

# Lecture 1

## 1. Defining Biotechnology

- **Core Definition:** The **manipulation or control** of living systems to solve problems.
- **Key Distinctions:** Simple *use* of nature (e.g., hunter-gatherer berry picking) is **not** biotechnology. True biotechnology requires control over reproduction, traits, and system-level manipulation.
- **Scope of Living Systems:** Applies not just to whole organisms, but to genes, enzymes, specific cells, or molecular pathways.

## 2. Biological Precedents (Evolutionary Biotech)

- **Ant Farming (50–60 Million Years Ago):** Leafcutter ants cultivate fungus for food using plant matter they cannot digest themselves.
  - **System Manipulation:** Ants practice **pest control** using symbiotic skin bacteria that produce targeted antibiotics against invasive species without harming their crop.
  - **Propagation:** Virgin queen ants carry fungus samples in their mouthparts to start new colonies.
- **Evolutionary vs. Human Biotech:** Ants exhibit domestication and growth management, but this behaviour evolved naturally without conscious design or mechanistic understanding.

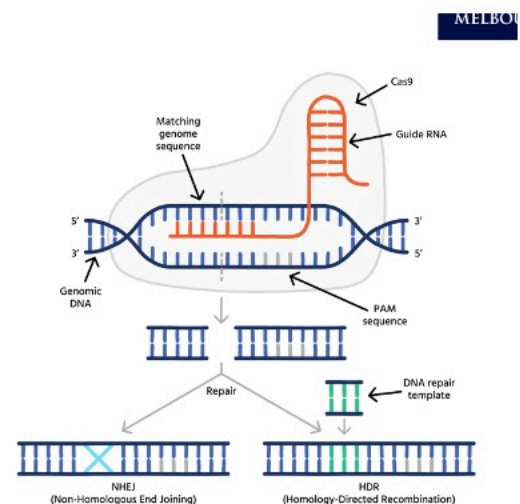
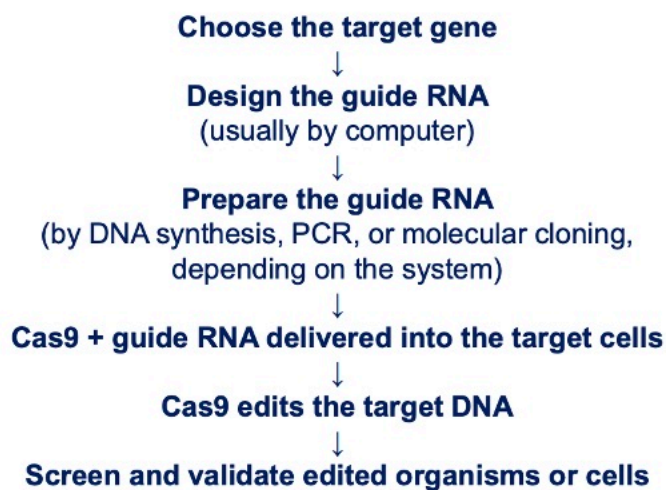
## 3. Ancient Human Biotechnology (Empirical & Observation-Based)

Ancient biotech relied on empirical observation, pattern recognition, and trial-and-error over thousands of years without any knowledge of DNA or cellular mechanisms:

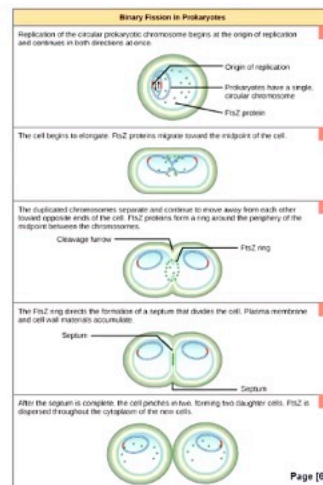
- **Dog Domestication (15,000–30,000+ years ago):** The oldest known human biotech application; selective breeding of calmer wolves for hunting, safety, and companionship.
- **Brewing & Fermentation (~13,000 years ago):** Predates agriculture. Ancient Natufian cultures used wild yeast (*Saccharomyces*) to ferment grains into alcoholic beverages in rock containers, likely pushing the eventual domestication of cereal crops.
- **Agriculture & Crop Selective Breeding (~10,000–12,000 years ago):** Emerging at the end of the last Ice Age across independent centers (Fertile Crescent, China, Mesoamerica).
  - *Example:* Teosinte to modern Maize in Mexico required selecting natural mutations in just a couple of key genes (e.g., *TB1*, *tga1*) over many generations.
- **Cheese Making (~7,000 years ago):** Discovered accidentally when storing milk in animal stomach containers containing leftover **rennet enzymes**.
  - *Impact on Human Evolution:* Consumption of dairy selected for a single base-pair mutation in the promoter region of the lactase gene, enabling lactase persistence in human adults.

## 5. CRISPR-Cas9 System

- **Natural Mechanism:** Adaptive immunity in bacteria and archaea against invading bacteriophages consisting of three phases: Acquisition (Memory), Expression (crRNA processing), and Interference (DNA cleavage).
- **Engineered Biotech Tool (Charpentier & Doudna, 2012):**
  - **Cas9:** Endonuclease that creates double-strand breaks (DSBs) in target DNA.
  - **Guide RNA (gRNA/sgRNA):** Engineered ~100-base RNA molecule containing a 20-base custom sequence targeting a specific DNA locus.
  - **PAM Sequence:** Short motif (e.g., 5'-NGG-3') scanned by Cas9 required for DNA unwinding and cleavage.
- **DNA Repair Pathways:**
  - **Non-Homologous End Joining (NHEJ):** Error-prone repair without a template; leads to insertions/deletions (indels) that knock out gene function.
  - **Homology-Directed Repair (HDR):** Template-driven repair utilizing an exogenous donor DNA sequence to precise edit or insert new genetic code.



#### 4. Cell Separation: Yields two equal daughter cells.



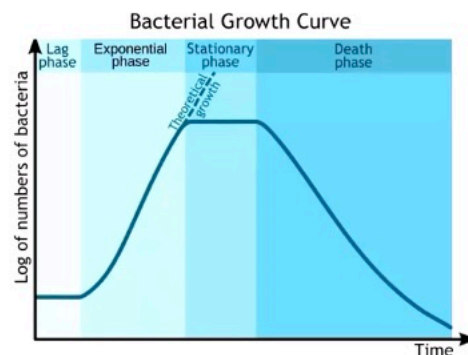
### C. Population Dynamics & The Bacterial Growth Curve

- **Generation Time ( $T_d$  / Doubling Time):** The time required for a population to double under optimal conditions.
  - *E. coli*: ~ 20 minutes.
  - *Mycobacterium leprae*: 12–14 days (due to large/complex metabolic pathways, cell wall complexity, and slow DNA replication).
- **Specific Growth Rate ( $\mu$ ):**

$$\mu = \frac{1}{N} \cdot \frac{\Delta N}{\Delta t}$$

(where  $N$  is cell number, cell concentration, or biomass per volume, normalized to account for starting population size).

- $\mu_{\max}$ : The theoretical maximum specific growth rate achieved when all conditions are non-limiting.



#### 1. Lag Phase:

- No net increase in cell number.
- High physiological activity: synthesizing enzymes, RNA, and metabolic intermediates to adapt to fresh medium.
- Length depends on previous culture conditions (e.g., inoculum taken from  $-80^\circ\text{C}$  requires extended recovery).

## 2. Comparative Analysis: Biologics vs. Small Molecules

| Feature                 | Biologics (e.g., Antibodies)                                  | Small Molecules (e.g., Kinase Inhibitors)            |
|-------------------------|---------------------------------------------------------------|------------------------------------------------------|
| Molecular Size & Weight | Very large (~ 150 kDa for standard IgG)                       | Very low (< 500–900 Da)                              |
| Target Specificity      | Exceptionally high; wide surface-area binding                 | Variable; risk of off-target kinase/receptor binding |
| Route of Administration | Parenteral injection only (IV, SC via <u>auto-injectors</u> ) | Often orally bioavailable (tablets/capsules)         |
| Tissue Penetration      | Poor diffusion into dense solid <u>tumors</u> /brain (BBB)    | High diffusion; can cross membranes readily          |
| Immunogenicity          | Risk of anti-drug antibodies (ADAs)                           | Non-immunogenic                                      |
| Dosing Frequency        | Long half-life (weeks to months)                              | Short half-life (daily or multiple daily doses)      |
| Manufacturing Cost      | High (bioreactors, strict cold-chain, purification)           | Lower (scalable chemical synthesis)                  |

## 3. The Drug Development Lifecycle & Target Discovery

### The Cost of De-risking

- Drug development requires over a decade and costs upward of hundreds of millions to over a billion dollars per approved drug.
- **Cost of Clinical Failure:** Estimated at ~ \$550 million per failed clinical program. Early-stage preclinical target validation is vital to de-risk late-stage attrition.

Target Discovery Strategy Matrix:

Unbiased / Discovery-Driven

- OMICS Profiling (Proteomics, Metabolomics, Transcriptomics)
- Functional Genetic Screens (CRISPR KO, RNAi)

Rational / Hypothesis-Driven]

- Literature Mining + NLP / Knowledge Graphs
- Structural Modeling & In Silico Virtual Screening

## 6. Next-Generation "Clean" Plant Biotechnology

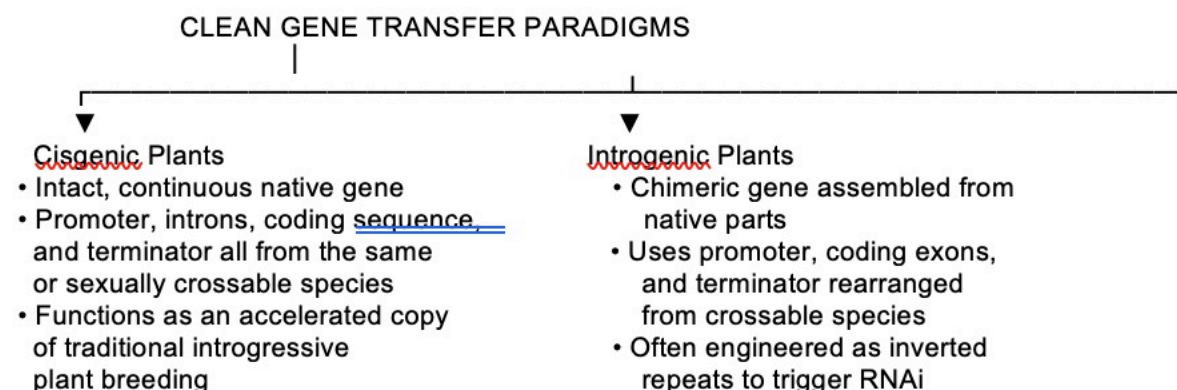
### A. Regulatory Hurdles and Public Perception

- First-generation transgenic crops carry foreign bacterial sequences—specifically antibiotic resistance selectable marker genes (e.g., *nptII*, *hpt*)—which are necessary only during in vitro tissue culture.
- Persistent antibiotic resistance genes raise consumer concerns and extend the time and expense of regulatory evaluations by agencies like the USDA, EFSA, and FSANZ.
- These considerations have driven the development of "clean" technologies designed to remove marker genes or utilize solely sexually compatible DNA.

### B. Strategies for Marker-Free Transformation

- **Co-Transformation (Two-T-DNA Approach):**
  - Employs two distinct T-DNAs: one carrying the gene of interest and the other carrying the selectable marker gene, hosted either in separate *Agrobacterium* strains or on independent plasmids.
  - Simultaneous infection occasionally integrates both T-DNAs into different chromosomes within the same plant cell.
  - Selected T<sub>0</sub> lines are self-fertilized; under Mendelian independent assortment, the two loci segregate in the T<sub>1</sub> generation. A standard dihybrid test cross predicts that roughly 3 out of 16 progeny will inherit the gene of interest while losing the unlinked antibiotic resistance marker.
- **Marker-Free Transformation via High-Throughput PCR Screening:**
  - T-DNA constructs containing the gene of interest are introduced without any selectable marker gene.
  - Tissues are regenerated on hormone media without antibiotics.
  - Although this produces many non-transgenic shoots, roughly 2% of regenerated plants integrate the T-DNA.
  - Automated, high-throughput polymerase chain reaction (PCR) screening identifies these rare, clean transformation events directly.

### C. Cisgenesis vs. Intragenesis



# Lecture 10

## 1. Global Micronutrient Malnutrition and Methodologies of Assessment

### A. Global Burden and Distribution

- Micronutrient deficiencies—principally involving **vitamin A, iron, iodine, and zinc**—disproportionately impact vulnerable populations in low- and middle-income regions, notably in South Asia, Central Africa, and parts of South America (e.g., Bolivia).
- The 2024 global dietary intake modeling study published in *The Lancet* (evaluating 34 countries using modeled consumption distributions versus estimated average requirements) reported substantial levels of **dietary micronutrient inadequacy**:
  - **Iodine**: ~68% of the assessed population failed to meet daily intake requirements.
  - **Iron**: ~65% inadequate intake.
  - **Vitamin A**: ~48% inadequate intake.
  - **Zinc**: ~46% inadequate intake.
- *Distinction in Terminology*: Dietary inadequacy represents an intake below physiological requirements, whereas clinical deficiency denotes pathological, biochemical, or functional tissue depletion.

### B. Analytical Methodologies and Methodological Confounders

Assessing micronutrient status across populations involves three primary methods, each subject to distinct limitations:

1. **Dietary Intake Surveys (e.g., 24-hour recalls, food frequency questionnaires)**:
  - *Limitations*: Relies on subjective recall and memory biases; high labor and administrative cost; fails to account for gut bioavailability, nutrient retention during cooking, or varying demographic demands.
2. **Biochemical Biomarkers (Blood/Serum Testing)**:
  - *Examples*: Serum ferritin (iron stores), serum retinol (vitamin A).
  - *Limitations*: Confounded by acute or chronic systemic inflammation; for example, ferritin acts as an acute-phase reactant that spikes during infections regardless of bone marrow iron stores.
3. **Functional and Clinical Indicators**:
  - *Examples*: Anemia (iron), night blindness and xerophthalmia (vitamin A), goiter (iodine).
  - *Limitations*: Clinical endpoints lack diagnostic specificity (e.g., anemia is driven by iron deficiency, parasitic infections, blood loss, hemoglobinopathies, and chronic disease).