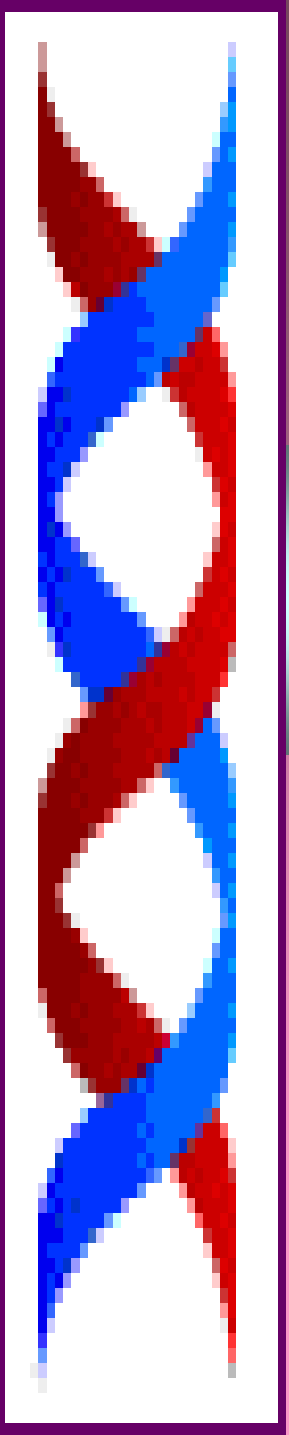


Genetic engineering

Molecular Biology course

May 2007

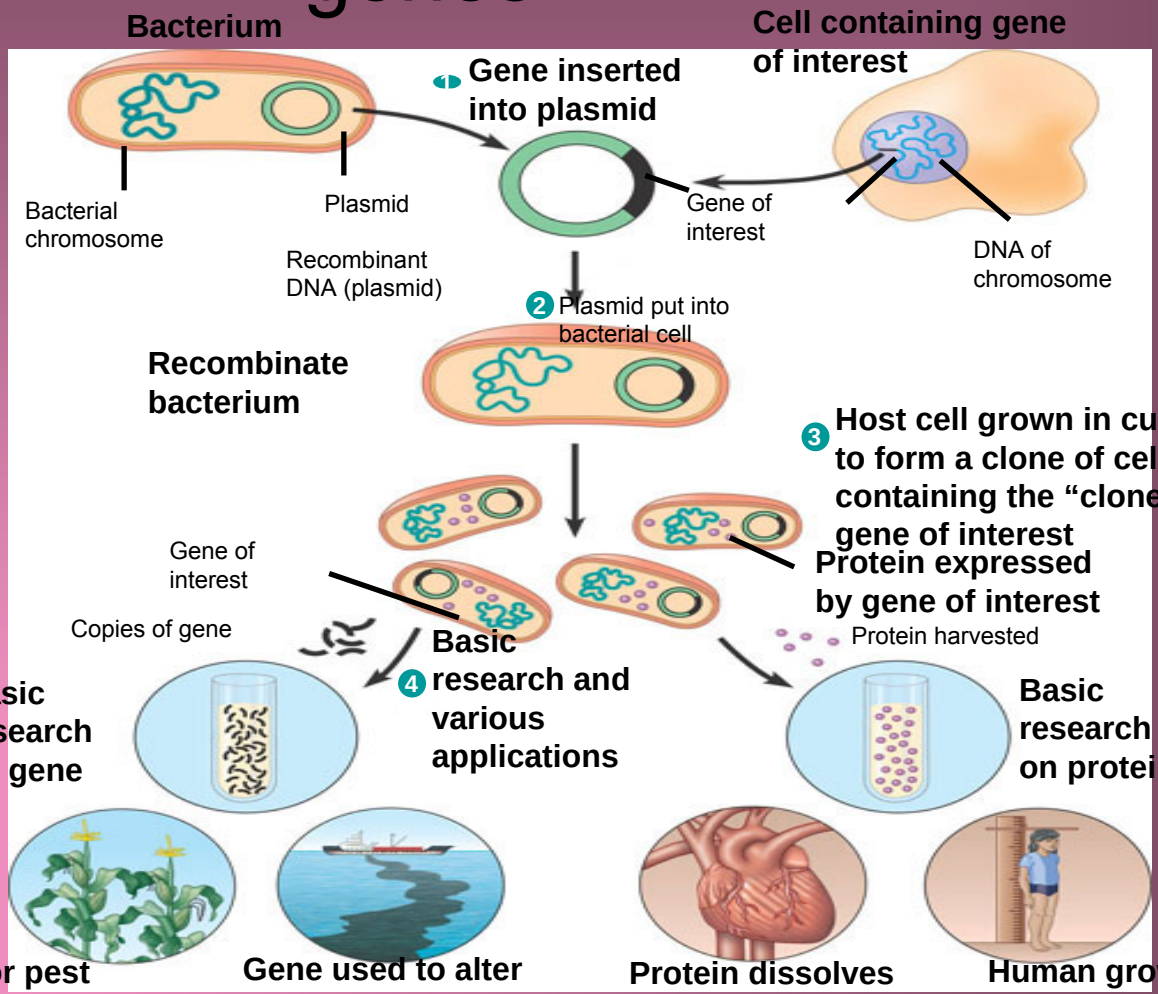
cloning

- DNA cloning permits production of multiple copies of a specific gene or other DNA segment
- To work directly with specific genes
  - Scientists have developed methods for preparing well-defined, gene-sized pieces of DNA in multiple identical copies, a process called gene cloning

DNA Cloning and Its Applications: *A Preview*

- Most methods for cloning pieces of DNA in the laboratory
  - –Share certain general features, such as the use of bacteria and their plasmids

Overview of gene cloning with a bacterial plasmid, showing various uses of cloned genes



- A restriction enzyme will usually make many cuts in a DNA molecule
  - Yielding a set of restriction fragments
- The most useful restriction enzymes cut DNA in a staggered way
  - Producing fragments with “sticky ends” that can bond with complementary “sticky ends” of other fragments
- DNA ligase is an enzyme
  - That seals the bonds between restriction fragments

Molecular Scissors and Glue

- There are 100's of restriction enzymes, each one with a different recognition site
  - These enzymes are 'molecular scissors' and can be used to specifically cut long DNA strands into smaller pieces

DNA ligase

- The T4 virus, which infects *E. coli*, has an enzyme, T4 DNA ligase, which can form a phosphodiester bonds between DNA molecules
  - Purified T4 DNA ligase can be used as ‘molecular glue’ to join pieces of DNA. This enzyme is widely used for DNA cloning

Using a restriction enzyme and DNA ligase to make recombinant DNA

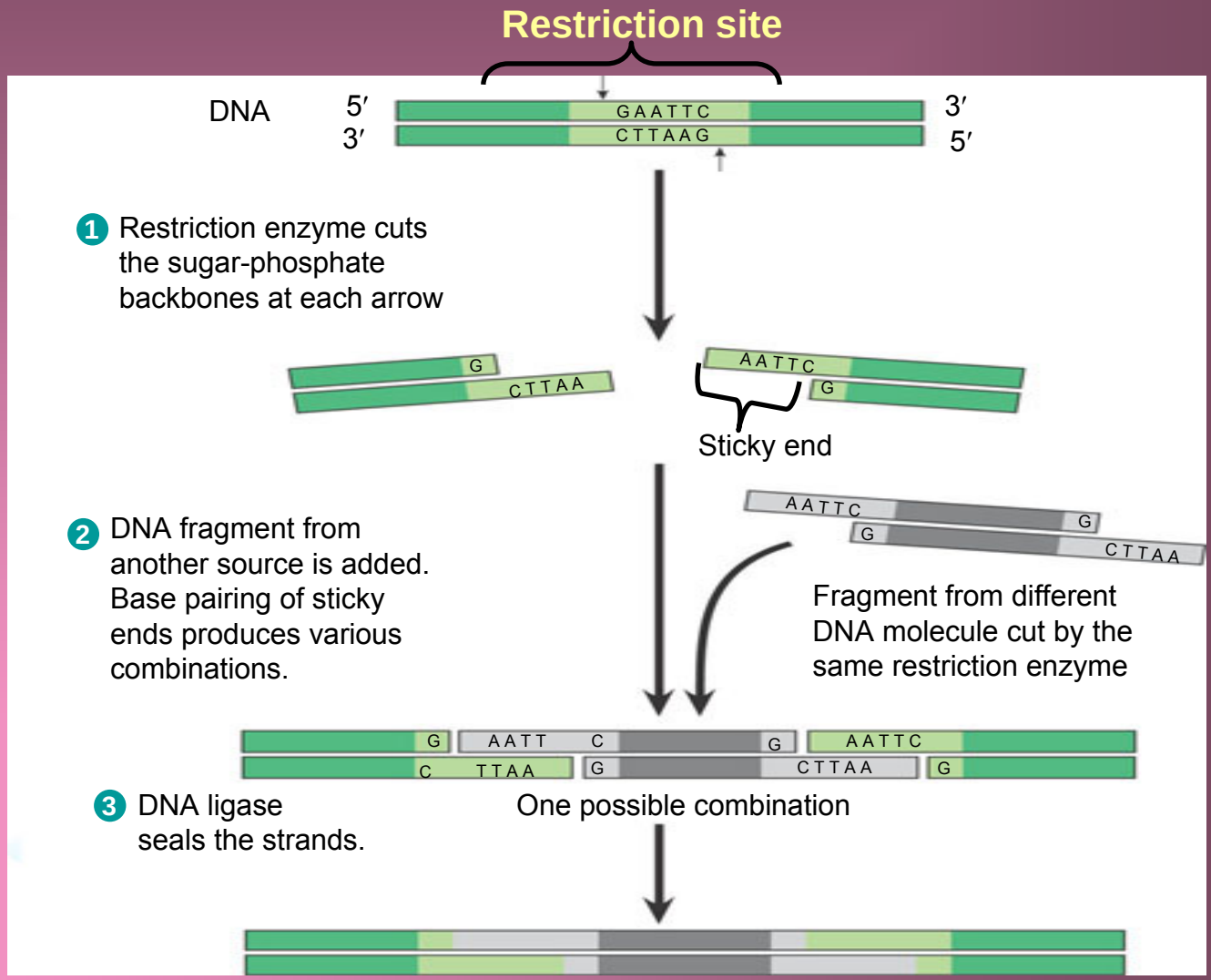
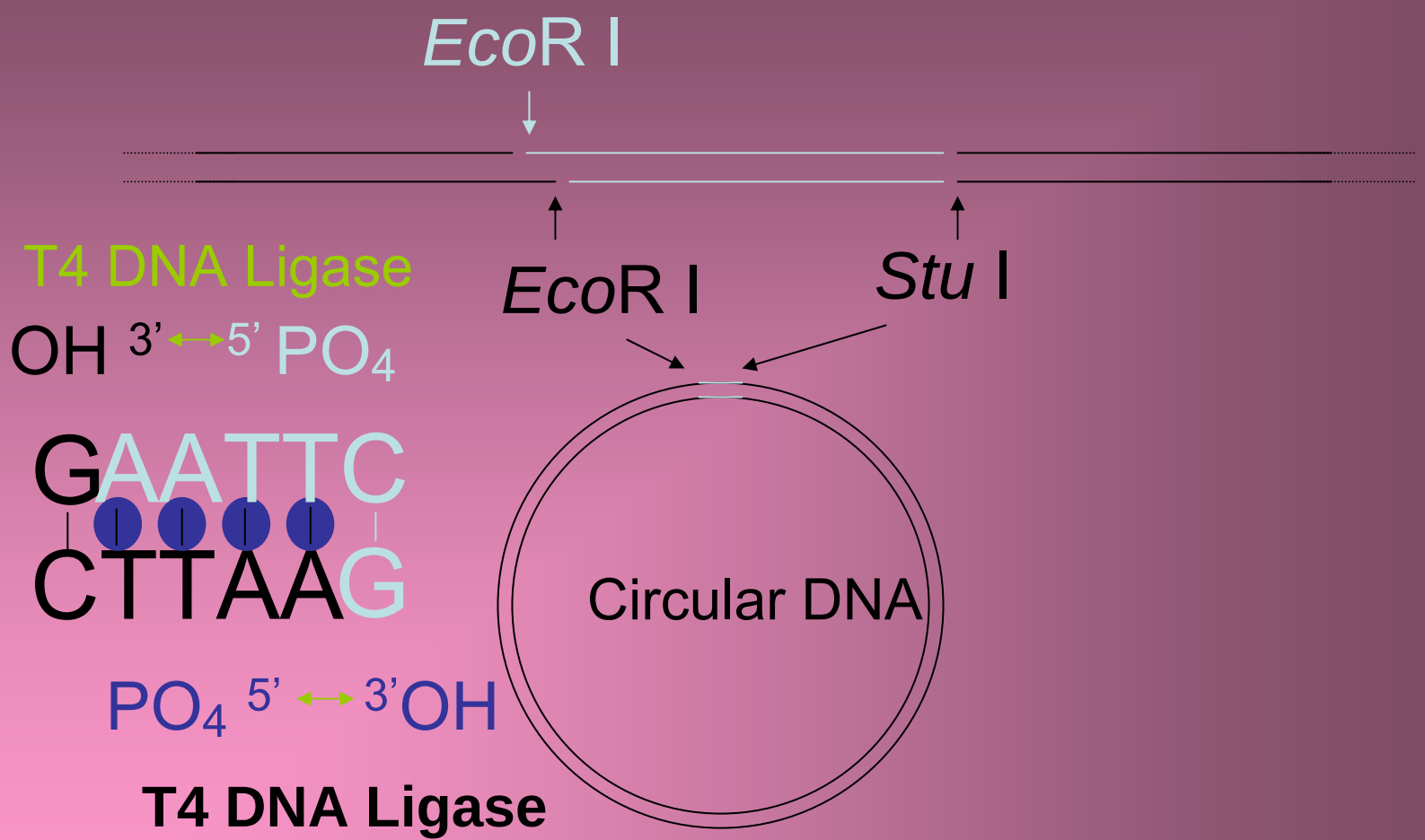


Figure 20.3

Ligation of DNA



T4 DNA ligase catalyses the formation of phosphodiester bonds

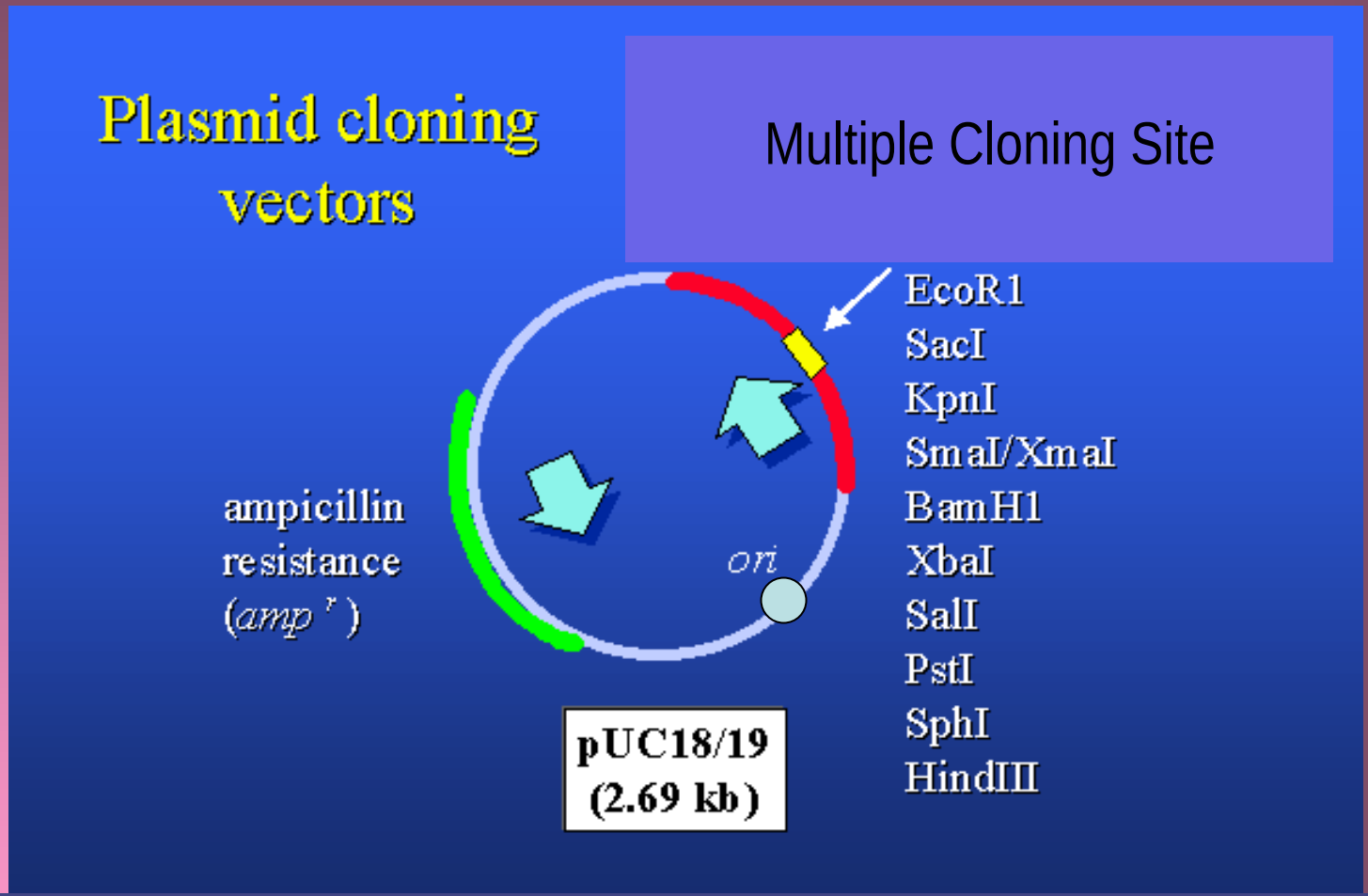
Methods of introducing DNA into cells

- Plasmids
- Viruses
  - DNA and RNA viruses
  - Phage vectors

Cloning DNA into Plasmids

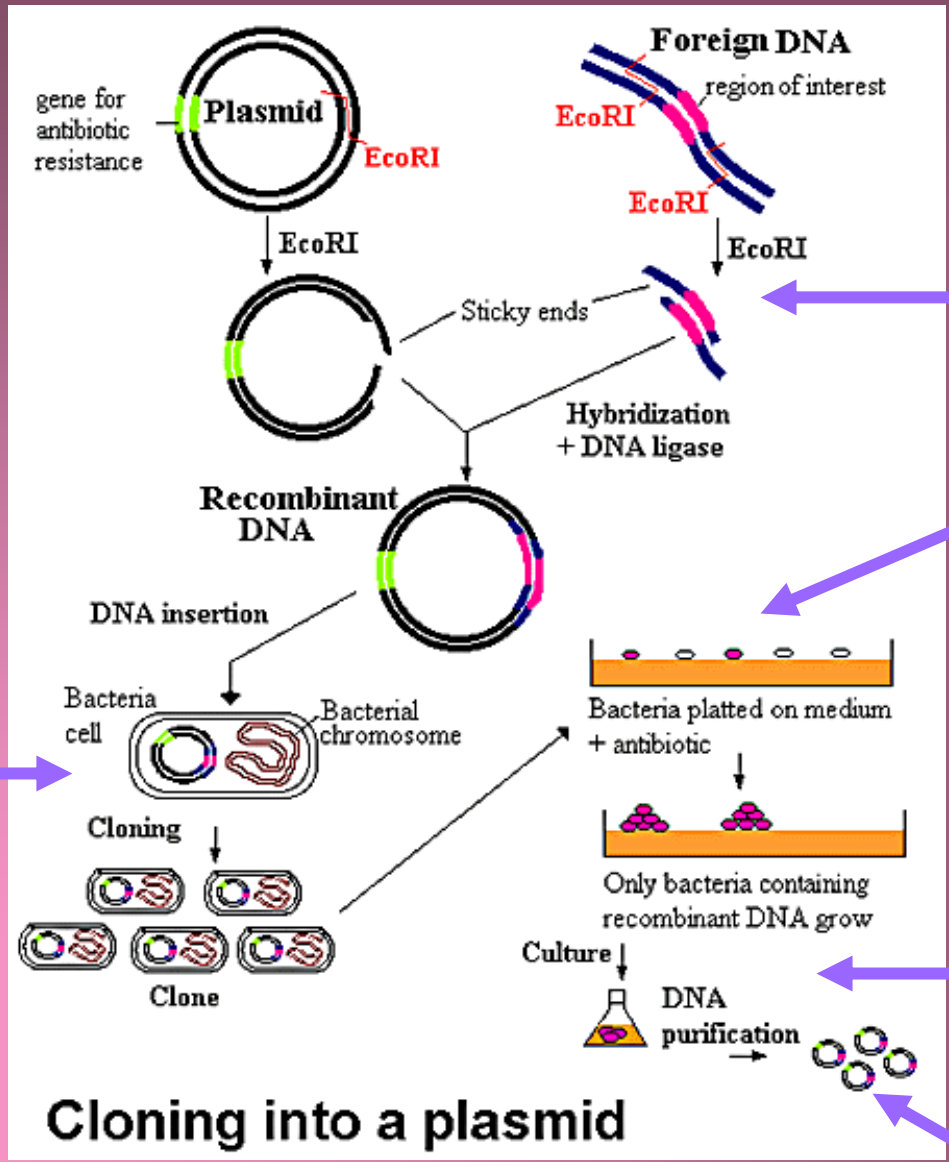
- Bacteria have a circular DNA genome
  - 5 to 10 million base pairs (bp) in size
- Many bacteria also contain plasmids
  - Small circular DNA molecules, ~3,000 to 50,000 bp
  - Note: The bacterial genome is not a plasmid
- Plasmids contain ‘extra’ genes which are often vital for the survival of the bacterium
  - Nutrient metabolism, antibiotic resistance
- Plasmids can be used as vectors in which foreign DNA can be ligated (cloned)

A General Laboratory Plasmid



A foreign gene can be ligated into a plasmid, and the genetically engineered plasmid introduced into *E. coli*.

Cloning DNA into a Plasmid



Both plasmid and foreign DNA have sticky *Eco*R I ends

Agar plates contain antibiotic. Grow at 37 °C

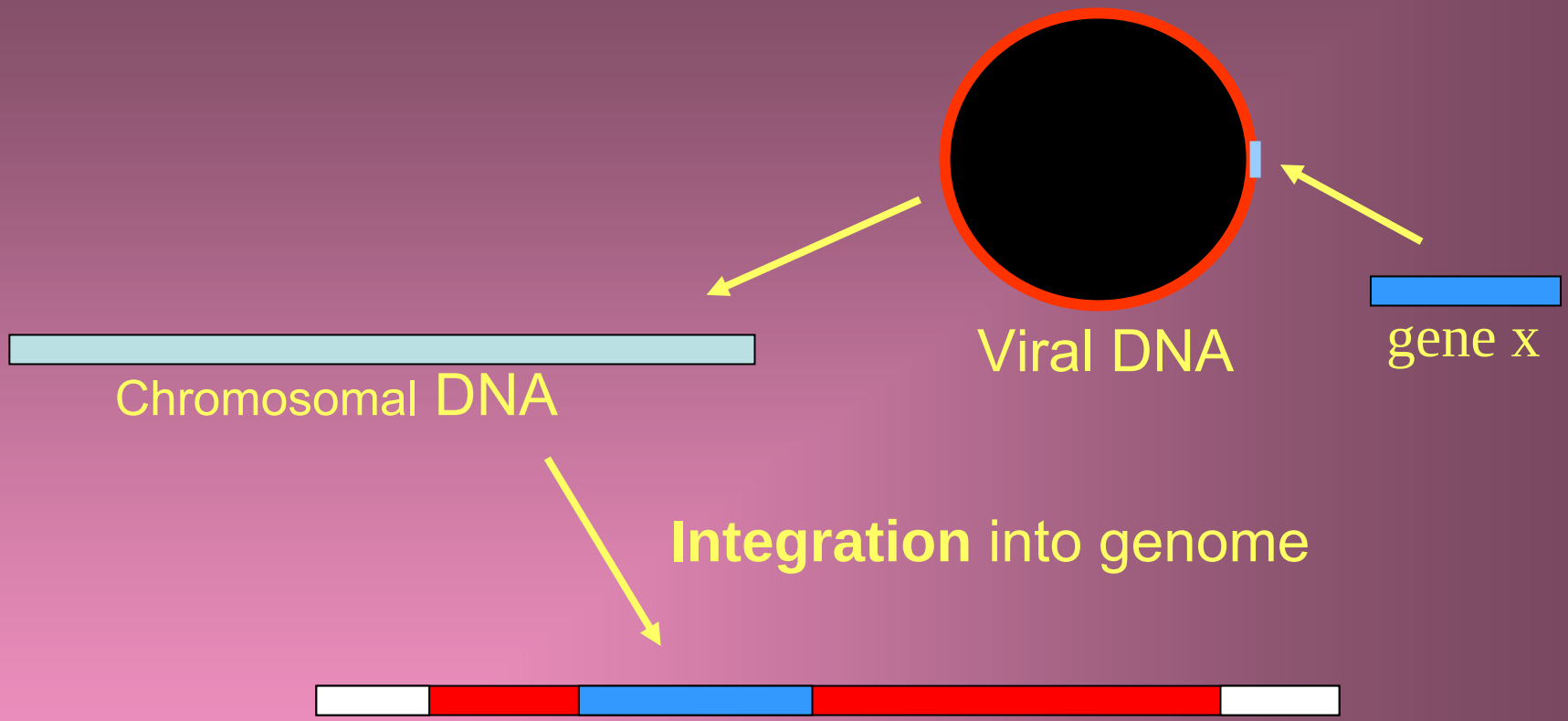
Insertion into *E. coli* (transformation)

Place 1 colony in liquid media + antibiotic. Grow at 37 °C

Purify Plasmid DNA (Billions of copies)

DNA and Retroviruses can serve as vehicles for the introduction of new DNA into a cell

DNA / RNA viruses as ‘vehicles’



Gene Therapy and Transgenics

Polymerase Chain Reaction (PCR)

- PCR generates multiple copies of DNA
  - Heat resistant DNA polymerase used to copy a section of DNA e.g *Taq*
- Very efficient copying
  - Billions of copies from a single ‘template’ DNA
- Small volume / quick analysis

Cloning a Eukraryotic Gene in a Bacterial Plasmid

- In gene cloning, the original plasmid is called a **cloning vector**
  - Defined as a DNA molecule that can carry foreign DNA into a cell and replicate there

Producing Clones of Cells

APPLICATION **Cloning is used to prepare many copies of a gene of interest for use in sequencing the gene, in producing its encoded protein, in gene therapy, or in basic research.**

TECHNIQUE In this example, a human gene is inserted into a plasmid from *E. coli*. The plasmid contains the *amp*^{R} gene, which makes *E. coli* cells resistant to the antibiotic ampicillin. It also contains the *lacZ* gene, which encodes β-galactosidase. This enzyme hydrolyzes a molecular mimic of lactose (X-gal) to form a blue product. Only three plasmids and three human DNA fragments are shown, but millions of copies of the plasmid and a mixture of millions of different human DNA fragments would be present in the samples.

1. **Isolate plasmid DNA and human DNA.**
2. **Cut both DNA samples with the same restriction enzyme**
3. **Mix the DNAs; they join by base pairing. The products are recombinant plasmids and many nonrecombinant plasmids.**

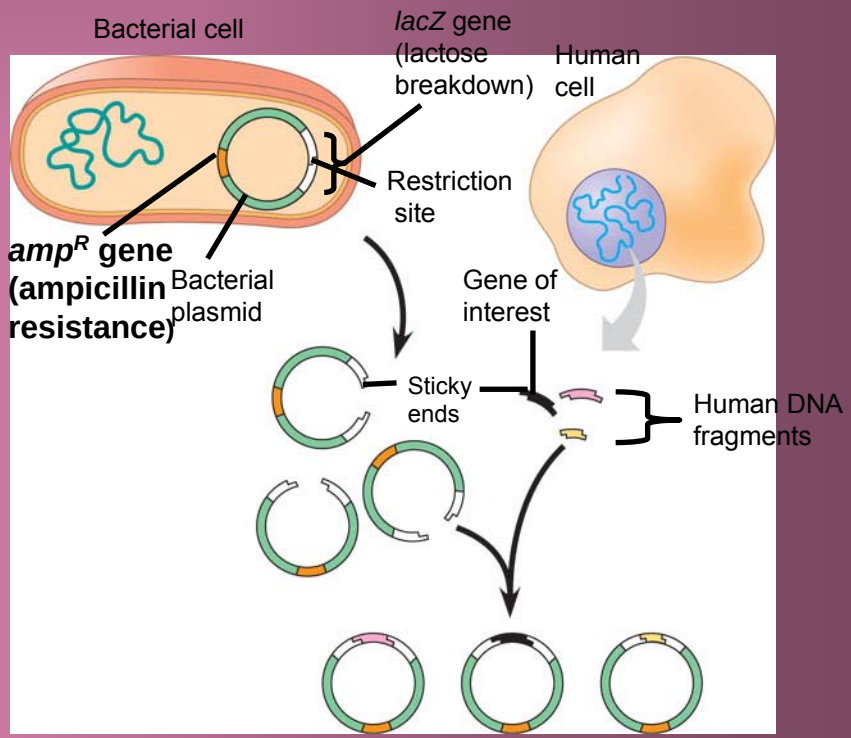
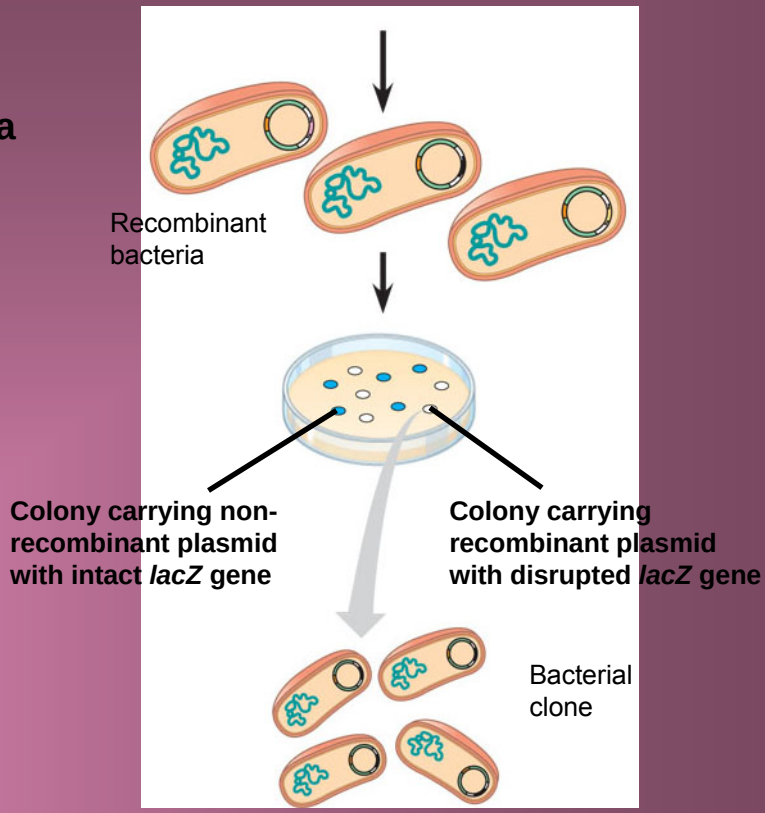


Figure 20.4

4 **Introduce the DNA into bacterial cells that have a mutation in their own *lacZ* gene.**

5 **Plate the bacteria on agar containing ampicillin and X-gal. Incubate until colonies grow.**



RESULTS

Only a cell that took up a plasmid, which has the *amp*^{R} gene, will reproduce and form a colony. Colonies with nonrecombinant plasmids will be blue, because they can hydrolyze X-gal. Colonies with recombinant plasmids, in which *lacZ* is disrupted, will be white, because they cannot hydrolyze X-gal. By screening the white colonies with a nucleic acid probe (see Figure 20.5), researchers can identify clones of bacterial cells carrying the gene of interest.

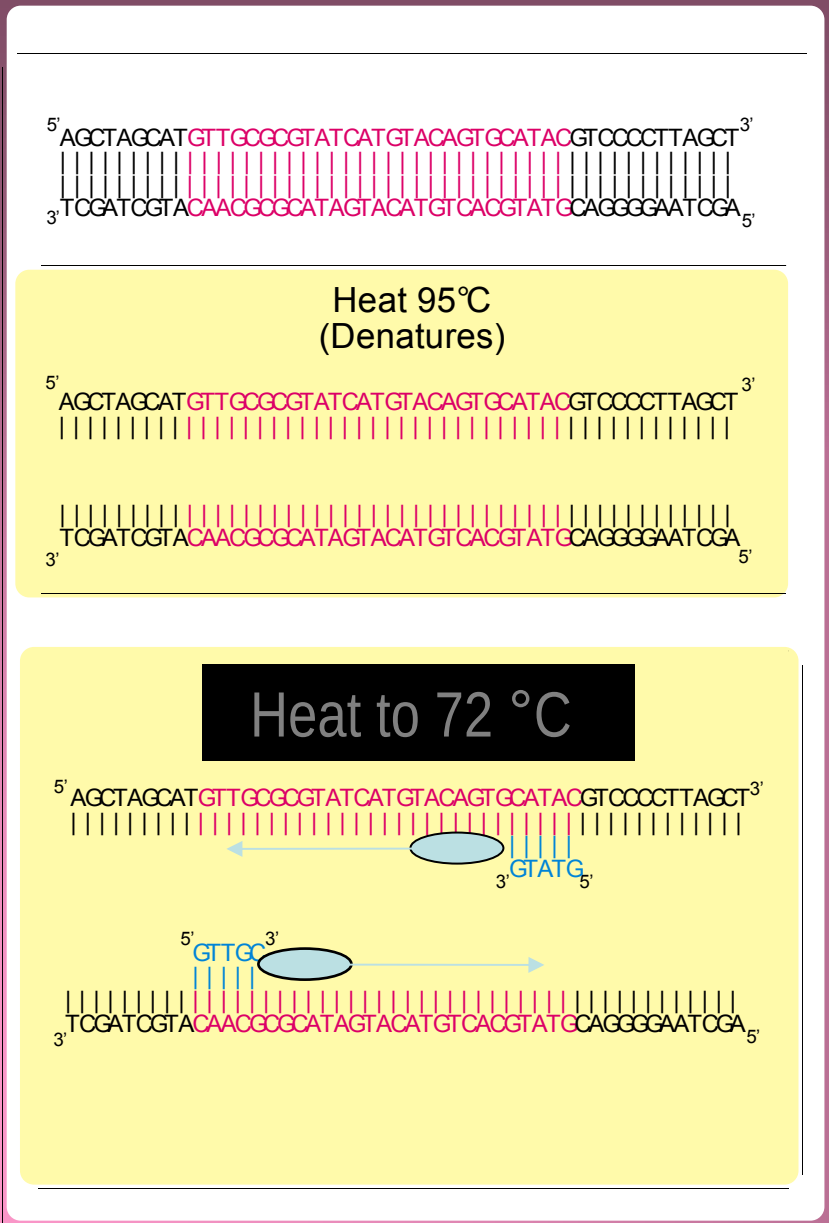
Polymerase Chain Reaction (PCR)

- Entire reaction performed in single tube
  - 20 to 50 µl volume
- Reaction contains
  - Template DNA, heat resistant DNA polymerase, a pair of specific DNA primers (in excess over the template), nucleotide bases, appropriate reaction buffer
- Reaction is repeatedly cycled through 3 temperatures (x30)
  - 95 °C (makes DNA single stranded)
  - 55 °C (primers anneal to template DNA)
  - 72 °C DNA polymerase copies DNA, starting from the primers

A Thermocycler

This thermocycler can accept 1500 reactions at a time, and complete them in 2 to 4 hours.

Principal of PCR



DNA (Double Stranded)

Heat Denature
(Becomes Single Stranded)

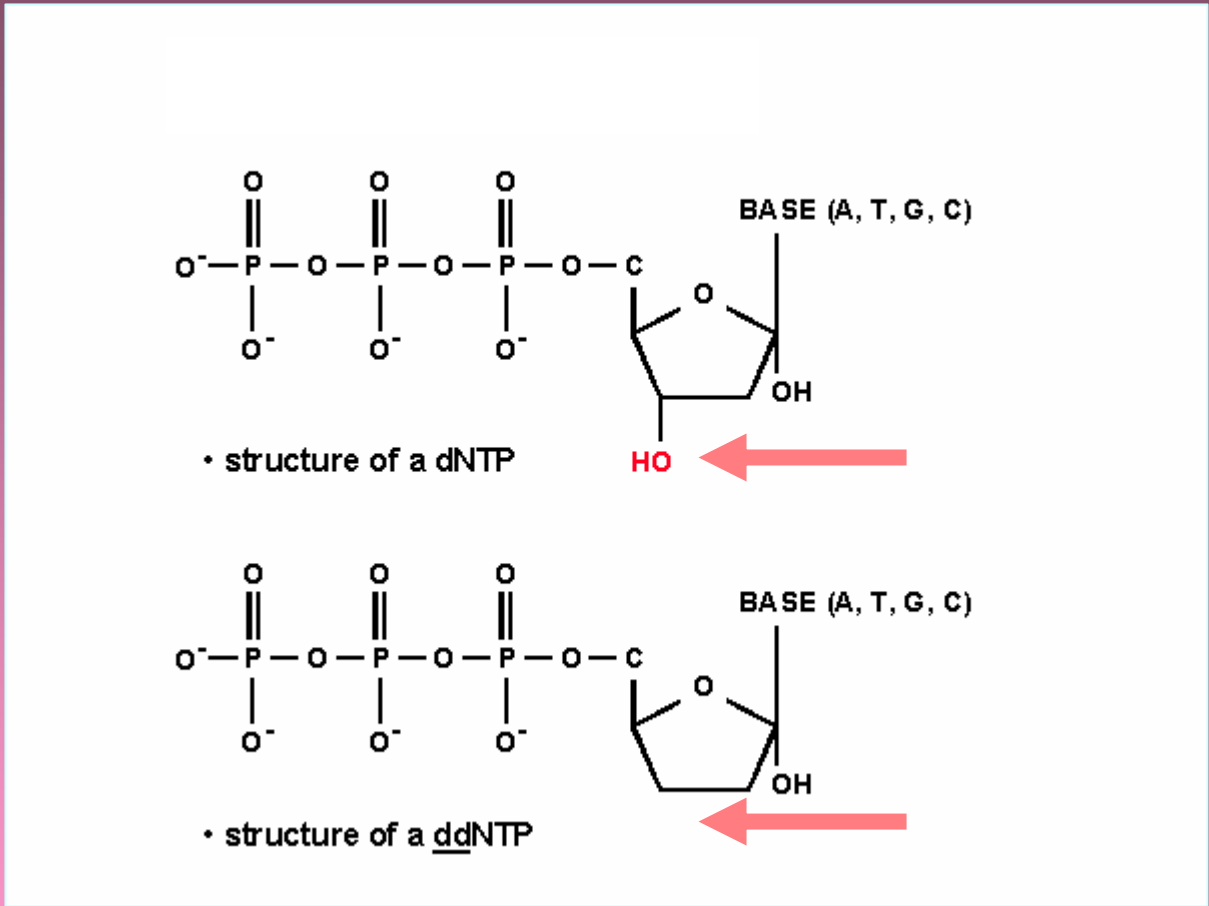
Cool. This allows specific 'primers' to anneal as shown

Heat to 72 °C. Heat resistant DNA polymerase extends new DNA from the primers

DNA Sequencing

- A specific primer binds to denatured DNA
- *Taq* DNA polymerase extends a new strand from this primer
- Complementary nucleotides are added as appropriate
- In the reaction are small quantities of coloured dideoxynucleotides
  - Colours: **ddTTP ddGTP ddATP ddCTP**
  - These prevent further additions (terminators)

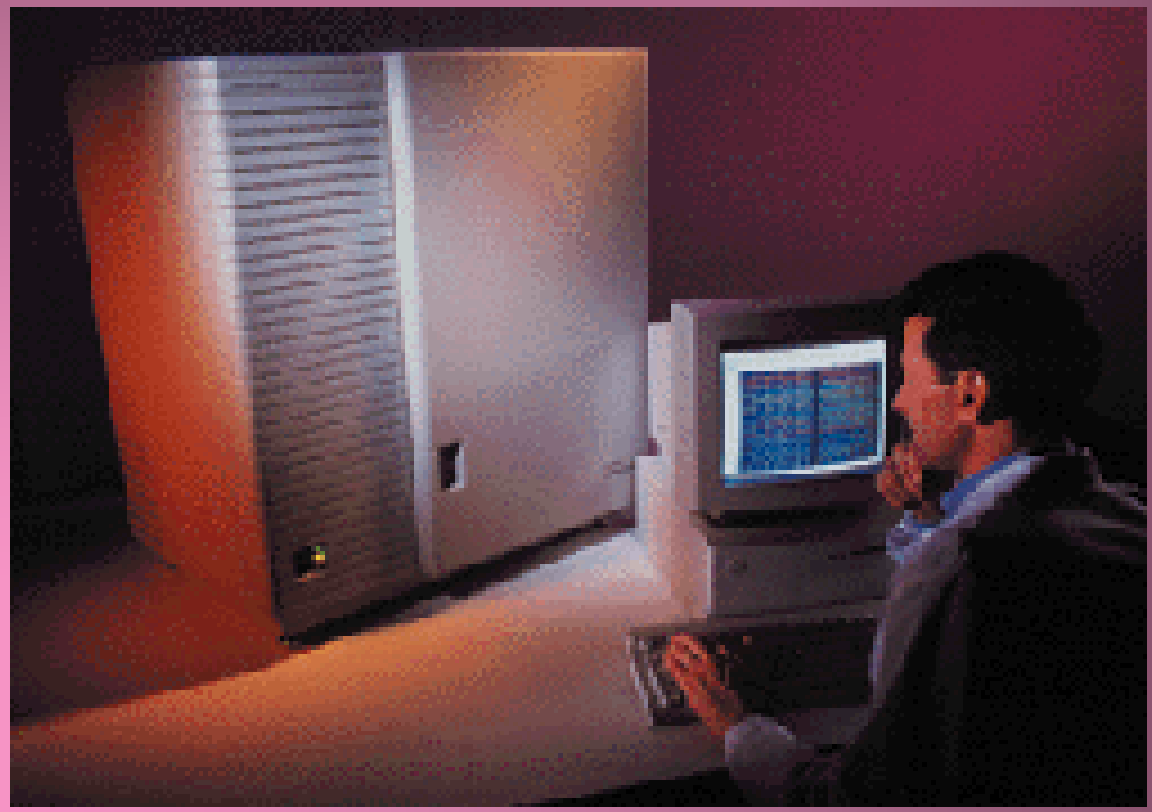
Dideoxynucleotides



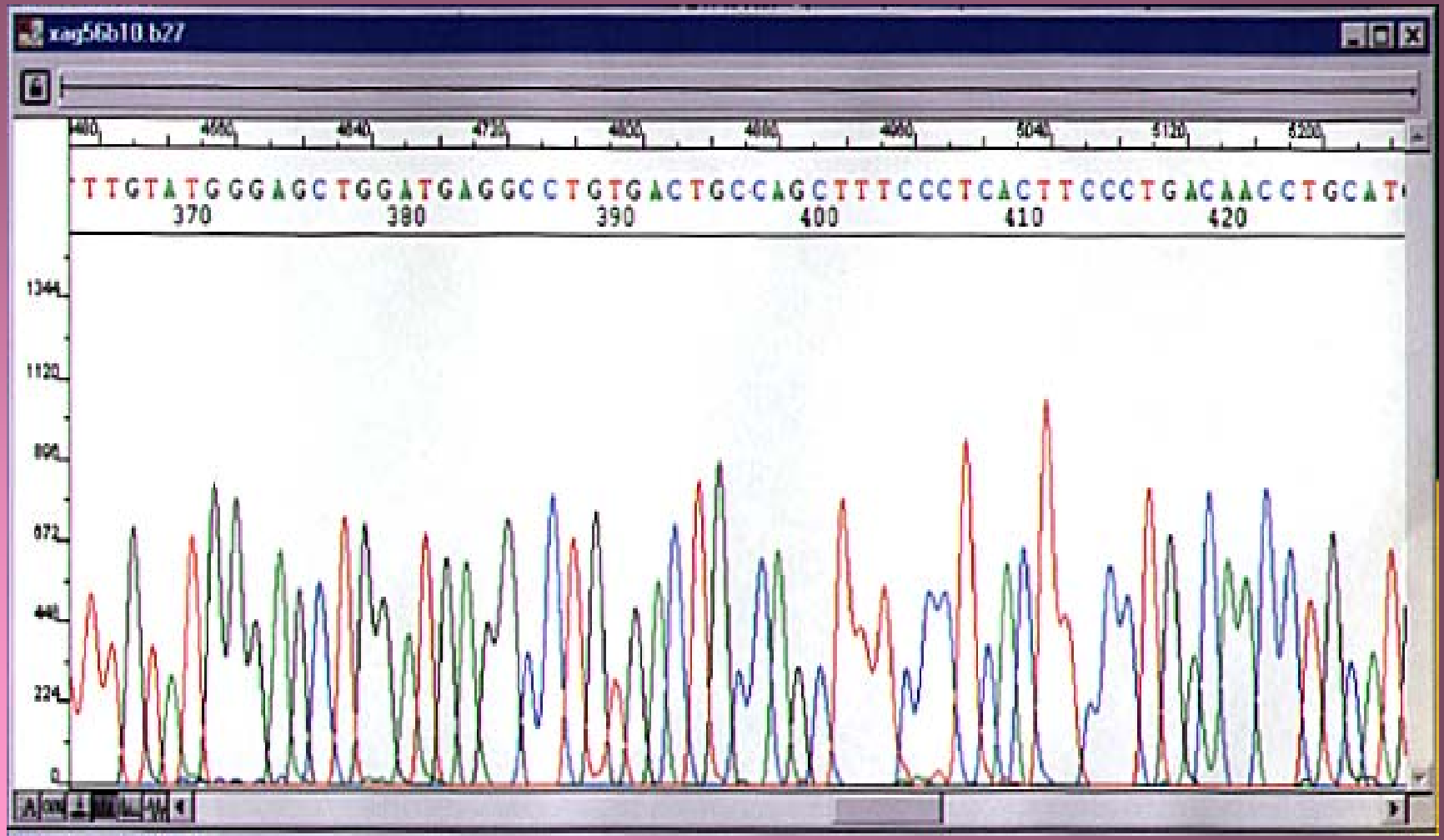
ddNTPs have no 3’ OH, so when added they cannot form the phosphodiester bond required to add the next nucleotide

DNA Sequencing Reaction

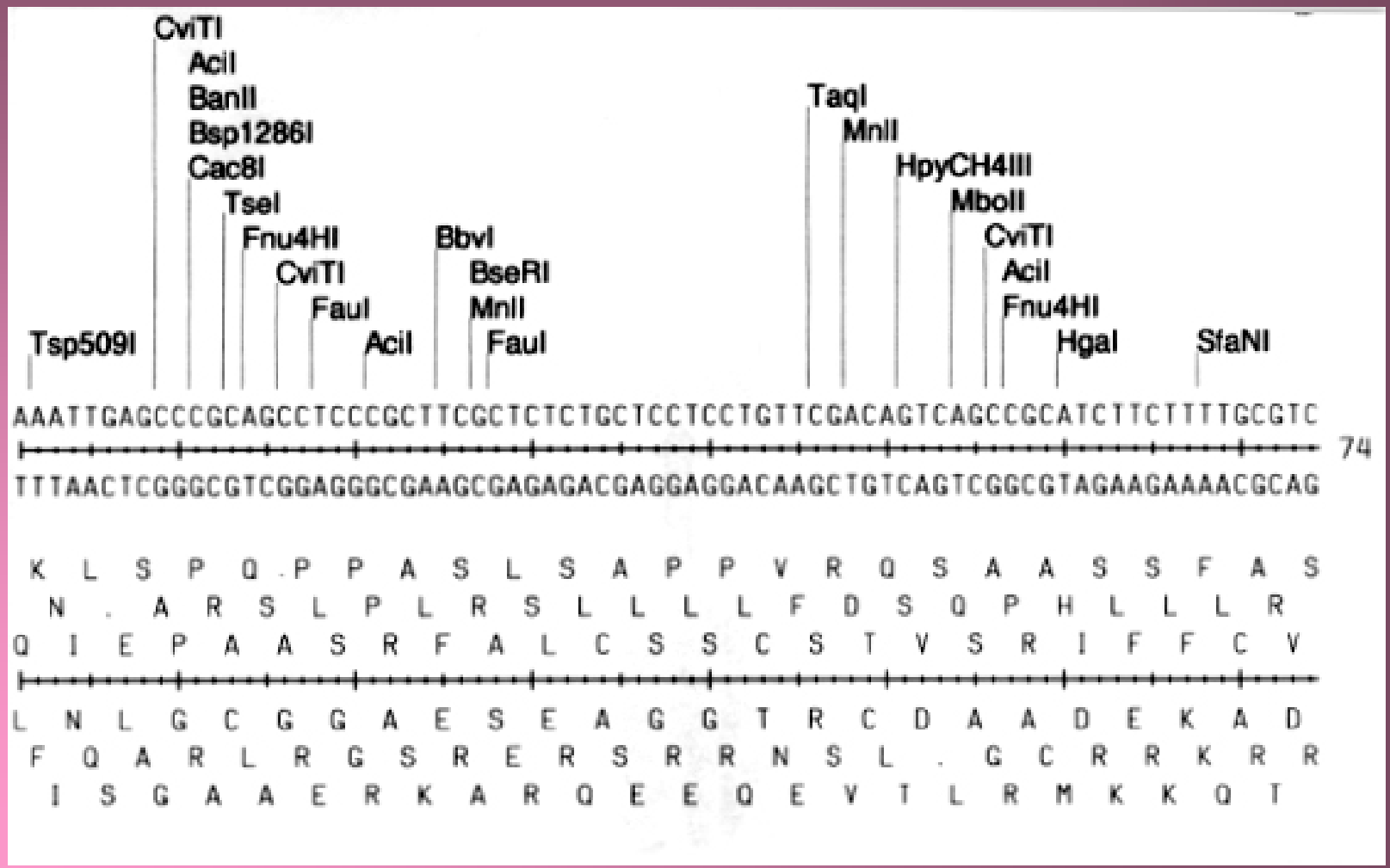
The reaction is boiled to make all the DNA single stranded and then the reaction is resolved on a long polyacrylamide or capillary gel in a DNA sequencer



Electropherogram of sequencing gel



Decoding DNA sequence data




THE
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