FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

- What is DNA Sequencing ?
- History of development
- Basic Methods- Chain termination and Chemical modification method

What is DNA Sequencing

- Determining the precise order of nucleotides in DNA.

- We need to determine the order of nucleotide bases in a strand of DNA for sequencing.

The Need for DNA Sequencing

- Gene isolation
- Sequence charaterization
- Forensics
- Molecular Archeology
- Gene Gene Interaction
- Gene Protein Interaction
- Cloning

DNA

- Deoxyribonucleic Acid Stores genetic information
- Four different nucleotides A,T,G,C
- DNA comprises of a long molecule analogous to a chain, while the links of the chain are called Nucleotides
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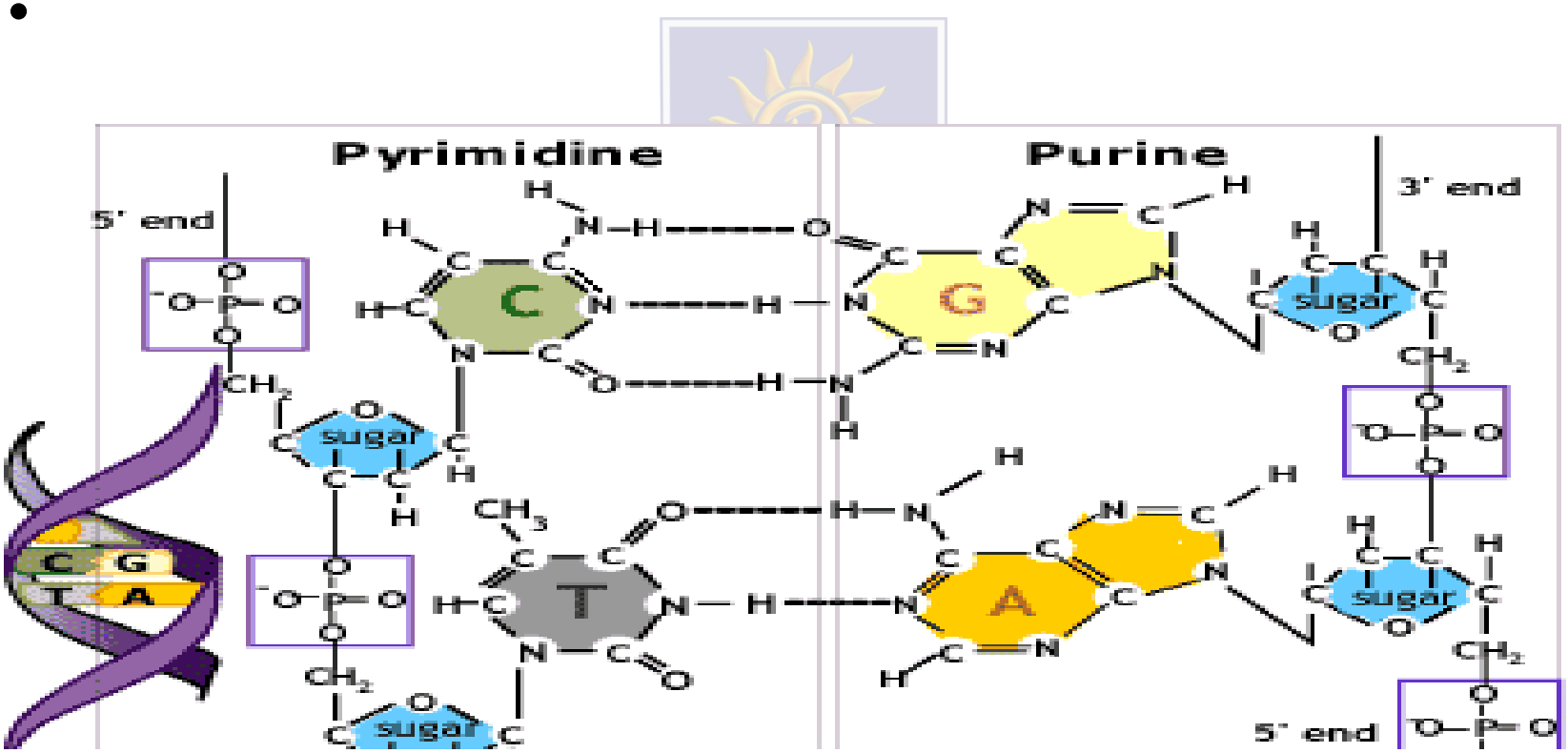


Historical Timeline

1870 – Miescher discovers DNA

1940 - Avery: Proposes DNA as 'Genetic Material' **1953** – Watson & Crick "double helical structure" **1970 -** Wu: Sequences λ Cohesive End DNA **1977** – Sanger: Dideoxy Chain Termination

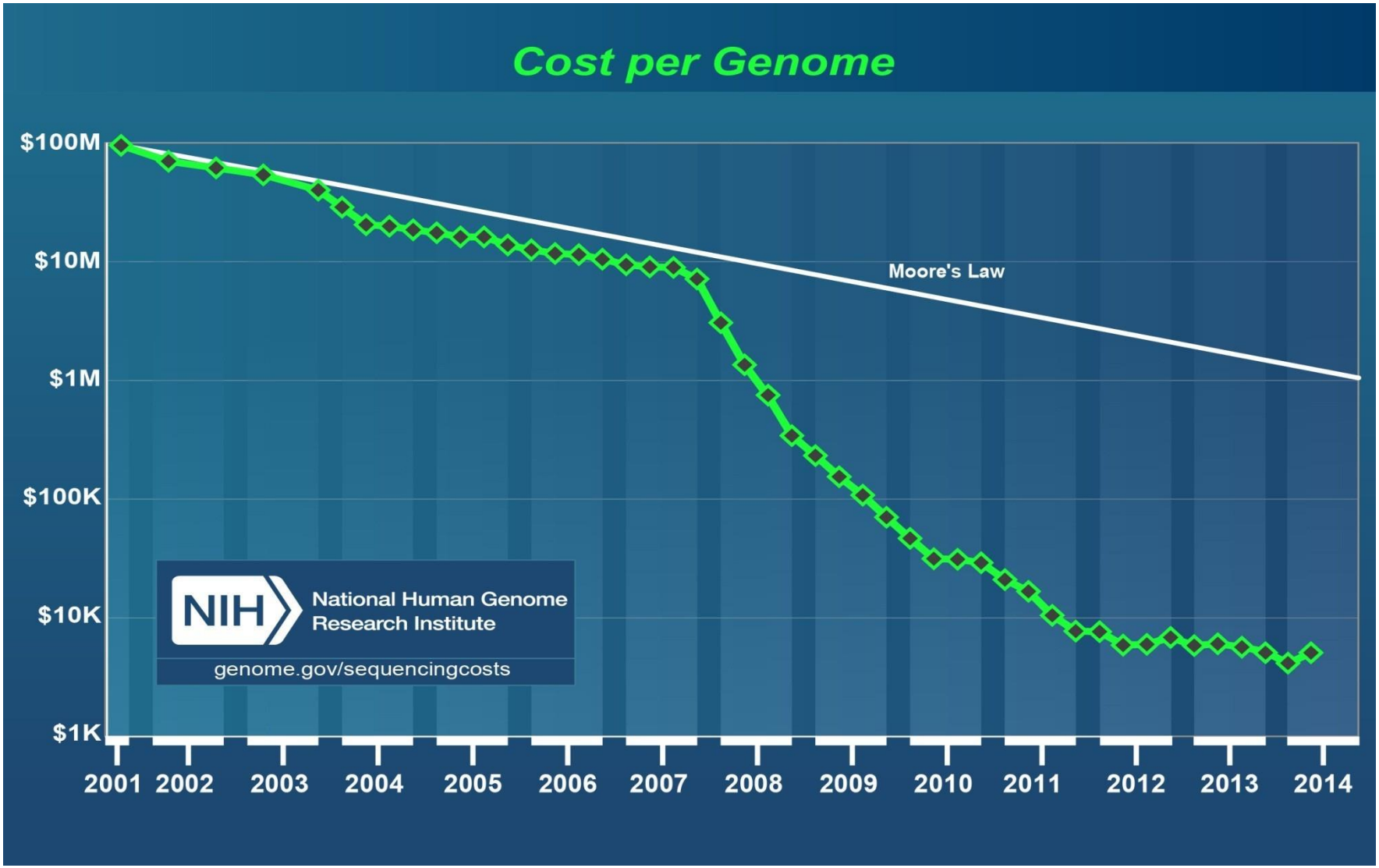
1977 – Gilbert: Chemical Degradation

1986 – Partial Automation

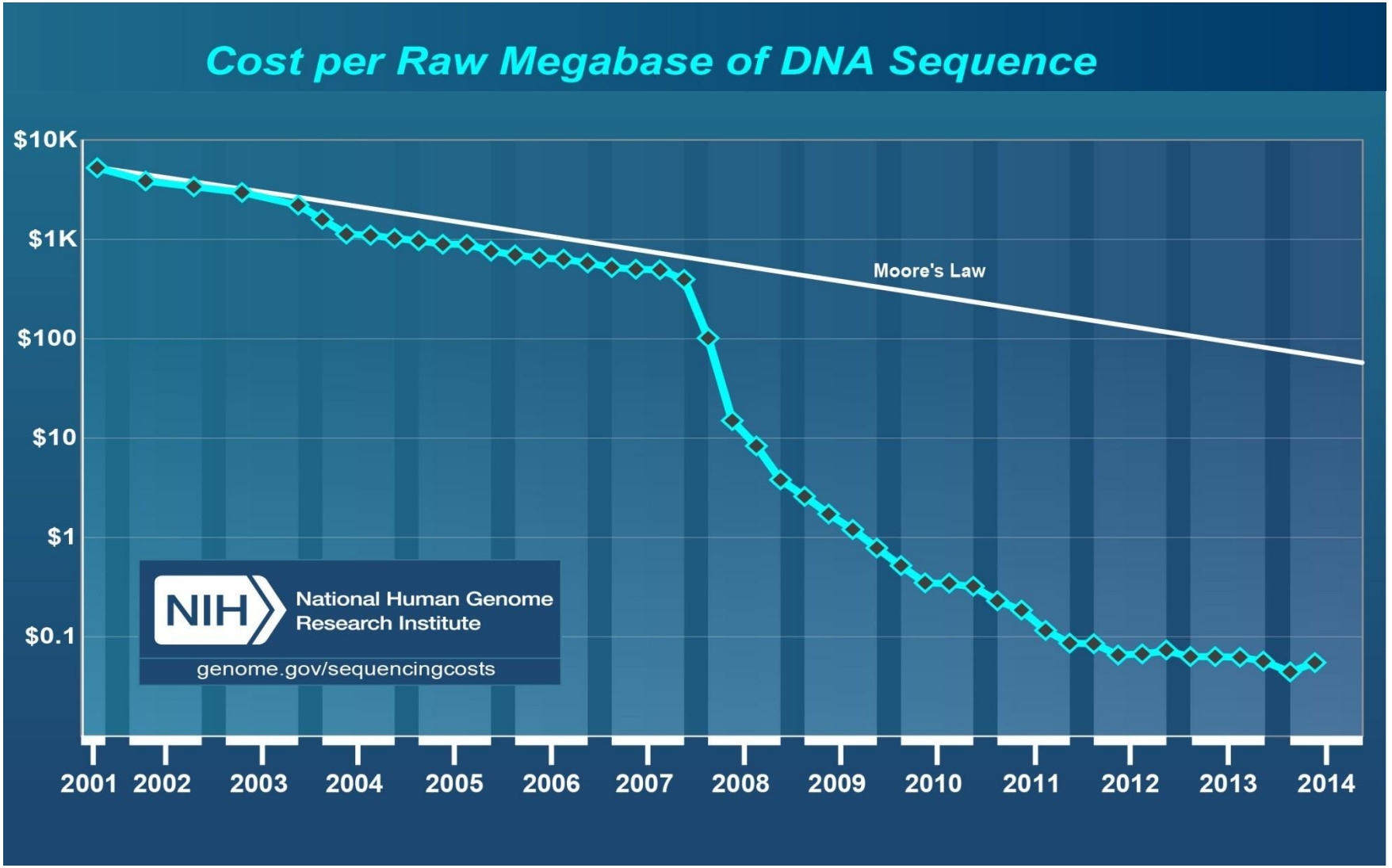
1990 – Cycle Sequencing, Improved Sequencing Enzymes, Improved fluorescent detection schemes

2002 – NGS: 454 , pyro sequencing

Cost per Genome



Cost per Megabases



Sequencing MethodS

- To determine the order of the nucleotide bases adenine, guanine, cytosine, and thymine in a molecule of DNA two methods were used
  1. Maxam and Gilbert; Chemical Sequencing
  2. Sanger; Chain Termination Sequencing
- These two are conventional methods
- Robotics and automated sequencing are based on these methods

Maxam and Gilbert Method

- In 1976–1977, Allan Maxam and Walter Gilbert developed a DNA sequencing method based on chemical modification of DNA and subsequent cleavage at specific bases

  I. Chemical Modification of DNA; radioactive labeling at one 5' end of the DNA (typically by a kinase reaction using gamma-^{32}P ATP)

  II. Purification of the DNA fragment to be sequenced

  III. Chemical treatment generates breaks in DNA

  IV. Run on the gel