Nucleic Acid Analysis

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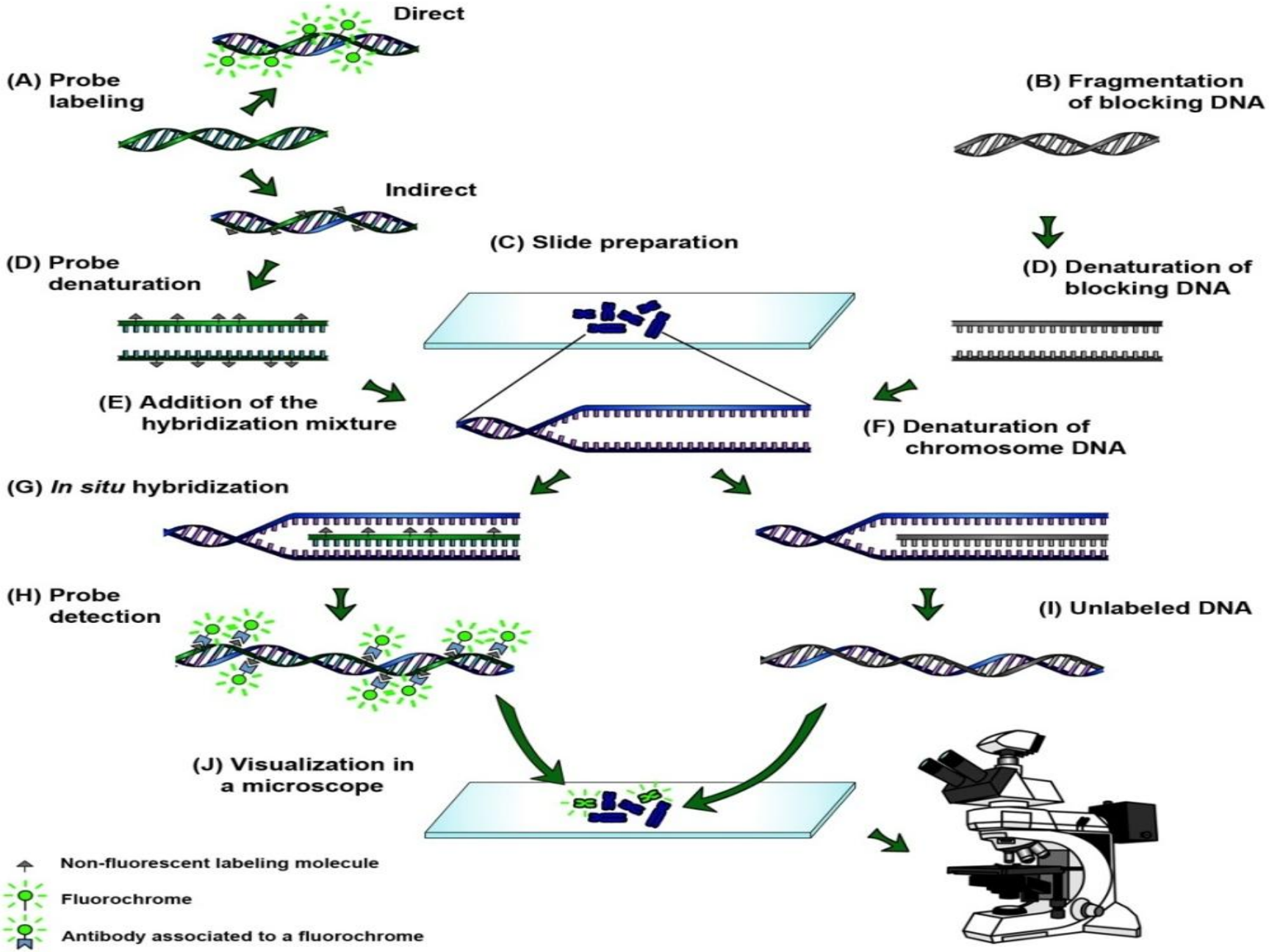
Methods of Nucleic Acid Analysis

- **DNA Hybridization and Probes**
- **Agarose Gel Electrophoresis**
- **Nucleic Acid Amplification**

DNA Hybridization and Probes

- If the DNA double helix is opened, leaving single-stranded (denatured) DNA, the nucleotide bases are exposed and thus available to interact with other single-stranded nucleic acid molecules.
- If complementary sequences of a second DNA molecule are brought into physical contact with the first, they hybridize to it, forming a new double-stranded molecule in that area.
- A probe is a cloned DNA fragment that has been labeled so that it can be detected if it hybridizes to complementary sequences in such a test system.
- The probe may be derived from the gene for a known protein of the pathogen or be empirically derived just for diagnostic purposes.


Direct
(A) Probe labeling
Indirect
(B) Fragmentation of blocking DNA
(C) Slide preparation
(D) Probe denaturation
(D) Denaturation of blocking DNA
(E) Addition of the hybridization mixture
(F) Denaturation of chromosome DNA
(G) In situ hybridization
(H) Probe detection
(I) Unlabeled DNA
(J) Visualization in a microscope
Non-fluorescent labeling molecule
Fluorochrome
Antibody associated to a fluorochrome

Agarose Gel Electrophoresis

- Nucleic acids may be separated in an electrophoretic field in an **agarose** (highly purified agar) gel. The speed of migration depends on size, with the smaller molecules moving faster and appearing at the bottom (end) of the gel.
- This analysis can be refined by the use of restriction endonucleases, which are enzymes derived from bacteria that recognize specific nucleotide sequences in DNA molecules and digest (cut) them at all sites where the sequence appears.
- Thus, plasmids of the same size may be differentiated by the size of fragments generated by endonuclease digestion of DNA.

PROCEDURE

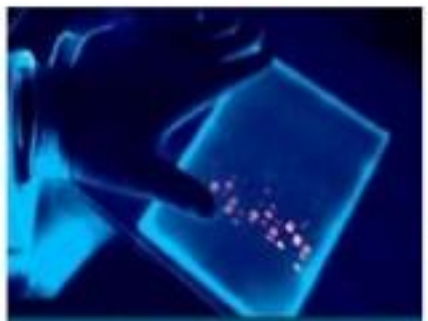
Set up gel

Load Buffer

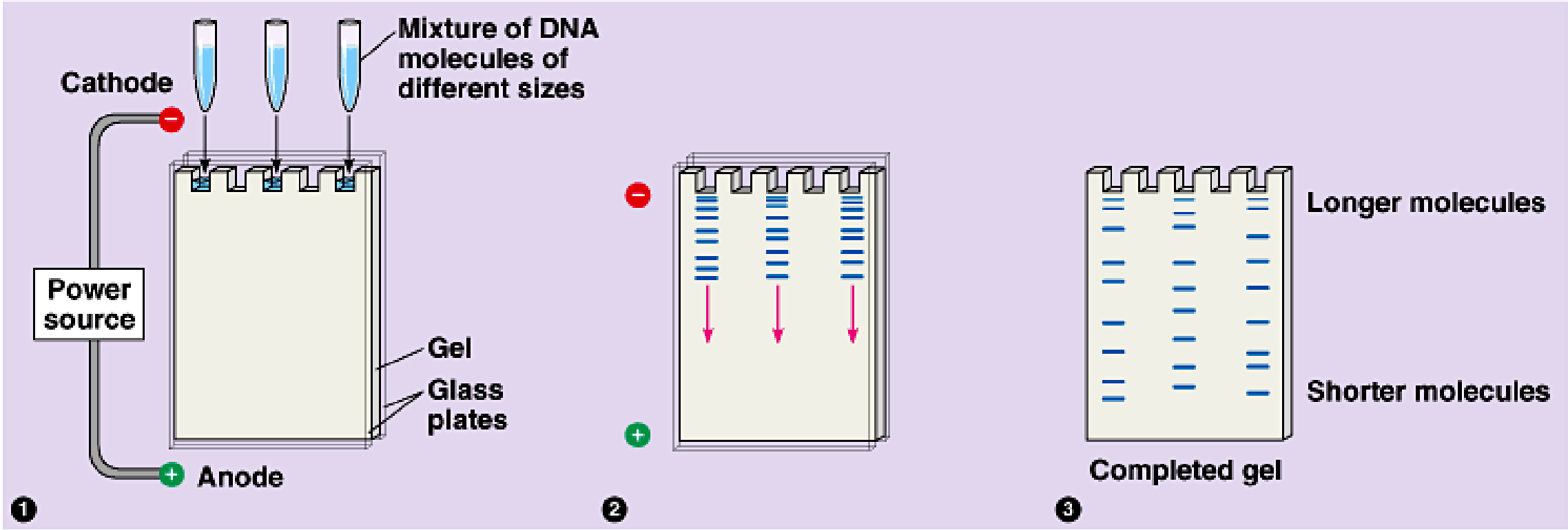
Get your sample obtained from previous purifying technique

Load Sample

Run Gel



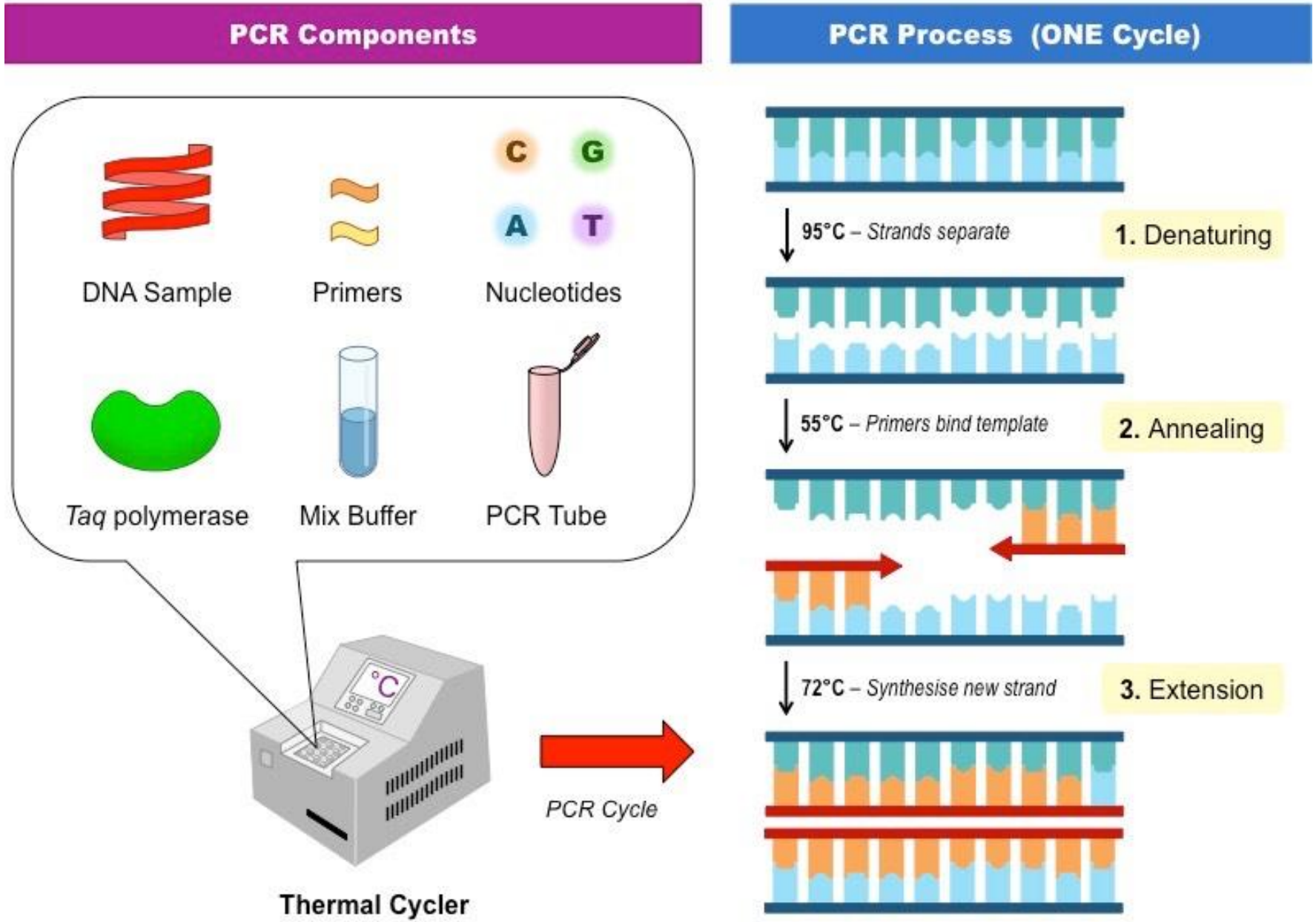
Stain and look at with UV light



Nucleic Acid Amplification

- Nucleic acid amplification (NAA) methods such as the polymerase chain reaction (PCR) allow the detection and selective replication of a targeted portion of the genome.
- The basic PCR technique uses synthetic oligonucleotide primers and special DNA polymerases in a way that allows repeated cycles of synthesis of only a segment of a targeted DNA molecule that may be as large as an entire genome.

- The specificity is provided by the sequence of approximately 20 nucleotides in each primer pair, which are crafted to flank the desired segment of the genome.
- The DNA polymerases used are ones that operate at unusually high temperatures. This allows the use of temperature to control shifts between separation of the complementary DNA strands (so primers can bind) and replication of the DNA sequence that lies between the two primers.
- Because each strand generates a new fragment, the increase is exponential. In a machine called a thermocycler, the targeted DNA can be amplified 1 million to 1 billion times in 20 to 30 cycles


PCR Components
PCR Process (ONE Cycle)
DNA Sample
Primers
C
G
A
T
Nucleotides
Taq polymerase
Mix Buffer
PCR Tube
°C
Thermal Cycler
PCR Cycle
95°C – Strands separate
1. Denaturing
55°C – Primers bind template
2. Annealing
72°C – Synthesise new strand
3. Extension