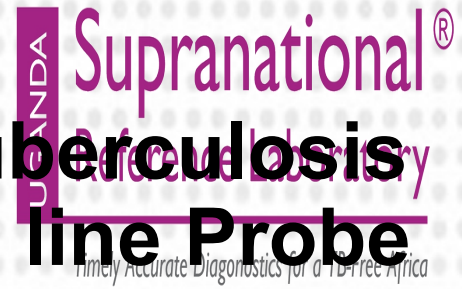




Training on New and rapid Tuberculosis diagnostics (first and second line Probe Assay)



Module 3: Molecular biology basics

**Uganda Supranational
Reference Laboratory**

Outline

- Definitions of DNA and RNA
- Functions of DNA
- Composition of DNA
- DNA translation and transcription
- Molecular identification of mycobacteria
- Assessment
- Summary

Definition of DNA

- DNA
 - deoxyribonucleic acid (DNA)
 - Present in all organisms
 - Eukaryotes
 - Animals
 - Prokaryotes
 - Bacteria
 - Mycobacteria
 - Archaea

Composition of DNA-1

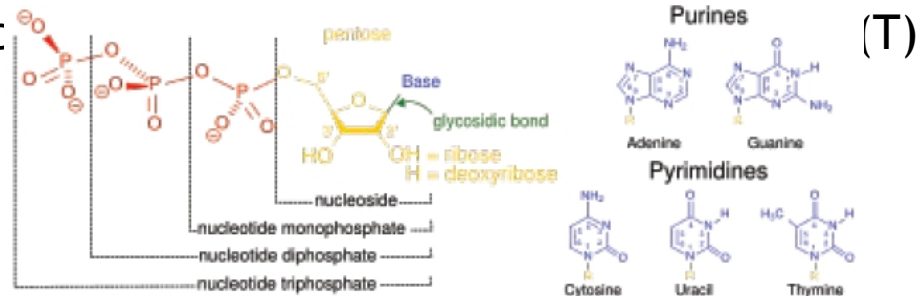
- Composed of two strands of nucleotide units bound together
- The order of the nucleic subunits in the structure represents all of the genetic information carried by a cell
- DNA contains genetic information and instructions for the development and functioning of all living organisms
- DNA location
 - Eukaryotes - nucleus
 - Prokaryotes - cytoplasm

Composition of DNA- 2

DNA is made of two long strands of polymers called nucleotides

Nucleotides are monomers, the building blocks of DNA and are composed of three parts:

1. Nucleobase or base
 - Cyclic nitrogen-containing compound
 - 4 types: Adenine (A), Cytosine (C), Guanine (G), and Thymine (T)
2. 5-carbon sugar
 - Deoxyribose

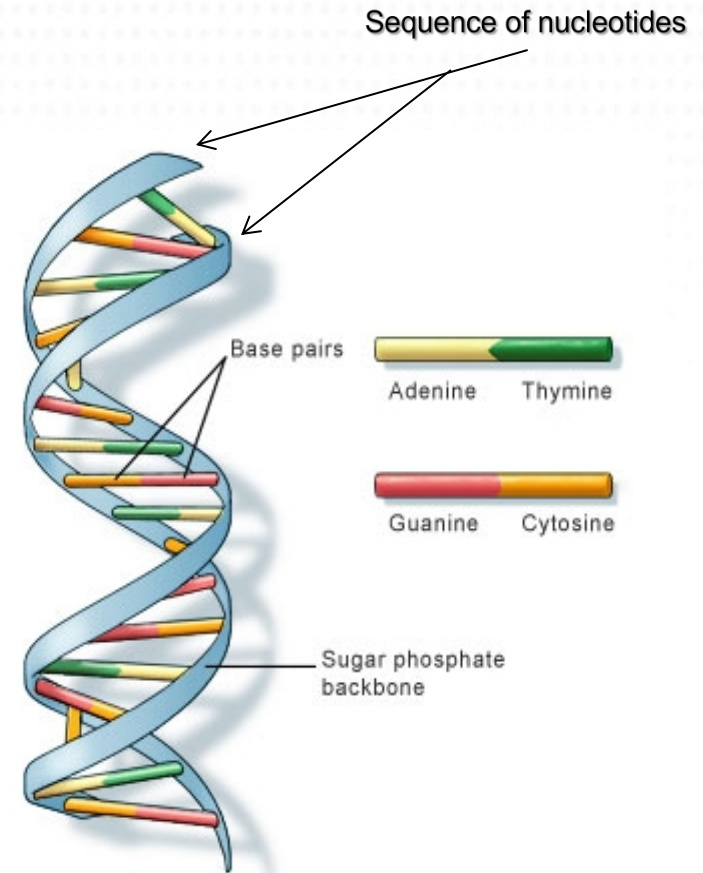


3. Phosphate group(s)

RNA has the same composition but a different 5-carbon sugar- ribose

Composition of DNA-3

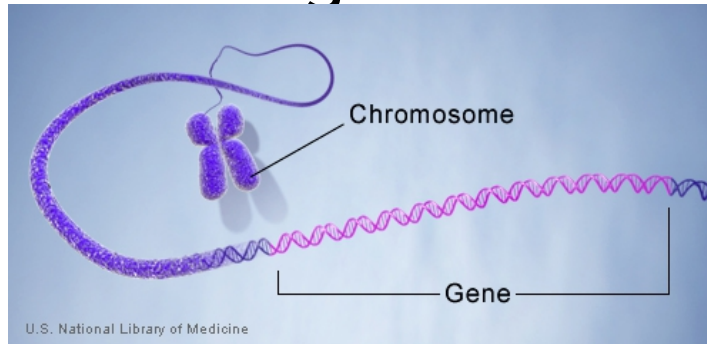
- Nucleotides
 - One base per nucleotide (A,T,G,C)
- The four bases are classified into two groups
 - Purines (A,G)
 - Pyrimidines (T,C)
- Any base can be next to each other on the strand
- Two DNA strands bind together to form a double helix
 - A only binds to T
 - C only binds to G
- The two strands are complementary to each other



U.S. National Library of Medicine

Functions of DNA

- Long-term storage of information
 - DNA Code
 - Instructions to build necessary components of cells
 - Proteins
 - RNA
 - Structural characteristics
 - Regulation
- Transmission of genetic information

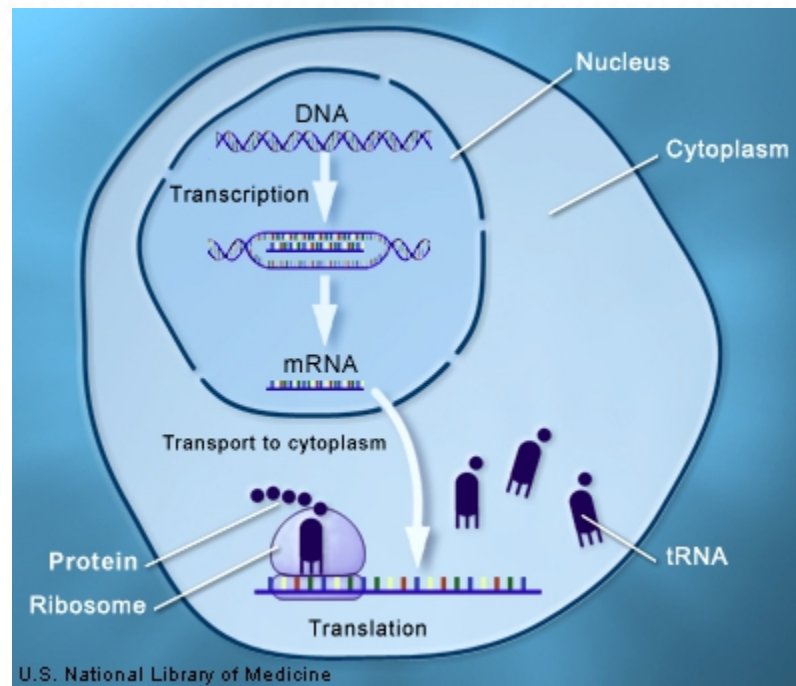


LPA/PP/003, Version 1.0, Effective
date: 01-Jun-2019

DNA translation-1

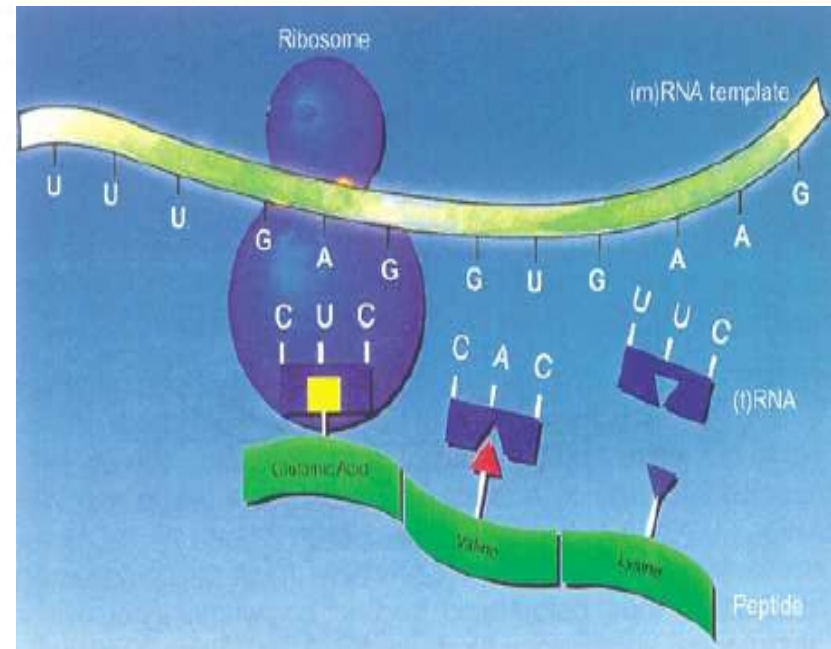
- Translation is the process of decoding DNA instructions for making proteins.
- A section of DNA is transcribed into RNA (mRNA)
 - mRNA is a working copy of the DNA section
 - A U G C where U replaces T
- mRNA is translated into amino acids at the ribosomes
 - Amino acids
 - A sequence of three nucleotide letters (triplets) codes for a specific amino acid
 - Proteins: a sequence of amino acids

DNA transcription - translation



DNA translation- 2

- mRNA passes through the sections of the ribosome
- Ribosome reads the code in 3 letter words (codon) in the genetic language
- Peptide chain is built as amino acids are added to the chain
- Proteins are made and have various functions





THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA
Supranational[®]
Reference Laboratory

Timely Accurate Diagnostics for a TB-Free Africa

Molecular identification of bacteria



Molecular identification of mycobacteria

- Highly conserved regions of DNA allow for species identification
- DNA sequences are conserved within a species or complex, but differ from other species
- Genes with sequence differences that are used for identification
 - 16S rRNA
 - 23S rRNA
 - 16S - 23S ITS (internal transcribed spacer) region

MTB complex can be differentiated from other mycobacterial species

- Members of the TB complex have identical DNA sequences in these regions
- Other mycobacterial species have sequence differences in these regions and this is the basis for molecular identification

Molecular identification methods

- DNA probes
 - Bind to DNA or RNA from growth-amplified cells (need isolates for testing)
 - GenProbe AccuProbes bind ribosomal RNA
- NAAT (nucleic acid amplification tests)
 - Use enzymatic amplification of DNA or RNA (PCR)
 - DNA sequencing
 - Line probe assays
 - Home brew assays, other commercial kits

Target and probe

- Target
 - Specific nucleic acid sequences or amplified product (amplicon) from the specimen or isolate to be identified
- Probe
 - A small molecule of nucleic acid (DNA or RNA) used to identify complementary nucleic acid sequences in the target by hybridization

Probe: Target detection

- Hybridization
 - Probe is labelled
 - Probe is in solution
 - Target is not labelled
 - Bound to a solid support (membrane or plate)
- Reverse hybridization
 - Probe is not labelled
 - Bound to a solid support (membrane or plate)
 - Target is labelled during amplification by PCR
 - Each copy is labelled
 - Amplicons are in solution

Assessment

1. What is the function of DNA
2. What are the nucleotide bases that make up DNA
3. What is DNA translation
4. What is a target and probe in relation to molecular biology

Summary

- DNA contains genetic information and instructions for the development and functioning of all living organisms.
- 4 bases make up the nucleotide that make up DNA: Adenine, Guanine, Cytosine & Thymine
- Translation is the process of decoding DNA instructions for making proteins.

References

- GLI TB training package
<http://www.stoptb.org/wg/gli/trainingpackages.asp>
- www.hain-lifesciences.com

Acknowledgments

