

INSTITUTE-UNIVERSITY INSTITUTE OF ENGINEERING

ACADEMIC UNIT-II

Computer Science Engineering
Subject Name-Biology For Engineers
Subject Code- 20SZT148

RECOMBINANT DNA TECHNOLOGY

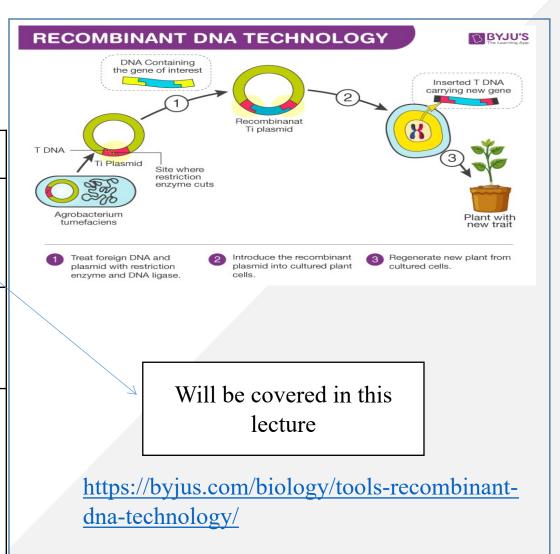
DISCOVER. LEARN. EMPOWER



RECOMBINANT DNA TECHNOLOGY

Course Outcome

CO Number	Title	Level
CO1	It gives an idea about the about the basic cell biology.	Understanding
CO2	It deals with the idea of uses of biology in engineering.	Understanding
CO3	It provide knowledge about the uses of softwares in biology field.	Remembering





BIOLOGY FOR ENGINEERS

Cell, Cell theory, Genetic information,
Cell death
(UNIT-1)

Medical instruments, Biosensors, Biosensors, Recombinant DNA technology and Immunology (UNIT-2)

Enzymes,
Nervous
system,Bioinfo
rmatics and
Disesaes
(UNIT-3)

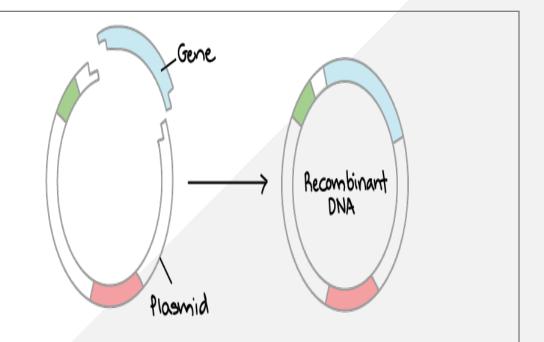




industry.

RECOMBINANT DNA TECHNOLOGY

•Recombinant DNA technology is the joining together of DNA molecules from two different species. The recombined DNA molecule is inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture, and





RECOMBINANT DNA TECHNOLOGY

- DNA cloning is the process of making multiple, identical copies of a particular piece of DNA.
- In a typical DNA cloning procedure, the gene or other DNA fragment of interest (perhaps a gene for a medically important human protein) is first inserted into a circular piece of DNA called a plasmid.
- The insertion is done using enzymes that "cut and paste" DNA, and it produces a molecule of recombinant DNA, or DNA assembled out of fragments from multiple sources.





RECOMBINANT DNA TECHNOLOGY

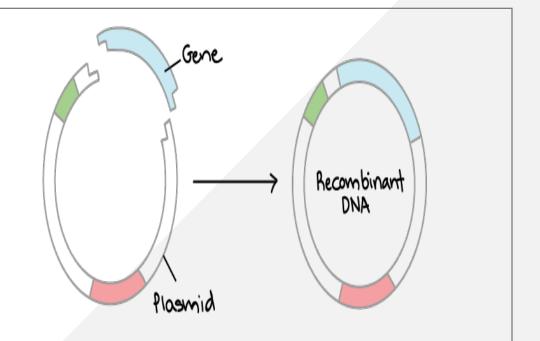
- DNA cloning is a molecular biology technique that makes many identical copies of a piece of DNA, such as a gene.
- In a typical cloning experiment, a target gene is inserted into a circular piece of DNA called a plasmid.
- The plasmid is introduced into bacteria via a process called transformation, and bacteria carrying the plasmid are selected using antibiotics.
- Bacteria with the correct plasmid are used to make more plasmid DNA or, in some cases, induced to express the gene and make protein.





RECOMBINANT DNA TECHNOLOGY

• DNA cloning is the process of making multiple, identical copies of a particular piece of DNA. In a typical DNA cloning procedure, the gene or other DNA fragment of interest is first inserted into a circular piece of DNA called a plasmid. The insertion is done using enzymes that "cut and paste" DNA, and it produces a molecule of recombinant DNA, or DNA assembled out of fragments from multiple sources.







DNA cloning is used for many purposes. As an example, let's see how DNA cloning can be used to synthesize a protein (such as human insulin) in bacteria. The basic steps are:

- Cut open the plasmid and "paste" in the gene. This process relies on restriction enzymes (which cut DNA) and DNA ligase (which joins DNA).
- Insert the plasmid into bacteria. Use antibiotic selection to identify the bacteria that took up the plasmid.
- Grow up lots of plasmid-carrying bacteria and use them as "factories" to make the protein. Harvest the protein from the bacteria and purify it.





1. Cutting and pasting DNA

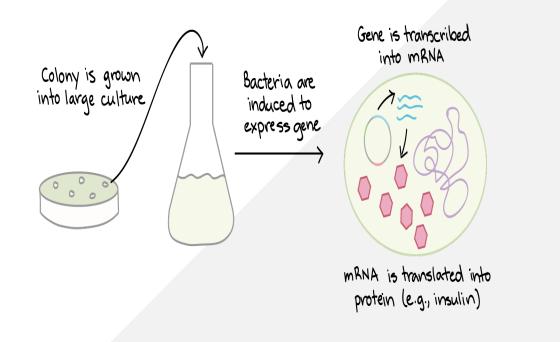
- How can pieces of DNA from different sources be joined together? A common method uses two types of enzymes: restriction enzymes and DNA ligase.
- A restriction enzyme is a DNA-cutting enzyme that recognizes a specific target sequence and cuts DNA into two pieces at or near that site.
- Many restriction enzymes produce cut ends with short, single-stranded overhangs.
- If two molecules have matching overhangs, they can base-pair and stick together. However, they won't combine to form an unbroken DNA molecule until they are joined by DNA ligase, which seals gaps in the DNA backbone.





- Our goal in cloning is to insert a target gene (e.g., for human insulin) into a plasmid. Using a carefully chosen restriction enzyme, we digest:
- The plasmid, which has a single cut site
- The target gene fragment, which has a cut site near each end
- Then, we combine the fragments with DNA ligase, which links them to make a recombinant plasmid containing the gene.

 https



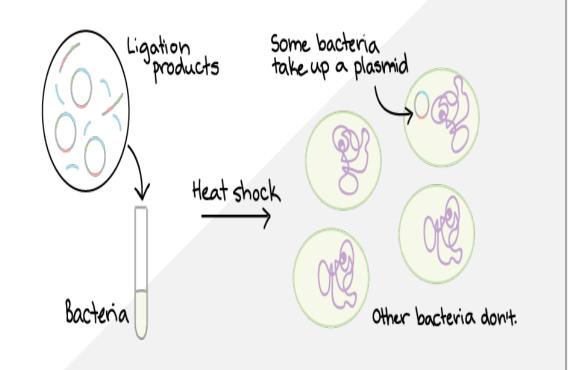




2. Bacterial transformation and selection

• Plasmids and other DNA can be introduced into bacteria, such as the harmless *E. coli* used in labs, in a process called transformation.

During transformation, specially prepared bacterial cells are given a shock (such as high temperature) that encourages them to take up foreign DNA.



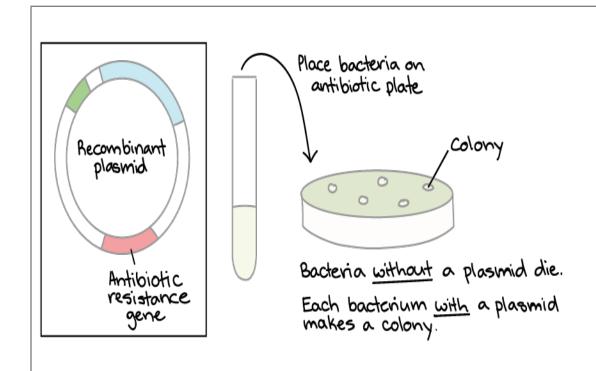




- A plasmid typically contains an antibiotic resistance gene, which allows bacteria to survive in the presence of a specific antibiotic.
- Thus, bacteria that took up the plasmid can be selected on nutrient plates containing the antibiotic.
- Bacteria without a plasmid will die, while bacteria carrying a plasmid can live and reproduce.
- Each surviving bacterium will give rise to a small, dot-like group, or colony, of identical bacteria that all carry the same plasmid.







 Not all colonies will necessarily contain the right plasmid. That's because, during a ligation, DNA fragments don't always get "pasted" in exactly the way we intend. Instead, we must collect DNA from several colonies and see whether each one contain the right plasmid. Methods like restriction enzyme digestion and PCR are commonly used to check the plasmids.



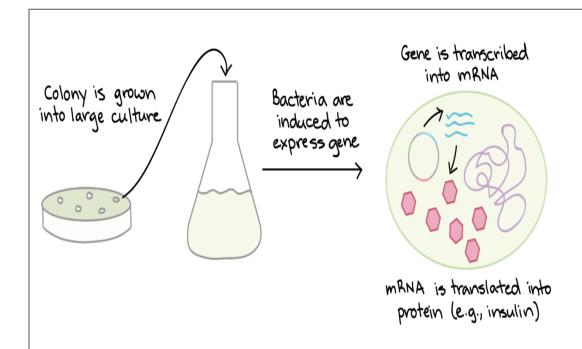


3. Protein production

- Once we have found a bacterial colony with the right plasmid, we can grow a large culture of plasmid-bearing bacteria.
- Then, we give the bacteria a chemical signal that instructs them to make the target protein.
- The bacteria serve as miniature "factories," churning out large amounts of protein.
- For instance, if our plasmid contained the human insulin gene, the bacteria would start transcribing the gene and translating the mRNA to produce many molecules of human insulin protein.







https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/overview-dna-cloning

• Once the protein has been produced, the bacterial cells can be split open to release it. There are many other proteins and macromolecules floating around in bacteria besides the target protein (e.g., insulin). Because of this, the target protein must be purified, or separated from the other contents of the cells by biochemical techniques. The purified protein can be used for experiments or, in the case of insulin, administered to patients.





USES OF DNA CLONING

- DNA molecules built through cloning techniques are used for many purposes in molecular biology. A short list of examples includes:
- **Biopharmaceuticals.** DNA cloning can be used to make human proteins with biomedical applications, such as the insulin mentioned above.
- Other examples of recombinant proteins include human growth hormone, which is given to patients who are unable to synthesize the hormone, and tissue plasminogen activator (tPA), which is used to treat strokes and prevent blood clots.
- Recombinant proteins like these are often made in bacteria.





USES OF DNA CLONING

- Gene therapy. In some genetic disorders, patients lack the functional form of a particular gene. Gene therapy attempts to provide a normal copy of the gene to the cells of a patient's body.
- For example, DNA cloning was used to build plasmids containing a normal version of the gene that's nonfunctional in cystic fibrosis.
- When the plasmids were delivered to the lungs of cystic fibrosis patients, lung function deteriorated less quickly.
- Gene analysis. In basic research labs, biologists often use DNA cloning to build artificial, recombinant versions of genes that help them understand how normal genes in an organism function.





CONCLUSION

- Recombinant DNA technology is the joining together of DNA molecules from two different species.
- The recombined DNA molecule is inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture, and industry.
- Recombinant DNA technology is used to make microbes, plants, and animals that carry genes from other species.
- Recombinant DNA technology can be used in the prenatal diagnosis of human genetic disease.





ASSESSMENT PATTERN

Assessment Pattern	Total Marks
1st Hourly Test	36
2 nd Hourly Test	36
Surprise Test	12
Assignment (3)	10
Quiz	4
End Semester Examination	60



REFERENCES

- C.B.Powar, 2010.Cell Biology.5th Ed,Himalyan Publishing House.
- Leshie Cromwell, Fred.J. Weibell and Erich.A.Pfeiffer. 2003. Biomedical instrumentation and measurements. 2nd edition, PHI.
- John G. Webster 1998. Medical Instrumentation: Applications and Design, 3rd edition, Jon Wiley and Sons, New York.
- Jeremy M. Berg, John L. Tymoczko and Lubert Stryer. 2006. "Biochemistry," 6th Ed. W.H. Freeman and Co. Ltd.
- Robert Weaver. 2012 "Molecular Biology," 5th Edition, MCGraw-Hill.
- Jon Cooper, , 2004. "Biosensors A Practical Approach" Bellwether Books.
- Martin Alexander, 1994 "Biodegradation and Bioremediation," Academic Press.







For queries

Email: subject_code_2020@gmail.com