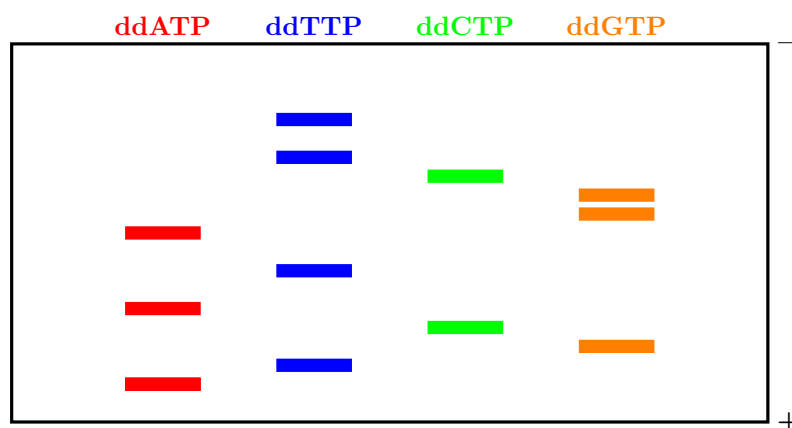


1. A single stranded DNA template, a DNA primer, DNA polymerase, normal dNTPs and ddNTPs.
2. Heat denature DNA and add primer and polymerase, in order to purify template DNA.
3. Add 1% mixtures of ddATP, ddTTP, ddCTP, ddGTP to 4 separate mixtures (1 per well.)
4. Add polymerase to each of the 4 mixtures.
5. Newly polymerized sequences will have varying lengths, based on where ddNTP was inserted.
6. Heat denature and place sequences in polyacrylamide gel and perform electrophoresis, short sequences migrate faster towards the positive anode placed at the bottom of the gel (due to the negative net charge of DNA.)
7. Gel will now have a series of bands, sorted by length, corresponding to the DNA sequence.
8. Reading the bands from bottom to top yields the sequence.



Here we can see the sequence represented is:

ATG   CAT   AGG   CTT

Next-gen sequencing is performed in a similar fashion, except that instead of radio-nucleotides, we use fluorescent ddNTP nucleotides all in a single capillary. The fluorescent pattern is then read with a laser detector and a color profile emerges, yielding the sequence.

