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Molecular Map of the *Chlamydomonas reinhardtii* Nuclear Genome

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We have prepared a molecular map of the *Chlamydomonas reinhardtii* genome anchored to the genetic map. The map consists of 264 markers, including sequence-tagged sites (STS), scored by use of PCR and agarose gel electrophoresis, and restriction fragment length polymorphism markers, scored by use of Southern blot hybridization. All molecular markers tested map to one of the 17 known linkage groups of *C. reinhardtii*. The map covers approximately 1,000 centimorgans (cM). Any position on the *C. reinhardtii* genetic map is, on average, within 2 cM of a mapped molecular marker. This molecular map, in combination with the ongoing mapping of bacterial artificial chromosome (BAC) clones and the forthcoming sequence of the *C. reinhardtii* nuclear genome, should greatly facilitate isolation of genes of interest by using positional cloning methods. In addition, the presence of easily assayed STS markers on each arm of each linkage group should be very useful in mapping new mutations in preparation for positional cloning.

Studies using the unicellular eukaryotic alga *Chlamydomonas reinhardtii* have yielded important insights into many cellular processes including photosynthesis (45, 123), flagellar assembly and motility (24, 28, 86, 112, 114, 124, 137, 140), basal body assembly and positioning (115), gametogenesis and fertilization (35, 42, 167), DNA repair (109), phototaxis (49, 139), cell wall assembly (1), circadian rhythms (91, 162), and the regulation of metabolic pathways (3, 14, 31, 50).

A major strength of *C. reinhardtii* as an experimental system is its usefulness for genetic experiments (45, 47, 74). Vegetative cells are haploid, facilitating the analysis of mutant phenotypes, but stable diploid strains can be easily produced for dominance and complementation tests. Gametes can be crossed to yield diploid zygotes that sporulate to produce four products of meiosis, allowing routine tetrad analysis. Over the past 50 years, hundreds of mutations have been isolated; more than 200 genetic loci have been mapped to 17 linkage groups (28, 29, 45, 46, 52). Mutations induced by chemical or UV mutagenesis have been supplemented recently by mutations induced by transposition of one of several transposable element families in the genome (17, 34, 130, 133) or by insertional mutagenesis (13, 101, 149).

Insertional mutagenesis has become the favored method for generating mutations since the development of procedures for efficient transformation of the nuclear genome (59, 132). Upon transformation, plasmid DNA inserts in random positions into the nuclear genome, facilitating cloning of affected genes by using the transforming plasmid as a hybridization probe. This method of gene tagging has led to the isolation of numerous genes identified by mutation over the past several years. Despite its usefulness, the insertional-mutagenesis approach has drawbacks, including the inability to clone essential genes, difficulty in analyzing the large deletions that occur in some cases, and a limitation in the types of phenotypes that can be found by using a method that generates mostly null mutations.

To increase the power of molecular genetic approaches using *C. reinhardtii*, we have developed a molecular map aligned with the genetic map. In this paper, we present a detailed map of the *C. reinhardtii* nuclear genome based on the analysis of restriction fragment length polymorphism (RFLP) and sequence-tagged site (STS) markers. The availability of such a physical map will facilitate the cloning of genes identified by any type of mutation in *C. reinhardtii*.

(A preliminary version of the molecular map of *C. reinhardtii* was published previously [133].)

MATERIALS AND METHODS

***C. reinhardtii* strains, growth conditions, and genetic crosses.** The *C. reinhardtii* standard laboratory wild-type strain 21gr mt^+ (CC-1690) and the interfertile field isolate strain S1-C5 mt^- (CC-1952) were used as parental strains. The 21gr strain and the other commonly used laboratory strain, 137c, are very closely related (64); almost all PCR amplifications of genomic DNA using primers predicted from the sequence of one of the strains amplify DNA from both strains. The S1-C5 strain is identical to the S1-D2 mt^- strain (CC-2290) (44); the two strains were isolated from the same soil sample. The 21gr and S1-C5 strains were crossed as described previously (75). Tetrad progeny from the resulting zygotes were separated; a total of 136 random progeny from 136 complete tetrads were used in the mapping experiments. Cells were grown in TAP medium (43) or M medium (125) by using the modification described by Schnell and Lefebvre (130).

Molecular markers. Several types of molecular markers were mapped in this study. Markers designated GP were obtained by digesting *C. reinhardtii* genomic DNA (strain 137c) with the restriction enzyme *Pst*I, size fractionating the DNA on an agarose gel, and preparing minilibraries of cloned fragments (0.5 to 6.0 kb) in plasmid vector pUC119 (119). Random cDNA clones constituting the CNA, CNB, and CNC series of markers were obtained from a *C. reinhardtii* cDNA library (143). Additional markers consisted of genomic DNA clones or cDNA clones provided by other laboratories and genomic DNA, cDNA, or expressed sequence tag (EST) sequences obtained from the GenBank database.

Scoring markers by RFLP detection. Genomic DNA was isolated by the method of Schnell and Lefebvre (130). DNA (1 μg per lane) was digested with restriction enzymes (*Pst*I, *Pvu*II, *Eco*RI plus *Xho*I, or *Hin*dIII). DNA fragments were separated by electrophoresis on a 1% agarose gel (12.7 by 20 by 0.5 cm) at 35 V for 18 to 20 h in TBE buffer (0.45 M Tris, 0.44 M boric acid, 0.01 M EDTA [pH 8.0]). The DNA was denatured and transferred to a MagnaGraph nylon

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membrane (Micron Separations Inc., Westborough, Mass.) by using the protocol of Sambrook et al. (126). DNA was cross-linked to the membrane by using a model 1800 UV Stratalinker (Stratagene, La Jolla, Calif.) at 1,200 μJ for 30 s. The membrane was baked at 80°C for 2 h in a vacuum oven. Membranes containing digested DNA from each of the 136 random progeny mapping strains were incubated with hybridization solution (50% formamide, 5× SSPE [1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA {pH 7.7}], 10× Denhardt's solution, 4% sodium dodecyl sulfate [SDS], and 300 μg of single-stranded salmon sperm DNA/ml) for 1 h at 42°C. Denatured, labeled probe (see below) was added, and the hybridization reaction mixture was incubated overnight at 42°C. Filters were washed with 2× SSPE–1% SDS for 20 min, followed by three washes in 0.2× SSPE–0.2% SDS for 20 min each at 68°C. Digoxigenin-labeled probes were detected according to the protocol from Roche Molecular Biochemicals (Indianapolis, Ind.) except that Tween 20 was increased to 0.3% in buffer A and 5% powdered milk (Carnation) was used in buffer B instead of Blocking Powder.

Preparation of hybridization probes. Hybridization probes were labeled using the digoxigenin nonradioactive system from Roche Molecular Biochemicals. Plasmids or purified plasmid inserts were labeled by random priming according to the manufacturer's instructions with the following modifications. Probe DNA in 13 μl of H_2O (200 to 500 ng of plasmid DNA or 50 to 75 ng of purified insert DNA) was denatured by boiling, and 4 μl of 5× OLB (0.225 M Tris-HCl [pH 8.0], 0.025 M $MgCl_2$, 0.02 M dithiothreitol, 1.36 A_{260} units of hexanucleotides [Pharmacia Biotech, Piscataway, N.J.]), 2 μl of DIG DNA labeling mixture (Roche), and 1 μl (2 U) of Klenow enzyme (Roche) were added. The reaction mixture was incubated overnight at 37°C, and the reaction was stopped with 2 μl of 0.2 M EDTA. The probe was mixed with 20 μl of 5% Blue Dextran and column purified by using Bio-Gel P-60 agarose (Bio-Rad Laboratories, Hercules, Calif.). PCR labeling of cloned DNA fragments utilized sets of plasmid-specific primers and the PCR DIG Probe Synthesis kit (Roche). The PCR mixture (50 μl) contained 5 ng of plasmid DNA, 200 mM digoxigenin deoxynucleoside triphosphates, 1× PCR Mg^{2+} buffer, 25 pmol of primers, 9% dimethyl sulfoxide, and 2.5 U of *Taq* polymerase. PCR program steps were as follows: (i) 94°C for 5 min, (ii) 29 cycles of 94°C for 1 min, 56°C for 45 s, and 72°C for 3 min, and (iii) 94°C for 1 min, 56°C for 45 s, and 72°C for 10 min. For amplification of cDNA clones in the λEx*lox* vector, phage DNA was prepared by resuspending a plaque in 200 μl of distilled water, freezing in liquid nitrogen, and thawing at room temperature. The freeze-thaw cycle was repeated, and the sample was boiled for 5 min. The crude DNA (25 μl) was used as template in a 100-μl PCR mixture containing PCR buffer with Mg^{2+} (Roche), 2 μl of DIG DNA labeling mixture, 20 pmol of primers, 2.5% dimethyl sulfoxide, and 2.5 U of *Taq* polymerase (Roche). PCR program steps were as follows: (i) 94°C for 4 min, 55°C for 2 min, and 72°C for 3 min, (ii) 35 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min, and (iii) 94°C for 1 min, 55°C for 2 min, and 72°C for 10 min.

Scoring markers by use of PCR. The PRIMER program (79) was used to design primers for amplification of fragments from 3′ untranslated regions (3′ UTR) of gene, cDNA, or EST sequences. The primer criteria included an optimal length of 22 nucleotides (range, 20 to 24), an optimal melting temperature (T_m) of 73°C (range, 71 to 75°C), and a GC content of 60 to 65%. The default settings of the program were used for other criteria. PCR mixtures (25 μl) contained buffer A (Fisher Biotech), 10% glycerol, 5% formamide, 200 μM each deoxynucleoside triphosphate, 12.5 pmol of each primer, 25 ng of genomic DNA, and 0.55 U of *Taq* DNA polymerase (Fisher Biotech). PCR program steps were as follows: (i) 94°C for 1 min, (ii) 30 cycles of 94°C for 1 min, 55°C (annealing temperature) for 1 min, and 72°C for 1 min, and (iii) 72°C for 10 min, followed by a 10°C hold. Amplified DNA fragments obtained from template DNA from the 21gr and the S1-C5 strain were sequenced by the Advanced Genetics Analysis Center, University of Minnesota. Nucleotide sequence polymorphisms between 21gr DNA and S1-C5 DNA provided the basis for manual design of allele-specific primers by using principles described by Dieffenbach et al. (25) and Kwok et al. (68). For some markers, ESTs obtained from strain S1-D2 (CC-2290) were available in the database; these sequences provided a source of nucleotide polymorphisms used in allele-specific primer design. The annealing temperature for PCRs using the allele-specific primers was optimized by using a gradient thermal cycler (DNA Engine Dyad; MJ Research, Waltham, Mass.) to amplify DNA from the 21gr and S1-C5 parent strains. DNA from progeny strains was amplified in 96-well format by using the optimal annealing temperature. Reaction products (7 μl) were fractionated on 1.5% agarose gels by using the Sunrise 96 apparatus (Life Technologies, Rockville, Md.) and visualized by ethidium bromide staining.

Linkage analysis. For all loci scored in this study, the data consisted of a scorable hybridization fragment or PCR product derived from each of the two parental strains. No plus-minus data sets were used in the analysis. Data were entered and annotated by using the Map Manager QTX program (version b12) (82) and were then exported to the mapping Mapmaker/QTL program (version 3.0) (69, 78) for linkage analysis and map construction. The F_2 backcross function of the program was used for map construction in this haploid organism by classifying one genotype as "homozygous" and the other as "heterozygous." The "group" command, at a Lod score threshold of 4.0, was used to place the markers in linkage groups. All loci mapped to one of the 17 known linkage groups in the *C. reinhardtii* genome. Map order within linkage groups was determined by using the multipoint mapping functions of the "order" and "build" commands. Markers placed with a confidence of at least Lod 2.0 are presented on the map (Fig. 1). For markers that could not be placed on the map at a Lod score greater than or equal to 2.0, the "try" command was used to establish the most likely position of the marker on the map. The Kosambi function was used to assign map distances in centimorgans.

RESULTS

Using a combination of RFLP and PCR-based markers, we have placed 264 molecular markers on the 17 linkage groups of the *C. reinhardtii* genome (Fig. 1; Table 1). These markers were mapped on a panel of 136 random progeny from a cross of strain 21gr (mt^+) with the field isolate S1-C5 (mt^-) (44). All of the markers map to the 17 known linkage groups, indicating that if other linkage groups exist in the *C. reinhardtii* genome, they must be very small. The total length of the molecular map (in Kosambi units) is 1,025 centimorgans (cM). Any point on the *C. reinhardtii* genome is, on average, 2 cM from one of the 264 mapped molecular markers. Given that the size of the genome is approximately 10^8 bp (46), 1 cM in *C. reinhardtii* should correspond to about 100,000 bp. This number is consistent with the centimorgan-to-base pair ratio found during the positional cloning of the *LF1* gene (R. Nguyen and P. A. Lefebvre, unpublished data).

Frequency of polymorphism. Previous efforts to develop an extensive molecular map for *C. reinhardtii* were hampered by the low frequency of DNA polymorphism observed for molecular markers by using *C. reinhardtii* and the interfertile strain *Chlamydomonas smithii* (120, 135). In this study, the laboratory strain 21gr and an interfertile field isolate strain (S1-C5) showed a high degree of polymorphism for many molecular markers. When DNA from the two strains was digested with *Pst*I or *Pvu*II, 94% of hybridization probes tested (n = 204) showed an RFLP with one or both of the enzymes. With three additional restriction enzymes, *Eco*RI plus *Xho*I and *Hin*dIII, the RFLP rate increased to 98%. Thus, it was possible to map almost all markers by using a set of standard filters prepared with genomic DNA digested with only a few different restriction enzymes.

The underlying variation in DNA sequence responsible for the high level of RFLP was confirmed by direct sequencing of a large number of 3′ UTR sequences from S1-C5 DNA. We chose to examine 3′ UTR sequences because they exhibit greater sequence variation than do coding sequences. In addition, 3′ UTR sequences are likely to be unique, even among genes in a multigene family. To obtain 3′ UTR sequences from S1-C5 genes, primers were designed to amplify 3′ UTR fragments from *C. reinhardtii* genes available in the GenBank database. Of 100 reactions that produced products by using 21gr DNA as a template, 82 also produced products by using S1-C5 DNA. Among these products, 16% showed a length polymorphism with the product obtained from the 21gr template DNA. We sequenced the amplified 3′ UTR regions from 62 S1-C5

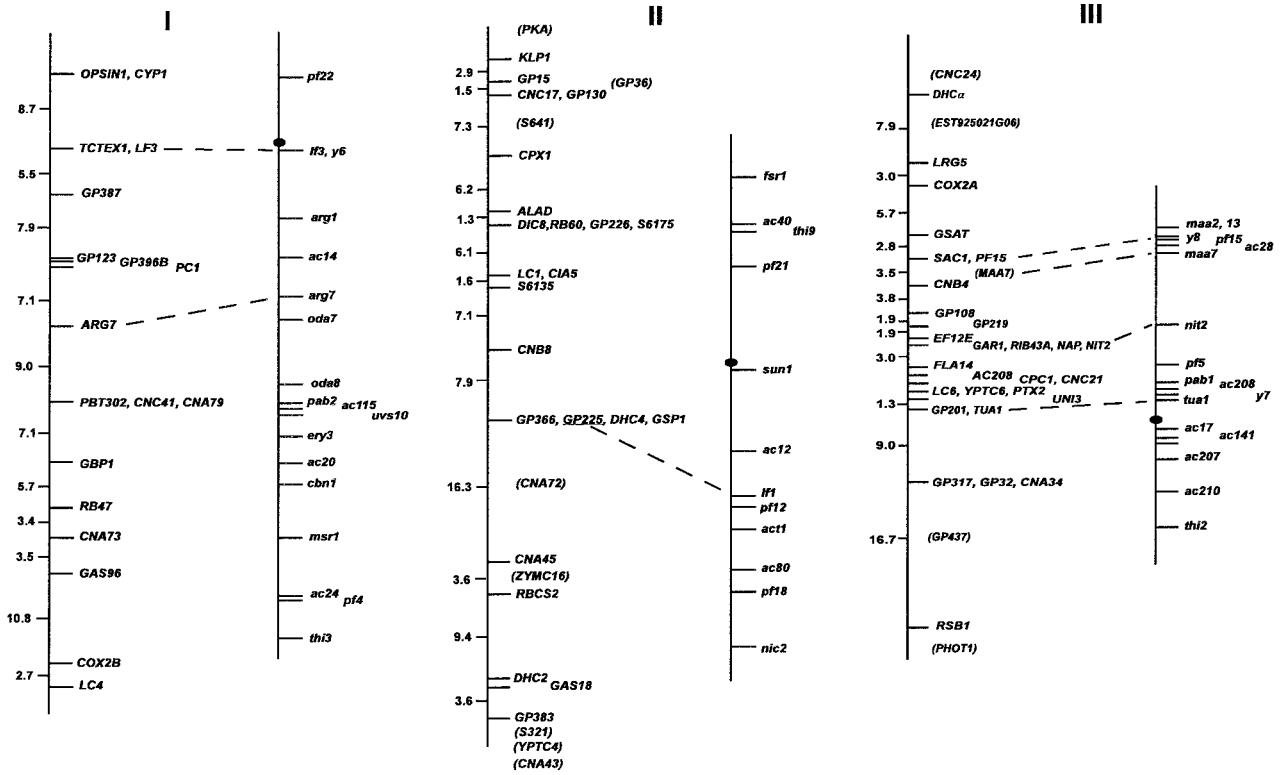

FIG. 1. *Chlamydomonas* molecular and genetic maps. For each of the 17 linkage groups, the genetic map (adapted from the work of Harris [46]) is shown on the right, with the centromere represented by the black oval, and the molecular map is shown on the left. For genetic maps, spaces between markers represent recombination units (percent recombination); the scale bar is to the right of the maps for linkage group XIX. For molecular maps, numbers to the left of the vertical line indicate centimorgans (Kosambi units). The order of molecular markers placed on the map is predicted to be accurate with a Lod score of at least 2.0 by use of MAPMAKER/QTL 3.0 (78). For markers that could not be ordered with a Lod score of at least 2.0, the "try" command was used to determine the most likely map position. Such markers are enclosed in parentheses. For markers separated by commas on the same line, the order was indistinguishable by recombination. Dashed lines connecting the genetic and molecular maps indicate a molecular marker corresponding directly to a previously mapped phenotypic marker. The orientation of the molecular map with respect to the genetic map has not been confirmed for linkage groups VIII, XV, and XVI/XVII. Further information on the markers is available at http://www.biology.duke.edu/chlamydb/.

genes, for a total of 29,053 bp. When these sequences were compared with the equivalent regions from strain 21gr or 137c (available in GenBank), single nucleotide substitutions were found at 793 positions (447 transitions and 346 transversions), for an average of 2.7 base substitutions per 100 bp of sequence. In addition, at 159 sites we found insertions or deletions of bases at a frequency of 0.54 per 100 bp.

The high level of sequence polymorphism in the S1-C5 gene sequences made it possible to design allele-specific primers based on single nucleotide polymorphisms (SNPs). We designed primers that yield PCR products of different lengths when template DNAs from the 21gr and S1-C5 parental strains are used (Table 2). These primer sets reproducibly generated reaction products when uniform reaction components and optimized annealing temperatures were used. The primer sets, corresponding to loci distributed over each of the linkage groups, were used to amplify PCR products ranging from 100 to 600 bp by using template DNA from the random progeny strains. The lengths of the resulting products were analyzed and scored by using agarose gel electrophoresis.

Anchoring the molecular map to the genetic map. For most linkage groups, the molecular map was anchored to the genetic map by using as mapping probes genes corresponding to mapped phenotypic markers. On linkage group I, for example, molecular probes for the *LF3* gene (149) and for the *ARG7* gene (19) were mapped, allowing the molecular map to be oriented relative to the genetically mapped *lf3* and *arg7* loci. Cloned genes corresponding to mapped mutations were not available for some linkage groups. For these six linkage groups, the molecular map was oriented relative to the preexisting genetic map by reference to earlier molecular mapping results that provided information about centromere linkage. The orientation of linkage group II, for example, is based on the observation that the centromere distances for markers *S6175* and *S6135* are 3 and 2 cM, respectively. For linkage group IV, the orientation of the map was supported by data from tetrads indicating that *TUA2* (α2 tubulin) and *PYR1* (the pyrithiamine resistance gene) are on opposite sides of the centromere and that *TUA2* maps within 7 cM of its centromere (120). For linkage group V, the orientation of the map was determined by examining the data from tetrad progeny showing that *DHC6* lies between the *PF26* marker and the centromere (113). The correspondence of the molecular and genetic maps for linkage group XVI/XVII is based on the demonstration that *DHC9* is

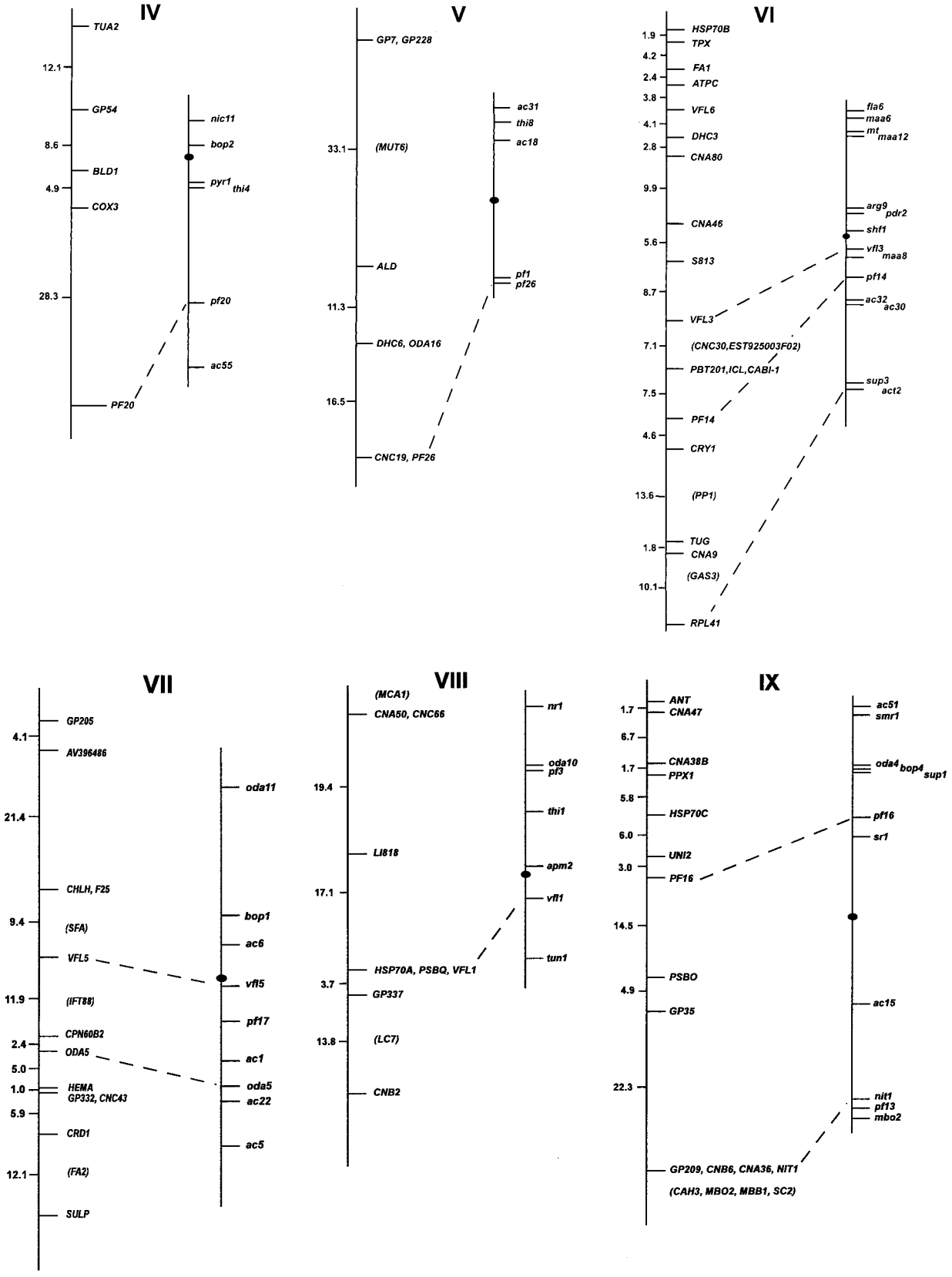

FIG. 1—*Continued*.

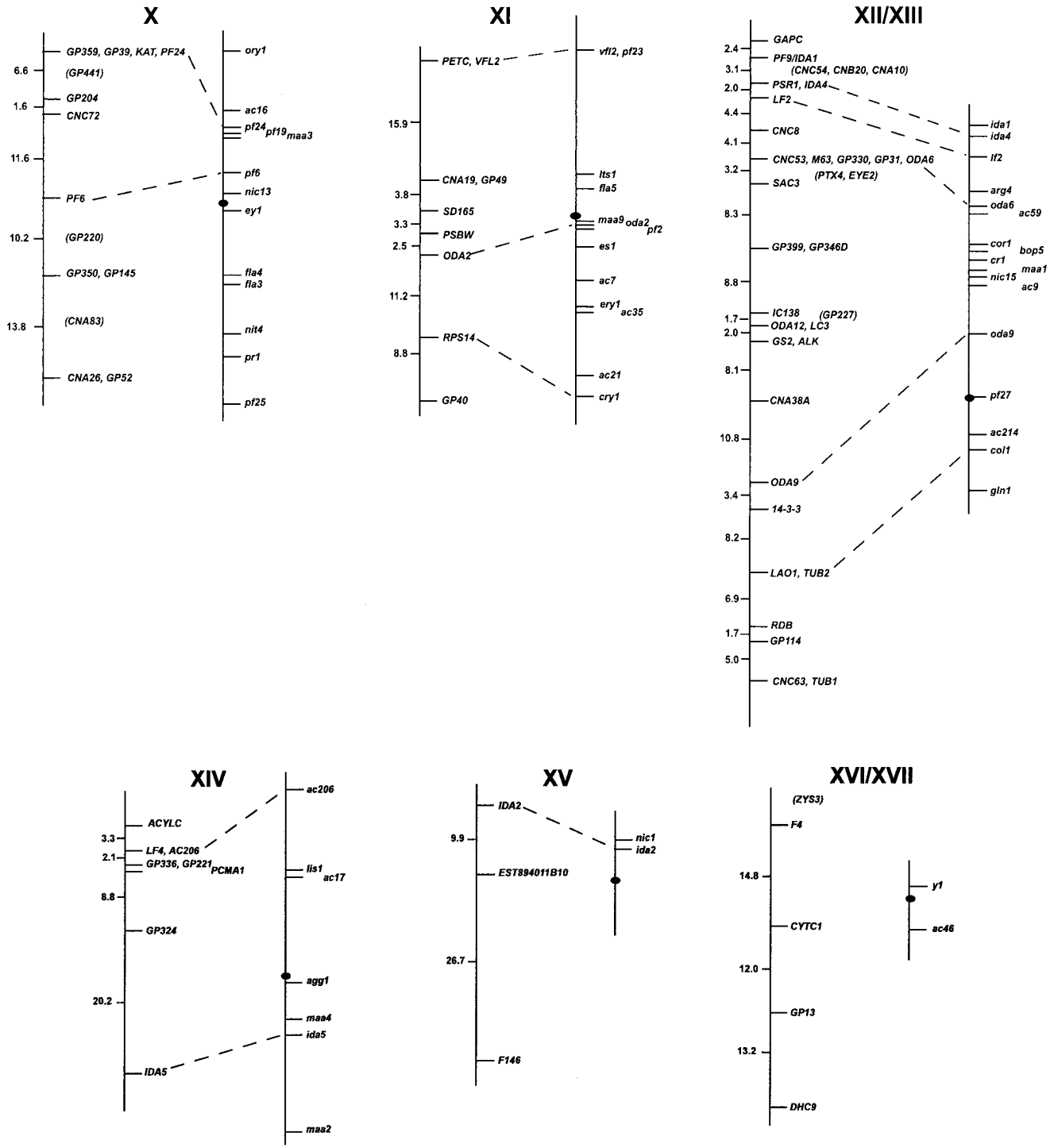

FIG. 1—*Continued*.

linked to the phenotypic marker *y1* (113). The orientation of the molecular map for linkage group XIX is supported by data showing that the *EF3A* marker maps within 3 cM of the centromere (120). For linkage groups VIII, XV, and XVI/XVII, the orientation of the anchored map relative to the genetic map is not known because there is only a single point of anchorage.

For most loci, cloned genes corresponding to previously mapped phenotypic loci were placed on the expected locations on the molecular map. The single exception was the *ac21* locus on linkage group XI. The *PETC* gene (encoding the chloroplast Rieske iron-sulfur center protein) has been shown to be the gene affected by the *ac21* mutation (5, 22, 23). When we mapped *PETC*, however, it was indistinguishable from *VFL2* by recombination. The map location of *ac21*

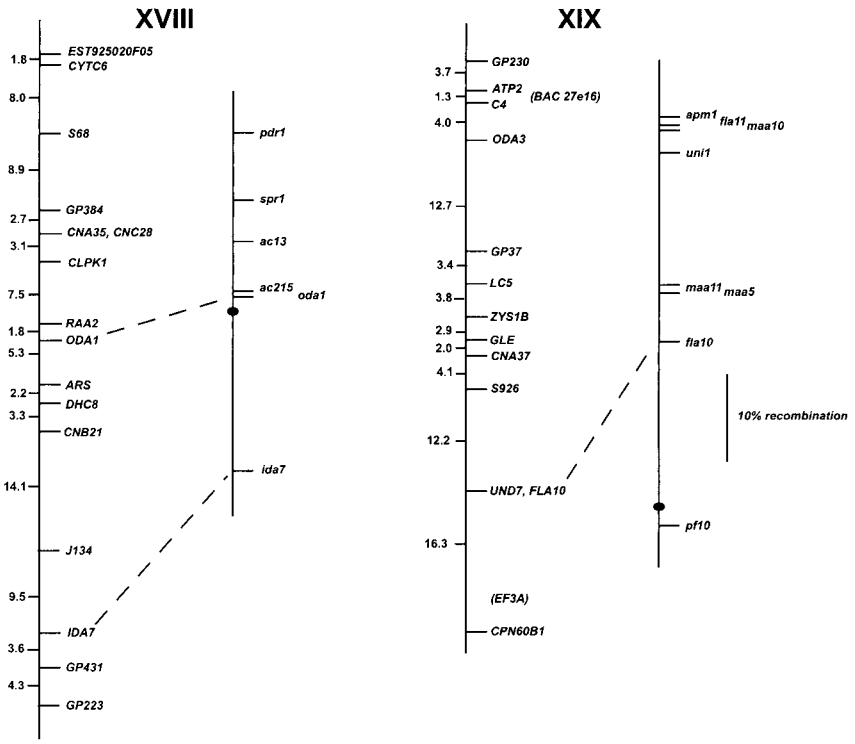

FIG. 1—*Continued*.

was previously determined to be on the opposite arm of linkage group XI.

DISCUSSION

The availability of the *C. reinhardtii* molecular map should enable researchers to take advantage of rapid advances in *C. reinhardtii* genomics to identify genes corresponding to mapped mutations. Sequences from more than 100,000 cDNA clones are publicly available (*Chlamydomonas* Genetics Center, Duke University [http://www.biology.duke.edu/chlamy_genome/]; Kazusa DNA Research Institute, Kazusa, Japan [http://www.kazusa.or.jp/en/plant/chlamy/EST/]). The ends of 15,000 bacterial artificial chromosome (BAC) clones have been sequenced by the Joint Genome Institute (JGI; Walnut Creek, Calif.) and are available for searching by use of BLAST algorithms (http://bahama.jgi-psf.org/prod/bin/chlamy/home.chlamy.cgi). The complete sequence of the nuclear genome is being completed by the JGI and should be available early in 2003.

All of these resources taken together should allow positional cloning and candidate gene approaches to be used to clone the genes identified by mapped mutations. BAC clone contigs anchored on each of the molecular markers have already been prepared. The total length of genomic DNA contained in these contigs represents more than 20% of the nuclear genome. By comparing the sequence of the nuclear genome to the sequences of the BAC clone ends, it will be possible to rapidly complete the coverage of the nuclear genome with mapped BAC clones.

For positional cloning, *C. reinhardtii* offers many technical advantages for efficient testing of whether a particular BAC clone corresponds to a mutation of interest. Transformation of *C. reinhardtii* involves simple and fast procedures such as vortexing with glass beads (59) or electroporation (132). The efficiency of cotransformation with BAC clones and a selectable marker gene on a separate plasmid is usually in the range of 1 to 2%, and it is easy to generate hundreds of transformants for screening. A BAC clone can be tested for rescue of a mutant phenotype in less than 2 weeks. Because screening of BAC clones for phenotypic rescue is so rapid and efficient, it should not be necessary to do extensive genetic mapping of new mutations in preparation for cloning. Numerous BAC clones, covering a large genetic interval, can be readily tested for phenotypic rescue of a mutation of interest upon transformation. As the sequence of the nuclear genome is completed and annotated, it should be possible to accelerate positional cloning and transformation using a candidate gene approach.

To use positional cloning or candidate gene approaches to clone genes corresponding to mutations, it is necessary to place these mutations on the genetic map. Hundreds of mutations have been mapped using phenotypic markers and multiply marked strains, but genetic mapping using phenotypic markers is tedious and is limited by the availability of useful mutations. A serious limitation is that many potentially useful genetic markers produce a similar phenotype, such as acetate auxotrophy or paralyzed flagella, so that only one mutant of each type can be used in an individual mapping cross. The molecular markers described in this report should greatly facilitate genetic mapping of new mutations in preparation for positional cloning.

To map the mutation in a new mutant strain, it should first

TABLE 1. Description of markers

Linkage group	Locus	Gene or marker description	Source or reference(s)	Accession no.
I	*CYP1*	Cyclophilin 1	145	AF052206
I	*OPSIN1*	Chlamyopsin 1	21	Z48968
I	*TCTEX1*	14-kDa dynein light chain	48	AF039437
I	*LF3*	LF3/ULF1 gene	L.-W. Tam and P. Lefebvre, unpublished data	
I	*GP387*	Random genomic fragment	This study	AY219891
I	*CNA13*	cDNA fragment	This study	
I	*GP123*	Random genomic fragment	This study	AF525919
I	*GP396B*	Random genomic fragment	This study	
I	*PC1*	NADPH: protochlorophyllide oxidoreductase	76	U36752
I	*ARG7*	Argininosuccinate lyase	19	X16619
I	*PBT302*	Genomic fragment	B. Tailon and J. Jarvik, unpublished data	
I	*CNC41*	cDNA corresponding to EST 1031062D08	This study	BI722495
I	*CNA79*	cDNA corresponding to EST 1031013A09	This study	BI994521
I	*GBP1*	G-strand binding protein	110	U10442
I	*RB47*	Poly(A) binding protein	173	AF043297
I	*CNA73*	cDNA corresponding to EST 894090B12	This study	BE726259
I	*GAS96*	Gene expressed during cell differentiation	C. F. Beck, unpublished data	
I	*COX2B*	Cytochrome *c* oxidase subunit II	104	AF305540
I	*LC4*	LC4, 17-kDa dynein light chain	60	U34345
II	*PKA*	Protein kinase A	N. Wilson and P. Lefebvre, unpublished data	AV390434
II	*KLP1*	Kinesin-like protein	6	X78589
II	*GP15*	Random genomic fragment	This study	
II	*GP36*	Random genomic fragment	This study	
II	*GP130*	Random genomic fragment	This study	
II	*CNC17*	cDNA corresponding to EST 1031071E11	This study	B1724441
II	*S641*	pcf 6-41 cDNA, upregulated after deflagellation	129	
II	*CPX1*	Coproporphyrinogen III oxidase precursor	117	AF133671
II	*ALAD*	Porphobilinogen synthase	84	U19876
II	*DIC8*	Genomic fragment	B. Williams and J. Rosenbaum, unpublished data	
II	*RB60*	Protein disulfide isomerase	153	AF036939
II	*GP226*	Random genomic fragment	This study	
II	*S6175*	pcf 6-175 cDNA, upregulated after deflagellation	129	
II	*LC1*	LC1, 22-kDa dynein light chain	4	AF112476
II	*CIA5*	Regulator of carbon concentrating mechanism	170	AF317732
II	*S6135*	pcf 6-135 cDNA, upregulated after deflagellation	129	
II	*CNB8*	cDNA corresponding to EST 1024021G10	This study	BG848462
II	*GP366*	Random genomic fragment	This study	AY220530
II	*GP225*	Random genomic fragment	This study	AY220531
II	*DHC4*	Dynein heavy chain 4	113	U81367
II	*GSP1*	Gamete specific protein 1	66	AF108140
II	*CNA72*	cDNA fragment	This study	
II	*CNA45*	cDNA corresponding to EST BI724982	This study	BI724982
II	*ZYMC16*	Gene expressed in zygotes	161	
II	*RBCS2*	Ribulose biphosphate carboxylase small subunit 2	41	X04472
II	*DHC2*	Dynein heavy chain 2	113	U61365
II	*GAS18*	Gene expressed during sexual differentiation	157	
II	*GP383*	Random genomic fragment	This study	
II	*S321*	pcf 3-21 cDNA, upregulated after deflagellation	129	
II	*YPTC4*	Small G protein	26	U13167
II	*CNA43*	cDNA fragment	This study	
III	*CNC24*	cDNA fragment	This study	AF525918
III	*DHC* α	Dynein heavy chain alpha	87, 88	L26049
III	EST 925021G06	EST	132a	BE442506
III	*LRG5*	Gene involved in blue light signaling	40	U73818
III	*COX2A*	Cytochrome *c* oxidase subunit II	104	AF305080
III	*GSAT*	Glutamate-1-semialdehyde aminotransferase	83	UC3632
III	*SAC1*	Sulfur limitation gene	15	U47541
III	*PF15*	Component of the central pair microtubule apparatus	E. F. Smith and P. Lefebvre, unpublished data	
III	*MAA7*	Tryptophan synthase beta-subunit	97	AF047024
III	*CNB4*	cDNA corresponding to EST 833013E07	This study	AW721386
III	*GP108*	Random genomic fragment	This study	
III	*GP219*	Random genomic fragment	This study	
III	*EF12E*	Genomic fragment	E. Fernandez, unpublished data	
III	*GAR1*	Gamete activation-regulated cobalamin-independent methionine synthase	67	U36197
III	*NAP*	Novel actin-like protein	57, 72	U68060
III	*NIT2*	NIT2 gene	130	
III	*RIB43A*	Microtubule ribbon protein	96	AF196576
III	*FLA14*	LC8, 10-kDa dynein light chain	61, 102	U19490
III	*AC208*	Apoplastocyanin	116	L07282
III	*CPC1*	Central pair-associated complex 1 protein	90	
III	*CNC21*	Random cDNA fragment	This study	
III	*PTX2*	Phototaxis-deficient gene	101	
III	*YPTC6*	YPTC6, small G protein	26	U13169
III	*LC6*	LC6, 13-kDa dynein light chain	61	U19484

Continued on following page

TABLE 1—*Continued*

Linkage group	Locus	Gene or marker description	Source or reference(s)	Accession no.
III	*UNI3*	δ-Tubulin	30	AF013108
III	*TUA1*	α-Tubulin	134	M11447
III	*GP201*	Random genomic fragment	This study	
III	*GP317*	Random genomic fragment	This study	
III	*GP32*	Random genomic fragment	This study	
III	*CNA34*	cDNA corresponding to EST 1031065B08	This study	BI722955
III	*GP437*	Random genomic fragment	This study	
III	*RSB1*	Radial spokehead polypeptide 1; corresponds to EST 1031064E02	165; this study	B1722838
III	*PHOT1*	Phototropin-like protein	52a	AJ416557
IV	*TUA2*	α2-Tubulin	134	M11448
IV	*GP54*	Random genomic fragment	This study	
IV	*BLD1*	Intraflagellar transport protein 52	9, 18	AF397450
IV	*COX3*	Cytochrome *c* oxidase subunit III	105	AF233515
IV	*PF20*	Protein required for flagellar central pair microtubule assembly	144	U78547
V	*GP7*	Random genomic fragment	This study	AF467707
V	*GP228*	Genomic fragment; corresponds to EST 1024061H08	This study	BG859190
V	*MUT6*	DEAH Box RNA helicase involved in gene silencing	168	AF305070
V	*ALD*	Plastid fructose-1,6-bisphosphate aldolase	103	X85495
V	*DHC6*	Dynein heavy chain 6	113	U61369
V	*ODA16*	Genomic fragment	D. Mitchell, unpublished data	
V	*CNC19*	cDNA corresponding to EST AV621283	This study	AV621283
V	*PF26 (S6187)*	pcf 6–187 cDNA, upregulated after deflagellation; radial spoke protein 6	12, 120, 129	M87526
VI	*HSP70B*	Chloroplast-localized heat shock protein	27	X96502
VI	*TPX*	Thioredoxin peroxidase	Y. Lee, S. H. Miller, and L. Keller, unpublished data	AF312025
VI	*FA1*	Flagellar autotomy protein	36, 37	AF246990
VI	*ATPC*	Chloroplast ATP synthase gamma subunit	142	M73493
VI	*VFL6*	VFL6 gene	K. Iyadurai and C. Silflow unpublished data	
VI	*DHC3*	Dynein heavy chain 3	113	U61366
VI	*CNA80*	cDNA corresponding to EST 1031002F08	This study	B1816487
VI	*CNA46*	cDNA fragment	This study	
VI	*S813*	G protein beta subunit-like protein	127	X53574
VI	*VFL3*	VFL3 gene	Iyadurai and Silflow, unpublished	
VI	*CNC30*	cDNA corresponding to cab II-1	54; this study	M24072
VI	EST 925003F02	EST	132a	BE441254
VI	*ICL*	Isocitrate lyase	111	U18765
VI	*CABI-1*	Light harvesting complex protein I-20	53	X65119
VI	*PBT201*	Genomic fragment	B. Tailon and J. Jarvik, unpublished data	
VI	*PF14*	Radial spoke polypeptide 3	166	X14549
VI	*CRY1*	DNA photolyase/blue light photoreceptor	141	L07561
VI	*PP1*	Axonemal type-1 phosphatase	172	AF156101
VI	*TUG*	γ-Tubulin	138	U31545
VI	*CNA9*	cDNA corresponding to EST 1024003B10	This study	BG843541
VI	*GAS3*	Gene expressed during sexual differentiation	157	
VI	*RPL41*	Ribosomal protein L41 (ACT2 locus)	146	AF130727
VII	*GP205*	Random genomic fragment	This study	AF467706
VII	*AV396486*	EST	2	AV396486
VII	*CHI H*	Magnesium chelatase H subunit	10	AJ307055
VII	*F25*	Class IV zygote-specific cDNA	33	
VII	*SFA*	SF-assemblin	70	U56982
VII	*VFL5*	VFL5 gene	Iyadurai and Silflow, unpublished data	
VII	*IFT88*	Intraflagellar transport protein 88	100	AF298884
VII	*CPN60B2*	Chloroplast chaperonin beta-like subunit	152	L27473
VII	*ODA5*	Outer dynein arm protein	M. Blomberg-Wirschell and G. Witman unpublished data	
VII	*HEMA*	Glutamyl-tRNA reductase	R. D. Willows et al.; unpublished data	AF305613
VII	*GP332*	Random genomic fragment	This study	
VII	*CNC43*	cDNA corresponding to EST 1024039D03	This study	BG854228
VII	*CRD1*	Copper response target 1 protein	92	AF226628
VII	*FA2*	Flagellar autotomy protein	81	AF479588
VII	*SULP*	Chloroplast sulfate transport system permease	H.-C. Chen, K. Yokthongwattana, and A. Melis, unpublished data	AF481828
VIII	*CNB2*	cDNA corresponding to EST AV626610	This study	AV626610
VIII	*LC7*	LC7, 11-kDa dynein light chain	8	AF140239
VIII	*GP337*	Random genomic fragment	This study	
VIII	*HSP70A*	70-kDa heat shock protein	93	M76725
VIII	*PSBQ*	OEE3 protein of photosystem II	85	X13832
VIII	*VFL1*	VFL1 gene	136	AF154916
VIII	*LI818*	Polypeptide related to CAB proteins	121	X95326
VIII	*CNA50*	cDNA corresponding to EST 833007A09	This study	AW676510

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TABLE 1—*Continued*

Linkage group	Locus	Gene or marker description	Source or reference(s)	Accession no.
VIII	*CNC66*	cDNA fragment	This study	
VIII	*MCA1*	RNA stability factor	A. Watson et al., unpublished data	AF330231
IX	*ANT*	Mitochondrial ADP/ATP translocator	131	X65194
IX	*CNA47*	cDNA corresponding to EST 1031051H10	This study	BI997794
IX	*CNA38B*	cDNA fragment	This study	
IX	*PPX1*	Protoporphyrinogen oxidase precursor	118	AF068635
IX	*HSP70C*	68-kDa heat shock protein	158	
IX	*UNI2*	UNI2 gene	W.-C. Wu and C. Silflow, unpublished data	
IX	*PF16*	Protein in C1 microtubule of flagellar central apparatus	143	U40057
IX	*PSBO*	OEE1 protein of photosystem II	85	X13826
IX	*GP35*	Random genomic fragment	This study	AF467704
IX	*GP209*	Random genomic fragment	This study	
IX	*CNB6*	cDNA corresponding to EST 1024027D07	This study	BG849900
IX	*CNA36*	cDNA fragment	This study	
IX	*NIT1*	Nitrate reductase	32	AH001336
IX	*CAH3*	Carbonic anhydrase, alpha type	39	U73856
IX	*MBO2*	MBO2 gene	150	AF394181
IX	*MBB1*	Required for expression of *psbB*/*psbT*/*psbH*	155	AJ296291
IX	*SC2*	Genomic fragment	A. Nguyen and W. Dentler, unpublished data	
X	*GP359*	Random genomic fragment	This study	AY219892
X	*GP39*	Random genomic fragment	This study	
X	*KAT*	p60 katanin subunit	80	AF205377
X	*PF24*	PF24 gene	P. Yang and W. Sale, unpublished data	
X	*GP441*	Random genomic fragment	This study	
X	*GP204*	Random genomic fragment	This study	
X	*CNC72*	cDNA fragment	This study	
X	*PF6*	PF6 gene	124a	AF327876
X	*GP220*	Random genomic fragment	This study	AF525922
X	*GP350*	Random genomic fragment	This study	AF525921
X	*GP145*	Random genomic fragment	This study	AF525920
X	*CNA83*	cDNA corresponding to EST 1031072D11	This study	BI724522
X	*CNA26*	cDNA corresponding to EST AV634482	2; this study	AV634482
X	*GP52*	Random genomic fragment	This study	
XI	*PETC*	Chloroplast Rieske Fe-S precursor protein	22, 23	X76299
XI	*VFL2*	Centrin/caltractin	73	X57973
XI	*CNA19*	cDNA fragment	This study	AF503637
XI	*GP49*	Random genomic fragment	This study	
XI	*SD165*	Genomic fragment	S. Dutcher, unpublished data	
XI	*PSBW*	Core subunit of photosystem II	7	AF170026
XI	*ODA2*	Dynein heavy chain gamma	163	U15303
XI	*RPS14*	Ribosomal protein S14 (CRY1 locus)	95	U06937
XI	*GP40*	Random genomic fragment	This study	AF525923
XII/XIII	*GAPC*	Glyceraldehyde-3-phosphate dehydrogenase, (NAD) cytosolic, subunit C	58	L27669
XII/XIII	*PF9/IDA1*	Dynein heavy chain 1	94	U61364
XII/XIII	*CNC54*	cDNA fragment	This study	AF174532
XII/XIII	*CNB20*	cDNA fragment	This study	AF486824
XII/XIII	*CNA10*	cDNA fragment	This study	
XII/XIII	*PSR1*	Phosphorus metabolism regulatory protein	169	AF174532
XII/XIII	*IDA4*	p28, dynein inner arm light chain	71	Z48059
XII/XIII	*LF2*	LF2/ULF2 gene	C. Amundsen and P. Lefebvre, unpublished data	
XII/XIII	*CNC8*	cDNA corresponding to EST 963109F12	This study	B1873562
XII/XIII	*CNC53*	cDNA corresponding to EST 894081D12	This study	BE725171
XII/XIII	*M63*	Gene involved in cytokinesis	J. Larsen, L.-W. Tam and C. Silflow, unpublished data	
XII/XIII	*GP330*	Random genomic fragment	This study	
XII/XIII	*GP31*	Random genomic fragment	This study	
XII/XIII	*ODA6*	IC70 dynein intermediate chain	89	X55382
XII/XIII	*PTX4*	Gene involved in phototaxis	G. Pazour, unpublished data	
XII/XIII	*EYE2*	Gene required for eyespot assembly	122	AF233430
XII/XIII	*SAC3*	Kinase regulating response to sulfur limitation	16	AF100162
XII/XIII	*GP399*	Random genomic fragment	This study	
XII/XIII	*GP346D*	Random genomic fragment	This study	
XII/XIII	*IC138*	Inner arm dynein I1 subunit	P. Yang and W. Sale, unpublished data	
XII/XIII	*GP227*	Random genomic fragment	This study	
XII/XIII	*ODA12*	LC2, 19-kDa outer arm dynein light chain	102	U89649
XII/XIII	*LC3*	LC3, 17-kDa dynein light chain	99	U43610
XII/XIII	*GS2*	Glutamine synthetase 2 (GS2)	11	U46208
XII/XIII	*ALK*	*Chlamydomonas* aurora-like kinase	98	AF199021
XII/XIII	*CNA38A*	cDNA fragment	This study	
XII/XIII	*ODA9*	IC78 dynein intermediate chain	164	U19120
XII/XIII	*14-3-3*	14-3-3 protein	77	X79445
XII/XIII	*LAO1*	L-Amino acid oxidase catalytic subunit	156	U78797
XII/XIII	*TUB2*	β2-Tubulin	174	K03281

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TABLE 1—*Continued*

Linkage group	Locus	Gene or marker description	Source or reference(s)	Accession no.
XII/XIII	*RDB*	Roadblock protein, EST 1024060G02	S. King, unpublished data	BG858985
XII/XIII	*GP114*	Random genomic fragment	This study	
XII/XIII	*CNC63*	cDNA fragment	This study	
XII/XIII	*TUB1*	β1-Tubulin	174	M10064
XIV	*ACYLC*	EST 832001D08	132a	AW676021
XIV	*LF4*	Genomic fragment	S. Berman and P. Lefebvre, unpublished data	
XIV	*AC206*	Ccs1, required for chloroplast C-type holocytochrome formation	55	U70999
XIV	*GP336*	Random genomic fragment	This study	AF467702
XIV	*GP221*	Genomic fragment; corresponds to EST 1031037H01	132a	BI996414
XIV	*PCMA1*	Genomic fragment	C. Asleson and P. Lefebvre, unpublished data	
XIV	*GP324*	Random genomic fragment	This study	AF467703
XIV	*IDA5*	Actin	147	D50838
XV	*IDA2*	Dynein heavy chain 1-β of I1 complex	108	AJ242525
XV	EST 894011B10	EST	132a	BE056715
XV	*F146*	Class VI zygote-specific cDNA	33	
XVI/XVII	*ZYS3*	Zygote-specific cDNA	65	AB004043
XVI/XVII	*F4*	Class I zygote-specific cDNA	33	
XVI/XVII	*CYTC1*	Mitochondrial cytochrome c_1	A. Atteia et al., unpublished data	AF245393
XVI/XVII	*GP13*	Random genomic fragment	This study	AF467701
XVI/XVII	*DHC9*	Dynein heavy chain 9	113	U61372
XVIII	EST 925020F05	EST	132a	BE442454
XVIII	*CYTC6*	Cytochrome c_6	51	M67448
XVIII	*S68*	pcf6–8 cDNA, upregulated after deflagellation	129	
XVIII	*GP384*	Random genomic fragment	This study	
XVIII	*CNA35*	cDNA corresponding to EST AV622556	This study	AV622556
XVIII	*CNC28*	cDNA fragment	This study	
XVIII	*CLPK1*	Cyclic nucleotide-dependent protein kinase	U. Kawabata et al., unpublished data	AB042714
XVIII	*RAA2*	Trans-splicing factor	106	AJ243394
XVIII	*ODA1*	cDNA corresponding to EST BG854636	148	AY039618
XVIII	*ARS*	Arylsulfatase	20	X52304
XVIII	*DHC8*	Dynein heavy chain 8	113	U61371
XVIII	*CNB21*	cDNA corresponding to EST 833007B10	This study	AW676519
XVIII	*J134*	Genomic fragment	J. Jarvik, unpublished data	
XVIII	*IDA7*	140-kDa inner arm dynein	107, 171	AF159260
XVIII	*GP431*	Random genomic fragment	This study	
XVIII	*GP223*	Random genomic fragment	This study	AF467708
XIX	*GP230*	Random genomic fragment	This study	
XIX	*ATP2*	ATP synthase mitochondrial F1 beta subunit	38	X61624
XIX	BAC 27e16	BAC end sequence	This study	
XIX	*C4*	Genomic fragment	W. Dentler, unpublished data	
XIX	*ODA3*	Genomic fragment	63	AF001309
XIX	*GP37*	Random genomic fragment	This study	
XIX	*LC5*	LC5, 14-kDa dynein light chain	99	U43609
XIX	*ZYS 1B*	Protein expressed during zygote formation	154	X76117
XIX	*GLE*	Gamete lytic enzyme	62	D90503
XIX	*CNA37*	cDNA corresponding to Lhcb4	151; this study	AB051211
XIX	*S926*	cDNA, upregulated after deflagellation	128	X62135
XIX	*UND7*	Genomic fragment	P. J. Ferris and U. W. Goodenough, unpublished data	
XIX	*FLA10*	Kinesin-homologous protein	160	L33697
XIX	*EF3A*	Genomic fragment	E. Fernandez, unpublished data	
XIX	*CPN60B1*	Chloroplast chaperonin beta-like subunit	152	L27471

be crossed to the S1-C5 strain, and 20 to 50 random progeny from different tetrads should be scored for the phenotype of interest. Small quantities of DNA from each progeny strain can then be used as template DNA for PCR-based mapping strategies. The primers for SNP scoring reported in this study (Table 2), because they produce PCR products of distinguishable sizes from the two parent strains, facilitate scoring of molecular markers in the progeny strains by using readily available thermal cycler and gel electrophoresis equipment. The marker set covers most of the genome, with multiple loci representing both arms of most linkage groups. Previously, primers for the scoring of SNPs using high-throughput methods were defined for 186 loci, including many of those mapped in this study (159).

A hierarchical approach to marker selection for mapping can be used to limit the number of PCRs to be performed. For each linkage group, the first marker to be tested for linkage analysis should be one that maps near the center of the group of markers. More than 50% of the molecular markers we developed map to only five linkage groups (I, II, III, VI, and XII/XIII). That the density of molecular markers corresponds roughly to the underlying gene density is suggested by the fact that slightly more than 50% of previously mapped mutations map to these five linkage groups as well. The first markers to be tested, therefore, should be from these linkage groups. If no linkage is detected between the mutant phenotype and the central marker on each linkage group, additional tests with markers spaced 20 to 30 cM from the first marker may be carried out until linkage is detected.

TABLE 2. Mapping primers[a]

Linkage group	Gene (3′ UTR)	Accession no.[b]	Primers[c]	*C. reinhardtii* allele (bp)	S1-C5 allele (bp)	Annealing temp (°C)
I	*CYP1*	AF052206	cyp1-R, GCATGCATTCCACAACGCACGC cyp1-F2, GGCGCGTACGCTCCGCGC[d] cyp1-F3, GGCTAATGGCTGTGCGGCTGG[e]	232	378	56.0
I	*ARG7*	X16619	ARG7-R, CGTCCCACACCTCCAAACGCCA ARG7-F2, GCCTTGACGTGAGGCTGCGCTG[e] ARG7-F3, TGGGGTACAAGGCGCTGTGAGAGA[d]	367	236	56.0
I	*CNA73*	BE726259	CNA73-R, CTTCTGCAGCCGTAGAAACCCGGC CNA73-F2, GCATAGGGGCTGTGCGGCG[e] CNA73-F3, TCTGTATGTGCCCCATGCGCACA[d]	436	379	63.9
I	*COX2B*	AF305540	cox2B-R, ATGGCTACGCCACCGCCGGTTT cox2BDNA-F3, TTGCTGGGCTGTGGCCGCG[d] cox2BDNA-F4, CAGCGGTCCCTCAGGGACGTTACA[e]	386	547	63.9
I	*LC4*	U34345	LC4-F, GCCGCGCGAGCTGGAAGAGTTT LC4-R, GTGCCGCCCACGAAACGTTCTG	565	~590	62.7
II	*CPX1*	AF133671	Cpx1-F, TTGCGTGCTAGCAGGCGTGGTG Cpx1-R2, GCTCCAAACCTGCTGCGGTCAGTC[e] Cpx1-R3, GCACGCACACACGCACACGA[d]	329	393	61.0
II	*RB60*	AF036939	RB60-R, GCGTTCGGAACCACGCACATCC RB60-F2, ACCAGCAGCAGCGCGTGATCCGG[d] RB60-F3, GCCAAAGAGGGACGCTGTCCACAG[e]	229	433	55.0
II	*CIA5*	AF317732	CIA5-F, TGGCTGCGTGCCACGACCGT CIA5-R2, GCTGAAGGTGAGTGCGGCAGCG[d] CIA5-R3, CCAGCTGCTGTTGGCGCCAGC[e]	354	314	55.0
II	*CNA45*	B1724982	CNA45-R, CGTGGTTCTTACATCACCCCAGCG CNA45-F2, TGTGGTGGGTGTTGATGGAGGAATG[d] CNA45-F3, TTGCGCGCATTGACAGATGTACAG[e]	244	328	58.9
II	*YPTC4*	U13167	YptC4-R, CGCCGTGATCAGCAGCAACAAGC YptC4-F2, TCCACATGATGGCTAGTGCGGACG[e] YptC4-F3, CCGTCAGCTACTGGGAAGGCCCG[d]	360	269	56.4
III	*DHCa*	L26049	DHC-alpha-F, AGGACATGCCCGCCAAGTGGGT DHC-alpha-R2, GCGGCACCTGGCTACTGCTGTACA	309	~290	58.9
III	*COX2A*	AF305080	cox2A-F, TGCGGAGAAGGCGCTGGTCAAG cox2A-R, GGCGTCTTGCGCCATTGCTGAA	522	~490	55.0
III	*GSAT*	U03632	GSAT-R, GAGGGTGCAATCAGAGCCCCCTTG GSAT-F2, CGCGTGCACAGCTTGCAGCAAA[e] GSAT-F3, CGGGCGGTGCCTGGTTCTTCG[d]	561	389	55.0
III	*MAA7*	AF047024	MAA7-F, CGGCGACAAGGACGTCAACAACG MAA7-F2, TGTGGGAGCGGGAGTGACTGCA MAA7-R, TGCAACCATCTCCCTTCGGCCC MAA7-R2, TAATCCGCCTCAGCCCCAACCG	114[f] 349 473	349	55.0
III	*GAR1*	U36197	MethSyn-R, GCAATGCGTTGGGTTACAAGCAGC MethSyn-F2, GCGAGCGGTACCGACTAGGCAGA[e] MethSyn-F3, GCTGAATTGTGTACGGTGCACACGG[d]	339	179	61.0
III	*FLA14*	U19490	LC8-F, TTCAAGTCGGGCTAAGCGGCCG LC8-R2, CATCCCTCCCCGCTATGTCCCG[d] LC8-R3, CCAGAGACCGCGCTCCGCC[e]	189	97	55.0
III	*CNA34*	B1722955	CNA34-F, GCAGCTGCCTGTCAATGCGCCT CNA34-R, GTCTGCGTAGCCGTACACGCGTCA	376	~350	55.0
III	*RSB1*	B1722838	RSB1-R3, CCGCCACCCATGTCACGGC[d] RSB1-F2, TGTTCCCGCCGGAGGAGG RSB1-R4, CACACCACACGCTGCCTACAGG[e]	109	146	55.0
III	*PHOT1*	AJ416557	PHOT1-F, CGCGAGGAAGGGTTTGAGGTGCTG[d] PHOT1-R, CGGATAACAGCTGCGTCCTTCCCC PHOT1-F2, CCGCCCCGGCTGCAGCTAA[e]	338	407	55.0
IV	*TUA2*	M11448	a2-tub-R, GCCAATAGAGGCACGGTCGTGGA a2-tub-F2, GGCGTGATCTGAGGCTTCGTTGG	130	150	55.0
IV	*COX3*	AF233515	cox3-F, ACGGCATCATCTACGTCGGCCAG cox3-R, ACATAAACCGTCCACGCGGCTGC	470	~500	55.0

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TABLE 2—*Continued*

Linkage group	Gene (3′ UTR)	Accession no.[b]	Primers[c]	*C. reinhardtii* allele (bp)	S1-C5 allele (bp)	Annealing temp (°C)
IV	*PF20*	U78547	PF20-F, TGTCTCTCCGTTCCCTTGCGCG[e] PF20-F4, GGACCCCGGTCCTCTGCTACCG[d] PF20-R2, ACACACCAAACCGCCCATGACCC	328	223	59.3
V	*GP228*	BG859190	GP228-F, CAACATGGTGGAGGAGCAGGAGGG GP228-R2, GGCAGCCATCACCTCACACCA[e] GP228-R3, GCTTCTCATCACCCCCTGCTCTTAA[d]	244	369	63.9
V	*ALD*	X85495	ald-F, CTGCCCAGGGCATGTACGAGAAGG ald-R, TCCCGACGCTCGATGGATAGGAGG[e] ald-R4, GTCGCAGGCTGCTGCGGCTG[d]	588	122	60.0
V	*PF26*	M87526	RSP6-R, AGATGAACCTCGTGCCTCAGCGGC RSP6-F2, GAAGAACGGATCAGAGCGGTGTGGG[d] RSP6-F3, GGAACGTGGGGGACATACCCGG[e]	206	529	61.0
VI	*FA1*	AF246990	FA1-F, ACGAGGAGGACATTCGGGAGCTGC FA1-R2, GCTCAGCCGTTCCAAGGAAGCAATG FA1-R3, GTCCAAGCAGGTCCAAGCCGTCAA	182	331	64.3
VI	*ATPC*	M73493	ATPC-R, TCGCCTCATGTCGGCACACAGG ATPC-F2, TAAATGCCTGGGCTCTTGGGCTCG[e] ATPC-F3, CGCCGTGAATTTGCGTGGCG[d]	578	411	55.0
VI	*S8-13*	X53574	S8-13-F, GCGCCCCGAGTTCAACATCACC S8-13-R2, CCTCAACACACCGCGACGCAGA[d] S8-13-R3, GCATCAACGCGTTACAGATCGCCA[e]	331	285	55.0
VI	*CRY1*	L07561	DNAph-R, GAGACCGAAAGGCAAGGCACAGGC DNAph-F3, AGCTAACCATGTCGGCCGGTCG[d] DNAph-F4, GCTGCATTGGGCGCACATGG[e]	443 147[f]	443	63.5
VI	*TUG*	U31545	g-tub-R, GTCGCCAGGAATTTTGCCCCTGG g-tub-F2, GCGCGCCTGGCGGTAGCACATA[d] g-tub-F3, AGCAGCGCTATGTTCGCTTCCCC[e]	281	443	61.0
VI	*RPL41*	AF130727	RPL41-R, TGCAACTTGCAATCCATCCGTTGC RPL41-F2, GCAACTAAACGTGGCGGCCTACCG[e] RPL41-F3, GGTAACCGATTCGAGCGTTCTGGA[d]	262	107	55.0
VII	*CHLH*	AJ307055	chlh-F, TTGGCGGGTTGTGGTTGGACTAGG chlh-R2, TCCTCGCGGAGCGCTCTCG[e] chlh-R3, CACAGCTCACACACACACGCACAA[d]	127	375	62.4
VII	*SFA*	U56982	SF-assem-R, ACAGCATGCCCTGCAAGCTCGC SF-assem-F2, TTGCATGGGCAGCACTGGTCGA[e] SF-assem-F3, GCCGTATAAATTCAGGGCAGGCGC[d]	330	211	65.0
VII	*IFT88*	AF298884	IFT88-F, TGCTGAGCTTTGGCTCGGCTGG IFT88-R2, ACATACACAAATGGCGGGCTGCAG[d] IFT88-R3, CTGGGGACCCCTGCAGCCAAAA[e]	191	91	55.0
VII	*CPN60B2*	L27473	cr2-R2, AGCTGCTTGGCAGCGGCTGTTG cr2-F4, TGGAATTGGCGGTGCGAGCG[d] cr2-F5, TGCAGCACAACTCCCGGCTGC[e]	260	621	61.0
VII	*FA2*	AF479588	fa2-F, GCACGTCGTACTACACCAGCGCCA fa2-R2, CCCCGTCAACCTGGGCCAATCA[e] fa2-R3, CCGTCAACACCTCGAGTGGACACGA[d]	140	396	55.0
VII	*SULP*	AF481828	SulP-R, TGCGTCCTTCGCTCAATCCCTGC SulP-F2, GTGGGAGGGGTGGGACTTTGGG[e] SulP-F3, GGTATGGGGATGTCCGCACGCTTC[d]	339	193	55.0
VIII	*MCA1*	AF330231	MCA1-F, CGCGGGCGAGTTTGCTGTTGCT MCA1-R2, CGGATCCCGAACAGCGGCAG[e] MCA1-R3, CCCCGTGACTCAATCAAGTCCCTG[d]	113	226	55.0
VIII	*LI818*	X95326	LI818-R, TCCGATGCACTCACGCTCACAGC LI818-F2, TGGGCATGCGGAAATGCGTGTC[e] LI818-F3, CTTGCTTGGCCGGCACGGG[d]	334	141	55.0
VIII	*PSBQ*	X13832	OEE3-R, CGTGCTGTTGCGAGCCACTCCA OEE3-F2, GCCGAGTTCTCAACCCTCTCGGC[d] OEE3-F3, GGGTGCAACCTCCGGTGGCCTA[e]	347	535	65.0
IX	*PPX1*	AF068635	Ppx1-R, CATGGCACTTATGGGCGAAGCCG Ppx1-F2, GGGCAAGCGGAGTGGAGGCGA[e] Ppx1-F3, TCGAAGTGCCTTCGAAAGTGGGCA[d]	526	181	55.0

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TABLE 2—*Continued*

Linkage group	Gene (3′ UTR)	Accession no.[b]	Primers[c]	*C. reinhardtii* allele (bp)	S1-C5 allele (bp)	Annealing temp (°C)
IX	*PSBO*	X13826	OEE1-R, CGCATGCACGACGAGAAGCGAG OEE1-F2, GTCGACCGCTGCGAGGAGGAGA[e] OEE1-F3, CGAGCGCCGTATCATCCGGCTTA[d]	510	161	55.0
IX	*MBO2*	AF394181	MBO2-F, CGTTAACAGCCCTGAACTCGGCCG MBO2-R2, TCACGCCACACCTGTACGTGCAA[d] MBO2-R3, ATGCGCCAAACCCGGAGCTACC[e]	516	406	65.0
IX	*CAH3*	U73856	CAH3-F, CATGCAGCCCATCAAGGTGCCC CAH3-R2, TCCCCACCGTGGGCCAAACC[e] CAH3-R3, CGTGCAGGCGATGCCTCCA[d]	307	359	55.0
IX	*MBB1*	AJ296291	mbb1 F, GGATGAGGCGGTGGAGGCACTACA mbb1-R2, GCGTGCCGCGTGTCACCAAGTA[d] mbb1-R3, GGCCATACGCCATCATAACCGAGG[e]	382	192	55.0
X	*KATANIN*	AF205377	Katan-F, ACGAGGAGTGGCTCAGCGTGTTCG Katan-R, GGACGCCCAAGCTTCAAATCCACG	394	~410	55.0
X	*PF6*	AF327876	PF6-R, GACAAACCCGTGTACCATCCGGCC PF6-F2, GCACAAGCAATGCATCGGTGTGC[e] PF6-F3, CGTTCCAGCGGCACTCACGGG[d]	362	229	55.0
X	*CNA83*	B1724522	CNA83-R, TGCACATACCTCTGCCGCTCCACC CNA83-F3, CGGATCGGGTATGCGGATGCCA[d] CNA83-F4, CGGCCGCTGAAGCTGCTGTGA[e]	278	324	55.0
XI	*VFL2*	X57973	vfl2-R, CCGCAGGCTGGCGATGGGAATA vfl2-F2, GACGCCGGGGCTTGCTTTCACC[e] vfl2-F4, TGCTGTGAAGGGTGGACACCCTGG[d]	463	283	58.9
XI	*ODA2*	U15303	ODA2-R, CACGCAGTGGCATCCTGCGC ODA2-F2, TTAGGGAGGCGGCACTGACGCA[e] ODA2-F3, AGCGTGCGATTGGCGTACGAGATTA[d]	298	197	55.0
XI	*RPS14*	U06937	CRY1-F, CATCCCCACCGACTCCACCCG CRY1-R2, CCCGCCGCCGCCACCTA[d] CRY1-R3, CCAGCCGCCAGGCGGGC[e]	268	248	54.2 (*C. reinhardtii*), 58.9 (S1-C5)
XII/XIII	*GAPC*	L27669	gapC-F, CAGATTGCTTCAGGGCTTCGGCG gapC-R, TTCACGCACCGTGTGGCAGTCC	572	~500	56.0
XII/XIII	*PSR1*	AF174532	Psr1-R, AGCACCCGTCCACACACCGCAA Psr1-F2, GCACCTGCGCATGCATCTGTTG[e] Psr1-F3, AGACAGCGGTTGGCCCTTGCTTG[d]	344	189	62.0
XII/XIII	*EYE2*	AF233430	EYE2-F, CGCGCGAGCTGACAGCTGAAGA EYE2-R2, TCACATACTGCGCAGCGCTCTCC[d] EYE2-R3, CGGGGTTGCCACAAGTTTCCTTCC[e]	525	211	64.8
XII/XIII	*SAC3*	AF100162	Sac3-R, ACTGCACAGCTCTGGGATGTCGCC Sac3-F2, ACGGAGCGCACTGGGTTCTTGCAA[d] Sac3-F3, TCGCGGTCCGGTCCCAGTATG[e]	324	508	55.0
XII/XIII	*IC138*		IC138-F, CGGGGCAGGCGTAGGACTGGAA IC138-R2, GCAAGCCTGGCCCCATCTGTTC[d] IC138-R3, CCTGGGCATCAGCACAGCACTTG[e]	287	171	55.0
XII/XIII	*ODA12*	U89649	LC2-F, GAGTAATGGTGCGGCCAAGCTGCC LC2-R, TTGCAACGGCAAGCCGCCAT	426	~450	55.0
XII/XIII	*14-3-3*	X79445	14-3-3R, AGTGCGCTTCAACACGCCTCACG 14-3-3F2, CGGCGTGAAGTGGCGTTACAGCTA[e] 14-3-3F3, TGACATTGTGTTGGCCATCACGGA[d]	317	205	55.0
XII/XIII	*TUB2*	K03281	b2-tub-R, CACGTGCACGAGTGTGTGGCCA b2-tub-F2, GGAGGGGGGCCCATTGCCC[d] b2-tub-F3, CGGCAGGGGCAGGTAACTGCC[e]	113	283	55.0
XII/XIII	*RDB*	BG858985	CRB-R, CGTCAATTTGGCCGACCTGACCG CRB-F2, CCCGAAGCCATGGGCATCGAA[e] CRB-F3, GCGTCGACAACCATCTGCGACCA[d]	558	257	55.0
XIV	*AC206*	U70999	CCS1-R, ACGAATGCTGGGTGGGCCAAGC CCS1-F2, GGGGTCACGACAGGGGTAGGGTG[e] CCS1-F3, CGTGCGGCAAACAAGCACCCTTG[d]	483	156	64.7

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TABLE 2—*Continued*

Linkage group	Gene (3′ UTR)	Accession no.[b]	Primers[c]	*C. reinhardtii* allele (bp)	S1-C5 allele (bp)	Annealing temp (°C)
XIV	*IDA5*	D50838	ACTIN-R, AAACCCCAGCGCTTTGGCGC ACTIN-F2, AAGCGCTTGTGAGTGCGCCAGA[d] ACTIN-F3, ACGCAGGTGGCAGGCCGAGG[e]	249	529	55.0
XV	EST 894011B10	BE056715	BE056715-R, CCCCCAAAATCAGCATGGGGTCC BE056715-F2, TGGATGAGGTGGGGTCGTTTGTCG[e] BE056715-F3, ACTGGCGTCGCGTCTGCAGG[d]	316	237	55.0
XV	*IDA2*	AJ242525	dhc10-F, TGCTGCTGTCGCTGGCCACGTA dhc10-R, TCACGGCAACCTGAAAGGACGCC	514	~550	55.0
XVI/XVII	*CYTC1*	AF245393	Cytc1-F, GCCCATCAAGTCGCAGCGCATC Cytc1-R3, CAGCTGAACAGCCTGTGCGGCA[d] Cytc1-R4, GCAAAGACACTCAGGCCGCGCTC[e]	131	436	66.0
XVI/XVII	*ZYS3*	AB004043	Zys3-F, AGCCGCCACGTGTTTGTGGAGG Zys3-R, ACTGCCTTCTGGCTCGTATGCGGG	344	~530	55.0
XVIII	*CYTC6*	M67448	CytC6-R, AAGCGCGTTCATGGTTCGGCC CytC6-F2, GTGTAGCTAGCTTTTGCCCCGGCA[d] CytC6-F3, CATCACGCAAATGGACACGTTCCG[e]	221	319	55.0
XVIII	*CLPK1*	AB042714	cpk1-F, GGATGGCAGCGTACCAGCTGTCAC[d] cpk1-R, CACCGCATGTGTATTGGAGGCGC cpk1-F2, ATGCAGCAACGGTAGGCGCTAGCG[e]	314	252	62.0
XVIII	*RAA2*	AJ243394	Maa2-F, ATTGACCACTGCGGCGCTAGCG Maa2-R, TAGTAGGGGCATCCGTGGCTCTCG	595	~530	59.0
XVIII	*ODA1*	AY039618	CDS-022-F, GACGCGGCGGTGATGGGC[d] CDS-022-R, CCCCGAGCGGATTGAGGTAATGG CDS-022-F2, CAAGGGTTCAGGGGCAGAATACCG[e]	160	277	55.0
XVIII	*IDA7*	AF159260	IC140-F, TGCTTATGGAAGGGCTGGGCGG IC140-R2, CTTGCGCCCGCCTCAGACACG[e] IC140-R3, GCGCAAGGCTTCGTCAGGCTGTC[d]	174	395	65.0
XIX	*ATP2*	X61624	atpB-R, TCGCAGTCCGTACCCTTGACACCG atpB-F2, GGCAGGGCGGTGCAGGCTTAA[d] atpB-F3, CGGGGCCATGTCAGCATGGGA[e]	102	255	55.0
XIX	BAC 27e16	PTQ10139.x1	BAC27e16-F, GTCTGTGCAGCGCTGCGCCTTT BAC27e16-R, AATGGCCAGGATGTGCGGGTAGC	296	~400	64.3
XIX	*ZYS1B*	X76117	zys1B-F, GCTTTGAGTGGAGCGAGGCGCA zys1B-R2, TAAATGCATCTCCGCAGTTTTCTCCG[b] zys1B-R3, GCATTGGGCATAACCAGTATGTGCCA[e]	466	303	61.0
XIX	*FLA10*	L33697	FLA10-F, CTGCGCGCCAGCAAGCTCAAGT FLA10-R, GGTAACAGCCCGTCTTCCAGGGCC	491	467	55.0
XIX	*CNB60B1*	L27471	cr40-F2, GATTGGGGGCAGTGGGCAGG[e] cr40-R2, CACCGCCATGCGAAAGTGCC cr40-F3, GCCTGTGCGGGATGGCGTGAG[e]	388	350	56.0

[a] All amplification reactions should be performed as described in Materials and Methods, at the annealing temperature given for each locus, and all of the primers listed should be mixed in a single tube for each amplification.
[b] Accession numbers refers to entries in the GenBank database (http://www.ncbi.nlm.nih.gov/), except for marker BAC 27e16 on linkage group XIX, which is from the JGI Chlamydomonas BAC-end sequence database (http://bahama.jgi-psf.org/prod/bin/chlamy/home.chlamy.cqi).
[c] When only two primers are listed they generate allele-specific product size differences.
[d] S1-C5-specific primers.
[e] 21gr-specific primers.
[f] The predominant product when more than two amplification products were observed.

As soon as a new mutation is mapped to an arm of one of the linkage groups, other molecular markers mapping to that arm can be used to attempt to place the mutation between pairs of flanking markers. Consulting the overlapping BAC contig map will then allow the choice of BAC clones to be tested for phenotypic rescue using cotransformation with a selectable marker gene (http://www.biology.duke.edu/chlamy_genome/BAC/index.html). A number of selectable markers are available for transformation of *C. reinhardtii* (47). If a project requires fine structure mapping of a genetic region, and multiple STS markers are not available, it is possible to generate additional STS loci. The sequences of all the BAC ends are available, and reference to the BAC contig map places these sequences at intervals of tens of thousands of bases along the genome. A BAC end sequence can be turned into an STS locus by sequencing the same region of genomic DNA from the S1-C5 strain and using sequence polymorphisms to design informative primers for PCR. The high degree of sequence poly-

morphism between the laboratory strains of *C. reinhardtii* and S1-C5 makes the task of finding useful sequence polymorphisms routine. One source of sequence polymorphisms is the set of ESTs derived from CC-2290 (strain S1-D2), available in the National Center for Biotechnology Information (NCBI) database. Many of these EST sequences were used to generate the markers reported in this study. Another possible source for additional molecular markers for mapping is microsatellite repeat sequences, which have been shown to be abundant in the *C. reinhardtii* genome (56).

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ADDENDUM IN PROOF

The sequence of the *Chlamydomonas* genome obtained by the DOE Joint Genome Institute is posted on the Internet at http://genome.jgi-psf.org/chlre1/chlre1.home.html.

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