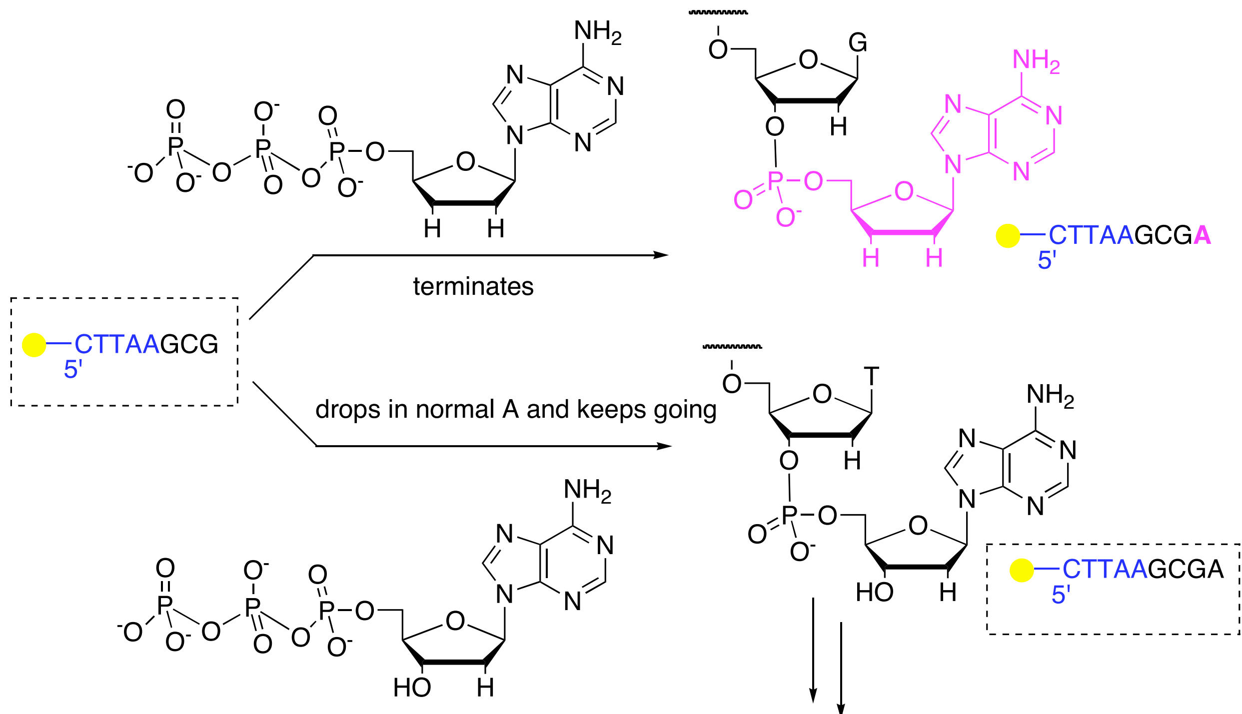
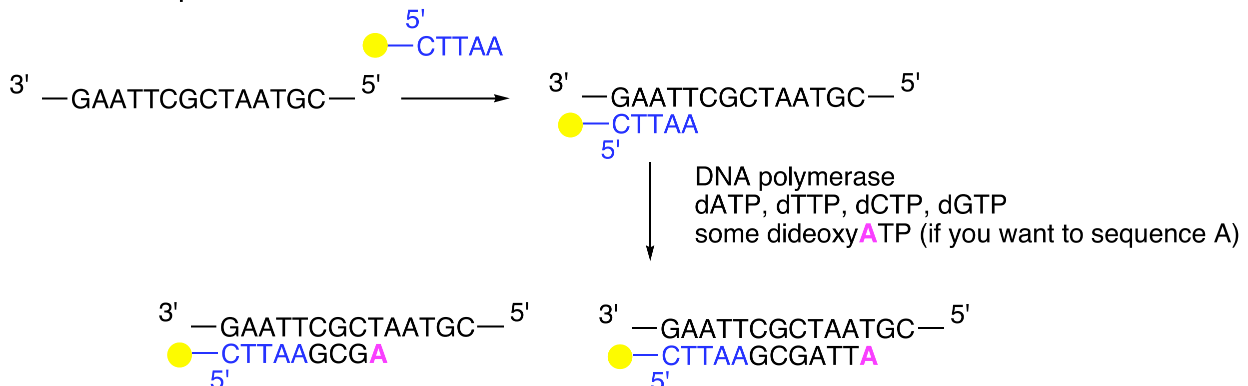
Sanger Dideoxy Sequencing

• Relies on generating DNA fragments whose length depends on the last base in the sequence using a technique of *Controlled Interruption of Enzymatic Replication*.
• Developed by Fred Sanger (same guy who worked out Peptide Sequencing) in 1977.
• Up to 500 bases at a time.
• Use 4 reaction mixtures at the same time (one for each chosen base):

1. Short ‘primer’ sequence (5’ to 3’), made through chemical synthesis, is added to DNA to be sequenced. This can be labelled in some way (fluorescence).
2. DNA polymerase used to extend the primer to make a complement strand (5’ to 3’) that complements the DNA to be sequenced (3’ to 5’).
3. The 4 different DNA-triphosphates that are used by enzyme are added. Alternatively, these can be labelled in some way (radioactive ^{32}P).
4. Small amount of 2’,3’-dideoxy-triphosphate of *one* (corresponding to chosen base) of the nucleotides is added too.
5. Reaction is run and when dideoxy sugar incorporated 5’ to 3’ joining stops (no 3’ OH). The low concentration means that some terminate at first location of this base, some at 2nd and so on.



6. This gives a mix of DNA strands all terminated at different places but that all correspond to that base.



7. The different length strands are separated and run on an electrophoresis gel that separates according to length.
8. An autoradiogram (radioactivity vs distance from origin of gel) or fluorimeter gives a trace that can be read off. Here, where the A's terminate corresponds to T's in original sequence.