Supplementary information

Accessing the genomic information of unculturable oceanic picoeukaryotes by combining multiple single cells

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Supplementary Tables

Table S1. Main physical-chemical characteristics of the two sampled stations.

Stations	Coordinates	Sampling date	Depth (m)	Temperature (°C)	Oxygen (µmol kg^{-1})	Salinity (psu)	Chlorophyll (mg $Chl.m^{-3}$)
23	42° 10’ 12" N, 17° 43' 12" E	18/11/2009	55	15.7	224.3	38.4	0.06
41	14° 33' 36" N, 70° 0' 36" E	30/03/2010	58	27.1	148.3	36.5	0.47

Table S2. General sampling and sequencing characteristics of the different individual SAGs of MAST-4A and MAST-4E.

Species	SAGs ID	Stations*	Sequencing depth (Gbp)	Sequencing platforms	CV**
MAST-4A	AA538-M19	23	6.9	Hiseq (Genoscope)	
	AA538-N22	23	8.5	Hiseq (Genoscope)	
	AA538-F10	23	6.0	Hiseq (Genoscope)	
	AA538-G04	23	4.7	Hiseq (Genoscope)	
	AA538-G20†	23	4.6	Hiseq (Genoscope)	
	AA538-K07	23	4.0	Hiseq (Genoscope)	
	AA538-E21	23	5.7	Hiseq (Genoscope)	
	AA538-C11	23	2.7	Hiseq (Oregon)	
	AA538-E15	23	6.4	Hiseq (Genoscope)	
	AB537-A17	41	4.0	Hiseq (Oregon)	
	AA538-E19	23	2.4	Hiseq (Oregon)	
	AA538-G20_bis†	23	6.8	Hiseq (Oregon)	
	AA538-J18	23	4.8	Hiseq (Genoscope)	
	AB537-K04	41	3.5	Hiseq (Oregon)	
MAST-4E	AA538-A02	23	4.5	Hiseq (Genoscope)	
	AA538-A03	23	4.5	Hiseq (Genoscope)	
	AA538-C05	23	4.6	Hiseq (Genoscope)	
	AA538-F08	23	4.0	Hiseq (Genoscope)	
	AA538-J09	23	4.7	Hiseq (Genoscope)	
	AA538-A11	23	6.8	Hiseq (Genoscope)	
	AA538-L23	23	4.4	Hiseq (Genoscope)	
	AA538-M11	23	4.2	Hiseq (Genoscope)	
	AA538-N16	23	4.9	Hiseq (Genoscope)	
Mean (SE)	all MAST-4A SAGs		5.1 (1.7)		34.0%
	all MAST-4E SAGs		4.7 (0.8)		17.3%
	all SAGs		4.9 (1.4)		28.8%

* Stations 23 and 41 are located in the Mediterranean sea (Adriatic Sea) and Indian Ocean (Arabic Sea), respectively (http://taraoceans.sb-roscoff.fr/EukDiv/#figureW1).

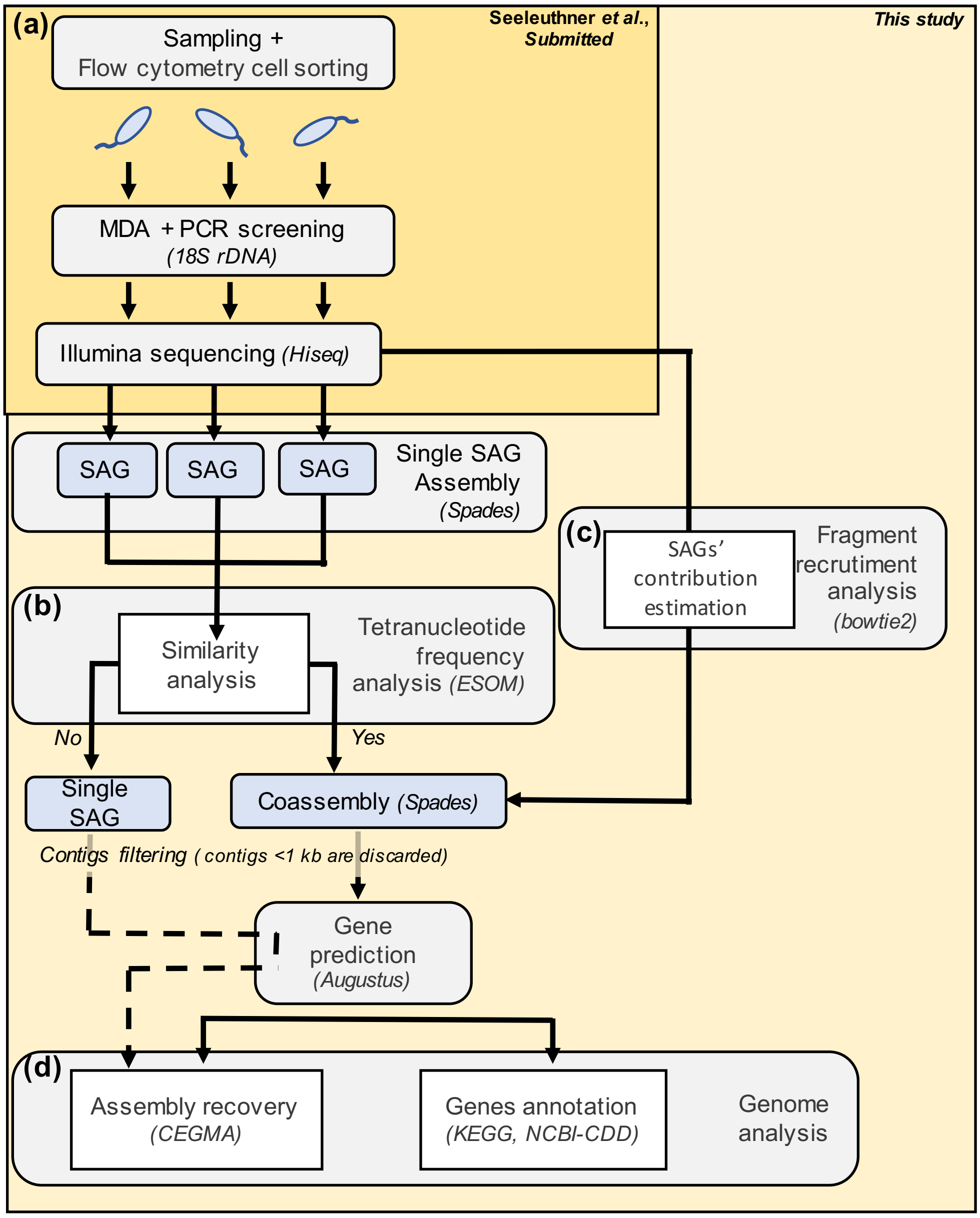
** CV: Coefficient of variation = Standard error/mean.

† SAG sequenced by two different sequencing centers, the two sequencing replicates (AA538_G20 and AA538_G20_bis) were kept for further analysis.

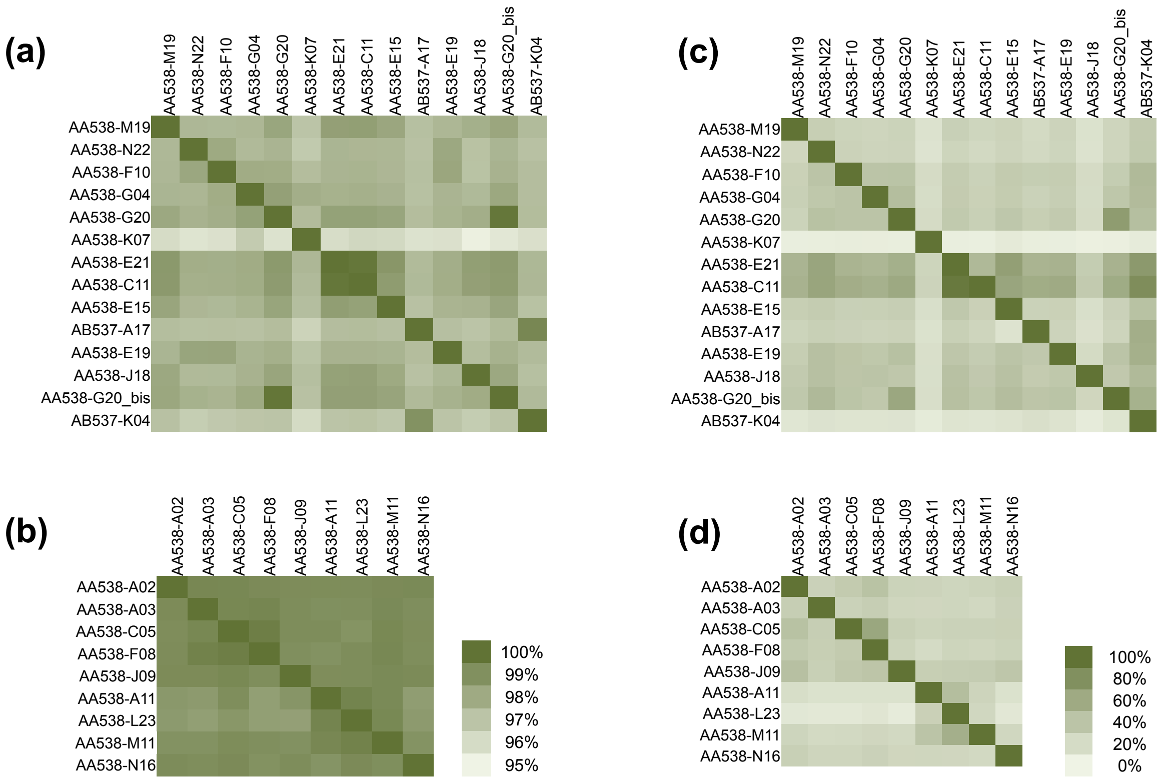
Table S3. General functions present in MAST-4A and MAST-4E genomes based on protein classification according to KOGs of the 248 universal CEGMA eukaryotic genes.

Functioning Process	General Functions	Number of COGs expected	Number of COGs in MAST-4A	Number of COGs in MAST-4E
Information storage and processing	Translation, ribosomal structure and biogenesis	34	29	25
	RNA processing and modification	23	18	16
	Transcription	13	6	9
	Replication, recombination and repair	10	9	7
	Chromatin structure and dynamics	0	0	0
	Shared functions	6	5	4
Cellular processes and signalling	Cell cycle control, cell division, chromosome partitioning	2	2	2
	Nuclear structure	0	0	0
	Defence mechanisms	0	0	0
	Signal transduction mechanisms	4	3	3
	Cell wall/membrane/envelope biogenesis	1	0	0
	Cell motility	0	0	0
	Cytoskeleton	3	2	2
	Extracellular structures	0	0	0
	Intracellular trafficking, secretion, and vesicular transport	17	13	13
	Posttranslational modification, protein turnover, chaperones	42	30	31
	Shared functions	6	3	2
Metabolism	Energy production and conversion	22	13	12
	Carbohydrate transport and metabolism	11	8	9
	Amino acid transport and metabolism	2	2	2
	Nucleotide transport and metabolism	6	4	4
	Coenzyme transport and metabolism	2	2	1
	Lipid transport and metabolism	5	3	2
	Inorganic ion transport and metabolism	3	3	0
	Secondary metabolites biosynthesis, transport and catabolism	1	1	1
	Shared functions	4	4	4
Poorly characterized	General function prediction only	19	15	13
	Function unknown	6	4	3
Shared between different functioning process		6	5	4

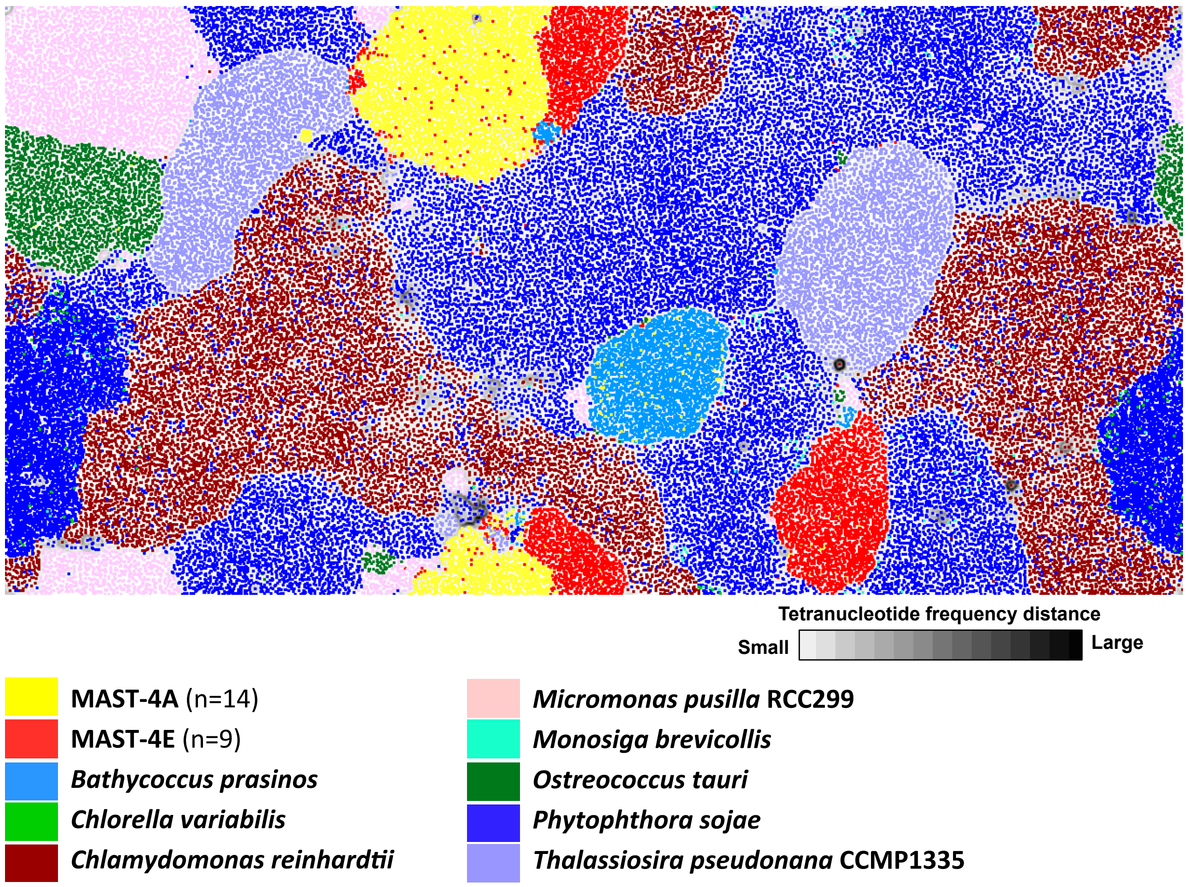
Supplementary Figures



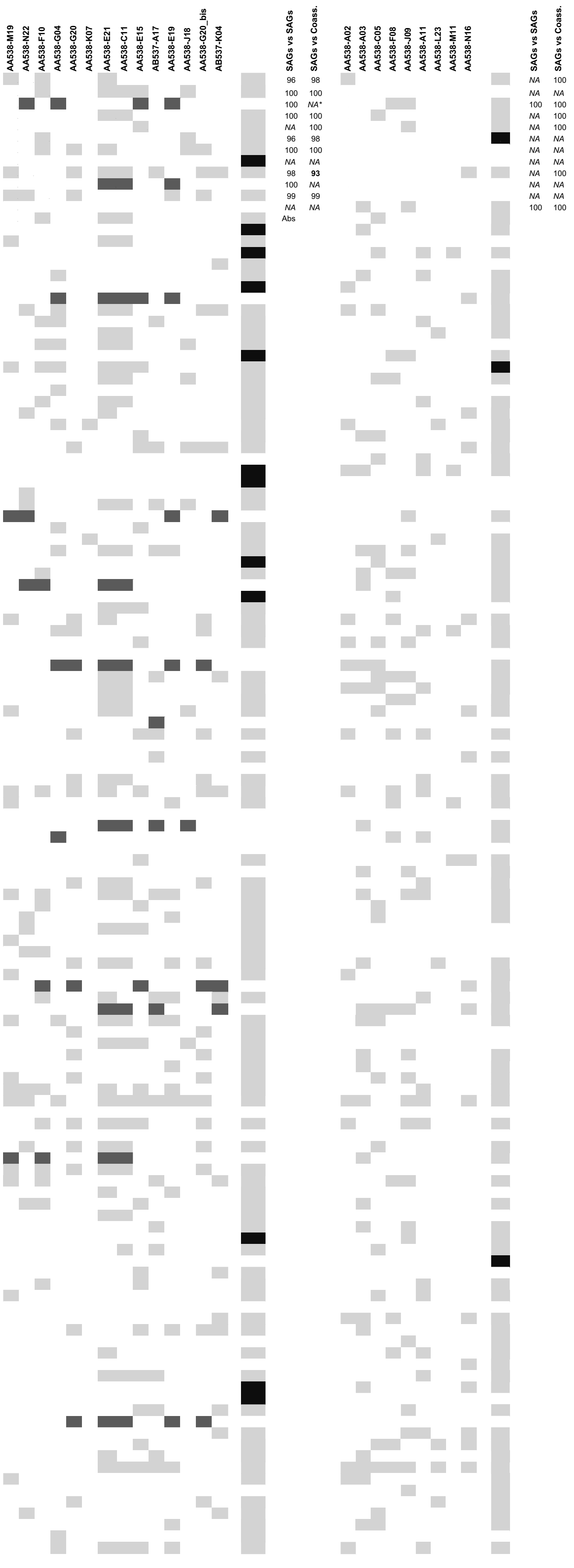
Supplementary Fig. S1. Schematic pipeline of a single-cells co-assembly performed in this study. Details on the sampling, single-cell sorting and SAG sequencing will be available in a concomitant study (Seeleuthner *et al.*, submitted). The rest of the main steps of our co-assembly strategy are described in this paper.



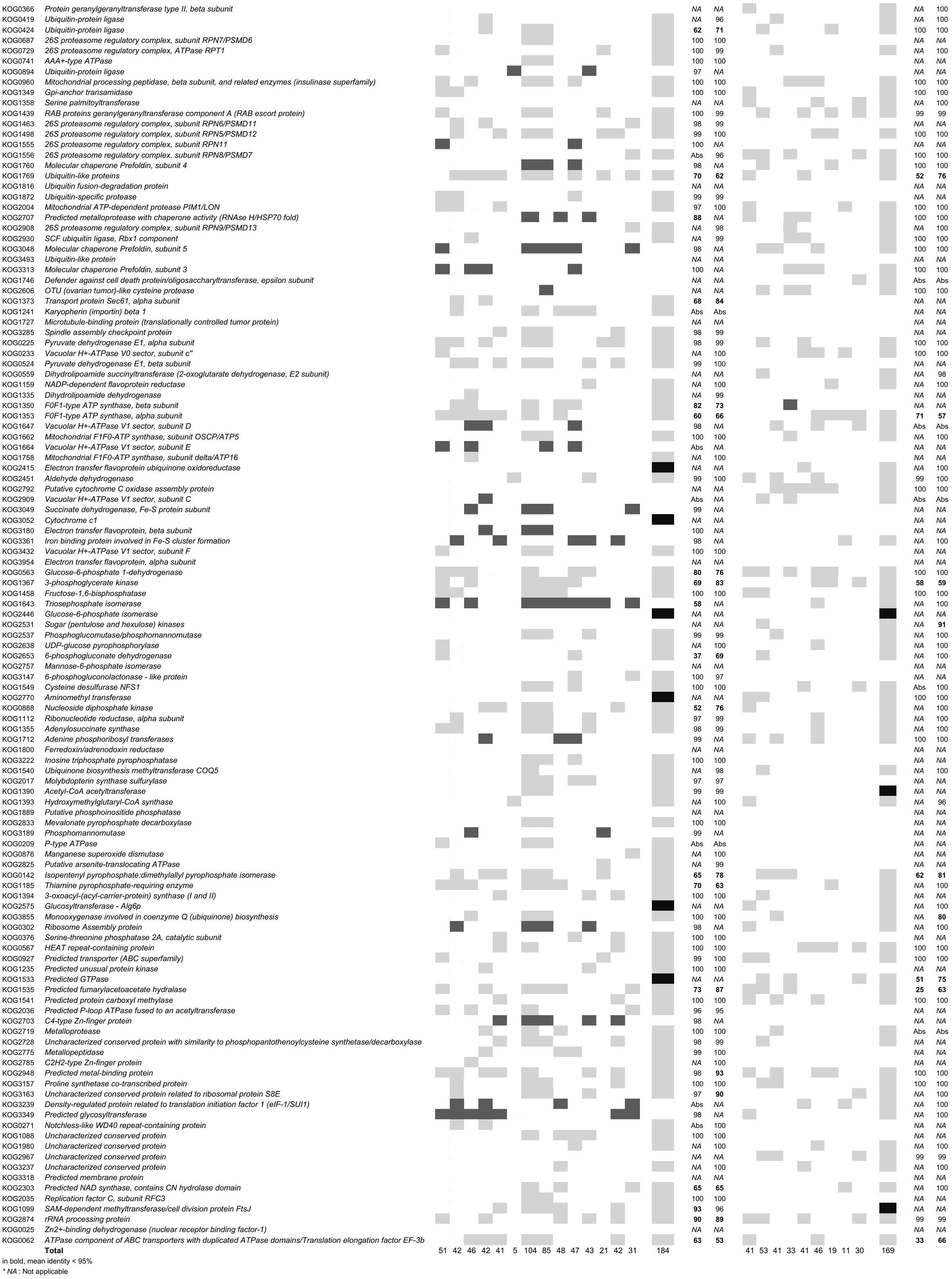
Supplementary Fig. S2. Cross-SAG Blast analysis between MAST-4A and MAST-4E SAGs. Mean pairwise genomic similarity of MAST-4A (**a**) and MAST-4E (**b**) SAGs are represented, together with the percentage of shared nucleotidic regions for MAST-4A (**c**) and MAST-4E (**d**). Values derive from blasting full-length contigs of each SAG against full-length contigs of each sister SAG. Query and subject SAGs are listed in the left and top of each heatmap, respectively.



Supplementary Fig. S3. Comparing tetranucleotide frequencies among selected genomes in an ESOM map. Published protist genomes belonging to separate supergroups are combined together with MAST-4A and MAST-4E SAGs. Bestmatches of contigs of 2.5-5 kbp in size are represented by individual points, coloured according to their provenance as MAST-4A (yellow), MAST-4E (red), *Ostreoccocus tauri* (dark green), *Micromonas pusilla* (light blue), *Bathycoccus prasinos* (blue), *Chlorella variabilis* (light green), *Chlamydomonas reinhardtii* (dark red), *Thalassiosira pseudonana* (purple), *Phytophtora sojae* (dark blue) and *Monosiga brevicollis* (pink). Large differences in tetranucleotide frequencies represent natural divisions between taxonomic groups.



KOG_ID	**Coding proteins**	**SAGs vs SAGs**	**SAGs vs Coass.**	**SAGs vs SAGs**	**SAGs vs Coass.**
KOG0002	*60s ribosomal protein L39*	96	98	*NA*	100
KOG0400	*40S ribosomal protein S13*	100	100	*NA*	*NA*
KOG0122	*Translation initiation factor 3 subunit g (eIF-3g)*	100	*NA**	100	100
KOG0188	*Alanyl-tRNA synthetase*	100	100	*NA*	100
KOG0434	*Isoleucyl-tRNA synthetase*	*NA*	100	*NA*	100
KOG0462	*Elongation factor-type GTP-binding protein*	96	98	*NA*	*NA*
KOG0466	*Translation initiation factor 2 gamma subunit (eIF-2gamma; GTPase)*	100	100	*NA*	*NA*
KOG0469	*Elongation factor 2*	*NA*	*NA*	*NA*	*NA*
KOG0556	*Aspartyl-tRNA synthetase*	98	**93**	*NA*	100
KOG0650	*WD40 repeat nucleolar protein Bop1, involved in ribosome biogenesis*	100	*NA*	*NA*	*NA*
KOG0688	*Peptide chain release factor 1 (eRF1)*	99	99	*NA*	*NA*
KOG0815	*60S acidic ribosomal protein P0*	*NA*	*NA*	100	100
KOG1068	*Exosomal 3'-5' exoribonuclease complex, subunit Rrp41 and related exoribonucleases*	Abs			

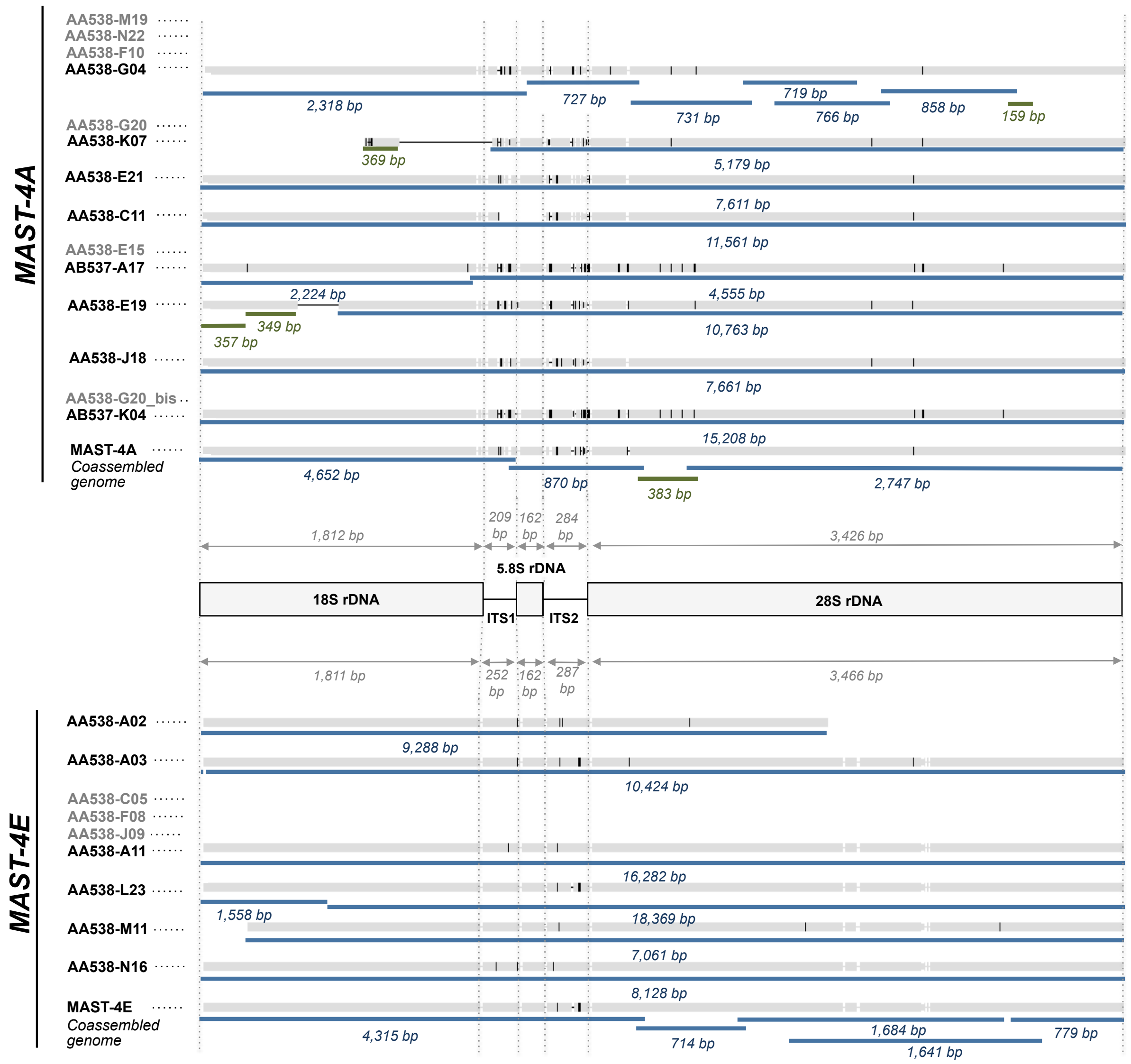


KOG0366	*Protein geranylgeranyltransferase type II, beta subunit*	*NA*	*NA*	*NA*	100
KOG0419	*Ubiquitin-protein ligase*	*NA*	96	*NA*	100
KOG0424	*Ubiquitin-protein ligase*	**62**	**71**	100	100
KOG0687	*26S proteasome regulatory complex, subunit RPN7/PSMD6*	100	100	*NA*	*NA*
KOG0729	*26S proteasome regulatory complex, ATPase RPT1*	100	99	*NA*	100
KOG0741	*AAA+-type ATPase*	100	100	*NA*	*NA*
KOG0894	*Ubiquitin-protein ligase*	97	*NA*	*NA*	*NA*
KOG0960	*Mitochondrial processing peptidase, beta subunit, and related enzymes (insulinase superfamily)*	100	100	100	100
KOG1349	*Gpi-anchor transamidase*	100	100	100	100
KOG1358	*Serine palmitoyltransferase*	*NA*	*NA*	*NA*	100
KOG1439	*RAB proteins geranylgeranyltransferase component A (RAB escort protein)*	100	99	99	99
KOG1463	*26S proteasome regulatory complex, subunit RPN6/PSMD11*	98	99	*NA*	*NA*
KOG1498	*26S proteasome regulatory complex, subunit RPN5/PSMD12*	99	100	100	100
KOG1555	*26S proteasome regulatory complex, subunit RPN11*	100	*NA*	*NA*	*NA*
KOG1556	*26S proteasome regulatory complex, subunit RPN8/PSMD7*	Abs	96	100	100
KOG1760	*Molecular chaperone Prefoldin, subunit 4*	98	*NA*	100	100
KOG1769	*Ubiquitin-like proteins*	**70**	**62**	**52**	**76**
KOG1816	*Ubiquitin fusion-degradation protein*	*NA*	*NA*	*NA*	*NA*
KOG1872	*Ubiquitin-specific protease*	99	99	*NA*	*NA*
KOG2004	*Mitochondrial ATP-dependent protease PIM1/LON*	97	100	100	100
KOG2707	*Predicted metalloprotease with chaperone activity (RNAse H/HSP70 fold)*	**88**	*NA*	100	100
KOG2908	*26S proteasome regulatory complex, subunit RPN9/PSMD13*	*NA*	98	*NA*	100
KOG2930	*SCF ubiquitin ligase, Rbx1 component*	*NA*	99	100	100
KOG3048	*Molecular chaperone Prefoldin, subunit 5*	98	*NA*	100	100
KOG3493	*Ubiquitin-like protein*	*NA*	*NA*	*NA*	*NA*
KOG3313	*Molecular chaperone Prefoldin, subunit 3*	100	*NA*	100	100
KOG1746	*Defender against cell death protein/oligosaccharyltransferase, epsilon subunit*	*NA*	*NA*	Abs	Abs
KOG2606	*OTU (ovarian tumor)-like cysteine protease*	*NA*	*NA*	100	100
KOG1373	*Transport protein Sec61, alpha subunit*	**68**	**84**	*NA*	*NA*
KOG1241	*Karyopherin (importin) beta 1*	Abs	Abs	*NA*	*NA*
KOG1727	*Microtubule-binding protein (translationally controlled tumor protein)*	*NA*	*NA*	*NA*	*NA*
KOG3285	*Spindle assembly checkpoint protein*	98	99	*NA*	*NA*
KOG0225	*Pyruvate dehydrogenase E1, alpha subunit*	98	99	100	100
KOG0233	*Vacuolar H+-ATPase V0 sector, subunit c''*	*NA*	100	100	100
KOG0524	*Pyruvate dehydrogenase E1, beta subunit*	99	100	*NA*	*NA*
KOG0559	*Dihydrolipoamide succinyltransferase (2-oxoglutarate dehydrogenase, E2 subunit)*	*NA*	*NA*	*NA*	98
KOG1159	*NADP-dependent flavoprotein reductase*	*NA*	100	*NA*	100
KOG1335	*Dihydrolipoamide dehydrogenase*	*NA*	99	*NA*	*NA*
KOG1350	*F0F1-type ATP synthase, beta subunit*	**82**	**73**	*NA*	*NA*
KOG1353	*F0F1-type ATP synthase, alpha subunit*	**60**	**66**	**71**	**57**
KOG1647	*Vacuolar H+-ATPase V1 sector, subunit D*	98	*NA*	Abs	Abs
KOG1662	*Mitochondrial F1F0-ATP synthase, subunit OSCP/ATP5*	100	100	*NA*	100
KOG1664	*Vacuolar H+-ATPase V1 sector, subunit E*	Abs	*NA*	*NA*	*NA*
KOG1758	*Mitochondrial F1F0-ATP synthase, subunit delta/ATP16*	*NA*	100	*NA*	*NA*
KOG2415	*Electron transfer flavoprotein ubiquinone oxidoreductase*	*NA*	*NA*	*NA*	100
KOG2451	*Aldehyde dehydrogenase*	99	100	99	100
KOG2792	*Putative cytochrome C oxidase assembly protein*	*NA*	*NA*	100	100
KOG2909	*Vacuolar H+-ATPase V1 sector, subunit C*	Abs	*NA*	Abs	Abs
KOG3049	*Succinate dehydrogenase, Fe-S protein subunit*	99	*NA*	*NA*	*NA*
KOG3052	*Cytochrome c1*	*NA*	*NA*	*NA*	*NA*
KOG3180	*Electron transfer flavoprotein, beta subunit*	100	*NA*	*NA*	*NA*
KOG3361	*Iron binding protein involved in Fe-S cluster formation*	98	*NA*	*NA*	100
KOG3432	*Vacuolar H+-ATPase V1 sector, subunit F*	100	100	*NA*	*NA*
KOG3954	*Electron transfer flavoprotein, alpha subunit*	*NA*	*NA*	*NA*	*NA*
KOG0563	*Glucose-6-phosphate 1-dehydrogenase*	**80**	**76**	100	100
KOG1367	*3-phosphoglycerate kinase*	**69**	**83**	**58**	**59**
KOG1458	*Fructose-1,6-bisphosphatase*	100	100	100	100
KOG1643	*Triosephosphate isomerase*	**58**	*NA*	*NA*	100
KOG2446	*Glucose-6-phosphate isomerase*	*NA*	*NA*	*NA*	*NA*
KOG2531	*Sugar (pentulose and hexulose) kinases*	*NA*	*NA*	*NA*	**91**
KOG2537	*Phosphoglucomutase/phosphomannomutase*	99	99	*NA*	100
KOG2638	*UDP-glucose pyrophosphorylase*	*NA*	100	*NA*	100
KOG2653	*6-phosphogluconate dehydrogenase*	**37**	**69**	*NA*	100
KOG2757	*Mannose-6-phosphate isomerase*	*NA*	*NA*	*NA*	*NA*
KOG3147	*6-phosphogluconolactonase - like protein*	100	97	*NA*	*NA*
KOG1549	*Cysteine desulfurase NFS1*	100	100	Abs	100
KOG2770	*Aminomethyl transferase*	*NA*	*NA*	100	100
KOG0888	*Nucleoside diphosphate kinase*	**52**	**76**	*NA*	100
KOG1112	*Ribonucleotide reductase, alpha subunit*	97	99	*NA*	100
KOG1355	*Adenylosuccinate synthase*	98	99	*NA*	100
KOG1712	*Adenine phosphoribosyl transferases*	99	*NA*	100	100
KOG1800	*Ferredoxin/adrenodoxin reductase*	*NA*	*NA*	*NA*	*NA*
KOG3222	*Inosine triphosphate pyrophosphatase*	100	100	*NA*	*NA*
KOG1540	*Ubiquinone biosynthesis methyltransferase COQ5*	*NA*	98	*NA*	100
KOG2017	*Molybdopterin synthase sulfurylase*	97	97	*NA*	*NA*
KOG1390	*Acetyl-CoA acetyltransferase*	99	99	*NA*	*NA*
KOG1393	*Hydroxymethylglutaryl-CoA synthase*	*NA*	100	*NA*	96
KOG1889	*Putative phosphoinositide phosphatase*	*NA*	*NA*	*NA*	*NA*
KOG2833	*Mevalonate pyrophosphate decarboxylase*	100	100	*NA*	*NA*
KOG3189	*Phosphomannomutase*	99	*NA*	*NA*	*NA*
KOG0209	*P-type ATPase*	Abs	Abs	*NA*	*NA*
KOG0876	*Manganese superoxide dismutase*	*NA*	100	*NA*	*NA*
KOG2825	*Putative arsenite-translocating ATPase*	*NA*	99	*NA*	*NA*
KOG0142	*Isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase*	**65**	**78**	**62**	**81**
KOG1185	*Thiamine pyrophosphate-requiring enzyme*	**70**	**63**	*NA*	100
KOG1394	*3-oxoacyl-(acyl-carrier-protein) synthase (I and II)*	100	100	*NA*	*NA*
KOG2575	*Glucosyltransferase - Alg6p*	*NA*	*NA*	*NA*	100
KOG3855	*Monooxygenase involved in coenzyme Q (ubiquinone) biosynthesis*	100	100	*NA*	**80**
KOG0302	*Ribosome Assembly protein*	98	*NA*	*NA*	100
KOG0376	*Serine-threonine phosphatase 2A, catalytic subunit*	100	100	*NA*	*NA*
KOG0567	*HEAT repeat-containing protein*	100	100	100	100
KOG0927	*Predicted transporter (ABC superfamily)*	99	100	100	100
KOG1235	*Predicted unusual protein kinase*	100	100	*NA*	*NA*
KOG1533	*Predicted GTPase*	*NA*	*NA*	**51**	**75**
KOG1535	*Predicted fumarylacetoacetate hydralase*	**73**	**87**	**25**	**63**
KOG1541	*Predicted protein carboxyl methylase*	100	100	100	100
KOG2036	*Predicted P-loop ATPase fused to an acetyltransferase*	96	95	*NA*	*NA*
KOG2703	*C4-type Zn-finger protein*	98	*NA*	*NA*	*NA*
KOG2719	*Metalloprotease*	100	100	Abs	Abs
KOG2728	*Uncharacterized conserved protein with similarity to phosphopantothenoylcysteine synthetase/decarboxylase*	98	99	*NA*	*NA*
KOG2775	*Metallopeptidase*	99	100	*NA*	*NA*
KOG2785	*C2H2-type Zn-finger protein*	*NA*	100	*NA*	*NA*
KOG2948	*Predicted metal-binding protein*	98	**93**	100	100
KOG3157	*Proline synthetase co-transcribed protein*	100	100	100	100
KOG3163	*Uncharacterized conserved protein related to ribosomal protein S8E*	97	**90**	*NA*	100
KOG3239	*Density-regulated protein related to translation initiation factor 1 (eIF-1/SUI1)*	Abs	*NA*	*NA*	100
KOG3349	*Predicted glycosyltransferase*	98	*NA*	*NA*	100
KOG0271	*Notchless-like WD40 repeat-containing protein*	Abs	100	*NA*	*NA*
KOG1088	*Uncharacterized conserved protein*	100	100	*NA*	*NA*
KOG1980	*Uncharacterized conserved protein*	*NA*	100	*NA*	100
KOG2967	*Uncharacterized conserved protein*	*NA*	*NA*	99	99
KOG3237	*Uncharacterized conserved protein*	*NA*	100	*NA*	100
KOG3318	*Predicted membrane protein*	*NA*	*NA*	*NA*	*NA*
KOG2303	*Predicted NAD synthase, contains CN hydrolase domain*	**65**	**65**	*NA*	100
KOG2035	*Replication factor C, subunit RFC3*	100	100	*NA*	*NA*
KOG1099	*SAM-dependent methyltransferase/cell division protein FtsJ*	**93**	96	*NA*	*NA*
KOG2874	*rRNA processing protein*	**90**	**89**	99	99
KOG0025	*Zn2+-binding dehydrogenase (nuclear receptor binding factor-1)*	*NA*	*NA*	*NA*	*NA*
KOG0062	*ATPase component of ABC transporters with duplicated ATPase domains/Translation elongation factor EF-3b*	**63**	**53**	**33**	**66**
	Total				

in bold, mean identity < 95%

* *NA* : Not applicable

Supplementary Fig. S4. Identification of the 248 CEGs within SAGs and co-assemblies of both lineages. The presence of CEGs among SAGs and co-assembly (light grey) or solely among SAGs (dark grey) or co-assembly (black) are here listed. The mean amino acid identities of the retrieved CEGs were calculated among SAGs (“SAGs vs SAGs”) and between SAGs and co-assembly (“SAGs vs Coass.”).



Supplementary Fig. S5. Retrieval of the rDNA operon in SAGs of the two MAST-4 lineages. Sequences of MAST-4A (top) and MAST-4E (bottom) individual SAGs containing the rDNA operon were aligned with their corresponding co-assembled genomes. SAGs without rDNA operon contigs are shown in grey. The position and length of contigs from each SAG and co-assemblies necessary to reconstruct the rDNA operon are shown (contigs <500 bp in green and >500 bp in blue). Differences in individual SAGs against the consensus sequence are marked.