# Biotechnology - Principles & Processes

Class 12 - Chapter 9 - Unit 8

EICB defn : large scale use of microbes, animal, plant cells (or parts/enzymes) to make useful products for humans - drugs, vaccines, etc.

## Principles of Biotechnology

Modern biotech rests on ~~two~~ TWO core techniques :

(i) Genetic Engineering (rDNA tech) - alter genetic make-up of cell/organism.

(ii) Bioprocess engineering - sterile growth of microbe/cell in large bioreactors.

| Identify + Cut + Join + Transfer |
|---|

<- core of rDNA tech

## Why Native DNA Won't Replicate in Host

Foreign DNA cannot multiply in alien host unless it is part of a chromosome with an ori site.

| ori - origin of replication |
|---|

<- sequence where DNA repln starts

So an alien gene is linked to ori-bearing DNA = this carrier is called a vector. Together they form recombinant DNA (rDNA).

First rDNA : Cohen & Boyer - 1972 - E. coli.

# Tools of rDNA Technology

Tool-kit : R. enzymes, polymerases, ligase, vectors, host cells, PCR primers.

## 1) Restriction Enzymes

Discovered : Smith, Arber, Nathans (1968-70).

First isolated = ~~EcoR2~~ Hind II (1968).

Belong to a class called NUCLEASES :

(a) Exonucleases - remove from DNA ends.

(b) Endonucleases - cut at internal sites.

## Naming Rule (e.g. EcoRI)

| Eco | R | I |
|---|---|---|

<- E = genus Escherichia
<- co = species coli

R = strain RY13. I = roman numeral - order of isolation from that strain.

## Palindrome & Sticky Ends

5' - G A A T T C - 3'

3' - C T T A A G - 5'

Reads same 5' to 3' on BOTH strands = palindrome.

Staggered cut - short single-strand overhangs called STICKY ENDS - H-bond with comp. ends = basis of joining vector + insert.

# Cloning Vectors

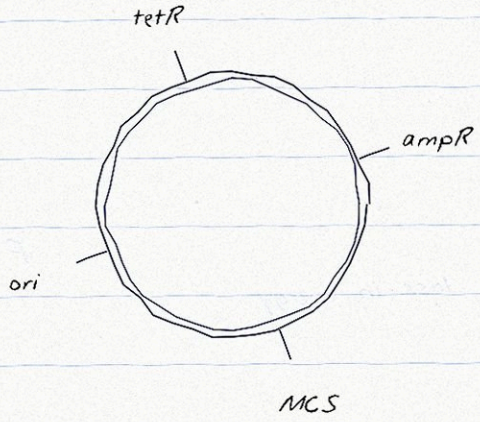
Vector = DNA molecule that ferries the gene of interest into a host cell & replicates it.

Examples : plasmids, bacteriophages (lambda), cosmids, YACs, BACs, Ti plasmid (plants).

## Features a Good Vector Needs

1. ori - origin of replication.
2. SM - selectable marker (antibiotic res. gene e.g. ampR, tetR, kanR).
3. MCS - cloning site / unique recogn. site.
4. size - small (easy to manipulate, transfer).

pBR322 - classic E. coli plasmid vector.




carries 2 antibiotic resistance genes - ampR & tetR.

## Insertional Inactivation

Insert gene WITHIN tetR = tetR is destroyed.

Recombinant colony = ampR+ , ~~tetR+~~ tetR-.

Replica plate on tet - used to screen out hits.

# Vectors for Plants & Animals

## Plant vector - Ti plasmid

Carried by Agrobacterium tumefaciens.
Naturally causes crown gall (tumour) in dicots.
Disarmed Ti = T-DNA replaced with our gene.
Now acts as a transformer of plant cells.

## Animal vector - Retroviruses

Retrovirus (after disarming) delivers gene into ~~plant~~ animal cells - becomes harmless and integrates into host genome.

## Competent Host

DNA is hydrophilic - cannot cross hydrophobic cell membrane normally. So cells are made 'competent' to take up DNA.

## 4 Methods to Force Entry

1. $CaCl_2$ + heat shock - chemical method.
2. Micro-injection - direct into nucleus.
3. Gene gun (biolistics) - DNA-coated gold/W particles fired into plant cells.
4. Disarmed pathogen - virus/Agrobacterium delivers the gene into host genome.

Heat shock : 42 oC for 90 s - then ice.

# Processes of rDNA Technology

Overall flow (memorise) :

Isolate > Cut > Amplify > Ligate >
Transfer > Culture > Purify (the rDNA pipe)

## Step 1 - Isolation of DNA

DNA is bound with RNA, proteins, polysaccharides and lipids. Cells are broken open by enzymes :

- Lysozyme (bacteria)
- Cellulase (plant cells)
- Chitinase (fungal cells)

Then Ribonuclease removes RNA, ~~Lipase~~ Protease removes proteins. Pure DNA precipitates out as fine threads when chilled ETHANOL is added - collect with a glass rod ('spool').

## Step 2 - Cutting + Gel Electrophoresis

Pure DNA + restriction enzyme -> fragments.
Separated on AGAROSE GEL by size :

DNA (-) -> (+) end <- size-based separation

Smaller fragments run faster (further).

# Step 3 - PCR Amplification

PCR = Polymerase Chain Reaction.

Invented by Kary Mullis (1983).

Makes ~~hundreds~~ billions of copies of a gene in vitro.

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## Ingredients of one PCR tube

(1) Template - the DNA to be copied.

(2) 2 Primers - short ssDNA ( 20 nt)
complementary to 3' ends of target.

(3) dNTPs - dATP, dTTP, dGTP, dCTP.

(4) Taq polymerase - from Thermus aquaticus
(thermostable - survives 95 oC).

(5) Mg2+ buffer - co-factor for Taq.

## 3 Steps - repeated 30-40 cycles

| | |
|---|---|
| (i) 94-95 oC | Denature - ds breaks into 2 ss DNA |
| (ii) 40-60 oC | Anneal - primers bind comp. site |
| (iii) 72 oC | Extend - Taq adds dNTPs 5' to 3' |

After n cycles : copies = $2^n$ <- exponential

# Step 4 - Ligation + Insertion

Cut vector + cut insert - joined by

**DNA LIGASE (T4 ligase)** <- seals nicks in <- sugar-PO4 backbone

Result = rDNA - ready for transfer to host.

## Step 5 - Bioreactor

Small flask culture is NOT enough for industry.

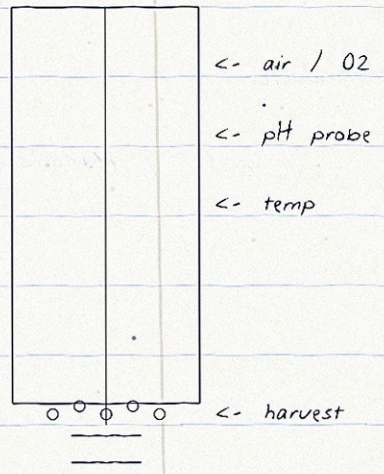
Use BIOREACTORS - 100 to 1000 L vessels.

Two common types :

(a) Simple stirred-tank bioreactor - has impeller for mixing + O2 transfer.

(b) Sparged stirred-tank - air sparged from below as bubbles.

stirred-tank bioreactor




## Conditions Maintained

Temperature, pH, substrate, salts, vitamins, O2 - all kept optimal = max product yield.

Output = large biomass + ~~DNA~~ desired protein.

Examples : insulin, hGH, hep-B vaccine.

# Downstream Processing

After biosynthesis - product is NOT ready yet. It must be separated and purified = this stage is called DOWNSTREAM PROCESSING (DSP).

## Typical DSP steps

1. Separation - cells separated from medium by centrifugation / filtration.
2. Purification - chromatography (gel filtration, ion-exchange, affinity column).
3. Formulation - preservatives added.
4. QC + clinical trial - esp. for drugs.

DSP cost = 60-70% of total <- biggest <- step

## Key Discoveries to Remember

1869 - Miescher - isolates 'nuclein'.
1968 - Smith - first restriction enzyme.
1972 - Cohen + Boyer - first rDNA molecule.
1983 - Mullis - PCR technique.

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## Quick Recap

Tools : R-enzymes, vectors, ligase, ~~RNA~~ host cells.
Steps : isolate, cut, amplify, ligate, transfer, culture, purify - the rDNA pipeline.