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An Insight into Advanced Genetic Manipulation Techniques

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Abstract

Genetic manipulation is the technological modification and manipulation of an organism's genes. It is sometimes referred to as genetic modification or genetic engineering. It is a collection of methods for altering a cell's genetic composition, including the transfer of genes both inside and between species to create new or enhanced organisms. Genetic engineering has been applied in numerous fields including research, medicine, industrial biotechnology and agriculture. This article describes briefly three genetic manipulation techniques which are implemented at a wider level in livestock research as well as in agricultural field.

Keywords: genetic manipulation, biotechnology, modification, genetic engineering

Introduction

Genetic manipulation, which involves modifying gene expression and expressing new genes, has emerged as a vital technique in modern genetic research. Methods for introducing, removing, and altering DNA at different levels, from a single base pair in a gene to whole genes, have been developed. DNA is modified in every genetic engineering procedure. In the past, DNA was extracted from the DNA of living cells. Genes were later artificially synthesized or cloned from a DNA segment following the establishment of a DNA library. After a gene is isolated, further genetic components are added to it to help in selection and enable it to be expressed in the host organism. Genetic engineering transfers a gene from one creature to another directly, in contrast to traditional animal and plant breeding, which entails making numerous crossings and then choosing the organism with the desired trait. This is far faster, keeps additional unwanted genes from being added, and allows any gene from any organism, even ones from other domains, to be inserted.

Introgressive Hybridization

The transfer of genetic material from one species into the gene pool of another by the repeated backcrossing of an interspecific hybrid with one of its parent species (Fig. 1).

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Introgression is a long-term process, even when artificial; it may take many hybrid generations before significant backcrossing occurs. This process is distinct from most forms of gene flow in that it occurs between two populations of different species, rather than two populations of the same species.

Introgression also differs from simple hybridization. Simple hybridization results in a relatively even mixture; gene and allele frequencies in the first generation will be a uniform mix of two parental species, such as that observed in mules. Introgression, on the other hand, results in a complex, highly variable mixture of genes, and may only involve a minimal percentage of the donor genome.

The introgression is considered 'adaptive' if the genetic transfer results in an overall increase in the recipient taxon's fitness (Suarez *et al.*, 2018). Ancient introgression events can leave traces of extinct species in present-day genomes, a phenomenon known as 'Ghost Introgression' (Jente, 2020).

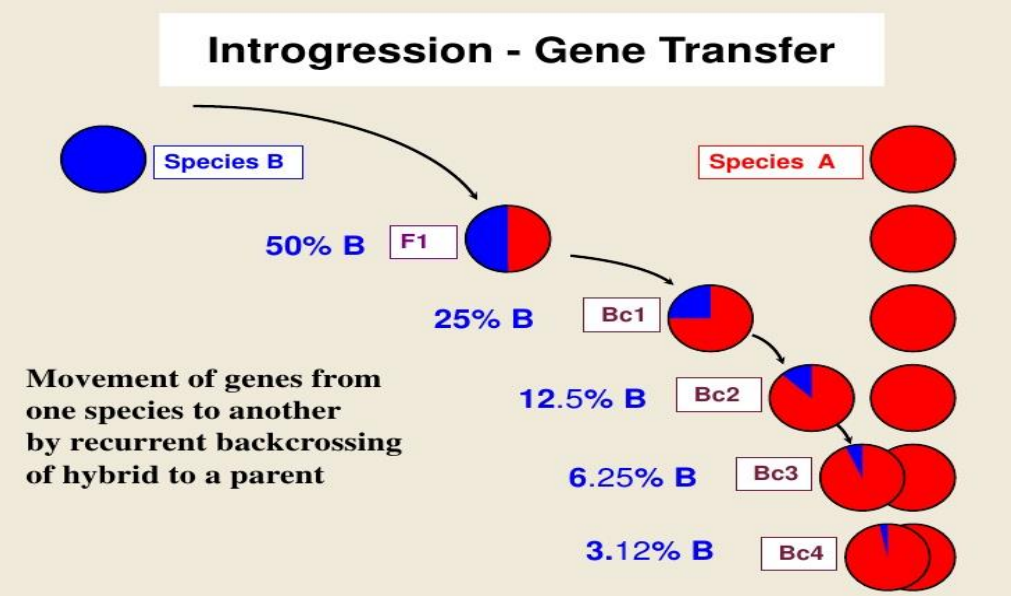

Fig. 1. Introgressive Hybridization

Utility of Introgression

- It is an important source of genetic variation in natural populations
- It may contribute to adaptation and even adaptive radiation (Grant *et al.*, 2005).
- Historically, introgression with wild animals is a large contributor to the wide range of diversity found in domestic animals, rather than multiple independent domestication events.

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Gene knockout

A gene knockout (KO) is a genetic technique in which one of an organism's genes is made inoperative ("knocked out" of the organism) (Fig. 2). Knocking out two genes simultaneously in an organism is known as **a double knockout** (DKO). Similarly, the terms **triple knockout** (TKO) and **quadruple knockouts** (QKO) are used to describe three or four knocked out genes, respectively.

Heterozygous KOs: Only one of two gene copies (alleles) is knocked out.

Homozygous KOs: Both of the gene copies are knocked out.

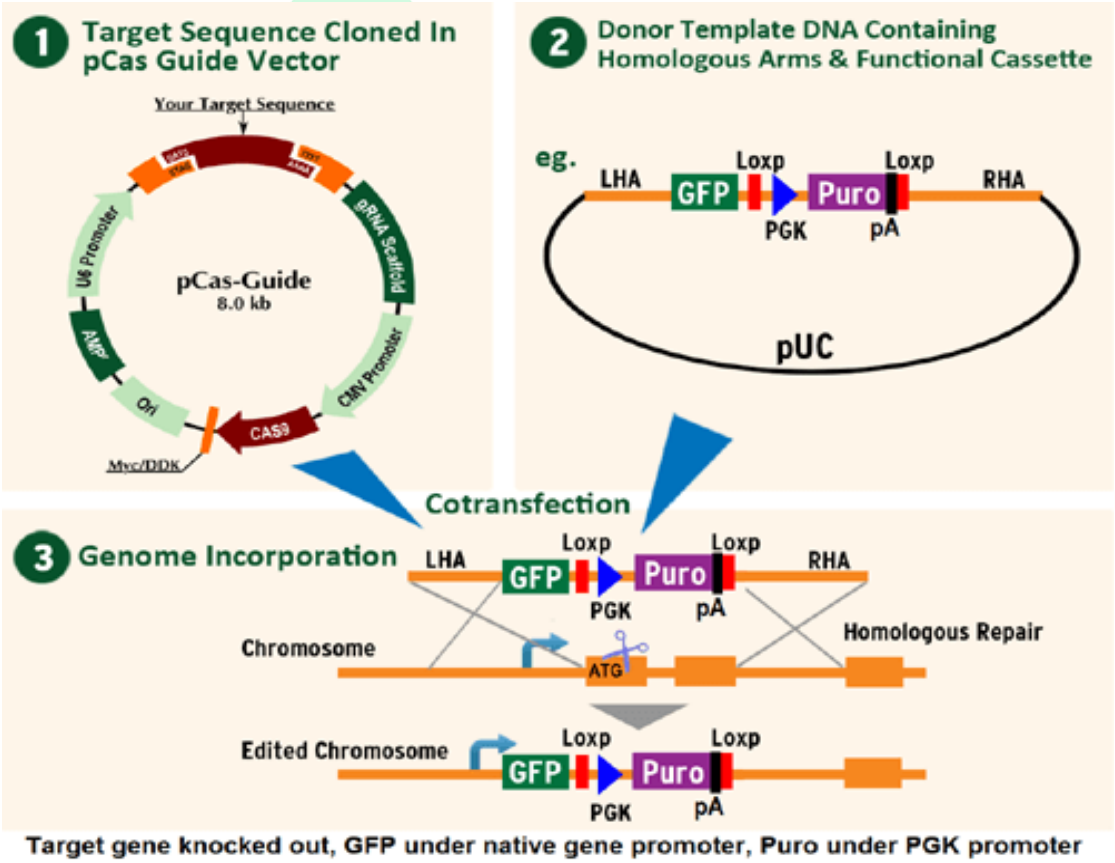

Fig. 2. Gene knockout

Utility of gene knockout individuals

- ✓ Knockout organisms or simply knockouts are used to study gene function, usually by investigating the effect of gene loss by drawing inferences from the difference between the knockout organism and normal individuals.
- ✓ Used as screening tools in the development of drugs, to target specific biological processes or deficiencies by using a specific knockout.

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✓ To understand the mechanism of action of a drug by using a library of knockout organisms spanning the entire genome, such as in *Saccharomyces cerevisiae.*

Methods of gene knockout

Originally, naturally occurring mutations were identified and then gene loss or inactivation had to be established by DNA sequencing or other methods.

a) Homologous recombination

- It is the main method for causing a gene knockout.
- This method involves creating a DNA construct containing the desired mutation. For knockout purposes, this typically involves a drug resistance marker in place of the desired knockout gene.
- The construct will also contain a minimum of 2kb of homology to the target sequence.
- The construct can be delivered to stem cells either through microinjection or electroporation.
- This method then relies on the cell's own repair mechanisms to recombine the DNA construct into the existing DNA.
- This results in the sequence of the gene being altered, and most cases the gene will be translated into a non-functional protein, if it is translated at all.
- However, this is an inefficient process, as homologous recombination accounts for only 10^{-2} to 10^{-3} of DNA integrations.

These stem cells now lacking the gene could be used in vivo, for instance in mice, by inserting them into early embryos. If the resulting chimeric mouse contained the genetic change in their germline, this could then be passed on to offspring.

In diploid organisms, which contain two alleles for most genes, and may as well contain several related genes that collaborate in the same role, additional rounds of transformation and selection are performed until every targeted gene is knocked out. Selective breeding may be required to produce homozygous knockout animals.

b) Site-specific nucleases

- There are currently three methods (ZFNs, TALENs and CRISPR/CAS9) in use that involve precisely targeting a DNA sequence in order to introduce a double-stranded break. Once

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this occurs, the cell's repair mechanisms will attempt to repair this double stranded break, often through non-homologous end joining (NHEJ), which involves directly ligating the two cut ends together.

- This may be done imperfectly, therefore sometimes causing insertions or deletions of base pairs, which cause frameshift mutations. These mutations can render the gene in which they occur nonfunctional, thus creating a knockout of that gene.
- This process is more efficient than homologous recombination, and therefore can be more easily used to create biallelic knockouts.

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat)

Short, partially palindromic repeating DNA sequences with a distinct organisation are present in the genomes of bacteria and other microbes (Pennisi, 2013). Despite their apparent harmlessness, CRISPR sequences are an essential part of these tiny life forms' immune systems. Similar brief variable sequences known as spacers are inserted in between the brief DNA repeats of bacterial CRISPRs (Fig. 3).

The DNA of viruses that have previously attacked the host bacterium is used to create these spacers. Spacers therefore function as a "genetic memory" of prior infections. The CRISPR defence mechanism will snip apart any viral DNA sequence that matches the spacer sequence in the event that the same virus should re-infect the bacterium. This will shield the bacteria from further viral assault. A new spacer is created, added to the chain of spacers, and repeats in the event that a previously undiscovered virus hit.

Three fundamental processes are used by the CRISPR immune system to shield bacteria from recurrent viral attacks:

Step 1: Adaptation

An invasive virus's DNA is cut into brief pieces and added to the CRISPR sequence as additional spacers.

Step 2: Creation of CRISPR RNA

Transcription, or the copying of DNA into RNA (ribonucleic acid), is carried out by CRISPR repeats and spacers in the bacterial DNA. The resultant RNA has a single-chain structure as opposed to DNA's double-chain helix. CRISPR RNAs are the small segments of this RNA chain that have been cut.

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Step 3: Targeting

The bacterial molecular machinery is guided by CRISPR RNAs to eliminate the viral material. CRISPR RNA sequences are precise fits to the viral genome and make great guides because they are derived from the viral DNA sequences that were acquired during adaptation.

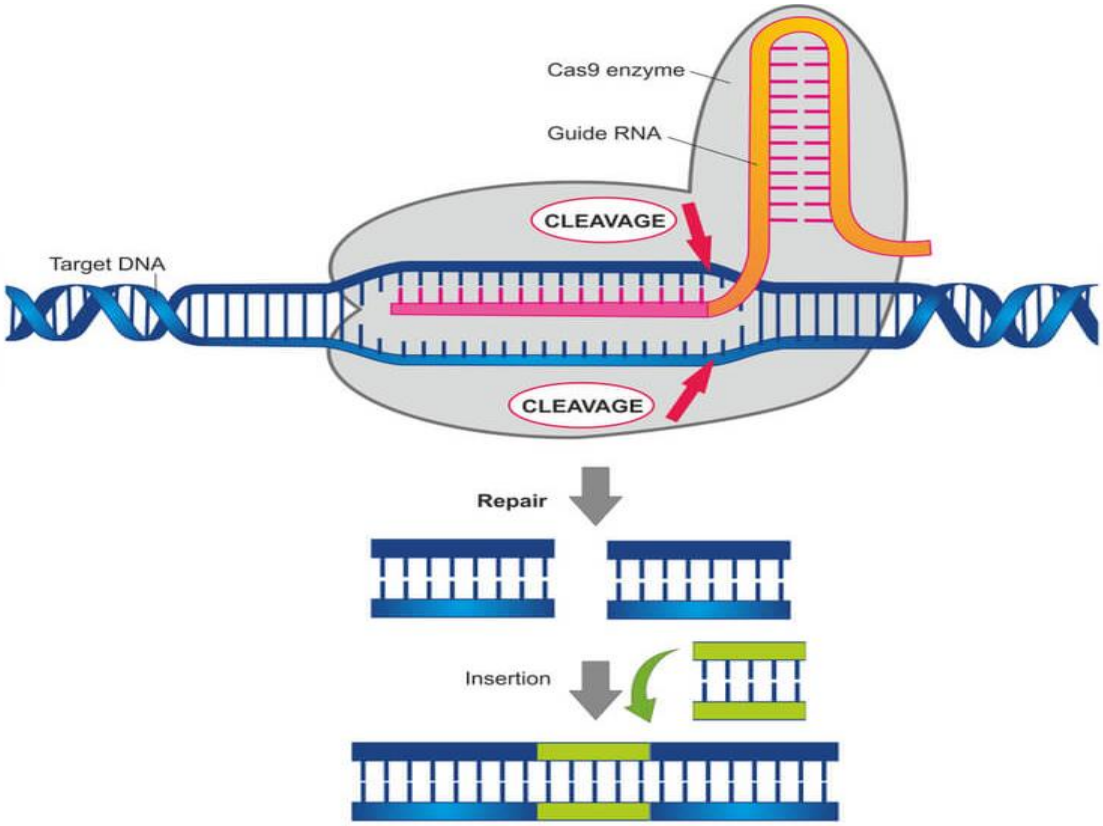

Fig. 3. Basic concept of CRISPR technique

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