



## Biotechnology

### IST-1.P.1

Genetic engineering techniques can be used to analyze and manipulate DNA and RNA—

- a. Electrophoresis separates molecules according to size and charge.
- b. During polymerase chain reaction (PCR), DNA fragments are amplified.
- c. Bacterial transformation introduces DNA into bacterial cells.
- d. DNA sequencing determines the order of nucleotides in a DNA molecule



**Which DNA tech separates fragments by size/charge?**

- A. Gel electrophoresis**
- B. PCR**
- C. Sequencing**
- D. Transformation**

**Which DNA tech separates fragments by size/charge?**

**A. Gel electrophoresis**



**Gel electrophoresis involves using an electrical current to draw DNA, RNA, or protein through a gel. The gel has pores which allows the small fragments to travel farther.**



**Why does the DNA move towards positive end?**

- A. Deoxyribose is negatively charged**
- B. Deoxyribose is positively charged**
- C. Phosphate is negatively charged**
- D. Phosphate is positively charged**

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**Why does the DNA move towards positive end?**

**C. Phosphate is negatively charged**



**Recall, DNA is made up of a nitrogenous base, deoxyribose, and a phosphate group.**

**The phosphate group is negatively charged with  $\text{PO}_4^{2-}$  so it will be attracted to the positive end.**

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**Which fragments travel farther?**

- A. Small**
- B. Large**

**Which fragments travel farther?**

**A. Small**



**Smaller fragments are able to pass through the pores quicker.**

**Another teacher describes it visually as comparing an adult to a kid in the ballpit of the playground. The child will be able to get out of the ballpit easier than the adult.**



**What cuts the DNA prior to running gel electrophoresis?**

- A. DNA polymerase**
- B. Helicase**
- C. Ligase**
- D. Restriction enzyme**



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**What cuts the DNA prior to running gel electrophoresis?**

**D. Restriction enzyme**



**Restriction enzymes will cut the DNA at specific restriction sites. This will form fragments that are run on a gel electrophoresis. When comparing two strands of DNA, researchers will use the same restriction enzymes.**

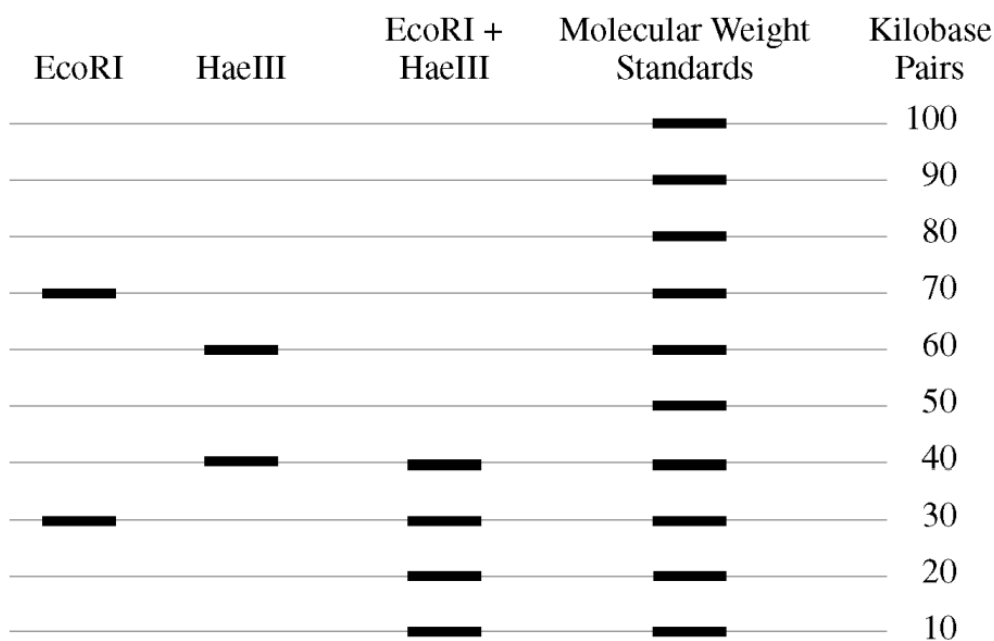
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RESULTS OF GEL ELECTROPHORESIS



**Minimum restriction sites for ECO on plasmid to get this?**

**A. 1**

**B. 2**

**C. 3**

**D. 4**

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**Minimum restriction sites for ECO on plasmid to get this?**

**B. 2**



**Plasmids are circular DNA. If you look at the gel electrophoresis, you would observe that ECO has 2 fragments which means it was cut 2 times. If it was cut 2 times, there must be 2 restriction enzymes**

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Evidence	A	B	C	D
—	—	—	—	—
==	—	==	—	—
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—



**Which suspect was at crime scene?**

**A. A**

**B. B**

**C. C**

**D. D**

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**Which suspect was at  
crime scene?**

**B. B**



**When comparing the banding  
pattern between the crime scene  
and the suspects, it is  
observable that section B has  
the same bands as the  
restaurant.**



**Which DNA tech is responsible for multiple copies of a gene?**

- A. Gel electrophoresis**
- B. PCR**
- C. Sequencing**
- D. Transformation**

**Which DNA tech is responsible for multiple copies of a gene?**

**B. PCR**



**PCR stands for polymerase chain reaction. This procedure will create multiple copies of a certain DNA to compare and run testing on.**

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**What does PCR stand for?**



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**What does PCR stand  
for?**



**Polymerase Chain Reaction**

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**PCR uses a specific type of polymerase, why and where does it come from?**

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**PCR uses a specific type of polymerase, why and where does it come from?**

**PCR involves a heating up then cooling cycle. Proteins (polymerase) denature in high temperatures so heat resistant polymerase from thermophile prokaryotes is needed to allow the enzymes to remain functional after heating phase.**

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**What is the function of the heating process in PCR?**

**What is the function of the heating process in PCR?**



- > Break the hydrogen bonds between the two strands**
- > Allows the strands to be single stranded to allow for the replication to take place in future steps**



**Which DNA tech involves adding plasmid to prokaryote?**

- A. Gel electrophoresis**
- B. PCR**
- C. Sequencing**
- D. Transformation**

**Which DNA tech involves adding plasmid to prokaryote?**

**D. Transformation**

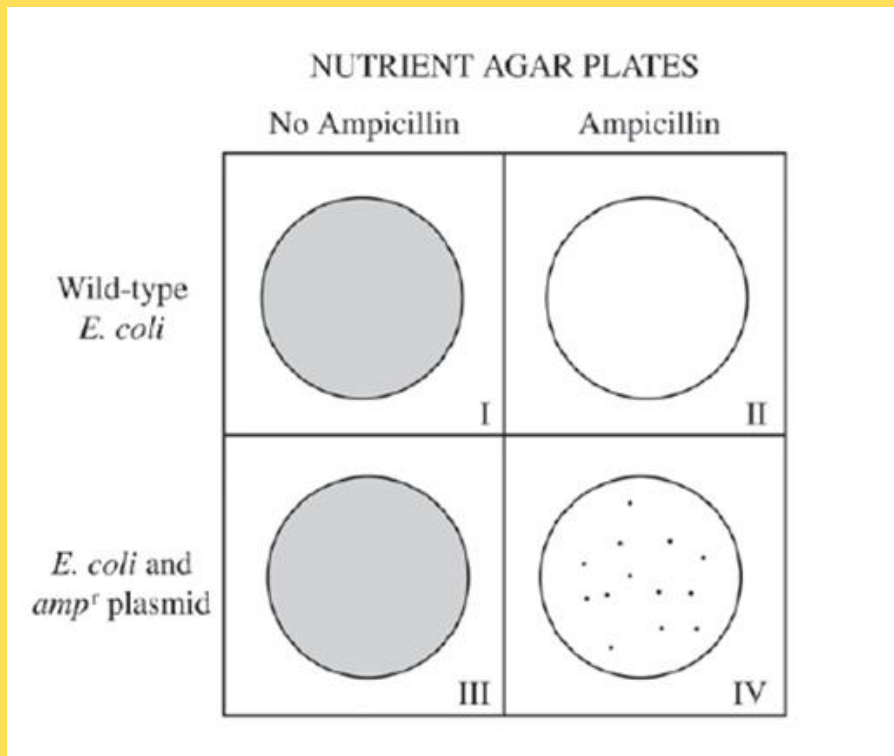


**Transformation is a process by which foreign DNA is introduced into a cell. During this lab, we added a plasmid to a solution with E. coli then heat shocked to introduce the plasmid into the bacteria. It is then transformed into an antibiotic resistant bacteria.**

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**Plate has non transformed bacteria & no antibiotics**

- A. I**
- B. II**
- C. III**
- D. IV**



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**Plate has non transformed bacteria & no antibiotics**

**A. I**

**Plate I has the wild-type E. coli (non-transformed bacteria) and no ampicillin (no antibiotics)**

**It has a lawn of growth because there is no antibiotic to inhibit the bacterial growth.**

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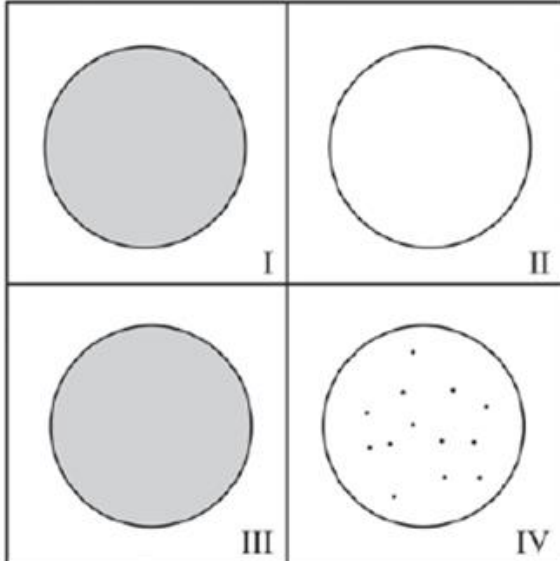


## NUTRIENT AGAR PLATES

No Ampicillin

Ampicillin

Wild-type  
*E. coli*



I

II

III

IV

**Why is there no growth  
on plate II?**

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**Why is there no growth  
on plate II?**

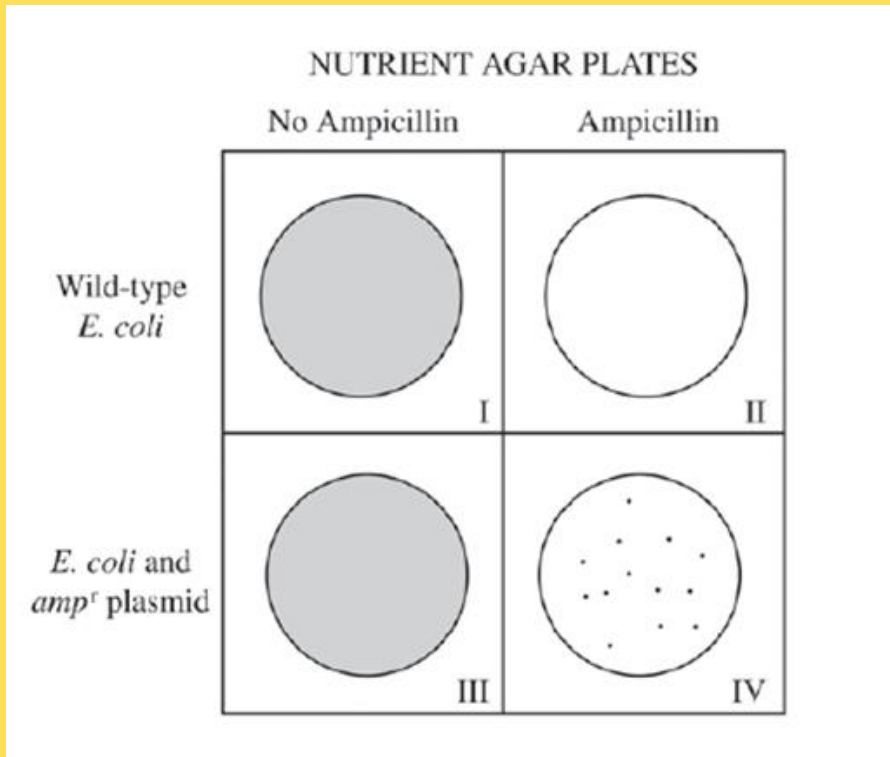


**Bacteria is not transformed and  
there is antibiotic in the agar  
which restricts growth.**

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**What is function of plates I and III?**



**What is function of plates I and III?**

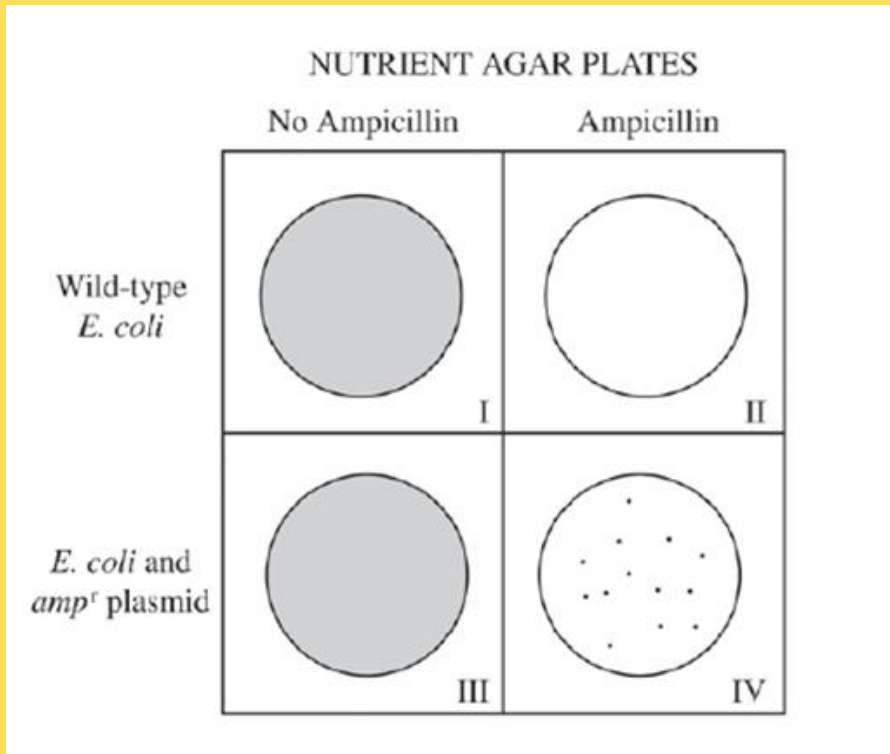
**Control:**

**Positive control- you are verifying that the bacteria is viable. You need to be sure that the bacteria would grow in the absence of the antibiotic to know that the antibiotic killed non-resistant bacteria (explains plates II and IV)**

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If plasmid also had the gene to synthesize insulin...

Which plate has the highest % of insulin producing bacteria?

- A. I
- B. II
- C. III
- D. IV

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If plasmid also had the gene to synthesize insulin...

Which plate has the highest % of insulin producing bacteria?

**D. IV**



Plate IV has *E. coli/amp* plasmid and ampicillin. The ampicillin will restrict the growth of the non-transformed bacteria. The only bacteria that will grow on this plate is the bacteria with the plasmid containing the gene to synthesize insulin, so **100%** of the bacteria are insulin producing bacteria.

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**DNA is \_\_\_ because  
of the \_\_\_\_.**

- A. Negatively charged; hydroxyl group**
- B. Negatively charged; phosphate group**
- C. Positively charged; hydroxyl group**
- D. Positively charged; phosphate group**



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**DNA is \_\_\_ because  
of the \_\_\_\_.**

**B. Negatively charged;  
phosphate group**



**DNA is negatively charged  
because of the phosphate group.  
Phosphate is  $\text{PO}_4^{2-}$  and DNA  
has an alternating sugar and  
phosphate backbone.**



**Gel electrophoresis separates molecules based on**

- A. Charge and Polarity**
- B. Polarity and Weight**
- C. Weight and Size**
- D. Size and Charge**

**Gel electrophoresis  
separates molecules based  
on**

**D. Size and Charge**



**Gel electrophoresis involves a gel with pores that allow for materials to be separated by size. Electrophoresis involves using an electrical current so the charge of the material will allow for movement through the gel.**



**In a gel electrophoresis...**

- A. More numerous sizes are near the wells**
- B. Less numerous sizes are near the wells**
- C. Larger molecules are near the wells**
- D. Shorter molecules are near the wells**

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In a gel electrophoresis...

**C. Larger molecules  
are near the wells**



**The large molecules will be unable to pass through the pores as quickly and will be located near the wells (where the DNA was added to the gel). Smaller molecules will travel faster and thus farther.**



**What does a thicker band represent in a gel?**

- A. There's more DNA material present in that band**
- B. There's more glucose available in that band**
- C. There's more mutation in the DNA fragment present**
- D. There's more radioactive material present in that band**

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**What does a thicker band represent in a gel?**

**A. There's more DNA material present in that band**



**The DNA that is the same length will travel the same distance. If multiple fragments travel the same distance, it will cause the band in the gel to be thicker.**



**What is used to cut the DNA into fragments for the gel?**

- A. Ligase from eukaryotes**
- B. Ligase from prokaryotes**
- C. Restriction enzymes from eukaryotes**
- D. Restriction enzymes from prokaryotes**



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**What is used to cut the DNA into fragments for the gel?**

**D. Restriction enzymes from prokaryotes**



**Restriction enzymes will cut the DNA at restriction sites. The prokaryotes use restriction enzymes to cleave foreign DNA.**



**What does PCR stand for?**

- A. Place for cellular respiration**
- B. Primase cytosine reactants**
- C. Polymerase chain reaction**
- D. Preferred chemical reaction**

**What does PCR stand for?**

**C. Polymerase chain reaction**



**PCR stands for Polymerase Chain Reaction. This is a process that will amplify a segment of DNA using a thermocycler, DNA polymerase, a primer and DNA nucleotides.**

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**What are the steps  
involved in PCR?**

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## What are the steps involved in PCR?

### PCR Components



DNA Sample



Primers



Nucleotides



Taq polymerase



Mix Buffer



PCR Tube



Thermal Cycler



PCR Cycle

### PCR Process (ONE Cycle)



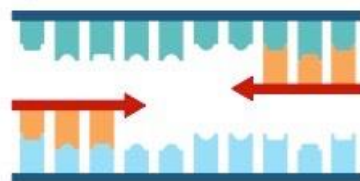
95°C – Strands separate

1. Denaturing



55°C – Primers bind template

2. Annealing



72°C – Synthesise new strand

3. Extension





**Where does the heat resistant polymerase come from?**

- A. Fire-resistant plants**
- B. Monkeys**
- C. Nucleus**
- D. Thermophiles**

**Where does the heat resistant polymerase come from?**

**D. Thermophiles**



**DNA polymerase is an enzyme (and a protein). Recall, heat will denature proteins. The process of PCR has repeated heating and cooling which would denature the enzyme. The thermophiles are bacteria that thrive at extremely high temperatures.**



**Plasmid makes bacteria resistant to ampicillin and able to synthesize the green glow protein. Which plate will have highest percent of growth?**

- A. Plate with nutrients and no ampicillin present**
- B. Plate with ampicillin and nutrient present**



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**Plasmid makes bacteria resistant to ampicillin and able to synthesize the green glow protein. Which plate will have highest percent of growth?**

**A. Plate with nutrients and no ampicillin present**

**Not all bacteria will be transformed, so the plate with nutrient only would have a lawn of growth while the plate with ampicillin would have colonies. Read the question carefully, it said highest percent of growth.**



**Plasmid makes bacteria resistant to ampicillin and able to synthesize the green glow protein. Which plate will have higher amount of glowing bacteria?**

- A. Plate with nutrients and no ampicillin present**
- B. Plate with ampicillin and nutrient present**

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**Plasmid makes bacteria resistant to ampicillin and able to synthesize the green glow protein. Which plate will have higher amount of glowing bacteria?**

**B. Plate with ampicillin and nutrient present**



**The plate with the ampicillin will restrict the growth to the transformed bacteria only. All of the bacteria on this plate will be transformed, thus will glow.**

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**What happens during DNA sequencing?**

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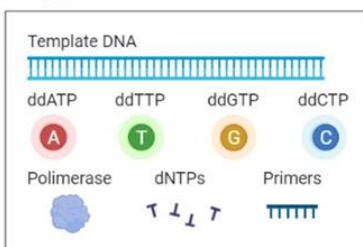
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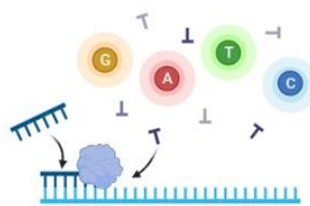


## What happens during DNA sequencing?

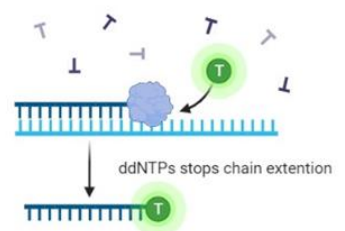
### Reagents



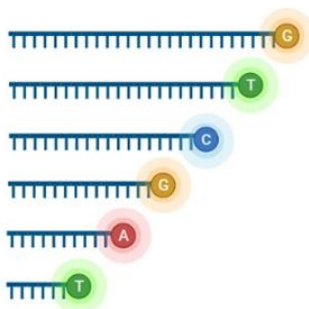
### ① Primer annealing and chain extension



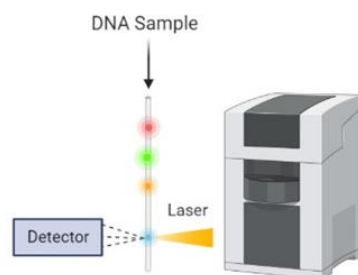
### ② ddNTP binding and chain termination



### ③ Fluorescently labelled DNA sample



### ④ Capillary gel electrophoresis and fluorescence detection



### ⑤ Sequence analysis and reconstruction

