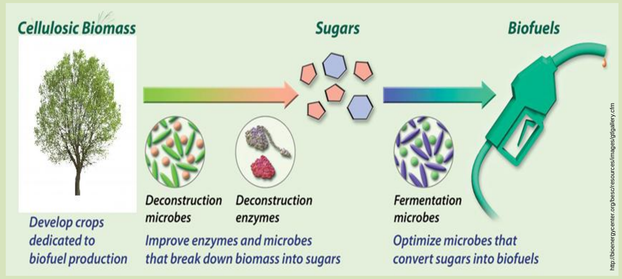
# Research Experience for High School Teachers in Sustainable Transportation Technologies

## Bioengineering Trees for Better Biofuels

**Jenn Coury (Lake Orion High School, Lake Orion, MI) and Robbie Olsen (Hesperia High School, Hesperia, MI)**

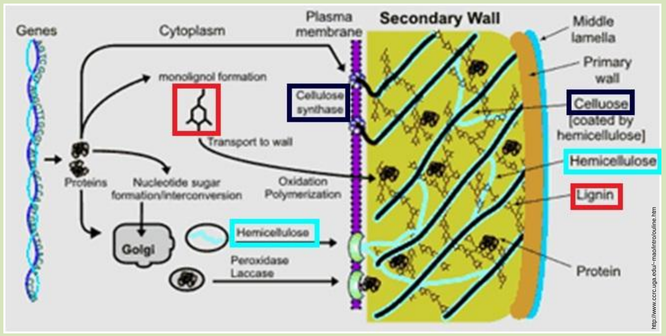
Sustainable Futures Institute

## Introduction to Sustainable Transportation Technologies




The Woods-to-Wheels program aims to understand biomass structure and how cellulose is broken down to optimize the ethanol produced.

## Plant Cell Wall Composition

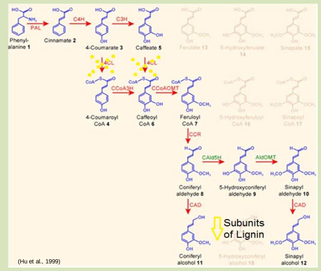


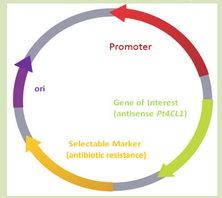

Wood (the secondary cell wall) is made up of three parts – cellulose, hemicellulose, and lignin. Scientists want to genetically engineer plants so they can be converted into ethanol easier – they want more cellulose and to make cellulose easier to break down into glucose. Hemicellulose and lignin interfere with enzymes that break down cellulose. By inserting genes, scientists can alter wood to have the desired composition to optimize the ethanol production.

## Research Methods: Transforming Plants

### 1. Construct a gene of interest.

GOAL: Decrease biomass recalcitrance (remove lignin to make cellulose more accessible). 4CL is an enzyme required in the process of producing lignin. If the amount of lignin is decreased then the proportion of cellulose will be higher. In order to repress 4CL, the gene of interest will make an antisense copy of the 4CL's mRNA. The cell then recognizes that there is double stranded RNA (RNA is single stranded) and destroys it, decreasing the amount of 4CL that is produced. With less 4CL, less lignin will be produced.



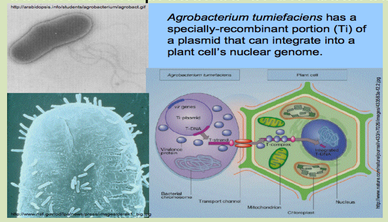

### 2. Clone gene of interest into bacteria

Using a heat shock method, clone the gene of interest into competent *E. coli* cells. Heat shock makes the cell membranes more permeable to plasmids, allowing the gene of interest and selectable marker in. The *E. coli* now contains the gene of interest and a selectable marker (in this case, antibiotic resistance).

### 3. Isolate plasmid DNA

Using a series of buffers, the cell membranes are broken down and the plasmid DNA is separated from the other cell contents.

**3a. PCR and Gel Electrophoresis:** The DNA is amplified in a thermal cycler by PCR. Gel electrophoresis allows the DNA to be visualized as bands. This step confirms the presence of the desired DNA.




### 4. Get plasmid into agrobacterium

Using a freeze-thaw method, the plasmid containing the gene of interest is inserted into agrobacterium. Agrobacterium is a species of bacteria that can infect plant cells and causes crown gall disease. Its ability to transfer DNA to plants allows scientist to use agrobacterium to transform plants.

### 5. Inoculate leaf with agrobacterium

Small pieces of leaves from the plant (in this case aspen) to be transformed are cut and placed on media. The leaves are injured and then exposed to agrobacterium. When the agrobacterium gets in, the transfer DNA is inserted into to the plant bringing the gene of interest with it. After 24 hours of exposure, the agrobacterium is killed with antibiotics.

### 6. Induce undifferentiated growth

The explant is now put on media with auxin and cytokinin (plant hormones) which cause a callus to grow. A callus is a mass of undifferentiated cell growth.

### 7. Induce shooting

The callus is transferred to new media containing cytokinin. Cytokinin is the hormone that induces shoot growth.

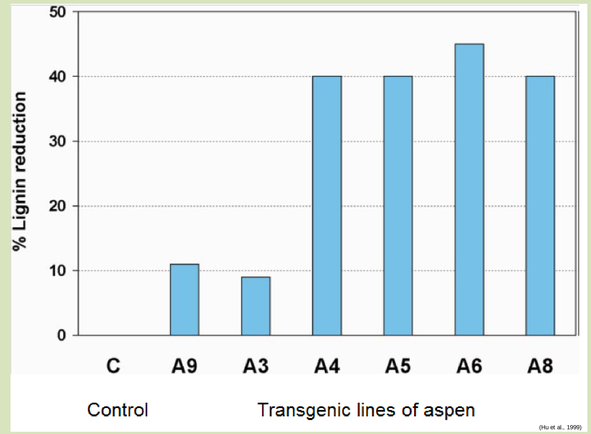
### 8. Induce rooting

The shoot is now placed in media containing auxin and the selectable marker in a sterile magenta box. Auxin is the hormone that induces root growth.

### 9. Planting

When the aspen outgrows the magenta box, it can be planted in soil and placed in the greenhouse. The plant is now considered transgenic. In this case, it is expected that the gene of interest will cause this plant to have decreased amounts of lignin.

## Conclusions




Levels of lignin were reduced (up to 45%) in stem wood of transgenic lines of aspen as compared with the control. This resulted in a 15% increase in cellulose.

## References Cited

Hu, W. et al. (1999). Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees. *Nature Biotechnology* 17: 808-812.

Watson, J.D., Gilman, J.W., Witkowsi, J., & Zoller, M. (1992). Recombinant DNA. New York: Scientific American Books.

**DNeasy Plant Handbook**: www.qiagen.com

**QIAprep Spin Miniprep:** www.qiagen.com

## Acknowledgements

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**Teacher Mentor:**
Joel Hockin
**Fellow Participants**:
JoEllen Hadel, Clint Heikkila, Lloyd Hilger, Sheri Kopka, Susan Stoll
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Dr. Brad Baltensperger, Lori Witting
**Outcomes Assessment:**
Kedmon Hungwe
**K-12 Education Leader**:
Shawn Oppliger
**Note:** Our RET position allowed us to learn the techniques used in a biotechnology laboratory. The research involving aspens (Hu, et al., 1999) was used to illustrate the application to biofuels.