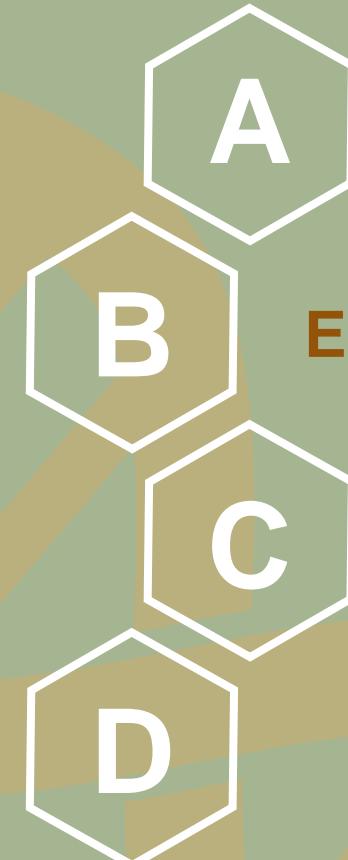




Uhamka
UNIVERSITAS MUHAMMADIYAH PROF. DR. HAMKA

“Diagnosis Berbagai Penyakit Melalui Peran Biomolekuler”

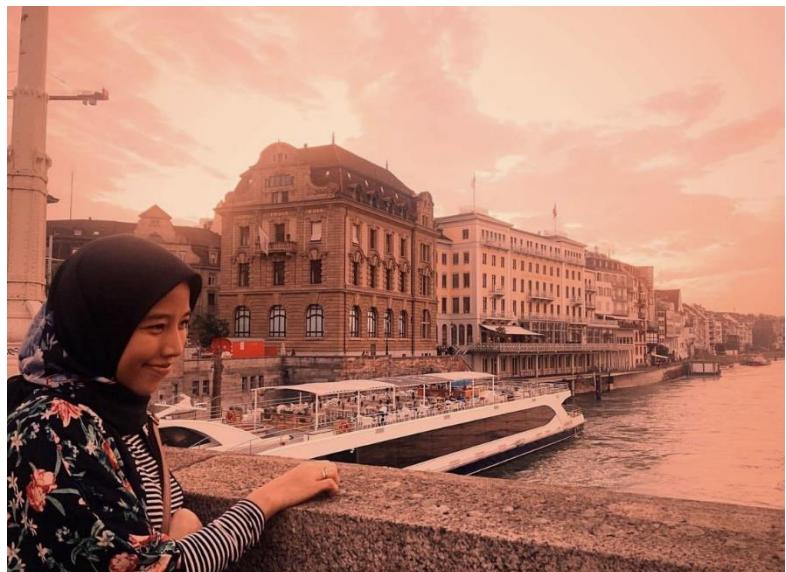


Engla Merizka, S.ST, M.Biomed

D 4 TLM / Analis Kesehatan
Fakultas Farmasi dan Sains
UHAMKA



Biodata



Nama Lengkap

Engla Merizka, S.ST, M.Biomed

Tempat, tanggal lahir

Pekanbaru, 18 September 1992

Email dan Hp

**engla.merizka@gmail.com
+6281296060118**

Research Interest

- ***Molecular Genetic***
- ***Genotyping***
- ***Monoclonal Antibody***

Pendidikan Terakhir

- **S2 Biomedik FK UI**
- **S3 Biomedik FK UI (sekarang)**

Apa itu “Biomolekuler”?



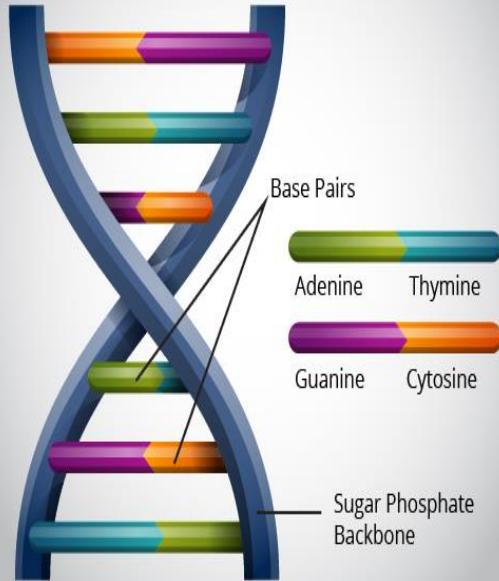
merupakan salah satu cabang biologi yang merujuk kepada pengkajian mengenai kehidupan pada skala molekul. Ini termasuk penyelidikan tentang interaksi molekul dalam benda hidup, terutama tentang interaksi berbagai sistem dalam sel, **termasuk interaksi DNA, RNA**, dan sintesis protein, dan bagaimana interaksi tersebut diatur. Bidang ini bertumpang tindih dengan bidang biologi (dan kimia) lainnya, terutama genetika dan biokimia



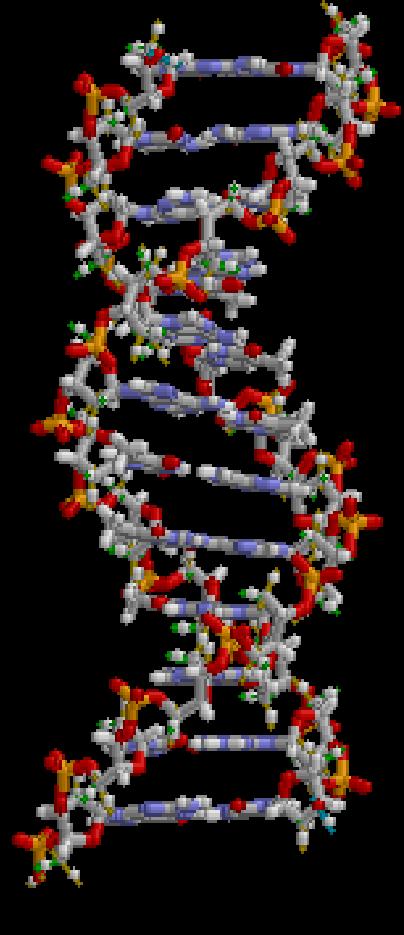
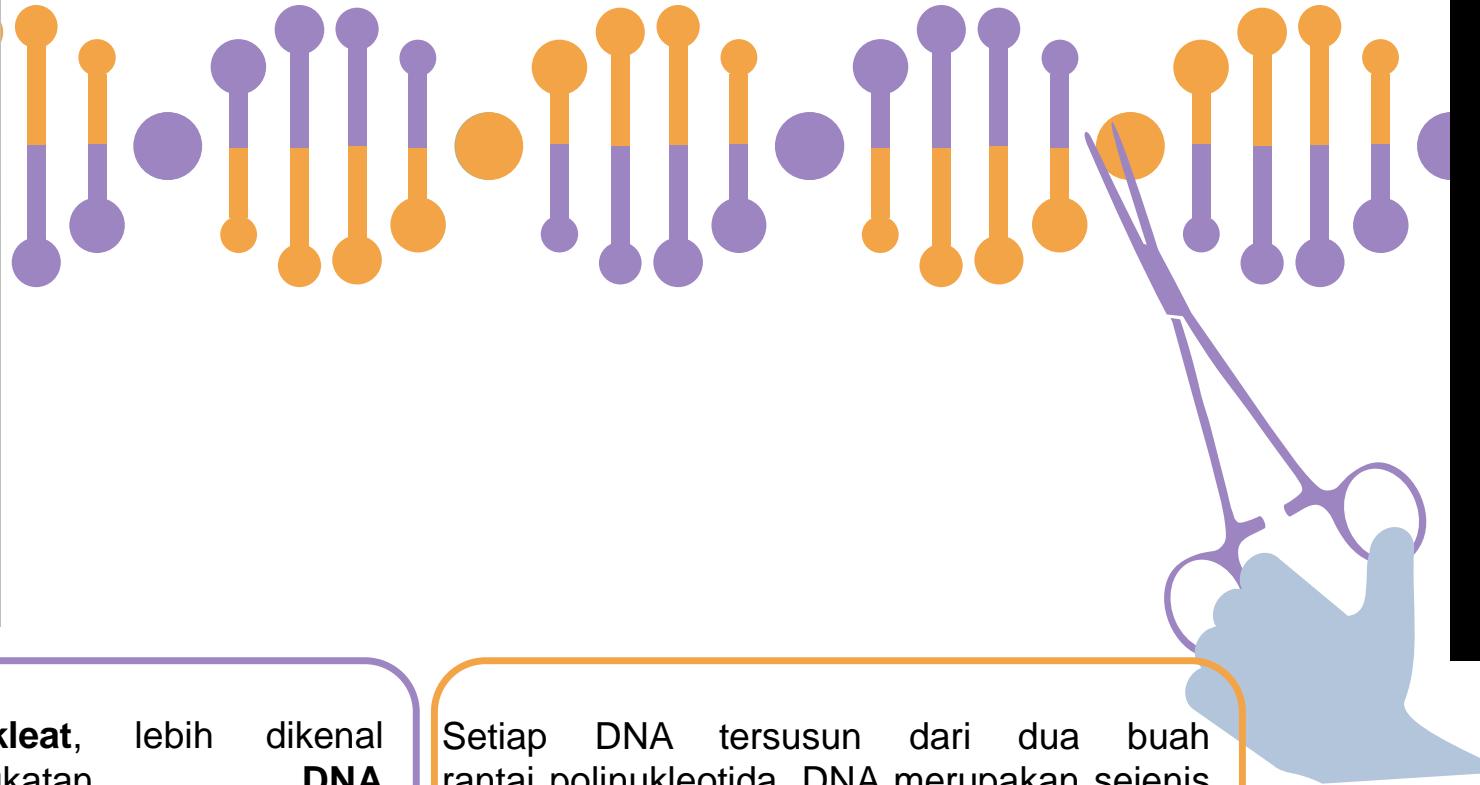
Biomolecular Sciences



DNA Structure



Apa itu DNA ?



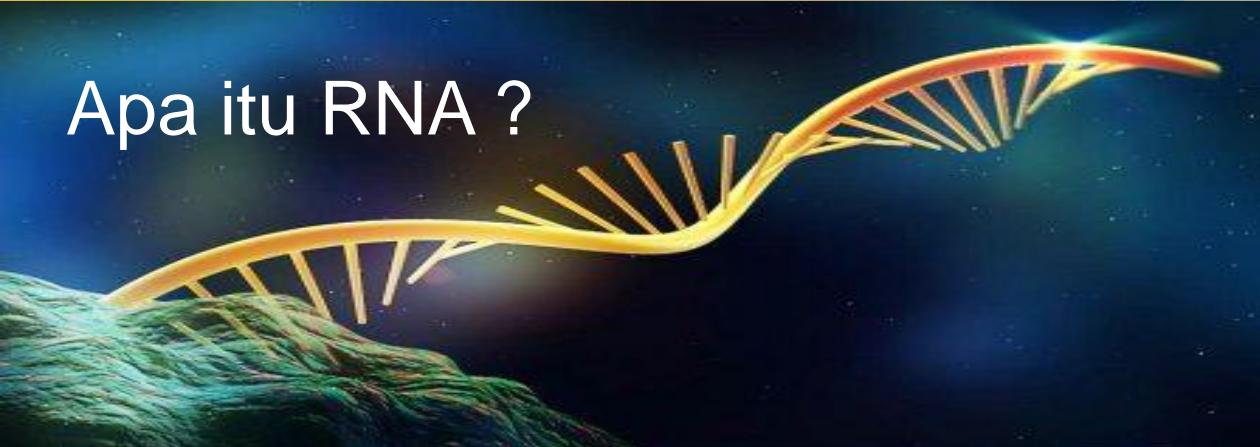
Asam deoksiribonukleat, lebih dikenal dengan singkatan **DNA** (*deoxyribonucleic acid*) merupakan salah satu jenis asam nukleat yang memiliki kemampuan pewarisan sifat. Keberadaan asam deoksiribonukleat ditemukan di dalam nukleoprotein yang membentuk inti sel.

Setiap DNA tersusun dari dua buah rantai polinukleotida. DNA merupakan sejenis biomolekul yang menyimpan dan menyandi instruksi-instruksi genetika setiap organisme dan banyak jenis virus. DNA terdiri dari dua unting biopolimer yang berpilin satu sama lainnya membentuk heliks ganda.

Asam ribonukleat (ARN/ ribonucleic acid, RNA) adalah molekul **polimer** yang terlibat dalam berbagai peran biologis dalam **ekspresi gen**. RNA adalah **asam nukleat**, dan, bersama dengan **protein** dan **karbohidrat**, merupakan empat **makromolekul** utama yang penting untuk semua bentuk kehidupan yang diketahui.

RNA lebih sering ditemukan di alam sebagai untai tunggal yang melipat ke dirinya sendiri

Apa itu RNA ?

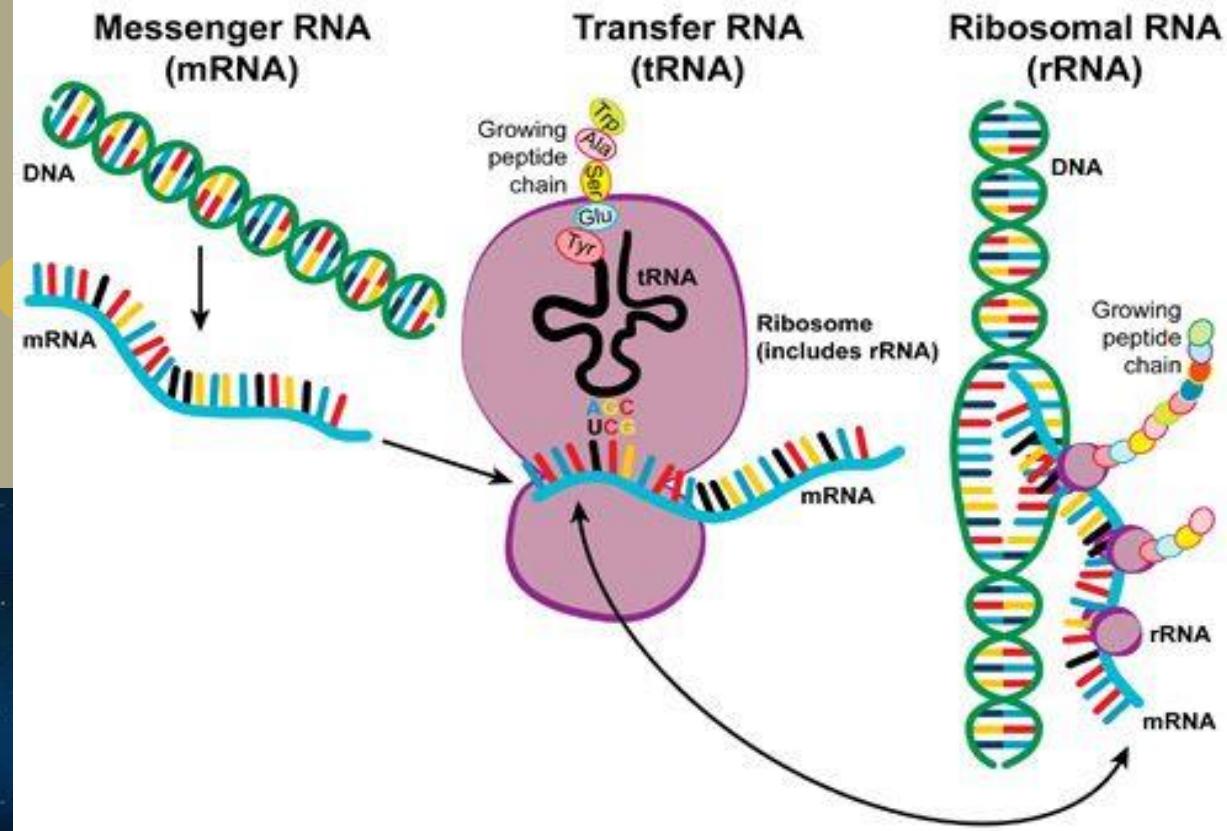


1.RNA duta (*messenger-RNA, mRNA*), yang disintesis dengan RNA polimerase I.

2.RNA ribosomal (*ribosomal-RNA, rRNA*), yang disintesis dengan RNA polimerase II

3.RNA transfer (*transfer-RNA, tRNA*), yang disintesis dengan RNA polimerase III

Ribonucleic acid (RNA)



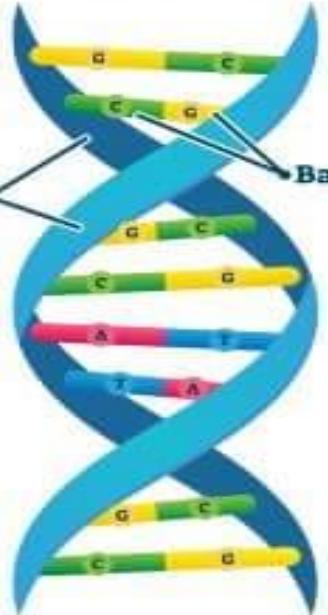
messenger RNA untuk menyampaikan informasi genetik (menggunakan huruf G, U, A, dan C untuk menunjukkan basa nitrogen guanin, urasil, adenin, dan sitosin)

DNA

Deoxyribonucleic Acid



Double-Stranded
Sugar Phosphate
DEOXYRIBOSE



VS

RNA

Ribonucleic Acid



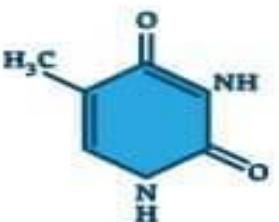
Single
Nucleobase



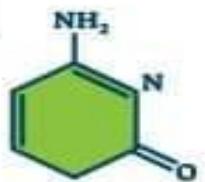
Single-Stranded
Sugar Phosphate
RIBOSE



Nucleobases



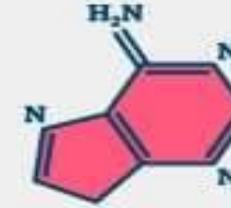
Thymine



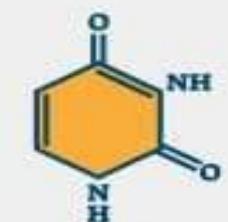
Cytosine



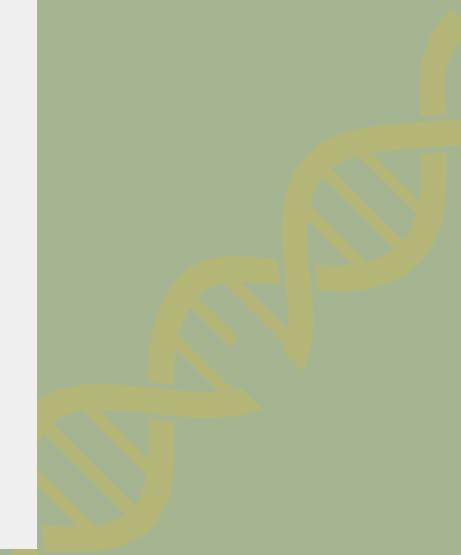
Guanine

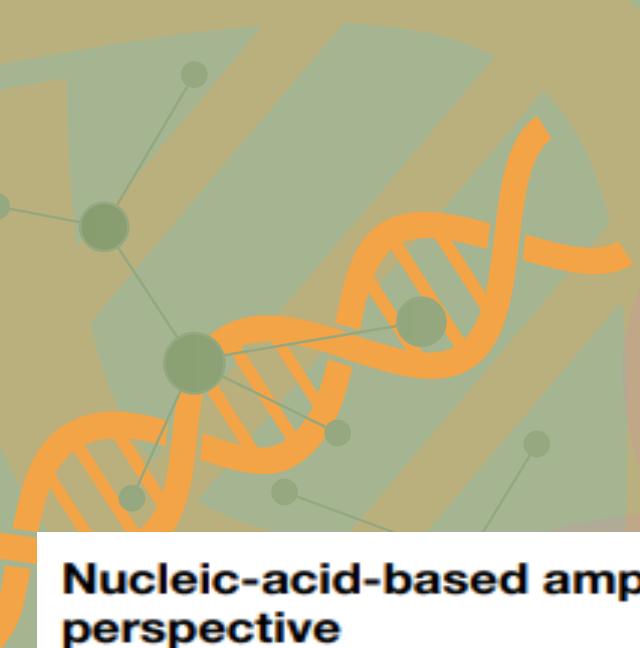


Adenine



Uracil





PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings

Samuel Yang and Richard E Rothman

Nucleic-acid-based amplification: historical perspective

The first nucleic-acid-based assays used DNA probe technology.^{14–16} DNA probes are short, labelled, single-strand segments of DNA that are designed and synthesised to hybridise targeted complementary sequences of microbial DNA. By contrast with traditional culture-based methods of microbial identification, which rely on phenotypic characteristics, this molecular fingerprinting technique relies on sequence-based hybridisation chemistry, which confers greater specificity to pathogen identification. Direct detection of target microbial DNA in clinical samples also eliminates the need for cultivation, drastically reducing the time required for reporting of results. In 1980, the description of DNA hybridising probes for detecting enterotoxigenic *Escherichia coli* in stool samples raised hopes that nucleic-acid-based technologies would eventually replace traditional culture techniques.¹⁷ Since that time, however, a more

PCR: basic principles and overview

PCR is an enzyme-driven process for amplifying short regions of DNA in vitro. The method relies on knowing at least partial sequences of the target DNA *a priori* and using them to design oligonucleotide primers that hybridise specifically to the target sequences. In PCR, the target DNA is copied by a thermostable DNA polymerase enzyme, in the presence of nucleotides and primers. Through multiple cycles of heating and cooling in a thermocycler to produce rounds of target DNA denaturation, primer hybridisation, and primer extension, the target DNA is amplified exponentially (figure

Apa beda PCR dan Real Time PCR?

PCR, atau Reaksi Rantai Polimerase, adalah proses untuk amplifikasi fragmen DNA tertentu. Ex: DNA HBV

Real-Time PCR teknik khusus yang memungkinkan reaksi PCR divisualisasikan "dalam waktu nyata" saat reaksi berlangsung.

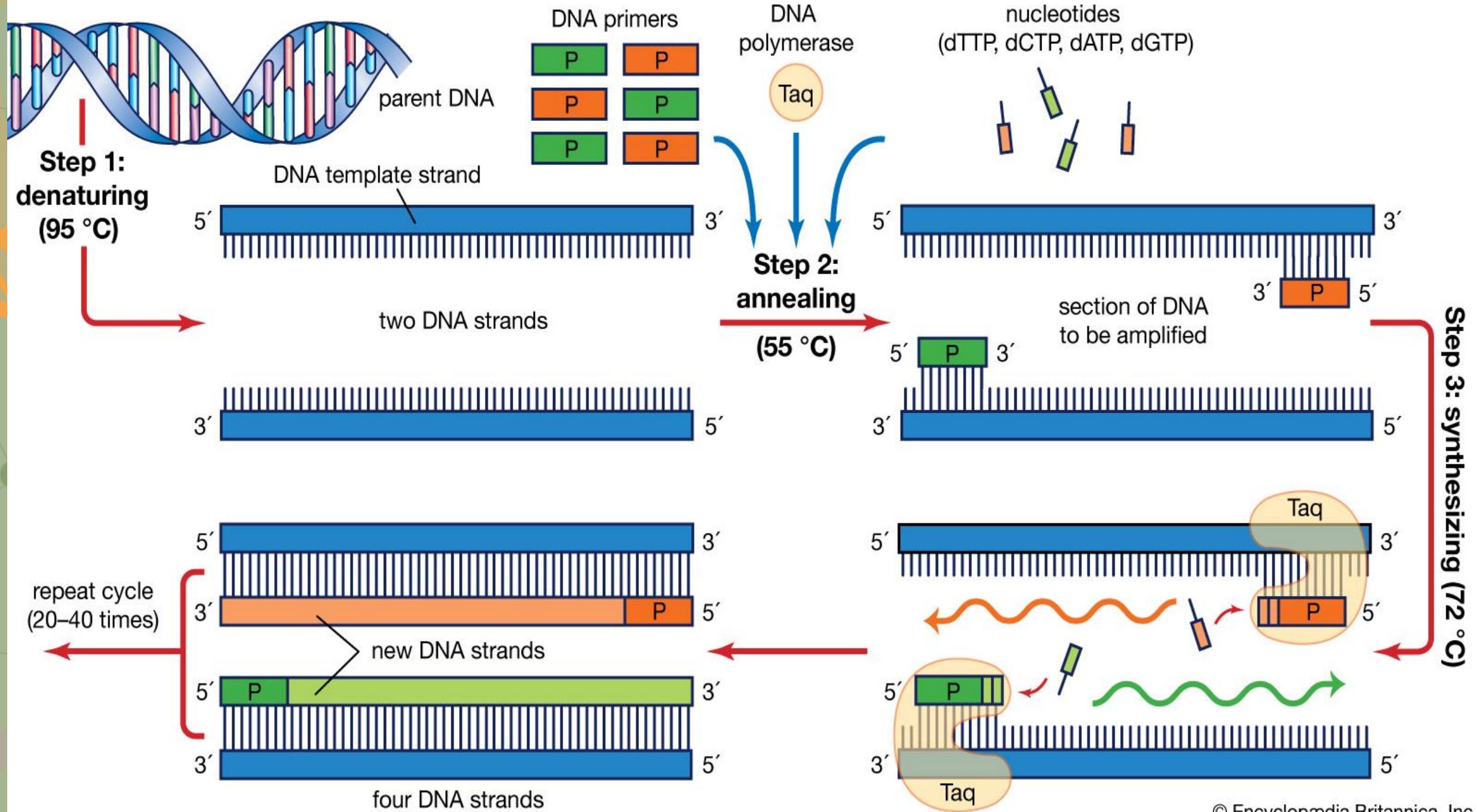
So, PCR Real-Time memungkinkan kita untuk mengukur sejumlah kecil urutan DNA dalam sampel.

Berikut tahapan PCR:

1. Denaturasi
2. Annealing
3. Elongasi

Conventional PCR
tells us “what”.

Real-Time PCR
tells us “how much”.



Penggunaan PCR dalam mendeteksi berbagai mikroorganisme

Table 1. FDA-approved nucleic-acid-based assays for detection of microbial pathogens

Organism detected	Trade name	Company/institution	Method	Clinical sensitivity	Clinical specificity
<i>Chlamydia trachomatis</i>	Amplicor	Roche	PCR	93·2 ¹²	98·4 ¹²
	LCX	Abbott	LCR	>95 ¹³	>99 ¹²
	AMP	Gen-Probe	TMA	86·7–99·2 ¹²	>99 ¹²
	PACE 2	Gen-Probe	Hybridisation	60·8–78·1 ¹²	>99 ¹²
	BDProbeTec	Becton Dickinson	SDA	94·0 ¹⁴	>99 ¹²
	Hybrid capture II CT-ID	Digene	Hybrid capture	95·4 ¹⁵	99 ¹⁷
	CMV pp67 mRNA	Organon Teknika	NASBA	95 ¹⁶	98 ¹⁸
	Hybrid capture CMV DNA test	Digene	Hybrid capture	95 ¹⁶	95 ¹⁸
	<i>Gardnerella vaginalis</i>	Affirm VIP III	Hybridisation	94 ¹⁹	81 ⁴⁰
	Group A streptococcus	GP-ST test	Gen-Probe	Hybridisation	88·6 ¹²
Group B streptococcus	IDI-StrepB	Infectio Diagnostics	Real-time PCR	97 ¹²	100 ¹²
	HCV	Roche	PCR	98 ¹²	NA
	Amplicor HCV	Bayer	TMA	NA	98 ⁴¹
	Versant HCV RNA qualitative assay	Bayer	BDNA	NA	98·2 ⁴²
	Versant HCV RNA 3·0	Roche	RT-PCR	NA	>99 ¹²
	Amplicor HIV-1 Monitor Test	Visible Genetics	DNA sequencing	NA	NA
	Trugene HIV drug resistance and OpenGene DNA sequencing	bioMerieux	NASBA	NA	>99 ¹²
	NucliSens EasyQ HIV-1	Chiron	TMA	>99 ¹²	>99 ⁴³
	Procleix HIV-1/HCV	Bayer	BDNA	NA	97·6 ⁴⁴
	Versant HIV-1 RNA 3·0	ViroSeq	DNA sequencing	NA	NA
HPV	Hybrid capture II HPV DNA	Applied Biosystems	Hybrid capture	>99 ¹²	85·90 ⁴⁵
	TB Amplicor	Digene	PCR	79·4–91·9 ¹²	>99 ¹²
	E-MTD	Roche	TMA	90·9–95·2 ¹²	>99 ¹²
	<i>Neisseria gonorrhoeae</i>	Gen-Probe	PCR	NA	NA
<i>Trichomonas vaginalis</i>	Amplicor	Roche	PCR	NA	>99 ¹²
	LCX	Abbott	LCR	>95 ¹²	>99 ¹²
	Hybrid capture II CT/GC	Digene	Hybrid capture	93 ¹⁶	98·5 ⁴⁶
	BDProbeTec	Becton Dickinson	SDA	88·9 ¹⁶	>99 ¹²
	PACE-2	Gen-Probe	Hybridisation	97 ¹⁶	99 ¹²
	Affirm VIP III	Becton Dickinson	Hybridisation	88–91·9 ¹²	100 ¹²

BDNA=branched DNA; LCR=ligase chain reaction; NASBA=nucleic-acid-sequence-based amplification; PCR=polymerase chain reaction; RT-PCR=reverse transcriptase PCR; SDA=strand displacement amplification; TMA=transcription mediated amplification; NA=not applicable. Adapted from reference 19.



Ex:

Penggunaan PCR dalam Forensik

Real-Time PCR in Forensics

What is it ??



Enough DNA to ID ??

STAIN IDENTIFICATION

The Use of Real-Time PCR for Forensic Stain Identification

By Trisha L. Noreault-Conti, Ph.D., and Eric Buel, Ph.D.
Vermont Forensic Laboratory, Department of Public Safety

Example: Real-Time PCR in Forensic Analysis!

Stain Identification:

New Real-Time methods can be directly used to identify the composition of unknown stains, with much better accuracy than traditional “color-change” tests.



DNA Quantification:

Since standard forensic STR Genotyping requires defined amounts of DNA, Real-Time PCR can be used to accurately quantify the amount of DNA in an unknown sample!

HIV-1 Viral Load Testing

Methods and Clinical Applications

Christine C. Ginocchio, PhD, MT(ASCP)

From North Shore-Long Island Jewish Health System Laboratories, Lake Success, and Department of Microbiology and Genetics, School of Medicine, State University of New York at Stony Brook, Stony Brook, NY

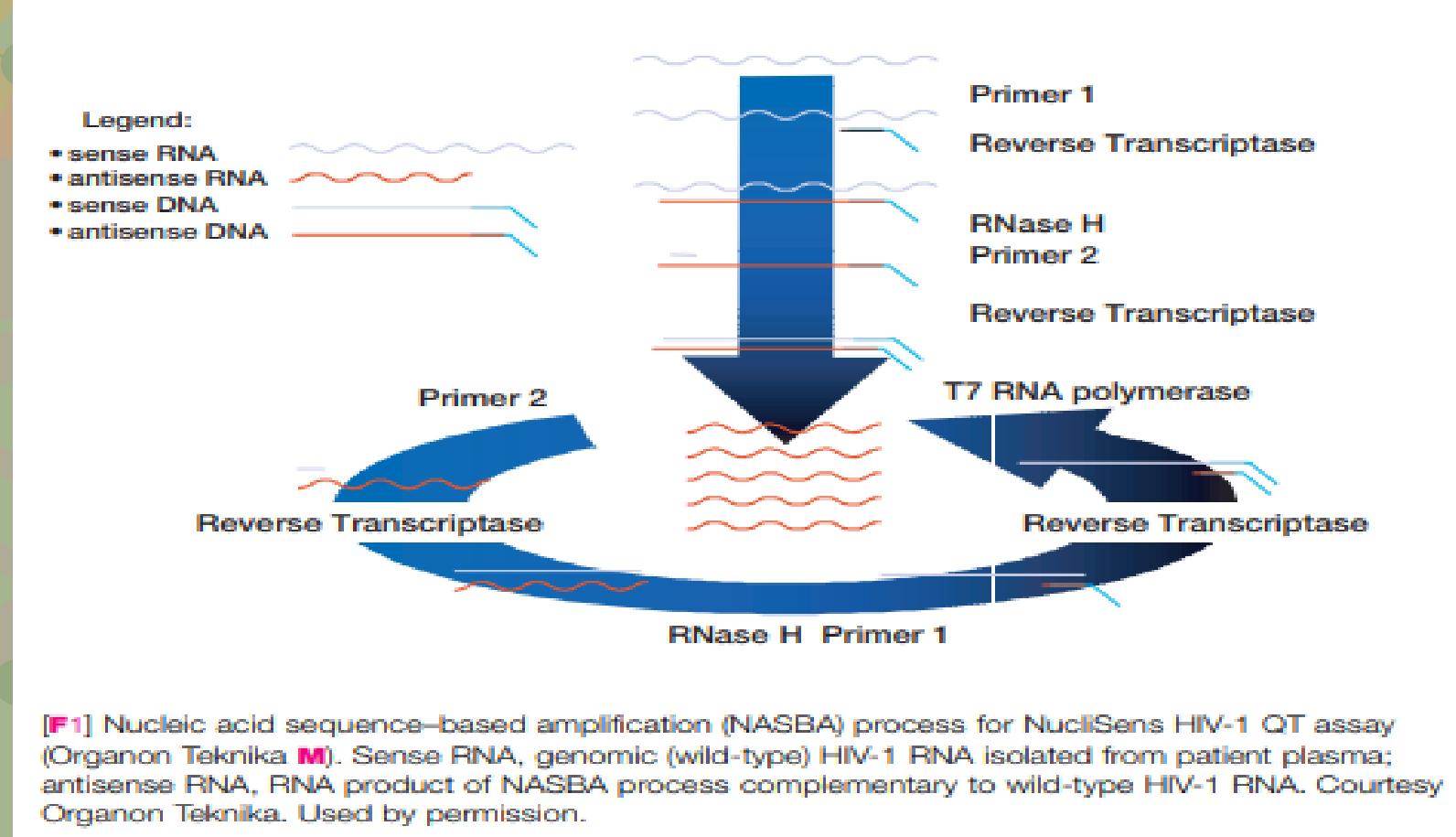
Ex:

Penggunaan PCR dalam mendeteksi Antigen HIV

Tujuan
Metode
Sampel

: untuk mengetahui jumlah virus HIV-1 pada suatu sampel

: RT-PCR
: Plasma

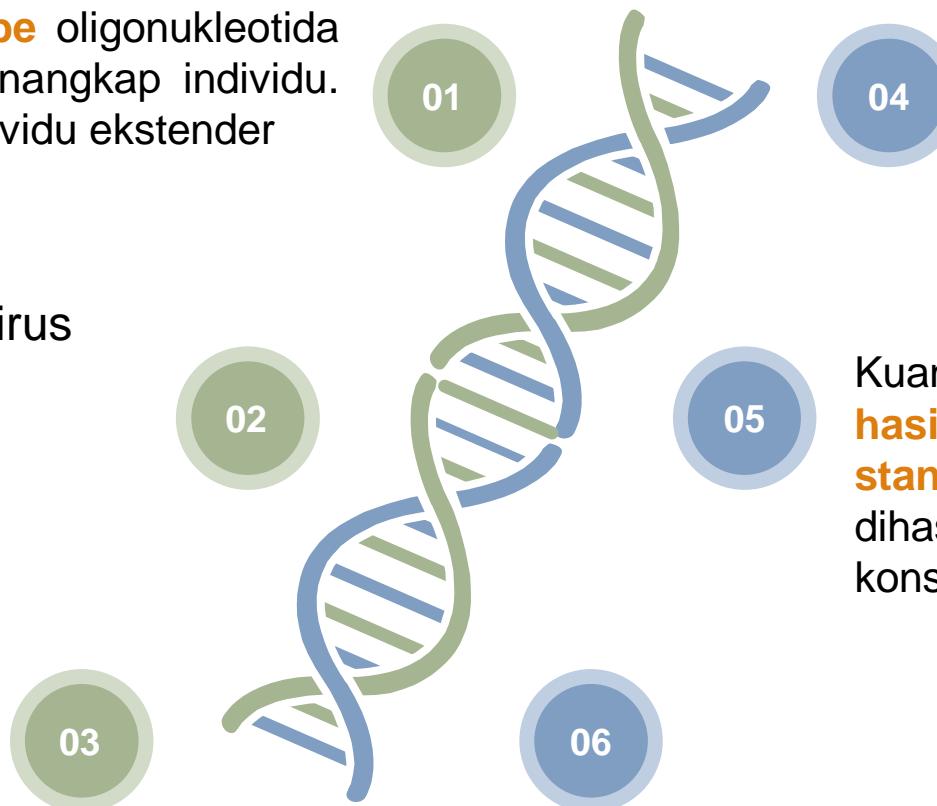


Prinsip

HIV-1 terdapat pada plasma dengan proses sentrifugasi, virion dilisiskan, dan **RNA HIV-1 ditangkap pada well dengan probe** oligonukleotida yang mengandung 17 ekstender penangkap individu. Target probe, terdiri dari 81 target individu ekstender

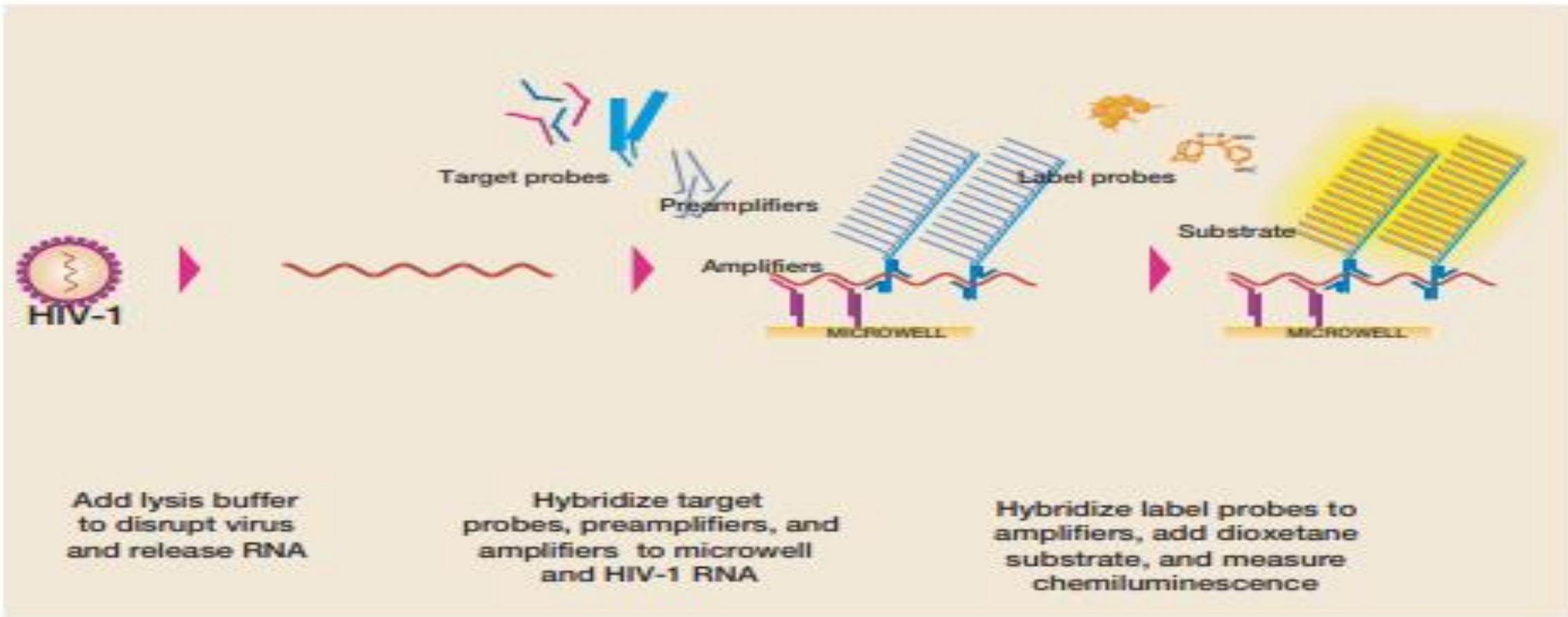
Lalu dihibridisasi ke kedua RNA virus dan probe preamplifier.

Probe preamplifier **mengikat probe amplifier**, membentuk kompleks bDNA yang berlabel dengan alkali fosfataseprobe kemudian dihibridisasi.

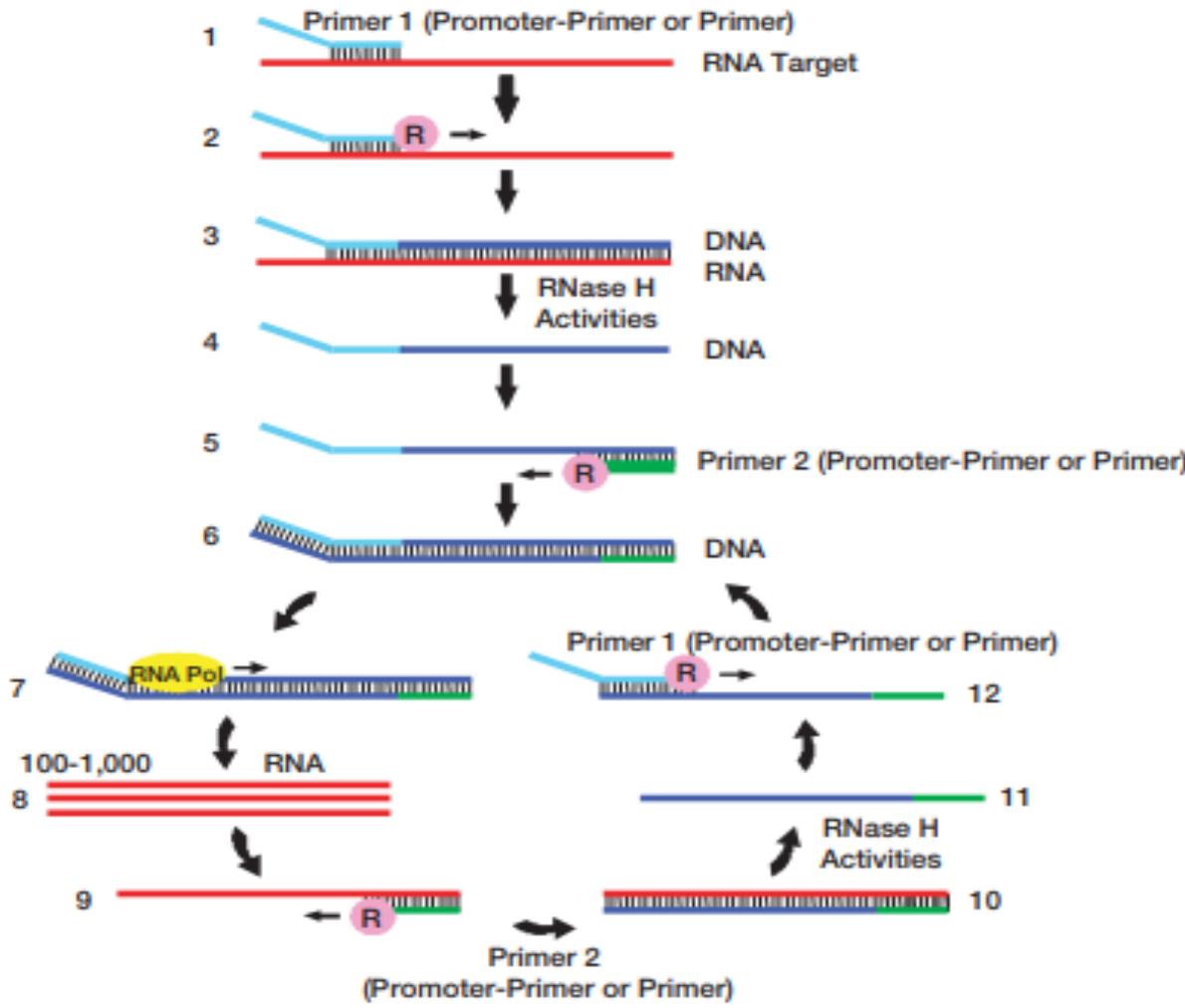


Tambahan dari substrat **chemiluminescent digunakan untuk deteksi keberadaan virus**, dengan hasil emisi cahaya (unit cahaya relatif [RLU]) **sebanding dengan jumlah RNA HIV-1 yang ada dalam spesimen**.

Kuantisasi dicapai dengan **membandingkan hasil spesimen pasien dengan kurva standar** yang ditentukan oleh RLU yang dihasilkan oleh 6 standar kit yang diketahui konsentrasi virus.

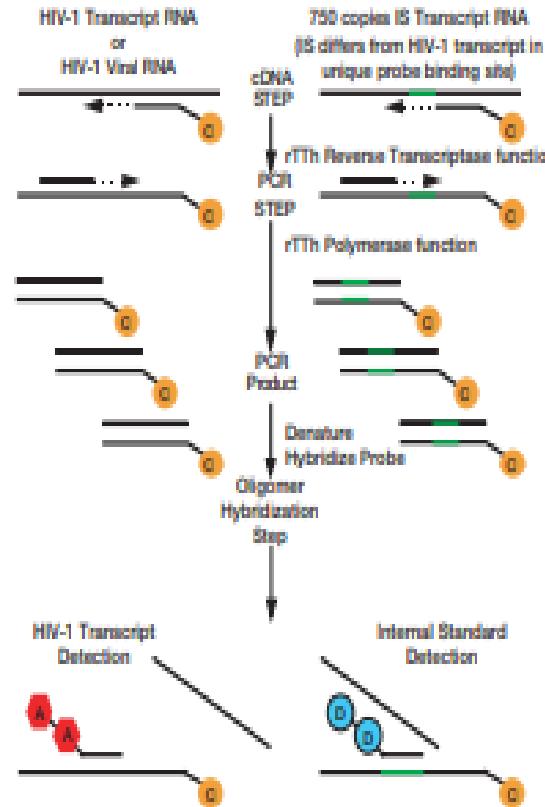


[F2] Branched DNA (bDNA) probe amplification method for HIV-1 RNA 3.0 bDNA assay (Bayer M). HIV-1 is concentrated from plasma by centrifugation, virions are lysed, and the HIV-1 RNA is captured on microtiter wells by specific synthetic oligonucleotide probes containing 17 individual capture extenders. The target probes, composed of 81 individual target extenders, hybridize to both the viral RNA and the preamplifier probes. The preamplifier probe binds to the amplifier probe forming a bDNA complex to which multiple copies of alkaline phosphatase-labeled probe are then hybridized. The addition of chemiluminescent substrate permits detection of the complex. Courtesy Bayer Diagnostics Division. Used by permission.

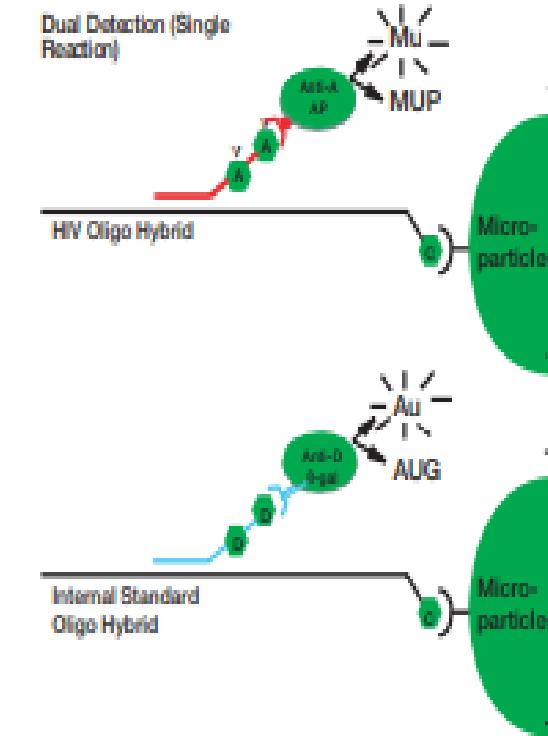


[F4] Transcription-mediated amplification process for Gen-Probe HIV-1 assay (Gen-Probe **M**). 1, primer 1 (containing the T7 RNA polymerase promoter sequence) binds to the target region on the HIV-1 RNA; 2, Reverse transcriptase generates a DNA copy of the HIV-1 RNA; 3, RNA-DNA hybrid; 4, RNase H degrades RNA from the RNA-DNA hybrid; 5, Primer 2 binds the DNA copy; 6, Reverse transcriptase generates a double-stranded DNA copy with the T7 RNA polymerase promoter; and 7-12, T7 RNA polymerase generates multiple copies of HIV-1 RNA that serve as new templates for sequential rounds of primer binding and amplification. Pol, polymerase. Used by permission.

Amplification



Detection

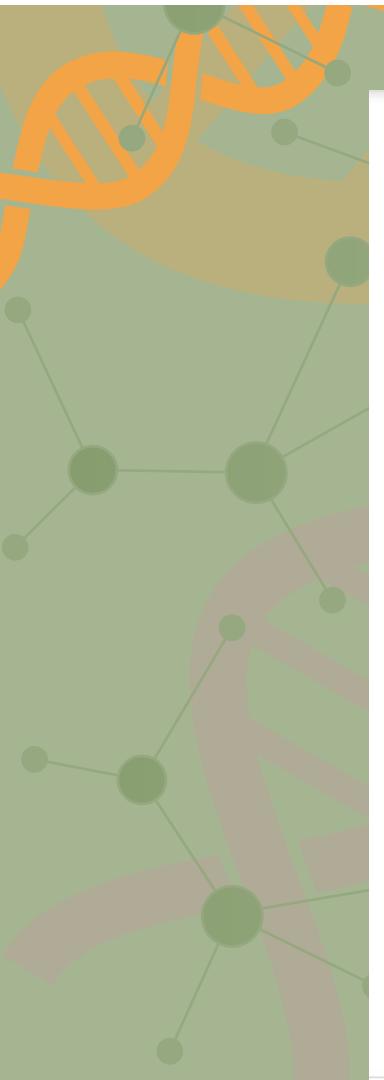


[F5] Amplification using competitive reverse transcription–polymerase chain reaction (RT-PCR) and detection by microparticle enzyme immunoassay (MEIA) for LCx HIV-1 assay (Abbott Laboratories **M**, not available in the United States). A and D, detection synthetic haptens; anti-A AP, alkaline phosphatase conjugate; anti-D beta-gal, beta-galactosidase conjugate; AUG, 7-beta-galactosidase coumarin-4-acetic [2-hydroxyethylamine] fluorescent substrate; C, primer synthetic haptens; cDNA, complementary DNA; IS, internal standard; MUP, 4-methylumbelliferyl phosphate fluorescent substrate; Courtesy Abbott Laboratories. Used by permission.



Diagnosing COVID-19: The Disease and Tools for Detection

Buddhisha Udugama,[◆] Pranav Kadhiresan,[◆] Hannah N. Kozlowski,[◆] Ayden Malekjahani,[◆] Matthew Osborne,[◆] Vanessa Y. C. Li,[◆] Hongmin Chen,[◆] Samira Mubareka, Jonathan B. Gubbay, and Warren C. W. Chan*



Ex:

Penggunaan PCR dalam mendeteksi Antigen COVID-19

Tujuan : untuk mendeteksi antigen COVID-19
Metode : RT-PCR
Sampel : Swab NAsofaring

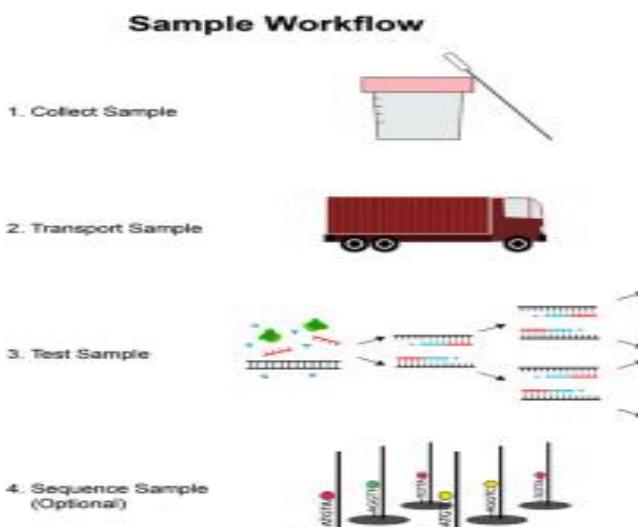
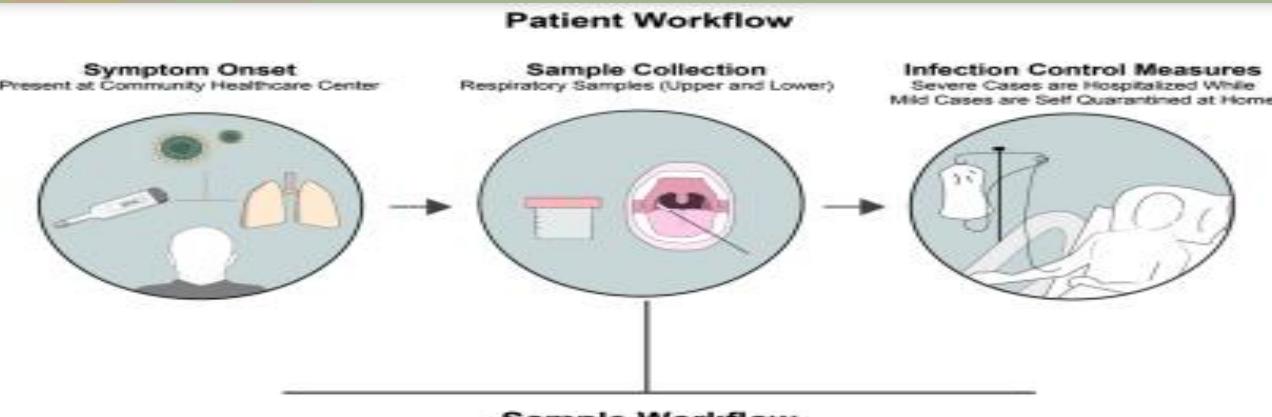
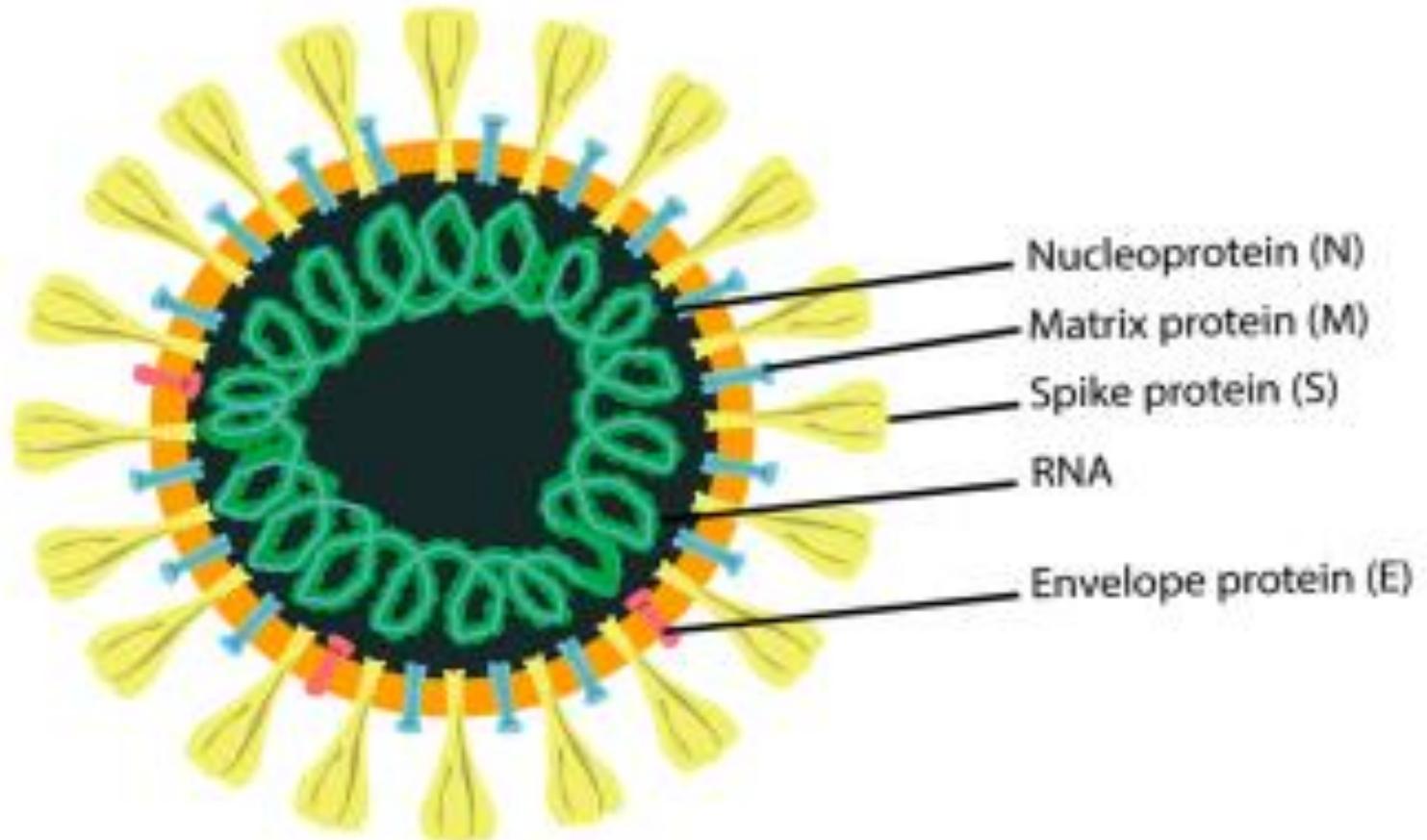


Figure 1. Example of patient and sample workflow during the COVID-19 outbreak. Patients present at a healthcare facility for triage. The collected samples are tested on-site if possible or transported for molecular testing and sequencing. Patients are then managed appropriately.



Morfologi

Figure 2. SARS-CoV-2 morphology. Transmission electron microscope image of SARS-CoV-2 spherical viral particles in a cell.¹³ The virus is colorized in blue (adapted from the US Centers for Disease Control). Representation of the viral structure is illustrated with its structural viral proteins.



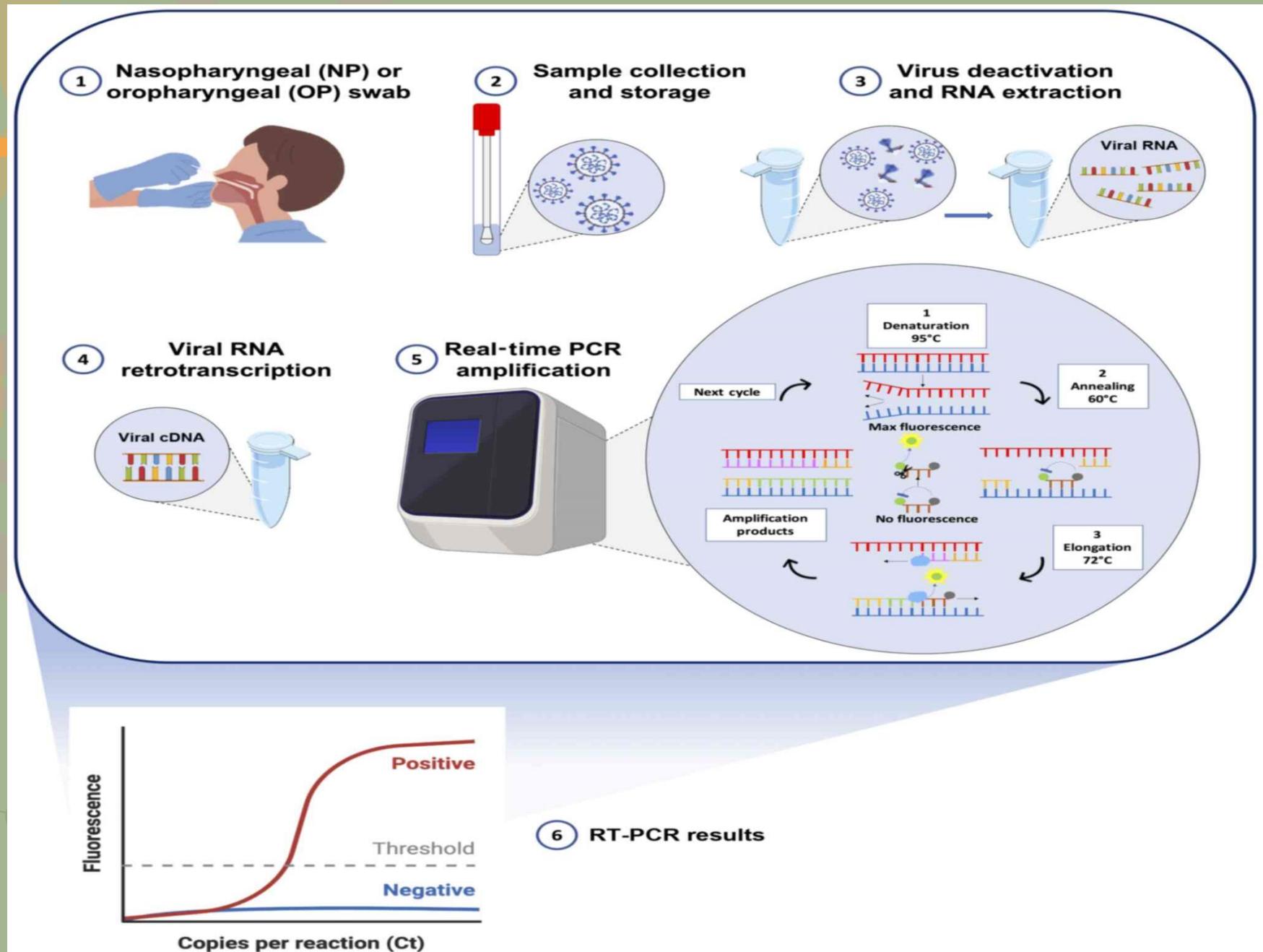
Primer

Table 1. Polymerase Chain Reaction (PCR) Tests/Primers for SARS-CoV-2

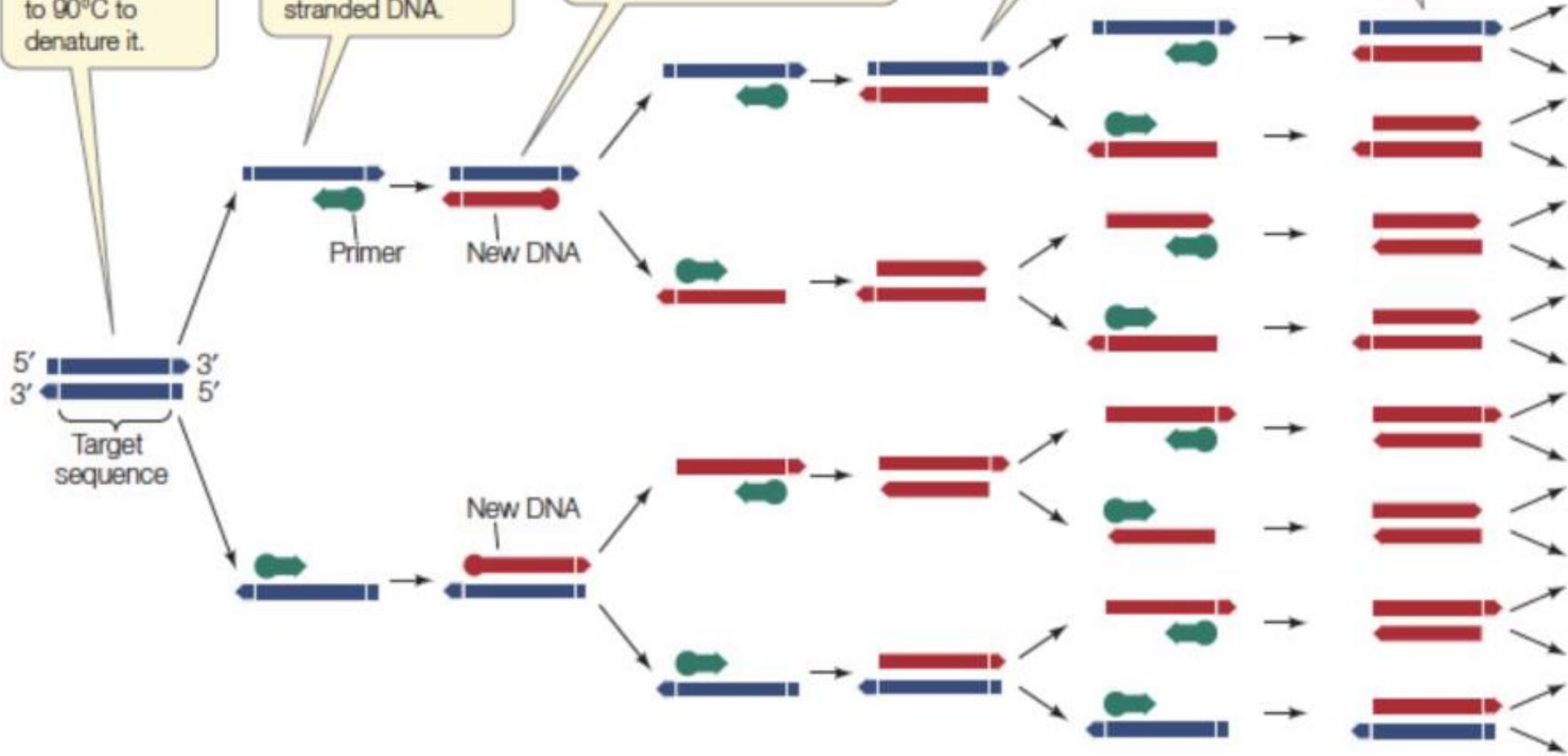
Institution	Gene target	Forward Primer (5'-3')	Reverse Primer (5'-3')	Probe (5'-3')
U.S. CDC ^a	N gene	N1: GACCCCAAAATCAGCGAAAT N2: TTACAAACATTGGCGCGAAA N3: GGGAGGCTTGAATAACACCAAAA RP-F RNase: AGATTGGAACCTGCGAGGG	N1: TCTGGTTACTGCCAGTTGAATCTG N2: GGGGGACATTCGGAAGAAA N3: TGTAGCACGATTGCAGCATTTG RP-RNase: GAGCCGCTGTCTCCACAAAGT	N1: FAM-AACCGGCAATTACGTTTG GTGGACCC-BHQ1 N2: FAM-ACAATTGCCCCCAGC GCTTCAG-BHQ1 N3: FAM-AYCACATTGGCAACCCGC AATCTG-BHQ1 RP-P RNase: FAM-TCTGAACCTGAAGGCTC TGCGCG-BHQ-1
China CDC ^a	ORF1ab and N gene	ORF1ab: CCCTGTGGTTTACACTTAA N: GGGGAACTTCTCCCTGAGAAT	ORF1ab: ACGATTGTGCATCAGCTGA N: CAGACATTTGCTCTCAAGCTG	ORF1ab: FAM- CCGTCTGGTATGTGGAAAG GTTATGG-BHQ1 N: FAM-TTGCTGCTGCTTGA CAGATT-TAMRA
Charité, Germany ^a	RdRp, E, N gene	RdRp: GTGARATGGCATGTGTGGGG E: ACAOGTACGTTAATAGTTAATAGGT	RdRp: CARATGTTAAASACACTATTAGCATA E: ATATTGAGCAGTAAGCACACA	RdRp 1: FAM-CAGGTGGAACCTCATC AGGAGATGC-BHQ RdRp 2: FAM-CAGGTGGWACRTCATC MGGTGTGATGC-BHQ E: FAM-ACACTAGGCATCCTTA CTGGCTTGG-BHQ
Hong Kong University ^a	ORF1b-nsp14, N gene	ORF1b-nsp14: TOGGGYTTAACGGTAACCT N: TAATCAGACAAGGAACGTGATTA	ORF1b-nsp14: AACCGCTTAACAAAAGCCTC N: CGAAGGTGTGACTTTCATG	ORF1b-nsp14: FAM-TAGTTGTGATGCWATC ATGACTAG-TAMRA N: FAM-GCAAATTGTGCA ATTTGCGG-TAMRA
National Institute of Infectious Diseases, Japan ^a	N gene	N: AAATTTGGGACCAGGAAC	N: TGGCAAGCTGTAGGTCAAC	N: FAM-ATGTCGGCGAT TGGCATGG-BHQ
National Institute of Health, Thailand ^a	N gene	N: CGTTTGGTGGACCGCTCAGAT	N: CGCCACTGGGTTCTCATT	N: FAM- CAACTGGCACTAACCAABHQ1



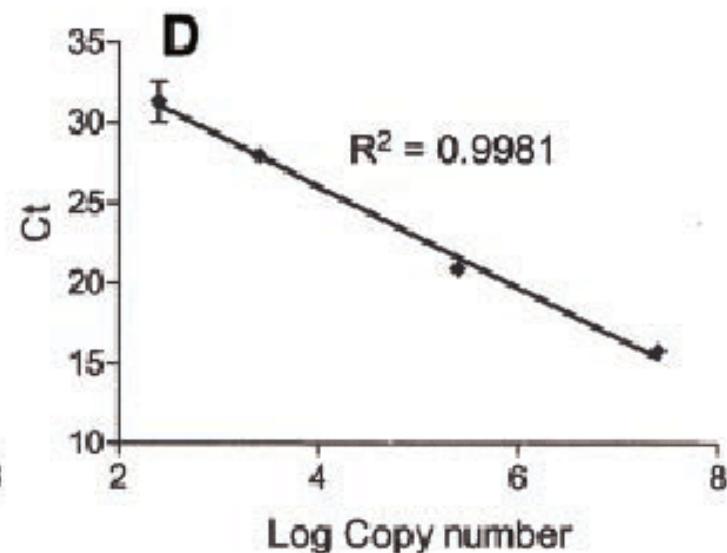
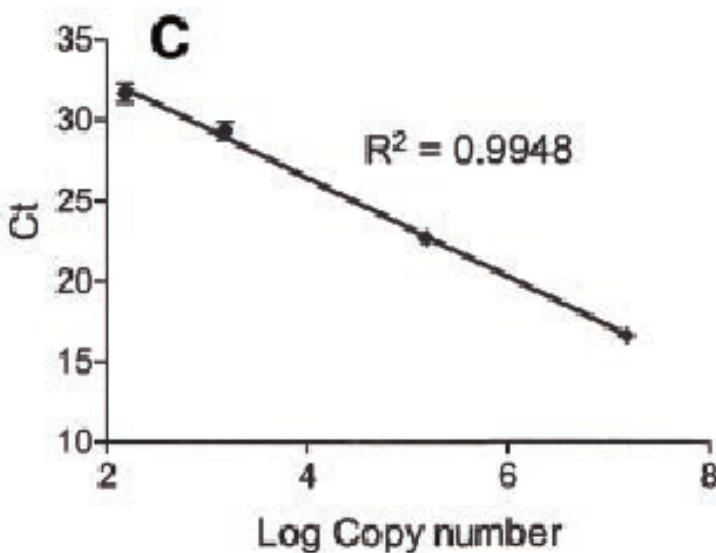
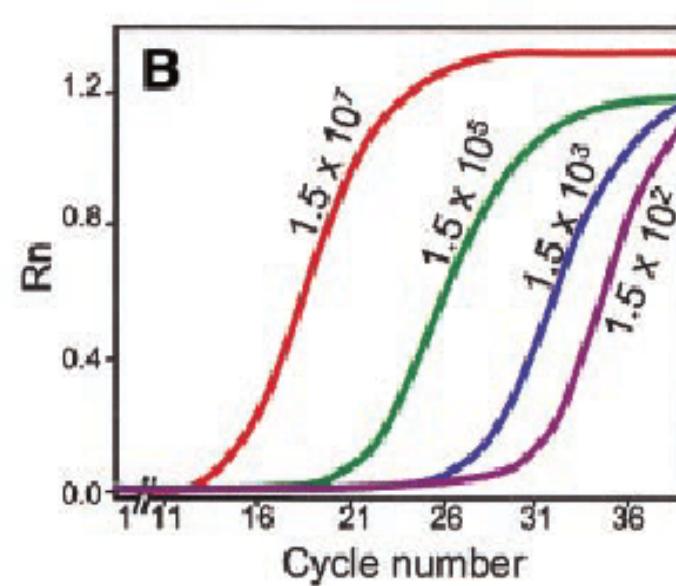
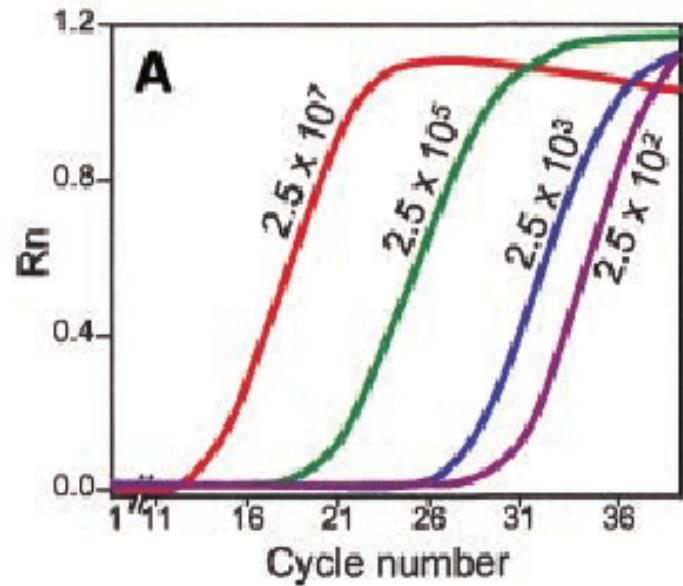
Alur



- 1 A DNA molecule with a target sequence to be copied is heated to 90°C to denature it.
- 2 When the mixture cools, artificially synthesized primers bond to the single-stranded DNA.
- 3 Heat-resistant DNA polymerase uses dNTPs to synthesize two new strands of DNA.
- 4 The process is repeated, doubling the amount of DNA.
- 5 By repeating the process, many copies of the original DNA can be produced in a short time.



Result



Tantangan sebagai teknisi laboratorium

- ❖ Harus menguasai teknologi terbaru
- ❖ Mampu mengerjakan ekstraksi DNA dan RNA
- ❖ Mampu dan memahami prindip molekuler
- ❖ Mampu mengerjakan pemeriksaan PCR sampel klinis
- ❖ Mampu memvalidasi hasil pemeriksaan sebelum diberikan ke dokter patologi klinik



Thank You