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(54) **NOVEL FATTY ACID DESATURASES AND ELONGASES AND USES THEREOF**

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(57) **ABSTRACT**

The invention provides isolated nucleic acid molecules which encode novel fatty acid desaturases and elongases from the organism *Emiliana huxleyi*. The invention also provides recombinant expression vectors containing desaturase or elongase nucleic acid molecules, host cells into which the expression vectors have been introduced, and methods for large-scale production of long chain polyunsaturated fatty acids (LCPUFAs), e.g. arachidonic acid (ARA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA).

Figure 1 : Schematical figure of the different enzymatic activities leading to the production of ARA, EPA and DHA.

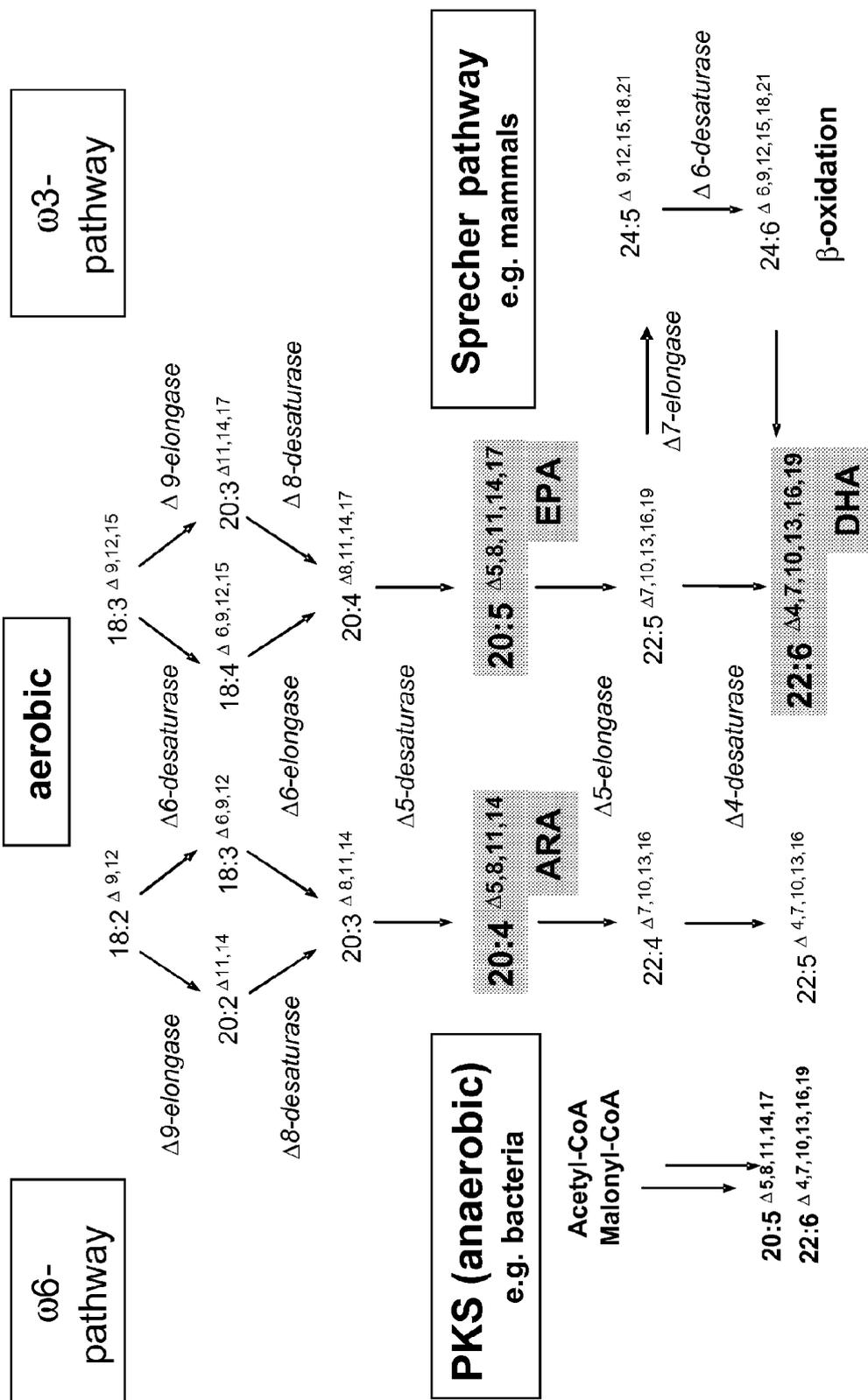


Figure 2 : Gas chromatograph of yeast expression experiment with feeding of 22:5n-3 in the presence (A) and absence (B) of d4Des(Eh). The arrow indicates the formation of 22:6, the product of d4Des(Eh) activity.

### Expression in yeast

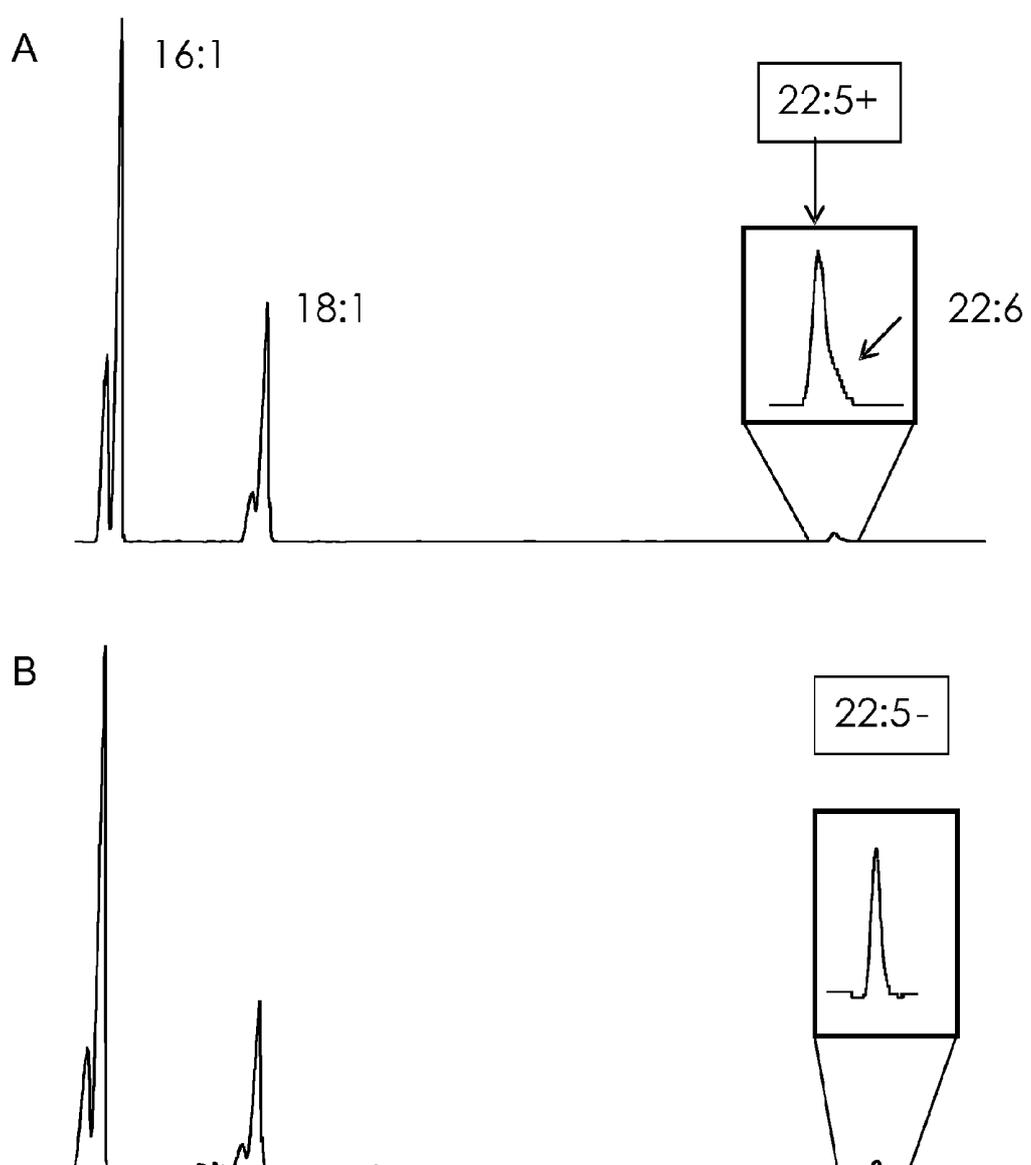


Figure 3 : Gas chromatograph of yeast expression experiment with feeding of 20:3n-3 in the presence (A) and absence (B) of d8Des(Eh). The arrow indicates the formation of 20:4n-3, the product of d8Des(Eh) activity.

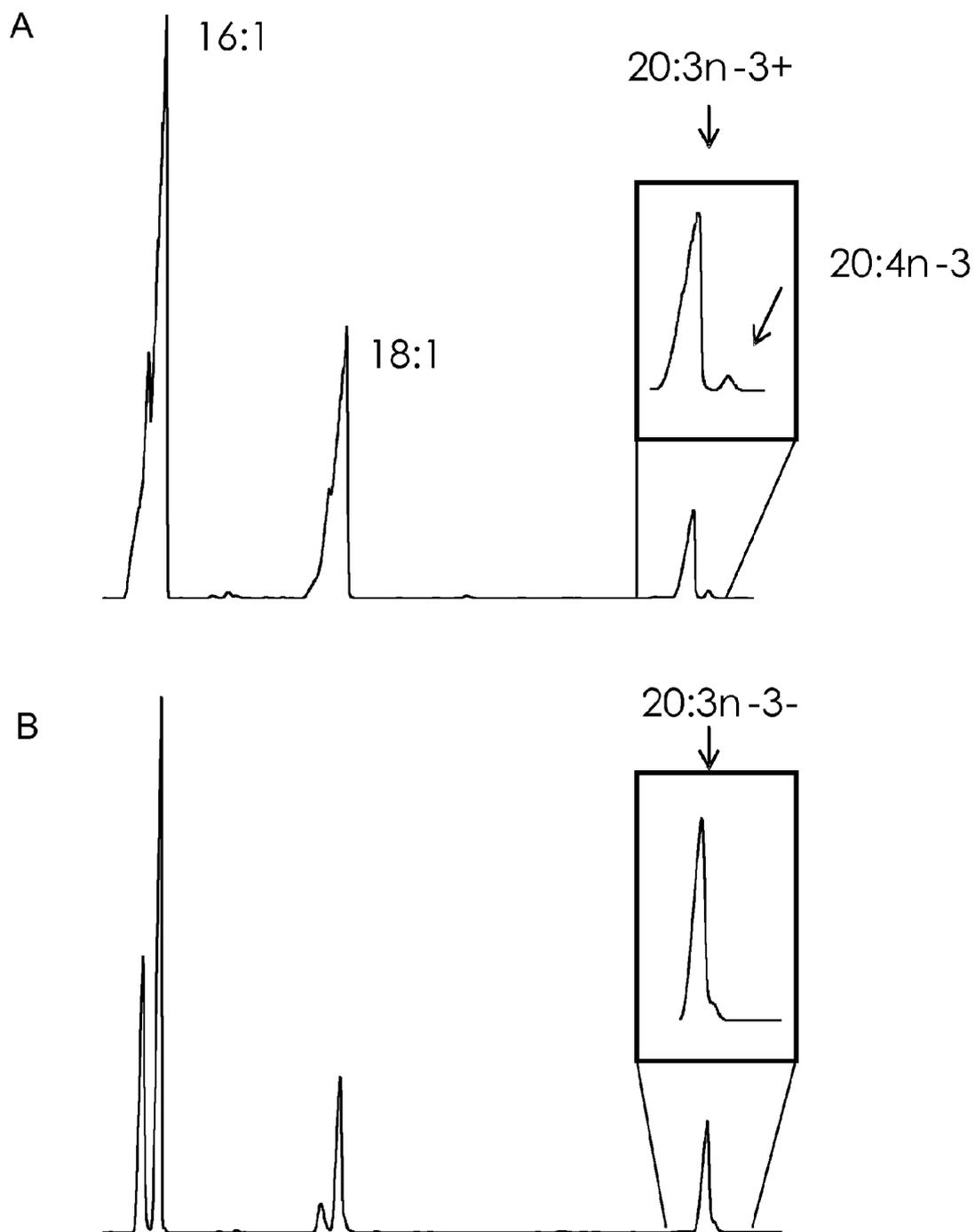


Figure 4 : Gas chromatograph of yeast expression experiment with feeding of 18:3n-3 (ALA) in the presence (A) and absence (B) of d9Elo(Eh). The arrow indicates the formation of 20:3n-3, the product of d9Elo(Eh) activity.

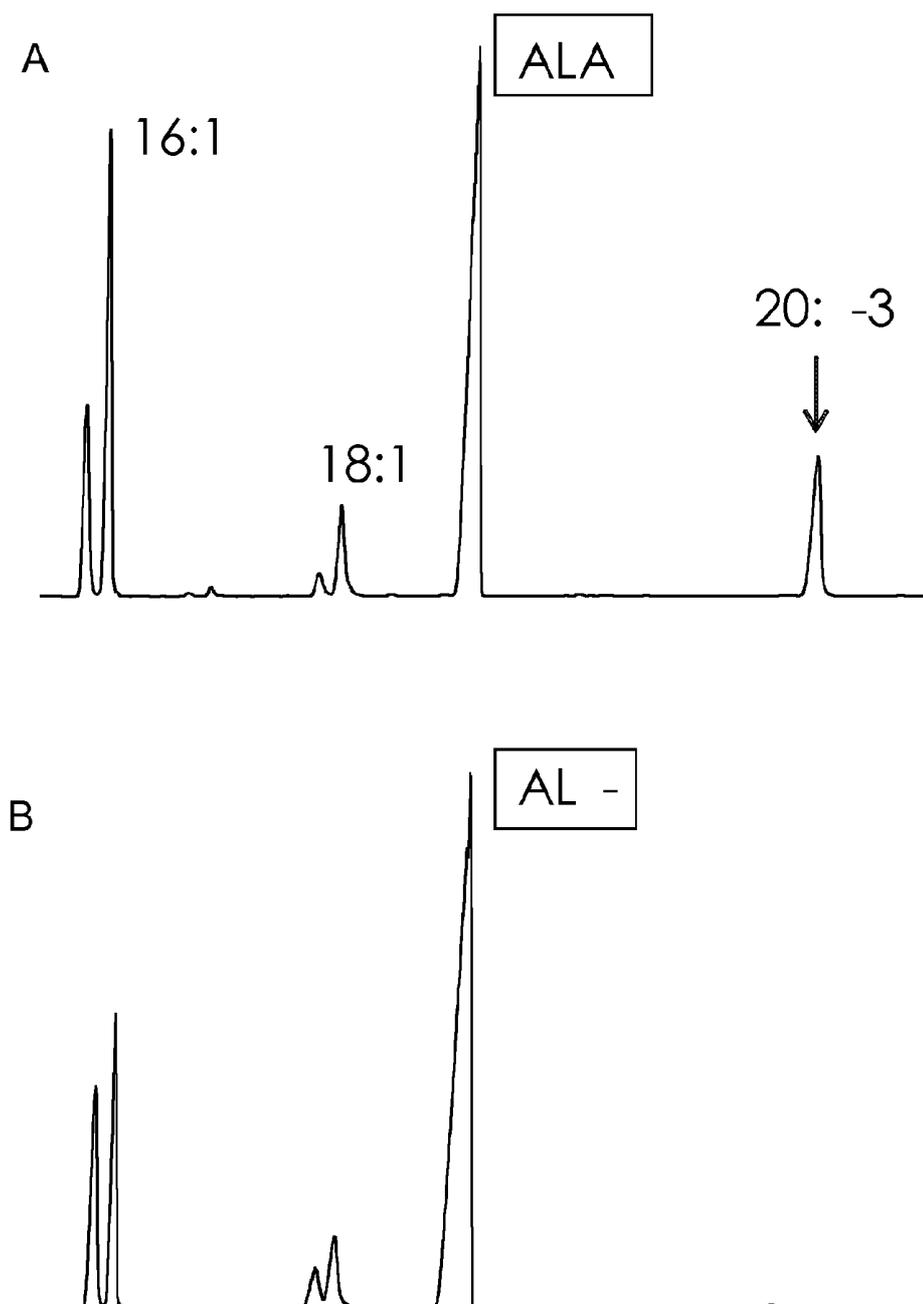


Figure 5 : Gas chromatograph of yeast expression experiment with feeding of 18:3n-6 (GLA) and 18:4n-3 (SDA) in the presence (A) and absence (B) of d5Elo(Eh). The arrow indicates the formation of 20:3n-6 or 20:4n-3, respectively, the products of d5Elo(Eh) activity

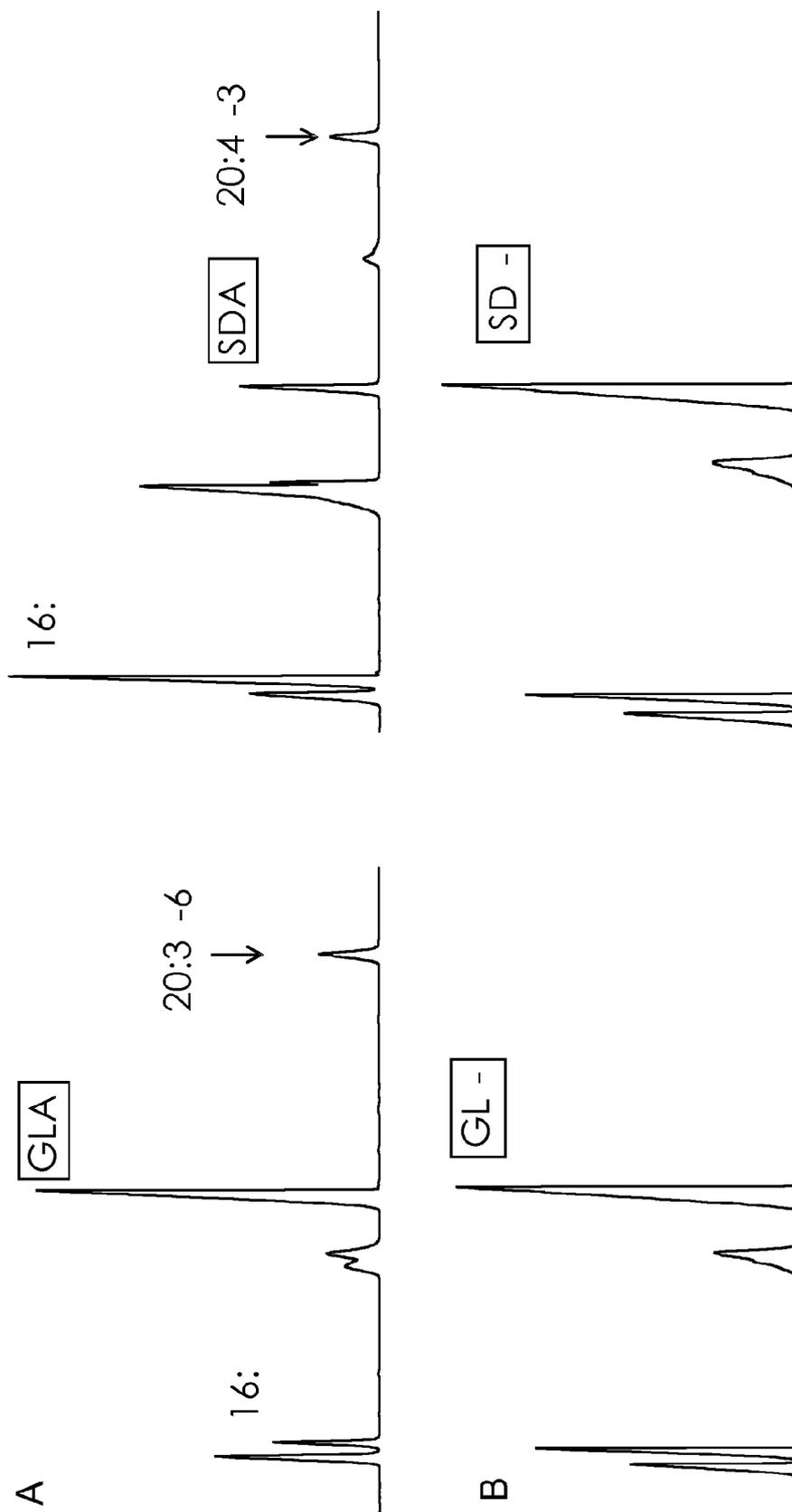


Figure 6 : Gas chromatograph of yeast expression experiment with feeding of 20:4n-6 (ARA) and 20:5n-3 (EPA) in the presence (A) and absence (B) of d5Elo(Eh). The arrow indicates the formation of 22:4n-6 or 22:5n-3, respectively, the products of d5Elo(Eh) activity.

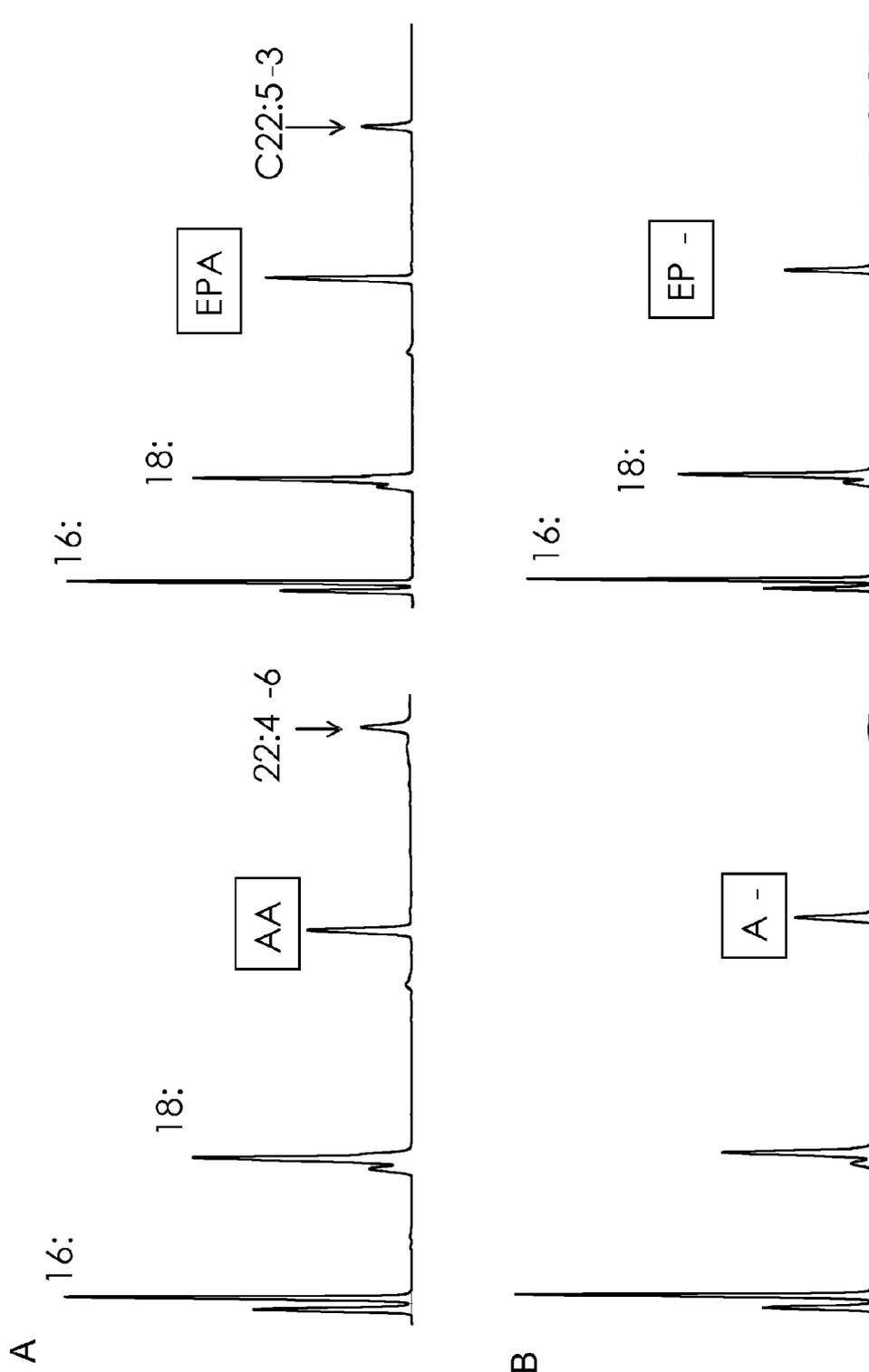


Figure 7 : Gas chromatograph analysis of mature Arabidopsis seeds. Peaks were quantified and listed in the table below. EmiElo91 and EmiElo92 are two selected events transformed with d9Elo(Eh). WT is a non-transformed control. The products of d9Elo(Eh) activity are 20:2 and 20:3n3, which are 10fold increased compared to the levels of the non-transformed control.

<b>Fatty</b>	16:0	18:0	18:1	18:2	ALA	18:4	20:0	20:1	20:2	20:3n3	22:1
EmiElo91	4.64	3.43	13.34	23.88	12.64	0.18	2.55	22	11.26	3.73	1.69
EmiElo92	4.21	3.04	12.63	24.63	14.57	0.34	2.28	21.56	10.94	4.02	1.8
WT	6.22	3.21	16.17	27.75	16.63	0.16	2.4	22.2	1.95	0.46	2.15

Figure 8 : Acyl-CoA analysis of mature Arabidopsis seeds of event EmiElo91 transformed with d9Elo(Eh). Col0 is seed material from a non-transformed plant. Δ9elo is seed material from EmiElo91. The product of d9Elo(Eh) activity is marked with a star (20:2), which is massively increased compared to the control, indicating the functional expression of d9Elo(Eh).

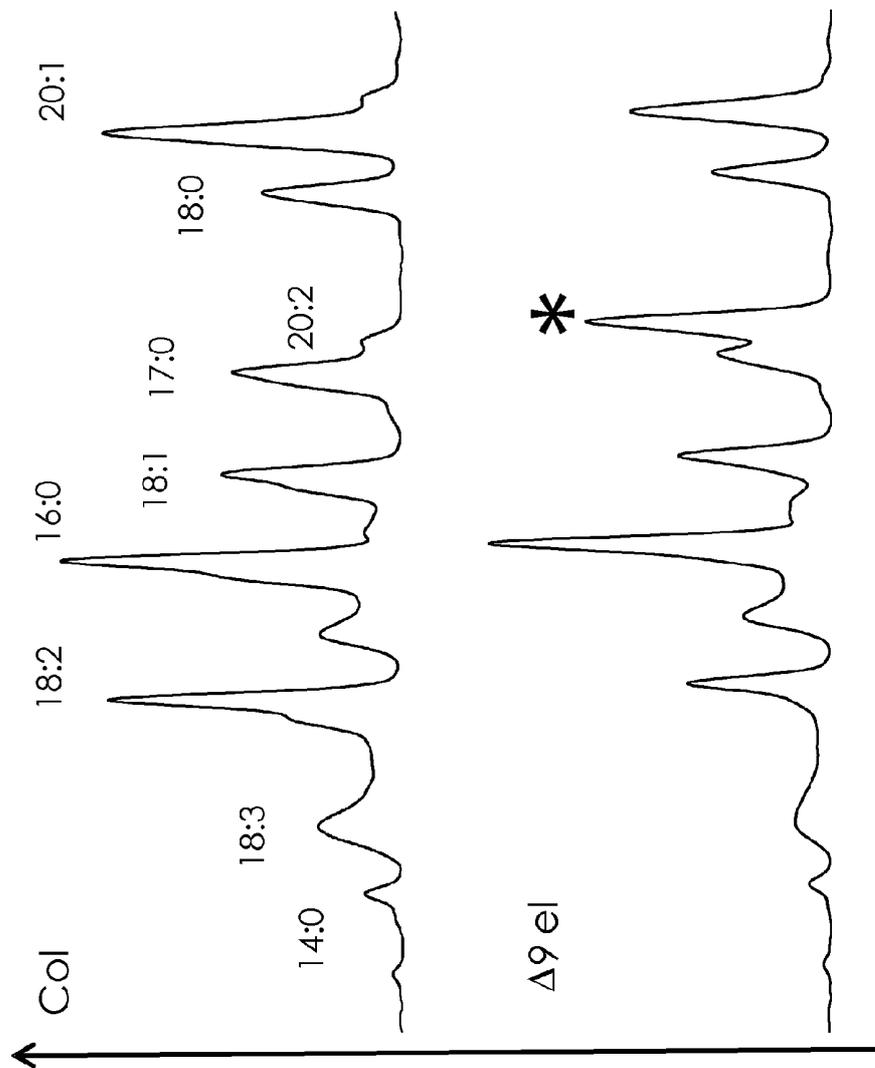


Figure 9 : Gas chromatographic analysis of mature Arabidopsis seeds transformed with the construct AP2 (gene combinations d9Elo(Eh)\_d8Des(Eh)\_d5Des(Eh)\_d12Des(Ps)\_o3Des(Pi). Peaks were quantified and listed in the table below. The products of d9Elo(Eh) activity are 20:2 and 20:3n3.

FA	16:0	18:0	18:1c9	18:1c11	18:2	18:3n3	20:0	20:1	20:2	20:3n3
AP2 1	6.5	3.4	12.9	1.8	27.4	13.2	2.0	15.9	7.5	4.5
AP2 5	5.7	3.2	14.9	1.5	26.4	13.1	2.0	17.9	6.8	3.2
AP2 11	5.9	3.2	13.5	1.6	26.6	13.3	2.0	17.1	7.7	4.0
AP2 16	6.5	3.1	11.9	1.7	26.9	13.5	2.0	16.2	7.8	5.0
AP2 17	7.6	3.4	12.2	3.1	30.6	13.8	2.0	14.9	3.8	2.2
AP2 18	6.5	3.3	11.4	1.9	27.2	14.2	2.3	16.7	6.7	3.9
AP2 21	6.6	3.1	10.9	1.8	24.9	15.5	2.2	15.9	7.4	5.7
AP2 22	6.2	3.2	9.7	1.7	25.9	14.4	2.1	15.9	8.7	6.4
AP2 23	6.3	3.1	10.8	1.9	27.1	14.6	2.1	16.0	7.5	5.2
AP2 24	5.9	3.1	10.3	1.6	25.4	14.2	2.1	16.1	9.4	5.9
AP2 25	6.2	3.0	12.0	1.6	26.0	14.8	2.0	17.1	7.7	3.9
AP2 26	6.2	3.3	11.1	1.8	26.6	13.1	2.1	16.0	8.6	5.7
AP2 27	5.8	3.1	9.8	2.0	24.1	12.2	2.2	15.8	9.5	8.6
AP2 28	6.0	3.2	9.8	1.8	25.0	13.0	2.2	15.7	8.9	7.6
AP2 29	6.6	3.3	10.1	2.0	26.5	14.1	2.2	15.8	7.9	5.8
AP2 30	5.6	3.0	10.7	1.5	26.7	13.2	2.0	16.6	10.5	4.7
AP2 31	5.5	3.2	11.2	1.8	24.5	12.6	2.1	16.5	10.6	6.0
AP2 32	5.7	3.2	11.1	1.6	25.5	13.5	2.2	17.4	8.8	4.9
AP2 33	6.5	3.2	9.2	1.9	26.7	15.4	2.3	15.8	7.6	5.5
AP2 34	6.4	3.3	10.1	2.0	26.1	13.7	2.3	16.7	8.0	5.7
AP2 42	5.7	3.4	13.1	1.8	25.6	12.8	2.3	18.0	7.6	4.2
AP2 46	6.3	3.3	10.3	2.4	27.2	12.8	2.4	17.0	6.7	5.0
AP2 47	5.7	3.5	13.3	2.0	26.7	12.4	2.3	17.5	7.3	3.8
AP2 48	5.6	3.5	12.0	2.0	25.5	12.2	2.3	16.6	8.9	5.7
AP2 49	6.0	2.9	12.9	1.6	26.7	14.2	2.2	18.4	6.5	3.2
AP2 50	5.8	3.0	10.8	2.5	26.6	13.9	2.2	17.1	6.7	5.1
average	6.1	3.2	11.4	1.9	26.3	13.6	2.2	16.6	7.9	5.1
std	0.5	0.2	1.4	0.3	1.2	0.9	0.1	0.8	1.4	1.4

Figure 10

FA	16:0		18:0		18:1		18:2		GLA		18:4		20:0		20:1		20:2		DHGLA		ARA		ETA		EPA		22:0		22:1		DPA		DHA	
			$\Delta 9$	$\Delta 11$	$\Delta 9,12$	$\Delta 18,3n6$	$\Delta 11$	$\Delta 11,14$	$\Delta 11$	$\Delta 11,14$	20:3n6	20:4n3	20:3n3	20:4n3	20:5n3	20:6n3	20:7n3	20:8n3	20:9n3	20:10n3	20:11n3	20:12n3	20:13n3	20:14n3	20:15n3	20:16n3	20:17n3	20:18n3	20:19n3	20:20n3	20:21n3	20:22n3	20:23n3	
OstELO5EmD4_1	6	3	10	19	25.5	0.7	0.3	2	17.9	2.0	0	1	0	1	2	0	1	0	1	1	0	1	2	0	0	1	1	1	2	3				
OstELO5EmD4_2	7	3	13	24	27.4	2.7	1.5	2	16.0	1.1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	1.2			
OstELO5EmD4_3	6	3	10	23	26.2	1.9	1.0	2	15.5	1.4	0	1	0	0	0	0	0	0	0	0	0	0	0	3	0	1	1	0	2	0	2.7			
OstELO5EmD4_4	7	3	8.7	2.8	23.7	5.7	3.2	2	15.2	1.1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	1	0	1	0	1.6			
OstELO5EmD4_5	7	3	10	24	24.6	3.2	1.8	2	16.3	1.1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	1	0	1	0	2.0			
OstELO5EmD4_6	8	3	9.0	2.2	23.2	6.1	4.0	2	15.7	1.3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1.4	1.4			
OstELO5EmD4_7	7	3	7.9	2.2	24.0	1.0	0.7	2	14.6	2.1	0	1	1	1	1	3	0	1	1	1	1	1	3	0	1	1	1	1	1	4.7	4.7			
OstELO5EmD4_8	9	3	10	1.7	26.8	1.1	0.5	1	13.7	1.6	0.7	1	0	0	1	2	0	1	2	0	1	0	2	0	1	1	0	1	0	2	2			
OstELO5EmD4_14	8	3	12	1.6	25.4	4.8	2	2	15.7	1.2	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0			
OstELO5EmD4_15	9	3	11	1.7	25.8	3.9	2	1	13.6	1.2	0.1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	1		
OstELO5EmD4_16	7	3	9.7	2.2	24.3	6.7	3	2	14.2	0.9	0.1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	1		
OstELO5EmD4_18	7	3	10	2.1	24.4	5.1	3	2	15.7	1.3	0.5	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	1	0	1	0	1		
OstELO5EmD4_20	6	3	8.8	2.0	24.4	1.0	0	2	16.5	1.8	0.7	1	1	1	1	3	0	1	3	0	1	0	3	0	1	0	1	0	3	0	3	3		
OstELO5EmD4_21	5	3	11	2.0	26.4	0.6	0	2	18.3	1.9	0.7	1	0	0	0	0	0	0	0	0	0	0	1	2	0	1	0	1	0	1	0	1		
OstELO5EmD4_25	6	3	10	1.9	24.9	2.1	1	2	17.4	1.9	0.5	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1		
OstELO5EmD4_26	6	3	11	2.1	25.5	5.0	3	1	15.6	1.3	0.2	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	1		
OstELO5EmD4_27	6	3	12	1.9	24.2	4.2	2	2	16.4	1.3	0.4	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	1	0	1	1		
OstELO5EmD4_28	8	3	8.2	2.4	23.5	3.7	2	2	14.2	1.1	0.8	1	1	1	1	4	0	1	4	0	1	1	1	1	1	1	1	1	1	2	2	2		
OstELO5EmD4_101	6	3	8.2	2.1	23.1	1.4	0	3	17.4	1.7	0.6	1	1	1	1	3	0	1	3	0	1	1	1	3	0	1	1	1	1	3	0	3		
OstELO5EmD4_103	6	3	10	2.2	26.7	1.1	0	2	17.1	2.0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1		
OstELO5EmD4_104	6	3	9.2	2.4	25.3	1.2	0	2	15.9	1.6	0.7	1	1	1	1	4	0	1	4	0	1	1	1	4	0	1	1	1	1	3	0	3		
OstELO5EmD4_105	6	3	9.3	2.0	24.9	3.5	1	2	15.1	1.4	0.7	2	0	0	0	0	0	0	0	0	0	0	0	3	0	1	1	1	1	1	1	1		
OstELO5EmD4_106	7	3	7.2	2.3	19.5	3.1	2	2	13.8	1.3	0.4	1	1	1	1	7	0	1	7	0	1	1	1	7	0	1	1	1	1	4	0	4		
OstELO5EmD4_109	7	3	7.5	2.4	21.7	1.8	1	2	14.7	1.7	0.3	1	1	1	0	0	0	0	0	0	0	0	0	5	0	1	1	2	4	0	4			
OstELO5EmD4_111	7	3	10	2.2	25.6	2.5	1	2	14.5	1.3	0.3	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1	1	1	2	0	2	2		
OstELO5EmD4_112	7	3	6.3	3.5	20.1	2.1	1	2	13.3	1.7	1.0	0	0	0	0	0	0	0	0	0	0	0	2	6	0	1	1	1	1	3	0	3		
OstELO5EmD4_115	8	3	7.8	2.7	24.1	2.6	1	2	13.8	1.5	0.3	1	1	1	0	0	0	0	0	0	0	0	4	0	1	1	2	3	0	3	0	3		
OstELO5EmD4_116	7	3	10	2.5	24.8	5.7	3	2	13.7	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	4	0	1	0	1	0	1	0	1		
OstELO5EmD4_117	6	3	9	2	26	1	0	2	15.2	1	0.9	1	1	1	1	3	0	1	3	0	1	1	1	3	0	1	1	1	3	0	3	0	3	
OstELO5EmD4_118	6	3	10.4	2	23	3	2	2	15.3	1	1.2	0	0	0	0	1.1	2	3	0	1	1	1	1	3	0	1	1	1	1	1	1	1		
OstELO5EmD4_119	7	3	9	2	25	4	2	2	14.2	1	0.2	0	0	0	0	1.0	0	2	0	0	0	0	2	0	0	1	0	0	2	0	2	0	2	
OstELO5EmD4_120	6	3	10.0	2	27	1	0	2	15.1	1	0.8	1	1	1	0.9	1	2	0	1	2	0	1	2	0	1	1	1	1	2	0	2	0	2	
average	7	3	9	2.2	24	3.0	1	2	15.4	1	0	0	0	0	1.0	0	3	0.4	1.3	1	2	0	3	0.4	1.3	1	2	0	2	0	2	0	2	
std	0	0	1	0.4	1	1.8	1	1	0	1	0	0	0	0	0.2	0	1	0.1	0.2	0	1	0	1	0.1	0.2	0	1	0	0	0	0	1	0	

Figure 11 : Gas chromatographic analysis of mature Arabidopsis seeds transformed with different constructs. Values are generated from the best individual Arabidopsis line. Following constructs are compared: BBC, OstELO5TcD4 and OstELO5EmD4. The construct with c4Des(Eh) delivered highest levels of DHA and highest ratio of DHA:DPA (2,9).

Line	16:	18:	18:	18:	18: n-6	GL	18: n-3	SD	20: n-9	A	EP	DP	DH
BBC	3.9	4.1	9.9	21.0	1.0	10.5	0.6	22.	1.6	10.	-	-	
OstELO TcD4	6.2	3.8	6.2	21.8	2.0	11.6	1.1	14.	1.5	5.4	4.4	3.8	
OstELO EmD4	7.1	3.3	7.9	24.0	1.0	13.6	0.7	14.	1.2	3.8	1.6	4.7	
BBC-OTEIc5- EhD4-2	6.9	3.2	7.1	21.5	1.2	11.8	0.6	13.	1.3	4.6	8.5	0.2	

Figure 12 : Gas chromatographic analysis of mature Arabidopsis seeds transformed with the construct EmELO5Tcd4. The production of 22:5 and 22:6 demonstrate the activity of d5Elo(Eh) in seeds.

FA	16:0	18:0	18:1	18:2	GLA	ALA	18:4	20:0	20:1	20:2	DHGLA	ARA	ETA	EPA	22:1	DPA	DHA		
	$\Delta 9$	$\Delta 11$	$\Delta 9,12$	18:3n6	18:3n3	20:1	$\Delta 11$	$\Delta 13$	$\Delta 11,14$	20:3n6	20:4n3	20:3n3	20:4n3	20:5n3	22:5	22:6			
EmELO5Tcd4_1	6.5	3.4	10.5	2.5	24.2	3.3	12.7	2.0	2.3	17.5	3.3	1.4	0.2	0.5	1.0	1.7	0.2	0.2	
EmELO5Tcd4_2	6.4	3.6	10.0	1.9	25.2	3.1	13.1	1.8	2.3	16.1	2.4	1.4	0.2	1.0	1.2	1.3	0.2	0.2	
EmELO5Tcd4_3	6.1	3.5	14.3	1.8	27.0	0.0	16.8	0.0	2.5	18.4	2.3	3.6	0.0	0.0	1.6	0.0	0.0	0.0	
EmELO5Tcd4_4	7.0	3.6	10.1	2.4	26.2	3.4	11.9	2.1	2.3	15.9	2.9	1.1	0.3	0.6	1.2	1.2	0.3	0.4	
EmELO5Tcd4_5	6.3	3.5	8.3	2.1	23.1	1.5	12.0	0.8	2.2	15.6	2.7	1.4	1.1	1.3	1.4	8.7	1.2	0.2	0.3
EmELO5Tcd4_6	6.1	3.6	10.6	2.1	24.9	1.6	13.3	0.8	2.3	16.6	2.5	1.8	0.9	0.7	1.3	4.8	1.3	0.2	0.2
EmELO5Tcd4_7	5.5	3.4	10.3	1.8	25.7	0.5	12.6	0.2	2.5	18.2	2.3	2.0	0.9	1.4	1.1	6.0	1.5	0.2	0.3
average	6.3	3.5	10.6	2.1	25.2	1.9	13.2	1.1	2.4	16.9	2.6	1.8	0.5	0.8	1.2	4.8	1.4	0.2	0.2
std	0.5	0.1	1.8	0.3	1.3	1.4	1.7	0.9	0.1	1.1	0.4	0.9	0.4	0.5	0.2	0.9	0.2	0.1	0.1

Figure 13 : Gas chromatographic analysis of mature Arabidopsis seeds transformed with four different constructs (A). The production of ARA and EPA demonstrates the activity of d5Des(Eh) in seeds (B). It could be shown that d5Des(Eh) has a preference for n-6 fatty acids (ARA).

A)

	Construct
Borage	Glycinin promoter + Borage
Borage Δ6:	Glycinin promoter + Borage Glycinin promoter
Ostr Δ6=	Glycinin promoter + Ostr
Ostr Δ6:PpELO6=	Glycinin promoter + Ostr Glycinin promoter

B)

Construct	1E0	1E0	1E1	1E2	1E3	GLA	16:3	SFA	10:1	20:2	BHGUA	AA	20:3-3	20:4-3	EPA
Bd6+elo6	6.2	4.1	17.3	24.8	4.4	9.5	1.8	15.4	4.3	2.6	-	1.3	0.9	-	-
Od6+elb6	6.6	4.3	12.9	26.3	0.9	9.5	0.4	11.4	1.7	9.1	-	0.5	4.2	-	-
B6/elb6/Ed5	10.8	4.3	9.3	26.0	4.4	10.7	2.0	10.6	5.0	2.0	1.4	1.7	1.0	0.5	0.5
O6/elb6/EdE	10.3	3.9	9.5	23.9	1.0	10.5	0.0	11.5	2.3	5.2	4.7	0.5	3.9	1.4	1.4

### NOVEL FATTY ACID DESATURASES AND ELONGASES AND USES THEREOF

**[0001]** The invention in principle pertains to the field of recombinant manufacture of fatty acids. It provides nucleic acid molecules which encode novel fatty acid desaturases and elongases. The invention also provides recombinant expression vectors containing desaturase and elongase nucleic acid molecules, host cells into which the expression vectors have been introduced, and methods for large-scale production of long chain polyunsaturated fatty acids (LCPUFAs), e.g. arachidonic acid (ARA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA).

**[0002]** Fatty acids are carboxylic acids with long-chain hydrocarbon side groups that play a fundamental role in many biological processes. Fatty acids are rarely found free in nature but, rather, occur in esterified form as the major component of lipids. As such, lipids/fatty acids are sources of energy (e.g.,  $\beta$ -oxidation). In addition, lipids/fatty acids are an integral part of cell membranes and, therefore, are indispensable for processing biological or biochemical information.

**[0003]** Fatty acids can be divided into two groups: saturated fatty acids formed of single carbon bonds and the unsaturated fatty acids which contain one or more carbon double bonds in *cis*-configuration. Unsaturated fatty acids are produced by terminal desaturases that belong to the class of nonheme-iron enzymes. Each of these enzymes are part of an electron-transport system that contains two other proteins, namely cytochrome  $b_5$  and NADH-cytochrome  $b_5$  reductase. Specifically, such enzymes catalyze the formation of double bonds between the carbon atoms of a fatty acid molecule, for example, by catalyzing the oxygen-dependent dehydrogenation of fatty acids (Sperling et al., 2003). Human and other mammals have a limited spectrum of desaturases that are required for the formation of particular double bonds in unsaturated fatty acids and thus, have a limited capacity for synthesizing essential fatty acids, e.g., long chain polyunsaturated fatty acids (LCPUFAs). Thus, humans have to take up some fatty acids through their diet. Such essential fatty acids include, for example, linoleic acid (C18:2), linolenic acid (C18:3). In contrast, insects, microorganisms and plants are able to synthesize a much larger variety of unsaturated fatty acids and their derivatives. Indeed, the biosynthesis of fatty acids is a major activity of plants and microorganisms.

**[0004]** Long chain polyunsaturated fatty acids (LCPUFAs) such as docosahexaenoic acid (DHA, 22:6(4,7,10,13,16,19)) are essential components of cell membranes of various tissues and organelles in mammals (nerve, retina, brain and immune cells). For example, over 30% of fatty acids in brain phospholipid are 22:6 (n-3) and 20:4 (n-6) (Crawford, M. A., et al., (1997) *Am. J. Clin. Nutr.* 66:1032 S-1041S). In retina, DHA accounts for more than 60% of the total fatty acids in the rod outer segment, the photosensitive part of the photoreceptor cell (Giusto, N. M., et al. (2000) *Prog. Lipid Res.* 39:315-391). Clinical studies have shown that DHA is essential for the growth and development of the brain in infants, and for maintenance of normal brain function in adults (Martinetz, M. (1992) *J. Pediatr.* 120:S129-S138). DHA also has significant effects on photoreceptor function involved in the signal transduction process, rhodopsin activation, and rod and cone development (Giusto, N. M., et al. (2000) *Prog. Lipid Res.* 39:315-391). In addition, some positive effects of DHA were

also found on diseases such as hypertension, arthritis, atherosclerosis, depression, thrombosis and cancers (Horrocks, L. A. and Yeo, Y. K. (1999) *Pharmacol. Res.* 40:211-215). Therefore, appropriate dietary supply of the fatty acid is important for human health. Because such fatty acids cannot be efficiently synthesized by infants, young children and senior citizens, it is particularly important for these individuals to adequately intake these fatty acids from the diet (Spector, A. A. (1999) *Lipids* 34:S1-S3).

**[0005]** Currently the major sources of DHA are oils from fish and algae. Fish oil is a major and traditional source for this fatty acid, however, it is usually oxidized by the time it is sold. In addition, the supply of fish oil is highly variable, particularly in view of the shrinking fish populations. Moreover, the algal source of oil is expensive due to low yield and the high costs of extraction.

**[0006]** EPA and ARA are both  $\Delta 5$  essential fatty acids. They form a unique class of food and feed constituents for humans and animals. EPA belongs to the n-3 series with five double bonds in the acyl chain. EPA is found in marine food and is abundant in oily fish from North Atlantic. ARA belongs to the n-6 series with four double bonds. The lack of a double bond in the  $\omega$ -3 position confers on ARA different properties than those found in EPA. The eicosanoids produced from AA have strong inflammatory and platelet aggregating properties, whereas those derived from EPA have anti-inflammatory and anti-platelet aggregating properties. ARA can be obtained from some foods such as meat, fish and eggs, but the concentration is low.

**[0007]** Gamma-linolenic acid (GLA) is another essential fatty acid found in mammals. GLA is the metabolic intermediate for very long chain n-6 fatty acids and for various active molecules. In mammals, formation of long chain polyunsaturated fatty acids is rate-limited by  $\Delta 6$  desaturation. Many physiological and pathological conditions such as aging, stress, diabetes, eczema, and some infections have been shown to depress the  $\Delta 6$  desaturation step. In addition, GLA is readily catabolized from the oxidation and rapid cell division associated with certain disorders, e.g., cancer or inflammation. Therefore, dietary supplementation with GLA can reduce the risks of these disorders. Clinical studies have shown that dietary supplementation with GLA is effective in treating some pathological conditions such as atopic eczema, premenstrual syndrome, diabetes, hypercholesterolemia, and inflammatory and cardiovascular disorders.

**[0008]** A large number of beneficial health effects have been shown for DHA or mixtures of EPA/DHA. DHA is a n-3 very long chain fatty acid with six double bonds.

**[0009]** Although biotechnology offers an attractive route for the production of specialty fatty acids, current techniques fail to provide an efficient means for the large scale production of unsaturated fatty acids. Accordingly, there exists a need for an improved and efficient method of producing unsaturated fatty acids, such as DHA, EPA and ARA.

**[0010]** Thus, the present invention relates to a polynucleotide comprising a nucleic acid sequence elected from the group consisting of:

**[0011]** a) a nucleic acid sequence having a nucleotide sequence as shown in SEQ ID NOs: 1, 3, 5, 7 or 9;

**[0012]** b) a nucleic acid sequence encoding a polypeptide having an amino acid sequence as shown in SEQ ID NOs: 2, 4, 6, 8 or 10;

**[0013]** c) a nucleic acid sequence being at least 70% identical to the nucleic acid sequence of a) or b), wherein said

nucleic acid sequence encodes a polypeptide having desaturase or elongase activity;

[0014] d) a nucleic acid sequence encoding a polypeptide having desaturase or elongase activity and having an amino acid sequence which is at least 82% identical to the amino acid sequence of any one of a) to c); and

[0015] e) a nucleic acid sequence which is capable of hybridizing under stringent conditions to any one of a) to d), wherein said nucleic acid sequence encodes a polypeptide having desaturase or elongase activity.

[0016] The term "polynucleotide" as used in accordance with the present invention relates to a polynucleotide comprising a nucleic acid sequence which encodes a polypeptide having desaturase or elongase activity. Preferably, the polypeptide encoded by the polynucleotide of the present invention having desaturase or elongase activity upon expression in a plant shall be capable of increasing the amount of PUFA and, in particular, LCPUFA in, e.g., seed oils or the entire plant or parts thereof. Such an increase is, preferably, statistically significant when compared to a LCPUFA producing transgenic control plant which expresses the present state of the art set of desaturases and elongases required for LCPUFA synthesis but does not express the polynucleotide of the present invention. Whether an increase is significant can be determined by statistical tests well known in the art including, e.g., Student's t-test. More preferably, the increase is an increase of the amount of triglycerides containing LCPUFA of at least 5%, at least 10%, at least 15%, at least 20% or at least 30% compared to the said control. Preferably, the LCPUFA referred to before is a polyunsaturated fatty acid having a C-20 or C-22 fatty acid body, more preferably, ARA, EPA or DHA. Suitable assays for measuring the activities mentioned before are described in the accompanying Examples.

[0017] The term "desaturase" or "elongase" as used herein refers to the activity of a desaturase, introducing a double bond into the carbon chain of a fatty acid, preferably into fatty acids with 18, 20 or 22 carbon molecules, or an elongase, introducing two carbon molecules into the carbon chain of a fatty acid, preferably into fatty acids with 18, 20 or 22 carbon molecules

[0018] More preferably, polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 1, 3, 5, 7 or 9 encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 2, 4, 6, 8 or 10 or variants thereof, preferably, exhibit desaturase or elongase activity.

[0019] Polynucleotides encoding a polypeptide having desaturase or elongase activity as specified above has been obtained in accordance with the present invention, preferably, from *Emiliana huxleyi*. However, orthologs, paralogs or other homologs may be identified from other species. Preferably, they are obtained from plants such as algae, for example *Isochrysis*, *Mantoniella*, *Ostreococcus* or *Cryptocodinium*, algae/diatoms such as *Phaeodactylum*, *Thalassiosira* or *Thraustochytrium*, mosses such as *Physcomitrella* or *Ceratodon*, or higher plants such as the Primulaceae such as *Aleuritia*, *Calendula stellata*, *Osteospermum spinescens* or *Osteospermum hyoseroides*, microorganisms such as fungi, such as *Aspergillus*, *Phytophthora*, *Entomophthora*, *Mucor* or *Mortierella*, bacteria such as *Shewanella*, yeasts or animals. Preferred animals are nematodes such as *Caenorhabditis*, insects or vertebrates. Among the vertebrates, the nucleic acid molecules may, preferably, be derived from *Euteleostomi*, *Actinopterygii*; *Neopterygii*; *Teleostei*; *Eute-*

*leostei*, *Protacanthopterygii*, *Salmoniformes*; *Salmonidae* or *Oncorhynchus*, more preferably, from the order of the Salmoniformes, most preferably, the family of the Salmonidae, such as the genus *Salmo*, for example from the genera and species *Oncorhynchus mykiss*, *Trutta trutta* or *Salmo trutta fario*. Moreover, the nucleic acid molecules may be obtained from the diatoms such as the genera *Thalassiosira* or *Phaeodactylum*.

[0020] Thus, the term "polynucleotide" as used in accordance with the present invention further encompasses variants of the aforementioned specific polynucleotides representing orthologs, paralogs or other homologs of the polynucleotide of the present invention. Moreover, variants of the polynucleotide of the present invention also include artificially generated muteins. Said muteins include, e.g., enzymes which are generated by mutagenesis techniques and which exhibit improved or altered substrate specificity, or codon optimized polynucleotides. The polynucleotide variants, preferably, comprise a nucleic acid sequence characterized in that the sequence can be derived from the aforementioned specific nucleic acid sequences shown in any one of SEQ ID NOs: 1, 3, 5, 7 or 9 or by a polynucleotide encoding a polypeptide having an amino acid sequence as shown in any one of SEQ ID NOs: 2, 4, 6, 8 or 10 by at least one nucleotide substitution, addition and/or deletion, whereby the variant nucleic acid sequence shall still encode a polypeptide having a desaturase or elongase activity as specified above. Variants also encompass polynucleotides comprising a nucleic acid sequence which is capable of hybridizing to the aforementioned specific nucleic acid sequences, preferably, under stringent hybridization conditions. These stringent conditions are known to the skilled worker and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y. (1989), 6.3.1-6.3.6. A preferred example for stringent hybridization conditions are hybridization conditions in 6x sodium chloride/sodium citrate (=SSC) at approximately 45° C., followed by one or more wash steps in 0.2xSSC, 0.1% SDS at 50 to 65° C. The skilled worker knows that these hybridization conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, with regard to the temperature and concentration of the buffer. For example, under "standard hybridization conditions" the temperature differs depending on the type of nucleic acid between 42° C. and 58° C. in aqueous buffer with a concentration of 0.1 to 5xSSC (pH 7.2). If organic solvent is present in the abovementioned buffer, for example 50% formamide, the temperature under standard conditions is approximately 42° C. The hybridization conditions for DNA: DNA hybrids are, preferably, 0.1xSSC and 20° C. to 45° C., preferably between 30° C. and 45° C. The hybridization conditions for DNA:RNA hybrids are, preferably, 0.1xSSC and 30° C. to 55° C., preferably between 45° C. and 55° C. The above-mentioned hybridization temperatures are determined for example for a nucleic acid with approximately 100 bp (=base pairs) in length and a G+C content of 50% in the absence of formamide. The skilled worker knows how to determine the hybridization conditions required by referring to textbooks such as the textbook mentioned above, or the following textbooks: Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (Ed.) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (Ed.) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford.

Alternatively, polynucleotide variants are obtainable by PCR-based techniques such as mixed oligonucleotide primer-based amplification of DNA, i.e. using degenerated primers against conserved domains of the polypeptides of the present invention. Conserved domains of the polypeptide of the present invention may be identified by a sequence comparison of the nucleic acid sequences of the polynucleotides or the amino acid sequences of the polypeptides of the present invention. Oligonucleotides suitable as PCR primers as well as suitable PCR conditions are described in the accompanying Examples. As a template, DNA or cDNA from bacteria, fungi, plants or animals may be used. Further, variants include polynucleotides comprising nucleic acid sequences which are at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the nucleic acid sequences shown in any one of SEQ ID NOs: 1, 3, 5, 7 or 9, preferably, encoding polypeptides retaining a desaturase or elongase activity as specified above. Moreover, also encompassed are polynucleotides which comprise nucleic acid sequences encoding a polypeptide having an amino acid sequences which are at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequences shown in any one of SEQ ID NOs: 2, 4, 6, 8 or 10, wherein the polypeptide, preferably, retains desaturase or elongase activity as specified above. The percent identity values are, preferably, calculated over the entire amino acid or nucleic acid sequence region. A series of programs based on a variety of algorithms is available to the skilled worker for comparing different sequences. In a preferred embodiment, the percent

sequences is determined using the needle program in the EMBOSS software package (EMBOSS: The European Molecular Biology Open Software Suite, Rice, P., Longden, I., and Bleasby, A, Trends in Genetics 16(6), 276-277, 2000), using the EDNAFULL scoring matrix and a gap opening penalty of 16, 14, 12, 10, 8, 6, or 4 and a gap extension penalty of 0.5, 1, 2, 3, 4, 5, or 6. A preferred, non-limiting example of parameters to be used in conjunction for aligning two amino acid sequences using the needle program are the default parameters, including the EDNAFULL scoring matrix, a gap opening penalty of 10 and a gap extension penalty of 0.5. The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the BLAST series of programs (version 2.2) of Altschul et al. (Altschul 1990, J. Mol. Biol. 215:403-10). BLAST using acyltransferase nucleic acid sequences of the invention as query sequence can be performed with the BLASTn, BLASTx or the tBLASTx program using default parameters to obtain either nucleotide sequences (BLASTn, tBLASTx) or amino acid sequences (BLASTx) homologous to acyltransferase sequences of the invention. BLAST using acyltransferase protein sequences of the invention as query sequence can be performed with the BLASTp or the tBLASTn program using default parameters to obtain either amino acid sequences (BLASTp) or nucleic acid sequences (tBLASTn) homologous to acyltransferase sequences of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST using default parameters can be utilized as described in Altschul et al. (Altschul 1997, Nucleic Acids Res. 25(17):3389-3402).

TABLE 1

Relation of sequence types of query and hit sequences for various BLAST programs				
Input query sequence	Converted Query	Algorithm	Converted Hit	Actual Database
DNA		BLASTn		DNA
PRT		BLASTp		PRT
DNA	PRT	BLASTx		PRT
PRT		tBLASTn	PRT	DNA
DNA	PRT	tBLASTx	PRT	DNA

identity between two amino acid sequences is determined using the Needleman and Wunsch algorithm (Needleman 1970, J. Mol. Biol. (48):444-453) which has been incorporated into the needle program in the EMBOSS software package (EMBOSS: The European Molecular Biology Open Software Suite, Rice, P., Longden, I., and Bleasby, A, Trends in Genetics 16(6), 276-277, 2000), using either a BLOSUM 45 or PAM250 scoring matrix for distantly related proteins, or either a BLOSUM 62 or PAM160 scoring matrix for closer related proteins, and a gap opening penalty of 16, 14, 12, 10, 8, 6, or 4 and a gap extension penalty of 0.5, 1, 2, 3, 4, 5, or 6. Guides for local installation of the EMBOSS package as well as links to WEB-Services can be found at <http://emboss.sourceforge.net>. A preferred, non-limiting example of parameters to be used for aligning two amino acid sequences using the needle program are the default parameters, including the EBLOSUM62 scoring matrix, a gap opening penalty of 10 and a gap extension penalty of 0.5. In yet another preferred embodiment, the percent identity between two nucleotide

**[0021]** A polynucleotide comprising a fragment of any of the aforementioned nucleic acid sequences is also encompassed as a polynucleotide of the present invention. The fragment shall encode a polypeptide which still has desaturase and elongase activity as specified above. Accordingly, the polypeptide may comprise or consist of the domains of the polypeptide of the present invention conferring the said biological activity. A fragment as meant herein, preferably, comprises at least 50, at least 100, at least 250 or at least 500 consecutive nucleotides of any one of the aforementioned nucleic acid sequences or encodes an amino acid sequence comprising at least 20, at least 30, at least 50, at least 80, at least 100 or at least 150 consecutive amino acids of any one of the aforementioned amino acid sequences.

**[0022]** The variant polynucleotides or fragments referred to above, preferably, encode polypeptides retaining desaturase or elongase activity to a significant extent, preferably, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the

desaturase and elongase activity exhibited by any of the polypeptide shown in any one of SEQ ID NOs: 2, 4, 6, 8 or 10. The activity may be tested as described in the accompanying Examples.

**[0023]** The polynucleotides of the present invention either essentially consist of the aforementioned nucleic acid sequences or comprise the aforementioned nucleic acid sequences. Thus, they may contain further nucleic acid sequences as well. Preferably, the polynucleotide of the present invention may comprise in addition to an open reading frame further untranslated sequence at the 3' and at the 5' terminus of the coding gene region: at least 500, preferably 200, more preferably 100 nucleotides of the sequence upstream of the 5' terminus of the coding region and at least 100, preferably 50, more preferably 20 nucleotides of the sequence downstream of the 3' terminus of the coding gene region. Furthermore, the polynucleotides of the present invention may encode fusion proteins wherein one partner of the fusion protein is a polypeptide being encoded by a nucleic acid sequence recited above. Such fusion proteins may comprise as additional part other enzymes of the fatty acid or PUFA biosynthesis pathways, polypeptides for monitoring expression (e.g., green, yellow, blue or red fluorescent proteins, alkaline phosphatase and the like) or so called "tags" which may serve as a detectable marker or as an auxiliary measure for purification purposes. Tags for the different purposes are well known in the art and comprise FLAG-tags, 6-histidine-tags, MYC-tags and the like.

**[0024]** The polynucleotide of the present invention shall be provided, preferably, either as an isolated polynucleotide (i.e. purified or at least isolated from its natural context such as its natural gene locus) or in genetically modified or exogenously (i.e. artificially) manipulated form. An isolated polynucleotide can, for example, comprise less than approximately 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid is derived. The polynucleotide, preferably, is provided in the form of double or single stranded molecule. It will be understood that the present invention by referring to any of the aforementioned polynucleotides of the invention also refers to complementary or reverse complementary strands of the specific sequences or variants thereof referred to before. The polynucleotide encompasses DNA, including cDNA and genomic DNA, or RNA polynucleotides.

**[0025]** However, the present invention also pertains to polynucleotide variants which are derived from the polynucleotides of the present invention and are capable of interfering with the transcription or translation of the polynucleotides of the present invention. Such variant polynucleotides include anti-sense nucleic acids, ribozymes, siRNA molecules, morpholino nucleic acids (phosphorodiamidate morpholino oligos), triple-helix forming oligonucleotides, inhibitory oligonucleotides, or micro RNA molecules all of which shall specifically recognize the polynucleotide of the invention due to the presence of complementary or substantially complementary sequences. These techniques are well known to the skilled artisan. Suitable variant polynucleotides of the aforementioned kind can be readily designed based on the structure of the polynucleotides of this invention.

**[0026]** Moreover, comprised are also chemically modified polynucleotides including naturally occurring modified poly-

nucleotides such as glycosylated or methylated polynucleotides or artificial modified ones such as biotinylated polynucleotides.

**[0027]** In the studies underlying the present invention, advantageously, polynucleotides were identified encoding desaturase and elongases from *Emiliana huxleyi*. In particular, the *Emiliana huxleyi* desaturases  $\Delta 4$ Des(Eh),  $\Delta 8$ Des(Eh) and  $\Delta 5$ Des(Eh) and elongases  $\Delta 9$ Elo(Eh) and  $\Delta 5$ EIo(Eh) have been identified. Each of the desaturases are capable of introducing a double bond into fatty acids. For example, the expression of the  $\Delta 8$ Des(Eh) leads to introduction of a double bond at position eight into C20:2n-6 fatty acid. The polynucleotides of the present invention are particularly suitable for the recombinant manufacture of LCPUFAs and, in particular, ARA, EPA and/or DHA.

**[0028]** In a preferred embodiment of the polynucleotide of the present invention, said polynucleotide further comprises an expression control sequence operatively linked to the said nucleic acid sequence.

**[0029]** The term "expression control sequence" as used herein refers to a nucleic acid sequence which is capable of governing, i.e. initiating and controlling, transcription of a nucleic acid sequence of interest, in the present case the nucleic sequences recited above. Such a sequence usually comprises or consists of a promoter or a combination of a promoter and enhancer sequences. Expression of a polynucleotide comprises transcription of the nucleic acid molecule, preferably, into a translatable mRNA. Additional regulatory elements may include transcriptional as well as translational enhancers. The following promoters and expression control sequences may be, preferably, used in an expression vector according to the present invention. The *cos*, *tac*, *trp*, *tet*, *trp-tet*, *Ipp*, *lac*, *Ipp-lac*, *lacIq*, *T7*, *T5*, *T3*, *gal*, *trc*, *ara*, *SP6*,  $\lambda$ -PR or  $\lambda$ -PL promoters are, preferably, used in Gram-negative bacteria. For Gram-positive bacteria, promoters *amy* and *SPO2* may be used. From yeast or fungal promoters *ADC1*, *AOX1r*, *GAL1*, *MF $\alpha$* , *AC*, *P-60*, *CYC1*, *GAPDH*, *TEF*, *rp28*, *ADH* are, preferably, used. For animal cell or organism expression, the promoters *CMV-*, *SV40-*, *RSV-promoter* (Rous sarcoma virus), *CMV-enhancer*, *SV40-enhancer* are preferably used. From plants the promoters *CaMV/35S* (Franck 1980, Cell 21: 285-294), *PRP1* (Ward 1993, Plant. Mol. Biol. 22), *SSU*, *OCS*, *lib4*, *usp*, *STLS1*, *B33*, *nos* or the ubiquitin or phaseolin promoter. Also preferred in this context are inducible promoters, such as the promoters described in EP 0 388 186 A1 (i.e. a benzylsulfonamide-inducible promoter), Gatz 1992, Plant J. 2:397-404 (i.e. a tetracyclin-inducible promoter), EP 0 335 528 A1 (i.e. an abscisic-acid-inducible promoter) or WO 93/21334 (i.e. an ethanol- or cyclohexenol-inducible promoter). Further suitable plant promoters are the promoter of cytosolic FBPase or the *ST-LSI* promoter from potato (Stockhaus 1989, EMBO J. 8, 2445), the phosphoribosyl-pyrophosphate amidotransferase promoter from *Glycine max* (Genbank accession No. U87999) or the node-specific promoter described in EP 0 249 676 A1. Particularly preferred are promoters which enable the expression in tissues which are involved in the biosynthesis of fatty acids. Also particularly preferred are seed-specific promoters such as the *USP* promoter in accordance with the practice, but also other promoters such as the *LeB4*, *DC3*, *phaseolin* or *napin* promoters. Further especially preferred promoters are seed-specific promoters which can be used for monocotyledonous or dicotyledonous plants and which are described in U.S. Pat. No. 5,608,152 (*napin* promoter from oilseed rape),

WO 98/45461 (oleosin promoter from *Arabidopsis*, U.S. Pat. No. 5,504,200 (phaseolin promoter from *Phaseolus vulgaris*), WO 91/13980 (Bce4 promoter from *Brassica*), by Baumlein et al., Plant J., 2, 2, 1992:233-239 (LeB4 promoter from a legume), these promoters being suitable for dicots. The following promoters are suitable for monocots: Ipt-2 or Ipt-1 promoter from barley (WO 95/15389 and WO 95/23230), hordein promoter from barley and other promoters which are suitable and which are described in WO 99/16890. In principle, it is possible to use all natural promoters together with their regulatory sequences, such as those mentioned above, for the novel process. Likewise, it is possible and advantageous to use synthetic promoters, either additionally or alone, especially when they mediate a seed-specific expression, such as, for example, as described in WO 99/16890. In a particular embodiment, seed-specific promoters are utilized to enhance the production of the desired PUFA or LCPUFA.

**[0030]** The term “operatively linked” as used herein means that the expression control sequence and the nucleic acid of interest are linked so that the expression of the said nucleic acid of interest can be governed by the said expression control sequence, i.e. the expression control sequence shall be functionally linked to the said nucleic acid sequence to be expressed. Accordingly, the expression control sequence and, the nucleic acid sequence to be expressed may be physically linked to each other, e.g., by inserting the expression control sequence at the 5' end of the nucleic acid sequence to be expressed. Alternatively, the expression control sequence and the nucleic acid to be expressed may be merely in physical proximity so that the expression control sequence is capable of governing the expression of at least one nucleic acid sequence of interest. The expression control sequence and the nucleic acid to be expressed are, preferably, separated by not more than 500 bp, 300 bp, 100 bp, 80 bp, 60 bp, 40 bp, 20 bp, 10 bp or 5 bp.

**[0031]** In a further preferred embodiment of the polynucleotide of the present invention, said polynucleotide further comprises a terminator sequence operatively linked to the nucleic acid sequence.

**[0032]** The term “terminator” as used herein refers to a nucleic acid sequence which is capable of terminating transcription. These sequences will cause dissociation of the transcription machinery from the nucleic acid sequence to be transcribed. Preferably, the terminator shall be active in plants and, in particular, in plant seeds. Suitable terminators are known in the art and, preferably, include polyadenylation signals such as the SV40-poly-A site or the tk-poly-A site or one of the plant specific signals indicated in Loke et al. (Loke 2005, Plant Physiol 138, pp. 1457-1468), downstream of the nucleic acid sequence to be expressed.

**[0033]** The present invention also relates to a vector comprising the polynucleotide of the present invention.

**[0034]** The term “vector”, preferably, encompasses phage, plasmid, viral vectors as well as artificial chromosomes, such as bacterial or yeast artificial chromosomes. Moreover, the term also relates to targeting constructs which allow for random or site-directed integration of the targeting construct into genomic DNA. Such target constructs, preferably, comprise DNA of sufficient length for either homologous or heterologous recombination as described in detail below. The vector encompassing the polynucleotide of the present invention, preferably, further comprises selectable markers for propagation and/or selection in a host. The vector may be incorpo-

rated into a host cell by various techniques well known in the art. If introduced into a host cell, the vector may reside in the cytoplasm or may be incorporated into the genome. In the latter case, it is to be understood that the vector may further comprise nucleic acid sequences which allow for homologous recombination or heterologous insertion. Vectors can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. The terms “transformation” and “transfection”, conjugation and transduction, as used in the present context, are intended to comprise a multiplicity of prior-art processes for introducing foreign nucleic acid (for example DNA) into a host cell, including calcium phosphate, rubidium chloride or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, carbon-based clusters, chemically mediated transfer, electroporation or particle bombardment. Suitable methods for the transformation or transfection of host cells, including plant cells, can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) and other laboratory manuals, such as Methods in Molecular Biology, 1995, Vol. 44, *Agrobacterium* protocols, Ed.: Gartland and Davey, Humana Press, Totowa, N.J. Alternatively, a plasmid vector may be introduced by heat shock or electroporation techniques. Should the vector be a virus, it may be packaged in vitro using an appropriate packaging cell line prior to application to host cells.

**[0035]** Preferably, the vector referred to herein is suitable as a cloning vector, i.e. replicable in microbial systems. Such vectors ensure efficient cloning in bacteria and, preferably, yeasts or fungi and make possible the stable transformation of plants. Those which must be mentioned are, in particular, various binary and co-integrated vector systems which are suitable for the T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they contain at least the vir genes, which are required for the *Agrobacterium*-mediated transformation, and the sequences which delimit the T-DNA (T-DNA border). These vector systems, preferably, also comprise further cis-regulatory regions such as promoters and terminators and/or selection markers with which suitable transformed host cells or organisms can be identified. While co-integrated vector systems have vir genes and T-DNA sequences arranged on the same vector, binary systems are based on at least two vectors, one of which bears vir genes, but no T-DNA, while a second one bears T-DNA, but no vir gene. As a consequence, the last-mentioned vectors are relatively small, easy to manipulate and can be replicated both in *E. coli* and in *Agrobacterium*. These binary vectors include vectors from the pBIB-HYG, pPZP, pBecks, pGreen series. Preferably used in accordance with the invention are Bin19, pBI101, pBinAR, pGPTV and pCAMBIA. An overview of binary vectors and their use can be found in Hellens et al, Trends in Plant Science (2000) 5, 446-451. Furthermore, by using appropriate cloning vectors, the polynucleotides can be introduced into host cells or organisms such as plants or animals and, thus, be used in the transformation of plants, such as those which are published, and cited, in: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Fla.), chapter 6/7, pp. 71-119 (1993); F. F. White, Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, 15-38; B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, vol. 1, Engineering

and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus 1991, *Annu. Rev. Plant Physiol. Plant Molec. Biol.* 42, 205-225.

**[0036]** More preferably, the vector of the present invention is an expression vector. In such an expression vector, i.e. a vector which comprises the polynucleotide of the invention having the nucleic acid sequence operatively linked to an expression control sequence (also called "expression cassette") allowing expression in prokaryotic or eukaryotic cells or isolated fractions thereof. Suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (Invitrogen) or pSPORT1 (GIBCO BRL). Further examples of typical fusion expression vectors are pGEX (Pharmacia Biotech Inc; Smith 1988, *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.), where glutathione S-transferase (GST), maltose E-binding protein and protein A, respectively, are fused with the recombinant target protein. Examples of suitable inducible nonfusion *E. coli* expression vectors are, inter alia, pTrc (Amann 1988, *Gene* 69:301-315) and pET 11d (Studier 1990, *Methods in Enzymology* 185, 60-89). The target gene expression of the pTrc vector is based on the transcription from a hybrid trp-lac fusion promoter by host RNA polymerase. The target gene expression from the pET 11d vector is based on the transcription of a T7-gn10-lac fusion promoter, which is mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is provided by the host strains BL21 (DE3) or HMS174 (DE3) from a resident  $\lambda$ -prophage which harbors a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter. The skilled worker is familiar with other vectors which are suitable in prokaryotic organisms; these vectors are, for example, in *E. coli*, pLG338, pACYC184, the pBR series such as pBR322, the pUC series such as pUC18 or pUC19, the M13 mp series, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1,  $\lambda$ gt11 or pBdCl, in *Streptomyces* pIJ101, pIJ364, pIJ702 or pIJ361, in *Bacillus* pUB110, pC194 or pBD214, in *Corynebacterium* pSA77 or pAJ667. Examples of vectors for expression in the yeast *S. cerevisiae* comprise pYep Sec1 (Baldari 1987, *Embo J.* 6:229-234), pMFa (Kurjan 1982, *Cell* 30:933-943), pJRY88 (Schultz 1987, *Gene* 54:113-123) and pYES2 (Invitrogen Corporation, San Diego, Calif.). Vectors and processes for the construction of vectors which are suitable for use in other fungi, such as the filamentous fungi, comprise those which are described in detail in: van den Hondel, C. A. M. J. J., & Punt, P. J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: *Applied Molecular Genetics of fungi*, J. F. Peberdy et al., Ed., pp. 1-28, Cambridge University Press: Cambridge, or in: *More Gene Manipulations in Fungi* (J. W. Bennett & L. L. Lasure, Ed., pp. 396-428: Academic Press: San Diego). Further suitable yeast vectors are, for example, pAG-1, YEep6, YEep13 or pEMBLYe23. As an alternative, the polynucleotides of the present invention can be also expressed in insect cells using baculovirus expression vectors. Baculovirus vectors which are available for the expression of proteins in cultured insect cells (for example Sf9 cells) comprise the pAc series (Smith 1983, *Mol. Cell. Biol.* 3:2156-2165) and the pVL series (Lucklow 1989, *Virology* 170:31-39).

**[0037]** The polynucleotide of the present invention can be expressed in single-cell plant cells (such as algae), see Falcione 1999, *Marine Biotechnology* 1 (3):239-251 and the

references cited therein, and plant cells from higher plants (for example Spermatophytes, such as arable crops) by using plant expression vectors. Examples of plant expression vectors comprise those which are described in detail in: Becker 1992, *Plant Mol. Biol.* 20:1195-1197; Bevan 1984, *Nucl. Acids Res.* 12:8711-8721; Vectors for Gene Transfer in Higher Plants; in: *Transgenic Plants*, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, p. 15-38. A plant expression cassette, preferably, comprises regulatory sequences which are capable of controlling the gene expression in plant cells and which are functionally linked so that each sequence can fulfill its function, such as transcriptional termination, for example polyadenylation signals. Preferred polyadenylation signals are those which are derived from *Agrobacterium tumefaciens* T-DNA, such as the gene 3 of the Ti plasmid pTiACH5, which is known as octopine synthase (Gielen 1984, *EMBO J.* 3, 835) or functional equivalents of these, but all other terminators which are functionally active in plants are also suitable. Since plant gene expression is very often not limited to transcriptional levels, a plant expression cassette preferably comprises other functionally linked sequences such as translation enhancers, for example the overdrive sequence, which comprises the 5'-untranslated tobacco mosaic virus leader sequence, which increases the protein/RNA ratio (Gallie 1987, *Nucl. Acids Research* 15:8693-8711). As described above, plant gene expression must be functionally linked to a suitable promoter which performs the expression of the gene in a timely, cell-specific or tissue-specific manner. Promoters which can be used are constitutive promoters (Benfey 1989, *EMBO J.* 8:2195-2202) such as those which are derived from plant viruses such as 35S CAMV (Franck 1980, *Cell* 21:285-294), 19S CaMV (see U.S. Pat. No. 5,352,605 and WO 84/02913) or plant promoters such as the promoter of the Rubisco small subunit, which is described in U.S. Pat. No. 4,962,028. Other preferred sequences for the use in functional linkage in plant gene expression cassettes are targeting sequences which are required for targeting the gene product into its relevant cell compartment (for a review, see Kermodé 1996, *Crit. Rev. Plant Sci.* 15, 4: 285-423 and references cited therein), for example into the vacuole, the nucleus, all types of plastids, such as amyloplasts, chloroplasts, chromoplasts, the extracellular space, the mitochondria, the endoplasmic reticulum, oil bodies, peroxisomes and other compartments of plant cells. As described above, plant gene expression can also be facilitated via a chemically inducible promoter (for a review, see Gatz 1997, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 48:89-108). Chemically inducible promoters are particularly suitable if it is desired that genes are expressed in a time-specific manner. Examples of such promoters are a salicylic-acid-inducible promoter (WO 95/19443), a tetracyclin-inducible promoter (Gatz 1992, *Plant J.* 2, 397-404) and an ethanol-inducible promoter. Promoters which respond to biotic or abiotic stress conditions are also suitable promoters, for example the pathogen-induced PRP1-gene promoter (Ward 1993, *Plant Mol. Biol.* 22:361-366), the heat-inducible hsp80 promoter from tomato (U.S. Pat. No. 5,187,267), the cold-inducible alpha-amylase promoter from potato (WO 96/12814) or the wound-inducible pinll promoter (EP 0 375 091 A). The promoters which are especially preferred are those which bring about the expression of genes in tissues and organs in which fatty acid, lipid and oil biosynthesis takes place, in seed cells such as the cells of endosperm and of the developing embryo. Suitable promoters are the napin gene

promoter from oilseed rape (U.S. Pat. No. 5,608,152), the USP promoter from *Vicia faba* (Baumlein 1991, Mol. Gen. Genet. 225 (3):459-67), the oleosin promoter from *Arabidopsis* (WO 98/45461), the phaseolin promoter from *Phaseolus vulgaris* (U.S. Pat. No. 5,504,200), the Bce4 promoter from *Brassica* (WO 91/13980) or the legumin B4 promoter (LeB4; Baumlein 1992, Plant Journal, 2 (2):233-9), and promoters which bring about the seed-specific expression in monocotyledonous plants such as maize, barley, wheat, rye, rice and the like. Suitable promoters to be taken into consideration are the Ipt2 or Ipt1 gene promoter from barley (WO 95/15389 and WO 95/23230) or those which are described in WO 99/16890 (promoters from the barley hordein gene, the rice glutelin gene, the rice oryzin gene, the rice prolamin gene, the wheat gliadin gene, wheat glutelin gene, the maize zein gene, the oat glutelin gene, the sorghum kasirin gene, the rye secalin gene). Likewise, especially suitable are promoters which bring about the plastid-specific expression since plastids are the compartment in which the precursors and some end products of lipid biosynthesis are synthesized. Suitable promoters such as the viral RNA-polymerase promoter, are described in WO 95/16783 and WO 97/06250, and the clpP promoter from *Arabidopsis*, described in WO 99/46394.

**[0038]** The abovementioned vectors are only a small overview of vectors to be used in accordance with the present invention. Further vectors are known to the skilled worker and are described, for example, in: Cloning Vectors (Ed., Pouwels, P. H., et al., Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018). For further suitable expression systems for prokaryotic and eukaryotic cells see the chapters 16 and 17 of Sambrook, loc cit.

**[0039]** It follows from the above that, preferably, said vector is an expression vector. More preferably, the said polynucleotide of the present invention is under the control of a seed-specific promoter in the vector of the present invention. A preferred seed-specific promoter as meant herein is selected from the group consisting of Conlinin 1, Conlinin 2, napin, LuFad3, USP, LeB4, Arc, Fae, ACP, LuPXR, and SBP. For details, see, e.g., US 2003-0159174.

**[0040]** Moreover, the present invention relates to a host cell comprising the polynucleotide or the vector of the present invention.

**[0041]** Preferably, said host cell is a plant cell and, more preferably, a plant cell obtained from an oilseed crop. More preferably, said oilseed crop is selected from the group consisting of flax (*Linum* sp.), rapeseed (*Brassica* sp.), soybean (*Glycine* sp.), sunflower (*Helianthus* sp.), cotton (*Gossypium* sp.), corn (*Zea mays*), olive (*Olea* sp.), safflower (*Carthamus* sp.), cocoa (*Theobroma cacao*), peanut (*Arachis* sp.), hemp, camelina, crambe, oil palm, coconuts, groundnuts, sesame seed, castor bean, lesquerella, tallow tree, sheanuts, tungnuts, kapok fruit, poppy seed, jojoba seeds and *perilla*.

**[0042]** Also preferably, said host cell is a microorganism. More preferably, said microorganism is a bacterium, a fungus or algae. More preferably, it is selected from the group consisting of *Candida*, *Cryptococcus*, *Lipomyces*, *Rhodospiridium*, *Yarrowia* and *Schizochytrium*.

**[0043]** Moreover, a host cell according to the present invention may also be an animal cell. Preferably, said animal host cell is a host cell of a fish or a cell line obtained therefrom.

**[0044]** More preferably, the fish host cell is from herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna.

**[0045]** Generally, the controlling steps in the production of LCPUFAs, i.e., the long chain unsaturated fatty acid biosynthetic pathway, are catalyzed by membrane-associated fatty acid desaturases and elongases. Plants and most other eukaryotic organisms have specialized desaturase and elongase systems for the introduction of double bonds and the extension of fatty acids beyond C18 atoms. The elongase reactions have several important features in common with the fatty acid synthase complex (FAS). However, the elongase complex is different from the FAS complex as the complex is localized in the cytosol and membrane bound, ACP is not involved and the elongase 3-keto-acyl-CoA-synthase catalyzes the condensation of malonyl-CoA with an acyl primer. The elongase complex consists of four components with different catalytic functions, the keto-acyl-synthase (condensation reaction of malonyl-CoA to acyl-CoA, creation of a 2 C atom longer keto-acyl-CoA fatty acid), the keto-acyl-reductase (reduction of the 3-keto group to a 3-hydroxy-group), the dehydratase (dehydration results in a 3-enoyl-acyl-CoA fatty acid) and the enoyl-CoA-reductase (reduction of the double bond at position 3, release from the complex). For the production of LCPUFAs including ARA, EPA and/or DHA the elongation reactions, beside the desaturation reactions, are essential. Higher plants do not have the necessary enzyme set to produce LCPUFAs (4 or more double bonds, 20 or more C atoms). Therefore the catalytic activities have to be conferred to the plants or plant cells. The polynucleotides of the present invention catalyze the desaturation and elongation activities necessary for the formation of ARA, EPA and/or DHA. By delivering the novel desaturases and elongases increased levels of PUFAs and LCPUFAs are produced.

**[0046]** However, person skilled in the art knows that dependent on the host cell, further, enzymatic activities may be conferred to the host cells, e.g., by recombinant technologies. Accordingly, the present invention, preferably, envisages a host cell which in addition to the polynucleotide of the present invention comprises polynucleotides encoding such desaturases and/or elongases as required depending on the selected host cell. Preferred desaturases and/or elongases which shall be present in the host cell are at least one enzyme selected from the group consisting of:  $\Delta$ -4-desaturase,  $\Delta$ -5-desaturase,  $\Delta$ -5-elongase,  $\Delta$ -6-desaturase,  $\Delta$ 12-desaturase,  $\Delta$ 15-desaturase, w3-desaturase and  $\Delta$ -6-elongase. Especially preferred are the bifunctional d12d15-Desaturases d12d15Des(Ac) from *Acanthamoeba castellanii* (WO2007042510), d12d15Des(Cp) from *Claviceps purpurea* (WO2008006202) and d12d15Des(Lg)1 from *Lottia gigantea* (WO2009016202), the d12-Desaturases d12Des(Co) from *Calendula officinalis* (WO200185968), d12Des(Lb) from *Laccaria bicolor* (WO2009016202), d12Des(Mb) from *Monosiga brevicollis* (WO2009016202), d12Des(Mg) from *Mycosphaerella graminicola* (WO2009016202), d12Des(Nh) from *Nectria haematococca* (WO2009016202), d12Des(O1) from *Ostreococcus lucimarinus* (WO2008040787), d12Des(Pb) from *Phycomyces blakesleeanus* (WO2009016202), d12Des(Ps) from *Phytophthora sojae* (WO2006100241) and d12Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d15-Desaturases d15Des(Hr) from *Helobdella robusta* (WO2009016202), d15Des(Mc) from *Microcoleus chthonoplastes* (WO2009016202), d15Des(Mf) from *Mycosphaerella fijiensis* (WO2009016202), d15Des(Mg) from *Mycosphaerella graminicola* (WO2009016202) and d15Des(Nh)2 from *Nectria haematococca* (WO2009016202), the d4-Desaturases

d4Des(Eg) from *Euglena gracilis* (WO2004090123), d4Des(Tc) from *Thraustochytrium* sp. (WO2002026946) and d4Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d5-Desaturases d5Des(OI)<sub>2</sub> from *Ostreococcus lucimarinus* (WO2008040787), d5Des(Pp) from *Physcomitrella patens* (WO2004057001), d5Des(Pt) from *Phaeodactylum tricornutum* (WO2002057465), d5Des(Tc) from *Thraustochytrium* sp. (WO2002026946), d5Des(Tp) from *Thalassiosira pseudonana* (WO2006069710) and the d6-Desaturases d6Des(Cp) from *Ceratodon purpureus* (WO2000075341), d6Des(OI) from *Ostreococcus lucimarinus* (WO2008040787), d6Des(Ot) from *Ostreococcus tauri* (WO2006069710), d6Des(Pf) from *Primula farinosa* (WO2003072784), d6Des(Pir)<sub>BO</sub> from *Pythium irregulare* (WO2002026946), d6Des(Pir) from *Pythium irregulare* (WO2002026946), d6Des(Plu) from *Primula luteola* (WO2003072784), d6Des(Pp) from *Physcomitrella patens* (WO200102591), d6Des(Pt) from *Phaeodactylum tricornutum* (WO2002057465), d6Des(Pv) from *Primula vitalii* (WO2003072784) and d6Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d8-Desaturases d8Des(Ac) from *Acanthamoeba castellanii* (EP1790731), d8Des(Eg) from *Euglena gracilis* (WO200034439) and d8Des(Pm) from *Perkinsus marinus* (WO2007093776), the  $\alpha$ 3-Desaturases  $\alpha$ 3Des(Pi) from *Phytophthora infestans* (WO2005083053),  $\alpha$ 3Des(Pir) from *Pythium irregulare* (WO2008022963),  $\alpha$ 3Des(Pir)<sub>2</sub> from *Pythium irregulare* (WO2008022963) and  $\alpha$ 3Des(Ps) from *Phytophthora sojae* (WO2006100241), the bifunctional d5d6-elongases d5d6Elo(Om)<sub>2</sub> from *Oncorhynchus mykiss* (WO2005012316), d5d6Elo(Ta) from *Thraustochytrium aureum* (WO2005012316) and d5d6Elo(Tc) from *Thraustochytrium* sp. (WO2005012316), the d5-elongases d5Elo(At) from *Arabidopsis thaliana* (WO2005012316), d5Elo(At)<sub>2</sub> from *Arabidopsis thaliana* (WO2005012316), d5Elo(Ci) from *Ciona intestinalis* (WO2005012316), d5Elo(II) from *Ostreococcus lucimarinus* (WO2008040787), d5Elo(Ot) from *Ostreococcus tauri* (WO2005012316), d5Elo(Tp) from *Thalassiosira pseudonana* (WO2005012316) and d5Elo(XI) from *Xenopus laevis* (WO2005012316), the d6-elongases d6Elo(OI) from *Ostreococcus lucimarinus* (WO2008040787), d6Elo(Ot) from *Ostreococcus tauri* (WO2005012316), d6Elo(Pi) from *Phytophthora infestans* (WO2003064638), d6Elo(Pir) from *Pythium irregulare* (WO2009016208), d6Elo(Pp) from *Physcomitrella patens* (WO2001059128), d6Elo(Ps) from *Phytophthora sojae* (WO2006100241), d6Elo(Ps)<sub>2</sub> from *Phytophthora sojae* (WO2006100241), d6Elo(Ps)<sub>3</sub> from *Phytophthora sojae* (WO2006100241), d6Elo(Pt) from *Phaeodactylum tricornutum* (WO2005012316), d6Elo(Tc) from *Thraustochytrium* sp. (WO2005012316) and d6Elo(Tp) from *Thalassiosira pseudonana* (WO2005012316), the d9-elongases d9Elo(Ig) from *Isochrysis galbana* (WO2002077213), d9Elo(Pm) from *Perkinsus marinus* (WO2007093776) and d9Elo(Ro) from *Rhizopus oryzae* (WO2009016208). Particularly, if the manufacture of ARA is envisaged in higher plants, the enzymes recited in Table 3, below (i.e. additionally a d6-desaturase, d6-elongase, d5-elongase, d5-desaturase, d12-desaturase, and d6-elongase) or enzymes having essentially the same activity may be combined in a host cell. If the manufacture of EPA is envisaged in higher plants, the enzymes recited in Table 4, below (i.e. additionally a d6-desaturase, d6-elongase, d5-desaturase, d12-desaturase, d6-elongase, omega 3-desaturase and d15-desaturase), or enzymes having essentially the same

activity may be combined in a host cell. If the manufacture of DHA is envisaged in higher plants, the enzymes recited in Table 5, below (i.e. additionally a d6-desaturase, d6-elongase, d5-desaturase, d12-desaturase, d6-elongase, omega 3-desaturase, d15-desaturase, d5-elongase, and d4-desaturase), or enzymes having essentially the same activity may be combined in a host cell.

**[0047]** The present invention also relates to a cell, preferably a host cell as specified above or a cell of a non-human organism specified elsewhere herein, said cell comprising a polynucleotide which is obtained from the polynucleotide of the present invention by a point mutation, a truncation, an inversion, a deletion, an addition, a substitution and homologous recombination. How to carry out such modifications to a polynucleotide is well known to the skilled artisan and has been described elsewhere in this specification in detail.

**[0048]** The present invention furthermore pertains to a method for the manufacture of a polypeptide encoded by a polynucleotide of any the present invention comprising

**[0049]** a) cultivating the host cell of the invention under conditions which allow for the production of the said polypeptide; and

**[0050]** b) obtaining the polypeptide from the host cell of step a).

**[0051]** Suitable conditions which allow for expression of the polynucleotide of the invention comprised by the host cell depend on the host cell as well as the expression control sequence used for governing expression of the said polynucleotide. These conditions and how to select them are very well known to those skilled in the art. The expressed polypeptide may be obtained, for example, by all conventional purification techniques including affinity chromatography, size exclusion chromatography, high pressure liquid chromatography (HPLC) and precipitation techniques including antibody precipitation. It is to be understood that the method may—although preferred—not necessarily yield an essentially pure preparation of the polypeptide. It is to be understood that depending on the host cell which is used for the aforementioned method, the polypeptides produced thereby may become posttranslationally modified or processed otherwise.

**[0052]** The present invention also encompasses a polypeptide encoded by the polynucleotide of the present invention or which is obtainable by the aforementioned method.

**[0053]** The term “polypeptide” as used herein encompasses essentially purified polypeptides or polypeptide preparations comprising other proteins in addition. Further, the term also relates to the fusion proteins or polypeptide fragments being at least partially encoded by the polynucleotide of the present invention referred to above. Moreover, it includes chemically modified polypeptides. Such modifications may be artificial modifications or naturally occurring modifications such as phosphorylation, glycosylation, myristylation and the like (Review in Mann 2003, Nat. Biotechnol. 21, 255-261, review with focus on plants in Huber 2004, Curr. Opin. Plant Biol. 7, 318-322). Currently, more than 300 posttranslational modifications are known (see full ABFRC Delta mass list at <http://www.abrf.org/index.cfm/dm.home>). The polypeptides of the present invention shall exhibit the desaturase or elongase activity referred to above.

**[0054]** Encompassed by the present invention is, furthermore, an antibody or fragments thereof which specifically recognizes the polypeptide of the invention.

**[0055]** Antibodies against the polypeptides of the invention can be prepared by well known methods using a purified polypeptide according to the invention or a suitable fragment derived therefrom as an antigen. A fragment which is suitable as an antigen may be identified by antigenicity determining algorithms well known in the art. Such fragments may be obtained either from the polypeptide of the invention by proteolytic digestion or may be a synthetic peptide. Preferably, the antibody of the present invention is a monoclonal antibody, a polyclonal antibody, a single chain antibody, a chimerized antibody or a fragment of any of these antibodies, such as Fab, Fv or scFv fragments etc. Also comprised as antibodies by the present invention are bispecific antibodies, synthetic antibodies or chemically modified derivatives of any of the aforementioned antibodies. The antibody of the present invention shall specifically bind (i.e. does significantly not cross react with other polypeptides or peptides) to the polypeptide of the invention. Specific binding can be tested by various well known techniques. Antibodies or fragments thereof can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988. Monoclonal antibodies can be prepared by the techniques originally described in Köhler 1975, Nature 256, 495, and Galfré 1981, Meth. Enzymol. 73, 3, which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized mammals. The antibodies can be used, for example, for the immunoprecipitation, immunolocalization or purification (e.g., by affinity chromatography) of the polypeptides of the invention as well as for the monitoring of the presence of said variant polypeptides, for example, in recombinant organisms, and for the identification of proteins or compounds interacting with the proteins according to the invention.

**[0056]** Moreover, the present invention contemplates a non-human transgenic organism comprising the polynucleotide or the vector of the present invention.

**[0057]** Preferably, the non-human transgenic organism is a plant, plant part, or plant seed. Preferred plants to be used for introducing the polynucleotide or the vector of the invention are plants which are capable of synthesizing fatty acids, such as all dicotyledonous or monocotyledonous plants, algae or mosses. It is to be understood that host cells derived from a plant may also be used for producing a plant according to the present invention. Preferred plants are selected from the group of the plant families Adolotheciaceae, Anacardiaceae, Asteraceae, Apiaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Caricaceae, Cannabaceae, Convolvulaceae, Chenopodiaceae, Cryptocodiniaceae, Cucurbitaceae, Ditrichaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Gramineae, Juglandaceae, Lauraceae, Leguminosae, Linaceae, Prasinophyceae or vegetable plants or ornamentals such as Tagetes. Examples which may be mentioned are the following plants selected from the group consisting of: Adolotheciaceae such as the genera *Physcomitrella*, such as the genus and species *Physcomitrella patens*, Anacardiaceae such as the genera *Pistacia*, *Mangifera*, *Anacardium*, for example the genus and species *Pistacia vera* [pistachio], *Mangifera indica* [mango] or *Anacardium occidentale* [cashew], Asteraceae, such as the genera *Calendula*, *Carthamus*, *Centaurea*, *Cichorium*, *Cynara*, *Helianthus*, *Lactuca*, *Locusta*, *Tagetes*, *Valeriana*, for example the genus and species *Calendula officinalis* [common marigold], *Carthamus tinctorius* [safflower], *Centaurea cyanus* [cornflower], *Cichorium intybus* [chicory], *Cynara scolymus* [arti-

choke], *Helianthus annuus* [sunflower], *Lactuca sativa*, *Lactuca crispa*, *Lactuca esculenta*, *Lactuca scariola* L. ssp. *sativa*, *Lactuca scariola* L. var. *integrata*, *Lactuca scariola* L. var. *integrifolia*, *Lactuca sativa* subsp. *romana*, *Locusta communis*, *Valeriana locusta* [salad vegetables], *Tagetes lucida*, *Tagetes erecta* or *Tagetes tenuifolia* [african or french marigold], Apiaceae, such as the genus *Daucus*, for example the genus and species *Daucus carota* [carrot], Betulaceae, such as the genus *Corylus*, for example the genera and species *Corylus avellana* or *Corylus colurna* [hazelnut], Boraginaceae, such as the genus *Borago*, for example the genus and species *Borago officinalis* [borage], Brassicaceae, such as the genera *Brassica*, *Melanosinapis*, *Sinapis*, *Arabidopsis*, for example the genera and species *Brassica napus*, *Brassica rapa* ssp. [oilseed rape], *Sinapis arvensis* *Brassica juncea*, *Brassica juncea* var. *juncea*, *Brassica juncea* var. *crispifolia*, *Brassica juncea* var. *foliosa*, *Brassica nigra*, *Brassica sinapioides*, *Melanosinapis communis* [mustard], *Brassica oleracea* [fodder beet] or *Arabidopsis thaliana*, Bromeliaceae, such as the genera *Anana*, *Bromelia* (pineapple), for example the genera and species *Anana comosus*, *Anana ananas* or *Bromelia comosa* [pineapple], Caricaceae, such as the genus *Carica*, such as the genus and species *Carica papaya* [paw-paw], Cannabaceae, such as the genus *Cannabis*, such as the genus and species *Cannabis sativa* [hemp], Convolvulaceae, such as the genera *Ipomea*, *Convolvulus*, for example the genera and species *Ipomea batatas*, *Ipomea pandurata*, *Convolvulus batatas*, *Convolvulus tiliaceus*, *Ipomea fastigiata*, *Ipomea tiliacea*, *Ipomea triloba* or *Convolvulus panduratus* [sweet potato, batate], Chenopodiaceae, such as the genus *Beta*, such as the genera and species *Beta vulgaris*, *Beta vulgaris* var. *altissima*, *Beta vulgaris* var. *Vulgaris*, *Beta maritima*, *Beta vulgaris* var. *perennis*, *Beta vulgaris* var. *conditiva* or *Beta vulgaris* var. *esculenta* [sugarbeet], Cryptocodiniaceae, such as the genus *Cryptocodinium*, for example the genus and species *Cryptocodinium cohnii*, Cucurbitaceae, such as the genus *Cucurbita*, for example the genera and species *Cucurbita maxima*, *Cucurbita mixta*, *Cucurbita pepo* or *Cucurbita moschata* [pumpkin/squash], Cymbellaceae such as the genera *Amphora*, *Cymbella*, *Okekenia*, *Phaeodactylum*, *Reimeria*, for example the genus and species *Phaeodactylum tricorutum*, Ditrichaceae such as the genera Ditrichaceae, *Astomiopsis*, *Ceratodon*, *Chrysoblastella*, *Ditrichum*, *Distichium*, *Eccremidium*, *Lophidion*, *Philibertiella*, *Pleuridium*, *Saelania*, *Trichodon*, *Skottsbergia*, for example the genera and species *Ceratodon antarcticus*, *Ceratodon columbiae*, *Ceratodon heterophyllum*, *Ceratodon purpureus*, *Ceratodon purpureus*, *Ceratodon purpureus* ssp. *convolutus*, *Ceratodon purpureus* spp. *stenocarpus*, *Ceratodon purpureus* var. *rotundifolius*, *Ceratodon ratodon*, *Ceratodon stenocarpus*, *Chrysoblastella chilensis*, *Ditrichum ambiguum*, *Ditrichum brevisetum*, *Ditrichum crispatisimum*, *Ditrichum difficile*, *Ditrichum falcifolium*, *Ditrichum flexicaule*, *Ditrichum giganteum*, *Ditrichum heteromallum*, *Ditrichum lineare*, *Ditrichum lineare*, *Ditrichum montanum*, *Ditrichum montanum*, *Ditrichum pallidum*, *Ditrichum punctulatum*, *Ditrichum pusillum*, *Ditrichum pusillum* var. *tortile*, *Ditrichum rhynchostegium*, *Ditrichum schimperii*, *Ditrichum tortile*, *Distichium capillaceum*, *Distichium hagenii*, *Distichium inclinatum*, *Distichium macounii*, *Eccremidium floridanum*, *Eccremidium whiteleggei*, *Lophidion strictus*, *Pleuridium acuminatum*, *Pleuridium alternifolium*, *Pleuridium holdridgei*, *Pleuridium mexicanum*, *Pleuridium ravenelii*, *Pleuridium subulatum*, *Saelania glaucescens*, *Tri-*

*chodon borealis*, *Trichodon cylindricus* or *Trichodon cylindricus* var. *oblongus*, Elaeagnaceae such as the genus *Elaeagnus*, for example the genus and species *Olea europaea* [olive], Ericaceae such as the genus *Kalmia*, for example the genera and species *Kalmia latifolia*, *Kalmia angustifolia*, *Kalmia microphylla*, *Kalmia polifolia*, *Kalmia occidentalis*, *Cistus chamaerhodendros* or *Kalmia lucida* [mountain laurel], Euphorbiaceae such as the genera *Manihot*, *Janipha*, *Jatropha*, *Ricinus*, for example the genera and species *Manihot utilisissima*, *Janipha manihot*, *Jatropha manihot*, *Manihot aipil*, *Manihot dulcis*, *Manihot manihot*, *Manihot melanobasis*, *Manihot esculenta* [manihot] or *Ricinus communis* [castor-oil plant], Fabaceae such as the genera *Pisum*, *Albizia*, *Cathormion*, *Feuillea*, *Inga*, *Pithecolobium*, *Acacia*, *Mimosa*, *Medicago*, *Glycine*, *Dolichos*, *Phaseolus*, *Soja*, for example the genera and species *Pisum sativum*, *Pisum arvense*, *Pisum humile* [pea], *Albizia berteriana*, *Albizia julibrissin*, *Albizia lebbek*, *Acacia berteriana*, *Acacia littoralis*, *Albizia berteriana*, *Albizia berteriana*, *Cathormion berteriana*, *Feuillea berteriana*, *Inga fragrans*, *Pithecolobium berterianum*, *Pithecolobium fragrans*, *Pithecolobium berterianum*, *Pseudalbizia berteriana*, *Acacia julibrissin*, *Acacia nemu*, *Albizia nemu*, *Feuillea julibrissin*, *Mimosa julibrissin*, *Mimosa speciosa*, *Sericandra julibrissin*, *Acacia lebbek*, *Acacia macrophylla*, *Albizia lebbek*, *Feuillea lebbek*, *Mimosa lebbek*, *Mimosa speciosa* [silk tree], *Medicago sativa*, *Medicago falcata*, *Medicago varia* [alfalfa], *Glycine max* *Dolichos soja*, *Glycine gracilis*, *Glycine hispida*, *Phaseolus max*, *Soja hispida* or *Soja max* [soybean], Funariaceae such as the genera *Aphanorrhagma*, *Entosthodon*, *Funaria*, *Physcomitrella*, *Physcomitrium*, for example the genera and species *Aphanorrhagma serratum*, *Entosthodon attenuatus*, *Entosthodon bolanderi*, *Entosthodon bonplandii*, *Entosthodon californicus*, *Entosthodon drummondii*, *Entosthodon jamesonii*, *Entosthodon leibergii*, *Entosthodon neoscoticus*, *Entosthodon rubrisetus*, *Entosthodon spathulifolius*, *Entosthodon tucsoni*, *Funaria americana*, *Funaria bolanderi*, *Funaria calcarea*, *Funaria californica*, *Funaria calvescens*, *Funaria convoluta*, *Funaria flavicans*, *Funaria groutiana*, *Funaria hygrometrica*, *Funaria hygrometrica* var. *arctica*, *Funaria hygrometrica* var. *calvescens*, *Funaria hygrometrica* var. *convoluta*, *Funaria hygrometrica* var. *muralis*, *Funaria hygrometrica* var. *utahensis*, *Funaria microstoma*, *Funaria microstoma* var. *obtusifolia*, *Funaria muhlenbergii*, *Funaria orcuttii*, *Funaria plano-convexa*, *Funaria polaris*, *Funaria ravenelii*, *Funaria rubriseta*, *Funaria serrata*, *Funaria sonora*, *Funaria sublimbatus*, *Funaria tucsoni*, *Physcomitrella californica*, *Physcomitrella patens*, *Physcomitrella readeri*, *Physcomitrium australe*, *Physcomitrium californicum*, *Physcomitrium collenchymatum*, *Physcomitrium coloradense*, *Physcomitrium cupuliferum*, *Physcomitrium drummondii*, *Physcomitrium eurystomum*, *Physcomitrium flexifolium*, *Physcomitrium hookeri*, *Physcomitrium hookeri* var. *serratum*, *Physcomitrium immersum*, *Physcomitrium kellermanii*, *Physcomitrium megalocarpum*, *Physcomitrium pyriforme*, *Physcomitrium pyriforme* var. *serratum*, *Physcomitrium rufipes*, *Physcomitrium sandbergii*, *Physcomitrium subsphaericum*, *Physcomitrium washingtoniense*, Geraniaceae, such as the genera *Pelargonium*, *Cocos*, *Oleum*, for example the genera and species *Cocos nucifera*, *Pelargonium grossularioides* or *Oleum cocois* [coconut], Gramineae, such as the genus *Saccharum*, for example the genus and species *Saccharum officinarum*, Juglandaceae, such as the genera *Juglans*, *Wallia*, for example the genera and species *Juglans regia*,

*Juglans ailanthifolia*, *Juglans sieboldiana*, *Juglans cinerea*, *Wallia cinerea*, *Juglans bixbyi*, *Juglans californica*, *Juglans hindsii*, *Juglans intermedia*, *Juglans jamaicensis*, *Juglans major*, *Juglans microcarpa*, *Juglans nigra* or *Wallia nigra* [walnut], Lauraceae, such as the genera *Persea*, *Laurus*, for example the genera and species *Laurus nobilis* [bay], *Persea americana*, *Persea gratissima* or *Persea persea* [avocado], Leguminosae, such as the genus *Arachis*, for example the genus and species *Arachis hypogaea* [peanut], Linaceae, such as the genera *Linum*, *Adenolinum*, for example the genera and species *Linum usitatissimum*, *Linum humile*, *Linum austriacum*, *Linum bienne*, *Linum angustifolium*, *Linum catharticum*, *Linum flavum*, *Linum grandiflorum*, *Adenolinum grandiflorum*, *Linum lewisii*, *Linum narbonense*, *Linum perenne*, *Linum perenne* var. *lewisii*, *Linum pratense* or *Linum trigyrum* [linseed], Lythrariceae, such as the genus *Punica*, for example the genus and species *Punica granatum* [pomegranate], Malvaceae, such as the genus *Gossypium*, for example the genera and species *Gossypium hirsutum*, *Gossypium arboreum*, *Gossypium barbadense*, *Gossypium herbaceum* or *Gossypium thurberi* [cotton], Marchantiaceae, such as the genus *Marchantia*, for example the genera and species *Marchantia berteriana*, *Marchantia foliacea*, *Marchantia macropora*, Musaceae, such as the genus *Musa*, for example the genera and species *Musa nana*, *Musa acuminata*, *Musa paradisiaca*, *Musa* spp. [banana], Onagraceae, such as the genera *Camissonia*, *Oenothera*, for example the genera and species *Oenothera biennis* or *Camissonia brevipes* [evening primrose], Palmae, such as the genus *Elaeis*, for example the genus and species *Elaeis guineensis* [oil palm], Papaveraceae, such as the genus *Papaver*, for example the genera and species *Papaver orientale*, *Papaver rhoeas*, *Papaver dubium* [poppy], Pedaliaceae, such as the genus *Sesamum*, for example the genus and species *Sesamum indicum* [sesame], Piperaceae, such as the genera *Piper*, *Artanthe*, *Peperomia*, *Steffensia*, for example the genera and species *Piper aduncum*, *Piper amalago*, *Piper angustifolium*, *Piper auritum*, *Piper betel*, *Piper cubeba*, *Piper longum*, *Piper nigrum*, *Piper retrofractum*, *Artanthe adunca*, *Artanthe elongata*, *Peperomia elongata*, *Piper elongatum*, *Steffensia elongata* [cayenne pepper], Poaceae, such as the genera *Hordeum*, *Secale*, *Avena*, *Sorghum*, *Andropogon*, *Holcus*, *Panicum*, *Oryza*, *Zea* (maize), *Triticum*, for example the genera and species *Hordeum vulgare*, *Hordeum jubatum*, *Hordeum murinum*, *Hordeum secalinum*, *Hordeum distichon*, *Hordeum aegiceras*, *Hordeum hexastichon*, *Hordeum hexastichum*, *Hordeum irregulare*, *Hordeum sativum*, *Hordeum secalinum* [barley], *Secale cereale* [rye], *Avena sativa*, *Avena fatua*, *Avena byzantina*, *Avena fatua* var. *sativa*, *Avena hybrida* [oats], *Sorghum bicolor*, *Sorghum halepense*, *Sorghum saccharatum*, *Sorghum vulgare*, *Andropogon drummondii*, *Holcus bicolor*, *Holcus sorghum*, *Sorghum aethiopicum*, *Sorghum arundinaceum*, *Sorghum caffrorum*, *Sorghum cernuum*, *Sorghum dochna*, *Sorghum drummondii*, *Sorghum durra*, *Sorghum guineense*, *Sorghum lanceolatum*, *Sorghum nervosum*, *Sorghum saccharatum*, *Sorghum subglabrescens*, *Sorghum verticilliflorum*, *Sorghum vulgare*, *Holcus halepensis*, *Sorghum miliaceum*, *Panicum militaceum* [millet], *Oryza sativa*, *Oryza latifolia* [rice], *Zea mays* [maize], *Triticum aestivum*, *Triticum durum*, *Triticum turgidum*, *Triticum hybernium*, *Triticum macha*, *Triticum sativum* or *Triticum vulgare* [wheat], Porphyridiaceae, such as the genera *Chrootheca*, *Flintiella*, *Petrovanella*, *Porphyridium*, *Rhodella*, *Rhodorus*, *Vanhoeffenia*, for example the genus and species

*Porphyridium cruentum*, Proteaceae, such as the genus *Macadamia*, for example the genus and species *Macadamia integrifolia* [macadamia], Prasinophyceae such as the genera *Nephroselmis*, *Prasinococcus*, *Scherffelia*, *Tetraselmis*, *Mantoniella*, *Ostreococcus*, for example the genera and species *Nephroselmis olivacea*, *Prasinococcus capsulatus*, *Scherffelia dubia*, *Tetraselmis chui*, *Tetraselmis suecica*, *Mantoniella squamata*, *Ostreococcus tauri*, Rubiaceae such as the genus *Coffea*, for example the genera and species *Coffea* spp., *Coffea arabica*, *Coffea canephora* or *Coffea liberica* [coffee], Scrophulariaceae such as the genus *Verbascum*, for example the genera and species *Verbascum blattaria*, *Verbascum chaixii*, *Verbascum densiflorum*, *Verbascum lagurus*, *Verbascum longifolium*, *Verbascum lychnitis*, *Verbascum nigrum*, *Verbascum olympicum*, *Verbascum phlomidis*, *Verbascum phoenicum*, *Verbascum pulverulentum* or *Verbascum thapsus* [mullein], Solanaceae such as the genera *Capsicum*, *Nicotiana*, *Solanum*, *Lycopersicon*, for example the genera and species *Capsicum annuum*, *Capsicum annuum* var. *glabriusculum*, *Capsicum frutescens* [pepper], *Capsicum annuum* [paprika], *Nicotiana tabacum*, *Nicotiana alata*, *Nicotiana attenuata*, *Nicotiana glauca*, *Nicotiana langsdorffii*, *Nicotiana obtusifolia*, *Nicotiana quadrivalvis*, *Nicotiana repanda*, *Nicotiana rustica*, *Nicotiana sylvestris* [tobacco], *Solanum tuberosum* [potato], *Solanum melongena* [eggplant], *Lycopersicon esculentum*, *Lycopersicon lycopersicum*, *Lycopersicon pyriforme*, *Solanum integrifolium* or *Solanum lycopersicum* [tomato], Sterculiaceae, such as the genus *Theobroma*, for example the genus and species *Theobroma cacao* [cacao] or Theaceae, such as the genus *Camellia*, for example the genus and species *Camellia sinensis* [tea]. In particular preferred plants to be used as transgenic plants in accordance with the present invention are oil fruit crops which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, sesame, *Calendula*, *Punica*, evening primrose, mullein, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, linseed, soybean, pistachios, borage, trees (oil palm, coconut, walnut) or crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassaya, pepper, *Tagetes*, Solanaceae plants such as potato, tobacco, eggplant and tomato, *Vicia* species, pea, alfalfa or bushy plants (coffee, cacao, tea), *Salix* species, and perennial grasses and fodder crops. Preferred plants according to the invention are oil crop plants such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, *Calendula*, *Punica*, evening primrose, pumpkin/squash, linseed, soybean, borage, trees (oil palm, coconut). Especially preferred are sunflower, safflower, tobacco, mullein, sesame, cotton, pumpkin/squash, poppy, evening primrose, walnut, linseed, hemp, thistle or safflower. Very especially preferred plants are plants such as safflower, sunflower, poppy, evening primrose, walnut, linseed, or hemp.

**[0058]** Preferred mosses are *Physcomitrella* or *Ceratodon*. Preferred algae are *Isochrysis*, *Mantoniella*, *Ostreococcus* or *Cryptothecodinium*, and algae/diatoms such as *Phaeodactylum* or *Thraustochytrium*. More preferably, said algae or mosses are selected from the group consisting of: *Emiliana*, *Shewanella*, *Physcomitrella*, *Thraustochytrium*, *Fusarium*, *Phytophthora*, *Ceratodon*, *Isochrysis*, *Aleurita*, *Muscarioides*, *Mortierella*, *Phaeodactylum*, *Cryptothecodinium*, specifically from the genera and species *Thalassiosira pseudonona*, *Euglena gracilis*, *Physcomitrella patens*, *Phytophthora infestans*, *Fusarium gramineum*, *Cryptocodium cohnii*,

*Ceratodon purpureus*, *Isochrysis galbana*, *Aleurita farinosa*, *Thraustochytrium* sp., *Muscarioides viallii*, *Mortierella alpina*, *Phaeodactylum tricornutum* or *Caenorhabditis elegans* or especially advantageously *Phytophthora infestans*, *Thalassiosira pseudonona* and *Cryptocodium cohnii*.

**[0059]** Transgenic plants may be obtained by transformation techniques as elsewhere in this specification. Preferably, transgenic plants can be obtained by T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they contain at least the vir genes, which are required for the *Agrobacterium*-mediated transformation, and the sequences which delimit the T-DNA (T-DNA border). Suitable vectors are described elsewhere in the specification in detail.

**[0060]** Also encompassed are transgenic non-human animals comprising the vector or polynucleotide of the present invention. Preferred non-human transgenic animals envisaged by the present invention are fish, such as herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna.

**[0061]** However, it will be understood that dependent on the non-human transgenic organism specified above, further, enzymatic activities may be conferred to the said organism, e.g., by recombinant technologies. Accordingly, the present invention, preferably, envisages a non-human transgenic organism specified above which in addition to the polynucleotide of the present invention comprises polynucleotides encoding such desaturases and/or elongases as required depending on the selected host cell. Preferred desaturases and/or elongases which shall be present in the organism are at least one enzyme selected from the group of desaturases and/or elongases or the combinations specifically recited elsewhere in this specification (see above and Tables 3, 4 and 5).

**[0062]** Furthermore, the present invention encompasses a method for the manufacture of polyunsaturated fatty acids comprising:

**[0063]** a) cultivating the host cell of the invention under conditions which allow for the production of polyunsaturated fatty acids in said host cell;

**[0064]** b) obtaining said polyunsaturated fatty acids from the said host cell.

**[0065]** The term "polyunsaturated fatty acids (PUFA)" as used herein refers to fatty acids comprising at least two, preferably, three, four, five or six, double bonds. Moreover, it is to be understood that such fatty acids comprise, preferably from 18 to 24 carbon atoms in the fatty acid chain. More preferably, the term relates to long chain PUFA (LCPUFA) having from 20 to 24 carbon atoms in the fatty acid chain. Preferred unsaturated fatty acids in the sense of the present invention are selected from the group consisting of DGLA 20:3 (8,11,14), ARA 20:4 (5,8,11,14), iARA 20:4(8,11,14, 17), EPA 20:5 (5,8,11,14,17), DPA 22:5 (4,7,10,13,16), DHA 22:6 (4,7,10,13,16,19), 20:4 (8,11,14,17), more preferably, arachidonic acid (ARA) 20:4 (5,8,11,14), eicosapentaenoic acid (EPA) 20:5 (5,8,11,14,17), and docosahexaenoic acid (DHA) 22:6 (4,7,10,13,16,19). Thus, it will be understood that most preferably, the methods provided by the present invention pertaining to the manufacture of ARA, EPA or DHA. Moreover, also encompassed are the intermediates of LCPUFA which occur during synthesis. Such intermediates are, preferably, formed from substrates by the desaturase or elongase activity of the polypeptides of the present invention. Preferably, substrates encompass LA 18:2 (9,12), ALA 18:3

(9,12,15), Eicosadienoic acid 20:2 (11,14), Eicosatrienoic acid 20:3 (11,14,17), DGLA 20:3 (8,11,14), ARA 20:4 (5,8,11,14), eicosatetraenoic acid 20:4 (8,11,14,17), Eicosapentaenoic acid 20:5 (5,8,11,14,17), Docosahexapentanoic acid 22:5 (7,10,13,16,19).

**[0066]** The term "cultivating" as used herein refers maintaining and growing the host cells under culture conditions which allow the cells to produce the said polyunsaturated fatty acid, i.e. the PUFA and/or LCPUFA referred to above. This implies that the polynucleotide of the present invention is expressed in the host cell so that the desaturase and/or elongase activity is present. Suitable culture conditions for cultivating the host cell are described in more detail below.

**[0067]** The term "obtaining" as used herein encompasses the provision of the cell culture including the host cells and the culture medium as well as the provision of purified or partially purified preparations thereof comprising the polyunsaturated fatty acids, preferably, ARA, EPA, DHA, in free or in -CoA bound form, as membrane phospholipids or as triacylglyceride esters. More preferably, the PUFA and LCPUFA are to be obtained as triglyceride esters, e.g., in form of an oil. More details on purification techniques can be found elsewhere herein below.

**[0068]** The host cells to be used in the method of the invention are grown or cultured in the manner with which the skilled worker is familiar, depending on the host organism. Usually, host cells are grown in a liquid medium comprising a carbon source, usually in the form of sugars, a nitrogen source, usually in the form of organic nitrogen sources such as yeast extract or salts such as ammonium sulfate, trace elements such as salts of iron, manganese and magnesium and, if appropriate, vitamins, at temperatures of between 0° C. and 100° C., preferably between 10° C. and 60° C. under oxygen or anaerobic atmosphere dependent on the type of organism. The pH of the liquid medium can either be kept constant, that is to say regulated during the culturing period, or not. The cultures can be grown batchwise, semibatchwise or continuously. Nutrients can be provided at the beginning of the fermentation or administered semicontinuously or continuously. The produced PUFA or LCPUFA can be isolated from the host cells as described above by processes known to the skilled worker, e.g., by extraction, distillation, crystallization, if appropriate precipitation with salt, and/or chromatography. It might be required to disrupt the host cells prior to purification. To this end, the host cells can be disrupted beforehand. The culture medium to be used must suitably meet the requirements of the host cells in question. Descriptions of culture media for various microorganisms which can be used as host cells according to the present invention can be found in the textbook "Manual of Methods for General Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981). Culture media can also be obtained from various commercial suppliers. All media components are sterilized, either by heat or by filter sterilization. All media components may be present at the start of the cultivation or added continuously or batchwise, as desired. If the polynucleotide or vector of the invention which has been introduced in the host cell further comprises an expressible selection marker, such as an antibiotic resistance gene, it might be necessary to add a selection agent to the culture, such as an antibiotic in order to maintain the stability of the introduced polynucleotide. The culture is continued until formation of the desired product is at a maximum. This is normally achieved within 10 to 160 hours. The fermentation broths can

be used directly or can be processed further. The biomass may, according to requirement, be removed completely or partially from the fermentation broth by separation methods such as, for example, centrifugation, filtration, decanting or a combination of these methods or be left completely in said broth. The fatty acid preparations obtained by the method of the invention, e.g., oils, comprising the desired PUFA or LCPUFA as triglyceride esters are also suitable as starting material for the chemical synthesis of further products of interest. For example, they can be used in combination with one another or alone for the preparation of pharmaceutical or cosmetic compositions, foodstuffs, or animal feeds. Chemically pure triglycerides comprising the desired PUFA or LCPUFA can also be manufactured by the methods described above. To this end, the fatty acid preparations are further purified by extraction, distillation, crystallization, chromatography or combinations of these methods. In order to release the fatty acid moieties from the triglycerides, hydrolysis may be also required. The said chemically pure triglycerides or free fatty acids are, in particular, suitable for applications in the food industry or for cosmetic and pharmacological compositions.

**[0069]** Moreover, the present invention relates to a method for the manufacture of poly-unsaturated fatty acids comprising:

**[0070]** a) cultivating the non-human transgenic organism of the invention under conditions which allow for the production of poly-unsaturated fatty acids in said non-human transgenic organism; and

**[0071]** b) obtaining said poly-unsaturated fatty acids from the said non-human transgenic organism.

**[0072]** Further, it follows from the above that a method for the manufacture of an oil, lipid or fatty acid composition is also envisaged by the present invention comprising the steps of any one of the aforementioned methods and the further step of formulating PUFA or LCPUFA as oil, lipid or fatty acid composition. Preferably, said oil, lipid or fatty acid composition is to be used for feed, foodstuffs, cosmetics or medications. Accordingly, the formulation of the PUFA or LCPUFA shall be carried out according to the GMP standards for the individual envisaged products. For example, an oil may be obtained from plant seeds by an oil mill. However, for product safety reasons, sterilization may be required under the applicable GMP standard. Similar standards will apply for lipid or fatty acid compositions to be applied in cosmetic or pharmaceutical compositions. All these measures for formulating oil, lipid or fatty acid compositions as products are comprised by the aforementioned manufacture.

**[0073]** The term "oil" refers to a fatty acid mixture comprising unsaturated and/or saturated fatty acids which are esterified to triglycerides. Preferably, the triglycerides in the oil of the invention comprise PUFA or LCPUFA as referred to above. The amount of esterified PUFA and/or LCPUFA is, preferably, approximately 30%, a content of 50% is more preferred, a content of 60%, 70%, 80% or more is even more preferred. The oil may further comprise free fatty acids, preferably, the PUFA and LCPUFA referred to above. For the analysis, the fatty acid content can be, e.g., determined by GC analysis after converting the fatty acids into the methyl esters by transesterification. The content of the various fatty acids in

the oil or fat can vary, in particular depending on the source. The oil, however, shall have a non-naturally occurring composition with respect to the PUFA and/or LCPUFA composition and content. It will be understood that such a unique oil composition and the unique esterification pattern of PUFA and LCPUFA in the triglycerides of the oil shall only be obtainable by applying the methods of the present invention specified above. Moreover, the oil of the invention may comprise other molecular species as well. Specifically, it may comprise minor impurities of the polynucleotide or vector of the invention. Such impurities, however, can be detected only by highly sensitive techniques such as PCR.

[0074] The contents of all references cited throughout this application are herewith incorporated by reference in general and with respect to their specific disclosure content referred to above.

#### FIGURES

[0075] FIG. 1 shows a schematical figure of the different enzymatic activities leading to the production of ARA, EPA and DHA.

[0076] FIG. 2 shows a yeast expression experiment with feeding of 22:5n-3 in the presence (A) and absence (B) of d4Des(Eh)

[0077] FIG. 3 shows a yeast expression experiment with feeding of 20:3n-3 in the presence (A) and absence (B) of d8Des(Eh)

[0078] FIG. 4 shows a yeast expression experiment with feeding of 18:3n-3 in the presence (A) and absence (B) of d9Elo(Eh)

[0079] FIG. 5 shows a yeast expression experiment with feeding of 18:3n-6 (GLA) and 18:4n-3 (SDA) in the presence (A) and absence (B) of d5Elo(Eh)

[0080] FIG. 6 shows a yeast expression experiment with feeding of 20:4n-6 (ARA) and 20:5n-3 (EPA) in the presence (A) and absence (B) of d5Elo(Eh)

[0081] FIG. 7 shows the expression of d9Elo(Eh) in seeds of two *Arabidopsis* events. As control seeds not expressing d9Elo(Eh) are shown (WT).

[0082] FIG. 8 shows the Acyl-CoA analysis of mature *Arabidopsis* seeds from both events expressing the d9Elo(Eh) in comparison to seeds not expressing d9Elo(Eh) (Col0).

[0083] FIG. 9 shows the expression of d9Elo(Eh), d8Des(Eh) and d5Des(Eh) in seeds of various *Arabidopsis* events.

[0084] FIG. 10 shows gas chromatographic analysis of mature *Arabidopsis* seeds transformed with the construct OstELO5EmD4. Peaks were quantified and listed in the table below. The products of d5Elo(Ot) and d4Des(Eh) activity are 22:6n-3 (DHA).

[0085] FIG. 11 is a comparison between two d4-desaturases (Tc and Eh) showing that d4Des(Eh) is different from known d4-desaturases in producing a high ratio of DHA: DPA.

[0086] FIG. 12 shows the expression of d5Elo(Eh) in seeds of various *Arabidopsis* events.

[0087] FIG. 13 is a comparison between three different d6-desaturases and the substrate specificity of d5Des(Eh).

[0088] This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent appli-

cations cited throughout this application, as well as the figures, are incorporated herein by reference.

#### EXAMPLES

##### Example 1

##### Organism and Culture Conditions

[0089] *Emiliana huxleyi* was grown as described in Scian-dra et al. (2003) Marine Ecology Progress Series 261:111-122 with following conditions:

[0090] Growth in 50 ml in conical flasks using K/2 medium (Keller et al. (1987) Journal of Phycology 23:633-638). The flasks were placed in a growth chamber at a temperature of  $17 \pm 0.1^\circ \text{C}$ . under 14L:10D irradiance. Light was provided by fluorescent lamps giving a photon flux density (400 to 700 nm) of  $170 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ .

##### Example 2

##### Cloning of Novel Desaturase and Elongase Sequences

[0091] RNA from cells grown as described under Example 1 was extracted using the RNA-extraction Kit from Qiagen, a RACE-library was generated using the RACE-Kit from Clontech. From the RACE-library sequences for desaturase and elongases were amplified with PCR using following primer pairs and PCR conditions.

[0092] PCR reaction (50  $\mu\text{L}$ ):  
5.00  $\mu\text{L}$  Template cDNA

5.00  $\mu\text{L}$  10 $\times$  Puffer (Advantage-Polymerase)+25 mM MgCl<sub>2</sub>

[0093] 5.00  $\mu\text{L}$  2 mM dNTP  
1.25  $\mu\text{L}$  je Primer (10  $\mu\text{mol}/\mu\text{L}$ )

0.50  $\mu\text{L}$  Advantage-Polymerase

[0094] The Advantage polymerase mix from Clontech was used.

[0095] Reaction conditions of the PCR:

Annealing: 1 min  $55^\circ \text{C}$ .

Denaturation: 1 min  $94^\circ \text{C}$ .

Elongation: 2 min  $72^\circ \text{C}$ .

Cycles: 35

[0096] Primer pairs used in PCR:

Name	Primer pair (5' orientation)	SEQ ID NO.
Eh4ff	CCATGGGAGGCGCCGGCCGCGAG	11
Eh4rv	CTAGTCCGCCTTGAGGTTCTC	12
Eh5ff	ACCATGTGCAAGGCGAGCGGCCT	13
Eh5rv	TCACCAATCATGAGGAAGGT	14
Eh8ff	CCATGGGCAAGGCGGCAACGC	15
Eh8rv	GGGCAGAGATGCCGCACTAG	16

-continued

Name	Primer pair (5' orientation)	SEQ ID NO.
Eh9ff	ACCATGCTCGATCGCGCCTCGTC	17
Eh9rv	TCACAGCGCCTTGGGGTAGC	18

[0097] The PCR reactions resulted in following polynucleotide sequences:

Gene	Activity	Length in bp	SEQ ID NO.
D4Des(Eh)	D4-desaturase	1280	5
D8Des(Eh)	D8-desaturase	1256	1
D9Elo(Eh)	D9-elongase	804	3
D5Elo(Eh)	Multi-elongase	921	7

[0098] A list of identified full-length coding sequences is shown in Table 1.

TABLE 1

List of full-length coding sequences and deduced amino acid sequences			
SEQ ID NO:	Gene	Coding sequence in bp	Amino acid sequence
1	D8Des(Eh)	1254	417
3	D9Elo(Eh)	801	266
5	D4Des(Eh)	1278	425
7	D5Elo(Eh)	918	305

[0099] Open reading frames as shown in Table 1 were cloned into the pESC(Leu) vector from Stratagene according to manufactures reaction conditions. Reactions were transformed into *E. coli* DH5a and plasmid DNA was isolated. The plasmids pESC-d4Des(Eh), pESC-d8Des(Eh), pESC-d9Elo(Eh), pESC-d5Elo(Eh) were then used for yeast transformation.

### Example 3

#### Yeast Transformation and Growth Conditions

[0100] *S. cerevisiae* strain INVSC from Invitrogen was transformed with the constructs pESC-d4Des(Eh), pESC-d8Des(Eh), pESC-d9Elo(Eh), pESC-d5Elo(Eh) and pESC using the S.C. EasyComp Transformation Kit (Invitrogen, Carlsbad, Calif.) with selection on leucine-deficient medium.

[0101] Yeast were grown after transformation in complete medium containing all amino acids and nucleotides. Then yeast were plated on different medium containing either the complete medium (SD) or the complete medium lacking leucine (SD-Leu). Only yeast containing pESC-d4Des(Eh), pESC-d8Des(Eh), pESC-d9Elo(Eh), pESC-d5Elo(Eh) or pESC vector can grow on this medium.

### Example 4

#### Functional Expression of Desaturases and Elongases in Yeast and Gas Chromatographic Analysis

[0102] Yeast cells containing the respective pESC plasmids as prepared above were incubated 12 h in liquid DOB-U

medium at 28° C., 200 rpm inkubiert and than additional 12 h in induction medium (DOB-U+2% (w/v) galactose+2% (w/v) raffinose). To the induction medium 250 µM of the respective fatty acids were added to check for enzyme activity and specificity.

[0103] Yeast cells were analyzed as following:

[0104] Yeast cells from induction medium were harvested by centrifugation (100×g, 5 min, 20° C.) and washed with 100 mM NaHCO<sub>3</sub>, pH 8,0, to remove residual fatty acids. From the yeast pellet a total extract of fatty acid methylesters (FAME) was generated by adding 2 ml 1 N methanolic sulfuric acid and 2% (v/v) Dimethoxypropan for 1 h at 80° C. FAME were extracted two times with Petrolether (PE). Not derivased fatty acids were removed by washing with 2 ml 100 mM NaHCO<sub>3</sub>, pH 8.0 and 2 ml Aqua dest. The PE-phases were dried with Na<sub>2</sub>SO<sub>4</sub> and eluted in 100 µl PE. The samples were then separated with a DB-23-column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850-machine with FID using following conditions: oven temperature 50° C. to 250° C. with a rate of 5° C./min and finally 10 min at 250° C.

[0105] The identification of the fatty acids was done using the retention times of known fatty acid standards (Sigma). The method is described e.g. in Napier and Michaelson, 2001, *Lipids*. 36(8):761-766; Sayanova et al., 2001, *Journal of Experimental Botany*. 52(360):1581-1585, Sperling et al., 2001, *Arch. Biochem. Biophys.* 388(2):293-298 and Michaelson et al., 1998, *FEBS Letters*. 439(3):215-218.

### Example 5

#### Functional Characterization of d4Des(Eh)

[0106] As described above d4Des(Eh) was functionally characterized in yeast. The result of the analysis is shown in FIG. 2. Yeast transformed with pESC-d4Des(Eh) was compared to yeast transformed with pESC (control) while feeding the yeast cells with the fatty acid DPA 22:5n-3. Based on this comparison pESC-d4Des(Eh) exhibits d4-desaturase activity as in the control no 22:6 is observed. Therefore d4Des(Eh) is a functional d4-desaturase.

### Example 6

#### Functional Characterization of d8Des(Eh)

[0107] As described above d8Des(Eh) was functionally characterized in yeast. The result of the analysis is shown in FIG. 3. Yeast transformed with pESC-d8Des(Eh) was compared to yeast transformed with pESC (control) while feeding the fatty acid 20:3n-3. Based on this comparison a new fatty acid was formed compared to the control, which is 20:4n-3. The formation of this fatty acid proves that d8Des(Eh) was functionally expressed and has d8-desaturase activity. The conversion rate of 20:3n-3 to 20:4n-3 was 5%.

### Example 7

#### Functional Characterization of d9Elo(Eh)

[0108] As described above d9Elo(Eh) was functionally characterized in yeast. The result of the analysis is shown in FIG. 4. Yeast transformed with pESC-d9Elo(Eh) was compared to yeast transformed with pESC (control) while feeding the fatty acids 18:3n-3 (ALA) or 18:2 (LA). Based on this comparison a new fatty acid was formed compared to the control, which is 20:3n-3 or 20:2n-6, respectively. The formation of these fatty acids proves that d9Elo(Eh) was func-

tionally expressed and has d9-elongase activity. The conversion rate of 18:3n-3 to 20:3n-3 was 17%, the conversion rate of 18:2n-6 to 20:2n-6 was 49%.

#### Example 8

##### Functional Characterization of d5Elo(Eh)

**[0109]** As described above d5Elo(Eh) was functionally characterized in yeast. The result of the analysis is shown in FIGS. 5 and 6. Yeast transformed with pESC-d5Elo(Eh) was compared to yeast transformed with pESC (control) while feeding the fatty acids 18:3n-6 (GLA), 18:4 (SDA) or 20:4n-6 (ARA), 20:5n-3 (EPA), respectively. Based on this comparison new fatty acids formation was observed when compared to the control, which is 20:3n-6 or 20:4n-3 when fed GLA or SDA and 22:4n-6 or 22:5n-3 when fed ARA or EPA, respectively. The formation of these fatty acids proves that d5Elo(Eh) was functionally expressed and has d5-elongase activity. The conversion rate of GLA was 13%, the conversion rate of 18:4n-3 was 30%, the conversion rate of ARA was 38% and the conversion rate of EPA was 30%. Surprisingly the elongase used a wide variety of substrates of elongation. The specification indicates a multifunctional elongase activity with higher specificities for omega3 fatty acids.

#### Example 9

##### Expression of Novel Elongases from *Emiliana Huxleyi* in Plants

**[0110]** The novel desaturases and elongases were cloned into a plant transformation vector as described in WO2003/093482, WO2005/083093 or WO2007/093776. Exemplary suitable combinations of genes are described in Table 2, 3 and 4.

TABLE 2

Gene combinations for the production of ARA.		
Gene	Aktivität	SEQ ID NO:
D6Des(Ot)	Δ6-Desaturase	19
D6Elo(Pp)	Δ6-Elongase	21
D5Des(Eh)	Δ5-Desaturase	9
D12Des(Ps)	Δ12-Desaturase	23
D6Elo(Tp)	Δ6-Elongase	25
D8Des(Eh)	Δ8-Desaturase	1
D9Elo(Eh)	Δ9-Elongase	3

TABLE 3

Gene combinations for the production of EPA.		
Gene	Aktivität	SEQ ID NO:
D6Des(Ot)	Δ6-Desaturase	19
D5Elo(Eh)	Δ5-Elongase	7
D5Des(Eh)	Δ5-Desaturase	9
D12Des(Ps)	Δ12-Desaturase	23
D6Elo(Tp)	Δ6-Elongase	25
ω3-Des(Pi)	Omega 3-Desaturase	27
D15Des(Cp)	Δ15-Desaturase	29
D8Des(Eh)	Δ8-Desaturase	1
D9Elo(Eh)	Δ9-Elongase	3

TABLE 4

Gene combinations for the production of DHA.		
Gene	Aktivität	SEQ ID NO:
D6Des(Ot)	Δ6-Desaturase	19
D5Elo(Eh)	Δ5-Elongase	7
D5Des(Eh)	Δ5-Desaturase	9
D12Des(Ps)	Δ12-Desaturase	23
D6Elo(Tp)	Δ6-Elongase	25
ω3-Des(Pi)	Omega 3-Desaturase	27
D15Des(Cp)	Δ15-Desaturase	29
D5Elo(Ot)	Δ5-elongase	31
D4Des(Eh)	Δ4-desaturase	5
D8Des(Eh)	Δ8-Desaturase	1
D9Elo(Eh)	Δ9-Elongase	3

**[0111]** Based on the gene combinations as described in Table 2, Table 3 or Table 4 following combinations were designed

**[0112]** AP2: LuCnl-d5Des(Eh)\_LuCnk-d8Des8Eh)\_Napin-ω3Des(PUNapin-d12Des(Ps)\_LuCnl -d9Elo(Eh)

**[0113]** OstELO5EmD4: VfUSP-d6Elo(Pp)\_LuCnl-d5Des8Tc)\_VfSBP-d6Des(Ot)\_Napin-ω3Des(PC-Napin-d12Des(Ps)\_LuCnl-d5Elo(Ot)\_LuCnl-d4Des(Eh)

**[0114]** OstELO5TcD4: VfUSP-d6Elo(Pp)\_LuCnl-d5Des8Tc)\_VfSBP-d6Des(Ot)\_Napin-ω3Des(PO\_Napin-d12Des(Ps)\_LuCnl-d5Elo(Ot)\_LuCnl-d4Des(Tc)

**[0115]** Transgenic rapeseed lines were generated as described in Deblaere et al, 1984, Nucl. Acids. Res. 13, 4777-4788 and seeds of transgenic rapeseed plants are analyzed as described in Qiu et al. 2001, J. Biol. Chem. 276, 31561-31566.

**[0116]** Transgenic *Arabidopsis* plants were generated as described in Bechtholdt et al. 1993 C. R. Acad. Sci. Ser. III Sci. Vie., 316, 1194-1199. Seeds of transgenic *Arabidopsis* plants expressing d9Elo(Eh) by using the seed-specific promoter Glycinin from soybean (Lelievre et al. (1992) Plant Physiol 98:387-391) were analyzed by gas chromatography (FIG. 7). Compared to non-transgenic control plants (WT) there are changes in the fatty acid profile, proving that d9Elo(Eh) was functionally expression in seeds. The major shifts in the fatty acid profile is directed to a 10 fold increase in the fatty acid 20:2n-6 and 20:3n-3 (FIG. 7). Therefore d9Elo(Eh) exhibits a Δ9-elongase activity, which is consistent with the yeast characterization. Further, the levels of 18:2 and ALA in the transgenic events expressing d9Elo(Eh) are lowered compared to WT, as these fatty acids are direct substrates for the d9Elo(Eh). Further, the endogenous elongation system in the plant is unchanged as levels of 20:1 and 22:1 are similar between transgenic plants expression d9Elo(Eh) and WT control. This indicates that the expression of d9Elo(Eh) does not disturb endogenous elongation process, but delivers additional activity.

**[0117]** To further prove the activity of d9Elo(Eh) expressed in seeds of *Arabidopsis thaliana* AcylCoA-measurements were done. Substrates and products of the d9Elo(Eh) elongation reaction are AcylCoA-esters, which are then further incorporated into triacylglycerides (oil). The analysis of the acylCoA-pool reveals the formation and flux of the elongation reaction. FIG. 8 summarizes the AcylCoA measurements for *Arabidopsis* event expressing d9Elo(Eh) in comparison to controls not expressing d9Elo(Eh) (Co10). The change in the chromatogram is indicated by a star. At this position a massive

amount of 20:2n-6 is detected, which is much lower in the control. The conditions for separation of the fatty acid CoA-esters does not allow the detection of 20:3n-3 as this CoA ester is not separated from 18:3 CoA.

**[0118]** The massive occurrence of 20:2n-6-CoA proves the expression of d9Elo(Eh) as this is the direct product of its enzymatic activity.

**[0119]** Further, transgenic *Arabidopsis* lines have been generated to validate the activity of d8Des(Eh) and d5Des(Eh). Vector AP2 has been constructed according to standard molecular biology steps as described in WO2003/093482, WO2005/083093, WO2007/093776 or WO2009/016202 and transformed into *Arabidopsis thaliana* as described above. Analysis of transgenic seeds is shown in FIG. 9. The products of d9Elo(Eh) are 20:2 and 20:3n-3.

**[0120]** Further, transgenic *Arabidopsis* lines have been generated to validate the activity of d4Des(Eh). Construct OstELO5EmD4 was transformed into *Arabidopsis* as described above and seeds of a number of individual lines have been analyzed by gas chromatography (FIG. 10). The activity of d4Des(Eh) is demonstrated by the formation of DHA 22:6 (last column). All lines show the production of DHA with levels of up to 4.7%. Of special interest is the ratio of DHA to DPA. Surprisingly the ratio of d4Des(Eh) is much higher than in d4-desaturases known in the art. A comparison against the d4-desaturase from *Thraustochytrium* ssp. of WO2002/026946 is shown in FIG. 11. The enzyme from *Thraustochytrium* ssp. showed so far highest levels of DHA (WO2005/083093), but with an unfavorable ratio of DPA to DHA. A high ratio of DHA:DPA is for the commercial use of such oils of importance.

**[0121]** Further, transgenic *Arabidopsis* lines have been generated to validate the activity of d5Elo(Eh). Construct EmELO5TcD4 was transformed into *Arabidopsis* as described above and seeds of a number of individual lines have been analyzed by gas chromatography (FIG. 12). The activity of d5Elo(Eh) is demonstrated by the formation of DPA 22:5 and DHA 22:6. Most lines show the production of these two fatty acids, proving that d5Elo(Em) is functionally expressed in the seeds.

**[0122]** Further, transgenic *Arabidopsis* lines have been generated to validate the activity and substrate specificity of d5Des(Eh). For this purpose two A6-desaturases were selected based on their different substrate specificity. The borageA6 is expected to use phosphatidylcholin-18:2 as substrate (WO96/21022), whereas the *Ostreococcus* A6 (OstrA6) uses Acyl-CoA ester (WO2005/012316). In combination with the d6-elongase from *Physcomitrella patens* (WO2001/059128) both d6-desaturases produce DGLA or 20:4n-3, respectively. The ratio of ARA to EPA is for the borageΔ6 2.9, for the OstrΔ6 2.3. It is noted that the use of OstrA6 results in 3-4 times higher levels of products compared to the borageA6. The further combination of the d5Des(Eh) resulted in the production of ARA and EPA, demonstrating the functionality of the d5Des(Eh). The conversion of d5Des(Eh) of DGLA to ARA is 29% (borageΔ6) or 47% (OstrΔ6). For 20:4n-3 to EPA it is 33% (borageΔ6) or 26% (OstrΔ6).

**[0123]** Based on these results it is concluded that for Acyl-CoA substrates d5Des(Eh) is specific for the omega6 fatty acid DGLA. This is a novel substrate specificity not observed in the state of the art d5-desaturases.

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- [0135]** All references cited in this specification are hereby incorporated by reference with respect to their entire disclosure content and the disclosure content specifically mentioned in this specification.

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 SEQUENCE LISTING
 

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<400> SEQUENCE: 2

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Met Gly Lys Gly Gly Asn Ala Asn Pro Arg Glu Leu Lys Gly Gly Lys
1           5           10           15
Ala Glu Gln Leu Thr Val Tyr Leu Tyr Gly Lys Ala Val Asp Val Ser
20           25           30
Lys Phe Ala Lys Leu His Pro Gly Gly Ala Lys Ala Leu Arg Ile Phe
35           40           45
Asn Asn Arg Asp Ala Thr Glu Gln Phe Glu Met Tyr His Ser Pro Ala
50           55           60
Ala His Lys Met Met Arg Ala Met Ser Lys Ser Ala Pro Glu Ala Pro
65           70           75           80

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-continued

Arg Glu Ser Glu Val Ala Thr Ser Val Val Gly Thr Asp Phe Ala Lys  
                   85                                  90                                  95  
 Leu Thr Gln Thr Leu His Asp Val Gly Cys Phe Asp Pro His Tyr Pro  
                   100                                  105                                  110  
 Asp Glu Ala Phe Lys Leu Gly Leu Thr Leu Leu Pro Gly Phe Leu Gly  
                   115                                  120                                  125  
 Phe Tyr Leu Leu Arg Ser Gly Met Pro Ala Leu Gly Ser Phe Leu Ile  
                   130                                  135                                  140  
 Ala Phe Ser Tyr Tyr Met Ser Gly Trp Thr Ser His Asp Tyr Leu His  
                   145                                  150                                  155                                  160  
 His Gly Cys Leu Lys Gly Gly Gln Lys Gln Leu Val His Trp Asn Asn  
                   165                                  170                                  175  
 Ala Val Gly Tyr Ala Ile Gly Ala Trp Gln Gly Tyr Ala Val Gly Trp  
                   180                                  185                                  190  
 Trp Arg Ala Arg His Asn Thr His His Leu Val Thr Asn Glu Glu Gly  
                   195                                  200                                  205  
 Asn Asp Pro Asp Ile Met Thr Ala Pro Val Leu Ile Phe Val Arg Asn  
                   210                                  215                                  220  
 Ser Pro Val Ile Ala Ala Ala Leu Asn Ala Ala Gln Arg Trp Gln Gln  
                   225                                  230                                  235                                  240  
 Tyr Tyr Tyr Val Pro Ala Met Ser Leu Met Asp Met Tyr Trp Arg Phe  
                   245                                  250                                  255  
 Glu Ser Met Gln Tyr Leu Ala Ala Arg Pro Phe Asn Lys Val Trp Ala  
                   260                                  265                                  270  
 Ser Trp Ala Leu Leu Ala Leu His Tyr Ser Phe Val Gly Tyr Met Phe  
                   275                                  280                                  285  
 His Gly Gln Tyr Gln Trp Leu Leu Leu Thr Met Leu Val Arg Gly Phe  
                   290                                  295                                  300  
 Leu Thr Gly Ile Val Val Phe Ser Thr His Tyr Gly Glu Glu Val Ile  
                   305                                  310                                  315                                  320  
 Pro Gly Asp His Gly Met Thr Leu Val Glu Gln Thr Ala Leu Thr Ser  
                   325                                  330                                  335  
 Arg Asn Ile Thr Gly Gly Tyr Leu Val Asn Leu Leu Thr Gly Tyr Ile  
                   340                                  345                                  350  
 Ser Leu Gln Thr Glu His His Leu Trp Pro Met Met Pro Thr Ala Arg  
                   355                                  360                                  365  
 Leu Glu Ala Ala Gln Pro Tyr Ala Arg Ala Phe Phe Lys Lys His Gly  
                   370                                  375                                  380  
 Phe Val Tyr Arg Glu Ser Asn Leu Val Glu Cys Val Lys Tyr Asn Ile  
                   385                                  390                                  395                                  400  
 Ala Ala Leu Asp Ile Arg Thr Arg Asn Gly Glu Trp Ala Glu Met Pro  
                   405                                  410                                  415

His

&lt;210&gt; SEQ ID NO 3

&lt;211&gt; LENGTH: 804

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Emiliana huxleyi

&lt;400&gt; SEQUENCE: 3

accatgctcg atcgcgcctc gtcgcagcgc gccatctggt ctgcgggtgc cgatccggaa

60

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atcctgatcg gcactttctc ctacctgctg ctcaagccgc tgctacgcaa ctcaaggctc 120
gtggacgagc ggaaaggcgc ctaccggacc tcgatgatct ggtacaacgt ggtgctcgcg 180
ctcttctccg cgacgagctt ctacgtgact gcgaccgcgc tcgggtggga caagggcacc 240
ggcgagtggc tcgcagctct cacggggcgc agcccgcagc agctgtggca atgcccgctg 300
agggtatggg actccaagct gttcctgtgg acggccaagg ccttctacta ctcaaagtac 360
gtggagtacc tcgacacggc gtggctcgtc ctcaagggga agaaggtctc cttcctgcag 420
ggcttccacc actttggcgc gccgtgggac gtgtacctgg gcattcggtt gaagaacgag 480
ggcgtgtgga tcttcatggt cttcaactcg ttcattccaca cggtcattgta cacgtactac 540
ggcctcaccg ccgcggggta caagatccgc ggcaagccga tcatcaccgc gatgcaaata 600
agccagttcg tcggcggcgtt tgcctagtgt tgggactaca tcaacgtgcc gtgcttccac 660
gccgacgcgc ggcaggtctt cagctgggtc tttactatg cttacgtcgg ctccgtcttt 720
ctgctctttt gccacttctt ctacatggac aacatcgca aggccaaggc caagaaggcc 780
gtcgtaccc gcaaggcgt gtga 804

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<210> SEQ ID NO 4
<211> LENGTH: 266
<212> TYPE: PRT
<213> ORGANISM: Emiliana huxleyi

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<400> SEQUENCE: 4

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```

Met Leu Asp Arg Ala Ser Ser Asp Ala Ala Ile Trp Ser Ala Val Ser
1          5          10          15
Asp Pro Glu Ile Leu Ile Gly Thr Phe Ser Tyr Leu Leu Leu Lys Pro
20         25         30
Leu Leu Arg Asn Ser Gly Leu Val Asp Glu Arg Lys Gly Ala Tyr Arg
35         40         45
Thr Ser Met Ile Trp Tyr Asn Val Val Leu Ala Leu Phe Ser Ala Thr
50         55         60
Ser Phe Tyr Val Thr Ala Thr Ala Leu Gly Trp Asp Lys Gly Thr Gly
65         70         75         80
Glu Trp Leu Arg Ser Leu Thr Gly Asp Ser Pro Gln Gln Leu Trp Gln
85         90         95
Cys Pro Ser Arg Val Trp Asp Ser Lys Leu Phe Leu Trp Thr Ala Lys
100        105        110
Ala Phe Tyr Tyr Ser Lys Tyr Val Glu Tyr Leu Asp Thr Ala Trp Leu
115        120        125
Val Leu Lys Gly Lys Lys Val Ser Phe Leu Gln Gly Phe His His Phe
130        135        140
Gly Ala Pro Trp Asp Val Tyr Leu Gly Ile Arg Leu Lys Asn Glu Gly
145        150        155        160
Val Trp Ile Phe Met Phe Phe Asn Ser Phe Ile His Thr Val Met Tyr
165        170        175
Thr Tyr Tyr Gly Leu Thr Ala Ala Gly Tyr Lys Ile Arg Gly Lys Pro
180        185        190
Ile Ile Thr Ala Met Gln Ile Ser Gln Phe Val Gly Gly Phe Val Leu
195        200        205
Val Trp Asp Tyr Ile Asn Val Pro Cys Phe His Ala Asp Ala Gly Gln
210        215        220

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-continued

Val Phe Ser Trp Val Phe Asn Tyr Ala Tyr Val Gly Ser Val Phe Leu  
 225 230 235 240  
 Leu Phe Cys His Phe Phe Tyr Met Asp Asn Ile Ala Lys Ala Lys Ala  
 245 250 255  
 Lys Lys Ala Val Ala Thr Arg Lys Ala Leu  
 260 265

<210> SEQ ID NO 5  
 <211> LENGTH: 1280  
 <212> TYPE: DNA  
 <213> ORGANISM: Emiliana huxleyi

<400> SEQUENCE: 5  
 ccattgggagg cgccggcgcg agcgaggctg aacggcccaa gtggaccacg atccacgggc 60  
 ggcacgtcga tgtgtcaaag ttccgccacc cgggtgggaa catcatcgag ctcttctatg 120  
 gcatggactc gaagagcgcg ttccagcagt tccacggcca ccacaagggc gcgtggaaga 180  
 tgctcaagge gctgcccacc aaggaggctg accccgccga cgtgcccag cagccgcagg 240  
 agcacgttgc cgagatgacg cgggtgatga cgtcgtggcg cgagcggcg ctctttaagc 300  
 cgccccctg cgctcgggac atctacggtc tegccgtcgt cgctgccatc gtcgctgca 360  
 tcgctgcgc gccgcacgcg ccggtgctga gcgggatcgg gctcggcagc tgctgggccc 420  
 agtgccgctt cctgcagcac atgggcccgc acccgagatg gggggtgccg tactccttcc 480  
 tctcgagca ctctctcgag ggcctcctca agggcgggtc cgctcgtgg tggcgcaacc 540  
 gccacaacia gcatcacgca aagactaacg tgctcggcga ggacggcgac ctgcccagca 600  
 ctcccttctt cgctcgggac ccgacgctcg ccaagaaggt tccagactgg tcgctcaaga 660  
 cgcaggcctt caccttcttc cccgccctcg gagcgtacgt ctttctctt gccttcacga 720  
 tccgcaagta tgcctcgtc aagaagctct ggcaagagct cgcactcatg atcggcact 780  
 acgcatggtt ctactacgcg ctgcagctcg ccggtgcgtc gctcggcagc ggcctcgcct 840  
 tttactgcac cggctacgcc tggcaaggca tctacctcgg cttctcttc ggcctgtccc 900  
 acttcgctg cgagcgagtc cctccaccg ccacctggct cgagctgtcc atgatcggca 960  
 ccgctcagct gggaggctcc tccgctttt gcggctacgt ctccggcttc cteaacatcc 1020  
 agatcgagca ccacatggcg ccgagatgc cgatggagaa cctgcccag atccgcccgc 1080  
 actgcaagge gagcgggag aagctcgggc ttccctatcg cgagctctcc ttcgcccggc 1140  
 cggtaagct gatgatggtc ggcctctggc gcacggggag ggacgagctg cagctcgcct 1200  
 ccgacaggcg caagtactcg cgcaccagg cctacatggc ggcgcccctg gcggtggtgg 1260  
 agaacctcaa ggcggactag 1280

<210> SEQ ID NO 6  
 <211> LENGTH: 425  
 <212> TYPE: PRT  
 <213> ORGANISM: Emiliana huxleyi

<400> SEQUENCE: 6  
 Met Gly Gly Ala Gly Ala Ser Glu Ala Glu Arg Pro Lys Trp Thr Thr  
 1 5 10 15  
 Ile His Gly Arg His Val Asp Val Ser Lys Phe Arg His Pro Gly Gly  
 20 25 30  
 Asn Ile Ile Glu Leu Phe Tyr Gly Met Asp Ser Thr Ser Ala Phe Glu

-continued

35				40				45							
Gln	Phe	His	Gly	His	His	Lys	Gly	Ala	Trp	Lys	Met	Leu	Lys	Ala	Leu
50						55					60				
Pro	Thr	Lys	Glu	Val	Asp	Pro	Ala	Asp	Val	Pro	Gln	Gln	Pro	Gln	Glu
65				70					75						80
His	Val	Ala	Glu	Met	Thr	Arg	Leu	Met	Thr	Ser	Trp	Arg	Glu	Arg	Gly
			85						90					95	
Leu	Phe	Lys	Pro	Arg	Pro	Val	Ala	Ser	Gly	Ile	Tyr	Gly	Leu	Ala	Val
			100						105				110		
Val	Ala	Ala	Ile	Val	Ala	Cys	Ile	Ala	Cys	Ala	Pro	His	Ala	Pro	Val
			115				120						125		
Leu	Ser	Gly	Ile	Gly	Leu	Gly	Ser	Cys	Trp	Ala	Gln	Cys	Gly	Phe	Leu
	130					135					140				
Gln	His	Met	Gly	Gly	His	Arg	Glu	Trp	Gly	Val	Arg	Tyr	Ser	Phe	Leu
	145				150					155					160
Leu	Gln	His	Phe	Phe	Glu	Gly	Leu	Leu	Lys	Gly	Gly	Ser	Ala	Ser	Trp
			165						170					175	
Trp	Arg	Asn	Arg	His	Asn	Lys	His	His	Ala	Lys	Thr	Asn	Val	Leu	Gly
			180						185				190		
Glu	Asp	Gly	Asp	Leu	Arg	Thr	Thr	Pro	Phe	Phe	Ala	Trp	Asp	Pro	Thr
		195					200						205		
Leu	Ala	Lys	Lys	Val	Pro	Asp	Trp	Ser	Leu	Lys	Thr	Gln	Ala	Phe	Thr
	210					215					220				
Phe	Leu	Pro	Ala	Leu	Gly	Ala	Tyr	Val	Phe	Val	Phe	Ala	Phe	Thr	Ile
	225				230					235					240
Arg	Lys	Tyr	Ala	Val	Val	Lys	Lys	Leu	Trp	His	Glu	Leu	Ala	Leu	Met
			245						250					255	
Ile	Ala	His	Tyr	Ala	Met	Phe	Tyr	Tyr	Ala	Leu	Gln	Leu	Ala	Gly	Ala
			260						265				270		
Ser	Leu	Gly	Ser	Gly	Leu	Ala	Phe	Tyr	Cys	Thr	Gly	Tyr	Ala	Trp	Gln
		275					280						285		
Gly	Ile	Tyr	Leu	Gly	Phe	Phe	Phe	Gly	Leu	Ser	His	Phe	Ala	Val	Glu
	290				295						300				
Arg	Val	Pro	Ser	Thr	Ala	Thr	Trp	Leu	Glu	Ser	Ser	Met	Ile	Gly	Thr
	305				310					315				320	
Val	Asp	Trp	Gly	Gly	Ser	Ser	Ala	Phe	Cys	Gly	Tyr	Val	Ser	Gly	Phe
			325						330					335	
Leu	Asn	Ile	Gln	Ile	Glu	His	His	Met	Ala	Pro	Gln	Met	Pro	Met	Glu
			340						345				350		
Asn	Leu	Arg	Gln	Ile	Arg	Ala	Asp	Cys	Lys	Ala	Ser	Ala	Glu	Lys	Leu
		355					360						365		
Gly	Leu	Pro	Tyr	Arg	Glu	Leu	Ser	Phe	Ala	Gly	Ala	Val	Lys	Leu	Met
	370					375					380				
Met	Val	Gly	Leu	Trp	Arg	Thr	Gly	Arg	Asp	Glu	Leu	Gln	Leu	Arg	Ser
	385				390				395						400
Asp	Arg	Arg	Lys	Tyr	Ser	Arg	Thr	Gln	Ala	Tyr	Met	Ala	Ala	Ala	Ser
			405						410					415	
Ala	Val	Val	Glu	Asn	Leu	Lys	Ala	Asp							
			420					425							

&lt;210&gt; SEQ ID NO 7

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&lt;211&gt; LENGTH: 921

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Emiliana huxleyi

&lt;400&gt; SEQUENCE: 7

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accatgtgta aggccttctgg tcttgcttca ggtgctaaac ctgctgctgc ttcaactatt    60
gatcagctcg ctggacttgg aagagttgct gttattgttg gatctttcac tgctgctatg    120
tgttatgctc ttcaacctct tgattcacct ggtactatct atcatgattc agctgttatg    180
ggtgctcttt tgccttggcc aatggtttac attgctcctc ttgcttacgt ttgtgctggt    240
atggtcggat gtgacctat gtcacaaaga gcttctatta agccattttt gaaacaatac    300
gttcagcctg tttacaatgt tttccaaatt gttatgtggt cttacatggt ttgggggttg    360
gctcctaaag ttgatgttct tggacttaac cctttcgcta tgaatacaga aagagataaa    420
aagactgagt ggtttatggt cgttcattac ctttctaaat tcggttgattg gacagatact    480
ttcttgatga ttggatctaa atctttttaga cagggttcat tcttgcaagt ttttcatcat    540
gctacagttg gtatgatttg ggggtccttg ttgagaaagg gatggggtgg aggtacttgt    600
gtttggggag cttttattaa ctctgttaca catggtctta tgtatacaca ttacttggtt    660
acatctcttg gtcttcataa cctcttaag tctcaactta ctaattttca acttgcctcaa    720
ttcgcttcat gtgttttgca tgcgtctttg gtttttgctt cagagacagt tcttctgct    780
agacttgctt atattcaatt ggtttaccat cctactcttt tgtttctttt cggttttcag    840
atgaagtggg ttcttctctg gatcactgga caaacaatca ctggtagaga gtcagaggct    900
cctgaaaaga aagttgcttg a                                     921

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&lt;210&gt; SEQ ID NO 8

&lt;211&gt; LENGTH: 305

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Emiliana huxleyi

&lt;400&gt; SEQUENCE: 8

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Met Cys Lys Ala Ser Gly Leu Ala Ser Gly Ala Lys Pro Ala Ala Ala
1           5           10           15
Ser Thr Ile Asp Gln Ser Ala Gly Leu Gly Arg Val Ala Val Ile Val
20          25          30
Gly Ser Phe Thr Ala Ala Met Cys Tyr Ala Leu Gln Pro Leu Asp Ser
35          40          45
Pro Gly Thr Ile Tyr His Asp Ser Ala Val Met Gly Ala Leu Leu Ser
50          55          60
Trp Pro Met Val Tyr Ile Ala Pro Leu Ala Tyr Val Cys Ala Val Met
65          70          75          80
Ala Gly Cys Arg Leu Met Ser Gln Arg Ala Ser Ile Lys Pro Phe Leu
85          90          95
Lys Gln Tyr Val Gln Pro Val Tyr Asn Val Phe Gln Ile Val Met Cys
100         105         110
Ser Tyr Met Val Trp Gly Leu Ala Pro Lys Val Asp Val Leu Gly Leu
115         120         125
Asn Pro Phe Ala Met Asn Thr Glu Arg Asp Lys Lys Thr Glu Trp Phe
130         135         140
Met Phe Val His Tyr Leu Ser Lys Phe Val Asp Trp Thr Asp Thr Phe
145         150         155         160

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Leu Met Ile Gly Ser Lys Ser Phe Arg Gln Val Ser Phe Leu Gln Val  
                   165                  170                  175  
 Phe His His Ala Thr Val Gly Met Ile Trp Gly Ala Leu Leu Arg Lys  
                   180                  185                  190  
 Gly Trp Gly Gly Gly Thr Cys Val Trp Gly Ala Phe Ile Asn Ser Val  
                   195                  200                  205  
 Thr His Val Leu Met Tyr Thr His Tyr Leu Val Thr Ser Leu Gly Leu  
                   210                  215                  220  
 His Asn Pro Leu Lys Ser Gln Leu Thr Asn Phe Gln Leu Ala Gln Phe  
                   225                  230                  235                  240  
 Ala Ser Cys Val Leu His Ala Ala Leu Val Phe Ala Ser Glu Thr Val  
                   245                  250                  255  
 Leu Pro Ala Arg Leu Ala Tyr Ile Gln Leu Val Tyr His Pro Thr Leu  
                   260                  265                  270  
 Leu Phe Leu Phe Gly Phe Gln Met Lys Trp Val Pro Ser Trp Ile Thr  
                   275                  280                  285  
 Gly Gln Thr Ile Thr Gly Arg Glu Ser Glu Ala Pro Glu Lys Lys Val  
                   290                  295                  300  
 Ala  
 305

&lt;210&gt; SEQ ID NO 9

&lt;211&gt; LENGTH: 1368

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Emiliana huxleyi

&lt;400&gt; SEQUENCE: 9

```

atgtcattgg ctgctaaga tgcagcctcg gccactcat cgtcttggga ccctaagtat    60
cacggagcta caaataagtc aagaactgat gcagcagacc ttacagttag ttctatcgac    120
acttctaagg agatgatcat aaggggtcgt gtgtatgatg tctctgattt tattaanaagg    180
caccggggag gaagcattat taaactctcc ttaggttctg atgcaacaga cgcttataac    240
aacttcata ttaggtctaa aaaagcggat aaatgttga gagctttgcc aagtaggcca    300
gtagcggatg gatttcgctag agacgctttg tctgcagact tgcaggccct gagagcccaa    360
ctcgaggccg aaggttactt cgaaccgaat ctgtggcatg tagcttatcg agttgaggaa    420
gtcgttgcta tgtactgggc gggattaga cttatctggg cgggttattg gtttttagga    480
gccattgtag caggaatagc tcaggggaga tgcggttggc ttcagcatga ggggtgcat    540
tattcgctca caggtaatat taaacttgat cgacacatgc aaatgattat ctatggatta    600
ggttgaggaa tgtccgggtg ttattggaga aaccaacata acaagcacca tgcgacaccg    660
caaaagttag gtgcagatcc agacctcaa acaatgcctc tggttgcgctt ccatggactc    720
atcggtgcta aggctagggg agcaggaaaag tegtggctag catggcaagc tccacttttc    780
tttgaggcgc ttatcacaac cctggtatct tttggttggc agttcgtcca acatccaaag    840
cacgcattga gagtaggaaa ccaactcgaa ttaggtata tggctttacg atatgcttta    900
tggtatgcag cattcgggtc tcttgggctt ggtggtgctt tcagattgta cgctttttat    960
gtggcagtcg gaggtacata tatcttcacg aactttgcgg tgtctcacac acataaggat    1020
gttggtccac acgataagca tatttcttgg accttgatt ctgcaaacca taccactaat    1080
caatctaaca cacctctagt caattggtgg atggcctatc tgaattttca aattgaacat    1140

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caccttttcc ctagcatgcc acaatataac catcctaaaa tctgcggaag agtgaacaaa 1200
ttgtttgaaa aacatggcgt agagtacgat gtcagaactt acgcgaagtc aatgcgtgat 1260
acatacgtga atctcttggc tgtgggaaat gcattctcatt cccttcatca gagaaacgag 1320
ggattaacga ctagggagtc tgcggctggt agagttacag gtcattga 1368

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<210> SEQ ID NO 10
<211> LENGTH: 455
<212> TYPE: PRT
<213> ORGANISM: Emiliana huxleyi

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<400> SEQUENCE: 10

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Met Ser Leu Ala Ala Lys Asp Ala Ala Ser Ala His Ser Ser Val Leu
1          5          10          15
Asp Pro Lys Tyr His Gly Ala Thr Asn Lys Ser Arg Thr Asp Ala Ala
20         25         30
Asp Leu Thr Val Ser Ser Ile Asp Thr Ser Lys Glu Met Ile Ile Arg
35         40         45
Gly Arg Val Tyr Asp Val Ser Asp Phe Ile Lys Arg His Pro Gly Gly
50         55         60
Ser Ile Ile Lys Leu Ser Leu Gly Ser Asp Ala Thr Asp Ala Tyr Asn
65         70         75         80
Asn Phe His Ile Arg Ser Lys Lys Ala Asp Lys Met Leu Arg Ala Leu
85         90         95
Pro Ser Arg Pro Val Ala Asp Gly Phe Ala Arg Asp Ala Leu Ser Ala
100        105        110
Asp Phe Glu Ala Leu Arg Ala Gln Leu Glu Ala Glu Gly Tyr Phe Glu
115        120        125
Pro Asn Leu Trp His Val Ala Tyr Arg Val Ala Glu Val Val Ala Met
130        135        140
Tyr Trp Ala Gly Ile Arg Leu Ile Trp Ala Gly Tyr Trp Phe Leu Gly
145        150        155        160
Ala Ile Val Ala Gly Ile Ala Gln Gly Arg Cys Gly Trp Leu Gln His
165        170        175
Glu Gly Gly His Tyr Ser Leu Thr Gly Asn Ile Lys Leu Asp Arg His
180        185        190
Met Gln Met Ile Ile Tyr Gly Leu Gly Cys Gly Met Ser Gly Cys Tyr
195        200        205
Trp Arg Asn Gln His Asn Lys His His Ala Thr Pro Gln Lys Leu Gly
210        215        220
Ala Asp Pro Asp Leu Gln Thr Met Pro Leu Val Ala Phe His Gly Leu
225        230        235        240
Ile Gly Ala Lys Ala Arg Gly Ala Gly Lys Ser Trp Leu Ala Trp Gln
245        250        255
Ala Pro Leu Phe Phe Gly Gly Val Ile Thr Thr Leu Val Ser Phe Gly
260        265        270
Trp Gln Phe Val Gln His Pro Lys His Ala Leu Arg Val Gly Asn Gln
275        280        285
Leu Glu Leu Gly Tyr Met Ala Leu Arg Tyr Ala Leu Trp Tyr Ala Ala
290        295        300
Phe Gly His Leu Gly Leu Gly Gly Ala Phe Arg Leu Tyr Ala Phe Tyr
305        310        315        320

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Val Ala Val Gly Gly Thr Tyr Ile Phe Thr Asn Phe Ala Val Ser His  
 325 330 335

Thr His Lys Asp Val Val Pro His Asp Lys His Ile Ser Trp Thr Leu  
 340 345 350

Tyr Ser Ala Asn His Thr Thr Asn Gln Ser Asn Thr Pro Leu Val Asn  
 355 360 365

Trp Trp Met Ala Tyr Leu Asn Phe Gln Ile Glu His His Leu Phe Pro  
 370 375 380

Ser Met Pro Gln Tyr Asn His Pro Lys Ile Cys Gly Arg Val Lys Gln  
 385 390 395 400

Leu Phe Glu Lys His Gly Val Glu Tyr Asp Val Arg Thr Tyr Ala Lys  
 405 410 415

Ser Met Arg Asp Thr Tyr Val Asn Leu Leu Ala Val Gly Asn Ala Ser  
 420 425 430

His Ser Leu His Gln Arg Asn Glu Gly Leu Thr Thr Arg Glu Ser Ala  
 435 440 445

Ala Val Arg Val Thr Gly His  
 450 455

<210> SEQ ID NO 11  
 <211> LENGTH: 22  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 11

ccatgggagg cgccggcgcg ag

22

<210> SEQ ID NO 12  
 <211> LENGTH: 21  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 12

ctagtccgcc ttgaggttct c

21

<210> SEQ ID NO 13  
 <211> LENGTH: 23  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 13

accatgtgca aggcgagcgg cct

23

<210> SEQ ID NO 14  
 <211> LENGTH: 20  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 14

tcaccaatca tgaggaaggt

20

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<210> SEQ ID NO 15
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 15

ccatgggcaa gggcggcaac gc                22

<210> SEQ ID NO 16
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 16

gggcagagat gccgcactag                20

<210> SEQ ID NO 17
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 17

accatgctcg atcgcgcctc gtc                23

<210> SEQ ID NO 18
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 18

tcacagcgcc ttgcgggtag c                21

<210> SEQ ID NO 19
<211> LENGTH: 1371
<212> TYPE: DNA
<213> ORGANISM: Ostreococcus tauri

<400> SEQUENCE: 19

atgtgtgttg agaccgagaa caacgatgga atccctactg tggagatcgc tttcgatgga    60
gagagagaaa gagctgaggc taacgtgaag ttgtctgctg agaagatgga acctgctgct    120
ttggctaaga ccttcgctag aagatacgtg gttatcgagg gagttgagta cgatgtgacc    180
gatttcaaac atcctggagg aaccgtgatt ttctacgctc tctctaacac tggagctgat    240
gctactgagg ctttcaagga gttccaccac agatctagaa aggctaggaa ggctttggct    300
gctttgcctt ctgacctgc taagaccgct aaagtggatg atgctgagat gctccaggat    360
ttcgctaagt ggagaaagga gttggagagg gacggattct tcaagccttc tctgtctcat    420
gttgcttaca gattcgctga gttggctgct atgtacgctt tgggaacctc cttgatgtac    480
gctagatacg ttgtgtcctc tgtgttggtt tacgcttgct tcttcggagc tagatgtgga    540
tgggttcaac atgaggggagg acattcttct ttgaccgaa acatctggtg ggataagaga    600
atccaagctt tcaactgctg attcggattg gctggatctg gagatatgtg gaactccatg    660

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cacaacaagc accatgctac tctcAAAAA gtgaggcagc atatggattt ggataccact 720
cctgctgttg ctttcttcaa caccgctgtg gaggataata gacctagggg attctctaag 780
tactggctca gattgcaagc ttggaccttc attcctgtga cttctggatt ggtgttgctc 840
ttctggatgt tcttctccca tcttctaag gctttgaagg gagaaaagta cgaggagctt 900
gtgtggatgt tggctgctca tgtgattaga acctggacca ttaaggetgt tactggatc 960
accgctatgc aatcctacgg actcttcttg gctacttctt gggtttccgg atgctacttg 1020
ttcgtcact tetctacttc tcacacccat ttggatgttg ttctgctga tgagcatttg 1080
tcttgggtta ggtacgctgt ggatcacacc attgatatcg atccttctca gggatgggtt 1140
aactggttga tgggatactt gaactgccaa gtgattcacc acctcttccc ttctatgect 1200
caattcagac aacctgaggt gtccagaaga ttcgttgctt tcgctaagaa gtggaacctc 1260
aactacaagg tgatgactta tgctggagct tggaaggcta ctttgggaaa cctcgataat 1320
gtgggaaagc actactacgt gcacggacaa cattctggaa agaccgettg a 1371

```

&lt;210&gt; SEQ ID NO 20

&lt;211&gt; LENGTH: 456

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Ostreococcus tauri*

&lt;400&gt; SEQUENCE: 20

```

Met Cys Val Glu Thr Glu Asn Asn Asp Gly Ile Pro Thr Val Glu Ile
1          5          10          15
Ala Phe Asp Gly Glu Arg Glu Arg Ala Glu Ala Asn Val Lys Leu Ser
20        25        30
Ala Glu Lys Met Glu Pro Ala Ala Leu Ala Lys Thr Phe Ala Arg Arg
35        40        45
Tyr Val Val Ile Glu Gly Val Glu Tyr Asp Val Thr Asp Phe Lys His
50        55        60
Pro Gly Gly Thr Val Ile Phe Tyr Ala Leu Ser Asn Thr Gly Ala Asp
65        70        75        80
Ala Thr Glu Ala Phe Lys Glu Phe His His Arg Ser Arg Lys Ala Arg
85        90        95
Lys Ala Leu Ala Ala Leu Pro Ser Arg Pro Ala Lys Thr Ala Lys Val
100       105       110
Asp Asp Ala Glu Met Leu Gln Asp Phe Ala Lys Trp Arg Lys Glu Leu
115       120       125
Glu Arg Asp Gly Phe Phe Lys Pro Ser Pro Ala His Val Ala Tyr Arg
130       135       140
Phe Ala Glu Leu Ala Ala Met Tyr Ala Leu Gly Thr Tyr Leu Met Tyr
145       150       155       160
Ala Arg Tyr Val Val Ser Ser Val Leu Val Tyr Ala Cys Phe Phe Gly
165       170       175
Ala Arg Cys Gly Trp Val Gln His Glu Gly Gly His Ser Ser Leu Thr
180       185       190
Gly Asn Ile Trp Trp Asp Lys Arg Ile Gln Ala Phe Thr Ala Gly Phe
195       200       205
Gly Leu Ala Gly Ser Gly Asp Met Trp Asn Ser Met His Asn Lys His
210       215       220
His Ala Thr Pro Gln Lys Val Arg His Asp Met Asp Leu Asp Thr Thr

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225		230		235		240									
Pro	Ala	Val	Ala	Phe	Phe	Asn	Thr	Ala	Val	Glu	Asp	Asn	Arg	Pro	Arg
				245					250					255	
Gly	Phe	Ser	Lys	Tyr	Trp	Leu	Arg	Leu	Gln	Ala	Trp	Thr	Phe	Ile	Pro
			260					265					270		
Val	Thr	Ser	Gly	Leu	Val	Leu	Leu	Phe	Trp	Met	Phe	Phe	Leu	His	Pro
		275					280				285				
Ser	Lys	Ala	Leu	Lys	Gly	Gly	Lys	Tyr	Glu	Glu	Leu	Val	Trp	Met	Leu
	290					295					300				
Ala	Ala	His	Val	Ile	Arg	Thr	Trp	Thr	Ile	Lys	Ala	Val	Thr	Gly	Phe
305					310					315					320
Thr	Ala	Met	Gln	Ser	Tyr	Gly	Leu	Phe	Leu	Ala	Thr	Ser	Trp	Val	Ser
			325						330					335	
Gly	Cys	Tyr	Leu	Phe	Ala	His	Phe	Ser	Thr	Ser	His	Thr	His	Leu	Asp
			340					345						350	
Val	Val	Pro	Ala	Asp	Glu	His	Leu	Ser	Trp	Val	Arg	Tyr	Ala	Val	Asp
		355					360					365			
His	Thr	Ile	Asp	Ile	Asp	Pro	Ser	Gln	Gly	Trp	Val	Asn	Trp	Leu	Met
	370					375						380			
Gly	Tyr	Leu	Asn	Cys	Gln	Val	Ile	His	His	Leu	Phe	Pro	Ser	Met	Pro
385					390					395					400
Gln	Phe	Arg	Gln	Pro	Glu	Val	Ser	Arg	Arg	Phe	Val	Ala	Phe	Ala	Lys
				405					410					415	
Lys	Trp	Asn	Leu	Asn	Tyr	Lys	Val	Met	Thr	Tyr	Ala	Gly	Ala	Trp	Lys
			420					425						430	
Ala	Thr	Leu	Gly	Asn	Leu	Asp	Asn	Val	Gly	Lys	His	Tyr	Tyr	Val	His
		435					440					445			
Gly	Gln	His	Ser	Gly	Lys	Thr	Ala								
	450					455									

&lt;210&gt; SEQ ID NO 21

&lt;211&gt; LENGTH: 873

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Physcomitrella patens

&lt;400&gt; SEQUENCE: 21

```

atggaagtgtg ttgagagggtt ctacggagag ttggatggaa aggtttccca aggagtgaac    60
gctttgttgg gatccttctgg agttgagttg actgataccc caactactaa gggattgcca    120
ctcgttgatt ctccaactcc aattgtgttg ggagtgtctg tttacttgac catcgtgatc    180
ggaggattgc tttggatcaa ggctagagat ctcaagccaa gagcttctga gccattcttg    240
ttgcaagctt tgggtgttgt gcacaacttg ttctgcttcg ctttgtctct ttacatgtgc    300
gtgggtatcg cttaccaagc tatcacctgg agatattcct tgtggggaaa cgcttataac    360
ccaaagcaca aggagatgga taccctctgt tacctcttct acatgtccaa gtacgtggag    420
ttcatggata ccgtgatcat gatcctcaag agatccacca gacagatttc tttcctccac    480
gtgtaccacc attcttctat ctcccttate tgggtgggcta ttgctcatca tgctccagga    540
ggagaggctt attggagtgc tgctctcaac tctggagtgc atgtgttgat gtacgcttac    600
tacttcttgg ctgcttgcct gagatcttcc ccaaagctca agaacaagta cctcttctgg    660
ggaagatacc tcaccaaat ccagatgttc cagttcatgc tcaacttggg gcaagcttac    720

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```
tacgatatga aaaccaacgc tccatatcca caatggctca tcaagatcct cttctactac 780
atgatctccc tctgttctct cttcggaaac ttctacgtgc aaaagtacat caagccatcc 840
gatggaaagc aaaagggagc taagaccgag tga 873
```

```
<210> SEQ ID NO 22
<211> LENGTH: 290
<212> TYPE: PRT
<213> ORGANISM: Physcomitrella patens

<400> SEQUENCE: 22
```

```
Met Glu Val Val Glu Arg Phe Tyr Gly Glu Leu Asp Gly Lys Val Ser
1 5 10 15
Gln Gly Val Asn Ala Leu Leu Gly Ser Phe Gly Val Glu Leu Thr Asp
20 25 30
Thr Pro Thr Thr Lys Gly Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
35 40 45
Val Leu Gly Val Ser Val Tyr Leu Thr Ile Val Ile Gly Gly Leu Leu
50 55 60
Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
65 70 75 80
Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
85 90 95
Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
100 105 110
Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
115 120 125
Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
130 135 140
Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gln Ile Ser Phe Leu His
145 150 155 160
Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
165 170 175
His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
180 185 190
Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
195 200 205
Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
210 215 220
Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
225 230 235 240
Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gln Trp Leu Ile Lys Ile
245 250 255
Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gly Asn Phe Tyr
260 265 270
Val Gln Lys Tyr Ile Lys Pro Ser Asp Gly Lys Gln Lys Gly Ala Lys
275 280 285
Thr Glu
290
```

```
<210> SEQ ID NO 23
<211> LENGTH: 1197
<212> TYPE: DNA
<213> ORGANISM: Phytophthora sojae
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&lt;400&gt; SEQUENCE: 23

```

atggctatth tgaacctga ggctgattct gctgctaacc tcgctactga ttctgaggct    60
aagcaaaagac aattggctga ggctggatac actcatgttg aggggtctcc tgctcctttg    120
cctttggagt tgctcattt ctctctcaga gatctcagag ctgctattcc taagcactgc    180
ttcgagagat ctttcgtgac ctccacctac tacatgatca agaactgtgt gacttgcgct    240
gctttgttct acgctgctac cttoattgat agagctggag ctgctgctta tgttttgtgg    300
cctgtgtact ggttcttcca gggatcttac ttgactggag tgtgggttat cgctcatgag    360
tgtggacatc aggcctattg ctctctcagag gtggtgaaca acttgattgg actcgtgttg    420
cattctgctt tgttggtgcc ttaccactct tggagaatct ctcacagaaa gcaccattcc    480
aacactggat cttgcgagaa cgatgaggtt ttcgttctctg tgaccagatc tgtgttggtc    540
tcttcttggg acgagacctt ggaggattct cctctctacc aactctaccg tatcgtgtac    600
atgttggttg ttggatggat gctcggatac ctctctctca acgctactgg acctactaag    660
tactggggaa agtctaggtc tcaactcaac ccttactccg ctatctatgc tgataggag    720
agatggatga tegtgtctc cgatatttct ttgggtggcta tgttggtgtt tttggctgct    780
ttggtgcaca ctttctcctt caacaccatg gtgaagtctt acgtggtgcc ttacttcatt    840
gtgaacgctt acttggtgtt gattacctac ctccaacaca cggataccta catccctcat    900
ttcagagagg gagagtggaa ttggttgaga ggagctttgt gcaactgtgga tagatcattt    960
ggtccattcc tcgattctgt ggtgcataga atcgtggata cccatgtttg ccaccacatc   1020
ttctccaaga tgcctttcta tcattgctgag gaggetacca acgctattaa gcctctcctc   1080
ggaaagtctt acttgaagga taccactcct gttcctgttg ctctctggag atcttacacc   1140
cattgcaagt tcgcttgagga tgatggaaaag gtggtgttct acaagaacaa gctctag    1197

```

&lt;210&gt; SEQ ID NO 24

&lt;211&gt; LENGTH: 398

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 24

```

Met Ala Ile Leu Asn Pro Glu Ala Asp Ser Ala Ala Asn Leu Ala Thr
1           5           10          15
Asp Ser Glu Ala Lys Gln Arg Gln Leu Ala Glu Ala Gly Tyr Thr His
20          25          30
Val Glu Gly Ala Pro Ala Pro Leu Pro Leu Glu Leu Pro His Phe Ser
35          40          45
Leu Arg Asp Leu Arg Ala Ala Ile Pro Lys His Cys Phe Glu Arg Ser
50          55          60
Phe Val Thr Ser Thr Tyr Tyr Met Ile Lys Asn Val Leu Thr Cys Ala
65          70          75          80
Ala Leu Phe Tyr Ala Ala Thr Phe Ile Asp Arg Ala Gly Ala Ala Ala
85          90          95
Tyr Val Leu Trp Pro Val Tyr Trp Phe Phe Gln Gly Ser Tyr Leu Thr
100         105         110
Gly Val Trp Val Ile Ala His Glu Cys Gly His Gln Ala Tyr Cys Ser
115        120        125
Ser Glu Val Val Asn Asn Leu Ile Gly Leu Val Leu His Ser Ala Leu

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130	135	140
Leu Val Pro Tyr His Ser Trp Arg Ile Ser His Arg Lys His His Ser		
145	150	155 160
Asn Thr Gly Ser Cys Glu Asn Asp Glu Val Phe Val Pro Val Thr Arg		
	165	170 175
Ser Val Leu Ala Ser Ser Trp Asn Glu Thr Leu Glu Asp Ser Pro Leu		
	180	185 190
Tyr Gln Leu Tyr Arg Ile Val Tyr Met Leu Val Val Gly Trp Met Pro		
	195	200 205
Gly Tyr Leu Phe Phe Asn Ala Thr Gly Pro Thr Lys Tyr Trp Gly Lys		
	210	215 220
Ser Arg Ser His Phe Asn Pro Tyr Ser Ala Ile Tyr Ala Asp Arg Glu		
225	230	235 240
Arg Trp Met Ile Val Leu Ser Asp Ile Phe Leu Val Ala Met Leu Ala		
	245	250 255
Val Leu Ala Ala Leu Val His Thr Phe Ser Phe Asn Thr Met Val Lys		
	260	265 270
Phe Tyr Val Val Pro Tyr Phe Ile Val Asn Ala Tyr Leu Val Leu Ile		
	275	280 285
Thr Tyr Leu Gln His Thr Asp Thr Tyr Ile Pro His Phe Arg Glu Gly		
	290	295 300
Glu Trp Asn Trp Leu Arg Gly Ala Leu Cys Thr Val Asp Arg Ser Phe		
305	310	315 320
Gly Pro Phe Leu Asp Ser Val Val His Arg Ile Val Asp Thr His Val		
	325	330 335
Cys His His Ile Phe Ser Lys Met Pro Phe Tyr His Cys Glu Glu Ala		
	340	345 350
Thr Asn Ala Ile Lys Pro Leu Leu Gly Lys Phe Tyr Leu Lys Asp Thr		
	355	360 365
Thr Pro Val Pro Val Ala Leu Trp Arg Ser Tyr Thr His Cys Lys Phe		
	370	375 380
Val Glu Asp Asp Gly Lys Val Val Phe Tyr Lys Asn Lys Leu		
385	390	395

&lt;210&gt; SEQ ID NO 25

&lt;211&gt; LENGTH: 819

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Thalassiosira pseudonana

&lt;400&gt; SEQUENCE: 25

atggatgctt ataacgctgc tatggataag attggagctg ctatcatcga ttggagtgat	60
ccagatggaa agttcagagc tgatagggag gattggtggt tgtgcgattt cagatccgct	120
atcaccattg ctctcatcta catcgctttc gtgatcttgg gatctgctgt gatgcaatct	180
ctcccagcta tggaccata ccoctatcaag ttcctctaca acgtgtctca aatcttctc	240
tgcgcttaca tgactgttga ggctggattc ctcgcttata ggaacggata caccgttatg	300
ccatgcaacc acttcaacgt gaacgatcca ccagttgcta acttgctctg gctcttctac	360
atctccaaag tgtgggattt ctgggatacc atcttcattg tgcctcgaaa gaagtggaga	420
caactctctt tcttgcacgt gtaccatcat accaccatct tctctcteta ctggttgaac	480
gctaacgtgc tctacgatgg agatatcttc ttgaccatcc tctcaacgg attcattcac	540

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accgtgatgt acacctacta cttcatctgc atgcacacca aggattctaa gaccggaaag      600
tctttgccaa tetgggtggaa gtcacttttg accgctttcc aactcttgca attcaccatc      660
atgatgtccc aagctaccta cttggttttc cacggatgcg ataaggtttc cctcagaatc      720
accatcgtgt acttctgtga cattctctcc cttttcttcc tcttcgctca gttctctgtg      780
caatcctaca tggctccaaa gaagaagaag tccgcttga                               819

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<210> SEQ ID NO 26
<211> LENGTH: 272
<212> TYPE: PRT
<213> ORGANISM: Thalassiosira pseudonana

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```

<400> SEQUENCE: 26

```

```

Met Asp Ala Tyr Asn Ala Ala Met Asp Lys Ile Gly Ala Ala Ile Ile
1          5          10          15
Asp Trp Ser Asp Pro Asp Gly Lys Phe Arg Ala Asp Arg Glu Asp Trp
20          25          30
Trp Leu Cys Asp Phe Arg Ser Ala Ile Thr Ile Ala Leu Ile Tyr Ile
35          40          45
Ala Phe Val Ile Leu Gly Ser Ala Val Met Gln Ser Leu Pro Ala Met
50          55          60
Asp Pro Tyr Pro Ile Lys Phe Leu Tyr Asn Val Ser Gln Ile Phe Leu
65          70          75          80
Cys Ala Tyr Met Thr Val Glu Ala Gly Phe Leu Ala Tyr Arg Asn Gly
85          90          95
Tyr Thr Val Met Pro Cys Asn His Phe Asn Val Asn Asp Pro Pro Val
100         105         110
Ala Asn Leu Leu Trp Leu Phe Tyr Ile Ser Lys Val Trp Asp Phe Trp
115         120         125
Asp Thr Ile Phe Ile Val Leu Gly Lys Lys Trp Arg Gln Leu Ser Phe
130         135         140
Leu His Val Tyr His His Thr Thr Ile Phe Leu Phe Tyr Trp Leu Asn
145         150         155         160
Ala Asn Val Leu Tyr Asp Gly Asp Ile Phe Leu Thr Ile Leu Leu Asn
165         170         175
Gly Phe Ile His Thr Val Met Tyr Thr Tyr Tyr Phe Ile Cys Met His
180         185         190
Thr Lys Asp Ser Lys Thr Gly Lys Ser Leu Pro Ile Trp Trp Lys Ser
195         200         205
Ser Leu Thr Ala Phe Gln Leu Leu Gln Phe Thr Ile Met Met Ser Gln
210         215         220
Ala Thr Tyr Leu Val Phe His Gly Cys Asp Lys Val Ser Leu Arg Ile
225         230         235         240
Thr Ile Val Tyr Phe Val Tyr Ile Leu Ser Leu Phe Phe Leu Phe Ala
245         250         255
Gln Phe Phe Val Gln Ser Tyr Met Ala Pro Lys Lys Lys Lys Ser Ala
260         265         270

```

```

<210> SEQ ID NO 27
<211> LENGTH: 1086
<212> TYPE: DNA
<213> ORGANISM: Phytophthora infestans

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<400> SEQUENCE: 27

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```

atggcgacga aggaggcgta tgtgttcccc actctgacgg agatcaagcg gtcgctacct    60
aaagactggt tcgaggcttc ggtgcctctg tcgctctact acaccgtgcg ttgtctggtg    120
atcgcggttg ctctaacctt cggctctcaac tacgctcgcg ctctgcccga ggtcgagagc    180
ttctgggctc tggacgccgc actctgcacg ggctacatct tegtgcaggg catcgtgttc    240
tggggcttct tcacggtggg ccacgatgce ggccaeggcg ccttctcgcg ctaccacctg    300
cttaacttcg tggtagggcac ttctcatgac tcgctcatcc tcacgccctt cgagtcgtgg    360
aagctcacgc accgtcacca ccacaagaac acgggcaaca ttgacctga cgaggtcttc    420
taccgcgaac gcaaggccga cgaccaccgc ctgtctcgca acctgattct ggcgctcggg    480
gcagcgtggc tcgcctatct ggtcgagggc ttccctcctc gtaaggtaa ccacttcaac    540
ccgttcgagc ctctgttctg gcgtcaggtg tcagctgtgg taatctctct tctcgcccac    600
ttcttcgtgg ccggactctc catctatctg agcctccagc tgggccttaa gacgatggca    660
atctactact atggacctgt ttttgtgttc ggcagcatgc tggtcattac caccttctca    720
caccacaatg atgaggagac cccatggtac gccgactcgg agtggacgta cgtcaagggc    780
aacctctcgt ccgtggaccg atcgtacggc gegctcattg acaacctgag ccacaacatc    840
ggcaagcacc agatccacca ctttttccct atcattccgc actacaaact caagaaagcc    900
actcggcctc tccaccaggc ttccctcgtg ctctgctcgc agagcgacga gcccaattatc    960
aaggctttct tccgggttgg acgtctctac gcaaaactac gcgttgtgga ccaggaggcg   1020
aagctcttca cgctaaagga agccaaggcg gcgaccgagg cggcggccaa gaccaagtcc   1080
acgtaa                                     1086

```

&lt;210&gt; SEQ ID NO 28

&lt;211&gt; LENGTH: 361

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora infestans*

&lt;400&gt; SEQUENCE: 28

```

Met Ala Thr Lys Glu Ala Tyr Val Phe Pro Thr Leu Thr Glu Ile Lys
 1             5             10             15

Arg Ser Leu Pro Lys Asp Cys Phe Glu Ala Ser Val Pro Leu Ser Leu
 20             25             30

Tyr Tyr Thr Val Arg Cys Leu Val Ile Ala Val Ala Leu Thr Phe Gly
 35             40             45

Leu Asn Tyr Ala Arg Ala Leu Pro Glu Val Glu Ser Phe Trp Ala Leu
 50             55             60

Asp Ala Ala Leu Cys Thr Gly Tyr Ile Leu Leu Gln Gly Ile Val Phe
 65             70             75             80

Trp Gly Phe Phe Thr Val Gly His Asp Ala Gly His Gly Ala Phe Ser
 85             90             95

Arg Tyr His Leu Leu Asn Phe Val Val Gly Thr Phe Met His Ser Leu
 100            105            110

Ile Leu Thr Pro Phe Glu Ser Trp Lys Leu Thr His Arg His His His
 115            120            125

Lys Asn Thr Gly Asn Ile Asp Arg Asp Glu Val Phe Tyr Pro Gln Arg
 130            135            140

Lys Ala Asp Asp His Pro Leu Ser Arg Asn Leu Ile Leu Ala Leu Gly
 145            150            155            160

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Ala Ala Trp Leu Ala Tyr Leu Val Glu Gly Phe Pro Pro Arg Lys Val  
 165 170 175  
 Asn His Phe Asn Pro Phe Glu Pro Leu Phe Val Arg Gln Val Ser Ala  
 180 185 190  
 Val Val Ile Ser Leu Leu Ala His Phe Phe Val Ala Gly Leu Ser Ile  
 195 200 205  
 Tyr Leu Ser Leu Gln Leu Gly Leu Lys Thr Met Ala Ile Tyr Tyr Tyr  
 210 215 220  
 Gly Pro Val Phe Val Phe Gly Ser Met Leu Val Ile Thr Thr Phe Leu  
 225 230 235 240  
 His His Asn Asp Glu Glu Thr Pro Trp Tyr Ala Asp Ser Glu Trp Thr  
 245 250 255  
 Tyr Val Lys Gly Asn Leu Ser Ser Val Asp Arg Ser Tyr Gly Ala Leu  
 260 265 270  
 Ile Asp Asn Leu Ser His Asn Ile Gly Thr His Gln Ile His His Leu  
 275 280 285  
 Phe Pro Ile Ile Pro His Tyr Lys Leu Lys Lys Ala Thr Ala Ala Phe  
 290 295 300  
 His Gln Ala Phe Pro Glu Leu Val Arg Lys Ser Asp Glu Pro Ile Ile  
 305 310 315 320  
 Lys Ala Phe Phe Arg Val Gly Arg Leu Tyr Ala Asn Tyr Gly Val Val  
 325 330 335  
 Asp Gln Glu Ala Lys Leu Phe Thr Leu Lys Glu Ala Lys Ala Ala Thr  
 340 345 350  
 Glu Ala Ala Ala Lys Thr Lys Ser Thr  
 355 360

<210> SEQ ID NO 29  
 <211> LENGTH: 1434  
 <212> TYPE: DNA  
 <213> ORGANISM: Claviceps purpurea

<400> SEQUENCE: 29

atggctgcta ctacctctgc tatgagcaag gatgctgttc ttagaagaac tgctgctgct 60  
 actactgcta tcgatcacga aagctctacc tctgcttctc cagctgattc tcttagactc 120  
 tctgcttctt ctacctctct ctcttctctc agctctctcg acgctaagga taaggatgat 180  
 gagtacgctg gacttcttga tacttacgga aacgctttca cccctcctga tttcaactatc 240  
 aaggatatca gagatgctat cctaagcacc tgcttcgagc gttctgctat caagggatac 300  
 gcttatatcc tcagagatgt ggcttgctt tetaccactt totacctctt ccacaacttc 360  
 gttaccctcg agaacgttcc ttacaccctc cttagagttt tctctgggg agtttactact 420  
 gctcttcagg gacttttcgg aactggactc tggattatcg ctcacgagtg tggacacggt 480  
 gctttctctc ctctaccct cactaacgat cttactggat gggttctcca ctctgctctt 540  
 ctctgctctt acttctcttg gaagtctct cactctgctc accacaaggg aaccggaaat 600  
 atggaaaagg atatggcttt cctccctaga actagggctc aatacgtac cagattcgga 660  
 agagctatgg atcagcttgg agatctttgc gaggaaccc ctatctacac tgctggatto 720  
 cttgttttcc agcagcttct tggatggcct tcttacttga tcgctaactg tactggacac 780  
 gatcttcacg agagacagag agaggaaga ggaaagggaa agaagaacgg attcggagga 840

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actgtaacc acttcgaccc tcgttctect atcttcgatg acaagcacgc taagtttato   900
gttctcagcg atateggact tggacttgct atcgetgctc ttgtttacct cggaaacaga   960
ttcggatggg ctaacggtgc tgtttggtag ttcgttcctt acctctgggt taaccactgg  1020
atcgttgcta tcactttect tcagcacact gatcctacto ttcctcacta cactgctgag  1080
gaatggaact tcgttcgtgg agctgctgct acaatcgata gagagatggg atttatcggg  1140
agacacctct tccacggaat cgttgagact cacgtgcttc accactacgt ttcttcaatc  1200
cctttctaca acgctgatga ggcttctgag gctatcaagc ctggttatggg aaagcactac  1260
cgttctgaga ctaaggatgg acctatgggt tttatcaggg ctttgggaa aactgctaga  1320
tggtgtcaat gggttgagcc ttctgctgat gctcaaggtg ctggtgaagg tgttctcttc  1380
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&lt;210&gt; SEQ ID NO 30

&lt;211&gt; LENGTH: 477

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Claviceps purpurea*

&lt;400&gt; SEQUENCE: 30

```

Met Ala Ala Thr Thr Ser Ala Met Ser Lys Asp Ala Val Leu Arg Arg
1           5           10           15
Thr Ala Ala Ala Thr Thr Ala Ile Asp His Glu Ser Ser Thr Ser Ala
20          25          30
Ser Pro Ala Asp Ser Pro Arg Leu Ser Ala Ser Ser Thr Ser Leu Ser
35          40          45
Ser Leu Ser Ser Leu Asp Ala Lys Asp Lys Asp Asp Glu Tyr Ala Gly
50          55          60
Leu Leu Asp Thr Tyr Gly Asn Ala Phe Thr Pro Pro Asp Phe Thr Ile
65          70          75          80
Lys Asp Ile Arg Asp Ala Ile Pro Lys His Cys Phe Glu Arg Ser Ala
85          90          95
Ile Lys Gly Tyr Ala Tyr Ile Leu Arg Asp Val Ala Cys Leu Ser Thr
100         105         110
Thr Phe Tyr Leu Phe His Asn Phe Val Thr Pro Glu Asn Val Pro Tyr
115         120         125
Thr Pro Leu Arg Val Phe Leu Trp Gly Val Tyr Thr Ala Leu Gln Gly
130         135         140
Leu Phe Gly Thr Gly Leu Trp Ile Ile Ala His Glu Cys Gly His Gly
145         150         155         160
Ala Phe Ser Pro Ser Thr Leu Thr Asn Asp Leu Thr Gly Trp Val Leu
165         170         175
His Ser Ala Leu Leu Val Pro Tyr Phe Ser Trp Lys Phe Ser His Ser
180         185         190
Ala His His Lys Gly Thr Gly Asn Met Glu Arg Asp Met Ala Phe Leu
195         200         205
Pro Arg Thr Arg Ala Gln Tyr Ala Thr Arg Phe Gly Arg Ala Met Asp
210         215         220
Gln Leu Gly Asp Leu Cys Glu Glu Thr Pro Ile Tyr Thr Ala Gly Phe
225         230         235         240
Leu Val Phe Gln Gln Leu Leu Gly Trp Pro Ser Tyr Leu Ile Ala Asn
245         250         255

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Val Thr Gly His Asp Leu His Glu Arg Gln Arg Glu Gly Arg Gly Lys  
 260 265 270

Gly Lys Lys Asn Gly Phe Gly Gly Thr Val Asn His Phe Asp Pro Arg  
 275 280 285

Ser Pro Ile Phe Asp Asp Lys His Ala Lys Phe Ile Val Leu Ser Asp  
 290 295 300

Ile Gly Leu Gly Leu Ala Ile Ala Ala Leu Val Tyr Leu Gly Asn Arg  
 305 310 315 320

Phe Gly Trp Ala Asn Val Ala Val Trp Tyr Phe Val Pro Tyr Leu Trp  
 325 330 335

Val Asn His Trp Ile Val Ala Ile Thr Phe Leu Gln His Thr Asp Pro  
 340 345 350

Thr Leu Pro His Tyr Thr Ala Glu Glu Trp Asn Phe Val Arg Gly Ala  
 355 360 365

Ala Ala Thr Ile Asp Arg Glu Met Gly Phe Ile Gly Arg His Leu Phe  
 370 375 380

His Gly Ile Val Glu Thr His Val Leu His His Tyr Val Ser Ser Ile  
 385 390 395 400

Pro Phe Tyr Asn Ala Asp Glu Ala Ser Glu Ala Ile Lys Pro Val Met  
 405 410 415

Gly Lys His Tyr Arg Ser Glu Thr Lys Asp Gly Pro Met Gly Phe Ile  
 420 425 430

Arg Ala Leu Trp Lys Thr Ala Arg Trp Cys Gln Trp Val Glu Pro Ser  
 435 440 445

Ala Asp Ala Gln Gly Ala Gly Glu Gly Val Leu Phe Phe Arg Asn Arg  
 450 455 460

Asn Gly Leu Gly Thr Lys Pro Ile Ser Met Arg Thr Gln  
 465 470 475

&lt;210&gt; SEQ ID NO 31

&lt;211&gt; LENGTH: 903

&lt;212&gt; TYPE: DNA

<213> ORGANISM: *Ostreococcus tauri*

&lt;400&gt; SEQUENCE: 31

```

atgtctgctt ctggagcttt gttgctgct attgctttcg ctgcttacgc ttacgctacc 60
tacgcttatg ctttcgagtg gtctcatgct aacggaatcg ataacgtgga tgctagagag 120
tggattggag ctttgccttt gagactccct gcaattgcta ccaccatgta cctcttgctc 180
tgccttgctg gacctagatt gatggctaag agggaggctt ttgatcctaa gggattcatg 240
ctcgtttaca acgcttacca aaccgcttcc aacgcttgctg tgctcggaat gttcgtetaga 300
gagatctctg gattgggaca acctgcttgg ggatctacta tgccttgagc cgataggaag 360
tccttcaaga ttttgttggg agtgtggctc cattacaaca ataagtacct cgagttgctg 420
gatactgtgt tcactgtggc taggaaaaag accaagcagc tctctttcct gcatgtgtac 480
catcatgctt tgttgatttg ggcttggttg cttgtttgtc atctcatggc taccaacgat 540
tgcacatgat cttatttcgg agctgcttgc aactctttca tccacatcgt gatgtactcc 600
tactacctca tgtctgcttt gggaaattaga tgccttgga agagatatat caccaggct 660
cagatgttgc aattcgtgat cgtgttcgct catgctgttt tctgtctcag acaaaagcac 720
tgcctgttta ctttgccttg ggcacaaatg ttcgtgatga caaatatggt ggtgctcttc 780

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ggaaacttct acctcaaggc ttactctaac aagtctaggg gagatggagc ttcttctggt      840
aagcctgctg agactactag agcaccttct gtgagaagaa ccaggtccag gaagatcgat      900
tga                                                                           903

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<210> SEQ ID NO 32
<211> LENGTH: 300
<212> TYPE: PRT
<213> ORGANISM: Ostreococcus tauri

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<400> SEQUENCE: 32

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Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Phe Ala Ala Tyr
1           5           10           15
Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Glu Trp Ser His Ala Asn Gly
20           25           30
Ile Asp Asn Val Asp Ala Arg Glu Trp Ile Gly Ala Leu Ser Leu Arg
35           40           45
Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
50           55           60
Pro Arg Leu Met Ala Lys Arg Glu Ala Phe Asp Pro Lys Gly Phe Met
65           70           75           80
Leu Ala Tyr Asn Ala Tyr Gln Thr Ala Phe Asn Val Val Val Leu Gly
85           90           95
Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gln Pro Val Trp Gly Ser
100          105          110
Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
115          120          125
Trp Leu His Tyr Asn Asn Lys Tyr Leu Glu Leu Leu Asp Thr Val Phe
130          135          140
Met Val Ala Arg Lys Lys Thr Lys Gln Leu Ser Phe Leu His Val Tyr
145          150          155          160
His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
165          170          175
Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
180          185          190
Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
195          200          205
Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gln Ala Gln Met Leu Gln
210          215          220
Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
225          230          235          240
Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
245          250          255
Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
260          265          270
Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
275          280          285
Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
290          295          300

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**1.** A polynucleotide comprising a nucleic acid sequence elected selected from the group consisting of:

- a) the nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7 or 9;
- b) a nucleic acid sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8 or 10;
- c) a nucleic acid sequence having at least 70% sequence identity to the nucleic acid sequence of a) or b), wherein said nucleic acid sequence encodes a polypeptide having desaturase or elongase activity;
- d) a nucleic acid sequence encoding a polypeptide having desaturase or elongase activity and comprising an amino acid sequence which has at least 82% sequence identity to an amino acid sequence encoded by any one of the nucleic acid sequences of a) to c); and
- e) a nucleic acid sequence which is capable of hybridizing under stringent conditions to any one of the nucleic acid sequences of a) to d), wherein said nucleic acid sequence encodes a polypeptide having desaturase or elongase activity.

**2.** The polynucleotide of claim **1**, wherein the polynucleotide further comprises an expression control sequence operatively linked to the nucleic acid sequence.

**3.** The polynucleotide of claim **1**, wherein the polynucleotide further comprises a terminator sequence operatively linked to the nucleic acid sequence.

**4.** A vector comprising the polynucleotide of claim **1**.

**5.** A host cell comprising the polynucleotide of claim **1** or a vector comprising said polynucleotide.

**6.** A method for the manufacture of a polypeptide encoded by the polynucleotide of claim **1** comprising:

- a) cultivating a host cell comprising said polynucleotide or a vector comprising said polynucleotide under conditions which allow for the production of said polypeptide; and

b) obtaining the polypeptide from the host cell of step a).

**7.** A polypeptide encoded by the polynucleotide of claim **1**.

**8.** A non-human transgenic organism comprising the polynucleotide of claim **1** or a vector comprising said polynucleotide.

**9.** The non-human transgenic organism of claim **8**, which is a plant, plant part, or plant seed.

**10.** A method for the manufacture of a polyunsaturated fatty acid comprising:

- a) cultivating the host cell of claim **5** under conditions which allow for the production of a polyunsaturated fatty acid in said host cell; and
- b) obtaining said polyunsaturated fatty acid from said host cell.

**11.** A method for the manufacture of a polyunsaturated fatty acids acid comprising:

- a) cultivating the non-human transgenic organism of claim **8** under conditions which allow for the production of a polyunsaturated fatty acid in said non-human transgenic organism; and
- b) obtaining said polyunsaturated fatty acid from said non-human transgenic organism.

**12.** The method of claim **10**, wherein said polyunsaturated fatty acid is arachidonic acid (ARA), eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA).

**13.** A method for the manufacture of an oil, lipid, or fatty acid composition comprising:

- a) cultivating the host cell of claim **5** under conditions which allow for the production of a polyunsaturated fatty acid in said host cell;
- b) obtaining said polyunsaturated fatty acid from said host cell; and
- c) formulating the polyunsaturated fatty acid as an oil, lipid, or fatty acid composition.

**14.** The method of claim **13**, wherein the oil, lipid, or fatty acid composition is used for feed, foodstuffs, cosmetics, or medicaments.

**15.** An antibody or a fragment thereof which specifically recognizes the polypeptide of claim **7**.

**16.** A polypeptide obtained by the method of claim **6**.

\* \* \* \* \*