Locomotion and Movement

Chapter 8

1 MOVEMENT AND LOCOMOTION

- **Movement** • Significant feature of living beings
- **Locomotion** • **Voluntary** movements resulting in change in place/location.
- Locomotion is performed by organisms for variable reasons, *e.g.,*
  - Their habitats
  - Demand of situation like search of food, mate, breeding ground, escape from enemies/predators

2 TYPES OF MOVEMENT / LOCOMOTION

Type	Structure	Examples and functions
Amoeboid	Pseudopodia involve microfilaments and streaming of protoplasm	Leucocytes, macrophages, *Amoeba*
Ciliary	Cilia	Removing dust particles from trachea Passage of ova through female reproductive tract
Flagellar	Flagella	Maintenance of water current in canal system of sponges Locomotion in *Euglena* Swimming of spermatozoa
Muscular	Muscles	Movement of limbs, jaws, tongue Running, walking, climbing, flying

- **All locomotions are movements but all movements are not locomotion.**
- In ***Paramoecium*** - Cilia helps in movement of food through cytopharynx and in locomotion as well.
- In ***Hydra*** - Tentacles are used for capturing of prey & also for locomotion.
- **Locomotion** requires a perfect coordinated activity of **muscular, skeletal** and **neural** systems.

3 MUSCLES

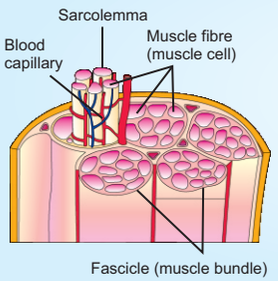
- **Muscle tissue:** Mesodermal in origin
- **40-50%** of body weight of a human adult is contributed by muscles.
- **Properties** • Excitability • Contractility • Extensibility • Elasticity
- Many cardiac muscle cells assemble in **branching** pattern to form a cardiac muscle

Classification of muscles

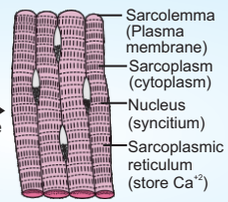
Basis	Location	Appearance	Regulation	Example
1.	Skeletal	Striated	Voluntary	Muscles of limbs
2.	Visceral	Non-striated/smooth	Involuntary	Inner walls of visceral organs
3.	Cardiac	Striated	Involuntary	Muscles of heart

4 SKELETAL MUSCLE FIBRES & ITS TYPES

- Skeletal muscles are closely associated with the skeletal components of the body.



Skeletal muscle
↓ Many
Muscle fibre bundles/ Fascicles
Held by ↓
Fascia (collagenous connective tissue)
→ Many muscle fibres



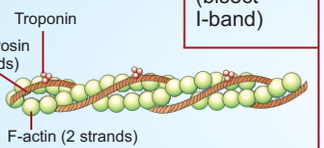
	Types	
	Red	**White**
Myoglobin	↑	↓
Mitochondria	↑	↓
SR	↓	↑
Respiration	Mainly aerobic	Mainly anaerobic

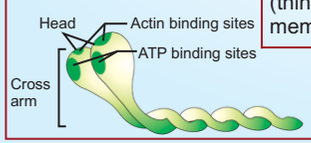
- Each muscle fibre have many parallelly arranged myofibrils / myofilaments.
- **Muscle fibre: Anatomical unit of muscle.**
- Skeletal muscles are primarily involved in locomotion and change in body posture.

5 MYOFILAMENTS AND STRUCTURE OF CONTRACTILE PROTEINS

Each myofibril has dark and light bands due to actin and myosin distribution that establish striated appearance.

	Filament	Held by	Protein	Monomer	Polymer	Typical
Light/I-band	Thin/actin	Z-line (bisect I-band)	Actin (contractile) Tropomyosin Troponin	Globular 'G' - actin — 3	Filamentous 'F'-actin —	F-actin helically arranged Tropomyosin run close to F-actin throughout its length Troponin distributed at regular intervals on tropomyosin Mask active binding sites for myosin on actin filaments
Dark/A-band	Thick	M-line (thin fibrous membrane)	Myosin (contractile)	Meromyosin (MM)	HMM (Heavy) - Head - **Short arm** LMM (Light) - Tail	Project outward at regular distance and angle from each other from the surface of polymerised myosin filament and is known as **cross arm**.





6 MECHANISM OF MUSCLE CONTRACTION/ SLIDING FILAMENT THEORY

- Contraction of muscle fibre takes place by the sliding of the **thin filaments over the thick filaments**.
  A motor neuron alongwith the muscle fibres connected to it constitute a **motor unit**.

CNS *via* → Motor neuron → Release → Neurotransmitter Acetylcholine

↓

At **Neuromuscular Junction** / **Motor end plate**, action potential is generated in sarcolemma that causes release of Ca^{+2} in sarcoplasm from SR leading to **Ca^{+2} increase** in sarcoplasm

↓

Ca^{+2} binds to troponin subunit, change in its confirmation, unmask active site for myosin binding on actin filament

↓

Energised myosin (Myosin – ADP + Pi) binds to actin

↓

Cross bridge = Actin-myosin-ADP + Pi

↓

Result (Shortening/ contraction of sarcomere)
- Pull thin filaments toward centre
- Pull Z-line
- Length of I-band reduced
- Length of A-band retained

↓

ADP+Pi released from myosin head

↓

New ATP binds to myosin head

↓

Cross bridge broken

↓

ATP hydrolysis on myosin head

↓

Cycle repeats

↓

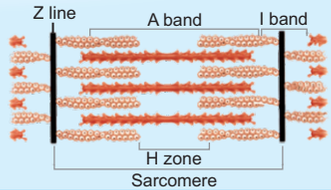
Process will continue till Ca^{2+} pumped back to sarcoplasmic cisternae

↓

Z-line return to original position

- **Reaction time** of fibres vary in different muscles.
- Repeated activity of muscle leads to accumulation of **lactic acid** due to **anaerobic** breakdown of **glycogen** in them, causing **fatigue**.

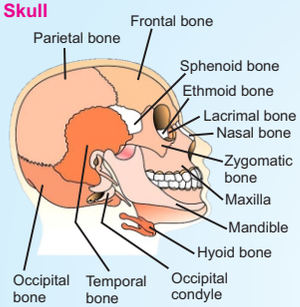
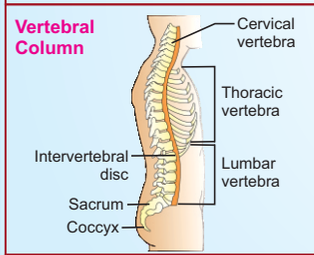
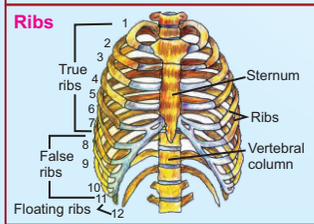
- **Globular head** is active **ATPase enzyme** and has binding sites for ATP and active sites for actin.
- Thin filaments make I/Isotropic band – actin
  Thick filaments make A/Anisotropic band – actin + myosin
  } Arranged alternately throughout the length of myofibrils parallel to each other and to longitudinal axis of myofibrils
- **Sarcomere: Functional unit** of contraction between 2 'Z' lines (elastic fibres) = **1 A-band + 2 half I-band**
- H-zone is non overlapped part of thick filament by thin filaments.



7 SKELETAL SYSTEM

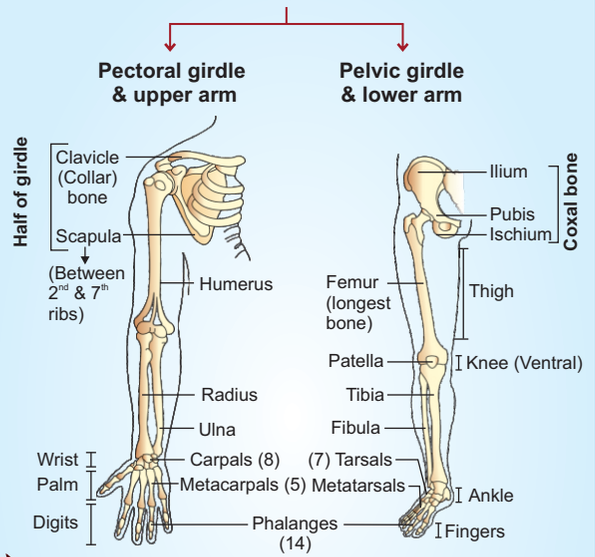
- This system has significant role in movement shown by the body.
- **Framework of 206 bones & few cartilages**
- **Principle division** • Appendicular skeleton • Axial skeleton

Axial skeleton (Bones-80)
Bones distributed along main axis

Structure	Bones included	No.	Name of bones	Typical feature or basic function
Skull 	Cranium	8	1- Frontal 1- Occipital 2- Parietal 1- Ethmoid 2- Temporal 1- Sphenoid	- Protect brain - Articulates with superior region of vertebral column by 2 occipital condyles **(Dicondylic skull)**
	Facial	14	2- Nasal 1- Mandible 2- Lacrimal 2- Maxilla 2- Zygomatic 5- others	- Form front part of skull
	Hyoid	1	1- U-shaped	- Present at the base of buccal cavity
	Ear ossicles	6	2-Malleus 2-Incus 2-Stapes	- Present in the middle ear
Vertebral Column 	Dorsal Vertebrae (serially arranged units)	26	7- Cervical 12- Thoracic 5- Lumbar 1- Sacral-Fused 1- Coccygeal-Fused	- Main framework of trunk - Protects spinal cord - Supports head - Point of attachment of ribs and muscles of back • 1st vertebra is **atlas** that articulates with **occipital condyles**. • **Seven cervical vertebrae** exist in almost all mammals. • **Neural canal** of vertebrae - site from where spinal cord passes
Sternum	Chest bone	1	1-Flat bone	- On ventral, midline of thorax
Ribs 	True ribs False ribs Floating ribs	24	14-Vertebrosternal 6- Vertebrochondral 4- Vertebral	- Attach dorsally to vertebrae and ventrally to sternum with **hyaline cartilage** - Not directly attached to sternum but to 7th rib with **hyaline cartilage** (8th to 10th pair) - Not connected ventrally (11th and 12th pair) • All ribs are **bicephalic thin flat bones** *i.e.*, they have 2 articulating ends on dorsal side • Vertebral column + Sternum + Ribs = **Rib Cage**

8 APPENDICULAR SKELETON (BONES - 126)

- Consists of bones of limbs (30 × 4 = 120) and girdles (6)



- Girdles helps in the articulation of limbs with axial skeleton
- Scapula, a dorsal triangular flat bone, have elevated ridge/spine, expanded to form **acromion process** that articulates with clavicle
- **Glenoid cavity** in scapula articulates with humerus head to form shoulder joint
- **Acetabulum**, formed by fusion of ilium, ischium and pubis, articulates with femur to form hip joint
- 2 halves of pelvic girdle meet ventrally to form **pubic symphysis** containing **fibrous cartilage**

9 JOINTS

- They are essential for all types of movements involving bony parts of the body.
- Point of contact between bones or bones and cartilages.
- Force generated by muscle is used to carry out movement through joint, where joint acts as **fulcrum**.
- **Types of joints** (Basis – Major structural forms)

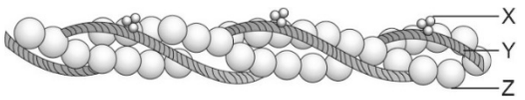
Types	Bones joined by	Movement	Examples
Fibrous	Dense fibrous connective tissue	Do not allow any movement	Flat skull bones fused end to end *via* **sutures** to form **cranium**
Cartilaginous	Fibrous cartilage	Limited movement	Adjacent vertebrae
Synovial	Fluid filled synovial cavity between 2 bones	Considerable movement, helps in locomotion and many other movements	Humerus & pectoral girdle (**Ball and socket joint**) Knee joint (**Hinge joint**) Atlas & axis (**Pivot joint**) Between carpals (**Gliding joint**) Carpal & metacarpal of thumb (**Saddle joint**)

10 DISORDERS

Disease	Causes	Impact
Myasthenia gravis	Autoimmunity	Affect neuromuscular junction Fatigue, weakening and paralysis of skeletal muscles
Muscular dystrophy	Genetic	Progressive degeneration of skeletal muscles
Tetany	Low Ca^{+2} in body fluid	Rapid spasms in muscle (wild contractions)
Arthritis		Inflammation of joints
Gout	Accumulation of uric acid crystals	Inflammation of joints
Osteoporosis	Age related Decreased levels of estrogen	Decreased bone mass, increased chances of fracture

Sharpen Your Understanding

1. Identify the structures labelled as X, Y and Z in the given figure [NCERT Pg. 306]



Which of the above structure(s) masks the myosin binding site on actin filament during resting state?

(1) X only (2) Y and Z

(3) X and Z (4) Y and Z

2. Which of the following when binds to the myosin head results in its detachment from actin? [NCERT Pg. 307]

(1) Ca^{+2} (2) Mg^{+2}

(3) ATP (4) ADP

3. Choose the **odd** one w.r.t. locomotion [NCERT Pg. 302]

(1) *Hydra* use tentacles for capturing prey

(2) Use of hind limbs for search of food in different fields

(3) Climbing a tree

(4) Flying to escape from predators

4. Pseudopodial movements are characteristic of all **except** [NCERT Pg. 303]

(1) Leucocytes (2) *Amoeba*

(3) Macrophages (4) Ova

NCERT Based MCQs

5. Which is **not** correct about muscle? [NCERT Pg. 303]

(1) It is mesodermal in origin

(2) 40-50% of body weight is formed by it

(3) They have properties like excitability and contractibility

(4) They are classified on the basis of reception of type of stimulus

6. The red and white muscle fibres can be distinguished from each other on the basis of all **except** [NCERT Pg. 308]

(1) Amount of myoglobin

(2) Dependence on aerobic process of energy

(3) Amount of sarcoplasmic reticulum

(4) Thickness & length of actin & myosin filaments

7. The central part of thick filaments, not overlapped by thin filament at relaxed state of muscle fibre is [NCERT Pg. 305]

(1) H-zone (2) I-band

(3) A-band (4) Sarcomere

8. During the mechanism of muscle contraction, following events occur.

A. Myosin head activated by ATP hydrolysis binds to exposed active sites to form cross bridge.

B. Pulling of actin filaments towards A-band leads to contraction.

C. Acetylcholine is released at neuromuscular junction which generate action potential.

D. Release of Ca^{+2} ions from SR, binds to subunit troponin of actin filaments.

Which of the following sequence of above events is **correct**? [NCERT Pg. 307]

(1) A, B, C, D (2) C, D, B, A

(3) C, D, A, B (4) C, B, D, A

9. In order to initiate muscle contraction, Ca^{+2} ions binds to which of the following muscle protein? [NCERT Pg. 307]

(1) Tropomyosin (2) Actin

(3) Troponin (4) Myosin

10. I-band lacks [NCERT Pg. 306]

(1) Actin (2) Tropomyosin

(3) Myosin (4) Troponin

11. Select the **incorrect** pair. [NCERT Pg. 309, 310]

(1) Axial skeleton – 80 bones

(2) Skull region – 22 + 1 + 6 bones

(3) Vertebrosternal ribs – 2 pairs

(4) Vertebrochondral ribs – 3 pairs

12. Which set of bones are single in human body? [NCERT Pg. 309, 311]
(1) Nasal, coxa
(2) Mandible, hyoid
(3) Temporal, sternum
(4) Frontal, maxilla

13. Match the appropriate number of the bones associated with given structures and choose the **correct** option [NCERT Pg. 310, 311]

a.	Limb bones	(i)	12
b.	Thoracic vertebrae	(ii)	2
c.	Hip girdle	(iii)	30
d.	Lumbar vertebrae	(iv)	5
		(v)	3

(1) a(iii), b(i), c(ii), d(iv)
(2) a(iii), b(i), c(v), d(iv)
(3) a(iv), b(i), c(ii), d(iii)
(4) a(iii), b(ii), c(i), d(iv)

14. Which of the following option include the bones whose number is **not** same in upper and lower limbs? [NCERT Pg. 311]
(1) Humerus and femur
(2) Metacarpals and metatarsals
(3) Radius and tibia
(4) Carpals and tarsals

15. Presence of a coxal bone formed by the fusion of three smaller bones is a feature of [NCERT Pg. 311]
(1) Pectoral girdle (2) Pelvic girdle
(3) Vertebral column (4) Tarsals

16. Acetabulum is bounded by [NCERT Pg. 311]
(1) Ischium and pubis only
(2) Ilium and pubis only
(3) Ilium, ischium and pubis
(4) Pubis only

17. Which pair of ribs do **not** articulate directly with the sternum but attach with hyaline cartilage of other ribs? [NCERT Pg. 310]
(1) 6^{th}, 7^{th}, 8^{th} (2) 8^{th}, 9^{th}, 10^{th}
(3) 7^{th}, 8^{th}, 9^{th} (4) 10^{th}, 11^{th}, 12^{th}

18. Which of the following joints does **not** allow any movement in our body? [NCERT Pg. 312]
(1) Between adjacent vertebrae
(2) Pubic symphysis in pelvic girdle
(3) Between skull and atlas
(4) Suture in skull bones

19. Which of the given disorder is commonly caused by decreased level of estrogen in aged woman? [NCERT Pg. 310]
(1) Arthritis
(2) Osteoporosis
(3) Myasthenia gravis
(4) Gout

20. During contraction and relaxation of skeletal muscle, size of which band remains unchanged? [NCERT Pg. 308]
(1) I-band
(2) A-band
(3) H-zone
(4) Z-line

Thinking in Context

1. Acromion process is the part of _____ bone. [NCERT Pg. 311]

2. Store house of Ca^{+2} ions in striated muscle fibres is _____. [NCERT Pg. 304]

3. Muscle bundles are held together by a common collagenous connective tissue layer called _____. [NCERT Pg. 304]

4. Spermatozoa show _____ movement. [NCERT Pg. 303]

5. M-line is present in the middle of _____ band. [NCERT Pg. 305]

6. Monomeric unit of thick myosin filament is called _____. [NCERT Pg. 306]

7. Ribs have two articulation surfaces on _____ side so are called _____.
[NCERT Pg. 310]

8. _____ is the autoimmune disorder affecting neuromuscular junction.
[NCERT Pg. 312]

9. _____ is the fundamental contractile unit of a skeletal myofibril. [NCERT Pg. 308]

10. Patella covers the knee _____.
[NCERT Pg. 311]

11. Our palm is formed of _____ bones.
[NCERT Pg. 311]

12. Maximum movement is possible at _____ joints. [NCERT Pg. 312]

13. Red colour of red muscle fibres is due to oxygen storing pigment called _____.
[NCERT Pg. 308]

14. Pliable nature of cartilage is due to _____.
[NCERT Pg. 309]

15. The cross arm of myosin head has two sites to bind with _____ and _____.
[NCERT Pg. 306]

16. _____ is the longest bone in our body.
[NCERT Pg. 311]

17. Pubic symphysis is a type of _____ joint.
[NCERT Pg. 311]

18. In a shoulder joint, head of humerus articulates with _____ cavity.
[NCERT Pg. 311]

19. Tetany, a rapid spasm in muscle is due to _____ ions in body fluid.
[NCERT Pg. 312]

20. Ventral side of thorax is supported by _____ bone. [NCERT Pg. 310]

❑ ❑ ❑

Biotechnology: Principles and Processes

Chapter 7

1 INTRODUCTION

- **Biotechnology** deals with techniques of using live organisms or enzymes from organisms to produce products and processes useful to humans.

Parameters	Traditional biotechnology	Modern biotechnology
Organisms involved	Microbes	Genetically modified organisms
Production	Small scale	Large scale
Examples/Technique include	Curd, bread or wine making	*In vitro* fertilisation leading to a 'test -tube' baby
		Synthesising a gene and using it
		Developing a DNA vaccine
		Correcting a defective gene

EFB (European Federation of Biotechnology)

- **'The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services'.**
- It encompasses both traditional view and modern molecular biotechnology.

2 PRINCIPLES OF BIOTECHNOLOGY/CORE TECHNIQUES INVOLVED IN MODERN BIOTECHNOLOGY

Parameters	Genetic engineering	Bioprocess engineering
Definition	Techniques to alter the chemistry of genetic material to introduce these into host organisms, and thus change the phenotype of host organism	Maintenance of sterile ambience in chemical engineering processes to enable growth of only the desired microbe/eukaryotic cell in large quantities
Include	Creation of rDNA Gene cloning Gene transfer	Manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.

The ability to multiply copies of antibiotic resistanse gene in *E.coli* was called **cloning** of antibiotic resistance gene in *E.coli*.

3 ADVANTAGES OF BIOTECHNOLOGY OVER OTHER TECHNIQUES

Methods	Advantage	Disadvantage
I. Asexual reproduction	Preserves genetic information	No variations
II. Sexual reproduction	Provides opportunities for variations and formulation of unique combinations of genetic setup	Some of which may be harmful to the organism as well as the population
III. Traditional hybridisation	Used in plant and animal breeding.	Very often lead to inclusion and multiplication of undesirable genes along with desirable genes.
IV. Genetic engineering	Allows us to isolate and introduce only one or a set of desirable genes without introducing undesirable genes into target organism.	—

4 THREE BASIC STEPS IN GENETICALLY MODIFYING ORGANISMS

(1) Identification of DNA with desirable genes (2) Introduction of the identified DNA into the host
(3) Maintenance of introduced DNA in the host and transfer of the DNA to its progeny

5 KEY TOOLS OF RECOMBINANT DNA TECHNOLOGY

(1) **Enzymes** (2) **Vectors** (3) **Competent host cells**

Enzymes - Most commonly used enzymes in genetic engineering are
- **Nucleases**
- **DNA polymerase**
- **Ligases**

Nucleases - Catalyse the cleavage of nucleic acids.

Types

Exonucleases
Remove nucleotides from the ends of the DNA

Endonucleases
Make cuts at specific positions within the DNA *i.e.* at **recognition/palindromic sequence**

- In the year 1963, the two enzymes responsible for **restricting the growth of bacteriophage** in *Escherichia coli* were isolated

Methylase
Add methyl groups to bacterial DNA

Restriction endonuclease / Molecular scissors
Cut the DNA of bacteriophage

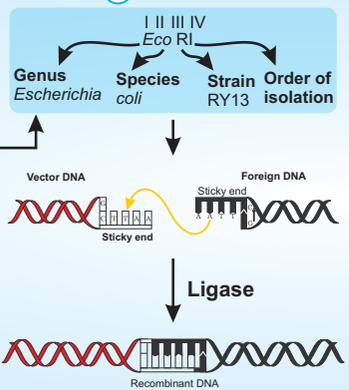
Palindromic sequence
reads same on the two strands (from 5′ → 3′ and 3′ → 5′ direction) when orientation of reading is kept same

6 ENZYMES

- **Restriction endonuclease**

More than **900** restriction enzymes have been isolated from over **230** strains of bacteria (**prokaryotic cell**) each of which recognise different recognition sequences.

- **Nomenclature/Naming of enzyme :**
- **Functions by:**
  - **'Inspecting'** the length of DNA sequence
  - **Binds** to the **"specific recognition sequence"**
  - **Cuts** the two strands of **ds DNA** at specific points in their **sugar-phosphate backbones** and leaves single stranded portions at the ends.
  - These **overhanging stretches** and called **sticky ends.**



Steps in formation of rDNA

- **Ligase**
  - When source DNA and vector DNA are cut by the same restriction enzyme the resultant DNA fragments have the same kind of 'sticky-ends'. Sticky ends are named so because they form hydrogen bonds with their complementary cut counterparts and this stickiness facilitates the action of the enzyme **DNA ligase**.

- First restriction endonuclease - ***Hind* II :** Isolated and characterised five years later, recognises sequence of **6 bp**.
- First recombinant DNA was prepared by **Stanley Cohen and Herbert Boyer, 1972** :
  - Antibiotic resistant gene
  - Plasmid of *Salmonella typhimurium*

→ Recombinant plasmid —Introduced into→ *Escherichia coli*

7 CLONING VECTORS

- **Vectors are vehicles** for delivering foreign DNA into recipient cells.
- Vectors used at present are engineered in such a way that they help **easy linking of foreign** DNA and selection of recombinants from non recombinants

Features of cloning vectors:

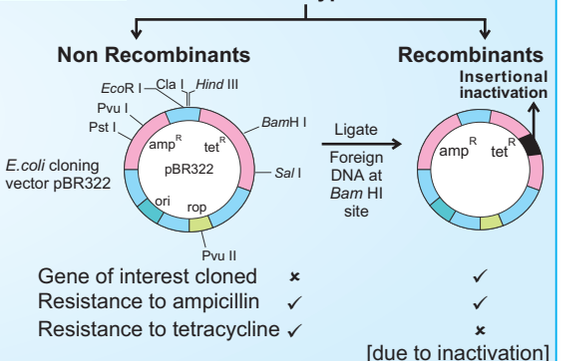
(1) **Origin of Replication (ori):**
- Sequence from where replication starts
- Responsible for **controlling copy number** of the linked DNA
- Those vectors are preferred which support **high copy number**

(2) **Selectable Marker:**
- Helps in selection of transformants
- Normally, the **genes encoding resistance to antibiotics** such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are considered useful selectable markers for *E.coli*
- The **normal *E.coli* cells do not carry resistance against any of these antibiotics**

(3) **Cloning Sites/Restriction Sites**
- **Single recognition site** for a restriction enzyme within the vector is a **preferable** feature.
- Presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning
- The ligation of alien DNA/**gene of interest (GOI)** is carried out at a restriction site present in one of the antibiotic resistant genes.

- **Transformation:** Procedure through which piece of foreign DNA is introduced in a host bacterium.
  - **Insertional inactivation:** Insertion of GOI within antibiotic resistance gene/selectable marker results in inactivation/formation of the coded product.
  - **Hypothesis:** Insertion of GOI at *Bam* HI site in tet^R.
  - If transformation **fail**s – Non transformants are obtained in antibiotic lacking agar medium but they don't grow on antibiotic rich medium.
  - If transformation **successful** – **Transformants** obtained are of **two types:**

- **All transformants are not recombinants but all recombinants are transformants.**
- One antibiotic resistant gene helps in selecting the transformants whereas the other antibiotic resistant gene helps in selection of recombinants
- *rop* → codes for the proteins involved in the replication of the plasmid



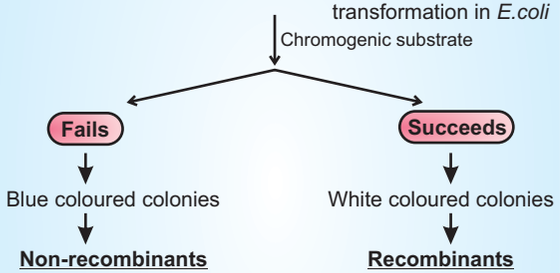
- **Plasmids as vectors:**
  - **Extra chromosomal**, circular, double stranded DNA.
  - Replicate independent of the control of chromosomal DNA (autonomously).
  - They may have 1 or 2 copies per cell or even15 - 100 copies per cell.

9 OTHER CLONING VECTORS

Selection of recombinants due to inactivation of antibiotic resistant gene as in pBR322 is a cumbersome procedure because it requires simultaneous plating of two plates having different antibiotics.

To **overcome the disadvantage of pBR322**, alternative selectable markers (***lac Z***) acting as **reporter enzyme** have been developed which differentiate recombinants from non-recombinants on the basis of their ability to produce colour in the presence of chromogenic substrate.

- *lac* Z gene coding for β-galactosidase acts as selectable marker in the plasmid
- **Experiment:** Insert foreign DNA at *lac* Z gene + transformation in *E.coli*



- **Ti plasmid of *Agrobacterium tumefaciens***
  - *Agrobacterium tumefaciens*, a pathogen of several **dicot plants** is able to deliver a piece of DNA known as '**T-DNA**' to transform normal plant cells into a tumor and direct the tumor cells to produce the chemicals required by the pathogen.
  - **Disarmed tumour inducing (Ti) plasmid** is used which is no more pathogenic to the plants but is still able to use the mechanism to deliver the genes of our interest into varieties of plants.
- **Bacteriophages**
  - **High copy number** than plasmid
- **Retroviruses**
  - Retroviruses in animals have the ability to transform normal cells into cancerous cells
  - **Disarmed retroviruses** are used to deliver desirable genes **into animal cells**

10 METHODS OF TRANSFORMATION

I. Micro - injection
- Recombinant DNA is **directly** injected into the nucleus of an **animal cell**.

II. Biolistic/Gene gun
- **Plant cells** are bombarded with high velocity micro-particles of **gold or tungsten coated with DNA**.

III. Heat shock method

IV. "Disarmed pathogen" vector

11 COMPETENT HOST FOR TRANSFORMATION WITH RECOMBINANT DNA

- DNA is **hydrophilic**, so it can not pass through cell membranes
- In order to force cell to take up alien DNA/rDNA, it must first be made '**competent**' by **treating** with ice cold **calcium chloride**.
- Entry of rDNA in host cell is due to transient pores created by heat shock (42°C) and not due to Ca^{+2} ions.
- Divalent cations **increases the efficiency** with which DNA enters the bacterium through pores in its cell wall.

12 PROCESS OF RECOMBINANT DNA TECHNOLOGY

Isolation of DNA
↓
Fragmentation of DNA by restriction endonucleases
↓
Isolation of desired DNA fragment (**electrophoresis**)
↓
Amplification of gene of interest (**PCR**)
↓
Ligation of the DNA fragment into a vector
↓
Transferring the alien DNA/recombinant DNA into the host
↓
Culturing the host cells in a medium at large scale (**Bioreactors**)
↓
Extraction and **purification** of the desired product

I. Isolation of the Genetic Material (DNA)
- In majority of organisms, DNA is the genetic material
- Since DNA is enclosed within the membranes, we have to break the cell open to release DNA along with other macromolecules → **Bacteria → Lysozyme**; **Fungi → Chitinase**; **Plant** cell → **Cellulase**
- In order to get DNA in pure form (free from other macromolecules), it is treated with different enzymes like RNase, protease etc.

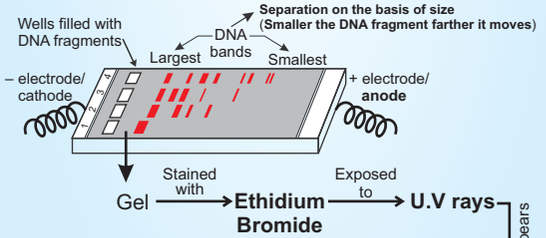

Pure DNA → Centrifuge → **Chilled ethanol to precipitate DNA**


Spooling ← Process

II. Fragmentation by restriction endonucleases

III. Separation and isolation of DNA fragments
- **Gel electrophoresis**
  - Separation of negatively charged DNA molecules under an electric field through a medium/matrix.
  - Most commonly used matrix for DNA separation is

Agarose → Natural polymer, obtained from **sea weeds**
→ Separate DNA fragments through **seiving effect**



Gel —Stained with→ **Ethidium Bromide** —Exposed to→ **U.V rays** —Appears→ **Bright orange bands** ← Process ← **Elution** → Removal of DNA fragment from gel

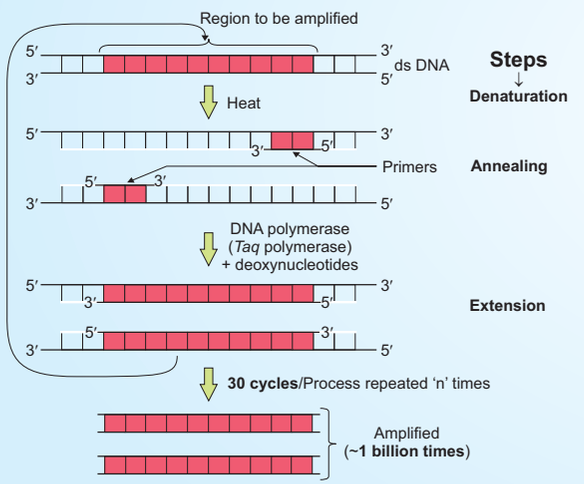
- Purified DNA fragments are generally amplified (PCR) before constructing rDNA by joining with cloning vector.

IV. PCR - Polymerase Chain Reaction

- *In vitro* amplification of DNA (gene of interest)

Reaction mixture	Work/Function
Nucleotides	Formation of DNA chain
Primers	2 sets of chemically synthesised oligonucleotides, complementary to the regions of DNA
***Taq* polymerase**	**Thermostable** DNA polymerase, isolated from bacterium, ***Thermus aquaticus***, remains active during high temperature induced denaturation of dsDNA. It extends the primers *i.e.* meant for chain elongation.
Genome DNA	Template DNA for gene of interest

- **Sequence of events**



- The amplified fragment if desired can now be used to ligate with a vector for further cloning.

V. Ligation of the DNA fragment into a vector by DNA ligase

↓

VI. Insertion of recombinant DNA into the host cell

- Transformed host cells are selected with the help of selectable marker genes.

VII. Culturing of recombinant host cells (Biosynthetic stage)

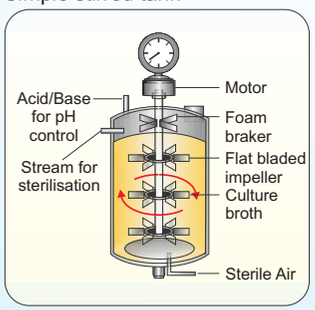
- The cells harbouring cloned genes of interest may be grown in **Laboratory/ Bioreactors**

Parameters	Laboratory	Bioreactors
Culture	Small volume	Large volumes (100 - 1000 lts)
Maintaining optimal conditions	Not possible	✓
Growth rate of cell	Never optimal	Optimum
Production	Small scale	Large scale

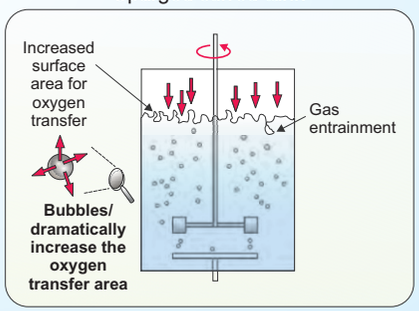
- Commonly used Bioreactors are **stirred type** having
  - Cylindrical or with curved base → Facilitate mixing of reactor contents
  - Stirrer → **Facilitate even mixing** and **oxygen availability** throughout the bioreactor
  - Agitator system
  - Oxygen delivery system
  - pH control system
  - Foam control system
  - Sampling ports → To withdraw small volumes of culture periodically

- **Types of stirred tanks**

Simple stirred tank



Sparged stirred tank



- **Bioreactors:** Vessels in which raw materials are biologically converted into specific products using microbial plant, animal human cells and provide optimal growth conditions (temperature, pH, substrate, salts, vitamins, oxygen)

In Open Culture System/ Continuous Culture System

- Used medium is drained out from one side while fresh medium is added from the other to maintain the cells in their **physiologically most active log/exponential phase**.
- Larger biomass → Higher yields of desired protein.

VIII. Downstream processing

- **Separation** and **purification** of the desired product/**recombinant protein** from **heterologous host** (non native host).
- Product has to be formulated with suitable **preservatives**.
- **Strict quality control testing** is done for each product
- The downstream processing and quality control testing vary from product to product.

IX. Product is subjected for marketing as a finished product

Sharpen Your Understanding

1. The definition given by EFB for biotechnology is [NCERT Pg. 193]
   (1) Techniques of using live organisms only
   (2) Techniques of using enzymes only to produce products and processes useful to humans
   (3) The integration of natural science and organisms, cells, parts there of and molecular analogues for product and services
   (4) Techniques which include only synthesising a gene and using it
2. The core technique of biotechnology which involves maintenance of sterile ambience is [NCERT Pg. 194]
   (1) Genetic engineering
   (2) Bioprocess engineering
   (3) Developing a DNA vaccine
   (4) Correcting a defective gene
3. _____ gene codes for the proteins required for the replication of plasmid [NCERT Pg. 199]
   (1) ori (2) rop
   (3) amp^R (4) tet^R
4. Genetic engineering is better over traditional hybridization in [NCERT Pg. 194]
   (1) Introducing desirable genes only
   (2) Introducing undesirable genes
   (3) Introducing both desirable and undesirable genes
   (4) Being easy to perform
5. First recombinant DNA was made by linking **A** with a **B** of **C**. Choose the option which correctly fill the blanks **A**, **B** and **C**. [NCERT Pg. 194]

	A	B	C
(1)	Antibiotic resistant gene	Plasmid	*Salmonella typhimurium*
(2)	Antibiotic resistant gene	Plasmid	*Escherichia coli*
(3)	Antibiotic sensitive gene	Chromosomal DNA	*Salmonella typhimurium*
(4)	Antibiotic sensitive gene	Chromosomal DNA	*Escherichia coli*

6. Restriction endonuclease and methylase were isolated from *E.coli* in the year [NCERT Pg. 195]
   (1) 1972 (2) 1963
   (3) 1997 (4) 1990
7. In *Eco* RI, the letter *R* is derived from [NCERT Pg. 195, 196]
   (1) Genus (2) Species
   (3) Strain RY 13 (4) Strain Rd

NCERT Based MCQs

8. Choose the **odd** one w.r.t. ends produced by *Eco* RI [NCERT Pg. 197]
   (1) Sticky ends
   (2) Single stranded portion on each strand of DNA
   (3) Overhanging stretches on each strand of DNA
   (4) Blunt ends
9. Restriction endonucleases cut which bonds? [NCERT Pg. 196]
   (1) Hydrogen bonds
   (2) Phosphodiester bonds
   (3) Ionic bonds
   (4) Disulphide bonds
10. The separated DNA fragments can be visualised only after staining the DNA with **A** and followed by exposure to **B**. Choose the option which correctly fill the blanks **A** and **B** respectively. [NCERT Pg. 198]

	A	B
(1)	Ethidium bromide	UV radiations
(2)	Bromophenol blue	UV radiations
(3)	Ethidium bromide	Visible light
(4)	Methylene blue	Visible light

11. The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This step is known as [NCERT Pg. 198]
(1) Precipitation (2) Elution
(3) Spooling (4) Fragmentation

12. If we ligate foreign DNA at *Bam* HI site of pBR322, then the resultant recombinants will show [NCERT Pg. 199]
(a) Resistance to ampicillin
(b) Sensitivity to tetracycline
(c) Resistance to tetracycline
(d) Sensitivity to ampicillin
(1) a and b (2) a and c
(3) b and d (4) c and d

13. Recombinant host cells in which foreign DNA is present in the coding sequence of enzyme β-galactosidase will produce [NCERT Pg. 200]
(1) Blue coloured colonies
(2) White coloured colonies
(3) Pink coloured colonies
(4) No colonies

14. Which vector is suitable to deliver desirable genes into dicot plants? [NCERT Pg. 200]
(1) pBR322
(2) Disarmed Ti plasmid of *Agrobacterium*
(3) Disarmed retrovirus
(4) Bacteriophage

15. Complete the analogy w.r.t. DNA isolation Bacteria : Lysozyme :: Fungus : _____ [NCERT Pg. 201]
(1) Cellulase (2) Chitinase
(3) Amylase (4) DNAse

16. All of the following are methods of direct gene transfer **except** [NCERT Pg. 201]
(1) Micro-injection
(2) Biolistics
(3) Gene gun
(4) Disarmed pathogen

17. To make the host cells competent, they are treated with specified concentration of [NCERT Pg. 200]
(1) Ca^{+2} (2) Na^{+}
(3) K^{+} (4) Cl^{-}

18. Which of the following is **incorrect** w.r.t. PCR? [NCERT Pg. 202, 203]
(1) *In vitro* amplification of DNA
(2) Single set of RNA primers
(3) Primers are chemically synthesized oligonucleotides
(4) Thermostable DNA polymerase is used

19. Select the **correct** sequence for the steps involved in PCR [NCERT Pg. 202]
(1) Denaturation → Annealing → Extension
(2) Annealing → Denaturation → Extension
(3) Denaturation → Extension → Annealing
(4) Extension → Annealing → Denaturation

20. What is **not** present in the simple stirred-tank bioreactor? [NCERT Pg. 204]
(1) Foam breaker
(2) Temperature control system
(3) Sampling port
(4) Sparger

Thinking in Context

1. EFB stands for _____. [NCERT Pg. 193]

2. The two core techniques that enabled birth of modern biotechnology are _____ engineering and _____ engineering. [NCERT Pg. 193, 194]

3. _____ sequence is responsible for initiating replication in plasmid. [NCERT Pg. 194]

4. First recombinant DNA was made by _____ and _____ in the year _____. [NCERT Pg. 194]

5. The cutting of DNA at specific locations became possible with the discovery of _____ enzymes, which are also called _____. [NCERT Pg. 194, 195]

6. The linking of antibiotic resistance gene with the plasmid vector became possible with the enzyme _____. [NCERT Pg. 195]
7. The first restriction endonuclease is _____. Its recognition sequence is _____ long. [NCERT Pg. 195]
8. First letter in the name of restriction endonuclease come from _____ and the second two letters come from the _____ of the prokaryotic cell from which they were isolated. [NCERT Pg. 195]
9. Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome sites, but between the _____ on the opposite strands. This leaves single stranded portions on each strand called _____. [NCERT Pg. 197]
10. DNA fragments are _____ charged molecules that move towards _____ during gel electrophoresis. [NCERT Pg. 198]
11. DNA fragments separate according to their _____ through _____ effect provided by the agarose gel. The _____ the fragment size, the farther it moves. [NCERT Pg. 198]
12. _____ sequence controls the copy number of the linked DNA. [NCERT Pg. 199]
13. *Agrobacterium tumefaciens*, a pathogen of several _____ plants is able to deliver an alien DNA through its _____ plasmid. [NCERT Pg. 200]
14. Disarmed _____ are now commonly used to deliver desirable genes into animal cells. [NCERT Pg. 200]
15. In a method known as _____, recombinant DNA is directly injected into the nucleus of an animal cell. [NCERT Pg. 201]
16. _____ cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA in a method known as _____ or _____. [NCERT Pg. 201]
17. Since DNA is a _____ molecule, it cannot pass through cell membranes. [NCERT Pg. 200]
18. If any protein encoding gene is expressed in a heterologous host, it is called a _____ [NCERT Pg. 203]
19. *Taq* polymerase, a _____ DNA polymerase, is isolated from _____. [NCERT Pg. 203]
20. Separation and purification of desired product in genetic engineering are included in _____ processing. [NCERT Pg. 204, 205]

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