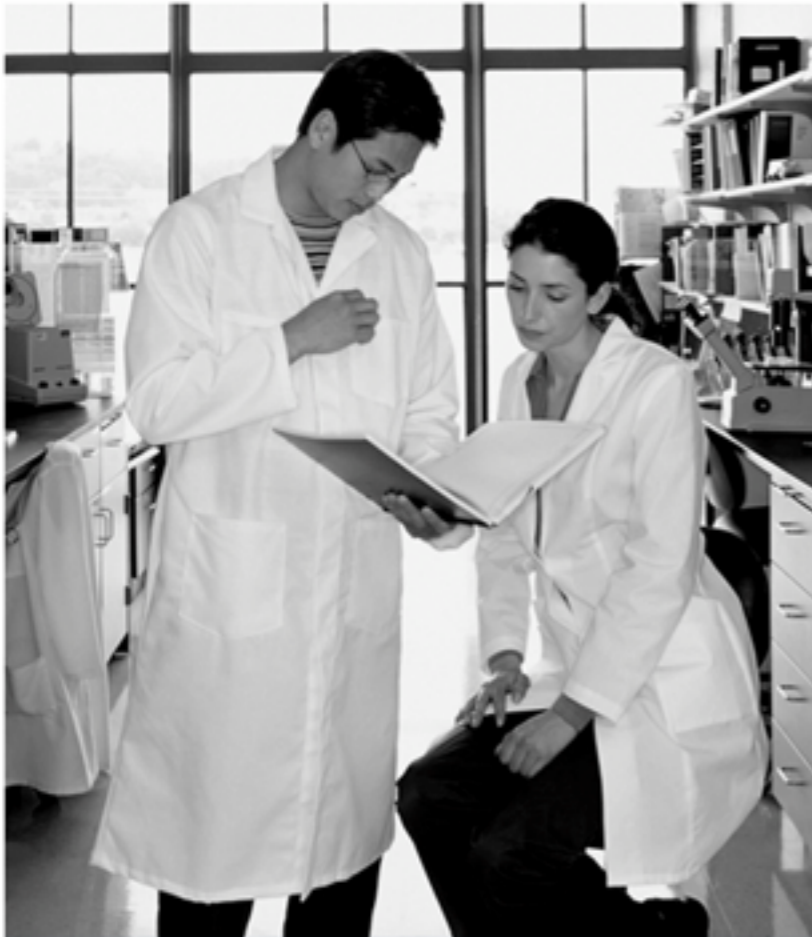


## SUMMER INSTITUTE - ONLINE MODALITY CALENDAR 2022

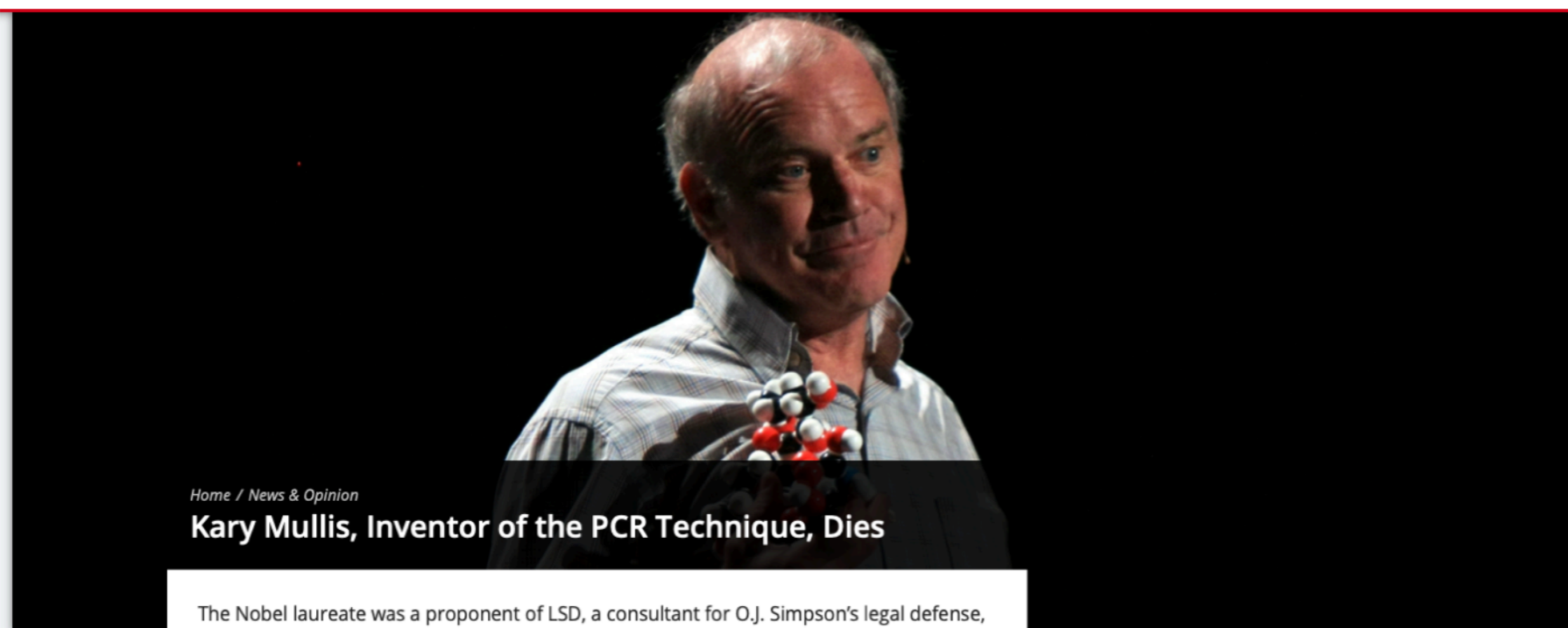
SUN	MON	TUE	WED	THU	FRI	SAT
June 26	27	28	29	30	31	July 02
	9:00-10:00am Virtual Program Orientation for Summer Institute Online Modality					
July 03	04	05	06	07	08	09
	Holiday (Independence Day)	8:30-10:00am -Welcome Reception and Buddy Meet & Greet Event	Free Day	Classes begin! 8:30-11am: BIOL4905 INTRODUCTION 8-10:20pm: Afternoon course	8:30-11am: BIOL4905 DNA PREPARATION 8-10:20pm: Afternoon course	
10	11	12	13	14	15	16
	8:30-11am:BIOL4905 PROTEOMICS I 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 PROTEOMICS II 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 PROTEOMICS III 8-10:20pm: Afternoon course	8:30-11am: BIOL4905 RNA PREPARATION 8-10:20pm: Afternoon course	Virtual Independence Day Activity	
17	18	19	20	21	22	23
	8:30-11am:BIOL4905 qPCR / ROBOTS 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 DNA Sequence Analysis 8-10:20pm: Afternoon course	Midterm Break	8:30-11am:BIOL4905 Next Gen. Sequencing 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Automated Microscopy /AFM	
24	25	26	27	28	29	30
	8:30-11am:BIOL4905 Microarray I 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Microarray II 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Nanostring 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Flow Cytometry 8-10:20pm: Afternoon course	FINALS	
31	August 01	02	03			
	9:00-10:00am: Closing Reception		Grades available in PAWS			

Legend:  
Orange: Courses    Blue: Activities



# Fundamentals of Real-Time RT-PCR

adapted from a PPT presentation  
by  
David Chappell, PhD  
ABI Field applications Specialist



Home / News & Opinion

## Kary Mullis, Inventor of the PCR Technique, Dies

The Nobel laureate was a proponent of LSD, a consultant for O.J. Simpson's legal defense, and the creator of a company that infused jewelry with celebrities' DNA.



**Kerry Grens**  
Aug 11, 2019



**K**ary Mullis, whose invention of the polymerase chain reaction technique earned him the Nobel Prize in Chemistry in 1993, died of pneumonia on August 7, according to [MyNewsLA.com](#). He was 74 years old.

ABOVE: FLICKR, ERIK CHARLTON

According to a 1998 profile in *The Washington Post*, Mullis was known as a "weird" figure in science and "flamboyant" philanderer who evangelized the use of LSD, denied the evidence for both global warming and HIV as a cause of AIDS, consulted for O.J. Simpson's legal defense, and formed a company that sold jewelry embedded with celebrities' DNA. The opening paragraph of his [Nobel autobiography](#) includes a scene depicting a visit from Mullis's dying grandfather in "non-substantial form."

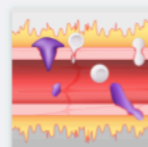
"He was personally and professionally one of the more iconic personalities science has ever witnessed," Rich Robbins, the founder and CEO of Wareham Development, a real estate developer for a number of biotech companies, tells the Emeryville, California-based paper, the *E'ville Eye*.

See "[PCR: Past, Present, & Future](#)"

Mullis was born in North Carolina in 1944 and earned a chemistry degree from Georgia Tech and a PhD in biochemistry from the University of California, Berkeley. In the early 1980s, when Mullis



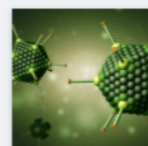
### Trending



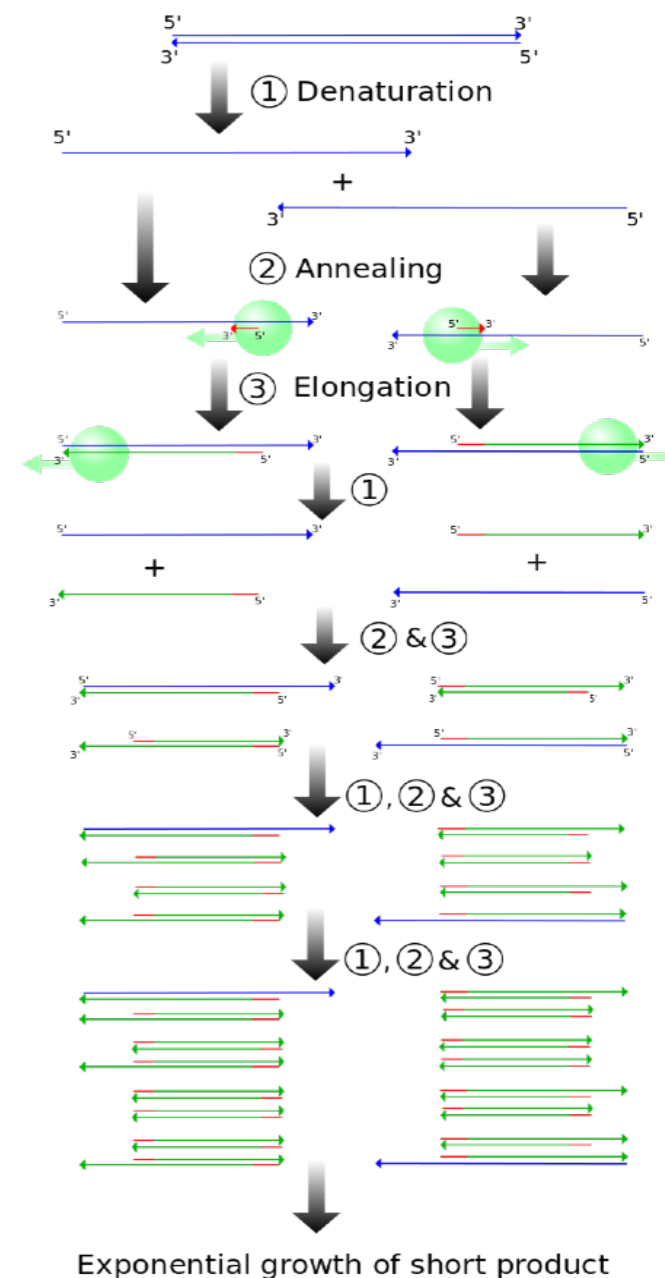
Is a Bradykinin Storm Brewing in COVID-19?



COVID-19 Vaccine Trial Pauses After Adverse Reaction



Scientists Voice Concerns over Russian COVID-19 Vaccine Study



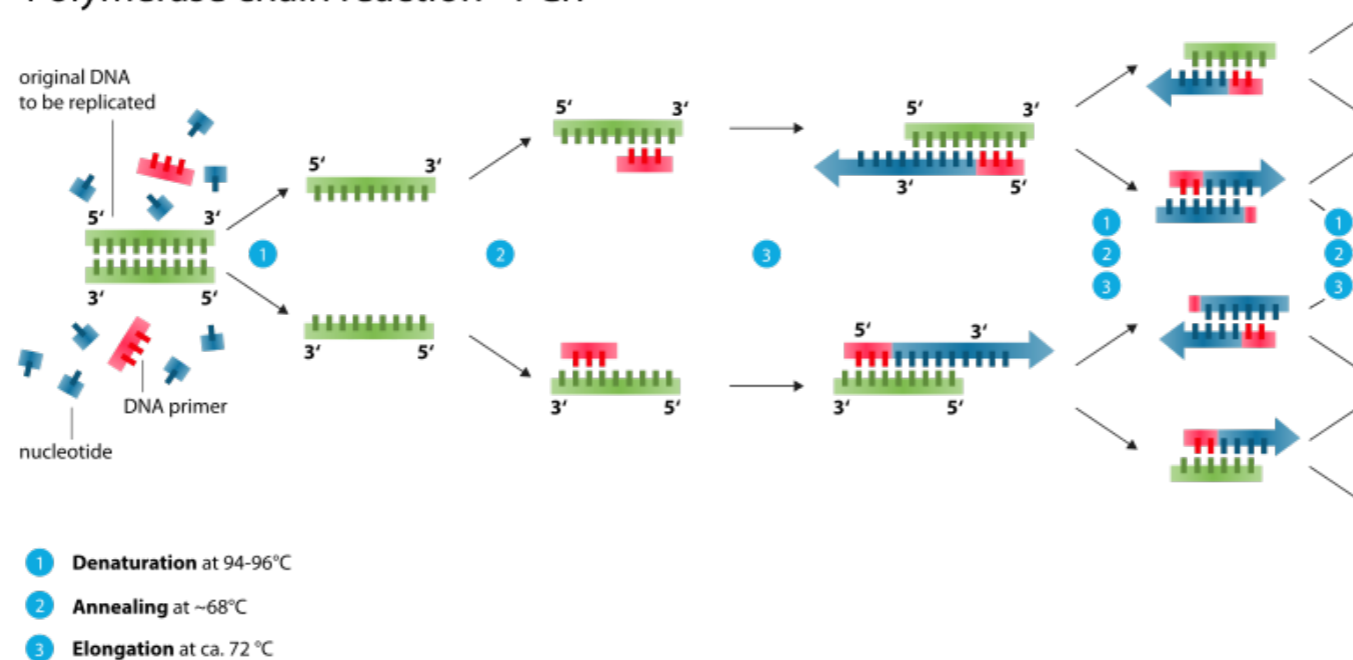
Exponential growth of short product

# PCR and other inventions

Main articles: [PCR](#) and [DNA polymerase](#)

In 1983, Mullis was working for [Cetus Corporation](#) as a chemist. Late one night while driving with his girlfriend, who was also a chemist at Cetus, he had the idea to use a pair of primers to bracket the desired DNA sequence and to copy it using DNA polymerase; a technique that would allow rapid amplification of a small stretch of DNA and become a standard procedure in molecular biology laboratories.<sup>[10]</sup> Cetus took Mullis off his usual projects to concentrate on PCR full-time. Mullis succeeded in demonstrating PCR December 16, 1983.<sup>[10]</sup> He received a \$10,000 bonus from Cetus for the invention.

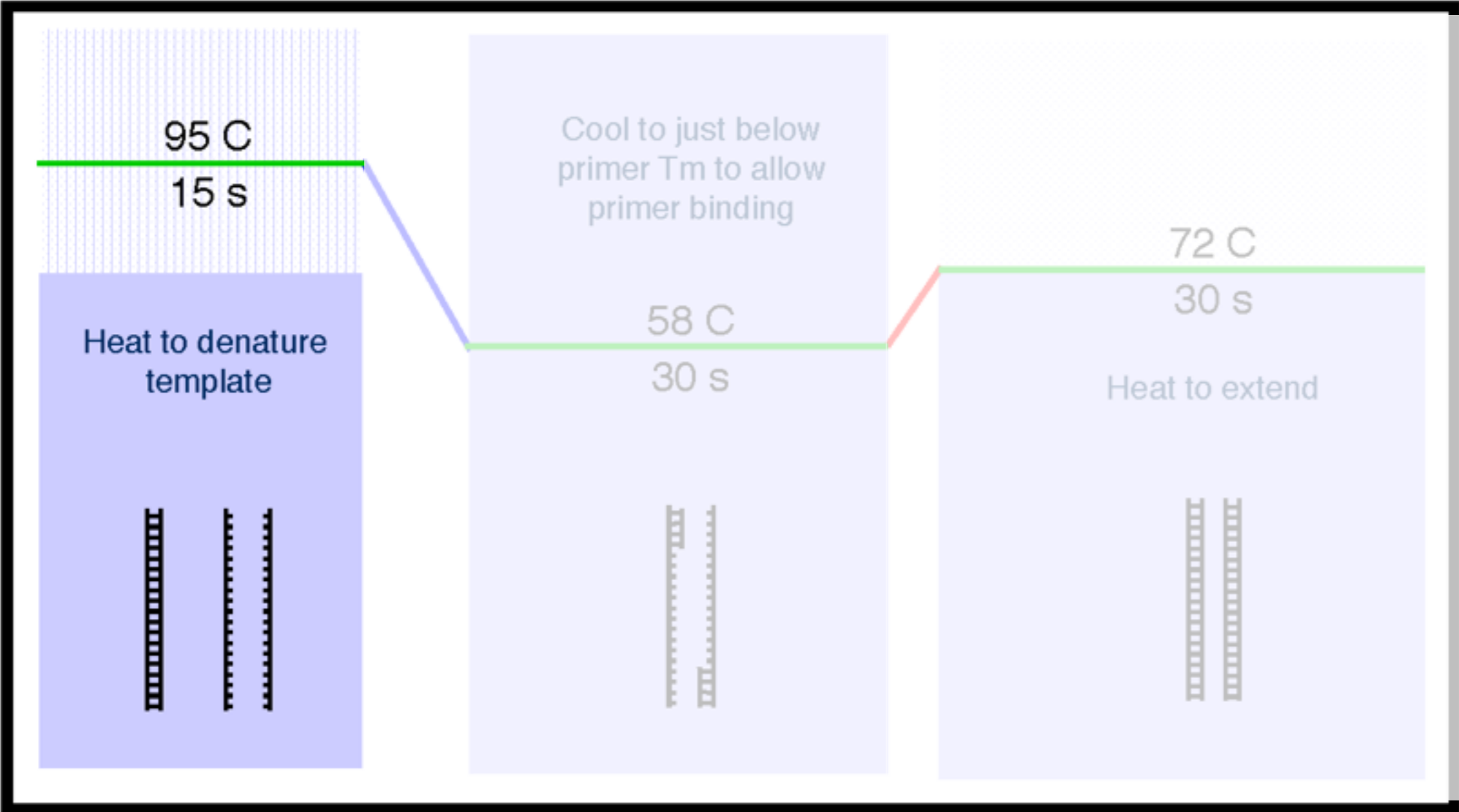
## Polymerase chain reaction - PCR



A drawback of the technique was that the DNA polymerase in the reaction was destroyed by the high heat used at the start of each replication cycle and had to be replaced. In 1986, Saiki started to use *Thermophilus aquaticus* (Taq) DNA polymerase to amplify segments of DNA. The Taq polymerase was heat resistant and only need to be added to the reaction once, making the technique dramatically more affordable and subject to automation. This modification of Mullis' invention revolutionized [biochemistry](#), [molecular biology](#), [genetics](#), [medicine](#), and [forensics](#).



ycles



# Traditional PCR



## 1990

Microcycler: Eppendorf introduces its first thermal cycler using water to heat and cool.



## 1993

Mastercycler 5330: Eppendorf introduces the first Mastercycler based on peltier technology.



## 2005

Mastercycler ep realplex: Extremely fast optics for rapid data acquisition.



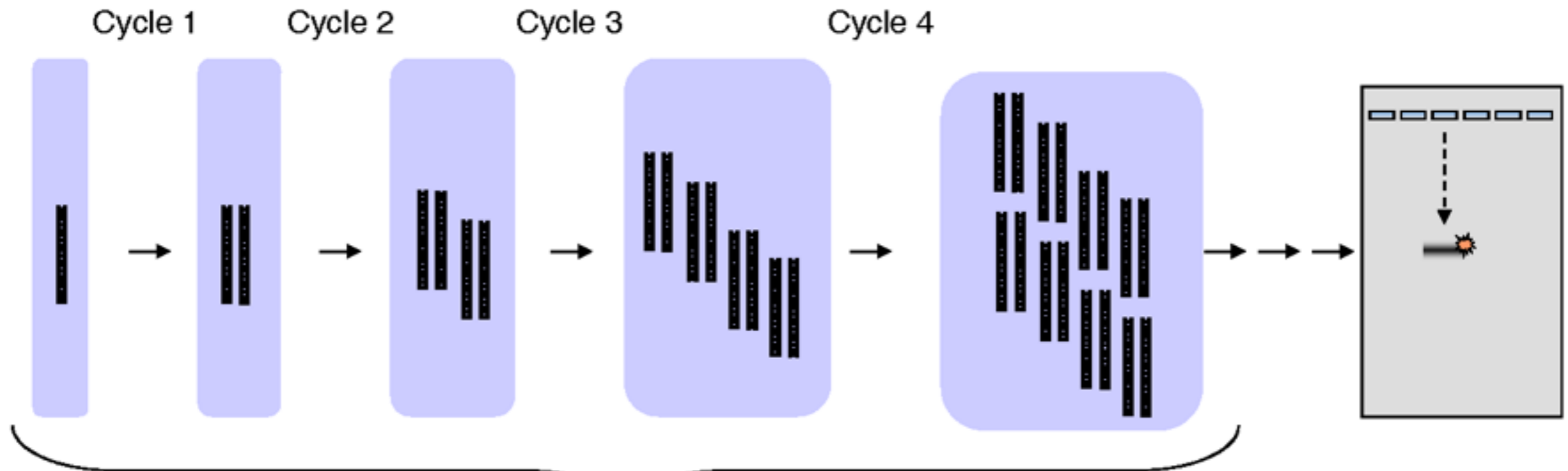
## 2008

Mastercycler pro: New vapo.protect™ technology reduces evaporation.

Temperature control range of the block	4–99 °C		
Temperature control mode	Fast, Standard, Safe		
Heating technology of the block	Peltier elements, Triple Circuit Technology		
Gradient block	over 12 columns	over 24 columns	over 24 columns
Gradient range	1–20 °C	1–24 °C	1–20 °C
Gradient temperature range	30–99 °C		
Lid temperature range	37–110 °C		
Lid descent and closing pressure	vapo.protect™ technology with Thermal Sample Protection		
Block homogeneity: 20 °C–72 °C	≤ ±0.3 °C		
95 °C	≤ ±0.4 °C		
Block temperature accuracy	± 0.2 °C		
Heating rate*	ca. 4 °C/s	ca. 6 °C/s	ca. 4 °C/s
Cooling rate*	ca. 3 °C/s	ca. 4,5 °C/s	ca. 3 °C/s
Interfaces	Centronics, USB, CAN in, CAN out		
Dimensions (W x D x H)	26 x 41.5 x 37 cm		
Weight	18.5 kg (40.8 lbs)		
Power supply	230 V, 50–60 Hz		
Max. power consumption	950 W		
Sound power levels	≤ 56 dB(A)		
<small>* Heating and cooling rates measured at block  **Unit can only be operated via a Mastercycler nexus unit (including flat, X1 versions) with control and display panel  Product appearance and/or specifications are subject to change without notice.</small>			

# Traditional PCR

– examine products at the **end** of the reaction



## Mastercycler Gradient Pro -Thermal Cycler

- Major reduction of evaporation in tubes
- Extremely fast heating and cooling rates
- Gradient blocks with SteadySlope technology
- Intuitive graphic programming
- Display to indicate cycler number in a network
- Optional self-test of peltier elements

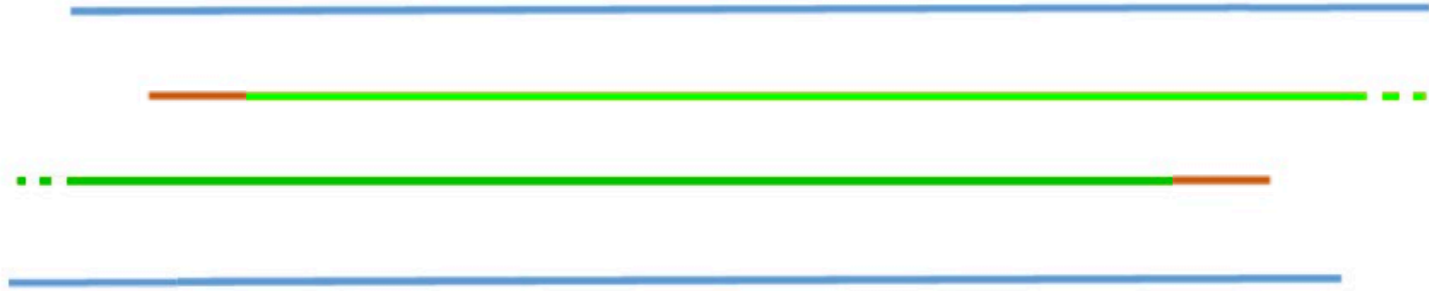


Denature

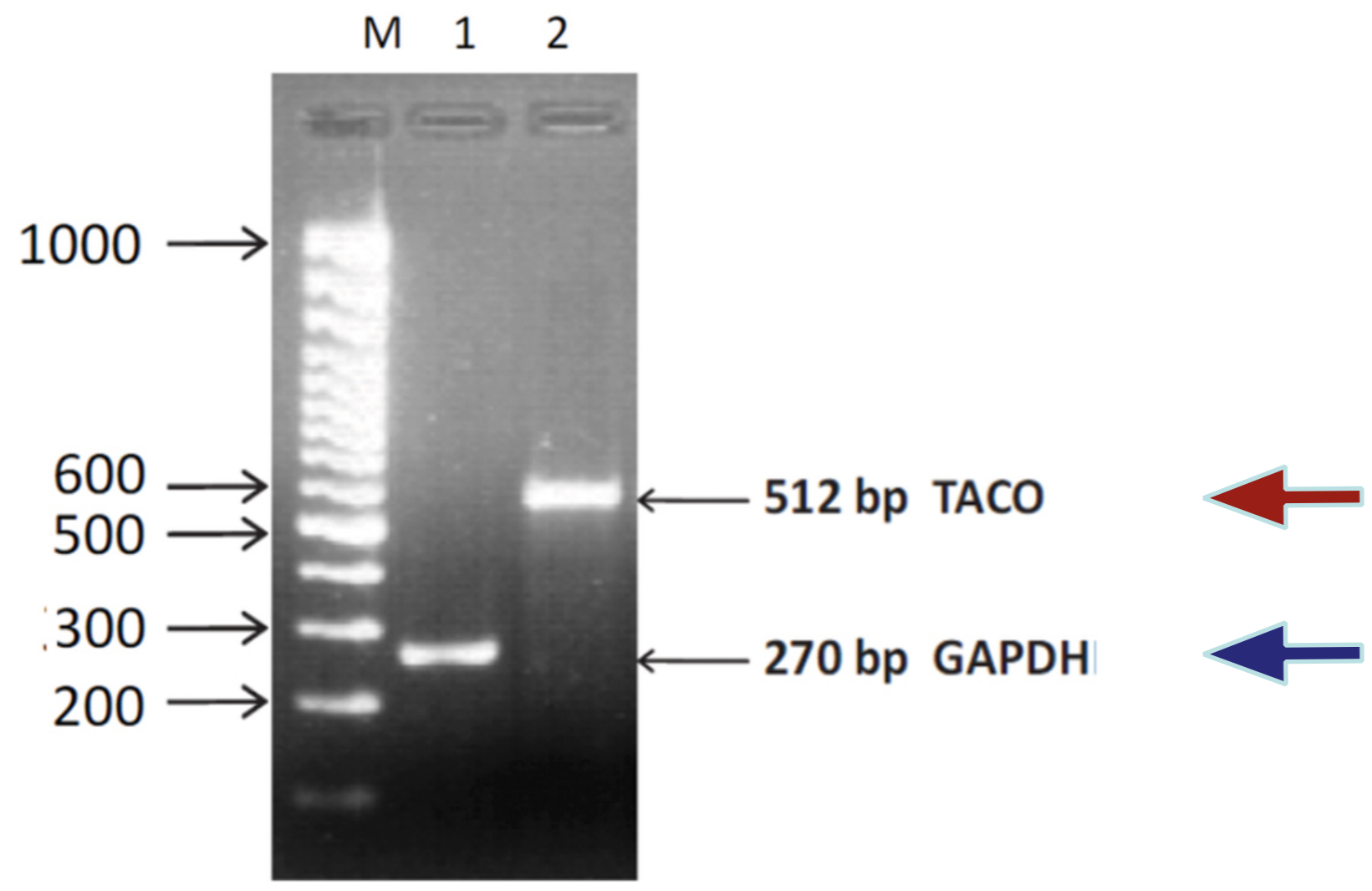
95 °C

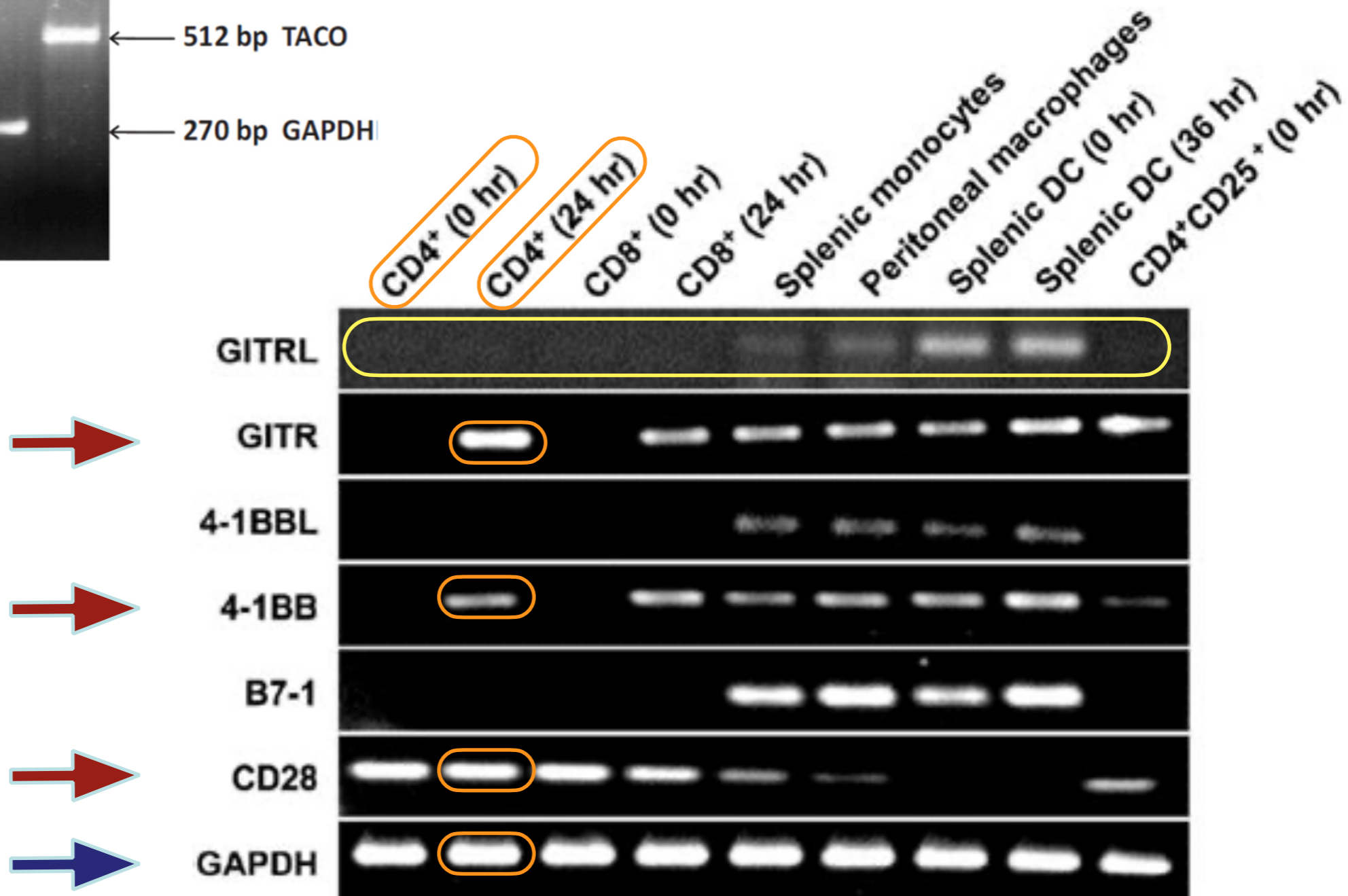
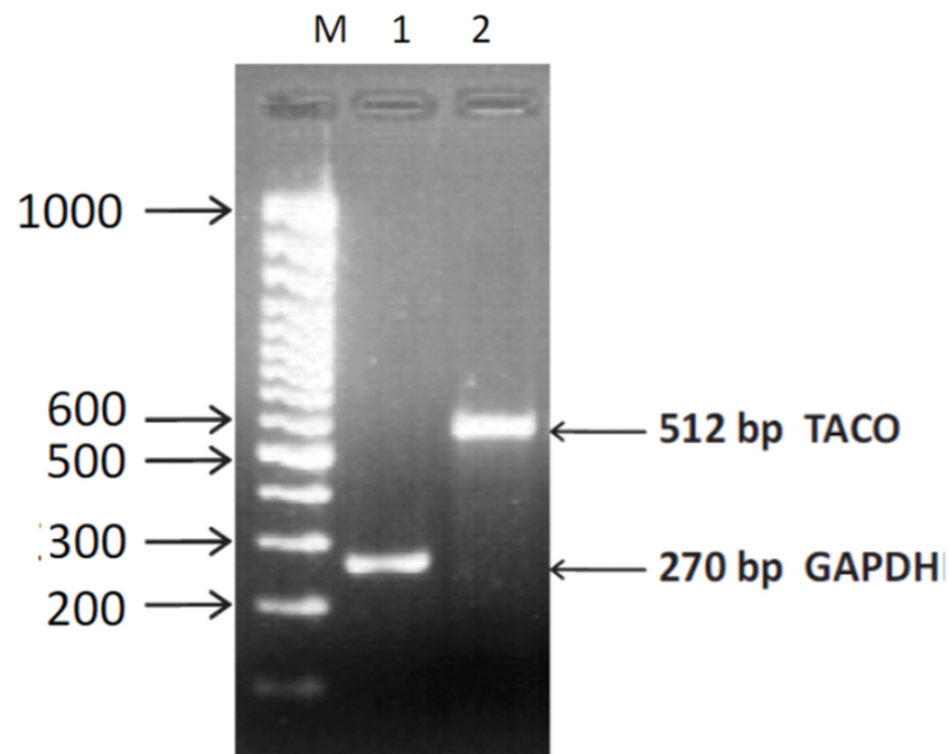






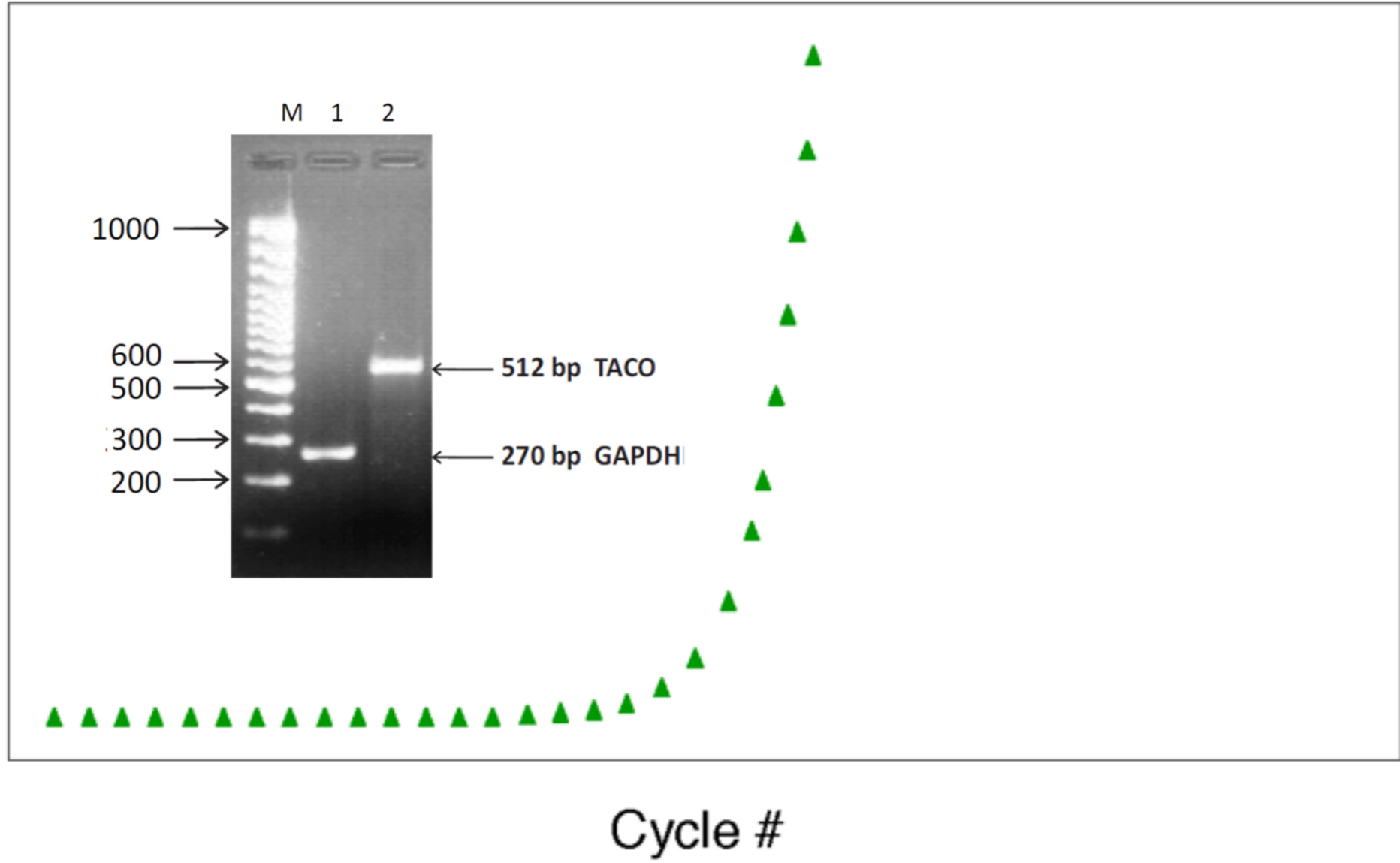






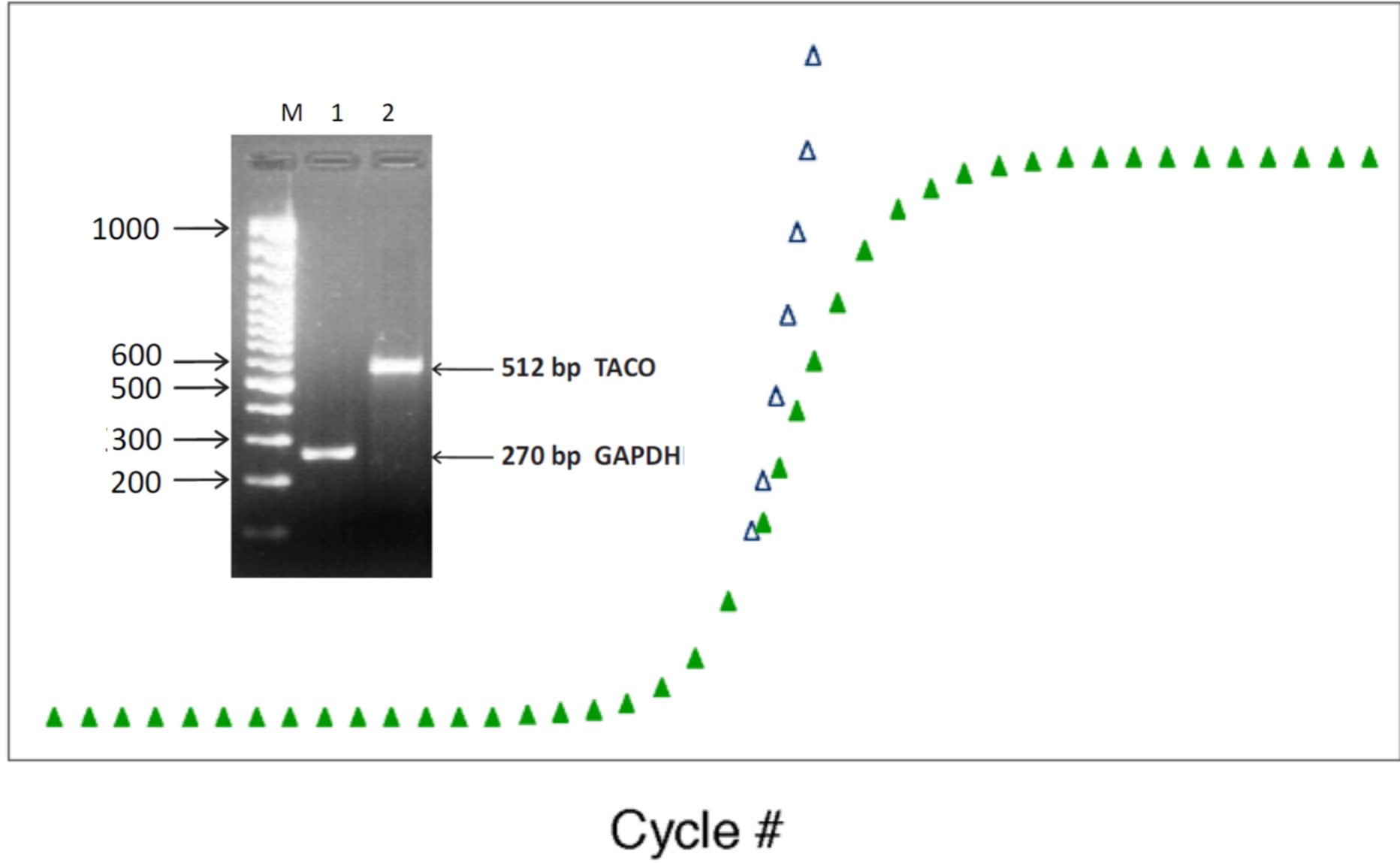


Fluorescence  
(Copy #)





Fluorescence  
(Copy #)



# Real-time PCR or qPCR

**SYBR<sup>®</sup> Green**

**TaqMan<sup>®</sup>**

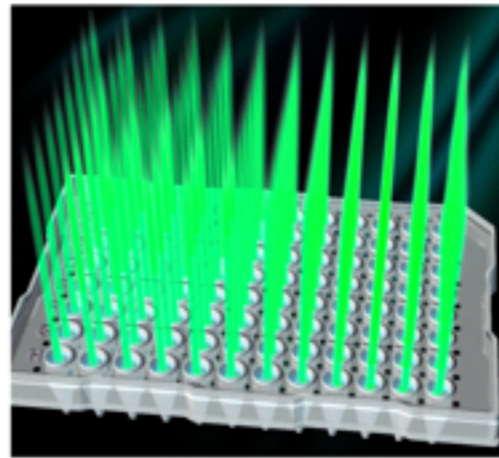
**MGB**

**ROX<sup>™</sup>**

**Multicomponenting**



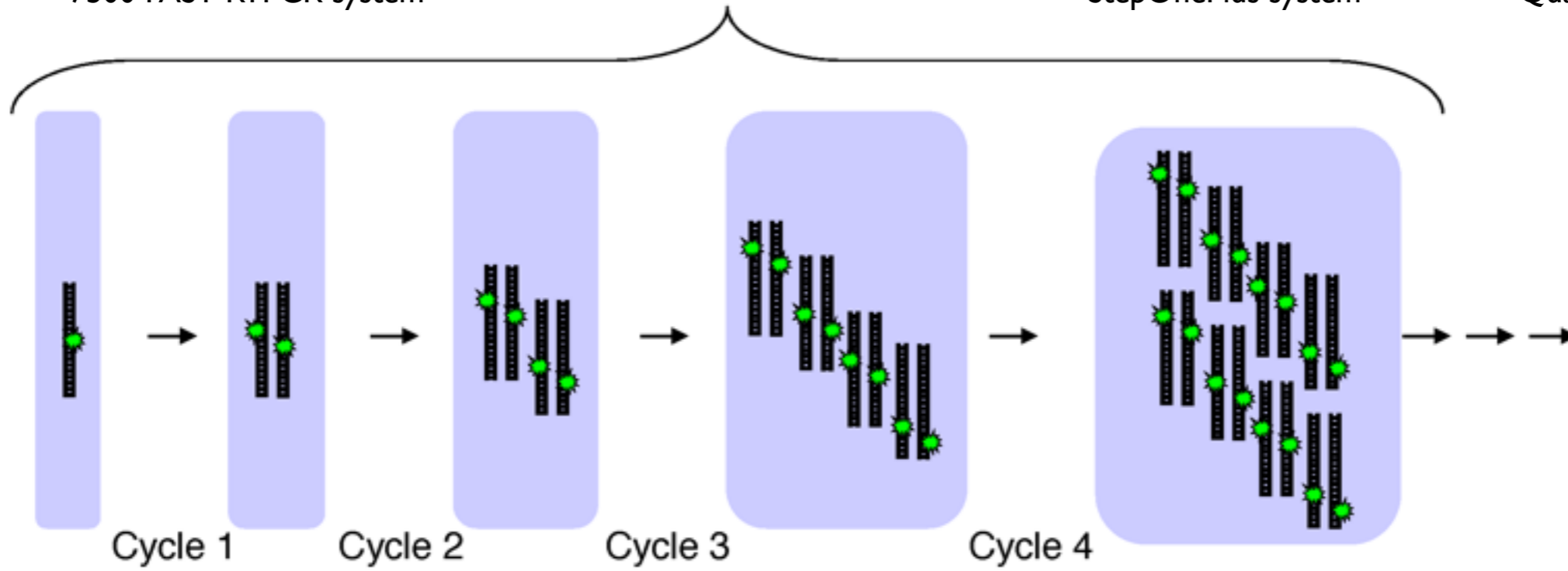
7500 FAST RTPCR system



StepOnePlus system



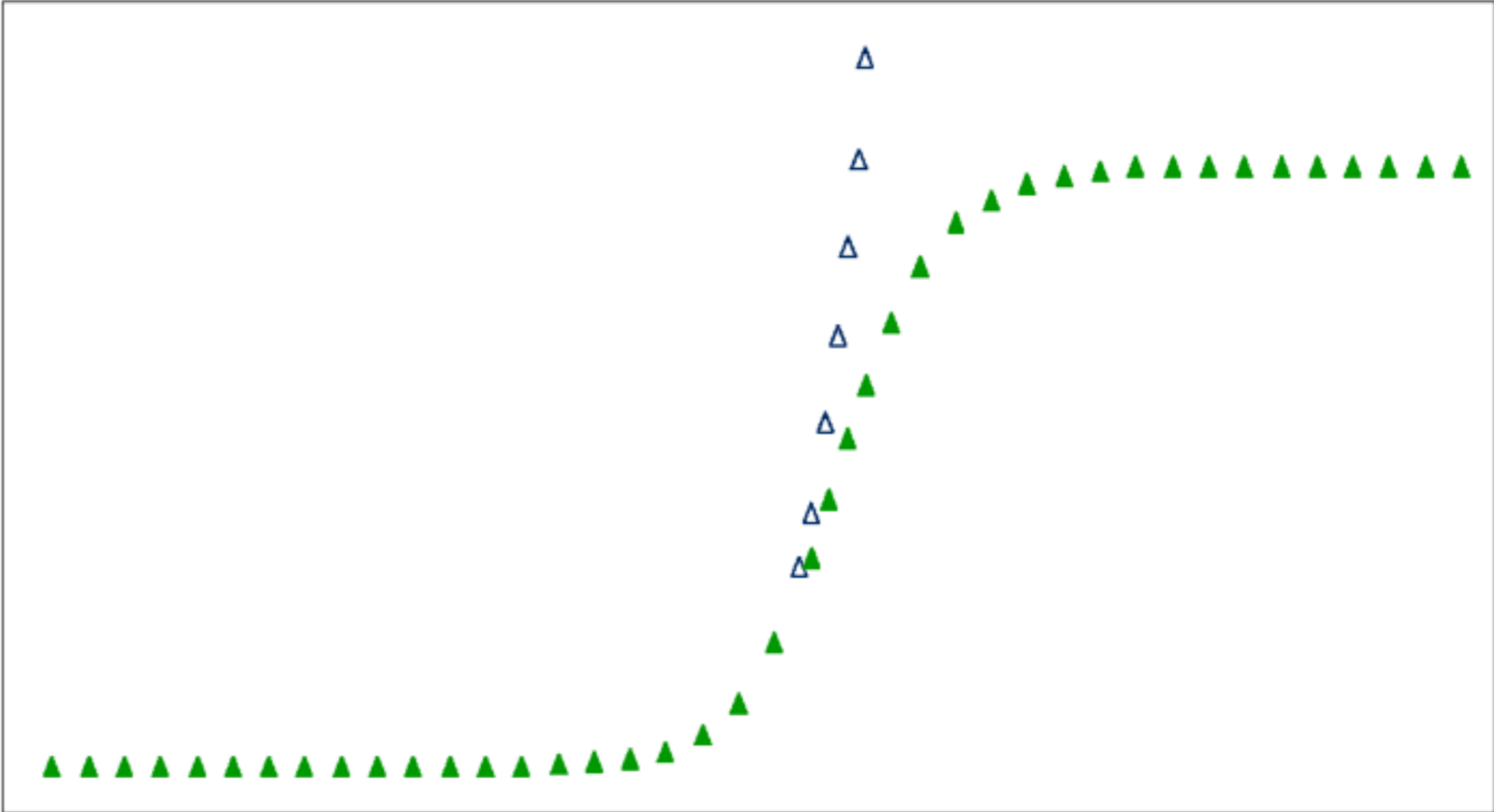
QuantStudio 3 system



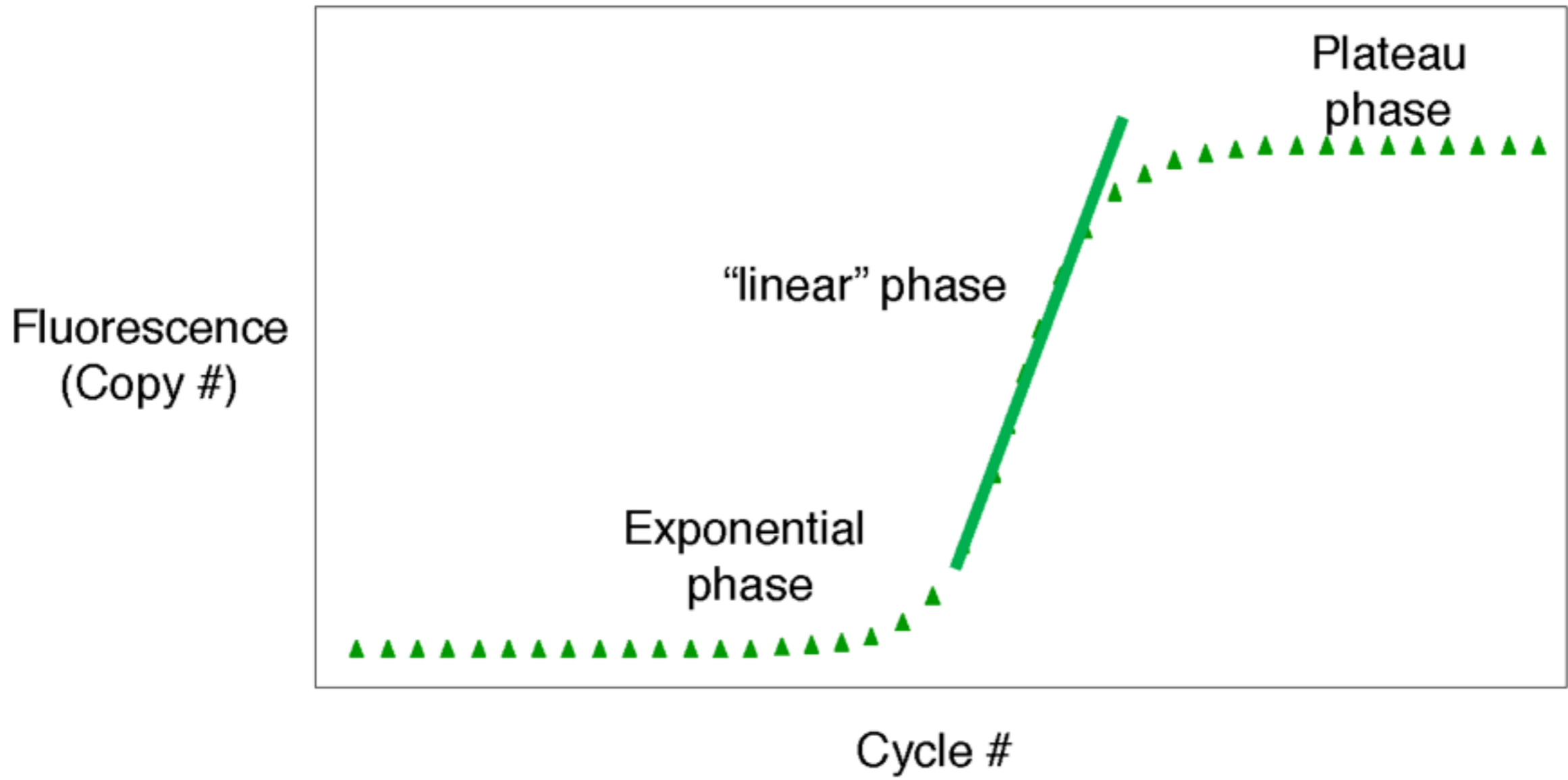


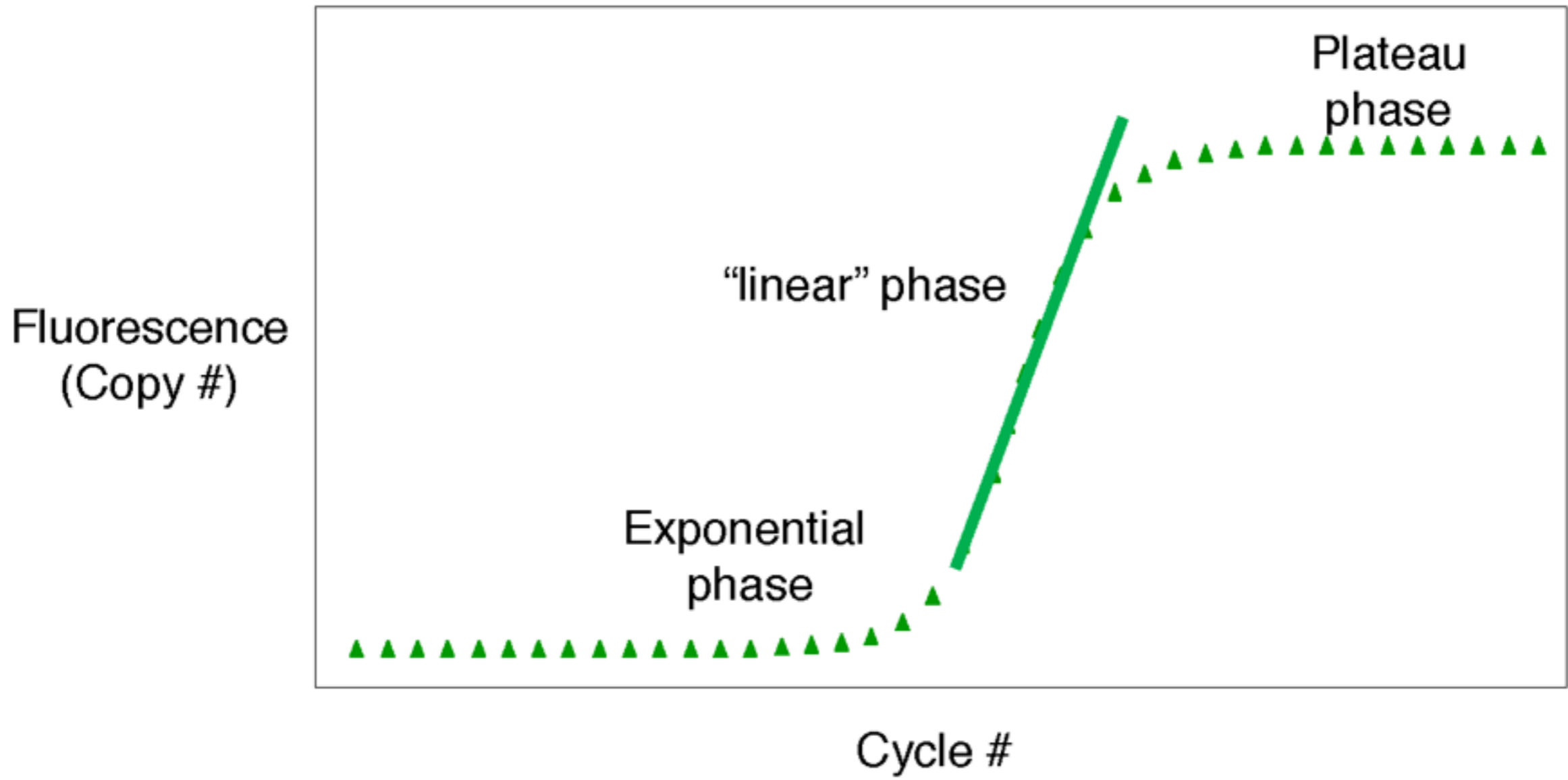


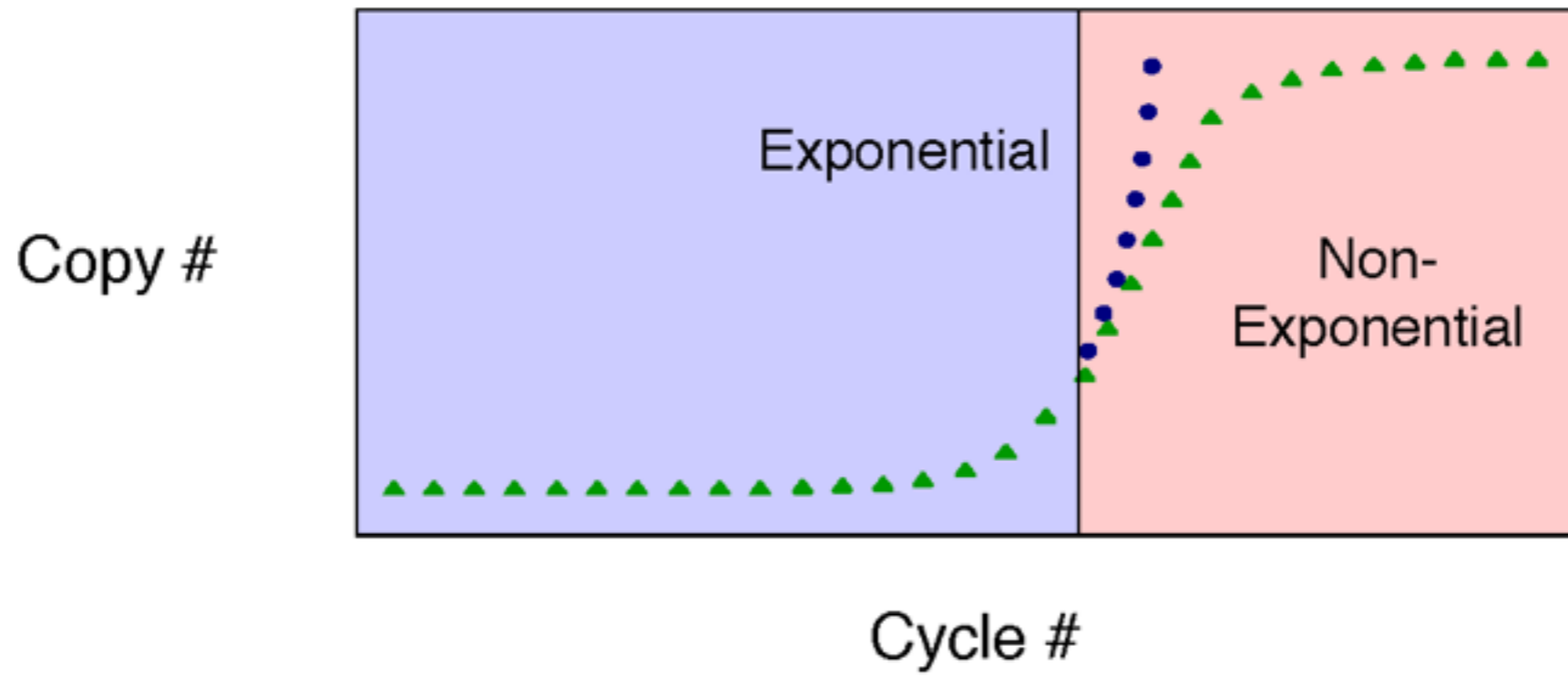
Fluorescence  
(Copy #)



Cycle #

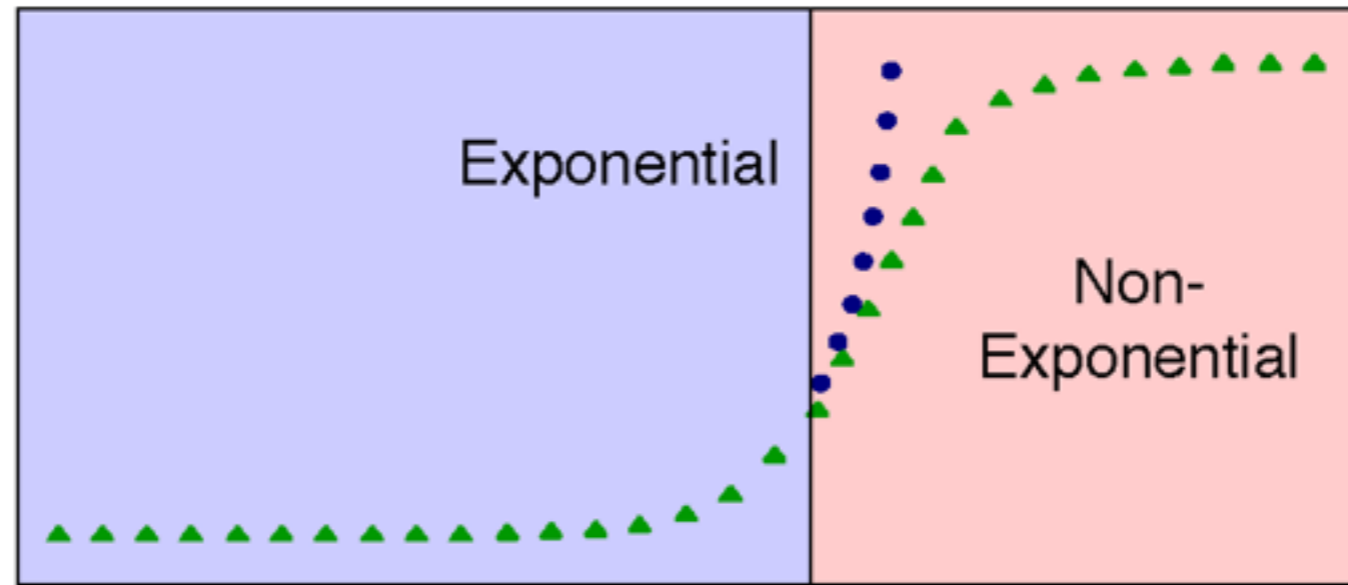




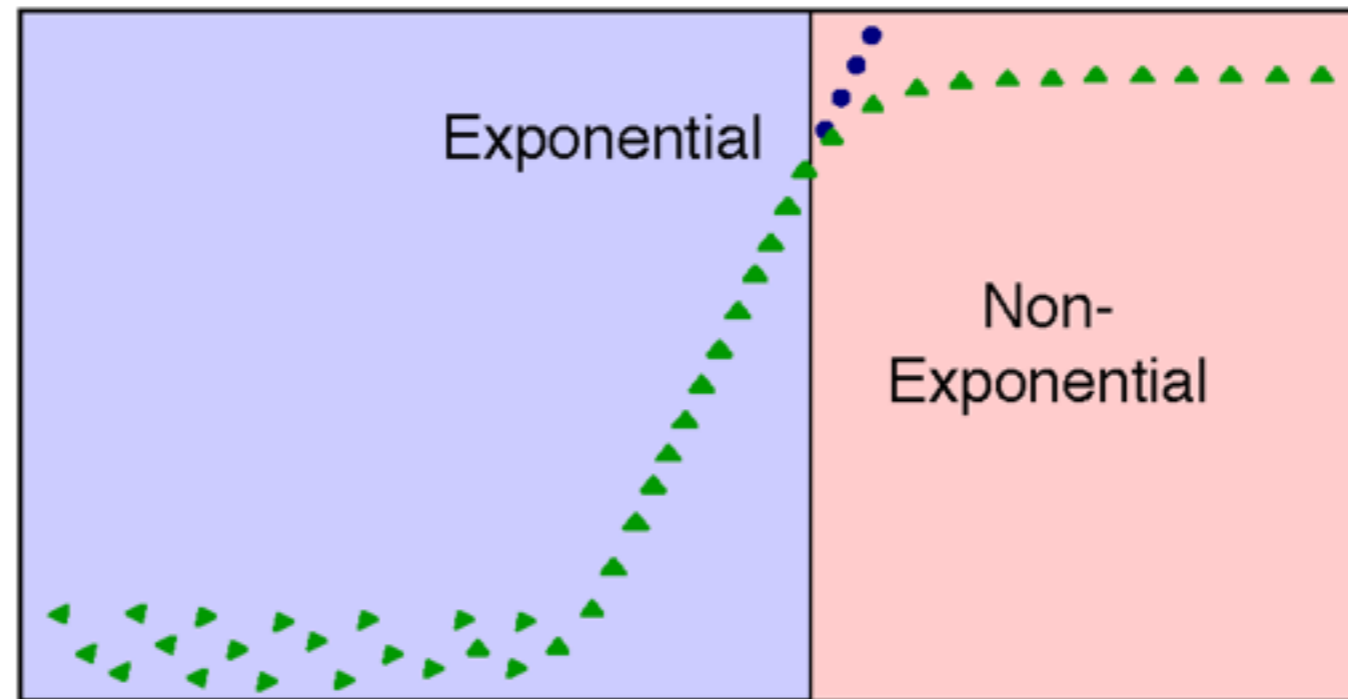




Copy #

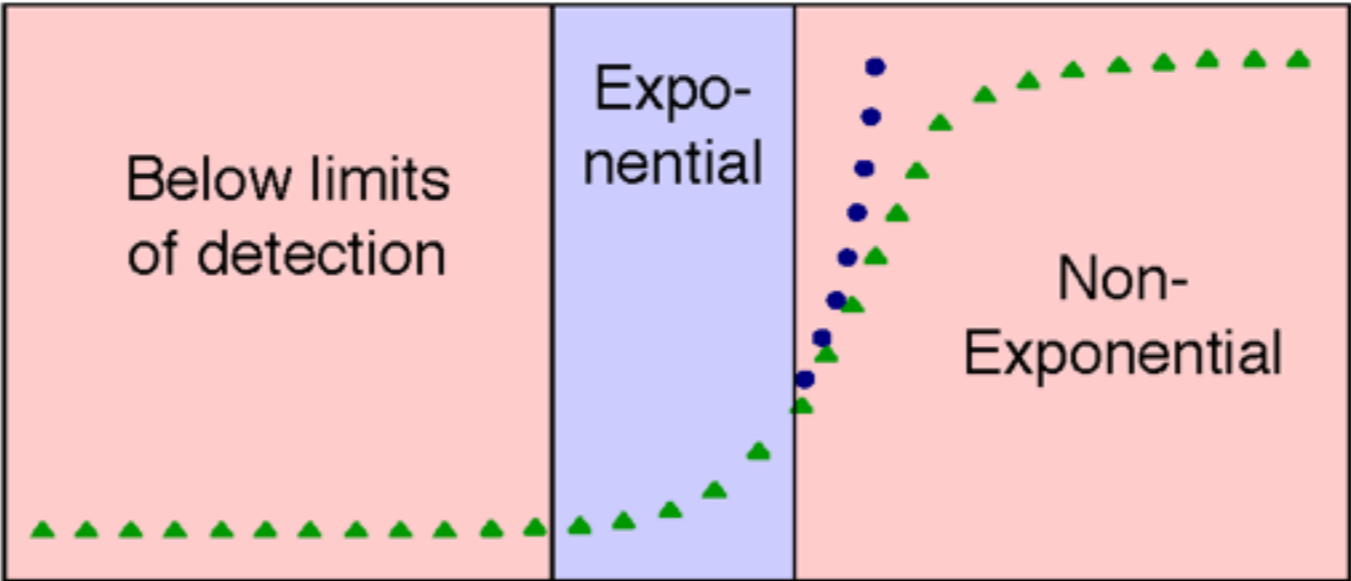


Log  
Copy #

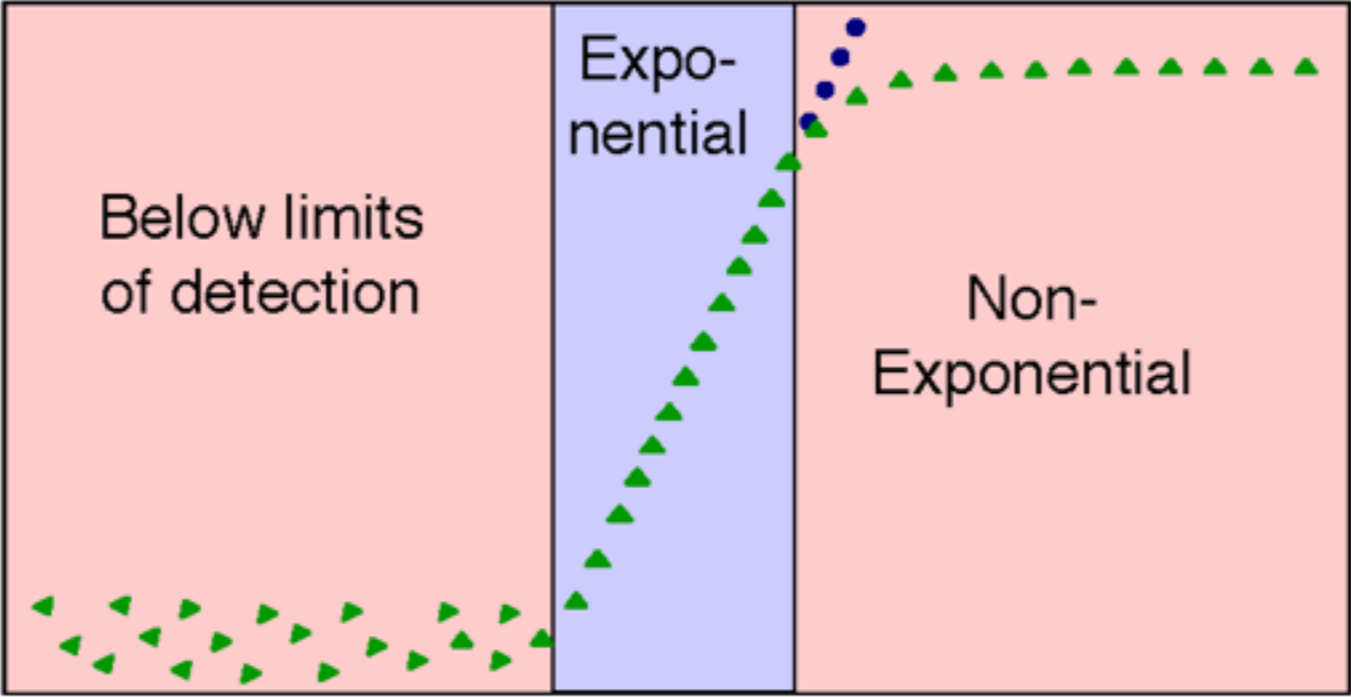


Cycle #

Copy #

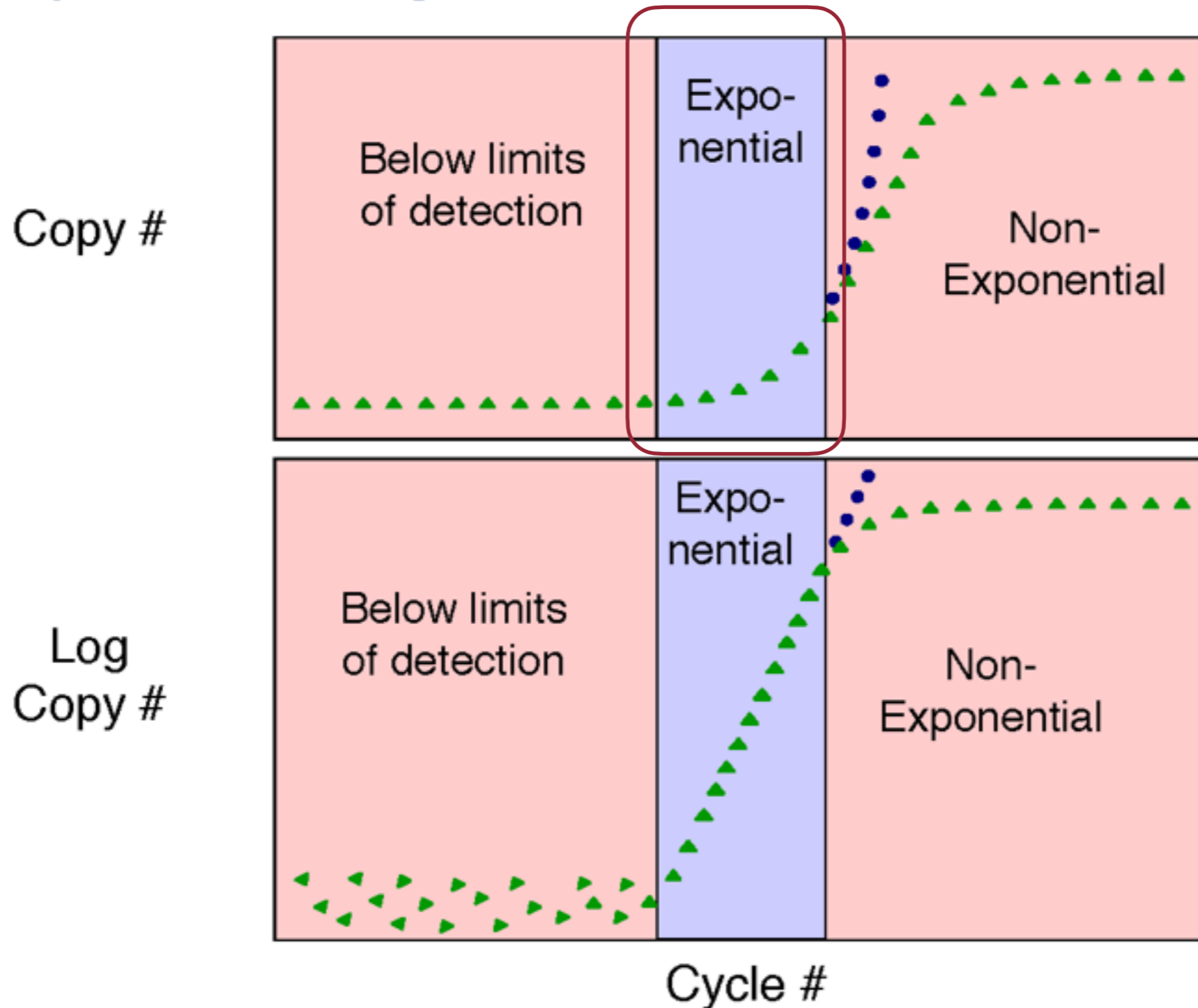


Log Copy #

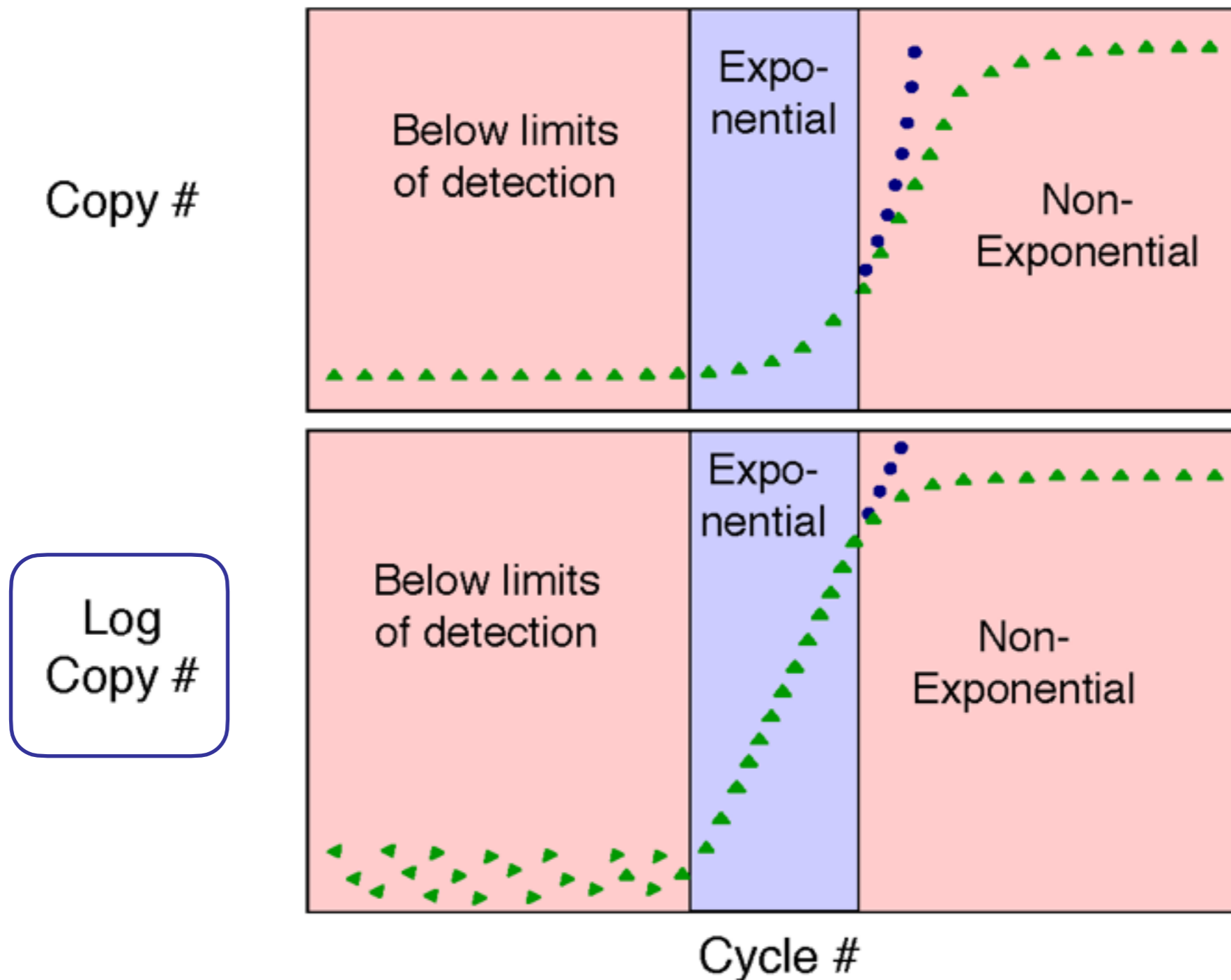


Cycle #

# The exponential region is easier to define in log phase

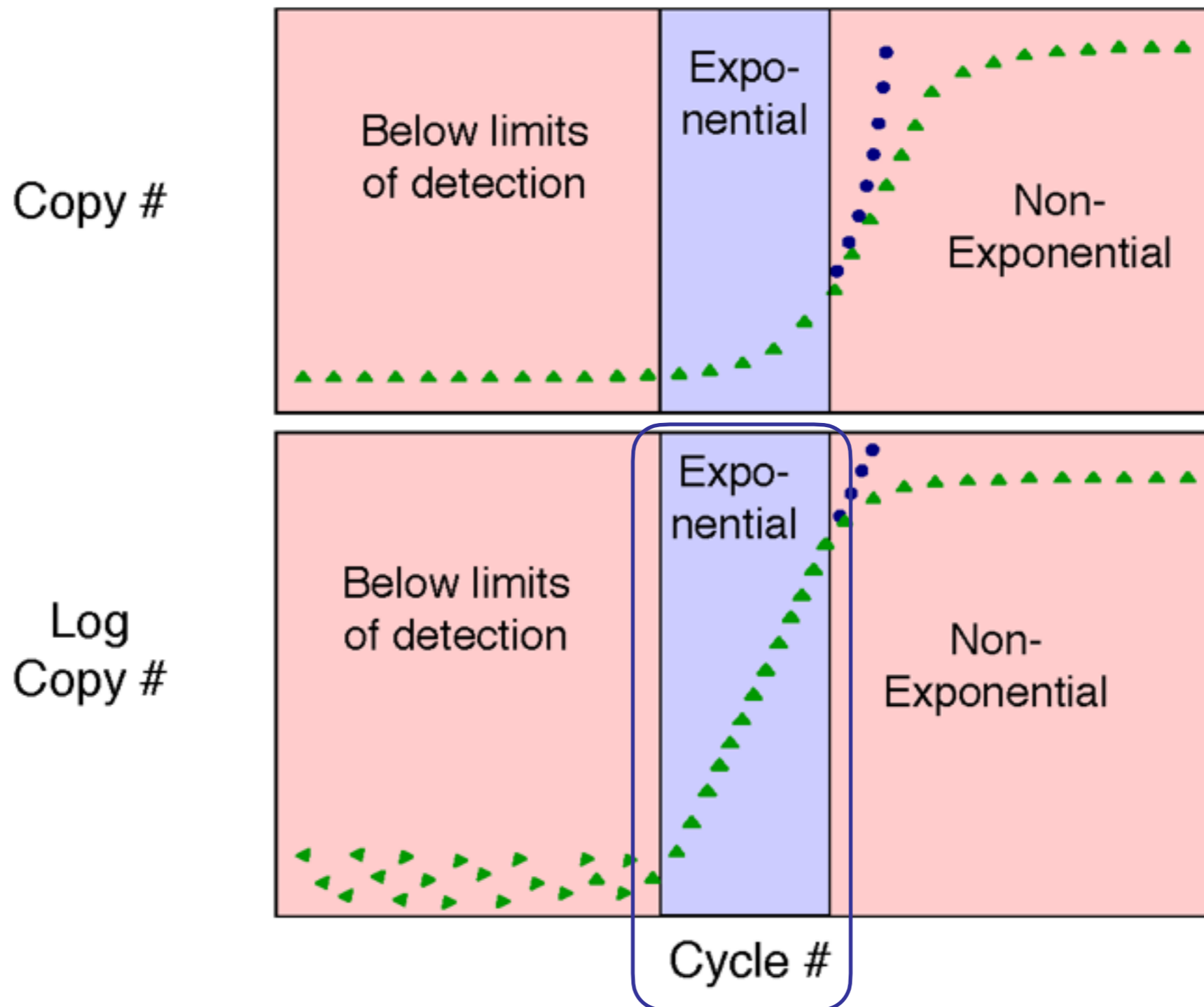


# The exponential region is easier to define in log phase

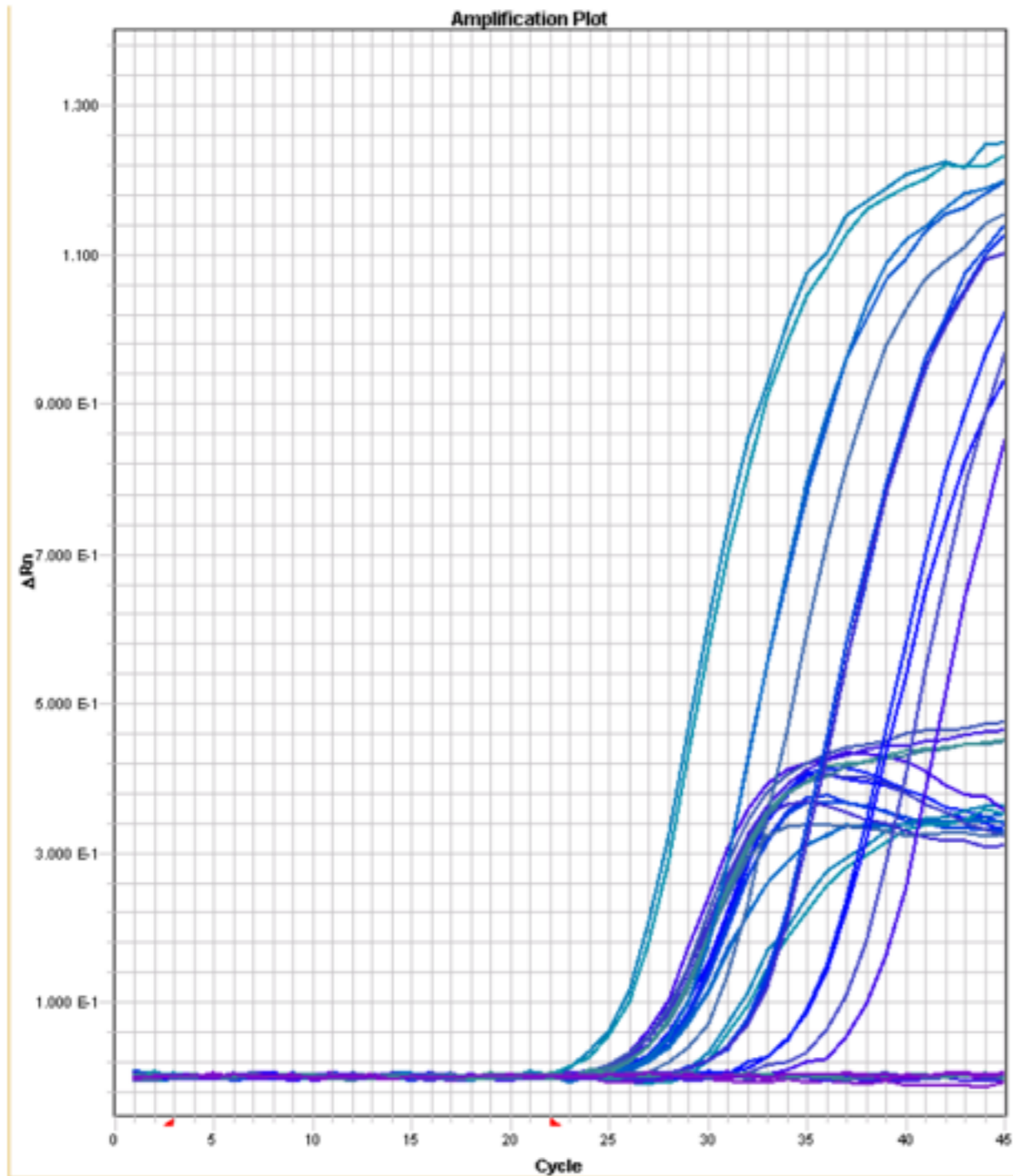




# The exponential region is easier to define in log phase

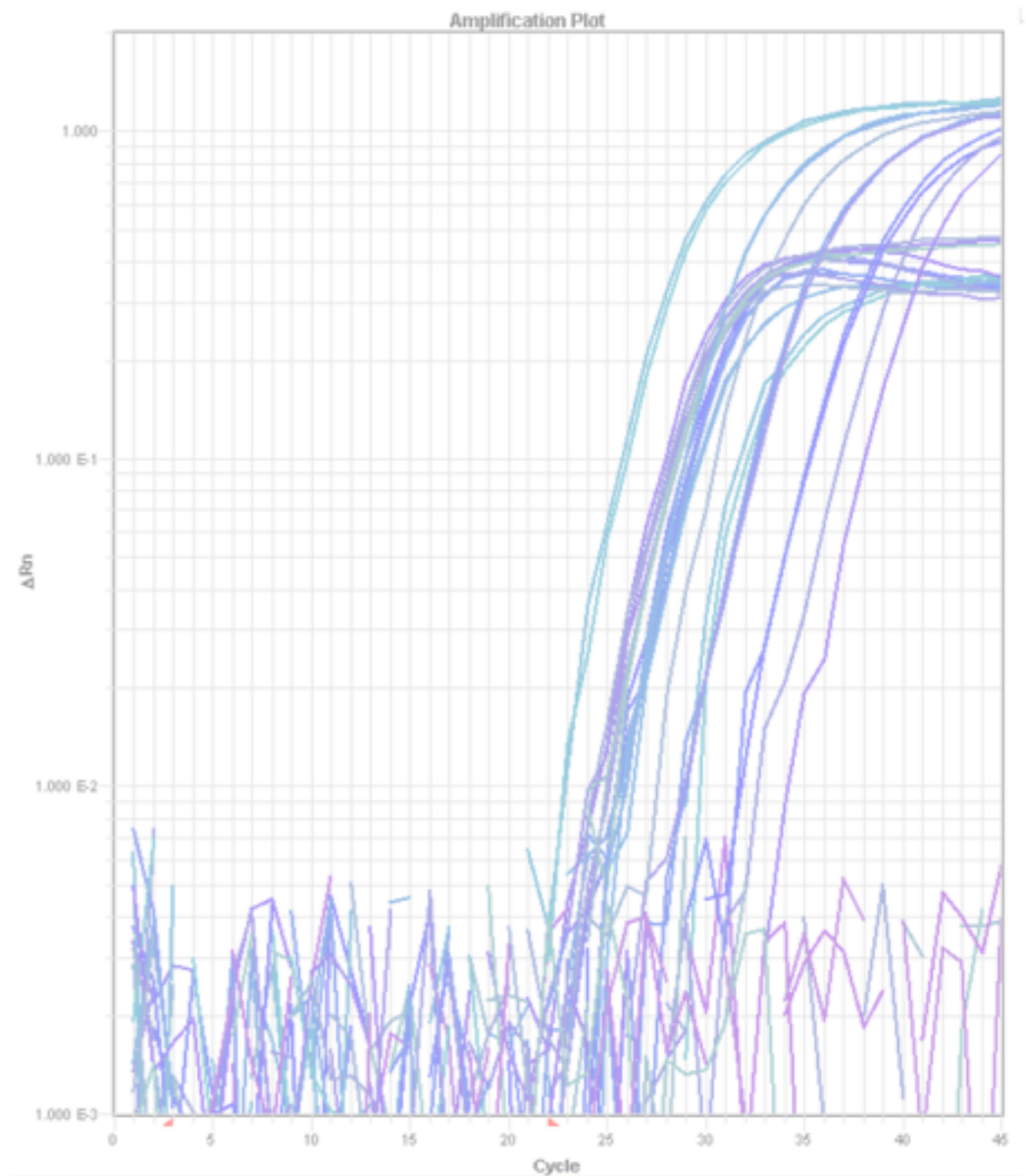


## Linear and Log view of the same data

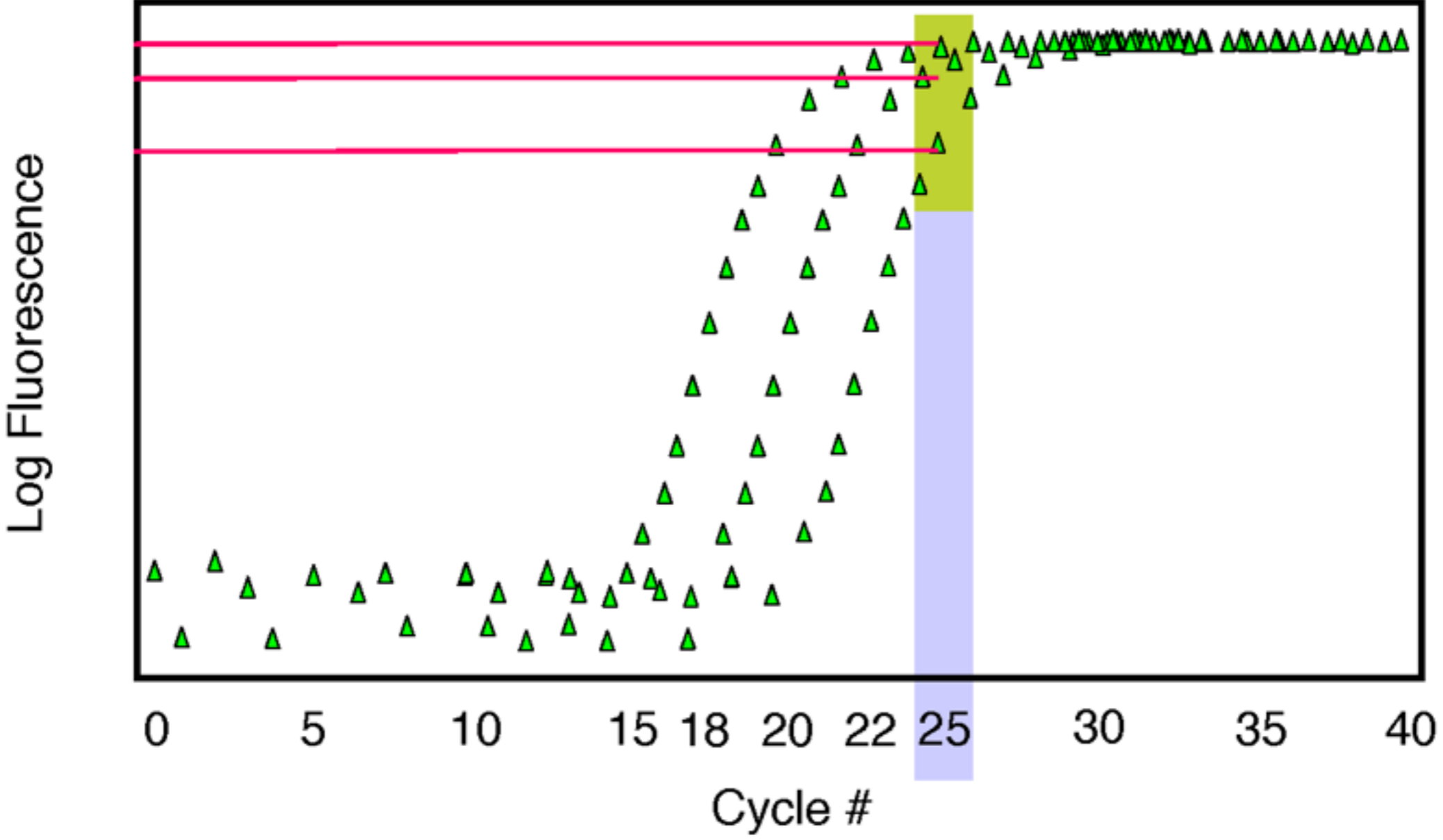


14

Linear Y axis

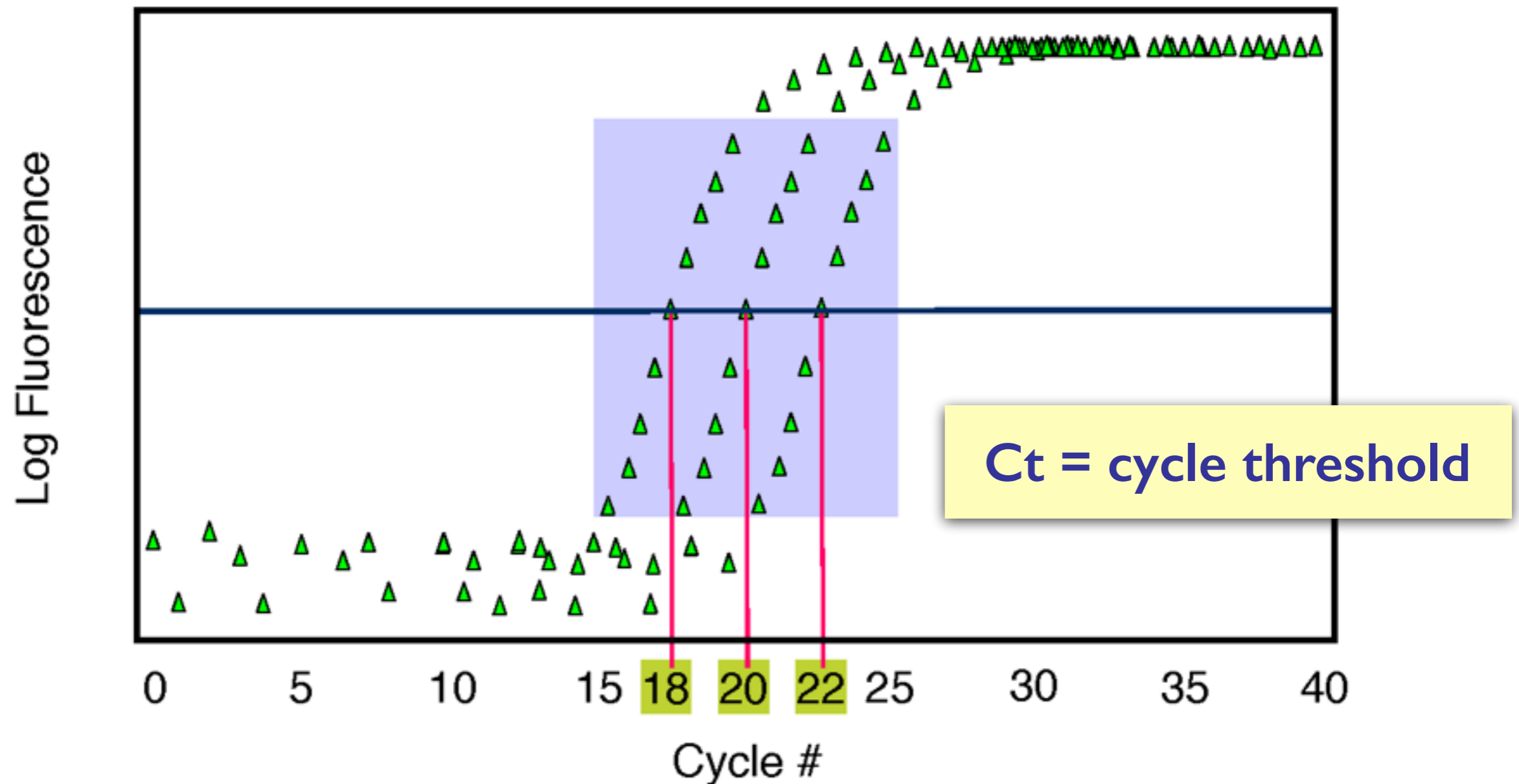


Log<sub>10</sub> Y axis

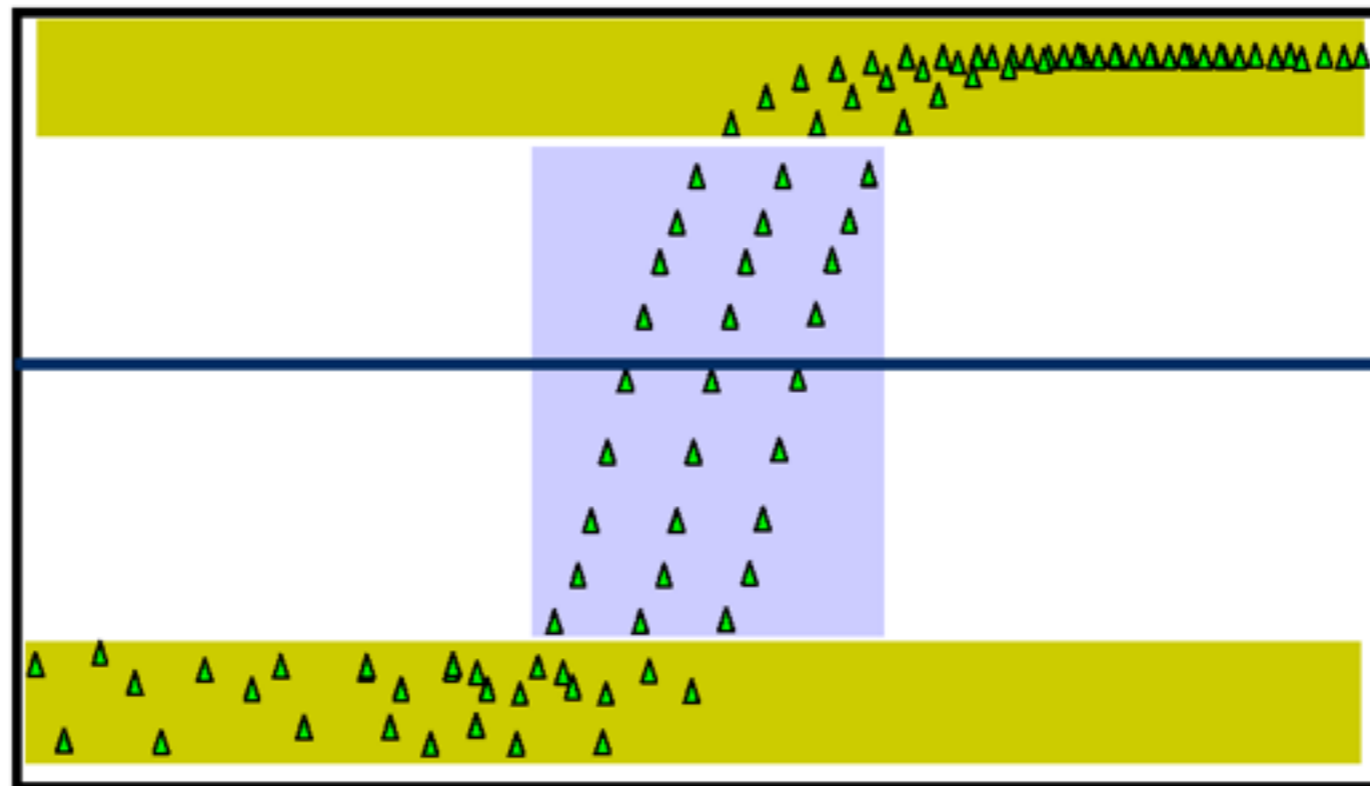


## Real-time PCR - Concept of Ct

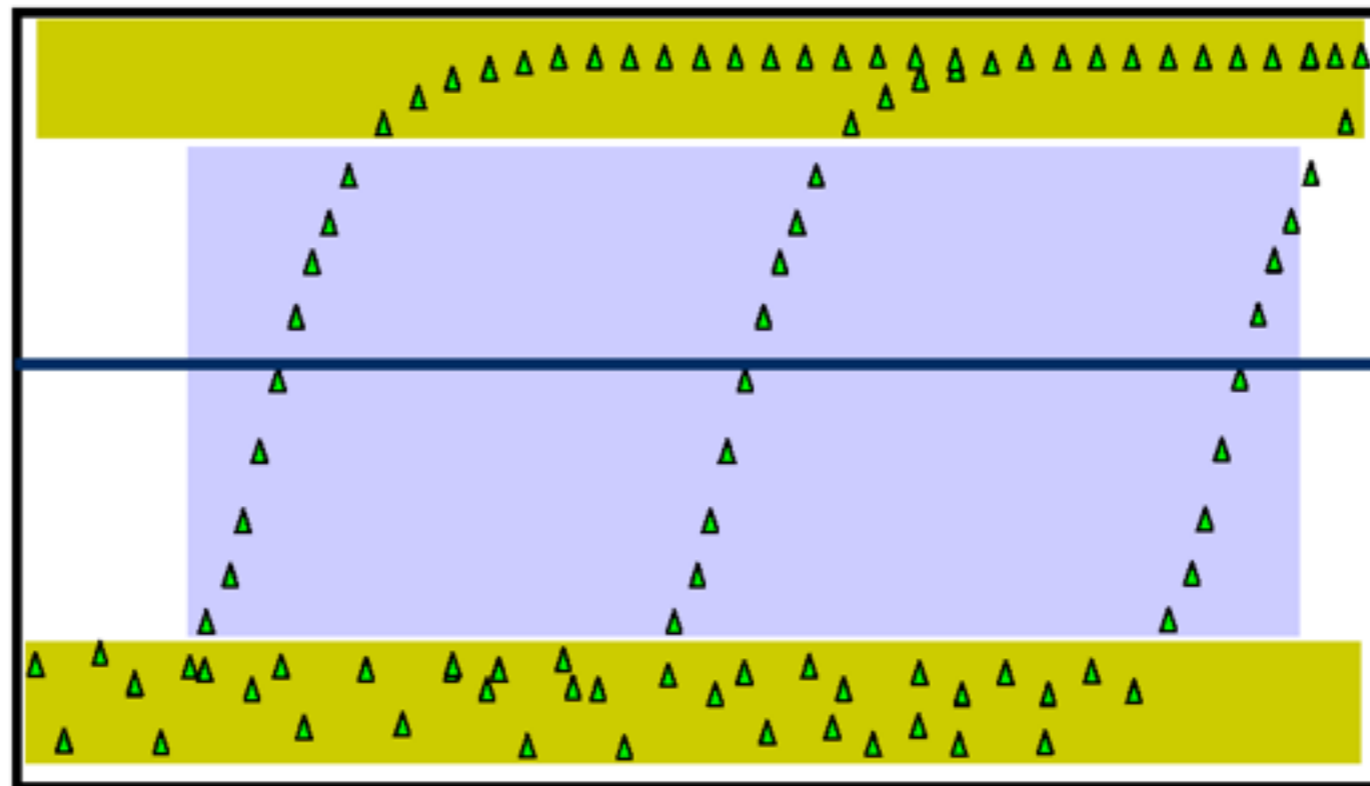
We measure the **number of cycles** it takes to reach a set fluorescence threshold (Ct)



Thus, real-time PCR is superior to regular PCR because:



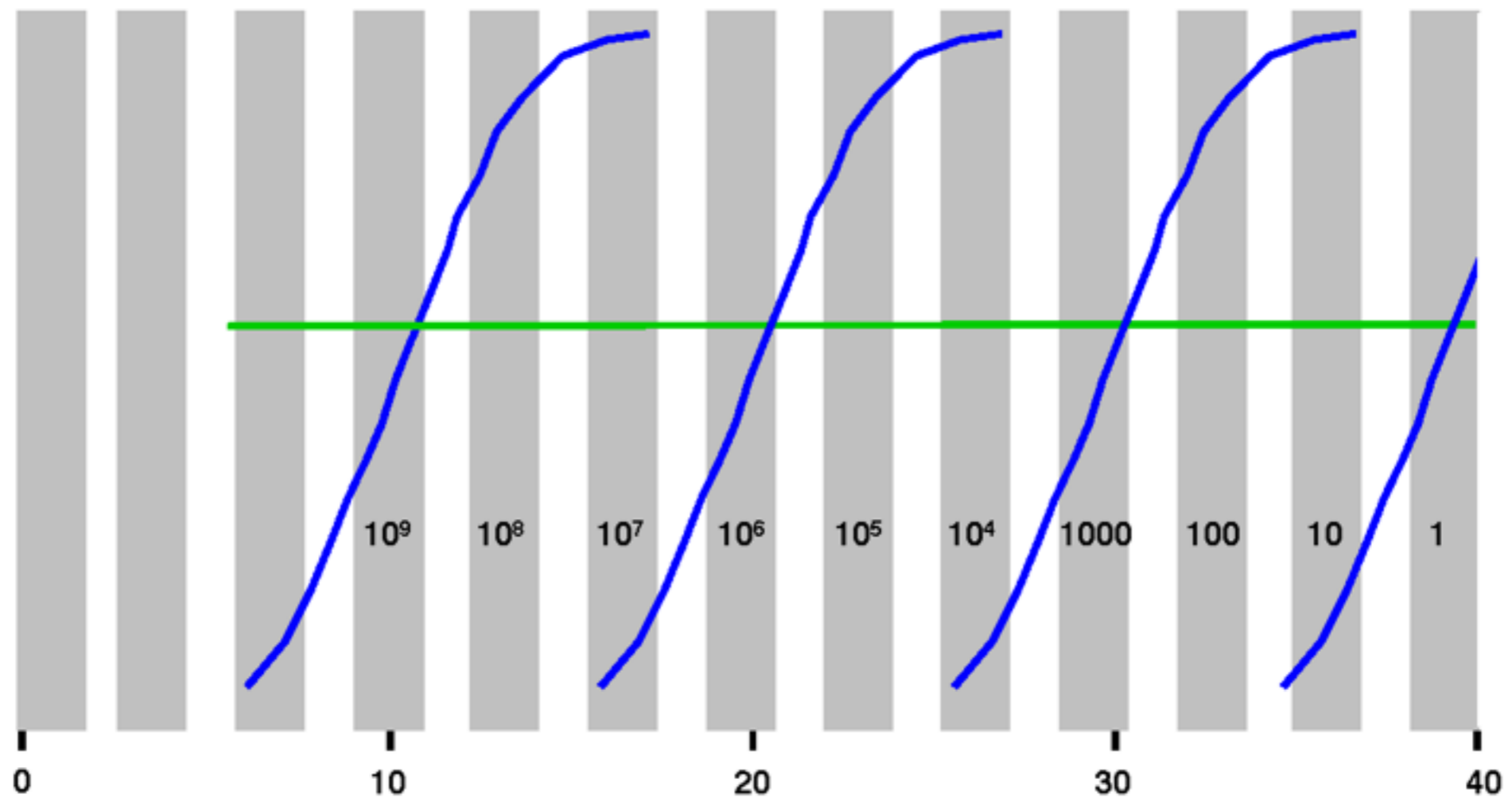
Thus, real-time PCR is superior to regular PCR because:



Results are usually given in table form  
 indicating Ct value  
 the higher the Ct, the lower the target copy number

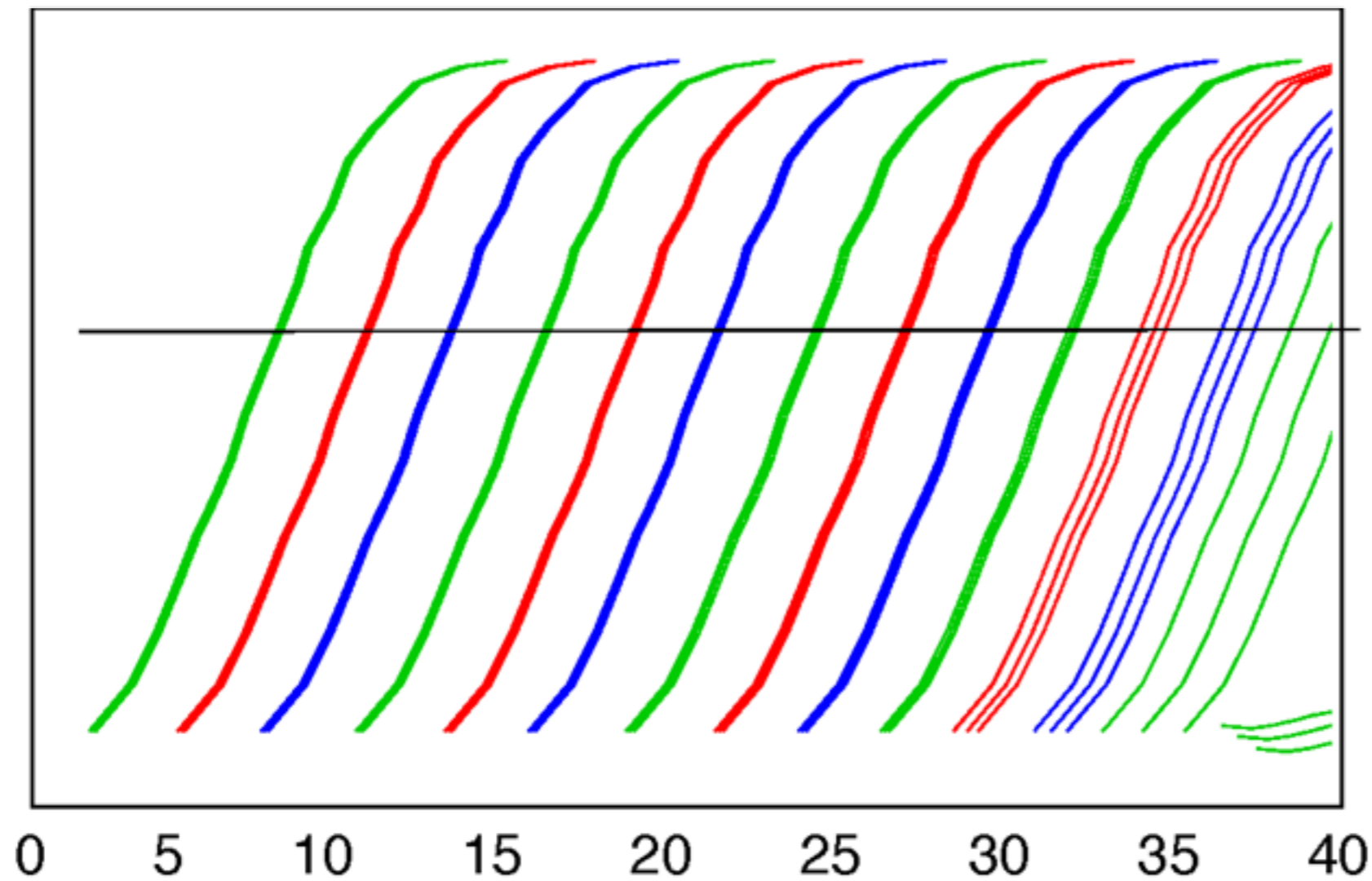
Well	Detector	ng RNA	Ct
A4	GAPDH 1	40	16.75
A5	GAPDH 1	40	16.89
A6	GAPDH 1	40	16.86
B4	GAPDH 1	4	20.27
B5	GAPDH 1	4	20.24
B6	GAPDH 1	4	20.24
C4	GAPDH 1	0.4	23.75
C5	GAPDH 1	0.4	23.71
C6	GAPDH 1	0.4	23.76
D4	GAPDH 1	0.04	27.21
D5	GAPDH 1	0.04	27.18
D6	GAPDH 1	0.04	27.17
E4	GAPDH 1	0.004	30.46
E5	GAPDH 1	0.004	29.98
E6	GAPDH 1	0.004	30.6

Well	Detector	ng RNA	Ct
A1	TNF-a 1	40	27.32
A2	TNF-a 1	40	27.34
A3	TNF-a 1	40	27.28
B1	TNF-a 1	4	30.83
B2	TNF-a 1	4	30.91
B3	TNF-a 1	4	30.87
C1	TNF-a 1	0.4	34.13
C2	TNF-a 1	0.4	34.32
C3	TNF-a 1	0.4	34.25
D1	TNF-a 1	0.04	38.46
D2	TNF-a 1	0.04	38.42
D3	TNF-a 1	0.04	37.18
E1	TNF-a 1	0.004	Undetermined
E2	TNF-a 1	0.004	Undetermined
E3	TNF-a 1	0.004	Undetermined



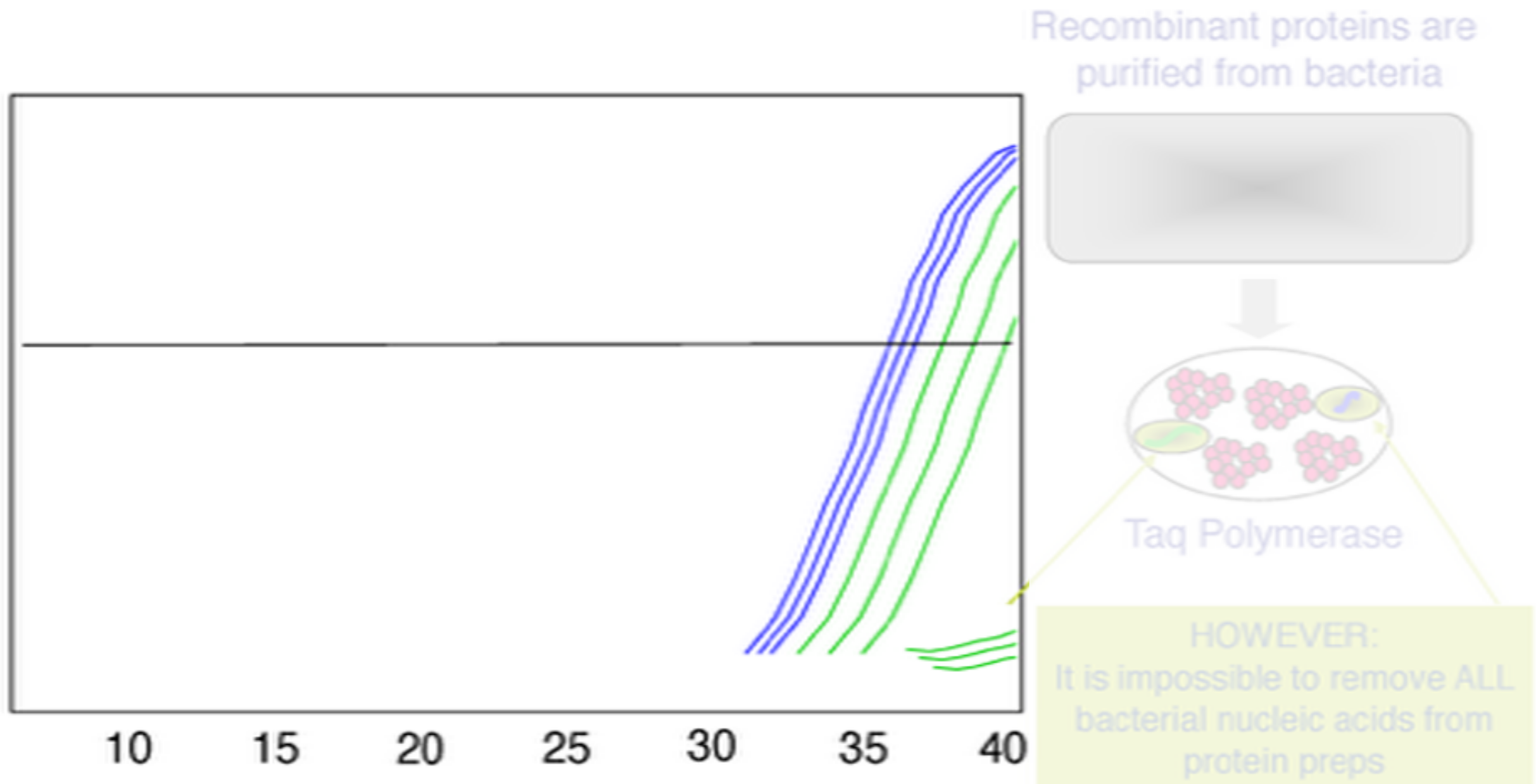


- how reproducible is the data?
- this is determined by replicates

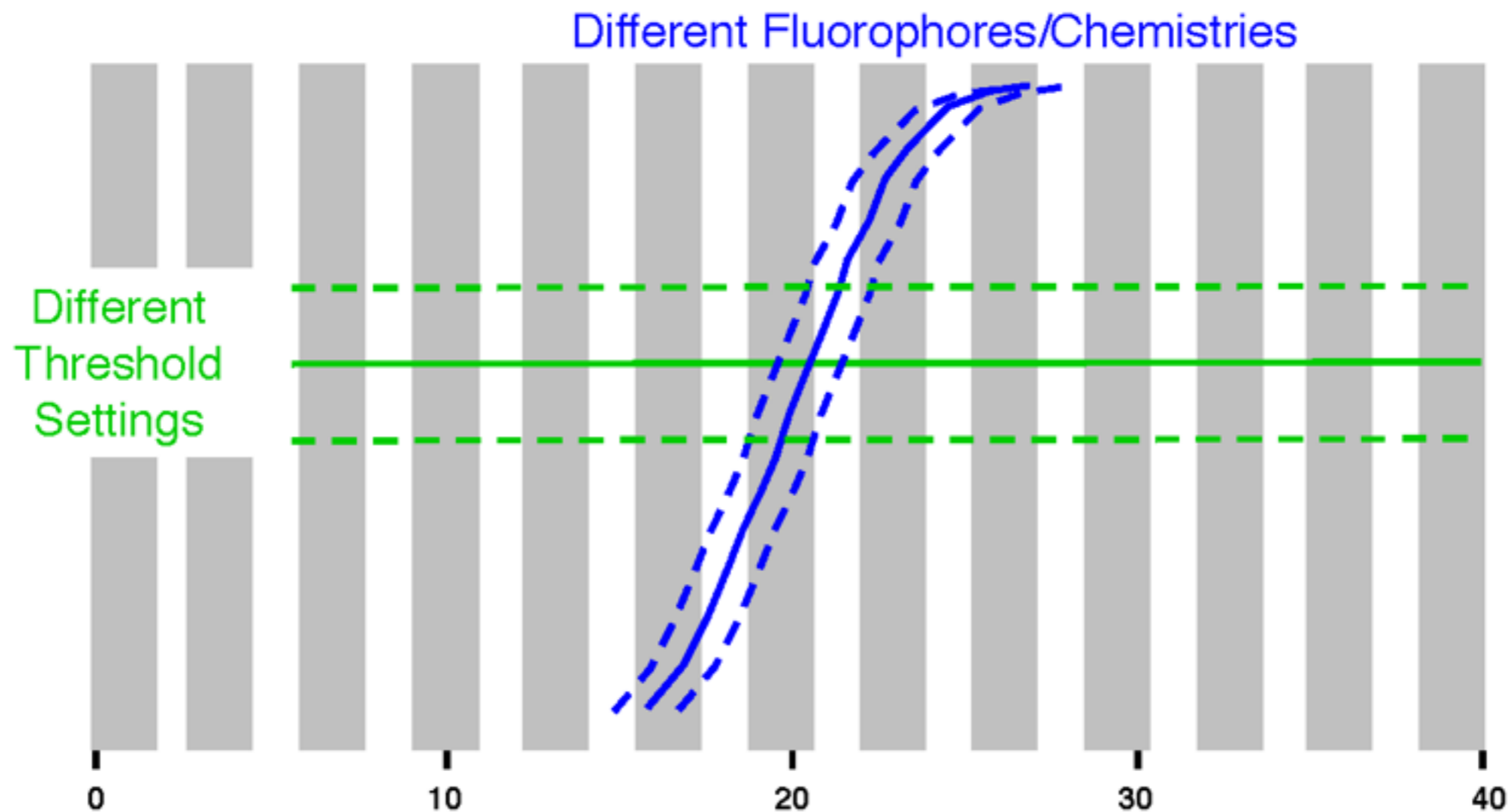


Precision is  
always reduced  
at high Cts

## NTCs may show some amplification



The **Exact** Ct value may vary due to:

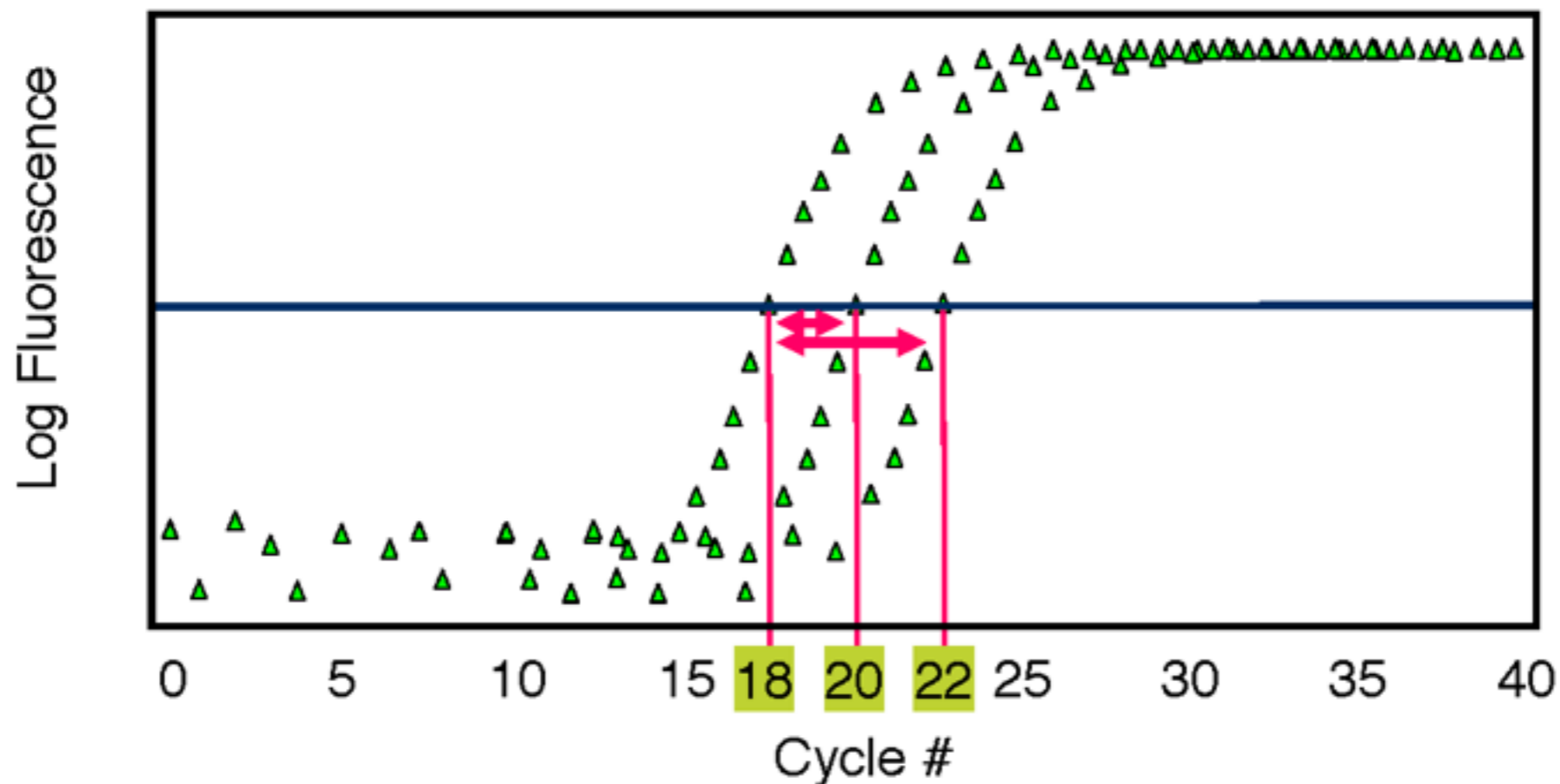


and therefore only indicates an approximate copy number.  
For this reason, Ct values are not normally published

However, if we compare Cts from the SAME PLATE,  
then we can be extremely accurate.

# Quantitative real-time PCR analysis measures the **DIFFERENCE** in the Cts

Either the difference between Sample Cts and Std Cve Cts (Absolute)  
Or, the difference between sample Cts directly (Relative)



# Real-time PCR

**SYBR<sup>®</sup> Green**

**TaqMan<sup>®</sup>**

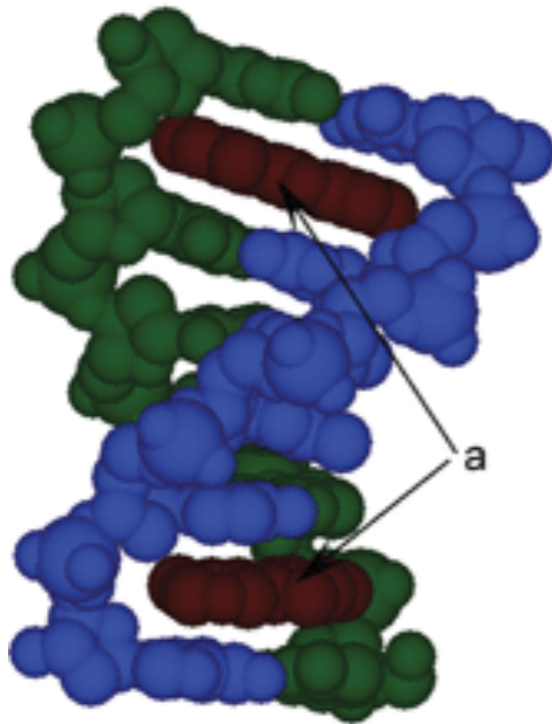
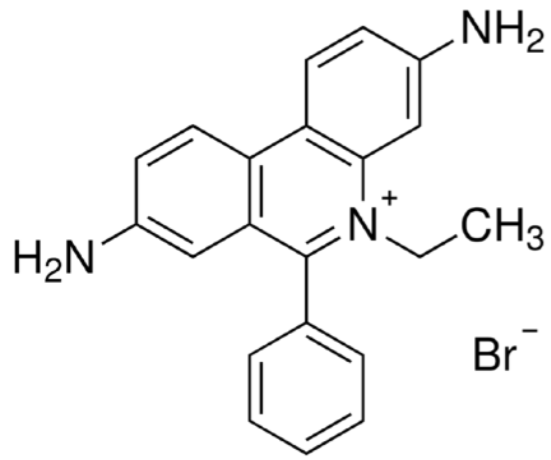
**MGB**

**ROX<sup>™</sup>**

**Multicomponenting**

# Visualization – Fluorescent dyes

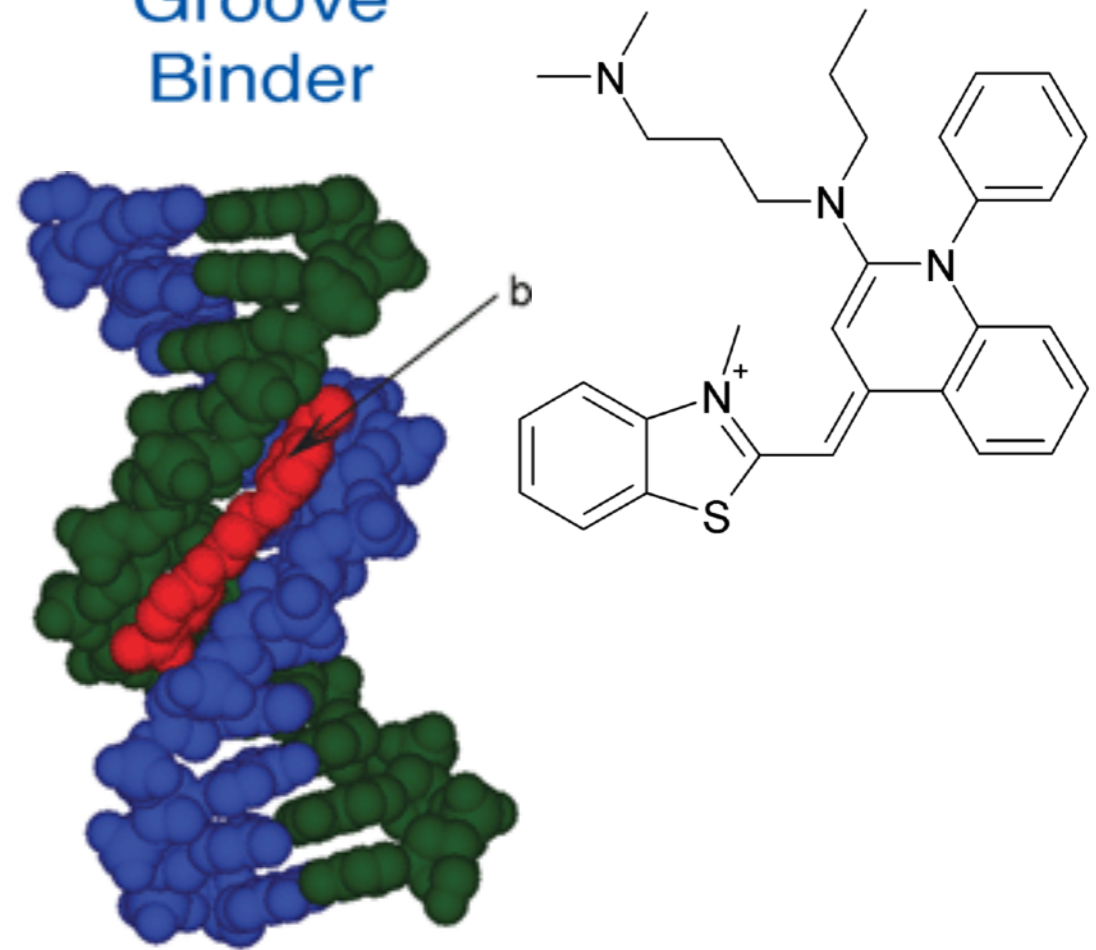
Intercalating agents



Intercalation

Ethidium Bromide

Minor Groove Binder

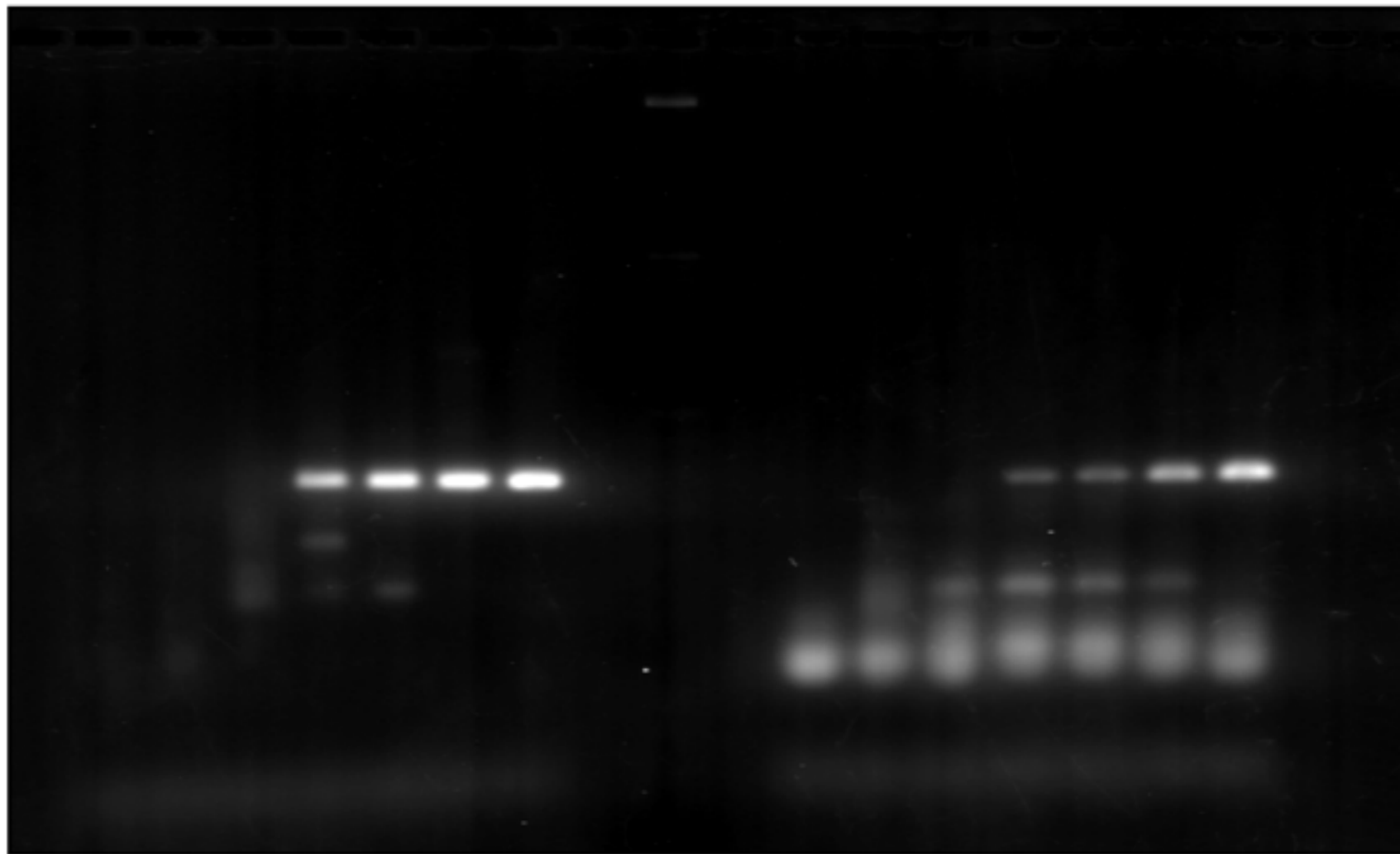


Groove binding

SYBR<sup>®</sup> Green

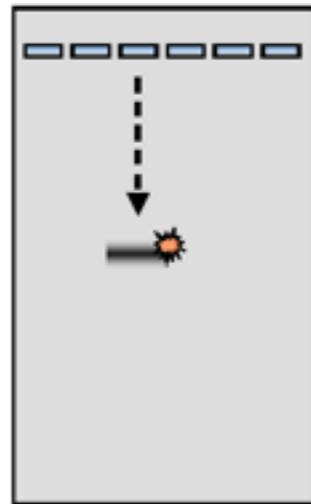
## Problem with DNA-binding Dyes

Bind non-specifically to *any* double-stranded DNA



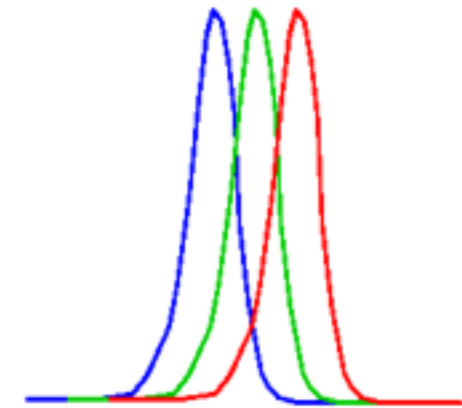
**Therefore specificity of the amplifications must be checked**

Exact position of  
band affected by:



1) size of fragment

Exact position of  
peak affected by:

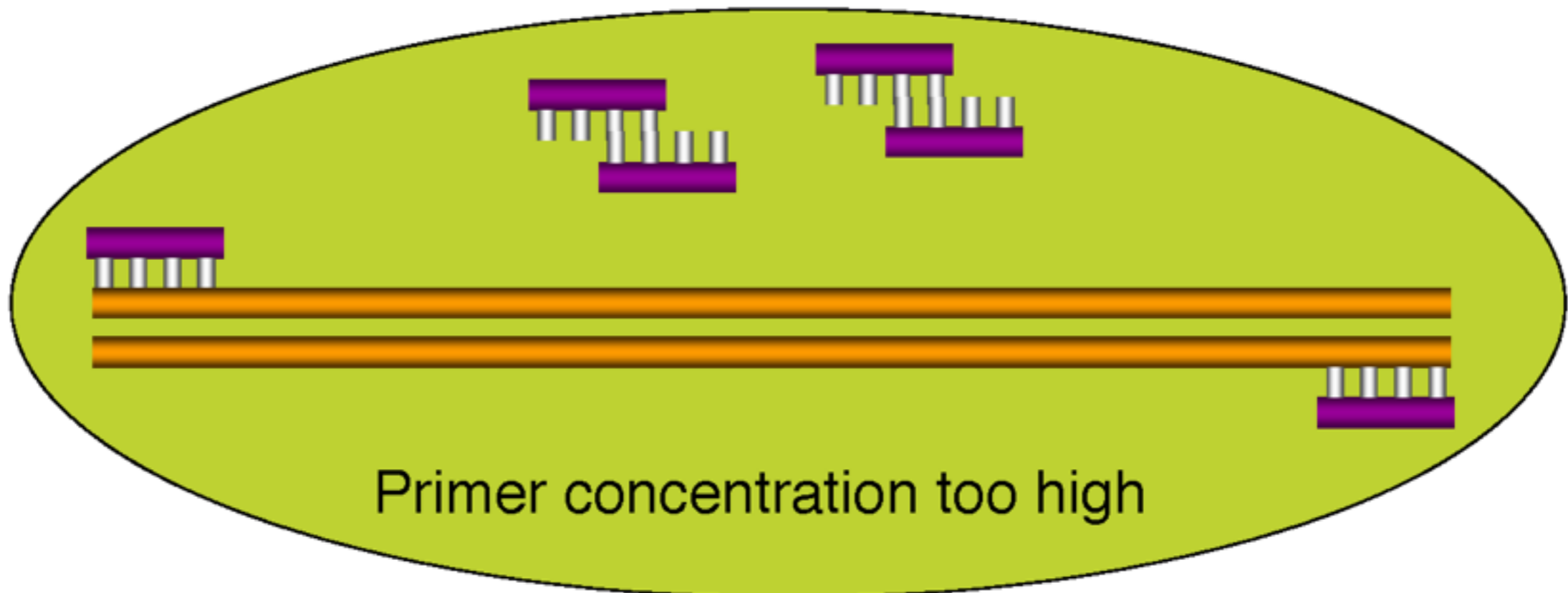
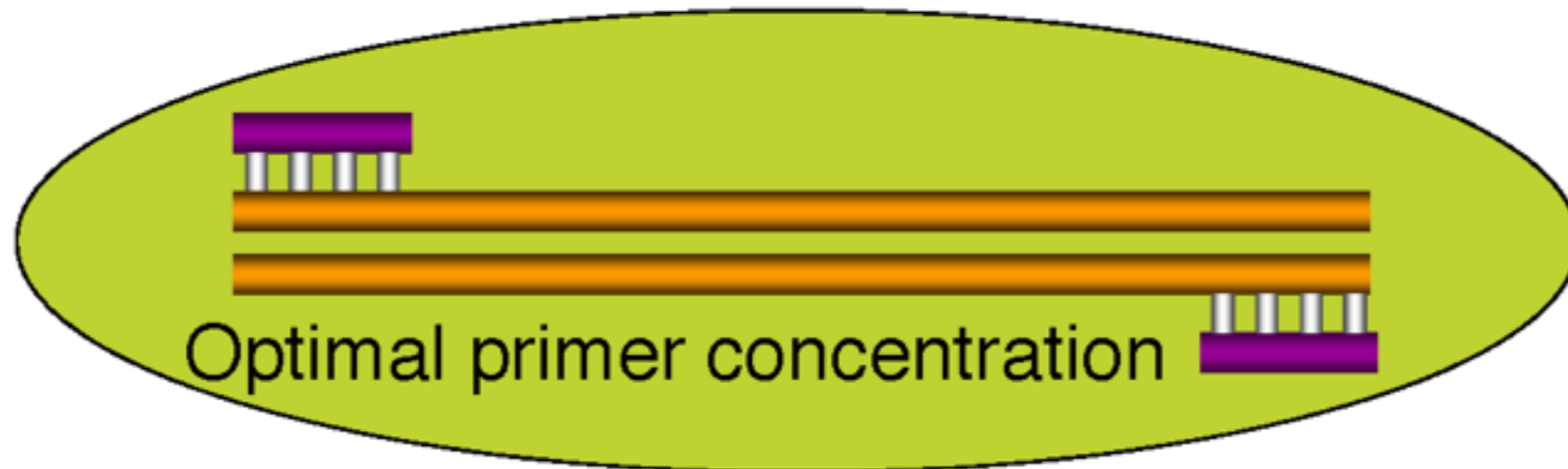


1) size of fragment

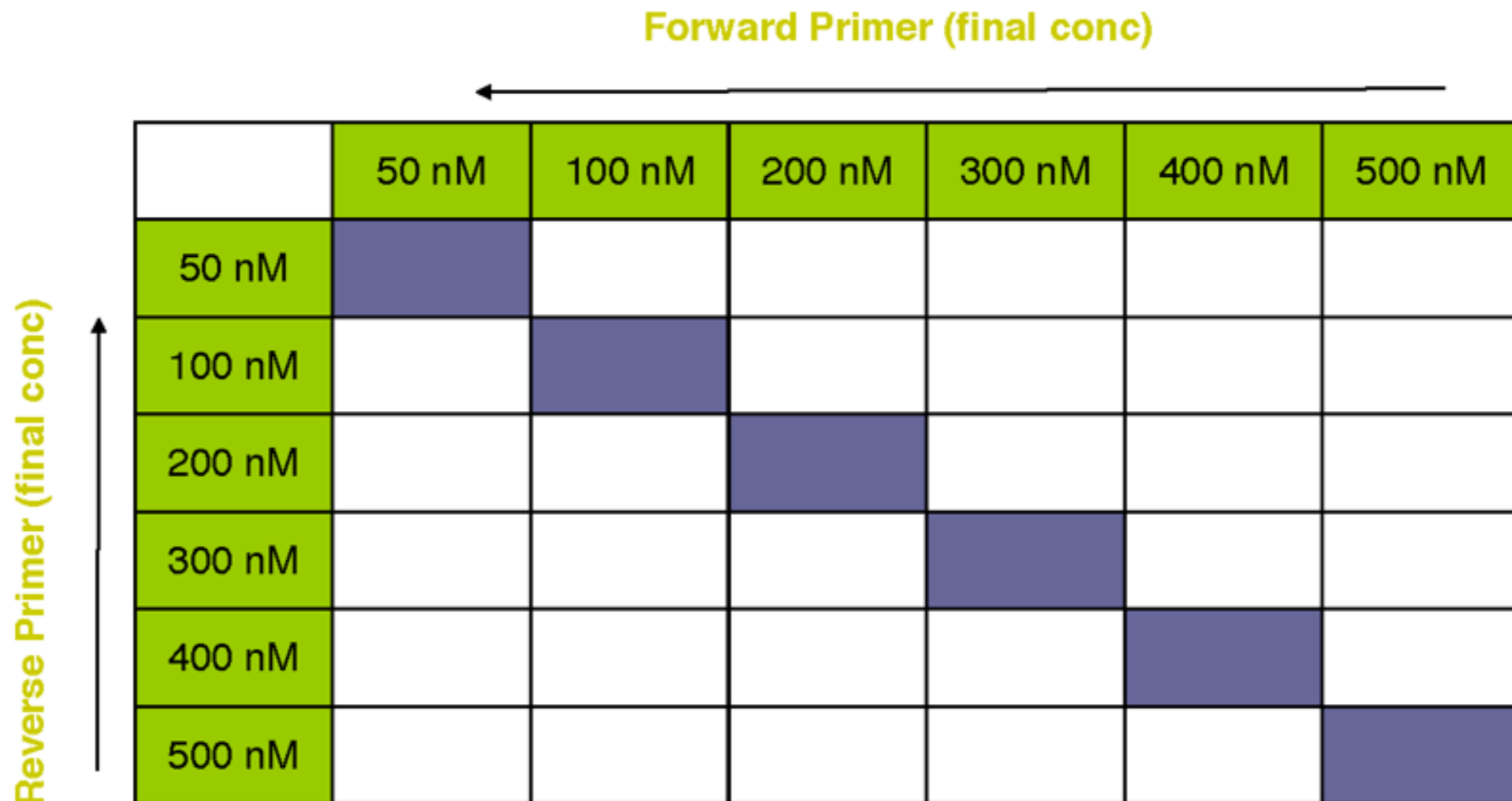
2) nucleotide composition



# Non-specific amplification promoted by high primer concentration



# Primer-dimer formation reduced by minimizing primer concentration





**Real-time PCR**

**SYBR<sup>®</sup> Green**

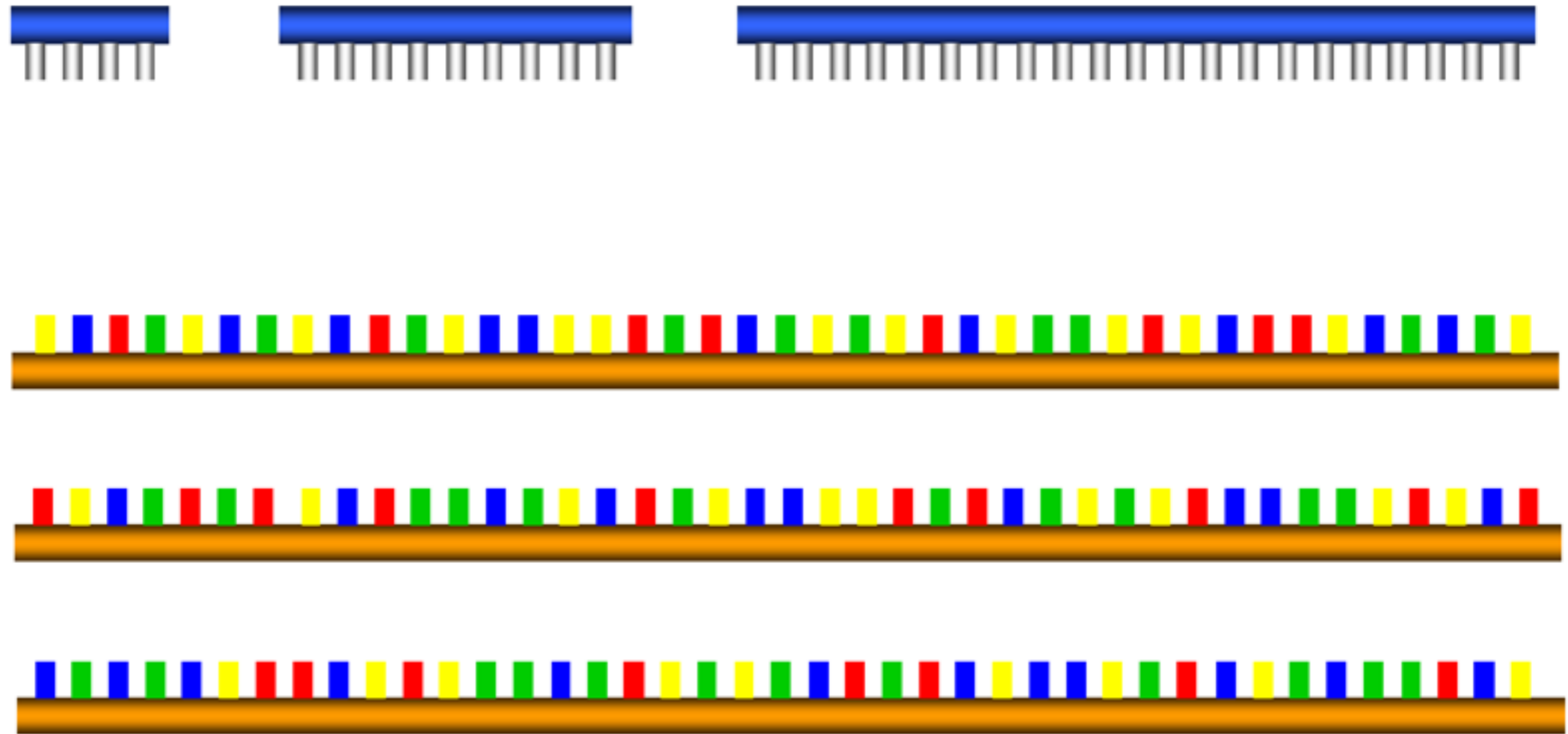
**TaqMan<sup>®</sup>**

**MGB**

**ROX<sup>™</sup>**

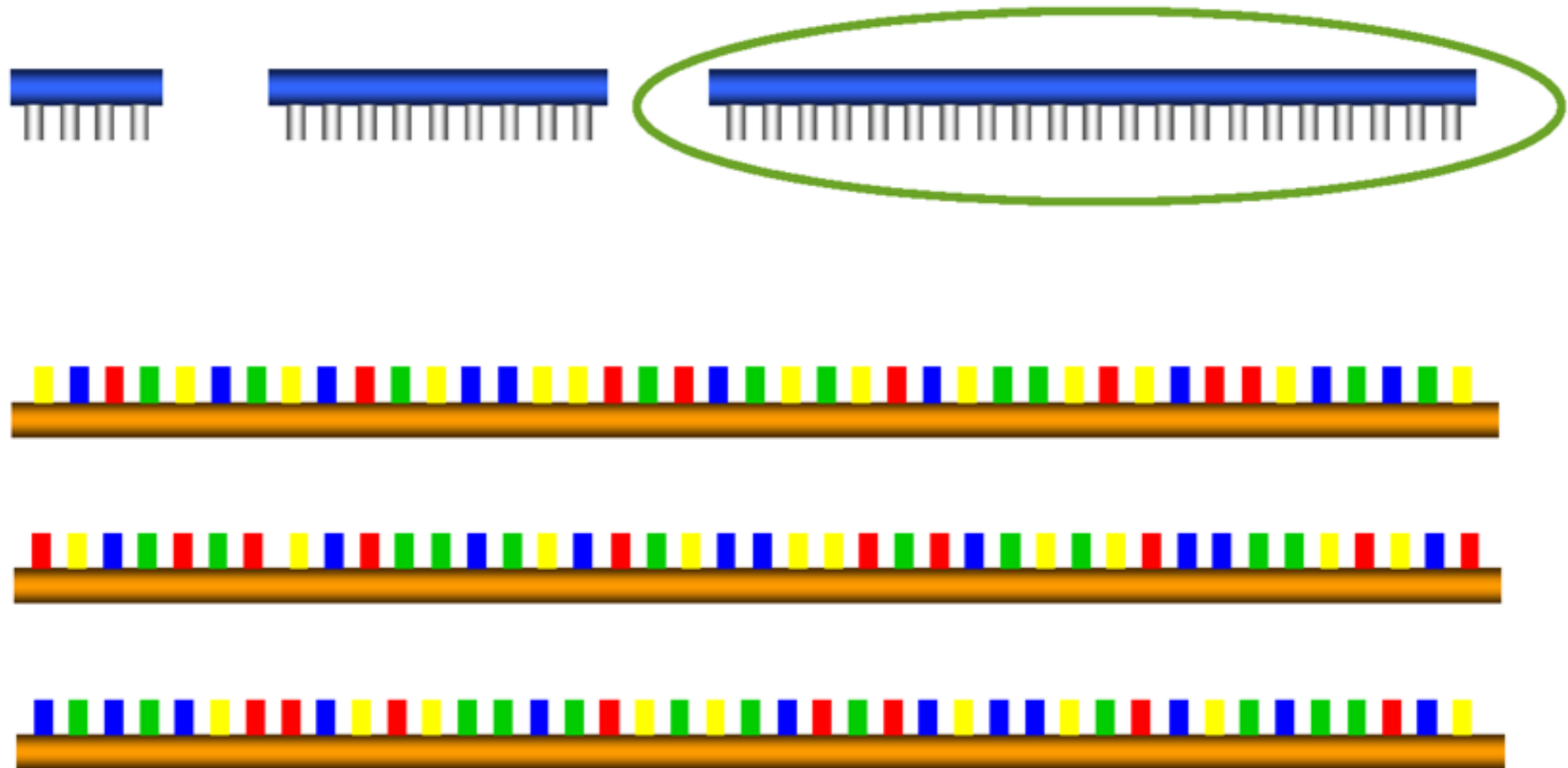
**Multicomponenting**

# Probing for specific sequence in a pool of very different sequences

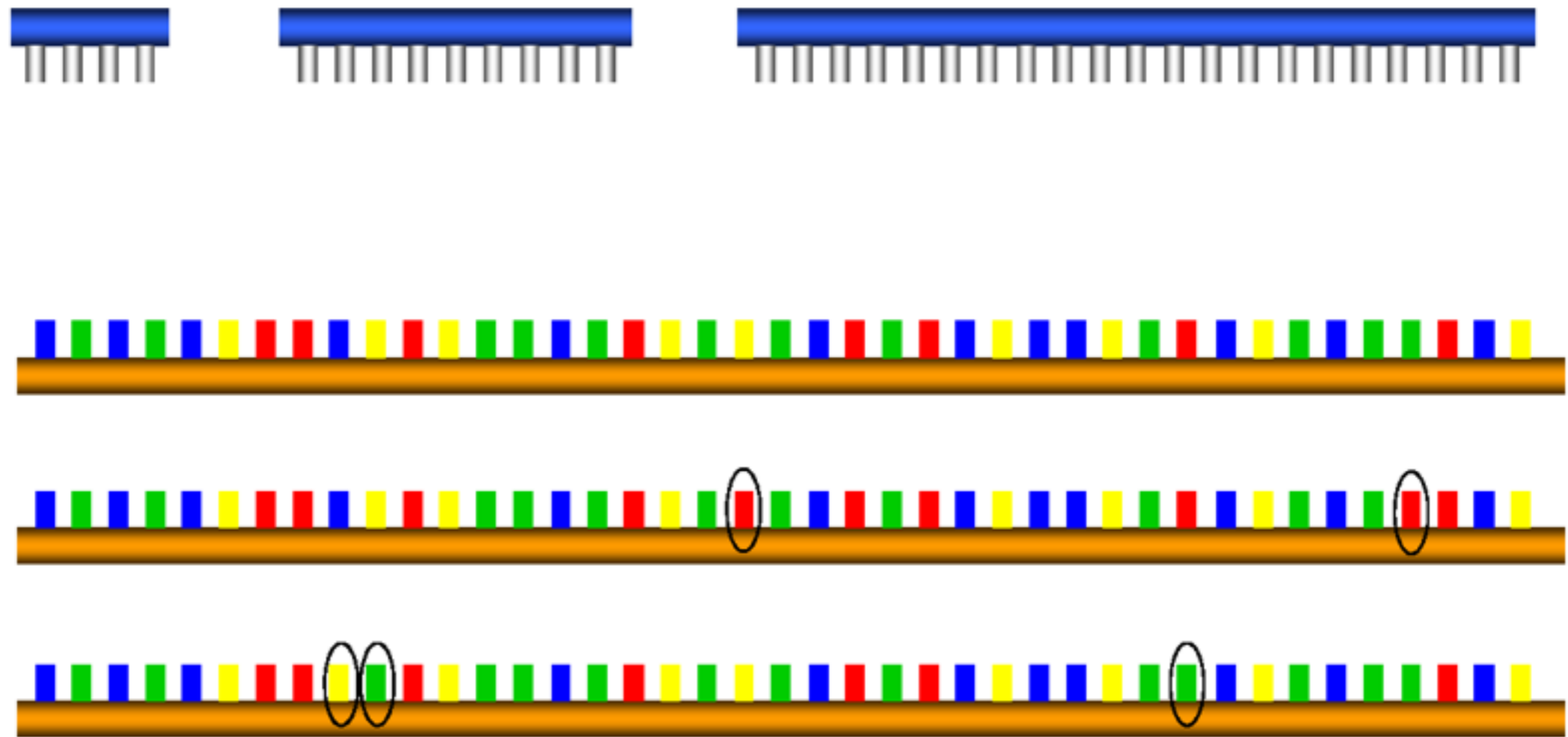


# Probing for specific sequence in a pool of very different sequences

Longer probes increase specificity between different sequences

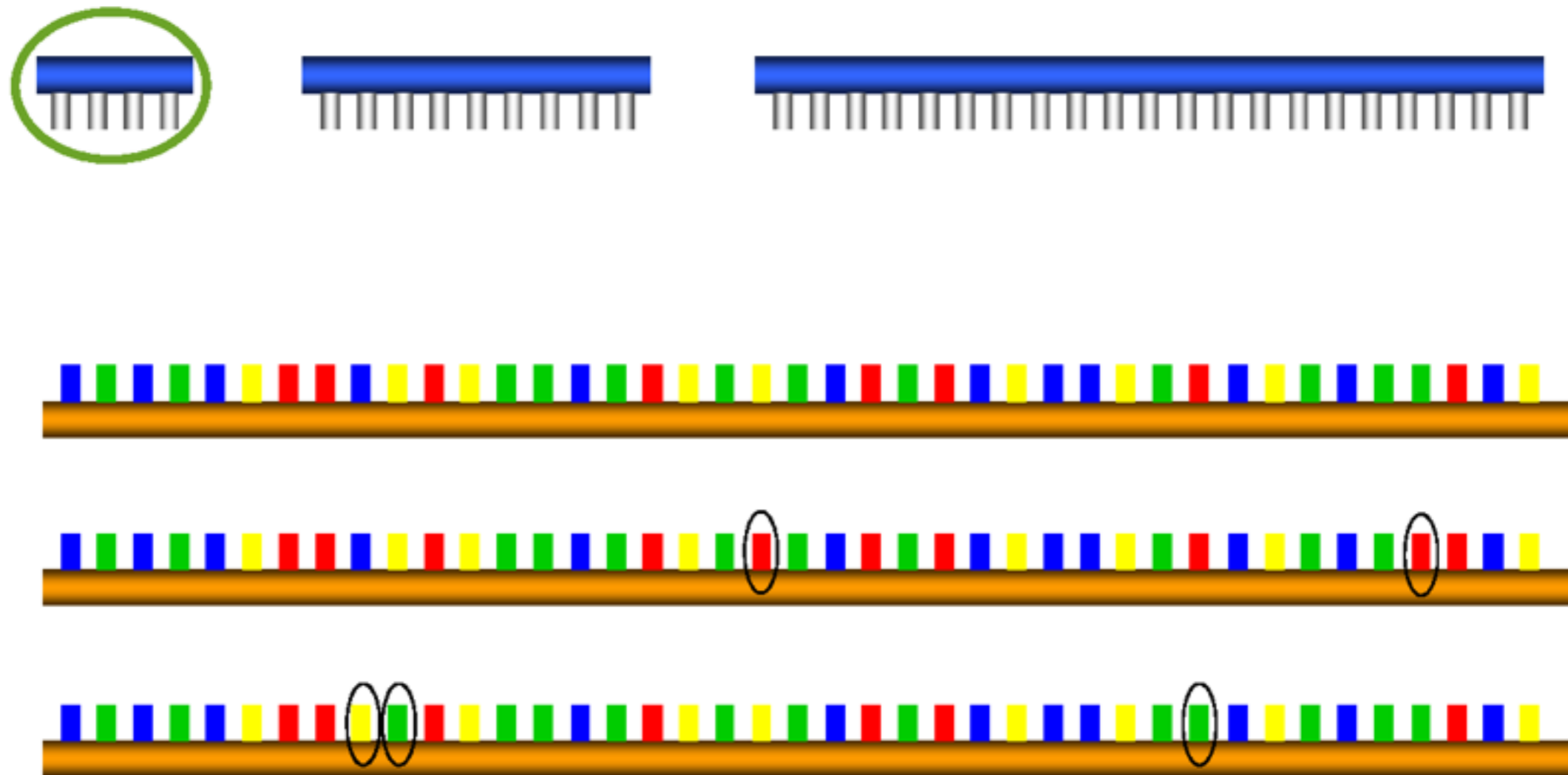


# Probing for specific sequence in a pool of very similar sequences



# Probing for specific sequence in a pool of very similar sequences

Shorter probes increase selectivity between similar sequences



Probe is made shorter by adding a minor-groove-binding molecule that increases probe  $T_m$

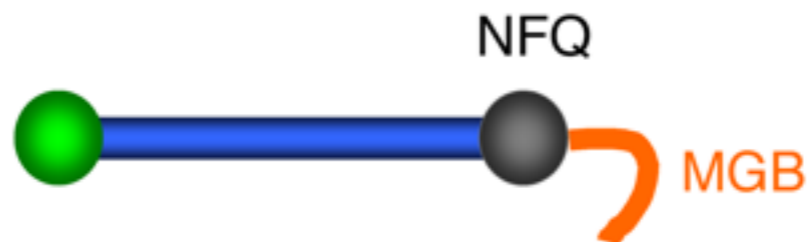
Primers ~ 20-30bp



regular probes ~30-40bp

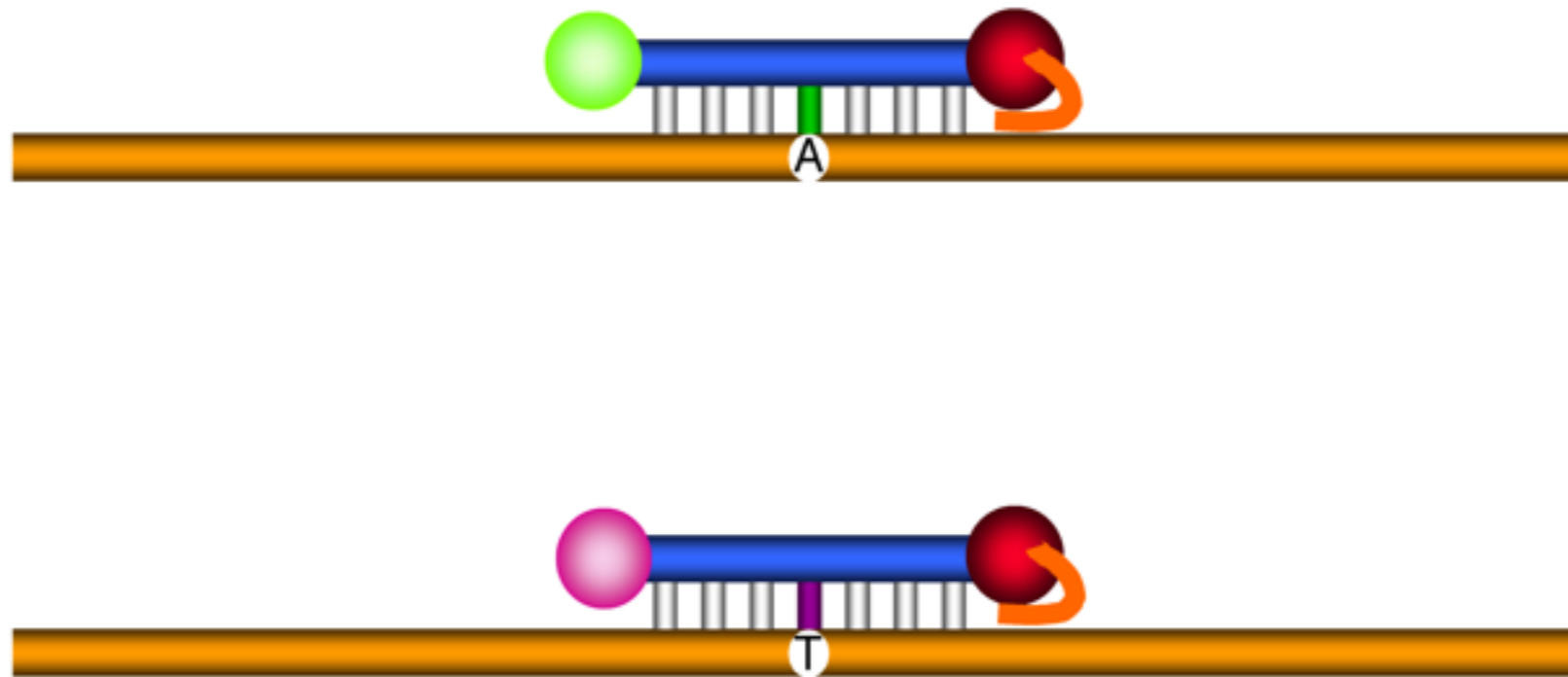


MGB probes ~13-22bp





Short MGB probes allow  
robust single nucleotide specificity  
ie: SNP assays





**Real-time PCR**

**SYBR<sup>®</sup> Green**

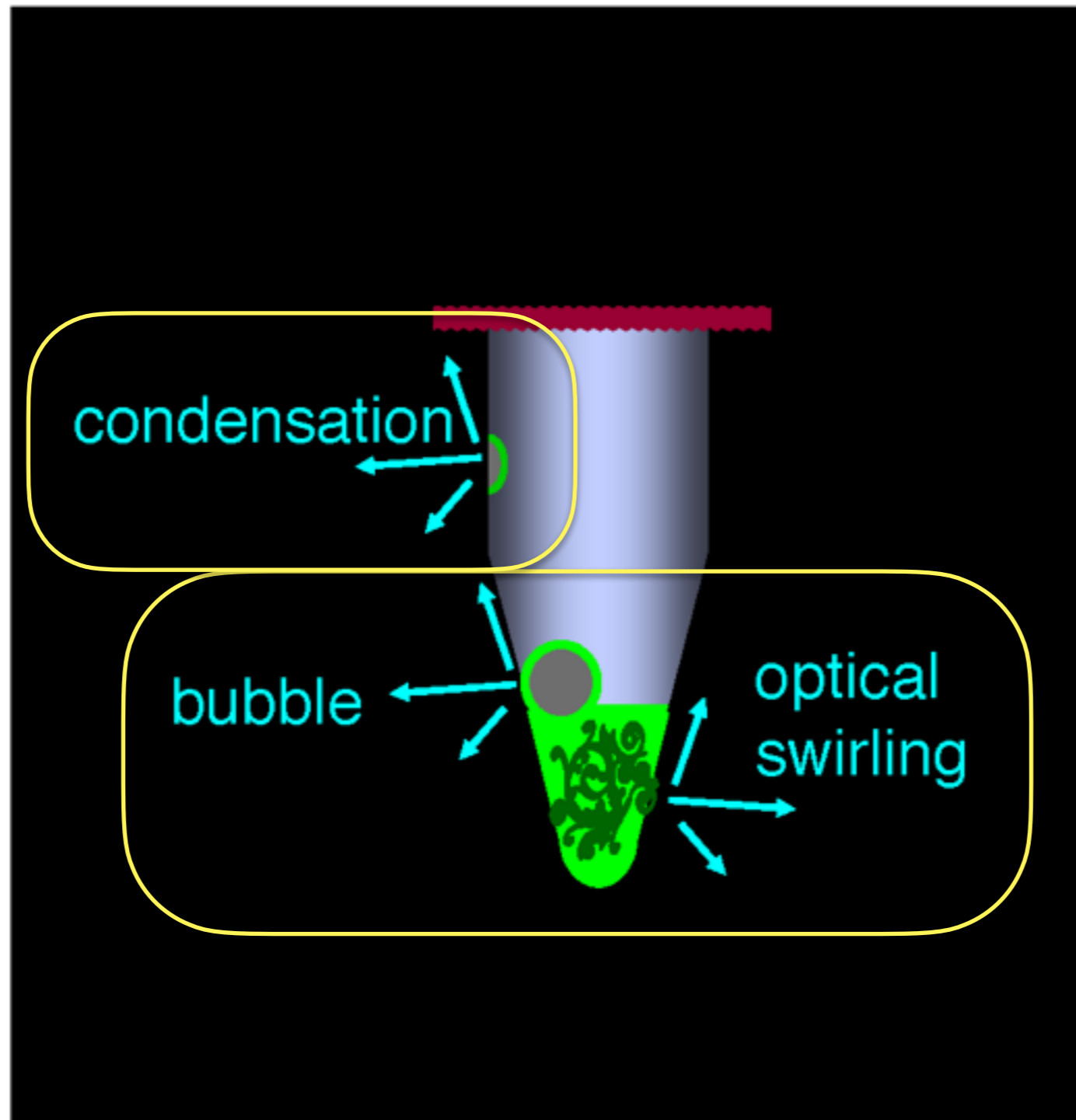
**TaqMan<sup>®</sup>**

**MGB**

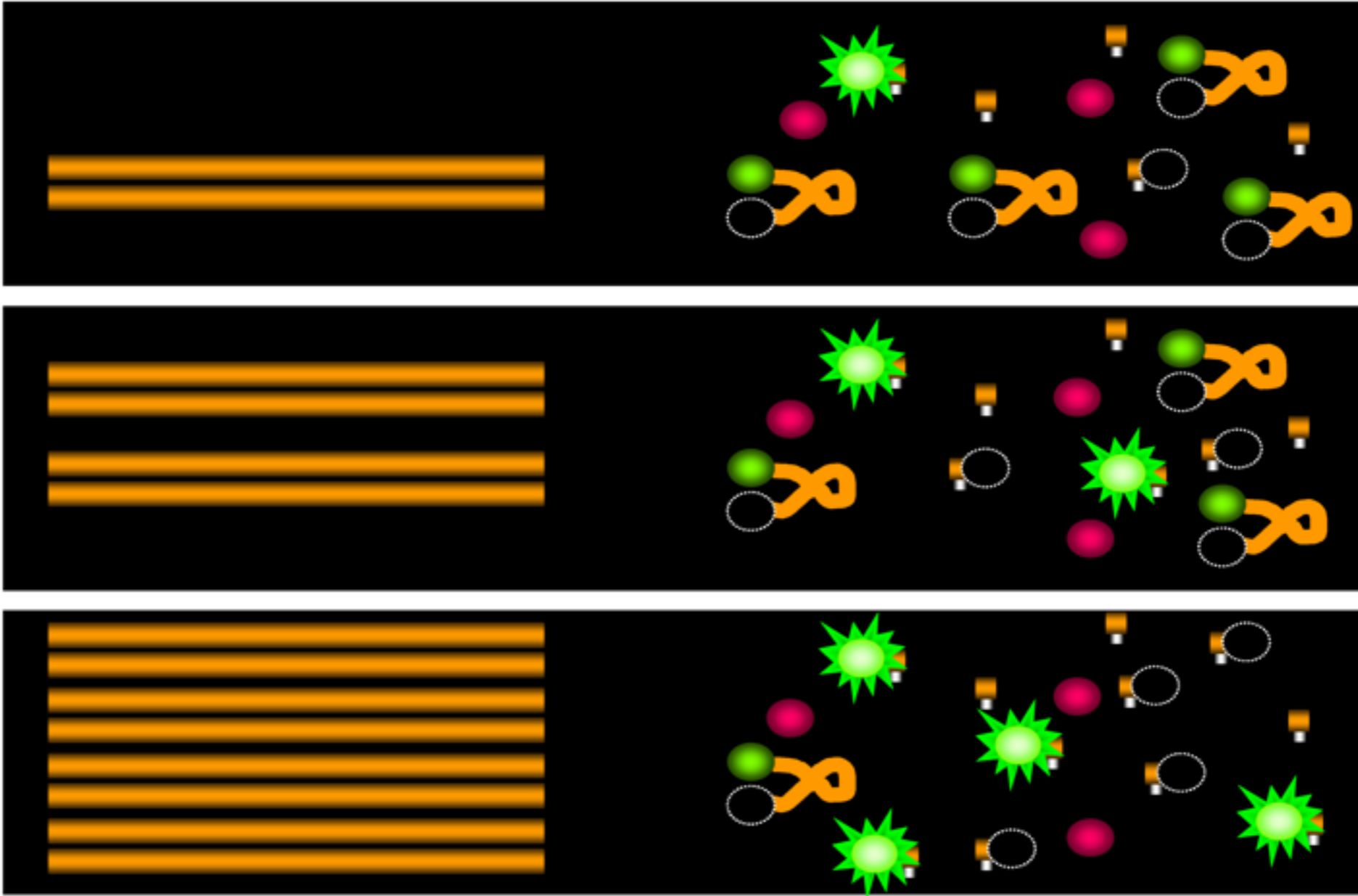
**ROX<sup>™</sup>**

**Multicomponenting**

# Common sources of **dynamic** variation of light signal



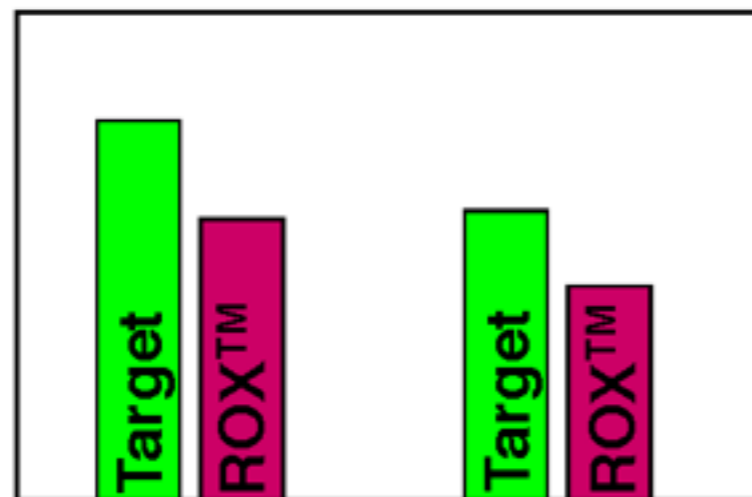
# Variation negated by normalizing to a Passive Reference dye



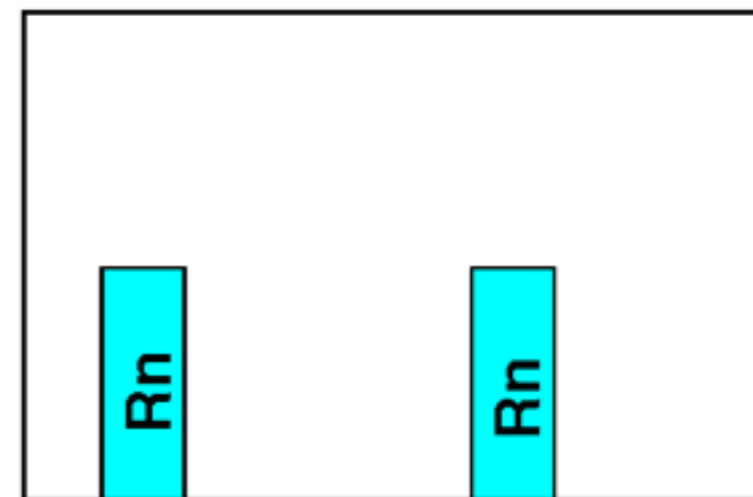
## ROX™ is a Passive Reference dye

Greatly improves precision of replicates.

$$R_n = \text{Normalization} = \frac{\text{Reporter}}{\text{Reference}}$$



Well 1      Well 2



Well 1      Well 2



**Real-time PCR**

**SYBR<sup>®</sup> Green**

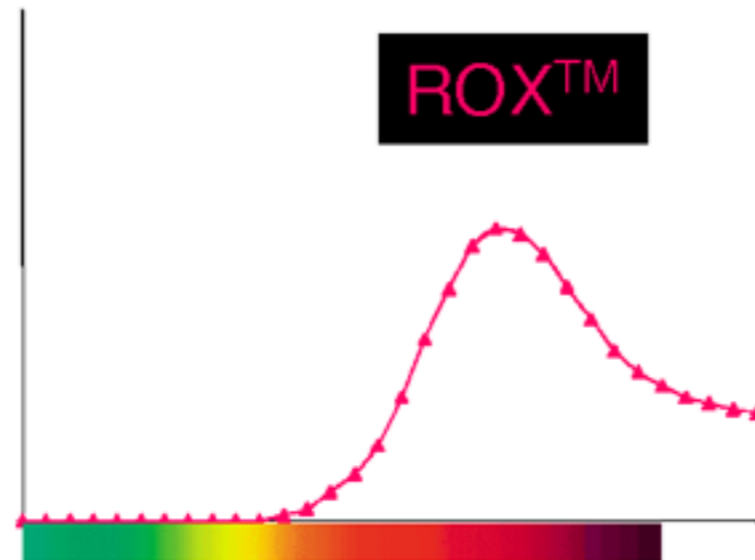
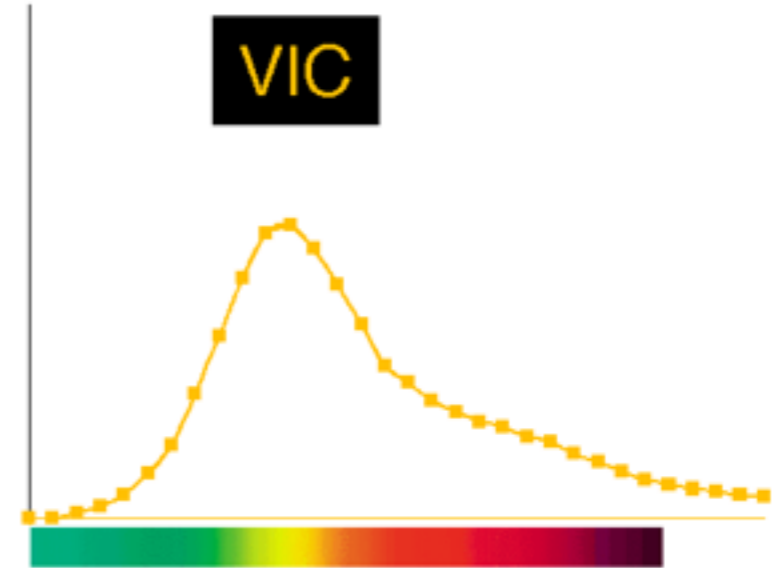
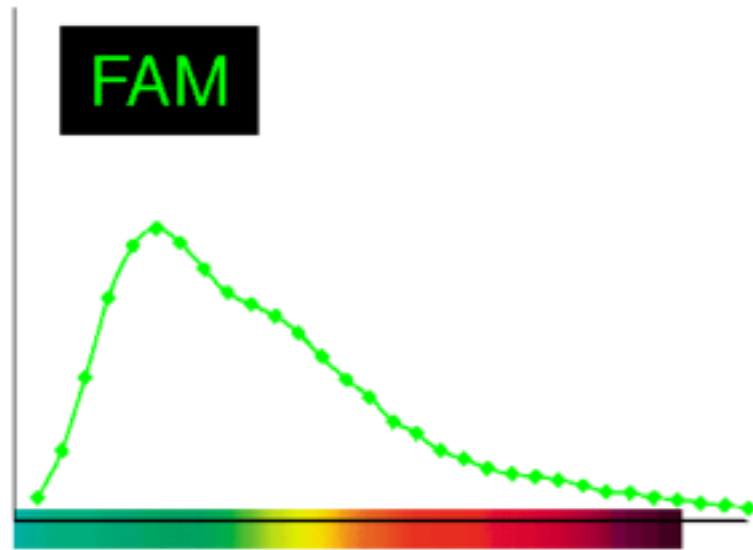
**TaqMan<sup>®</sup>**

**MGB**

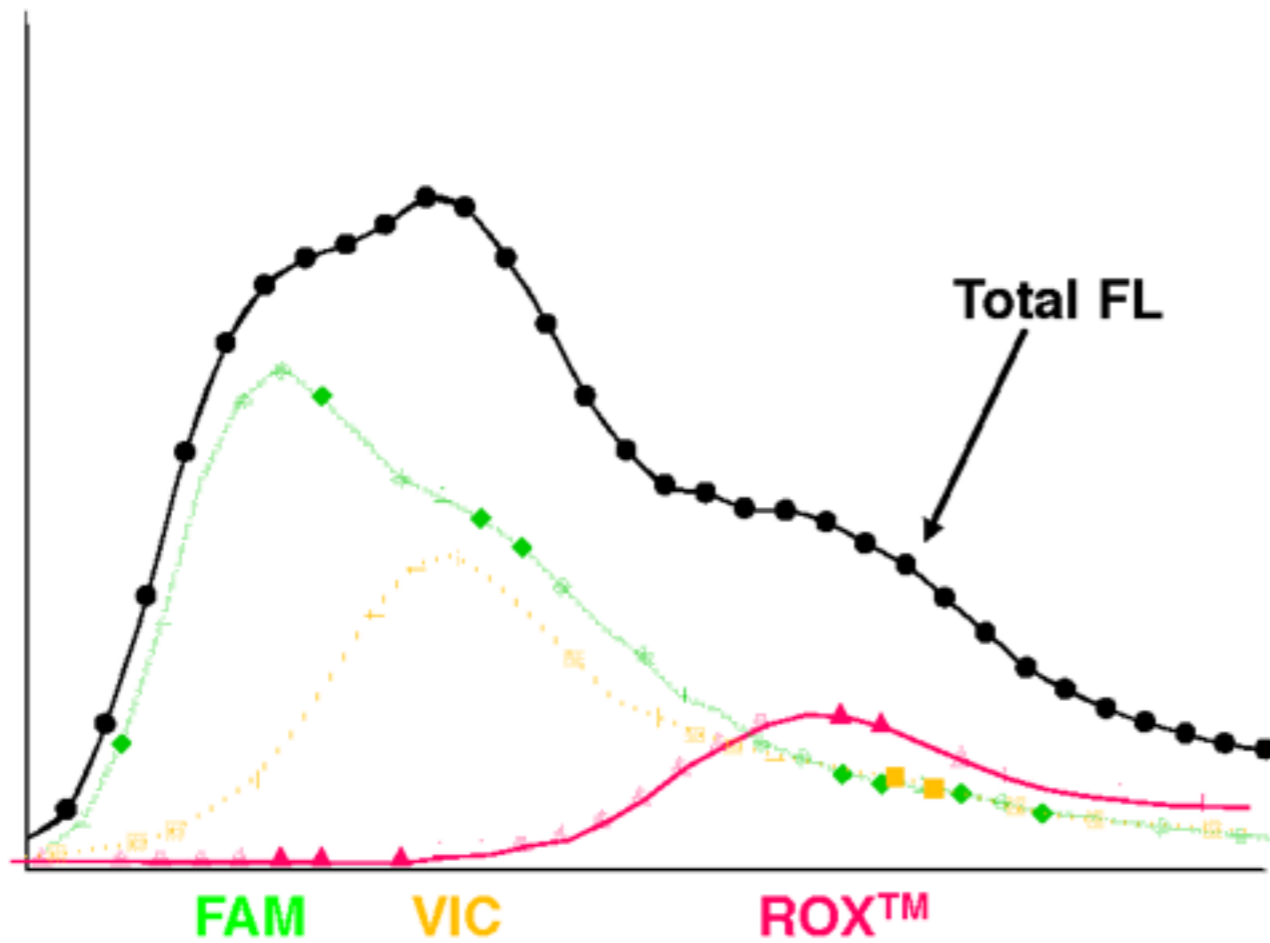
**ROX<sup>™</sup>**

**Multicomponenting**

Dyes have specific fluorescence spectra with specific peaks



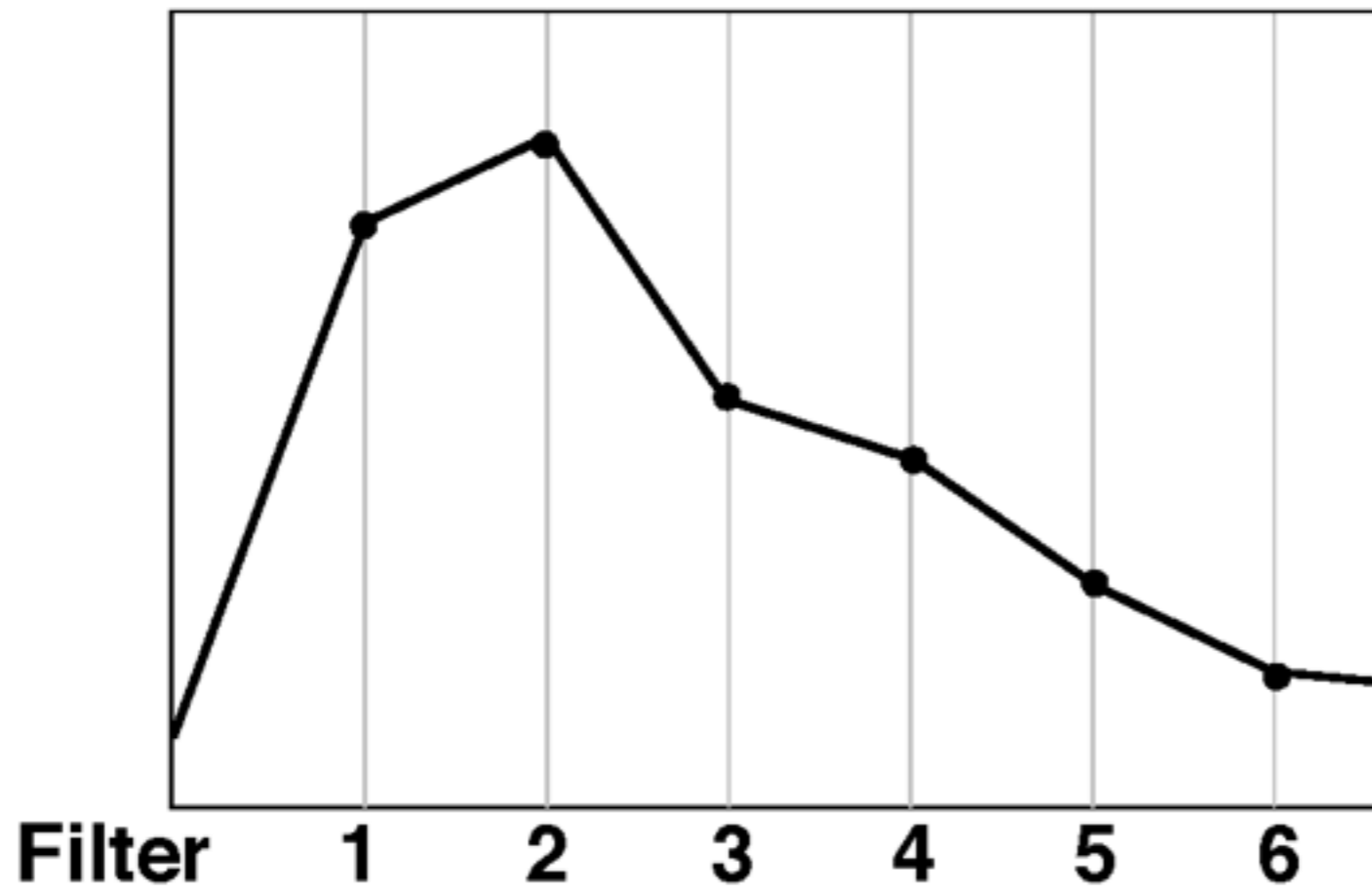
However, if more than one dye is present, there is spectral overlap



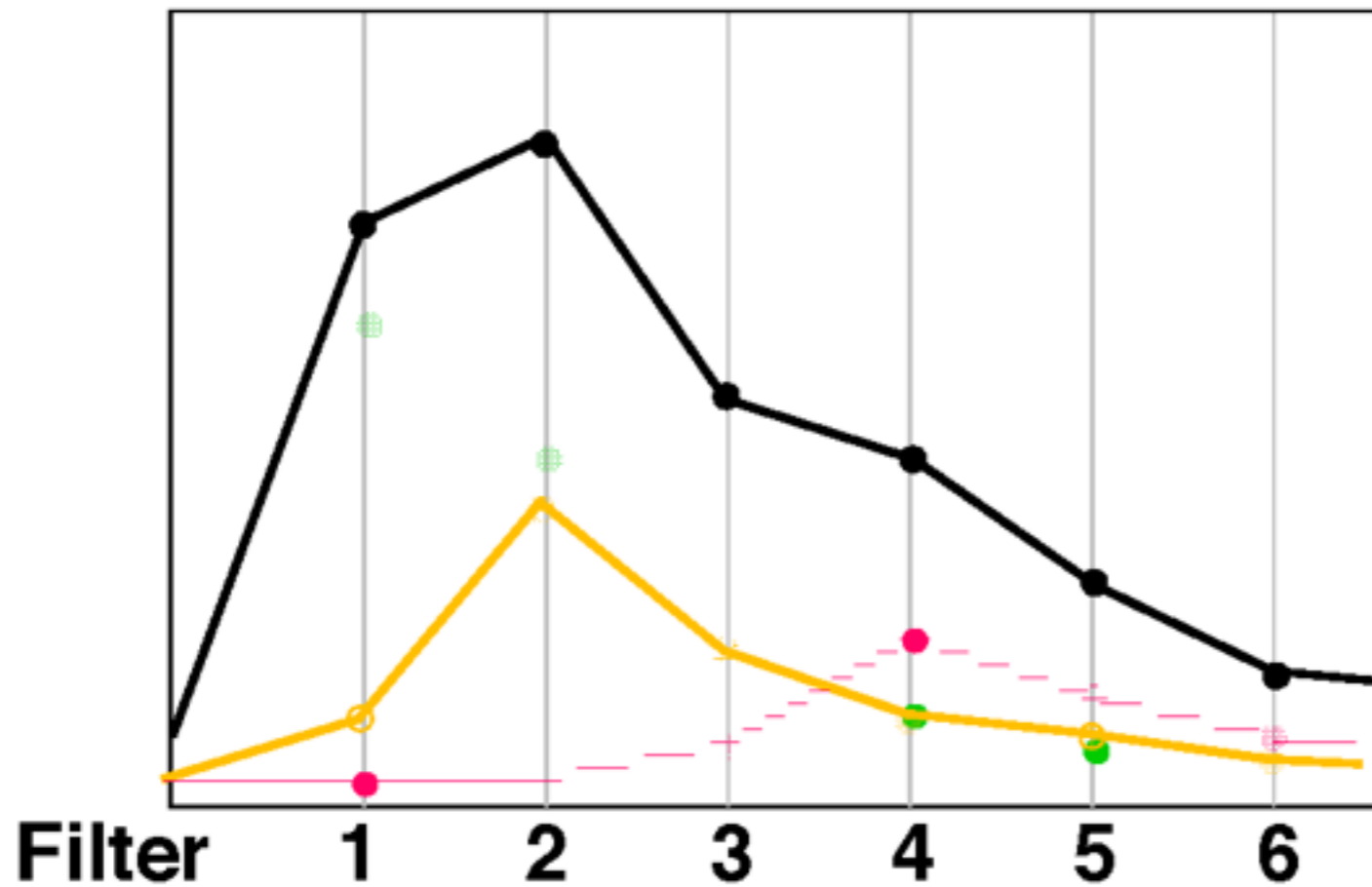
If not addressed, this would introduce large inaccuracies



On the ViiA7, this is depicted as a 5 or 6-point spectral curve

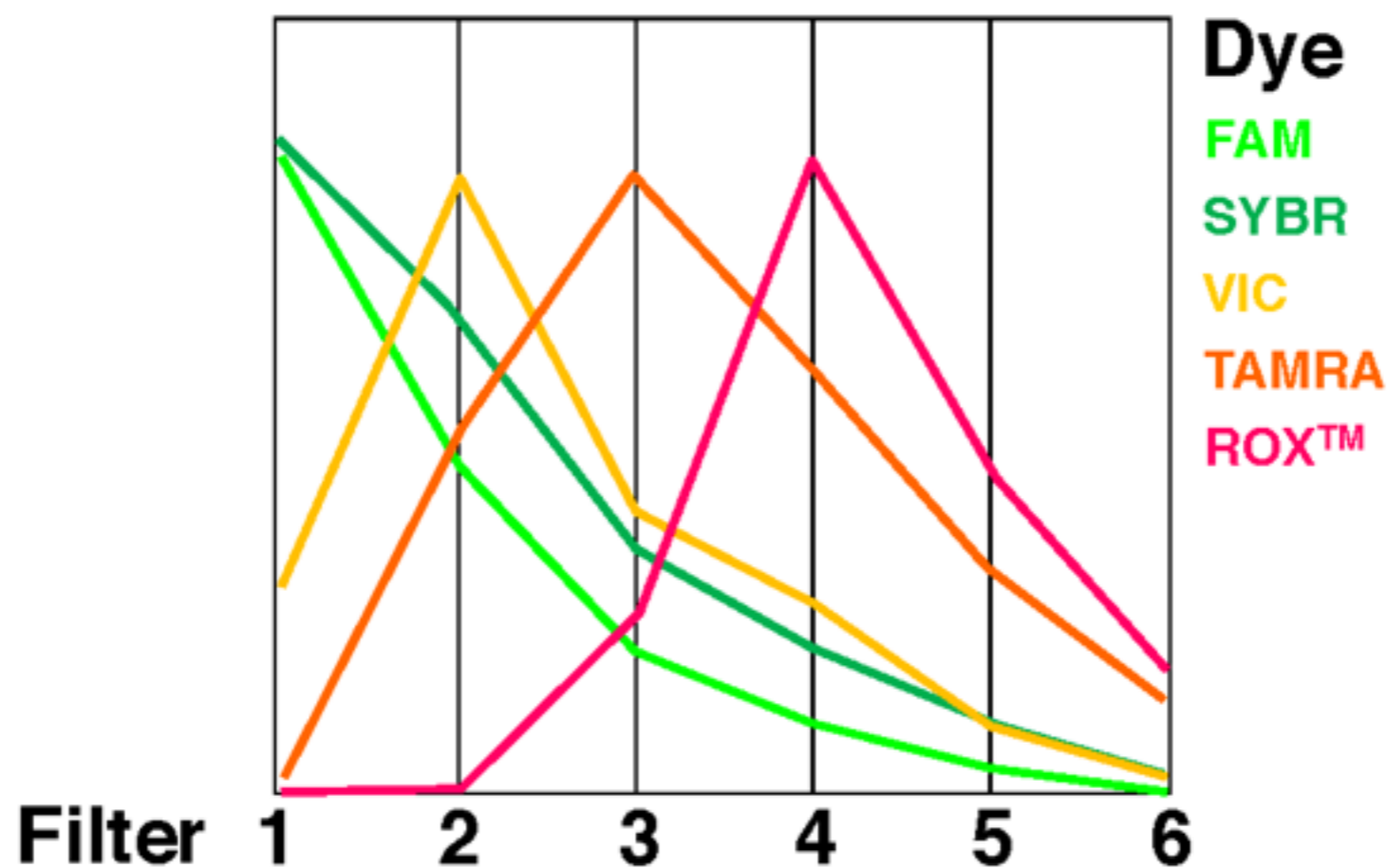


What we see is the Total Fluorescence at each wavelength – this is not the same as the individual dye fluorescence



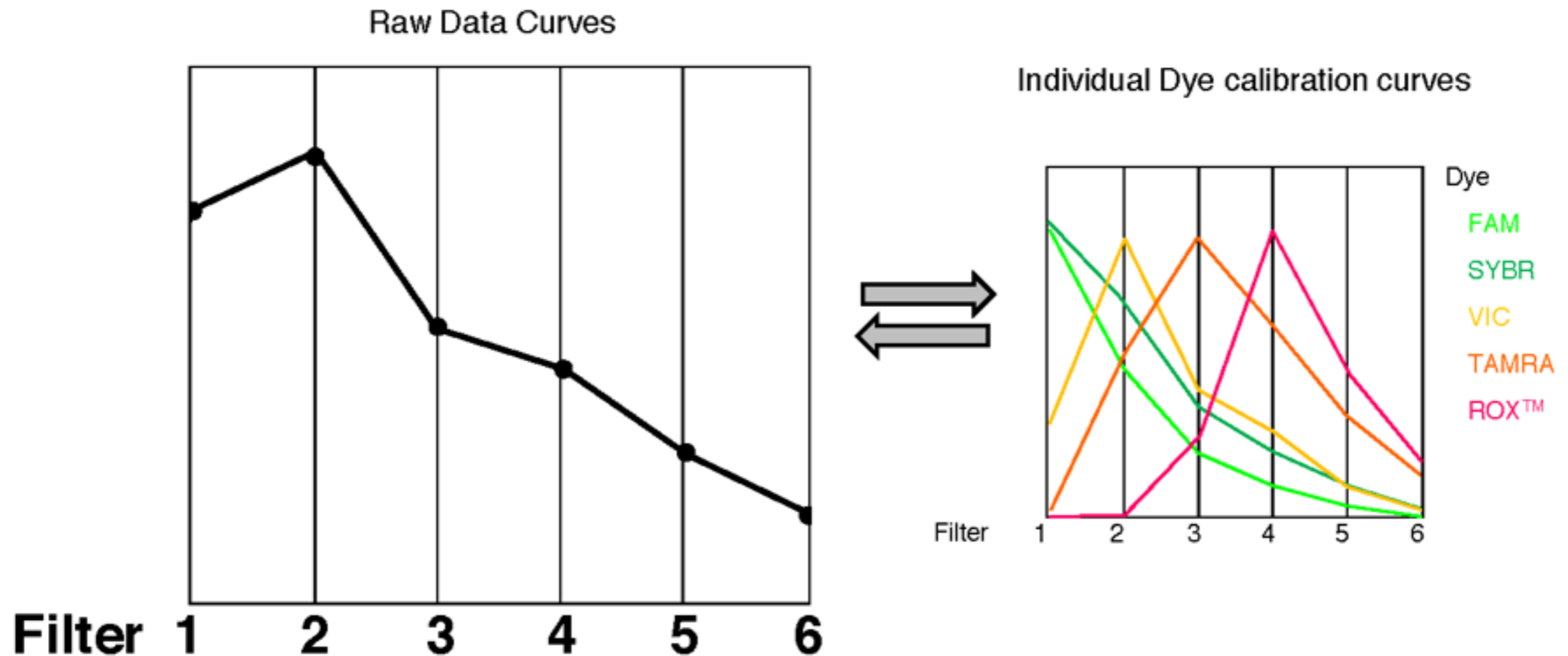
How do we adjust for this?

## Answer: Dye Calibration

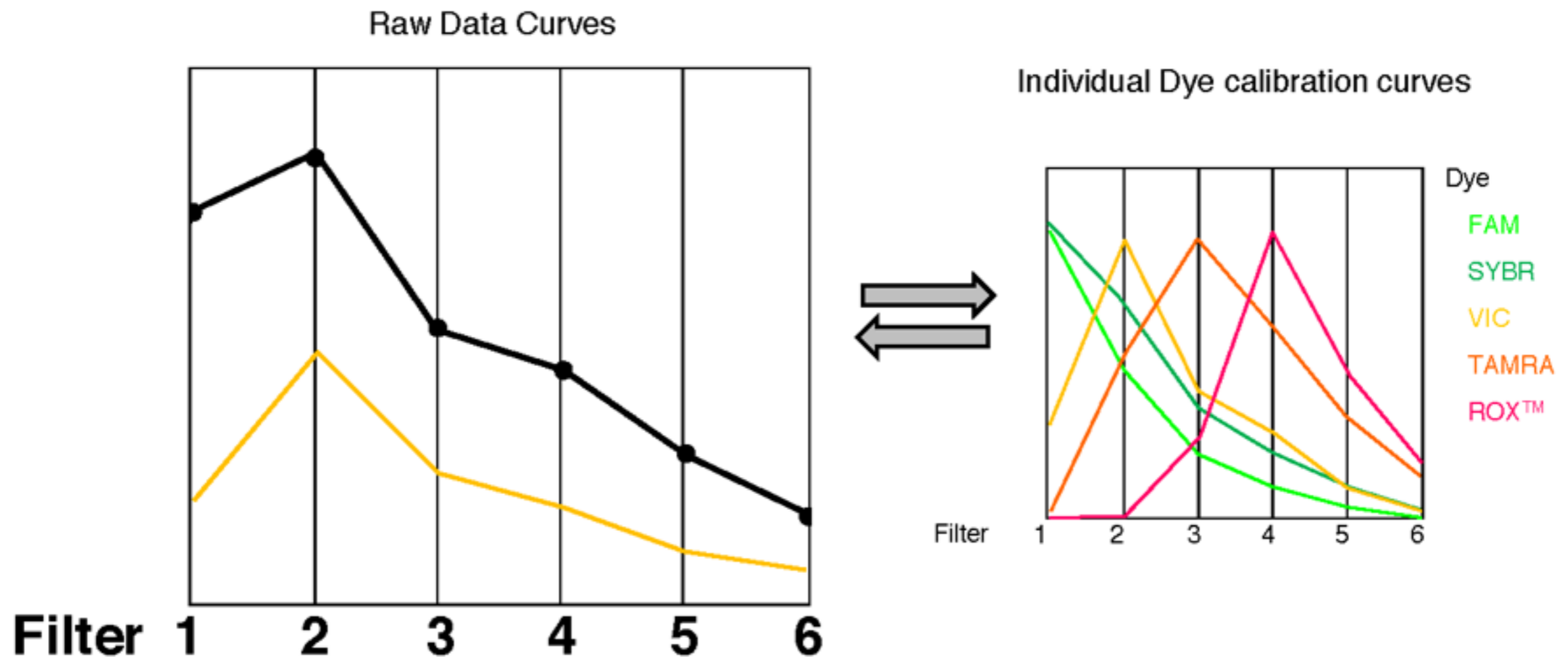


At installation, a dye calibration plate is read. This contains dilutions of pure dye. So the instrument records what each dye “looks like”.

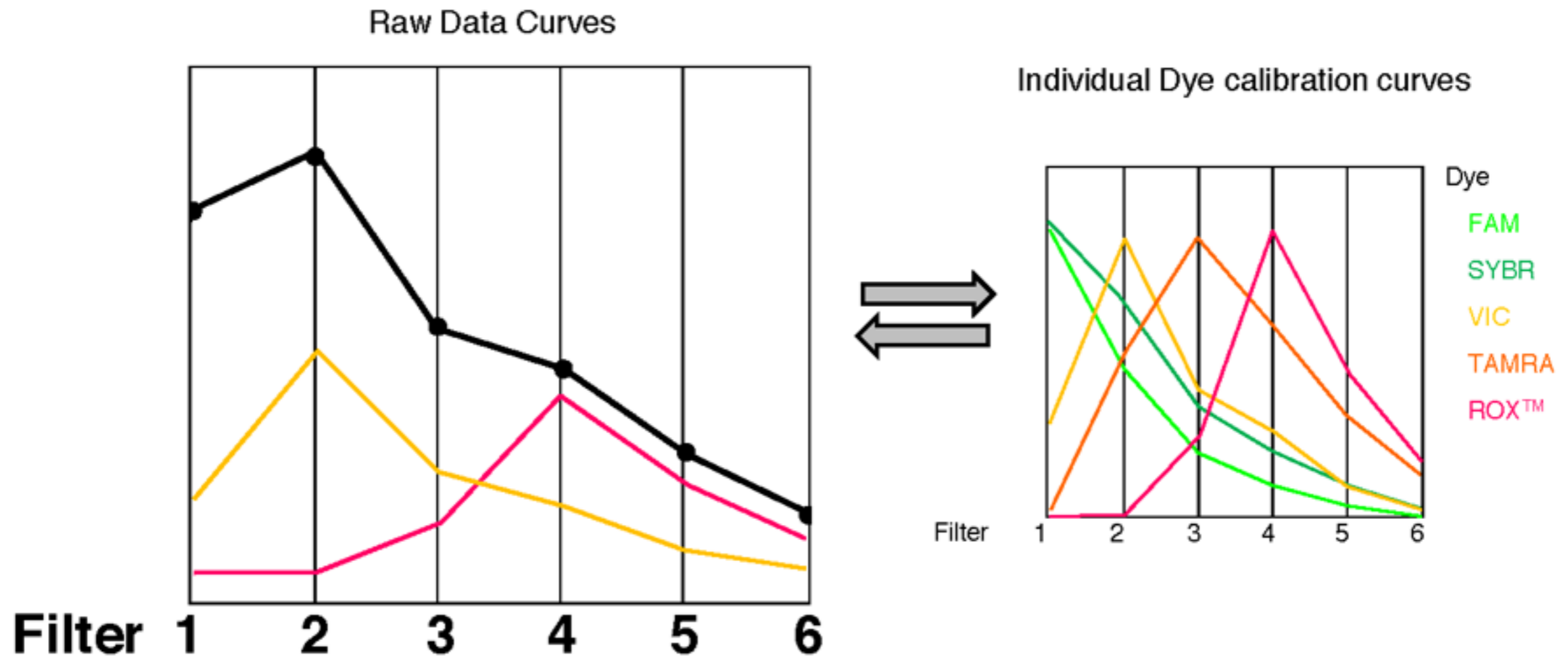
Then, the multicomponenting software reconstructs the Total FL curve from the individual dye curves



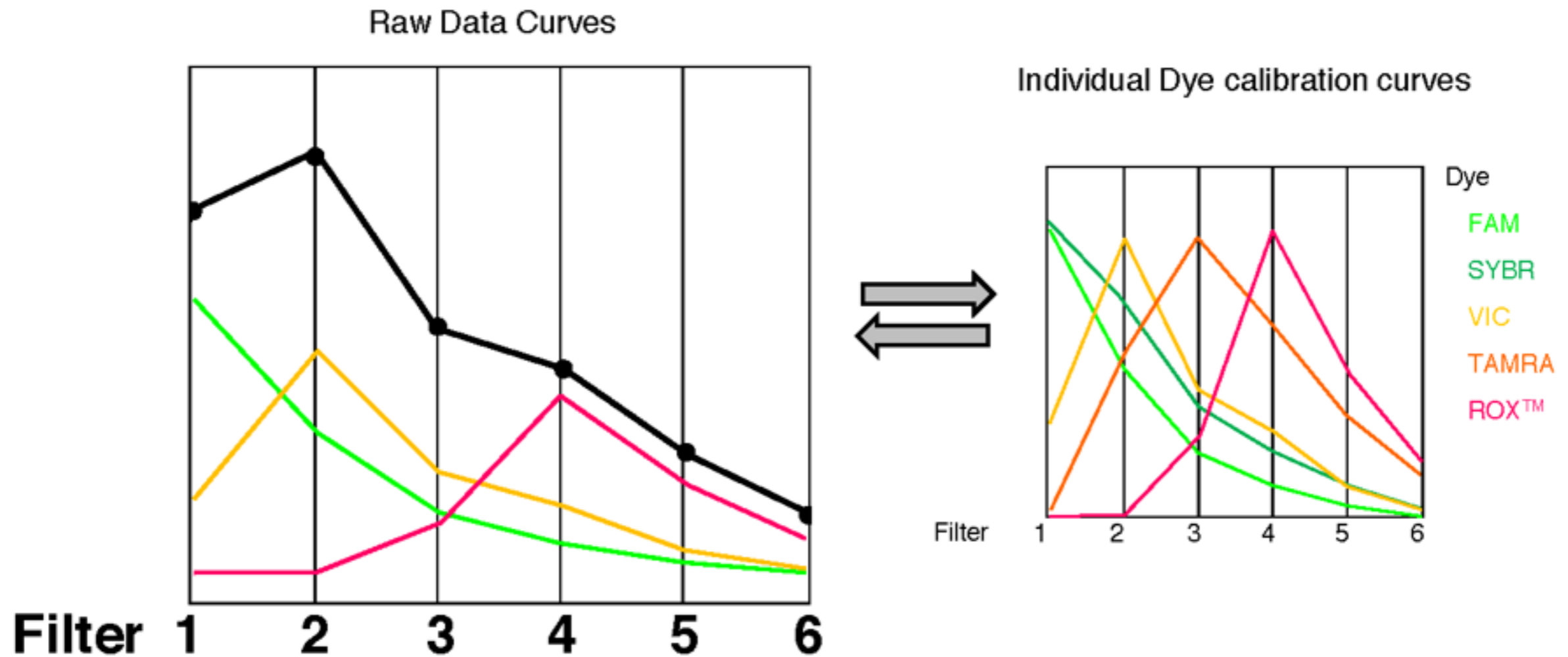
Then, the multicomponenting software reconstructs the Total FL curve from the individual dye curves



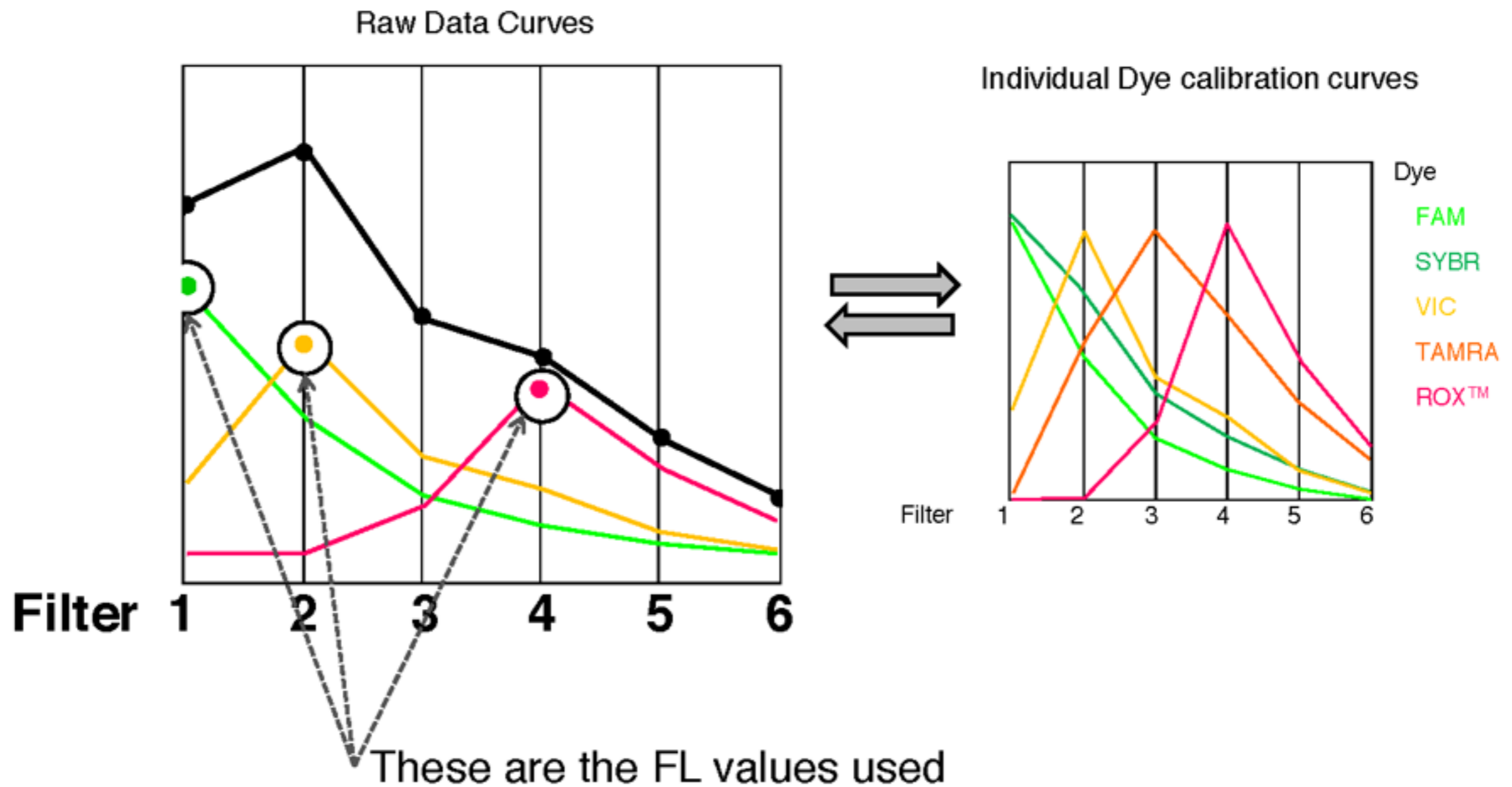
Then, the multicomponenting software reconstructs the Total FL curve from the individual dye curves



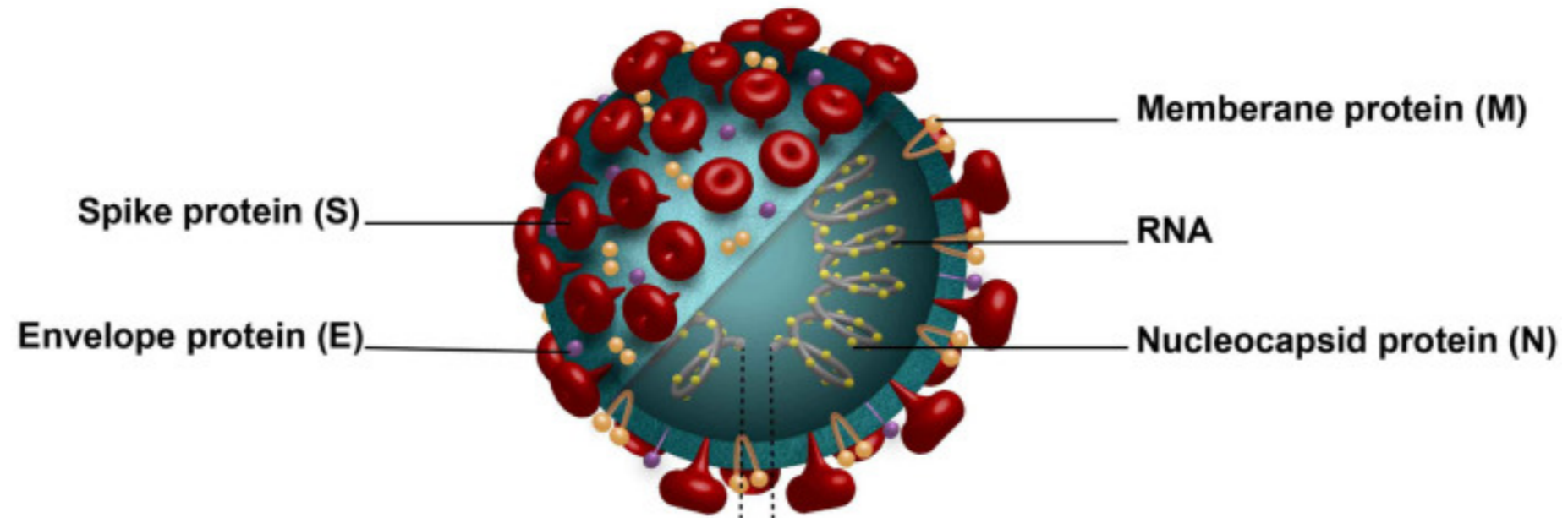
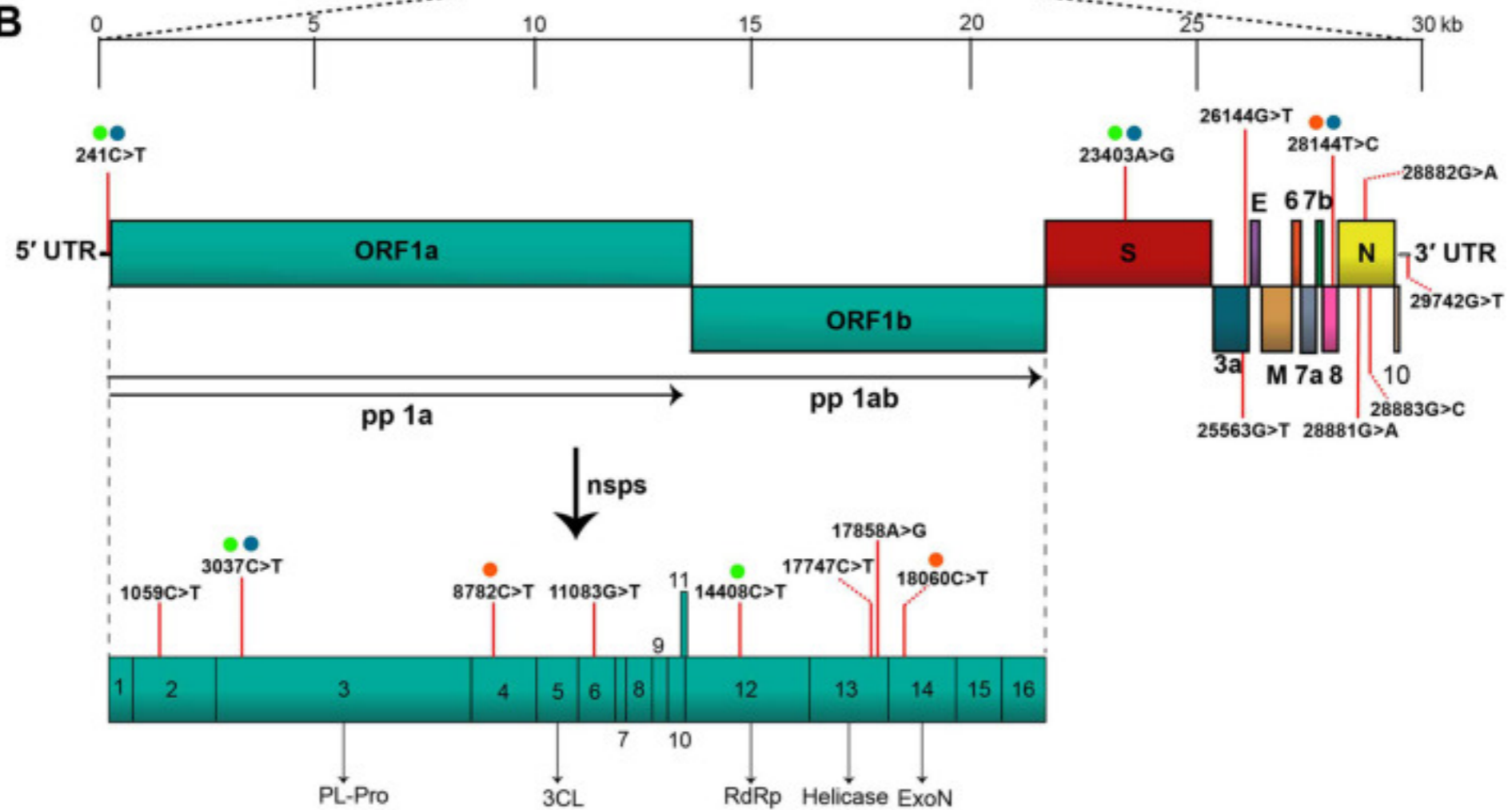
Then, the multicomponenting software reconstructs the Total FL curve from the individual dye curves



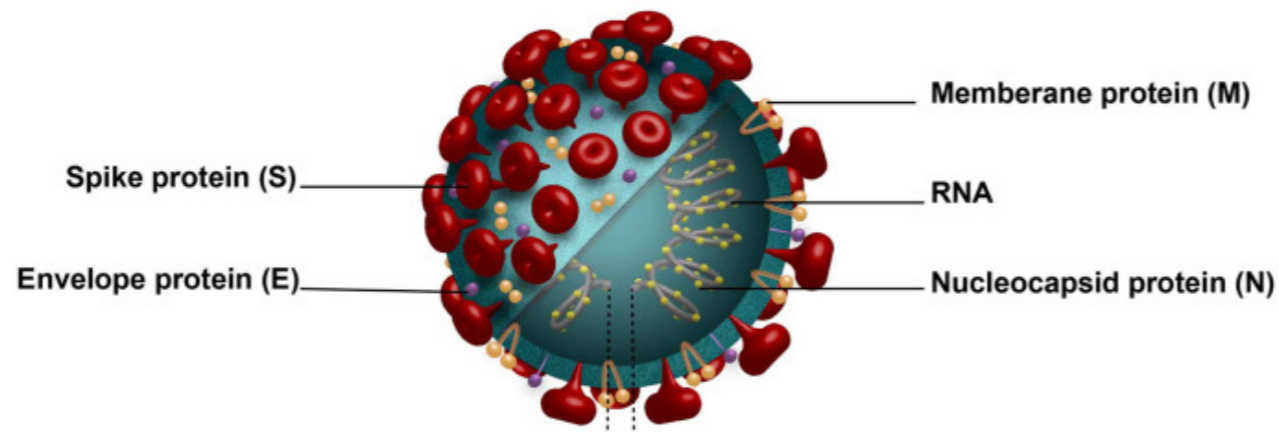
Then, the multicomponenting software reconstructs the Total FL curve from the individual dye curves



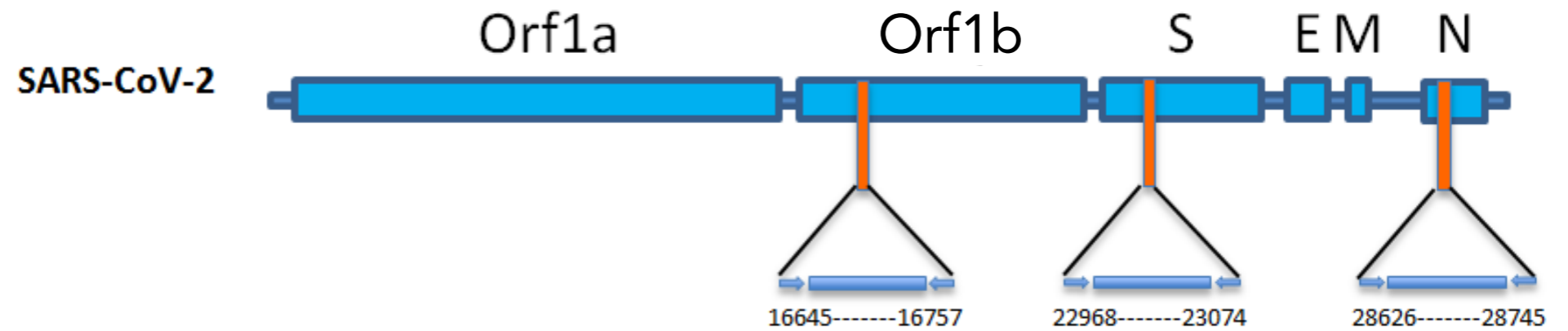
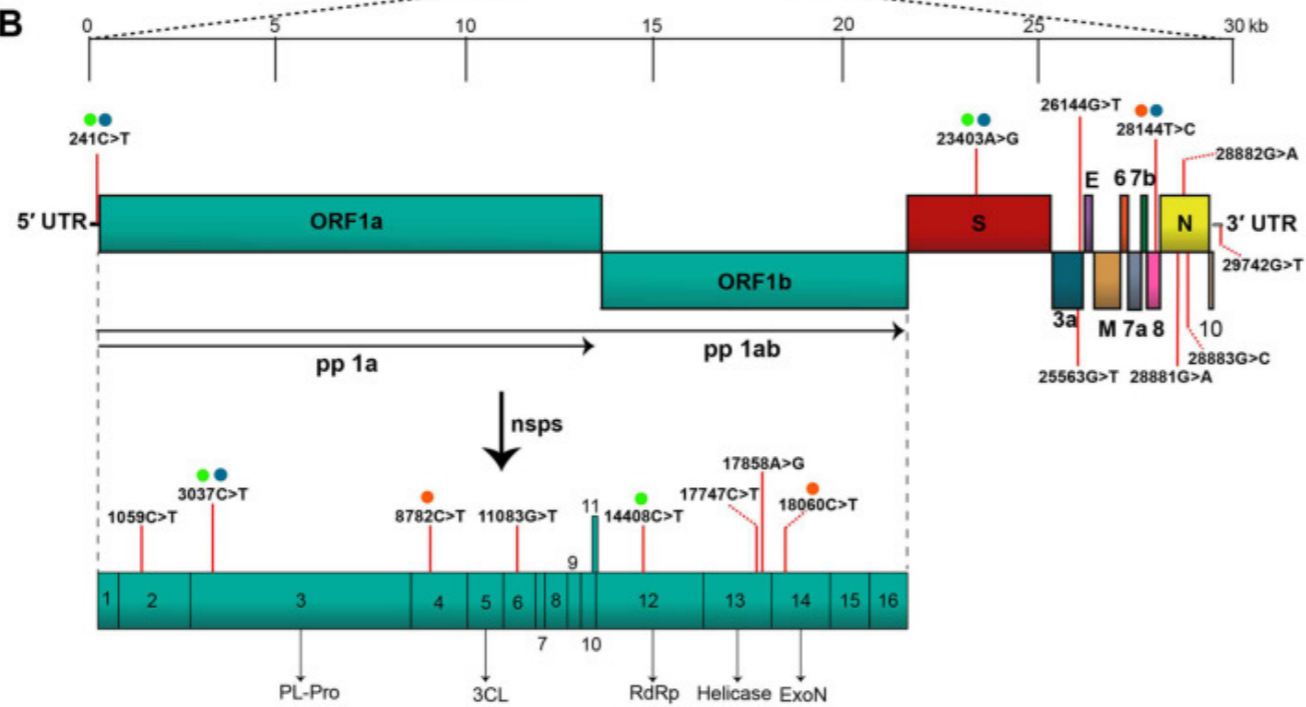


**A****B**

A



B

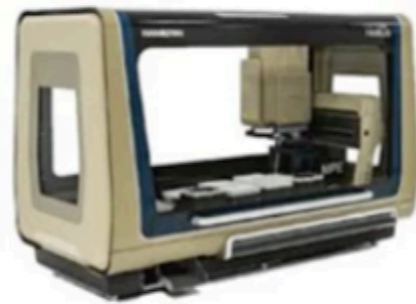


# HealthTrackRX – SARS-CoV-2 Diagnostic Test EUA

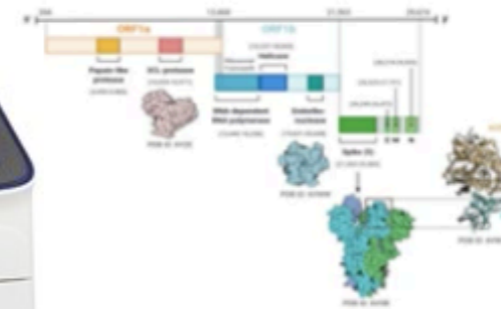
Sample Collection



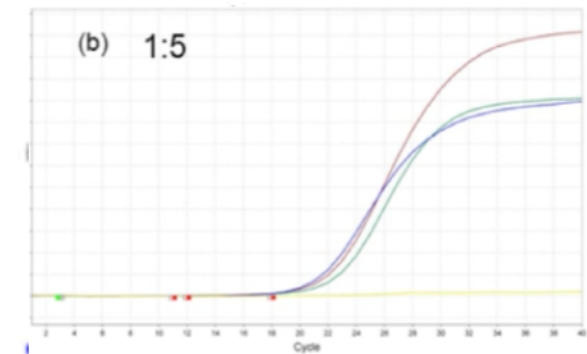
RNA Extraction



Real Time-PCR

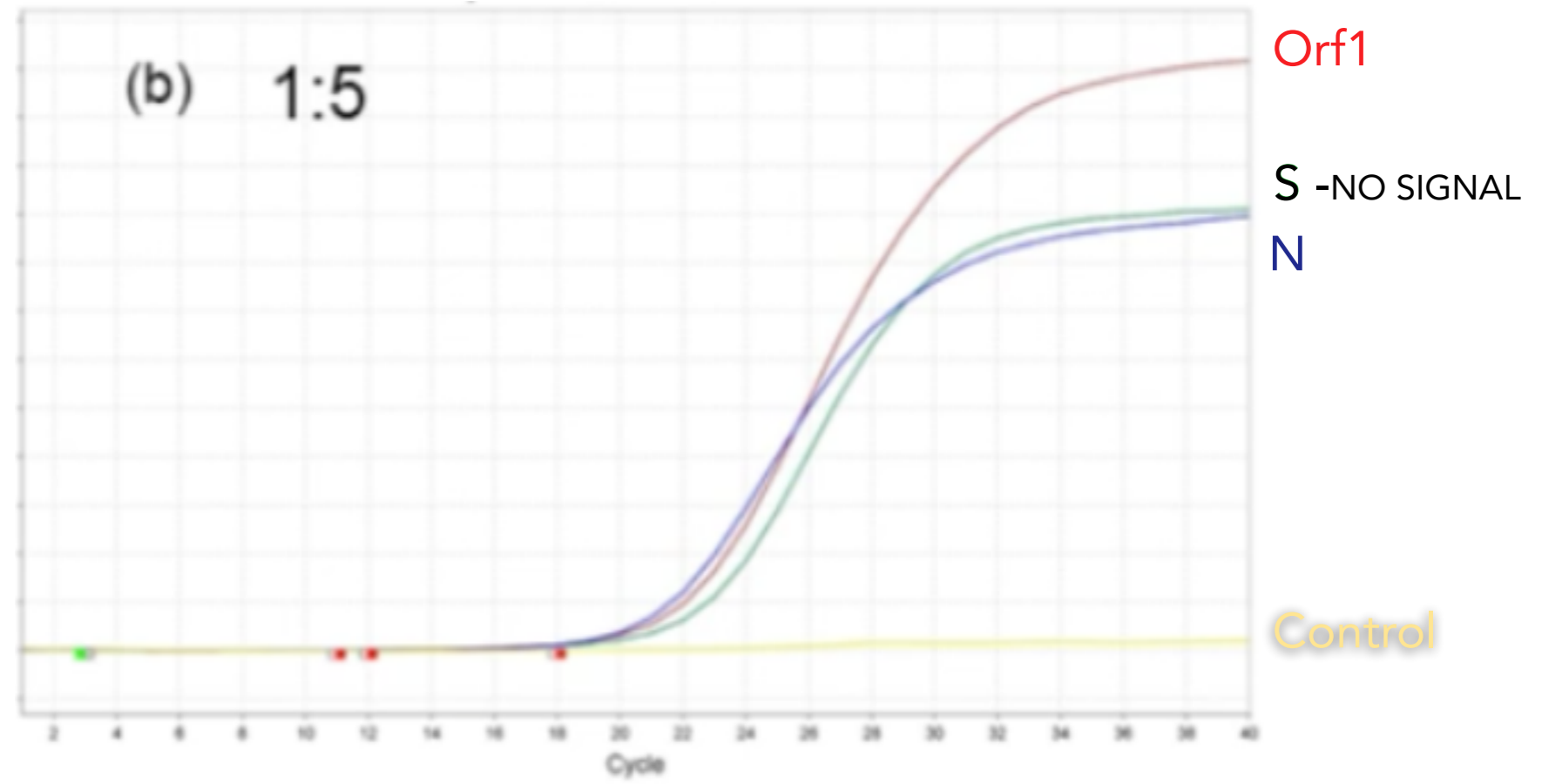


Results

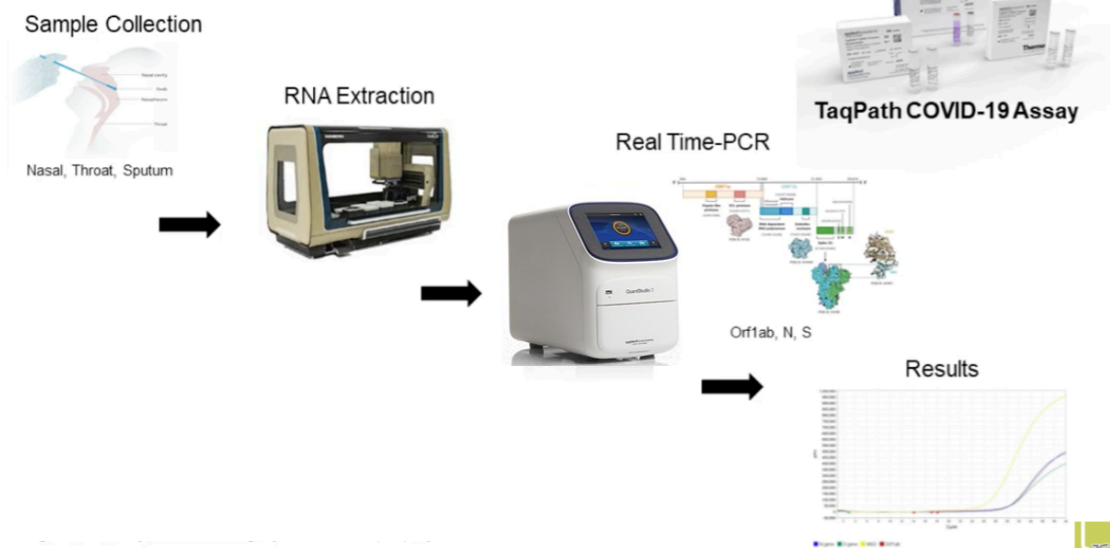




TaqPath COVID-19 Assay






### HealthTrackRX – SARS-CoV-2 Diagnostic Test EUA



Search All  Search by catalog number, product name, keyword, application 

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 In order to support the growing demand for this product, an additional Thermo Fisher Scientific manufacturing site has begun producing these products. [Please click here for more information.](#)

Applied Biosystems™

## TaqPath™ 1-Step RT-qPCR Master Mix, CG



**Catalog number:** A15299

**Related applications:** [Diagnostic Development](#) | [Real Time PCR \(qPCR\)](#)  
| [miRNA & Non-Coding RNA Analysis](#) | [Real Time PCR-Based Gene Expression Profiling](#)  
| [SARS-CoV-2 Pathogen Research Solutions](#)

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	Catalog number	Unit Size	Price (USD)	Qty
☆	A15299	5 x 1 mL	2,085.00 Your price: <a href="#">Sign In</a> ⓘ	<input type="text"/>
☆	<a href="#">A15300</a>	1 x 10 mL	3,925.00 Your price: <a href="#">Sign In</a> ⓘ	<input type="text"/>

Save to list

Add To Cart

Volume

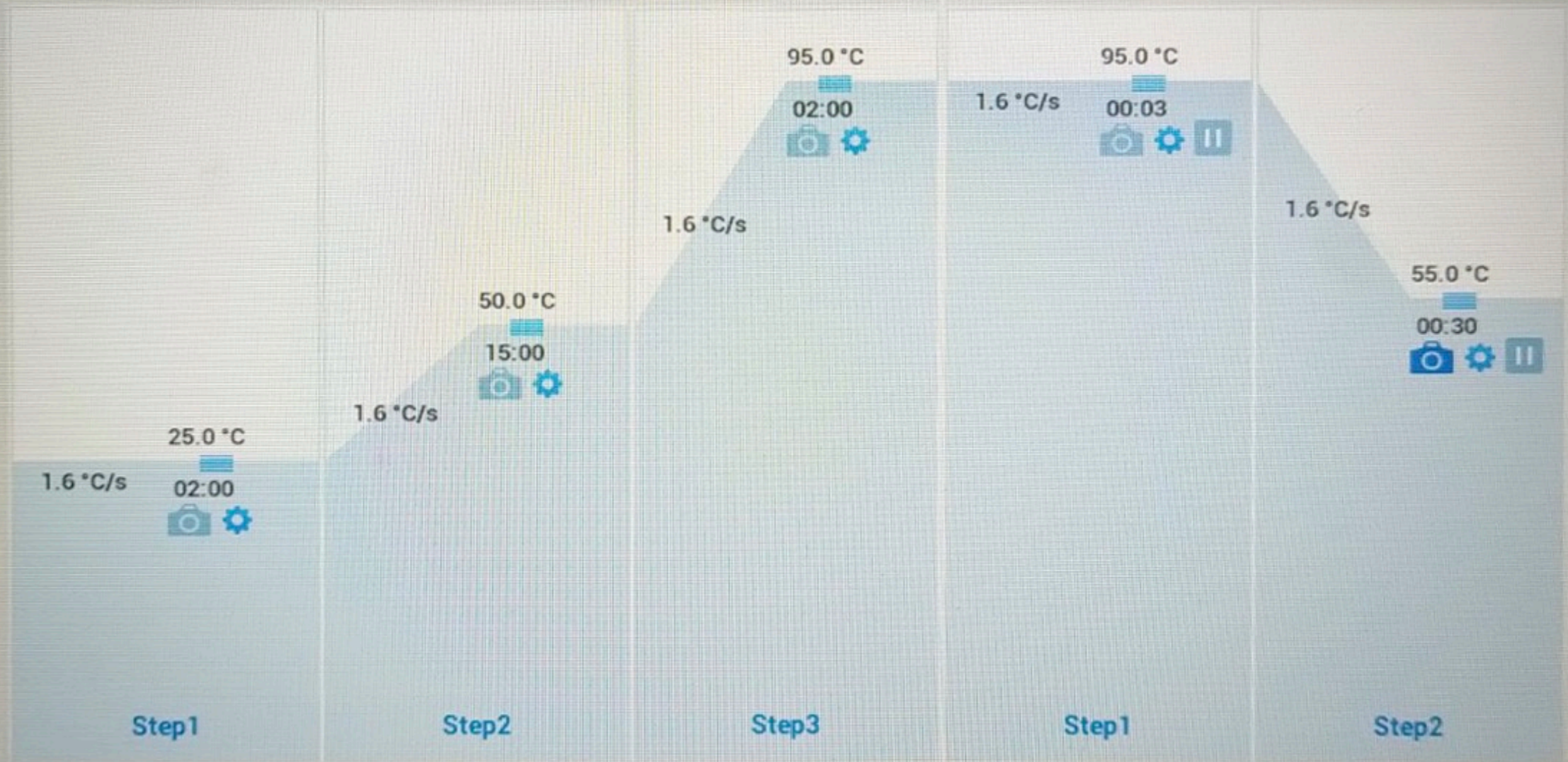
20  $\mu$ L

Cover

105.0  $^{\circ}$ C

### Hold Stage

### PCR Stage



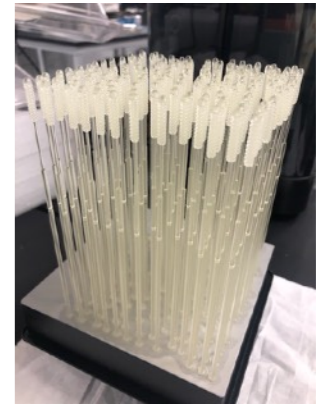
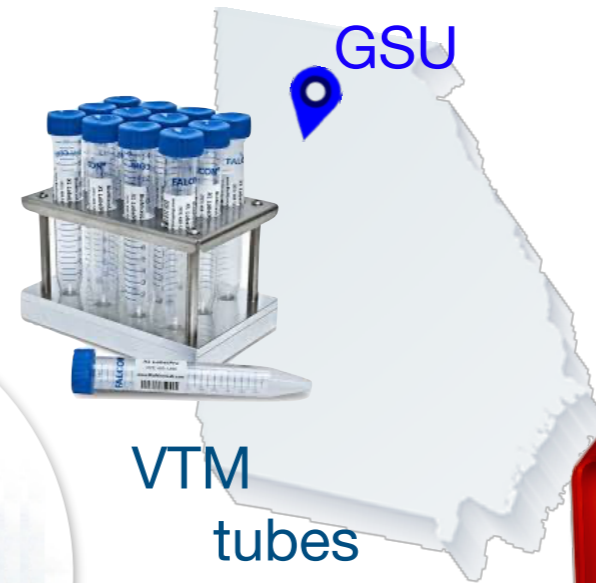
45 x

nds: Data Collection On Data Collection Off Pause On Pause Off Advanced Settings VeriFlex

# SARS-CoV-2 Pandemic

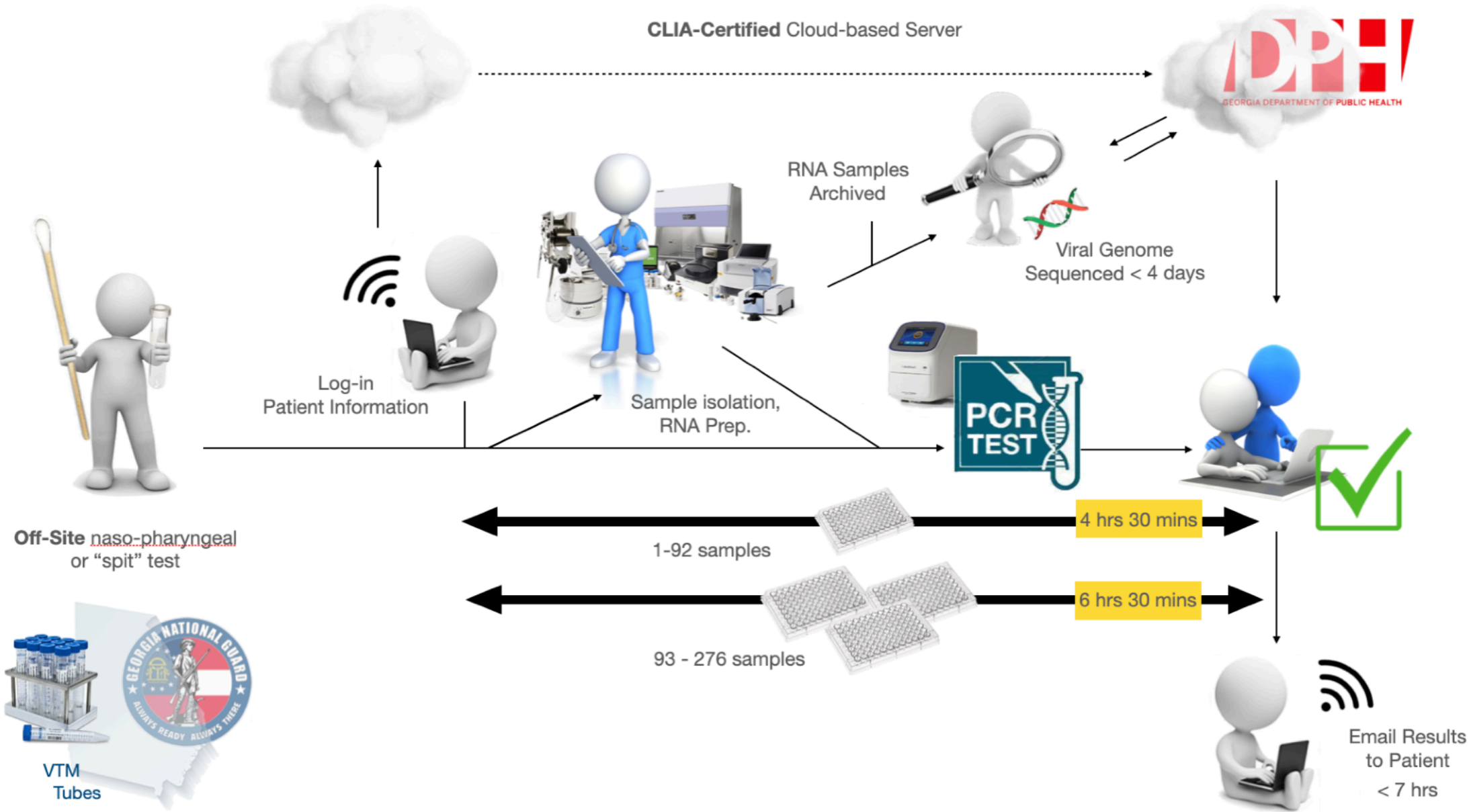
## CLIA-certified COVID-19 PCR testing lab

# 2020-22



**F**acilities

# CLIA-certified COVID-19 PCR testing lab





# SARS-CoV-2 Pandemic

## SARS-CoV-2

## Pfizer / Moderna Vaccine Storage

# 2021-22



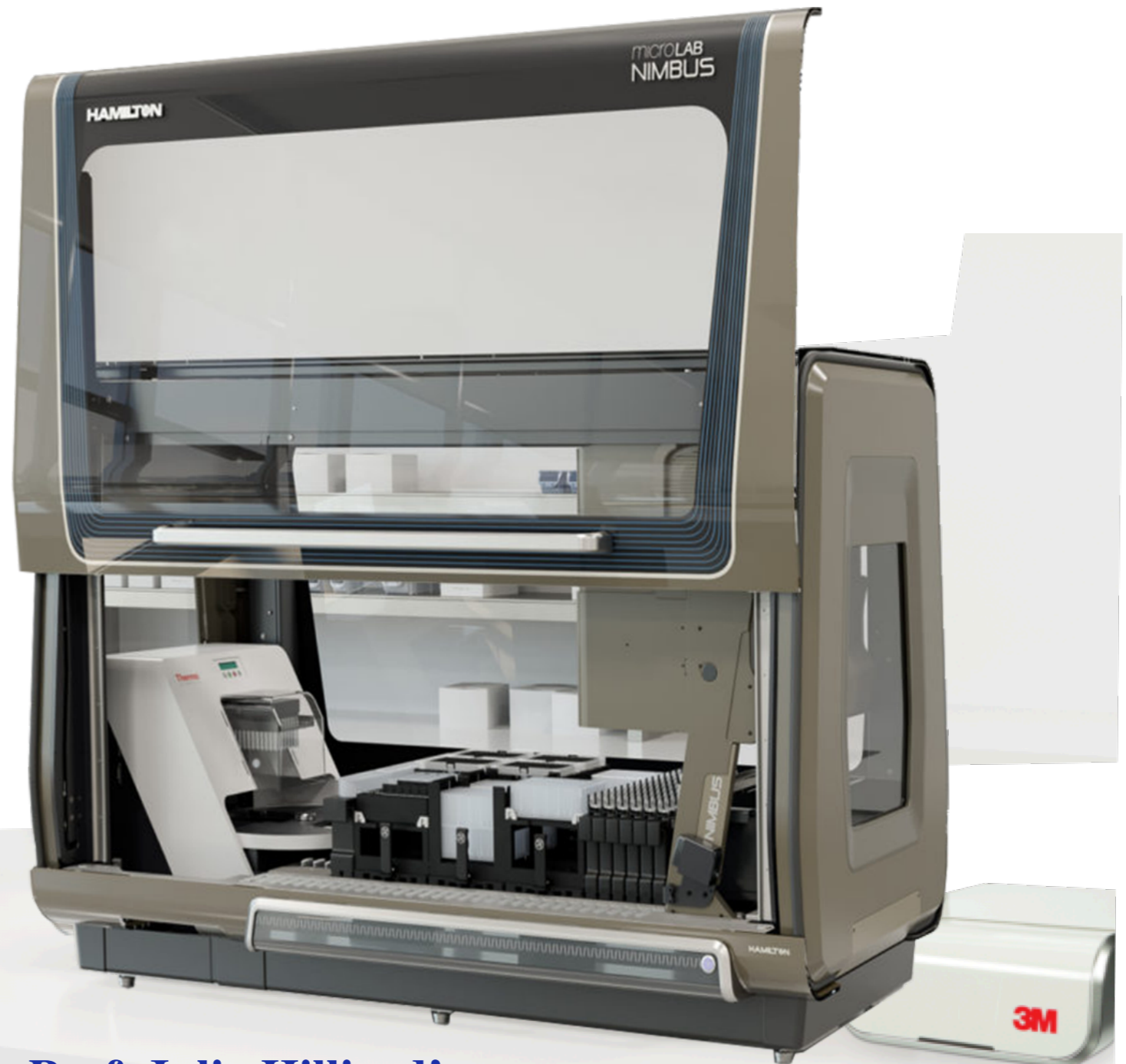
Dr. Azonobi  
GSU Student Health Ctre.



**F**acilities



**Automated Cell  
Disrupter**  
**Kingfisher /Presto**  
**(Thermo)**

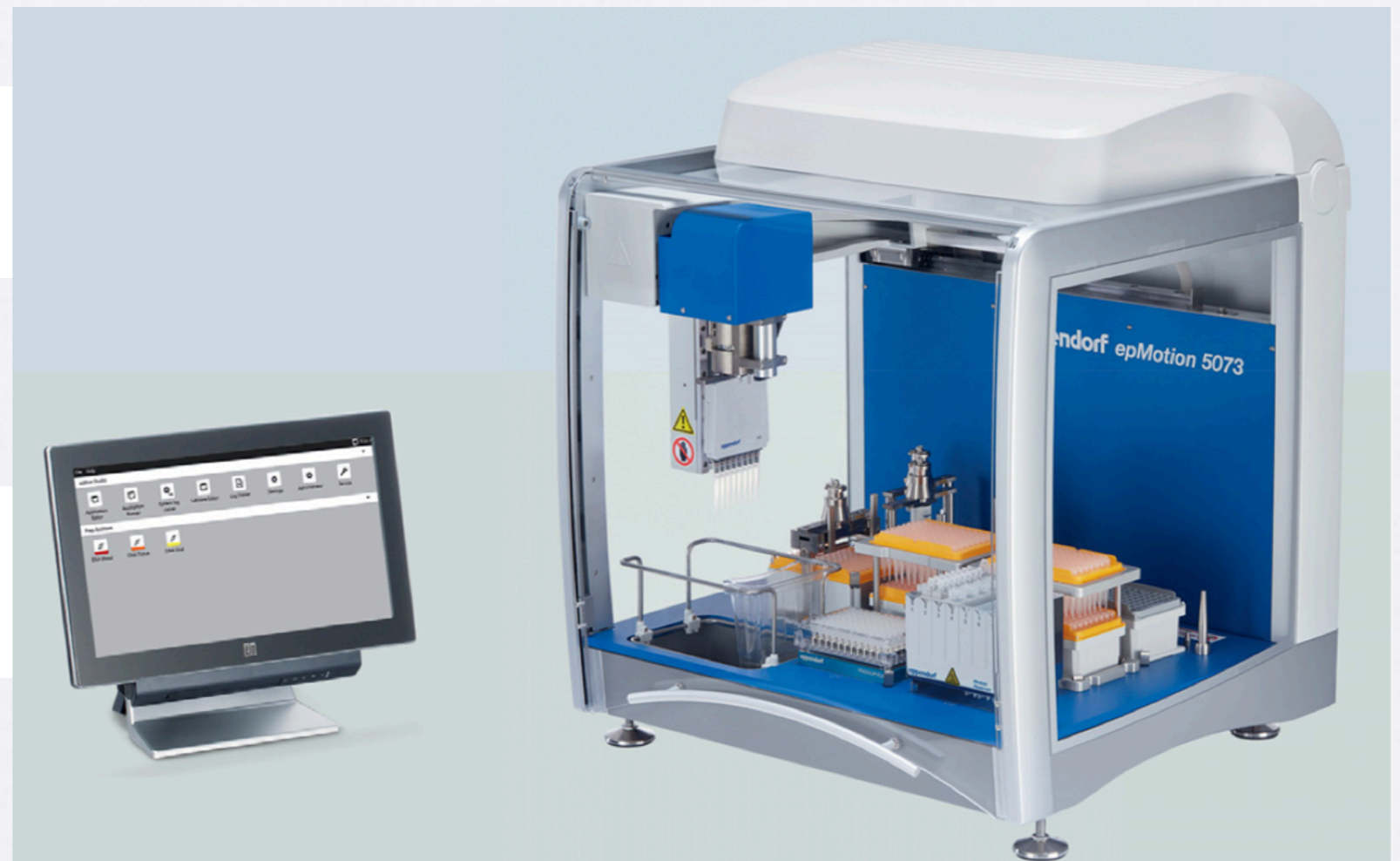


**Robotic  
Workstation**  
**Nimbus**  
**(Hamilton)**

**Prof. Julia Hilliard's  
COVID-19 Testing Facilities**



**Robotic  
Workstation  
Integra Assist Plus  
(Integra)**



**EpMotion 5073  
(Eppendorf)**