

Growing Needs for Practical Molecular Diagnostics: Indonesia's Preparedness for Current Trend


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Essential Practical Molecular Diagnostics Seminar
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Outline

- ▶ History
- ▶ Molecular Diagnostics Now and Future
- ▶ Readiness of the Molecular Diagnostics Laboratory in Indonesia

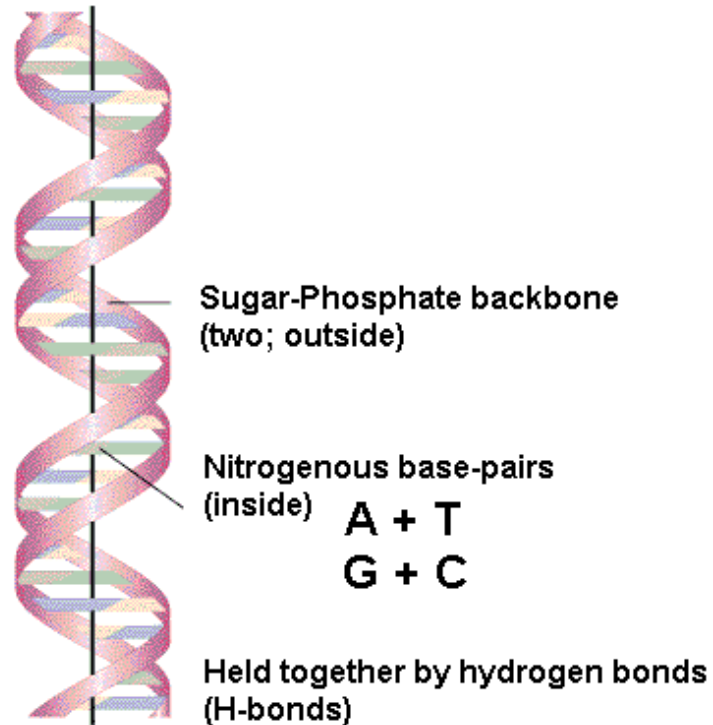
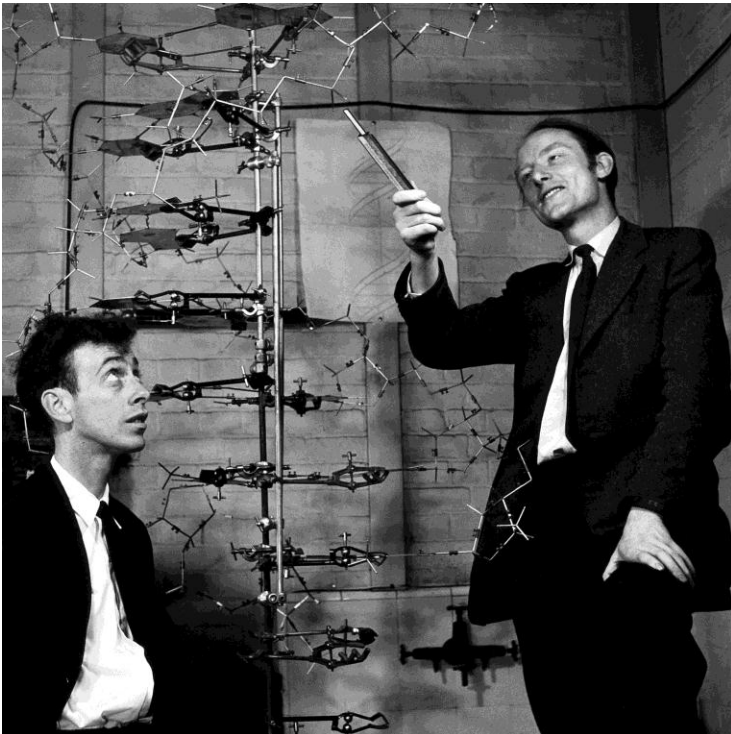
Discovery of Molecular Biology



1865	Law of Hereditary - Gregor Mendel
1953	Structure of DNA - Watson & Crick
1970	Recombinant DNA Technology
1977	DNA Sequencing
1985	Amplification of DNA (PCR) - Kary B Mullis
2001	Human Genome Project
2005-11	Sequencing Technology & Genome Sequencing
2010-	Next Generation Sequencing
2010-	“Omics” Technology

DNA Structure

- ▶ **J.D. Watson and F.H.C. Crick (1953) : A structure for deoxyribose nucleic acid. *Nature* 171:737**
- ▶ **One of the most important biological discovery in the 20th century**

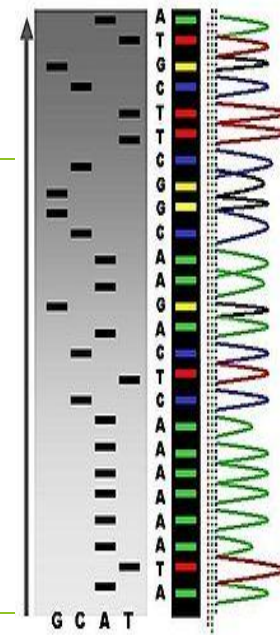


Recombinant DNA Technology

- ▶ **Recombinant DNA** - DNA that has been artificially manipulated to combine genes from **two different** sources.
- ▶ Genes transferred - **among unrelated species** via laboratory manipulation.
- ▶ **Genetic engineering** - human manipulation of an organism's genetic material in a way **that does not occur under natural conditions**

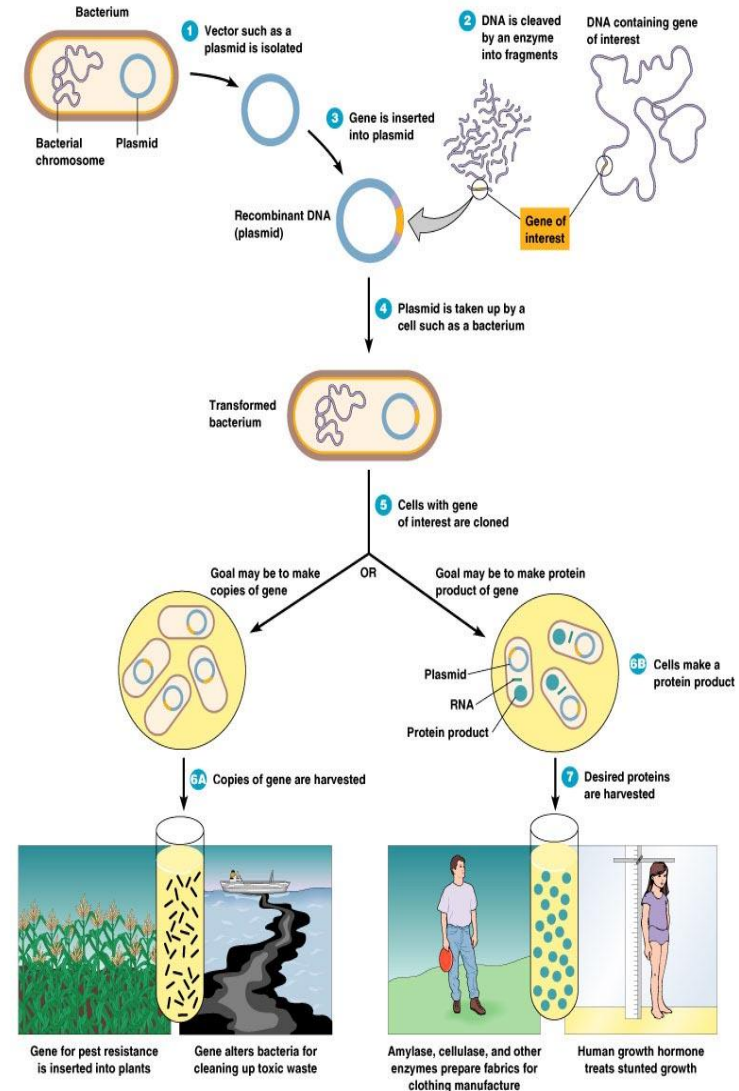
DNA Sequencing

- ▶ **DNA sequencing** - is the process of determining the precise order of nucleotides within a DNA molecule
(A, G, C and T in a molecule of DNA)



An Overview of Recombinant DNA Technologies

1. **Gene of interest (DNA)** is isolated
(DNA fragment)
2. A **desired gene** is inserted into a DNA molecule - **vector**
(plasmid, bacteriophage or a viral genome)
3. **The vector** inserts the DNA into a **new cell**, which is grown to form a **clone**.
(bacteria, yeast, plant or animal cell)
4. Large quantities of the **gene product** can be harvested from **the clone**.



Nucleic Acid Amplification Test (PCR)

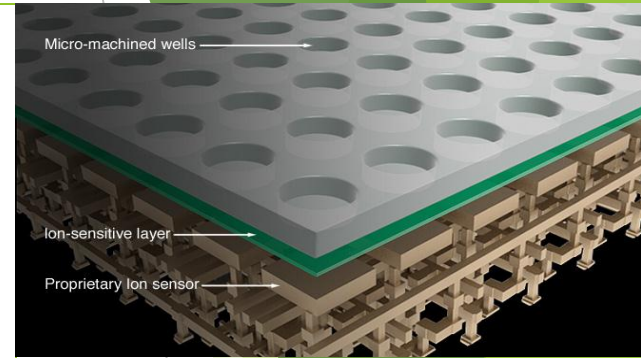
- PCR has greatly facilitated and revolutionized molecular diagnostics.
- Its most powerful feature - large amount of copies of the target sequence generated by its exponential amplification, which allows the identification of a known mutation within a single day.
- PCR markedly decreased need for radioactivity, allowed molecular diagnostics to enter the clinical laboratory.
- PCR either is used for the generation of DNA fragments to be analyzed, or is part of the detection methods



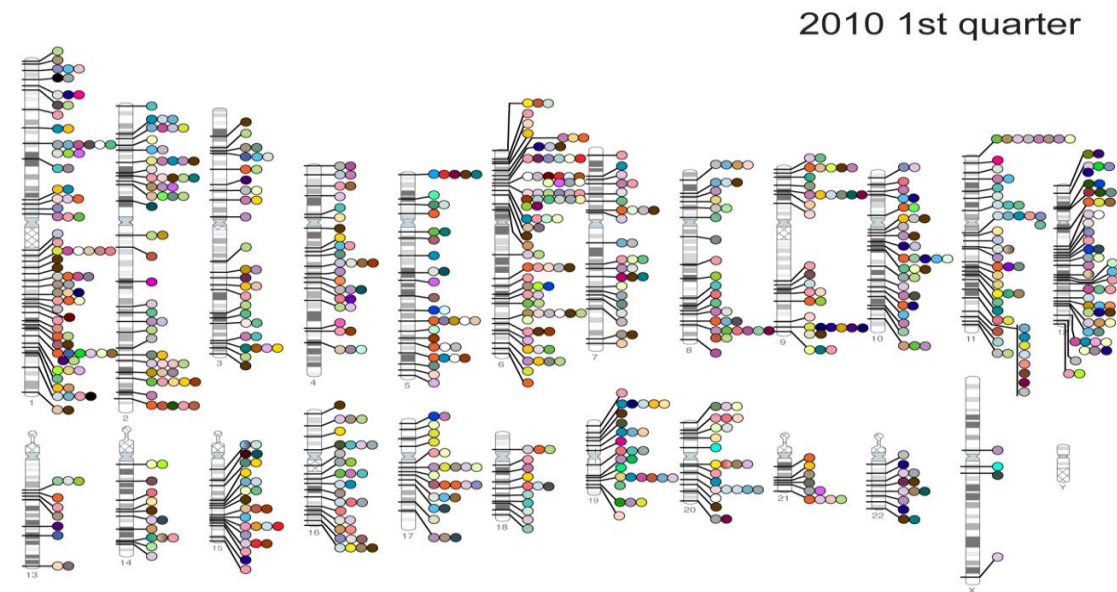
Kary B. Mullis, inventor of the Polymerase Chain Reaction

Next Generation Sequencing

- ▶ Takes advantage of miniaturization to engage in massively parallel analysis
 - ▶ Essentially carrying out millions of sequencing reactions simultaneously in each of 10 million tiny wells
- ▶ Sophisticated computer analysis of huge amounts of information allows “assembly” of a given sequence



- ▶ An avalanche of Genome Wide Association Studies (GWAS) in common diseases
- ▶ WGS is now a practical reality
- ▶ Will shed light on the genetic underpinnings of every disease imaginable



“Omics” Technology

- ▶ 'Omics' **technology** are primarily aimed at the universal detection of
 - ▶ genes (genomics)
 - ▶ mRNA (transcriptomics)
 - ▶ proteins (proteomics)
 - ▶ metabolites (metabolomics)in a specific biological sample
- ▶ Omics **technology** have a broad range of applications.

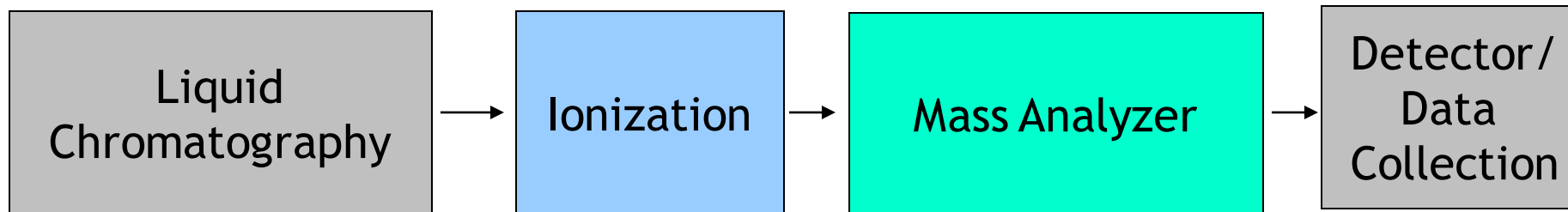
Configuration of LC/MS/MS

► Why Liquid Chromatography?

- Analysis of labile analytes
- Analysis of more polar compounds without derivatization.
- Analysis of significantly higher masses
- Reduction of lengthy clean-up

► Why MS/MS?

- Additional structural elucidation
- Further reduction of clean-up (?)
- Specificity
- Useful MS modes



- Very important!
- Many columns
- Many solvent systems

- ESI
- APCI
- APPI

- Triple Quadrupoles
- Ion-Traps
- Hybrids

Application of Molecular Technologies

- ▶ **Scientific applications**
 - ▶ Identify **mutations** in DNA; **Alter the phenotype** of an organism
 - ▶ **Bioinformatics** is the use of computer applications to study genetic data
- ▶ **Shotgun sequencing** - Recombinant DNA techniques were used to map the human genome through the **Human Genome Project**
- ▶ **Diagnose genetic disease**
 - ▶ **RFLP analysis** (Restriction fragment length polymorphism)
 - ▶ **Southern blotting** is used for detection of a specific DNA sequence in DNA sample
 - ▶ **PCR analysis** with specific primers

Application of Molecular Technologies

▶ Genetic fingerprinting identification

- ▶ **Forensic microbiology** - use **DNA fingerprinting** to identify the source of bacterial or viral pathogens.

▶ Therapeutic Applications

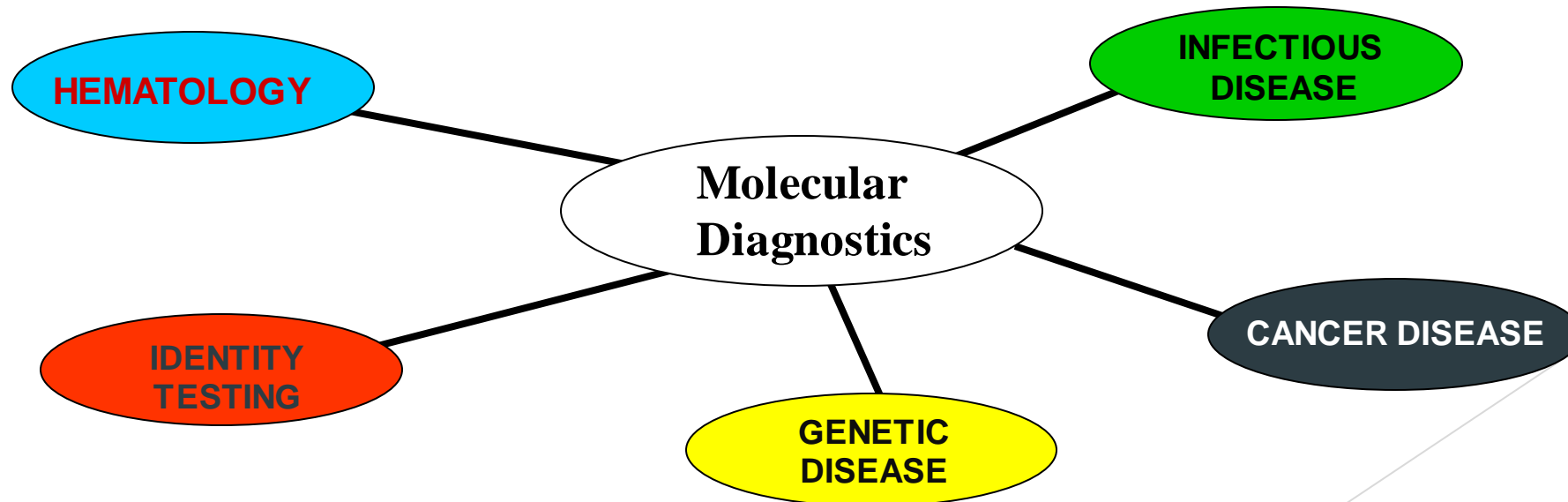
- ▶ Produce human proteins – hormones and enzymes
- ▶ **Vaccines**
 - ▶ **Cells** and **viruses** can be modified to produce a pathogen's surface protein
 - ▶ Nonpathogenic viruses carrying genes for pathogen's antigens as **DNA vaccines**
 - ▶ DNA vaccines consist of circular rDNA
- ▶ **Gene therapy** can be used to cure genetic diseases by **replacing the defective or missing gene**.
- ▶ **Gene silencing** – RNA interference - siRNA or microRNA

Molecular Diagnostics

- ▶ **The use of molecular biology techniques to expand scientific knowledge of the natural history of diseases, identify people who are at risk for acquiring specific diseases, and diagnose human diseases at the nucleic acid level**
- ▶ **Goal:**
 - ▶ **identification of novel markers of human diseases**
 - ▶ **develop and apply useful molecular assays to monitor disease, determine appropriate treatment strategies, and predict disease outcomes**

Practical Application in Human Diseases

- ▶ Diagnostic-Identity of a disease
- ▶ Prognostic-Outcome of a disease
- ▶ Predictive-Possibility of a disease
- ▶ Therapeutic-Response of a disease to treatment



Characteristic of Ideal Diagnostics Test

- ▶ **AFFORDABLE** by those at risk of infection
- ▶ **SENSITIVE** (few false-negative)
- ▶ **SPECIFIC** (few false-positives)
- ▶ **USER-FRIENDLY** (simple to perform and requiring minimal training)
- ▶ **RAPID** (to enable treatment at first visit) and **ROBUST** (does not require refrigerated storage)
- ▶ **EQUIPMENT-FREE**
- ▶ **DELIVERED** to those who need it

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Practical Molecular Diagnostics

Now

- ▶ PCR (Polymerase Chain Reaction)

- ▶ Infectious diseases: TB, HIV, Malaria, Thypoid, STD, Dengue, Hepatitis, Influenza, etc
- ▶ Cancer marker
- ▶ Genetic disorder
- ▶ Paternity
- ▶
- ▶ Psychiatry disorder

Molecular Diagnostics

Future

- ▶ **Genome: Deep catalog of Human Genetic Variation**
 - data quality assessment
 - project design (# samples depth of read coverage)
 - read mapping
 - SNP calling
 - structural variation discovery
- ▶ **Personalised analysis base on genome analysis**
 - ▶ Prediction - prevention
 - ▶ Detection and monitoring treatment
 - ▶ Prognostic test

PRENATAL SCREENING

- ▶ In vitro Fertilization
 - ▶ Pre-implantation Genetics Screens
- ▶ Pregnant Women
 - ▶ 10 weeks (first trimester pregnancy)
 - ▶ Specimen: Whole blood

DNA Amplification	Library Preparation	Sequencing	Data Analysis	Generate Report
Extract and amplify DNA using the SurePlex DNA Amplification Kit	Prepare libraries for sequencing on the MiSeq System using the VeriSeq PGS Kit - MiSeq	Start MiSeq instrument Add library to the ready-to-use flow cell	MiSeq System demultiplexes samples and aligns reads to the genome	Analyze data using BlueFuse Multi analysis software Generate report



Indonesia's readiness

(1)

- ▶ Man power
 - ▶ Improve knowledge, base on availability molecular diagnostics test/kit
 - ▶ On the job training in laboratory - hands on
 - ▶ Continuing education and practical training
 - ▶ Regular twice / year
 - ▶ Peer group discussion

Indonesia's readiness

(3)

- ▶ Facility
 - ▶ Molecular room; separate 3 rooms
 - ▶ DNA/RNA extraction room
 - ▶ Amplification room
 - ▶ Detection room
 - ▶ Modify/simplify →

Indonesia's readiness

(2)

► Method

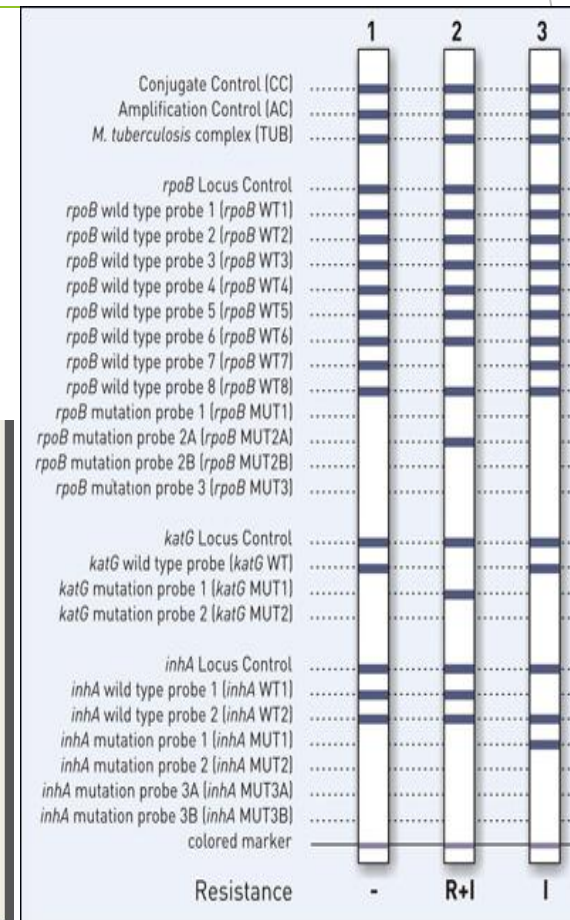
► PCR konvensional / real time

► Line Probe Assay

► POCT

► Xpert

► LAMP



Indonesia's readiness

(4)

Primary HC

- ▶ PCR & detection system
 - ▶ POCT
 - ▶ Simple
 - ▶ Small volume
 - ▶ Cheap
 - ▶ Infectious disease marker
- ▶ Xpert MTB/RIF

Secondary HC

- ▶ Real Time PCR
 - ▶ Lab Molecular
 - ▶ Regular size
 - ▶ Infectious disease marker & DST
 - ▶ Cancer marker
- ▶ LPA
- ▶ Hybridization method

Tertiary HC

- ▶ PCR, Sequencing n more
 - ▶ Lab Molecular
 - ▶ High tech
 - ▶ Huge sample as a referral lab
 - ▶ Inf disease, cancer, genetic marker
- ▶ Research

Indonesia's readiness

(5)

- ▶ Translation research
- ▶ Developing new diagnostics marker

Thank you

The background features abstract, overlapping geometric shapes in various shades of green, ranging from light lime to dark forest green. These shapes are primarily located on the right side of the frame, creating a modern, layered effect against the white background.