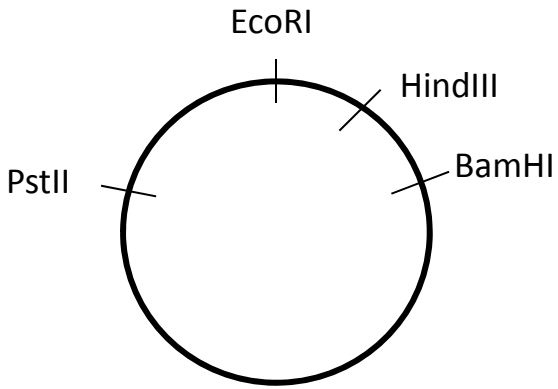
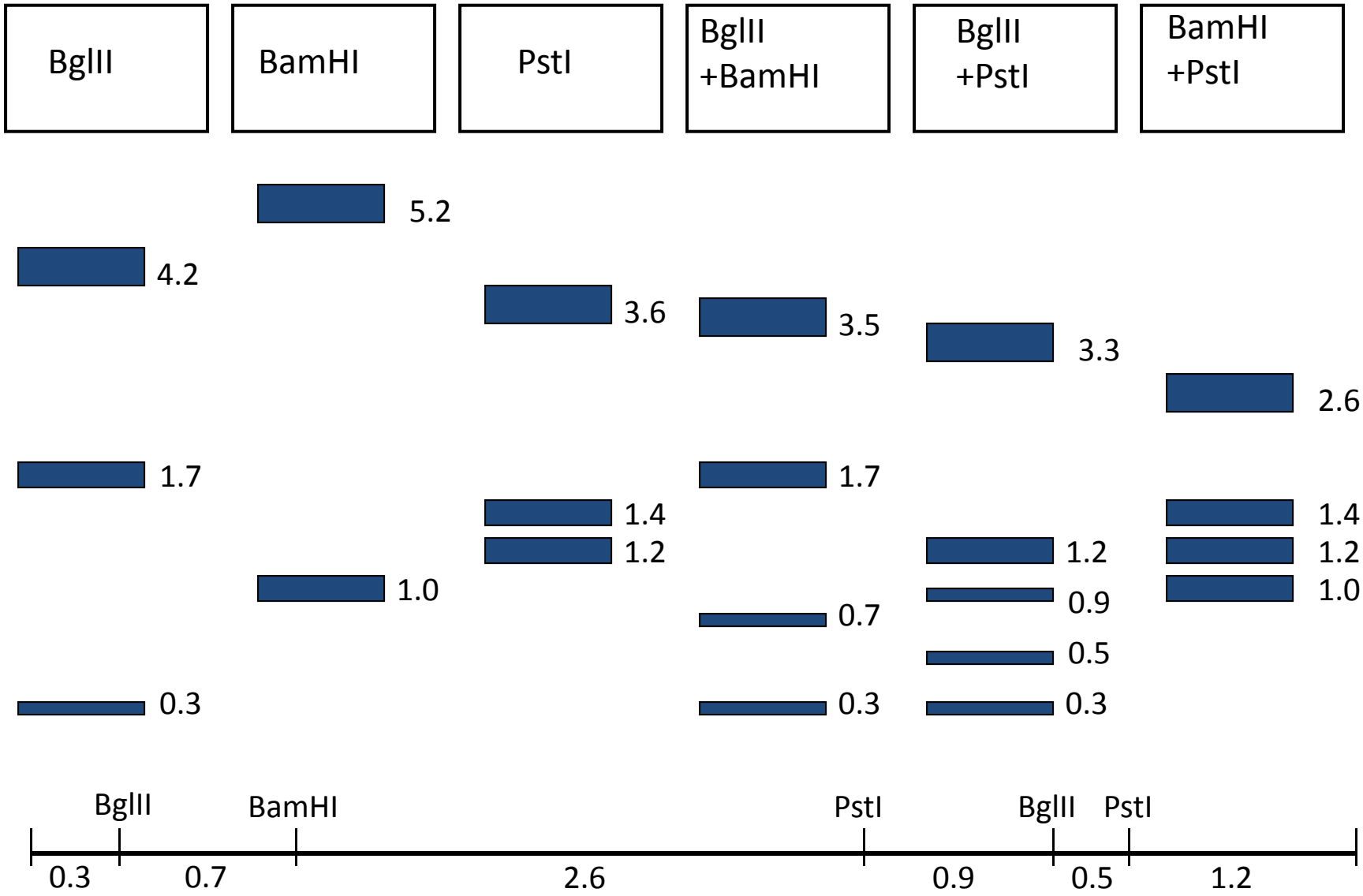
Restriction Mapping

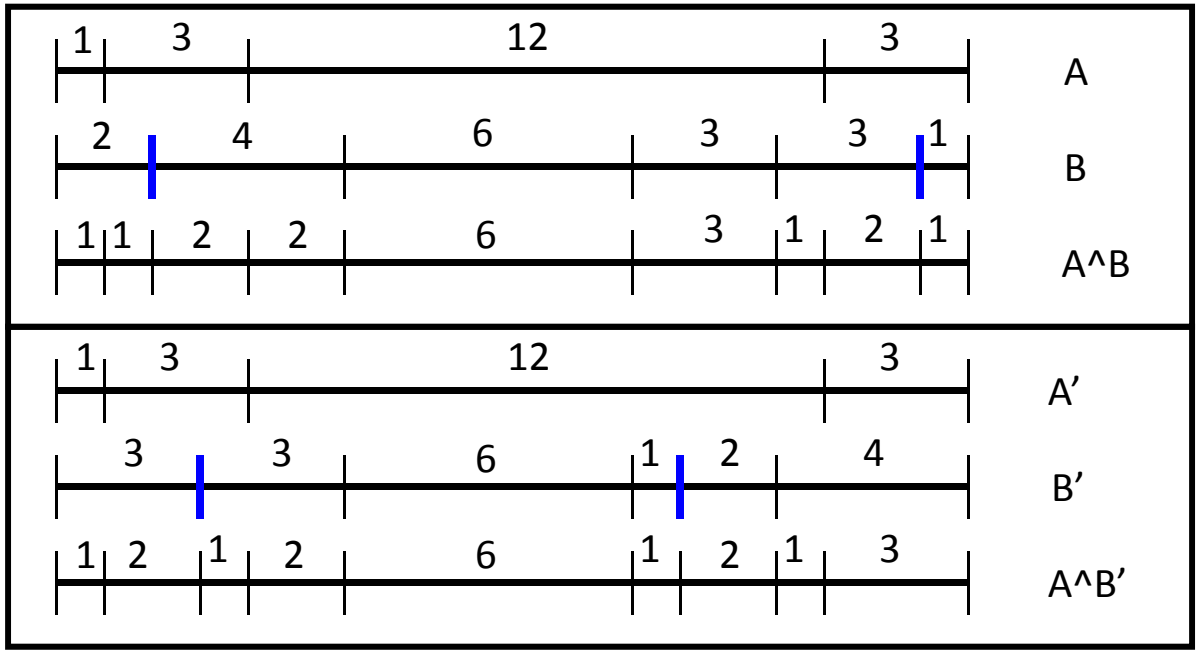
Restriction maps show the relative location of a selection of restriction sites along linear or circular DNA.



Restriction Mapping

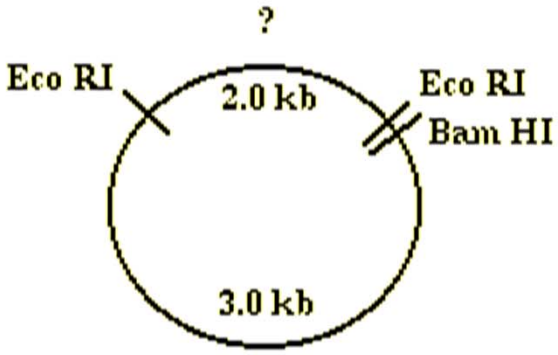


Restriction Mapping

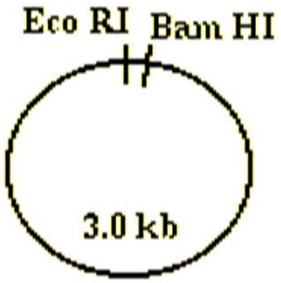


For Example

- Take a pBluescript portion of plasmid (3kb) with a known;
  - ✓ Restriction site (Eco RI & Bam HI)
  - ✓ Insert of 2 kb long



pBluescript with unknown insert in the Eco RI site



pBluescript without insert

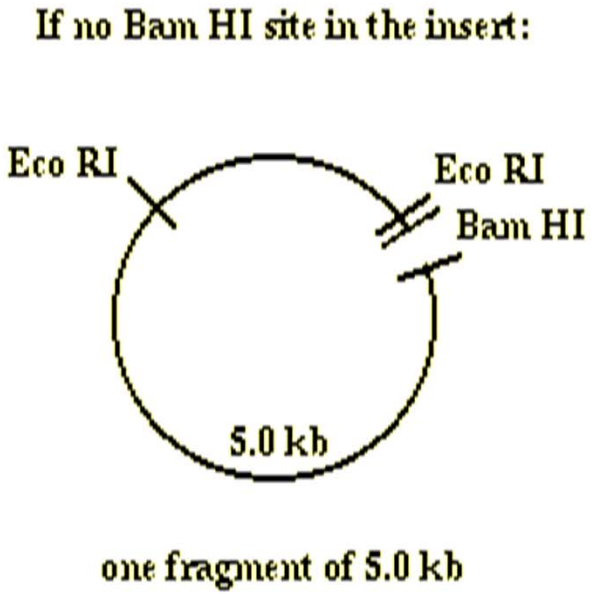
- Digest plasmid with an enzyme that you know is in the pBluescript plasmid. For example, you know that there is only one Bam HI site in pBluescript, and it is in the multiple cloning site next to the Eco RI site

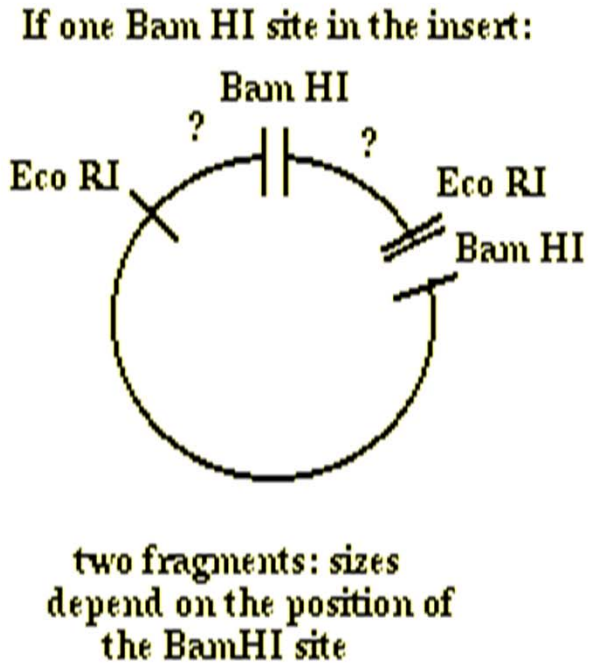
- If you digest this plasmid with Bam HI, there are two possibilities:

1) There are no Bam HI sites in the insert. If this is the case, when you run this digestion on a gel you will see only one DNA fragment, and it will be 5.0 kb long (3.0 kb of pBluescript DNA and 2.0 kb of insert DNA).

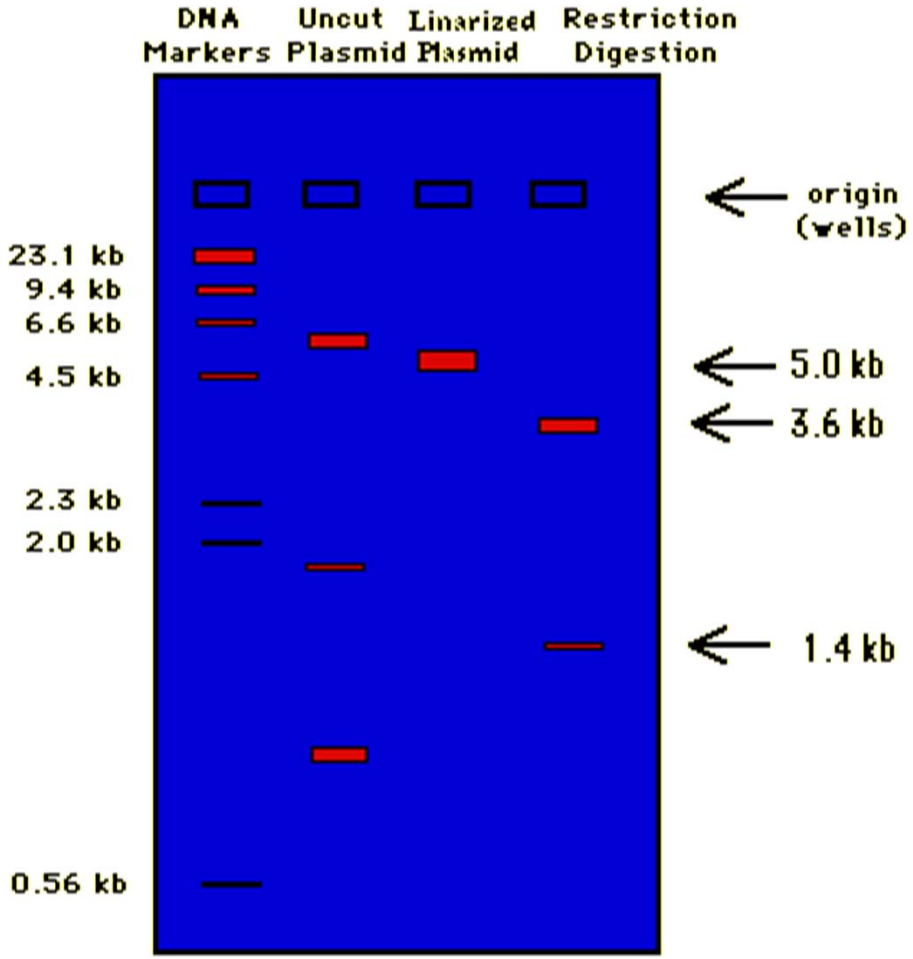
2) There is a Bam HI site in the insert. If this is the case, then the enzyme will cut the circular plasmid in two places, in the pBluescript part of the plasmid and in the insert.

- In 2nd case, you will end up with two fragments of DNA. One will be pBluescript with some of the insert still attached and the other will be just insert. The sizes of the two fragments (determined by electrophoresis) will tell you where the site is. These two possibilities are shown below





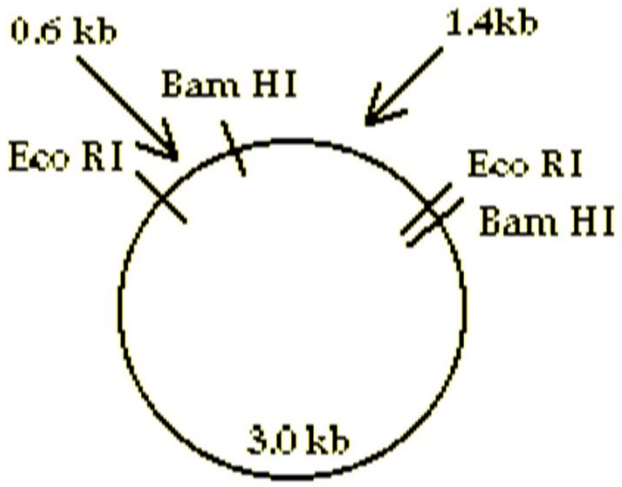
- In the second case, where there is a site in the insert, the gel might look like this:



- This case,show two pieces of information:
  - ✓ That there is a Bam HI site in the insert,
  - ✓ where the site is in relation to the one end of the insert.

- When the Bam HI digestion is separated on an agarose gel, the sizes of the two fragments can be determined. In the above gel, the fragments are 3.6 kb and 1.4 kb. Therefore, we know that the Bam HI site is 1.4 kb away from the right hand side of the insert . In this way, you have "mapped" the Bam HI site:

- By testing the insert for the presence and location of sites of many different restriction enzymes,a "restriction map" of the clone is made.



pBluescript + insert

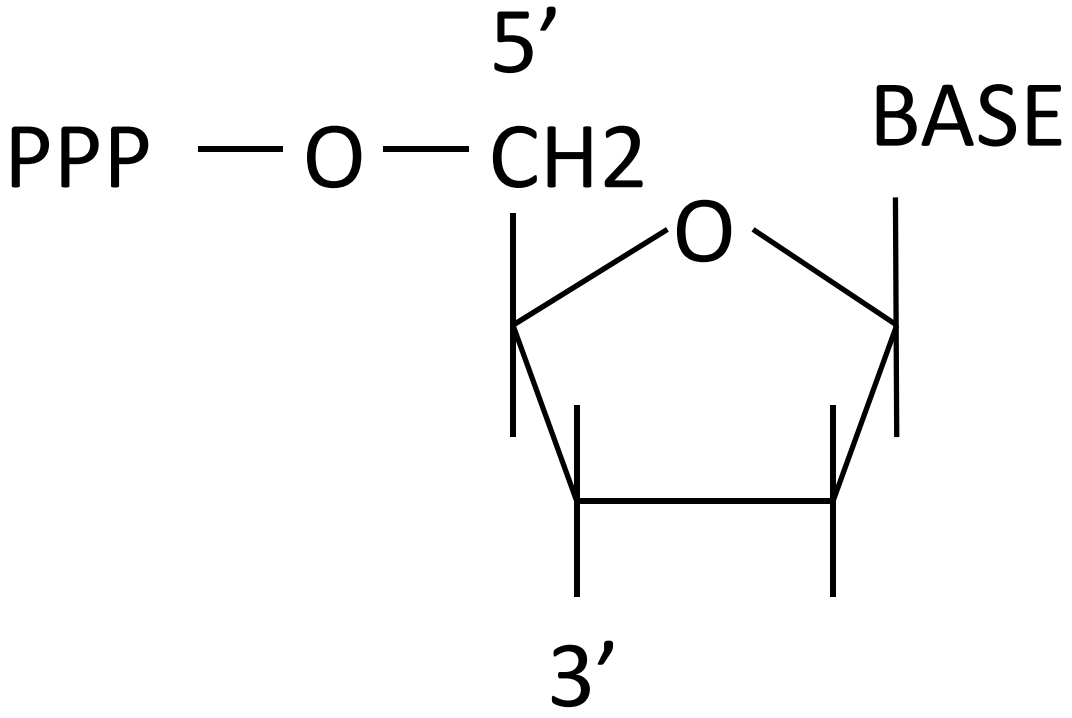
Purified DNA Molecules Can Be Sequenced Rapidly

- DNA Sequencing
  - Important to determine the sequence of nucleotides of the cloned gene
  - Chain termination sequencing (Sanger method)
  - Computer automated sequencing
    - ddNTP's are each labeled with a different fluorescent dye
    - Samples are separated on a single-lane capillary gel that is scanned with a laser beam
    - Creates different color patterns for each nucleotide
    - Converted by computer to the sequence

Sanger Method

- ✓ in-vitro DNA synthesis using ‘terminators’, use of dideoxi-nucleotides that do not permit chain elongation after their integration
- ✓ DNA synthesis using deoxy- and dideoxynucleotides that results in termination of synthesis at specific nucleotides
- ✓ Requires a primer, DNA polymerase, a template, a mixture of nucleotides, and detection system
- ✓ Incorporation of di-deoxynucleotides into growing strand terminates synthesis
- ✓ Synthesized strand sizes are determined for each di-deoxynucleotide by using gel or capillary electrophoresis
- ✓ Enzymatic methods

Dideoxynucleotide

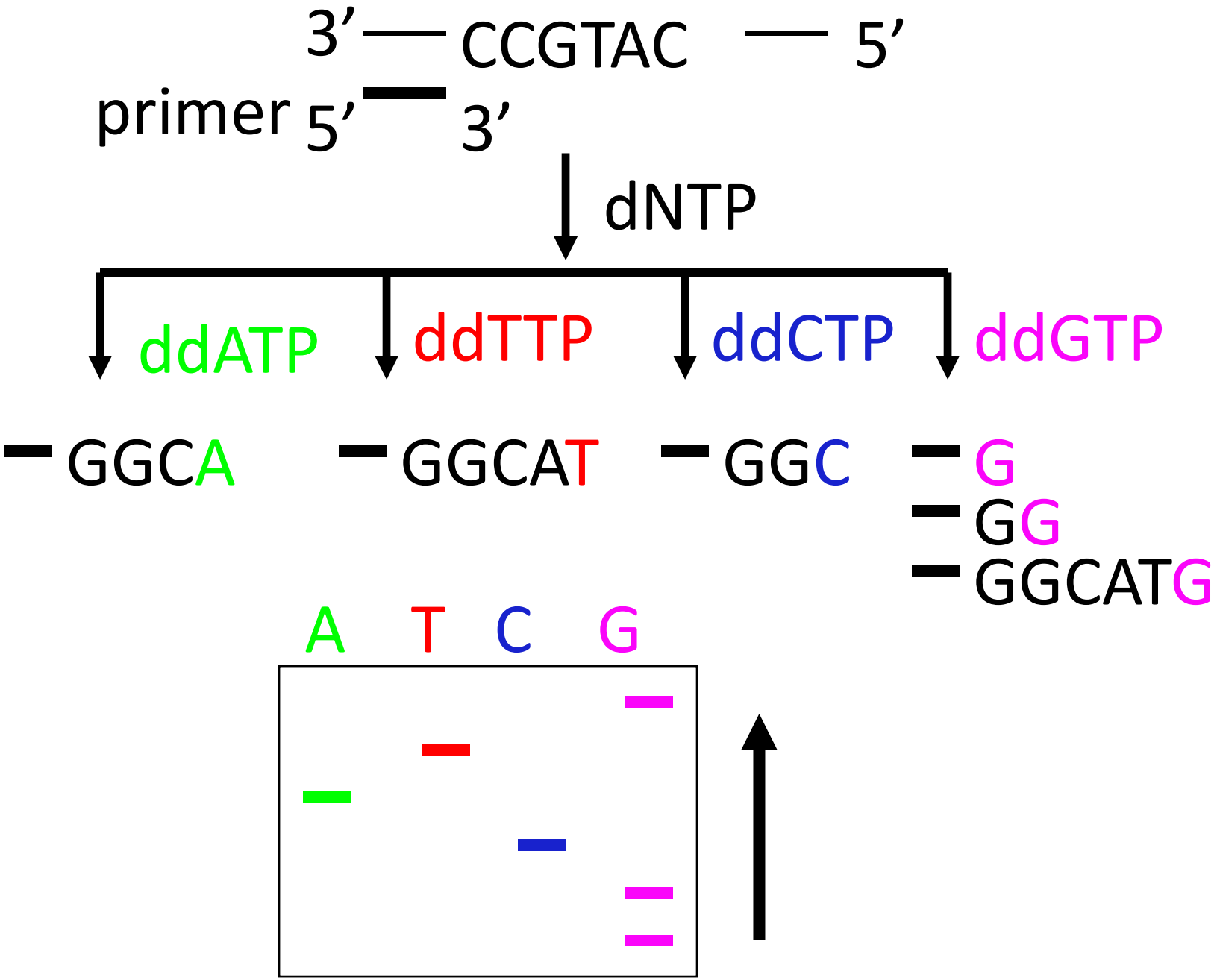


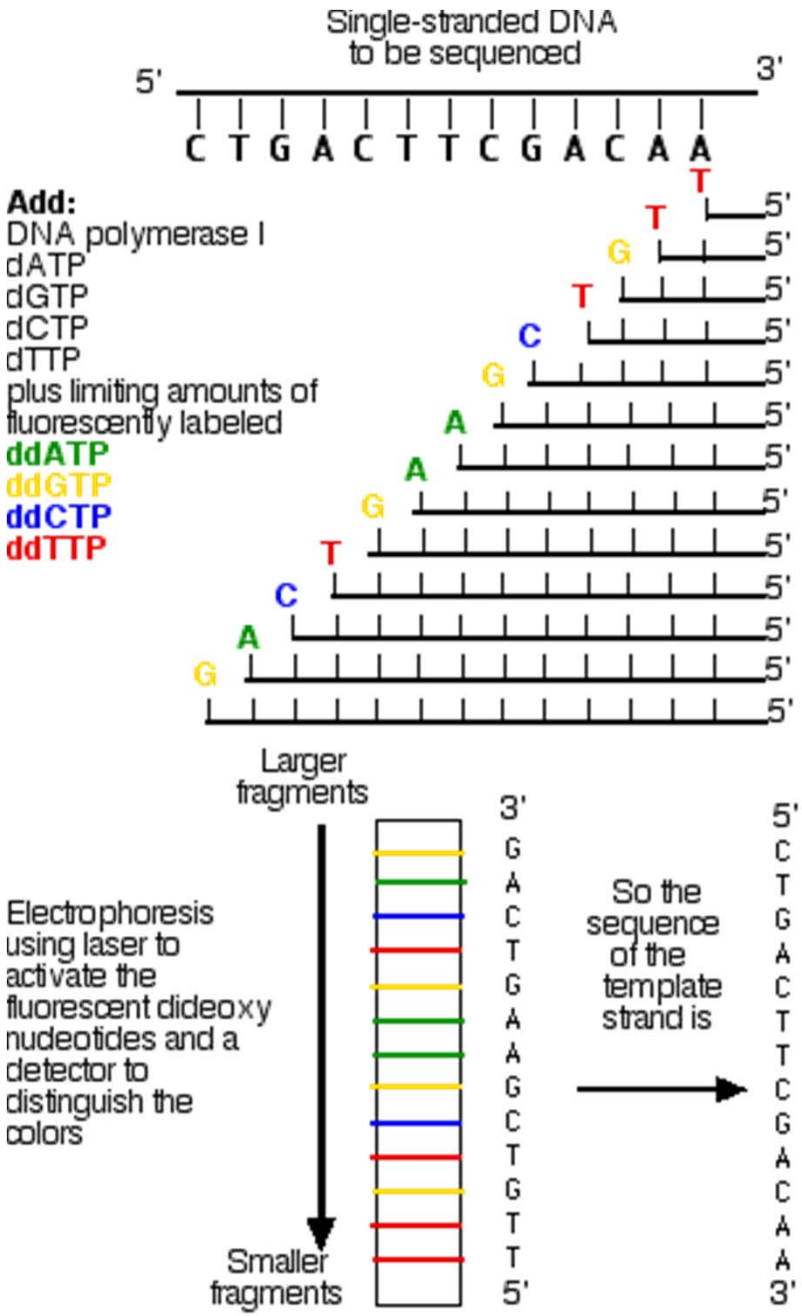
no hydroxyl group at 3’ end

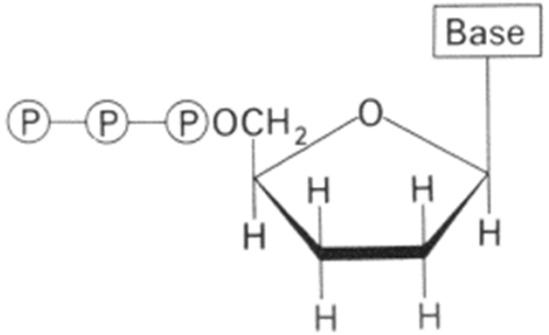
prevents strand extension

The principles

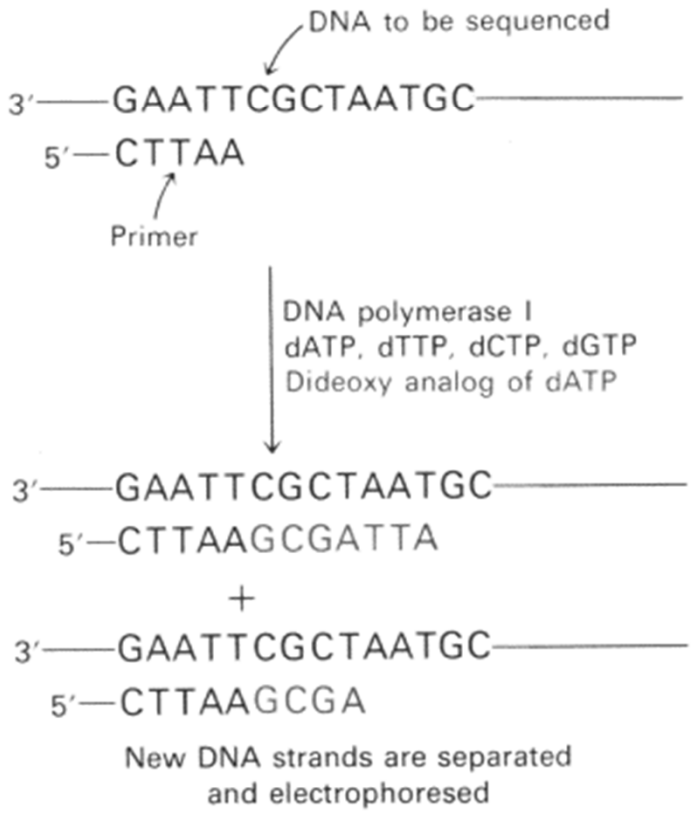
- Partial copies of DNA fragments made with DNA polymerase
- Collection of DNA fragments that terminate with A,C,G or T using ddNTP
- Separate by gel electrophoresis
- Read DNA sequence


3’ CCGTAC 5’
primer 5’ 3’
dNTP
ddATP
ddTTP
ddCTP
ddGTP
GGCA
GGCAT
GGC
G
GG
GGCATG
A T C G



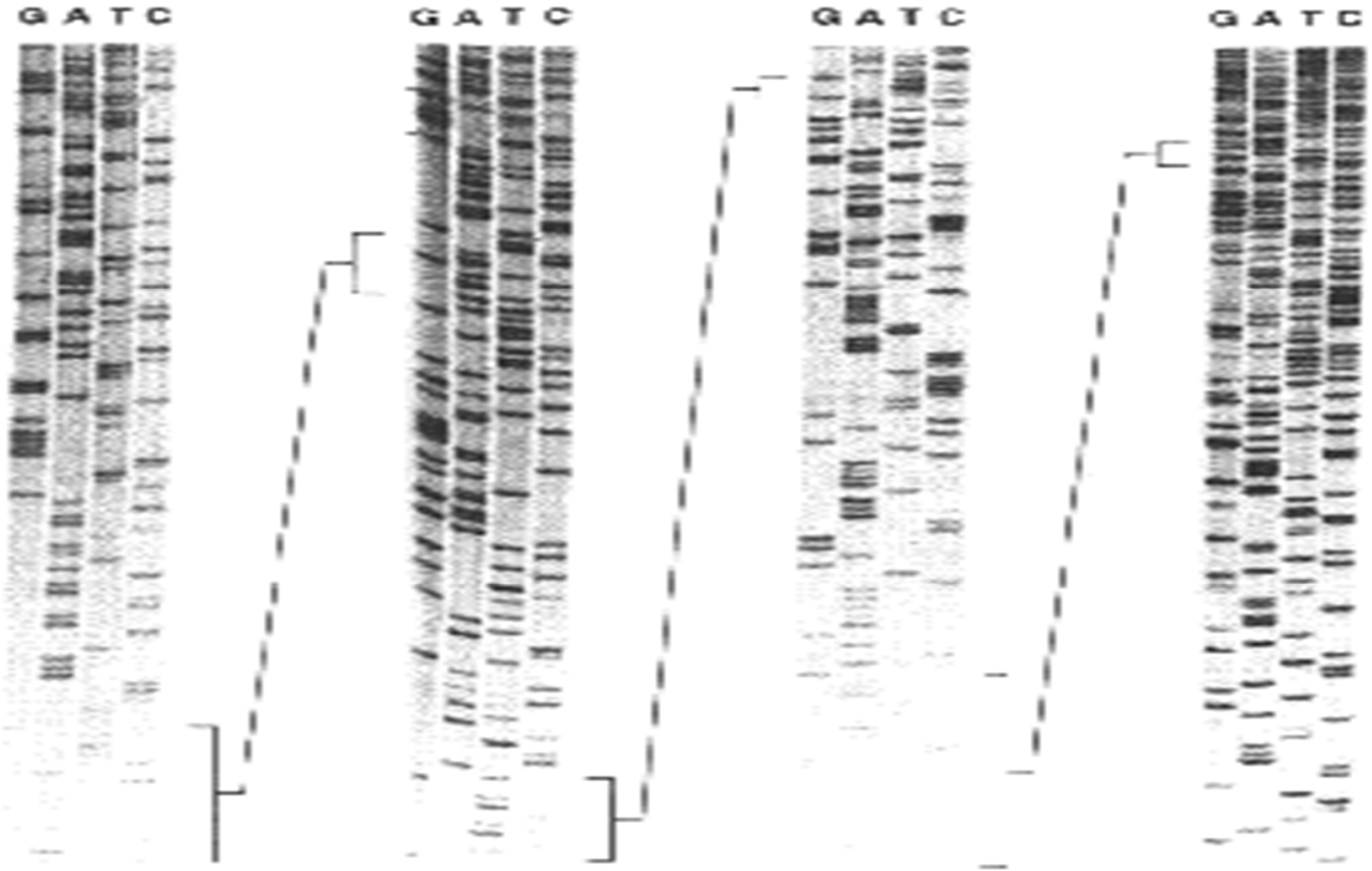


2′,3′-Dideoxy analog

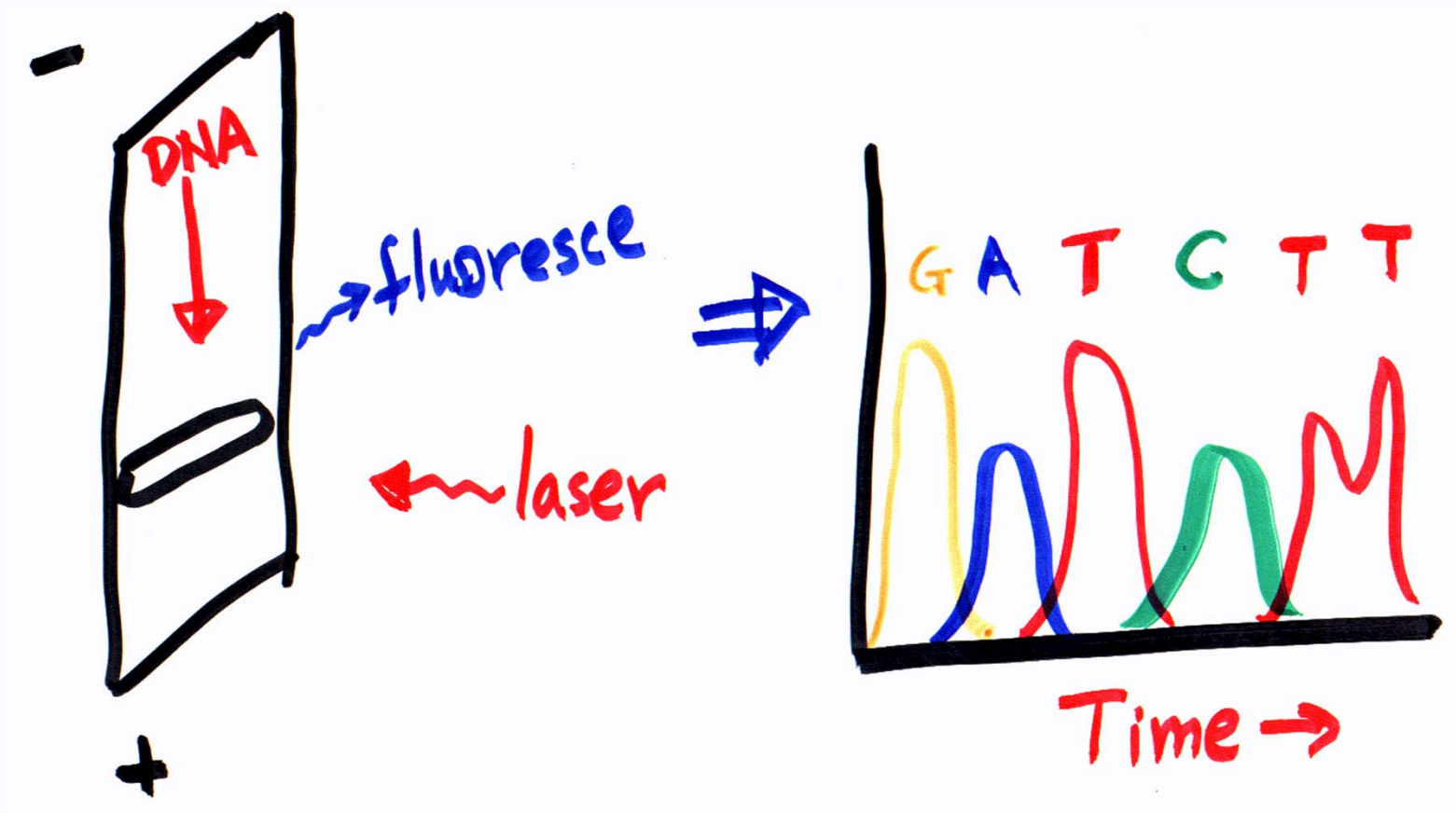


New DNA strands are separated
and electrophoresed

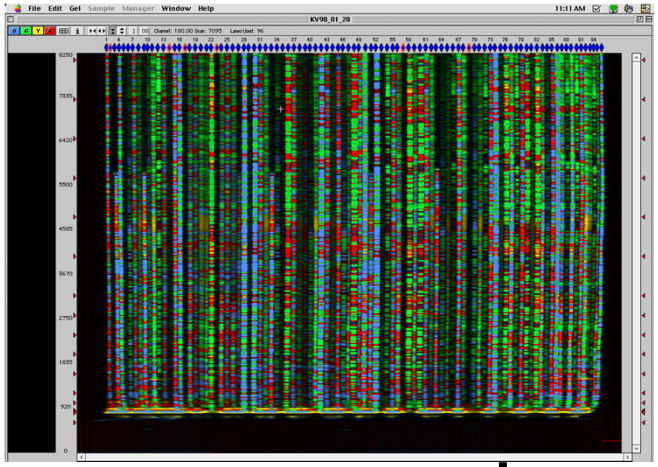
Sanger Method Sequencing Gel

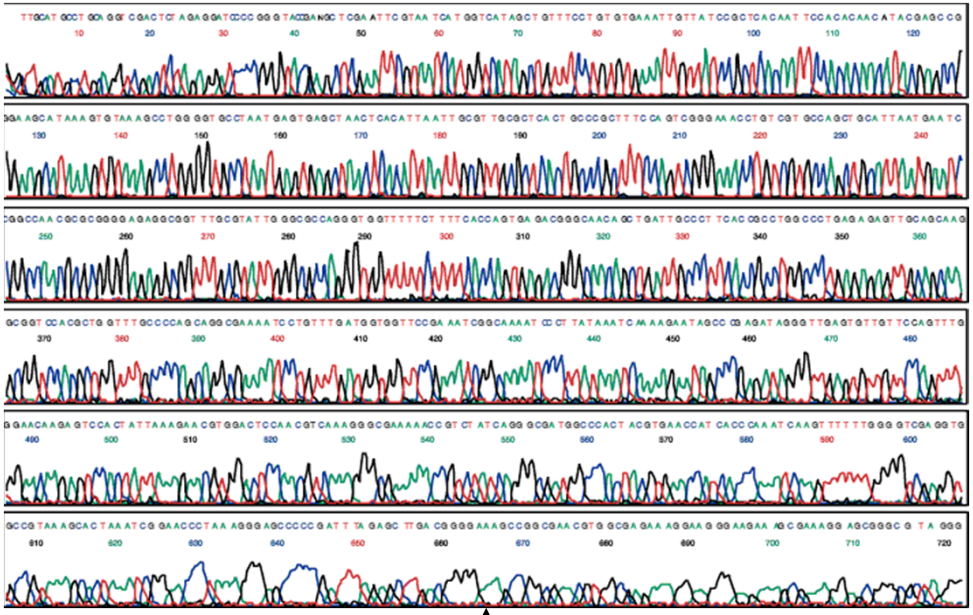


Electrophoresis



Sample Output



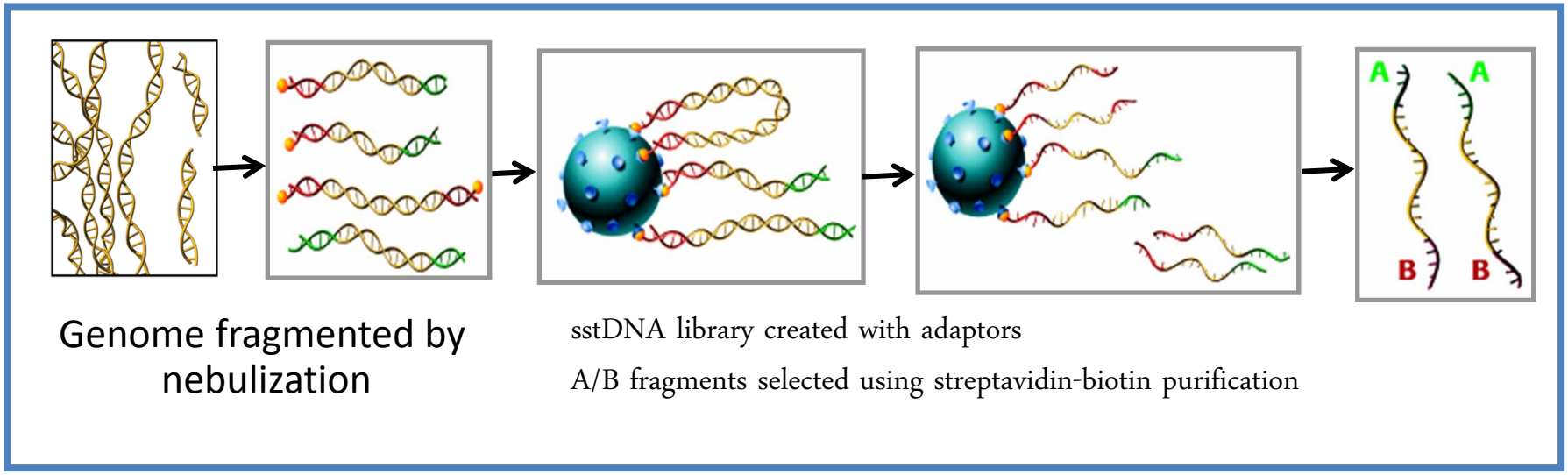


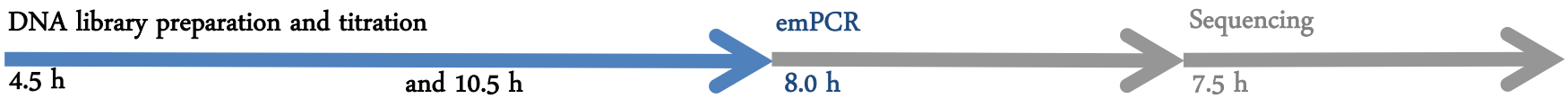
1 lane

454 Pyrosequencing System

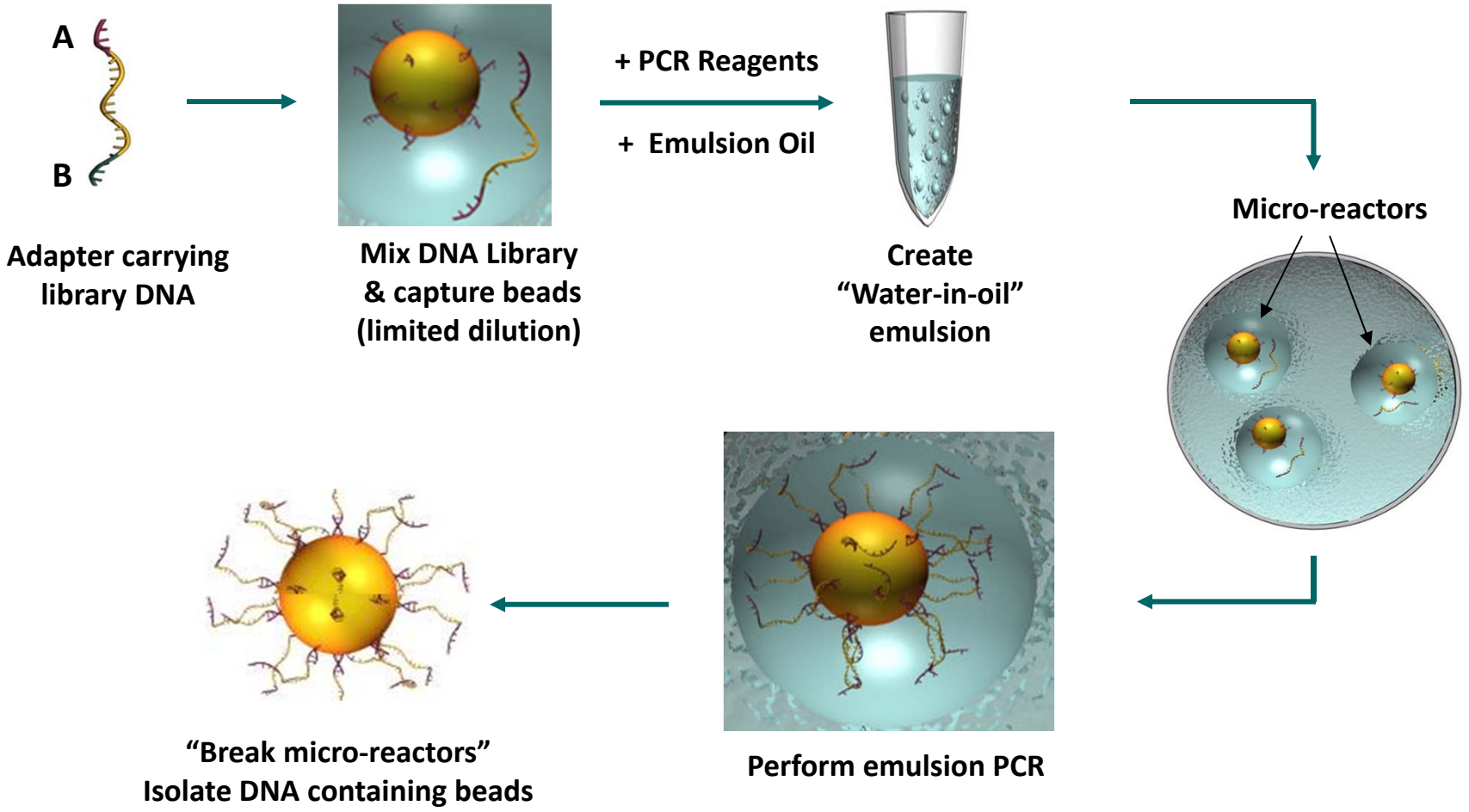
Sequencing Workflow

Library Preparation





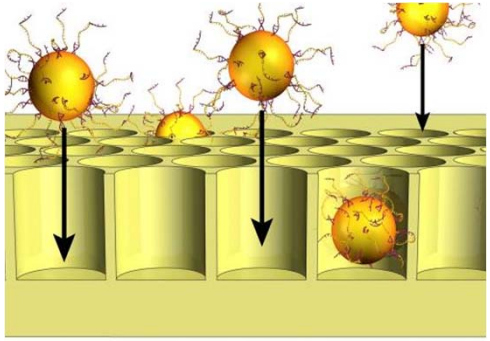
Emulsion Based Clonal Amplification



- Generation of millions of clonally amplified sequencing templates on each bead
- No cloning and colony picking

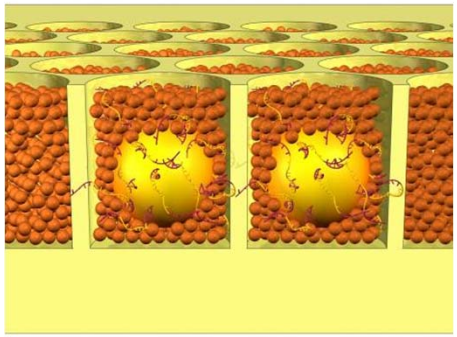
Depositing DNA Beads into the PicoTiter™Plate

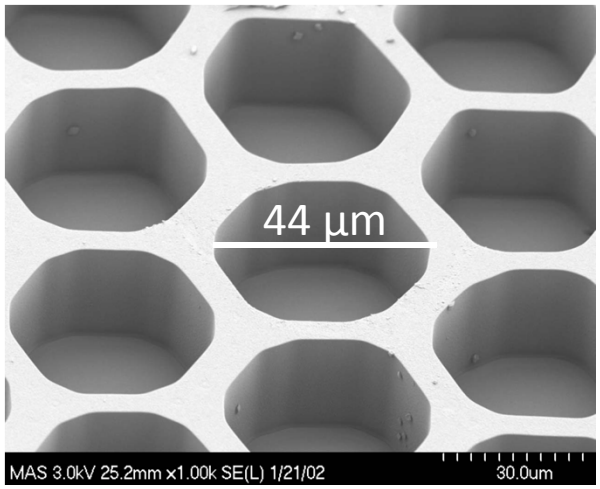
Load beads into PicoTiter™Plate

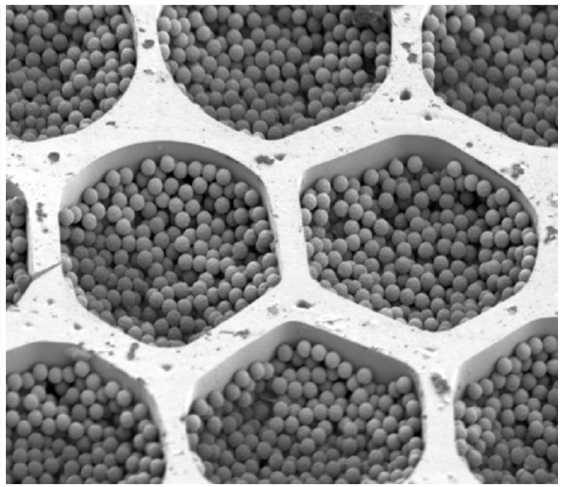


Load Enzyme Beads

Centrifuge Step

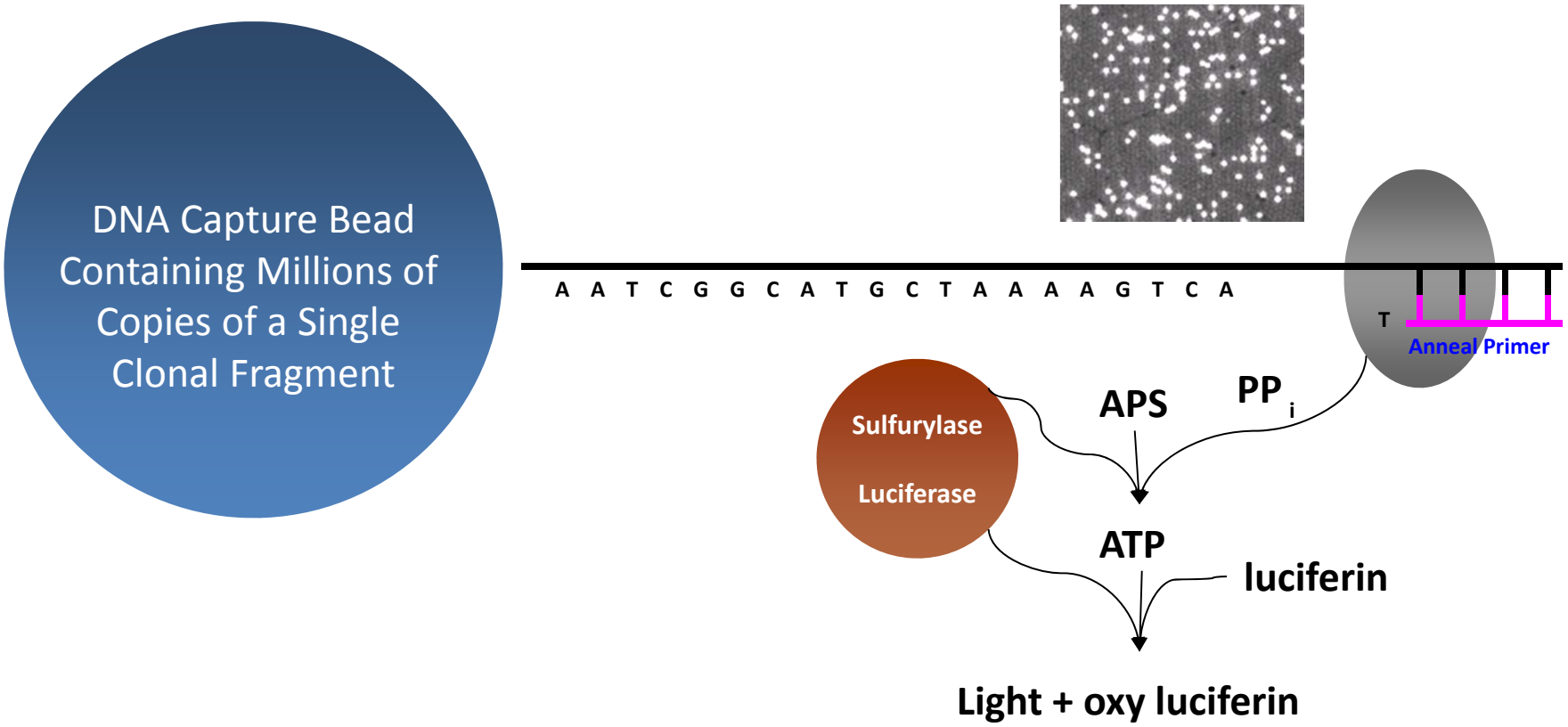






Sequencing-By-Synthesis

- Simultaneous sequencing of the entire genome in hundreds of thousands of picoliter-size wells
- Pyrophosphate signal generation



GS FLX Data Analysis

Flowgram Generation