which can prediction tumor behavior, progression and prognosis. Over expression of ABCG2 protein lead to treatment failure, tumor relapse and tumor metastasis by

Iraqi Journal of Cancer and Medical Genetics

Confirming intrinsic pathway apoptosis event in cervical carcinoma cells (HeLa) treated with hybrid nanoliposomes

Noor A. Awad[1], Nahi Y. Yassen[2], Amer T. Tawfeeq[2], Kismat M. Turki[1]

1 Clinical biochemistry division\ College of Medicine \ Baghdad University.
2 Molecular Biology Dept.\ Iraqi Center for Cancer and medical Genetics Research\ Al-Mustansiriya University.

Abstract:

Cancer targeted nanotherapy represent an exciting field in the search for new cancer specific therapies to avoid conventional chemotherapy side effects. Because cancer cells usually have malfunctioning apoptotic machinery which favors survival pathways and drug resistance. Cancer cell apoptosis is the favorable event to be induced in any new anticancer agent development. Nanotherapy goals are to elevate therapeutic efficiency, selectivity, and overcome drug resistance as major obstacle in cancer treatment. Hybrid nanoliposomes (nHLs) may fulfill all these features in cancer therapeutics. We have previously demonstrated the ability of in house synthesized nHLs to inhibit HeLa cell line proliferation and study preliminary the induction of apoptosis as a consequences of that inhibition. In order to confirm the event of apoptosis in HeLa cell line incubated with the synthesized nHLs we exposed HeLa cells to inhibition concentration 50 (IC50) of previously synthesized hybrid nanoliposomes. Mechanism of apoptosis induction was determined using mitochondrial membrane potential disruption, caspase-3 activity and single cell gel electrophoresis as well as DNA fragmentation assay. All apoptosis detection procedures used gave a clear defined significant indication that nHLs was capable of induce apoptosis in HeLa cells through intrinsic pathway. This result needs further investigation to confirm nHLs as potential nanotherapy.

Key words: *apoptosis, nanoliposome, liposome, nanobiotechnology, HeLa cells.*

DOI : 10.35124/bca.2019.19.1.1741

Biochem. Cell. Arch. Vol. 19, No. 1, pp. 1741-1746, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

ROLE OF GENETIC VARIATIONS IN *MTHFR* GENE ASSOCIATED WITH PCOS IN A SAMPLE OF IRAQI PATIENTS

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[1]Department of Medical Genetics, Iraqi Center for Cancer and Medical Genetic Research, Almustansiriyah University, Iraq.
[2]Department of Biology/College of Science for Women/ Baghdad University, Iraq.
[3]Madinat Al-Amamin Al-Kazimin Al-Tibbia Hospital, Iraq.
e-mail : asmaa.m.salih@gmail.com

(Accepted 23 February 2019)

ABSTRACT : This study was attempting to determine the role of *MTHFR* gene from exon8 regionin polycystic ovarian syndrome, using specific primers result in 164bp fragment then it was investigated by direct sequencing. Two of genetic variation were found in this region rs1801131 and rs484605. The rs484605 polymorphism revealed the mutant homozygous genotype TT among all samples of study (patients and controls), however, this substitution was silent mutations. While, rs1801131 polymorphism showed significance with PCOS patients ,revealed AG heterozygote genotype the common genotype in Iraqi female population.

Key words : *MTHFR* gene, PCOS, polymorphism.

INTRODUCTION

Polycystic ovary syndrome is the disorder that affects the women in reproductive age and result in subfertility and fertility. The etiology of polycystic ovary syndrome is not clearly understood. Even that many studies consider it as combination ofgenetic, environmental factors and life style lead to spectrum of endocrine, metabolic, reproductive disorders (Organerubo, 2016). The 1998). One of the common genetic polymorphisms was observed in MTHFR, including rs1801131(A1298C) that located at exon 8. The present study focuses on the correlation between rs1801131 genetic polymorphisms and metabolic disturbances development of polycystic ovary syndrome. Based on this, it was aimed at determining allele frequencies and genotype distributions of MTHFR rs1801131 polymorphisms in sample of Iraqi patients with

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ISSN-0972-3005

INVESTIGATION FOR VARIATION IN *MTHFR* GENE IN IRAQI ARAB FEMALE WITH PCOS

ASMAA A. ALMUKHTAR[1] AND ASMAA M. SALIH ALMOHAIDI*[1]

[1]Medical Genetics Department, Iraqi Center for Cancer and Medical Genetic Research, University of Almustansiriyah, Iraq
*[1]Biology Department /College of Science for Women/ University of Baghdad, Iraq

(Received 12 April, 2019; accepted 20 June, 2019)

Key words : MTHFR gene, PCOS, Polymorphism

Abstract – In current study, the role of MTHFR gene, especially the rs1801133, rs7533315 and rs2274976 polymorphism, has been determined in polycystic ovarian syndrome. The studyinvolved 100 samples, 50 as polycysticovarian syndrome patients and 50 controls. After DNA extraction,samples undergo to PCR-RFLP analysis to investigate the rs1801133, rs7533315 and 2274976. Rs1801133 showed three genotypes CC,CT,TT. No statically significant differences among the three genotypes between patients and controlswere found. The three genotypesdistribution was agree with Hardy-Weinberg Equilibrium theory, the observed genotype freqaencies had no significant differences than those predicted. Odds ratio referred that C allelecould be consider as protective allelewhile T allele could be consider as susceptible factor for disease. While rs7533315 showed three genotypes with no significant differences among the three genotypes between patients and controls.However, AG genotype was the common in both patients and controls which indicate the likely ofundergo population of study to genetic variation that makes AG the common genotype. In other hand, the odds ratio of both A allele and G allele were close to each other 's given the same susceptibility to association the disease. The further sequencing analysis for some samples revealed the association of rs2066071, rs3546336 and 17037390 with patients. This indicates to the necessary of further investigation for these SNPs with larger sample size. Moreover, somepatients' samples revealed by sequencing analysis new insertions that may lead to frame shift reading. Finally, rs2274976 showed only GG

Stem Cells Cloning. 2016; 9: 1–15.
Published online 2016 Apr 18. doi: 10.2147/SCCAA.S94545
PMCID: PMC4846075
PMID: 27143939

Characterization of neural stemness status through the neurogenesis process for bone marrow mesenchymal stem cells

Maeda H Mohammad,[1] Ahmed M Al-shammari,[1] Ahmad Adnan Al-Juboory,[2] and Nahi Y Yaseen[1]

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Abstract

Go to:

The in vitro isolation, identification, differentiation, and neurogenesis characterization of the sources of mesenchymal stem cells (MSCs) were investigated to produce two types of cells in culture: neural cells and neural stem cells (NSCs). These types of stem cells were used as successful sources for the further treatment of central nervous system defects and injuries. The mouse bone marrow MSCs were used as the source of the stem cells in this study. β-Mercaptoethanol (BME) was used as the main inducer of the neurogenesis pathway to induce neural cells and to identify NSCs. Three types of neural markers were used: nestin as the immaturation stage marker, neurofilament light chain as the early neural marker, and microtubule-associated protein 2 as the maturation marker through different time intervals in the neurogenesis process starting from the MSCs, (as undifferentiated cells), NSCs, production stages, and toward neuron cells (as differentiated cells). The results of different exposure times to BME of the neural markers analysis done by immunocytochemistry and real time-polymerase chain reaction helped us to identify the exact timing for the neural stemness state. The results showed that the best exposure time that may be used for the production of NSCs was 6 hours. The best maintenance media for NSCs were also identified. Furthermore, we optimized exposure to BME with different times and concentrations, which

Stem Cell Discovery, 2016, 6, 1-12
Published Online January 2016 in SciRes. http://www.scirp.org/journal/scd
http://dx.doi.org/10.4236/scd.2016.61001

Scientific Research Publishing

Production of Neural Progenitors from Bone Marrow Mesenchymal Stem Cells

Maeda Mohammad[1*], Nahi Yaseen[1], Ahmad Al-Joubory[2], Rafal Abdullah[1], Noah Mahmood[1], Aesar A. Ahmed[1], Ahmed Al-Shammari[1*]

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Received 1 August 2015; accepted 25 December 2015; published 29 December 2015

Abstract

In the brain, there are hundreds of types of specialized neurons and to generate one type of them we need to have neural progenitors for differentiation to specific neuron type. Mesenchymal stem cells (MSCs) are easily isolated, cultured, manipulated ex vivo, showing great potential for therapeutic applications. The adult MSCs have the potential to produce progeny that differentiate into a

Veterinary Medicine and Science
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ORIGINAL ARTICLE | Open Access

Clinical, molecular and cytopathological characterization of a Newcastle disease virus from an outbreak in Baghdad, Iraq

Ahmed M. Al-Shammari, Mohammed A. Hamad, Murtadha A. AL-Mudhafar, Khansaa Raad, Aeser Ahmed

First published:31 March 2020 | https://doi.org/10.1002/vms3.262

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.262

Abstract

Background

The frequent outbreaks of Newcastle disease virus (NDV) in Iraq pose a constant threat to commercial poultry, despite the introduction of routine vaccination programmes. Several factors, particularly stress factors and coinfections, might play a role in increasing NDV outbreaks in poultry species.

Objectives

The current study was aimed to characterize an NDV isolate from an outbreak in North Baghdad, Iraq.

Methods

Clinical pathogenicity of the isolate was determined experimentally in chickens. In vitro studies included cytopathological examination, as well as molecular and phylogenetic

Front Mol Biosci. 2019; 6: 90.
Published online 2019 Sep 27. doi: 10.3389/fmolb.2019.00090
PMCID: PMC6777003
PMID: 31612140

2-Deoxyglucose and Newcastle Disease Virus Synergize to Kill Breast Cancer Cells by Inhibition of Glycolysis Pathway Through Glyceraldehyde3-Phosphate Downregulation

Ahmed Majeed Al-Shammari,[1,*†] Amer Hasan Abdullah,[1] Zainab Majid Allami,[2] and Nahi Y. Yaseen[1]

▸ Author information ▸ Article notes ▸ Copyright and License information Disclaimer

Abstract

Go to:

Targeting cancer cells metabolism is promising strategy in inhibiting cancer cells progression that are known to exhibit increased aerobic glycolysis. We used the glucose analog 2-Deoxyglucose (2-DG) as a competitor molecule of glucose. To further enhance the effectiveness of 2-DG, the Newcastle disease virus (NDV) was used as a combination virotherapy to enhance the anti-tumor effect. Human and mouse-breast cancer cells were treated by NDV and/or 2-DG. The effect was analyzed by study cell viability, apoptosis and level of glyceraldehyde3-phosphate (GAPDH) by ELISA and QPCR assays. Synergistic cytotoxicity was found after a 72-h treatment of human- and mouse-breast cancer cells with 2-DG in combination with NDV at different concentrations. The synergistic cytotoxicity was accompanied by apoptotic cell death and GAPDH downregulation and inhibition to glycolysis product pyruvate. The combination treatment showed significant tumor growth inhibition compared to single treatments *in vivo*. Our results suggest the effectiveness of a novel strategy for anti-breast cancer therapy through glycolysis inhibition and GAPDH downregulation.

Keywords: glycolysis inhibition, virotherapy, cancer metabolism, breast cancer model, Warburg effect

Detection of Infectious Laryngotracheitis by real-time PCR in Baghdad

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Abstract

The present study is concerned with developing detection method for avian infectious laryngotracheitis (ILT) virus in Iraq using real-time PCR assay. Total of 64 clinically suspected birds believed to be infected with ILTV , were chosen from 6 diverse commercial poultry farms in several areas of North Baghdad. The infected birds were examined and the tissue samples collected from the lungs, spleens, tracheas of each farm. The positive collected samples were used for DNA extraction using High Pure Viral Nucleic Acid Kit (Patho Gene-spin DNA/RNA Extraction Kit) according to the manufacturer's instructions. The extracted DNA was immediately assayed via RT-PCR.

Tissues were presented to a real-time PCR assay for detection of ILTV. The test is based on a fast and sensitive real-time PCR method with the use of TaqMan

creatures or potentially diminished weight gain and egg production.[11] Sporadic instances of ILT happen in all classes of winged creatures including pastime/appear/amusement chickens, ovens, overwhelming reproducers and business laying hens. Rapid detection methods such as Realtime PCR assays are required in the field for fast pathogen detections.[2] So, we aimed to create this assay to help in accurate and rapid detection for the viral diseases.

Material and Methods

Collection of samples: A total number of 64 clinically suspected birds believed to be infected with ILTV, were chosen from 6 different commercial poultry farms in various areas of North Baghdad, brought to "Uruk Lab for the molecular and serological test" by the owner, in Middle of Baghdad. The infected birds were examined and the tissue samples collected from the lungs, spleens, tracheas of each farm were removed and transferred into sterile tubes separately. The samples were kept at -20°C for later analysis.

Hope it will be easy for you

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Thank you for attending this workshop