

The Big Picture

The continuous consumption of nonrenewable energy

Increases gas price & retail food price

Renewable energy: Biofuel

Pros: Secure America's energy future

Cons: Current method of converting starch crops to sugar triggers competition between food and biofuel

Lignocellulose is the most abundant renewable and inedible material for the production of biofuel

Anaerobic gut fungi that live in the digestive track of large herbivores are among the most efficient degrader of lignocellulose

The contributor to fungi enzymatic efficiency is the large macro molecular structure called **cellulosome complex**, which delivers all enzymatic components in a concentrated and targeted manner to the lignocellulose

Cellulosome's architecture remains elusive



Research Goal

Cellulosome complex = Enzymes + Dockerin + Cohesin + Scaffoldin

Interaction between Dockerin and its binding partner is essential to the construction of cellulosome complex

Knowledge regarding dockerin's binding partner is limited

Research goal:

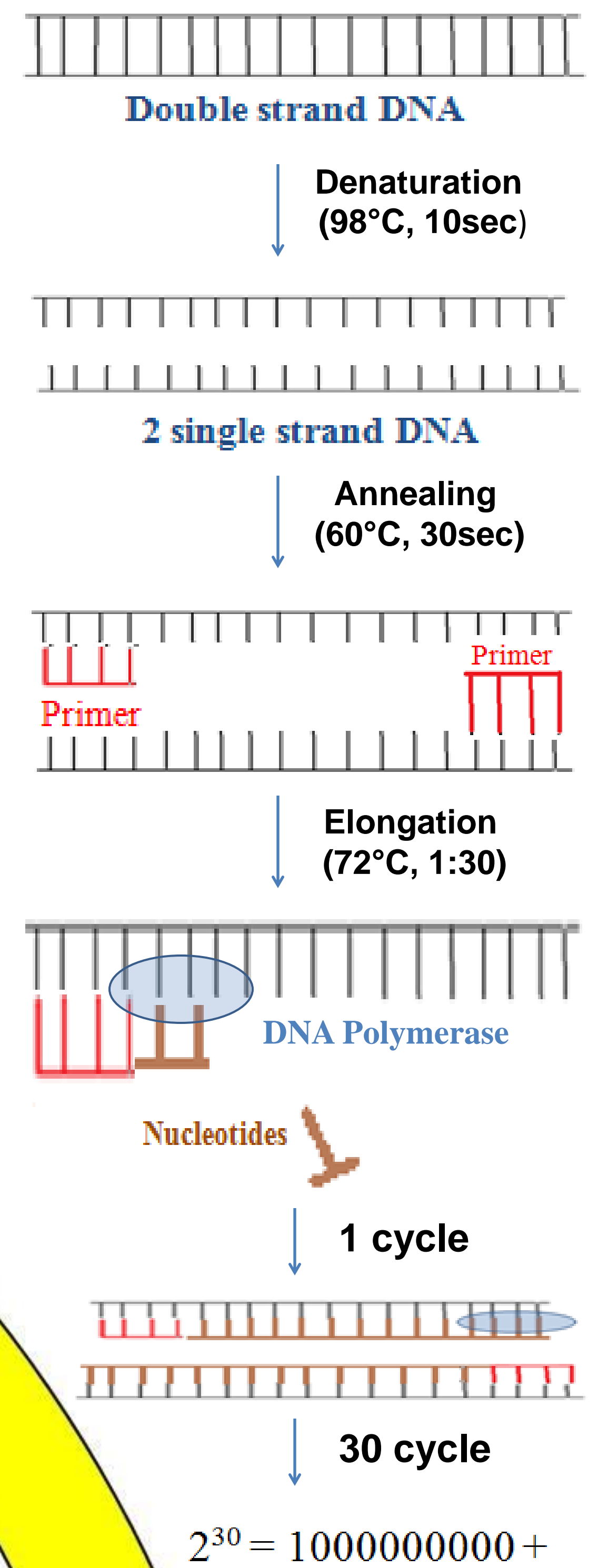
Decipher Fungal Cellulosome Architecture by Identifying Dockerin Binding Partner

Experimental Method

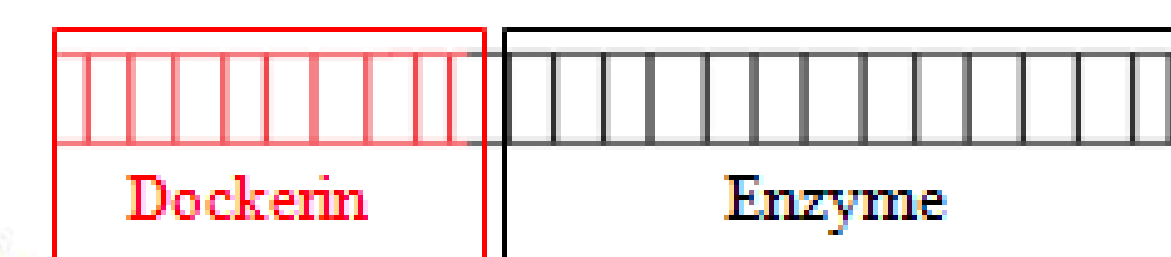
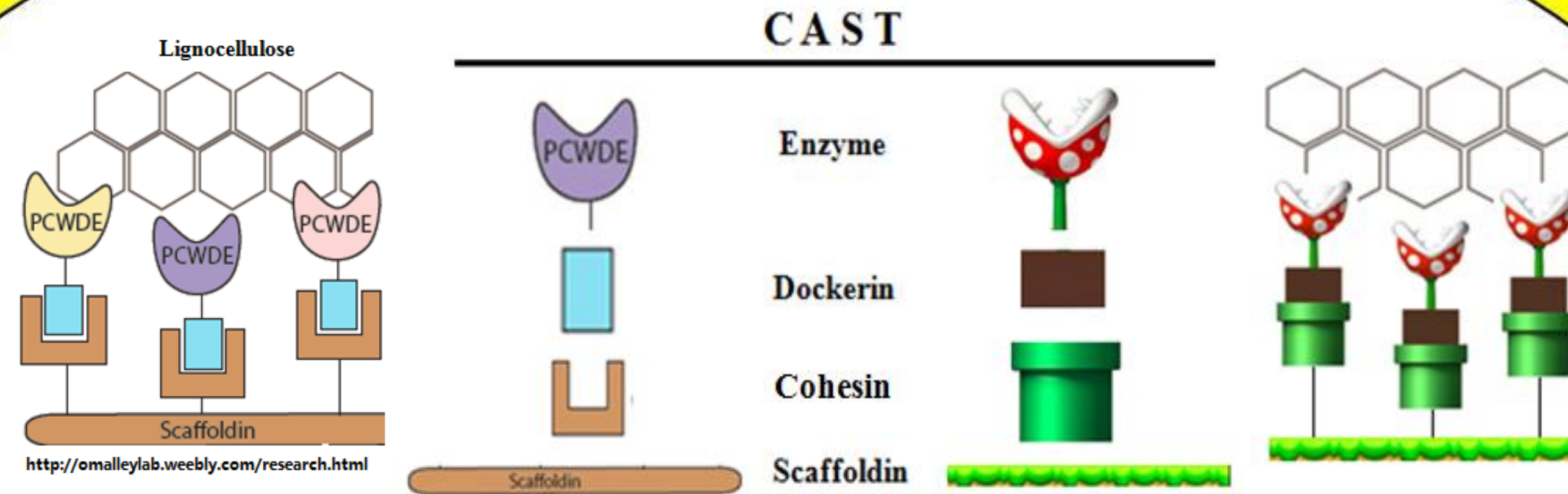
--- polymerase chain reaction (PCR)



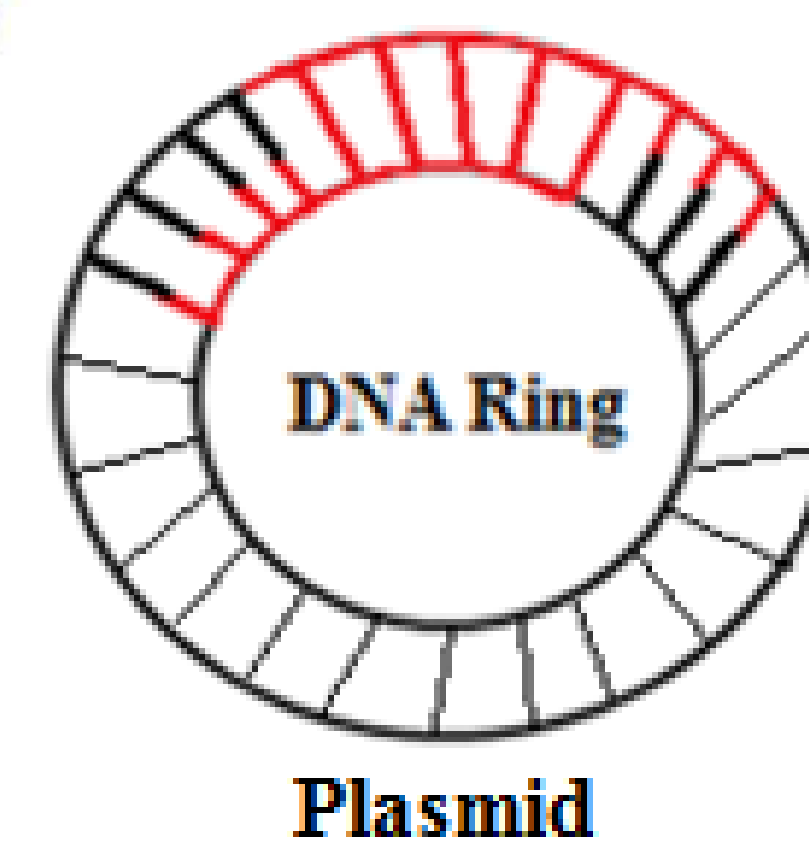
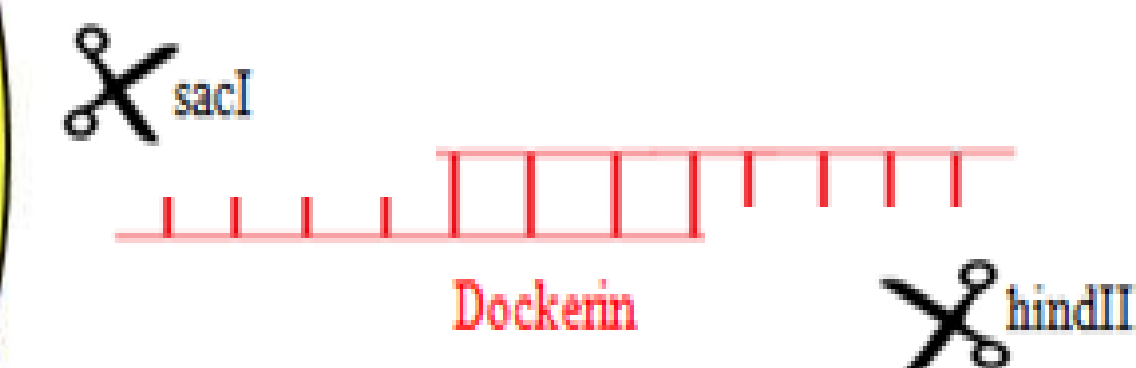
PCR machine: Thermal Cycler



The Experimental Procedure



Cel6A



Plasmid

E.Coli (Origami)

Engineering Anaerobic Gut Fungi For Lignocellulose Breakdown

Jiehao Chen, Charles Haitjema, Michelle O'Malley
Department of Chemical Engineering, UCSB

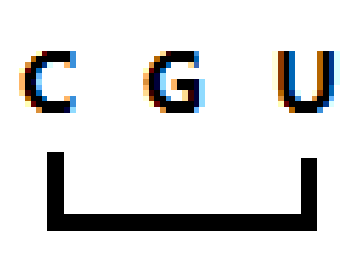
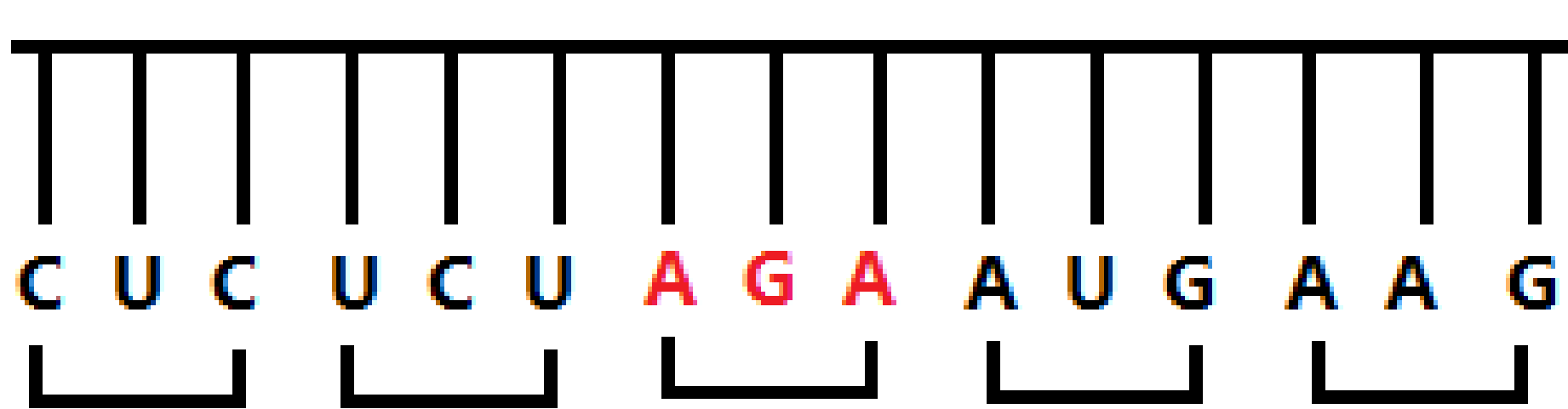
Future Works

Whichever of the following strategies works, the well expressed dockerin protein will be put together with the cellulosome complex to reveal which part of it the dockerin will bind to

Use a different E.Coli strain -Tuner

Replace the rare codons

- Tuner is capable of recognizing rare code
- Dockerin proteins may not be folded properly



Revenge is sweet

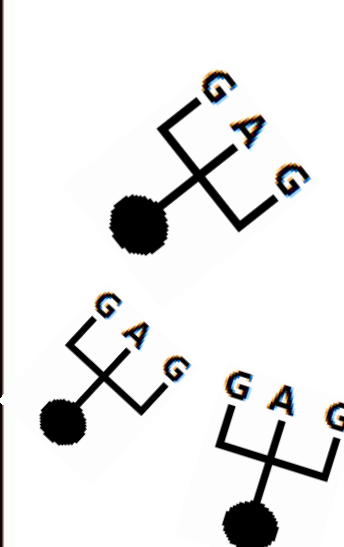
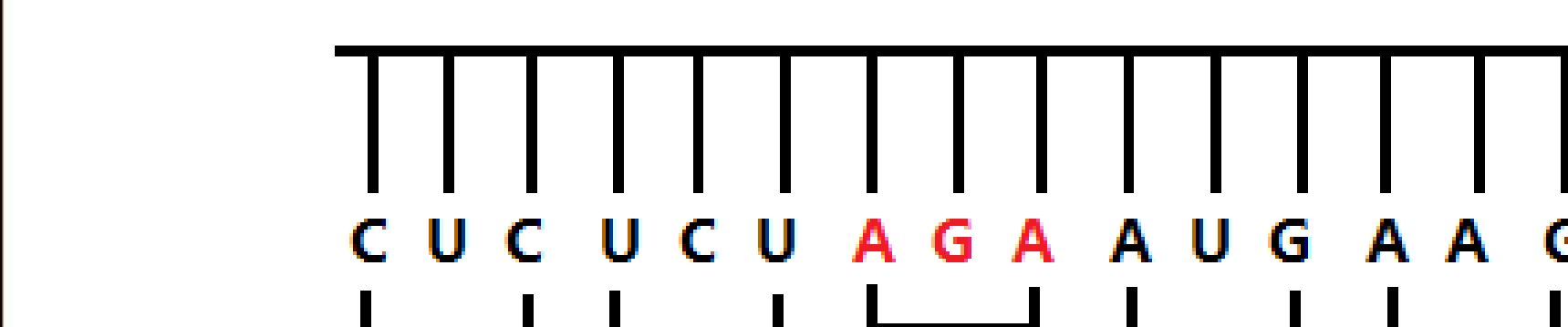
Try E.Coli strain that contain both features of Origami and Tuner

Conclusion

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

- Cel6a has rare codons
- tRNA for rare codons are not sufficient

⇒ Dockerin protein wasn't expressed well



amino acid: Leucine

Arginine

I'm lonely!

Data: Protein Gel Electrophoresis

Principle: The gel was put in the buffer solution, which will coat the proteins with - charges so when apply electrical field, the proteins will move to the + side and smaller the size the further down it will travel, so different types of proteins could be separated.

Elution: Extracting dockerin proteins from other proteins by washing with elution solution

