Polymerase Chain Reaction Laboratory

The purpose of the polymerase chain reaction (PCR) is to amplify specific segments of DNA. If one knows the DNA sequence of regions of DNA that flank a DNA segment of interest, it can be amplified.

In this lab we will exploit the PCR process to amplify a segment of the \textit{nptII} gene in putative transgenic plants.

The use of PCR to detect transgenes in our plants is an efficient screen because only small amounts of DNA need be isolated (DNA quickprep), then the thermocycling is performed (3 hours), and then the sample is electrophoresed. This whole process can be completed in 1 workday for up to 96 samples. To conduct this PCR, we have synthesized flanking primers for the \textit{nptII} gene of 26 and 25 bases each. These NPTII primers will be combined with the template DNA, Amplitaq polymerase, buffers and nucleotides and placed in a thermocycler for 30 cycles of amplification. If a 255bp fragment is amplified, we can assume (with some level of confidence) that the gene is inserted into the plant genome. We visualize the amplified fragment via DNA electrophoresis.

As part of the laboratory exercise, we will set up PCR amplifications to test putative transgenic tobacco and corn. (A positive PCR provides strong evidence that the plant is transgenic, while no amplification indicates it is non-transgenic.)