

Recombinant dna pkan and pamp



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DNA is an essential part of organisms. It is the director of many aspects of the body. The DNA consists of a sugar base, phosphate and a nitrogen base. There are only four nitrogen bases to choose from. They are guanine, adenine, thymine and cytosine. They are grouped under two groups: the purine and the pyridimines. The purines consist of guanine and adenine while the pyridimines consist of thymine and cytosine. During DNA synthesis, the purines pair up with the pyridimines. The sugar which is used in the DNA is referred to as the deoxyribose which is different from the sugar present in the RNA which is ribose. DNA's most important task in the body is to make proteins. The body runs on proteins. Proteins are needed for almost every task. They play a role as hormones, enzymes, transport carriers, signal proteins, and many others. A recombinant DNA is a DNA which is not found ordinarily in the nature. There are three ways with which recombinant DNA can be made: Transformation, phage introduction and non-bacterial transformation (An Introduction to Recombinant DNA 1).

There are many steps involved in the process of transformation. The first step consists of selecting a piece of DNA which can be inserted as a vector. After this, the piece is to be cut with a restriction enzyme and then with the help of DNA ligase, should be inserted into the vector. After this process, the vector can be inserted into the host cell and this process is referred to as transformation. Recombinant DNA is said to work when the cell in which it is inserted into starts to express its recombinant genes. Recombinant DNA is used more and more each day because of the growing need of certain proteins. One major example is the insulin for humans. There are many people who are diabetics and need the insulin from the recombinant DNA in order to survive otherwise they become weak and feeble and eventually die. Recombinant DNA is needed for better crops which are heat resistance and drought resistance because of the changing weathers over the past couple of years. We also need recombinant vaccines such as in the case of hepatitis B to eradicate certain diseases from the human population. There are many other fields of medicine which need the technique of recombinant DNA. We need recombinant DNA for somatic gene therapies, production of the clotting factors, and insecticides (An Introduction to Recombinant DNA 1).

Objectives:

- to adequately follow the rules of the lab.
- to understand the concept of recombinant DNA.
- to obtain a pKan plasmid and add the amp gene.
- to grow the bacteria in growth medias.

to understand the concept of sticky ends and restriction enzymes.

Hypothesis:

-Plasmids obtained, only, contain a single recognition site for each enzyme, producing only two restriction fragments.

-Cleavage of pAMP yields a 3755-base pair fragment containing the ampr gene and another fragment of 784 bp.

- pKAN yields an 1875 bp fragment containing the kan r gene and another fragment of 2332 bp.

- Plasmid fragments are mixed with DNA ligase.

-Complementary BamHI and HindIII “ sticky ends” hydrogen bond to align restriction fragments.

-Ligase catalyzes the formation of phosphodiester bonds that covalently link the DNA fragments to form stable recombinant DNA molecules.

Results:

Results for our recombination= For this lab, we were responsible for the pKan plasmid. We added the amp gene properly and followed the procedure as stated in the procedure. Unfortunately, we were unable to obtain the results that we were suppose to get. Our recombinant did not yield any colonies. This could have been due to many factors. This could have been due to human error, for example, we could have had some contamination which could have killed the colonies or we could have incubated them for too

long a period of time. There could have been many other reasons for this experiment to have not gone the way we intercepted it to go

Results and conclusion:

This lab was a very interesting lab. This lab took us about 2 weeks to do because it required a long incubation period. For this lab, we obtained pKan as our plasmid. We followed the procedure as directed but unfortunately, we did not obtain the results that we were supposed to get. This could have been due to human errors such as accidental contamination. We were supposed to obtain pKan colonies which had taken up the amp gene. This did not occur. Besides that fact, we learned a number of things from doing this lab. We learned about restriction enzymes and ligases. Restriction enzymes cut DNA at specific sites leaving either sticky ends or blunt ends. Sticky ends form overhanging sides. They can be hydrogen bonded to DNA fragments complements. Blunt ends cut straight down without leaving any overhanging sides. Two ends can be reconnected by the use of ligases. During the ligation processes hydrogen bonds form between the bases. The sugar molecules are held together by the covalent bonds. Ligases, then, come into action. They form phosphodiester linkages between molecules.

Questions:

1. Explain what is meant by “ sticky ends.” Why are they so useful in creating recombinant DNA molecules?

Sticky ends are DNA sequences which are created using an restriction enzyme. This enzyme cuts off at the center of its recognition sequence.

Afterwards, the sticky ends are used to ligate two fragments together using ligase.

2. Why is ATP essential for the ligation reaction?

ATP is a very powerful molecule and used all over the body. During the ligation reaction, it is used for energy which is needed for the condensation reactions when connecting bonds.

3. Ligation of the four Bam HI/HindIII restriction fragments of pAMP and pKan produces many types of hybrid molecules, including plasmids composed of more than two fragment. However, only those constructs possessing an origin of replication will be maintained and expressed. Three different replicating plasmids with selectable antibiotic resistance, are created by ligating combinations of two (2) BamHI/HindIII fragments:

a. Ligation of the 784-bp fragment to the 3755-bp fragment regenerates pAMP.

b. Ligation of the 1875-bp fragment to the 2332-bp fragment regenerates pKAN.

Ligation of the 1875-bp fragment to the 3755-bp fragment produces the “simple recombinant” plasmid, pAMP/KAN, in which the kanamycin resistance gene has been fused into the pAMP backbone. Make a scale drawing of the simple recombinant molecule pAMP/KAN. Include fragment sizes, locations of BamHI and Hind III restriction sites, location of origin(s), and location of antibiotic resistance gene(s).

4. Make scale drawings of other two-fragment recombinant plasmids having the following properties. Whenever possible, include fragment sizes, locations of BamHI and HindIII restriction sites, location of origin(s), and location of antibiotic resistance gene(s).

a. Three kinds of plasmids having two origins.

Three kinds of plasmids having no origin

5. What rule governs the construction of plasmids composed of more than two restriction fragments?

There is a formula to how the restriction fragments align. Restriction fragments always have to align BamHI fragments-to-BamHI fragments and HindIII fragments-to-HindIII fragments. It seems that the recombinant plasmids must be made from even numbers of BamHI and HindIII fragments. The ones which are composed of odd numbers have a BamHI end and a HindIII end. These unfortunately do not align.

Ligation of the 784-bp fragment, 3755-bp fragment, 1875-bp fragment, and the 2332-bp fragment produces a “ double plasmid” pAMP/pKAN. Make a scale drawing of the double plasmid pAMP/KAN.

Make scale drawings of several recombinant plasmids composed of any three of the four BamHI/HindIII fragments of pAMP and pKAN. Include fragment sizes, locations of BamHI and HindIII restriction sites, location of origin(s), and location of antibiotic resistance gene(s).

What kind of antibiotic selection would identify E. coli cells that have been transformed with each of the plasmids drawn in Questions 3, 4, 6, and 7?

To test if the E-coli took up the plasmids, they should be grown in Media's lack that particular substances and then one should check if E-coli produced that particular substance.

9. Competent cells often take up more than one kind of recombinant plasmid. Consider double transformations, where the transformed cell contains two different plasmids. Double transformations of which of the molecules described in Questions 3, 4, 5, and 7 would result in ampicillin and kanamycin resistance?

Work cited:

“ An Introduction to Recombinant DNA.” Rensselaer Polytechnic Institute (RPI) :: Architecture, Business, Engineering, IT, Humanities, Science. Web. 23 Nov. 2010.