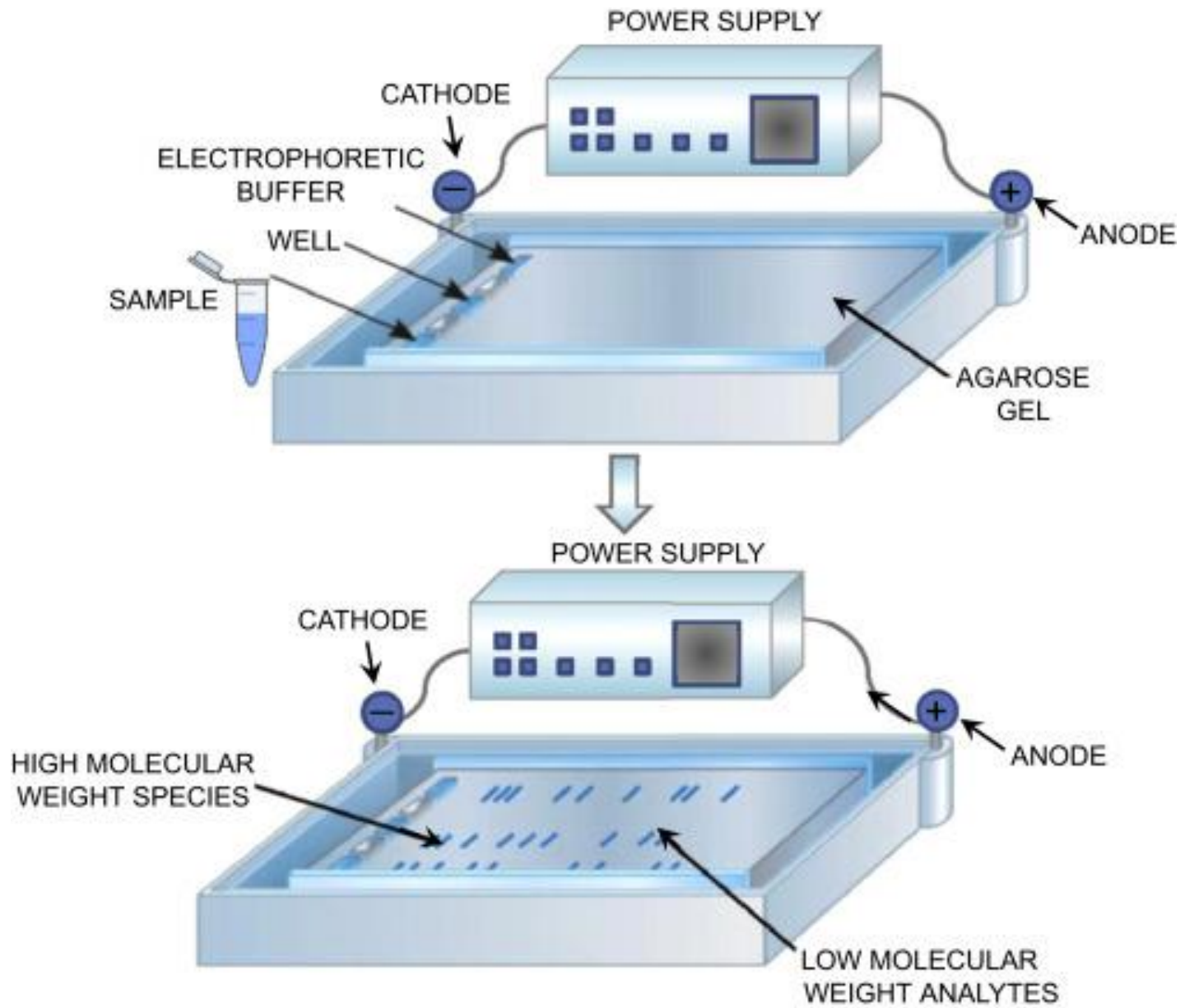


Aquaculture Biotechnology

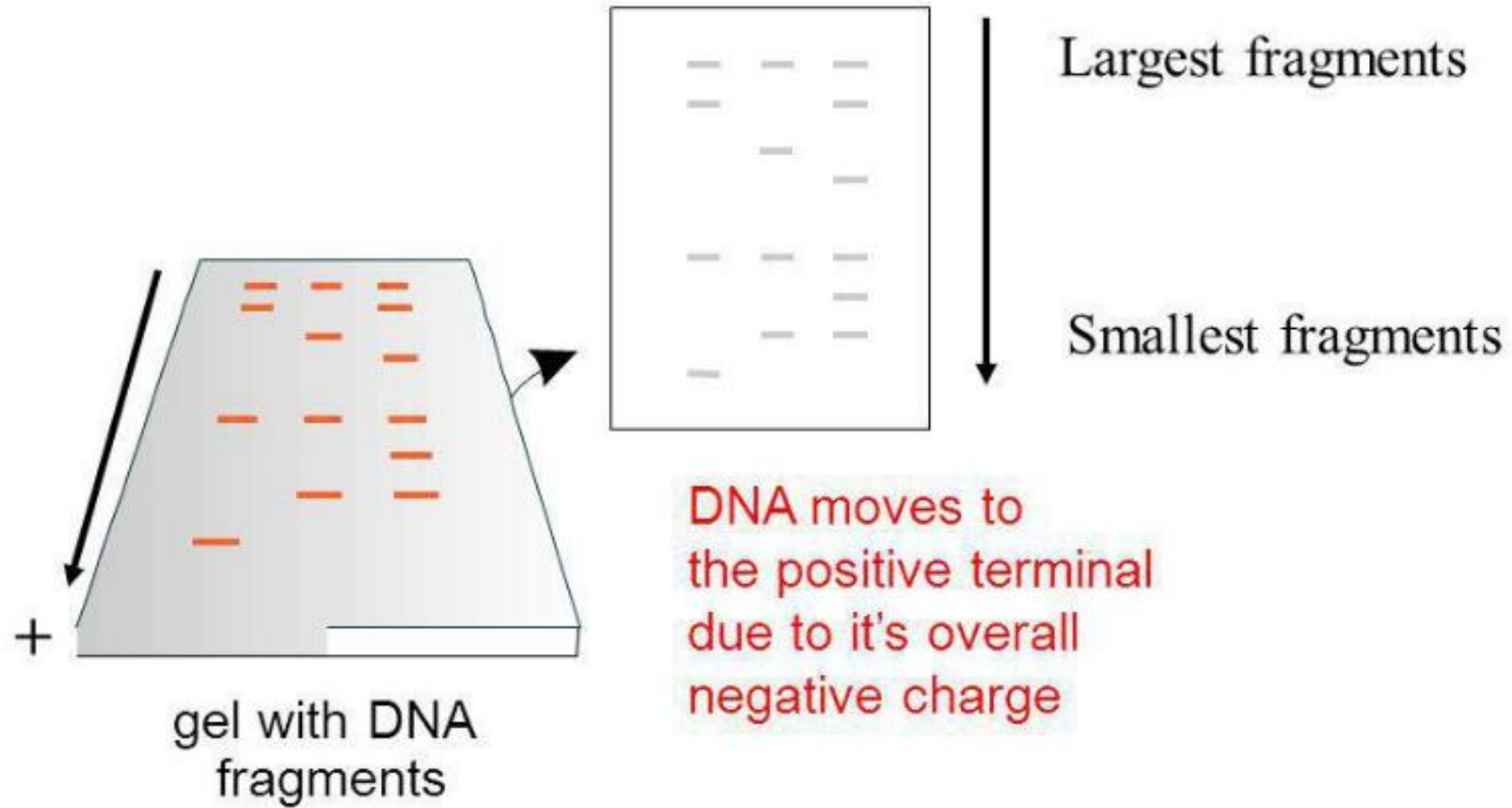
Diagnostic Techniques

Gel electrophoresis

- ✓ Gel electrophoresis
- ✓ DNA fragments of different sizes can be separated by an electrical field applied to a “gel”.
- ✓ The negatively charged DNA migrates away from the negative electrode and to the positive electrode.
- ✓ The smaller the fragment the faster it migrates.



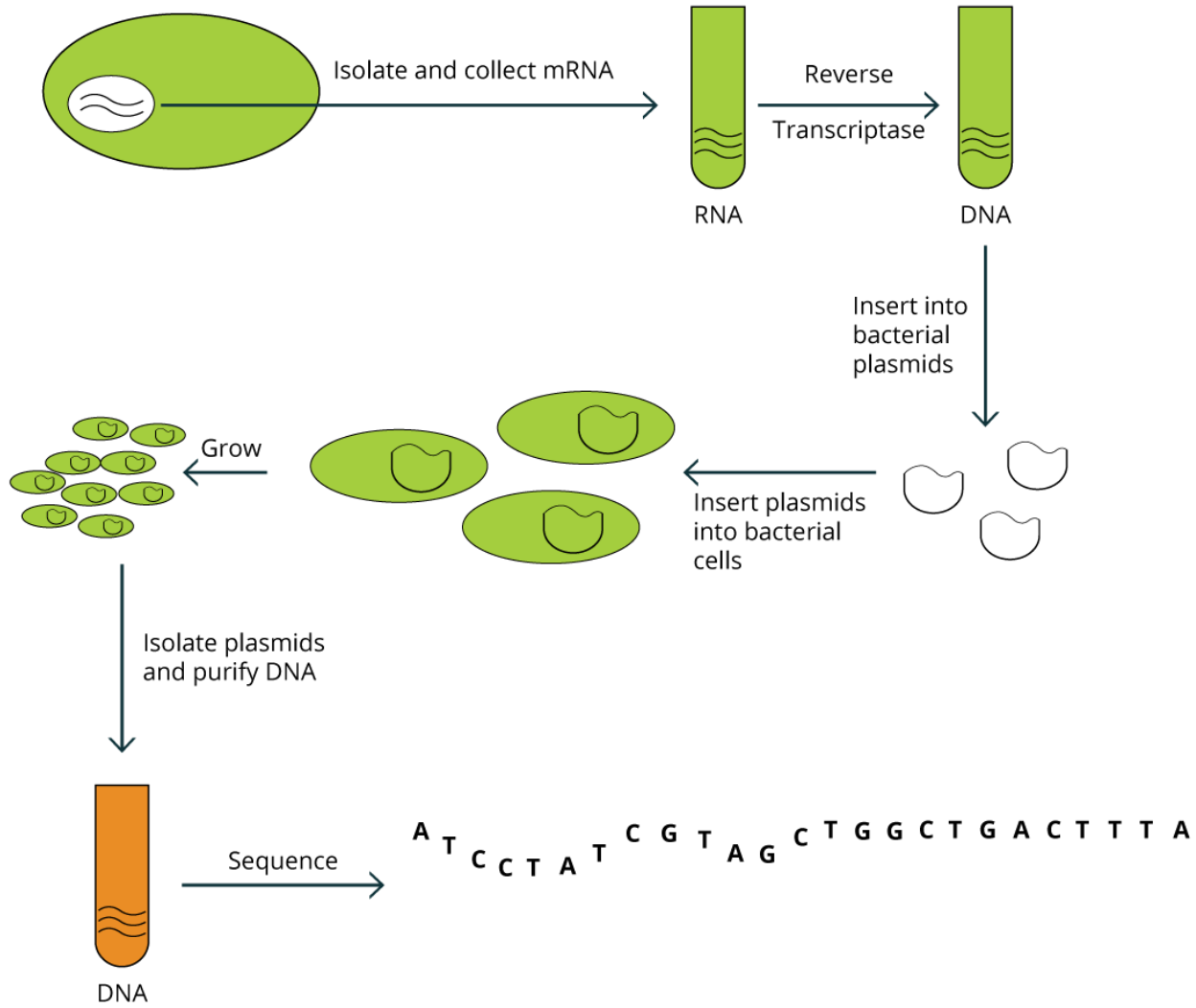
DNA fragments can be separated by gel electrophoresis



Cloning Libraries

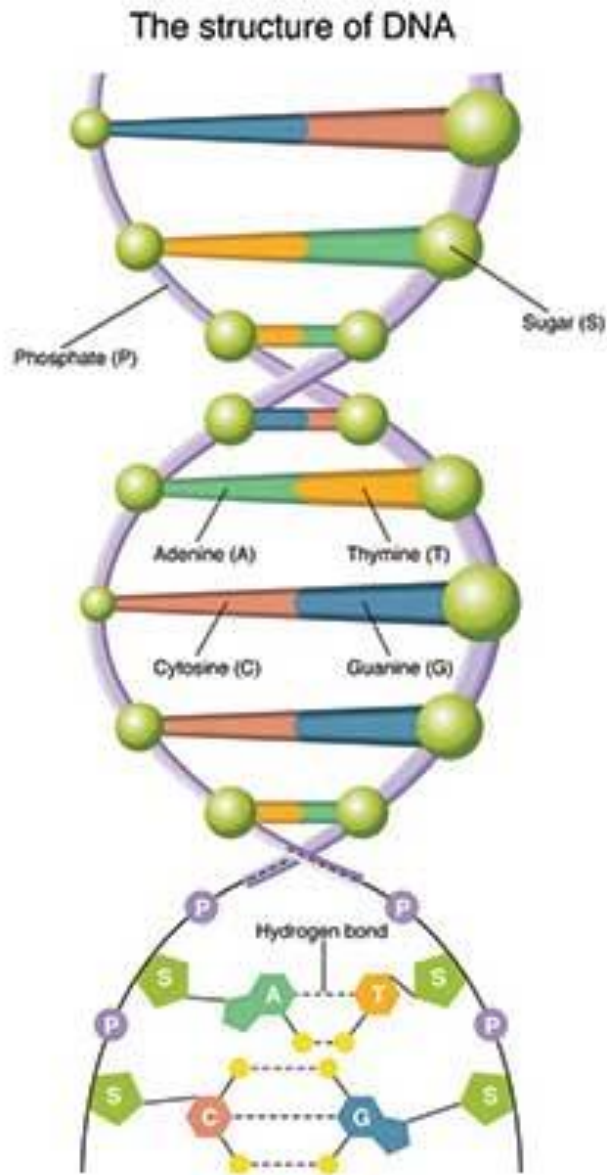
- ✓ Libraries are collection of DNA clones in a certain vector.
- ✓ The goal is to have each gene represented in the library at least once.
- ✓ Genomic - made from RE DNA fragments of total genomic DNA
- ✓ cDNA (complementary DNA) – made from DNA synthesized from mRNA

Formation of a cDNA Library

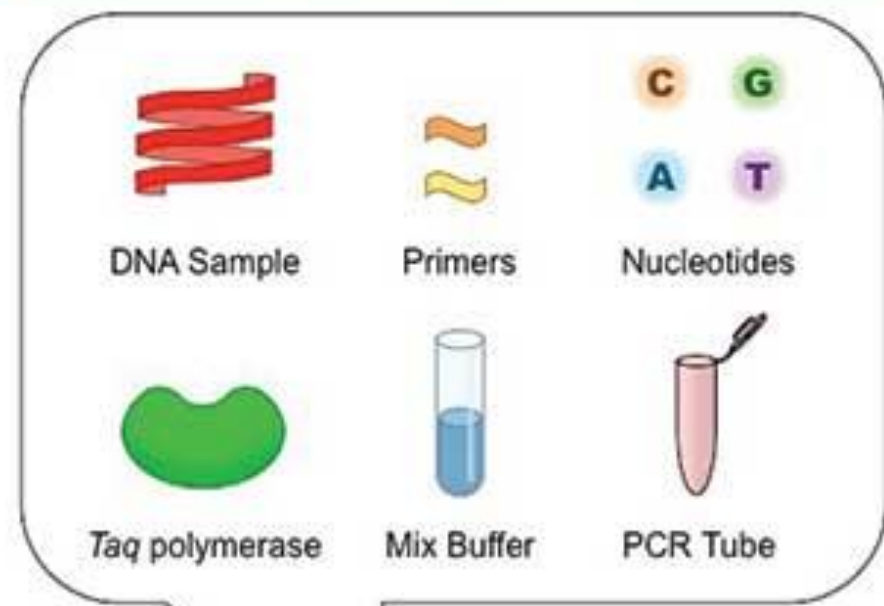


Polymerase chain reaction (PCR)

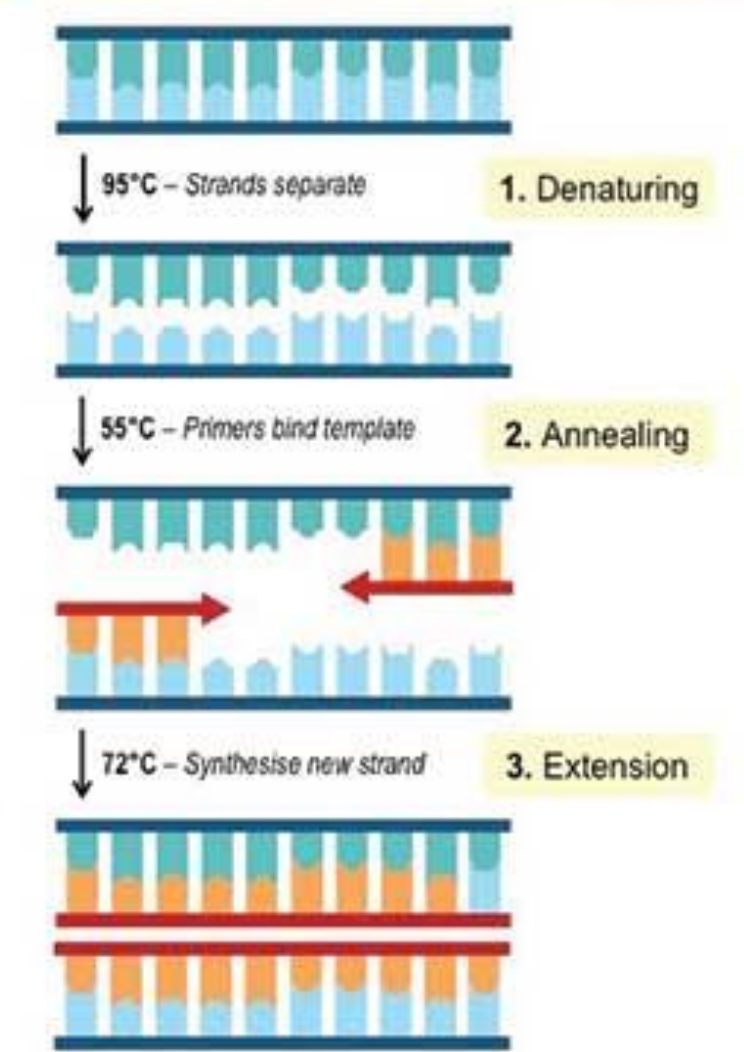
- ✓ **Polymerase chain reaction**, is a technique to make many copies of a specific DNA region
- ✓ This is in vitro (in a test tube rather than an organism) DNA synthesis
- ✓ PCR relies on a thermostable **DNA polymerase, Taq polymerase, and requires DNA primers** designed specifically for the DNA region of interest.



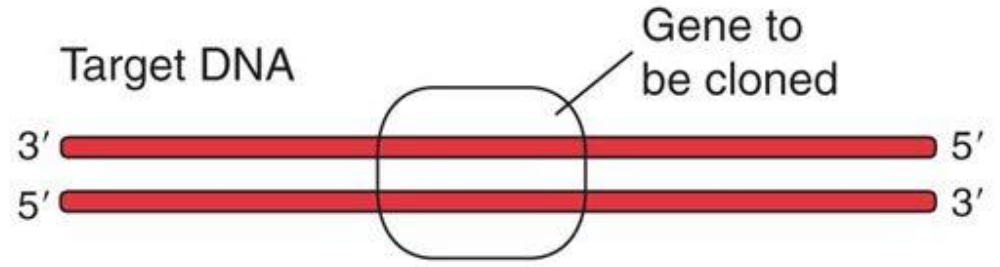
PCR Components



PCR Process (ONE Cycle)

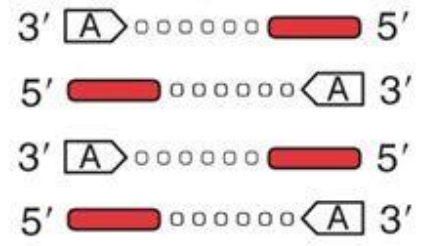


Cloning a gene by PCR



Denature DNA, anneal primers

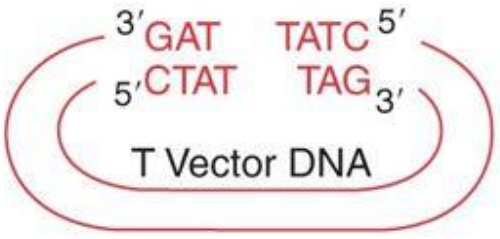
Amplify DNA with *Taq* DNA polymerase, which adds "A" nucleotide to end of PCR product



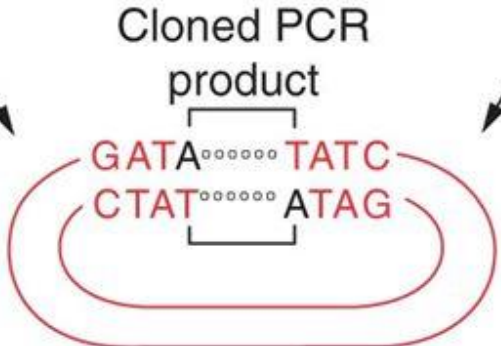
Amplify DNA

Ligate and subclone PCR product into T vector

Uses a restriction enzyme that recognizes A-T restriction site for cutting T vector for insertion of gene



T vector with single-stranded thymine nucleotide at each end.

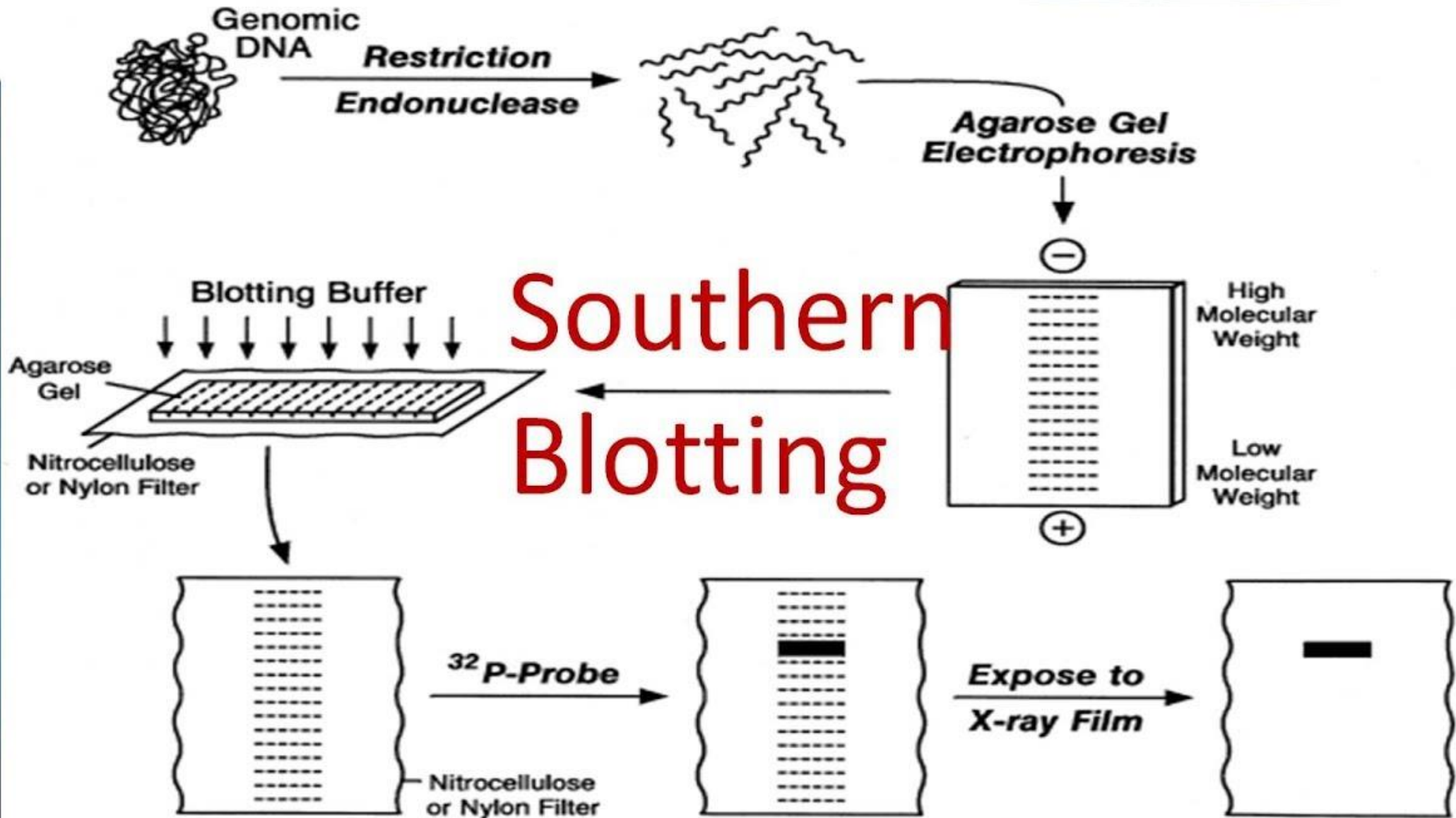


Transform bacteria

DNA Hybridization

Southern blot

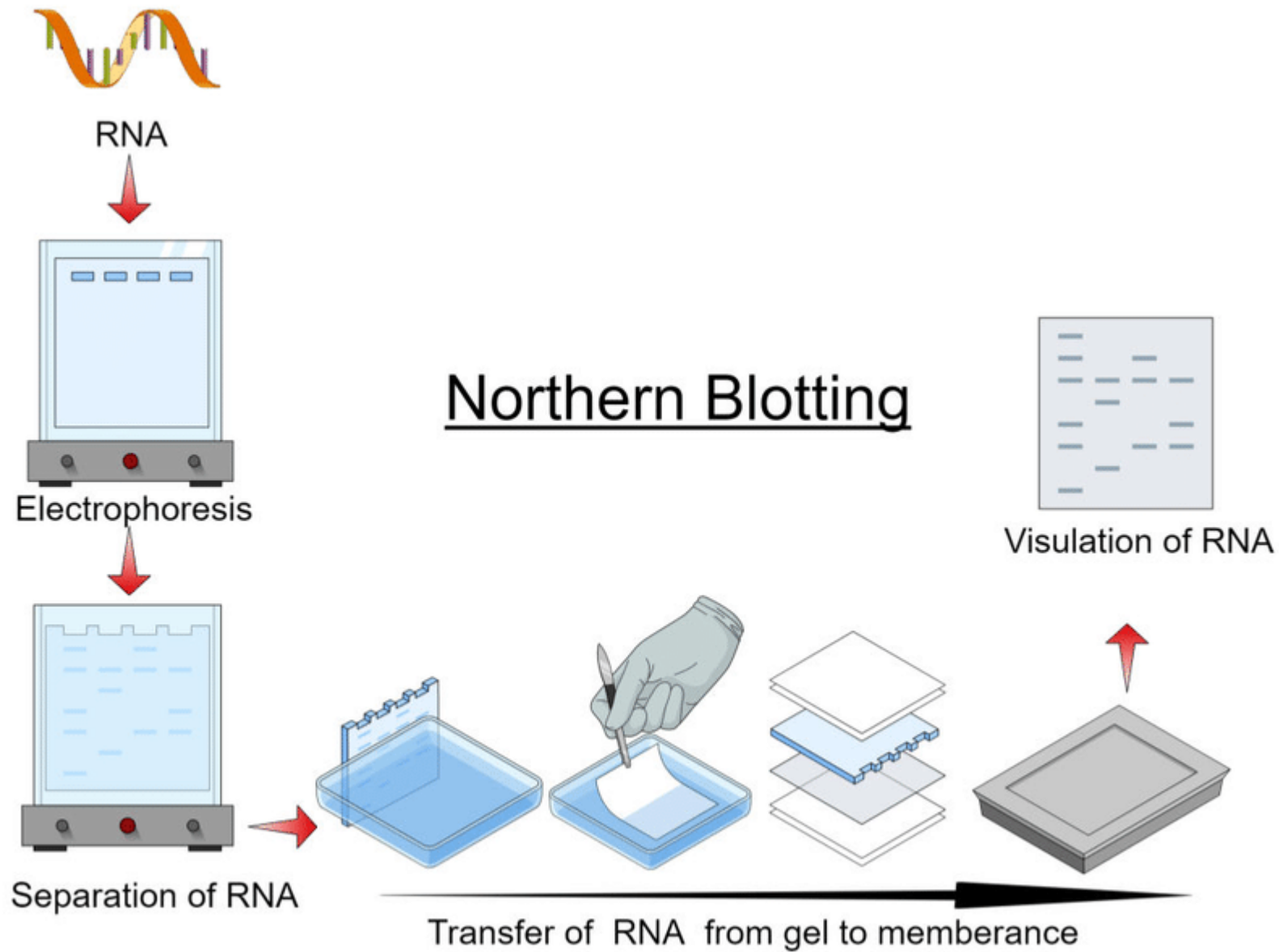
- ✓ allows the detection of a gene of interest by probing DNA fragments that have been separated by gel electrophoresis with a “labeled” probe.
- ✓ It is used to detect a specific DNA sequence in blood or tissue sample of the animal.



DNA Hybridization

Northern Blot

- ✓ probes RNA on a gel with a DNA probe
- ✓ It is used to study gene expression by detection of RNA (or isolated mRNA) in a sample.



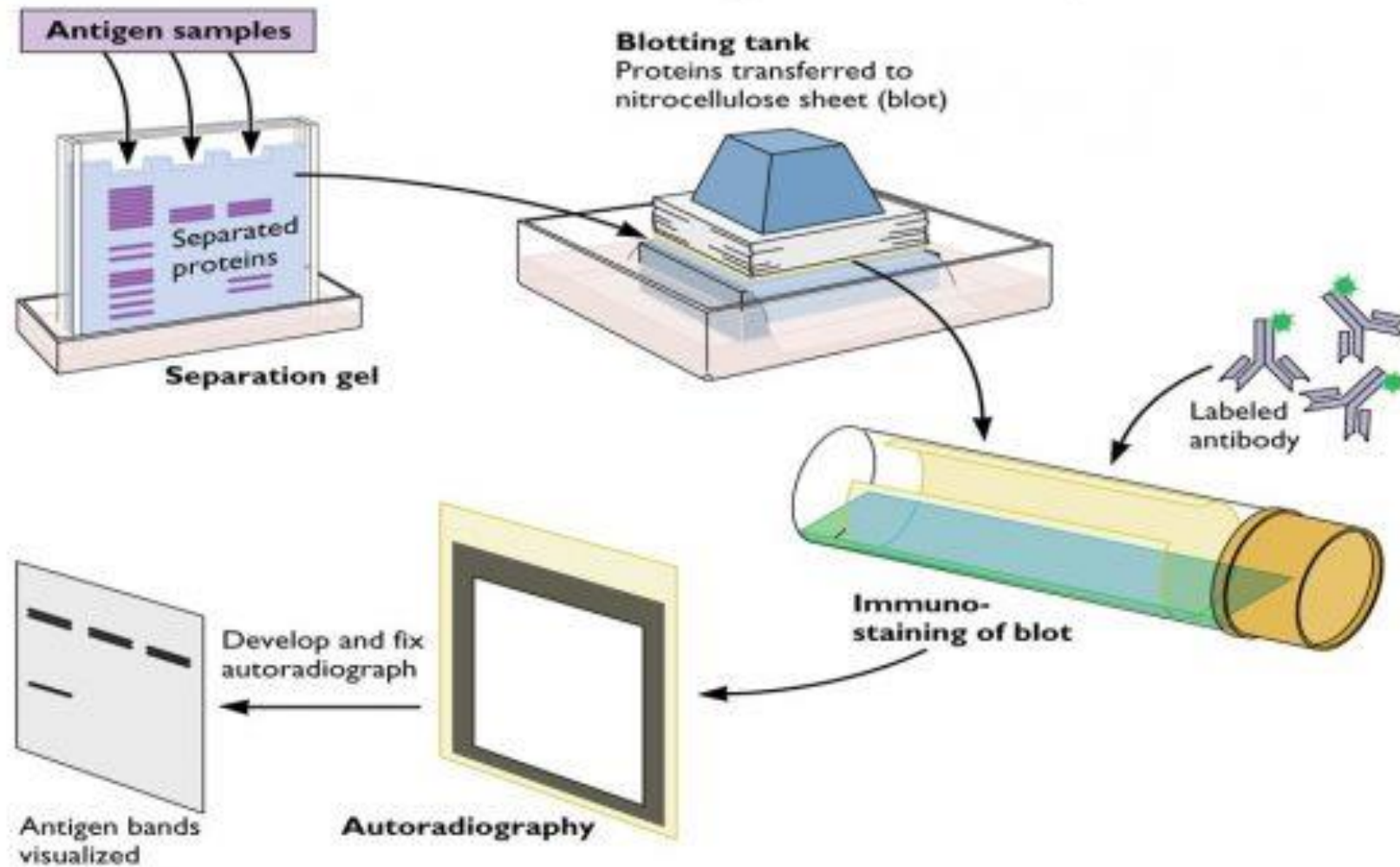
<https://www.researchgate.net/publication/318305214/figure/fig1/AS:514025217851392@1499564570665/General-schematic-of-Northern-blotting.png>

DNA Hybridization

Western Blot

- ✓ probes proteins on a gel with an antibody
- ✓ It is used to detect specific proteins in the given sample.
- ✓ Also called protein blotting or immunoblotting

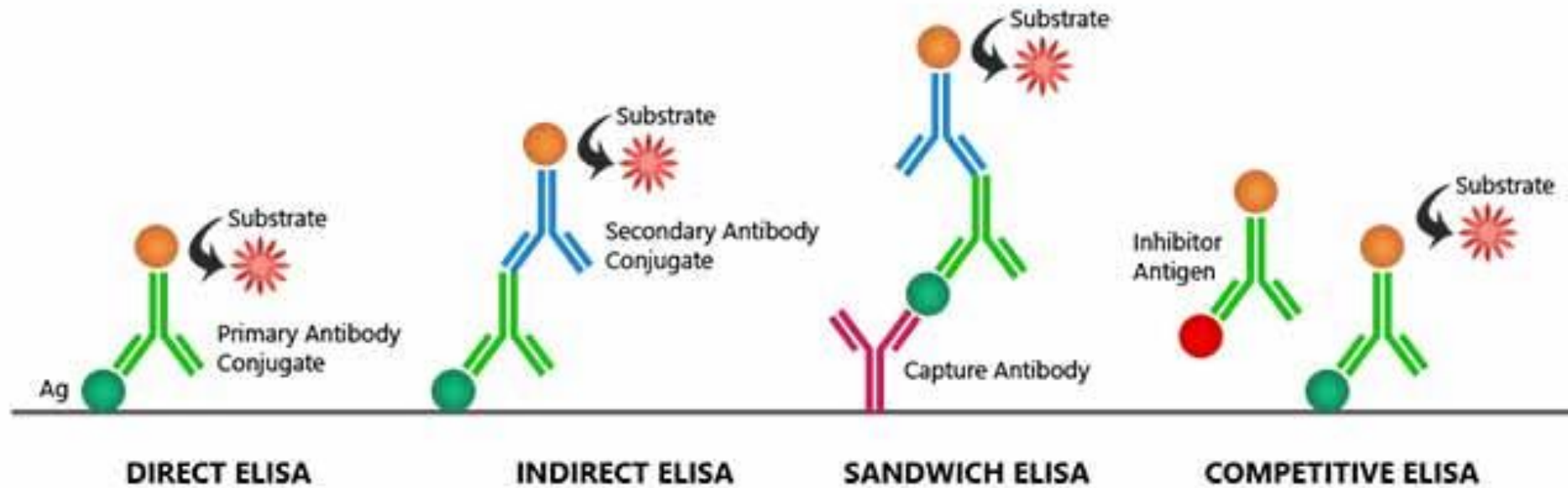
Western Blotting Technique



Enzyme-linked immunosorbent assay (ELISA)

- The enzyme-linked immunosorbent assay (ELISA) can be used to measure either the pathogen or the host antibody response from any aquatic species
- It has the advantage of high throughput, and can be automated (and is quantitative for which standards are required).
- Normally, the serum is screened using an indirect antibody capture ELISA to measure the fish's antibody response.
- The specificity of the test is dictated by the pathogen used to coat the ELISA plate, and the fish species screened by the availability of anti-fish species antibody

Types of ELISA



<https://www.asiaradiosales.com/wp-content/uploads/2019/05/Enzyme-linked-immunosorbent-assay-ELISA-and-its-types.jpg>