

Saul Purton, University College London

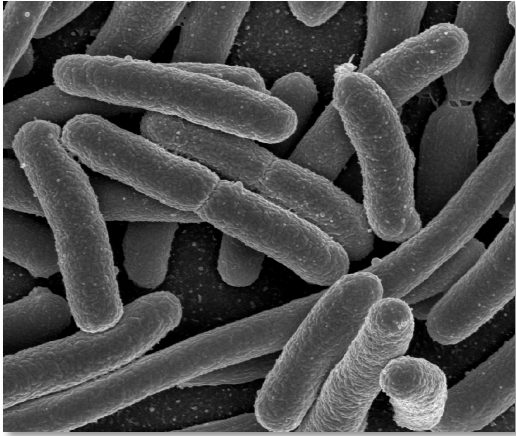


Synthesis of antibacterial proteins in the chloroplast of the green microalga *Chlamydomonas reinhardtii*

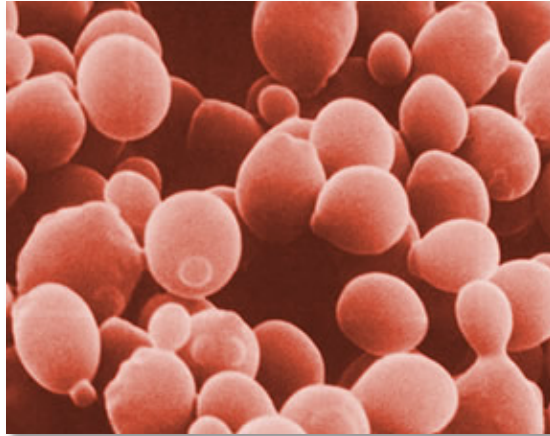
Outline

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

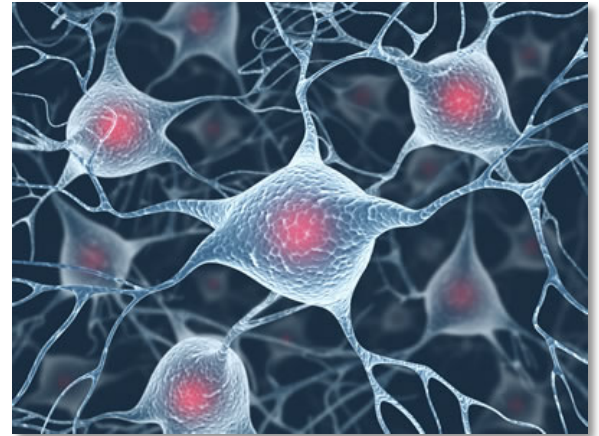
Platforms for production of high value recombinant proteins



bacteria



yeasts



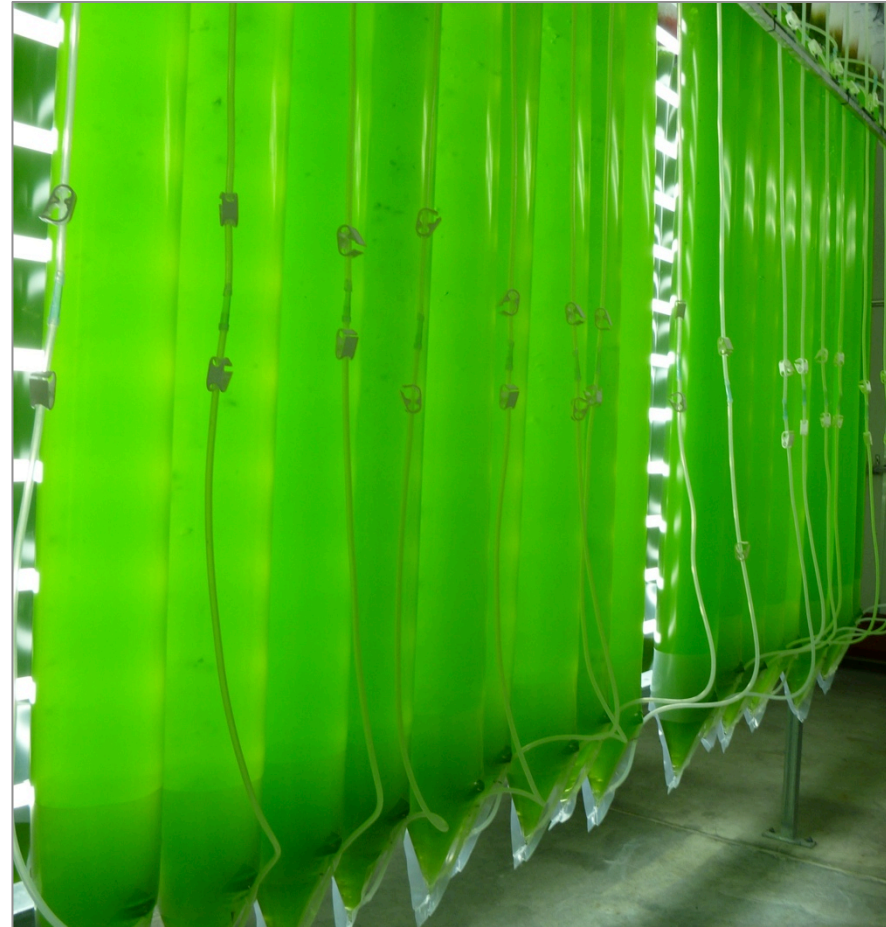
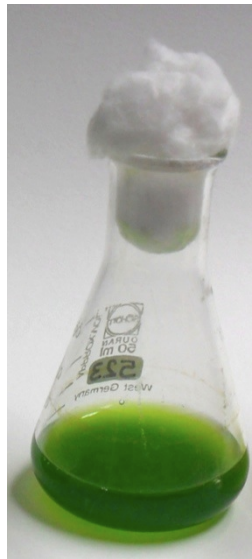
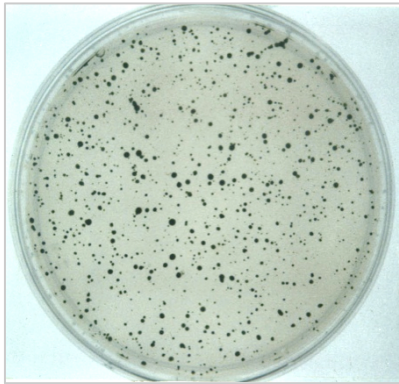
mammalian cells



algae ?

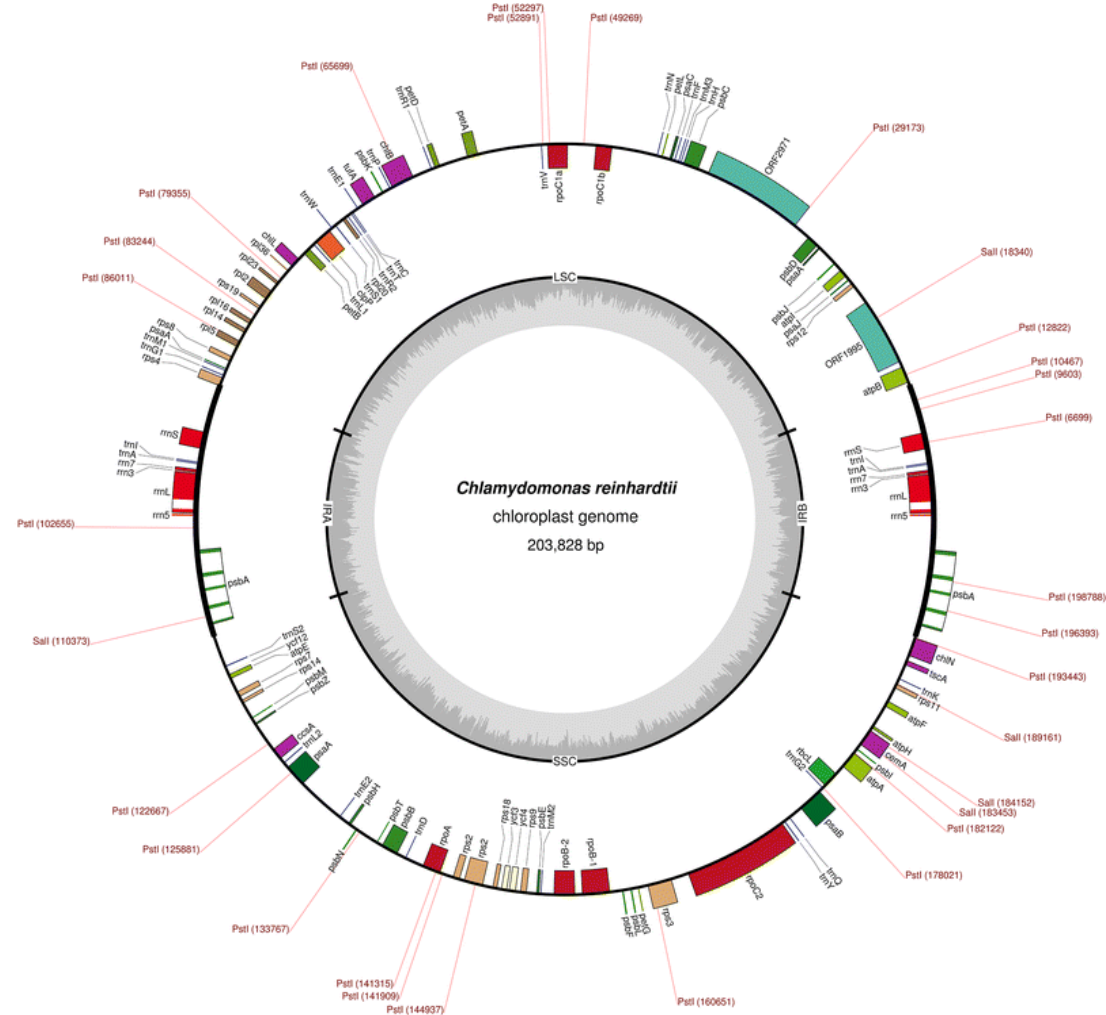
Microalgae: an attractive platform

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



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

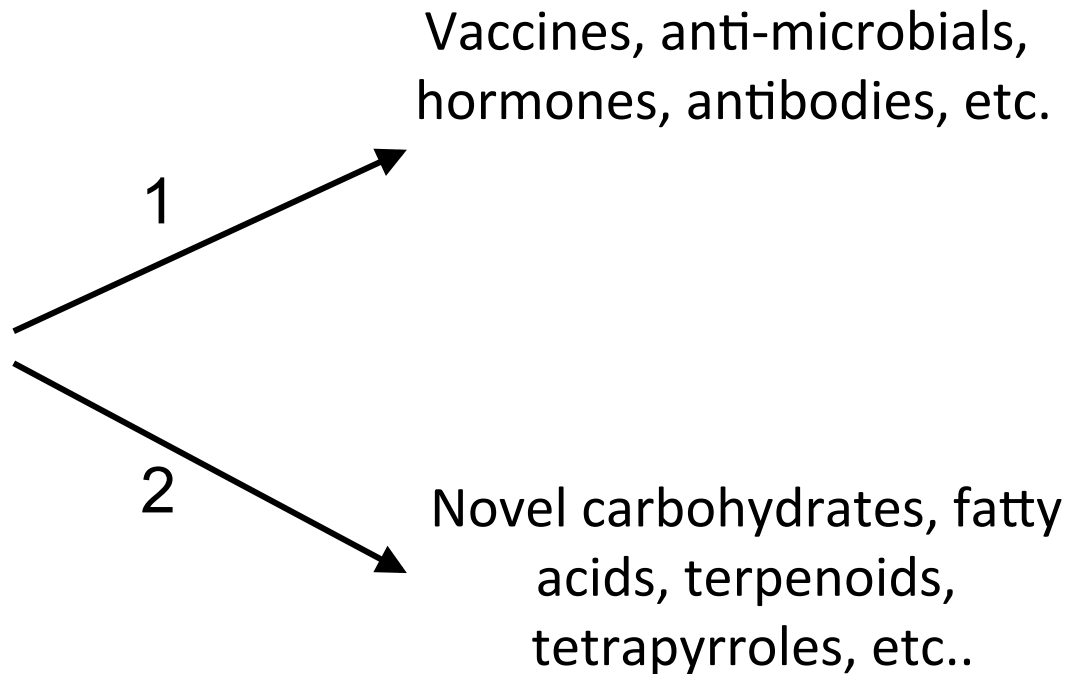


Porphyridium sp.

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



*Chlamydomonas
reinhardtii*



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

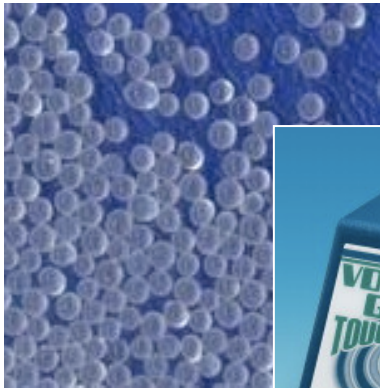
Engineering the chloroplast genome: Techniques and capabilities for chloroplast transformation in *Chlamydomonas reinhardtii*

(cotransformation/particle gun/bombardment/algae)

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Communicated by André T. Jagendorf, November 19, 1990 (received for review October 5, 1990)



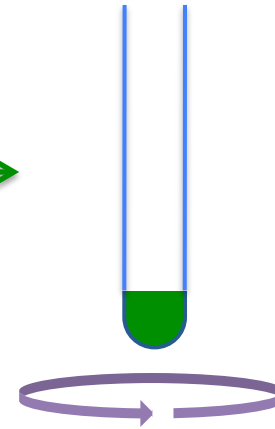
Vortexing cell/DNA suspension with glass beads

Cheap, quick and simple

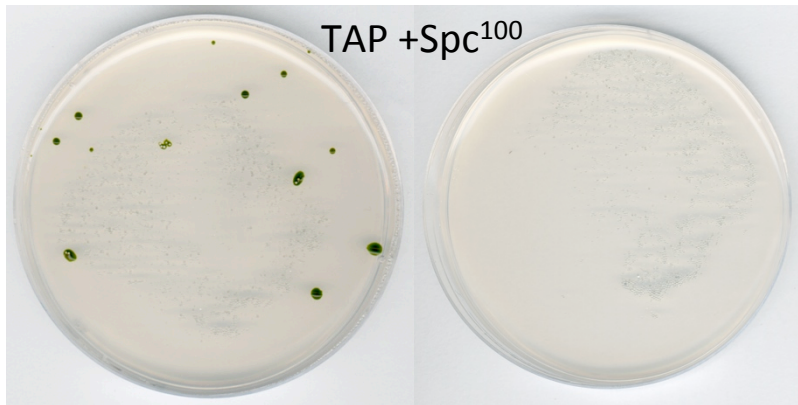


Remove cell wall
with autolysin

... or use a cell wall
deficient mutant

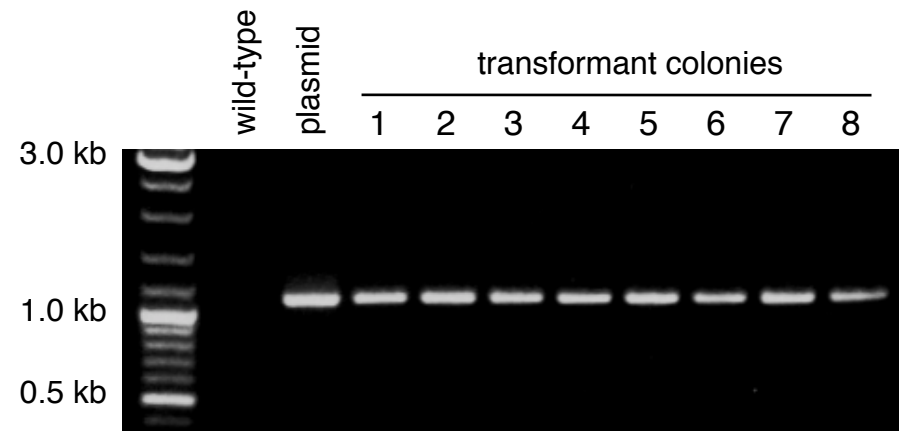
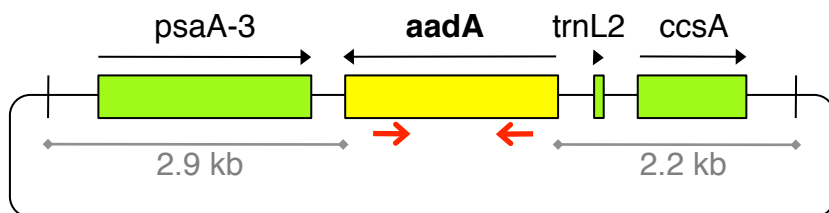


Vortex cell suspension
with 5 μ g DNA and glass
beads, 15 s

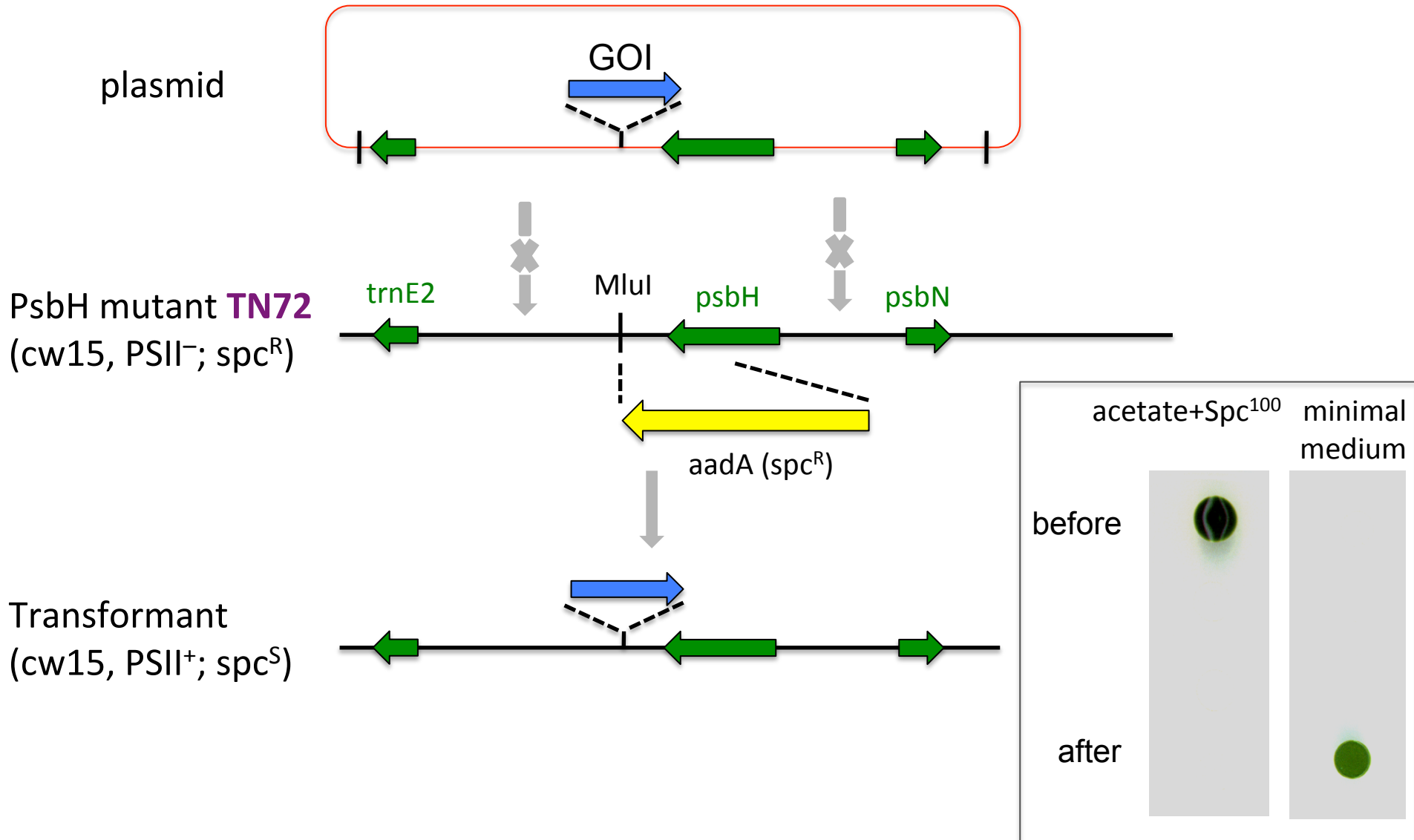


+ DNA

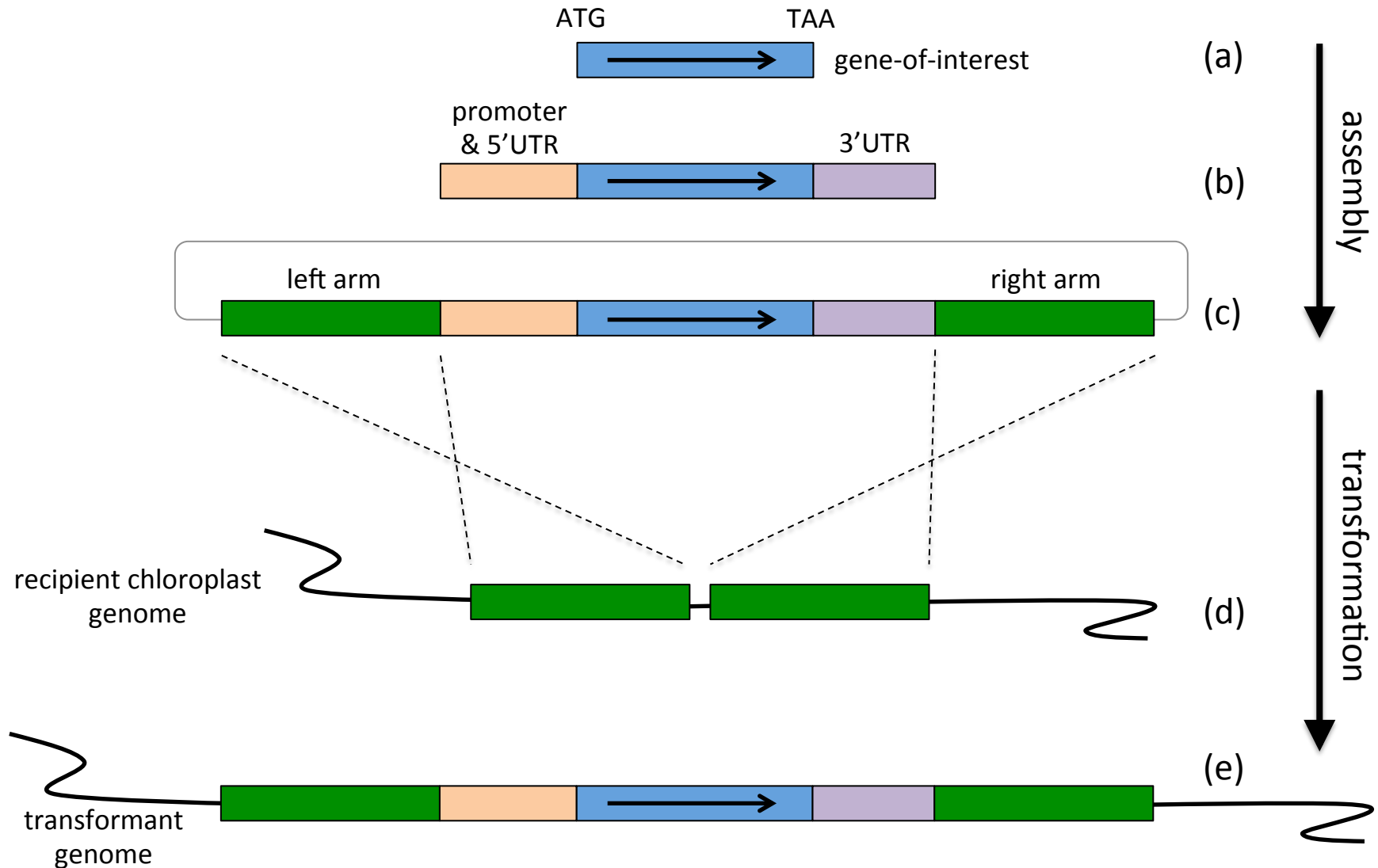
- DNA



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

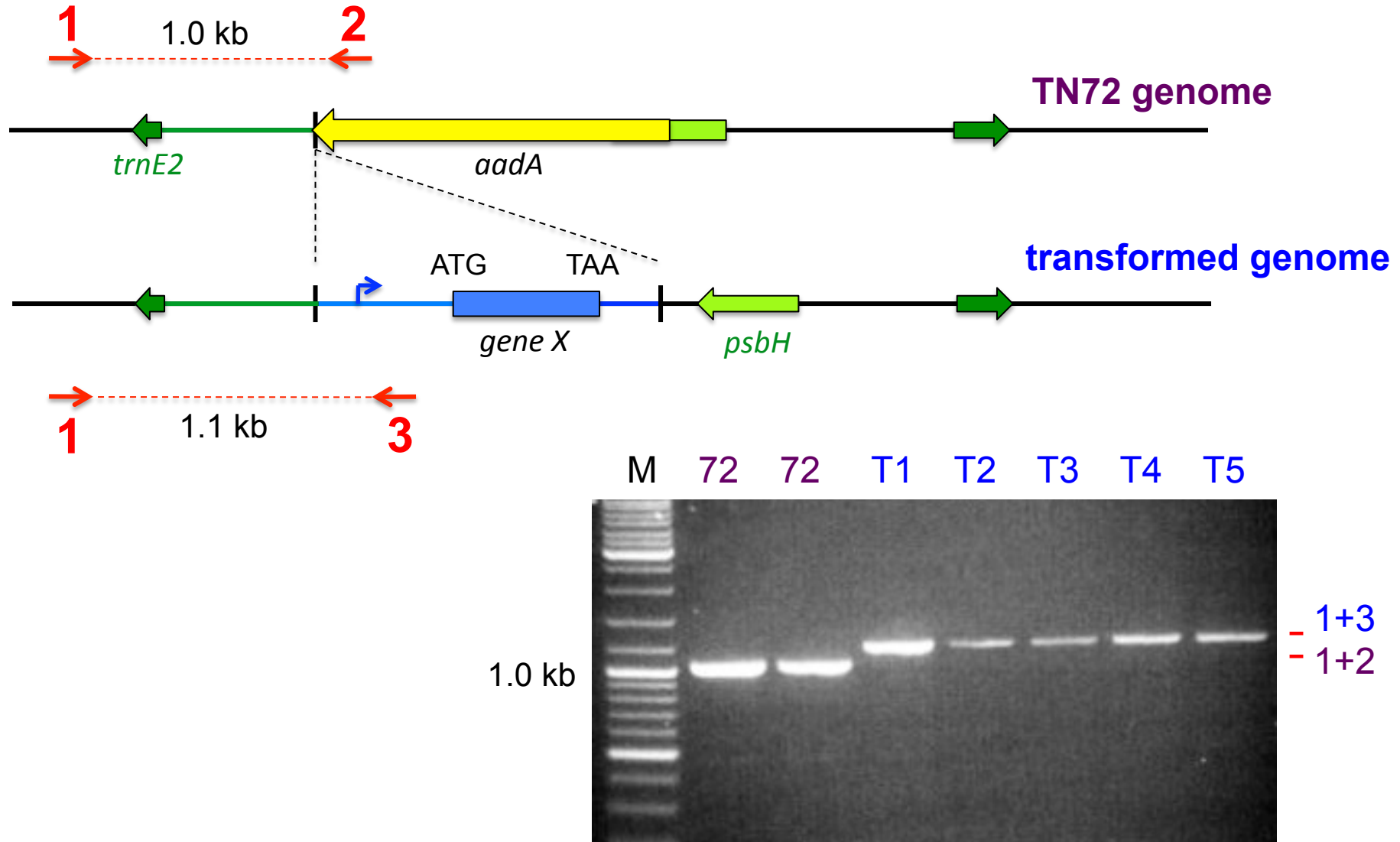


Transforming DNA requires assembly of multiple parts

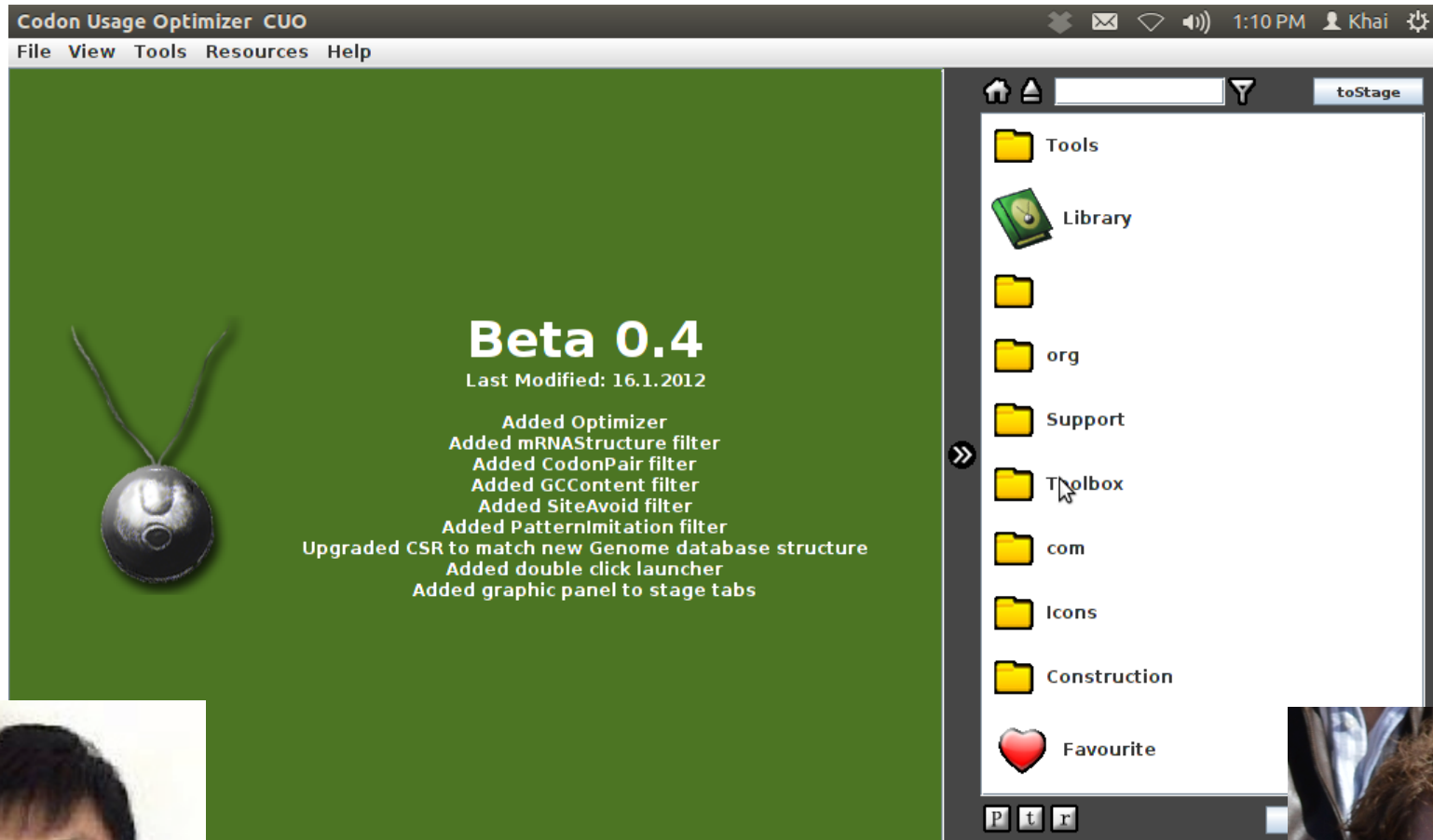


Gibson method allows seamless one-step assembly of all the parts without using any restriction enzymes.

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

Codon Usage Optimizer - CUO

File Tools Help Version

chlamydomonas chloroplast Nucleotide

1576 found. 20 shown.

Beta 0.92
Last Modified: 14.10.2012

Kasuzo Table parser added
Edit Table function added
View Table function added
Translator added
Plot graph function in Moptimizier
Operation Panel improved
Tutorials added

New developers on board!

Coding Sequence Retriever

Pinus thunbergii chloroplast genes for tRNA-Gln, tRNA-Lys, tRNA-Ile, tRNA-His and photosystem II D1
Length: 7440 RefSeq: D11467 GI: 344007
Create Date: 1993/08/04 Update Date: 2013/04/19

Arabidopsis thaliana chromosome 2, complete sequence
Length: 18585056 RefSeq: CP002687 GI: 332656411
Create Date: 2011/05/11 Update Date: 2013/04/12

Sequence 136 from Patent EP2477478
Length: 2996 RefSeq: J8048496 GI: 475797135
Create Date: 2013/04/04 Update Date: 2013/04/04

Chlamydomonas sp. ICE-L chloroplast stearyl-ACP-desaturase (FAB2) mRNA, complete cds; nuclear ge
Length: 1223 RefSeq: KC012988 GI: 451988480
Create Date: 2013/02/26 Update Date: 2013/02/26

Mycobacterium tuberculosis H37Rv complete genome
Length: 4411532 RefSeq: NC_000962 GI: 448814763
Create Date: 2001/09/07 Update Date: 2013/02/15

Chlamydomonas reinhardtii chloroplast cytochrome c synthesis 2 (Ccs2) mRNA, complete cds; nuclear ge
Length: 5163 RefSeq: KC292647 GI: 443910008
Create Date: 2013/02/02 Update Date: 2013/02/02

Mycobacterium tuberculosis H37Rv complete genome
Length: 4411532 RefSeq: AL123456 GI: 444893469
Create Date: 2002/07/18 Update Date: 2013/01/31

Chlamydomonas reinhardtii chloroplast SRP43 (cpSRP43) mRNA, complete cds; nuclear gene for chlorop
Length: 1293 RefSeq: KC331036 GI: 442571675
Create Date: 2013/01/21 Update Date: 2013/01/21

TPA inf: Chlamydomonas reinhardtii chloroplast, complete genome
Length: 203828 RefSeq: BK000554 GI: 32880373
Create Date: 2003/02/07 Update Date: 2006/02/06

Syntrophobotulus glycolicus DSM 8271 chromosome, complete genome
Length: 3406739 RefSeq: NC_015172 GI: 325288201
Create Date: 2011/03/04 Update Date: 2012/12/31

Pantoea ananatis LMG 20103 chromosome, complete genome
Length: 4703373 RefSeq: NC_013956 GI: 332139403
Create Date: 2010/03/26 Update Date: 2012/12/24

Halomonas elongata DSM 2581 chromosome, complete genome
Length: 4061296 RefSeq: NC_014532 GI: 307543589
Create Date: 2010/09/17 Update Date: 2012/12/24

Select All Compact Retrieve

toStage

Chlamydomonas reinhardtii chloroplast, complete genome
Length: 203828 Create: 23-JAN-2004
Update: 01-JAN-1900

Chlamydomonas reinhardtii mitochondrion, complete genome
Length: 15758 Create: 02-NOV-1994
Update: 01-FEB-2010

debug
Length: 32

FKBP_F36V
Length: 321

qCVN
Length: 309

qCVN
Length: 309

test
Length: 309

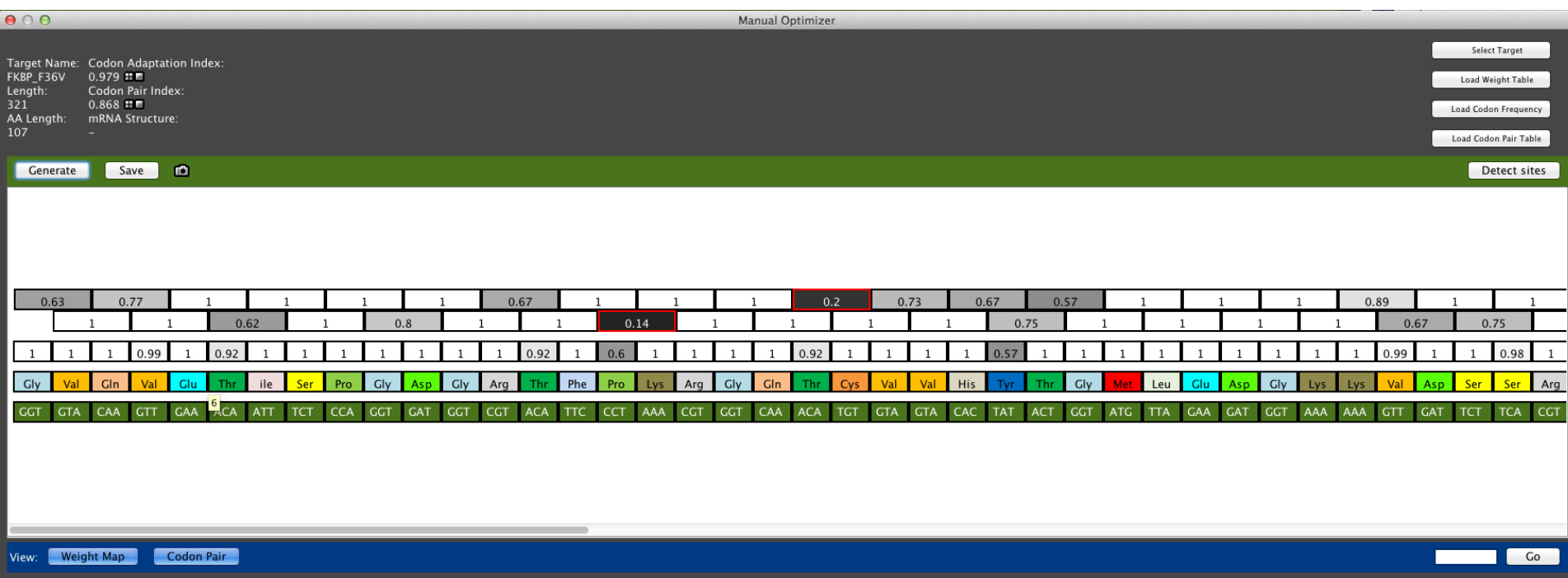
prot
Length: 57

Sample 3
Length: 4167 Fragments: 7

qCVN
Length: 309

11 found Select Inverse

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



Also takes into account:

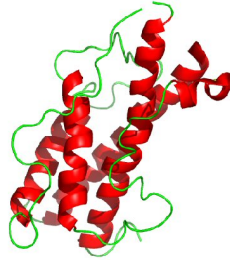
- codon pair thresholds
- Unwanted runs of the same codon
- Any unwanted restriction sites

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

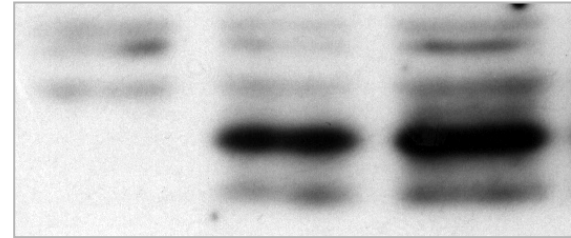
0.08	0	1	0.8				
	0	1	1				
1	0.03	0.03	1	1	1	1	0.3
Met	Gly	Arg	Ala	Thr	Pro	Trp	ile
ATG	GGA	AGA	GCT	ACT	CCA	TGG	ATC

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

human growth hormone

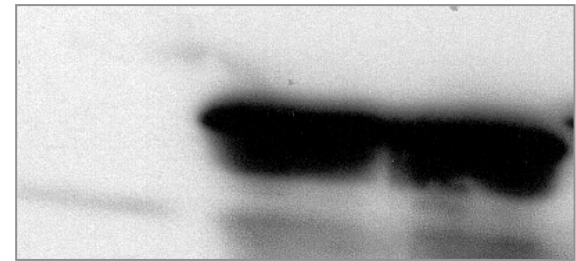
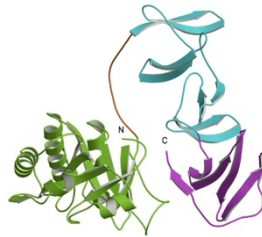


Δ psbH T1 T2

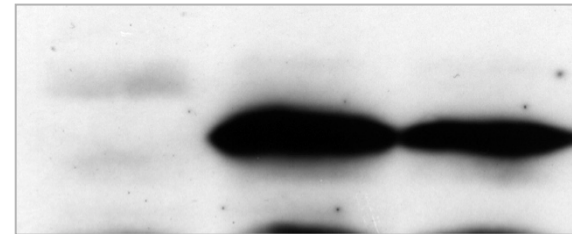
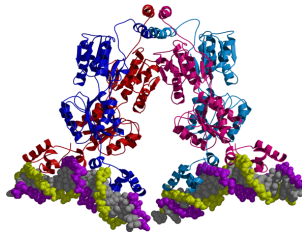


bacteriophage endolysin

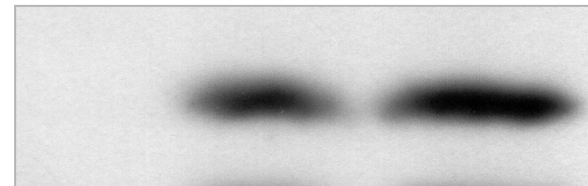
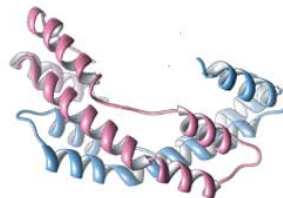
A new class of antibiotics ?



bacterial repressor

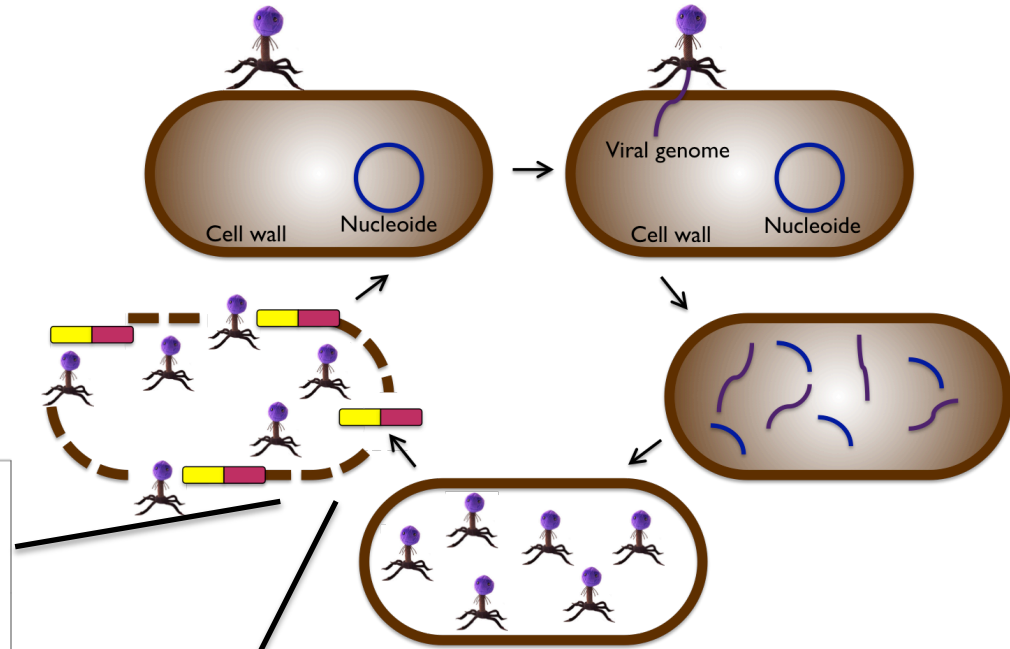
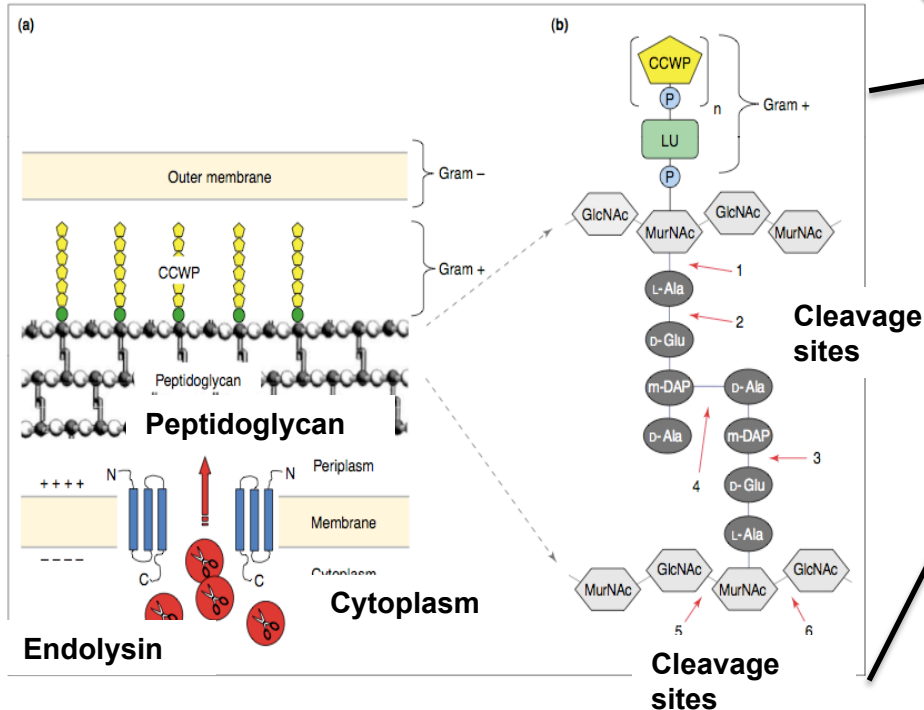


bacterial chaperone



Bacteriophage endolysins as potential antibiotics

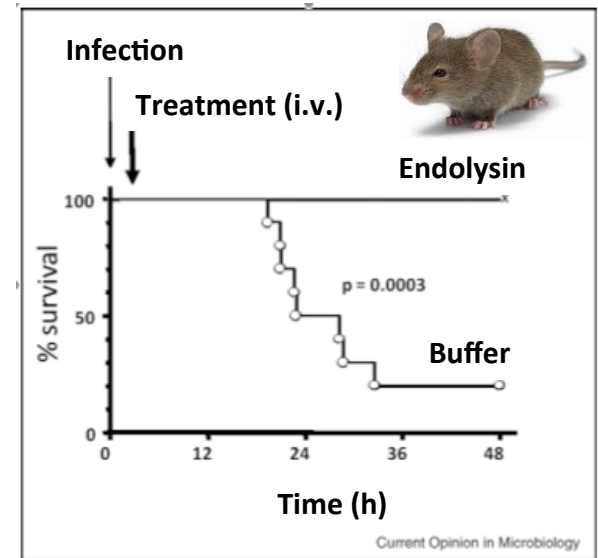
- Proteins produced in bacteriophage infected cells
- Cleave the peptidoglycan for phage progeny release at the end of the lytic cycle



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

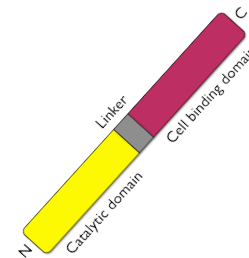
- Can act extrinsically against Gram +ve bacteria

- *In vitro*
- *In vivo* mouse models
- Active against biofilms



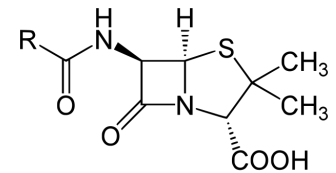
- Advantages over antibiotics

- 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

