AL-FARABI KAZAKH NATIONAL UNIVERSITY
FACULTY OF BIOLOGY AND BIOTECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

Lecture 3

GENERAL SCIENTIFIC METHODS IN BIOLOGY

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Aim of the Lecture

To introduce students to the general scientific methods used in biological research, explain their principles and applications, and develop an understanding of how these methods contribute to the study of biological processes and phenomena.



By the end of this lecture, students will be able to:

1. Identify the main general scientific methods used in biology.
2. Explain the principles of observation, comparison, analysis, and synthesis.
3. Describe the role of induction and deduction in biological research.
4. Understand the importance of modeling and experimentation in biology.
5. Select appropriate scientific methods for investigating biological phenomena

Agenda & Presentation Plan

Foundations of Biological Inquiry: Slides3–5:The nature of biological methodology, classification of methods, and the scientific method cycle as the core framework of inquiry.

Empirical Methods: Slides 6–10: Observational and experimental methods, scientific observation requirements, controls, variable isolation, and lab vs. field experiments.

Comparative, Historical & Instrumental Methods: Slides 11–18: Comparative and historical approaches, microscopy techniques, centrifugation, electrophoresis, and chromatography.

Quantitative, Molecular & Advanced Methods: Slides 19–30: Modeling, biostatistics, bioinformatics, PCR, DNA sequencing, CRISPR-Cas9, ethics, synthesis, workshop task, and references.

The Nature of Biological Methodology

Qualitative Observation: Systematic recording and description of biological phenomena across organisms, behaviors, and ecosystems, forming the foundational layer of biological inquiry.

Controlled Experimentation: Manipulating variables under controlled laboratory or field conditions to establish cause-effect relationships and test hypotheses at cellular and organismal levels.

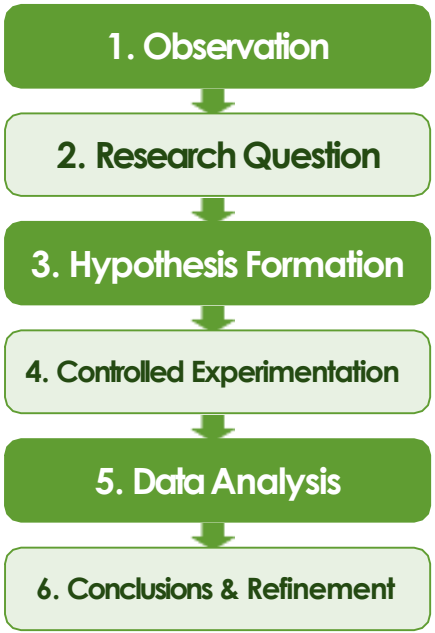
Mathematical Modeling: Applying quantitative frameworks and computational simulations to represent complex biological systems spanning molecules, cells, organisms, and ecosystems.



Classification of Biological Methods

Method / concept	Description / application
Observational Method	Systematic recording and description of biological phenomena — qualitative (behavior, morphology) or quantitative (counts, measurements). Applied across ecology, ethology, and taxonomy.
Experimental Method	Active manipulation of independent variables to establish cause-effect relationships. Comparison of experimental vs. control groups ensures valid, reproducible results across biological disciplines.
Comparative & Modeling Methods	Comparative analysis identifies evolutionary patterns across species. Mathematical and computational modeling simulates complex systems — ecosystems, metabolic pathways, and population dynamics.
Specialized Techniques	Subfield-specific tools requiring advanced instrumentation: patch- clamp electrophysiology in neurobiology, X-ray crystallography in structural biology, and flow cytometry in cell biology.

The Scientific Method in Biological Inquiry



Systematically record biological phenomena using qualitative and quantitative methods, forming the empirical foundation of all scientific inquiry.

Formulate a focused, testable question arising from observations, defining the scope and direction of the investigation.

Propose a falsifiable hypothesis predicting the relationship between variables, based on prior knowledge and observed patterns.

Design and conduct controlled experiments manipulating independent variables while measuring dependent variables to test the hypothesis.

Apply statistical and analytical tools to interpret collected data, identifying patterns, significance, and sources of variation.

Draw evidence-based conclusions, evaluate the hypothesis, and refine the model — feeding new observations back into the cycle.

The Observational Method

Systematic Recording &Description:Involvescareful, structured documentationof biological phenomena withoutmanipulating variables. Observers record data using standardized protocols, field notes, photographs, and measurement instruments to ensure repeatability and objectivity.

Qualitative vs. Quantitative Observation: Qualitative observations describe characteristics such as behavior patterns, morphological features, or species interactions. Quantitative observations yield numerical data including population counts, body measurements, and activity frequencies for statistical analysis.

Applications: Ethology, Ecology & Taxonomy: Ethology relies on behavioral observation to study animal communication and social structures. Ecology uses field surveys and transect sampling to assess biodiversity.
Taxonomy depends on morphological observation to classify and describe new species.



Requirements for Scientific Observation

Objectivity: Minimize observer bias by applying consistent criteria, blind protocols where possible, and peer verification to ensure data reflects true biological phenomena rather than researcher expectations.

Systematic Approach: Follow structured, repeatable protocols with defined sampling intervals and documented procedures so that observations can be independently replicated by other researchers.

Standardized Instruments: Employ calibrated, validated tools such as microscopes, sensors, and measuring devices with standardized units to ensure accuracy and comparability across studies.

Environmental Control: Record and regulate environmental factors such as temperature, humidity, light intensity, and time of day that may influence observations and confound biological data.



The Experimental Method

Variable Manipulation: The independent variable is deliberately manipulated by the researcher (e.g., light wavelength, temperature, or drug dosage) while all other conditions are held constant to isolate its specific effect on the biological system.

Dependent Variable Measurement: The dependent variable is systematically measured and recorded in response to changes in the independent variable (e.g., photosynthesis rate, enzyme activity, or cell growth), providing quantifiable evidence of biological effects.

Cause-Effect Establishment: Unlike observational studies, controlled experiments allow researchers to establish direct cause-and-effect relationships by comparing experimental groups against control groups under identical baseline conditions.



Experimental Controls and Variable Isolation

Method / concept	Description / application
Independent Variable	The factor deliberately manipulated by the researcher. Example: light wavelength applied to plant samples during a photosynthesis experiment.
Dependent Variable	The measured outcome that responds to changes in the independent variable. Example: photosynthesis rate recorded as O_2 production per hour.
Controlled Variables	All conditions kept constant across experimental and control groups. Example: temperature, CO_2 concentration, soil moisture, and pot size.
Control Group	The baseline group receiving no experimental treatment. Example: plants grown under natural white light, providing a reference for comparison.

Laboratory vs. Field Experiments

Method / concept	Description / application
Laboratory Environment	Controlled, artificial setting with regulated temperature, light, humidity, and nutrients; allows precise manipulation of variables.
Field Environment	Natural habitat setting preserving ecological interactions, seasonal cues, and realistic organism behavior in authentic conditions.
Advantages of Lab Experiments	High internal validity, repeatable conditions, precise measurements, and elimination of confounding environmental factors.
Advantages of Field Experiments	High ecological validity, realistic population dynamics, and results that are directly applicable to natural biological systems.

The Comparative Method

Morphological Comparison: Examineshomologousanatomical structures across species to reveal evolutionary relationships and shared ancestry. Forelimb bones in mammals demonstrate descent with modification from a common ancestor.

Comparative Genomics: Aligns DNA and protein sequences across species to identify conserved functional regions and divergence points. Reveals evolutionary pressures and enables reconstruction of phylogenetic relationships.

Comparative Physiology: Investigates how different organisms adapt physiologically to their environments. Comparing metabolic rates, thermoregulation, and oxygen transport across taxa uncovers adaptive strategies.



Applications of the Comparative Method

Method / concept	Description / application
Comparative Genomics	Aligns DNA and protein sequences across species to identify conserved gene functions, regulatory regions, and evolutionary divergences at the molecular level.
Comparative Physiology	Records complete sampling procedure, including technique, sample size rationale, and selection criteria, for transparency in the methodology chapter.
Sequence Alignment Analysis	Compares homologous DNA, RNA, and protein sequences across taxa to reconstruct phylogenies and pinpoint conserved versus mutated genomic regions.
Adaptation Studies	Examines physiological traits across species to reveal how organisms adapt biochemically and structurally to extreme or varied environmental conditions.

The Historical Method

FossilRecord Analysis: Fossils provide morphological evidence and absolute dating of extinct organisms, enabling reconstruction of anatomical transitions across geological time periods.

Biogeography: Studies the geographic distribution of species across continents and islands to infer historical dispersal routes, vicariance events, and evolutionary divergence
pat ter ns.

Molecular Clock Estimation: Molecular clocks use known mutation rates in DNA or protein sequences to estimate divergence times between lineages, calibrated against radiometric fossil dating.



Integrating Paleontological and Molecular Data

Method / concept	Description / application
Fossil Morphology & Preservation	Examine preserved hard and soft tissue structures in fossils to reconstruct ancestral morphologies, body plans, and phenotypic transitions across geological time periods.
Absolute Dating Methods	Apply radiometric dating (e.g., uranium-lead, carbon-14) to fossil- bearing strata to establish absolute age constraints and temporal calibration points for evolutionary lineages.
Molecular Clock Analysis	Estimate divergence times between lineages by measuring accumulated mutations in conserved gene sequences and applying known substitution rates as a molecular clo ck.
Calibrated Phylogenetic Trees	Integrate fossil calibration points with molecular phylogenies to produce time-calibrated trees that accurately reflect both morphological and genetic divergence events.

Microscopic Methods: Light Microscopy

Brightfield Microscopy: The simplest and most widely used light microscopy technique. Uses transmitted white light to illuminate stained or naturally pigmented specimens. Ideal for observing fixed tissue sections, cell morphology, and histological preparations.

Fluorescence Microscopy: Uses high-intensity light to excite fluorescent dyes or proteins (e.g., GFP) bound to specific cellular structures. Enables visualization of proteins, organelles, and dynamic processes in both fixed and living cells.

Confocal Microscopy: Uses a pinhole aperture to eliminate out-of-focus light, producing high-resolution optical sections. Enables 3D reconstruction of cellular structures and precise localization of fluorescently labeled molecules in living cells.



Electron Microscopy Methods

Method / concept	Description / application
Transmission Electron Microscopy (TEM)	Electrons pass through ultrathin specimen slices (50–100 nm). Reveals internal cell ultrastructure including organelles, membranes, and macromolecular complexes at sub-nanometer resolution.
Scanning Electron Microscopy (SEM)	Focused electron beam scans the specimen surface. Secondary electrons emitted create detailed 3D topographic images of cell surfaces, tissues, and material structures.
TEM	Internal Ultrastructure: Applications: visualizing mitochondrial cristae, nuclear pores , ribosome structure, viral particles, and synaptic vesicles within fixed and stained biological sections.
SEM	Surface Imaging: Applications: imaging cell surface morphology, pollen grain texture, insect compound eyes, nanoparticle coatings, and biofilm architecture at nanometer-scale resolution.

Differential Centrifugation Fractionation Process



Cells are disrupted by mechanical or chemical lysis to release organelles into a suspension buffer, forming the starting homogenate for fractionation.

The homogenate is centrifuged at low speed for 10 minutes. Dense cell debris and intact nuclei sediment to form the first pellet, leaving other organelles in the super nat ant.

The supernatant is spun at high speed for 20 minutes. Mitochondria, chloroplasts, and lysosomes pellet out, isolating these energy- producing organelles for biochemical analysis.

The final supernatant is ultracentrifuged for 60–90 minutes. Ribosomes, microsomes, and membrane fragments sediment, enabling isolation of translational machinery and vesicle components.

Electrophoresis and Chromatography

Method / concept	Description / application
Gel Electrophoresis Principle	Separates DNA, RNA, or proteins through a gel matrix using an electric field. Molecules migrate based on size and charge — smaller fragments move faster toward the positive electrode.
Agarose Gel for Nucleic Acids	Agarose gels resolve DNA and RNA fragments. A molecular ladder provides size reference. Ethidium bromide or SYBR stains visualize bands under UV light for size compar ison.
Chromatography Principle	Separates mixture components by differential affinity between a mobile phase and stationary phase. Variants include TLC, HPLC, ion-exchange, and size-exclusion chromatography.
Applications in Biology	Used to purify proteins, isolate metabolites, and analyze complex biological mixtures. Affinity chromatography exploits specific binding interactions to isolate target molecules with high purity.

The Modeling Method in Biology

EmpiricalData Input: Collect quantitative data from field observations, laboratory experiments, or sensor measurements to serve as the foundation for model construction and calibration.

Computational Model Construction: Translate biological processes into mathematical equations or algorithms. Models may represent ecosystems, metabolic pathways, population dynamics, or disease spread.

Predictive Output & Validation: Generate predictions about biological system behavior. Validate outputs against independent datasets and refine the model iteratively to improve accuracy.



Mathematical and Population Modeling

Method / concept	Description / application
Lotka-Volterra Predator-Prey. Model	Differential equations describe oscillating predator and prey populations: $dN/dt = \alpha N - \beta NP$ and $dP/dt = \delta NP - \gamma P$, revealing cyclical dynamics in ecosystems.
SIR Epidemiological Model	Compartmental model divides population into Susceptible, Infectious, and Recovered groups. Transmission rate (β) and recovery rate (γ) determine epidemic peak and herd immunity threshold.
Population Growth Equations	Exponential growth ($dN/dt = rN$) and logistic growth ($dN/dt = rN(1-N/K)$) model unconstrained and carrying- capacity-limited population dynamics respectively across biological systems.
Model Validation & Application	Models are validated against empirical field data, calibrated with real parameters, and used to predict outcomes, guide conservation policy, and forecast disease outbreaks.

Statistical Methods and Biostatistics

Method / concept	Description / application
Hypothesis Testing	Formulate null (H_0) and alternative (H_1) hypotheses. Statistical tests determine whether observed biological differences exceed what chance variation alone would produce.
t-Tests and ANOVA	t-tests compare means between two groups; one- way and two-way ANOVA extend this to multiple groups, identifying significant differences in biological measurements.
Regression Analysis	Linear and multiple regression model relationships between biological variables, enabling prediction of outcomes and quantification of effect sizes.
Statistical Significance (p □ 0.05)	The p-value threshold ($p < 0.05$) indicates a less than 5% probability that results are due to chance, establishing confidence in biological findings.

Sampling and Data Representation

Method / concept	Description / application
Random Sampling	Every individual in the population has an equal probability of selection, minimizing systematic bias and ensuring a representative sample for statistical inference.
Stratified Sampling	Population is divided into subgroups (strata) by shared characteristics; samples are drawn from each stratum proportionally to improve accuracy across diverse populations.
Transect Sampling	Linear transects and quadrat grids systematically record species presence and abundance along environmental gradients in field ecology studies.
Data Visualization Techniques	Biological data are displayed using scatter plots (correlations), box plots (distribution and outliers), and histograms (frequency distribution) to reveal patterns and variation.

Bioinformatics & Computational Biology

Raw Data Acquisition: High-throughput sequencers and biosensors generate massive volumes of raw genomic, transcriptomic, and proteomic data from biological samples.

Data Processing & Quality Control: Raw reads are filtered, trimmed, and aligned to reference genomes using bioinformatics pipelines to remove noise and ensure data integrity.

Algorithm Analysis & Interpretation: Machine learning algorithms and statistical models interpret processed datasets to identify gene expression patterns, variants, and biological functions.

Cloud Database Storage: Processed datasets are uploaded to cloud- based repositories such as NCBI, Ensembl, and UniProt for secure storage, sharing, and large-scale comparative analysis.

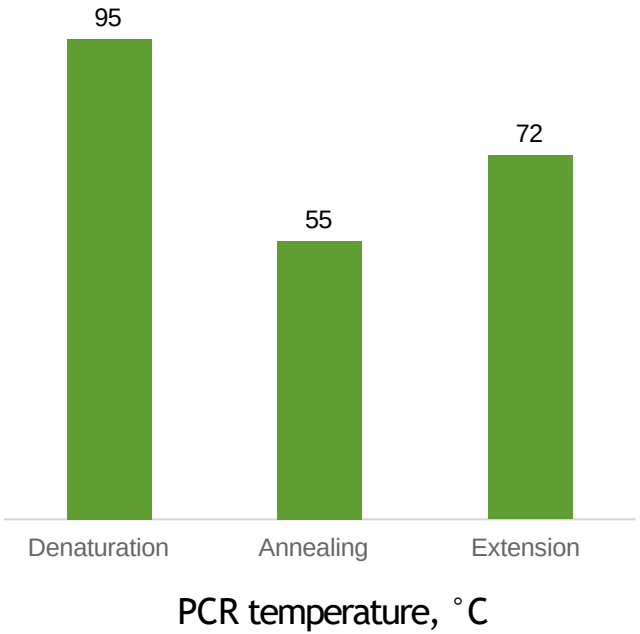


PCR Cycle Temperature Phases

Step1: Denaturation (95°C):The double-stranded DNA template is heated to 95°C, breaking hydrogen bonds between base pairs and separating the two strands to expose single-stranded templates for copying.

Step 2: Annealing (55°C): The reaction is cooled to ~55°C, allowing short DNA primers to bind (anneal) to their complementary sequences on each single- stranded template, defining the target region to be amplified.

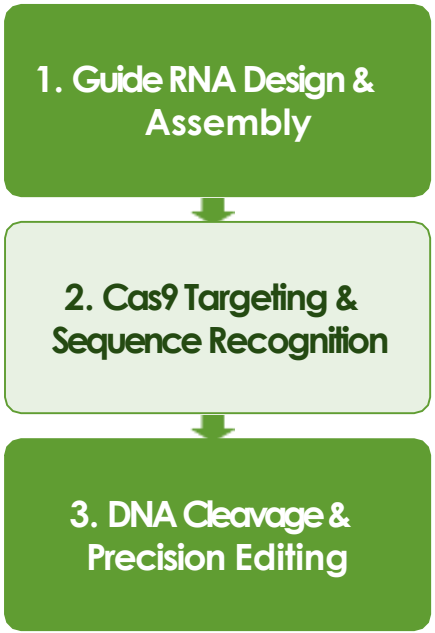
Step 3: Extension (72°C): Temperature rises to 72°C, the optimal activity point for Taq polymerase, which synthesizes a new complementary DNA strand from each primer, extending in the 5' to 3' direction.



DNA Sequencing Methodologies

Method / concept	Description / application
Sanger Sequencing	Chain-termination method developed by Frederick Sanger (1977). Uses dideoxynucleotides (ddNTPs) to terminate replication at specific bases. Reads ~800–1000 bp per reaction with high accuracy. Ideal for targeted single-gene sequencing and mutation verification.
Next-Generation Sequencing (NGS)	Massively parallel sequencing of millions of DNA fragments simultaneously. Enables whole- genome, transcriptome, and epigenome sequencing. Dramatically higher throughput at lower cost per base. Platforms include Illumina, Ion Torrent, and Oxford Nanopore.
Cost & Throughput Trends	Human Genome Project (1990–2003) cost ~$3 billion using Sanger. By 2023, NGS reduced whole- genome sequencing to under $200. Throughput increased from kilobases per day (Sanger) to terabases per run (NGS), enabling population-scale genomics.
Applications & Future Directions	Sanger: clinical diagnostics, forensic DNA profiling, plasmid verification. NGS: cancer genomics, metagenomics, GWAS studies, personalized medicine. Long-read platforms (PacBio, Nanopore) bridge gaps by resolving complex structural variants.

CRISPR-Cas9 Gene Editing Mechanism



A short guide RNA (sgRNA) is engineered to match the target DNA sequence. The 20-nucleotide spacer sequence is designed complementary to the genomic locus, directing the Cas9 protein with high specificity to the intended cut site.

The sgRNA-Cas9 complex scans the genome and binds to the complementary DNA sequence adjacent to a PAM motif (5'-NGG-3'). Watson-Crick base pairing between the guide RNA and target strand triggers Cas9 conformational activation.

Activated Cas9 introduces a double-strand break (DSB) at the target locus. The cell repairs the break via NHEJ (causing insertions/deletions) or HDR using a donor template, enabling precise gene knockout or correction.

Ethical Considerations in Biological Methodology

Refinement: Improve procedures to minimize pain, suffering, and distress in animal subjects. Use anesthesia, humane endpoints, and enhanced care standards to ensure animal welfare throughout the study.

Reduction: Minimize the number of animals used by optimizing experimental design, applying statistical power analysis, and sharing data across studies to avoid unnecessary duplication.

Replacement: Replace animal models with alternatives such as cell cultures, computer simulations, or organoids wherever scientifically valid. Human research requires informed consent and privacy safeguards.



Synthesis of General Biological Methods

Observational Foundation: Systematic field and microscopic observation generates the empirical questions and baseline data that drive all subsequent biological inquiry across scales.

Experimental Validation: Controlled laboratory and field experiments establish cause-effect relationships, testing hypotheses generated from observational and comparative evidence.

Molecular Sequencing: PCR, NGS, and CRISPR-based genomic methods reveal molecular mechanisms underlying the patterns identified through observation and experimentation.

Computational Modeling: Mathematical, statistical, and bioinformatic models integrate multi-scale data to predict system behavior and generate new testable biological hypotheses.



Practical Workshop Task: Microplastic Experiment Design

Method / concept	Description / application
Formulate Hypotheses	H_1: Microplastic exposure significantly reduces photosynthetic efficiency in marine phytoplankton. H_0: Microplastic exposure has no effect on photosynthetic efficiency.
Identify Variables	Independent Variable: microplastic concentration. Dependent Variable: photosynthetic efficiency. Controlled Variables: light intensity, temperature, and nutrient levels.
Define Experimental Groups	Experimental Group: phytoplankton cultures exposed to varying microplastic concentrations. Control Group: phytoplankton cultures with no microplastic exposure.
Select Measurement Technique	Measure oxygen production rate via dissolved oxygen sensor or chlorophyll fluorescence (Fv/Fm ratio) using a pulse amplitude modulation fluorometer.

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Method / concept	Description / application
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