SUBSURFACE BIOSPHERE INIATIVE
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PsaE knockout mutant in *T. elongatus*

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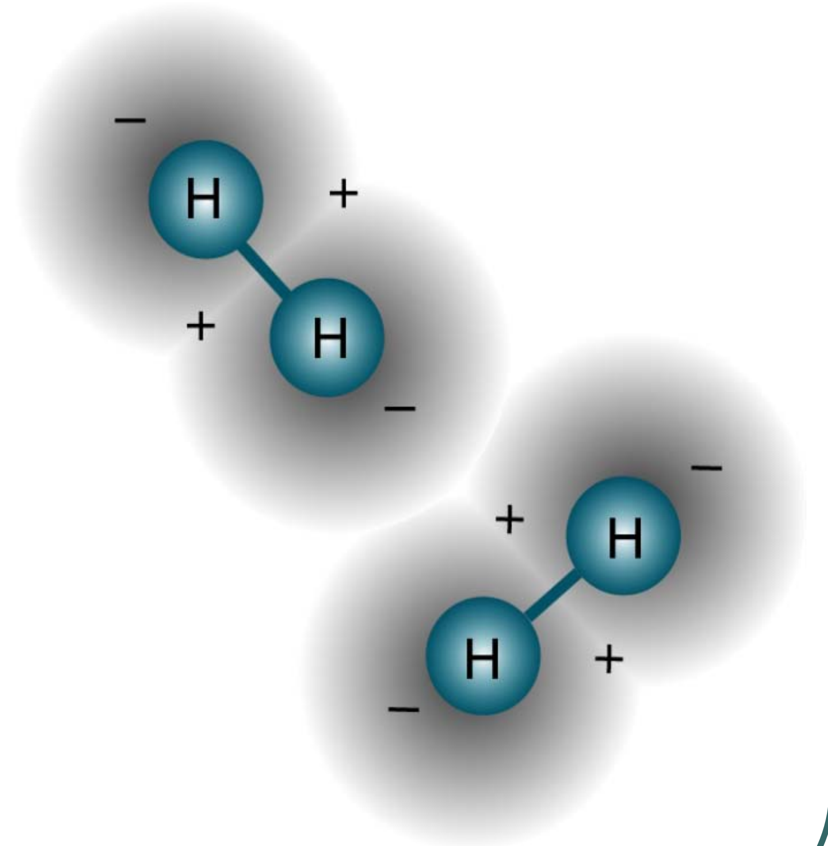
Bio-based hydrogen energy

Lab goal:

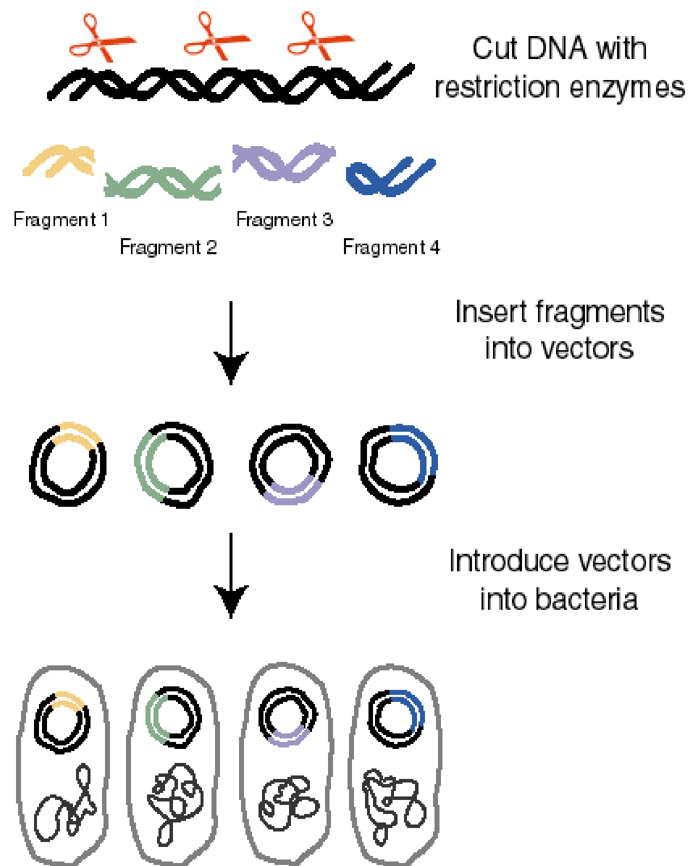
- Hydrogen – The cleanest fuel of the future
- Optimize hydrogen production from cyanobacteria – energy carrier for fuel cells or ICE



Why PsaE deletion.

- Knockout PsaE:
  - PsaE is a protein subunit in photosystem I.
  - To replace native PsaE with PsaE-hydrogenase hybrid.
  - So, electrons will flow from PSI directly to H_2-ase
- Bio-solar-panel for Hydrogen, temperature goes over 45°C.
- *Thermosynecoccus elongatus* is the solution.
- It is thermophillic cyanobacteria that can live up to 50°C.

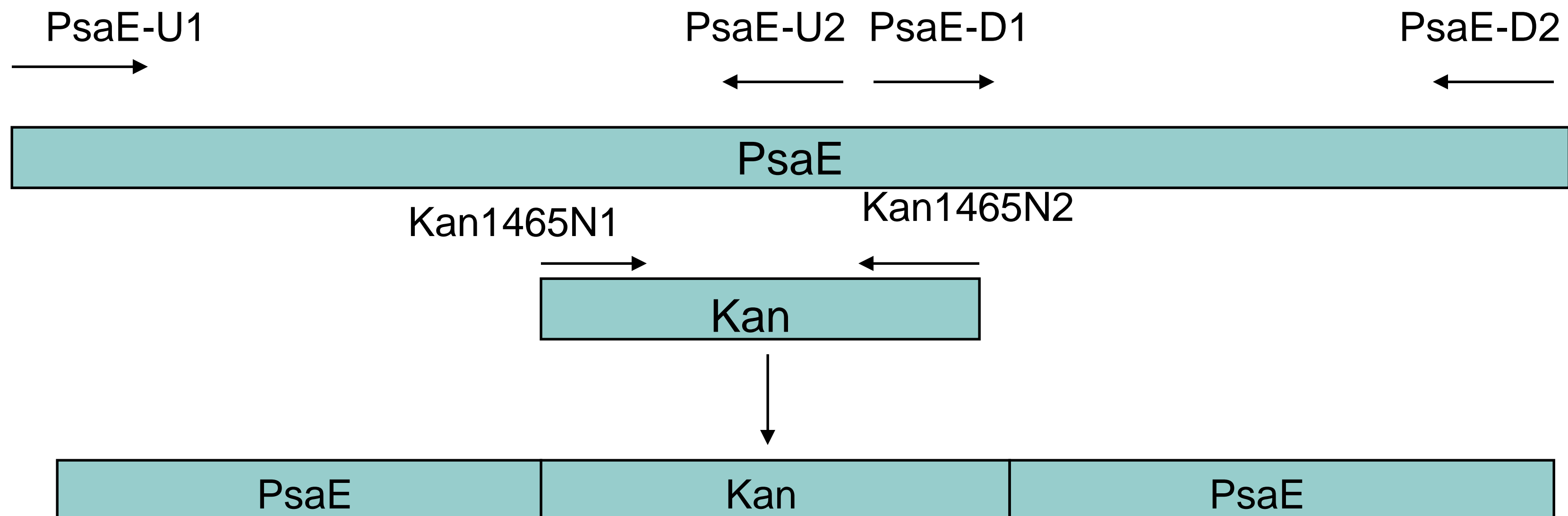
Overview for cloning



- **PCR: Gene amplification.**
- **Ligation:** Cut the insert with appropriate endonucleases. Then, ligate the linear pieces of DNA.
- **Ligate the vector and insert together.**
- **Transform *E. coli***
- **Screen for the correct insert-vector construct.**
- **Final construct will be used to transform cyanobacteria (*T. elongatus*).**

PCR Amplification of PsaE for knockout mutant

- PCR: amplify upstream, downstream regions of PsaE and Kan resistant cassette.



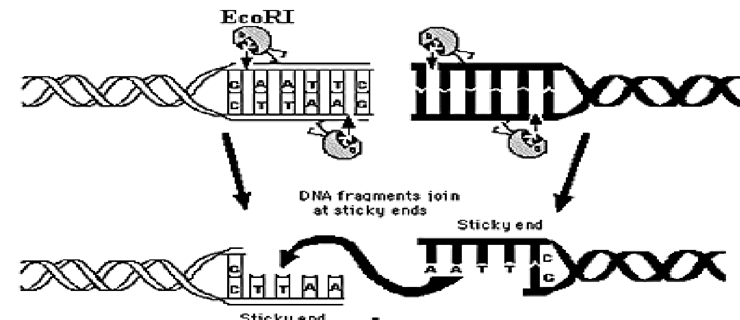
Restriction Digest of PsaE PCR Products

After upstream and downstream regions of PsaE were PCR amplified, DNA fragmentation was done with restriction endonucleases XbaI and EcoRI.

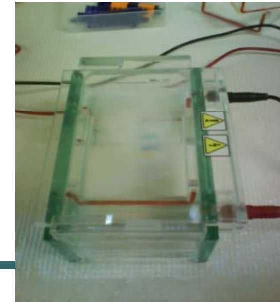
PsaE upstream region (PsaE-U):
Digested with *Xba* I restriction enzyme

PsaE down stream region (PsaE-D):
Digested with *EcoR* I restriction enzyme

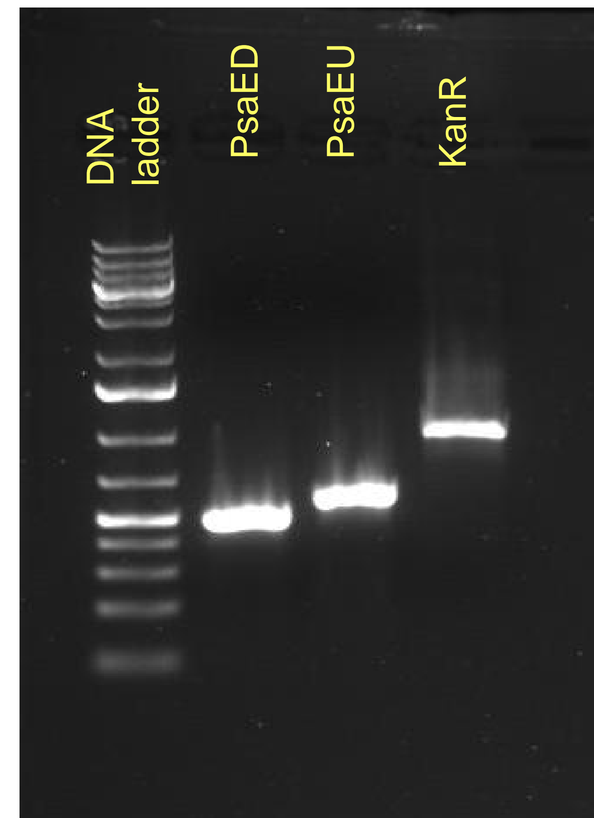
Kan^r:
Double digested with *Xba* I and *EcoR* I.



DNA gel electrophoresis

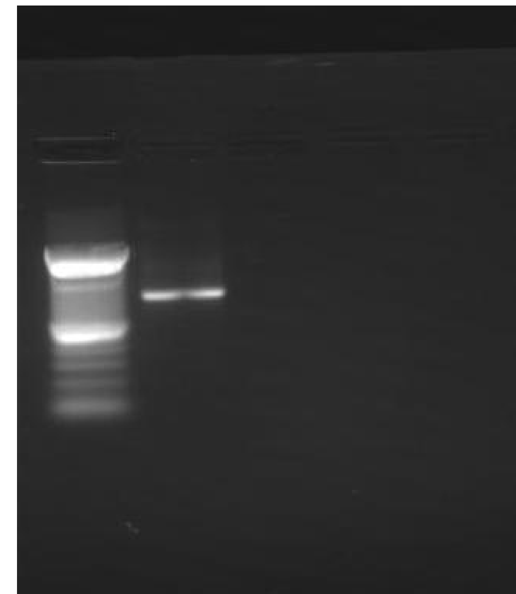


- Use gel electrophoresis to identify and isolate the DNA fragments.
- Slice DNA fragments of interest out of a gel and use in recombinant DNA application, which is the ligation step.
- The right DNA fragments after the restriction digest:
  - PsaED : 494 bp
  - PsaEU : 572 bp
  - KanR : 896 bp



Recombinant DNA

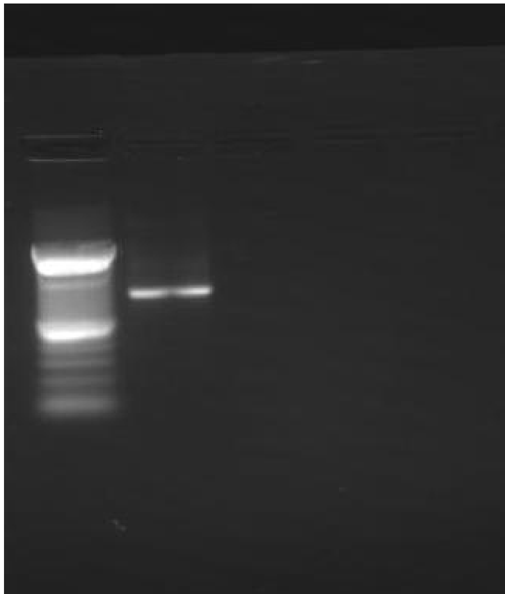
- Two DNA fragments of PsaE flank a kanamycin resistance cassette.
- The final product is 1962 bp.



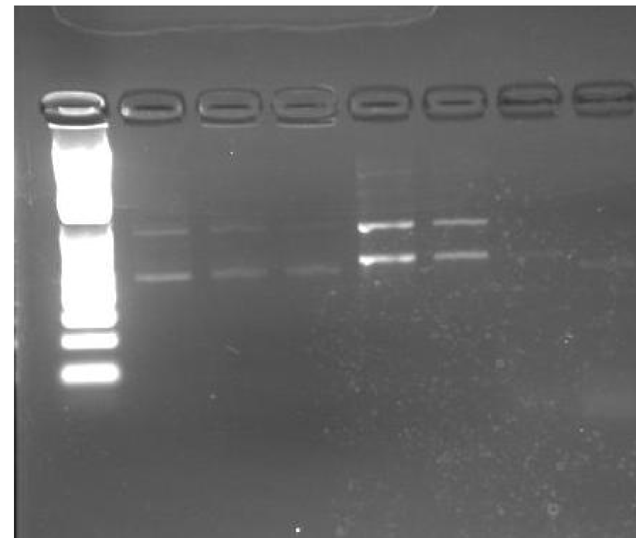
Identification of recombinant DNA

- Gel electrophoresis

ligation that works.

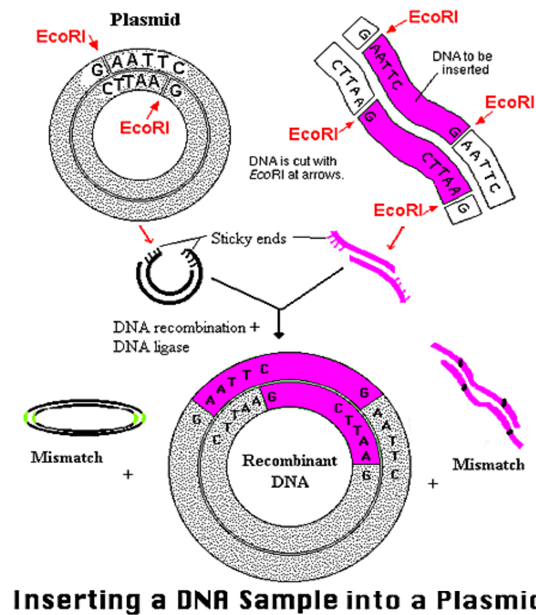


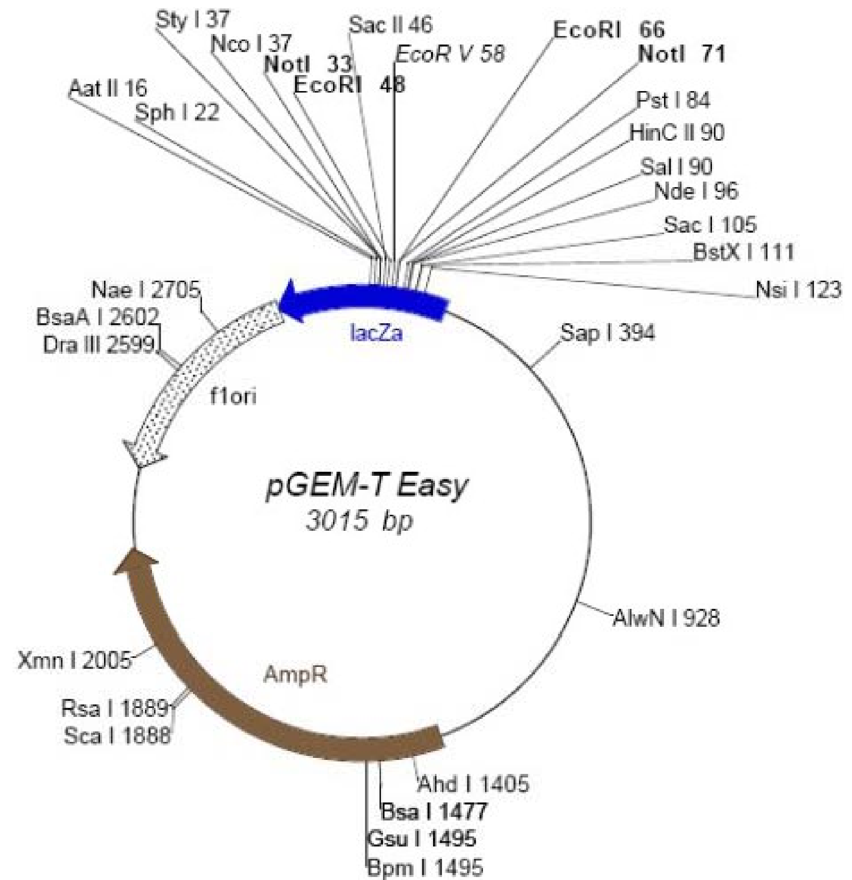
Fail ligation.



Ligation to the vector

- Ligate the DNA fragment to a vector.

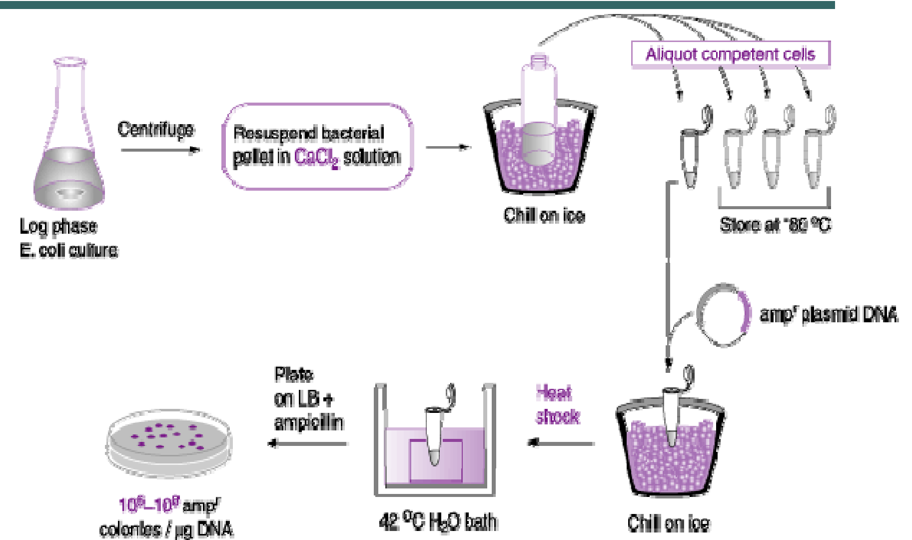


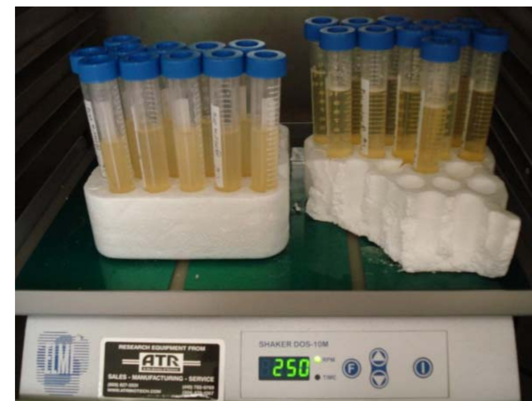


Transformation

- Insert the recombinant DNA molecule that I created (PsaE knockout) into *E. coli* bacteria.

- *E. coli* will take up DNA by heat shock.



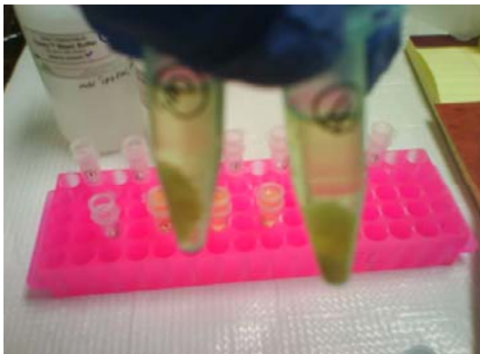


Blue and white selection.

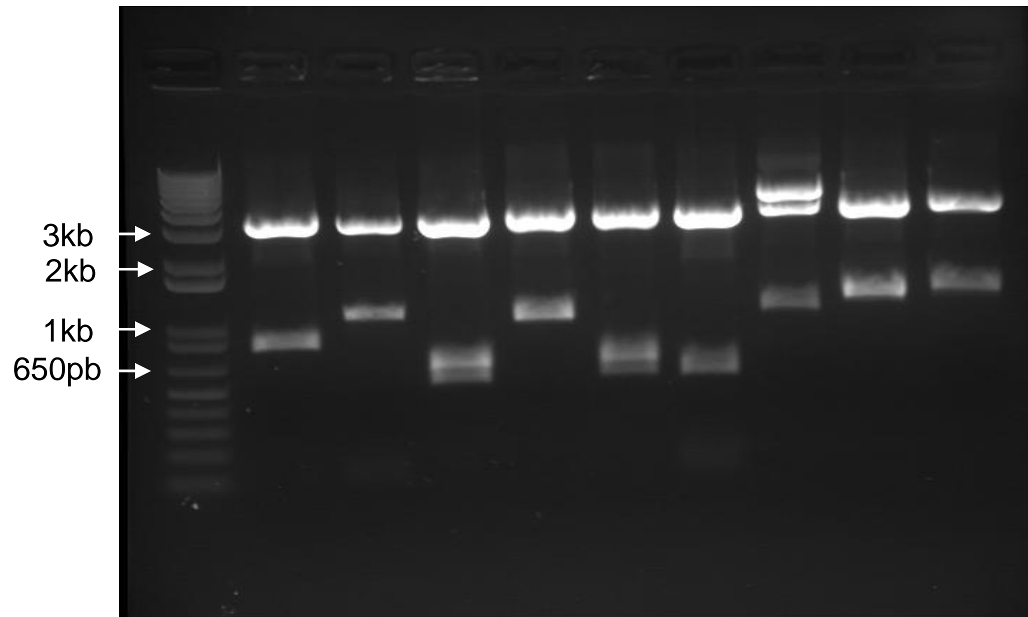
- Blue, - insert (+ Lac Z)
- White, + insert (- Lac Z).
- Pick individual colonies and test for the desired insert.

Plasmid extraction and screening for insert

- Extract plasmid from selected colonies
- Double digest: - NaeI
  - PstI



- Run the plasmid on DNA gel to check for the correct clone.



Conclusion

- Confirming the sequence of the constructed DNA *(PsaEU-Kan-PsaED)*.
- Transform *T. elongatus* to delete PsaE.
- Replace the deleted native peptide with modified PsaE fused with H_2-ase.
- **Soon, we looking to construct a new strain with PsaE-H_2ase hybrid.**

Acknowledgment

- Subsurface Biosphere Initiative
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