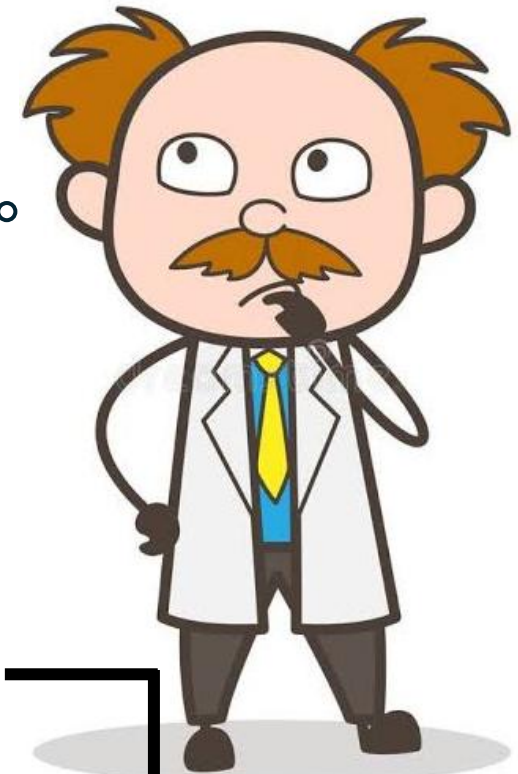


Pertanyaan konservasi



Pengambilan Sampel



Analisis Laboratorium

Isolasi DNA

Uji Kuantitas
dan Kualitas
DNA

Amplifikasi (PCR)¹

*Library Preparation*²



Sekuensing

Sanger
sequencing¹

Next
Generation
Sequencing²



Bioinformatika dan
Analisis Data



Interpretasi Data

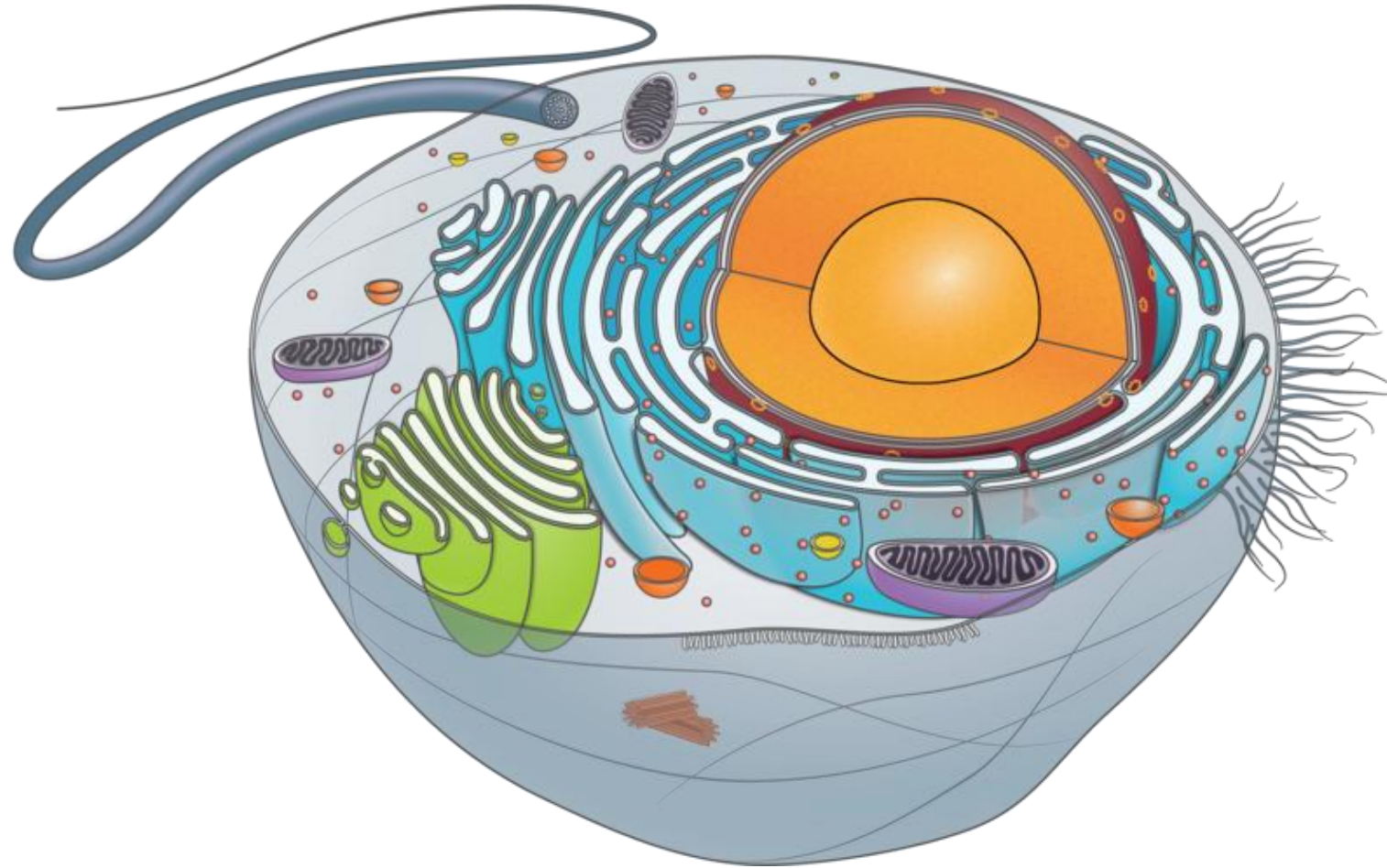


Pengenalan Isolasi DNA

Alvin Wiwiet Susanto

Praktikum Biokonservasi
Rabu, 16 September 2026

Di mana DNA dapat ditemukan?



Marka genetik: DNA nukleus

— GATAGATAGATAGATAGATAGATAGATA —

(GATA)₈

— GATAGATAGATAGATAGATAGATAGATAGATA —

(GATA)₁₀

Mikrosatelit

Sekuens 2-10 basa yang berulang dan dapat bervariasi dalam jumlah pengulangan.

Indv 1

Indv 2

Marka genetik: DNA nukleus

Diagram illustrating DNA sequences for two individuals (Indv 1 and Indv 2) with highlighted Single Nucleotide Polymorphisms (SNPs). The sequences are shown within a box, with the top sequence for Indv 1 and the bottom sequence for Indv 2. The SNPs are highlighted in yellow boxes.

Indv 1: CGATATTCC**T**ATCGAATGTCATG**C**CTGATGT**A**TTCT**A**TCA

Indv 2: CGATATTCC**C**ATCGAATGTCATG**A**CTGATGT**G**TTCT**G**TCA

**Single Nucleotide
Polymorphisms (SNPs)**
Variasi satu pasang basa

Indv 1

Indv 2

Apa itu isolasi DNA?

- Proses yang dilakukan untuk mendapatkan DNA dari sampel yang didapatkan.
- Ada empat tahapan, yaitu ***lysis***, ***binding***, ***washing***, dan ***elution***.

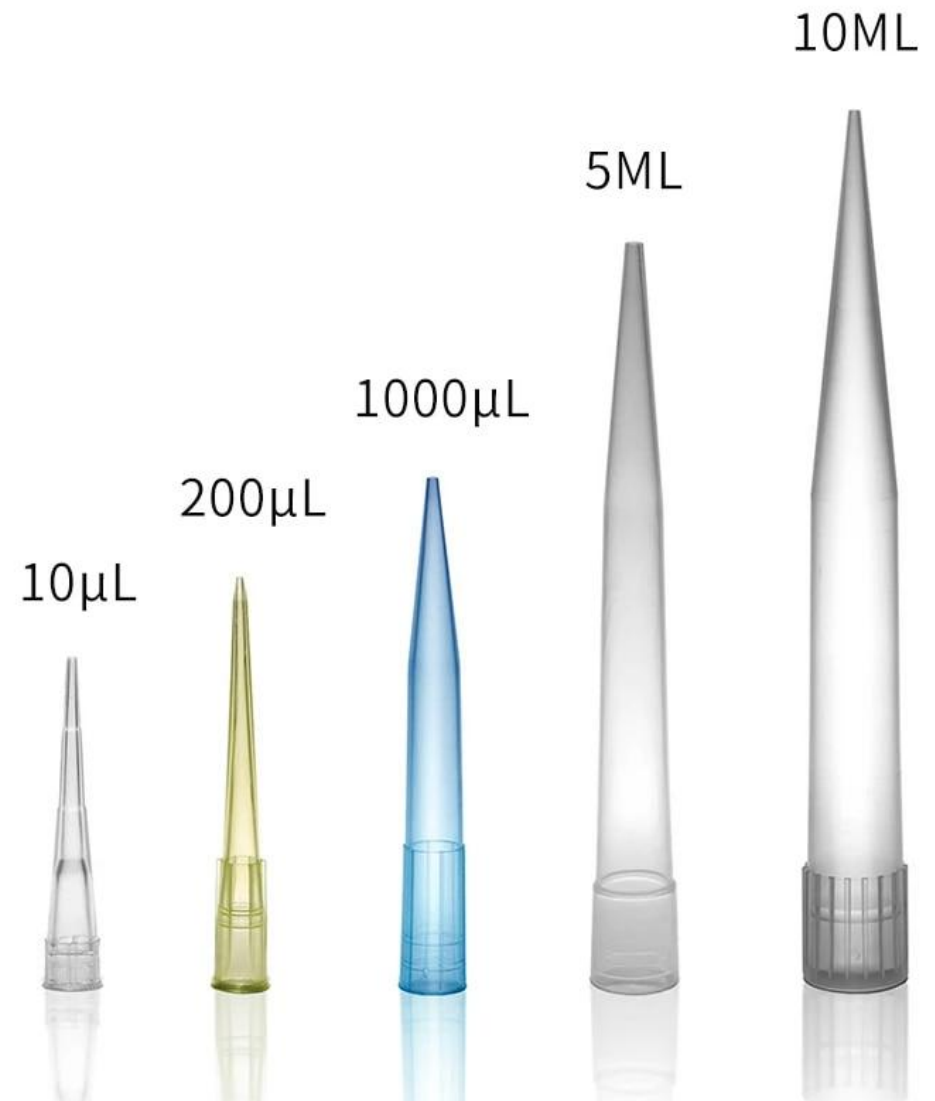
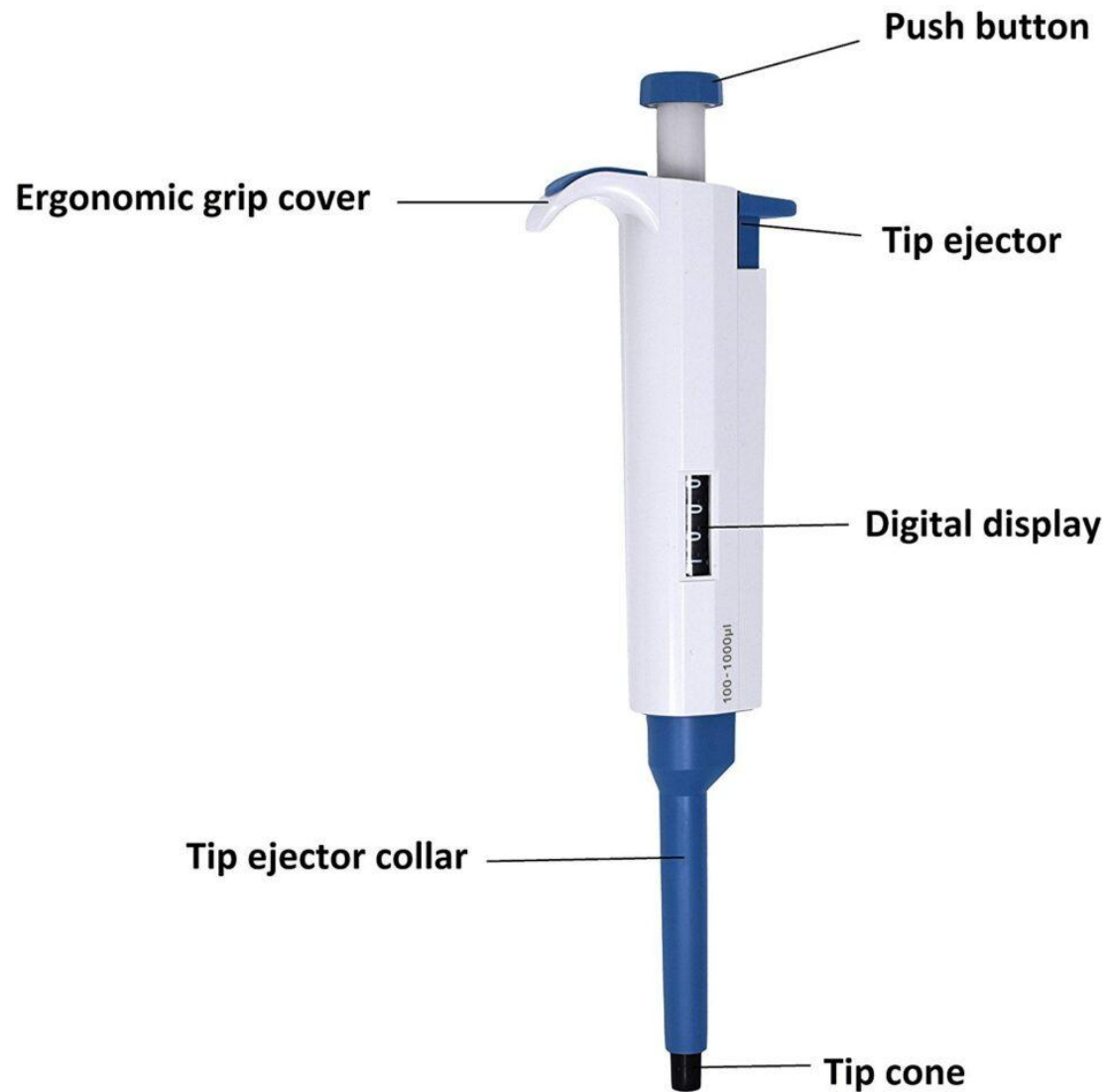


Bahan: Reagen Isolasi DNA



Spin column* hanya digunakan untuk isolasi DNA dengan **metode kolom

Alat: Mikropipet



Alat



Alat



Tata Letak Alat dan Bahan



Prosedur Isolasi DNA

DNeasy® Blood & Tissue Handbook

DNeasy Blood & Tissue Kit
DNeasy 96 Blood & Tissue Kit
For purification of total DNA from
animal blood
animal tissue
rodent tails
ear punches
cultured cells
fixed tissue
bacteria
insects

Sample to Insight

July 2020



Received: 7 January 2021 | Revised: 9 May 2021 | Accepted: 14 May 2021
DOI: 10.1111/1755-0998.13433

RESOURCE ARTICLE

MOLECULAR ECOLOGY
RESOURCES WILEY

Successful application of ancient DNA extraction and library construction protocols to museum wet collection specimens

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Michael V. Westbury⁸ | Célio F. B. Haddad³ | Axel Barlow⁵ | Michael Hofreiter⁵

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Axel Barlow, School of Science and Technology, Nottingham Trent University, Nottingham, UK

Abstract

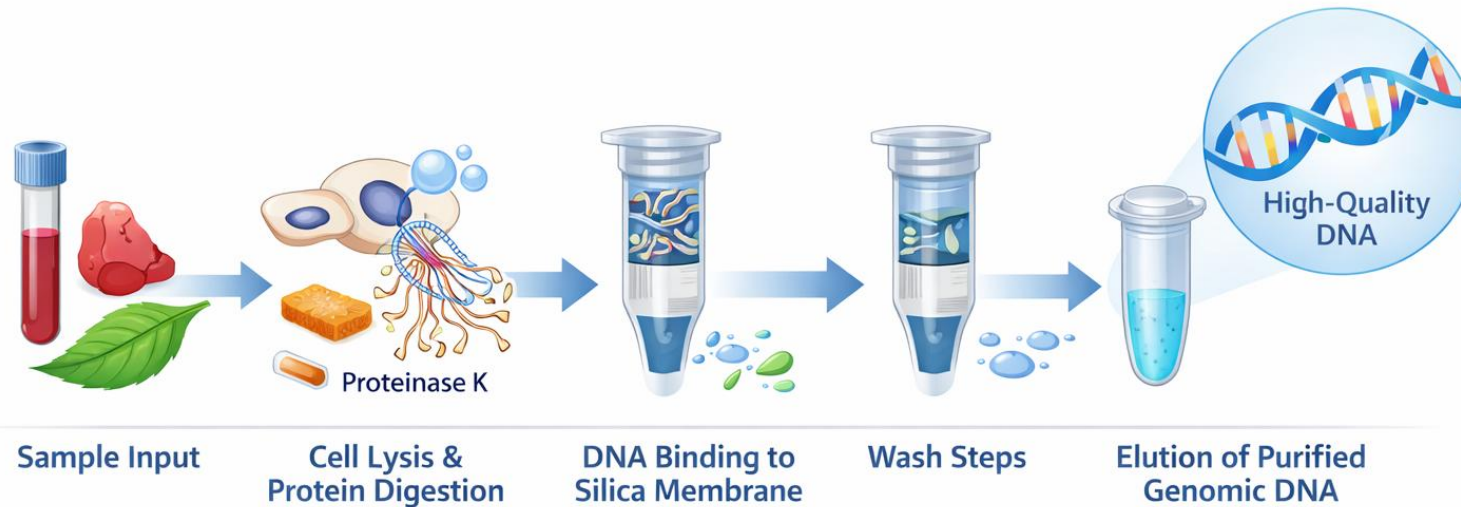
Millions of scientific specimens are housed in museum collections, a large part of which are fluid preserved. The use of formaldehyde as fixative and subsequent storage in ethanol is especially common in ichthyology and herpetology. This type of preservation damages DNA and reduces the chance of successful retrieval of genetic data. We applied ancient DNA extraction and single stranded library construction protocols to a variety of vertebrate samples obtained from wet collections and of different ages. Our results show that almost all samples tested yielded endogenous DNA. Archival DNA extraction was successful across different tissue types as well as using small amounts of tissue. Conversion of archival DNA fragments into single-stranded libraries resulted in usable data even for samples with initially undetectable DNA amounts. Subsequent target capture approaches for mitochondrial DNA using homemade baits on a subset of 30 samples resulted in almost complete mitochondrial genome sequences in several instances. Thus, application of ancient DNA methodology makes wet collection specimens, including type material as well as rare, old or extinct species, accessible for genetic and genomic analyses. Our results, accompanied by detailed step-by-step protocols, are a large step forward to open the DNA archive of museum wet collections for scientific studies.

Straube and Lyra - Joint first authors. Barlow and Hofreiter - joint senior authors.

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Tahapan Isolasi DNA

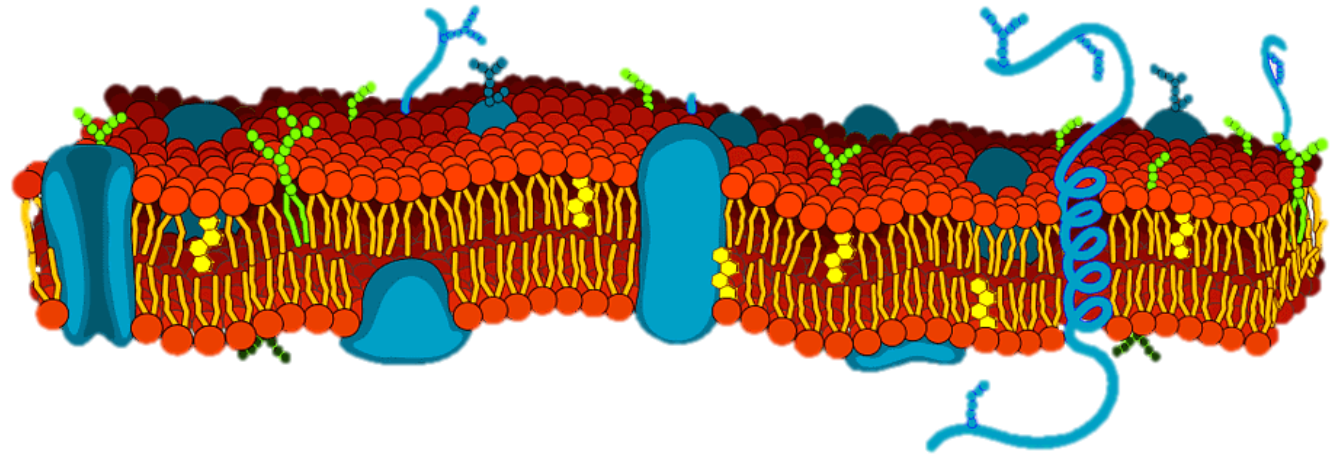
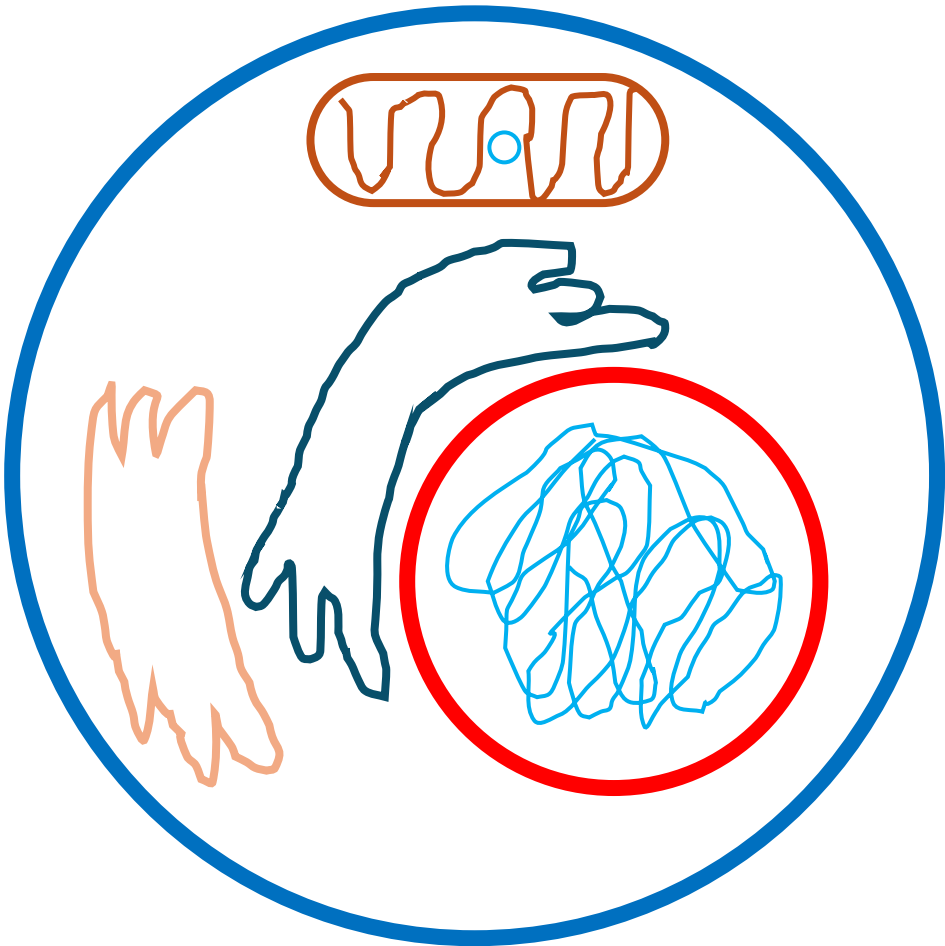
Genomic DNA Extraction Kit Workflow



High-quality genomic DNA for PCR, sequencing, and cloning.

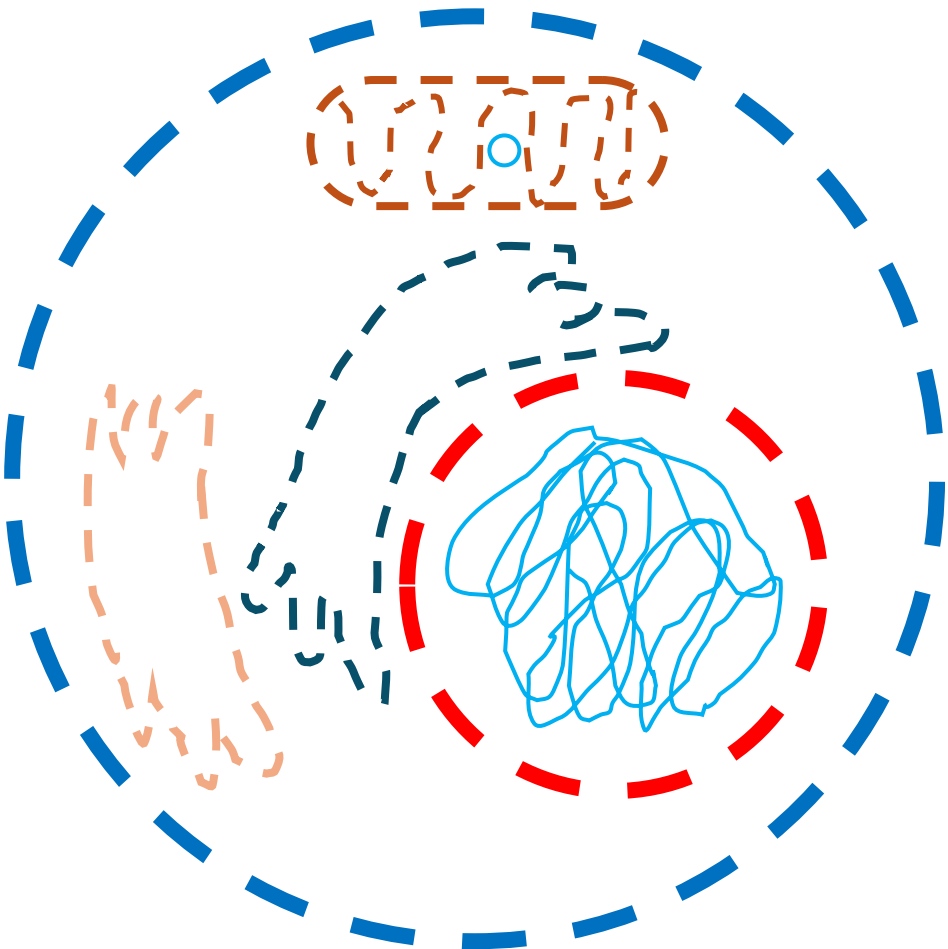
Tahapan 1: *Lysis*

Proses yang bertujuan untuk menghancurkan struktur sel.



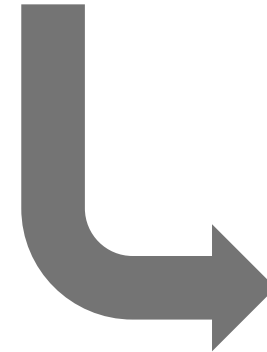
Tahapan 1: *Lysis*

Proses yang bertujuan untuk menghancurkan struktur sel.



Bahan

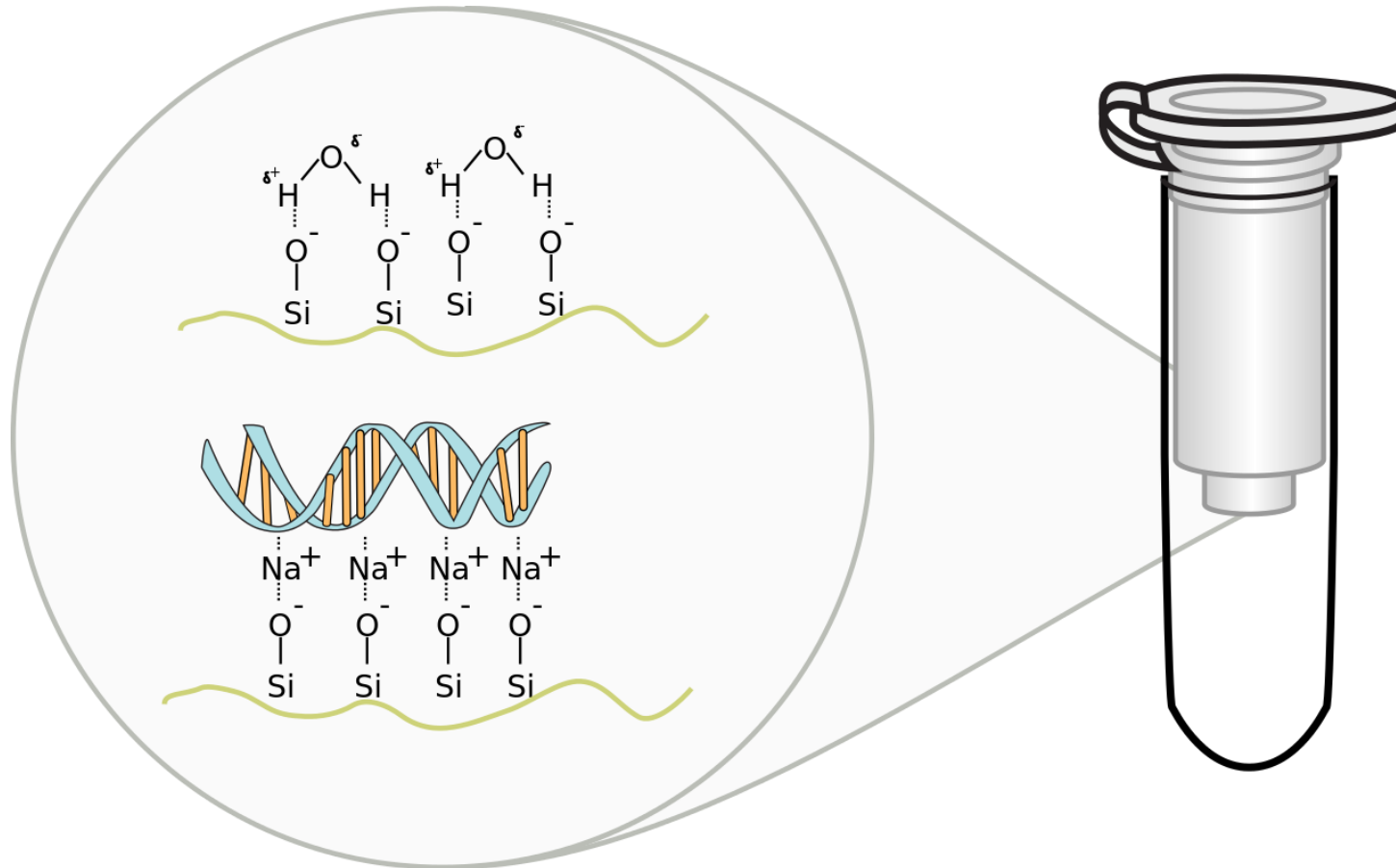
- Enzim Proteinase K
- *Lysis Buffer*
- Sampel



Inkubasi

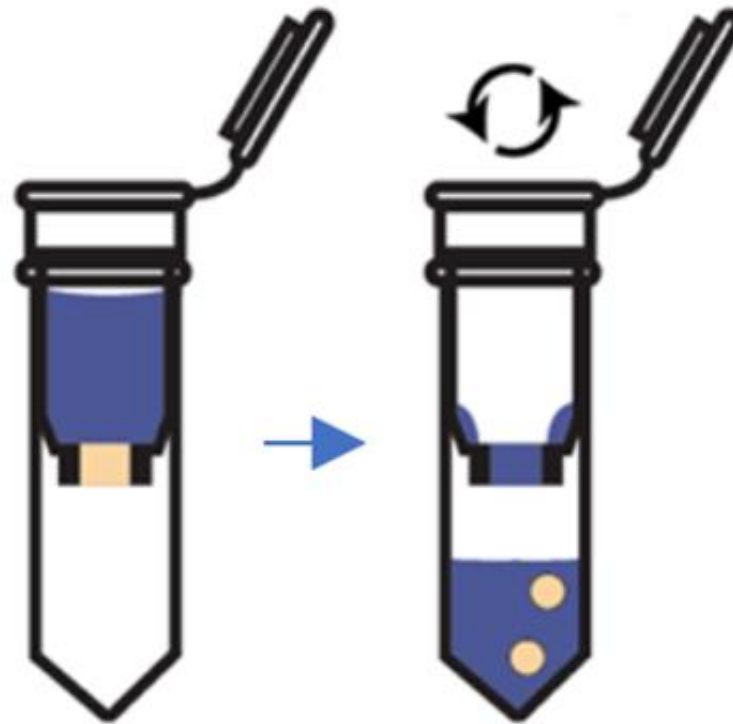
Tahapan 2: *Binding*

Proses yang bertujuan untuk mengikat molekul DNA, memisahkannya dari senyawa yang lain.



Tahapan 3: *Washing*

Proses yang bertujuan untuk menghilangkan kontaminan.

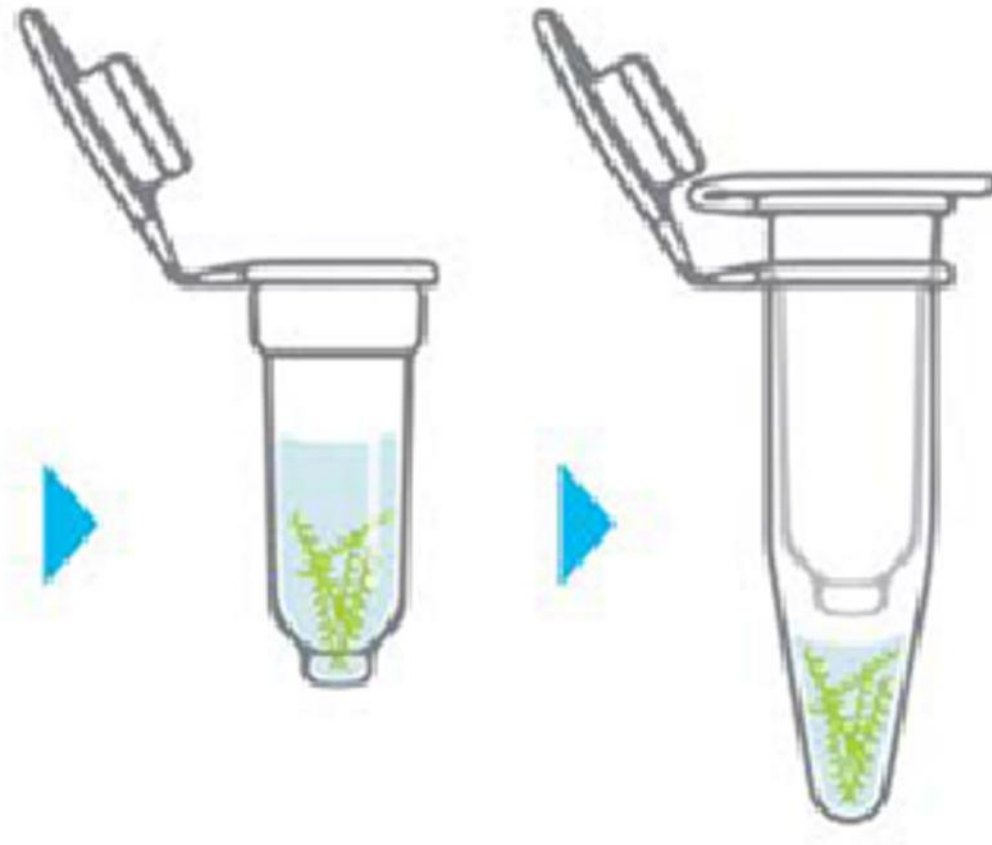


Wash

Centrifuge

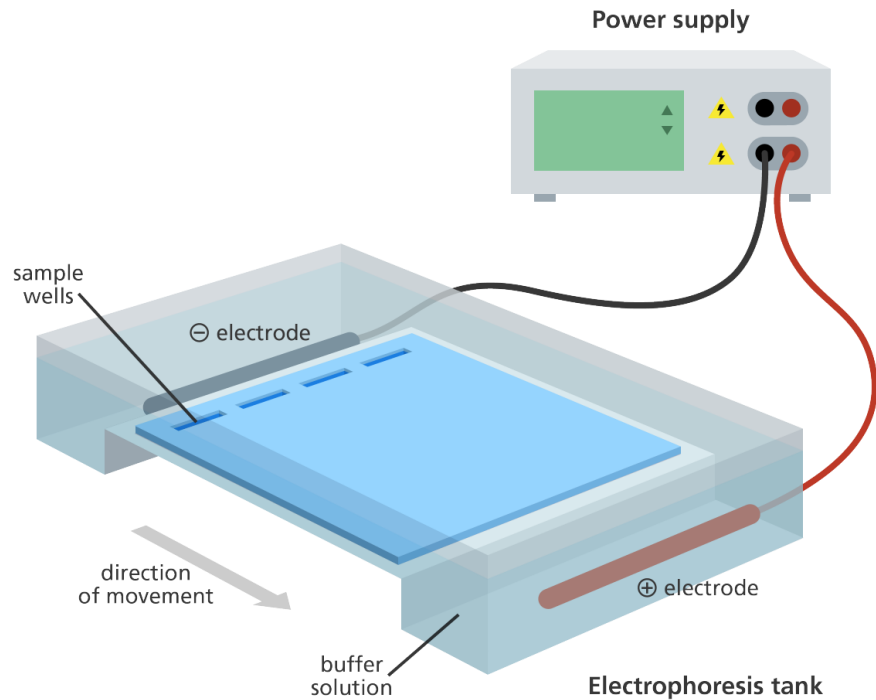
Tahapan 4: *Elution*

Proses yang bertujuan untuk melarutkan DNA menggunakan *elution buffer*.



Bagaimana cara menilai hasil isolasi DNA?

Elektroforesis



Kualitas

Nanodrop Spectrophotometer



Kuantitas dan
kemurnian

Qubit Fluorometer



Kuantitas

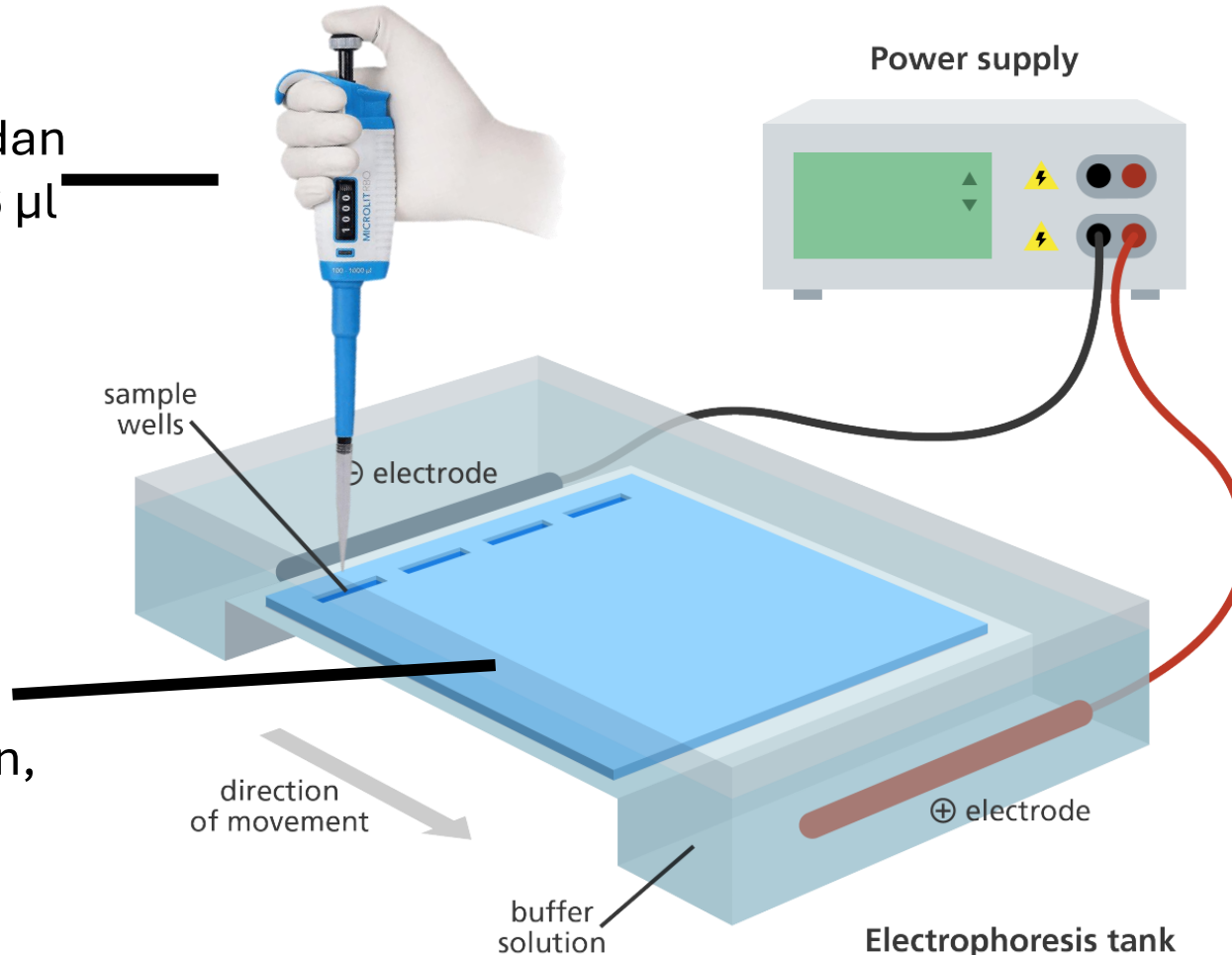
Cara 1: Elektrofesis

2

Marker/penanda (7 μ l) dan sampel + *loading dye* (6 μ l + 2 μ l) dimasukkan ke dalam sumur

1

Buat gel agarose 1% menggunakan cetakan, masukkan ke dalam *chamber/tank*



3

Pengaturan

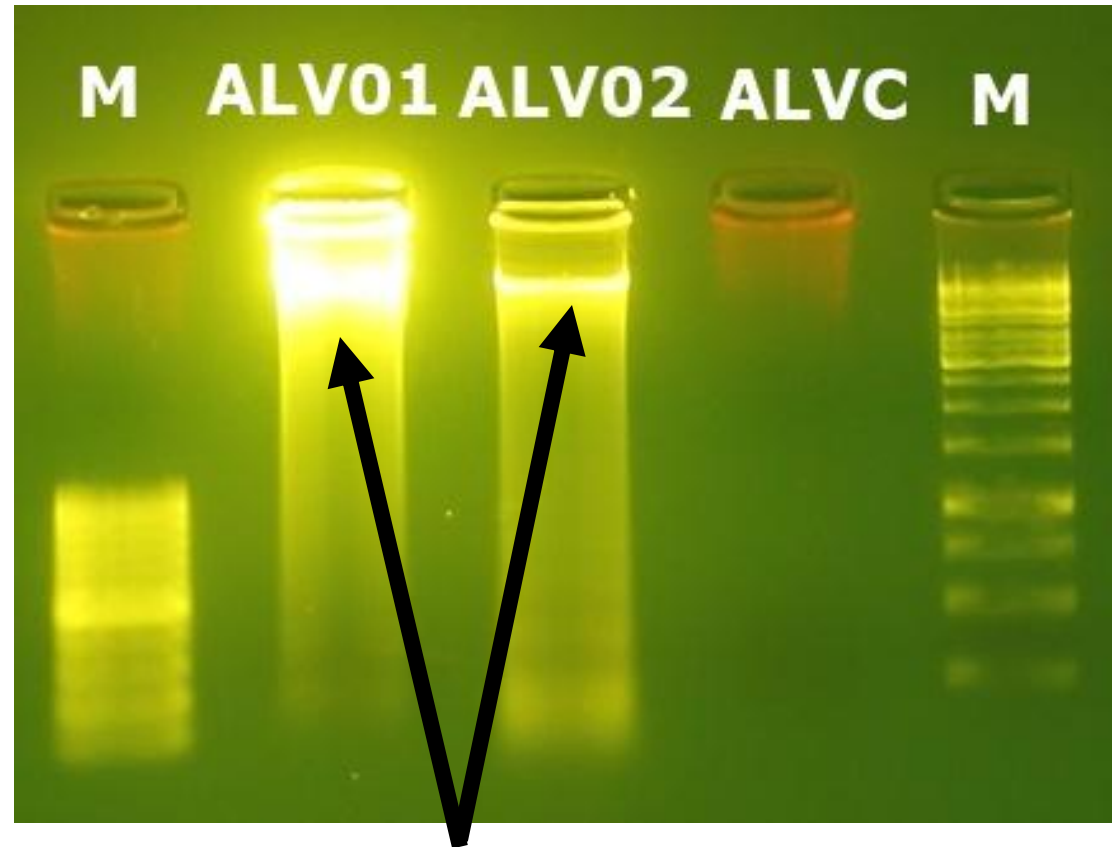
- 30 menit
- Tegangan 100 V
- Kuat arus 300 A

4

Visualisasikan hasil menggunakan transiluminator.

Cara 1: Elektrofesis

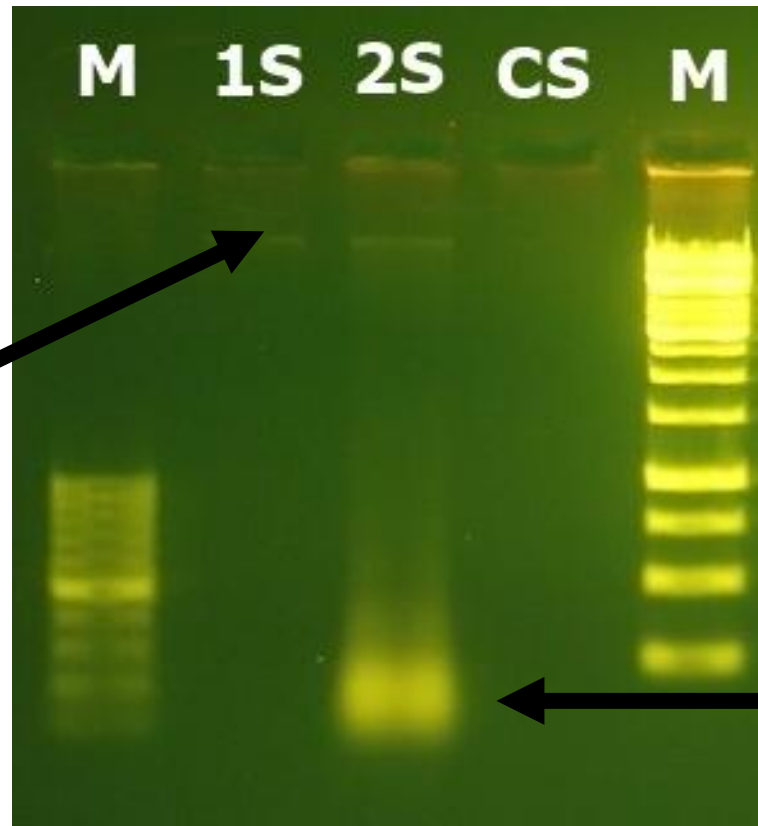
M adalah marker/penanda (kiri: 100 bp, kanan: 1000 bp)



Pita DNA tebal dan jelas → kualitas bagus

Cara 1: Elektroforesis

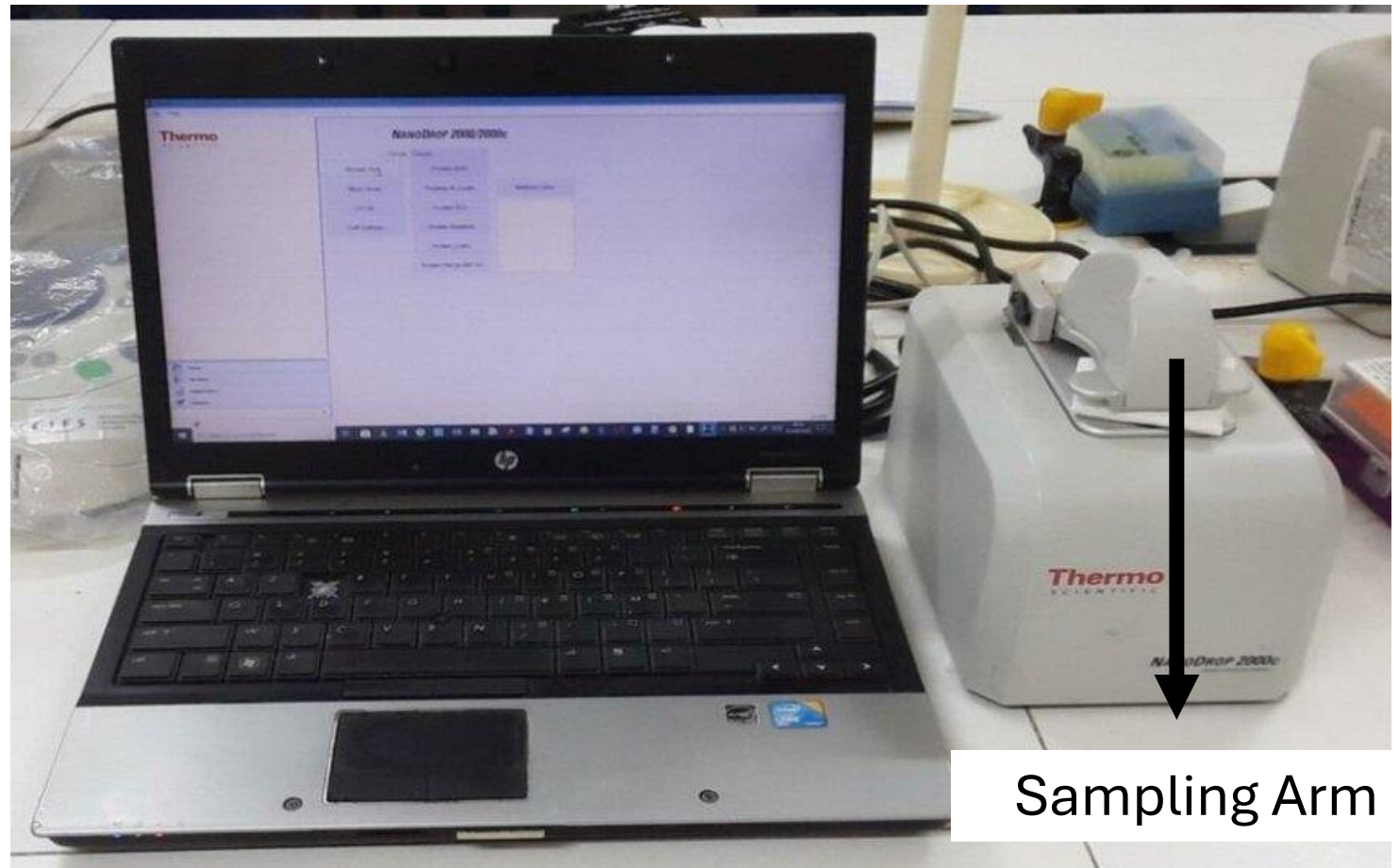
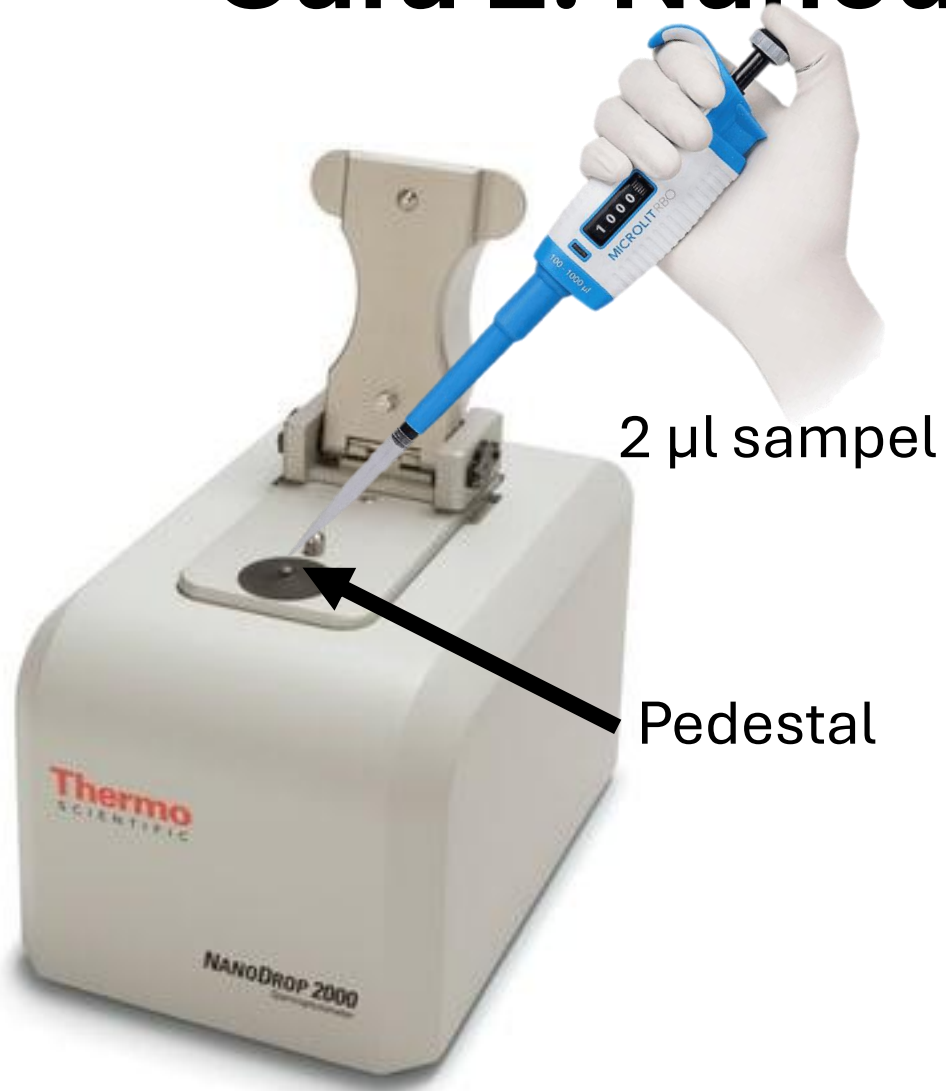
M adalah marker/penanda (kiri: 100 bp, kanan: 1000 bp)



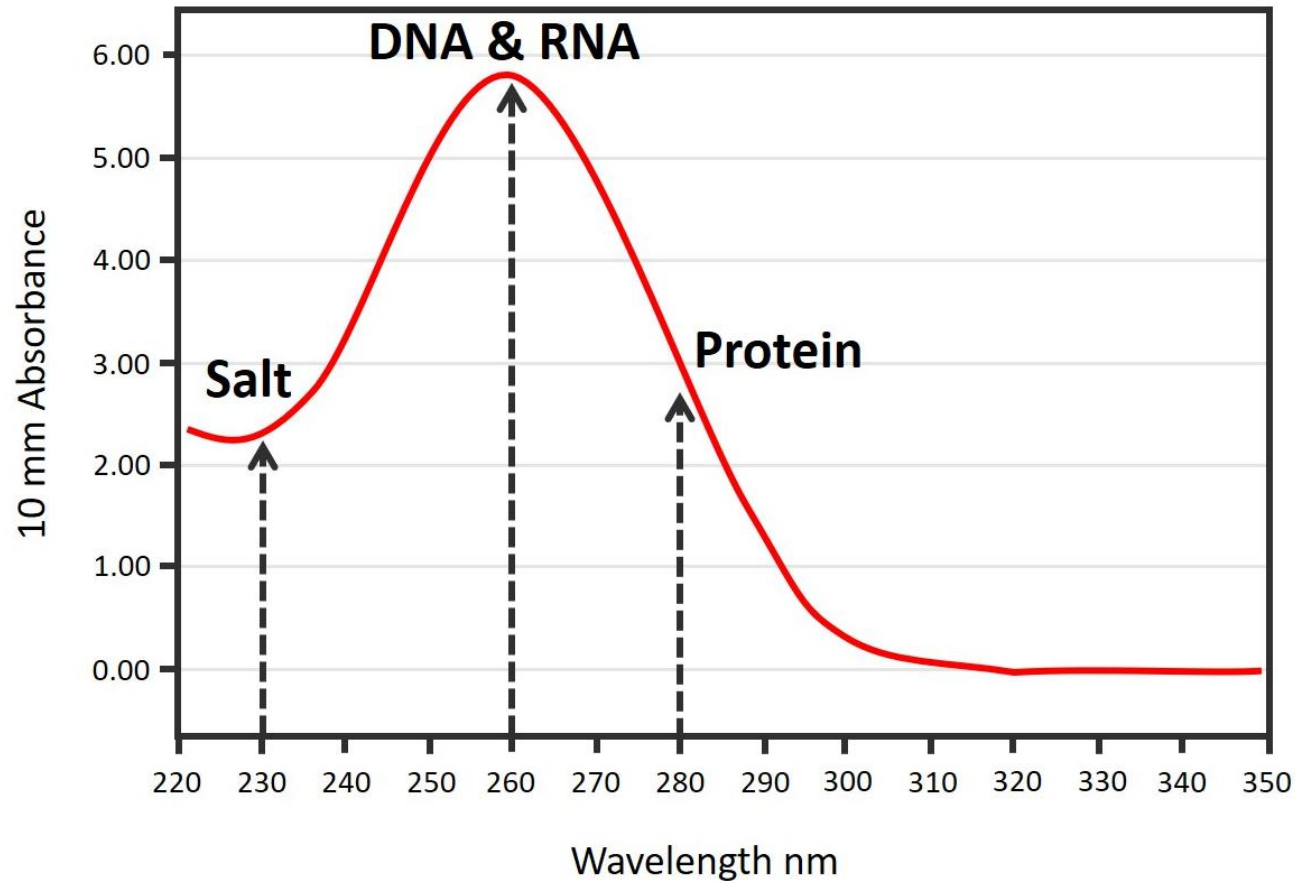
Pita DNA tipis/tidak terlihat sama sekali → kualitas kurang baik

Pita DNA tebal namun kurang jelas → DNA terfragmentasi

Cara 2: Nanodrop Spectrophotometer



Cara 2: Nanodrop Spectrophotometer



Contoh hasil

DNA conc. (ng/ μ l)	260/280	260/230
580.1	1.77	1.65

- Konsentrasi (ng/ μ l)
- Rasio 260/280 dengan nilai 1.8 (DNA) atau 2.0 (RNA)
- Rasio 260/230 dengan nilai 2.0 – 2.2

Cara 3: Qubit Fluorometer

Sample Chamber

Digital Display



Reagen

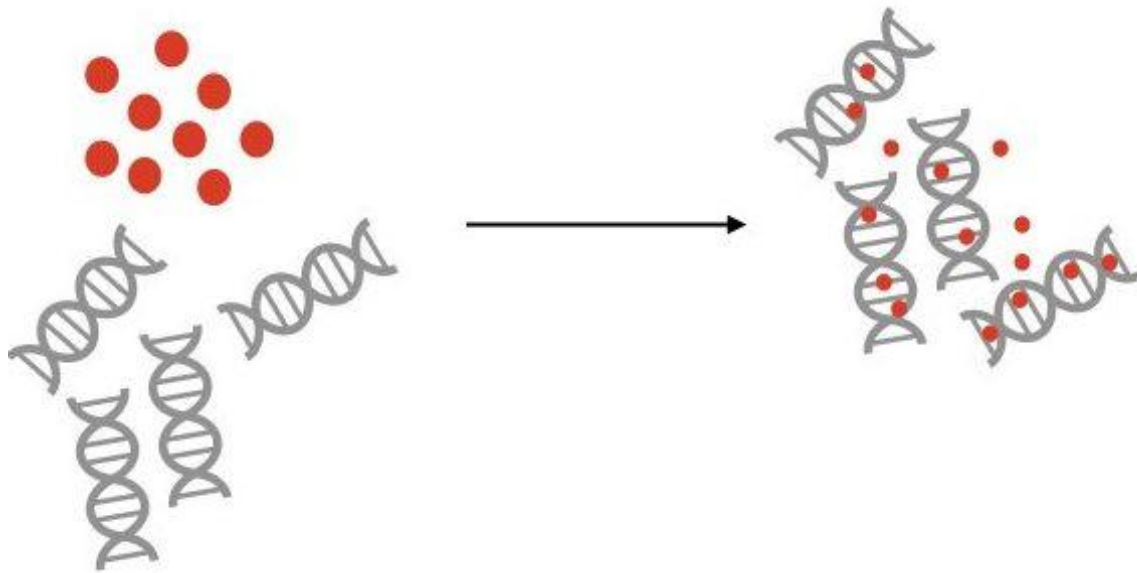
- Working Solution
- Standard 1
- Standard 2

Campuran:

Sampel (1 μ l) + working solution (199 μ l)

Standard 1 / 2 (10 μ l) + working solution (190 μ l)

Cara 3: Qubit Fluorometer



Simulasi cara pakai

[Qubit 4 Fluorometer 3D Tour](#)



Apabila DNA tidak terdeteksi:

- Too low, out of range
- Too high, out of range



Terima Kasih

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