****Final focuses heavily on lecture 20-25 but below is a quick summary of these lectures. Besides this I suggest studying your old exams****

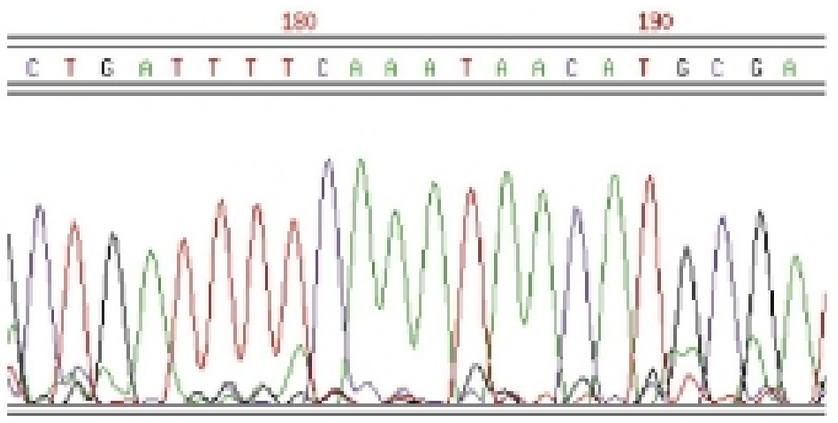
BSCI330 FINAL EXAM REVIEW

Lecture 20 Manipulating DNA, RNA, and Proteins:

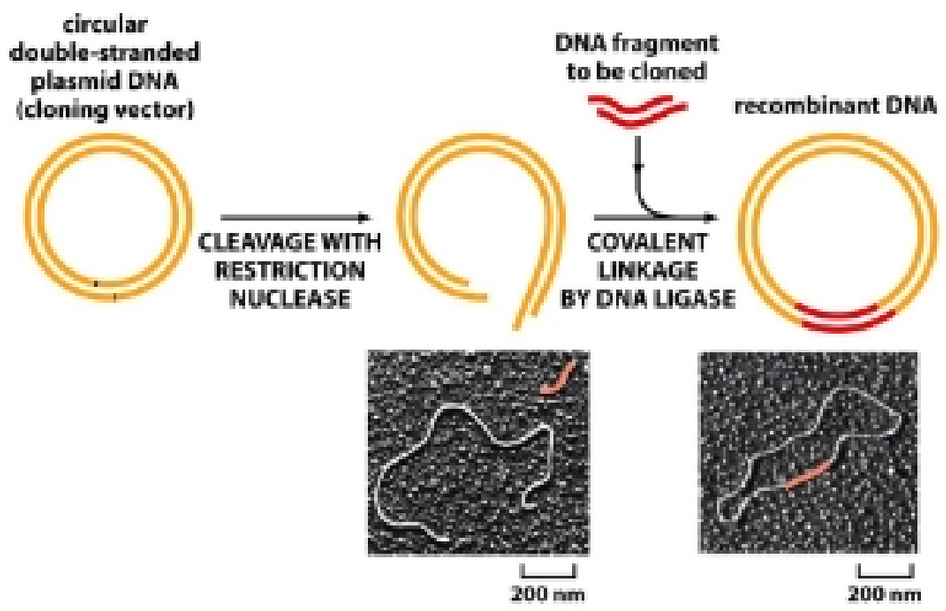
- Cells retain morphological and functional characteristics in culture (removed from body)
- Embryonic stem cells can either
    - Reproductive- placed in foster mother (calf) or
    - Therapeutic- transferred from early embryo to culture dish
        - To differentiate ES cells, gene regulatory proteins and other factors added
- Centrifugation
    - Low speed
        - Pellet contains whole cells, nuclei, cytoskeletons
    - Particles higher density and larger in size travel at faster rate
        - Hence faster the centrifugation speed the smaller particles settle
- Difference between different types of gels
    - Pore size (ie. Small= acrylamide gel)
- PCR- This is a very important topic and likely question on Exam
    - Amplify DNA
    - Several cycles of changing temp to cleave and anneal
    - Each cycle 2X double stranded DNA produced
- Hypervariable microsatellites
    - Used DNA fingerprinting
    - Created by variable number of tandem repeats (VNTR)
        - Ex. 2 nucleotides A and C repeated variable number of times
            - Generally di, tri, tetra nucleotides
        - In noncoding DNA regions
        - On each side of repeat are flanking regions of unordered DNA→ allows specific loci for primers to amplify the microsatellites with PCR
        - Primers for PCR from these unique flanking regions
            - Useful genetic markers

    - Highly polymorphic
    - Created through slippage mechanism
- Dideoxy sequencing (aka Sanger method)
    - 1 of many methods to sequence DNA
    - uses 2'-3' dideoxynuclotide triphosphates (ddNTPs)
        - this method takes advantage of the H bonded at the 3' hydrogen rather than usual OH bonded there
    - this sequencing terminates DNA chain elongation because can not form phosphodiester bond with next nucleotide
    - once completed PAGE gel electrophoresis performed
        - sequence produced from this read from bottom to top to get sequence (a)
        - can also do this with fluorescent dye detection (b)

(a) (b)



- Restriction nucleases
    - Cut DNA
    - Blunt- cohesive
    - Cohesive-cohesive
        - Cohesive ends to be ligated have to be capable of base pairing with each other
        - Blunt ends can be ligated to other blunt ends
- Ligation by DNA ligase
    - Recombinant DNA
        - Compatible ends do not have to be on different molecules

- 

  Recombinant plasmid→into bacterial cell→cell culture→millions of new bacteria→ purified amplified plasmid obtained from isolated lysed bacterial cells
- Human Genomic DNA library
  - Total genomic DNA from single organism
  - Recombinant human DNA plasmids in bacterial cells
- mRNA→ complement (cDNA) for analysis and storage
  - used to ID mRNA expressed in a tissue
  - converted to DNA because greater stability and DNA can be amplified by PCR or bacteria
- DNA molecules labeled with radioactivity or chemical modification invitro
  - "end label" radioactivity
    - DNA labeled at 5' end with polynucleotide kinase and p-labeled ATP
    - Transferred phosphate is gamma-phosphate
  - "body label" radioactivity
    - denature and anneal with hexanucleotides
    - add DNA polymerase and labeled nucleotides
    - DNA polymerase incorporates labeled nucleotides resulting in a population of DNA molecules with labeled
      - Ex. Of a sequence on both strands
      - Incorporated phosphate = alpha phosphate
  - Chemical modification
    - Antibodies and fluorescent markers
    - Temperature changes
    - Labeled probes (RNA and DNA localization)
- Southern Blot= DNA, Northern Blot= RNA