Biotechnology

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The most important biotechnologies are :

1-MAb technology.

2-Cell Culture (Animal and plant cells).

3-Bioprocessing technology تقنية المعالجة الحيوية

Biodegradation التدرك الحيوي

- ✓ Using microbes to degrade unwanted substances helps to lessen the impact human activities have had on the environment.

- ➢ *Bioremediation*: the use of microbial populations to clean up pollution.

- ➢ *Phytoremdiation*:

- ✓ Use certain plants to treat contamination caused by sources of pollution, such as waste-water from certain industrial manufacturing facilities.

4-Biosensors

5-Genetic Engineering: (Recombinant DNA technology).

- Recombinant DNA is made by joining genetic material from two different sources.
- Nature joins genetic material from two sources:

✓ Crossing over: between homologous maternal and paternal chromosomes during gamete formation

6-Protein Engineering.

(often in conjunction with genetic engineering).

Oligonucleotide-directed mutagenesis

7-Cloning.

Technology to generate a population of genetically identical molecules, cells, plants, or animals.

8-Antisense technology

- Antisense technology is being used to block or decrease the production of certain proteins.

9-Microarray technology

- analyze tens of thousands of samples simultaneously.

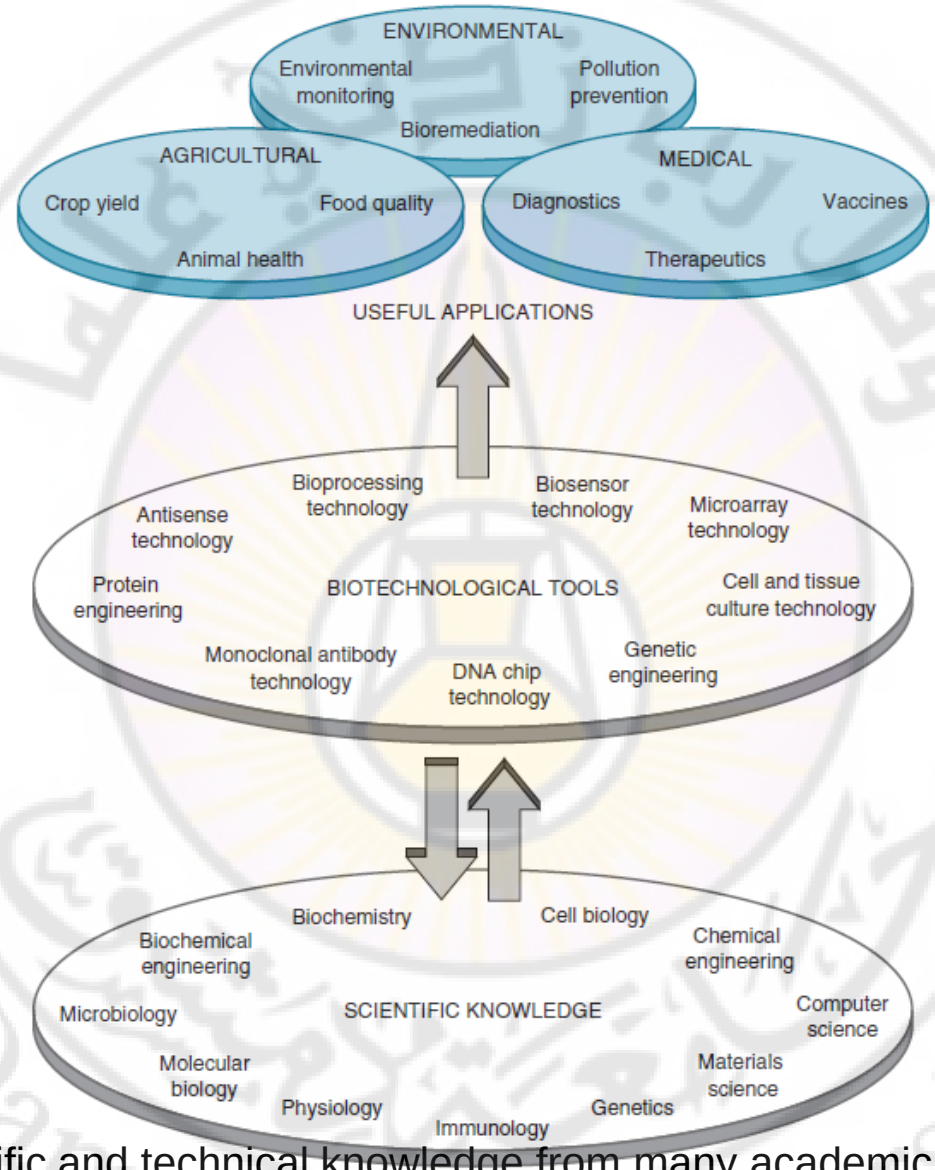
For example, thousands of different DNA, RNA, or protein molecules are placed on glass slides in a grid-like array to create DNA chips, RNA chips, and protein chips.

10-Bioinformatics technology

Bioinformatics is the use and organization of information about biology.

11-Nanobiotechnology

- ✓ the study, manipulation, and manufacture of ultrasmall structures and machines made of as few as one



Synthesis of scientific and technical knowledge from many academic disciplines has produced a set of enabling technologies—the biotechnologies. Any one technology will be applied to a number of industries to produce an even broader array of products.

Medical biotechnology

- **Diagnostics**
- **Therapeutics**

MONOCLONAL-ANTIBODY (MAB) TECHNOLOGY

M o n o c l o n a l A n t i b o d i e s :

- Antibodies that are derived from only one cell and recognize only one portion of a molecule.
- A mouse is immunized by injection of an antigen X to stimulate the production of antibodies targeted against X.
- The antibody forming cells are isolated from the mouse's spleen. Fusing single antibody-forming cells to tumor cells grown in culture produces monoclonal antibodies. The resulting cell is called a hybridoma (ورم هجين).
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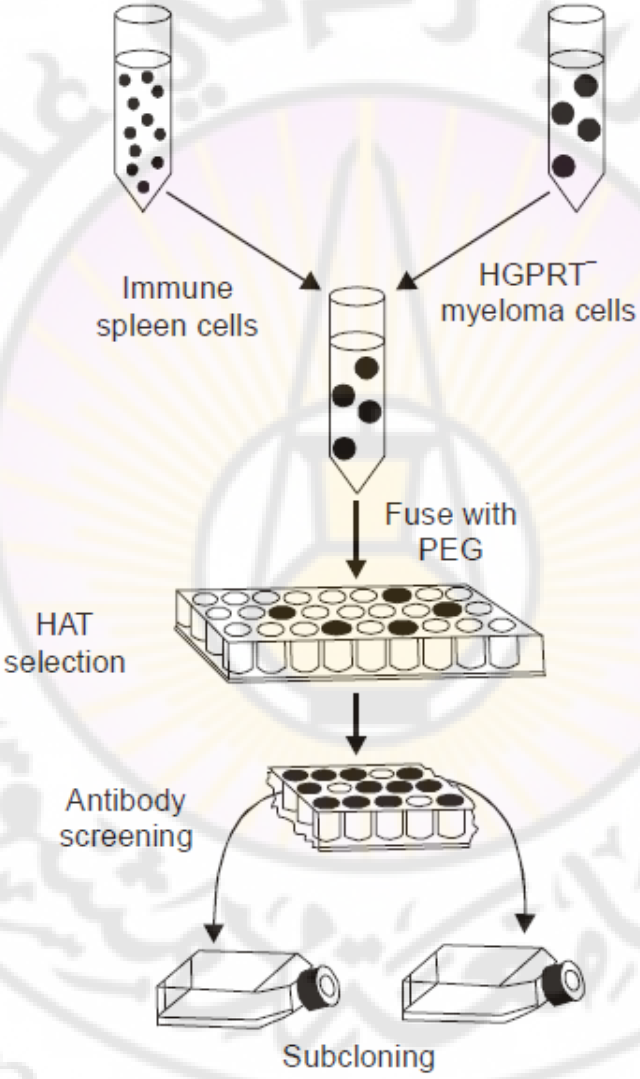
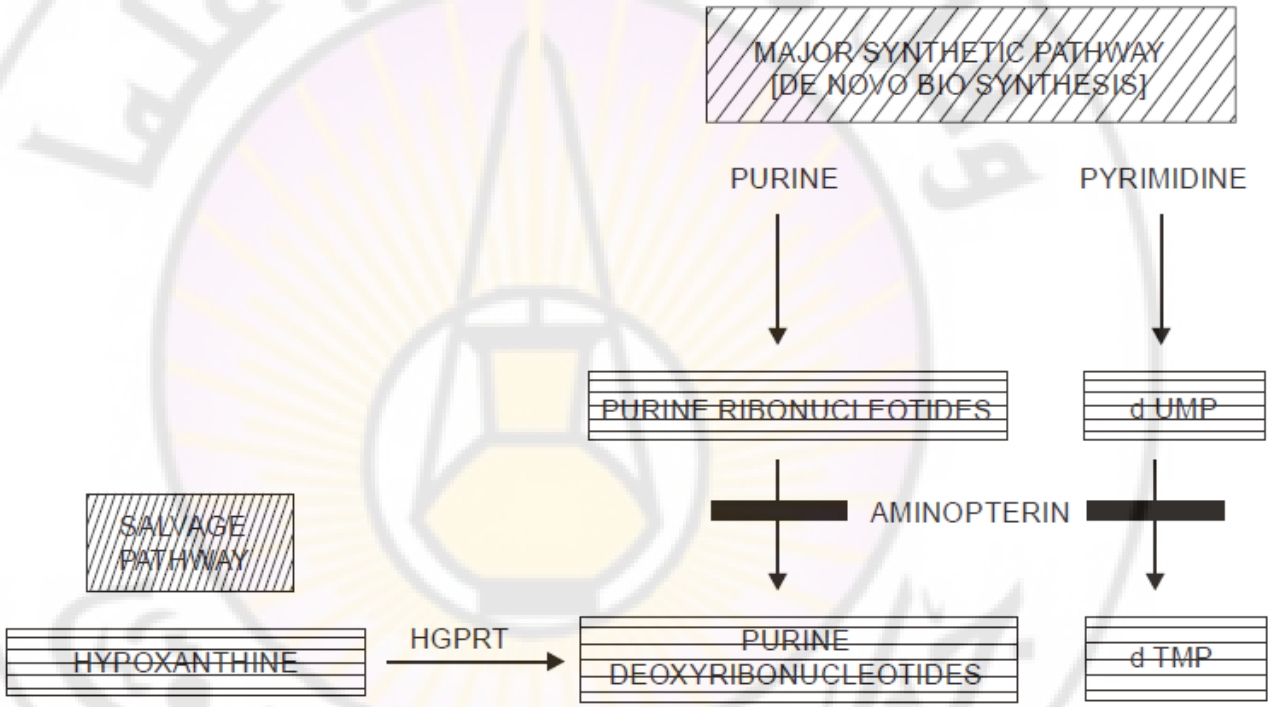


Immune spleen cells and HGPRT myeloma cells are fused with polyethylene glycol resulting in hybrid cells.

Cells are distributed in microwell plates and grown in HAT medium. Unfused myeloma cells die because they have no HGPRT to use the salvage pathway. Unfused spleen cells are unable to grow *in vitro* ; only fused cells grow[●].

Fused cells (hybridomas) grown in microwells and supernate of each well tested for specific antibody production. Wells making antibody of interest are subcloned and grown in larger volumes.


MAJOR SYNTHETIC PATHWAY
[DE NOVO BIO SYNTHESIS]
PURINE
PYRIMIDINE
PURINE RIBONUCLEOTIDES
d UMP
AMINOPTERIN
SALVAGE PATHWAY
HYPOXANTHINE
HGPRT
PURINE DEOXYRIBONUCLEOTIDES
d TMP

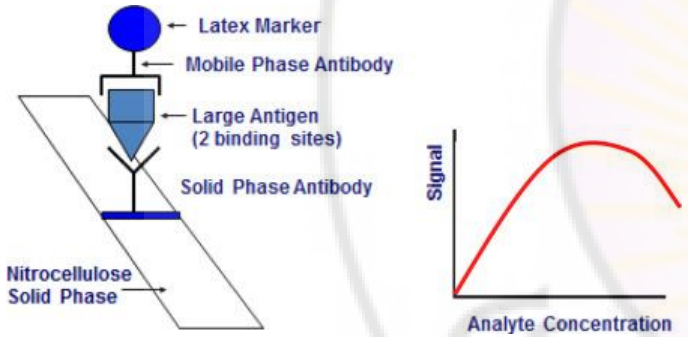
Genetically engineering: For example, the humanized monoclonal antibody drug Herceptin (trastuzumab)

Using Monoclonal Antibodies to Combat Ebola

- ZMapp is a combination of three mAbs produced by incorporating the antibody genes into tobacco plants (plantibodies—plant-produced mAbs) using a viral vector.

Diagnostic and Therapeutic Uses

- MAb can bind to hCG, to diagnose a number of infectious diseases.



- Immunoprecipitation.

Cancer therapies.

- A MAb that specifically binds to tumors and is tagged with a radioisotope or toxin (**immunotoxins**).
- **Abzymes:** a new type of monoclonal antibody has been designed to behave like enzymes.

Immunosuppressive therapies.

- In organ transplant rejections and autoimmune diseases, suppressing our immune system would be in our best interest.
- **OKT3 (Otelixizumab, teplizumab, visilizumab)**
- In **autoimmune diseases**, such as rheumatoid arthritis and multiple sclerosis.

Monoclonal antibodies directed against the pathogenic cytokine and cellular elements within the RA synovium

1-Initial studies using mAbs in RA utilized anti-CD4, anti-CD7

2-against TNF-a, CD20-positive B cells, IL-1 and IL-6.

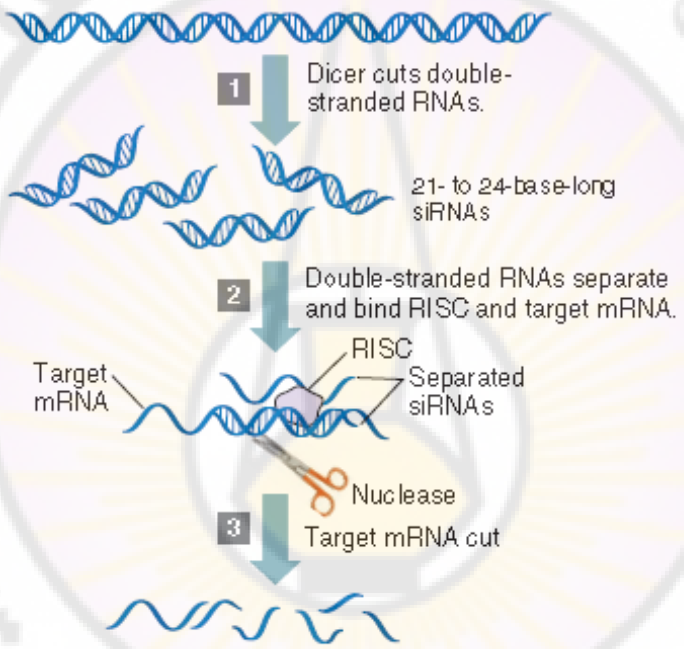
- Infliximab
- Golimumab
- Tocilizumab TCZ

Applications

- Immunoblot Assay: The Western Blot Western blotting.
- Soluble antigens can be detected in a patient's serum
- One powerful use of EIA is immunostaining, in which antibody-enzyme conjugates enhance microscopy.
- **Flow Cytometry** : uses fluorescent mAbs against cell-membrane proteins to quantify specific subsets of cells in complex mixtures.

RNA interference

- ✓ miRNA (micro-RNA), dsRNA (double-stranded RNA), siRNA (small interfering RNA), and shRNA (short hairpin RNA), all of which are involved in RNAi (RNA interference).
- ✓ RNA interference (RNAi) is a process of post-transcriptional gene silencing mediated by short double-stranded RNA molecules called siRNA (small interfering RNAs).



RNA interference. Dicer cuts doublestranded

parts of RNA molecules, which then associate with RNA-induced silencing complexes (RISCs). The RNA is open, revealing single strands that locate and bind specific mRNAs. Nucleases then break down the targeted mRNAs, preventing their translation into protein

Bioinformatics

Primer selection

Critical variables are:

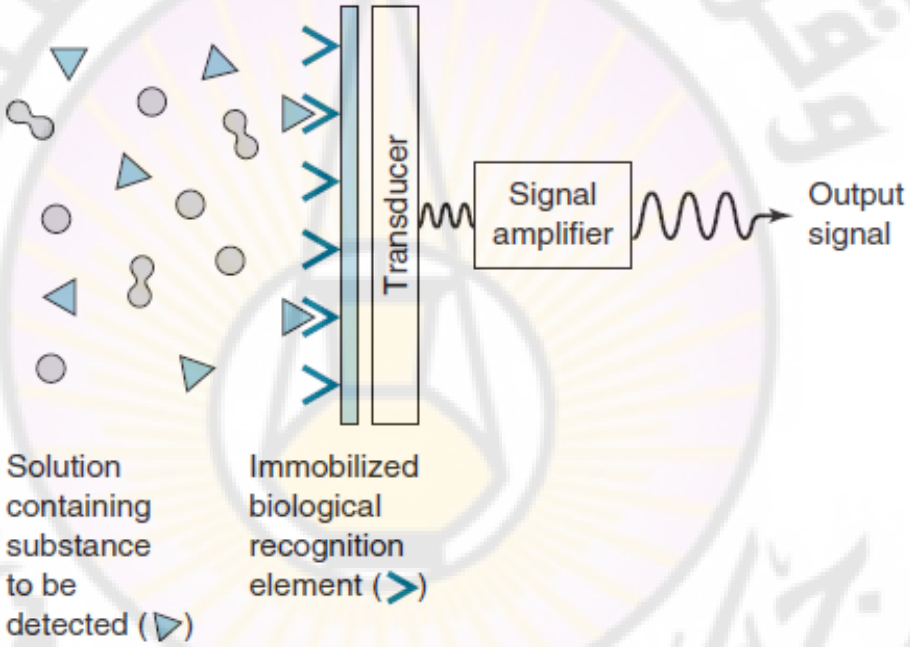
- primer length
- melting temperature (T_m)
- complementary primer sequences
- GC% content
- 3'-end sequence

The Basic Local Alignment Search Tool (**BLAST**) finds regions of local similarity between sequences.

Primer Design Using Software (Primer Premier Software)

$$T_m = ((\text{Number of G+C}) \times 4°C + (\text{Number of A+T}) \times 2°C)$$

BIOSENSORS



Schematic drawing of a simple biosensor

WHAT IS BIOSENSOR?

- Biosensors are small devices that utilize biological reactions for detecting target analytes.
- The analytical device essentially consists of a *biocatalyst* and a *transducer*.
- The transducer effectively converts the biological or biochemical signal produced by the catalyst into a quantifiable signal.
- The common transducing elements including optical and electrochemical generate light and current signals, respectively.

TRANSDUCERS

- The key component of a biosensor is the transducer, which utilizes a physical change accompanying the given reaction. The change may be heat absorbed or released by the reaction: **Calorimetric** biosensors,
- or may refer to the distribution of charges leading to an electrical potential to be produced: **Potentiometric** biosensors.
- Alternatively, it may represent the movement of electrons produced in a redox reaction: **Amperometric** biosensors.
- In addition, transducers also signify light output during the course of the reaction or difference in light absorbance between the reactants and products (**optical** biosensors),
- and those denoting effects are attributable to the mass of the reactants or products (piezo – **electric** biosensors).

CLASSIFICATION OF BIOSENSORS

- *Bioaffinity devices*: These rely on the selective binding of target analyte to a surface-restricted ligand partner (e.g., antibody, oligonucleotide, DNA, cell…)
- *Biocatalytic devices:* An immobilized enzyme is used to help recognize target substrate. e.g., Sensor strips with immobilized GOX have been in vogue for personal/home monitoring of diabetes mellitus.
- Glucose biosensors account for 90% of the market worldwide.

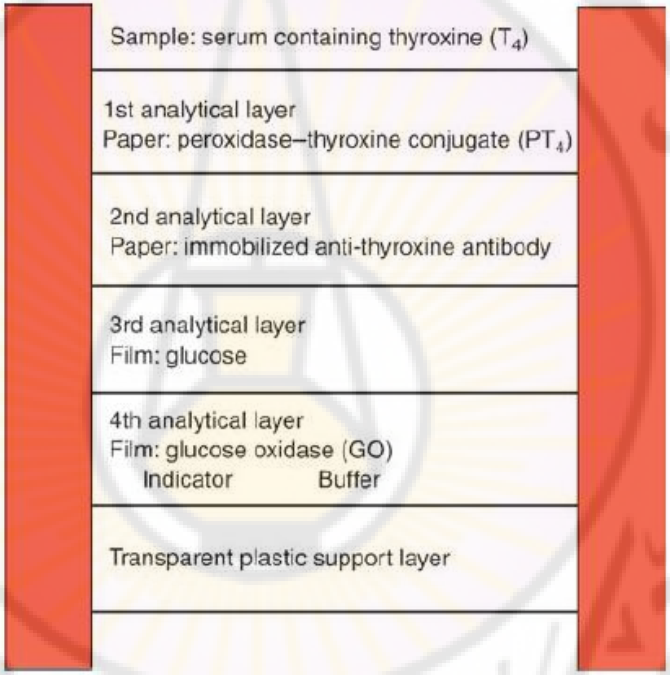


Figure 43.4 The structure of a Fuji® thyroxine reagent strip