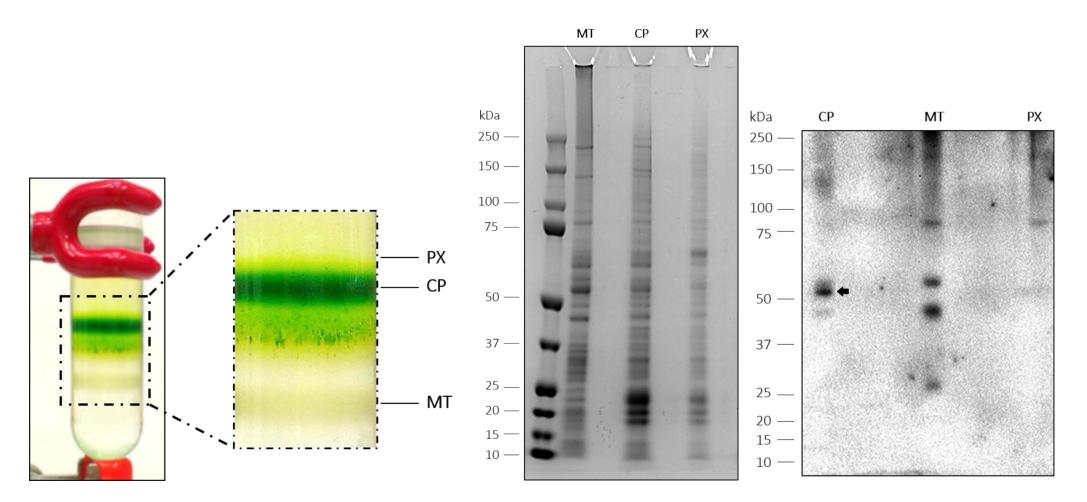
Supplementary data

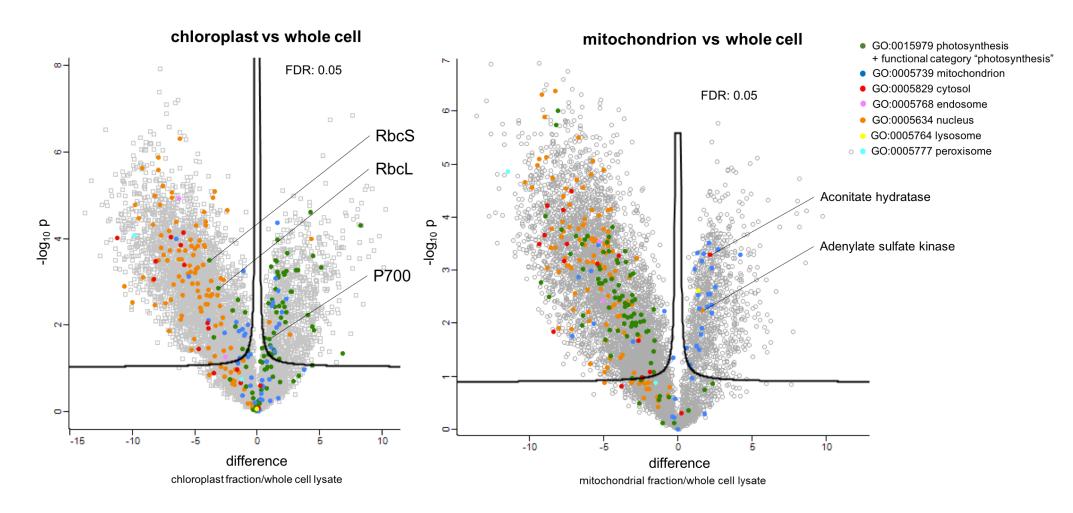


S1.1: The result of cell fractionation on a sucrose gradient: the plastidial fraction is marked as CP, while the mitochondrial and peroxisomal fractions are marked MT and PX, respectively. S1.2: Coomasie-stained gel (Nupage Bis-Tris Mini Gels 4-12%, IM-8042, Life Technologies) of the cellular fractions with Precision Plus Protein[™] Dual Color Standards (#1610374, Bio-Rad). S1.3: Immunoblot of the cellular fractions with rabbit anti-RbcL (1:5000, AS03 037, Agrisera, protein size: 52 kDa) as primary antibody and goat anti-Rabbit (1:2000, A6154-5x, Sigma) as secondary antibody, and Protein Ladder (161-0374, Biorad), the positive band of expected size is marked by arrow.

	Cytosol	Acidocalcisome	Endosome	Peroxisome	Lysosome	Golgi	Nucleus	Surface
Protein ID EG_transcript_	21524	2633	181	15991	10514	5712	53416	32527
Annotation	Aldolase	VP1 Vacuolar proton translocating pyrophosphatase	CHC Clathrin heavy chain	PEX2 Peroxisomal biogenesis factor 2	Lysosomal aspartic protease	Coatomer subunit γ2	Histone H4	Articulin 80 kDa
Ratio CP/W	0.01	1.95	0.21	W only	3.30	0.01	0.04	0.01
-log10p	5.9	1.2	3.1	1.4	2.0	3.4	3.3	4.7
Ratio Mt/W	0.02	1.46	0.53	W only	7.4	0.01	0.02	0.04
-log10p	3.3	0.5	1.9	2.2	2.6	3.3	3.6	4.4
Ratio CP/Mt	0.26	1.33	0.40	NaN	0.44	0.74	2.0	0.15
-log10p	1.5	0.6	2.8	NaN	1.5	0.3	0.8	2.9
Unique peptides	19	31	90	1	7	40	20	13

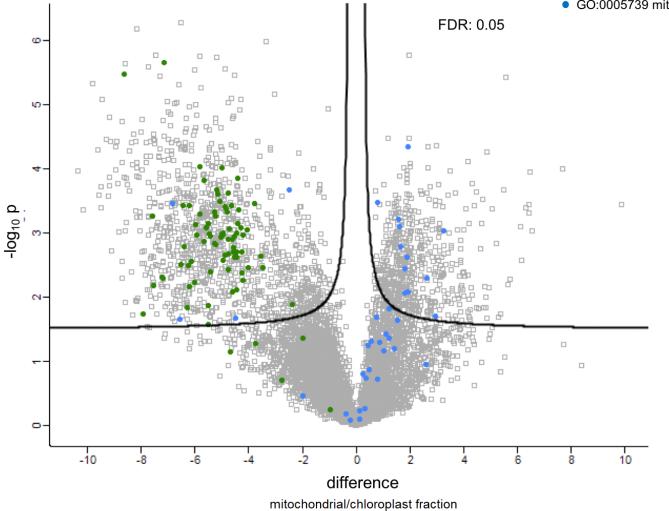
		Chloroplast			Mitochondrion	
Protein ID EG_transcript_	40006	158	25897	2112	23844	8912
Annotation	light-harvesting complex I protein precursor LhcB5	Photosystem I P700 chlorophyll <u>a</u> apoprotein A1	light-harvesting complex I protein precursor Lhca2	Pyruvate dehydrogenase [<u>NADP(</u> +)], mitochondrial	ubiquinol-cytochrome c reductase iron-sulfur subunit	F-type H+-transporting ATPase subunit beta
Ratio CP/W	4.5	1.95	CP only	1.31	3.10	4.6
-log10p	3.2	1.7	1.9	0.6	4.4	3.8
Ratio Mt/W	0.2	0.10	NaN	4.7	4.27	4.8
-log10p	1.8	2.3	NaN	2.6	3.5	3.4
Ratio CP/Mt	24.2	20.5	CP only	0.28	0.73	0.95
-log10p	2.7	2.8	2.2	2.1	1.3	0.1
Unique peptides	4	30	2	76	35	32

S2.1: Assessment of the purity of isolated fractions using selected marker proteins and their relative abundance compared against the whole cell lysate and between the organellar fractions.



S2.2: Volcano plots from p-values versus the corresponding t-test difference of 8216 protein groups quantified in the two organellar fractions and whole cell lysate. Green and blue dots represent proteins assigned to "photosynthetic" and "mitochondrial" GO categories, respectively. The remaining colours represent other selected GO categories (indicated at the top right) associated with other cellular compartments. Stringent cutoff curves for statistically significant enrichment (black curves) were calculated from the estimated false discovery rate (FDR).

mitochondrion vs chloroplast



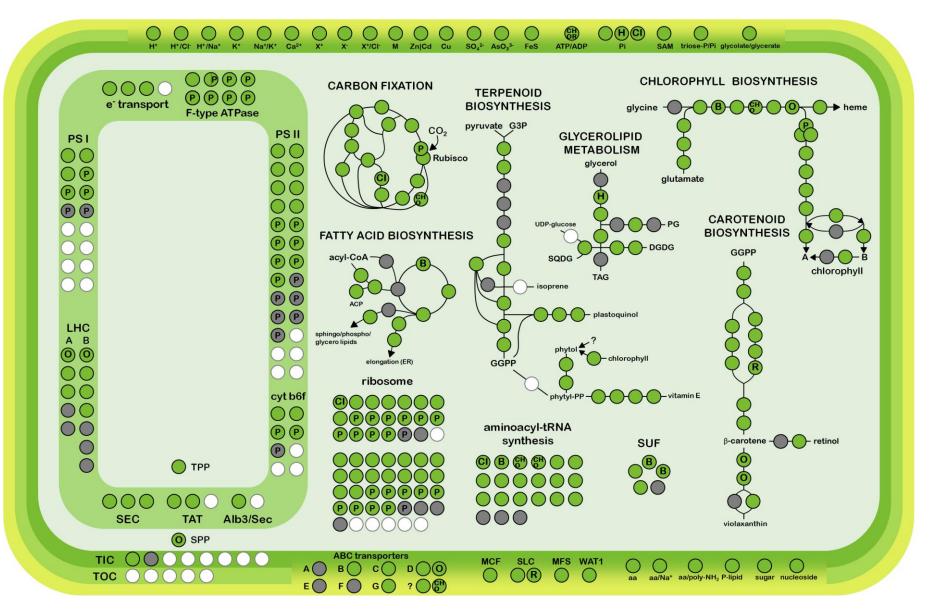
GO:0015979 photosynthesis
 + functional category "photosynthesis"

• GO:0005739 mitochondrion

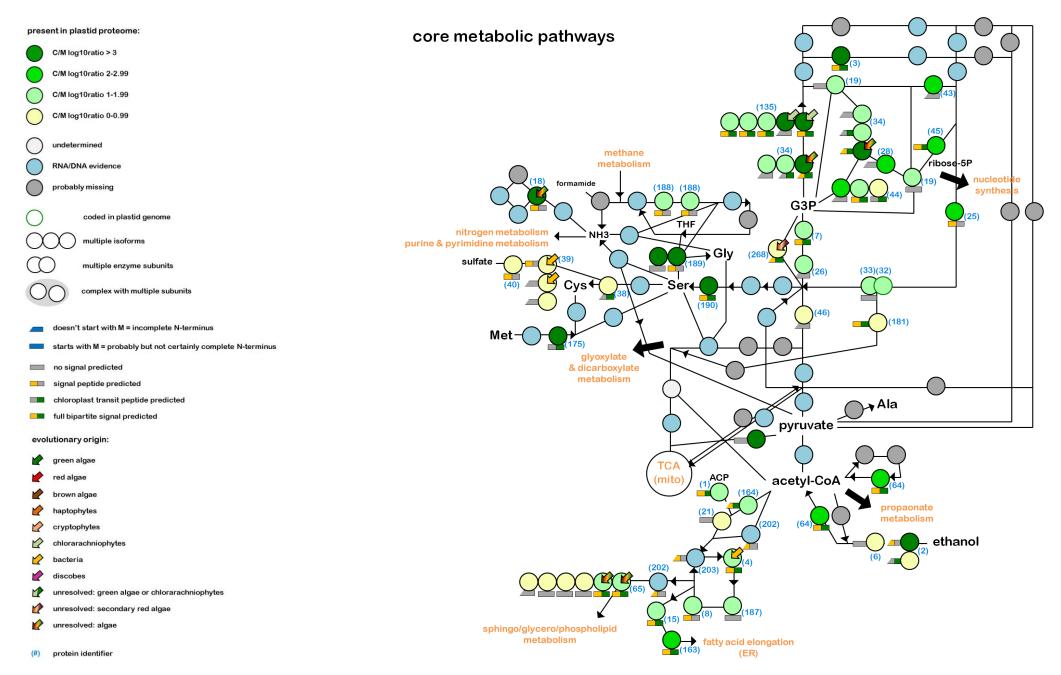
S2.3: Volcano plot from p-values versus the corresponding t-test difference of 3,736 protein groups quantified in the mitochondrial and chloroplast fraction. Green and blue dots represent proteins assigned to "photosynthetic" and "mitochondrial" GO categories, respectively. Stringent cutoff curves for statistically significant enrichment (black curves) were calculated from the estimated false discovery rate (FDR).

custom category name	description
protein transport, folding, processing, and degradation	protein translocases of plastid envelope and thylakoid membranes, signal peptidases, heat shock proteins, enzymes of post-translational protein modifications such as methylation, acetylation, glycosylation, proline cis/trans isomerisation, disulfide bond formation and breakage, and components of protein degradation systems
metabolite and ion transport	proteins involved in transport of all non-protein compounds, including predicted membrane transporters of undetermined substrates
photosynthesis	components of photosystems, light-harvesting antennae, cytochrome b6/f complex and proteins involved in their biogenesis
ribosome, aminoacyl-tRNA biosynthesis and translation	ribosomal proteins and proteins involved in ribosome biogenesis, aminoacyl-tRNA synthetases, translation regulators
regulation and signal transduction	proteins involved in other than transcriptional and translational regulation, signaling molecules and their receptors, not-further-specified protein kinases, phosphatases, adenylate
	cyclases and similar enzymes typically involved in signal transduction
metabolism of cofactors and vitamins	mostly proteins involved in chlorophyll biosynthesis, several enzymes of metabolism of ubiquinone and retinol
lipid metabolism	enzymes of fatty acid biosynthesis, elongation, modification and degradation, synthesis of glycerolipids and glycerophospholipids
core metabolic pathways	proteins of glycolysis, pentose phosphate pathway, pyruvate metabolism, carbon fixation, one-carbon and acetyl-CoA metabolism
oxidative phosphorylation and electron transport	components of ATP synthase and electron transport chain
transcription and transcription regulation	transcription and translation factors and other proteins involved in gene expression regulation
RNA processing and degradation	RNAses and other enzymes responsible for RNA splicing, maturation and degradation
metabolism of terpenoids and polyketides	proteins involved in biosynthesis of terpenoids and carotenoids
DNA replication, recombination and repair	DNA polymerases, ligases, helicases, proteins involved in DNA maintenance and repair
reaction to oxidative and toxic stress	enzymes involved in detoxification of xenobiotics and protection from reactive oxygen species and photo oxidative damage, proteins responsible for redox balance
amino acid metabolism	proteins involved in amino acid synthesis and interconversions, enzymes of shikimate pathway
FeS cluster assembly and sulfur metabolism	components of SUF pathway, proteins involved in metabolism of sulfur compounds
carbohydrate metabolism	enzymes of starch and other saccharides metabolism
other	members of other pathways which were minor in comparison to other functions

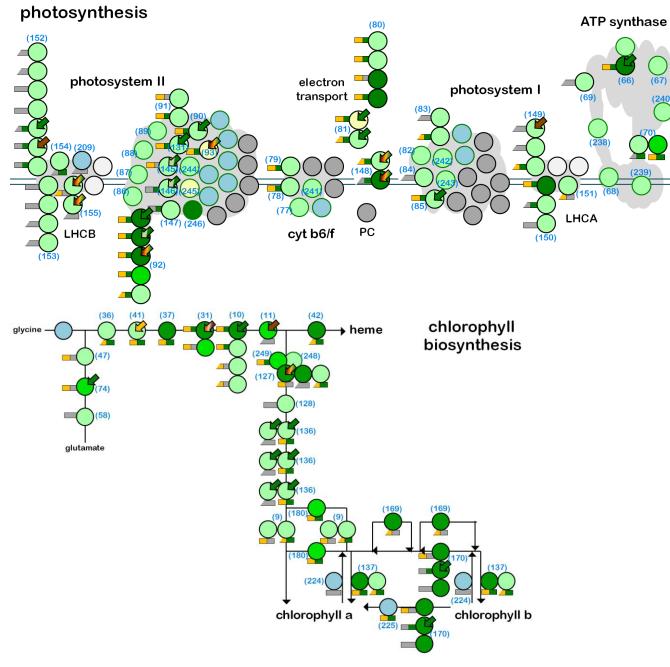
S3: List of the custom protein categories with their descriptions and examples.



S4: Overview of the *E. gracilis* chloroplast metabolism as reconstructed from mass spectrometry-based proteome: Enzymes present in the plastid proteome in at least one isoform are marked as green circles, grey circles represent enzymes which were identified on the RNA or DNA level (in this study or previously) but are absent from the proteome; white circles represent genes completely absent in Euglena; circles marked by the letter "P" represent genes coded in the plastid genome while rest of the circles represent genes coded in the nucleus; circles marked by other letters represent genes with at least one of their isoforms gained via lateral transfer from one of the following donor groups: "Ch" for chlorarachniophytes, "Cr" for cryptophytes, "H" for haptophytes, "O" for ochrophytes, "R" for rhodophytes, "CHO" for unresolved secondary algae (cryptophytes, haptophytes or ochrophytes), CHOR for unresolved primary or secondary red algae (cryptophytes, haptophytes, ochrophytes, ochrophytes), and "B" for bacteria. Genes related to green algae and discobes are not marked, as well as genes with either "green or chlorarachniophyte" or completely unresolved algal origin. Multiple overlapping circles represent multiple subunits of certain enzymes.

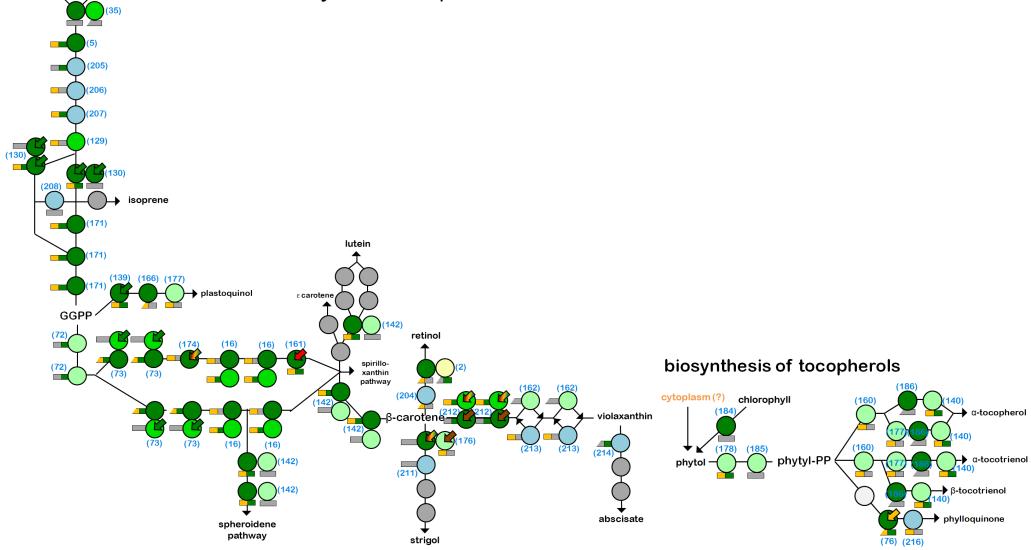


S5.0: Legend for following (S5.1-11) detailed metabolic maps; proteins used in these maps can be searched in supplemental table **supplementary-dataset-1.xlsx** by the identifier in brackets; the identifier is also specified for some proteins with mere DNA/RNA-level evidence which could, however, localize to plastid and fill the gaps in the otherwise plastidial pathways or complexes. S5.1: Metabolic map of *E. gracilis* chloroplast core metabolic pathways

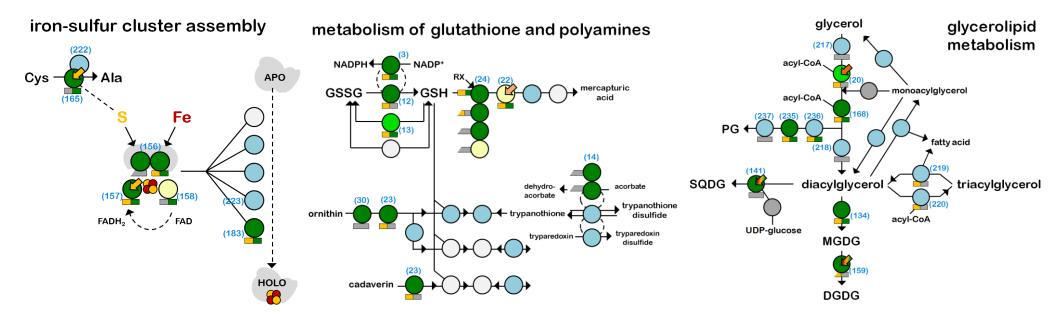


pyruvate + glyceraldehyde-3P

biosynthesis of terpenoids and carotenoids



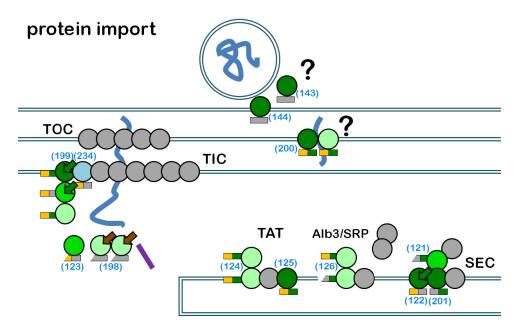
S5.4: Metabolic map of *E. gracilis* chloroplast terpenoid and carotenoid biosynthesis S5.5: Metabolic map of *E. gracilis* chloroplast tocopherol biosynthesis



S5.6: Metabolic map of *E. gracilis* chloroplast SUF system

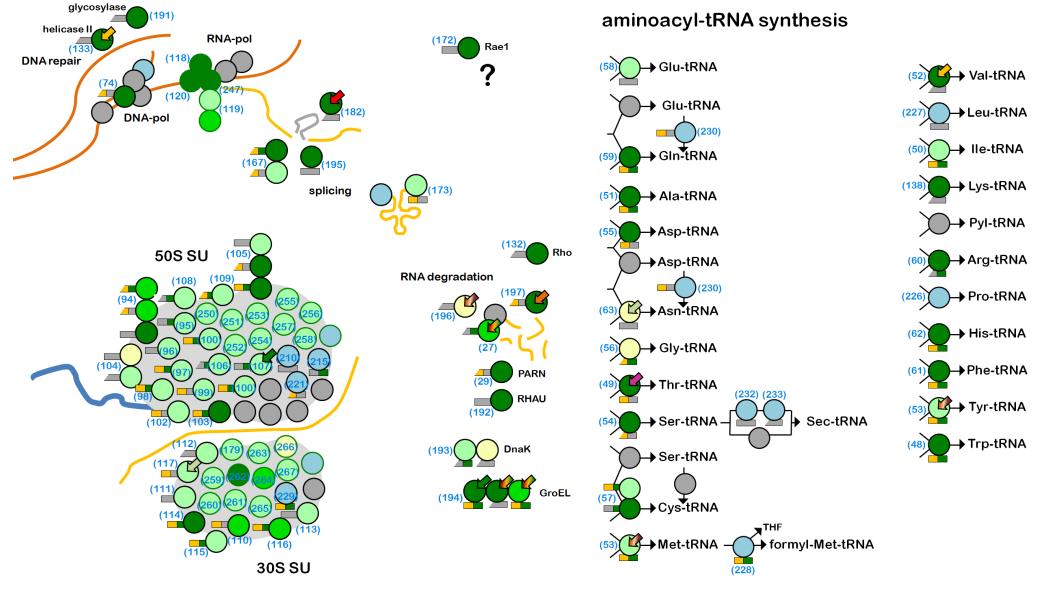
S5.7: Metabolic map of *E. gracilis* chloroplast part of metabolism of glutathione and polyamines

S5.8: Metabolic map of E. gracilis chloroplast metabolism of glycerolipids



S5.9: Map of *E. gracilis* chloroplast protein importing machinery

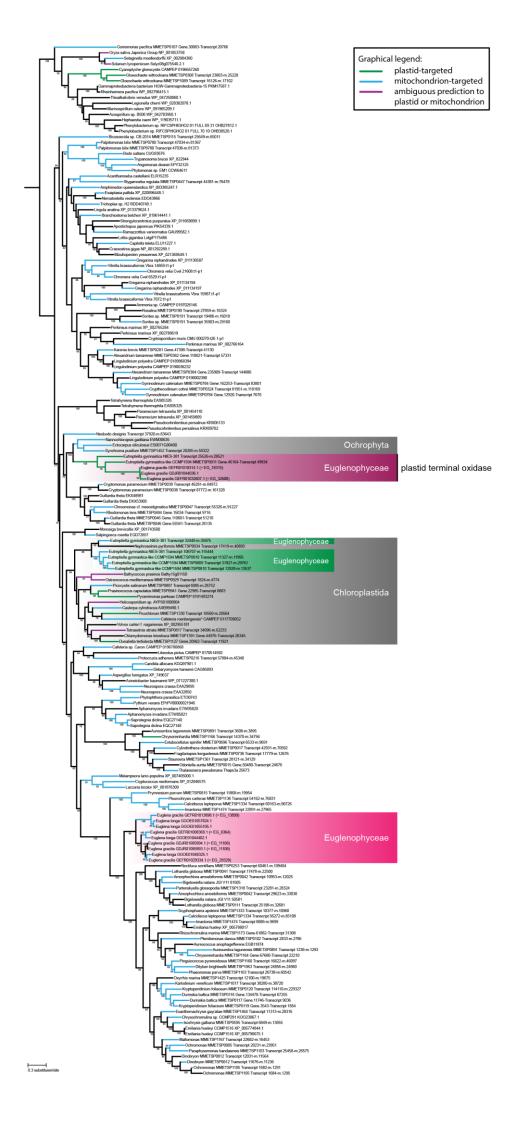
genetic information processing



S5.10: Map of *E. gracilis* chloroplast transcription, translation, RNA processing and degradation S5.11: Map of *E. gracilis* chloroplast aminoacyl-tRNA synthesis

S6: Phylogenetic trees showing positions of the two plastid terminal oxidases (PTOX) identified in transcriptomic data of the three euglenophytes. While the PTOX1 position suggests conventional enzyme inherited from algae, the PTOX2 falls among mitochondrial alternative oxidases.

	Prasinococcus capsulatus MMETSP0941 Gene, 14464-Transcript 5625			
	Anabaena variabilis WP_011317903.1	1		
	Picocystis salinarum MMETSP0807 Transcript 7364-m .32407 Nephroselmis pyriformis MMETSP0034 Transcript 11710-m.27171		Graphical legend:	
	91 Selaginella moellendorffil XP_002980057		plastid-ta	ngeted adrion-targeted
100	100 Physcomitrella patens XP_001766430 100 Physcomitrella patens XP_001774094			us prediction to
	100 Amborella trichopoda ERM96920 Oryza sativa Japonica Group NP .001054199			mitochondrion
	Arabidopsis thaliana Q56X52			
	Solanum lycopersicum Solyc11g011990.1.1			
	Bathycoccus prasinos Bathy13g01840			
	Ostreococcus mediterraneus MMETSP0938 Gene.12006-Transcript 5069 Micromonas pusilla wiab 210149.1			
	100 Micromonas pusilla MMETSP1327 Transcript 11897-m.29280			
	99 Micromonas pusila MMETSP1401 Transcript 1177-m.3975 Chroomonas cf. mesostigmatica MMETSP0047 Transcript 11731-m.19334			
	100 Eutreptiella gymnastica-like CCMP1594 MMETSP0811-Transcript 42304	Euglenophyc	eae	Cryptophyceae
	Ohroomonas cf. mesostigmatica MMETSP0047 Transcript 58076-m.96029 79 Rhodomonas lens MMETSP0484 Gene.15146-Transcript 9762			cryptopriyceae
	pe Dinobryon MMETSP0019 Transcript 20540-m.20891			
	91 Nannochloropsis gaditana EWM22362 81 Vaucheria litorea MMETSP0946 Transcript 14210-m. 13258			
	Rhizochromulina marina MMETSP1173 Gene.35700-Transcript 17933			
	Thalassiosira pseudonana Thapa3a 24800 100 Thalassionema nitzschioides MMETSP0156 Transcript 13882-m.14595			
	Extubocellulus spinifer MMETSP0696 Transcript 12962-m.19076 Bit Ditylum brightwellii MMETSP1012 Transcript 43663-m.43865			
	Odontella aurita MMETSP0015 Gene.123905-Transcript 59410			
	Galdieria sulphuraria XP_005704943 Gloeochaete wittrockiana MMETSP0308 Transcript 31619-m.32940			
	Pyramimonas parkeae MMETSP0059 Transcript 39038-m.48989			
	100 - Tetraselmis astigmatica MMETSP0804 Transcript 10985-m.21022			
	97 Cyanidioschyzon merolae XP_005536397			
	Cyanidioschyzon merolae XP_005536398 Porphyridium aerugineum MMETSP0313 Transcript 6895-m.8178			
	The Chondrus crispus XP_005712073			
	100 Chondrus crispus XP_005712075 S7 Compsopogon caeruleus MMETSP0312 Transcript 6098-m.7534			
	99 Madagascaria erythrocladioides MMETSP1450 Gene.74747-Transcript 43690			
	Dunallella salina AFR58666.1			
	Raphidocells subcapitata GBF99932.1			
	Chlamydomonas leiostraca MMETSP1391 Gene.56058-Transcript 32956			
	Volvox carteri f. nagariensis XP_002950383			
	Chlamydomonas reinhardtii XP 001692600.1			
	Alexandrium tamarense MMETSP0384 Gene.6340-Transcript 3976			
	100 Dinophysis acuminata CAMPEP 0179293410 100 Eutreptiella gymnastica-like CCMP1594 MMETSP0811-Transcript 65428			
	100 Euglena gracilis GEFR01002890.1 (= EG_2887) 100 Eutreptiella gymnastica NIES-381 Transcript 18229-m.19913			
	Lingulodinium polyedra CAMPEP 0190052922			Dinophyceae
	100 Noctiluca scintillans MMETSP0253 Transcript 58889-m.106740 Alexandrium tamarense MMETSP0382 Gene.14965-Transcript 7601			
	77 Gymnodinium catenatum MMETSP0784 Gene.22514-Transcript 12092			
	Durinskia baltica CAMPEP 0199928928 94 Kryptoperidinium foliaceum MMETSP0120 Transcript 25553-m.51159			
	Chromera vella Cvel 13636.t1-p1			
	100 Chromera vella Cvel 25704.11-p1 Dinobryon MMETSP0019 Transcript 32125-m.32722			
	Ochromonas MMETSP1105 Transcript 20589-m.16365			
	100 Aureoumbra lagunensis MMETSP0891 Transcript 17888-m.19126 Extubocellulus spinifer MMETSP0696 Transcript 21791-m.32352			
	98 Chrysoreinhardia MMETSP1164 Gene.10196-Transcript 3483			
	Calcidiscus leptoporus MMETSP1334 Transcript 45626-m.70558			
	Thalassionema nitzschloides MMETSP0156 Transcript 5034-m.5178 Imantonia MMETSP1474 Transcript 20743-m.25332			
	Pelagomonas calceolata MMETSP0889 Transcript 12193-m.26265			
	Isochrysis galbana MMETSP0595 Transcript 18076-m.36226 Emiliania huxleyi XP_005787845			
	Tetraselmis astigmatica MMETSP0804 Transcript 139-m.361			
	Picochlorum MMETSP1330 Transcript 1454-m.3364 Pyramimonas parkeae MMETSP0059 Transcript 16086-m.20792			
	ez Polytomelia parva MMETSP0052 Gene.39612-Transcript 31591			
	Volvox carteri f. nagariensis XP_002946776 Exanthemachrysis gayraliae MMETSP1464 Transcript 19562-m.48089			
	Ectocarpus siliculosus ES0003G01770			
	Vitrella brassicaformis Vbra 14000.t1-p1			
	Chattonella subsalsa MMETSP0948 Transcript 25090-m.22083			
	Historic GAQ78184.1			
	Selaginella moellendorffi XP_002973024 Synchroma pusillum MMETSP1452 Transcript 4052-m.10048			
	Chroomonas cf. mesostigmatica MMETSP0047 Transcript 66155-m. 109735			
	100 Guillardia theta MMETSP0046 Gene.8355-Transcript 3505 100 Rhodomonas lens MMETSP0484 Gene.39599-Transcript 24832			
	Bigelowiella natans JGI V11 47352			
	Lotharella globosa MMETSP0041 Transcript 32648-m.42224 Tetraselmis astigmatica MMETSP0804 Transcript 21450-m.40177			
	No Tetraselmis striata MMETSP0818 Transcript 32546-m.58179			
	Bolidomonas pacifica MMETSP1319 Transcript 25648-m.36154 Nitzschia MMETSP0014 Gene.50872-Transcript 31532			
	Detonula confervacea MMETSP1058 Transcript 9695-m.14644			
	 Thalassiosira pseudonana Thaps3a 25462 Control aurita MMETSP0015 Gene.43329-Transcript 21236 			
	Fragilariopsis kerguelensis MMETSP0906 Transcript 17950-m.12564			
	 100 Staurosira MMETSP1361 Transcript 22641-m.27626 60 Chlamydomonas eustigma GAX73707.1 			
	Pleurochrysis carterae MMETSP1138 Gene.30303-Transcript 22371			
	Prymnesium parvum CAMPEP 0191232478 Dinophysis acuminata CAMPEP 0179312104			
	100 Karenia brevis MMETSP0649 Gene.60098-Transcript 51563			
	Aureococcus anophagefferens MMETSP0915 Gene.26744-Transcript 11301			
	Chrysoreinhardia MMETSP1166 Transcript 26709-m.64124			
	Phaeomonas parva MMETSP1163 Transcript 20141-m.43994 Pinguiococcus pyrenoidosus MMETSP1160 Transcript 2076-m.6733			
	Mallomonas MMETSP1167 Transcript 18747-m.13573			
	100 Dinobryon MMETSP0812 Transcript 14276-m.13669			
	Cchromonas MMETSP0004 Transcript 21512-m.24816			
	Phaeccystis MMETSP1444 Gene.46352-Transcript 29576 Pyramimonas parkeae MMETSP0059 Transcript 46982-m.58381			
	Ostreococcus mediterraneus MMETSP0930 Transcript 7556-m. 11460			
	Micromonas pusilla MMETSP1327 Transcript 11315-m.27692			
	⁹² Micromonas pusilia MMETSP1401 Transcript 5628-m. 19028 100 Micromonas pusilia MMETSP1402 Transcript 2975-m.7207			
0.1 substitution	/site 100 Micromonas pusilla MMETSP1403 Transcript 2776-m.8754			
	¹⁰⁰ LMicromonas pusilia MMETSP1404 Transcript 10006-m.29757			



S7: Overview of SUF subunits identified in transcriptomes of *Euglena gracilis*, *Euglena longa* and *Eutreptiella gymnastica* with their sources and accession numbers and proteins corresponding to the *E. gracilis* transcripts with their respective log10 CP/MT ratios representing the credibility of plastidal localization. In case of SufB1, SufE1 and SufS1, the protein was captured in one replicate of mass spectrometry analysis of the plastidal fraction only, suggesting the protein is of lower abundance but plastid-localized. In case of SufD1 and SufS2, the protein was not captured by mass spectrometry and its localization was inferred based on the N-terminal signals and/or localizations of their putative interaction partners.

pathway	annotation	E. gracilis protein ID	log10 CP/MT	E. gracilis (GenBank)	Eut. gymnastica NIES-381 (MMETSP)	Eut. gymnastica-like CCMP1594 (MMETSP)	E. longa (Záhonová et Füssy et al. 2018)
	SufB1	6397	>0, one replicate only	GDJR01089002	CAMNT_0000679585 + reads	CAMNT_0046511287	Contig18694 + Contig38168 + PCR
SUF1	SufC1	13141	3+	GDJR01041264	CAMNT_0000683911	CAMNT_0046510015	Contig4493 + SL-PCR
	SufD1	6034, 33281	not captured by MS	GDJR01038170	MMETSP0039-Transcript_4862	CAMNT_0046444101	Contig15035
	SufE1	23255	>0, one replicate only	GDJR01007756	MMETSP0039-Transcript_116548	CAMNT_0046518931	Contig14296
	SufS1	12732	>0, one replicate only	GDJR01047871	CAMNT_0000708793 corrected + reads	CAMNT_0046452723	Contig3452
	SufB2	8044	3+	GEFR01008046	-	-	Contig15117
	SufC2	24338	3+	GDJR01028245	-	-	Contig63589
SUF2	SufD2	13381	0,750	GDJR01049034	MMETSP0039-Transcript_74532	MMETSP0809-Transcript_58667 + MMETSP0810-Transcript_89787 + MMETSP0811-Transcript_48154 + MMETSP0811-Transcript_48153 + MMETSP0811-Transcript_48158 + reads	Contig36957
	SufE2	32911	not captured by MS	GDJR01012039	MMETSP0039-Transcript_109175	-	Contig52519 + reads
	SufS2	9032	3+	GDJR01072295	MMETSP0039-Transcript_75235	CAMNT_0046485773 + CAMNT_0046488253 + CAMNT_0046492131 + reads	Contig31 + Contig43468

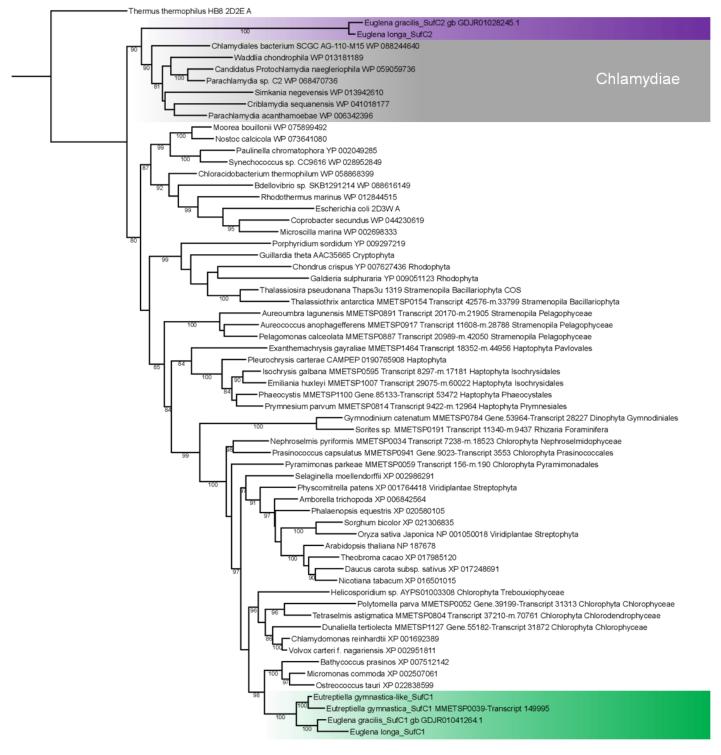
S8: Phylogenetic trees showing positions of SUF subunits identified in transcriptomic data of the three euglenophytes; the algaerelated genes are highlighted in green while the genes of prokaryotic origin are highlighted in purple.

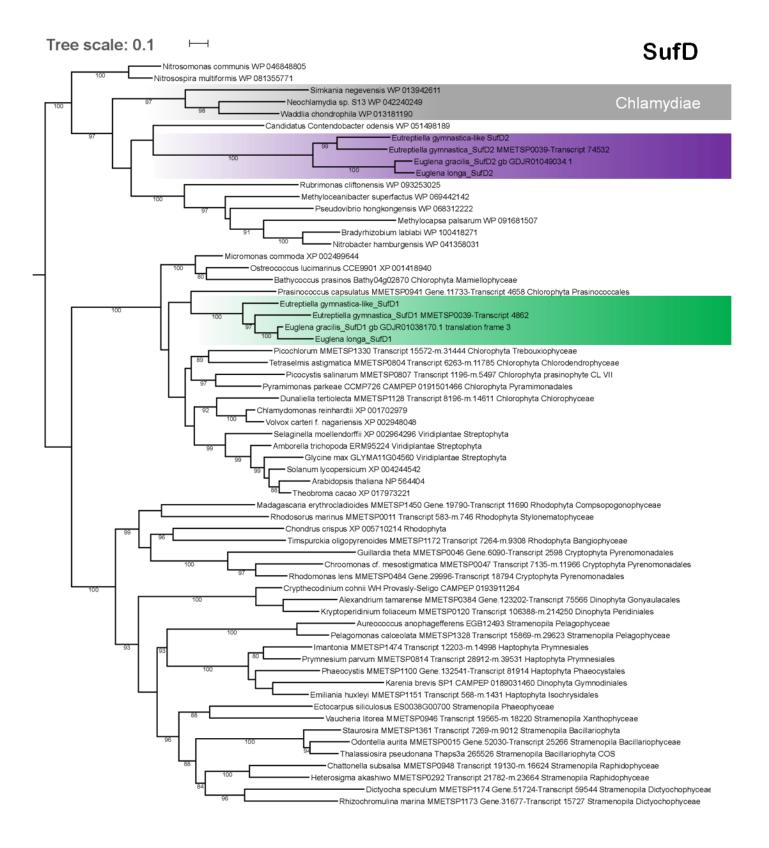
Tree scale: 0.1	SufB
Chloracidobacterium thermophilum WP 058868398	
Escherichia coli K-12 5AWF A 100 Raineya orbicola WP 101358564	
100 Ignavibacterium album WP 014561735	
81 Melioribacter roseus WP 041356184 Chromera velia Cvel 4495.t1-p1 Apicomplexa	
89 Euglena gracilis_SufB2	
100 Euglena longa_SufB2 93 Chlamydiales bacterium SCGC AG-110-M15 WP 088244641	
99 Simkania negevensis WP 013942609	
100 Waddia chondrophila WP 013181188 Estrella lausannensis WP 098037251	Chlamydiae
99 Parachlamydiaceae bacterium HS-T3 WP 042105548	Chlamydiae
⁹⁸ Neochlamydia sp. TUME1 WP 039386721	
100 Parachlamydia sp. C2 WP 068470738	
Oxyrrhis marina MMETSP0470 Gene.36950-Transcript % Sarlodinium veneficum MMETSP1017 Transcript 46880-m.47630 Dinophyta G	
99 Gymnodinium catenatum MMETSP0784 Gene.91075-Transcript 47033 Dinophyta Gy	
Alexandrium tamarense MMETSP0384 Gene.2316-Transcript 1123 Dinophyta 88 Kryptoperidinium foliaceum MMETSP0120 Transcript 133317-m.2685	,
Lingulodinium polyedra CCMP 1738 CAMPEP 0190068322	o Diriophyta i endiniales
Aureococcus anophagefferens YP 003002043 Stramenopila Pelagophyceae	
Guillardia theta AAC35664 Cryptophyta	Stylonematophyceae
100 82 Chondrus crispus YP 007627437 Rhodophyta	
Galdieria sulphuraria XP 005705039 Rhodophyta	Jophyta Bangiophyceae
Thalassiosira pseudonana Thaps3u 1360 Stramenopila Bacillariophyta COS	
Thalassionema nitzschioides MMETSP0158 Transcript 19552-m.2118 Ectocarpus siliculosus ESCG00290 Stramenopila Phaeophyceae	J Stramenopila Bacillariophyta
Heterosigma akashiwo MMETSP0416 Transcript 32157-m.30803 Stramenopila F	
Nannochloropsis gaditana B31 AFZ64224 Stramer Pseudanabaena sp. PCC 6802 WP 019502103	nopila Eustigmatophyceae
99 Synechococcus sp. PCC 7502 WP 015169618	
Cyanobacterium aponinum WP 015220402	
95 Phormidium ambiguum WP 073591789	
Big Gloeocapsa sp. PCC 7428 WP 015190665	
90 Synechocystis sp. PCC 7509 WP 009632056	
Calothrix sp. NIES-2098 WP 096593907 93 — Anabaena cylindrica WP 015213829	
⁸⁷ —Nostoc sp. PCC 7524 WP 015140225	
Eutreptiella gymnastica-like_SufB1	
100 Euglena gracilis_SufB1 gb GDJR01089002.1	
Euglena longa_SufB1 Nephroselmis pyriformis MMETSP0034 Transcript 40481-m.93858 Chlorophyta Nephroselmidophyc	eae
100 Amborella trichopoda ERN16060 Viridiplantae Streptophyta	
Physcomitrella patens XP 001751223 Selaginella moellendorffii XP 002973895 Viridiplantae Strep	tophyta
95 100 Oryza sativa Japonica NP 001044693 Viridi	
99 Solanum lycopersicum Solyc02g080440.2.1 Viridiplantae Strep 100 Arabidopsis thaliana Q9ZS97 Viridiplantae Streptoph	
95Glycine max GLYMA13G23260 Viridiplantae Streptophy	
Pyramimonas parkeae MMETSP0059 Transcript 36570-m. 46005 Chlorophyta Pyramimonae 88 Tetraselmis astigmatica MMETSP0804 Transcript 8632-m. 16836 Chlorophyta Chlor	
100 Tetraselmis striata MMETSP0819 Transcript 3437-m.6115 Chlorophyta Chlorodendrog	
Picocystis salinarum MMETSP0807 Transcript 10830-m.47668 Chlorophyta prasinophyte CL V Micromonas pusilla CCMP1545 XP 003057567	il .
Bathycoccus prasinos XP 007513604	
Ostreococcus tauri XP 022840887 Coccomyxa subellipsoidea C-169 XP 005647845	
95 Chlorella variabilis XP 005852172	
90 Auxenochlorella protothecoides XP 011401414 99 grnHELIs AYPS01000064 Chlorophyta Trebouxioph	vceae
Polytomella parva CAMPEP 0175054296 Chlorophyta Ch	,
94 Dunaliella tertiolecta MMETSP1128 Transcript 18800-m.35432 Chloro 97 Volvox carteri f. nagariensis XP 002952498	phyta Chlorophyceae
Chlamydomonas leiostraca MMETSP1391 Gene.56611-Transcript 33362	Chlorophyta Chlorophyceae

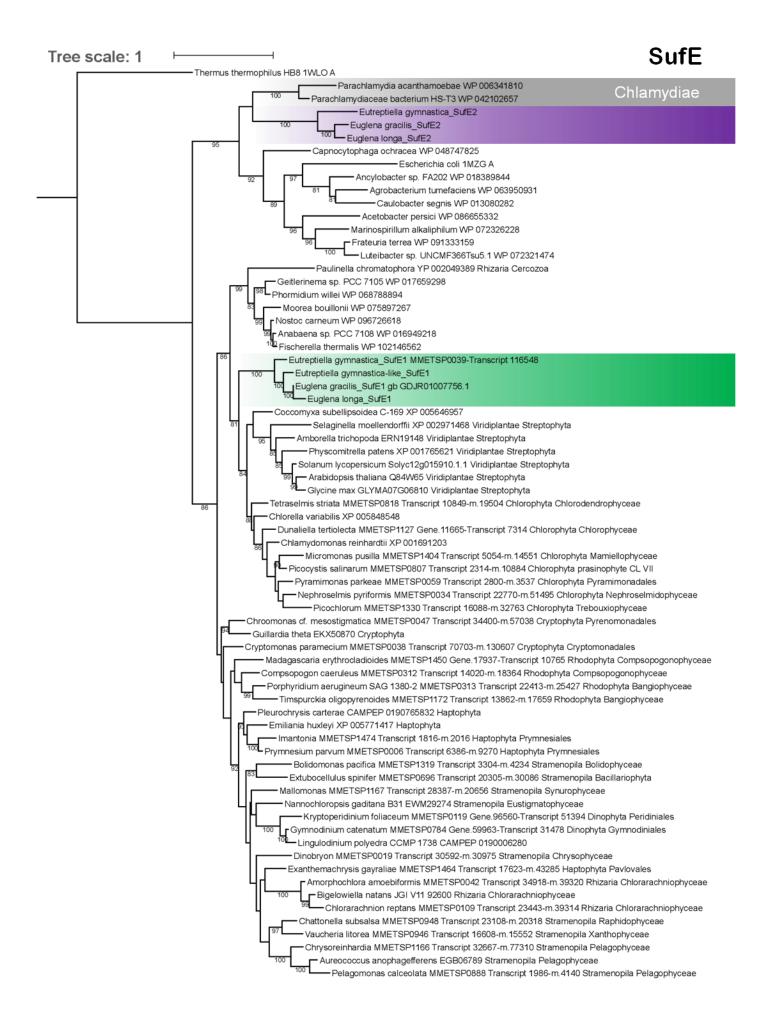
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Tree scale: 0.1 ⊢

SufC

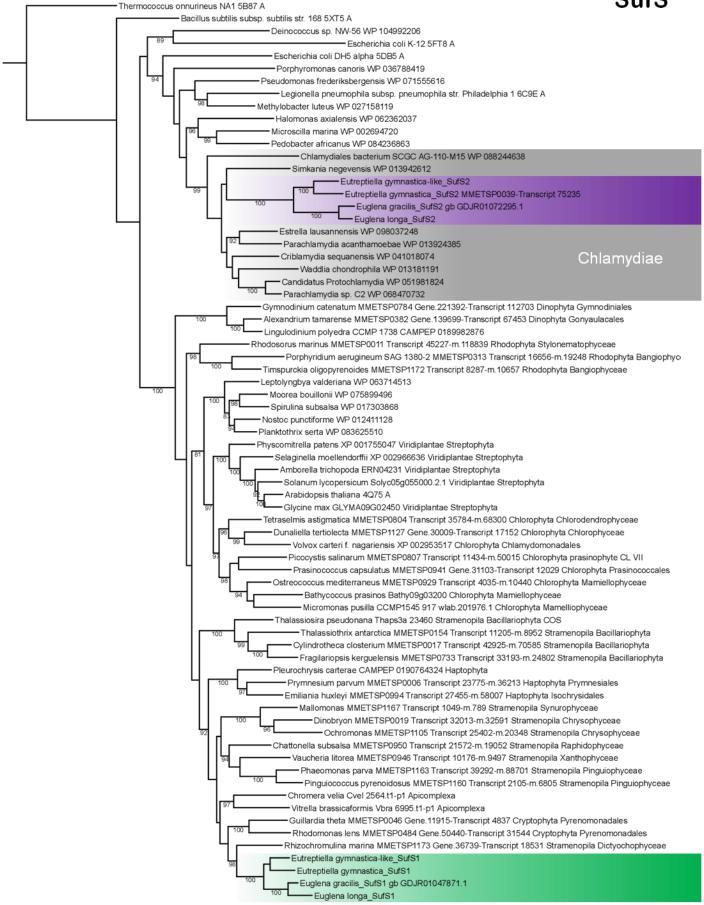


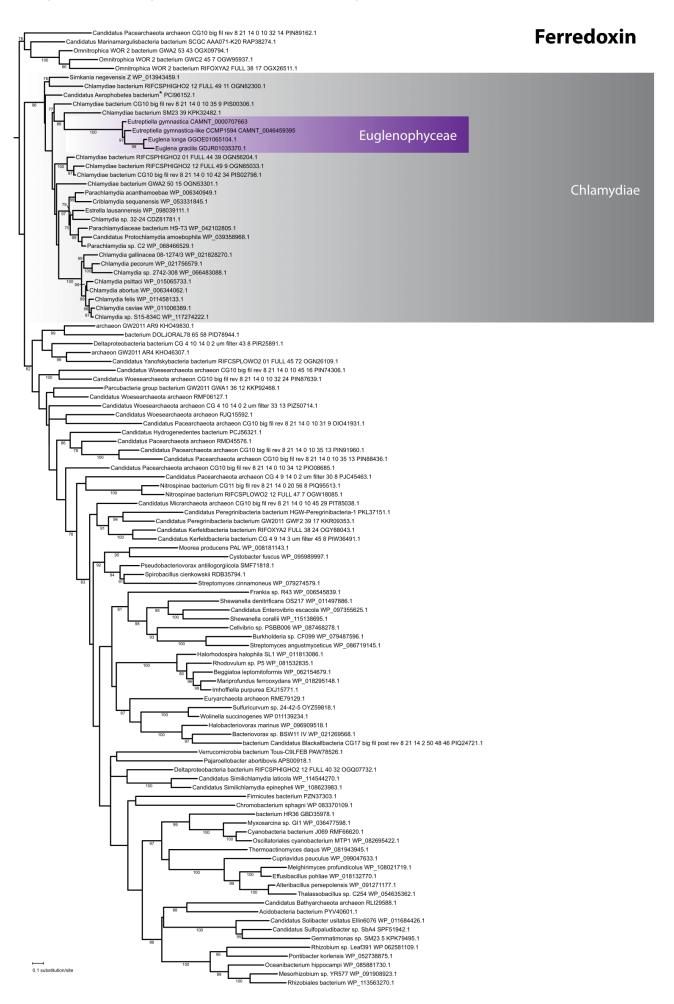




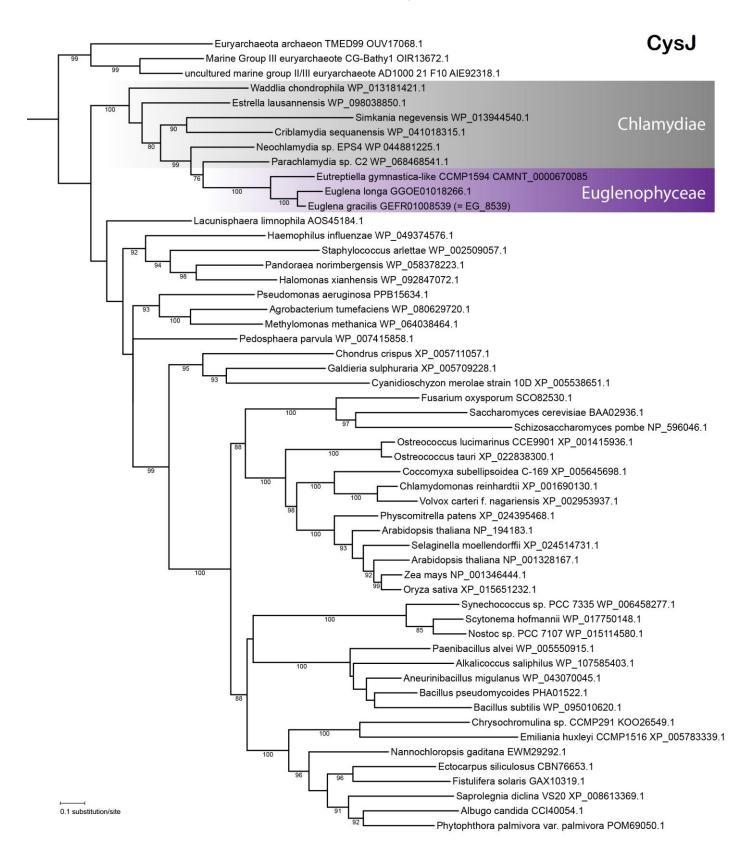


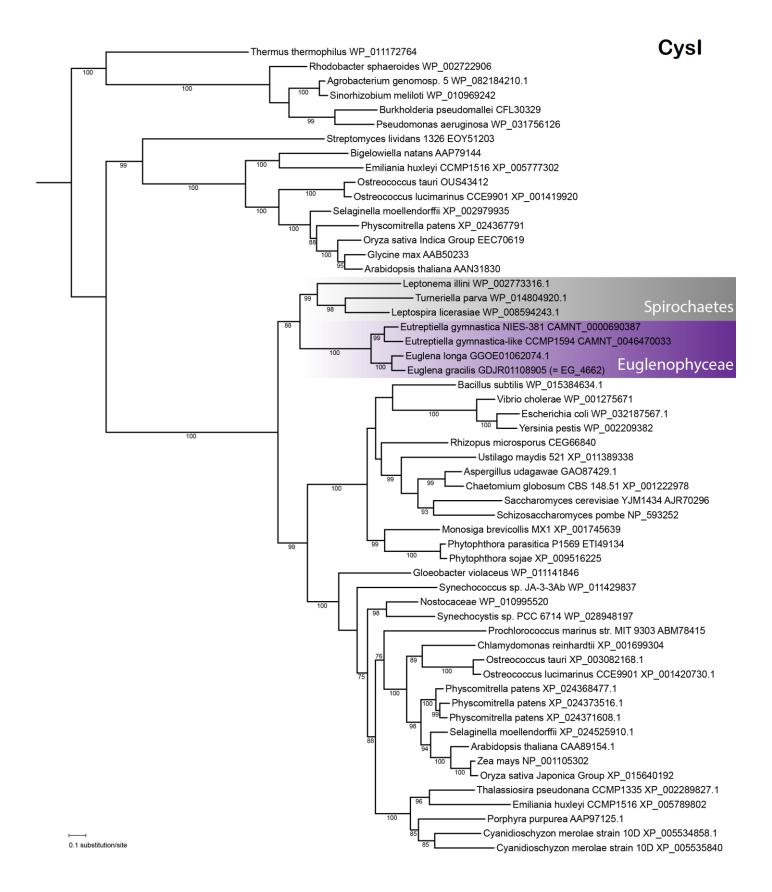
SufS





S10: Phylogenetic trees showing positions of sulfite reductase subunits. While the subunit alpha (CysJ) sits inside chlamydiae, the subunit beta (CysI), which is also of putative prokaryotic evolutionary origin, is sister to spirochaetes.



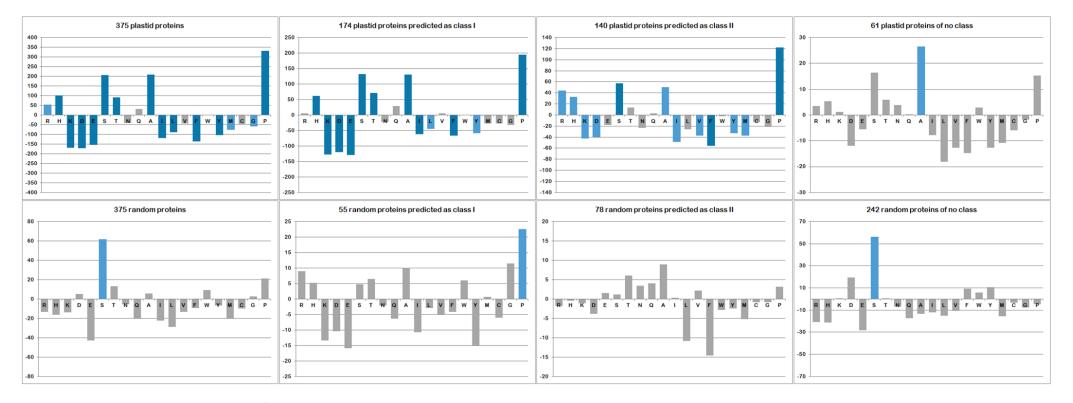


S11: MAFFT alignment of the conserved region (positions 310-650 of the fasta alignment derlins.fasta provided in supplementary files) of Der-like plastid proteins of *E. gracilis, E. longa, Eutreptiella gymnastica,* and *Eutreptiella sp.,* reffered to as DerL1 and DerL2, host (ER) and symbiont (SELMA) specific Der1 proteins of *Guillardia theta, Phaeodactylum tricornutum,* and *Plasmodium falciparum* (as identified in Sommer et al. 2007), Der of a red alga *Gracilariopsis chorda,* and three human derlins and a human RHBDL4 rhomboid-like protease. In comparison to the more canonical derlins, the euglenophyte homologs contain approximately 200 aa long N-terminal extension not shown in this view. Conserved positions are highlighted in light and dark grey.

	1, 10, 20, 30, 40, 50, 60, 70, 80 XGIKGAMSGFIDWXKNIPPMTRIYLTASXLTTLLWALGLINPLXLXLSG-X-FYCFXIWRLITNFLFFG
1. Hsa_RHBL4 2. Hsa_DERL1	
3. Hsa_DERL2 4. Hsa_DERL3	AYQSLR LEYLQI P PWSRAYTTACVLTTAAVQL ELIT - PFQLYFNPELIFKHFQTWRLITNFLFF GPV AWQGLAAEFLQVPAWTRAYTAACVLTTAAVQL ELLS - PFQLYFNPHLVFRKFQVWRLVTNFLFF GPL
5. Gch_Derlin-2 6. Ptr_hDer1	MTLFEEWWNSIPPWTRSYICLSWLTTSAVALEVIT-PYKLYLDWALILTQAHVWRUFTNFLEFGNF GGHAGAGVDLMSWYMEIPPWSRLYLTGAFLTSAACA
7. Gth_sDer1 8. Pfa_hDer1-2 9. Gth_hDer1	IDSIKNYPPMIRIPYIISILFNIASFA
10. Pfa_hDer1-1 11. Ptr_hDer1 2	I SGPEVWYNNLPNMTKYVIITLIFLVTLLITCNLLNVVYILLDWNLIYYKYHIWRIFLNFLYVGKF VADAAGPDQWFKISLPVMTRYWFGATIAVTLAANFEIISSGQCAFVWPLVRYKFELWRLLSCFLWAGPF
12. Ptr_sDer1-2 13. Ptr_sDer1-1 14. Pfa_sDer1-2	<pre>FHVGINNIPPWTLATLALNIWFFLNPQKP</pre>
15. Pfa_sDer1-1 16. Egr_DerL1	Y FNNLKSSFIYKLKNTKITTKLFLSSSLLILTTNVIGLKPREDIALHSKRVLRAFEFYRTYTSALFYGDI WVMGVVNGFIRWFRG-SPTEVVMILIFLCSWALWINPGLEPLFLVQGFRN-PLAFVFSGFACFNIYOLYTDIFYV
17. Elo_DerL1 18. Ecg_DerL1 19. Egy_DerL1	W LSDAMEAIADWISN-KPIDAAMLAIFVCSVMIWKIPSLESVFLVQGFRNPLAFIMSGFACFNIYOLFSDIFVV GIVNFFNGFVRWVRN-HPIDVVMILIFLCSWALWLNPSLEPVFLVQGFRNPLAFIFSGFTCFNIYOLYSDIFVV GILNFFRGFVRWIKN-SPIDVIMLSIFLCSWALWLKPNLTPLFLVEGFSNPLAFIGSGFACFNIYOLYSDIFV
20. Egr_DerL2 21. Elo_DerL2	PIGGVVGKTVDHLMNLHPAHQLGLIHIVCTALLWLVPALYPLFLANGLPN - VLGFVLSG FSCVNPIALINQVFSY GSLGGKFIHDILSLHPAYLVGLTNLISTALLRREPAYYOLFLARGLGN - PLGFLISG FSCVNPIALVGEVFSF
22. Egy_DerL2 23. Ecg_DerL2	RRKGLWGSIVAQLDAFHPMQQTIAAINLFTTVLLYAWPTLYPLFIADGFRN PEGFVLSG FISCMNPIAFINE NEIFEF N
1. Hsa_RHBL4 2. Hsa_DERL1 3. Hsa_DERL2	DWHLYFNMASMLWKGINLERKLGSRWHAYMIIAFSVLIGWYLLLQFAVAEFMD
4. Hsa_DERL3 5. Gch_Derlin-2	GFSFFFNMLFVFRWCRMLEEGSFRGRTADEVFMFLFGGVLMTLLGLLG
6. Ptr_hDer1 7. Gth_sDer1 8. Pfa_hDer1-2	SVDENLYRMYFLURWISR LLEDGDFRGRTAN MYNFLLFGI I GUISIWA SYM
9. Gth_hDer1 10. Pfa_hDer1-1	GWP FILMN L I FM V Q Y S K T L E K D FNG S A S D F L W C L I MG G A L L C G I N H W T
11. Ptr_hDer1 2 12. Ptr_sDer1-2 13. Ptr_sDer1-1	SMNTMISCYMLVTHISRQYHAGGPYNTGAGGGTADHAANHAMIMFGAAIMLUTFPLVTAI GLGYLMTAHFVWTYMATLERLNHDRPYDFWIMIFFGQLSMVVGYPIF
14. Pfa_sDer1-2 15. Pfa_sDer1-1	Y LQYIII M FNY LN I WMS SVEI SH Y KK PEDELI FLTFGY I SNLL FT I WANMYNEN I MNVKLY I HNFKN FFI KDCVSKYTSRS SLYVII TN I YMLYVQSNQLEN I L G S SIML SYYII SQ I SI LSII I CSYI
16. Egr_DerL1 17. Elo_DerL1 18. Ecg_DerL1	YILGKAYCVEI
19. Egy_DerL1 20. Egr_DerL2	YILGKAYCVME
21. Elo_DerL2 22. Egy_DerL2 23. Ecg_DerL2	
25.208_50122	180 190 200 210 220 220 240 250
	X-YL2WYLLXFS-X-X-
2. Hsa_DERL1	
2. Hsa_DERL1 3. Hsa_DERL2 4. Hsa_DERL3	G S P FLIG - C A X T A ALLEY A M S R R N P X T P V N W X - Y L D W V L L X F S - X - X -
2. Hsa_DERL1 3. Hsa_DERL2 4. Hsa_DERL3 5. Gch_Derlin-2 6. Ptr_hDer1 7. Gth_spar1	
2. Hsa_DERL1 3. Hsa_DERL2 4. Hsa_DERL3 5. Gch_Derlin-2 6. Ptr_hDer1 7. Gth_spar1	
2. Hsa_DERL1 3. Hsa_DERL2 4. Hsa_DERL3 5. Gch_Derlin-2 6. Ptr_hDer1 7. Gth_spar1	
2. Hsa_DERL1 3. Hsa_DERL2 4. Hsa_DERL3 5. Gch_Derlin-2 6. Ptr_hDer1 7. Gth_spar1	
1. Hsa_NBEL 2. Hsa_DERL1 3. Hsa_DERL3 5. Gch_Derlin-2 6. Ptr_hDer1 9. Gch_hDer1-2 9. Gch_hDer1 10. Pfa_hDer1-1 10. Pfa_hDer1-1 12. Ptr_sDer1-2 13. Ptr_sDer1-2 13. Ptr_sDer1-2 15. Pfa_sDer1-2 15. Pfa_sDer1-1 16. Egr_DerL1 17. Elo_DerL1	
2. Hsa_DERL1 3. Hsa_DERL3 4. Hsa_DERL3 5. Gch_Derlin-2 6. Ptr_hDer1-2 6. Ptr_hDer1-2 9. Gch_hDer1-1 10. Pfa_hDer1-1 10. Pfa_hDer1-1 11. Ptr_hDer1 2 12. Ptr_sDer1-2 13. Ptr_sDer1-2 13. Ptr_sDer1-2 14. Pfa_sDer1-2 15. Pfa_sDer1-1 16. Egr_DerL1 18. Ecg_DerL1 19. Ecg_DerL1	
2. Hsa_DERL1 3. Hsa_DERL3 4. Hsa_DERL3 5. Gch_Derlin-2 6. Ptr_hDer1 9. Gth_DDer1-2 9. Gth_DDer1 10. Pfa_hDer1-1 10. Pfa_hDer1-1 11. Ptr_hDer1 2 12. Ptr_sDer1-2 13. Ptr_sDer1-2 13. Ptr_sDer1-1 14. Pfa_sDer1-2 15. Pfa_sDer1-2 15. Pfa_sDer1-1 16. Egr_DerL1 18. Ecg_DerL1 19. Egy_DerL1 20. Egr_DerL2 21. Elo_DerL2 22. Egy_DerL2	
2. Hsa_DERL 3. Hsa_DERL 4. Hsa_DERL3 5. Gch_Derlin-2 6. Ptr_hDer1 9. Gth_DDer1 10. Pfa_hDer1-2 9. Gth_hDer1 11. Ptr_hDer1 12. Ptr_sDer1-2 13. Ptr_sDer1-2 13. Ptr_sDer1-2 13. Ptr_sDer1-1 14. Pfa_sDer1-2 15. Pfa_sDer1-1 16. Egr_DerL1 18. Ecg_DerL1 19. Egr_DerL1 20. Egr_DerL2 21. Elo_DerL2 22. Egr_DerL2 23. Ecg_DerL2 23. Ecg_DerL2	
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	ALL	chisq sum	p-val chisq sum	norm chisq sum	p-val norm chisq sum	CLASS I	chisq sum	p-val chisq sum	norm chisq sum	p-val norm chisq sum	CLASS II	chisq sum	p-val chisq sum	norm chisq sum	p-val norm chisq sum	no class	chisq sum	p-val chisq sum	norm chisq sum	p-val norm chisq sum
	R	175,086	1	53,1541	0,00605	R	68,4805	1	5,5466	0,67413	R	76,4798	1	44,0918	0,00019	R	30,1258	0,99969	3,51573	0,65261
	н	196,786	1	100,417	1,59E-07	Н	103,726	0,99999	62,2853	2,04E-06	Н	68,4743	1	32,7265	0,00485	Н	24,5851	0,99999	5,40481	0,48533
	K	295,634	0,99906	-165,47	0	K	165,277	0,66994	-125,249	0	K	97,0751	0,99779	-41,4843	0,00045	K	33,2821	0,99854	1,26311	0,87152
	D	270,683	0,99999	-168,934	0	D	146,398	0,93696	-118,104	0	D	74,2336	1	-38,8899	0,00101	D	50,0515	0,84044	-11,9406	0,1263
	E	331,85	0,94694	-150,778	6,88E-15	E	181,6	0,33097	-127,026	0	E	91,39	0,9995	-18,2782	0,1224	E	58,8598	0,55389	-5,47392	0,48339
	S	401,66	0,16465	206,083	0	S	232,689	0,00196	132,049	0	S	124,561	0,82088	57,6567	1,10E-06	S	44,4105	0,94553	16,377	0,03601
	Т	220,009	1	90,9184	2,67E-06	Т	139,829	0,97327	71,7089	5,44E-08	Т	49,0246	1	13,2613	0,26238	Т	31,1553	0,99947	5,94825	0,4463
	N	127,755	1	-42,4131	0,0283	N	62,6668	1	-23,0973	0,07908	N	42,1703	1	-23,2282	0,04963	N	22,9175	1	3,91239	0,61642
SAMPLE	Q	171,625	1	31,1712	0,10747	Q	109,334	0,99997	28,1758	0,03268	Q	44,4741	1	2,79381	0,81334	Q	17,8174	1	0,20158	0,97941
	A	550,223	8,93E-09	207,823	0	A	322,218	6,09E-11	130,78	0	A	141,913	0,43895	50,5491	1,94E-05	A	86,0922	0,01889	26,4941	0,00069
		198,963	1	-116,336	1,79E-09		94,6478	1	-60,7521	4,11E-06		75,0626	1	-47,7654	5,09E-05		29,2522	0,99981	-7,81817	0,31682
	L	193,851	1	-87,2043	6,69E-06	L	84,6792	1	-43,2203	0,00105	L	69,5416	1	-25,8399	0,02897	L	39,6304	0,98461	-18,1441	0,02017
	V F	197,749	1	-44,6475	0,02113	V F	77,5955	1	4,64804	0,72456	V F	80,1055	0,99999	-36,5342	0,00202	V F	40,0481	0,98254	-12,7614	0,10227
	W	208,752	1	-134,126	4,32E-12	w	101,429 33,1091	1	-64,428 -0.69202	1,04E-06	w	84,0705 32,2305	0,99995	-54,9889 -2,38569	3,36E-06	w	23,253 13,5939	1	-14,7091	0,05966 0,70318
	Y	78,9335 131,868	1	-0,15093 -101,205	0,9937 1,43E-07	Y	60,5727	1	-0,69202 -56,6235	0,95755 1,58E-05	Y	32,2305 41,1337	1	-2,36569 -31,9114	0,83849 0,0066	Y	30,1611	0,99954	2,92677 -12,6704	0,70318
	M	106,69	1	-74,8029	9,86E-05	M	37,7605	1	-27,7899	0,03357	M	45,2825	1	-36,2452	0,0000	M	23,6471	0,99904	-12,0704	0,10189
	C	69,7508	1	-45,7416	9,00 <u></u> -05 0,01298	C	22,9606	1	-27,7899	0,03354	C	45,2625 27,8388	1	-30,2432	0,24666	C	18,9514	1	-5,93654	0,108
	G	203,731	1	-55,8315	0,01298	G	97,7821	1	-32,365	0,03334	G	64,0528	1	-21,6138	0,24000	G	41,8965	0.97065	-1,85269	0,43100
	P	794.957	0	331,381	0,00394	P	425,55	0	-32,303	0,01414	P	300,299	1,00E-13	122,179	0,00773	P	69,1068	0,97003	15,2626	0,05068
		101,001	v	001,001	v		120,00	, v	, -			000,200	,	,	v	•	00,1000	,	,	
			n-val chisa	norm	n-val norm			n-val chisa	norm chisa	n-val norm			n-val chisa	norm chisa	n-val norm			n-val chisa	norm chisa	n-val norm
	ALL	chisq sum	p-val chisq sum	norm chisq sum	p-val norm chisq sum	CLASS I	chisq sum	p-val chisq sum	norm chisq sum	p-val norm chisq sum	CLASS II	chisq sum	p-val chisq sum	norm chisq sum	p-val norm chisq sum	no class	chisq sum	p-val chisq sum	norm chisq sum	p-val norm chisq sum
	R	177,641		chisq sum -13,522	chisq sum 0,48501	R	27,4672	sum 0,99931	sum 9,04872	chisq sum 0,22242	R	42,351		sum -1,7648	chisq sum 0,84162	R	107,823	sum 1	sum -20,8059	chisq sum 0,18107
	R H	177,641 104,238		chisq sum -13,522 -16,265	chisq sum 0,48501 0,40095	R H	27,4672 30,0401	sum 0,99931 0,99756	sum 9,04872 5,29145	chisq sum 0,22242 0,47554	R H	42,351 11,4542	sum	sum -1,7648 -0,46625	chisq sum 0,84162 0,9579	R H	107,823 62,7435	sum 1	sum -20,8059 -21,0902	chisq sum 0,18107 0,17519
	R H K	177,641 104,238 150,159	sum 1 1 1	chisq sum -13,522 -16,265 -13,8913	chisq sum 0,48501 0,40095 0,47079	R H K	27,4672 30,0401 16,3777	sum 0,99931 0,99756 1	sum 9,04872 5,29145 -13,4226	chisq sum 0,22242 0,47554 0,06776	R H K	42,351 11,4542 22,1346	sum	sum -1,7648 -0,46625 -1,06981	chisq sum 0,84162 0,9579 0,90297	R H K	107,823 62,7435 111,647	sum 1 1 1	sum -20,8059 -21,0902 0,60112	chisq sum 0,18107 0,17519 0,96905
	R H K D	177,641 104,238 150,159 139,253	sum 1 1 1 1 1	chisq sum -13,522 -16,265 -13,8913 5,40623	chisq sum 0,48501 0,40095 0,47079 0,78011	R H K D	27,4672 30,0401 16,3777 25,0269	sum 0,99931 0,99756 1 0,99983	sum 9,04872 5,29145 -13,4226 -10,3959	chisq sum 0,22242 0,47554 0,06776 0,16098	R H K D	42,351 11,4542 22,1346 24,2952	sum	sum -1,7648 -0,46625 -1,06981 -3,77318	chisq sum 0,84162 0,9579 0,90297 0,66921	R H K D	107,823 62,7435 111,647 89,9305	sum 1 1 1 1	sum -20,8059 -21,0902 0,60112 19,5753	chisq sum 0,18107 0,17519 0,96905 0,20827
	R H K D E	177,641 104,238 150,159 139,253 207,541	sum 1 1 1 1 1 1	chisq sum -13,522 -16,265 -13,8913 5,40623 -42,7289	chisq sum 0,48501 0,40095 0,47079 0,78011 0,02735	R H K D E	27,4672 30,0401 16,3777 25,0269 23,9834	sum 0,99931 0,99756 1 0,99983 0,99991	sum 9,04872 5,29145 -13,4226 -10,3959 -15,9465	chisq sum 0,22242 0,47554 0,06776 0,16098 0,03154	R H K D E	42,351 11,4542 22,1346 24,2952 25,6805	sum 0,99967 1 1 1 1	sum -1,7648 -0,46625 -1,06981 -3,77318 1,61052	chisq sum 0,84162 0,9579 0,90297 0,66921 0,8553	R H K D E	107,823 62,7435 111,647 89,9305 157,877	sum 1 1 1 1 0,99999	sum -20,8059 -21,0902 0,60112 19,5753 -28,3929	chisq sum 0,18107 0,17519 0,96905 0,20827 0,06798
	R H K D E S	177,641 104,238 150,159 139,253 207,541 278,938	sum 1 1 1 1 1 0,99994	chisq sum -13,522 -16,265 -13,8913 5,40623 -42,7289 62,0444	chisq sum 0,48501 0,40095 0,47079 0,78011 0,02735 0,00136	R H K D E S	27,4672 30,0401 16,3777 25,0269 23,9834 29,437	sum 0,99931 0,99756 1 0,99983 0,99991 0,99815	sum 9,04872 5,29145 -13,4226 -10,3959 -15,9465 4,80842	chisq sum 0,22242 0,47554 0,06776 0,16098 0,03154 0,51675	R H K D E S	42,351 11,4542 22,1346 24,2952 25,6805 40,2188	sum	sum -1,7648 -0,46625 -1,06981 -3,77318 1,61052 1,16952	chisq sum 0,84162 0,9579 0,90297 0,66921 0,8553 0,89465	R H K D E S	107,823 62,7435 111,647 89,9305 157,877 209,282	sum 1 1 1 0,99999 0,93691	sum -20,8059 -21,0902 0,60112 19,5753 -28,3929 56,0665	chisq sum 0,18107 0,17519 0,96905 0,20827 0,06798 0,00031
	R H K D E S T	177,641 104,238 150,159 139,253 207,541 278,938 145,228	sum 1 1 1 1 1 1 0,99994 1	chisq sum -13,522 -16,265 -13,8913 5,40623 -42,7289 62,0444 13,1402	chisq sum 0,48501 0,40095 0,47079 0,78011 0,02735 0,00136 0,49742	R H K D E S T	27,4672 30,0401 16,3777 25,0269 23,9834 29,437 25,8714	sum 0,99931 0,99756 1 0,99983 0,99991 0,99815 0,99972	sum 9,04872 5,29145 -13,4226 -10,3959 -15,9465 4,80842 6,54869	chisq sum 0,22242 0,47554 0,06776 0,16098 0,03154 0,51675 0,37722	R H K D E S T	42,351 11,4542 22,1346 24,2952 25,6805 40,2188 16,4064	sum 0,99967 1 1 1 1	sum -1,7648 -0,46625 -1,06981 -3,77318 1,61052 1,16952 6,0564	chisq sum 0,84162 0,9579 0,90297 0,66921 0,8553 0,89465 0,49287	R H K D E S T	107,823 62,7435 111,647 89,9305 157,877 209,282 102,95	sum 1 1 1 1 0,999999 0,93691 1	sum -20,8059 -21,0902 0,60112 19,5753 -28,3929 56,0665 0,53511	chisq sum 0,18107 0,17519 0,96905 0,20827 0,06798 0,00031 0,97256
	R H K D E S T N	177,641 104,238 150,159 139,253 207,541 278,938 145,228 101,854	sum 1 1 1 1 1 1 0,99994 1 1	chisq sum -13,522 -16,265 -13,8913 5,40623 -42,7289 62,0444 13,1402 -5,51324	chisq sum 0,48501 0,40095 0,47079 0,78011 0,02735 0,00136 0,49742 0,7747	R H K D E S T N	27,4672 30,0401 16,3777 25,0269 23,9834 29,437 25,8714 16,039	sum 0,99931 0,99756 1 0,99983 0,99991 0,99815 0,99972 1	sum 9,04872 5,29145 -13,4226 -10,3959 -15,9465 4,80842 6,54869 -2,09914	chisq sum 0,22242 0,47554 0,06776 0,16098 0,03154 0,51675 0,37722 0,77514	R H K D E S T N	42,351 11,4542 22,1346 24,2952 25,6805 40,2188 16,4064 14,2222	sum 0,99967 1 1 1 1	sum -1,7648 -0,46625 -1,06981 -3,77318 1,61052 1,16952 6,0564 3,48531	chisq sum 0,84162 0,9579 0,90297 0,66921 0,8553 0,89465 0,49287 0,69123	R H K D E S T N	107,823 62,7435 111,647 89,9305 157,877 209,282 102,95 71,5924	sum 1 1 1 0,99999 0,93691 1 1	sum -20,8059 -21,0902 0,60112 19,5753 -28,3929 56,0665 0,53511 -6,89941	chisq sum 0,18107 0,17519 0,96905 0,20827 0,06798 0,00031 0,97256 0,65606
CONTROL	R H K D E S T N Q	177,641 104,238 150,159 139,253 207,541 278,938 145,228 101,854 127,543	sum 1 1 1 1 1 0,99994 1 1 1	chisq sum -13,522 -16,265 -13,8913 5,40623 -42,7289 62,0444 13,1402 -5,51324 -19,6212	chisq sum 0,48501 0,40095 0,47079 0,78011 0,02735 0,00136 0,49742 0,7747 0,31095	R H K D E S T N Q	27,4672 30,0401 16,3777 25,0269 23,9834 29,437 25,8714 16,039 12,2343	sum 0,99931 0,99756 1 0,99983 0,99991 0,99815 0,99972 1 1	sum 9,04872 5,29145 -13,4226 -10,3959 -15,9465 4,80842 6,54869 -2,09914 -6,35625	chisq sum 0,22242 0,47554 0,06776 0,16098 0,03154 0,51675 0,37722 0,77514 0,3914	R H K D E S T N Q	42,351 11,4542 22,1346 24,2952 25,6805 40,2188 16,4064 14,2222 24,9884	sum 0,99967 1 1 1 1 0,99988 1 1 1	sum -1,7648 -0,46625 -1,06981 -3,77318 1,61052 1,16952 6,0564 3,48531 4,05136	chisq sum 0,84162 0,9579 0,90297 0,66921 0,8553 0,89465 0,49287 0,69123 0,64643	R H K D E S T N Q	107,823 62,7435 111,647 89,9305 157,877 209,282 102,95 71,5924 90,3204	sum 1 1 1 0,99999 0,93691 1 1 1	sum -20,8059 -21,0902 0,60112 19,5753 -28,3929 56,0665 0,53511 -6,89941 -17,3163	chisq sum 0,18107 0,17519 0,96905 0,20827 0,06798 0,00031 0,97256 0,65606 0,26565
CONTROL	R H K D E S T N	177,641 104,238 150,159 139,253 207,541 278,938 145,228 101,854 127,543 275,145	sum 1 1 1 1 0,99994 1 1 1 0,99997	chisq sum -13,522 -16,265 -13,8913 5,40623 -42,7289 62,0444 13,1402 -5,51324 -19,6212 5,67527	chisq sum 0,48501 0,40095 0,47079 0,78011 0,02735 0,00136 0,49742 0,7747 0,31095 0,76947	R H C D E S T N Q A	27,4672 30,0401 16,3777 25,0269 23,9834 29,437 25,8714 16,039 12,2343 56,5231	sum 0,99931 0,99756 1 0,99983 0,99991 0,99815 0,99972 1 1 1 0,41786	sum 9,04872 5,29145 -13,4226 -10,3959 -15,9465 4,80842 6,54869 -2,09914 -6,35625 10,0478	chisq sum 0,22242 0,47554 0,06776 0,16098 0,03154 0,51675 0,37722 0,77514 0,3914 0,17547	R H K D E S T N	42,351 11,4542 22,1346 24,2952 25,6805 40,2188 16,4064 14,2222 24,9884 36,8012	sum 0,99967 1 1 1 1	sum -1,7648 -0,46625 -1,06981 -3,77318 1,61052 1,16952 6,0564 3,48531 4,05136 8,98436	chisq sum 0,84162 0,9579 0,90297 0,66921 0,8553 0,89465 0,49287 0,69123 0,64643 0,30902	R H K D E S T N Q A	107,823 62,7435 111,647 89,9305 157,877 209,282 102,95 71,5924 90,3204 181,821	sum 1 1 1 0,99999 0,93691 1 1	sum -20,8059 -21,0902 0,60112 19,5753 -28,3929 56,0665 0,53511 -6,89941 -17,3163 -13,3569	chisq sum 0,18107 0,17519 0,96905 0,20827 0,06798 0,00031 0,97256 0,65606 0,26565 0,39055
CONTROL	R H K D E S T N Q	177,641 104,238 150,159 139,253 207,541 278,938 145,228 101,854 127,543 275,145 131,641	sum 1 1 1 1 0,99994 1 1 1 0,99997 1	chisq sum -13,522 -16,265 -13,8913 5,40623 -42,7289 62,0444 13,1402 -5,51324 -19,6212 5,67527 -22,2844	chisq sum 0,48501 0,40095 0,47079 0,78011 0,02735 0,00136 0,49742 0,7747 0,31095 0,76947 0,2492	R H C D E S T N Q A I	27,4672 30,0401 16,3777 25,0269 23,9834 29,437 25,8714 16,039 12,2343 56,5231 24,5822	sum 0,99931 0,99756 1 0,99983 0,99991 0,99815 0,99972 1 1 0,41786 0,99987	sum 9,04872 5,29145 -13,4226 -10,3959 -15,9465 4,80842 6,54869 -2,09914 -6,35625 10,0478 -10,6552	chisq sum 0,22242 0,47554 0,06776 0,16098 0,03154 0,51675 0,37722 0,77514 0,3914 0,17547 0,15079	R H K D E S T N Q A I	42,351 11,4542 22,1346 24,2952 25,6805 40,2188 16,4064 14,2222 24,9884 36,8012 21,1974	sum 0,99967 1 1 1 1 0,99988 1 1 1	sum -1,7648 -0,46625 -1,06981 -3,77318 1,61052 1,16952 6,0564 3,48531 4,05136 8,98436 0,31489	chisq sum 0,84162 0,9579 0,90297 0,66921 0,8553 0,89465 0,49287 0,69123 0,64643 0,30902 0,97156	R H K D E S T N Q A I	107,823 62,7435 111,647 89,9305 157,877 209,282 102,95 71,5924 90,3204 181,821 85,8615	sum 1 1 1 0,99999 0,93691 1 1 1 0,99852 1	sum -20,8059 -21,0902 0,60112 19,5753 -28,3929 56,0665 0,53511 -6,89941 -17,3163 -13,3569 -11,9441	chisq sum 0,18107 0,17519 0,96905 0,20827 0,06798 0,00031 0,97256 0,65606 0,26565 0,39055 0,44166
CONTROL	R H K D E S T N Q	177,641 104,238 150,159 139,253 207,541 278,938 145,228 101,854 127,543 275,145 131,641 197,538	sum 1 1 1 1 0,99994 1 1 1 0,99997	chisq sum -13,522 -16,265 -13,8913 5,40623 -42,7289 62,0444 13,1402 -5,51324 -19,6212 5,67527 -22,2844 -28,5685	chisq sum 0,48501 0,40095 0,47079 0,78011 0,02735 0,00136 0,49742 0,7747 0,31095 0,76947 0,2492 0,14014	R H C D E S T N Q A I L	27,4672 30,0401 16,3777 25,0269 23,9834 29,437 25,8714 16,039 12,2343 56,5231 24,5822 22,4132	sum 0,99931 0,99756 1 0,99983 0,99991 0,99815 0,99972 1 1 0,41786 0,99987 0,99997	sum 9,04872 5,29145 -13,4226 -10,3959 -15,9465 4,80842 6,54869 -2,09914 -6,35625 10,0478 -10,6552 -2,84671	chisq sum 0,22242 0,47554 0,06776 0,16098 0,03154 0,51675 0,37722 0,77514 0,3914 0,17547 0,15079 0,70109	R H K D E S T N Q A I L	42,351 11,4542 22,1346 24,2952 25,6805 40,2188 16,4064 14,2222 24,9884 36,8012 21,1974 34,1793	sum 0,99967 1 1 1 0,99988 1 1 0,99998 1 0,99998 1 1	sum -1,7648 -0,46625 -1,06981 -3,77318 1,61052 1,16952 6,0564 3,48531 4,05136 8,98436 0,31489 -10,7669	chisq sum 0,84162 0,9579 0,90297 0,66921 0,8553 0,89465 0,49287 0,69123 0,64643 0,30902 0,97156 0,2228	R H K D E S T N Q A	107,823 62,7435 111,647 89,9305 157,877 209,282 102,95 71,5924 90,3204 181,821 85,8615 140,945	sum 1 1 1 0,99999 0,93691 1 1 1	sum -20,8059 -21,0902 0,60112 19,5753 -28,3929 56,0665 0,53511 -6,89941 -17,3163 -13,3569 -11,9441 -14,9549	chisq sum 0,18107 0,17519 0,96905 0,20827 0,06798 0,00031 0,97256 0,65606 0,26565 0,39055 0,44166 0,33638
CONTROL	R H K D E S T N Q A I L	177,641 104,238 150,159 139,253 207,541 278,938 145,228 101,854 127,543 275,145 131,641 197,538 164,256	sum 1 1 1 1 0,99994 1 1 1 0,99997 1 1	chisq sum -13,522 -16,265 -13,8913 5,40623 -42,7289 62,0444 13,1402 -5,51324 -19,6212 5,67527 -22,2844 -28,5685 -13,4159	chisq sum 0,48501 0,40095 0,47079 0,78011 0,02735 0,00136 0,49742 0,7747 0,31095 0,76947 0,2492 0,14014 0,48844	R H C D E S T N Q A I	27,4672 30,0401 16,3777 25,0269 23,9834 29,437 25,8714 16,039 12,2343 56,5231 24,5822 22,4132 23,582	sum 0,99931 0,99756 1 0,99983 0,99991 0,99815 0,99972 1 1 0,41786 0,99987	sum 9,04872 5,29145 -13,4226 -10,3959 -15,9465 4,80842 6,54869 -2,09914 -6,35625 10,0478 -10,6552 -2,84671 -4,91324	chisq sum 0,22242 0,47554 0,06776 0,16098 0,03154 0,51675 0,37722 0,77514 0,3914 0,17547 0,15079 0,70109 0,50765	R H K D E S T N Q A I	42,351 11,4542 22,1346 24,2952 25,6805 40,2188 16,4064 14,2222 24,9884 36,8012 21,1974 34,1793 35,4584	sum 0,99967 1 1 1 1 0,99988 1 1 1	sum -1,7648 -0,46625 -1,06981 -3,77318 1,61052 1,16952 6,0564 3,48531 4,05136 8,98436 0,31489 -10,7669 2,2388	chisq sum 0,84162 0,9579 0,90297 0,66921 0,8553 0,89465 0,49287 0,69123 0,64643 0,30902 0,97156 0,2228 0,79989	R H K D E S T N Q A I L	107,823 62,7435 111,647 89,9305 157,877 209,282 102,95 71,5924 90,3204 181,821 85,8615 140,945 105,216	sum 1 1 1 0,99999 0,93691 1 1 0,99852 1 1 1	sum -20,8059 -21,0902 0,60112 19,5753 -28,3929 56,0665 0,53511 -6,89941 -17,3163 -13,3569 -11,9441 -14,9549 -10,7415	chisq sum 0,18107 0,17519 0,96905 0,20827 0,06798 0,00031 0,97256 0,65606 0,26565 0,39055 0,44166 0,33638 0,48989
CONTROL	R H K D E S T N Q A I L V F	177,641 104,238 150,159 139,253 207,541 278,938 145,228 101,854 127,543 275,145 131,641 197,538 164,256 145,28	sum 1 1 1 1 0,99994 1 1 1 0,99997 1 1 1 1 1	chisq sum -13,522 -16,265 -13,8913 5,40623 -42,7289 62,0444 13,1402 -5,51324 -19,6212 5,67527 -22,2844 -28,5685 -13,4159 -9,19004	chisq sum 0,48501 0,40095 0,47079 0,78011 0,02735 0,00136 0,49742 0,7747 0,31095 0,76947 0,2492 0,14014 0,48844 0,63464	R H K D E S T N Q Q A I L V F	27,4672 30,0401 16,3777 25,0269 23,9834 29,437 25,8714 16,039 12,2343 56,5231 24,5822 22,4132 23,582 20,2589	sum 0,99931 0,99756 1 0,99983 0,99991 0,99815 0,99972 1 1 0,41786 0,99987 0,99997 0,99993	sum 9,04872 5,29145 -13,4226 -10,3959 -15,9465 4,80842 6,54869 -2,09914 -6,35625 10,0478 -10,6552 -2,84671 -4,91324 -4,13733	chisq sum 0,22242 0,47554 0,06776 0,16098 0,03154 0,51675 0,37722 0,77514 0,3914 0,17547 0,15079 0,70109 0,50765 0,57693	R H K D E S T N Q A I L V F	42,351 11,4542 22,1346 24,2952 25,6805 40,2188 16,4064 14,2222 24,9884 36,8012 21,1974 34,1793 35,4584 24,7953	sum 0,99967 1 1 1 0,99988 1 1 0,99998 1 0,99998 1 1	sum -1,7648 -0,46625 -1,06981 -3,77318 1,61052 1,16952 6,0564 3,48531 4,05136 8,98436 0,31489 -10,7669 2,2388 -14,6199	chisq sum 0,84162 0,9579 0,90297 0,66921 0,8553 0,89465 0,49287 0,69123 0,64643 0,30902 0,97156 0,2228 0,79989 0,09785	R H K D E S T N Q A I L V F	107,823 62,7435 111,647 89,9305 157,877 209,282 102,95 71,5924 90,3204 181,821 85,8615 140,945 105,216 100,225	sum 1 1 1 0,99999 0,93691 1 1 0,99852 1 1 1 1 1 1 1 1 1 1 1 1 1	sum -20,8059 -21,0902 0,60112 19,5753 -28,3929 56,0665 0,53511 -6,89941 -17,3163 -13,3569 -11,9441 -14,9549 -10,7415 9,5672	chisq sum 0,18107 0,17519 0,96905 0,20827 0,06798 0,00031 0,97256 0,65606 0,26565 0,39055 0,44166 0,33638 0,48989 0,53771
CONTROL	R H K D E S T N Q A I L V	177,641 104,238 150,159 139,253 207,541 278,938 145,228 101,854 127,543 275,145 131,641 197,538 164,256	sum 1 1 1 1 0,99994 1 1 1 0,99997 1 1 1 1	chisq sum -13,522 -16,265 -13,8913 5,40623 -42,7289 62,0444 13,1402 -5,51324 -19,6212 5,67527 -22,2844 -28,5685 -13,4159	chisq sum 0,48501 0,40095 0,47079 0,78011 0,02735 0,00136 0,49742 0,7747 0,31095 0,76947 0,2492 0,14014 0,48844 0,63464 0,63305	R H K D E S T N Q A I L V	27,4672 30,0401 16,3777 25,0269 23,9834 29,437 25,8714 16,039 12,2343 56,5231 24,5822 22,4132 23,582	sum 0,99931 0,99756 1 0,99983 0,99991 0,99815 0,99972 1 0,41786 0,99987 0,99997 0,99993 1	sum 9,04872 5,29145 -13,4226 -10,3959 -15,9465 4,80842 6,54869 -2,09914 -6,35625 10,0478 -10,6552 -2,84671 -4,91324 -4,13733 6,06124	chisq sum 0,22242 0,47554 0,06776 0,16098 0,03154 0,51675 0,37722 0,77514 0,3914 0,17547 0,15079 0,70109 0,50765 0,57693 0,41376	R H K D E S T N Q A I L V	42,351 11,4542 22,1346 24,2952 25,6805 40,2188 16,4064 14,2222 24,9884 36,8012 21,1974 34,1793 35,4584	sum 0,99967 1 1 1 0,99988 1 1 0,99998 1 0,99998 1 1	sum -1,7648 -0,46625 -1,06981 -3,77318 1,61052 1,16952 6,0564 3,48531 4,05136 8,98436 0,31489 -10,7669 2,2388 -14,6199 -2,84992	chisq sum 0,84162 0,9579 0,90297 0,66921 0,8553 0,89465 0,49287 0,69123 0,64643 0,30902 0,97156 0,2228 0,79989	R H K D E S T N Q A I L V	107,823 62,7435 111,647 89,9305 157,877 209,282 102,95 71,5924 90,3204 181,821 85,8615 140,945 105,216	sum 1 1 1 0,99999 0,93691 1 1 0,99852 1 1 1 1 1 1 1 1 1 1 1 1 1	sum -20,8059 -21,0902 0,60112 19,5753 -28,3929 56,0665 0,53511 -6,89941 -17,3163 -13,3569 -11,9441 -14,9549 -10,7415	chisq sum 0,18107 0,17519 0,96905 0,20827 0,06798 0,00031 0,97256 0,65606 0,26565 0,39055 0,44166 0,33638 0,48989 0,53771 0,69863
CONTROL	R H K D E S T N Q A I L V F W	177,641 104,238 150,159 139,253 207,541 278,938 145,228 101,854 127,543 275,145 131,641 197,538 164,256 145,28 81,3488	sum 1 1 1 1 0,99994 1 1 0,99997 1 1 1 1 1 1 1 1 1 1 1 1 1	chisq sum -13,522 -16,265 -13,8913 5,40623 -42,7289 62,0444 13,1402 -5,51324 -19,6212 5,67527 -22,2844 -28,5685 -13,4159 -9,19004 9,13389	chisq sum 0,48501 0,40095 0,47079 0,78011 0,02735 0,00136 0,49742 0,7747 0,31095 0,76947 0,2492 0,14014 0,48844 0,63464	R H K D E S T N Q Q A I L V F W	27,4672 30,0401 16,3777 25,0269 23,9834 29,437 25,8714 16,039 12,2343 56,5231 24,5822 22,4132 23,582 20,2589 14,2621	sum 0,99931 0,99756 1 0,99983 0,99991 0,99815 0,99972 1 0,41786 0,99987 0,99997 0,99993 1 1	sum 9,04872 5,29145 -13,4226 -10,3959 -15,9465 4,80842 6,54869 -2,09914 -6,35625 10,0478 -10,6552 -2,84671 -4,91324 -4,13733	chisq sum 0,22242 0,47554 0,06776 0,16098 0,03154 0,51675 0,37722 0,77514 0,3914 0,17547 0,15079 0,70109 0,50765 0,57693	R H K D E S T N Q A I L V F W	42,351 11,4542 22,1346 24,2952 25,6805 40,2188 16,4064 14,2222 24,9884 36,8012 21,1974 34,1793 35,4584 24,7953 11,9919	sum 0,99967 1 1 1 0,99988 1 1 0,99998 1 0,99998 1 1	sum -1,7648 -0,46625 -1,06981 -3,77318 1,61052 1,16952 6,0564 3,48531 4,05136 8,98436 0,31489 -10,7669 2,2388 -14,6199	chisq sum 0,84162 0,9579 0,90297 0,66921 0,8553 0,89465 0,49287 0,69123 0,64643 0,30902 0,97156 0,2228 0,79989 0,09785 0,74535	R H K D E S T N Q A I L V F W	107,823 62,7435 111,647 89,9305 157,877 209,282 102,95 71,5924 90,3204 181,821 85,8615 140,945 105,216 100,225 55,0948	sum 1 1 1 0,99999 0,93691 1 1 0,99852 1 1 1 1 1 1 1 1 1 1 1 1 1	sum -20,8059 -21,0902 0,60112 19,5753 -28,3929 56,0665 0,53511 -6,89941 -17,3163 -13,3569 -11,9441 -14,9549 -10,7415 9,5672 5,92257	chisq sum 0,18107 0,17519 0,96905 0,20827 0,06798 0,00031 0,97256 0,65606 0,26565 0,39055 0,44166 0,33638 0,48989 0,53771
CONTROL	R H K D E S T N Q A I L V F W Y	177,641 104,238 150,159 139,253 207,541 278,938 145,228 101,854 127,543 275,145 131,641 197,538 164,256 145,28 81,3488 137,628	sum 1 1 1 1 0,99994 1 1 0,99997 1 1 1 1 1 1 1 1 1 1 1 1 1	chisq sum -13,522 -16,265 -13,8913 5,40623 -42,7289 62,0444 13,1402 -5,51324 -19,6212 5,67527 -22,2844 -28,5685 -13,4159 -9,19004 9,13389 -6,54392	chisq sum 0,48501 0,40095 0,47079 0,78011 0,02735 0,00136 0,49742 0,7747 0,31095 0,76947 0,2492 0,14014 0,48844 0,63305 0,73405	R H K D E S T N Q A I L V F W Y	27,4672 30,0401 16,3777 25,0269 23,9834 29,437 25,8714 16,039 12,2343 56,5231 24,5822 22,4132 23,582 20,2589 14,2621 19,4967	sum 0,99931 0,99756 1 0,99983 0,99991 0,99915 0,99972 1 0,41786 0,99987 0,99997 0,99993 1 1 1 1	sum 9,04872 5,29145 -13,4226 -10,3959 -15,9465 4,80842 6,54869 -2,09914 -6,35625 10,0478 -10,6552 -2,84671 -4,91324 -4,13733 6,06124 -14,9079	chisq sum 0,22242 0,47554 0,06776 0,16098 0,03154 0,51675 0,37722 0,77514 0,3914 0,17547 0,15079 0,70109 0,50765 0,57693 0,41376 0,04441	R H K D E S T N Q A I L V F W Y	42,351 11,4542 22,1346 24,2952 25,6805 40,2188 16,4064 14,2222 24,9884 36,8012 21,1974 34,1793 35,4584 24,7953 11,9919 19,1168	sum 0,99967 1 1 1 0,99988 1 1 0,99998 1 0,99998 1 1	sum -1,7648 -0,46625 -1,06981 -3,77318 1,61052 1,16952 6,0564 3,48531 4,05136 8,98436 0,31489 -10,7669 2,2388 -14,6199 -2,84992 -2,46614	chisq sum 0,84162 0,9579 0,90297 0,66921 0,8553 0,89465 0,49287 0,69123 0,64643 0,30902 0,97156 0,2228 0,79989 0,09785 0,74535 0,77868	R H K D E S T N Q A I L V F W Y	107,823 62,7435 111,647 89,9305 157,877 209,282 102,95 71,5924 90,3204 181,821 85,8615 140,945 105,216 100,225 55,0948 99,0148	sum 1 1 1 0,99999 0,93691 1 1 0,99852 1 1 1 1 1 1 1 1 1 1 1 1 1	sum -20,8059 -21,0902 0,60112 19,5753 -28,3929 56,0665 0,53511 -6,89941 -17,3163 -13,3569 -11,9441 -14,9549 -10,7415 9,5672 5,92257 10,8301	chisq sum 0,18107 0,17519 0,96905 0,20827 0,06798 0,00031 0,97256 0,65606 0,26565 0,39055 0,44166 0,33638 0,48989 0,53771 0,69863 0,48359
CONTROL	R H K D E S T N Q A I L V F W Y M	177,641 104,238 150,159 139,253 207,541 278,938 101,854 127,543 275,145 131,641 197,538 164,256 145,28 81,3488 137,628 85,777	sum 1 1 1 1 0,99994 1 1 0,99997 1 1 1 1 1 1 1 1 1 1 1 1 1	chisq sum -13,522 -16,265 -13,8913 5,40623 -42,7289 62,0444 13,1402 -5,51324 -19,6212 5,67527 -22,2844 -28,5685 -13,4159 -9,19004 9,13389 -6,54392 -19,9739	chisq sum 0,48501 0,40095 0,47079 0,78011 0,02735 0,00136 0,49742 0,7747 0,31095 0,76947 0,2492 0,14014 0,48844 0,63464 0,63305 0,73405 0,29778	R H K D E S T N Q Q A I L V V F W Y Y	27,4672 30,0401 16,3777 25,0269 23,9834 29,437 25,8714 16,039 12,2343 56,5231 24,5822 22,4132 23,582 20,2589 14,2621 19,4967 17,7007	sum 0,99931 0,99756 1 0,99983 0,99991 0,99915 0,99972 1 0,41786 0,99987 0,99997 0,99993 1 1 1 1 1 1	sum 9,04872 5,29145 -13,4226 -10,3959 -15,9465 4,80842 6,54869 -2,09914 -6,35625 10,0478 -10,6552 -2,84671 -4,91324 -4,13733 6,06124 -14,9079 0,666401	chisq sum 0,22242 0,47554 0,06776 0,16098 0,03154 0,51675 0,37722 0,77514 0,3914 0,17547 0,15079 0,70109 0,50765 0,57693 0,41376 0,04441 0,928	R H K D E S T N Q Q A I L V F W Y Y	42,351 11,4542 22,1346 24,2952 25,6805 40,2188 16,4064 14,2222 24,9884 36,8012 21,1974 34,1793 35,4584 24,7953 11,9919 19,1168 14,7746	sum 0,99967 1 1 1 0,99988 1 1 0,99998 1 0,99998 1 1	sum -1,7648 -0,46625 -1,06981 -3,77318 1,61052 1,16952 6,0564 3,48531 4,05136 8,98436 0,31489 -10,7669 2,2388 -14,6199 -2,84992 -2,46614 -5,19602	chisq sum 0,84162 0,9579 0,90297 0,66921 0,8553 0,89465 0,49287 0,69123 0,64643 0,30902 0,97156 0,2228 0,79989 0,09785 0,74535 0,77868 0,55376	R H K D E S T N Q A I L V V F W Y Y	107,823 62,7435 111,647 89,9305 157,877 209,282 102,95 71,5924 90,3204 181,821 85,8615 140,945 105,216 100,225 55,0948 99,0148 53,3018	sum 1 1 1 0,99999 0,93691 1 1 0,99852 1 1 1 1 1 1 1 1 1 1 1 1 1	sum -20,8059 -21,0902 0,60112 19,5753 -28,3929 56,0665 0,53511 -6,89941 -17,3163 -13,3569 -11,9441 -14,9549 -10,7415 9,5672 5,92257 10,8301 -15,4419	chisq sum 0,18107 0,17519 0,96905 0,20827 0,06798 0,00031 0,97256 0,65606 0,26565 0,39055 0,44166 0,33638 0,48989 0,53771 0,69863 0,48359 0,31583

S12: Putative transit peptide region amino acid composition analysis results: The predicted "TP" region was compared to the predicted mature chain of the same sequence. This was performed a) for both experimental and control protein sets (two rows of tables), b) for the whole sets of 375 proteins regardless of their classification as well as for each subset representing a class of pre-proteins (four columns of tables), and c) for each amino acid (twenty rows in each table). χ^2 sums and their respective *p*-values, as well as normalized χ^2 sums (sum of χ^2 with plus or minus sign depending on the positive or negative value of its residual) and their *p*-values are shown in each table. *P*-values lower than 0.01 are colored in yellow, *p*-values lower than 0.00001 are colored in light orange.



S13: Graphical representation of normalized χ^2 sums for each amino acid in the TP vs. mature chain comparison in the two sets: positive or negative value reflects whether the amino acid frequency in the "TP" region is higher or lower than expected, colored bars represent statistically significant differencies with *p*<0.01 (lighter blue) and *p*<0.00001 (darker blue).