PCR and DNA diagnostics

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DNA

- DNA contains loads of information neatly stacked and compressed to small sizes that can fit into the cell nucleus.
- A single DNA molecule has two strands that wrap around one another to form a double helix.
- Every single strand of DNA is composed of the sequences of four nucleotides which the individual letters were building blocks of DNA
- Nucleotides of DNA made up of sugar deoxyribose, phosphate, and one of the four nuclear bases, adenine (A), cytosine (C), guanine (G), and thymine (T).
- The nucleotide of the one strands forms hydrogen bonds to complementary nucleotides and other strands, specifically A bonds to T by two hydrogen bonds, and C bonds with G with three hydrogen bonds.
- Two DNA strands also have a direction, which means one of them runs from 3' end 5' end while the other runs from 5' end to 3' end.
- Every single protein of our body is encoded in combinations of just four nucleotides.

Polymerase chain reaction (PCR)

- PCR is its technique in molecular biology to amplifies a segmented DNA.
- To analyze it, we need lots and lots of copies of DNA to make it much easier.
- PCR is based on DNA replication, a process used to duplicate the genetic material dividing into two identical daughter cells by using thermal cycling.
- DNA that we wish to multiply and enzyme Taq polymerase, primer, nucleotides (A, T, C, and G).
- Thermal cycle of 96°C (denaturation), 55°C (annealing), 72°C (extension).
- The whole process lasts for 10 min.
- About an hour is enough to analyze with electrophoresis.

Gel electrophoresis

- DNA is chopped up into smaller fragments using restriction enzymes, which are enzymes that break the DNA at specific nucleotide sequences.
- Then the DNA fragments are poured into a well within a piece of agarose gel.
- An electrical current is passed through the gel and pulls the negatively charged DNA fragments through the gel towards the positive end.
- Each band would represent a different size of DNA fragments.
- Use the PCR ladder to read the result.
- Do not forget to make a negative and positive control as well.
- Keep in mind that the restriction enzyme doesn't always cut the nucleotide sequence that we primarily intended to.

Restriction fragments length polymorphism (RFLP)

- Depending on DNA sequence on an individual, cleaving off the chromosomal DNA with restriction enzymes leads to DNA fragments of the variable lengths.
- The cleavage of DNA in specific sequences can be used as a polymorphic marker.
- These markers out of specific spots identify polymorphism using a restrictive enzyme.
- The fragments are analyzed by Southern blot and detected by the probe.

Single nucleotide polymorphisms (SNPs)

- DNA sequence variants in a population that differ by only a single base pair.
- They are usually caused by errors during DNA replication and are point mutations.
- The difference can be seen from the number of bands on the gel electrophoresis, which helps us to identify the gene mutations.

Microsatellites (short tandem repeats ; SRTs)

- The repetitive sequence of several base pairs in DNA that are highly polymorphic.
- Belong to the variable number tandem repeats (VNTRs), which are short nucleotide sequences in the genome of an individual that are repeated a variable number of times.
- The common pattern of VNTRs in the repetition of trinucleotides for about 5~50 times or even more.
- DNA polymerase may copy more and changes the length of the allele.
- Then restriction enzyme added, and the fragments would be differed by showing up using gel electrophoresis.

References

https://en.wikipedia.org/wiki/Polymerase_chain_reaction

https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-sequencing-pcr-electrophoresis/a/gel-electrophoresis

https://www.osmosis.org/

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