Mid-Term Assignment (Spring-2020) (BS-MLT 4th)

Course Title: Molecular Biology **Instructor: Mr. Fazli Zahir Mian**

Time: 48 Hours **Tariq Khan : 14547**

Q1: Fill in the Blanks.

1) Watson and Francis Crick discovered the double helical structure of the DNA molecule.
2) Watson and Crick were awarded Nobel Prize in 1962.
3) Nucleic acids store, transmit, and help express hereditary information.
4) The amino acid sequence of a polypeptide is programmed by a unit of inheritance called a gene.
5) Hundreds of Y-shaped regions of replicating DNA molecules where new strands are growing called replication forks.
6) Topoisomerase type I & type II are enzyme which relieves stress on the DNA molecule by allowing free rotation around a single strand.
7) Genetic Code is a dictionary that corresponds with sequence of nucleotides and sequence of amino acids.
8) Translation is the process of covalently attaching an amino acid to the tRNA.
9) Helicase are proteins which attach and help keep the separated strands apart.

Q2: Write short notes on the following

1) Common tools of molecular biology

List of equipment / apparatus used in microbiology laboratory

1. Autoclave
2. Incubator
3. Hot air oven
4. Inoculating loop
5. Vortex mixer / shaker
6. Water bath

7. Heating mantle

8. Hot plate with magnetic stirrer

9. UV chamber

10. Inoculation chamber

11. PH meter

12. Colony counter

13. Microscope

14. Refrigerator

15. Bunsen burner

16. Spirit lamp

17. Micrometer (stage and ocular)

18. Balance (Digital and 4-beam)

19. Thermometer

20. Membrane filter set

DESCRIPTION

1. Autoclave

It is a robust, electrically heated steam vessel meant for sterilizing 'thermostable' culture media, glassware, and other materials that are not spoiled by moist heat. Autoclave runs on the principle of pressure cooker. The moist heat (steam) has a very good penetrating power. Microorganisms / cells are killed as a result of denaturation of cellular constituents (protein and nucleic acids). In routine process, sterilization can be achieved by operating the autoclave at 121°C (15 psig) for 15 min. In its simplest form, the equipment has a removable lid for the delivery of materials to be sterilized. It is necessarily equipped with a gasket, pressure-cum-temperature gauge, a vent for letting out air or excess pressure, a safety valve, and a drain.

2. Incubator

This an insulated, electrically heated cabinet meant for providing microorganisms with optimum temperature for growth. The cabinet is insulated and thermostatically controlled. For routine purposes, the temperature is maintained at 28-30°C for bacteria, about 25°C for molds, and 35-37°C for mesophilic bacteria. A temperature as high as 100°C can also be maintained for extremely thermophilic organisms (stereothermophiles).

3. Hot air oven

This is similar to incubator in make except that it can operate at temperatures up to 300°C and has a fan for circulating hot air. Hot air oven is used for sterilization of glassware and materials that are spoiled by moist heat. The death of cells occurs due to the oxidation of

cellular constituents by the dry heat. For routine purpose, sterilization can be achieved by running the equipment at 180°C for 1.5 hours. Hot air oven is less effective than autoclave.

4. Inoculating loop

This is a tool for transferring and streaking cultures. It consists of a thin nichrome wire whose one end is twisted into a small loop while the other end is fixed to a thermoset plastic handle. Sometimes, the looped end is straightened out to form what is called inoculating needle. Inoculating needles are used for preparing ‘stab’cultures. shows inoculating loops and needles.

5. Vortex mixer

This equipment is used for mixing liquids kept in a test tube. It has one or more cup-like depressions at the top to receive the bottom of the test tube. The machine is electrically powered. When actuated, the machine moves the bottom of the test tube in a gyratory motion, thereby affecting a thorough mixing of the solution. The speed of the mixer can be varied.

6. Water bath / Boiling water bath

Water bath is used for heating and melting of media, solutions, samples etc. at temperatures below 100°C. It can also be used to maintain constant temperature that is required in microbiology lab work. Several models and types of water bath are available.It is electrically heated and thermostatically controlled.

7. Heating mantle

It is an electrically heated and thermostatically controlled unit used to heat or melt samples and reagents. The inner lining is made of asbestos and therefore gives an indirect heat to the materials to be heated.

8. Hot plate with magnetic stirrer

This is an electrically powered equipment performs the dual function of heating and agitation. The agitation occurs by magnetic arrangement. Any type of glassware can be used for the heating and agitation. Magnetic beads are used for the agitation.

9. UV chamber / UV viewing cabinet

This equipment is used for analyzing fluorescent materials, spots in thin layer chromatography, etc. The equipment has two lamps for long- and short wavelength UV radiation. Since UV radiation is genotoxic(mutagenic) its exposure to skins and eyes must be avoided. A viewport with colored glass is provided for safety.

10. Inoculation chamber / Sterile chamber This is an enclosed box in which culture transfers, plating, etc. can be carried out aseptically. The chamber is equipped with UV lamp for periodic disinfection of the chamber. While working, the UV light must be turned off and day-light bulb is turned on.

11 PH meter

PH meter is an electrical instrument used for measuring hydrogen ion concentration of solutions and mixtures. In microbiology lab, it is used for maintaining pH of the medium and diluents. The pH meter must be standardized with buffer solutions before operation. Since the instrument is very sensitive, it must not be used for stirring and it must not be dipped in hot or very cold solutions. The electrodes must always be kept immersed in suitable solutions. Read the manual carefully before using the instrument.

12. Colony counter

It is used for counting microbial colony (bacterial and yeast). The instrument is equipped with a backlight source, gridlines and a magnifying lens. It also has a sensor for digitally registering the number of colonies counted

13. Microscope

It is an instrument for observing microscopic items such as cells, crystals and cell organelles. It has the dual function of magnification and resolution. For routine microbiological works, bright field compound microscope with oil immersion objective is adequate.

14. Refrigerator

This is a common household equipment for keeping foods and beverages cool. This equipment is used in microbiology laboratory for storing / preserving cultures, media, and many sensitive materials. The equipment is electrically powered and uses ammonia as the refrigerant.

15. Bunsen burner

Bunsen burner is a common tool used in science lab . In microbiology lab, it is used for sterilizing inoculating loop, plating out cultures, transferring cultures, heat-fixing of smears and creating a sterile zone for aseptic operation.

16. Spirit lamp

The function of spirit lamp is the same as the Bunsen burner but is portable. It uses rectified spirit as the fuel (produces smoke-free flame). The lamp must be covered with a lid when not in use to prevent loss of spirit.

17. Micrometers (stage and ocular)

These are graduated glass pieces used for the measurement of size of the cells. Stage micrometer is a slide on which etching is done with 0.001 mm spacing. The ocular micrometer, which is place on the eyepiece, has an arbitrary scale and must be calibrated against the stage micrometer. During measurements, the ocular micrometer is retained while the stage micrometer is replace with the specimen slide.

18. Balance

Balance is needed in microbiology lab for weighing chemicals, samples, media, etc. Digital balances are fast to work with but needs frequent calibration. The triple-beam and 4-beam balances are robust equipment that need little care and maintenance. Beam balances run on

mechanical principles while the principles on which electronic balances run is quite complicated .

19. Thermometer

Thermometers are required to ensure the heating equipment is running at the correct temperature. The temperature of the medium, incubator, etc., need to be frequently checked. Mercury in glass thermometers are standard thermometers, the temperature measurement is based on the expansion of mercury present in the bulb. Digital thermometers use probes for measurement of temperatures.

20. Coliform membrane filter

This glass equipment is used for the testing of coliforms in water. 100 ml of test water is poured in the funnel and filtered through a special Millipore filter through external application of suction. The filter retains the microorganisms. The filter is then aseptically transferred to a selective-cum-differential semisolid medium.

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2) Nucleic acids

Nucleic acids are the biopolymers, or small biomolecules, essential to all known forms of life. The term nucleic acid is the overall name for DNA and RNA. They are composed of nucleotides, which are the monomers made of three components: a 5-carbon sugar, a phosphate group and a nitrogenous base.

Nucleotides join together through phosphodiester linkages between the 5' and 3' carbon atoms to form nucleic acids. The 3' -OH of the sugar group forms a bond with one of the negatively charged oxygen of the phosphate group attached to the 5' carbon of another sugar. When many of these nucleotide subunits combine, the result is the large single-stranded polynucleotide or nucleic acid, DNA

The two sides of the nucleic acid strand shown above are different, resulting in polarity. At one end of the large molecule, the carbon group is unbound and at the other end, the -OH is unbound. These different ends are called the 5'- and 3'-ends, respectively. The Helical Structure of DNA shows a single strand of DNA. However, as stated earlier, DNA exists as a double-helix, meaning two strands of DNA bind together.

One strand is oriented in the 5' to 3' direction while the complementary strand runs in the 3' to 5' direction. Because the two strands are oppositely oriented, they are said to be anti-parallel to each other. The two strands bond through their nitrogen bases (marked A, C, G, or T for adenine, cytosine, and guanine). Note that adenine only bonds with thymine, and cytosine only bonds with guanine. The nitrogen bases are held together by hydrogen bonds: adenine and thymine form two hydrogen bonds; cytosine and guanine form three hydrogen bonds.

An important thing to remember about the structure of the DNA helix is that as a result of anti-parallel pairing, the nitrogen base groups face the inside of the helix while the sugar and phosphate groups face outward. The sugar and phosphate groups in the helix therefore make

up the phosphate backbone of DNA. The backbone is highly negatively charged as a result of the phosphate groups.

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3) Chargaff's rule

Chargaff's rules states that DNA from any cell of all organisms should have a 1:1 ratio (base Pair Rule) of pyrimidine and purine bases and, more specifically, that the amount of guanine is equal to cytosine and the amount of adenine is equal to thymine. This pattern is found in both strands of the DNA. They were discovered by Austrian chemist Erwin Chargaff.

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4) Wobble hypothesis

Crick (1996) proposed the 'wobble hypothesis' to explain the degeneracy of the genetic code. Except for tryptophan and methionine, more than one codons direct the synthesis of one amino acid.

There are 61codons that synthesis amino acids, therefore, there must be 61 tRNAs each having different anticodons. But the total number of tRNAs is less than 61.

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5) Names of main steps in Translation and Transcription

Translation & Transcription proceeds in three phases:

a. Initiation
b. Elongation
c. Termination

Q3: Explain the process of DNA Replication.

Step 1: Replication Fork Formation

Before DNA can be replicated, the double stranded molecule must be "unzipped" into two single strands. DNA has four bases called adenine (A), thymine (T), cytosine (C) and guanine (G) that form pairs between the two strands. Adenine only pairs with thymine and cytosine only binds with guanine. In order to unwind DNA, these interactions between base pairs must be broken. This is performed by an enzyme known as DNA helicase. DNA helicase disrupts the hydrogen bonding between base pairs to separate the strands into a Y shape known as the replication fork. This area will be the template for replication to begin.

DNA is directional in both strands, signified by a 5' and 3' end. This notation signifies which side group is attached the DNA backbone. The 5' end has a phosphate (P) group attached, while the 3' end has a hydroxyl (OH) group attached. This directionality is important for replication as it only progresses in the 5' to 3' direction. However, the replication fork is bi-directional; one strand is oriented in the 3' to 5' direction (leading strand) while the other is oriented 5' to 3' (lagging strand). The two sides are therefore replicated with two different processes to accommodate the directional difference.

Replication Begins Step 2: Primer Binding

The leading strand is the simplest to replicate. Once the DNA strands have been separated, a short piece of RNA called a primer binds to the 3' end of the strand. The primer always binds as the starting point for replication. Primers are generated by the enzyme DNA primase.

DNA Replication: Elongation

DNA polymerases (blue) attach themselves to the DNA and elongate the new strands by adding nucleotide bases.DNA polymerases (blue) attach themselves to the DNA and elongate the new strands by adding nucleotide base

Step 3: Elongation

Enzymes known as DNA polymerases are responsible creating the new strand by a process called elongation. There are five different known types of DNA polymerases in bacteria and human cells. In bacteria such as E. coli, polymerase III is the main replication enzyme, while polymerase I, II, IV and V are responsible for error checking and repair. DNA polymerase III binds to the strand at the site of the primer and begins adding new base pairs complementary to the strand during replication. In eukaryotic cells, polymerases alpha, delta, and epsilon are the primary polymerases involved in DNA replication. Because replication proceeds in the 5' to 3' direction on the leading strand, the newly formed strand is continuous.

The lagging strand begins replication by binding with multiple primers. Each primer is only several bases apart. DNA polymerase then adds pieces of DNA, called Okazaki fragments, to the strand between primers. This process of replication is discontinuous as the newly created fragments are disjointed.

Step 4: Termination

Once both the continuous and discontinuous strands are formed, an enzyme called exonuclease removes all RNA primers from the original strands. These primers are then replaced with appropriate bases. Another exonuclease “proofreads” the newly formed DNA to check, remove and replace any errors. Another enzyme called DNA ligase joins Okazaki fragments together forming a single unified strand. The ends of the linear DNA present a problem as DNA polymerase can only add nucleotides in the 5′ to 3′ direction. The ends of the parent strands consist of repeated DNA sequences called telomeres. Telomeres act as protective caps at the end of chromosomes to prevent nearby chromosomes from fusing. A special type of DNA polymerase enzyme called telomerase catalyzes the synthesis of telomere sequences at the ends of the DNA. Once completed, the parent strand and its complementary DNA strand coils into the familiar double helix shape. In the end, replication produces two DNA molecules, each with one strand from the parent molecule and one new strand.

Replication Enzymes

DNA polymerase molecule

DNA polymerase molecule.

DNA replication would not occur without enzymes that catalyze various steps in the process. Enzymes that participate in the eukaryotic DNA replication process include:

DNA helicase - unwinds and separates double stranded DNA as it moves along the DNA. It forms the replication fork by breaking hydrogen bonds between nucleotide pairs in DNA.

DNA primase - a type of RNA polymerase that generates RNA primers. Primers are short RNA molecules that act as templates for the starting point of DNA replication.

DNA polymerases - synthesize new DNA molecules by adding nucleotides to leading and lagging DNA strands.

Topoisomerase or DNA Gyrase - unwinds and rewinds DNA strands to prevent the DNA from becoming tangled or supercoiled.

Exonucleases - group of enzymes that remove nucleotide bases from the end of a DNA chain.

DNA ligase - joins DNA fragments together by forming phosphodiester bonds between nucleotides.

GOOD LUCK.