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Unit -4
Cloning Strategies

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Confirmation of Transgenic Animals

Confirmation of Transgenic Animals

Transgenic animals are those that have been genetically modified by the introduction of foreign genes, or transgenes, into their genomes.

These genetic modifications can result in new traits, such as disease resistance, improved growth rates, or the production of valuable proteins.

The confirmation of transgenic animals is a crucial step in the development and characterization of genetically modified organisms (GMOs).

This process ensures that the transgene has been successfully integrated, is being expressed properly, and is stable through subsequent generations.

The confirmation of transgenic animals generally involves several steps, ranging from molecular biology techniques to phenotypic analysis.

Confirmation of Transgenic Animals

1. Molecular Confirmation (DNA-Based Techniques)

a. Polymerase Chain Reaction (PCR)

PCR is one of the most common methods for detecting the presence of a transgene in an animal's genome. It is used to amplify a specific region of DNA that corresponds to the transgene.

•**Procedure**: A primer pair designed to amplify a region of the transgene or surrounding genomic sequence is used. The PCR products are then analyzed by gel electrophoresis or other detection methods.

•**Advantages**: PCR is a fast and highly sensitive method for detecting transgenes even at low copy numbers. It can be used for confirmation at the DNA level.

b. Southern Blotting

Southern blotting is a technique that allows for the detection and analysis of specific DNA sequences within a genome. It can confirm both the integration and the copy number of the transgene.

•**Procedure**: Genomic DNA is digested with restriction enzymes, separated by gel electrophoresis, transferred to a membrane, and probed with a labeled DNA sequence complementary to the transgene.

•**Advantages**: This technique can also provide information about whether the transgene is integrated into the animal's chromosome in a stable form, and it helps determine how many copies of the transgene are present.

c. Fluorescence in situ Hybridization (FISH)

FISH allows for the localization of transgenic sequences within chromosomes using fluorescently labeled probes.

•**Procedure**: The probe, which is complementary to the transgene, hybridizes with the target DNA within the cells. The chromosomes are then observed under a fluorescence microscope.

•**Advantages**: This method provides detailed information on the chromosomal location of the transgene.

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2. Expression Confirmation (RNA and Protein-Based Techniques)

a. Reverse Transcription PCR (RT-PCR)
RT-PCR is used to detect mRNA expression from the transgene. This confirms whether the transgene is being transcribed into RNA.
•**Procedure**: RNA is extracted from tissues (e.g., liver, muscle, or other target tissues), reverse-transcribed into cDNA, and then amplified using PCR with primers specific to the transgene.
•**Advantages**: RT-PCR confirms not only the presence of the transgene but also its expression.

b. Western Blotting
Western blotting is used to detect the presence and quantify the expression of transgenic proteins in the animal's tissues.
•**Procedure**: Protein extracts from tissues are separated by gel electrophoresis, transferred to a membrane, and probed with antibodies specific to the transgenic protein.
•**Advantages**: This technique directly detects the protein encoded by the transgene, confirming its expression in the animal.

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c. Immunohistochemistry (IHC)

IHC is a technique used to visualize the spatial localization of a transgenic protein within tissue sections.

•Procedure: Tissue samples are stained with antibodies that specifically recognize the transgenic protein. The presence of the protein is visualized using a colorimetric or fluorescent detection system.

•Advantages: IHC provides information on where in the tissue the transgene is expressed.

d. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA can be used to quantify transgenic protein levels in biological samples, such as blood, urine, or tissue lysates.

•Procedure: The specific protein of interest is captured using a monoclonal or polyclonal antibody on a plate, followed by a secondary antibody that binds to the protein. The presence of the protein is detected via a color change.

•Advantages: This method is highly sensitive and allows for quantification of protein expression.

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3. Phenotypic Confirmation

Phenotypic analysis is often used as a secondary confirmation step, particularly when the transgene is associated with a visible or measurable trait.

a. Observing Trait Expression

Many transgenic animals are developed for specific phenotypic traits, such as enhanced growth rates, resistance to diseases, or the ability to produce therapeutic proteins. Monitoring the animal for these traits is a simple but effective method for confirming that the transgene is functional.

- Examples: Transgenic animals producing human insulin, growth hormones, or other therapeutic proteins can be analyzed for protein production or improved traits.

b. Breeding and Inheritance Studies

Transgenic animals are bred to determine whether the transgene is inherited by offspring. This can be performed by mating transgenic animals with non-transgenic animals and then testing the offspring for the presence of the transgene.

- Procedure: PCR, Southern blotting, or other genetic tests are used on offspring to verify inheritance patterns.
- Advantages: Ensures that the transgene is stably inherited and passed down to future generations.

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Long-Term Confirmation (Stability and Copy Number Analysis)

For transgenic animals used in research or for commercial purposes, ensuring the stability of the transgene over time is critical.

a. Genetic Stability Analysis

Long-term stability of the transgene is confirmed by breeding the transgenic animal through several generations. PCR, Southern blotting, or RT-PCR can be performed periodically to confirm that the transgene remains intact and expressed.

b. Copy Number Analysis

Determining how many copies of the transgene are present in the genome is important, especially for animals engineered to express a specific amount of protein. Southern blotting or qPCR (quantitative PCR) can be used to determine the copy number of the transgene.

Thank you