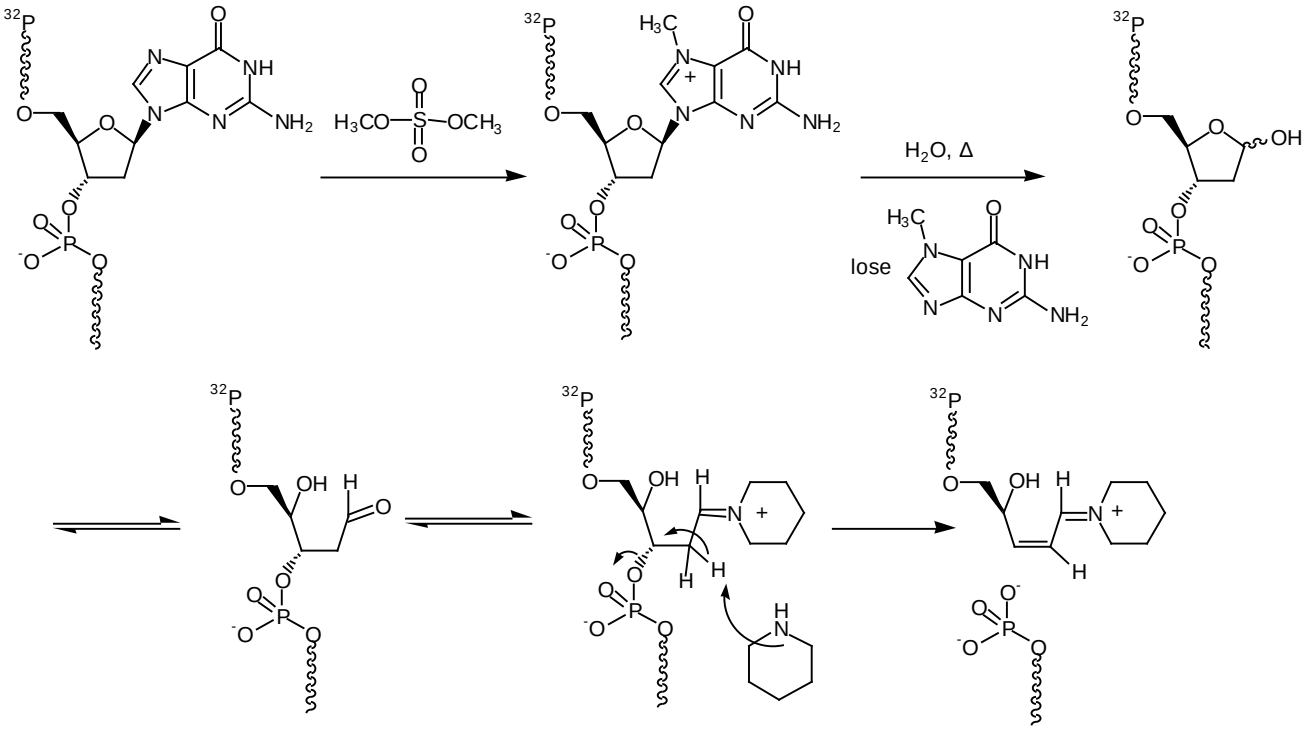
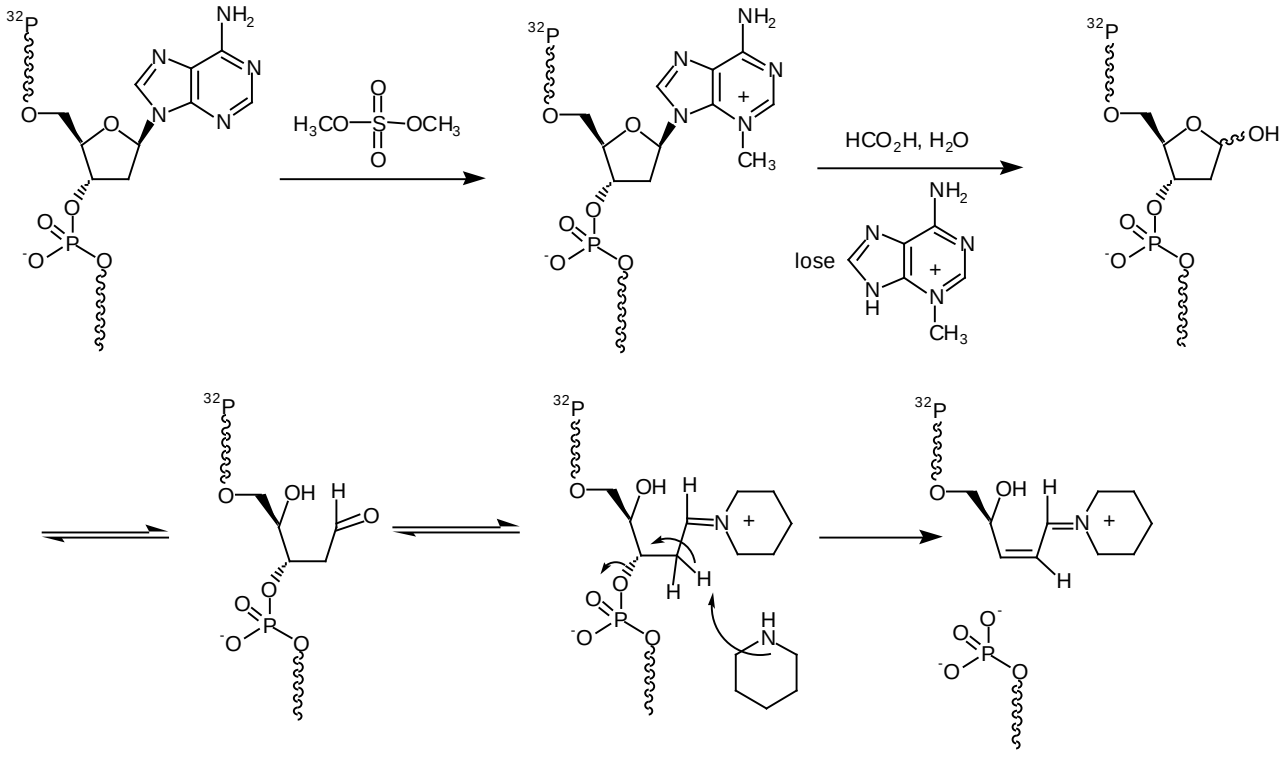
Maxim-Gilbert Sequencing

Detection: 5'-^{32}P-labeling

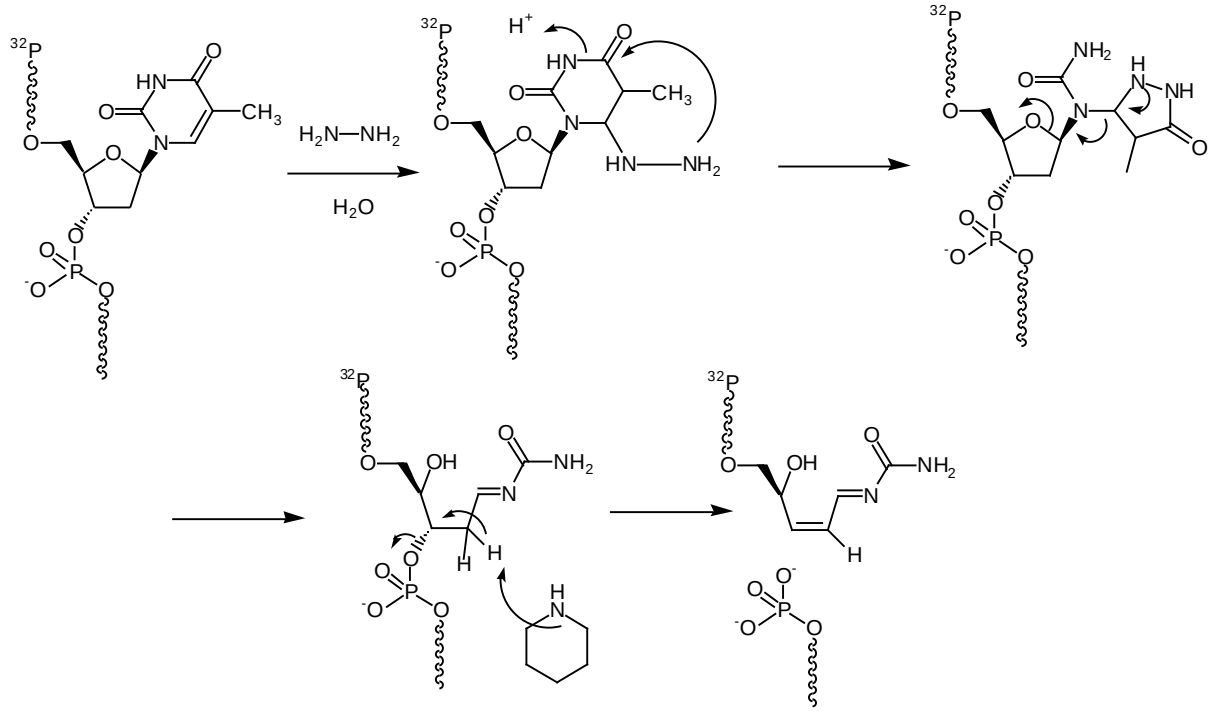
A. Cleavage at the 3'-side of guanosines with a) dimethylsulfate b) Δ, c) piperidine.



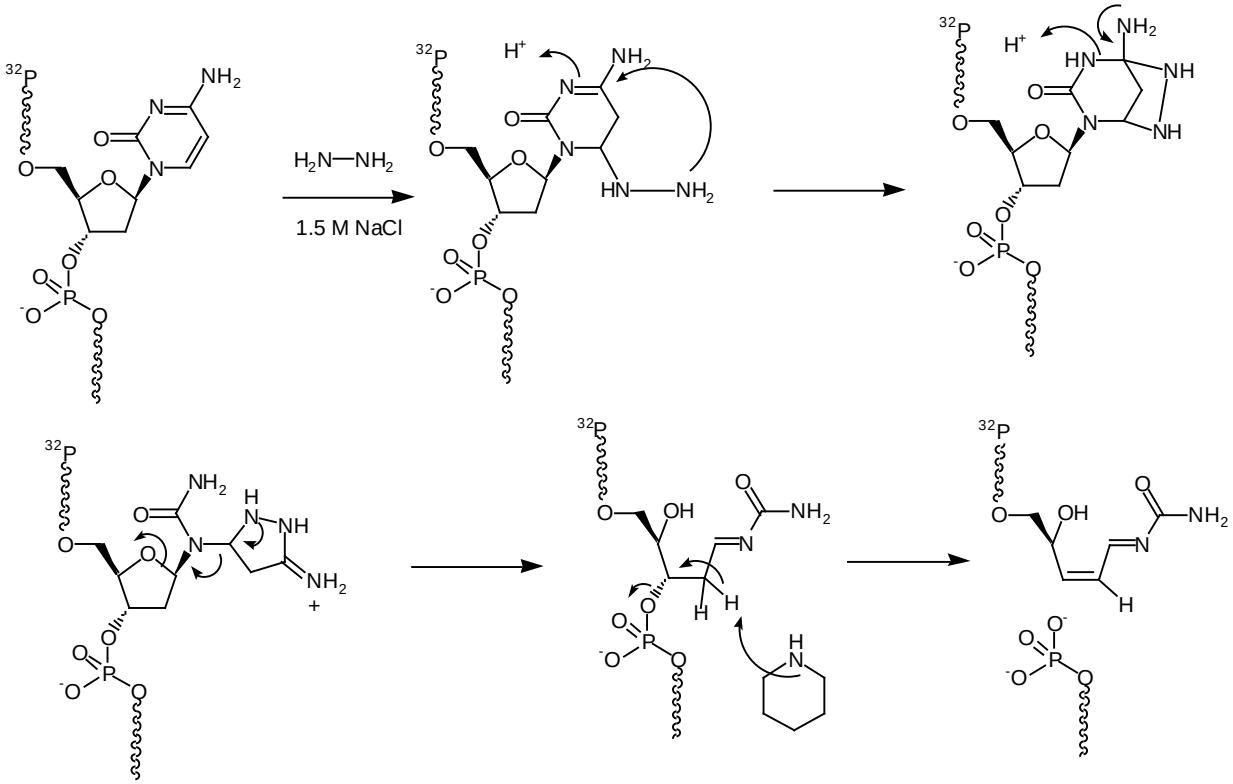
B. Cleavage at the 3'-side of adenosines and guanosines with a) dimethyl sulfate b) HCO_2H, c) piperidine.



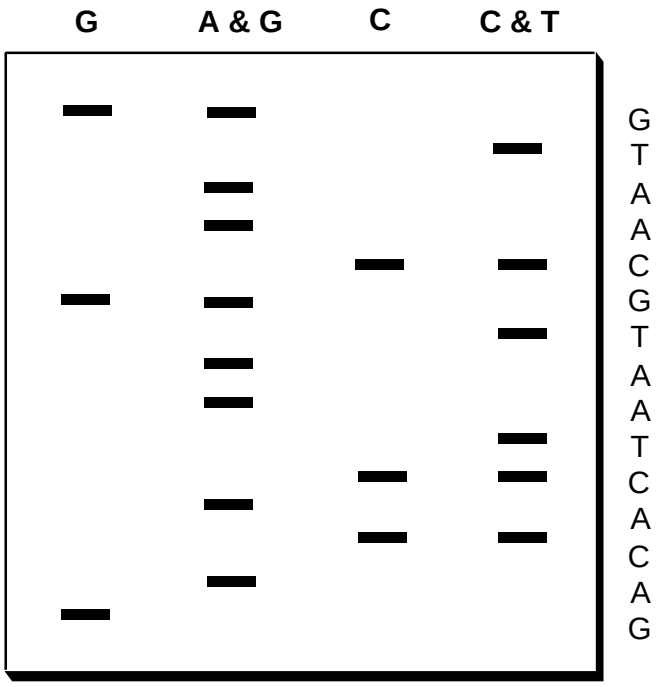
C. Cleavage at the 3'-side of thymidines and cytidines with a) hydrazine, b) Δ, c) piperidine.



D. Cleavage at the 3'-side of cytidines with a) hydrazine, 1.5 M NaCl, b) Δ, c) piperidine.



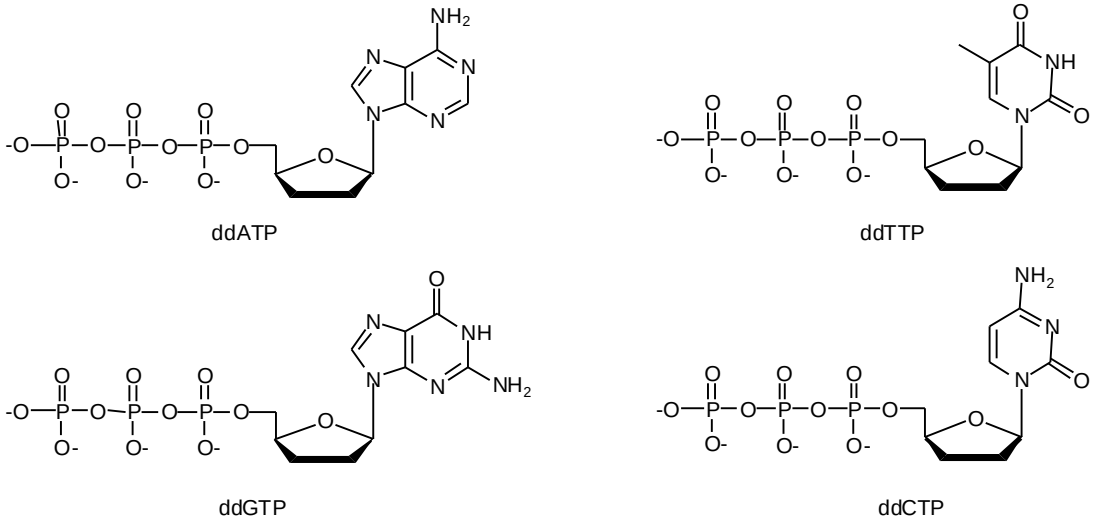
Separation and detection of ^{32}P-labeled DNA fragments by polyacrylamide gel electrophosesis (PAGE). DNA fragments separated based upon charge, which is proportional to the length of the DNA fragment.



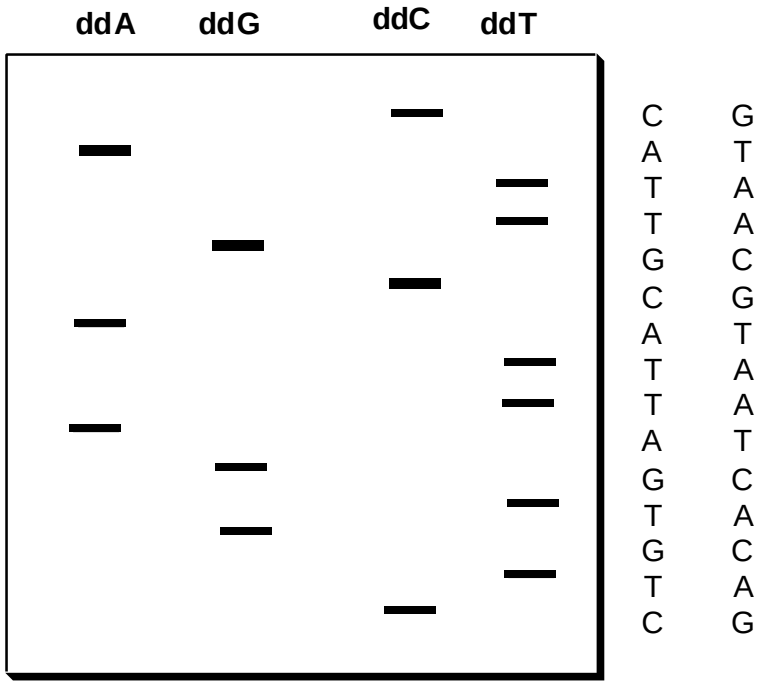
Sanger Sequencing

Enzymatic replication of the DNA fragment to be sequnced with DNA polymerase, Mg(+2), dNTP's, ddNTP's and ^{32}P-labeled pimers.

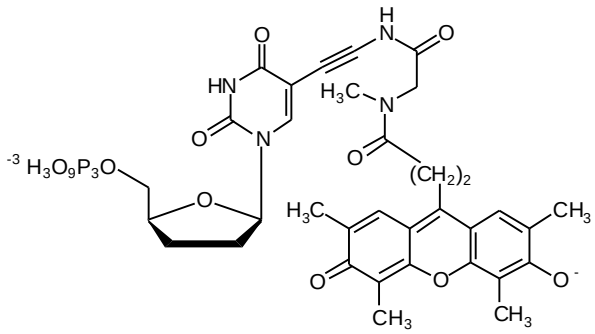
ddNTP's



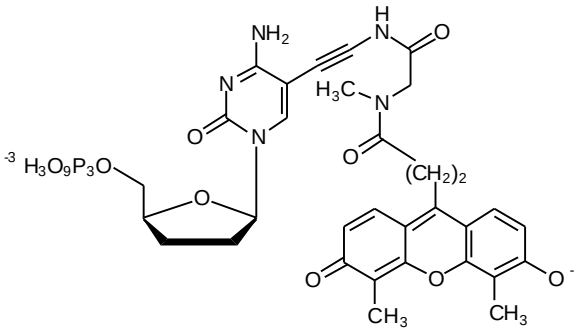
The extended ^{32}P-labeled primers are separated by PAGE



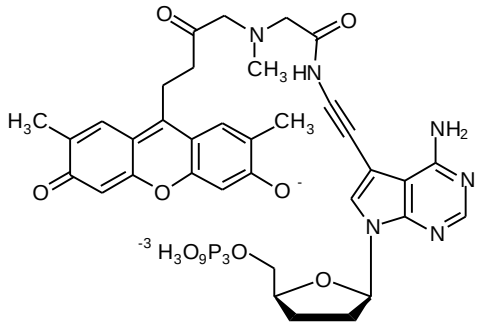
Sanger sequencing with flourescent ddNTP's



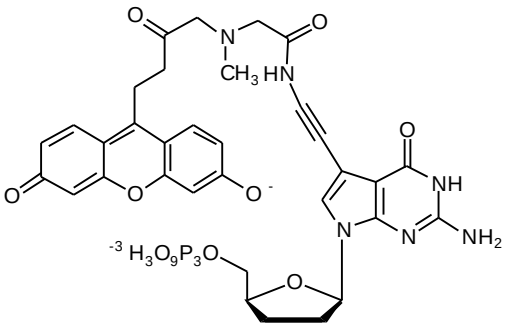
ddT



ddC

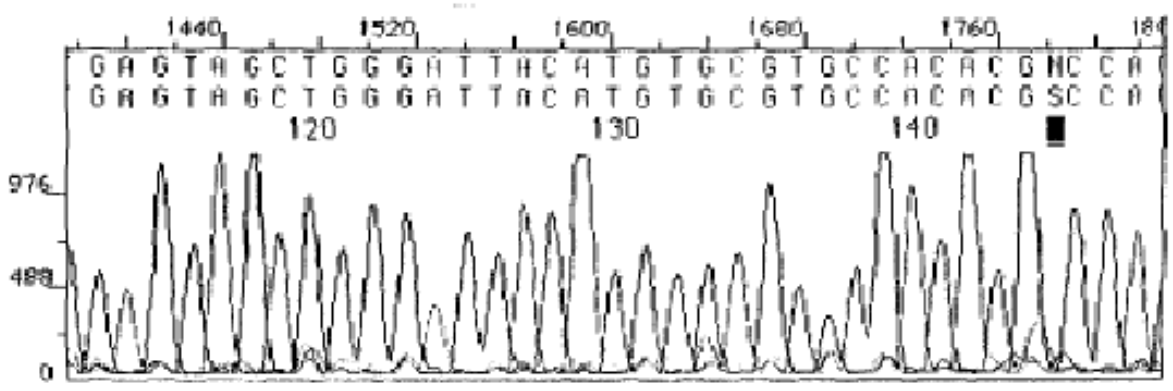


ddA



ddG

Excitation: ~ 490 nM, Emmission: ddT= 526 nm. ddC= 519 nm, ddA= 512 nm, ddG= 505 nm



ouput is color coded by base