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Understanding the Importance of the Zinc Binding Domain in CESA Protein Interaction: Some Assembly Required

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Understanding the Importance of the Zinc Binding Domain in CESA Protein Interaction: Some Assembly Required

By Al Schupp

What is a CESA protein?

CESA = Cellulose Synthase

Transmembrane Protein

Makes one strand of polymer…maybe

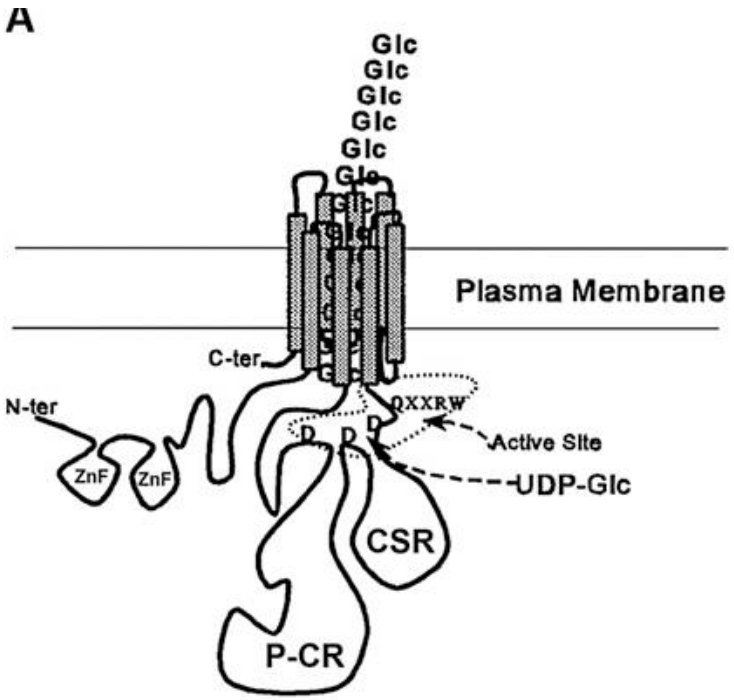
Two CESAs might be needed to make a single strand of polymer

Domains

Regions of the protein that serve specific functions

Catalysis, protein binding

Important for proper function



Carpita, N. (2011). Update on Mechanisms of Plant Cell Wall Biosynthesis: How Plants Make Cellulose and Other (1,4) –B-D-Glycans. *Plant Physiology* Vol 155 p 174

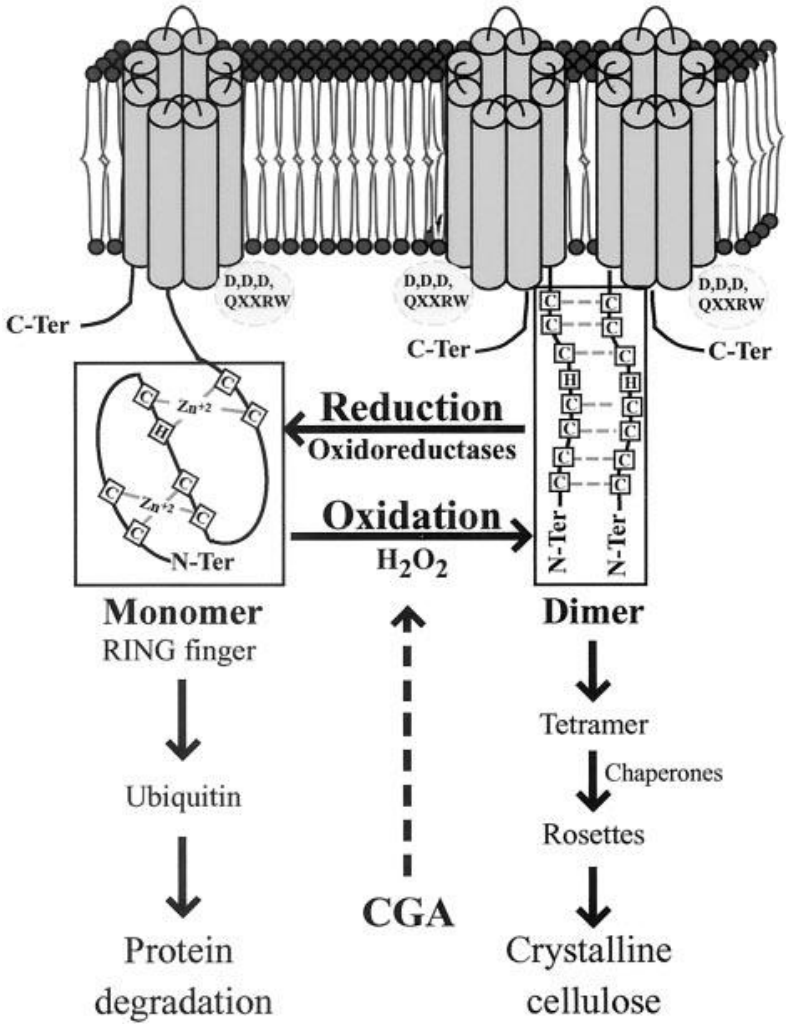
End Domains

Kurek, I *et* al. (2002). Dimerization of cotton fiber cellulose synthase catalytic subunits occurs via oxidation of the zinc-binding domains. *PNAS*. Vol 99 no.17 p. 11114

N-terminal zinc binding domain

Contains amino acid residues capable of forming strong covalent bonds

Cysteine contains Sulfer



Zinc Binding Domain Function

Provides strong interactions between CESA proteins

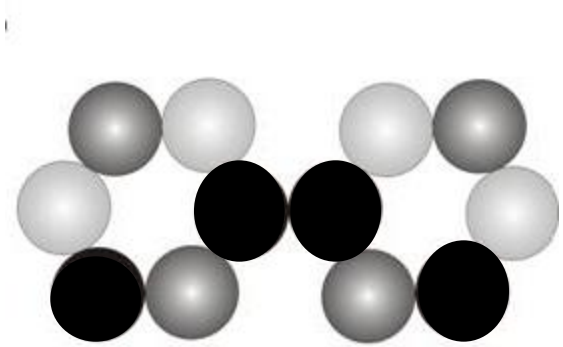
In the oxidized form, the domains dimerize

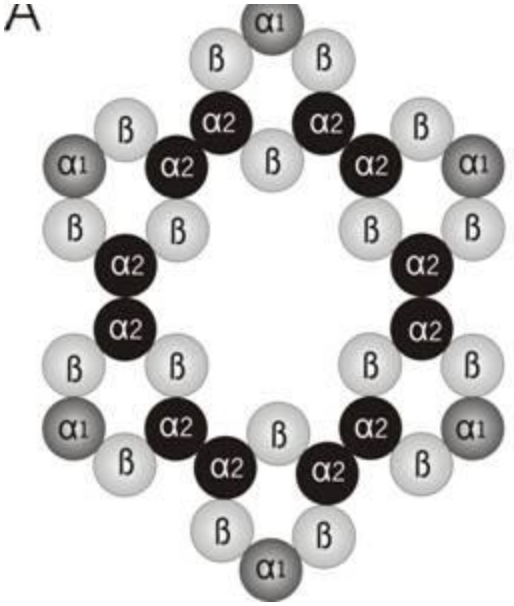
In the reduced form, the proteins exist as monomers and are quickly degraded

Protein Assembly

http://www.plantbreeding.wur.nl/Images/group_biobased_cellwalls_clip_image002.jpg

- Not completely understood
- 3 different CESAs make up a complex
- Hypothesis: 6 CESAs join together to form a six-membered "ring"
- Six "rings" join to form a Cellulose Synthase Complex





Purpose

Determine the importance of the zinc binding domain

Will a protein without the zinc binding domain rescue a knockout line of moss?

Create a line of moss that could make cellulose regardless of the cellular redox state

Facilitate protein binding without relying on covalent bonds

Basic Plan of Action

Delete the DNA encoding the zinc binding domain from *PpCESA5*

See if this DNA, when put into moss in which the *CESA5* gene has been knocked out (Goss *et al*, 2012), can rescue the moss

If the KO moss is not rescued, try another form of protein interaction instead

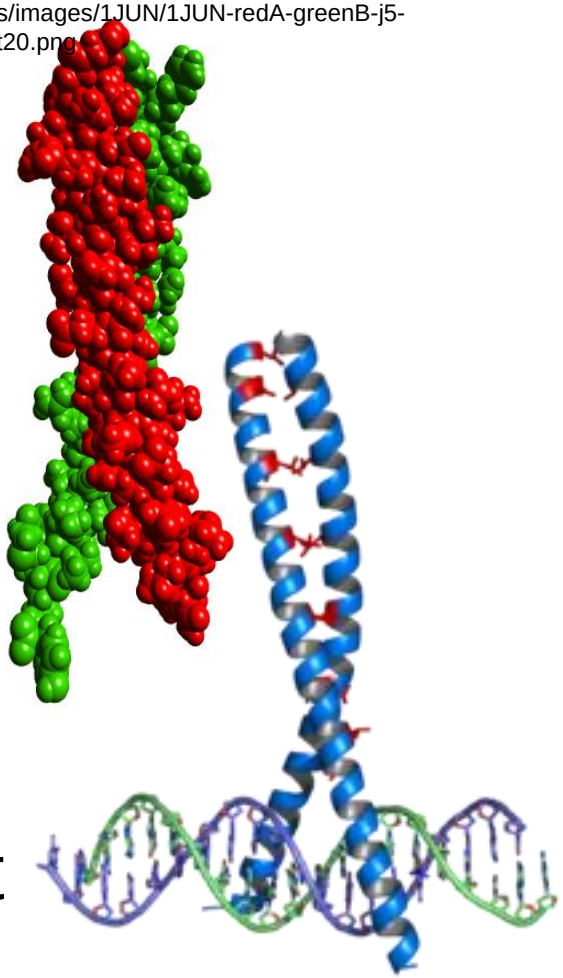
Leucine Zipper

Zinc Fingers and Leucine Zippers are typically parts of transcription factors

Leucine Zippers are dimers joined by a coiled-coil

Coiled-coils are stabilized by hydrophobic forces, which aren't as strong as covalent bonds

http://proteinshader.sourceforge.net/tutorials/images/1JUN/1JUN-redA-greenB-j5-t20.png

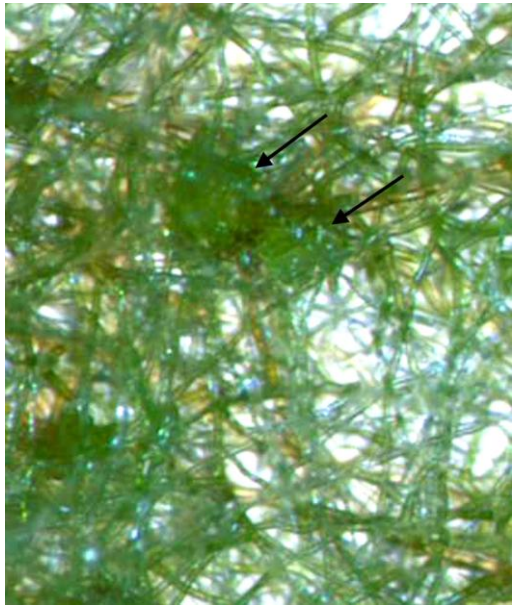


http://upload.wikimedia.org/wikipedia/commons/thumb/e/e8/Leucine_zipper.png/220px-Leucine_zipper.png

Results

Transformed moss maintained mutant phenotype, but for the wrong reasons.

Wrong peptide product



Goss, C.A. *et al* (2012). A *Cellulose Synthase (CESA)* gene essential for gametophore morphogenesis in the moss *Physcomitrella patens.* Planta.

Methods

PCR and Gel Electrophoresis

Restriction digestion

DNA ligation

Bacterial Transformation and Selection

DNA sequencing

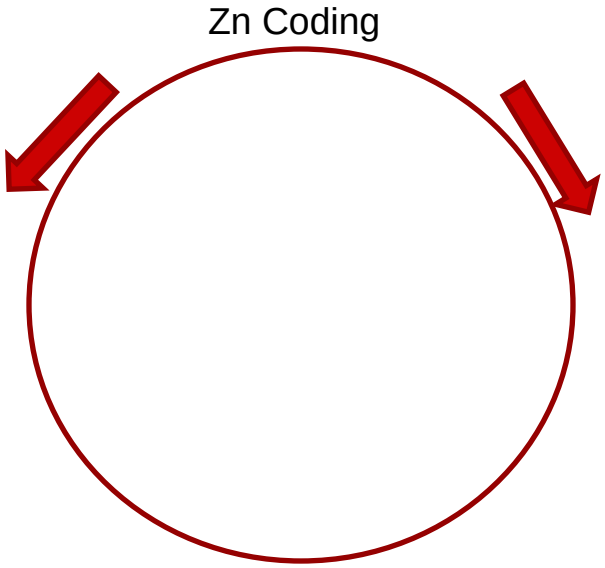
PCR Screening

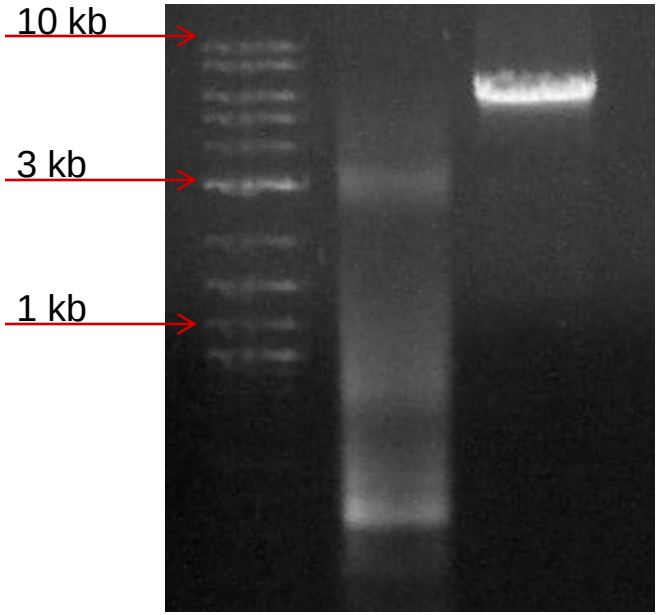
Moss Transformation

PCR and Gel Electrophoresis

Design primers that amplify DNA, but exclude the zinc coding region

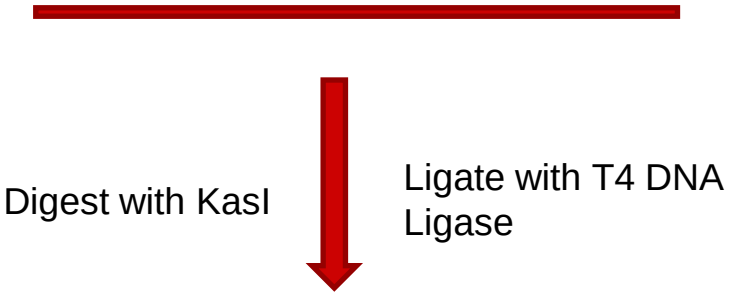
Include restriction sites



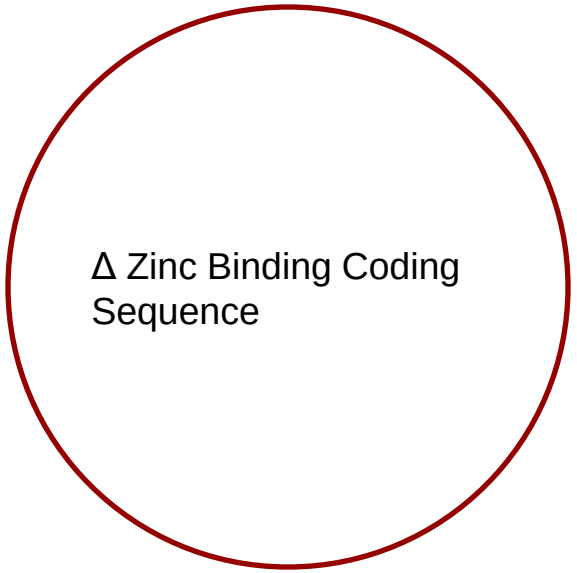


Restriction Enzymes and Ligation

Molecular Scissors that cut DNA at specific sequences



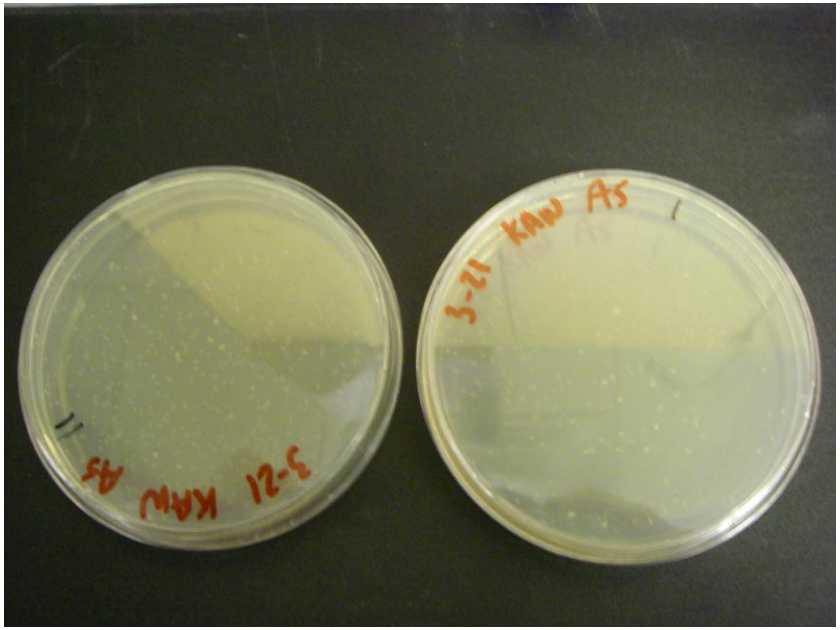
Fuse the cut DNA back together with DNA Ligase



Bacterial Transformation

Technique to amplify plasmid DNA

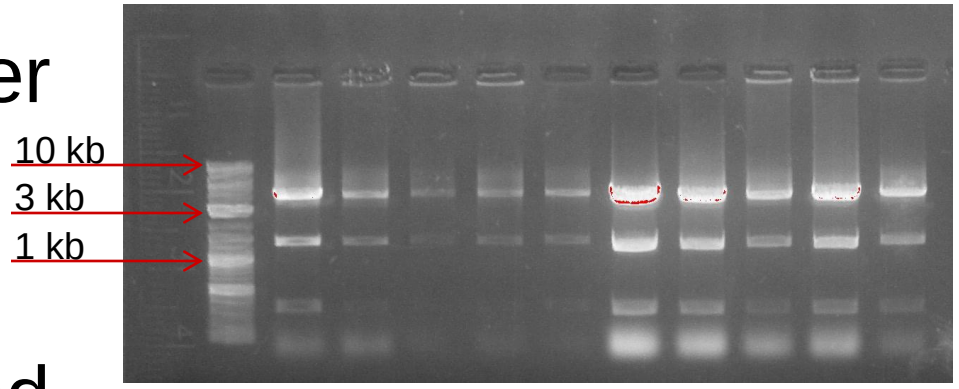
Harness the power of billions of bacteria, then lyse them for their precious plasmid



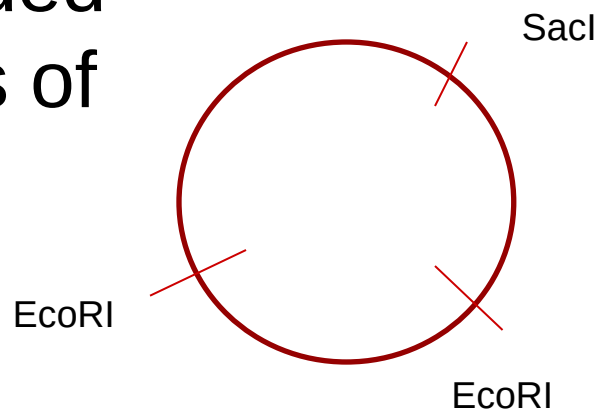
Two bacterial plates from my second transformation attempt. Plates are on KAN rather than AMP

More Restriction Digestion

Assessment for proper plasmid size



Multiple bands needed for proper diagnosis of the plasmid



These appear to be correct, but were actually off by 4 bases

DNA Sequencing

Requires a template, primers, and special nucleotides

Allows for comparison of actual sequence to expected sequence

Beware of frame-shift mutations, insertions, and deletions

The Sequence

The plasmid sequence had four extra nucleotides

Created a frame-shift mutation

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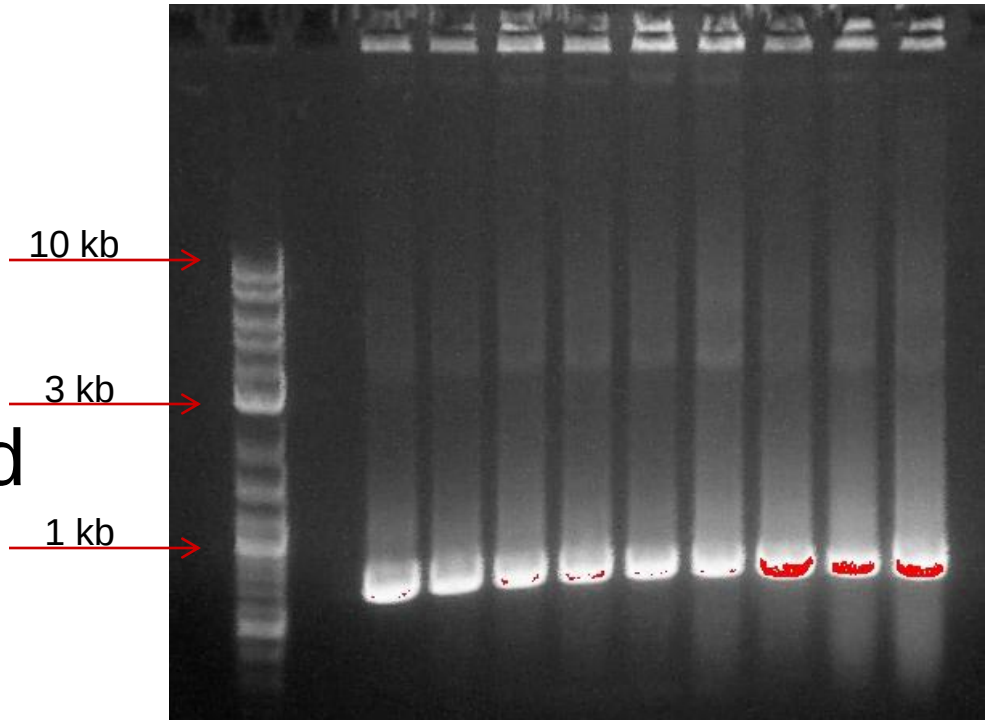
PCR Screening

Using primers to find DNA of interest

Only DNA with the right sequence should be amplified

10 colonies per lane

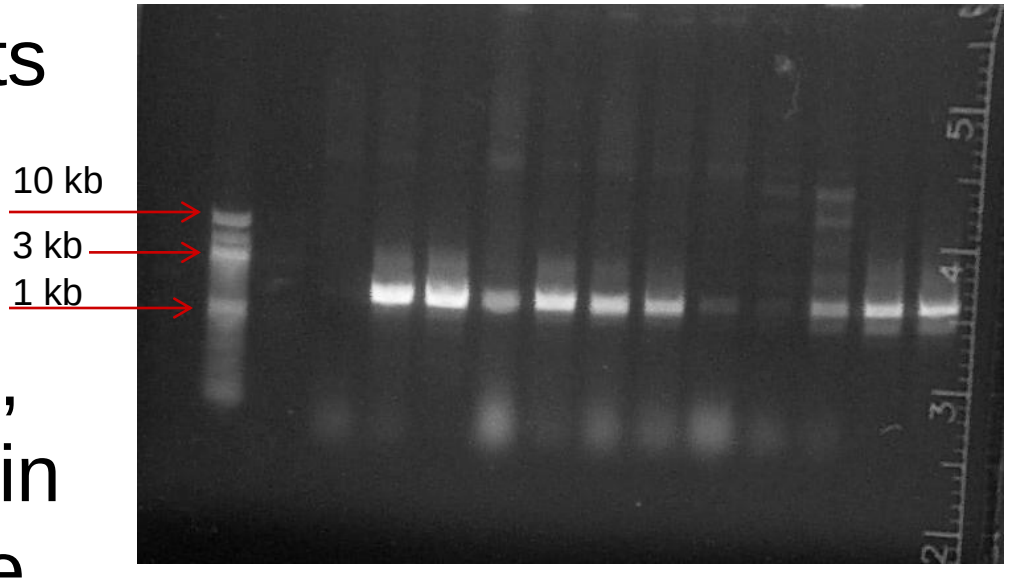


More Specific Screening

Each lane represents one colony

According to the gel, most colonies contain the proper sequence

Negative Controls

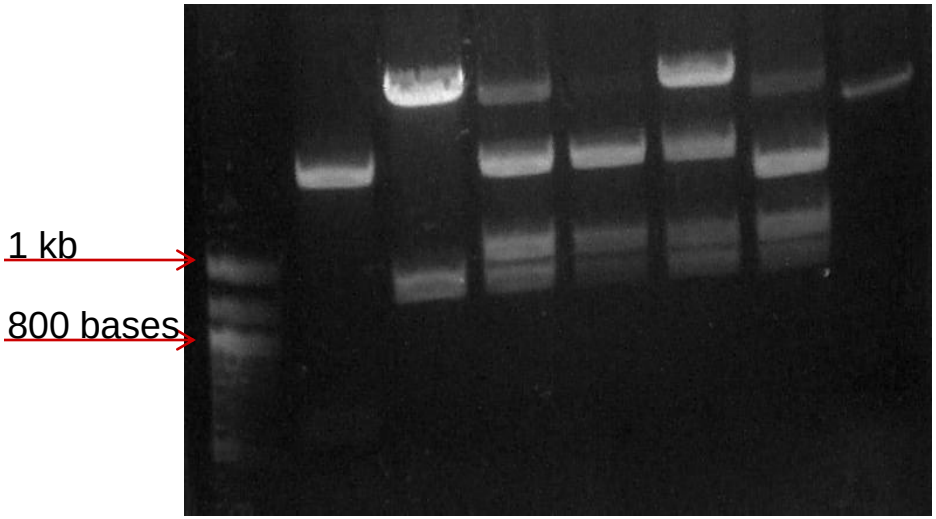


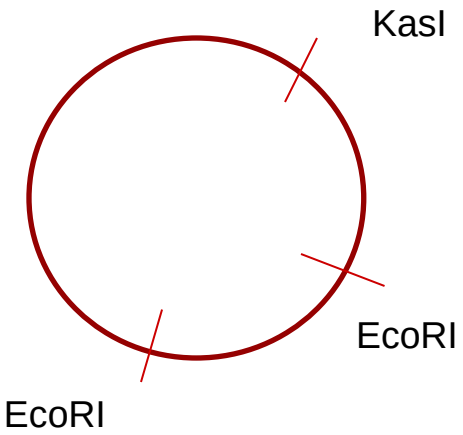
Back to Square One

Repeat the digestion and ligation steps

Decrease Ligation Time

Utilize different restriction digestion strategy





For Future Research

Modify plan to utilize the Leucine Zipper

One copy of the coiled-coil will not be enough

Multiple copies will need to be added to the CESA5 protein for successful interaction and assembly

Lessons

Double check each procedure

Carefully examine results

Learn from your mistakes

Look on the bright side

Thank You

Dr. Roberts and Dr. Norris:

For guidance, lab space, and materials

The Undergraduate Research Initiative:

For funding and support

Audience:

For listening!