

A microscopic image showing numerous cells, likely bacteria or yeast, that have been stained with a blue fluorescent dye. The cells are scattered across the field of view, with some appearing as individual dots and others as small clusters. The background is dark, making the bright blue fluorescence of the cells stand out.

Chemiluminescence

BY

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LUMINESCENCE

- ❖ “Cold light” that can be emitted at **lower temperature**
- ❖ Source kicks an electron of an atom out of its lowest energy “ground” state into a higher energy “excited” state
- ❖ Finally electron returns the **energy** in the form of **light** so it can fall back to its “ground” state

TYPES LUMINESCENCE

Excitation event		process
Chemicals	Luminol Isoluminol acridinium ester	Chemiluminescence
Biochemical	Luciferin aequorin	Bioluminescence
Electromagnetic	Ruthenium Tris (bipyridly) chelate	Electroluminescence
Photons	inorganic phosphors	Photoluminescence

CHEMILUMINESCENCE

Emission of light with limited emission of heat (luminescence), as the result of a chemical reaction.



[A], [B]: reactants

[\diamond]: excited intermediate

For example, if [A] is luminol and [B] is hydrogen peroxide in the presence of a suitable catalyst we have:



Where:

- 3-APA is 3-aminophthalate
- 3-APA[\diamond] is the excited state producing light as it decays to a lower energy level.

CHEMILUMINISCENCE



Luminol and peroxidase
before adding H_2O_2



Chemiluminescence after
addition H_2O_2

Application of Chemiluminescence

- **Chemiluminescence immunoassay**
- **DNA hybridization detection**
- **Western blotting**
- **Forensic science**
- **Food analysis**

CHEMILUMINESCENCE IMMUNOASSAY

Provides a **sensitive, high throughput** alternative to conventional colorimetric methodologies

Principle: -same as ELISA

- uses chemiluminescent substrate, hydrogen peroxide, enhancers
- stopping reagent is not required
- Incubation period is small

Monoclonal antibody coated well



Test specimen (serum)



HRP labelled antibody conjugate



Test antigen: sandwich between solid phase Ab
and enzyme labelled Ab



Incubate for 1 hr at 37° C



Remove unbound enzyme labeled Ab



Chemiluminescence reagent added



Read **relative light unit** with luminometer

- **USES**

- ✓ Hormones: insulin, thyroxin, estradiol, testosterone, progesterone
- ✓ Vitamin: vit B12
- ✓ Tumor markers: bone morphogenic protein-2, carcino embryonic antigen (CEA), alpha fetoprotein (AFP)
- ✓ Human beta chorionic gonadotropin
- ✓ C-reactive protein
- ✓ Tumor necrosis factor

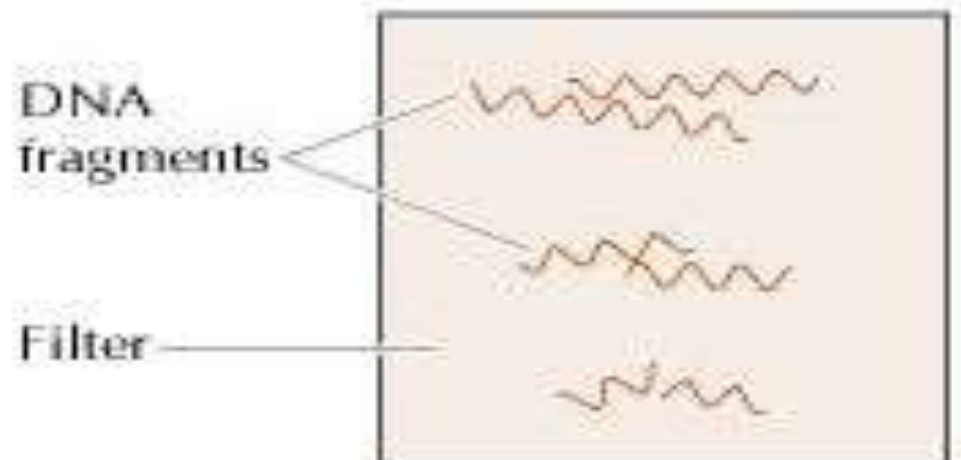
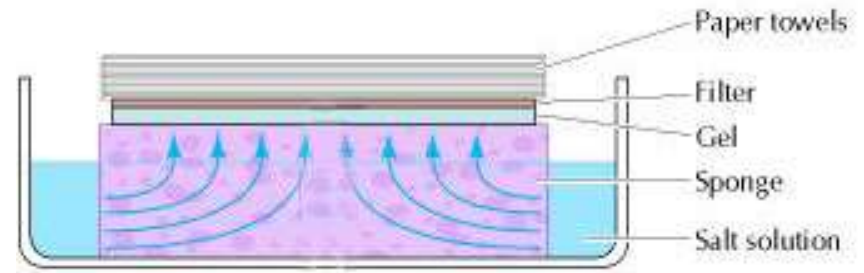
DNA hybridization detection

Southern blotting Involves DNA separation, transfer and hybridization

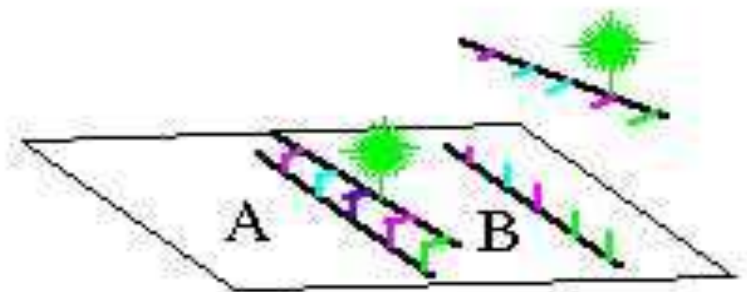
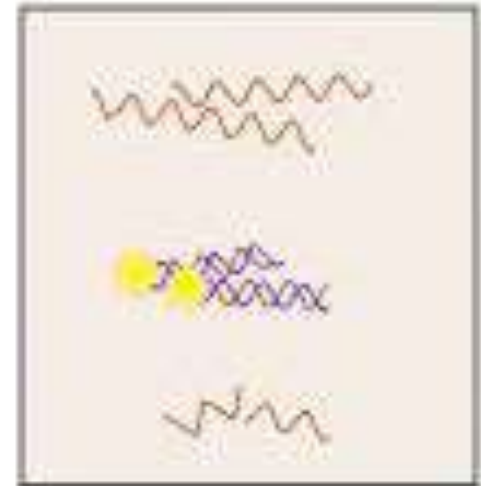
Hybridization - Process of forming a double-stranded DNA molecule between a single-stranded DNA probe and a single-stranded target DNA

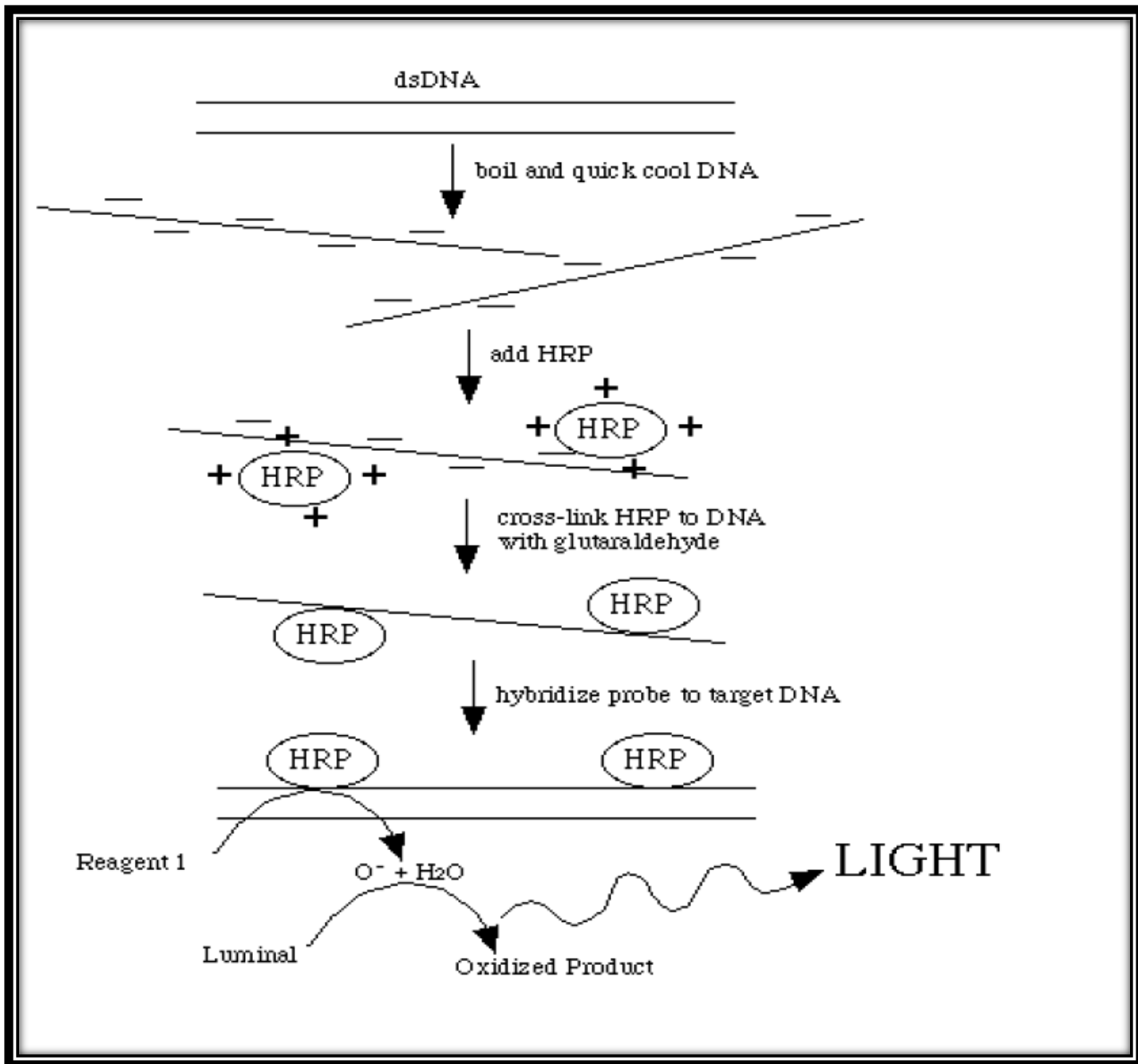
3. The restriction fragments present in the gel are denatured with alkali and transferred onto a nitrocellulose filter or nylon membrane by blotting.

- This procedure preserves the distribution of the fragments in the gel, creating a replica of the gel on the filter.



4. The filter is incubated under hybridization conditions with a specific HRP labelled DNA probe.
- The probe hybridizes to the complementary DNA restriction fragment.
 - Detection reagent containing H_2O_2 & luminol is added onto the membrane



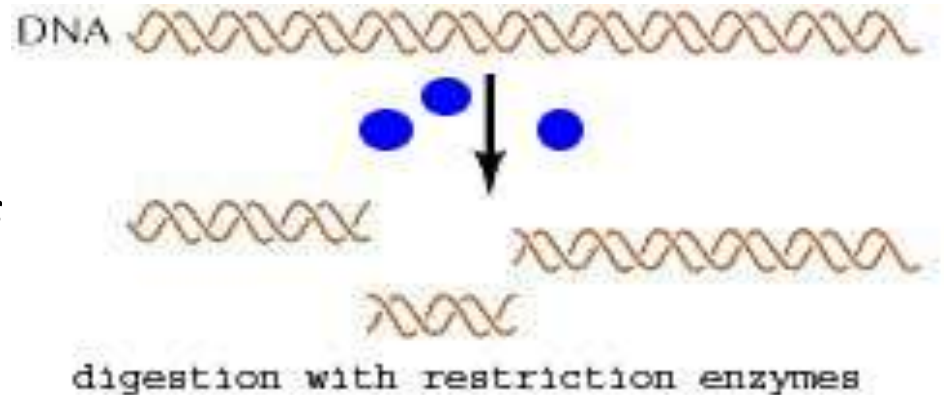


- **USES**

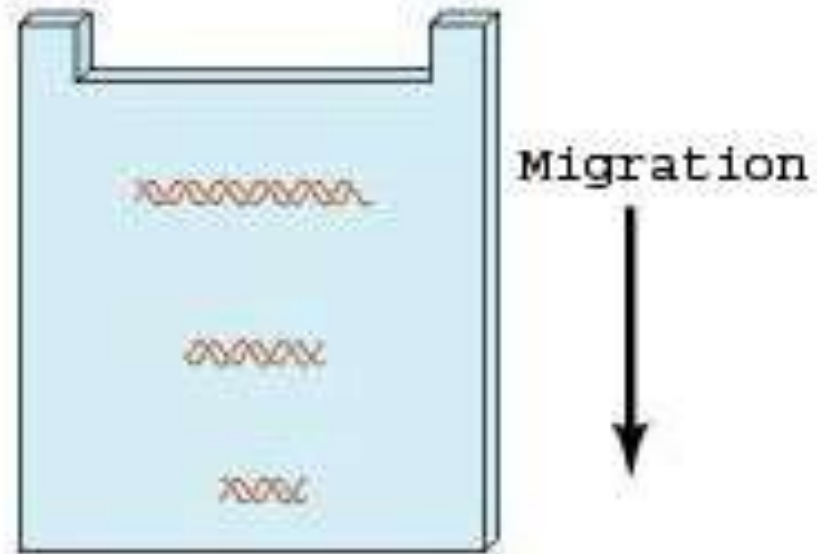
- ✓ Identifying DNA in crime case
- ✓ Paternal Dispute
- ✓ Classify DNAs of various organism

Steps in southern blotting

1. The DNA to be analyzed, such as the total DNA of an organism, is digested to completion with a restriction enzyme.



2. The complex mixture of fragments is subjected to gel electrophoresis to separate the fragments according to size.



Western blotting

➤ Western blotting (or protein immunoblotting) is a technique widely used to detect specific proteins in samples of tissue homogenate, cell lysates, cell culture supernatants or body fluids.

Western blotting



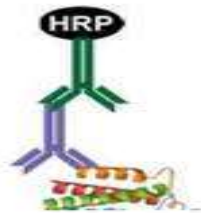
Sample preparation



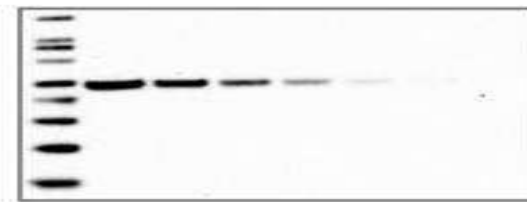
Gel electrophoresis



Protein transfer



Immunodetection



Imaging

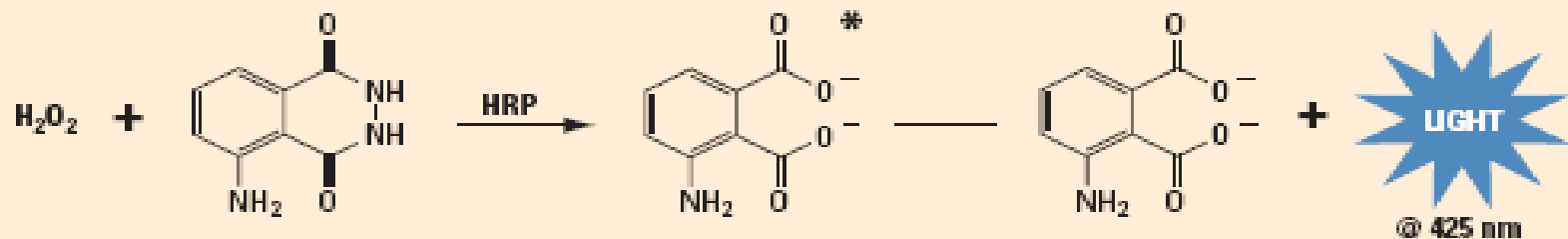


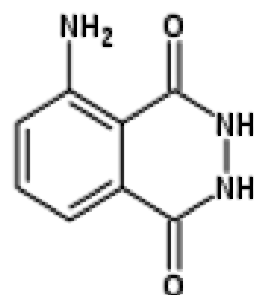
Figure 1. Luminol is oxidized in the presence of HRP and hydrogen peroxide to form an excited state product (3-aminophthalate). The 3-aminophthalate emits light at 425 nm as it decays to the ground state.

Forensic science

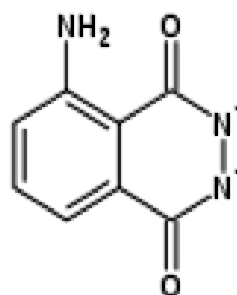
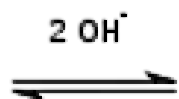
Chemiluminescence is used by criminalists to detect traces of blood at crime scene

Solution: luminol powder ($C_8H_7O_3N_3$), hydrogen peroxide, and a hydroxide (eg. KOH) sprayed where blood might found

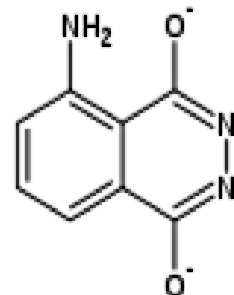
Tiny amount of iron from Hb in blood serves as **catalyst** for the chemiluminescence reaction, luminol to glow



Luminol



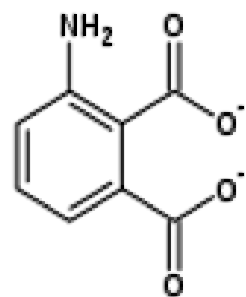
Dianion



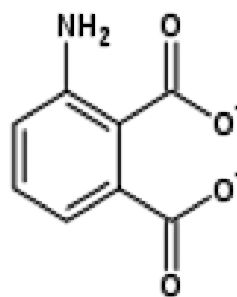
Dianion



Reaction with Q
produces unstable peroxide



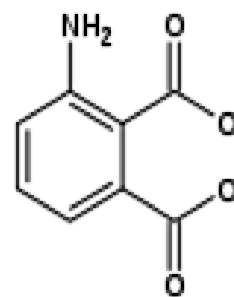
Ground state dianion (S_0)



Singlet dianion (S_1)

Excited state

Intersystem
crossing



Triplet dianion (T_1)

Excited state





A trail of blood made visible with the use of the reagent luminol.

Food analysis

- Organophosphorous most popular pesticide
- Most commonly used organophosphorous:
QUINALPHOS (O,O diethyl-o-quinoxaliny
phosphorothioate)

Food analysis

Quinalphos + H₂O₂ \longrightarrow peroxyphosphonate

Peroxyphosphonate + luminol \longrightarrow 3-aminophthalate anion*

3-aminophthalate* \longrightarrow 3-aminophthalate +
observed emission

Limitations

- Light leaks from assay reagent & reaction vessels
- Ultra sensitive – stringent controls on purity of reagents
- High intensity light emission leads to pulse pileup in photomultiplier tubes leads to underestimation

THANK YOU

