Production of Very Long-Chain n-3 and n-6 PUFAs in Plants: Strategies and Open Questions

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Journées Chevreul 2007 - Lipids and Brain

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NAPUS 2000
Functional food from transgenic rapeseed

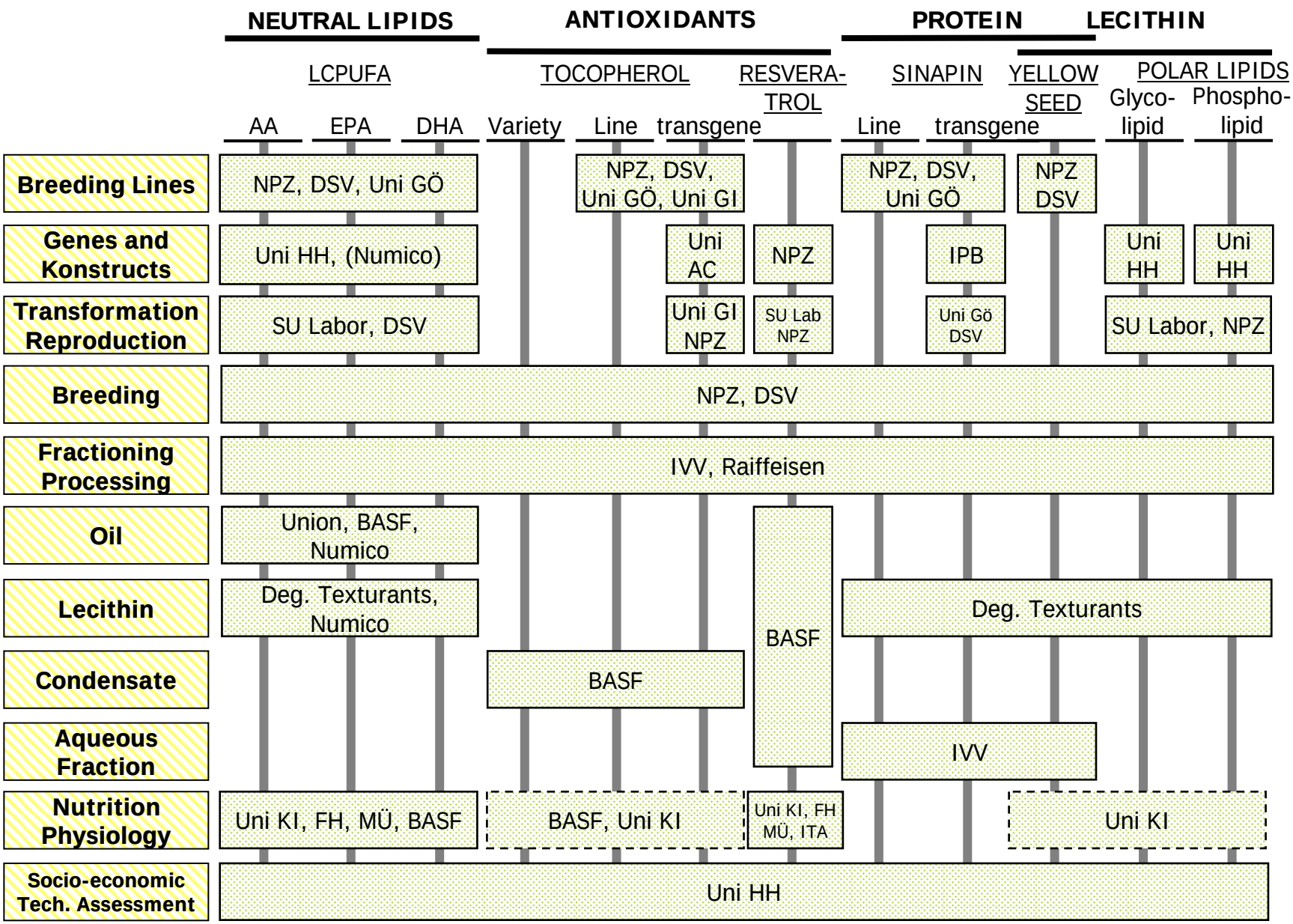
- **Leading Project Initiative of the German Federal Ministry of Education and Research** „Nutrition – modern processes for food production“
- Optimum use of the **whole** rapeseed kernel with improved quality for healthy food and as functional food
- Combine modern methods of genetic engineering with classical plant breeding to develop new varieties
- 20 partners from science, private plant breeding and industry

- Duration of sub-projects: between 2 – 5 years
- Duration of the entire project: October 1999 – November 2005
- Financial volume: 20,5 Mio. €
- Support volume: 13,6 Mio. €

Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Hohenlieth, 24363 Holtsee
Dr. Martin Frauen, Prof. Dr. Wolfgang Friedt;
Dr. Gunhild Leckband

NAPUS 2000

NAPUS 2000 - Functional Food from Transgenic Rapeseed



NAPUS 2000 – Thematic groups

Neutral lipids / LCPUFA

Fish oils and fatty acids

Optimising the fatty acid pattern of rapeseed

Tocopherol

Vitamins and oils

Accumulation of vitamin E in rapeseed oil

Resveratrol

Rapeseed and red wine

Integration of highly effective resveratrol in rapeseed

Protein

Yellow Seed and low sinapine

Use of rapeseed protein for human nutrition

Polarlipids / Lecithin

Use of rapeseed lecithin as emulsifier

Market for LCPUFA in Europe

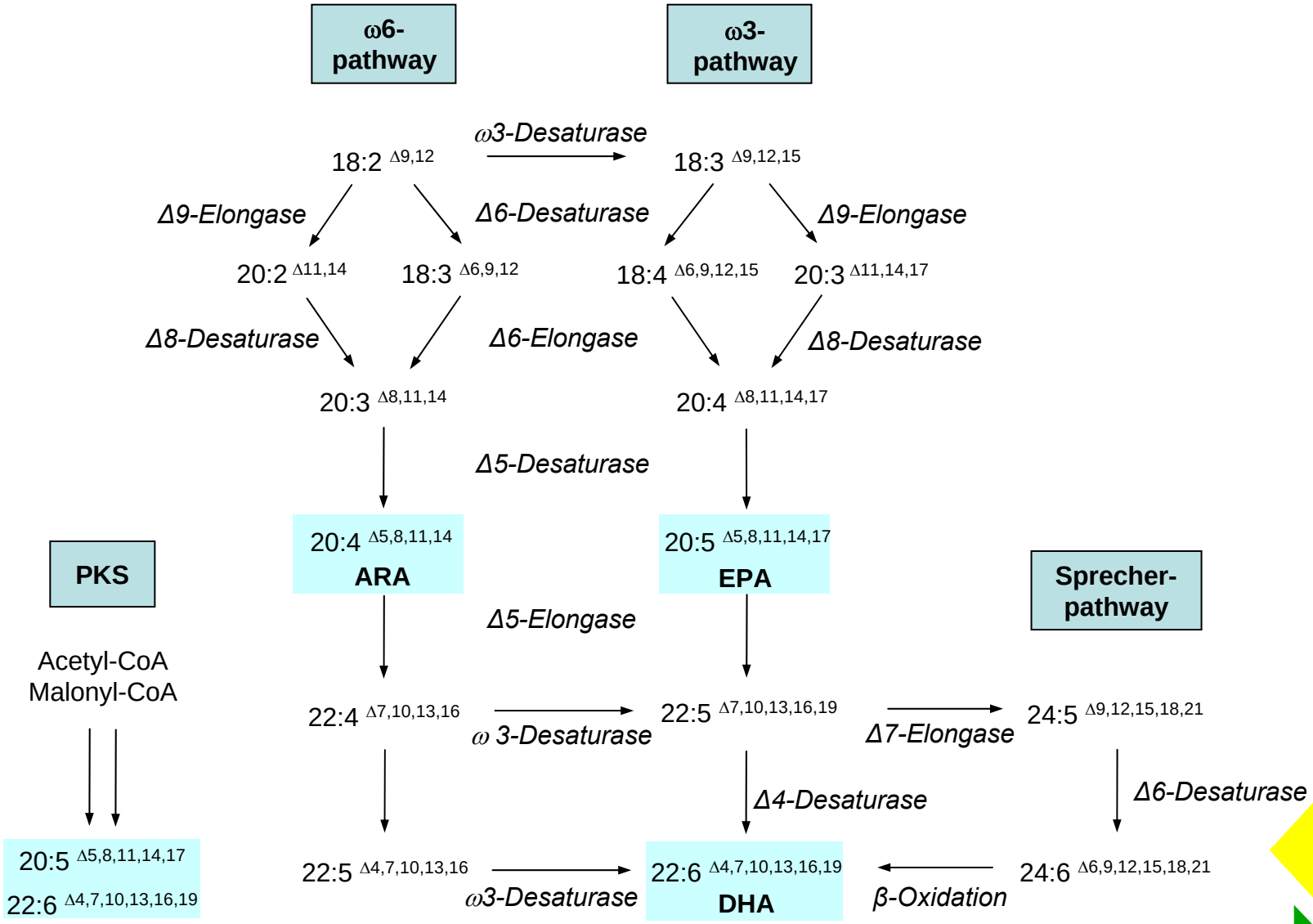
European Market for Omega-3- und Omega-6-LCPUFA Forecast 2010; in Million US-Dollar

Year	Return (in Million US-Dollar)
2003	176
2004	195
2005	216
2006	240
2007	250
2008	266
2009	276
2010	298

Source: Frost & Sullivan Report B329 (07/04)

US-Market (2006): 549,6 M $

Alternative routes for LCPUFA biosynthesis



Alternatives for ARA/EPA biosynthesis (DHA biosynthesis requires another elongation cycle)

Δ9-elo/Δ8-des

Δ5,8,11,14-20:4-PC

Δ5-Des

Δ8,11,14-20:3-PC

Δ8-Des

Δ11,14-20:2-CoA

Δ11,14-20:2-PC

Δ9-Elo

18:2-CoA

18:2-PC

Δ6-Des

Δ6,9,12-18:3-CoA

Δ6-Elo

Δ8,11,14-20:3-CoA

Δ5-Des

Δ5,8,11,14-20:4-CoA

Δ6-Des

Δ6,9,12-18:3-PC

Δ6,9,12-18:3-CoA

Δ6-Elo

Δ8,11,14-20:3-PC

Δ8,11,14-20:3-CoA

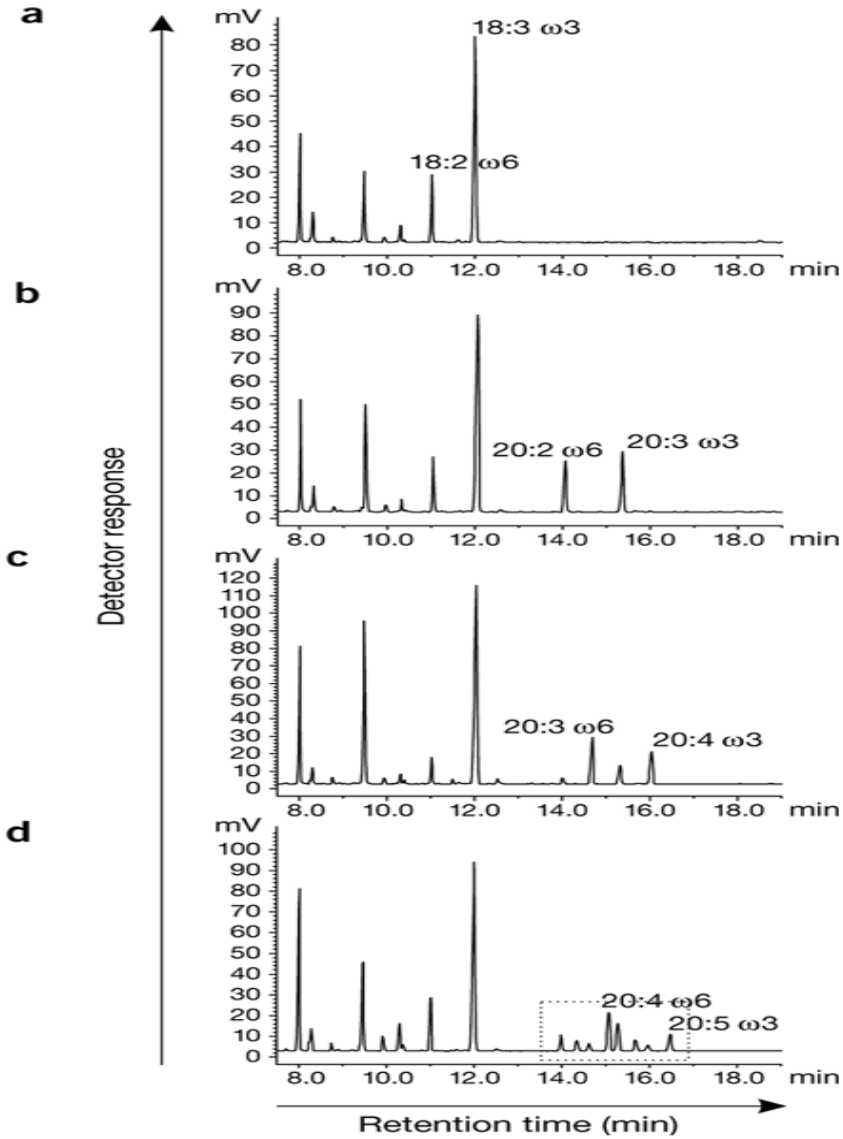
Δ5-Des

Δ5,8,11,14-20:4-PC

thioester desaturation

lipid-linked desaturation

LCPUFA biosynthesis in *Arabidopsis* leaves *via* the D9-elongase-dependent alternative



wt

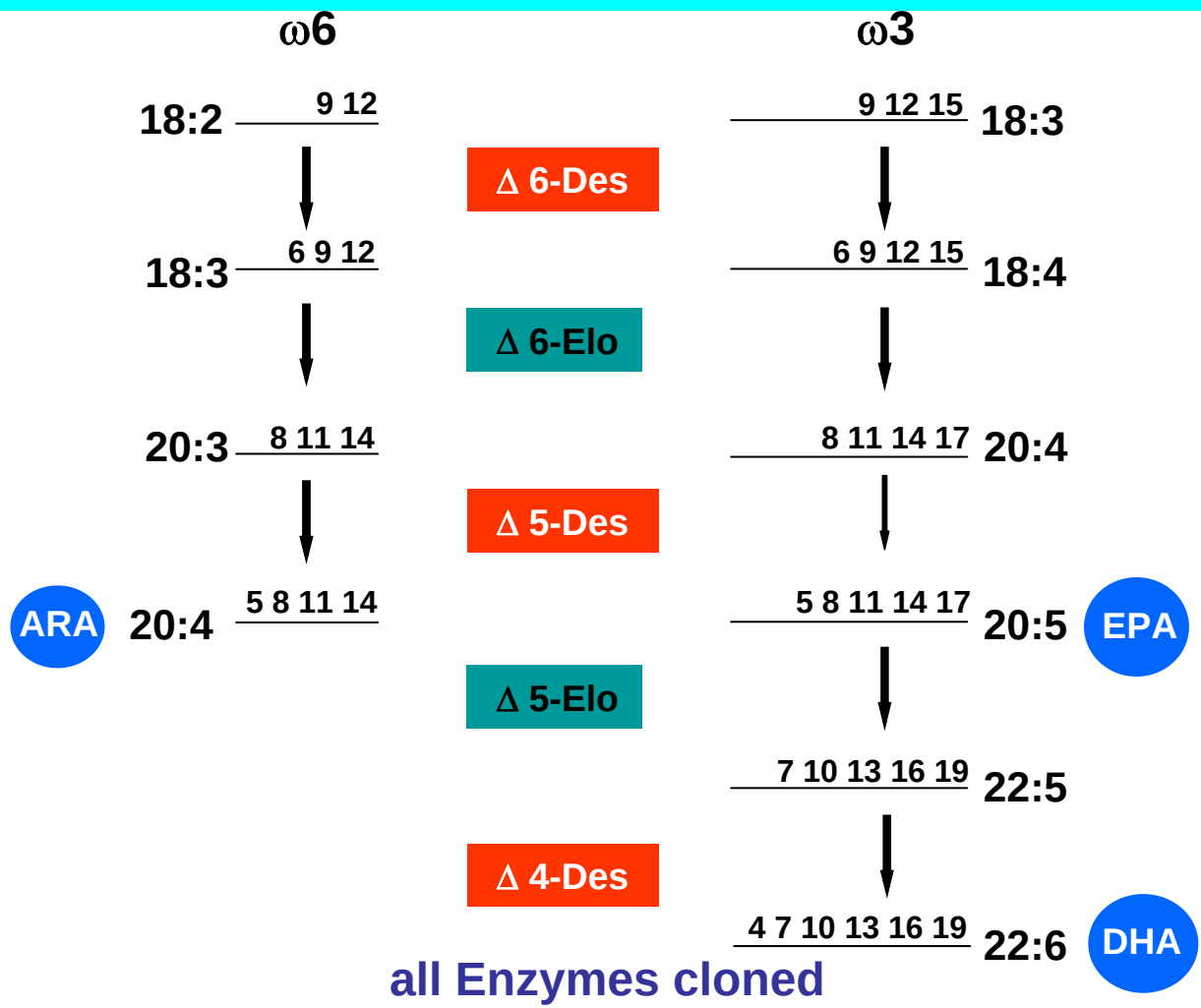
+ Δ9Elo

+ Δ9Elo + Δ8Des

+ Δ9Elo + Δ8Des + Δ5Des

Qi et al., Nature Biotechnol.2004

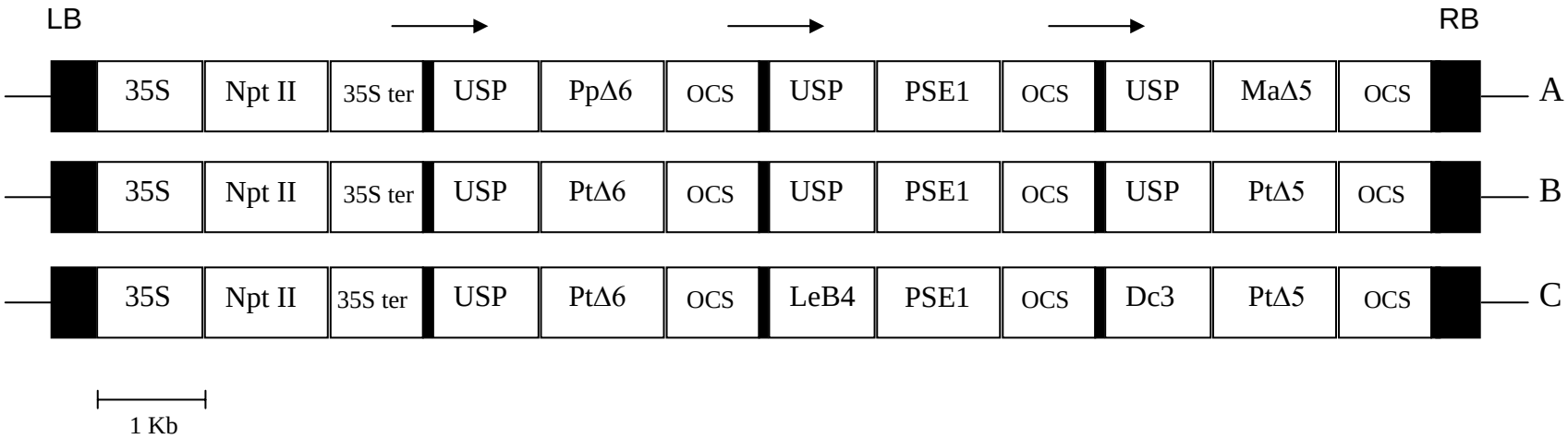
LCPUFA–Biosynthesis: The D6-Desaturase Pathway



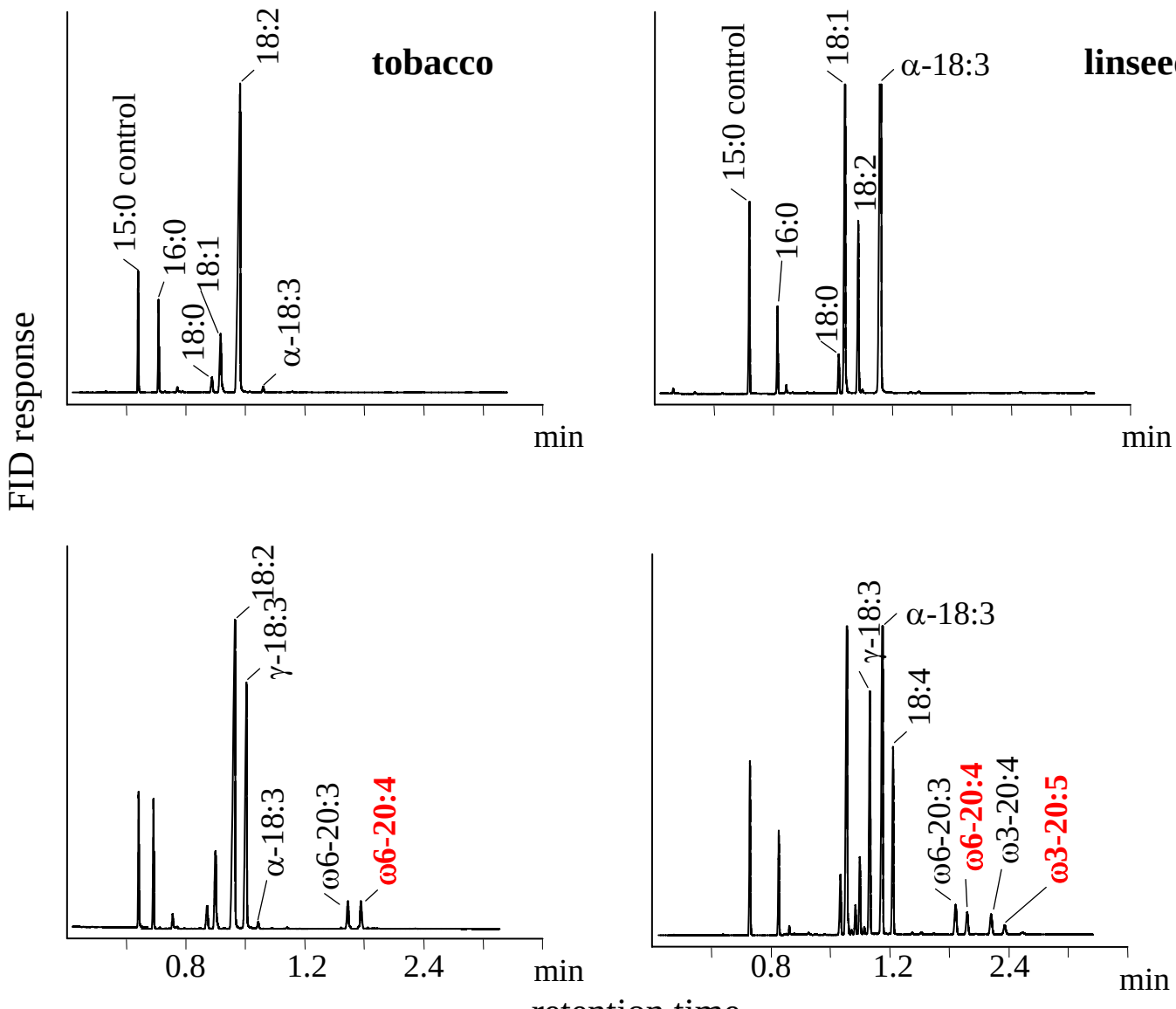
all Enzymes cloned

Δ9-Desaturase	ω3-Desaturase	Δ5-Desaturase
Δ12-Desaturase	Δ6-Desaturase	Δ5-Elongase
Δ15-Desaturase	Δ6-Elongase	Δ4-Desaturase

LCPUFA – The Strategy



Seeds from homozygous single-copy transformants



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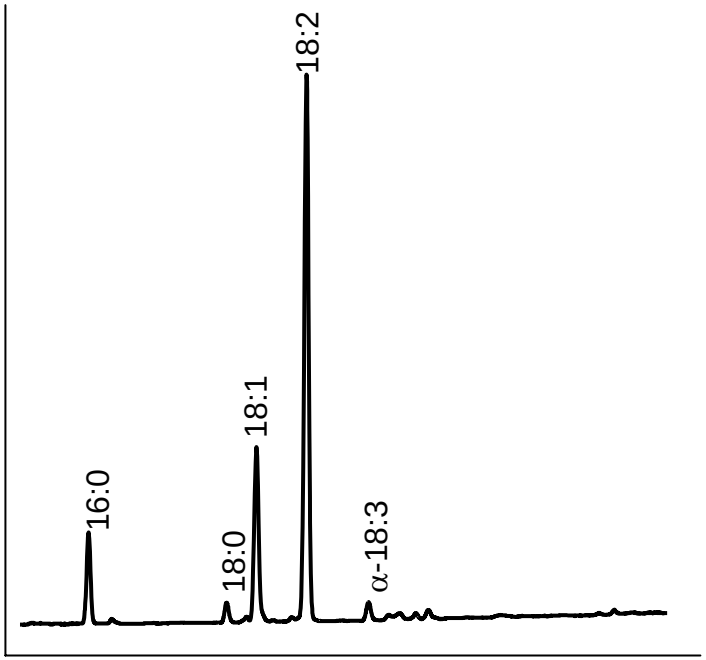
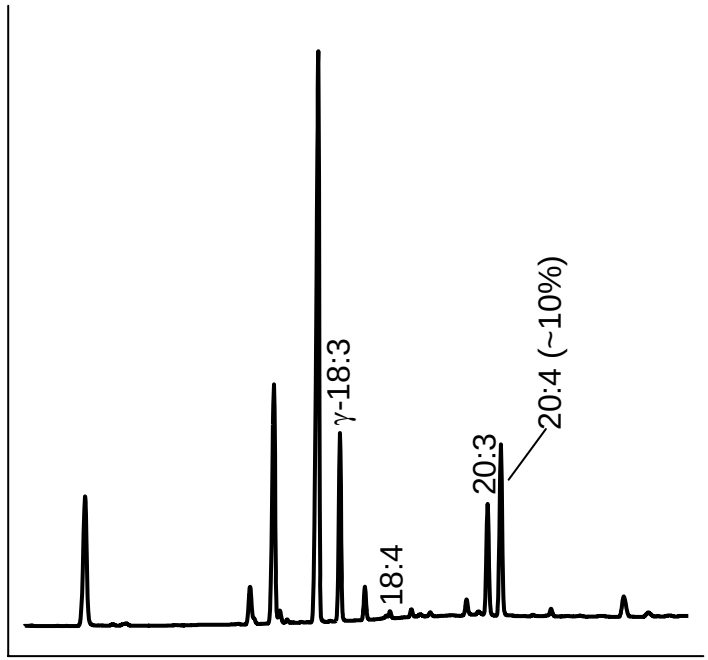
LCPUFA in Plants

Wt (Solin high 18:2)

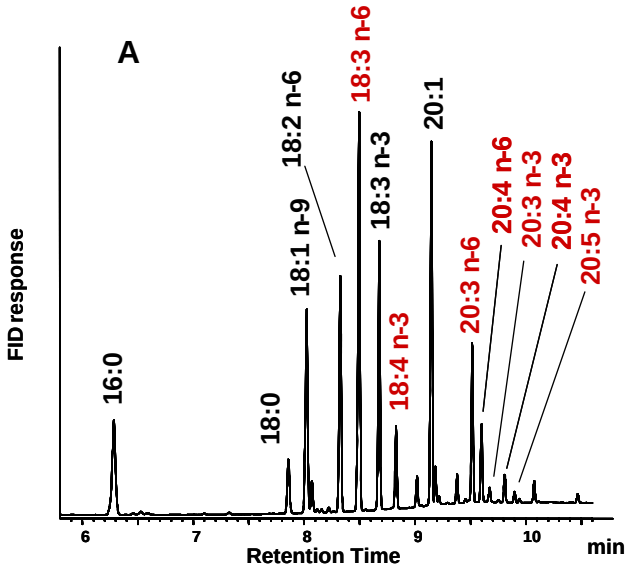


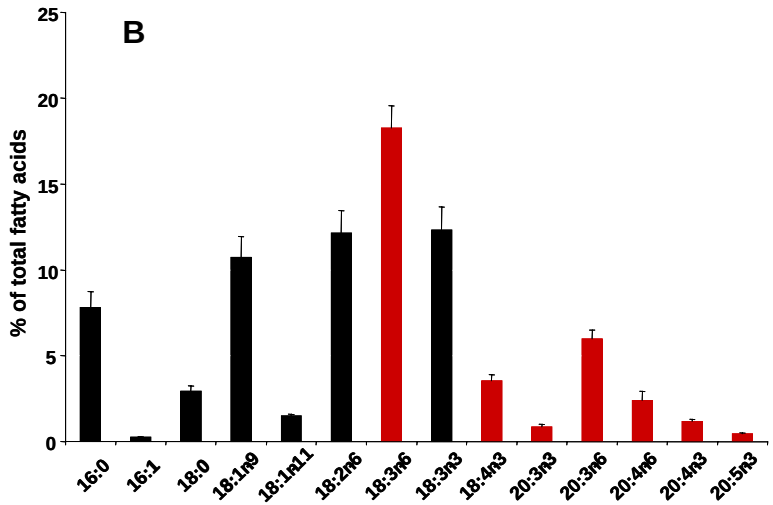
Transgenic (Linseed)



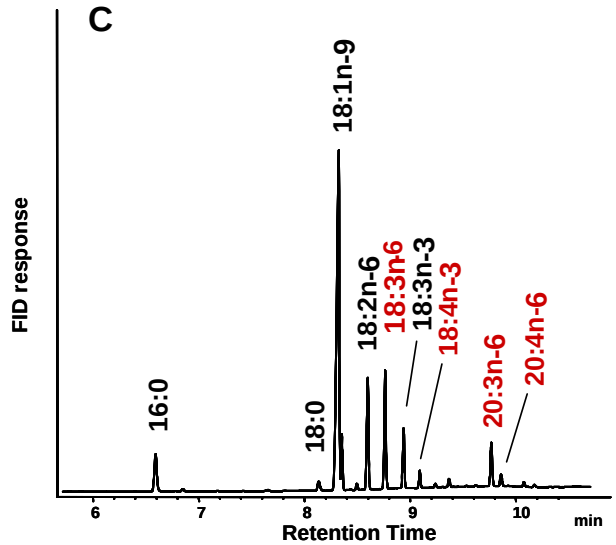
LCPUFA in Plants

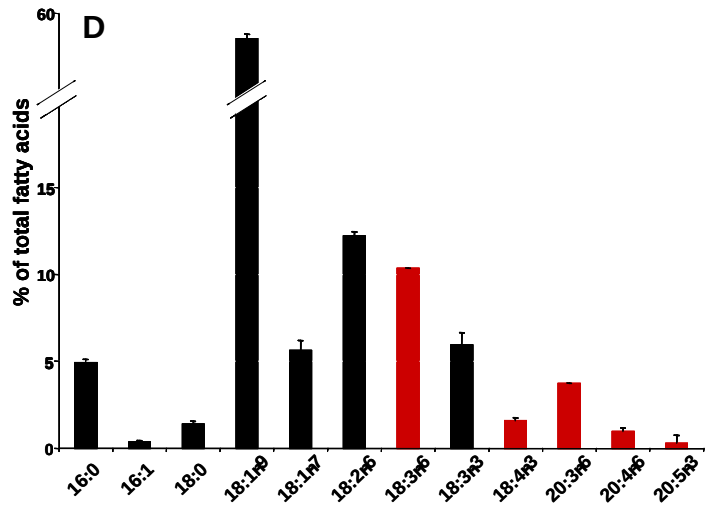
Arabidopsis



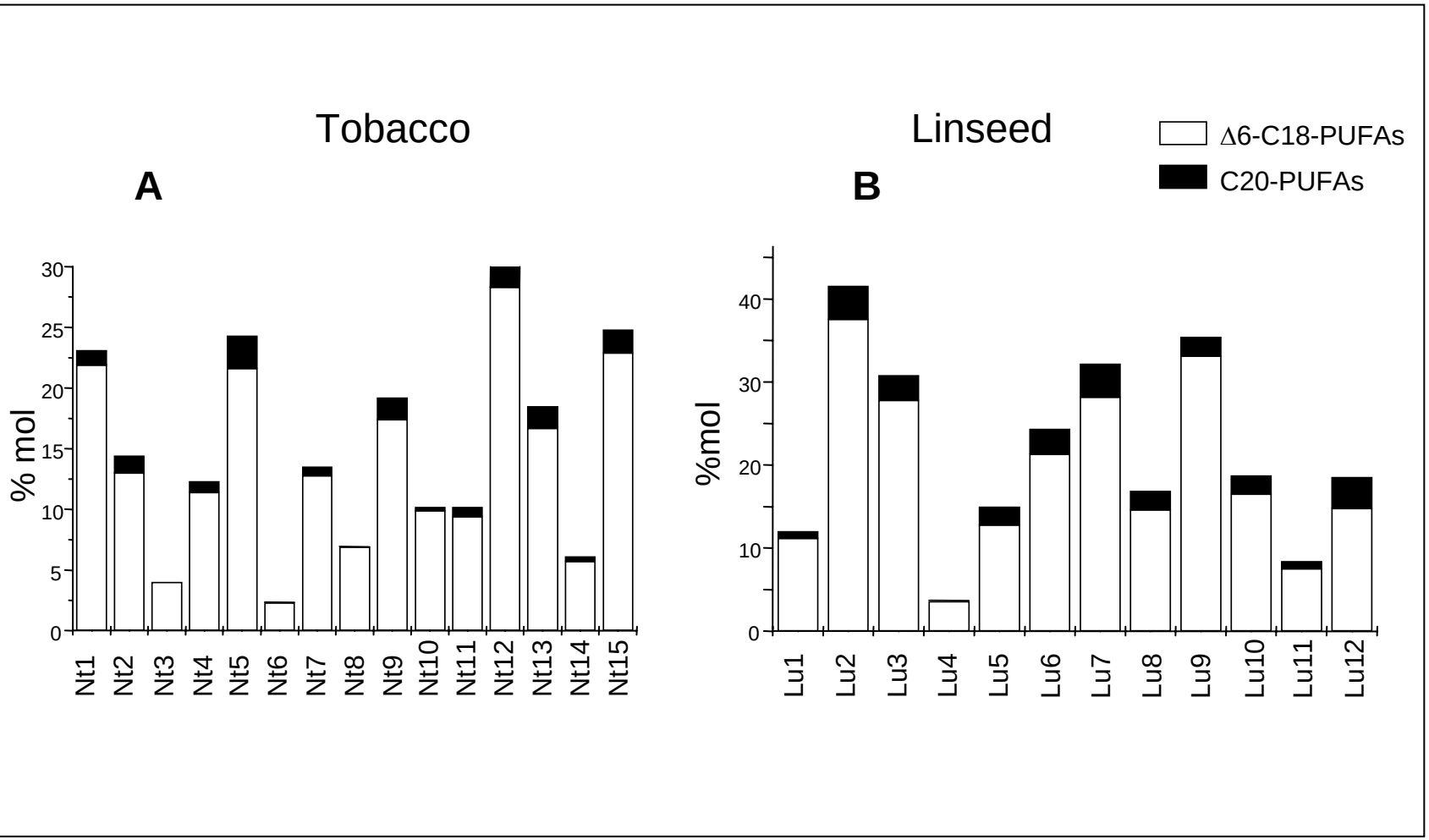


Rapeseed



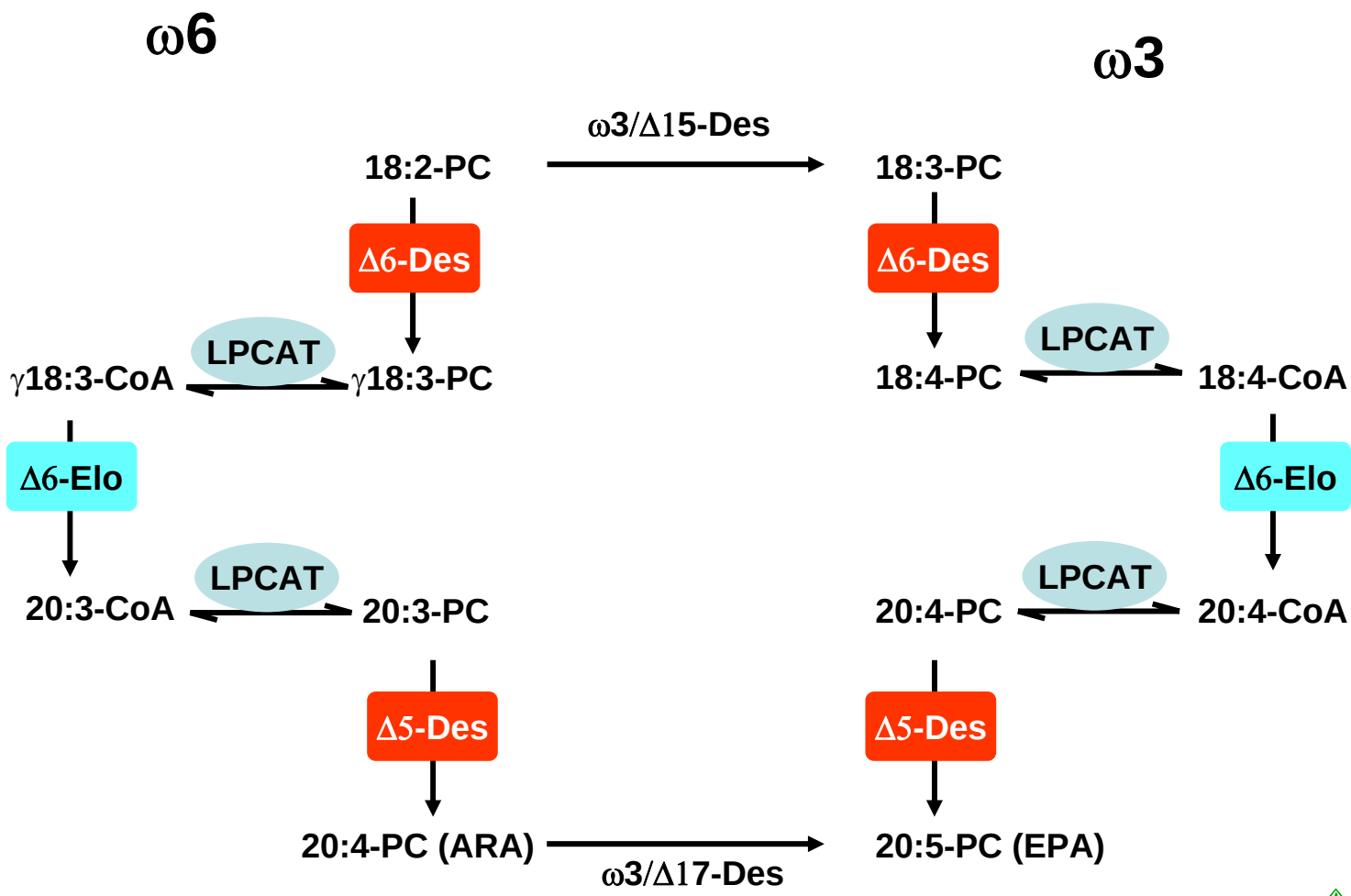


LCPUFA – Results

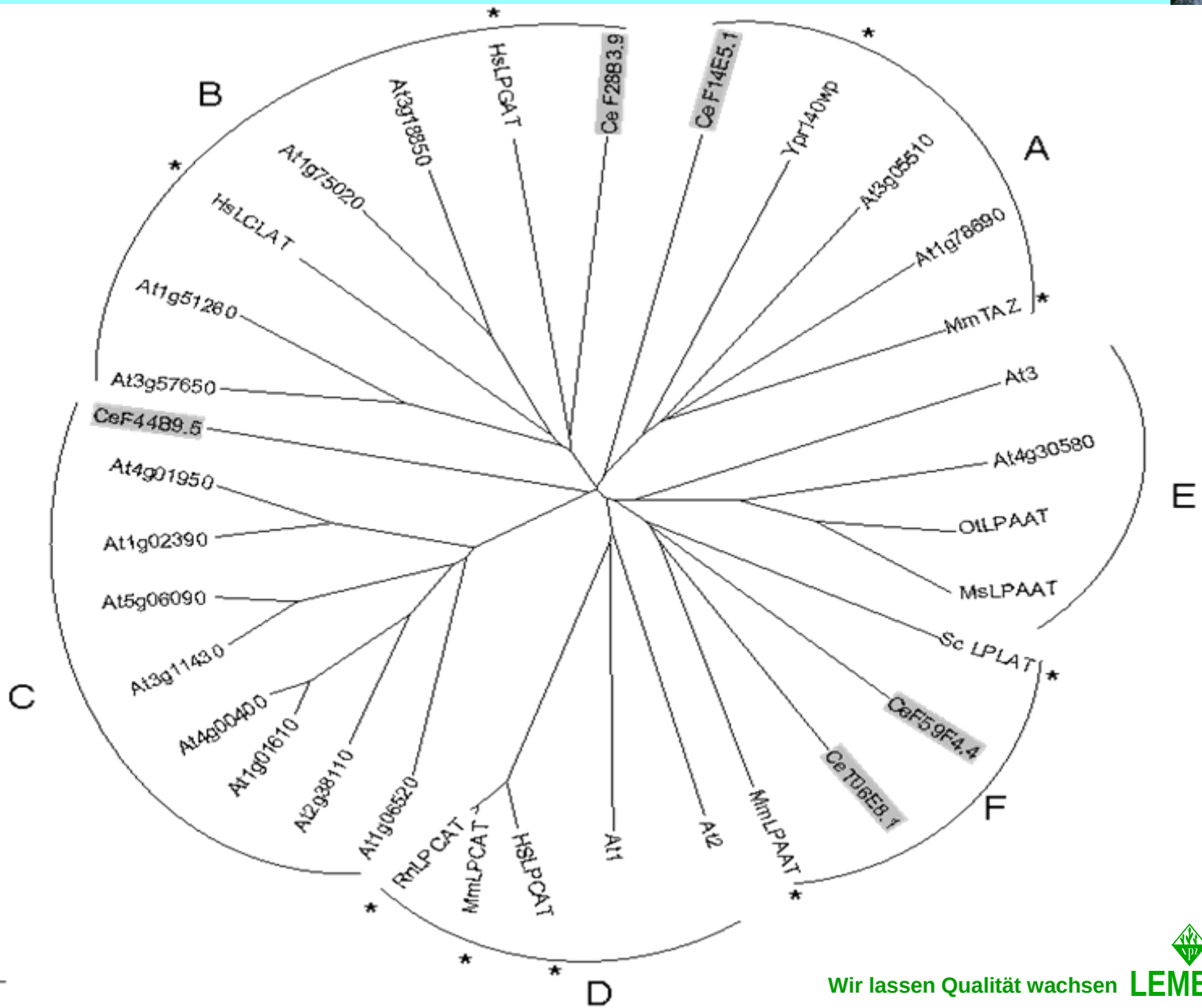


Correction of the LCPUFA biosynthesis pathway

LCPUFA biosynthesis requires desaturases, elongases and acyltransferases operating with different substrates



LPCAT Phylogeny



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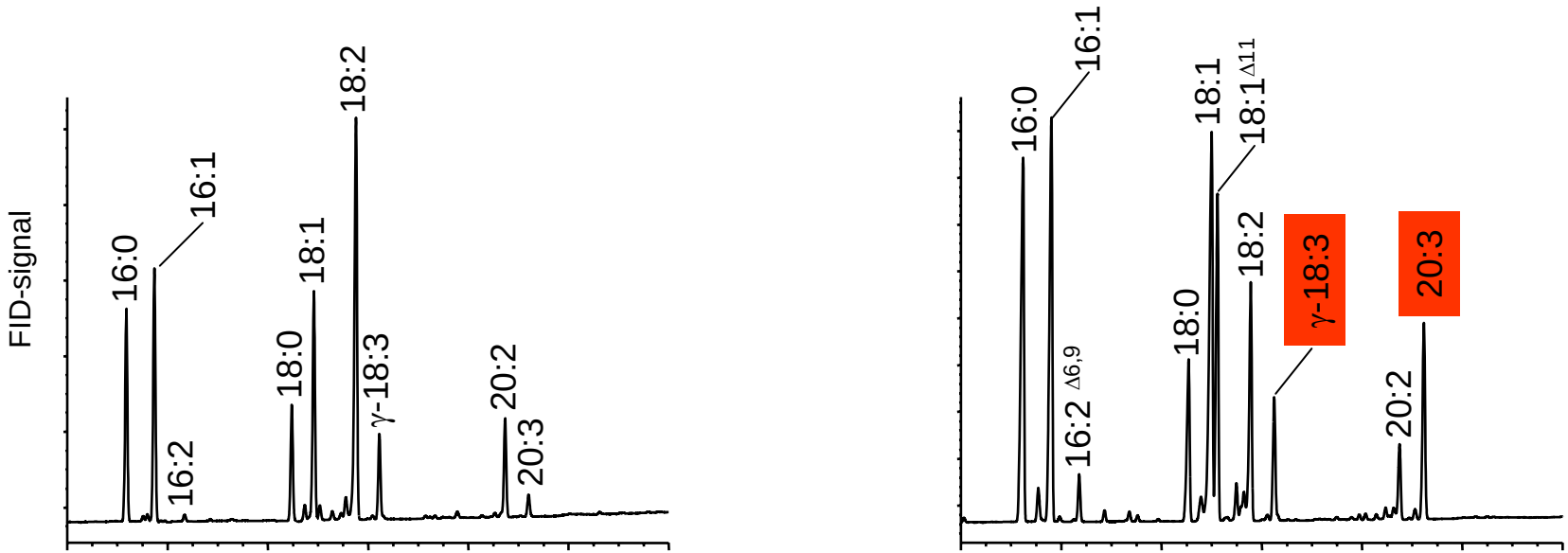
Bottleneck in elongation: acyl-CoA:lysophosphatidylcholine acyltransferase (CeLPCAT)

18:2 → γ-18:3 → 20:3

yeast transformant with (PpΔ6Pse1+pYes2) +18:2

total fatty acids

yeast transformant with (PpΔ6Pse1+ LPCAT) +18:2



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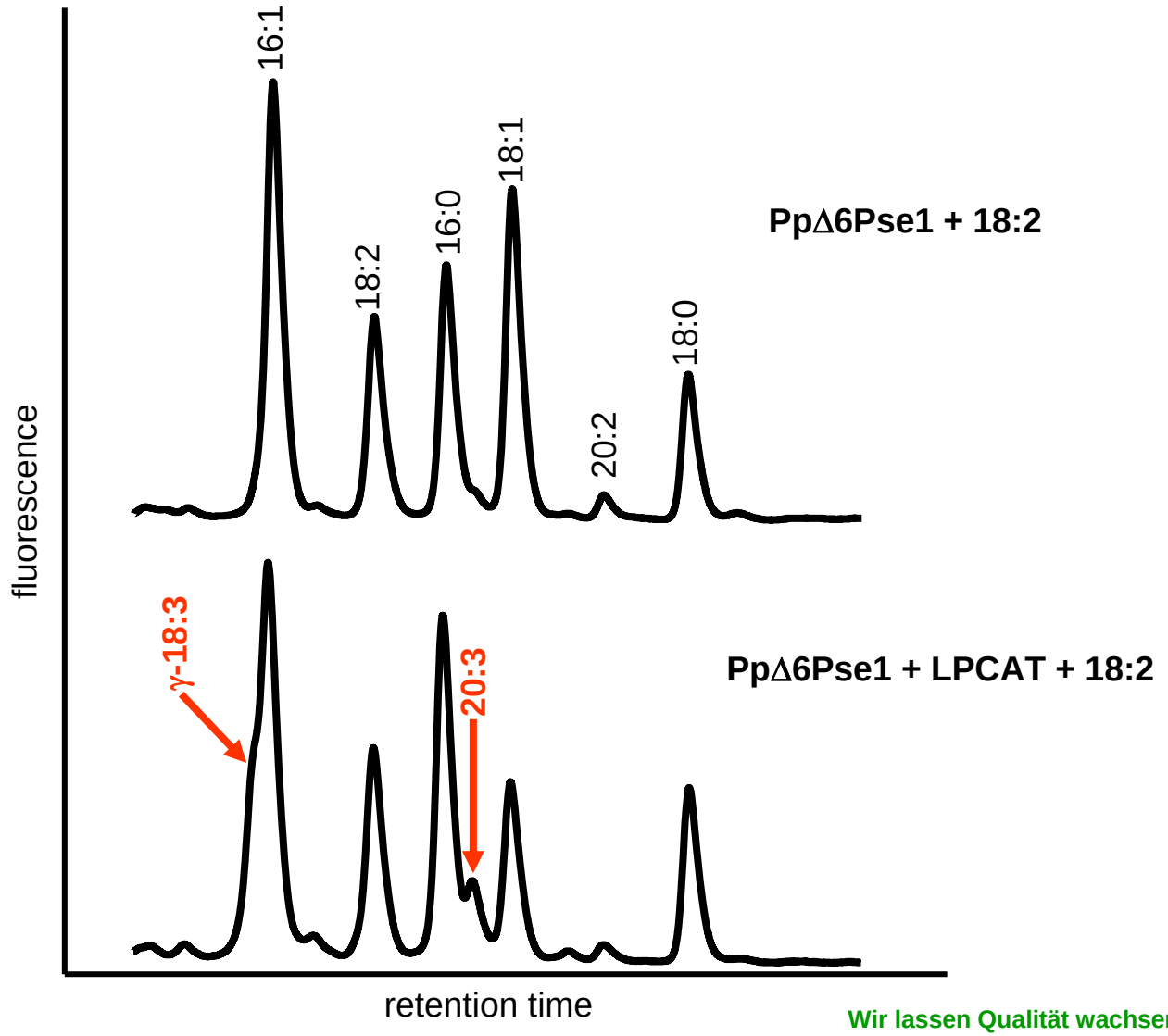
Acyl-CoA analysis of the yeast transformants



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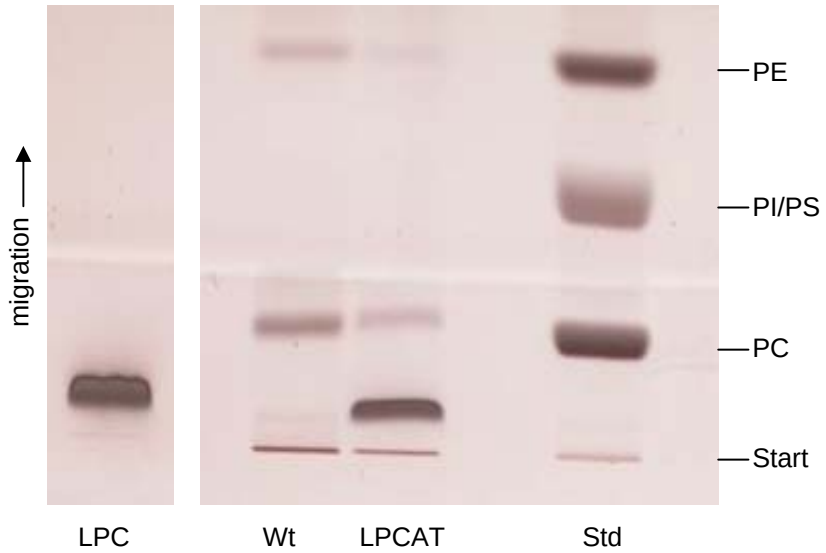
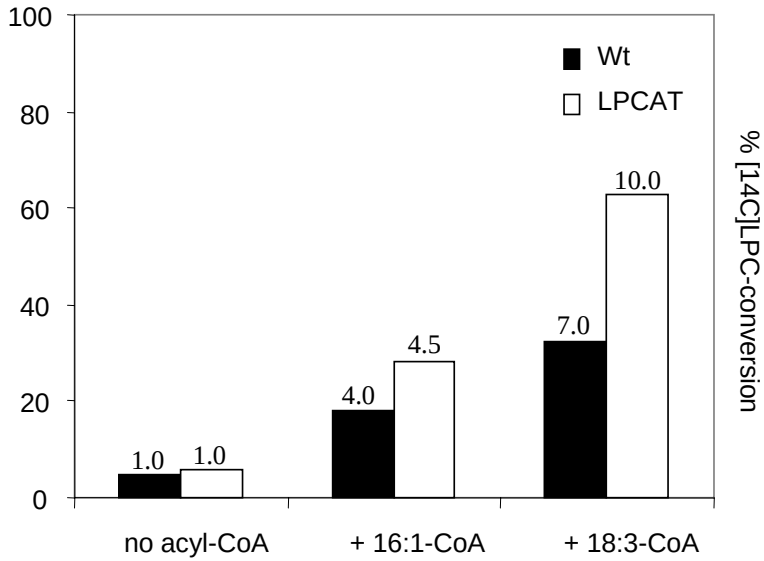
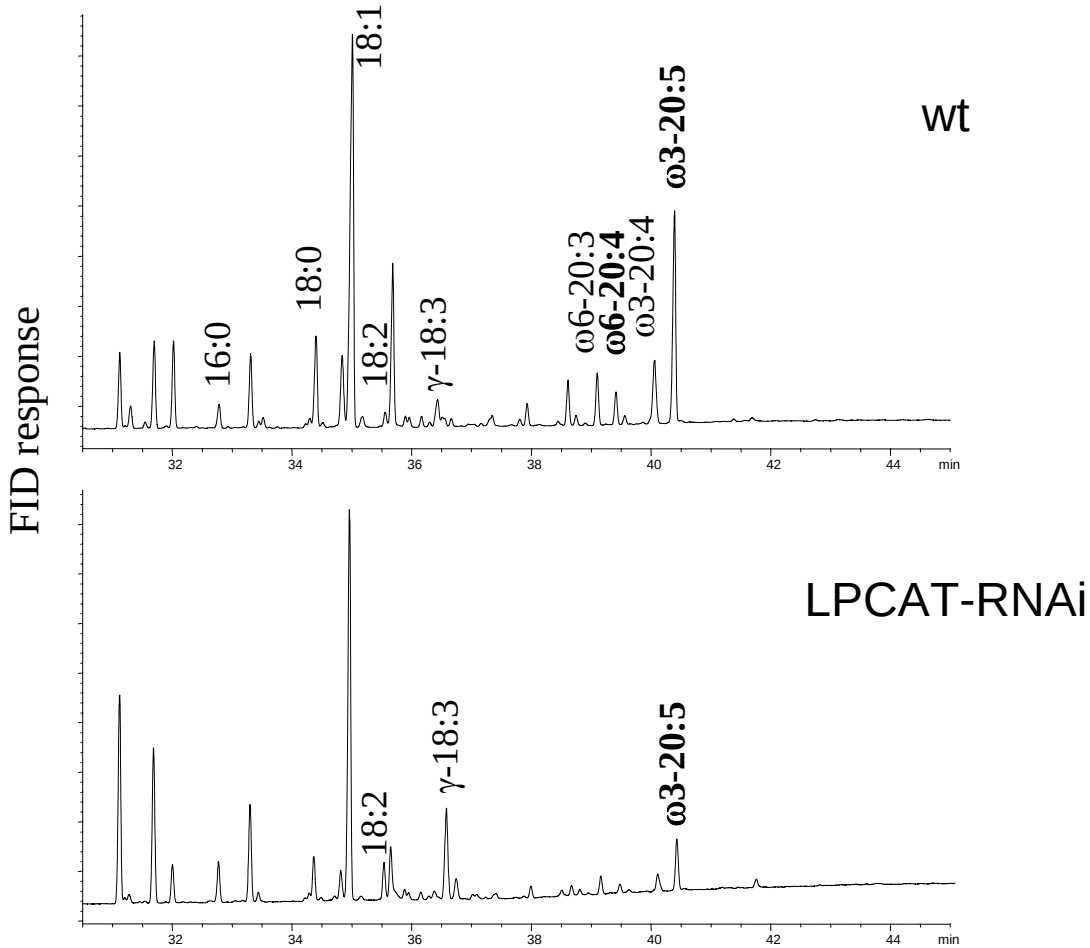
C. elegans LPCAT (activity test)

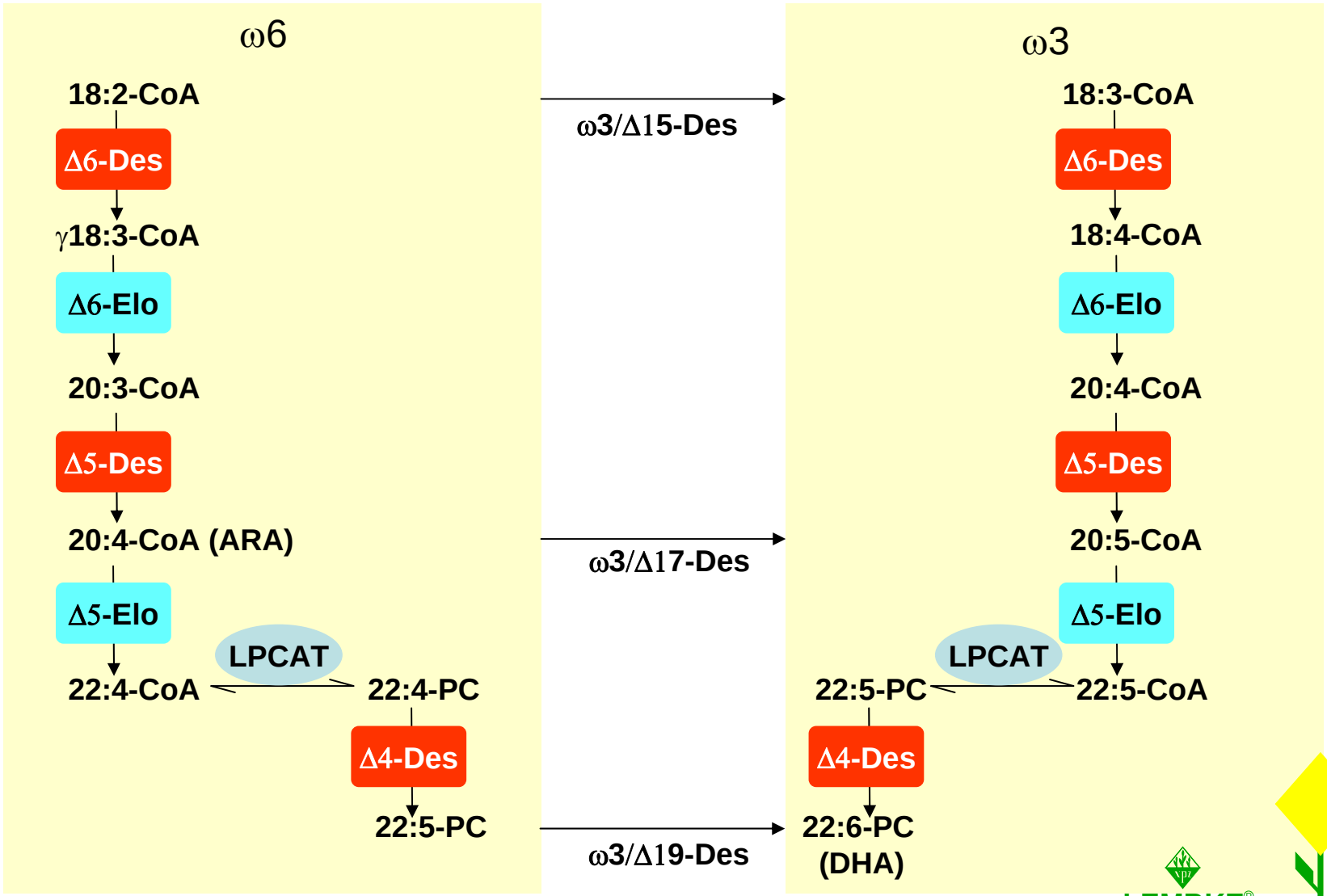




RNAi of the LPCAT in *C. elegans*



Acyl-CoA-Pathway



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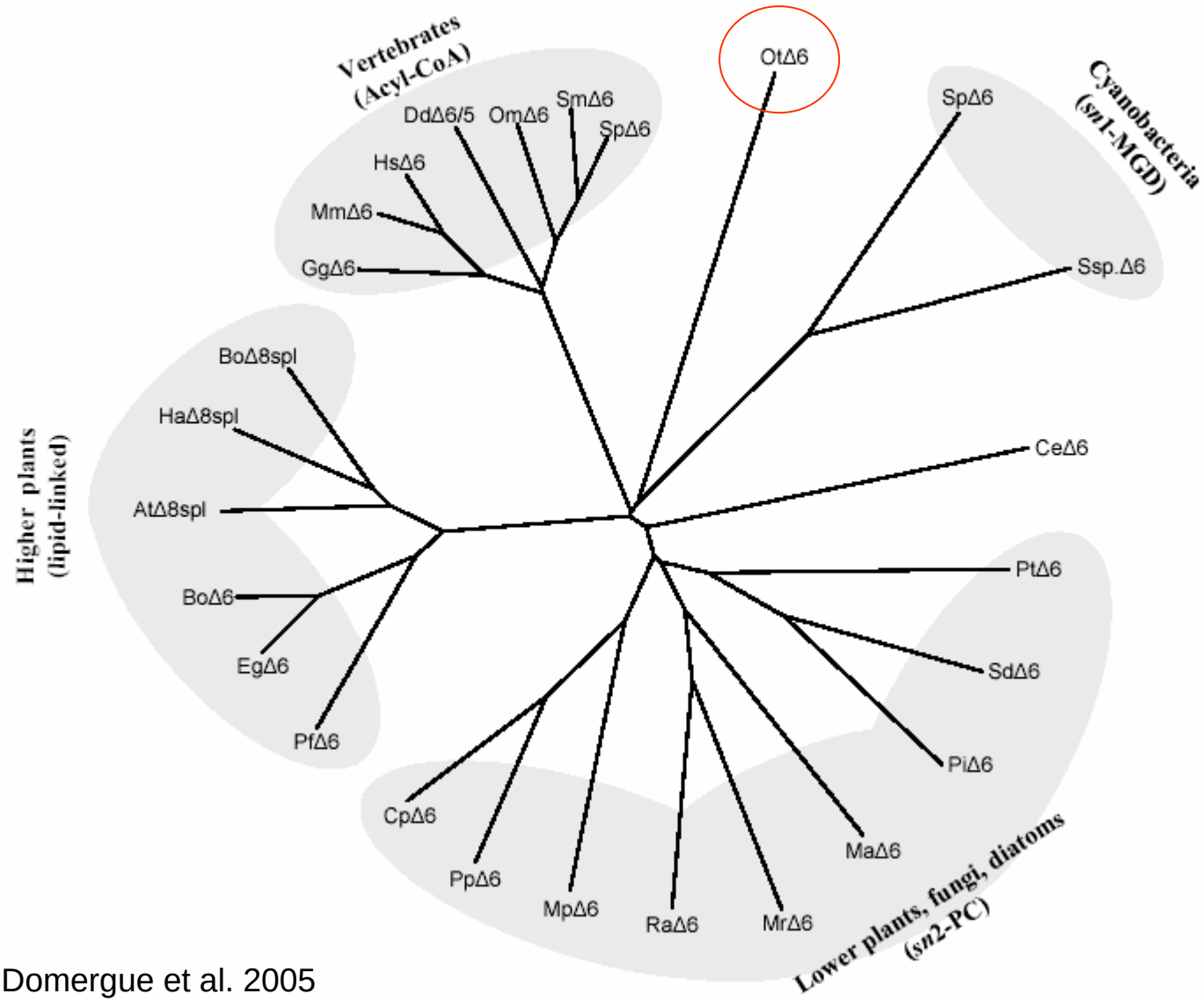
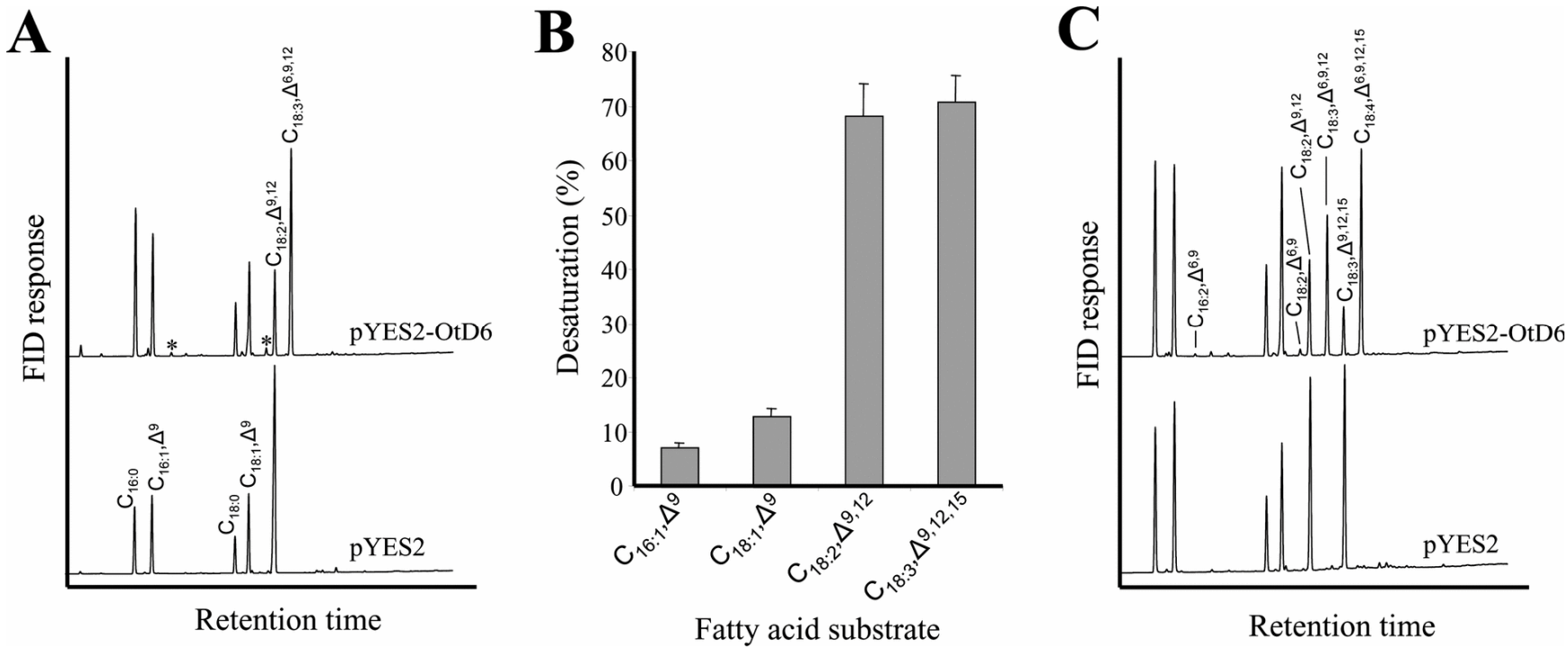
Acyl-CoA Desaturases from Algae



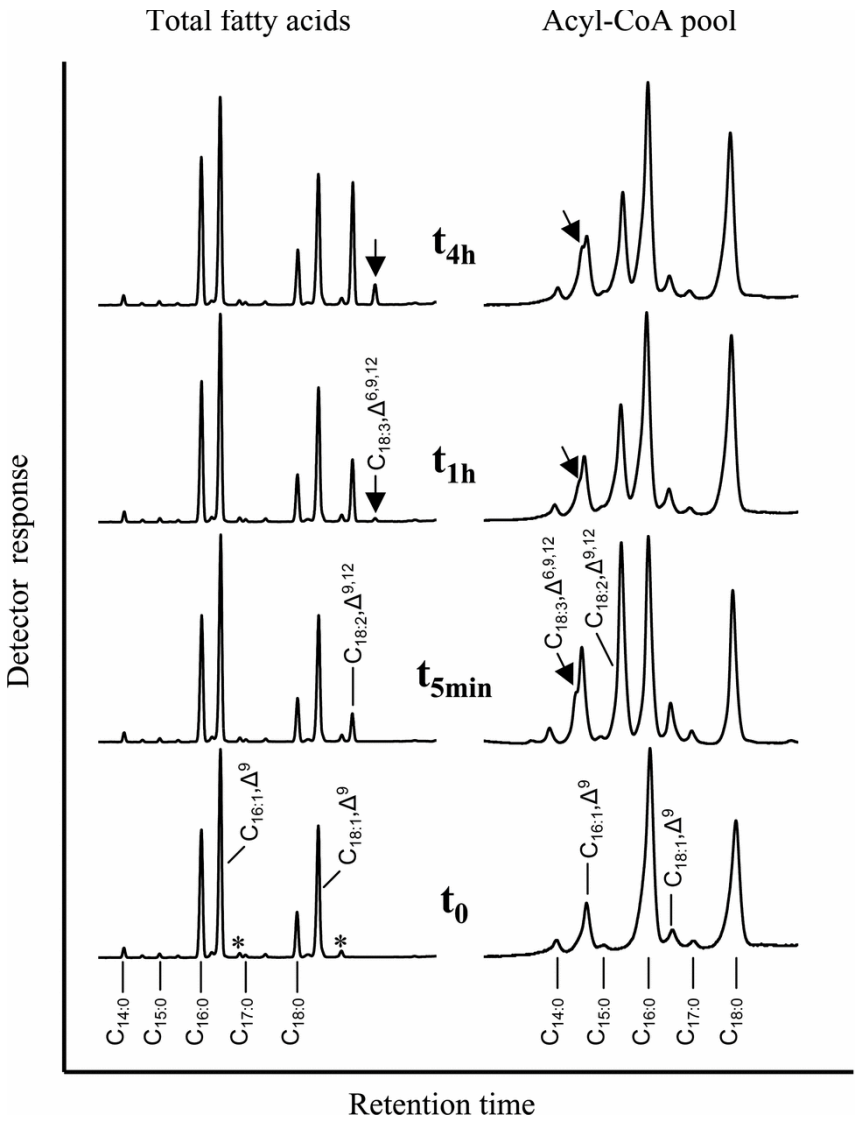
Domergue et al. 2005

Expression of OtΔ6 in yeast



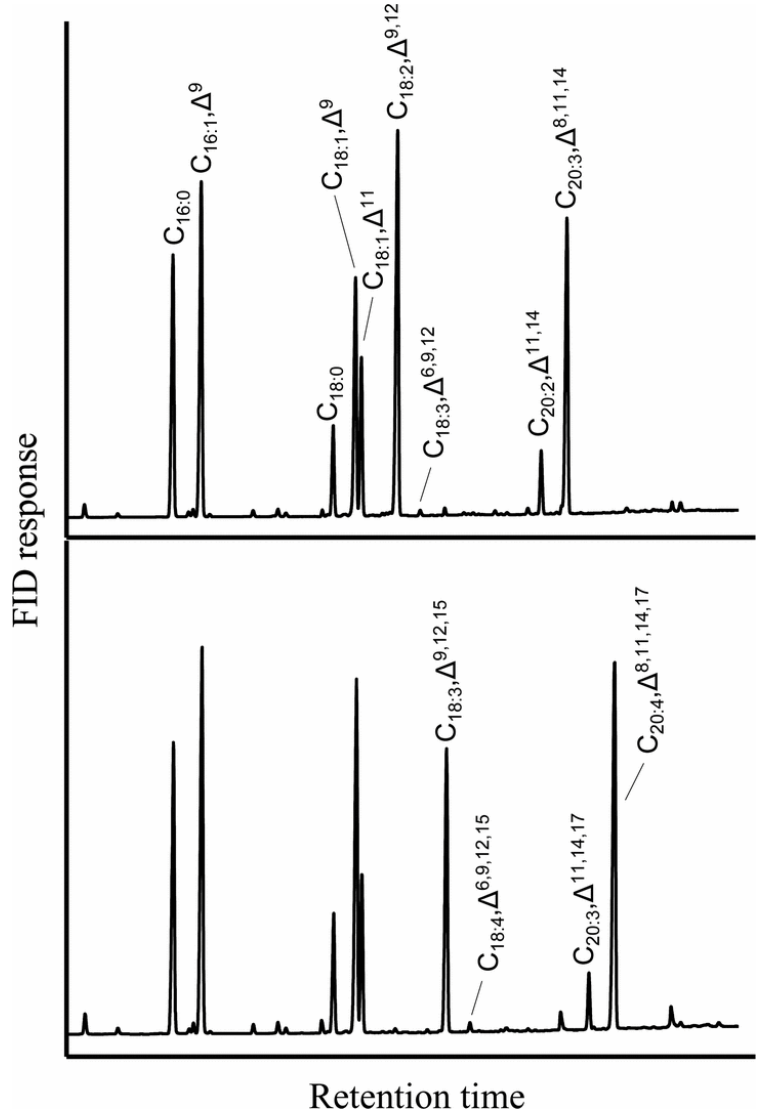
Domergue et al. 2005

Time-course expression of OtΔ6



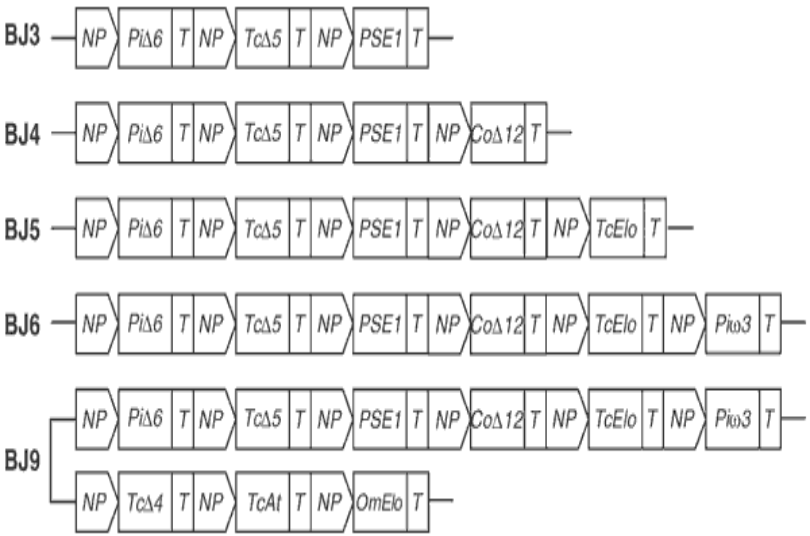
Domergue et al. 2005

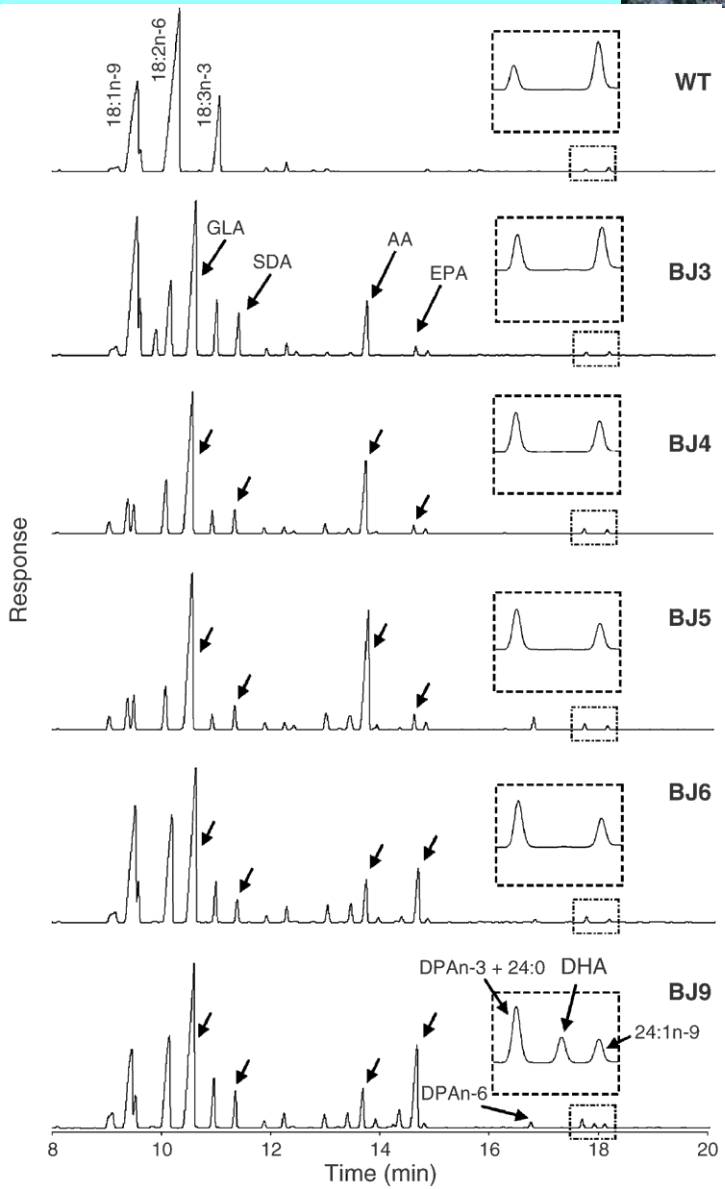
Coexpression of OtΔ6 and Pse1



Domergue et al. 2005

LCPUFAs in *Brassica juncea*

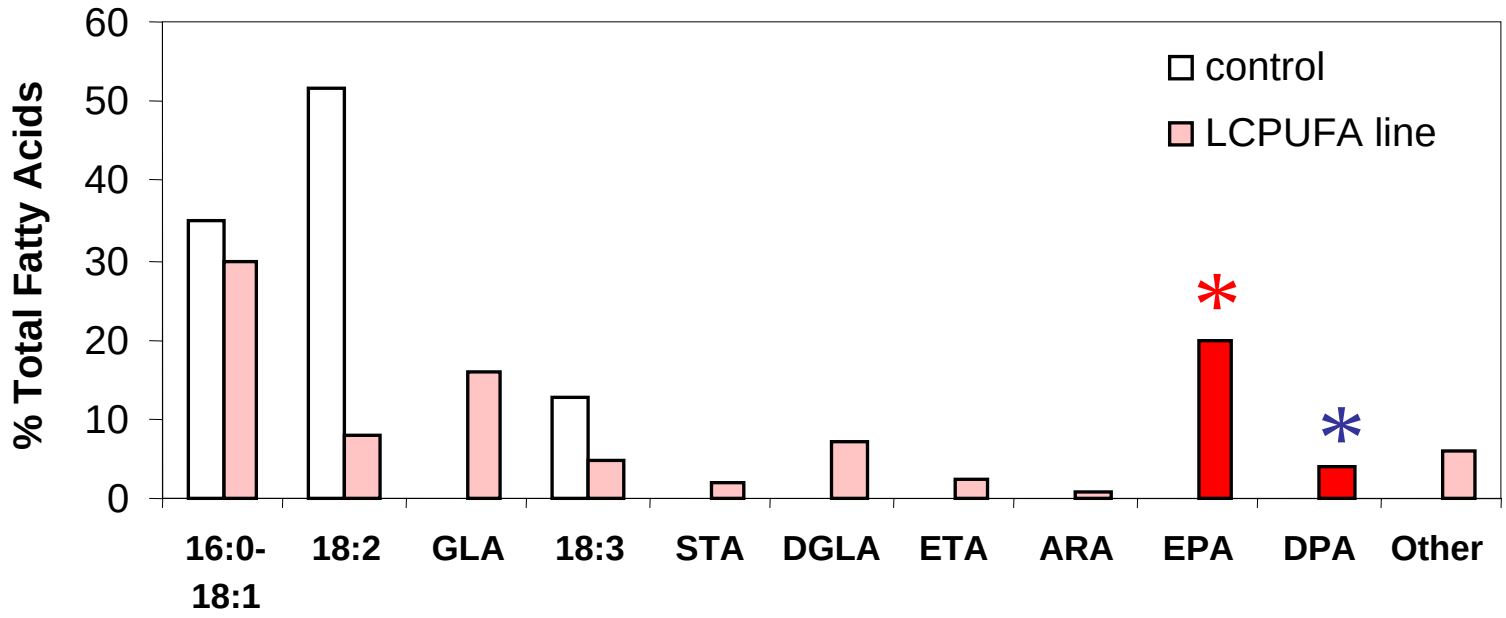




Wu et al., Nature Biotechnol. 2005

Transgenic, Somatic Embryos of Soybean:

Expression of 4 Desaturases (Δ6, Δ5, Δ15, Δ17 from *Saprolegnia* and *Arabidopsis*) and 1 Elongase (elo1 from *Mortierella*)



20 % EPA* und 4 % 7,10,13,16,19 – DPA*

Δ5 Elo+Δ4 Des: 3% DHA

A. J. Kinney et al., 2004

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Summary

- Green biotechnology will provide novel sources of the high valuable LCPUFA and will open new markets

- Change the acceptance of the consumer is the severe bottleneck. This should be the next task in Europe