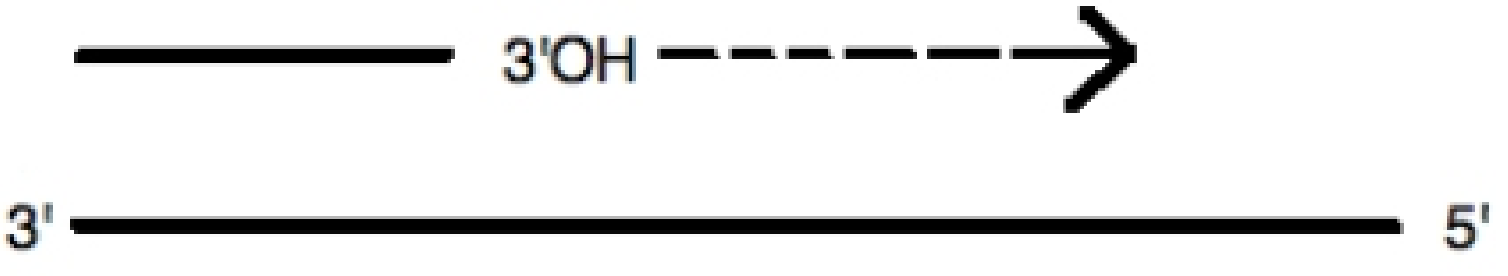
Lecture 8

- Difference between a Northern blot and microarry is?
  - Involve RNA
  - Involves determining whether a particular RNA species is present in a particular sample and at what level it is expressed at.
  - Quantitative methods for looking at the relative expression pattern of various RNA that are encoded by a certain gene
  - The difference in a northern blot is the actual experimental sample are run on the agarose gel, then transfered to filter paper, and the gene whose expression your interested in is the probe.
    - have to look at each gene separately
  - Microarray experiment is reversed, all the genes you are interested in and want to examine are spotted on the chip
    - either short oligos that are complementary to the message or the cDNAs of those message. And the RNAs are probed.
      - probe - who ever has the tag that your experimental design is detecting
  - in both experiments the goal is to determine the level of RNA for a specific gene
  - Microarray allows you to examine tens of thousands of genes at once where Northern blot allows you to look at many tissues but only one gene at a time.
    - Both give quantitative information, so the signal shows greater hybridization in that band or spot, so there had to be more RNA in the sample
    - To make it quantitative which nucleic acid should be in excess for a chip, should it be the oligonucleotide on the chip or should it be the RNA your hybridizing onto the chip?
      - RNA, otherwise it would saturate the chip so you wouldn't have a quantitative result
    - Microarray some gene is covalently attached to the slide.
- Why does every spot in microarray have a different intensity?
  - the intensity correlates to the level of expression.
    - greater intensity means that there is higher expression
    - tells how much of the probe hybridized to the given gene
- In any given genome, a replicon of the unit between the origin and the termination
  - In phage or e. coli there is only one origin, and the replicon is the entire genome

- In larger organisms, it is required to have many replicons growing at the same time to ensure that replication can occur in the alloted time. So the human genome is broken into many replicons

- Some genomes have an origin that has a unidirectional fork, meaning the direction that your unwinding the parental DNA (fork) is moving in one direction. (usually viral genome)
  - In other types of genomes replications is bidirectional, meaning that there is a fork movement in both directions. And have two different replication apparatus assembled on two different sides moving in two different directions simultaneously. (usually bacterial)
- RNA polymerase requires NTPs and a template
  - needs a template to tell the polymerase what should I synthesize and where is the complementary base. Without a template it does not know how to synthesize the polynucleotide chain.
  - Uses 4 ribotriphosphates
  - does not need a primer
- DNA polymerase requires dNTPs, a template and a primer with a free 3' OH
  - uses deoxyribonucleotides where the 3' OH has been replaced by H.
  - have the property that a primer is required



- The primer for DNA polymerase can be either RNA or DNA
- RNA Polymerase that is used for either transcription or laying down the primer for DNA replication
  - sometimes RNA polymerases are used to recognize an origin of replication and may lay down an initial 10 to 12 nucleotide start replication, then the DNA polymerase continues. Then the RNA primer is deleted and the gap is filled by DNA polymerase.
- To start replication a 3' OH is required to extend the DNA
- RNA Polymerase can start de novo, meaning that they do not need a primer. The problem is that they don't know where to start.
- Primer ensures that the event is regulated
  - The primer is needed because DNA polymerase has a proof reading function that RNA polymerase does not

- DNA polymerases decides if the base is complementary, if the base is not then a exonuclease removes the base.
    - Advancement along the DNA cannot occur unless the incorrect base is corrected.
    - The error correction subunit which means that DNA polymerase cannot occur de novo.
- How many origins would it take to synthesize all of the DNA in the human genome in 6 hours during s phase?
    - The haploid human is 3.3×10^9 bp, and eukaryotic DNA polymerase add base pairs at 2000 bp/min. Therefore if there was only one origin, and humans are diploid, it would take 3.3×10^6 min or 60,000 hr
- Reverse transcriptase does not have error correction so there is a high error rate
- Fig 15.11 Each eukaryotic chromosome contains many replicons
    - eukaryotic replicons are 40 to 100 kb in length
        - this is the distance between any origin
    - A chromosome is divided into many replicons. Eukaryotes may contain as many as 10,000 origins. Only 15% are active at the same time.
        - means that some how the cell is keeping track which are going at once. Can see that, in general, repetitive DNA that is packaged as heterochromatin tends to be later in the later part of S phase.
- Fig 15.15 Origins can be mapped by auto radiography and electrophoresis
    - replication fork movement can be detected by autoradiography using radioactive pulses
        - amount to the radioactive nucleotide relative to the cold (un-labeled) nucleotides is the specific activity
    - In unidirectional forks, doing the pulse the first time shows that the path of the label goes in one direction, in the second pulse you would see the same thing
    - In bidirectional forks, the first signal would go in both direction, and the second would go in both directions.
- All polymerases always add nucleotides to the free 3'OH
    - means that polymerization is always growing from the 5' to 3' end
    - first nucleotide in the chain is a 5' triphosphate and always has a free 3'-OH so that when you write the sequence of nucleic acids, the convention is that write the one at the 5' end and the last is the 3'-OH even though it is both strands
    - Why is it that you must add to the free 3' end and cannot add to the 5' end?
        - The triphosphate is located at the 5' end, which is the high energy bond. You have to add via the triphosphate.