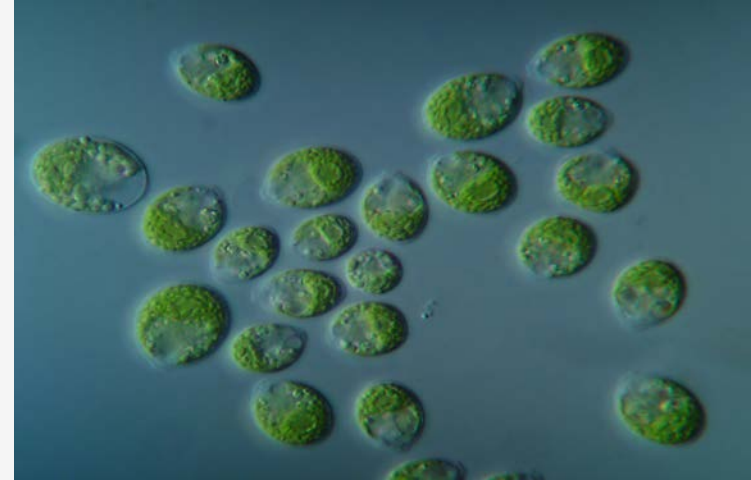
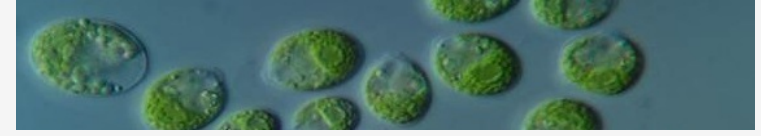


Chloroplast Transformation biolistic Targeting of the Chloroplast Plasmid in *Chlamydomonas reinhardtii*

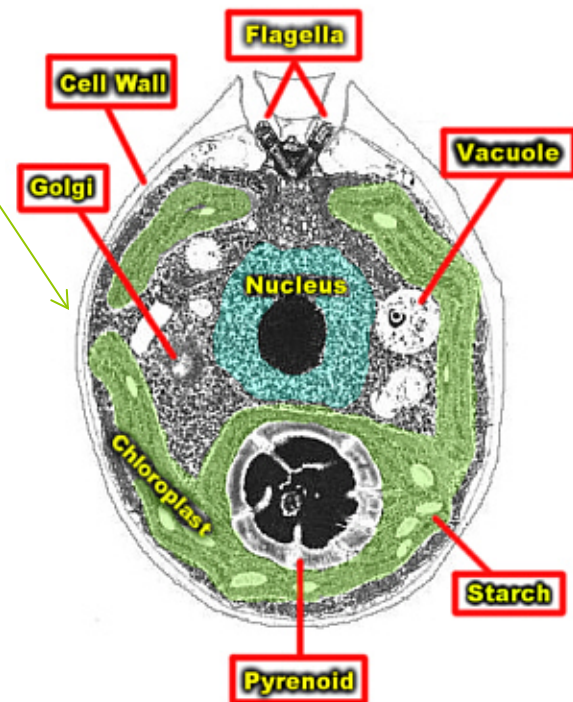


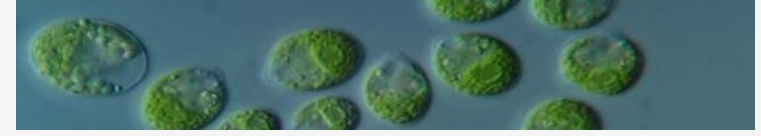
Presented by
Patrick Lopez
&
Geri Long



Chlamydomonas reinhardtii

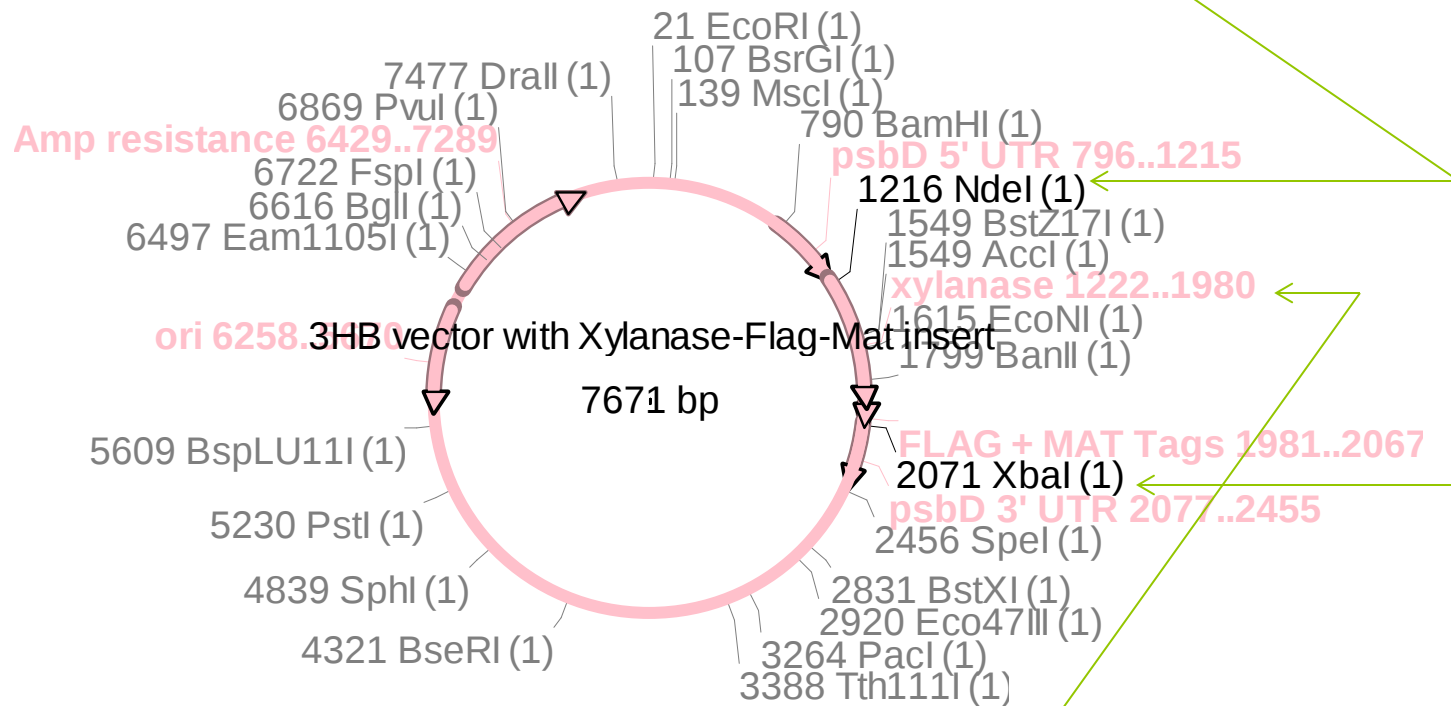
- One Chloroplast
- Approximately 50-80 plasmids coding for the chloroplast
- Nuclear genome codes for everything else
- Normally in vegetative state, but can be induced to mating strains with nutrient deprivation



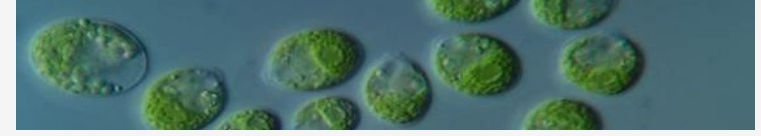


The 3HB Carrier/Vector Plasmid

Restriction Enzymes Sites Targeted



Transcription Sites Targeted



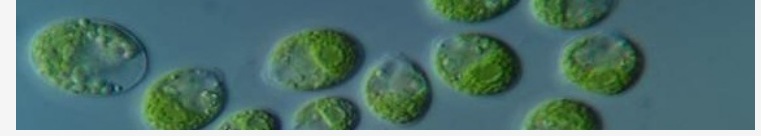
The Hypothesis:

- We can use transcription and ligation enzymes to construct a new plasmid.
- We can insert this new plasmid into a chloroplast's DNA by means of a biolistic gene gun pellet coated with the plasmid.
- The Chloroplast will uptake the foreign DNA, incorporating it through repair function via homologous end joining.
- Using selective media, we can then screen for the new transforms
- Using PCR, SDS-PAGE, and Western Blot Transfer, we then can confirm of the presence of the insert, the protein, and enzyme activity that it transcribes.



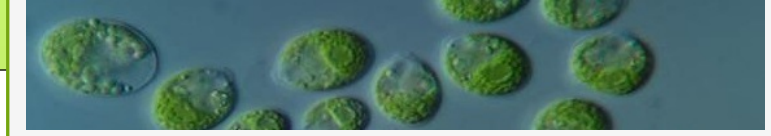
*gaining new abilities
via gene insertion*





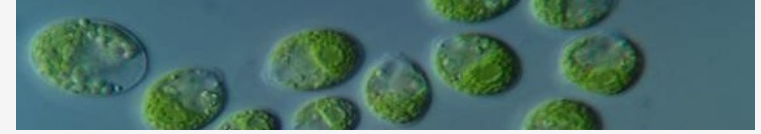
General outline to vector assembly, purification, and insertion

- Prepared bacterial mini-preps
- Obtained digests of 3HB carrier vector & pUC19 gene of interest
- Agarose gel purification with restriction analysis
- Ligation using T4 DNA Ligase enzyme for homologous end joining in competent E. Coli cells
- Screened colonies with target digest
- Stacked mini-preps to gather plasmid
- Prepared biolistic gene gun gold bullets, shot Chlamydomonas reinhardtii algae, incubated



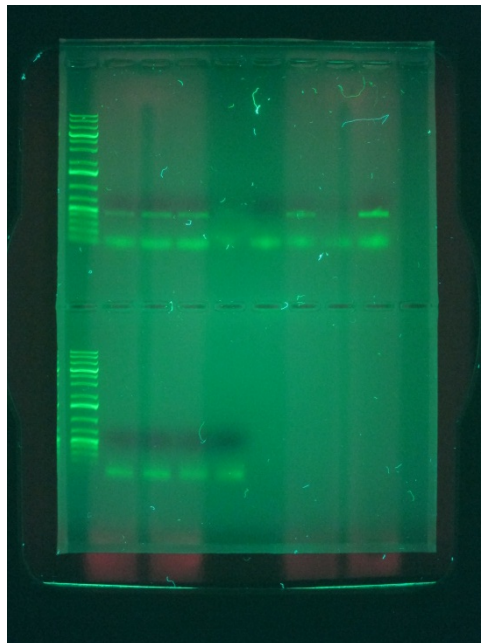
General Outline to Expression, strain isolation, and protein confirmation

- Streak colonies on High Salt Medium to promote homoplasmy
- Screen for the gene integration and homoplasmy by PCR
- Modified plasmid is inserted, test for protein
- Ran on SDS-polyacrylamide gel for protein isolation
- Transferred SDS-PAGE to Western Blot membrane
- Expose to specific antibodies for xylanase
- Target protein presence confirmation, but Is it active?
- Enzyme activity assay in the platereader

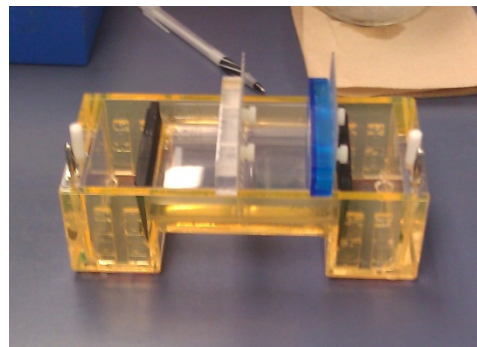
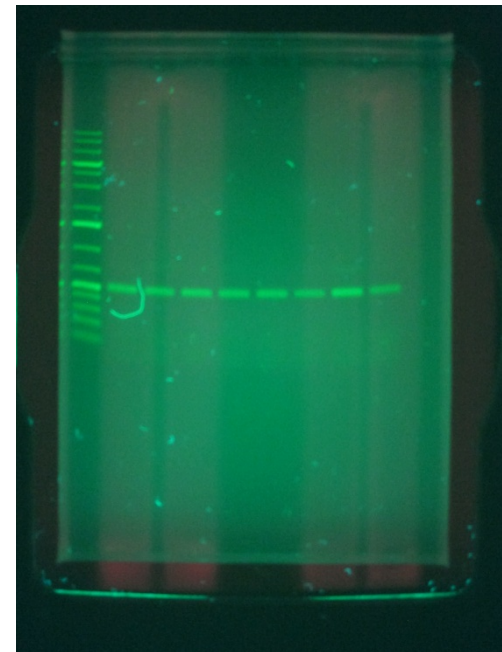


Data: Gels

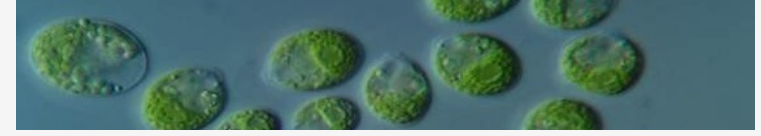
Presence of Introduced
Chloroplast genes



Screening for Gene
Homoplasmy

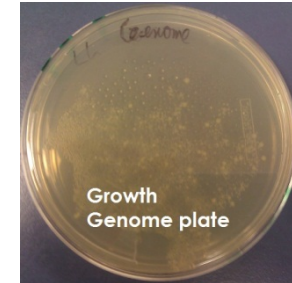
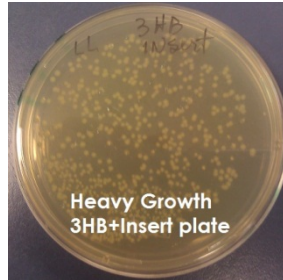
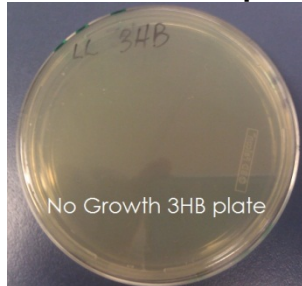


Agarose gel
Apparatus



Data: Plating for Confirmation

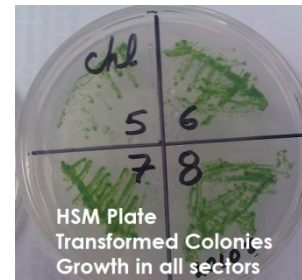
1. Initial competent E. Coli 3HB vector + pUC19 insert colonies:

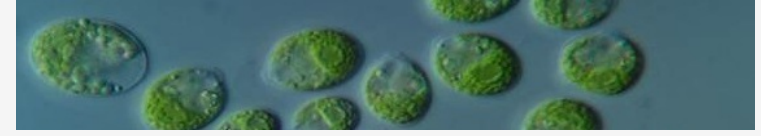


2. Initial gene gun colonies only confirms an existing colony:

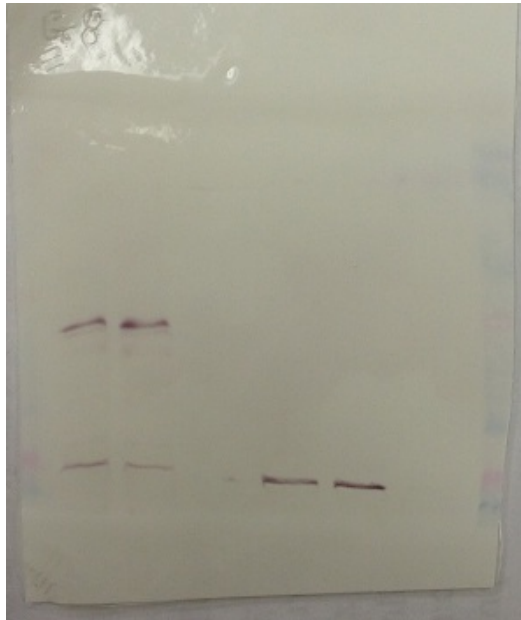


3. Plating gene gun colonies on High Salt Medium "HSM" to confirm photosynthesis capability, all eight sectors with growth:

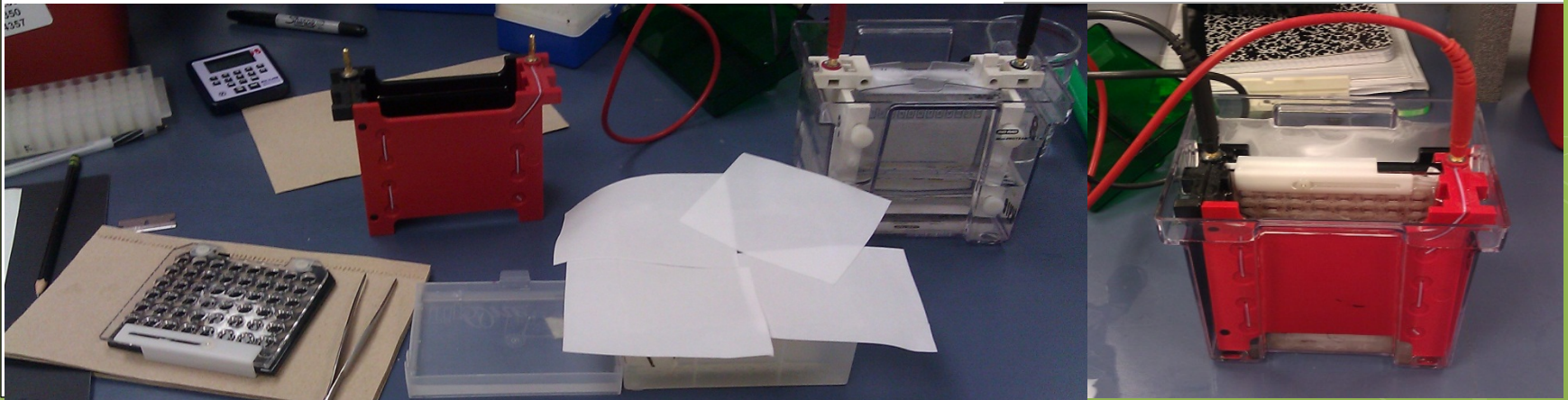
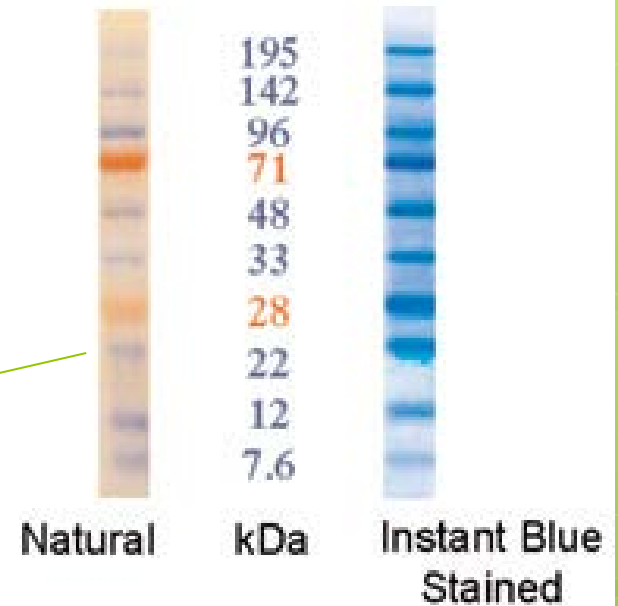


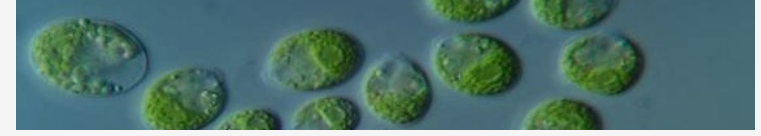


Data: Western Blot Confirmation



Xylanase protein
bands appeared
at ~ 22 kDa





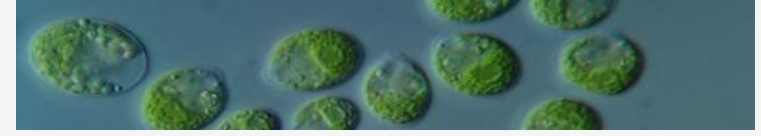
Data Analysis: Confirmations

The agarose gels confirmed Insertion digests.

The plating confirmed gained photosynthetic ability.

The Western Blot confirmed the presence of xylanase.

The Xylanase enzymatic assays confirmed actual protein activity.



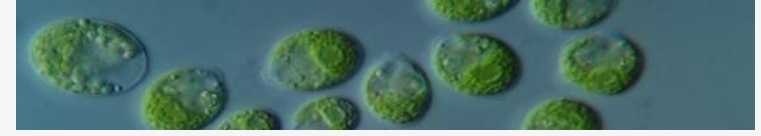
Future Applications: Biofuels

Chlamydomonas is a good subject to study structure/function of photosystem II, which is important in maximizing biofuel algae production.

ADV-integration of the transgene in plastid genome preceded by homologous recombination is precise and predictable.

Primary interest in algae for growth rates, growth density, lipid accumulation, resistance to predators, ease of harvest, and co-products.

Technologies formed through Chlamydomonas can be applied to other algal strains.



Future Applications: Other Fields

Transformation can be used for photo autotrophy, resistance to antibodies, tolerance to herbicides, and resistance to other metabolic inhibitors

Production platform for recombinant proteins w/ therapeutic uses

Algae chloroplasts can assemble and fold complex mammalian proteins

Algae can be used for food for fish, mollusks, shrimp

Chlorella produces β -1,2-glucan which is an immuno-stimulator, free radical scavenger, and reduced blood lipids

Chloroplasts are bioreactors for molecular farming proteinaceous biopharmaceuticals include vaccines, antimicrobials, antibodies.

