

Engineering Anaerobic Gut Fungi for Lignocellulose Breakdown

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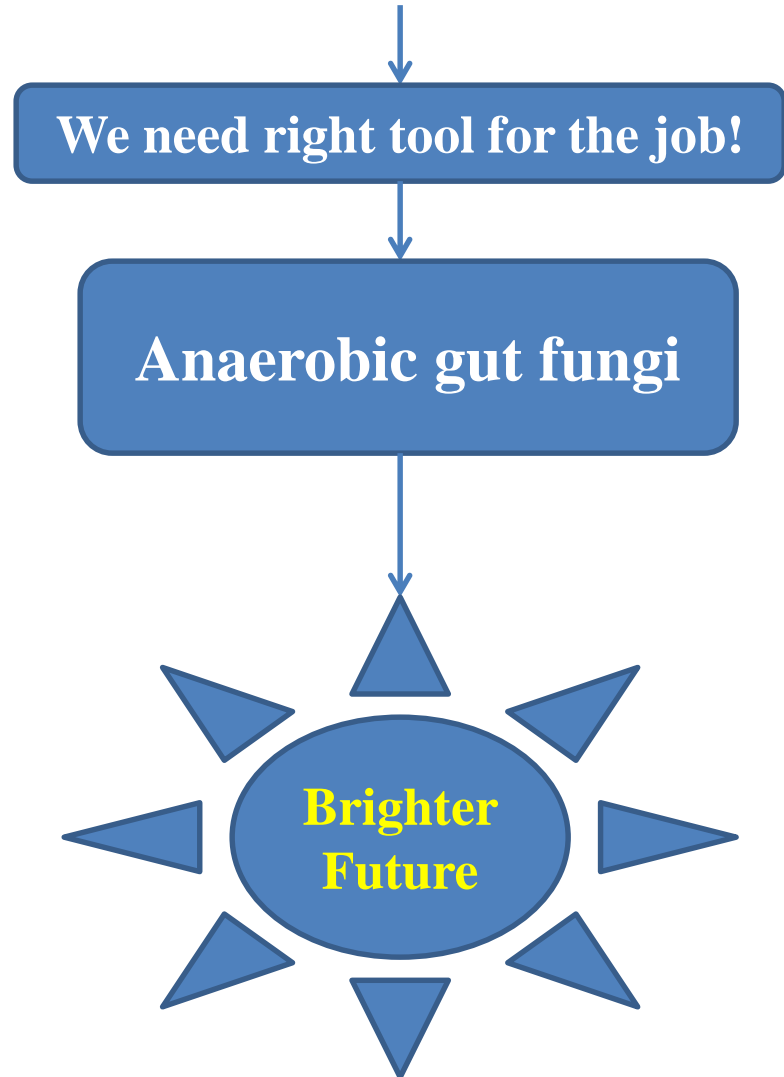
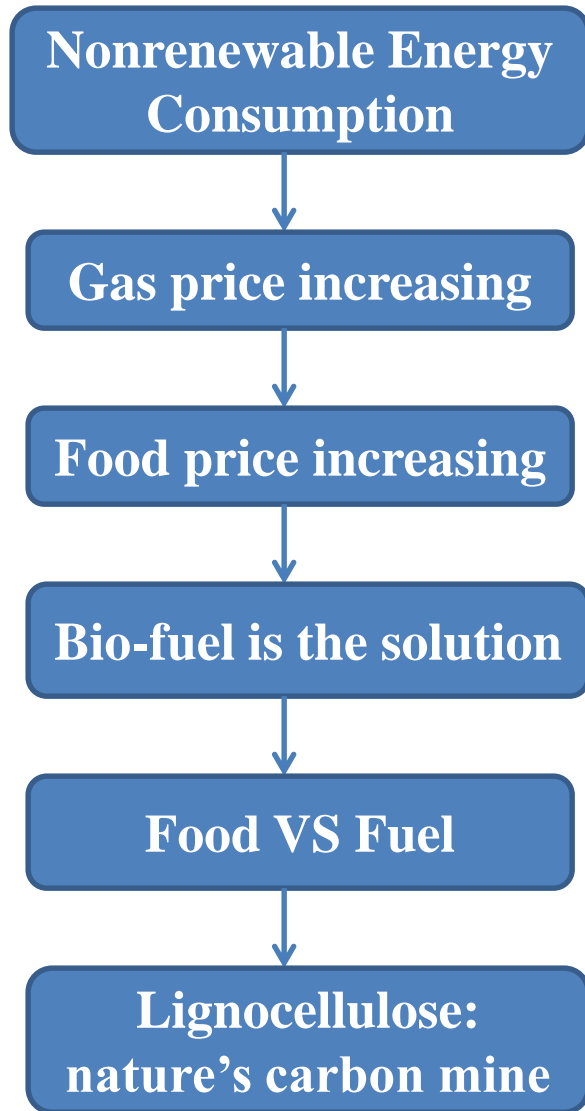
Mentor: Charles Haitjema

Funding Source:

The U.S. Army



The Big Picture



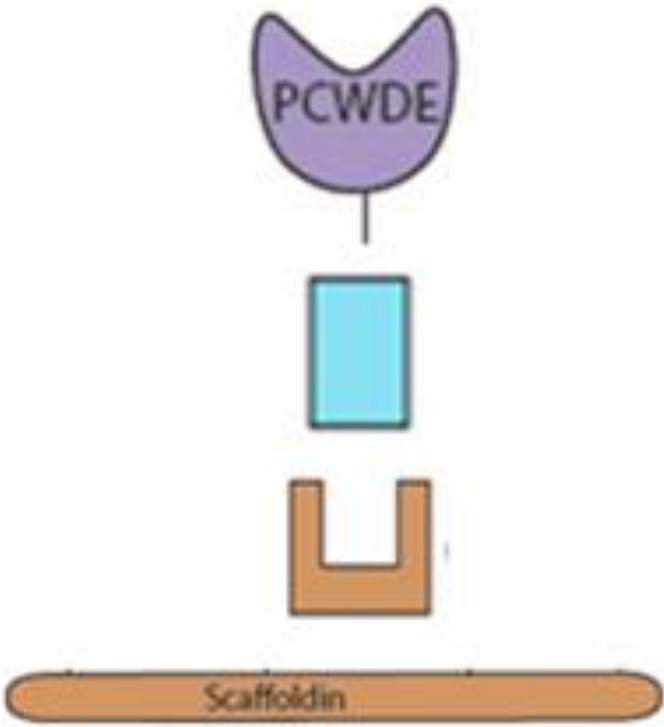
Assemble

Anaerobic Gut Fungi

Enzyme: Cellulase

Cellulosome Complex

CAST



Enzyme

Dockerin

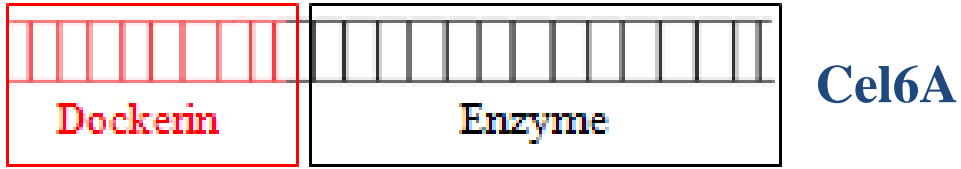
Cohesin

Scaffoldin

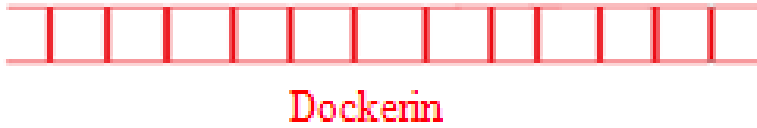


Binding Partner

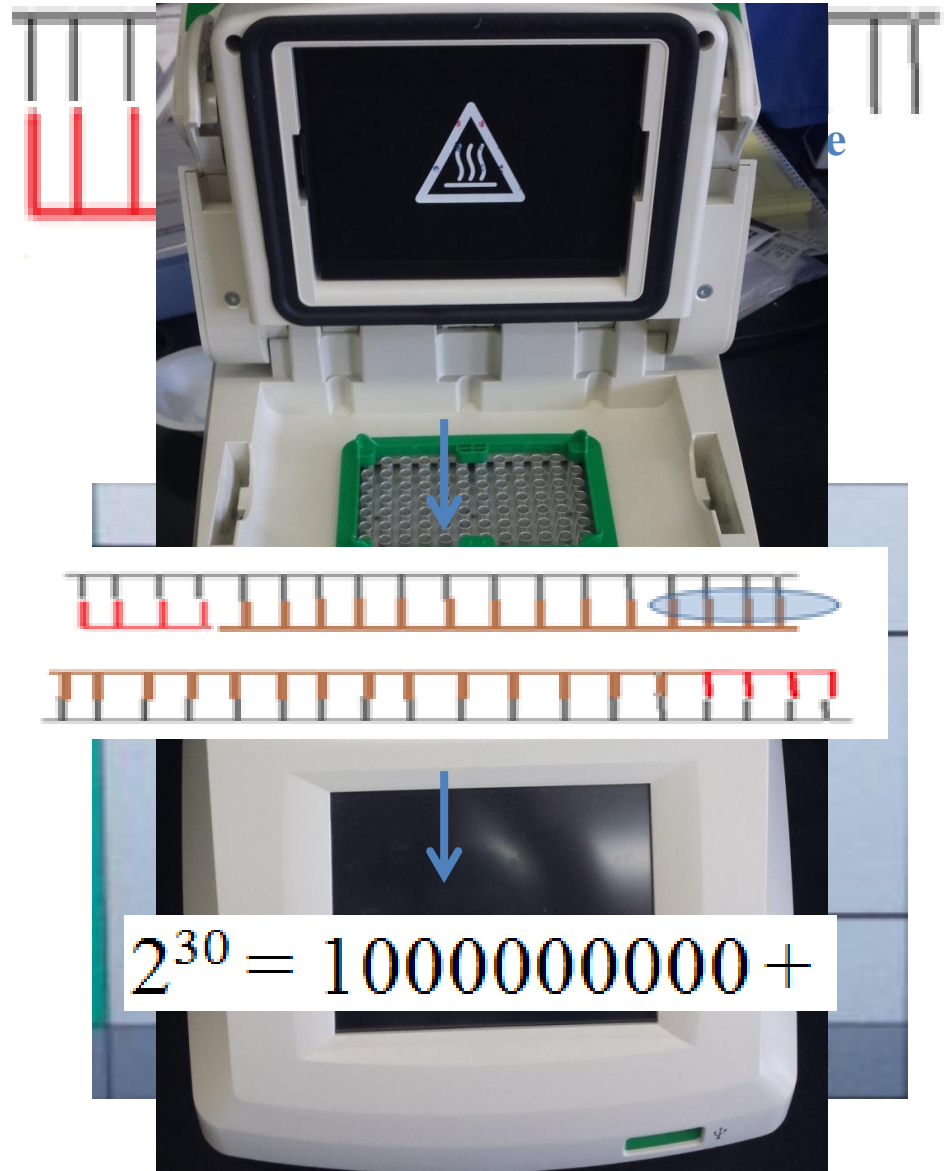
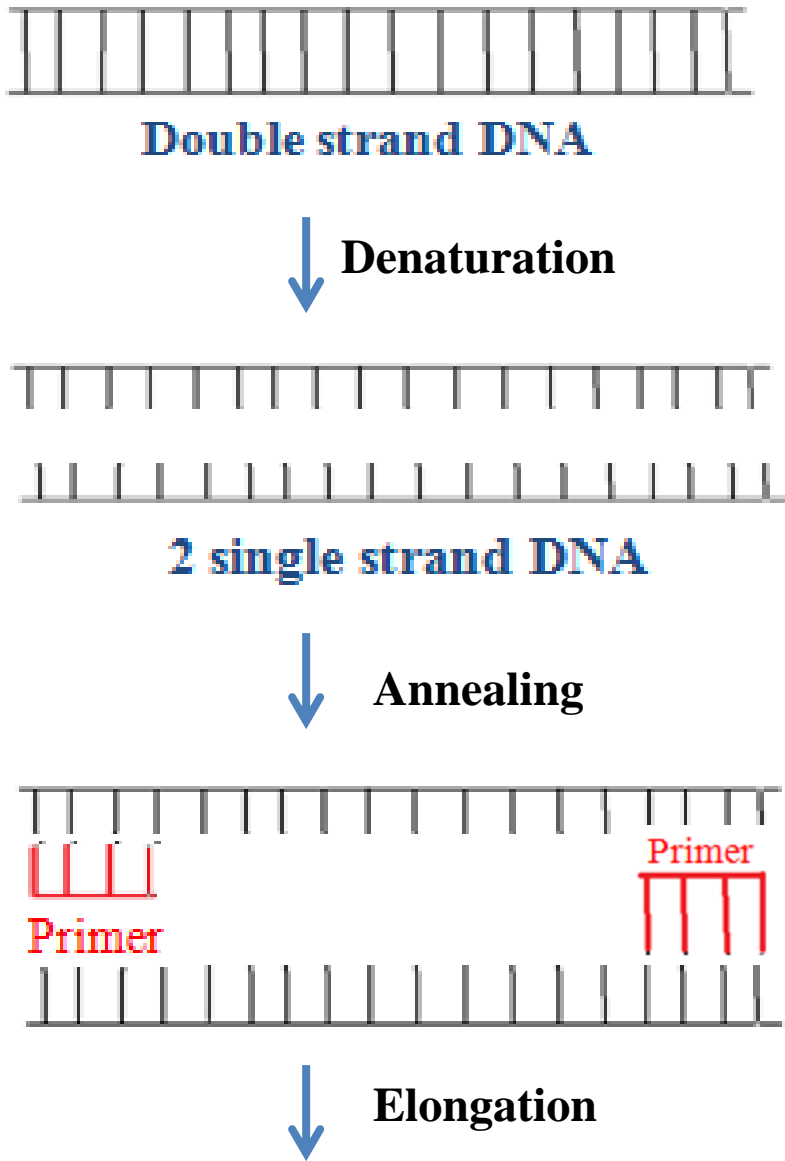
The Project Procedure



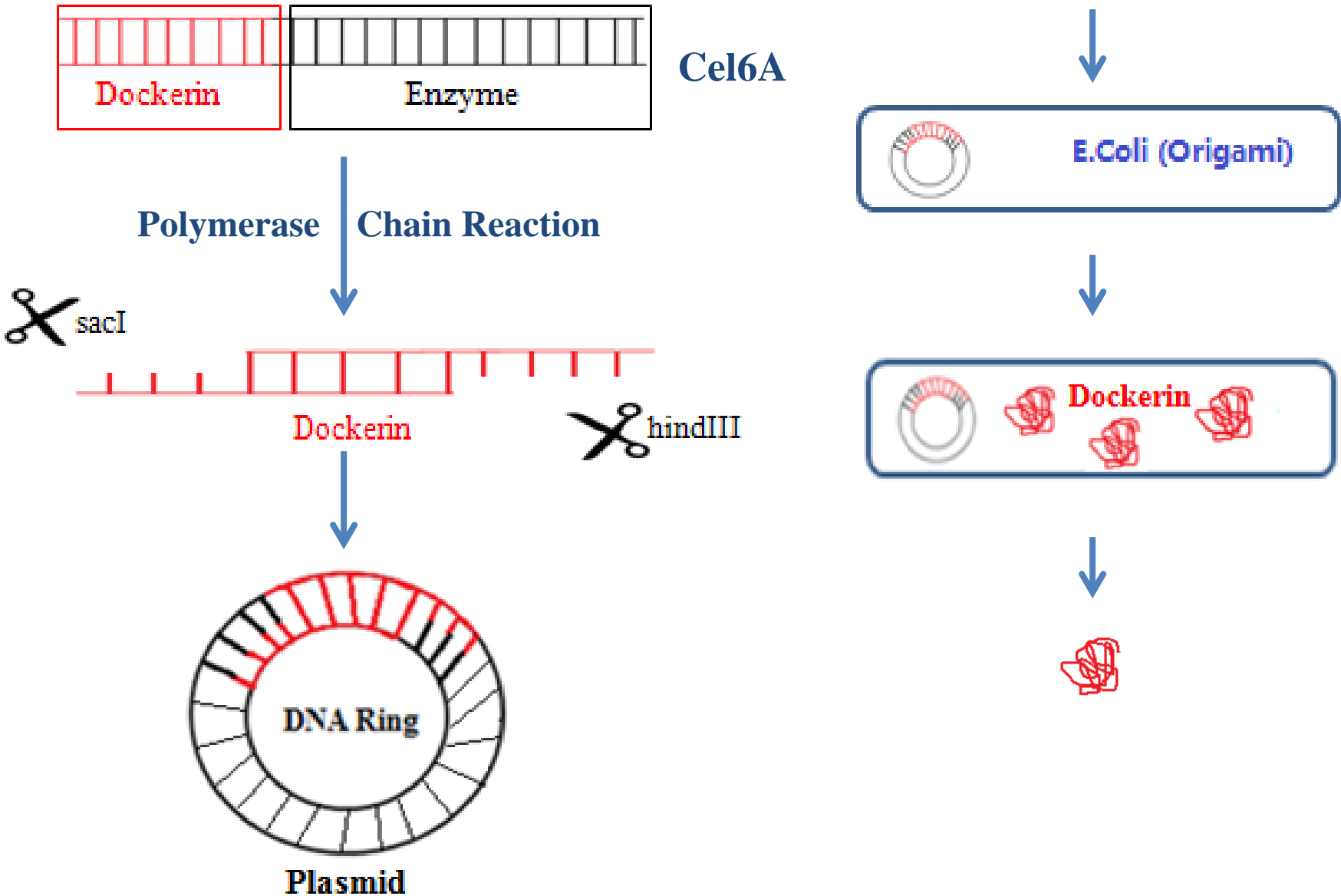
Polymerase Chain Reaction



Experimental Method

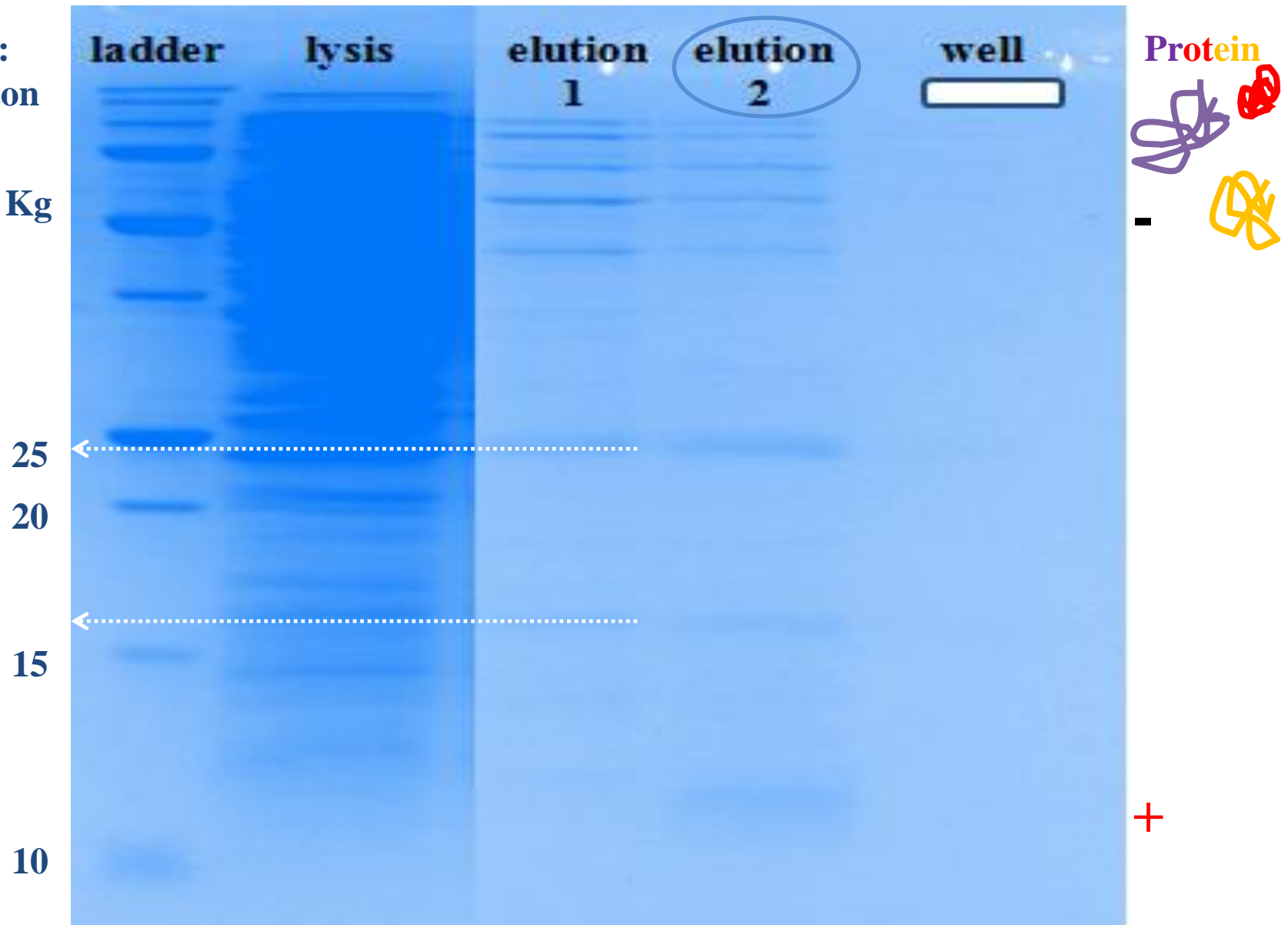


The Project Procedure



Protein Gel Electrophoresis

Unit:
Kilodalton
||
 1.7×10^{-24} Kg



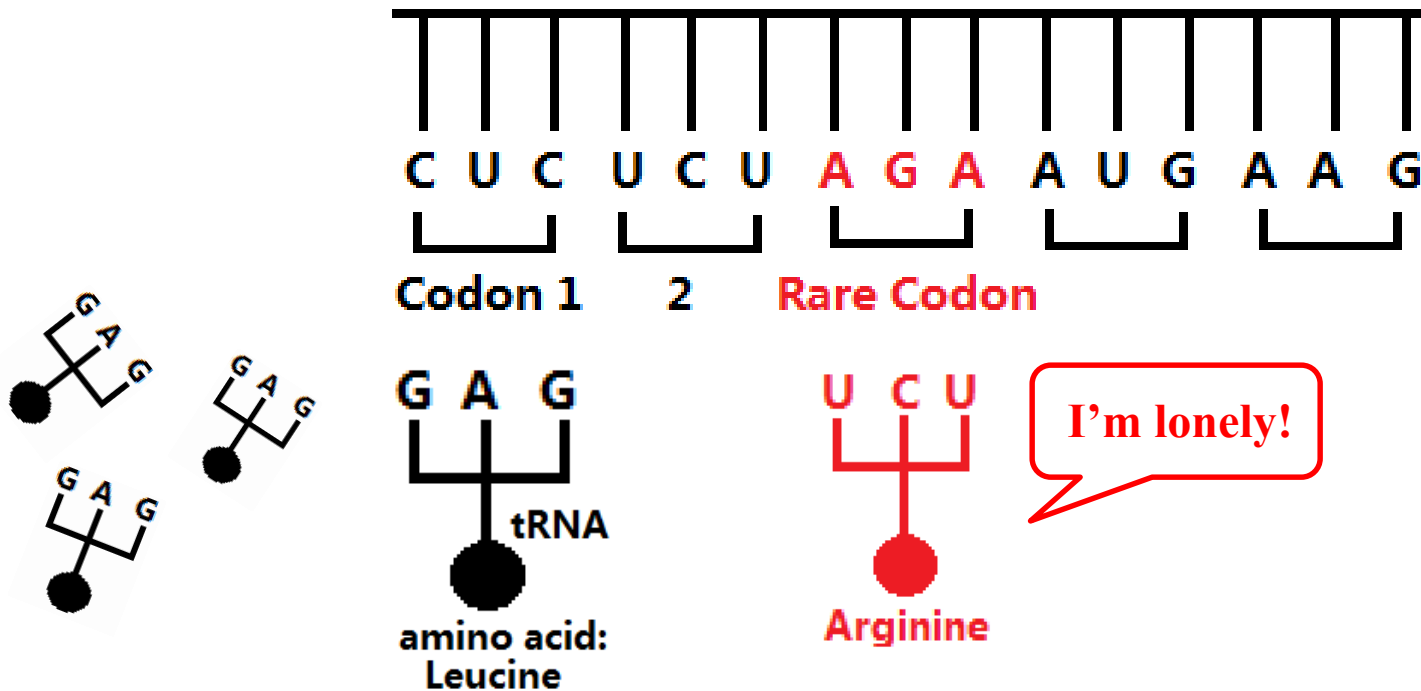
Conclusion

➤ Origami is not the best host for Cel6A dockerin protein expression

- Cel6a has rare codons
- In origami, transport RNA for rare codons are not efficient



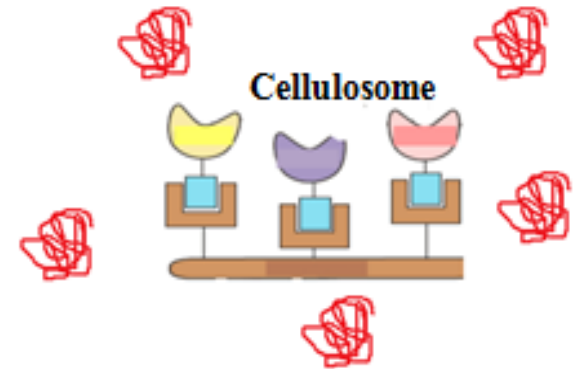
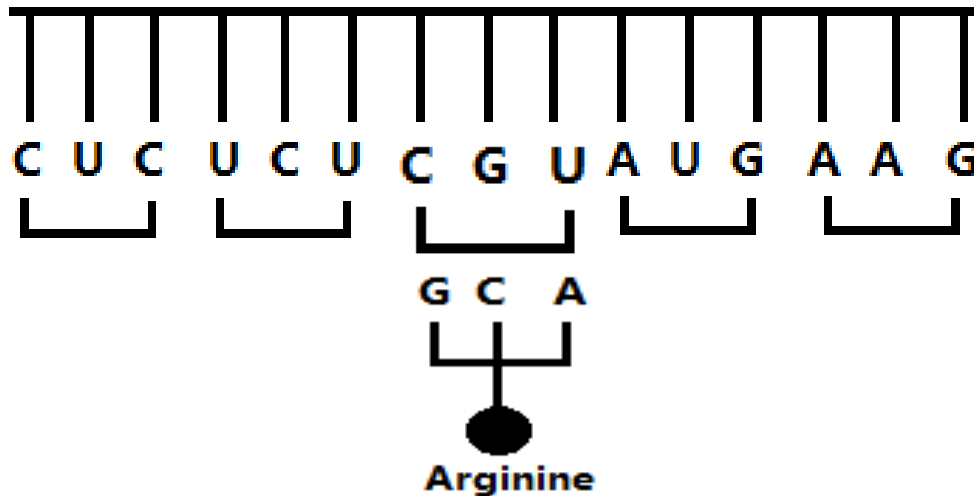
Dockerin protein wasn't expressed very well



What the future holds

- Use a different E.Coli strain -Tuner
 - Tuner is capable of recognizing rare code
 - Dockerin proteins may not be folded properly

- Replace the rare codons



- Try E.Coli strain that contain both features of Origami and Tuner

Time line



Acknowledgement

- **The O'Malley Lab**
 - ❖ **PI: Dr. Michelle O'Malley**
 - ❖ **Mentor: Dr. Charles Haitjema**
 - ❖ **Co-worker: Kane Nania**

- **INSET staff**

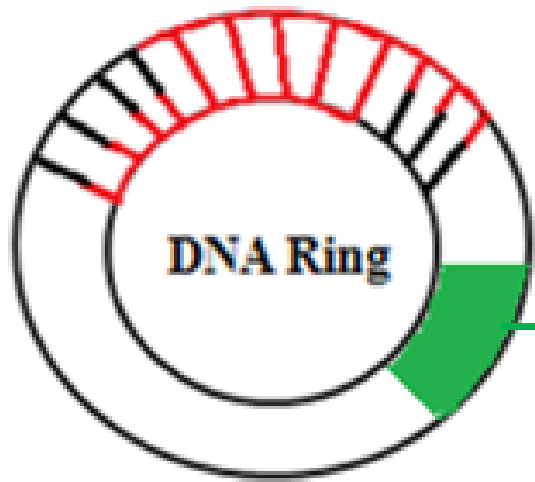
- **Friends**

- **ICB**

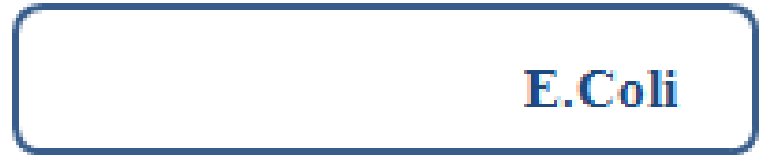
- **U.S. Army**



Arg	AGG	0.022	0.003
Arg	AGA	0.039	0.006
Arg	CGG	0.098	0.008
Arg	CGA	0.065	0.011
Arg	CGU	0.378	0.643
Arg	CGC	0.398	0.330
Gly	GGG	0.151	0.044
Gly	GGA	0.109	0.020
Gly	GGU	0.337	0.508
Gly	GGC	0.403	0.428
Ile	AUA	0.073	0.006
Ile	AUU	0.507	0.335
Ile	AUC	0.420	0.659
Leu	UUG	0.129	0.034
Leu	UUA	0.131	0.055
Leu	CUG	0.496	0.767
Leu	CUA	0.037	0.008
Leu	CUU	0.104	0.056
Leu	CUC	0.104	0.080
Pro	CCG	0.525	0.719
Pro	CCA	0.191	0.153
Pro	CCU	0.159	0.112
Pro	CCC	0.124	0.016

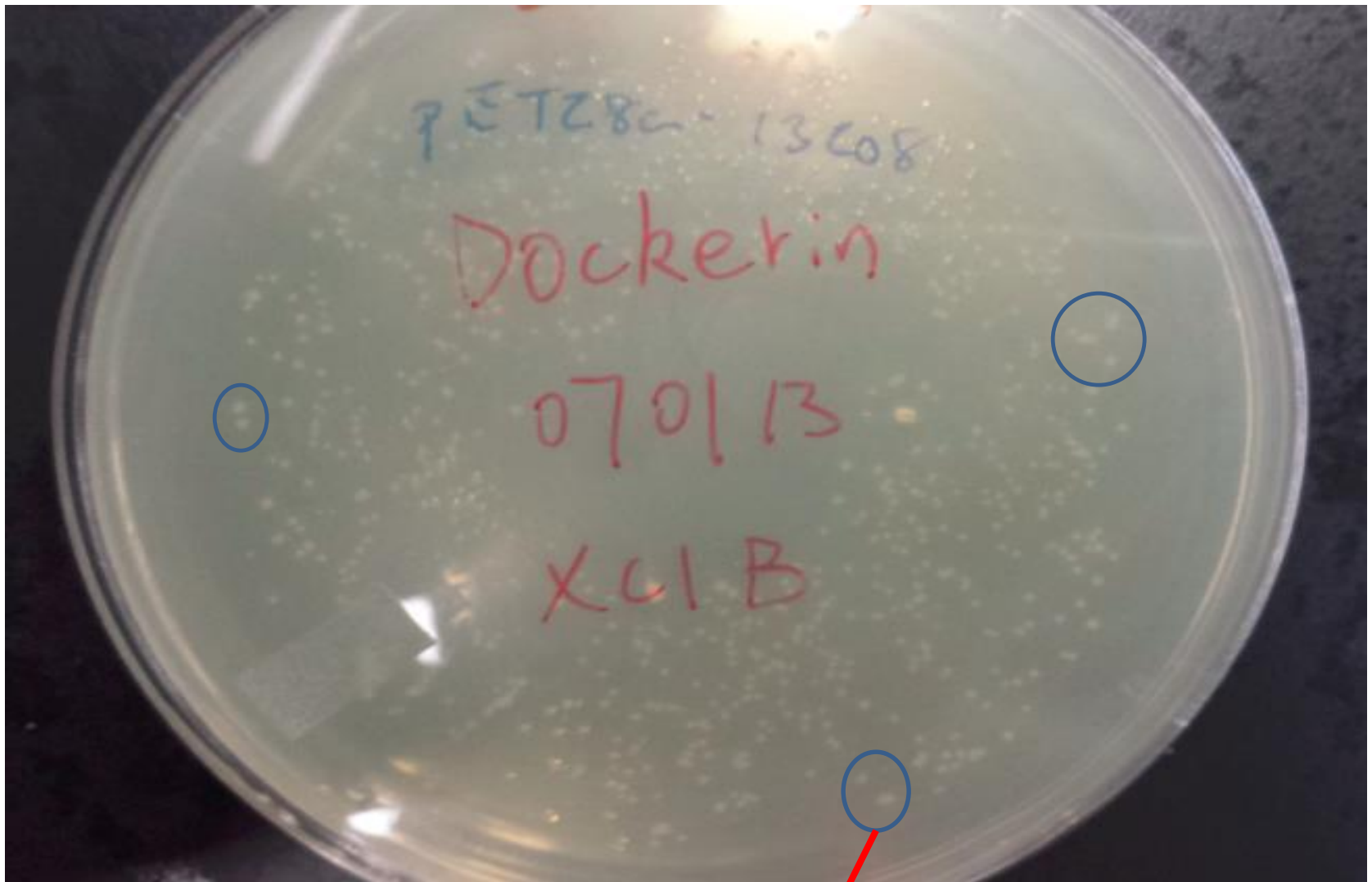


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Kanamycin Resistance





 **E.Coli**