Isolation of Plasmid DNA

One of the most basic and key steps in DNA manipulation involves the isolation of DNA. DNA does not exist as a free molecule in a cell, but rather as a complex association of DNA, RNA, and proteins. There are three basic steps in the purification of DNA. First, the high molecular weight DNA must be released from the disrupted cell wall and membranes. Then, the DNA-protein complexes must be disassociated by denaturation or proteolysis. Lastly, the DNA must be separated from other macromolecules.

In this laboratory exercise, each laboratory group will isolate a circular double-stranded DNA element called a plasmid from six cultures of *E. coli*, using the alkaline-lysis procedure (Birnboin & Doley 1979). Each culture of *E. coli* (DH5α) has been previously transformed with a different plasmid/insert each for a specific purpose (Table 1). The first plasmid, Bluescript (BS) is used for cloning of DNA fragments. We will use the BS vectors later in the term to construct a genomic library. T8P423 is a BS plasmid that contains a fragment of the potato ribosomal gene selected out of a potato genomic library at MSU. BS, T8P423 and pBSCryV are also high copy number plasmids. We will use pBSCryV as DNA probe in our Southern analysis experiment later in the term. The plasmid pBI121 is a vector used for *Agrobacterium*-mediated transformation. The plasmid carries a TDNA region in which a selectable marker (NPTII) and promoter with a polyclonal site. Genes of interest can be inserted at this site and then expressed in plants following transformation. The pBI121-derived plasmids do not have a high copy number compared to BS, therefore the yield of DNA of these plasmids will be lower. We will also isolate the plasmids from pBICryV and pBlpat/CryV.