2021

MICROBIOLOGY — HONOURS — PRACTICAL

Paper : CC-10P

(Recombinant DNA Technology)

Full Marks : 30

The figures in the margin indicate full marks.

Candidates are required to give their answers in their own words as far as practicable.

1. (a) 100 ng of plasmid DNA (carrying ampicillin resistance gene) was added to 100 µl of competent *E.coli* cells. After introduction of the plasmid DNA, the cells were allowed to express the antibiotic resistance phenotype by addition of 900 µl of sterile broth and growing further for a period of 1hr. Subsequently, 50 µl and 100 µl of transformed cells were plated in presence of the antibiotic. Calculate the transformation efficiency from the number of CFUs provided corresponding to the two plates.

(b) In which phase of growth can artificial competence be induced in an *E.coli* culture? How can you ascertain this growth phase?

(c) In a certain cloning experiment, the plasmid containing the insert is being screened by blue-white screening. You have made agar plates with dextrose together with other nutrients, antibiotic as well as X-gal and IPTG to identify your clones. Several colonies have appeared. However, neither of them is blue in colour. Assuming that you have executed all the steps and added all other reagents, do all of your colonies contain the cloned insert. —Explain. 4+(1+2)+3

2. (a) Formulate a typical reaction mix (20 µl) for digestion of 2·4 µg of lambda DNA with 15 units Eco RI. Also mention the time and temperature of digestion.

Stock concentrations

EcoRI = 10 units/µl

Lambda DNA = 0·8 µg/µl

EcoRI digestion buffer = 10X

(b) There are five recognition sites of EcoRI in the lambda genome. Draw a representative picture of the agarose gel showing the band patterns of three consecutive lanes of (i) Linear lambda DNA digested with EcoRI (ii) Circular lambda DNA digested with EcoRI and (iii) Uncut lambda DNA.

(c) You have planned to amplify a certain sequence of the bacterial genome by Polymerase Chain Reaction. Schematically show the different steps which are carried out by the thermal cycler and explain the role of each step. How will you ascertain that the amplification of your desired sequence had occurred? (2+1)+3+(3+1)

3. viva voce. 5

4. Laboratory notebook. 5