Biology

Introduction to Biotechnology

Module 7

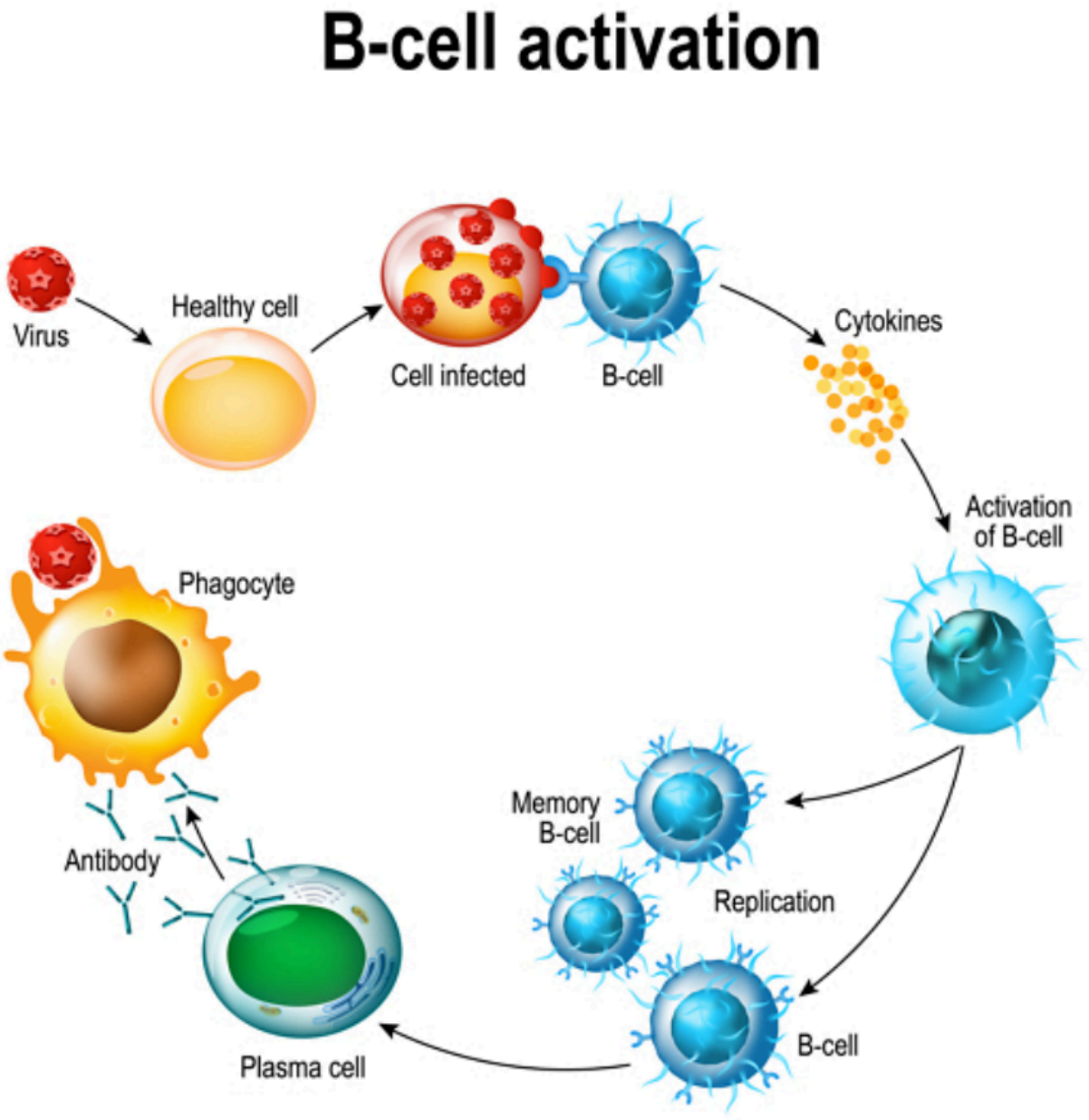
Imuune technology

Antibody structure and classification

- Shape
  Y - shaped , consist of two light chains and two heavy chains.
- Types
  - IgA : Found in mucous, saliva, tears, and breast milk. Protects against pathogens.**Monomer or dimer**
  - IgD : Part of B cell receptor. Activates basophils and mast cells.
  - IgE : Protects against parasitic worms. Resposnsible for allergic reactions.
  - IgG : Secreted by plasma cells in the blood. Able to cross the placenta into the fetus.
  - IgM : May be attached to the surface of a B cell or secreted into the blood. Responsible for early stages of immunity. **Pentamer**

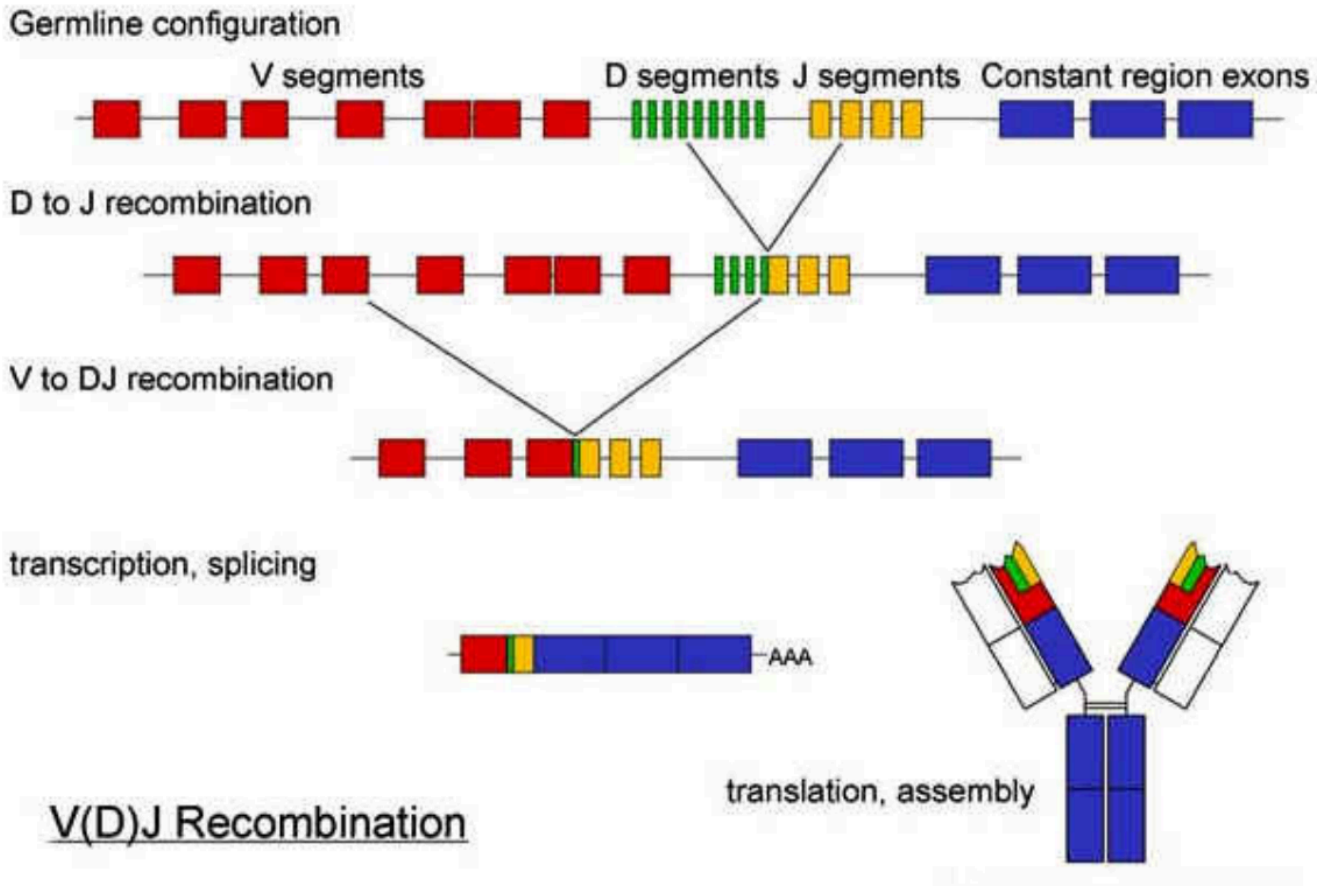
Adaptive immmunity

immunity that has memory and occurs after exposure to an antigen either from a pathogen or vaccination



V(D)J recombination

During B cell development, the antibody encoding genes are underwant V(D)J recombina



Phage display

when phage displays antibody library, it can be used for antibody screening.

Biopanning cycles

- Antibody library displayed by phage
- Antigen immobilized on platform
- Phage-antigen binding
- Wash out non-specific and unbound phage
- Elution of bound phage (the one displays antigen-specific antibody)
- Amplify the bound phage with specific antibody displayed

Monoclonal antibody and polyclonal antibody

- Monoclonal Antibody
    - Expensive to produce
    - Single antibody species

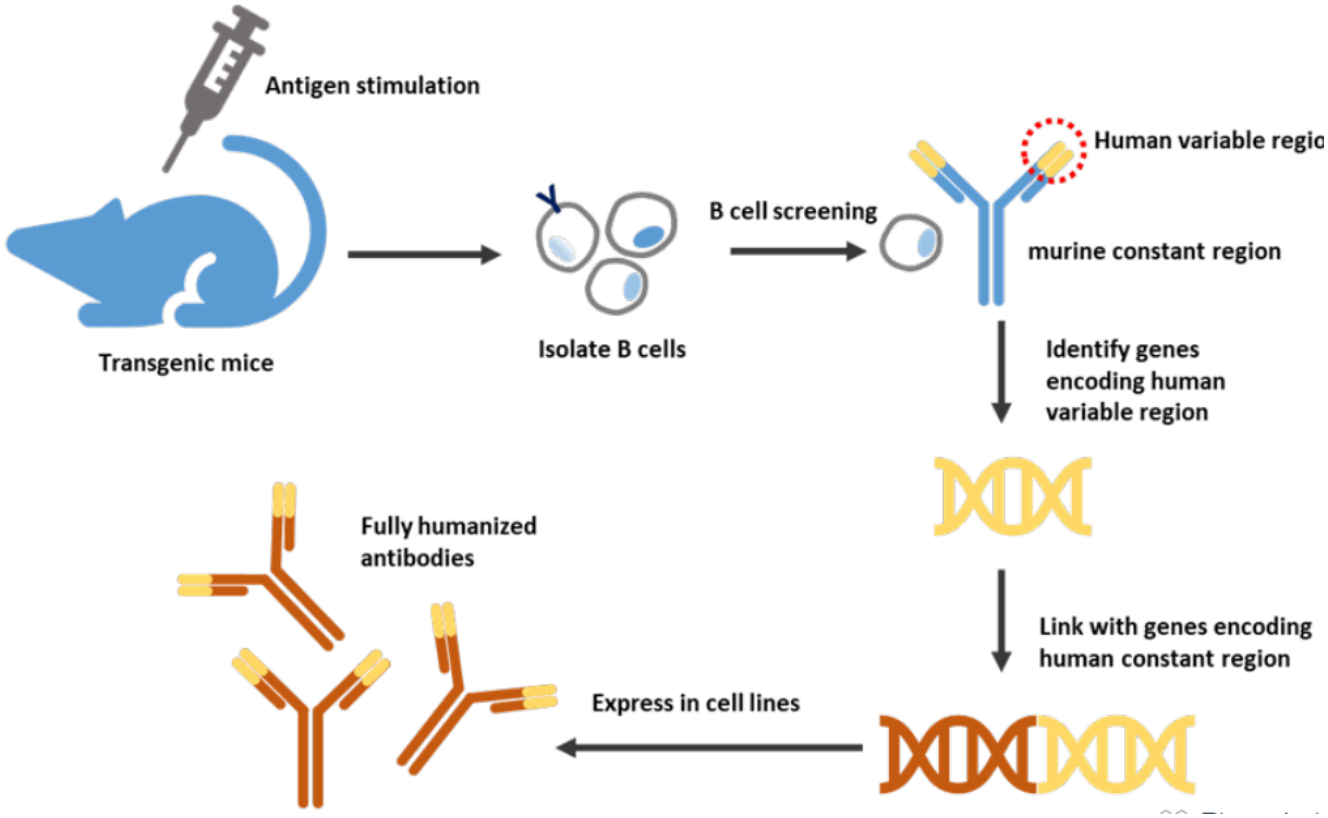
        - Will only bind single specific site
        - May recognise a particular protein form
- Polyclonal Antibody
        - Cheap to produce
        - Mixed population of antibodies
        - May bind to different areas of the target molecule
        - Tolerant of small changes in protein structure

Hybridoma technology for monoclonal antibody production

1. **Immunization:** the antigen is first injected into a mouse to provoke an immune response.
2. **B cell harvest:** the spleen is harvested because it harbors many activated B cells.
3. **Cell fusion:** the spleen cells are short-lived in culture, so they are fused to immortal myeloma cells.
4. **Hybridoma cell growth:** the hybridoma cells are cultured and isolated so each hybrid is separate from the other.
5. **Screening:** each hybrid clone can then be screened for the best antibody to the target protein.

Humanization of monoclonal antibodies

Antibodies from a mouse can be altered to become more like a human antibody.



Fab antibody and nanobodies

- Fab fragments are produced by protease digestion of the hinge region.
- dsFv fragment: Fv fragment with disulfide bonds engineered into the two halves.
- scFv fragment: Fv fragment with a linker added to hold the VH and VL domains together.

特征	Nanobody (VHH)	Fab	scFv	IgG (全抗体)
分子量	~15 kDa	~50 kDa	~25–30 kDa	~150 kDa
结构	单VHH结构	VH+CH1 + VL+CL	VH + VL（连接肽）	2重链 + 2轻链
是否有Fc区	✕	✕	✕	✓
稳定性	✓✓✓	✓	中等（不稳定）	✓
抗原亲和力	高	高	高	高

特征	Nanobody (VHH)	Fab	scFv	IgG (全抗体)
表达系统	简单（细菌）	中等（哺乳细胞）	容易（细菌）	复杂（哺乳细胞）

Different types of vaccination and their key principles

- Whole Vaccine
    - **Benefits**:immune system reacts very well and will typically remember the pathogen for a very long time. Usually, one dose immunization.It can be mass-produced and are relatively inexpensive to make.
- Subunit Vaccine
    - **Benefits**:safer as only contain pieces of a pathogen. Suitable for people who should not receive “live” vaccines, such as young children, older people, and immunocompromised people.
- Nucleic acid Vaccine
    - **Benefits**:fast and adaptable.
- Vector Vaccine
    - **Benefits**:strong immune response. One does immunization.
- Edible Vaccine
    - **Benefits**:can be mass-produced with low expense.Easy storage and distribution.

ELISA

Enzyme Linked Immunosorbent Assay

- High sensitivity, suitable for the detection of trace amounts of specific antibodies or antigens in body fluids.
- Suitable for mass screening.
- Suitable for qualitative and quantitative measurements.

Flow cytometry technique

- Cells are firstly labelled with antibodies or dyes.
- Cells flow in suspension through a measuring device one by one.
- laser beam strikes the single cell, light scattered, and fluorescence light emitted by the cell.

- Light scattering gives the structural and morphological properties of the cell.
- Fluorescence emission derived from the antibody or dye reflects the amount of antibody or dye bound to the cell, i.e. the detecting target of the cell.
- The detecting target can be membrane, cytoplasmic and nuclear antigens, or cellular components such as organelles, nuclei, DNA, RNA, chromosomes, cytokines, hormones or proteins.
- Analysis of cell proliferation and cell cycle are the commonly used examples for flow cytometry.

Module 8

Stem cells and cloning

Stem cell potency

类型	中文名称	可分化范围	举
Totipotent	全能性	可形成**完整的个体**，包括所有**胚胎和胎盘细胞**	受精卵、早期胚胎2-4细胞
Pluripotent	多能性	可分化为**三胚层（内/中/外）所有体细胞，但不能形成胎盘**	胚胎干细胞（ESCs）、诱
Multipotent	多潜能	可分化为**某一组织系统内多种细胞类型**	造血干细胞（HSCs）、间
Oligopotent	寡能性	可分化为**少数几种特定类型细胞**	淋巴系干细胞（仅产生T/
Unipotent	单能性	只能形成**一种特定类型细胞**	肌卫星细胞（形成肌肉细

Principle of cell reprogramming

Cell reprogramming is the process of reverting mature, specialised cells into induced pluripotent stem cells (iPSCs, 诱导多能干细胞).

- **Yamanaka factors:** Oct3/4,Sox2,Klf4,c-Myc (OSKM)

Cell reprogramming methods

1. **Somatic cell nuclear transfer (SCNT),** established in 1962： transplanting nuclei of adult somatic cells into enucleated (去核的) eggs containing endogenous

reprogramming factors allows the reprogramming of somatic cell nuclei and the generation of pluripotent stem cells.

2. **Yamanaka factors-based reprogramming,** established in 2006: using four defined cell transcription factors to reprogram somatic cells to pluripotent stem cells, known as iPSCs.9
3. **Chemical reprogramming,** established in 2013: using exogenous chemical compounds mainly small molecules to reprogram somatic cells to pluripotent stem cells. 18 small molecules can penetrate in cells and reprogram somatic cells.

Stem cell therapy

- Stem cell therapy utilizes stem cells to regenerate damaged cells and tissues in the human body or replace these cells with new, healthy and fully functional cells by delivering exogenous cells into a patient.
- It includes:
    - The creation and use of therapeutic stem cells to repair a problem area in our body.
    - Tissue engineering
    - Production of artificial organs.
- The iPSCs can be genetically engineered before differentiation and ransplanting for medical purpose.

iPSCs-based drug screening

- iPSCs generated in a disease- and patient- specific fashion
- iPSCs differentiated into functional phenotypes
- Set up disease models
- Performing drug screening or toxicology screening
- Validation of the screening result
- Development of personalized cell therapies

somatic cell nuclear transfer and reproductive cloning

- Reproductive cloning is defined as the deliberate production of genetically identical individuals.
- Each newly produced individual is a clone of the original.
- Cells from two clones have the same DNA and the same genes in their nuclei.
- There are two methods used for reproductive cloning:

    - somatic cell nuclear transfer (SCNT) 体细胞核移植
    - embryo splitting/artificial embryo twinning 胚胎分裂

somatic cell nuclear transfer (SCNT)

- Somatic cell nuclear transfer (SCNT) involves the culture of donor somatic cells and oocytes, transplantation of donor cell nuclei into enucleated oocytes, activation of reconstructed embryos, and transfer of cloned embryos into surrogates (代孕者).
- Successful cloning of more than 20 mammalian species has been reported using SCNT technology.
- In addition to cloning new organisms (reproductive cloning), SCNT can be used to clone new stem cells (therapeutic cloning)

Cell reprogramming and therapeutic cloning

- Therapeutic cloning refers to the use of SCNT to reprogram somatic cells into undifferentiated cells (embryonic stem cells) for different therapeutic purposes.
- The difference between reproductive cloning and therapeutic cloning:
    - reproductive cloning aims to clone new organisms
    - therapeutic cloning aims to clone new stem cells for differentiation and disease treatment

Module 9

Transgenic animals and plants

Trangene

- Transgenesis consists of introducing an exogenous (外源的)DNA sequence into the genome of an organism, which then becomes present in most cells and is transmitted to progeny (后代).
- Transgenesis is capable of expressing foreign gene in an organism or edit the genome of an organism.

- Transgenic technology involves introducing exogenous DNA into the genome of an organism.
- Most of transgenic animals produced so far are mice. It was then followed by rabbits, pigs, sheep, chicken, fish and cattle, or even primates.
- There are mainly 3 transgenic strategies:
    - retrovirus mediated gene transfer
    - DNA microinjection (显微注射)
    - Embryonic stem cell method, which is an approach that is routinely used to achieve gene-targeted (基因靶向) transgene

Embryonic stem cell method

This method refers to the introduction of foreign genes into fertilized eggs or embryonic stem cells, through random recombination to insert foreign genes into the chromosomal DNA of cells, and then implant fertilized eggs or embryonic stem cells into the uterus of the recipient animal, so that the foreign genes can be inherited to offspring with cell division.

Key steps:

1. Obtain embryonic stem cells from donor blastocysts
2. Construction of transgene expression vectors
3. Introduction of exogenous genes into ES cells
4. Modified ES cells into blastocysts (囊胚)
5. Implant the blastocyst to surrogate animal
6. Acquisition and identification of transgenic animals
7. Establishment of transgenic animal strains

Definition of gene targeting

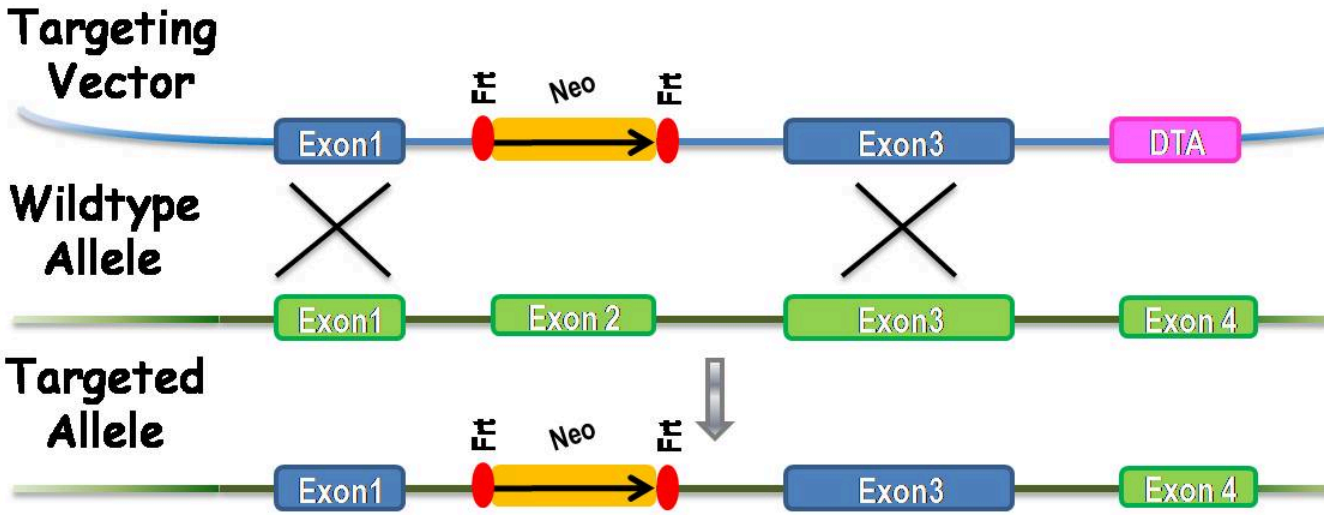
- Gene targeting is the process of altering a specific sequence or gene at its location in a genome.
- Gene targeting is made possible through homologous recombination (HR, 同源重组) which involves the exchange of nucleotides between two similar or identical DNA sequences.
- Gene targeting enables the tremendous ability of modifying essentially any endogenous gene at will in a site-specific (位点特异) manner and even in a single nucleotide accuracy.
- Potential modifications include deletion, insertion or replacement of endogenous

sequence with alternative sequences.

Principle of knockout and knock-in techniques

Gene Knockout(KO):

- Gene knockout (KO) is a genetic engineering method to make genes inoperative or deleted from the organism's genome.
- Gene knockout uses stem cell method to generate breeding animals.
- The introduced exogenous DNA can homologously recombinant with chromosomal DNA in the same sequence region, therefore destroying endogenous genes in ES cells at a fixed site.
- Gene knockout can result in a complete loss of function of a gene and allows scientists to understand the importance of a gene by functional analysis.
- Gene knockout is also a type of gene targeting technology.



Gene Knockin(KI):

- Gene knockin (KI) is a genetic engineering method to make insertion, deletion and substitution of DNA sequences within the organism's genome.
- Gene knockin uses stem cell method to generate breeding animals.
- The introduced exogenous DNA can homologously recombinant with chromosomal DNA in the same sequence region, therefore replacing endogenous genes in ES cells at a fixed site.
- Gene knockout can result in a complete loss of function of a gene and allows scientists to understand the importance of a gene by functional analysis.

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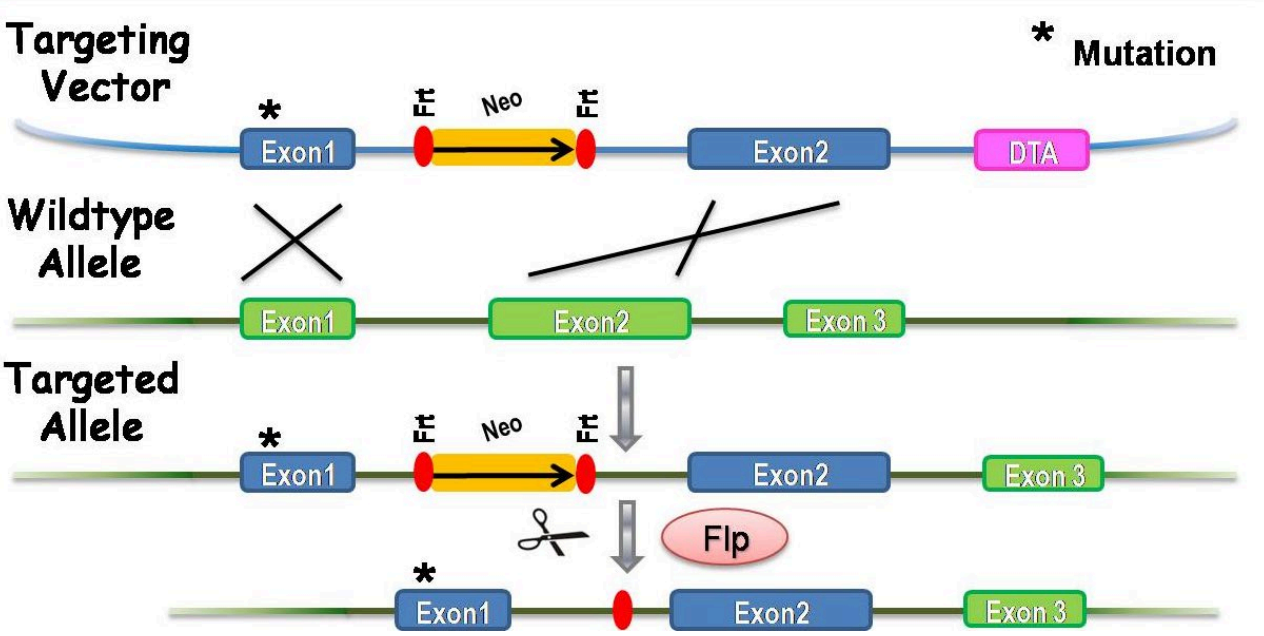
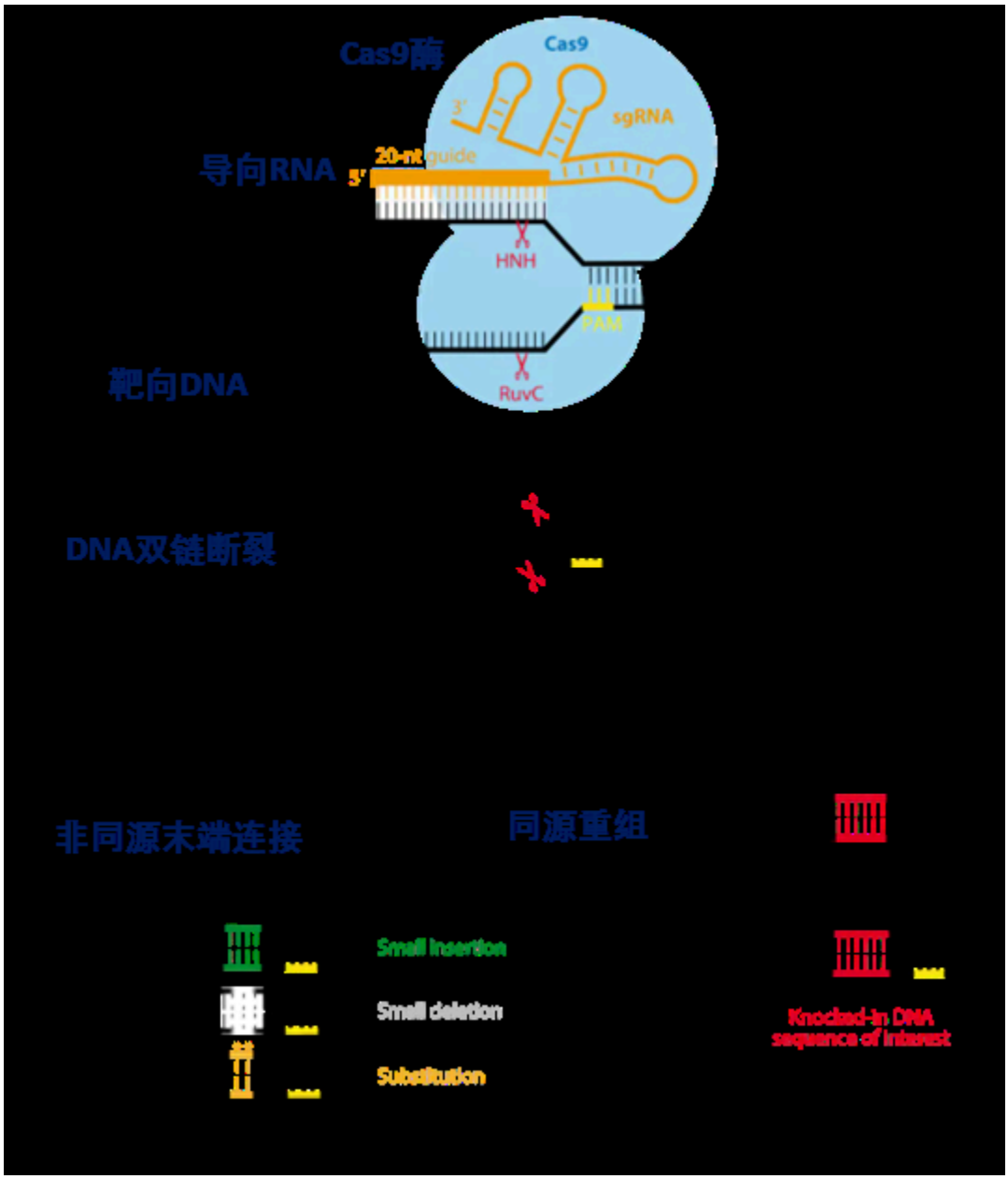


CRISPR/CAS9 technique



Agrobacterium tumefaciens mediated transfromation method

Application of transgenic animals and plants

Module 10

Biotechnology in human health

Principle of DNA finger printing

Techniques of DNA finger printing

Paternity and maternity determination

Strategies of gene therapy

Pros and cons of using viral vectors in gene therapy

Applications of gene therapy in various diseases

Module 11

The future biotechnology

Why is personalized medicine important

How can AI assist in drug screening and drug development

The common types of big data in biotechnology and the significance of these omics analys

The five functional classes for ecological engineering designs

The potential uses of brain-computer interfaces

The main steps involved in the functioning of Neuralink's brain-machine