DNA-based Diagnostics

This is the topic that takes you to the current edge of what Molecular Biology is doing to the practice of Medicine in concrete terms. What was impossible 40 years ago is now routine. What cost 1.5 billion dollars 30 years ago, now costs 1000. What took 5 days, now takes 2 hours. A test you would not be allowed to request 15 years ago, will now go unchallenged. The pace of change has not yet slowed. One of the cutting edge sequencing technologies (**MBoC(6th)** pgs. 479-481) will likely soon replace pyrosequencing to push the cost of sequencing a human genome down to 100 USD.

Reading:

MBoC(6th) Ch8: *ANALYZING AND MANIPULATING DNA*. pgs: 463-482.

Lehninger (4th): DNA BASED INFORMATION TECHNOLOGIES. pgs. 319--323.

Lehninger (4th): FRET. pgs. 446-448.

Vanni et al (2020) **The Current State of Molecular Testing in the BRAF-Mutated Melanoma Landscape**

Mamotte (2006) **Genotyping of Single Nucleotide Substitutions**

Hawkins (2017) **Analysis of Human Genetic Variations using DNA sequencing** in Basic Science Methods for Clinical Researchers.

Zhu (2020) **PCR past, present and future**

Need to know and understand

- Sanger (aka chain-terminator, aka di-deoxy) DNA sequencing, Next generation sequencing (NGS), pyrosequencing, RNAseq
- Shotgun sequencing and genome assembly
- PCR
  - Screening for heritable diseases, for pathogens, for cancer-associated mutations
  - Probing disease alleles vs. allele-linked polymorphisms
  - Allele Specific PCR Amplification
  - Melt curve tests for product size and heterozygosity
  - Realtime quantitative PCR (qPCR).
    - Fluorescence Resonance Energy Transfer (FRET)- based techniques
    - TaqMan
    - RT-qPCR (An initial Reverse Transcriptase reaction to prepare a DNA template from extracted RNA.
- Microarray assays (Gene Chips)
  - Copy Number Variation (CNV) - Comparative Genomic Hybridization microarrays
- Forensics
  - Fingerprinting - Variable Number of Tandem Repeat (VNTR) assays
- Largely historic methods
  - Southern Blot
  - Restriction Fragment Length Polymorphism (RFLP)