

Name:-

1- The main two aims of using sterilization methods when dealing with the bacterial cultures are:

- To prevent any contaminant from entering to your culture.
- To prevent any germs from escaping from your culture.

2- There are two different methods to prepare competent bacterial cells, mention them:

- Electroporation method. - Calcium Chloride method.

3- Before starting the procedures of preparing the bacterial competent cells, the bacteria should be brought in the lag phase, and this done because the bacteria in this growth phase is more able to withstand to the harsh conditions of the high salt treatment.

4- Tracking dye like Bromophenol blue is usually used in the SDS-PAGE experiment to track the samples movement in the gel.

5- Reducing agents such as DTT or beta-mercaptoethanol/BME is usually added to the protein sample when preparing the samples to SDS-PAGE running, what is the aim of this step?

DTT used to break the disulfide bonds in the protein.

6 - What is the definition of the His-tag protein?

7- In the western blot experiment a blocking solution such as TBS contains non-fat dry milk or TBS contains being used to block the membrane.

[BSA]

8 - How do you be sure of that your transformation process is successful?

If there are bacterial colonies growing in the petri dish which contains antibiotic, this means the transformation is successful.

9 - Why using the blocking solution is necessary in the Western blot experiment?

To block the unwanted targets and then preventing or reducing the false results, reducing noise.

10- Taking up the DNA by bacteria can be happen by three different methods, transformation and conjugation method which requires sex pilus and transduction which requires Bacteriophage intermediate .

11- There are several stains can be used to stain the samples in the SDS-PAGE experiments, mention two of these stains:-

- Silver staining
- Coomassie Brilliant Blue

12- In the western-blot experiment the protein sample will be transferred from the gel to a membrane which is usually PVDF or... Nitrocellulose...

13 - Mention two examples of the protein tags:-

- MBP
- His

14- In the western-blot experiment the secondary antibody must be specific to... primary antibody.

15- Before freezing the competent bacteria in -70 degree the Glycerol should be added to the competent bacterial cells to make the bacteria able to withstand under the freezing conditions.

16- Briefly mention the steps of the transformation experiment:-

- The frozen bacterial competent cells will thaw in ice for several minutes, this is to make thawing the bacteria happens gradually.
- Then, the plasmid will be added to the competent bacteria.
- Heat shock process will be made by heating the competent cells with the plasmid to 42°C for approximately 90 seconds.
- The sample will be put back into ice for another few minutes.
- The sample will be grown in LB media to regain and activate the bacterial growth again.
- Then, the bacteria will be spreaded on petri dish contains appropriate antibiotic and incubated overnight.
- * Finally, the bacterial colonies being noticed.