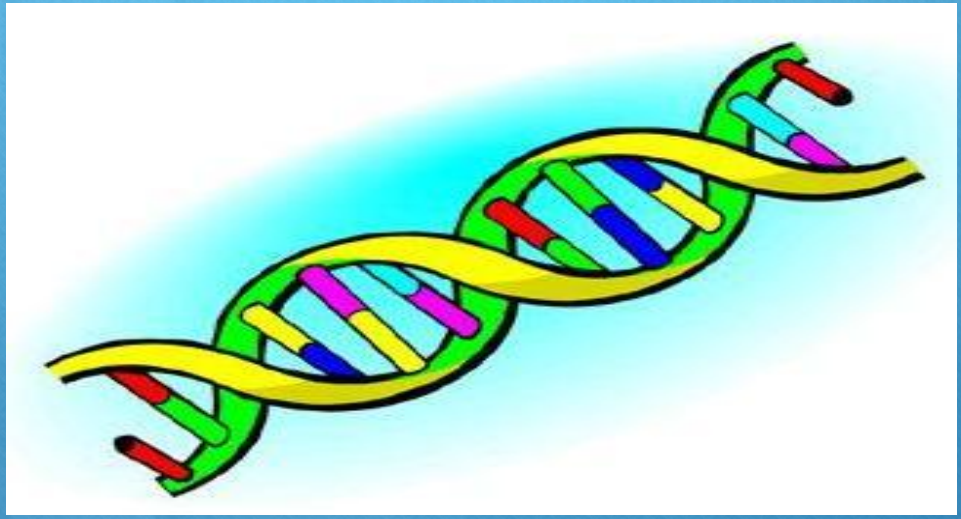
SAMPLE PREP
Plant DNA extraction

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 [1]



1.http://serc.carleton.edu/microbelife/research_methods/genomics/dnaext.html

What is it used for…?

- 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

1.http://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.
Open Microbiol J. 2011;5:14-7. Epub 2011 Apr 22.
PMID: 21643498 [PubMed - in process] **Free PMC Article**

B

Extraction of high-quality genomic DNA from latex-containing plants.
Michiels A, Van den Ende W, Tucker M, Van Riet L, Van Laere A.
Anal Biochem. 2003 Apr 1;315(1):85-9.
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.
Mol Ecol Resour. 2008 May;8(3):551-60. doi: 10.1111/j.1471-8286.2007.02042.x.
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]

http://plantandsoil.unl.edu/croptechnology2005/pages/index.jsp?what=topicsD&informationModuleId=957882007&topicOrder=6&max=7&min=0&

Break open the cells - CTAB

- Cetyltrimetyl 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]
- <u>WHY DO NEED TO USE CTAB</u>….?
  - Plant cells are surrounded by a polysaccharide-rich primary wall[4]

http://www.ccrc.uga.edu/~mao/intro/ouline.htm

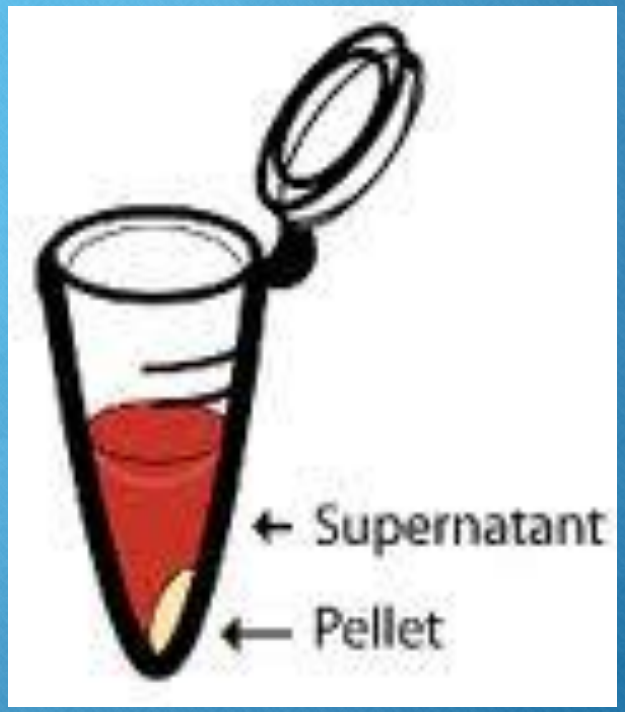
Removing the Junk

DNA isolation

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.


Supernatant
Pellet