PCR

- **Sequence-specific amplification** of DNA
- **Exponential** replication
- Based on thermal cycling; possible because of thermostable (heat-tolerant) DNA polymerase (***Taq*** from *Thermus aquaticus*, organism from hot springs 72^{o}C optimum)
- PCR requires **primers**

  Two of them, "upstream" and "downstream", usually around 30 nt long

  Primer sequence determines specificity: the primers define the ends of the DNA sequence that will be amplified

  Primers are complementary to ***opposite strands*** of the DNA
- PCR cycling: **1. Denaturation (94^{o}C)**

  2. Primer hybridization (50-65^{o}C). Use the highest temperature that works to minimize nonspecific products. Melting temperature depends on:

  Primer length (longer, will still hybridize at higher Temp.)

  Primer sequence (AT base pairs only 2 H bonds; GC form 3)

  Desired specificity (greater specificity at higher Temp)

  3. Extension/polymerization (72^{o}C)
- Reactions require these components:
  1. **Target DNA** which is to be amplified
  2. **Single-stranded DNA Primers (oligonucleotides)**
  3. **DNA polymerase (*Taq*)**
  4. **All 4 dNTPs (deoxynucleotide triphosphates) (dATP, dCTP, dTTP, dGTP)**
  5. **Appropriate buffer (including Mg++)**
- Mineral oil to prevent evaporation

Main problems with PCR:

- **Nonspecific amplification:**
  1. Primers hybridize to unexpectedly similar region of the DNA, and an unwanted sequence is amplified.
  2. Primers partially hybridize (with mismatches) and prime DNA synthesis inappropriately

  Possible solutions: Increase primer length; raise hybridization temperature; change primer sequence entirely
- **Contamination:** PCR is *exquisitely* sensitive; in principle, a single copy of the target DNA is all that is required for amplification. Therefore, trace contaminants can amplify into big problems.

  WEAR GLOVES in the real world; use tips with plugs to prevent aerosols
- **Error rate is high:** *Taq* polymerase, acting in a PCR reaction, lacks proofreading function. *In vivo*, DNA replication has an error rate of less than 1 per 10^9 nucleotides; in PCR, the rate is more like 1 in 10^4. If a PCR product is cloned, then any error made in that one clone becomes permanent in all DNAs made from it.

  Possible solutions: Minimize number of cycles of amplification for less total DNA synthesis; use a different thermotolerant polymerase with higher fidelity (such as *Pfu*)

DNA synthesis of all kinds, including PCR, proceeds 5' to 3'. This means new nucleotides are added to the growing DNA chain at the 3' end, where the OH group is. DNA polymerases attach a fresh nucleotide using deoxynucleotide triphosphates; two of the phosphates are cleaved off, and the third phosphate binds to the 3' OH. Also, sequences are always written 5' to 3'.

This is very important for understanding primer design.

Draw the first cycles of amplification to see that it isn't until the 3rd cycle that the desired product is made with the proper ends.