

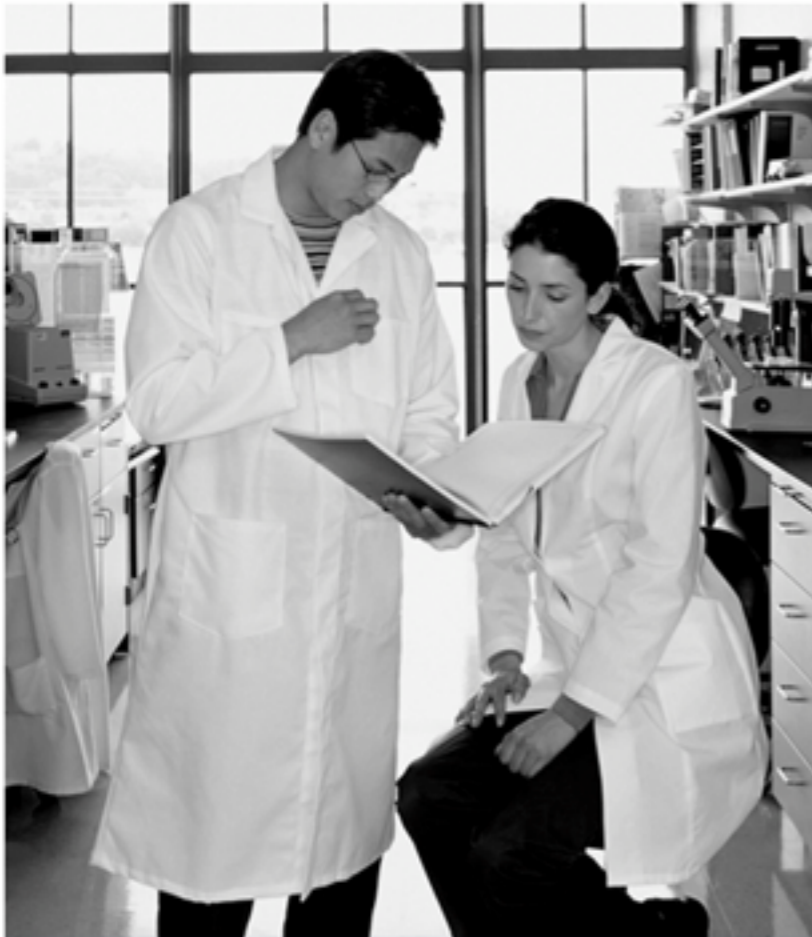
SUMMER INSTITUTE CALENDAR 2022

SUN	MON	TUE	WED	THU	FRI	SAT
						July 02
						Early Arrival Airport Arrivals and Check-in
July 03	04	05	06	07	08	09
Early Arrival Airport Arrivals and Check-in	Airport Arrivals and Check-in 6:00pm: 4th of July Celebrations	9:30am-12pm: Campus tour, Panther ID & ISSS Check-in 12-2pm Lunch 2:00-6:00pm, Shuttle to local grocery store	9:30am-11:30am ISSS, OII, & Housing Orientation & Presentation 2:30-4:30pm:-Welcome Reception and Buddy Meet & Greet Event	Classes begin! 9-11:20am: Morning course 11:20am-2:00pm: Lunch break 1:30-4:30pm: BIOL4905 INTRO - TRAINING	9-11:20am: Morning course 11:20am-2:00pm: Lunch break 1:30-4:30pm: BIOL4905 DNA PREPARATION	Free Day
10	11	12	13	14	15	16
12:00-4:00pm: The World Coca-Cola and Georgia Aquarium	9-11:20am: Morning course 11:20am-2:00pm: Lunch break 1:30-4:30pm: BIOL4905 PROTEOMICS I	9-11:20am: Morning course 11:20am-2:00pm: Lunch break 1:30-4:30pm: BIOL4905 PROTEOMICS II	9-11:20am: Morning course 11:20am-2:00pm: Lunch break 1:30-4:30pm:BIOL4905 PROTEOMICS III 6:00-10:00pm: Atlantic Station Shopping & Movie (Sign up)	9-11:20am: Morning course 11:20am-2:00pm: Lunch break 1:30-4:30pm: BIOL4905 PROTEOMICS IV ?	9-11:20am: Morning course 11:20am-2:00pm: Lunch break 1:30-4:30pm: BIOL4905 RNA PREPARATION	6:00-9:00pm: Dinner in America (Sign-up)
17	18	19	20	21	22	23
Free Day	9-11:20am: Morning course 11:20am-2:00pm: Lunch break 1:30-4:30pm: BIOL4905 qPCR & AUTOMATION	9-11:20am: Morning course 11:20am-2:00pm: Lunch break 1:30-4:30pm: BIOL4905 DNA SEQUENCING	MINI BREAK	9-11:20am: Morning course CDC TRIP 1:30-4:30pm: BIOL4905 MICROSCOPY / AFM	9-11:20am: Morning course 11:20am-2:00pm: Lunch break 1:30 - 4:30pm: BIOL4905 NEXT GEN SEQ. 5:30-7:30pm: Meet & Greet BBQ event @ The Commons	9:00am - 6:00pm: Outlet Mall
24	25	26	27	28	29	30
Free Day	9-11:20am: Morning course 11:20am-2:00pm: Lunch break 1:30-4:30pm: BIOL4905 MICROARRAY I	9-11:20am: Morning course 12:30 - 1:30pm: Lunch and LearnGrad School Info Session 2:00 - 5:00pm: BIOL4905 MICROARRAY II	9-11:20am: Morning course 11:20am-2:00pm: Lunch break 1:30-4:30pm: BIOL4905 NANOSTRING	Last day of classes 9-11:20am: Morning course 11:20am-2:00pm: Lunch break 1:30-4:30pm: BIOL4905 FLOW CYTOMETRY	FINALS	Free Day
31	August 01	02	03	04		
Free Day	Activity Day at the Recreation Center (Sign-up)	Free Day	9:30-11:00am: Georgia Capitol Tour (Sign-up) 2:00-4:00pm: Closing Reception	Departures (check-out at 12:00pm)		

Note: Students may arrive prior to the program date with an extra charge of \$35 per night. Earliest day to check-in to University Commons is July 2.

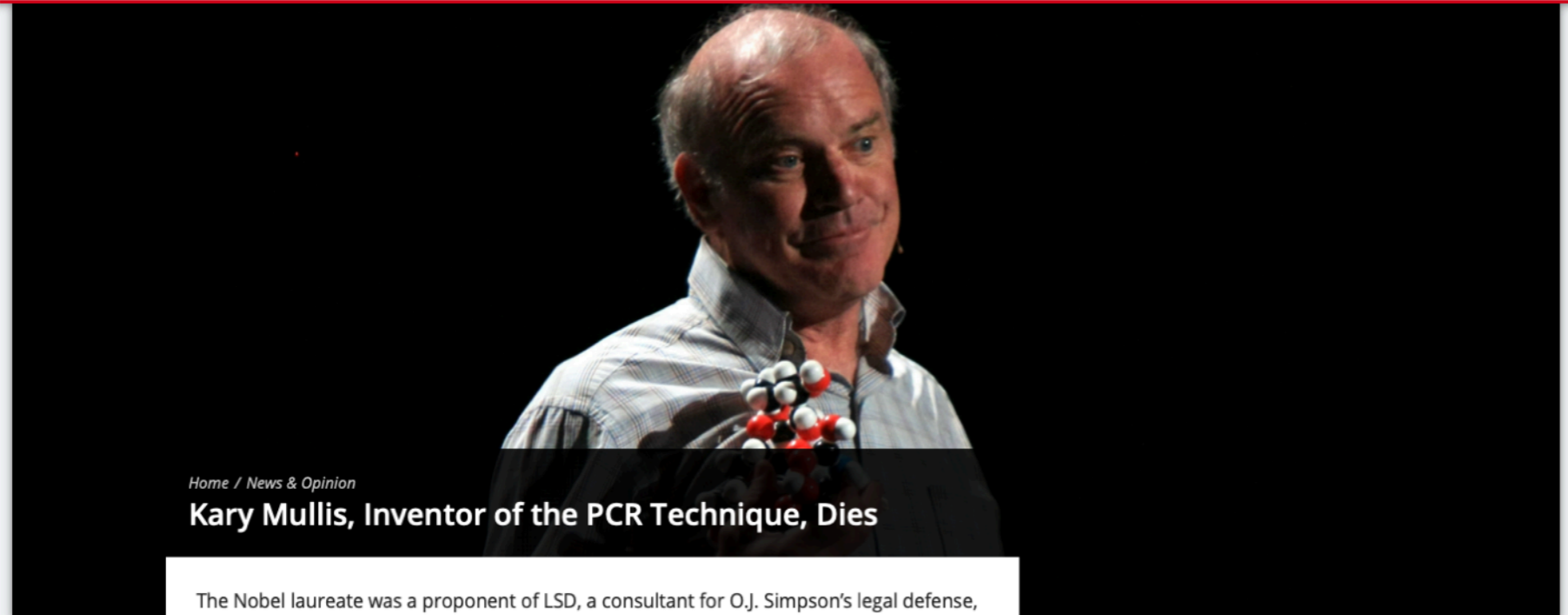
Legend:

Orange: Courses Blue: Lunch Break Red: Sign-up events



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

47K

Kary 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."

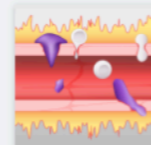
"He was personally and professionally one of the more iconic personalities science has ever witnessed," Rich Robbins, the founder and CEO of Wareham Development, a real estate developer for a number of biotech companies, tells the Emeryville, California-based paper, the *E'ville Eye*.

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



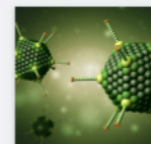
Trending



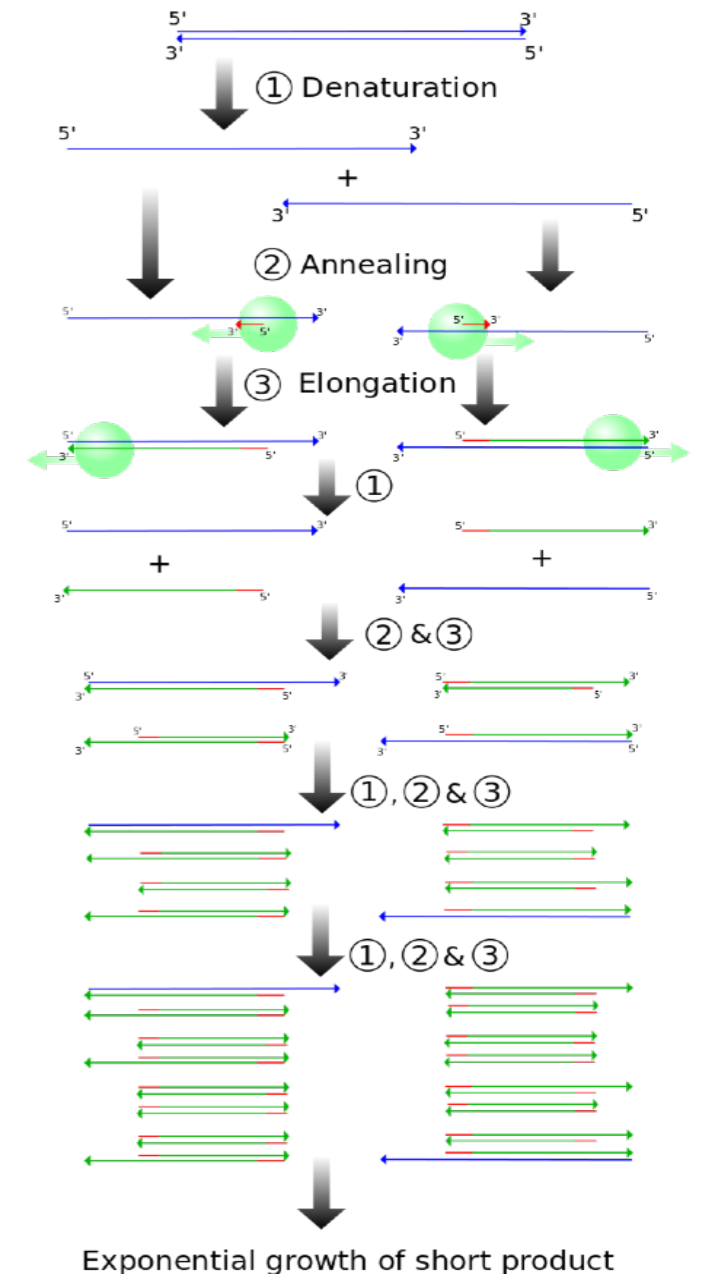
Is a Bradykinin Storm Brewing in COVID-19?



COVID-19 Vaccine Trial Pauses After Adverse Reaction



Scientists Voice Concerns over Russian COVID-19 Vaccine Study



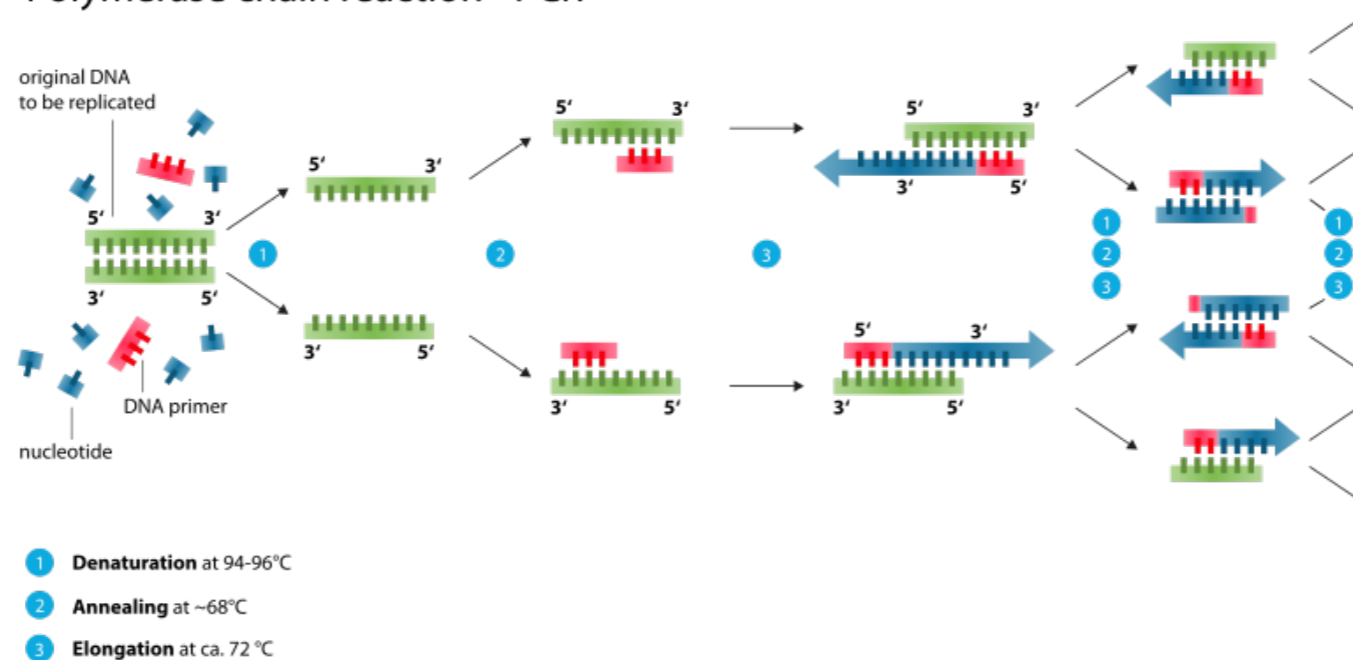
Exponential growth of short product

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.^[10] Cetus took Mullis off his usual projects to concentrate on PCR full-time. Mullis succeeded in demonstrating PCR December 16, 1983.^[10] He received a \$10,000 bonus from Cetus for the invention.

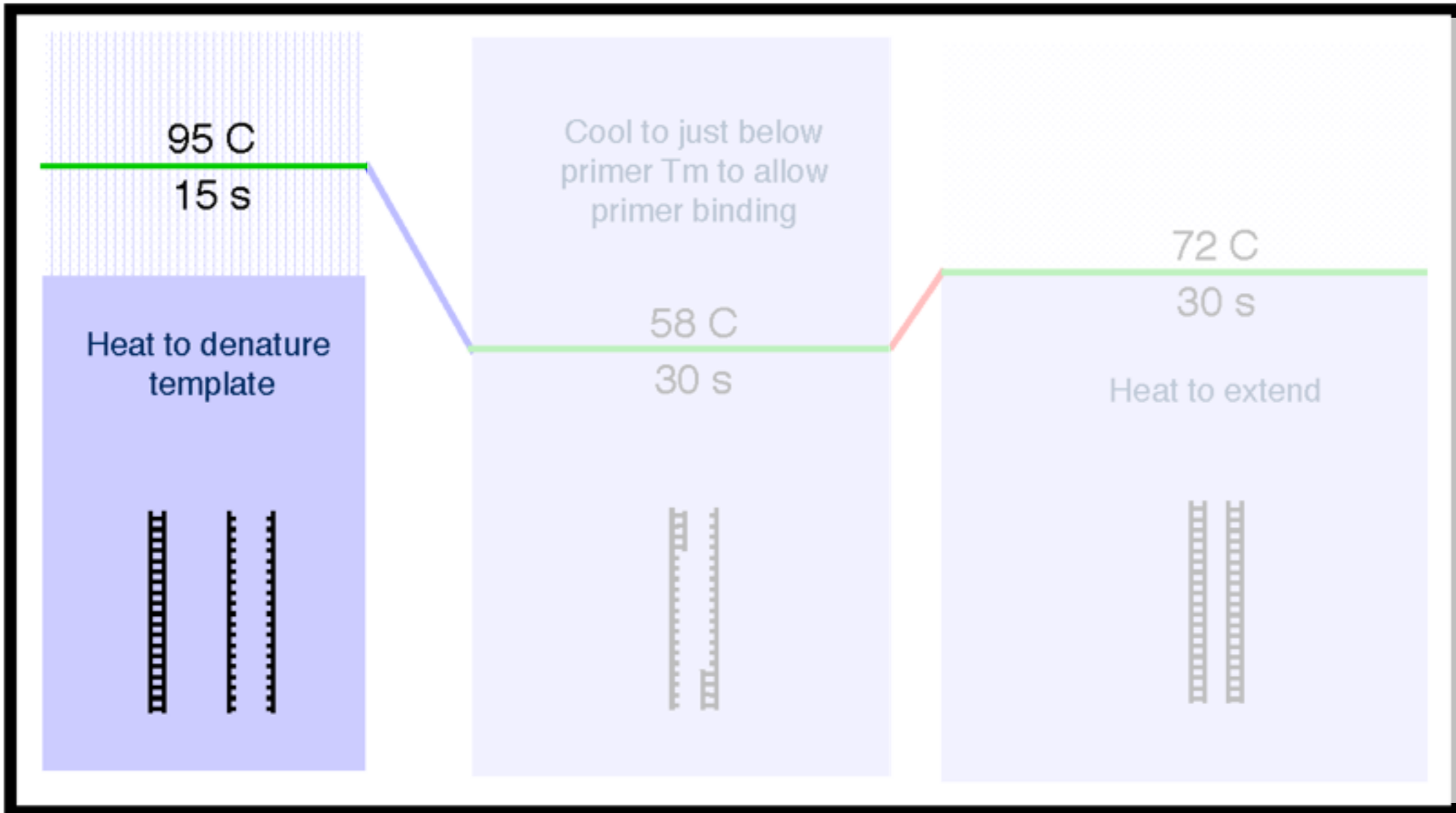
Polymerase chain reaction - PCR



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

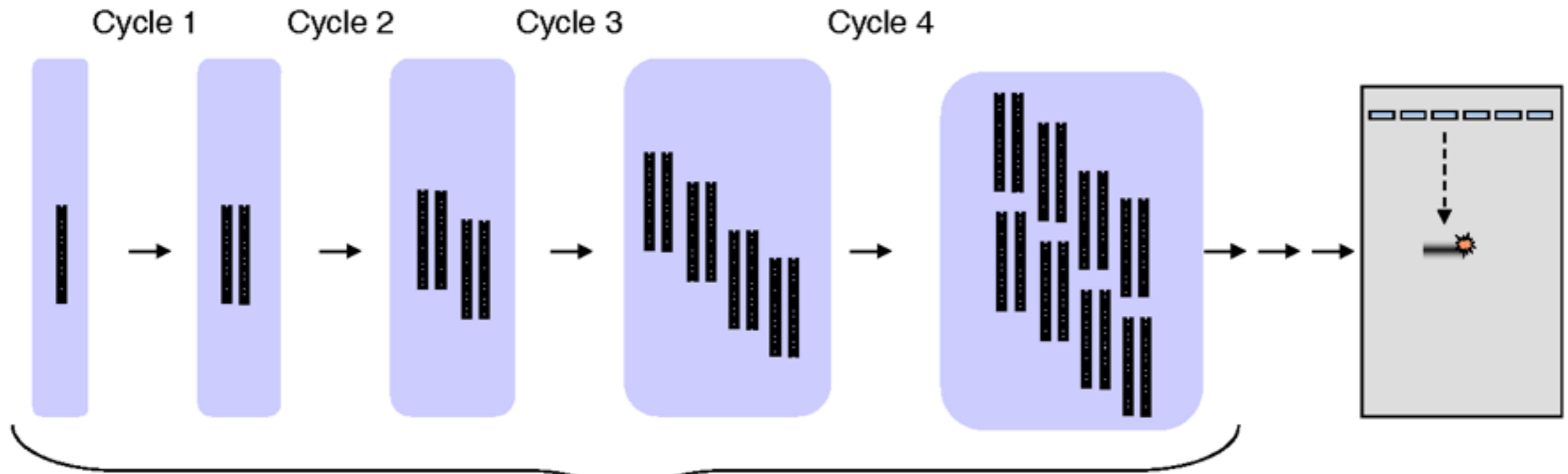
Mastercycler pro: New vapo.protect™ technology reduces evaporation.

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)		

* 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.

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



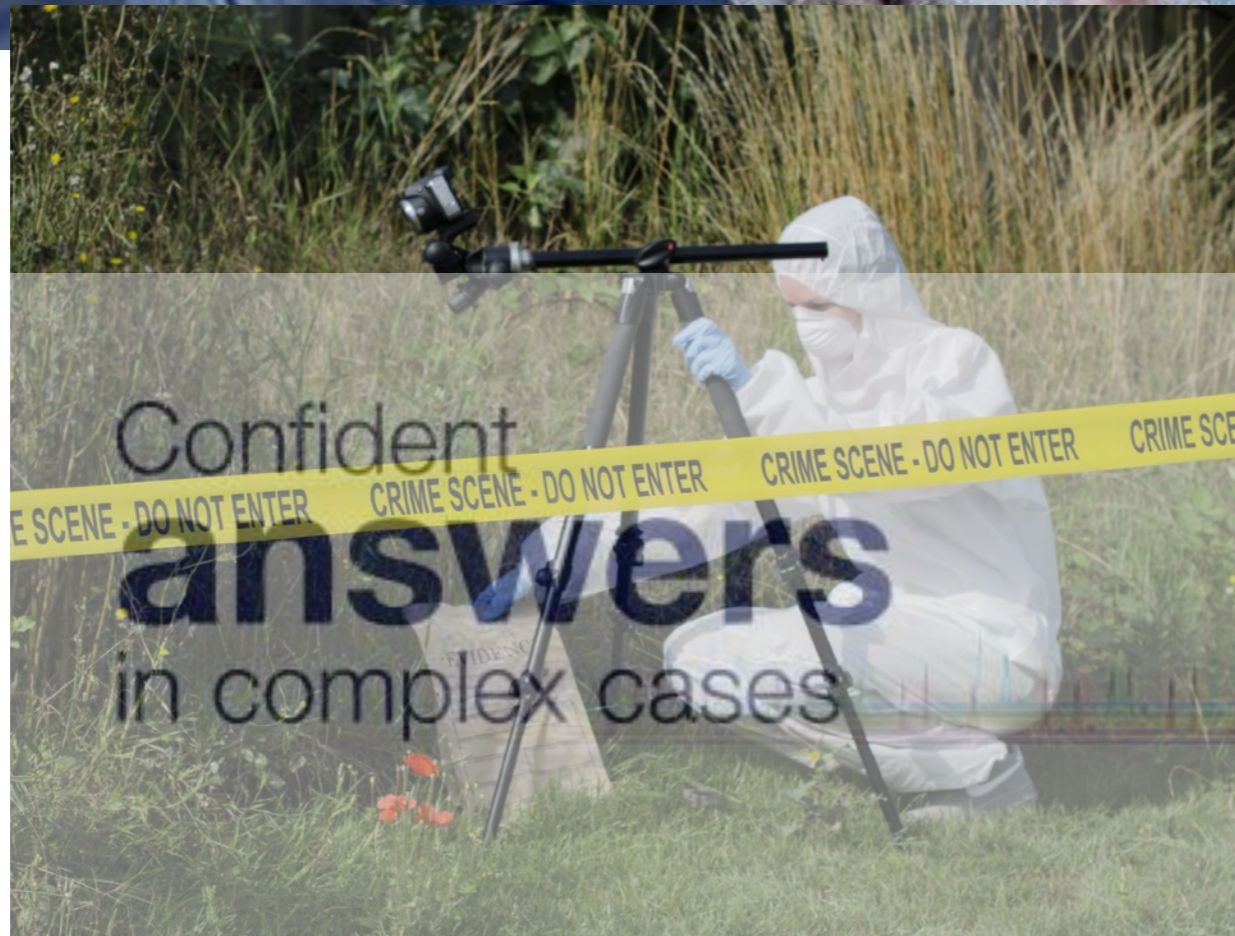




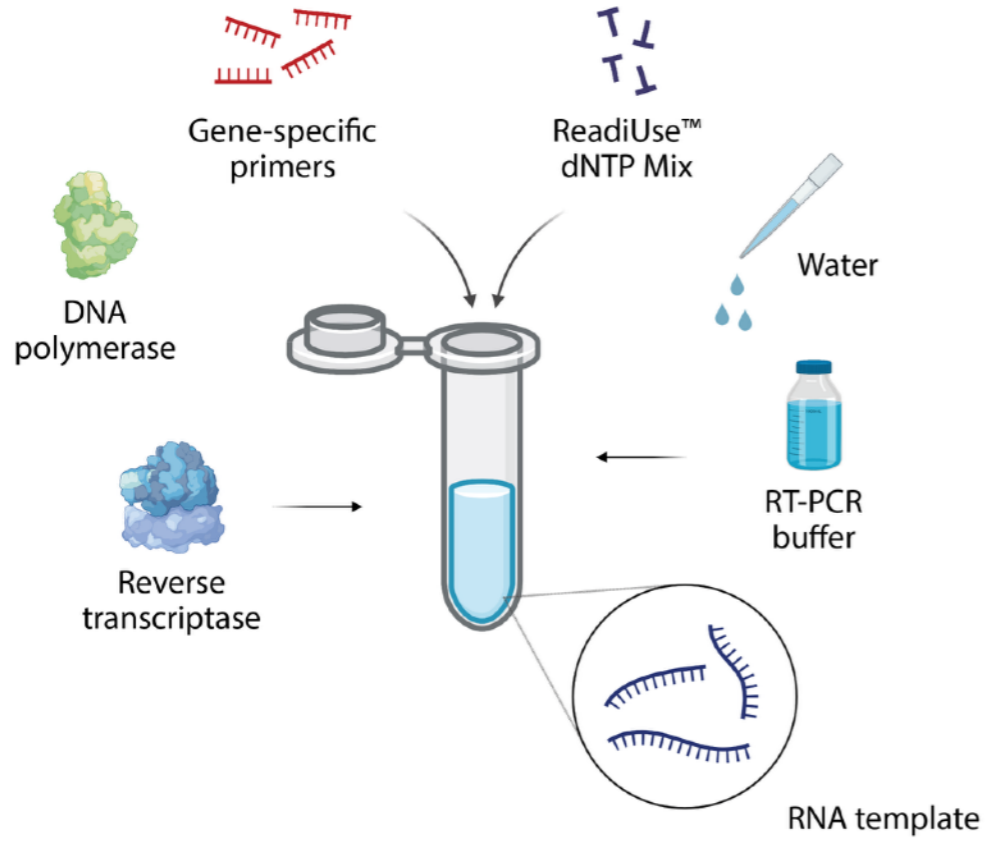
Forensic PCR amplification: of small amounts of DNA



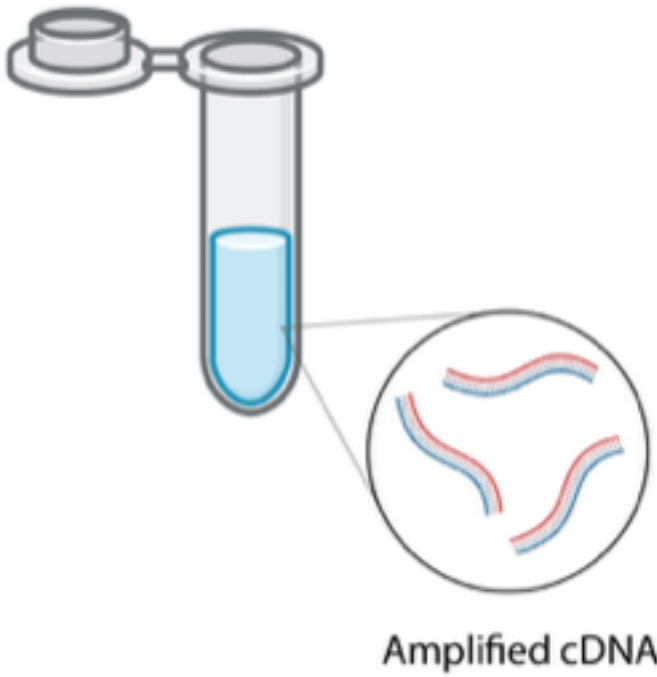
Forensic PCR amplification: Paternity Testing



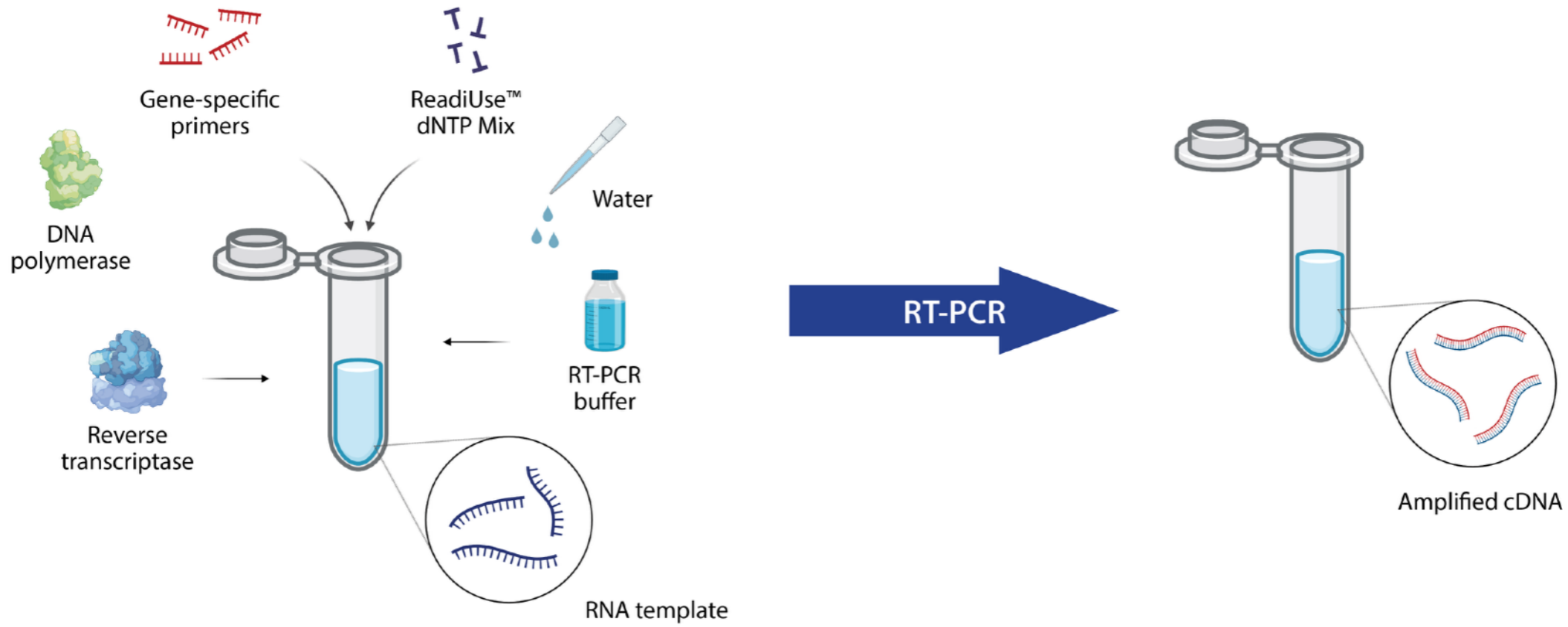
One-Step RT-PCR



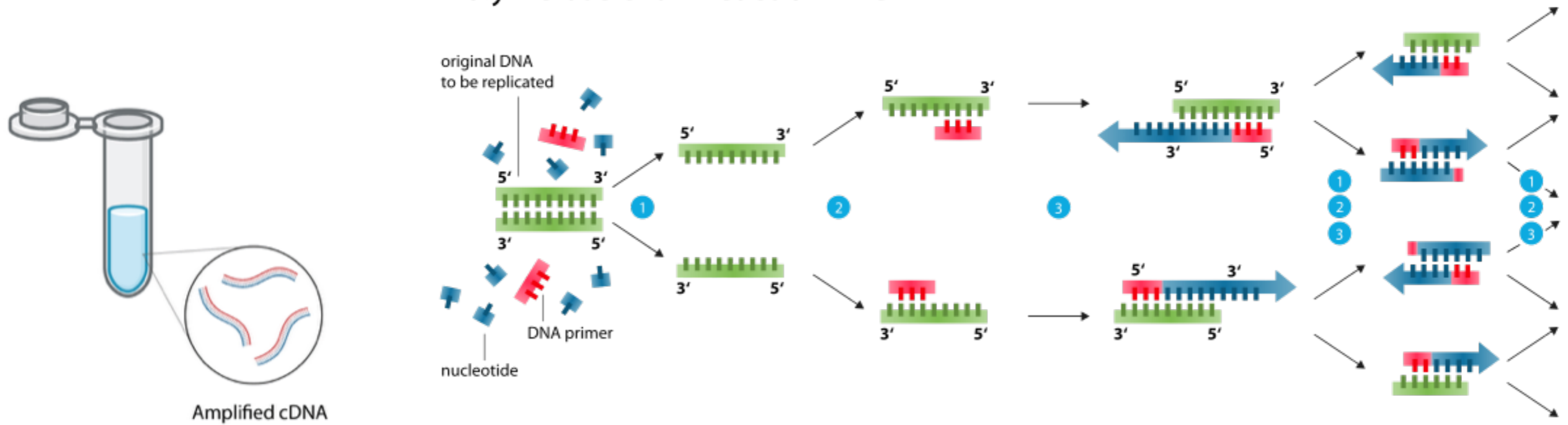
RT-PCR



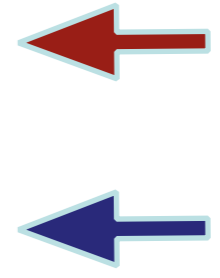
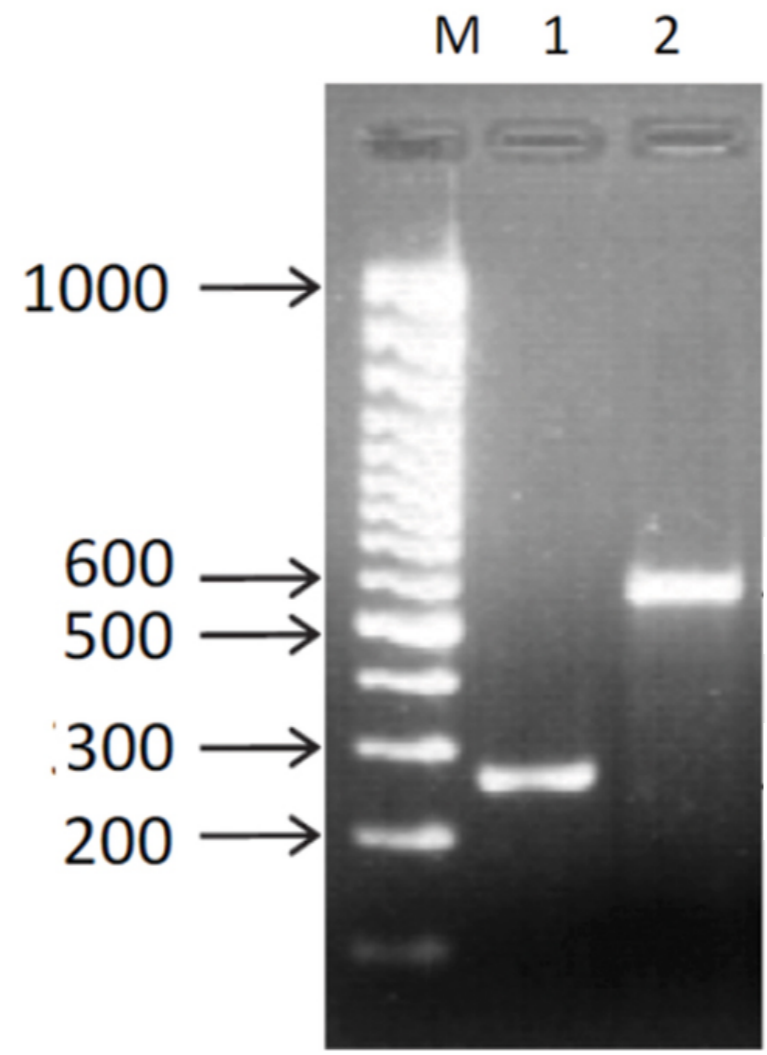
One-Step RT-PCR

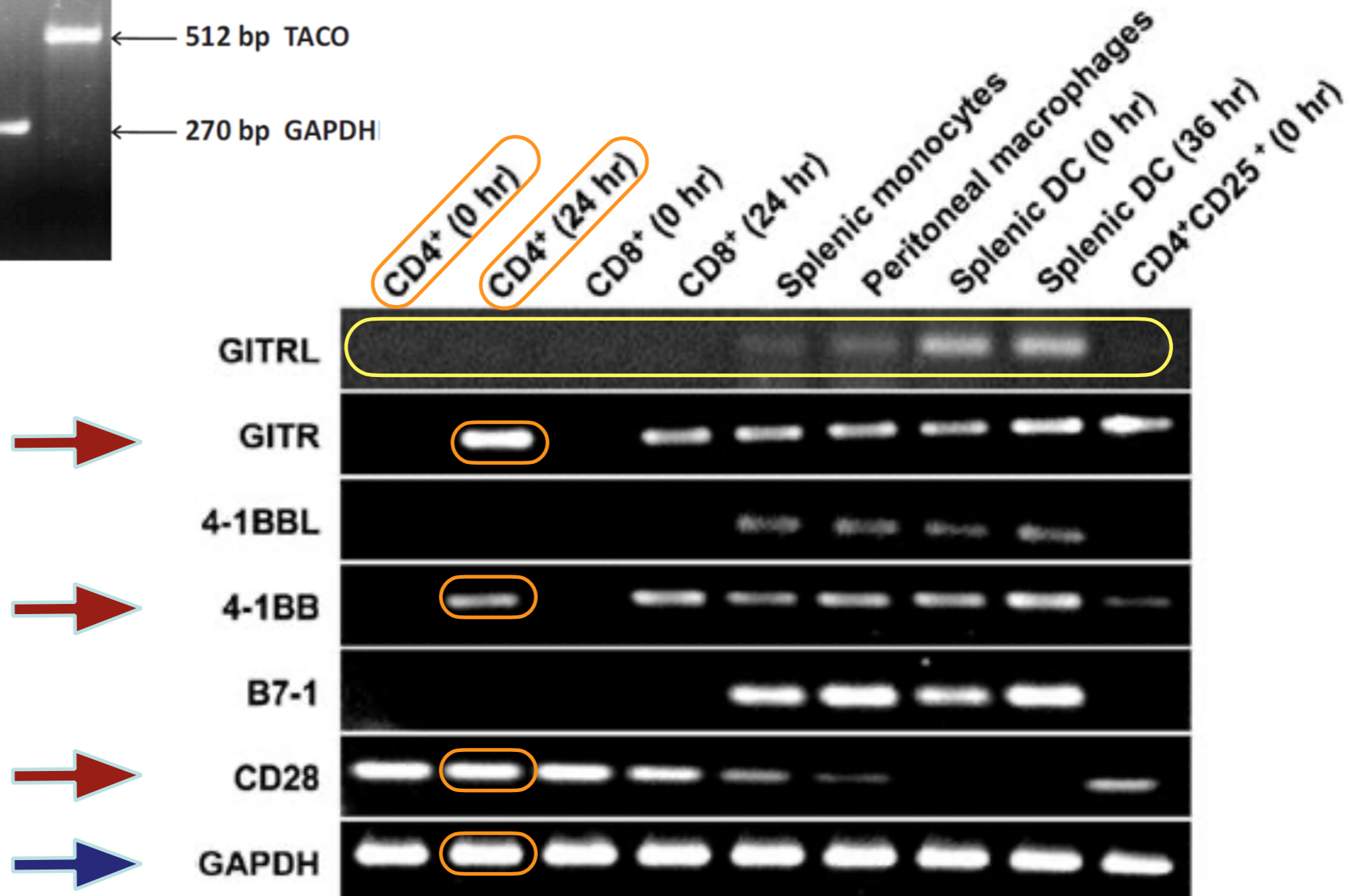
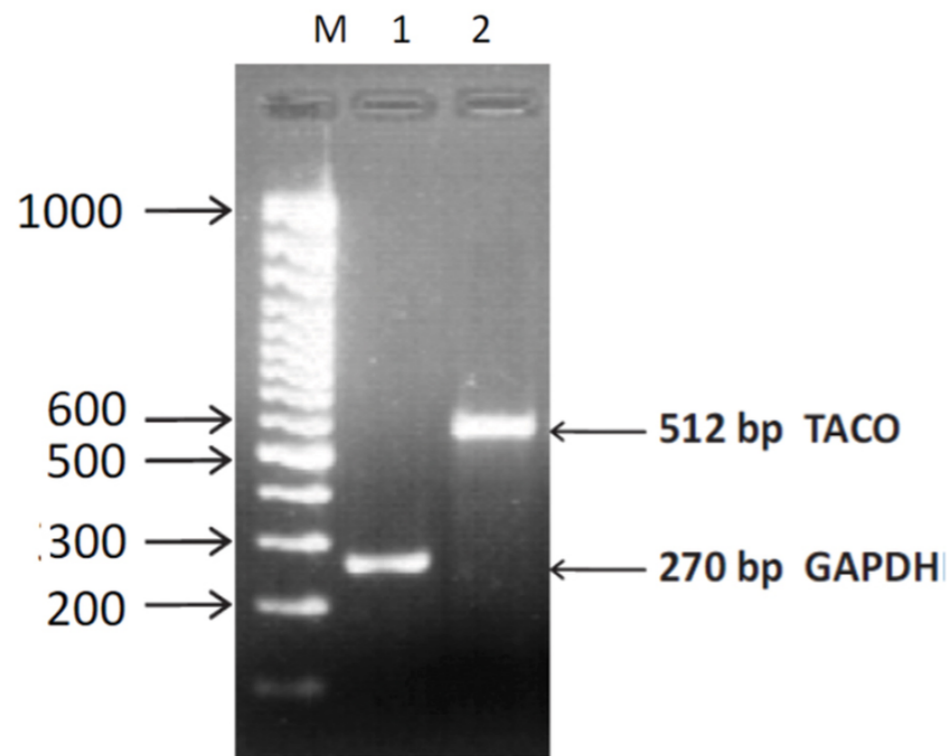


Polymerase chain reaction - PCR



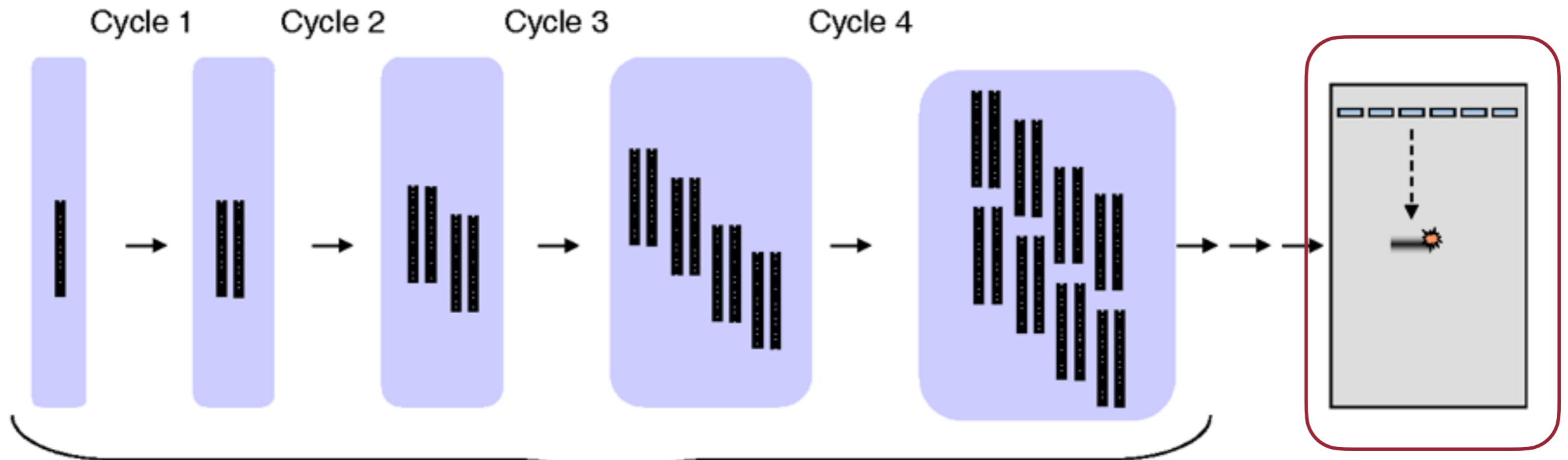
- 1 Denaturation at 94-96°C
- 2 Annealing at ~68°C
- 3 Elongation at ca. 72 °C





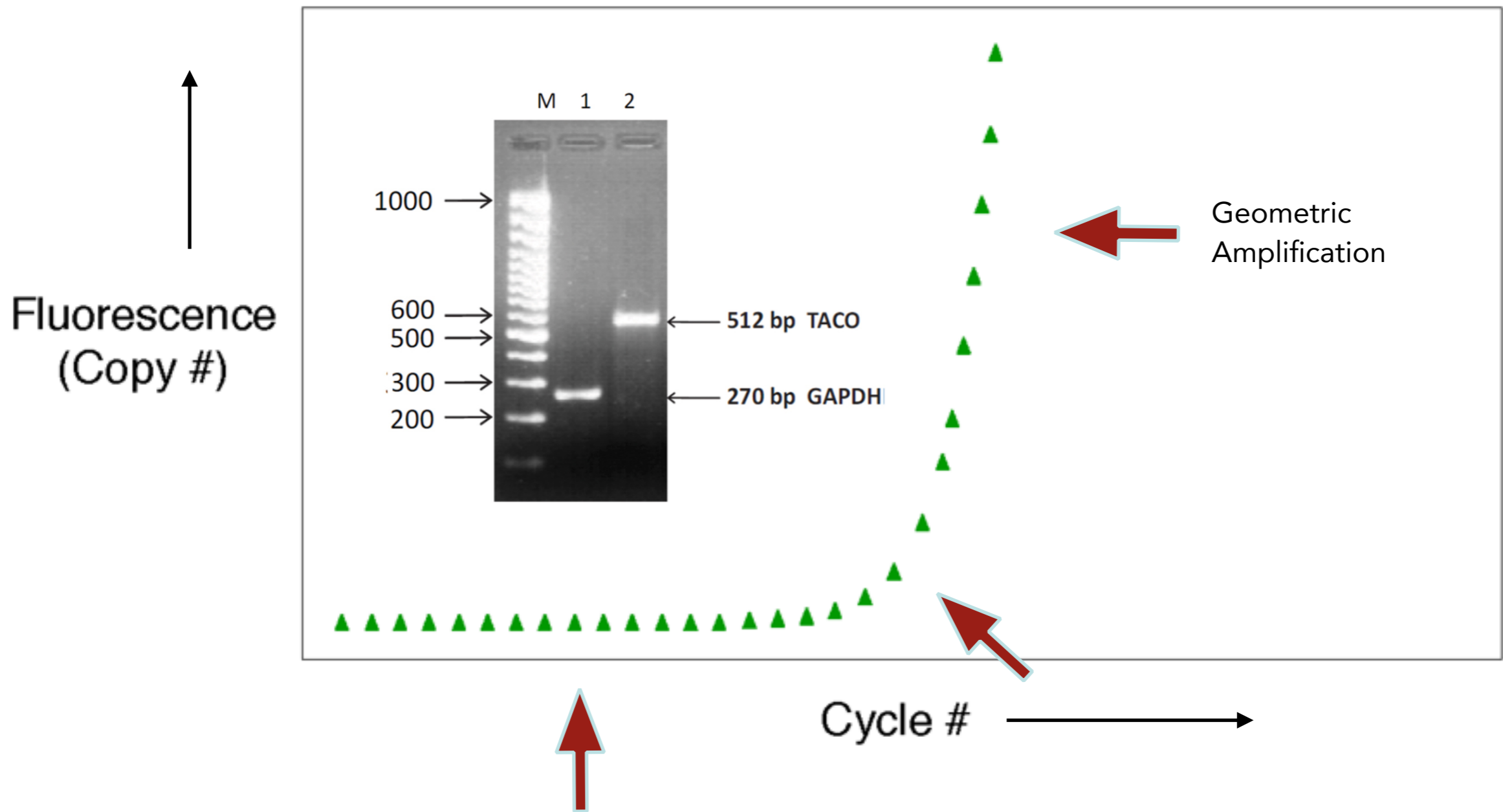
Traditional PCR

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



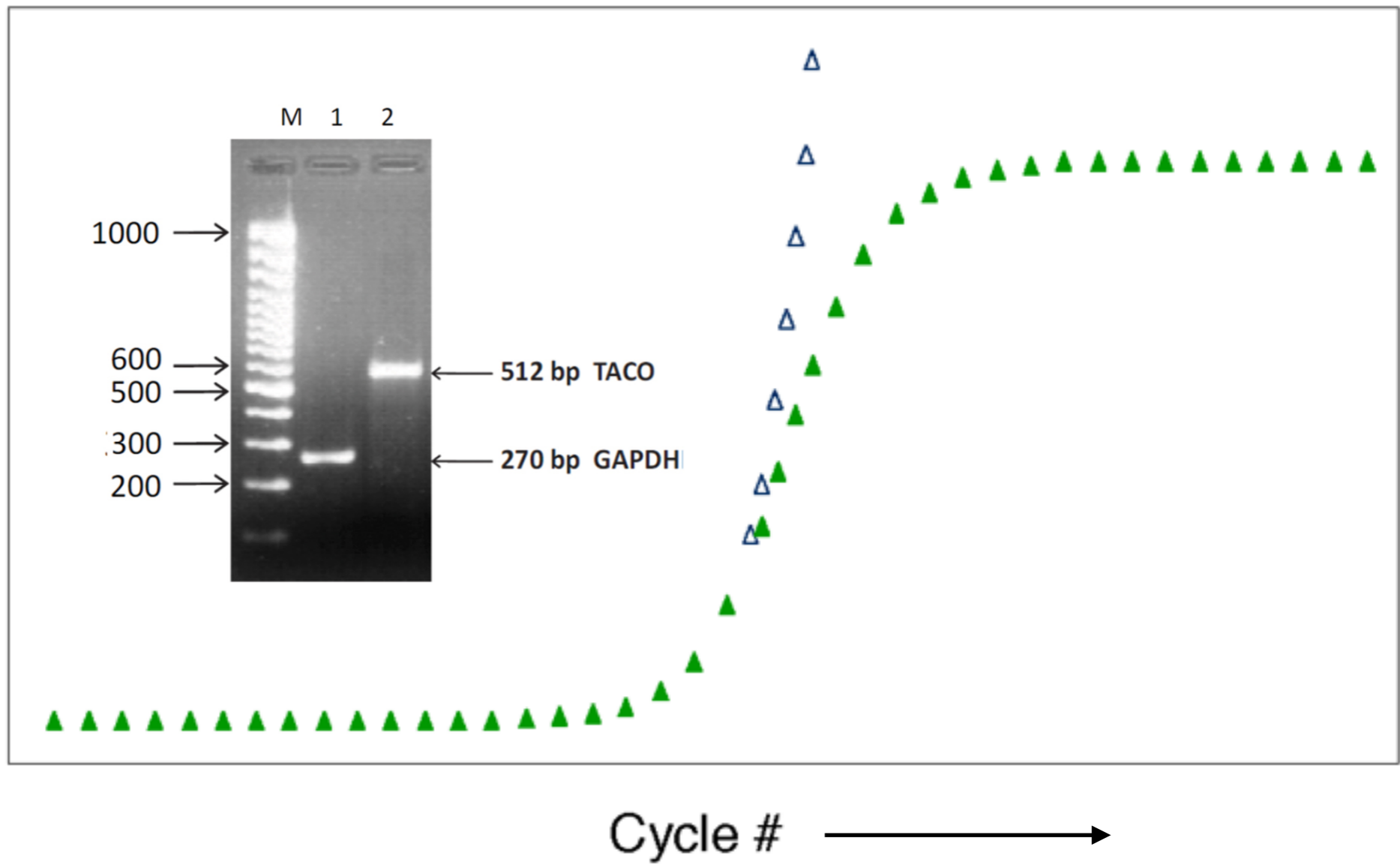
Mastercycler Gradient Pro -Thermal Cycler

- Major reduction of evaporation in tubes
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- Intuitive graphic programming
- Display to indicate cycler number in a network
- Optional self-test of peltier elements





↑
Fluorescence
(Copy #)



Real-time PCR or qPCR

SYBR[®] Green

TaqMan[®]

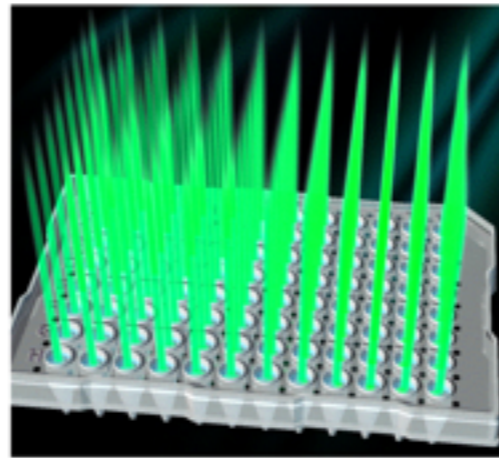
MGB

ROX[™]

Multicomponenting



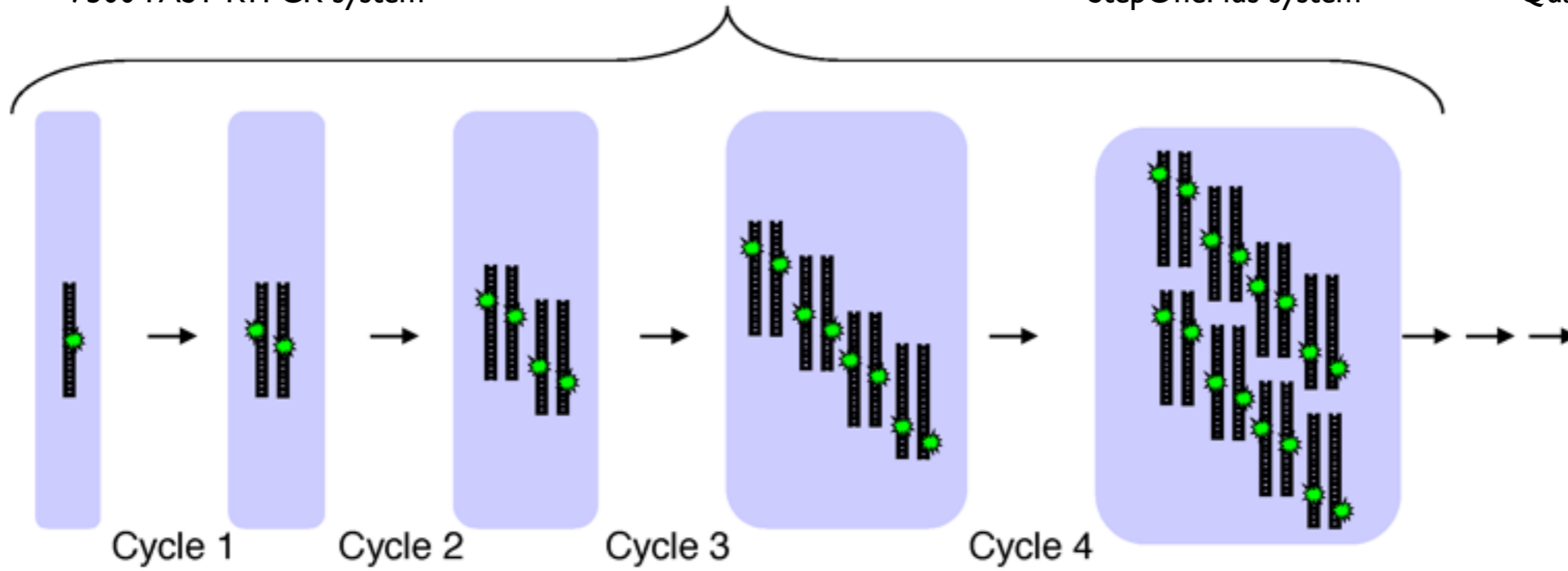
7500 FAST RTPCR system

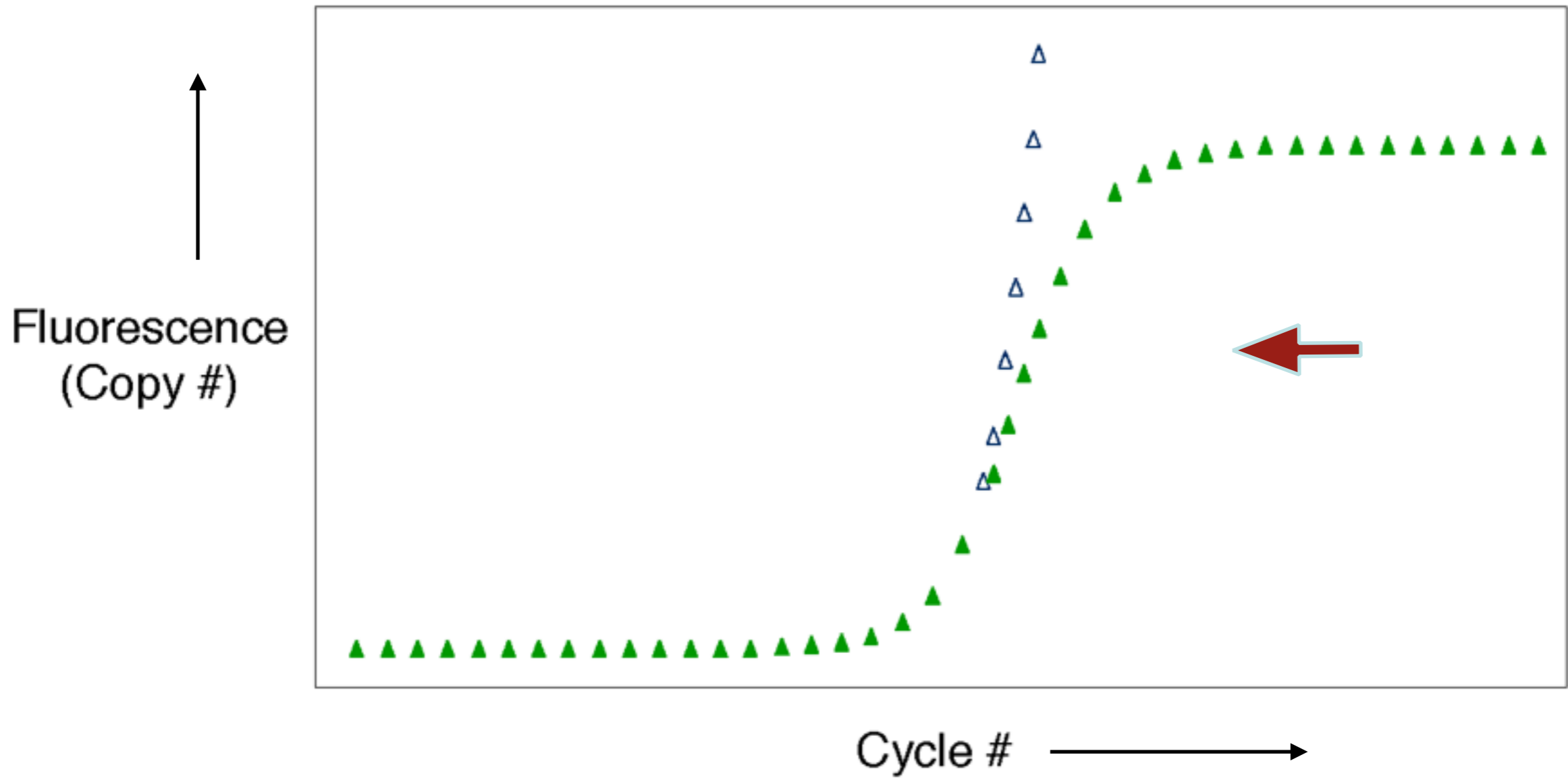


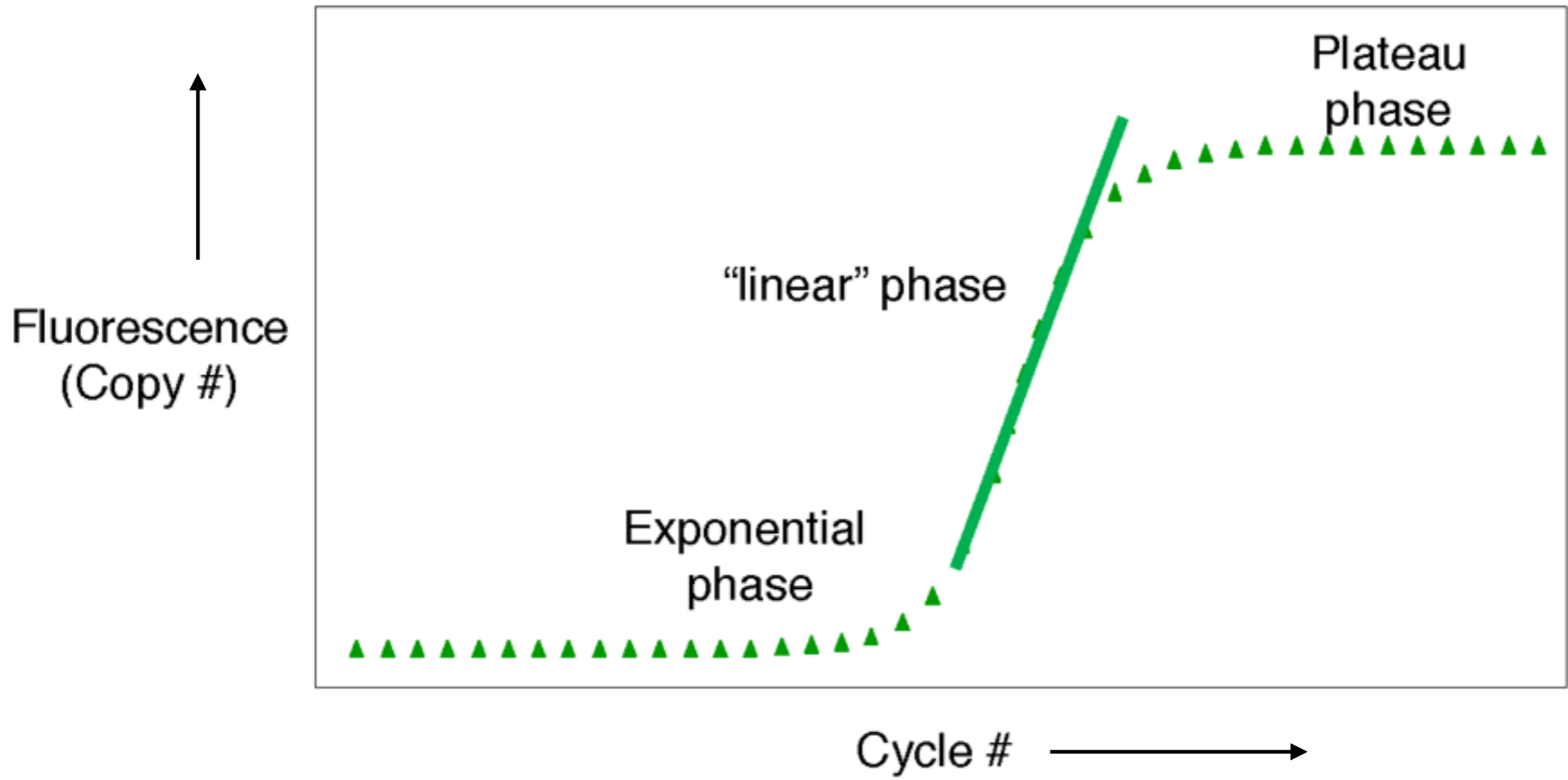
StepOnePlus system

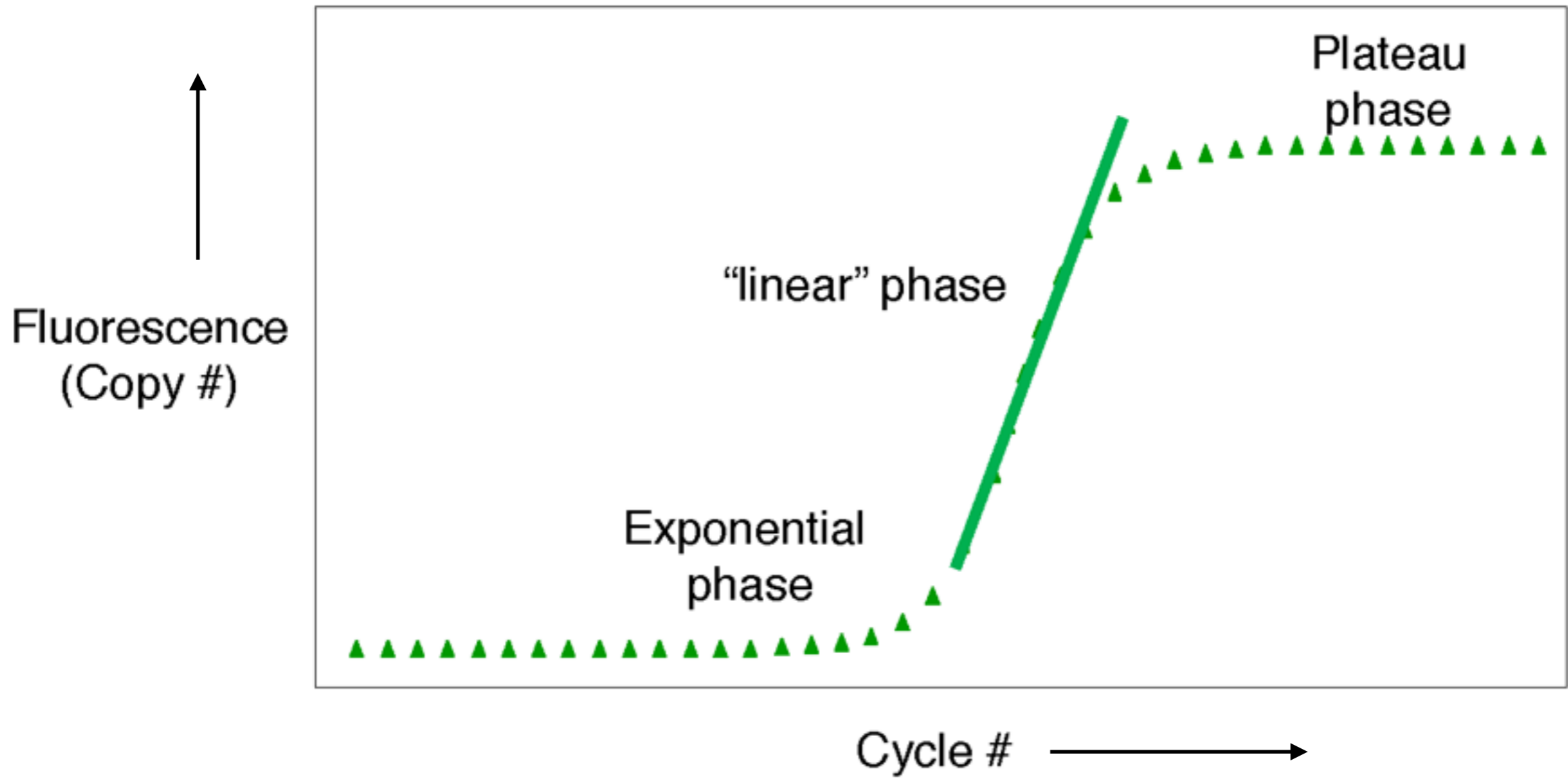


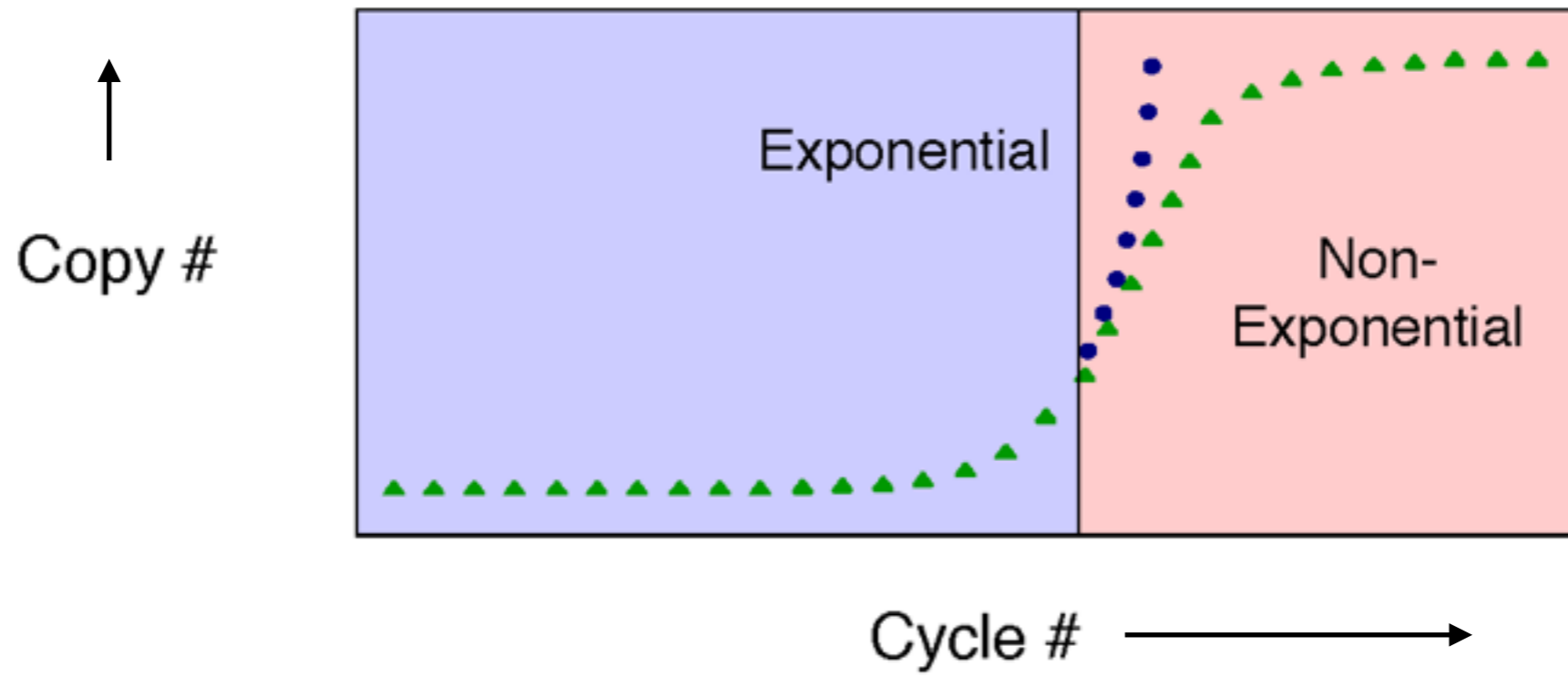
QuantStudio 3 system

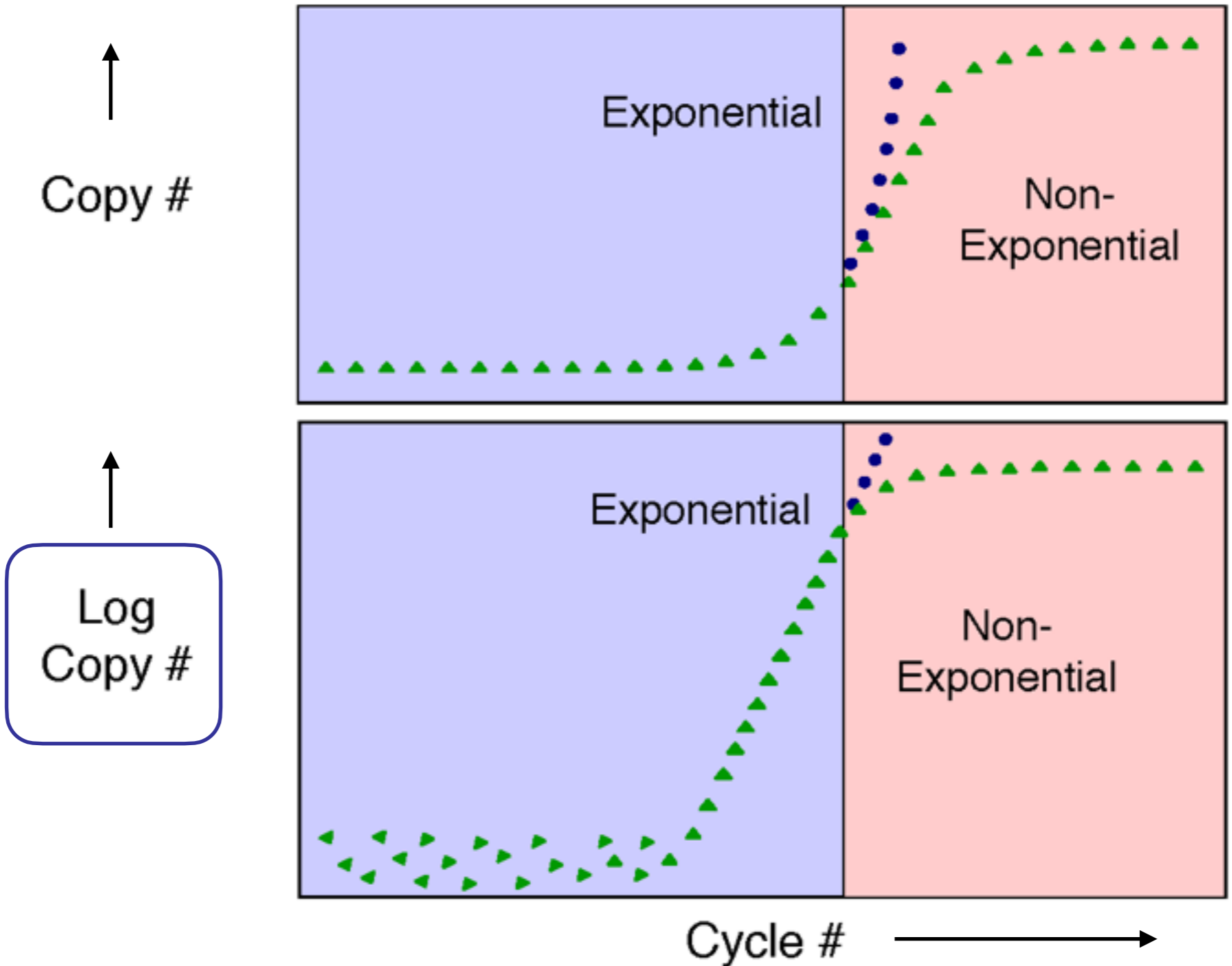




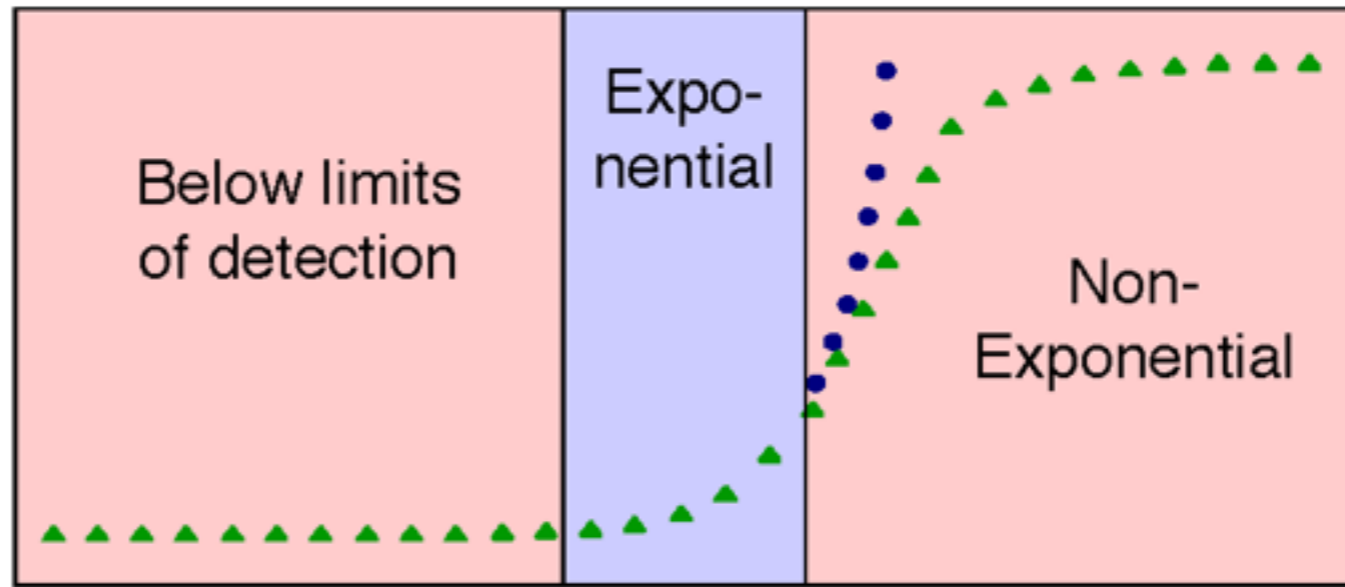




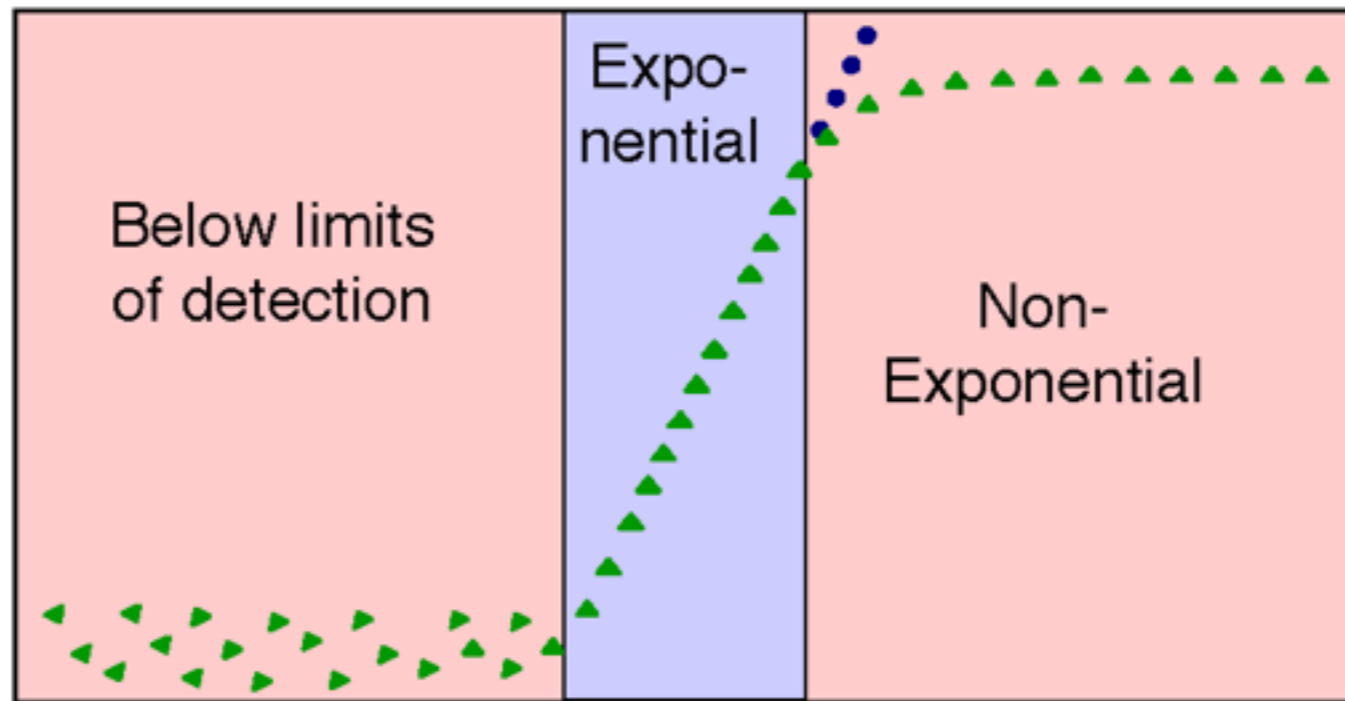




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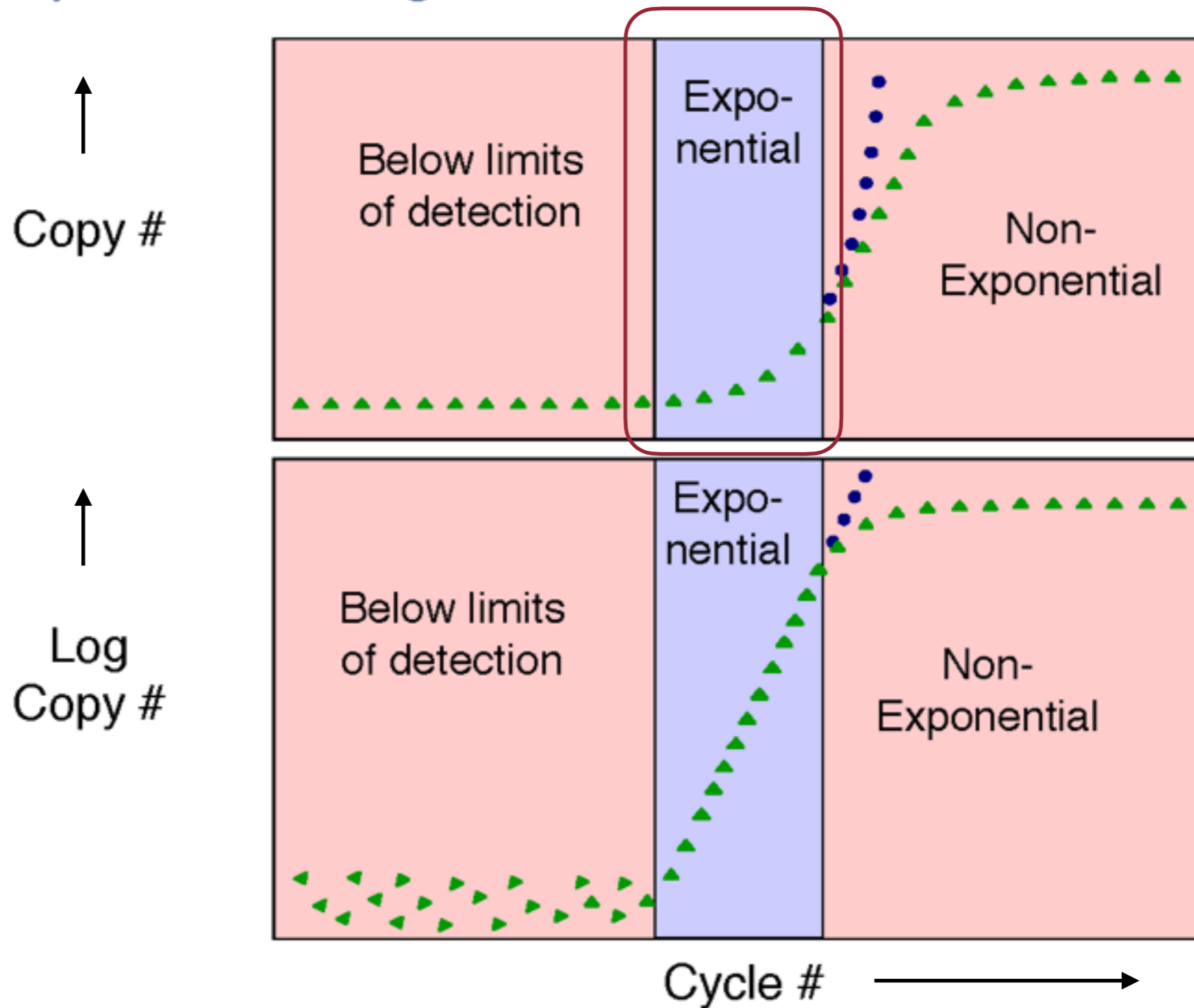


↑
Log
Copy #

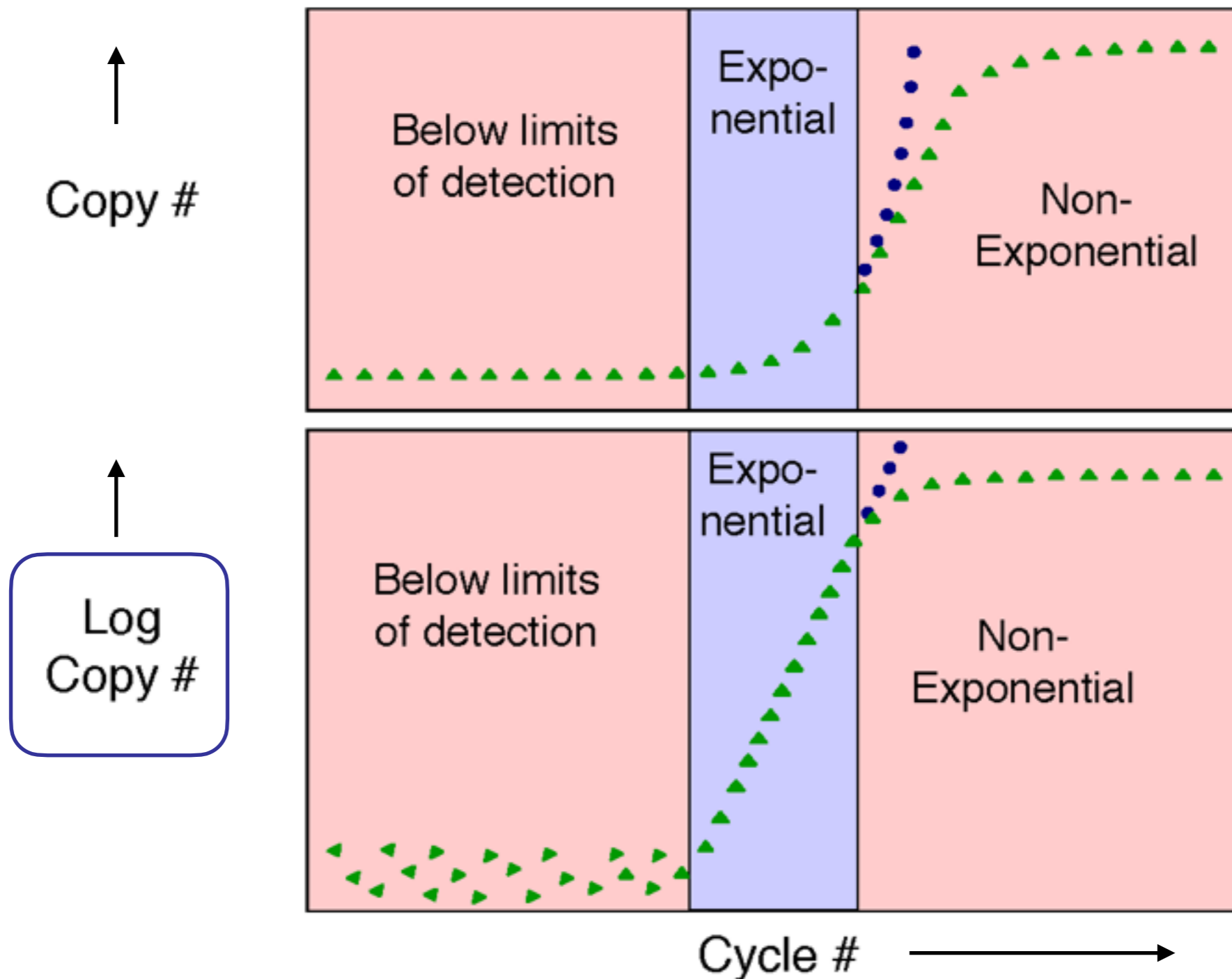


Cycle # →

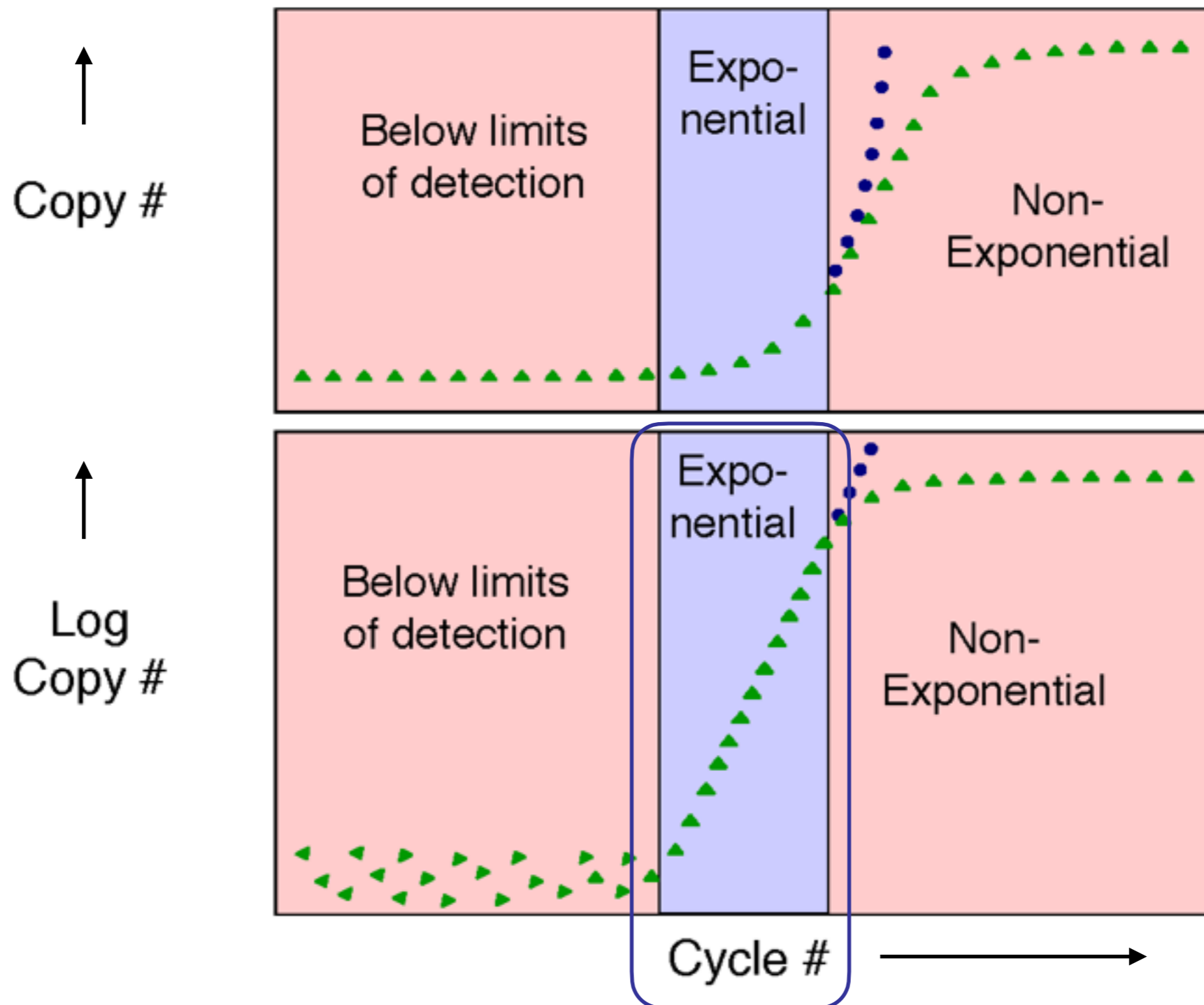
The exponential region is easier to define in log phase



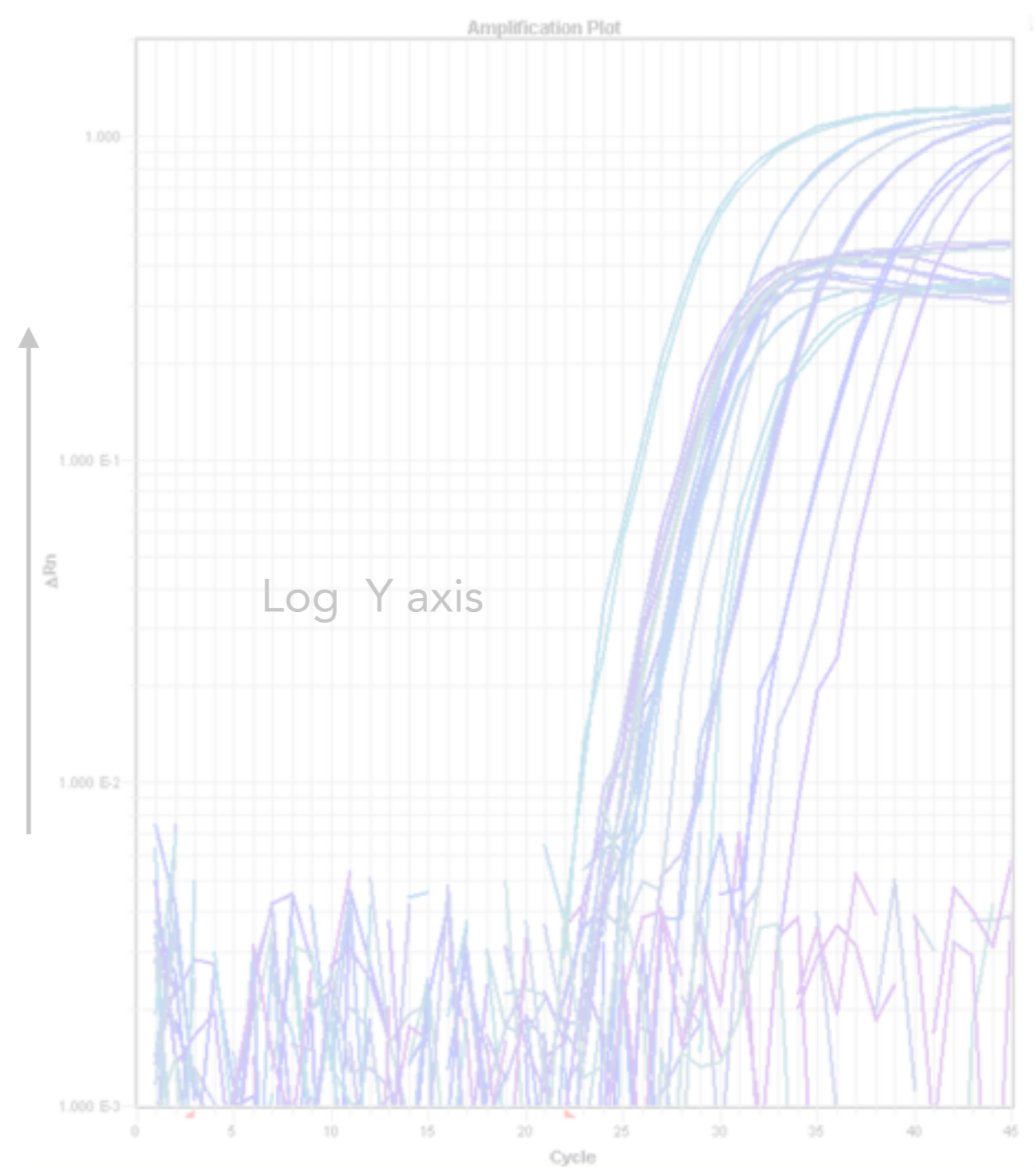
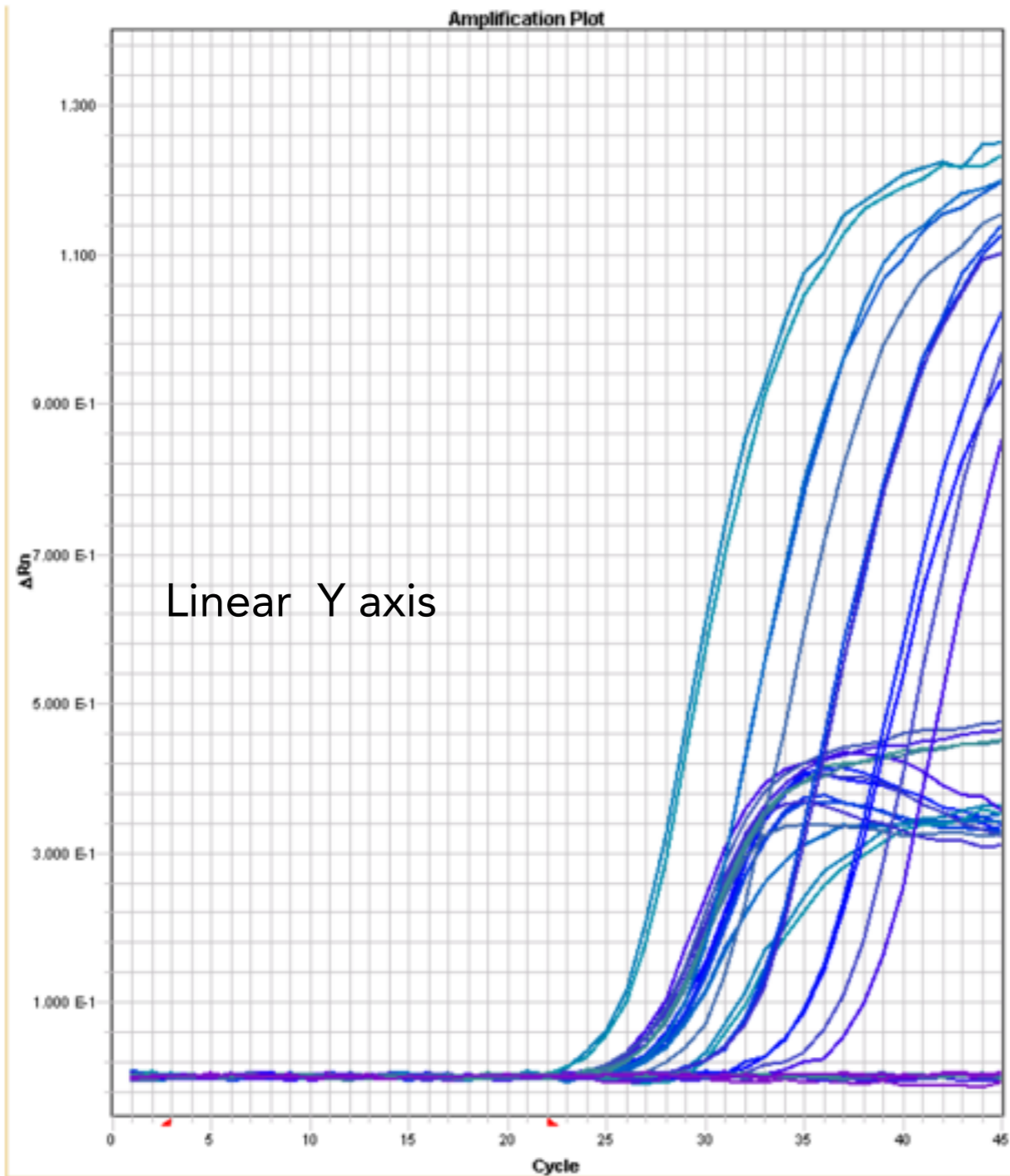
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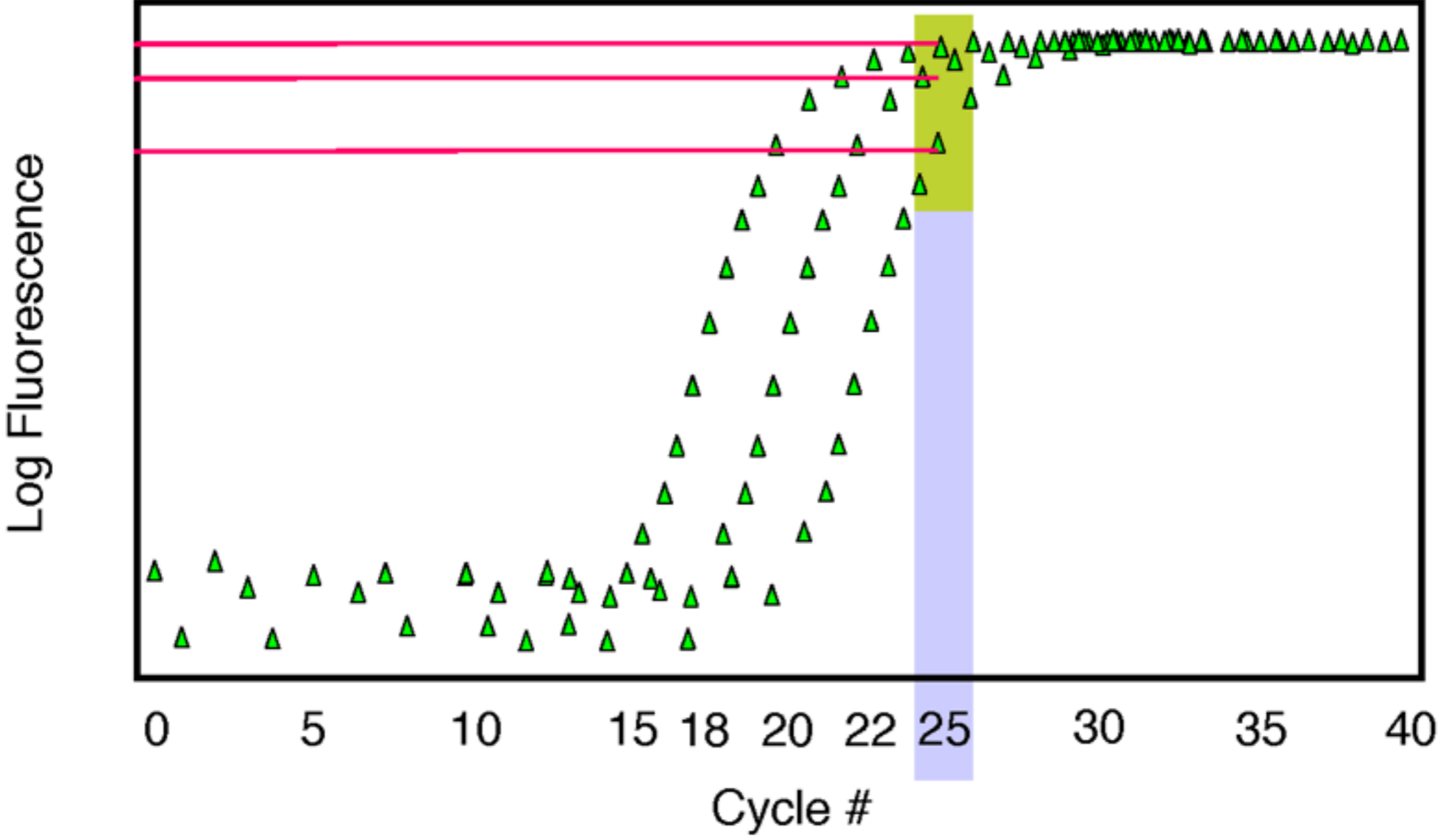


The exponential region is easier to define in log phase



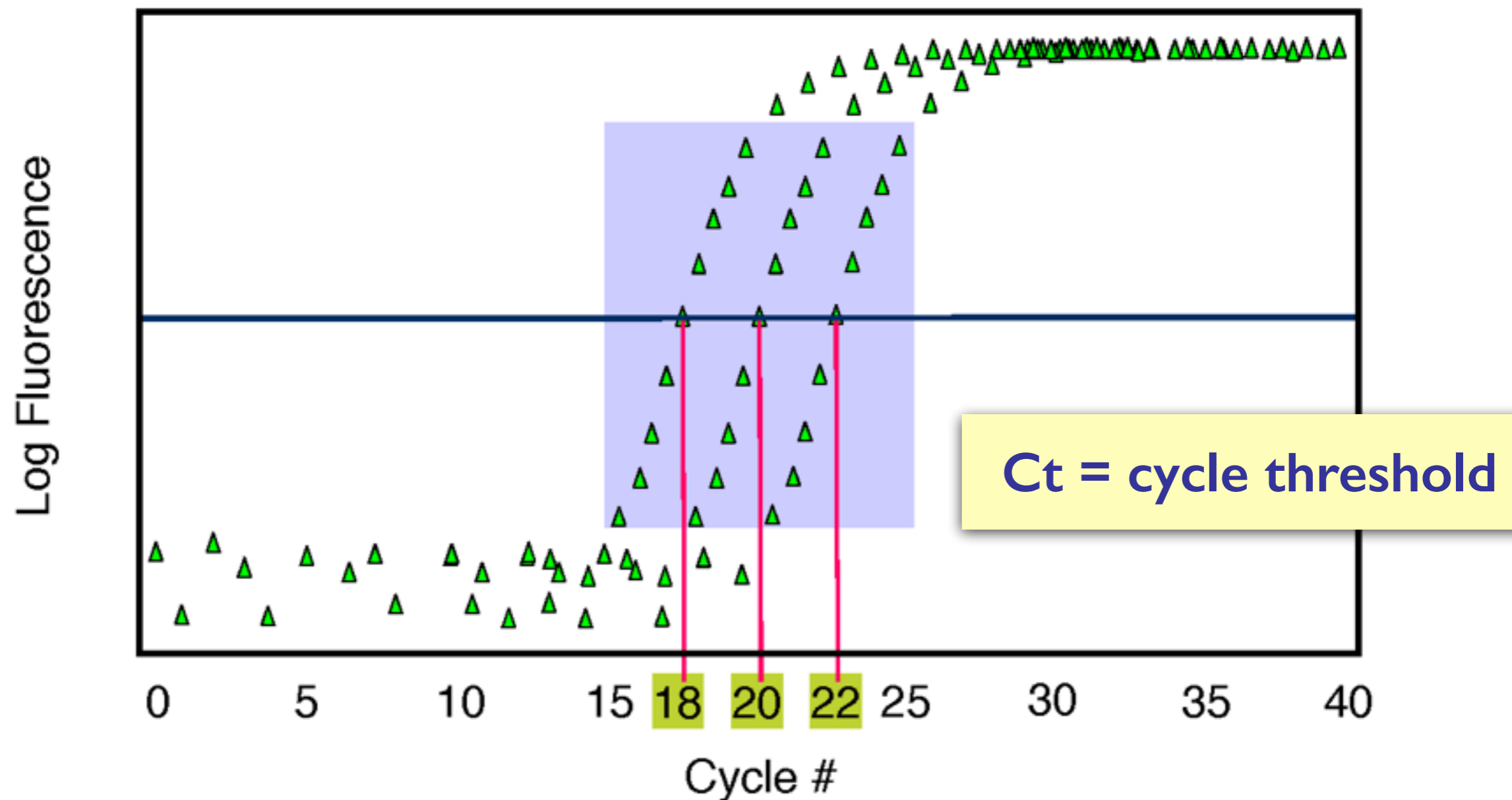
Linear and Log view of the same data



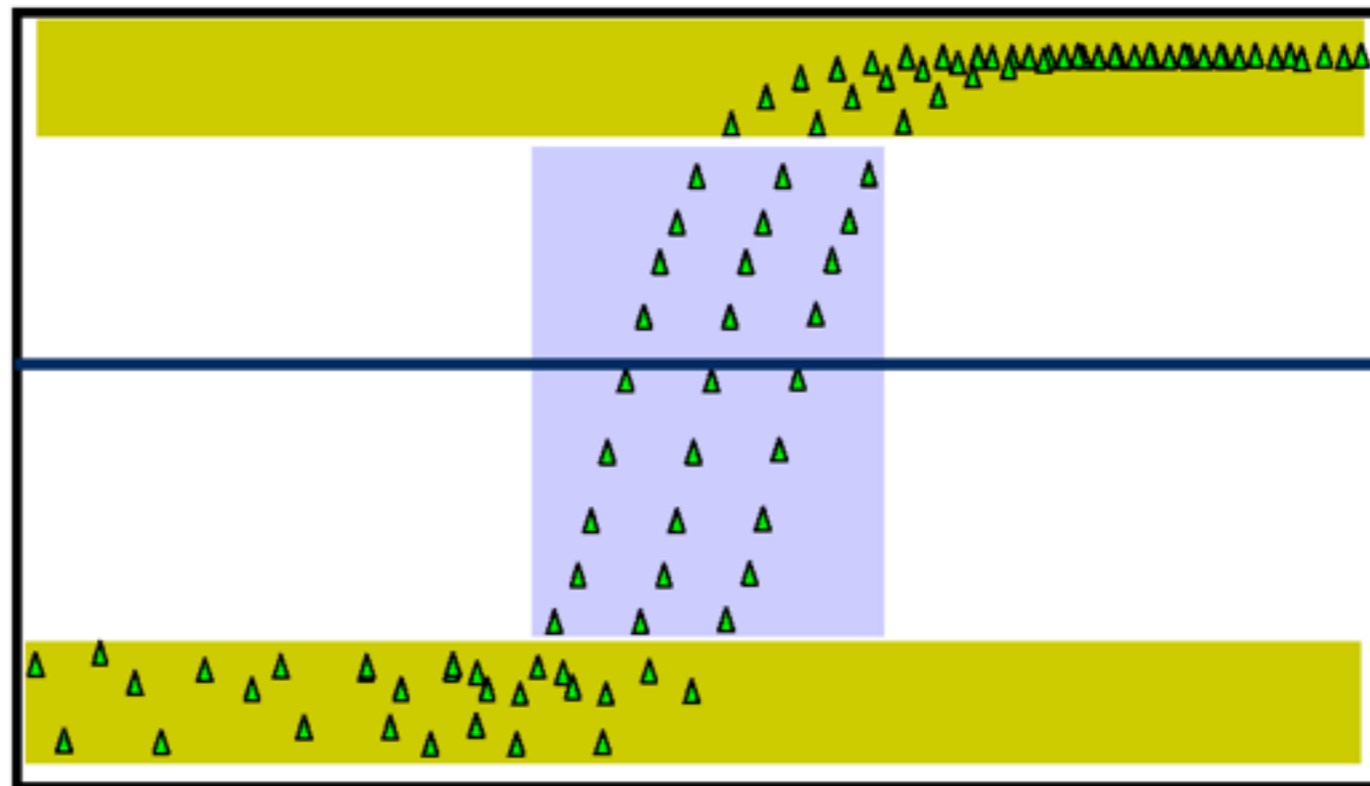


Real-time PCR - Concept of Ct

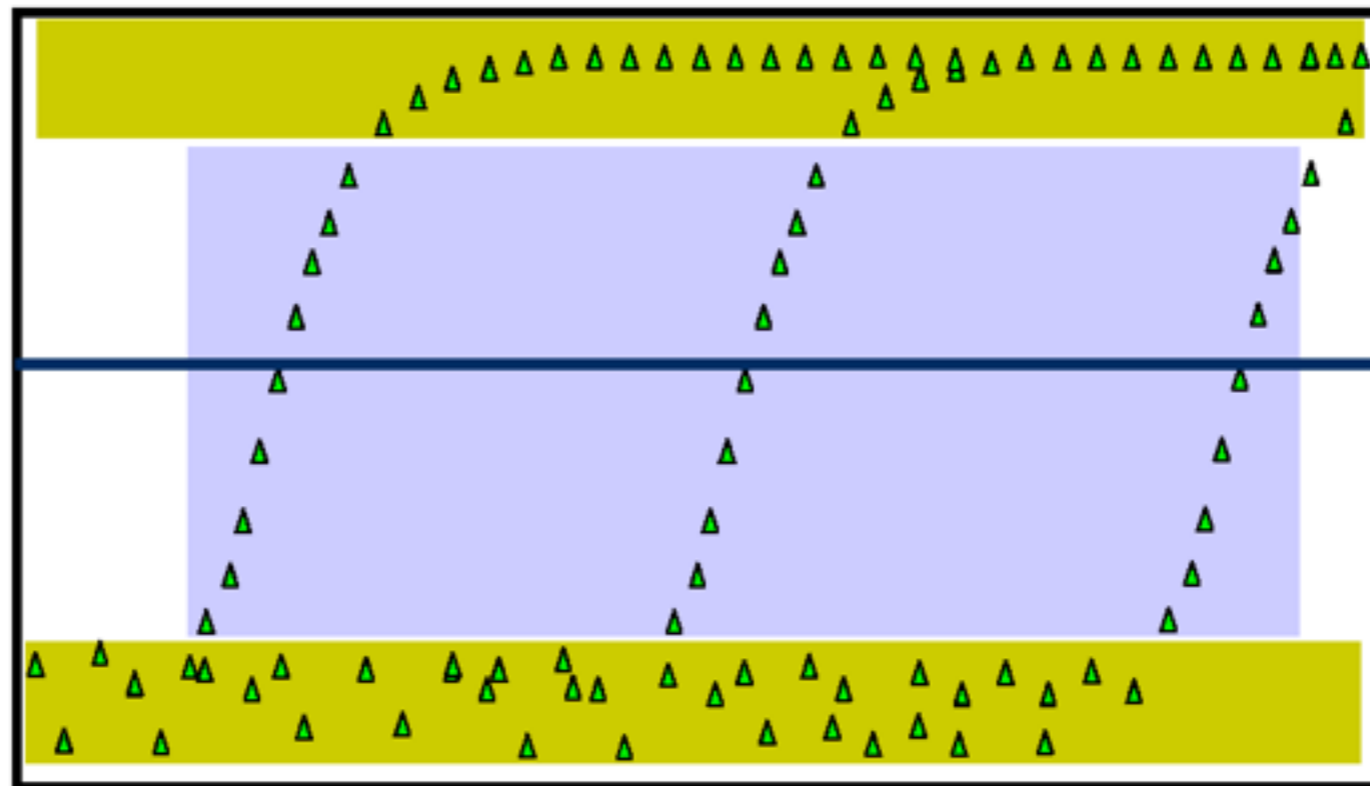
We measure the **number of cycles** it takes to reach a set fluorescence threshold (Ct)



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



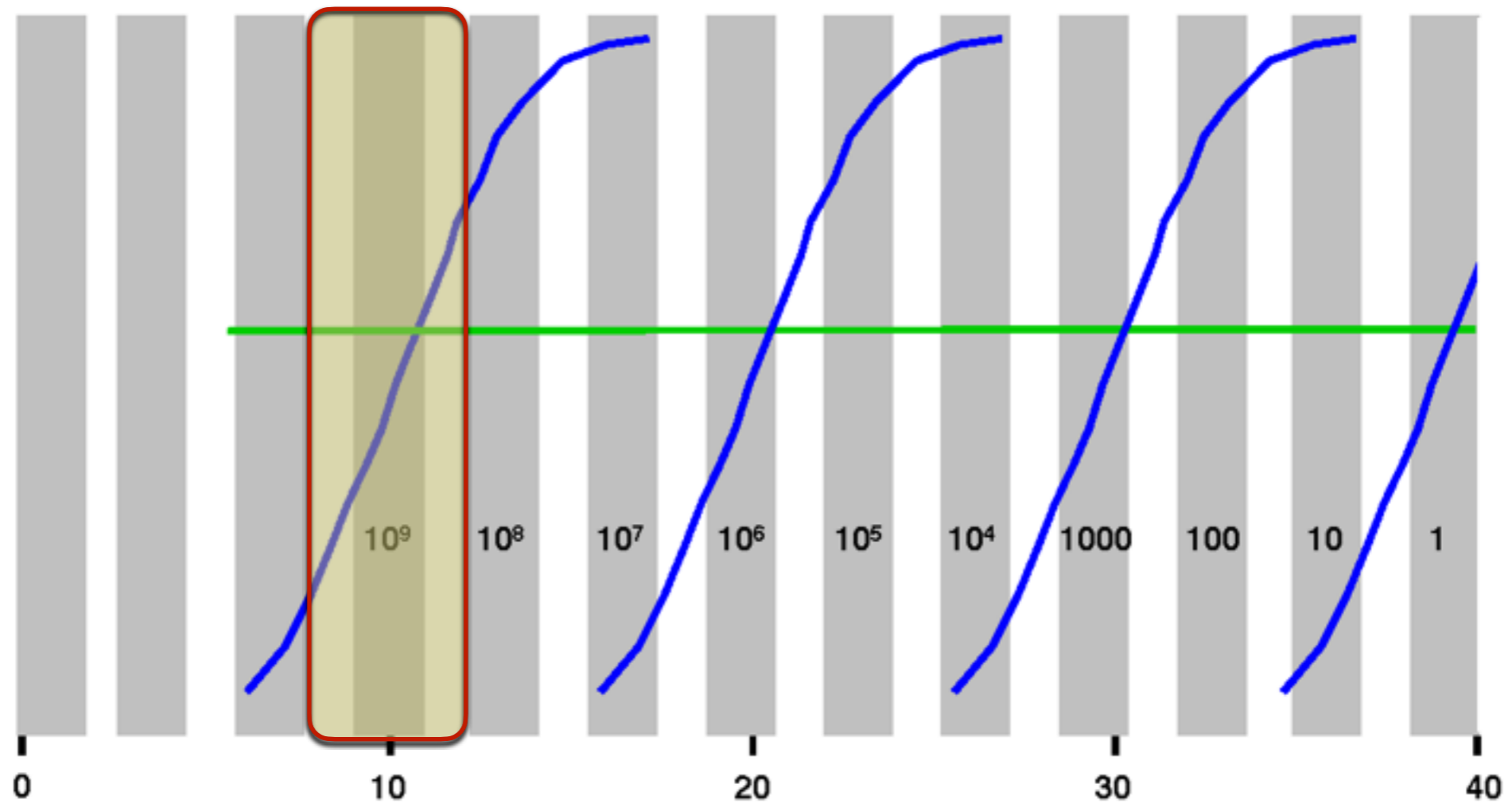
Thus, real-time PCR is superior to regular PCR because:

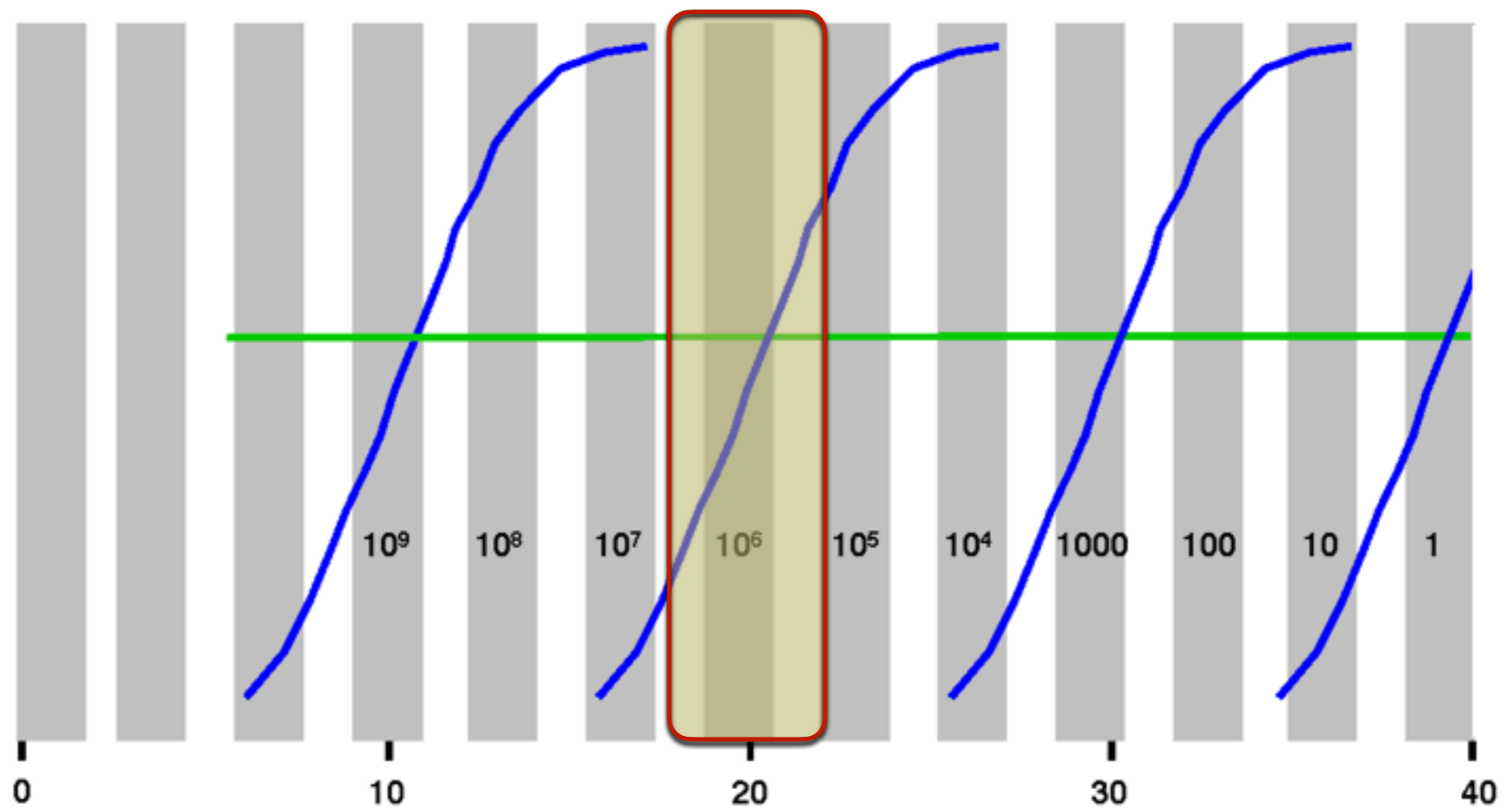


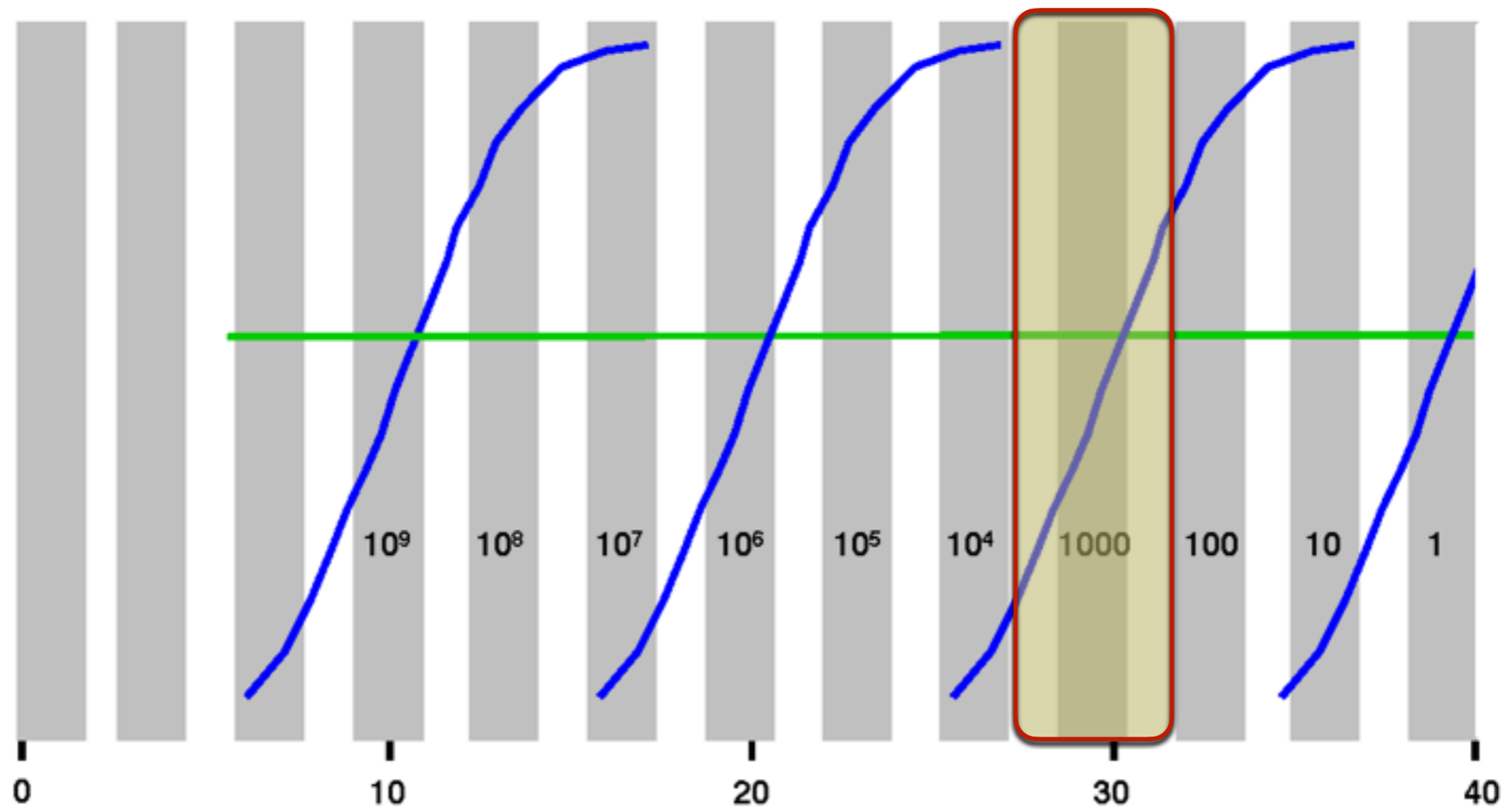
Results are usually given in table form
 indicating Ct value
 the higher the Ct, the lower the target copy number

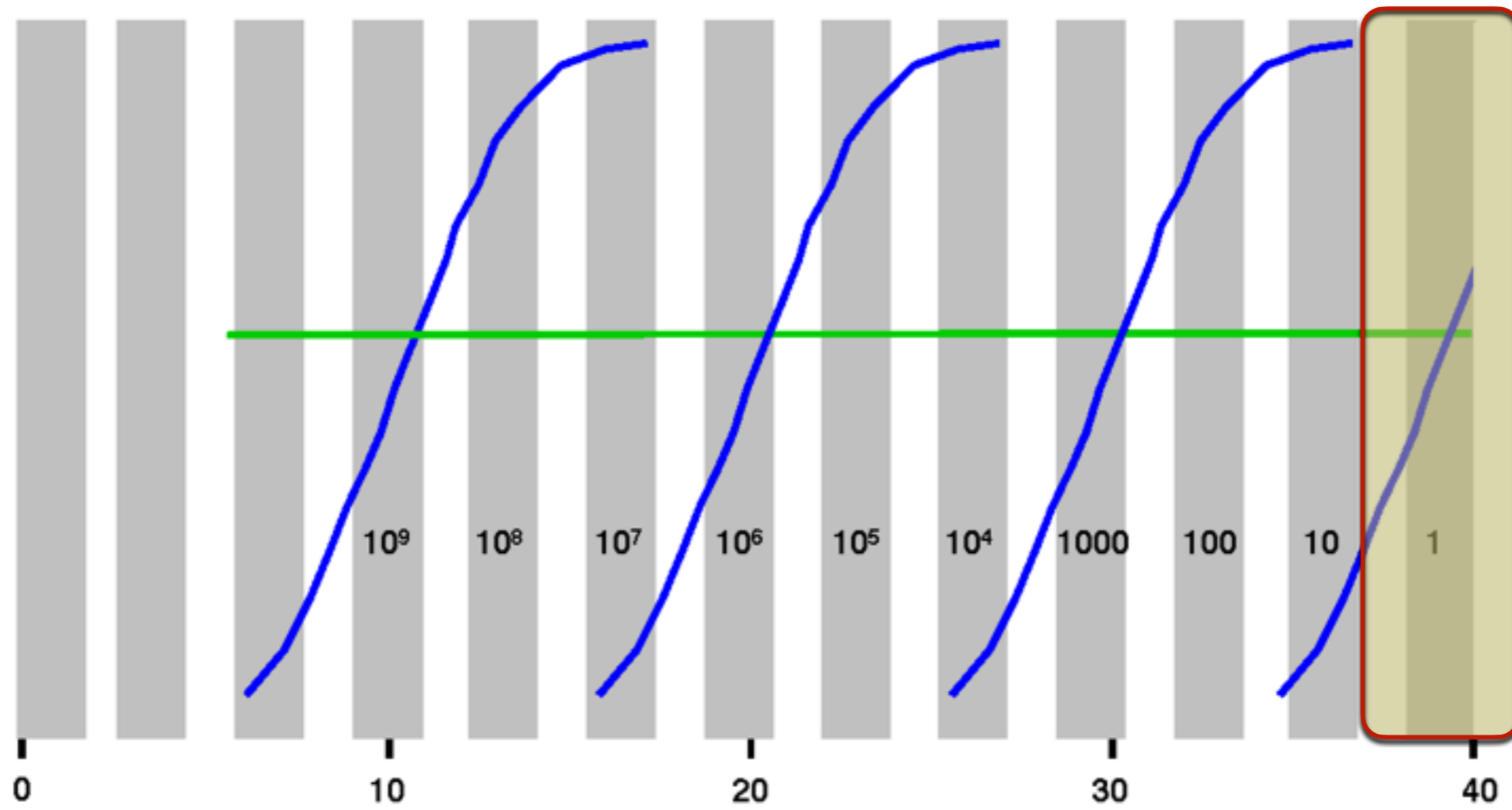
Well	Detector	ng RNA	Ct
A4	GAPDH 1	40	16.75
A5	GAPDH 1	40	16.89
A6	GAPDH 1	40	16.86
B4	GAPDH 1	4	20.27
B5	GAPDH 1	4	20.24
B6	GAPDH 1	4	20.24
C4	GAPDH 1	0.4	23.75
C5	GAPDH 1	0.4	23.71
C6	GAPDH 1	0.4	23.76
D4	GAPDH 1	0.04	27.21
D5	GAPDH 1	0.04	27.18
D6	GAPDH 1	0.04	27.17
E4	GAPDH 1	0.004	30.46
E5	GAPDH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

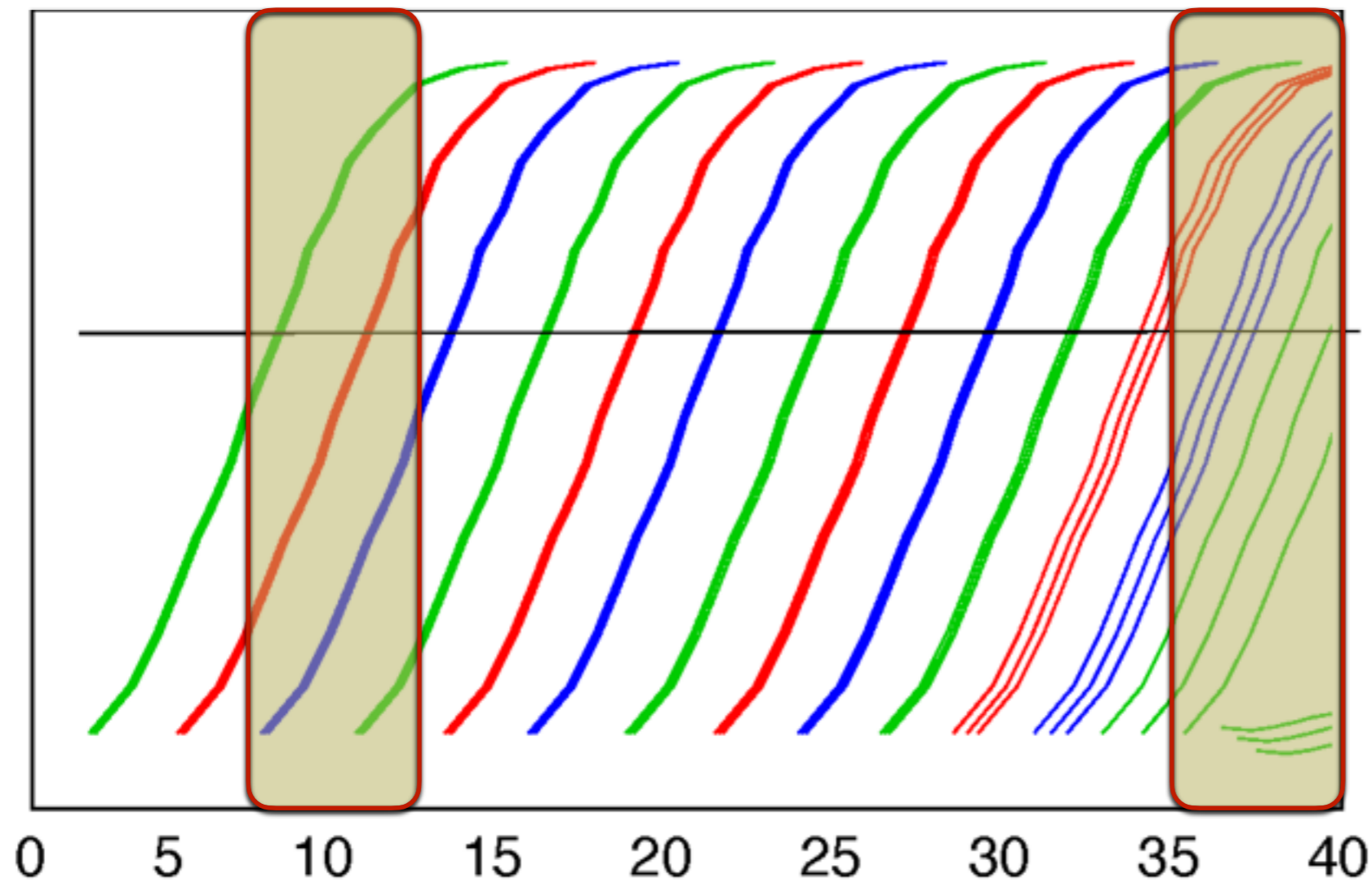






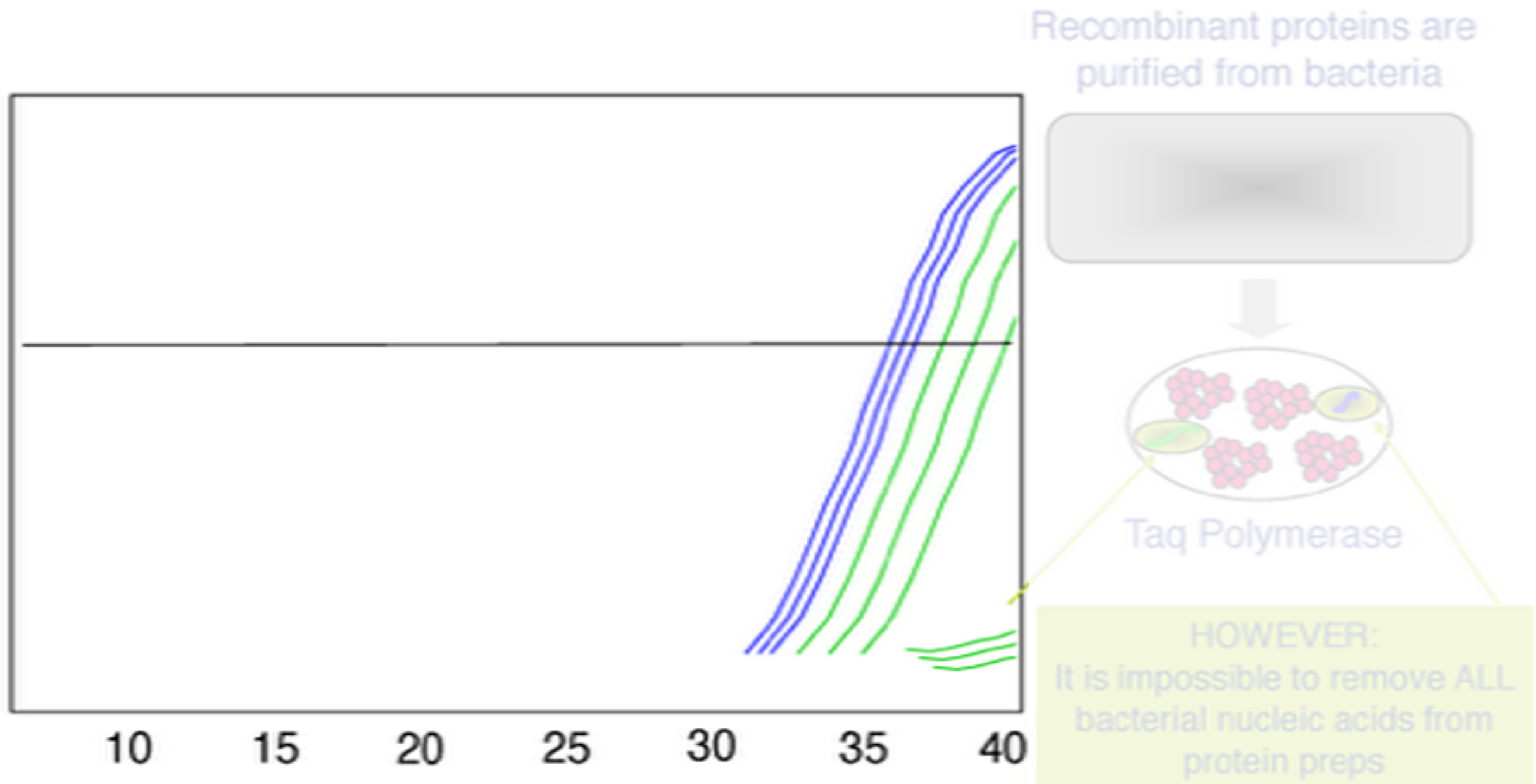


- how reproducible is the data?
- this is determined by replicates

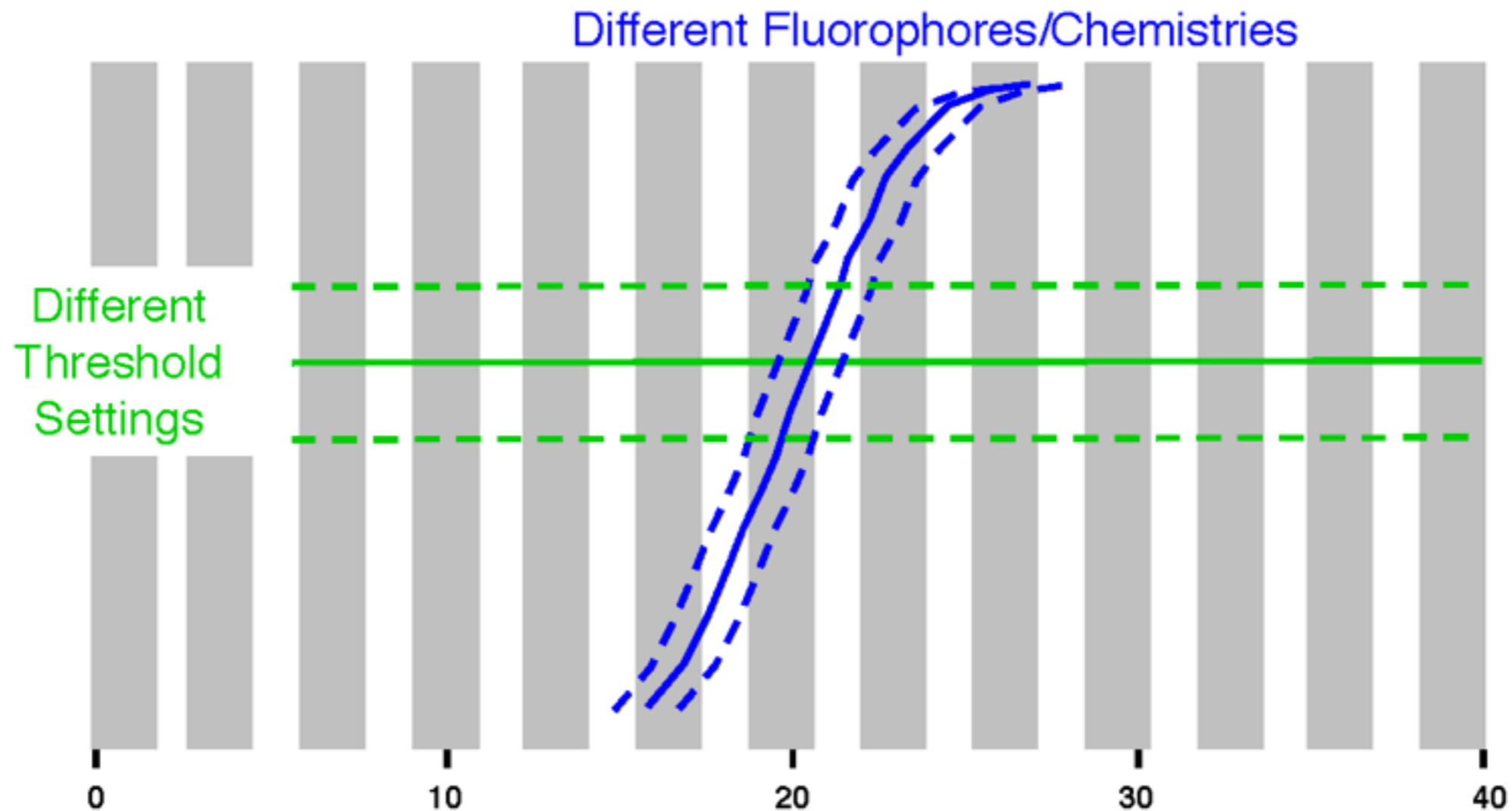


Precision is always reduced at high Cts

NTCs may show some amplification



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

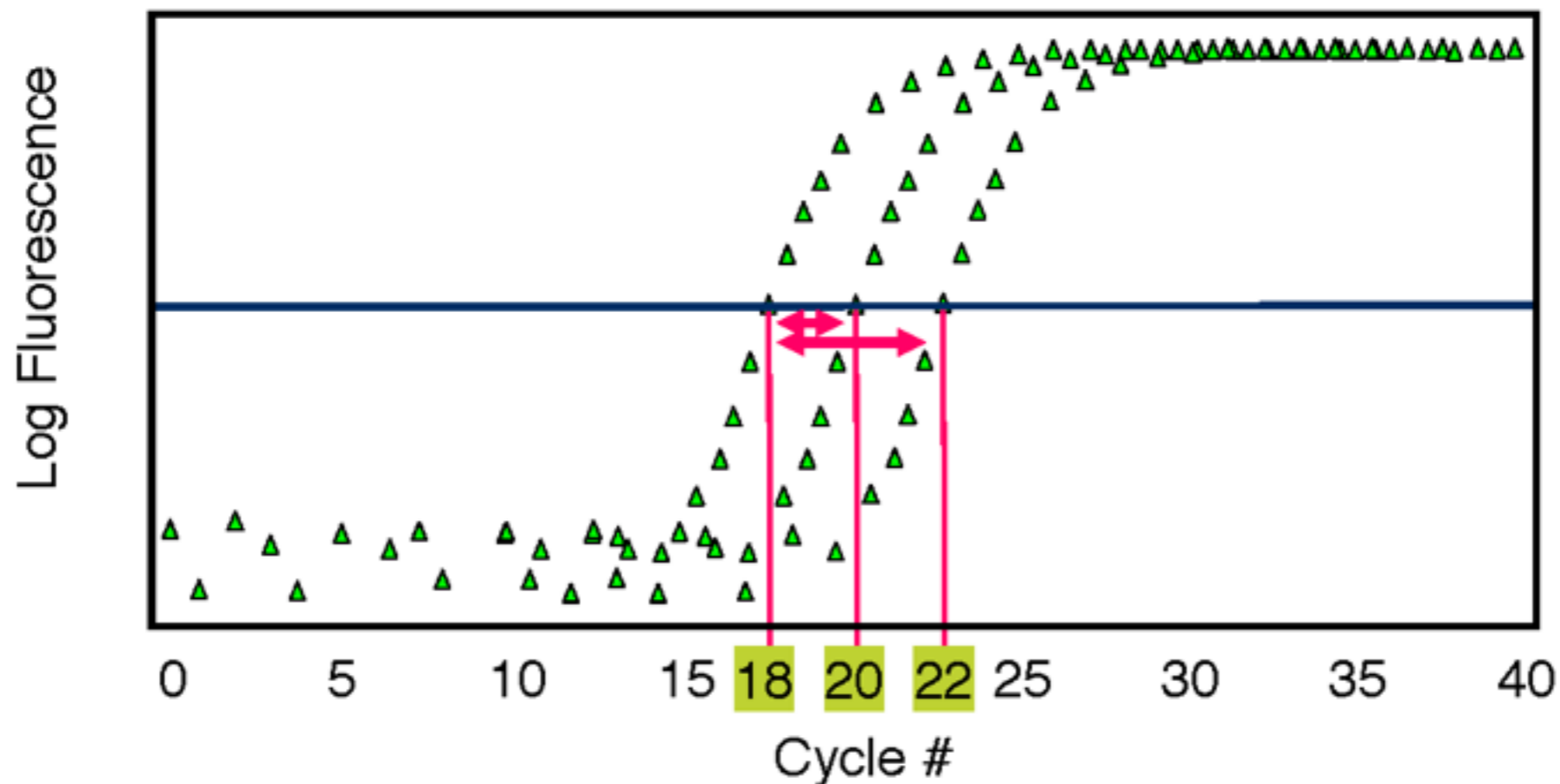


and therefore only indicates an approximate copy number.
For this reason, Ct values are not normally published

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[®] Green

TaqMan[®]

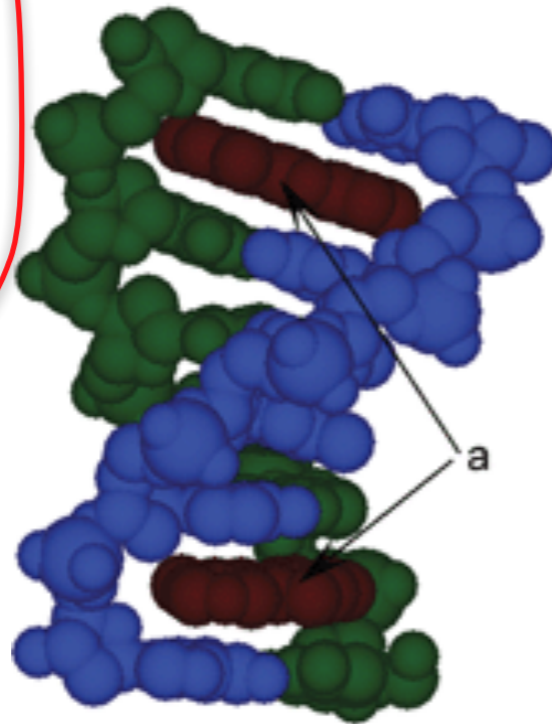
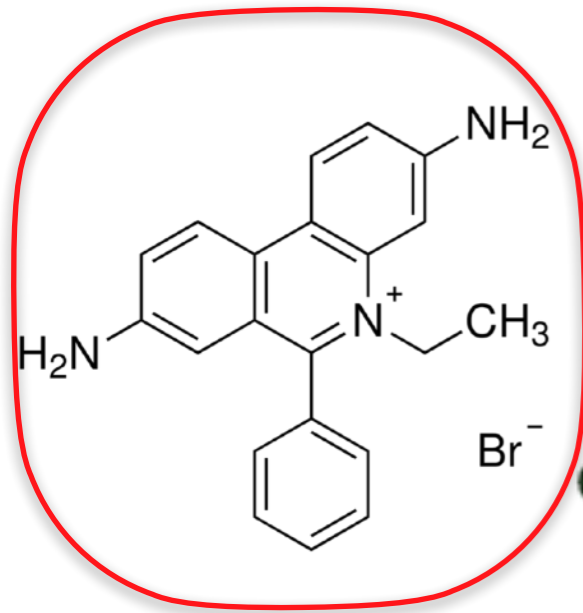
MGB

ROX[™]

Multicomponenting

Visualization – Fluorescent dyes

Intercalating agents



Intercalation

Ethidium Bromide

Minor Groove Binder

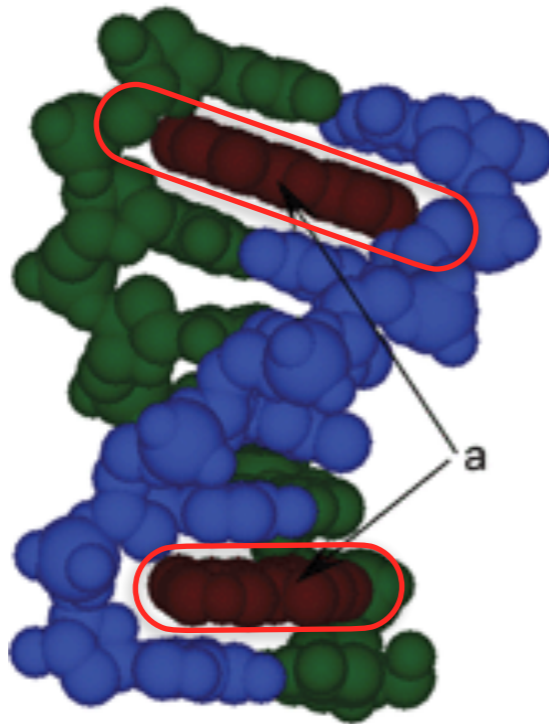
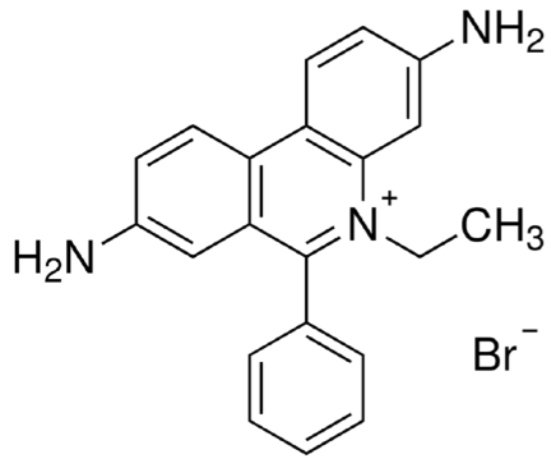


Groove binding

SYBR[®] Green

Visualization – Fluorescent dyes

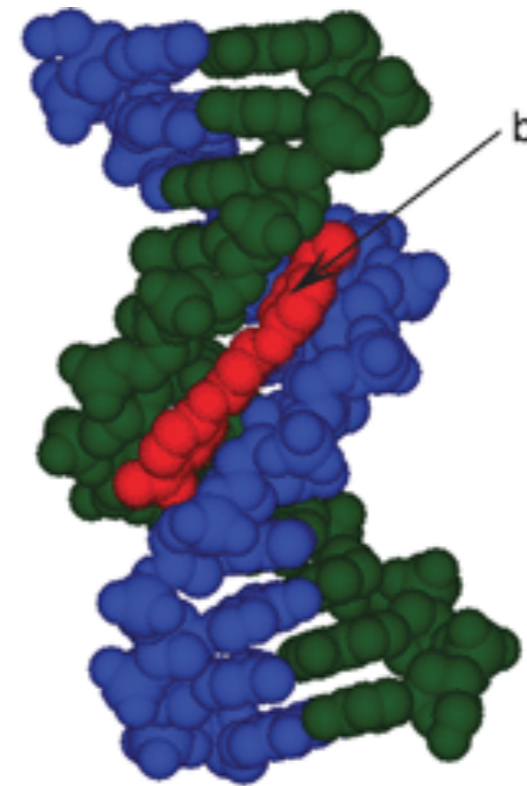
Intercalating agents



Intercalation

Ethidium Bromide

Minor Groove Binder

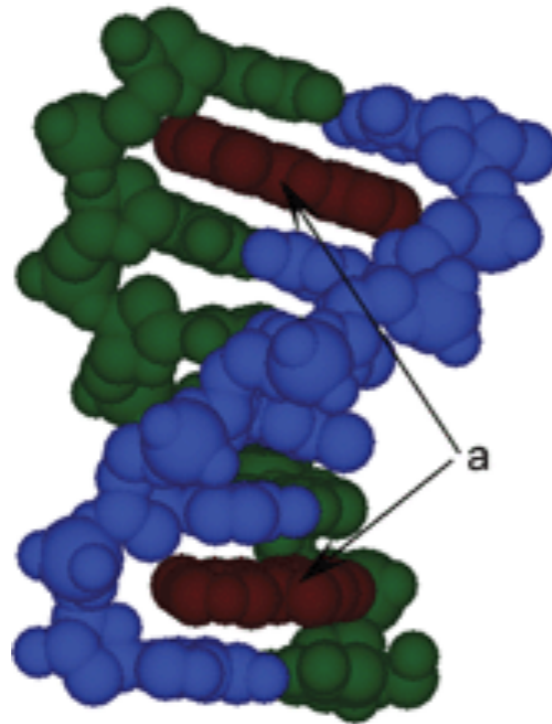
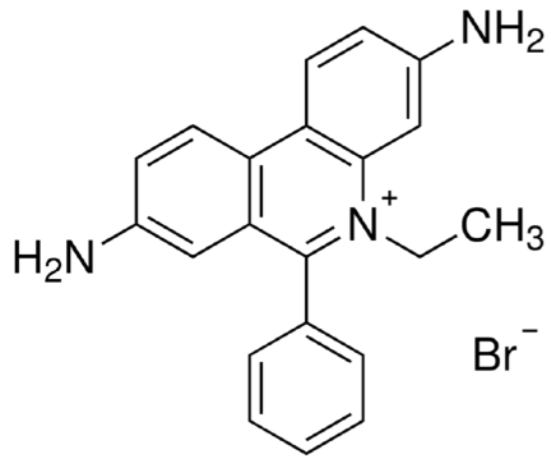


Groove binding

SYBR[®] Green

Visualization – Fluorescent dyes

Intercalating agents



Intercalation

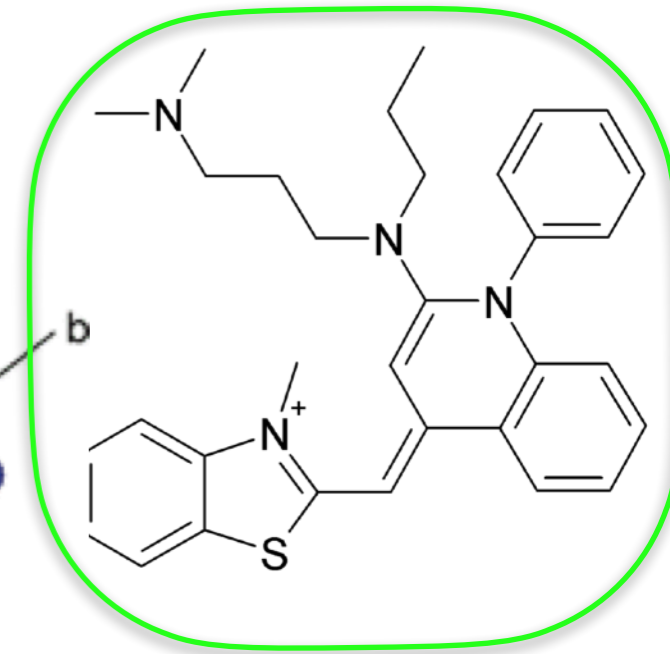
Ethidium Bromide

Minor Groove Binder



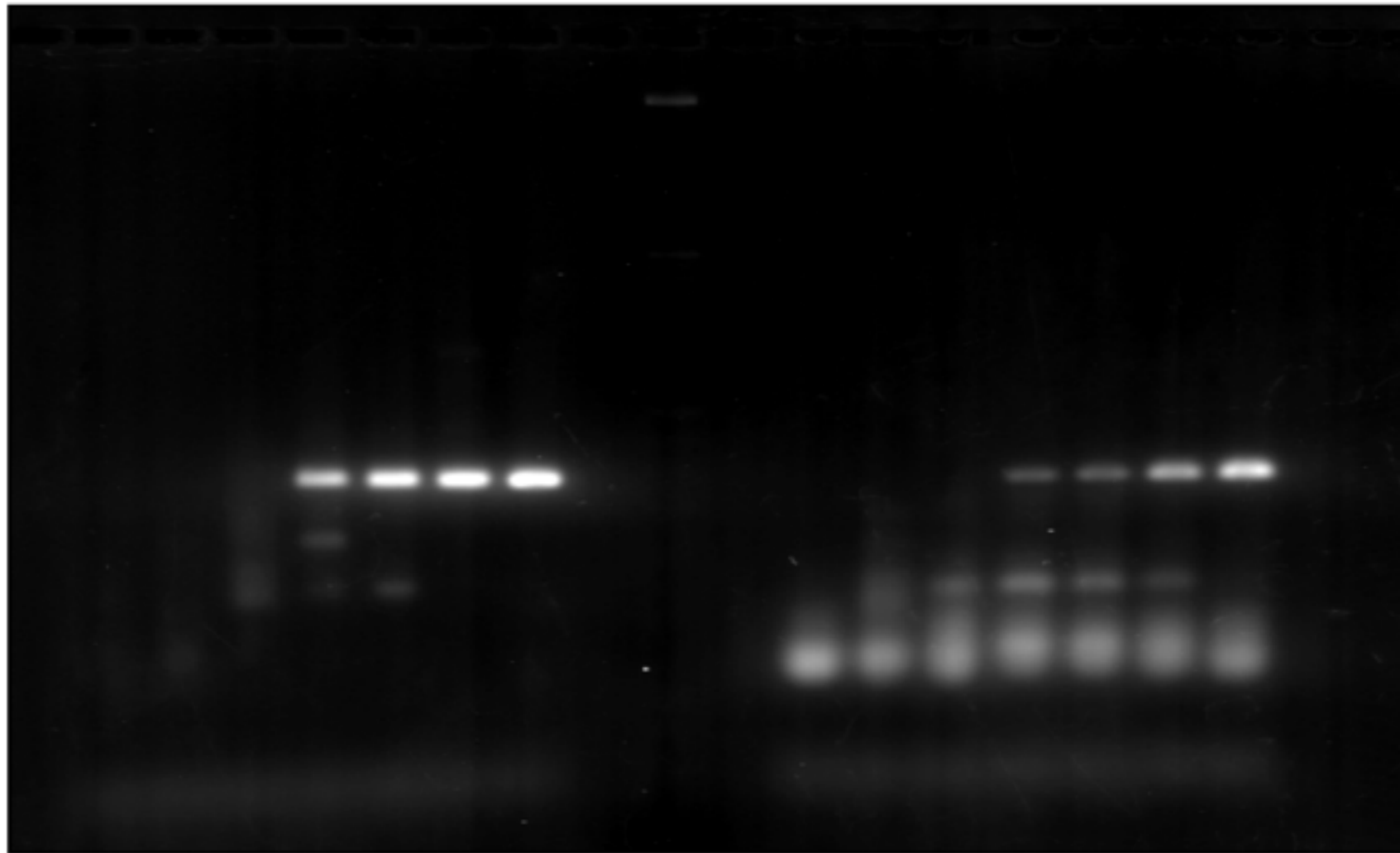
Groove binding

SYBR[®] Green



Problem with DNA-binding Dyes

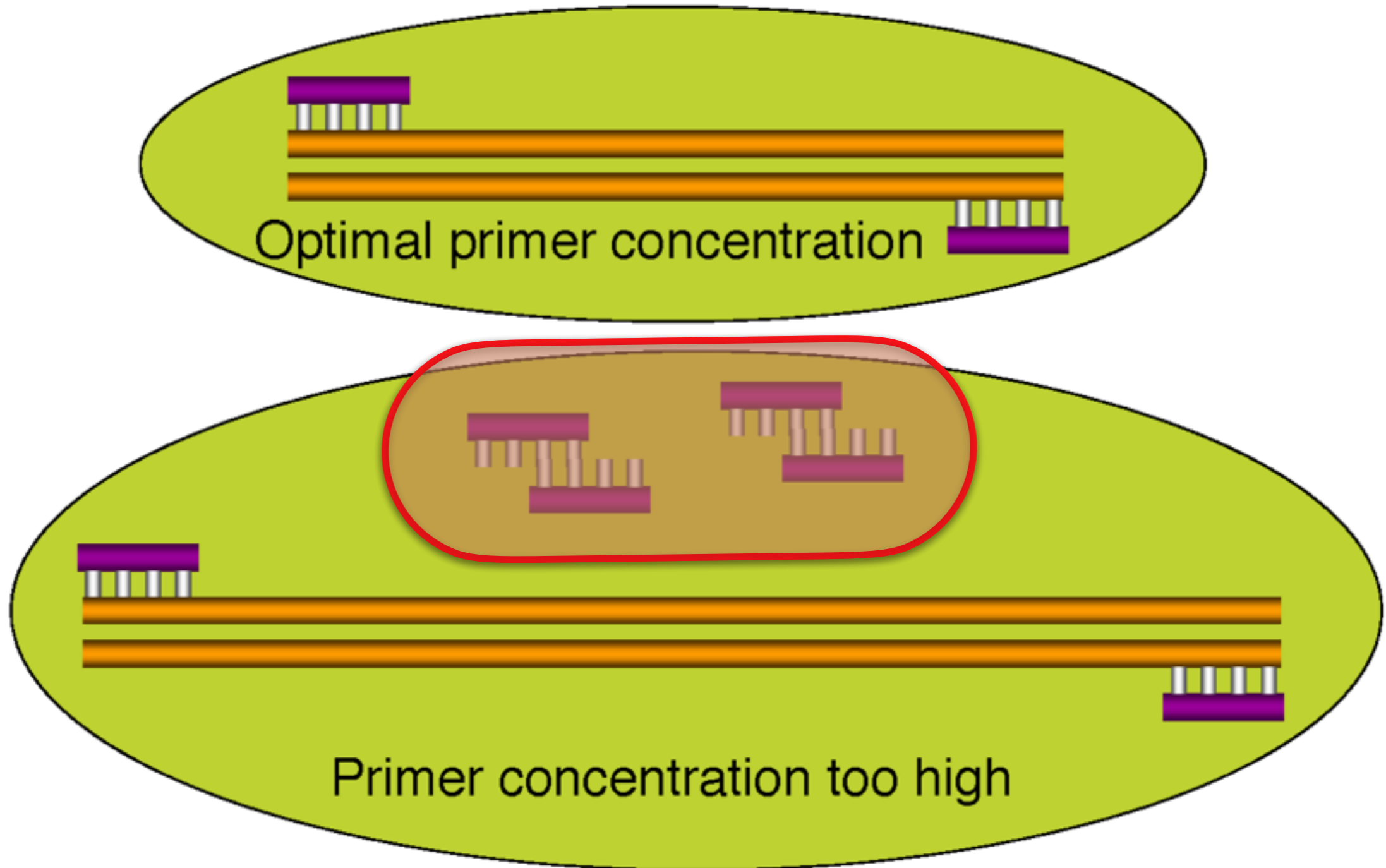
Bind non-specifically to *any* double-stranded DNA



Therefore specificity of the amplifications must be checked

Potential Problems !!

Non-specific amplification promoted by high primer concentration



Potential Solution

Primer-dimer formation reduced by minimizing primer concentration

Forward Primer (final conc)

←

	50 nM	100 nM	200 nM	300 nM	400 nM	500 nM
50 nM						
100 nM						
200 nM						
300 nM						
400 nM						
500 nM						

↑

Reverse Primer (final conc)



Real-time PCR

SYBR[®] Green

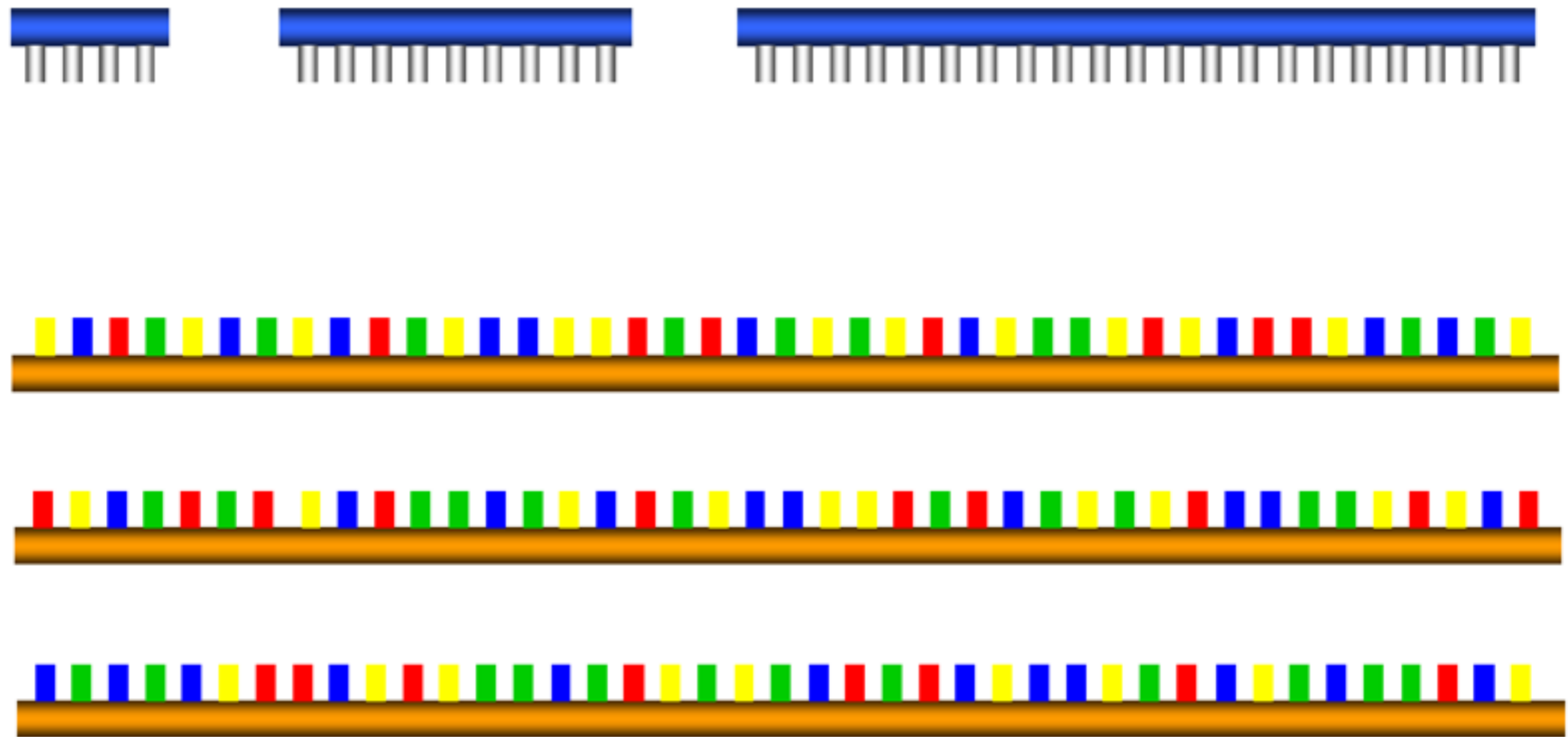
TaqMan[®]

MGB

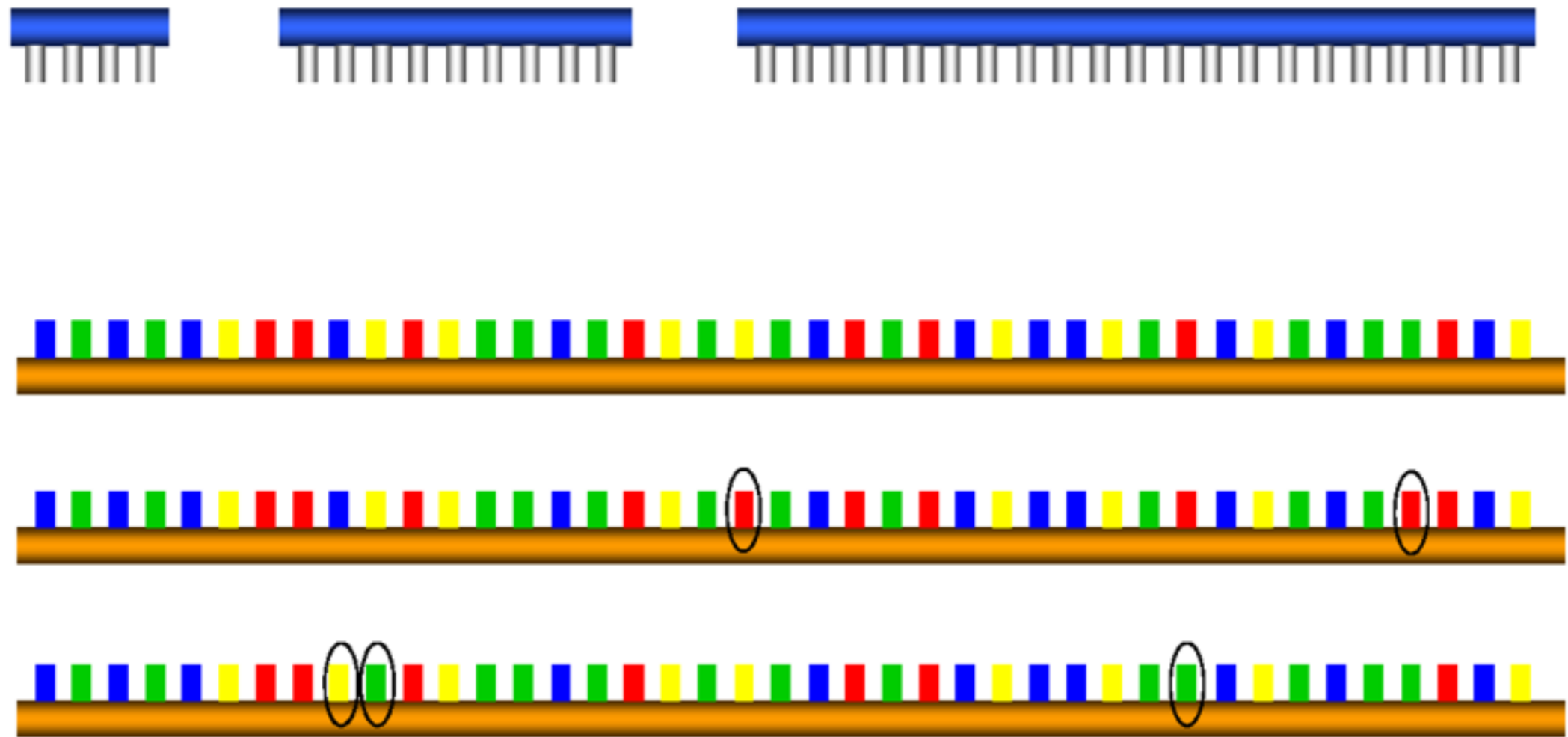
ROX[™]

Multicomponenting

Probing for specific sequence in a pool of very different sequences

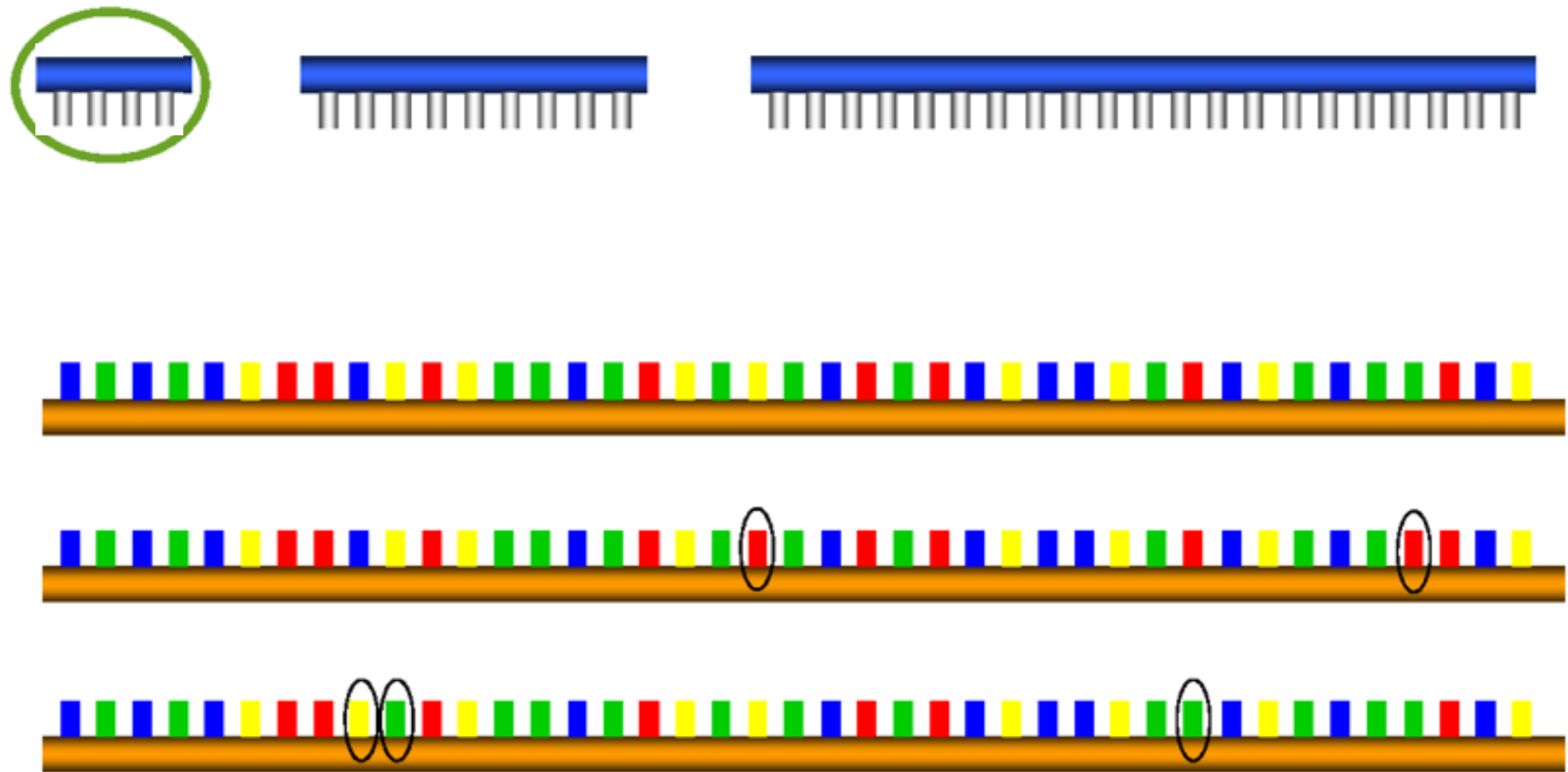


Probing for specific sequence in a pool of very similar sequences



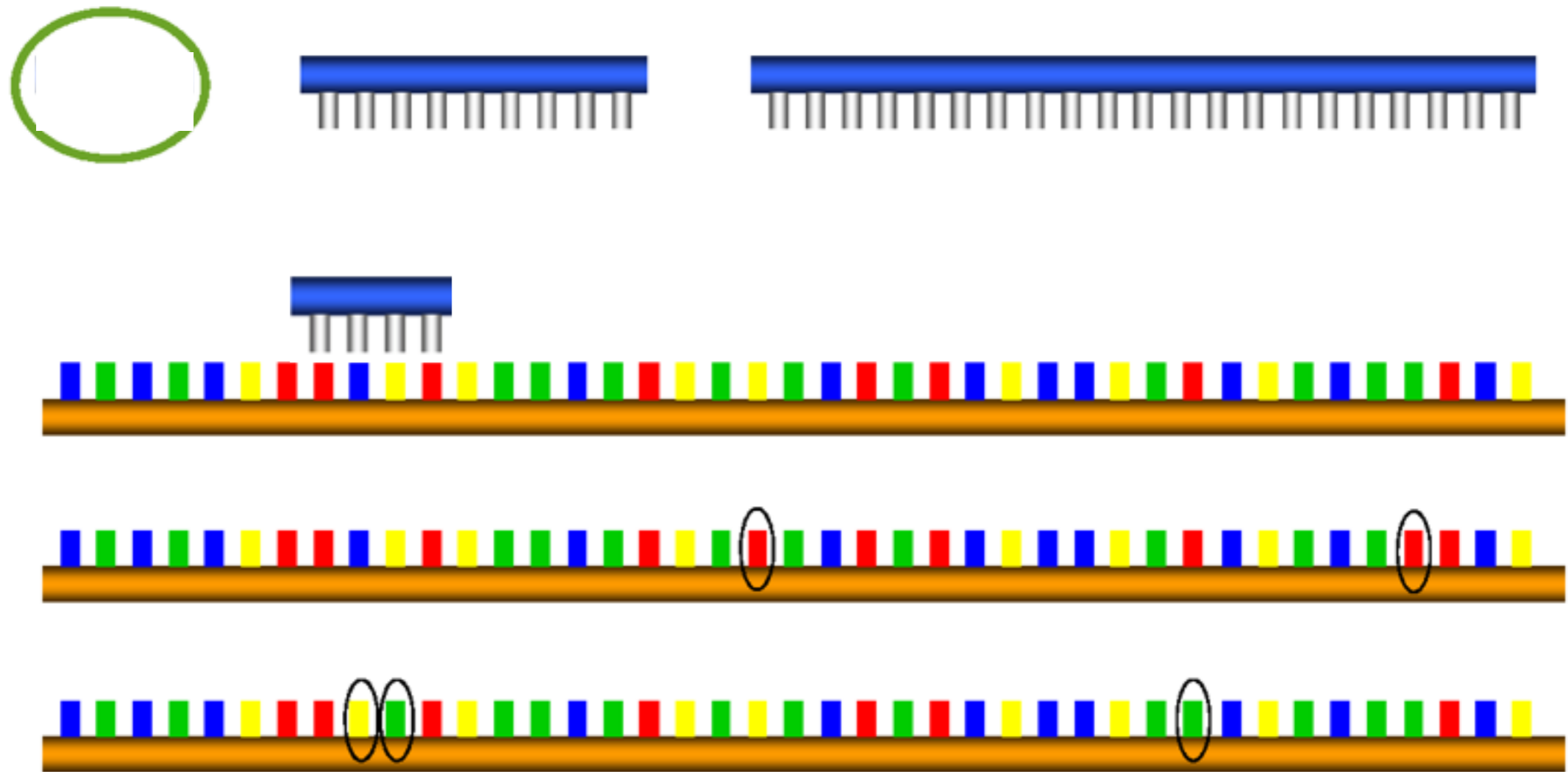
Probing for specific sequence in a pool of very similar sequences

Shorter probes increase selectivity between similar sequences



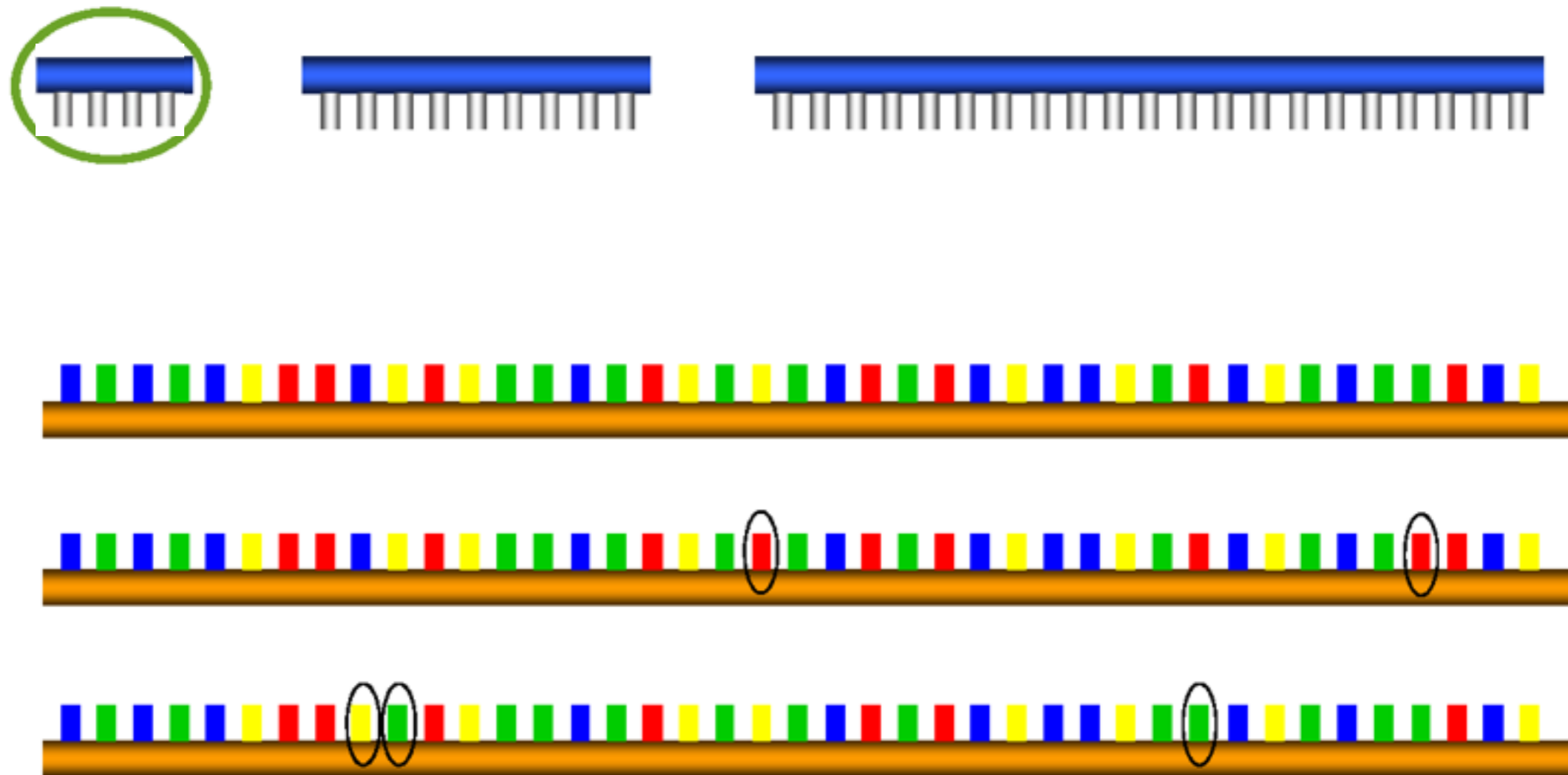
Probing for specific sequence in a pool of very similar sequences

Shorter probes increase selectivity between similar sequences



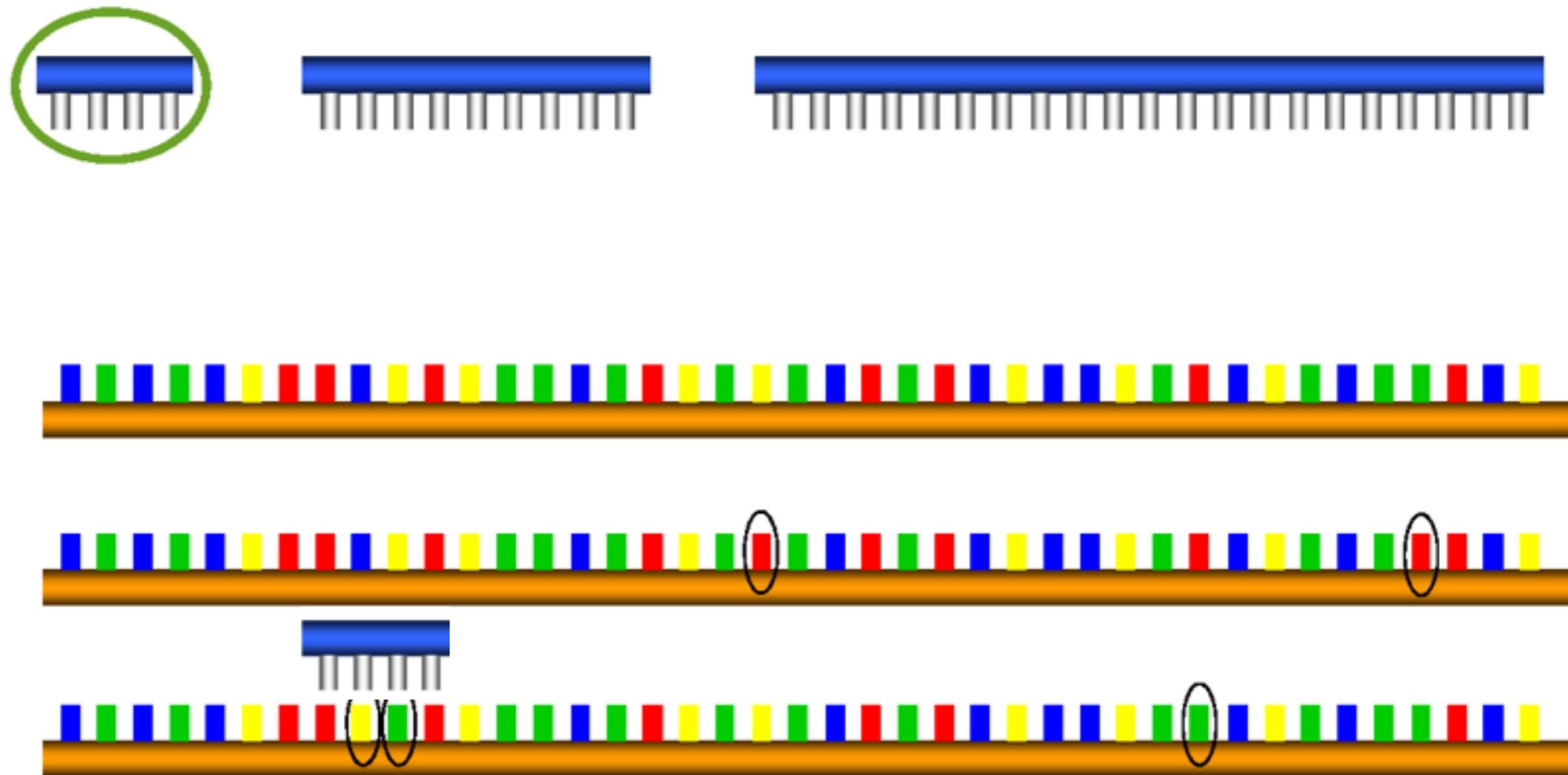
Probing for specific sequence in a pool of very similar sequences

Shorter probes increase selectivity between similar sequences



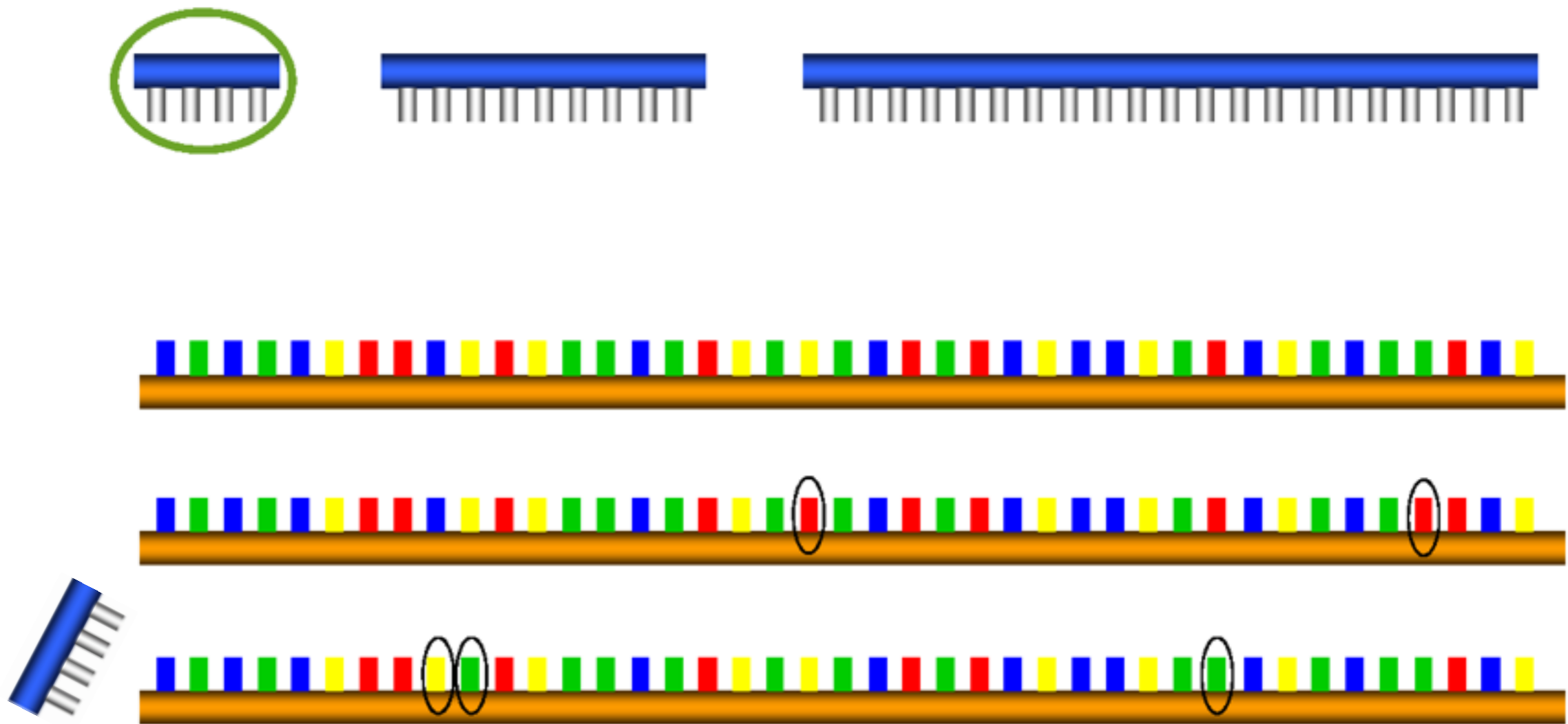
Probing for specific sequence in a pool of very similar sequences

Shorter probes increase selectivity between similar sequences



Probing for specific sequence in a pool of very similar sequences

Shorter probes increase selectivity between similar sequences



Potential Problem / Solved !!

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

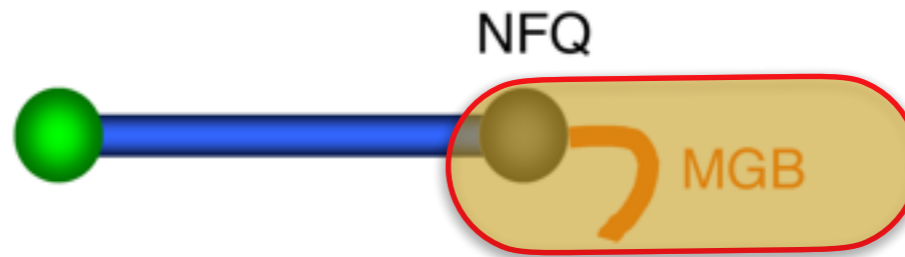
Primers ~ 20-30bp



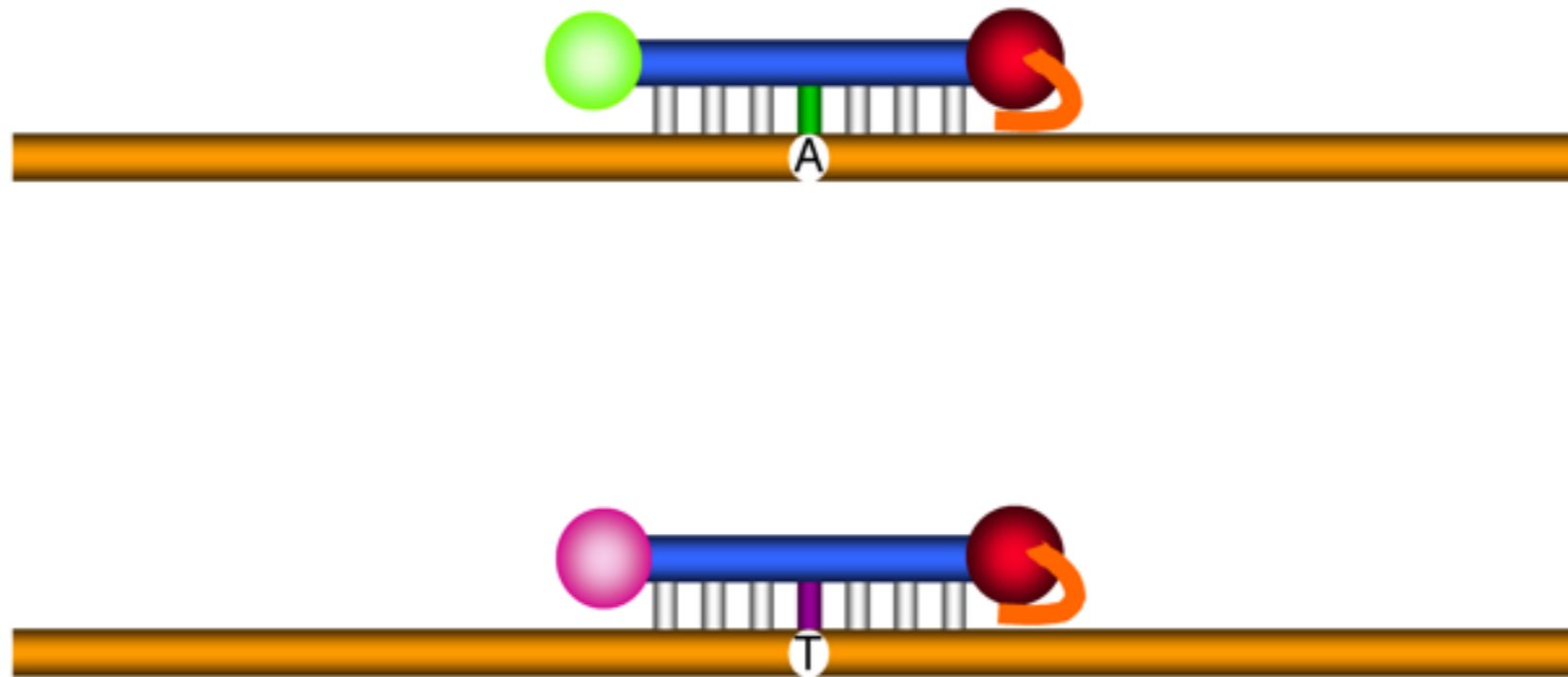
regular probes ~30-40bp



MGB probes ~13-22bp



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



Real-time PCR

SYBR[®] Green

TaqMan[®]

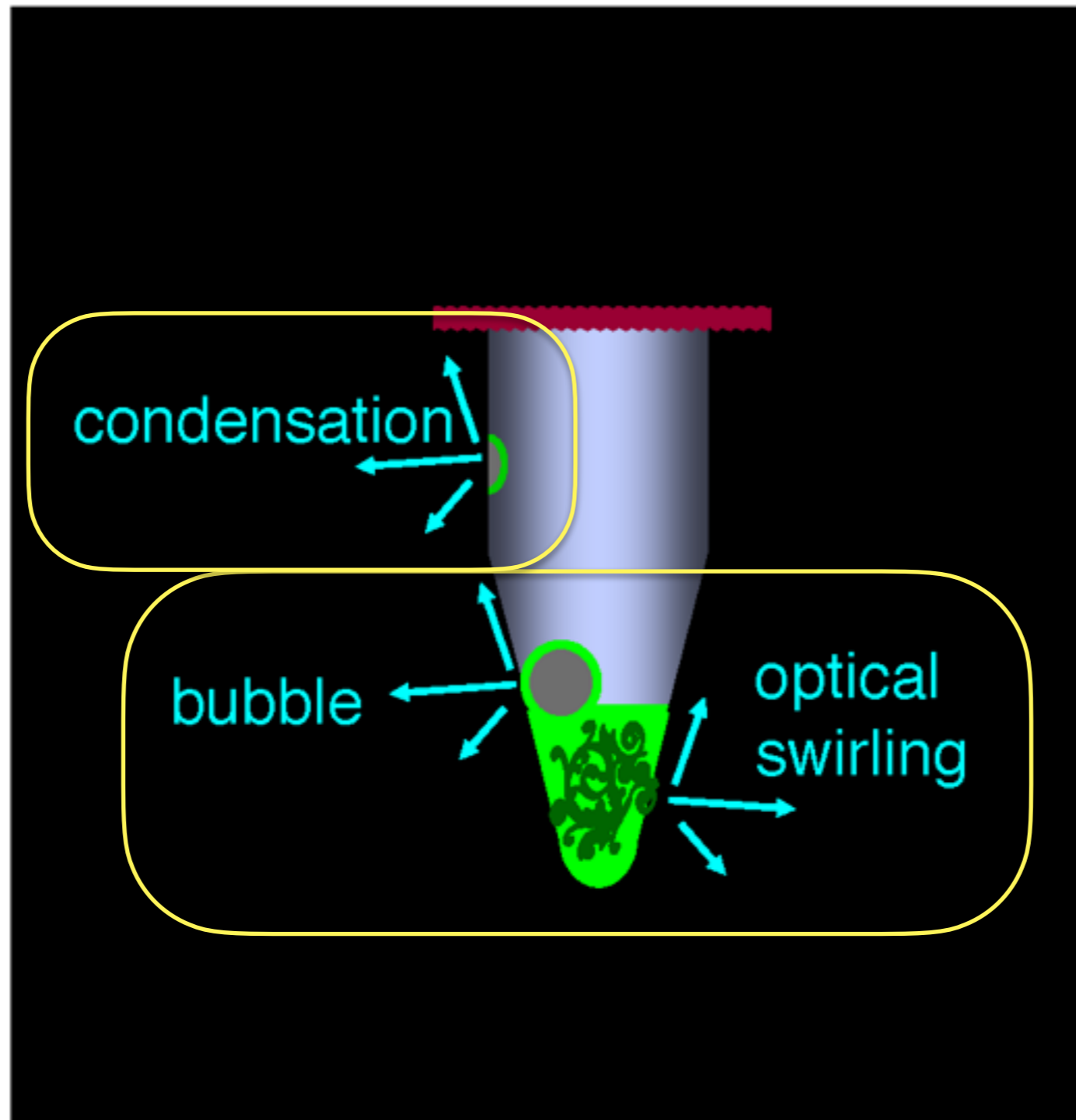
MGB

ROX[™]

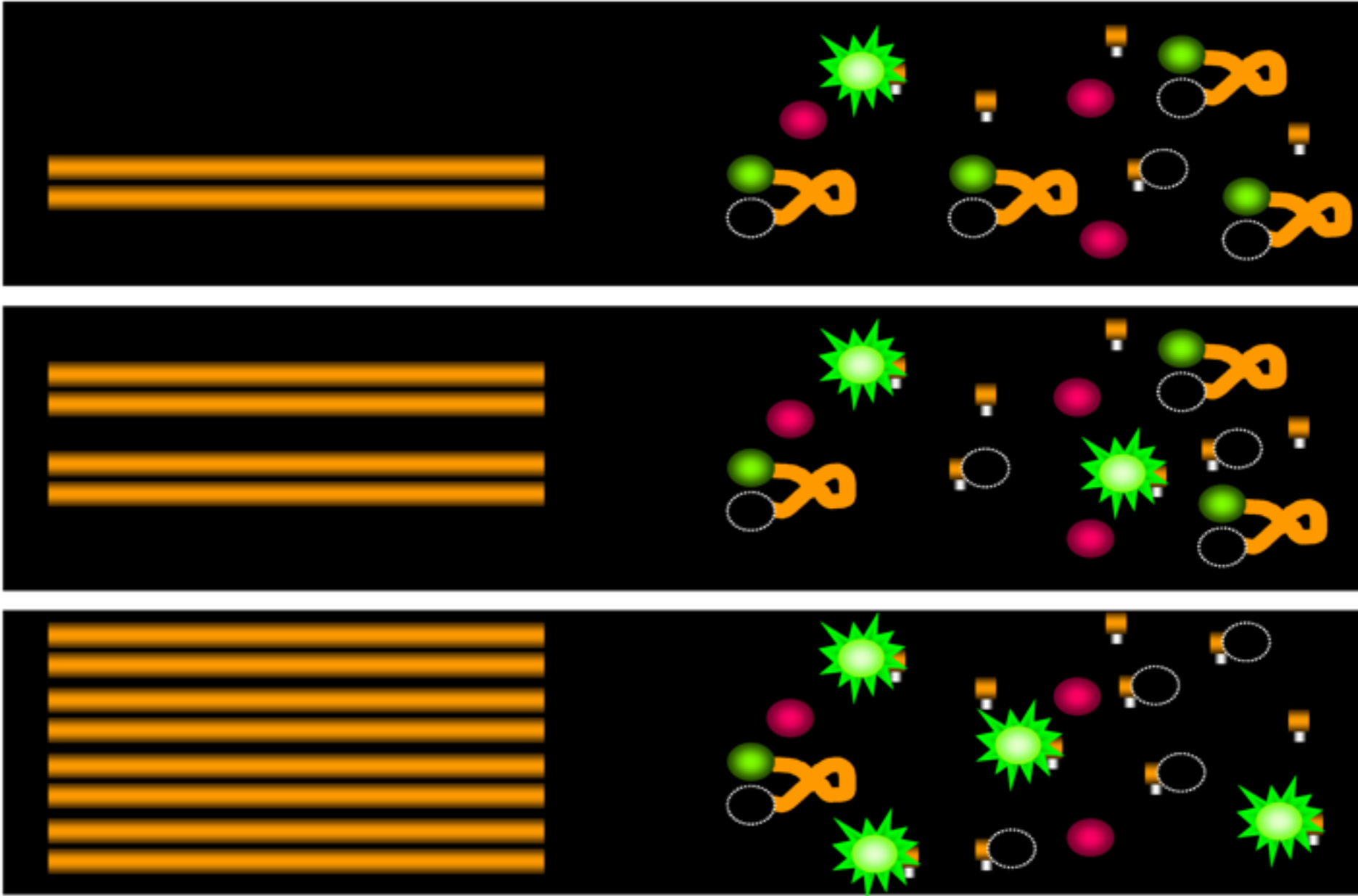
Multicomponenting

Potential Problem !!

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



Variation negated by normalizing to a Passive Reference dye

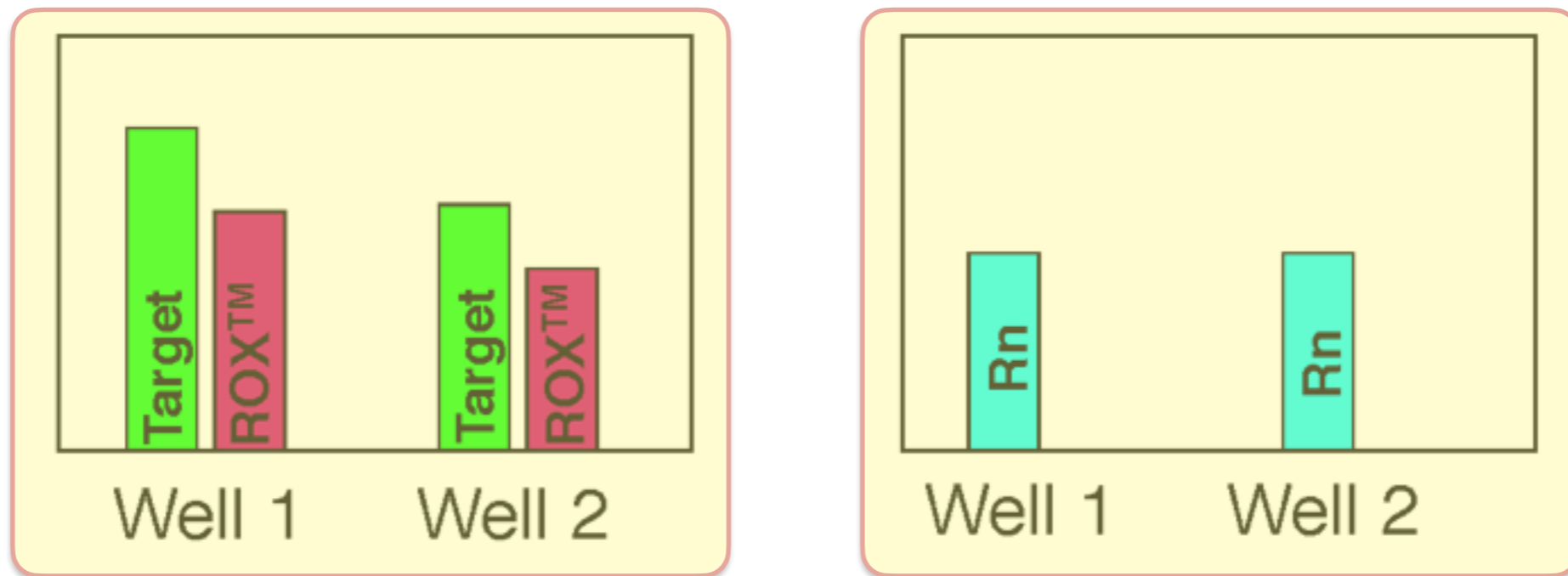


Potential Solution

ROX™ is a Passive Reference dye

Greatly improves precision of replicates.

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





Real-time PCR

SYBR[®] Green

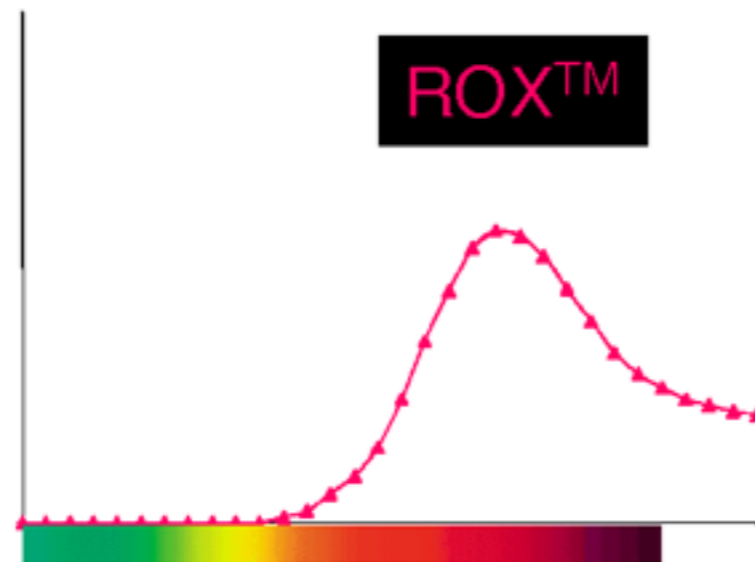
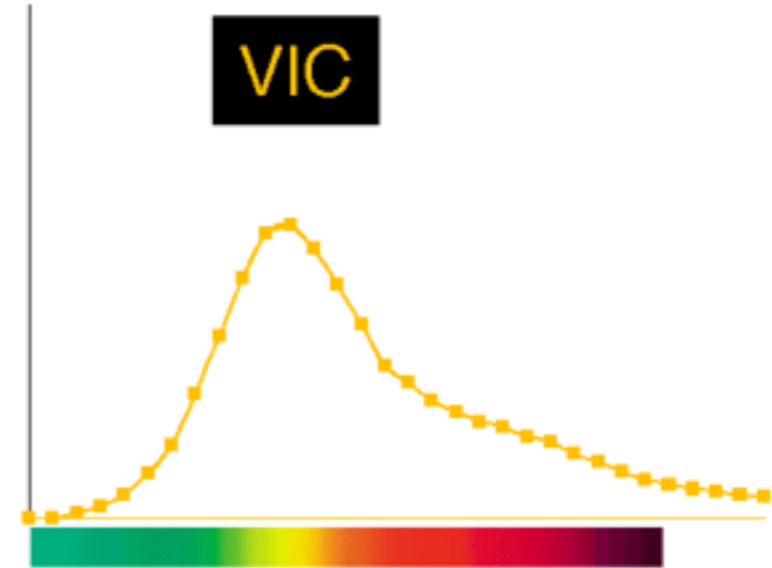
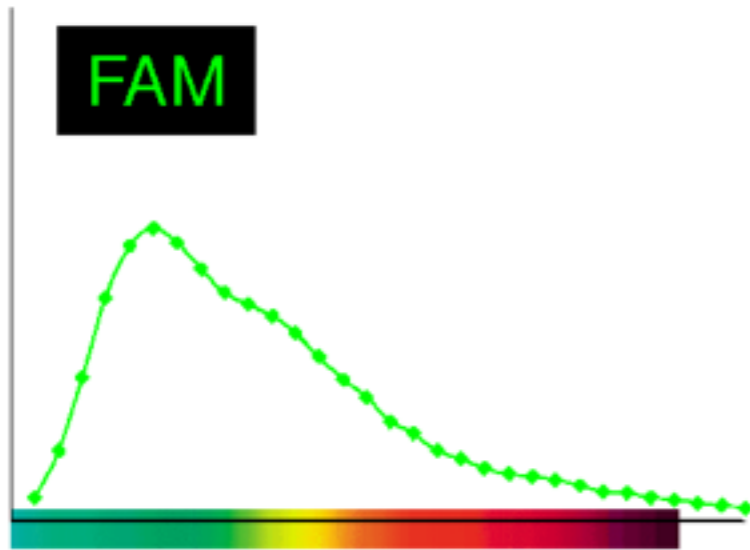
TaqMan[®]

MGB

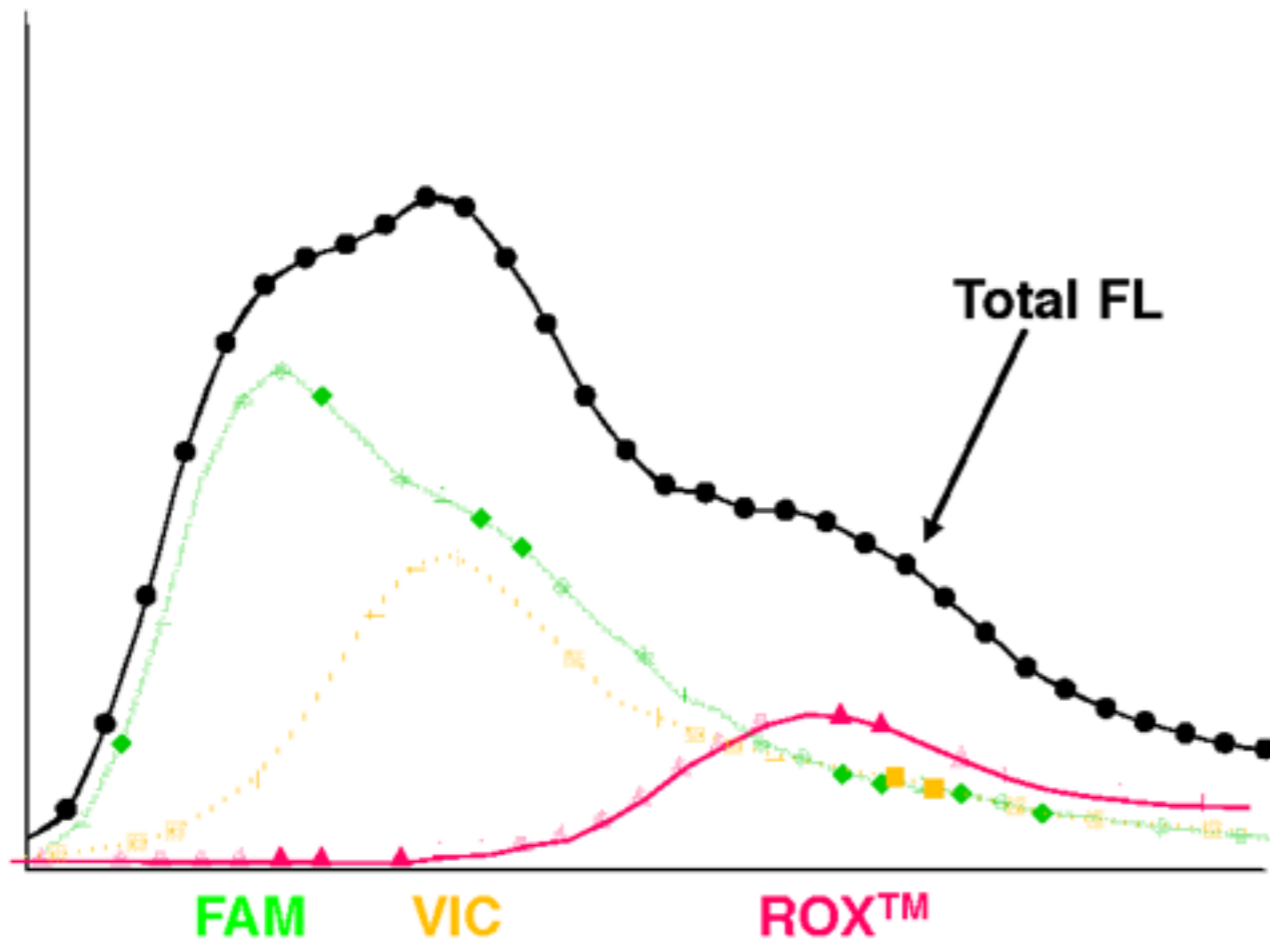
ROX[™]

Multicomponenting

Dyes have specific fluorescence spectra with specific peaks

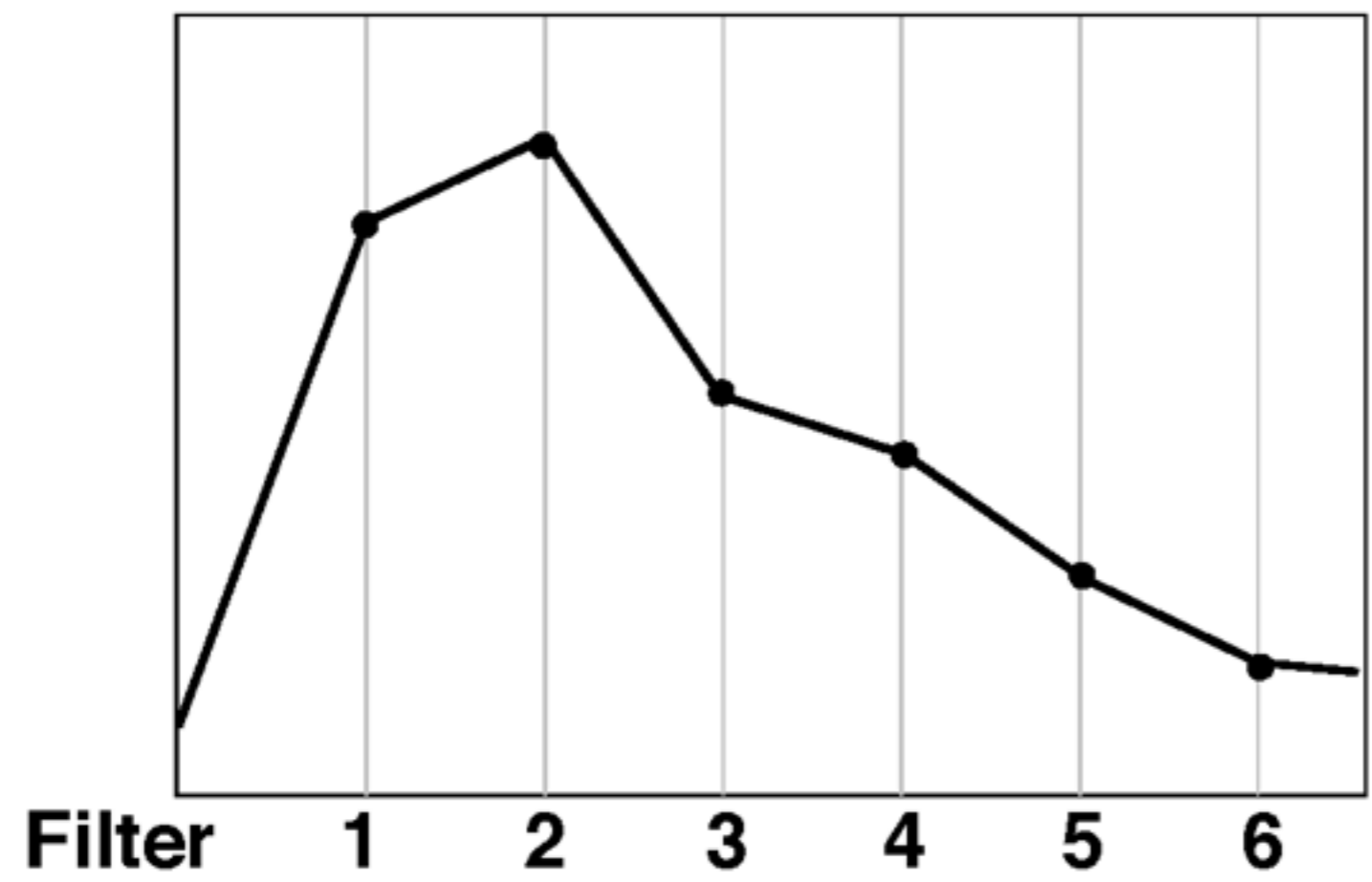


However, if more than one dye is present, there is spectral overlap

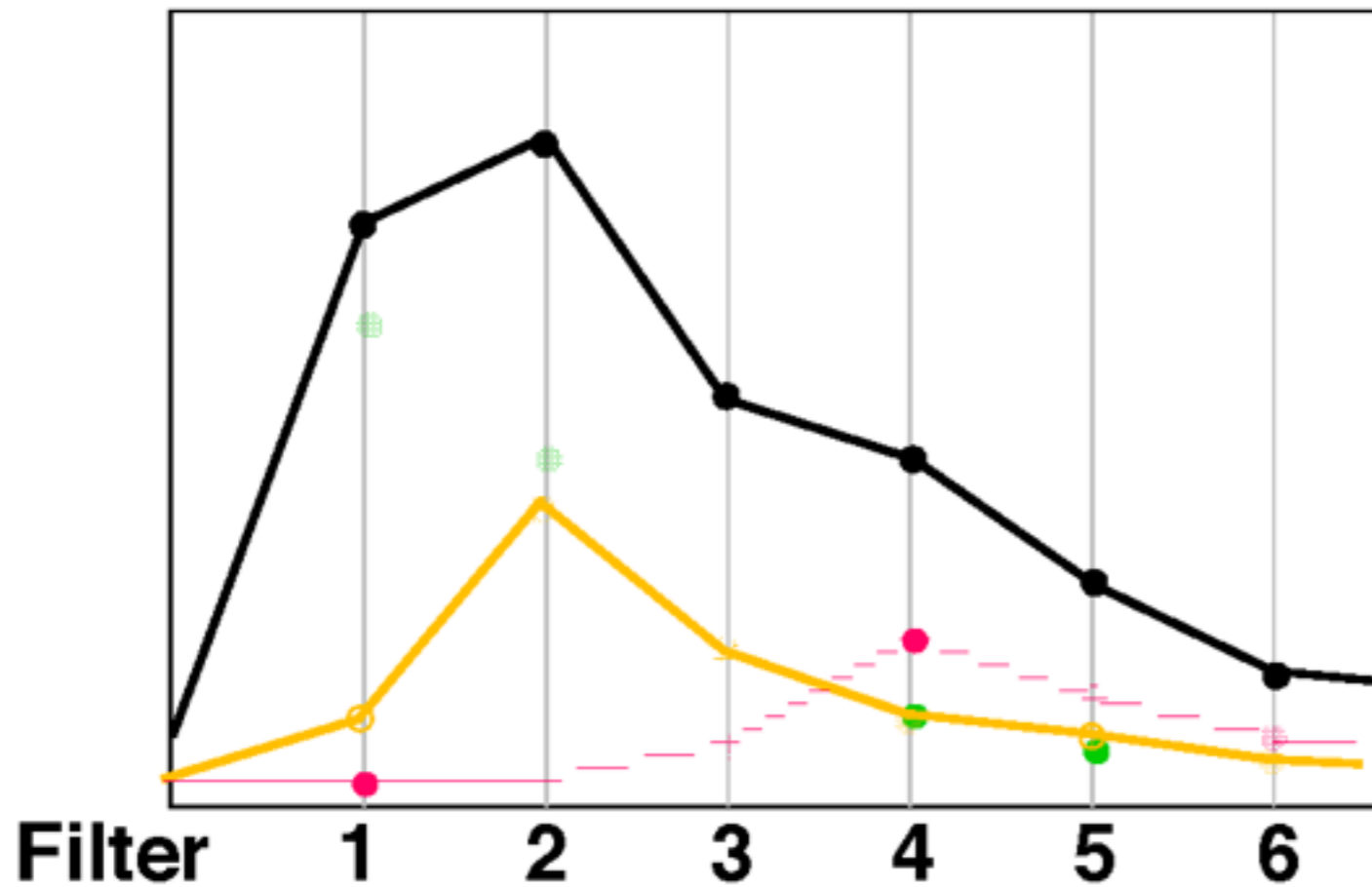


If not addressed, this would introduce large inaccuracies

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

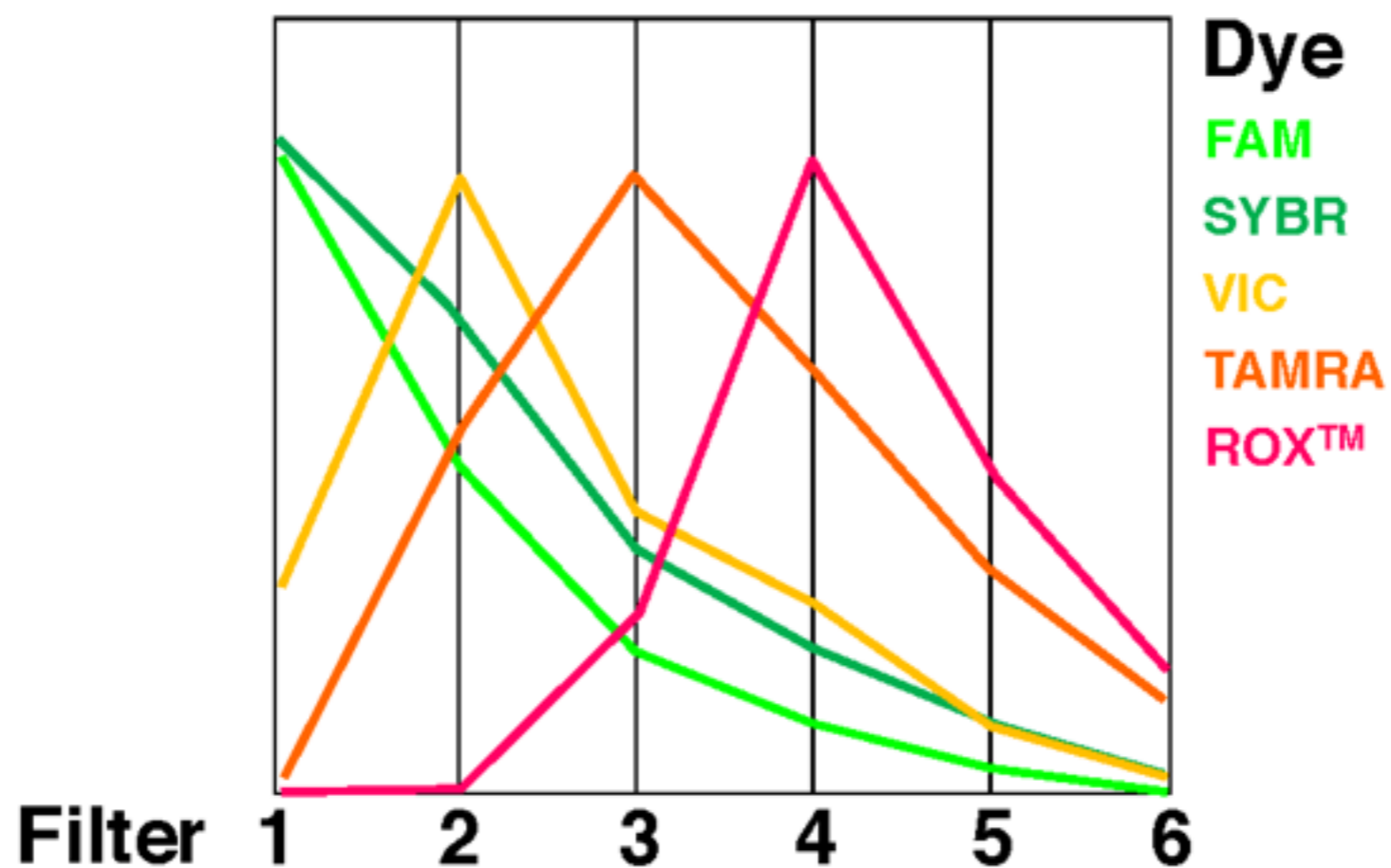


What we see is the Total Fluorescence at each wavelength – this is not the same as the individual dye fluorescence



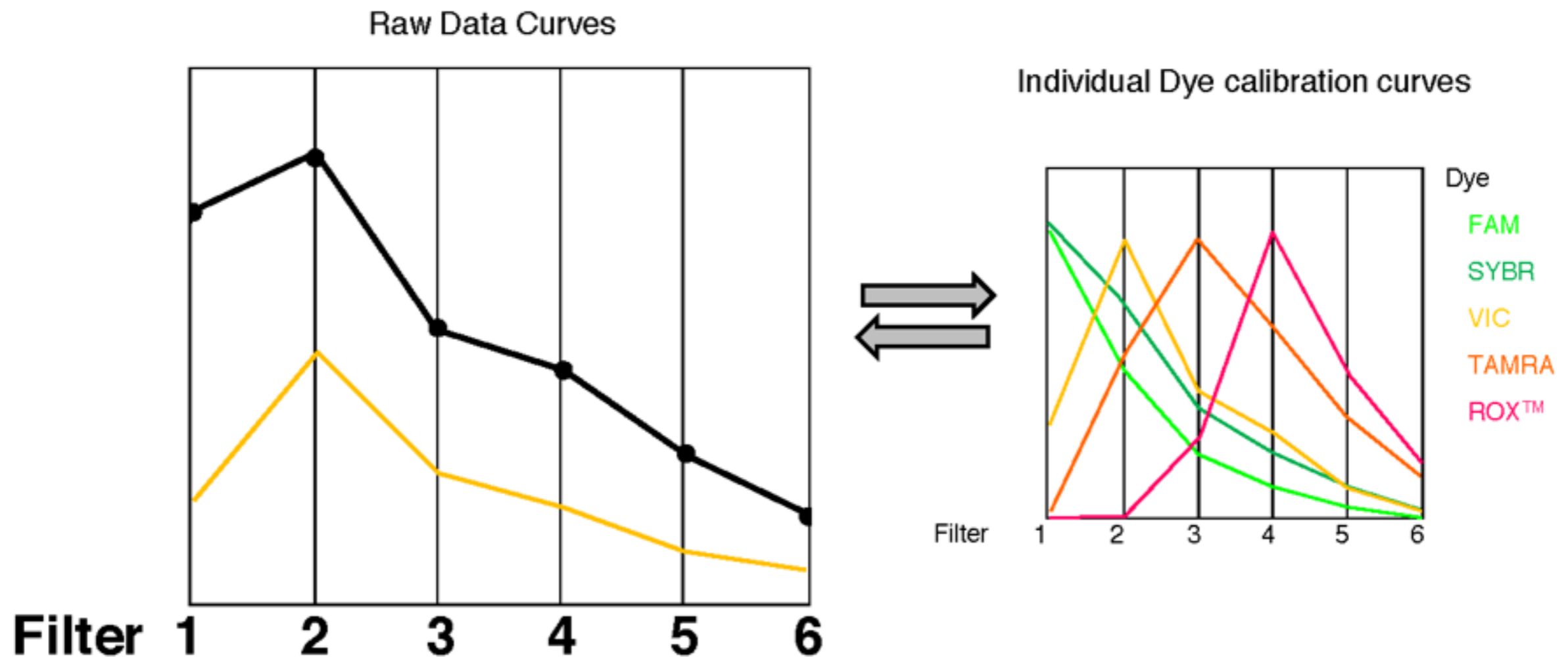
How do we adjust for this?

Answer: Dye Calibration

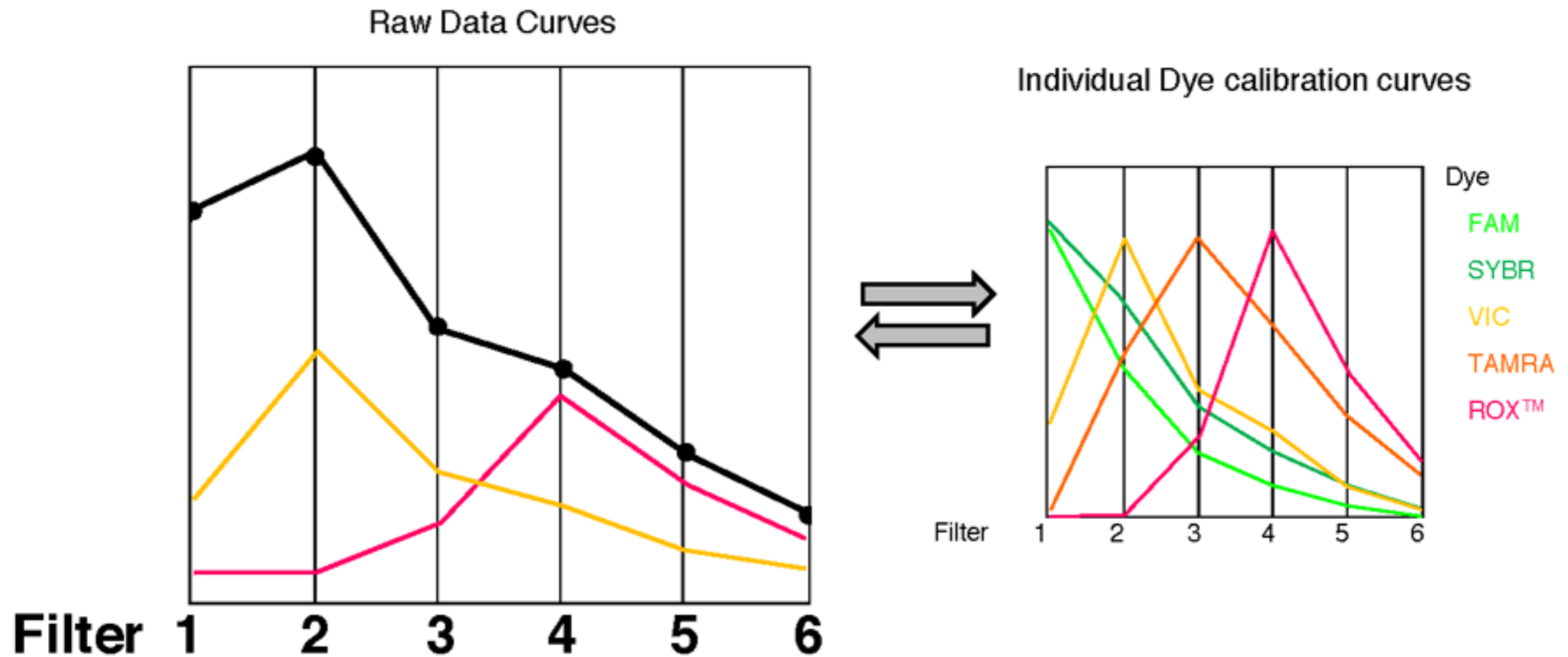


At installation, a dye calibration plate is read. This contains dilutions of pure dye. So the instrument records what each dye “looks like”.

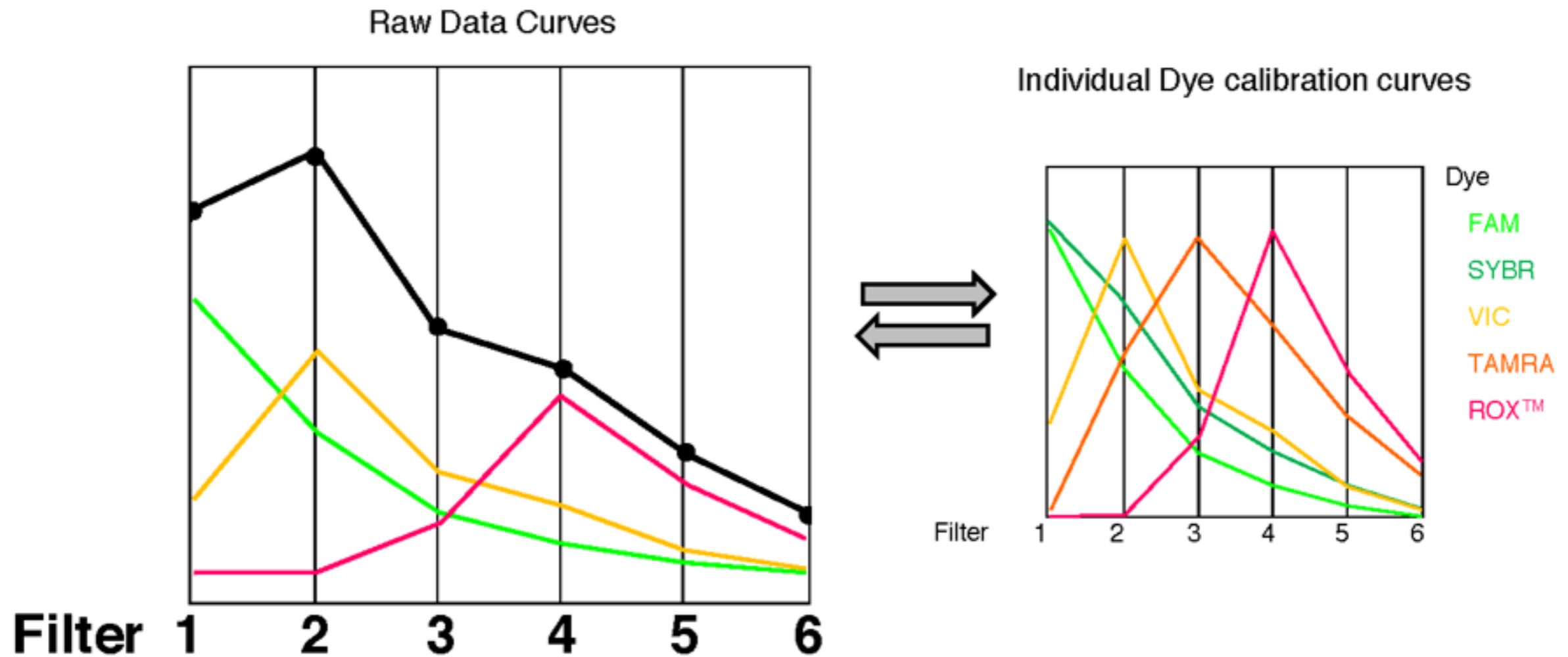
Then, the multicomponenting software reconstructs the Total FL curve from the individual dye curves



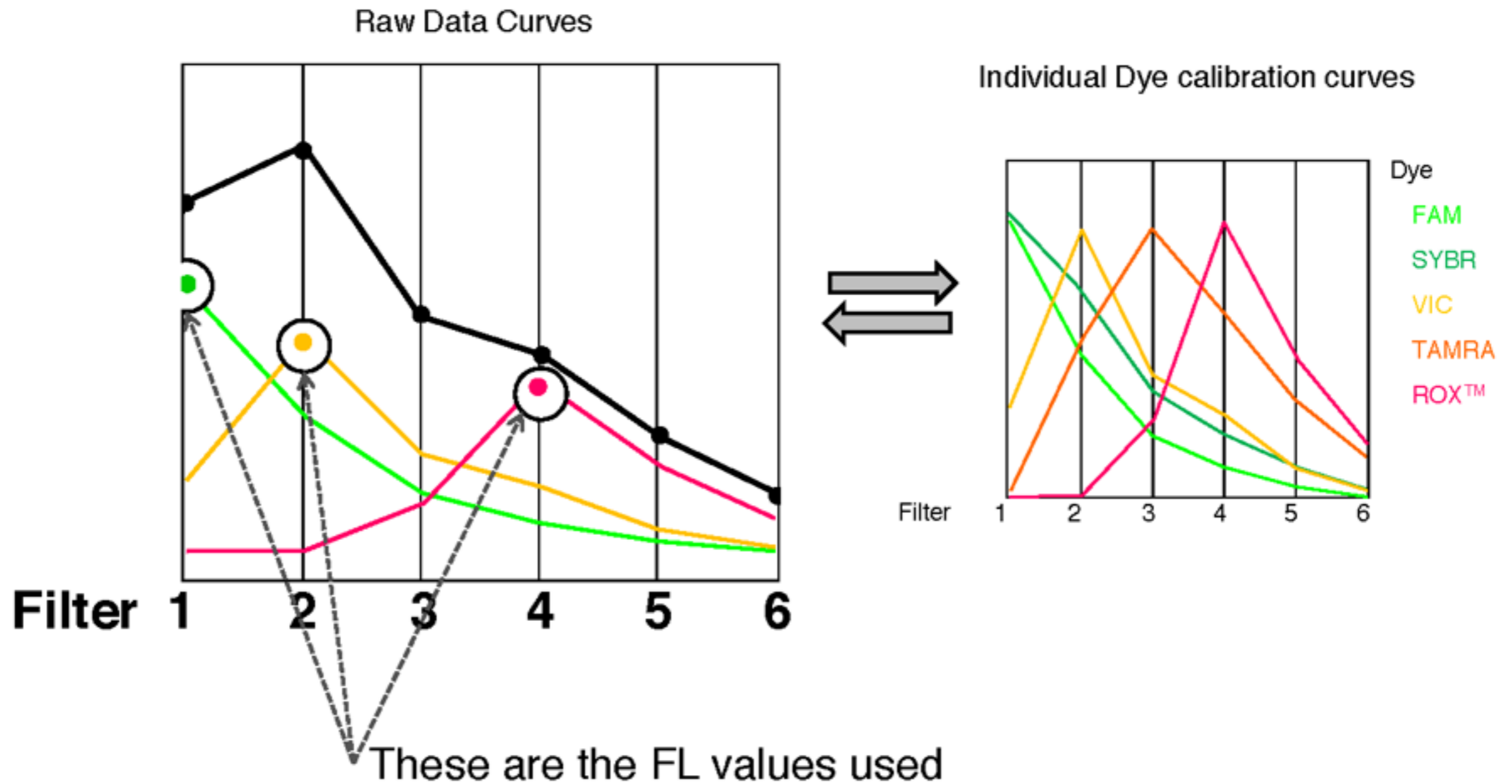
Then, the multicomponenting software reconstructs the Total FL curve from the individual dye curves



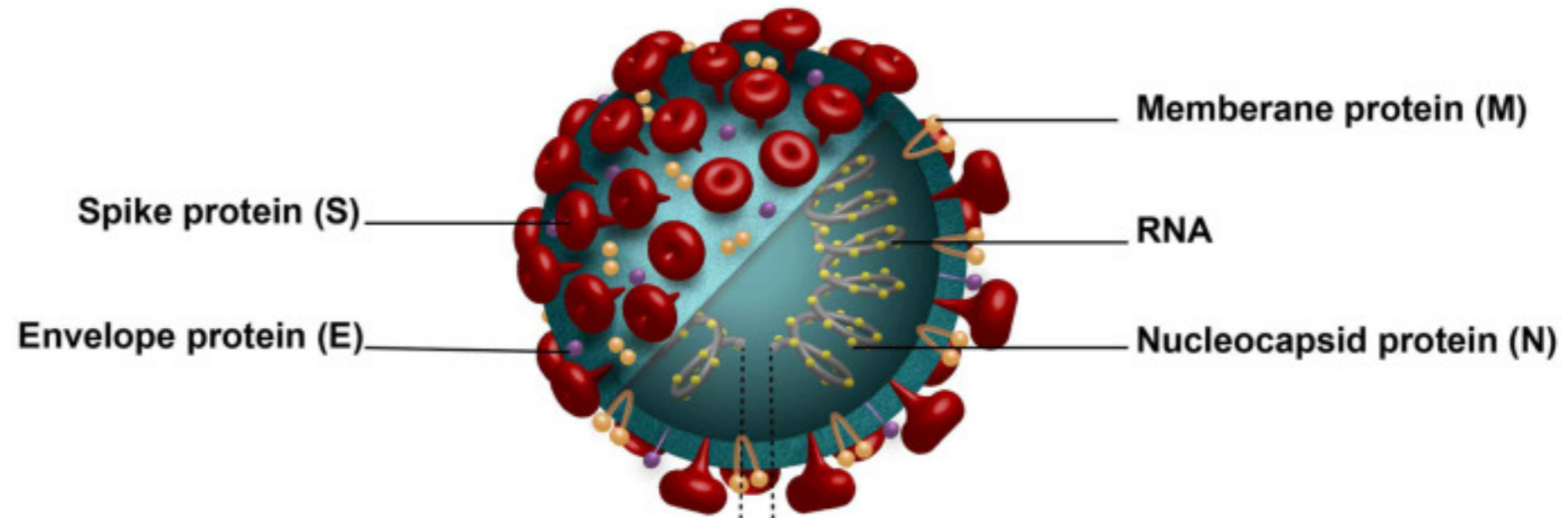
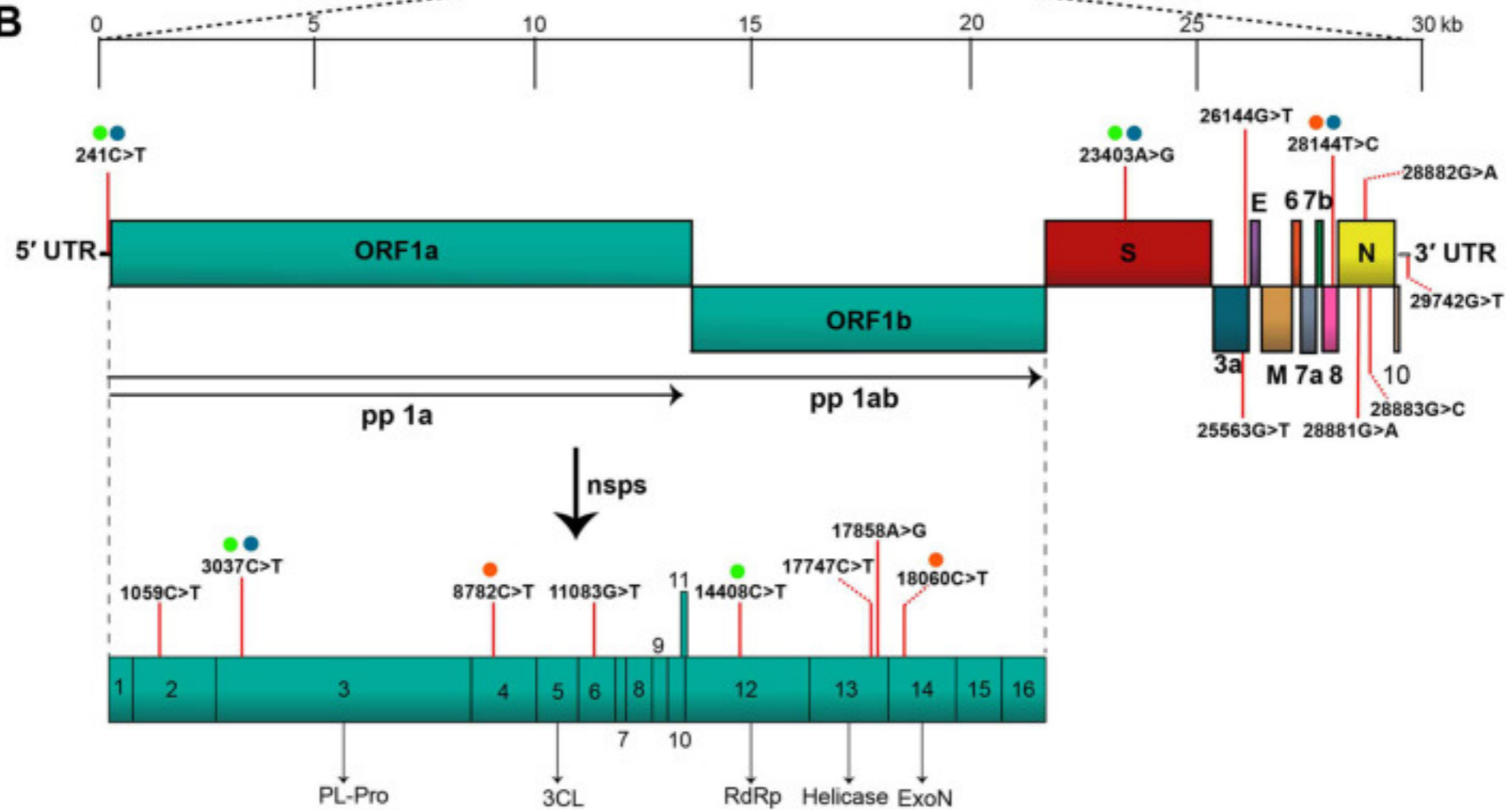
Then, the multicomponenting software reconstructs the Total FL curve from the individual dye curves



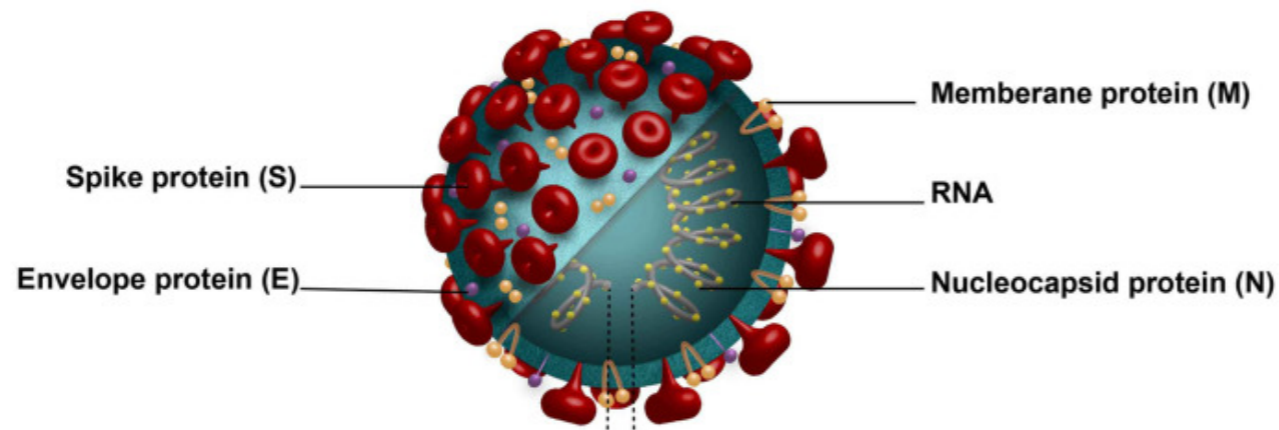
Then, the multicomponenting software reconstructs the Total FL curve from the individual dye curves



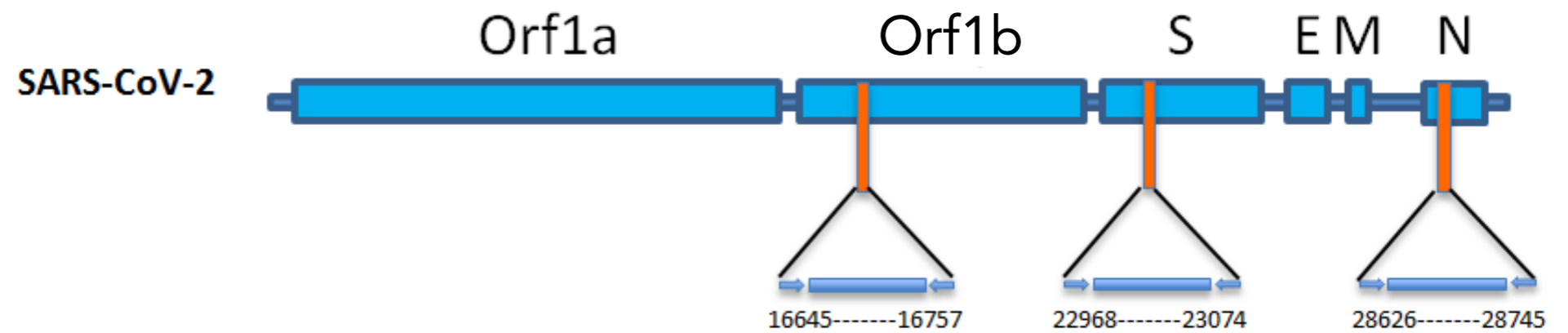
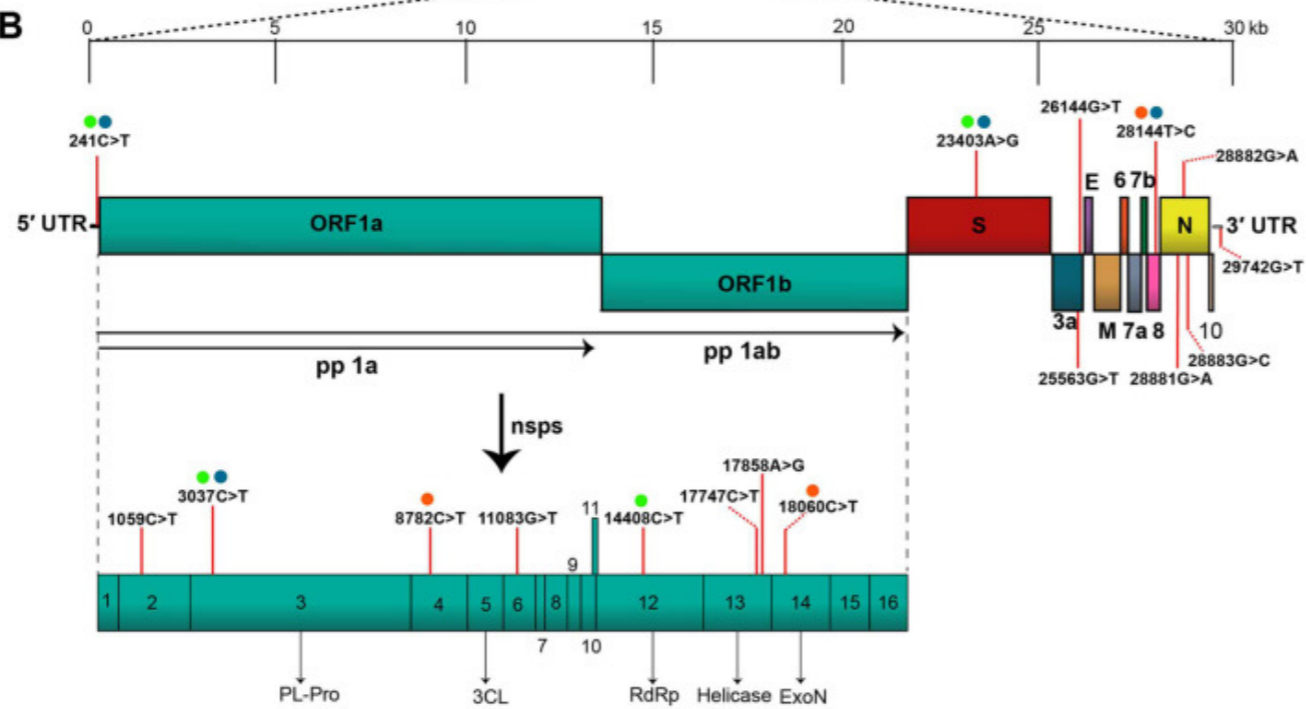
Example..

A**B**

A



B



HealthTrackRX – SARS-CoV-2 Diagnostic Test EUA

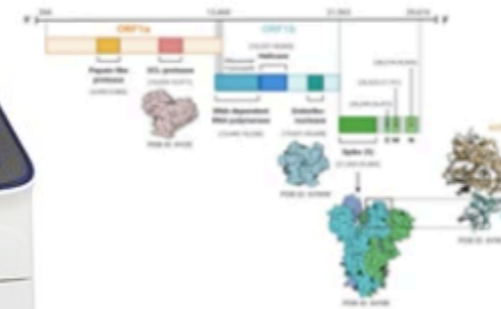
Sample Collection



RNA Extraction



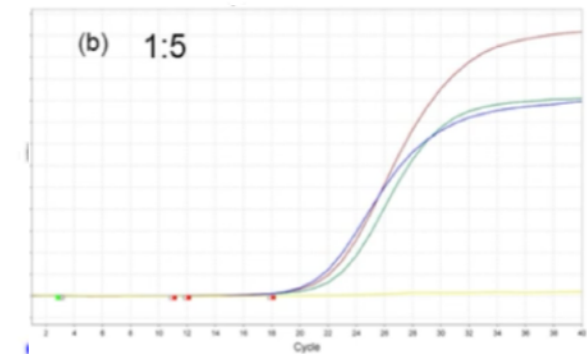
Real Time-PCR



Orf1ab, N, S

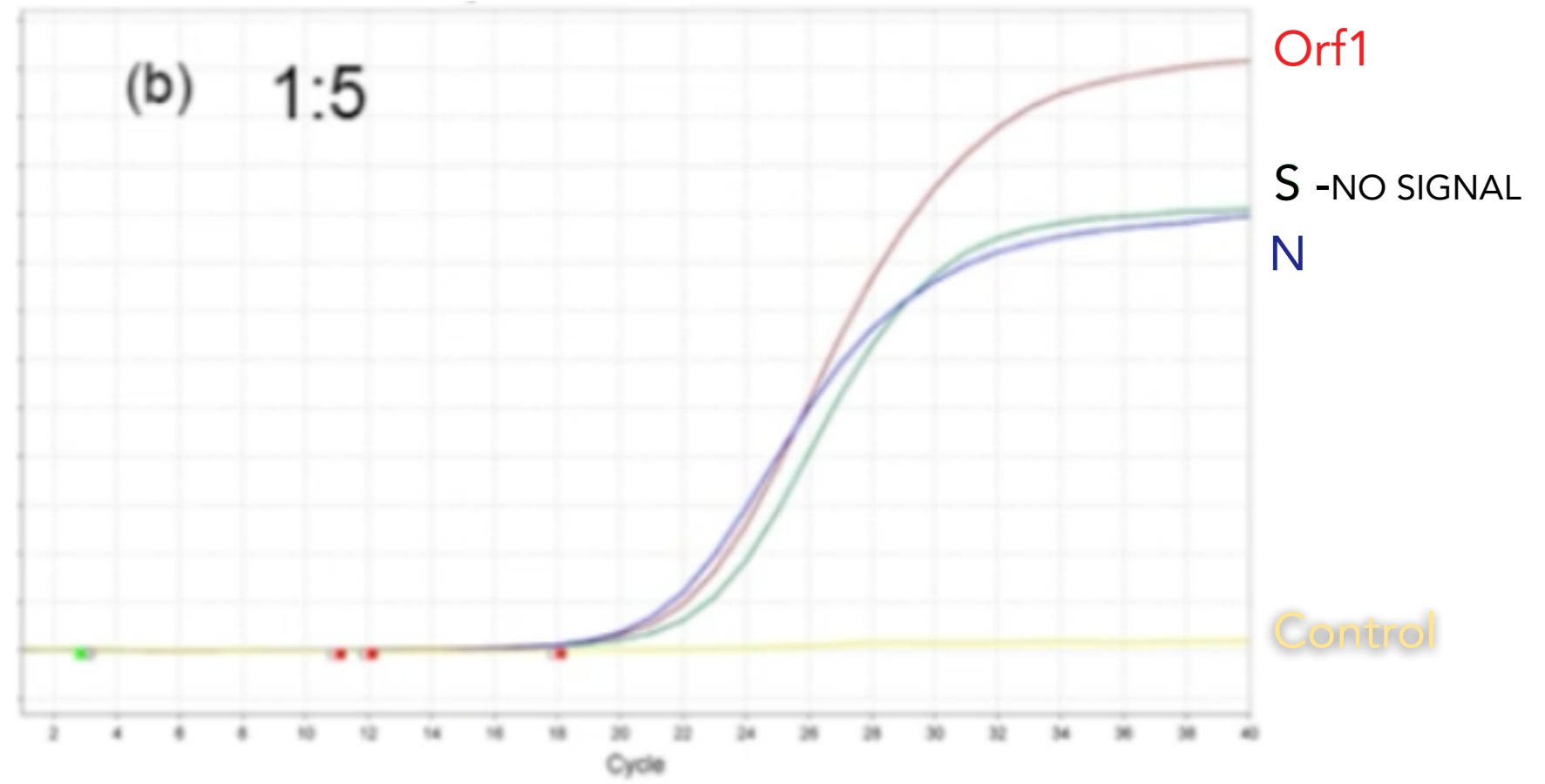


Results

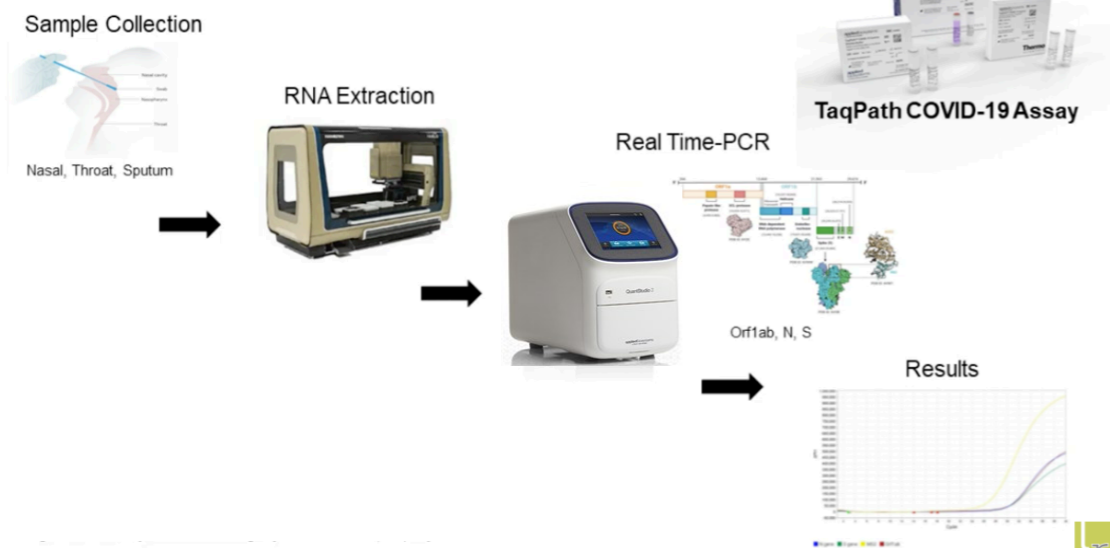




TaqPath COVID-19 Assay






HealthTrackRX – SARS-CoV-2 Diagnostic Test EUA



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

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Kits enhance Reproducibility

Volume

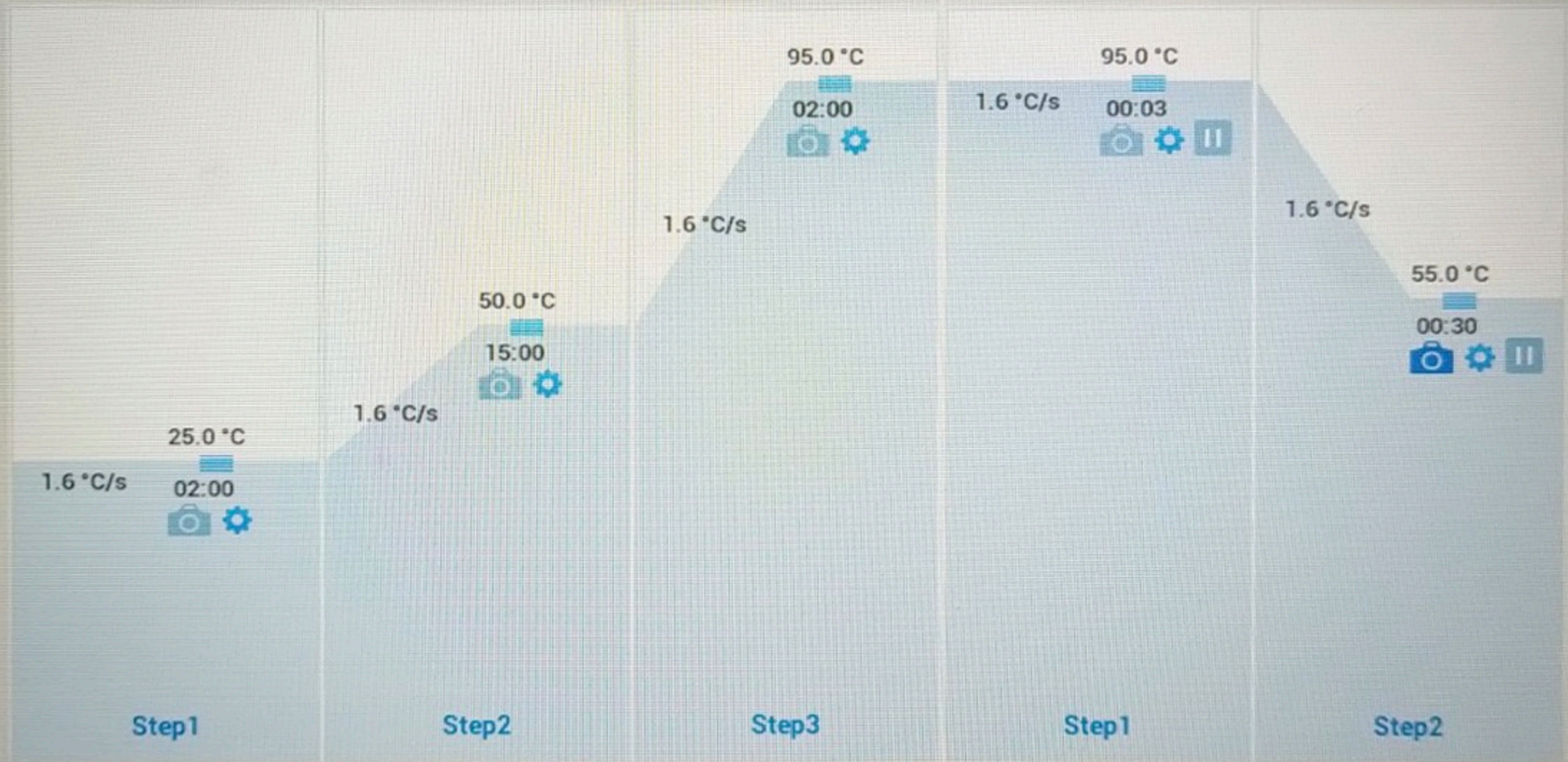
20 μ L

Cover

105.0 $^{\circ}$ C

Hold Stage

PCR Stage



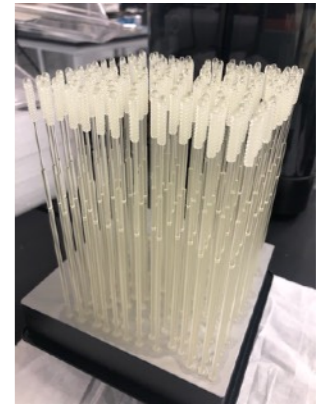
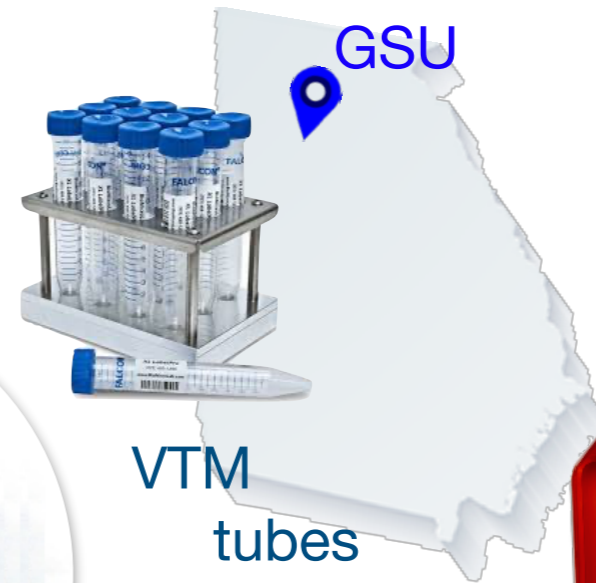
45 x

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

SARS-CoV-2 Pandemic

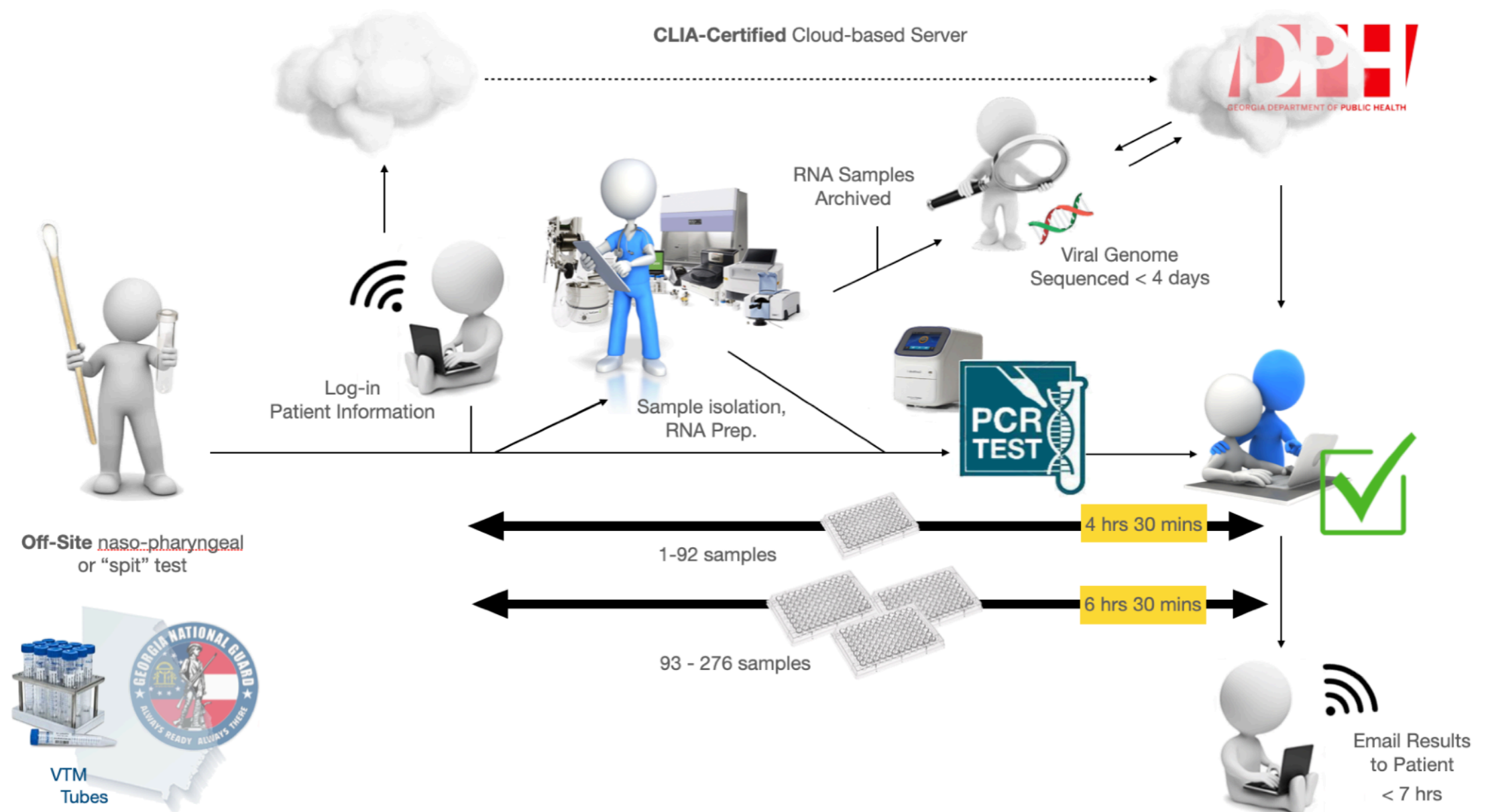
CLIA-certified COVID-19 PCR testing lab

2020-22



Facilities

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.



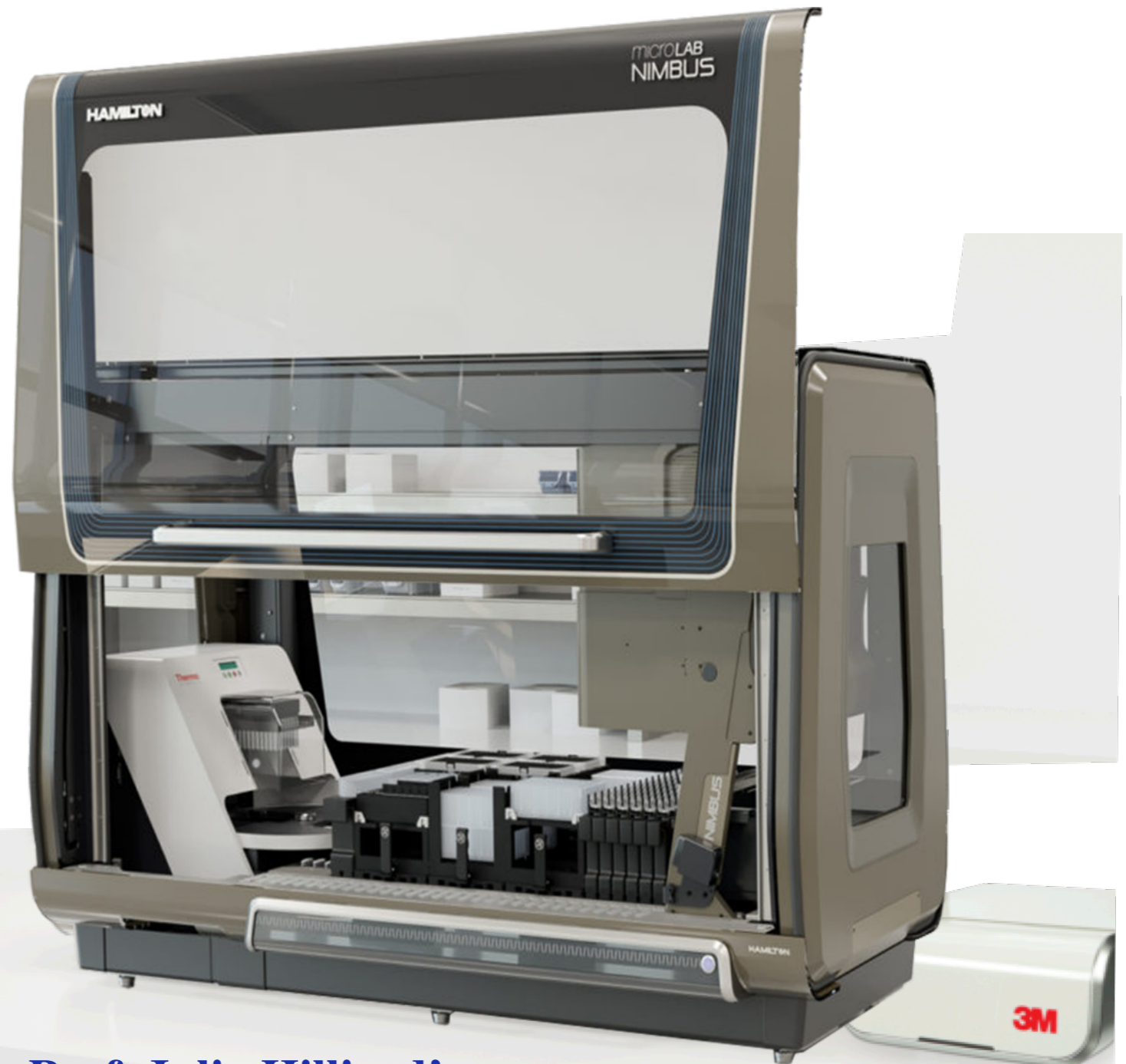
Facilities

Experiments go faster...
Higher throughput...
More accuracy,
Better Reproducibility

AUTOMATION



**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)**

