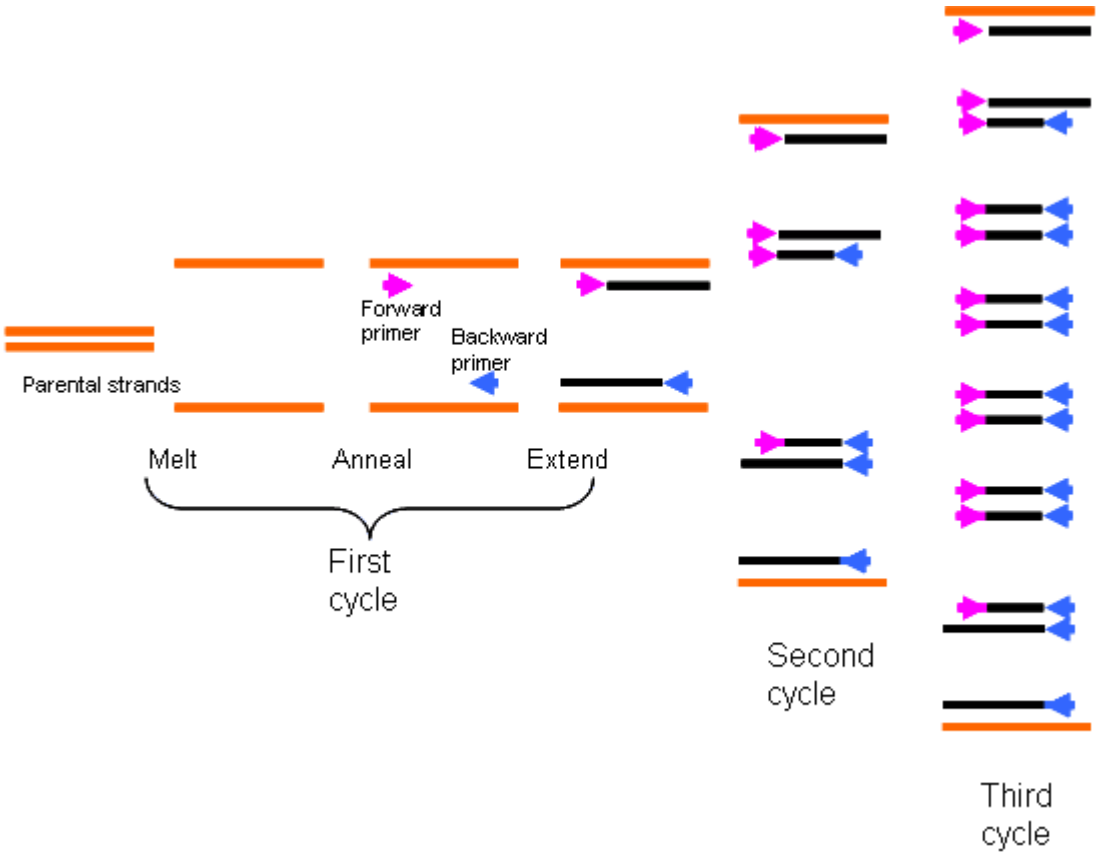
Polymerase chain reaction (PCR)

DNA can be replicated in the lab (in vitro) by isolating the individual components required, such as enzymes, and adding a **template DNA** to the mix.

If we have obtained a DNA sample or a few, what next? Well, nothing much can be done with that. We must obtain exponentially more DNA to use for any purpose. And it all of course must be identical. We must essentially **clone our DNA**. Considered the very staple of molecular biology, this technique for multiplying DNA many-fold was invented by a chap Kary Mullis who believes in astrology.

The DNA template to be amplified can be extracted from a field sample (a leaf, human saliva, cultured microorganisms, etc.) or synthesised chemically, on order.

Essentially the DNA is denatured so the 2 strands break apart, short complementary bits called **primers** attach to the strands, the enzyme **DNA polymerase** binds to the primers and initiates the assembly of a new DNA strand, and finally the process is repeated many times over in a chain reaction. This is the **polymerase chain reaction, PCR**.



Soon enough, the few bits of DNA become thousands, and hundreds of thousands, and millions…

The components of PCR can fit in a very small tube which is placed in a specialised **thermocycler** or **water bath** in order to expose it to these fluctuating temperatures. Thermocyclers can be programmed to run automatically on a cycle along the lines of (degrees Celsius) 90-60-70 each for a few minutes, repeated many times over e.g. 30 times. Overall, this can take a few hours to complete.

The fluctuations in **temperature** correspond to each step in PCR. The highest temperature is required to separate the strands. The lower, **annealing temperature** bring the strands closer again, in order to bind the **primers** required to kick-start replication by DNA polymerase, while the temperature lower than the denaturing step, but higher than the annealing step is required for the addition of nucleotides by the polymerase – **extending**.

These temperatures are well above most physiological

conditions where enzymes like polymerase would be functional, so special polymerases are used in PCR which are **heat-resistant**. They were isolated from microorganisms found living in hot springs and such extreme environments.

Other ingredients of PCR include the **nucleotides** themselves (free and ready to be added to new DNA strands by polymerase), other optimising agents such as magnesium ions for the DNA polymerase, and water.

Controls and applications

Positive and **negative controls** are used for PCR. Controls ensure that the outcome of the experiment is what it seems to be.

Positive controls give a reference point for what the result would look like if it worked, while negative controls give a reference point for what the result would look like if it didn’t work.

A **positive control** for PCR might be a PCR reaction **identical to the one we are running** as an experiment, but instead of the test template DNA we add a **different template DNA** that we know will definitely work based on previous data. If the experiment fails, but the positive control works, we can be sure that the PCR reaction was correct but there was an issue with the test template DNA.

A **negative control** for PCR requires a little less sophistication, and might involve using **the same PCR reaction** while **omitting any template DNA** at all. If we seem to get something that looks like it worked in our experiment using our template DNA, but it looks the same as the negative control, then we can be sure that it actually hasn’t worked, and the result is because of another reason e.g. contamination, background signal, PCR ingredients themselves, etc.