

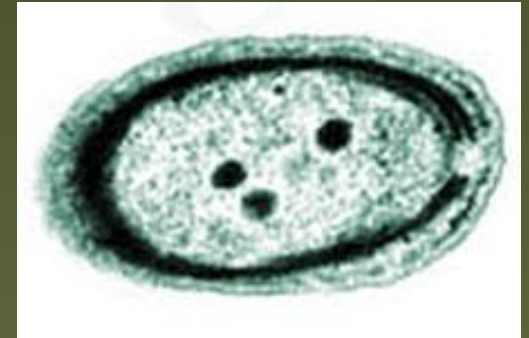
Cloning PMT1149 from
Prochlorococcus marinus str.
MIT 9319

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Project Goals

- Clone the Antifreeze gene PMT1149 from *Prochlorococcus marinus* str. MIT 9319 into *E. coli*.

Source Organism



- Prochlorococcus marinus str. MIT 9313
 - These bacteria belong to the photosynthetic picoplankton and are probably the most abundant photosynthetic organism on Earth.
- Obtained from the **National Center for Marine Algae and Microbiota (NCMA)**

Introduction of Initial Project

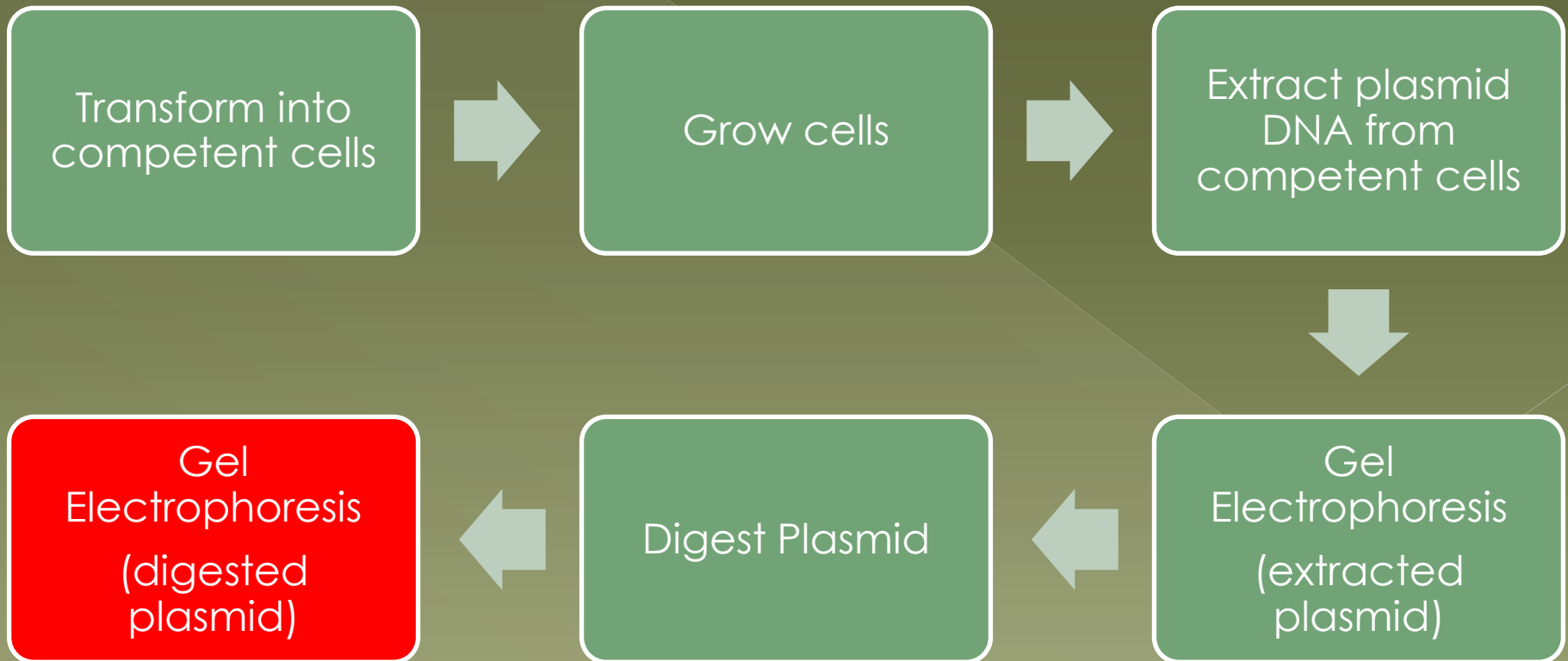
- GOI: PMT1149
 - > Antifreeze Protein from *Prochlorococcus marinus* str. MIT 9319
- Size: 372 bp, 124 aa
 - > No introns
- BioBrick part: Bba_I0500
 - > Pbad
 - Inducible by L-arabanose

Initial setback

- We were unable to obtain a sample of *Prochlorococcus marinus* and the special medium needed pro99 until almost mid-October.

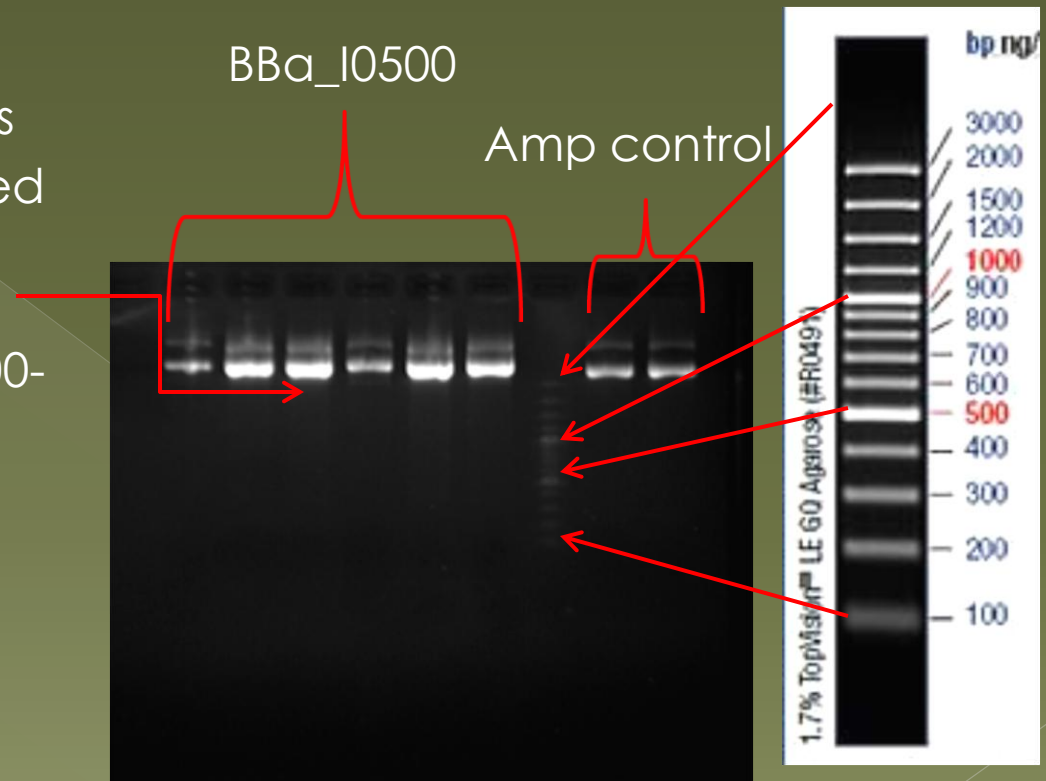
While waiting...

- We began work on our Biobrick part
 - > BBa_I0500



Extracted plasmid

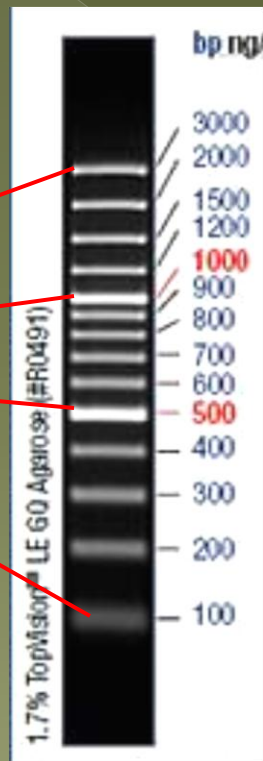
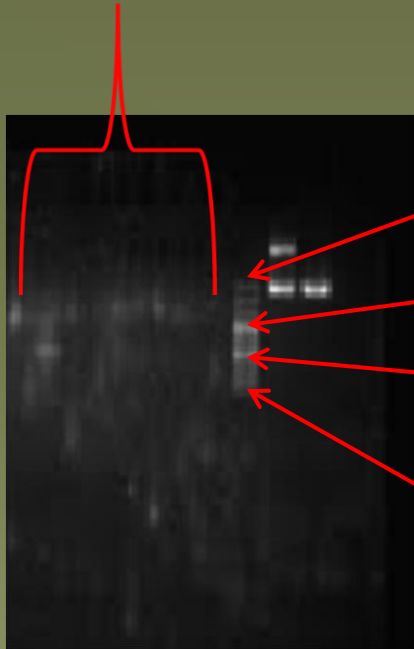
- 1% agarose gel-electrophoresis
- 2.0 μ L EtBr added to gel- allowed visualization under UVB light
- GeneRuler™ 100bp Plus DNA Ladder used for calibration [100-3000bp]



SUCCESS!!!!

Digested Plasmid

Inconclusive results



The results were sketchy, the gel wasn't very clear (lots of marking)

The results were quite faint as well overall a **failure**

Digested plasmid, Round 2

Clear gel, absolutely no results.

Somewhere in the digest we appeared to have lost all DNA

Wrong ladder used.
Overall a failure!

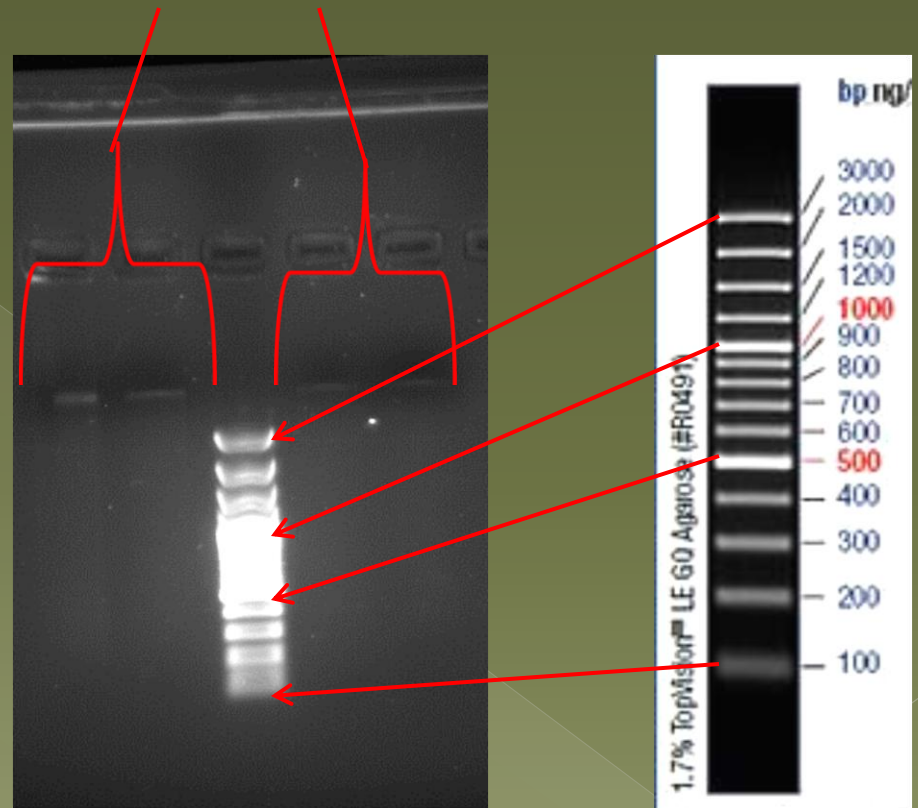


Nothing at all

Extracted Plasmid Round 2

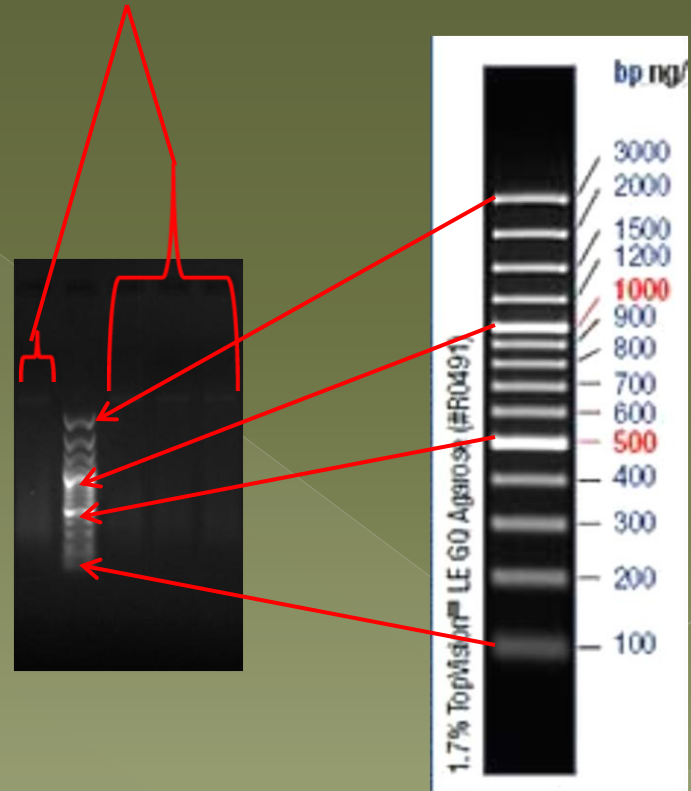
- We attempted new extraction in hopes of better yields of DNA
- Very faint results.
 - Possibly due to low levels of DNA used in gel.

Extracted Bba_I0500



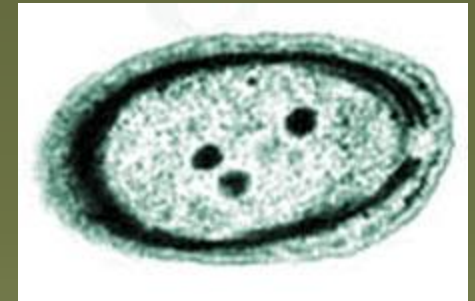
Finally some results, they're faint but this most likely due to the large size

Light bands of Digested plasmid part Bba_I0500



Growing prochlorococcus

- We attempted to grow this bacteria
- Ordered from the NCMA
- Grows in Pro99 medium
 - Which is a natural sea water medium



Growing Prochlorococcus

- First attempt:

- > Pro99 mixture was produced using natural autoclaved sea water
- > Flask was placed in natural light near a window
 - So it would have a natural light cycle

- FAIL!

- > No growth what so ever

Growing prochlorococcus

- Second attempt:

- Pro99 mixture was produced using natural autoclaved sea water
- Flask was placed under the hood to have a constant source of light to depend on,
 - less temperature fluctuation as the window sample

- Failure again!

Growing Prochlorococcus

- Third attempt:
 - > Pro99 mixture was produced using natural autoclaved sea water
 - > Flask was placed in temperature and light controlled unit
- Again Nothing.

Growing Prochlorococcus

- Fourth attempt

- Pro99 mixture was produced using natural autoclaved sea water
- Flask was placed under the hood like our second attempt was
 - This time it was placed on a rocker in hopes the motion would assist the organism in growth

- And another failure

Growth Conclusion

- Possible reasons there was no growth
 - > The Pro99 medium is a complex mixture
 - > Light/temperature conditions
- We hit a dead end on this project.
 - > No bacteria to work with means
 - No DNA
 - No transformation

Conclusion

● Setbacks

- Very late arrival of *Prochlorococcus marinus* and growth medium pro99
- Failed attempts at digesting plasmid.
- Failed to grow *Prochlorococcus marinus* when we did receive it.