Targeted mutagenesis of the *Phaeodactylum tricornutum* urease gene using TALENs

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Viva la genetic revolution!

- Eukaryotes have become genetically accessible through programmable nucleases
  - Zn-Finger Nucleases (ZFN)
  - Transcription Activator-Like Effector Nuclease (TALEN)
  - Custom meganucleases
  - CRISPR-Cas9
TALEN = TAL effector + nuclease

- TAL Effectors originally identified in *Xanthomonas* plant pathogens
- 34 amino acid repeats that come in 4 “flavors”: one to bind each nucleotide
- Order of repeats designed in TALEN = DNA sequence recognized

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Two paths to mutants

• Non-homologous end joining (NHEJ)
  o “Cut and repair” (often with random insertions or deletions)
    - Meganucleases and TALENs

• Homologous recombination (HR)
  o Cut and insert specific sequence (e.g. antibiotic resistance gene) based on presence of homologous sequence
  o We pursued this approach with TALENs
Targeting TALENs to *P. tricornutum* urease

![Diagram showing the targeting of TALENs to *P. tricornutum* urease with specific DNA sequences highlighted.](image)

- **TGGACCGGTACGTTGCTAAA**
- **ATTTGTCCAGCTCATTA**
- **CGATCTTCCGCAAGGTAAGA**
6x ~100bp assembly

3x ~600bp assembly

(Parallel assembly process)

Co-transform P.t. with TALEN plasmid + KO-plasmid via particle bombardment

E. ShBle
Urease insertions identified in ~20% of BleoR Pt lines

A. Urease KO-05 to BleoR to Urease KO-06

TALEN + KO plasmid

KO plasmid only

B. 4 kb, 3 kb, 2 kb

C. 4 kb, 3 kb, 2 kb

Mut

WT

WT
Most lines do not maintain TALEN cassettes or plasmid.

[Diagram showing genetic analysis results with lanes labeled 9-1, 11-1, 11-6, WT, 9-7, 11-3, 11-4, 11-5, NTC. Bands are labeled with sizes: 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb for Urease, and 3 kb, 2 kb, 1 kb, 0.5 kb for KmR and TALEN.]
Mutants have expected phenotype

+Nitrate

+Urea
Western blot shows urease protein absent in biallelic mutants
Knockout of urease leads to accumulation of ornithine-urea cycle intermediates

- Arginine
- Ornithine
- Urea
- Arginase (EC: 3.5.3.1, Pt38509)
- Arginosuccinate lyase (EC: 4.3.2.1, Pt34526)
- Arginosuccinate synthase (EC: 6.3.4.5, Pt21116)
- Ornithine carbamoyltransferase (EC: 2.1.3.3, Pt30514)
- UnCPS (EC: 6.3.4.16, Pt24195)
- ATP, AMP, PPI, H⁺, CO₂, NH₄⁺
Applying synthetic genomics to algae

- TALENs, etc. can be used for single gene mutations
- Next step: large fragment “swaps” (replace wild type with modified/synthetic)
- Near future: whole chromosome swaps?

Working at the chromosome scale

Synthetic 1 Mb Mycoplasma mycoides genome:
- Built in yeast
- Transplanted to M. capricolum
- “Booted up”

See Poster by Bogumil Karas

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