BSEH MARKING SCHEME

CLASS- XII Biotechnology (March-2024) Code: A

- **The answer points given in the marking scheme are not final. These are suggestive and indicative. If the examinee has given different, but appropriate answers, then he should be given appropriate marks.**

Q. No.	Answers	Marks
1.	a) *Haemophilus influenza*	1
2.	c) Co-ordinate bonds	1
3.	a) K	1
4.	b) Urea	1
5.	a) Leaf culture	1
6.	b) G. Gay	1
7.	Double	1
8.	Latex of green fruit and leaves of papaya tree	1
9.	Thomas Roder	1
10.	Vacuum	1
11.	transgenic plants or their tissues producing antigens can be eaten for vaccination/immunization.	1
12.	Christmas disease	1
13.	a) Both A and R are true, and R is the correct explanation of A.	1
14.	c) A is true but R is false	1

<table>
<tr><td>15.</td><td>c) A is true but R is false</td><td>1</td></tr>
<tr><td>16.</td><td>variation in size (length) of the restriction enzyme generated fragments among individuals within a given species is termed RFLP.
(1 mark)
A major application of this technique is DNA fingerprinting analysis.
(1 mark)</td><td>2</td></tr>
<tr><td>17.</td><td>ADA deficiency, Hemophilia B
(1 mark each)</td><td>2</td></tr>
<tr><td>18.</td><td>
<table>
<tr><th>Characteristics</th><th>Batch culture</th><th>Fed-batch culture</th></tr>
<tr><td>Cultivation system</td><td>Closed type</td><td>Semi-closed type</td></tr>
<tr><td>Addition of fresh nutrition</td><td>No</td><td>Yes</td></tr>
<tr><td>Volume of culture</td><td>Constant</td><td>Increases</td></tr>
<tr><td>Chance of contamination</td><td>minimum</td><td>Intermediate</td></tr>
<tr><td>Log phase</td><td>Shorter</td><td>longer</td></tr>
<tr><td>Product yield</td><td>Low</td><td>Medium</td></tr>
</table>
(any two, 1 mark each)
Or
• Continuous culture provides a better degree of control than a batch culture.</td><td>2</td></tr>
</table>

	• Growth rates are regulated and maintained for extended periods. • By varying the dilution rate, biomass concentration may be controlled. • Secondary metabolite production may be sustained simultaneously together with growth. (½ mark each)	
19.	• The non-coding region of eukaryotic gene must be excised. This requires use of reverse transcription of mRNA into cDNA. • Additionally, the recombinant protein may not be secreted into the medium or its incorrect folding and accumulation intracellularly may generate inclusion bodies. (1 mark each)	2
20.	Monoclonal antibodies (mAbs) bind specifically to an epitope on an antigen (1 mark) Herceptin (trastuzumab) is a monoclonal antibody approved for therapy of early-stage breast cancer. (1 mark) Or	2

	tPA is a serine protease that catalyzes the conversion of plasminogen to plasmin which is responsible for dissolving blood clots. (1 mark) tPA is produced by introducing plasmid, containing tPA gene, in mammalian cells. (1 Mark)	

21.			
	• In roller bottles, the cells adhere to the total curved surface area of the micro carrier beads, thereby markedly increasing the available space for growth.	• Spinner cultures are used for scaling up the production of suspension cells. They consist of a flat surface glass flask with a suspended central teflon paddle that turns and agitates the medium when placed on a magnetic stirrer.	2

	• After each complete rotation of the bottle, the entire cell monolayer has transiently been exposed to the medium.	• The cells are not allowed to settle to the bottom of the flask and thus cell crowding occurs only at very high densities.
	• The volume of medium need only be sufficient to provide a shallow covering over the monolayer	• Stirring the medium improves gas exchange
	(Any two, 1 mark each)	
22.	1. Isolation of a DNA fragment containing a gene of interest that needs to be cloned (called as insert). (½ mark) 2. Generation of a recombinant DNA (rDNA) molecule by insertion of the DNA fragment into a carrier DNA molecule called vector (e.g. plasmid) that can self-replicate within a host cell. (1 mark)	3

	3. Transfer of the rDNA into an E. coli host cell (process called transformation). (1 mark) 4. Selection of only those host cells carrying the rDNA and allowing them to multiply thereby multiplying the rDNA molecules. (½ mark) Or 1. It must contain an origin of replication (ori) so that it is independently able to replicate within the host. 2. It should incorporate a selectable marker, a gene whose product can identify the host cells containing the vector. 3. The vector must also have one unique restriction enzyme recognition site which can be used for cutting and introducing an insert. (1 mark each)	
23.	Volume of the E.coli (V) = $\pi r^2 h$ $= \frac{22}{7} \times 0.5 \times 0.5 \times 2$ $= 1.57 \mu m^3$ $= 1.57 \times 10^{-12} ml$ (½ mark) Mass of the E.coli cell = Density of cell × volume of the cell $= 1.28 \times 1.57 \times 10^{-12}$ $= 2.0096 \times 10^{-12}$ 13.5% of the wet weight of E.coli is soluble proteins. now we can calculate the mass of the protein ∴ Mass of the soluble protein (M)= $\frac{13.5}{100} \times$ mass of the cell $= 0.271296 \times 10^{-12}$	3

(1 mark)

The desired protein represent 0.1 % of all soluble proteins in the cell.

$\therefore$ mass of desired enzyme (Menz) = $\frac{0.1}{100} \times M$

$= 0.2713 \times 10^{-15}$

moler mass of enzyme = mass of the enzyme / molecular weight of the enzyme

$= 0.2713 \times 10^{-15} / 100000$

$= 0.2713 \times 10^{-20}$

(1 mark)

molecules of enzymes in the cell = no. of moles × Avogadro's number

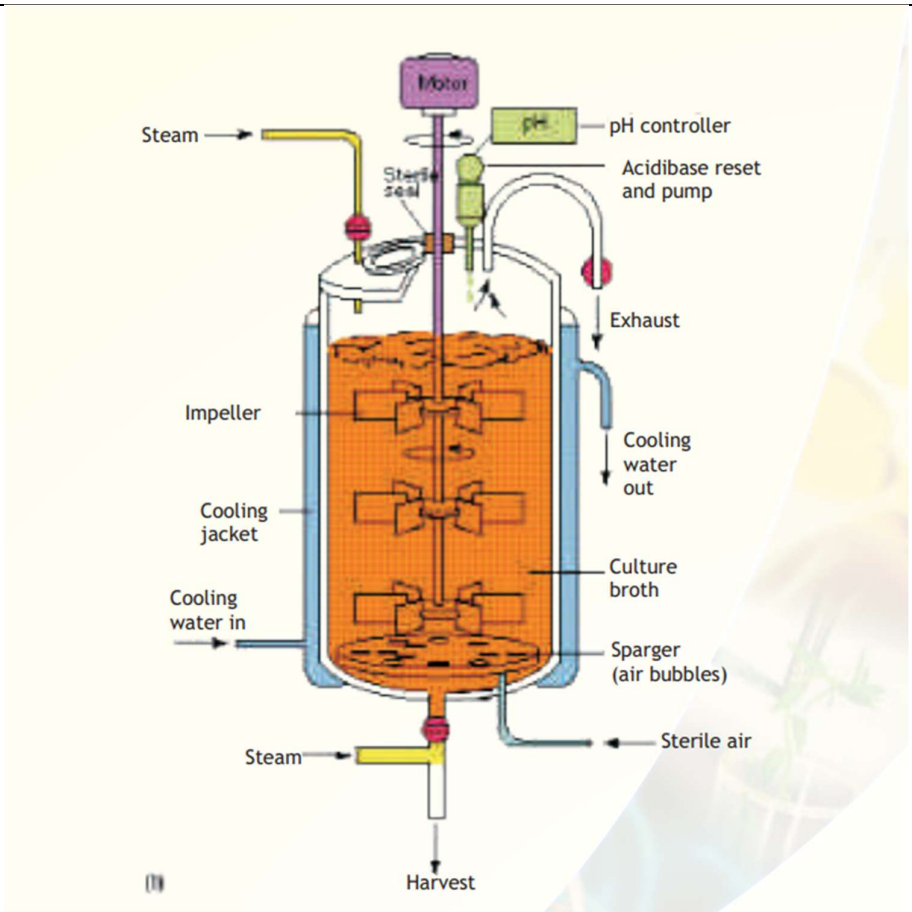
$= 0.2713 \times 10^{-20} \times 6.023 \times 10^{23}$

$= 1634$

Therefore, 1634 molecules of the enzyme are produced in one cell.

(½ mark)

24.



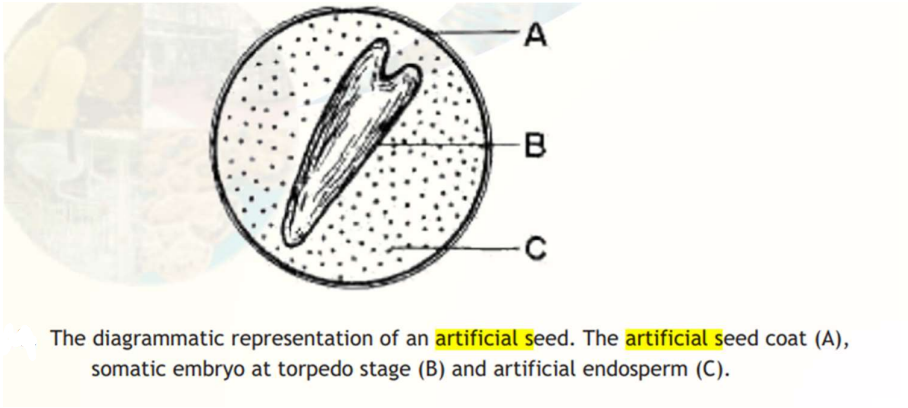
3

(proper diagram; 1 mark, any 4 correct labeling; ½ mark each)

25.	Shotgun sequencing is when a genomic library is constructed by ligating random genomic DNA fragments into a vector, and then randomly sequencing these clones. The sequence data are then assembled into contigs using computers that determine regions of overlap. (1 mark) The difficulties with assembling sequences with repeats are as: • Requires computer processing power beyond what an ordinary laboratory would possess • Can introduce errors in the assembly process • Requires a reference genome • May not be able to assemble repetitive sequences (½ mark each)	3
26.	The artificial seeds (also called as synthetic seeds or somatic seeds) can be utilized for the rapid and mass propagation of elite plant species as well as hybrid varieties. (1 mark) Artificial seeds are produced either by encapsulating the somatic embryos in a protective coating, i.e.,	3

calcium alginate beads or by desiccating the somatic embryos with or without coating.

(1 mark)



The diagrammatic representation of an artificial seed. The artificial seed coat (A), somatic embryo at torpedo stage (B) and artificial endosperm (C).

(1 mark)

Or

A popular and widely used direct gene transfer method for delivering foreign genes into virtually any tissues and cells or even intact seedlings.

(½ mark)

In this method, the foreign DNA (containing the genes of interest) is coated (precipitated) onto the surface of minute gold or tungsten particles (1-3 micrometers)

(1 mark)

and bombarded (shot) onto the target tissue or cells using a particle gun (also called as gene gun/ shotgun/ microprojectile gun).

(1 mark)

	Then, the bombarded cells or tissues are cultured on selection medium to regenerate plants from the transformed cells. (½ mark)	
27.	Cryopreservation - this utilizes the long-term preservation of cells and tissues (e.g. shoot tips, axillary buds, meristems, somatic embryos, etc.) at ultra-low temperature for indefinite time by using cryoprotectants. (1 mark) For example: dimethylsulfoxide, glycerol, proline and mannitol (Any two, 1 mark each)	3
28.	The non-covalent interactions involved in organising the structure of protein molecules can be broadly divided into four categories: • Ionic bonds • Hydrogen bonds • Van der Waals forces • Hydrophobic interactions (1 mark) 1. Ionic bonds: These involve interactions between the oppositely charged groups of a molecule. For example, the positively charged amino acid side chains of lysine	5

and arginine can form salt bridges with the negatively charged side chains of aspartate and glutamate. Ionic bonds are highly sensitive to pH and salt concentration.

(1 mark)

2. Hydrogen bonds:

   Hydrogen bonds are formed by "sharing" of a hydrogen atom between two electronegative atoms such as Nitrogen and Oxygen. In this case strongly polarised bonds between hydrogen and a small, very electronegative atom (N, O or F) allow a strong dipole-dipole bond to be formed with another small very electronegative element.

   (1 mark)

3. Van der Waals forces:

   These forces are weak attractions (or repulsions) which occur between atoms at close range. The Van der Waals types of forces are essentially contact forces, proportional to the surface areas in contact. Even though weak, these bonds can be important in macromolecules because the large surface areas involved can result in reasonably large total forces.

   (1 mark)

4. Hydrophobic interactions:

Hydrophobic interactions can be best explained by taking an example of oil in water. The oil tends to separate out fairly quickly, not because the oil molecules "want to get together", but because the water forces them out. The hydrophobic interaction is thus a manifestation of hydrogen bonding network in water.

(1 mark)

Or

Chymotrypsin, a proteolytic enzyme:

Chymotrypsin, which hydrolyses peptide bonds following bulky aromatic amino acid residues in polypeptides is actually synthesised in the pancreas and through the pancreatic duct released into the duodenum.

(½ mark)

This enzyme being a powerful proteolytic enzyme does not end up cutting cellular proteins within the pancreas itself.

(½ mark)

Nature has ensured that chymotrypsin and other proteolytic enzymes are synthesised as inactive harmless precursors known as zymogens which are

	then activated when required only in the duodenum, their site of activity, a process called in-situ activation. (½ mark) This activation in molecular terms results in an alteration in its shape so that it may now be able to interact with its substrate. The inactive precursor enzyme is termed chymotrypsinogen and the fully active enzyme is called chymotrypsin. (½ mark) The enzyme chymotrypsin is made up of a linear chain of 245 amino acids interrupted into three peptides A, B, C. (½ mark) The protein folds into a globular structure. In the 3-D structure of the enzyme three important amino acid residues, his57, asp102 and ser195 come close together in space which allows a "charge relay system" to operate. (½ mark) The negatively charged asp102 is able to hydrogen bond with the adjacent his57 partially borrowing the hydrogen ion from the latter. The his57 makes good its partial hydrogen ion loss to aspartate by attracting a hydrogen ion from the adjacent ser195 through the his57 residue much like a relay race where the baton	

	is passed from one member to another, the difference here being that the baton is a charge. (½ mark) Normally the hydroxyl group of a serine residue is not acidic (pKa 12) and this is true for all other serine residues of chymotrypsin; only ser195 becomes acidic due to the unique constellation of the three amino acid residues because the protein has folded uniquely in space. (½ mark) The specific site of chymotrypsin is a large space created within the enzyme active site and lined by hydrophobic residues which therefore only allow bulky aromatic, hydrophobic amino acids to bind. This binding brings the susceptible peptide bond close to the attacking ser195 residue. In chymotrypsinogen, the substrate binding site is blocked and hence the enzyme is inactive. (½ mark) In-situ activation of trypsin involves a proteolytic cut in chymotrypsinogen which results in a conformational change, exposing the substrate binding pocket. (½ mark)	
29.	FISH is Fluorescence in situ hybridization. (½ mark)	5

	It is possible to introduce colours into DNA by a technique called Nick Translation developed in 1977 by Rigby and Paul Berg. (½ mark) The enzymes, DNA polymerase I makes DNA and DNase I, which cuts DNA are combined in a buffered reaction with dNTP's, including dUTP labelled with a red or green fluorescence. (½ mark) The DNA polymerase I adds nucleotide residues to the 3-prime hydroxyl terminus that is the result of nicks (breaks) created by the DNase I in the DNA. (½ mark) In the process, the fluorescence labelled nucleotide in the free nucleotide mixture becomes incorporated into the newly synthesized strands of DNA. (½ mark) The application of FISH can be illustrated by taking an example of chronic mylogenous leukemia (CML). It was observed from the karyotype analysis of the lymphocyte preparation made from blood samples of CML patients that there was a 9-22 translocation in the chromosome. (½ mark)	

	Although by counting the number of such cells it was possible to find out the severity of the disease, it was not an easy procedure. The regions on the chromosomes involved in translocation were identified on chromosomes 9 and 22. (½ mark) From the DNA library it was possible to pick up clones carrying the particular genes involved in CML. Using nick translation, it was possible to fluorescently label chromosome 9 region with red colour and chromosome 22 region with green colour and prepare the probe. (½ mark) It was observed that when CML lymphocytes smear cells were hybridized with the two probes in situ and when observed under fluorescent microscope, the cells, which were affected, appeared yellow (mixing of green and red colour produces yellow colour). The unaffected cells appeared as red and green. (½ mark) This technique known as Fluorescence in situ Hybridization (FISH) allows knowing the status in the interphase unlike in karyotyping where you need a metaphase chromosome. The status of the disease	

	could easily be identified by counting the number of cells, which appeared yellow. (½ mark) Or Microarray Technology: This technology promises to monitor the whole genome on a single chip so that researchers can have a better picture of the interactions among thousands of genes simultaneously. (½ mark) Principle: Microarrays consist of large numbers of DNA molecules spotted in a systematic order on a solid substrate, usually a slide. The base pairing or hybridization is the underlying principle of DNA microarray. Microarray exploit the preferential binding of complementary single-stranded nucleic acids. (1 mark) Microarrays are made from a collection of purified DNA molecules typically using an arraying machine. The choice of DNA to be used in the spots on a microarray determines which genes can be detected in a comparative hybridization assay. (½ mark) This microarray technology promises to monitor the whole genome on a single chip so that researchers	

	can have a better picture of the interactions among thousands of genes simultaneously. In the case of gene chips, the substrate for immobilization is a silicon wafer and the probes are oligonucleotides spotted through photolithographic etching. (1 mark) This technique has been used to study the following: 1. Tissue specific genes 2. Regulatory gene defects in a disease 3. Cellular responses to environment 4. Cell cycle variations. (½ mark each)	
30.	The basic technique of plant tissue culture involves the following steps: 1. Selection of suitable explants like shoot tip, leaf, cotyledon and hypocotyls. (½ mark) 2. Surface sterilization of the explants by disinfectants (e.g. sodium hypochlorite) and then washing the explants with sterile distilled water. (1 mark) 3. Inoculation (transfer) of the explants onto the suitable nutrient medium (shoot regeneration medium, which is sterilized by autoclaving or	5

filter-sterilized to avoid microbial contamination) in culture vessels under sterile conditions (i.e., in laminar flow cabinet).

(1 mark)

4. Growing the cultures in the growth chamber or plant tissue culture room having the appropriate physical conditions [i.e., artificial light photoperiod), temperature and relative humidity].

(1 mark)

5. Regeneration of shoots from cultured plant tissues and their elongation.

(½ mark)

6. Rooting of regenerated shoots on rooting medium.

(½ mark)

7. Transfer of plants to the transgenic green-house or field conditions following the acclimatization (tissue hardening) of the regenerated plants.

(½ mark)

Or

In the direct gene transfer methods, the foreign gene of interest is delivered into the host plant cell without the help of a vector. The following are some of the common methods of direct gene transfer in plants.

	Chemical mediated gene transfer: Certain chemicals like polyethylene glycol (PEG) and dextran sulphate induce DNA uptake into plant protoplasts. (1 mark) Microinjection: Here, the DNA is directly injected into plant protoplasts (specifically into the nucleus) using fine tipped (0.5-10 micrometer diameter) glass needle or micropipette to transform plant cells. The protoplasts are immobilized on a solid support (e.g. agarose on a microscopic slide) or held with a holding pipette under suction, and the DNA is injected into the protoplasts with the help of a fine microscope. (1½mark) Electroporation: In this case, a pulse of high voltage is applied to protoplasts/cells/tissues, which makes transient (temporary) pores in the plasma-membrane which facilitates the uptake of foreign DNA. The DNA is mixed with the plant protoplasts in a specially made cuvettes before the application of the electric field. (1 mark) Particle gun: A popular and widely used direct gene transfer method for delivering foreign genes into virtually any tissues and cells or even intact seedlings.	

	In this method, the foreign DNA (containing the genes of interest) is coated (precipitated) onto the surface of minute gold or tungsten particles (1-3 micrometers) and bombarded (shot) onto the target tissue or cells using a particle gun (also called as gene gun/ shotgun/ microprojectile gun). Then, the bombarded cells or tissues are cultured on selection medium to regenerate plants from the transformed cells. (1½ mark)	