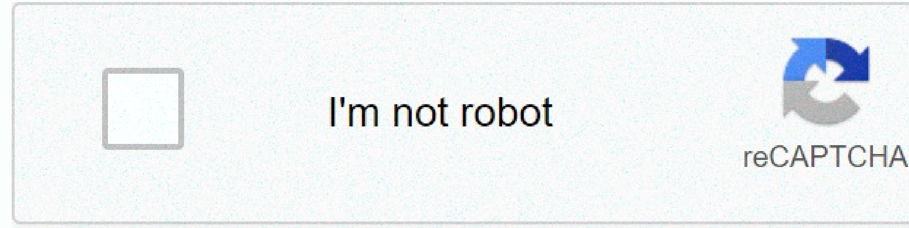

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Laboratory 6 molecular biology answers

Paul Andersen explains the two major parts of the molecular biology laboratory in AP Biology. He starts by discussing the process of transformation. He explains how you can use pGLO plasmid to produce glowing E. coli bacteria. He then describes how you can use limiting enzymes and the process of gel electrophoresis to cut and separate DNA. Education ResourcesMoecular Biology Lab Review Spreadsheet – Winnie Litten Pearson, as an active contributor to the biology learning community, is happy to provide free access to the classic edition of The Biology Place to all educators and their students. The purpose of the activities is to help you review material you have already studied in class or have read in your text. Some of the material will expand your knowledge beyond your class work or textbook reading. At the end of each activity, you can rate your progress through a Self-Quiz.To clicking on an activity title. AP Lab 6 Molecular Biology Introduction: Exercise 6A: Recombinant DNA Technology To study the structure and function of a single protein coding gene, prepare the gene in a purified form. Vertebrate cells contain enough DNA to code for more than I 00,000 proteins; Therefore, isolating a gene using conventional biochemical procedures is not very practical. That is why recombinant DNA technology is so important; it can be used to isolate and amplify a particular gene relatively simply.   Plasmids, small circular DNA molecules, are usually extra chromosomal; they are found with the exception of the chromosomes of most bacterial species. Plasmids are not necessary for the survival of host bacteria, but they may contain genes that allow the bacteria to survive in certain environments. If a bacterial cell contains a plasmid that carries a gene that provides resistance to antibiotics, then that cell can survive in the presence of the drug. Plasmids can be introduced into bacterial cells by the process of transformation. Bacteria placed in a calcium chloride solution can take in plasmid DNA molecules. In this way, large amounts of specific plasmid DNA can be produced because a transformed cell gives rise to duplicate cells that also contain the plasmid DNA molecule. Plasmids are very important to the molecular biologist because they serve as gene carrier molecules called cloning vectors. A gene of interest can be assembled into vector DNA to form a hybrid or recombinant molecule that can replicate in bacteria. In preparing a recombinant DNA molecule, a procedure is required to cut cloning vectors and cellular DNA molecules into precise positions.   Limiting nuclisses are important for recombinant DNA technology because they cut DNA in specific locations. These enzymes are usually made of bacterial species where they break down invading DNA in the bacterial cell. Most restriction enzymes recognize a specific sequence of nucleotides in DNA and cut a long DNA double healing to restriction fragments, which are measured in the process of agarose gel electrophoresis. Introduction: Exercise 6B: DNA Fingerprint Electrophoresis is the movement of charged particles in solution under the influence of an electric field. In gel electrophoresis, agarose gel is the stabilizing medium that acts as a matrix for the buffer in which the sample molecules move. The gel is immersed in buffer in the electrophototic gel cell. The samples are loaded into the sample wells of the gel, and electrical current passes through the gel.   Molecules of DNA are negatively charged due to negative charges on the phosphate group. In this exercise, nucleic acids migrate through the gel's pores from the negative end towards the positive end. The large DNA molecules move more slowly than smaller molecules, therefore molecules are sorted by size. Objective: Exercise 6A Examine basic genetic concepts by transforming bacterial cells by inserting an ampicillin-resistant gene into E. coli cells. Purpose: Exercise 6B Examine basic genetic concepts using restrictive enzymes to digest faglamda DNA and separate and identify the DNA fragments using gel electrophoresis. Materials and methods: Exercise 6A The materials used in this exercise included: 2 Luria agar plates, 2 Luria agar plates with ampicillin, 2 microcentrifuge tubes, 1 swab loop, 1 Bacti-Spreader, sterile micropipes, calcium chloride, Luria broth, plasmid pUC8, Bunsen burner, hob, boiling plate, ice cream, water bath. The two microcentrifuge tubes were marked ! I+ and !1-!1, and 250IJI cold calcium chloride was added to each using a pipet. A large colony of bacteria was added to each tube with a sterile inoculating loop. A micropipet was used to transfer 10IJI of plasmid pUCS solution to ! I+ tube. Both tubes were incubated on ice for 15 minutes, and in the meantime, the two Luria agar plates were labeled + and - and so were the two Luria plates with ampicillin. The pipes were removed from the ice and placed in a 42°C hot water bath for 90 seconds. The pipes were then removed from the water bath and put on ice for two minutes. A micropipet was used to add 250IJI Luria broth to each tube. Another micropipet was used to add 100IJI of ! I + solution for the two! I+ plates and 1oomicroliters of the solution on the two - plates. The bacteria were flamed to sterilize, and after cooling, were used to disperse the cells all over the surface of the plates. After five minutes, the plates were placed in a 37°C incubator, inverted, overnight. Materials and methods: Exercise 6B The materials used in this exercise included: 8% agarose gel, 2 electrophoresis chambers, package, running buffer- Tris, micropipettes and tips, dyeing tray, methylene blue dye, gloves, aprons, 4 DNA samples cut with restrictive enzymes, vial tray, microcentrifuge, paper, pencil, distilled water, spatula, plastic container for destaining, masking tape, light box, ruler, semi-Iog graph paper. The gel, on the gel tray, was placed in the center of the chamber, with good side of the gel near the black electrode. Approximately 350 ml of running buffer was added to the chamber. Of each DNA sample, 10 microlitres were loaded into the corresponding gel path with a micropipe. The power cords were attached to the relevant connections and the power supply was switched on, set to 50 volts. The samples were allowed to migrate for three hours. The gel was then removed, spotted, and destained overnight. The gel was seen on a light table and the band's migration distances were measured. Results: Exercise 6A Question 1. Based on your experimental results, did transformation happen? Why or why not? Yes, transformation took place. Colonies of E. coli grew in the presence of ampicillin. 2. What other methods can be used to verify that the transformation took place? DNA Fingerprint Results: Exercise 6B Question 1. Compare the band patterns. Do you think the DNA samples were the same? No, the samples were different sizes 2. Which of the two suspects was the real burglar? Suspect #2 3. Explain the function of each of these steps in DNA fingerprints: a. Limiting enzyme digest – used to cut DNA b. Gel electrophoresis – used to separate different sized pieces of DNA c. Denaturing in single-stranded DNA - the process used to only see a strand of DNA double helix d. Southern Blot - DNA tape transferred to a nitrocellulose paper e. Radioactive DNA probe – used to find & bind to the additional sequence in one or more RLFP's f. Autoradiograph – used to show similarities in DNA samples Error Analysis Lab 6A: Not enough agar was poured on the plates. Lab 6B: Base pair counts for DNA tapes could have been inaccurate as suggested by the most suitable line on the graph. Conclusion Lab 6A: This laboratory showed that genes can be inserted into living bacterial cells, thus transforming the cells and giving them new properties such as ampicillin resistance. Lab 6B: Through gel electrophoresis of DNA samples, it was determined that the suspect #2 was guilty. Their DNA banding pattern matched the banding pattern taken by DNA at the crime scene. Scene.

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