Advances in the development of genetic tools for microalgae research and biotechnology

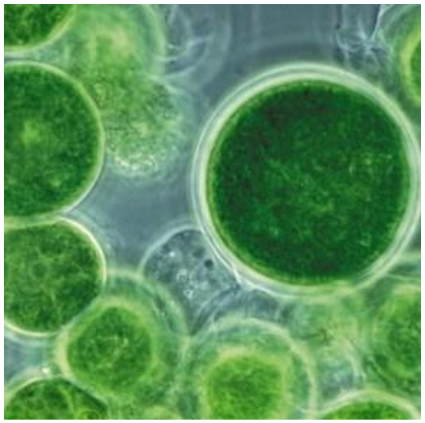
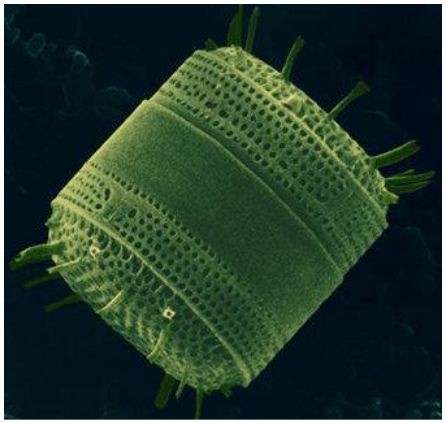
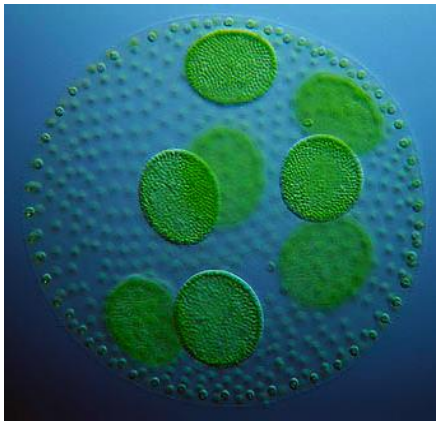
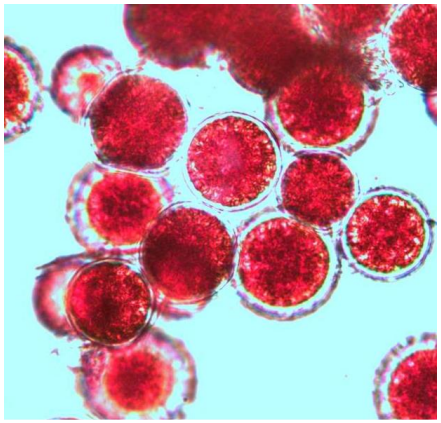
BIO Pacific Rim Summit
2013

Beth A. Rasala, Ph.D.
Prof. Stephen Mayfield's Lab/
Triton Algae Innovations

UC San Diego
Local Impact, National Influence, Global Reach

Cal-CAB
CALIFORNIA CENTER FOR ALGAE BIOTECHNOLOGY

Microalgae as a biomanufacturing platform

Biofuels
bio-diesel
bio-gasoline
jet fuel
bio-hydrogen
ethanol

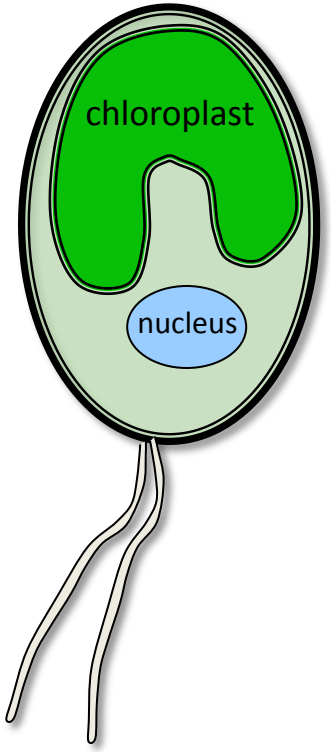
Nutraceuticals
carotenoids
omega-3 fatty acids
astaxanthin
other vitamins and supplements

Chemicals
cosmetics
industrial
lubricants
bio-plastics
and more...

Proteins
therapeutics
oral vaccines
antimicrobials
industrial enzymes
animal feed

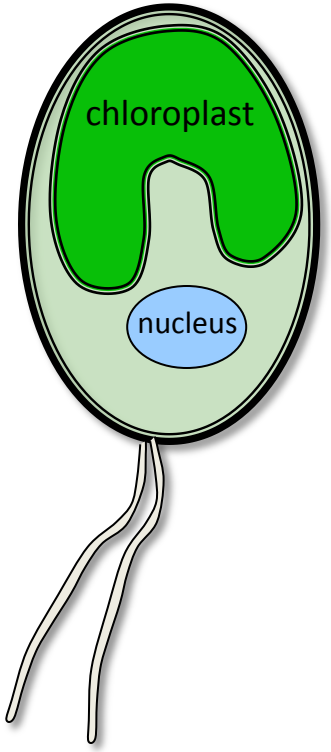
Genome engineering will likely enhance algal biotechnology.

Engineering *Chlamydomonas reinhardtii*



	Nucleus	**Chloroplast**
Transformation	Random integration	Homologous recombination
Stability of transgene	Susceptible to gene silencing	Highly stable once homoplasmic
Levels of heterologous protein accumulation	Low Levels susceptible to position effect	High Levels consistent across clones
Protein modification	Disulfide bond formation Glycosylation	Disulfide bond formation
Protein localization	Cytosol, organelle targeting, secretion	Protein retained in chloroplast

Chloroplast engineering – a success



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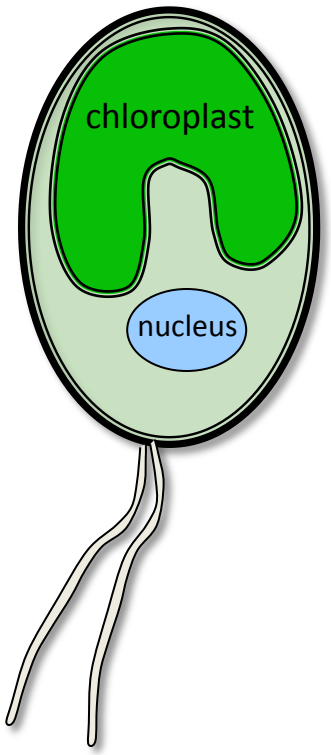
Nuclear genome engineering



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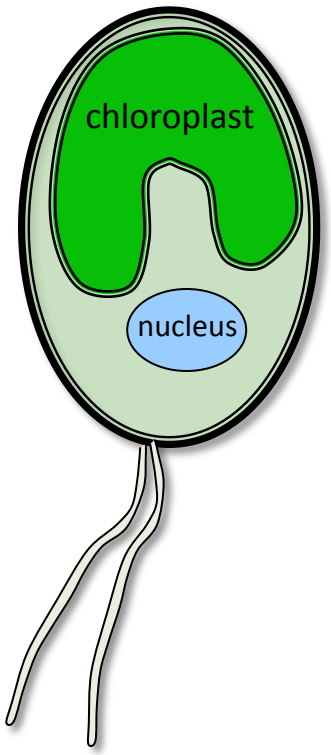

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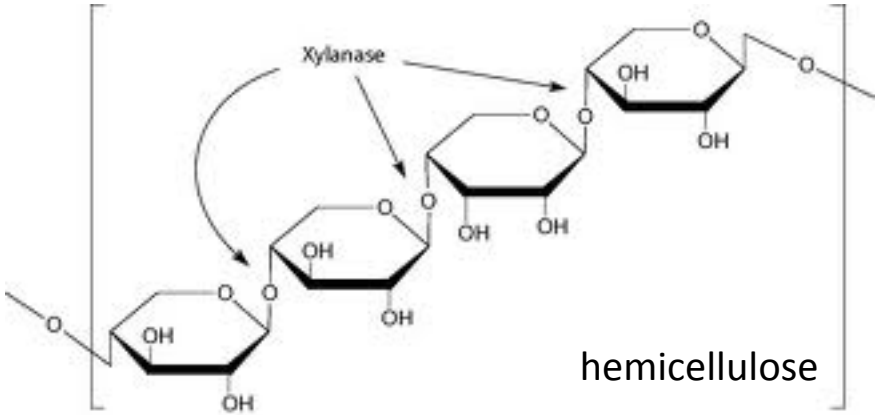
Improved methods for nuclear genome engineering in green microalgae

Case Study: Heterologous expression of Xylanase (*T. reesei*)



- **Endo-1,4-beta-xylanase (*Trichoderma reesei*)**
- hydrolysis of xylosidic linkages in hemicellulose polysaccharides
- industrial applications including paper manufacturing, **animal feed**, bread-making, juice and wine industries, and more
- potential large-scale use in **cellulosic ethanol**

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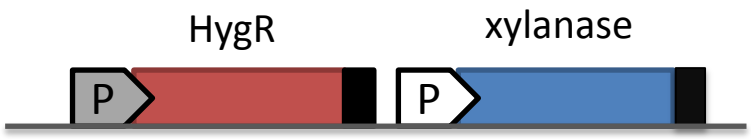
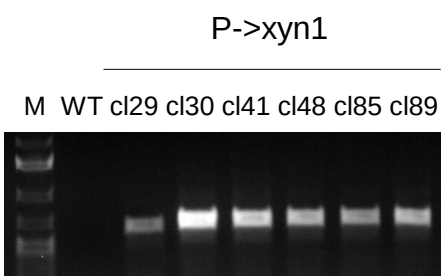
Sapphire Energy

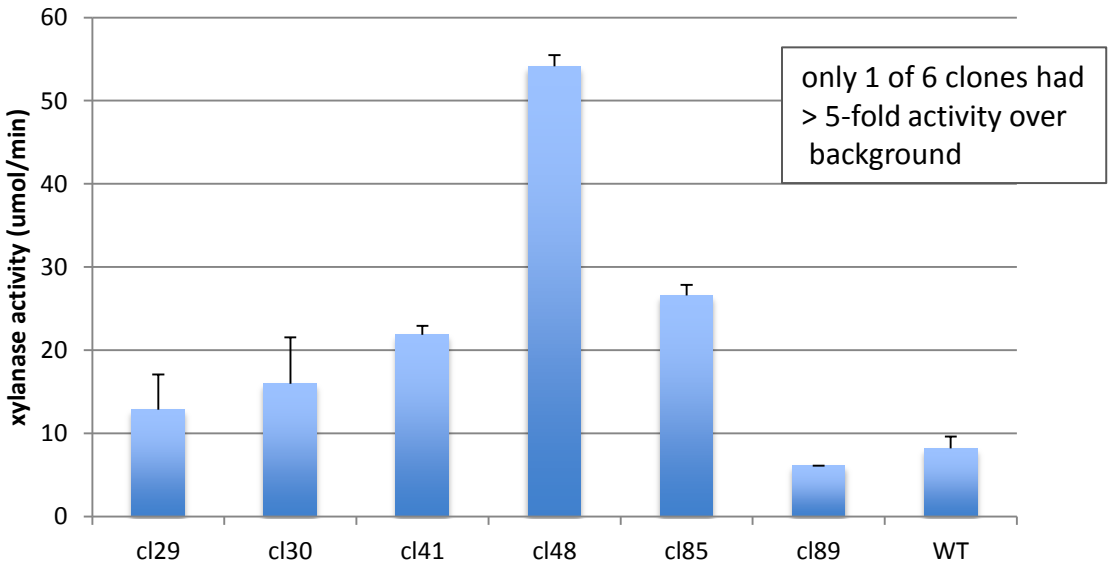
Heterologous expression of Xylanase



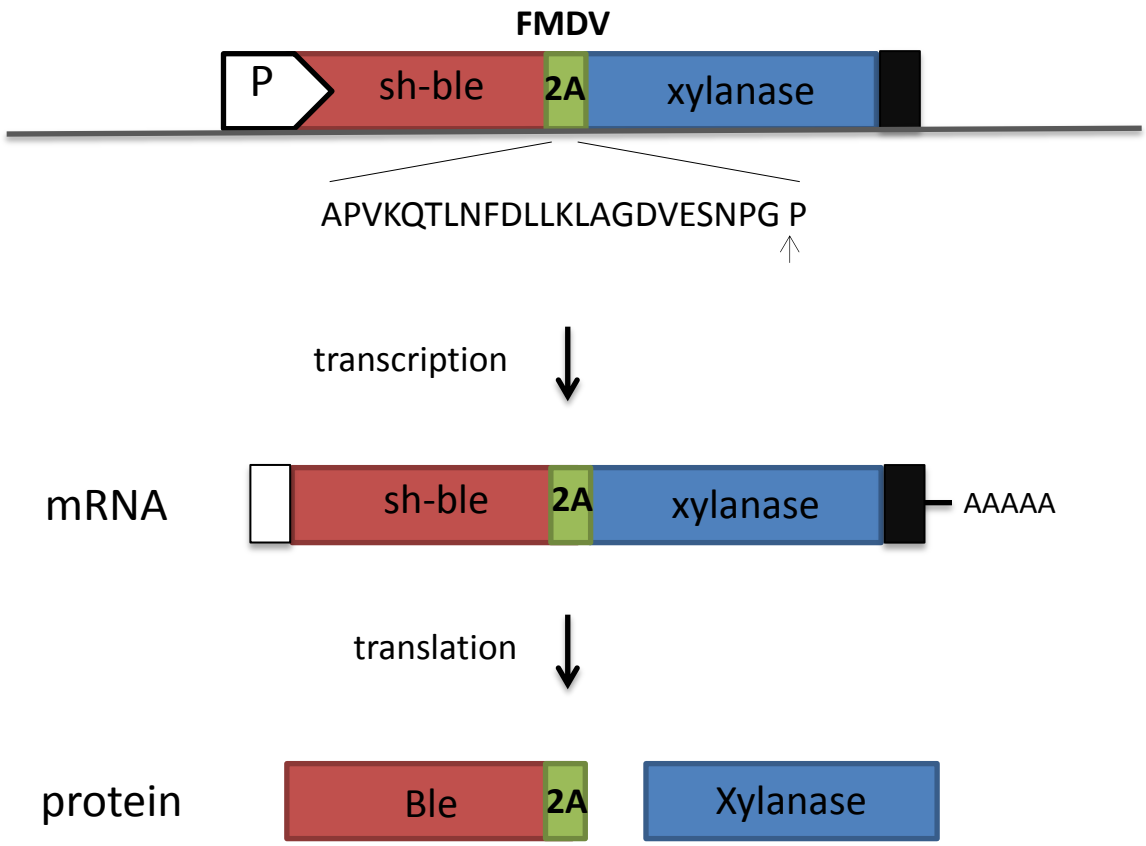
PCR: gene positive



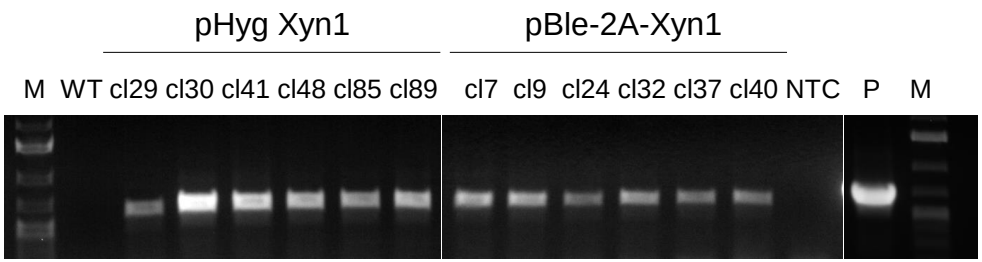
xylanase activity



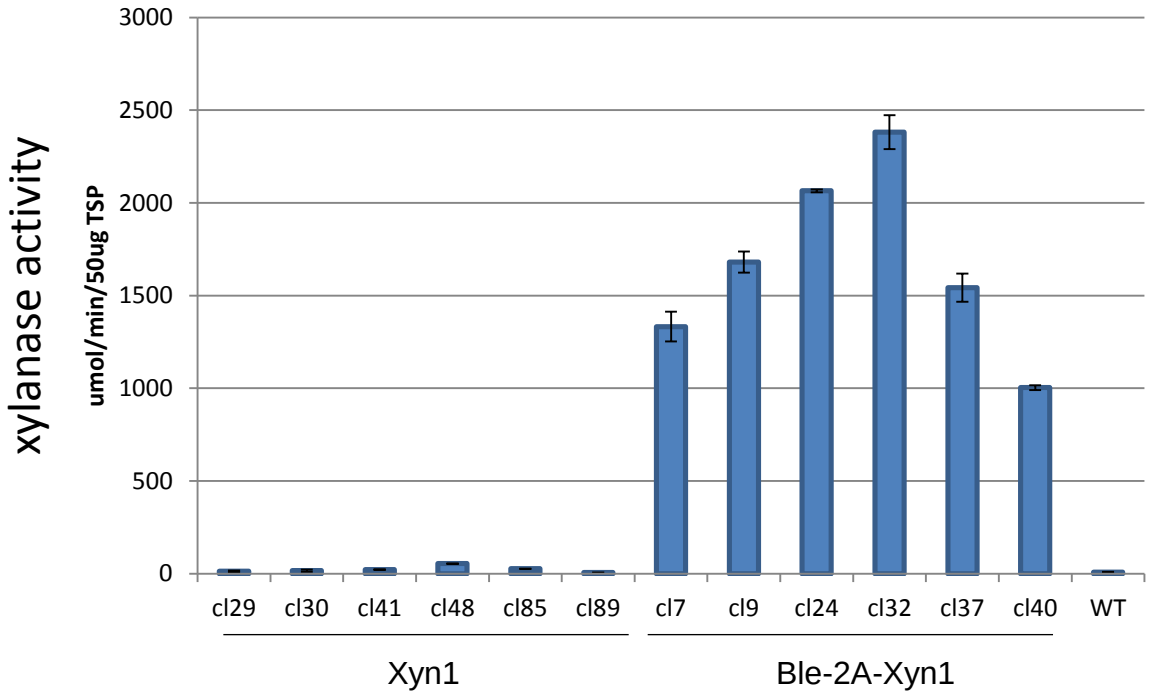
Transcriptionally linking *xylanase* expression to antibiotic resistance using the self-cleaving 2A sequence



Transcriptionally fusing Xyn1 to Ble2A leads to the selection of transformants with higher xylanase activity



PCR: gene+

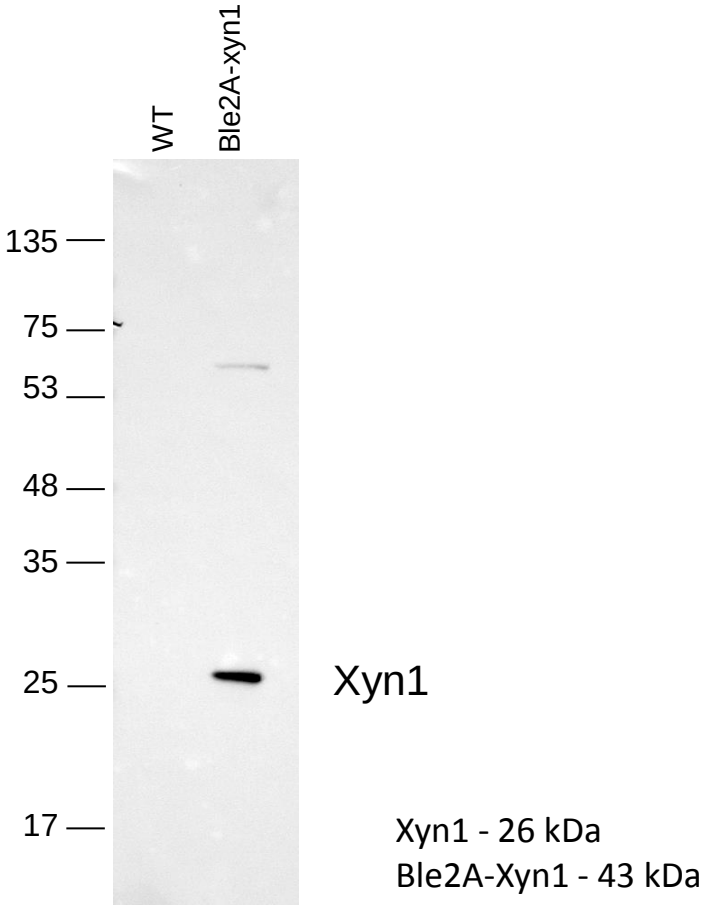


~ 100-fold higher activity

Rasala et al., PLoS One, 2012

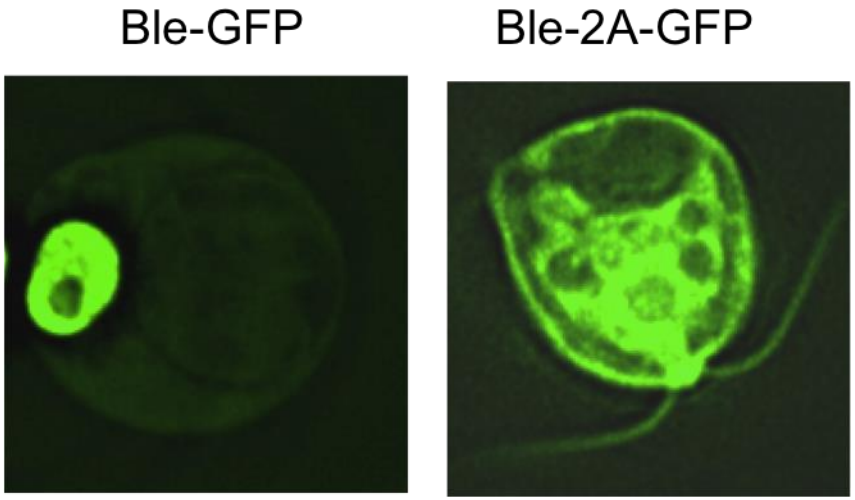
FMDV 2A sequence is ‘cleaved’ in microalgae

Western Blot



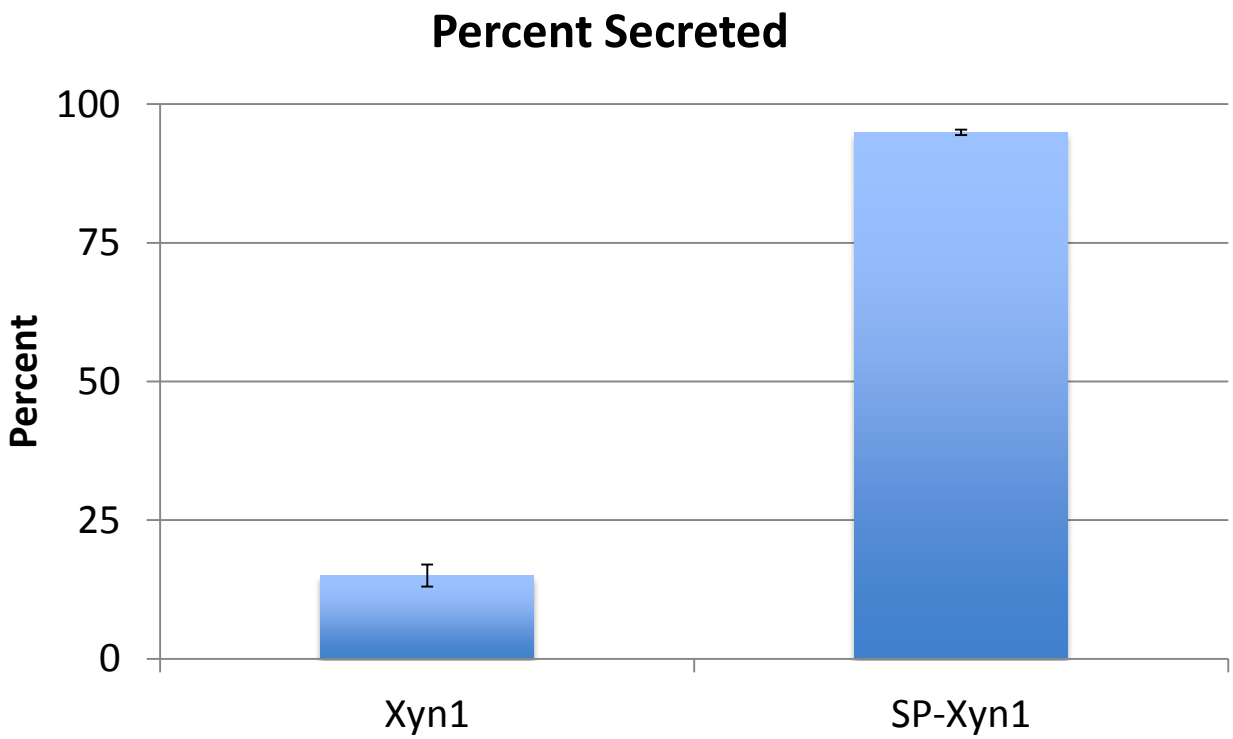
Xyn1 - 26 kDa
Ble2A-Xyn1 - 43 kDa

live cell microscopy



Rasala et al., PLoS One, 2012

The Ble-2A vector supports xylanase secretion



Rasala et al., PLoS One, 2012

Chlamydomonas Protein Expression Kit

‹ Algae Engineering Kits from GeneArt®

Chlamydomonas Protein Expression Kit

Synechococcus Protein Expression Kit

Request GeneArt® Algae Kit Information

Transformation Products for Algal Expression

GeneArt® Cryopreservation Kit for Algae

The GeneArt® *Chlamydomonas* Protein Expression Kit is a second-generation *Chlamydomonas* cloning and expression system from Life Technologies. Like the first-generation GeneArt® *Chlamydomonas* Engineering Kit, this new system offers transgene expression from the nuclear genome of eukaryotic green alga *Chlamydomonas reinhardtii* 137c, but is optimized for high-level expression, provides selection against gene silencing, and offers dual protein tags for detection and/or purification of your gene of interest. It includes frozen *Chlamydomonas reinhardtii* 137c cells, MAX Efficiency® Transformation Reagent for Algae, expression vector, OneShot® TOP10 Competent *E. coli* cells, and easy-to-follow protocols. Our Gibco® TAP Growth Media, offered separately, is optimized for the growth and maintenance of *Chlamydomonas*.

- Express up to 1% total soluble protein of your gene of interest
- Select against gene silencing, even over multiple passages
- Detect and purify your gene of interest with 6His TEV and/or V5-His epitope tags
- Use (optional) seamless assembly to create your constructs
- Enables reliable results with exceptional strain viability and purity

Enabling fluorescent protein tools and technologies

UC San Diego life technologies™ University of Nebraska Lincoln Johns Hopkins University U.S. Department of Energy

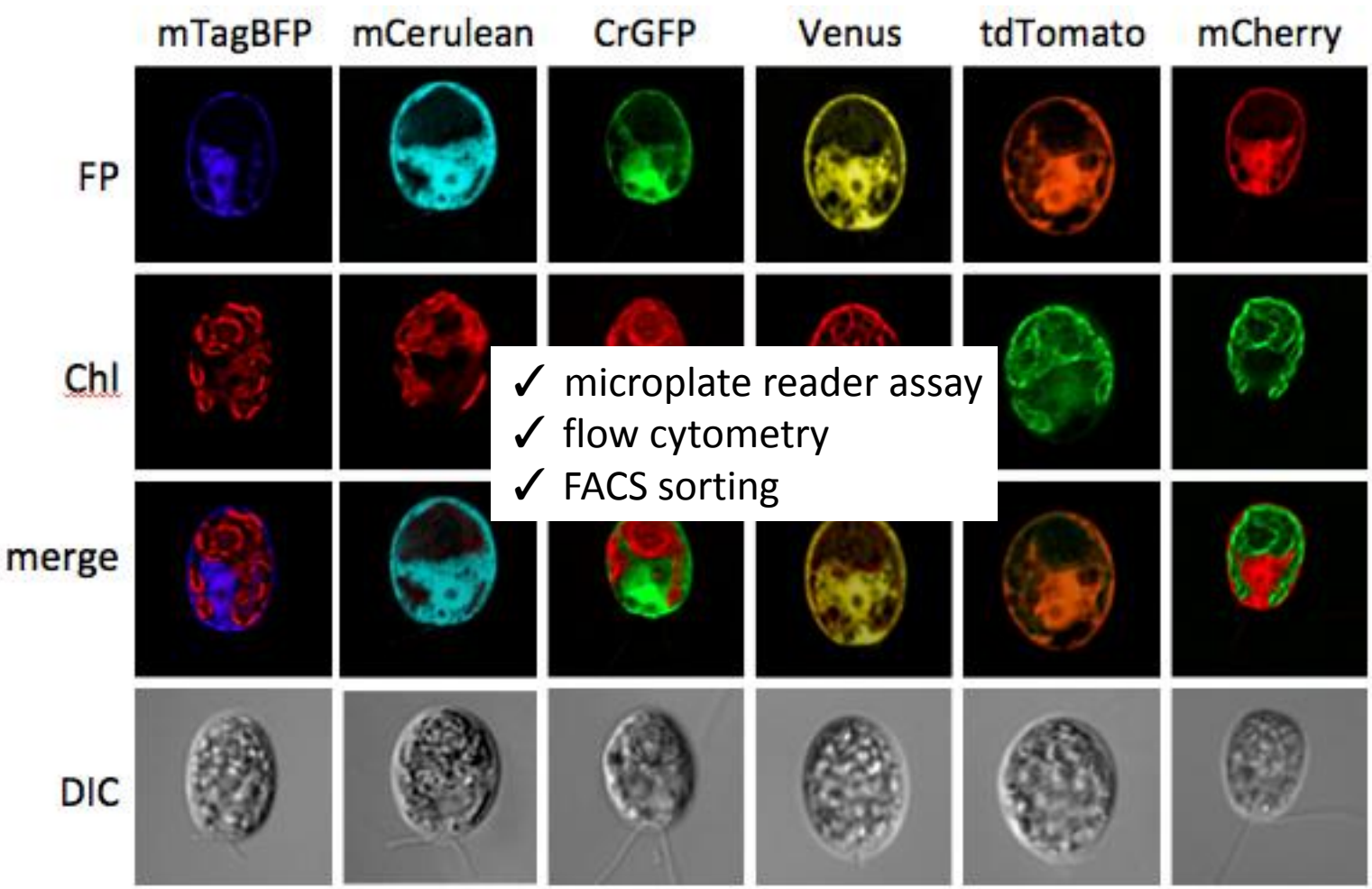
Development of **fluorescent reporters** in microalgae

Fluorescent protein (FP) technology has revolutionized many fields of biology, with an ever-expanding list of applications, including the investigation of:

- promoter function
- protein localization
- protein-protein interactions
- cell cycle progression
- organelle labeling
- and as biosensors for monitoring small molecules and second messengers.

However, due to poor transgene gene expression from the nuclear genome, FPs have not been widely developed for microalgal research.

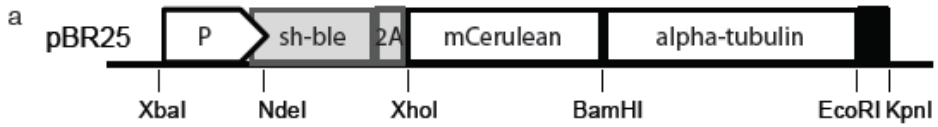
Live Cell Microscopy: FP expression in *C. reinhardtii*

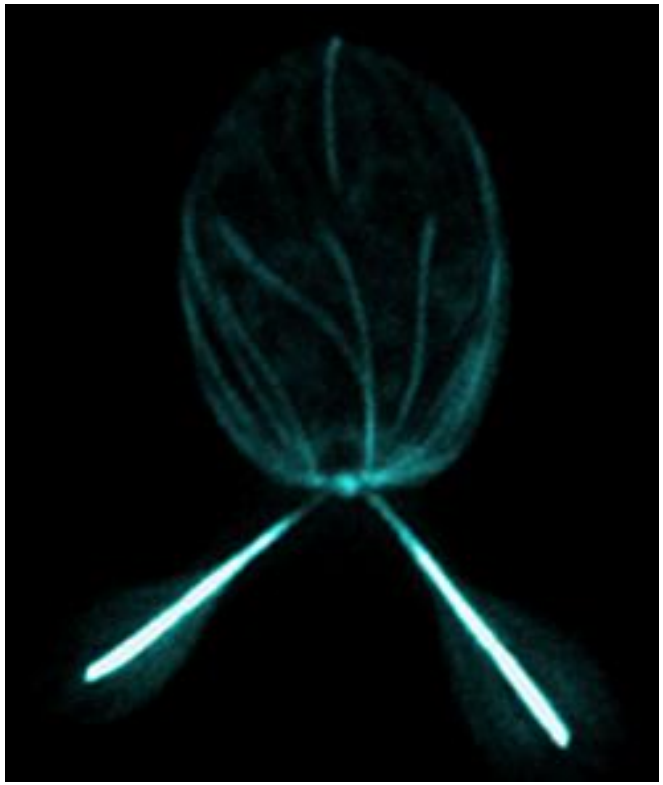


- ✓ microplate reader assay
- ✓ flow cytometry
- ✓ FACS sorting

Rasala et al., Plant Journal, 2013

Expression of a mCerulean–α-tubulin fusion protein in *Chlamydomonas*



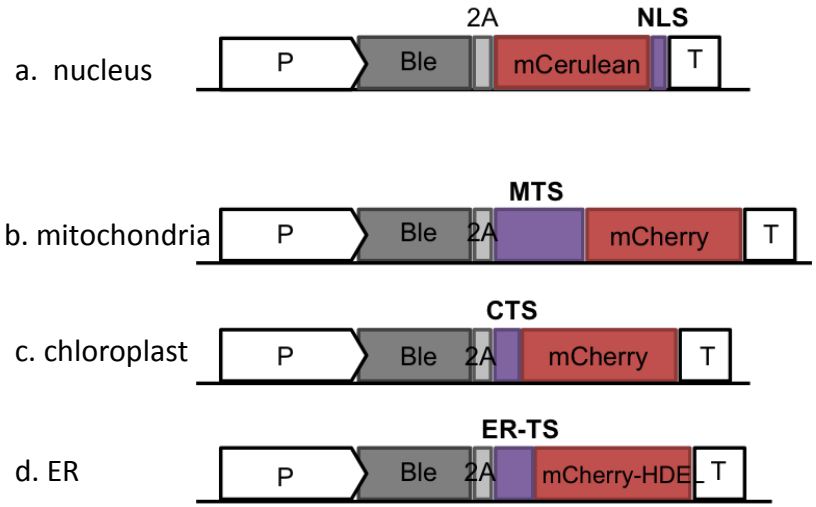


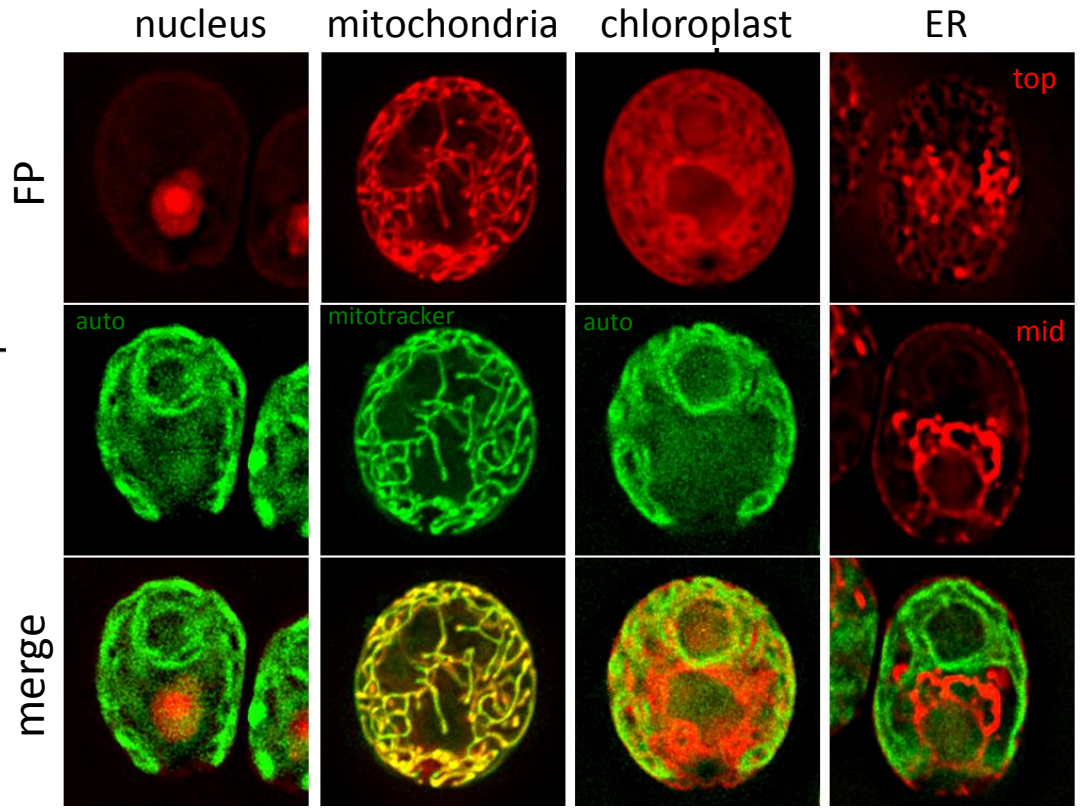
Rasala et al., Plant Journal, 2013

Enabling metabolic engineering

- Subcellular targeting vectors
- Coordinated expression of multiple transgenes within a single cell

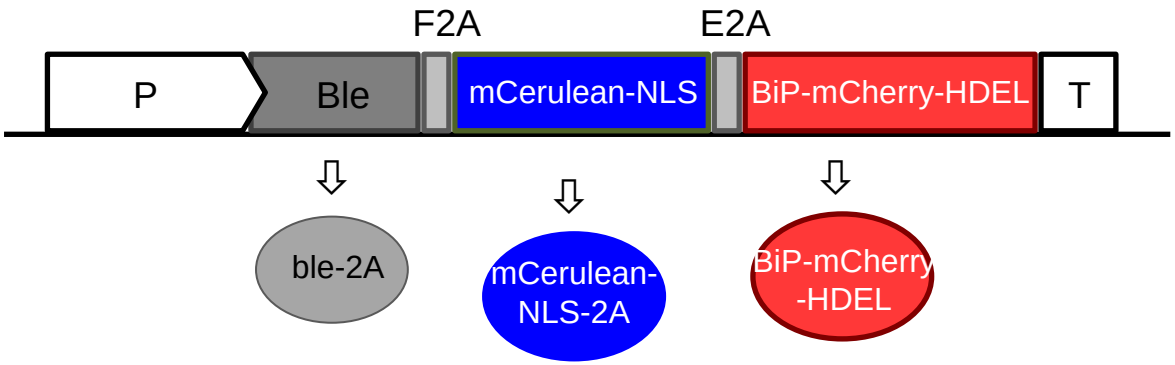
Organelle targeting vectors for advanced genetic engineering

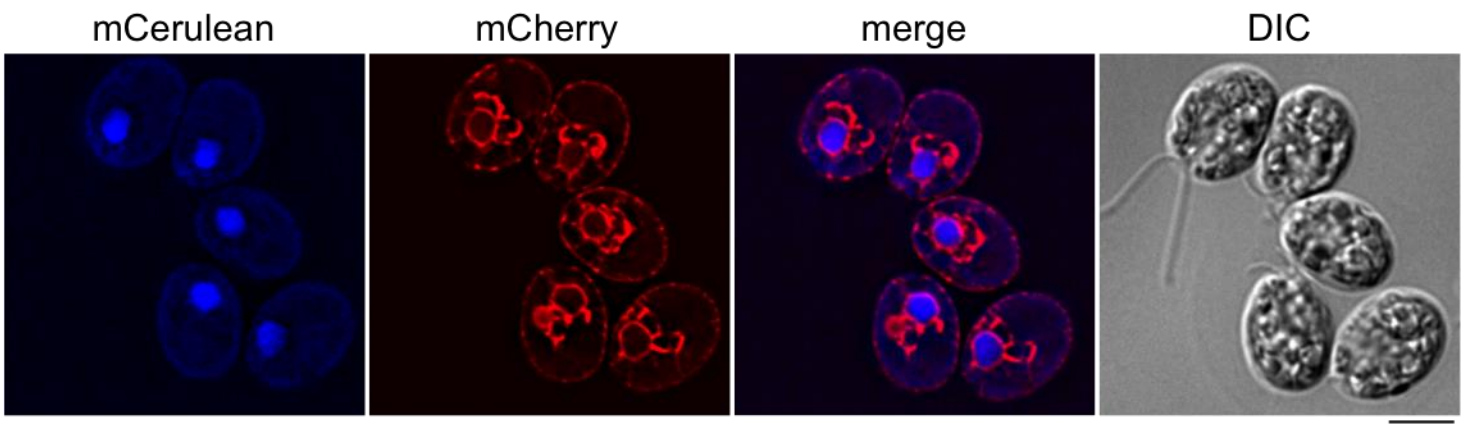




live cell microscopy

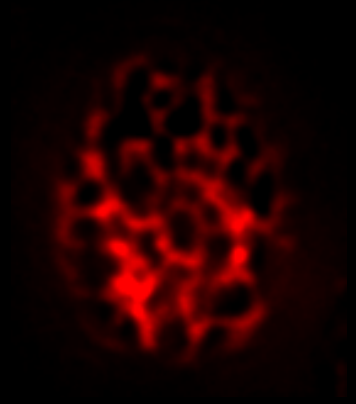
Gene stacking using a single transformation vector



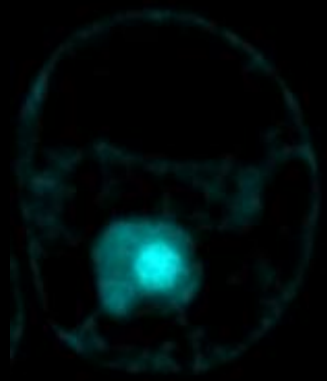


Gene stacking through mating

mCherry -> ER

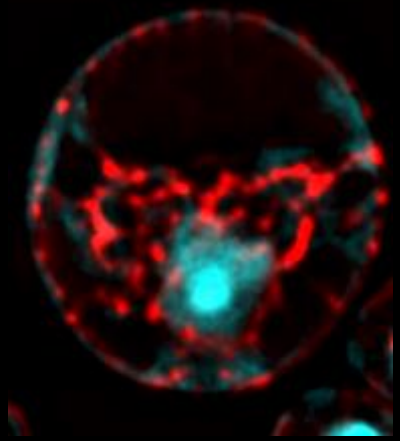


mCerulean -> nucleus



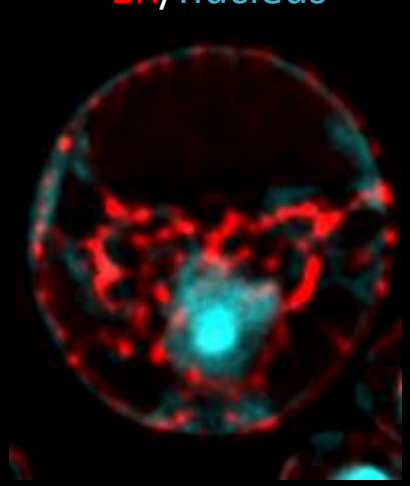
1st

X

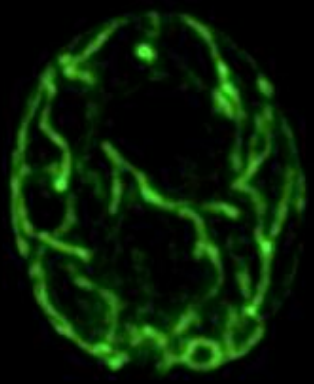


Gene stacking through mating

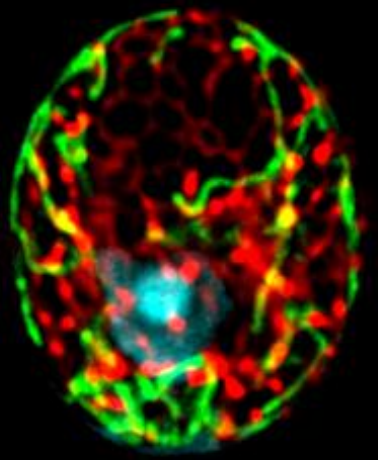
ER/nucleus



Venus -> MT

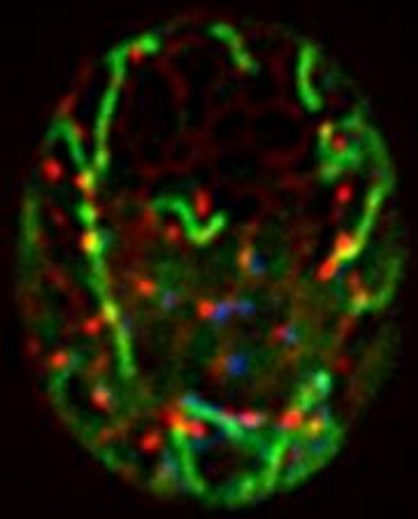


2nd
X



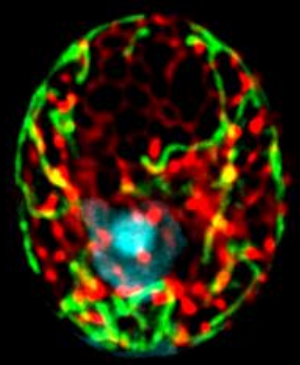
A Z-stack through *C. reinhardtii*

ER
nucleus
mitochondria

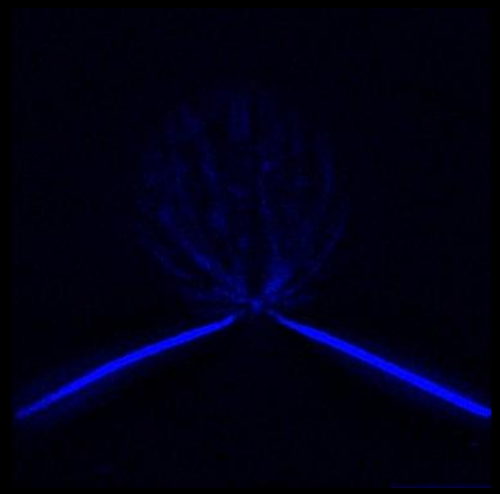


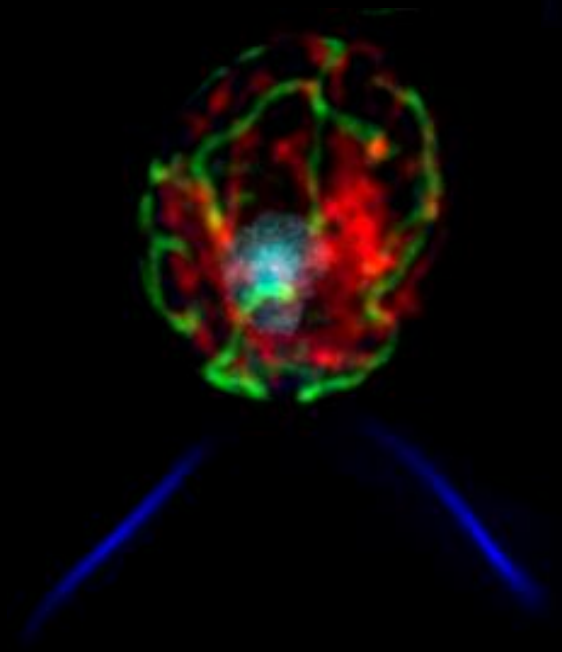
Gene stacking through mating

mTagBFP-TUA1



3rd
X



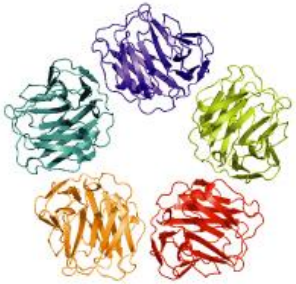


Enabling microalgae biotechnology and commercialization

Triton uses algae to create orally-available, advanced biologic molecules for animal and human health

TRITON HEALTH & NUTRITION

1 Identify biologic

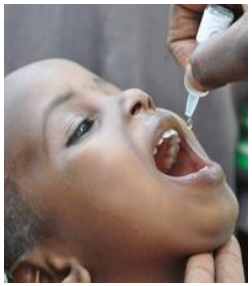


2 Create algae that produces target biologic

3 Grow algae

4 Harvest algae, dry, convert to bulk products

5 Formulate in retail product for consumption



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PhycoShield™ MAA

TRITON HEALTH & NUTRITION

www.tritonhn.com

- A powerful therapy to address intestinal disease in humans, livestock, and companion animals
- A natural product found in colostrum and milk, never before available at large scale
- Stimulates the production of mucin in the small intestines to block pathogenic bacterial infections
- Potential to replace the prophylactic use of antibiotics in livestock
- Produced at a cost that is affordable to people in the developing world
- Sales will begin in 2014, in partnership with heliae®

Acknowledgements

UC San Diego

The Mayfield Lab:

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James Golden
Susan Cohen
Federico Unglaub

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Stephen Mayfield
William Julien
Nick Hofmeister
Daniel Sachs
Miller Tran

life technologies™

Todd Peterson
Farzad Haerizadeh
Lisa Stillwell

University of Nebraska Lincoln

George Oyler
Donald Weeks
Julian Rosenberg

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