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Chapter 1 : Recombinant DNA - Definition and Examples | Biology Dictionary

International, practice-oriented and detailed, the book is divided into four parts: Choosing a production system - Propagation of recombinant microorganisms and animal cells - Purification of recombinant proteins - Health care and veterinary recombinant products Moreover, the book is interdisciplinary.

A method of cutting DNA from one organism and inserting the DNA fragments into a host organism of the same or different species. What is a Transgenic Organism? Transgenic organisms are created using recombinant DNA. DNA fragments cannot function all by themselves. They must become part of the genetic material of living cells before the genes they contain can be activated. DNA fragments may be combined with bacterial DNA so they can later be inserted into a bacterial cell. Bacteria contain small circular DNA molecules known as plasmids in addition to their chromosomes. These plasmids can be removed from the bacterial cells and cut with the same restriction enzyme used to produce the DNA fragments. These sticky ends are the sites at which the DNA fragment and the plasmid can be joined end to end paste , thereby forming a new plasmid that contains a piece of foreign DNA recombinant DNA. Step three of genetic engineering involves the recombinant DNA plasmid being inserted back into the bacterial cell mixed with millions of bacteria suspended in a dense salt solution. After a few minutes, the bacteria will take up the recombinant DNA. These bacteria can be isolated and grown into large colonies that contain recombinant DNA. The term for a large number of cells grown from a single cell is a clone; therefore this technique is often referred to as DNA cloning. Note recombinant DNA can be inserted into other cells other than bacteria including: Why would you want to create transgenic Organisms? Pharmaceutical use, to treat human disorders. Diabetes â€" condition where people cannot make insulin Cut the gene out of humans and put it into bacteria. The bacterial can make human insulin using this gene and the insulation can be collected to give to people with diabetes. Hemophilia â€" use transgenic pig to make missing clotting factor. Recombinant bacteria are used in the production of human growth hormone to treat pituitary dwarfism. Vaccine â€" a harmless form of the pathogen. Made from recombinant DNA. Tells your body what a specific pathogen looks like â€" if your body knows what it looks like it can fight it. To make a vaccine, scientists find the gene for the proteins on the outside of a harmful pathogen. Then they put the gene into a pathogen that is harmless to humans. The harmless pathogen can then make the same protein that are on the outside of the harmful pathogen. They tell your body what the harmful pathogen looks like without making you sick. Transgenic Organisms in Indusrty 2. Industry Create bacteria that can break down oil from oil spills faster than normal bacteria. They are the first patented organisms. Scientists have modified the bacterium E. Production of cheese, laundry detergent, pulp and paper products, and sewage treatment. High protein corn with protein levels similar to beef. Automobile fuel from discarded corn stalks. Various plants have been made resistant to herbicides used to rid fields of unwanted weeds. Canola plants have been modified so they make a higher yield of oil. Crops that are better tasting, stay fresher longer, and are protected from disease and insect manifestations. Gene Therapy Gene Therapy is the insertion of normal genes into human cells to correct genetic disorders. This is in the trial stages i. Used first with cystic fibrous. How does gene therapy work? Put the normal gene the one that makes the normal transport protein in a cold virus. Introduce the virus to the lungs the organ manly affected by cystic fibrosis using a nasal spray. Hope that the lung cells take up the normal gene into their chromosomes and make up normal protein. Powered by Create your own unique website with customizable templates.

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Chapter 2 : Recombinant vaccines and the development of new vaccine strategies

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What is the difference between Bacterial Cell and Animal Cell What is a Bacterial Cell Bacterial cells are prokaryotes, which can be considered as simple, unicellular microorganisms. They are lacking membrane-bound organelles like nucleus and mitochondria. They live in either symbiotic or parasitic relationships with plants and animals. By attaching to surfaces, bacteria form dense aggregations like a mat. These bacterial mats are called biofilms. Cellular Structure of Bacterial Cell Bacterial cells are 0. The cell is surrounded by a cell membrane. The membrane-enclosed cytoplasm contains nutrients, proteins, DNA and other essential components of the cell. Small 70S ribosomes are present for the protein synthesis. Protein localization is carried out by their primitive cytoskeleton. A single, circular chromosome is found in the nucleoid. Murein forms a cell wall outside the bacterial cell membrane. The cell wall provides protection to the cell, maintains the shape and prevents the cell from dehydration. The thicker cell wall is classified as gram-positive and the thinner cell wall is classified as gram-negative in the gram staining of bacteria. Flagella are used for the mobility of the cell. The entire cell is covered by glycocalyx which forms the capsule. Some genera of gram-positive bacteria form a resistant, dormant structures called endospores. Endospores contain some parts of the cytoplasm , DNA and ribosomes covered by a cortex. They are resistant to radiation, detergents, disinfectants, heat, freezing, pressure and desiccation. Bacterial cells reproduce asexually by binary fission and sexually by conjugation. A generalized structure of a gram-positive bacterial cell is shown in figure 1. Generalized gram-positive bacterial cell Classification of Bacterial Cell Bacteria can be categorized depending on their morphology: Cocci are the spherical-shaped bacteria. Bacillus are the rod-shaped bacteria. Vibrio are the comma-shaped bacteria Spirilla are the spiral-shaped bacteria Spirochaetes are tightly coiled bacteria Some bacteria live as single cells. But, some of them live in pairs called diploids. Streptococcus are the bacterial chains. Filaments are the elongated bacteria like Actinobacteria. Some are branched filaments such as Nocardia. Metabolism Depending on the carbon source, bacteria can be divided into two groups: The carbon source is organic compounds in heterotrophps whereas the carbon source is carbon dioxide in autotrophs. Depending on the energy source, bacteria can be divided into three groups: In the phototrophs, the energy source is sunlight. Organic compounds are used as the energy source in organotrophs. In lithotrophs, the energy source is inorganic compounds. What is an Animal Cell Animal cell can form either unicellular or multicellular eukaryotic organisms, containing membrane-enclosed organelles such as nucleus, mitochondria and Golgi apparatus. Multicellular eukaryotes contain specialized tissues made by different types of cells. Approximately distinct animal cell types can be found in the adult human body. Animal cells are heterotrophs. They are irregular in shape due to the lack of a cell wall. The outer boundary of an animal cell is the plasma membrane , which is considered as semi-permeable. Semi-permeable membranes only allow selected molecules to move across it. Plasma membrane is composed of phospholipids containing polor heads and non-polor tails. It is described by the lipid bi-layer model. Cytoskeleton of the animal cell is composed of microfilaments, microtubules and intermediate filaments. Cytoskeleton plays a vital role in cellular organization and its shape. Animmal cells are composed of a variety of membrane-bound organelles. The nucleus is enclosed by two membranes called nuclear membrane or nuclear envelop. Nuclear membrane forms the endoplasmic reticulum ER which is involved in protein maturation and transportation. Ribosomes are large, 80S in size and are bound to the ER. Ribosome-bound ER is referred to as rough ER. Vesicles are present for the transformation of various molecules within the cell such as golgi bodies, lysosomes and peroxizomes. Lysosomes store digestive enzymes. Mitochondria is also surrounded by two phospholipid bilayers. They covert sugar into ATP s in order to use it as energy. Animal cells contain structures like cilia,

centrioles, flagella and lysosomes. A generalized animal cell is shown in figure 2. Generalized animal cell Usually, animal cells are composed of more than one chromosomes in the nucleus. These chromosomes are linear and often exist in multiple copies called homologous. Animal cells reproduce asexually by mitosis and sexually by meiosis , followed by the fusion of gametes. Bacterial cell is a prokaryotic cell. Animal cell is a eukaryotic cell. Bacterial cells are 0. Cell wall Bacterial Cell: The bacterial cell wall is made up of murein. Animal cells do not have a cell wall. The plasma membrane is the outer boundary. Bacterial cells consist of several shapes like coccui, bacillus, vibrio, spirilla. Animal cells are irregular in shape due to the lack of a cell wall. Cell Nucleus Bacterial Cell: Bacterial cells do not possess a nucleus. Animal cells are composed of a membrane-bound nucleus. Bacterial cytoplasm has plasmids. Animal cells do not have plasmids. Animal cells contain mitochondria in the cytoplasm. Bacterial cells contain 70S, small ribosomes. Animal cells contains 80S, larger ribosomes. Bacterial cells do not contain centrioles. Animal cells contain centrioles. Bacterial cells do not contain lysosomes. Animal cells contain lysosomes. Bacterial cells can be either heterotrophs or autotrophs. Animal cells reproduce asexually by mitosis and sexually by meiosis, followed by the fusion of gametes. Conclusion Bacterial cells and animal cells can be considered as independent units, carrying out cellular metabolism and reproduction without the aid of other cells. Bacterial cells contain a primitive origin compared to animal cells. Also, animal cells contain membrane-bound organelles like nucleus, mitochondria, Golgi apparatus and ER. On the contrary, bacterial cells lack membrane-bound organelles. Bacterial chromosomes are localized to an area in the cytoplasm called as nucleoid. The main difference between bacterial cell and animal cell is their cellular organization. Accessed 01 March 3. Wikipedia, the free encyclopedia, Accessed 01 March Image Courtesy: Image renamed from Image:

Chapter 3 : Biologicals from Recombinant Microorganisms and Animal Cells: Production and - Google Boo

On the other hand, important cell-mediated immunity against intracellular pathogens which in most cases leads to chronic infections is much more difficult to obtain using current vaccine strategies. The live attenuated pathogen vaccines, which are capable of eliciting this type of response, although not often, may offer potential risks that cannot be overlooked, such as virulence in susceptible hosts and potential reversal of attenuation. Recombinant protein vaccines permit the avoidance of several potential concerns raised by vaccines based on purified macromolecules, such as the risk of co-purification of undesired contaminants or reversal of the toxoids to their toxigenic forms, if considering diphtheria or tetanus toxoid vaccines, for example. Another fundamental issue overcome by this technology is the complexity involved in obtaining sufficient quantities of purified antigenic components. However, one of the main challenges in the development of these new strategies of immunization consists of designing vaccines that elicit the appropriate kind of immune response to confer immunity mainly to intracellular pathogens and especially to those that establish chronic, often lifelong infections. For this, the knowledge of the biology of highly conserved antigens involved in pathogenesis and of the immune mechanisms that should be elicited for protection must be obtained to rationally design vaccine strategies that can overcome the low protective immunity naturally generated by infection reviewed in Ref. Substantial efforts have been made towards the identification of protective antigens, which have been selected by several rational and experimental approaches 5 , 6. However, the use of these antigens as vaccines goes beyond their discovery. The use of novel biotechnological tools has provided a new arsenal of strategies and possibilities to the field of vaccinology. Here we review some of these strategies being currently used and discuss their potential for the generation of new human vaccines, as well as the challenges that remain to be solved for their development and use 5. Recombinant vaccine strategies Several genes from different etiologic agents have been cloned, expressed and purified to be tested as vaccines. There are a variety of expression systems for antigenic protein components, such as bacteria, yeast, mammalian cells and insect cells, in which the DNA encoding the antigenic determinant can be inserted and expressed. However, several factors must be taken into account before selecting the system for antigen expression. The level of expression obtained using each specific expression vector and promoter, the selection marker of choice, the presence or absence of post-translational modification by the recombinant vector, among others, are essential features that interfere in the efficacy of production of recombinant antigens as vaccines. Bacterial expression systems are the most used due to the ease of handling and to their capacity for high level expression. However, for antigens in which post-translational modifications e. Recombinant protein vaccines Most of the vaccines under investigation today are based on highly purified recombinant proteins or subunits of pathogens 9. The classical example of recombinant protein vaccines currently in use in humans is the vaccine against hepatitis B Table 1 Hepatitis B virus HBV infection is a chronic liver disease occurring worldwide. HBV presents a marked tropism for human liver cells, partially due to a specific receptor that is expressed on the surface of infected cells. The current vaccines are produced by expressing the hepatitis B surface antigen HBsAg in yeast cells. The yeast expression system may secrete the antigen into the culture supernatant that can facilitate its purification 11 , Furthermore, yeast cells offer some of the eukaryotic cellular machinery responsible for the post-translational modification of proteins, being capable of rendering proteins glycosylated. The technology of production of the HBV vaccine has been transferred to several manufacturers and the prices have decreased due to competition, which has rendered this vaccine affordable to most developing countries. A more recently developed example of recombinant vaccine is the vaccine against human papillomaviruses HPVs 13 Table 1. HPV is one of the most common sexually transmitted diseases and this infection is associated with many types of mucocutaneous diseases in humans, including cervical, vulva,

and vaginal cancers, and genital warts. These vaccines utilize the L1 recombinant proteins of each subtype, produced either in yeast or in an insect-cell system. The L1 is the major capsid protein and its expression in vitro results in the assembly of VLPs. The vaccines are given in a three-dose regimen, using aluminum potassium sulfate as adjuvant, which induces high titers of virus-neutralizing serum antibodies These vaccines are proprietary and extremely expensive, and therefore will have limited accessibility for low-income countries for some time. Even though vaccines based on recombinant proteins offer several advantages when compared with traditional vaccines, such as safety and production cost, most of them present weak or poor immunogenicity when given alone, and thereby require the use of adjuvants to elicit a protective and long-lasting immune response The successful use of recombinant proteins as vaccines, including hepatitis B and, more recently, HPV, was possible due to the use of aluminium salt as adjuvant 9 , Therefore, the investigation of new adjuvants is an extremely important field in vaccinology. The main difficulties for the development of new adjuvants involve understanding their molecular complexity and the mechanisms by which they operate to stimulate or induce the immune response. For example, the mechanism of action of the aluminum salts, which are the most commonly used adjuvants in human and animal vaccines worldwide, remains unknown. An alternative path for antigen presentation has been the use of live vectors, such as bacteria and viruses, in which their natural adjuvant properties are explored. Formulation and safety, among other concerns, are also important aspects to be considered Live recombinant vaccines using bacterial or viral vectors As a result of advances in the fields of molecular biology and genetic engineering it is now possible to create live recombinant vectors capable of delivering heterologous antigens by the introduction of antigen-encoding genes. The idea behind this approach is to use the capacity of infection and the immunological properties of the live vector to elicit an immune response against its own proteins, as well as towards the heterologous protein being presented The use of live-attenuated bacterial vaccines is not novel. However, their utilization as carriers or delivery vehicles for heterologous antigen expression represents a technology with broad applicability that may have a significant impact on vaccine development. Significant advances in molecular biology have enabled precise deletions of genes encoding important virulence factors, as well as the introduction of recombinant DNA into avirulent yet immunogenic vaccine strains. Bacterial vectors have many advantages that make them attractive systems for heterologous antigen presentation. Most are antibiotic-sensitive strains, which allow antibiotic treatment if any adverse reaction occurs. In general, they display very favorable cost-effectiveness 9. Several bacteria have been used as vectors, such as Mycobacterium bovis BCG 18 , Listeria monocytogenes 20 , Salmonellae spp 17 and Shigellae spp 21 , all of which have been shown to be capable of eliciting immune responses against important viral, bacterial, protozoan, and metazoan pathogens in animal models 9. Although such bacterial vectors present similar features, they have distinct characteristics that should be considered before making a choice for any one of them. Among these bacterial vectors, M. BCG offers several features that render it an attractive vaccine vector. It is safe and has been administered to over 3 billion individuals with minimum side effects, it can be administrated soon after birth, it is a potent adjuvant, and it provides the possibility of generating T cell-mediated immunity against the cloned heterologous antigen. This last feature is considered to be an essential element of a successful vaccine against intracellular pathogens. These were demonstrated to induce both humoral and cellular immune responses and, in some cases, protection against challenge with the infective microorganism 18 , This strain was shown to induce a cellular immune response in adult and neonate mice that protected them against a lethal challenge with virulent B. An antibiotic-free strain has been constructed by autotrophic complementation to be investigated in clinical trials Recently, many studies have focused on the use of rBCG as a means of increasing the protection against TB 28 , Recombinant BCG expressing important M. The idea was to improve the BCG vaccine by overexpressing an immunodominant antigen that had been demonstrated to be protective. Another BCG-based vaccine that is also in clinical trials involves a more sophisticated approach in technical terms. TB vaccines based on the attenuation of M. However, this strategy has occasionally rendered M. In general, similar approaches could be applicable to

recombinant S. Another important class of presentation systems for heterologous antigens is based on viral vectors. The use of viral vectors in vaccine development has been recently reviewed 19 , 38 , Vaccines based on viral vectors represent an important strategy against infectious diseases caused by intracellular pathogens, partially due to the fact that they localize in the same compartment that may mimic a natural viral infection. By delivering antigens within the host cells, processing and antigen presentation via major histocompatibility complex MHC class I molecules on the surface of infected cells can occur, facilitating the induction of cellular immune responses, which are known to be important in the control of intracellular infections. There are a wide variety of viral vectors under investigation as vaccine delivery vehicles. Some characteristics are desirable for a virus to be used as a delivery vehicle, such as: There are many differences between the viral vectors available. They can be classified according to the virion type DNA or RNA , particle size, transgene capacity, and cell tropism 40 , Viral vectors can be replicating or non-replicating viruses; the replication-defective viruses being the most tested in clinical trials, partly due to their higher safety. However, some groups are focusing on the use of replicating vectors in clinical trials as they are more likely to elicit stronger cellular and mucosal immune responses, as well as antibodies against the expressed proteins, depending on their cell tropism and sites of replication Several studies have demonstrated that recombinant viral vectors encoding genes from important pathogens, such as malaria, HIV and TB are able to induce both humoral and cell-mediated immune responses against their expressed antigens in immunized animals and, in some cases, may even protect the animals from lethal challenge 19 , Co-expression of immunomodulating cytokines in viral vectors has also been used in order to enhance their immunogenicity, also with the above-cited restrictions This strategy has been used extensively in the development of vaccines against HIV. Similar to other viral vaccines or viral vector-based vaccines developed, a vaccine against HIV infection could be devised based on its attenuation. However, due to the possible risk of reversion or recombinant events, which can lead to a pathogenic HIV phenotype, vaccines based on HIV virus attenuation have been avoided. Therefore, live recombinant viral vectors such as Ad and MVA have been proposed as safer and less concern raising approaches. Ad and MVA are among the most promising live viral vector systems and, besides having been tested as vaccines against HIV 19 , are currently being used in clinical trials against other important infectious diseases such as TB 43 and malaria Adenoviruses are non-enveloped icosahedral viruses containing a linear double-stranded DNA in their genome, which can infect and replicate in different locations in the body, such as the respiratory tract and the urinary bladder. There are over 50 subtypes of human Ad, with Ad serotype 5 Ad5 being the best characterized and most used in several vaccination trials. Ad5 is a stable, non-replicating virus, characteristics that contribute to its safe application. Replication-competent adenovirus vectors are also under development as vaccine carriers for HIV. The advantages of this type of adenoviruses vector are the lower doses necessary for inducing immune responses and longer persistence in the host, which may be associated with a more prolonged immune response. Noteworthy, both systems elicit a potent and long-lasting immune response carrying the same gene inserts Antigens from HIV such as gag, pol, env, and nef have been expressed in adenovirus vectors, particularly Ad5, showing promising results in diverse animal models and in phase I trials However, the same results were not observed in phase II trials in humans USA has been considered to be the most promising vaccine against HIV-1 to date; however, the clinical trial of this vaccine was interrupted after it was demonstrated not to be protective against HIV infection. Moreover, an increase was observed in the rate of HIV infection in vaccinees that had pre-existing immunity to Ad5 New immunization strategies have been developed to overcome this problem, including the use of other adenovirus serotypes and heterologous vector prime-boost regimens reviewed in Ref. Similar to Ad5 and other viral vaccine vectors, MVA has also been used as a vaccine platform in the development of HIV and TB vaccines, as well as for other infections. MVA is an attenuated strain derived from vaccinia virus, which was obtained after passages in chicken embryo fibroblasts. This process resulted in several deletions, making the MVA strain unable to replicate in mammalian cells and inefficient in evading the immune system of the host. In addition, this process modified the host range of the virus. Even though promising results have been obtained

using vaccines based on viral vectors in recent clinical trials, the use of this technique alone has not been shown to be sufficient to induce a protective immune response. Other approaches are therefore under investigation to be used in combination with this technique. A promising technique that will be discussed in more detail below is called heterologous prime boost. It combines the use of two methods of immunization sequentially, for example, first an immunization with viral vectors, followed by a recombinant protein or live bacterial vaccines. DNA vaccines The direct injection of a naked DNA plasmid into muscle as a vaccine system with the ability to induce an immune response and protection after challenge is now well established, since this approach has been used to express numerous antigens from different pathogens with promising results 50 - A DNA vaccine or genetic vaccine as it is also called consists of a plasmid containing:

Chapter 4 : An Introduction to Recombinant DNA

Biologicals from recombinant microorganisms and animal cells: production and recovery: 34th Oholo conference on "novel strategies in production and recovery of biologicals from recombinant microorganisms and animal cells": Papers.

Before we get to the "r" part, we need to understand DNA. DNA is the keeper of the all the information needed to recreate an organism. All DNA is made up of a base consisting of sugar, phosphate and one nitrogen base. There are four nitrogen bases, adenine A , thymine T , guanine G , and cytosine C. The sugar used in DNA is deoxyribose. The four nitrogen bases are the same for all organisms. The sequence and number of bases is what creates diversity. This leads to either a different protein, or an inactive protein. Now that we know what DNA is, this is where the recombinant comes in. Thus, the name recombinant! Recombinant DNA is also sometimes referred to as "chimera. The most common recombinant process involves combining the DNA of two different organisms. How is Recombinant DNA made? There are three different methods by which Recombinant DNA is made. Each are described separately below. Transformation The first step in transformation is to select a piece of DNA to be inserted into a vector. The insert contains a selectable marker which allows for identification of recombinant molecules. An antibiotic marker is often used so a host cell without a vector dies when exposed to a certain antibiotic, and the host with the vector will live because it is resistant. The vector is inserted into a host cell, in a process called transformation. One example of a possible host cell is E. The host cells must be specially prepared to take up the foreign DNA. Selectable markers can be for antibiotic resistance, color changes, or any other characteristic which can distinguish transformed hosts from untransformed hosts. Different vectors have different properties to make them suitable to different applications. Some properties can include symmetrical cloning sites, size, and high copy number. This is a process very similar to Transformation, which was described above. The only difference between the two is non-bacterial does not use bacteria such as E. Coli for the host. In microinjection, the DNA is injected directly into the nucleus of the cell being transformed. In biolistics, the host cells are bombarded with high velocity microprojectiles, such as particles of gold or tungsten that have been coated with DNA. Phage Introduction Phage introduction is the process of transfection, which is equivalent to transformation, except a phage is used instead of bacteria. In vitro packagings of a vector is used. This uses lambda or MI3 phages to produce phage plaques which contain recombinants. The recombinants that are created can be identified by differences in the recombinants and non-recombinants using various selection methods. How does rDNA work? Recombinant DNA works when the host cell expresses protein from the recombinant genes. A significant amount of recombinant protein will not be produced by the host unless expression factors are added. Protein expression depends upon the gene being surrounded by a collection of signals which provide instructions for the transcription and translation of the gene by the cell. These signals include the promoter, the ribosome binding site, and the terminator. Expression vectors, in which the foreign DNA is inserted, contain these signals. Signals are species specific. In the case of E. Coli, these signals must be E. Coli signals as E. Coli is unlikely to understand the signals of human promoters and terminators. Problems are encountered if the gene contains introns or contains signals which act as terminators to a bacterial host. This results in premature termination, and the recombinant protein may not be processed correctly, be folded correctly, or may even be degraded. Production of recombinant proteins in eukaryotic systems generally takes place in yeast and filamentous fungi. The use of animal cells is difficult due to the fact that many need a solid support surface, unlike bacteria, and have complex growth needs. However, some proteins are too complex to be produced in bacterium, so eukaryotic cells must be used. Why is rDNA important? Recombinant DNA has been gaining in importance over the last few years, and recombinant DNA will only become more important in the 21st century as genetic diseases become more prevelant and agricultural area is reduced. Hepatitis B Prevention and cure of sickle cell anemia Prevention and cure of cystic fibrosis Production of clotting factors.

Chapter 5 : Difference Between Bacterial Cell and Animal Cell | Cellular Structure, Classification, Metabolis

Recombinant DNA and biotechnology can be used to form proteins not normally produced in a cell to make drugs or vaccines or to promote human health. In addition, bacteria that carry recombinant DNA can be released into the environment under carefully controlled conditions to increase the fertility of the soil, serve as an insecticide, or.

Every living organism is made up of cells or just one in the case of bacteria. Your body is composed of microsopic cells that are only visible if viewed under a microsocope. All the animals, trees and plants are made up of cells that share many similar characteristics. They all have cell membrane s separating them from the outside environment, DNA to store information, RNA to pass this information to the ribosome- and ultimately protein that is translated from the RNA. The cell membrane , or plasma membrane separates the cell from exterior environment and is composed of a phospholipid bilayer. It is composed of phospholipids which each have a polar hydrophillic head and a polar hydrophobic tail. The polarity of the phospholipids helps them self assemble into a structure where the hydrophobic tails all face inward away from the aqueous interior and exterior of the cell. Transmembrane proteins pass all the way through the membrane, while peripheral proteins only pass through one side of the bilayer. Transmembrane proteins are often are involved in the transport of compounds and nutritents across the lipid bilayer since only small hydrophobic molecules, water and gas can diffuse freely through the hydrophobic interior. Organelles of the Cell Eukaryotic cells all organisms except bacteria and archaea prokaryotes have complex organelles which are surrouned by their own membrane similar to the cell membrane. Each cell has one nucleus. Within the nucleus is a structure called the nucleolus which is the site of ribosome assembly. The image below is an image of a cell with the nucleus stained blue and the multiple mitochondria stained red Mitochondria - often refered to as the "powerhouse" of the cell, this is the organelle that generates ATP the energy currency of the cell. Mitochondria have a highly folded inner membrane that provides surface area for the enzymatic reactions that produce ATP. The interior of the two membranes is called the matrix, the space in between the two membranes is called the intermembrane space and the folds created by the inner membrane are called cristae. Mitochondria also contain their own DNA which encodes some of the enzymes that are used inside the mitochondria. Endoplasmic reticulum - the system of membranes used for the folding and transport of proteins. Golgi apparatus - used for modifying and packaging of proteins Chloroplast - in plants this organelle is responsible for the reactions of photosynthesis Cell Parts There are other important components of the cell that are not considered organelles since they are not surrounded by their own lipid bilayer. Lysosomes - where the breakdown of nutrients can occur using enzymes Cell Membrane - this is the structure composed of a lipid bilayer that separates the cell from the outside environment Cell Wall - found only in plant and bacteria this structure is found outside the cell membrane and serves as a more rigid protective barrier Differences Between Eukaryotes and Prokaryotes Bacteria and archaea which are seldomly mentioned are prokaryotes. The term prokaryotes is derived from pro before and karya nucleus: This is because it is thought that bacteria are still very similar to their primitive ancestors which did not have a nucleus. So- bacteria prokaryotes do not have a nucleus, while all eukaryotic cells do have a nucleus this is a popular question for exams- and a common mistake. Bacteria also lack all other membrane bound organelles. Bacteria do not have:

Chapter 6 : Types of Recombinant Vaccines: 3 Types

(i) Bacterial cells or plant cells or animal cells are treated with enzymes like lysozyme for bacteria cellulose for plant cells and chitinase for fungus. This will break the cell envelope open and like DNA, RNA, proteins, polysaccharides and lipids will be released.

Gene gun or biolistic delivery involves pressure delivery of DNA-coated gold beads. DNA Vaccine and Immunity: An illustration of a DNA vaccine and the mechanism of its action in developing immunity is given in Fig. The plasmid vaccine carrying the DNA gene for antigenic protein enters the nucleus of the inoculated target cell of the host. The antigen can act directly for developing humoral immunity or as fragments in association with major histocompatibility class MHC molecules for developing cellular immunity. As the antigen molecules bind to B- lymphocytes, they trigger the production of antibodies which can destroy the pathogens. Some of the B-lymphocytes become memory cells that can protect the host against future infections. The protein fragments of the antigen bound to MHC molecules can activate the cytotoxic T-lymphocytes. They are capable of destroying the infected pathogenic cells. Some of the activated T-lymphocytes become memory cells which can kill the future infecting pathogens. Some workers have successfully used cDNA as vaccines e. DNA vaccines for production of antigens and antibodies: A novel approach for the production of antigens as well as antibodies by DNA vaccine was developed in In the experiments conducted in mice, researchers injected plasmids containing the genes for malarial parasite and also the genes for the antibodies against malarial parasite. The B-lymphocytes 4 of these mice performed a double duty, and produced antigens for and antibodies against malarial parasite. The antigens stimulate to produce more and more antibodies. The antibodies so produced react with malarial parasite. The generation of antigens and antibodies by using a DNA vaccine is a recent development in immunology, and is referred to as antigenic antibody approach of DNA vaccine. Screening of pathogenic genome for selecting DNA vaccines: The ultimate goal of scientists is to choose the right DNA fragment from the pathogen to serve as a vaccine for the strongest immune response against the invading pathogen. The immune response for each one of the DNA vaccines can be studied by injecting the pathogen. By screening the DNA fragments of the pathogenic genome, it is possible to choose one or few DNA vaccines that can offer maximal immune protection. Advantages of DNA vaccines: There are several advantages of using DNA vaccines in immunization: The tedious and costly procedures of purifying antigens or creating recombinant vaccines are not necessary. DNA vaccines are very specific in producing the target proteins antigens or antibodies. Thus, they trigger immune response only against the specific pathogen. In general, DNA vaccines elicit much higher immune response compared to other kinds of vaccines. DNA vaccines are more stable for temperature variations low or high than the conventional vaccines. Thus, the storage and transport problems associated with vaccines are minimal. The delivery methods to the host are simpler for DNA vaccines. Disadvantages of DNA vaccines: The fate of the DNA vaccine in the host cells is not yet clear. There is a possibility of this DNA getting integrated into the host genome and this may interrupt the normal functions. There also exists a danger of cancer due to DNA vaccines. The post-translational modification of the gene DNA vaccine product in host cells may not be the same as that found in the native antigen. Present status of DNA vaccines: Since , several groups of workers world-over have been trying to develop DNA vaccines against various diseases in experimental animals. Genetic immunization has been done against a number of pathogenic organisms. These include influenza A virus, rabies virus, hepatitis B virus, bovine herpes virus, HIV type I, and Plasmodium species malarial parasite. It must be noted that DNA vaccines have not been tried in humans for obvious reasons. The most important being the unknown risks of these foreign DNAs in human subjects. Several workers are trying to use RNA molecules as vaccines. These RNAs can readily synthesize the antigenic proteins and offer immunity. This poses a big problem for RNA vaccine manufacture and distribution. Plants as Edible Subunit Vaccines: Plants serve as a cheap and safe production systems for subunit vaccines. The

edible vaccines can be easily ingested by eating plants. This eliminates the processing and purification procedures that are otherwise needed. Transgenic plants tomato, potato have been developed for expressing antigens derived from animal viruses rabies virus, herpes virus. A selected list of recombinant vaccines against animal viruses produced in plants is given in Table Edible vaccine production and use: The production of vaccine potatoes is illustrated in Fig. The bacterium, Agrobacterium tumefaciens is commonly used to deliver the DNA genetic material for bacterial or viral antigens. A plasmid carrying the antigen gene and an antibiotic resistance gene are incorporated into the bacterial cells A. The cut pieces of potato leaves are exposed to an antibiotic which can kill the cells that lack the new genes. The surviving cells i. This callus is allowed to sprout shoots and roots, which are grown in soil to form plants. In about three weeks, the plants bear potatoes with antigen vaccines. The first clinical trials in humans, using a plant- derived vaccine were conducted in This involved the ingestion of transgenic potatoes with a toxin of E. In the early years of vaccine research, attenuated strains of some pathogenic organisms were prepared by prolonged cultivation â€" weeks, months or even years. Although the reasons are not known, the infectious organism would lose its ability to cause disease but retains its capability to act as an immunizing agent. This type of approach is almost outdated now. It is now possible to genetically engineer the organisms bacteria or viruses and use them as live vaccines, and such vaccines are referred to as attenuated recombinant vaccines. The genetic manipulations for the production of these vaccines are broadly of two types: Deletion or modification of virulence genes of pathogenic organisms. Genetic manipulation of non-pathogenic organisms to carry and express antigen determinants from pathogenic organisms. The advantage with attenuated vaccines is that the native conformation of the immunogenic determinants is preserved; hence the immune response is substantially high. This is in contrast to purified antigens which often elicit poor immunological response. Some of the important attenuated vaccines developed by genetic manipulations are briefly described. Cholera is an intestinal disease characterized by diarrhea, dehydration, abdominal pain and fever. It is caused by the bacterium, Vibrio cholera. This pathogenic organism is transmitted by drinking water contaminated with fecal matter. Cholera epidemics are frequently seen in developing countries where the water purification and sewage disposal systems are not well developed. On entering the small intestine, V. This enterotoxin stimulates the cells lining intestinal walls to release sodium, bicarbonate and other ions. Water accompanies these ions leading to severe diarrhea, dehydration, and even death. The currently used cholera vaccine is composed of phenol-killed V. The immuno-protection, lasting for months is just moderate. Attempts are being made to develop better vaccines. The DNA technologists have identified the gene encoding enterotoxin toxic protein. Enterotoxin, an hexamer, consists of one A subunit and five identical B subunits. The A subunit has two functional domains-the A1 peptide which possesses the toxic activity and A2 peptide that joins A subunit to B subunits. By genetic engineering, it was possible to delete the DNA sequence encoding A1 peptide and create a new strain of V. This strain is non-pathogenic, since it cannot produce enterotoxin. The genetically engineered V. Creating a new strain of V. The development of a new strain of Vibrio cholera that can effectively serve as an attenuated recombinant vaccine is depicted in Fig. A tetracycline resistance gene was inserted into the A1 peptide sequence of V. This destroys the DNA sequence encoding for A1 peptide, besides making the strain resistant to tetracycline. Unfortunately, the tetracycline resistant gene is easily lost and the enterotoxin activity is restored. Because of this, the new strain of V. The DNA sequence of A1 peptide is incorporated into a plasmid, cloned and digested with restriction enzymes Clal and Xbal. In this manner, the A1 peptide coding sequence is deleted the DNA encoding for of the amino acids of the A1 peptide is actually removed. By using T4 DNA ligase, the plasmid is re-circularized. This plasmid contains a small portion of A1 peptide coding sequence. The plasmid, containing the deleted A1 peptide sequence is transferred by conjugation into the V. Recombination can occur between the plasmid containing a small portion of peptide A1 coding sequence and the chromosome of V.

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a DNA fragment can be replicated inside a bacterial cell; a double-stranded recombinant plasmid DNA introduced into a bacterial cells, they are grown in cell culture, producing hundreds of millions of new bacteria, and many copies of purified plasmid are isolated from lysed bacteria;.

Blood products and related biologicals Cell substrates A number of biological medicinal products are derived from cells that either: Traditionally animal cells have been used for the production of viral vaccines, although one of the commercially available recombinant human papillomavirus virus-like particle vaccines is produced in insect cells. Cell substrates may be microbial cells e. Within the animal cell group, there are a number of cell types use for production: Cell substrates also differ in their host range ability to be infected or transfected by microorganisms and their degree of differentiation. Primary cells are obtained by dissociating cells from specific tissues, and many vaccines continue to be produced on such highly differentiated cell substrates e. Diploid cell lines contain the full complement of genetic material, often retain many characteristics of the cell types from which they were derived, and normally cease to replicate in cell culture conditions after a limited number of generations. On the other hand, continuous cell lines replicate in cell culture for indefinite periods, but often lose the characteristics of their parent cells. To reduce variability and avoid potential contaminants, various diploid and continuous cell lines have been established and characterized. Because these well characterized cells can be stored, they have improved the consistency of production of biological products including vaccines. Cell substrates differ in the degree to which they can be adapted to grow in defined culture media such as serum-free media. This is desirable for production in order to avoid potential contaminants from serum. Cell substrates also have different characteristics regarding their adhesiveness to solid supports. For example, some cell substrates can grow on microcarrier beads suspended in a large fermenter, a technique capable of generating high yields of consistent products. The challenge in standardizing cell substrates is to balance the desire for a very efficient production system with the goal of minimizing risks. The objectives of cell line characterization are to confirm the identity and purity of the cell substrate and to provide a high level of confidence in its safety as a component in the manufacture of biologicals. To facilitate this, WHO regularly reviews current practices in the use of cells as production substrates, evaluates the risks inherent in specific cell types, and makes recommendations to manufacturers and regulatory authorities on their correct handling and use. WHO has generated stocks of the widely used Vero cell line Vero for use as a cell seed from which master cell banks may be established through additional qualification. This is distributed to qualified vaccine manufacturers upon request. Cell Substrates standardization Written Standards The first WHO requirements for cell cultures used in the production of biologicals were formulated in as part of the production of inactivated poliomyelitis vaccine. The revision describes the characterization and testing of continuous-cell line and diploid cell substrates, with the general manufacturing requirements applicable to primary cell substrates as well. In , the ECBS adopted the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks.

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In animal cells like mouse cells, special animal viruses were used as cloning vehicles. Simian virus 40 (SV40) is one such virus, in which globin gene could be integrated. This gene integrated in SV40 could be transcribed and translated in mouse kidney cells.

DNA Fingerprinting and other Forensics applications Human identical twins are clones; the original single embryo separate to become two individuals. If the inserted gene is replicated and expressed, we can recover the cloned gene or protein product. Cloned genes have many research purposes: Humans can be treated with gene therapy: Recombinant DNA Technology 1. To make rDNA, technician selects a vector. A vector is a plasmid or a virus used to transfer foreign genetic material into a cell. Plasmids were discovered in research on reproduction of intestinal bacteria Escherichia coli. Analyzing DNA Segments 1. Mitochondria DNA sequences in modern living populations can decipher the evolutionary history of human populations. DNA fingerprinting is the technique of using DNA fragment lengths, resulting from restriction enzyme cleavage and amplified by PCR, to identify particular individuals. DNA is treated with restriction enzymes to cut it into different sized fragments. During gel electrophoresis, fragments separate according to length, resulting in a pattern of bands. DNA fingerprinting can identify deceased individuals or perpetrators of crimes 3. Genetically engineered organisms can produce biotechnology products. Organisms that have had a foreign gene inserted into them are transgenic. Bacteria are grown in large vats where they can make products such as insulin and human growth hormone, and vaccines 2. Transgenic bacteria have been produced to improve the health of plants and degrade substances, such as oil 3. Transgenic bacteria can produce chemical products, such as phenylalanine artificial sweeteners B. Foreign genes now give cotton, corn, and potato strains the ability to produce insect toxin s 2. Plants are being engineered to produce human proteins including hormones, clotting factors, and antibodies C. Transgenic Animals Animal use requires methods to insert genes into eggs of animals early in development. Using this technique, many types of animal eggs have been injected with bovine growth hormone bGH to produce larger fishes, cows, pigs, rabbits, and sheep. Cloning Transgenic Animals 1. The cloning of Dolly in showed that mammals could be cloned. Cloning of mammals involves injecting the nucleus of an adult cell into an enucleated egg. The cloned eggs begin development in vitro and are then returned to host mothers until the clones are born. A well-loved horse named Barbero breaks his leg in a race. Many people were praying for his well being and thousands of dollars were spent trying to get him to recover. Mail and flowers poured into the animal hospital and stable where Barbero lived. Alas, after a year of poor recovery, the decision was made to euthanize Barbero. The owners save sample of his DNA so that Barbero can be cloned. Do you think they should clone him? Why or why not. Melissa is a happy 5 year old who is loved by her family. She becomes ill and is diagnosed with childhood leukemia. A desperate search ensues to find a bone marrow donor whose type matches Melissa. Her family decides to clone Melissa so that her clone could be the bone marrow donor. Do you think this is a god idea? Sequencing the Bases The Human Genome Project has produced a working draft of all the base pairs in all our chromosomes. Other organisms are being sequenced to compare genes and located genes responsible for disorders. DNA profiles can determine if a person has an increased risk for a particular disease The genetic profile can be used to determine if a particular drug therapy is appropriate in a specific clinical condition. Proteomics Proteomics is the study of the structure, function, and interaction of cellular proteins. The information obtained from proteomic studies can be used in designing better drugs, and to correlate drug treatment to the particular genome of the individual. Bioinformatics Bioinformatics is the application of computer technologics to the study of the genome. Information obtained from computer analysis of the genome can show relationships between genetic profiles and genetic disorders. Gene therapy involves procedures to give patients healthy genes to make up for a faulty gene. Gene therapy also includes the use of genes to treat genetic disorders and various human illnesses. There are ex vivo outside body and in vivo inside body methods of gene therapy.

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For single-celled organisms such as yeast, and cultured cells, bacterial plasmids containing foreign genes can be transformed into the cells. The plasmid DNA gets into the nucleus, and inserts into random locations in the host cell's chromosomes.

Isolation of Genetic Material: Following steps are required for isolation of genetic material DNA in pure form: Additional Protein can be removed by treating with enzyme protease. RNA can be removed by treating with enzyme ribonuclease. Agarose gel electrophoresis is employed to check the progression of restriction enzyme digestion. DNA being negatively charged moves towards anode positive electrode. Same technique is used for vector DNA. PCR technique was developed by Kary Mullis in If one knows the sequence of at least part of a DNA segment to be cloned, a number of copies of that DNA Sent can be hugely amplified using polymerase chain reaction. The technique is based on principle that when a DNA molecule is subjected to high temperature due to denaturation the two DNA strands separate. As a result two single stranded DNA molecules appear. DNA polymerase can copy these single stranded molecules. This leads to the formation of original DNA double stranded molecule. Due to repetition of this process several copies of DNA sequences can be formed. Steps involved in PCR reaction: Basic requirements for PCR reaction are: These primers are oriented with their ends facing each other permitting formation of DNA between them. It leads to separation of two DNA strands. Next step is of annealing. In annealing two oligonucleotide primers anneal or hybridize to each of single stranded template DNA. It is followed by extension step. The primers extend towards each other so that DNA segment lying between two primers is copied. The enzyme extends the primers using the genomic DNA as template and nucleotides made available in reaction. Here DNA replication occurs several times. Segment DNA gets amplified to approximately billion times. As a result 9 billion copies can be formed. Repeated amplification is possible by use of thermostable DNA polymerase. It is isolated from bacterium, Thermus aquaticus. This enzyme remains active during high temperature induced denaturation of double helix DNA. Preparation of the Gene: Gene coining in bacteria is achieved by cleaving the purified DNA with enzyme restriction endonuclease which produces small fragments approximately 4 kilobase pairs. In cases where nucleotide or amino acid sequences are known, synthetic DNA may be produced. The vector is an agent which is used to transfer DNA into a host cell e. The vector is cut with the same enzyme restriction endonuclease as that used to generate the chromosomal DNA fragments. Those plasmids which contain an inserted fragment are called recombinant plasmid. Transformation of Host Cell: The ligated plasmid mixture is introduced into the bacterial cell where they take up DNA through transformation process. Transformation is generally carried out by placing actively growing cells of a bacterium in cold, dilute solution of CaCl2 which enhances the ability of bacterial cells to take up foreign DNA. In majority of the cases, E. There are many methods to introduce the ligated DNA into recipient competent cell. Suppose a recombinant DNA having ampicillin antibiotic resistant gene is transferred to E. When such cells are spread over agar plates containing ampicillin on transformants will grow there. Detection of the Cloned Gene Recombinants: Cells with recombinant DNA rDNA are selected on the expression or non-expression of some traits like resistance to antibiotic chloramphenicol. Direct selection of recombinants is made due to encoding of these traits by vector or cloned DNA sequence. Various methods for identification of recombinants are: Transformants host cells with foreign DNA can be selected by: It is based on basic principle that cloned DNA fragment disrupts the coding sequence of gene. To identify recombinants, one of the important approaches is to use DNA probe. In a DNA molecule, the two complementary strands are held together by hydrogen bonds. If two similar DNA pieces are mixed together and hydrogen bonds broken by heating the strands will separate. Upon lowering the temperature, the hydrogen bonds are formed again. Some of the resultant double-stranded DNA will be hybrids i. The transformed colonies are replica plated to a nitrocellulose filter and are lysed to release the DNA. This DNA is denatured by raising the temperature and fixed to the nitrocellulose so as to produce a

DNA print corresponding exactly to the position of the colonies on the original plate. The DNA print is then hybridized with the probe which has been previously radioactively labelled. After washing off unhybridized DNA, the position of the radioactive spots on the filter is indicated by autoradiography in order to identify the presence of the required DNA. Obtaining the Foreign Gene Product: Due to expression of this foreign gene, proteins can be formed. Such target proteins recombinant proteins are to be produced in large scale. The cells having cloned genes may be grown on small scale in laboratory. Such cultures are used to make proteins with required characters. For multiplication of cells continuous culture for system is used. Here medium is drained out and fresh medium is added. To produce this product in large quantities bioreactors are needed. In such bioreactors litres of culture can be processed. Most commonly used in stirring type bioreactor, whose details are given below? The basic design of a stirred-tank fermenter is shown in Fig. It consists of a large stainless steel vessel with a capacity of upto , dm3 around which there is a jacket of circulatory water used to control the temperature within the fermenter. There is also an agitator, comprising of a series of flat blades, which can be rotated with the help of a motor. This ensures the thoroughly mixing of the contents so that nutrients come in close with the micro-organisms. The agitator also prevents settling out of the cells at the bottom. Fermenter also has adequate arrangement for aeration, temperature and pH control. For proper aeration, air can be forced in at the bottom of the tank through a porous ring, called sparger, by the process called sparging, while there is an outlet to remove air and waste gases at the top of the tank. The top of the tank also a number of inlet tubes called ports, through which materials can be introduced or withdrawn e. Inoculation port for introducing initial inoculum; ii. Nutrient port for introducing more nutrients; iii. Antifoam port for introducing antifoaming agents; and iv. At the base of the tank, there is a harvest line to extract culture medium and microbial products. To regularly detect the pH and temperature changes, tank is fitted with certain probes. The stirred-tank fermenter is a well- tried and tested design for large-scale production of micro-organisms under aseptic and controlled environment for a number of days. Small-scale fermenters of litres capacity are used in research laboratories. It is also provided with many controls for the monitoring of physical, chemical and biological parameters that affect the growth of cells. It is relatively costly to run largely due to high energy requirements to drive the agitators and introduce the compressed air. Products formed are separated and purified. Steps are collectively called as down streaming processing. Suitable preservatives are used. For medicinal purposes, clinical trials are carried out. Quality control is also maintained.