ZOO101 Current Solved

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1) Ethidium Bromide?

• Intercalation of Et. br. causes a decrease in the negative charge of the ds DNA and an increase in both its stiffness and length.

• The rate of migration of the linear DNA-dye complex through gels is retarded by a factor

of - 15%.

2) Parts of microscope

(i) Eyepiece:

An **eyepiece** is that part of an optical system, which is directed to the viewer. It is a construction of at least one or more lenses. The function of the eyepiece in a microscope is to convert the real-enlarged-intermediate-image from the objective into an enlarged-virtual-image. It contains the ocular lens, which the user looks through to see the magnified specimen. The ocular lens has a magnification that can range from 5x to 30x, but 10x or 15x is the most common setting.

(ii) Objective lenses:

- It combines with the eyepiece lens to increase magnification levels. Microscopes generally feature three or four objective lenses, with magnification levels ranging 4x to 100x.

- Objective revolvers are used in microscopes with multiple objective lenses that have different magnification factors.
- An objective (lens) is that part of an optical system, which is directed to the object. It´s task is to collect the light rays that are reflected from the observed item. The objective generates a real-optical image.

1) Limitations….?

• Very low contrast of most biological samples.
• The practical limit to magnification with a light microscope is **around 1300X.** Although higher magnifications are possible, it becomes increasingly difficult to maintain image
Clearity as the magnification increases
• Low apparent **OPTICAL RESOLUTION** due to the material used.
• Samples that are naturally colorless and transparent cannot be seen well, e.g. many types of mammalian cells. These samples often have to be stained before viewing. Samples that do have their own color can be seen without
Preparation

2) Homogeneous mixtures and heterogeneous?

mixtures. A homogeneous mixture is a mixture that is uniform throughout. A
heterogeneous mixture is a mixture that is not uniform throughout, i.e., ingredients

of the mixture are distributed unequally. Air is a homogeneous mixture of different
gases, including oxygen, nitrogen, carbon dioxide, and water vapour.

3) Saw microtome and Laser microtome? long

• It is especially used for hard materials such as teeth or bones.
• The microtome of this type has a recessed rotating saw, which slices through the sample.
• The minimal cut thickness is approximately 30 µm and can be made for comparatively large samples.

Laser microtome

• It cuts tissue or other material with the help of photons instead of steel blades.
• The method is contact-free and enables to cut tissue in its native state.
• Special preparation techniques are not required.
• Cutting by optical breakdown.
• The thickness achievable is between 10 and 100 µm.

4) Multilayer filtration differ b/w hot and cold filtration? Long

• This can refer to multiple layers of different material, including sand, gravel, or

charcoal, where the different layers contain different particle sizes of that
material. In this type of filtration, a mixture of liquid and insoluble solid
particles is poured over the layers, and the solid particles are caught
throughout, resulting in a filtered liquid.

Cold filtration:

• Cold filtration makes use of very low temperatures, often by using an ice bath.
Some substances, such as fatty acid particles, become suspended in the mixture as they cool down, which then allows us to filter them out more easily.

Hot filtration:

• This is often used for crystalline compounds that contain impurities. The way this filtration is done is by melting down the crystalline compound, removing the impurities as the substance is still in liquid form, and finally recrystallizing the now pure substance. Often, it is recommended that the apparatus used in this filtration be heated up so that the filtered substance doesn't crystallize in the funnel and block the flow.

5) Advantages of SEM?

• The scanning electron microscope has many advantages over traditional microscopes.

• The SEM has a large depth of field, which allows more of a specimen to be in focus at one time.
• The SEM also has much higher resolution, so closely spaced specimens can be magnified at much higher levels.
• Because the SEM uses electromagnets rather than lenses, the researcher has much more control in the degree of magnification.
• Provides strikingly clear images, make the scanning electron microscope one of the most useful instruments in research today.

6) Cationic and anionic exchangers?

Cationic exchangers:

• Cationic exchangers possess negatively charged group, and these will attract positively charged cations. These exchangers are also called “Acidic ion exchange” materials, because their negative charges result from the ionization of acidic group.

Anionic exchangers:

Anionic exchangers have positively charged groups that will attract negatively charged anions. These are also called “Basic ion exchange” materials. Ion exchange chromatography is most often performed in the form of column chromatography. However, there are also thin layer chromatographic methods that work basically based on the principle of ion exchange.

7) Manual and automates Processing? Long

Manual Processing:

Slow, most labor intensive method since transfer of specimens or changing reagents is done by hand. With an advance of automation this method is almost obsolete.

Automated Processing:

Automated Processing Specimen-transfer or “dip and dunk” processors: instruments which transfer cassettes from station to station in a rotary or linear configuration.

Fluid-transfer or “enclosed” instruments hold the specimens in a process chamber or retort and the reagents are pumped in and out during processing. Microwave assisted processing: might require manual transfer of specimen or reagents, it accelerates processing by heating reagents.

8) **The rate of migration of DNA through agarose gels? long**

Factors affecting the rate of DNA migration

Different factors determine the rate of migration of DNA through agarose gels.

Molecular Size of DNA:

• Molecules of double stranded DNA migrate through gel matrices at rates that are inversely proportional to the log10 of the number of base pairs.

Conformation of the DNA:

• Super helical circular (form I),
• Nicked circular (form II),
• Linear (form III) DNAs migrate through agarose gels at different rates
• The relative mobilities of the three forms depend primarily
• On the concentration and type of agarose
• Strength of the applied current
• The ionic strength of the buffer
• Density of superhelical twists in the form I DNA

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Voltage:

• At low voltages, the rate of migration of linear DNA fragments is proportional to the voltage applied.
• However, as the strength of the electric field is raised, the mobility of high-molecular-weight fragments increases differentially.

• Thus, the effective range of separation in agarose gels decreases as the voltage is increased.
• To obtain maximum resolution of DNA fragments >2 kb in size, agarose gels should be run at no more than 5-8 V/cm.

9) Factors affecting resolution?

• **d= λ/2 NA**
• The resolution of a microscope is a function of two factors as given below:
1. Numerical aperture (NA) of the optical components
2. Wavelength of light (λ)which is used to examine a specimen.

LIMIT OF RESOLUTION (LR): The minimum distance between any two closest points on the microscopic object, which can be resolved into two separate and distinct points on the enlarged image.
• **d= λ/2 NA**
Wavelength of light and RESOLUTION:

• The smaller is the wavelength of light (λ), the greater is its ability to resolve the points on the object into distinctly visible finer details in the image.
• Thus, the smaller is the wavelength of light, the greater is its resolving power.

10) Bright field microscopy and light path? Long

Bright-field microscopy:

• In Bright-field microscopy the Sample is illuminated from below and observed from above through the eye piece)

• white light and contrast in the sample is caused by absorbance of some of the transmitted light in dense areas of the sample.

• Staining is often required to increase contrast, which prevents use on live cells in many situations

• Bright field illumination is useful for samples which have an intrinsic colour, for example chloroplasts in plant cells.

The light path consists of:

1. A trans illumination light source, commonly a **halogen lamp** in the microscope stand;
2. A **condenser lens** which focuses light from the light source onto the sample;
3. Objective lens which collects light from the sample and magnifies the image.
4. Oculars and/or a camera to view the sample image.

11) TLC components? Long

• TLC system components consists of:

TLC plates, preferably ready made with a stationary phase: These are stable and chemically inert plates, where a thin layer of stationary phase is applied on its whole surface layer. The

stationary phase on the plates is of uniform thickness and is in a fine particle size.

TLC chamber- This is used for the development of TLC plate. The chamber maintains a uniform environment inside for proper development of spots. It also prevents the evaporation of solvents, and keeps the process dust free.

Mobile phase- This comprises of a solvent or solvent mixture The mobile phase used should be particulate-free and of the highest purity for proper development of TLC spots. The solvents recommended are chemically inert with the sample, a stationary phase.

A filter paper- This is moistened in the mobile phase, to be placed inside the chamber. This helps develop a uniform rise in a mobile phase over the length of the stationary phase.

12) Embedding?

• Embedding is the process in which the tissues or the specimens are enclosed in
a mass of the embedding medium using a mould.
• Since the tissue blocks are very thin in thickness they need a supporting medium in which the tissue blocks are embedded.
• This supporting medium is called embedding medium.

13) What is chromatography? Long

Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis. A wide range of chromatographic procedures makes use of differences in size, binding affinities, charge, and other properties to separate materials.

It is a powerful separation tool that is used in all branches of science and is often the only means of separating components from complex mixtures

Applications of Chromatography:

Pharmaceutical sector:

- To identify and analyze samples for the presence of trace elements or chemicals.
- Separation of compounds based on their molecular weight and element composition.
- Detects the unknown compounds and purity of mixture.
- In drug development.

Chemical industry:

In testing water samples and also checks air quality.

Forensic Science:

In forensic pathology and crime scene testing like analyzing blood and hair samples of crime place.

Molecular Biology Studies:

Various hyphenated techniques in chromatography such as EC-LC-MS are applied in the study of metabolomics and proteomics along with nucleic acid research.

•HPLC is used in Protein Separation like Insulin Purification, Plasma Fractionation, and Enzyme Purification and also in various departments like Fuel Industry, biotechnology, and biochemical processes.

Q.16 Condenser?

- Usually, the condenser is cooled to promote condensation of the vapor, which is collected.
- The condenser ensures that any solvent vapors cool, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent.
- a **condenser lens** which focuses light from the light source onto the sample;

Q.17 Sample Preparation for TEM?

• Since electrons are deflected by air molecules and are easily absorbed and scattered by solid matter, only extremely thin slices (20 to 100 nm) of a microbial specimen can be viewed in the average TEM. Such a thin slice cannot be cut unless the specimen has support of some kind; the necessary support is provided by plastic.

• After fixation with chemicals like glutaraldehyde or osmium tetroxide to stabilize cell structure, the specimen is dehydrated with organic solvents (e.g., acetone or ethanol). Complete dehydration is essential because most plastics used for embedding are not water soluble.

• Next the specimen is soaked in unpolymerized, liquid epoxy plastic until it is completely permeated, and then the plastic is hardened to form a solid block.
• Thin sections are cut from this block with a glass or diamond knife using a special instrument called an ultramicrotome