SAMPLE PREP
Plant DNA extraction

B3 Summer Camp – Olympic High School
June 21, 2011
What is DNA extraction...?

- DNA extraction is the removal of deoxyribonucleic acid (DNA) from cells or viruses.

Extraction of DNA is often an early step in many diagnostic processes used to detect bacteria and viruses in the environment as well as diagnosing disease and genetic disorders.¹

- PCR experiments – Gene presence/absence
- Sequencing experiments – Human genome
- Validation and verification – quality control

¹http://serc.carleton.edu/microbelife/research_methods/genomics/dnaext.html
Protocol Development for Plant DNA extraction

- What is the first step in research....? Tons of background literature research
- Research literature contain sections called Experimental Methods and Materials which can help guide you in your protocol development
Protocol Development for Plant DNA extraction

Based on our experimental hypothesis, what would be the best literature article to use to help us in our sample prep protocol...

A. Optimising bacterial DNA extraction from faecal samples: comparison of three methods.
   Smith B, Li N, Andersen AS, Slotved HC, Krogfelt KA.

B. Extraction of high-quality genomic DNA from latex-containing plants.
   PMID: 12672415 [PubMed - indexed for MEDLINE]

C. Extracting DNA from museum bird eggs, and whole genome amplification of archive DNA.
   Lee PL, Prys-Jones RP.
   PMID: 21585832 [PubMed - in process]

Basic steps in DNA extraction

- Break open the cells – cell disruption or cell lysis
- Remove large junk from the mix (i.e. organelles)
- Get the DNA to form a solid state (to precipitate) to remove most other contaminants
Break open the cells

The plant tissue must be ground with a mortar and pestle to break the plant cells open allowing the DNA to freely leave the cell\(^2\)
Break open the cells - CTAB

◊ Cetyltrimethyl ammonium bromide (CTAB) is a surfactant useful for isolation of DNA from tissues containing high amounts of polysaccharides.³

◊ Surfactants are compounds that lower the surface tension of a liquid, the interfacial tension between two liquid, or that between a liquid and solid. (i.e. Gulf of Mexico oil spill – controversial dispersants)

http://cshprotocols.cshlp.org/cgi/content/abstract/2009/3/pdb.prot5177
Break open the cells - CTAB

- CTAB – binds to the polysaccharides, removing them from the solution\(^3\)
- The presence of polysaccharides in DNA prep can inhibit use of techniques (i.e. PCR) \(^3\)
- **WHY DO NEED TO USE CTAB....?**
  - Plant cells are surrounded by a polysaccharide-rich primary wall\(^4\)

http://www.cccrc.uga.edu/~mao/intro/ouline.htm
Removing the Junk
Your DNA should be in the aqueous layer. Careful removal of this layer is important to prevent contamination.

Adding Isopropyl alcohol is an effective DNA precipitant that render insolubility of DNA in an aqueous medium.

** If it is cold it helps to minimize DNA degradation.