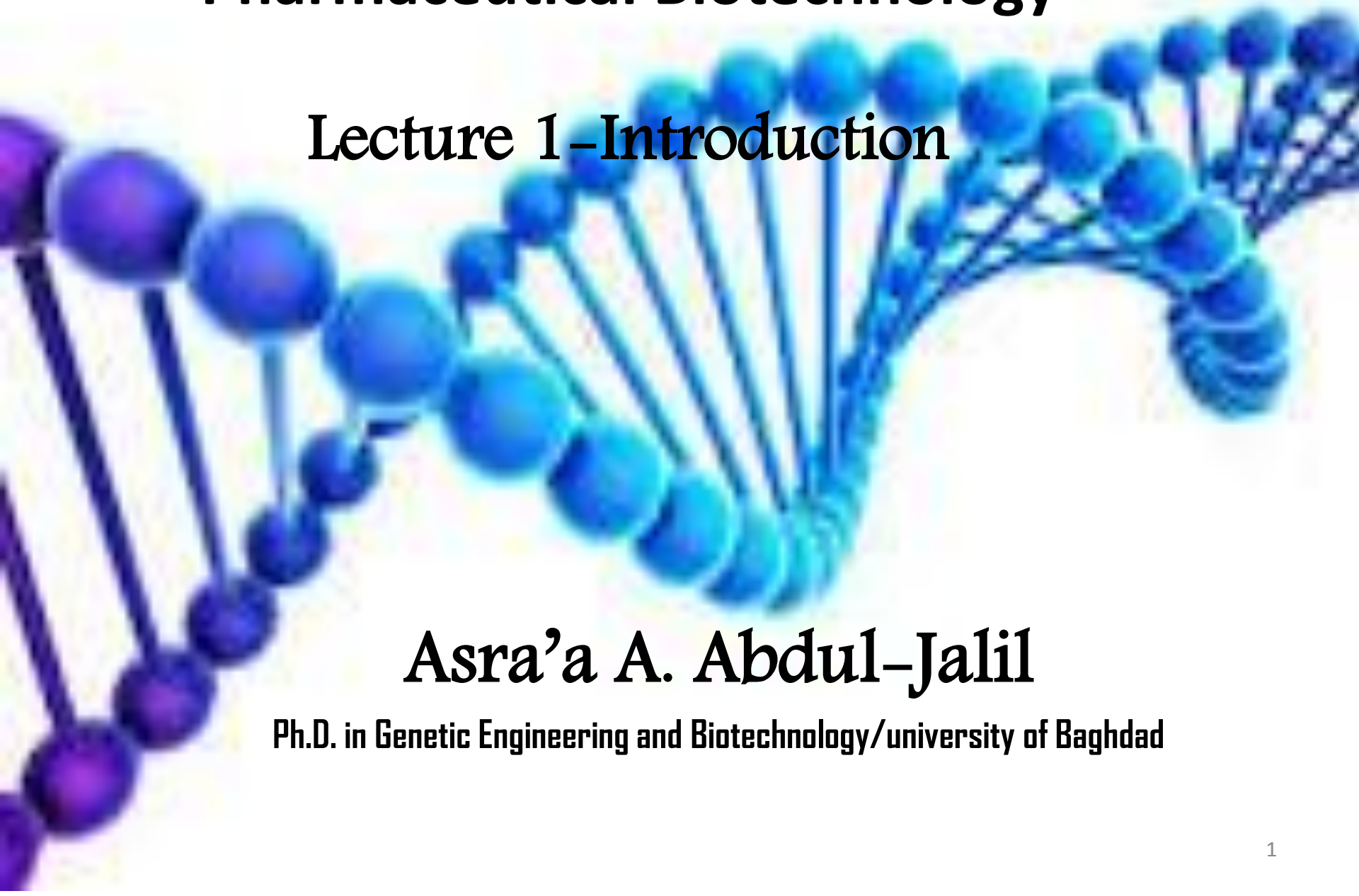
Pharmaceutical Biotechnology

Lecture 1-Introduction



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- ✓ General concepts of Molecular Biology
- ✓ Recombinant DNA technology
- ✓ Production of Human Insulin by Synthetic DNA

References

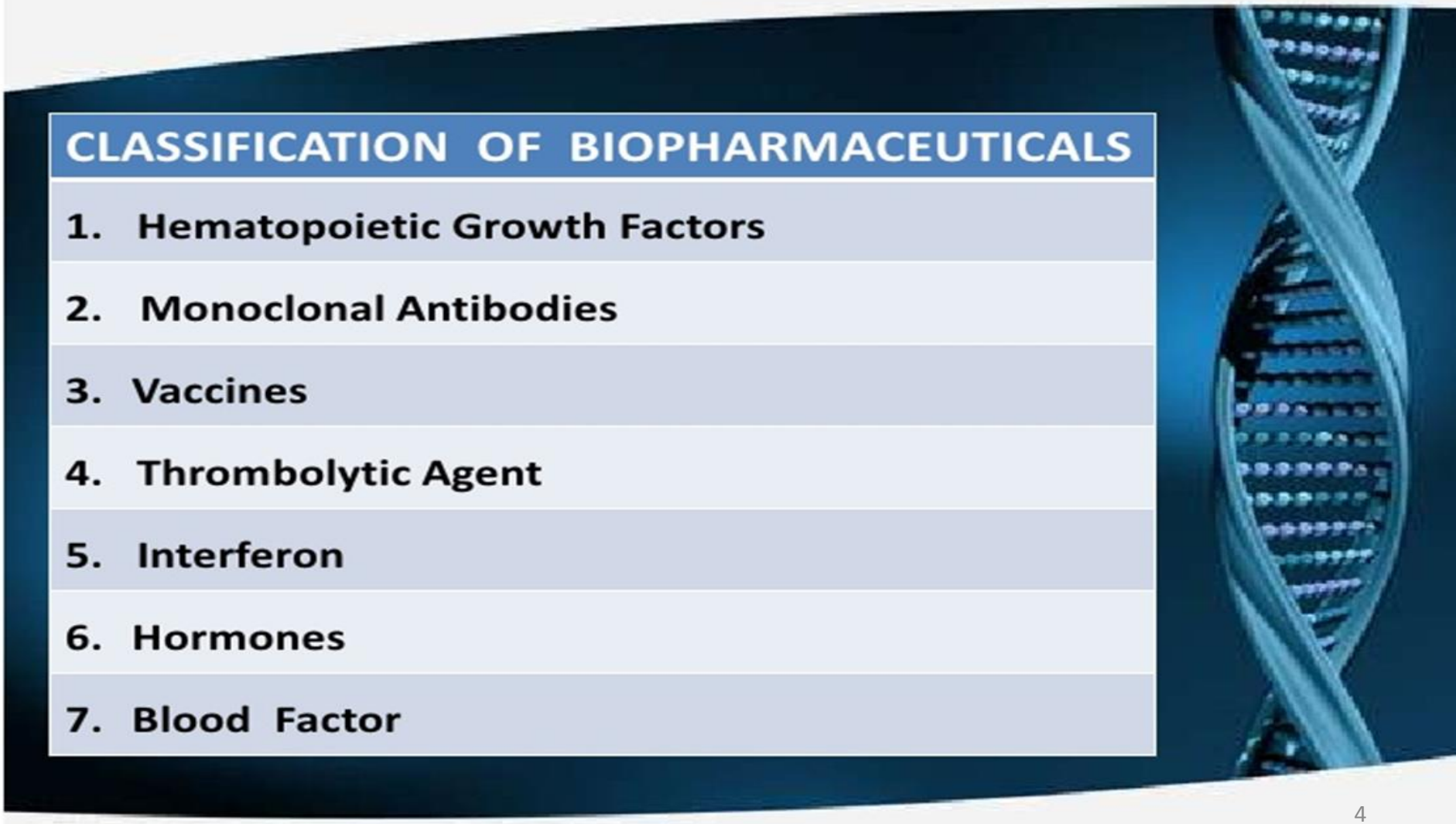
1. **Pharmaceutical biotechnology**

J . A . Crommelin , Robert D. Syinder

2. Biophrmaceuticals

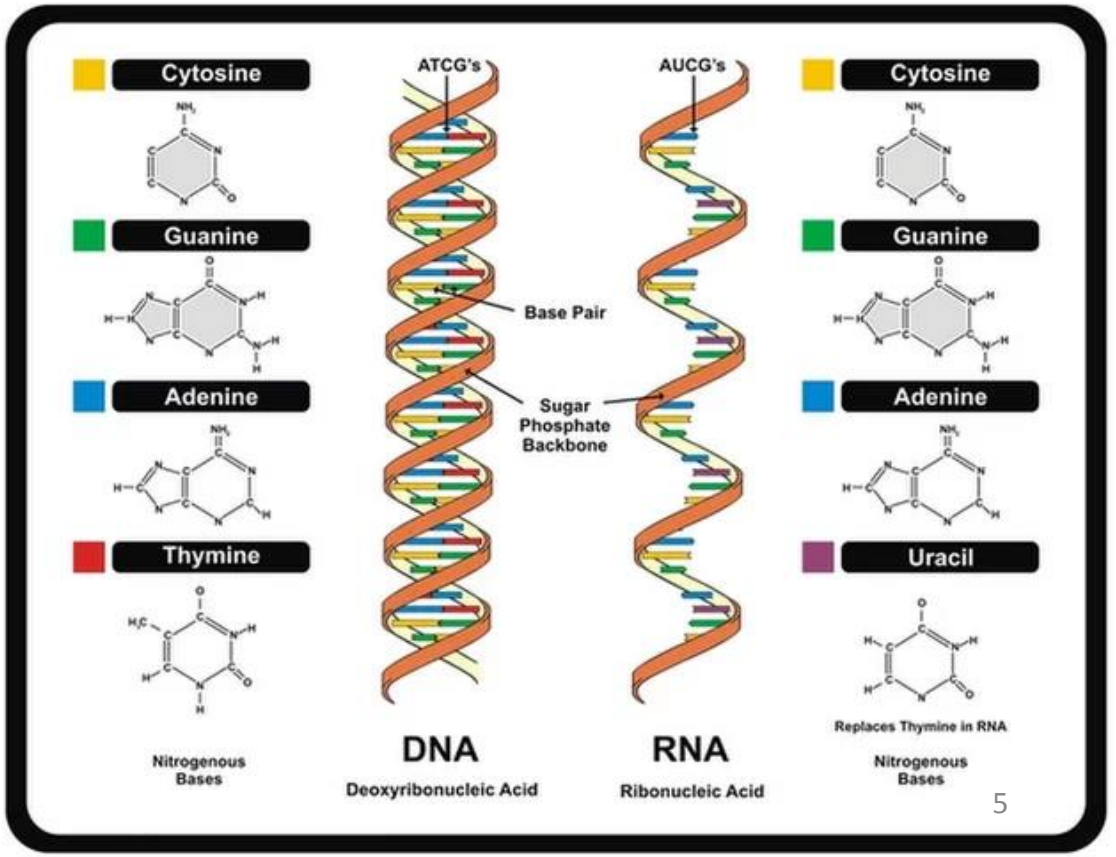
Walsh G.

REPORTS


CLASSIFICATION OF BIOPHARMACEUTICALS
1. Hematopoietic Growth Factors
2. Monoclonal Antibodies
3. Vaccines
4. Thrombolytic Agent
5. Interferon
6. Hormones
7. Blood Factor

General concepts

- **Genetic material!!!**
- **Building Blocks!!**
- **Gene expression**
- **Codons**



Abbreviations

AA	amino acid(s)
Ab	Antibody
Ag	Antigen
BRCA1	breast cancer gene 1
cDNA	copy DNA/ complementary deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EMBL	European Molecular Biology Laboratory
FDA	Food and Drug Administration
DDBJ	DNA Data Bank of Japan
FSH	follicle-stimulating hormone
GF	Growth Factor
HPLC	high-performance liquid chromatography
IV	intravenous
Kb	thousand base pairs of DNA
HIV	Human immunodeficiency virus

PHARMACEUTICAL BIOTECHNOLOGY

INTRODUTION:

Pharmaceutical biotechnology consist of the combination of two branches which Are "PHARMACEUTICAL SCIENCE" AND "BIOTECHNOLOGY".

DEFINATION:

PHARMACEUTICAL SCIENCE: Can simply be define as the branch of science that deals with the formulation and dispensing of drugs

BIOTECHNOLOGY: Can simply be define as the application of biological system, living organisms, or their derivatives in making or modifying products or processes for specific use.

PHARMACEUTICAL BIOTECHNOLOGY : **Can simply be define as the science that covers all technologies required for the production, manufacturing and registration of biological drugs.**

The aim of this pharmaceutical biotechnology is to design, produce drugs that are adapted to each persons genetic make up,which can give the maximum therapeutic effect.

Biotechnology plays an important role in pharmaceutical science most especially in the pharmaceutical industries by creation of genetically modified organisms that can be used in industrial production.

Recombinant DNA technology

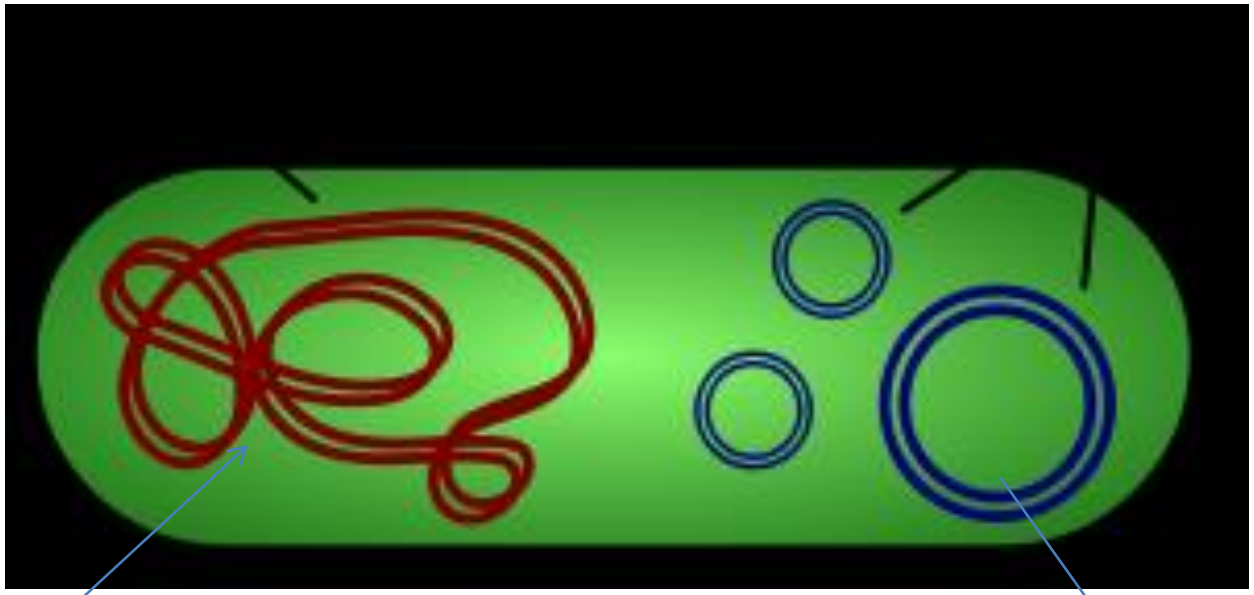
Other names:

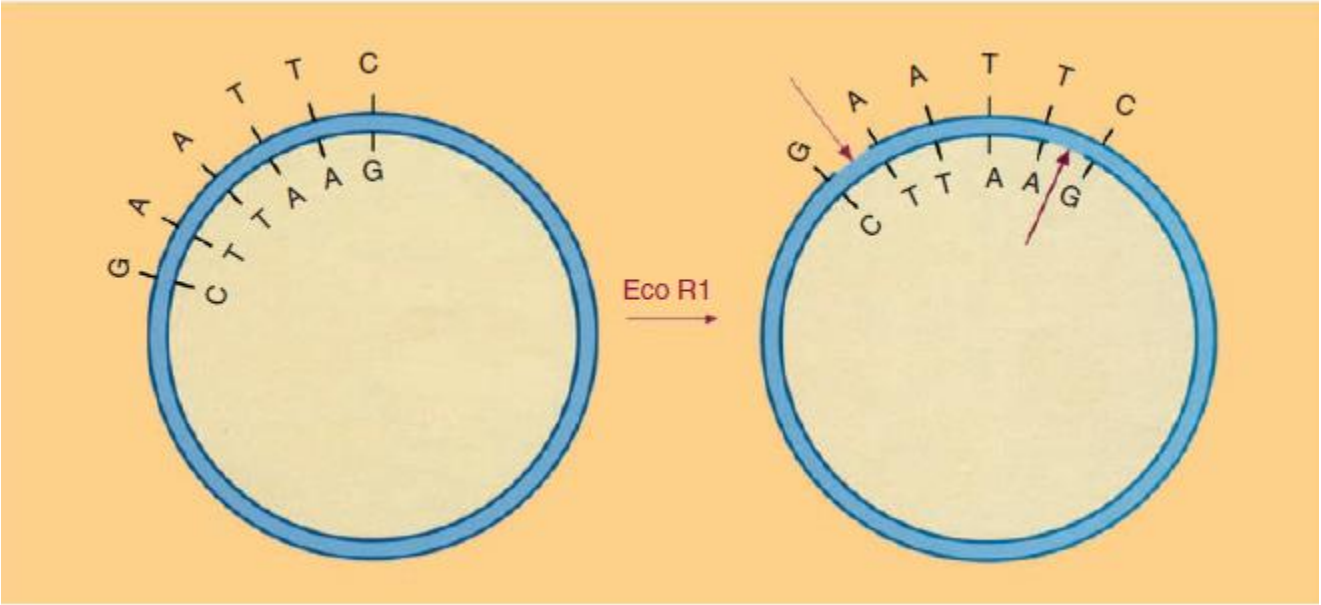
(Genetic modification) OR (DNA cloning) OR (genetic engineering)

Adding of foreign DNA(desired gene) to microbial cells, plant cells or animal cells to form genetically Modified Organism(GMO)

Plasmids usually used as a vector for a foreign DNA after treating them with Restriction enzymes such as ECORI.

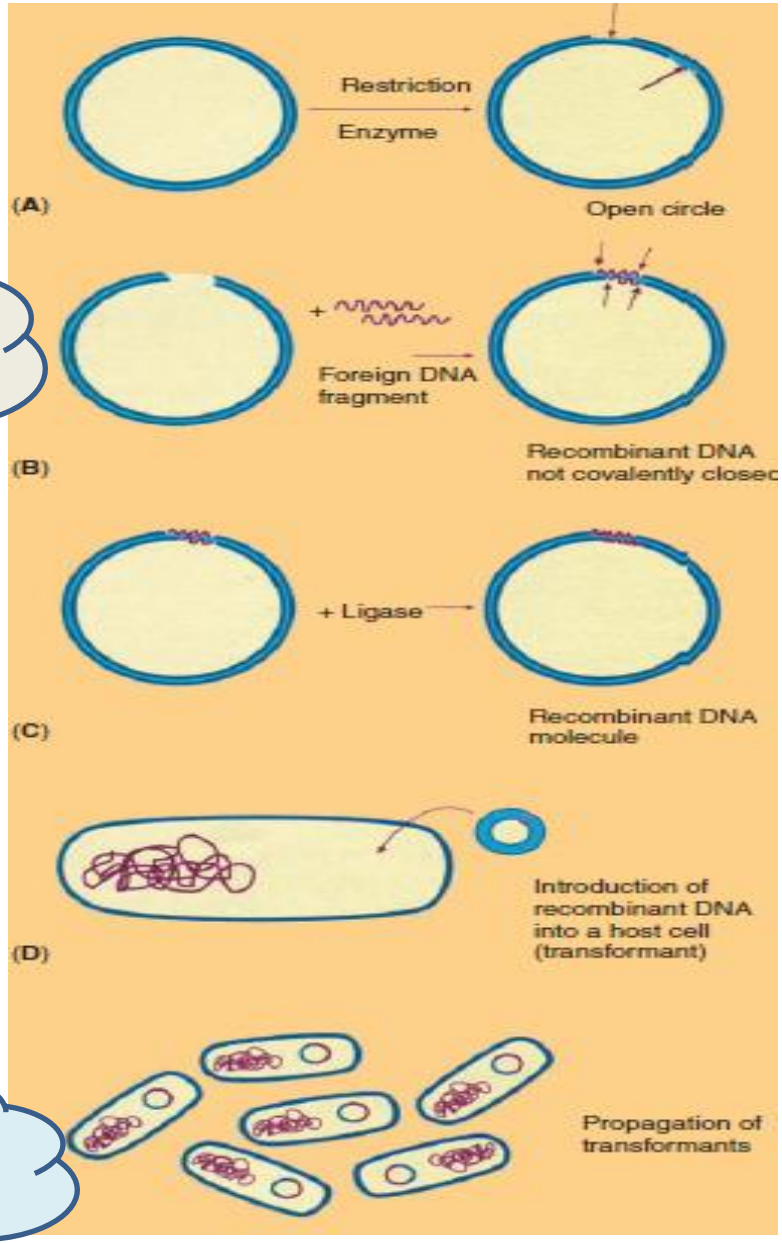
Plasmids





Treatment of a plasmid with an unique EcoR1 site. This restriction enzyme will open the plasmid and make it amenable for manipulation.

Bovine albumin



Bacillus subtilis

Principle of cloning a foreign DNA fragment

Recombinant DNA technology

Benefits:

The cloning technique is very suitable to obtain large amounts of a specific DNA fragment, by fusing such a fragment to an appropriate vector and transferring the construct to a host that can easily be cultivated to high cell densities. The recombinant DNA molecules, which can then be isolated from the cell mass, form an abundant source for the specific DNA fragment and it is important for pharmaceutical biotechnology.

Enzyme	Source	Cutting sequence[a]
EcoR1	*Escherichia coli*	G↓AATT C
		C TTAA↑G
Pst1	*Providencia stuartii*	C TGCA↓G
		G↑ACGT C
Taq1	*Thermus aquaticus*	T↓CG A
		A GC↑T
Hinf1	*Hemophilus influenzae*	G↓ANT C
		C TNA↑G
Msp1	*Moraxella species*	C↓CG G
		G GC↑C
HaeIII	*Hemophilus aegyptus*	GG↓CC
		CC↑GG

[a] N, no base preference.
Note: Open space in the recognition site indicates the endonucleolytic cut by the enzyme.

Some restriction enzymes, their origin, and their recognition site.

Foreign DNA fragment may be synthetic DNA(Oligonucleotides linked at laboratory and under certain conditions)

Recombinant DNA transfer

Transfer of a recombinant DNA molecule to a cell (host cell) is an essential step in DNA technology.

Bacillus subtilis frequently used in industrial biotechnology.

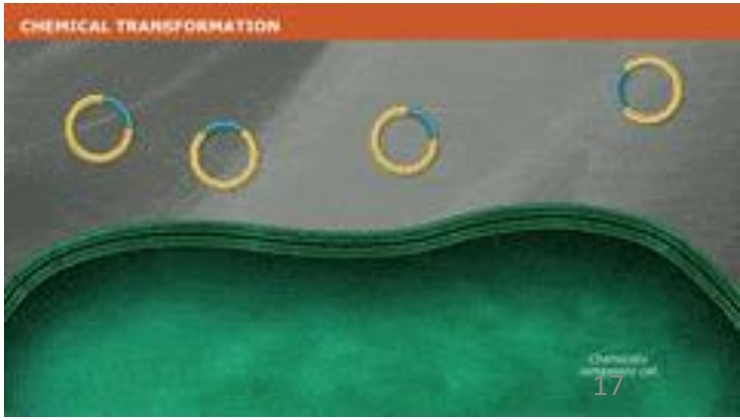
I. Natural transformation: DNA are taken up under physiological conditions.

II. Creation of Non physiological conditions:

1. heat shock to the host cells in the presence of high amounts of Ca+2 ions.

Recombinant DNA transfer

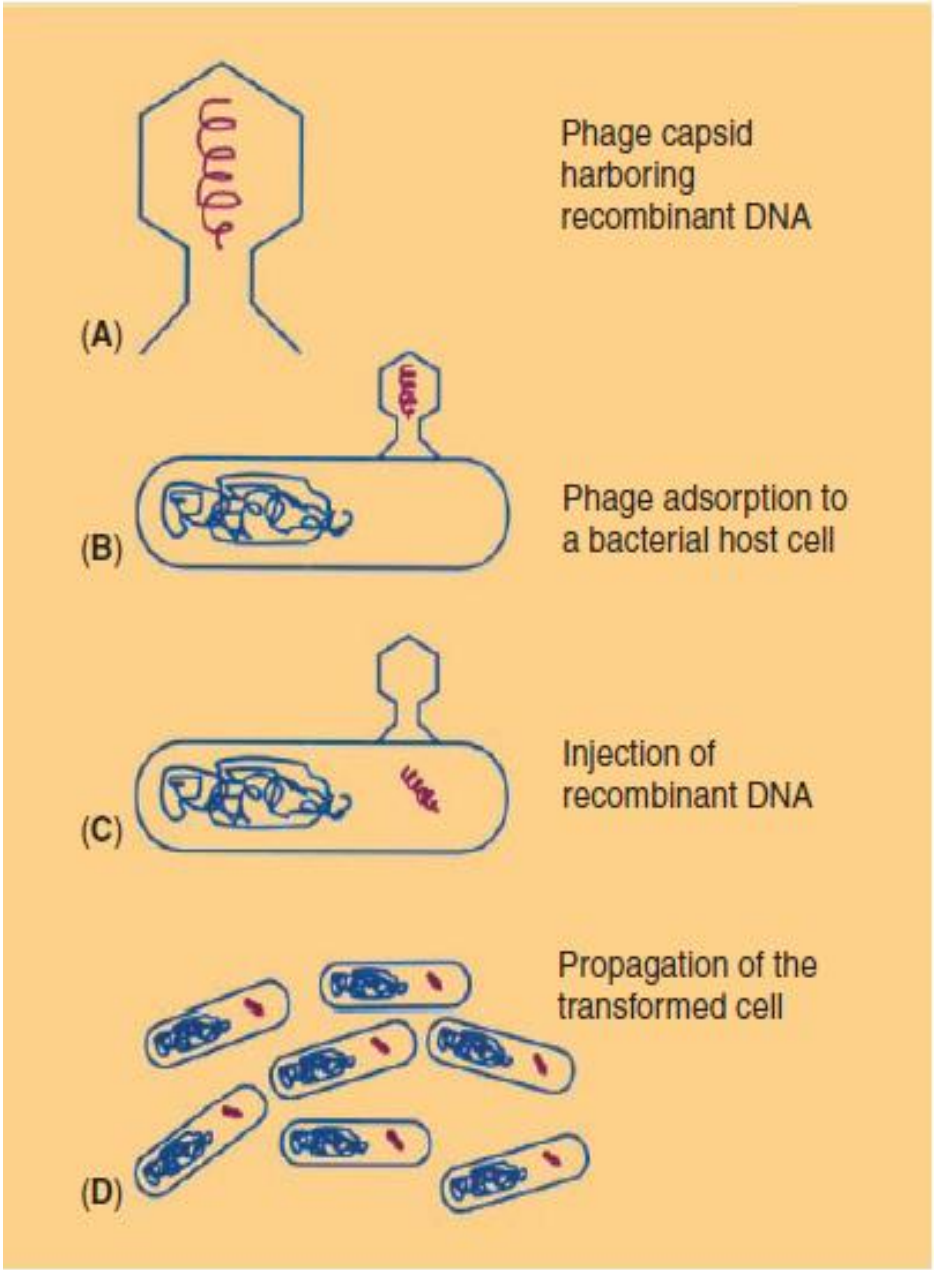
2. **Electroporation**: DNA and cells are brought together in a cuvette which is then subjected to a vigorous electrical discharge. Under those artificial conditions the cell envelope is forced to open itself, after which DNA may enter through the“holes” that are created, The technique of electroporation is widely applicable and frequently used.



DNA transfer

III. Transduction:

package DNA in a bacteriophage capsid and then to mimic the normal bacteriophage infection procedure.



Phage as a mediator for transfer of recombinant DNA

IV. **Conjugation:**

is a process where DNA transfer takes place by cell–cell mating conjugation a special class of plasmids is required, socalled conjugative plasmids. If a cell with such a plasmid—the donor—meets a cell without such plasmid—the recipient—they may form together cell aggregates.

Production by Recombinant DNA Technology

production of human insulin

The structural gene for human insulin is 1430 nucleotides long

The protein encoded by the gene is 110 amino acids in length and called Preproinsullin.

Processing steps, enzymes

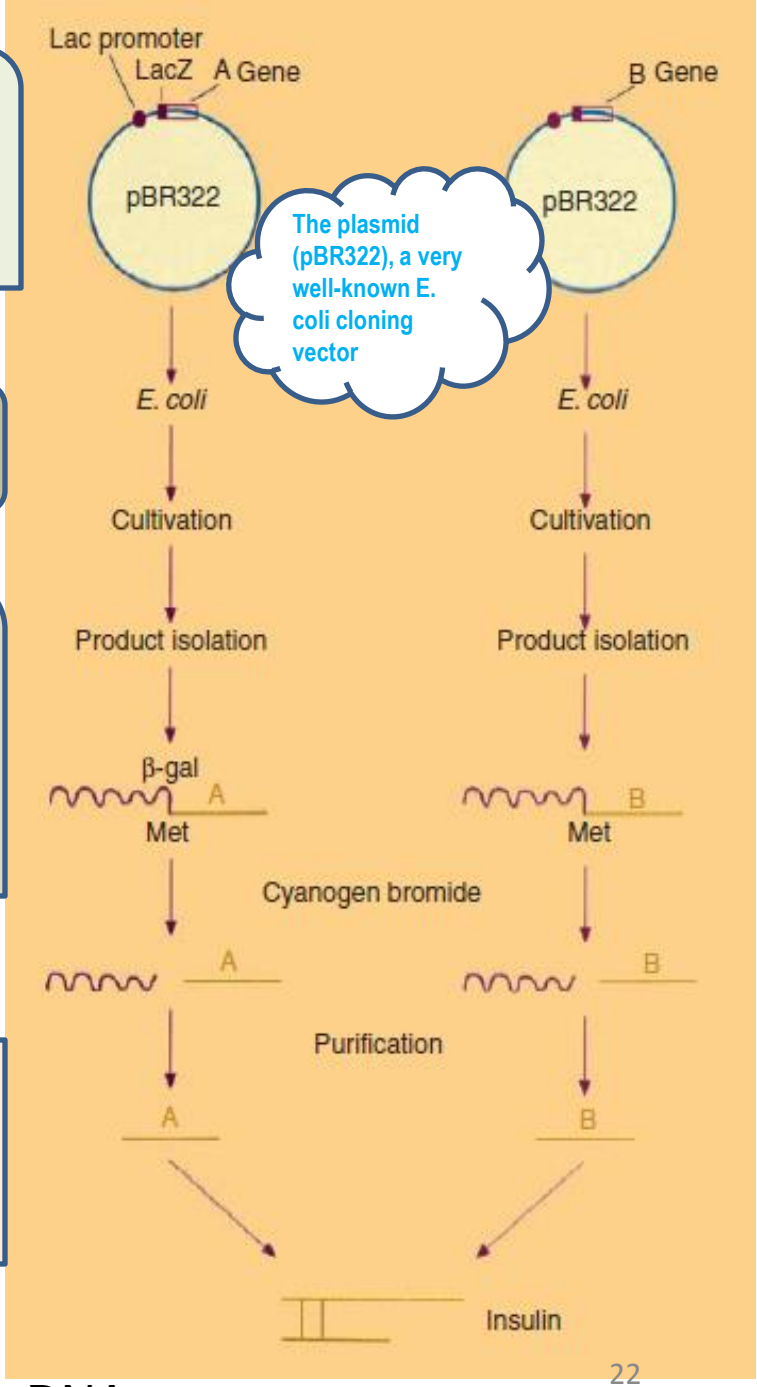
mature protein encompasses a total of 51 amino acids. It consists of two separate chains: an A chain of 21 amino acids and a B chain of 30 amino acids. Chains A and B are held together by S bonds between the amino acids cysteine on the adjacent chains.

Synthesis of A-chain gene and B-chain gene from Oligonucleotides
Both parts were linked together and fused at the end of the lacZ gene in the plasmid pBR322. Again the codon for the amino acid methionine was built in at the fusion point

the peptides A and B are synthesized as products fused to ß-galactosidase

Cynogen Bromide
This agent has the ability to cleave peptides whenever the amino acid methionine is present and cleaves immediately after this amino acid. Since neither fragment A nor B of insulin contain methionine and the cloning strategy guaranteed the presence of methionine at the fusion point.

The final step consists of mixing A and B and allowing the S bonds to form spontaneously



Synthesis of insulin by synthetic DNA.


THANK YOU