

Pharmaceutical Biotechnology

Lecture 2–biopharmaceutical consideration

5th Stage , Pharmacy Students

Topics

- ☐ Genetic Engineering
- ☐ Formulation of pharmaceutical proteins

Abbreviations

MI	Myocardial infarction
LPS	lipopolysaccharide
rDNA	Recombinant deoxyribonucleic acid/recombinant-DNA
HSA	Human serum albumin
IP	Intra peritoneal
LDL	low-density lipoprotein
SOD	Superoxide dismutase
WHO	World Health Organization
IA	Intra-arterial
IC	Intracoronary

Recombinant DNA technology

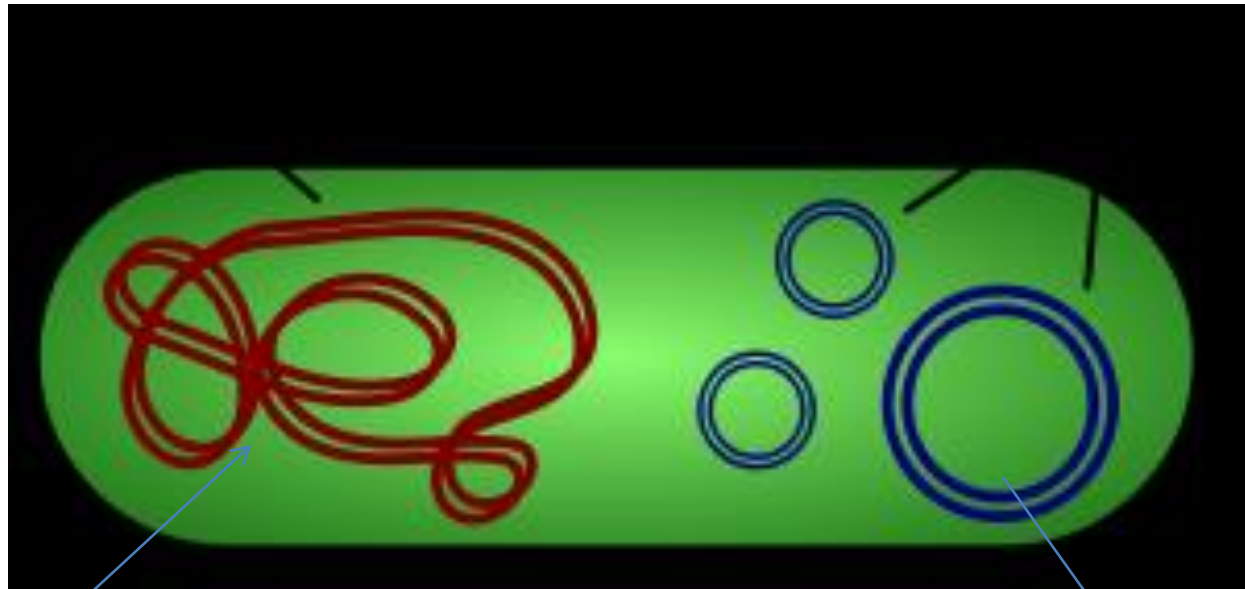
Other names:

(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)

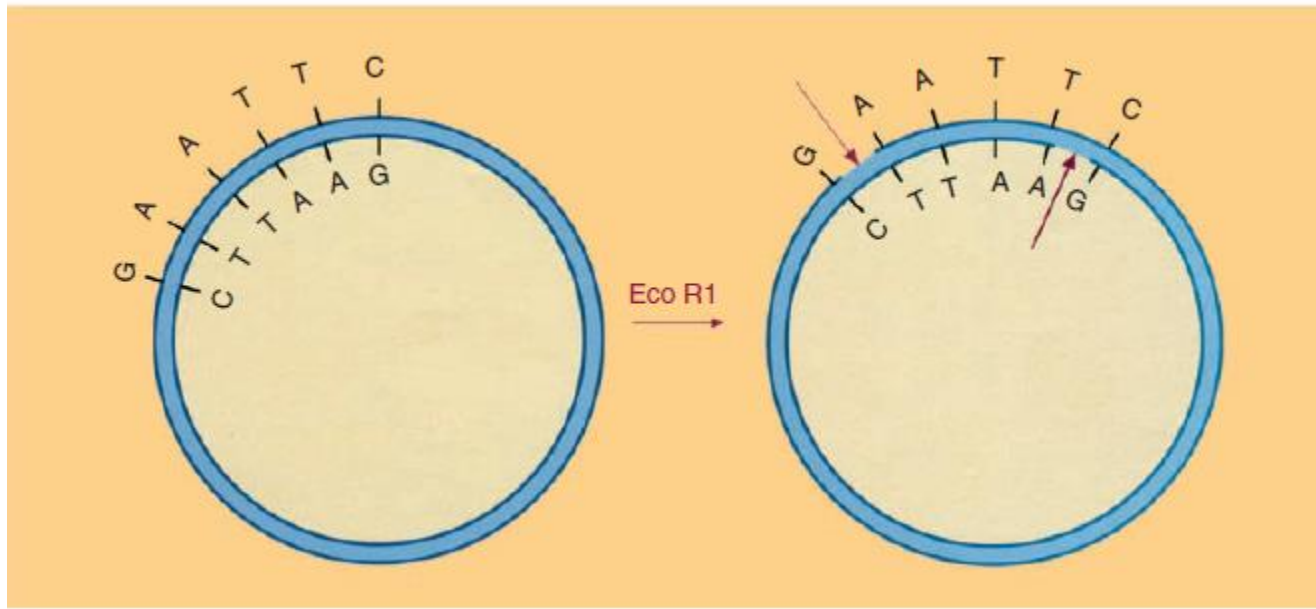
Plasmids usually used as a vector for a foreign DNA after treating them with Restriction enzymes such as ECORI.

Plasmids



Bacterial DNA

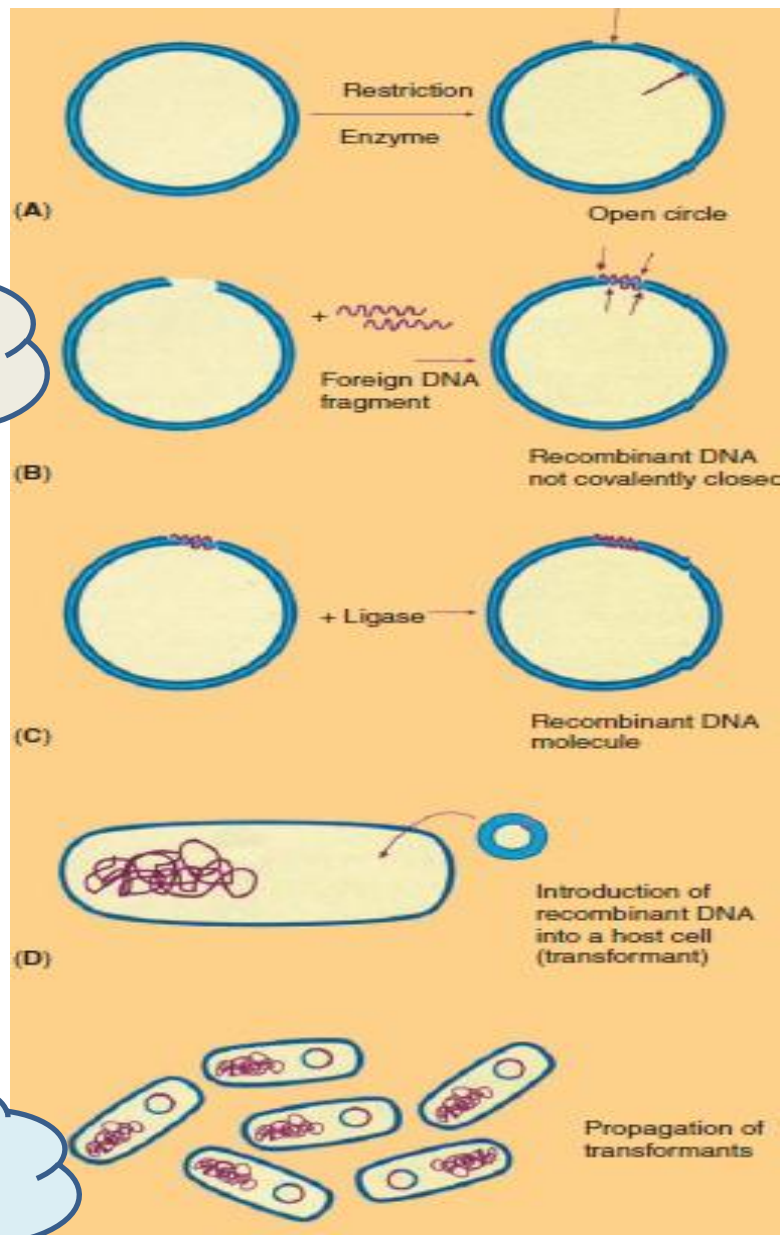
Plasmids



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	<i>Escherichia coli</i>	G↓AATT C
		C TTAA↑G
Pst1	<i>Providencia stuartii</i>	C TGCA↓G
		G↑ACGT C
Taq 1	<i>Thermus aquaticus</i>	T↓CG A
		A GC↑T
Hinf1	<i>Hemophilus influenzae</i>	G↓ANT C
		C TNA↑G
Msp1	<i>Moraxella species</i>	C↓CG G
		G GC↑C
HaeIII	<i>Hemophilus aegyptus</i>	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.



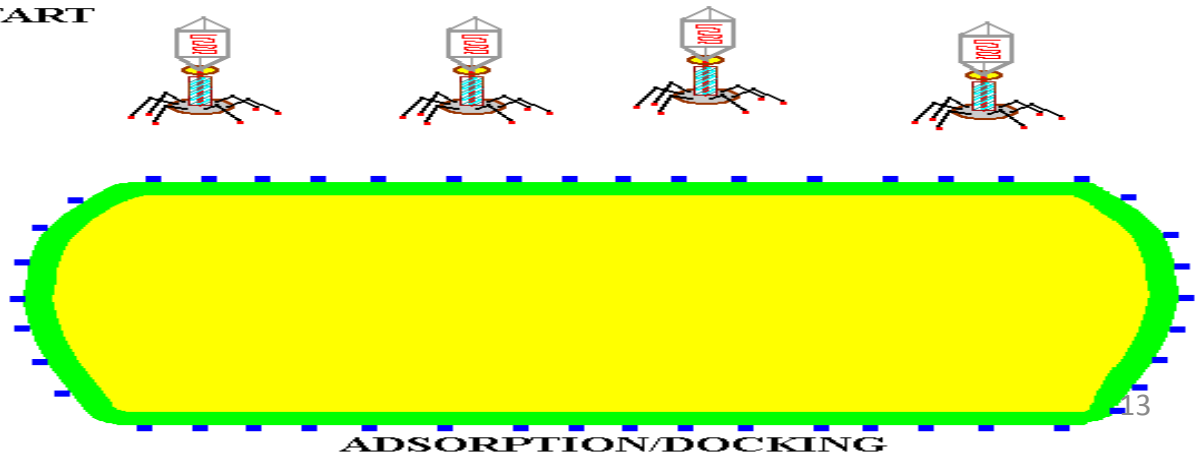
DNA transfer

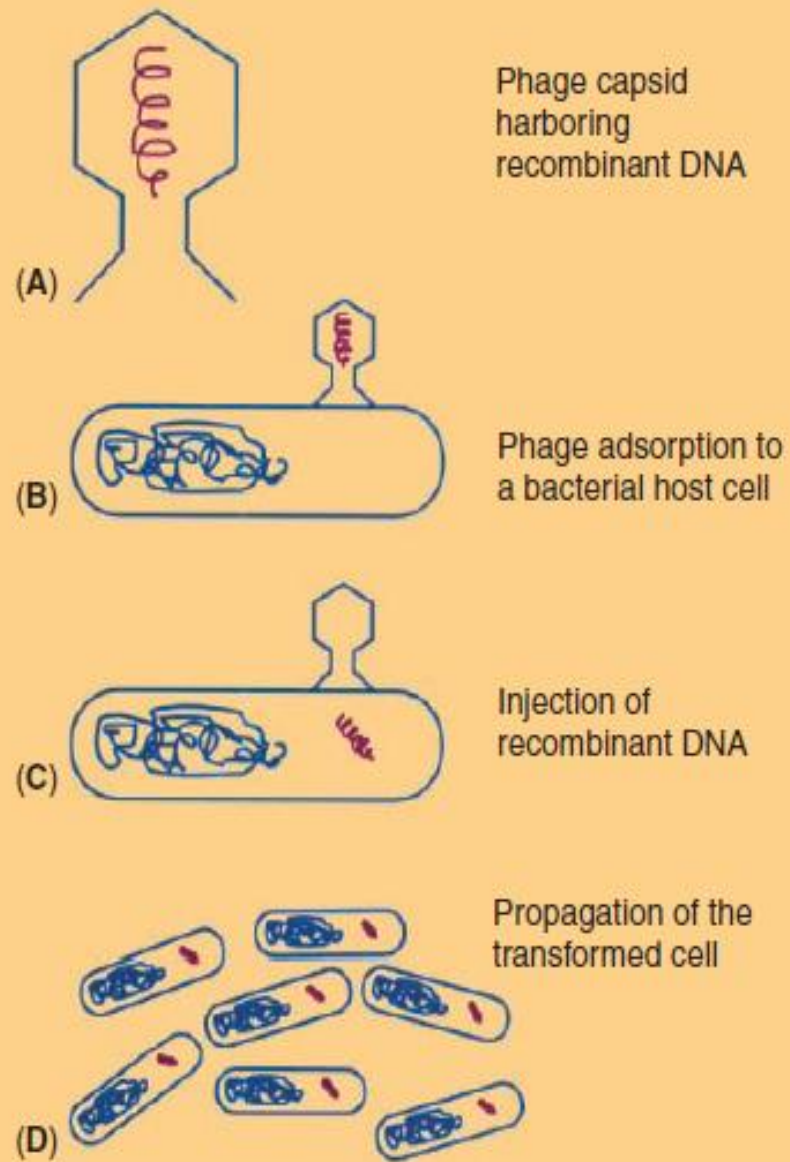
III. Transduction:

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

Lytic or Lysogenic?? H.W

START





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, so called 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.

The term “**biopharmaceuticals**” is used to describe biotechnologically derived drug products. Biopharmaceuticals are protein-based macromolecules and include, insulin, human growth hormone, the families of the cytokines and of the monoclonal antibodies, antibody fragments, and nucleotide based systems such as antisense Oligonucleotides , siRNA and DNA preparations for gene delivery.

These are large complex molecules and are often heterogeneous mixtures compared to synthetically manufactured, pure small molecules.

Biopharmaceuticals



By mid-2002, some 120 biopharmaceutical products had gained marketing approval in the USA and/or EU.

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 Preproinsulin.



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.

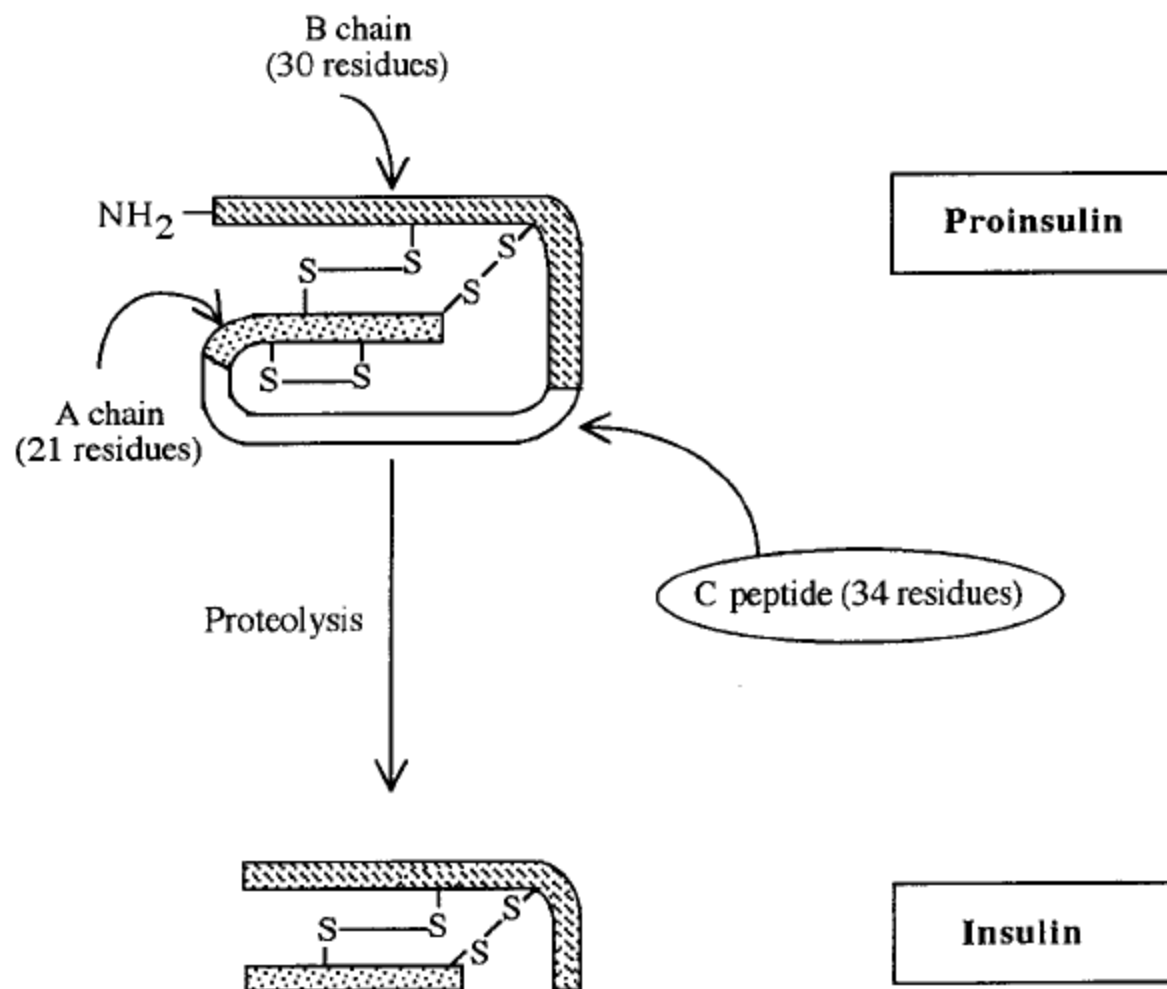


Figure 8.1. Proteolytic processing of proinsulin, yielding mature insulin, as occurs within the coated secretory granules

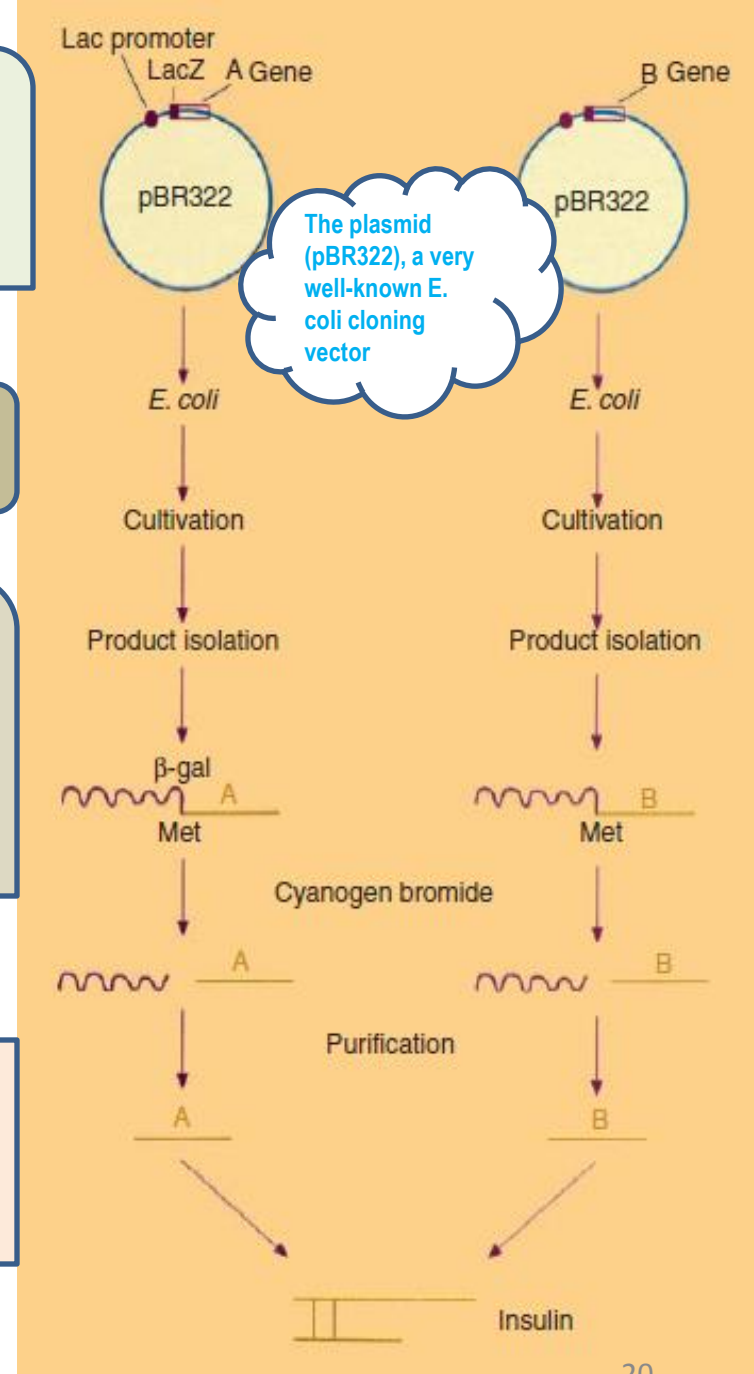
Synthesis of A-chain gene and B-chain gene from Oligonucleotides
Both parts were linked together and fused at the end of the lacZ gene in the plasmid pBR322. Again the codon for the amino acid methionine was built in at the fusion point

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

Cynogen Bromide

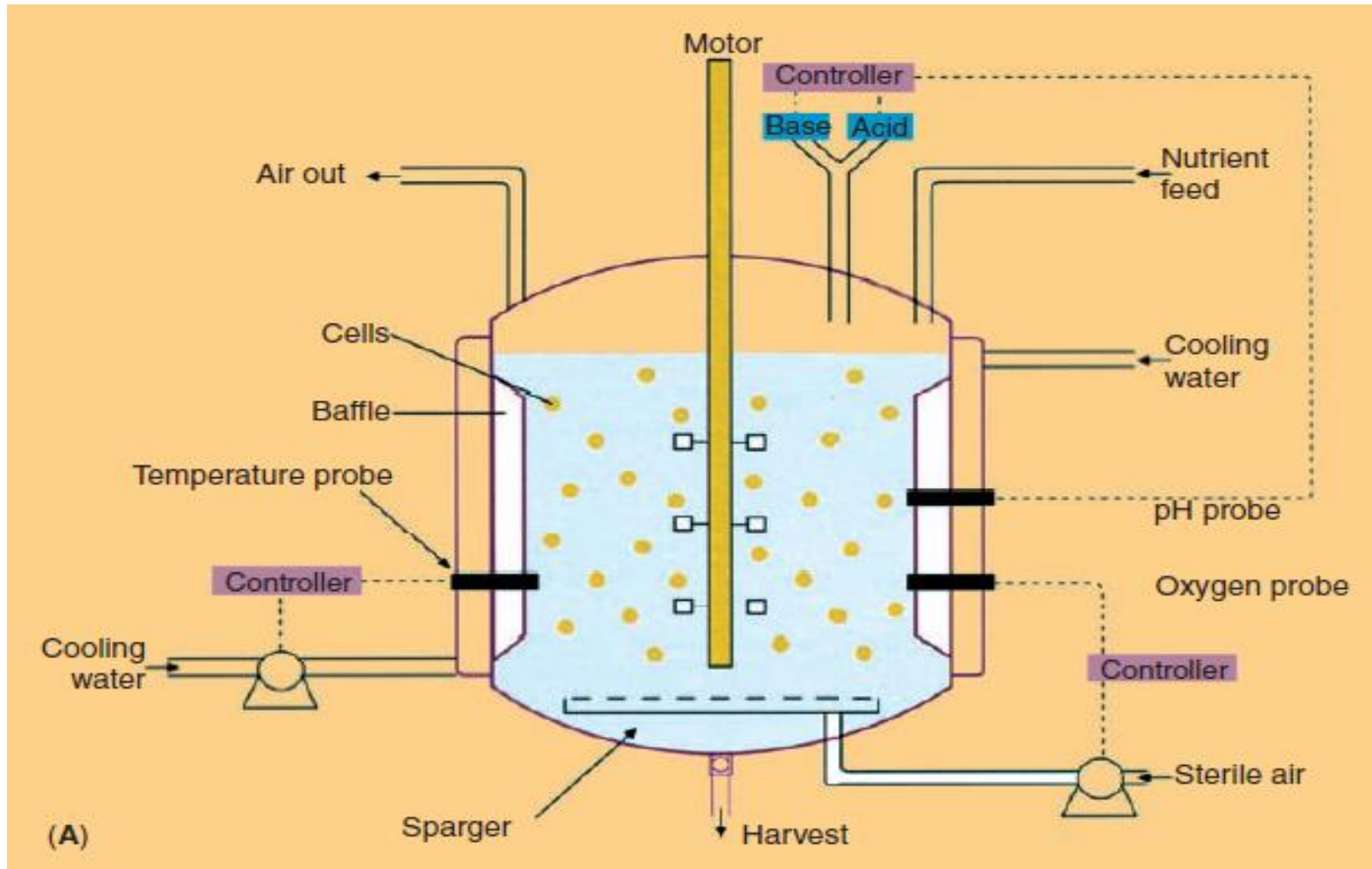
This agent has the ability to cleave peptides whenever the amino acid methionine is present and cleaves immediately after this amino acid. Since neither fragment A nor B of insulin contain methionine and the cloning strategy guaranteed the presence of methionine at the fusion point.

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



Cultivation Systems

production-scale cultivation is commonly performed in fermenters or bioreactors. Bioreactor systems can be classified into four different types: stirred-tank, airlift, microcarrier (e.g., fixed-bed bioreactors) and membrane bioreactors (e.g., hollow fiber perfusion bioreactors)



Schematic representation of stirred –tank bioreactor

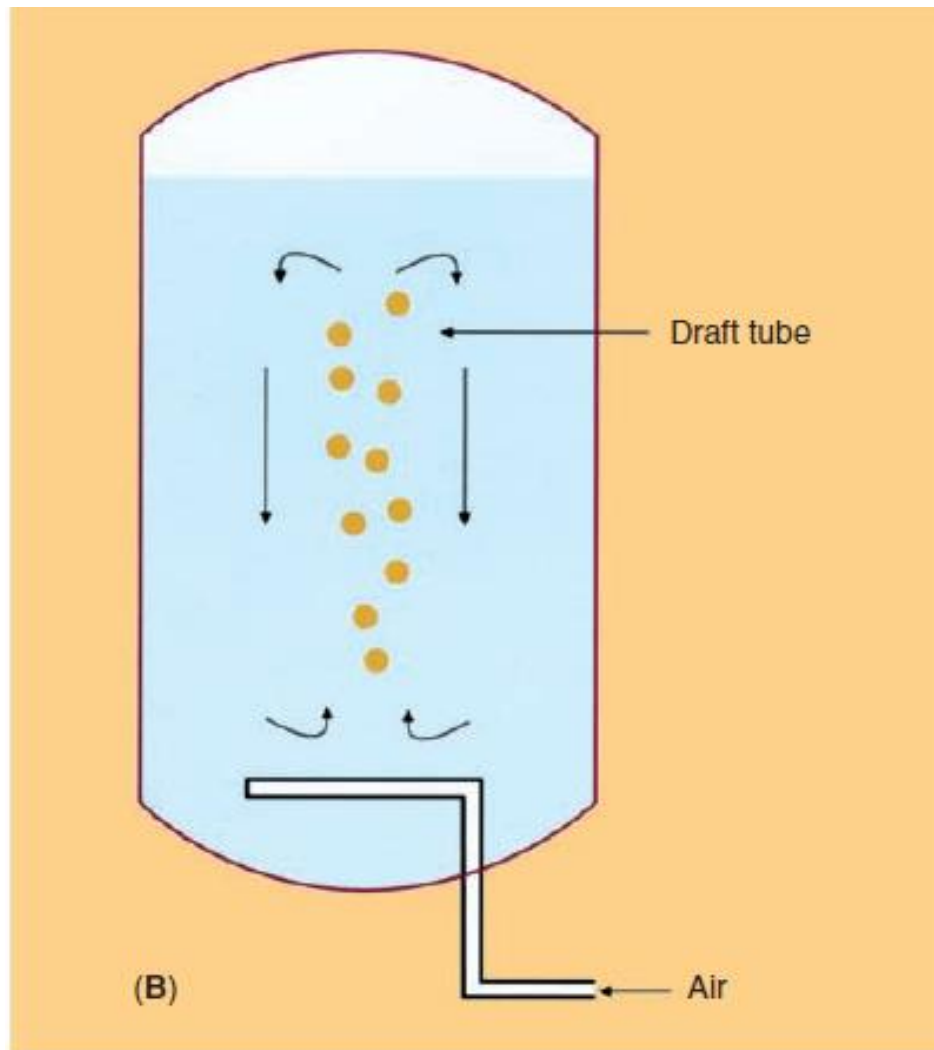


Figure 1B ■ Schematic representation of airlift bioreactor.
Source: Adapted from Klegerman and Groves, 1992.

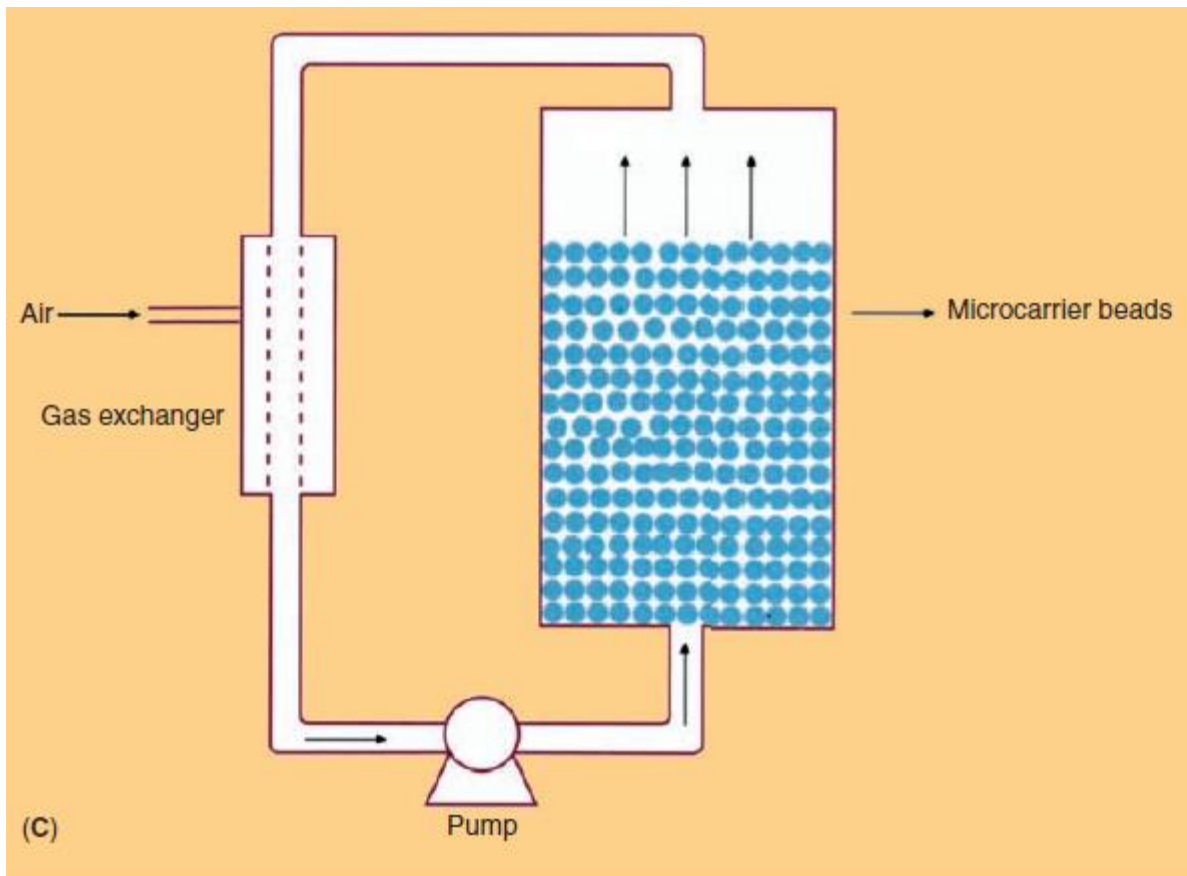


Figure 1C ■ Schematic representation of fixed-bed stirred-tank bioreactor. *Source:* Adapted from Klegerman and Groves, 1992.

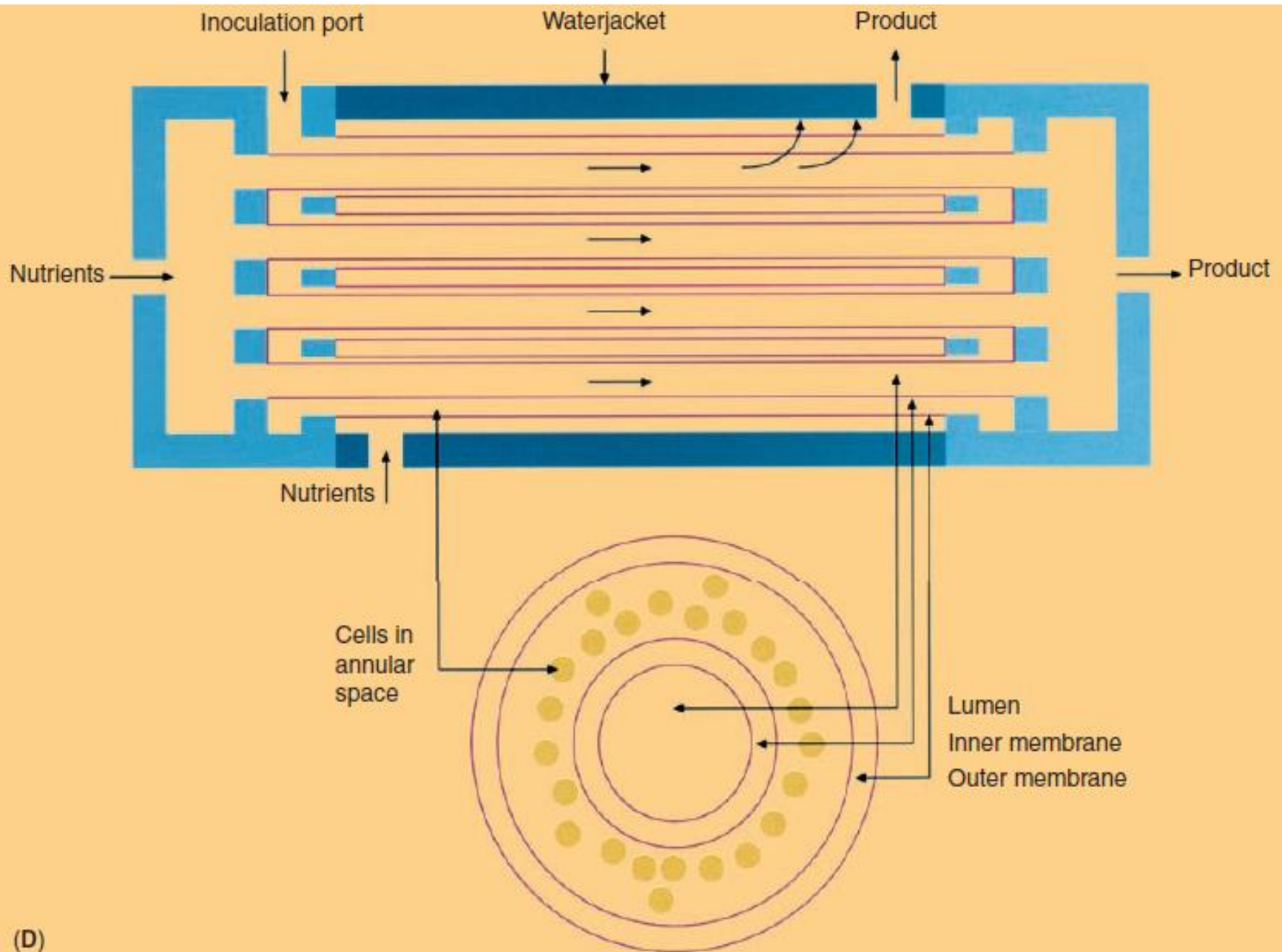


Figure 1D ■ Schematic representation of hollow fiber perfusion bioreactor. *Source:* Adapted from Klegerman and Groves, 1992.