

Setting up your Sequencing reaction

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What is DNA sequencing?

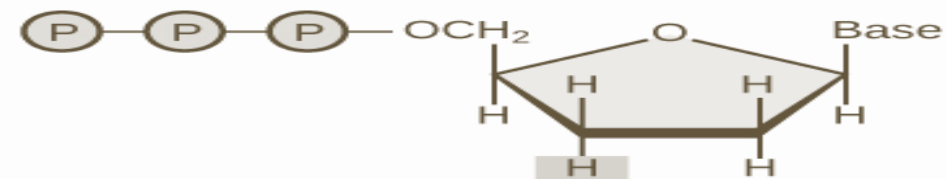
Determining the precise order of nucleotides in DNA.

DNA sequencing is useful in applied fields such as diagnostic or forensic research.

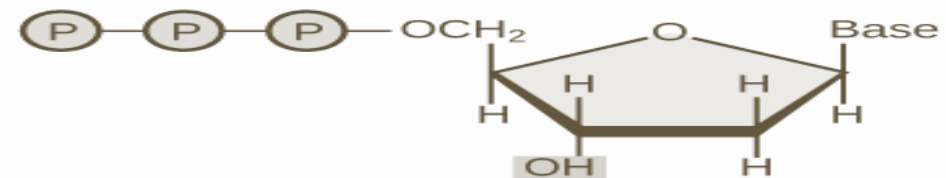
The Sanger method: Frederick Sanger, 1977

Uses dideoxy or (chain terminating) nucleotides to terminate DNA synthesis in addition to the normal nucleotides.

Dideoxy nucleotide similar to deoxy nucleotides but they lack a hydroxyl group on the 3' carbon of the sugar ring.



Dideoxynucleotide (ddNTP)



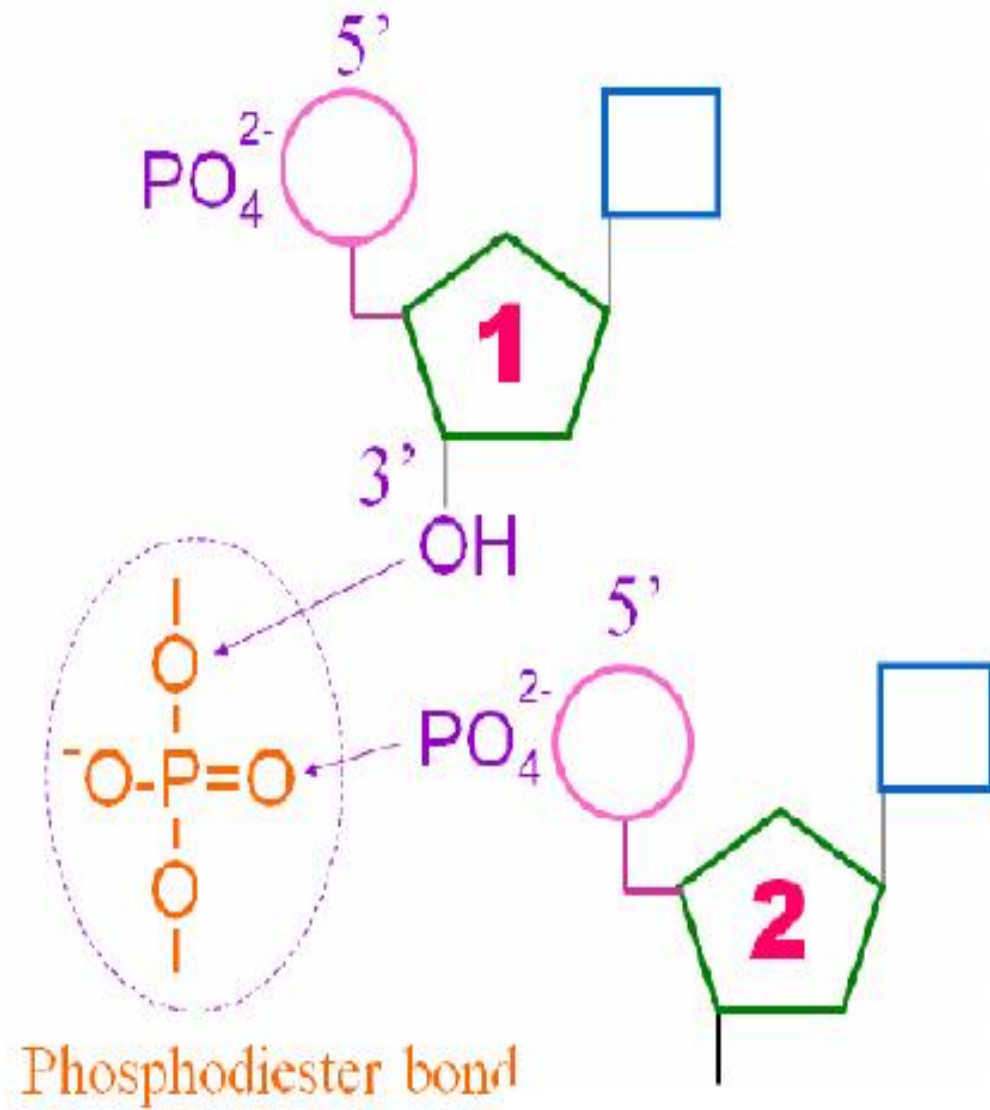
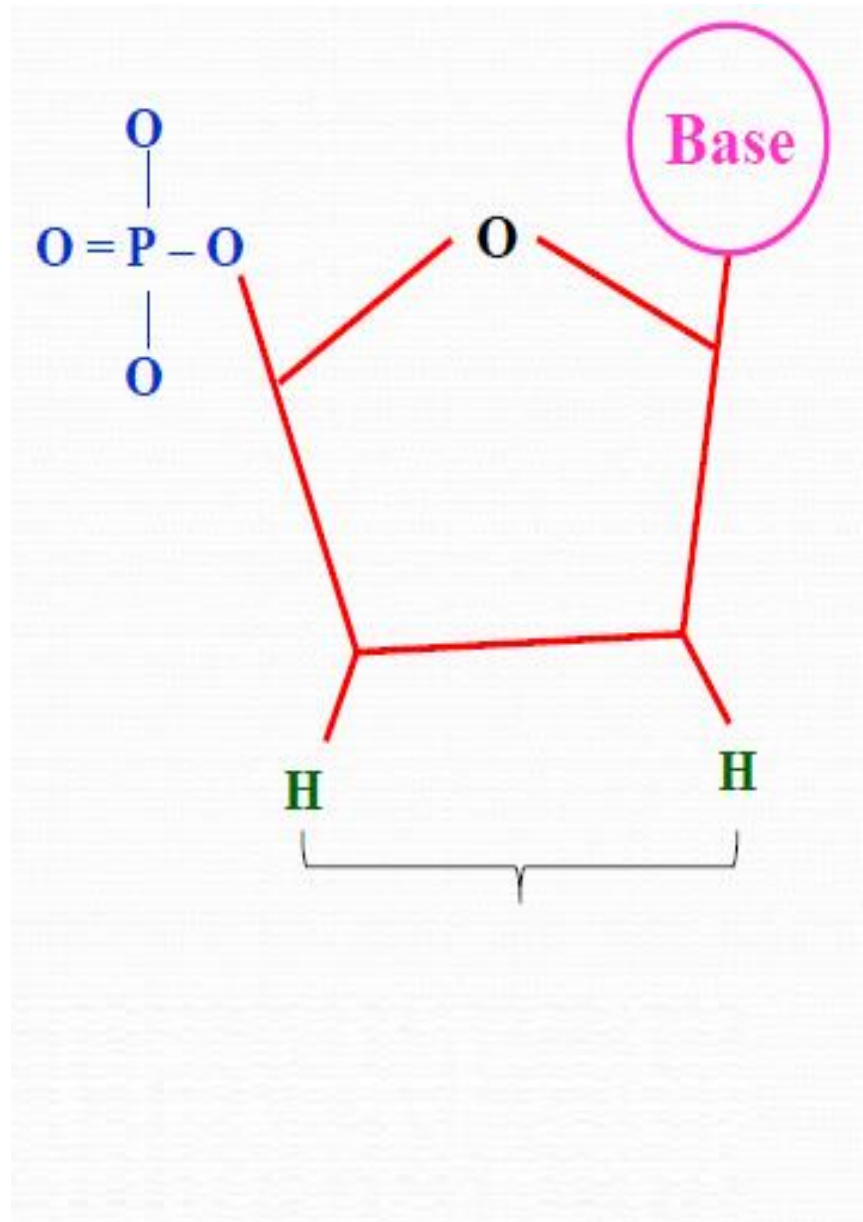
Deoxynucleotide (dNTP)

Dideoxy nucleotide

In the regular nucleotide the 3' hydroxyl group acts as a **hook** allowing a new nucleotide to be added to an existing chain.

Once dideoxy nucleotide has been added, the chain ends and can't form phosphodiester bond with the second coming dNTP.

Dideoxy nucleotide is marked with a particular color of dye depending on the base (A, T, G, or C).

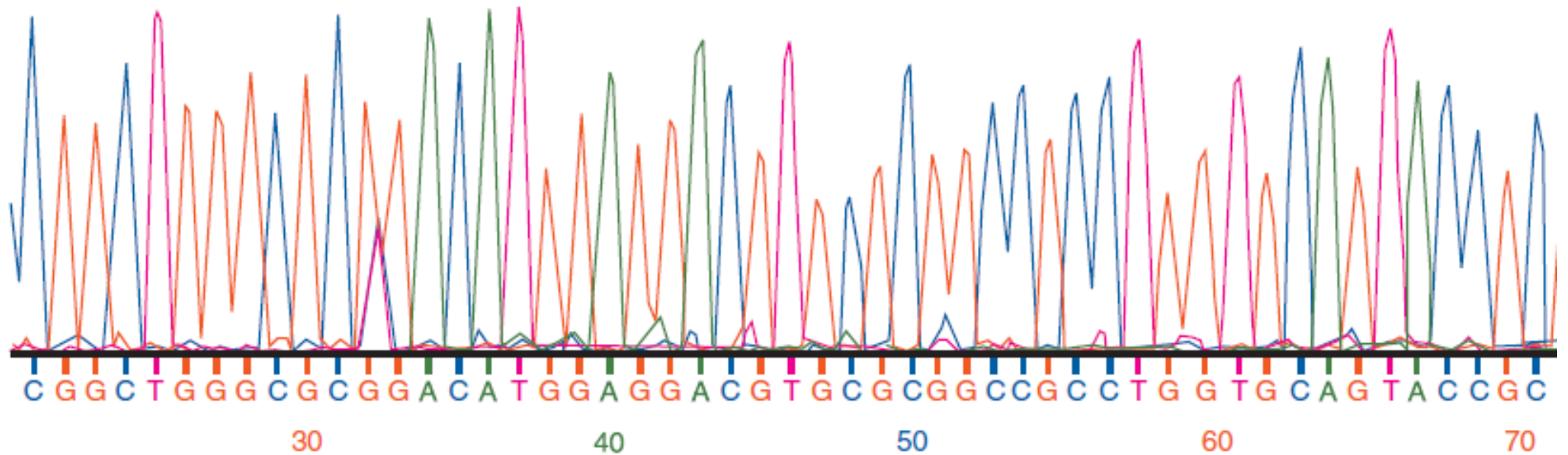


Because they lack the -OH, replication stops

How do we go from this . . .

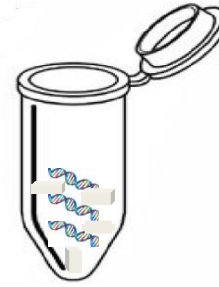
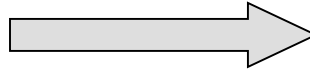


. . . To this?

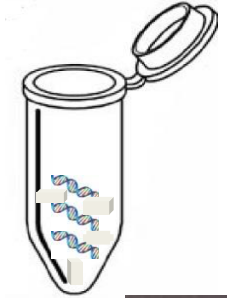
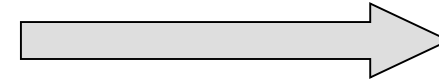


**Samples: Blood
Tissue
Plant,
etc.....**

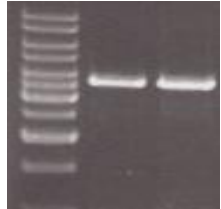
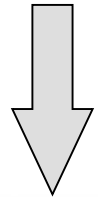
1. DNA isolation



2. PCR amplification



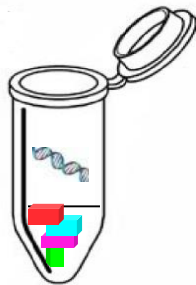
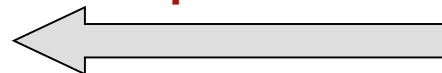
**3. Purify PCR
product**



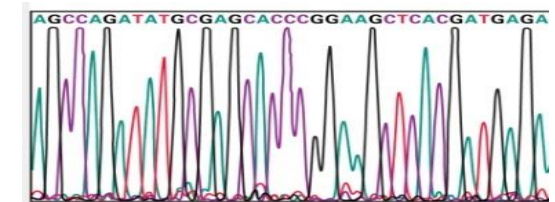
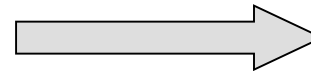
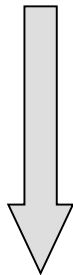
**4. Set up and Perform
sequencing reaction**



**5. Purification PCR
product**

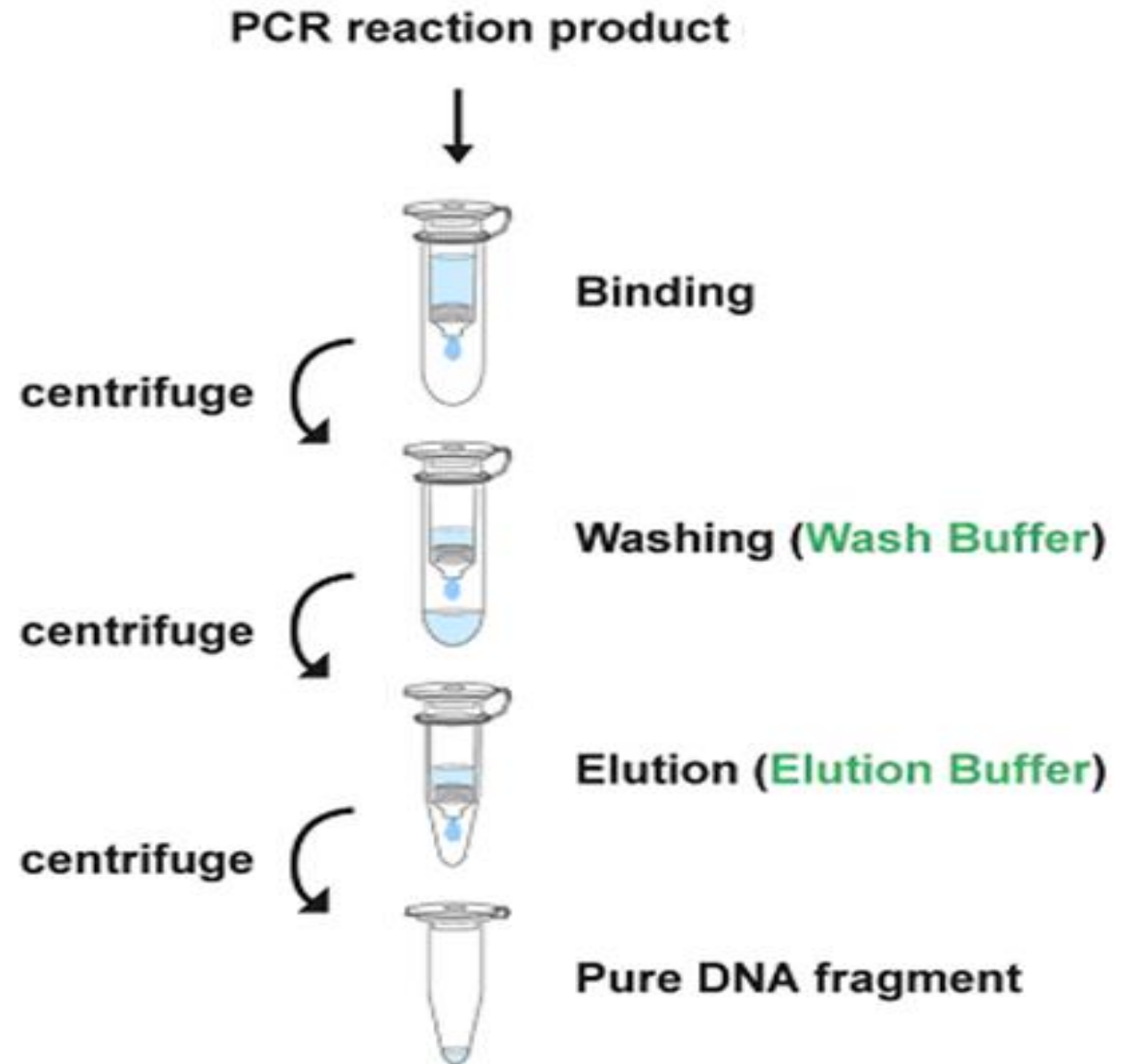
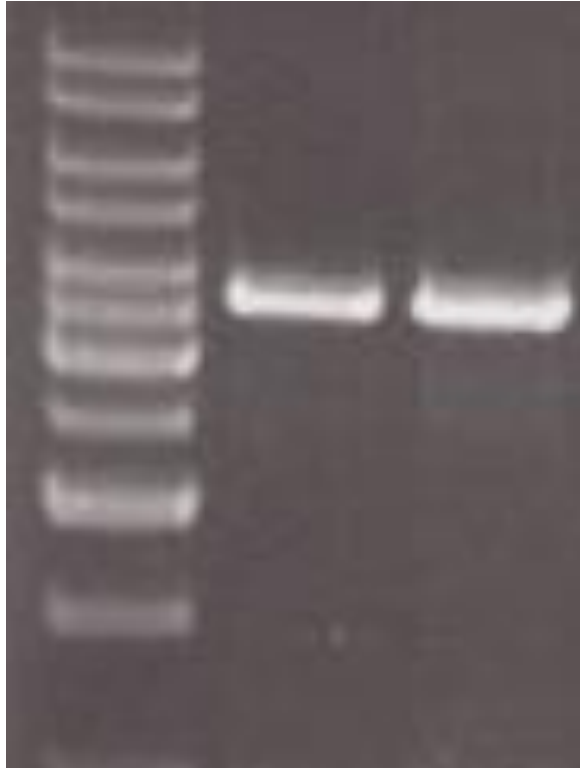


**6. Resolve sequence
fragments**



**7. Read of terminators
(DNA sequence)**

PCR product purification



DNA Quantity

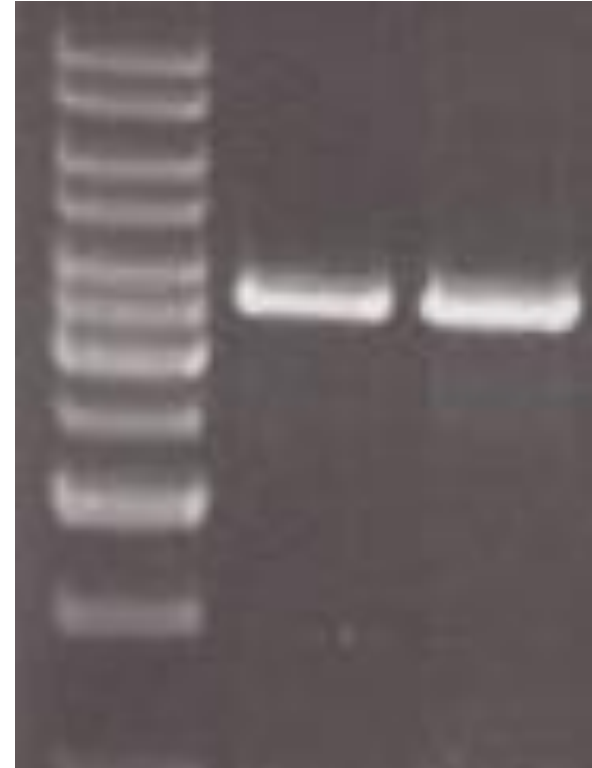
Spectrophotometer 260 nm

PCR product

100-200 bp	1-3 ng
200-500 bp	3-10 ng
500-1000 bp	5-20 ng

Gel electrophoresis

According to the marker bands molecular weight



Preparation of Sequencing Reaction

■ Big-dye terminator sequencing kit (Applied Biosystems)

- 20 μ l reaction:

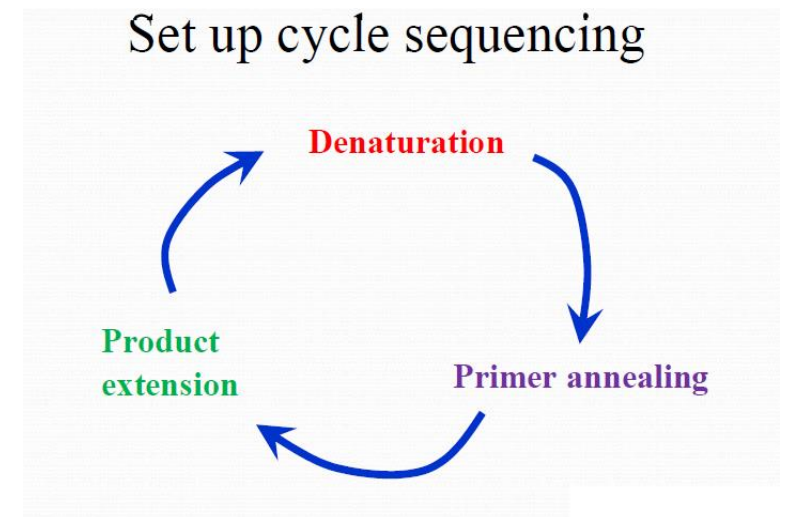
- 4 .0 μ l BigDye Terminator
- 2 .0 μ l Sequencing buffer
- 3.2 μ l Primer (1 pmol)
- - μ l Purified product
- - μ l Deionized water

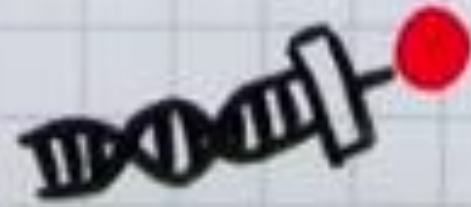
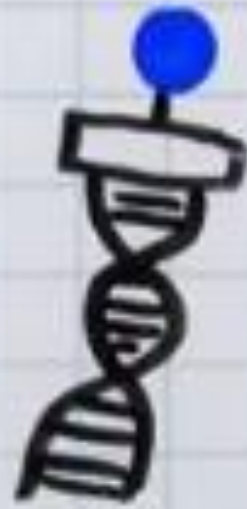
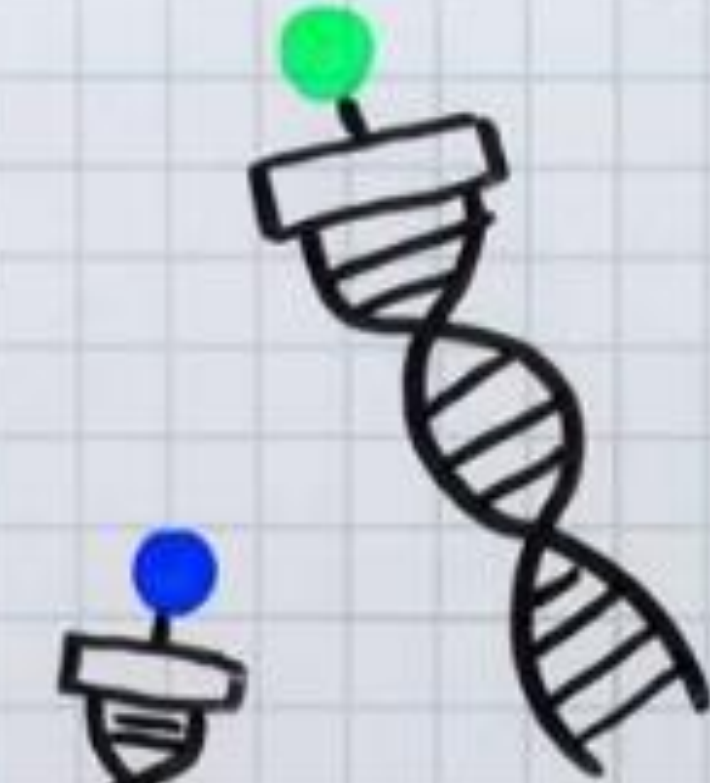
- Program:

- 96 °C 1 min
 - 96 °C 10 sec
 - 50 °C 5 sec
 - 60 °C 4 min
- } 25 cycle

- Fresh reagents
- Shield from light
- Divide reagents
(avoid thawing freezing)

Set up cycle sequencing





ATGCACTGATGCATGAACTAG

TACGTGACTA**A**

TACGTGACTA**C**

TACGTGACTA**CG**

TACGTGACTACGT

TACGTGACTACGT**A**

TACGTGACTA

TACGTGACTAC

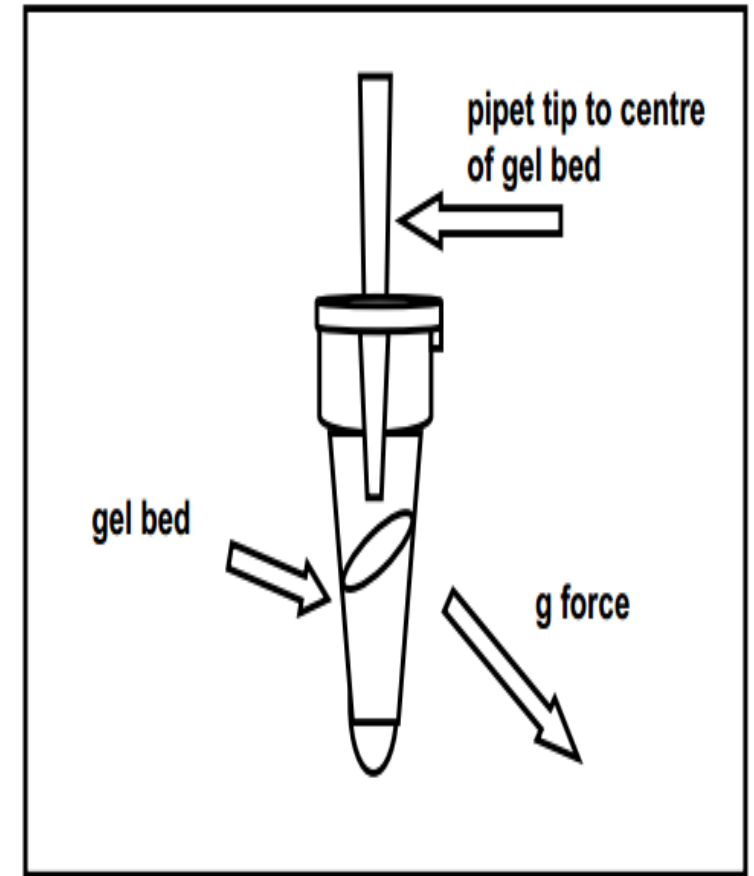
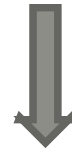
TACGTGACTACG

TACGTGACTACGT

Sequencing Reaction Purification



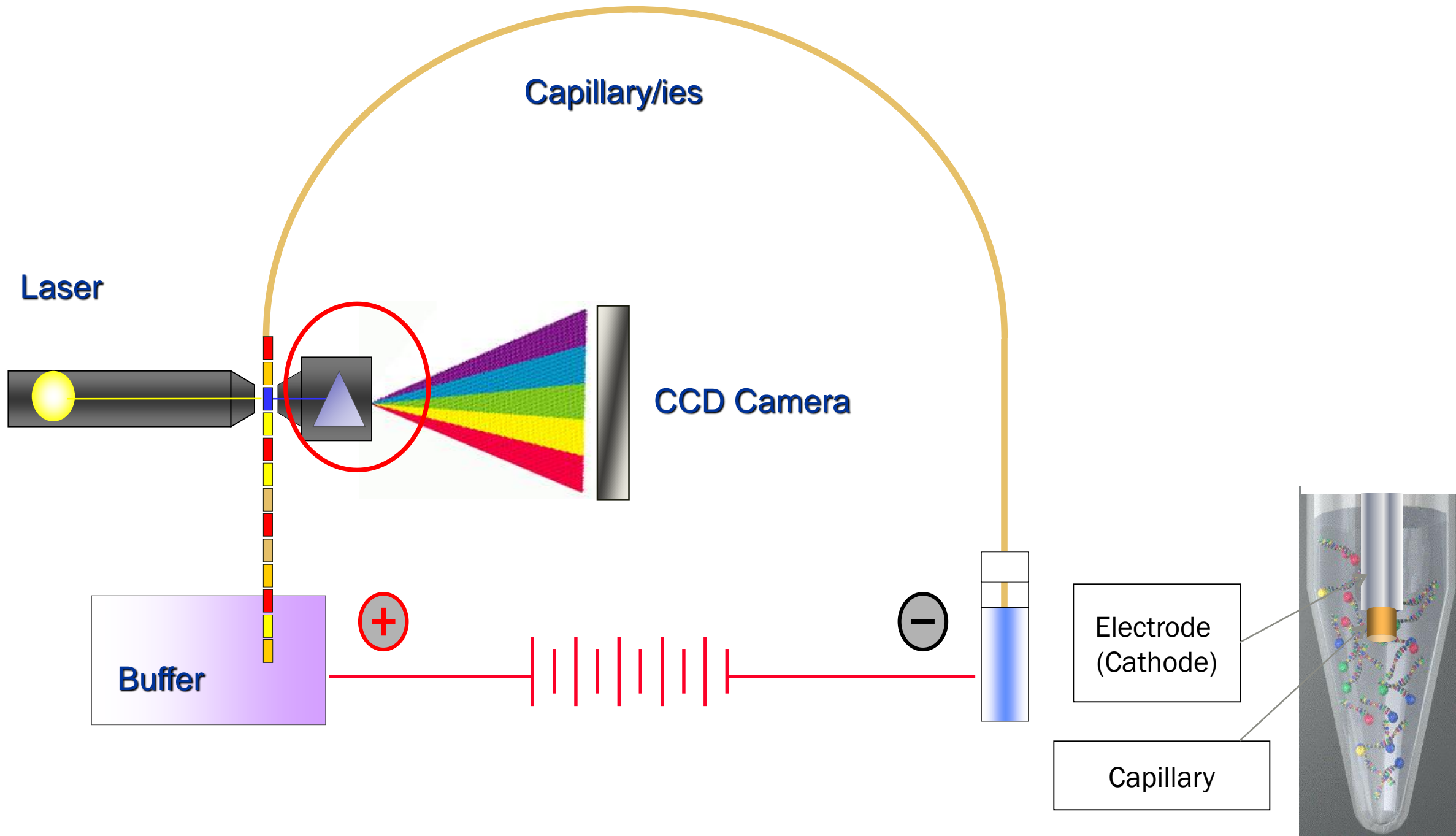
Centri-Sep Protocol

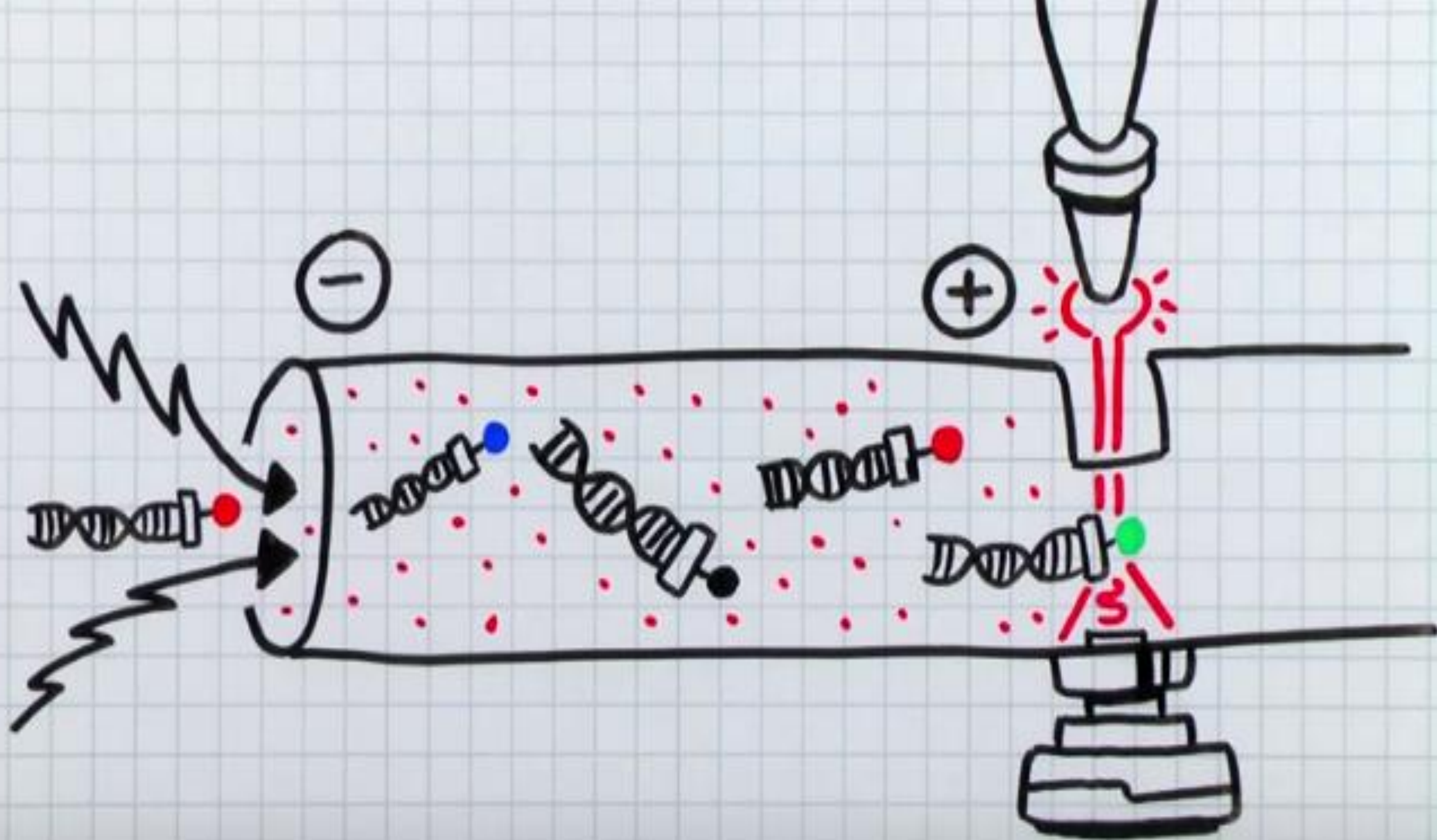


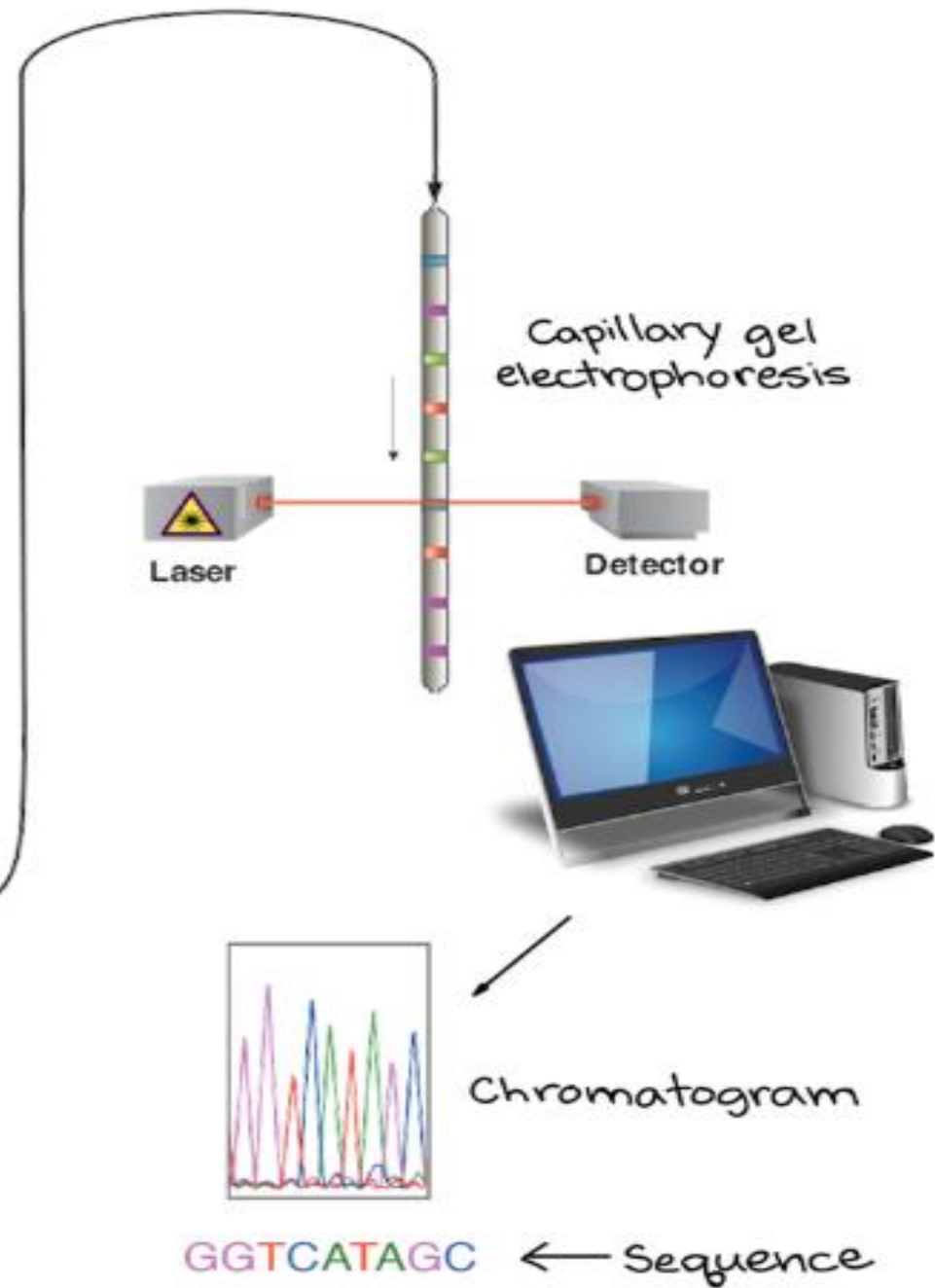
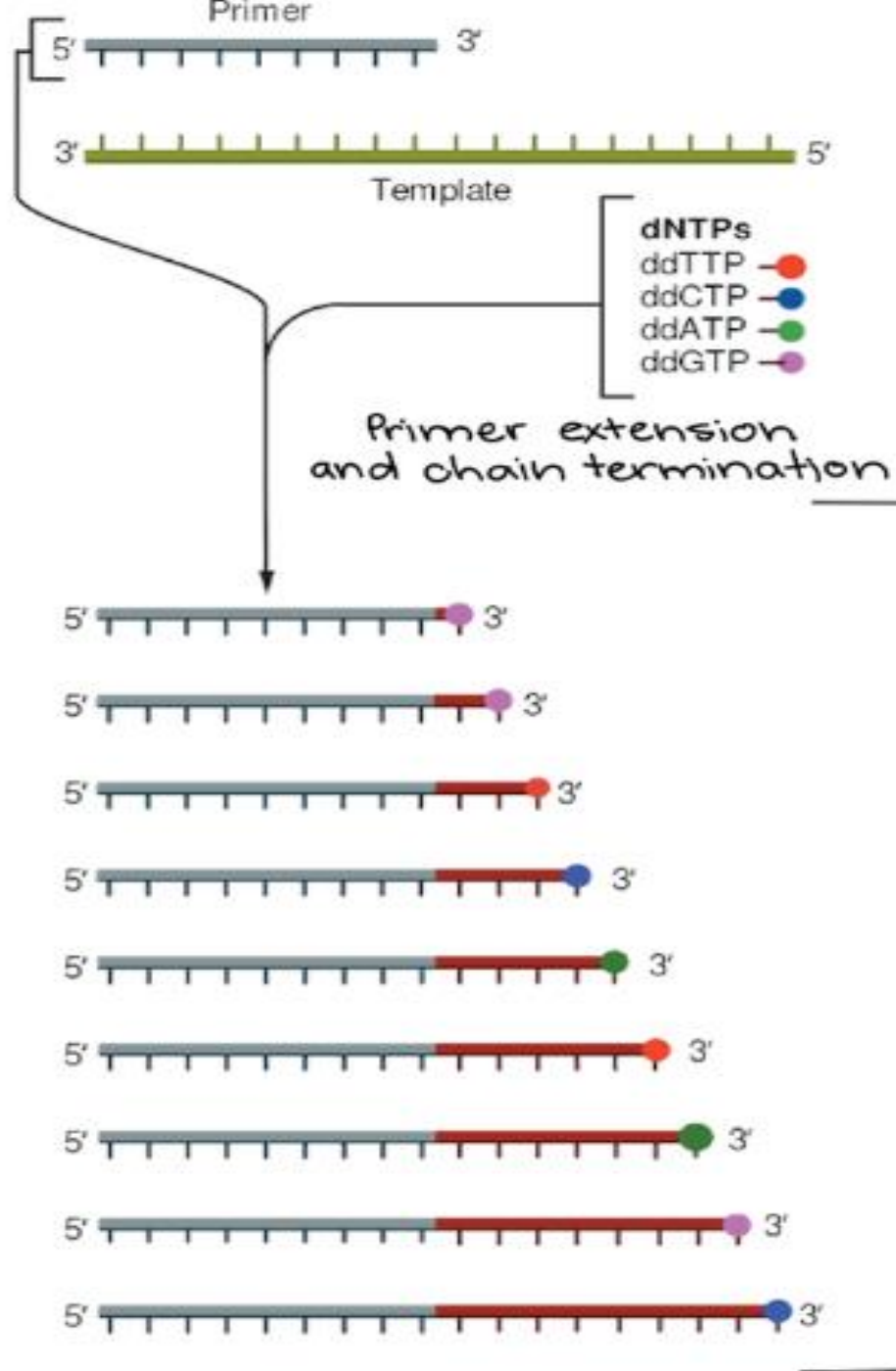
Elution with Hi-Di Formamide

Loading the Reaction on the 310 ABI sequencer



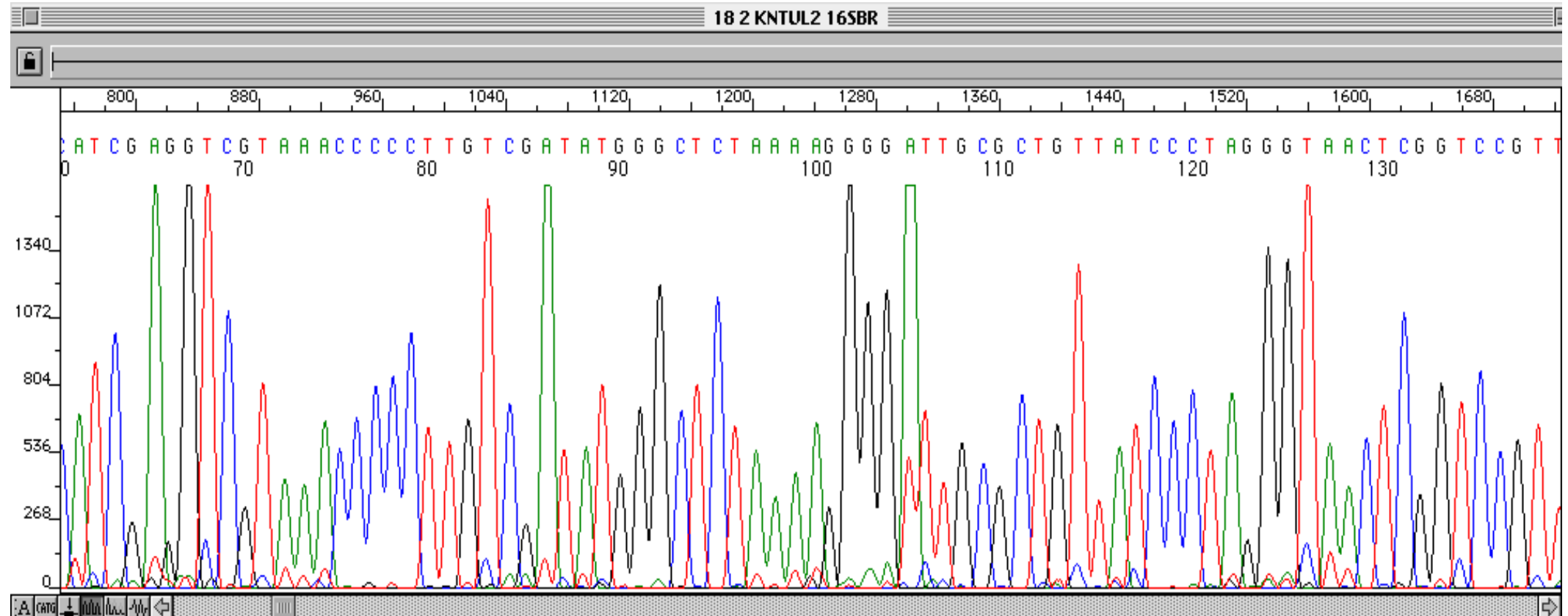






Data output

Data in electropherogram format shows peaks





Thank you