Genetics and Molecular Biology: Lecture 5

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12 September 2024

- DNA Replication Technology
  - Polymerase Chain Reaction (PCR) - 1983
    - In vitro (test tube) method for making copies of DNA sequences using DNA polymerase
    - USes: molecular cloning, diagnostics, forensics, DNA sequencing, gene expression analysis, etc.
    - E.g. Covid testing
    - PCR amplifies specific 'target' DNA sequences
  - PCR Primers Initiate Replication
    - Short DNA sequences (oligonucleotides) produced synthetically and added to PCR Reaction
    - Fufill same role as RNA primase (but are DNA)
    - Primers can be designed to target regions of desire (region amplifed between primers)
  - PCR Components
    - Template (source) DNA
    - DNA nucleotides (dNTPS) e.g. any nucleotide triphosphates
    - oligonucleotides primers
    - Buffers/salts - magnesium buffers triphosphate charge: need proper envrionment for DNA polymerase to work
    - DNA polymerase
  - PCR cycle
    1. Denature - 95 deg C: heat to separate DNA strands
    2. Anneal (prime) - $50 - 60$ deg C: cool to allow primers to bind to template DNA
    3. Extend - 72 deg C: heat to allow DNA polymerase to synthesize new DNA strand
    4. Repeat
  - Heat stable polymerase greatly improves PCR
    - Thermus aquaticus (Taq): bacteria that lives in Yellowstone hot springs has DNA polymerase that will not denature during heating of DNA to separate strands
  - The product of one cycle being used as a template in next cycle leads to an exponential increase in DNA - only works if the product of one primer contains the binding site for the other primer
  - Primer Sequence influences annealing temperature
    - GC base pairs are stronger than AT pairs, so they can anneal at higher temps
    - Larger sequences anneal at higher temps
- DNA Sequencing

- Reasosn to know a DNA molecule's nucleotide sequences
  - Find and interpret genes
  - Asses evoluation connections
  - Discover and diagnose genetic disorders
- Sanger Sequencing - 1977
  - Similar to PCR, but only one primer is used and no chain reaction occurs
  - Dideoxynucleotides (ddNTPs) included to prematurely teminate strand synthesis (they do not have a 3' OH group)
  - Flourescently labeled ddNTPS + unlabled dNTPS + primer and polymerase + DNA template are added to sequence
  - Goal to creaate nucleotide stands of each length then sort by size to determine nucelotide at each location
  - Gel Electrophoresis
    - Uses electricity to separate DNA molecules by size (number of nucelotides)
    - Larger molecules move through the gel slower than small moleculear and get stuck sooner
    - Add a DNA sample to electric field and it slowly moves toward the postive end (nucleic acids are negatively charged)
  - Capillary Electrophoresis
  - Sanger sequencing can only sequence 100s to 1000s of base pairs at a time (a single read)
  - Human genome has 3.2 billion base pairs (shotgun sequencing used to sequnce many disjointed small portions)
- Contigs and Coverage (Genome Sequencing Assembly)
  - Contig: continuous segment of sequence created through overalapping reads (sequences)
  - Coverage: number of copies of genome sequnced, more coverage is needed for new genome assembly