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## (Most Important Questions on Methods In Biology )

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Sahi Prep Hai Toh Life Set Hai

1. During Western Blotting various chemicals are used. Match each chemical with their corresponding use.

1) TEMED a Recognizes protein of interest 2) Bovine serum albumin b Positive control 3) Beta actin (antibody) c Catalyst for polymerization of acrylamide 4) Primary antibody d Blocking agent

A) 1 (d), 2 (c), 3 (b), 4 (a)

B) 1 (c), 2 (a), 3 (b), 4 (d)

C) 1 (d), 2 (c), 3 (a), 4 (b)

D) 1 (c), 2 (d), 3 (b), 4 (a)

2. If one wishes to separate organelles of similar size which differ in density, then he/she must use which of the following?

A) Differential centrifugation

C) Ultracentrifuge

D) Isopycnic centrifugation

3. Which of the following microscopic techniques can be used without the requirement of staining the samples/specimen?

A) Phase contrast and DIC microscopy

B) Only Phase contrast microscopy

C) Transmission electron microscopy

D) Scanning electron microscopy

4. Which of mentioned properties is correct regarding the use of Green Fluorescent Protein?

A) Can be used to stain living cells

B) Requires No fixation, substrates or co-enzymes

C) Both A and B are true

D) The fluorescence of GFP is susceptible to photobleaching.

5. Which of the following is incorrect for Transmission electron microscopy (TEM).

A) Electrons that pass through the specimen are imaged.

B) Electrons that are reflected back from the specimen are collected to form an image.

C) Surface structure can be viewed by TEM by using negative stains. D) The magnitude of resolution is enhanced due to the shorter wavelength of electrons

6. Match constituents required during DNA isolation with their appropriate counterparts.

1. EDTA a) Denature proteins

2. Phenol-chloroform b) Precipitation of DNA

3. TAE buffer c) Chelation of $Mg^{2+}$ ions

4. Ethanol d) Dissolving DNA

5. TE buffer

A) 1 (c), 2 (a), 4 (b), 5 (d)

B) 1 (c), 2 (d), 4 (b), 3 (a)

C) 1 (a), 2 (c), 4 (b), 5 (d)

D) 1 (c), 3 (d), 4 (b), 5 (a)

7. If you were to separate a mixture of DNA of different sizes by agarose gel electrophoresis then which of the following statement is incorrect? A) DNA will be loaded at Cathode

B) Shorter DNA fragments show maximum movement

C) DNA will be loaded at Anode

D) TBE buffer is used to run the samples under an electric field

8. While the results for the coronavirus samples are tested using Real-time PCR, what you obtain are the CT (cycle threshold values). What is the significance of CT values in analyzing the results?

A) The cycle at which a fluorescent signal is detected is termed as CT value B) High CT values indicate weak expression of target gene

C) Lower CT values indicate a high amount of viral load

D) All are correct

9. Mark the wrong statement for Pulse field gel electrophoresis A) Used to separate only large fragments of DNA

B) Electric field direction is periodically changed

C) used to produce a DNA fingerprint for a bacterial isolate.

D) DNA with large size take lesser time to realign according to the altered electric field direction

10. The correct arrangement of the general steps involved in the preparation of cDNA library.

A) Produce cDNA, insert each cDNA into a vector, Cloning of cDNA, Extract mRNA

B) Extract mRNA, produce cDNA, insert each cDNA into vector, Cloning of cDNA

C) Insert each cDNA into a vector, produce cDNA, Cloning of cDNA, Extract mRNA

D) Extract mRNA, produce cDNA, cloning of cDNA, Insert each cDNA into a vector

## Answers:

1. D
2. D
3. A
4. C
5. B
6. A
7. C
8. D
9. D
10. B

## Solution:

Explanation of TEMED- It binds with APS (Ammonium persulfate) and results in the solidification of acrylamide gels for electrophoresis.

Explanation of Bovine serum albumin- BSA blocks the unwanted area on the nitrocellulose or PVDF membrane where no protein is present. This is done before the addition of primary antibody, to ensure antibody binds with the proteins only.

Explanation of beta-actin- It is a housekeeping gene and used as a positive control to ensure there was no error while loading protein samples for western blotting. These genes are essential while drugs screening for various diseases as their expression is not affected even after drug treatment.

Explanation of primary antibody- It binds with the specific amino acid sequence of the protein of our interest and enables us to identify the amount of expression of that protein. After primary antibody secondary antibodies are added and finally detected using fluorescent molecules.

**Solution 2:**

The explanation for A- separation occurs due to differences in the sedimentation rate of particles of different sizes and densities.

The explanation for B- used for separation of a large volume of samples at low-speed

Explanation for C- used to separate particles that require high speed

The explanation for D- It is a type of density gradient centrifugation. Particles of the same size but with different densities stop at a point where their density is equal to the surrounding medium.

**Solution 3:** The explanation for A- Phase contrast- image is formed based on the differences in the refractive index of cellular structures. The light that passes through thicker parts of the cell is held up as compared to the light that passes through thinner parts of the cytoplasm.

DIC (Differential interference contrast) also does not require staining, but it produces pseudo-3-dimensional images which are not seen in phase-contrast microscopy.

The explanation for B- Phase contrast- image is formed based on the differences in the refractive index of cellular structures. The light that passes through thicker parts of the cell is held up as compared to the light that passes through thinner parts of

the cytoplasm.

Explanation for C- In TEM positive or negative staining is required. Explanation for D- Uranyl acetate, osmium tetroxide staining is required.

**Solution 4:** Explanation A and B- The green fluorescent protein (GFP) is used as a reporter of gene expression in living cells.

Explanation B- No fixation is required as fixation of cells results in cell death.

Explanation D-The fluorescence of GFP is extremely bright and is not susceptible to photobleaching.

**Solution 5.** In the Scanning electron microscopy electrons that are reflected back from the specimen (secondary electrons) are collected, and the surfaces of specimens are imaged.

**Solution 6:**

Explanation for EDTA (1)- EDTA chelates the $Mg^{2+}$ ions needed for enzymes that degrade DNA termed DNase.

Explanation for Phenol chloroform- protein, is removed by shaking the solution gently with water-saturated phenol, or with a phenol/chloroform mixture, either of which will denature proteins but not nucleic acids. Centrifugation of the emulsion formed by this mixing produces a lower, organic phase, separated from the upper, aqueous phase by an interface of denatured protein.

Explanation for TAE buffer- Tris Acetate EDTA Buffer is used for running the samples in agarose gel electrophoresis

Explanation for Ethanol- Ethanol precipitates the DNA

Explanation for TE Buffer- TE buffer (PH-8) helps in dissolving DNA and protects it from degradation.

**Solution 8:**

DNA is negatively charged so it is loaded at the cathode (negatively charged) and moves towards the positively charged anode on applying electric field.

Explanation B- Short DNA molecules can easily move large distances through the agarose gel due to their smaller size and mass.

Explanation C- DNA is negatively charged so it is loaded at the cathode (negatively charged) and moves towards the positively charged anode on applying electric field. If it is loaded at the anode (positive charge) than it would move backwards and soon

will come out of the gel into the buffer.

Explanation D- Both agarose gel and buffer in the tank consists of TAE or TBE buffer. If we use water in place of these buffers than gel will melt on applying electric field.

**Solution 9:** In real-time PCR, a positive reaction is determined by the accumulation of a fluorescent signal. Generally, real-time assays undergo 40 cycles of amplification. The cycle at which the fluorescent signal can be detected is termed cycle threshold (CT) value

CT values are inverse to the amount of the target gene expression in samples. Lower CT values indicate high amounts of target sequence while higher CT values correspond to weak expression of the target gene.

**Solution 10:** cDNA (complementary DNA) is made from mRNA so first it should be isolated.

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