

- by replication

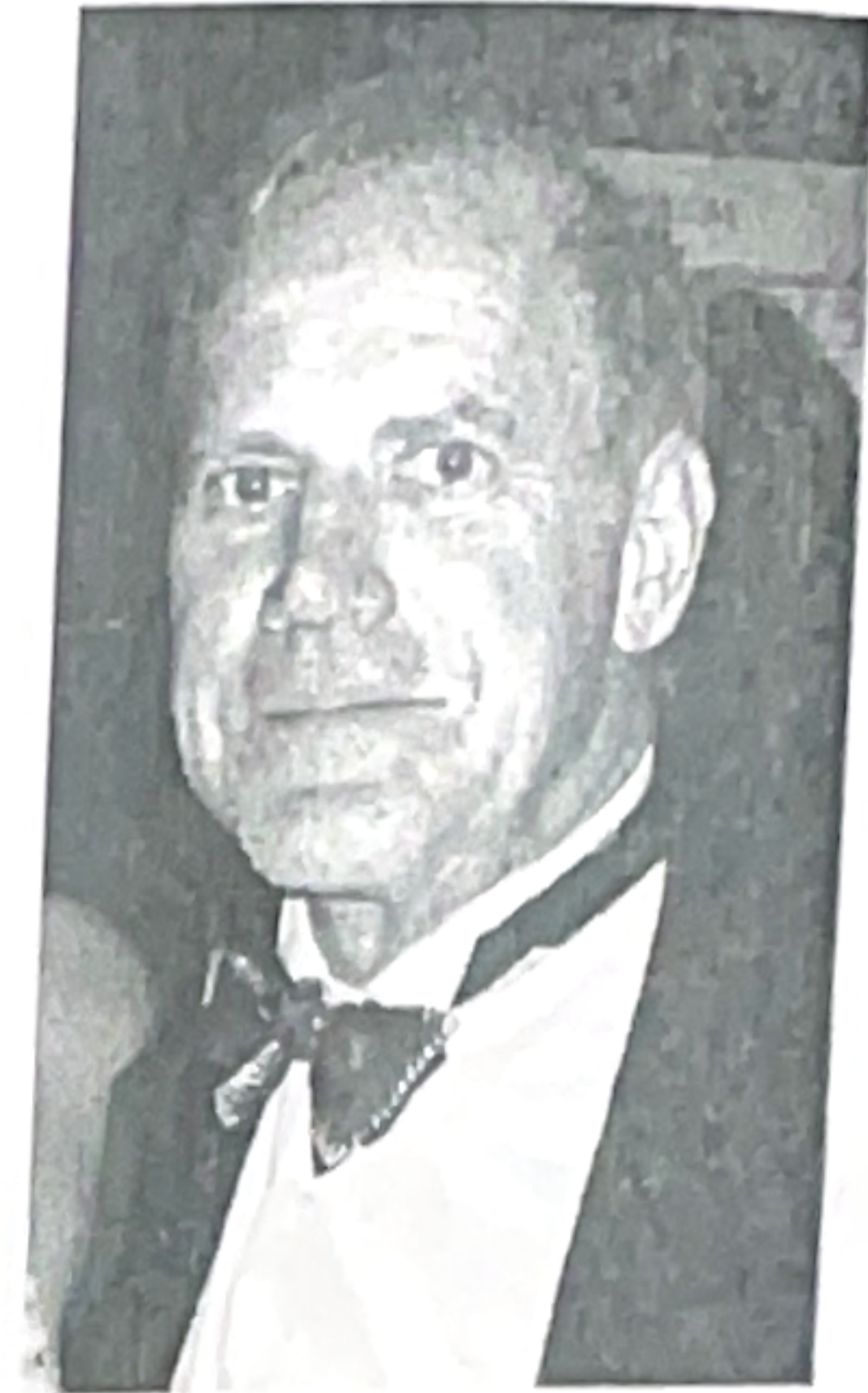
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Why is it important?

- Revolutionized molecular biology and biotechnology.
- Its applications are vast and PCR is now an integral part of Molecular Biology
- Essential for diagnostics, drug development, and genetic research.
- Inventor:** Invented by Kary Mullis in 1985, earning him the Nobel Prize.



Kary Mullis (1944-2019)

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← اكتشف استغل على فكرة PCR وهو

PCR PRINCIPLES AND STEPS



- The basis of PCR is temperature changes and the effect that these temperature changes have on the DNA.
- In a PCR reaction, the following series of steps is repeated 20-40 times
(note: 25 cycles usually takes about 2 hours and amplifies the DNA fragment of interest 100,000 fold)
- The Three Main Steps of PCR:
 - Denaturation** (94-98°C): Double-stranded DNA separates.
 - Annealing** (50-65°C): Primers bind to target DNA sequence.
 - Extension** (72°C): Taq polymerase synthesizes new DNA strands.



من أهم العوامل بالتجربة
هو درجة الحرارة، كيت
Dr Ala Abuhammad, PhD
مهم بالخطوات الرئيسية
بال PCR

نوع ال polymerase يمينياً
بنسخته من PCR، اسمه
(Taq) نسبة للبكتيريا التي أخذوه
منها

② في حال DNA fluorescent لا ، لكن بقدر أصف dyes أو probes يرتبطو بهاد او وظيفته معرفة تركيز DNA specifically as a function of fluorescence

PCR PRINCIPLES AND STEPS

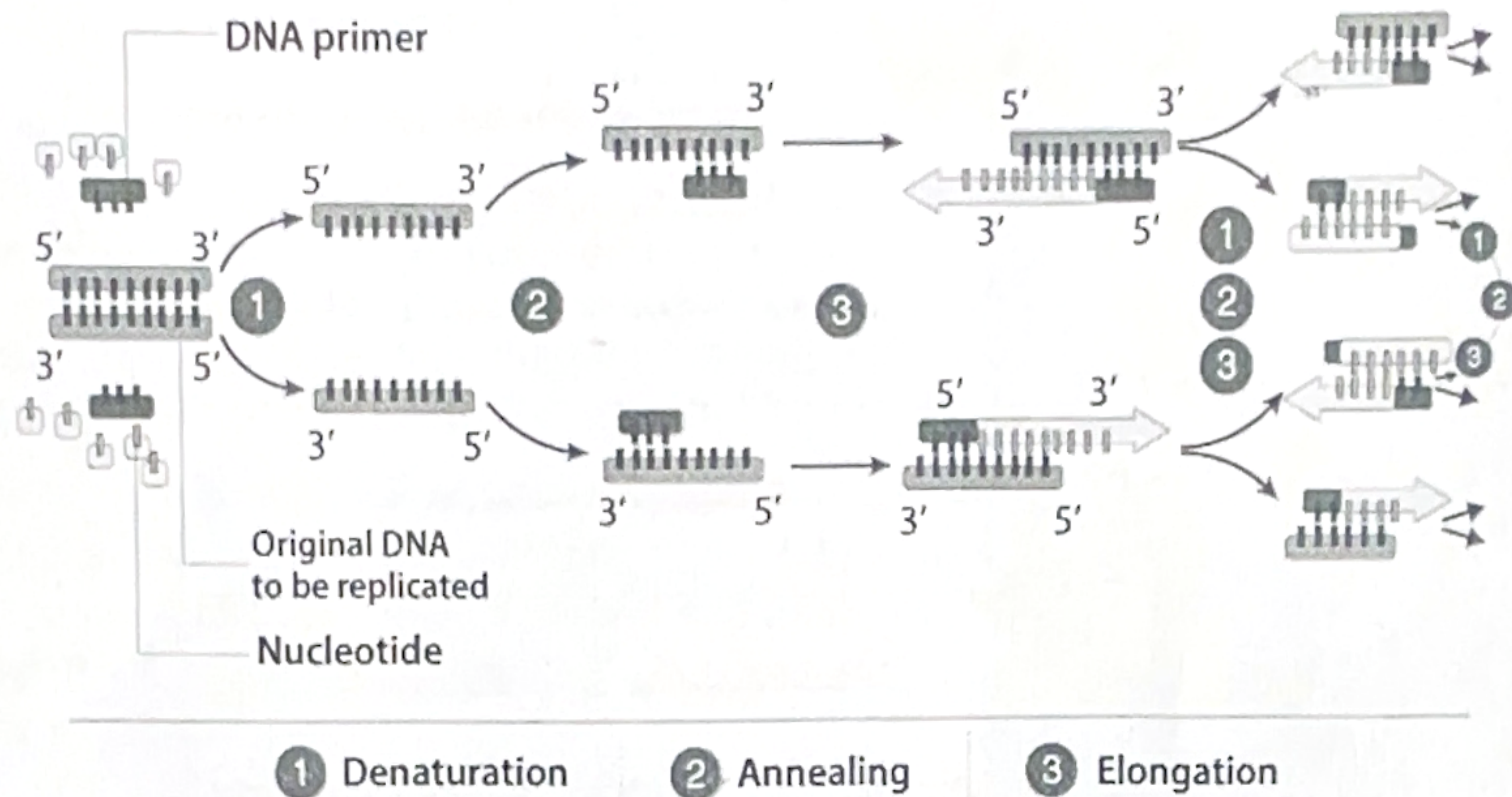


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POLYMERASE CHAIN REACTION (PCR)

BYJU'S
The Learning App



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← عبارة عن cycle بتفعلها تنفاد لكل ال resulting DNA موجود عندي ، كل م أحصل على مجموعة من ال Copies في نهاية كل cycle م دول ال Copies بربع ~~بربع~~ بغير الهم نفس الخطوات exponential increase/build up

for DNA copies DETECTION AND READOUT IN PCR



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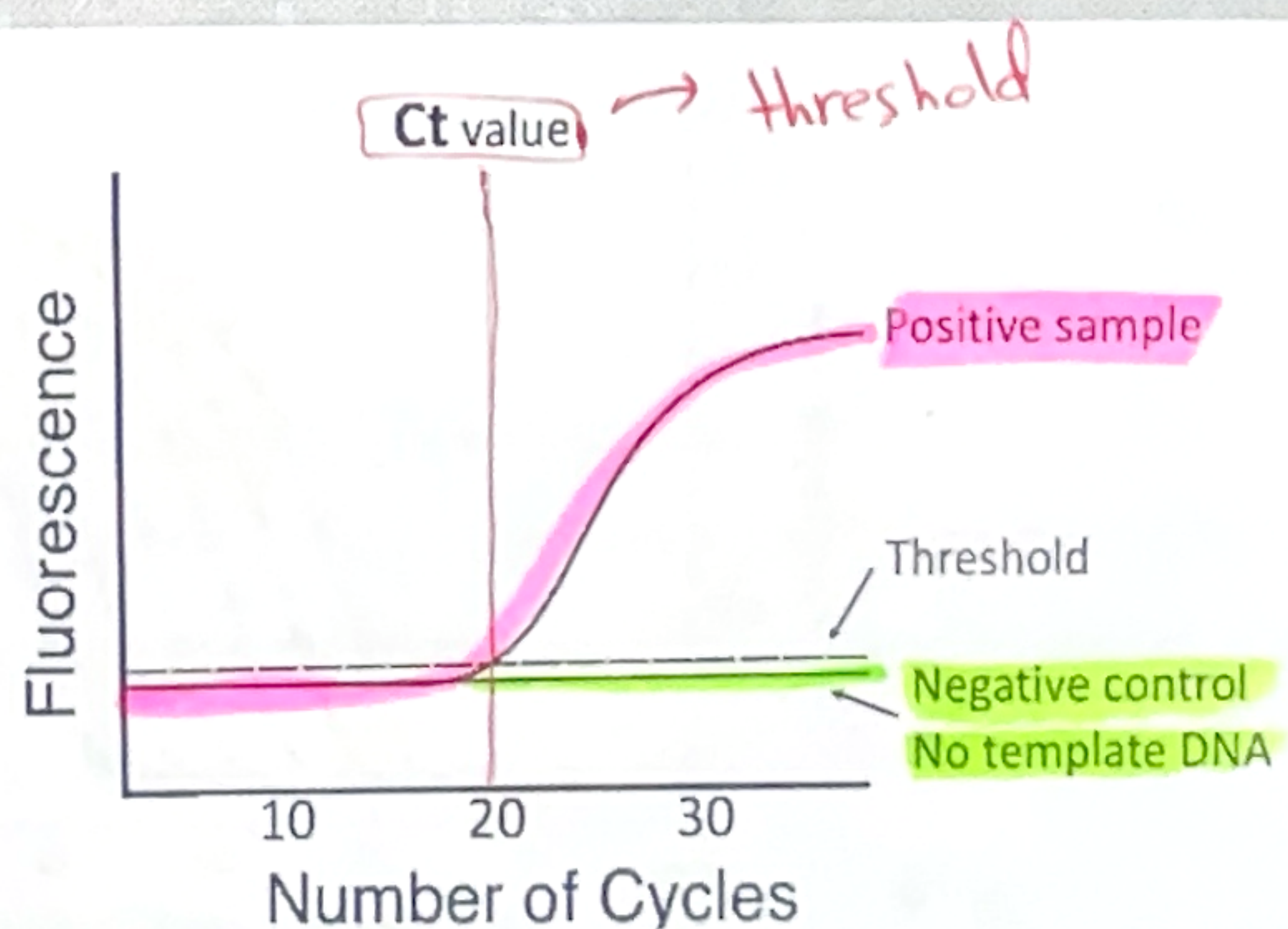
PCR detection and readout allow us to identify, measure, and analyze amplified DNA.

Common Detection and Readout Methods in PCR:

① Fluorescence-Based Detection:

- Used in qPCR and Multiplex PCR.
- Dyes (e.g., SYBR Green) or probes (e.g., TaqMan) bind to double-stranded DNA. As DNA amplifies, fluorescence increases, and the amount of fluorescence is proportional to the amount of DNA present.

Readout: Amplification curves and cycle threshold (Ct) values.



A typical graph where fluorescence intensity is plotted against reaction cycle number. qPCR reaction graph showing fluorescence plotted against the number of cycles.

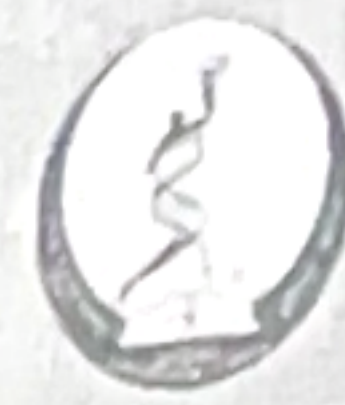
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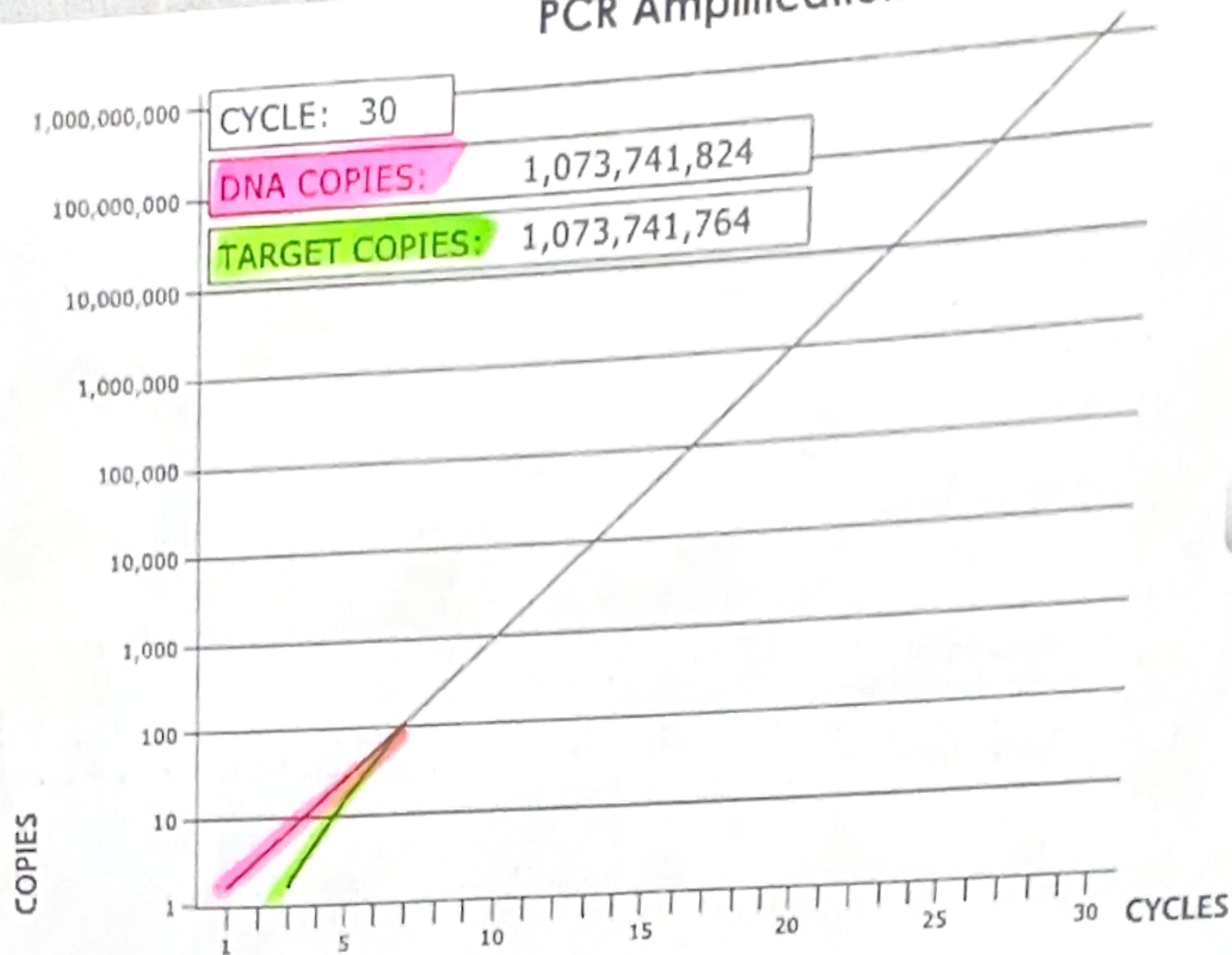
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تقرأها وتعرف شو عم بغير داخل reaction mixture detection ، بندق for DNA sequence of interest
تحت مفهومين identification ← quantification ← PCR tech بالنهاية ، اح نحصل عن نتائج بذكر

DETECTION AND READOUT IN PCR



PCR Amplification Graph



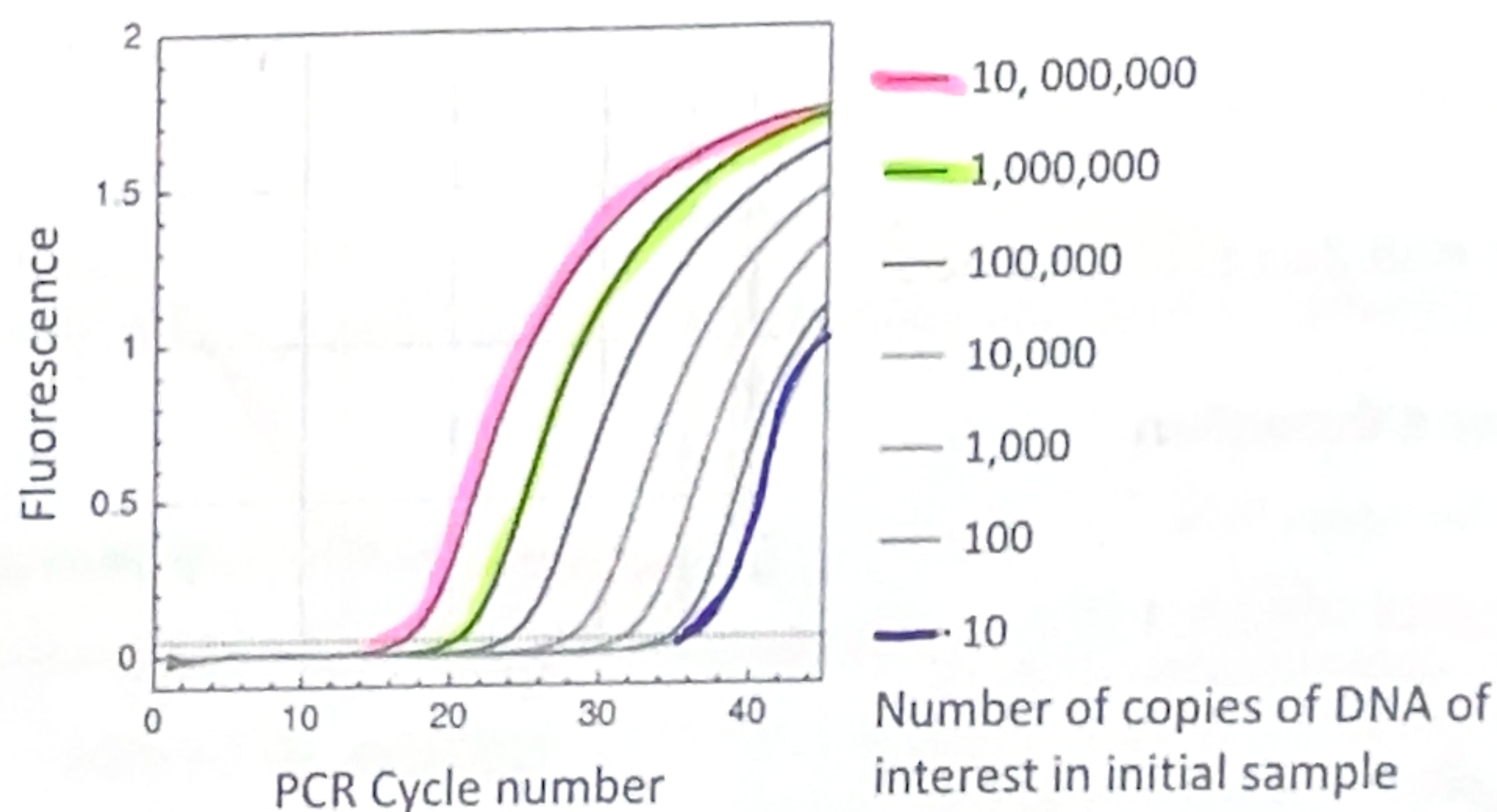
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← بعض الأجهزة ممكن يعطيك readout مباشر (بقية) ، another way of readout (بقية) ،
calculations (based on fluorescent versus number, Copies of cycles)

DETECTION AND READOUT IN PCR



qPCR plot showing the effect on Ct of increasing starting template DNA copy numbers in the starting reaction mix. The sample with 10 millions copies of target DNA (red line) has a lower Ct value than the sample with 10 copy numbers (green line to the right of the graph)

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← اذا كنا بنحكي عن Real time PCR شكل plot زي هيك (multiple curves) ،
Continuous over time (number of cycles)

① Gel ... الهدف الرئيسي منها هو identification ، بعد استخدامها for quantification نعم لكن غالباً هي تستخدم for visualizing amplified DNA

يعني العينة بعد ما طلعها من PCR بافها بالطريقة بالgel electrophoresis

DETECTION AND READOUT IN PCR



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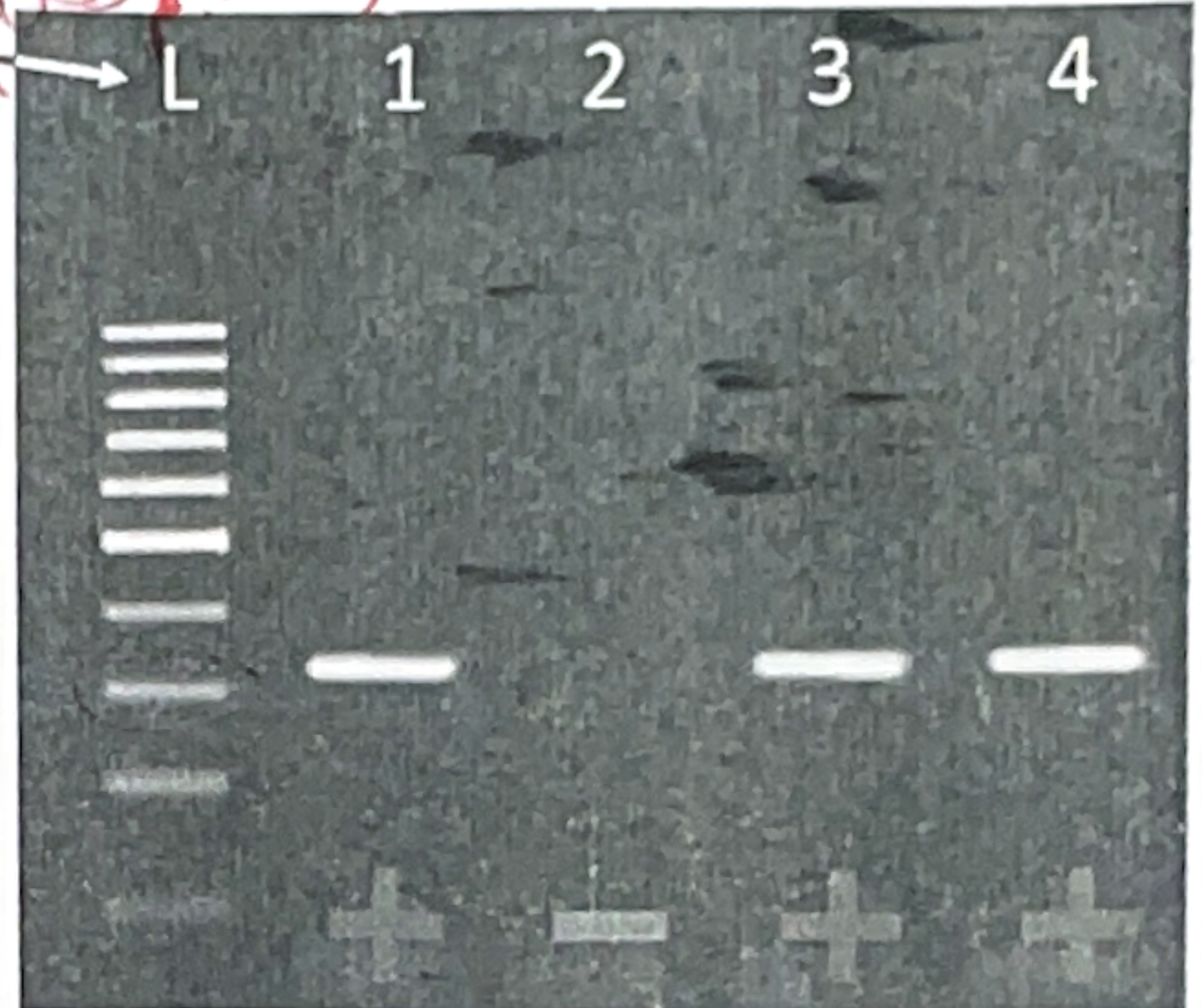
another way of detection

عبارة عن Standard (يعني عارفة شو الحجم الذي)

2. Gel Electrophoresis:

Ladder to calibrate amplified fragment size

(band)



- Used in conventional and RT-PCR.
- Visualizes amplified DNA as bands based on size.
- After the PCR process, the amplified DNA is separated using gel electrophoresis. The DNA fragments are stained with a dye (e.g., ethidium bromide) and visualized under UV light. Bands corresponding to the target DNA indicate successful detection.
- The figure shows: End point PCR gel electrophoresis showing the results of four samples tested. From these results it can be seen that Samples 1, 3 and 4 tested positive for the DNA of interest. Sample 2 was negative for the DNA of interest.

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② عشان اتشوف هل تكونت ولا ما تكونت ال fragment of interest
رطبنا برضو بستخدم dyes ، لما اشغل الكهراز على العينة ، DNA راح يصير يتحرك بسبب التيار الكهربائي بين يمر من خلال ال agar ، فكرة ال
انه يصير عندي ← Gel electrophoresis [migration (movement) of the DNA fragment through the agar gel after application of electrical current]

Slide (13)

② ← الـ fragment الذي صغر حجماً راح يتلوع أسرع
والأكبر راح يكون أبطأ بالـ migration, فالأصغر
يسير بقدر أسرع من elec... gel electrophoresis

③ ← ويقارن الـ sample إلى عدي بها Ladders
إذا طلع ← corresponding size of sample
to the size of standard
معناه أنا فعلاً حصلت على DNA
fragment of interest
إلى بي أعمالها amplification

④ ← هل هي specific؟ لا هي مارح تأكدي
(identification method) بالزبط أيسر هو الـ
(DNA of interest) sequence
نسبة كثير عالية هل تكون ولا ما تكون بالعز
إلى بي أياها,
فالنتائج من الـ gel electrophoresis
ليكن تعطين indication كثير كبير عن نجاح
PCR, فالتأكد إلى مستقل عن PCR غالباً
technique مستخدمه another confirmatory
الـ gel electrophoresis يتأكدو تماماً من
fragment of interest
إلى عملها amplification

they confirm it with another technique

DNA sequencing : $\downarrow$

OR

hybridization using probes

KEY COMPONENTS OF PCR

amplification عملية تضاعف

المفروغ عنها استهدافه
البيئة داخل الخلية

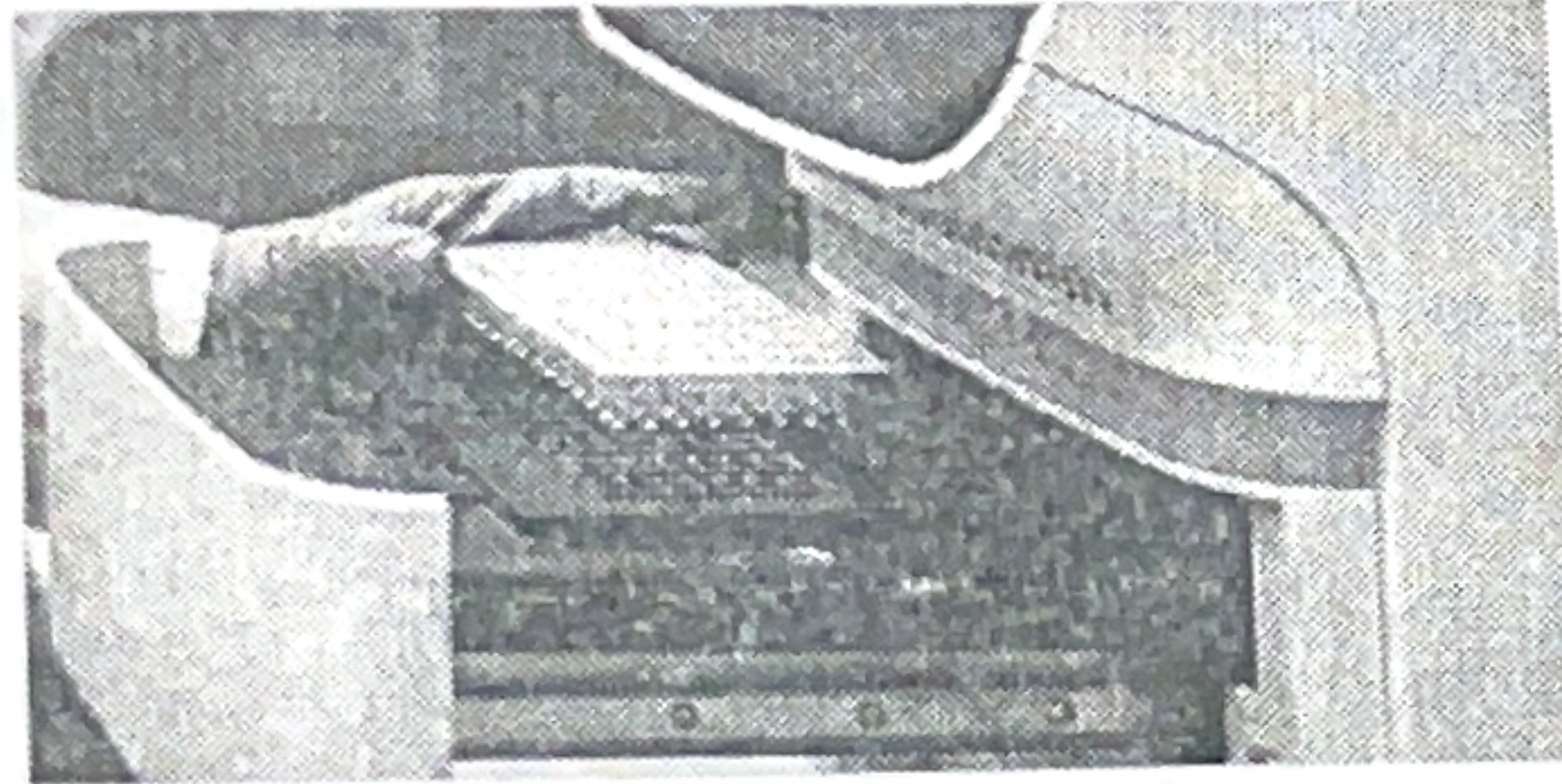


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- 1 **Template DNA:** The DNA of interest to be amplified; that contains the target sequence we want to copy.
- 2 **Primers:** Short oligonucleotides for targeting specific DNA regions (short, single-stranded DNA molecules).
- 3 **Taq Polymerase:** A Heat-stable enzyme for DNA synthesis.
- 4 **Nucleotides (dNTPs):** All four deoxynucleotide triphosphates building blocks of DNA.
- 5 **Buffer:** Maintains the optimal conditions for the reaction/ the DNA polymerase to function effectively.
- 6 **Thermal cycler** (a device that can change temperatures dramatically in a very short period of time)
 - Thin walled tubes

يغير داخلة الحرارة بسرعة وبكل دقة



DNA Template



Primers



DNA Polymerase



dNTPs



Buffer/Cofactors

Slide (14)

③ Taq polymerase :-

سَوْ بَمِيْزِهِ عَنْ polymerase يِي مِنْ جَسْمِنَا
لَيْسَتْ مَا حَصِيَتْ فِي التَّفَاعُلِ بَيْنَ polymerase يِي
مَوْجُودٍ فِي خَلَايَانَا ؟ لِأَنَّهُ زِي مِمَّ مَكِينَا
هِيَ الرِّقْمَةُ techniq تحتاج إِلَى دَرَجَاتٍ عَرَارَةٍ
عَالِيَةٍ (هِيَ مَوْبَالِزِيْبُو بَيْنَ يِي بِصِيْرٍ دَاخِلِ
الْخَلِيَّةِ أَيْضًا يَسْتَابِيْهَةً مَعَ شَوِيَّةِ adaptation)
مِنْ هِيَ adaptation أَنَّهُ نَوْعُ polymerase عِشَانِ
تَعْمَلُ فِي دَرَجَاتٍ اِكْرَارَةٍ الْعَالِيَةِ جَابُوهُ مِنْ بَكْتِيْرِيَا
تَتَكُونُ heat resistance

PCR VARIANTS AND MODIFICATIONS



هناك نوعان من PCR: النوع الأول لا وهو التجربة شغالة وهو amplification قاي ريسر

2 **Quantitative PCR (qPCR):** Measures DNA concentration in real-time during amplification using fluorescent dyes or probes. Also known as real-time PCR.

- The name "real-time" highlights the ability to observe and measure DNA synthesis dynamically, without delay, as the reaction progresses. This characteristic is vital for applications requiring precise and rapid nucleic acid quantification, such as diagnostics and gene expression studies.

- **Therapeutic Monitoring:** Measuring viral load in patients undergoing antiviral treatments (e.g., HIV or hepatitis).

- **Pharmacogenomics:** Quantifying gene copy numbers to predict patient response to drugs like warfarin (gene target - CYP2C9) or trastuzumab (gene target - HER2).

- **Quality Control:** Ensuring consistency in biologics and biosimilars production by quantifying genetic content.

① يمكننا اننا انواع من PCR , ممكن يكون نفس experiment set up

يكون يشتغل على one أو more than one variant

② يلي ما يشتغل أو ما في مجال للخطأ , لازم مباشرة أقيس التركيز

ممكن اذا خليت العينة مع جنب ممكن يغيرا شياء (لغرون ما) تغيير النتائج وتحسين false result

Slide (15)

③ * therapeutic monitoring :-

في بعض أنواع viral treatment ما أظنهم
للرئف يكون مهم اني أفتي viral load
من الرئف خلال Course of treatment مثل
HIV / hepatitis (كثير مهم استوف اذا الرئف
عم يتحسن وال viral load يقل عنده)
بنقي viral load عن طريق RNA تاج العزوك
(بغول RNA الي DNA باستخدام reverse
transcriptase)

* pharmacogenomics :- مثلاً warfarin, trastuzumab
يكونو يستغلو على receptor معين وهاذا receptor
مين جدد expression لا له؟ مين معين
مثلاً Her2 receptor في Breast Cancer Cell

مين يقرر وجود هاد receptor على السطح؟

وهو د صحت مسؤول عنه ، فأنا أتوقع قبل
من أن تطرح أن تعريف الدواء ، أشوف هذا التعريف هاد
ممكنت يستجيب للدواء يلي عندي ولا لا ؟ يعني
هل الكمية المسؤول عن expression of the
target receptor of the drug
هو هو د عن التعريف ولا mutated ولا سوا
وصفه

* quality Control :-

المصانع والمختبرات يلي بتستغل على biologics
و ~~biologics~~ biosimilars عشان يقدرو يحددو
أو يعرفو تركيز ال biologic يلي عندهم ويعملو
identification ويعملو قسمة ال quality control

The Advantages Of Real-time PCR Include:



1. Ability to monitor the progress of the PCR reaction as it occurs in real time
2. Ability to precisely measure the amount of amplicon at each cycle, which allows highly accurate quantification of the amount of starting material in samples
3. An increased dynamic range of detection
4. Amplification and detection occurs in a single tube, eliminating post-PCR manipulations.

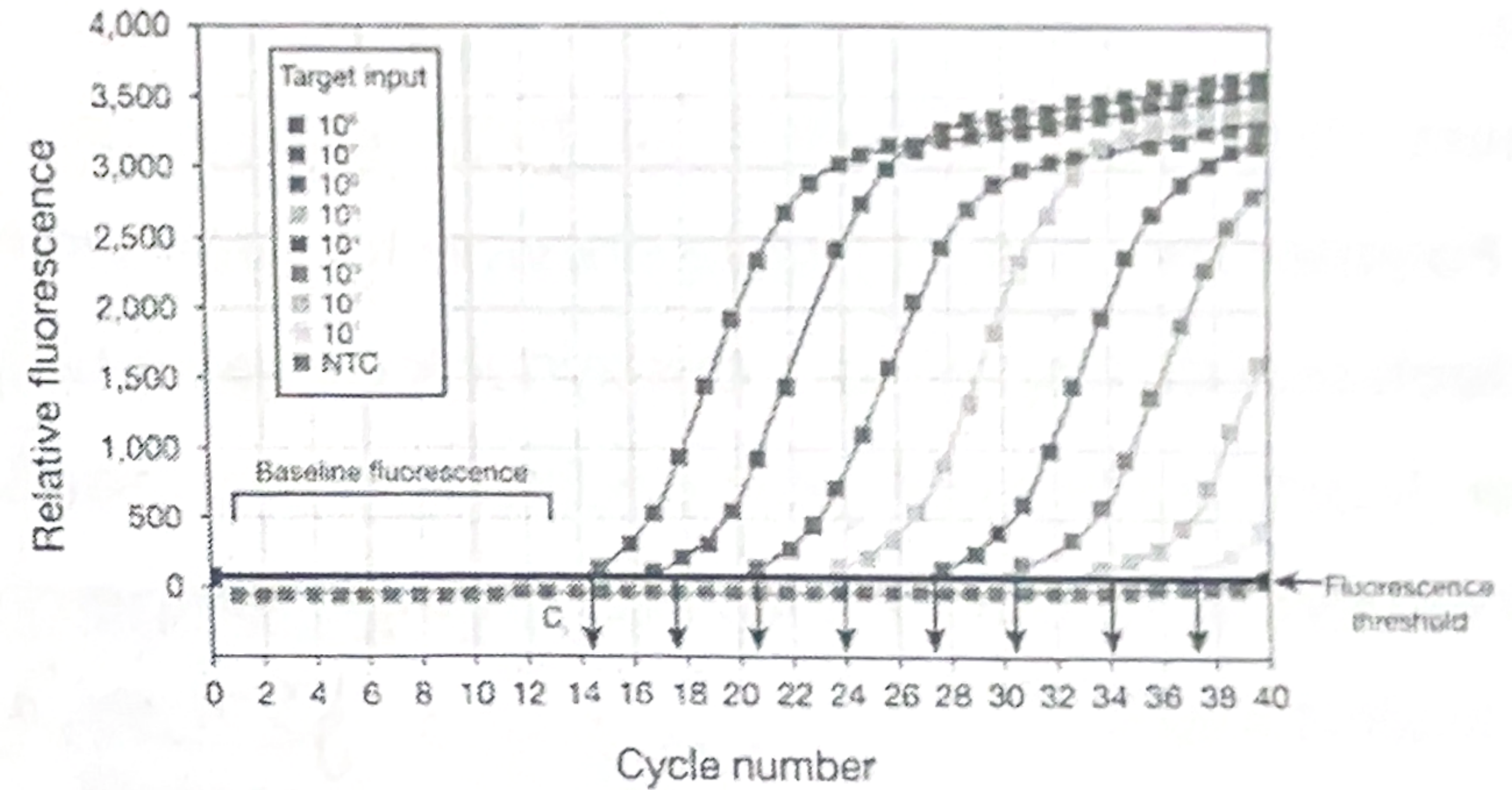


Figure 1. **Relative fluorescence vs. cycle number.** Amplification plots are created when the fluorescent signal from each sample is plotted against cycle number; therefore, amplification plots represent the accumulation of product over the duration of the real-time PCR experiment. The samples used to create the plots are a dilution series of the target DNA sequence.

نقطة 4 ← لا ينبغي إجراء عمل measurement قبل
ما يحتاج زي هو الحال بـ الأنواع الأخرى من PCR التي تعمل
في الـ gel electrophoresis (يعني ما يحتاج عمل gel electrophoresis)
بعد الـ real time PCR
ممكن استقراءها لا Confirmation بس مو ضروري
reaction mixture

PCR VARIANTS AND MODIFICATIONS



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Reverse Transcriptase PCR (RT-PCR): For RNA to DNA conversion. Detecting RNA viruses like influenza or SARS-CoV-2 (e.g., mRNA COVID-19 vaccines were validated using RT-PCR).

- Note:** RT-PCR (Reverse Transcriptase PCR) and qPCR (Quantitative PCR) can be combined, and this technique is commonly referred to as **real-time RT-PCR** or **qRT-PCR**.

① الفرق الرئيسي بينه وبين Conventional PCR انه يكون عندي reverse transcriptase (يعني لازم يكون عندي هاد الانزيم عشان يحول RNA الى DNA) PCR بالكل المعتمد

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② زي م حينا بالبداية انو هذول ال variant الانواع صحت معناها اني ممكن يكون نفس الجهاز او نفس ال experimental set up يسمح اني اعمل RT-PCR و reverse PCR مع بعض ، حسب نوع العينة والهدف بيبي انا

PCR VARIANTS AND MODIFICATIONS



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Multiplex PCR: Amplifies multiple targets simultaneously by multiplying several fragments in a single DNA sample by using a number of primers.

- Reduces time and resources by providing multi-target results in one assay, useful for high-throughput drug screening and diagnostics.
- Pathogen Detection:** Identifying multiple pathogens in a single diagnostic test (e.g., simultaneous detection of drug-resistant genes in tuberculosis).
- Genetic Screening:** Testing for mutations in multiple genes related to diseases or drug metabolism (e.g., BRCA1/2 mutations for cancer risk).

Long-range PCR uses a variety of polymerases, to generate larger DNA ranges. DNA fragment

- Amplifies large DNA fragments (>5-10 kb).
- Uses high-fidelity polymerases for accuracy and completeness.
- Enables study of large genomic regions, structural variations, and sequencing templates.

Touchdown PCR: Optimizes specificity through gradual temperature decrease.

- Improves amplification accuracy and specificity, essential for critical pharmaceutical and clinical studies.
- Precision Drug Development:** Amplifying genes with high GC content or challenging regions, aiding in the identification of druggable targets.
- Rare Mutation Detection:** Enhancing specificity for low-abundance or rare genetic variations critical in cancer drug discovery

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بعيت انك تضيف عدد اكبر او انواع اكثر من polymerase عشان تغطي هاد ال range الطويل

موقع في ال drug development

Slide (18)

Touchdown PCR :

هنا النوع من PCR لما يكون عندك specificity and accuracy

المطلوبة كثير عالية وكثير مهمة، من مسجون
لدي نوع من الأخطاء وهذا غالباً يكون بال clinical studies

(نزي يبي رعلوها فذل) (drug development)

* precision drug development

من مناهت يكون فيها GC content high، هاي
المناهت بتقل زي herpan (من متأكدة)

بترقوع على بعض وبتكرمش ال DNA fragment
وبمتنع primer انه يروح يرتبط بها دايكان،

وكيف انا ا منع هذا distortion، بالعبوديات

اكرارة بصيرو ينزلوها أو يرفعوها gradually

بتساعدهم يحصلو على high accuracy

and specificity

تلخيصه (ما شرمه)

DETECTION AND READOUT IN PCR VARIANTS



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- Fluorescence-based methods provide real-time insights, while traditional methods like gel electrophoresis confirm the final product.

Type of PCR	Purpose	Detection Method	Readout/Output
Conventional PCR	DNA amplification for downstream applications.	Gel electrophoresis.	DNA bands indicating presence/size.
Reverse Transcriptase PCR	Converts RNA to DNA for amplification.	Gel electrophoresis.	DNA bands derived from RNA.
Quantitative PCR (qPCR)	Measures DNA concentration in real-time.	Fluorescent dyes (e.g., SYBR Green) or probes.	Amplification curves (Ct values).
Multiplex PCR	Amplifies multiple targets simultaneously.	Fluorescent probes or gel electrophoresis.	Multiple DNA bands or fluorescence curves.
Touchdown PCR	Enhances specificity through temperature steps.	Gel electrophoresis or fluorescence detection.	Target-specific bands or curves.
Digital PCR	Absolute quantification of DNA molecules.	Partition-based fluorescence.	Digital quantification (copies/ μ L).

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من مال كنت تستغل على أكثر من region أو target، راجع ربحناج
التي تضيف أكثر من نوع primer فلازم تنبئه انوه دول ال primers
ما جلو dimers مع بعض (بدل برومو-يرتبطو بال DNA بسرو
يرتبطو مع بعض)

KEY DECISIONS WHEN DESIGNING A PCR EXPERIMENT



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ستوالا يبي تاخذها بعين الاعتبار لما نفل PCR

What's the Goal?

Think about why you're doing PCR. Is it to clone a gene, diagnose a disease, or sequence DNA? The purpose shapes the experiment.

DNA Quality Matters:

Use clean, high-quality DNA. If the DNA is damaged or contains contaminants, PCR might fail.

Good Primer Design:

Primers are like guides for the polymerase. They should be the right length, match the DNA sequence you're targeting, and not stick to themselves (to avoid primer-dimers).

ال materials والأدوات مهم
الها كيت وتؤثر على النتائج لازم تنقده quality

من معادرا مواد وتخيرينها، والكان والأدوات

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يكونو ربحناج

CHOOSING THE RIGHT DNA POLYMERASE

DNA POLYMERASE: THE 4 KEY FEATURES



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① Thermostability

- PCR heats up to high temperatures. Polymerases must stay active at the high temperatures used in PCR.
- For example, the thermostable polymerase (Taq) lasts 40 minutes at 95°C, while Deep Vent polymerase can stay active for hours at even higher temperatures.

most common

② Accuracy (Fidelity):

- Some experiments need super-accurate DNA copying (e.g., for cloning) without mistakes.
- High-fidelity enzymes like Pfu or Q5® are great for cloning because they proofread and fix errors.

③ Speed and Length (Processivity):

- If you're amplifying a long DNA fragment, use a polymerase designed for long-range PCR.
- Refers to how fast and efficiently the polymerase adds DNA bases.
- Some polymerases, like KAPA2G, work faster and handle tricky DNA regions (like GC-rich areas) better than standard Taq.

④ Specificity: بضائ polymerase عند الحاجة انه يميز صح ويصح correction

- Ensures the correct DNA sequence is amplified without errors.
- Hot-start Taq polymerase helps by staying inactive until heated, preventing unwanted reactions during preparation.

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② في التجارب التي تتطلب درجة عالية من الدقة، فإن أنواع polymerase تستخدمها تعمل proofreading بحيث انه لو صار عندي اي خطأ بار build up of the DNA strand during polymerization they can detect any distortion

HOW TO GET THE BEST PCR RESULTS



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■ Set the Right Annealing Temperature:

tem at which the annealing occurs

- This is when primers attach to the DNA.

- Too low? You'll get random results.
- Too high? Primers won't bind. Try a gradient to find the sweet spot.

■ Adjust Mg^{2+} Levels:

Magnesium helps the polymerase work. Too much or too little can mess up the reaction.

polymerase ما يشتغل بدون

SOLVING COMMON PCR PROBLEMS



Nothing Shows Up:

Check if the DNA is good, primers are correct, or if cycling conditions are wrong.

Extra Bands on the Gel:

The primers might stick to the wrong places. Try adjusting the temperature or using a specialized polymerase.

Weak Bands:

Use more DNA or run the reaction for more cycles.

ترتبط بـ
different fragment
غير ال target

gel electrophoresis
weak bands
يمكن تكون ال fluorescent dyes
يحيى ضيقها عن ال
visualization for the band
يمكن تكون هاي ال dye من
سغالة مع

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TIPS FOR SUCCESSFUL PCR



Avoid Contamination:

Use clean tools, separate workspaces, and include a control reaction with no DNA to check for contamination.

Set Up Reactions Carefully:

Use a master mix to avoid pipetting errors and ensure all reactions are consistent.

Check Results:

Run your PCR product on a gel to confirm it's the right size and pure.

يفضل تشتري kits جاهزة
عشان نضمن ان
نتائج
يعني كل م قدرت تختصر خطوات من ال mixing
(إضافة ال شيء على شيء) يكون احسن

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master : يكون بجوتى على أكبر قدر ممكن من ال شيء
يحيى بدما تنضاف على التجربة , هيك بقلل من ال خطأ

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■ Diagnostics:

- Detecting genetic mutations (e.g., BRCA1/BRCA2 in breast cancer).
- Pathogen detection (e.g., COVID-19 testing).

■ Drug Development:

- Genotyping for personalized medicine.
- Screening for genetic diseases.

■ Therapeutics:

- Identifying therapeutic targets.
- Generating DNA for gene therapy.

(قرائه قرا د ه)

LIMITATIONS OF PCR



they may combine it to other techniques to overcome these limitations

1. Requires precise temperature cycling equipment.
2. Sensitive to contamination, leading to false positives.
3. Amplifies DNA errors if present in the template.

لـ اي error بـيـمـمـكـن يـخـرب كـل الشـقـل