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.

Choose the target gene

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Design the guide RNA
(usually by computer)

↓

Prepare the guide RNA
(by DNA synthesis, PCR, or molecular cloning, depending on the system)

↓

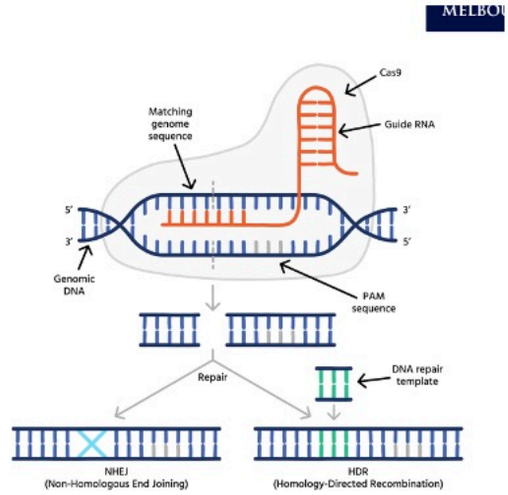
Cas9 + guide RNA delivered into the target cells

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Cas9 edits the target DNA

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Screen and validate edited organisms or cells



Practice Multiple-Choice Questions

Question 1

During bacterial binary fission, what is the precise mechanical role of the FtsZ protein?

A) It synthesizes the complementary strand of circular DNA at the replication fork.

B) It anchors the single circular chromosome to the polar peptidoglycan wall.

C) It polymerizes into a contractile ring at the cellular midpoint to direct inward membrane invagination.

D) It acts as an active efflux pump to remove toxic metabolic waste before division.

Question 2

Why does *Mycobacterium leprae* display an exceptionally long generation time (12--14 days) compared to *Escherichia coli* (20 minutes)?

A) *M. leprae* reproduces exclusively via eukaryotic sexual sporulation.

B) *M. leprae* lacks an origin of replication in its circular genome.

C) *M. leprae* possesses complex metabolic requirements, an elaborate cell wall, and slower DNA replication dynamics.

D) *M. leprae* lacks functional FtsZ proteins during cell division.

Question 3

A culture of *Bacillus subtilis* transitions from the exponential growth phase into the stationary phase. What change occurs in the net specific growth rate (μ)?

A) μ reaches its absolute maximum value ($\mu_{\max}$).

B) The rate of binary fission matches the rate of cell death, resulting in a net growth rate of zero.

C) Cell death completely ceases, and only primary metabolism continues.

D) The optical density at 600 nm (OD_{600}) immediately drops to zero.

Question 3

Which of the following definitions correctly describes an Expression Quantitative Trait Locus (eQTL)?

A) A genetic variant, such as a SNP, that is statistically associated with quantitative differences in the expression level of a specific gene.

B) An artificial fluorescent dye that binds irreversibly to 18S and 28S ribosomal subunits during bridge amplification.

C) A specialized microfluidic channel that separates live nucleated cells from anucleated erythrocytes using shear stress.

D) A bacterial restriction endonuclease that degrades unmethylated foreign DNA during library preparation.

Question 4

Why is ribosomal RNA (rRNA) depletion commonly performed prior to constructing whole-transcriptome RNA sequencing libraries?

A) Ribosomal RNA contains high levels of thymine bases that permanently poison Illumina DNA polymerases on the flow cell.

B) Modern bioinformatic aligners like STAR cannot process single-stranded RNA molecules.

C) Ribosomal RNA comprises more than 80% to 90% of total cellular RNA, which would otherwise consume the vast majority of sequencing reads.

D) Ribosomal RNA actively degrades messenger RNA within minutes of cell lysis.

Question 5

What is the primary technical disadvantage of using nucleic acid microarrays compared to next-generation RNA sequencing?

A) Microarrays can only sequence short contiguous DNA fragments under 15 base pairs in length.

B) Microarrays require the complete absence of all biological salts and buffers in the hybridization chamber.

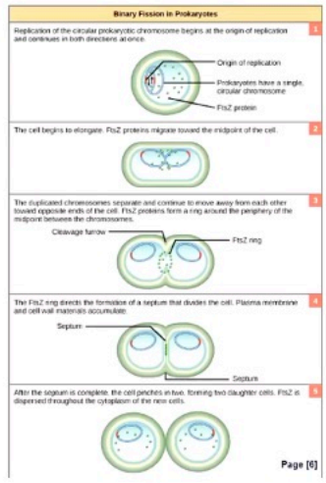
C) Microarrays are limited to single-cell suspensions and cannot analyze bulk tissue homogenates.

D) Microarrays rely on pre-designed immobilized oligonucleotide probes, preventing the discovery of novel or unannotated transcripts.

Answer Key & Detailed Explanations

1. **B** — The initial phase of salinity stress is osmotic: high concentrations of dissolved ions in the soil lower external water potential, making it difficult for root cells to extract water and causing rapid loss of turgor and wilting within minutes.
2. **D** — Sodium (Na^+) is structurally similar to potassium (K^+). When cytosolic Na^+ levels rise, it competes with and displaces K^+ from enzyme binding pockets; unlike K^+, Na^+ cannot activate these enzymes, causing protein misfolding and impaired protein synthesis.
3. **B** — Deep-rooted native perennial trees maintained deep water tables. Replacing them with shallow-rooted annual crops reduced water consumption, allowing excess seasonal rainfall to recharge groundwater; the rising water table dissolved subsoil NaCl and brought it to the surface, forming salt scalds.
4. **D** — In sodium-excluding plants, HKT transporters located on the plasma membrane of root xylem parenchyma cells extract Na^+ from the ascending xylem sap, sequestering it in the root and preventing toxic accumulation in photosynthetic leaf tissues.
5. **B** — Ubiquitous, constitutive overexpression of *HKT* causes Na^+ to be transported into cells throughout the entire plant. This turns the crop into an indiscriminate salt sponge, causing tissue toxicity, severe stunting, and sterility.
6. **A** — Generating transgenic rice takes over six months and involves significant expense. Combining the promoterless GAL4 gene and the UAS-GFP reporter into a single T-DNA construct allowed researchers to perform enhancer trapping efficiently in one transformation step.
7. **D** — The single isolated line that successfully conferred sodium exclusion drove expression specifically in the xylem parenchyma cells lining the mature xylem stream, exactly where Na^+ needs to be retrieved before reaching the shoot.
8. **B** — An intragenic plant is generated by recombining native genetic sequences (such as a native tissue-specific promoter fused to a native coding sequence from the same species) without retaining foreign bacterial genes. A cisgenic plant requires using an intact native gene with its own natural promoter.
9. **C** — *MerB* (organomercurial lyase) breaks the stable covalent carbon-mercury bond in organic methylmercury (CH_3Hg^+), converting it into the less toxic inorganic divalent ion (Hg^{2+}).
10. **B** — *MerA* (mercuric ion reductase) is an NADPH-dependent enzyme that reduces divalent inorganic mercuric ions (Hg^{2+}) to uncharged elemental mercury (Hg^0), which is volatile at physiological temperatures and diffuses out through stomata into the atmosphere.

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



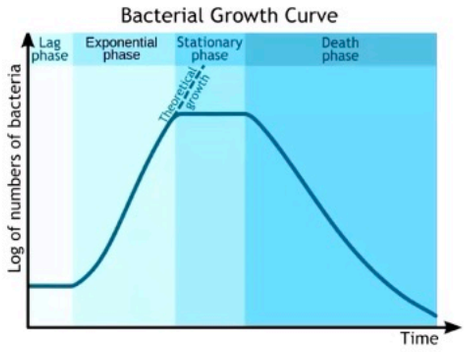
C. Population Dynamics & The Bacterial Growth Curve

- **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 (μ):**

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

(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 $-80°C$ 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 auto-injectors)	Often orally bioavailable (tablets/capsules)
Tissue Penetration	Poor diffusion into dense solid tumors/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 $\sim \$550$ 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

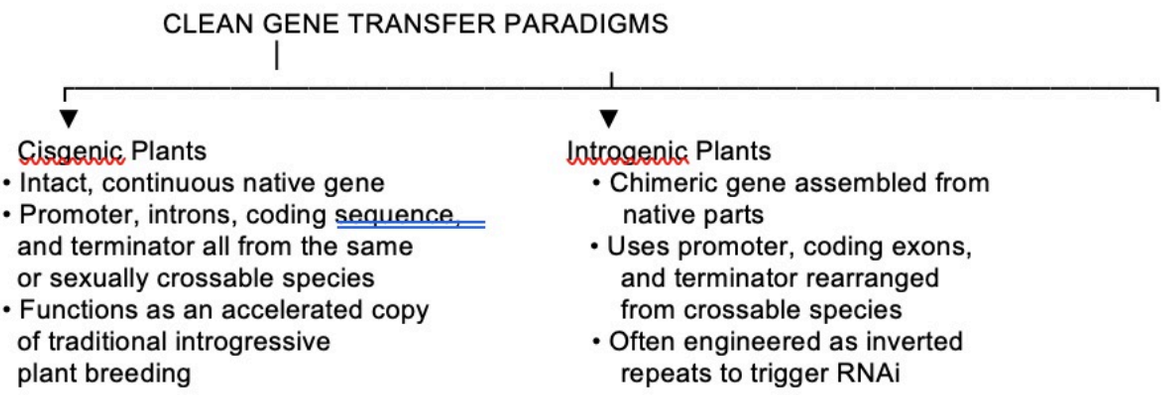
A. Regulatory Hurdles and Public Perception

- 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_0 lines are self-fertilized; under Mendelian independent assortment, the two loci segregate in the T_1 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).