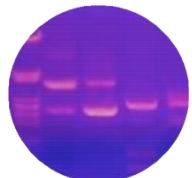


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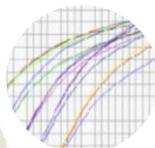
Evolusi PCR serta Pemanfaatannya dalam Bidang Kesehatan

Sylvia Sance M, M.Biomed

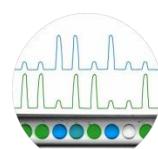
1st



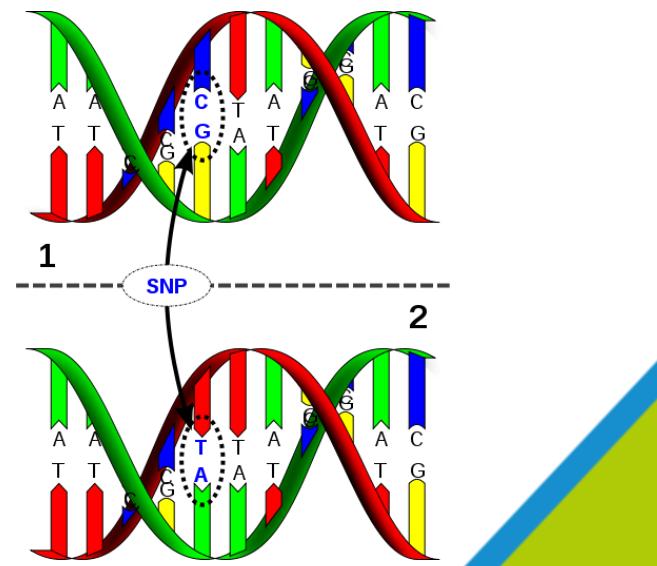
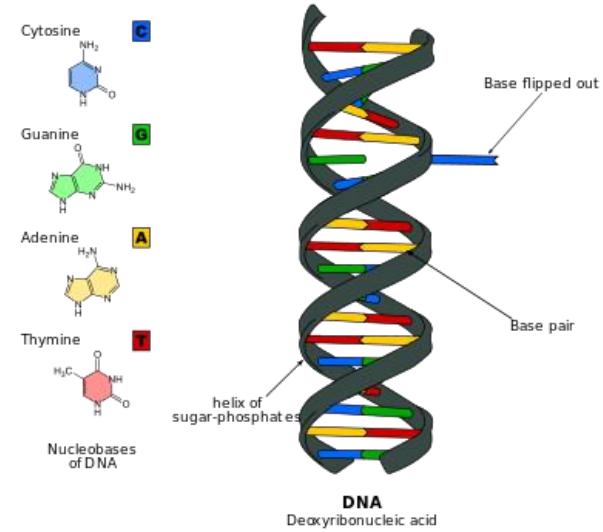
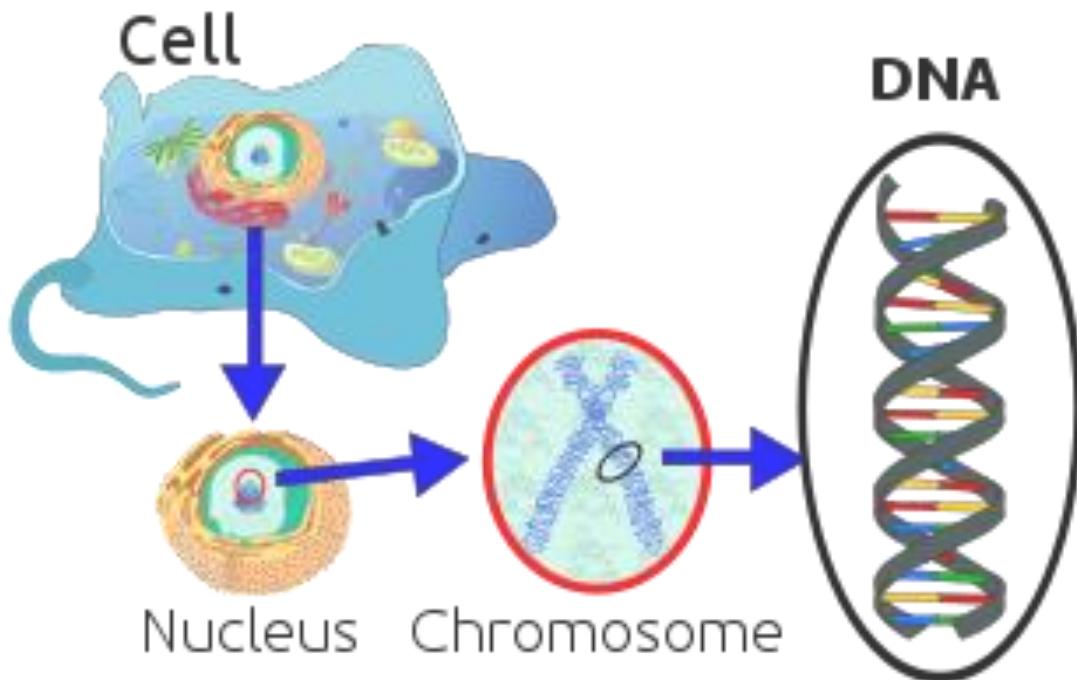
2nd



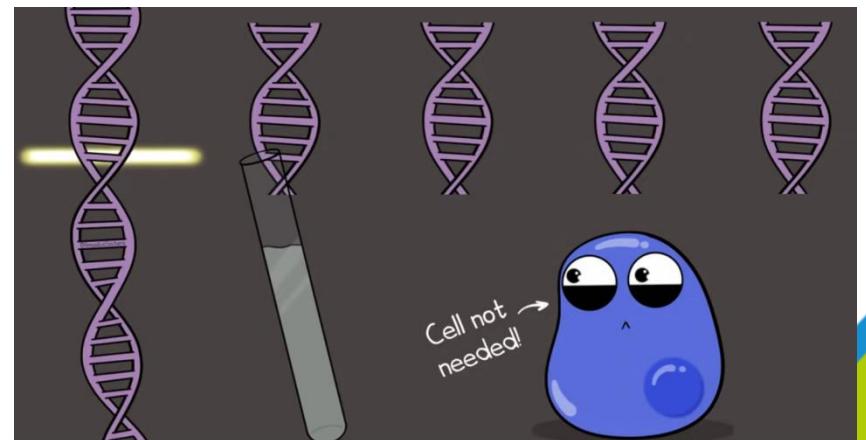
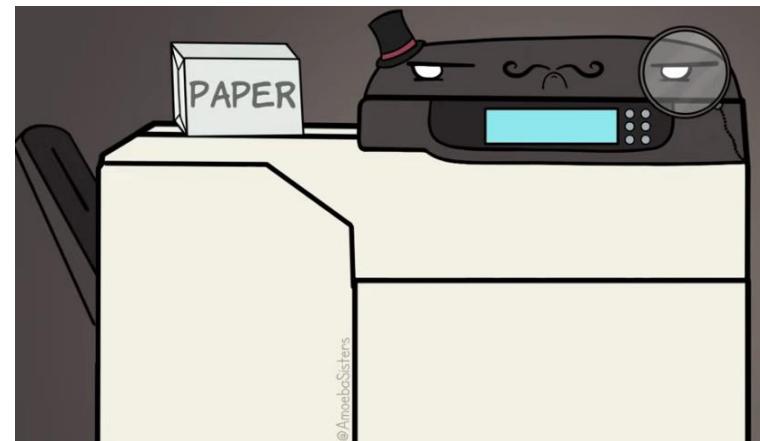
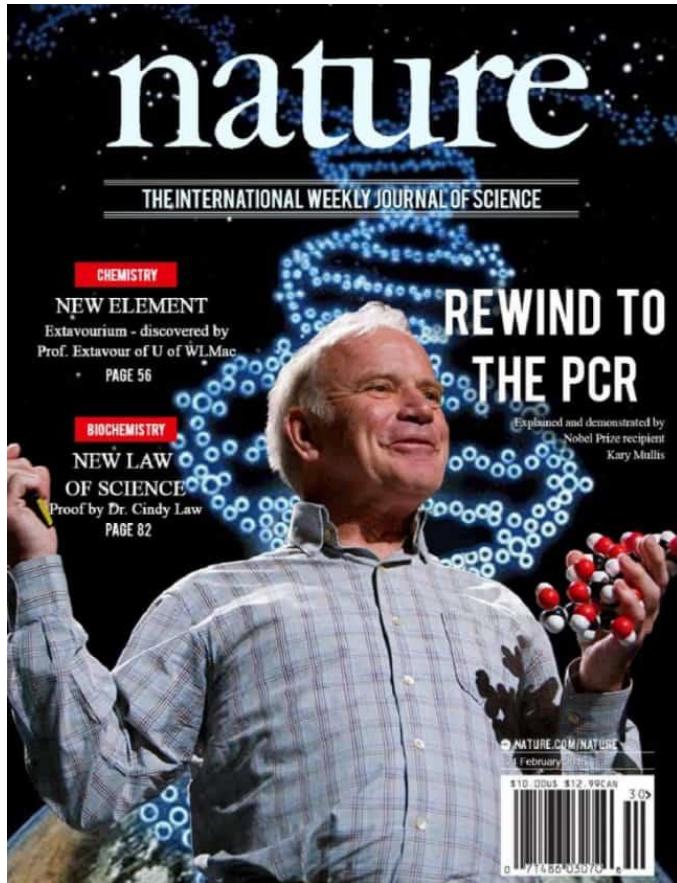
3rd



Apa itu DNA?

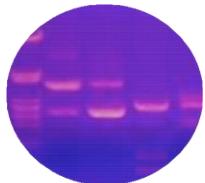


Polymerase Chain Reaction (PCR)



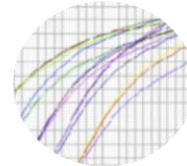
Evolusi PCR

Qualitative



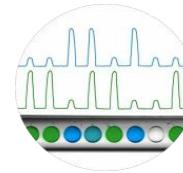
Thermal Cycler
PCR

Relative Quantitative



Real time (qPCR)

Absolute Quantitative



Droplet Digital PCR (ddPCR)

Features & Sensitivity

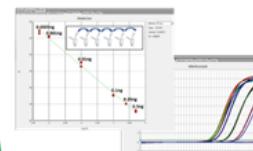
Generasi PCR



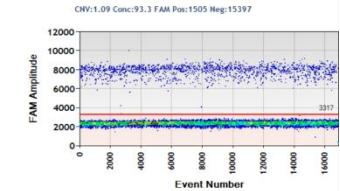
Generasi 1st
PCR



Generasi 2nd
Real-Time PCR (qPCR)



Generasi 3rd
Digital PCR (dPCR)

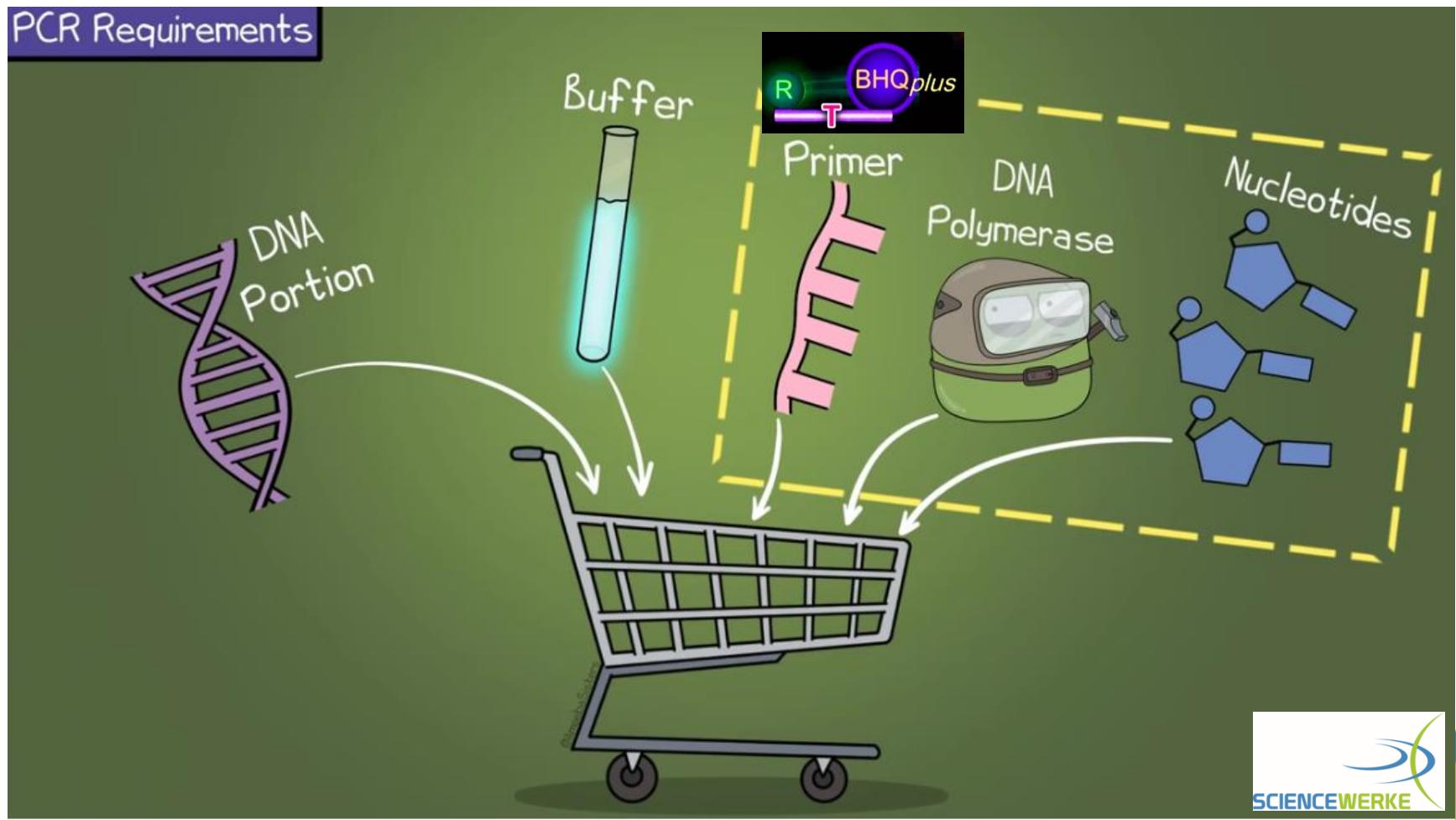


- ■ — ■ — ■
- 1. Bersifat Kualitatif
- 2. Membutuhkan instrumen tambahan untuk analisanya

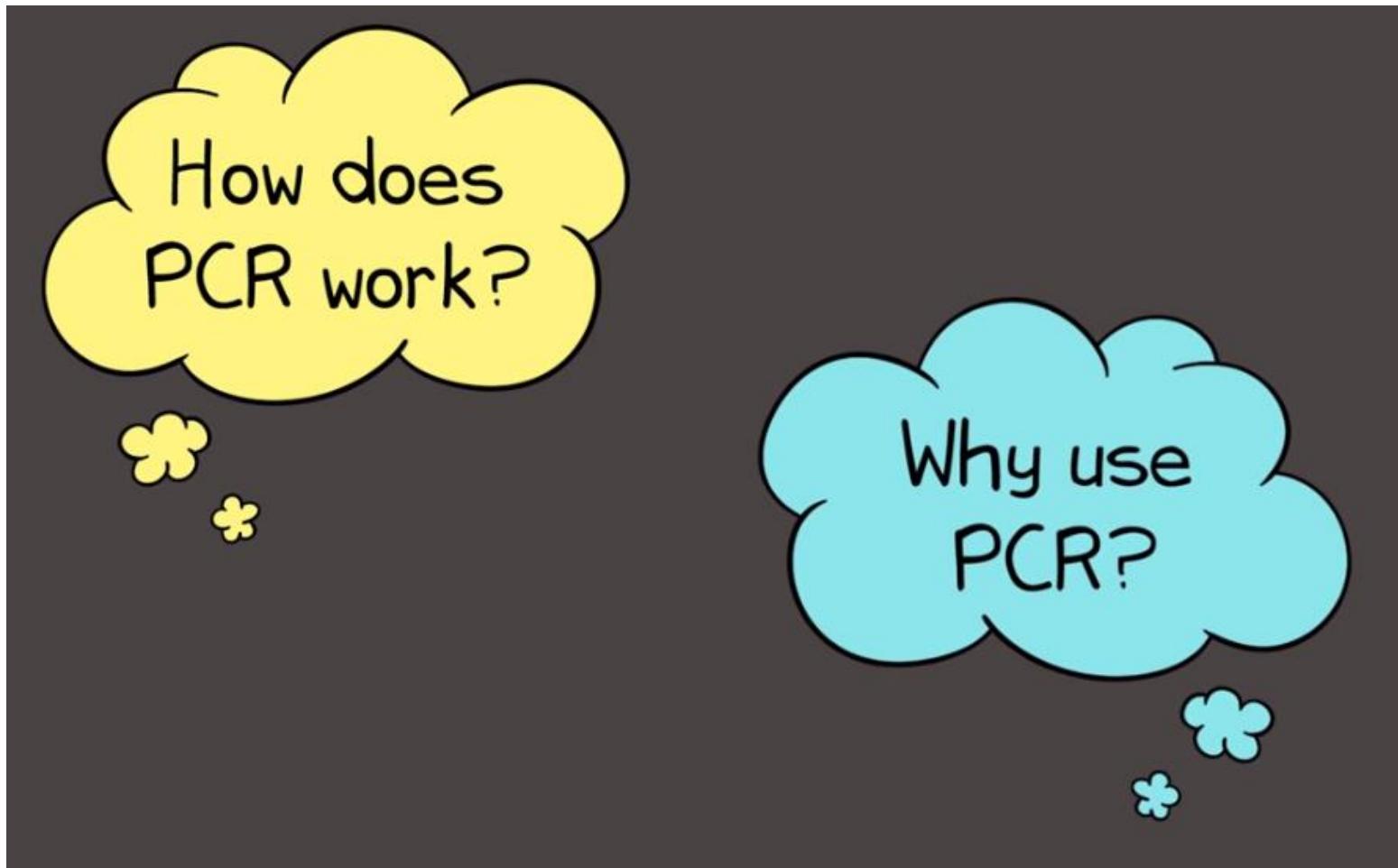
- ■ — ■ — ■
- 1. Bersifat Semi-kuantitatif
- 2. Tidak memerlukan instrumen tambahan untuk analisanya
- 3. Deteksi secara real-time

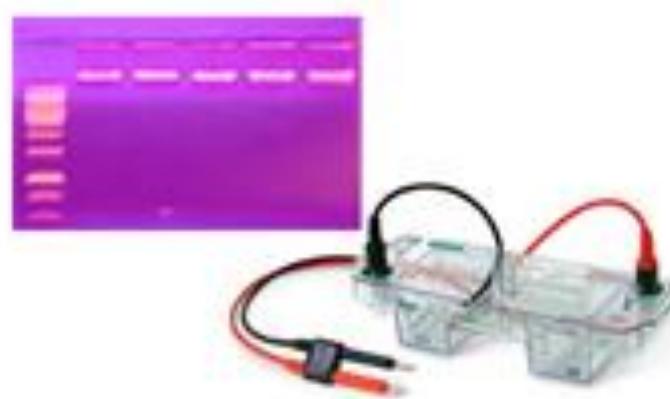
- ■ — ■ — ■ →
- 1. Bersifat Absolut kuantifikasi
- 2. Tidak memerlukan instrumen tambahan untuk analisanya
- 3. Deteksi secara real-time
- 4. Sensitifitas tingkat tinggi (1/20,000 x)

PCR Requirements



Polymerase Chain Reaction (PCR)

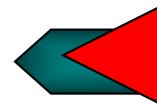
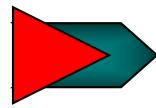




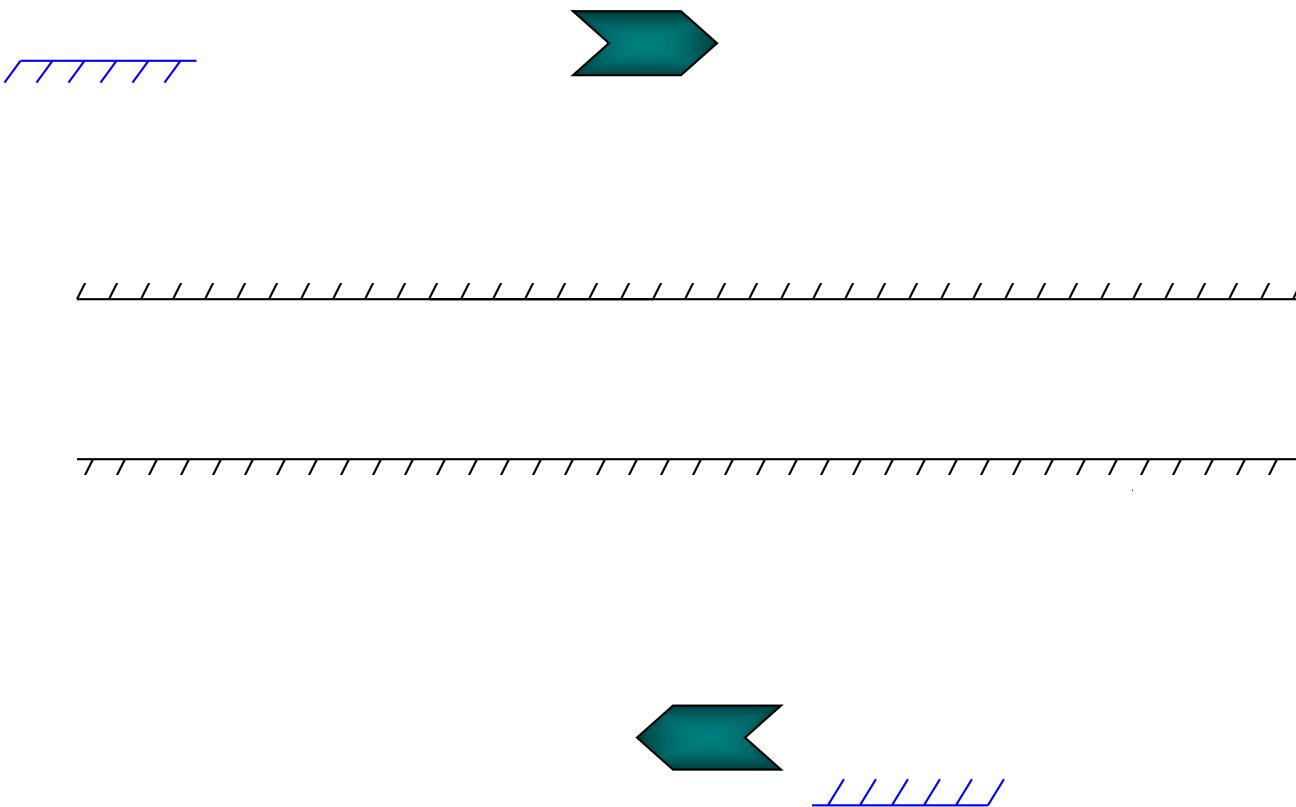
Generasi 1st PCR



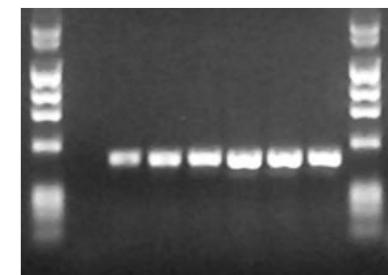
Hot Start

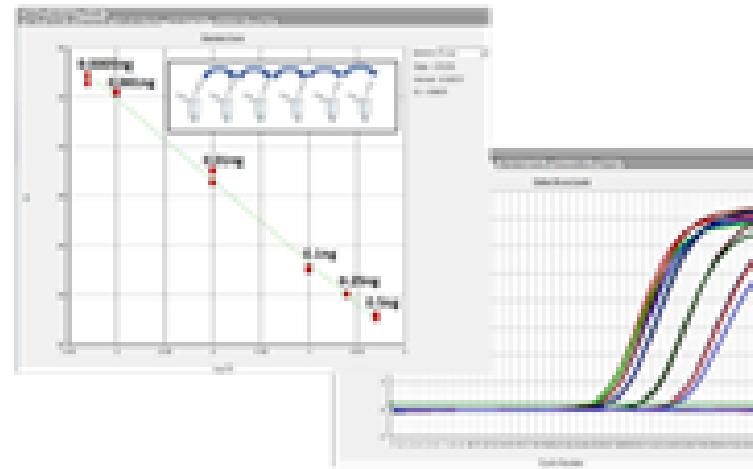


Classic PCR



End-point analysis of
amplification product
(30 cycles)

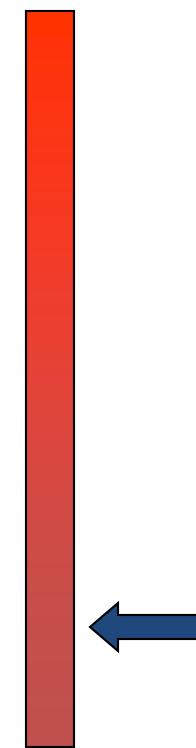
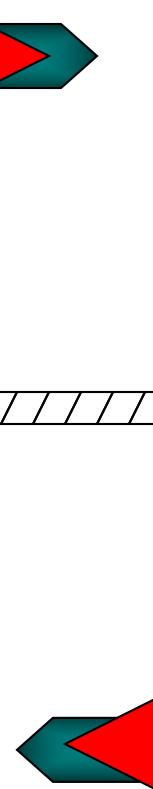
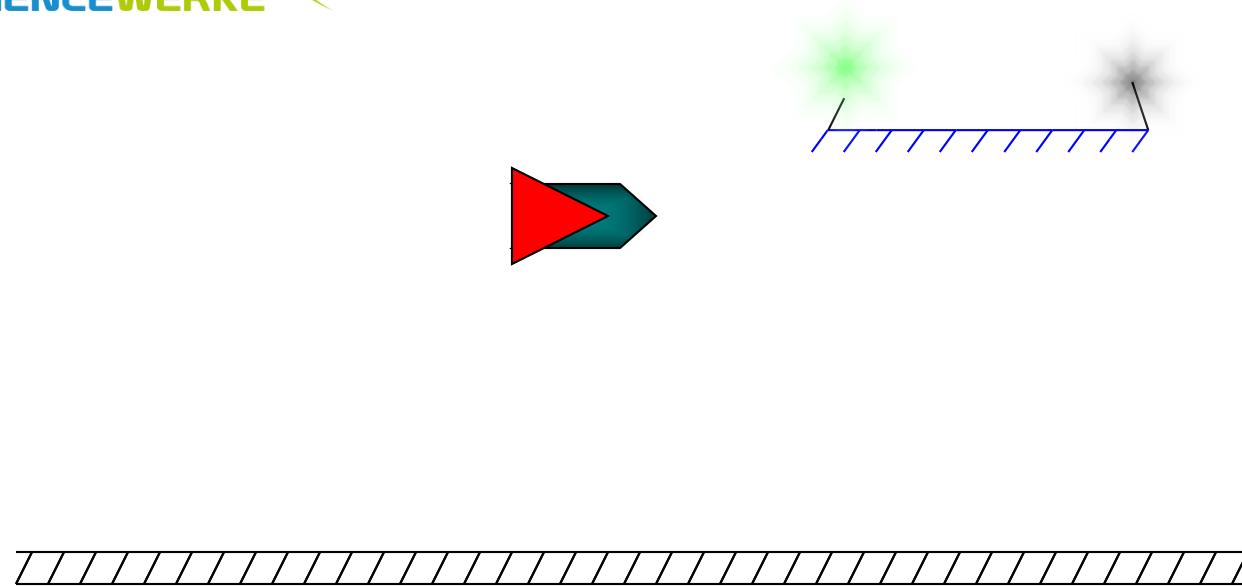




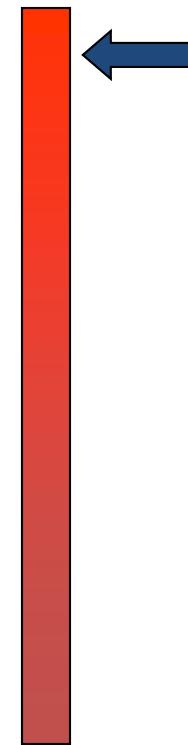
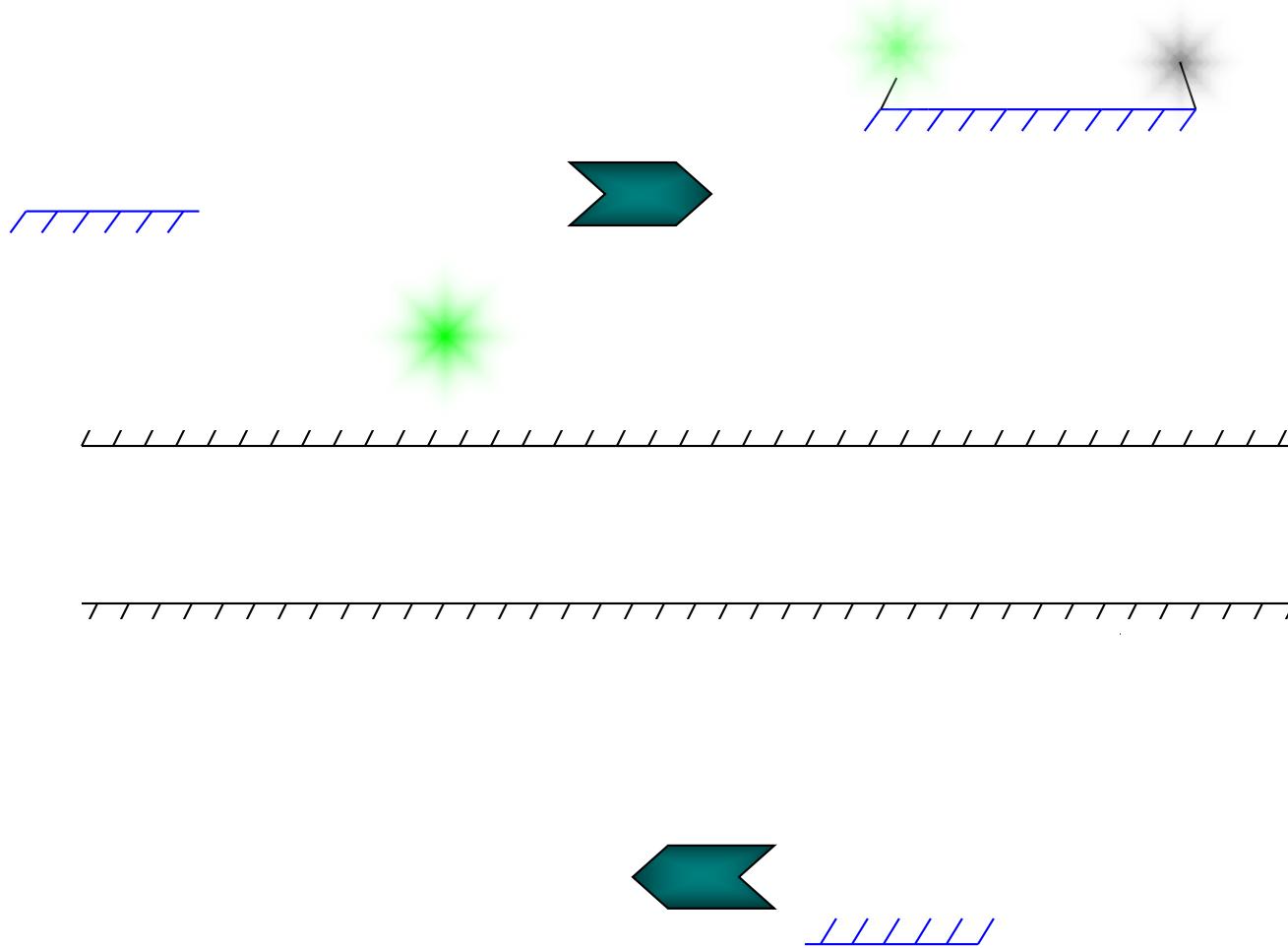
Generasi 2nd Real-Time PCR (qPCR)

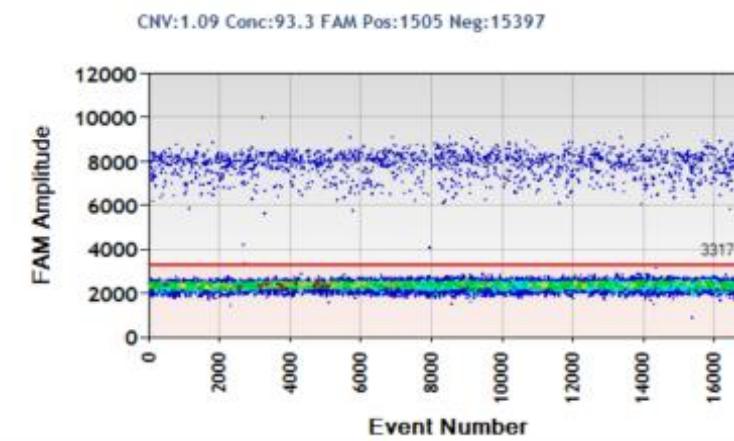


Real-Time PCR: Probes



Real-Time PCR: Probes





Generasi 3rd Digital PCR (dPCR)

What is Wrong with Conventional or Real-time PCR?

I need to
prepare
standards!

So MANY wells
so LITTLE
sample

I'm gonna spend
months optimizing
this multiplex
assay!

Low PCR efficiency -
urghhh inhibitors!

My standards
are inconsistent!



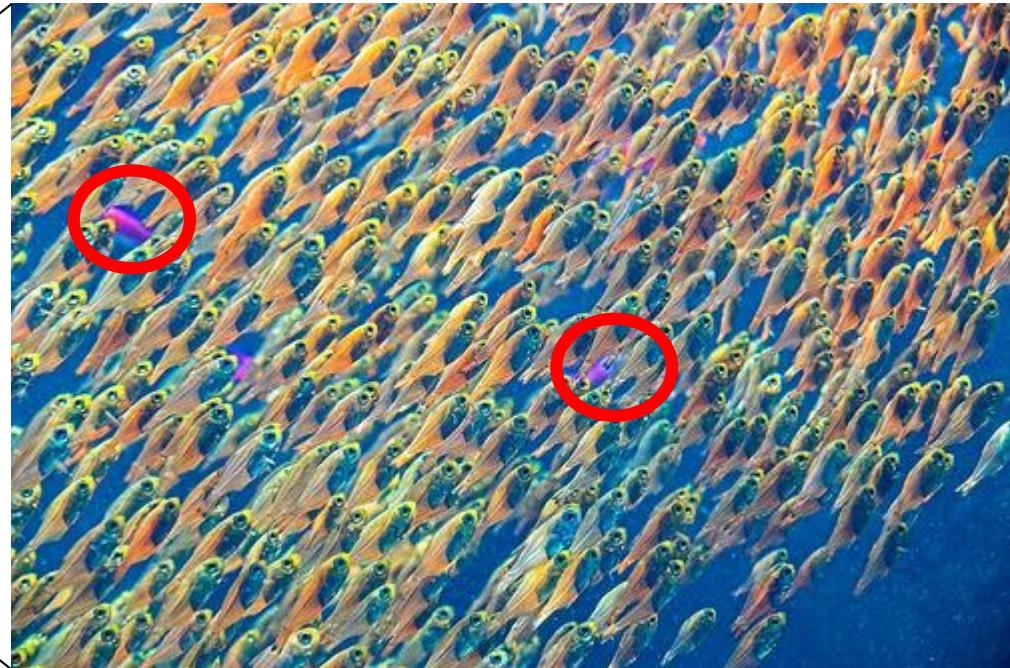
Is this really
cheaper?

My colleague got
a different data!

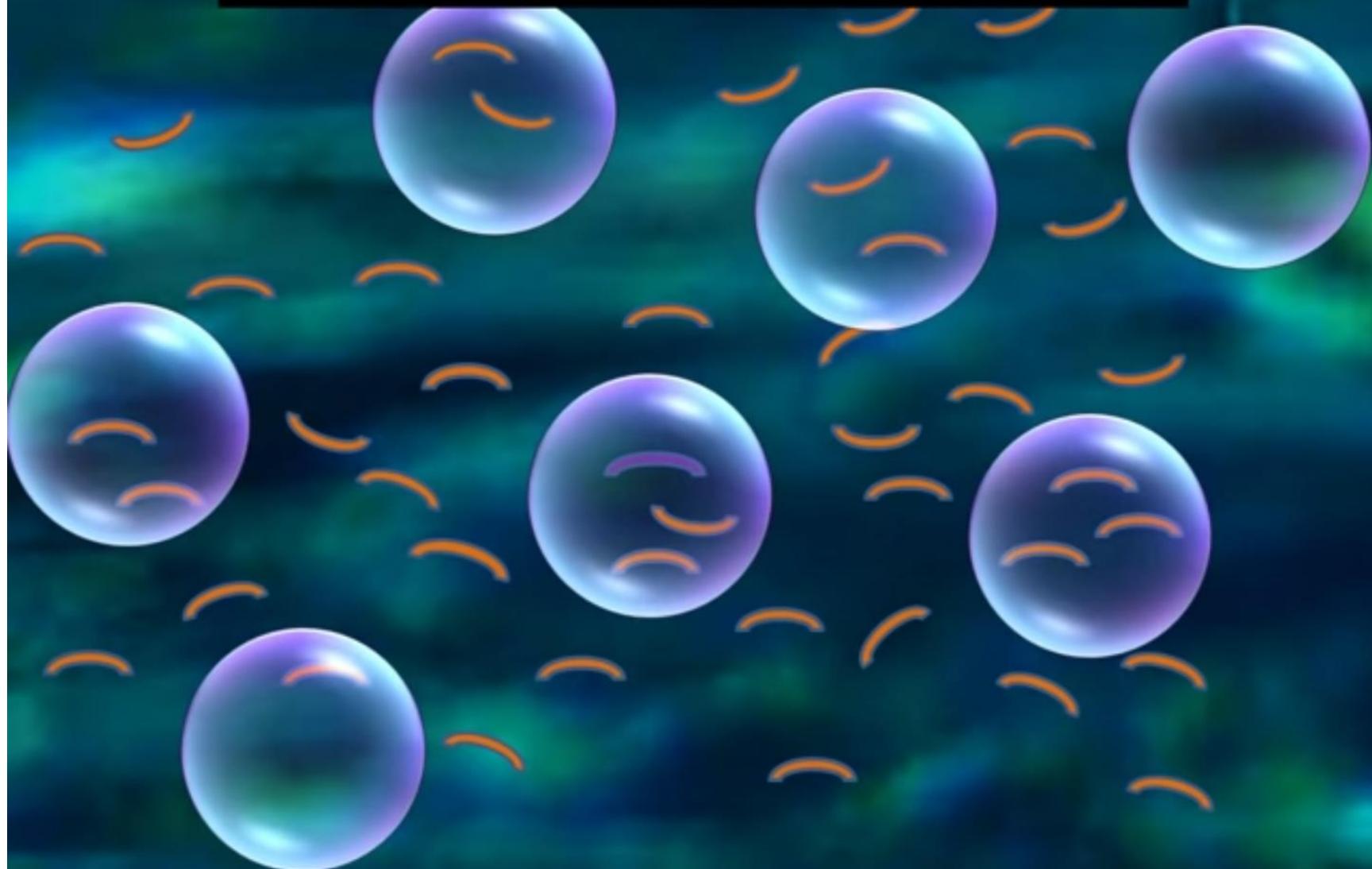
Is this really
reliable data?

WHAT?! Not
detected?

The Power of Partitioning into Droplets



Separate the sample into compartments so that
only a few molecules are present in each partition

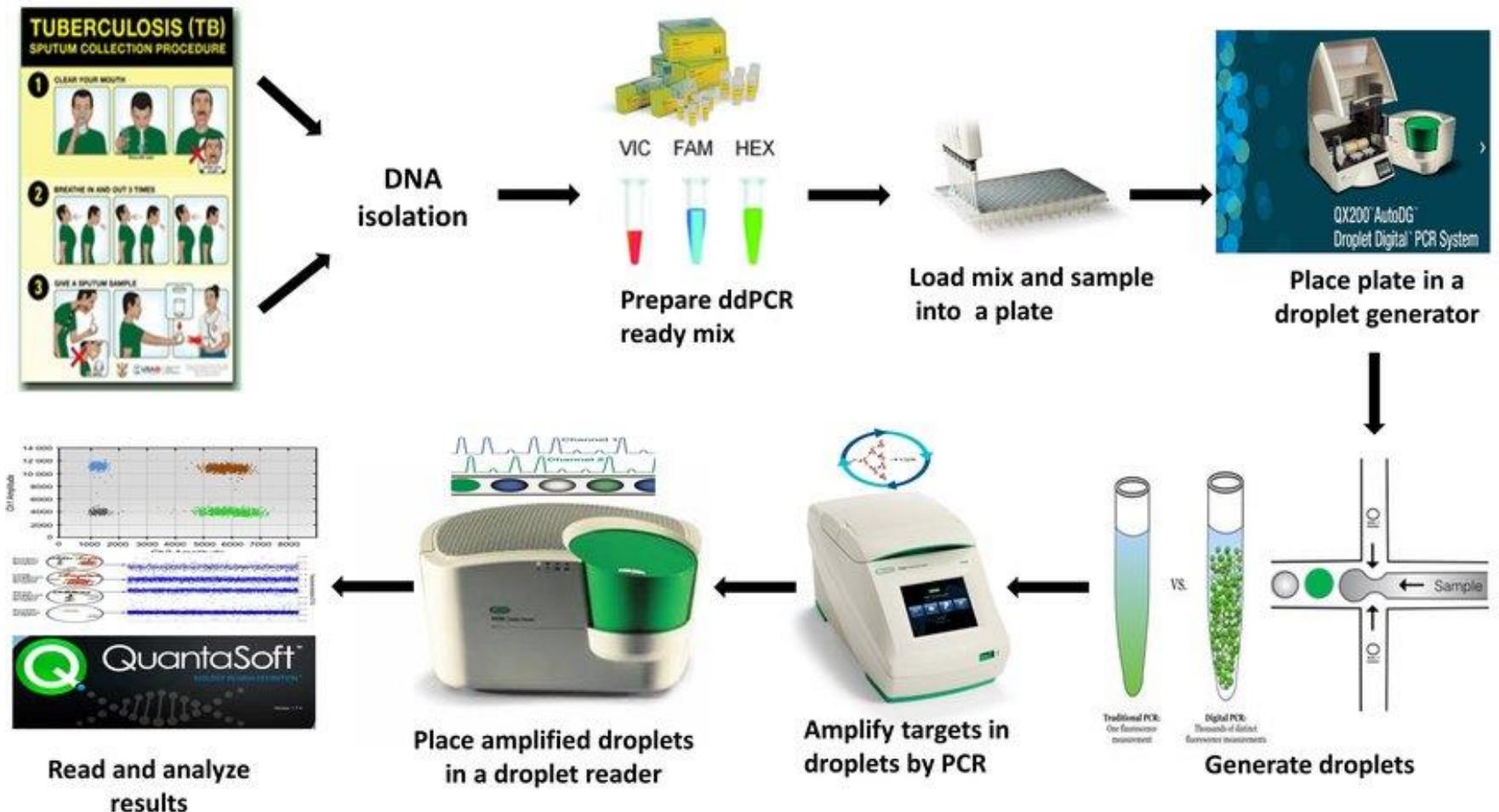


so

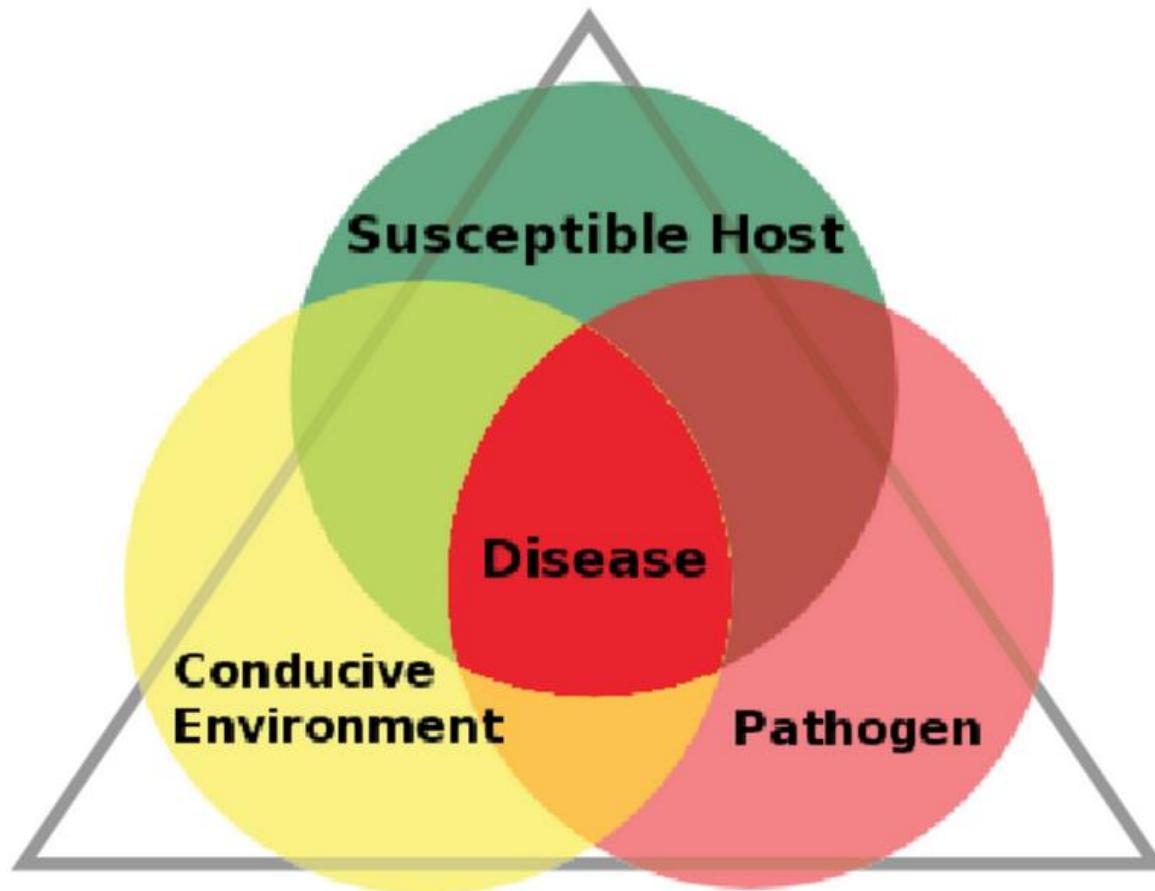
— 0.033 molecules per compartment

— 2.30 molecules per compartment

Alur Kerja ddPCR

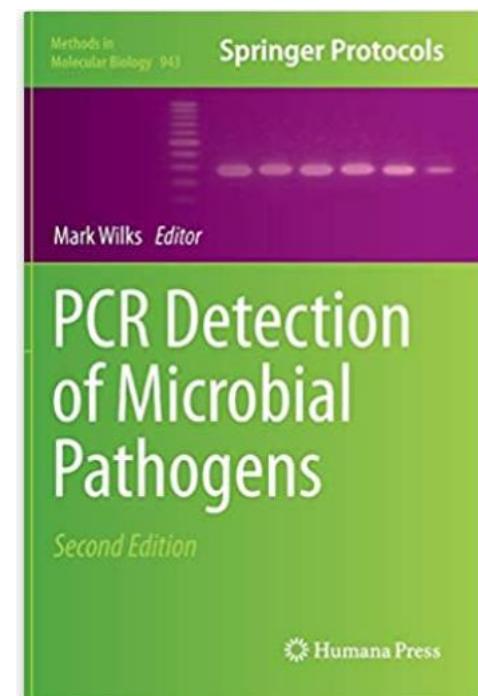


Pemanfaatan 3 Generasi PCR dalam Bidang Kesehatan

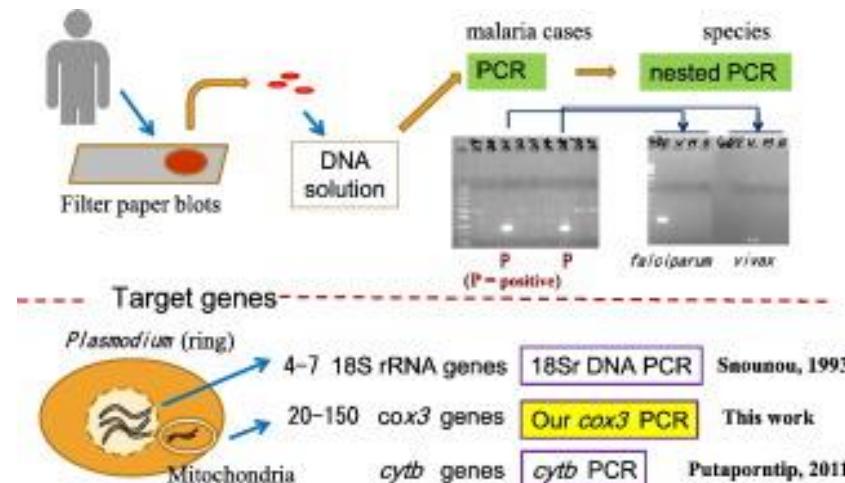


Pemanfaatan 3 Generasi PCR dalam Bidang Kesehatan

Aplikasi	Thermal Cycler PCR	Real-Time PCR	Droplet Digital PCR
Deteksi Patogen	**	*****	***
Deteksi Mutasi/SNP	*	***	*****
Analisis Copy Number Variation		*	****
Analisis miRNA		****	**
Analisis Ekspresi Gen		***	*****
Kuantifikasi Patogen		***	*****



Deteksi Patogen



PCR Method	Efficiency*
18Sr DNA	1.00 (standard)
cox3	1.54 (better)
cytb	0.42 (worse)

* estimated for microscopically undetected samples

Can Primary PCR detect *Plasmodium*?

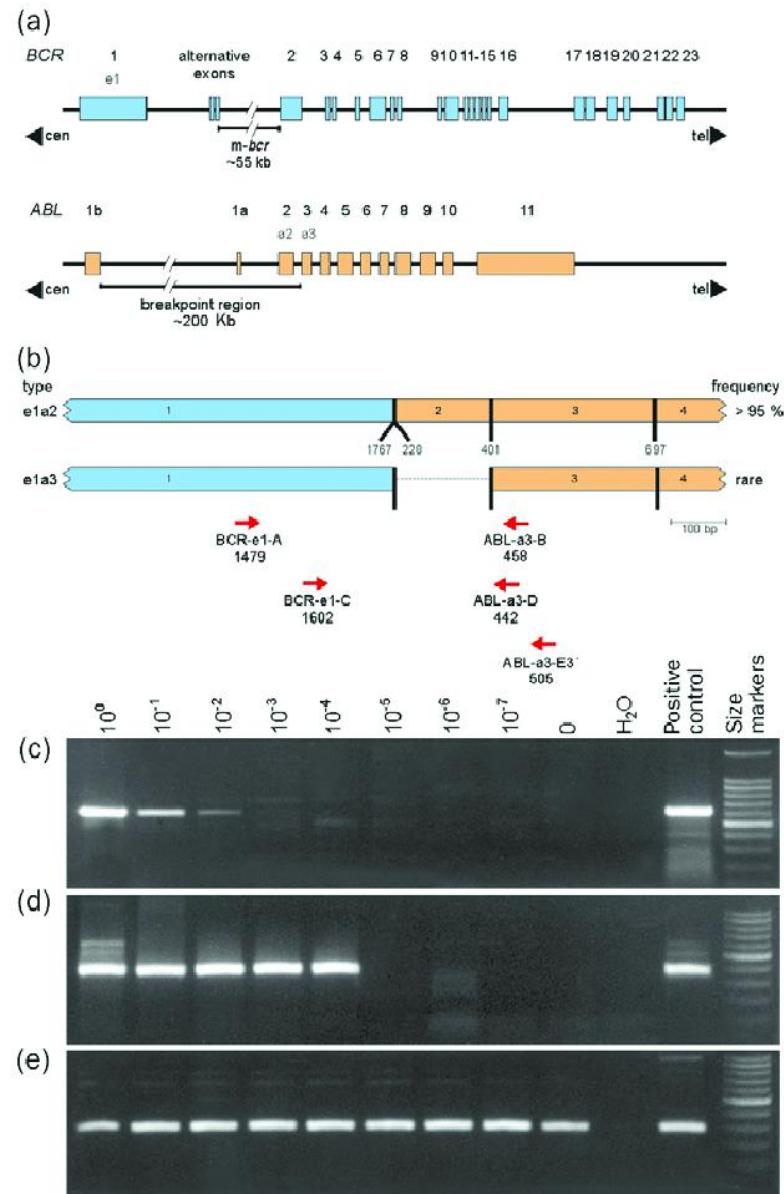
18Sr DNA	No
cox3	Yes**

**good feature in low endemic settings

Deteksi infeksi *Plasmodium* sp. untuk diagnosis malaria

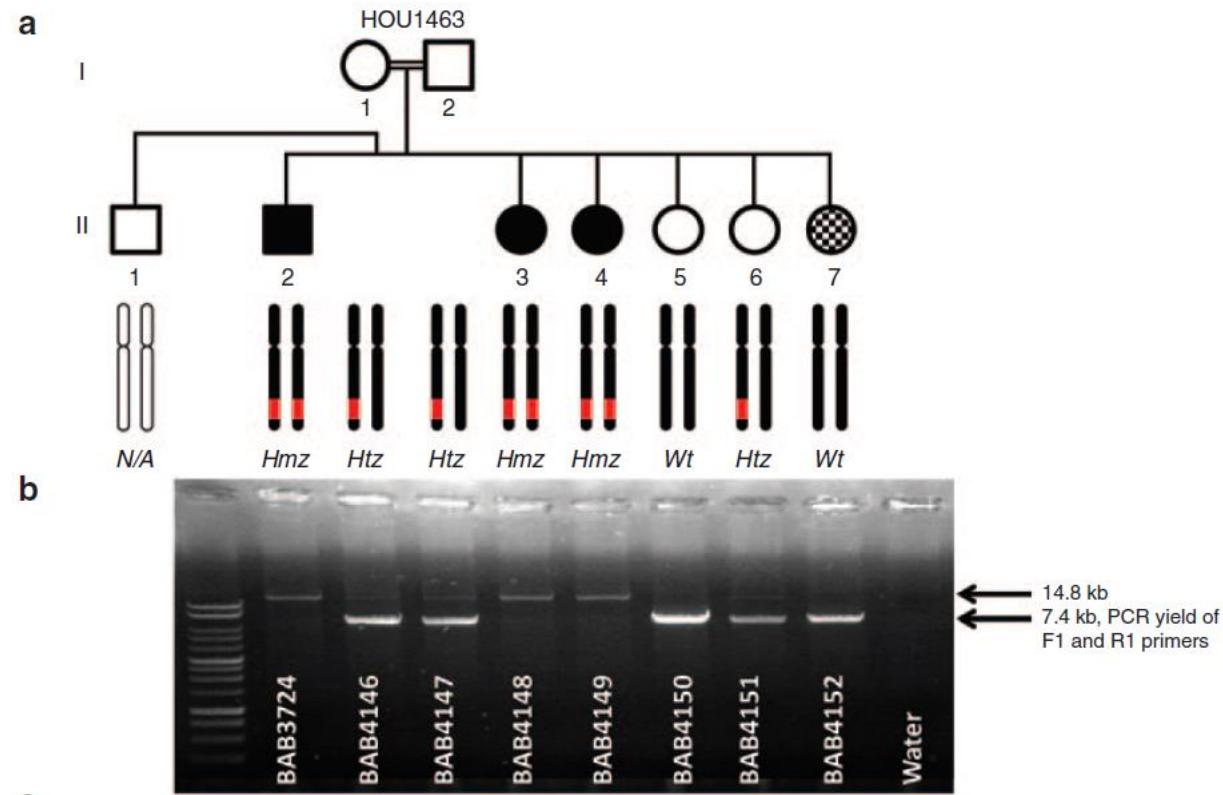
Deteksi Mutasi

Fusi gen BCR-ABL penting untuk patogenesis pada *Chronic Myloid Leukemia (CML)*



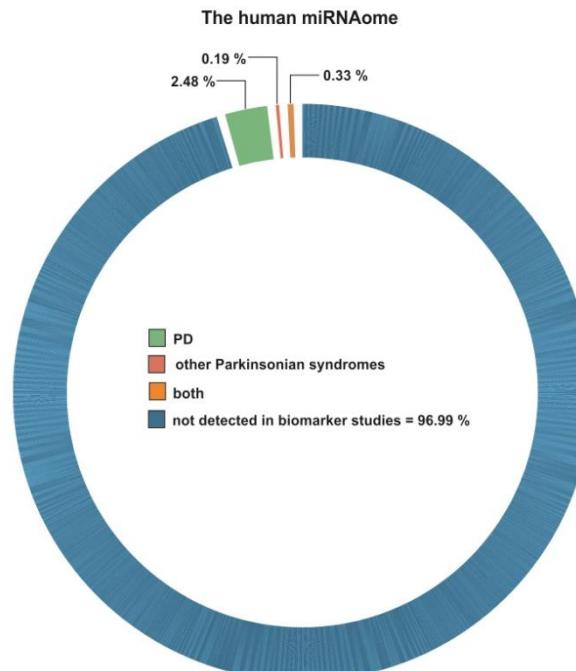
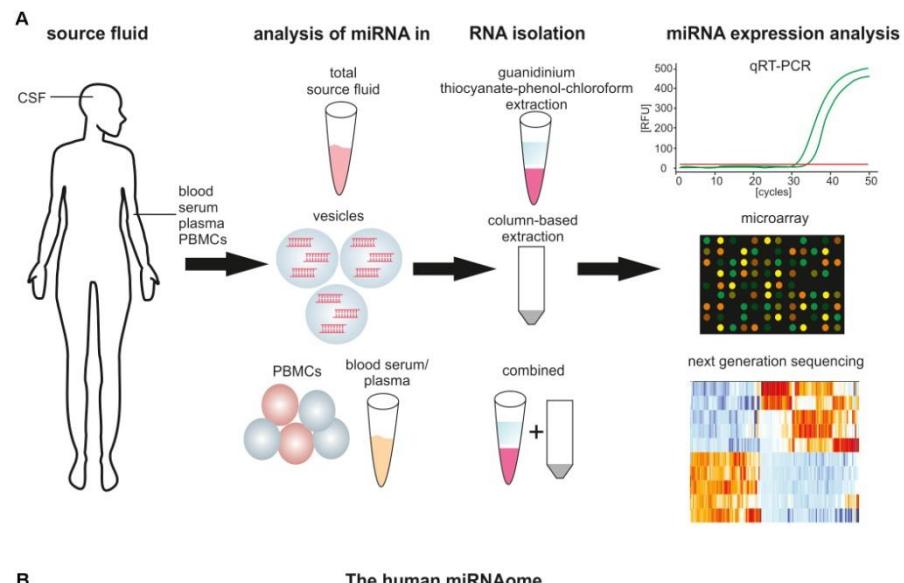
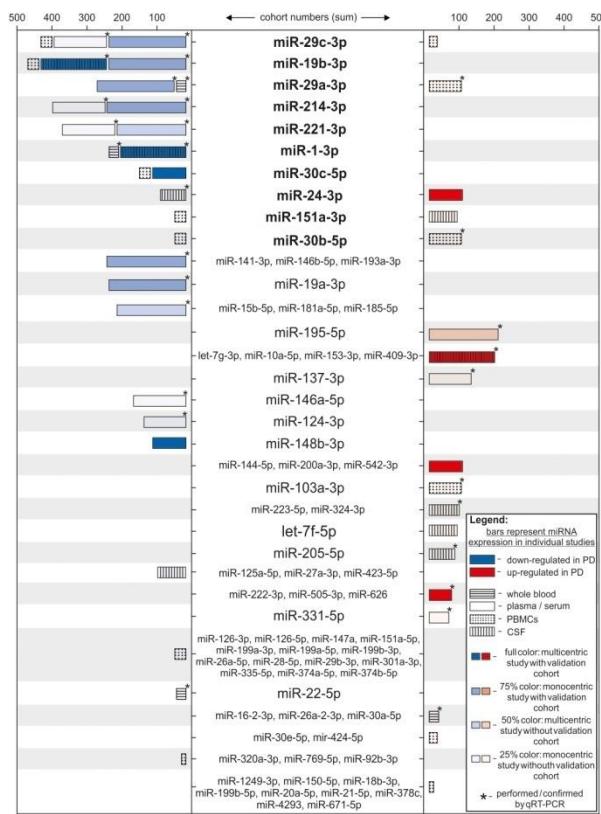
Analisis CNV

50% dari pasien dengan penyakit Charcot-Marie-Tooth (CMT) memiliki 1,4Mb duplikasi CNV sebagai penyebab neuropati

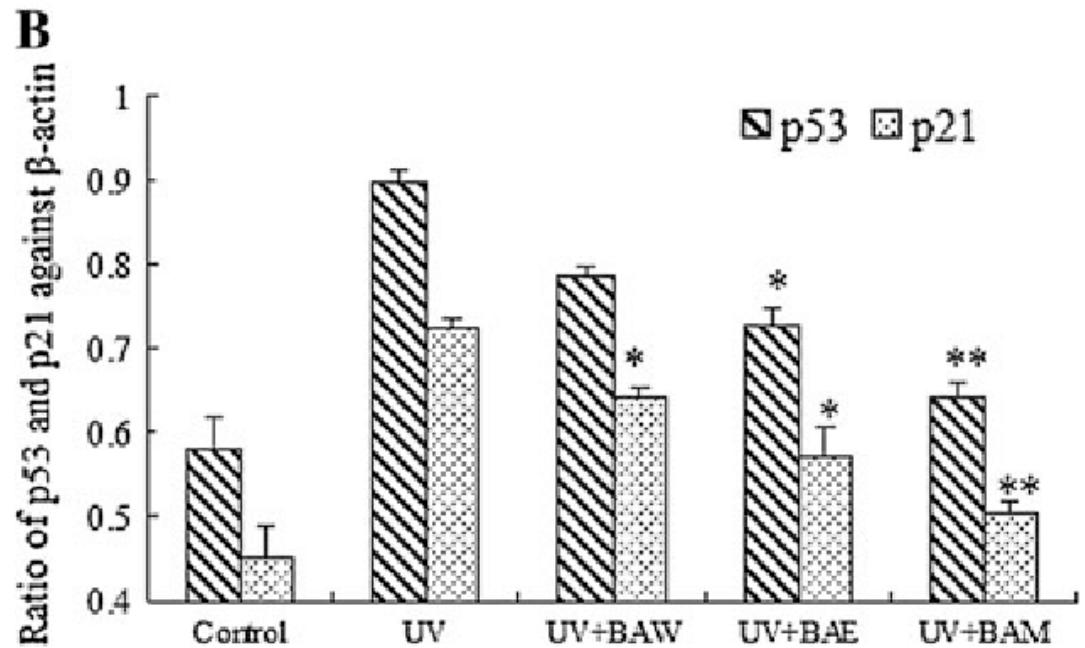
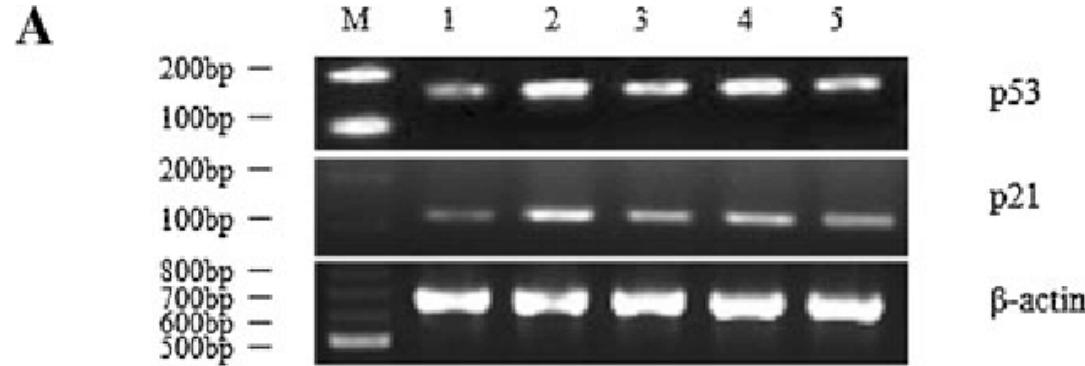
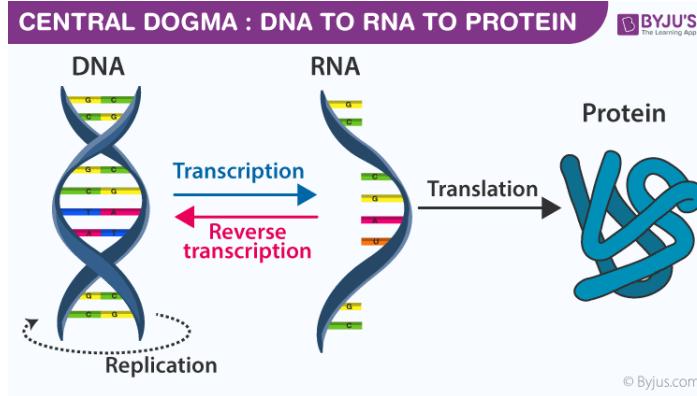


Analisis miRNA

Perubahan ekspresi miRNA
dikaitkan dengan proses
patofisiologis yang relevan
dengan Penyakit Parkinson



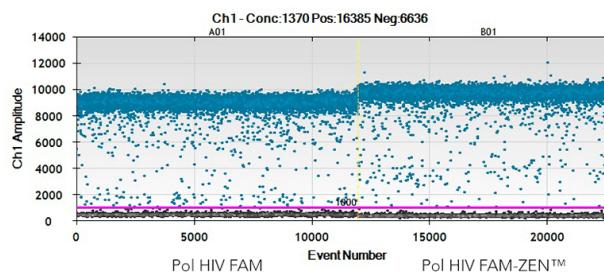
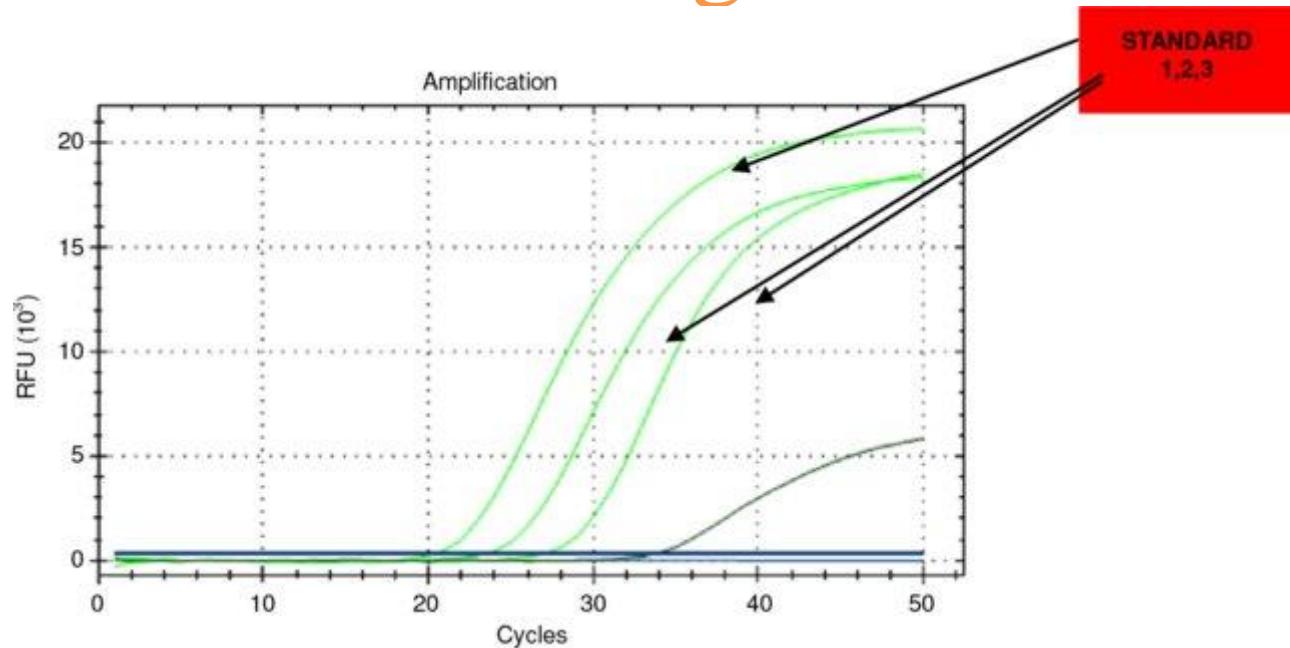
Analisis Ekspresi Gen



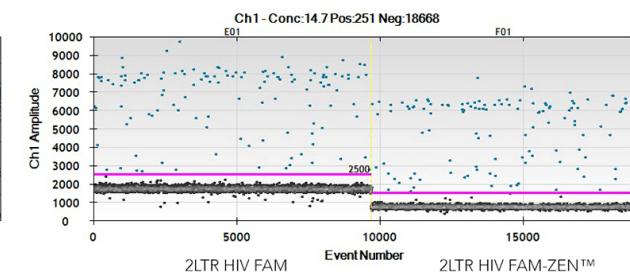
p53 merupakan gen supresor *tumor* dan mutasi pada gen ini berperan dalam karsinogenesis, aktivitas proliferasi serta invasi dan metastasis sel *tumor*

Kuantifikasi Patogen

Analisis *Viral Load* HIV sangat penting untuk pemantauan efikasi pengobatan dan prognosis



A. ddPCR Pol HIV RNA Assay.



B. ddPCR 2LTR HIV DNA Assay.



The over-all point is that new technology will not necessarily replace old technology, but it will date it. By definition. Eventually, it will replace it. But it's like people who had black-and-white TVs when color came out. They eventually decided whether or not the new technology was worth the investment.

— *Steve Jobs* —

AZ QUOTES