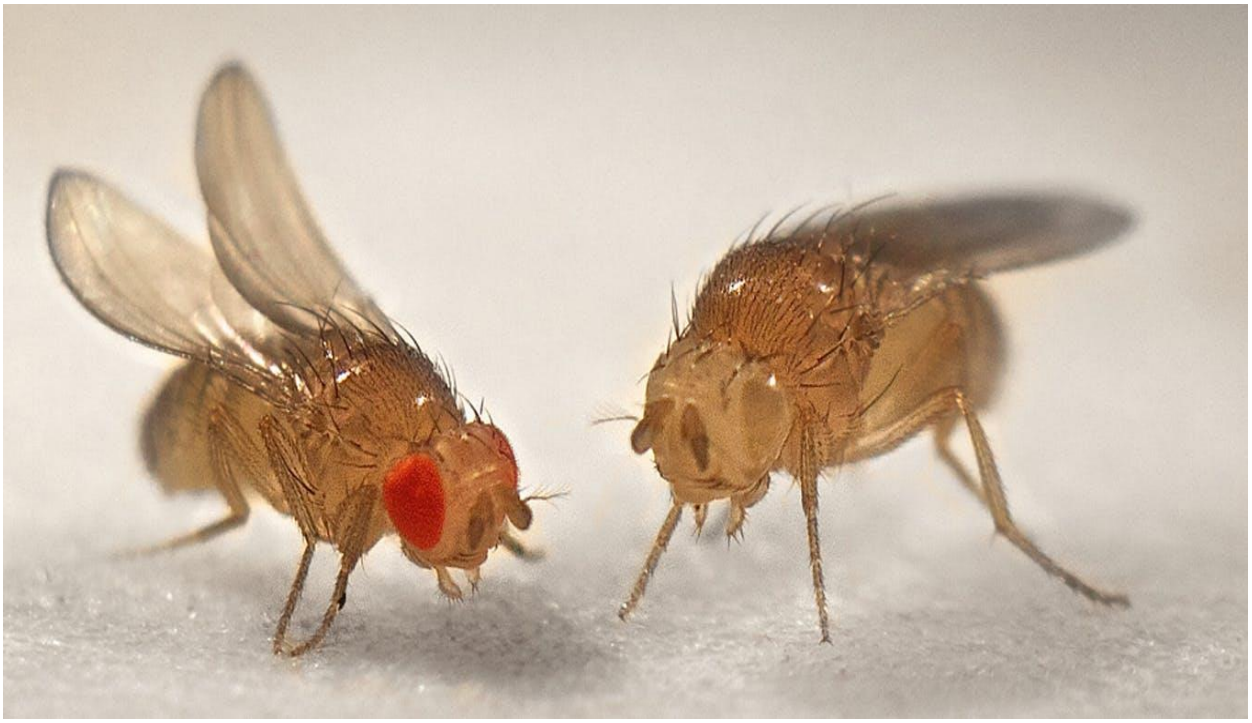
A project on

Cloning of the scarlet Gene of Drosophila melanogaster



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Introduction

The birth of Dolly the sheep in 19961 heralded an explosion in the cloning of animals. Within the past decade we have seen the production of a cloned mouse,cow, goat, pig, rabbit, cat, zebrafish, mule, horse, rat and dog. Decades before Dolly, frogs and mice derived from transplanted embryonic cells, illustrated the totipotency of embryonic nuclei, however most assumed that the epigenetic restriction of cell fates during mammalian development was not flexible enough to allow for the production of a viable adult from a differentiated somatic cell. With the successful cloning of a sheep from an adult somatic cell this assumption was removed1 and epigenetics and nuclear transplanta- tion technology were catapulted into the forefront of developmental, biotechnological and medical research. Nuclear transplantation is the basis of both reproductive and therapeutic cloning. Reproductive cloning allows for the generation of live offspring possessing the complete and unrecombined genetic material of an existing animal. In the decade following the production of Dolly the sheep, reproductive cloning of mammals engineered to produce biologically important compounds has become an established technology and has allowed for the reconstitution of an organism from cryogenically frozen nuclei. Although reports of the generation and birth of human clones remain unsubstantiated, there is no obvious technical barrier to the creation of viable cloned human embryos through nuclear transplantation. Therapeutic cloning, the culturing of cloned embryos to produce individually tailored stem cells, tissues or organs, represents the forefront of medical research. To date, various tissues and organs, including skin, liver, kidney, cornea and breast, have been grown from stem cells., In the longer term, cloning may provide a unique opportunity to readdress classical lines of biological inquiry such as the origin of sexual reproduction, the ongoing debate over the contributions of nature versus nurture to one's individuality and the nature of individuality itself. For all its promise, cloning is an inefficient technique and organisms generated by nuclear transplantation often suffer from a high rate of associated defects, in part due to the loss of epigenetic gene silencing. Thus, the rapid progress in nuclear transplantation technology has meant that the question "should we clone?" has been replaced by a new question - "can we clone effectively?" Examination of the ethical issues raised by cloning technology is a profession in itself; one that has called for a universal moratorium on new biotechnologies until these issues can be satisfactorily addressed.

CLONING at the organismal level refers to the creation of a genetically identical individual from an existing individual, generally through nuclear transfer. This technology can be exploited to create stem cells for use in therapeutic cloning and is being used to increase the production of transgenic mammals producing pharmacologically important compounds. In many cases, the technology is constrained by a lack of fundamental understanding of the nuclear reprogramming events that occur following transplantation, resulting in a high frequency of developmental defects in the cloned offspring. We have successfully used embryonic nuclear transfer to create viable adult Drosophila clones. Embryos that hatch but fail to develop to adulthood exhibit characteristic developmental defects; hence we can potentially use this system to identify gene mutations or conditions that encourage complete nuclear reprogramming. The developmental programming of nuclei is a fundamental epigenetic process based, in part, on histone

modification and packaging so the events involved in nuclear reprogramming in Drosophila are likely conserved across taxa. The method outlined herein provides a straightforward, cost-effective means of studying the effects of epigenetic interactions on nuclear transplants.

Host embryos laid by white-eyed w^{1118} females were fertilized by homozygous *ms(3)K81* males. These males generate sperm incapable of participating in pronuclear fusion and thus the resulting embryos are unable to complete embryogenesis under control of their own DNA. Embyros donating nuclei possessed green-fluorescent-protein-labeled histon so donor nuclei were easily distinguishable from those of the host. Less than 2 μl of cytoplasm was aspirated from preblastoderm stage embryos 70–100 min after egg laying. Nuclei were drawn laterally from the ventral face of the embryo and 5–15 nuclei were transplanted to the ventral area of a 10- to 30-min-old recipient embryo. Nuclei drawn from a single donor embryo were injected into one to six recipients, potentially allowing for the generation of more than one clone from a single donor embryo. In the trial reported here, two of the five adult clones, both females, originated from adjacent embryos, suggesting that they may have been derived from one donor. Recipient embryos were incubated at 18° until the completion of embryogenesis at which point larvae were raised on standard Drosophila culture medium.

Cloning

Cloning is the process of producing individuals with identical or virtually identical DNA, either naturally or artificially. In nature, many organisms produce clones through asexual reproduction. Cloning in Biotechnology refers to the process of creating clones of organisms or copies of cells or DNA fragments.

Why cloning is used

Cloning is commonly **used to amplify DNA fragments containing whole genes**, but it can also be used to amplify any DNA sequence such as promoters, non-coding sequences and randomly fragmented DNA.

Mainly cloning is used for modify or to increase the productivity of a animal, tree, bacteria and organism.

CAUSE OF TAKING DOSOPHILLA:-

The fruit fly Drosophila melanogaster is a versatile model organism that has been used in biomedical research for over a century to study a broad range of phenomena. There are many technical advantages of using Drosophila over vertebrate models; they are easy and inexpensive to culture in laboratory conditions, have a much shorter life cycle, they produce large numbers of externally laid embryos and they can be genetically modified in numerous ways. Research using Drosophila has made key advances in our understanding of regenerative biology and will no doubt contribute to the future of regenerative medicine in many different ways. Many obvious practical and ethical obstacles severely limit the scope for experiments using humans in biomedical science, thus much of what we know about the underlying biology of cells and tissues comes from studies using model organisms such as mice, and the focus of this article, the fruit fly Drosophila melanogaster. Drosophila has been used productively as a model organism for over a century to study a diverse range of biological processes including genetics and

inheritance, embryonic development, learning, behavior, and aging. Although humans and fruit flies may not look very similar, it has become well established that most of the fundamental biological mechanisms and pathways that control development and survival are conserved across evolution between these species. The first documented use of Drosophila in the laboratory was by William Castle's group at Harvard in 1901, although the "father" of Drosophila research is undoubtedly Thomas Hunt Morgan1. Morgan greatly refined the theory of inheritance first proposed by Gregor Mendel, by using Drosophila to define genes and establish that they were found within chromosomes (long before it was even established that DNA is the genetic material). Morgan won the Nobel Prize in Physiology or Medicine in 1933 "for his discoveries concerning the role played by the chromosome in heredity"2. One of Morgan's protégés, Hermann Muller, won the Nobel Prize in Physiology or Medicine in 1946 "for the discovery of the production of mutations by means of x-ray irradiation"3. Using Drosophila in the 1920s, Muller discovered that x-rays caused a massive increase in the mutation rate of genes, and could actually break chromosomes4. Although irradiated flies looked normal, their offspring . frequently showed the effects of mutation. This led to the realization that radiation causes harmful genetic defects in the offspring of exposed humans – a timely observation given that this was at the advent of man's attempts to harness and exploit nuclear fission. Over the past four decades, Drosophila has become a predominant model used to understand how genes direct the development of an embryo from a single cell to a mature multicellular organism. In 1995,Christiane Nüsslein-Volhard, Eric Wieschaus, and Ed Lewis won the Nobel Prize in Physiology or Medicine "for their discoveries concerning the genetic control of early embryonic development"5. Many of the genes that they defined as being important for fly development have since been shown to be critical for all animal development, including humans. Although the final architecture of a fly and a human differs greatly, many of the underlying building blocks and engineering processes have been conserved through evolution and are strikingly similar. In 1999, Craig Venter and colleagues used the Drosophila genome as a test bed to prove the practicality of the "shot-gun" approach for sequencing the human genome. This approach worked well and the first release of the sequence of the Drosophila genome was released in March 2000, just 11 months ahead of the human genome6. The sequence and annotation of the Drosophila genome is freely available to all and can be accessed via "Flybase", the outstanding online database dedicated to Drosophila7. Comparisons between the fully sequenced Drosophila and human genomes revealed that approximately 75 % of known human disease genes have a recognizable match in the genome of fruit flies consolidating its legitimacy as a model organism for medical research8. Currently, it is estimated that there are around 14 000 genes in Drosophila6 and each of these has a dedicated page on Flybase that contains links to practically everything known about that gene including sequence, gene product sequence, known mutations, and related literature Drosophila – a genetic "work horse" There has been a long history of using Drosophila genetics as a tool for understanding biology dating back to Morgan's experiments over 100 years ago. One convention in Drosophila, which Morgan initiated, is naming mutations to reflect the nature of their phenotype (the observable effects of the mutation). For example, one of the first mutations Morgan isolated is known as white; flies with this mutation have white eyes (instead of red)9. Genes are usually named after the first mutation isolated within them (e.g., white). This convention has lead to many genes with somewhat entertaining names. One

example is groucho. Flies carrying the first mutant allele (variant) of have extra bristles above their eyes resembling the bushy eyebrows sported by Groucho Marx, a famous film star and entertainer at the time the mutation was discovered10. Other genes include tribbles (cells divide ectopically in mutants; the name refers to an alien species in "Star Trek" that reproduced uncontrollably11), wingless , Notch and hedgehog. Many genes first characterized in flies have subsequently been isolated and studied in mice and humans, and the fly names have been adopted or adapted. Examples of these include Notch, "sonic hedgehog" (related to Drosophila hedgehog14) and Wnt. However, if a related gene (orthologue) has been characterized in other model systems before mutant alleles are isolated in the fly, then the established name is adopted in Drosophila. All Drosophila genes were given a "CG number" during the annotation of the genome sequence, and there are still many uncharacterized genes with no known mutation or orthologues that are referred to by a CG number7. Genetic analysis gives essential insight into the role played by individual factors in a given biological process. The range of genetic tools that have become available for Drosophila over the past century far surpass those for any other multicellular organism. The vast assortment of strains containing endogenous mutations is collated in Flybase, and these are usually available from the labs that generated them or from stock centers. It is now relatively simple to generate transgenic flies carrying DNA that activates or inhibits the expression of individual genes of interest, either throughout the organism or in defined tissues. Furthermore, they relatively short life cycle of the fly means that genetic experiments that would take months or even years in vertebrate models such as mouse or zebrafish can be completed in a matter of weeks. It is possible to reduce expression of individual genes in Drosophila cells by expressing double-stranded RNA corresponding to that gene's sequence; this is known as RNA interference or RNAi. The Vienna Drosophila RNAi Center houses a collection of transgenic fly lines, each carrying an inducible UAS-RNAi construct against a single protein coding gene. Currently they accommodate over 22 000 different transgenic fly lines, which provide knockdowns for over 88 % of Drosophila genes. Expression of these transgenic RNAi constructs can be driven in a tissue specific manner using the "GAL4 system"21,22 providing a simple, yet powerful strategy to study the role of individual genes in diverse biological processes. Other valuable resources include collections of transgenic lines that allow inducible overexpression of individual genes and transgenic lines expressing green fluorescent protein (GFP) or βgalactosidase (LacZ) in specific tissues or in the pattern of specific genes7,18. Life cycle of Drosophila Like butterflies and moths, Drosophila undergo a four stage life cycle; egg, larva, pupa, and fly. Once fertilized, the embryo develops in the egg for around one day (at 25 °C) before hatching as a larva. The larva eats and grows (and goes through three molts) over five days until it pupates and undergoes metamorphosis into the adult fly over the course of four days. During metamorphosis, most of the embryonic and larval tissue is destroyed. The adult tissues (e.g., wing, leg, eye) develop from groups of cells known as "imaginal discs" that have been set-aside since early embryonic development. Like humans, adult tissues generally do not regenerate in Drosophila; if you pull the wings off a fly, they will never grow back. However, imaginal discs do have the capacity to regenerate if damaged in certain conditions (see below), and over recent years have provided an invaluable model system to study the genetics of tissue regeneration. Working with Drosophila are relatively inexpensive and easy to keep; indeed they are commonly used in high school biology classes as a teaching aid for

demonstrating the basic principles of genetics and inheritance. Furthermore, there are generally very few restrictions on their use in the laboratory as there are minimal ethical and safety issues (although some Drosophila strains are considered to be genetically modified organisms). Each female fly can lay up to ~100 eggs per day for up to 20 days. It takes approximately 10 days at 25 °C for an embryo to develop into a fertile adult fly. Thus it is relatively easy to generate large numbers of embryos or flies for an experimental approach if required. Although commonly referred to as a fruit fly and often found in the wild hanging around vineyards and orchards, Drosophila melanogaster actually eat the yeasts growing on the fruit rather than the fruit itself. Initially lab flies were kept in bottles containing rotting banana pulp1, but these days it is most common to keep them in bottles containing a jelly-like food that is typically made from a mix of water, cornmeal, yeast, soy flour, malt extract, corn syrup, and agar24. The jelly needs to be hard enough so that the flies don't get stuck in it, but soft enough for the larvae to crawl around and feed in. There aremany variations on the basic recipe and pre-mixed formulations are available. Bottles and vials are plugged with foam or cotton wool to prevent the flies escaping and also to keep mites and other nasties out. Drosophila can be safely anaesthetized in carbon dioxide if it is necessary to manipulate individual flies (e.g., to set up a genetic cross). In most established fly labs, flies are placed on porous pads connected to a source of carbon dioxide and moved around with a fine tipped paintbrush while viewed with a stereomicroscope. Ether can be used as an alternative to carbon dioxide, and a magnifying glass used in place of a stereomicroscope. To date, it has not been possible to find an efficient way to freeze down Drosophila gametes or embryos, thus it is necessary to keep fly strains as living stocks. Typically stocks of flies are kept at 18 °C as this slows the life cycle down to approximately 28 days. This means that each fly stock needs to be turned over on to fresh food just once every month, leaving enough time between feeds for a decent vacation. Drosophila as a model for regenerative biology and medicine Most of our understanding of the biology underlying tissue regeneration comes from experiments using model organisms, including Drosophila. In order to either stimulate tissue regeneration at the site of injury or to generate tissues anew, it is necessary to understand which gene products are involved and how they interact with each other. Not surprisingly, the pathways and processes that we know to be activated during tissue regeneration are first used during embryonic development. In both contexts, cells must divide to increase in number, and then stop dividing and differentiate into specific cell types in specific locations. These events must be highly regulated to give rise to the complicated organs and tissues found in animals and to also avoid uncontrolled cell division and improper differentiation, i.e., cancer. The regenerative capacity of Drosophila imaginal discs has been studied for over 40 years. Fragments of imaginal discs transplanted into the abdomens of adult female flies can survive for a number of days. The cells in these discs do proliferate, but do not differentiate. In contrast, fragments of imaginal discs transplanted into larvae (just before pupation) do differentiate into the tissue for which they were originally destined. However, discs that have been cultured repetitively in different fly abdomens , can differentiate into an alternative tissue demonstrating "transdetermination". Thus, it was deduced that although the future identity of the discs is determined early in embryonic development, it is not fixed until the differentiation. Another interesting observation is that imaginal discs that have been cultured in adult hosts before being allowed to differentiate in larvae will either regenerate or duplicate the tissue depending on the precise origin of the disc

fragment. These classic observations were made long before any understanding of the underlying molecular patterning pathways that have since been deduced to some extent from genetic analysis. Drosophila and bioengineering Drosophila embryos have the potential to provide a valuable system for studying the biological safety of bioengineering technologies since they are relatively small (~500 μm) and can readily be produced in large numbers. Animal development is particularly sensitive to adverse environmental conditions that cause mechanical or genetic damage. Embryogenesis involves the precise coordination of numerous cellular processes including cell communication, cell division, gene expression, cell death, cell movement, and cell shape changes. Disruption to any of these processes can have devastating consequences for the embryo including severe deformities, infertility, or death. Cultured mammalian cells, which are probably the most common system for analysis, provide a much less sophisticated model since they usually contain few, often only one, type of cell. Thus, it is difficult to evaluate potential damage to physical interactions and communication processes amongst the assortment of cells that are found in the very 3-dimensional environment of animal tissues. One goal of regenerative medicine is to be able to produce artificial tissues by precise manipulation of cells and growth factors. Various methods are currently under development to do this, including bioelectrosprays. We used Drosophila embryos to show that this technique does not induce genetic or physical damage that significantly affects development or fertility, thus verifying that this procedure is safe for handling sensitive biological material.

Steps of cloning

In standard molecular cloning experiments, the cloning of any DNA fragment essentially involves seven steps: (1) Choice of host organism and cloning vector, (2) Preparation of vector DNA, (3) Preparation of DNA to be cloned, (4) Creation of recombinant DNA, (5) Introduction of recombinant DNA into host organism, (6) Selection of organisms containing recombinant DNA, (7) Screening for clones with desired DNA inserts and biological properties.

Notably, the growing capacity and fidelity of DNA synthesis platforms allows for increasingly intricate designs in molecular engineering. These projects may include very long strands of novel DNA sequence and/or test entire libraries simultaneously, as opposed to of individual sequences. These shifts introduce complexity that require design to move away from the flat nucleotide-based representation and towards a higher level of abstraction.

Choice of host organism and cloning vector

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Preparation of vector DNA

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Preparation of DNA to be cloned

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Creation of recombinant DNA with DNA ligase

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Introduction of recombinant DNA into host organism

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Selection of organisms containing vector sequences

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Screening for clones with desired DNA inserts and biological properties

1.Choice of host organism and cloning vector

Although a very large number of host organisms and molecular cloning vectors are in use, the great majority of molecular cloning experiments begin with a laboratory strain of the bacterium *E. coli* (Escherichia coli) and a plasmid cloning vector. *E. coli* and plasmid vectors are in common use because they are technically sophisticated, versatile, widely available, and offer rapid growth of recombinant organisms with minimal equipment. If the DNA to be cloned is exceptionally large (hundreds of thousands to millions of base pairs), then a bacterial artificial chromosome or yeast artificial chromosome vector is often chosen.

Specialized applications may call for specialized host-vector systems. For example, if the experimentalists wish to harvest a particular protein from the recombinant organism, then an expression vector is chosen that contains appropriate signals for transcription and translation in the desired host organism. Alternatively, if replication of the DNA in different species is desired (for example, transfer of DNA from bacteria to plants), then a multiple host range vector (also termed shuttle vector) may be selected. In practice, however, specialized molecular cloning experiments usually begin with cloning into a bacterial plasmid, followed by subcloning into a specialized vector.

Whatever combination of host and vector are used, the vector almost always contains four DNA segments that are critically important to its function and experimental utility:

- DNA *replication origin* is necessary for the vector (and its linked recombinant sequences) to replicate inside the host organism
- one or more unique *restriction endonuclease recognition sites* to serves as sites where foreign DNA may be introduced
- a *selectable genetic marker* gene that can be used to enable the survival of cells that have taken up vector sequences
- a *tag* gene that can be used to screen for cells containing the foreign DNA

2.Preparation of vector DNA

The cloning vector is treated with a restriction endonuclease to cleave the DNA at the site where foreign DNA will be inserted. The restriction enzyme is chosen to generate a configuration at the cleavage site that is compatible with the ends of the foreign DNA (see DNA end). Typically, this is done by cleaving the vector DNA and foreign DNA with the same restriction enzyme, for example EcoRI. Most modern vectors contain a variety of convenient cleavage sites that are unique within the vector molecule (so that the vector can only be cleaved at a single site) and are located within a gene whose inactivation can be used to distinguish recombinant from non-recombinant organisms at a later step in the process. To improve the ratio of recombinant to non-recombinant organisms, the cleaved vector may be treated with an enzyme that dephosphorylates the vector ends. Vector molecules with dephosphorylated ends are unable to replicate, and replication can only be restored if foreign DNA is integrated into the cleavage site.

3.Preparation of DNA to be cloned

For cloning of genomic DNA, the DNA to be cloned is extracted from the organism of interest. Virtually any tissue source can be used (even tissues from extinct animals), as long as the DNA is not extensively degraded. The DNA is then purified using simple methods to remove contaminating proteins (extraction with phenol), RNA (ribonuclease) and smaller molecules (precipitation and/or chromatography). Polymerase chain reaction (PCR) methods are often used for amplification of specific DNA or RNA (RT-PCR) sequences prior to molecular cloning.

DNA for cloning experiments may also be obtained from RNA using reverse transcriptase (complementary DNA or cDNA cloning), or in the form of synthetic DNA (artificial gene synthesis). cDNA cloning is usually used to obtain clones representative of the mRNA population of the cells of interest, while synthetic DNA is used to obtain any precise sequence defined by the designer. Such a designed sequence may be required when moving genes across genetic codes (for example, from the mitochrondria to the nucleus) or simply for increasing expression via codon optimization.

The purified DNA is then treated with a restriction enzyme to generate fragments with ends capable of being linked to those of the vector. If necessary, short double-stranded segments of DNA (*linkers*) containing desired restriction sites may be added to create end structures that are compatible with the vector.

4.Creation of recombinant DNA with DNA ligase

The creation of recombinant DNA is in many ways the simplest step of the molecular cloning process. DNA prepared from the vector and foreign source are simply mixed together at appropriate concentrations and exposed to an enzyme (DNA ligase) that covalently links the

ends together. This joining reaction is often termed ligation. The resulting DNA mixture containing randomly joined ends is then ready for introduction into the host organism.

DNA ligase only recognizes and acts on the ends of linear DNA molecules, usually resulting in a complex mixture of DNA molecules with randomly joined ends. The desired products (vector DNA covalently linked to foreign DNA) will be present, but other sequences (e.g. foreign DNA linked to itself, vector DNA linked to itself and higher-order combinations of vector and foreign DNA) are also usually present. This complex mixture is sorted out in subsequent steps of the cloning process, after the DNA mixture is introduced into cells.

5.Introduction of recombinant DNA into host organism

The DNA mixture, previously manipulated in vitro, is moved back into a living cell, referred to as the host organism. The methods used to get DNA into cells are varied, and the name applied to this step in the molecular cloning process will often depend upon the experimental method that is chosen (e.g. transformation, transduction, transfection, electroporation).

When microorganisms are able to take up and replicate DNA from their local environment, the process is termed transformation, and cells that are in a physiological state such that they can take up DNA are said to be competent. In mammalian cell culture, the analogous process of introducing DNA into cells is commonly termed transfection. Both transformation and transfection usually require preparation of the cells through a special growth regime and chemical treatment process that will vary with the specific species and cell types that are used.

Electroporation uses high voltage electrical pulses to translocate DNA across the cell membrane (and cell wall, if present). In contrast, transduction involves the packaging of DNA into virus-derived particles, and using these virus-like particles to introduce the encapsulated DNA into the cell through a process resembling viral infection. Although electroporation and transduction are highly specialized methods, they may be the most efficient methods to move DNA into cells.

6.Selection of organisms containing vector sequences

Whichever method is used, the introduction of recombinant DNA into the chosen host organism is usually a low efficiency process; that is, only a small fraction of the cells will actually take up DNA. Experimental scientists deal with this issue through a step of artificial genetic selection, in which cells that have not taken up DNA are selectively killed, and only those cells that can actively replicate DNA containing the selectable marker gene encoded by the vector are able to survive.

When bacterial cells are used as host organisms, the selectable marker is usually a gene that confers resistance to an antibiotic that would otherwise kill the cells, typically ampicillin. Cells harboring the plasmid will survive when exposed to the antibiotic, while those that have failed to take up plasmid sequences will die. When mammalian cells (e.g. human or mouse cells) are used, a similar strategy is used, except that the marker gene (in this case typically encoded as part of the kanMX cassette) confers resistance to the antibiotic Geneticin.

7.Screening for clones with desired DNA inserts and biological properties

Modern bacterial cloning vectors use the blue-white screening system to distinguish colonies (clones) of transgenic cells from those that contain the parental vector (i.e. vector DNA with no recombinant sequence inserted). In these vectors, foreign DNA is inserted into a sequence that encodes an essential part of beta-galactosidase, an enzyme whose activity results in formation of

a blue-colored colony on the culture medium that is used for this work. Insertion of the foreign DNA into the beta-galactosidase coding sequence disables the function of the enzyme so that colonies containing transformed DNA remain colorless (white). Therefore, experimentalists are easily able to identify and conduct further studies on transgenic bacterial clones, while ignoring those that do not contain recombinant DNA.

The total population of individual clones obtained in a molecular cloning experiment is often termed a DNA library. Libraries may be highly complex (as when cloning complete genomic DNA from an organism) or relatively simple (as when moving a previously cloned DNA fragment into a different plasmid), but it is almost always necessary to examine a number of different clones to be sure that the desired DNA construct is obtained. This may be accomplished through a very wide range of experimental methods, including the use of nucleic acid hybridizations, antibody probes, polymerase chain reaction, restriction fragment analysis and/or DNA sequencing.

Cloning Applications

The ability to replicate or duplicate the animal genome has had an enormous impact on the potential promise of the agricultural animal biotechnology industry. Prior to the publication of the techniques used to clone the first animal, a sheep named Dolly, using cells derived from mammary tissue of an adult sheep, it was thought that the developmental program acquired by cells as they differentiate from early embryonic stages to form specialized tissues that make up the fetus was irreversible. In the following few years, researchers have rapidly shown that the same techniques, or with slight modifications, can be used to clone other species including cattle, pigs, goats, mice, rabbits, cats, endangered species, equines, and most recently rats and flies. The rush to perfect these techniques in these other species is reflective of the unique benefits of the basic technology and its broad impact. The scope of this article will be to outline the potential benefits of cloning technology to the animal agricultural industry and highlight some of the possibilities for combining the tools offered by nuclear transfer, animal genomics, and genetic engineering to make improved animal agricultural products.

1.Preservation and Dissemination of Genetic Value

The donor cells used in nuclear transfer to create a copy of an animal can be propagated in vitro to produce millions of cells that can be stored frozen indefinitely. Therefore, in theory, cloning could be used to generate many copies of valuable animals at any time in the future. The ability to gain maximum benefit from this attribute relies on the foresight of individuals that manage genetically valuable animals to cryopreserve cells. Furthermore, progress made in animal genomics in the future may be capitalized on by the availability of a frozen gene pool from which to screen for valuable traits identified by genomic markers. For example, if genetic markers are identified that correlate to traits such as meat tenderness, parasite resistance, reproductive soundness, or other desired attributes, the screening process could be applied to a genetic base that far exceeds the number of animals alive at the time. In fact, this can be viewed as a potential method for broadening the gene pool to incorporate desired traits into breeding programs, as opposed to narrowing it.

2.Genetic Modifications

Thus far, this discussion has centered on using cloning and genomics to capitalize on genetic traits that occur through natural breeding or mutations induced by the environment. The ability to clone an animal also offers the unique capability to alter the genome to greatly accelerate the rate at which desirable traits are acquired. This is accomplished using transgenic technology.

Cloning technology offers the unique ability to replicate highquality production animals and disseminate their genetic value more broadly than ever before possible. It also affords the ability to preserve genetic seed stock indefinitely for potential use in the future. These attributes have value in their own right. However, cloning also represents a tool with which to capitalize on the knowledge gleaned from animal genomics research and the power of transgenic technology. Genetic gains have been realized through conventional selective breeding and culling of domestic animals for centuries. However, those improvements are based strictly on trial and error due to the nature of chromosome recombination events during meiosis, thus, offspring that have desirable traits are produced alongside those that do not. Applying these new biotechnologies in the agricultural animal industry will permit deliberate and precise modifications with highly predictable outcomes avoiding the trial and error of selective breeding programs.

Ethical problems

Researchers have observed some adverse health effects in sheep and other mammals that have been cloned. These include **an increase in birth size and a variety of defects in vital organs**, such as the liver, brain and heart. Other consequences include premature aging and problems with the immune system

Conclusion

Cloning technology offers the unique ability to replicate highquality production animals and disseminate their genetic value more broadly than ever before possible. It also affords the ability to preserve genetic seed stock indefinitely for potential use in the future. These attributes have value in their own right. However, cloning also represents a tool with which to capitalize on the knowledge gleaned from animal genomics research and the power of transgenic technology. Genetic gains have been realized through conventional selective breeding and culling of domestic animals for centuries. However, those improvements are based strictly on trial and error due to the nature of chromosome recombination events during meiosis, thus, offspring that have desirable traits are produced alongside those that do not. Applying these new biotechnologies in the agricultural animal industry will permit deliberate and precise modifications with highly predictable outcomes avoiding the trial and error of selective breeding programs.

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