Introduction to CRISPR

Creating Double Stranded Breaks

- Protein-based recognition of DNA
  - ZFNs
    - Zinc Finger Nucleases
    - Recognizes 3-6 nucleotide triplets
    - Zinc fingers are the most common DNA binding domains in eukaryotes
      - Small protein motif that has zinc ions to help stabilize folding
  - TALENs
    - Transcription Activator-Like Effector Nucleases
    - Recognizes single nucleotides
    - Easier to make
- Now…CRISPR-Cas9
  - RNA-based recognition of DNA

Repairing DNA breaks

- Cells have ways to repair double stranded DNA breaks:
  - NHEJ
    - Non-homologous End Joining
      - Ends of the DNA are chemically ligated with introduction of insertion or deletion
  - HDR
    - Homology Directed Repair
      - Donor DNA molecule has sequences that match the ones flanking the site of the break
      - This leads to the donor DNA being integrated into the break

CRISPR

- **C**lusters of (or Clustered) **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats
- Short segments of DNA (20-40 nucleotides)
  - Palindromic
  - The resulting transcription of these regions forms hairpin turns
  - These repeats are interspaced- with spacer DNA in between the segments
    - Segments of spacer DNA are going to be unique
    - Spacer DNA matches up with bacteriophage DNA (2000s)
- CRISPR associated genes (cas)
  - Cas proteins
    - Helicases
    - Nucleases
- CRISPR system in bacteria

- Cas protein complex formed by cas genes
- CRISPR DNA is transcribed and fits into Cas complex
    - The CRISPR-Cas complex will bind to viral DNA and destroy it
- If the bacterial cell does not have a spacer that matches up with bacteriophage DNA that is injected…
    - Class 1 Cas protein is made that will bind to bacteriophage DNA, cut it, and more importantly takes resulting fragment(s) and incorporates it into the CRISPR region
- So, CRISPR is spacer-repeat-spacer-repeat-spacer, with the spacers being a running history of ”old infections”.

- CRISPR in biotech
    - Use it to either inactivate genes or insert new genes

- CRISPR-Cas9 system
    - Doudna & Charpentier
        - Found in *S. pyogenes*
        - Only one Cas protein (Cas 9)
            - Nuclease
        - Two long strips of RNA are created
            - CRISPR RNA (crRNA)
            - Trans-activated crRNA (tracrRNA)
                - These two parts are what recruit Cas9 (crRNA:tracrRNA duplex)
                - These form a complex that can break DNA

- Doudna & Charpentier
    - Use Cas9 but replace spacer with desired DNA sequence
        - Then find a way to link it to tracrRNA
            - Linker loop
        - tracrRNA-crRNA chimera (guide RNA)
    - So, CRISPR-Cas9 system
        - CRISPR part= guide RNA that has the information of where we want to cut the DNA
        - Cas9 part= nuclease that does the editing (cutting)

- So, if there is DNA that we want to cut:
    - Create a guide RNA (gRNA) that will have a region complementary to the DNA
        - gRNA recruits Cas9
            - Cas 9 causes a double stranded break.
        - Since guide RNA is designed, we can decide where DNA is cut
            - Why?
            - **To cause mutations or insert gene of interest!**

- Inserting gene of interest
    - CRISPR-Cas9 + DNA we want inserted

    - Modified DNA is added and repair enzymes use that DNA to repair the other strand (Homology Directed Repair)
    - This system results in in vivo gene editing and editing in several places

Applications

- Fighting cancer
- Knocking out HIV
- Knocking out other diseases
- Improving IVF
- Protecting plants
- Biofuels
- Dinosaurs?