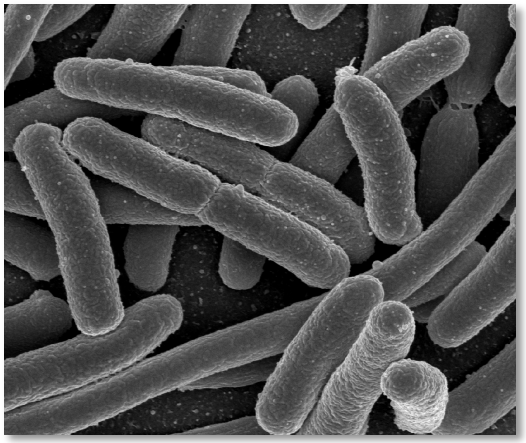
Saul Purton, University College London

Synthesis of antibacterial proteins in the chloroplast of the green microalga *Chlamydomonas reinhardtii*

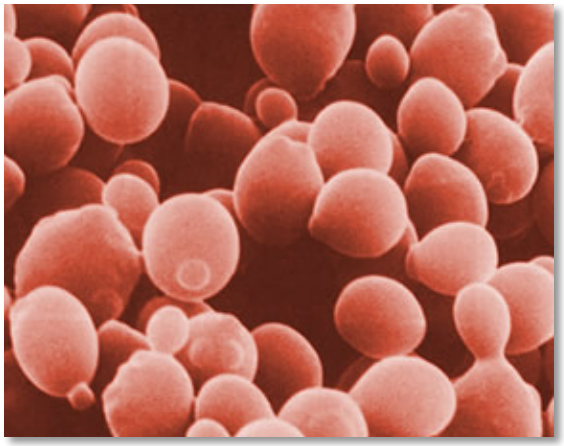
Outline

- The algal chloroplast as an attractive chassis
- Development of molecular tools for chloroplast engineering in *Chlamydomonas*
- Production of recombinant endolysins

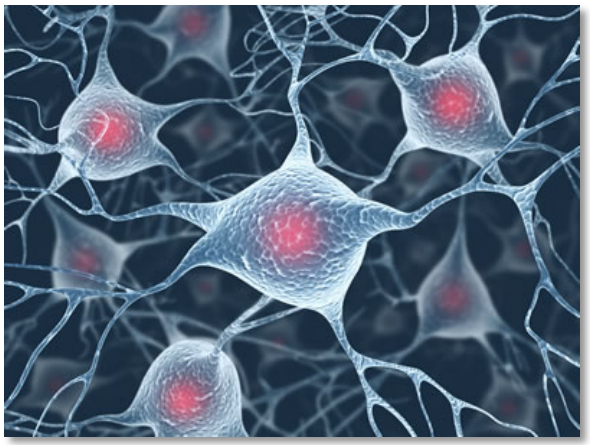
Platforms for production of high value recombinant proteins



bacteria



yeasts

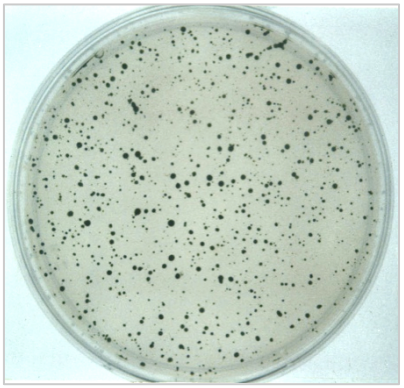


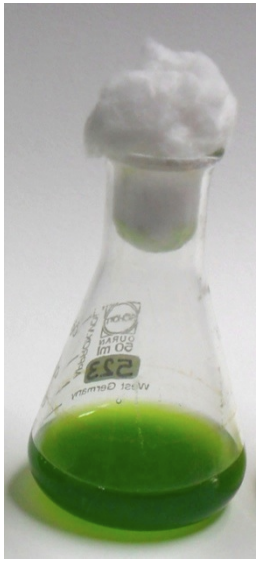
mammalian cells

algae ?

Microalgae: an attractive platform

- Single cell type
- Fast growth
- Controlled and contained production
- Scalable
- Some species have GRAS status

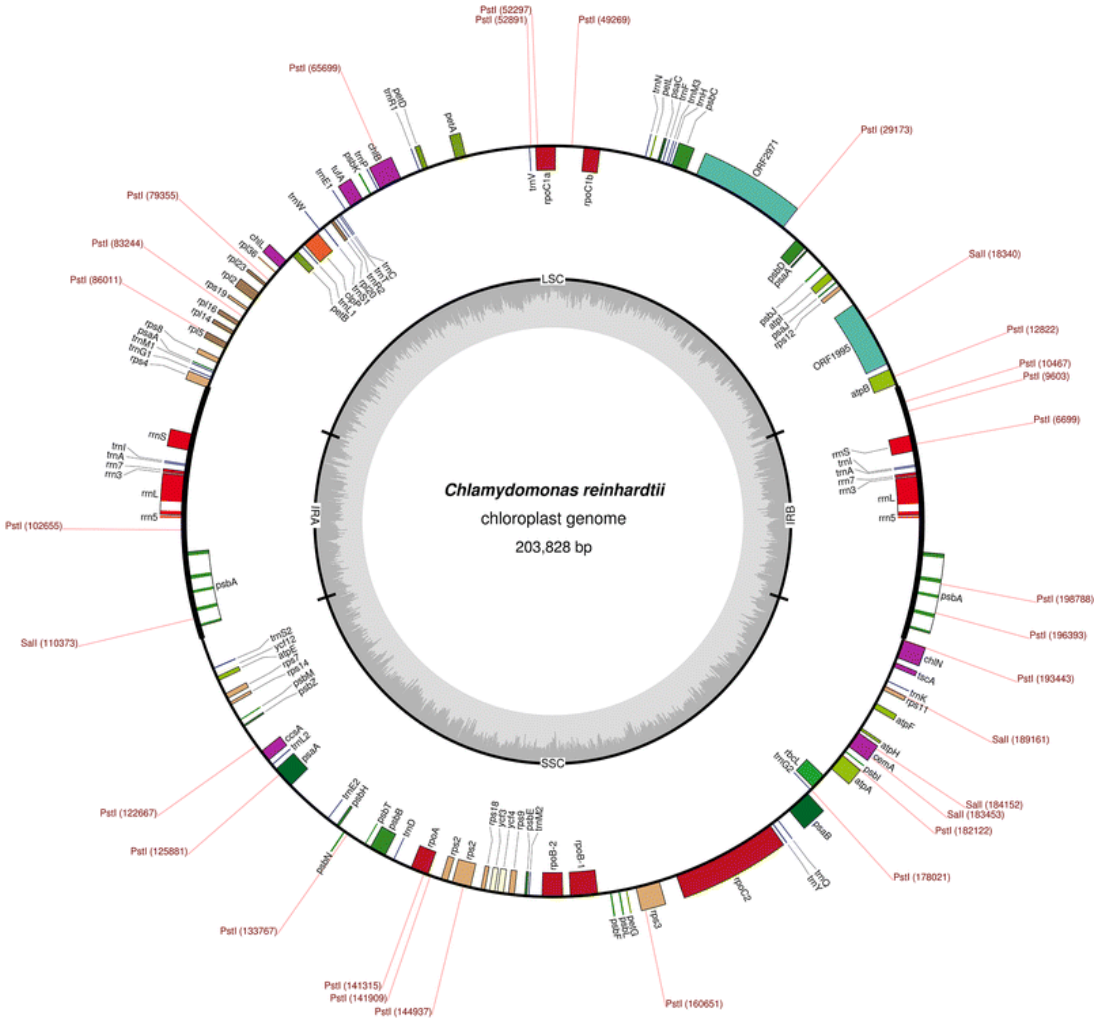




supreme biotech

Advantages of the chloroplast genetic system

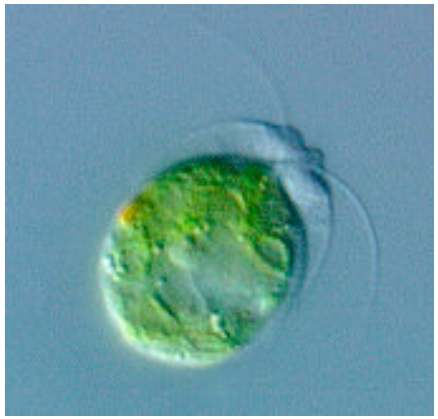
- Foreign DNA can be targeted to a specific locus.
- High copy number since chloroplast has multiple copies of genome.
- No gene silencing mechanisms.
- High level expression (up to 5% total soluble protein).
- Proteins appear to fold correctly and form disulphide bonds.



To-date, chloroplast transformation has been reported in only five algal species

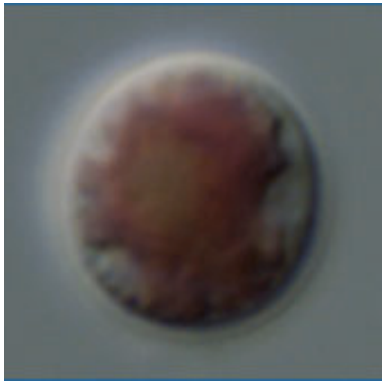
Chlamydomonas reinhardtii

Dunaliella tertiolecta



Haematococcus pluvialis

Euglena gracilis



Porphyridium sp.

Advanced chloroplast engineering tools needed for both platform development, and metabolic engineering of key pathways

Chlamydomonas reinhardtii

1

Vaccines, anti-microbials, hormones, antibodies, etc.

2

Novel carbohydrates, fatty acids, terpenoids, tetrapyrroles, etc..

Metabolic engineering requires regulated expression of multiple trans-genes

A simple method for DNA delivery into the chloroplast

Microparticle bombardment

Expensive. Licensing/patent issues

Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 1721–1725, March 1991
Botany

Engineering the chloroplast genome: Techniques and capabilities for chloroplast transformation in *Chlamydomonas reinhardtii*

(cotransformation/particle gun/bombardment/algae)

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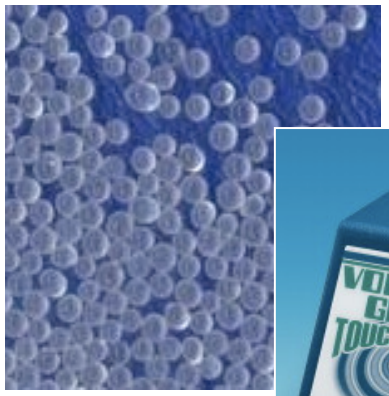
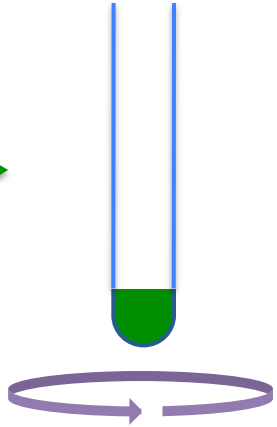


Vortexing cell/DNA suspension with glass beads

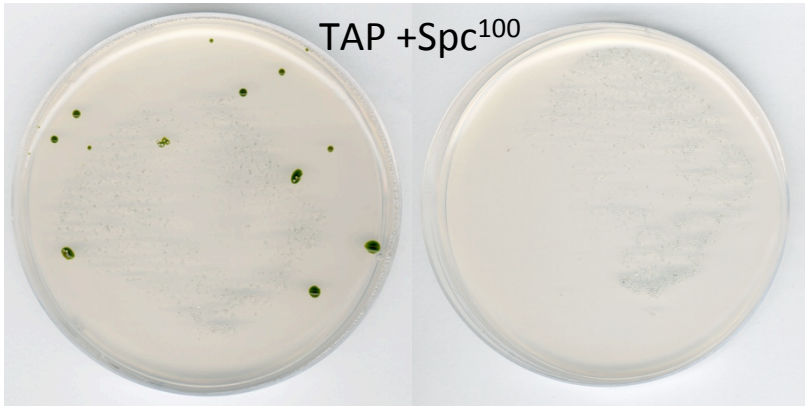
Cheap, quick and simple

Remove cell wall
with autolysin

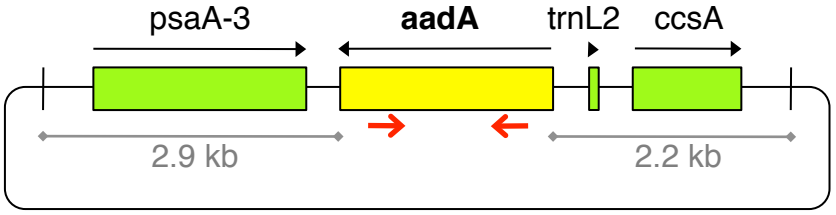
… or use a cell wall
deficient mutant

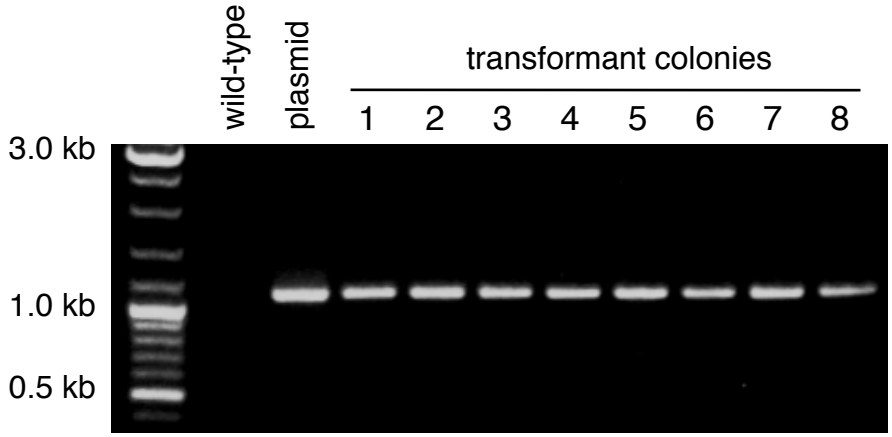


Vortex cell suspension
with 5 µg DNA and glass
beads, 15 s

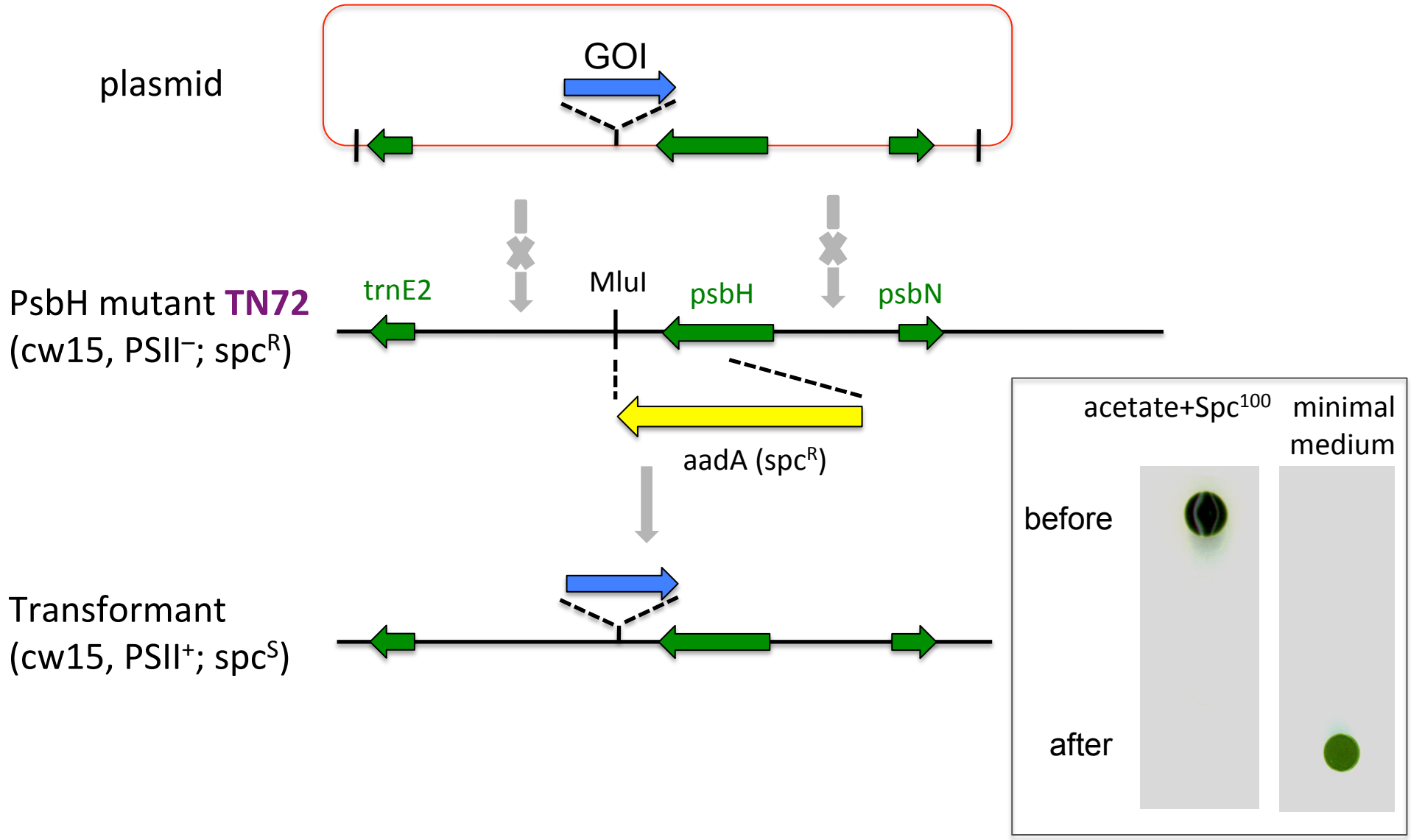


+ DNA – DNA

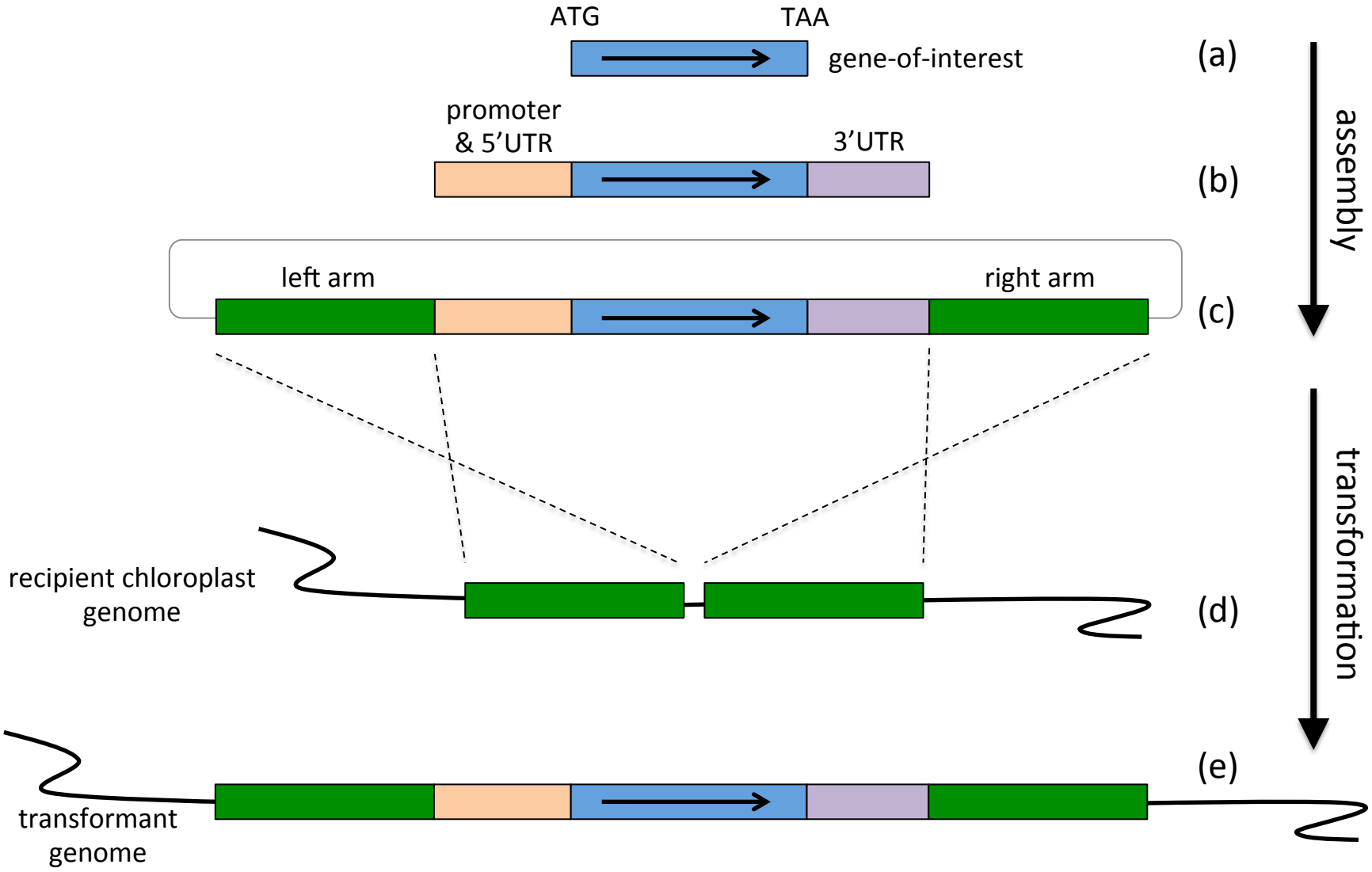




A recipient strain and selection method that generates transformant lines containing only the GOI

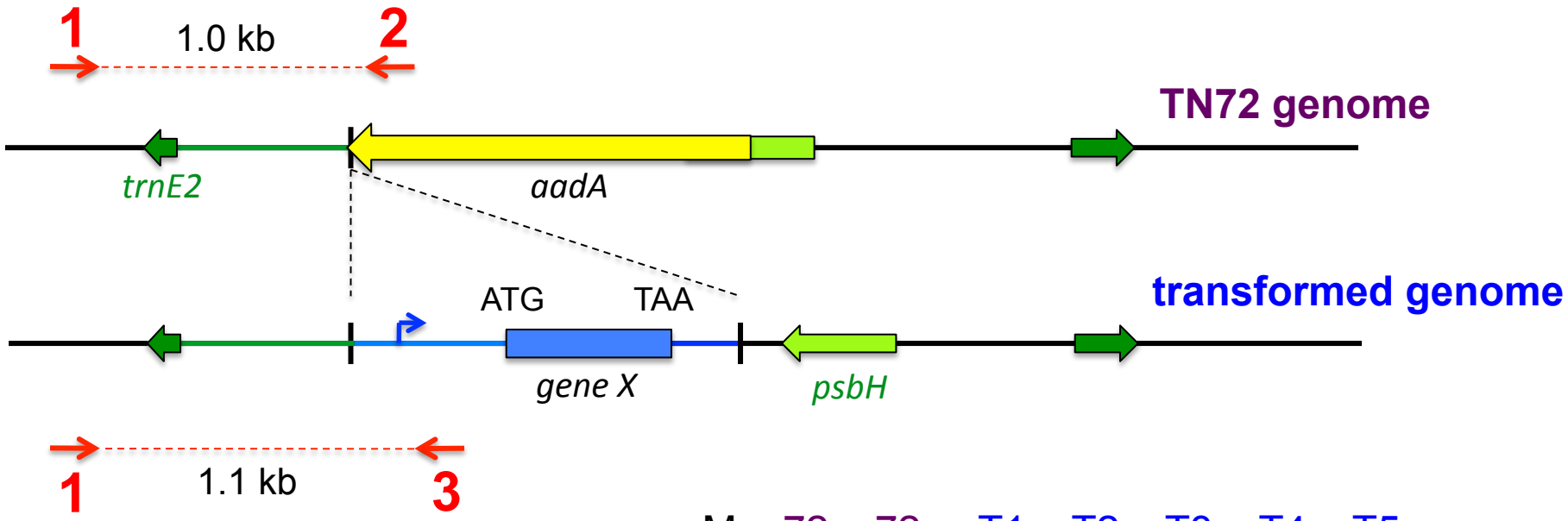


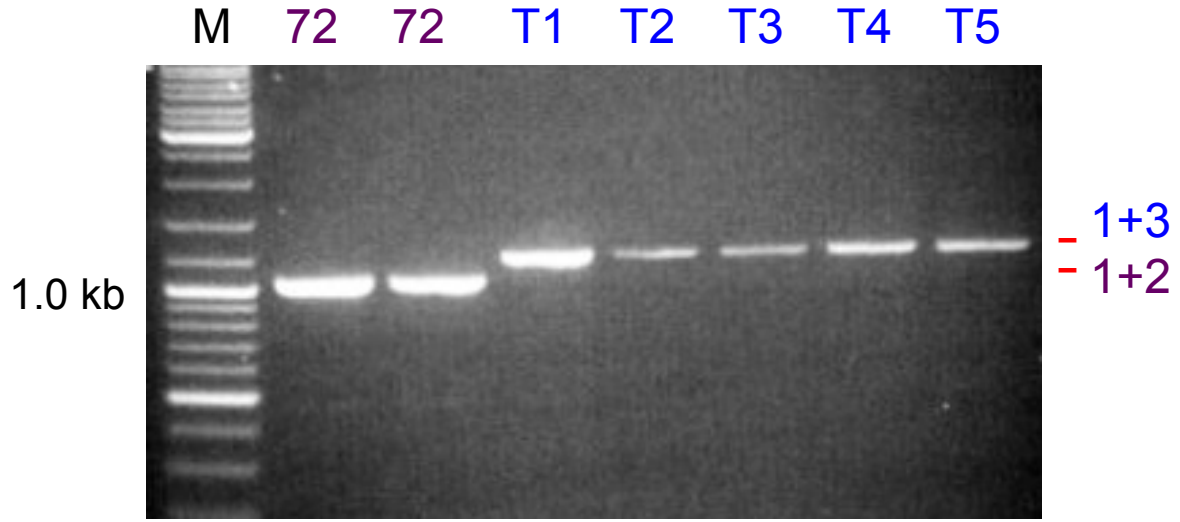
Transforming DNA requires assembly of multiple parts



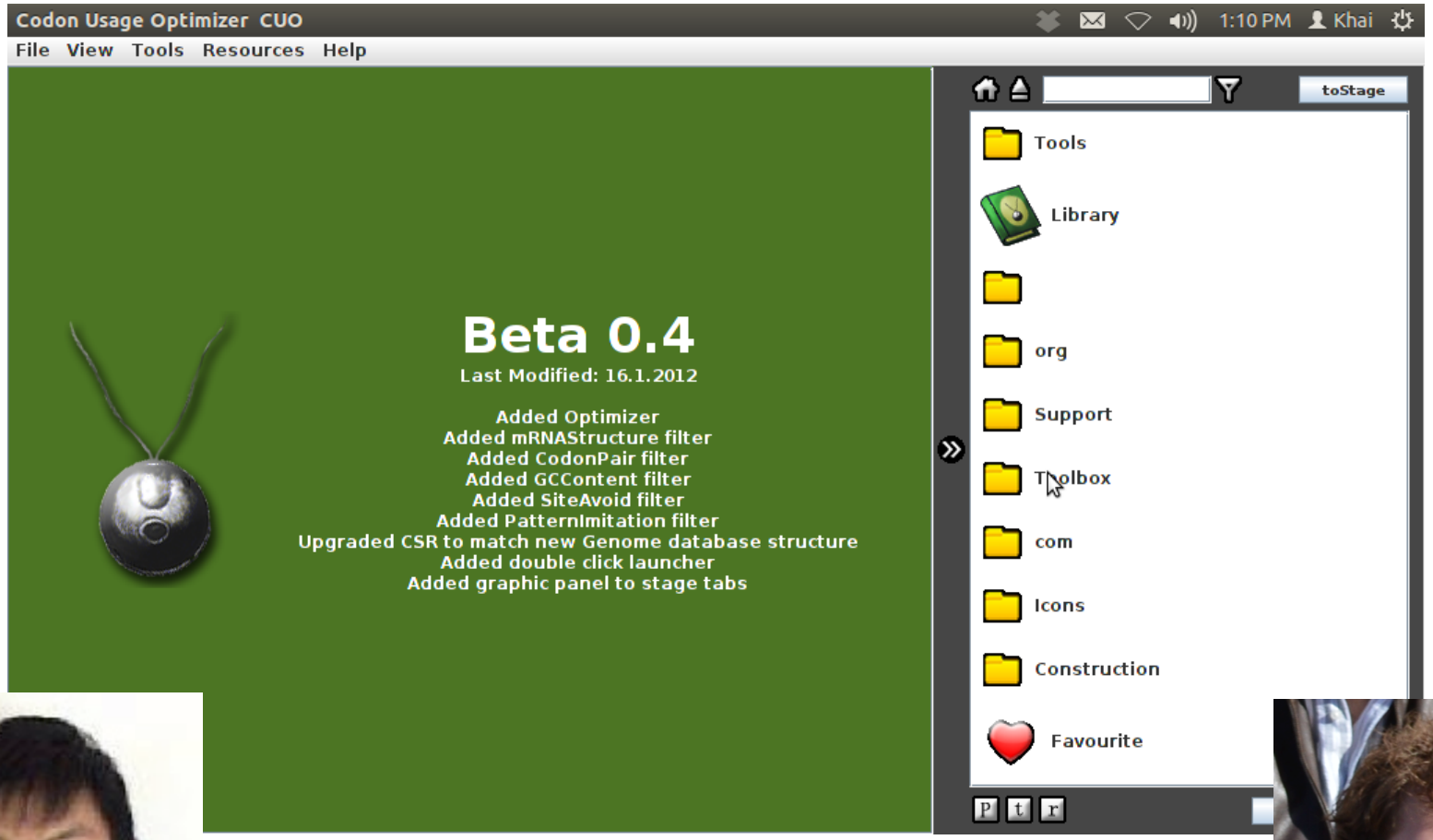
Gibson method allows seamless one-step assembly of all the parts without using any restriction enzymes.

Prototrophic selection is so strong that homoplasmy is obtained after only one round of single-colony isolation





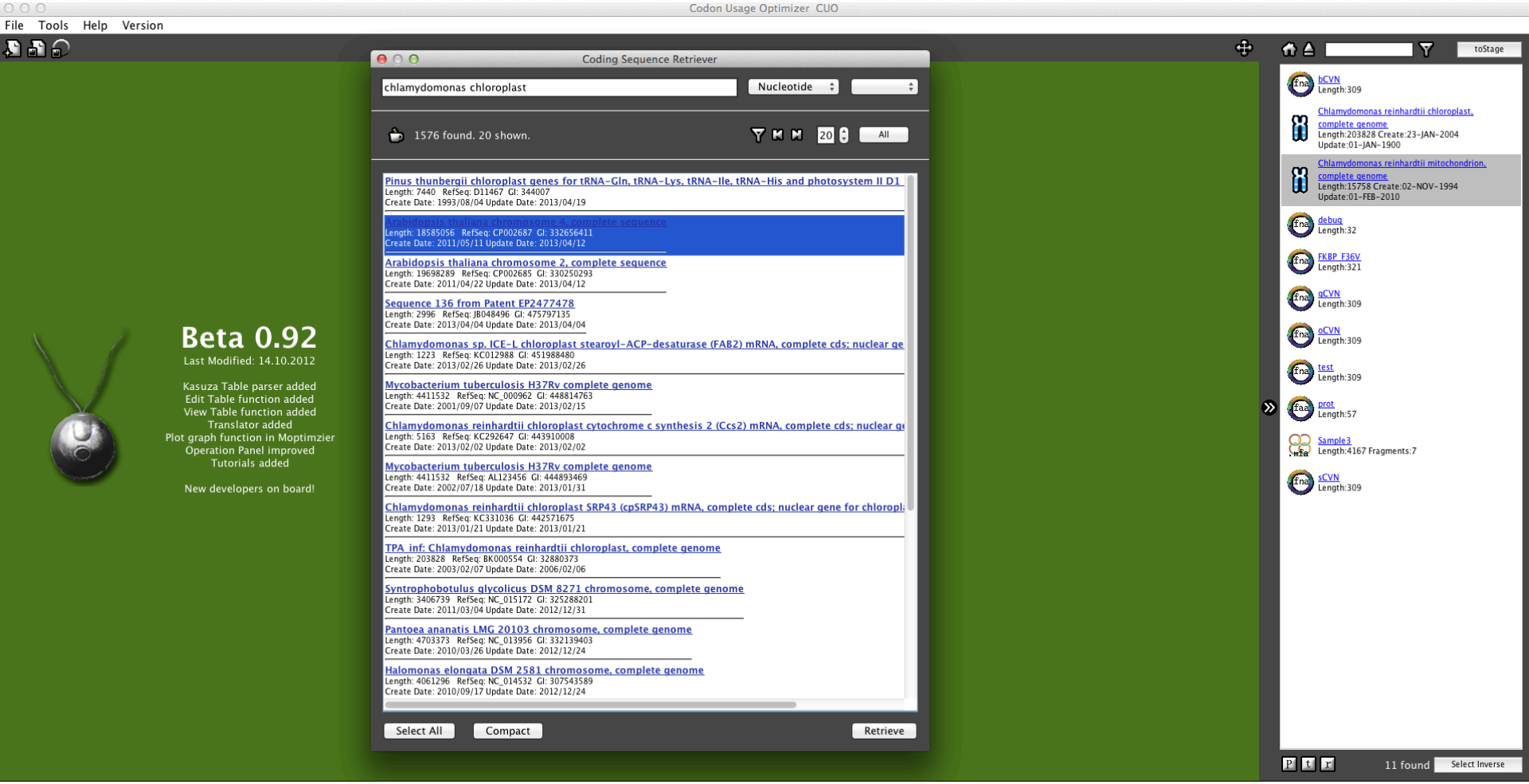
Codon optimisation software



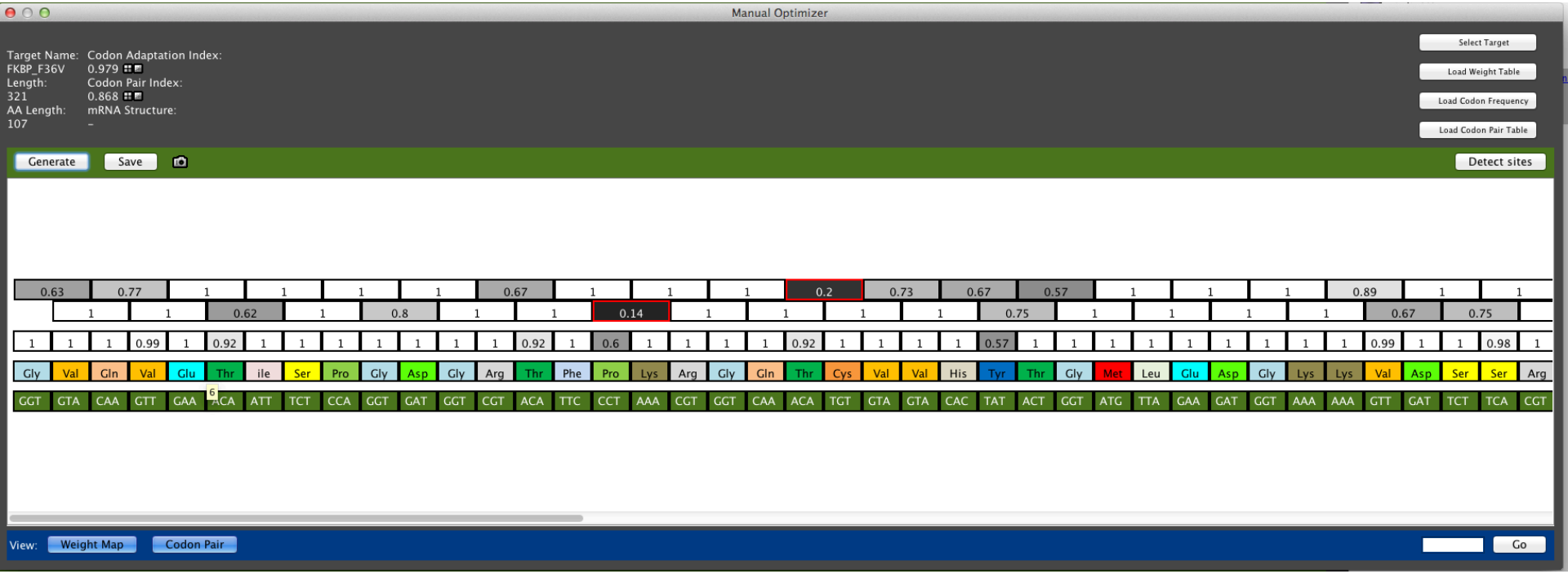
Khai Kong

Henry Taunt

Software allows codon analysis of subsets of genes and creation of bespoke codon tables



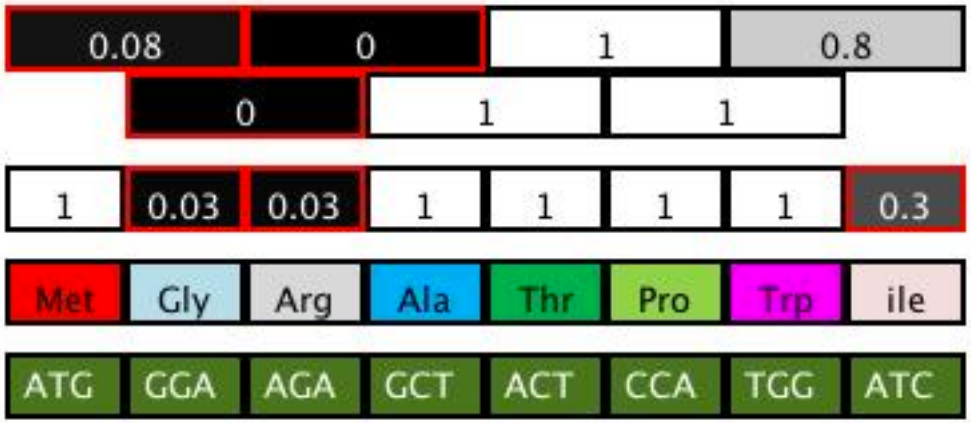
Program then designs your gene based on a chosen codon usage threshold



Also takes into account:

- codon pair thresholds
- Unwanted runs of the same codon
- Any unwanted restriction sites

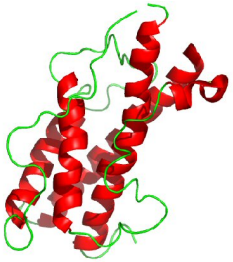
The ‘manual optimiser’ function allows you to modify the design by changing codons or codon pairs

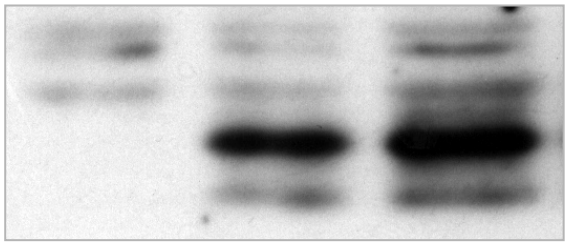


Some examples of recombinant proteins produced in the *Chlamydomonas* chloroplast

ΔpsbH T1 T2

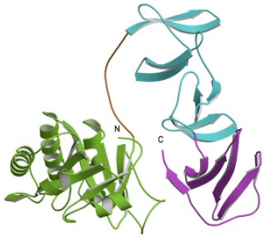
human growth hormone

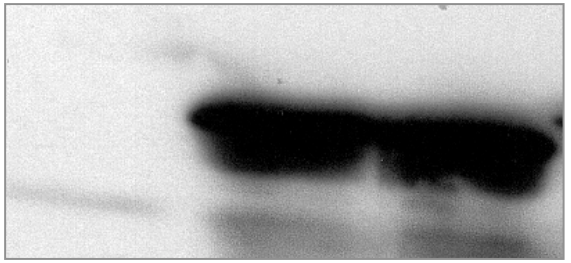




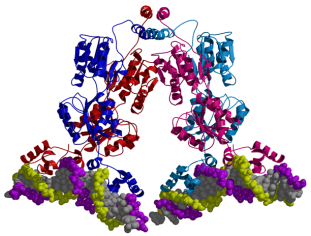
bacteriophage endolysin

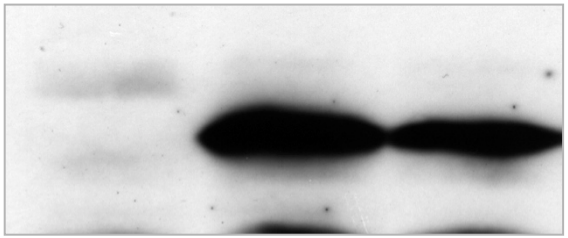
A new class of antibiotics ?



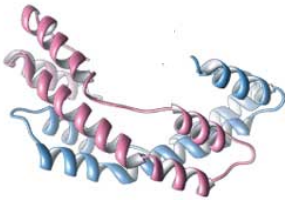


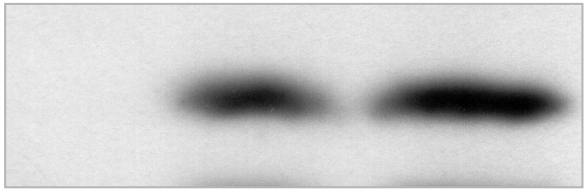
bacterial repressor





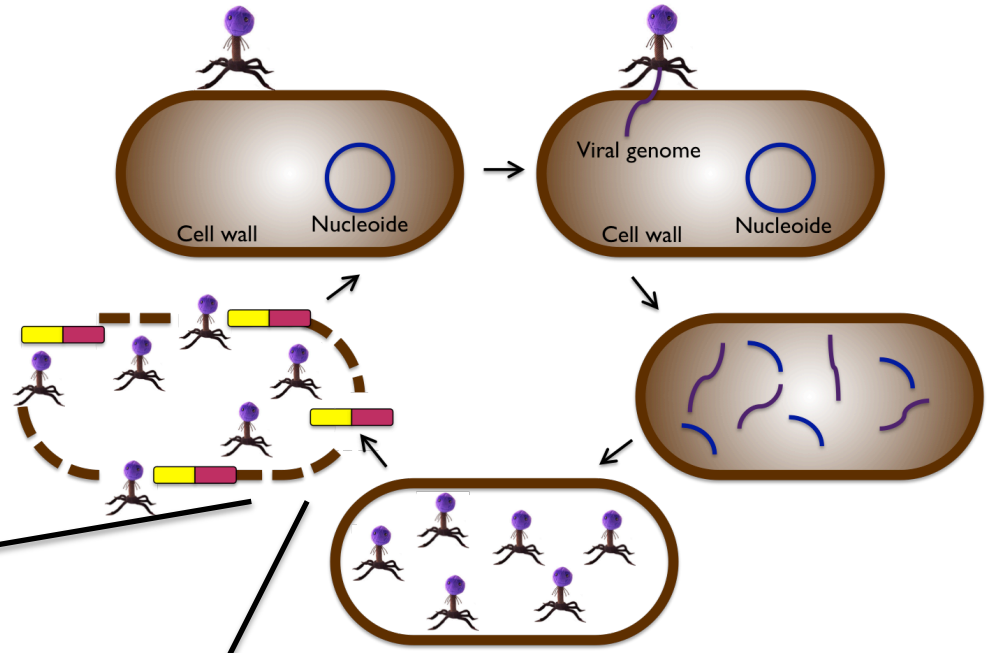
bacterial chaperone

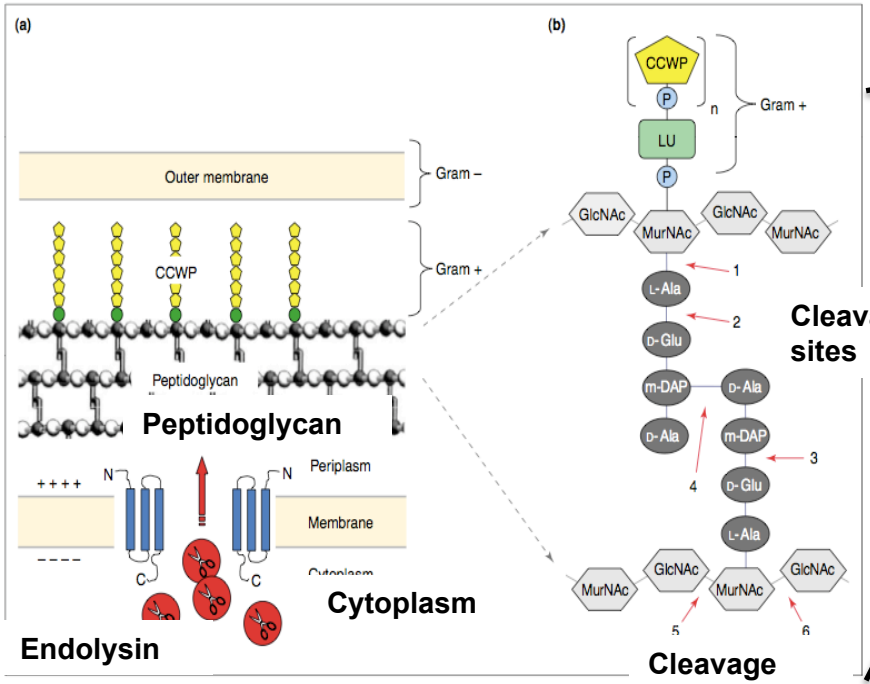




Bacteriophage endolysins as potential antibiotics

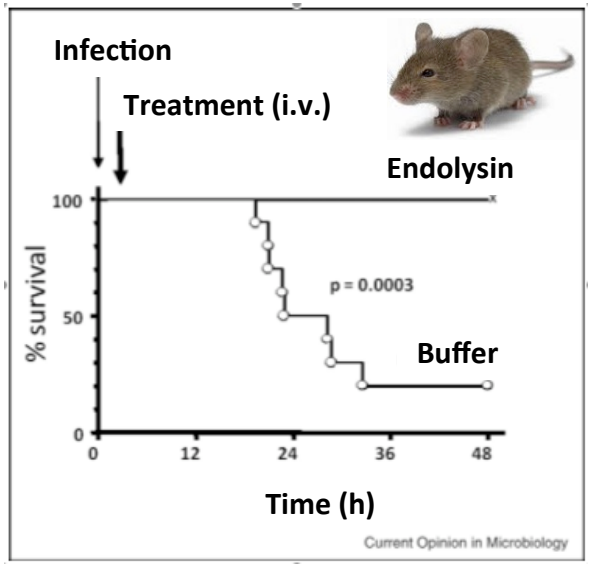
- Proteins produced in bacteriophage infected cells
- Cleave the peptidoglycan for phage progeny release at the end of the lytic cycle





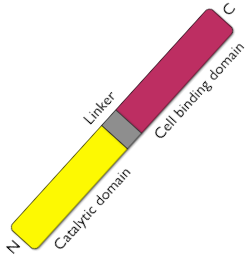
- Simple monomeric proteins
- Typically, a two-domain structure
- No-cofactors

Antimicrobial activity in an *in vivo* experiment



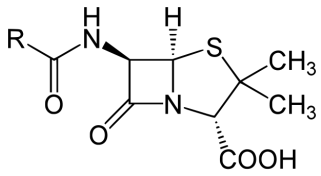
- **Can act extrinsically against Gram +ve bacteria**
  - *In vitro*
  - *In vivo* mouse models
  - Active against biofilms

- **Advantages over antibiotics**
  - Highly specific
  - Development of resistance is very rare
  - Active against both dividing and non-dividing cells



Endolysin

vs



Antibiotic

We have synthesized several lysins in the chloroplast and shown that they are highly active against major human pathogens.

Conclusions

- ➔ New molecular tools allow rapid genetic engineering of the *Chlamydomonas* chloroplast (design to transformant line in ~6 weeks).

- ➔ High value recombinant proteins can accumulate to >1% total soluble protein.

- ➔ Scale-up and purification straight-forward.

- ➔ Endolysins against major human pathogens have been successfully expressed in the *C. reinhardtii* chloroplast and show promise as anti-bacterials.

Thank you for your attention