

Supplemental Figure 1. Growth Phenotype of Iron Limitation on Additional Wild Type Strains

The indicated strains were inoculated to a density of 1 x 10^4 cells / mL in 100 mL cultures of TAP medium containing iron concentrations ranging from 0.1 to 200 μ M.

- (A) A first batch of cultures was photographed after 4 d of growth.
- (B) A second batch of cultures was photographed after 5 d of growth.



Supplemental Figure 2. Callable Loci

The quality of the base calls for each of the 109 million total nucleotides in the *C. reinhardtii* genome were categorized and plotted by GATK CallableLoci for each strain. Callable loci were those base calls that were based on four or more quality control-passing reads. The remaining reads were evaluated by the software to be uncallable due to insufficient coverage, low mapping quality, or a lack of sequence information in the reference genome.



Supplemental Figure 3. Evidence for Recombination Between Haplotypes Nucleotides the same as reference (i.e. haplotype 1) are gray. SNVs relative to reference are color-coded: green = A, blue = C, brown = G, red = T. Regions that were identified as haplotype 2 based on their pattern of SNVs are indicated by a yellow box below the alignment. Haplotype 1 regions are indicated by a blue box. The double-headed arrow indicates the 52-bp region that is the likely site of a meiotic recombination event in the cross that produced CC-3269.



Supplemental Figure 4. Comparison of Haplotype Patterns for Selected Strains

Subsets of strains from Figure 5 are presented separately for easier comparison. Blue indicates haplotype 1, and yellow indicates haplotype 2. The mating locus is depicted with + or -.

(A) Strain 2137. Comparison of three examples of strain 2137 compared to CC-4532.

(B) CC-4532 cannot result from a cross of CC-124 and CC-1690.

(C) CC-4349 cannot be the parental strain of CC-4348.

(**D**) CC-124 backcrossed strains. CC-4402 and CC-4403 remain distinct from CC-124, despite 10 backcrosses to that strain.

(E) CC-4246 and CC4287. The parents of these two strains are not as reported, and remain to be identified.

(**F**) CC-4051 and CC-4603. These two strains are nearly isogenic. They differ in haplotype only in those blocks that include, or are adjacent to, the mating locus. (**G**) *cw15* mutant strains. Five different *cw15* strains have dramatically different haplotypes.



Supplemental Figure 5. Allele-Specific Amplification to Identify Haplotype
Allele-specific amplification primers were designed to amplify a product in one haplotype, but not the other, for all 41 defined haplotype blocks (Table 2). The sequences of all 82 primer pairs are included in Supplemental Dataset 6.
(A) Strains CC-1009 and CC-1010 have alternate haplotypes in all 41 regions.
(B) Haplotype scoring by analytical gel electrophoresis. DNA from CC-1009 and CC-1010 was subjected to PCR amplification with all 82 primer pairs, and scored by analytical gel electrophoresis. In each panel, the haplotype 1 specific product is on the left, and the haplotype 2 product is on the right. The position of 100, 200, and 300 bp markers is indicated to the right of each panel.



Supplemental Figure 6. Examples of Transposon Position Jumping in Chromosome 16

(A) Transposons in Chromosome 16. The position of 10 transposons identified in the reference sequence in chromosome 16 are depicted in blue. Large deletions (greater than 40 bp) identified by sequencing the standard laboratory strains are depicted in red. The scale bar indicates 1 Mbp.

(**B**) An expanded view of three transposons. The scale bar indicates 10 kbp. An additional track in green indicates the loci of genes likely to be affected by these transposons.



Supplemental Figure 7. Predicted Effects of Haplotype 2 Variants on Gene Models

The likely effect of the haplotype 2 SNVs and small InDels on the gene models was predicted computationally. Each class of variant is presented in a proportionately size pie chart.

(**A**) All haplotype 2 variants. 164,606 out of 592,554 haplotype 2 small variants were localized within coding regions.

(**B**) Coding region-localized variants. These were classified based on their predicted effect on the relevant gene model.

(**C**) Non-synonymous codon changes. The 64,685 non-synonymous codon changes are further subdivided based on the predicted change to the encoded amino acid.

CGL49 (Cre12.g487850)							
<u> </u>							
Hap2 12-D							
FAL18 (Cre16.g652400)							
Hap2 16-B							
DUR1 (Cre08.g360050)							
Han2 8-A							
HSP90A (Cre09.g386750)							
Нар2 9-А							
RSEP1 (Cre17.g730100)							
Hap2 17-F							
large deletion							
inversion							
in frame InDel							
frame-shift InDel							

Supplemental Figure 8. Variants in Genes Attributable to Haplotype 2

The predicted effects of haplotype 2 variants on gene models are color coded according to the legend, and a black scale bar representing 1 kbp of DNA is included for each.



Supplemental Figure 9. Laboratory-Originated Mutations in Commonly Studied Genes

Presented here are mutations that we identified in three commonly studied genes. A black scale bar indicating 1 kbp of DNA is included for each gene. Mutations are color coded according to the legend, and an example strain that carries the mutation is indicated for each variant.



Supplemental Figure 10. Determining Haplotype from RNA-seq Data Identifies Mislabeled "2137" Strain

Reads from RNA-seq experiments performed by this group in 2007 were realigned to the reference genome in order to identify the haplotypes of the strains used in those experiments.

(A) A representative region of chromosome 17 from 4,359,642 to 4,359,871 that has a distinctive pattern of SNVs between the two haplotypes. The coverage of RNA-seq reads to the *SDR28* locus is shown for the indicated experiments. Nucleotides the same as reference (i.e. haplotype 1) are gray. SNVs are color-coded: green = A, blue = C, brown = G, red = T. "2137" RNA from Fe data indicates reads from an RNA-seq study in a strain that was believed to be 2137 that was grown in limiting iron (Urzica et al., 2012). "2137" RNA from Cu data indicates reads from an independent RNA-seq study performed with the same strain grown in limiting copper (Castruita et al., 2011). *crr1-2* RNA from Cu data indicates reads from the same copper study performed in mutant strain *crr1-2*. (B) For comparison, genomic data from the current study is included to demonstrate the pattern of SNVs in the two haplotypes at this locus. Haplotype 1 reads are from CC-1010 and haplotype 2 reads from CC-1009.

(**C**) Using this approach, the "2137" and *crr1-2* data were scored for haplotype in each block. The resulting haplotypes from the RNA-seq data are compared to the indicated DNA haplotypes.

Transversions

A -> C	29,483
G -> T	29,715
A -> T	17,238
T -> A	17,468
C -> A	29,294
T -> G	29,098
C -> G	32,082
G -> C	31,725

Transitions

A -> G	77,280
C -> T	77,003
G -> A	76,862
T -> C	77,392

Supplemental Table 1. Transversions and Transitions for all SNVs

The 524,640 SNVs that were identified in this study were subdivided into each of the possible transversions and transitions.

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	REM1	chromosome_17	4416165	4418249	2084	86%																													

Supplemental Table 2. Transposon Position Jumping at 84 Loci.

The coordinates of eight different *C. reinhardtii* transposons were identified in the reference genome sequence. Those that had substantial overlap with the coordinates of large deletions identified in one or more of the sequenced strains are presented. Gray boxes indicate that the transposon sequence is present in that strain, and white boxes indicate that the transposon sequence is absent.

Gene ID	Gene Name	Gene Description	Hanlotype 2 Variant
	BLIG25	Basal body protein and	9112 bp deletion removes all of even 1 and part of
Creo 1.9009850	60023	putative AP2 domain	exon 2 in BUG25. 1 other gene is completely removed
		transcription factor	and 1 other gene is partially removed.
Cre01.g009800	FAP289	Flagellar Associated Protein, coiled-coil	79,864 bp deletion completely removes FAP289 and 6 other genes, and partially removes 2 other genes.
Cre01.g012100	ARS4	Arylsulfatase	77 bp deletion removes part of exon 6 in ARS4. Also, 1 frame shift InDel, 1 in-frame InDel, and 17
Cre01.g012800	FAP230	Flagellar Associated Protein	nonsynonymous codons. 15,260 bp deletion causes complete loss of FAP230 and partial loss of 2 other genes.
Cre01.g013300	DEG10	DegP-type protease	1 in frame InDel, 11 non-synonymous codons
Cre01.g016500	DLD2	Dihydrolipoamide dehydrogenase	1 in frame InDel, 21 non-synonymous codons
Cre01.q044100	AMYB3	Beta-amylase	10 non-synonymous codons
Cre02.g113850	PBGD2 /	Porphobilinogen deaminase	2 frame shift InDels, 2 non-synonymous codons
Cre02.g118950	PRPS17	Plastid ribosomal protein S17	1 in frame InDel
Cre06 g252200	T0C34	Translocon at the outer	14 non-synonymous codons
0.000.g_000		envelope membrane of	
Cre06.g255450	MAT3	Retinoblastoma protein	6 non-synonymous codons
Cre06.g260450	LCI20	2-oxoglutarate/malate	1 in frame InDel
Cre08.g360050	DUR1	Urea carboxylase/allophanate	1 in frame InDel, 16 non-synonymous codons
0.00.000000	01102	hydrolase	
Cre08.g360100	DUR2	Allophanate hydrolase	7 non-synonymous codons
Cre09.g386750	HSP90A	Heat shock protein 90A	I frame shift
Cro09.g394200	A0V1	Alternative exidence	1 in frame InDel 2 non synonymous codons
Cre09.g396650	PAT2	Phosphate acetyltransferase	1626 bp deletion removes exon 17. A second 105 bp
			deletion removes part of exon 18.
Cre09.g396700 Cre09.g399400	ACK1 FAP199 /	Acetate kinase Triacylglycerol lipase	9 non-synonymous codons 265 bp deletion removes most of exon 1.
Cro00 #403000	TGL15	Elegallar Associated Protein	12 CC1 bp delation removes EAD204 and 2 other
Cre09.g403900	FAP294	Flagellar Associated Protein	genes, and partially removes 2 more genes.
Cre10.g419050	ATP1B	Mitochondrial F1F0 ATP synthase, alpha subunit	3 in frame InDels, 3 non-synonymous codons
Cre10.g465550	CLPD1	ClpD chaperone, Hsp100 family	1 in frame InDel, 3 non-synonymous codons
Cre10.g465900	CDKA1	Cyclin dependent protein kinase	21,642 bp deletion removes CDKA1 and 5 other genes, and partially removes 1 other gene.
Cre12.g487850	CGL49 /	ARF/SAR superfamily small	27,736 bp deletion removes all of CGL49 and 3 other
	ARL11	monomeric GTP binding	genes, and partially removes 1 other gene.
Cre12.g488500	ARC6	unannotated in Phytozome10	12 non-synonymous codons
Cre12.q490700	MIN1	Mini-evespot protein	1 in frame InDel. 3 non-synonymous codons
Cre12.g495100	PSR1	Phosphorus starvation response protein, Myb-like	1 in frame InDel, 4 non-synonymous codons
Cre12.g547100	CGL2 /	S-adenosyl-L-methionine-	23,595 bp deletion removes all of CGL2 and 4 other
Cre12.g554250	LPB1	Low Photochemical Bleaching	2 in frame InDels, 7 non-synonymous codons
		protein	
Cre12.g554300	NSS6	Sodium:solute symporter	5 in trame InDels, 24 non-synonymous codons
Cre16.g652400	FAL18	Similar to Flagellar Associated Protein FAP183	A 579 bp inverion reverses the orientation of exon 22. A second 762 bp inversion reverses the orientation of
Cre16.g654150	FAP63	Flagellar Associated Protein	lexon 23. 17,916 bp deletion removes 5' UTR of FAP63 and
Cre17.g698850	ISA2	Isoamvlase-type starch	completely removes 4 other genes. 19.265 bp inversion disrupts ISA2 and 1 other gene. 2
		debranching enzyme	other genes in reverse orientation. Also, 1 in frame
Cre17.g699500	TTLL9 / FAP267 / TTL8	Tubulin tyrosine ligase	2412 bp inversion disrupts TTLL9.
Cre17.g713200	OMT2	Chloroplast oxoglutarate-	17,076 bp deletion removes part of OMT2 and
Cre17.g720400	HMA1	Heavy metal transporting	5 in frame InDels, 24 non-synonymous codons
Cre17.g728900	FSA1 / TAL3	Transaldolase	22,593 bp deletion completely removes FSA1 and 1
Cre17.g730100	RSEP1 / RSE1	Intramembrane	2 frame shifts, 2 in frame InDels, 21 non-synonymous
0 17 700050		metalloprotease	codons
Cre17.g739650	MFT1 / MAE1	MATE efflux family protein	4 in trame InDels, 28 non-synonymous codons

Supplemental Table 3. Examples of Genes with Predicted Haplotype 2-Specific Variants

	Gene		
Gene ID	Name	GO Identifiers	GO Names
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre01.g053450	CYA1	GO:0023052, GO:0044700	signaling, single organism signaling
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre02.g100500	CYG22	GO:0023052, GO:0044700	signaling, single organism signaling
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre02.g103200		GO:0023052, GO:0044700	signaling, single organism signaling
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre04.g217450		GO:0023052, GO:0044700	signaling, single organism signaling
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre05.g237800	CYG64	GO:0023052, GO:0044700	signaling, single organism signaling
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre06.g290200	CYG39	GO:0023052, GO:0044700	signaling, single organism signaling
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre07.g318551	CYA8	GO:0023052, GO:0044700	signaling, single organism signaling
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre07.g342350		GO:0023052, GO:0044700	signaling, single organism signaling
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre08.g362100		GO:0023052, GO:0044700	signaling, single organism signaling
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre08.g373200		GO:0023052, GO:0044700	signaling, single organism signaling
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre09.g386900		GO:0023052, GO:0044700	signaling, single organism signaling
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre10.g429750		GO:0023052, GO:0044700	signaling, single organism signaling
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre11.g467651		GO:0023052, GO:0044700	signaling, single organism signaling
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre13.g567800		GO:0023052, GO:0044700	signaling, single organism signaling
		GO:0007154, GO:0007165,	cell communication, signal transduction,
Cre13.g605100		GO:0023052, GO:0044700	signaling, single organism signaling

Supplemental Table 4. Enrichment of Gene Ontology Terms in Laboratory-Originated Loss-of-Function Mutations

Original Strain	Sequence-Verified Clone
CC-124	CC-5074
CC-125	CC-5075
CC-425	CC-5076
CC-503	CC-5077
CC-620	CC-5078
CC-621	CC-5079
CC-1009	CC-5080
CC-1010	CC-5081
CC-1690	CC-5082
CC-1691	CC-5083
CC-2290	CC-5084
CC-3269	CC-5085
CC-4051	CC-5086
CC-4286	CC-5087
CC-4287	CC-5088
CC-4348	CC-5089
CC-4350	CC-5090
CC-4351	CC-5091
CC-4402	CC-5092
CC-4403	CC-5093
CC-4425	CC-5094
CC-4504	CC-5095
pcc1-1	CC-5096
CC-4603	CC-5097
IAM C-9	CC-5098
SAG 73.72	CC-5099
S24-	CC-5100
T222+	CC-5101
g1	CC-5102
CJU10-	CC-5103

Supplemental Table 5. Sequence-Verified Clones Available from the Chlamydomonas Resource Center