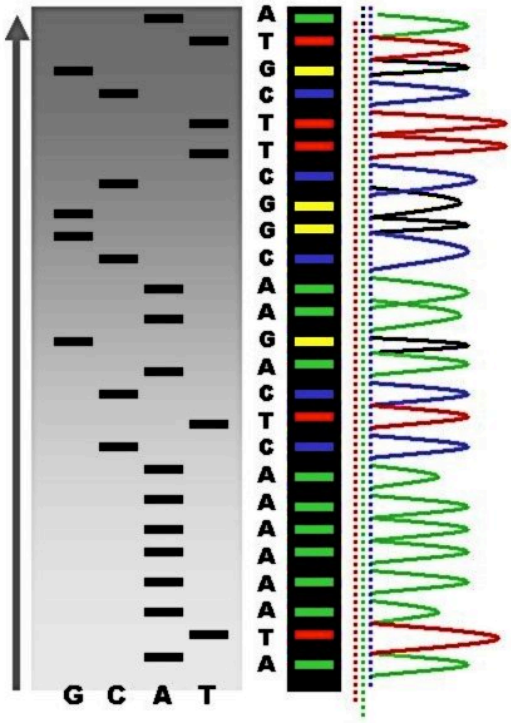
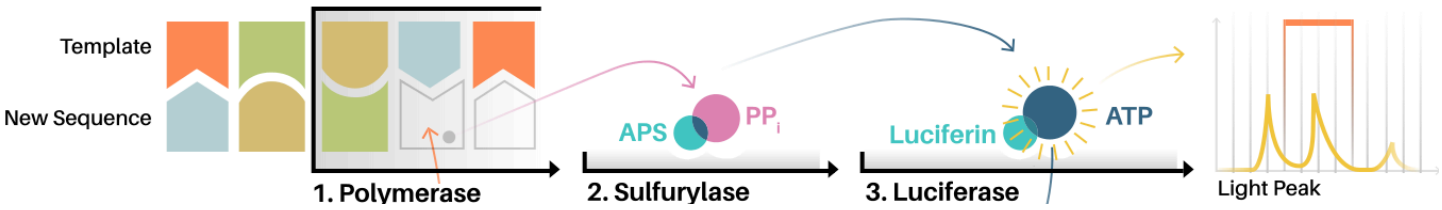
CONCEPT: DNA SEQUENCING

- Sanger sequencing – dideoxy sequencing using radiolabelled ddNTP's
    - □ mM conc. of dNTP's and uM conc. of ddNTP's
        - Only use one type of ddNTP (A, T, C, or G) per synthesis
    - □ Use gel electrophoresis to separate the segments of varying length
- Radiolabelled ddNTP's eventually replaced with fluorescent ones
    - □ Capillary gel electrophoresis column used with fluorescent detector to separate strands and determine sequence



- Pyrosequencing (454) – uses the pyrophosphate that is released by DNA polymerase to determine sequence
    - □ Beads in well with sequence of interest, along with enzymes sulfurylase and luciferase
    - □ Try each base in sequence, only a match will result in the release of pyrophosphate
    - □ Detect light flashes due to sulfurylase converting pyrophosphate to ATP, which activates luciferase



- Ion torrent sequencing – uses the H^+ that is released by DNA polymerase to determine sequence
    - □ Similar concept to pyrosequencing, but monitoring pH changes instead of light emission