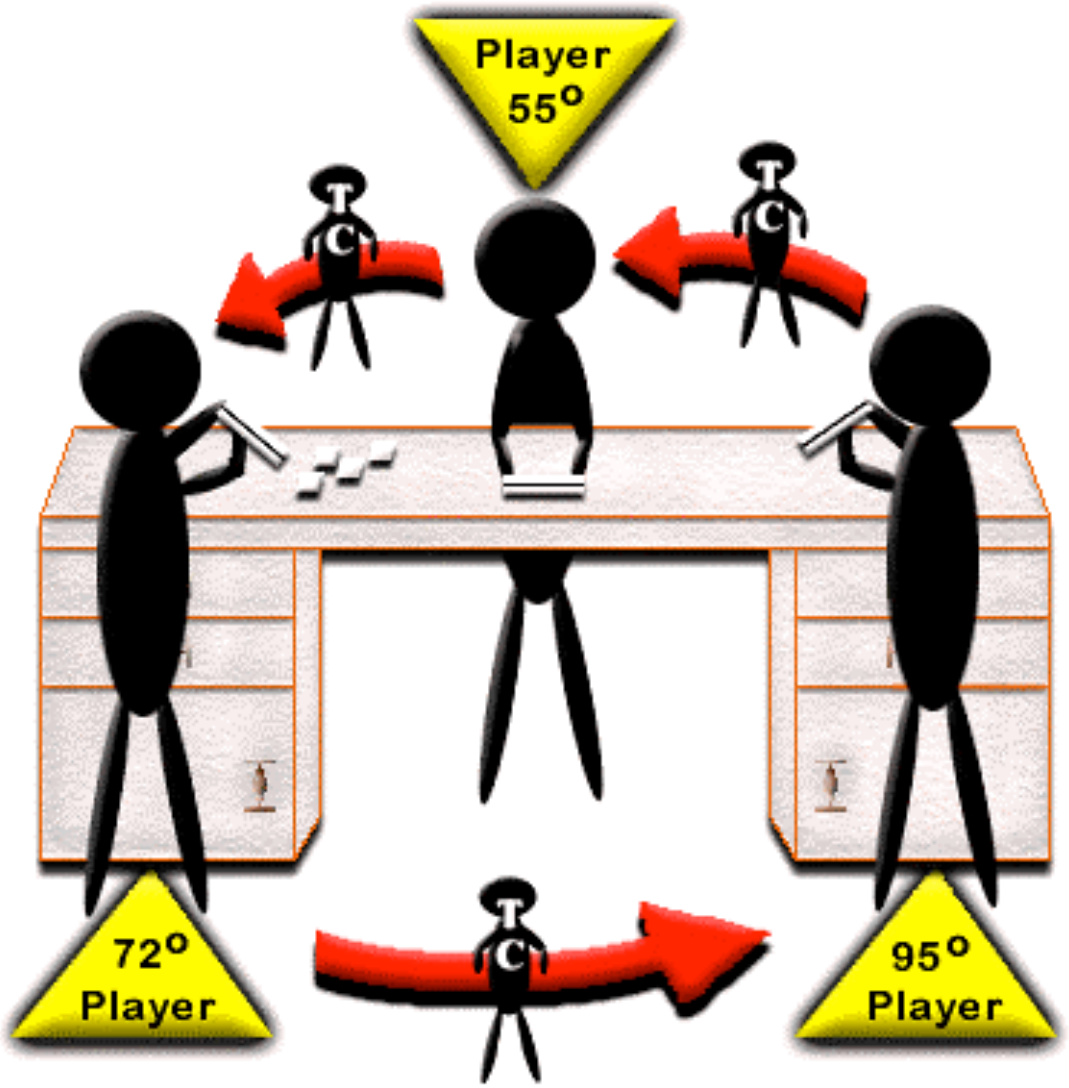
The PCR Dash



http://research.nmsu.edu/molbio/bioinfo/k-12/pcr_dash/index.html
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The PCR Dash

The Polymerase Chain Reaction is a technique used very extensively in molecular genetics, forensics and other fields to greatly amplify a specific DNA sequence. Kary Mullis who received the 1993 Nobel Prize in Physiology and Medicine for the discovery invented it in 1983.

A PCR amplification reaction includes: the source DNA containing the sequence of interest (as few as 1 copy), two unique single-stranded DNA primers that bracket the desired sequence, deoxyribonucleotides (the building blocks of DNA), and Taq DNA polymerase (from the thermophilic organism *Thermus aquaticus*). The reaction is cycled between three temperatures that facilitate the construction of new copies of the desired DNA sequence. At 95°C, the double stranded source (or template) DNA is denatured into single strands. Then, at 55°C, the primers bind (anneal) to complimentary sequences on the template DNA that bracket the sequence to be amplified. Last, at 72°C, the Taq DNA polymerase adds deoxyribonucleotides in sequence to the primer as it builds a complementary strand to the template DNA. This cycle is repeated until a very large number of copies are obtained. The reaction is usually carried out in a machine called a thermal cycler that quickly changes the temperature of the reaction mixture between the three necessary temperatures. Good graphic illustrations of the process can be found at http://darwin.cshl.org/pcranwhole.html and http://www.accessexcellence.org/AB/GG/polymerase.html.

The PCR Dash is a game that will help illustrate the PCR process for students. The game is best played by several teams in a fun competition. Each team should have 4 people.

Materials:
The instructor or a designated timekeeper will need a stop watch or a watch or clock with a second hand.

The following set of materials is needed for each team.
Paper DNA strands from the accompanying template pages
1 marker
2 plastic grocery sacks
2 rolls of scotch tape
1 pair of scissors
4 nametags – 1 labeled 95°C, 1 labeled 55°C, 1 labeled 72°C, and the last labeled thermal cycler.

Preparation:
Make one photocopy of the template pages for each group. Cut out the DNA template strands, the primers, the polymerase product strands and the decoy strands. The template strands have

complimentary DNA sequences with purines (A & G) lightly shaded and pyrimidines (C & T) more darkly shaded. There are two different primers, one matching the primer binding site on one template strand and the other matching the primer binding site on the other template strand. There are also two different polymerase products, one complimentary to each of the template strands.
Tape the DNA template strands together. Place one role of tape and the primers into one plastic sack labeled 55°C. Place the other role of tape, the polymerase product strands and the decoy strands into the other plastic sack labeled 72°C.
Give each of the 4 players a nametag. Place the 95°C, 55°C, and72°C players in a line a little over arm's length apart. They may be seated or standing. Give the 95°C player the DNA template strands and the scissors, the 55°C player the sack with the primers and tape, and the 72°C player the sack with the tape, polymerase products, and decoy strands. The fourth player is the thermal cycler and will transfer the DNA from one temperature player to another during the game.

Game Play:
The teams are given 3 minutes in which to properly synthesize as many copies of the template DNA as possible.

Steps:
1. The timekeeper starts the game with 3 minutes to play.
2. The 95°C player denatures (cuts apart) the template DNA strands.
3. The thermal cycler (TC) takes them and gives them to the 55°C player.
4. The 55°C player matches the complimentary primer to each DNA strand and attaches them with tape.
5. The TC then takes the primed strands to the 72°C player.
6. The 72°C player matches the complimentary polymerase product to each DNA strand and tapes it to the primer.
7. The TC then takes the finished double stranded products back to the 95°C player who denatures them and the cycle is repeated as many times as possible during the time allowed.

Winners:
The winning team is the one that has synthesized the most finished copies of the desired DNA sequence when time is called.

Note:
There are only two real deviations from what normally happens in a PCR reaction that have been incorporated into the game for convenience. Usually the DNA template strands are much longer than the desired product strands. And, the primers are usually in the range of 15-20 base pairs in length and the region to be copied can range from the 100's to the 1000's of base pairs.

The game may be played with 3 players on each team if the thermal cycler is left out and the other players are positioned in a circle so that they can pass the "reaction" to each other. PCR is sometimes carried out by moving a tube holding the reaction mixture between three water baths at different temperatures.