

DNA sequencing by Sanger's method

→ Single stranded DNA of unknown sequence used as template

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Add primer & DNA polymerase + dATP, dGTP, dCTP, dTTP

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Split sample into four tubes & add one of the four dideoxynucleotide (fluorescent dye labelled) to each [ddNTP]

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Synthesis proceeds until dideoxynucleotide is incorporated into DNA strand. DNA terminating in a dideoxynucleotide cannot be elongated because it lacks 3'-OH, which is required for formation of 3' → 5' phosphodiester bond

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Take product formed in all 4 sample tube & perform gel electrophoresis using polyacrylamide gel, acco. to size

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Read sequence of newly synthesized strand [complementary to original DNA sample]

→ primer is radiolabeled & complementary to 3' end of target DNA