

# Biotechnology: Principles and Processes



cut, copy, paste a gene, then grow enough cells to matter

## Genetic engineering vs Bioprocess technology

### Genetic engineering

- changes a cell's genetic makeup by cutting a gene out of it
- then joins the gene to a vector and inserts it in a host
- gives an organism a trait it lacked, e.g. a bacterium making human insulin

### Bioprocess technology

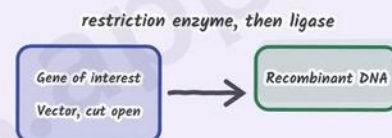
- keeps that modified cell alive at large, contamination-free scale so its product is worth harvesting
- sterile ambient conditions, controlled growth, a way to extract the product at the end

the two together are what the word biotechnology actually means: engineer the change, then scale it up cleanly

### Restriction enzymes: molecular scissors

- cuts a phage's DNA, spares the host's own; found by Arber, Nathans, Smith
- restriction endonucleases cut within a strand; exonucleases only trim from the ends, a different job
- each enzyme recognises its own palindromic sequence: same read on both strands, reversed
- the cut is usually staggered, leaving short single-stranded overhangs called sticky ends
- sticky ends let a cut gene pair with a cut vector
- the pairing works by complementary base pairing, then ligase seals the join

### Joining a gene to a vector



the enzyme that cuts both pieces open leaves matching sticky ends, and ligase is what actually seals the join

### Restriction enzymes and their recognition sites

| Enzyme  | Recognition sequence |
|---------|----------------------|
| EcoRI   | GAATTC               |
| BamHI   | GGATCC               |
| HindIII | AAGCTT               |
| PstI    | CTGCAG               |
| SalI    | GTCGAC               |

each sequence is a palindrome: PstI sits inside pBR322's ampR gene, BamHI and SalI inside its tetR gene

### Cloning vectors

- a vector carries a foreign gene into a host, copied with the host's own DNA
- every usable vector needs an origin of replication, so it can copy itself inside the host
- it needs a selectable marker, e.g. antibiotic resistance, to show which host cells took it up
- it needs a cloning site: unique restriction sites for inserting a gene
- insertion there does not wreck the vector's own genes

