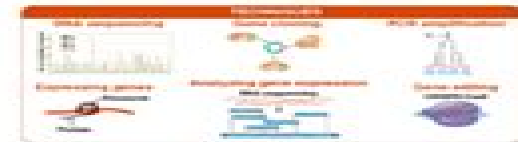


DNA Tools Part I

- There are many tools for manipulating DNA
 - DNA sequencing* - to directly sequence a site of variation
 - Vectors* - to carry specific DNAs and make identical copies
 - Restriction endonucleases* - to cut and join different DNAs
 - Gel electrophoresis* - to visualize the size of PCR products
 - PCR* - to amplify specific regions of a person's genome
 - RT PCR* - to characterize mRNAs expressed in a cell
 - Allele specific oligonucleotides* - to genotype SNPs

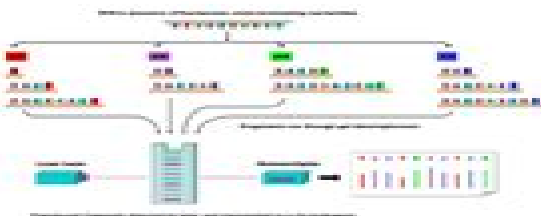


DNA sequencing

- A useful tool
 - A gene's complete nucleotide sequence can be determined using this process
 - The first automated procedure was based on a technique called dideoxy or chain termination sequencing
 - It was developed by Frederick Sanger
 - In the last 15 years, "next generation sequencing" techniques have been developed that are much faster



- Sanger sequencing is by chain termination
 - This is still used for sequencing single genes and shorter fragments
 - Put all components in a tube and send it out
 - If we have the sequence on the top, a ten base sequence, we're going to do the PCR in presence of fluorescent chain terminating nucleotides
 - Most of our nucleotides are dNTPs, but some are ddNTPs (dideoxynucleic acids) and they have two effects: fluorescent and stop PCR chain
 - Added at a low concentration, so sporadically added in and not extremely frequently
 - When this PCR occurs, when the first base is added it complements our A and in many of them it will get the normal dNTP, but in small percentage some get ddNTP and stop the chain and be one nucleotide long and it will fluoresce
 - We'll add more base pairs once again and most will be the normal base pair but some get the ddNTP A, and what happens is that every position gets a small amount of PCR product that is terminated → run the fragments through gel electrophoresis which separates them out from smallest to biggest and that can be run across a laser beam which will excite it and it can be read
 - In every position the different fluorescent tag will be read → in this case we got a red T, purple A, blue C, green G, etc
- Entire genomes can be sequenced
 - If we did this with Sanger sequencing it would take forever → there are other techniques that can be used



Manipulating Dna Study Guide

AN Whitehead



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