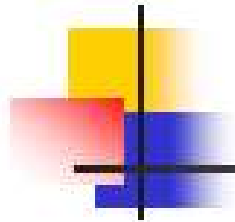


Part VI New molecular technologies

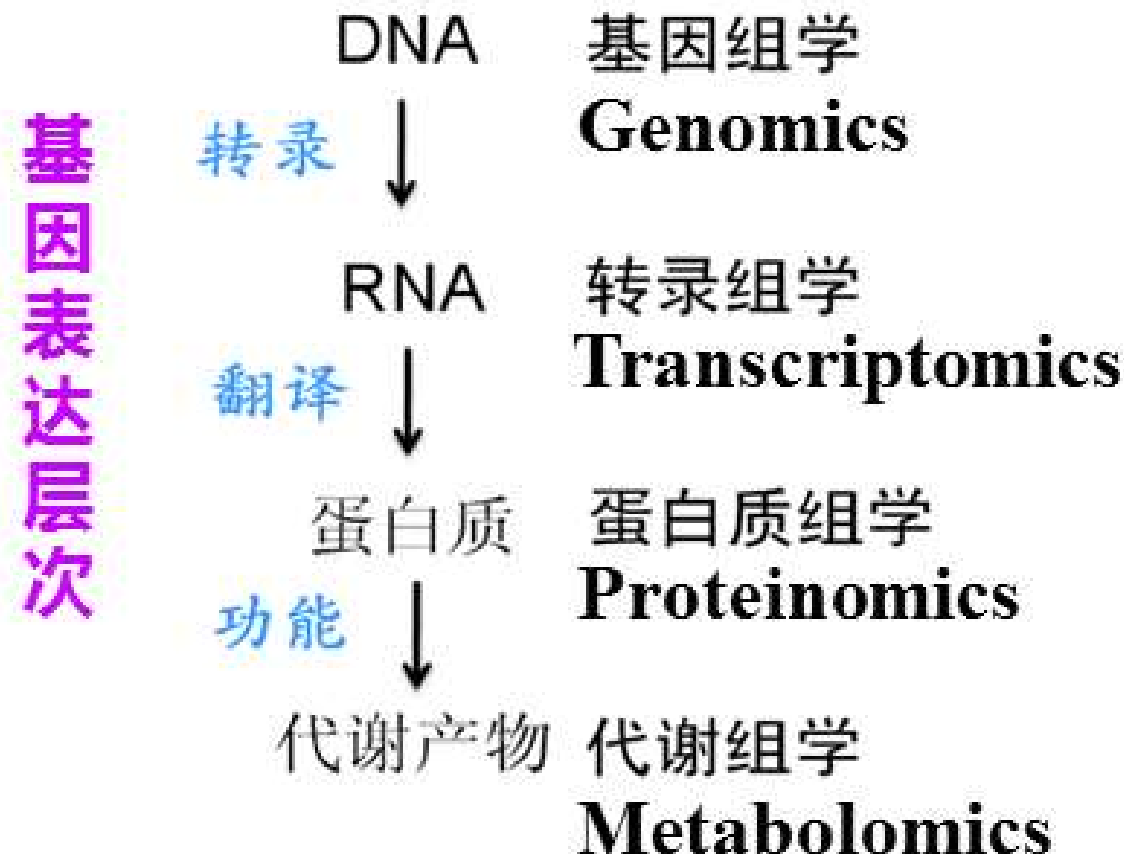
Chapter 13 Functional genomics and new technologies

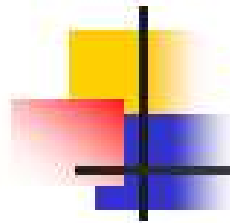


- ❑ **Introduction to the 'omics**
- ❑ **Global gene expression analysis**
- ❑ **Proteomics**
- ❑ **Cell and molecular imaging**
- ❑ **Transgenics and stem cell technology**

1. Introduction to the 'omics (组学)

Omics refers to the comprehensive analysis of biological systems. **组学**是指对生物系统的全面分析。





1.1 Genomics

- **Genome** is the complete set of sequences in the genetic material of an organism (**haploid cell**).
- **Genomics** is the study of determining and understanding the structure and function of whole genomes.

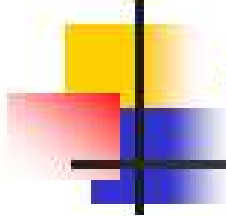
基因组学是阐明整个基因组的结构（所有DNA序列）与功能的一门学科。

{ **Structural genomics** (结构基因组学)
Functional genomics (功能基因组学)



1.1.1 Structural genomics

- **Research content:**
 - The **sequence** of the complete genome
 - A complete **structural description** of all the proteins and macromolecular complexes within a cell
- **Techniques:**
 - Sequencing
 - X-ray crystallography
 - NMR spectrometry



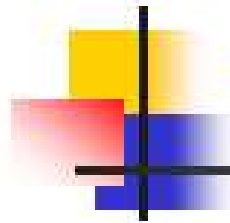
1.1.2 Functional genomics

- **Research content:**

- Determine the functions of all the genes (genomics) and gene products (transcriptomics & proteomics)
- The interactions between molecules, that define cellular and biological function

- **Techniques:**

- High-throughput technologies. e.g. DNA microarrays (微阵列)/chips (芯片), protein microarrays/chips



1.2 Transcriptomics

- The **transcriptome** is the full set of **RNA** transcripts produced from the genome at any given time.
转录组是指在任意给定时间内由基因组产生的全套**RNA**转录物。
- mRNA and nontranslated (noncoding) RNA
- The composition of the transcriptome varies markedly depending on **cell type, growth or developmental stage, and on environmental signals and conditions.**

- **Transcriptomics** is the systematic and quantitative analysis of all the transcripts present in a cell or a tissue under a defined set of conditions.

转录组学是对一定条件下细胞或组织中所有转录物的系统和定量分析。

- **Techniques:**
 - Nucleic acid hybridization
 - PCR
 - Transcription profiling (**转录 / 表达谱**)
/DNA microarrays



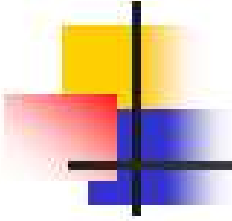
1.3 Proteomics

- The **proteome** is the total set of **proteins** encoded in the genome of a cell or the various subsets that are expressed from the transcriptome at any one time.
蛋白质组是指由细胞基因组所编码的一整套**蛋白质**，或者是任意一段时间内由特定转录组所表达出的各种蛋白质的群体。
- Varies in composition depending on conditions
- Post-translational modifications to proteins make the proteome highly complex.

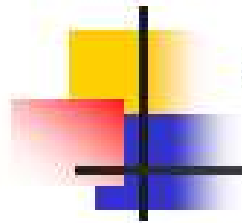
- **Proteomics** is the quantitative study of the proteome using techniques of high resolution protein separation and identification.

蛋白质组学是采用高分辨率蛋白质分离技术和鉴定技术对蛋白质组进行定量分析的学科。

- Research content:
 - Identification of proteins from the proteome of a given cell
 - Structural description of all the proteins
 - Protein modification, function, subcellular localization, and the interactions of proteins (interactome) in complexes



- **Techniques:**
 - Two-dimensional (2D) gel electrophoresis / high performance liquid chromatography (HPLC, 高效液相色谱) + mass spectrometry (MS, 质谱)
 - Imaging of biological molecules
 - Co-Immunoprecipitation (Co-IP)



1.4 Metabolomics

- The **metabolome** is the entire set of **small molecules** – amino acids, nucleotides, sugars etc. and all the intermediates that exist within a cell during their synthesis and degradation.

代谢组是指一整套**小分子**，如氨基酸、核苷酸、糖等，以及细胞内在合成与降解时存在的所有中间产物。

- **Metabolomics, or metabolic profiling, is the quantitative analysis of all cellular metabolites at any one time under defined conditions.**

代谢组学或代谢谱是对特定条件下任意一段时间内所有这些细胞代谢物的定量分析。

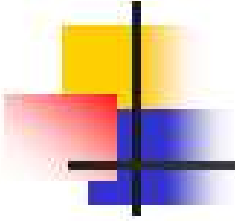
- **Techniques:**
 - Gas chromatography (GS, 气相色谱)
 - HPLC
 - Capillary electrophoresis (毛细管电泳)
 - nuclear magnetic resonance (NMR)
 - MS



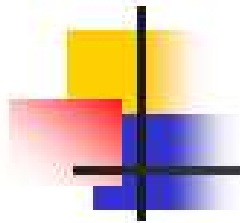
1.5 Other 'omics

- **Glycomics** is the study of **carbohydrates** (mostly extracellular poly saccharides, glycoproteins and proteoglycans) and their involvement in cell-cell and cell-tissue interactions.

糖组学是对**碳水化合物**（主要是细胞外多糖、糖蛋白和蛋白聚糖）及其在细胞-细胞相互作用、细胞-组织相互作用中的功能研究。



- **Lipidomics (脂质组学)**
- **Kinome (激酶组)**
- **Degradome (降解组)**
- **Phosphoproteome (磷酸化蛋白组)**
- **Pseudogenome (假基因组)**



Pseudogene (假基因)

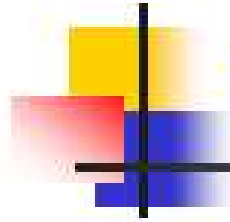
基因家族的某些成员由于碱基顺序发生突变而**失去功能**，不能表达或表达异常的无活性多肽，这些DNA序列称为**假基因**。

加工型假基因

加工成熟的mRNA经反转录产生cDNA，再整合到染色体DNA中。**无内含子**。

非加工型假基因

基因某些功能部位突变而来



2. Global gene expression analysis

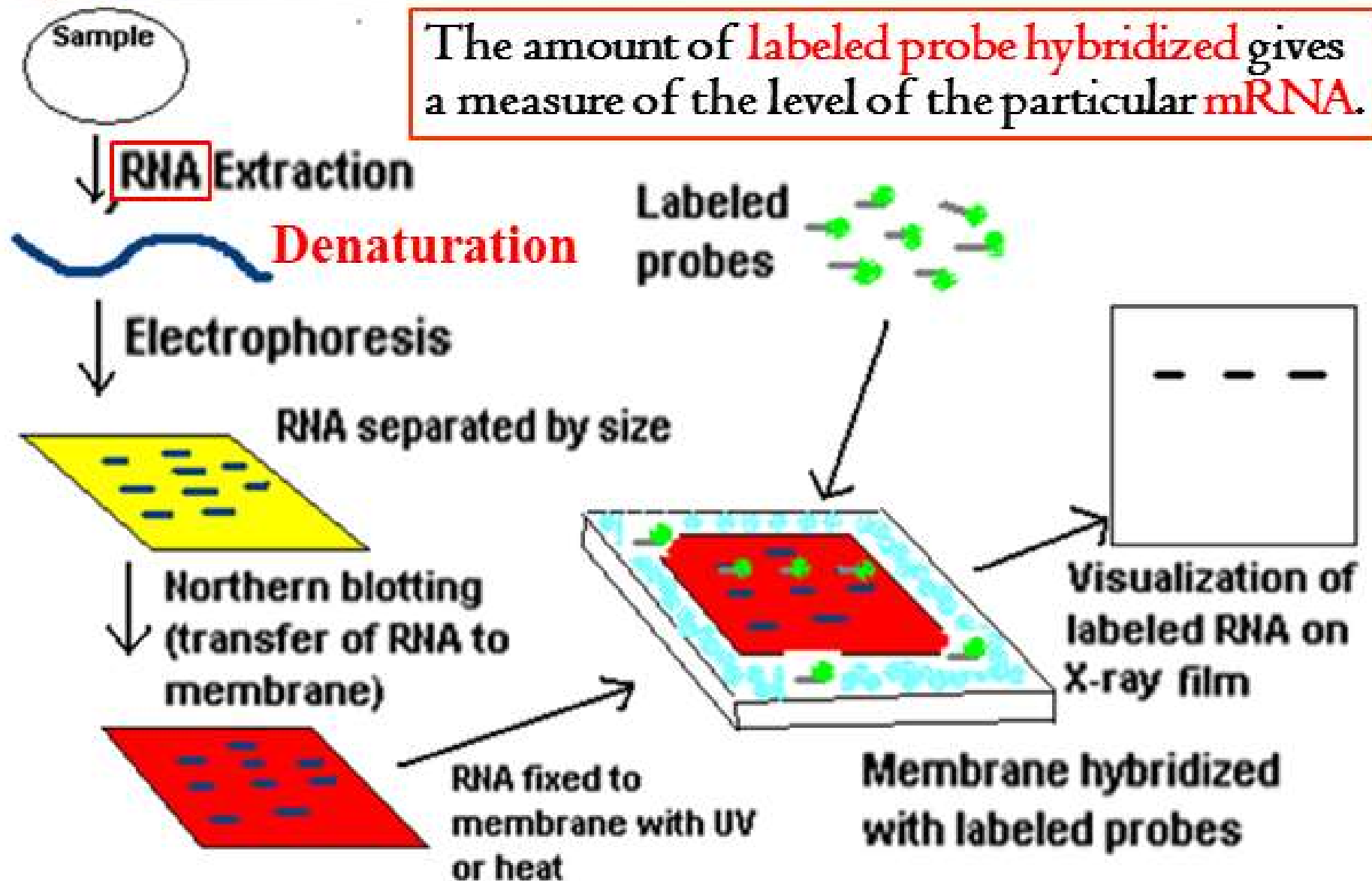
2.1 Detection of mRNA levels

Northern blotting

**Ribonuclease protection assay
(核糖核酸酶保护测定)**

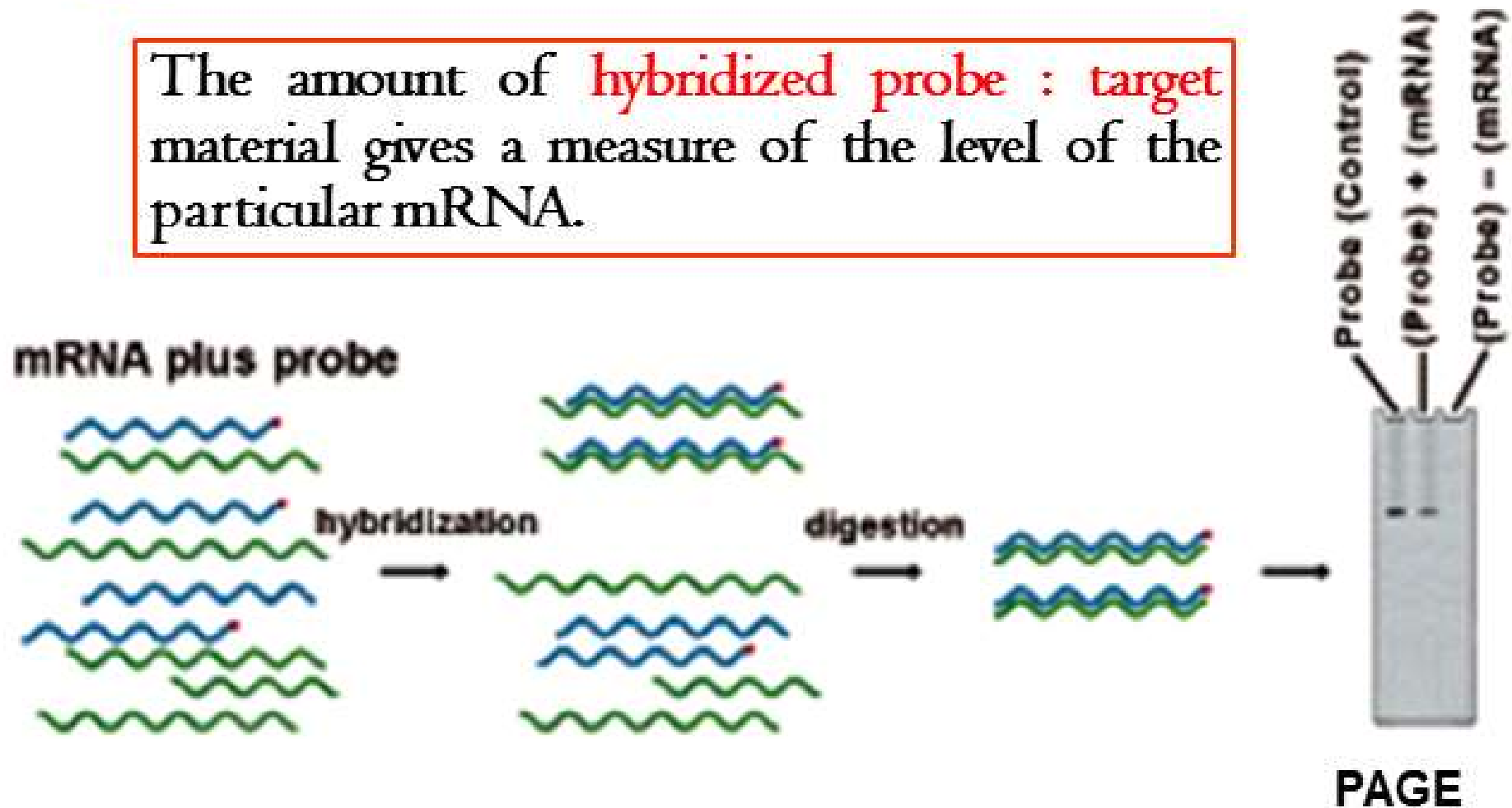
**Reverse transcription coupled with the
polymerase chain reaction (RT-PCR)**

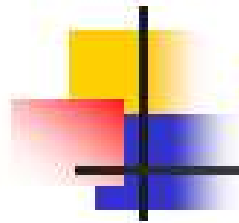
2.1.1 Northern blotting



2.1.2 Ribonuclease protection assay

The amount of **hybridized probe** : target material gives a measure of the level of the particular mRNA.





2.2 Genome-wide analysis

Subtractive cloning (减法克隆)

Differential display (差异显示)

**Serial analysis of gene expression
(SAGE, 基因表达的系列分析)**

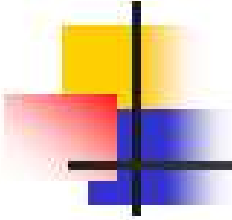
DNA microarray analysis

RNA-seq (RNA测序)



2.2.1 DNA microarray analysis

- **DNA microarray (DNA chips)** are **small, solid supports** on to which DNA samples corresponding to **thousands of different genes** are attached at known locations in a regular pattern of rows and columns. **DNA微阵列 (DNA芯片)** 是在**小型固体支持物**上将**上千种**对应于**不同基因**的DNA样本以规则的阵列方式点样在**已知位置上**。

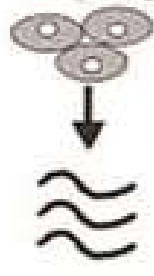


- Follows the principles of **Southern** and **Northern blot**, but with the sample in solution and the **gene probes immobilized**.
- Supports may be made of **glass**, **plastic** or **nylon** and are typically the **size of a microscope slide (显微镜载玻片)**.

Sample A



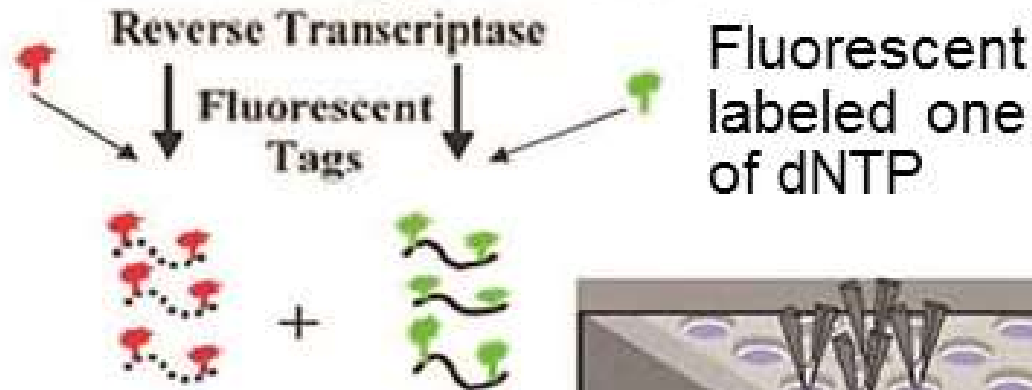
Sample B



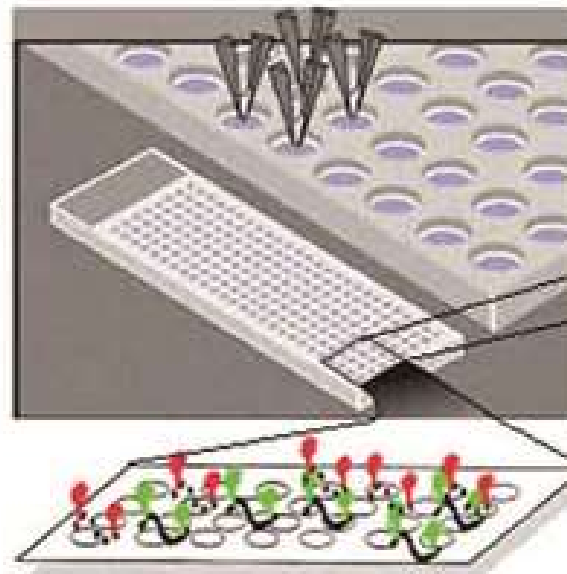
A. RNA Isolation

B. cDNA Generation

C. Labeling of Probe

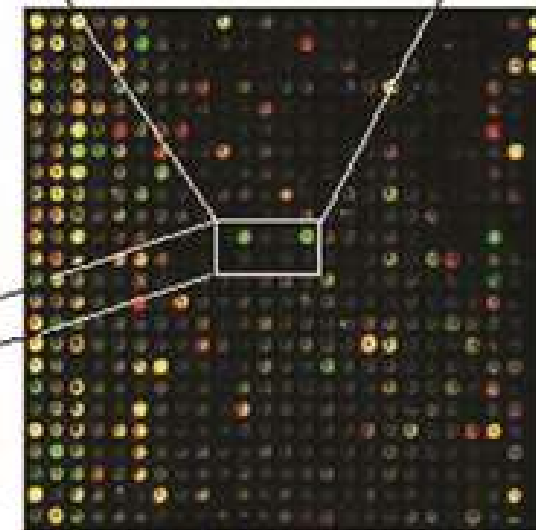
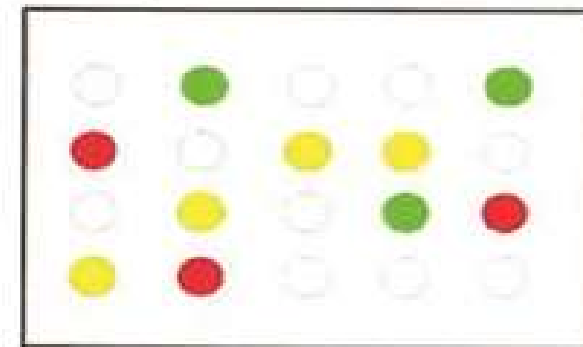


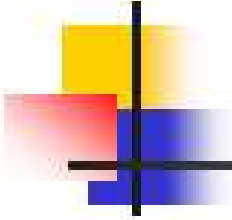
D. Hybridization to Array



E. Imaging

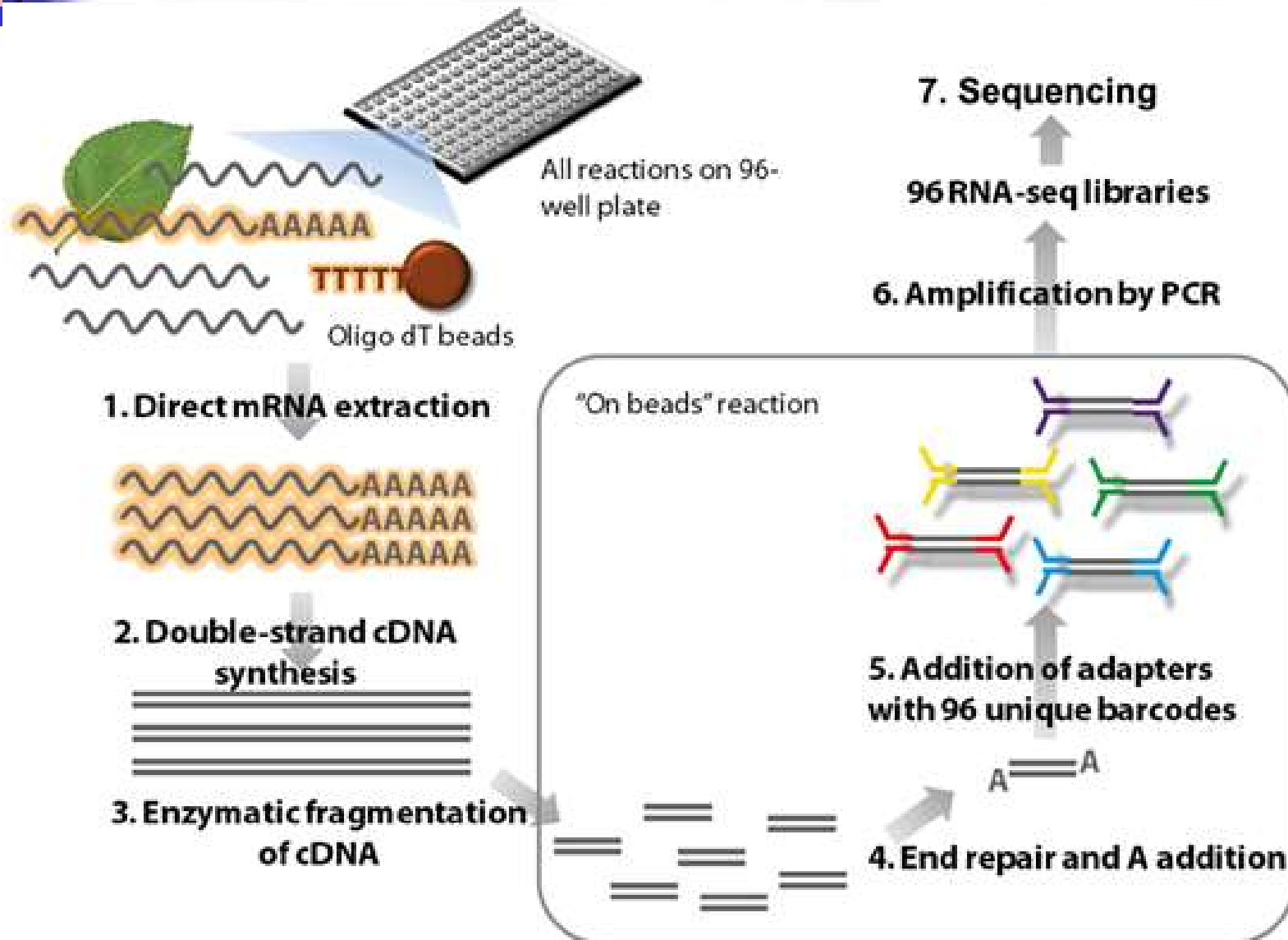
● Sample A > B
● Sample B > A
● Sample A = B

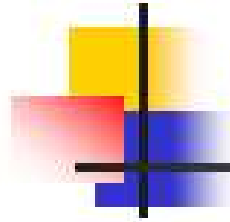




- **Expression microarray** – measures gene expression.
- **Comparative genomic microarray** – detects loss or gain of genomic DNA that may be associated with certain genetic disorders.
- **Mutation microarray** – detects SNPs

2.2.5 RNA-Seq (RNA sequencing)





2.3 Gene knockouts

- A powerful method for determining **gene function**

Gene function {
Gene knockin or overexpression
Gene knockout or knockdown

- Inactivation of the gene by **mutation, disruption or deletion**



2.3.1 Strategy for deleting yeast genes

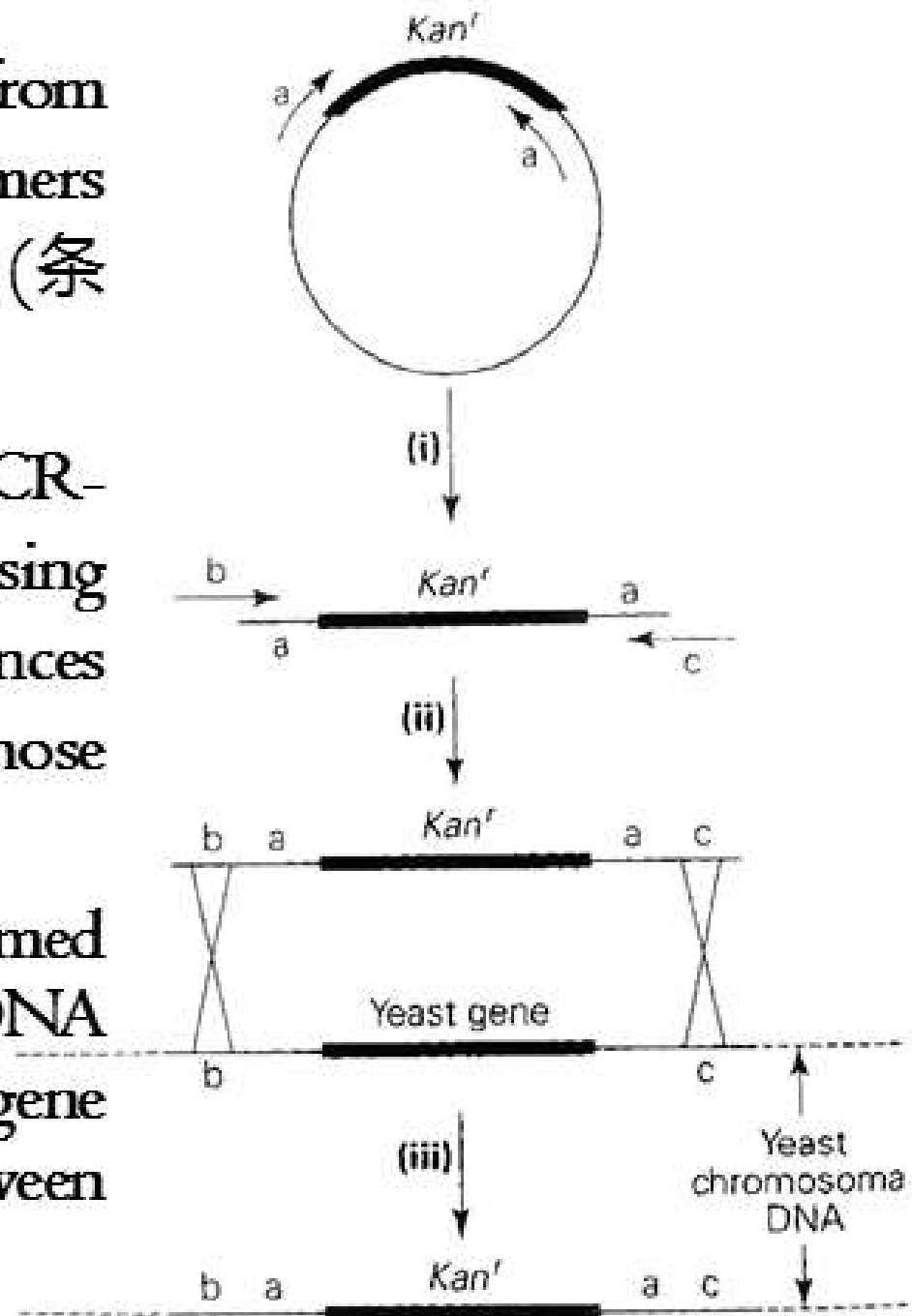
- **Efficient system for homologous recombination**
- **Systematic targeted gene disruption (or targeted insertion mutagenesis, or gene knockout)**

目标基因中断/定向嵌入式诱变/基因敲除

I. *Kan^r* is PCR-amplified from a plasmid using primers containing barcode tag (条形码) sequences (a).

II. PCR product is PCR-amplified again using primers containing sequences (b & c) homologous to those flanking the target gene.

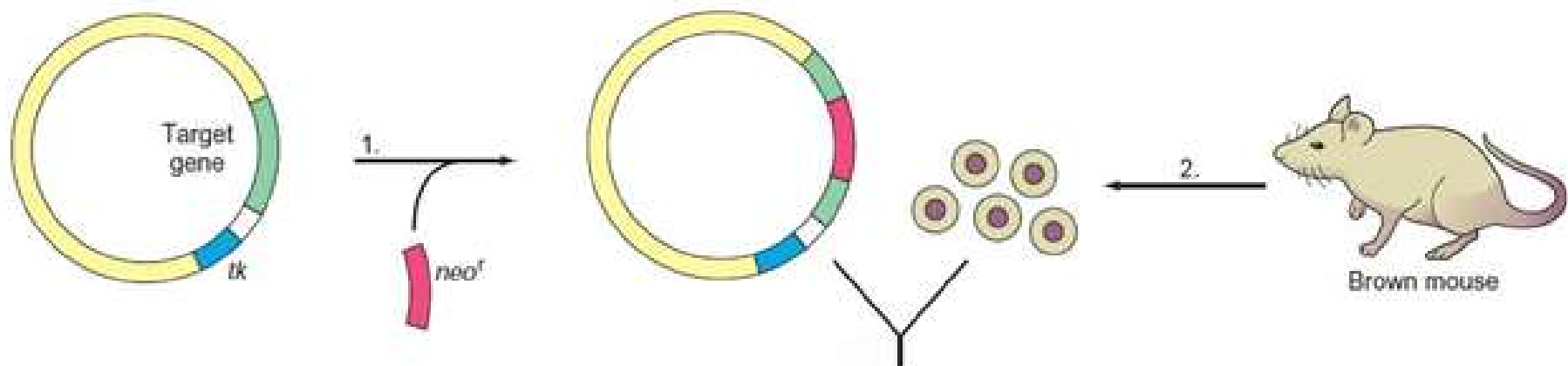
III. Yeast cells are transformed with a marker gene DNA fragment and the target gene is replaced by HR between sequences (b & c).

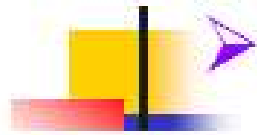


2.3.2 Gene knockout mice

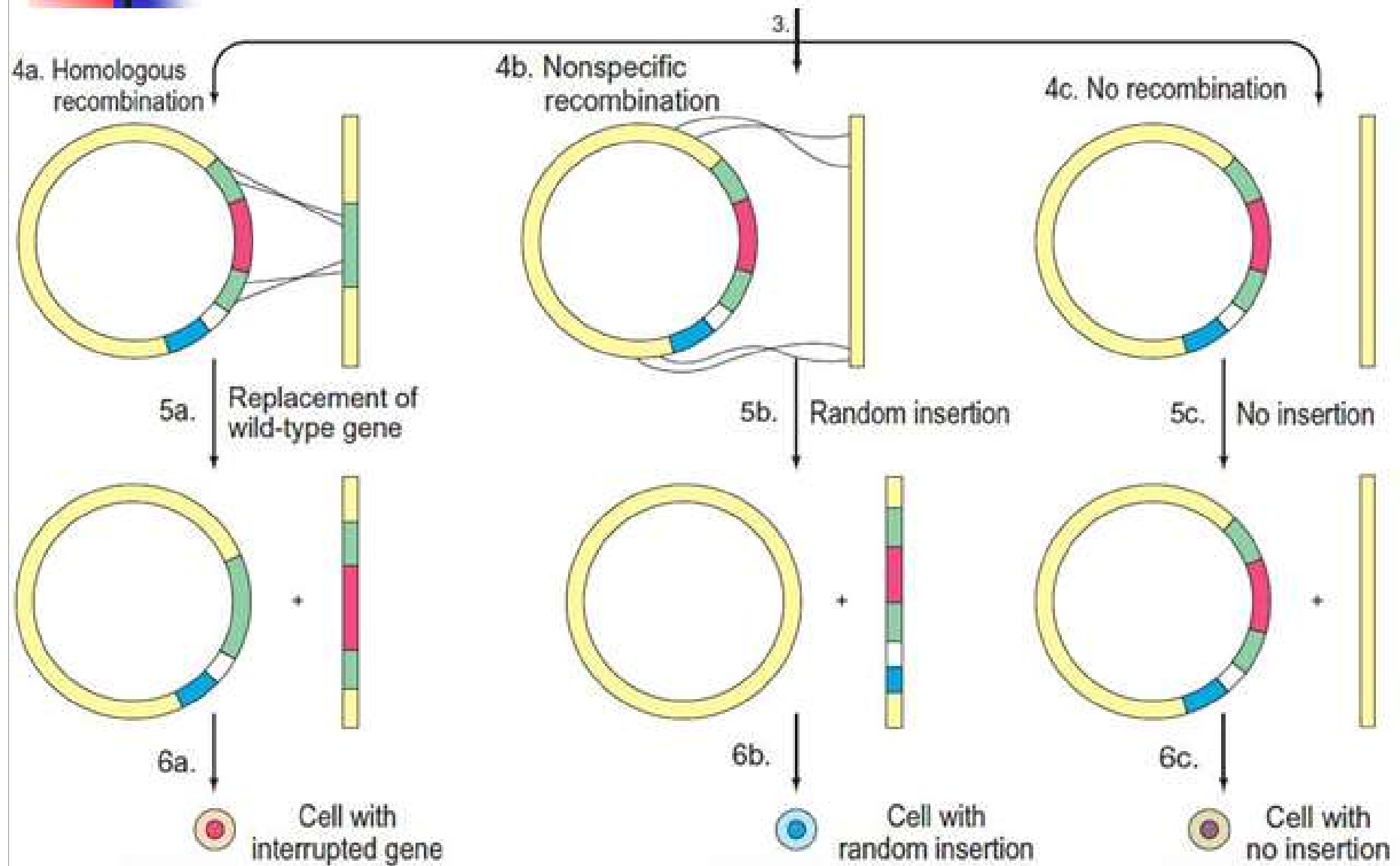
(1) 构建带有干扰基因的胚胎干细胞

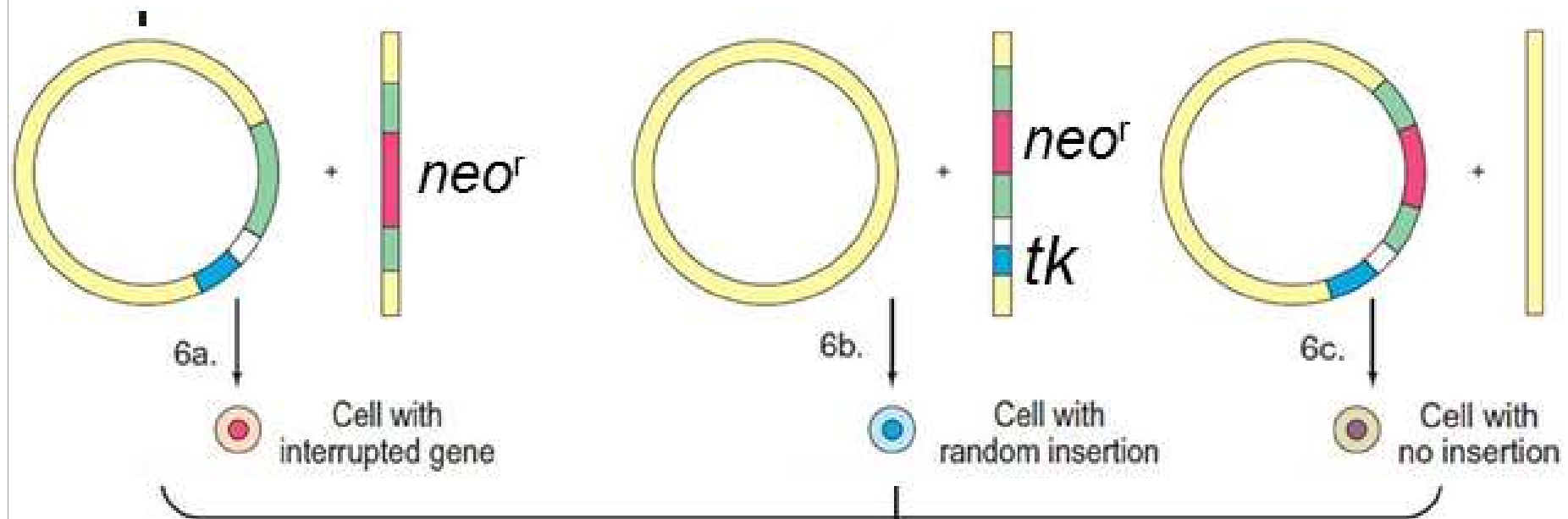
- 构建含有待敲除基因（靶基因）和胸苷激酶基因（ tk^+ ）的质粒。将新霉素抗性基因（ neo^r ）插入靶基因内部。
- 收集小鼠（褐色）的胚胎干细胞（embryonic stem cells, ESC）。





用含有断开靶基因的质粒转染ESC



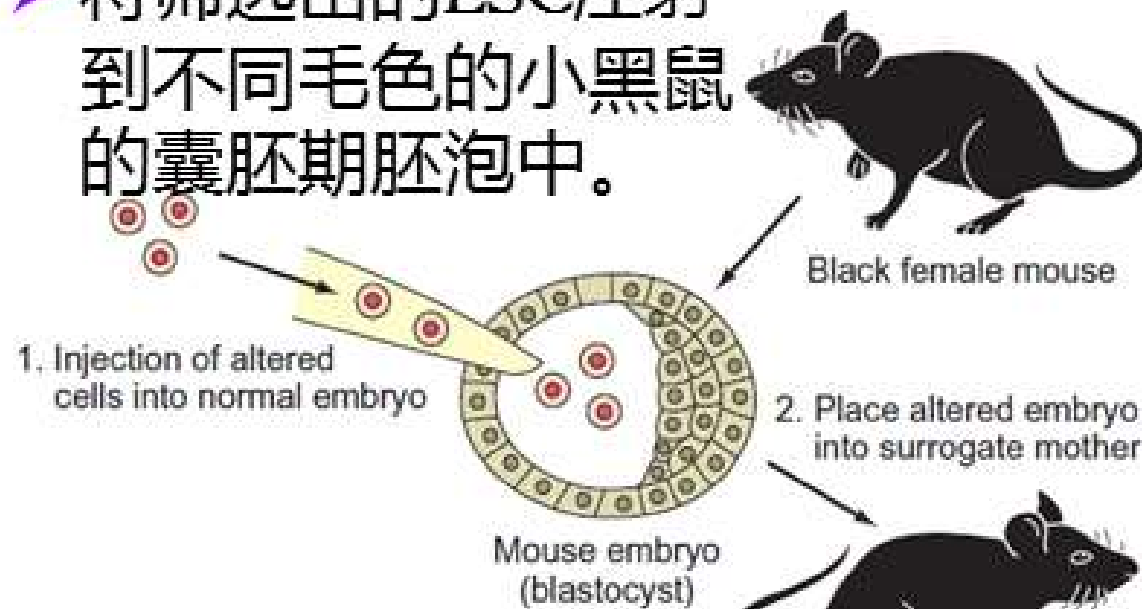


- 收集转化的ESC
- 在含有新霉素类似物 G418（杀死无新霉素抗性细胞）和丙氧鸟苷（杀死含 tk 基因的细胞）的培养基中培养。

可以用CRISPR/Cas9

(2) 将含有断开靶基因的ESC置入动物体

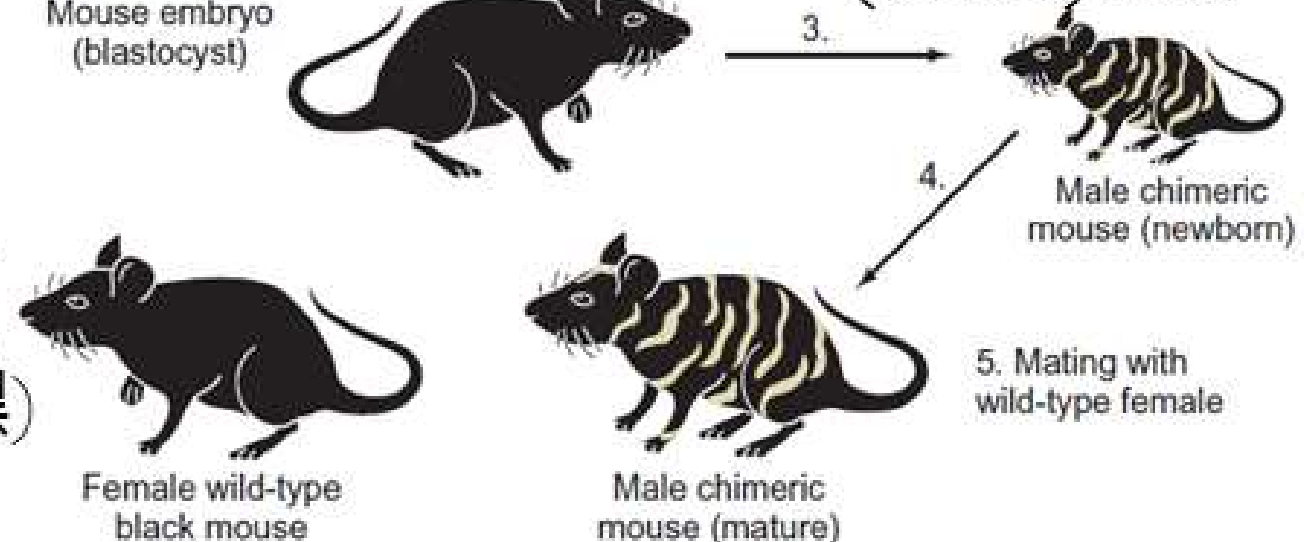
- 将筛选出的ESC注射到不同毛色的小黑鼠的囊胚期胚泡中。

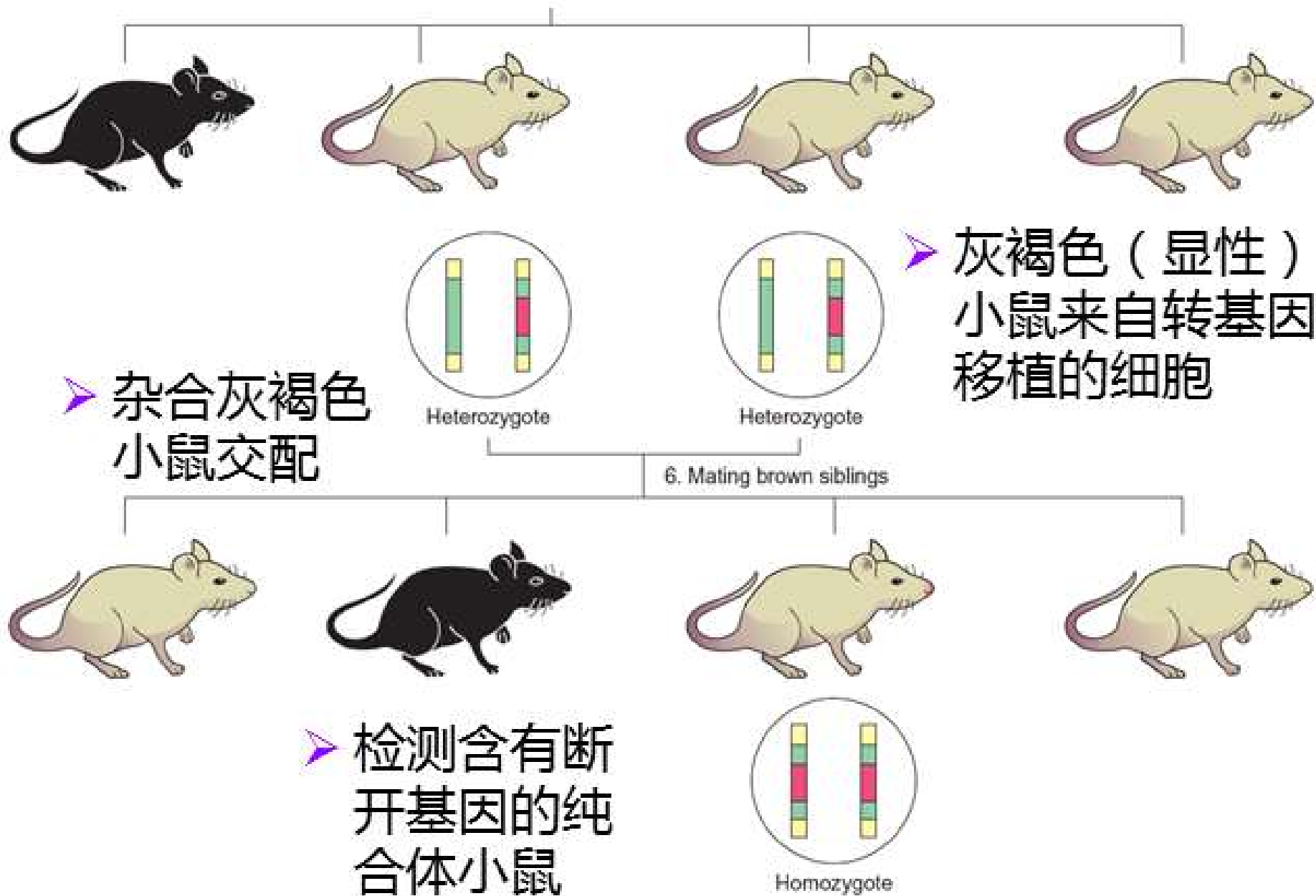


- 将混合胚胎置入代孕母鼠子宫。

- 产下嵌合体 (mosaic) 小鼠

- 嵌合体雄性小鼠与野生型雌性小鼠(黑)交配

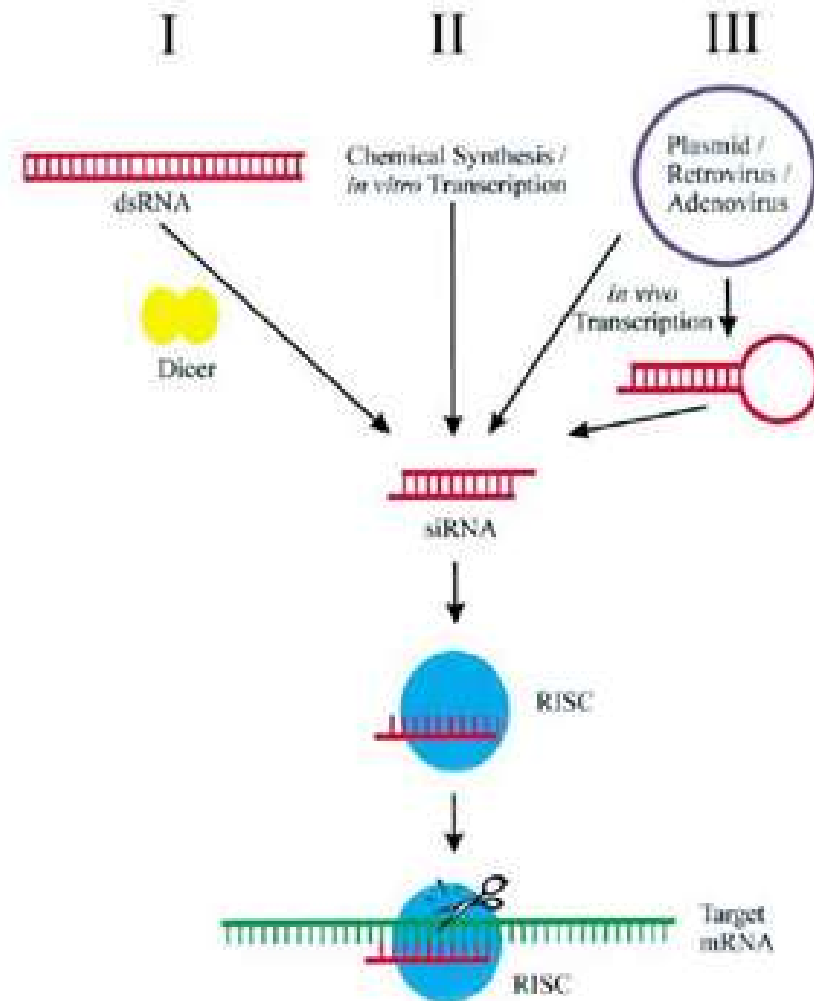




2.4 RNA knockdown (减量表达/敲降)

- RNAi / gene knockdown

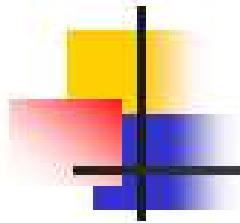
Introduction of certain types of double-stranded RNA into cells promotes the degradation of homologous mRNA transcripts.





2.5 Gene editing (基因编辑)

- **基因编辑**是一种可以在基因组水平上对DNA序列进行改造的遗传操作技术。
- **原理**：构建一个**人工内切酶**，在预定的基因组位置切断dsDNA，在被细胞内的DNA修复系统（NHEJ或同源重组）修复过程中达到定点改造基因组的目的（基因敲除、定点突变、转基因）。

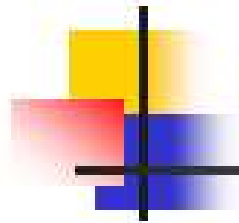


- **ZFN** (Zinc-finger nucleases , 锌指**核酸酶**)
- **TALEN** (transcription activator-like effector nucleases , 转录激活因子样效应物**核酸酶**)
- **CRISPR/Cas9** (clustered regulatory interspaced short palindromic repeat /Cas9-based RNA-guided DNA endonucleases , 成簇规律间隔的短回文重复序列/基于Cas9的RNA指导的DNA内切**核酸酶**)

特异性
DNA识别域

+

非特异性
核酸内切酶



3. Proteomics

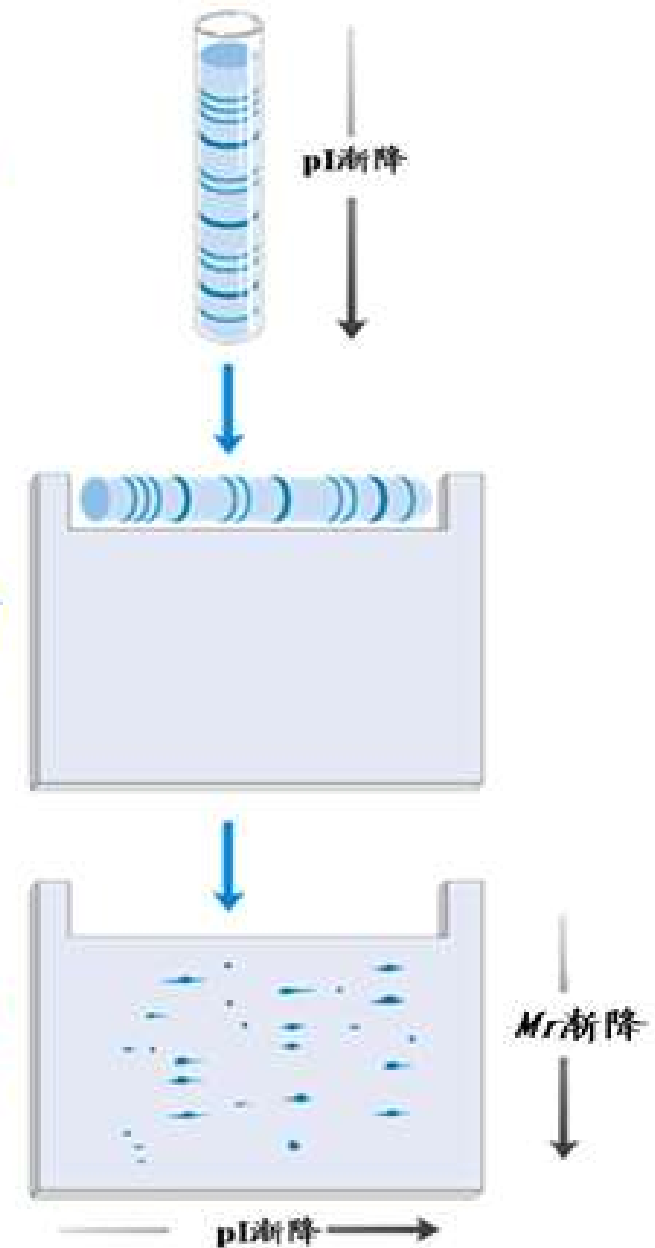
3.1 Proteomics

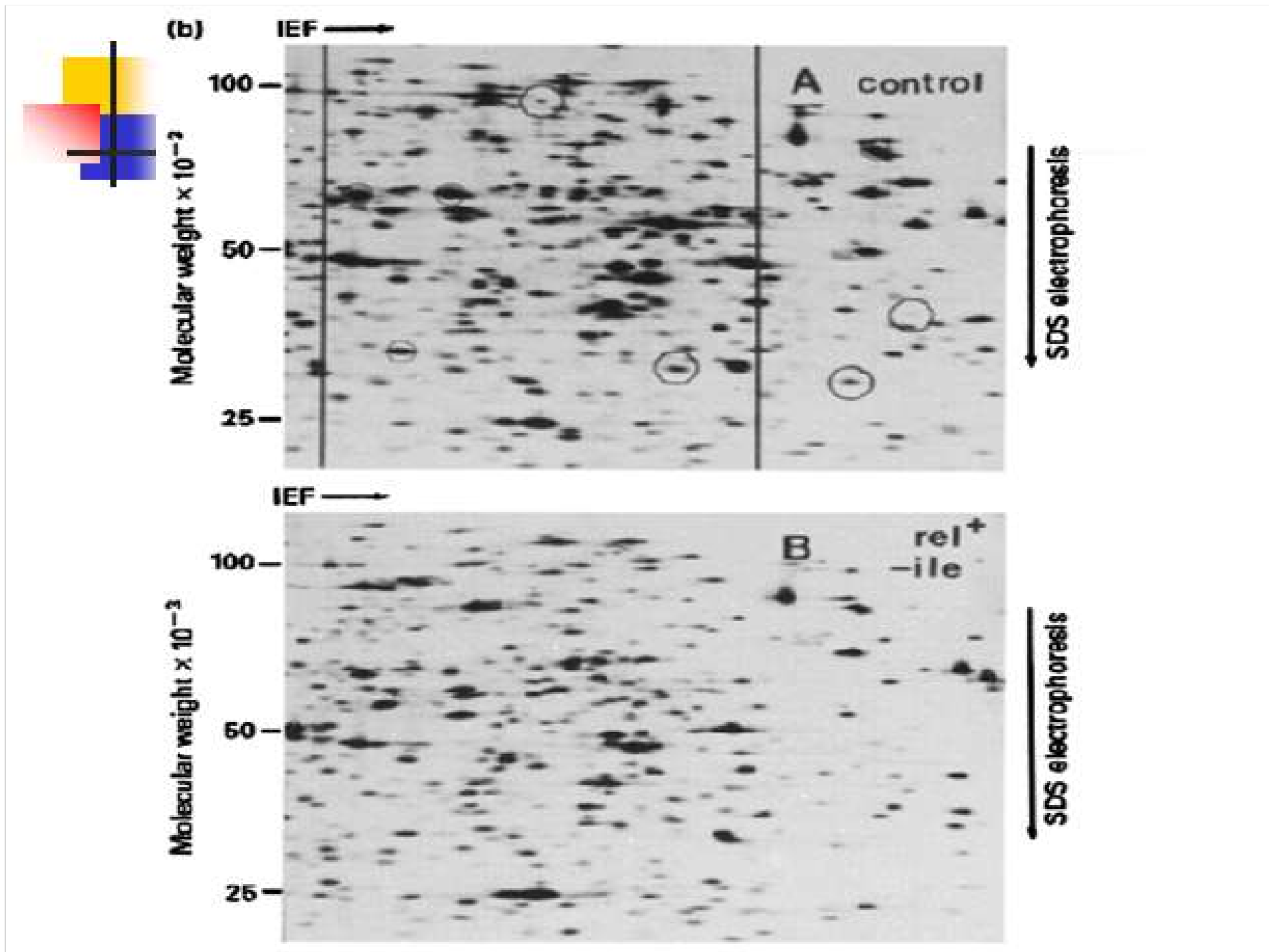
双向电泳

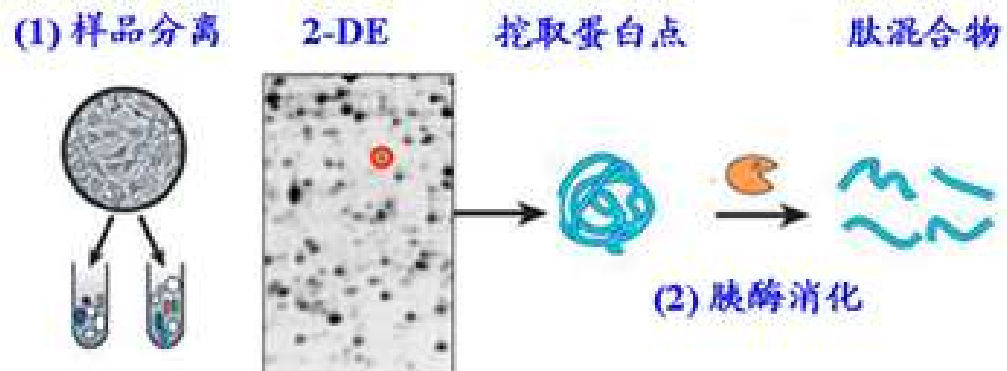
一维电泳
等电聚焦

IEF胶条置于
SDS凝胶上

二维电泳
SDS-PAGE



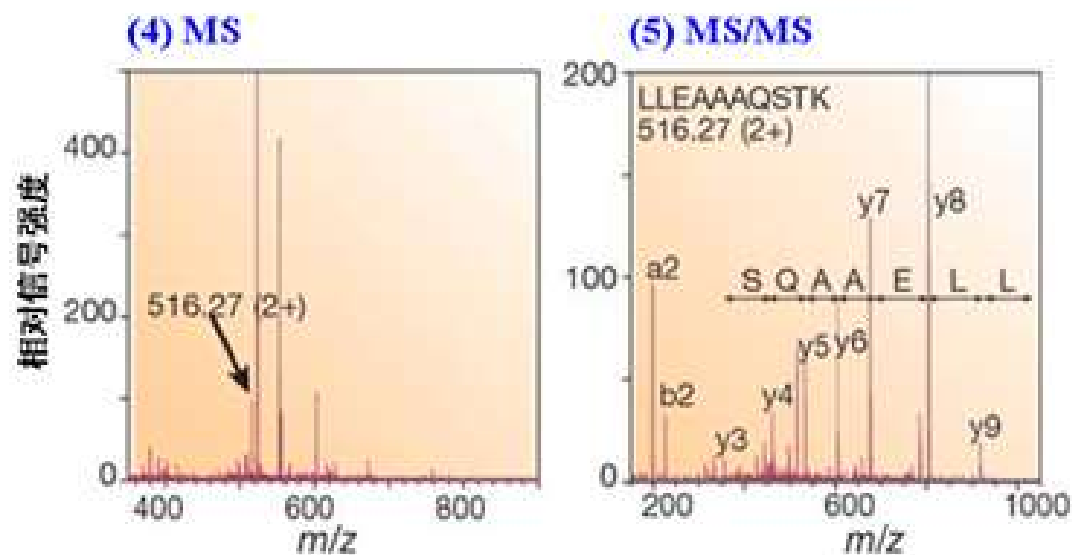


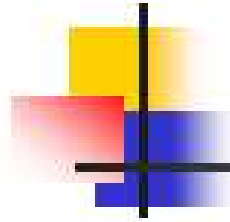


(2) 胰酶消化



蛋白质的质谱分析



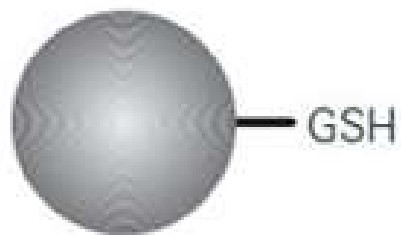


3.2 Protein–protein interactions

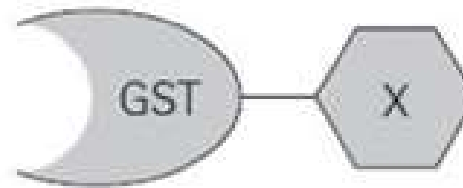
- Co-immunoprecipitation (co-IP)
- Two-hybrid analysis
- **GST pull-down**
- **Protein arrays/chips**

3.2.1 GST pull-down

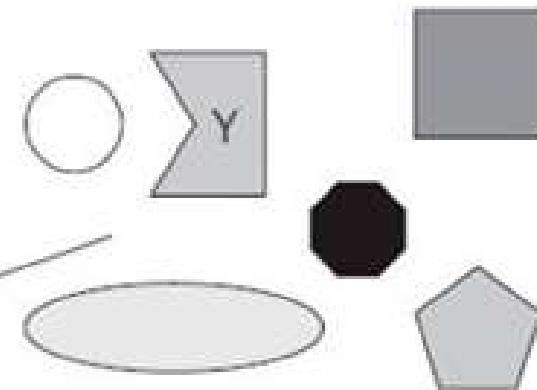
Agarose bead with
attached glutathione
(GSH)



Fusion protein containing
glutathione-S-transferase
(GST) and bait ('X')

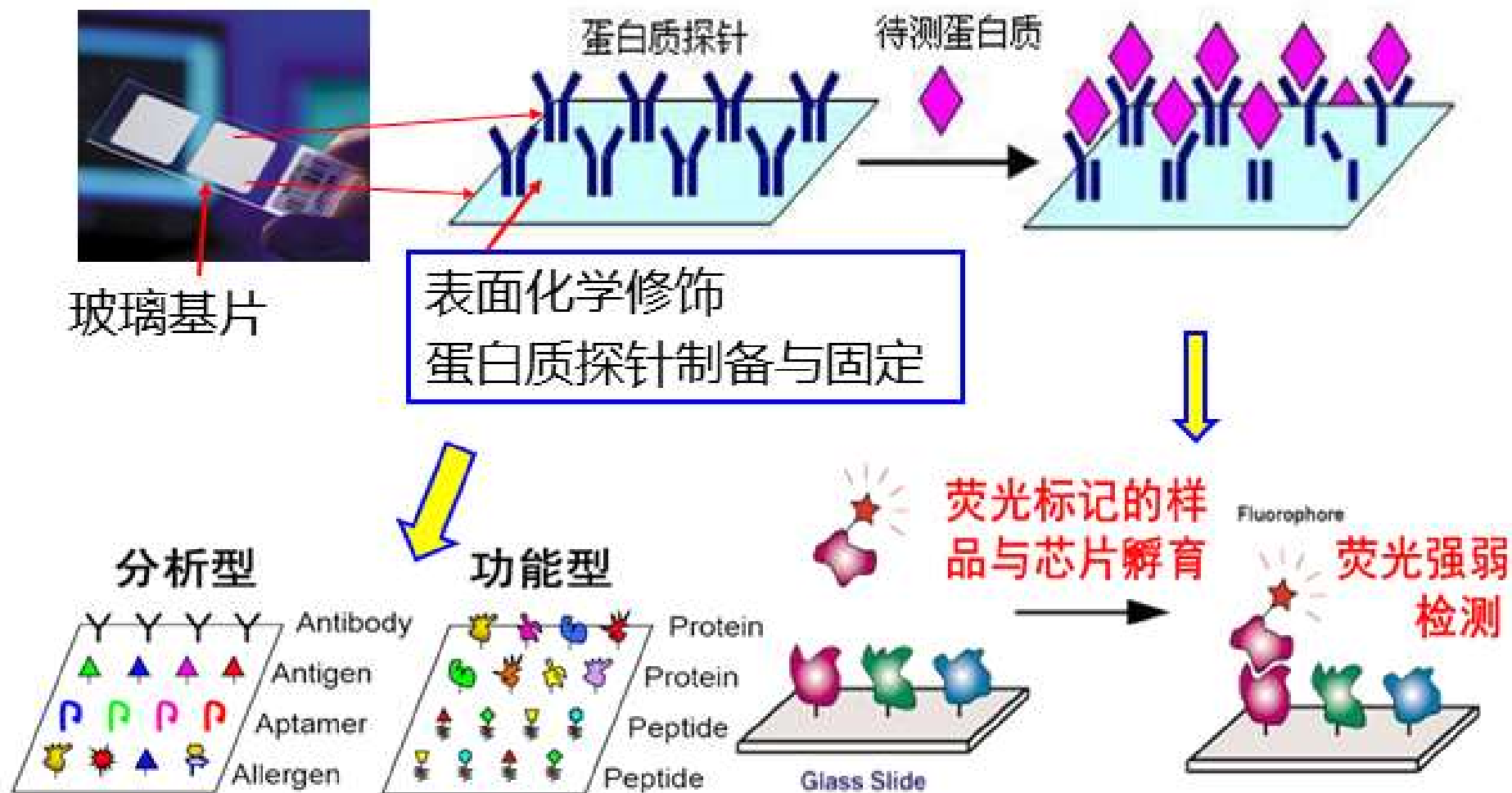


Cell extract containing
the interacting prey ('Y')



Complex isolated by centrifugation

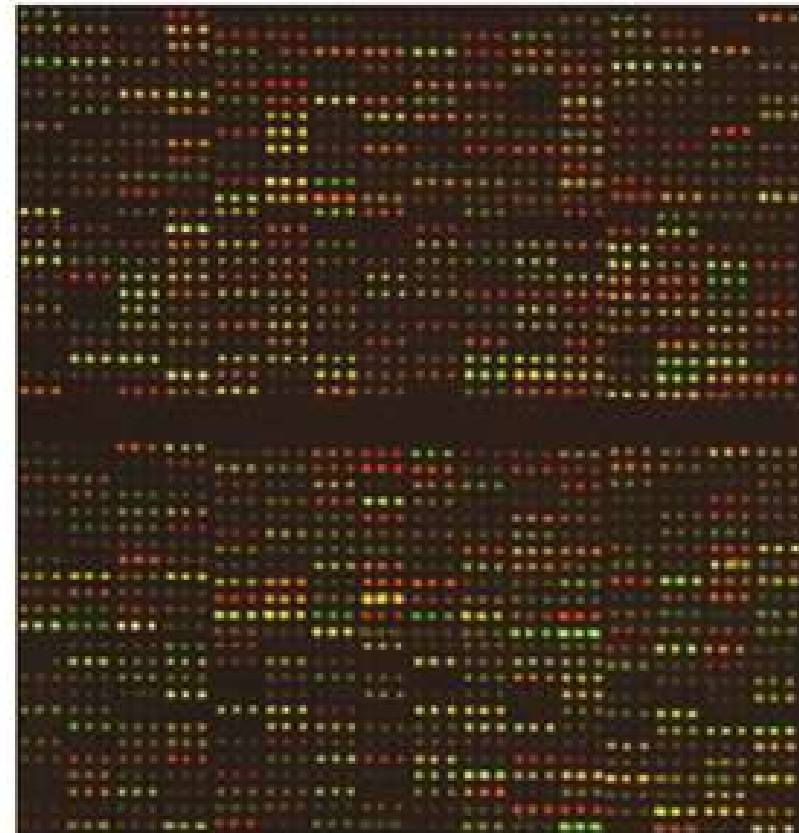
3.2.2 Protein arrays/chips



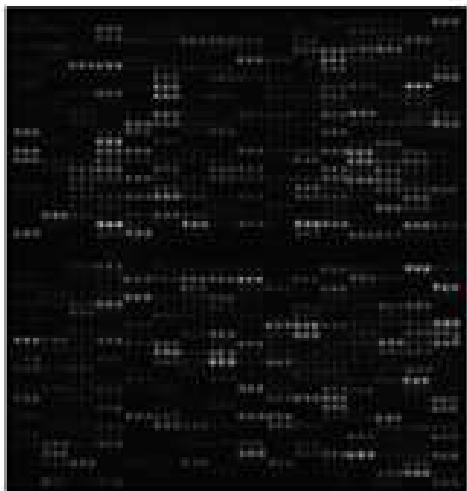
Cy3 channel (control)



False-color overlay



Cy5 channel (treatment)



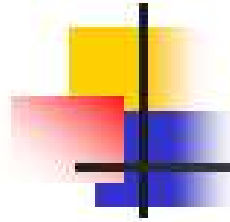
4. Cell and molecular imaging

4.1 Cell imaging (细胞成像)

- **Cell imaging** is the visualization of cells, subcellular structures, or molecules within them to follow events or processes they undergo.

Fluorescence microscope
(荧光显微镜)



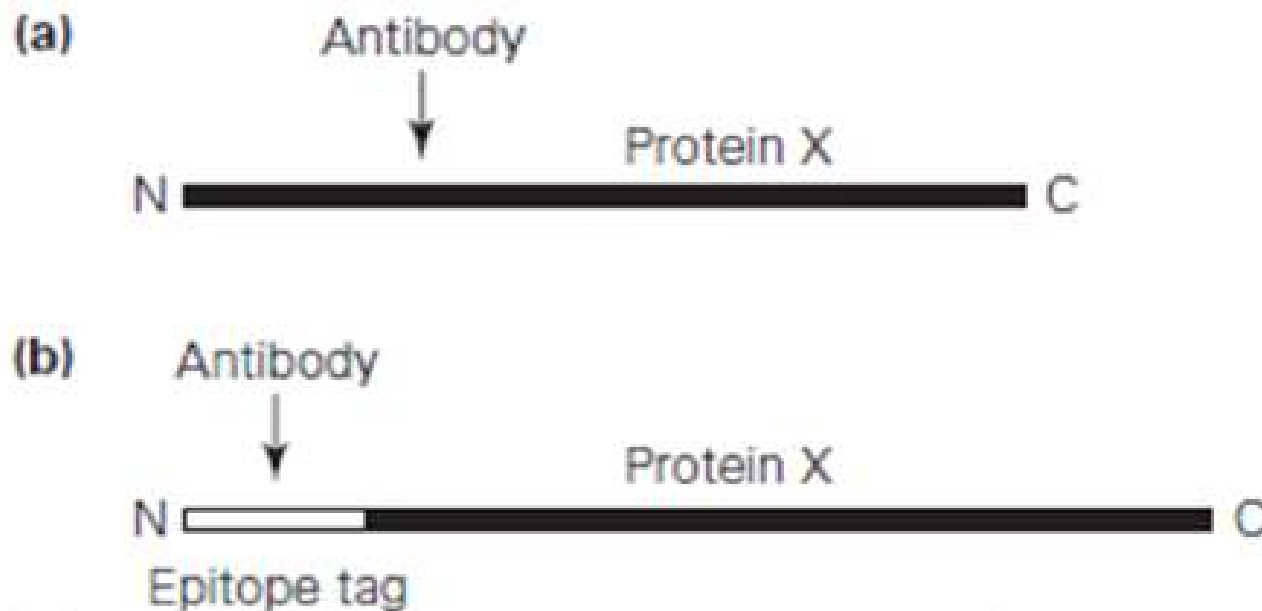


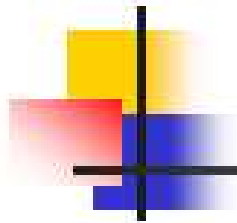
4.2 Detection technology

- **Labeling (标记)** – assists with visualization of the detected molecules and cells.
 - Radioactive (放射性的) labels
 - Colored (显色的) labels
 - Luminescent (发光的) labels
 - nuclear magnetic resonance (NMR)
 - **Fluorescent (荧光的) labels** – most commonly

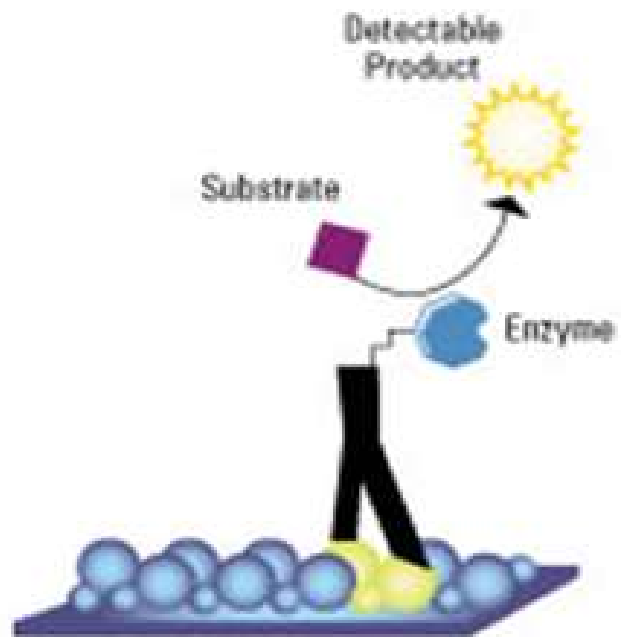
4.3 *In vitro* (体外) detection

- **Nucleic acid probes or antibodies** are often used to detect biological molecules in cells and tissues.

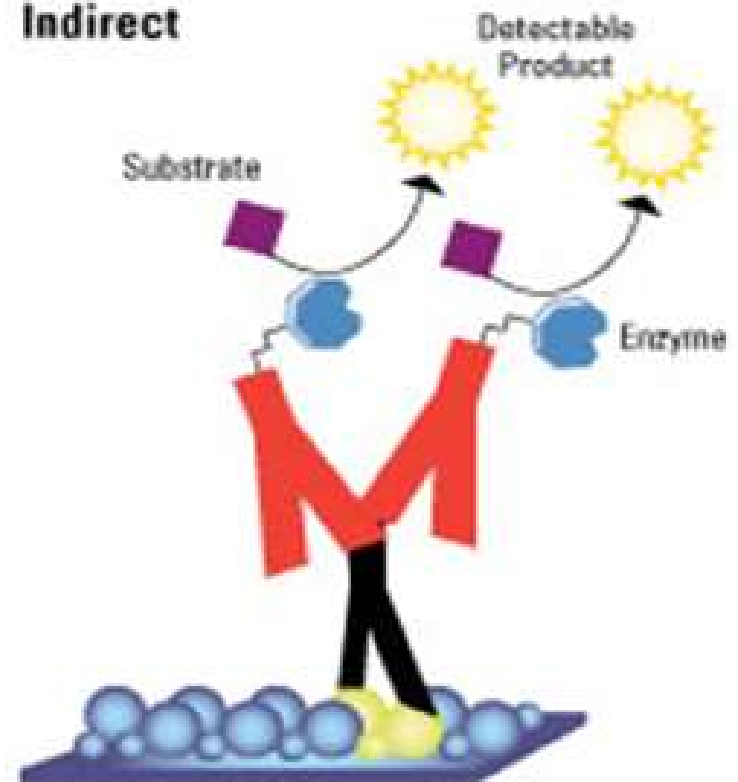




Direct



Indirect



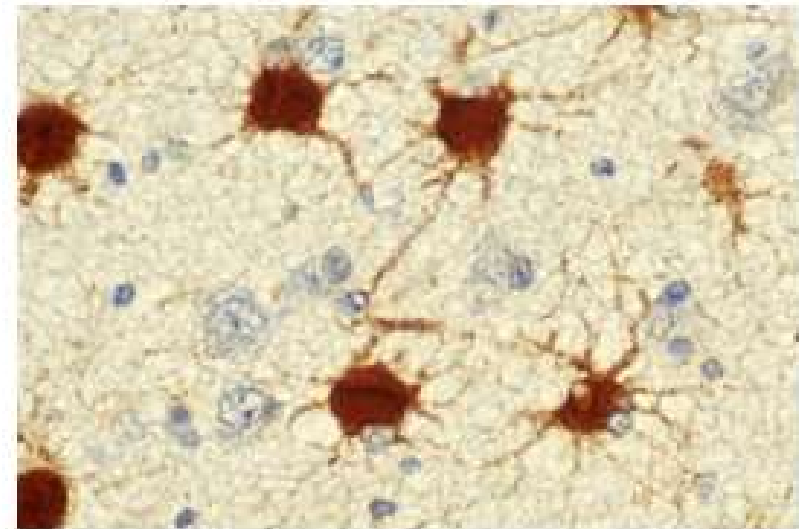
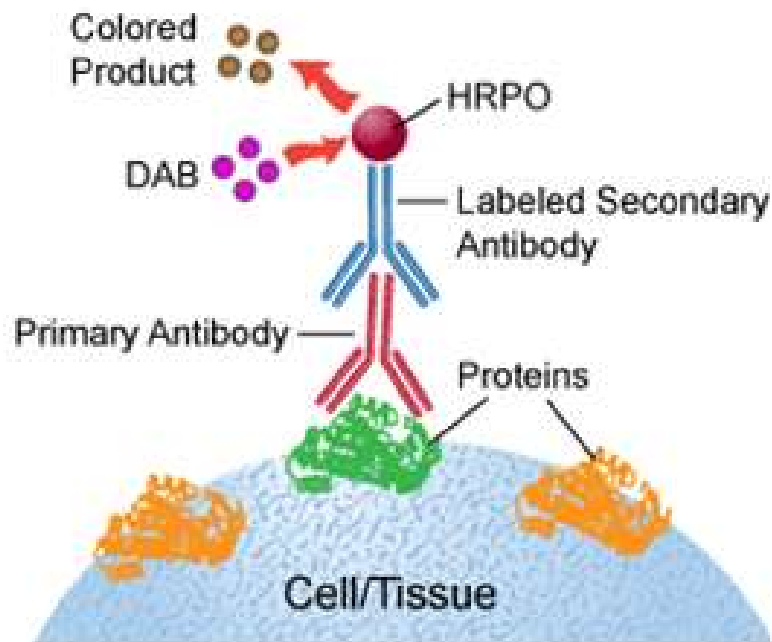
4.4 Imaging of biological molecules in fixed cells

- **Cell fixation (细胞固定)**
 - Maintain cell structure
 - Allowing permeability of antibody or nucleic acid probes
- ***in situ* hybridization (ISH, 原位杂交) - Detecting complementary nucleic acids using either radioactive or fluorescent (FISH) probes.**

- Immunocytochemistry (ICC, 免疫细胞化学)
& Immunohistochemistry (IHC, 免疫组织化学)

➡ Detecting proteins with antibodies

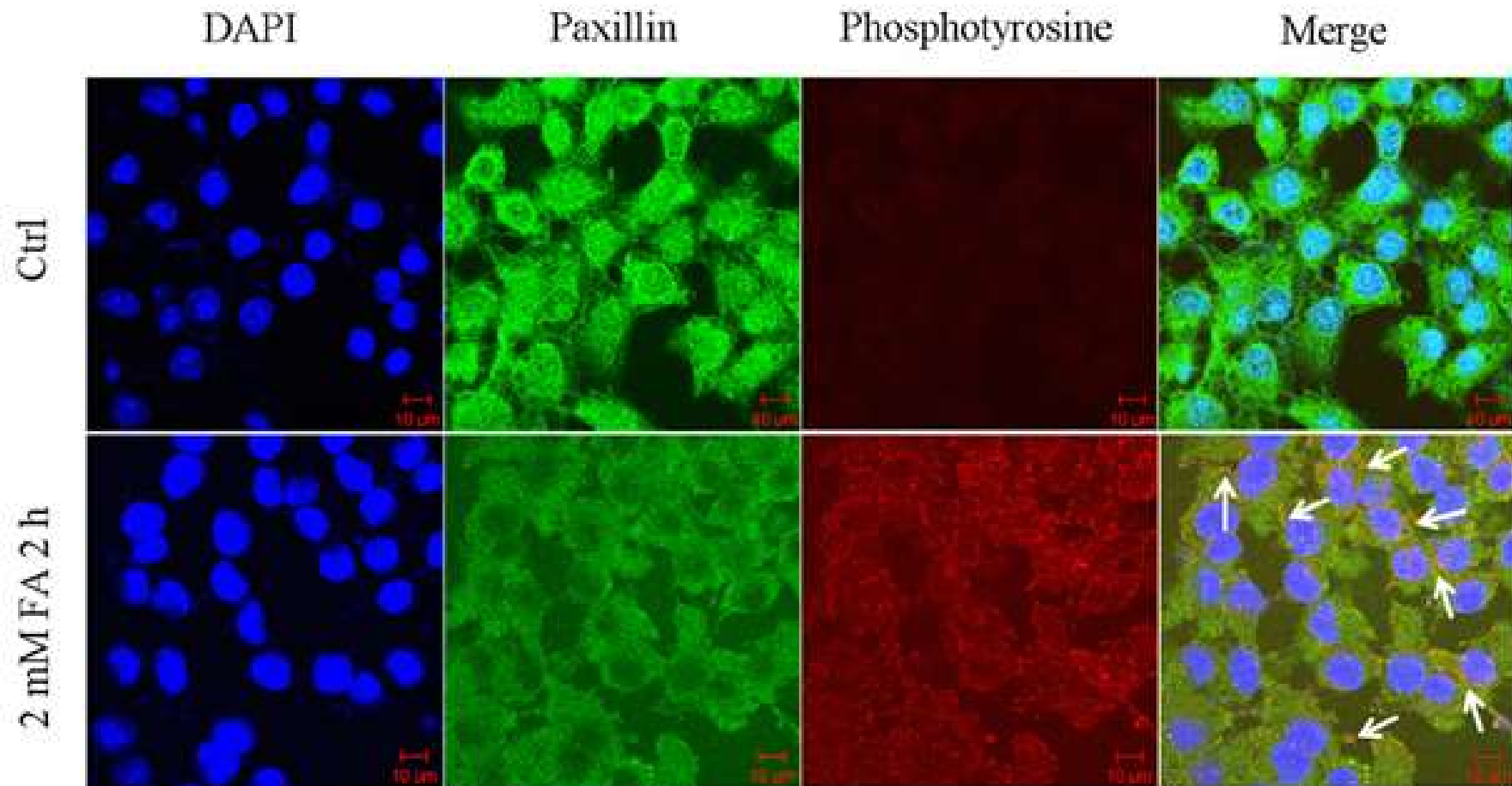
Indirect Immunohistochemistry



Astrocytes (星形胶质细胞) in brain tissue; immunostain for GFAP (胶质纤维酸性蛋白)

- IHC generally involves colorimetric rather than fluorescent detection due to the high level of background fluorescence in many tissue

➤ Immunofluorescence (免疫荧光)



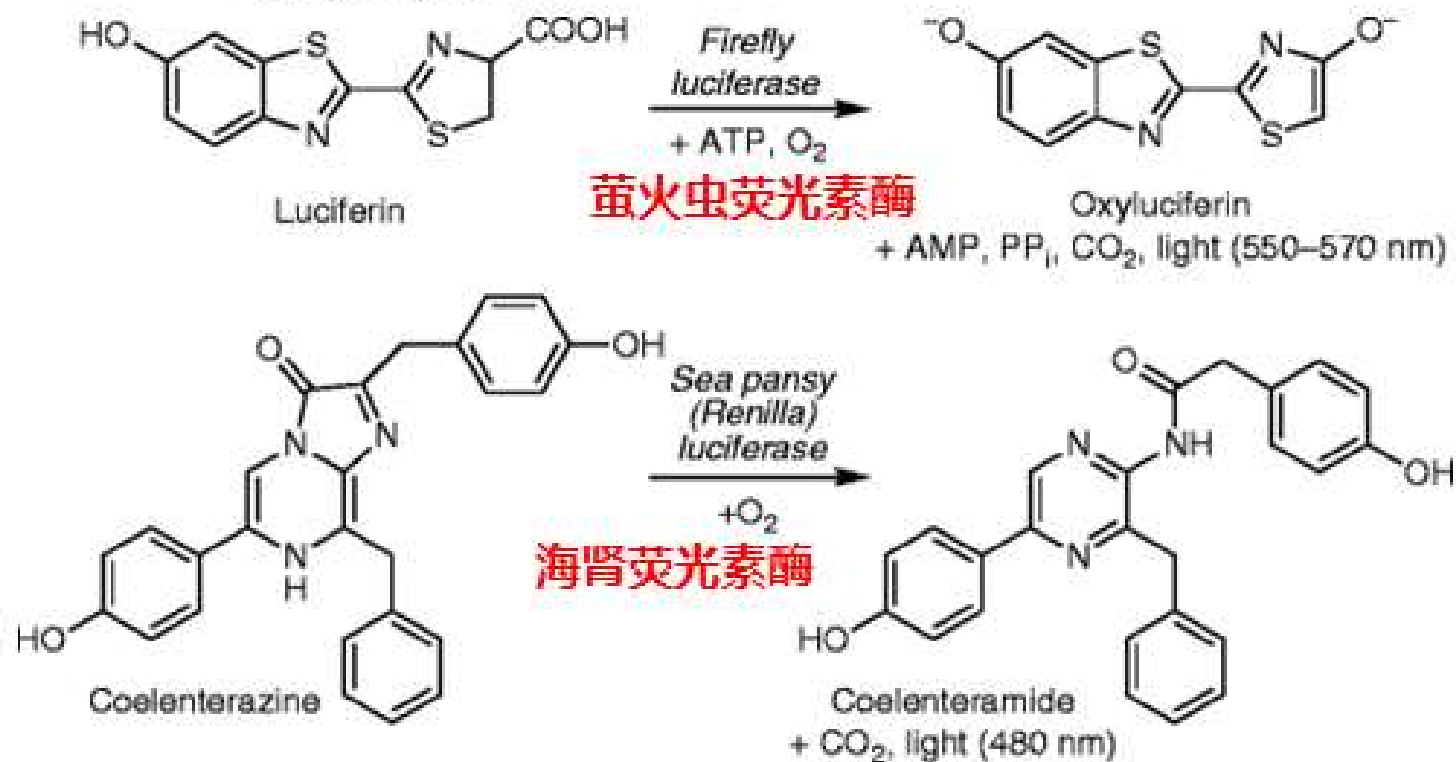
4.5 Detection of molecules in living cells and tissues

- **Noninvasive (无创的)**
 - Not kill the cells
 - Not damage the cells
 - Not perturb (干扰) the cells
- **Reporter genes (报告基因)** express protein products (reporter proteins) that are not normally expressed in the cells of interest.

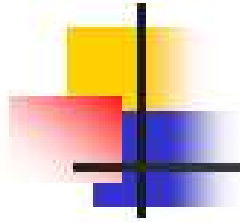
Chloramphenicol acetyl transferase (CAT, 氯霉素乙酰转移酶)

LacZ (β -galactosidase)

Luciferase (荧光素酶)



Fluorescent protein (荧光蛋白)



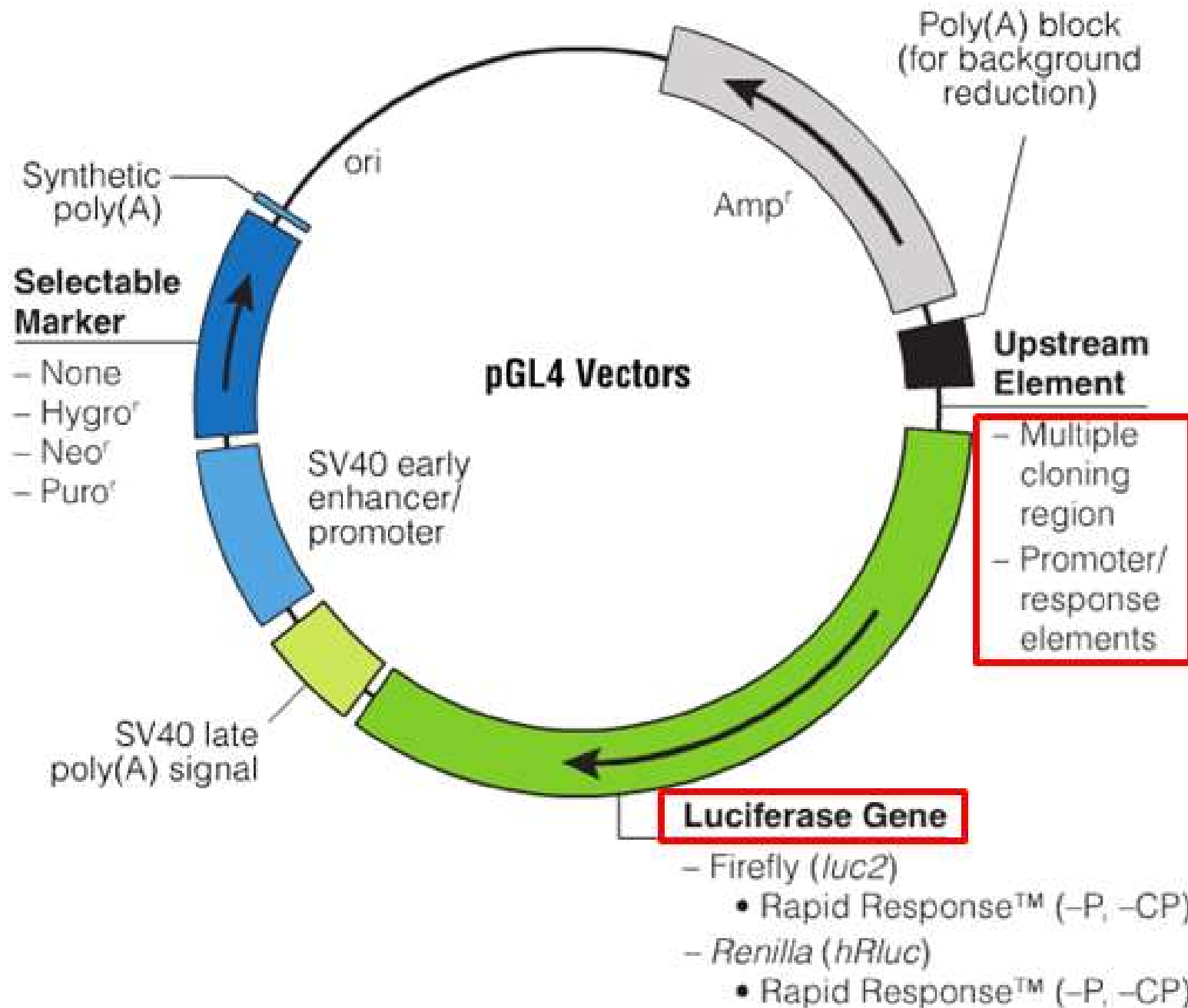
Promoter

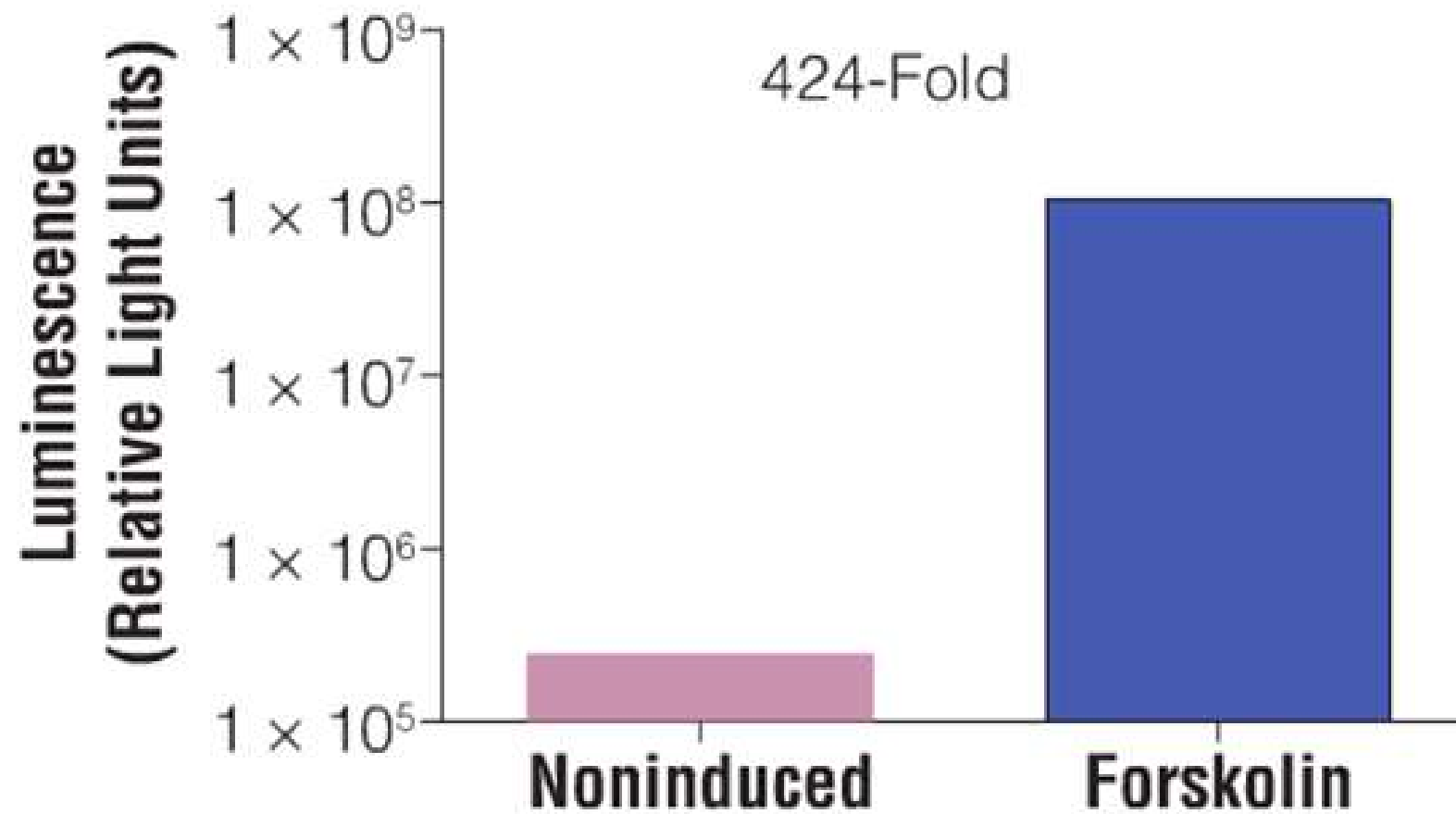
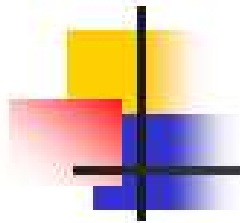
Reporter gene

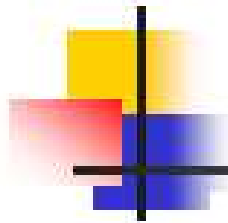
Protein X



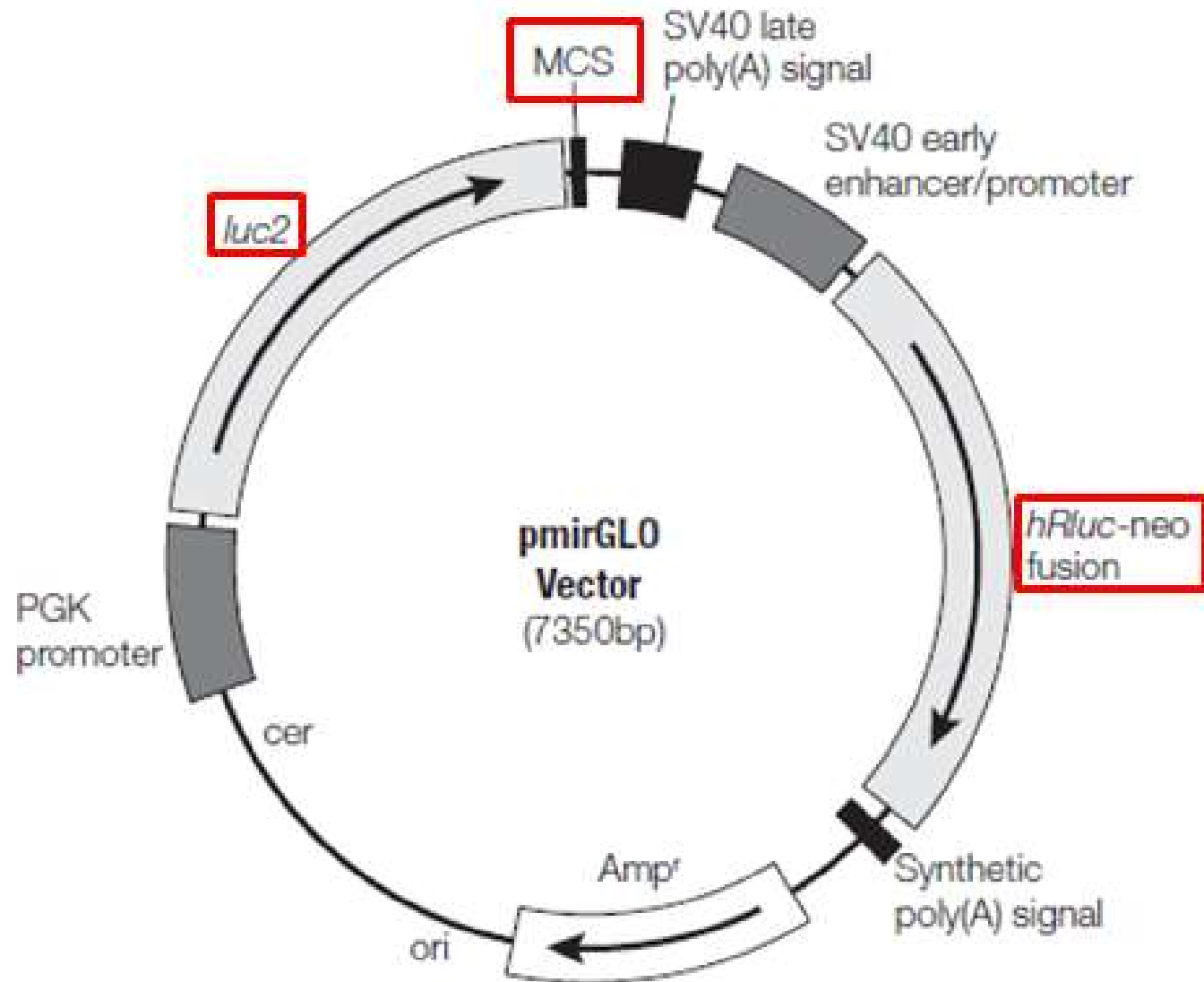
- Measure indirectly the expression of specific gene
- Measure the activity of the promoter of interest

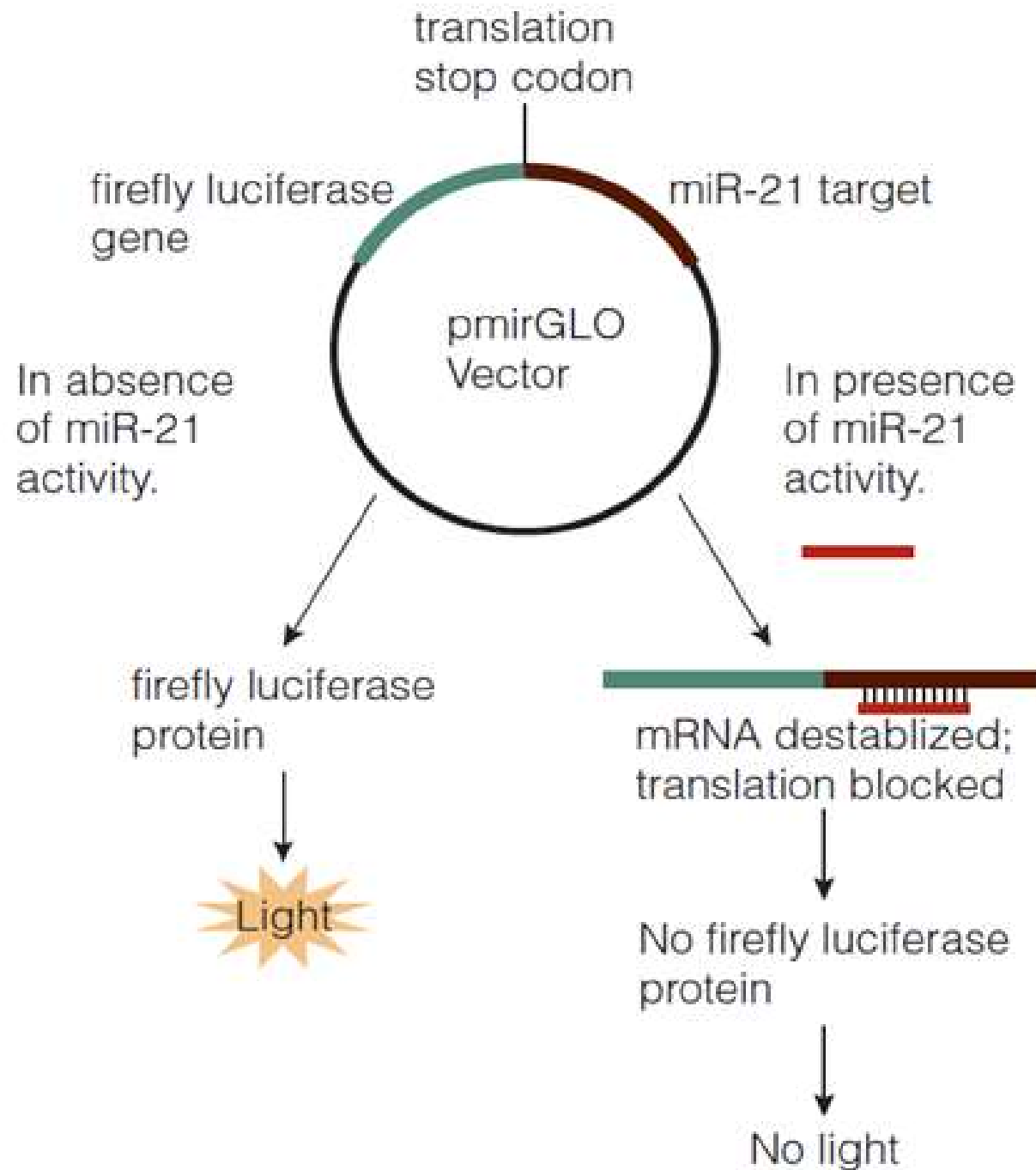




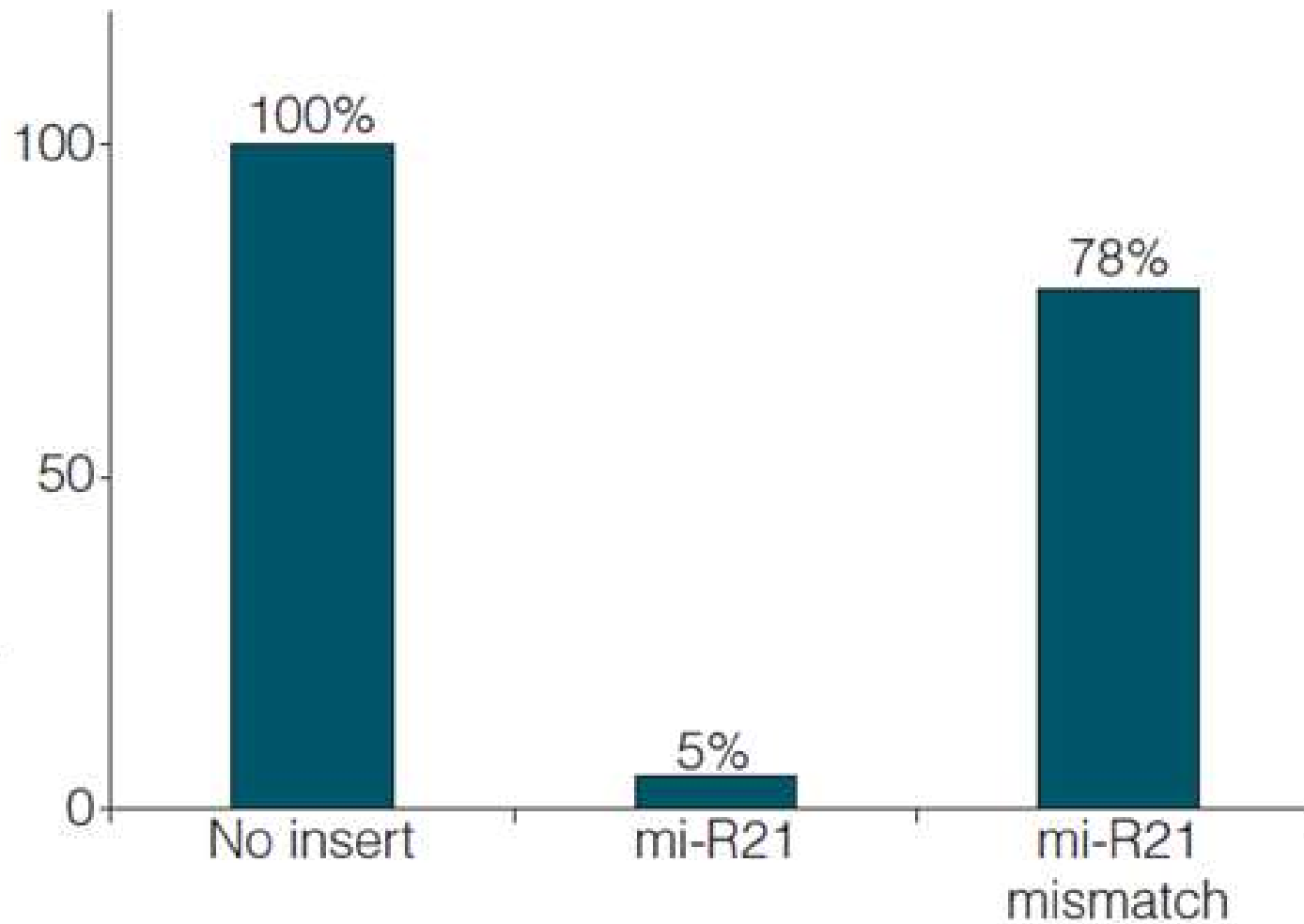


➤ Evaluate miRNA activity

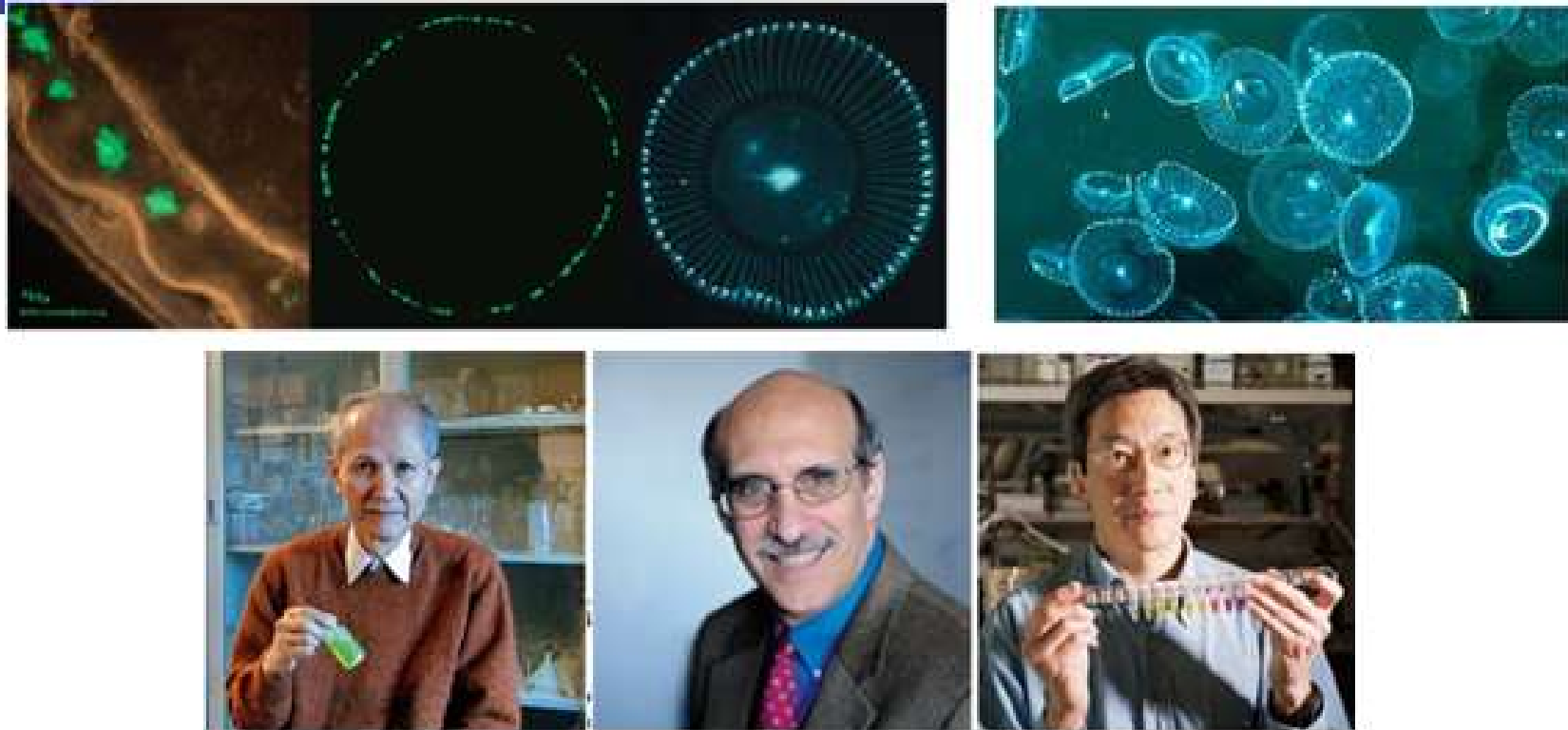




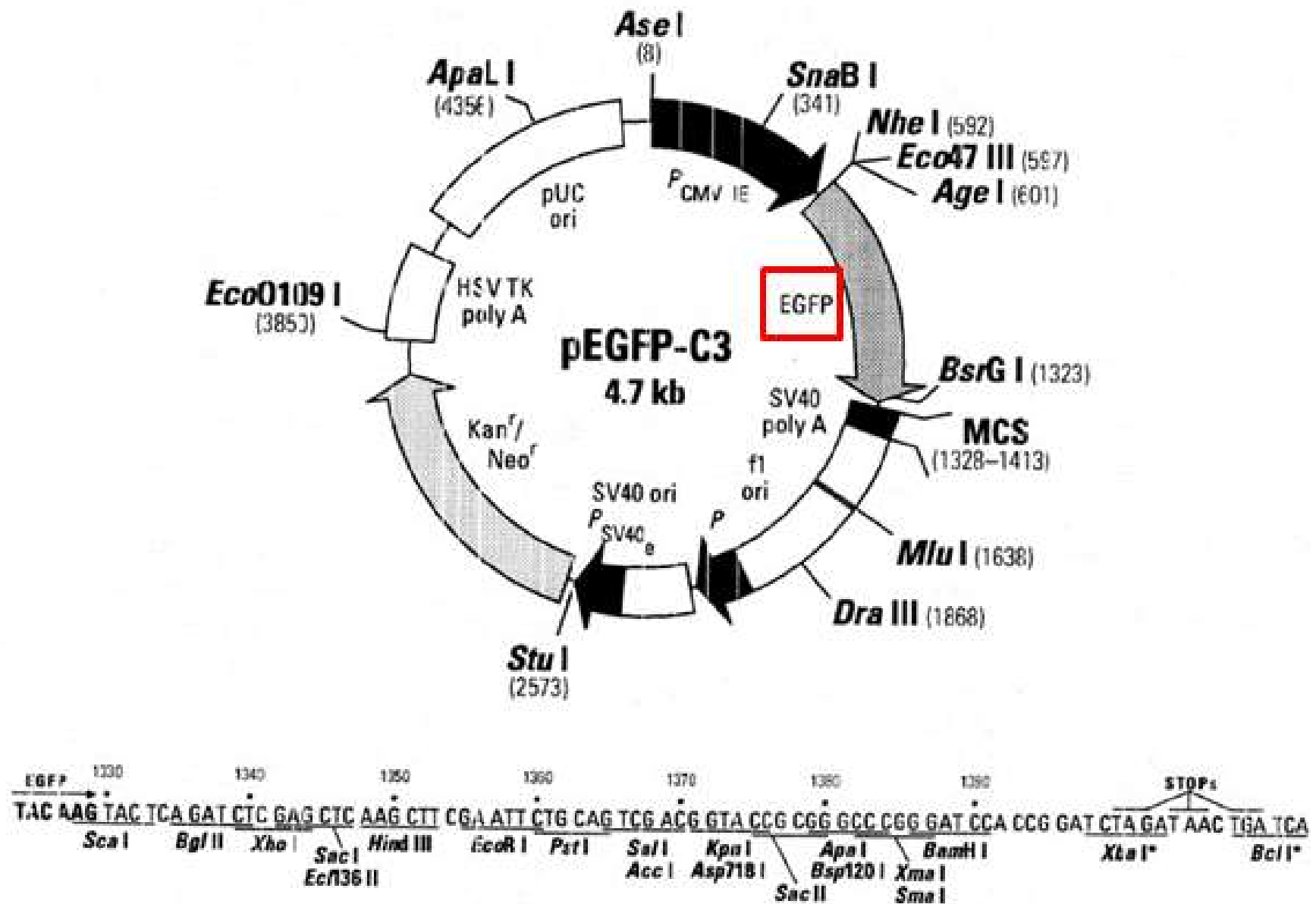
Percent firefly:*Renilla* luciferase activity
compared to no-insert control

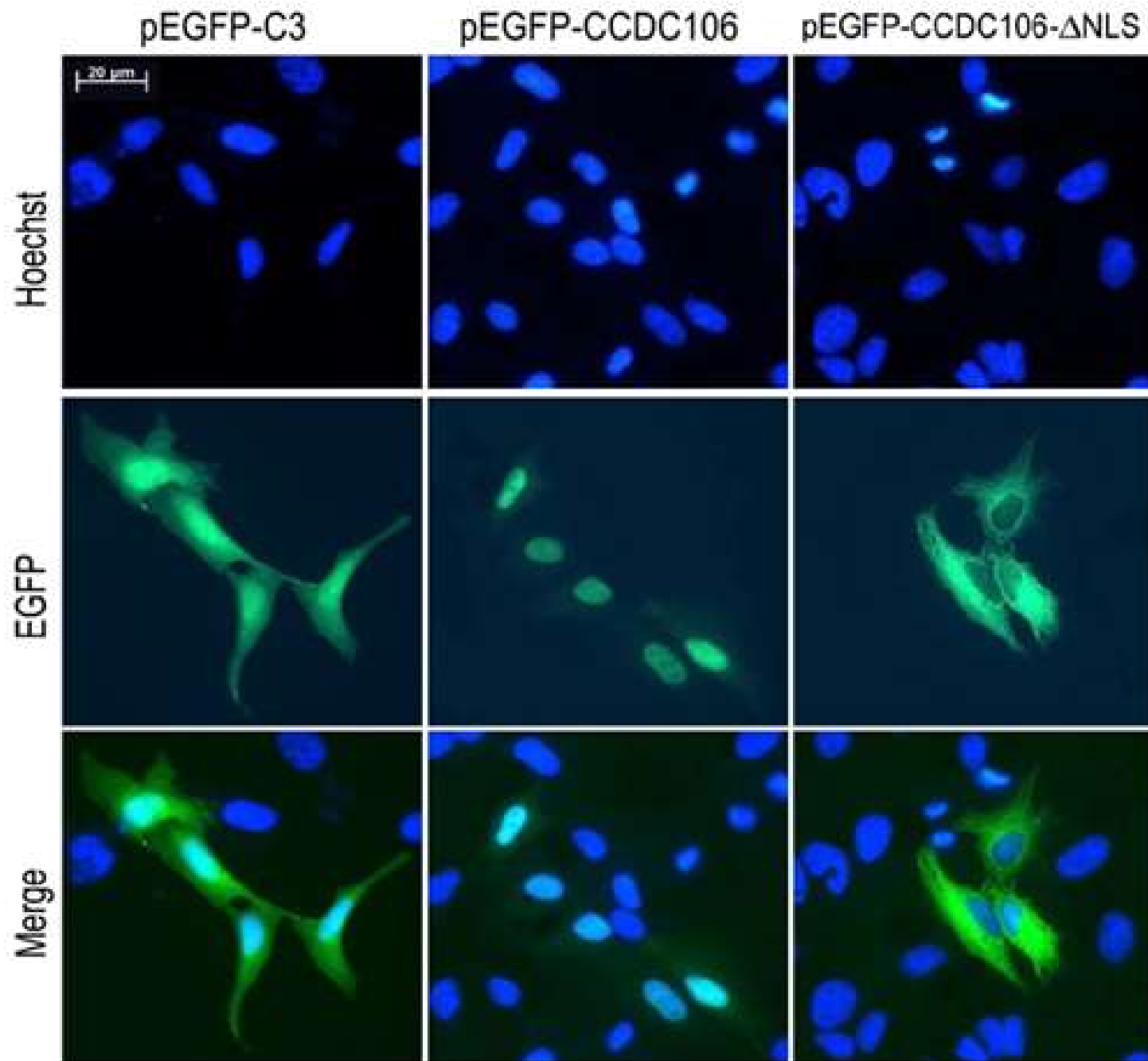


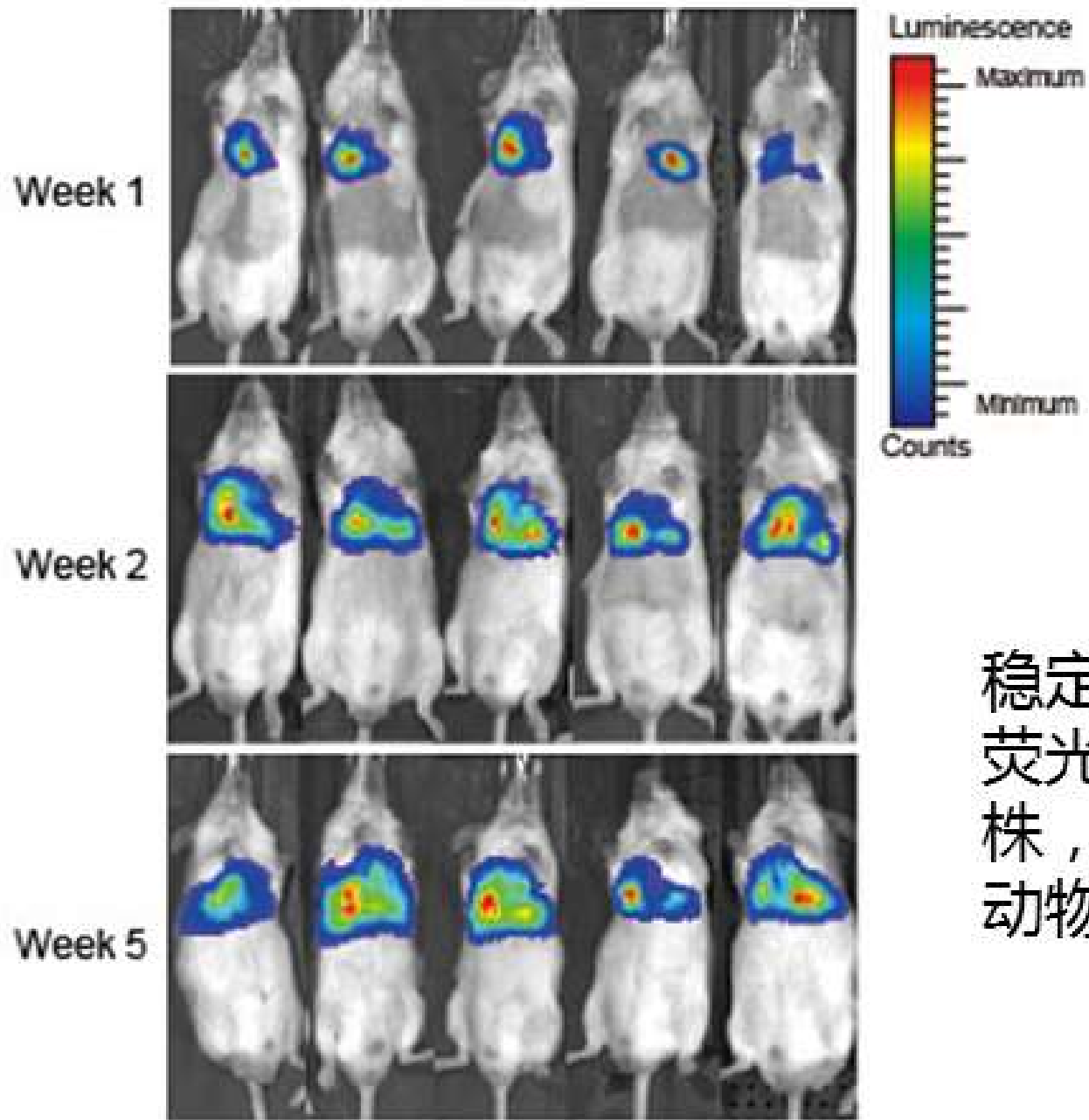
Fluorescent protein



On December 10, 2008 Osamu Shimomura, Martin Chalfie and Roger Tsien were awarded the Nobel Prize in Chemistry for “the discovery and development of **the green fluorescent protein, GFP**”.







稳定表达萤火虫
荧光素酶的细胞
株，可用于活体
动物成像实验。



5. Transgenics and stem cell technology

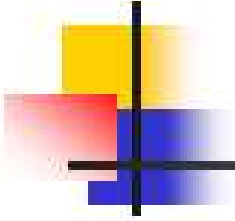
5.1 Transfection

- The introduction of DNA (normally plasmids) into **mammalian cells** is called **transfection**.

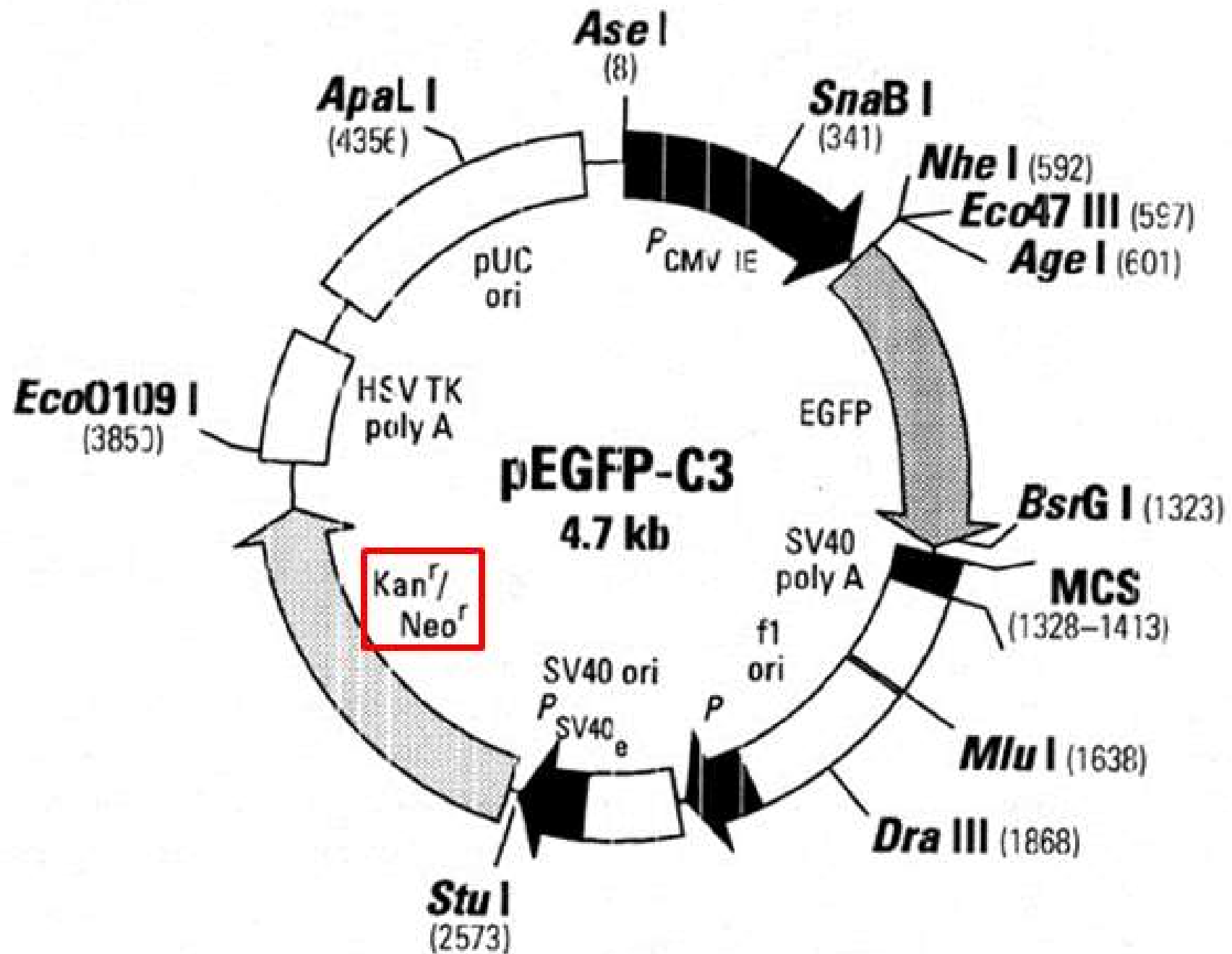
转染是指将DNA (通常是质粒)导入到**哺乳动物细胞**。

- **Bacteria** can be transformed with DNA extracted from a **bacterial virus (bacteriophage)**, a process known as **transfection**.

外源DNA通过**细菌病毒（噬菌体）**的感染转移到宿主（**细菌**）细胞的过程也称为**转染**。



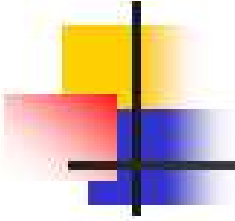
- **Transient transfection (瞬时转染)** - Shorter-term introduction of foreign DNA
- **Stable transfection (稳定转染)** - Longer-term introduction of foreign DNA
 - Vector with *neo^r*
 - Screening with G418





5.2 Transduction

- **Transduction** is the process of genetic recombination caused by a bacterial virus (bacteriophage) transferring DNA from a bacterium to another bacterium.
转导是指通过细菌病毒（噬菌体）将一个细菌的DNA转移到另一个细菌中而引起的基因重组。
- **Transduction** also refers to the process whereby foreign DNA is introduced into a cell via a viral vector.
转导还指通过病毒载体将外源DNA导入细胞的过程。



- **DNA viruses**

- Longer-term transient expression
- e.g. recombinant adenoviruses (重组腺病毒),
vaccinia viruses (牛痘病毒)

- **RNA viruses**

- Permanently integrated (永久性整合) cell lines
- e.g. retroviruses (逆转录病毒)



5.3 Transgenic organisms

- **Transgenic organisms (also genetically modified organisms or GMO)** are multicellular organisms expressing a foreign gene.

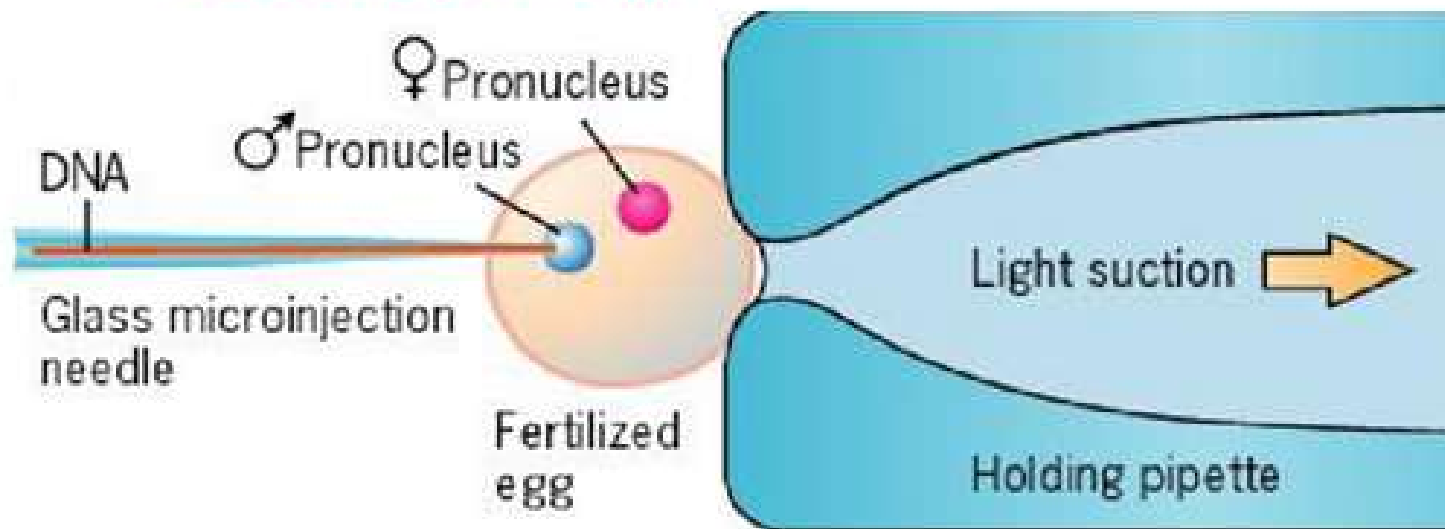
转基因生物 (也称为遗传修饰生物或GMO)
是指表达外源基因的多细胞生物。

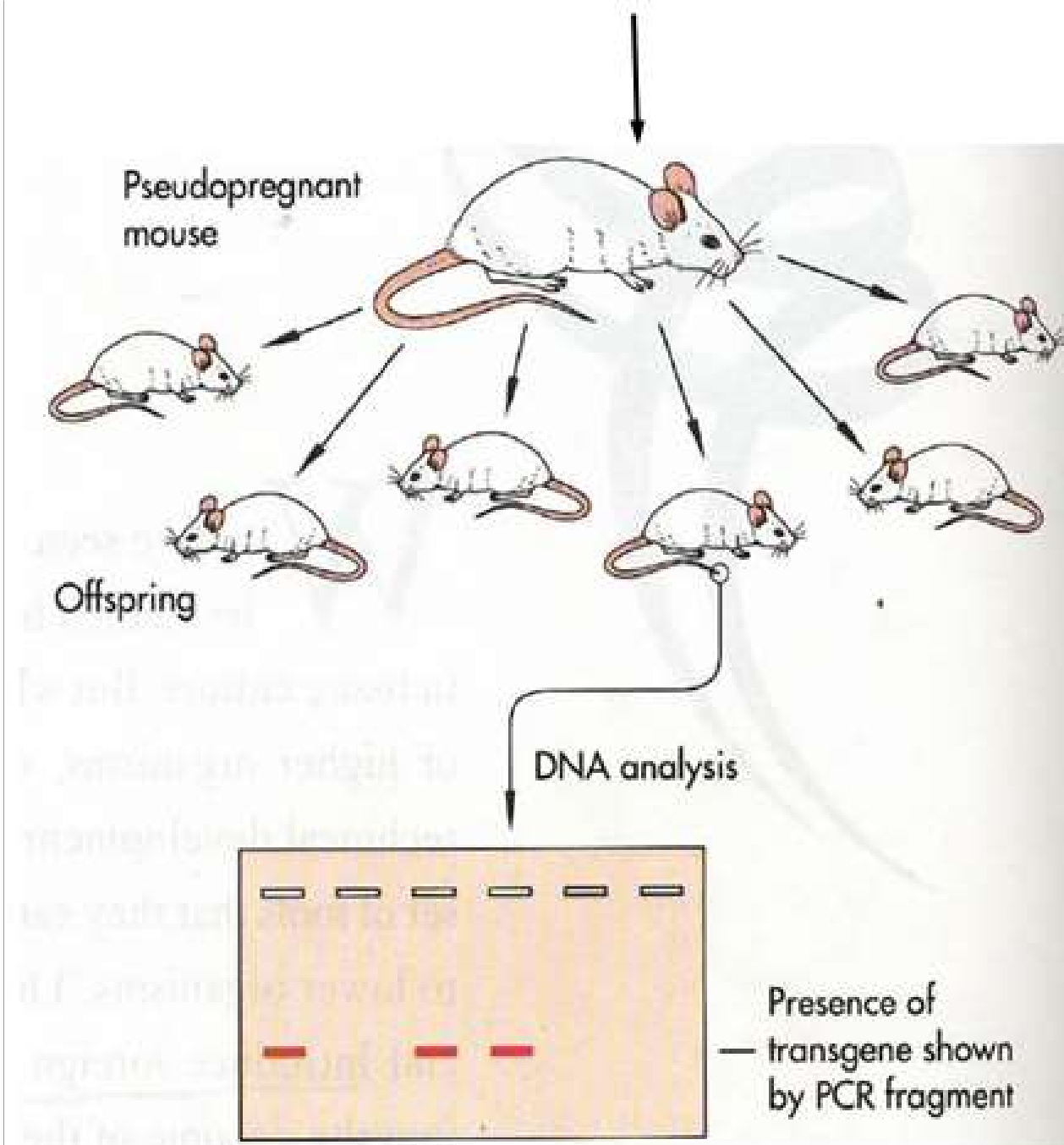
5.3.1 Transgenic animals

- Preparation methods

(1) Microinjection (显微注射)

- 利用显微操作技术将外源基因直接注射到**受精卵的雄原核**中。





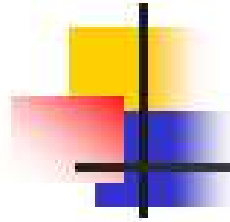
➤ 通过胚胎移植技术将整合有外源基因的受精卵移植到受体动物子宫内发育。

➤ PCR鉴定。

(2) Sperm-mediated gene transfer (SMGT, 精子介导的基因转移)

- 精子头部后方（核后帽区）易吸收外源DNA。**
- 刚受精的精卵细胞的核尚未融合，允许外源DNA插入受精卵的DNA中。**
- 将整合有外源基因的受精卵移植到受体动物子宫内发育。**

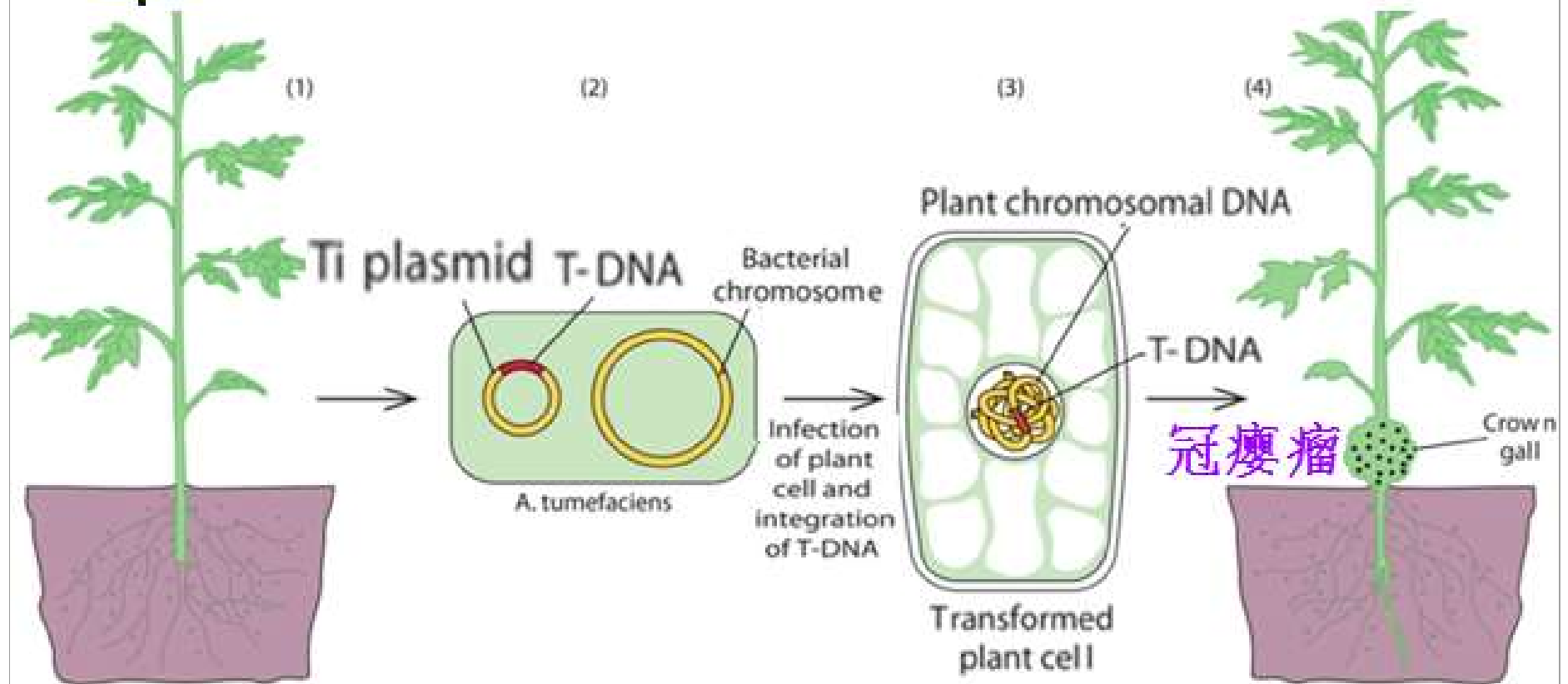
(3) Embryonic stem cells-mediated gene transfer (胚胎干细胞介导的基因转移)



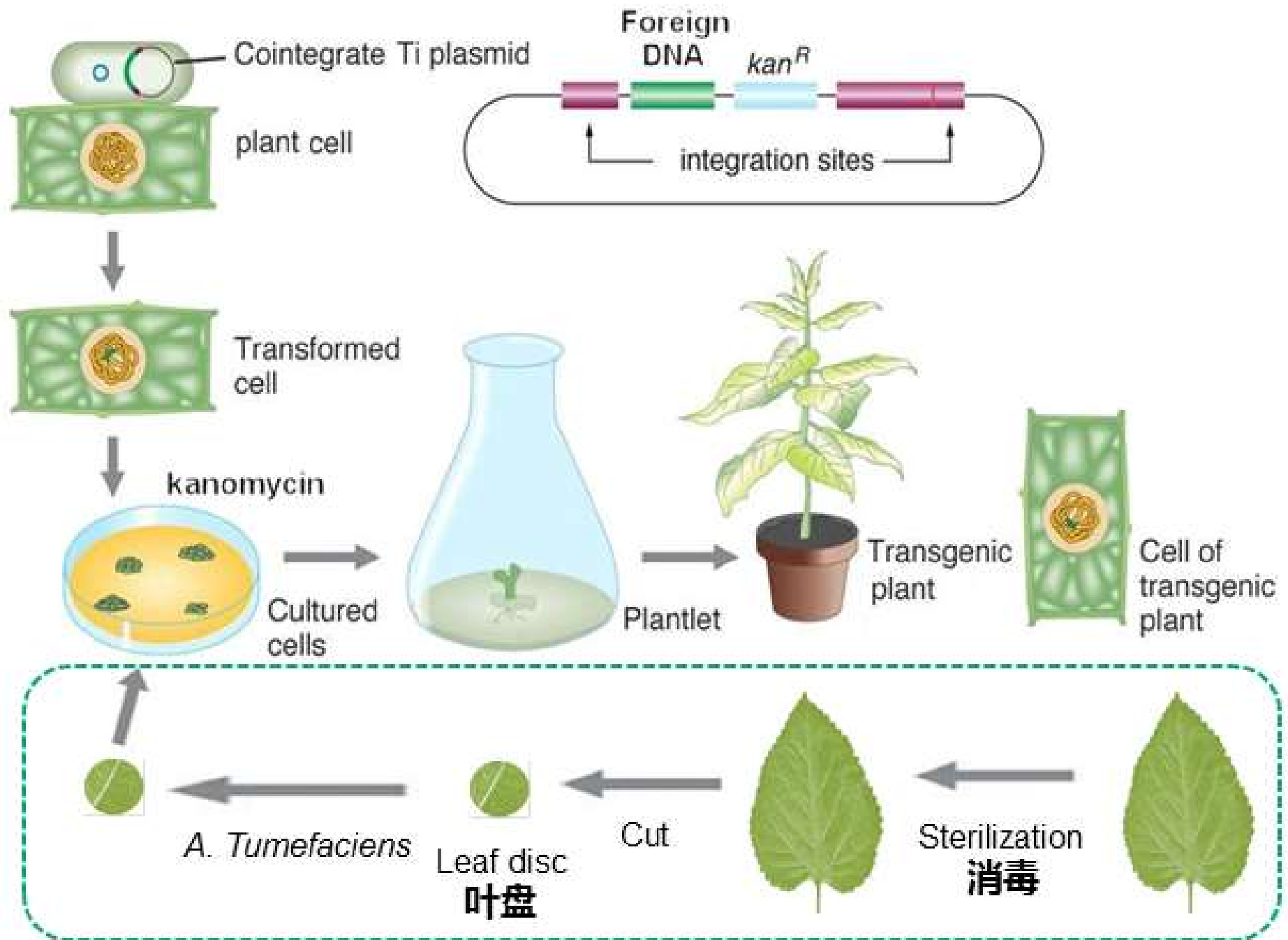
• Application

- (1) Establishment of animal models of human diseases**
- (2) Xenotransplantation (异种移植)**
- (3) Mammary bioreactor (乳腺生物反应器)**
- (4) Farm animals with good traits (优良性状)**

5.3.2 Transgenic plants



土壤农杆菌感染受损伤的植株，**Ti质粒**中的T-DNA转移并插入植物细胞的基因组中。





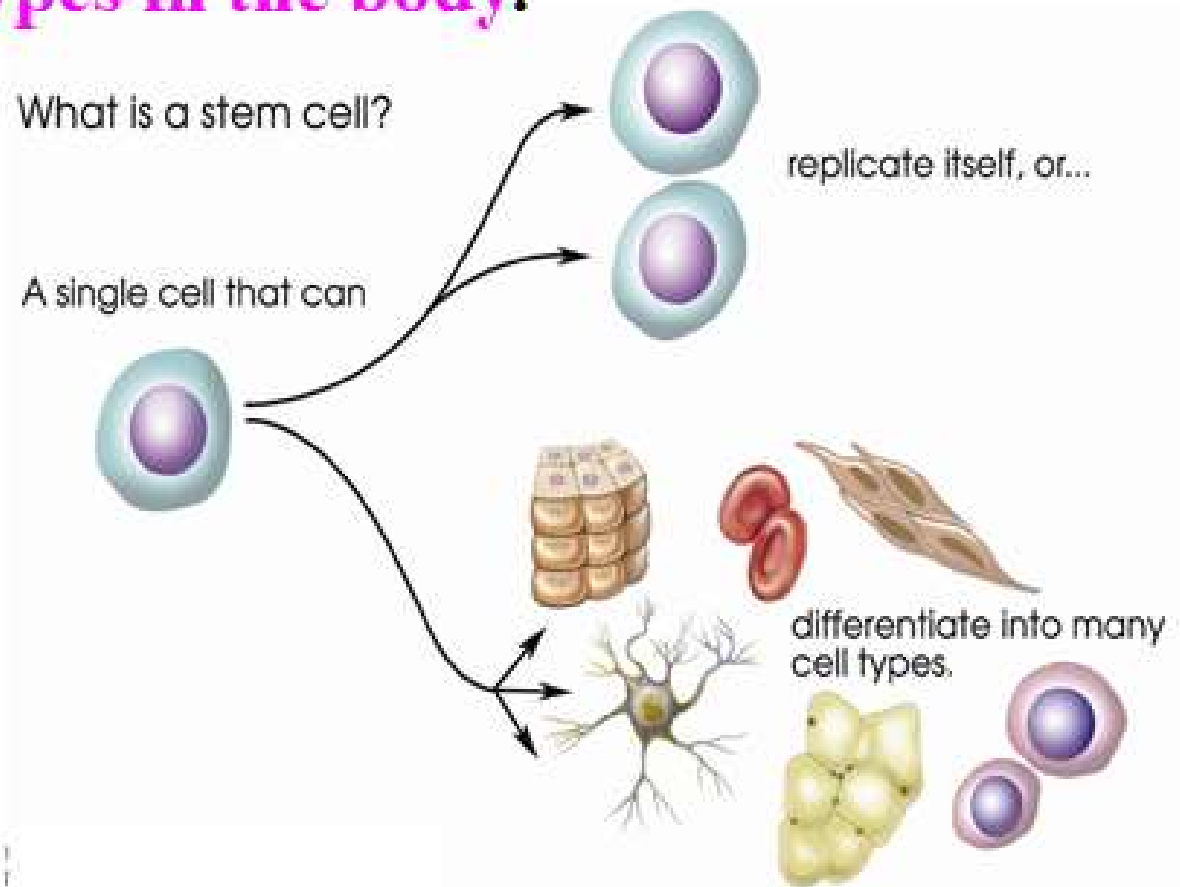
• Application

- (1) Resistance to pests and diseases**
苏云金芽孢杆菌的毒蛋白基因
- (2) Resistance to herbicide (除草剂)**
转入对除草剂不敏感的细菌EPSPS基因（芳香族氨基酸合成必需）
- (3) Extended shelf life (延长保存期)**
反义ACC基因（参与乙烯合成）
- (4) High yield and high quality (高产和高品质)**
提高必需氨基酸和维生素含量，延长棉花纤维的长度

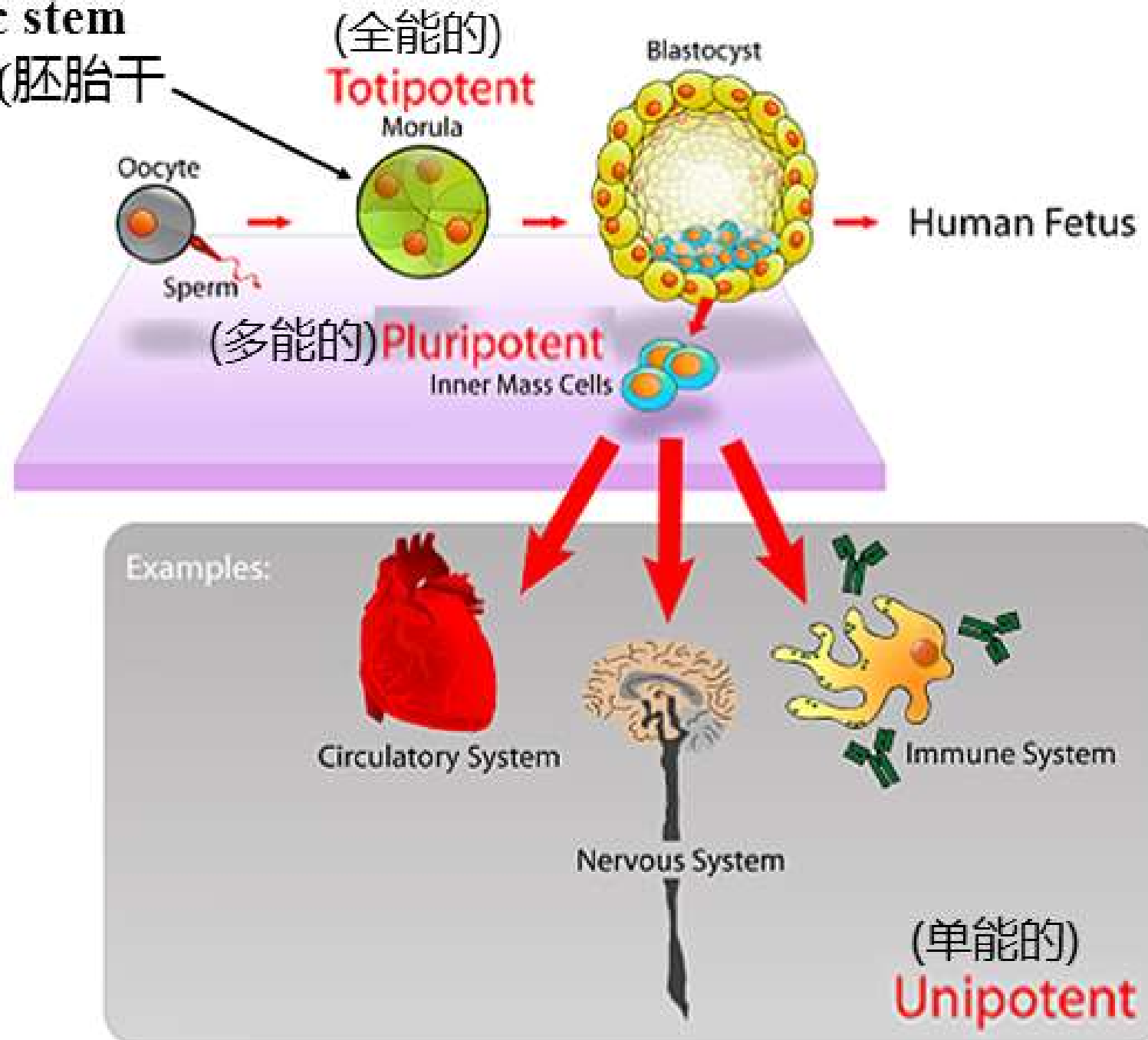
5.4 Stem cell

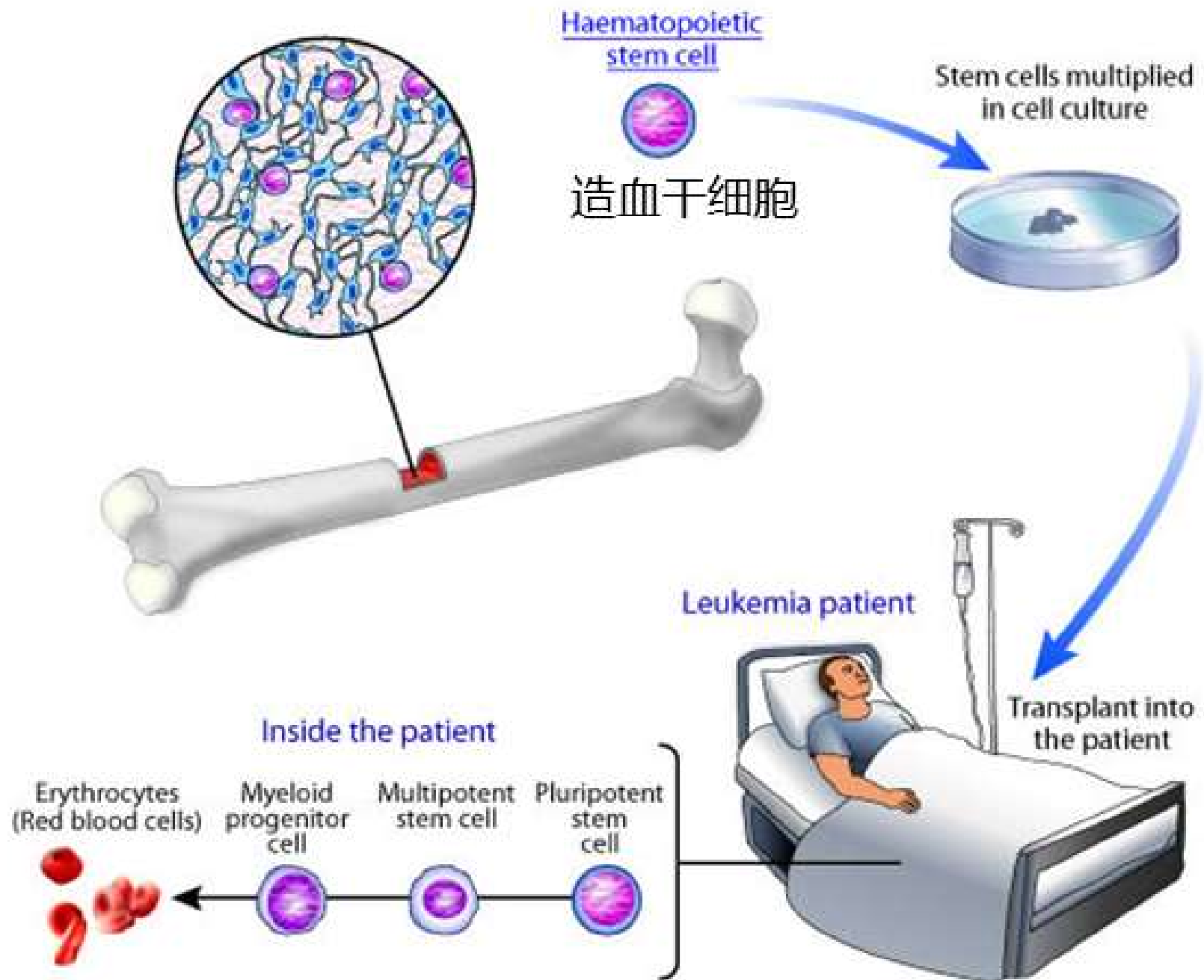
- **Stem cells** are **unspecialized or undifferentiated cells** that have the ability to develop into many different cell types in the body.

干细胞是一种非特化或未分化的细胞，具有发育成体内多种不同类型细胞的潜能。



embryonic stem
(ES) cells (胚胎干
细胞)







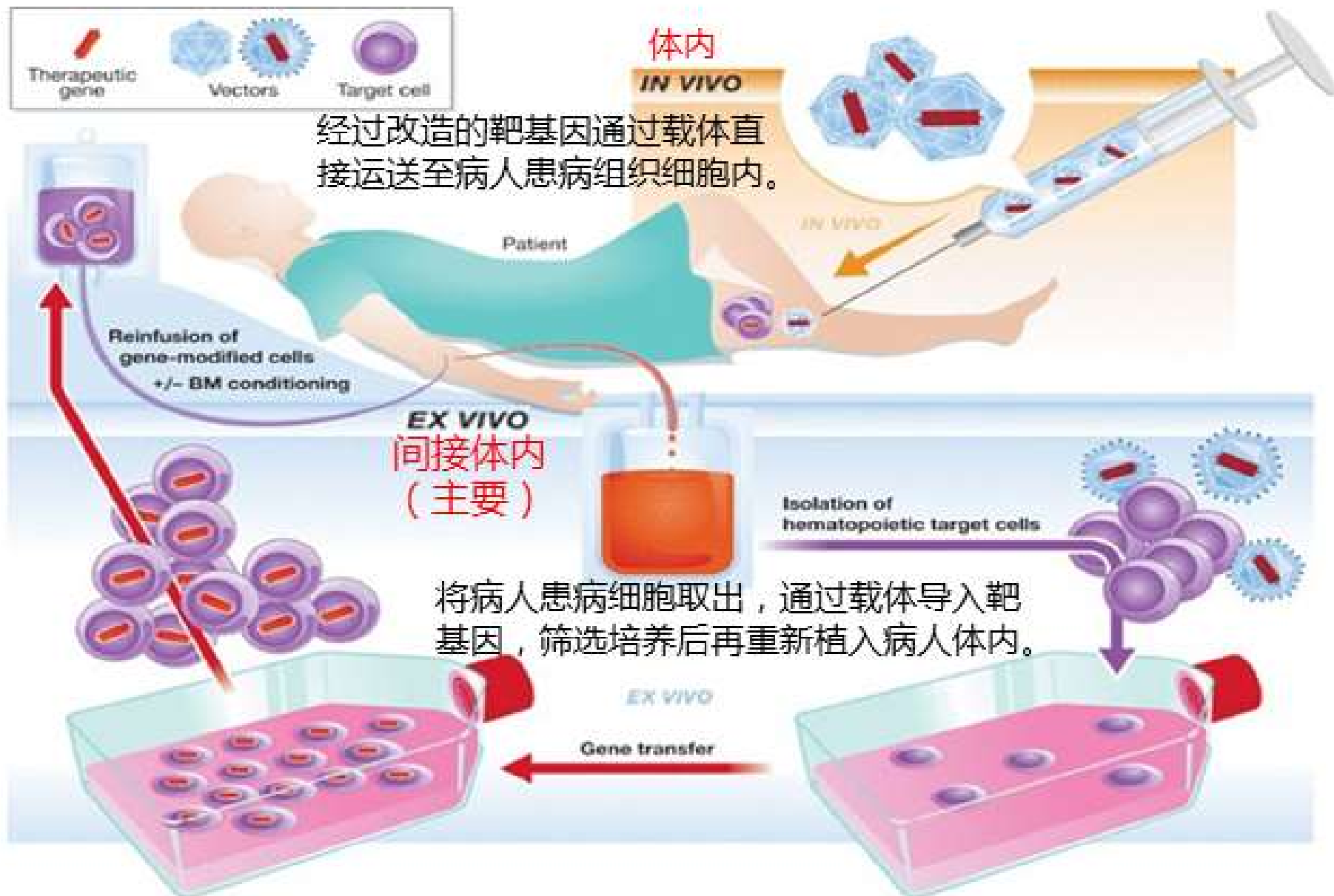
5.5 Gene therapy (基因治疗)

- **Gene therapy** uses **transgenic technology** to transfer a specific gene of interest into the human body, allowing it to **express products *in vivo* and exert biological activity** to correct or ameliorate **diseases caused by certain genetic abnormalities**.

基因治疗是利用**转基因技术**，将某种特定的目的基因转移到人体内，使其在**体内表达产物并发挥生物学活性**，以纠正或改善某种**基因异常**所导致的疾病。

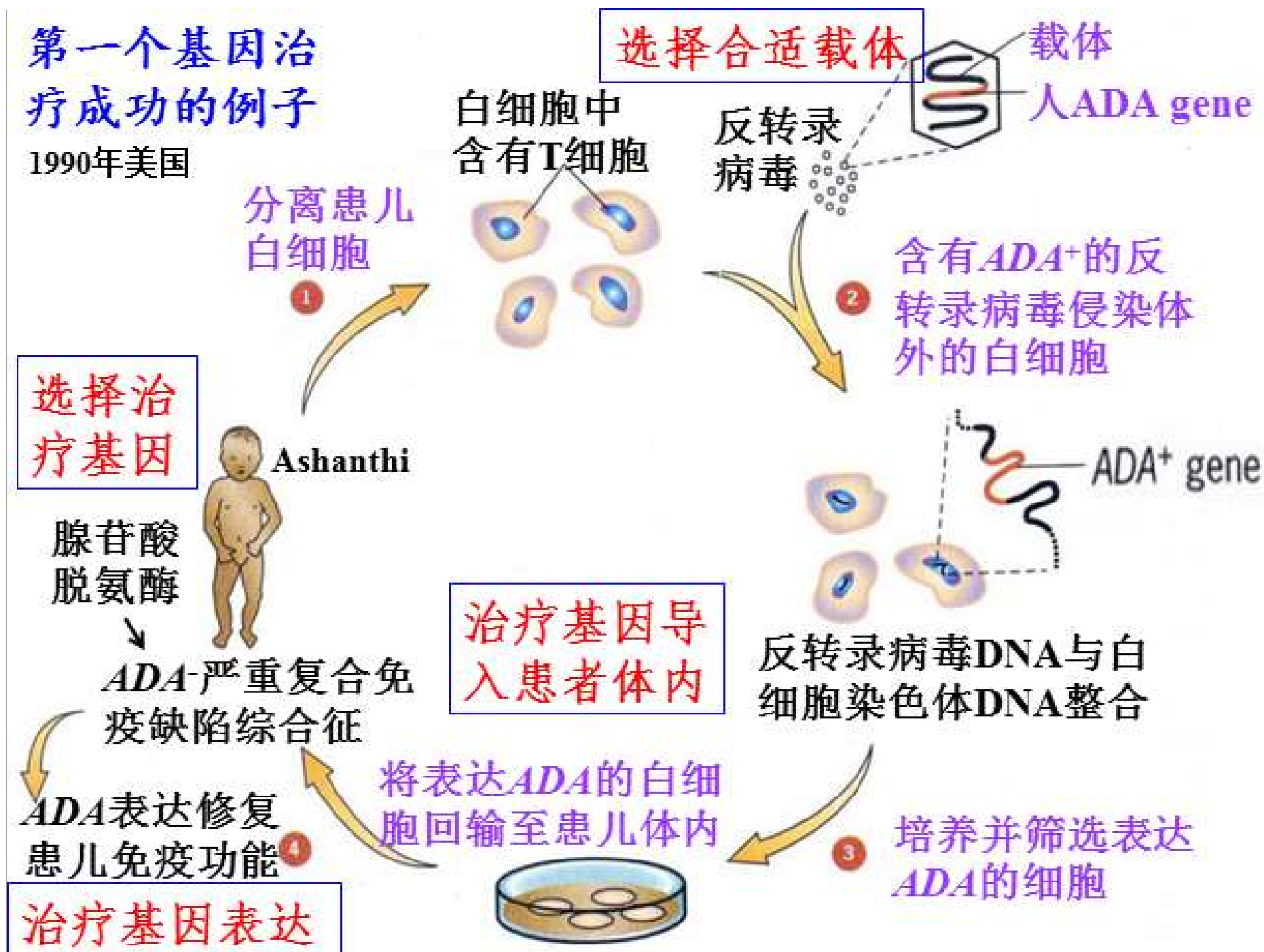
In vivo gene therapy (体内基因治疗)

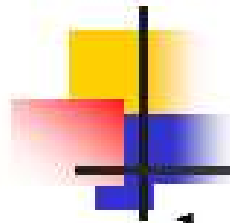
Ex vivo gene therapy (间接体内基因治疗)



第一个基因治疗成功的例子

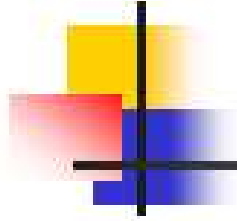
1990年美国





Summary

- 1. Definition of genomics, transcriptomics, proteomics and metabolomics**
- 2. Research contents and techniques of the 'omics**
- 3. Methods for genome-wide analysis (especially the mechanism of DNA microarray)**
- 4. The main methods and principles for studying the interaction between proteins and proteins**
- 5. Report gene and its application**
- 6. Definition of transfection and transduction**
- 7. Preparation methods of transgenic animals and plants**
- 8. Two strategies for gene therapy**



Exams

期末题型： 名词解释 (专业英文单词)
 填空题 (部分英文)
 选择题
 简答题 } or 问答题
 论述题