目的基因的分离
基因序列的测定

倪培华

http://www.shsmu.edu.cn/

第一节

分离目的基因的方法及原理

一、目的基因的定义

对基因组中某一基因成分或该基因片段进行研究或应用，此基因（或片段）称为目的基因。

Genome of a particular genes or gene fragments of the study or application of this gene (or fragments) as the target gene.

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二、外源性基因（或DNA）
cloned DNA in vitro

插入至载体、并导入宿主细胞内复制的基因

To insert the carrier and into the host cell's genetic replication

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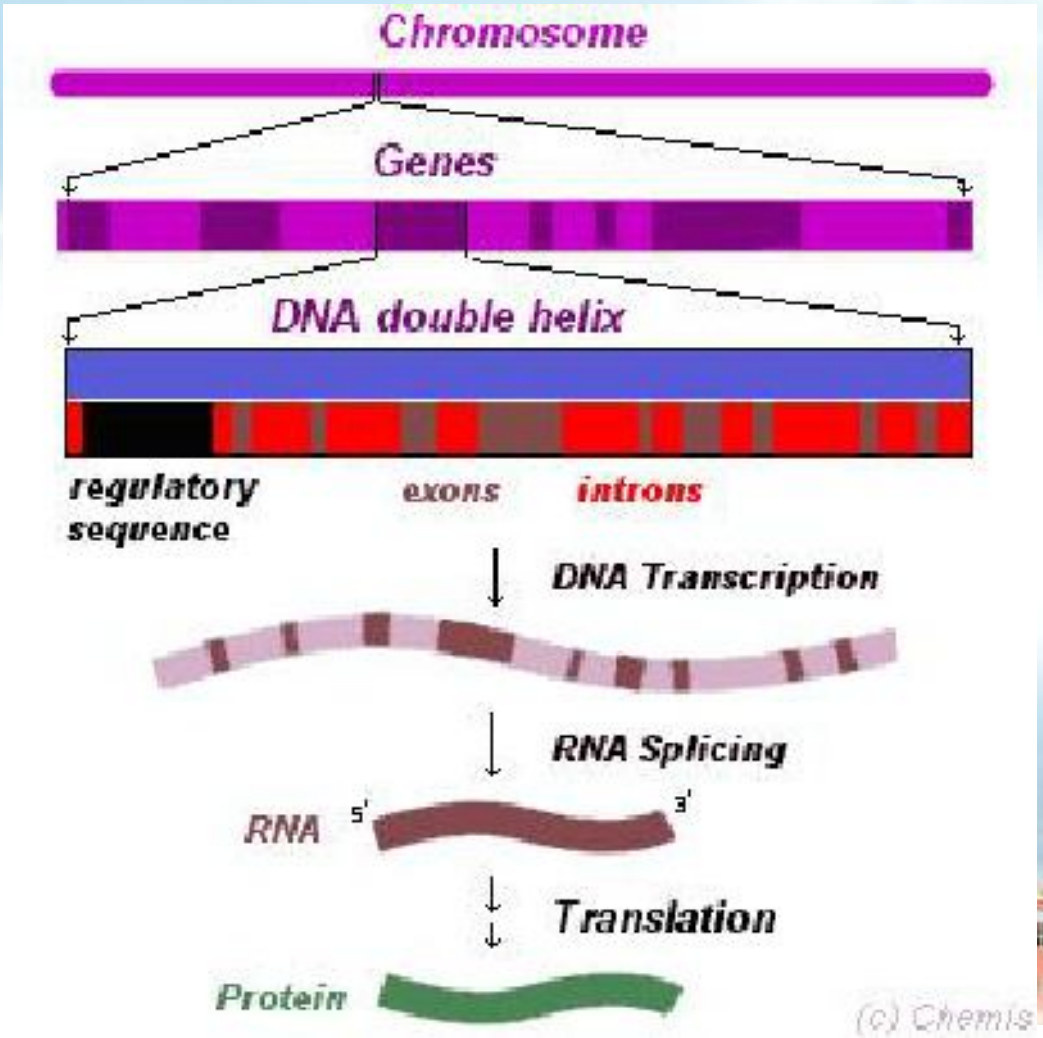
- 对于高等真核生物，基因组DNA十分庞大,基因可达数万个，且基因组成结构复杂
  - 编码序列　coding sequence
  - 非编码序列　non-coding sequence
  - 调控序列　regulator sequence
- 基因间还存在
  - 间隔序列　intervening sequences
  - 重复序列　repeat sequence
- 单个目的基因在整个基因组中所占的比例极其微小，除少数例外，绝大多数基因难以直接分离得到。



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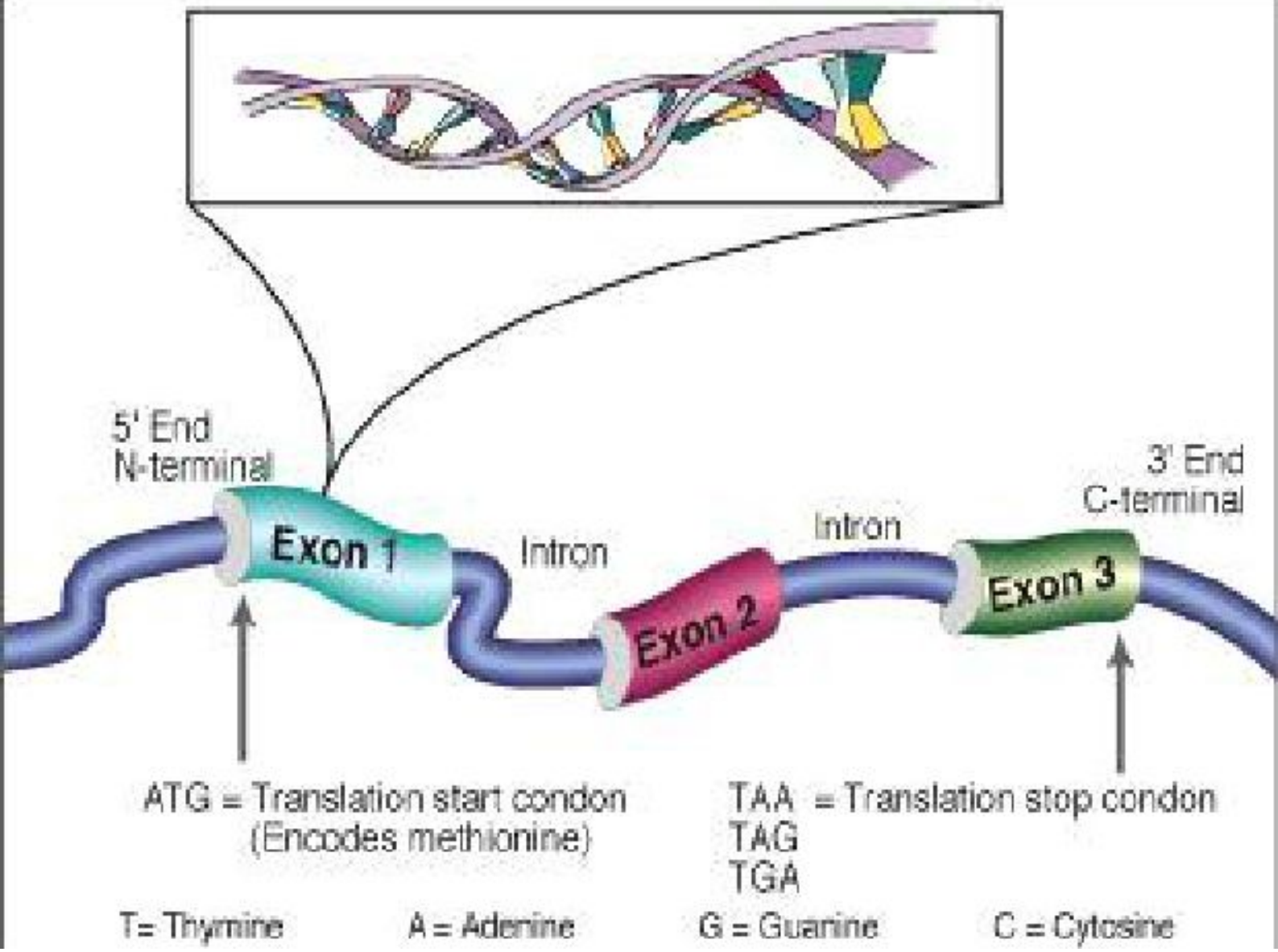
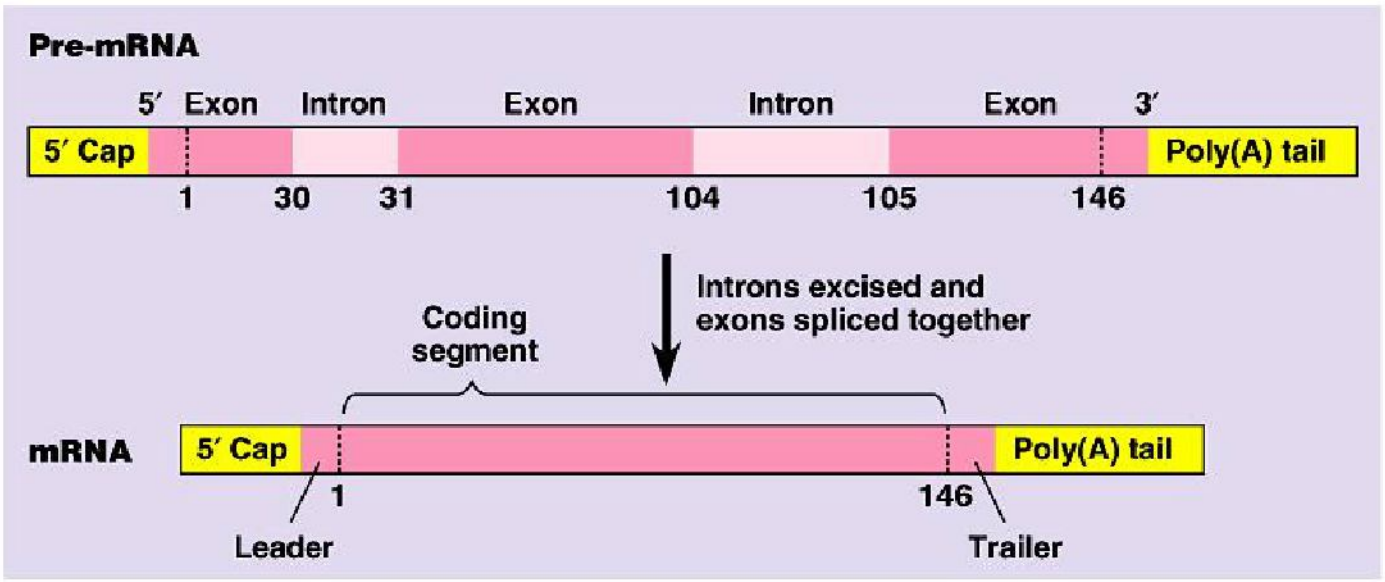

5' End
N-terminal
Exon 1
Intron
Exon 2
Intron
Exon 3
3' End
C-terminal
ATG = Translation start condon
(Encodes methionine)
TAA = Translation stop condon
TAG
TGA
T= Thymine
A = Adenine
G = Guanine
C = Cytosine

间隔序列intervening sequences

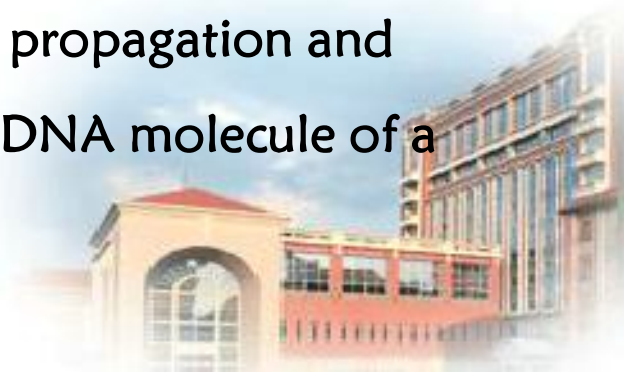


The long DNA template of one **gene** contains long stretches of a nucleotide sequence that **DO NOT CODE FOR ANY PART OF THE POLYPEPTIDE.** These nontranslated portions are called **intervening sequences** or **introns**.

❖ Recombinant DNA technology

按照人的意愿在体外对 DNA 分子进行重组，再将重组分子导入受体细胞，使其在细胞中扩增和繁殖，以获得该DNA分子的大量拷贝。

In accordance with the wishes of the people in vitro of recombinant DNA molecules, and then re-import molecular receptor cell to cell in the propagation and amplification in order to obtain the DNA molecule of a large number of copies.



三、目的基因的来源

要求

①纯度高；②数量多

原核生物（Procaryote）

-基因组较小，可直接分离获得（较易获得目的基因）

真核生物（Eukaryote）

一个单拷贝基因仅占整个基因组的10^{-5}甚至更少，不易获得

原核细胞

基因组

限制性内切酶

水解成若干片段

探针筛选

提　　纯

扩　　增

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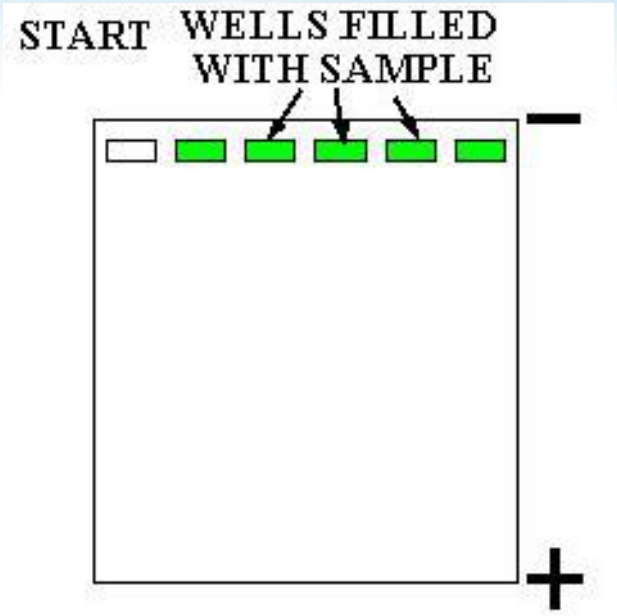
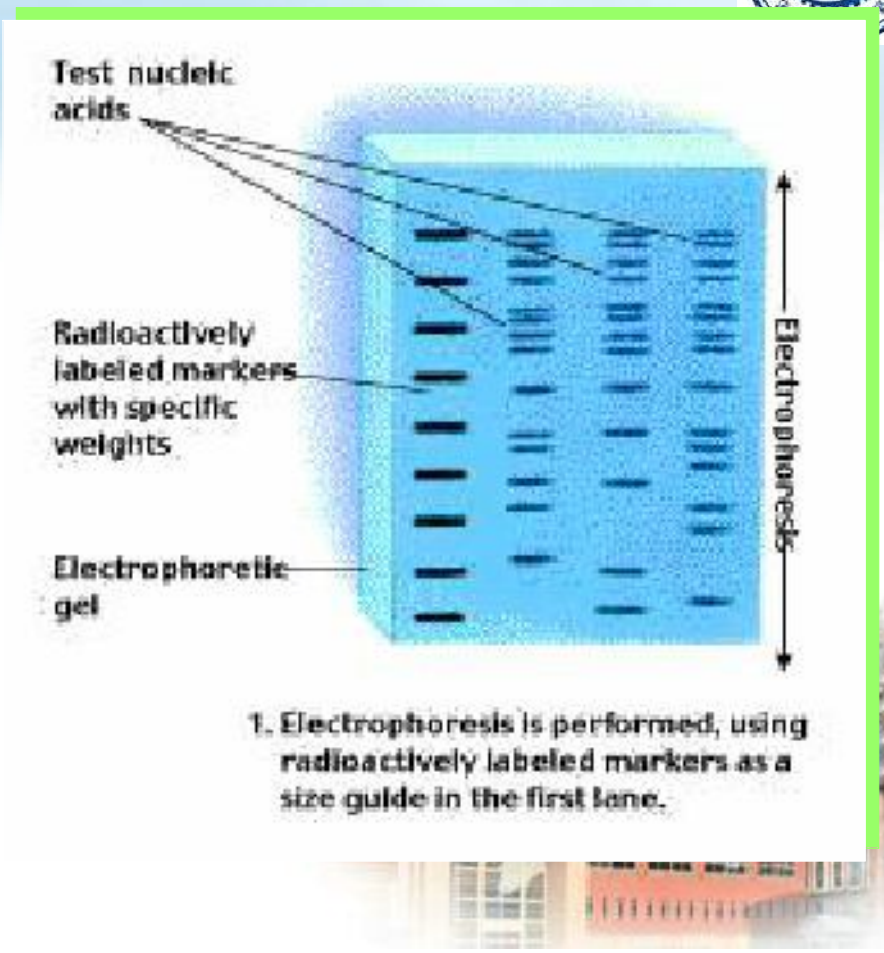

START
WELLS FILLED
WITH SAMPLE
−
+


Test nucleic acids
Radioactively labeled markers with specific weights
Electrophoretic gel
Electrophoresis
1. Electrophoresis is performed, using radioactively labeled markers as a size guide in the first lane.

真核生物目的基因的来源

构建 cDNA 文库

construct cDNA library

构建基因组文库

construct genomic library

人工合成

Synthesize DNA in vitro

聚合酶链反应

Polymerase chain reaction,PCR

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四、基因文库(gene library)概念

1、定义：

又称DNA文库，是指某个生物的基因组DNA或cDNA片段与适当的载体在体外重组后，转化宿主细胞，并通过一定的选择机制筛选后得到大量的阳性菌落(或噬菌体)，所有菌落或噬菌体的集合即为该生物的基因文库。

2、组成

外源DNA片段

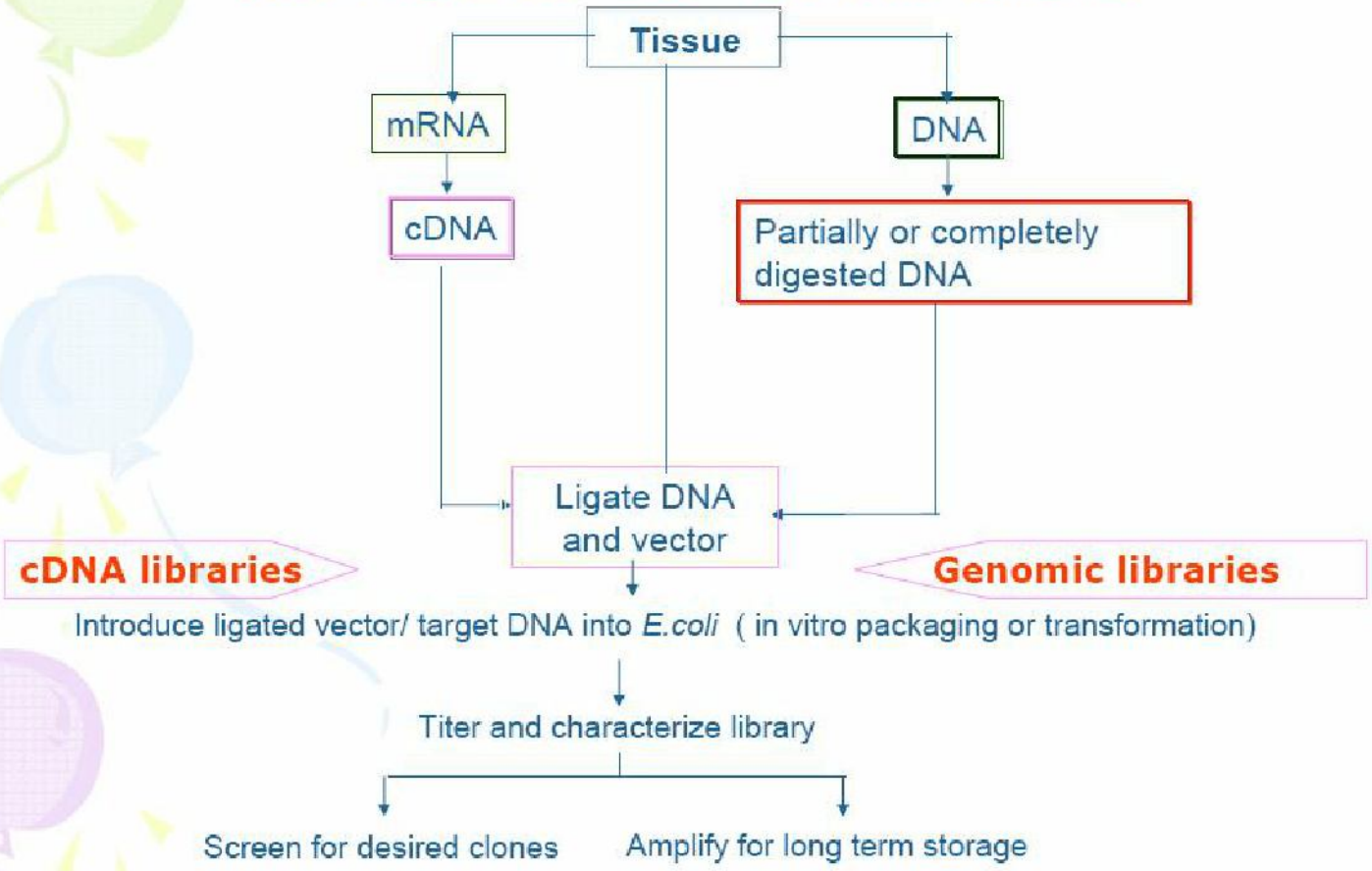
载体

宿主

3、构建基因文库的基本程序

- 提取研究对象基因组DNA，制备合适大小的DNA片段，或提取组织或器官的mRNA并反转录成cDNA
- DNA片段或cDNA片段与经特殊处理的载体连接形成重组DNA
- 重组DNA转化宿主细胞或体外包装后侵染受体菌
- 阳性重组菌落或噬菌斑的选择

Library construction
cDNA libraries and Genomic libraries



4、种类

cDNA文库

基因组文库

cDNA文库(cDNA library)

是指将某种生物体基因组转录的全部mRNA经反转录产生的cDNA片段分别与克隆载体重组，储存于某种受体菌中，该群体就称该生物基因组的cDNA文库(C-文库)。

代表该种生物（或组织）的全部mRNA序列

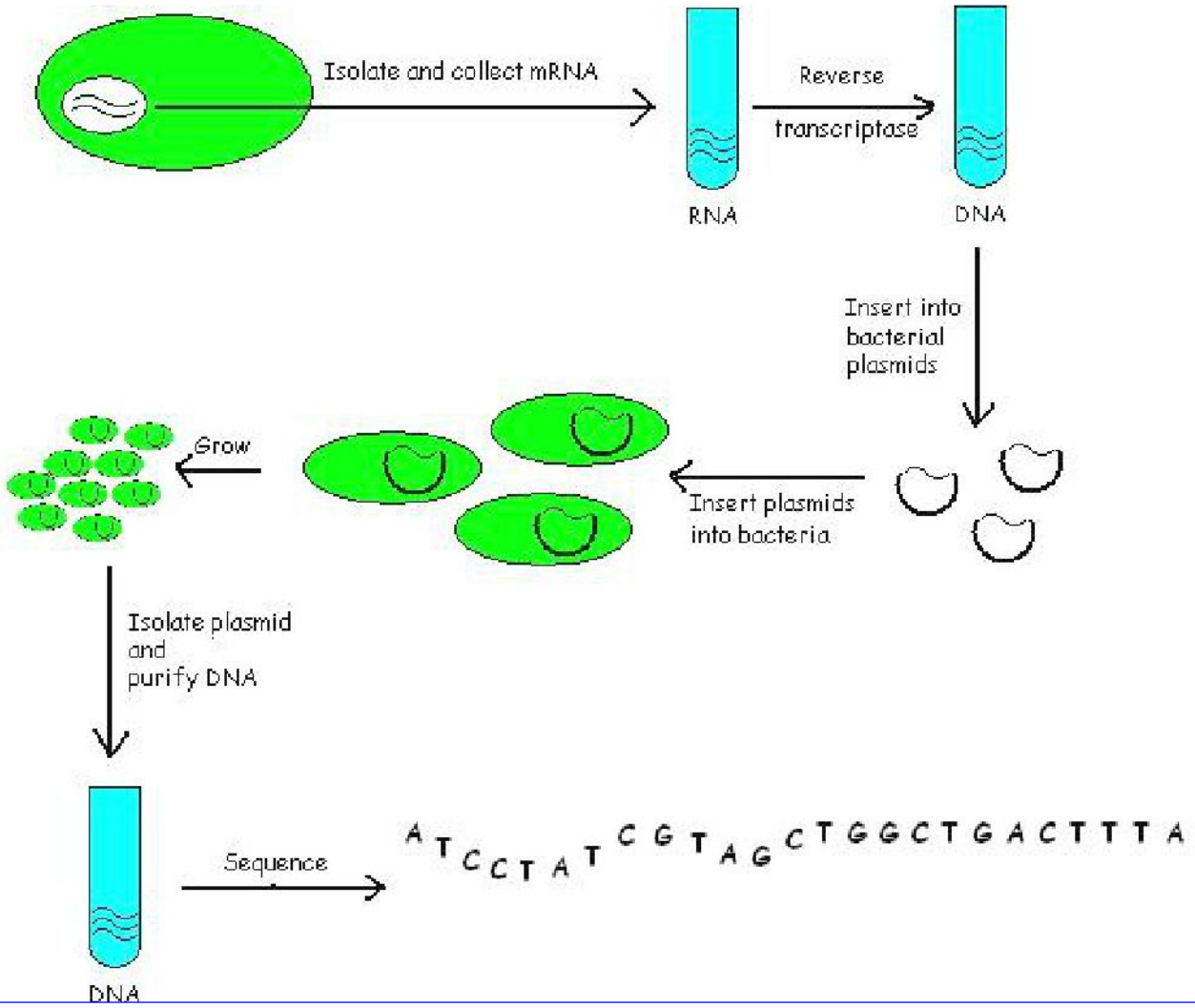
具有组织细胞特异性

- **A cDNA library is a collection of cloned cDNA (complementary DNA) fragments.**

- **cDNA is produced from fully transcribed mRNA found in the nucleus and therefore contains only the coding regions of an organism.**

the libraries lack information about enhancers, introns, and other regulatory elements found in a genomic DNA library.

Formation of a cDNA Library



•cDNA is created from mRNA with reverse transcriptase.

Process

❑A poly-(A) tail can be used as a primer site for reverse transcription.

❑Several methods exist for purifying RNA （ trizol extraction and column purification）.

❑ oligo-dT and random primers can be used with reverse transcriptase to create cDNA templates.

❑Restriction endonucleases and DNA ligase are then used to clone the sequences into bacterial plasmids.

The cloned bacteria are then selected , commonly through the use of antibiotic selection. Once selected, stocks of the bacteria are created which can later be grown and sequenced to compile the cDNA library.

基因组文库（Genomic library）

是指将某种生物体的全部基因组DNA用限制性内切酶或机械力量切割成一定长度范围的DNA片段，再与合适的载体在体外重组并转化相应的宿主细胞获得的所有阳性菌落，这个群体就称为该生物基因组文库。

其目的是分离有用的目的基因和保存某种生物的全部基因。

代表该种生物的全部基因组序列

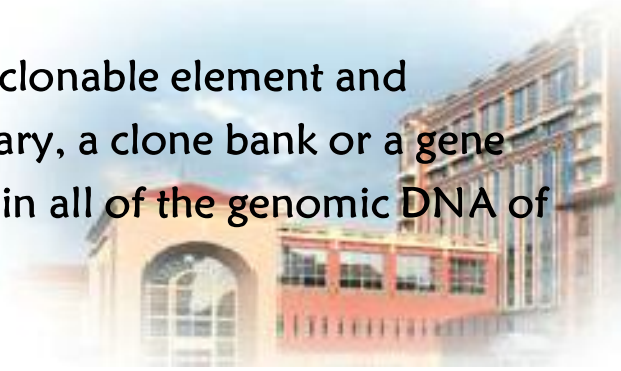
➢**Gene library** carries a DNA molecule that was inserted into a cloning vector.

➢ all of the cloned DNA molecules represent the entire genome of the organism.

➢A gene library is also called *gene bank*.

- The DNA molecules of an organism in interest are isolated.
- The DNA molecules are partially digested by an endonuclease restriction enzyme. the DNA molecules are digested to different lengths of time in order to ensure that all the genes have been digested to manageable sizes.
- The digested DNA molecules are run on agarose electrophoresis for which a suitable range of lengths of DNA pieces are isolated and ligated to vector plasmids.
- 99% of the genes will be incorporated into the plasmids.
- The plasmids can be taken up by suitable

The process of subdividing genomic DNA into clonable element and inserting them into host is called creating a library, a clone bank or a gene bank. A complete library of host cell will contain all of the genomic DNA of the source organism.



Types of Gene Libraries

Plasmid libraries质粒文库:

Have a capacity of 10 kb.

Phage libraries噬菌体文库:

Have a capacity of 23 kb.

Cosmid libraries粘粒文库:

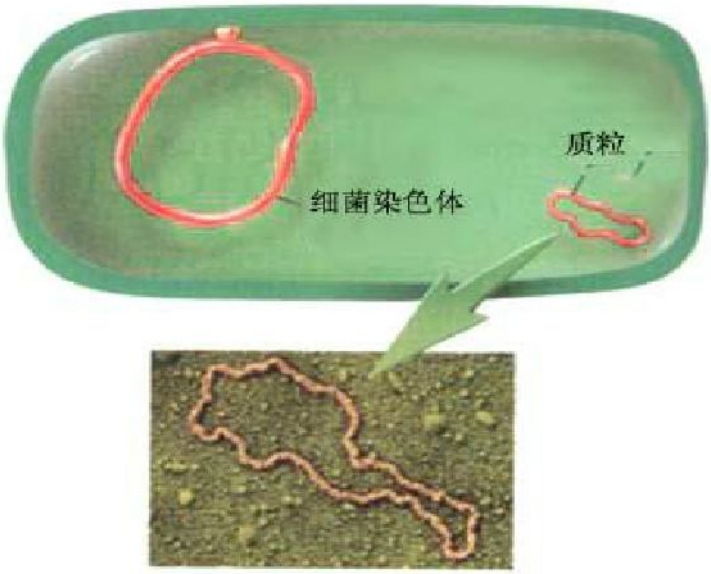
Have a capacity of 45 kb.

Yeast artificial chromosome (YAC) libraries 人工染色体文库:

Have a capacity of 1000 kb.

Vectors	Plasmid	phage λ	cosmid	YAC
insert (kb)	10	23	45	1000

质粒 (plasmid)



- 质粒是细菌细胞内独立于细菌染色体而自然存在的、能自我复制、易分离和导入的环状双链DNA分子。
- 适应范围广，拷贝数多。进入宿主细胞复制后，每个细胞的质粒拷贝数可高达1000个。

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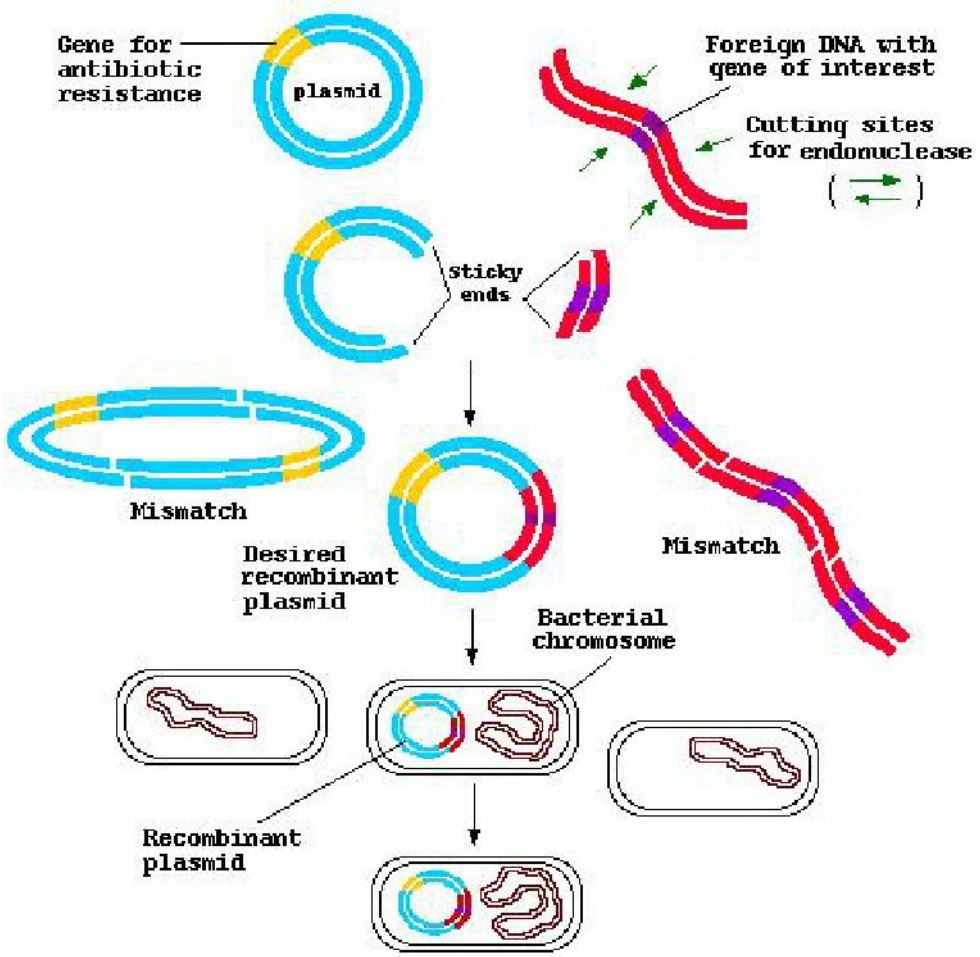
Plasmid Insertion



噬菌体(phage)

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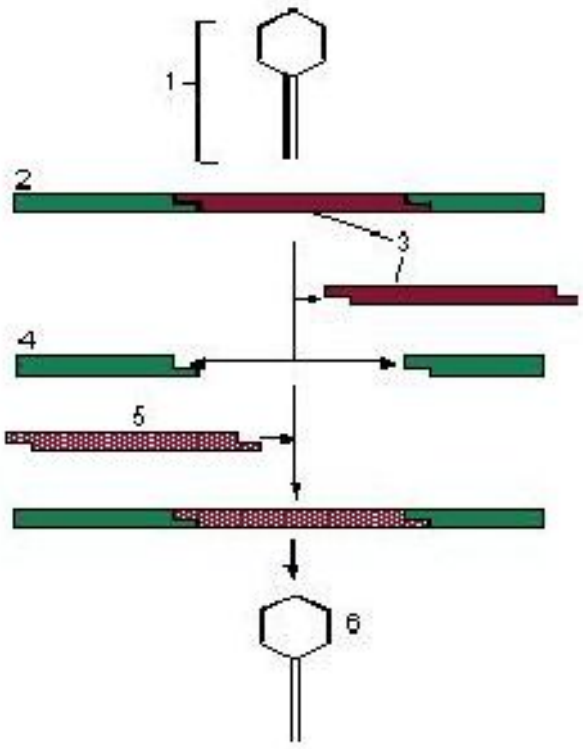
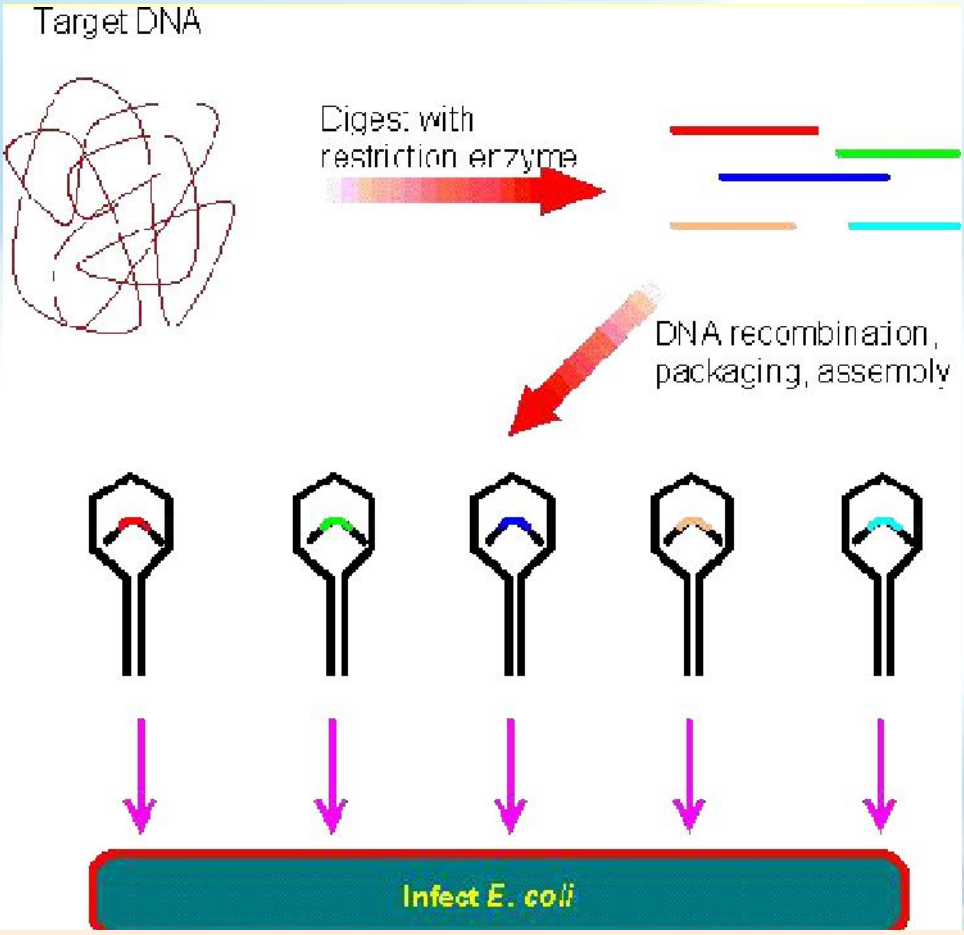


图 n—4 λ噬菌体载体

1、λ噬菌体； 2、λ噬菌体 DNA； 3、λ噬菌体 DNA 中间基因簇；4、将连接物体外包装后感染细菌，制备基因库。

◆ λ噬菌体基因组全长49kb。λ噬菌体DNA中间约2/3的序列为中间基因簇(central gene cluster)，位于两端的为DNA左、右臂。λ基因组的中间基因簇序列可被外源DNA片段取代，而不影响噬菌体感染细菌及形成噬菌斑的能力。

◆ λ噬菌体载体可接受15kb-23 kb的外源DNA片段，在基因库筛选中，λ噬菌体作载体与细菌质粒相比，具有易操作、阳性克隆数多等特点。



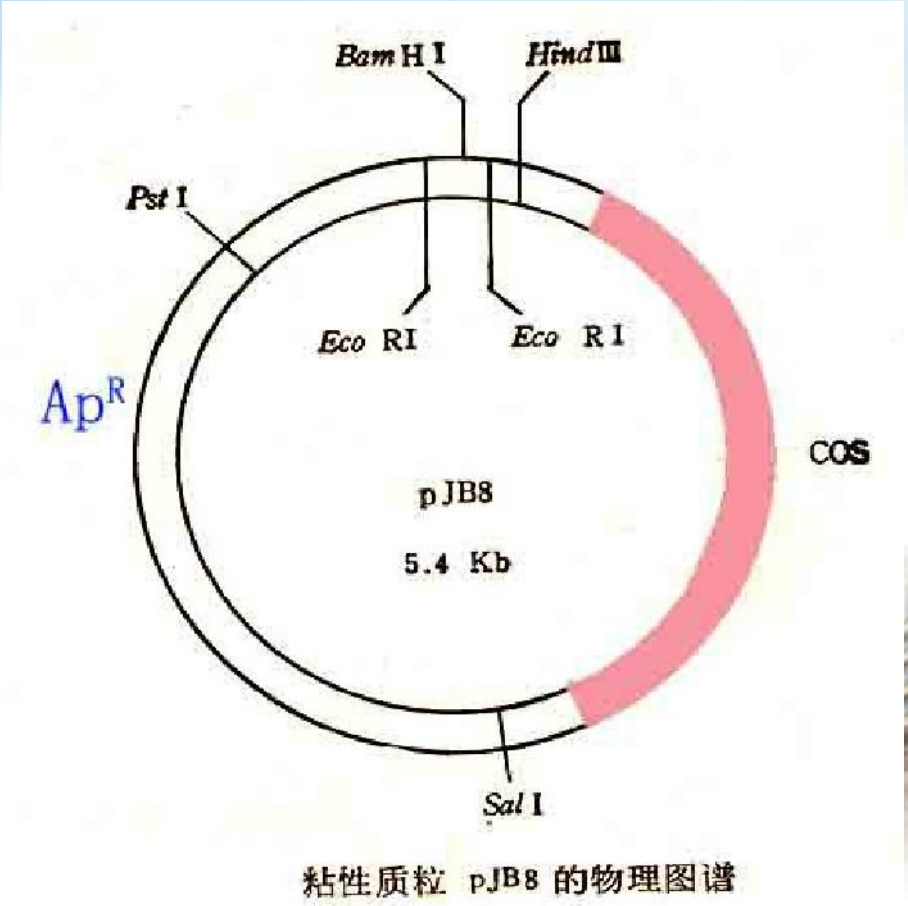
Preparation of the genomic library using l phage vectors. It is basically the cloning of all DNA fragments representing the entire genome.

柯斯质粒(cosmid)

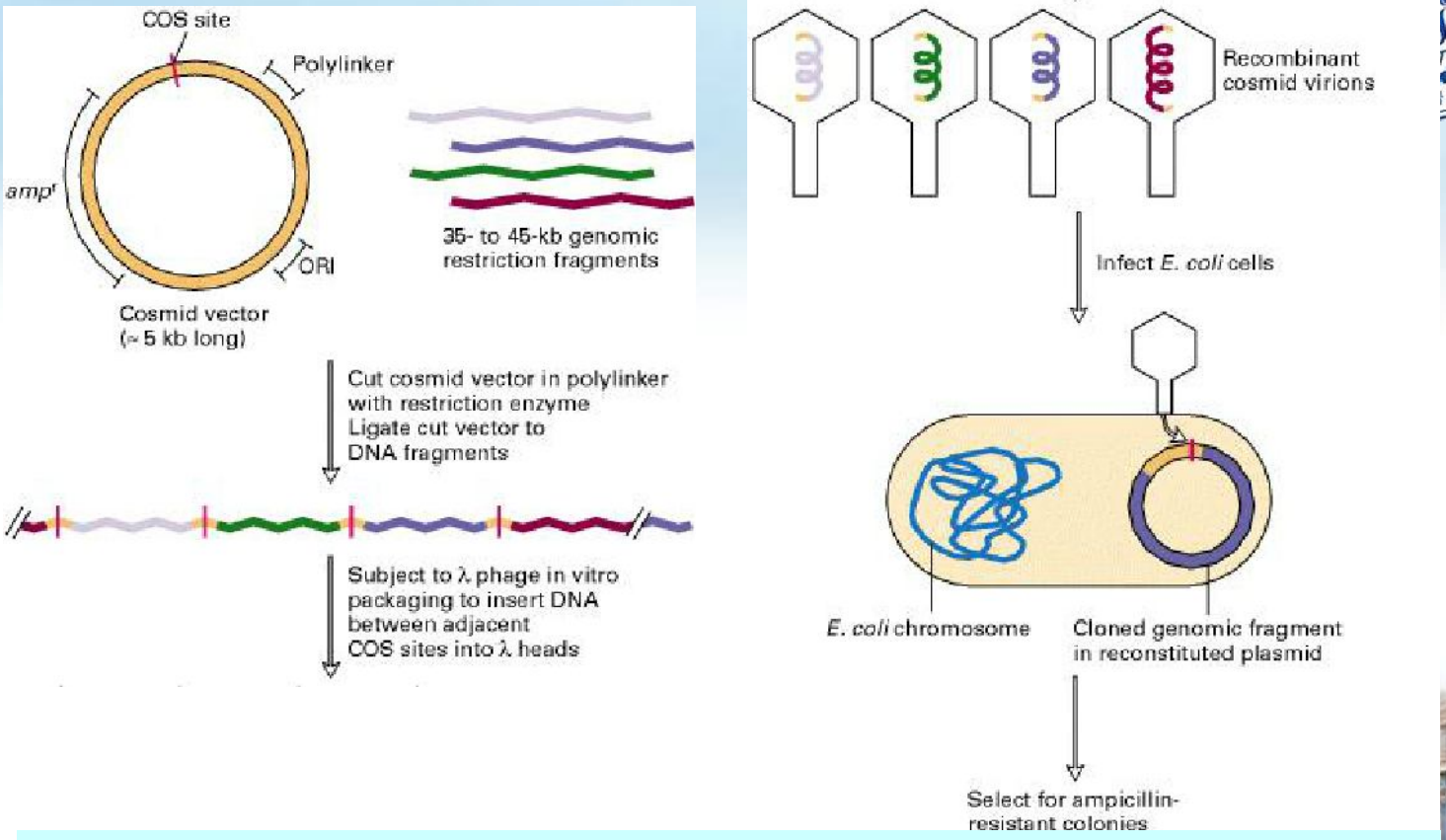
一种人工构建的克隆载体，包含了λ噬菌体的COS基因。

柯斯质粒能被包装到λ噬菌体粒子中，感染大肠杆菌；

携带入宿主细菌的DNA片段要比质粒载体携带的要大



粘性质粒 pJB8 的物理图谱



This procedure has the high efficiency associated with λ phage cloning and permits cloning of restriction fragments up to ≈45 kb long. In this example, four different types of recombinant cosmid virions could be generated, each carrying one of the genomic fragments indicated by different colors.

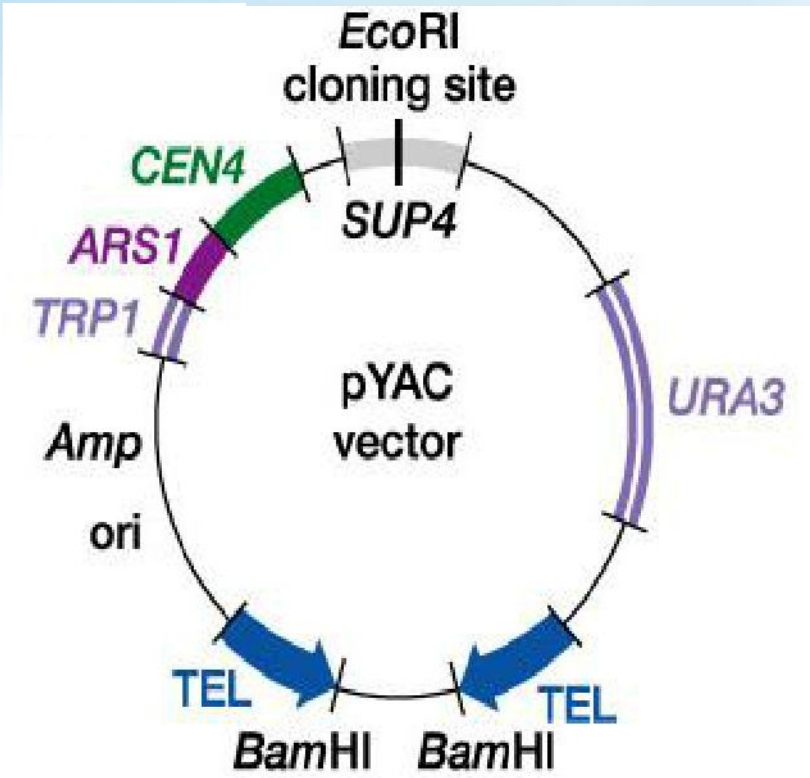
Cosmids, Phages, and plasmids as DNA carriers

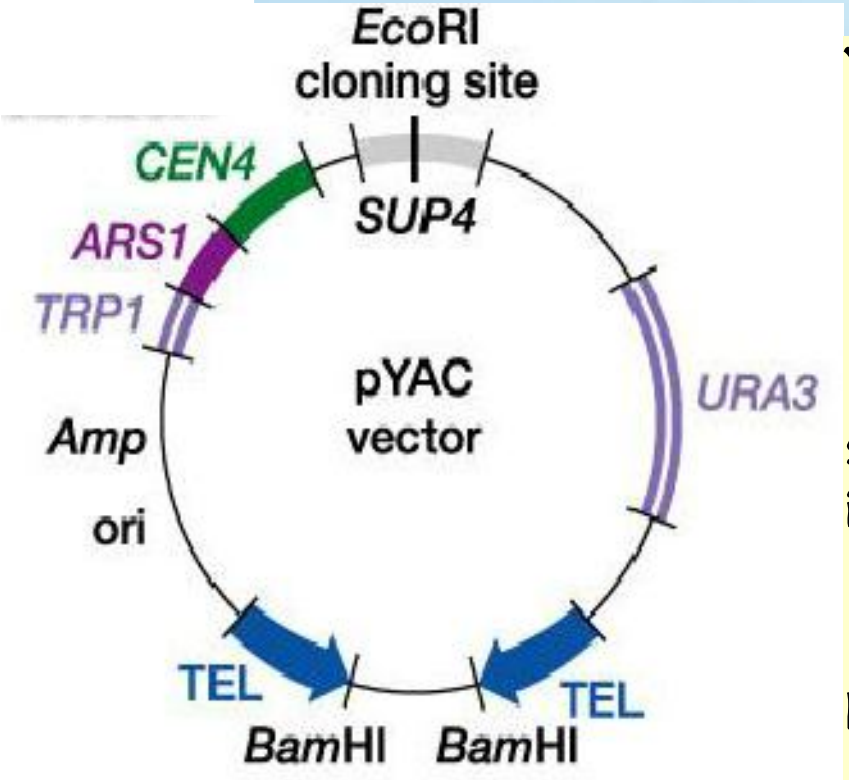
	Cosmid	Phage	Plasmid
Host	Bacteria	Bacteria	Bacteria
Insert size	Up to 45kb	~ 23 kb	~ 10kb
Entry into Cells	Infection	Infection	Transformation
Efficiency	Very efficient	Very efficient	Less efficient
Outcome	Multiply	Multiply and kill	Multiply
Appearance of infected cells	Colonies	Plaques	Colonies
Application	Genomic library	Genomic library	Cloning

◆酵母人工染色体（yeast artificial chromosome，YAC）

具有自主复制序列、克隆位点以及可在细菌和酵母菌中选择的标记基因。

YAC可以接受100-1000 kb的外源DNA片段，使YAC成为人类基因组计划及克隆分离基因的重要工具

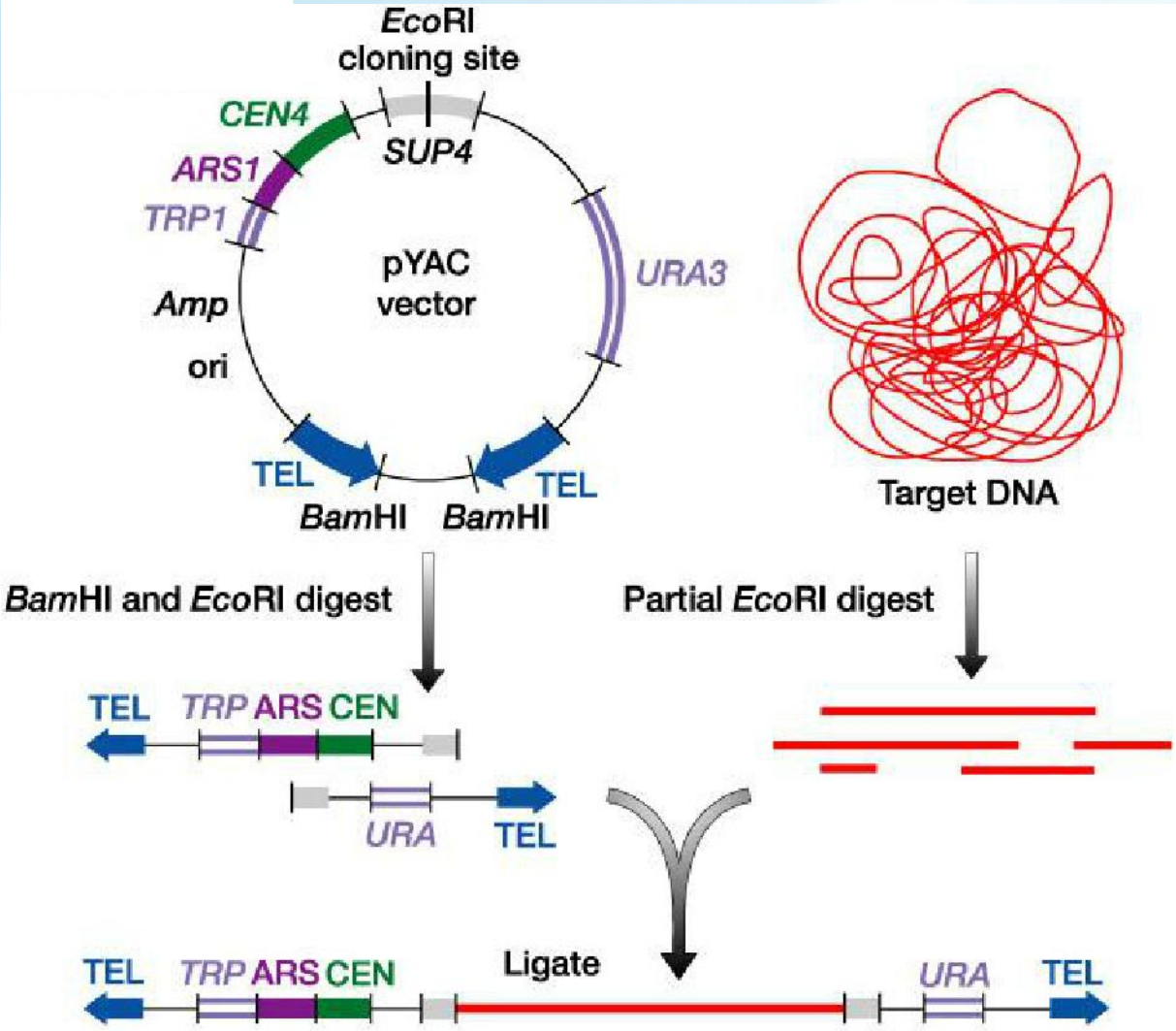




YACs must include:

- centromere sequences (CEN)
  着丝点
- Telomere sequences (TEL)
  端粒
- Autonomous replicating sequences (ARS) for replication in the yeast nucleus.

- Ampicillin resistance for propagation in E. coli


EcoRI
cloning site
CEN4
SUP4
ARS1
TRP1
pYAC
vector
URA3
Amp
ori
TEL
TEL
BamHI
BamHI
Target DNA
BamHI and EcoRI digest
Partial EcoRI digest
TEL
TRP ARS CEN
URA
TEL
Ligate
TEL
TRP ARS CEN
URA
TEL

基因组DNA文库的质量标准

一个理想的基因组DNA文库应具备下列条件：

- 重组克隆的总数不宜过大，以减轻筛选工作的压力
- 载体的装载量最好大于基因的长度，避免基因被分隔克隆
- 克隆与克隆之间必须存在足够长度的重叠区域，以利克隆排序
- 克隆片段易于从载体分子上完整卸下
- 重组克隆能稳定保存、扩增、筛选

cDNA Library vs. Genomic DNA Library

A cDNA library lacks the non-coding and regulatory elements found in genomic DNA.

Genomic DNA libraries provide much more detailed information about the organism, but are much more resource-intensive to generate and maintain.

Advantages and disadvantages

Genomic Library

- Relatively large
- Relatively easy to construct
- Contains non-coding sequences
- Better for organisms lacking introns (prokaryotes)
- Useful in sequencing genomes

cDNA Library

- Relatively small
- Difficult to construct, many steps and reagents
- Contains only transcribed sequences
- Useful in understanding gene expression

cDNA基因文库构建的步骤

- 细胞总RNA的提取和mRNA的分离
- 第一条cDNA合成
- 第二条cDNA合成
- 双链cDNA克隆进质粒或噬菌体载体并导入宿主中繁殖

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cDNA以mRNA为模板经逆转录而成

In genetics, complementary DNA (cDNA) is DNA synthesized from a mature mRNA template in a reaction catalyzed by the enzyme reverse transcriptase.

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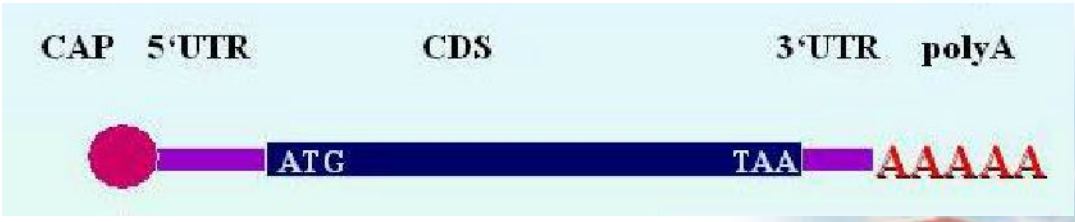
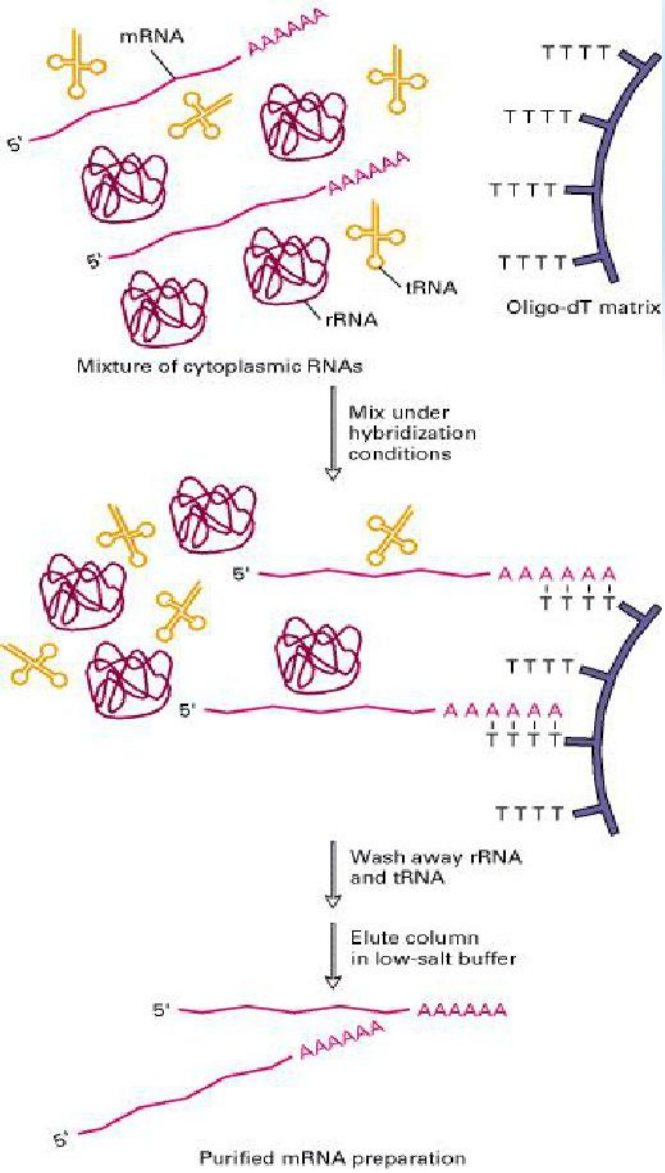
真核细胞mRNA的分离和纯化

- 提取细胞内总RNA
- 分离和纯化mRNA

  mRNA 3’端有polyA尾，用oligo-dT纤维素亲和柱分离纯化mRNA

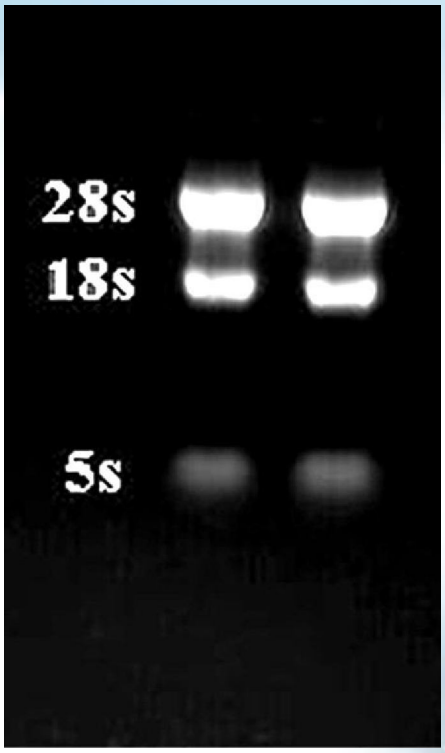




RNA : ribosomal RNAs (rRNAs)、transfer RNAs (tRNAs) and the much less abundant mRNAs (red) have 3' poly(A) tails, which hybridize to oligo-dT covalently coupled to the column matrix.

After hybridization, the rRNAs and tRNAs are washed out of the column; then the mRNAs are eluted.

The resulting purified mRNA preparation contains many different mRNA molecules encoding different proteins.



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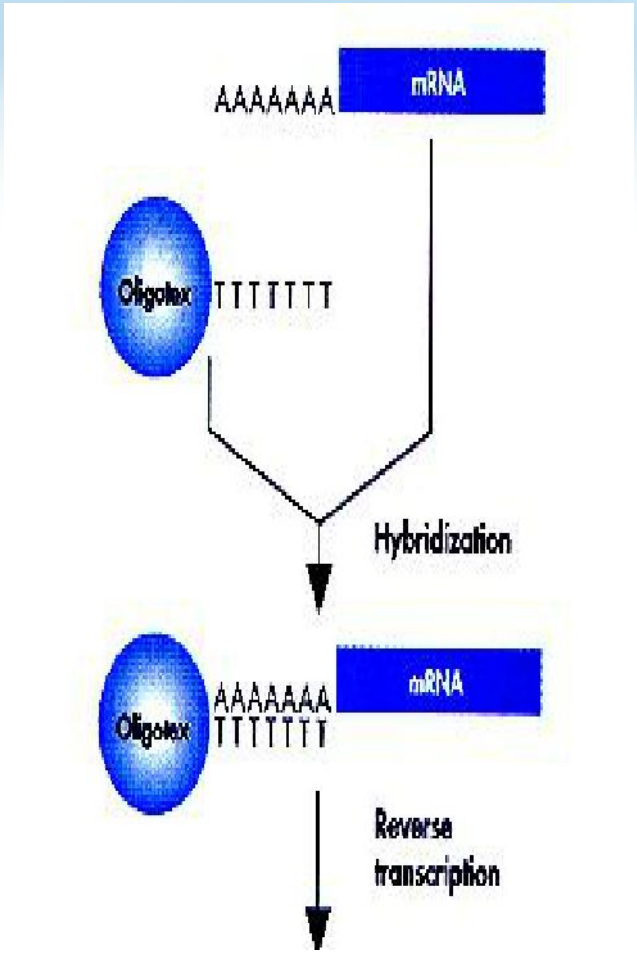
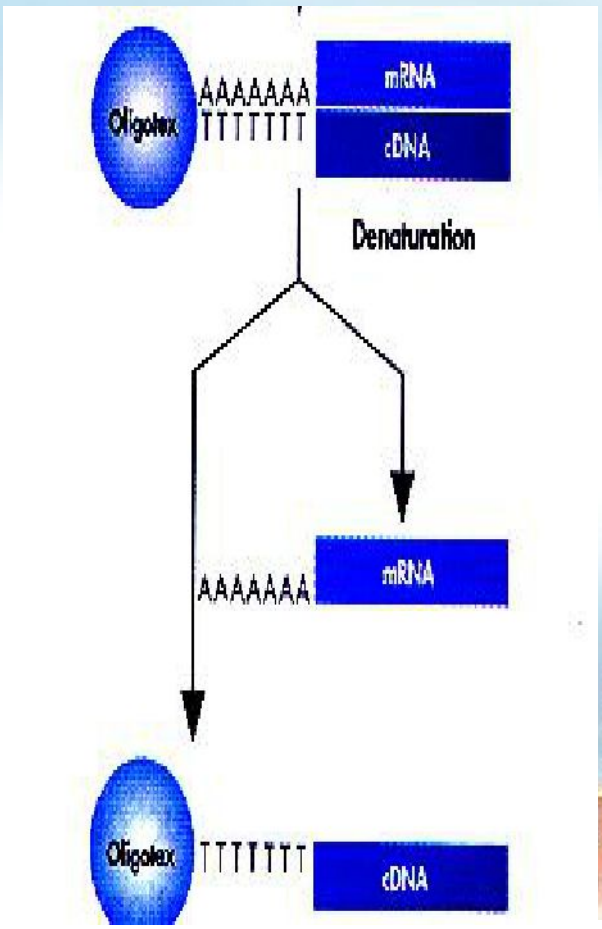
逆转录合成cDNA





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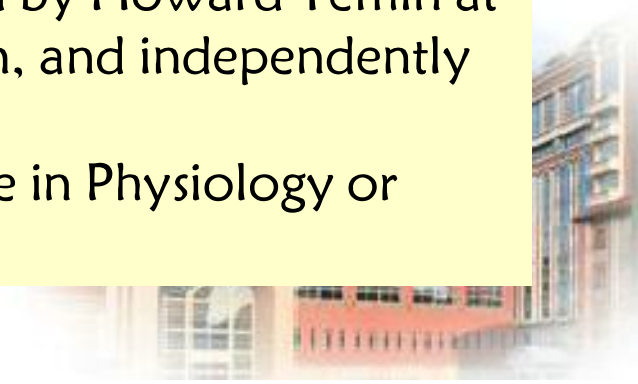
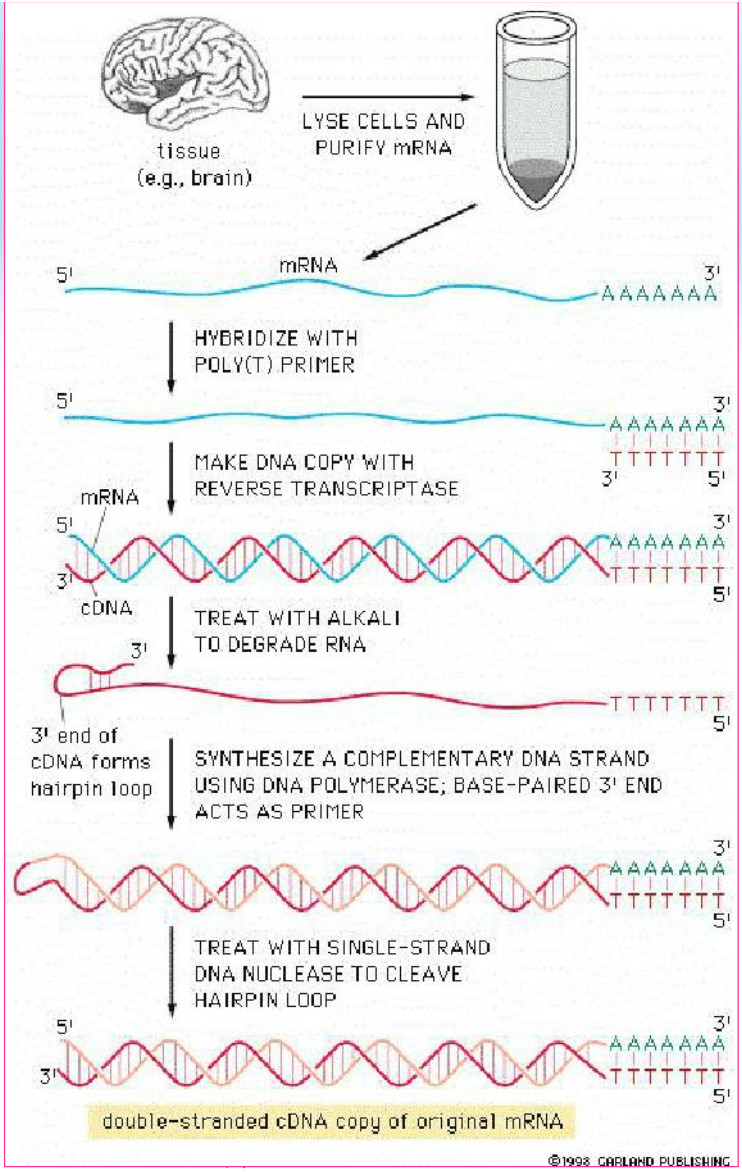
Reverse transcriptase, also known as **RNA-dependent DNA polymerase**, is a DNA polymerase enzyme that transcribes single-stranded RNA into double-stranded DNA. It also helps in the formation of a double helix DNA once the RNA has been reverse transcribed into a single strand cDNA. Normal transcription involves the synthesis of RNA from DNA.

Reverse transcriptase was discovered by Howard Temin at the University of Wisconsin-Madison, and independently by David Baltimore in 1970 at MIT.
The two shared the 1975 Nobel Prize in Physiology or Medicine.





Isolate poly(A) mRNA on oligo dT column

Reverse transcribe the mRNA to DNA

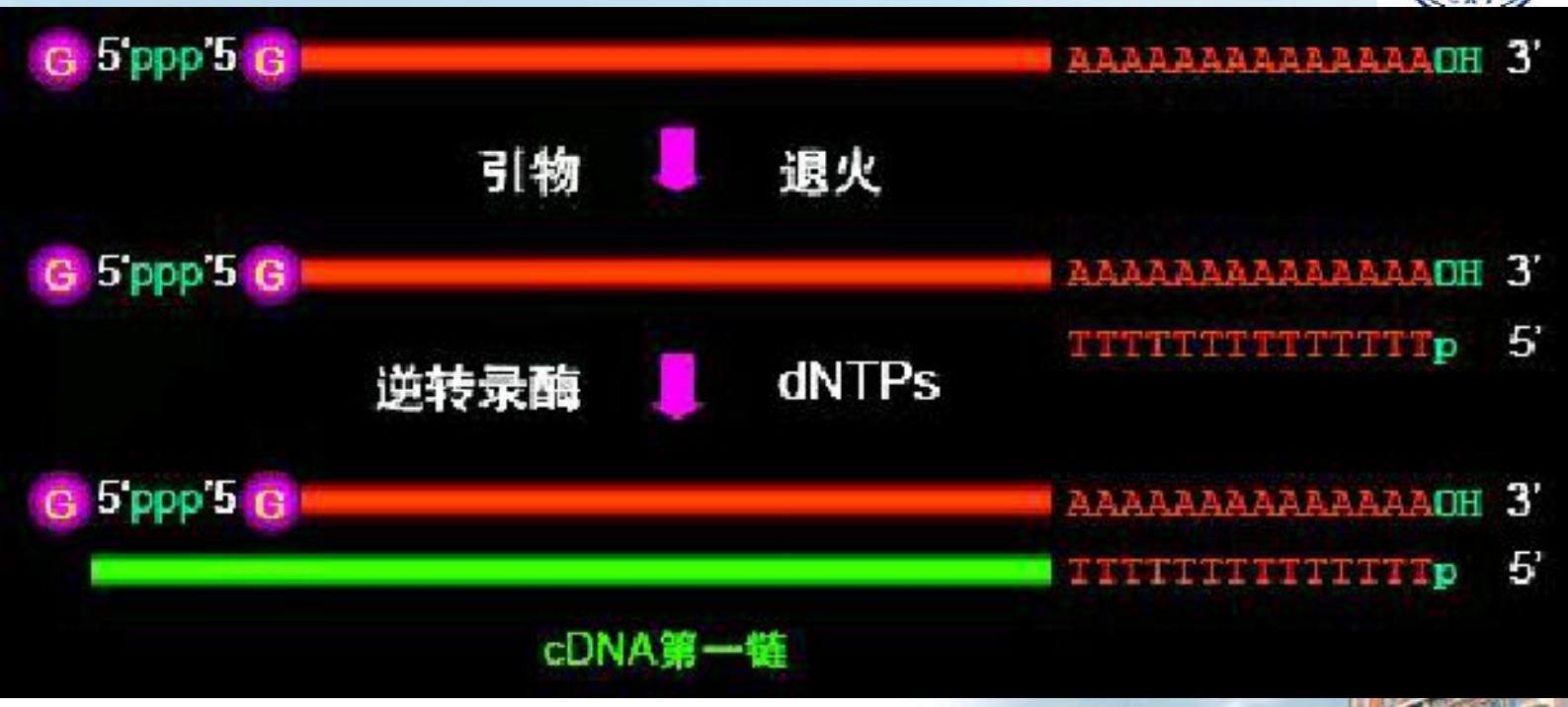
Second strand synthesis of DNA

Result: ds DNA copy of the mRNA

oligo(dT)引导的DNA合成法：

- 利用真核mRNA分子所具有的poly(A)尾巴的特性，加入12-20个脱氧胸腺嘧啶核苷组成的oligo(dT)短片段，由反转录酶合成cDNA的第一链。

- 缺陷：逆转录酶无法到达mRNA分子的5’－末端，必须从3’－末端开始合成cDNA。对于大分子量的较长的mRNA分子而言，特别麻烦



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随机引物引导的cDNA合成法 (randomly primed cDNA synthesis)：

- 根据许多可能的序列，合成出6-10个核苷酸长的寡核苷酸短片段（混合物），作为合成第一链cDNA的引物。
- 在应用这种混合引物的情况下，cDNA的合成可以从mRNA模板的许多位点同时发生，而不仅仅从3'-末端的oligo(dT)引物一处开始。

第二链cDNA的合成

- cDNA第二链的合成就是将上一步形成的mRNA-cDNA杂合双链变成互补双链cDNA的过程。
- cDNA第二链的合成的方法：

  自身引导合成法

  置换合成法

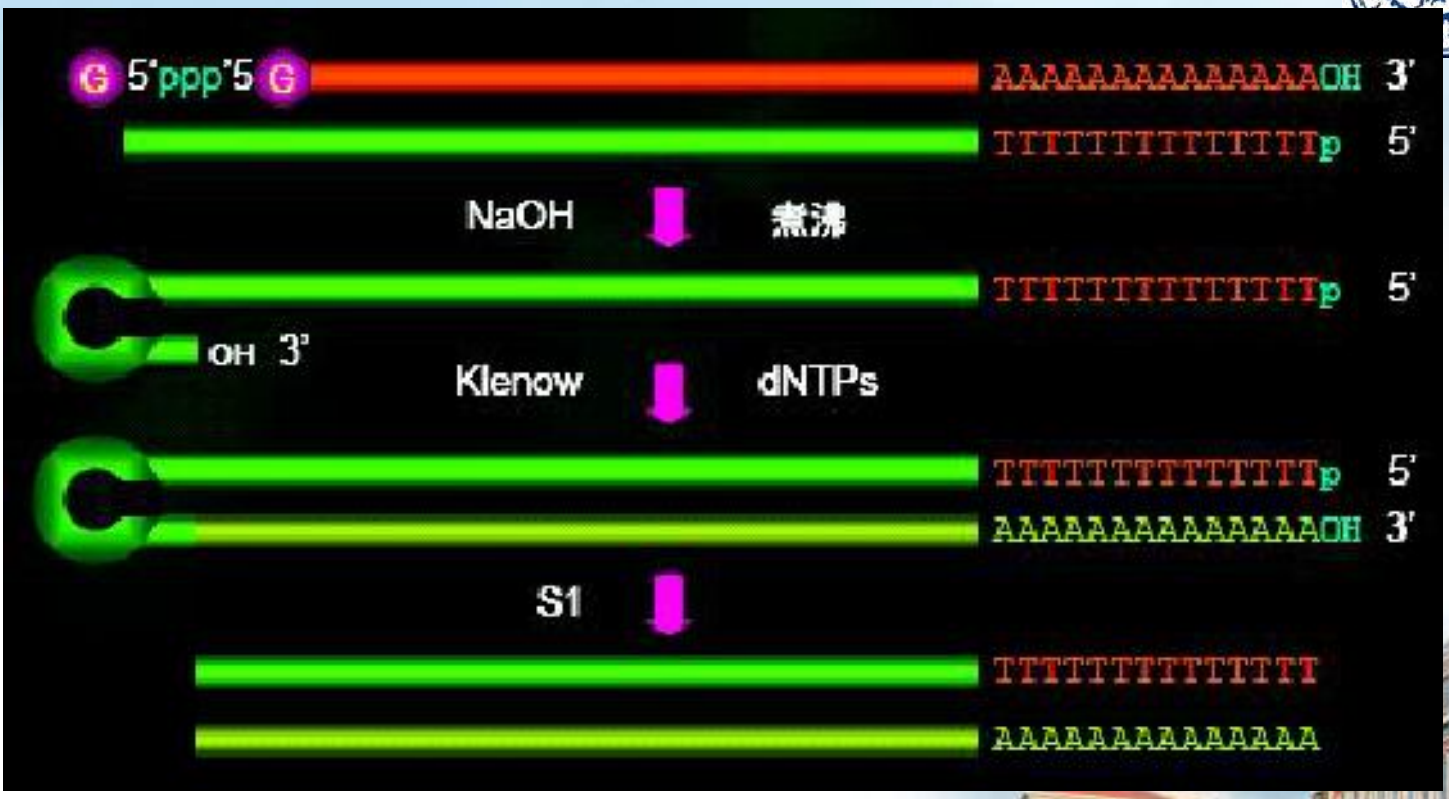
  引导合成法

  引物－衔接头合成法

自身引导合成法：

获得的单链cDNA3’端会形成发夹结构的能力，以此作为第二链合成的引物，在大肠杆菌聚合酶I Klenow或反转录酶的作用下，合成cDNA的第二链。

缺点：在以S1核酸酶切割cDNA的发夹状结构时，会导致对应于mRNA 5’端的地方的序列出现缺失和重排。S1核酸酶的纯度不够时，会偶尔破坏合成的双链cDNA分子。



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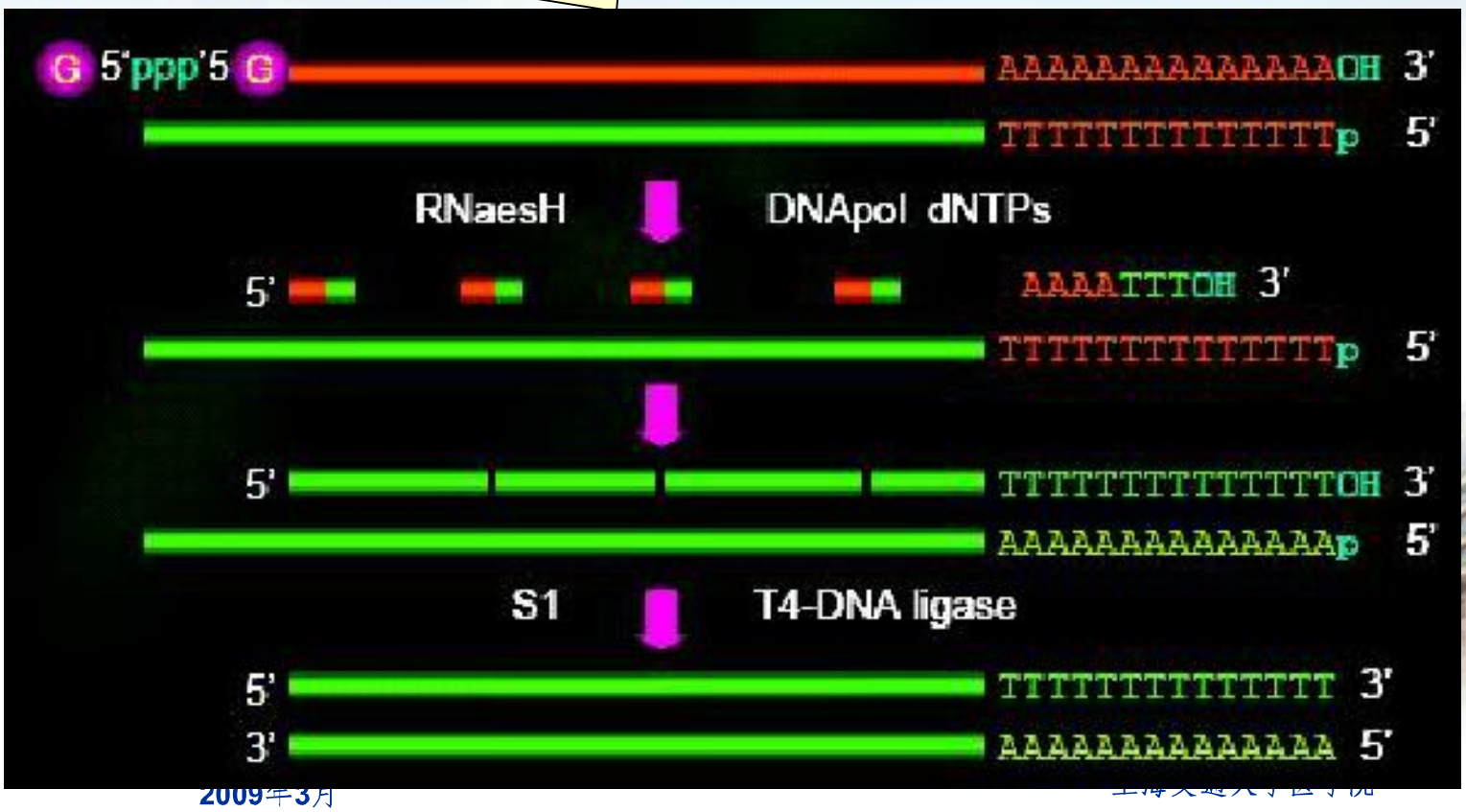
置换合成法：

获得的双链cDNA 5’端也会有几对碱基缺失

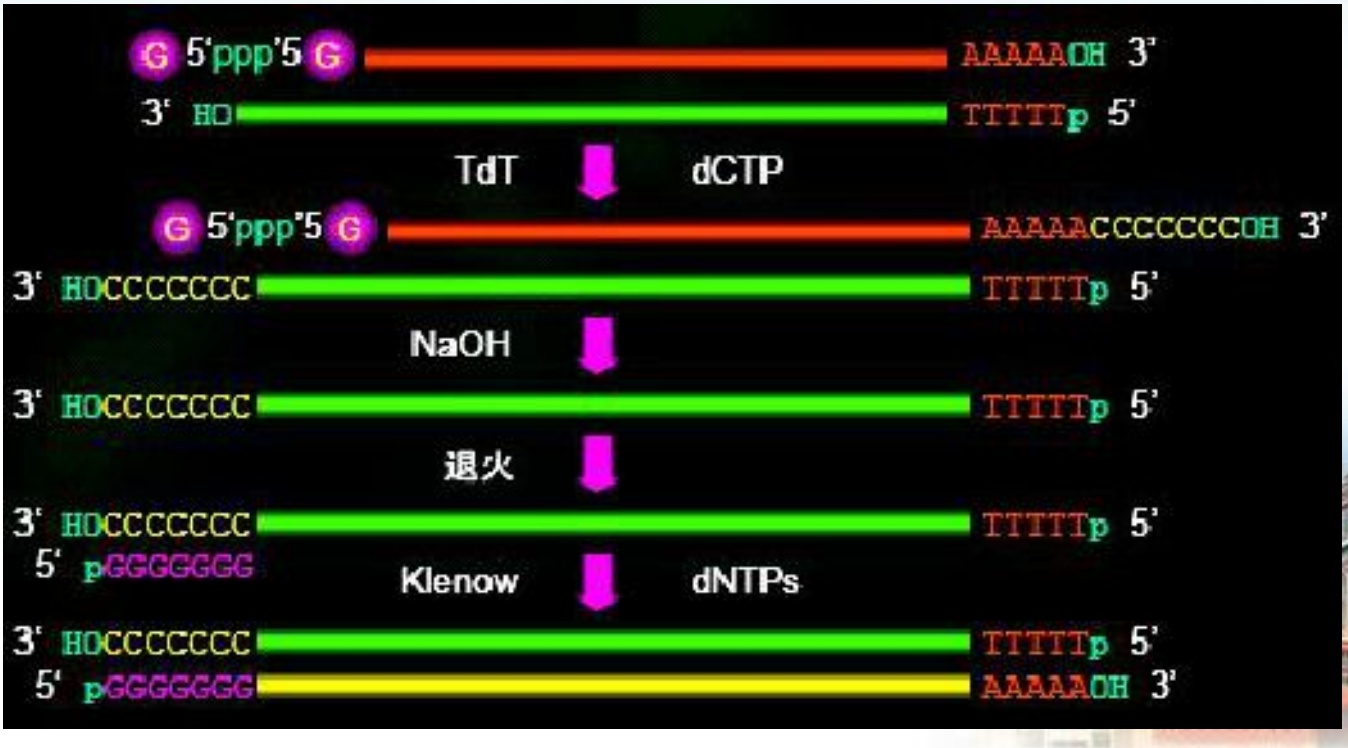
原　理：

以第一链合成产物cDNA：mRNA杂交体作为切口平移的模板，RNA酶H在杂交体的mRNA链上造成切口和缺口，产生一系列RNA引物，在大肠杆菌DNA聚合酶I的作用下合成cDNA的第二链。

优点：a）合成cDNA的效率高
b）直接利用第一链的反应产物，不需纯化
c）避免使用S1核酸酶来切割双链cDNA



❖ 引导合成法：获得的双链cDNA 能保留完整的5’端序列



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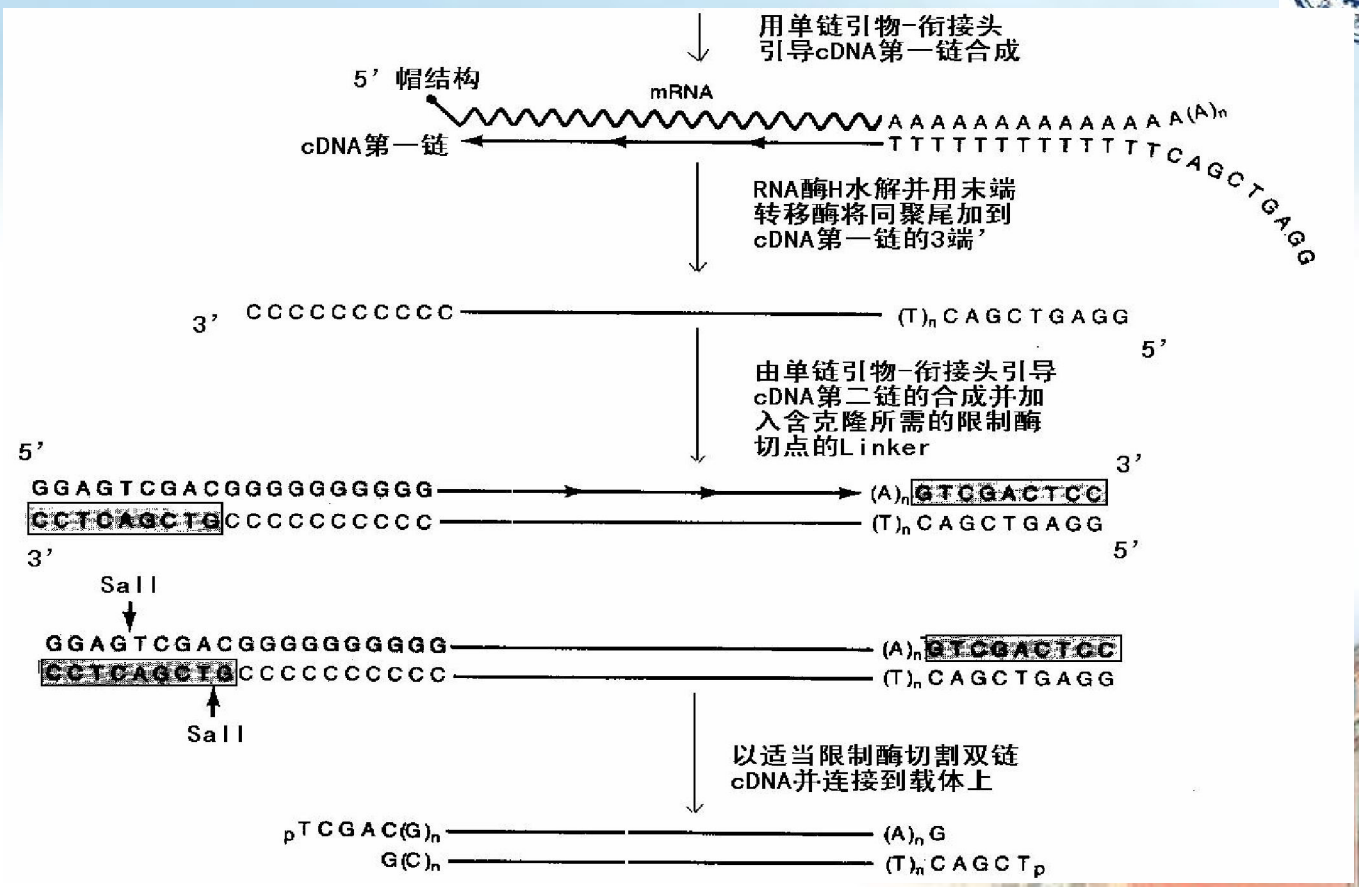
引物-衔接头法



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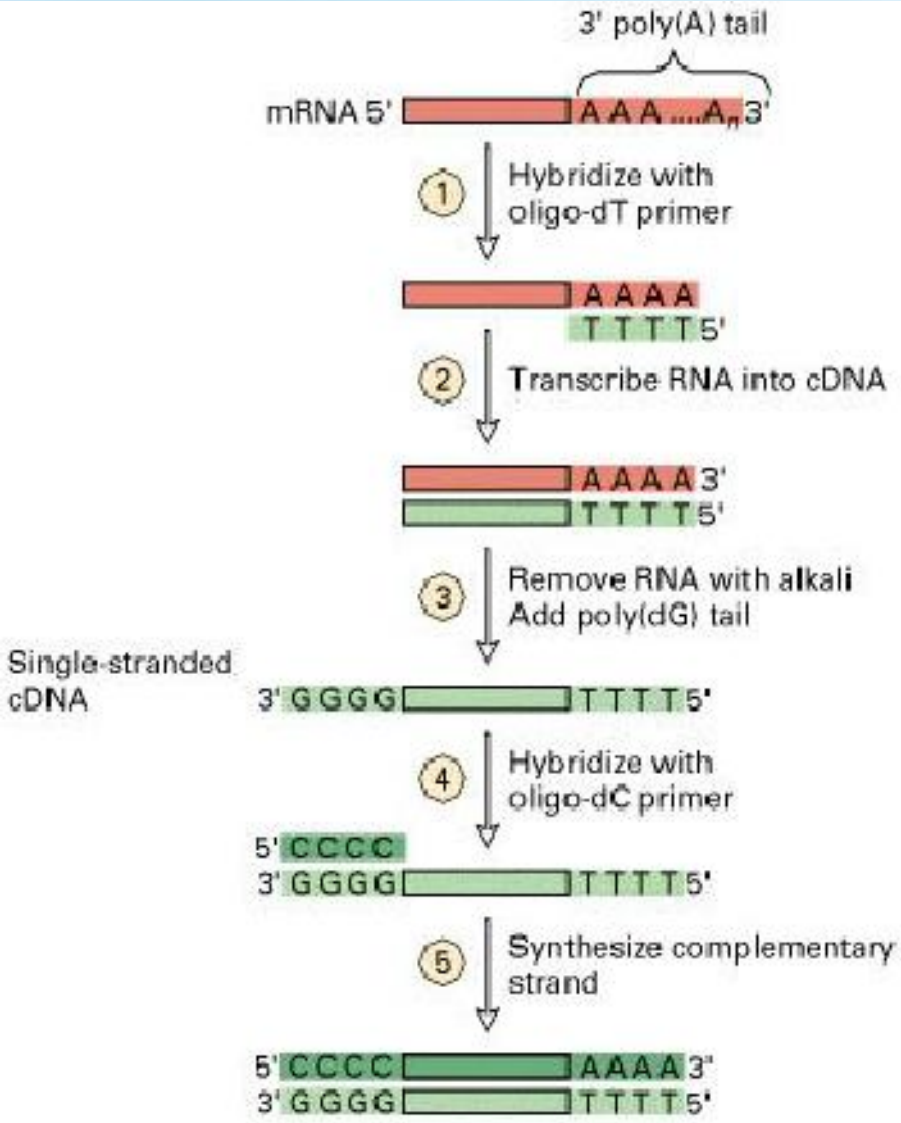
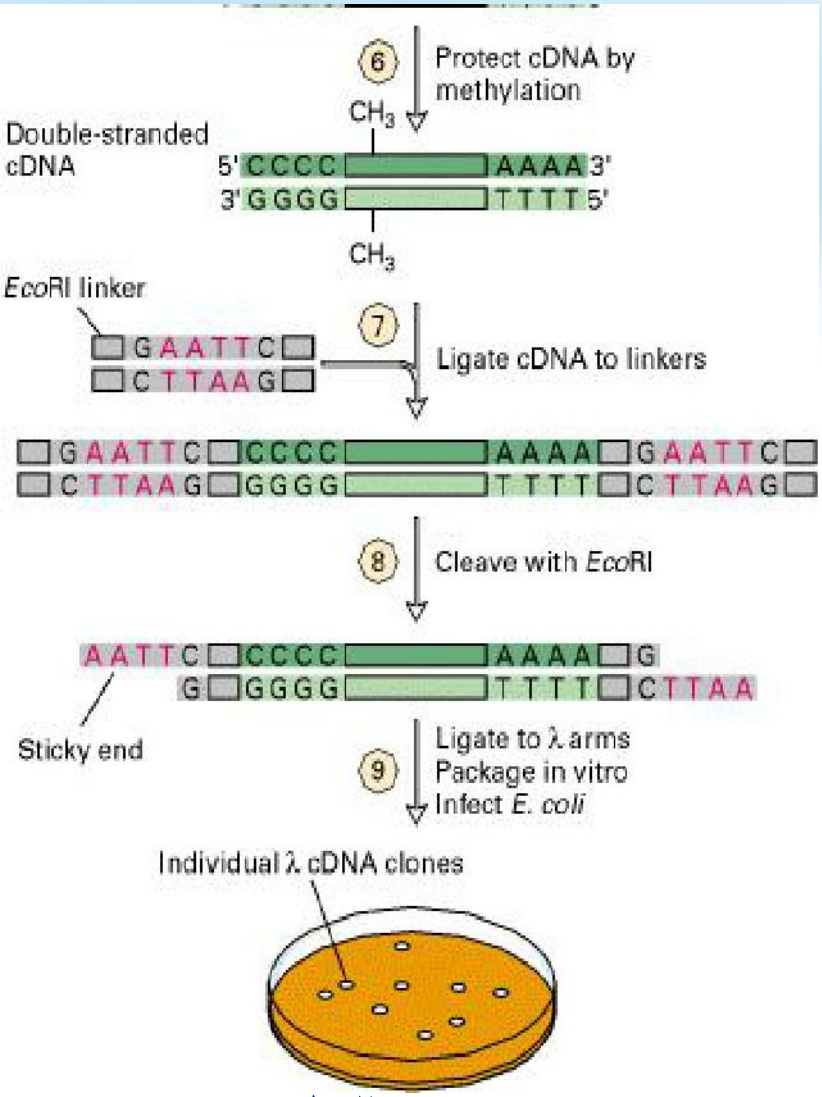
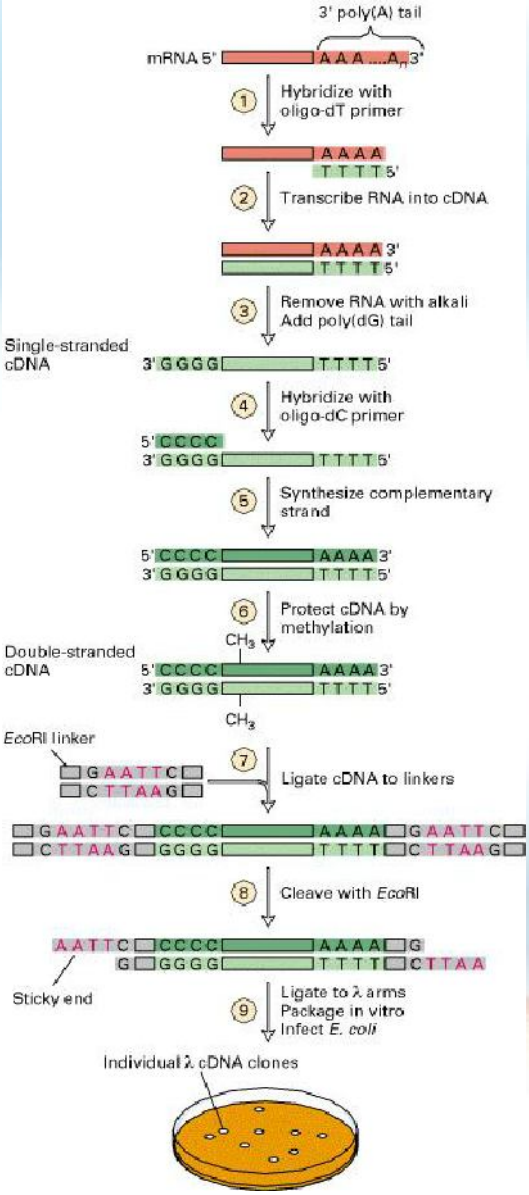


A mixture of mRNAs is used to produce cDNAs corresponding to all the cellular mRNAs (steps **1** – **3**). These single-stranded cDNAs (light green) are then converted into double-stranded cDNAs, which are treated with *Eco*RI methylase to prevent subsequent digestion by *Eco*RI (steps **4** – **6**).



The protected double-stranded cDNAs are ligated to a synthetic double-stranded *Eco*RI-site linker at both ends and then cleaved with the corresponding restriction enzyme, yielding cDNAs with sticky ends (red letters); these are incorporated into λ phage cloning vectors, and the resulting recombinant λ virions are plated on a lawn of *E. coli* cells .



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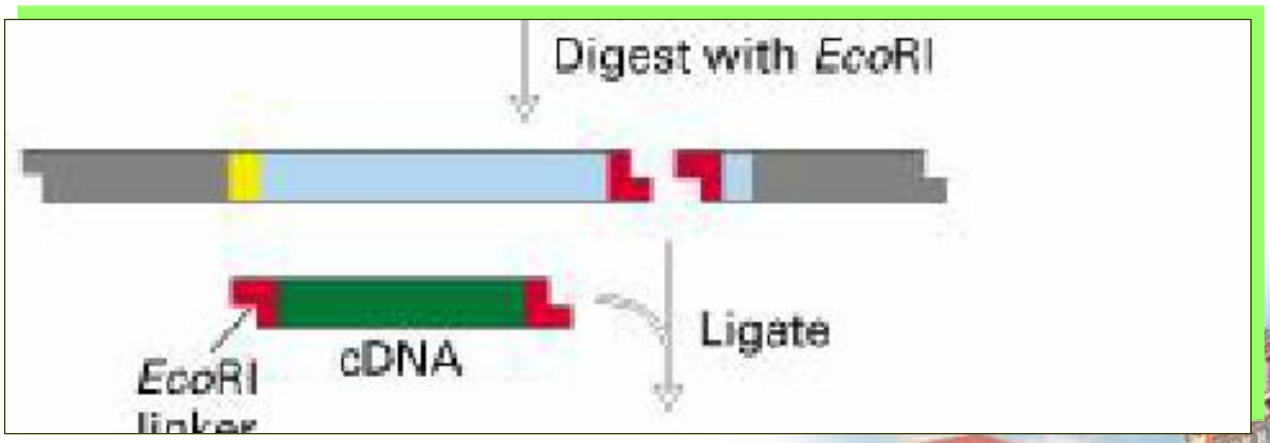
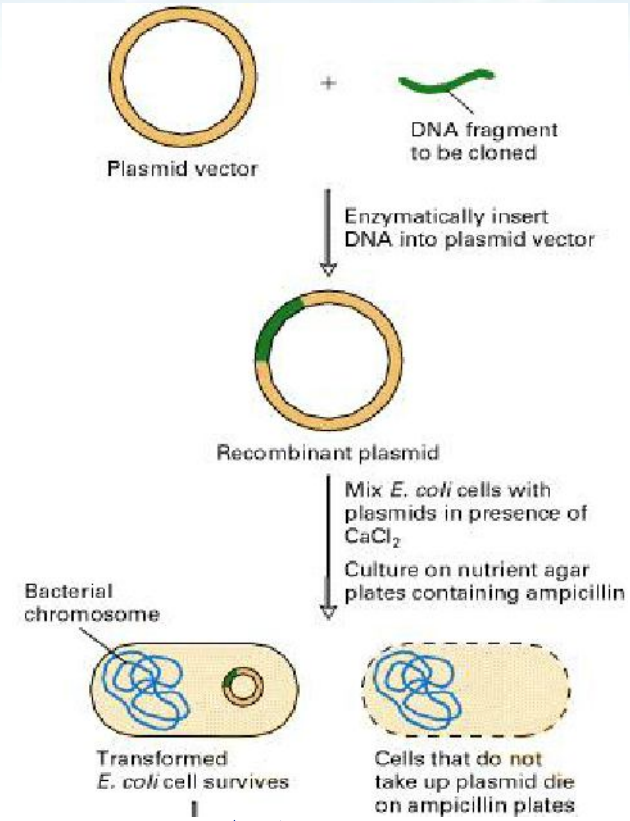
cDNA与载体相连

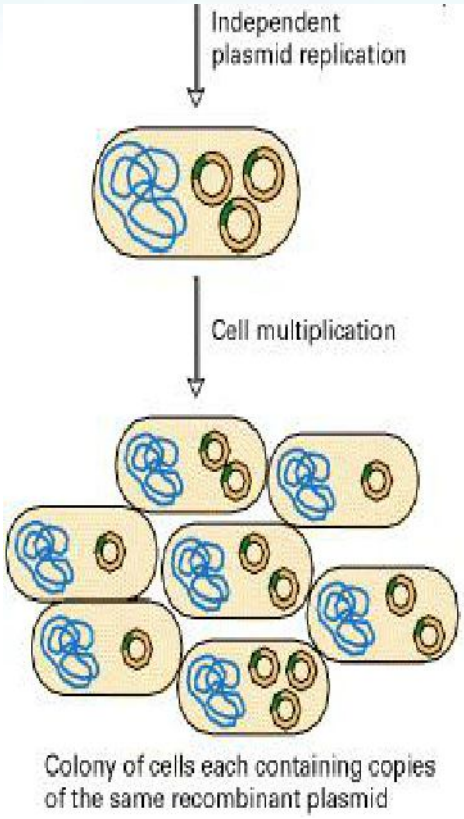
各cDNA各自与一个载体在T_4连接酶作用下以粘末端互相连接，即为重组DNA分子。



导入宿主细胞

重组体在一定条件下导入宿主细胞培养或保存。





筛选目的基因

- **核酸杂交法：**

cDNA克隆（菌斑或噬菌斑）

硝酸纤维素膜

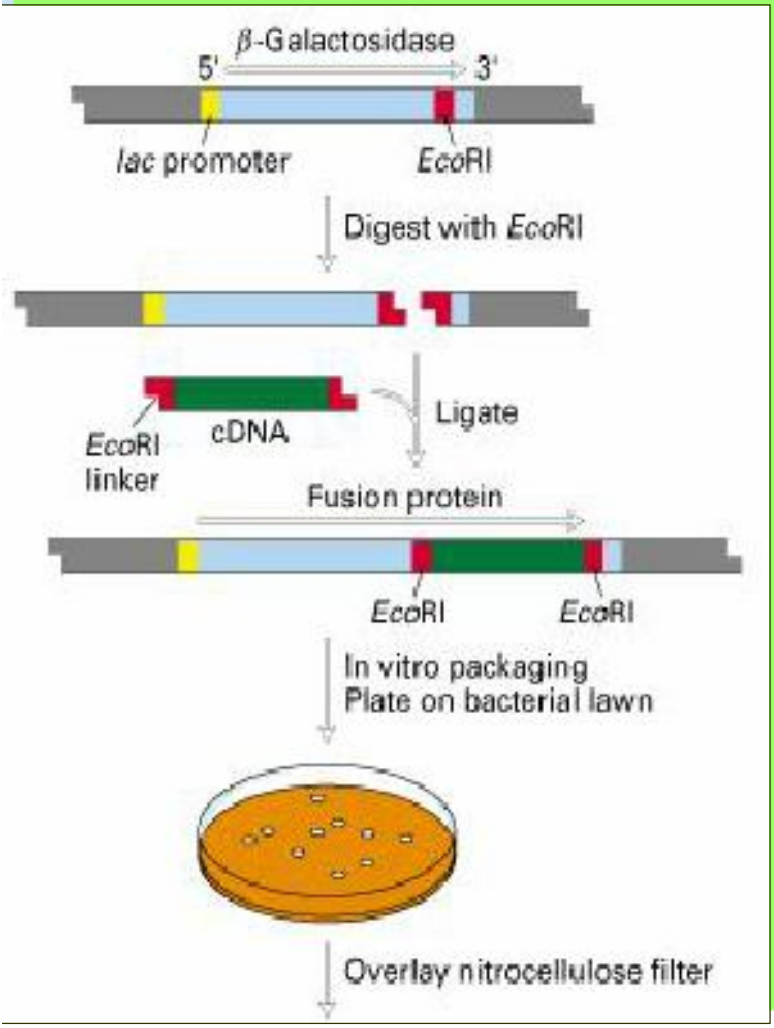
杂交（DNA探针）

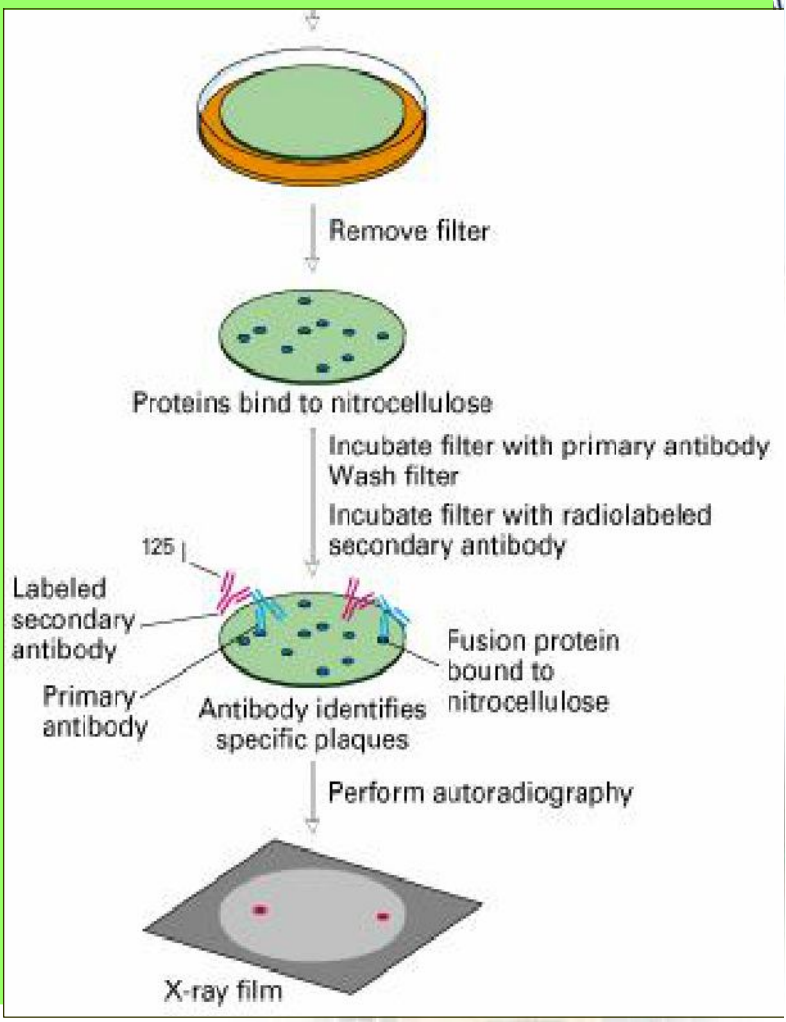
显影定位

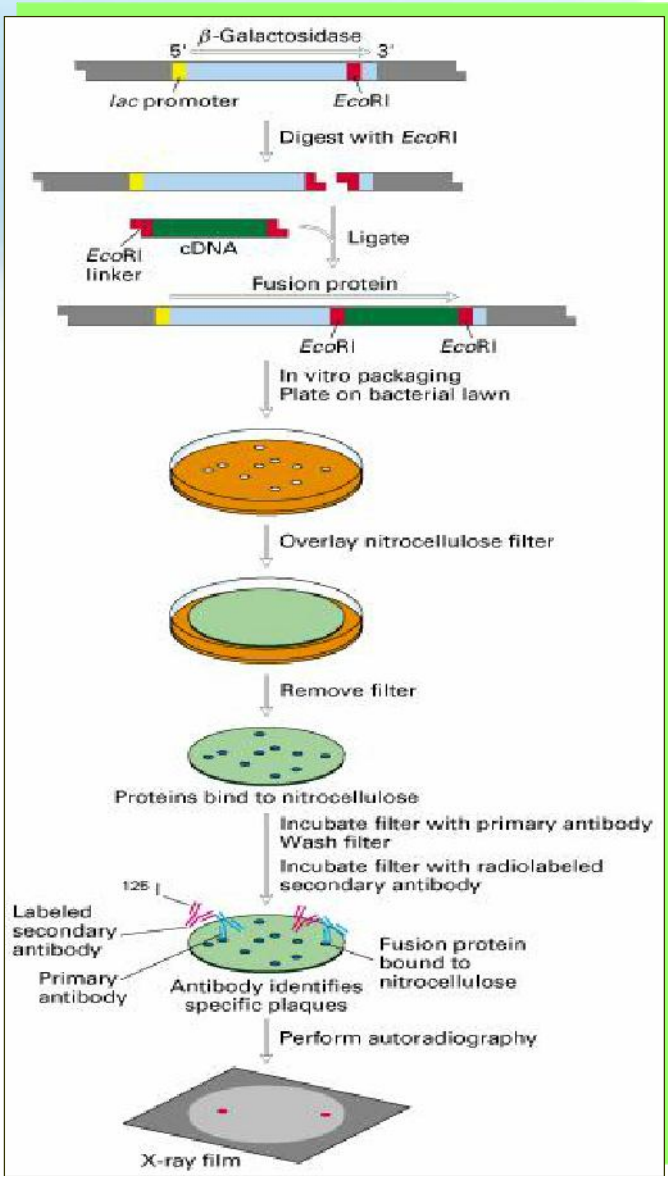
挑选

培养扩增

纯化


β-Galactosidase
5'
3'
lac promoter
EcoRI
Digest with EcoRI
EcoRI linker
cDNA
Ligate
Fusion protein
EcoRI
EcoRI
In vitro packaging
Plate on bacterial lawn
Overlay nitrocellulose filter


Remove filter
Proteins bind to nitrocellulose
Incubate filter with primary antibody
Wash filter
Incubate filter with radiolabeled secondary antibody
125
Labeled secondary antibody
Primary antibody
Antibody identifies specific plaques
Fusion protein bound to nitrocellulose
Perform autoradiography
X-ray film



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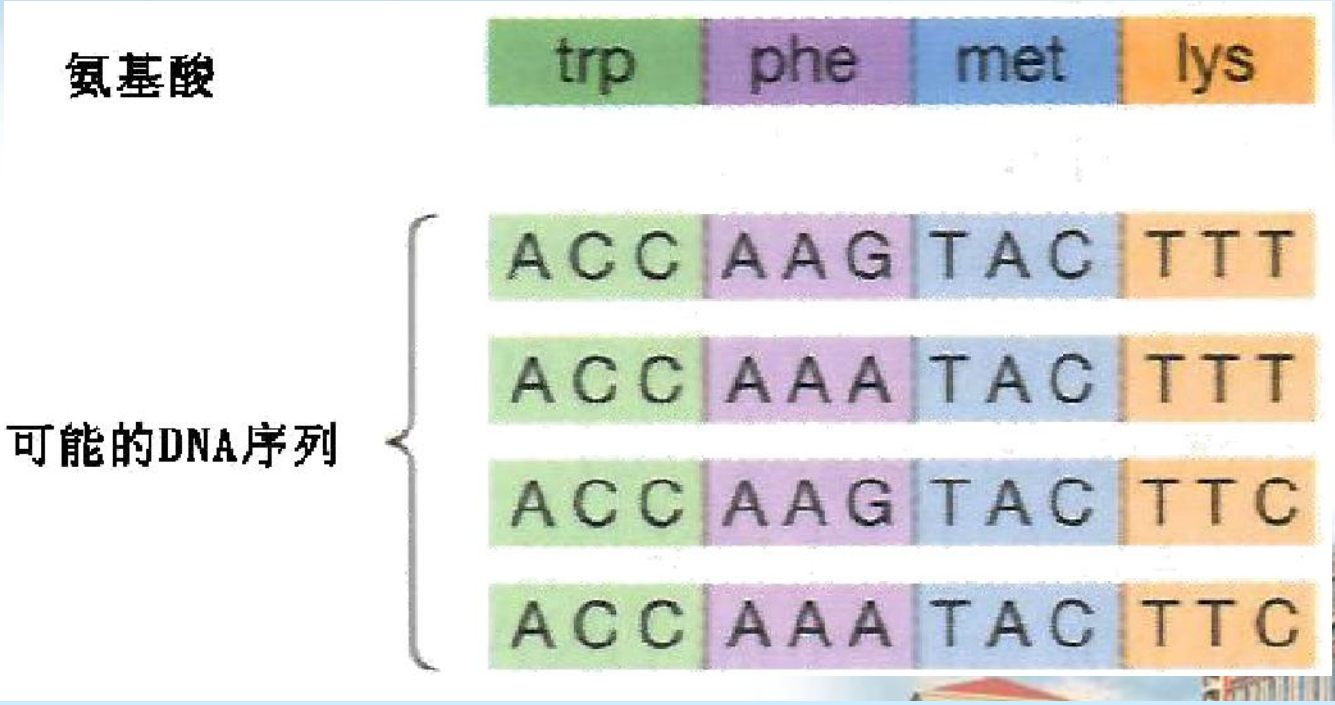
三、人工合成目的基因DNA片段

人工合成
Synthesize DNA in vitro

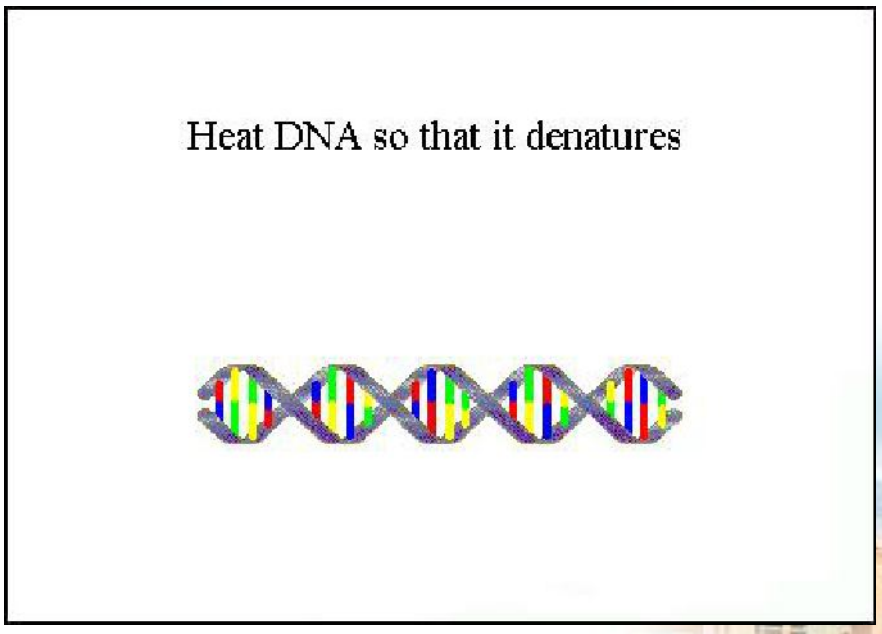


要求：已知目的基因的核苷酸序列或其产物的氨基酸序列 。

一般用于小分子基因的合成

四、聚合酶链反应合成DNA片段

（见PCR章）



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第二节 目的基因序列测定

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一、双脱氧链终止法（Sanger法）
The dideoxy chain termination method of DNA sequencing

- 原理(principle)

DNA链中的戊糖在3’位碳原子上有-OH基团，在DNA合成、链的延长过程中，新掺入的脱氧核苷酸5’-p与前一核苷酸的戊糖3’-OH以磷酸二酯键相连。

Sanger法是在反应体系中加入2’,3’-ddNTP，由于其没有3’-OH而不能与下一个核苷酸相连，于是DNA链的合成便终止。

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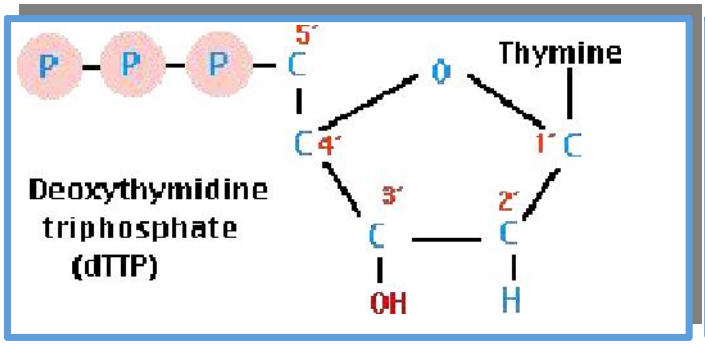
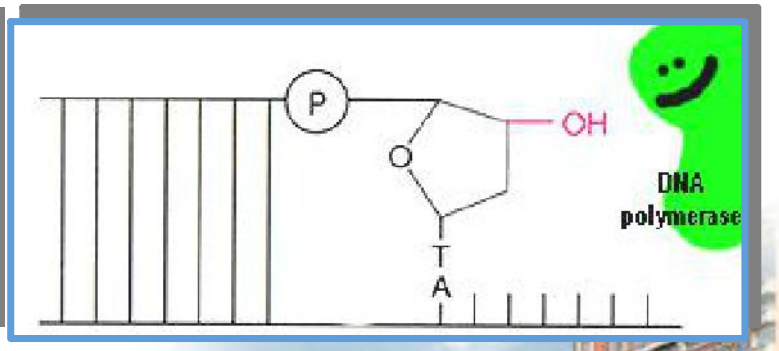
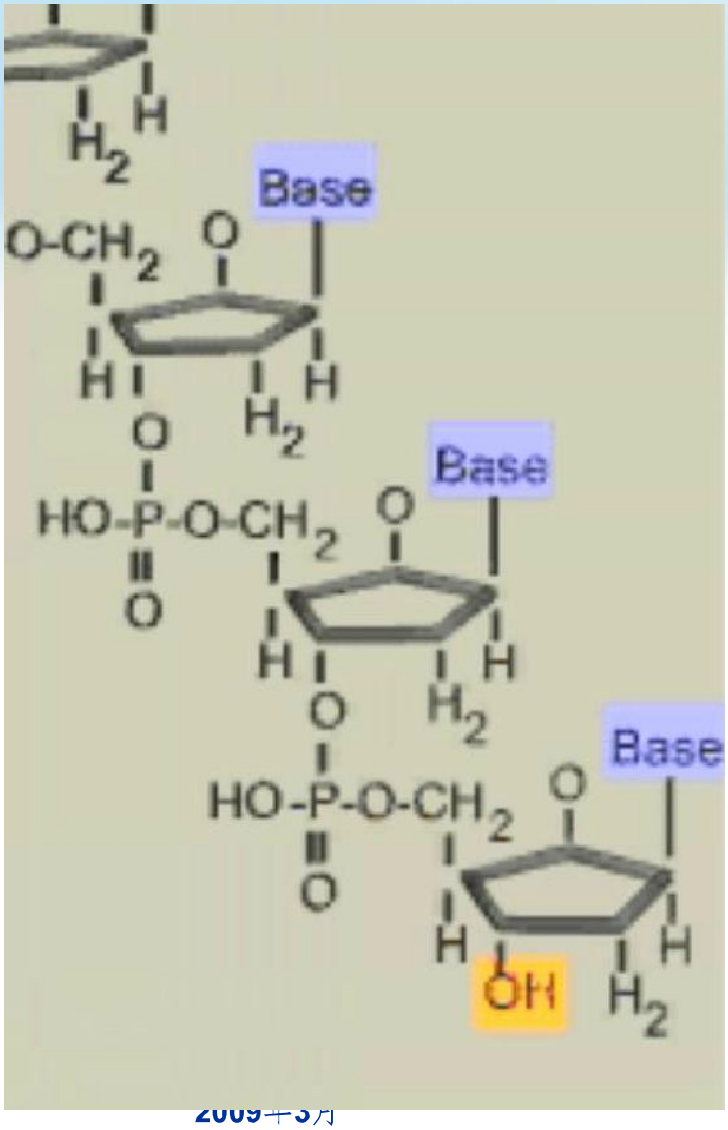
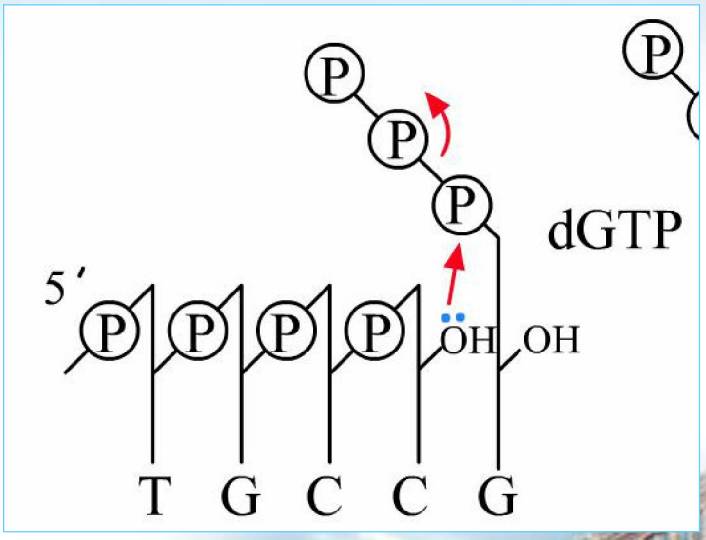
- Sanger法是在反应体系中加入2', 3' -ddNTP，由于其没有3'-OH而不能与下一个核苷酸相连，于是DNA链的合成便终止。









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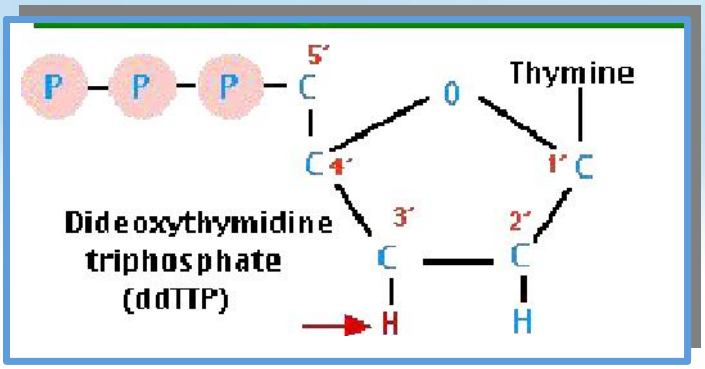

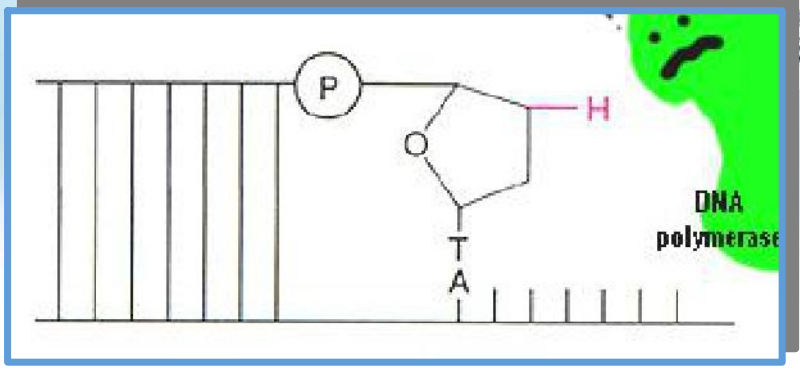

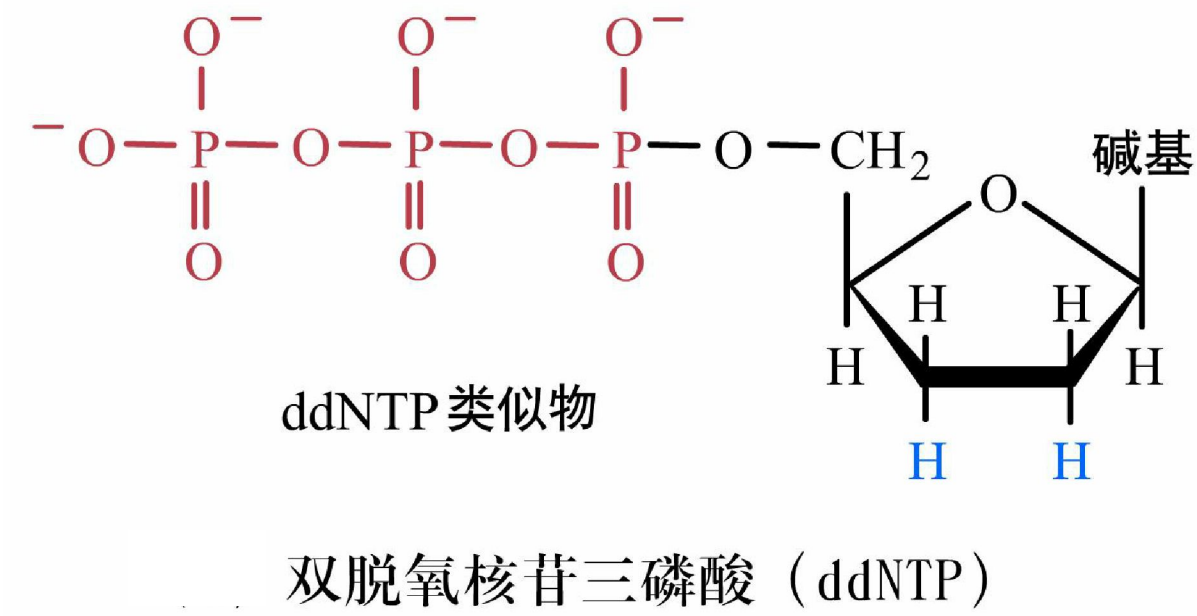

ddNTP类似物

双脱氧核苷三磷酸（ddNTP）

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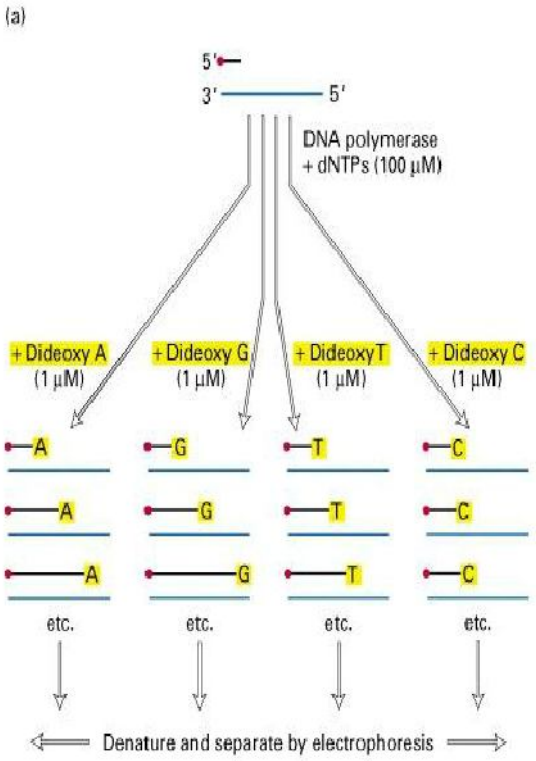
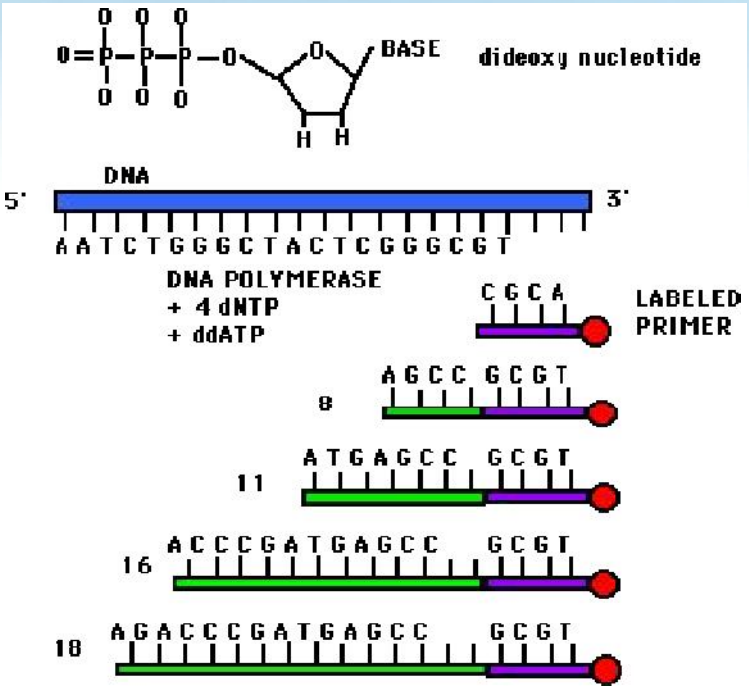
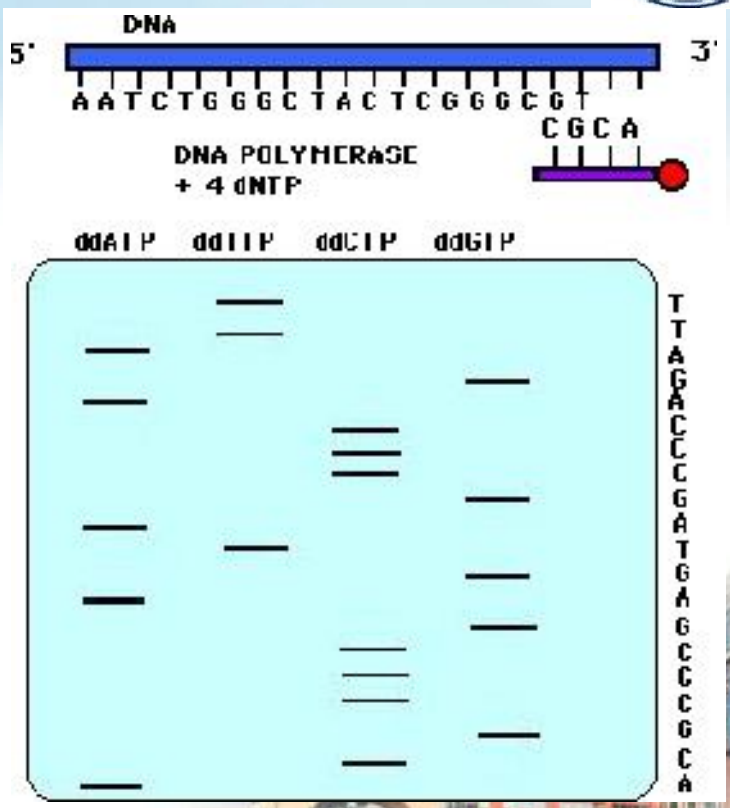


A single strand of the DNA to be sequenced is hybridized to a 5′ -end-labeled synthetic deoxyribonucleotide primer. The primer is elongated in four separate reaction mixtures containing the four normal deoxyribonucleoside triphosphates (dNTPs) plus one of the four dideoxyribonucleoside triphosphates (ddNTPs) in a ratio of 100 to 1. A ddNTP molecule can add at the position of the corresponding normal dNTP, but when this occurs, chain elongation stops because the ddNTP lacks a 3′ hydroxyl. In time, each reaction mixture will contain a mixture of prematurely terminated chains ending at every occurrence of the ddNTP (yellow).





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5′ GAGTCACACTTGAC——3′ 待测单链DNA

3′ CTG— 5′ 引物、Klenow酶、dATP 、 dGTP 、 dCTP、dTTP 和少量α -^{32}P-dATP

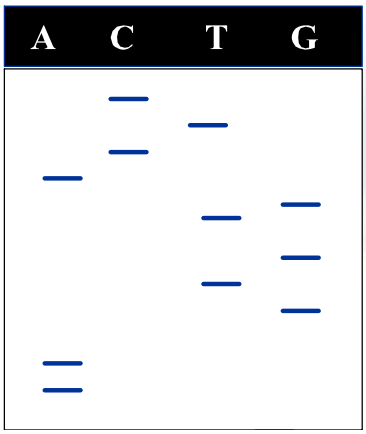
加ddATP反应
3'ACTG— 5'
3'AACTG— 5'
3'AGTGTGAACTG 5'

加ddCTP反应
3'CAGTGTGAACTG— 5'
3'CTCAGTGTGAACTG 5'

加ddTTP反应
3'TGAACTG— 5'
3'TGTGAACTG— 5'
3'TCAGTGTGAACTG5'

加ddGTP反应
3'GAACTG— 5'
3'GTGAACTG— 5'
3'GTGTGAACTG 5'

变性凝胶电泳、放射自显影



GAGTCACACTT

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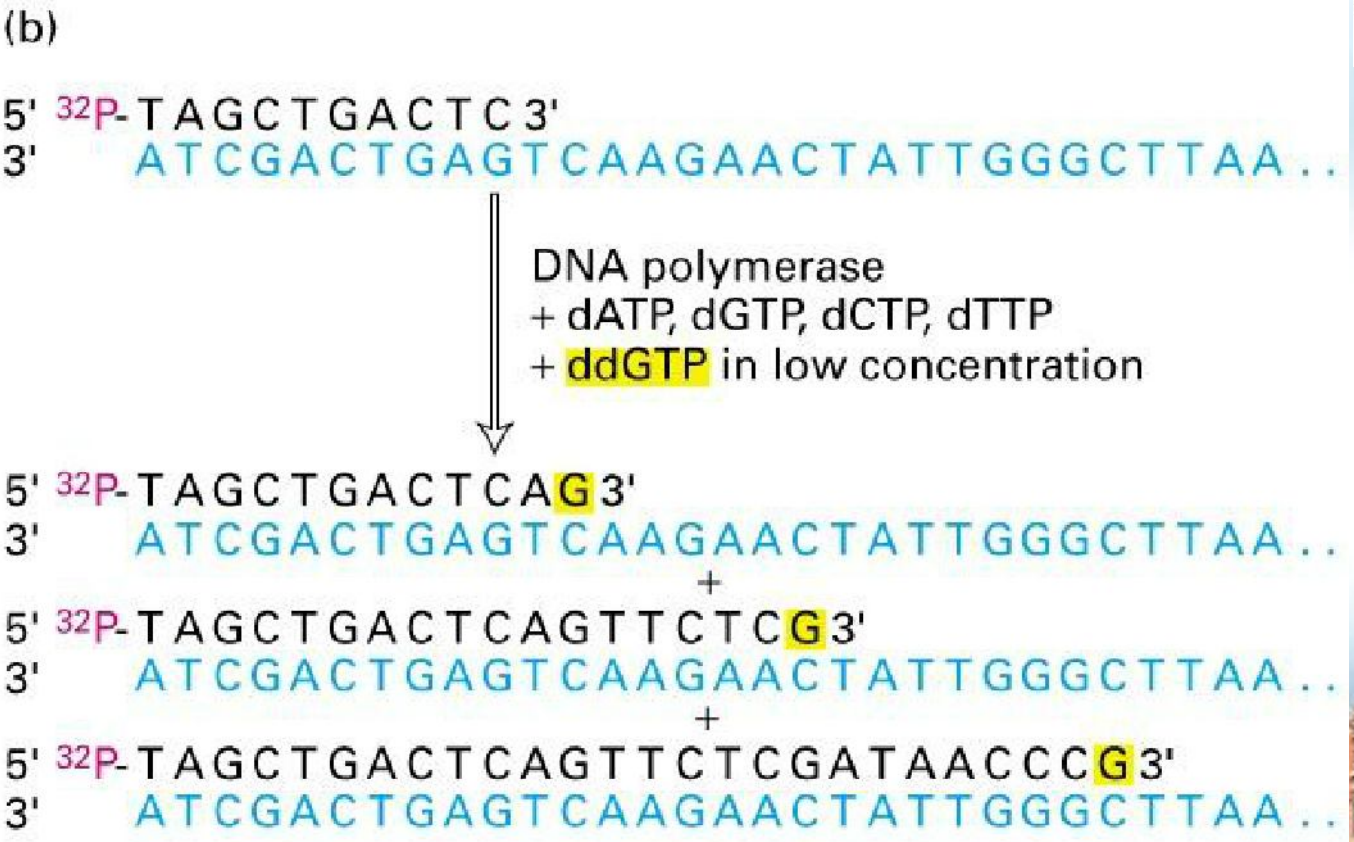
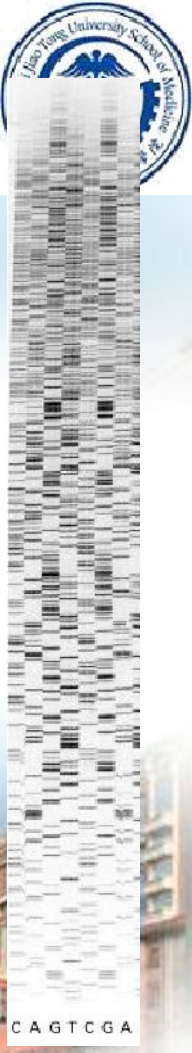
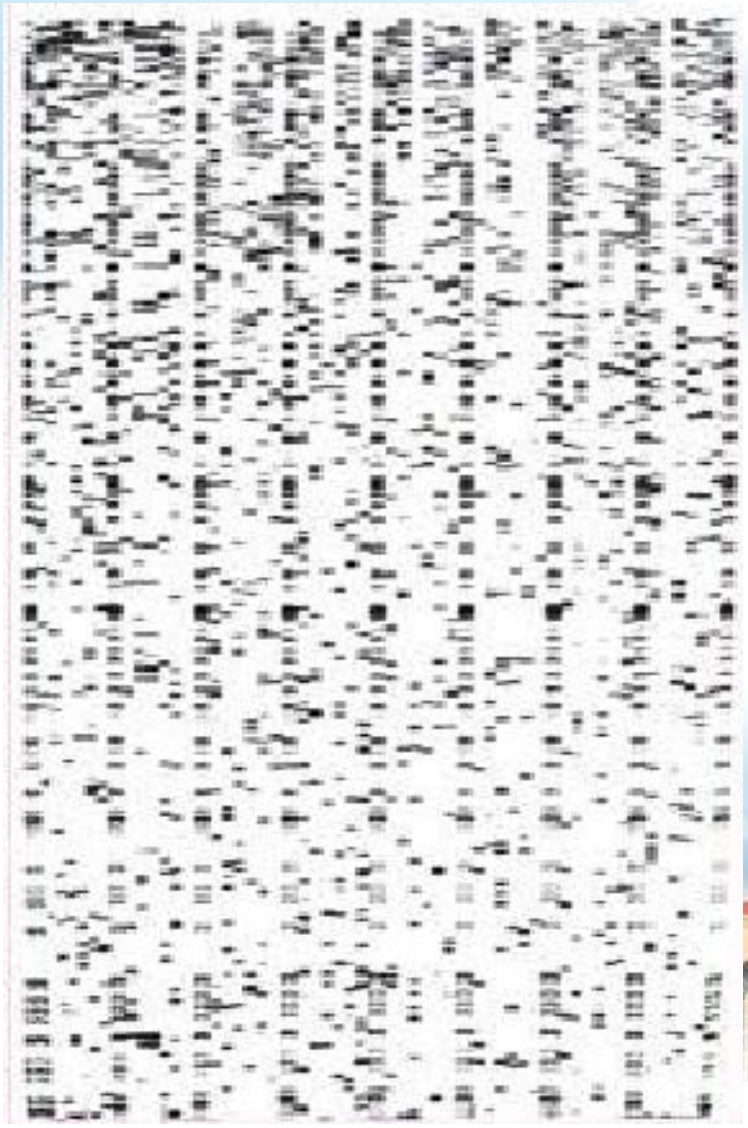

(b)
5' 32P-TAGCTGACTC 3'
3' ATCGACTGAGTCAAGAACTATTGGGCTTAA..
DNA polymerase
+ dATP, dGTP, dCTP, dTTP
+ ddGTP in low concentration
5' 32P-TAGCTGACTCAG 3'
3' ATCGACTGAGTCAAGAACTATTGGGCTTAA..
+
5' 32P-TAGCTGACTCAGTTCTCG 3'
3' ATCGACTGAGTCAAGAACTATTGGGCTTAA..
+
5' 32P-TAGCTGACTCAGTTCTCGATAACCCG 3'
3' ATCGACTGAGTCAAGAACTATTGGGCTTAA..


CAGTCGA



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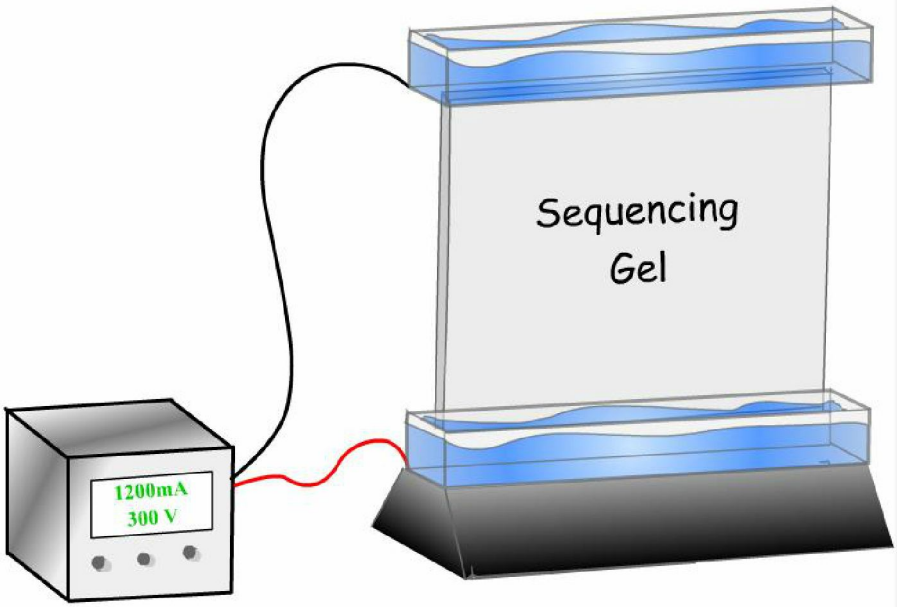

The chain-terminator, or dideoxy method of DNA sequencing

Click play to begin

BACK PLAY PAUSE REPLAY NEXT

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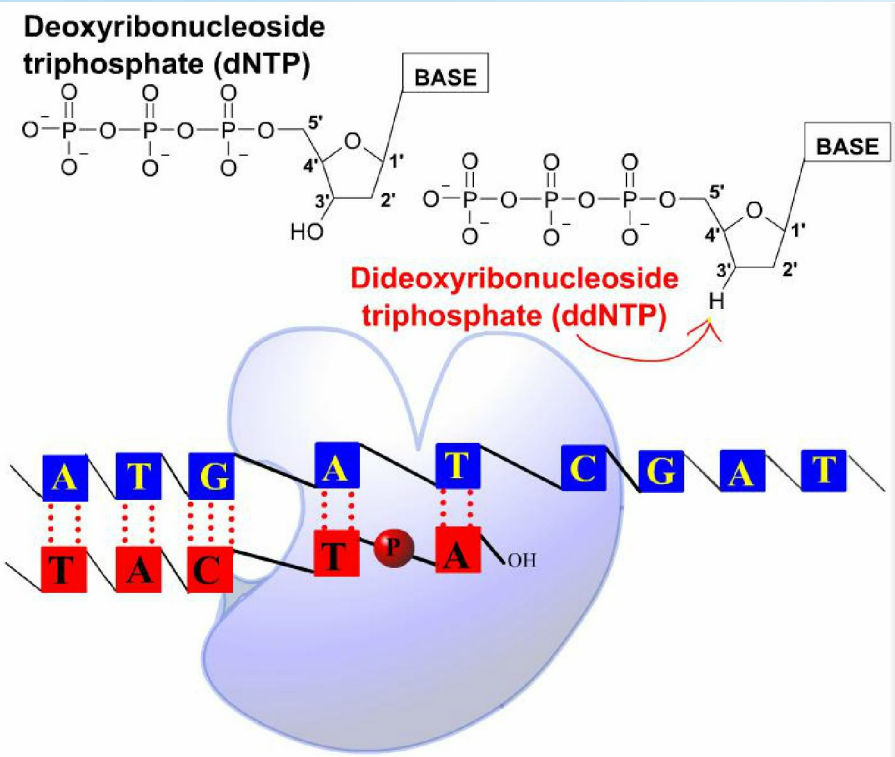
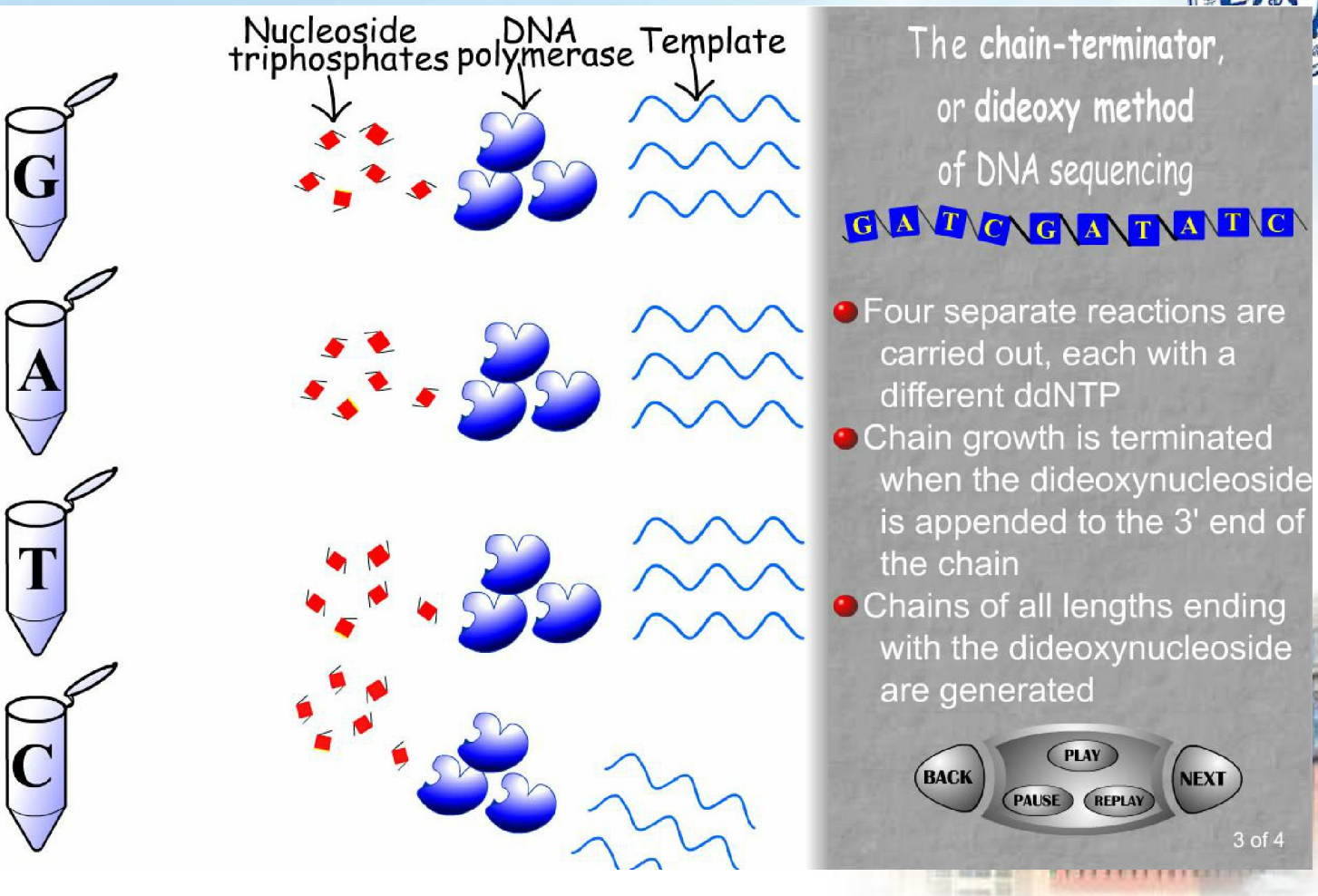


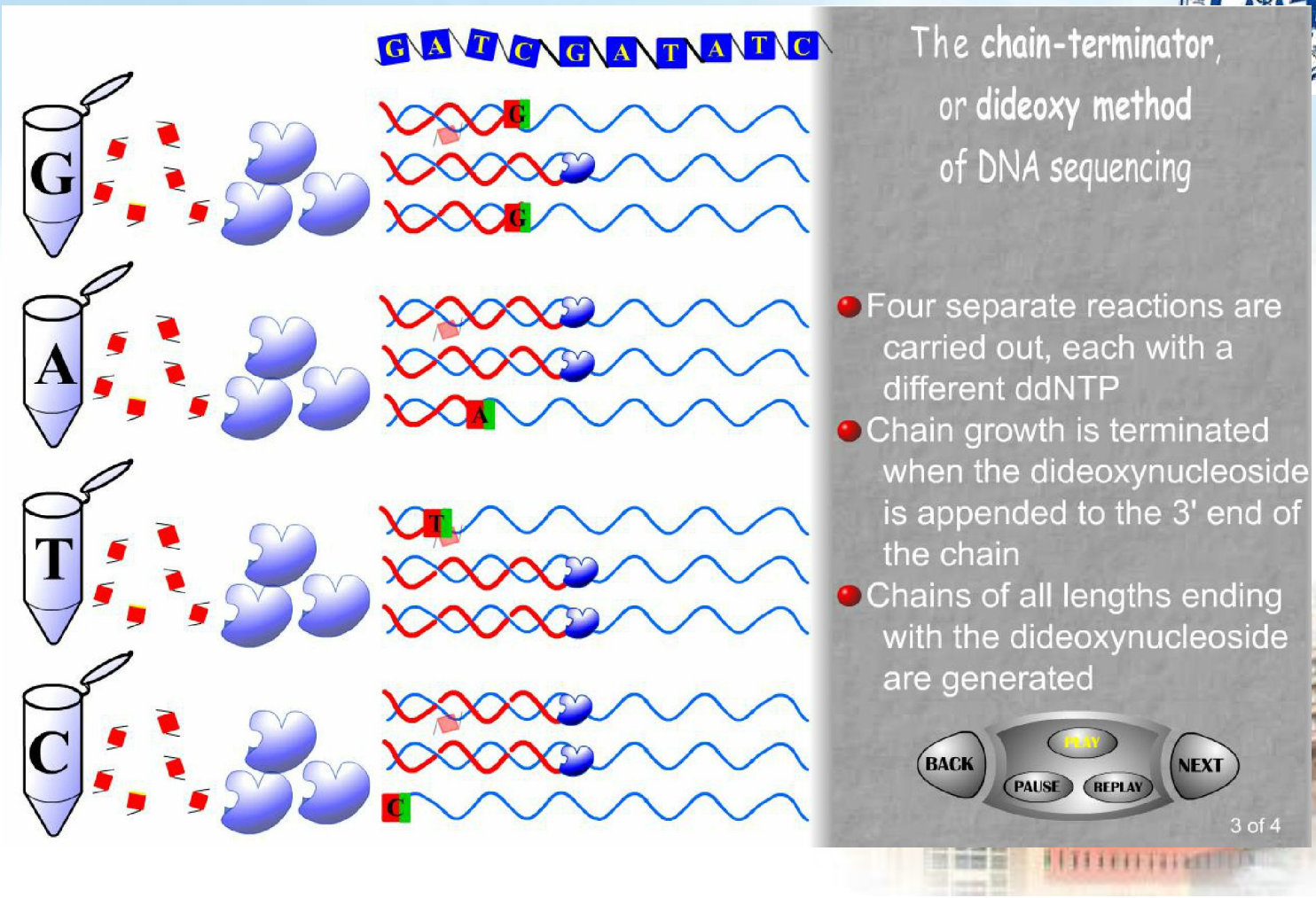
The chain-terminator, or dideoxy method of DNA sequencing

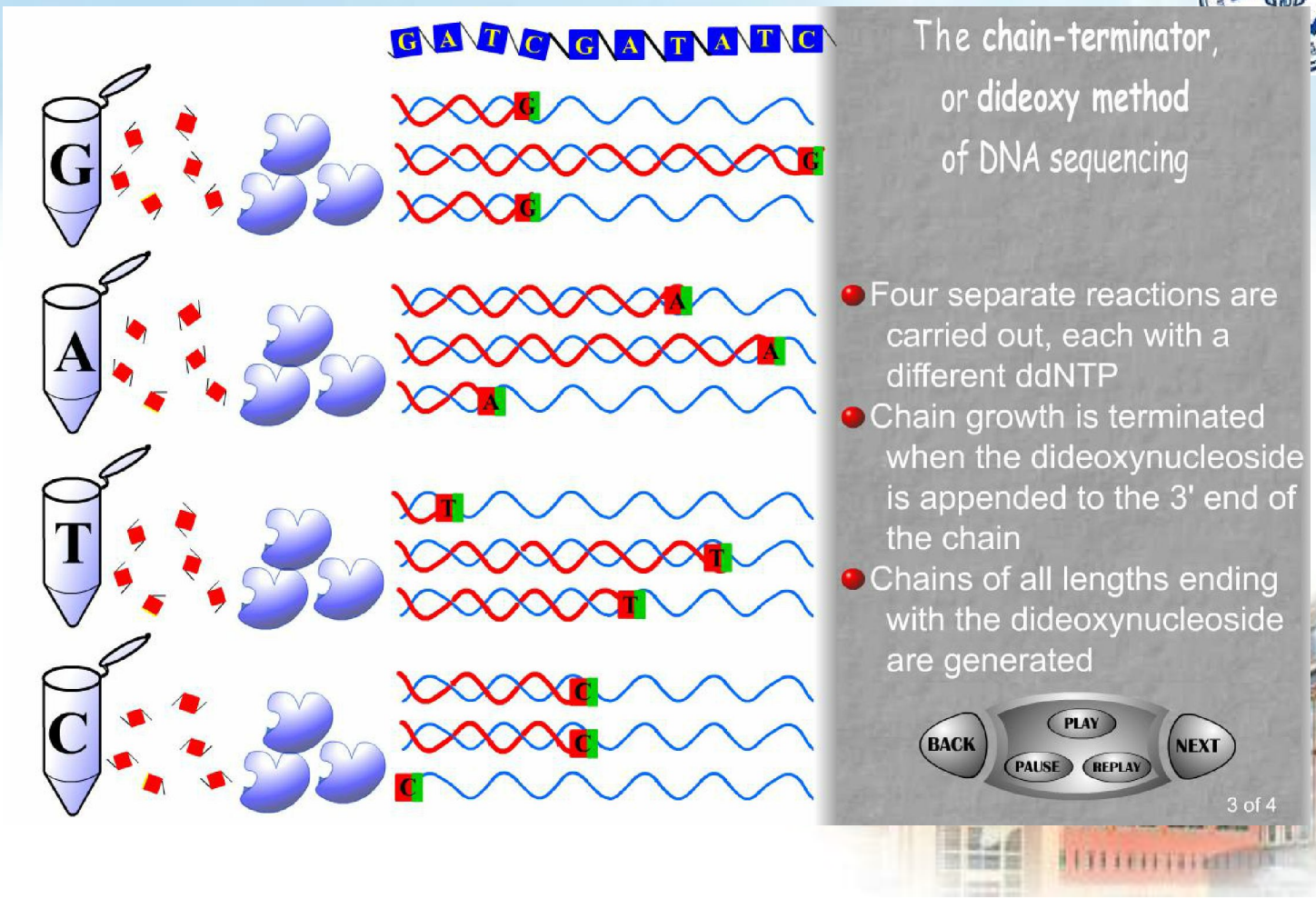
- Dideoxynucleoside triphosphates (ddNTPs) are introduced into the reaction mixture
- Dideoxynucleotides lack 3'-hydroxyl groups
- Dideoxynucleotides terminate the growth of DNA chains

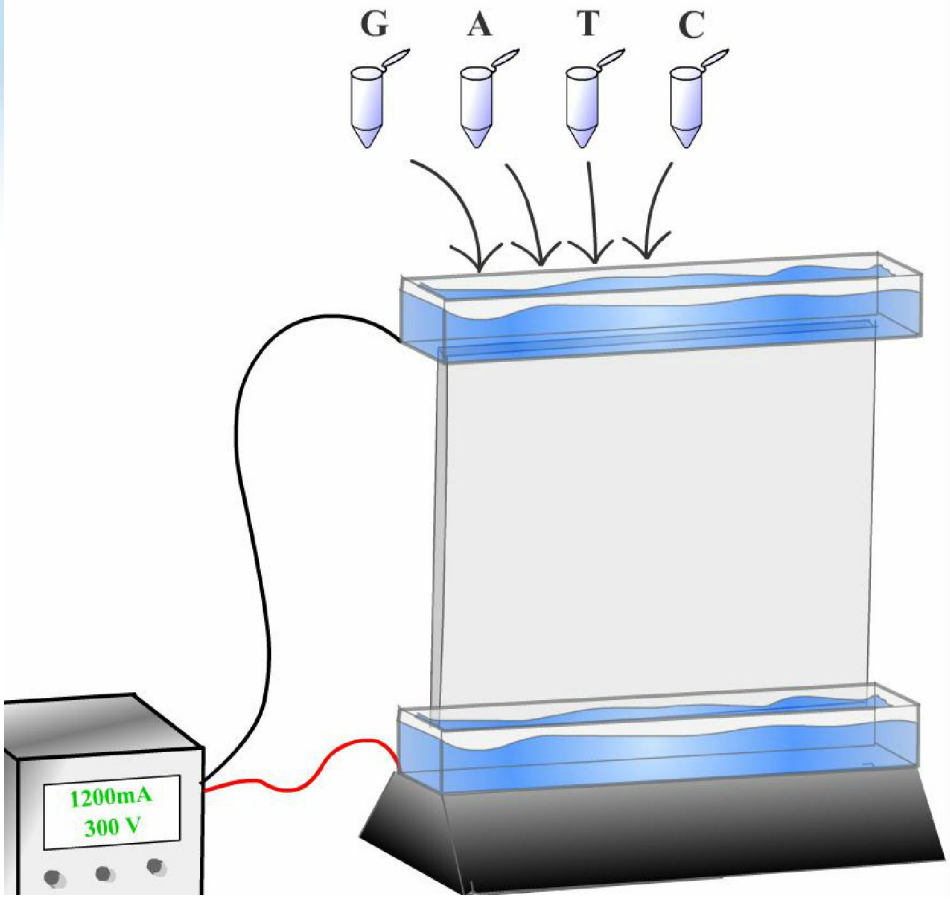
BACK PLAY PAUSE REPLAY NEXT

2 of 4


Nucleoside triphosphates
DNA polymerase
Template
G
A
T
C
The chain-terminator, or dideoxy method of DNA sequencing
G A T C G A T A T C
Four separate reactions are carried out, each with a different ddNTP
Chain growth is terminated when the dideoxynucleoside is appended to the 3' end of the chain
Chains of all lengths ending with the dideoxynucleoside are generated
BACK
PLAY
PAUSE
REPLAY
NEXT
3 of 4


G A T C G A T A T C
G
A
T
C
The chain-terminator, or dideoxy method of DNA sequencing
Four separate reactions are carried out, each with a different ddNTP
Chain growth is terminated when the dideoxynucleoside is appended to the 3' end of the chain
Chains of all lengths ending with the dideoxynucleoside are generated
BACK
PLAY
PAUSE
REPLAY
NEXT
3 of 4


G A T C G A T A T C
G
A
T
C
The chain-terminator, or dideoxy method of DNA sequencing
Four separate reactions are carried out, each with a different ddNTP
Chain growth is terminated when the dideoxynucleoside is appended to the 3' end of the chain
Chains of all lengths ending with the dideoxynucleoside are generated
BACK
PLAY
PAUSE
REPLAY
NEXT
3 of 4

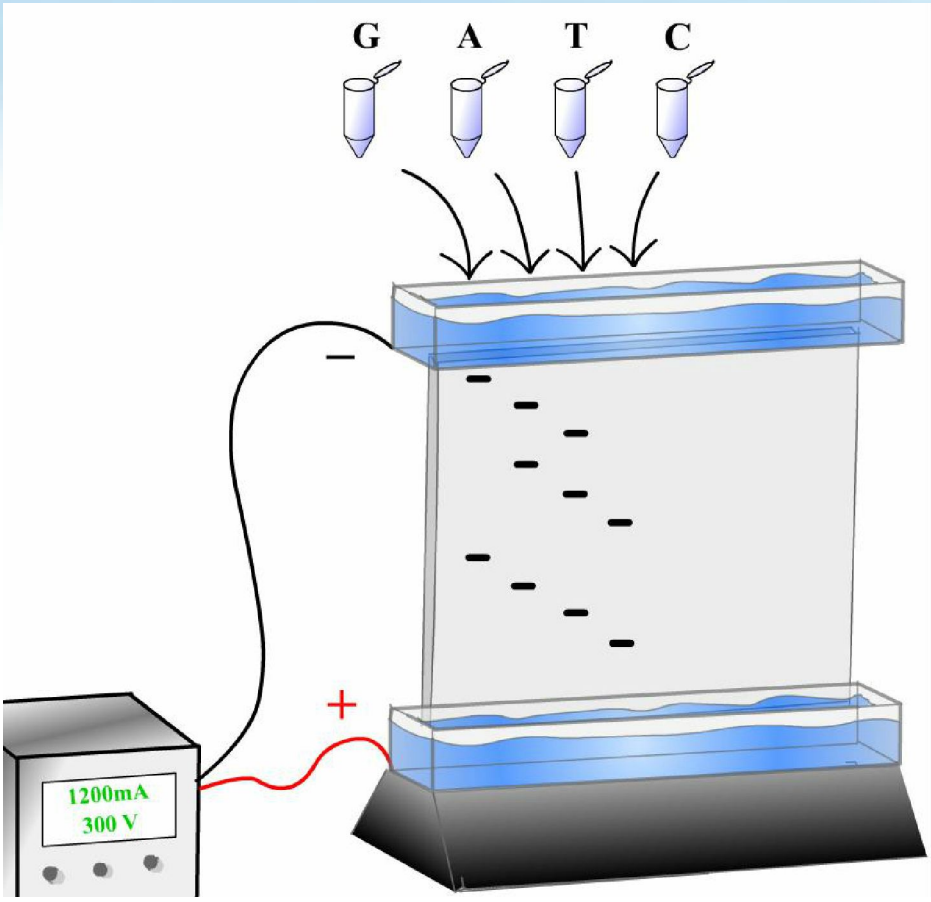


The chain-terminator, or dideoxy method of DNA sequencing

- The DNA is separated by size using gel electrophoresis
- The bands are visualized by radioactively or fluorescently labeling one of the dNTPs or the primer
- Short chains migrate faster than long chains producing a ladder of DNA bands
- The sequence is read bottom to top

BACK PLAY PAUSE REPLAY NEXT

4 of 4

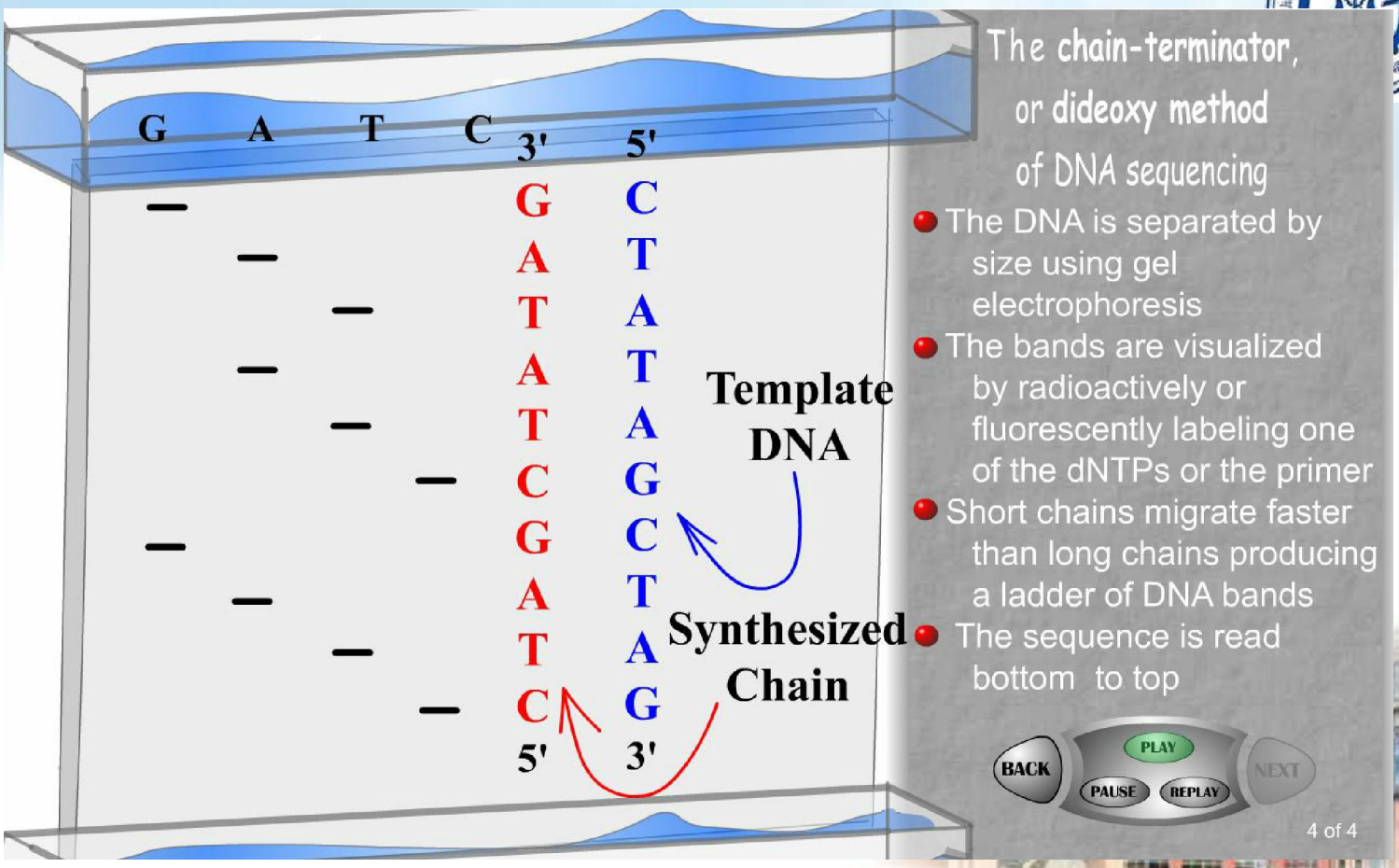


The chain-terminator, or dideoxy method of DNA sequencing

- The DNA is separated by size using gel electrophoresis
- The bands are visualized by radioactively or fluorescently labeling one of the dNTPs or the primer
- Short chains migrate faster than long chains producing a ladder of DNA bands
- The sequence is read bottom to top

BACK PLAY PAUSE REPLAY NEXT

4 of 4

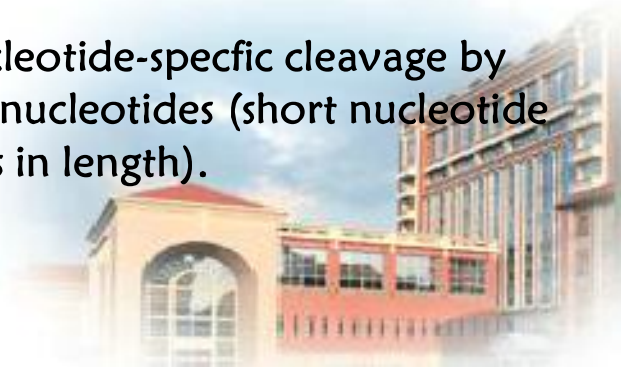

G A T C
3' 5'
G C
A T
T A
A T
T A
C G
G C
A T
T A
C G
5' 3'
Template DNA
Synthesized Chain
The chain-terminator, or dideoxy method of DNA sequencing
The DNA is separated by size using gel electrophoresis
The bands are visualized by radioactively or fluorescently labeling one of the dNTPs or the primer
Short chains migrate faster than long chains producing a ladder of DNA bands
The sequence is read bottom to top
BACK
PLAY
PAUSE
REPLAY
NEXT
4 of 4

二、化学裂解法（Maxam-Gilbert法）

- 原理

使特定的碱基首先被化学修饰，然后再经化学处理。如：用硫酸二甲酯使鸟嘌呤甲基化，然后再加哌啶，使甲基化的鸟嘌呤丢失，DNA链从鸟嘌呤修饰处后的3’，5’-磷酸二酯键断裂。

The Maxam-Gilbert method is based on nucleotide-specfic cleavage by chemicals and is best used to sequence oligonucleotides (short nucleotide polymers, usually smaller than 50 base-pairs in length).



三、自动测序仪测序法 (ABI)

自动测序仪基于2，3-双脱氧末端终止法的原理，标记系统由**放射性核素改为发特定荧光信号的荧光染料**，预先将四种荧光染料与四种双脱氧核苷三磷酸共价相连，使其带有不同的荧光标记。当某一种ddNTP掺入到正在合成的DNA单链分子中时，该链的延伸便终止并带有相应的荧光染料。

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自动测序系统

电泳分离系统

荧光检测装置

计算机成像系统

分析软件

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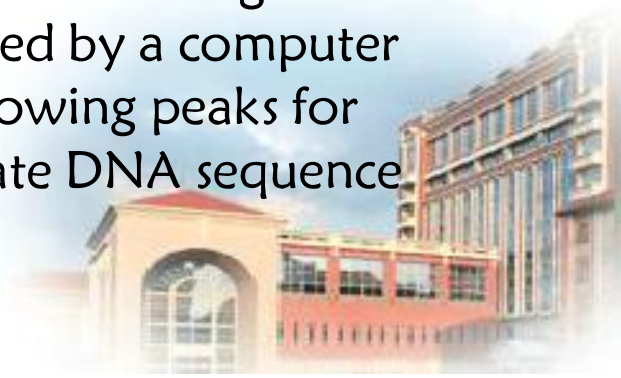
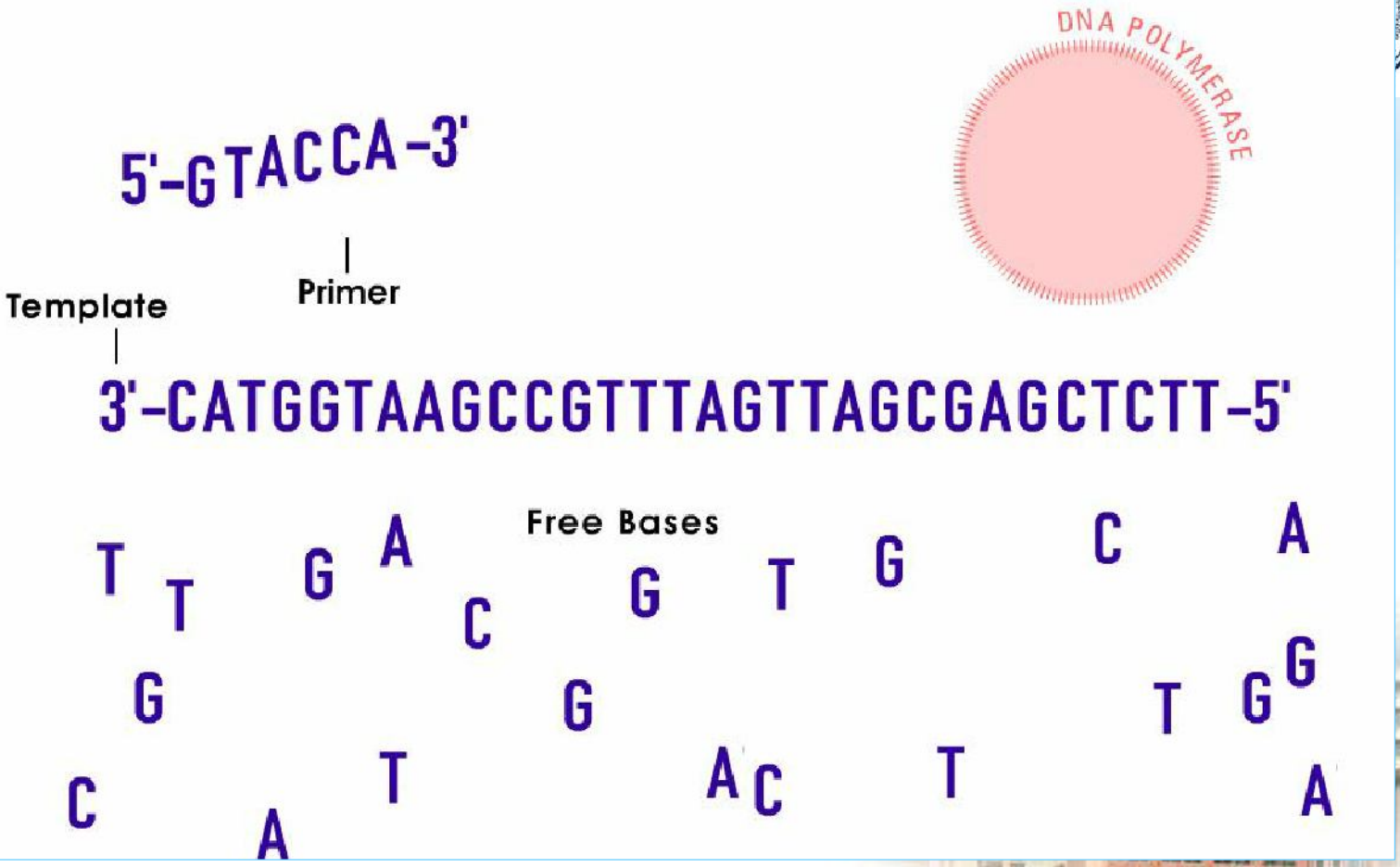
- In the automated Sanger reaction, primers are used that are labeled with four different coloured fluorescent tags.
- PCR reactions, in the presence of the different dideoxy nucleotides, are performed as described above.
- the four reaction mixtures are then combined and applied to a single lane of a gel.
- The colour of each fragment is detected using a laser beam and the information is collected by a computer which generates chromatograms showing peaks for each colour, from which the template DNA sequence can be determined.





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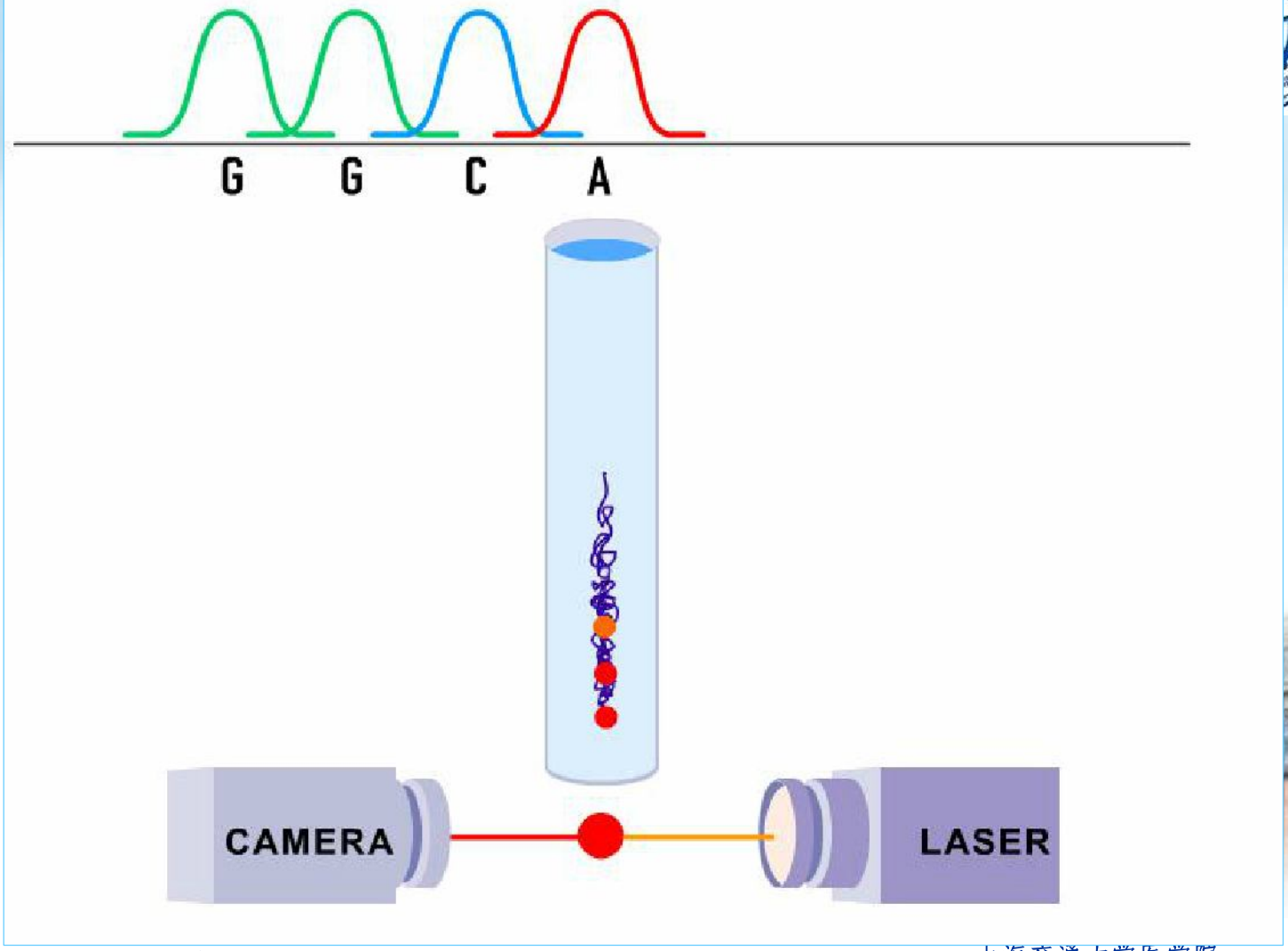
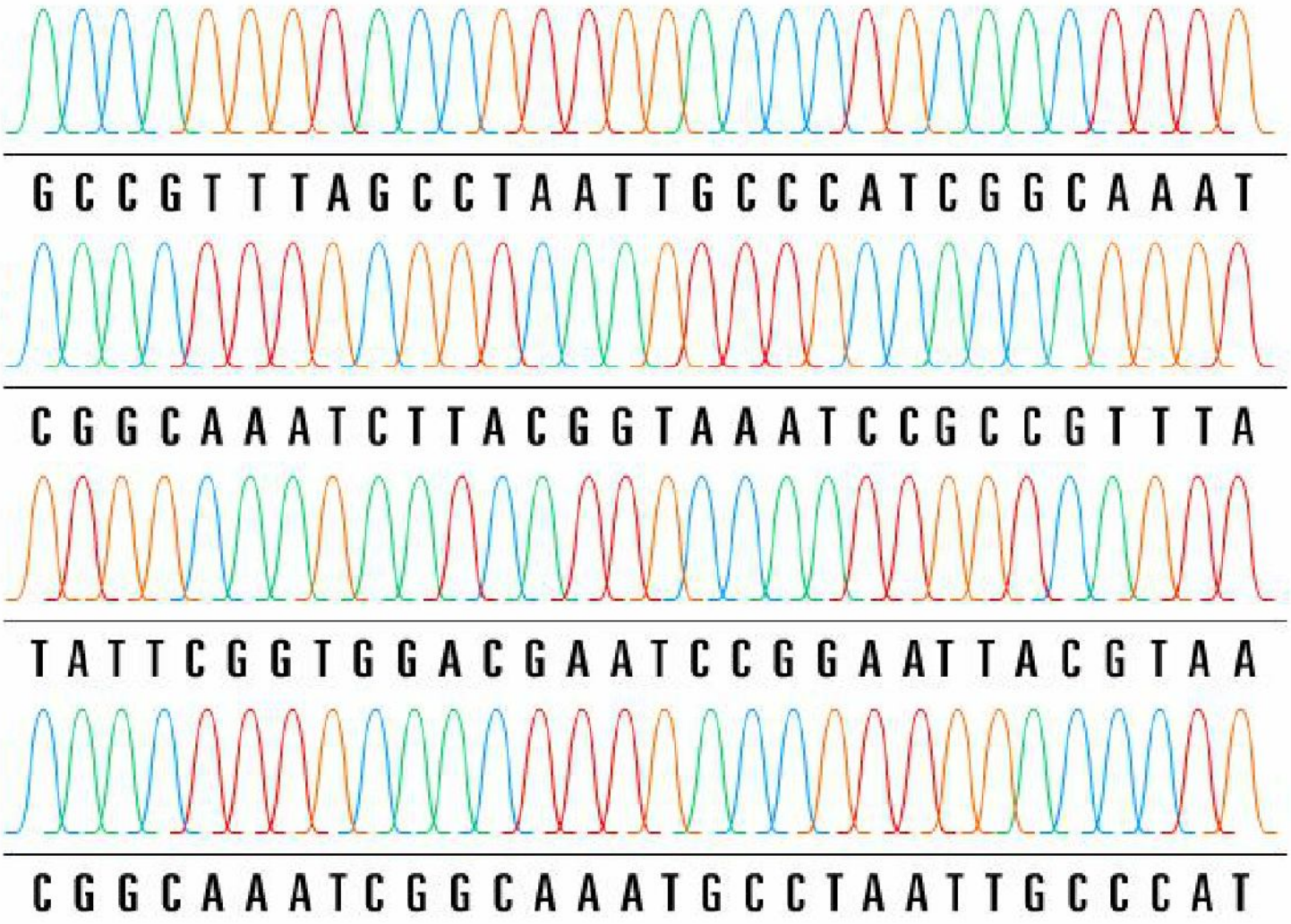

G
G
C
A
CAMERA
LASER


GCCGTTTAGCCTAATTGCCCATCGGCAAAT
CGGCAAATCTTACGGTAAATCCGCCGTTTA
TATTCGGTGGACGAATCCGGAATTACGTAA
CGGCAAATCGGCAAATGCCTAATTGCCCAT

ABI PRISM™

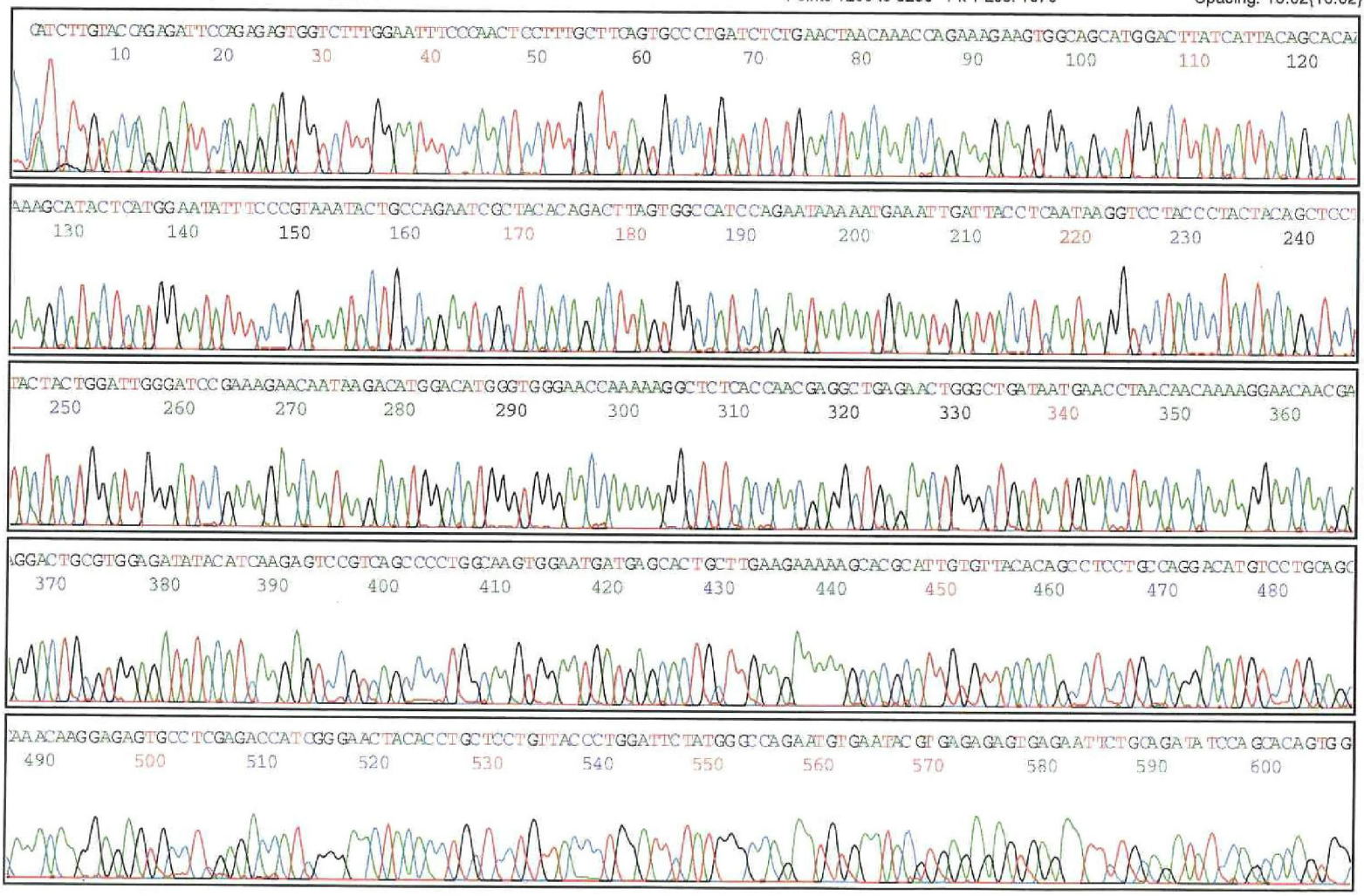
Model 377
Version 3.3
SemiAdaptive
Version 3.2

GC8000.LIE-6.PL1(+)

GC8000.LIE-6.PL1(+)
Lane 20

Signal G:245 A:362 T:225 C:398
DT {BD Set Any-Primer}
#2_dRhod Matrix
Points 1200 to 8256 Pk 1 Loc: 1070

Thu, Mar 22, 2001 9:16 AM
Wed, Mar 21, 2001 4:44 PM
Spacing: 10.02{10.02}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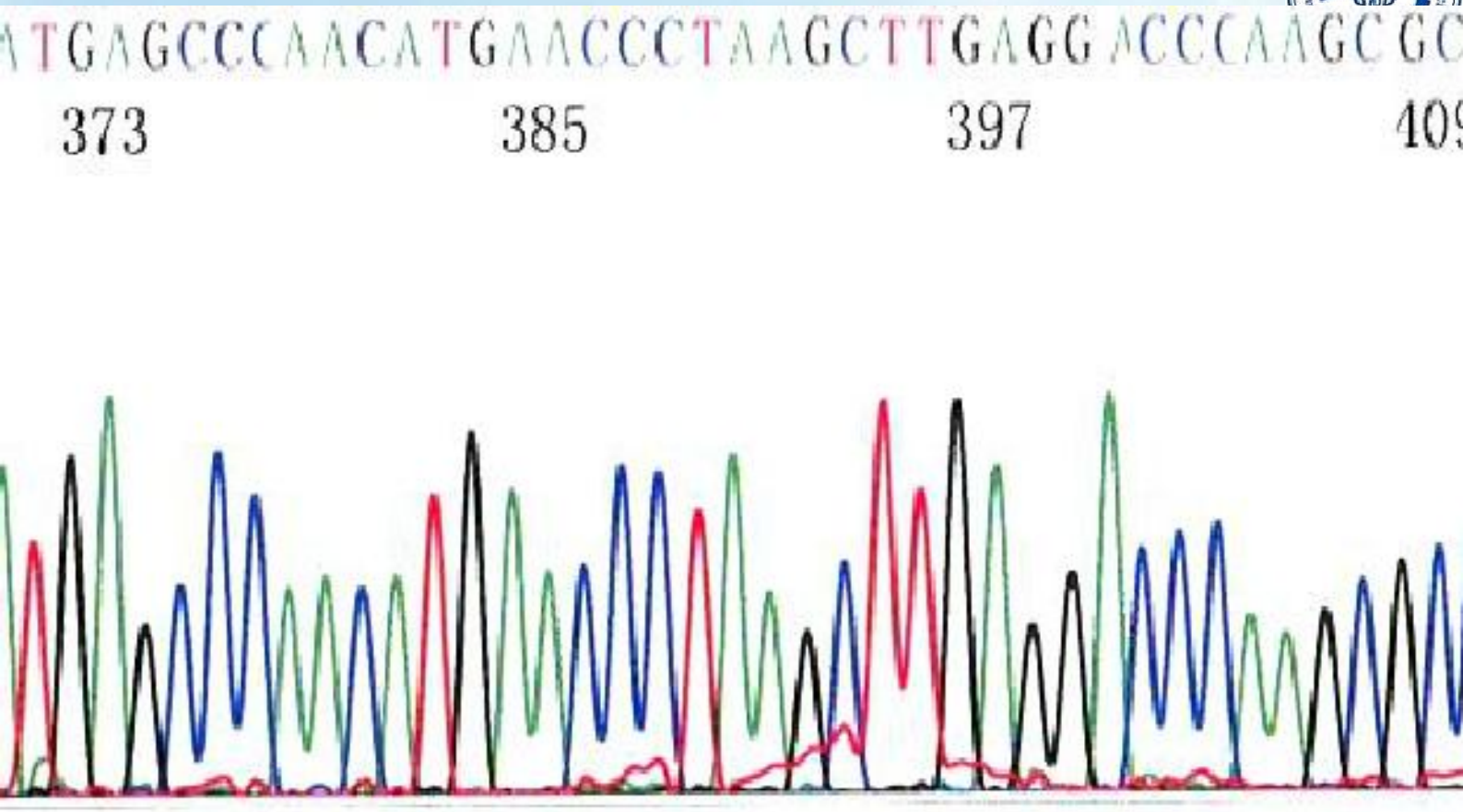
10 20 30 40 50 60 70 80 90 100 110 120
AAAGCATACTCATGGAATATTTCCCGTAAATACTGCCAGAATCGCTACACAGACTTAGTGGCCATCCAGAATAAAAATGAAATTGATTACCTCAATAAGGTCCTACCCTACTACAGCTCCT
130 140 150 160 170 180 190 200 210 220 230 240
TACTACTGGATTGGGATCCGAAAGAACAATAAGACATGGACATGGGTGGGAACCAAAAAGGCTCTCACCAACGAGGCTGAGAACTGGGCTGATAATGAACCTAACAACAAAAGGAACAACGA
250 260 270 280 290 300 310 320 330 340 350 360
AGGACTGCGTGGAGATATACATCAAGAGTCCGTCAGCCCCTGGCAAGTGGAATGATGAGCACTGCTTGAAGAAAAAGCACGCATTGTGTTACACAGCCTCCTGCCAGGACATGTCCTGCAGC
370 380 390 400 410 420 430 440 450 460 470 480
AAACAAGGAGAGTGCCTCGAGACCATCGGGAACTACACCTGCTCCTGTTACCCTGGATTCTATGGGCCAGAATGTGAATACGTGAGAGAGTGAGAATTCTGCAGATATCCAGCACAGTGG
490 500 510 520 530 540 550 560 570 580 590 600



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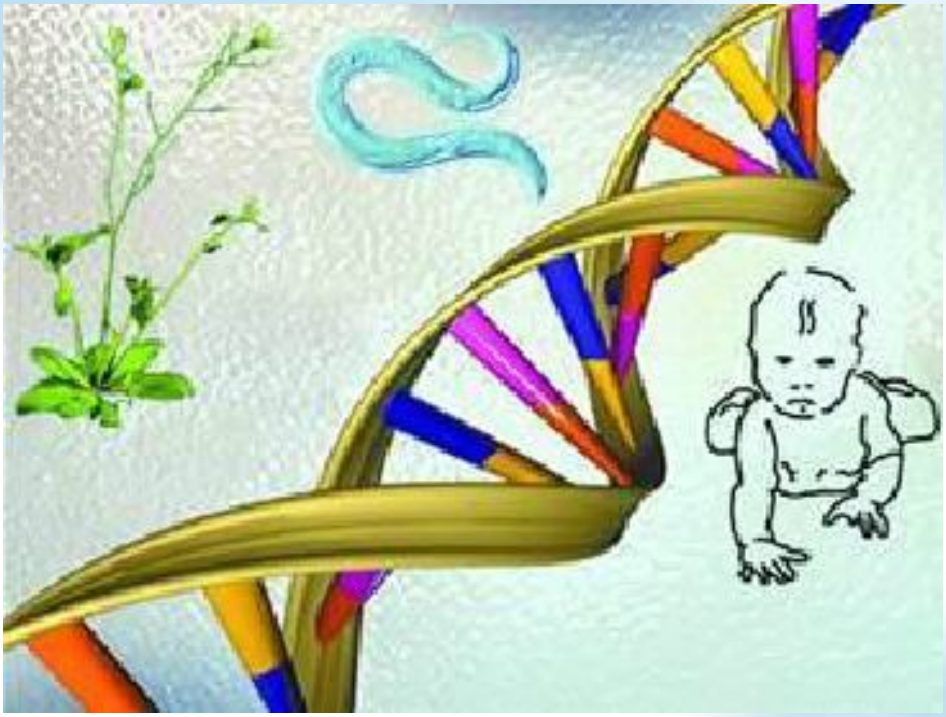

1990年7月，人类基因组计划（Human Genome Project，HGP）

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人类基因组计划(human genome project，HGP)

1996年，构建了每个标记的密度为0.6 Mb (1Mb= 一百万个碱基)的人类基因组遗传图谱，0.1 Mb的物理图谱。

2000年完成了人类基因组草图的构建，并已测定基因组的大量核苷酸序列。

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鸟枪射击法(shotgun) 基因组序列测定

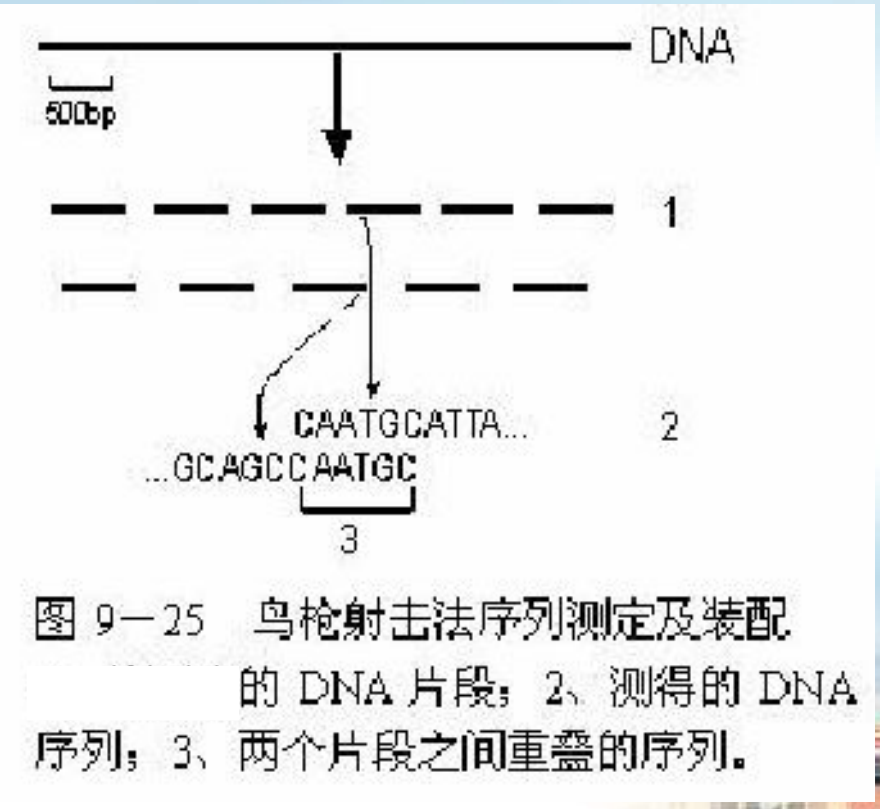
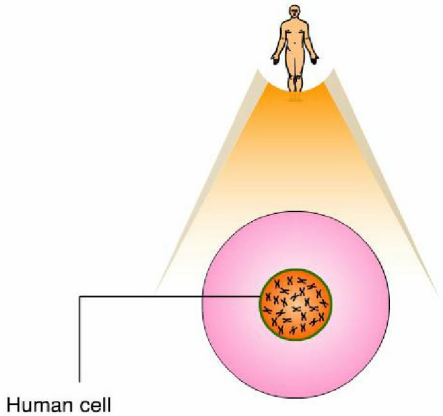


图 9—25 鸟枪射击法序列测定及装配
的 DNA 片段；2、测得的 DNA
序列；3、两个片段之间重叠的序列。

Sequencing the Human Genome



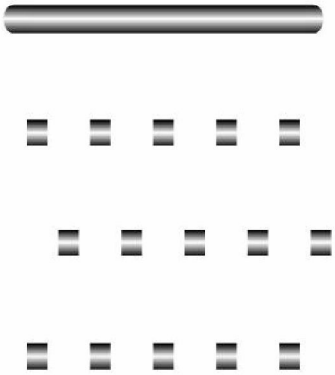
Human cell nucleus containing 23 pairs of chromosomes

Sequencing the Human Genome

23 pairs of chromosomes make up the human genome

Sequencing the Human Genome

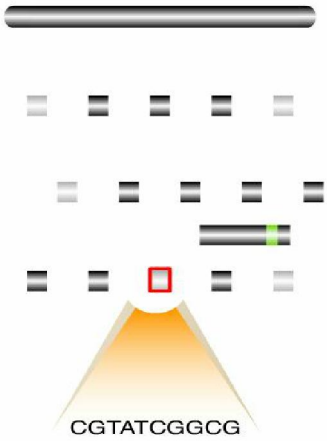
Shotgun Approach



Genome is randomly fragmented into thousands of small fragments that can be sequenced.

Sequencing the Human Genome

Shotgun Approach

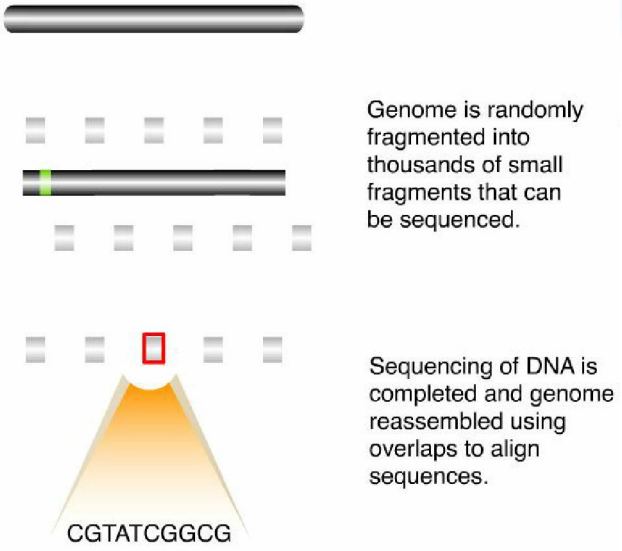


Genome is randomly fragmented into thousands of small fragments that can be sequenced.

Sequencing of DNA is completed and genome reassembled using overlaps to align sequences.

Sequencing the Human Genome

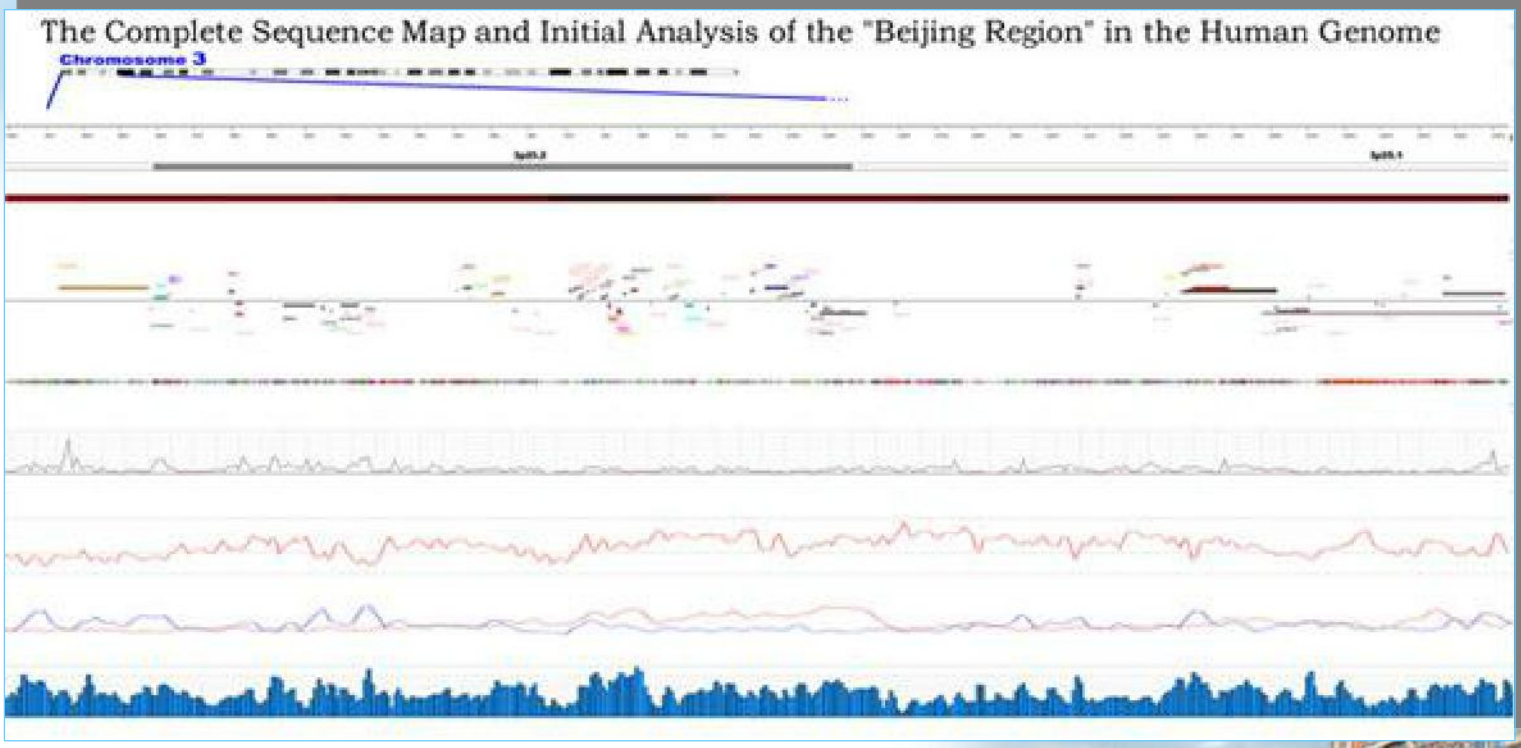
Shotgun Approach



Sequencing the Human Genome

Shotgun Approach

CGTATCGGCG



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