DNA Digestion

One of the prerequisites of an experiment with recombinant DNA is the availability of DNA fragments. These fragments can be generated four ways: cleavage by restriction endonucleases, mechanical shearing, RNA-directed synthesis, and chemical synthesis. The method of choice to fragment DNA is with restriction endonucleases. Their discovery (along with cloning vectors and ligation) and application has helped revolutionize molecular biology. The class II restriction endonucleases are enzymes that cut DNA into discrete fragments by cleaving only at specific DNA sequences. The site at which an enzyme cuts is called its recognition sequence and the resulting pieces are referred to as restriction fragments. These enzymes are stable and require only Mg$^{++}$ as a cofactor. In addition, comparatively crude enzyme preparations can be used effectively. Over 300 restriction enzymes with over 100 different recognition sequences and specificities are available. Restriction enzyme digestions can be performed analytically (usually small-scale to analyze a digestion pattern) or preparatively (usually large scale for the isolation of a particular DNA fragment resulting from digestion).

A DNA digestion includes the following components:

1) DNA in appropriate quantity
2) Restriction endonuclease buffer (usually a 10x concentration)
3) Sterile distilled water to bring the reaction to a predetermined volume
4) Restriction endonuclease in appropriate quantity.

The purpose of this laboratory exercise is to digest the plasmid and plant DNA that you isolated in the earlier laboratory exercises. These digestions will be loaded into an agarose gel in the following laboratory exercises for electrophoretic separation (size fractionation), then Southern transferred to a nylon membrane, and hybridized with dig-labelled DNA probes.

The lab groups will digest the potato DNA from the transgenic potatoes (and control Spunta) using two different restriction enzymes (BamHI and XbaI) along with pBICryV. With these digests, we will be able to check for gene insertion and transgene copy number.