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Chapter 14

Gene Recombination and Genetic Engineering

Section Ⅰ Gene Transfer and Recombination in Nature

1 Conjugation

plasmid DNA transfer from one cell (germ)to another cell(germ)

$F^+ \rightarrow F^-$

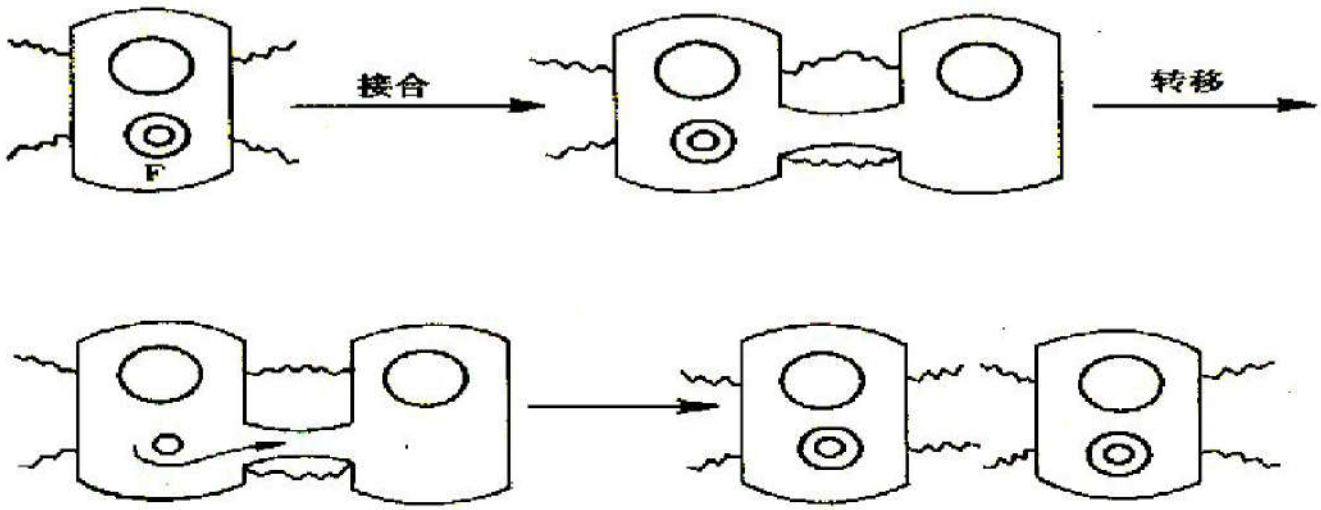


图 15-1　接合作用

2 Transformation and Transduction

2.1 Transformation

2.2 Transduction

Virus, Phage

lysis pathway

lysogenic pathway

图 15-2　转化作用

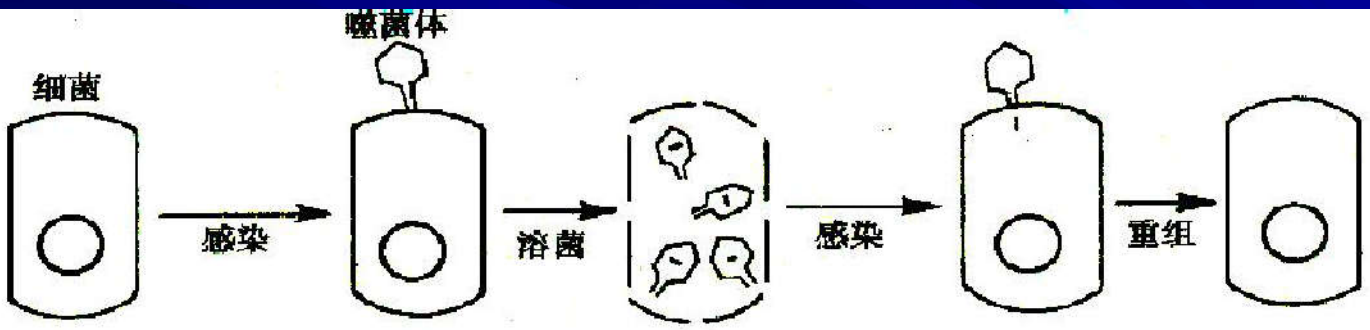
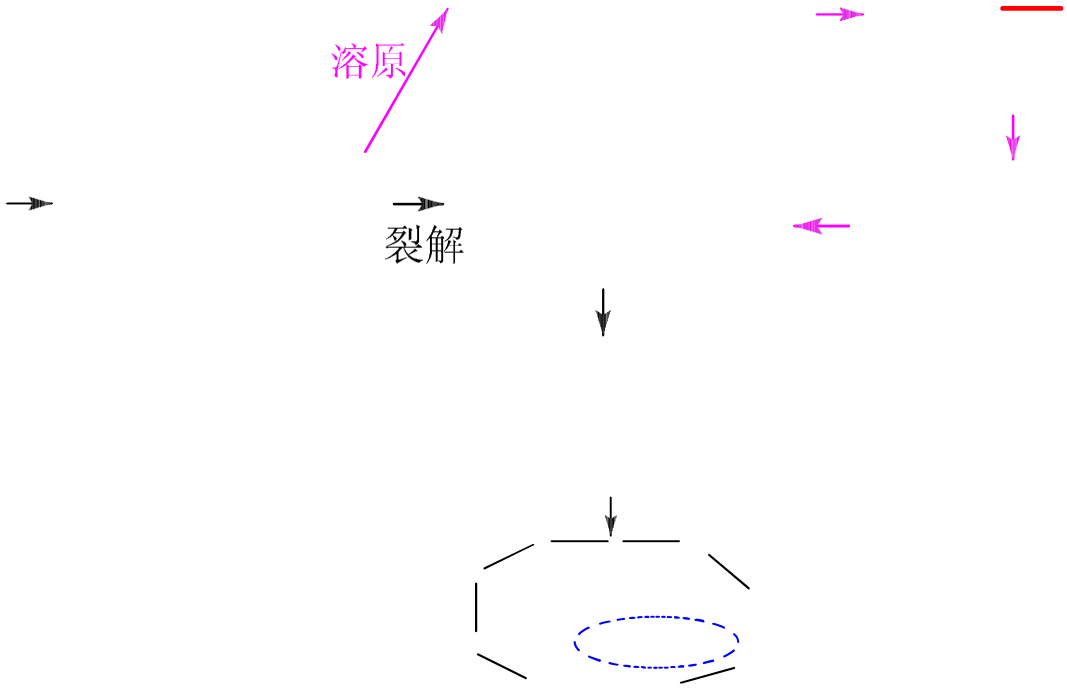


图 15-3　转导作用


溶原
裂解

3 Transposition

IS

transposons

4 Gene Recombination

- Covalent ligation of different DNA molecule

Section Ⅱ Recombinant DNA Technology

1 Concepts

1.1 DNA cloning(genetic engineering, molecular cloning)

- **Clone:** assembly of same copies from the same ancestor
- **Cloning**
- **DNA cloning:**

1973 Cohen第一例成功的克隆实验

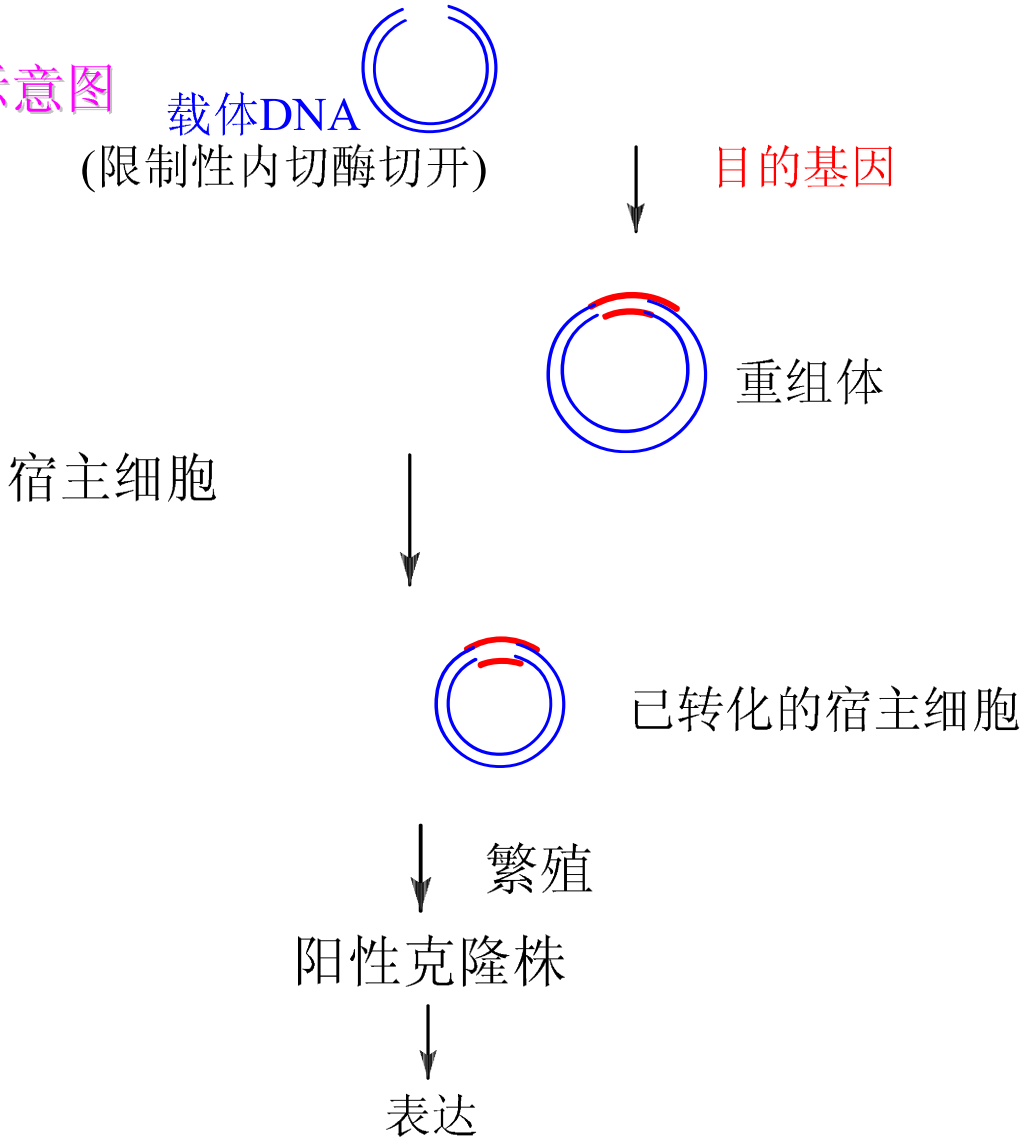
1978 Genentech公司 人胰岛素 世界上第一种基因工程蛋白药物

1982 第一个基因工程药物--重组人胰岛素在英、美获准使用

1985 第一批转基因家畜（兔、猪和羊），中国 转基因鱼

- **1993** 基因工程西红柿在美国上市
- **1997** 英国罗斯林研究所 多莉羊
- **1999.9** 中国获准加入人类基因组计划,负责测定人类基因组全部序列的**1%**
- **2000.6.26** 科学家公布人类基因组工作草图
- **2001.2.11** 公布人类基因组基本信息
- 生物技术工程 基因工程、蛋白质工程、酶工程、细胞工程

基因克隆示意图



1.2 Tool Enzymes

❖ common tool enzymes

限制性核酸内切酶	切割DNA
DNA连接酶	生成3′ - 5′ 磷酸二酯键
DNA聚合酶 I	探针标记、补平3′ 末端
反转录酶	cDNA合成
多聚核苷酸激酶	5′ 磷酸化、探针标记
末端转移酶	3′ 末端多聚尾
碱性磷酸酶	切除末端磷酸基

❖ Restriction endonuclease

①Concept

② Features

1）Cut DNA at specific site

4-8bp base palindrome

2）In germ： restriction-modification system

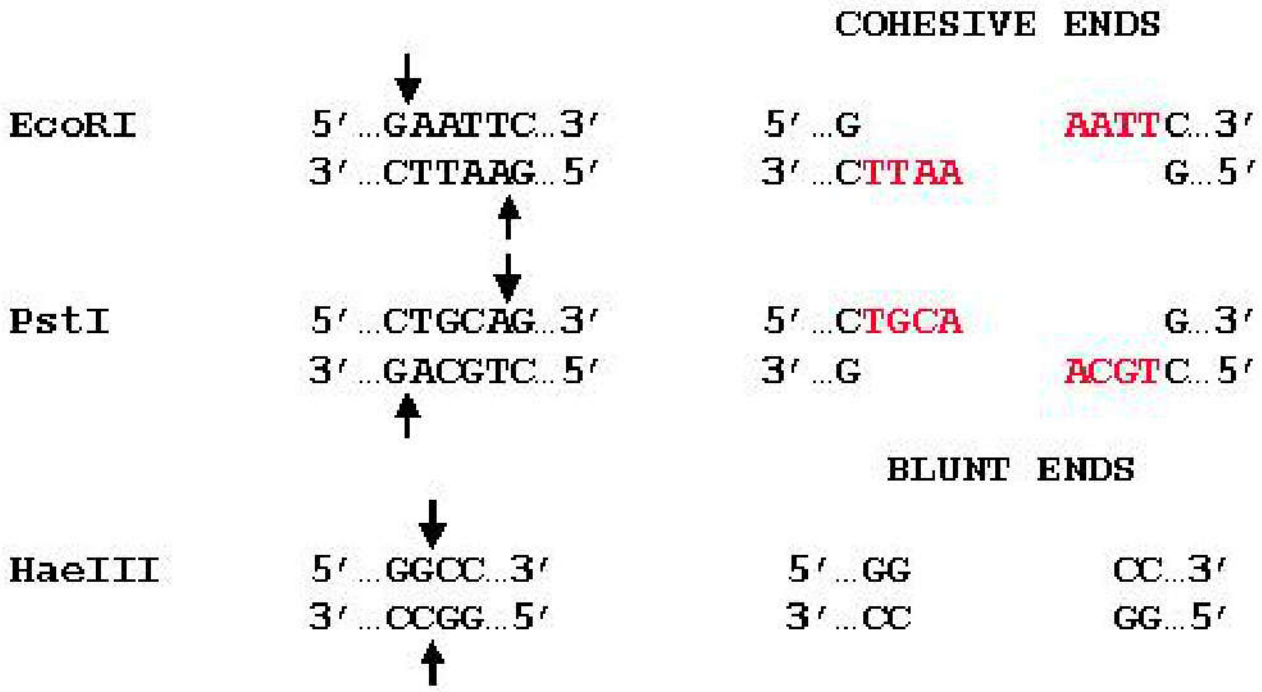
3) Product blunt end

cohesive end 5’-

3’-

compatible end

Products generated by restriction enzymes



Restriction endonucleases

- Restriction enzymes cut DNA into specific fragments
- Restriction enzymes recognize specific base sequences in double-stranded DNA and cleave both strands of the duplex at specific places
- Characteristics of restriction enzymes:

1. Cut DNA sequence-specifically
2. Bacterial enzymes; hundreds are purified and available commercially
3. Restriction-modification system

   Bacteria have enzymes that will cleave foreign DNA; hence, "restrict" the entry of viral DNA. To prevent the bacteria's own DNA from being cut, there is a second enzyme that methylates the same sites recognized by the restriction enzyme (modifies that site).
4. Named (e.g., EcoRI) for bacterial genus, species, strain, and type
5. Recognize specific 4-8 bp sequences
   - sequences have symmetry (they are palindromes)
   - after cutting the DNA, the cut ends are either
     - blunt
     - staggered (overhangs) - cohesive ends facilitate cloning the DNA
6. Frequency of cutting
   - 4-base cutter 4^4 = 256 bp
   - 5-base cutter 4^5 = 1,024 bp
   - 6-base cutter 4^6 = 4,096 bp
   - 8-base cutter 4^8 = 65,536 bp

1.3 Target gene

① Concept: target DNA

cDNA(complementary DNA)

② Types RNA → cDNA → dscDNA

genome DNA

1.4 Cloning vector

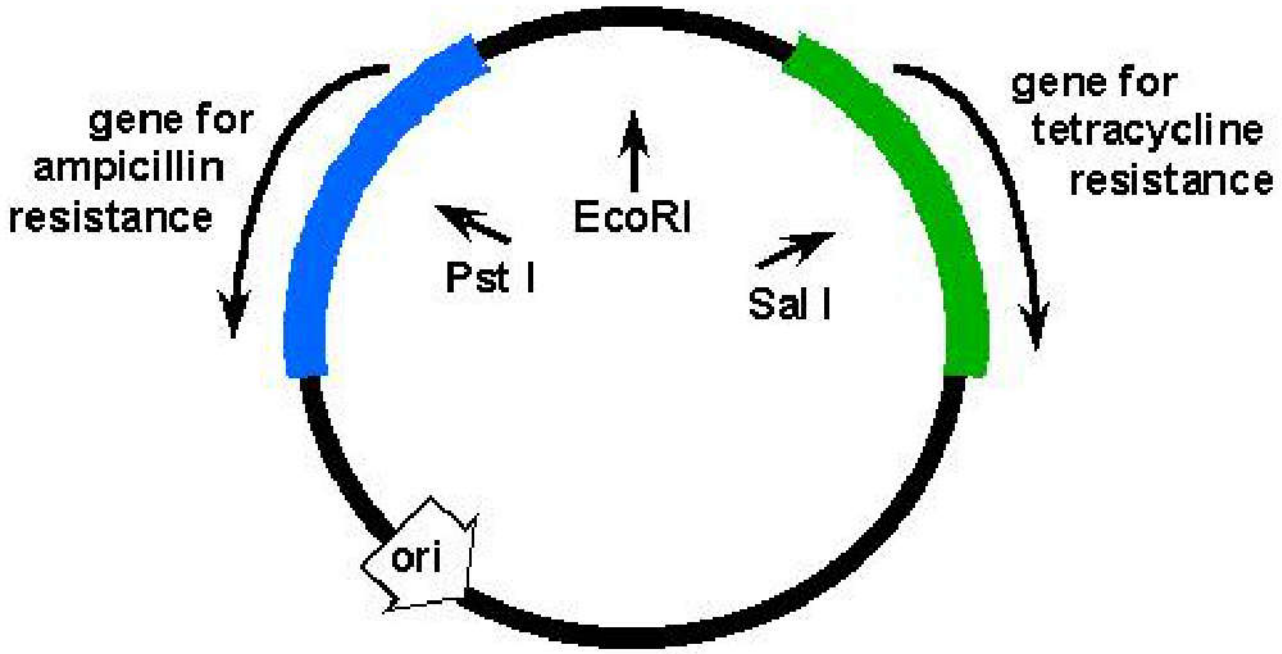
- DNA that can carry target gene into acceptor
- Types: Plasmid Phage Virus

① Plasmid

- outside chromosome, small , circle , double-strand DNA
  - can replicate (ori)
  - site of restriction endonuclease
  - ter^r, amp^r
- pBR322：4.5Kb

Structure of pBR322 - a common cloning vector

- derived from a naturally occurring plasmid
- has antibiotic resistance genes for selection of transformants containing the plasmid
- has unique restriction enzyme cleavage sites for insertion of foreign DNA
- has origin of DNA replication (ori) for propagation in E. coli



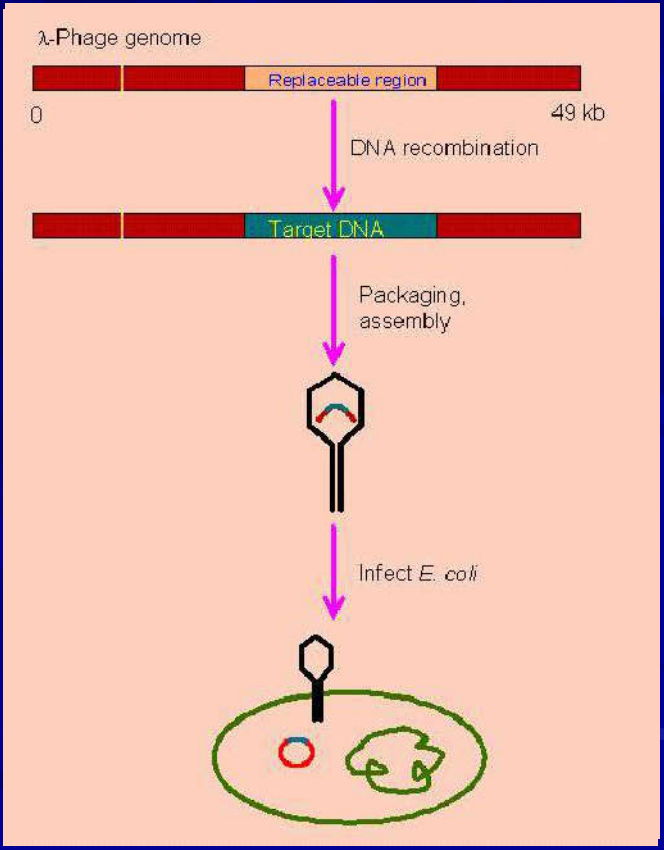
② Phage

λ phage: λgt EMBL

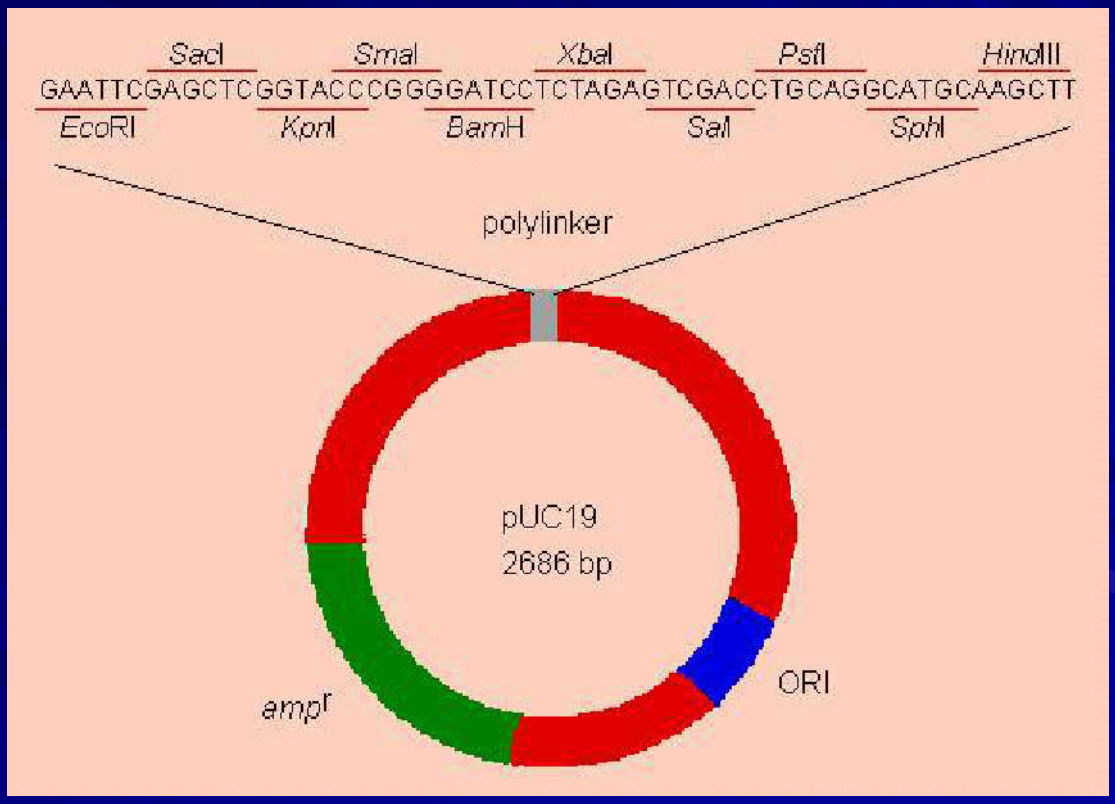
M13: M13mp pUC

③ Virus

expression vector



pUC19的分子结构


SacI
SmaI
XbaI
PstI
HindIII
GAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTT
EcoRI
KpnI
BamH
SalI
SphI
polylinker
pUC19
2686 bp
ampr
ORI

2 Basic Principle of Recombinant DNA Technology

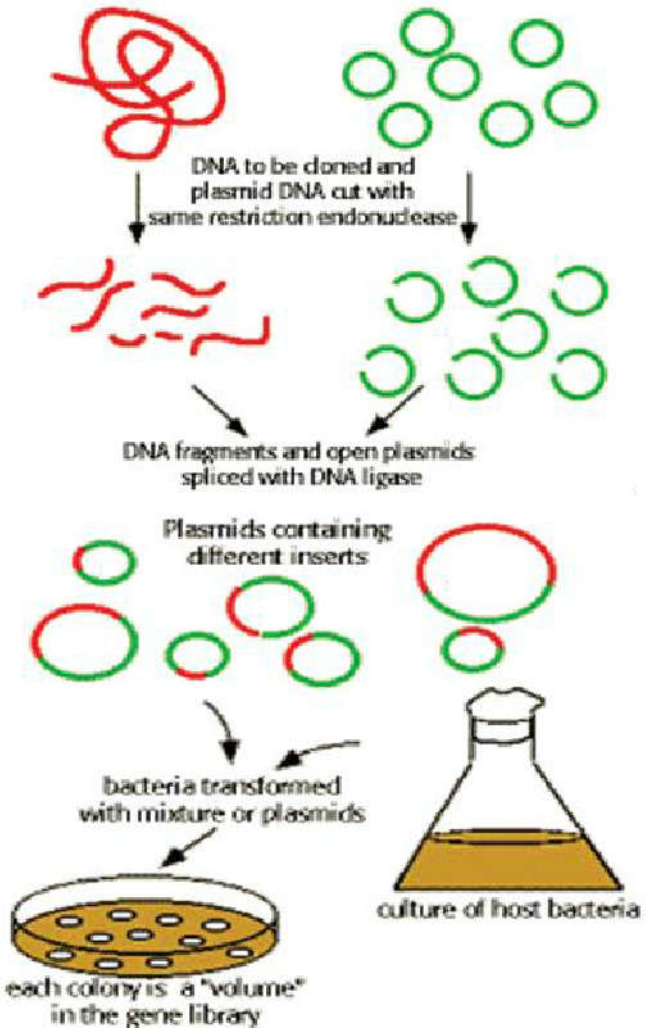
2.1 Obtaining of target gene(separation)

① Chemical synthesis

② Screening from gene library

- **Gene library**

Total DNA → restriction endonuclease cutting → ligation with vector to form recombinant DNA → E.coli → clone of all DNA fragments


DNA to be cloned and
plasmid DNA cut with
same restriction endonuclease
DNA fragments and open plasmids
spliced with DNA ligase
Plasmids containing
different inserts
bacteria transformed
with mixture or plasmids
culture of host bacteria
each colony is a "volume"
in the gene library

③ Screening from cDNA library

mRNA → cDNA → dscDNA → ligation with vector →

- Genes that can code for protein

④ PCR: polymerase chain reaction

Target DNA primers DNA-pol dNTP

2.2 Cutting by restriction endonuclease (cutting)

target gene vector complementary ends

2.3 Ligation of target gene and vector (ligation)

DNA recombination DNA ligase

4 models

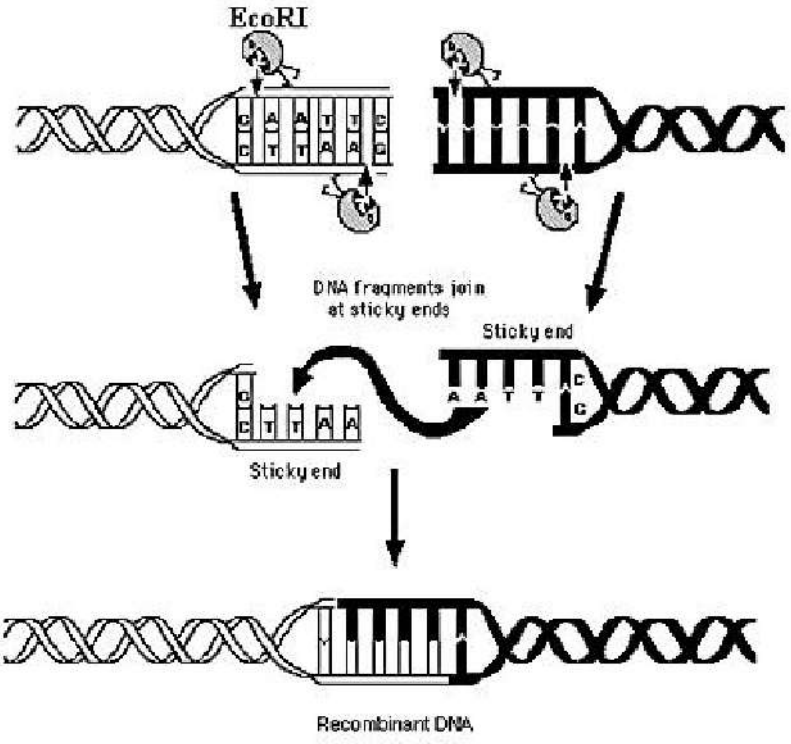
① Ligation of sticky ends

Same RE (EcoR Ⅰ)→same sticky ends

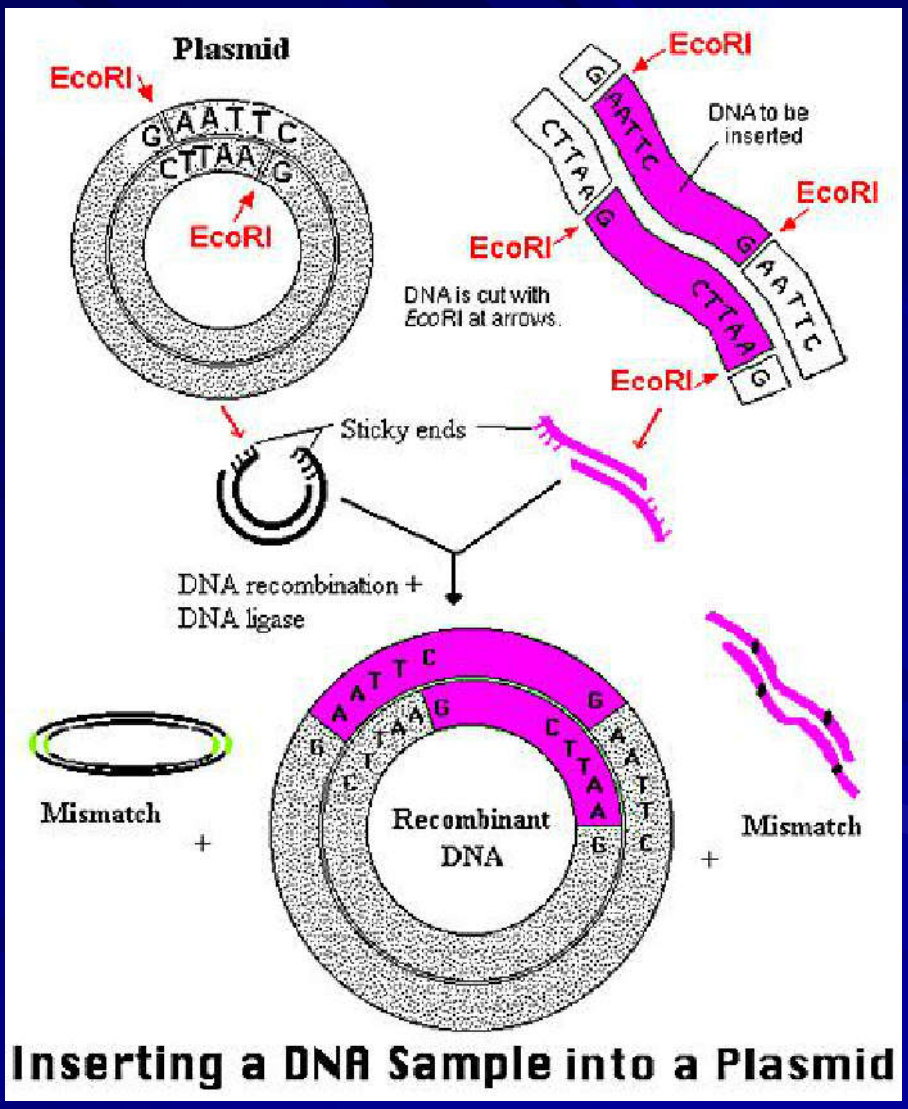
Different RE(Mbo Ⅰ,BamH Ⅰ) →compatible ends

② Ligation of blunt ends

- same/different RE (EcoR Ⅴ) →blunt ends
- low ligation efficiency



Restriction Enzyme Action of EcoRI



Inserting a DNA Sample into a Plasmid

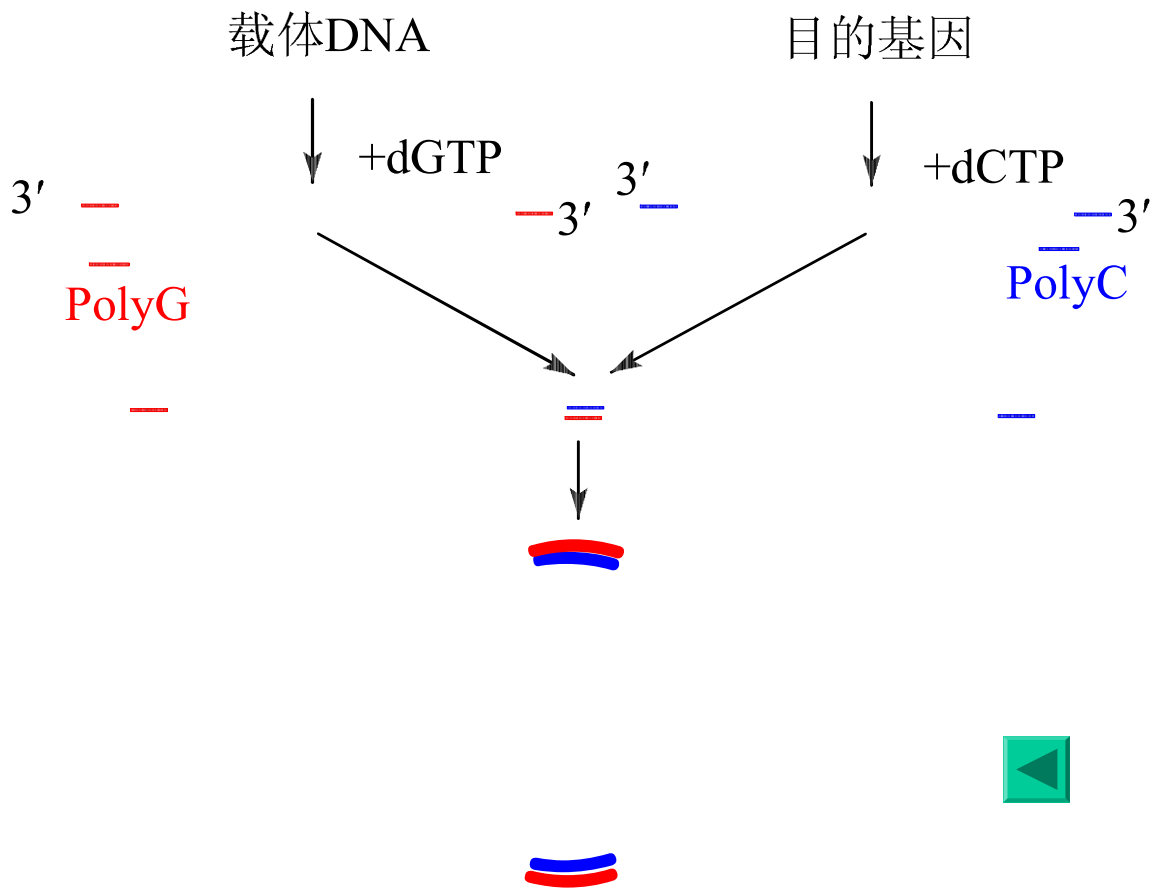
③ Homopolymer add tail: terminal transferase

3’-end（polydA/polydT)→sticky ends

④Artificial linker

linker: double-strand DNA （8~12bp）, RE site

Linker--target gene and vector→RE cutting → sticky ends


载体DNA
目的基因
+dGTP
+dCTP
3′
3′
3′
3′
PolyG
PolyC

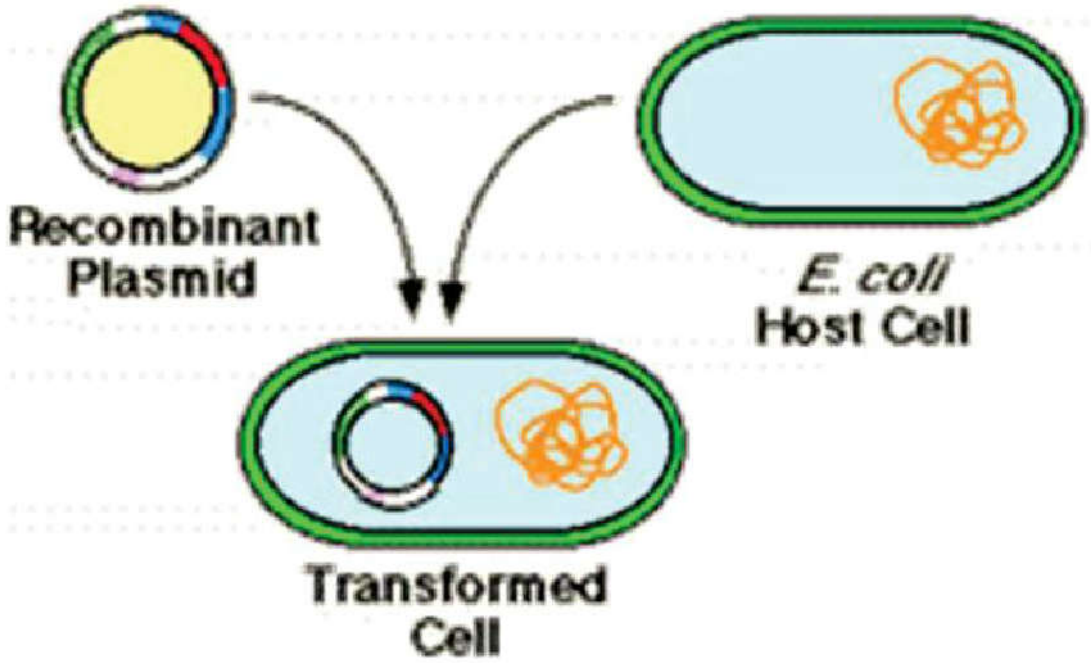
2.4 Transfer of recombinant DNA into germ(transfer)

- Recombinant DNA→germ（E.coli）

  Transformation plasmid

  Transfection phage,virus

- Competent cell


Recombinant
Plasmid
E. coli
Host Cell
Transformed
Cell

2.5 Screening of recombinants（screening）

❖ **recombinant**:recombinant DNA with target gene

①Direct selection

A. Antibiotics: transformed /non-transformed

amp^r,tet^r,kan^r

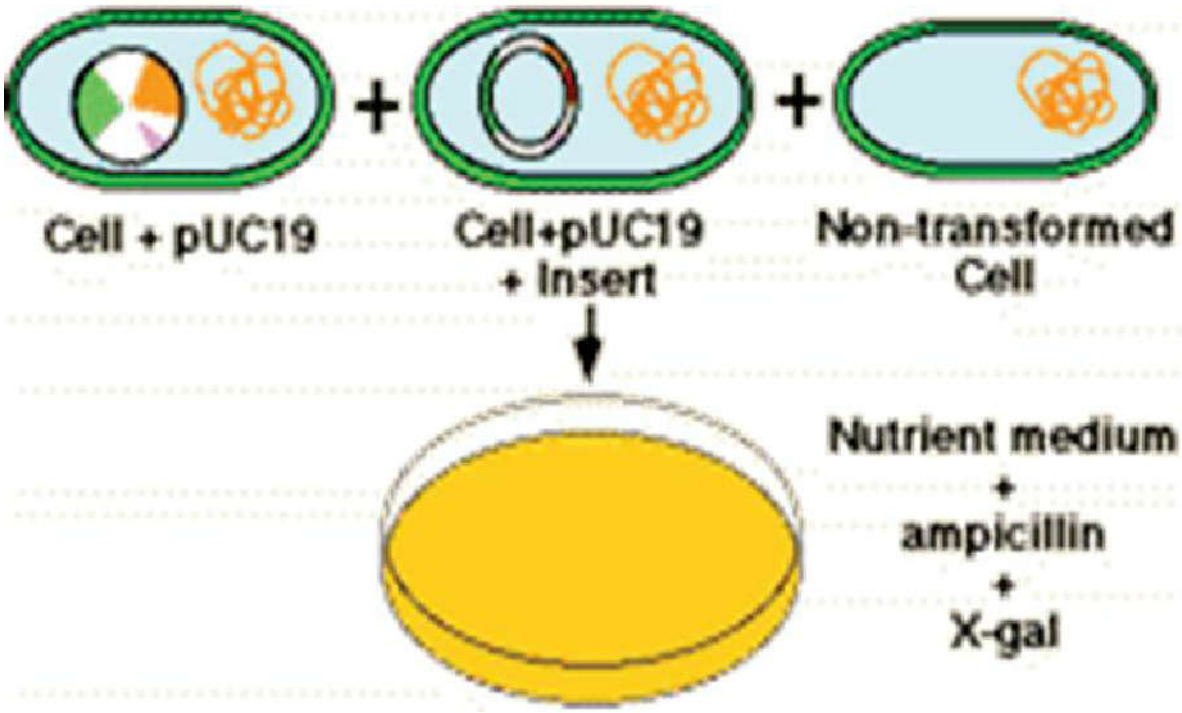
B. α -complementation: X-gal →blue

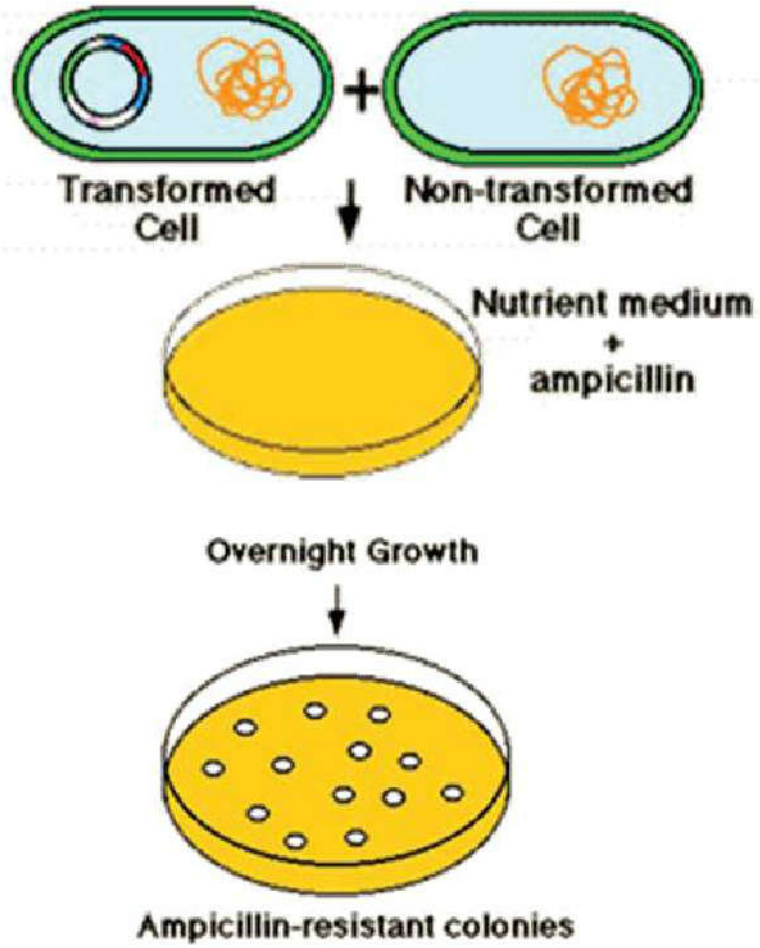
pUC: N end of β -galactosidase /lac^-E.coli—C end

（α fragment） （ω fragment ）

White clone:with target gene

Blue clone:without target gene


Cell + pUC19
Cell+pUC19
+ Insert
Non-transformed
Cell
Nutrient medium
+
ampicillin
+
X-gal


Transformed Cell
Non-transformed Cell
Nutrient medium
+
ampicillin
Overnight Growth
Ampicillin-resistant colonies

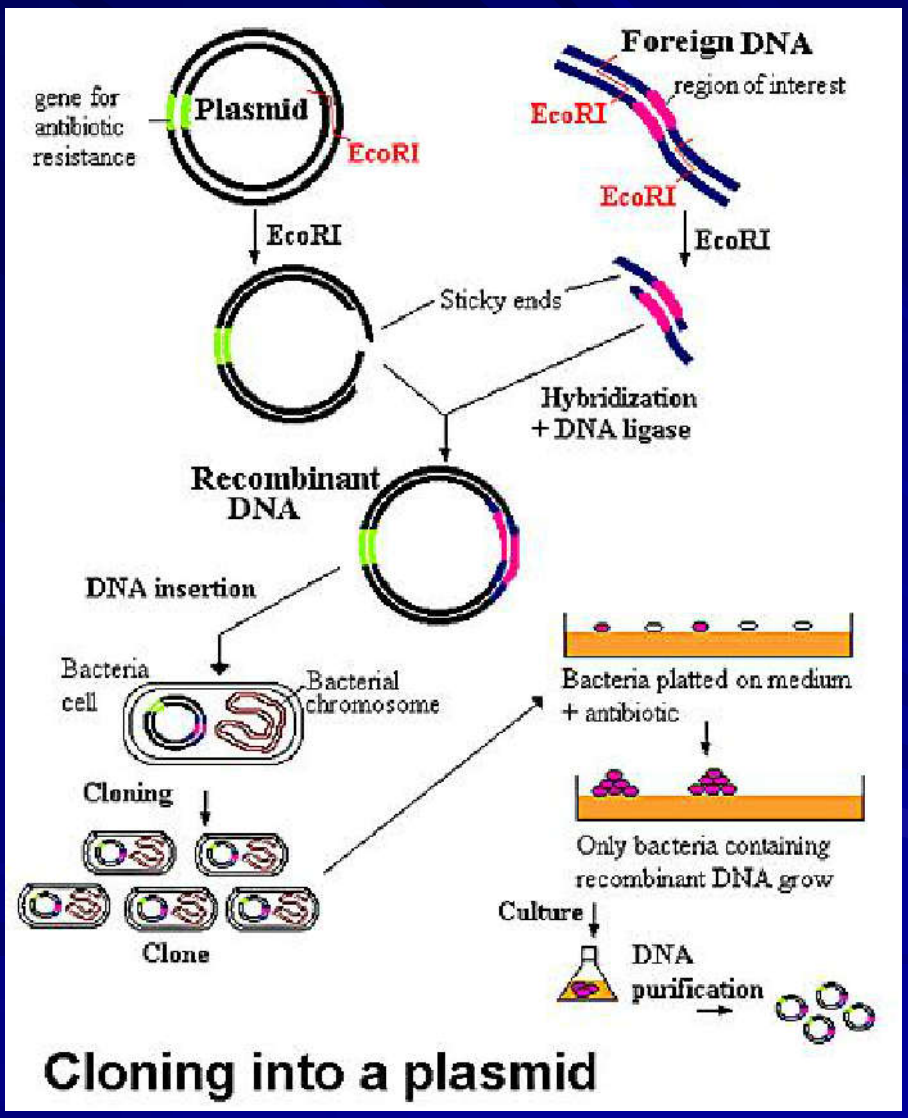
C. in situ hybridization

②Indirect selection—immunological method

antigen-antibody

2.6 Expression of cloned gene

- Expression system
  - prokaryotic expression system
  - eukaryotic expression system


gene for antibiotic resistance
Plasmid
EcoRI
EcoRI
Foreign DNA
region of interest
EcoRI
EcoRI
EcoRI
Sticky ends
Hybridization + DNA ligase
Recombinant DNA
DNA insertion
Bacteria cell
Bacterial chromosome
Cloning
Clone
Bacteria platted on medium + antibiotic
Only bacteria containing recombinant DNA grow
Culture
DNA purification
Cloning into a plasmid

小　结

1. 了解自然界的基因转移和重组。
2. 掌握克隆，DNA克隆的概念；限制性核酸内切酶的概念和作用特点；目的基因的概念和类型；基因载体的概念和类型，质粒载体的特点。
3. 掌握重组DNA技术的基本原理及过程（分，切，接，转，筛），了解克隆基因的表达。

①Prokaryotic expression system--E.coli

- **Expression vector**
  - selection marker
  - strong promoter(lac promoter)
  - regulation sequences (RBS+initiation site）
  - polylinker cloning sites
- Target gene-vector→E.coli→screening→expression
- Advantages: simple，quick，economic，large-scale
- Disadvantages

② Eukaryotic expression system

mammalian cell（COS，CHO）

- **Transfection: expression vector → eukaryotic cell**

  transient transfection/ stable transfection

- **Methods: calcium phosphate ~ , DEAE dextran-mediated~,**

  electroporation, lipofection ~, microinjection

- **Advantages /Disadvantages**

3 Recombinant DNA Technology and Medicine

Molecular medicine

3.1 基因工程药物：重组人胰岛素,重组促红细胞生成素，重组干扰素,乙肝疫苗，丙种球蛋白

3.2 基因治疗

- **1990.9.14**　　首例基因治疗

  4岁　女孩　严重免疫缺陷症**(SCID)**

  缺乏腺苷酸脱氨酶**(ADA)**

  2--脱氧腺苷含量升高　　毒性

  严重破坏免疫功能

  ADA基因　　**LN**逆转录病毒载体

  靶细胞为病人淋巴细胞　　回输

小　结

1. 了解自然界的基因转移和重组。
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3. 掌握重组DNA技术的基本原理及过程（分，切，接，转，筛），了解克隆基因的表达。
4. 了解重组DNA技术与医学的关系。