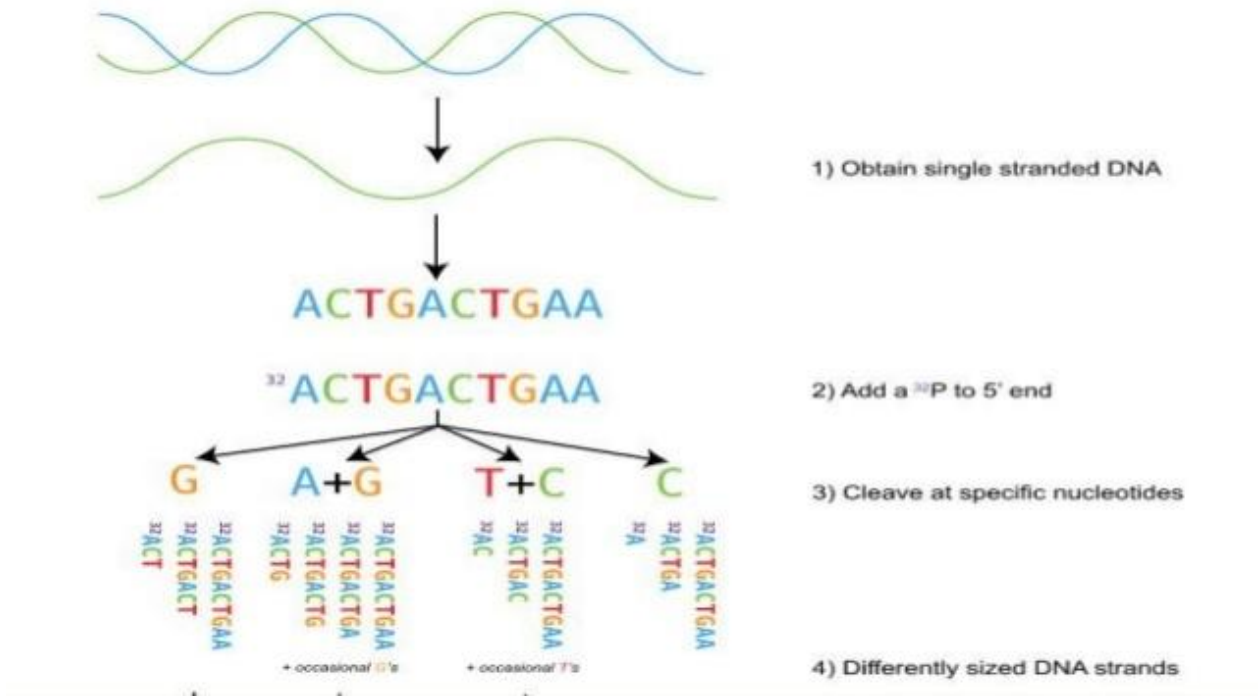
DNA sequencing

Methods

- First methods for DNA sequencing were developed in 1970-ies:

1. Maxam-Gilbert “sequencing by chemical degradation”
2. Sanger’ method of “chain termination”

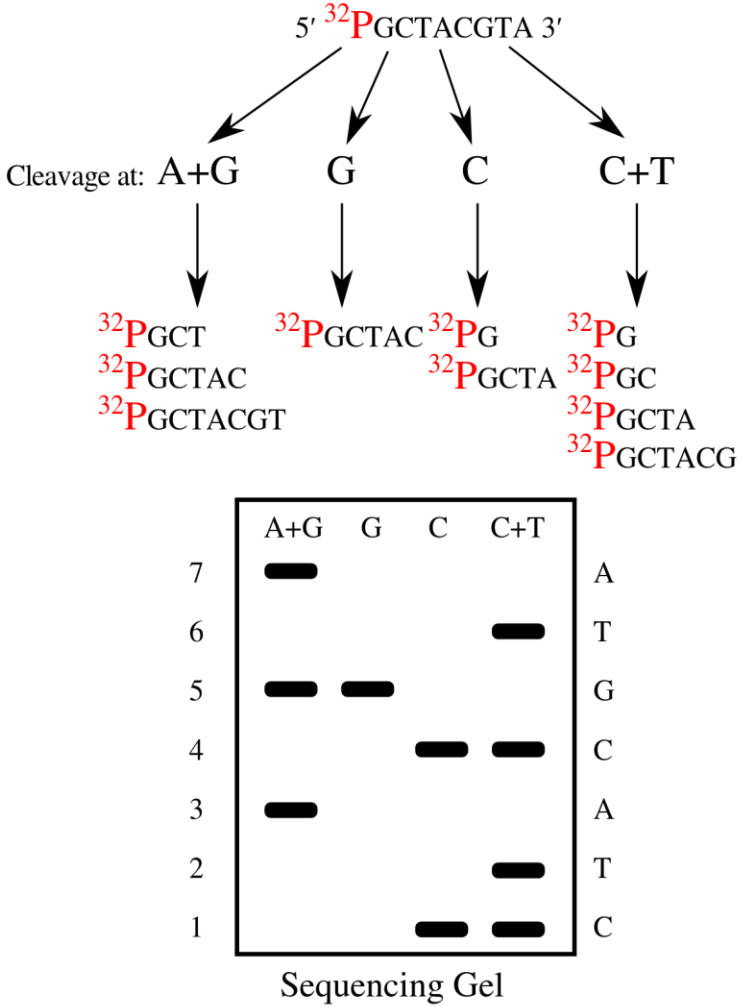
Maxam-Gilbert sequencing



Conditions for cleavage are chosen to introduce on average one modification per DNA molecule.

Maxam-Gilbert sequencing

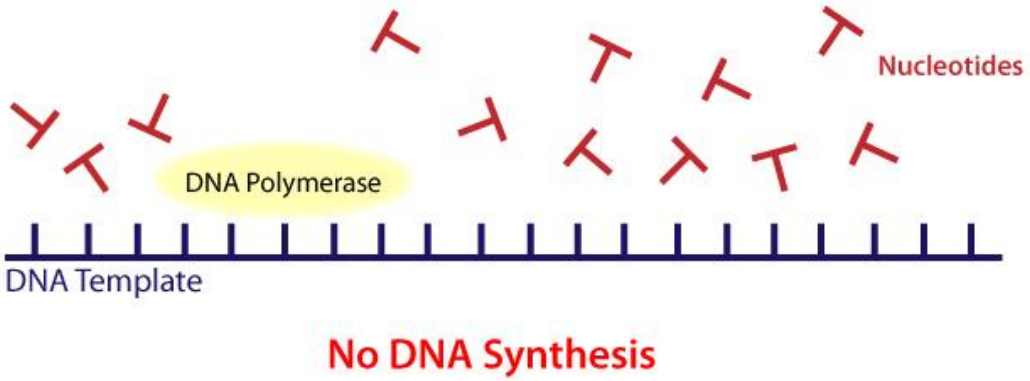
C+T C G A+G



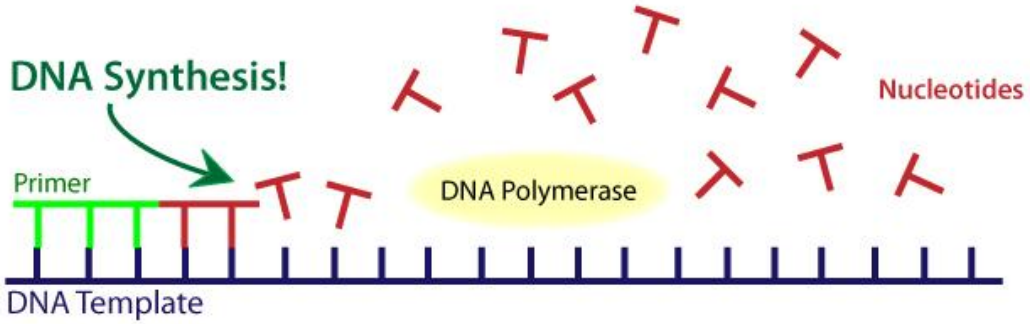
Sanger sequencing

- Method is based on *in vitro* replication of DNA
- Primer extension reaction is performed using regular deoxynucleotide triphosphates (dNTPs), and modified di-deoxynucleotidetriphosphates (ddNTPs)
- The DNA sample is divided into four separate sequencing reactions, containing all four of the standard deoxynucleotides (dATP, dGTP, dCTP and dTTP) and the DNA polymerase. To each reaction is added only one of the four dideoxynucleotides (ddATP, ddGTP, ddCTP, or ddTTP), while the other added nucleotides are ordinary ones.

Primer extension reaction.



No DNA Synthesis



Sanger sequencing

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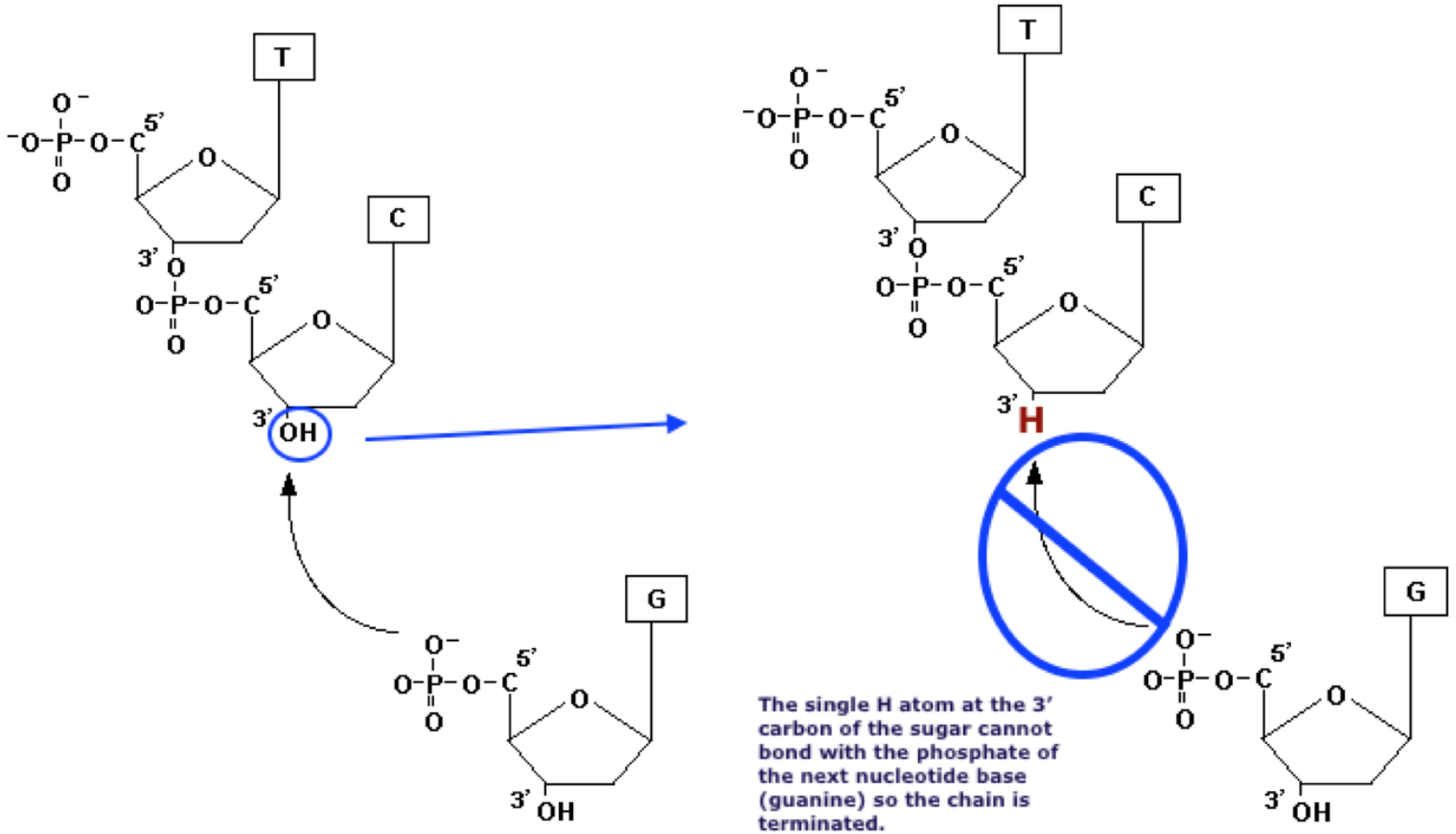
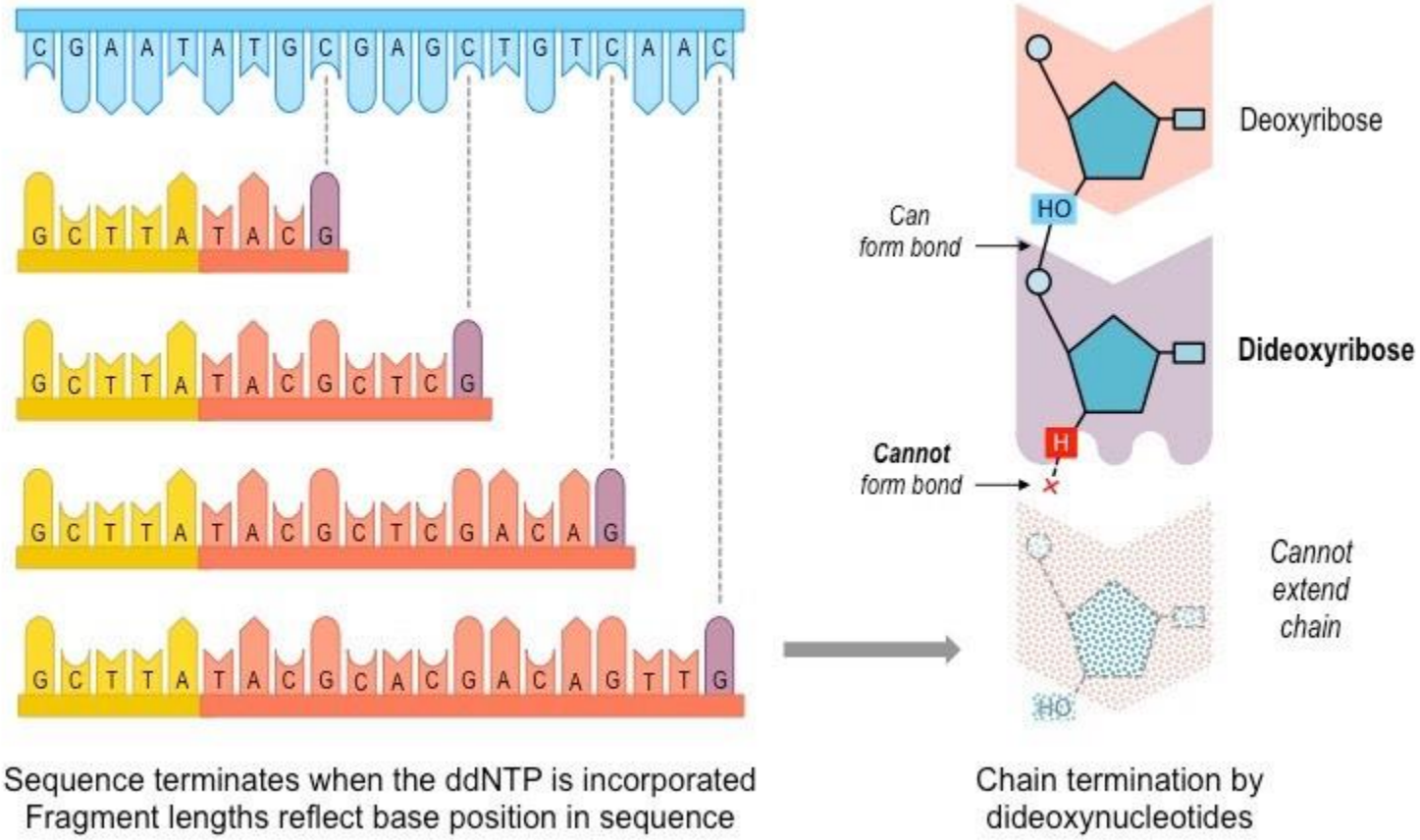
- he dideoxynucleotide is added to be approximately 100-fold lower in concentration than the corresponding deoxynucleotide

DNA chain termination



DNA chain termination

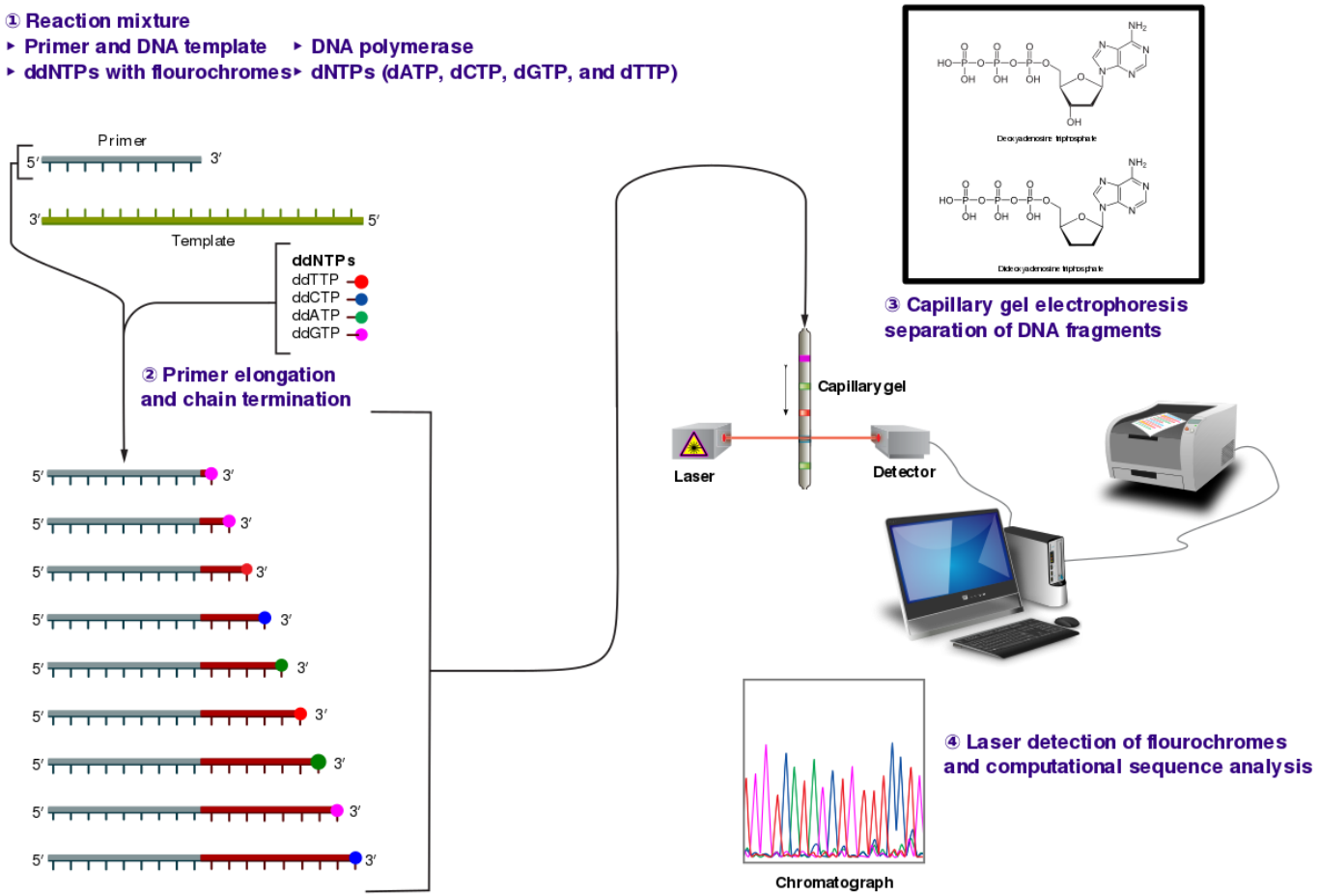


Sequence terminates when the ddNTP is incorporated
Fragment lengths reflect base position in sequence

Chain termination by
dideoxynucleotides

- Traditional Sanger sequencing method used labeling of the growing DNA chain by utilizing a radioactively labeled primer
- Alternatively α-labeled deoxynucleotide triphosphates could be used.

Modern Sanger sequencing

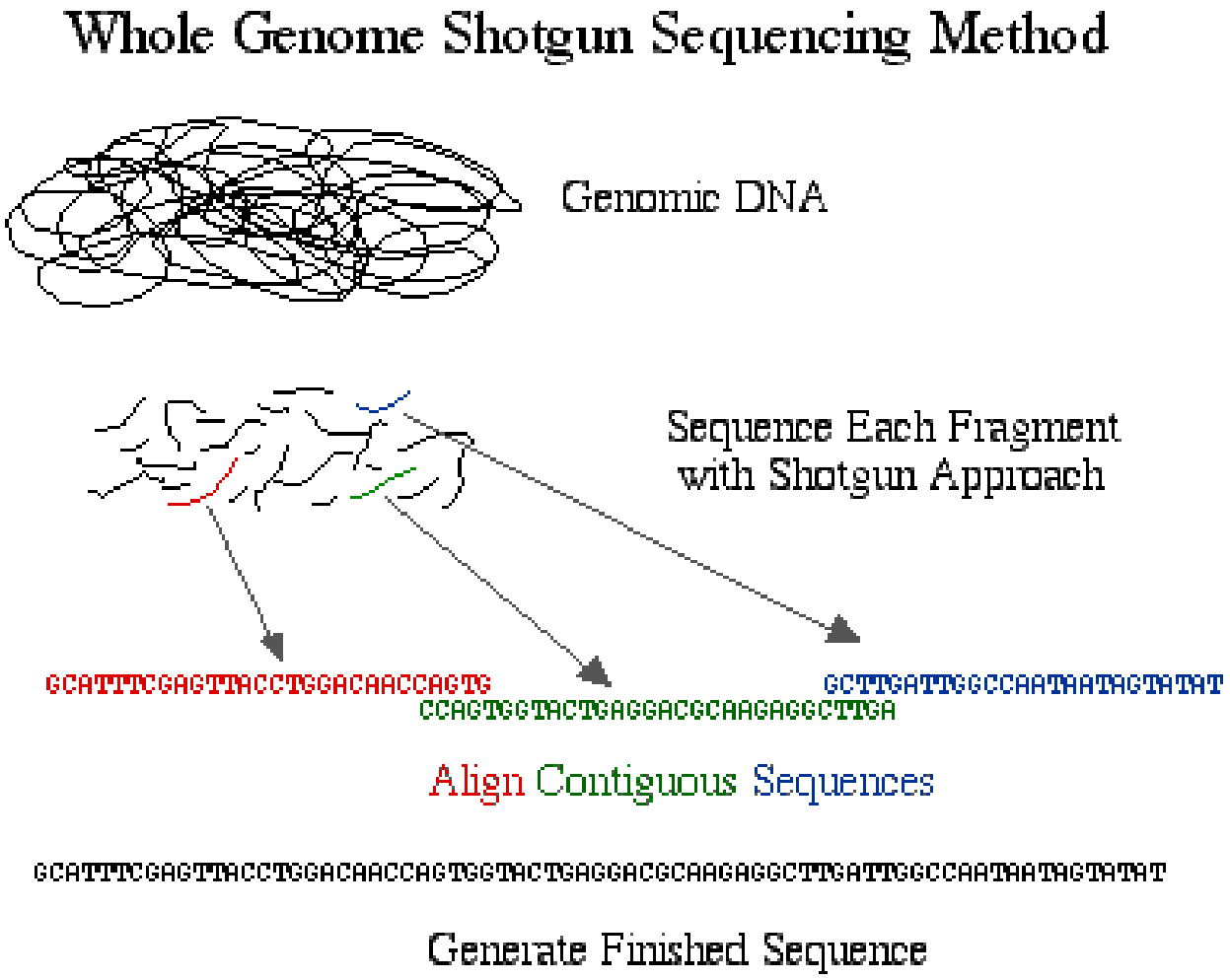


Traditional sequencing methods limitations

- The length of the DNA fragment that could be reliably read from a specific primer is about 700-800 bp
- If we want to read longer fragments we can:

1. Use consecutive primers – “chromosome walk”
2. Divide the fragment in several, read each sub-fragment individually then combine sequences.
3. Use the “shotgun sequencing” approach.

Shotgun sequencing



“Next-generation” sequencing

- New high-throughput sequencing methods were developed in 1990-s and 2000-s producing thousands or millions of sequences concurrently. The generated sequences are analyzed by complex computer programs.
- New methods are currently developed
- Cost of whole genome sequencing experiments has dropped dramatically.