

All-in-one kit solutions for simple, rapid, consistent algae engineering

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Workshop Overview

- **Synthetic Biology**
 - Definition
 - Integrated technologies
- **Host Systems**
 - Microalgae opportunities and challenges
- **Development of microalgae engineering kits**
 - Host strains
 - Vector systems
 - Media
 - Algae engineering examples

Synthetic Biology

- Engineering life for useful purposes
- A rapidly growing field of research: a new approach to life sciences
- Multi-disciplinary: Engineering, biology & informatics converge
- Cutting edge research and development tools
- Enable broad industrial applications

Standardized Parts
Engineered Hosts
Synthesis & Assembly Tools
Computational Design Software
Analytical Tools

Healthcare



Energy



Chemicals



Agriculture



Bio-Remediation



Leading the Way in Synthetic Biology

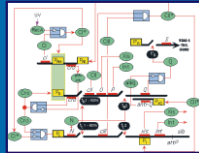
Industry Leading Portfolio



DNA Parts
& Devices



Specialty Media



BioDesign
Software



Optimized Hosts

Generating
synthetic
solutions to
improve
the human
condition

Energy



Healthcare



Chemicals



Building a complete synthetic biology toolkit

NGS Analysis Engine

Modified PGM™ Sequencer



LifeScope Genomic
Analysis Software

Galaxy

De Novo
Assemblers

- Velvet
- Abyss
- SOAP deNovo
- Trans-ABYSS

Tablet – assembly
editing

Classification -
NCBI/EBI
The Seed
KEGG
Reactome

Visualization -
Vector NTI Advance
IGV (Broad
Institute)

SOLiD™ NGS Platform

Primary Analysis

Imaging &
Base Calling

Secondary Analysis

De Novo & Reference
Mapping Algorithms

Tertiary Analysis

Gene Calling,
Classification,
Pathway Mapping

Jatropha

- Collaboration with SG Biofuels
- > 55M single and mate pair reads on Solid 4 platform
- Transcriptome analysis
- Genome: ~30k Contigs, mapped to known homologs
- N50 ~ 3500 bp, Max Contig ~14k

Sugar Cane

- Collaboration with Dr. Glaucia Souza, Instituto di Química, Universidade de São Paulo
- Full-Length transcriptome analysis
- 2 cultivars, ~440M Single reads on Solid 4
- ~20k Clusters
- N50 ~900 bp, Max Contig ~1500 bp

Synechococcus elongatus

- 2 single read runs on PGM, one afternoon
- *De novo* genome analysis: 87% complete
- 34x & 57x coverage of genome
- 4 hours per run
- 16 contigs, N50 ~100kb, Max Contig 450 kb

Chlamydomonas reinhardtii

- 1.5 kbp mate pair library on Solid 4 platform
- *De novo* genome analysis partially completed
- 530M total reads, 26.5 Bbp bases of sequence, 50 bp/read
- ~265 x coverage



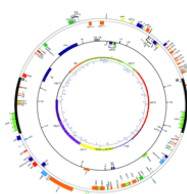
Genomic DNA



RNA



Hybrid
Assembly



Genome



Transcriptome



Metabolic
Models

See Poster P0123: *De novo* assembly of a novel filamentous blue-green algal genome for *Leptolyngbya* sp. strain BL0902 enabled by a novel, extra-long read sequencing protocol

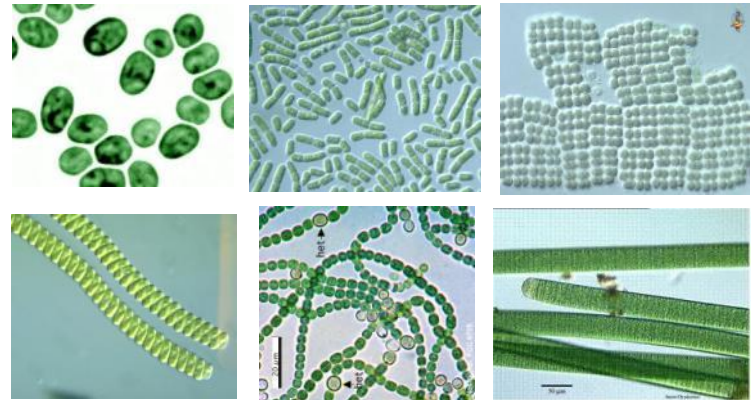
What are microalgae?

- Algae: Very diverse group of organisms dating back ~3 billion years and spanning several kingdoms of life of which only green algae are in the plant kingdom
- Utilize light and CO₂ to make reduced C compounds (starch and lipids)
- 200,000-800,000 species estimated to exist
- Higher photosynthetic efficiency than plants and little recalcitrant biomass
- Cyanobacteria (blue green algae) are bacteria – origin of the algal chloroplast (3.5 billion years old)

Eukaryotic

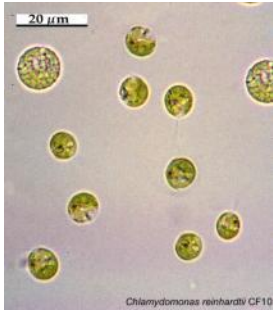


Prokaryotic



Addressing algae pain points with innovative solutions

| | Current source | Pain points | Solution |
|-----------|------------------------------------|--|---|
| Cells | Culture collections, academic labs | Genetic drift, contamination | Frozen format, single use |
| Media | make it yourself | Batch to batch variability, long protocols | Validated and QC'd media formulated by GIBCO® |
| Vectors | academic labs | Elements not optimized, poor expression | Optimized elements, TOPO® cloning |
| Protocols | Web, publications | Suboptimal protocols, non-standardized | Fast workflows, robust and reliable |



Tools for model algal hosts

Chlamydomonas reinhardtii 137c

- Model eukaryotic algae
- 121 Mb genome (64% GC content)
- Facultative heterotroph
- Research focus: flagellar motility, photosynthesis, protein expression, lipid metabolism



Synechococcus elongatus PCC7942

- Model cyanobacterium
- 2.7 Mb genome (55% GC content)
- Facultative phototroph
- Research focus: circadian rhythms, nutrient regulation, environmental response, lipid metabolism

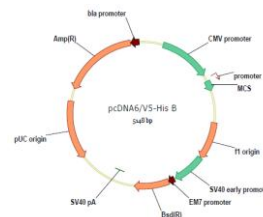
in the kit:



frozen cells



media



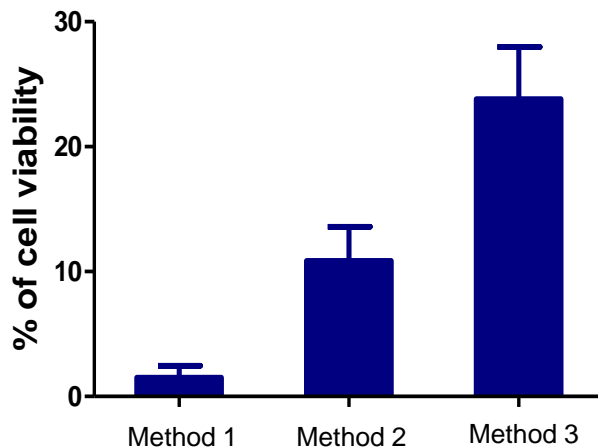
vectors



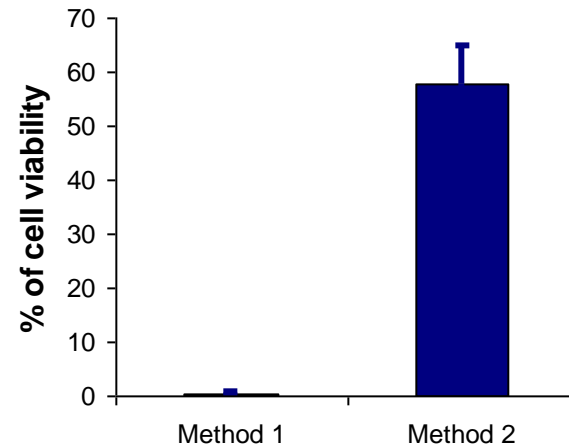
protocols

Development of cryopreservation methods to enable frozen cell format, storage & shipping

Chlamydomonas reinhardtii
cryopreservation viability

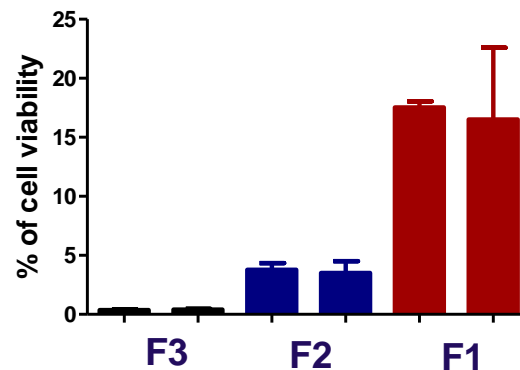
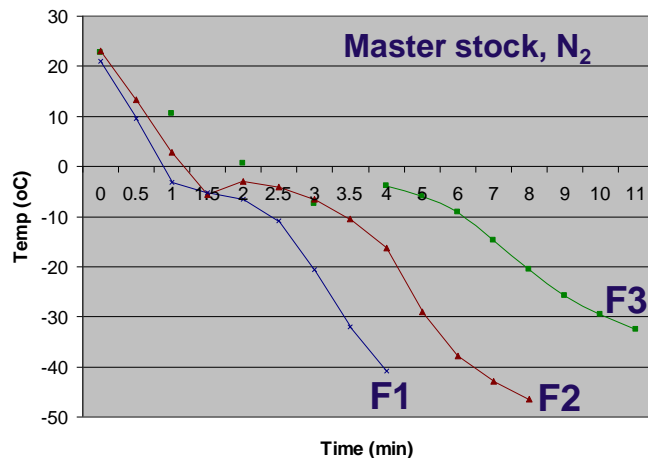


Synechococcus elongatus
cryopreservation viability



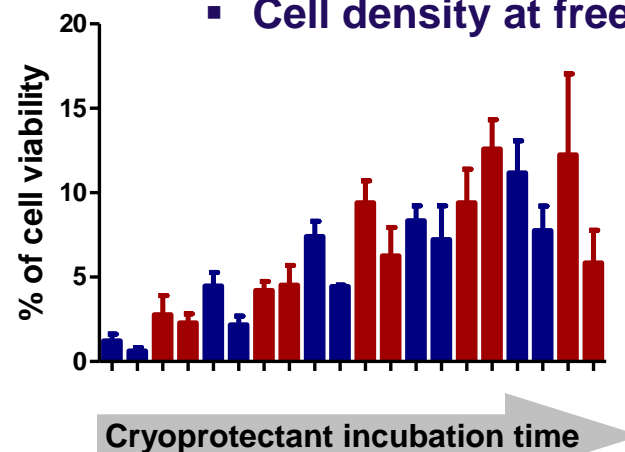
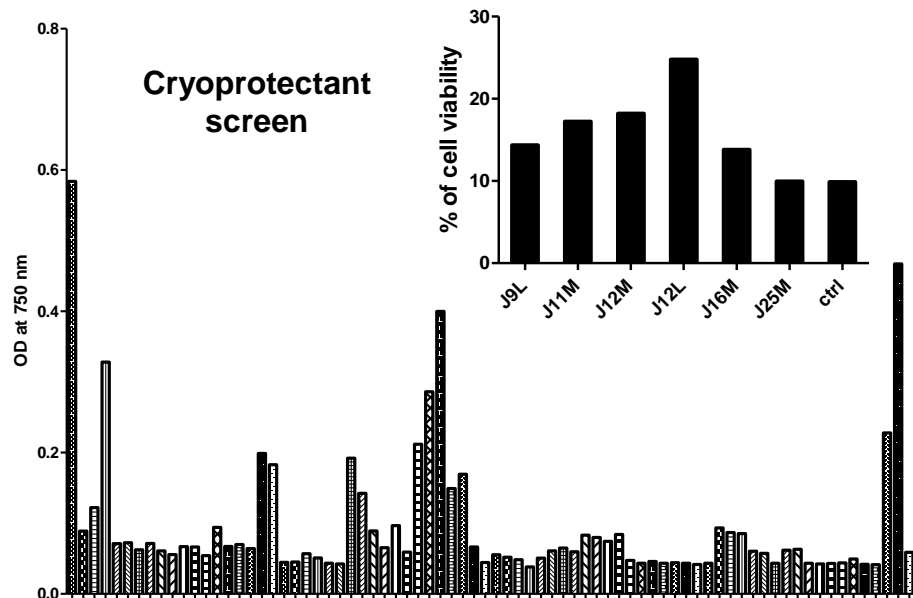
- Single use vial One Shot format frozen cells, -80 °C storage for both hosts
- Guaranteed to be axenic and to resuscitate
- Minimal batch to batch variation

Development of *Chlamydomonas* cryopreservation methods

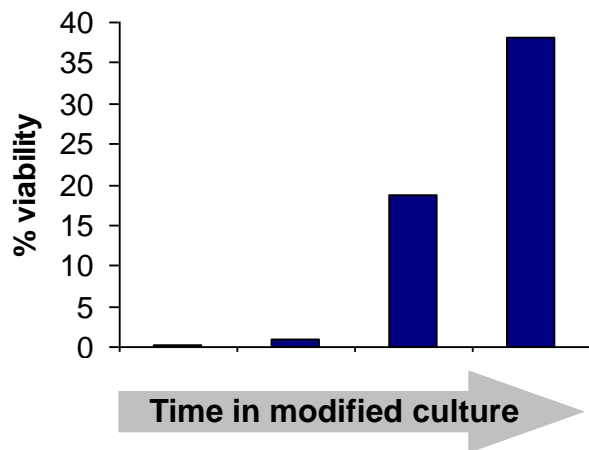


Factors affecting viability:

- Freeze rate
- Cryoprotectant formulation
- Cryoprotectant incubation time
- Pre-freeze culture conditions
- Cell density at freeze

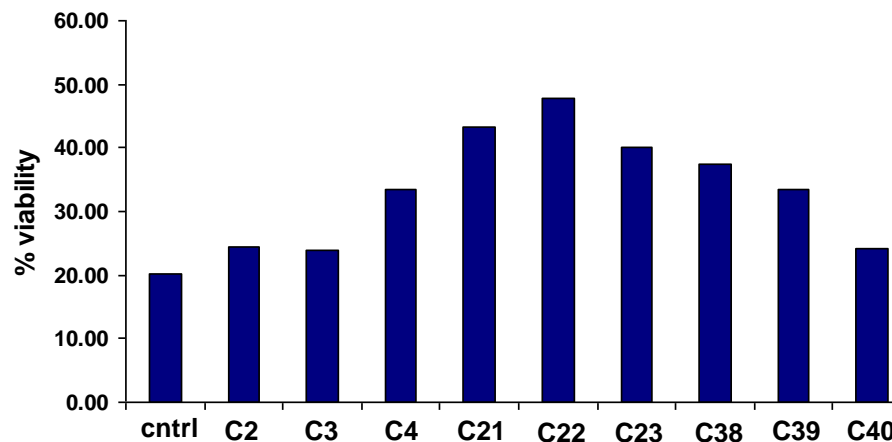
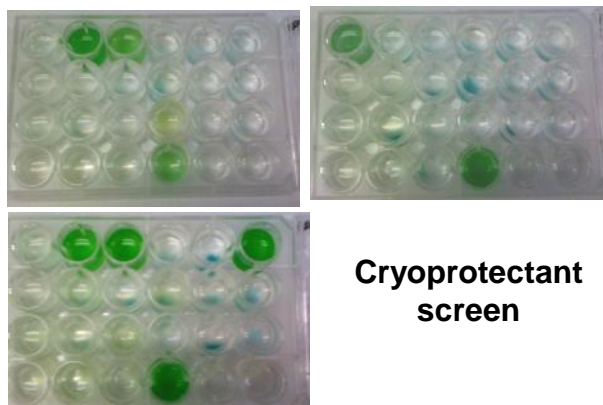


Development of *Synechococcus* cryopreservation methods



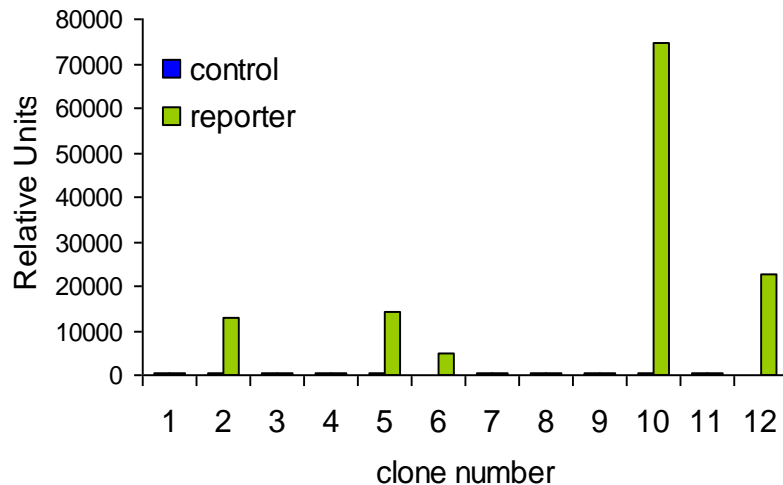
Factors affecting viability:

- Cryoprotectant formulation
- Pre-freeze culture conditions
- Freeze rate

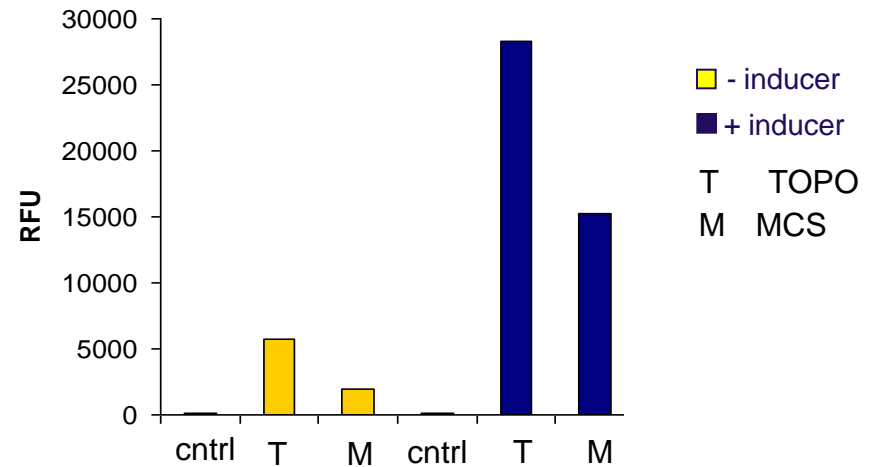


Validated vector elements for robust selection and expression

Expression of luciferase reporter in *Chlamydomonas reinhardtii*

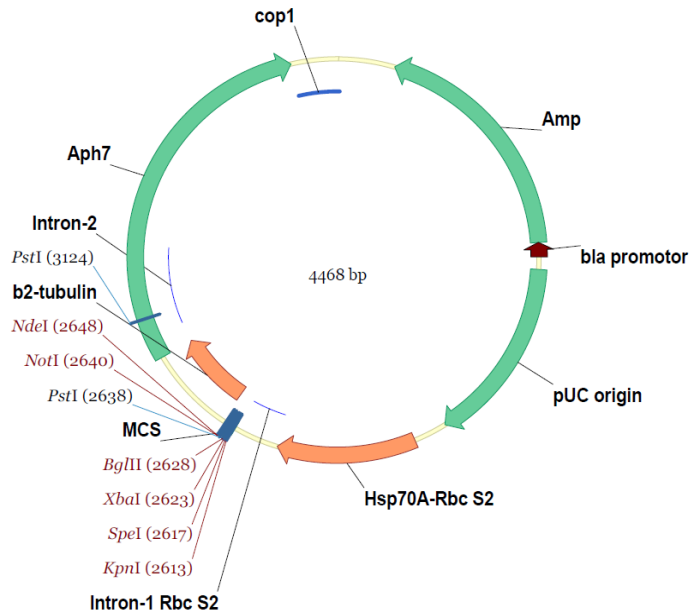


Expression of GUS reporter in *Synechococcus elongatus*



- Traditional restriction enzyme cloning or TOPO® cloning
- *Chlamydomonas* – random integration into the nuclear genome
- *Synechococcus* – targeted integration into the genome
- Robust selection markers and validated promoters

Chlamydomonas nuclear expression vectors

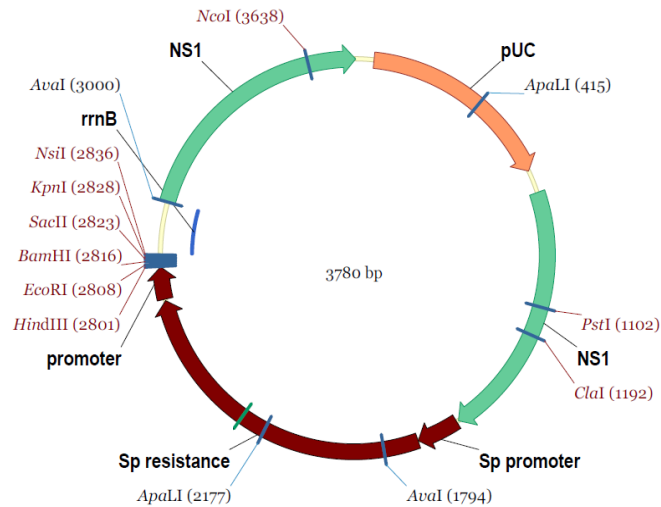


- Vectors construction:
 - Geneart gene synthesis
 - Seamless cloning and assembly technologies
- pUC and Amp for *E. coli* propagation
- Hsp70A-RbcS2 constitutive promoter (+1st Rubisco intron)
- Multiple cloning site
- Hygromycin resistance marker (+2nd Rubisco intron), driven by beta tubulin promoter
- Cop1 3'UTR

| | | | | | | | | | | | |
|---|------------|------------|------------|------------|------------|------------|------------|-------------|------------|------------|------------|
| 2401 | TATGTTCTTT | ACTTTTTTAC | AAGAGAAGTC | ACTCAACATC | TTAAATG | GC | CAGGTGAGTC | GACGAGCAAG | CCCGGCGGAT | CAGGCAGCGT | GCTTGCAGAT |
| | ATACAAGAAA | TGAAAAAATG | TTCCTTCAG | TGAGTTGTAG | AAATTTAC | CG | GTCCACTCAG | CTGCTCGTTC | GGGCGGCCIA | GTCCGTGCA | CGAACGTCTA |
| 2501 | TTGACTTGCA | ACGCCCGCAT | TGTGTCGACG | AAGGCTTTTG | GCTCCTCTGT | CGCTGTCTCA | AGCAGCATCT | AACCCCTGCGT | CGCCGTTTCC | ATTTCAGGA | |
| | AACTGAACGT | TGCGGGCGTA | ACACAGCTGC | TTCCGAAAAC | CGAGGAGACA | GCGACAGAGT | TCGTCTAGA | TTGGGACGCA | GCGGCAAAGG | TAAACGTCCT | |
| <div style="text-align: center;"> XbaI BglII NotI NdeI </div> | | | | | | | | | | | |
| 2601 | GATTCGAGGT | ACCATACTAG | TTCTAGAGAT | CTCTGCAGCG | GCCGCCATAT | GATTCGAATG | TCTTTCTTGC | GCTATGACAC | TTCCAGCAAA | AGGTAGGGCG | |
| | CTAAGCTCCA | TGGTATGATC | AAGATCTCTA | GAGACGTCGC | CGCGGTATA | CTAAGCTTAC | AGAAAGAACG | CGATACTGIG | AAGGTCGTTT | TCCATCCCGC | |

Synechococcus expression vectors

- Vectors construction:
 - Geneart gene synthesis
 - Seamless cloning & assembly technologies
- pUC and Spec for *E. coli* propagation
- Metal inducible promoter
- Spec – Syn selection
- NS1 or targeted recombination
- multiple cloning site / TOPO®
- Strong RBS in TOPO® vector
- rrnB terminator



insert

EcoRI
CGAAT TCCGAAGGAG CCCTT
GCTTA AGGCTTCCTC GGGAAAGTGG

A AGGGTGTACC AT
T TCCCACATGG TA

10 ug/mL
chloramphenicol



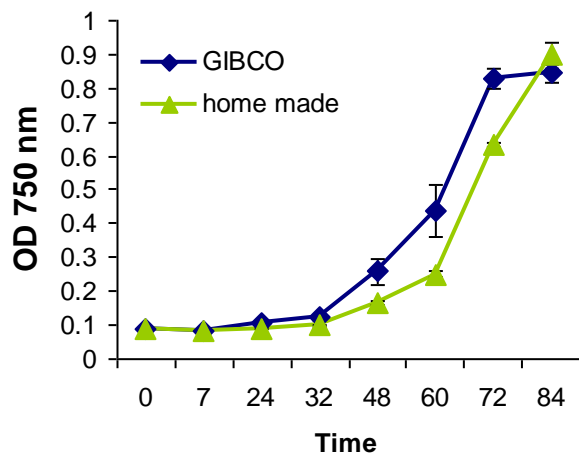
Empty vector

NS1-mcs-CAT

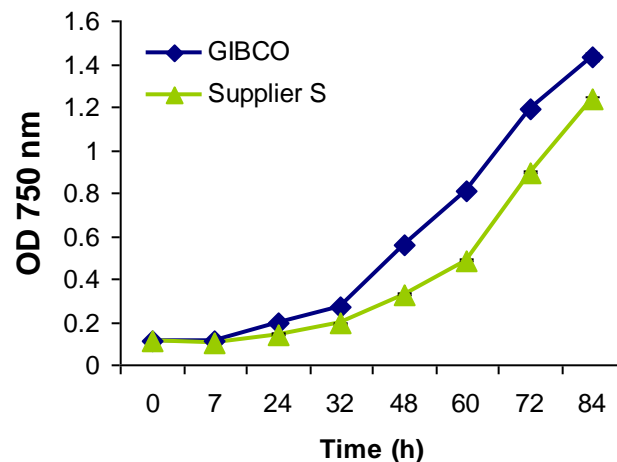
NS1-TOPO® dir-CAT

Validated GIBCO® media for reproducible results

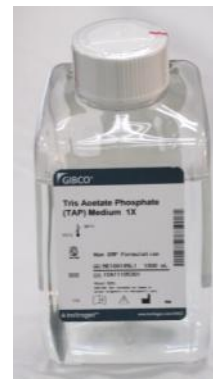
Chlamydomonas reinhardtii
TAP media performance



Synechococcus elongatus
BG11 media performance

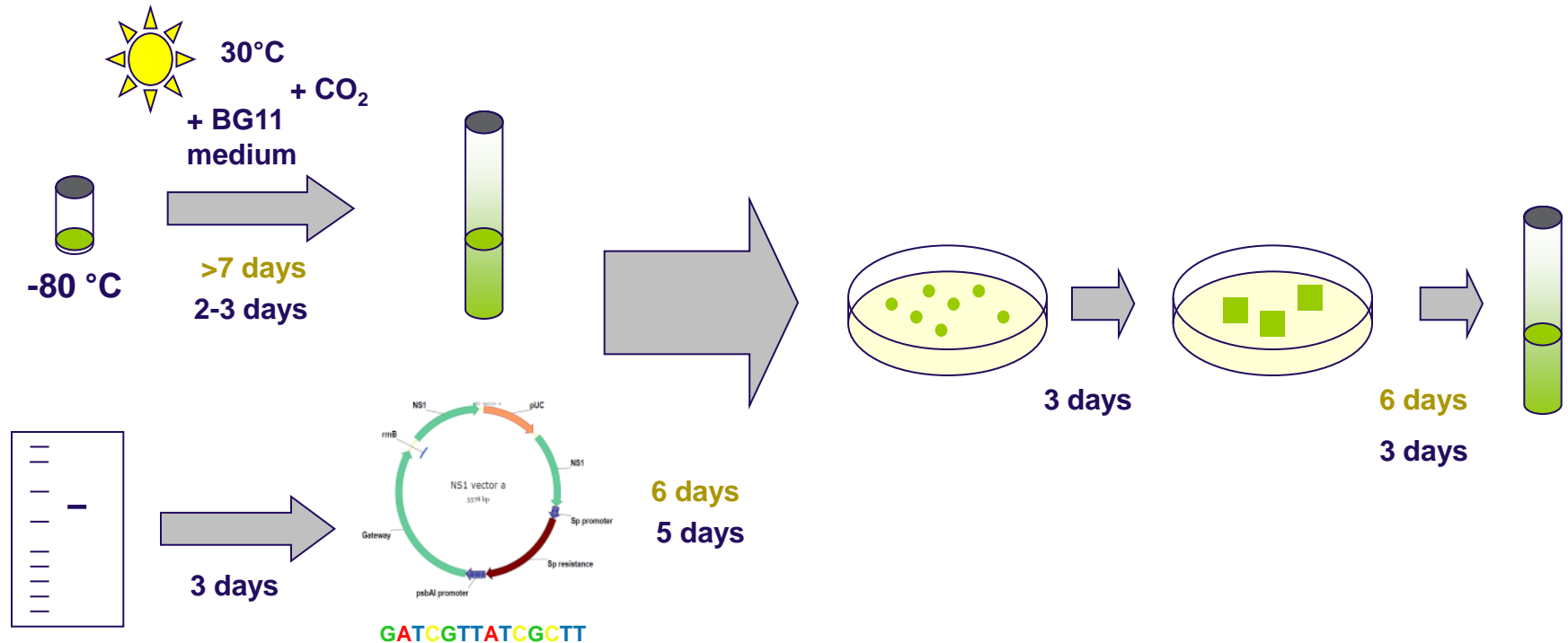


- Convenient 1x formulation of TAP and BG-11
- Minimal batch to batch variability
- Rigorously tested for stability and functionality
- Formulated by an industry media leader - GIBCO®



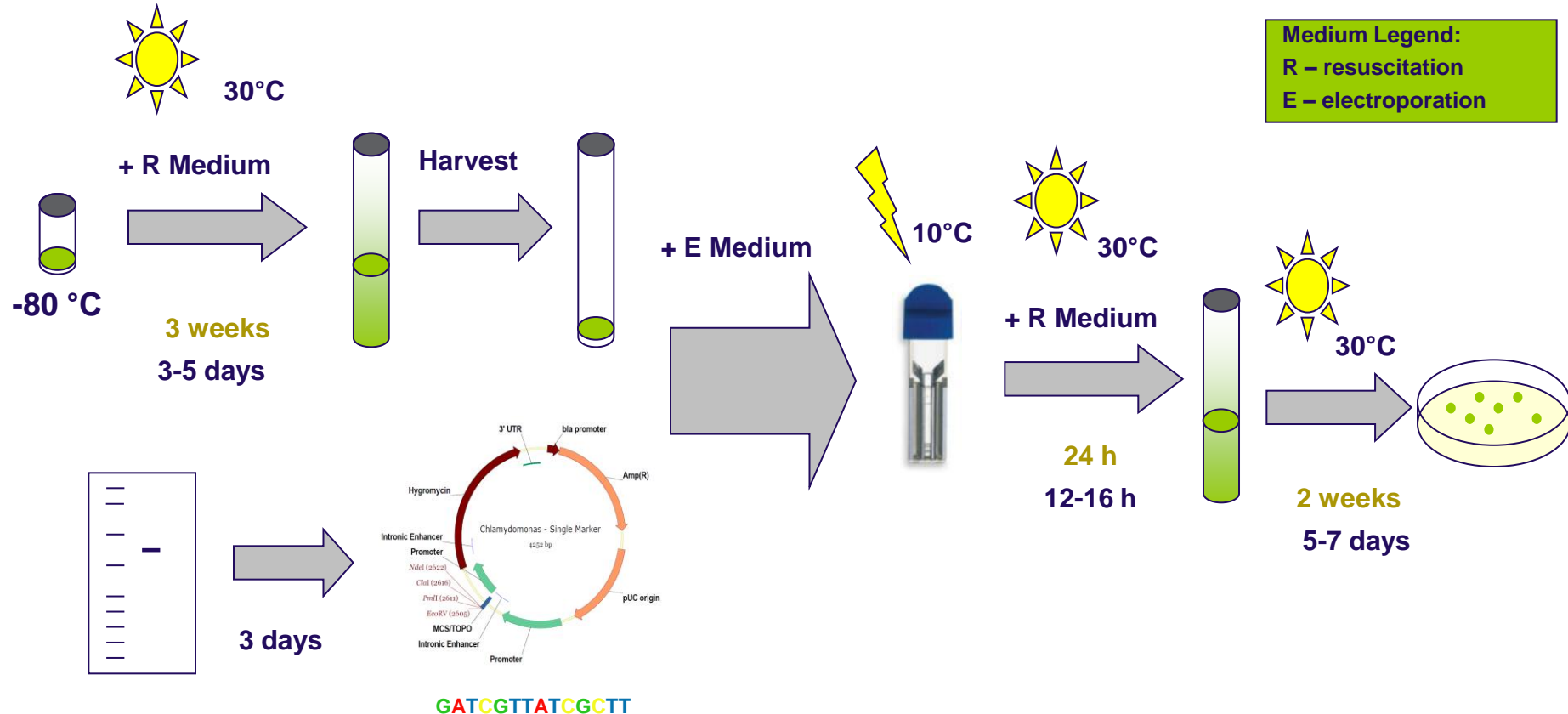
Algae Engineering Workflow Improvements:

Synechococcus elongatus

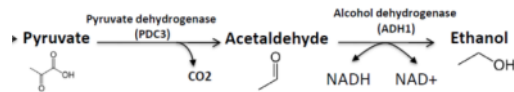
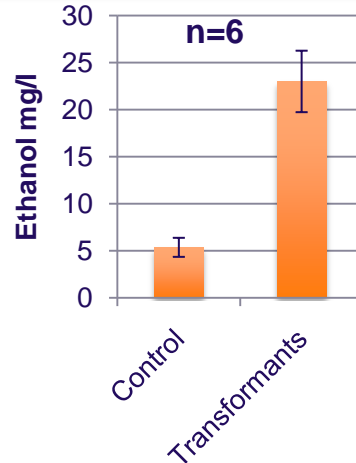
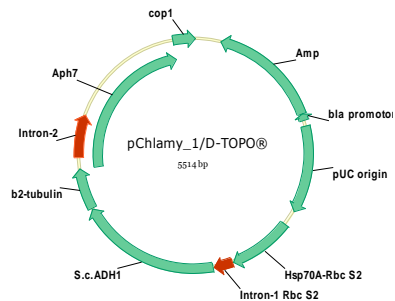


Algae Engineering Workflow Improvements:

Chlamydomonas reinhardtii

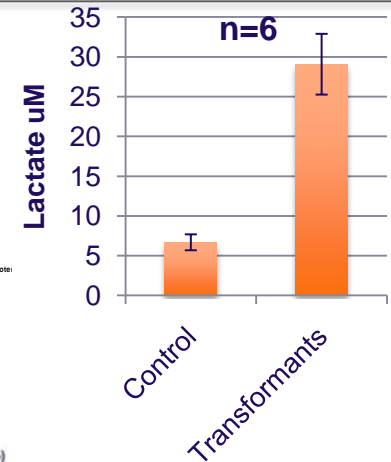
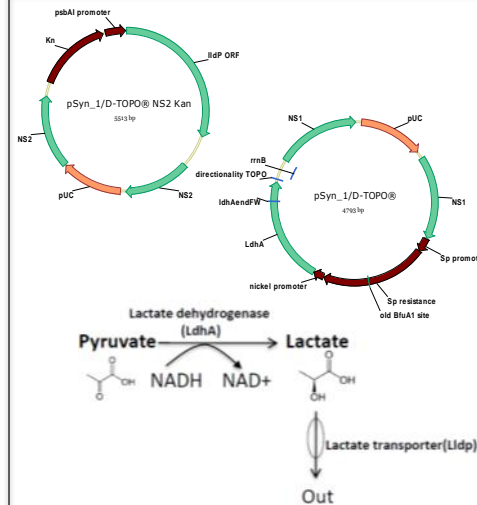


Algae Engineering Examples



EtOH production in *C. reinhardtii*

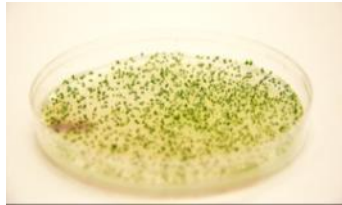
- The *adh1* was cloned from *Saccharomyces cerevisiae* into the pChlamy_1/D-TOPO® vector
- Transformants were selected on Gibco® TAP medium plates containing hygromycin
- Colonies were picked into TAP medium containing hygromycin, grown for 5 days
- Ethanol production was measured in medium by enzymatic, colormetric assay



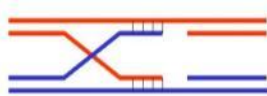
Lactate production in *S. elongatus*

- *S. elongatus* was transformed with pSyn_1/D-TOPO® vector containing *LdhA* and re-transformed with pSyn_1/D-TOPO® vector containing the *Lldp* (lactate permease) genes from *E. coli*
- Transformants were selected BG-11 plates containing spectinomycin
- Colonies were picked into BG-11 spec medium, grown for 6 days
- Lactate production was measured by an enzymatic, colormetric assay

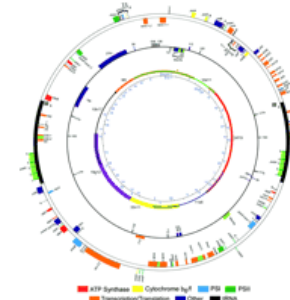
Addressing future interests through collaborations and partnerships



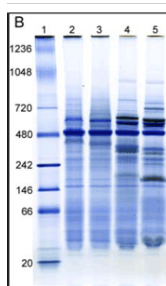
Enhanced DNA delivery



Homologous recombination



Chloroplast engineering



Regulated expression



Additional hosts

SD-CAB



Partnerships

Acknowledgements

Life Technologies

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Lisa Stillwell

Declan Donovan

UCSD

Steve Mayfield

Susan Golden

Jim Golden