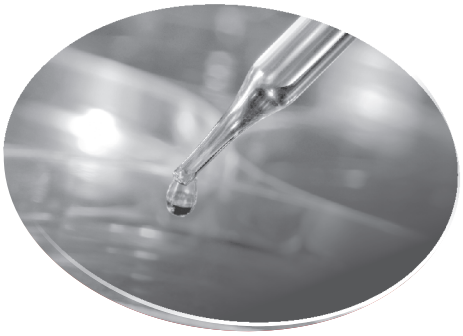

Department of
Life and Consumer Sciences

BIOTECHNOLOGY

Only study guide for BIT2601

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Florida

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CONTENTS

GENERAL INTRODUCTION

Welcome to Biotechnology 2601 (BIT2601). This module is a 12-credit course, meaning you will need at least 120 study hours for this module. Before starting on the first unit of this study guide, some details of the course are given.

The **prescribed textbook** to study in conjunction with this study guide is:

Introduction to Biotechnology, 2nd Ed (2009); Thiemann, W.J. & Palladino, A., Pearson/ Benjamin Cummings, London, Toronto, New York.

ISBN-10: 0-321-58903-3
ISBN-13: 978-0321-58903-3

Time allocation

You need at least **120 study hours** in total for this module:

At least **50 hours** should be spent on reading and studying the content.

About **30 hours** should be spent on assignments.

About **40 hours** should be spent on revision, studying and answering the competency assessment.

Purpose of the course

This introductory course deals with the fundamentals of biotechnology, where the disciplines of biochemistry, microbiology, molecular biology, physiology and genetics (Unit 1) are applied and adapted as foundation technologies to further investigate biological phenomena in our environment. These biological phenomena include various aspects of health and disease in plants, animals and humans (Unit 2). Unit 3 is a basic introduction to some processes and instrumentation used to manipulate genetic material as well as Best Practice Principles involved in applying this kind of technology. The purpose of this module is to enable you, the individual learner, to identify and apply practices, processes and principles of biotechnology to solve problems in health and disease, including maintaining an environment that is suitable for growing crops and farming with animals.

We hope that you will find this course interesting and that it will give you an overview of some basic principles of biotechnology that is applicable to Life Science. To make it even more exciting, you will be able to apply this knowledge in other courses and in your daily life. Try to identify and explain the principles applied to, for example, the impact of manipulating genetic material of plants for enhanced resistance to insect attack.

Outcomes of this course

This course has been designed to provide you, the student, with the ability to:

- demonstrate understanding of the applications of biotechnology in society.
- acquire insight into how biotechnology impacts on the environment, agriculture, nutrition, industry and health.
- show understanding of how biotechnology depends on other disciplines, especially biochemistry, microbiology, molecular biology, physiology and genetics.
- show an appreciation of the material and equipment required for conducting research in biotechnology, especially with regard to manipulation of nucleic acids
- demonstrate an understanding of why it is important to adhere to the principles and methods of quality control when applying biotechnology as a problem-solving tool.

Knowledge, skills and values assumed in place

What do you need to know before starting this module? As this is a second-year module presented by a distance education institution, it is firstly expected of you to have self-discipline. You need to have good study, reading and writing skills, with Grade 12 (university entrance). Secondly, it will be helpful if you had secondary education in chemistry, physics and physiology/biology, although this module can be completed without having passed these subjects at secondary school level.

Participative critical evaluation

Excellent as any book is, it is often not perfect. It will be a part of our task to approach this study guide very critically. You are welcome to send us your comments on any part of it. Your evaluation of the course gives us the opportunity to improve the quality of our study material. The contact details of the lecturers for this course are indicated in Tutorial Letter 101.

Success in distance learning

Distance studies are unique, with particular requirements for success that should not be underestimated. Once you have received your study material plan ahead by drawing up study schedule with reasonable timelines to guide you through the whole module. Take into consideration the due dates of the assignments as indicated in Tutorial Letter 101 for this module.

Independent study

A crucial phase in the process of understanding and learning the basics of biotechnology is to articulate your ideas about these principles, both *orally and in writing*. Only when you have tried this process will you understand the full value of this exercise.

The assessment following your studies measures an aspect of success. This course will have formative (ongoing) and summative (final) assignments and examinations in the form of mainly written work. Your reflections on your process of learning are therefore also an important part of your work. Since the focus in this module is to understand and apply the basic principles regarding chemistry, physics and physiology, your assessment will focus on the imbedded abilities to be competent in meeting these requirements.

Work through the study guide by following the guidelines in the next section ("Improved study skills"). This involves drawing up mind maps and making your own summaries of the objectives and contents of chapters. Limit summaries to one page. Be conscientious about this: it is vital to keep closely in touch with this book if you are to master its contents. Additional textbooks or articles give alternative views or provide more insight into issues under discussion and are optional additional reading.

Be focused in your study. Build up your own study and exam preparation **portfolio** (with your assignments, activities, reflections, summaries, self-evaluations and notes) throughout the period of academic and/or experiential learning. *This portfolio won't be assessed by the lecturer, but you will need to prepare such a document in order to be able to complete the assignments and ultimately pass the final examination*. It is also very important to use this **portfolio,** in combination with your **assignments** and subsequent feedback **(tutorial letters),** for your **exam preparation.** The advantage of having a study and exam preparation portfolio is that it enables you to engage personally in your learning, to set goals, and to evaluate your progress through reflective actions, as well as your ability to realise the learning outcomes, therefore, becoming more independent and self-directed learners.

What is a portfolio? A portfolio is a folder/file with additional and/or summarised information gathered and compiled during the year while studying through the study manual/guide. This portfolio will help you to prepare for the examination by focussing on the most important facts and issues.

Your portfolio should comprise of:

- Answers for each activity for every unit
- A mind map/summary of every unit
- Your returned assignments (or a copy of the assignment which you submitted)
- Your reflections of every unit
- Extra reading material from books, journals or news papers
- New vocabulary or glossary of new terms in your own words

To ensure you master this module, you are advised to use the following guidelines on studying skills.

Improved study skills

As a student enrolled at an open distance learning institution, it is essential to become familiar with being able to search for research/scientific articles via the internet:

How to search for

Research/Scientific Articles

As a general source of information, the **Google** website has now created an additional search engine under "advanced search", called **"Google Scholar",** which provides an option "advanced search" where a number of linked fields exist. This allows a brief description of your subject query, say in four to six words, to be coupled to specific phrases. By pressing "search-enter", a variety of websites relating to the query is displayed. The advantage of using this portal is that you can access most of the journal references from any internet site, other than *my*Unisa. In the latter instance, certain journals for example, *Science Direct* at the Elsevier site, can only be accessed through tertiary academic institutions, such as Unisa. To assess this journal, you need to proceed as follows:

1) Go to Unisa online at **http://www.unisa.ac.za/**.
2) Click on **Library** at the top of the page.
3) In the maroon area on the top of the page, click on "**search for information resources**".
4) Follow the guidelines if you are a first-time user.
5) Click on the option **"a-z list of the names of all electronic resources"** on the right hand side of the page.
6) Various links for databases will now be displayed on the screen. Click on any database to do a search. For biotechnology we recommend ***Science Direct***, ***Nature*** or ***SpringerLink.*** (Remember, if you choose *Science Direct*, click on **s** on top, and if you choose *Nature*, click on **n.**).
7) When you have entered one of these databases, you can search for scientific articles by typing in the relevant keywords in the "search" box. Be very specific when entering keywords. One word will usually give too much information and not the specific topic you are looking for.
8) You will need to do some independent searches as part of your portfolio, assignments and exam preparation, especially since this is a distance education course, which needs to be supplemented with information from internet sources.

Contact the Unisa Library if you have any difficulties or for assistance:

+27 12 4293206 or visit the library website to obtain the telephone number of the library's local branch.

A variety of **strategies for studying** are available and a combination of the following can be used to help you study throughout the course, ensuring optimal education. Make sure you use these methods as you approach each unit:

Skimming, scanning, study-reading process (SSS-process)

The three techniques included in this strategy are **s**kimming, **s**canning and **s**tudy-reading. The exercise comprises six steps. In order to understand what these steps involve, you need to study the example given below.

Example for SSS-process:

SKIMMING

1. **Page through and explore.** Read the section or paragraph quickly, forming a rough idea of the contents. Concentrate on headings and subheadings, bold and italic type, boxes, tables and illustrations, and – in the case of a chapter – introductions and summaries. The objectives set for a chapter are important. (Think of how you would page through a magazine. When starting a new unit, scan it and concentrate on the concepts that catch your eye).

2. **Make a cursory survey.** Ask yourself this while you read: What key terms occur in this division or chapter? Stop when you identify a key term and read carefully what is said about it. Mark it in the book. What you are trying to ascertain is: **Where** is it? In other words, where the information you will need later, is discussed.

SKIMMING AND REFLECTING

3. **Scan** the section or chapter.

4. **Start a mind map** (for the whole or for parts of it, as in starting a summary). You are looking for items and concepts while reading the information in the section or chapter in a more evaluative way. Reflect on interrelationships between concepts. The question now is: **What** is it? What is the meaning and the purpose? Visualisation is important and you are certainly going to start writing down key concepts. You can omit parts of the text.

5. **Deeper reflection.** Start building a structure in your mind map, work towards an entirety. As you work through the prescribed activities of the section or chapter, keep returning to the mind map to fill in the detail. Reflect on the value and meaning or categories, concepts, motivations, variables and key terms.

6. **Study-read.** This follows directly from stages 2, 4 and 5 and is done carefully, thoroughly and thoughtfully. The key terms and concepts you pinpointed have to be linked up, and in this the mind map and summaries are important. (Remember to put your detailed mind map in your portfolio.) Pause while reading, consolidate what you remember and consider how new information fits in with what you already have.

7. **Activity-based approach**

   Whenever you get to an activity in your study guide, complete it in full on loose pages which you then insert into your portfolio, grouped together per section and unit. Supplement this with your own notes from your notebook. Proceed, using your study guide. (You don't need to submit activities or the portfolio to the lecturer, but these are essential for exam preparation.)

8. Understand what you read

Take time to note new vocabulary. Use a dictionary to understand the meaning of new words. You could compile a page for each unit and add it to your portfolio.

9. Manage your self-paced study time

In order to keep up with the required amount of study, an average student requires at least 120 study hours to master this module. However, this time may vary substantially, depending on students' individual learning abilities. Therefore, you may plan to use eight study hours per week per module, meaning one module should be finished in 15 weeks. Remember, if you have registered for more than one module you should plan enough study time for each module. We advise you to keep a study schedule or diary so that you have a clear idea of the time you have available to study. This will enable you to manage your studies within your available time and balance study with work and family life. For example, in Tutorial Letter 101 you will find a list of due dates for various assignments, so note these dates in your normal diary. Divide the large assignments into a series of smaller tasks to complete one step at a time.

In order to manage your workload, your best working method would be to work frequently and regularly in this subject. Establish a routine environment with low noise and good lighting. Reward yourself after a productive session.

Academic specialist guidance

Contact me, as the lecturer responsible for this course, whenever you have any difficulties with the course. A positive, encouraging attitude towards the course as a whole will keep you motivated to persevere in your studies.

Hopefully my feedback to you will be helpful and encouraging in sustaining your improvement and learning for the course.

Plagiarism

Do not try to pass off other people's work (or our lecture notes and tutorial matter) as your own. If you wish to use other people's words and ideas or our notes when formulating an answer, you must use quotation marks and acknowledge the source which you consulted (use the Harvard method). If unsure about the correct way of referencing, contact Unisa's Library Information Desk. Students who fail to use appropriate referencing for quotations obtained from lecture notes and outside sources or who copy someone else's answers may be refused permission to write the examination or may get penalised in the assignment.

The course development team

The work of writing study materials is always undertaken with the help of a group of people. The people who worked together to design a learning experience for you that follows a transformative paradigm for education, a text that is well written and pleasing to the eye. The team consisted of Dr MS Myer (subject specialist), Ms H Wilson (learning developer) and Ms DC Hyman (graphic designer). These people are creative, knowledgeable, and

dedicated; they worked together as a team to make this a special learning experience for you because they are passionate about science education and innovative distance learning.

In conclusion

After reading the General introduction you should now have a better understanding of what the course involves and what the aims are for completing an introductory course to biotechnology.

Study Unit 1 deals with the overall scope of Biotechnology, while the origins of Biotechnology as well as issues pertaining to the impact of this technology, on the Environment and Society, are covered in Study Unit 2. Study Unit 3 provides a basic introduction to some of the processes and instrumentation used to manipulate genetic material, as well as quality control measures that need to be applied.

STUDY UNIT 1

Time allocation

You should spend at least 14 hours on this study unit.

WHAT IS BIOTECHNOLOGY?

Textbook reference

This study unit is based on Introduction to Biotechnology, 2nd Ed (2009); Thiemann, W.J. & Palladino, A., Pearson/Benjamin Cummings, London, Toronto, New York. Chapter 1: Types of Biotechnology, pages 8–14; Chapter 2: An Introduction to Genes and Genomes, pages 27–43; Internet Sources, as indicated in the text of this Study Guide.

STUDY UNIT CONTENT

This study unit is divided into the following study sections:

Study section 1.1 Required background to understand procedures in biotechnology

Study section 1.2 Modern applications of biotechnology in a biological setting.

Outcomes

After completing this study unit you should be able to:

- compare and contrast the structures of prokaryotic and eukaryotic cells.
- briefly describe the process of translation, including the roles of mRNA, tRNA and rRNA.
- briefly describe what plasmid DNA is and why it is important in recombinant DNA technology.
- briefly explain how transformation and selection of recombinant bacteria occurs.
- briefly explain how screening of cDNA libraries takes place.

- briefly explain how the polymerase chain reaction works.
- briefly discuss how the efficiency of bioremediation can be improved using either microbes (bacteria) or certain plants.
- briefly explain what biosensors are and some of their applications.
- critically discuss various applications of biotechnology in an environmental setting.

STUDY SECTION 1.1: Required background to understand the procedures used in biotechnology

1.1.1 The nature of genetic material in plant and animal cells

The outer surface of **eukaryotic** cells (for example, animal and plant cells) consists of a double-layer structure of lipids and proteins, known as the plasma membrane. The plasma membrane encloses the cytoplasm containing the nucleus and various organelles. **Prokaryotic** cells (for example, bacteria) lack a nucleus and distinct organelles.

Various organelles, such as the nucleus, mitochondria, lysosomes, Golgi apparatus and endoplasmic reticulum are depicted in Text Figure 1. The nucleus contains the genetic material, or deoxyribose nucleic acid (DNA), which codes for the assembly of different kinds of proteins that are assembled on the ribosomes and subsequently packaged inside the Golgi apparatus.

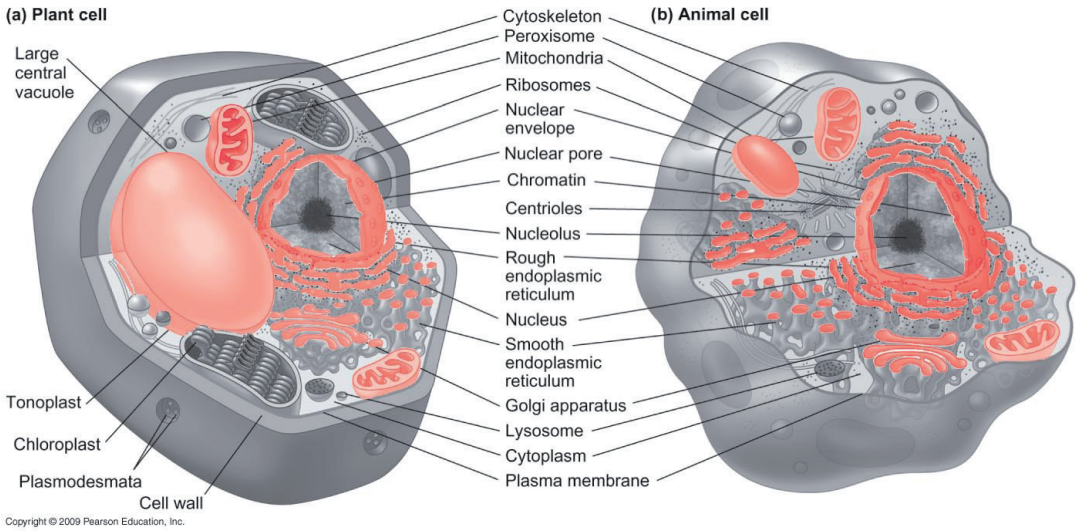

Figure 1: Organelles
(Reference Figure 2.2 p.28 of prescribed textbook)

Text Table 1 below summarises the essential differences between prokaryotic and eukaryotic cells.

Table 1: Essential differences between prokaryotic and eukaryotic cells

Table 2.1 PROKARYOTIC AND EUKARYOTIC CELLS

	Prokaryotic Cells	Eukaryotic Cells
Cell Types	True bacteria (eubacteria), Archaebacteria	Protists, fungi, plant, animal cells
Size	100 nm–10 µm	10–100 µm
Structure	No nucleus; DNA located in the cytoplasm. Lack organelles.	DNA enclosed in a membrane-bound nucleus. Many organelles.

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DNA of both prokaryotic and eukaryotic cells consists of basic building blocks called **nucleotides**, each being composed of a 5-carbon (pentose) sugar called deoxyribose, a phosphate molecule and one of four possible nitrogenous bases, either:

Adenine (A), thymine (T), guanine (G) or cytosine (C) (see Text Figure 2.)

Nucleotides are joined to form long strands of DNA, where each DNA molecule consists of two strands that join together and wrap around each other to form a double helix, as depicted in Text Figure 2:

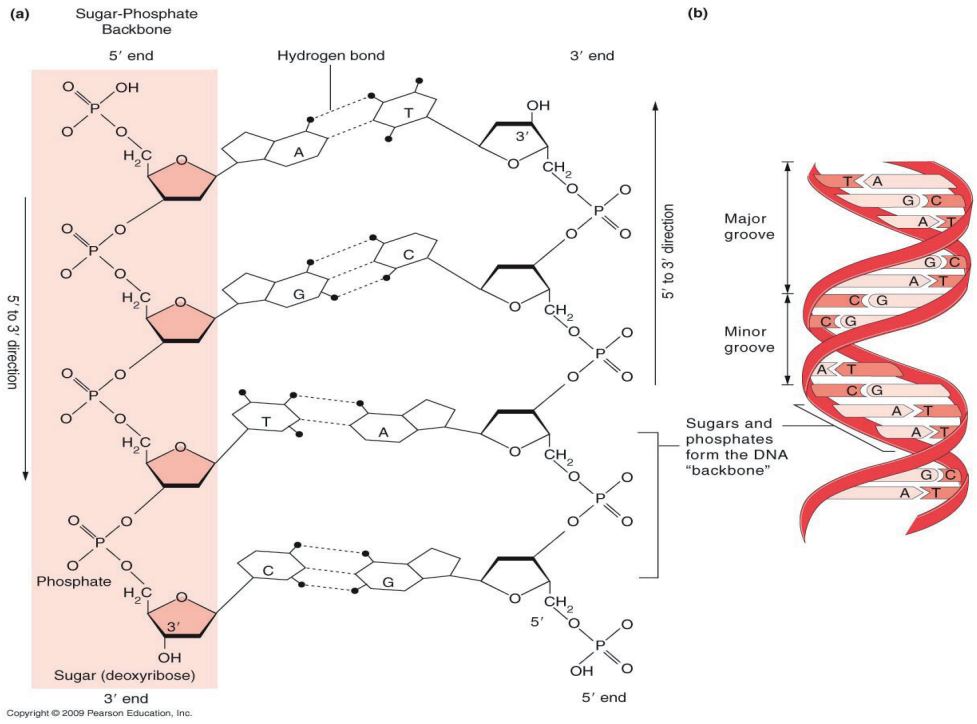


Figure 2: Structure of DNA Molecule

Within each DNA molecule, nucleotides of both strands are held together by **phosphodiester bonds** (like a string of beads), so that each strand has a **polarity –** a 5' end and a 3' end. The two strands of the DNA molecule are in turn held together by **hydrogen bonds.** These form between complementary base pairs such as adenine (A), which pairs with thymine (T), and guanine (G), which pairs with cytosine (C). As a result of the polarity, which is reversed relative to each other, the two strands are **antiparallel.** In eukaryotic cells, the DNA is also associated with binding proteins called histones. In this form the DNA is known as **chromatin,** which in turn forms the **chromosomes.**

A chromosome consists of two thin, rodlike structures of DNA called **sister chromatids,** which are exact replicas of each other copied during a process of DNA replication. During cell division, each sister chromatid is separated.

RNA and protein synthesis

The information packets or genes, which code for the assembly of different kinds of proteins, is contained in the arrangement of nucleotides which are **transcribed** into ribonucleic acid (RNA) via an enzyme known as **RNA polymerase.** This enzyme unwinds the DNA helix and copies one strand of DNA into RNA. At end of a gene, the RNA polymerase encounters a **termination sequence,** which causes a newly formed strand of RNA to be released from the DNA molecule. Such an RNA strand is called **messenger RNA (mRNA)** and multiple copies of mRNA are transcribed from each gene during transcription.

A diagram which summarises this process is depicted in Text Figure 3.

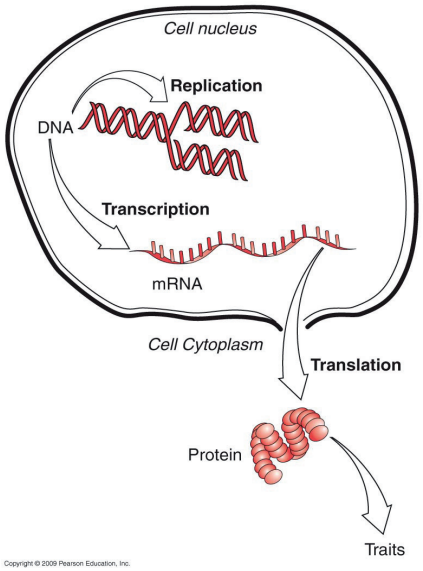


Figure 3: RNA and protein synthesis

The process is illustrated in more detail in **Figure 2.11** (Transcription) on **page 41** of the prescribed textbook. The initial mRNA produced is not fully functional until RNA splicing, polyadenylation and addition of a 5′ cap have taken place. Each three nucleotide units of mRNA is called a **codon,** and each codon codes for a single **amino acid.** Proteins are made up of these amino acids, which can be coded for by more than one codon. Segments of mRNA have start and stop codons for each protein. See Figure 2.13 (Stages of Protein Synthesis) on page 45 of the prescribed textbook. The actual process of assembling amino acids into proteins is known as **translation.**

Translation, which occurs in the cytoplasm, requires three kinds of RNA. These are **mRNA**, which is an exact copy of the gene and carries the genetic code from nucleus to the cytoplasm, **rRNA,** a component of **ribosomes,** the organelles responsible for protein synthesis, and finally **tRNA,** which transports amino acids to the ribosome.

A summary of the **translation process** is as follows:

1. **Initiation –** small ribosome subunit binds to 5' end of mRNA
   Moves along the mRNA until the start codon is found
2. **Elongation –** tRNAs, carrying the correct amino acid, enter the ribosome, one at a time, as the mRNA code is read
3. **Termination –** ribosome encounters the stop codon

Thereafter, newly formed protein is released from the ribosome into the surrounding cytoplasm or passed onto the Golgi apparatus for further processing.

Study Chapter 2 from page 26 to 56 of the prescribed textbook. Pay particular attention to the figures indicated below. Please note, that in an introductory course to biotechnology, such as this one, you will not be required to have knowledge of all the detailed biochemistry of DNA replication. You are only required to understand the applications and kind of instrumentation used in biotechnology.

- Familiarise yourself with Figure 2.1 on page 27 of the prescribed textbook which illustrates a prokaryotic cell. Compare the structure of the prokaryotic cell with the much more detailed eukaryotic cell structure illustrated in Figure 2.2 on page 28, as well as table 2.2 on page 29. Please consult the "Recommended reading" section below to obtain additional information.

ACTIVITY 1.1.1

In view of the fact that Biotechnology has to do with the manipulation of genetic material, it is essential to familiarise yourself with the basic makeup of genes.

1.1.1.1 With reference to Figures 2.4 (Nucleotide Structure), 2.5 (DNA is a Double-Stranded Helix), 2.6 (Chromosome organisation) and related text, make a **summary** of the basic structure of DNA. The summary must not exceed **two pages.**

1.1.1.2 According to the "central dogma" of molecular biology, genetic material is transcribed from DNA into RNA, which in turn codes for protein formation in the cytoplasm of eukaryotic cells. As already indicated, it is not necessary to study all the detail of DNA replication and protein synthesis, but Figures 2.10 (The Flow of Genetic Information in Cells) p.39 and 2.14 (Levels of Gene Expression) p.46 are important. Use these two figures and related text, as well as the three bullet points of text on page 43, "Translating the code: Protein Synthesis", "messenger RNA, ribosomal RNA and transfer RNA", to make a **1½ page summary (including diagrams)** of the "central dogma".

Reflection on Activity 1.1.1

- Review the first two questions and activities on page 95. Make sure you can distinguish between gene cloning, recombinant DNA technology and genetic engineering, as well as the importance of DNA ligase.

Recommended reading

You might find it beneficial to investigate the following websites of the Microbial World Website which has some excellent illustrations of cell components, particularly of eukaryotic cells:

http://www.microbiologytext.com/index.php?module=Book&func=displayarticle&art_id=266

Have a look at sections 2 to 11, 18, 19, 20, 21, 22, 40 and 43.

1.1.2 An overview of some of the techniques used to manipulate genetic material in plant and animal cells

Introduction to recombinant DNA technology and DNA cloning

In the 1970s, gene cloning became a reality with the discovery of **restriction enzymes** which cut DNA, together with **plasmid DNA vectors,** which are a circular form of self-replicating DNA, found primarily in bacteria, and which can accept, carry and replicate other pieces of DNA. DNA itself, when cut, has the phosphodiester bond, which joins adjacent nucleotides in a DNA strand, cleaved. As such, the binding to, recognition and subsequent cutting of DNA within specific sequences of bases is called a **recognition sequence** or **restriction site** (See Text Figure 4).

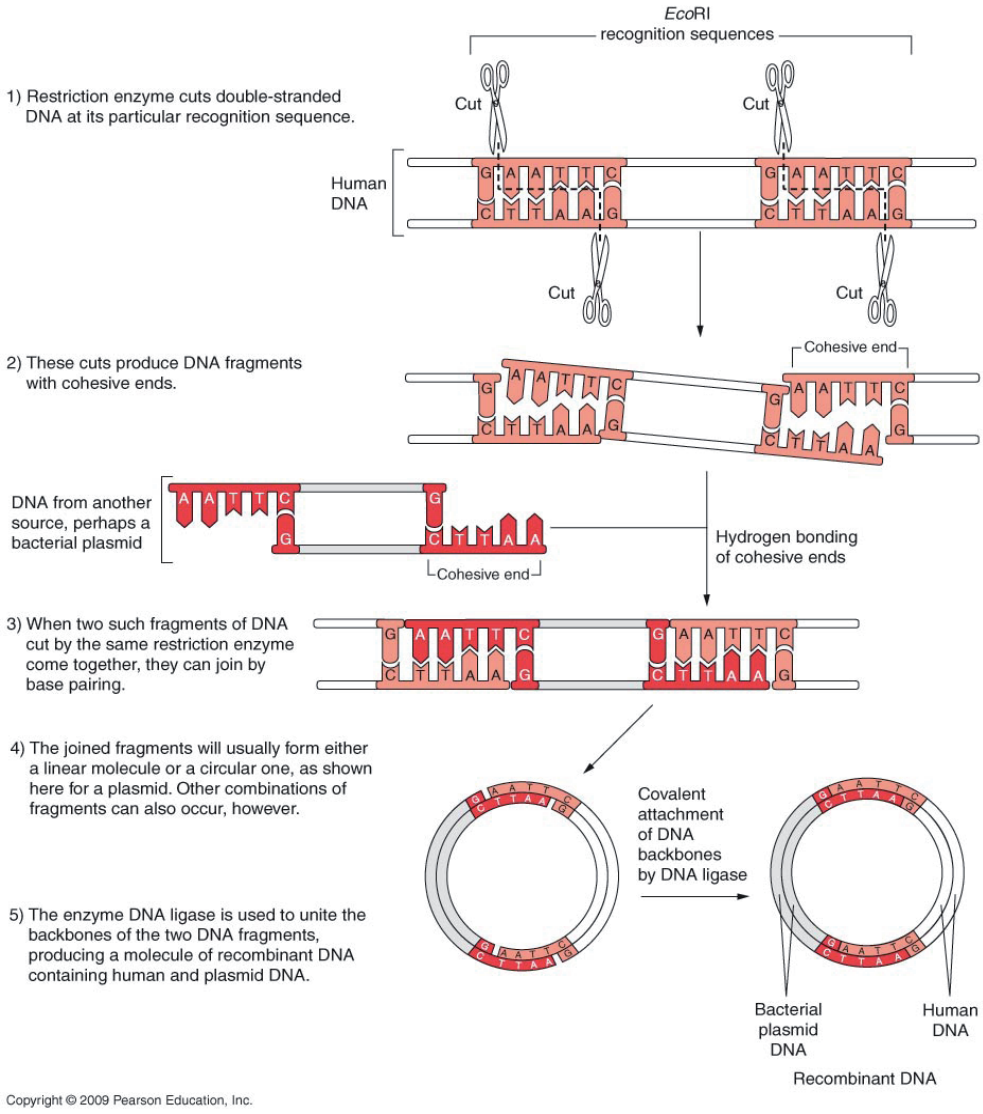


Figure 4: Restriction and ligase enzymes used in gene cloning

Study the section on Introduction to Recombinant DNA Technology and DNA Cloning on page 58 to 65 of the prescribed textbook.

- Refer to Figure 3.1 (Restriction Enzyme Recognition Sequence and Enzyme Action) p.59 and Figure 3.2 (Creating Recombinant DNA) p.62 and Figure 3.3 (Cloning a Gene in a Plasmid and Blue-white Selection) p.64, together with related text.

ACTIVITY 1.1.2

A brief historical account on the discovery of the first restriction enzymes as well as an explanation of how these enzymes work are given on page 58 to 61 of the prescribed textbook (Introduction to Recombinant DNA Technology and DNA Cloning).

1.1.2.1 Briefly explain in your own words how these enzymes work, what plasmid DNA is and why it is important in recombinant DNA technology. The explanation, **including diagrams,** must not exceed **two pages.**

An account of how recombinant bacteria can be created is given on page 61 to 63 in the prescribed textbook (Transformation of Bacterial Cells and Antibiotic Selection of Decombinant Bacteria).

1.1.2.2 Briefly explain in your own words how transformation and selection of recombinant bacteria occurs. The explanation, **including diagrams,** must not exceed **two pages.**

Reflection on Activity 1.1.2

- Review the Q&A textbox on p.63 (Why do bacteria have plasmids?)

Recommended reading

You might find it beneficial to investigate the following websites:

http://www.microbiologytext.com/index.php?module=Book&func=displayarticle&art_id=266

- National institutes of health clinical centre

Transformation of bacterial cells

This is a process for inserting foreign DNA into bacterial cells which have been treated with calcium chloride. Thereafter, plasmid DNA is added to the cells, which have been chilled on ice. The mixture of cells and DNA is then heated to facilitate entry of the plasmid into the bacteria, followed by replication and expression of the introduced gene. A more modern method of transformation can be effected via *electroporation*.

Selection of recombinant bacteria

Selection is a process designed to facilitate the identification of recombinant bacteria while preventing the growth of non-transformed bacteria. This can be done via antibiotic or blue-white selection, as shown in Figure 5.

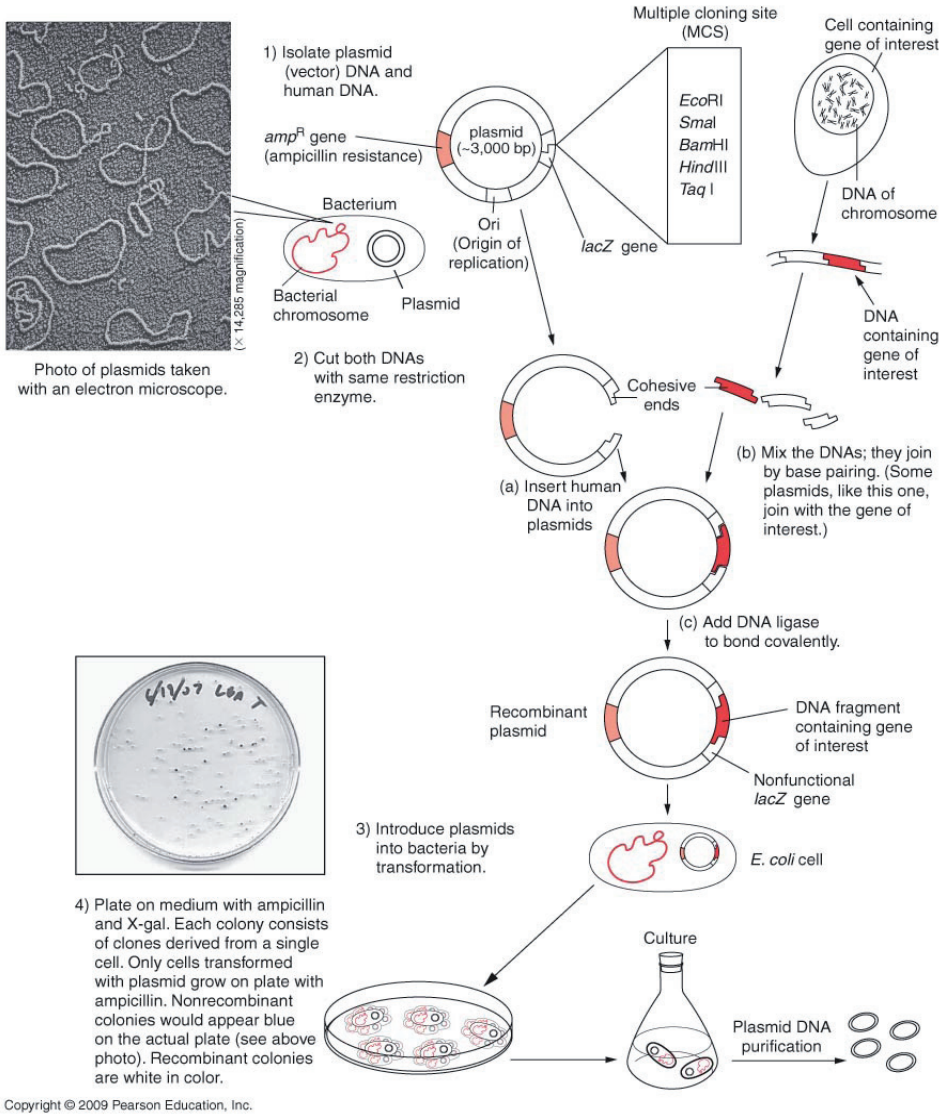


Figure 5: Selection protocol to identify recombinant bacteria using blue-white selection

Practical features of DNA cloning vectors

Any good cloning vector requires an origin of replication (ori), multiple cloning site (MCS), selectable marker genes and RNA polymerase promoter sequences.

Identification and subsequent cloning of a gene of interest

DNA libraries are created with collections of cloned DNA fragments, from a particular organism, contained within bacteria or viruses, as the host. These host cells are then screened to pick out different genes of interest.

Two types of libraries are available, namely **genomic DNA libraries** and **complementary DNA libraries (cDNA libraries).**

Genomic libraries

Chromosomal DNA, from the tissue of interest, is isolated and digested with one or more restriction enzymes. Similarly, a suitable vector is digested with the same enzyme and DNA ligase is used to ligate genomic DNA fragments with vector DNA. These recombinant vectors are used to transform other bacteria.

Disadvantages

Non-protein coding pieces of DNA (introns) are cloned in addition to exons; majority of genomic DNA is introns in eukaryotes, which means the majority of the library will contain non-coding pieces of DNA.

Many organisms have a very large genome and, therefore, searching for the gene of interest is difficult.

cDNA Libraries

Complementary DNA (cDNA) is an exact copy of the mRNA.

mRNA from tissue of interest is isolated and converted to double-stranded DNA by using the enzyme **reverse transcriptase.** Residual mRNA is degraded and DNA polymerase used to create the second strand of DNA. Short linker sequences, which contain restriction enzyme recognition sites, are added to the end of the cDNA.

Both the cDNA and vector are then cut with the same restriction enzyme, so that fragments can be ligated to create recombinant vectors that can be used to transform suitably prepared bacterial hosts.

Advantage

Only actively expressed genes are collected from physiologically active cells or tissues, from which the mRNA is isolated. As such, introns are not cloned.

Disadvantage

Can be difficult to make the cDNA library if a source tissue with an abundant amount of mRNA for the gene is not available.

Screening of Libraries to identify the gene of interest

Bacterial colonies containing recombinant DNA are grown on an agar plate. Thereafter, a nylon or nitrocellulose filter is placed over the plate and some of the bacterial colonies stick to the filter at the exact location they were on the plate. The filter is then treated with an alkaline solution to lyse the cells and denature the DNA, which subsequently binds to the filter as single-stranded DNA.

This filter is then incubated with a **probe,** which is a suitably tagged DNA fragment that is complementary to the gene of interest. The DNA probe binds by hydrogen bonding, to complementary sequences on the filter, which is then washed to remove excess unbound probe and is subsequently exposed to film. This is known as autoradiography, a process whereby light will be emitted and will expose silver grains in the film anywhere probe has bound.

After being developed, the film is compared to the original agar plate to identify which colonies contained recombinant plasmid with the gene of interest.

Study the section on identification and cloning a gene of interest on page 68 to 74 of the prescribed textbook.

Refer to Figure 3.6 dealing with colony hybridisation and Figure 3.7 dealing with the polymerase chain reaction, together with related text.

ACTIVITY 1.1.3

A brief account on the creation of a copy or cDNA library is given on page 68 (Creating DNA Libraries: Building a Collection of Cloned Genes) in the prescribed textbook. Read through this part, but focus on the actual methods involved in the screening of these libraries.

1.1.3.1 Briefly explain in your own words how screening of cDNA libraries takes place. Pay particular attention to the role of **probes** in this process. An account of how recombinant bacteria can be created is given on page 61 to 63 (Transformation of Bacterial Cells and Antibiotic Selection of Recombinant Bacteria) in the prescribed textbook. The explanation, **including diagrams,** must not exceed **two pages.**

The polymerase chain reaction is explained on page 71 to 73 (Polymerase Chain Reaction) of the prescribed textbook.

1.1.3.2 Briefly explain in your own words how the polymerase chain reaction works. Pay particular attention to the role of **primers** and **Taq DNA polymerase**. The explanation, **including diagrams,** must not exceed **two pages.**

Reflection on Activity 1.1.3

- Review the Q&A textbox on page 73 of the prescribed textbook (How do scientists determine what primer sequence and temperature conditions should be used for a PCR experiment).

Polymerase chain reaction (PCR)

Developed in the 1980s by Kary Mullis, this is a technique for making copies, or amplifying, a specific sequence of DNA, in a short period of time. The process involves making up a

mixture of target DNA to be amplified, which is added to a tube, containing nucleotides (dATP, dCTP, dGTP, dTTP), buffer, and DNA polymerase.

The type of DNA polymerase used is very important. *Taq* DNA polymerase is isolated from a species known as *Thermus aquaticus* that thrives in hot springs. As such, *Taq* polymerase is one of the few enzymes that can function at temperatures above 68°C and puts a single adenine nucleotide on the 3' end of all PCR products.

Primers, consisting of short single-stranded DNA oligonucleotides (20–30 bp long) are added to the mix, and the reaction tube is placed in an instrument, called a thermocycler. This thermocycler will then take the DNA in the tube through a series of reactions, called a **PCR cycle**.

Each cycle consists of three stages, namely denaturation (heating solution to above 68°C), annealing (hybridisation) and extension (elongation). At the end of one cycle, the amount of DNA in the tube has doubled. Cycles are repeated 20 to 30 times until sufficient DNA is available for detection and analysis.

Advantage of PCR

Millions of copies of target DNA can be amplified from a very small amount of starting material in a short period of time.

Applications

The PCR technique can be used for making DNA probes, studying gene expression, detecting viral and bacterial infections, diagnosing genetic conditions, detecting trace amounts of DNA from tissue found at a crime scene, and cloning PCR products rapidly and effectively.

Disadvantage

Knowledge is required about the DNA sequence that flanks the gene of interest, in order to design or select the appropriate primers, which also needs to include restriction enzyme recognition sequences.

See Figure 3.7 on page 72 of the prescribed textbook. A copy is included below.

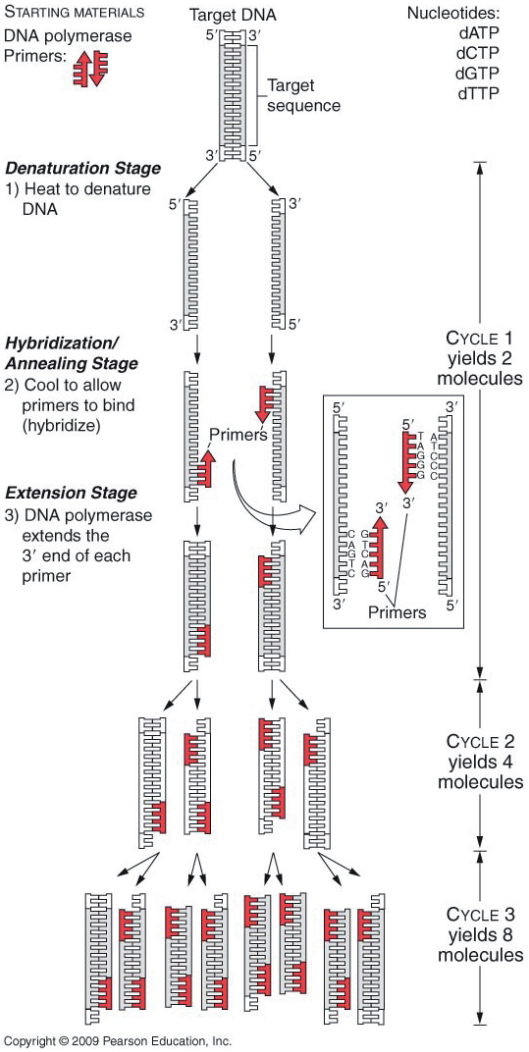


Figure 6: The polymerase chain reaction

STUDY SECTION 1.2: Modern applications of biotechnology in a biological setting

1.2.1 Applications of biotechnology in an ecological setting

Biotechnology can be applied in a **Bioremediation** process, whereby contaminated environmental sites have their chemical pollutants degraded into less toxic substances by using living organisms, such as certain bacteria.

Natural biodegradation occurs when living organisms such as bacteria, fungi, and plants to degrade biological material and other chemical compounds. Bioremediation as a process was started in the USA to counteract careless and even negligent practices of chemical dumping and storage in view of the concern over how these pollutants might affect human health and the environment.

In essence, bioremediation is used to counteract careless and even negligent practices of chemical dumping and storage in view of the concern over how these pollutants might affect human health and the environment. Biotechnological approaches are essential for detecting pollutants, restoring ecosystems, learning about conditions that can result in human diseases and converting waste products into valuable energy.

The Basics of bioremediation processes

Microbes convert chemicals into harmless substances by either **aerobic metabolism** which requires the presence of oxygen, or **anaerobic metabolism,** which does not require oxygen. Both processes involve **oxidation** and **reduction reactions. Oxidation** results from the removal of one or more electrons from an atom or molecule, while **reduction** results in the addition of one or more electrons to an atom or molecule.

These reactions are illustrated in Figures 7 and 8.

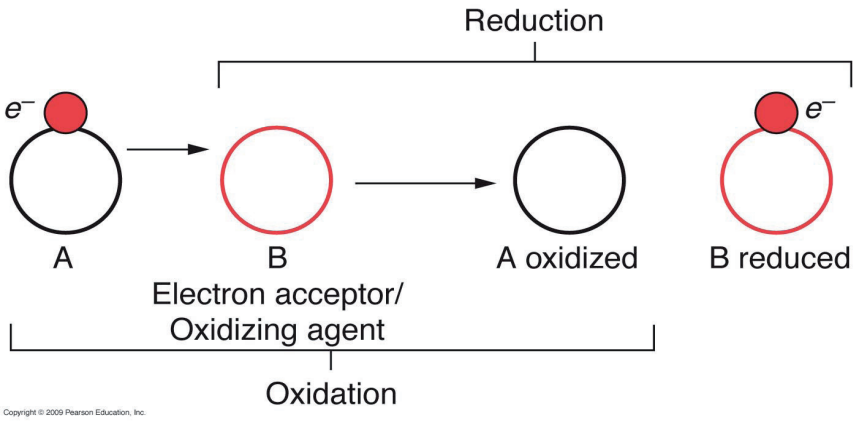


Figure 7: Oxidation and reduction reactions

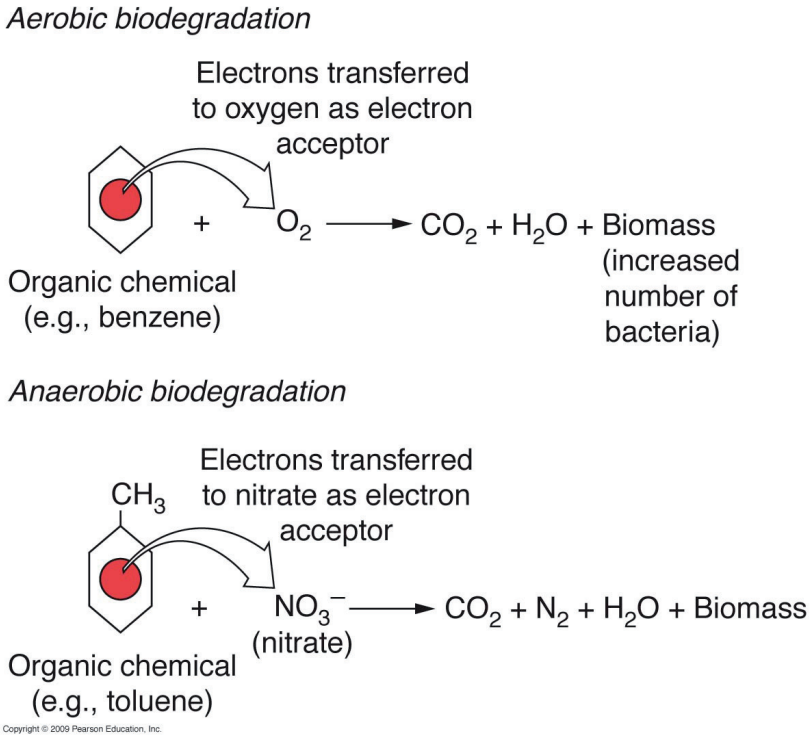


Figure 8: Aerobic and Anaerobic Biodegradation

Aerobic bacteria (aerobes) use oxygen as an electron acceptor molecule to oxidise organic chemical pollutants such as benzene. During this process, oxygen is reduced to produce water and carbon dioxide is derived from the oxidation of benzene. Energy from downgrading the pollutant is used to stimulate bacterial cell growth (biomass). Similar reactions occur during anaerobic biodegradation, except that anaerobic bacteria (anaerobes) rely on iron, sulphate and nitrate molecules as electron acceptors to oxidise stimulating bioremediation.

Two other applications of this technology include the following:

Nutrient enrichment (fertilisation) – fertilisers are added to a contaminated environment to stimulate the growth of indigenous microorganisms that can degrade pollutants, and

Bioaugmentation (seeding) – bacteria are added to the contaminated environment to assist indigenous microbes with biodegradative processes

Study the section on Bioremediation Basics on page 210 to 217 as well as page 222 to 224 (Applying Genetically Engineered Strains to Clean up the Environment) of the prescribed textbook.

Refer to Figure 9.1 dealing with Treatment Environments and Contamination Zones p.211, Figure 9.2 on Oxidation and Reduction Reactions, Figures 9.3 and 9.5 on Aerobic and Anaerobic Biodegradation and Figure 9.16 (Phytoremediation of Toxic Explosives using Transgenic Plants) p.225 on Phytoremediation.

ACTIVITY 1.2.1.1

Bioremediation deals with biological repair of an environment, which has usually become damaged by some form of chemical pollution.

1.2.1.1.1 Make a summary in which you explain in your own words how chemical pollutants generally enter the environment, as well as how oxidation and reduction reactions contribute to aerobic and anaerobic biodegradation of these pollutants. The summary, **including diagrams,** must not exceed **two pages.**

Many scientists are studying the genomes/metabolic pathways of organisms, in order to find ways of stimulating bioremediation.

1.2.1.1.2 Study pages 215 to 217 (Bioremediation Genomics Program) and 222 to 224 (Applying Genetically Engineered Strains to Clean up the Environment) of the prescribed textbook. Make a summary in which you explain in your **own words** how the efficiency of bioremediation can be improved using either microbes (bacteria) or certain plants, where the process is referred to as phytoremediation. Pay particular attention to bacteria used to process **petroleum** and **heavy metals,** bacteria which can be used as **biosensors** of toxic chemicals and genetically modified plants for **phytoremediation.** The summary, **including diagrams,** must not exceed **three pages.**

Reflection on Activity 1.2.1.1

- Review the contents of the textbox 'Tools of the Trade' on page 223 of the textbook, and make sure you understand the principles behind optimising bioremediation processes that can also include a new chemical released into the environment.

ACTIVITY 1.2.1.2

Read the contents of this textbox which contain excerpts from the IANCAS website:

http://iancas.org/bulletins/Jan%202005.pdf, is a special bulleting dealing with aspects of

Environmental Biotechnology

In nature, biofilms attached to the plant roots of some crops help cycle nutrients to and from the plant, resulting in increased agricultural productivity. Biofilms can also be used to produce a wide variety of biochemicals that are then purified and used, including medicines, food additives, or chemical additives for cleaning products.

Bioremediation

Bioremediation, which uses materials of microbial and plant origin, has been recognized as an alternate method of environmental clean-up compared to conventional methods. Plants have the inherent ability to absorb and translocate essential and non-essential elements and organic chemicals from the soil through roots.

Phytoremediation

Phytoremediation is an umbrella term, which include the following subsets

(a) Phytoextraction: Use of high biomass metal-accumulating plants to remove and concentrate metals/organic pollutants in harvestable plant parts such as shoots, which are harvested using conventional agricultural methods.

(b) Rhizofiltration: Use of plant roots to adsorb/absorb pollutants – mainly metals from water and aqueous waste streams.

(c) Phytodegradation: The use of plants and associated microorganisms to degrade organic pollutants.

(d) Phytostabilization: The use of plants to reduce the bioavailability of pollutants in the environment by stabilizing them in soils.

(e) Phytovolatilization: The use of plants to volatilize volatile metals through foliage (e.g. mercury, selenium).

(f) Removal of pollutants from air.

The potential of certain species of plants called as "hyperaccumulators" to accumulate high concentrations of metals in the above ground parts has been recognized for potential exploitation for phytoremediation.

Genetically engineered plants tailored for phytoremediation can be developed by introduction of genes from plant, bacterial and animal sources, which can enhance metal accumulation or degradation of organics. Introduction of genes for secretion of organic acids such as citric acid, malic acid and oxalic acid and other compounds such as phenolics, flavanoids or coumarins that induce rhizospheric bacteria to degrade xenobiotics and the the introduction of genes for degradation of organic pollutants are some of the strategies which can be employed for improving phytoremediation.

Phytoremediation : Prospects and Limitations

The main advantages of phytoremediation are that it is far less disruptive to the environment, lack of requirement for huge disposal sites, high public acceptance, avoidance of excavation, lack of noise and frequent worker activity and its potential versatility to treat a diverse range of hazardous materials.

The disadvantages of phytoremediation include limitation of growth of plants under extreme conditions, possibility of contaminants being released back into the environment by litter fall and enhancement in solubility of some contaminants resulting in pollutant migration.

Large-scale Cultivation of Cell Cultures in Bioreactors and production of Bioactive Compounds

The application of bioreactor systems for large-scale cultivation of plant cells for the production of valuable bioactive compounds is an active field, whose application holds great promise for the pharmaceutical industry. Large-scale cultivation of plant cells increases biomass production much more than whole plants grown in the field.

Many multinational pharmaceutical companies have started their production units in India with a view to producing bulk amounts of plant-based drugs. Currently, field grown plants are being used for extraction and purification processes. The continuous production of anti-cancer, anti-AIDS and other important life-saving drugs requires a large number of plants, thus, leading to the problem of depletion of several elite varieties of medicinal plants. Automated, bioreactor-based systems may be the method of choice for the mass propagation of shoots and hairy roots for the production of bioactive compounds.

Biodegradable waste

1. Environmentally friendly disposal of waste.
2. Generation of fuel gas would support dwindling energy resources.
3. Generation of high-quality manure which would be weedless and an excellent soil conditioner. This is very important for replenishing fast-decreasing resources of productive soils. It may be noted that need for replenishing the soil with high quality organic manure has been identified.
4. It would reduce the menace of street dogs and other nuisance animals.
5. It would help in removing the garbage hills from rural and urban areas.

Thermophiles

They can thrive in extreme environments where ordinarily no one can even imagine that there would be life. An organism that can happily grow in an extreme environment is an extremophile. The discovery of extremophiles has pumped new life into the biotech industry and dreams of stock options in the minds of field biologists. Extreme environments include physical extremes like pressure, temperature and radiation and geochemical extremes like desiccation, salinity, pH and low redox potentials.

Environmental Biosensors

The term biosensor is defined as a sensor incorporating biological elements such as enzymes, antibodies, receptors proteins, nucleic acids, cells, or tissue sections – as the recognition element, coupled to a transducer.

Biosensor Technology

The two main elements in a biosensor are a biological recognition element or bioreceptor and a signal transducer. The bioreceptor is a biomolecule that recognizes the target analyte and can be divided into three distinct groups: biocatalytic, bioaffinity, and microbe-based systems. Biocatalysis-based biosensors depend on the use of pure or crude enzymes to moderate a biochemical reaction. For environmental applications, enzyme-based reactions involve enzymatic transformation of a pollutant or inhibition of enzyme activity by the pollutant. Enzyme inhibition approaches tend to cater for a larger number of environmental pollutants, usually of a particular chemical class such as pesticides and heavy metals. However, such methods require the use of substrates and in some cases the biosensor may need to be reactivated due to the inhibition.

A signal transducer is the second essential component of a biosensor. It converts the recognition event into a measurable signal. Amperometric biosensors are based on monitoring the current associated with oxidation or reduction of an electroactive species involved in the recognition process. The current produced is linearly proportional to the concentration of the electroactive product, which in turn is proportional to the non-electroactive enzyme substrate. Compounds of environmental concern, measured using amperometric and electrochemical electrodes include 2,4-Toluene diamine (2,4-T) [4] polychlorinated biphenyls (PCBs), triazines and various toxins such as serin and soman [5].

Eco-friendly Methods for the Management of Insect Pests of Agricultural Importance

Biopesticides

Like human beings, insects are subjected to infections by an entire array of micro-organisms like bacteria, virus, protozoa, fungi and nematodes. Perhaps the greatest potential for future progress in biological control lies in improving the success of microbial pathogens. Many of these organisms are highly desirable as biocontrol agents and attack a narrow range of insect hosts. They are non-hazardous to humans or domestic animals and pose no threat to the environment.

In the past few years, there has been significant progress in the development of microbial insecticides. These are commercial suspensions of spores, toxins, or virus particles that can be mixed with water and sprayed onto crops, just like conventional

insecticides. In many cases, microbial insecticides are better than conventional insecticides because they suppress pest populations without eliminating natural populations of predators and parasites.

Insect-resistant crop varieties

Breeding plants for resistance to insects is just another form of biological pest control. Breeders look for genetic traits (or combinations of traits) that reduce crop susceptibility to attack or injury by its insect pests. Recent advances in plant biotechnology have made it possible to transfer and express the novel foreign insect resistance genes in to crop plants. To develop insect resistant crop plants the first Bt toxin gene was cloned and transgenic plants were produced in the mid 1980s. Since then several crop species have been genetically engineered to produce insect resistant crop varieties.

Autocidal control or Genetic control

Some pest control tactics are designed to suppress a pest population by altering its genetic makeup and/or reducing its reproductive potential. These are also known as genetic controls because they affect the accuracy or efficiency with which a pest species passes its genetic material (DNA) from one generation to the next. Genetic control usually works in one of two ways: either by causing (inducing) reproductive sterility, or by incorporating new and potentially deleterious genes into the genetic makeup of a pest population. In effect, some members of a pest species are transformed into biological time bombs that eventually destroy other members of their own species. Because of the self-destructive nature of these tactics, they are sometimes called autocidal control.

Reflection on Activity 1.2.1.2

- Review the contents of the Activity 1.2.1.2 of the textbox in this study guide pp.40–45. Write a critical essay, specifically stating your own point of view, referring to at least **four** of the **headings** in the textbox. In order to do this, you will need to make use of 'Google Scholar', to find out more information under the different headings. (See introduction under "Improved study skills"). The essay must not exceed **three typed pages.**

ACTIVITY 1.2.1.3

1.2.1.3.1 Make a summary in which you explain in your **own words** how chemical pollutants generally enter the environment, as well as how oxidation and reduction reactions contribute to aerobic and anaerobic biodegradation of these pollutants. The summary, **including diagrams,** must not exceed **two pages.**

Many scientists are studying the genomes/metabolic pathways of organisms, in order to find ways of stimulating bioremediation.

ACTIVITY 1.2.1.4

Bioremediation deals with biological repair of an environment, which has usually become damaged by some form of chemical pollution.

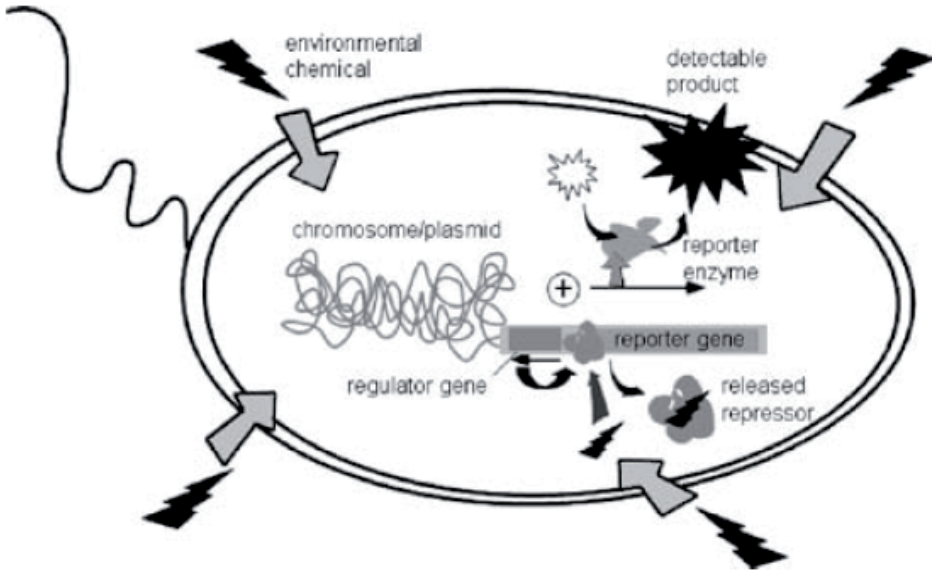
1.2.1.4.1 Make a summary in which you explain in your **own words** how chemical pollutants generally enter the environment, as well as how oxidation and reduction reactions contribute to aerobic and anaerobic biodegradation of these pollutants. The summary, **including diagrams,** must not exceed **two pages.**

Many scientists are studying the genomes/metabolic pathways of organisms, in order to find ways of stimulating bioremediation.

ACTIVITY 1.2.1.5

Read through the excerpt from the Springer-Verlag website:

http://www.springerlink.com/content/57425571834378q4/fulltext.pdf, published in "Applied Microbiology and Biotechnology (2006)" which deals with whole cell biosensors and their applications.



Schematic representation of a class I bioreporter bacterium for an environmental chemical (drawn as a flash). Upon entering the cell, the chemical induces the transcription of a reporter gene, in the case depicted here by causing a repressor protein to leave the promoter/operator region. The accumulation of a visible reporter enzyme product results in signal amplification.

This shows that bioreporter bacteria were not just a fancy laboratory trick, but could be used to measure aqueous phase chemical concentrations. Although some of those constructions were driven by the desire to understand bacterial signalling chains, they propelled bioreporter analysis forward as an alternative for certain types of measurements.

In view of future commercial use, it was seen as advantageous that bacterial cells are rapidly self-producing, hence making bioreporter production potentially very cheap. In addition, various toxicologically or environmentally important chemicals could be targeted while maintaining the same kind of reporter output (e.g. luminescence, green fluorescence or discoloration), thus allowing multianalytics with a single apparatus (such as a luminometer or fluorimeter).

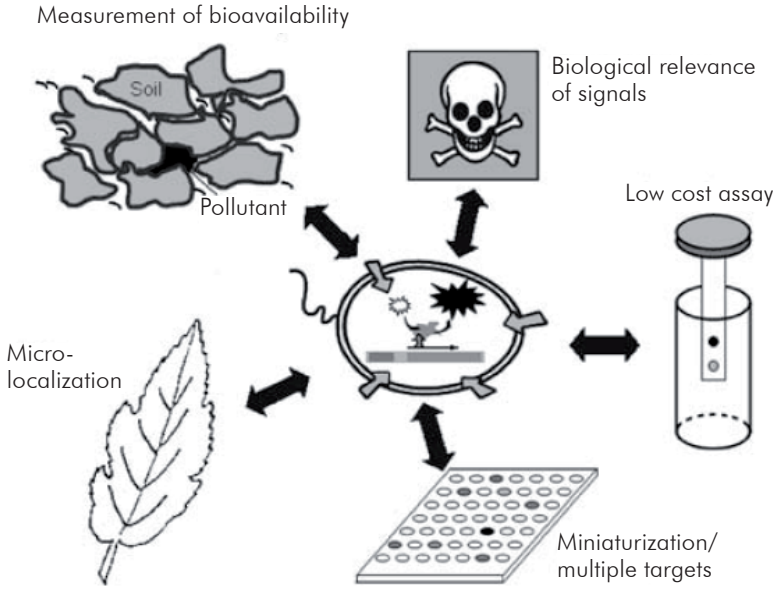


Illustration of frequent arguments for bioreporter developments, including (clockwise) the measurement of bioavailable fractions of compounds, the inherent biological relevance of bioreporter reactions to toxicants or food compounds, the potential for low-cost analytics not requiring apparatus, the potential for miniaturization and multiple target analytics and the high spatial resolution of micron-sized bioreporters facilitating the microlocalization of target compounds and the access to spatially restricted environments.

Uptake and metabolism of the target compound by a bacterial cell (biodegradation) automatically creates a further chemical flux and thus potentially changes the bioavailable compound, for instance, in the case of sorbed or crystalline pollutants. However, also in this case, by careful choice and design of the bioreporter host cell, it is possible to obtain a bioreporter which senses or modifies the freely available compound like the bacteria performing the bioremediation do. For example, bioreporter bacteria are often constructed from natural isolates to degrade pollutants. When the same organism is equipped with the biosensor construction while maintaining its degradation capacity or surfactant production, the resulting bioreporter will perform similar to wild-type degraders. Future bioreporters may be further refined by using other host cells which are more suitable for measurements in certain specific environments and which have different ways of modifying the bioavailable compound.

(Reference see p.48 of this study guide).

Reflection on Activity 1.2.1.5

- Review the contents of the Activity 1.2.6.1 textbox and make sure you can answer a short exam question requiring an explanation about the makeup of biosensors and some of their applications.

1.2.2 Applications of biotechnology in a biomedical setting

ACTIVITY 1.2.2.1

Read the following excerpt which was taken from the OECD International Futures Programme:

http://www.oecd.org/dataoecd/12/57/40920458.pdf

which specifically deals with the future of agricultural biotechnology to 2030:

Climate Change and Population Increases Driving Technological Advancement

By 2030, both the global temperature and the frequency of severe weather conditions have increased more than had been predicted earlier in the century. Many previously productive agricultural centres (*e.g.* Australia, Brazil, the United States, and countries in southern Europe) have struggled to maintain productivity in the face of the increasingly unpredictable weather. These environmental pressures, coupled with surging global demand for higher value food commodities, have led private industry to invest heavily in new technologies to keep pace with markets. Biotechnology has provided the means to increase yield in drought conditions and provide the value-added traits (such as enhanced nutrition, taste, and ease of preparation) demanded by consumers in both crop commodities and livestock varieties.

With the increased temperatures associated with climate change, the conditions ripened for pest infestations and disease. Molecular biology advances such as viral coat protein technologies provided protection from viruses found in wheat, rice, and potatoes. As a large portion of the major and minor crops used in agriculture had known DNA profiles, because of continued investment in DNA databanks and sharing among trading partners, some minor crops also benefited from virus reduction technologies through adoption of the new technologies with simple and cost-effective practices. Additionally, gene sequence transfers worked to confer resistance to plant nematode infestations and fungal infections that had increased in frequency in the most used global crops (soybeans, maize, rice, potatoes). These molecular biology and genetics advances enabled the agricultural sector to maintain stability in agricultural production in spite of increased pest and disease challenges.

The early concerns in 2005 to 2015 regarding the limited availability of biomass and societal tension over crops for food versus fuel diminished as biotech and seed development industry invested in new technologies that made available high-yield food crops adapted to grow in the changing climate, and dedicated energy crops much like *Jatropha*, *Miscanthus*, and Switchgrasses that were not suitable for eating, yet grew on marginal lands. The introduction of more effective processing technologies and refineries that required less energy and provided maximum product increased the efficiency of "whole usage" crops and decreased the volume grown in the field.

The forestry industry also increased investments in biotechnology to develop tree varieties adapted for drought and increased temperature conditions. Additionally, industry developed varieties adapted for salinity, to cope with saline soil developing in drought conditions, and adapted to combat pest and disease brought upon with warmer climate and stressed trees. Tree varieties grown on company plantations in southern locals (in South America) were destined for the global pulp and paper industry, while varieties grown on plantations in northern locals with successful forestry industries (such as Canada and Russia) were destined for high value structural timber and composite wood products.

In specific areas along coastal China, eastern India, and the Gulf of Mexico, regions moved forward with the purchase of generic brands of genetically modified (GM) marine plants to remediate surrounding coastal waters. Marine areas that had become "dead zones" through industrial pollution or agricultural run-off were revitalised through the use of adapted marine plants. While countries bordering the Adriatic and Baltic Seas, in addition to coastal Argentina and Brazil, would have benefited from this technology, public concern over the spread of such plants into adjacent coastal waters was too voracious, so regional governments banned the use of such plants.

Driven by the tangible evidence of climate change and significant waste disposal challenges, affluent consumers began increasing demand for so-called "green" products, produced from renewable materials (*e.g.* agricultural crops) and the use of sustainable production methods by industry. By 2030, there is wide-scale market acceptance of these renewable products as biotech has provided the tools to produce and market them in a cost competitive manner.

We Knew it was Coming – Zoonotic Disease Hits Vietnam and Spreads

The first influenza outbreak of the 21st century had been long overdue, but when its third wave hit Southeast Asia in 2020 not enough research and precaution had been taken to prevent the spread of this highly infectious viral disease setting off a global panic. The World Health Organisation (WHO) and international organisations like the early International Partnership on Avian and Pandemic Influenza had tried to prepare for this virulent strain (next generation post H5N1), but society had been distracted by global tensions and health biotechnology had been focussed on the spread of pathogens and contaminants most likely to be introduced purposefully.

The avian influenza was among many infectious livestock diseases that received modest research. The influenza started in Vietnam in domestic duck and spread rapidly to poultry and humans in close proximity reaching pandemic status when it began spreading between humans. Fortunately, the number of people quickly falling victim with similar symptoms set off alarm bells which were heard all over the globe. The WHO, the United Nations Children's Fund (UNICEF), and the US Centers for Disease Control and Prevention (CDC) immediately enacted an emergency response plan, restricting movement in/out of Asia, Europe, and North Africa, enacting world scale diagnostics and therapeutics to contain and treat the disease. The immediate action contained the outbreak to Cambodia, China, Indonesia, Thailand, and Vietnam.

Overall, there were nearly 5 million deaths, a large proportion in China, where the outbreak started in rural agricultural areas and then spread to densely populated urban areas. Immense damage was done to the perception of Southeast Asia as a safe place to visit and do business and their trading relationships suffered as a result of this outbreak. It was apparent to global trading powers that there was a distinct difference between the poultry raising practices in Southeast Asian countries and those in Europe and North America. For example, in Europe when the H5 and H7 strains had appeared the disease was contained and eradicated without creating a disaster. This distinction was not lost on these countries or their trading partners as trade and commerce with affected countries temporarily halted and caused a serious re-examination of food and agriculture infrastructure and regulations.

The short-term economic impacts of the "Southeast Asian flu" as it became known were significant as trade halted while the illness was contained. Once containment and treatment had begun, trade continued to be impacted in the short term. Countries who formerly imported food commodities from South- East Asia (such as meats, oils, shrimp, molluscs, and rice) were very wary of such goods and trade fell by 75% for some products. Technology and manufacturing industries (such as electronics, computers, household items, and industrial parts) suffered lost productivity, causing an opportunity for other suppliers (most notably India and Japan) to take advantage of supply shortages.

Wary of any future epidemics, the so-called ASEAN+3 (China, Japan, Korea, and the Association of Southeast Asian Nations (ASEAN)) developed an immediate action plan to get "back to business" within 6 months. This goal was modestly achieved and a longer-term action plan was put in place comprising diplomatic and communications efforts and a restructuring of the regulatory oversight of food commodities. Pressure remained on government budgets because of expenditures on hospital care, vaccinations, public safety and subsidies to businesses who sustained lost revenues. These budgetary constraints were felt over the longer term as governments worked to restructure food safety and security, and tourism continued to suffer for the region.

Although, the infected areas were quarantined with millions of poultry being culled and hospitalisation/quarantined zones for affected humans, this outbreak highlighted the susceptibility of developing nations that did not have the basic safety measures to keep up with their growth. While a number of the these countries were seen as effectively dealing with the situation, the WHO, the Food and Agriculture Organisation (FAO), the World Organisation for Animal Health and NGOs continued to emphasise the need for comprehensive growth bringing in line health and safety with development. The WHO also emphasised health and safety risk mitigation strategies to deal with possible additional effects of the "Southeast Asian flu", while the IMF and World Bank used support that had been pledged to counteract the fiscal challenges faced by these countries.

Regional organisations like ASEAN were the most effective players dealing with the crisis and worked as a strong intermediary balancing the interests of individual countries, with those of the region, and the international community. Once the initial impacts of the flu pandemic subsided, a global shift took place with more citizens demanding greater individual health and safety, with increased recognition of the social and economic reasons to improve animal welfare. With a greater emphasis on human and animal health and safety, all countries, particularly developed countries, began to expect increased detection, investigation, and containment capacity from their trading partners.

Reflection on Activity 1.2.2.1

- Review the contents of the Activity 1.2.2.1 textbox in this study guide pp.52–57 dealing with Environmental Biotechnology. Write a critical essay, specifically stating your own point of view, in which you refer to at least four of the headings in the textbox. In order to do this, you will need to make use of "Google Scholar", to find out more information under the different headings. (Refer to the introduction under "Improved study skills".)

STUDY UNIT 2

Time allocation

You should spend at least 12 hours on this study unit.

ISSUES PERTAINING TO THE IMPACT OF BIOTECHNOLOGY ON THE ENVIRONMENT AND SOCIETY

Textbook reference

This study unit is based on Introduction to Biotechnology, 2nd Ed (2009); Thiemann, W.J. & Palladino, A., Pearson/Benjamin Cummings, London, Toronto, New York. Chapter 6: Plant Biotechnology, pages 155–170; Chapter 7: Animal Biotechnology, pages 171–186; Chapter 9: Bioremediation, pages 208–230; Chapter 10: Aquatic Biotechnology, pages 231–259; internet sources as indicated in the text of this study guide.

STUDY UNIT CONTENT

This study unit is divided into the following study sections:

Study section 2.1 An overview of general issues pertaining to the impact of biotechnology on the environment and society

Study section 2.2 A general perspective of specific impacts of biotechnology in the biosphere

Study section 2.3 The origins of biotechnology

Outcomes

After completing this study unit you should be able to:

- critically discuss arguments for and against various applications of biotechnology in the environment.

- briefly discuss the methods used in plant transgenesis, paying particular attention to protoplast fusion, leaf fragment technique and gene guns.
- describe practical field applications in plant biotechnology, with specific reference to genetic pesticides and herbicide resistance, enhanced nutrition and fuel.
- briefly discuss various methods used in creating transgenic animals, with special attention being paid to retrovirus-mediated transgenics, pronuclear microinjection, embryonic stem-cell method and sperm-mediated transfer.
- review some of the ethical concerns about using animals in research.
- review the discovery and applications of novel genes from aquatic organisms, paying particular attention to antifreeze proteins and "green genes".
- briefly discuss the role of biofilms and biosensors in aquatic remediation issues.

Review the birth of molecular biology from a number of basic disciplines, such as biochemistry and cell biology.

STUDY SECTION 2.1: An overview of general issues pertaining to the impact of biotechnology on the environment and society

2.1.1 Biotechnology issues in the environment

Microbial Biotechnology involves the manipulation of microorganisms, such as yeast and bacteria, in order to generate better enzymes for food processing, or for more efficient decontamination processes for industrial waste product removal.

Agricultural Biotechnology makes use of genetically engineered, pest-resistant plants, as well as to produce foods with higher protein or vitamin content. In some cases, certain drugs can be developed and subsequently grown as plant products.

Animal Biotechnology makes use of animals as a source of medically valuable proteins, such as antibodies. In addition, certain animals, such as laboratory rats, can be used as important models in basic research, especially in the design and testing of new medicines and genetic therapies.

Bioremediation involves the use of biotechnology to process and degrade a variety of natural and manmade substances, particularly those that contribute to pollution. For example, bacteria that degrade components in crude oil were used to control the spread of the 1989 Exxon Valdez oil spill in Alaska.

Aquatic Biotechnology is used in Aquaculture, such as when finfish or shellfish are raised in controlled conditions for use as food sources. Disease-resistant strains of oysters have been produced as well as vaccines against viruses that infect salmon and other finfish. As such, Aquaculture can provide a rich and valuable source of new genes, proteins and metabolic processes with important applications for the benefit of mankind.

In addition, marine plankton and snails have been found to be rich sources of antitumor and anticancer molecules.

Figure 9 provides a good summary of the various applications of biotechnology. As such, the roots are formed out of the so-called "basic sciences" such as Microbiology, Biochemistry and Cell Biology. Integration of these elements leads to the multidisciplinary nature of a biotechnological approach to solving environmental problems.

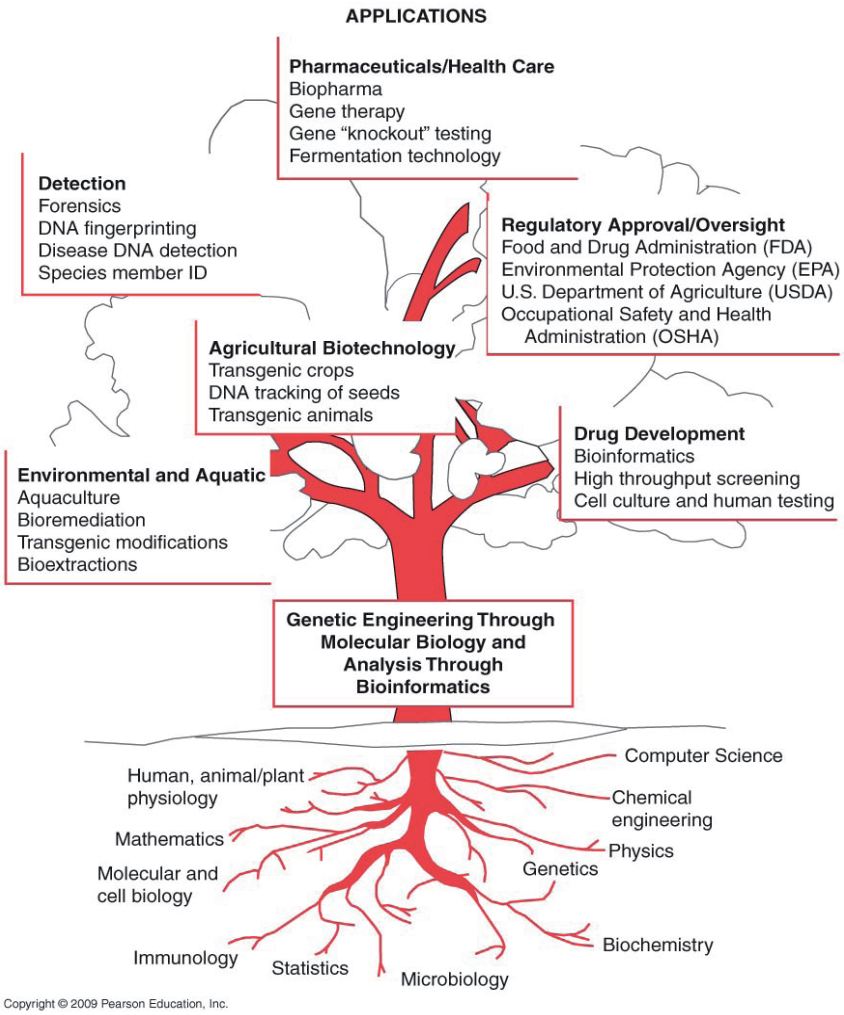


Figure 9: Applications of biotechnology

ACTIVITY 2.1.1

Biotechnology is a science of many disciplines.

2.1.1.1 Make a summary in which you discuss the scope covered by Microbial, Agricultural, Animal, Bioremediation and Aquatic Biotechnology in your **own words.** The summary must not exceed **two pages.**

2.1.1.2 Visit the "About Biotech" section of the Access Excellence website at www.accessexcellence.org/AB and find a topic on biotechnology, with sufficient content, that interests you, on which you can write a summary in your **own words.** The summary, including **diagrams,** must not exceed **two pages.**

Reflection on Activity 2.1.1

- Biotechnology involves teams made up of scientists from different disciplines, often working together on a specific project. Make use of the myUnisa website Biotechnology Bulletin Board to share your thoughts and participate in group discussions, particularly in the current context of Biotechnology issues in the environment.

Recommended reading

You might find it beneficial to investigate the following website:

http://www.eng-consult.com/BEN/papers/Paper-mizan.PDF.

This website provides a brief historical account of the development of biotechnology and subsequent applications in:

- **agriculture and food,** especially with regard to richer nutrient content and more attractive taste and flavour and the use of genetically modified (GM) bacteria to produce a variety of food substances.
- **medicine,** where GM microorganisms are producing human and animal antibodies, hormones, vaccines, enzymes, interferons and many other compounds needed for diagnosis and therapeutics.
- **Environment,** where GM microorganisms have been created to degrade environmentally obnoxious agents, including engine oil and heavy metals. Agricultural plants have been created to be resistant to common pests resulting in reduced use of chemical pesticides. GM bacteria might replace chemical fertilisers and thus reduce the environmental pollution.

2.1.2 The impact of biotechnology on society

ACTIVITY 2.1.2

Study the following excerpt which was taken from an Australian Biotechnology website:

http://www.biotechnology.gov.au/index.cfm?event=object.showContent&objectID=CDF6F671-BCD6-81AC-1022E789A37D0643

which, although no longer being updated, contains useful information and ideas pertaining to the following:

Arguments for and against gene technology

Gene technology refers to the direct alteration of the genetic material of living things so they may produce new or modified substances, or perform new or changed functions. As with any technology, gene technology carries with it risks as well as benefits. This fact sheet lists some of the current questions raised when considering gene technology, and summarises the key arguments for and against gene technology and its applications.

Is Gene Technology Natural?

The arguments for

- Gene technology is another step along the path of genetic improvement, which began with people selectively breeding plants and animals for desirable characteristics thousands of years ago.
- Many plants and animals have tens or hundreds of thousands of genes. Adding one or a few new genes using gene technology is a tiny change to the overall genetic makeup of a living thing. In contrast, conventional breeding technology and other methods of genetic modification (including mutagenesis by chemical or radiation treatment) involves the change and/or transfer of many genes. Gene technology allows for the specific manipulation of one or two genes. In contrast, conventional breeding technology and other methods of genetic modification (including mutagenesis by chemical or radiation treatment) involves the change and/or transfer of many genes in a less specific way.

The arguments against

- Gene technology is quite different from historical genetic modification techniques that involve breeding within a species or between very closely-related species. Gene technology enables the transfer of genes across species barriers, and this has virtually never happened before, even over evolutionary timescales.
- Species have evolved with their genes working together in complex systems. We cannot predict what might happen when an existing gene is broken up by the insertion of foreign genes into an organism, or what the effects of a foreign gene might be on other apparently unrelated genes or traits. Also, rapid alteration of a species by gene technology could have unforeseen consequences in ecosystems that are not adapted to the new version.

Will it affect my health?

The arguments for

- There are strict legal requirements that control the development, release and use of genetically modified organisms (GMOs) in Australia.
- The Office of the Gene Technology Regulator (OGTR) protects the health and safety of people and the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.

- Food Standards Australia New Zealand (FSANZ) carries out a thorough risk assessment of all food derived from or containing ingredients from GMOs before they can be sold. This includes examining whether the food has additional allergens or toxins as a result of the GM process.
- While genes that provide for antibiotic resistance have been used as markers in GM plants, the antibiotics involved are not usually those used in medicine. It is extremely unlikely that these genes could transfer from plants to bacteria as there are a number of barriers to such transfer. There are several common marker genes that do not confer antibiotic resistance.

The arguments against

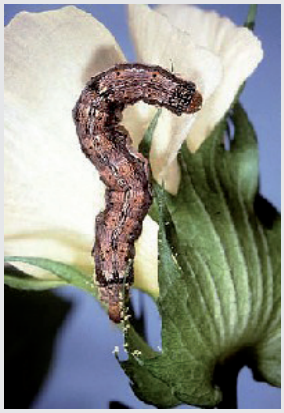
- Added genes could make 'safe' plants produce toxins or allergy-causing substances.
- GM foods are quite new, and there have been no studies of the long-term effects on human health.

Antibiotic resistance marker genes are a type of marker gene derived from bacteria. They provide the bacteria with natural resistance to particular antibiotics. They are sometimes used in GM plants to find out whether the added gene has been taken up by the plant. If such an antibiotic resistance gene moved from a GM plant to a bacterium that causes human disease, the antibiotic to which the marker gene provides resistance may no longer be useful for treating the disease.

- The use of GM crops and weed species under Australian conditions is actually very low. In addi-tion, precautionary steps, such as the use of 'buffer' zones around GM crops, can be used to further reduce the risks of gene transfer.
- Gene technology can produce crop plants that have genes from hardier plants added to them, allowing them to tolerate, for example, salinity, drought or poor soil.
- Gene technology may produce plants that are better at taking up soil nutrients and so do not need as much artificial fertiliser, reducing run-off to the environment.

How will gene technology affect the environment?

The arguments for



- GM crops such as insect-resistant Bt cotton reduce pesticide use by farmers and are therefore less harmful to the environment and to some beneficial insects than synthetic chemical insecticides. Bt cotton contains a gene from the bacterium *Bacillus thuringiensis*. This makes the cotton produce bacterial toxin in the plants, which acts as a pesticide designed to kill only target insect pests. Herbicide-tolerant crops allow farmers to spray less toxic herbicides than commonly-used herbicides.
- Herbicide-tolerant crops allow farmers more weed control choices, including in some cases to spray less toxic herbicides and employ conservation tillage practices which can decrease soil erosion and water loss.

- Transfer of herbicide tolerance to weeds is a concern not only with some GM crops, but also with conventionally-bred crops.

The arguments against

- Bt pesticides may 'leak' out of GM plant roots, harming non-target insects and soil microbes in addition to the pests they are designed to kill.
- Farmers will still be faced with pests becoming resistant to pesticides, even if pesticides are engineered into crops. Therefore, GM crops do not offer much advantage over existing ways of dealing with pests.
- Some herbicide-tolerant plants may transfer their tolerance to related plants, creating 'superweeds' that are not easily controlled by environmentally friendly herbicides. This would increase the use of more toxic herbicides.
- The use of herbicide-tolerant crops encourages farmers to use more herbicide because the risk of killing post-emergent crops is reduced. This will have a damaging effect on the environment. In addition, the companies that produce herbicide-tolerant crops often manufacture the corresponding herbicide, which locks farmers into a single supplier.
- GM plant varieties may not be 'stable' in the long-term. Their gene combinations may be more susceptible to sudden changes.
- Growing GM crops that are more tolerant to drought or poor soils may encourage farmers to encroach on lands not suited to agriculture, resulting in more environmental damage than leaving these lands unused.

Are there markets for GM crops?

The arguments for

- It appears that yields from GM crops are generally higher, so a farmer may earn more from the same crop area.
- Research shows that most export markets for Australia (such as Japan) are prepared to buy GM crops.
- Markets that ban GM products can be replaced with those that tolerate low levels of GM content, e.g. Canada's GM canola is now sold to Japan rather than to the European Union (EU).
- To date, GM-free crops have not generally commanded a higher market price than GM crops.

The argumenst against

- Australia's 'clean, green' image may be harmed by the introduction of GM crops.

- Many countries, such as in the EU, do not want to buy GM products so farmers may lose markets if they grow GM crops. Farmers may also be able to get a price premium in some markets if they can certify that their crops are GM-free.
- One hundred per cent segregation of GM and non-GM products will be difficult to achieve and the costs may outstrip any potential benefits to farmers from GM crops.

The costs of identity preservation processes may also deter buyers of GM products.

Will genetically modified crops help feed the world?

The arguments for

- Gene technology has the potential to develop plants that are more nutritious and yield bigger harvests than conventional plants and are at the same time more resistant to diseases and to stresses like drought. One example is golden rice, modified to contain the molecule from which the body forms vitamin A. The vitamin, which is often deficient in people with poor diets, is essential for good health and prevents blindness.
- Gene technology may help produce animals that are more productive or resistant to parasites and diseases, thus improving livestock quality in developing nations.
- GM plants that can grow in poor soils will enable countries with poor lands to be able to grow more of their own food and reduce land-clearing.

The arguments against

- Feeding the world is more to do with politics, economics and population than hi-tech developments. Poor countries often cannot afford to buy from the food surpluses in rich countries. Also, poorer nations are often encouraged to clear land and grow cash crops and animals for export, rather than subsistence crops to feed their population. GM crops will not alleviate these issues.
- GM crops such as 'golden' rice may be considered an unnecessary way to achieve the same result as, for example, programs to grow pumpkins in communities, to provide vitamin A as well as many other nutrients.

Poor farmers will not be able to afford the GM seeds and related herbicides they need from multinational companies, who are primarily interested in making a profit.

Will GM technology benefit us?

The arguments for

- Farmers may benefit because of the potential for increased yields of crops, healthier animals, fewer diseases and lower costs of production.
- There could be some environmental benefits because of the reduction in the use of toxic chemicals, as well as the potential to grow more on less land.
- Humans and animals will benefit through better quality, more specific drugs to treat diseases and genetic conditions.

- Multinational companies have already freely shared some of their GM technology with developing nations.

The arguments against

- As with introduced exotic species, once GM crops and animals are out in the environment they cannot be recalled; they may cause ecosystem changes that cannot be reversed.
- Farmers could be tied in to multinational companies selling patented seed and associated chemicals at greater cost. Farmers are already being sued by multinationals for allegedly growing GM crops on their land without a licence.
- Multinationals will increasingly own intellectual property in agriculture, thus dominating world markets.
- Increased costs for segregation of crops and animals will probably lead to higher prices for consumers.

Reflection on Activity 2.1.2

- Briefly review the various arguments for and against the various issues raised in the textbox of this study guide pp.64–71, and indicate which point of view you support. Provide reasons why you support a specific viewpoint. In order to do this properly, you will need to make use of "Google Scholar" to find more information under the various headings in the textbox. The answer must not exceed **three pages.**

Biotechnology

STUDY SECTION 2.2: A general perspective of specific impacts of biotechnology in the biosphere

2.2.1 The impact of biotechnology in industry and agriculture

Plant biotechnology

Agriculture: The next revolution

Plant transgenesis refers to the direct transfer of genes to plants and is used for the development of plant vaccines, or where plants end up producing their own pesticides or develop resistance to herbicides.

Cloning of transgenic plants involves gene gun technologies as well as protoplast fusion, leaf fragmentation, genetic manipulation of chloroplasts and creation of antisense RNA.

Methods used in plant transgenesis

Although conventional selective breeding and hybridisation programmes using sexual crossing between two lines and repeated backcrossing between hybrid offspring and parent have been used to transfer whole chromosomes rather than single genes, **cloning** and the use of Ti plasmids have become the norm.

Cloning involves growing plants from a single cell, which has been suitably "engineered" via **protoplast fusion** from cells of different species. A protoplast cell is a callus cell whose cell wall has been dissolved by the enzyme cellulose, so that fusion of the two protoplast cells creates a new cell that can grow into a hybrid plant, such as the broccoflower. Cloning via the **leaf fragment technique** involves small discs being cut from a leaf and subsequently cultured in a medium containing genetically modified *Agrobacter (Agrobacterium tumefaciens)*. This is a soil bacterium that infects plants and which contains a plasmid, known as the Ti plasmid, that can be genetically modified to integrate with DNA of the host cell. Leaf discs are then treated with plant hormones to stimulate shoot and root development.

Figure 10 shows how the transfer of genetically modified Ti plasmid to susceptible plants through tissue culture takes place.

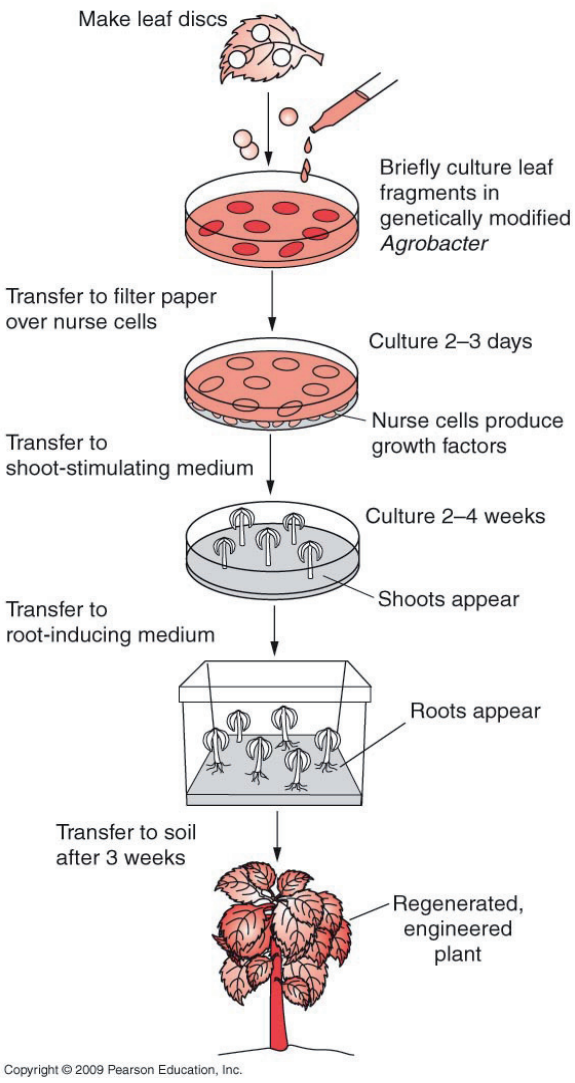


Figure 10: Transfer of genetically modified Ti plasmid

Gene guns are used to blast tiny metal beads coated with DNA into an embryonic plant cell, specifically at the nucleus or the chloroplast of the cell. Marker genes coding for antibiotic resistance are used to distinguish between genetically transformed cells.

Chloroplast engineering is useful, not only because the DNA in a chloroplast can accept several new genes at once, but also a high percentage of genes will remain active. In addition, the DNA in chloroplasts is completely separate from DNA released in pollen, so there is no chance that transformed genes will be carried on wind to distant crops.

Antisense technology involves a process of inserting a complementary copy of a gene into a cell. Specifically, a gene which encodes an mRNA molecule, called an antisense molecule, binds to normal mRNA (sense molecule) and inactivates it, such as is the case with the *Flavr Savr* tomato.

The rate at which tomatoes rot is controlled by an enzyme known as polygalactose (PG). By using antisense technology, where a complementary strand of DNA (cDNA) which encodes for this enzyme, is produced and inserted into other tomato plants (transgenic plants) via a suitable vector, the activity of the PG gene complex can be 'switched off'

with antisense mRNA, coded for by the inserted cDNA. This slows the rotting process and results in a tomato that usually lasts for three weeks after ripening.

Figure 11 summarises the procedure.

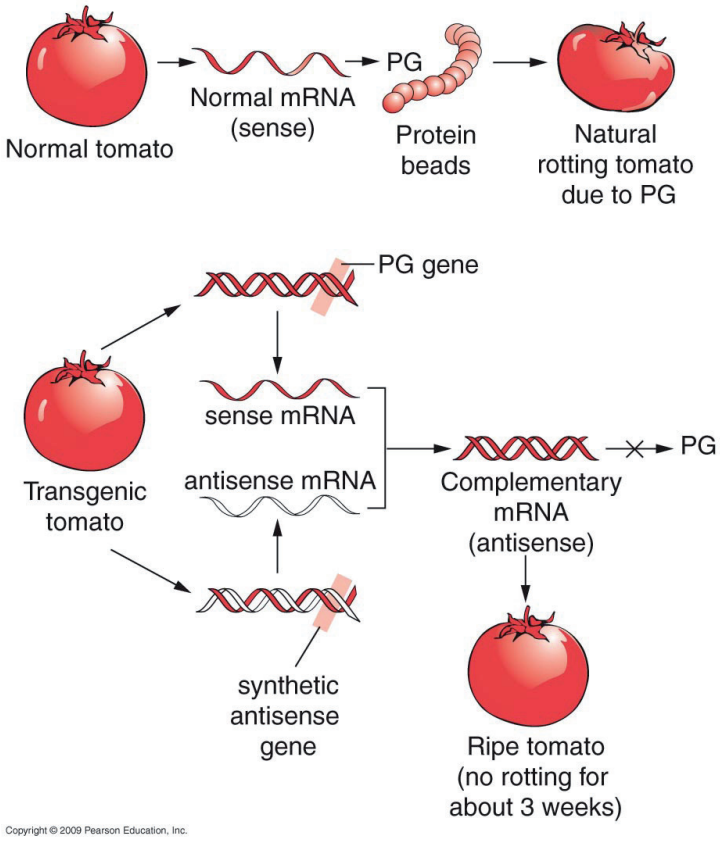


Figure 11: Summary of antisense technology to delay ripening of tomatoes

Transgenic plants expressing the TMV coat protein (CP) were produced by *Agrobacterium* mediated gene transfer. When the generated plants expressing the viral CP were infected with TMV, they exhibited increased resistance to infection. Whereas control plants developed symptoms within three to four days, transgenic CP-expressing plants resisted infection for 30 days.

Figure 12 summarises this procedure.

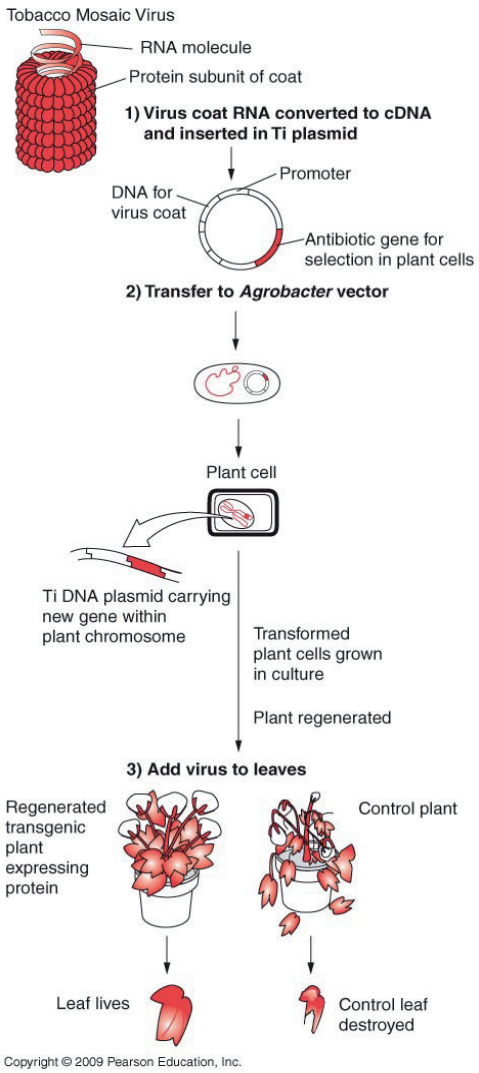


Figure 12: Summary of procedure resulting in transgenic plants expressing TMV coat protein

With regard to the engineering of **genetic pesticides,** Bt genes from *Bacillus thuringiensis* (Bt), a bacterium that produces a protein that kills harmful insects and their larvae, can be inserted into a plant's DNA. This process creates a built-in defense against certain insects. In a similar manner, transgenic corn can expresses avidin, making them highly resistant to pests, such as the Monarch Butterfly, during storage. Avidin blocks the availability of biotin, a vitamin required by insects to grow.

Herbicide resistance involves the modification of crops to be resistant to common herbicides, thereby allowing farmers to control weeds with chemicals that are milder and more environmentally friendly than typical herbicides.

Genetic modification for stronger fibres allows for increasing the strength of one variety of cotton by 60%, which also results in softer, more durable clothes for consumers and greater profits for farmers.

Genetic modification for enhanced nutritional potential, such as in the case of Golden Rice, has resulted in a foodstock containing large amounts of beta carotene, which the body converts to vitamin A.

In general, future applications could include some kinds of plants becoming ideal protein factories to grow medicines, including human vaccines, antibodies and insulin, as well as plant-based petroleum for fuel, alternatives to rubber, nicotine-free tobacco, caffeine-free coffee, biodegradable plastics and stress-tolerant plants. In addition, metabolic manipulation of the biochemistry of certain plants could result in the production of a variety of other products, including alkaloids, lipids, polyterpenes, pigment production, and biodegradable plastics.

However, despite all these future developments, there are opponents to this new technology that fear the effects of foreign genes. Examples include bits of DNA not naturally found in plants which might cause allergic reactions, or antibiotic-resistance marker genes that could spread to disease-causing bacteria in humans, or unexpected carcinogens could be introduced into the diet. Similarly, from an environmental perspective, genes for pest or herbicide resistance could spread to weeds. In order to address these concerns, a number of regulatory bodies exist in the USA, such as the FDA which regulates foods on the market, the USDA, which oversees growing practices and the EPA, which controls use of Bt proteins and other pesticides.

Study the section on Methods Used in Plant Transgenesis from page 157 to 159, as well as Practical Applications in the Field from page 161 to 170 in the prescribed textbook.

Refer to text and Figure 6.2 Protoplast Fusion and Regeneration of a Hybrid Plant p.158, Figure 6.3 Process of Crown Gall Formation in Plants p.159, Figure 6.4 Transfer of Genetically Modified Ti Plasmid to Susceptible Plants Through Tissue Culture p.159, Figure 6.5 Gene Guns p.160 of the prescribed textbook, before doing Activity 2.2.1.1.

ACTIVITY 2.2.1.1

Plant transgenesis, or the technologies employed in the direct transfer of genes to plants, is considered to be an integral part of the next revolution in Agriculture.

2.2.1.1.1 Make a summary in which you describe the methods used in plant transgenesis in your **own words.** Pay particular attention to protoplast fusion, leaf fragment technique and gene guns. The summary, **including diagrams,** must not exceed **three pages.**

As a result of the development of plant transgenesis technologies, a number of practical field applications are contributing to the revolution in agriculture.

2.2.1.1.2 Make a summary in which you discuss the practical field applications in plant biotechnology in your own words. Pay specific attention to genetic pesticides and herbicide resistance, enhanced nutrition and fuel. The summary, **including diagrams,** must not exceed **three pages.**

Reflection on Activity 2.2.1

- Review **all 10** Q&A activities on page 170 and make sure you are able to answer these questions in an exam situation. Please consult Appendix 1 of the textbook for model answers to these questions.

ADDITIONAL REFERENCE MATERIAL FOR BACKGROUND READING

RNAi-mediated crop protection against insects

Daniel R.G. Price and John A. Gatehouse

Trends in Biotechnology Vol.26 No.7 (2008) 393–400

Downregulation of the expression of specific genes through RNA interference (RNAi), has been widely used for genetic research in insects. The method has relied on the injection of double-stranded RNA (dsRNA), which is not possible for practical applications in crop protection. By contrast, specific suppression of gene expression in nematodes is possible through feeding with dsRNA. This approach was thought to be unfeasible in insects, but recent results have shown that dsRNA fed as a diet component can be effective in downregulating targeted genes. More significantly, expression of dsRNA directed against suitable insect target genes in transgenic plants has been shown to give protection against pests, opening the way for a new generation of insect-resistant crops.

The insect gut is divided into three regions; foregut, midgut and hindgut. Of these, the first two are continuations of the 'outside' of the insect and are chitin-lined, so that their surfaces do not present areas of exposed cells (although receptors and transporters are present to allow processes such as taste recognition in the mouth cavity and water transfer in the hindgut to occur). The midgut region is the only part of the gut that contains surfaces of exposed cells, and it is the main site of exchange between the circulatory system (haemolymph) and the gut contents. The midgut itself is responsible for nutrient absorption, whereas excretion and water balance take place primarily in the Malpighian tubules attached to the hind end, which carry out a function similar to that of the kidney in higher animals. RNAi effects occurring in insects as a result of oral delivery of dsRNA are presumably mediated by the midgut surfaces through exposure of cells of the midgut epithelium and the Malpighian tubules to dsRNA in the gut contents as shown in Text Figure 13.

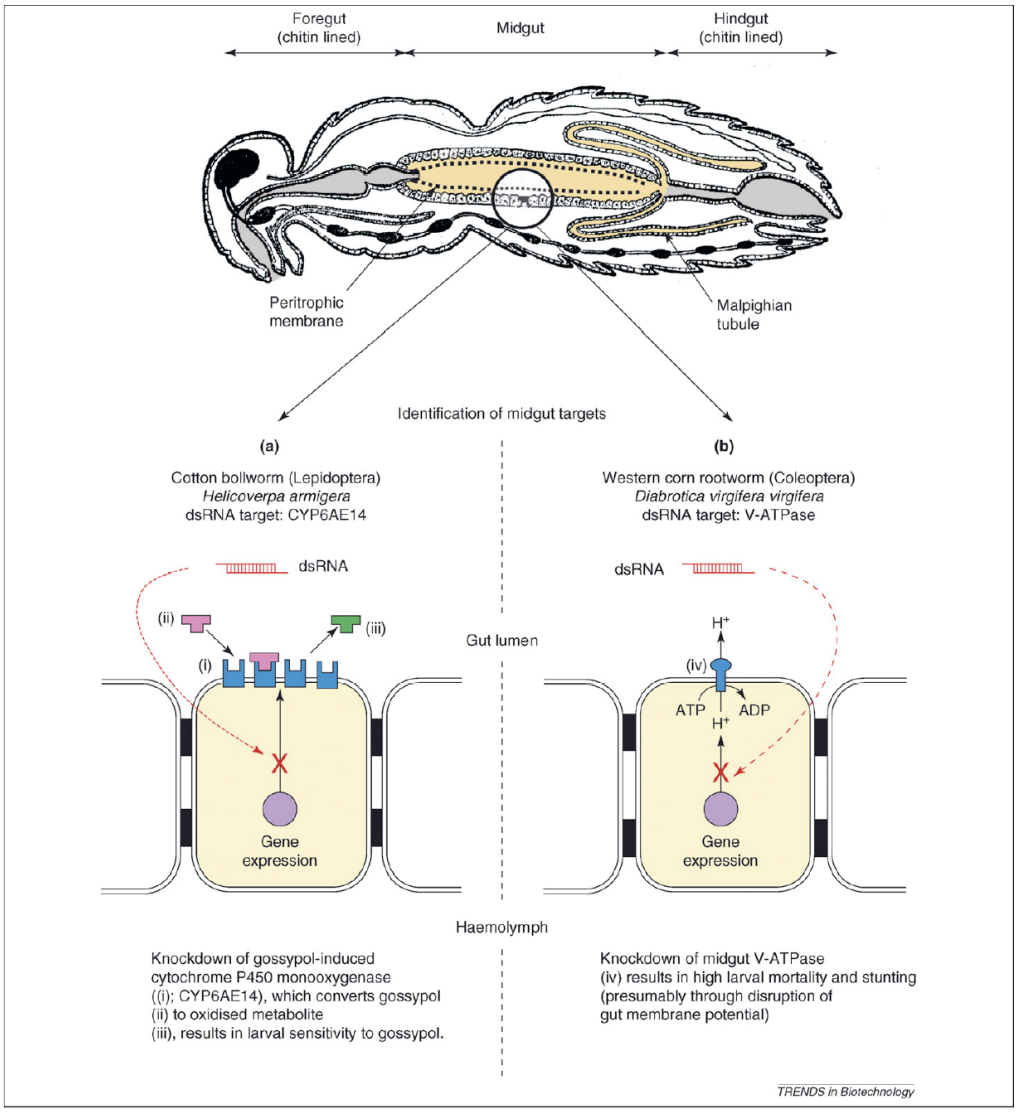


Figure 13: Identification of midgut targets in insects

Figure 13. Overview of RNAi approaches for insect-resistant transgenic plants. Double-stranded RNA (dsRNA) produced *in planta* can lead to targeted gene silencing in Lepidoptera and Coleoptera pest species. dsRNAs corresponding to specific insect targets are expressed *in planta* and are cleaved by endogenous plant Dicer enzymes to produce short interfering RNAs (siRNAs) of around 21 nucleotides. Large dsRNA and siRNA cleavage products are expressed throughout plant tissues and are orally delivered to insect herbivores feeding on transgenic plant material. For gene-silencing to initiate in targeted insect pests, large dsRNAs and siRNAs must persist in the insect gut, and sufficient quantities must be present for uptake into cells in contact with RNAs (the exact uptake mechanism in target insects remains unknown). Approach (a): a gut-specific cytochrome monooxygenase, CYP6AE14, has been identified (i) whose expression correlates with larval growth on diets containing gossypol (ii), a cotton secondary metabolite. CYP6AE14 is presumably involved in detoxification of gossypol (iii) because specific knockdown of this gene product by dsRNAs delivered in artificial diet and by transgenic plant material increases larval sensitivity to gossypol. Approach (b): a related study used a screening approach to identify a lethal phenotype in *Diabrotica virgifera* when midgut V-type ATPase A (V-ATPase) (iv) was downregulated by dsRNAs delivered in artificial diet feeding trials and transgenic corn. Although no

direct evidence was presented for the deleterious effects observed in larvae, it is tempting to speculate that knockdown of V-type ATPase A results in disruption of electrochemical gradient across the gut epithelia, which results in high larval mortality.

Recommended reading

You might find it beneficial to investigate the following websites:

Biotechnology: the gene revolution

http://www.biodevelopments.org/ip/ipst2hr.pdf

This website analyses biotechnological crop improvement in an agro-ecosystem rather than just the plant species. They consider that what might be considered valuable biotechnology in the industrial world is not necessarily directly beneficial to the developing world, and vice versa. A good example is vitamin A rice, or so-called Golden Rice. This rice is genetically engineered to produce vitamin A, which gives the rice grains a light gold colour. In many areas of the world where rice is a basic staple, thousands of poor people every year lose their eyesight because of vitamin A deficiency. This new rice variety could deliver vitamin A directly to the poor in their staple food. Vitamin A deficiency is not a problem in the industrial world, but it is obviously of great benefit to Asia's rural poor.

This article also considers genetic engineering for pest and disease resistance, cell and tissue culture techniques, molecular-aided analyses and DNA markers.

Plant resistance towards insect herbivores

http://www3.interscience.wiley.com/cgi-bin/fulltext/118909139/PDFSTART

Considers plant defences against insect herbivores both locally and systemically by signaling pathways involving systemin, jasmonate, oligogalacturonic acid and hydrogen peroxide.

Endophytic MO's vs insect resistance

http://www.scielo.cl/pdf/ejb/v3n1/a04.pdf

In this review, the authors address the major topics concerning the control of Insects pests by endophytic microorganisms. Several examples of insect control are described, notably those involving the interactions between fungi and grazing grasses from temperate countries. The mechanisms by which endophytic fungi control insect attacks are listed and include toxin production as well as the influence of these compounds on plant and livestock and how their production may be affected by genetic and environmental conditions.

Transformation of crops for insect resistance

http://pdfserve.informaworld.com/448004_751308860_713610871.pdf

Transgenic resistance to insects has been demonstrated in plants expressing insecticidal genes such as δ-endotoxins from *Bacillus thuringiensis* (*Bt*), protease inhibitors, enzymes, secondary plant metabolites, and plant lectins. Since transgenic plants with introduced *Bt*

genes have been deployed in several crops on a global scale, the alternative genes have received considerably less attention. The protease inhibitor and lectin genes largely affect insect growth and development and, in most instances, do not result in insect mortality. The effective concentrations of these proteins are much greater than the *Bt* toxin proteins.

Co-evolution and plant resistance to natural enemies

http://www.biology.duke.edu/rausher/evgen/reprints/Nature2001.pdf

Co-evolution between plants and their natural enemies is generally believed to have generated much of the Earth's biological diversity. A process analogous to co-evolution occurs in agricultural systems, in which natural enemies adapt to crop resistance introduced by breeding or genetic engineering. Because of this similarity, the investigation of resistance mechanisms in crops is helping to elucidate the workings of co-evolution in nature, while evolutionary principles, including those derived from investigation of co-evolution in nature, are being applied in the management of resistance in genetically engineered crops.

Insect resist vs transgen v strophic levels

http://www3.interscience.wiley.com/cgi-bin/fulltext/118957038/PDFSTART

As food web components, the authors distinguish target herbivores, non-target herbivores, pollinators, parasitoids and predators. Below-ground organisms such as Collembola, nematodes and earthworms should also be included in risk assessment studies, but have received little attention. The toxins produced in Bt plants retain their toxicity when bound to the soil, so accumulation of these toxins is likely to occur. Earthworms ingest the bound toxins, but are not affected by them. However, earthworms may function as intermediaries through which the toxins are passed on to other trophic levels. In studies where effects of insect-resistant (Bt) plants on natural enemies were considered, positive, negative and no effects have been found. So far, most studies have concentrated on natural enemies of target herbivores.

Phytoremediation of soil pollutants

http://www.springerlink.com/content/yay8urhcnbvh7nqu/fulltext.pdf

This is a more technical article dealing with various aspects of phytoremediation, including *rhizodegradation*, where the contaminant is transformed by microbes in the rhizosphere (which means the microbe-rich zone is in intimate contact with the vascular root system of the plant), *contaminant metabolism during phytoremediation in plants*, where organic compounds can be translocated to other plant tissues and subsequently volatilised and *phytoremediation with genetically engineered plants*. In the latter instance, several questions concerning the effect of introduced genetically modified plants arise whenever the intended introduction differs substantially from that with an established record of safety. The release of transgenic plants into the environment will depend on a number of factors, including their familiarity, the nature of the genetic modification, the ability to confine plants, and the perceived environmental impact.

Bioaugmentation as remediation technology

http://pdfserve.informaworld.com/944601_751308860_713903278.pdf

Bioaugmentation is commonly employed as a remediation technology. Several new approaches may increase the persistence and activity of exogenous microorganisms and/or genes following introduction into the environment. These techniques include:

(1) bioaugmentation with cells encapsulated in a carrier such as alginate;

(2) gene bioaugmentation where inoculants are added to transfer remediation genes to indigenous microorganisms;

(3) rhizosphere bioaugmentation where the microbial inoculant is added to the site along with a plant that serves as a niche for the inoculant's growth; and

(4) phytoaugmentation where the remediation genes are engineered directly into a plant for use in remediation without a microbial inoculant.

Animal biotechnology

Genetically engineered animals can be used to develop new medical treatments, improve our food supply as well as enhancing our understanding of all animals as well as human beings. However, this technology presents tough scientific and ethical challenges.

The use of animals in research

Animal models: many genetic and physiological similarities exist between animals and humans, which have allowed many medical breakthroughs to take place in the past century, including the polio vaccine, cataract surgery techniques and kidney dialysis.

FDA regulations in the USA state that new drugs, medical procedures, and cosmetic products must pass safety tests which involve phase testing, where a significant number of trials have to be conducted on cell cultures, in live animals, and on human research participants. Purebred mice and rats are most often used, followed by Zebrafish, fruit flies and nematodes.

Dogs, monkeys, chimpanzees and cats make up less than 1% of the total number of research animals. Alternatives to animal models include cell culture (mainly to check for toxicity of substances) and computer-generated models (mainly to simulate specific molecular and chemical structures and their interactions).

The use of animals in research is regulated, especially where funding is concerned. Researchers must follow standards of care set out in documents, such as *The Guide for the Care and Use of Laboratory Animals*.

Cloning technology

This is a technology where the donor cell must come from a living organism. Cloning usually begins with removal of the egg nucleus from a fertilised egg and replacement with another nucleus (possibly from an adult animal like a cow). If the process is successful,

then desirable characteristics can be obtained with a high degree of certainty. Success is not always easy, particularly if it takes 278 days to find out if the experiment worked!

As such, clones are not exactly identical and the present success rate is quite low. The sheep Dolly was the result of 277 efforts to implant a cloned embryo. Cloning is not a practical solution to transplant organ shortages and in some cases there are profound ethical questions. In future, clones may provide a unique window on the cellular and molecular secrets of development, ageing, and diseases, as well as sustaining breeding populations of endangered species.

New genetic material can be introduced into animals via **retrovirus-mediated transgenics,** where mouse embryos can be infected with retroviruses before the embryos are implanted. **Pronuclear microinjection** involves the introduction of transgene DNA at the earliest possible stage of development of the zygote, where DNA is injected directly into the nucleus of the egg or sperm.

Figure 14 summarises the procedure.

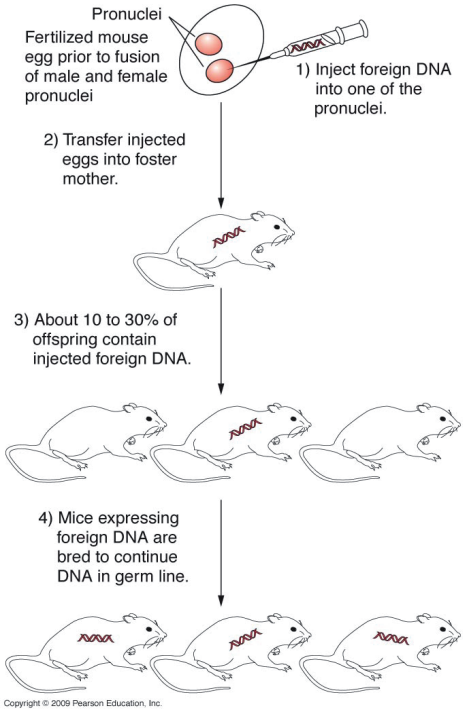


Figure 14: Process Flow for retrovirus-mediated transgenics

Transgenic animals have been used as **bioreactors,** where whole animals can serve as bioreactors to produce milk or eggs which are enriched with the required proteins.

Genetic knockout technology involves a special case of transgenics for example, mice that have been genetically engineered so that a specific gene is disrupted.

DNA is modified and added to the embryonic stem (ES) cells, where it recombines with the existing gene on a chromosome, called homologous recombination. Modified ES cells are introduced into normal embryo and embryo is implanted into a mother.

The mouse pup is a **chimera –** some cells are normal and some are knockouts. Two generations of breeding are required to produce complete knockouts, where some have a human gene inserted to replace their own counterpart.

Study the section on Transgenic Animals from page 180 to 186 in the prescribed textbook.

Refer to Figure 14 p.48 of this study guide (Reference Figure 7.9 Pronuclear Microinjection Cloning) as well as Figure 7.10 depicting human-like plasma from cattle in the prescribed textbook before starting Activity 2.2.1.2.

ACTIVITY 2.2.1.2

2.2.1.2.1 Make a summary in which you explain in your **own words** the various methods used in creating transgenic animals. Pay particular attention to retrovirus-mediated transgenics, pronuclear microinjection, embryonic stem cell method and sperm-mediated transfer. The summary, **including diagrams,** must not exceed **three pages.**

As a result of the development of transgenic animal technologies, a number of animal bioreactor systems are currently in use.

2.2.1.2.2 Make a summary in which you describe in your **own words** three practical applications of this technology in the pharmaceutical and related industries. The summary must not exceed **one and a half pages.**

2.2.1.2.3 Discuss some of the ethical concerns, including their justifications, about using animals in research in your **own words.** Your answer must not exceed **one and a half pages.** Refer to section 7.2 Animals in Research pp.172–176 in the prescribed textbook.

Recommended reading

You might find it beneficial to investigate the following websites:

Global diffusion of plant and animal biotechnology

http://www.economia.uniroma2.it/conferenze/icabr2005/papers/Runge_Ford_paper.pdf

Broadly defined, biotechnology is a wide array of technologies that includes techniques that use living organisms or substances from these organisms to make or modify a biological product or to improve plants, animals, or microorganisms for specific uses. It can be divided into two major categories: molecular genetics and genetic engineering.

Molecular genetics focuses on the use of molecular markers and genetic fingerprinting to allow us to identify the presence of specific genes already present in an organism that govern traits of interest. Genetic engineering involves the insertion of native or foreign gene(s) into a host organism (microorganism, plant or animal) in order to increase the value or usefulness of the organism. Products of genetic engineering are called genetically modified organisms (GMOs).

Transgenic animals versus human health versus animal production

http://www3.interscience.wiley.com/cgi-bin/fulltext/118659503/PDFSTART

Transgenic animals are more widely used for various purposes. Applications of animal transgenesis may be divided into three major categories: (i) to obtain information on gene function and regulation as well as on human diseases, (ii) to obtain high value products (recombinant pharmaceutical proteins and xeno-organs for humans) to be used for human therapy, and (iii) to improve animal products for human consumption. All these applications are directly or not related to human health.

Transgenesis versus farm animal welfare

http://jas.fass.org/cgi/reprint/79/7/1763

Evidence is discussed showing that treatments imposed in the context of farm animal transgenesis are by no means biologically neutral and may compromise animal health and welfare. Factors posing a risk for the welfare of transgenic farm animals include integration of a transgene within an endogenous gene with possible loss of host gene function (insertional mutations), inappropriate transgene expression and exposure of the host to biologically active transgene-derived proteins, and in vitro reproductive technologies employed in the process of generating transgenic farm animals that may result in an increased incidence of difficult parturition and fetal and neonatal losses and the development of unusually large or otherwise abnormal offspring (large offspring syndrome).

Ethics of animal genetic manipulation

http://www3.interscience.wiley.com/cgi-bin/fulltext/118950559/PDFSTART

The agricultural biotechnology industry uses transgenic research in pursuit of quantitative and qualitative changes in animal products. Potential quantitative changes include more milk, more meat and more wool, while potential qualitative changes include altered milk composition (for example, to make cow's milk more suitable for human babies), leaner meat and pest-resistant wool. Truly novel uses of transgenic animals are also under development. For example, transgenic animals as 'bioreactors' are able to produce human proteins. Such proteins, produced in the milk, have potential medical uses. Another example is research aimed at producing transgenic animals with human-compatible organs for human transplantation, referred to as "xenotransplantation").

This article also deals with MORAL OBJECTIONS TO TRANSGENESIS and considers the following:

1. Transgenesis is objectionable, because it is intrinsically wrong to deliberately alter genetic sequences.

2. Transgenesis is objectionable, because it may lead to the genetic manipulation of humans.

3. Transgenesis is objectionable, because it necessitates the killing of animals.

4. Transgenesis is objectionable, because it involves the infliction of suffering on animals.

2.2.2 The impact of biotechnology in nutrition and health

Aquatic biotechnology

Aquaculture involves increasing the world's food supply through biotechnology and as such involves the cultivation of aquatic animals and aquatic plants for recreational or commercial purposes.

Molecular probes and PCR-based assays are being developed for detecting bacteria, viruses, and a host of parasites that infect finfish and shellfish. Disease often results due to difficulty in maintaining water with the proper flow rate to deliver adequate concentration of nutrients and to remove accumulating waste products.

In future, this biotechnology will be used to increase growth and productivity, as well as improving disease resistance. This will require a basic knowledge of gene expression and regulation in aquatic organisms, as well as understanding the genes involved in processes such as reproduction, growth, development, and survival at extreme conditions. Specifically, transgenic fish and plants could be bred with enhanced resistance to cold temperatures and freezing.

The use of "Green Genes" which code for green fluorescent protein (GFP), as well as bioluminescence (red, yellow, and orange) genes have been used to create **reporter gene** constructs to allow for detection of the expression of genes of interest.

Figure 15 (Reference on page 245 The GFP Gene is a Valuable Reporter Gene of the prescribed textbook) describes how DNA coding for green fluorescent protein (GFP) originally isolated from a bioluminescent jellyfish, *Aequorea victoria*, can be ligated (connected) to other genes of interest. If these hybrid genes are introduced into suitable receptive cells, subsequent expression of these hybrid genes will generate a recombinant or fusion protein that can fluoresce in UV light. Only cells producing the GFP fusion protein fluoresce.

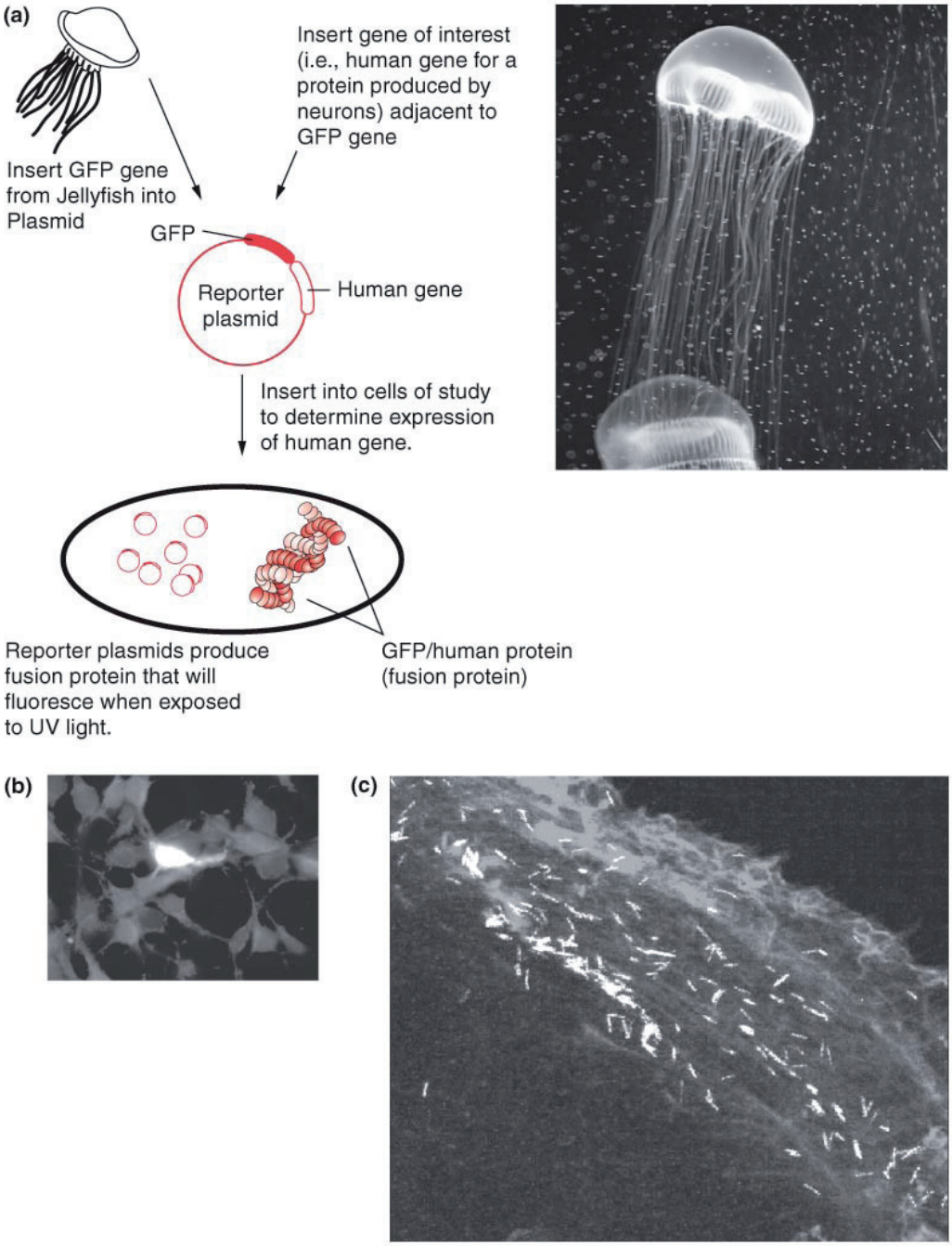


Figure 15: Ligation of GFP into fusion proteins

Medical applications of aquatic biotechnology

A large number of marine species contain or are suspected to contain compounds of biomedical interest, such as antibiotics, antiviral molecules, anticancer compounds, and insecticides.

Nutritional applications

Chemical energy can be harvested from biomass such as seaweeds, marine grasses and planktons. Algae may be valuable for expressing recombinant proteins as part of a food bioprocessing initiative.

Environmental applications of aquatic biotechnology

Biofilming (biofouling) involves the attachment of organisms (for example, barnacles, algae, mussels, clams, and bacteria) to surfaces, such as hulls of ships, inner lining of pipes, cement walls, and pilings used around piers, bridges, and buildings.

Traditional antifouling agents employed toxic chemicals, such as copper-rich or mercury-rich paints. Marine organisms use natural antifouling agents to protect their own surfaces from biofilms. In addition, these organisms can also be used as biosensors and also in environmental remediation.

Study the section on Molecular Genetics of Aquatic Organisms from page 242 to 246 in the prescribed textbook.

Refer to Figure 10.5 p.244 depicting gene expression in transgenic fish and Figure 10.7 p.245 illustrating the connection of the GFP gene from jellyfish to other genes of interest plus related text on pp.242–246 before starting Activity 2.2.2.1.

ACTIVITY 2.2.2.1

An understanding of the molecular complexities of aquatic organisms is central to understanding the methodologies and applications of aquatic biotechnology.

2.2.2.1.1 Make a summary in which you discuss the discovery and applications of novel genes from aquatic organisms in your own words. Pay particular attention to anti-freeze proteins and "green genes". The summary, **including diagrams,** must not exceed **three pages.**

Sustained dumping of industrial and related effluent in the world's oceans has resulted in serious pollution issues that affect fish stocks, marine organisms and the environment in general.

2.2.2.1.2 Make a summary in which you discuss the environmental applications of aquatic biotechnology in your own words. (Refer to section 10.6 pp. 256–259 of the prescribed textbook.) Pay particular attention to biofilms, biosensors and aquatic environmental remediation issues. The summary must not exceed **one and a half page.**

Reflection on Activity 2.2.2.1

- Review questions 3 and 9 of the Questions and Activities on page 259, making sure that you can answer similar questions in the exams according to the examples given in Appendix 1 of the textbook.

Recommended reading

You might find it beneficial to investigate the following websites:

Biotechnology of microalgae

http://www.springerlink.com/content/gbbkaptetj1n76h3/fulltext.pdf

The idea to genetically engineer microalgae for example, to increase their content of valuable compounds, is very tempting. Because of the usual absence of cell differentiation, microalgae represent a much simpler system for genetic manipulations compared with higher plants. In addition, allelic genes are usually absent because of the haploid nature of most vegetative stages of microalgae. Diatoms are especially interesting for biotechnology because they have the physiological potential to accumulate high proportions of lipid. Improving the oil-producing characteristics of these algae naturally is a prime target of genetic engineering.

In the area of **human nutrition,** functional food or nutraceuticals produced with microalgal biomass are sensorily much more convenient and variable, thus combining health benefits with attractiveness to consumers. The market of functional foods is believed to be the most dynamic sector in the food industry and could constitute up to 20% of the whole food market within the next few years. Food supplemented with microalgal biomass might have other positive influences, e.g., prebiotic effects or mineral fortification.

In the field of **medicine development,** cytotoxic activity is important in anticancer medicine. Antiviral activities have been found mainly in cyanobacteria. However, these are also present in apochlorotic diatoms and the conjugaphyte Spirogyra, where certain sulfolipids are active, for example, against the herpes simplex virus. Antimicrobial activity is also under investigation to find new antibiotics. Although the success rate is about 1%, there seem to be some promising substances from microalgae, for example, the cyanobacterium Scytonema. Antifungal activity is found in different extracts of cyanobacteria while antihelmintic effects are known for Spirogyra and Oedogonium algae.

Finally, a scheme for CO2 fixation from industrial exhaust gas by microalgae, is also presented.

Microalgae and cyanobacteria

http://www3.interscience.wiley.com/cgi-bin/fulltext/119393484/PDFSTART

In this article, the authors review data on the use of cyanobacteria and microalgae in human nutrition and searched for available information on legislature that regulates the use of these products. They have found that, although the quality control of these products is most often self-regulated by the manufacturers, different governmental agencies are introducing strict regulations for placing novel products, such as algae and cyanobacteria,

on the market. The existing regulations require these products to be tested for the presence of toxins, such as microcystin. However, other, sometimes novel, toxins remain undetected, and their long-term effects on human health remain unknown.

STUDY SECTION 2.3: The origins of biotechnology

2.3.1 Biotechnology has its roots embedded in bio-chemistry, physiology, genetics, microbiology and molecular biology

ACTIVITY 2.3.1.1

Read through the following excerpt taken from:

Genetic engineering and biotechnology

Scientific convergence in the birth of molecular biology, 2006, By: Sunny Y. Auyang

http://www.creatingtechnology.org/biomed/dna.pdf

"Molecular biology, then, is the study of how DNA, RNA, and protein are interrelated," summarized David Baltimore in his forward to *Nobel Lectures in Molecular Biology*.

In this sense, "molecular biology" refers to a focused science. It is narrower than the casual sense of the phrase meaning the study of life at the molecular level, which applies to most if not all areas in life science. Only the narrow sense is used here. Knowledge gained in molecular biology may contribute to embryology, physiology, and other scientific areas, but these are not part of molecular biology as far as we are concerned. Organisms come in dazzling varieties, but chemically they are rather homogeneous. Of the millions of chemical compounds available, they are made up of only water, a few hundred kinds of small molecules, and four classes of large macromolecules. A macromolecule is a long chain of linked small molecules, which together consist of thousands to tens of thousands of atoms. Two classes of macromolecules, fats and carbohydrates, serve mainly as foodstuff and building material.

The other two classes of macromolecules, proteins and nucleic acids (deoxyribonucleic acid, DNA and ribonucleic acid, RNA), perform heredity and dynamic functions that are the essence of life. Anything but monotonous; their variety underlies the diversity of life forms.

A strand of DNA is a long sequence of subunits, called nucleotide bases, attached consecutively to a backbone made of sugar and phosphate. All nucleotide bases in DNA fall into only four kinds: adenine (A), guanine (G), thymine (T), and cytosine (C). Their irregular order in the sequence constitutes the primary structure of DNA. Two DNA strands running opposite to each other constitute a DNA molecule in the form of a double helix. The bases in the two strands form complementary base pairs: the base A in one strand is always paired to the base T in the other, and G to C. The two members of a base pair bind to each other. The bond in one base pair is rather weak, but millions of base pairs acting together hold the two DNA strands together firmly, not unlike the weak teeth of a zipper acting together to form a strong bind.

DNA accounts for only a fraction of a percent of a cell by weight, but its minute amount belies its vast importance. It performs three vital functions. Its first function, to carry enormous information for specifying heritable traits, is performed by the irregular ordering of its numerous base pairs. Its second function, to serve as a stable template for the almost exact replication of itself, is facilitated by its double helical structure. DNA's third function is to provide detail recipes for the synthesis of proteins. Protein synthesis is a complicated process. Roughly, some DNA base pairs serve as codes that are transcribed into RNA that, after editing if necessary, is translated into a protein or a polypeptide chain.

A protein is made of one or more polypeptide chains. A polypeptide chain is a long sequence of amino acids, each belonging to one of twenty kinds, linked to each other by strong peptide bonds. The linear order of the amino acids in a chain, which constitutes the primary structure of a protein, is specified by DNA. A chain's primary structure determines how it folds up to form higher order structures. These three-dimensional structures have highly specific shapes that are crucial for proteins to perform their specific functions.

If DNA is like the queen bee in a bee colony, then proteins are the worker bees. They are more varied than DNA and more abundant, accounting for more than half the dry weight of most organisms. They constitute the core of life dynamics and perform a host of biological functions. Structural proteins such as elastin endow tissues with strength. Transport proteins such as hemoglobin carry molecules to various parts of the body as needed. Regulatory proteins such as hormones convey signals among cells and maintain them at favorable conditions. Defense proteins such as antibodies recognize and neutralize foreign bodies and pathogens. The most varied and specialized proteins are enzymes, which direct and catalyze almost all chemical reactions in the body. Enzymes are amazingly efficient and can speed up a reaction by ten billion folds. Without them, metabolism and life itself grind to a halt.

DNA molecules, chromosomes, base pairs, proteins, and polypeptide chains are clearly physical entities individuated by their spatial separations or physical characteristics. The same cannot be said of genes. To say genes are DNA is saying only that DNA is the stuff genes are made of. It does not answer what counts as *a* gene. To that question no consensual answer exists, but that in no way implies that genes are fictitious constructions. Most criteria for individuating genes now invoke both material and function, and they overlap in essential points. Some definitions designate as a structural gene the DNA regions that contain the codes for a functional polypeptide, and a regulatory gene the DNA regions containing regulatory sites. Other definitions combine the two and include in a gene all DNA regions, coding and regulatory, that are responsible for the synthesis of a functional product, notably a polypeptide that is a functional part of a protein.

The number of human proteins is considerably higher than the number of human genes for several reasons. A gene's transcription can be spliced and combined in several ways in the process of protein synthesis. The polypeptide encoded by a gene can function in several proteins. A polypeptide can also acquire small ions and molecules to perform new functions. On top of sheer numbers, the location, timing, and abundance of each type of protein make crucial differences to the welfare of cells and organism itself. All these make proteomics, the study of all proteins and their functions, far more complex than genomics.

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Even at the basic level of macromolecules, life exhibits awesome complexity. Molecular biology approaches genes and proteins from four sides: their proximate and remote functions, their properties and interactions, their microscopic structures, and the processes of manipulating them. For these it integrates and develops, among others, four major disciplines: genetics for functions, biochemistry for properties, crystallography for structures, and biotechnology for processes. The first three disciplines have traditions of their own.

Biology, which lacks broad fundamental principles, is more fragmented than physics and chemistry. It has roots in medicine, natural history that emphasizes description and classification, and natural philosophy that emphasizes experimentation, generalization, and underlying mechanisms. Ironically, it was the tradition of natural history that produced the most important arching biological theory, Charles Darwin's theory of evolution by natural selection. It provides the philosophical underpinning for molecular biology. The evolutionary concept of descent by modification from a common ancestor helps to justify several practices in molecular biology. One practice is to investigate simple organisms such as microbes and judiciously extrapolating results to higher organisms. Another is to draw inference from comparing genes and proteins from various organisms. Conversely, the discovery that all organisms share similar molecular structures in their genetic materials lends empirical support to the theory of evolution.

Chemistry, genetics, and microbiology all have stronger inclinations towards applications than physics. Although molecular biology concentrated on basic science during its first two decades, the practical tradition of its roots showed up in Tatem's 1958 Nobel lecture. "With a more complete understanding of the functioning and regulation of gene activity in development and differentiation, these processes may be more efficiently controlled and regulated, not only to avoid structural or metabolic errors in the developing organisms but also to produce better organisms. This may permit the improvement of all living organisms by processes which we might call biological engineering." The four aspects are present equally in molecular biology.

We have discussed how molecular biology addresses the properties, structures, and functions of proteins and nucleic acids. Instruments and processing techniques, although sometimes left out of the core of scientific content, have been indispensable to science ever since Galileo turned his telescope toward the heavens and Hooke peered down the microscope at cells. Molecular biology is no exception. Since its inception, it has depended on ultracentrifuge, electrophoresis, X-ray diffraction, and electron microscope.

Most of these instruments, which make essential use of physical properties such as mass and electric charge, are applicable to many materials besides the biological.

As molecular biologists acquired more knowledge about the properties and interactions of biological macromolecules, they began to use these as tools peculiar to their topics of investigation. Synthesis, manipulation, and processing of macromolecules, the fourth aspect of molecular biology, accelerated scientific progress. In the 1970s, it engendered genetic engineering, which aims to turn natural functions of macromolecules into desired performances for serving human needs and wants. A biotechnology industry grew, marked by social controversies and volatile stock markets. Industrial-styled scientific research emerged, exemplified by the Human Genome Project. The rank of molecular biologists swelled almost a hundred fold. Sensational coverage in the popular media, however, missed much of the basic science involved, which boasted a slew of Nobel Prizes.

Central to genetic engineering, also called recombinant DNA technology, is the abilities to design and manufacture specific DNA segments and insert them into living cells to perform certain desired functions. These abilities take decades to develop and refine, as new scientific discoveries lead to new technological inventions. They are mainly biochemical treatments informed by the requirements of gene functions, especially the function of producing proteins that perform desired physiological functions.

Manipulation of DNA began in the 1950s with Kornberg's attempt to synthesize it. The research led to the discovery of several enzymes that work on DNA. One enzyme, DNA polymerase, assembles nucleotides on an exposed strand of DNA to form a complementary strand. Another, ligase, fuses the backbones of two DNA strands to form an integral DNA. More enzymes were discovered in two other Nobel-prized works. Bacteria yield enzymes that can cut DNA molecules at specific nucleotide sequences. They are called restriction enzymes because they restrict the growth of viruses in host bacteria by cutting up viral DNA. Many restriction enzymes leave exposed nucleotides at the cut ends, which can stick DNA fragments with complementary ending sequences. Hundreds of restriction enzymes enable scientists to cut DNA at desirable places and paste together selected pieces. Retrovirus, a kind virus of which HIV is the most well known, yields another potent chemical tool. Unlike other organisms, retroviruses carry their genetic information not in DNA but in RNA. They also produce an enzyme, the reverse transcriptase, which transcribes their RNA into DNA so that their genes can merge into their hosts' DNA for reproduction. Using reverse transcriptase, scientists can start with the messenger RNA of a desired protein and turn it into DNA.

Enzymes enable molecular biologists to cut and paste DNA at will. For practical purposes, the designed DNA has to be mass-produced. Two general methods of multiplying DNA, introduced a decade apart, won Nobel Prizes. The first uses living cells as tiny DNA factories. Pieces of designed DNA are inserted via retrovirus or bacteria genetics into hosts, which can be single-celled bacteria or cultured mammalian cells. As the cells grow and reproduce, they not only replicate the inserted DNA but also produce the protein that DNA coded for. The proteins, harvested from the cell culture, are desirable products. At first, genetic engineering generated much anxiety about the possibility that the engineered microbes could escape from the laboratory and cause biological catastrophe. When proved safe, it got the biotechnology industry off the ground in the late 1970s.

Cellular amplification of DNA met a stiff competitor in 1983. Polymerase chain reaction can amplify trace amount of any DNA fragments in test tubes by using polymerase and other biochemical means. It rapidly found many uses, such as diagnostics kits for genetic diseases or genome sequencing.

Genetic engineering has many applications, from genetically modified crops that prolong the green revolution to DNA fingerprinting that revolutionizes forensics. It has also changed the pace of basic research in molecular biology. Restriction enzymes once kept hundreds of front-line research scientists busy; now they are sold as reagents in bottles. It took years to sequence the first gene, most of time spent in preparing suitable DNA fragments. Now thousands of sequenced DNA fragments are individually identified, arranged in an array, and fixed on a chip, commercial available for experiments. All these provide the technological infrastructure for the science. Despite the acceleration in discoveries it affords, however, the wait for envisioned biomedicine is far from over. Life is far more complex than molecular biologists first anticipated.

Reflection on Activity 2.3.1.1

- Review the contents of the Activity 2.3.1.1 textbox in this Study Guide dealing with scientific convergence in the birth of molecular biology. Write a summary essay in your own words on the birth of molecular biology from a number of basic disciplines, such as biochemistry and cell biology. In order to do this, you will need to make use of 'Google Scholar', to find out more information under the different headings. (See introduction under "Improved study skills".)

STUDY UNIT 3

Time allocation

You should spend at least 14 hours on this study unit.

AN INTRODUCTION TO SOME OF THE PROCESSES AND INSTRUMENTATION USED IN BIOTECHNOLOGY

Textbook reference

This study unit is based on Introduction to Biotechnology, 2nd Ed (2009); Thiemann, W.J. & Palladino, A., Pearson/Benjamin Cummings, London, Toronto, New York. Chapter 3: Recombinant DNA Technology and Genomics, pages 57–92; Chapter 12: Biotechnology Regulations, 305–323; Internet Sources, as indicated in the text of this Study Guide.

STUDY UNIT CONTENT

This study unit is divided into the following study sections:

Study section 3.1 Specific processes and equipment required to manipulate genetic material

Study section 3.2 Criteria and Best Practice Trends used in the biotechnology industry

Outcomes

After completing this study unit you should be able to:

- briefly explain how and why the Sanger method has been replaced with that of computer-automated DNA sequencing methods.
- compare and contrast how and why the Southern Blot methodology differs from that of the northern Blot methodology, especially where applications of SYBR Green and TaqMan probes used in Real-time PCR (polymerase chain reaction) experiments are concerned.

- briefly explain what gene microarray analysis is as well as some of its applications.
- compare and contrast the mechanisms and applications of size exclusion, ion exchange and affinity chromatography.
- briefly discuss criteria and Best Practice Trends used in the biotechnology industry.

STUDY SECTION 3.1: Specific processes and equipment required to manipulate genetic material

3.1.1 Processes and equipment used to isolate, digest, reform and manipulate genetic material

What can you do with a cloned gene? Applications of recombinant DNA technology

Gel electrophoresis is used as a basic technology to map genes which are biochemically "dissected" with restriction enzymes. The exact location of these enzyme cutting sites allow for a restriction map to be created using **agarose gel electrophoresis:**

To run an agarose gel, it is submerged in a buffer solution that conducts electricity. DNA is then loaded into wells at the top of the gel and an electric current is subsequently applied to the ends of the gel so that DNA migrates according to its charge and size. The rate of migration of DNA through the gel depends on the size of the fragments generated by the restriction enzymes. Large fragments migrate slowly, while smaller fragments migrate faster. A tracking dye is added to the samples to monitor DNA migration during electrophoresis, which can finally be visualised after electrophoresis by the addition of DNA staining dyes.

DNA Sequencing

It is important to be able to determine the sequence of nucleotides of a cloned gene, which is usually done according to the chain termination sequencing (Sanger method). In the computer-generated sequencing method, ddNTPs are each labelled with a different fluorescent dye and samples are separated on a single-lane capillary gel that is scanned with a laser beam. Different colour patterns for each nucleotide are created and converted by computer to the sequence.

Fluorescence *in situ* hybridisation (FISH) is a technique where chromosomes are isolated from cells and spread out on a glass slide. A cDNA probe for gene of interest is labelled with fluorescent nucleotides and incubated with these slides. The probe will hybridise with complementary sequences on the slide, which is washed and exposed to fluorescent light. Wherever probe bound, it is illuminated to indicate the presence of that gene.

Southern blotting

Chromosomal DNA is digested into small fragments with restriction enzymes, which are then separated by agarose gel electrophoresis. The gel is treated with alkaline solution to denature the DNA and fragments are subsequently transferred onto a nylon or nitrocellulose filter (called blotting). The filter (blot) is incubated with a probe and exposed to film by autoradiography, where the number of "bands" on the processed film represents gene copy number.

A number of methods can be used to study *expression of genes in cells* and includes the following:

- **Northern blot analysis,** which is similar to Southern blotting, except that RNA is isolated from a tissue of interest, separated by gel electrophoresis, blotted onto a membrane, and hybridised to a probe.
- **Reverse transcription PCR** involves the conversion of mRNA into double-stranded cDNA which is then amplified with a set of primers specific for the gene of interest. Products are then electrophoresed on an agarose gel as described above.
- ***In situ* hybridisation.** The tissue of interest is preserved in a fixative solution and embedded in a wax-like substance. Tissue can be sliced into very thin sections attached to microscope slides which are then incubated with a probe which hybridises to the gene of interest.
- **Gene microarrays** is a relatively new technology where single-stranded DNA molecules are attached onto a slide using a robotic arrayer fitted with tiny pins which can have more than 10,000 spots of DNA. Extracted mRNA from the tissue of interest can be tagged with fluorescent dye, and incubated overnight with the slide. The tagged mRNA will then hybridise to spots on the microarray that have complementary DNA sequences. The slide is subsequently scanned with a laser that causes the spots to fluoresce wherever hybridisation between the probe and the bound complementary DNA has occurred.

Study the section on Gel Electrophoresis and Mapping Gene Structure with Restriction Enzymes on page 75 to 80 in the prescribed textbook.

Refer to Figure 3.11 (Agarose Gel Electrophoresis) p.77 dealing with mechanisms and methodology underlying gel electrophoresis and, in particular, why DNA fragments of varying sizes can be separated and visualised by using this technique.

ACTIVITY 3.1.1

DNA sequencing methodology, with particular emphasis on the Sanger method, is discussed.

3.1.1.1 Briefly explain how and why the Sanger method has been replaced with that of computer-automated DNA sequencing methods. The answer must not exceed one page.

3.1.1.2 Tools of the Trade are discussed on p.80 of the prescribed textbook specifically regarding increasing availability and use of restriction enzymes, particularly in recombinanat DNA technology. Visit the website www.BioTechniques.com.

3.1.1.3 Review questions and activities on page 95 of the prescribed textbook and make sure that you are able to answer these questions in an exam situation. Please consult Appendix 1 of the textbook for model answers.

Reflection on Activity 3.1.1

- Review the first two questions and activities on page 95, making sure that you can distinguish between gene cloning, recombinant DNA technology and genetic engineering, as well as the importance of DNA ligase.

Study the section on Chromosomal Location and Gene Copy Number on page 80 to 87 in the prescribed textbook.

Refer to Figure 3.14 in the prescribed textbook dealing with *fluorescence in situ* hybridisation (FISH) technique that can be used to identify a chromosome containing a gene of interest.

ACTIVITY 3.1.2

Study Southern Blot analysis of DNA fragments as illustrated in Figure 3.15 on p.82 of the prescribed textbook.

3.1.2.1 Discuss in detail how and why the Southern Blot methodology differs from that of the northern Blot methodology (Figure 3.16 p.83 of the prescribed textbook). In the latter instance, include applications of SYBR Green and TaqMan probes used in Real-time PCR (polymerase chain reaction) experiments. The answer must not exceed two typed pages.

3.1.2.2 Briefly summarise gene microarray analysis as depicted in Figure 3.18 and also as described on page 84 to 86 of the prescribed textbook. The summary must not exceed one page.

Protein purification methods

Similarities between proteins allow the separation of proteins from nonprotein material. Protein precipitation makes use of various salts to cause proteins to settle out of solution.

Filtration (size-based) separation methods involve *centrifugation, membrane filtration, microfiltration and ultrafiltration.*

Separating the components in the extract using various forms of **chromatography:**

Differences in proteins allow the separation of the target protein from other proteins. **Chromatography** allows the sorting of proteins based on size or by how they cling to or dissolve in various substances.

Size exclusion chromatography uses gel beads with pores. Larger proteins move quickly around the beads and smaller proteins slip through the pores and therefore move more slowly through the beads.

Ion exchange chromatography which relies on the charge of the protein moving through a charged resin. Opposite charged proteins will stick to resin beads and can be eluted by changing the charge with salts of increasing concentration.

Affinity chromatography relies on the ability of proteins to bind specifically and reversibly to uniquely shaped compounds called *ligands*.

Hydrophobic interaction chromatography sorts proteins on the basis of their repulsion of water.

Iso-electric focusing used in QC to identify two similar proteins that are difficult to separate by any other means. Each protein has a specific number of charged amino acids on its surface in specific places and creates a unique electric signature known as its isoelectric point (IEP) where charges on the protein match the pH level of the solution.

Other analytic methods

High-performance liquid chromatography (**HPLC**) uses high pressure to force the extract through the column in a shorter time.

Mass spectrometry (mass spec) is a highly sensitive method used to detect trace elements and can be used to indicate the size and identity of most protein fragments.

Verification: The presence and concentration of the protein of interest must be verified at each step of the purification process, and will include SDS-PAGE (polyacrylamide gel electrophoresis), western blotting and ELISA techniques.

Preserving proteins: Lyophilization (freeze-drying), where a protein, usually a liquid product, is first frozen. A vacuum is used to hasten the evaporation of water from the fluid, thereby maintaining protein structure, with subsequent storage at room temperature, for long periods of time.

Postpurification analysis methods

Protein sequencing: Must determine the primary structure, the sequence of amino acids

X-ray crystallography: Used to determine the complex tertiary and quaternary structures

Proteomics: A new scientific discipline dedicated to understanding, amongst others, protein folding and the complex relationship between disease and protein expression. Protein microarrays may test variation in protein expression between healthy and diseased states.

Study the section on Protein Purification Methods on page 110 to 115 in the prescribed textbook.

Various ways of separating proteins in an extract, usually via chromatographic processes, are illustrated in Figures 4.10 Protein Anrification by Size Exclusion Chromatography p.112, Figure 4.11 Ion Exchange Chromatography p.112 and Figure 4.12 Affinity chromatography p.113 in the prescribed textbook. These basic methods can be incorporated into more sophisticated analytical systems as indicated in Figure 4.14 High Performance Liquid Chromatography, p.114. Every step in the purification process can be verified using gel electrophoresis, as illustrated in Figure 4.15, SDS-PAGE, p.115 of the prescribed textbook.

ACTIVITY 3.1.3

Study the various types of chromatography which are used to separate proteins.

3.1.3.1 Tabulate the mechanisms and applications of size exclusion, ion exchange and affinity chromatography. The size of the table must not exceed one page.

3.1.3.2 Briefly explain how high-performance liquid chromatography (HPLC) and Mass Spectrometry (MS) can be used to analyse a mixture of different proteins. The answer must not exceed one page.

3.1.3.3 Briefly summarise the methodology and mechanism of SDS-PAGE (poly-acrylamide gel electrophoresis). The summary must not exceed one page.

ADDITIONAL READING:

Plant gene expression profiling with DNA microarrays

Shu-Hsing Wu§, Katrina Ramonell, Jeremy Gollub, Shauna Somerville*

Plant Physiol. Biochem. 39 (2001) 917–926

Researchers in this age face revolutionary challenges in the pursuit of biological questions. The availability of vast amounts of genome sequence information has dramatically altered both the experimental scale and strategies for conducting research. Experimental designs allowing high through-put screening, measurement and cross-referencing will benefit the most from this treasure of sequence data. The microarray is one of the technologies permitting simultaneous interrogation of complex nucleic acid mixtures. The genome-wide examination of gene expression with this technology has provided transcript profiles of numerous biological samples. When coupled with a microarray expression database and data analysis and viewing tools, these data can serve as a foundation for proposing hypotheses and performing dedicated research.

The sequencing of the complete genomes of dozens of organisms has opened a new avenue for biologists to explore these organisms at the genome level. The most obvious immediate challenge is how to dissect gene function once sequence information is known. The microarray is one of the revolutionary technologies enabling comprehensive and high through-put surveys of DNA or RNA molecules on a genome-wide scale.

In what is essentially a reverse northern procedure, microarrays allow the simultaneous analysis of thousands or tens of thousands of genes in a single experiment. Coupled with bioinformatic tools and internet-based infrastructure, this technology greatly extends the scope of biological research in the post-genomics era.

The two most common platforms for microarrays are DNA microarrays, the preparation of which involves robotic deposition of DNA fragments onto slides, and oligonucleotide microarrays (e.g. Affymetrix GeneChip™; Affymetrix, Sunnyvale, CA, USA, http://www.affymetrix.com), which are manufactured by synthesis of oligonucleotides in situ on solid supports. The most popular usage of microarrays is to monitor transcript abundance patterns of biological samples. This has yielded fruitful discoveries of yeast genes involved in the diauxic metabolic shift in the cell cycle and in responses to environmental stimuli.

A step-by-step procedure for preparing DNA microarrays is illustrated in *Text Figure 16*. First, the cloned DNA template collection, which is maintained in a 96-well format, is used for PCR amplification of the clone inserts with universal primers complementary to the clone vector. Quality control of the purified PCR product includes DNA quantitation (e.g. PicoGreen; Molecular Probes, Eugene, OR, USA; http://www.probes.com) and DNA gel electrophoresis. The gels are manually scored as to whether PCR amplification led to the production of a single band as expected.

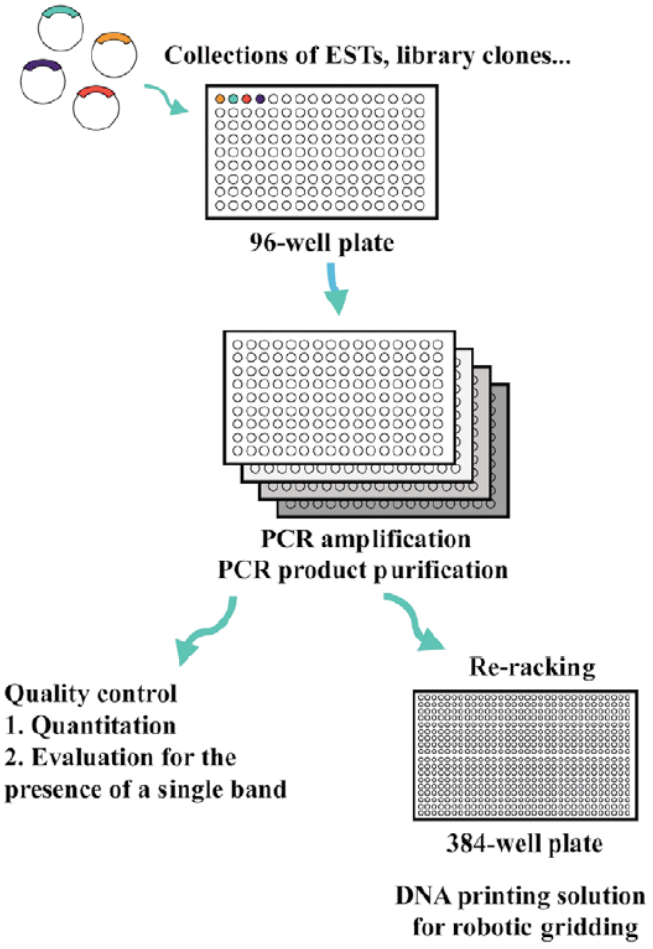

Figure 16: DNA sample preparation for printing DNA microarrays

PCR products that fail to meet the quality control criteria are noted and this information is stored in a database for future reference at the time of data analysis. Four 96-well plates of PCR products are usually re-racked into one single 384-plate for high through-put robotic deposition onto glass slides. Detailed protocols for the preparation of DNA samples for DNA microarray preparation are available at the DNA Protocols page (http://afgc.stanford.edu/afgc_html/AFGCProtocols-fev2001.pdf) at the AFGC web site.

DNA MICROARRAY HYBRIDIZATION AND DATA ACQUISITION

The core of DNA microarray hybridization is the ability to compare relative gene expression levels of mRNAs prepared from two distinct samples. In essence, the hybridization is a scale-up of the reverse-northern procedure with thousands or tens of thousands of probe DNAs immobilized on a slide surface (*figure 2*).

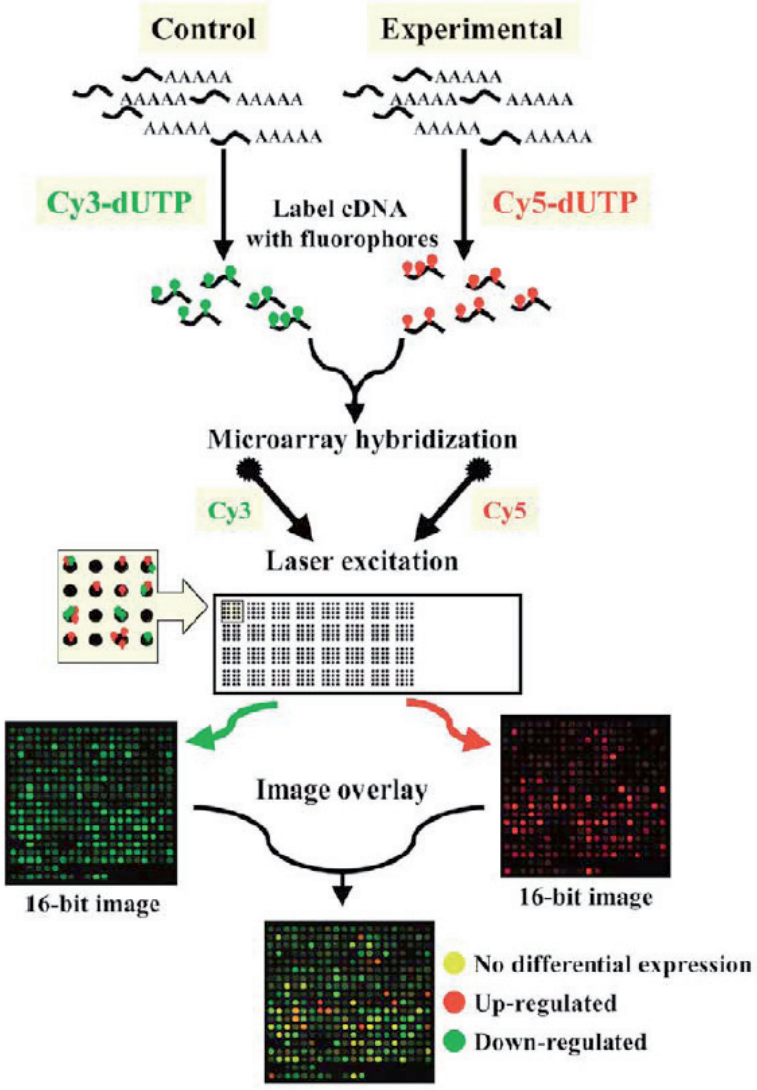

Figure 17: DNA microarray hibridization and data requisition

This is achieved by labeling whole mRNA populations (targets) from two samples with different fluorophores, usually Cy3 (control, in green) and Cy5 (experimental, in red). The two targets are then mixed together and hybridized to a single DNA microarray slide. The fluorescence intensity of each gene is measured individually after laser or white light excitation at the appropriate wavelength for each fluorophore. The ratio of the fluorescence readings of each gene for the two samples correlates with the relative abundance of its mRNA from the two samples. By this process, one can identify genes that are expressed differentially between the two samples.

STUDY SECTION 3.2: Criteria and Best Practice Trends used in the Biotechnology Industry

3.2.1 Launching of new agricultural products is supported by six criteria

There are various regulatory bodies in each country where biotechnology is practiced, including bodies to regulate agriculture, environment, food and medicine development and registration of patents.

In the USA, their National Institute of Health (NIH) was the first federal agency to assume regulatory responsibility over biotechnology. In 1974, the NIH published research guidelines for recombinant DNA techniques. Advancement and Regulations of Agriculture for regulating plant pests, plants, and veterinary biologics followed. Animal and Plant Health Inspection Service (APHIS) was created to be responsible for protecting agriculture from pests and diseases.

A permitting process was instituted, where several years of field trials were required to investigate everything about a given plant's disease resistance, drought tolerance and reproductive rates. In the process, precautions needed be taken to prevent accidental cross-pollination.

An alternative system to fast-track new agricultural products, called **notification,** was introduced. In terms of this system, new agricultural products were required to meet the following **six criteria:**

- The new agricultural product must be one of only a limited number of eligible plant species.
- The new genetic material must be confined to the nucleus of the new plant.
- The function of the genes being introduced must be known.
- If to be used for food, the new genes cannot cause the production of a toxin, an infectious disease, or any substance used medically.
- If the gene is derived from a plant virus, it cannot have the potential to create a new virus.
- The new genetic material must not be derived from animal or human viruses.

The Environmental Protection Agency (EPA) was also established in the USA in 1970. The responsibilities of the Agency include the protection of endangered species, establishing emission standards for cars, regulation of the use of pesticides and herbicides, as well as

the regulation of any plant that is genetically engineered to express proteins that provide pest control or herbicide resistance.

The EPA spends about one year reviewing the data collected, concentrating on four areas of concern:

- Source of gene, how it is expressed, and the nature of the pesticide-protein produced
- Health effects of the bioengineered plant
- The environmental fate of the pesticide protein
- The effects on nontarget species

The **Food and Drug Administration** (FDA) serves as a consultant to study unexpected or undesirable effects of food additives. The new medicine approval process or **Investigational New Drug (IND) application** is considered by the FDA in terms of previous experiments, the nature of the substance, and the plans for additional testing.

3.2.2 Quality control in the manipulation of genetic material is supported by three Best Practice Trends

- Good Laboratory Practice **(GLP)** and Good Manufacturing Practice **(GMP)** include regulations instituted by the FDA to govern animal studies of pharmaceutical products, including written protocols and evidence for the provision of adequate facilities for proper care of the animals, proper recording of data, and the conduction of valid toxicity tests.
- Phase testing of medication
  - **Phase I (safety) –** between 20 and 80 healthy volunteers take the medicine
  - **Phase II (efficacy) –** test new treatment on 100 to 300 patients who actually have the illness
  - **Phase III (comparative benefit to other current medicines) –** new treatment tested on 1,000 to 3,000 patients in double-blinded tests
- FDA approval as an **NDA** (new drug authorisation)

Biotechnology and nature

As we have seen, there are a number of issues surrounding the use of genetically modified (GM) crops, including

- The plant itself **(species integrity)**
- Possible effect of altered plants on the ecosystem and on overall **biodiversity**
- Effects on nontarget species
- How will the crop be used? Is it safe to feed to animals? Is it safe for humans?

ACTIVITY 3.2.2.1

Read the following excerpt taken from:

Livestock, ethics and quality of life

By: J. Hodges

http://jas.fass.org/cgi/content/full/81/11/2887

J. Anim. Sci. 2003. 81:2887-2894 © 2003 American Society of Animal Science European Association for Animal Production, Mittersill, Austria

Recent Unethical Behavior in the Food Chain

Several large-scale cases have recently occurred where the public perceives unethical behavior on the part of some leading scientists, multinational companies and governments. Here are two major examples – there are others.

Case A. Bovine Spongiform Encephalopathy (BSE)

This new and fatal cattle disease became an epidemic in the UK as a result of livestock intensification. The formal independent report to the UK government (Phillips et al., 2000) said "A vital industry has been dealt a body blow" and referring to new variant Creutzfeldt-Jakob Disease (nvCJD), "BSE has caused a harrowing fatal disease for humans." Here is a brief history.

Following unrecognized cases in the early 1980s, BSE was diagnosed in 1986 as a new, lethal condition in cattle. It was subsequently thought (Horn et al., 2001) that earlier cases and cycles probably took place over several decades. Following diagnosis, high profile scientists said repeatedly in public that BSE could not affect humans and that beef was safe to eat. In 1988, meat and bone meal (MBM) containing bovine offal was suspected as the vector (Wilesmith et al., 1988). This led slowly to the UK government banning MBM totally from animal feed by 1996. However, there was indecision and uncertainty for several years. Regrettably exports of MBM continued to other EU countries and when the EU banned MBM it was then exported from the UK to more distant markets. The full consequences of this unethical behavior by agribusiness have yet to be revealed.

In 1997, scientists announced that nvCJD was a new fatal human condition caused by eating beef from cows affected with BSE (Bruce et al., 1997). One hundred thirty-three people had died by the end of summer 2003. Uncertainty on exposure/dose, incubation period and susceptibility leads to estimates between a few hundred and several thousands who do not know they are infected (Horn et al., 2001). To date, there is no early diagnosis and no treatment.

Summary Statistics of BSE in the UK

There have been 190,000 cows with BSE on 36,000 UK farms between 1986 and 2002, peaking in 1992 with 37,289 cases and slowly declining to 900 cases in 2002. BSE cases have occurred in 19 European countries plus Israel, Japan, and Canada.

Why Credibility of Scientists Was Undermined by BSE and nvCJD

1. Scientists had never heard of, nor anticipated this new disease;
2. After diagnosis scientists remained ignorant for several years of how it was transmitted between cows;
3. At time of writing, summer 2003, the origin of BSE remains unknown to scientists;
4. For 11 yr, leading scientists stated that BSE was specific to cattle and posed no threat to human health. This was a conclusion beyond available data and knowledge. They should have said: "We do not know."

Case B. Genetically Modified Food (GM Food)

1. GM Food appeared unannounced in food stores in Europe in 1997;
2. GM Food ingredients were mainly imported;
3. Food products containing GM ingredients were not labeled;
4. Consumers were offered no choice between GM and non-GM Food.

With BSE as a background, safety assurances from scientists working for multinational companies fell on very skeptical ears. The companies and their scientists, mainly from the USA and Switzerland, then made a serious mistake by seeking to attribute European consumer concerns to environmental fringe groups, or to government trade protectionism, or to a superstitious view of science. These statements further inflamed consumers. Based upon the testimony of scientists that there is no difference (substantive equivalence) between GM and non-GM Foods the US government made the original decision not to label. After protests from consumers in Europe the labeling issue moved from scientists to consumers where it rightly belongs.

Consequently the major European supermarkets responded to consumer concerns by not stocking GM Food products. European governments and the EU require labels thus supporting market economy principles of choice. This scenario is described in Hodges (1999).

Unethical behavior is central to this GM Food debacle, which concerns the attitude of the multinational companies who evidently thought consumers of food were a captive market. In the market economy it is the consumers' privilege, not the manufacturers' right, to decide whether to buy a new product. This approach has backfired and brought discredit on the multinational companies and their scientists.

The suspicions and ire of the European consumers were aroused by the following items:

1. Apparent intention of gaining corporate control over the world's food supplies;
2. Terminator gene technology;
3. Choice of global staple foods for the first generation of genetically modified crops;

4. First generation GMF only equipped to increase tolerance of the growing crop to chemicals marketed by the same company;

5. Absence of benefit to the consumer;

6. Fears that second generation GM products with transgenes designed to affect plant and animal physiological processes will cause human physiological mis-balances;

7. The argument that Western society has to eat GM Foods so that the hungry people of the developing world do not face famine;

8. Scientific assurances that GMF is equivalent to Non-GM Food, seen as attempts to remove the right of the consumer to know and to choose;

9. Statements by some scientists that all GMF is safe – thus seeking to give a generic clearance – but seen as dissembling. Each new genetically modified product is unique and must be tested just as each pharmaceutical product is tested (Hodges, 2000).

Implications: The challenge for Leaders in Animal Science

Agricultural and animal scientists now face the task of formulating a larger vision of their contribution to society. It will not be easy as the goal of biological efficiency has been the foundational assumption of a whole generation of animal scientists. How can animal scientists contribute to the new Quality of Life Agricultural Era? Here are some suggestions for starters.

Animal scientists need to decouple themselves from:

1. The single-minded pursuit of biological efficiency.

2. Advocacy of techniques that encourage further intensification and scale of production.

3. Profit and shareholder value as a primary research aim.

4. A posture that promotes higher consumption of animal products per capita as an end in itself.

Animal scientists need to make new contributions to society by:

1. Regaining and maintaining scientific objectivity, not only in the learned journals, but in the eyes of the consumer.

2. Positioning themselves as objective, thoughtful servants of the whole of society.

3. Listening to what the public as consumers and as citizens are saying about food related to quality of life.

4. Ensuring science is independent of self-serving interests.

5. Rethinking the nature of risk in the context of radical biotechnologies. This means evaluating risk in terms of the consequences flowing from one failure rather than from small probabilities of failure.

6. Designing and researching new hypotheses and questions that will enrich life quality.

7. Engaging in dialogue with other components in the food chain community, including business, to ensure that information flows in both directions with the aim of improving transparency and accountability (Hodges, 2002).

Literature Cited

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Wilesmith J W, G A Wells, M P Cranwell and J P Ryan. 1988. Bovine spongiform encephalopathy: Epidemiological studies. Vet. Rec. 25:638–644.

Reflection on Activity 3.2.2.1

- Review the contents of the Activity 3.2.2.1 textbox in this study guide dealing with Livestock, Ethics and the Quality of Life pp.123–128. Write a summary essay in your own words on the ethical issues surrounding the ethical challenges of applying biotechnology. The summary essay must not exceed **three pages**. Some additional reading sources have been given below to assist in this final portfolio exercise.

Agricultural Biotechnology And Societal Decision-Making: The Role Of Risk Analysis

Jeffrey D. Wolt and Robert K.D. Peterson

http://www.agbioforum.org/v3n1/v3n1a06-wolt.htm

The Journal of Agrobiotechnology Management and Economics, Vol 3, #6, 1997

This article discusses the role of risk analysis in public policy decision-making, specifically as it relates to the framework for characterizing, communicating, and managing the risks

imposed by agricultural biotechnology. Inasmuch as scientists and public policy experts have not successfully implemented risk-based paradigms in dealing with agricultural biotechnology, the authors additionally address the ascendancy of the precautionary principle in policy circles. Finally, we consider how rediscovering and reinstituting risk-based approaches for agricultural biotechnology may allow knowledge to be effectively used in dealing with public attitudes and perceptions.

Changing Nature's Course, a 207 page document which focuses on "The Ethical Challenge of Biotechnology" http://lib.hku.hk/Press/9622094031.pdf and contains a collection of articles dealing with:- the human genome project and gene therapy, transgenic animals, biotechnology and the environment, including genetically engineering resistance to plant virus diseases, genetic markers for severe human heredity disorders, biotechnology and medicine.