Principles of DNA Amplification & Sequencing.





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DNA Polymerase binds at the primer

Cattcgggatccaga

CGGGATCCAGACATTCGGGATCCAGA

6

G C NUG A 11 C AAPICGGGATCCA **C C A A C I A G**

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> DNA Polymerase moves along DNA strand adding extra nucleotides



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> GGUUGAATTCGGGATCCAGACATTCGGGATCCAGA GGAAGUAGUU

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GGUUGUAGIUA

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> **<u>G</u>GUUGA**ATTCGGGATCCAGACATTCGGGATCCAGA <u>C</u>CAGAGCGCCAGACATTCGGGGCCAGA <u>C</u>CAGGCGCCAGACATTCGGGGCGCCAGA



MODERN DNA SEQUENCING

Sequencing" means finding the order of nucleotides on a piece of DNA

 Nucleotide order determines Amino acid order, and by extension, protein structure and function (proteomics)

 An alteration in a DNA sequence can lead to an altered or non functional protein, and hence to a harmful effect in a plant or animal





Methods of DNA Sequencing

Sanger Method DNA sequencing by enzymatic synthesis Nobel Prize 1958, seq.of insulin Nobel Prize 1980, DNA seq.



Maxam-Gilbert Method DNA sequencing by chemical degradation Nobel Prize 1980, DNA sequence



Modern sequencing equipment uses the principles of the Sanger technique





➢ Direction of replication 5[`]→ 3[`]
 ➢ DNA has proofreading

Termination during Replication

DNA SEQUEN CE 3'	G	С	A	Т	Т	G	G	G	A	A	С	С
PRIMER 5'	С	G	Т	A								
NO OF BASES	1	2	3	4	5	6	7	8	9	10	11	12

G terminated

CGTA ACCTTG CGTA ACCTTGG

Aterminated

CGTAA

Tterminated

CGTAACC T CGTAACC T T

Cterminated

CGTAAC CGTAACC CGTAACCC

























GGTTGATCGGGA

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> **Series of pieces** of DNA of ځا different lengths each \٧ labelled with a fluorescent dye 5 Δ ' Ğ Δ G G Δ $|\Delta\rangle$ (CΔ G Δ Δ Δ ح

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DNA sequencing: Importance

- Location of genes.
- Location of coding region.
- Location of promoters, start codons and terminators.
- The nature and distribution of specific DNA sequences such as:
 - STRs (Short Tandem Repeats).
 - VNTR (Variable Number of Tandem Repeats)
 - SNP (Single Nucleotide Polymorphism)



 SNPs account for about 90% of all human genetic variation and are believed to occur every 100 to 300 bases along the 3-billion-base human genome.

- Approximately 5 million of the ~10 million human SNPs have been catalogued.
- SNPs may occur in exons (coding regions), introns (non coding regions between exons) and intergenic regions (regions between genes).
- SNPs may lead to coding or amino acid sequence changes (non-synonymous) or they may leave the sequence unchanged (synonymous)



DNA sequencing: Importance

- Gene and protein.
 - Function
 - Structure
 - Evolution

Genome-based diseases-

- Genetic disorders
- Genetic predispositions to infection
- Diagnostics
- Therapies

