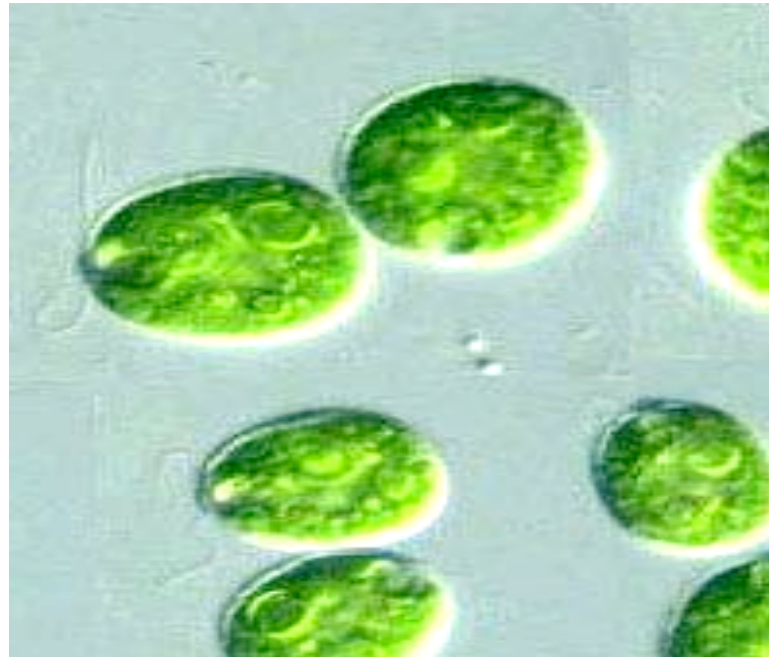


Algal Model Systems

Stephen Mayfield

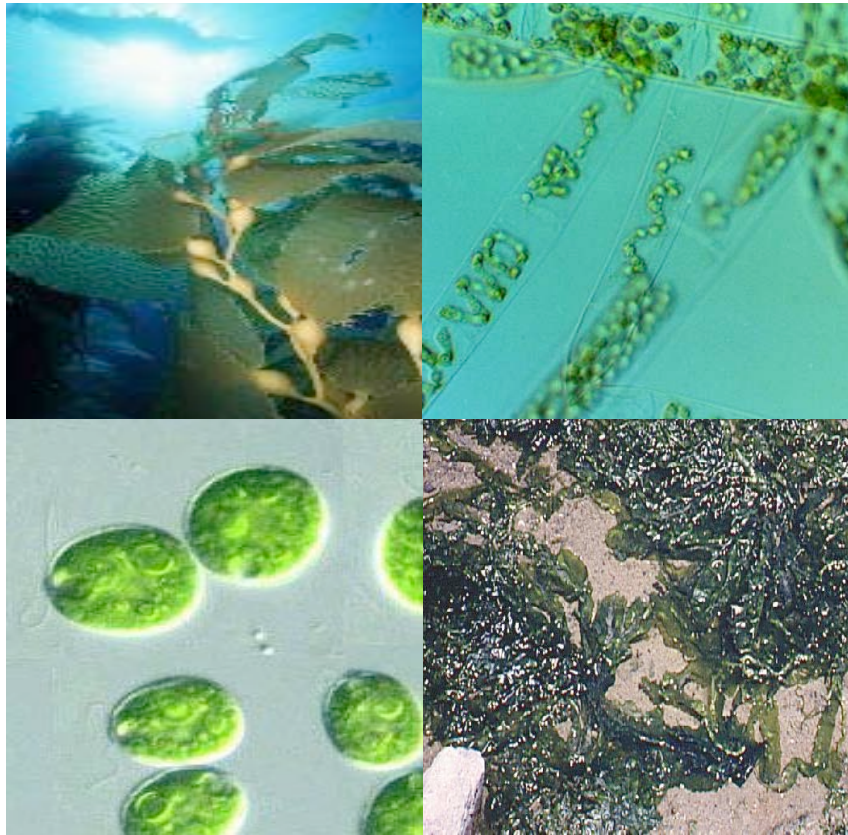
Department of Cell Biology and
The Skaggs Institute for Chemical Biology
The Scripps Research Institute



Alga: Latin “Seaweed”

- Aquatic *eukaryotic* organisms that contain chlorophyll and other pigments and can carry on photosynthesis
 - Range from microscopic single cells to very large multicellular structures resembling stems and leaves
 - Further categorized as brown algae, red algae, green algae, also dinoflagellates and diatoms
 - Some prokaryotes are incorrectly called blue-green algae

Why algae as a biofuel platform?



Cost

Scalability

Sustainability

Superior Fuels

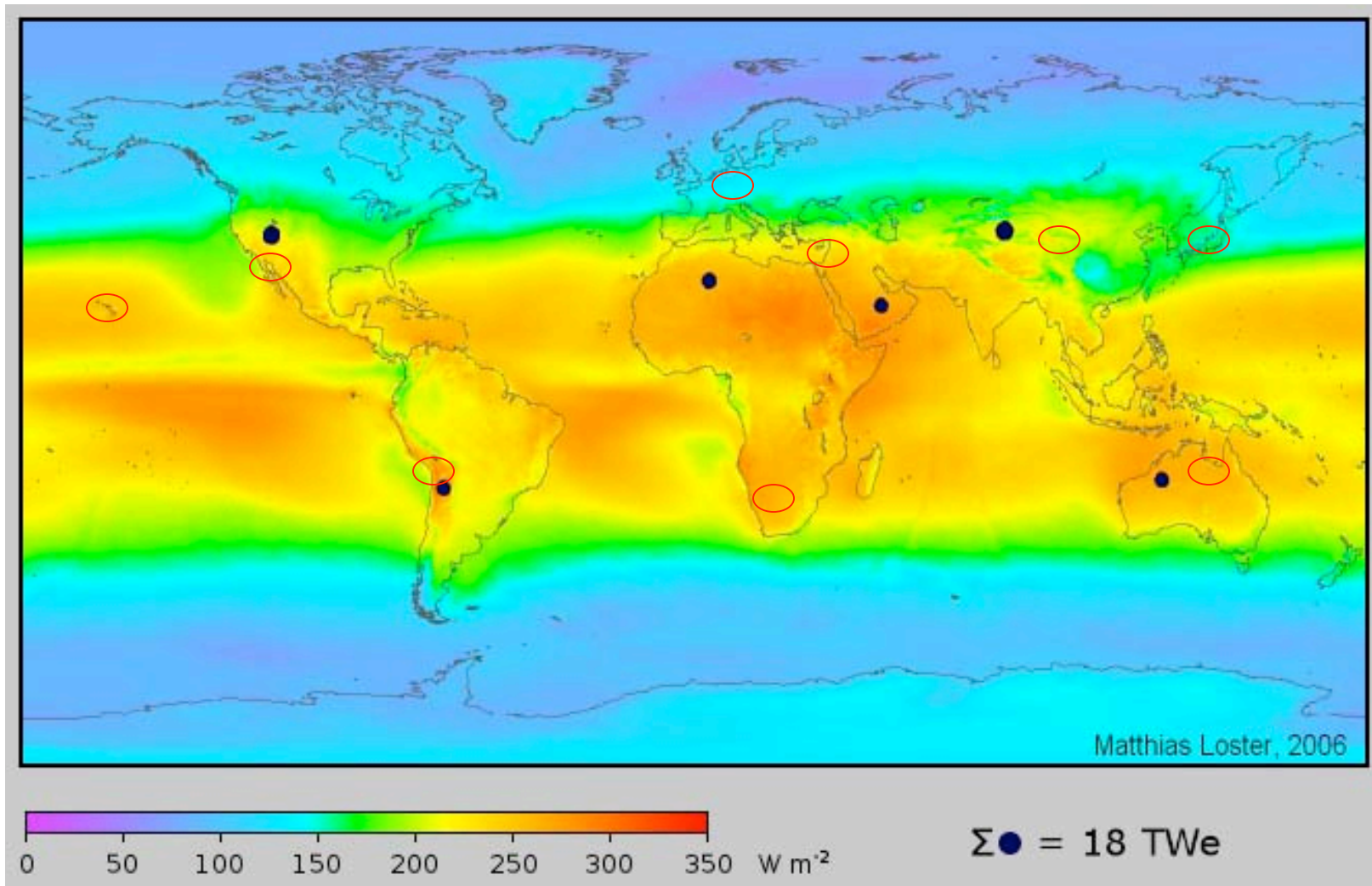
A few numbers to consider for biofuel production from algae

- 140 billion gallons/year of liquid fuel consumed in US
- If algae can produce 50 tons biomass or 1,600 gallon/acre-year
- 90 million acres needed to fill liquid fuel requirements
 - 90 million in corn (\$52 B) and 67 million in soybeans (\$26 B) in 2008
- Cost of algae oil estimated between \$6 and \$60 /gallon

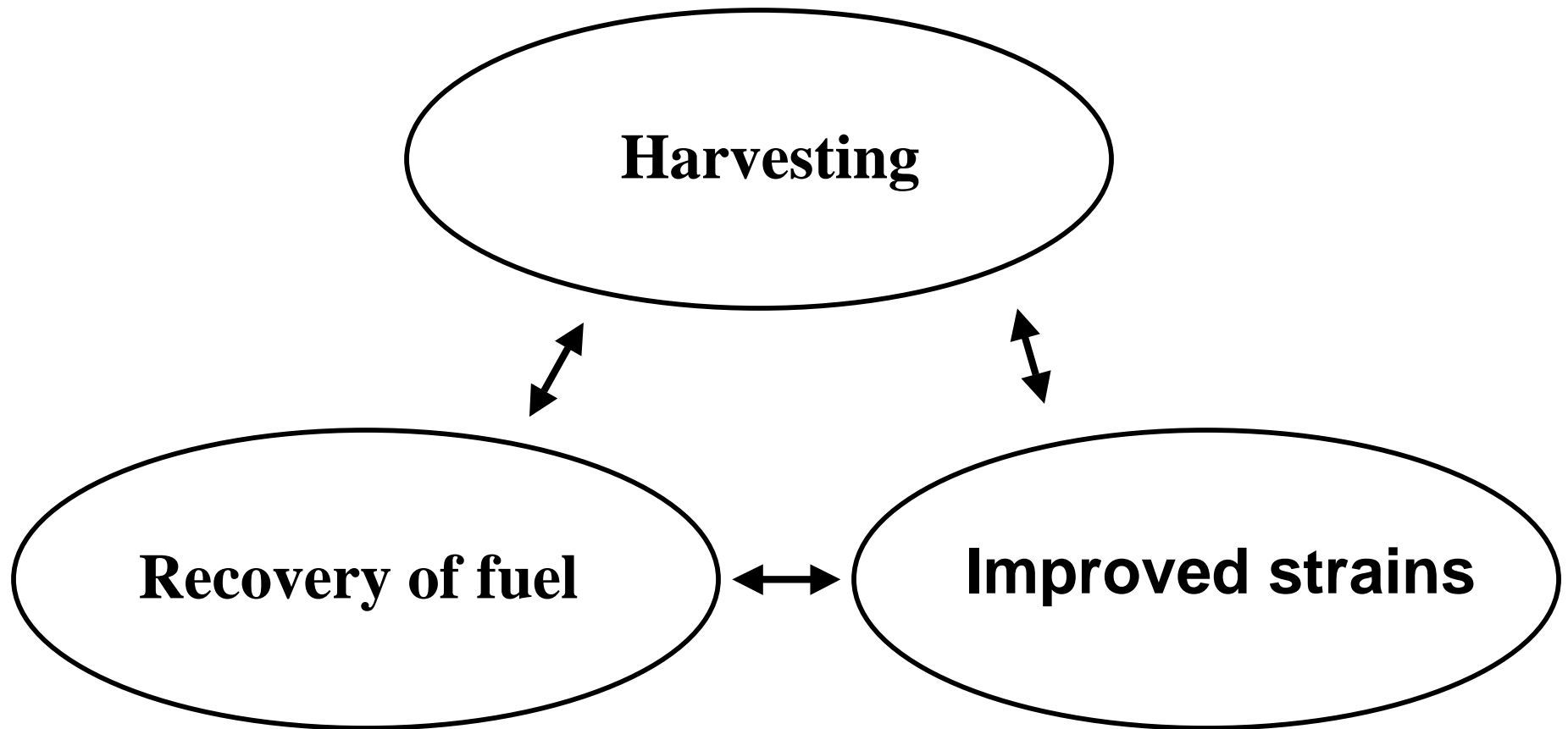
What should an algal biofuel solution look like?

- Sunlight energy converted directly to fuel
- No use of agricultural land or products
- Highly scalable process to meet demand
- Commodity energy prices
- Carbon neutral process

Sunlight is the original source of crude oil and all biofuels



Challenges of producing fuels from algae



Biofuels produced by algae

Biodiesel, triglycerides and fatty acids

Lipids, long chain hydrocarbons - botryococcene

Carbohydrates: sugars and starches

Ethanol or other alcohols

Cellulose or other biomass

Production of each of these is likely from a different species

Guesses* about how to realize biofuel production from algae

- Identify strains with desired traits
 - one strain unlikely to have everything we want
 - one strain unlikely to grow ever where we need it to
- Need to modify those strains
 - to produce high levels of desired molecule
 - to fit harvest and fuel recovery requirements
 - Probably not naturally occurring traits
 - **Require genetic modification on an accelerated time frame**

*To predict without sufficient information

Need an accelerated time frame for “domestication” of algae

Corn Domesticated 4000 B.C.

Steel plow, large scale agriculture 1837

Corn “varieties” 1863

Green Revolution 1944

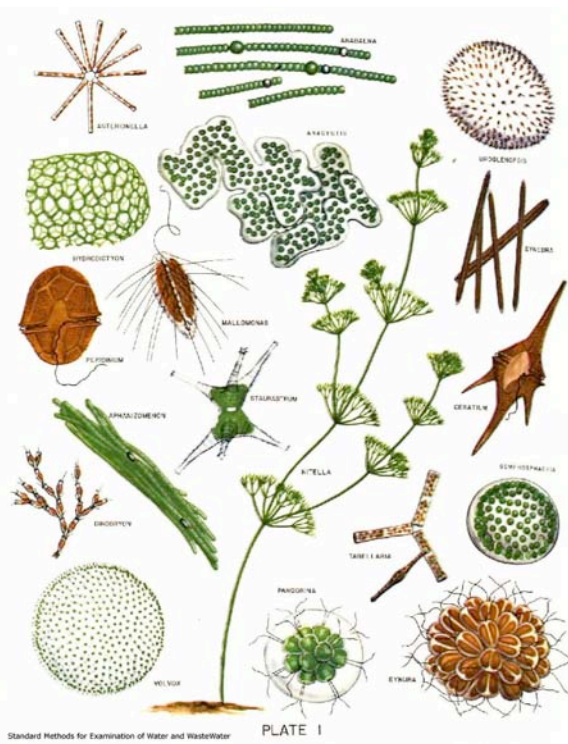
Genetically modified corn 2000

- Need the same for algae only quicker

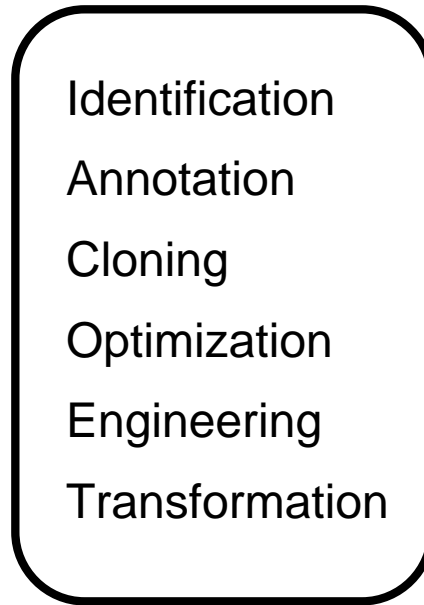
What do we need to achieve rapid domestication of algae for biofuels?

- We need a much bigger and better knowledge base on algae
- We need to identify and characterize a large number of diverse algal species
 - Genomic, proteomic and metabolic profiles
- *We need to develop genetic tools for breeding*
- *We need to develop molecular tools for engineering*
- *We need to develop agricultural practices for algal growth, harvesting and processing*

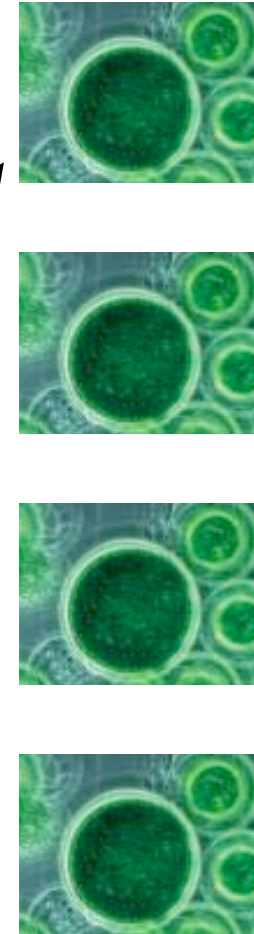
Domestication will require source genes, engineering and hosts strains



Source genes



Engineering

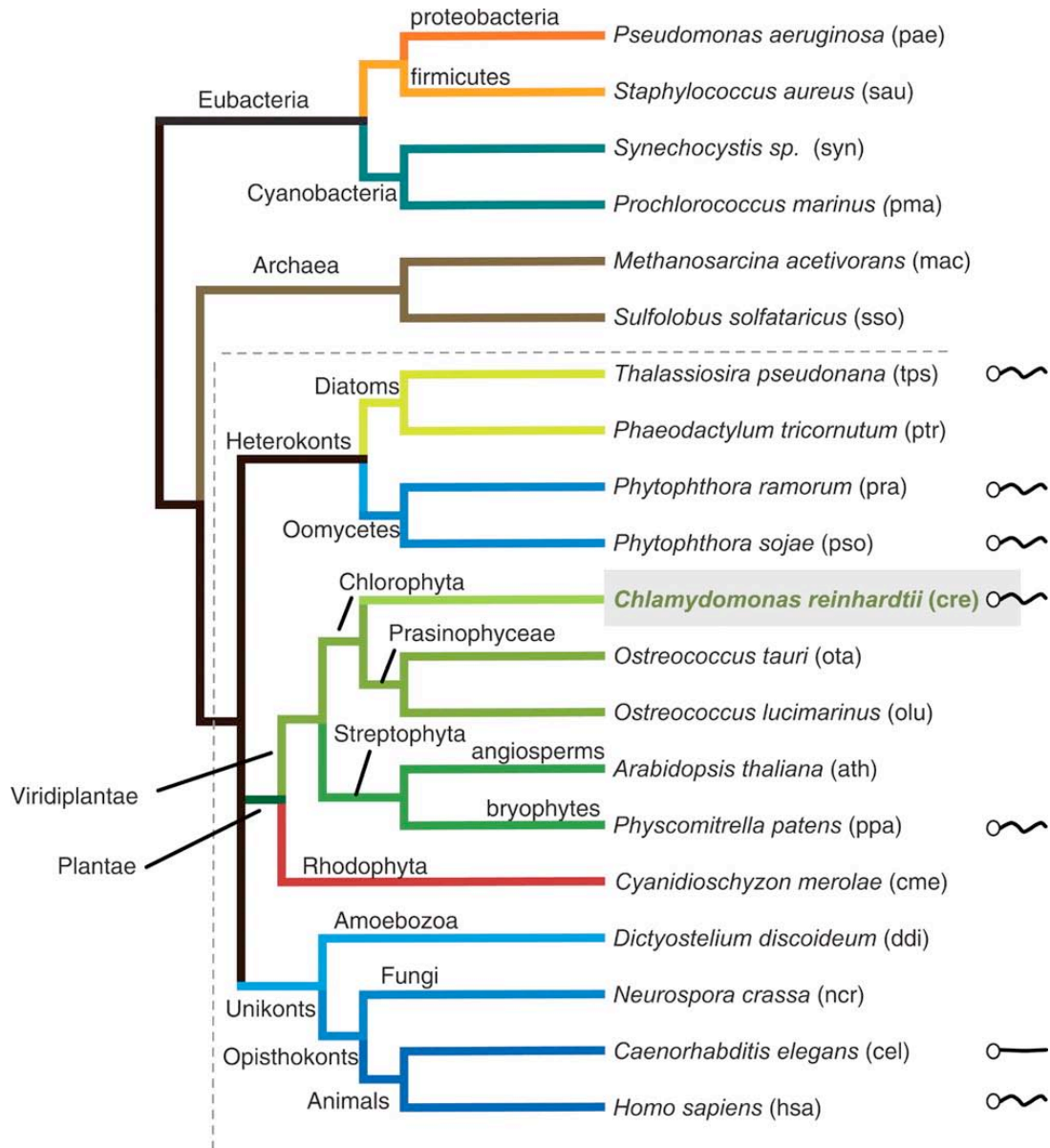


Production strains

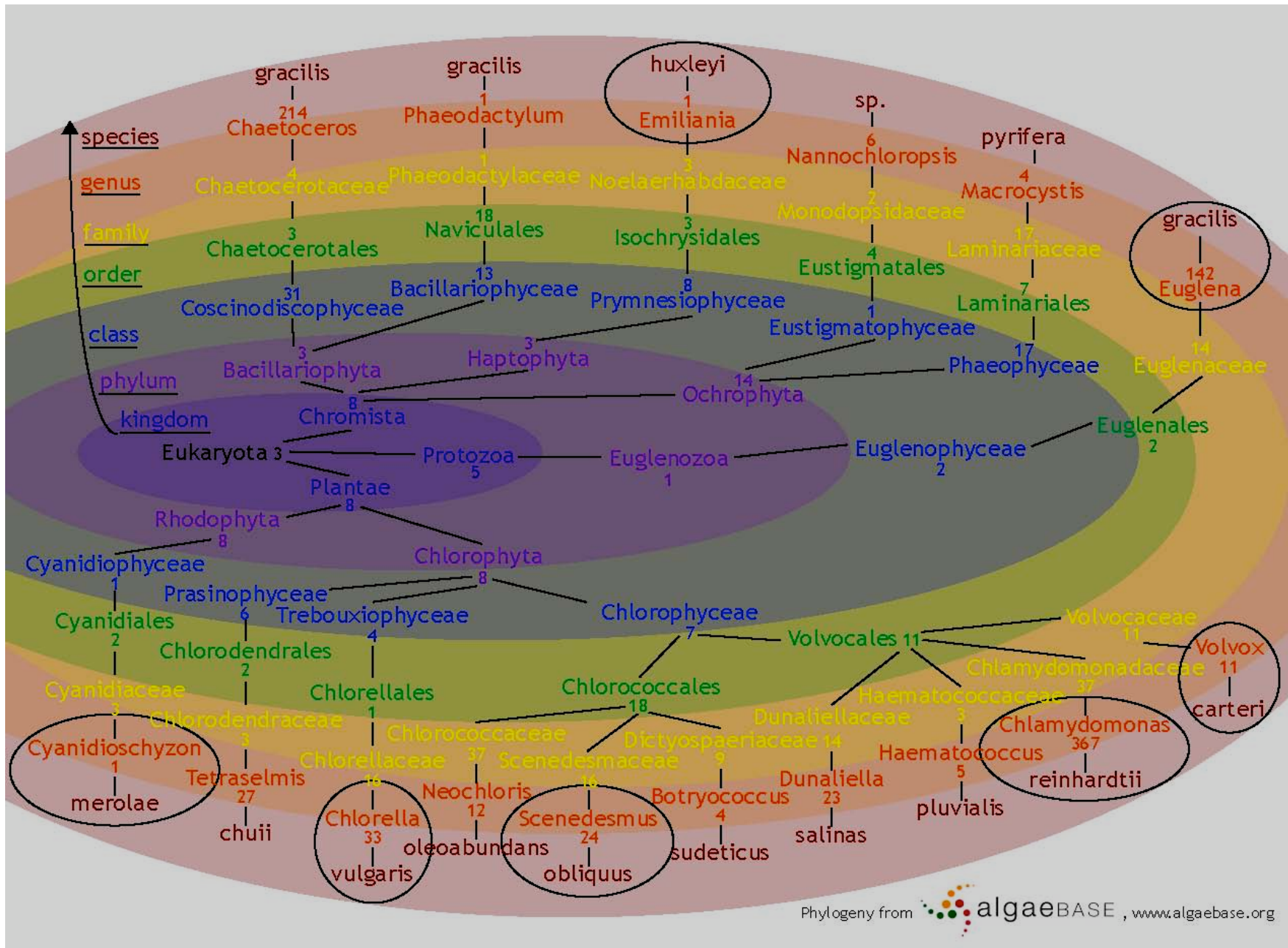
What do we have so far?

- We have many species identified with limited characterization, but showing the potential
- We know how to grow algae at a modest scale
- We have a few algal genomes sequenced and annotated
- We have nuclear and chloroplast transformation for a handful of species

Phylogenetic tree



Algae are the most diverse organisms in the world



Completely sequenced chloroplast genomes (as of 10/07)

	organism		strain	GenBank	size (bp)
chlorophyta	<i>Chlamydomonas</i>	<i>reinhardtii</i>		BK000554	203828
chlorophyta	<i>Chlorella</i>	<i>vulgaris</i>	C-27	AB001684	150613
rhodophyta	<i>Cyanidioschizon</i>	<i>merolae</i>	10D	AY286123	149987
rhodophyta	<i>Cyanidium</i>	<i>caldarium</i>	RK1	AF022186	164921
glaucocestophyceae	<i>Cyanophora</i>	<i>paradoxa</i>	Pringsheim strain LB 555	CPU30821	135599
haptophyceae	<i>Emiliana</i>	<i>huxleyi</i>	CCMP 373	AY741371	105309
euglenozoa	<i>Euglena</i>	<i>gracilis</i>	Pringsheim strain Z	X70810	143171
rhodophyta	<i>Gracilaria</i>	<i>tenuistipitata</i>	liui	AY673996	183883
chlorophyta	<i>Leptosira</i>	<i>terrestris</i>	UTEX 333	EF506945	195081
chlorophyta	<i>Mesostigma</i>	<i>viride</i>		AF166114	118360
chlorophyta	<i>Nephroselmis</i>	<i>olivacea</i>		AF137379	200799
stramenophiles/diatom	<i>Odontella</i>	<i>sinensis</i>		Z67753	119704
chlorophyta	<i>Oltmannsiellopsis</i>	<i>viridis</i>		DQ291132	151933
chlorophyta	<i>Ostreococcus</i>	<i>tauri</i>		CR954199	71666
stramenophiles/diatom	<i>Phaeodactylum</i>	<i>tricornutum</i>		EF067920	117369
rhodophyta	<i>Porphyra</i>	<i>purpurea</i>	avonport	U38804	191028
rhodophyta	<i>Porphyra</i>	<i>yezoensis</i>		AP006715	191952
chlorophyta	<i>Pseudendoclonium</i>	<i>akinetum</i>		AY835431	195867
cryptophyta	<i>Rhodomonas</i>	<i>salina</i>	CCMP1319	EF508371	135854
chlorophyta	<i>Scenedesmus</i>	<i>obliquus</i>	UTEX 393	DQ396875	161452
chlorophyta	<i>Stigeoclonium</i>	<i>helveticum</i>	UTEX 441	DQ630521	223902
stramenophiles/diatom	<i>Thalassiosira</i>	<i>pseudonana</i>		EF067921	128814

How do we choose the species to isolate the source genes



Oil content of selected algae species

Species	Oil content (% dw)	Reference
<i>Ankistrodesmus TR-87</i>	28-40	Ben-Amotz and Tomabene (1985)
<i>Botryococcus braunii</i>	29-75	Sheehan et al. (1998); Banerjee et al. (2002); Metzger and Largeau (2005)
<i>Chlorella sp.</i>	29	Sheehan et al. (1998)
<i>Chlorella protothecoides (autotrophic/ heterotrophic)</i>	15-55	Xu et al. (2006)
<i>Cyclotella DI-35</i>	42	Sheehan et al. (1998)
<i>Dunaliella tertiolecta</i>	36-42	Kishimoto et al. (1994); Tsukahara and Sawayama (2005)
<i>Hantzschia DI-160</i>	66	Sheehan et al. (1998)
<i>Isochrysis sp.</i>	7-33	Sheehan et al. (1998); Valenzuela-Espinoza et al. (2002)
<i>Nannochloris</i>	31 (6-63)	Ben-Amotz and Tomabene (1985); Nagoro et al. (1991); Sheehan et al. (1998)
<i>Nannochloropsis</i>	46 (31-68)	Sheehan et al. (1998); Hu et al. (2006)
<i>Nitzschia TR-114</i>	28-50	Kyle DJ, Gladue RM (1991) Eicosapentaenoic acids and methods for their production. International Patent Application, Patent Cooperation Treaty Publication WO 91/14427, 3 October 1991.
<i>Phaeodactylum tricornutum</i>	31	Sheehan et al. (1998)
<i>Scenedesmus TR-84</i>	45	Sheehan et al. (1998)
<i>Stichococcus</i>	33 (9-59)	Sheehan et al. (1998)
<i>Tetraselmis suecica</i>	15-32	Sheehan et al. (1998); Zittelli et al. (2006); Chisti (2007)
<i>Thalassiosira pseudonana</i>	(21-31)	Brown et al. (1996)

Photosynthetic efficiency and yield

Plant system	Photosynthetic efficiency of PAR (%)	Typical productivity range (g m ⁻² day ⁻¹)	Typical productivity (t ha ⁻¹ y ⁻¹) (Maximum)	Comment	Reference
Land plants					
C3 land plants	< 6.6 (theor.)	Not applicable	10 – 18 (24) 8 -10 (30)	Sugarbeet (temperate climate) Willow (max. on test plots)	Kanter et al. (2006) Keoleian and Volk (2005)
C4 land plants	< 13.4 (theor.)	Not applicable	10 – 30 (72) 10 – 20 (50) 15 – 20 (40)	Sugarcane Sorghum Miscanthus	Muchow et al. (1994), Samson et al. (2005) Habyanimana et al. (2004), Clifton-Brown et al. (2001), Heaton et al. (2004),
Macro-algae					
<i>Laminaria</i> offshore	Not reported	1 – 5	7 - 16	Natural populations and commercial harvesting	Mann (1973); Chynoweth (2002), page 39
<i>Macrocystis</i> , <i>Gracilaria</i> , <i>Laminaria</i> and <i>Chondrus</i> in culture chambers	Not reported	3 – 10	10 – 34 (127)	"probably not achievable on a commercial scale" (Chynoweth 2002)	Chynoweth (2002), page 11-15
<i>Laminaria</i> in offshore farm	Not reported	Not reported	28 – 46 (expected values, prevented by storm!)	High values can only be obtained by supplying nutrients at excessive costs	Brinkhuis and Levin (1987)
Uncultivated brown algae	Not reported	Not reported	10 - 36	Review	Gao and McKinley (1994)
Micro-algae in open ponds					
Micro-algae in commercial raceway ponds	Not reported	3 - 8	10 – 30	<i>Chlorella</i> , <i>Arthrospira</i> , and <i>Dunaliella</i> sp.	Jimenez et al. (2003)
Algae in experimental raceway ponds (Aquatic Biomass Program)	< 10	3 – 40 (winter to summer)	30 – 50	Summary of ABP-program run from 1978 – 1996	Benemann and Oswald (1996); Sheehan et al. (1998)
<i>Haematococcus pluvialis</i>	3 – 4.4	10 – 15 (uncorrected)	20 – 30	Annual yield corrected for space occupied by PBRs	Huntley and Redalje (2007)
<i>Arthrospira</i> (<i>Spirulina</i>)	Not reported	2 – 15	30	450 m ²	Jimenez et al. (2003)

Photosynthetic efficiency and yield

Table 4 continued.

Plant system	Photosynthetic efficiency of PAR (%)	Typical productivity range (g m ⁻² day ⁻¹)	Typical productivity (t ha ⁻¹ y ⁻¹) (Maximum)	Comment	Reference
<i>Dunaliella salina</i>	Not reported	2	Not reported	Small outdoor photo-bioreactor, 55l, 2.2 m ²	García-González et al. (2005)
<i>Pleurochrysis carterae</i>	Not reported	3 – 33 (winter to summer)	60	Small system (1 m ²), 13 months 21.9 t/ha/y lipids 5.5 t/ha/y CaCO ₃	Moheimani and Borowitzka (2006) (see Table 3 for a list of similar experiments)
<i>Scenedesmus obliquus</i>	Not reported	48 (3 months in summer)	Not applicable	20 m ² raceway pond unpublished results	Grobbelaar (2000)
<i>Tetraselmis suecica</i>	6 – 7 average 13 – 18 max	20 60 – 70	Not applicable	Duration less than 1 month Single day result	Laws et al. (1986)
Micro-algae in photobioreactors					
<i>Chlorella vulgaris</i>	Not reported	Not reported	130 – 150 (claimed)	Tubular PBR (700 m ³) in 1.2 hectare greenhouse	Moore (2001); Pulz (2001)
<i>Phaeodactylum tricomutum</i>	15 - 20	61 – 73 (depending on tube diameter) 14 – 17 (calculated for total area)	Not applicable	PBR with optimised dilution rates, extrapolated yields	Acien Fernandez et al. (1998)
<i>Chlorella vulgaris</i>	5.1 – 6.4	0.57 – 0.97	Not applicable	Helical bioreactor, artificial light	Scragg et al. (2002)
<i>Chlorella</i> sp.	7.1	43	Not applicable	Low level artificial light Turbulent culture	Tamiya (1957)
<i>Chlorella</i> sp.	< 47	Not reported	Not applicable	Value obtained under extremely low light with alternative photosystems	Pirt et al. (1980); Richmond (2000)
<i>Arthrospira (Spirulina)</i>	5.45	5.44	Not applicable	Helical bioreactor, artificial light	Watanabe et al. (1995)
<i>Arthrospira (Spirulina)</i>	2 - 5	7 – 25	33	215 days outdoor cultivation period in central Italy	Torzillo et al. (1986)

What are essential criteria for selecting hosts strains?



Kelp



Euglena



Cyanobacteria

Should commercial algae be the host species

Species/group	Product	Application areas	Prod. facilities	References
<i>Spirulina</i> (<i>Arthrospira platensis</i>) / Cyanobacteria)	Phycocyanin, biomass	Health food, cosmetics	Open ponds, natural lakes	Lee (2001); Costa et al. (2003)
<i>Chlorella vulgaris</i> / Chlorophyta	Biomass	Health food, food supplement, feed surrogates	Open ponds, basins, glass-tube PBR	Lee (2001)
<i>Dunaliella salina</i> / Chlorophyta	Carotenoids, β -carotene	Health food, food supplement, feed	Open ponds, lagoons	Jin and Melis (2003); Del Campo et al. (2007)
<i>Haematococcus pluvialis</i> / Chlorophyta	Carotenoids, astaxanthin	Health food, pharmaceuticals, feed additives	Open ponds, PBR	Del Campo et al. (2007)
<i>Odontella aurita</i> / Bacillariophyta	Fatty acids	Pharmaceuticals, cosmetics, baby food	Open ponds	Pulz and Groß (2004)
<i>Porphyridium cruentum</i> / Rhodophyta	Polysaccharides	Pharmaceuticals, cosmetics, nutrition	Tubular PBR	Fuentes et al. (1999)
<i>Isochrysis galbana</i> / Chlorophyta	Fatty acids	Animal nutrition	Open ponds, PBR	Molina Grima et al. (1994); Pulz and Gross (2004)
<i>Phaedactylum tricorutum</i> / Bacillariophyta	Lipids, fatty acids	Nutrition, fuel production	Open ponds, basins, PBR	Yongmanitchai and Ward (1991); Acien-Fernandez et al. (2003)
<i>Lyngbya majuscula</i> / Cyanobacteria	Immune modulators	Pharmaceuticals, nutrition		Singh et al. (2005)
<i>Muriellopsis</i> sp. / Chlorophyta	Carotenoids, Lutein	Health food, food supplement, feed	Open ponds, PBR	Blanco et al. (2007); Del Campo et al. (2007)

Could macro-algae be a host species

Seaweed genus	Remarks
<i>Alaria</i>	Possesses floating structure, occurs in arctic waters
<i>Corallina</i>	Calcareous, spread widely, small, can possibly be grown together with other species
<i>Cystoseira</i>	Moderate climate zone, floating reproduction structures
<i>Ecklonia</i>	Subtropical and moderate climate zone, one floating species
<i>Egregia</i>	Moderate climate zone, floating structure, very robust species
<i>Euचेumia</i>	Already cultivated in tropical areas, relatively small size
<i>Gracillaria</i>	Widely occurring, often cultivated, high productivity
<i>Laminaria</i>	Extensively grown in moderate climate zones
<i>Macrocystis</i>	In semi-culture, seasonal harvest, moderate climate zone
<i>Pterygophora</i>	Moderate climate zone, very robust species
<i>Sargassum</i>	Widely occurring (including Sargasso Sea), many species, floating structures, in moderate and tropical climate zones

What model species for engineering?

Do we take one strain and devote substantial resources to develop it faster? The *E. coli* paradigm

Do we bet on several horses and see who wins?

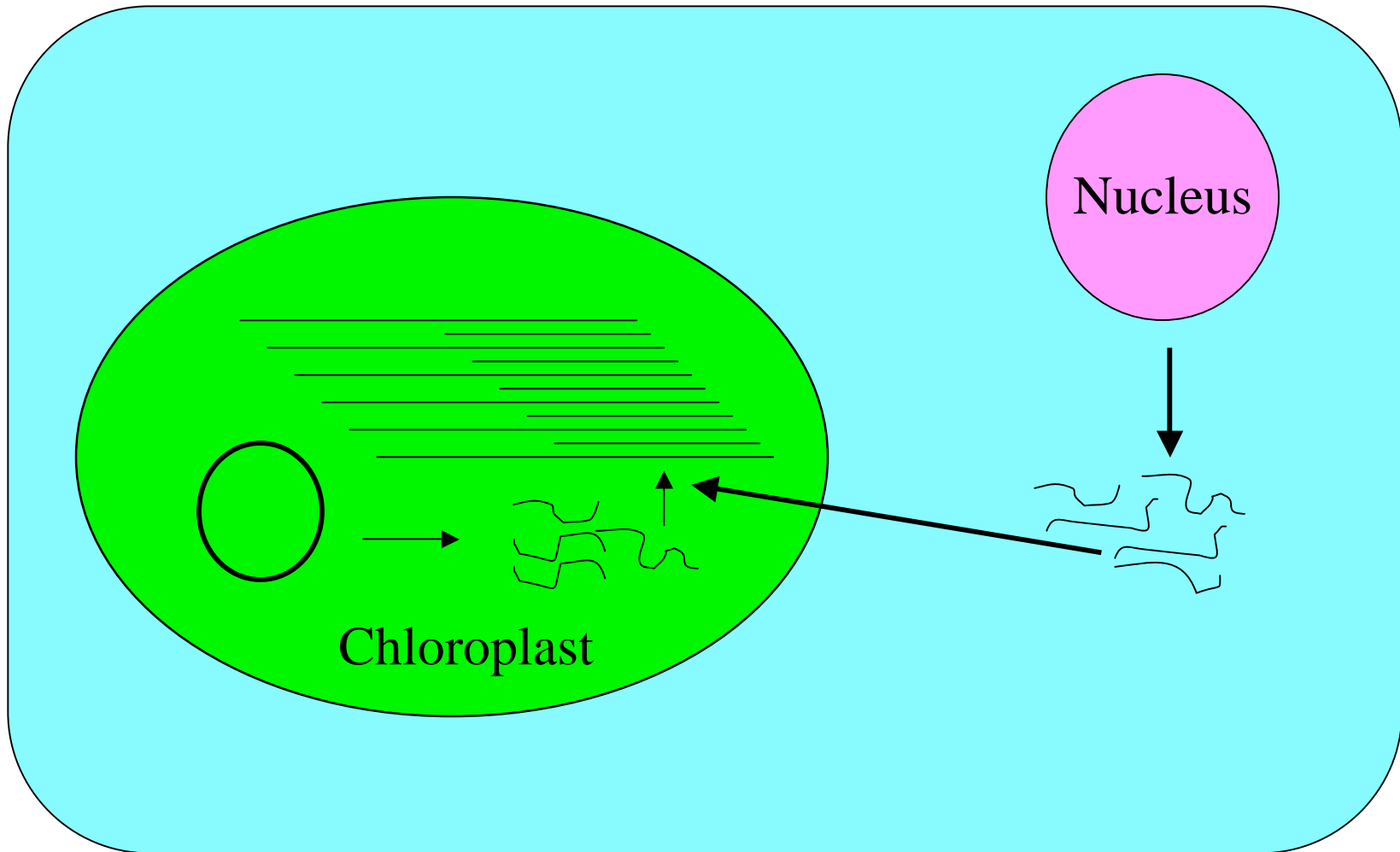
Do we already have the model organisms we need?

Model Algal Species by Citation

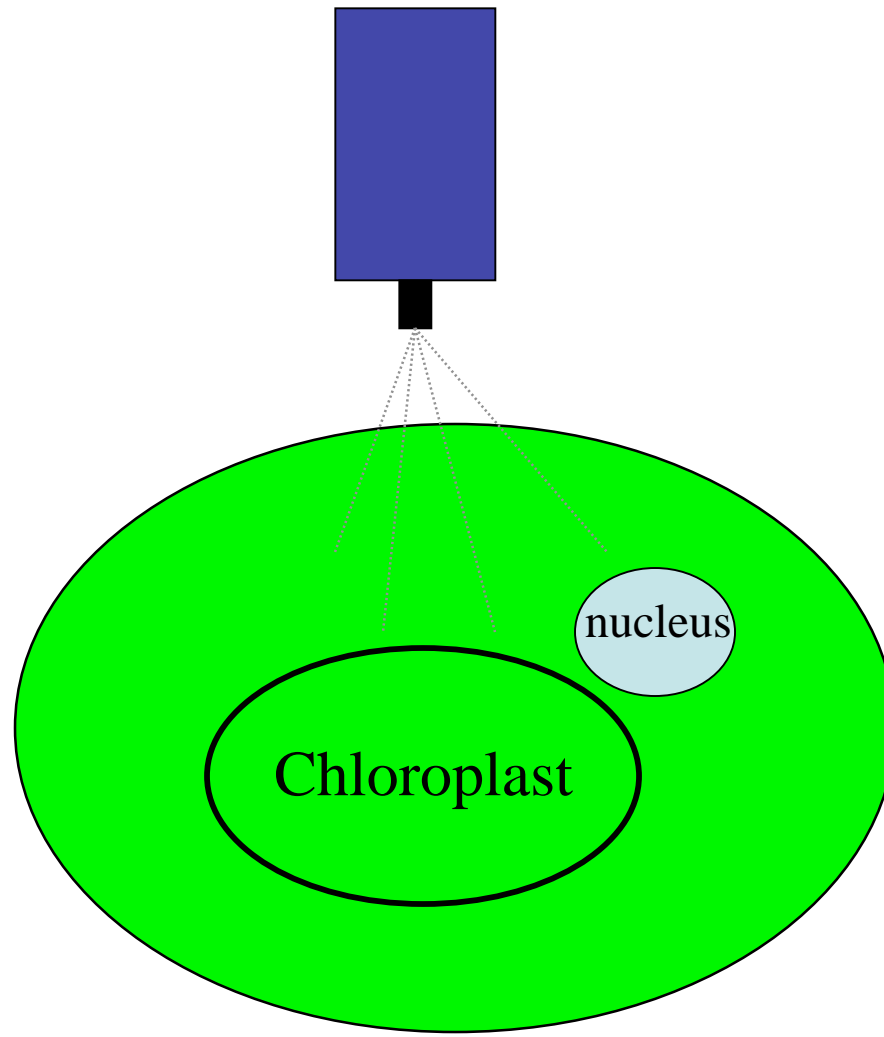
Genus species	Nuclear Genome	Chloroplast Genome	Transformation	Papers
<i>Chlamydomonas reinhardtii</i>	complete	complete	Nuc/Ct (269)	4611
<i>Chorella vulgaris</i>	in progress	complete	Nuc (1)	2901
<i>Euglena gracilis</i>	In progress	complete	Ct (1)	2291
<i>Scenedesmus obliquus</i>	none	complete		642
Laminaria japonica	none	complete	Nuc (1)	623
<i>Dunaliella salina</i>	minimal	partial	Nuc (3)	499
<i>Volvox carteri</i>	draft	complete	Nuc (8)	257
Porphyra sp.	none	complete	Nuc (1)	221
<i>Phaeodactylum tricornutum</i>	minimal	complete	Nuc (3)	192
<i>Porphyridium sp.</i>	minimal	minimal	Ct (1)	184
<i>Thalassiosira pseudonana</i>	complete	complete	Nuc (1)	158
<i>Cyanidium caldarium</i>	none	complete		155
<i>Cyanophora sp.</i>	none	complete		129
<i>Haematococcus pluvialis</i>	some	minimal	Nuc (1)	119
<i>Tetraselmis chunii</i>	minimal	minimal		93
<i>Isochrysis galbana</i>	none	none		90
<i>Cyanidioschyzon merolae</i>	complete	complete	Nuc (1)	71
<i>Emiliana huxleyi</i>	complete	complete		70
<i>Chaetoceros gracilis</i>	minimal	minimal		67
<i>Nannochloropsis sp.</i>	minimal	minimal		48
Macrocystis pyrifera	none	none		56
<i>Rhodomonas sp.</i>	none	complete		40
<i>Botryococcus braunii</i>	minimal	minimal		36
<i>Ostreococcus tauri</i>	draft	complete		33
<i>Nannochloris oculata</i>	minimal	minimal		29
<i>Odontella sinensis</i>	none	complete		18
<i>Leptosira sp.</i>	none	complete		4
<i>Neochloris oleoabundans</i>	none	none		3
<i>Arabidopsis</i>	complete	complete	880	23564
<i>Saccharomyces</i>	complete		3077	84960
<i>E. coli</i>	complete		7407	220222

Developing the tool for algal engineering

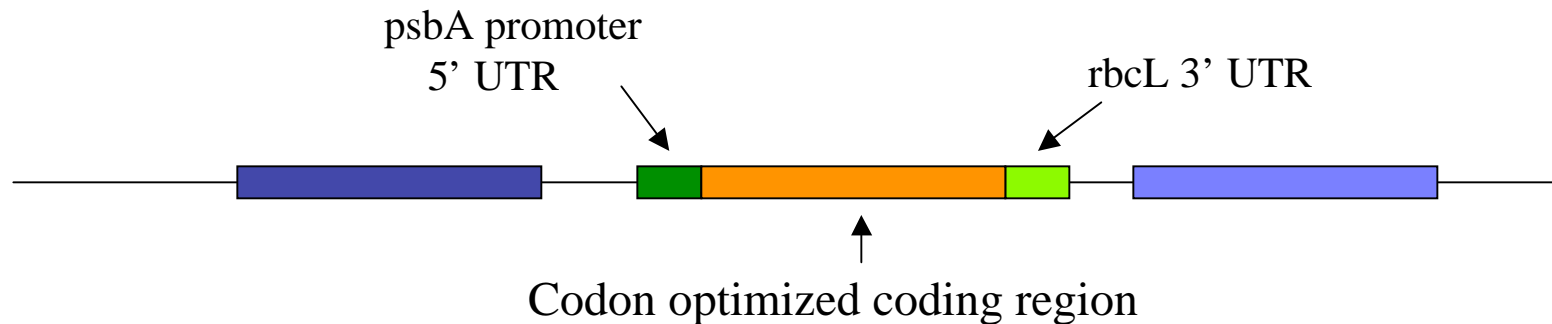
Biofuels are all made in the chloroplast from photosynthesis
- most of enzymes responsible are nuclear encoded



Genetic transformation of algae is relatively easy
Although you need selectable markers for each species

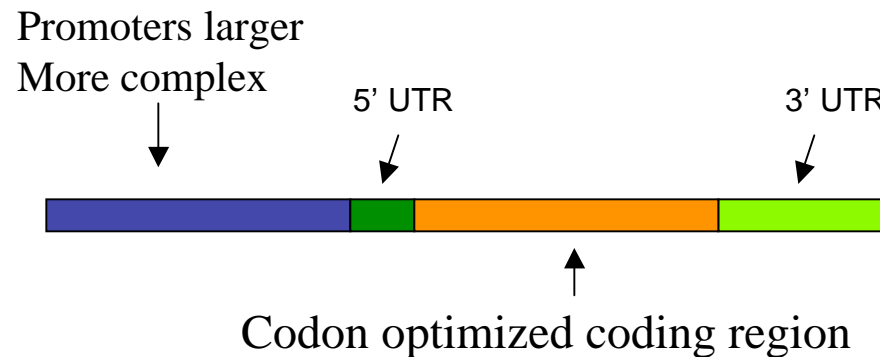


Chloroplast transformation proceeds by homologous recombination



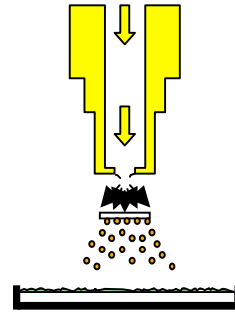
- need promoter and UTRs flanking region of homology
- recombinant proteins can accumulate to very high levels
- chloroplast can express complex proteins
- less sophisticated gene regulation in plastids

Nuclear transformation proceeds by random integration

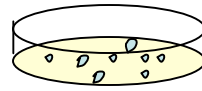


- Need more transformation events to get good expression
- Gene expression more complex, regulation potential greater
- Can target proteins to plastids, cytoplasm or export
- Gene silencing is presently a limiting factor

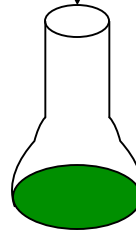
1 Initial Transformation
Day 1



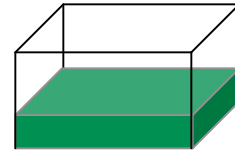
2 Selection of Primary Transformants
Day 10



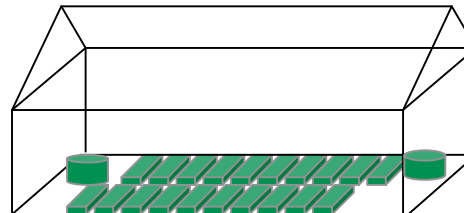
3 Homoplasmic Lines with High Expression Levels
Day 14



4 Scale-up to Multi-liter Volumes
4 weeks

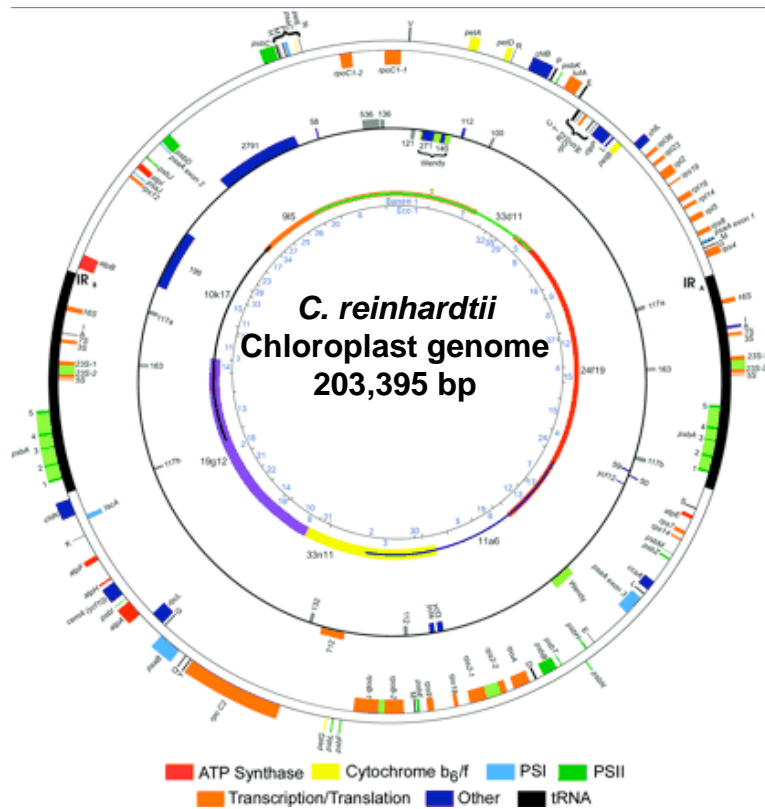


5 Scale-up to 64,000 Liters
6 weeks



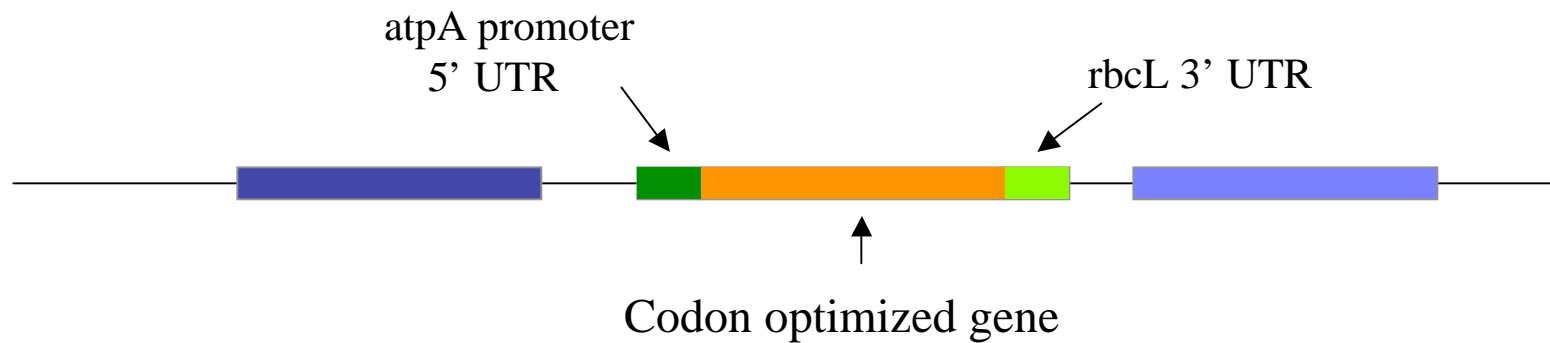
6 Grow out to 1.2 Million L/acre
12 weeks

Chloroplast Genome

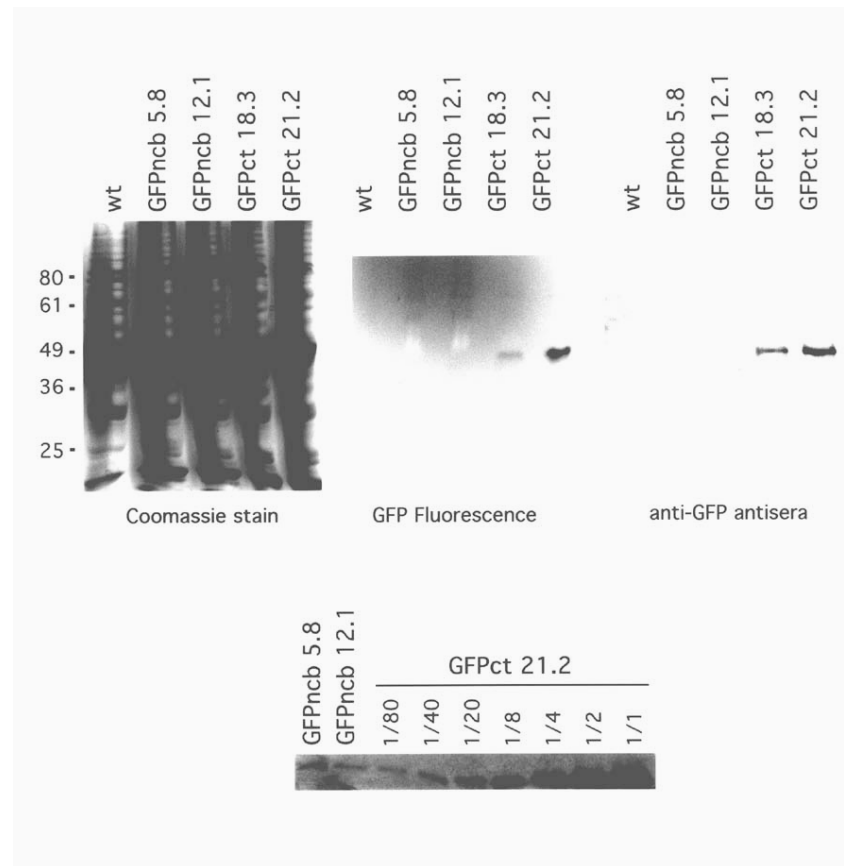


- Complete set of genetic material
- Simple prokaryotic promoters
- Stable uncapped non-polyA mRNAs
- Bacteria-like ribosomes
- Easily transformed

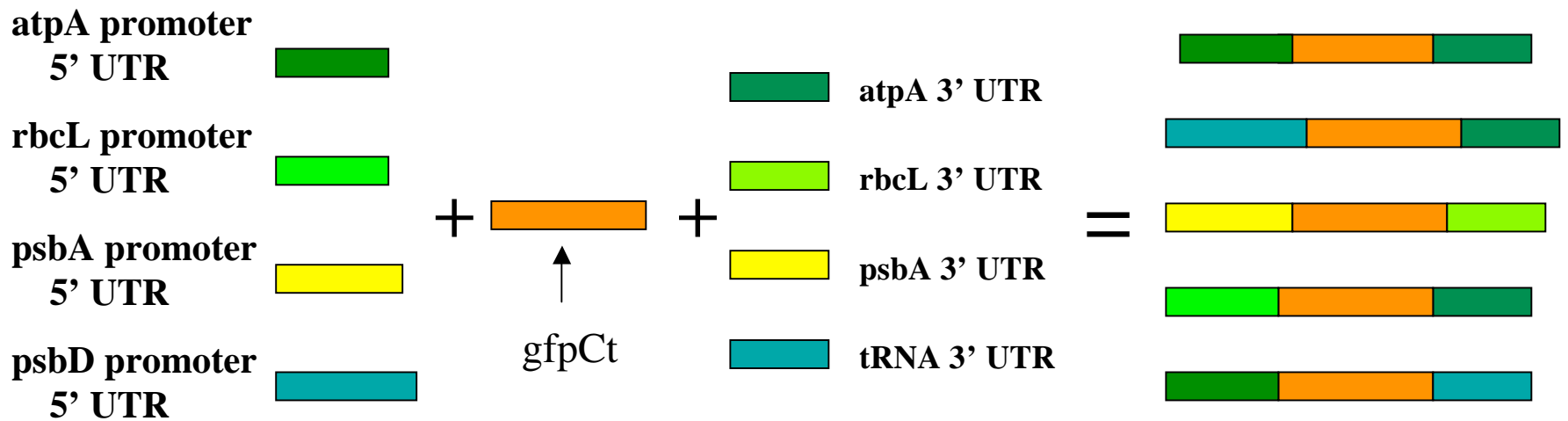
Expression of recombinant protein in *C. reinhardtii* chloroplasts



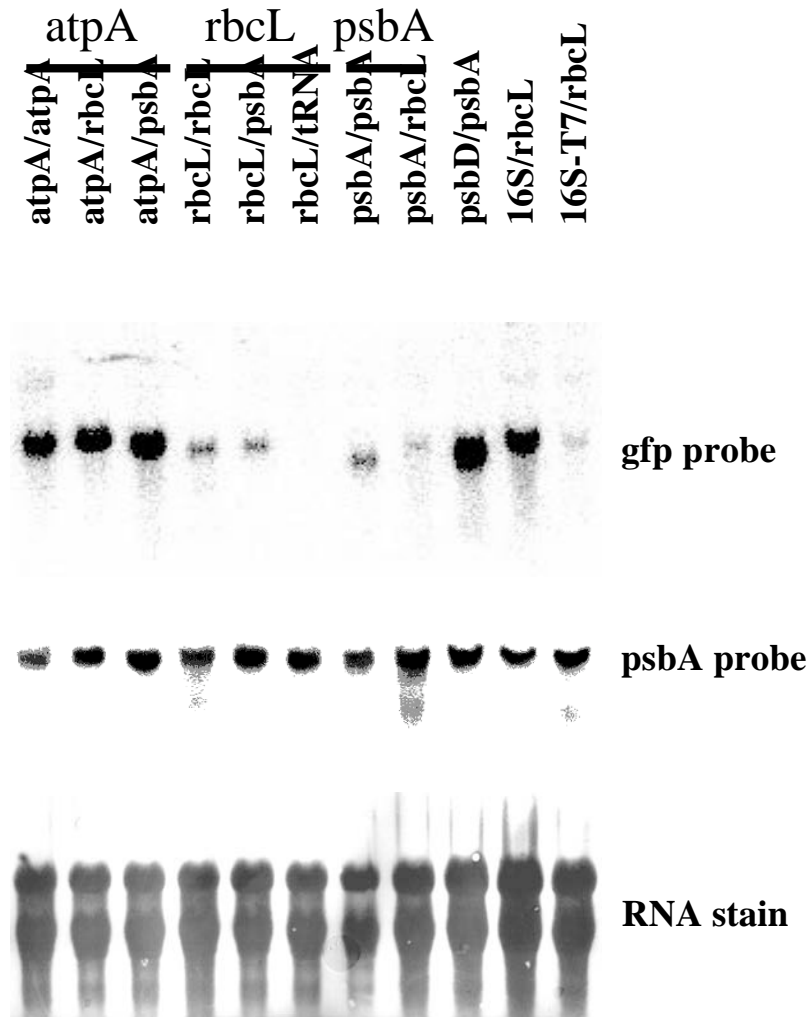
Analysis of codon optimized gfp expression in transgenic chloroplast



Promoter and UTR combinations for increased chloroplast expression

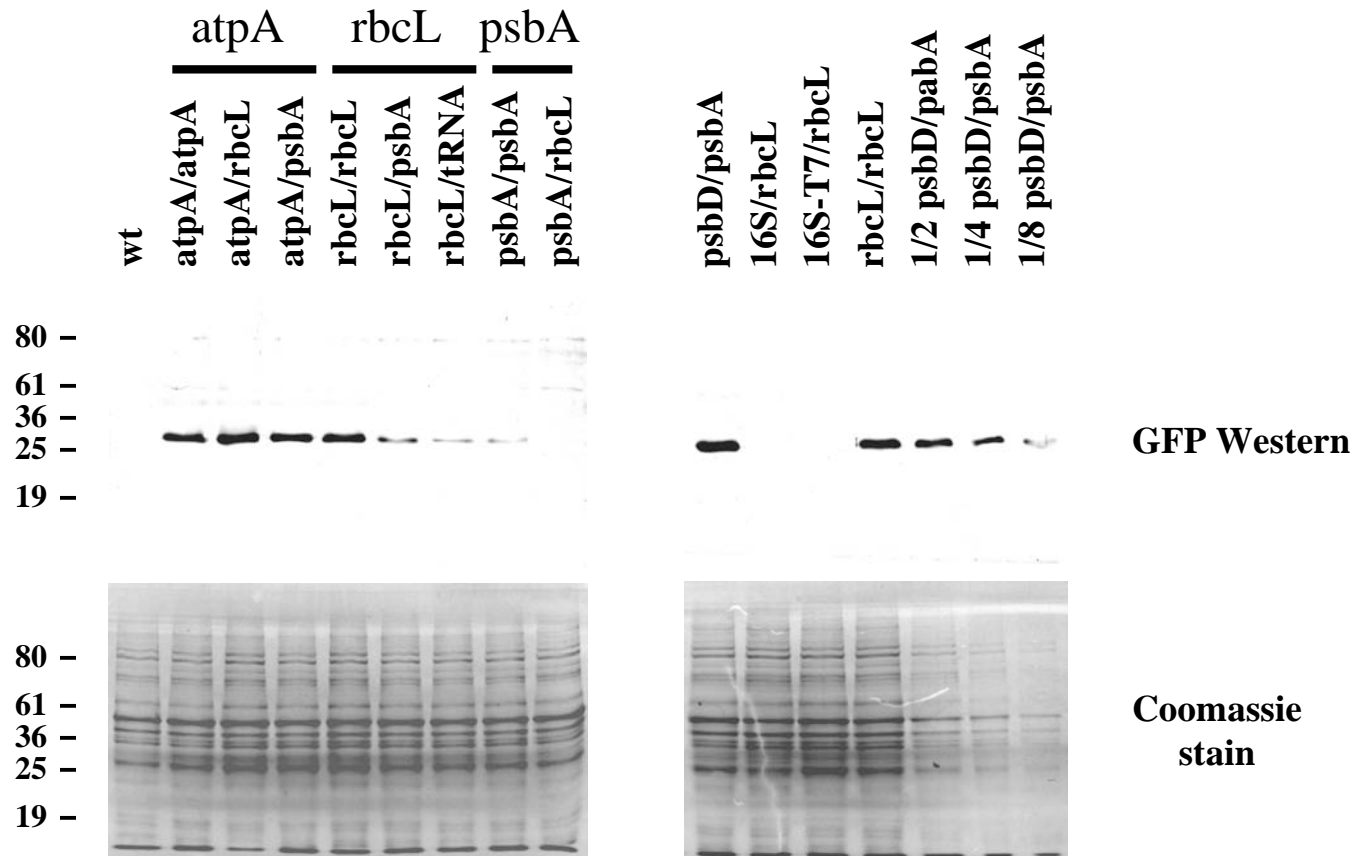


Accumulation of chimeric gfp mRNAs in *C. reinhardtii* chloroplast

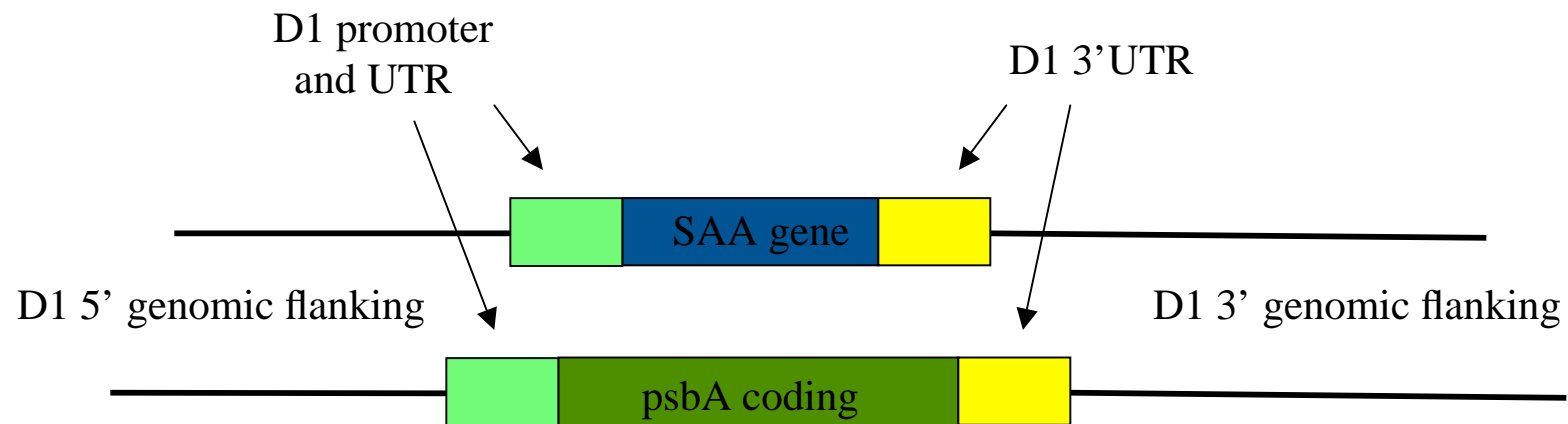


Northern blot

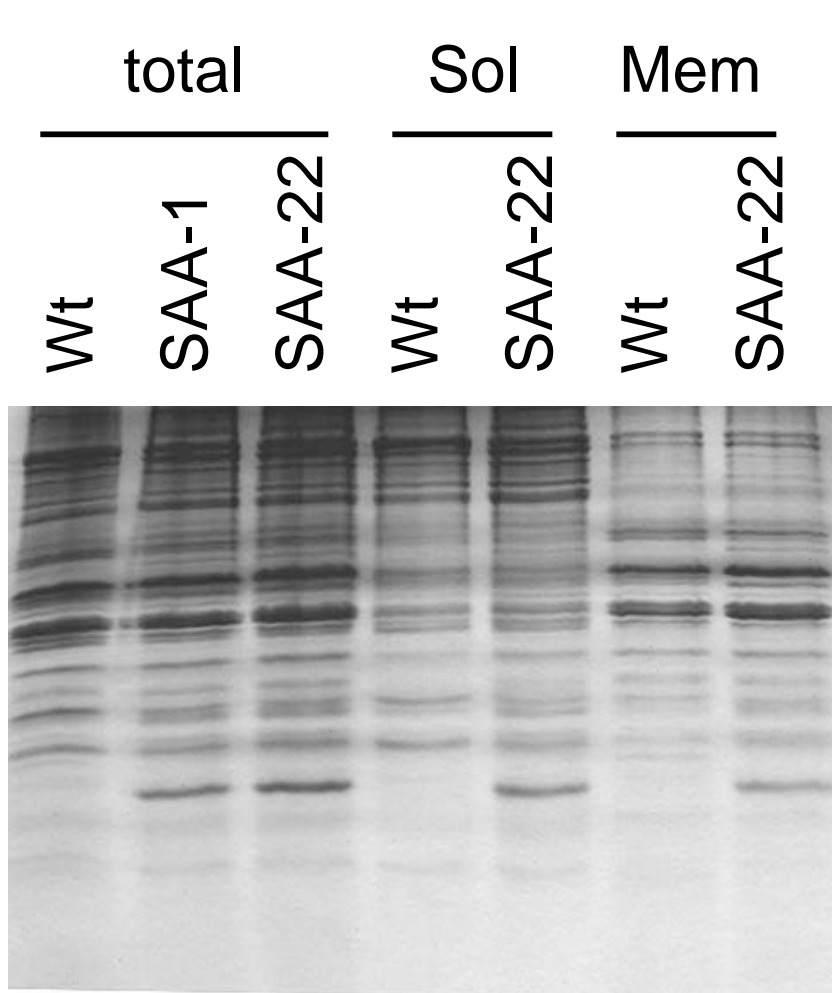
GFP accumulation in transgenic lines



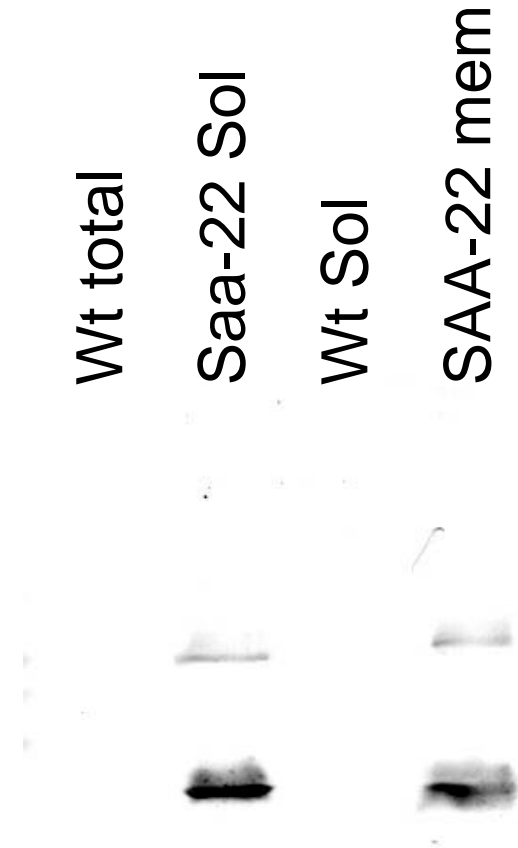
Gene replacement vector for improved Recombinant protein expression



Expression of SAA from *psbA* KO vector

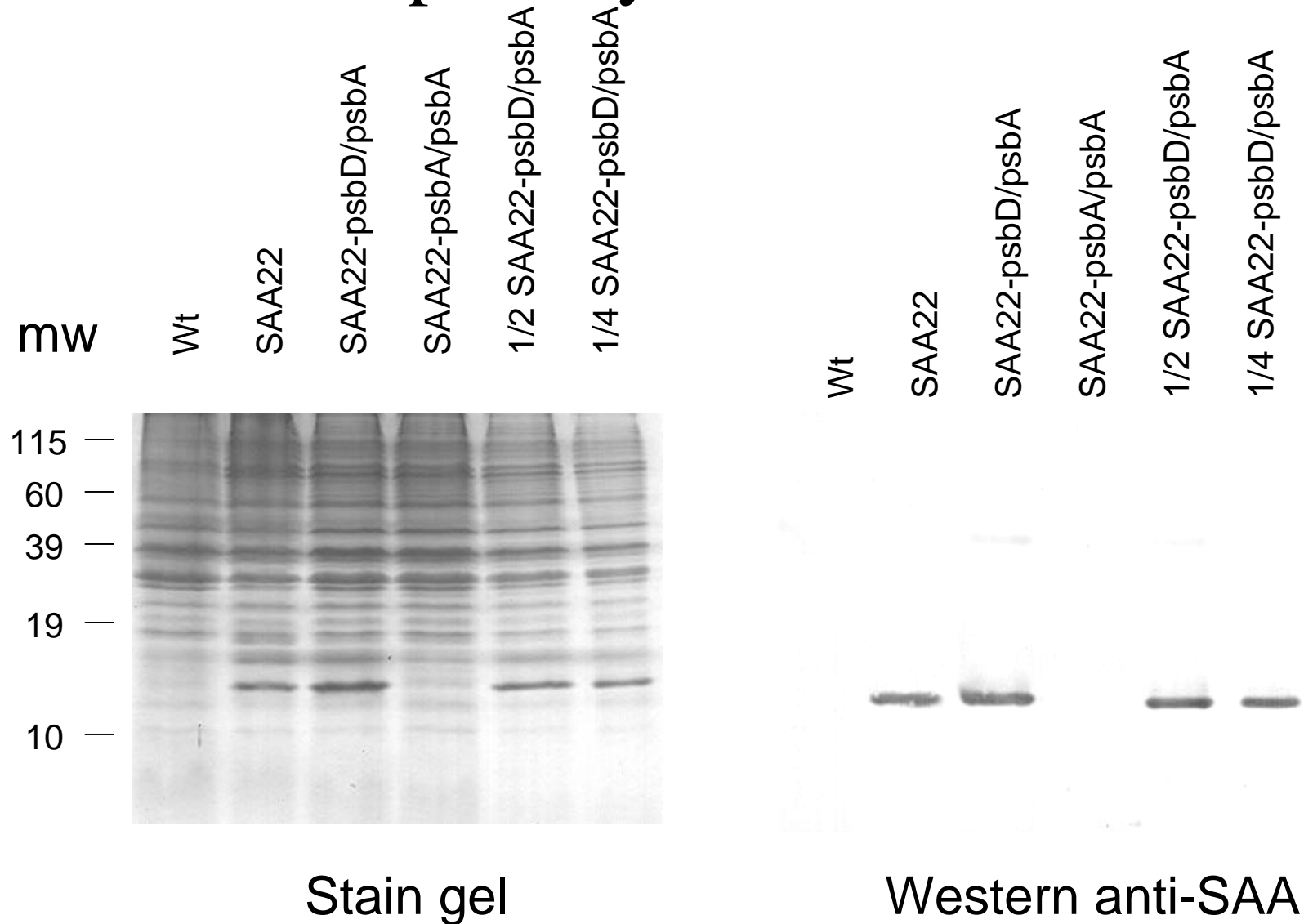


Coomassie stain gel

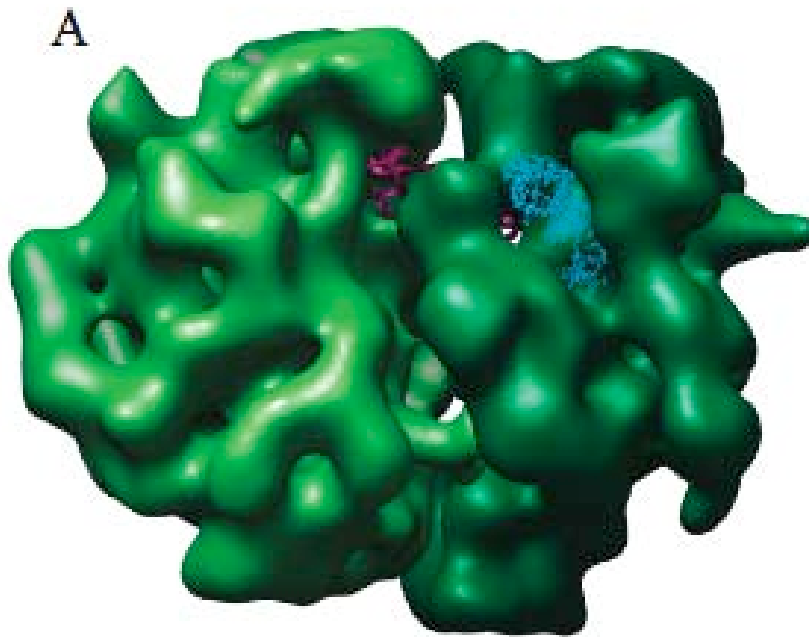


Western anti-SAA3

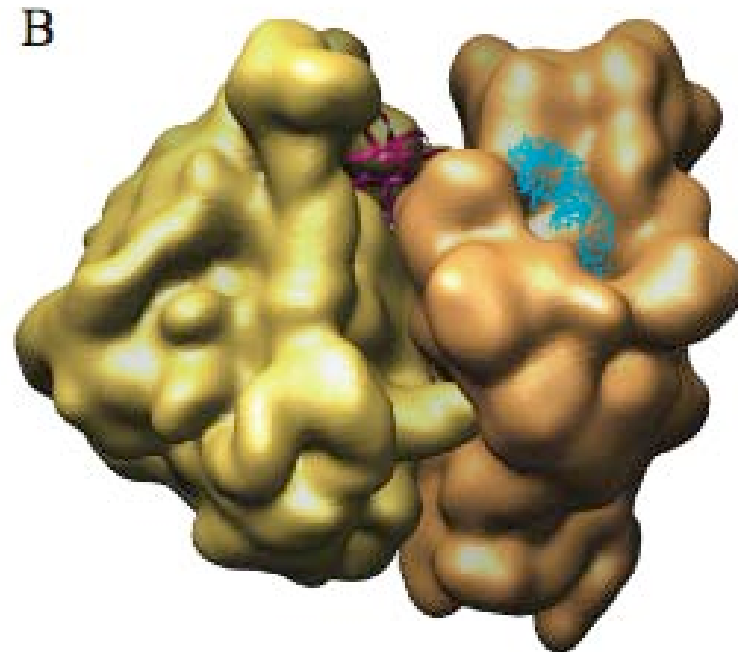
Robust expression of recombinant proteins in a photosynthetic strain



C. reinhardtii and *E. coli* mRNA binding site



Chloroplast



Bacteria

Where do we go from here?



- We need a national center for algal research
- Develop the knowledge base of algal
- Develop the molecular tools to make algae a biotechnology platform
- Develop strains of algae for economic biofuel production
- Develop industrial practices for growth harvest and recovery of biofuel