

DNA sequencing

Sanger's method
dideoxy sequencing
or chain termination



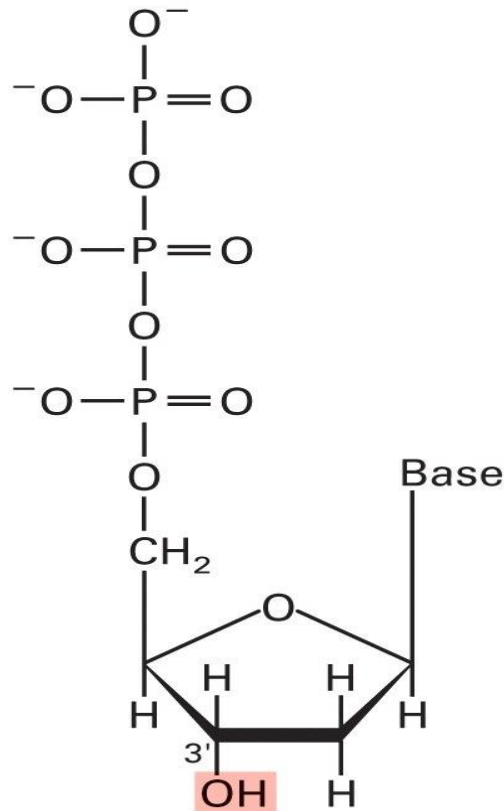
Dr. Suheir Ereqat 2016

Dideoxy (Sanger) Method

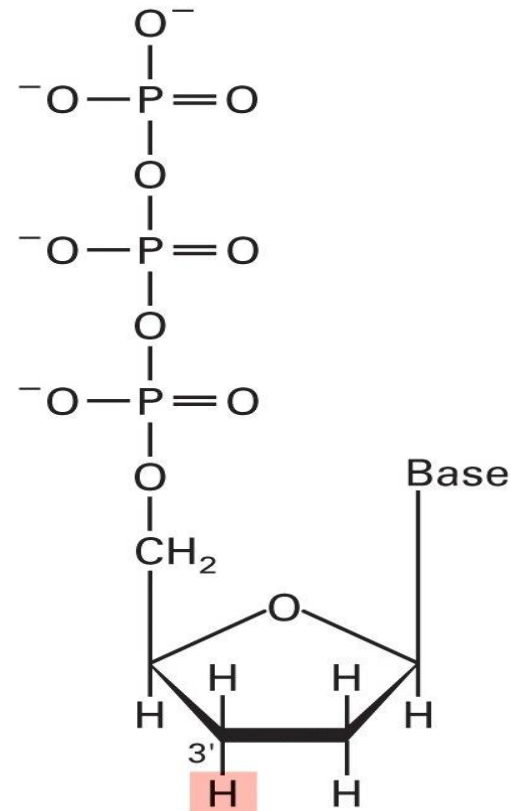
- 4 Steps:
 1. Denaturation
 2. Primer attachment and extension of bases
 3. Termination (ddNTP)
 4. Gel electrophoresis

DNA polymerase will incorporate nucleoside monophosphates,

- ddNTP- 2',3'-dideoxynucleotide (modified)
- No 3' hydroxyl
- **Terminates** chain when incorporated
- Add enough so each ddNTP is randomly and completely incorporated at each base



Deoxyribonucleoside triphosphate (dNTP)

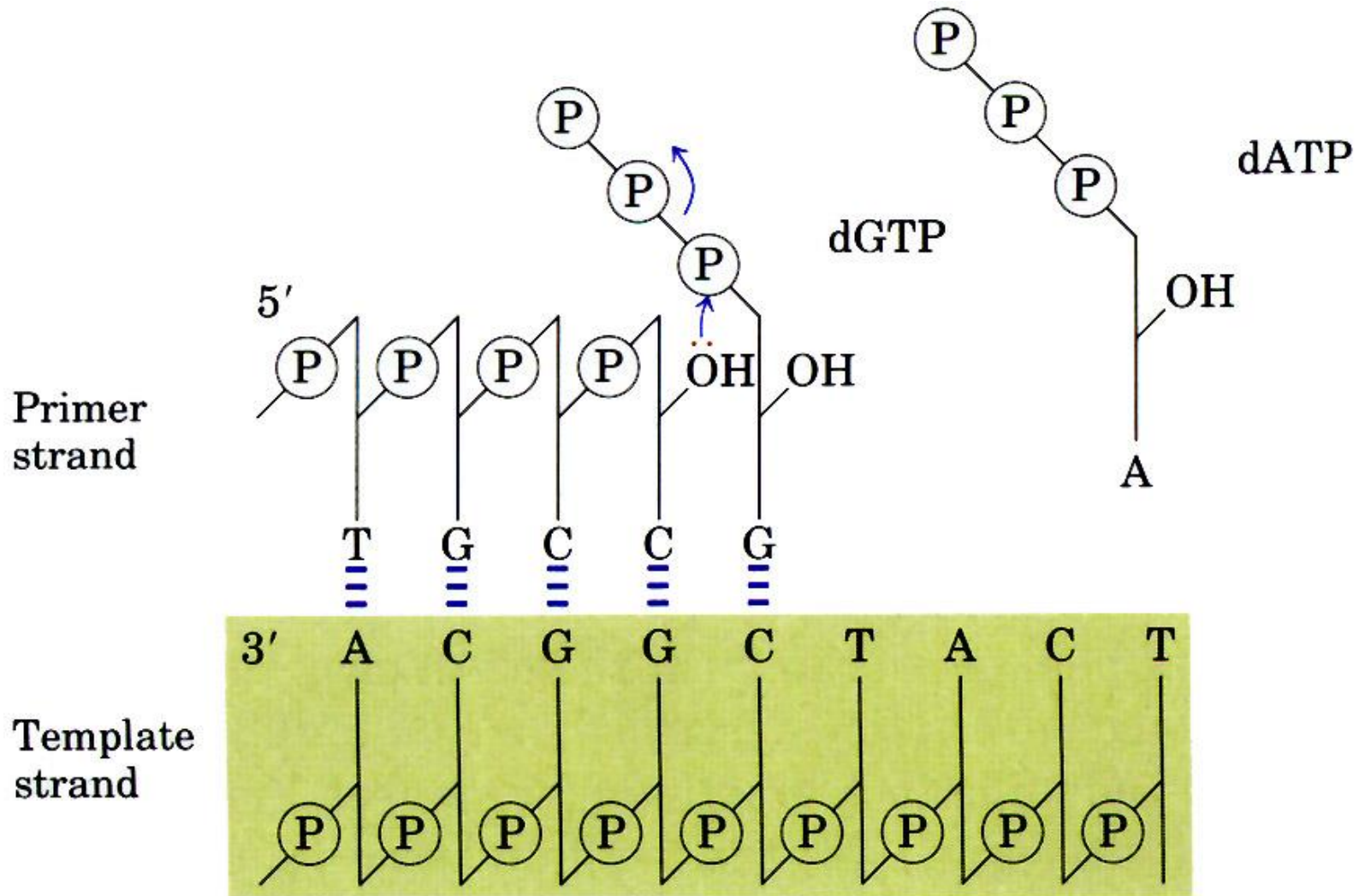


Dideoxynucleoside triphosphate (ddNTP)

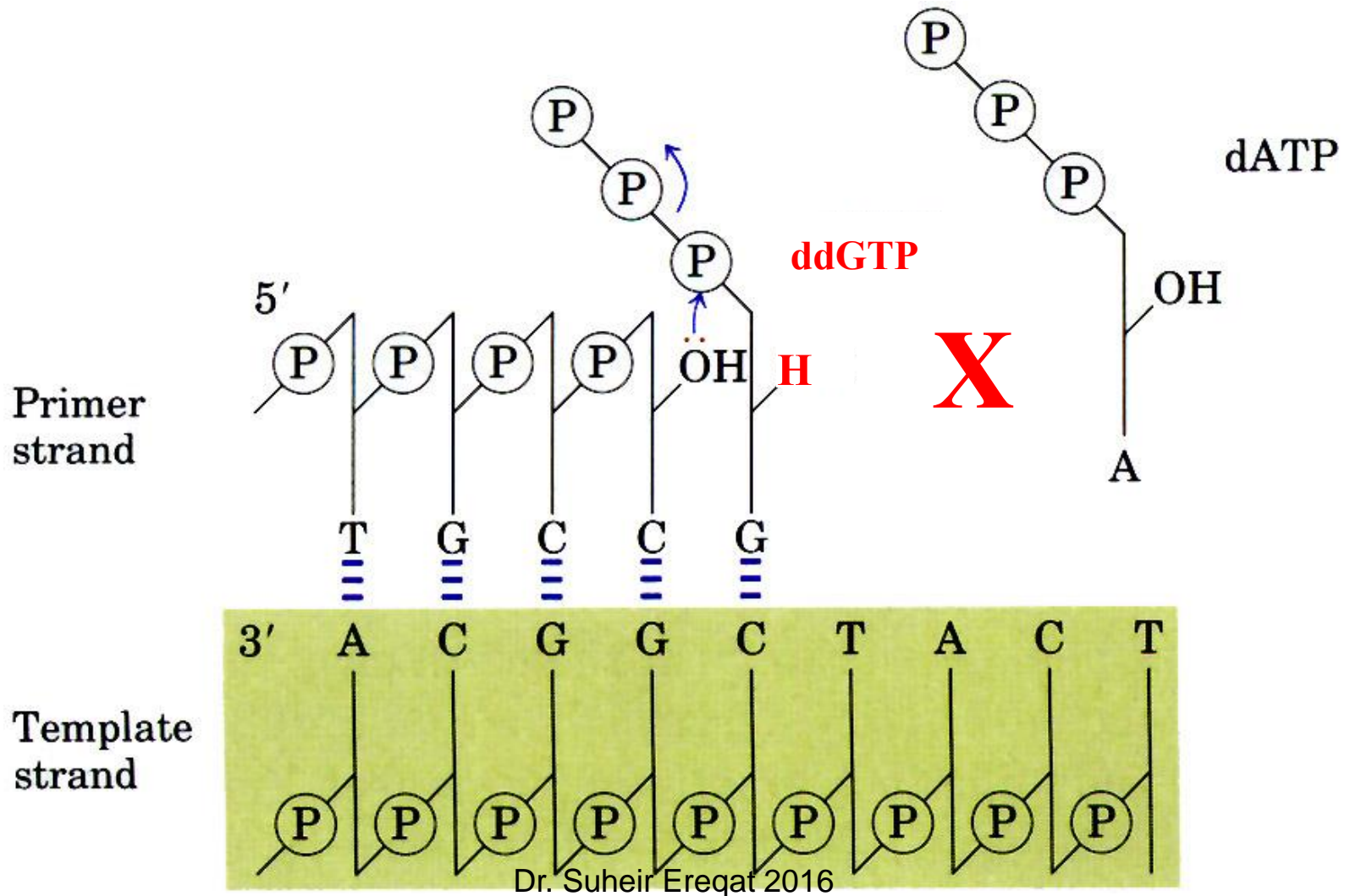
3' OH can be used for phosphodiester bond

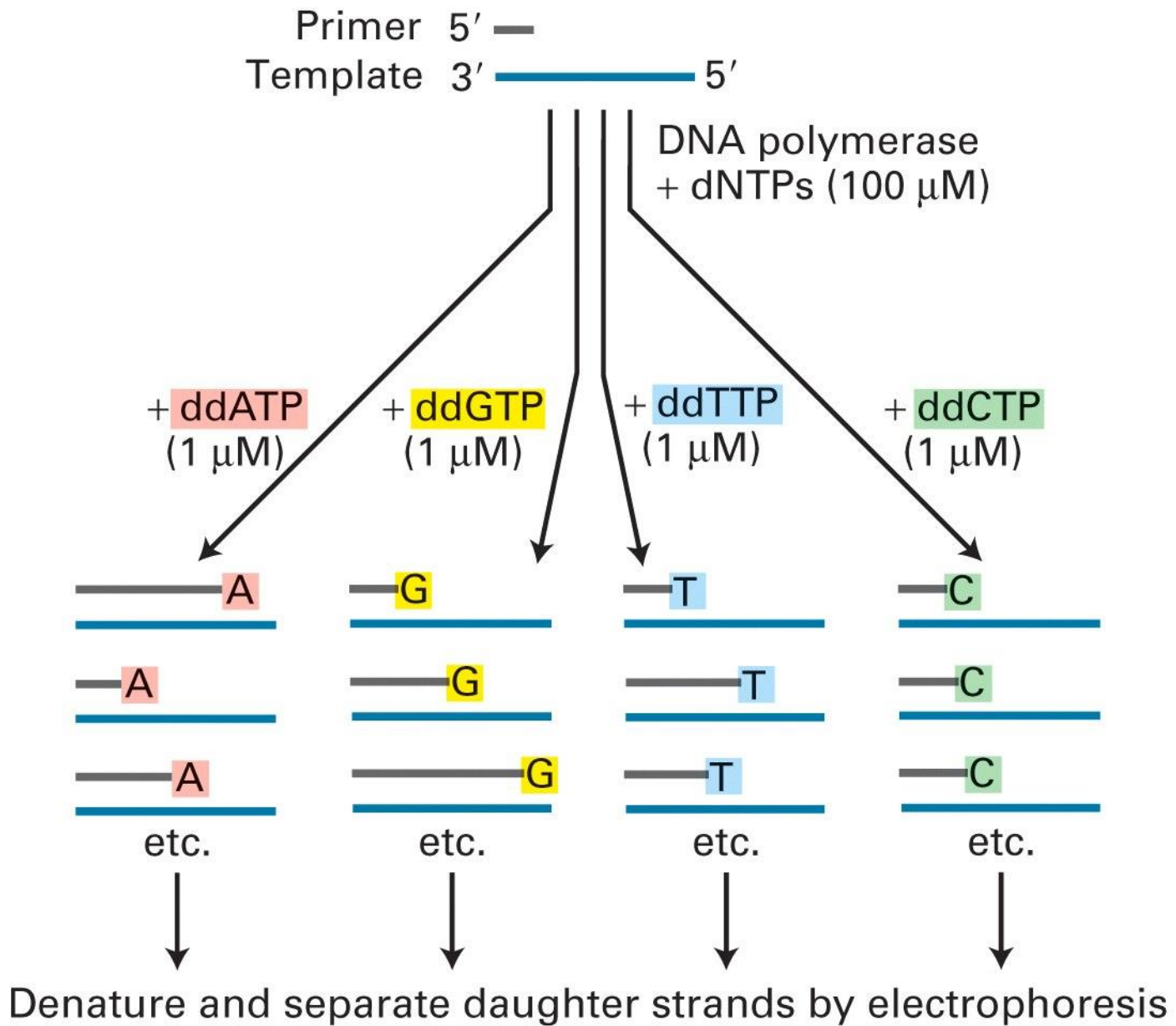
No 3' OH: DNA synthesis terminates

DNA Polymerase Action

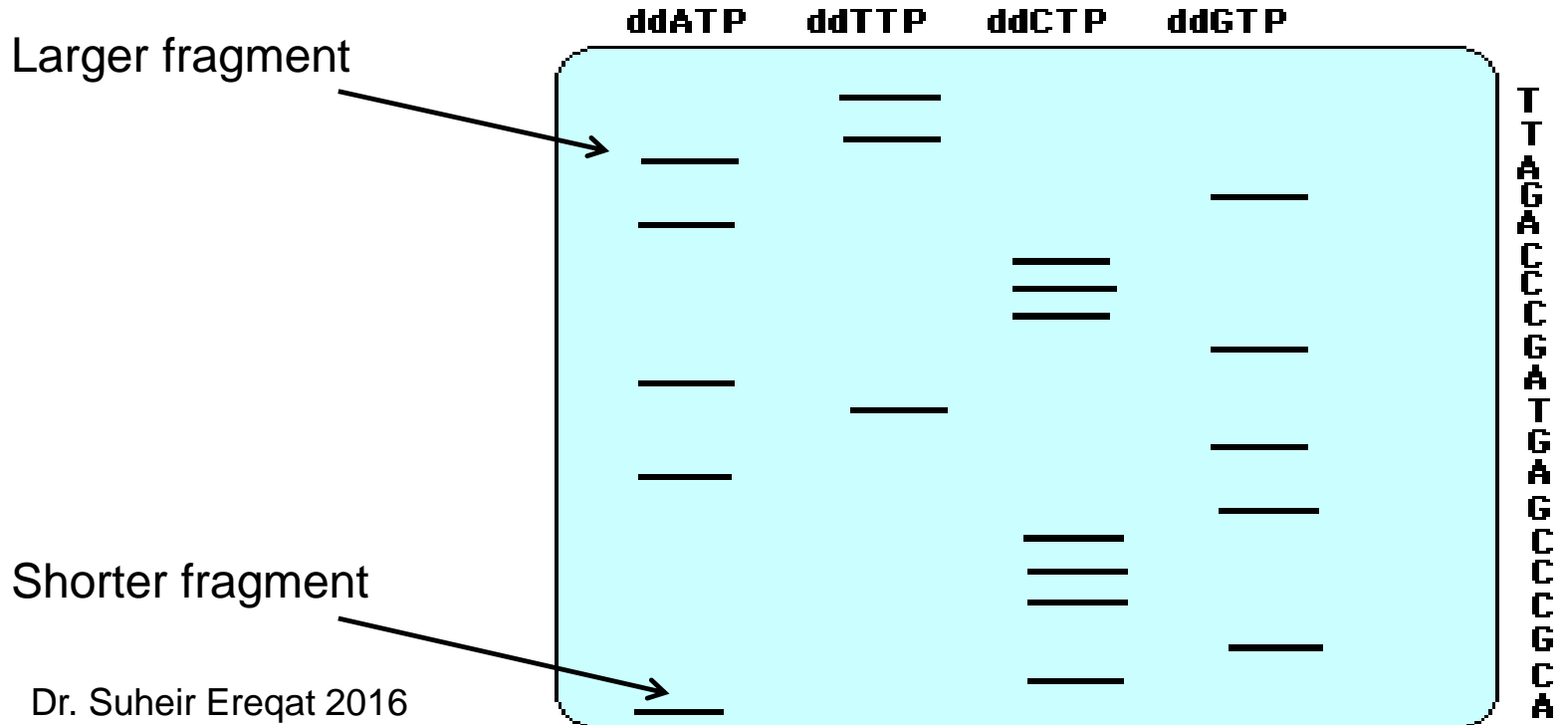
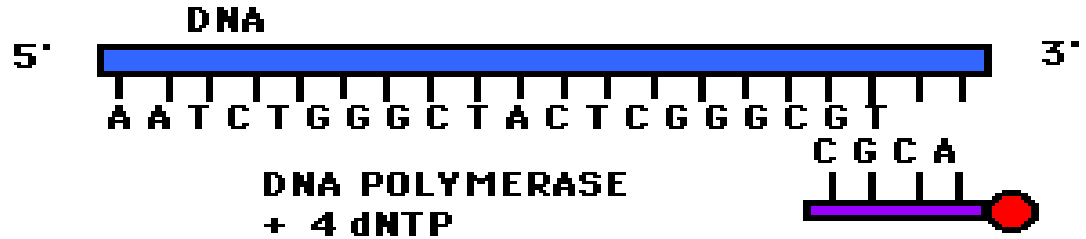


Dideoxies block elongation

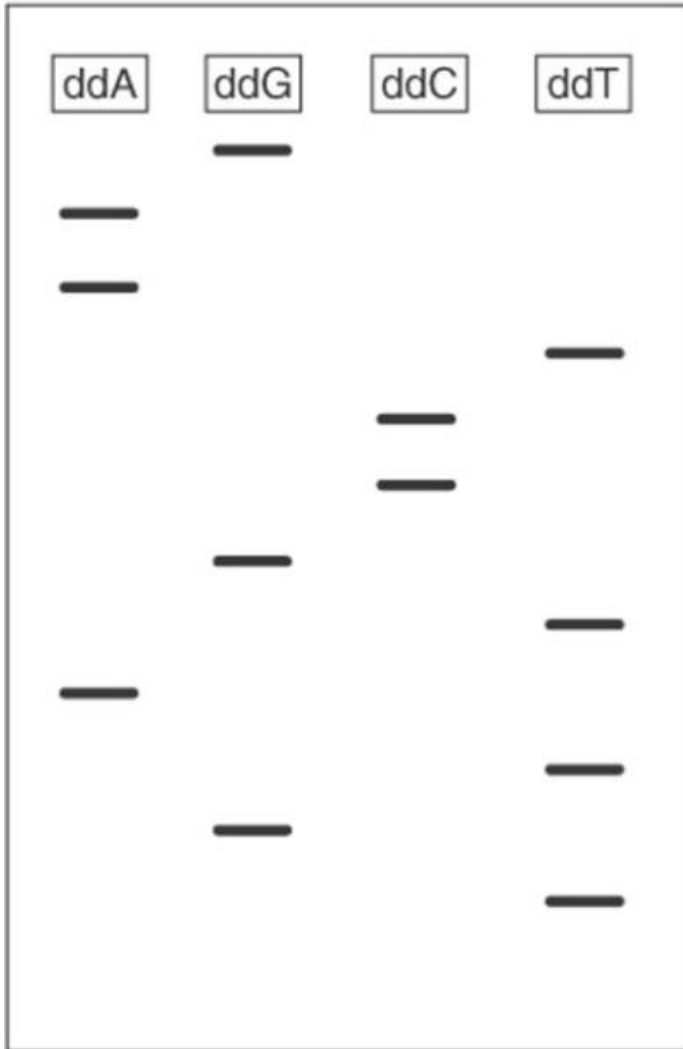




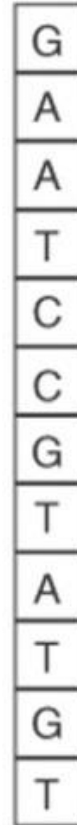
- **G" tube:** all four dNTP's, **ddGTP** and DNA pol
- **"A" tube:** all four dNTP's, **ddATP** and DNA pol
- **"T" tube:** all four dNTP's, **ddTTP** and DNA pol
- **"C" tube:** all four dNTP's, **ddCTP** and DNA pol



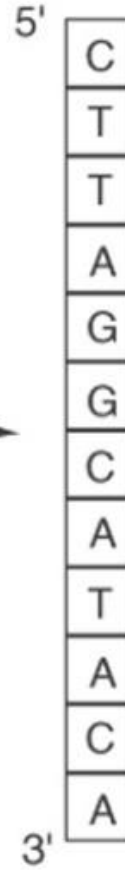
Gel analysis of fragments



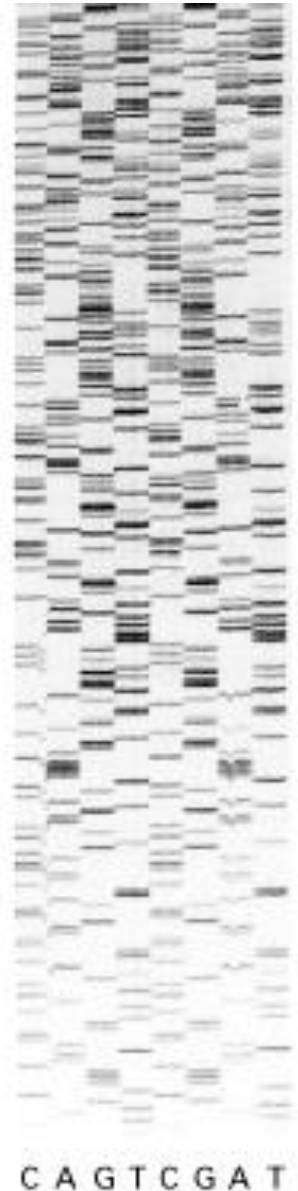
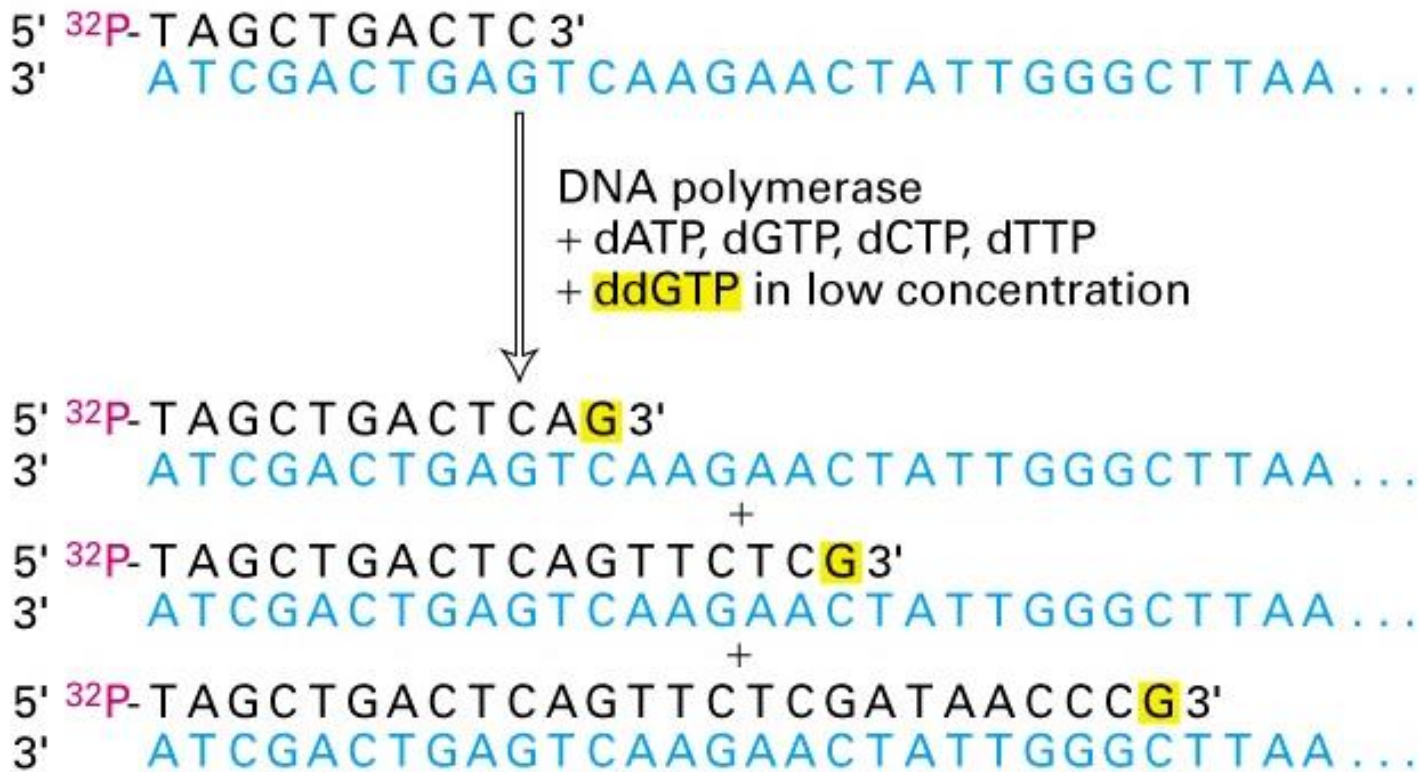
Sequence of synthesized DNA



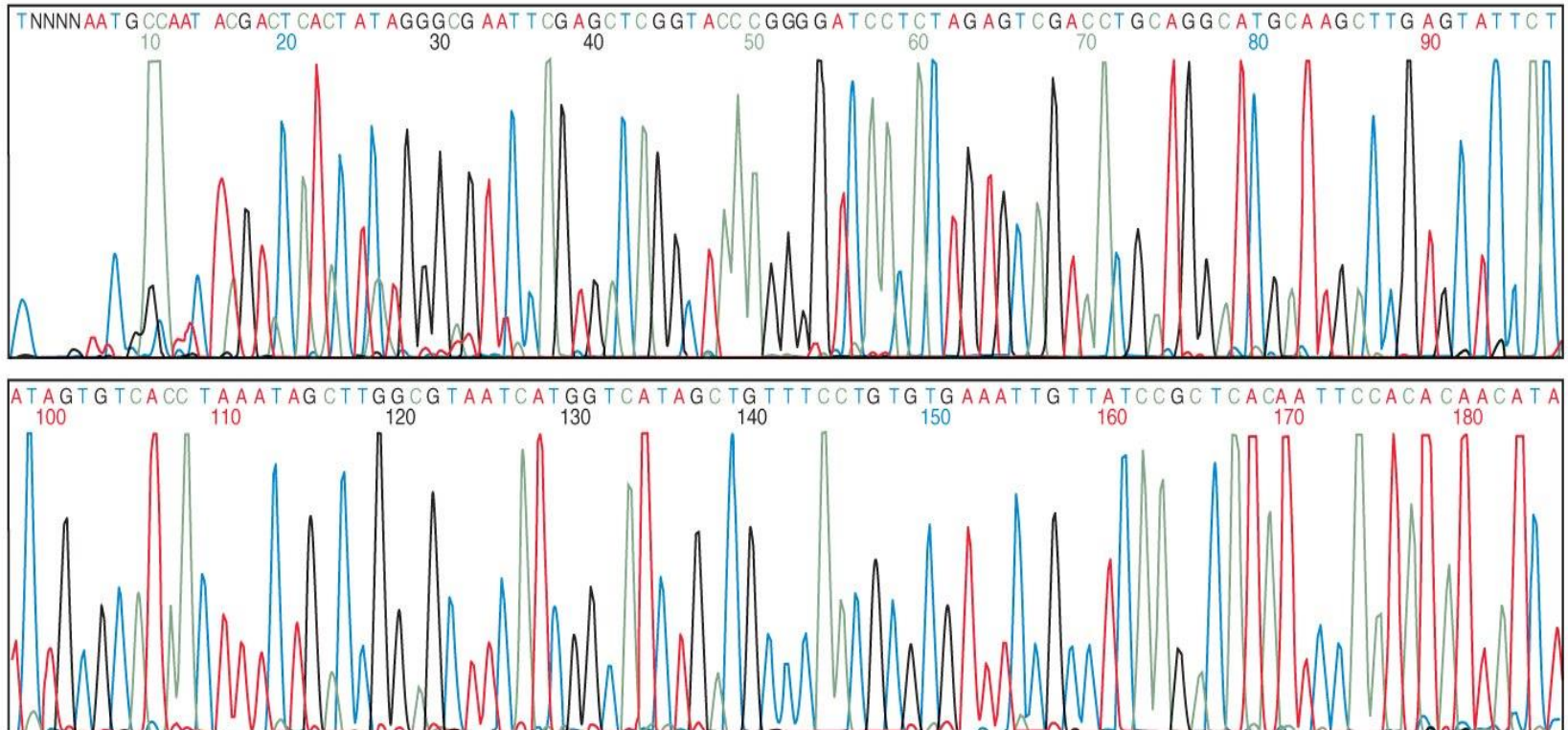
Sequence of template DNA



DNA sequencing: the Sanger (dideoxy) method



Automated DNA sequencing involves use of four different fluorescent ddNTP allowing the simultaneous detection of all four reactions in one sample.



Dr. Suheir Ereqat 2016