SUPPLEMENTARY RESULTS

Archaeplastids

The prasinophyte *Micromonas pusilla* (CCMP1545) was selected for reverse genetics, as members of the genus *Micromonas* are abundant in marine ecosystems and have larger genomes than *Bathycoccus* and *Ostreococcus*, in addition to having a flagellum[1,2]. Although *M. pusilla* is typically less stable in culture in an axenic state than *M. commoda* (RCC299/NOUM17; deposited at the NCMA after being rendered axenic and genome sequenced as CCMP2709), it was initially selected for manipulation due to its interesting genomic features[3]. The procedures that worked for *Ostreococcus*[4] and *Bathycoccus* (this work) failed with *M. pusilla*. Furthermore, we made multiple attempts to use electroporation and bacterial conjugation (ATCC agrobacterium strain AGL-1) where we also monitored cells to ensure they were in exponential growth at the start of each experiment. We constructed plasmids with a codon-optimized chloramphenicol acetyltransferase (CAT) or eGFP-SV40NLS genes driven by *M. pusilla* RPS9 elements in between Ti-plasmid elements required for *Agrobacterium*-mediated transfer in the pOSCAR plasmid system[5]. We determined *M. pusilla* sensitivity to chloramphenicol by testing a range of concentrations (**Suppl. Table 3**). While it is difficult to say exactly why these efforts failed, we did not observe GFP fluorescence nor did chloramphenicol resistant cells emerge from culture. Because of the persistence of *Agrobacterium* in the culture after transfer, analysis by flow cytometry and recovering axenic cultures was challenging. In all assays where DNA was added directly to cultures, it was in supercoiled form as isolated from *E. coli*. Lonza nucleofection was not attempted with *M. pusilla* due to toxicity to all three Lonza buffers (SF, SG, SE), at least under the conditions tested herein, while buffers SF and SG allowed growth of *M. commoda* (SE was toxic). It should be noted that *M. commoda* and *M. pusilla* have major differences, for example, the latter retains the peptidoglycan pathway (peptidoglycan layer surrounding the plastid), while the former has lost most of this pathway like *Arabidopsis thaliana*[2]. This influences antibiotic usage as many of them work on this pathway. Furthermore, *M. pusilla* has some known RNAi genes while *M. commoda* does not.

Tetraselmis striata (KAS-836) is a member of the class Chlorodendrophyceae but the phylogenetic relationship of this group within the green algae remain unclear[6]. Recent chloroplast phylogenomic analyses support the notion that Chlorodendrophyceae is an early lineage of the core Chlorophyta (and no longer part of Prasinophyceae), although its precise placement relative to other chlorophyte lineages could not be resolved[7,8]. Therefore, we selected *T. striata* for genetic transformation, since the development of genetic tools may eventually contribute to understanding its biological features and provide additional evidence for its phylogenetic position. *T. striata* has been successfully transformed using microprojectile bombardment (**Suppl. Fig. 3**). Two selectable markers were tested, consisting of a putative promoter and 5' UTR sequences from the *T. striata* actin gene and either the coding sequences of the *Streptoalloteichus hindustanus* bleomycin gene (conferring resistance to zeocin) or the *Streptomyces hygroscopicus* bar gene (conferring resistance to glufosinate) (**Suppl. Table 3; Suppl. Fig. 3)**. The terminator sequence was obtained from the *T. striata g*lyceraldehyde-3-phosphate dehydrogenase gene. Linearized plasmids were coated on gold particles and introduced into *T. striata* cells by using the PDS-1000/He Particle Delivery System (BioRad). Transformants were successfully selected on half-strength f/2 (seawater-distilled water in a 1:1 volume ratio) agar plates containing either 150 μg/ml zeocin or 150 μg/ml glufosinate. In contrast, despite repeated attempts to introduce these markers

into the *T. striata* cells by square wave electric pulses using the NEPA21 electroporator (Nepa Gene), we were unable to recover surviving colonies on the selective plates.

Pyramimonas parkeae (SCCAP K-0007), class I prasinophyte alga[9], was targeted by mRNA encoding firefly luciferase and by two circular plasmids. The cells were electroporated by Gene Pulser xCell (BioRad) with 5 μg of *in vitro*-synthesized firefly luciferase mRNA using exponential pulse at 8 different combinations of voltage and capacitance settings (**Suppl. Table 4**). Light emission was measured after 4.5, 5.5 and 22 h, yet no specific luciferase activity could be detected. Before we conducted the genetic transformations, the sensitivity to 5 antibiotics was tested. Puromycin and geneticin were identified to be most effective at a concentration of 30 μg/ml each (**Suppl. Table 3**). Two plasmids containing 5' and 3' UTRs from the *P. parkeae* histone H2 gene were synthesized, with one containing the geneticin resistance gene, while the other plasmid carried the firefly luciferase and puromycin resistance genes. The former gene was flanked by the 5′and 3 UTR's of the native histone H4 gene. Both circular and linearized plasmids were delivered by electroporation using Gene Pulser xCell or Amaxa Nucleofector II (Lonza) (**Suppl. Table 4**). However, no light emission was measured in the cultures post DNA delivery, and no cells were selected even after 4 weeks of growth on selective liquid F2 medium. Subsequently, we have tried to deliver fluorescein- or tetramethylrhodamine-labelled DNA by electroporation or nucleofection as described above, with various settings (**Suppl. Table 4**), as well as by biolistics (PDS-1000/He [BioRad]; 0.6 μm particles; 1250 psi) to test the DNA delivery efficiency. Nucleofection resulted in fluorescent signal from inside the cells suggesting the DNA delivery was succesful (**Suppl. Fig. 4**), although expression was not achieved.

Haptophytes *(incertae sedis)*
Emiliania huxleyi (see Transformation of natural protist communities)

Rhizarians
Bigelowiella natans (CCMP 2755) is a widespread chlorarachniophyte[10], which we have attempted to transform independently with 2 circular plasmids. One plasmid contained the firefly luciferase gene, while the other carried both the luciferase and geneticin resistance cassette. Approximately 300 nt 3' and 5' UTRs of the *B. natans*–derived histone H4 were used as endogenous promoters and terminators for driving the geneticin resistance gene. As for the firefly luciferase gene, the native regulatory sequences were derived from the up- and downstream flanking regions of an endogenous pyruvate kinase gene. Although 3 methods of delivery (electroporation of circular plasmid, lipofection, and biolistics - **Suppl. Table 4**) were tested, no transformants were obtained and no light emission was measured in the culture post-DNA delivery. Finally, we have also attempted delivery of tetramethylrhodamine-labelled DNA using electroporation and biolistics (**Suppl. Table 4**), yet also with an unsuccessful outcome.

Stramenopiles
Although genetic transformation of diatoms using biolistics has been ongoing for decades[11,12], it has only recently accelerated, partially fuelled by the availability of more genome sequences and numerous transcriptomes[13], as well as by the development of novel genome editing methods. Indeed, new model diatoms are being developed to address questions that cannot be investigated with their more established representatives *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*[14]. Different diatom species have been transformed using a variety of DNA delivery methods including electroporation,

biolistics, and conjugation. Targeted gene expression and genome editing have also been achieved using overexpression[15], RNAi[16], meganucleases, TALENs[17] and CRISPR/Cas9[18]. Building on theses successes and extensive resources, we here extend this work to other ecologically and economically important diatom species. Our primary goal was to increase the repertoire of tools available for their genetic manipulation, as well as to describe what was tried with the other species.

Seminavis robusta (D6 and VM3-4), a benthic pennate diatom with a well-defined life cycle, is accessible to genetic analysis due to the presence of 2 defined mating types[19]. We maintained strains D6 and VM3-4 in autoclaved filtered artificial sea water at 12 h light-dark cycle at 18 °C in F/2 medium supplemented with Guillard's F/2 nutrients, and with 250 µg/ml penicillin, 250 µg/ml ampicillin, 50 µg/ml streptomycin sulfate, and 25 µg/ml gentamicin for regular maintenance. Antibiotic sensitivities were established by following for 3 days the division of small number of cells deposited in arrays of 40 µl drops on Petri dishes, which was both faster and more reliable than screening in bulk cultures. In such liquid cultures, we found that 250 µg/ml of glyphosate or 10 µg/ml of puromycin were sufficient to arrest growth, thus qualifying as selectable marker for transformation (**Suppl. Table 3**). To create 3 transformation constructs (pBS_Act_Agro4GlyphR, pBS_Act_PurR and pBS_ActEF-AgroCP4GlyphR), the puromycin N-acetyltransferase and agrobacterium CP4 glyphosate resistance genes were codon optimized (**Suppl. Table 5**) and cloned into different plasmids. To drive their expression, each antibiotic resistant marker gene was 5' flanked with 1.2 kb promoter from a highly expressed actin 1, while the 3' flanking region was represented by either 1.64 kb 3' region derived again from actin 1, or 1.0 kb 3' region derived from the elongation factor 1 of *S. robusta*. However, transformation failed with either biolistic bombardment PDS1000 (BioRad) or electroporation (BTX ECM 2001 and NEPA21) (**Suppl. Table 4**). Following biolistic bombardment, we found that the recovered cultures, which were of only one mating type, contained many apparent exconjugants. This implied that the cultures had undergone self-mating[20]. This observation may be useful for future genetic studies, as it might allow inducing the formation of homozygotes from a clonal population.

Heterosigma akashiwo (CCMP2393) is a harmful algal species from the raphidophyte group, causing fish mortality. Although no specific toxin has been isolated, reports indicate that some raphidophytes produce a suite of compounds that contribute to their toxicity, including reactive oxygen species[21], polyunsaturated fatty acids[22] and brevetoxin-like compounds[23]. Research on *H. akashiwo* suggests that toxicity is strain-specific and linked to genetic markers[24]. *H. akashiwo* is also a candidate for biodiesel production[25] and its non-toxic strain is being developed for biofuel production coupled to bioremediation of industrial emissions[26]. While the nuclear genome has not been fully sequenced yet, transcriptomes and the plastid genome are available[27].

Antibiotic sensitivities of *H. akashiwo* are listed in **Suppl. Table 3**. Plasmids were constructed from *Chlamydomonas reinhardtii* Chlamy_3 and 4, and the diatom pPhat_T1 plasmids[28]. pChlamy_3 was modified with cLuc or cGFP reporter genes[29], while pChlamy_4 and pPhat_T1 vectors were modified with pAES-Luc[30]. All reporter genes were codon optimized for *C. reinhardtii*. Three transformation methods were compared: a glass bead beating protocol, electroporation and *Agrobacterium*-mediated transformation (**Suppl. Table 4**). Of these, an optimized bead-beating protocol successfully and repeatedly produced cultures transformed with a plasmid conferring hygromycin resistance. For this protocol, cells were harvested, washed and resuspended in MAX Efficiency Transformation Reagent for Algae or with 384 mM sorbitol. Cells were mixed with 1-10 µg of linearized plasmid and

glass beads (425-600 μm), vortexed at high speed for 10 s and transferred to a fresh medium with cefotaxime to control bacterial growth. Transformation and expression were verified up to 9 months after the experiment by PCR and RT-PCR amplifications of antibiotic-resistance genes, respectively, but we were not successful in visualizing the fluorescent reporter by microscopy. An optimized electroporation protocol achieved lower efficiency than the bead beating protocol. Cells resuspended as described above with at least 1 μg of linearized plasmid were electroporated using Gene Pulser xCell with a 10-15 ms pulse at 50/75 V, 25 μF and ∞Ω. Finally, when transformation with *Agrobacterium* carrying cGFP was attempted, *H. akashiwo* survived the co-cultivation step but there was little or no viability apparent after several days of hygromycin selection. A major constraint of all methods was that *H. akashiwo* could not be cultured on solid media and hence single-cell transformants were not selected for further analysis. Therefore, all work was done with cells suspended in culture medium. A comparison of methods revealed that PEG-mediated bead beating produced the best results.

Fluorescein isothiocyanate conjugated to dextran, a fluorescent DNA analog, has been successfully introduced into *Caecitellus* sp. using a high voltage exponential decay pulse (1000 V, 10 μF, ∞ Ω) from a GenePulser xCell (cuvette width 0.2 cm). Cell viability was confirmed by observing motility and fluorescence 24 h after electroporation. Lower voltage pulses did not result in DNA delivery.

For *P. tricornutum* (CCAP1055/1), we adapted the CRISPR/Cas9 system for multiplexed gene mutagenesis. Bacterial conjugation was used to deliver an episome that contained a Cas9 cassette and 2 single-guide RNA (sgRNA) expression cassettes designed to mutate glutamine synthetase II and a nuclear-encoded chloroplastic glutamate synthase. The GoldenGate assembly was used to clone two expression cassettes carrying sgRNAs into a *P. tricornutum* episome that contained a Cas9-2A-ShBle expression cassette and the centromeric region CenArsHis (**Suppl. Fig. 6A**). A successfully assembled episomal plasmid with 2 sgRNA and one LacZ insert produced blue *E. coli* colonies (**Suppl. Fig. 6B**). After their addition to a *P. tricornutum* culture, plates were incubated in a growth chamber under standard growth conditions for 2 days and transformed *P. tricornutum* colonies begun to appear after 14 days. Only colonies maintaining Cas9-2A-ShBle sequence on the delivered episome were able to grow on selection plates because Cas9 and the antibiotic-resistant gene (ShBle) were transcriptionally fused by the 2A peptide[31] (**Suppl. Fig. 6C**). Sequencing of PCR products from target genes confirmed the deletions (**Suppl. Fig. 6D**).

Alveolates

For introduction see Results. *Euplotes crassus* (txid5936) and *Euplotes focardii* (txid36767) are marine ciliates carrying a micronuclear genome that represents the germ line, and a macronuclear genome containing single-gene nanochromosomes amplified to thousands of copies for their somatic life[32]. Both ciliates are suitable for studying DNA rearrangements and transposition mechanisms, which have been observed in macronuclei after completion of the sexual cycle[33,34]. *E. crassus* and *E. focardii* are resistant to antibiotics usually used for genetic transformations such as ampicillin (100 μg/ml). However, geneticin (200 μg/ml), paromomycin (200 μg/ml) and puromycin (40 μg/ml) are effective (**Suppl. Table 3**) when cells were grown in a modified medium (10% artificial seawater and 90% 0.3 M glucose). Here, we describe conditions for growing both *Euplotes* species in the presence of bacteria, which are being used as vectors for transmitting RNAi to knockdown genes in ciliates. Plasmids and artificial nanochromosomes ending with telomeres that contain either eGFP and/or geneticin as selectable marker, both optimized according to the *Euplotes* codon usage,

have been generated. They resemble the *Euplotes* nanochromosome structure with the flanking non-coding regions belonging to the constitutive highly expressed *Euplotes* genes. Telomeres have been added via PCR using primers containing a specific sequence and ending with C_4A_4 repeats. The 940 nt eGFP artificial nanochromosomes are composed of *E. crassus* 5' UTR from a highly expressed gene as promoter, eGFP coding sequence, *E. crassus* 3' UTR from a highly expressed gene, and telomeres on both ends. The 3.2 kb artificial nanochromosomes containing eGFP and resistance to geneticin are composed of β-tubulin 5' UTR, β-tubulin coding sequence, a spacer, eGFP coding sequence, β-tubulin 3' UTR, α-tubulin 3' UTR, geneticin resistance gene, α-tubulin 5' UTR, and telomeres on both ends. The DNA delivery protocols (**Suppl. Table 5**) were successful, since we could confirm the presence of Cy3-labelled plasmids inside the cells following electroporation, lipofectamine transfection, and microinjection (**Suppl. Table 4**). However, there is no evidence for either incorporation of the plasmids into the nuclear genome or for the expression of the introduced marker genes (eGFP and/or resistance to geneticin).

Chromera velia (CCMP2878) is a marine photosynthetic alveolate associated with corals[35]. We were not able to identify antibiotics suitable for its selection (**Suppl. Table 3**), although a range of antibiotics at different concentrations was tested. Nevertheless, we developed 2 plasmids for the transformation of *C. velia*. The first one contained the firefly luciferase gene, while the other plasmid carried both luciferase and the puromycin resistance genes. The *C. velia*-derived ~300 nt 5' UTR of the enolase gene and equally long 3' UTR of its H4 histone gene were used as regulatory sequences of the puromycin resistance gene. In the case of the firefly luciferase gene, the native regulatory sequences were derived from the 5' and 3' UTRs of histone H1 and pyruvate kinase, respectively (**Suppl. Table 5**). Electroporation with 5 different settings was tested to deliver circular and linear plasmids into the cells (**Suppl. Table 4**), yet there was no evidence of successful transformation, as no luciferase activity was detected by Sirius luminometer after 5 or 24 h. The same negative result was obtained following the delivery of tetramethylrhodamine-labelled DNA using electroporation and biolistics. Finally, 1 μg of *in vitro* synthesized firefly luciferase mRNA was used for electroporation with 4 different settings (**Suppl. Table 4**), and light emission was measured after 24 h, yet again, no specific luciferase activity has been detected.

Hematodinium sp. belongs to the order Syndiniales and infects the hemolymph of many marine decapod crustaceans. The genus is considered an emerging pathogen for both natural populations and aquacultured crustaceans, with an expanding host range[36,37]. The parasite has multiple stages in the hemolymph[38] and can be continuously maintained in the laboratory[39]. *Hematodinium* cells were transformed using electroporation (Amaxa Nucleofector D-023; BioRad X-100), as well as *via* the glass beads abrasion protocol, the latter having a much higher survival rate (**Suppl. Table 4**). Cells were transformed with 150 kDa FITC-dextran and HEM or Cas9/sgRNA with GFP sequence as DNA donor. We have also tested the use of a synthetic viral RNA derived from a virus known to infect the dinoflagellate *Heterocapsa circularisquama*[40]. Originally, the RNA molecule had 2 ORFs: one that codes for a putative polyprotein bearing a protease and an RNA-dependent RNA polymerase, while the other codes for a capsid protein[41]. We have exchanged the second ORF with an eGFP reporter and *in vitro* transcribed RNA was used to transform *Hematodinium* **(Suppl. Table 5).** However, based on microscopy and PCR verification no clear positive result was observed. Still, *Hematodinium* appears to be a promising organism to transform since in comparison to many dinoflagellates, its cell does not have any theca or cell wall.

Fugacium (*Symbiodinium*) *kawagutii* (CCMP 2468) and *Alexandrium catenella* (CCMP BF-

5) are both dinoflagellates. *F. kawagutii,* (formerly known as *Symbiodinium)* is a symbiont originally isolated from the scleractinian coral *Montipora capitate* and has a draft genome available[42]. *A. catenella* is a free-living dinoflagellate and produces potent neurotoxins resulting in paralytic shellfish poisoning[43]. Both species were tested for antibiotic sensitivities (**Suppl. Table 3**). Subsequently, an *Agrobacterium*-mediated transformation method[44] was applied on *F. kawagutii* using the published plasmids, and Basta as the selecting agent. However, this method proved to be difficult to reproduce. Thus, for both species, we developed an electroporation protocol using MicroPulser (BioRad) (**Suppl. Table 4**). Centrifugation protocols that would result in concentrated cells with minimal damage were found to be species-dependent (*F. kawagutii* 800 g for 5 min and *A. catenella* 800 g for l min). Through extensive testing, a suitable transfection solution (10% glycerol in Milli-Q water) that resulted in minimal mortality for both dinoflagellates was identified. Several pulse conditions were tested for each species: Sc2 and ShS performed best for *F. kawagutii* and *A. catenella*, respectively (**Suppl. Table 4**). Plasmids described elsewhere[44] were used in addition to our DinoIII-gfp/DinoIII-pat/DinoIII-bsr vectors[45]. Each plasmid contained either a eGFP reporter, a selection marker, or both. They were introduced into cells as circular or linearized plasmids, and their presence was tested by PCR. The results were inconclusive, species-dependent, with generally very low efficiency. Hence, we tested other methods such as biolistics on *F. kawagutii* and *A. catenella* and electroporation with the Nucleofector (Lonza) on *A. catenella* (**Suppl. Table 4**). For biolistics, a method developed previously[17] was appied using various rupture discs and microcarriers. Due to the difficulty of growing dinoflagellates on agar, 3 μm Millipore filters were used to collect cells and were placed on agarose plates during the biolistic procedure and were then transferred to liquid media for recovery and selection. While *A. catenella* and *F. kawagutii* performed best with 900 PSI and 1550 PSI rupture discs, respectively, the results were inconclusive.

The pyrimidine analog 5-fluoroorotic acid (5FOA) has been shown to successfully suppress the growth of *Breviolum (Symbiodinium)* sp. (**Suppl. Table 3**). A 5FOA-resistant uracil-requiring mutant strain was isolated, which possesses a splice variant mutation in the URA3 gene[46]. We therefore tried to develop a method to complement the phenotypes (i.e., uracil requirement and 5FOA resistance) of this URA3 mutant with the wild type homolog. The full-length URA3 gene was amplified by PCR and cloned into pBlueScript. So far multiple attempts to transform the mutant with this plasmid, either linearized or circular, using electroporator NEPA21 failed (**Suppl. Table 4**). However, we have successfully introduced fluorescein into *Breviolum* sp., using the same method as the DNA-based transformation (**Suppl. Table 5**), suggesting that homologous recombination alone might not be effective enough to replace the mutated version with the wild type sequence.

Discobids

Eutreptiella gymnastica (SCCAP K-0333) is a marine green euglenophyte distantly related to the recently sequenced model euglenid, *Euglena gracilis*[47]. All attempts to transform *E. gymnastica* with either the firefly luciferase mRNA or 2 circular plasmids failed. Four methods for DNA delivery were tested, and their efficiency was checked by labelled DNA (**Suppl**. **Fig. 4; Suppl. Table 4**). Following electroporation with 5 μg of *in vitro* synthesized mRNA for firefly luciferase (10 different settings tested) (**Suppl. Table 4**), light emission was measured after 3.5, 5.5, and 22 h, yet no specific luciferase activity has been detected.

The antibiotic selection by 10 μg/ml of puromycin was used for transformations based on plasmids (**Suppl. Table 3**). One plasmid contained the firefly luciferase gene inserted between *E. gymnastica*-derived 300 nt 5' and 3' UTRs of the histone 2A gene. The other plasmid contained this region complemented with the puromycin resistance gene flanked

upstream by 5' UTR region of histone-lysine N-methyltransferase and downstream by 3' UTR region of chloroplast light-harvesting complex II gene from *E. gymnastica*. Four methods for delivery of these 2 plasmids were performed: electroporation, lipofection and particle bombardment-mediated delivery of circular or linearized plasmids (**Suppl. Tables 4** and **5**). However, we were unable to identify any transformants under selective conditions and no light emission was measured in cultures post-DNA delivery. Since none of the transformation methods was successful, we have proceeded to the delivery of fluorescein- or tetramethylrhodamine-labelled DNA using all 4 delivery methods listed above. In case of electroporation and lipofection, a seemingly intracellular fluorescent signal could be detected suggesting the DNA delivery succeeded, although expression was not achieved (**Suppl. Fig. 4**).

Opisthokonts

For the ichthyosporean *Pirum gemmata* we performed electroporation by Neon (Invitrogen) and calcium phosphate transfection approaches using heterologous promoters from another ichthyosporean *Sphaeroforma artica*, yet with no success. Although transfection of *S. arctica* did not result in successful DNA delivery (**Suppl. Table 4**), we identified 4 antibiotics (benomyl, carboxine, phleomycin and puromycin), which might be used for future work with *S. arctica* (**Suppl. Table 3**).

Transformation of natural protist communities

Generally, genetic transformation has been achieved in a relatively small number of marine protists, with stable transformation reliably achieved in even fewer of them[48]. Model organisms are typically selected based on well-known criteria, such as relative ease of isolation, cultivation in the laboratory and small genome size. However, these attributes may not correlate with the capacity for uptake and expression of exogenous DNA which, nevertheless, is key for genetic tractability. Thus, using a culture-independent, we explored the ability of natural marine planktonic pico- and nanoeukaryotic communities to incorporate and express exogenous DNA.

We used a strategy based on electroporation and flow cytometry, to identify cells from natural communities, which had incorporated and expressed DNA (using FITC-dextran) tagged to the gene encoding the blue-fluorescent protein (mTagBFP2). We constructed plasmids expressing mTagBFP2 under the control of the cauliflower mosaic virus 35S (35S) and the cytomegalovirus (CMV) promoters reported to function in a broad range of eukaryotes including phytoplankton[49], as well as a VCP1 promoter previously described from a pico-stramenopile. Plasmids with mTagBFP2 driven by the 35S and CMV promoters were first validated in human embryonic kidney cells and yeast, allowing their functionality to be confirmed prior to testing micro-algae cultures and natural communities. The electroporation protocol was developed using both cultured *Nannochloropsis oceanica*, *Isochrysis galbana*, and *Emiliania huxleyi*, as well as natural communities collected and concentrated by tangential flow filtration (Vivaflow). Electroporation efficiency was monitored with 10 mg/ml of fluorescein-conjugated 2000 kDa dextran (for *I. galbana* the concentration of dextran was lowered to 0.5 mg/ml), and cells were analyzed using an InFlux flow cytometer equipped with a 488 nm principal laser (for determining forward and side scatter as well as green fluorescence from fluorescein at 530 ± 20 nm and red fluorescence from chlorophyll at 692 ± 20 nm), a 405 nm laser (for blue fluorescence at 460 ± 25 nm), and a 640 nm laser (for red fluorescence from chlorophyll at 692 ± 20 nm). Cells were washed over polycarbonate filters with different concentrations of sorbitol, in which they were resuspended. Highest recovery of both natural and cultured phytoplankton was obtained with 800 mM sorbitol.

Electroporation was performed with a Gene Pulser II (BioRad) in 0.2 cm cuvettes. Voltages of between 4 and 10 kV/cm with time constants of 8 ms and 20 ms, respectively, were tested. For *N. oceanica*, 5 and 9 kV/cm with time constant of 20 ms was used. For *I. galbana*, 5 kV/cm with a 20 ms time constant was chosen after preliminary tests. For natural communities, as it is not possible to optimize for all potential members, 5 kV/cm with an 8 ms time constant and 10 kV/cm with a 20 ms time constant were usually used in parallel and on a set of sub-samples. Electroporated samples were allowed to recover in moderate (<50 µmol phot/m/s) light at about 15˚ C and followed for up to 72 h.

The protocol was first tested with plasmids and cultures of *N. oceanica*, *I. galbana*, and *E. huxleyi*, always comparing cells electroporated with plasmids to those electroporated with the same protocol but without plasmids (negative control). In both *N. oceanica* and *I. galbana*, blue fluorescent cells appeared within several hours. However, the percentage of cells successfully transformed varied greatly, ranging from a few percent to >70%, and, similarly, the percentage of blue fluorescent cells varied from ~1% to over 20%. In *N. oceanica*, the CMV promoter was effective at driving blue fluorescence, with >20% transformants when a linearized plasmid was used (**Fig. 4**; **Table 1; Suppl. Table 5**), but only <2% when the plasmid was circular (**Table 1**). The 35S promoter resulted in inconsistent results with both linearized and circular plasmids, and in two trials there was no change in blue fluorescence with the VCP1 promoter. In *I. galbana*, the 35S promoter resulted in blue fluorescence peaking in up to 20% of cells at 4 h and declining thereafter. In calcified *E. huxleyi* cultures, electroporation both with and without plasmid resulted in inconsistent survival, and fluorescence and light scatter signatures became much dispersed, making interpretation impossible.

In 3 out of 6 trials with natural communities, blue-fluorescent pico-eukaryotes appeared in samples electroporated with plasmids encoding mTagBFP2. In one of these natural communities, an important portion of a particular pico-phytoeukaryote (grouped based on optical properties) exhibited blue fluorescence. Indeed, 58 to 68% cells fluoresced when electroporated at 5 kV/cm after 2 h with a plasmid containing the CMV promoter. Curiously, a portion of these cells (up to 18% at 12 h after electroporation) showed blue fluorescence when exposed to plasmid DNA even in the absence of electroporation, although the time course was markedly slower (**Suppl. Figure 7**). Unfortunately, when sorting for culturing was prepared at the 24 h time point, the signal of blue fluorescence was so diminished that we could only sort pico-phytoplankton with similar light scattering and pigment autofluorescence characteristic for live cultures. However, based on two replications, our initial results were not reproducible.

Taken together, transforming natural marine planktonic protist communities is challenging because of the following issues. First, the natural abundance of protist cells is usually very low (< 1000 per ml), and therefore they should be concentrated by i.e. tangential flow filtration. Second, the salts from seawater need to be removed for electroporation, resuspending the cells in sorbitol. Finally, it is not possible to optimize the electroporation protocol for a natural community, as even when the same location is sampled repeatedly, the community will likely be different due to the dynamics of the marine system. For the latter reason, in most trials we used 2 different electroporation protocols in parallel on distinct sub-samples. The variable and low percentage of apparently transformed cells presents a challenge for cell sorting, for transfer into media, for determining identity-independent culturing, and for confirming the expression of the eGFP gene. A recently published method based on single cell sorting and subsequent culturing may help to address these issues[51].

Although amplification of barcoding genes (e.g. 18S rRNA) from sorted populations is becoming more routine, we were unable to obtain amplicons from populations of pico-phytoeukaryotes that exhibited a high percentage of blue fluorescent cells, suggestion an alternative protocol is required for transformed populations.

Open Material Transfer Agreement (OpenMTA)

For sharing biological material (e.g. cells and DNA), the available Material Transfer Agreement (MTA)[52] is not well suited for the needs of collaborative projects aiming at the development of community-sourced toolkits. The shortcomings of traditional MTAs have recently been addressed[52]. Although MTA barriers could be bypassed by *de novo* synthesis of DNA, this would be very impractical and costly. Instead, we highly recommend the adoption of the OpenMTA; https://www.openplant.org/openmta/) for future community-wide efforts. An OpenMTA enables more expedited processing times, decreased administrative transaction costs, broader sharing, use and redistribution of biological materials, while maintaining the rights of creators and promoting safe practices and responsible research. Perhaps most importantly, OpenMTA allows tracking the provenance and impact of projects[53]. OpenMTA is more compatible with public and philanthropic funding policies as well as with open science practices.

Supplementary References

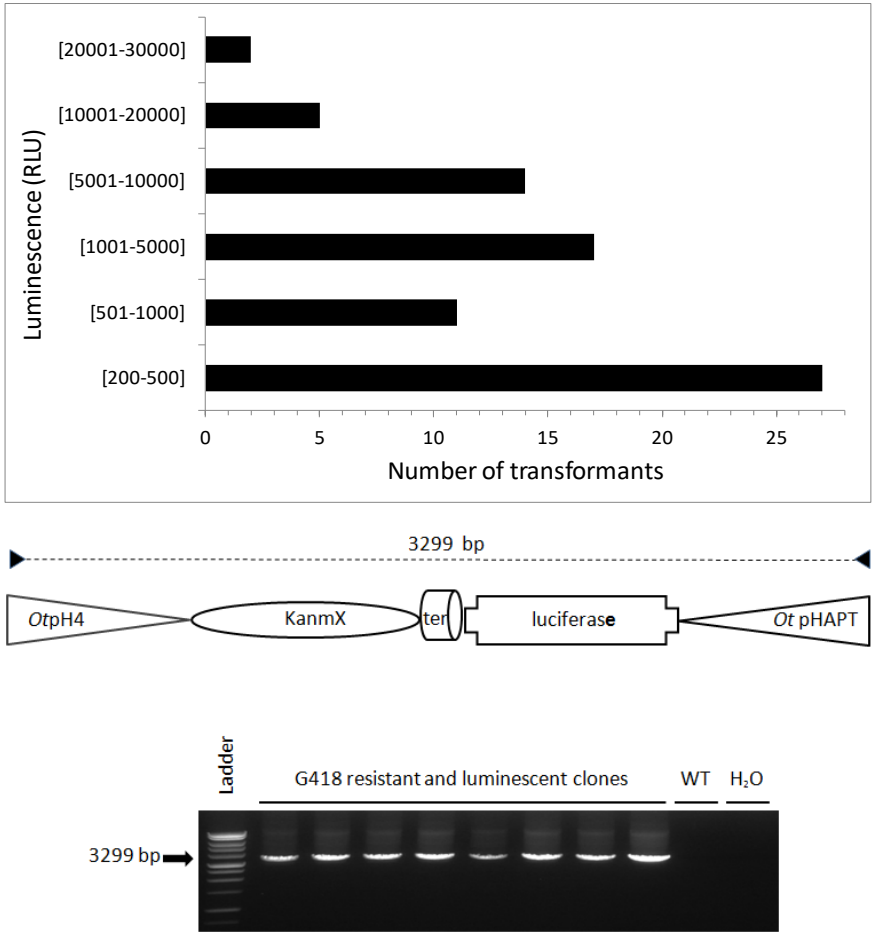
1. Simmons, M. P. et al. Intron invasions trace algal speciation and reveal nearly identical Arctic and Antarctic *Micromonas* populations. *Mol. Biol. Evol.* **32,** 2219-2235 (2015).
2. Van Baren, M. J. et al. Evidence-based green algal genomics reveals marine diversity and ancestral characteristics of land plants. *BMC Genomics* **17,** (2016).
3. Worden, A.Z. et al. Green evolution and dynamic adaptations revealed by genomes of the marine picoeukaryotes *Micromonas*. *Science* **324**, 268-272 (2009).
4. Van Ooijen, G., Knox, K., Kis, K., Bouget, F-Y. & Millar, A. J. Genomic transformation of the picoeukaryote *Ostreococcus tauri*. *J. Vis. Exp.* **65**, e4074 (2012).
5. Paz Z. et al. One step construction of *Agrobacterium*-recombination-ready-plasmids (OSCAR), an efficient and robust tool for ATMT based gene deletion construction in fungi. *Fungal Genet. Biol.* **48**, 677–684 (2011).
6. Duanmu, D. et al. Marine algae and land plants share conserved phytochrome signaling systems. *Proc. Natl. Acad. Sci. USA* **111,** 15827-15832 (2014).
7. Turmel, M., de Cambiaire, J. C., Otis C. & Lemieux, C. Distinctive architecture of the chloroplast genome in the Chlorodendrophycean green algae *Scherffelia dubia* and *Tetraselmis sp.* CCMP 881. *PLoS One* **11,** e0148934 (2016).
8. Fang, L. et al. Improving phylogenetic inference of core Chlorophyta using chloroplast sequences with strong phylogenetic signals and heterogeneous models. *Mol. Phylogenet. Evol*. **127,** 248-255 (2018).
9. Leliaert, F. et al. Chloroplast phylogenetic analyses reveal the deepest-branching lineage of the Chlorophyta, Palmophyllophyceae class. nov. *Sci. Rep.* **6,** 25367 (2016).
10. Rogers, M. B., Gilson, P. R., Su, V., McFadden, G. I. & Keeling, P. J. The complete chloroplast genome of the chlorarachniophyte Bigelowiella natans: Evidence for independent origins of chlorarachniophyte and euglenid secondary endosymbionts. *Mol. Biol. Evol.* **24,** 54-62 (2006).
11. Dunahay, T. G., Jarvis, E. E. & Roessler, P. G. Genetic transformation of the diatoms *Cyclotella cryptica* and *Navicula saprophila*. *J. Phycol.* **3,** 1004–1012 (1995).

12. Poulsen, N. & Kroger, N. A new molecular tool for transgenic diatoms. Control of mRNA and protein biosynthesis by an inducible promoter-terminator cassette. *FEBS J.* **272,** 7413-3423 (2005).
13. Mock, T. et al. Evolutionary genomics of the cold-adapted diatom *Fragilariopsis cylindrus. Nature* **541,** 536–540 (2017).
14. Russo, M. T., Cigliano, R. A., Sanseverino, W. & Ferrante, M. I. Assessment of genomic changes in a CRISPR/Cas9 *Phaeodactylum tricornutum* mutant through whole genome resequencing. *PeerJ*. **6,** e5507 (2018)
15. Vardi, A., Bidle, K. D., Kwityn, C., Hirsh, D. J. & Thompson, S. M. A diatom gene regulating nitric-oxide signaling and susceptibility to diatom-derived aldehydes. *Curr. Biol.* **18,** 895-899 (2008).
16. De Riso, V., Raniello, R., Maumus, F., Rogato, A. & Bowler, C. Gene silencing in the marine diatom *Phaeodactylum tricornutum*. *Nucleic Acids Res.* **37,** e96 (2009).
17. Daboussi, F. et al. Genome engineering empowers the diatom *Phaeodactylum tricornutum* for biotechnology. *Nat. Commun.* **5,** 3831 (2014).
18. Hopes, A., Nekrasov, V., Kamoun, S. & Mock, T. Editing of the urease gene by CRISPR-Cas in the diatom *Thalassiosira pseudonana*. *Plant Methods* **12,** 49 (2016)
19. Moeys, S. et al. A sex-inducing pheromone triggers cell cycle arrest and mate attraction in the diatom *Seminavis robusta*. *Sci. Rep.* **6,** 19252 (2016).
20. Chepurnov, V. A., Chaerle, P., Vanhoutte, K. & Mann, D. G. How to breed diatoms: examination of two species with contrasting reproductive biology. In: R. Gordon and J. Seckbach (Eds.), The Science of Algal Fuels, *Springer* **323-340,** (2012).
21. Marshall, J. A., de Salas, M., Oda, T. & Hallegraeff, G. Superoxide production by marine microalgae. *Marine Biol.* **147,** 533-540 (2005).
22. de Boer, M. K. et al. Haemolytic activity within the species *Fibrocapsa japonica* (Raphidophyceae). *Harmful Algae* **8,** 699-705 (2009).
23. Khan, S., Arakawa, O. & Onoue, Y. Neurotoxins in a toxic red tide of *Heterosigma akashiwo* (Raphidophyceae) in Kagoshima Bay, Japan. *Aquacult. Res.* **28**, 9-14 (1997).
24. Fredrickson, K. A., Strom, S. L., Crim, R. & Coyne, K. J. Interstrain variability in physiology and genetics of *Heterosigma akashiwo* (Raphidophyceae) from the West Coast of North America. *J. Phycol.* **47,** 25-35(2011).
25. Fuentes-Grunewald, C. et al. Improvement of lipid production in the marine strains *Alexandrium minutum* and *Heterosigma akashiwo* by utilizing abiotic parameters. *J. Ind. Microbiol. Biotechnol.* **39,** 207-16 (2012).
26. Stewart, J. J., Bianco, C. M., Miller, K. R. & Coyne, K. J. (2015) The marine microalga, *Heterosigma akashiwo*, converts industrial waste gases into valuable biomass. *Front. Energy Res.* **3**, 12 (2015).
27. Keeling P. J., Burki, F., Wilcox, H. M., Allam, B. & Allen, E. E. The Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP): illuminating the functional diversity of eukaryotic life in the oceans through transcriptome sequencing. *PLoS Biol.* **12,** e1001889 (2014).
28. Zaslavskaia, L. A., Lippmeier, J. C., Kroth, P. G., Grossman, A. R. & Apt, K. E. Transformation of the diatom *Phaeodactylum tricornutum* with a variety of selectable marker and reporter genes. *J. Phycol.* **386,** 379–386 (2000).
29. Fuhrmann, M. et al. Monitoring dynamic expression of nuclear genes in *Chlamydomonas reinhardtii* by using a synthetic luciferase reporter gene. *Plant. Mol. Biol.* **55,** 869-88 (2004).
30. Heitzer, M. & Zschoernig, B. Construction of modular tandem expression vectors for the green alga *Chlamydomonas reinhardtii* using the Cre/lox-system. *Biotechniques* **43**, 324-32 (2007).

31. Duda, K. et al. High-efficiency genome editing via 2A-coupled co-expression of fluorescent proteins and zinc finger nucleases of CRISPR/Cas9 nicase pairs. *Nucl. Acids. Res.* **42,** e84 (2014).
32. Lobanov, A.V. et al. Position-dependent termination and widespread obligatory frameshifting in *Euplotes* translation. *Nat. Struct. Mol. Biol*. **24**, 61-68 (2017).
33. Prescott, D.M. The DNA of ciliated protozoa. *Microbiol. Rev.* **58,** 233–67 (1994).
34. Klobutcher, L. A. & Jahn C. L. Developmentally controlled genomic rearrangements in ciliated protozoa. *Curr. Opin. Genet. Dev.* **1,** 397–403 (1991).
35. Oborník, M. & Lukeš, J. Cell biology of chromerids, the autotrophic relatives to apicomplexan parasites. *Int. Rev. Cell. Mol. Biol.* **306**, 333-369 (2013).
36. Gornik, S. G., Cranenburgh, A. & Waller, R. F. New host range for *Hematodinium* in southern Australia and novel tools for sensitive detection of parasitic dinoflagellates. *PLoS One* **8**, (12): e82774 (2013).
37. Ryazanova, T. V., Eliseikina, M. G. & Semenchenko, A. A. A new host for *Hematodinium* infection among lithodid crabs from the Sea of Okhotsk. *J. Invertebr. Pathol.* **153**, 12-19 (2018).
38. Stentiford, G. D. & Shields, J. D. A review of the parasitic dinoflagellates *Hematodinium* species and *Hematodinium*-like infections in marine crustaceans. *Dis. Aquat. Organ.* **66**, 47-70 (2005).
39. (Li, 2011).
40. Tomaru, Y. et al. Isolation and characterization of two distinct types of HcRNAV, a single-stranded RNA virus infecting the bivalve-killing microalga *Heterocapsa circularisquama*. *Aquat. Microb. Ecol*. **34,** 207–218 (2004).
41. Nagasaki, K. et al. Comparison of genome sequences of single-stranded RNAviruses infecting the bivalve-killing dinoflagellate *Heterocapsa circularisquama*. *Appl. Environ. Microbiol*. **71**, 8888–8894 (2005).
42. Lin, S. et al. The *Symbiodinium kawagutii* genome illuminates dinoflagellate gene expression and coral symbiosis. *Science* **350**, 691-694 (2015).
43. Anderson, D. M. Bloom dynamics of toxic *Alexandrium* species in the Northeastern US. *Limnol. Oceanogr.* **42**, 1009-1022 (1997).
44. Ortiz-Matamoros, M. F. et al. Heterologous DNA uptake in cultured *Symbiodinium* spp. aided by *Agrobacterium tumefaciens*. *PLoS ONE* **10**, e0132693–16 (2015).
45. Sprecher, B. N., Zhang, H. & Lin, S. Nuclear gene transformation in a dinoflagellate. *bioRxiv* 602821z (2019).
46. Ishii, Y., et al. Isolation of uracil auxotroph mutants of coral symbiont alga for symbiosis studies. *Sci. Rep*. **8,** 3237 (2018).
47. Ebenezer, T. et al. Transcriptome, proteome and draft genome of *Euglena gracilis*. *BMC Biol.* **17,** 11 (2019).
48. Waller, R. F. et al. Strength in numbers: collaborative science for new experimental model systems. *PLOS Biol.* **16,** e2006333 (2018).
49. Sakaue et al. 2008
50. Kilian, O., Benemann, C. S., Niyogi, K. K. & Vick, B. High-efficiency homologous recombination in the oil-producing alga *Nannochloropsis sp. Proc. Natl. Acad. Sci. USA* **108,** 21265-21269 (2011).
51. Marie, D., Le Gall, F., Edern, R., Gourvil, P. & Vaulot, D. Improvement of phytoplankton culture isolation using single cell sorting by flow cytometry. *J. Phycol.* **53,** 271-282 (2017).
52. Nielsen, J., Bubela, T., Chalmers, D. R. C., Johns, A. & Kahl, L. Provenance and risk in transfer of biological materials. *PLoS Biol*. **16,** e2006031 (2018).

53. Kahl, L. et al. Opening options for material transfer. *Nat. Biotechnol*. **36,** 923-927 (2018)

Suppl. Fig. 1



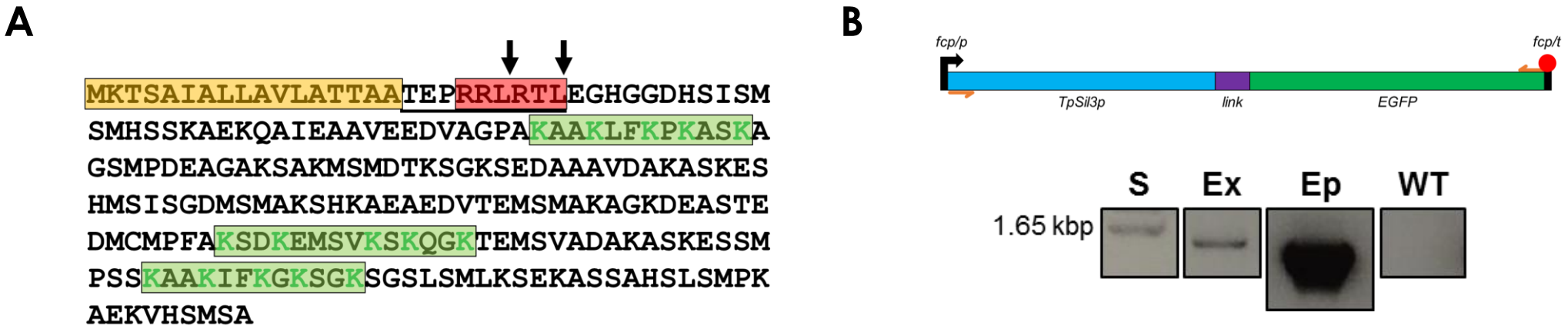
Stable transformation of *Ostreococcus lucimarinus* RCC802
Top: Luminescence of 76 RCC802 transformed by HAPT:luc transgene, exhibiting luminescence levels at least two fold above RCC802 WT control transformed without DNA (80 RLU). The range of luminescence (RLU) is shown on the Y axis.
Bottom: PCR amplification of the transgene (includiing *O. tauri* histone H4 and HAPT promoters) in 8 RCC802 luminescent lines resistant to G418 . A band at the expected size is amplified ii all transformants. WT : control cells electroporated without DNA, H_20: PCR control.

Transient expression and stable transformation of selected Mamiellophyceae

Species (Transgene)	Transient luciferase expression (RLU/mg of protein)	Stable transformants resistant to G418 (clones/ µg of transgene)	Stable transformants expressing luciferase[1]
O tauri RCC745 (pH4 :KanMX-pHAPT :Luc)[2]	400	480 (100)	80
O tauri RCC 1110 (pH4 :KanMX-pHAPT :Luc)[2]	600	32 (32)	32
O lucimarinus (pH4 :KanMX-pHAPT :Luc RCC 802)[2]	650	480 (96)	76
B. prasinos RCC 4222 (pH4 :KanMX-pHAPT :Luc)[3]	Nd	48 (20)	14
B. prasinos RCC 4222 (pH4 :KanMX-BCCT)[4]	Na	384 (77)[5]	Na

[1]Luminescence two fold above background
[2]O. tauri RCC745 Histone (H4) and high affinity phosphate transporter (HAPT) promoters
[3]B prasinos RC4222 Histone (H4) and high affinity phosphate transporter (HAPT) promoters
[4]B prasinos BCCT gene encoding putative DMSP transporter interrupted by pH4:KanMX selection marker to test homologous recombination
[5]No BBCT knock out by homologous recombination were obtained

Suppl. Fig. 2



Suppl. Fig. 2 | TpSil3p features and positive exconjugant confirmation with colony PCR. (A) TpSil3p amino acid sequence. Orange: signal peptide; underlined: pro-peptide with consecutive RxL motifs (red)—known cleavage sites in biosilicification proteins possibly recognized by signal-1 protease in endoplasmic reticulum or Golgi apparatus (arrows indicate putative cleavage sites); green: pentalysine clusters—12-14 amino acid residues long motifs with 5 non-consecutive lysines—known to promote biosilica association. **(B)** Top: expression cassette scheme. *fcp/p*: *fcp* promoter; *TpSil3p*: silaffin 3 precursor; *link*: KGSGSTSGSG linker; *EGFP*: enhanced GFP. The whole ORF was optimized for expression in *Tp* using IDT's Codon Optimization Tool (choosing "*Thalassiosira pseudonana*" in the drop down menu). Depicted in orange are colony PCR primers. The expression cassette was inserted into pTpPuc3 32 bp downstream of the HIS3 gene. Bottom: colony PCR result. S: DNA standard; Ex: *Tp* exconjugant; Ep: positive control (episome used for conjugation); WT: wild type *Tp*. 0.5 µL cells were genotyped with Phire Plant Direct PCR Master Mix according to the protocol described here. Annealing temperature and extension time were set to 57.5 °C and 40 sec, respectively. Expected ~1.45 kb long amplicon was observed in the exconjugant strain corresponding to the full length of designed ORF. Verification primers: Fwd: 5'-ATGAAGACTTCTGCCATTGC-3', Rev: 5'-GATTCACCAATCCAGTATTTACTTATAGAGCTCGTCCATAC-3'.

Suppl. Fig. 2 - References:

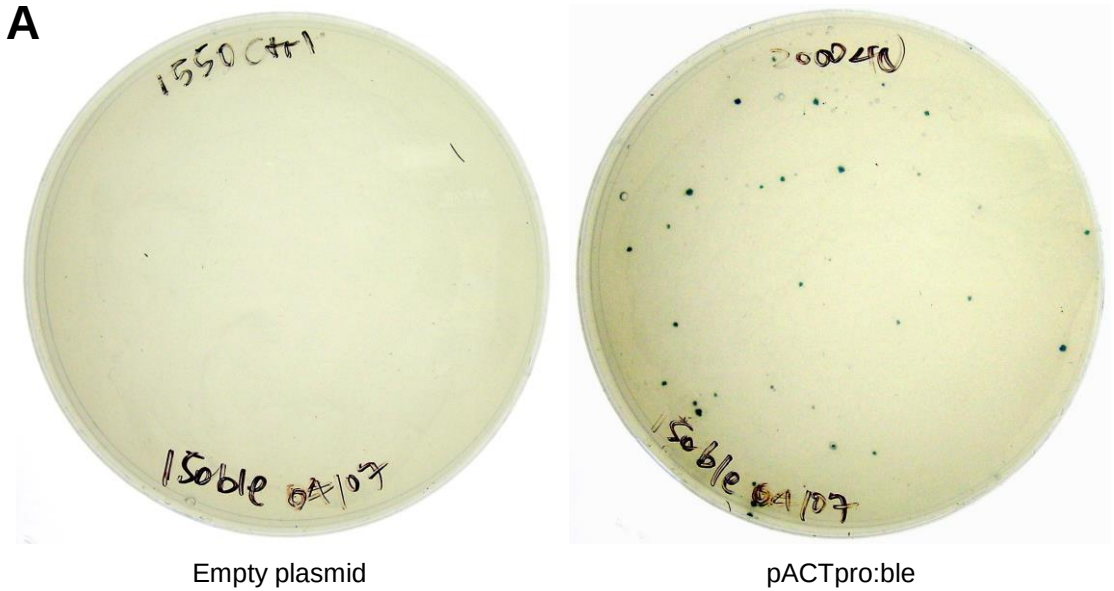
Kröger, N, Bergsdorf, C. & Sumper, M. Frustulins: domain conservation in a protein family associated with diatom cell walls. *Eur. J. Biochem.* **239**, 259–264 (1996).

Kröger, N., Deutzmann, R. & Sumper, M. Polycationic Peptides from Diatom Biosilica That Direct Silica Nanosphere Formation. *Science* **286**, 1129–1132 (1999).

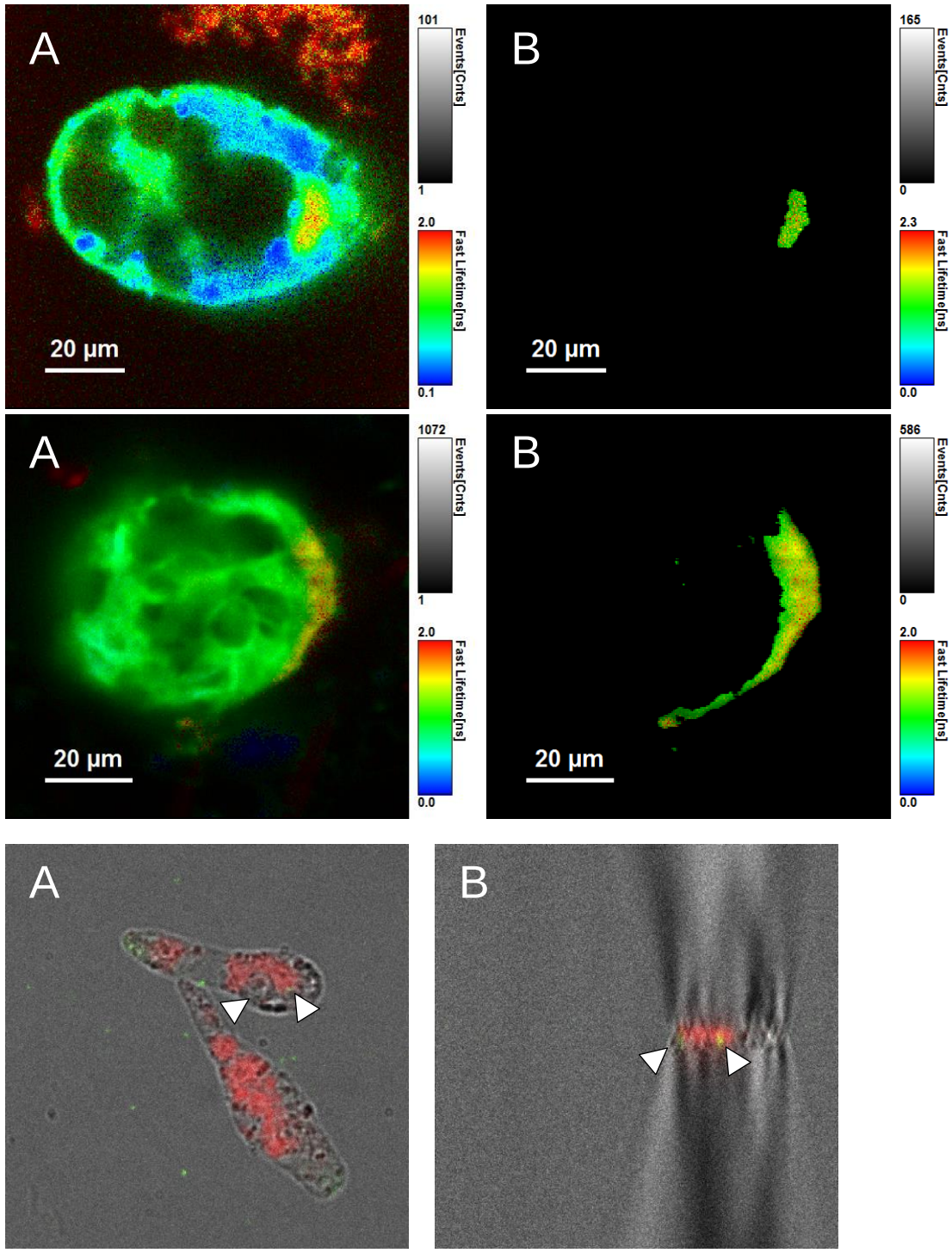
Pamirsky, I. E. & Golokhvast, K. S. Silaffins of diatoms: from applied biotechnology to biomedicine. *Mar. Drugs* **11**, 3155–3167 (2013).

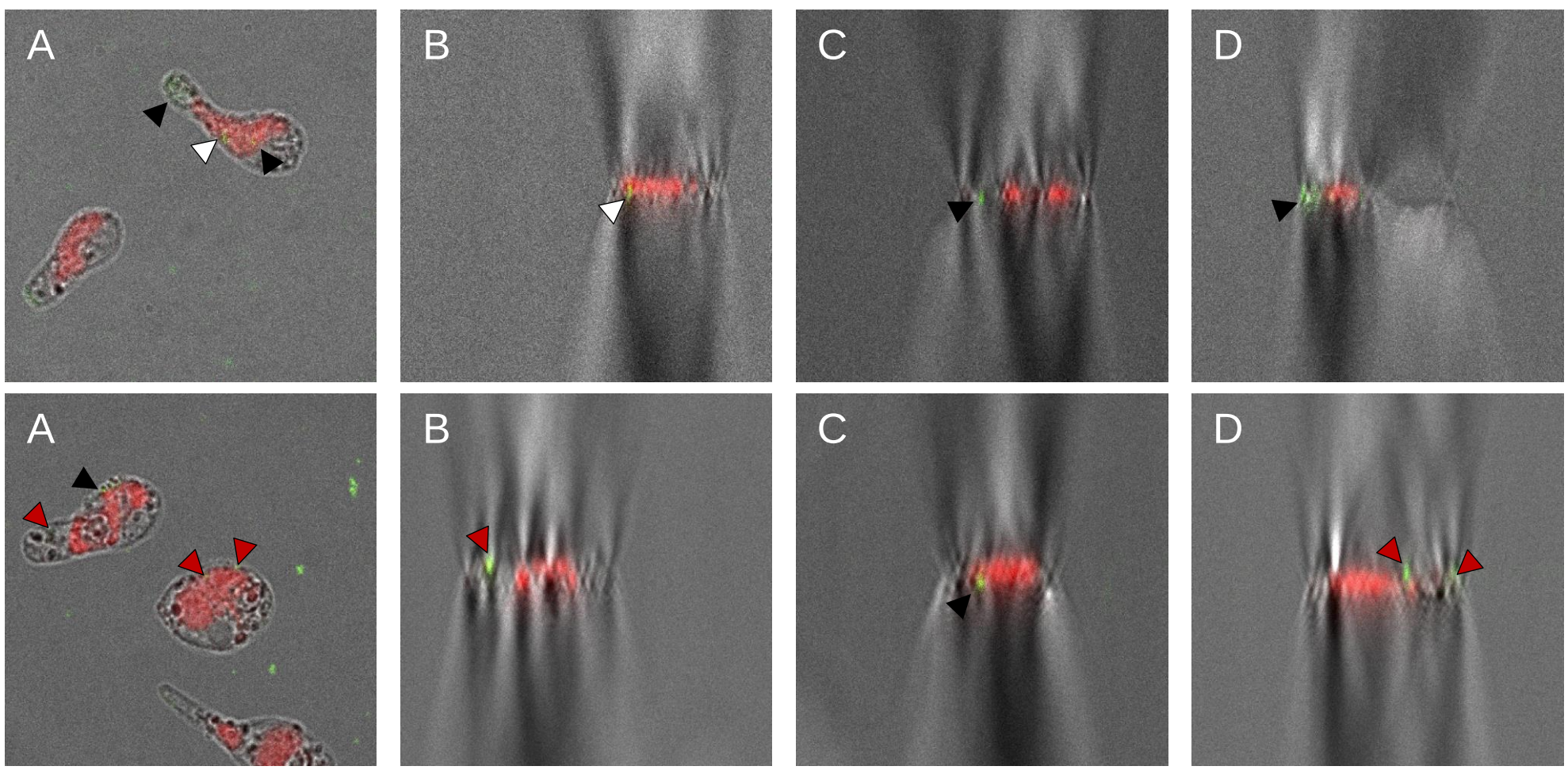
Poulsen, N., Scheffel, A., Sheppard, V. C., Chesley, P. M. & Kröger, N. Pentalysine Clusters Mediate Silica Targeting of Silaffins in *Thalassiosira pseudonana*. *J. Biol. Chem.* **288**, 20100–20109 (2013).

Zhang, K. *et al.* Endoplasmic Reticulum Stress Activates Cleavage of CREBH to Induce a Systemic Inflammatory Response. *Cell* **124**, 587–599 (2006).

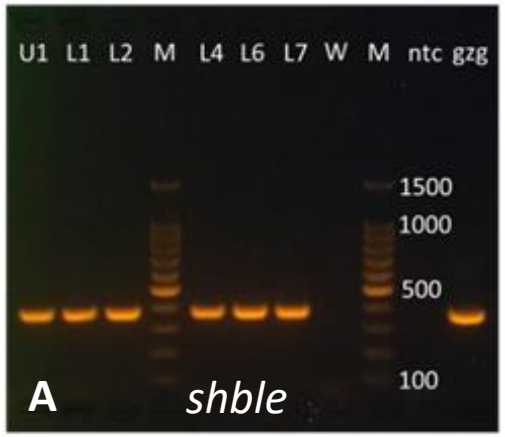


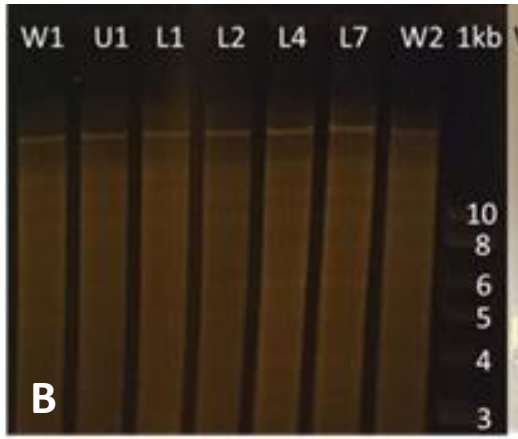
Suppl. Fig. 3. Transformation of *Tetraselmis striata* (KAS-836), by microprojectile bombardment, with a plasmid (pACTpro:ble) containing a selectable marker consisting of the coding sequence of the *Streptoalloteichus hindustanus Ble* gene (conferring resistance to zeocin) under the control of regulatory sequences from the *T. striata Actin* gene. (**A**) Transformants were selected on half-strength f/2 agar plates containing 150 μg/ml zeocin. (**B**) Colonies surviving on the selective plates (or non-transgenic control colonies) were examined by PCR amplification of the *Ble* coding sequence using total genomic DNA as the template. The promoter of the *T. striata RPS12* gene (encoding 40S Ribosomal Protein S12) was also amplified as a control for equivalent amount of input DNA in the PCR reactions. The panels show reverse images of ethidium bromide stained PCR products amplified from the non-transgenic recipient strain and from five putative transformants.

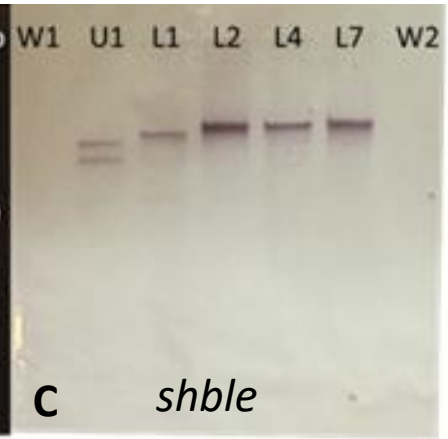




Suppl. Fig. 4. Fluorescent signal of the plasmid DNA in *E. gymnastica* (1, 3-5) **and *P. parkeae*** (2). In case of delivery by nucleofection (1-2), the DNA was labelled by fluorescein and its signal was distinquished from the chlorophyll autofluorescence using fluorescence-lifetime imaging microscopy (FLIM); lifetimes in the whole cells (A) and in the area with longest component (B) is shown. In the case of delivery by standard electroporation (3-5), the DNA was labelled by tetramethylrhodamine and its localization was observed by confocal microscopy (A); XZY scan was performed to determine whether the suspect signal is localized inside the cell or on its surface (B-D): the seemingly successful deliveries are marked by white arrows, the probable extracellular DNA localizations are marked by red arrows, and the ambiguous cases are marked by black arrows.

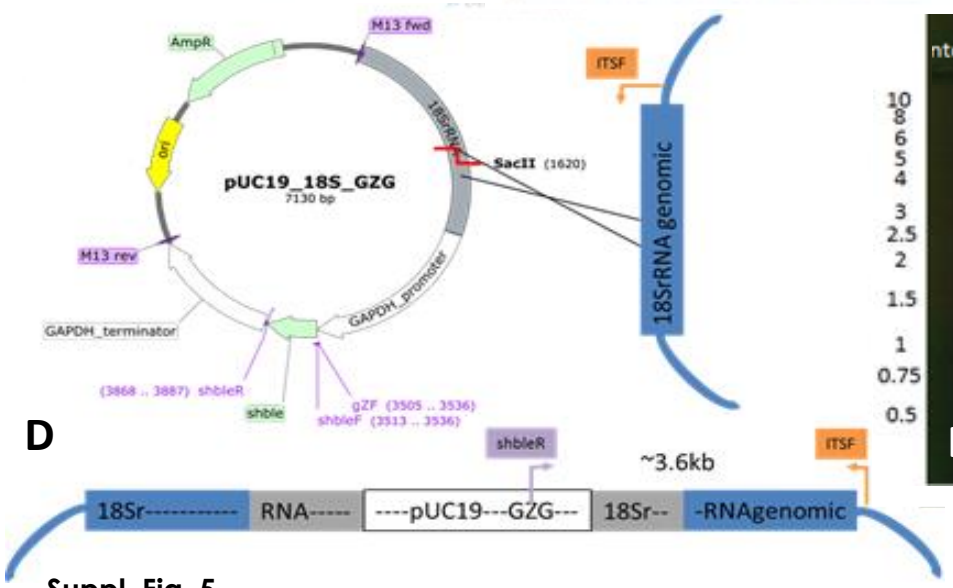


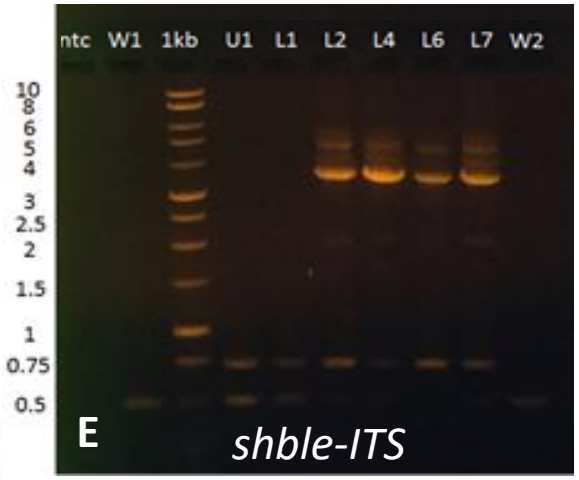


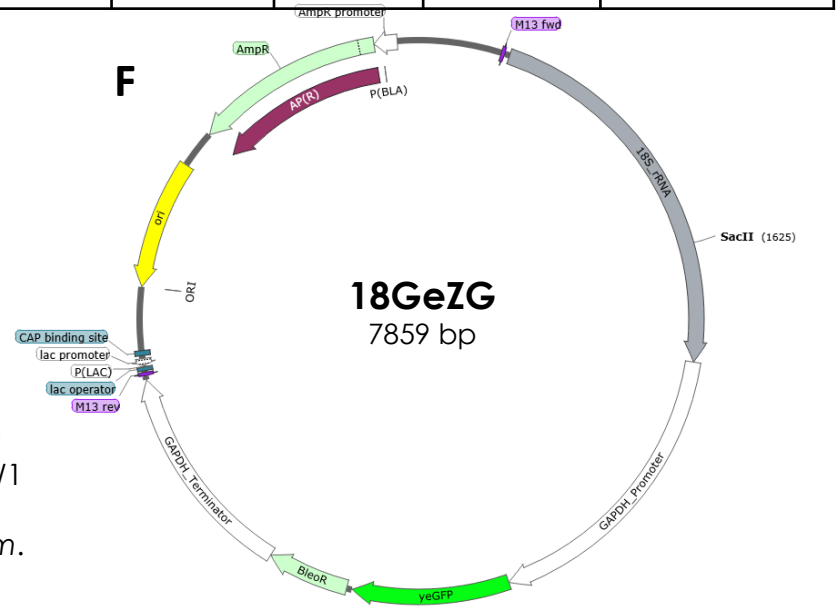


Transformation conditions and efficiencies for *Aurantiochytrium limacinum*.

Instrument	Condition	pUC19_18 GZG DNA	Initial Colonies	Stable Transform-ants	Efficiency (transformant/ µg DNA)
Bio-Rad Gene Pulser	300 V, 1 pulse	10 µg	10	1	0.1
	450 V, 2 pulses	1 µg	54	44	44
		2.5 µg	62	52	20.8
		5 µg	50	39	7.8
		10 µg	75	68	6.8
NEPA	250 V, 4 ms, 2 pulses	1 µg	25	18	18
	275 V, 8 ms, 2 pulses	1 µg	24	13	13
	300 V, 12 ms, 2 pulses	1 µg	11	7	7



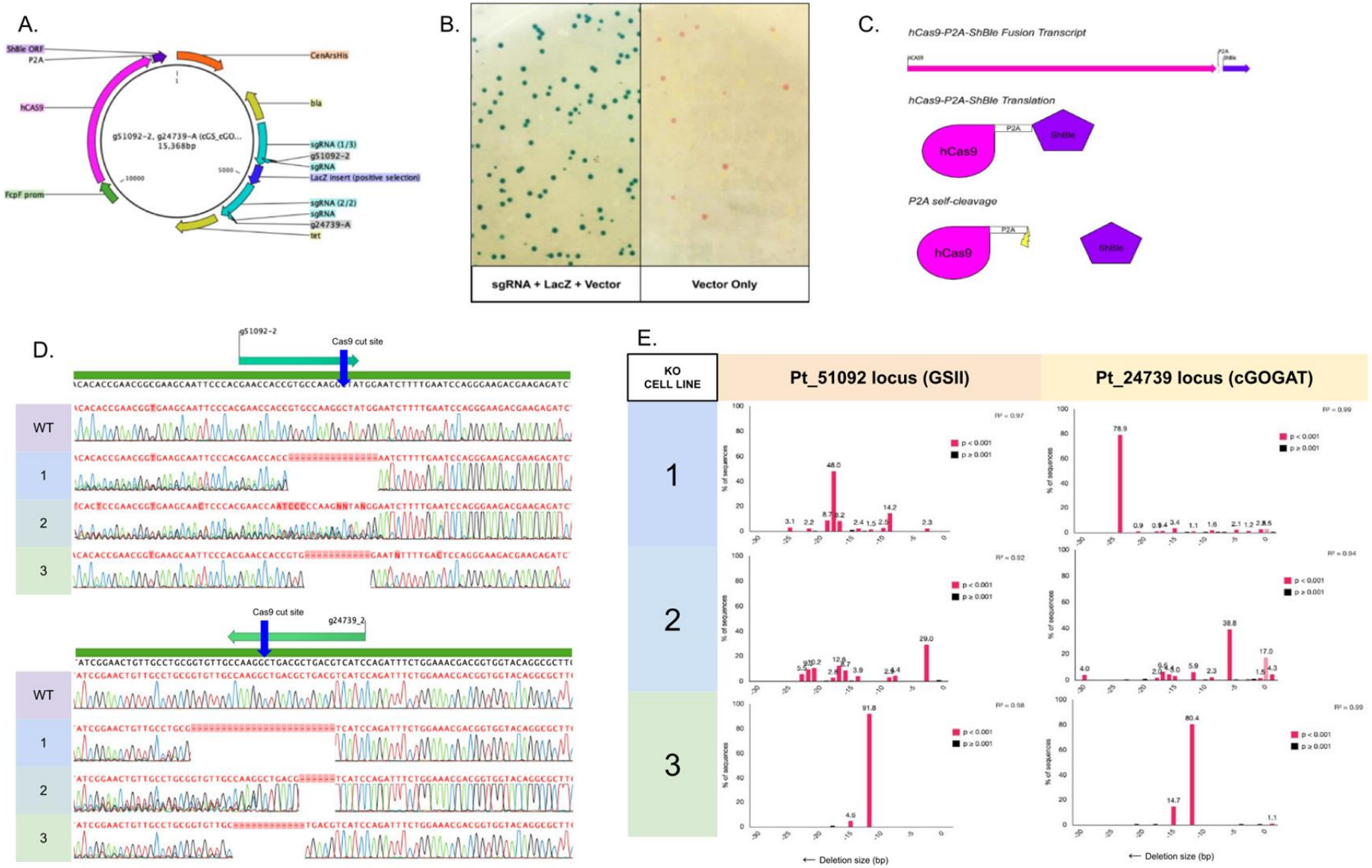




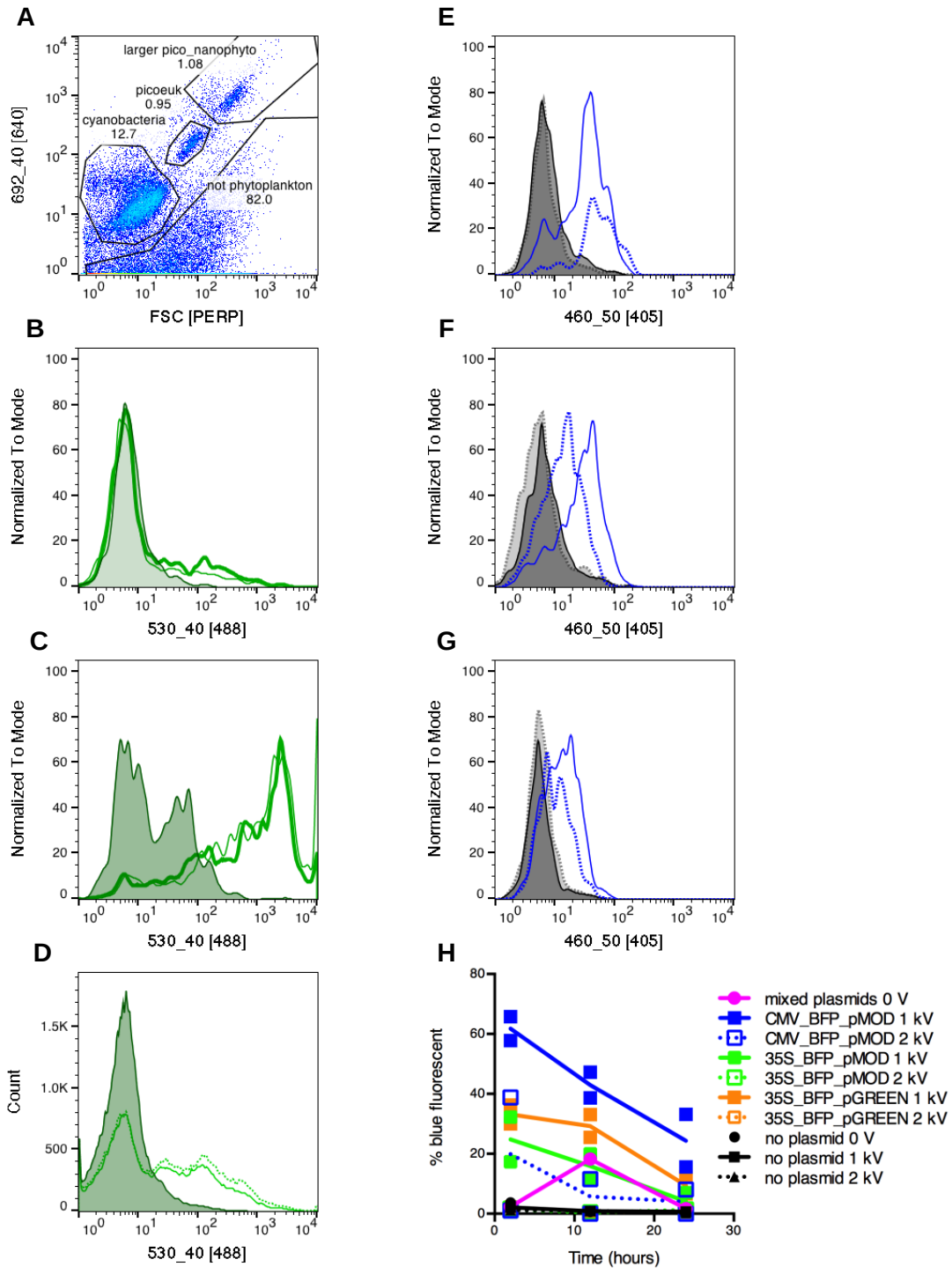
Suppl. Fig. 5

(**A**) Genomic DNA PCR confirmation of *shble* (375 bp) in six 18GZG transformants (U1, L1, L2, L4, L6, L7). W is the wild-type, gzg is the plasmid control, ntc is the no template control, and M is a 100 bp marker. (**B**) Gel electrophoresis of XhoI digested genomic DNA from five transformants (U1, L1, L2, L4, L7) and two wildtypes (W1, W2). (**C**) Developed southern blot using digoxigenin labeled probe of shble. (**D**) Diagram of expected 18GZG plasmid (addgene.org/117228/) integration into *Aurantiochytrium limacinum* genome. The SacII restriction endonuclease site located in the 18S rRNA region was used for linearization. Plasmid primers annotated in purple, and ITS specific primer in orange (ITSF). (**E**) Genomic DNA PCR of shble-ITS region (3.6 kb) in six putative transformants (U1, L1, L2, L4, L6, L7). W1 & W2 are wild-type and ntc is a no template control. L2, L4, L6 and L7 produce the band expected from single homologous recombination. (**F**) 18GeZG plasmid map, used in yeGFP::*shble* expression in *Aurantiochytrium limacinum*. SacII restriction site was used in linearization.

Suppl. Fig. 6



Suppl. Fig. 6: CRISPR-Cas9 Methodology in *Phaeodactylum tricornutum*.
A. Vector map of *Phaeodactylum* CRISPR-Cas9 episome. Golden Gate assembly was employed to clone two sgRNA targets and a LacZ positive selection cassette in the episome that contains hCas9. The two sgRNAs are designed to target glutamine synthetase II (GSII, Gene ID: 51092) and glutamate synthase (cGOGAT, Gene ID: 24739) **B.** Red-white-blue screening of golden gate assembled episomes. Positive clones appear blue when the sgRNA and LacZ inserts are supplied (left panel). The vector only control produces red and white colonies since there is no added inserts (right panel). **C.** After transformation of the episome, hCas9 and ShBle are transcriptionally co-expressed and separate upon translation in vivo. The 2A peptide that spans hCas9 and ShBle cleaves itself after translation. **D.** Sequencing alignment analysis of target loci. (Top) Three mutant cell line (1,2,3) and one wild-type sequencing alignment of the Pt_51092 target locus compared to a reference genomic sequence (green bar). Nucleotides for mutant cell lines are colored red. (Bottom) Three mutant cell lines (1,2,3) and one wild-type sequencing alignment of the Pt_24739 target locus compared to a reference genomic sequence (green bar). For both alignments, the sgRNA is represented by the teal arrow (arrow direction is 5' to 3') and the blue arrow indicates the predicted hCas9 cut site. **E.** TIDE analysis results. Each sequencing read was compared to a wild-type (shown in D) using TIDE software analysis. The bar plots show the predicted deletion genotype indicated by the dark pink bars ($p<0.001$) along the x-axis. Each called indel has a number above each bar in the plot that indicates the percentage of the total deconvolved sequence read that individual indel represents.



Suppl. Fig. 7. **Transient expression of BFP in natural sea water communities.** Flow cytogram of red fluorescence from chlorophyll excited by the 488 nm laser (692_40 [488]) versus forward scatter of natural community obtained by concentration of 50 liters of sea water by tangential flow filtration after which cells were washed with sorbitol 800 mM (A). Histograms of green fluorescence (from fluorescein 2000 kD dextran and autofluorescence) in picophytoeukaryote (picoeuk) (B), larger pico-nanophytoeukarotes (C), and nonphytoplankton (D) 2 hours after were electroporated with a pulse of 1 kV for 20 ms (solid line) or a pulse of 2 kV for 8 ms (dashed line). Shaded histogram shows sub-sample incubated with dextran but w/o electroporation. Picophytoeukaryotes electroporated also with (blue) the CMV_BFP_pMOD plasmid (blue) or without plasmid (grey) at 1 kV (solid lines) or 2 kV (dashed lines) at 2 hours (E), 12 hours (F), and 24 hours (G). Time course of percentage blue fluorescent cells in picoeukaryotic phytoplankton over time in all treatments (2 replicas per treatment) (H).

Suppl. Table 1. Major representative previous algal genetic transformation studies.

Phylum	Species	Transformation method	Construct (promoter)	Selectable marker; selecting agent	Reporter	Reference
Alveolates	*Symbiodinium microadriaticum*	Si carbide whiskers (SiCaW)	pMT Npt/GUS (nos, CaMV35S); pMT Hpt/GUS *(Agrobacterium* [*Agro]* p1'2')	*nptII, hpt*; kanamycin, hygromycin*, G418	Beta-glucuronidase (GUS)	ten Lohuis & Miller (1998)
	Symbiodinium spp.	Agitation with Glass beads	*p*CB302-*gfp-AtRACK1C*, *p*CAMBIA-*FABD2-gfp* (CaMV35S)	*bar, hpt; Basta*, hygromycin	Green fluorescent protein (GFP)	Ortiz-Matamoros et al. (2015a)
		Glass beads and co-incubation with *Agro*	pCB302-*gfp-AtRACK1C*, pCB302-*gfp-MBD*, pCB302-*gfp-FABD2* (CaMV35S)	*bar*; Basta	Green fluorescent protein (GFP)	Ortiz-Matamoros et al. (2015b)
	Amphidinium carterae	SiCaW	pMT Npt/GUS (nos, CaMV35S); pMT Hpt/GUS (*Agro* p1'2')	*nptII, hpt*; hygromycin*, G418, kanamycin	GUS	ten Lohuis & Miller (1998)
Stramenopiles	*Phaeodactylum tricornutum*	Biolistics	(*fcp* [fucoxanthin chlorophyll-a or -c binding protein])	*ShBle* (phleomycin/zeocin)	Luciferase (LUC)	Falciatore et al. (1999)
		Biolistics	*p*fcpA (fcp [fucoxanthin chlorophyll-a or -c binding protein])	*ShBle* (phleomycin/zeocin)	Chloramphenicol acetyltransferase (CAT)	Apt et al. (1996)
		Electroporation	*p*HY11-*cat* (native NR promoter)	*cat*; chloramphenicol	CAT	Niu et al. (2012)
		Conjugation	*p*0521S/CEN6-ARSH4-HIS3/*p*fcpA	*shble*	Cyan fluorescence protein (CFP), GFP, Yellow fluorescence protein (YFP)	Karas et al. (2015)

	Cyclotella criptica	Biolistics	plasmids with nptII (diatom acetyl-CoA carboxylase promoter)	*nptII;* G418	Expression of NPTII	Dunahay et al. (1995)
	Navicular saprophila	Biolistics	plasmids with nptII (diatom acetyl-CoA carboxylase promoter)	nptII; G419	Expression of NPTII	Dunahay et al. (1995)
	Cylindrotheca fusiformis	Biolistics	pHUPtag-*fcp* (fucoxanthin chlorophyll-a or -c binding protein)	*ble*/*frue, ble*/HUPtag; zeocin, kanamycin, hygromycin B		Fischer et al. (1999)
	Conticribra (Thalassiosira) weissflogii	Biolistics	(*fcp* [fucoxanthin chlorophyll-a or -c binding protein])		Luciferase (LUC)	Falciatore et al. (1999)
	Pseudo-nitzschia multistriata, P. arenysensis	Bioballistic	Histone H4	*Shble* , zeocin	Growth on selective medium	Sabatino et al. 2015
	Thalassiosira pseudonana	Biolistics	pTpNR/GFP (nitrate inducible NR promoter)	*Nat* (nourseothricin)	EGFP	Poulsen & Chesley (2006)
	Thalassosira pseudonana	Conjugation	pTpExpPEPCK-YFP (LHCF9 promoter_	*Nat* (nourseothricin)	Yellow fluorescent protein (YFP)	Karas et al. (2015)
	Nannochloropsis sp.	Electroporation	*pVCP1*	*shble*		Kilian et al. (2011)
	Nannochloropsis gaditana	Electroporation	*p*TUB/*p*HSP/*p*UEP/	*shble*		Radakovits et al. (2013)
	Nannochloropsis oceanica	Electroporation	*p*CMV-EM7	*sheble*		Osorio et al. (2019)
Archaeplastids		Glass beads	*p*MN24 (containing native fragments)	*nr;* nitrate	Growth on selective medium (nitrate)	Kindle (1990)
	Chlamydomonas reinhardtii	Electroporation	*p*JD67 carrying ARG7	*arg7*	Growth on corn starch	Shimogawara et al. (1998)
		Si carbide whiskers	*p*MN24 based on pUC19 (containing native NR)	*nr*, nitrate	Growth on selective medium (nitrate)	Dunahay (1993)

	Biolistics	*p*U12.6 (containing ASL)	argininosuccinate lyase (ASL); AS	Growth on selective medium	Debuchy et al. (1989)
	Biolistics	*p*UC19	OEE1, oxygen-evolving enhancer protein 1	Growth on selective medium (-acetate)	Mayfield & Kindle (1990)
	*Agrobacterium tumefaciens***	T-DNA	*hpt*; hygromycin	GUS, GFP	Kumar et al. (2004)
Chlorella ellipsoidea	Biolistics	*p*DO432		LUC	Jarvis & Brown (1991)
	Electroporation	(Ubil-Ω)		GUS	Chen et al. (2001)
Chlorella saccharophila	Electroporation	PBI221 (CaMVS35)		GUS	Maruyama et al. (1994)
Chlorella vulgaris	Protoplast transformation		*kanr*; G418	Expression of human growth hormone (hGH)	Hawkins & Nakamura (1999)
	Electroporation	pMD18-Ap*cat* (NR promoter)	*cat*; chloramphenicol		Niu et al. (2011)
Haematococcus pluvialis	Biolistics	*p*SV40-*LacZ* (SV40)		β-galactosidase (LacZ)	Teng et al. (2002)
Ostreococcus tauri	Electroporation	*Potluc* *Potox*	KanMX, G418 Nat1, cloNat	LUC	Corellou et al. [2009]
	Electroporation	PCR product including 1kbp of ferritin homologous sequence	KanMx, G418	LUC (Knock in)	Lozano et al. [2014]
Dunaliella salina	Electroporation	PBI221, pUGUS, pUΩGUS, P35UΩGUS (CaMVS35, Ubil-Ω)		GUS	Geng et al. (2003)
	Electroporation		*ble*; Zeocin		Sun et al. (2005)***
	Biolistics	(CaMV35S promoter)	*bar*; Basta	GUS	Tan et al. (2005)

	Biolistics	*p*DM307 (native CA [carbonic anhydrase] promoter)	*bar*; Basta	Nitric oxide synthase (NOS)	Lü et al. (2005)

*found to be most effective; ** shown to work better than glass beads; *** some introduced DNA stayed as episomal plasmid DNA

References for Suppl. Table 1:

ten Lohuis. M. R. & Miller, D. J. Genetic transformation of dinoflagellates (*Amphidinium* and *Symbiodinium*): expression of GUS in microalgae using heterologous promoter constructs. *Plant J*. **13**, 427-435. (1998).

Ortiz-Matamoros, M. F., Villanueva, M. A., & Islas-Flores, T. Transient transformation of cultured photosynthetic dinoflagellates (*Symbiodinium* spp.) with plant-targeted vectors. *Ciencias Marinas* **41**, 21-32. (2015a).

Ortiz-Matamoros, M. F., et al. Heterologous DNA Uptake in Cultured *Symbiodinium* spp. Aided by *Agrobacterium tumefaciens*. *PLoS ONE* **10**, e0132693. (2015b).
Falciatore, A., Casotti, R., Leblanc, C., Abrescia, C. & Bowler, C. Transformation of nonselectable reporter genes in marine diatoms. *Mar. Biotechnol.* **1**, 239-251 (1999).

Apt, K. E., Kroth-Pancic, P. G. & Grossman, A. R. Stable nuclear transformation of the diatom *Phaeodactylum tricornutum*. *Mol. Gen. Genet.* **252**, 572-579 (1996).

Niu, Y.-F., et al. Transformation of diatom *Phaeodactylum tricornutum* by electroporation and establishment of inducible selection marker. *BioTechniques* **52**, 1-3 (2012).

Karas, B. J. et al. Designer diatom episomes delivered by bacterial conjugation. *Nat. Commun.* **6**, 6925 (2015).

Dunahay, T., Jarvis, E. & Roessler, P. Genetic transformation of the diatoms *Cyclotella cryptica* and *Navicula saprophita*. *J. Phycol.* **31**, 1004**–**1012 (1995).

Fischer, H., Robl, I., Sumper, M. & Kröger, N. Targeting and covalent modification of cell wall and membrane proteins heterologously expressed in the diatom *Cylindrotheca fusiformis. J. Phycol.* **35**, 113-120 (1999).

Falciatore, A., Casotti, R., Leblanc, C., Abrescia, C. & Bowler, C. Transformation of nonselectable reporter genes in marine diatoms. *Mar. Biotechnol.* **1**, 239-251 (1999).

Sabatino, V., Russo M. T., Patil S., d'Ippolito G., Fontana A., and Ferrante M. I. Establishment of genetic transformation in the sexually reproducing diatoms *Pseudo-nitzschia multistriata* and *Pseudo-nitzschia arenysensis* and inheritance of the transgene. *Marine Biotechnology* **17**: 452-462 (2015).

Poulsen, N., Chesley, P. M. & Kröger, N. MOLECULAR GENETIC MANIPULATION OF THE DIATOM *THALASSIOSIRA PSEUDONANA* (BACILLARIOPHYCEAE). *J. Phycol.* **42**, 1059–1065 (2006).

Kilian, O,, et al. High-efficiency homologous recombination in the oil-producing alga *Nannochloropsis* sp. *Proc. Natl. Acad. Sci. USA* **108**, 21265–21269 (2011)

Radakovits, R. et al. Draft genome sequence and genetic transformation of the oleaginous alga *Nannochloropsis gaditana*. *Nature Communications* **3**, 686 (2013).

Osorio, H., et al. High-efficiency nuclear transformation of the microalgae *Nannochloropsis oceanica* using Tn5 Transposome for the generation of altered lipid accumulation phenotypes. Biotechnology for Biofuels **12**, 134 (2019).

Kindle, K. L. High-frequency nuclear transformation of *Chlamydomonas reinhardtii. Proc. Natl. Acad. Sci. USA* **87**, 1228-1232 (1990).

Shimogawara, K., Fujiwara, S., Grossman, A. and Usuda, H. High-efficiency transformation of *Chlamydomonas reinhardtii* by electroporation. *Genetics*, **148**, 1821-1828 (1998).

Dunahay, T. G. Transformation of *Chlamydomonas reinhardtii* with silicon carbide whiskers. *BioTechniques* **15**, 452-460 (1993).

Debuchy, R., Purton, S. and Rochaix, J.D. The argininosuccinate lyase gene of *Chlamydomonas reinhardtii*: an important tool for nuclear transformation and for correlating the genetic and molecular maps of the ARG7 locus. *The EMBO journal*, **8**, 2803-2809 (1989).

Mayfield, S. P., Kindle, K. L. Stable nuclear transformation of *Chlamydomonas reinhardtii* by using a *C. reinhardtii* gene as the selectable marker. *Proc. Natl. Acad. Sci. USA* **87**, 2087-2091 (1990).

Kumar, S. C., Misqitta, R. W., Reddy, V. S., Rao, J. B. & Rajam, M. V. Genetic transformation of the green alga *Chlamydomonas reinhardtii* by *Agrobacterium tumefaciens*. *Plant Sci*. **166**, 731-738 (2004).

Jarvis, E.E. and Brown, L.M. Transient expression of firefly luciferase in protoplasts of the green alga *Chlorella ellipsoidea*. *Current genetics*, **19**, 317-321. (1991).

Chen, Y., Wang, Y., Sun, Y., Zhang, L. and Li, W. Highly efficient expression of rabbit neutrophil peptide-1 gene in *Chlorella ellipsoidea* cells. *Current genetics*, **39,** 365-370 (2001).

Maruyama, M., et al. Introduction of foreign DNA into *Chlorella saccharophila* by electroporation. *Biotechnol. Techn.* **8**, 821-826 (1994).

Hawkins, R.L. and Nakamura, M. Expression of human growth hormone by the eukaryotic alga, *Chlorella*. *Current Microbiology*, **38**, 335-341 (1999).

Niu, Y. F., et al. A new inducible expression system in a transformed green alga, *Chlorella vulgaris*. *Genet. Mol. Res.* **10**, 3427-3434 (2011).

Teng, C., et al. Transient expression of lacZ in bombarded unicellular green alga *Haematococcus pluvialis*. *Journal of Applied Phycology*, **14,** 497-500 (2002).

Corellou et al. Clocks in the green lineage: comparative functional analysis of the circadian architecture of the picoeukaryote *Ostreococcus*. Plant Cell, **21,** 3436-3449 (2009).

Lozano et al. Efficient gene targeting and removal of foreign DNA by homologous recombination in the picoeukaryote *Ostreococcus*. Plant J. **78**, 1073-1083. (2014).

Geng, D., Wang, Y., Wang, P., Li, W. & Sun, Y. Stable expression of hepatitis B surface antigen gene in *Dunaliella salina* (Chlorophyta). *J. Appl. Phycol.* **15**, 451-456 (2003).

Sun, Y., et al. Expression of foreign genes in *Dunaliella* by electroporation. *Mol. Biotechnol*. **30**, 185-192 (2005).

Tan, C., Quin, S., Zhang, Q., Jiang, P. & Zhao, F. Establishment of a micro-particle bombardment transformation system for *Dunaliella salina*. *J Microbiol*. **43**, 361-365 (2005).

Lü, Y. M., et al. Cloning and functional analyses of promoters of two carbonic anhydrase genes from *Dunaliella salina*. *Acta Genet. Sin.* **31**, 1157-1166 (2004).

Suppl. Table 2. Selected physiological and ecological characteristics of the protists targeted in this study.

Species	Feeding (auto/hetero/mixo)	Habitat	Cell walls	Cell structure	Life cycle	Planktonic?
Archaeplastids						
Ostreococcus lucimarinus	photoautotroph	marine	no visible cell wall, just thin membrane	small (~1 µm); atypical prasinophyte (no flagella, no scales) (Worden et al., 2004)	asexual by binary fission, possible sexual cycle	yes
Bathycoccus prasinos	photoautotroph	marine	organic scales	small (~2-3 µm); cells covered by scales of unknown organic material. no flagellum. (Limardo et al., 2017)	asexual by binary fission, possible sexual cycle	yes
Micromonas commoda	photoautotroph	marine	no visible cell wall, just thin membrane	small (~1-2 µm); single flagellum, no scales; phototactic (positive)	asexual by binary fission, possible sexual cycle	yes
Micromonas pusilla	photoautotroph	marine	no visible cell wall, just thin membrane	small (~1-2 µm); single flagellum; phototactic (positive); Encodes most of peptidoglycan pathway from cyanobacterial endosymbiont that formed plastid.	asexual by binary fission, possible sexual cycle	yes
Tetraselmis striata	photoautotroph	marine coastal; most free-living but some animal symbionts	complex polysaccharide scales fuse to form a wall; cell anchored to wall by microtublues in four places	3-25 µm, two pairs of flagella with complex scales etc covering, one large chloroplast; accumulates HUFAs	flagellated stage, vegetative no-motile stage (usually dominant), cyst	yes
Pyramimonas parkeae	photoautotroph	marine	wall-less	20 x 15 µm	four flagella	yes
Haptophytans						
Isochrysis galbana	photoautotrophic	marine	thin layer of organic scales, non-calcified	5-6µm, two flagella, 2 chloroplasts, stigma, oil droplets.	Asexual by binary fission, cyst	yes
Emiliana huxleyi	photoautotrophic	marine, broadly distributed	calcium carbonate plates (coccoliths)	~5 µm	diploid calcified stage, motile non-calcified haploid stage	yes
Rhizarians						
Amorphochlora (Lotharella) amoebiformis	photomixotrophic (eat bacteria)	marine	none	Amoeboid cell (8-15 µm) with many filopodia.	asexual by binary fission	no

Bigelowiella natans	photomixotrophic (eat bacteria)	marine, broadly distributed	vegetative cells usually naked; this cell wall only in 'cysts' in old cultures	Amoeboid, thin filopodia (thread-like pseudopodia; sometimes reticulopodia); or coccoid cells with multilayered cell wall; or uniflagellated zoospores. store b-1,3 glucan (not starch). Mitochondria with tubular cristae. Nucleomorph genome ~400 kb (3 linear chromosomes), coding 17 plastid genes and ~350 housekeeping genes with lots of small (~20 bp) introns. Secondary endosymbiosis between cercozoan host and green algal symbiont.	some species have all three cell types, many are missing one or another; which form is the main vegetative stage differs among species. Chlorarachnion may have amoeboid gamete!	yes
Stramenopiles						
Fragilariopsis cylindrus	photoautotrophic	polar oceans and sea ice; ice edge blooms	silica			yes
Thalassiosira pseudonana	photoautotrophic	oceanic, coastal temperate, possible bacterial symbiont	silica and organics (long-chain polyamines, chitin, proteins)	~5-10 µm	size reduction–restitution cycle (SRRC): asexual by binary fission accompanied by cell size reduction, cell size restored in a sexual cycle	yes
Seminavis robusta	photoautotrophic	benthic	silica	~10x50 µm; pennate		no
Pseudo-nitzscha multiseries	photoautotrophic	HAB-forming but otherwise rare; toxic; coastal neretic	silica	~5x100 µm; pennate, chain-forming;		yes
Pseudo-nitzscha australis	photoautotrophic	HAB-forming but otherwise rare; toxic; coastal neretic	silica	~5x100 µm; pennate, chain-forming;		yes
Heterosigma akashiwo	photoautotrophic	diverse; some form HAB	naked	~50-100 µm, heterokont flagella		yes
Aurantiochytrium limacinum MYA-1381	osmoheterotrophic; stores PUFAS	marine (plant detritus)	sulfated polysaccharide scales	4 - 20 µm; grow attached by ectoplasmic net elements or in suspension	probably asexual reproduction by zooospores	mero-[1]
Caecitellus sp.	phagoheterotrophic		none?	<5 µm; gliding motility, raptorial feeding		yes

Nannochloropsis oceanica	photoautotroph	marine waters worldwide	Smooth cell wall, composed of principally cellulose and algaenan, with papilla	<5 µm ovoid cells, non-motile	asexual reproduction; may be ameiotic	yes
Phaeodactylum tricornutum	photoautotrophic	brackish and marine waters worldwide	oval morphotype silicified, other morphotypes very lightly silicified, sulfated α-mannan decorated with glucuronic residues	polymorphic (exists as oval, fusiform, triradiate, cruciform morphotype), ~5x30 µm (fusiform), ~10 µm ovoiate gliding motility (oval), can form chains	asexual by binary fission, possible sexual cycle	yes (oval type benthic)
Alveolates						
Euplotes crassus	phagoheterotrophic	marine	rigid pellicle	~50-100 µm; hypotrichous ciliate	asexual by binary fission and sexual by conjugation or autogamy	yes
Euplotes focardi	phagoheterotrophic	marine f=antarctic	rigid pellicle	~50-100 µm; hypotrichous ciliate	asexual by binary fission and sexual by conjugation	yes
Chromera velia	photoautotroph?	coral reef coral-associated, possibly non-facultative symbiont	thick cell wall	7.0 × 7.6 µm, coccoid stage with no flagella	sex not observed, forms flagellate stages	mero[1]
Perkinsus marinus	osmoheterotroph, obligate parasite	parasite of marine molluscs	Yes	2-10 µm; apicoplasts;	zoospores and trophozooites	Both as trophozoite and flagellated stage
Oxyrrhis marina	heterotrophic (phagotrophic; omnivorous)	plankton; global coastally except polar; can form blooms	naked (no theca) except has scales	20-30 µm		yes
Hematodinium sp.	parasite	crustacean hemolymph	naked at trophont stage	Cultured as asexual multinucleated trophonts (up to 50 µm)	Complex asexual stages, non-flagellate; mononucleated sexual zoospores	Only at zoospore stage
Fugacium (Symbiodinium) kawagutii	photoautotrophic symbionts (maybe some phagotrophy?)	intra- or intercellular symbionts of marine invertebrates	naked swimming cell but cellulose wall in nonmotile phase	~10 µm	motile (free-swimming) and non-motile (in host) phases; only the latter grows and divides	
Alexandrium catenella	photoautotroph	HAB-forming phytoplankton; produce saxitoxin (PSP)	cellulose plates	~25 µm, usually chains of 2, 4, 8	asexual reproduction by binary fission; sexual reproduction to form a cyst	yes

Breviolum (Symbiodinium) sp.	photoautotrophic symbionts (maybe some phagotrophy?)	intra- or intercellular symbionts of marine invertebrates	naked swimming cell but cellulose wall in nonmotile phase	~10 µm	motile (free-swimming) and non-motile (in host) phases; only the latter grows and divides	
Crypthecodinium cohnii	hetertrophic (glucose, acetate, propionic acid); may predate via peduncle	brackish, littoral, neritic; often around macrophytes like Fucus. temperate and tropical	very thin cellulose	~15-20 µm; produce carotenoids in the light	stores starch during log phase, DHA mainly in cysts?; swimming cells and cysts.	
Amphidinium carterae	photoautotroph	HAB-forming phytoplankton	naked?	~25 µm, solitary	Asexual by binary fission	yes
Karlodinium veneficum	mixotroph (predatory)	HAB-forming, toxic, marine, planktonic	naked	~10 µm		yes
Discobans						
Bodo saltans	phagoheterotrophic	broadly distributed	none?	saltans is 4-5 µm with two flagellae		no? saltans attaches to surfaces by tip of long (posterior) flagellum
Diplonema papillatum	phagoheterotrophic	free-living planktonic?	no pellica	~16 µm, two flagella of equal lenth, two subapical openings		yes
Eutreptiella gymnastica	Photoautotrophic	neritic, cosmopolitan	flexible pellicle	15-20 µm, two flagella, reddish eyespot, paramylon granules in cytoplasm, vigorous metaboly often observed	can form a cyst with layered cell wall	yes
Naegleria gruberi	phagoheterotrophic	wet soil and freshwater	naked	amoeboflagellate; the amoeba lacks microtubule cytoskeleton, flagellate has elaborate one including the flagella; de novo synthesis of basal body durin transformation from former to latter;	apparently the genome revealed two distinct haplotypes	
Opisthokonts						
Pirum gemmata ATCC PRA-279? 96% identical to Abeoforma	osmoheterotroph; stores glycogen	peanut worm (Phascolosoma agassizii) gut contents, BC, 2004	composition unknown but fibrous and woven; contain membrane-bound tubular extensions of the cytoplasm with tubules.	vegetative cells ~50-150 µm; large central vacuole with cytoplasm mostly pressed against periphery; multinucleate with nuclei 2 to 4 µm; sporulation happens in half an hour!; endospores ~5 µm and 'weakly amoeboid'.	walled cells divide internally to produce lots of endospores, which are released through parental cell wall in spurts	no

Sphaeroforma arctica	phagoheterotrophic	invertebrate symbiont; isolated from Gammarus; arctic	carbohydrate, mostly N-acetyl-glucosamine (chitin?)	simple round cells, lots of DHA and EPA in cell membranes but not accumulated to high levels in lipid bodies	very simple growth from 5-7 µm cells for 48 hr to 35-40 µm, then releasing ~120 new cells	no
Abeoforma whisleri ATCC PRA-280? 96% identical to Pirum	osmoheterotroph; stores glycogen	mussel (Mytilus sp.) gut contents, BC, 2007	have both cell wall and extracellular matrix; wall sometimes very thick; composition unknown but fibrous and woven; contain membrane-bound tubular extensions of the cytoplasm with tubules.	vegetative cells mostly spherical ~50 µm but some plasmodial; endospores, plasmodia, and hyphae-like structures observed; say dispersal amoebae 'function in post reproductive dispersal' and are uninucleate.	walled spherical cells, plasmodia, amoebae; asexual reproduction by dispersal amoebae, endospores, binary fission and budding.	no
Salpingoeca rosetta	phagoheterotrophic	free-living planktonic and benthic thecate	a proteinaceous and polysaccharide matrix	cell body 3-10 µm; single apical flagellum surrounded by a collar of 30-40 actin-filled microvilli	asexual by longitutidnal fission; sexual reproduction triggered by nutrient deprivation and a secreted chondroitin lyase from *Vibrio* bacteria; dynamic life history includes unicellular and multicellular forms	yes

[1] Meroplanktonic organisms spend only a portion of their lives as plankton.

References for Suppl. Table 2:

Worden, A. Z., Nolan, J. K., & Palenik, B. (2004). Assessing the dynamics and ecology of marine picophytoplankton: the importance of the eukaryotic component. *Limnology and Oceanography* **49**, 168–179.

Limardo AJ, Sudek S, Choi CJ, Poirier C, Rii YM, Blum M, Roth R, Goodenough U, Church MJ & AZ Worden. (2017)Quantitative biogeography of picoprasinophytes establishes differential ecotype distributions and significant contributions to marine phytoplankton. *Environmental Microbiology* **19**, 3219-3234.

Suda S, Atsumi M, & Miyashita H. (2002). Taxonomic characterization of a marine *Nannochloropsis* species, *N. oceanica* sp. nov.(Eustigmatophyceae). *Phycologia*, **41**, 273-279.

Scholz MJ, Weiss TL, Jinkerson RE, Jing J, Roth R, Goodenough U, Posewitz MC & Gerken HG. (2014). Ultrastructure and composition of the Nannochloropsis gaditana cell wall. *Eukaryotic cell*, **13**, 1450-1464.

Pan K, Qin J, Li S, Dai W, Zhu B, Jin Y, Yu W, Yang G. & Li D. (2011). Nuclear monoploidy and asexual propagation of *Nannochloropsis oceanica* (eustigmatophyceae) as revealed by its genome sequence1. *Journal of phycology*, **47**, 1425-1432.

Suppl. Table 3

	5-Fluoroorotic Acid	Amphotericin B	Ampicillin	Atrazine	Basta	Benomyl	Blasticidin	Carboxine	Chloramphenicol	Cycloheximide	Formaldehyde	Geneticin (G418)	Glufosinate	Glyphosate	Hygromycin B	Kanamycin	Neomycin	Nourseothricin	Paromomycin	Penicillin	Phleomycin	Phleomycin	Puromycin	Rifampicin	Streptomycin	Tetracycline	Zeocin
Abeoforma whisleri	n/s					1-20		20-300										n/s			n/s		100-500				
Alexandrium catenella		n/s	n/s		60		25		20		20	n/s			n/s	n/s			n/s					200	n/s		
Amorphochlora amoebiformis (CCMP2058)																											300
Amphidinium carterae				2.5					10																		
Aurantiochytrium limacinum			n/s									500			200											n/s	100
Bathycoccus prasinos (RCC4222)												1000															
Bigelowiella natans												400															
Bodo saltans												5															
Breviolum (Symbiodinium) sp.	200																										
Crypthecodinium cohnii (CCMP316)							50		100-400			100-800															
Diplonema papillatum							50					75			125			400				500	20			n/s	
Emiliania huxleyi												250						50									
Euplotes crassus and E. focardii												200							200				40				
Eutreptiella gymnastica							10					200			200								10				200
Fragilariopsis cylindrus																											100
Heterosigma akashiwo															10							0.05					0.5
Isochrysis galbana												175						150									250
Karlodinium veneficum		n/s	n/s		100		30		10		20	n/s			n/s	100			n/s					300	500		
Micromonas commoda (CCMP2709)									2	0.001																	
Micromonas pusilla (CCMP1545)									3	0.001										1500							
Naegleria gruberi															300		700										
Ostreococcus lucimarinus (RCC802)												1000							n/s		2.5						
Oxyrrhis marina		n/s	n/s		n/s		n/s		50		40	n/s			n/s	n/s			n/s	n/s			1	225	n/s		
Phaeodactylum tricornutum																		200			20						50
Perkinsus marinus							50-200																10-50				50-200
Pirum gemmata	n/s					1-20		20-300										n/s			10-300		100-500				
Pseudo-nitzschia australis											3							100									1
Pseudo-nitzschia multiseries											3							100									1
Pyramimonas parkeae												400											40				100
Salpingoeca rosetta																							80				
Seminavis robusta														250									10				
Sphaeroforma arctica	n/s					1-20		20-300										n/s			10-300		100-500				
Tetraselmis striata													150														150
Thalassiosira pseudonana (CCMP1335)																		100-200*									

1500 1000 500 100 50 1
concentration (µg/mL)

*we recommend 100 µg/mL in pour plates and 200 µg/mL in liquid medium

Suppl. Table 4. Tested transformation techniques.
A. Electroporation, B. Biolistics, C. Microinjection, D. Chemical transformation, E. Conjugation and F. Glass beads abrasion conditions. Clades of the species are following the order from Fig. 1.

A. Used electroporation machines, programs and survival rate of cells after electroporation.

Species	Electroporation machine (type of transformation)	Used program/setting	Survival rate
Archaeplastids			
Ostreococcus lucimarinus (RCC802)	Gene Pulser Xcell	1200V, 25µF	30-50%
Bathycoccus prasinos (RCC4222)	Gene Pulser Xcell	1500V, 25µF	50%
Micromonas commoda (CCMP2709, genome sequenced, axenic version of RCC299)	LONZA®	SF Buffer with pulse EH-100, EO-100, EN-138 and EW-113	10-11%
Micromonas pusilla (CCMP1545)	BioRad Gene Pulser	1000V, 10µF, 400Ω 800V, 25µF,400Ω 600V, 50µF,400Ω	n/a
Pyramimonas parkeae	Gene Pulser Xcell	1500V, 25µF	n/a
	Gene Pulser Xcell	300V, 500µF	n/a
	Gene Pulser Xcell	2500V, 25µF	n/a
	Gene Pulser Xcell	310V, 960µF	n/a
	Gene Pulser Xcell	420V, 960µF	n/a
	Gene Pulser Xcell	300V, 200µF	n/a
	Gene Pulser Xcell	100V, 100µF	n/a
	Gene Pulser Xcell	100V, 300µF	n/a
	Amaxa Nucleofector II	preset program X-001	n/a
	Amaxa Nucleofector II	preset program T-020	n/a
	Amaxa Nucleofector II	preset program T-023	n/a
	Amaxa Nucleofector II	preset program U-035	n/a
Rhizarians (Chlorarachniophytes)			
Amorphochlora (Lotharella) amoebiformis (CCMP2058)	Gene Pulser Xcell	120 V, 25 ms square wave, 0.2 cm cuvette	20-30%
Bigelowiella natans	Amaxa Nucleofector II	preset program X-001	n/a
	Amaxa Nucleofector II	preset program T-020	n/a
	Amaxa Nucleofector II	preset program T-023	n/a
	Amaxa Nucleofector II	preset program U-035	n/a

	Gene Pulser Xcell	7× poring pulse: 300V, 5ms; 5× transfer pulse: 10V, 50ms	n/a
	Gene Pulser Xcell	7× poring pulse: 250V, 5ms; 5× transfer pulse: 10V, 50ms	n/a
	Gene Pulser Xcell	7× poring pulse: 350V, 5ms; 5× transfer pulse: 10V, 50ms	n/a
Stramenopiles (Diatoms, Bacillariophytes, Raphidophytes)			
Seminavis robusta	BTX ECM 2001	Single pulse conditions: 5 V AC for 5 sec, 300 V pulse for 5, 2.5, 0.5, or 0.1 msec. 5 pulse conditions: 5 V AC for 5 sec, 300 V pulse for 5, 2, 1, or 0.1 msec 10 pulse conditions: 5 V AC for 5 sec, 300 or 500 V pulse for 0.1 or 0.05 msec (only for 500 V)	n/a
	NEPA21	Poring pulse: 150, 200, 225, 250, 275, or 300 V, 10% decay rate; 5 ms length, 50 ms interval Transfer pulse: 8 V, 40% decay rate, 50 ms length, 50 ms interval, alternating polarity	n/a
Heterosigma akashiwo	Gene Pulser Xcell	50/75 V, 25 µF and ∞Ω	80%
Aurantiochytrium limacinum	Gene Pulser (BIO-RAD)	2 pulses: 450V, 25 µF, 1000 Ω (~5 ms)	2.6%
	NEPA	2 pulses: 250 V, 4 ms	1.55%
	NEPA	2 pulses: 275 V, 8 ms	8.2%
	NEPA	2 pulses: 300 V, 12 ms	4.7%
Nannochloropsis oceanica	Gene Pulser II	1800V, 50 µF, 20 ms	n/a
	Gene Pulser II	1000V, 50 µF, 20 ms	n/a
Alveolates			
Euplotes crassus	BioRad Gene Pulser	200V, 25µF, 100Ω	50-60%
Chromera velia	Amaxa Nucleofector II	preset programs X-001	n/a
	Amaxa Nucleofector II	preset program T-020	n/a
	Amaxa Nucleofector II	preset program T-023	n/a
	Gene Pulser Xcell	1500V, 25µF	n/a
	Gene Pulser Xcell	300V, 500µF	n/a
	Gene Pulser Xcell	2500V, 25µF	n/a
	Gene Pulser Xcell	310V, 960µF	n/a
Perkinsus marinus	Amaxa Nucleofector II	preset program D-023	n/a
Oxyrrhis marina	BioRad's MicroPulser	DIC	n/a
	BioRad's MicroPulser	DIC -2 shocks	n/a
	BioRad's MicroPulser	SHS	n/a

	BioRad's MicroPulser	SC2	n/a
Hematodinium sp.	Amaxa Nucleofector II	D-023 and X-001	<1%
Fugacium kawagutii	BioRad's MicroPulser	DIC	n/a
	BioRad's MicroPulser	DIC -2 shocks	n/a
	BioRad's MicroPulser	SHS	n/a
	BioRad's MicroPulser	SC2	n/a
Alexandrium catenella	BioRad's MicroPulser	DIC	n/a
	BioRad's MicroPulser	DIC -2 shocks	n/a
	BioRad's MicroPulser	SHS	n/a
	BioRad's MicroPulser	SC2	n/a
Breviolum (Symbiodinium) sp.	NePA21 Electro-kinetic transfection system	Poring pulse: 300V, 10ms Transfer pulse: 8V, 50ms	n/a
Crypthecodinium cohnii	Amaxa Nucleofector II	preset program D-023	79%
	Amaxa Nucleofector II	preset program A-020	85%
	Amaxa Nucleofector II	preset program T-020	67%
	Amaxa Nucleofector II	preset program T-030	53%
	Amaxa Nucleofector II	preset program X-001	62%
	Amaxa Nucleofector II	preset program X-001	71%
	Amaxa Nucleofector II	preset program L-029	77%
	Amaxa Nucleofector II	preset program X-003	n/a
	Amaxa Nucleofector II	preset program X-005	n/a
	Amaxa Nucleofector II	preset program X-033	n/a
	Amaxa Nucleofector II	preset program Y-003	n/a
	Amaxa Nucleofector II	preset program Y-005	n/a
	Amaxa Nucleofector II	preset program Y-033	n/a
	Amaxa Nucleofector II	preset program Z-007	n/a
	Amaxa Nucleofector II	preset program Z-023	n/a
	Amaxa Nucleofector II	preset program Z032	n/a
	Amaxa Nucleofector II	preset program BAC1	n/a
	Amaxa Nucleofector II	preset program BAC2	n/a
	Amaxa Nucleofector II	preset program BAC3	n/a
	Amaxa Nucleofector II	preset program BAC4	n/a
	Amaxa Nucleofector II	preset program BAC5	n/a
	Amaxa Nucleofector II	preset program BAC6	n/a
	Amaxa Nucleofector II	preset program BAC7	n/a
	Amaxa Nucleofector II	preset program L-029	n/a
	Amaxa Nucleofector II	preset program L-029	n/a
	Microfluidics	Straight 313 V	n/a
	Microfluidics	Straight 625 V	n/a
	Microfluidics	Straight 938 V	n/a
	Microfluidics	Straight 1250V	n/a

	Microfluidics	Straight 1563 V	n/a
	Microfluidics	Divergent 333 V	n/a
	Microfluidics	Divergent 667 V	n/a
	Microfluidics	Divergent 1000 V	n/a
	Microfluidics	Divergent 1333 V	n/a
	Microfluidics	Divergent 1667 V	n/a
	Microfluidics	Divergent 5000 V	n/a
	Lipofectamine		90-100%
Amphidinium carterae	Amaxa Nucleofector 4D	X-100, D-023, L-029 and EH 100.	n/a
	NEPA electroporator	Poring pulse: 150V, length: 5 ms, interval 50 ms, number 7 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 200V, length: 5 ms, interval 50 ms, number 7 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 225V, length: 5 ms, interval 50 ms, number 7 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 250V, length: 5 ms, interval 50 ms, number 7 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 275V, length: 5 ms, interval 50 ms, number 7 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 300V, length: 5 ms, interval 50 ms, number 1 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 300V, length: 5 ms, interval 50 ms, number 4 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 300V, length: 5 ms, interval 50 ms, number 7 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
	NEPA electroporator	Poring pulse: 300V, length: 5 ms, interval 50 ms, number 9 Transfer pulse: 8V, length 50 ms, interval 50 ms, number 5	n/a
Karlodinium veneficum	BioRad's MicroPulser	DIC	n/a
	BioRad's MicroPulser	DIC -2 shocks	n/a
	BioRad's MicroPulser	SHS	n/a
	BioRad's MicroPulser	SC2	n/a

Discobans (Euglenozoans and Heteroloboseans)			
Bodo saltans	NePA21 Electro-kinetic transfection system	Poring pulse: 250V, 25ms Transfer pulse: 60V, 99ms	30-50%
Diplonema papillatum	BTX	1600V, 25W, 50mF	10 %
	Amaxa Nucleofector II	preset program X-001	80-90 %
	Amaxa Nucleofector II	preset program X-014	40-50 %
Eutreptiella gymnastica	Gene Pulser Xcell	1500V, 25µF	n/a
	Gene Pulser Xcell	300V, 500µF	n/a
	Gene Pulser Xcell	2500V, 25µF	n/a
	Gene Pulser Xcell	310V, 960µF	n/a
	Gene Pulser Xcell	420V, 960µF	n/a
	Gene Pulser Xcell	300V, 200µF	n/a
	Gene Pulser Xcell	100V, 100µF	n/a
	Gene Pulser Xcell	100V, 300µF	n/a
	Gene Pulser Xcell	200V, 100µF	n/a
	Gene Pulser Xcell	200V, 300µF	n/a
	Gene Pulser Xcell	350V, 1000µF	n/a
	Gene Pulser Xcell	7× poring pulse: 300V, 5ms; 5× transfer pulse: 10V, 50ms	n/a
	Gene Pulser Xcell	7× poring pulse: 250V, 5ms; 5× transfer pulse: 10V, 50ms	n/a
	Amaxa Nucleofector II	preset program X-001	n/a
	Amaxa Nucleofector II	preset program T-020	n/a
	Amaxa Nucleofector II	preset program T-023	n/a
Naegleria gruberi	BioRad Gene Pulser xCell	175V, 500µF, 400Ω	10-20 %
	Amaxa Nucleofector II	preset program X-29	40-50%
Opisthokonts			
Sphaeroforma arctica	Neon	1000-2500V, 10-40 ms, 1-3 pulses	n/a (not successful)
	Lipofectamina		n/a (not successful)
	LONZA®	16 preset codes P3/P4/P5 buffer	n/a (not successful)
Abeoforma whisleri	Neon (invitrogen)	1300V, 25ms, pulse	60%
	LONZA®	preset program EN-138 P3 buffer	70%
	CaCl+Glycerol		n/a (not successful)
	Lipofectamine		100% (not successful)
Salpingoeca rosetta	LONZA®	SF Buffer with pulse CM156	50%

B. Used biolistic programs.

Species	Biolistic machine	Used setting	Survival rate

Archaeplastids			
Tetraselmis striata	Bio-Rad Biolistic PDS-1000/He Particle Delivery System	0.6µm AuNPs, rupture disc 1550 or 2000 psi, 6cm gap	n/a
Pyramimonas parkeae	PDS-1000/He	0.6 or 1µm AuNPs, rupture disc 1350 psi, 6cm gap	n/a
Haptophytes			
Isochrysis galbana	PDS-1000/He	0.7µm Tungsten beads rupture disc 1350 psi, 6cm gap	n/a
Rhizarians (Chlorarachniophytes)			
Amorphochlora (Lotharella) amoebiformis	PDS-1000/He	1 µm AuNPs, rupture disc 450 psi, 4cm gap	n/a
Bigelowiella natans	PDS-1000/He	0.6µm AuNPs, rupture disc 1350 psi, 6cm gap	n/a
Stramenopiles (Diatoms, Bacillariophytes, Raphidophytes)			
Fragilariopsis cylindrus			
Pseudo-nitzschia multiseries			
Pseudo-nitzschia robusta			
Seminavis robusta	PDS-1000/He	0.55 µm AuNPs or 1.1 µm WNPs, 1550 psi, 3 µg/mL DNA non-linearized and linearized (but not CIP-treated), at 3, 6, 9, and 12 cm gap distances	n/a
Alveolates			
Euplotes crassus	Bio-Rad Biolistic PDS-1000/He Particle Delivery System	0.6 µm or 1.6 µm AuNPs, rupture disk 1550 psi, helium pressure 1750 psi, vacuum 26 inches Hg, gap distance ⅜ inches, in 10 mM HEPES pH 7.4	80-90%
Chromera velia	PDS-1000/He	0.6µm AuNPs, rupture disc 1350 psi, 6cm gap	n/a
Hematodinium sp.	Bio-Rad Biolistic PDS-1000/He Particle Delivery System	rupture disk 1550 psi 550 nm diameter gold particles	10%
*Fugacium kawagutii**	Bio-Rad Biolistic PDS-1000/He Particle Delivery System	0.7 or 1.1 µm Tungsten; rupture disc 450, 650, 900, 1100, 1350, 1550 psi; vacuum 28 inches Hg; 7.5cm gap	n/a
Alexandrium catenella*	Bio-Rad Biolistic PDS-1000/He Particle Delivery System	0.7 or 1.1 µm Tungsten; rupture disc 450, 650, 900, 1100, 1350, 1550 psi; vacuum 28 inches Hg; 7.5cm gap	n/a

Crypthecodinium cohnii	Bio-Rad Biolistic PDS-1000/He Particle Delivery System	rupture disk 1550 psi 550 nm diameter gold particles	70-80%
Amphidium carterae	Bio-Rad Biolistics PDS-1000/He	rupture disk 1550 psi 550 nm diameter gold particles	n.d.
Discobans (Euglenozoans and Heteroloboseans)			
Eutreptiella gymnastica	PDS-1000/He	0.6 or 1μm AuNPs, rupture disc 1350 psi, 6cm gap	n/a

*****This part of work was assisted by Kaidian Zhang from Xiamen University, China.

C. Used microinjection setting.

Species	Microinjection machine	Used setting	Survival rate
Alveolates			
Euplotes crassus	Eppendorf InjectMan NI 2	With Eppendorf Femtotips Microinjection Capillary Tip	2-10%

D. Used chemical transformation reagents.

Species	Transfection reagent	Used setting	Survival rate
Archaeplastids			
Pyramimonas parkeae	Lipofectamine® 3000 Transfection Reagent (Invitrogen)	DNA–Lipofectamine complex prepared according to the supplier.	n/a
Rhizarians (Chlorarachniophytes)			
Bigelowiella natans	Lipofectamine® 3000 Transfection Reagent (Invitrogen)	DNA–Lipofectamine complex prepared according to the supplier.	n/a
Alveolates			
Euplotes crassus	Lipofectamine® 2000 or Lipofectamine® 3000 Transfection Reagent (Invitrogen)	DNA–Lipofectamine complex prepared according to the supplier.	100%
	Effectene Transfection Reagent (QIAGEN)	DNA–Effectene complex prepared according to the supplier with a double amount of DNA	10-20%
	FuGENE HD Transfection Reagent (Promega)	DNA–FuGENE complex prepared according to the supplier.	50-60%
Discobans (Euglenozoans and Heteroloboseans)			
Eutreptiella gymnastica	Lipofectamine® 3000 Transfection Reagent (Invitrogen)	DNA–Lipofectamine complex prepared according to the supplier.	n/a

E. Used conjugation.

Species	Coincubation (species co-incubated)	Survival rate	Efficiency
Stramenopiles (Diatoms, Bacillariophytes, Raphidophytes)			
Thalassiosira pseudonana	*E. coli* EPI300	n/a	~10%
Heterosigma akashiwo	Agrobacterium	10-15%	n/a
Alveolates			
Oxyrrhis marina	*E. coli*	100%	1-5%
Karlodinium veneficum	*E. coli*	100%	n/a
Alexandrium catenella	*E. coli*	100%	n/a

F. Used glass beads abrasion.

Species	Coincubation (species co-incubated)	Survival rate	Efficiency
Stramenopiles (Diatoms, Bacillariophytes, Raphidophytes)			
Heterosigma akashiwo	n/a	80%	n/a
Alveolates			
Perkinsus marinus	n/a	80-90%	0.01%-1%
Hematodinium sp.	n/a	40-50%	0%
Amphidinium carterae	None	No data	0%
	Polyethylene glycol	No data	0%

Suppl. Table 5: List of used organisms, including name and link to www.protocols.io webpage

Species	PI	Investigators	protocols.io links	Sequences submitted (Accession No.)
Archaeplastids				
Ostreococcus lucimarinus	François-Yves Bouget	Jean-Claude Lozano Valérie Vergé	Link	*p*HAPT:luc vector (Djouani-Tahri *et al.*, 2011)was used as a template for preparation of linear construct
Bathycoccus prasinos	François-Yves Bouget	Jean-Claude Lozano Valérie Vergé	Link Link Link	*p*HAPT:luc vector (Djouani-Tahri *et al.*, 2011)was used as a template for preparation of linear construct
Micromonas commoda	Alexandra Z. Worden	Manuel Ares Jian Guo Lisa Sudek	Pending	
Micromonas pusilla	François-Yves Bouget Alexandra Z. Worden	Manuel Ares Jian Guo Jean-Claude Lozano Lisa Sudek Valérie Vergé	Link	
Tetraselmis striata	Heriberto Cerutti Thomas Clemente	Patrick Beardslee Fulei Luan Xiaoxue Wen	Link	GenBank (KY886895)
Pyramimonas parkeae	Vladimír Hampl	Natalia Ewa Janowicz Anna M.G. Novák Vanclová	Link	
Haptophytes				
Isochrysis galbana	Colin Brownlee	Cecilia Balestreri Andrea Highfield Rowena Stern Glen Wheeler	Link Link	MK903009 (pIgNAT construct) MK903010 (PCR product of transgene)
Emiliania huxleyi	Colin Brownlee	Cecilia Balestreri Andrea Highfield Rowena Stern Glen Wheeler	Link Link	
Rhizarians				
Amorphochlora (Lotharella) amoebiformis	Yoshihisa Hirakawa	Kodai Fukuda	Link	
Bigelowiella natans	Vladimír Hampl	Natalia Ewa Janowicz Anna M.G. Novák Vanclová	Link	
Stramenopiles				
Fragilariopsis cylindrus	Thomas Mock	Amanda Hopes	Link	

		Nigel Belshaw Reuben Gilbertson		
Thalassiosira pseudonana	Christopher L. Dupont Pamela Silver	Jernej Turnsek	Link Link	
Seminavis robusta	Aaron Turkewitz	Luke Noble Matthew Rockman Lev Tsypin	Link	
Pseudo-nitzschia multiseries	G. Jason Smith	Deborah Robertson April Woods	Link	
Pseudo-nitzschia australis	G. Jason Smith	Deborah Robertson April Woods	Link Link	
Heterosigma akashiwo	Kathryn Coyne	Pamela Green	Link Link	
Aurantiochytrium limacinum	Jackie Collier	Joshua Rest Mariana Rius	Link Link Link	
Caecitellus sp.	Patrick Keeling	Liz Cooney Nicholas Irwin	Link	
Nannochloropsis oceanica	Peter von Dassow	Fernan Federichi Isaac Nuñez Tamara Matute Albane Ruaud	Link Link	
Phaeodactylum tricornutum	Andrew E. Allen	Mark Moosburner	Link Link Link	
Alveolates				
Euplotes crassus	Cristina Miceli	Rachele Cesaroni Lawrence A. Klobutcher Mariusz Nowacki Angela Piersanti Sandra Pucciarelli Estienne Swart	Link	
Euplotes focardii	Cristina Miceli	Angela Piersanti Sandra Pucciarelli	Link	
Chromera velia	Vladimír Hampl	Natalia Ewa Janowicz Anna M.G. Novák Vanclová	Link	
Perkinsus marinus	José A. Fernández Robledo Senjie Lin Ross Waller	Duncan B. Coles Elin Einarsson Nastasia J. Freyria Sebastian Gornik Imen Lassadi Arnab Pain	Link Link Link Link	GenBank (EF632302, EF632303, KX423758–60)

Oxyrrhis marina	Patrick Keeling Claudio Slamovits Senjie Lin	Elizabeth Cooney Nicholas A. T. Irwin Elisabeth Hehenberger Yoshihisa Hirakawa Brittany Sprecher Lu Wang Huan Zhang	Link Link Link Link Link	
Hematodinium sp.	Ross Waller	Sebastian Gornik I Ian Hu Imen Lassadi Arnab Pain	Pending	
Fugacium (Symbiodinium) kawagutii	Senjie Lin	Brittany Sprecher Lu Wang Huan Zhang	Link	
Alexandrium catenella	Senjie Lin	Brittany Sprecher Lu Wang Huan Zhang	Link Link	
Karlodinium veneficum	Senjie Lin	Brittany Sprecher Huan Zhang	Link Link	
Breviolum (*Symbiodinium*) sp.	Jun Minagawa	Yuu Ishii Konomi Kamada Shinichiro Maruyama	Link	
Crypthecodinium cohnii	José Fernández Robledo Ross Waller	Duncan B. Coles Nastasia J. Freyria Paulo A. García Imen Lassadi	Link	
Amphidinium carterae	Christopher Howe	Adrain Barbrook Isabel Nimmo Ellen Nisbet	Link	
Discobans (Euglenozoans and Heteroloboseans)				
Bodo saltans	Virginia Edgcomb	Miguel A. Chiurillo Roberto Decampo Fatma Gomaa Noelia Lander Zhuhong Li	Link Link Link Link Link	
Diplonema papillatum	Julius Lukeš	Drahomíra Faktorová Ambar Kachale Binnypreet Kaur Getraud Burger Matus Valach	Link	GenBank (MN047315)
Eutreptiella gymnastica	Vladimír Hampl	Natalia Ewa Janowicz Anna M.G. Novák Vanclová	Link	

Naegleria gruberi	Anastasios Tsaousis	Veronica Freire-Beneitez Eleanna Kazana Jan Pyrih Tobias von der Haar	Link Link Link
Opisthokonts			
Pirum gemmata	Elena Casacuberta Iñaki Ruiz-Trillo	Cristina Aresté	Link
Sphaeroforma arctica	Elena Casacuberta Iñaki Ruiz-Trillo	Cristina Aresté Omaya Dudin	Link Link
Abeoforma whisleri	Elena Casacuberta Iñaki Ruiz-Trillo	Elena Casacuberta Cristina Aresté Sebastián Najle	Link Link
Salpingoeca rosetta	Nicole King	David Booth Monika Sigg	Link

Reference to Suppl. Table 5:

Djouani-Tahri el B, Sanchez F, Lozano JC, Bouget FY (2011). A phosphate-regulated promoter for fine-tuned and reversible overexpression in *Ostreococcus*: application to circadian clock functional analysis. PLoS One **6**: e28471.