6. Cross-linking acrylamide with N,N methylene-bis-acrylamide (bis-acrylamide)
    1. Initiated by addition of TEMED and ammonium persulfate added
    2. Ammonium persulfate (APS) + TEMED → help polymerization of acrylamide
    3. Don't want oxygen to get in → hinders polymerization

b. Know which direction the current flows, DNA flows, and why

1. Current goes from cathode to anode → DNA moved along with it
2. Run to red → DNA goes to anode/electrode (positive charge)
3. DNA has relatively negative charge
    1. Due to phosphate groups in sugar-phosphate backbone
4. Current flows from positive to negative (anode to cathode)
    1. Because opposite of electron flow; electrons going from negative to positive

c. Know by what characteristic DNA gets separated through electrophoresis

1. Travels to anode with charge
2. Larger fragments have slower rate of travel
    1. Physically hindered
3. Smaller fragments have higher rate of movement
4. Bigger fragments nearer starting point, smaller fragments travel further
5. Also conformation
    1. Supercoiled DNA travels quickest
    2. Linear typically travels faster than supercoiled, but slower than nicked circles
        1. Sometimes same as circles
    3. Nicked circles: usually slowest

d. Know the purpose of a DNA marker

1. Can make standard curve to estimate sizes of unknown DNA fragments
2. Proves gel was running properly
    1. Can compare to how it's supposed to look

3. **<u>Sequencing</u>**

a. Know what sequencing allows us to do

1. Can find sequences of DNA fragments
2. Unknown DNA sample → can sequence to find code
3. Useful for classifying organisms/crime investigations
    1. Compare sample of known and unknown DNA

b. Know why we clean sequences

1. Computer program can't always figure out what base is supposed to be where
    1. "messy reads"
        1. Ex. Could be A, could be C
    2. Clean reads based on which base looks most promising
        1. Allows one to get actual useful sequence
2. Need clean reads to create contig/run BLAST