Team ENERGYneering

Growing Green

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Goal: How can we help solve the world's energy problems??

- Find a way to maximize the production of hydrocarbons
- Find a way to maximize the harvest (yield) of hydrocarbons

Novelty of Our System… Why Algae

E coli

Must be forced to produce fuels

Algae

Optimized for the production of fuel

Yeast

Produces ethanol (inferior)

Problem: export is extremely difficult

Novelty of Our System… Possible Solutions

How do we harvest the hydrocarbons?

Option 1:
Lyse the cells
(possibly with a phage gene)

Option 2:
Continuous export
(through metabolic engineering)

Novelty of Our System… Metabolic engineering

- Goal: force hydrocarbons to be secreted from the algae
- How?
  - Unblock the path by minimizing cell wall
  - Increase the production of hydrocarbons
    - Ramp up hydrocarbon pathway
    - Cut off carbon diversion to other pathways

Which species of algae?

- *C. reinhardtii*
- Use as a model for our system
- Is already sequenced
- Successful homologous recombination

- *B. braunii*
- Our ideal species
- Has most productive fuel pathways
- Produces hydrocarbons vs fatty acids
- Problem: has not been sequenced

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Source: http://protist.i.hosei.ac.jp/PDB5/PCD0074/htmls/27.html

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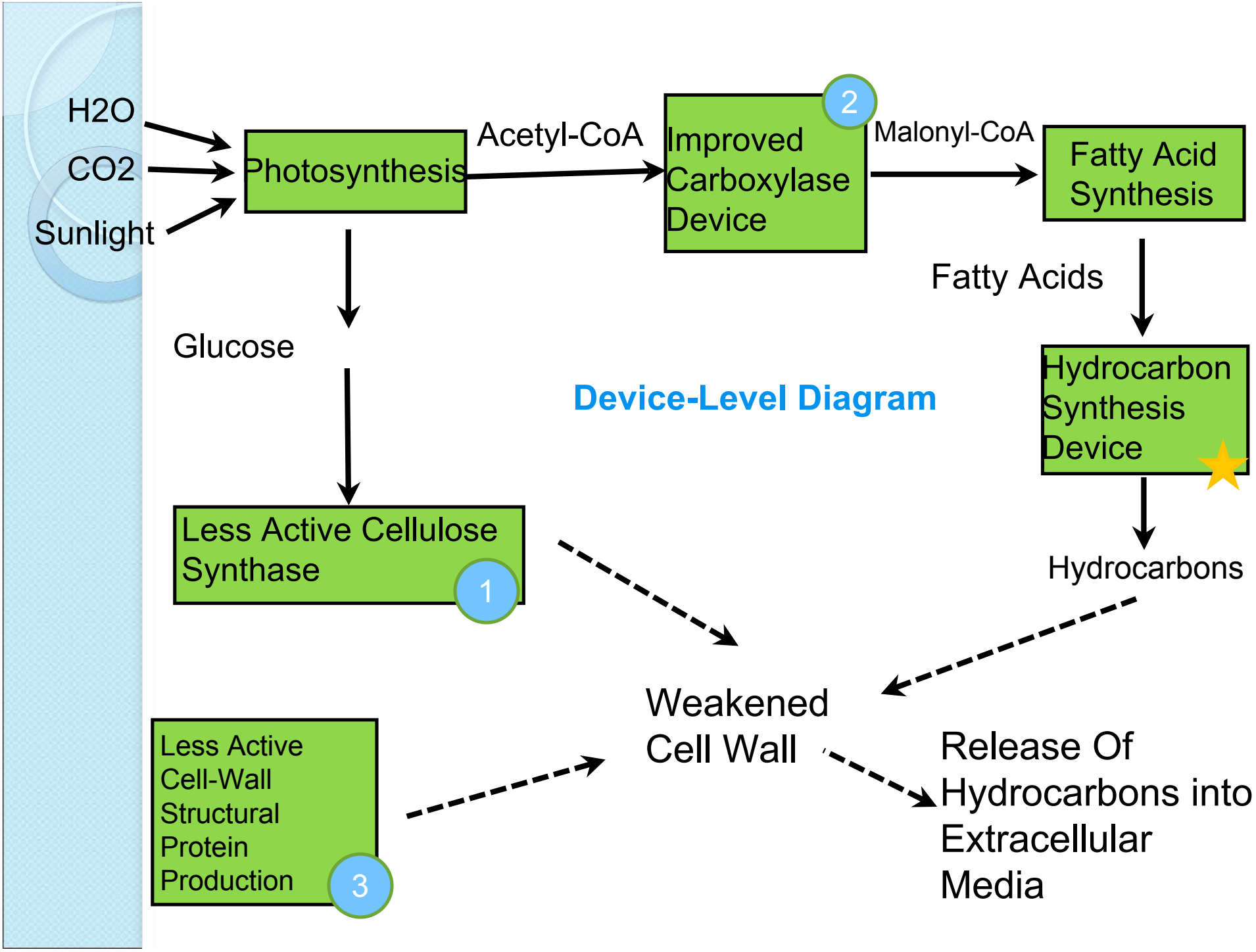
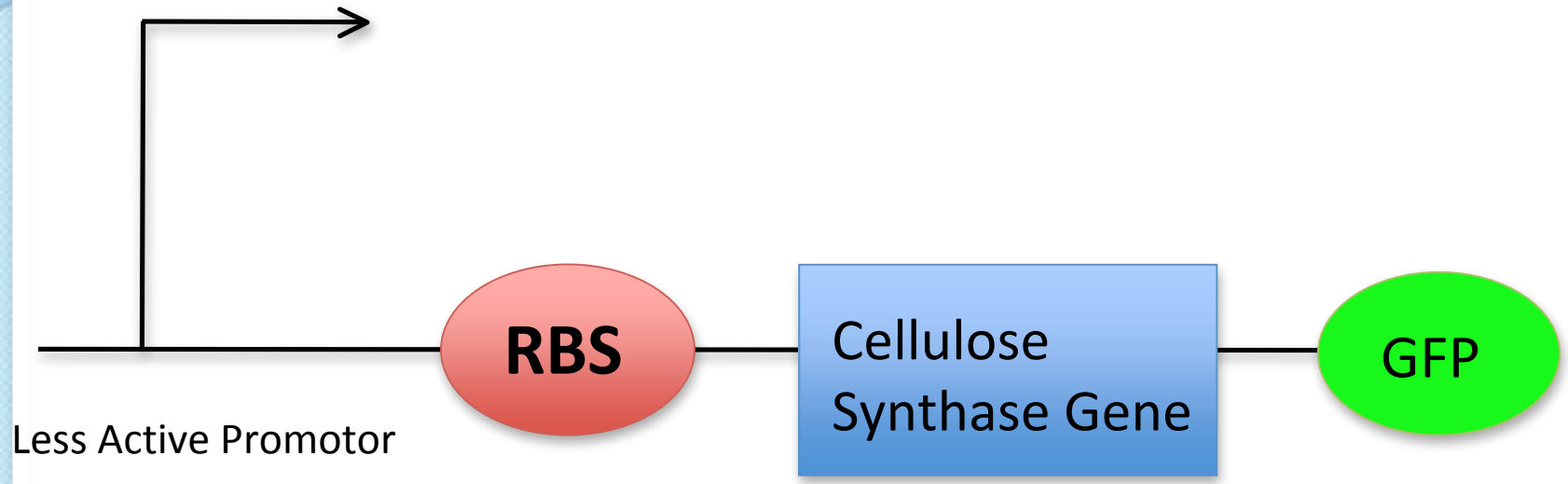
Source: DFCI *Chlamydomonas reinhardtii* Gene Index,
http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/tc_report.pl?gudb=C.reinhardtii&tc=TC40277


H2O
CO2
Sunlight
Photosynthesis
Acetyl-CoA
Improved Carboxylase Device
2
Malonyl-CoA
Fatty Acid Synthesis
Fatty Acids
Glucose
Device-Level Diagram
Hydrocarbon Synthesis Device
Less Active Cellulose Synthase
1
Hydrocarbons
Weakened Cell Wall
Less Active Cell-Wall Structural Protein Production
3
Release Of Hydrocarbons into Extracellular Media

Parts-Level Diagram: Less Active Cellulose Synthase Device

1



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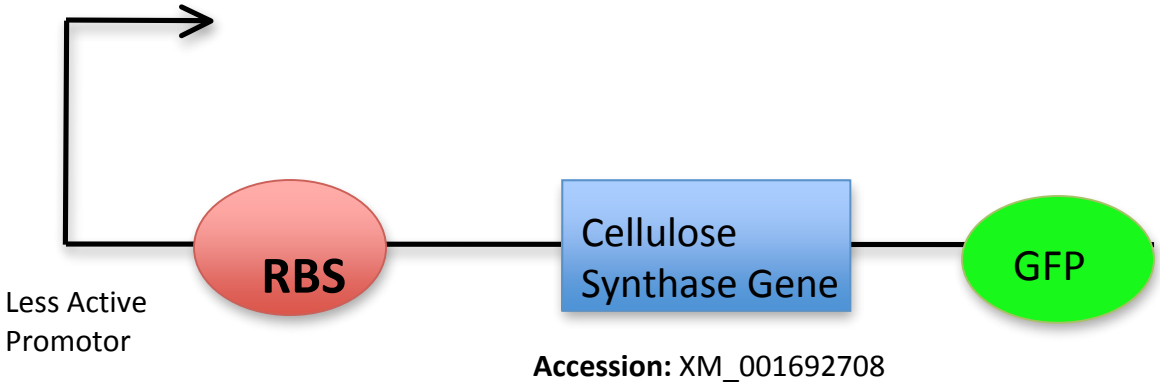
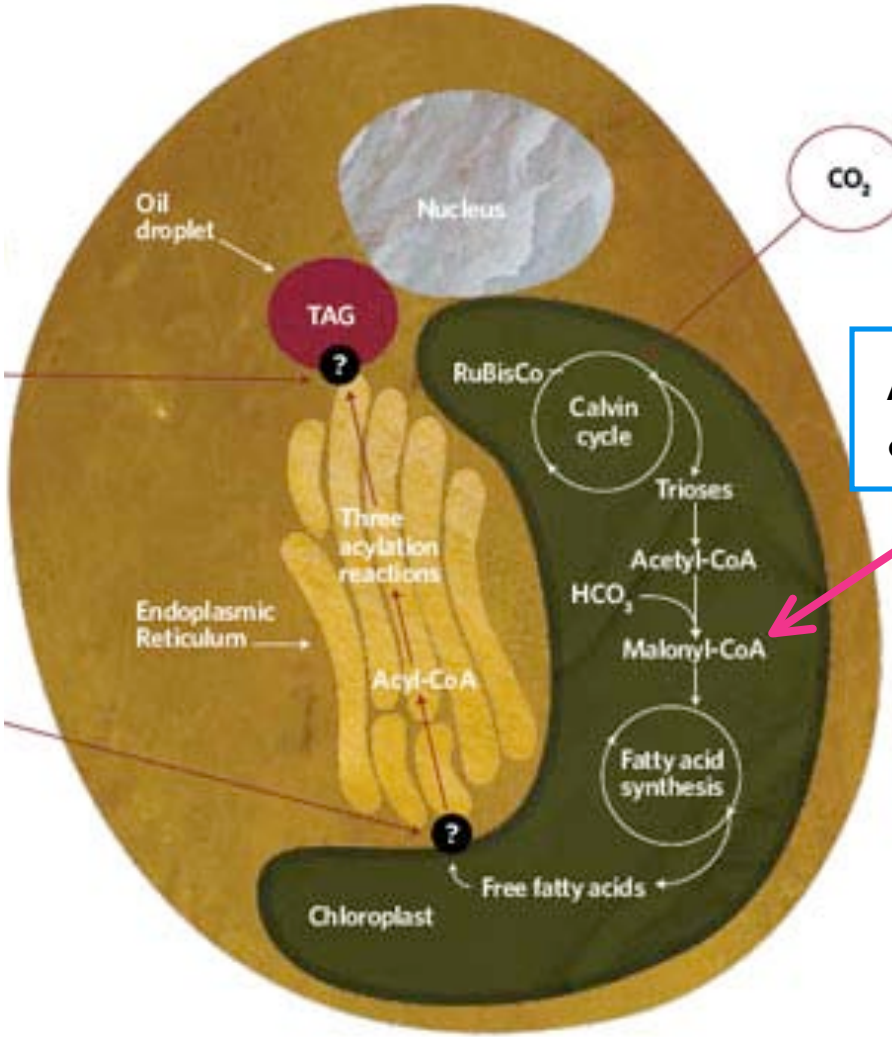
Parts-Level Diagram: Less Active Cellulose Synthase Device

1

- Hydrocarbons are stored in the cell wall
- Decrease cellulose to increase cell wall permeability
    - Test algae promoters (through GFP tagging) to find the least active promoter
    - Ribosome Binding Site: algae specific
- Engineer through homologous recombination



How to increase hydrocarbon production



Acetyl CoA & HCO_3 + Acetyl CoA Carboxylase

Device 2: increases Production of Acetyl CoA Carboxylase

http://www.the-scientist.com/article/display/55376/

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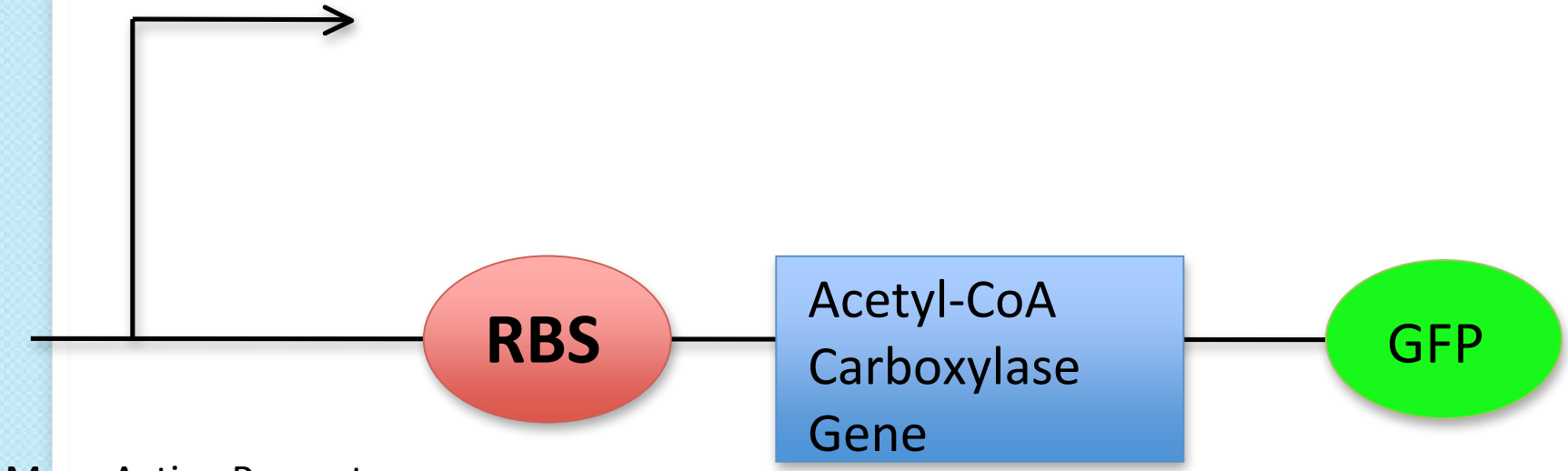
Parts Level Diagram: Improved Carboxylase Device



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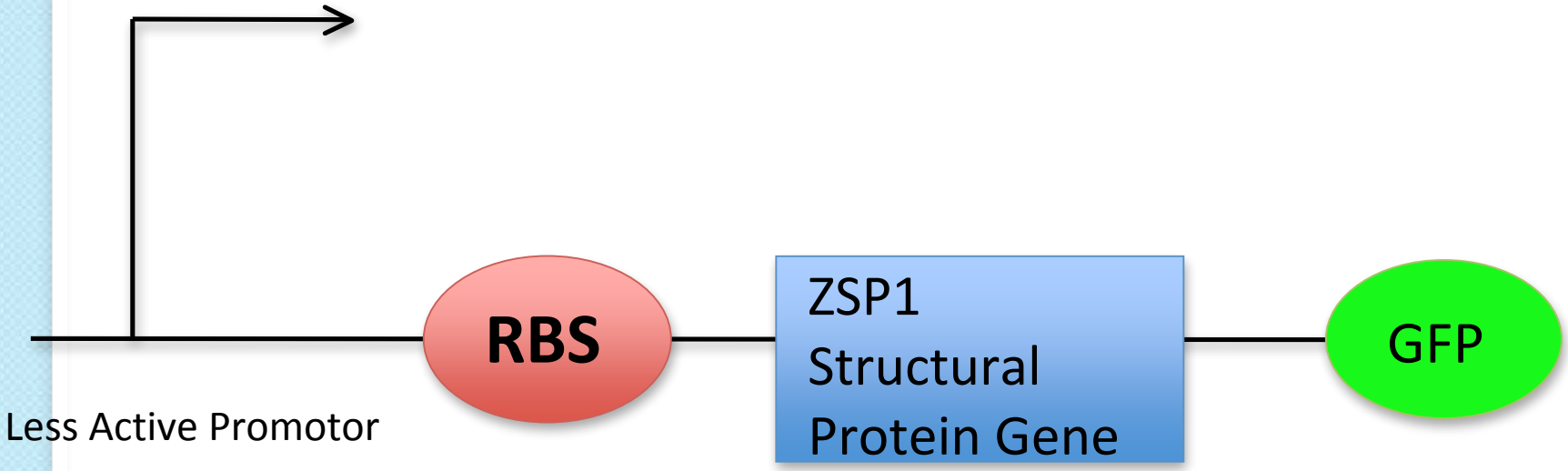
- Test to find the most active promoter
  - Use GFP to measure the different levels of protein expression
- Incorporate through homologous recombination

Parts Level Diagram: Less Active Structural Protein Production Device



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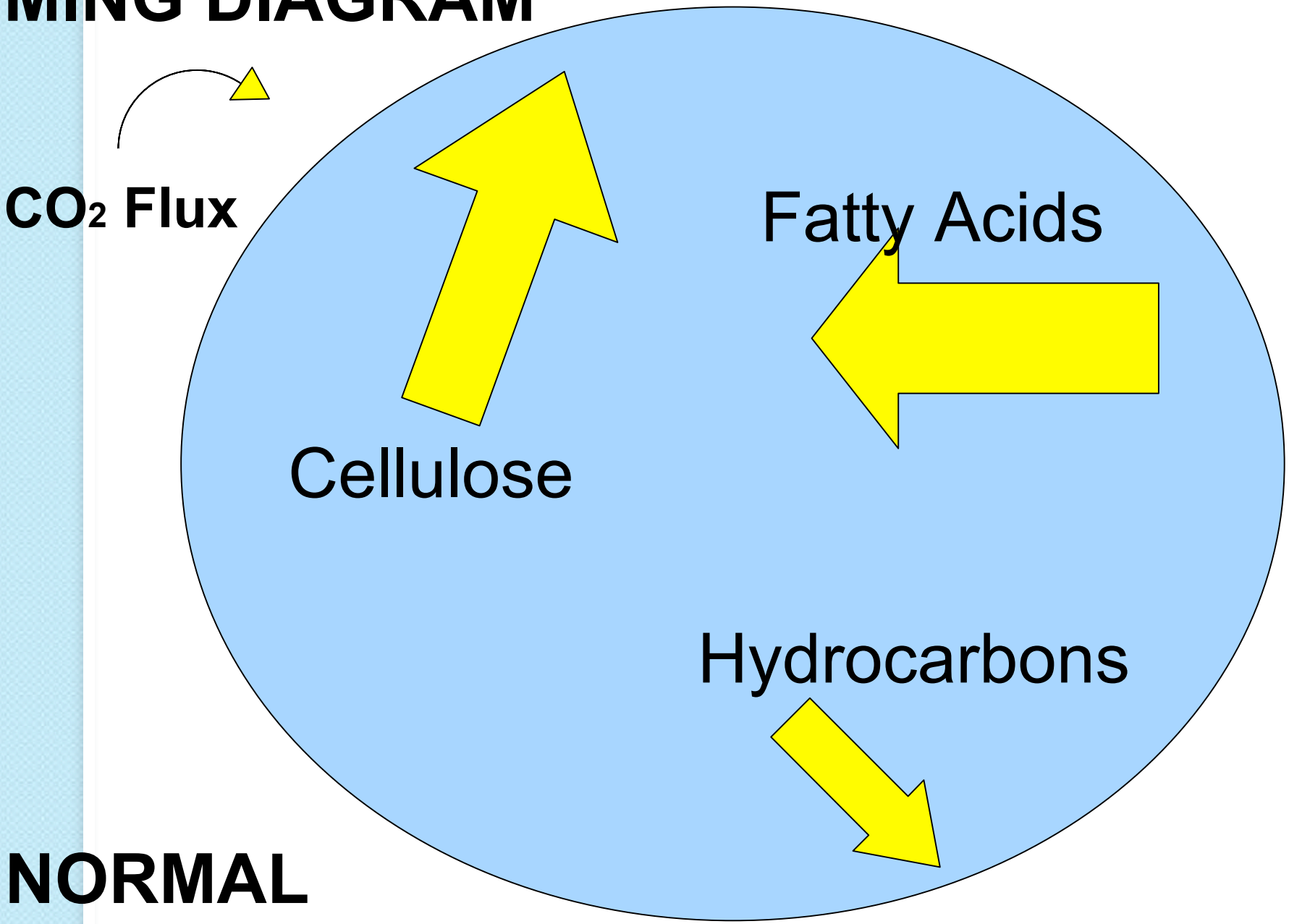
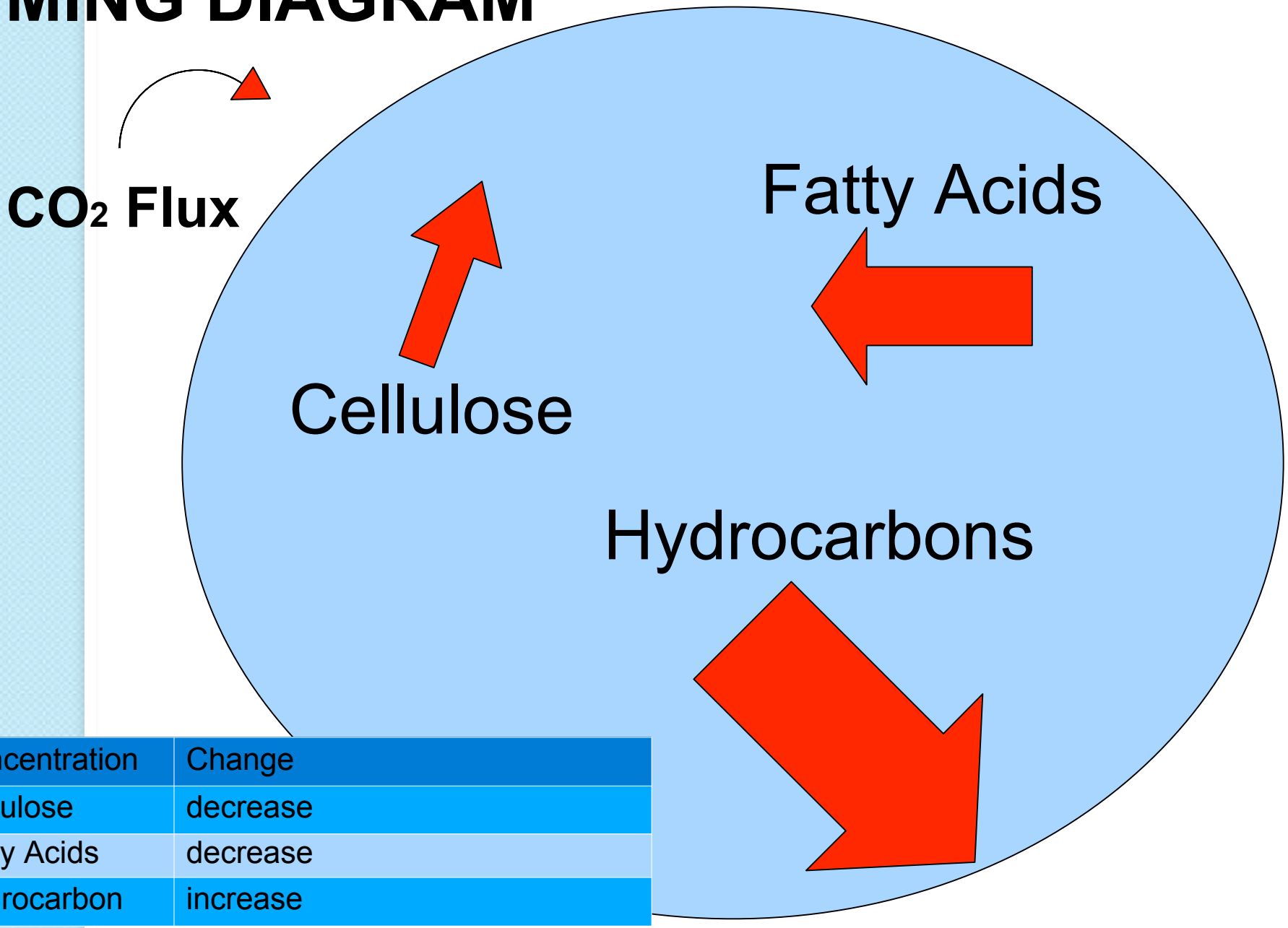
- Decrease presence of structural protein in the cell wall
- Similarly test for least active promoter and incorporate using homologous recombination

TIMING DIAGRAM



NORMAL

TIMING DIAGRAM



Concentration	Change
Cellulose	decrease
Fatty Acids	decrease
Hydrocarbon	increase

Testing and Debugging

- Since we would have established a range of different promoters, we can tune our system to ensure optimum oil production and secretion while maintaining cell's viability
- We can test each device separately to check if they work since they are independent
- Couple the genes of the altered enzymes to different fluorescent genes and test relative expression to see if the device is working according to plan.

Impact of Our System

- Maximize total yield of hydrocarbons
  - Improving rate of production
  - Increasing harvest
- Saving the environment by not using solvents to harvest hydrocarbons
- Decreasing CO_2 emissions

Open Issues

- Ideal species (*B. braunii*) has not yet been sequenced
- Hope to eventually transfer our system into *B. braunii* but we can't be certain that our model will work in both species

Concerns

- **Is it buildable?**

  The *C. Reinhardtii* Model is buildable, *B.Braunii* is only buildable after genome sequencing.
- **Time?**

  Not a very time intensive project(~3-6 months)
- **Cost:**

  Testing the primers could be an expensive process.

  However, Overall it shouldn't be too expensive(~15,000$)
- **Safety:** The algae will be contained in tanks. They should not cause public health issues. Not pathogenic. No new biological material.
- **Security:** Cannot be used to inflict harm. No more of a threat than normal algae.


GO

SOURCES

http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=5716257http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=5716257&ordinalpos=4&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum

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MIT OpenCourseWare
http://ocw.mit.edu

20.020 Introduction to Biological Engineering Design

Spring 2009

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