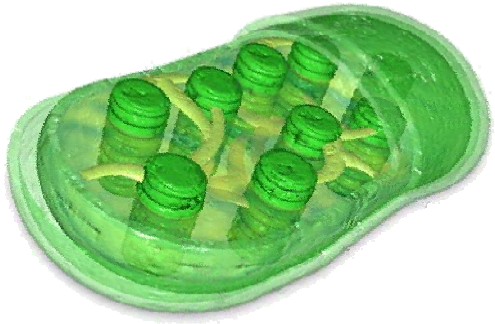
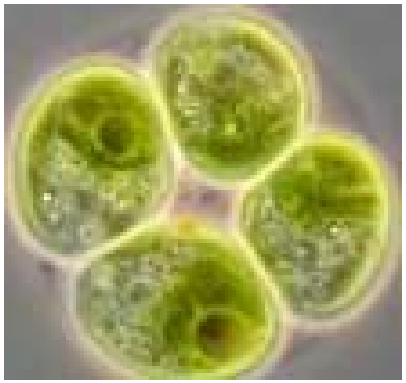
Algae Chloroplast Manipulation for the production of valuable products including biofules

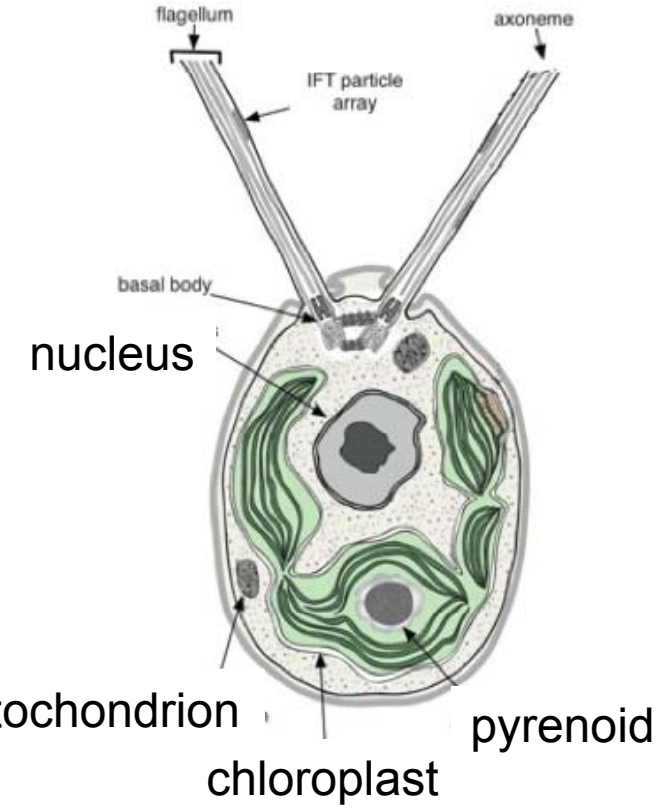
Dr. Panagiotis Madesis

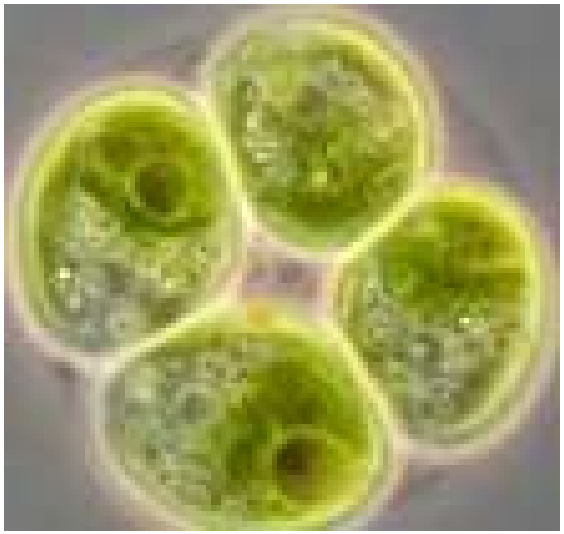
CERTH / INA





Algae structure



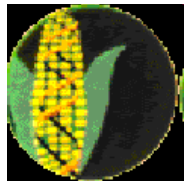


Chlamydomonas reinhardtii

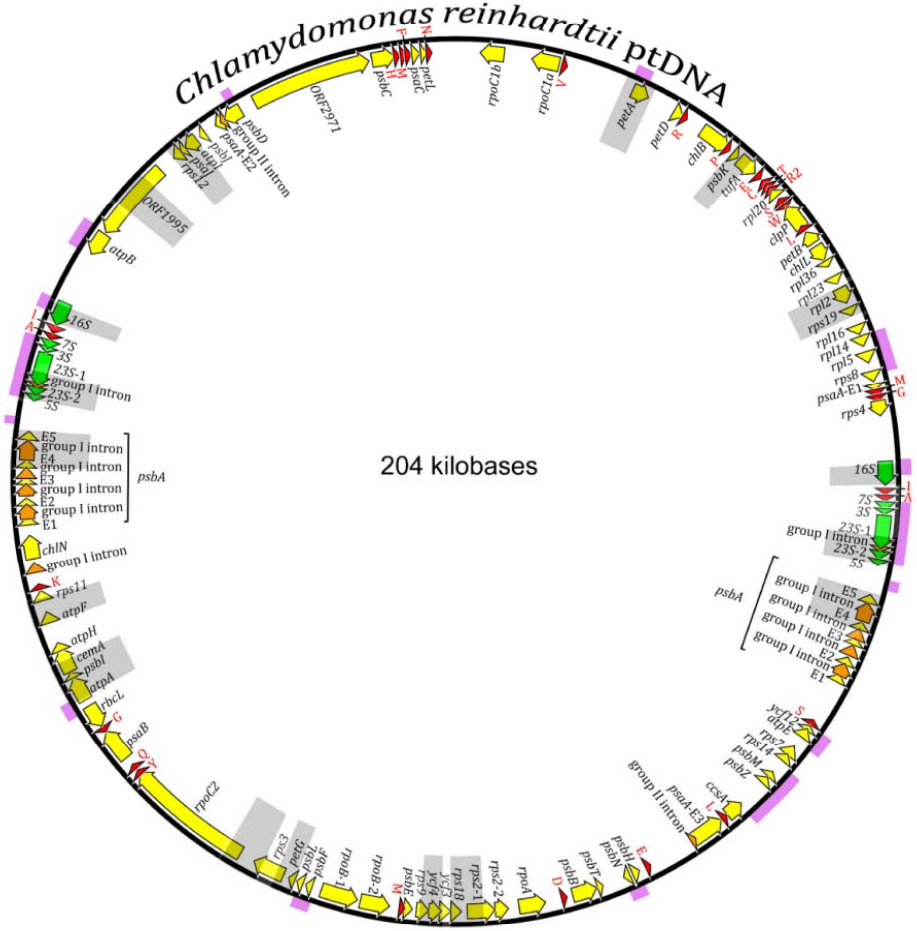
Chloroplast is the biosynthetic factory of cells

Algal cell is a big chloroplalst

Chloroplast Genome

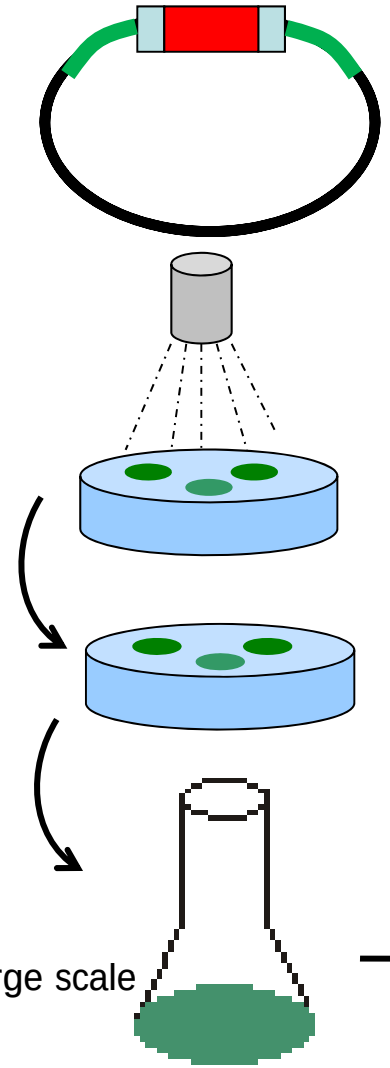


- *C. reinhardtii* contains a single large chloroplast
- The chloroplast contains its own genome, which is a circular molecule of approximately 200 kb
- each chloroplast contains approximately **<u>80</u>** identical copies of the genome
- 170 plastid genomes are available from NCBI



Algal chloroplast genomes are much more variable in organisation and gene content than those of land plants but many features are conserved

Algae chloroplast manipulation: the method



Plasmid stock purified and
used for particle bombardment
of leaf tissue

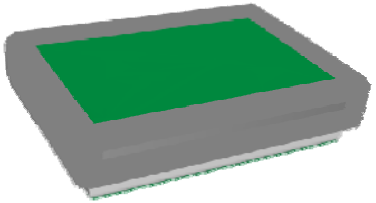
Initial transformation

Explants on regeneration medium without selection for 1- 2 days

Followed by rounds of selection to obtain homoplasmic cells

Homoplasmy - uniform population of transgenic chloroplast genomes

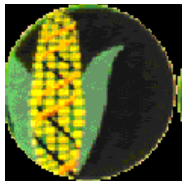
Transfer to large scale

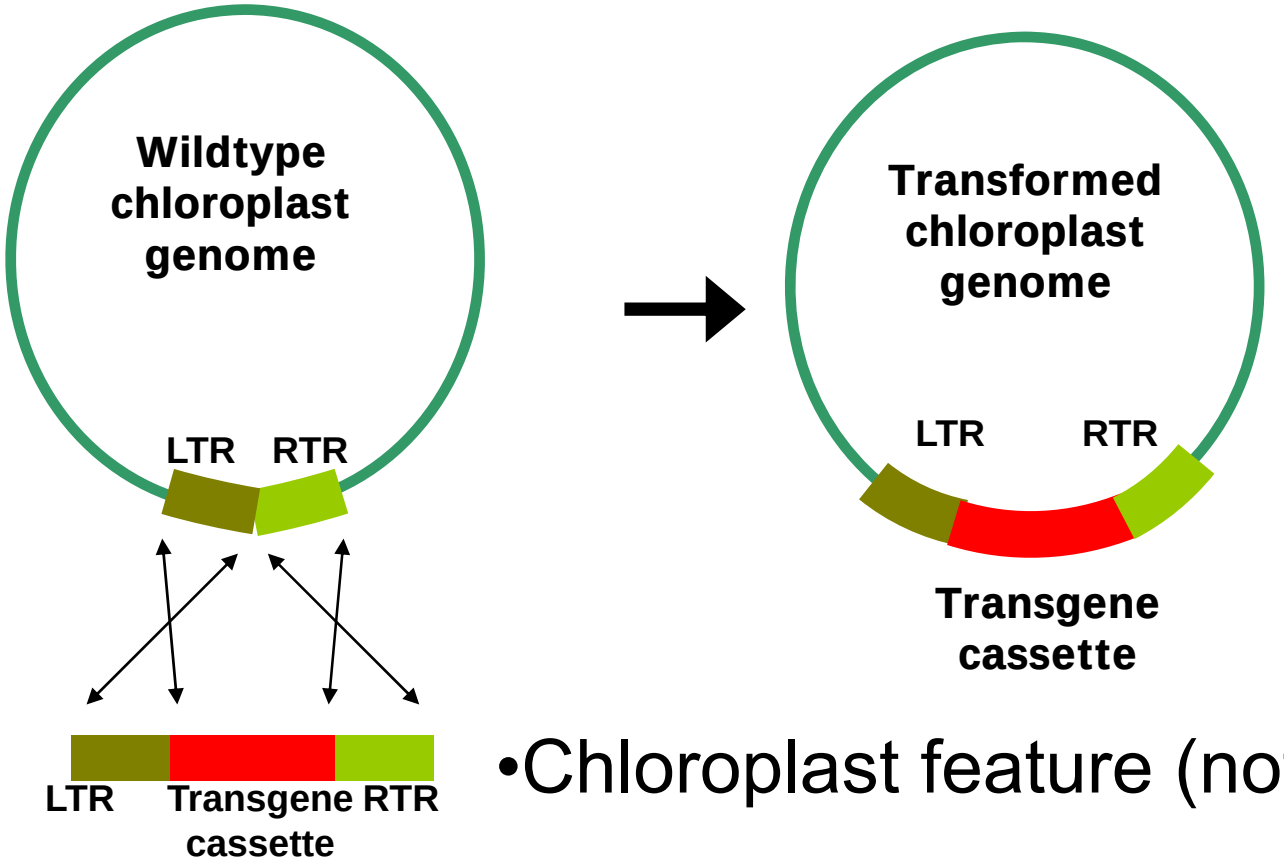


Transfer to large scale

Open or closed systems

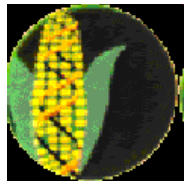
Transgene insertion proceeds by homologous recombination



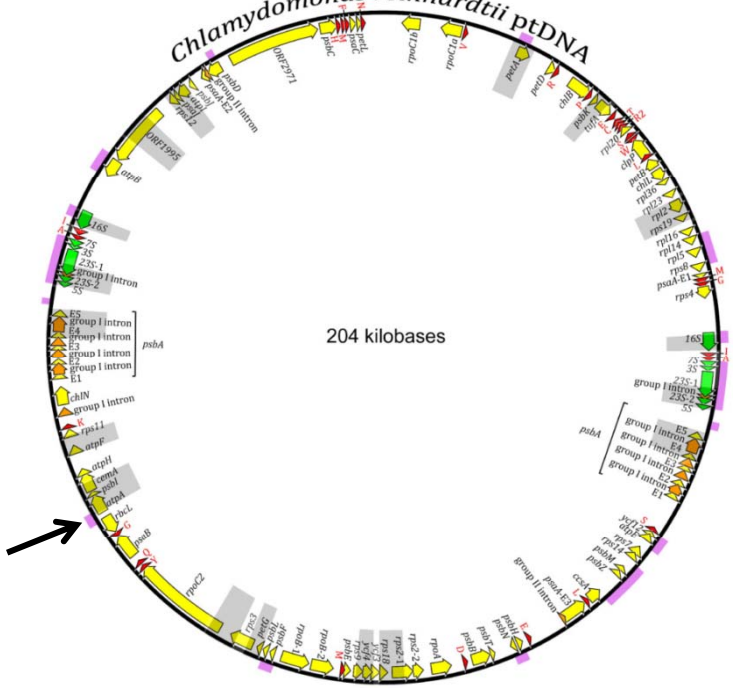


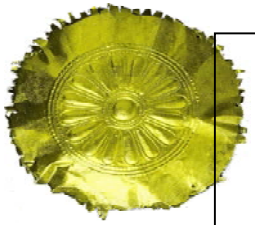
- Chloroplast feature (not nuclear)
- Targeted and stable integration
- Reproducible and precision of results

Advantages of chloroplast engineering

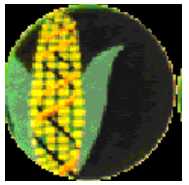


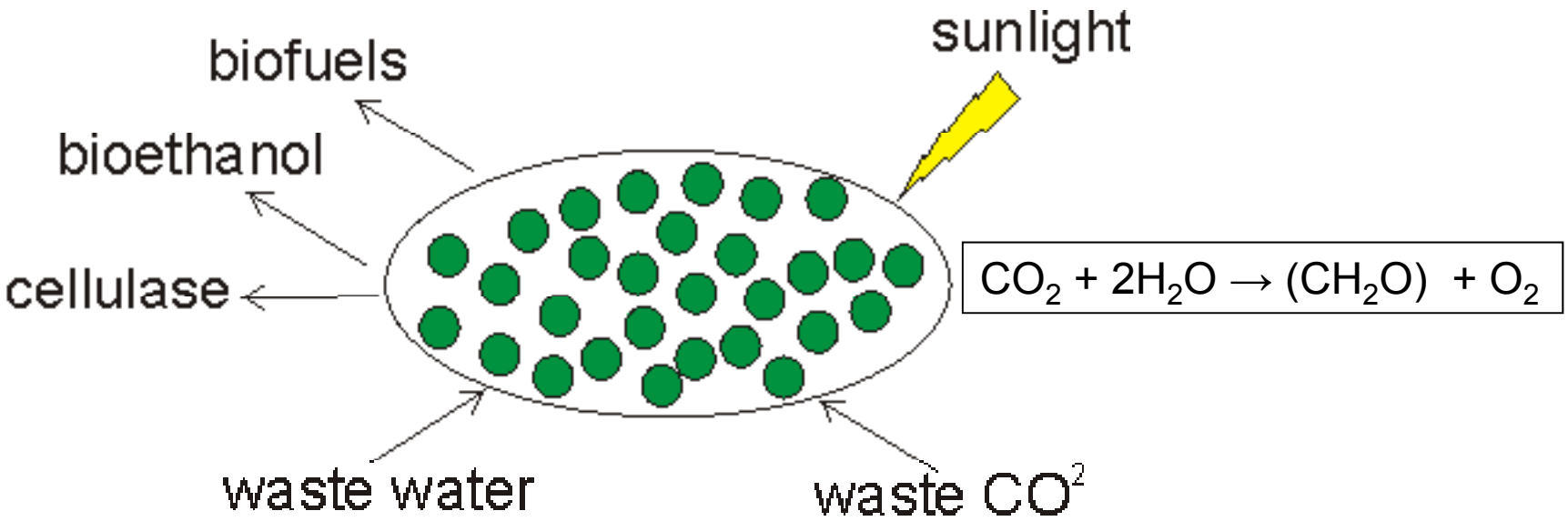
- **Very high and stable expression levels**
- **Homologous recombination enables precise engineering**
- **Gene silencing and position effects are not detected in chloroplasts – stable expression**
- **Multiple genes in a single event**
- **Clean technology**



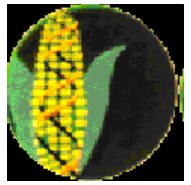


Algae chloroplast manipulation for biofuel production



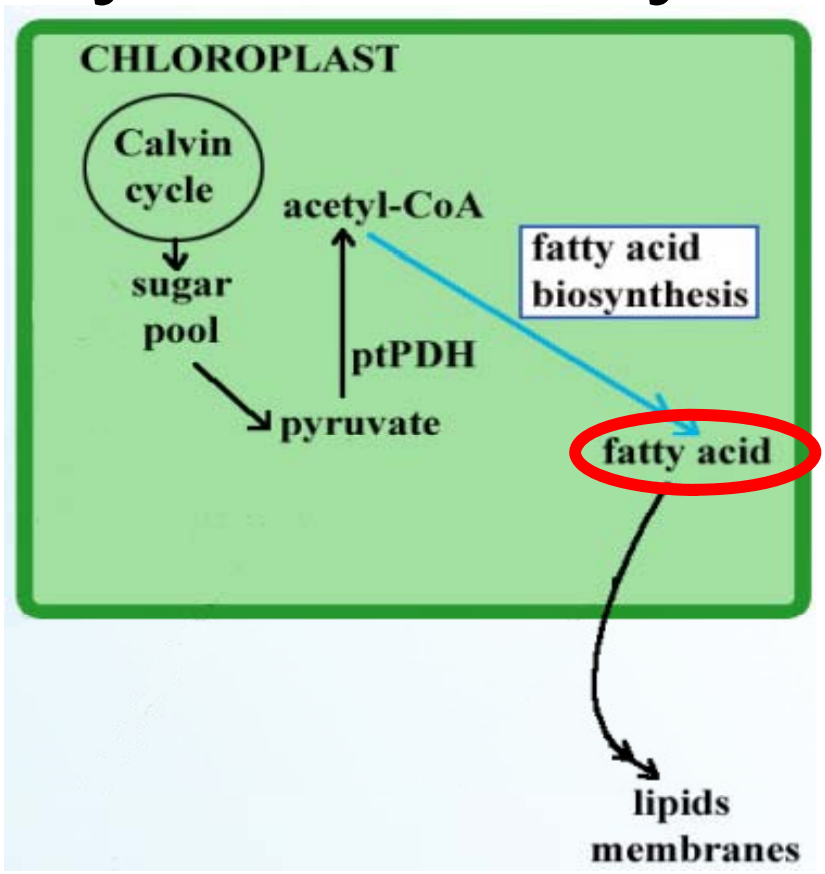


What could Chloroplast manipulation do?



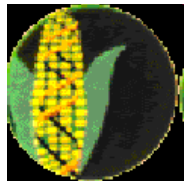
- Enhance the photosynthetic efficiency
- Increase biomass yield on light
- Increase biomass growth rate
- Increase oil content of the desired type

Chloroplast is a major site of fatty acid biosynthesis



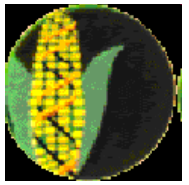
In algae de novo synthesis of fatty acids occurs primary in the chloroplast
Chloroplast transformation could fine tune the fatty acid synthesis

What could Chloroplast manipulation do?



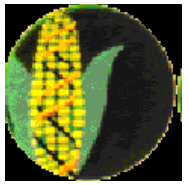
- Enhance the photosynthetic efficiency
- Increase biomass yield on light
- Increase biomass growth rate
- Increase oil content of the desired type
- Improve temperature tolerance of algae to high temperatures
- Reduce photoihibition
- Use Algae as bioreactors to produce enzymes to break down plant waists and produce bioethanol
- Alter algae to produce Hydrogen

Were the chloroplast transformation has been achieved



Stable transplastomic species	Year of publication
Chlamydomonas reinhardtii	Boynton et al 1998
Euglena gracilis	Doetsch et al 2001
Porphyridium sp.	Lapidot et al 2002

INA has experience in Chloroplast Transformation and expressing proteins in plastids



A hepatitis C virus core polypeptide expressed in chloroplasts detects anti-core antibodies in infected human sera.
Madesis P, Osathanunkul M, Georgopoulou U, Gisby MF, Mudd EA, Nianiou I Tsitoura P, Mavromara P, Tsaftaris A and Day A (in press)

Binding and Glutathione Conjugation of Porphyrinogens by Plant Glutathione Transferases[*S]

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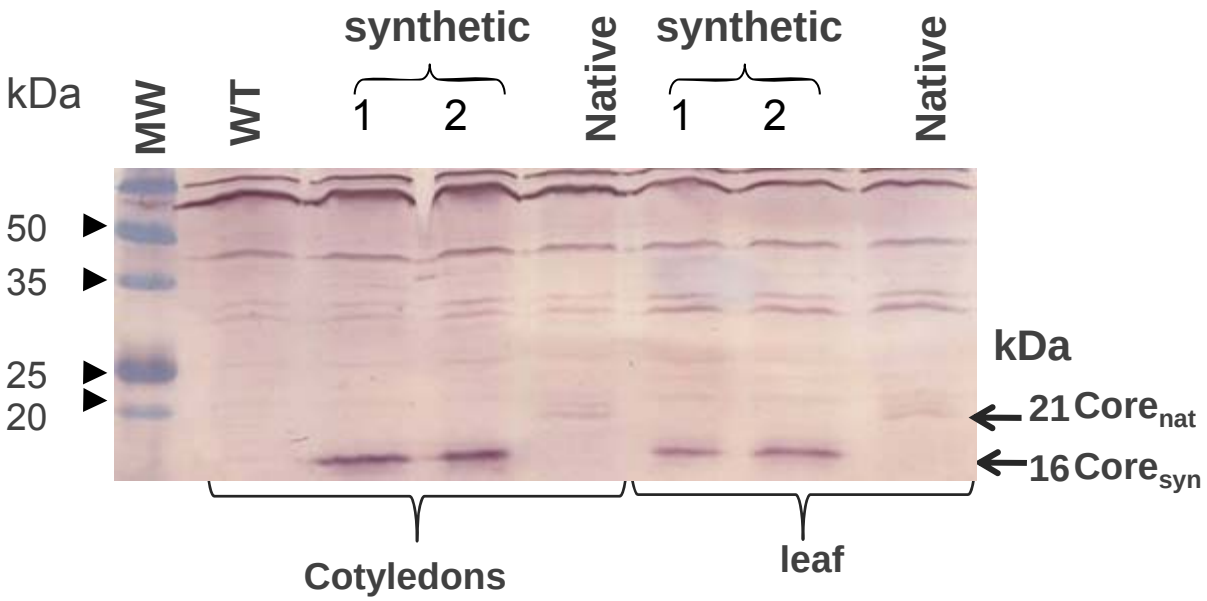
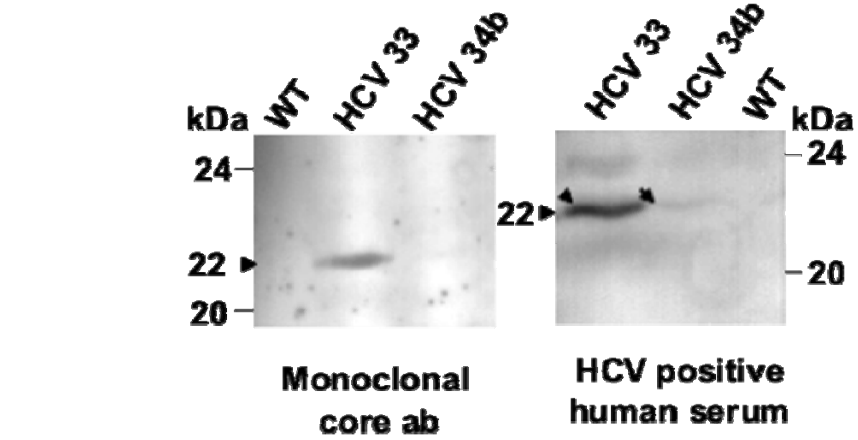
The HCV core protein

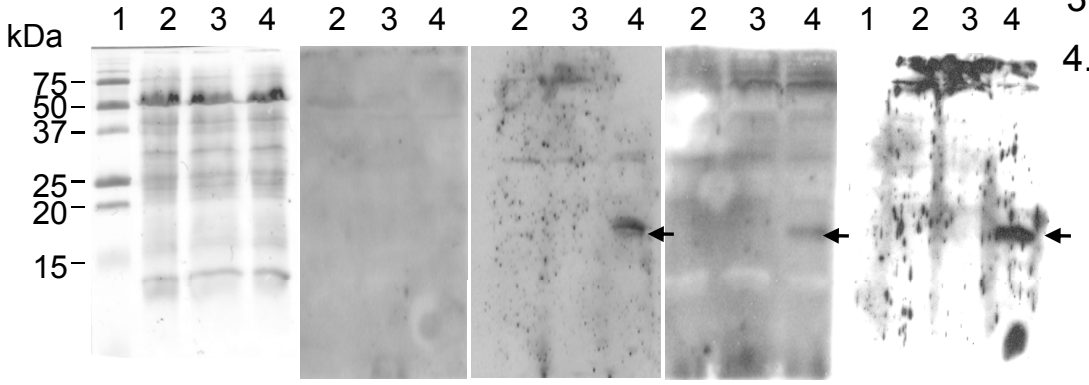


Synthetic core protein is accumulated 4 fold higher than the wt

The HCV core protein



Lanes
1. MW
2. WT
3. core^{nat}
4. core^{syn}



Ponceau red | healthy | carrier S6 HCV 1a | carrier S8 HCV 1a | carrier S HCV 3

Core protein recognizes the virus in human serum

Chloroplast manipulation in new species

Pepper (*Capsicum annum*)

Chloroplast manipulation in new species

Cucurbitaceae

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Thank you for your attention