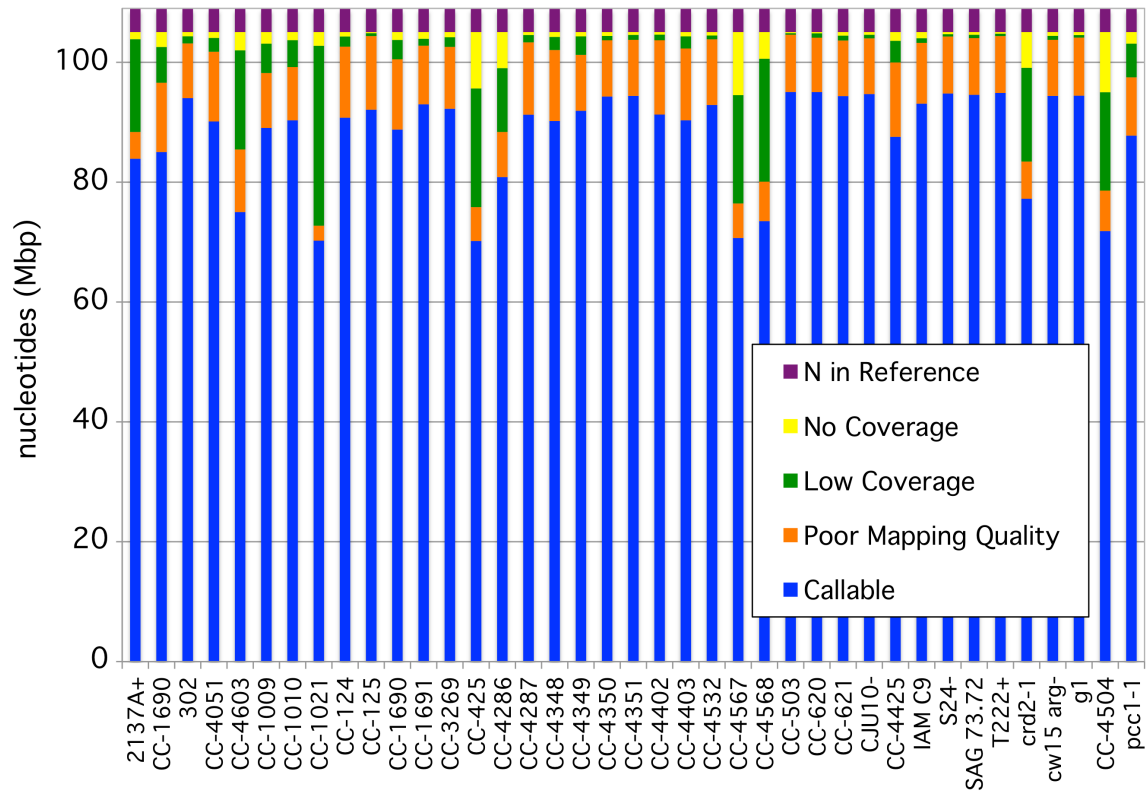


Supplemental Figure 1. Growth Phenotype of Iron Limitation on Additional Wild Type Strains

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

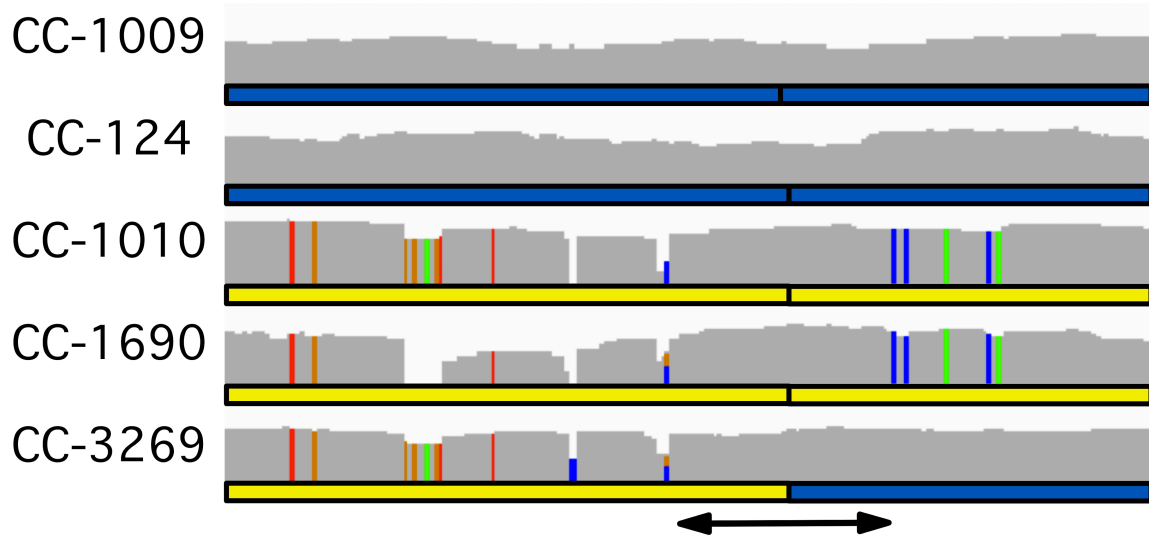
(A) A first batch of cultures was photographed after 4 d of growth.

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

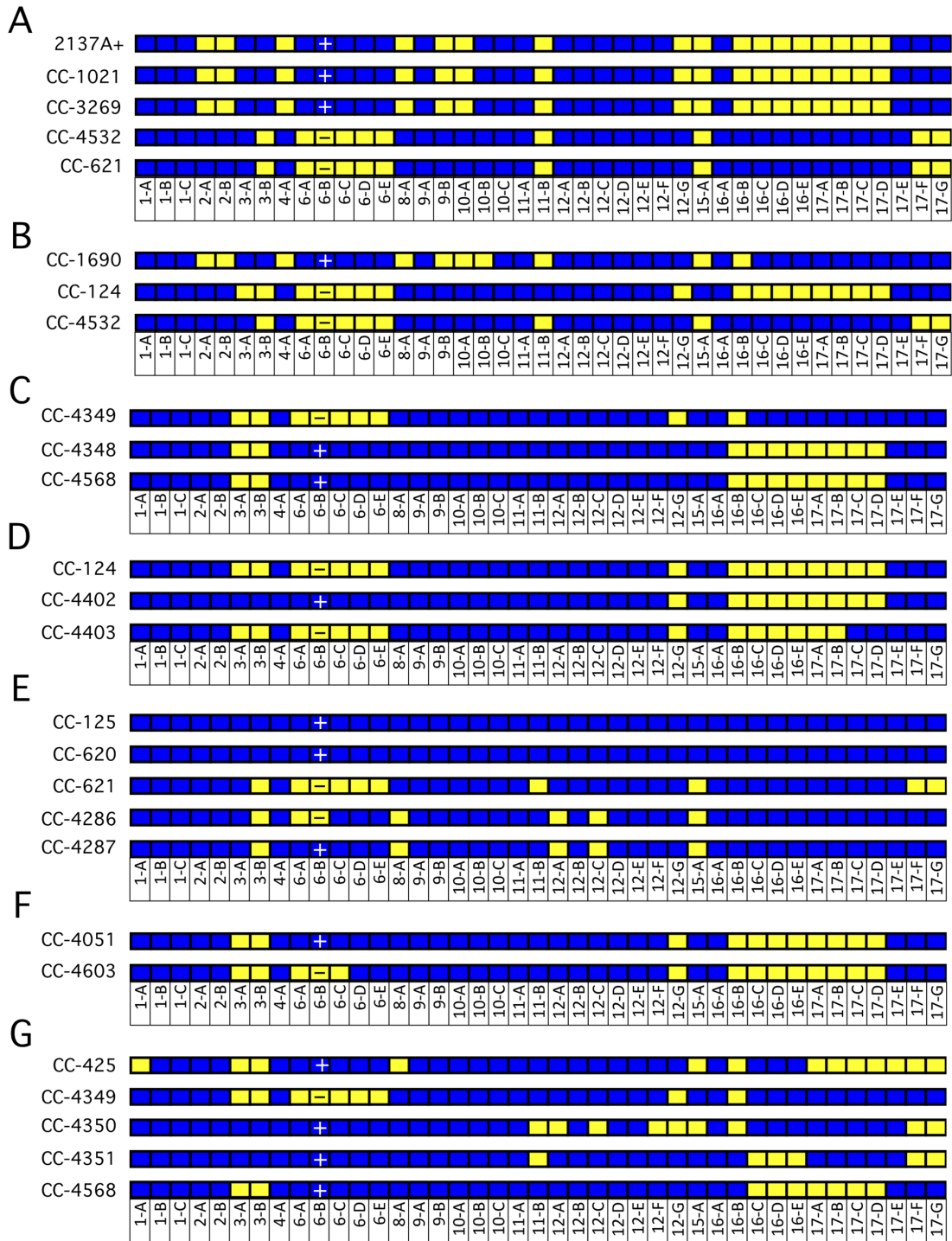


Supplemental Figure 2. Callable Loci

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.

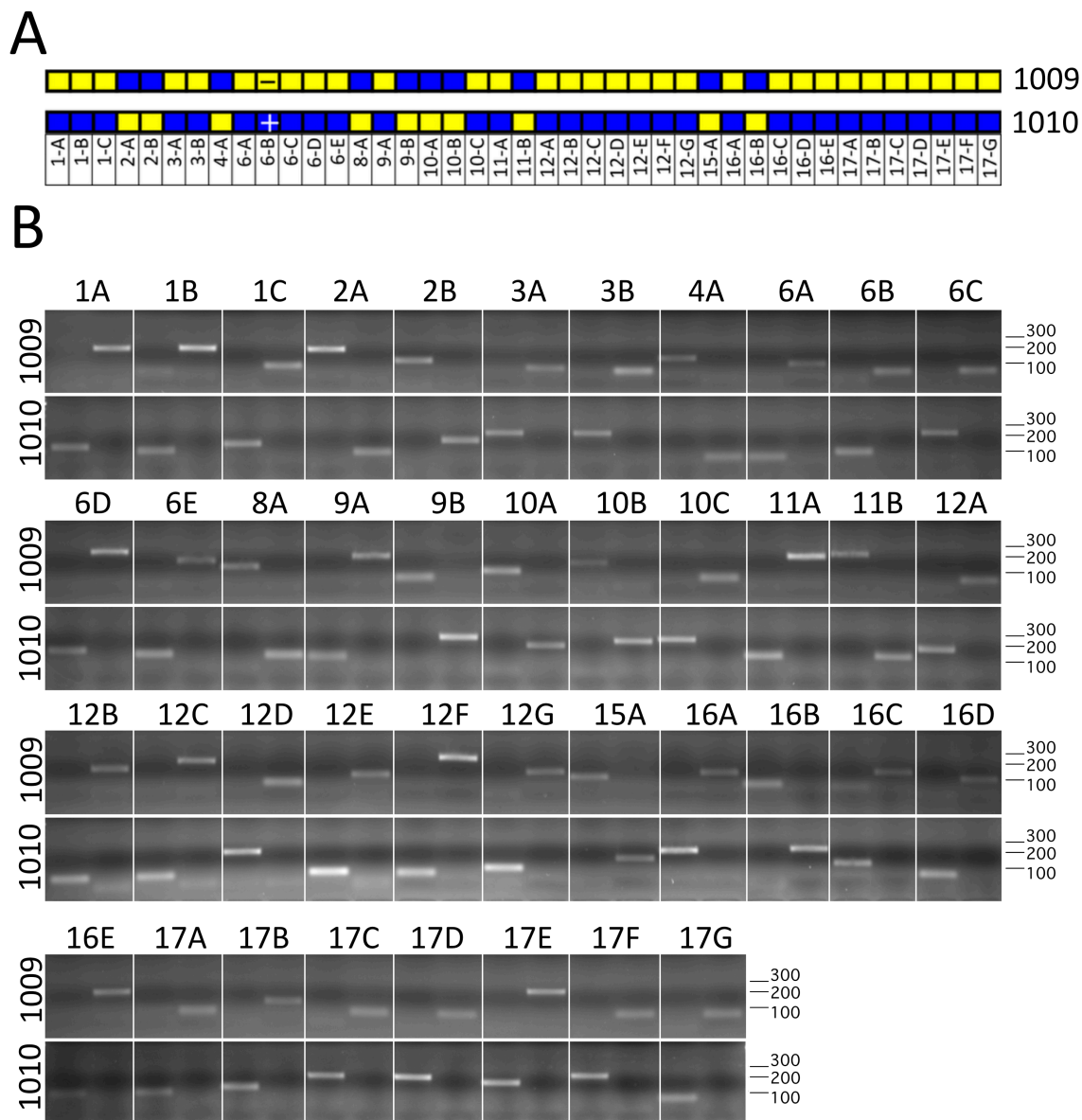
(C) CC-4349 cannot be the parental strain of CC-4348.

(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.

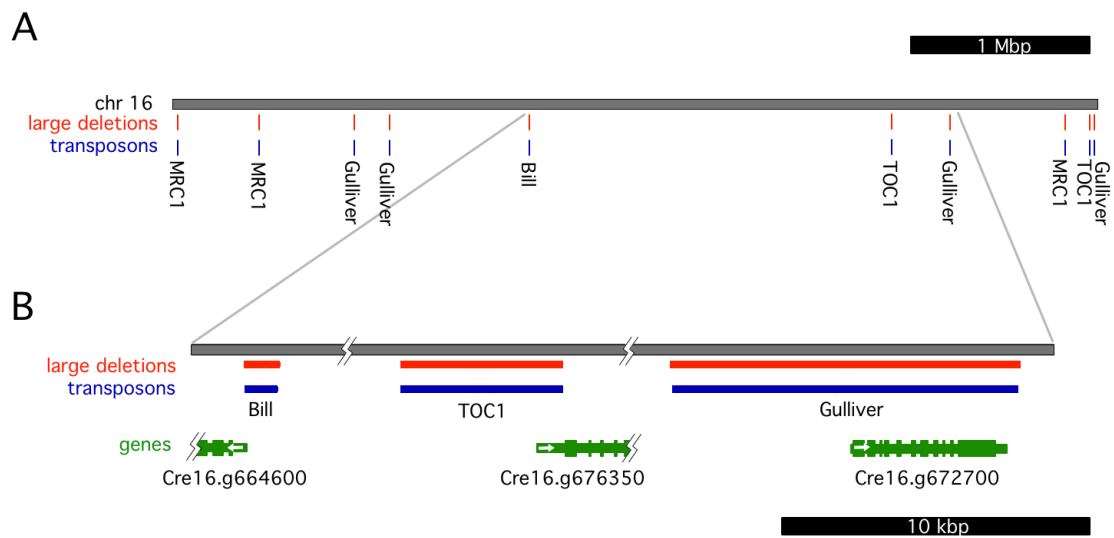


Supplemental Figure 5. Allele-Specific Amplification to Identify Haplotype

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.

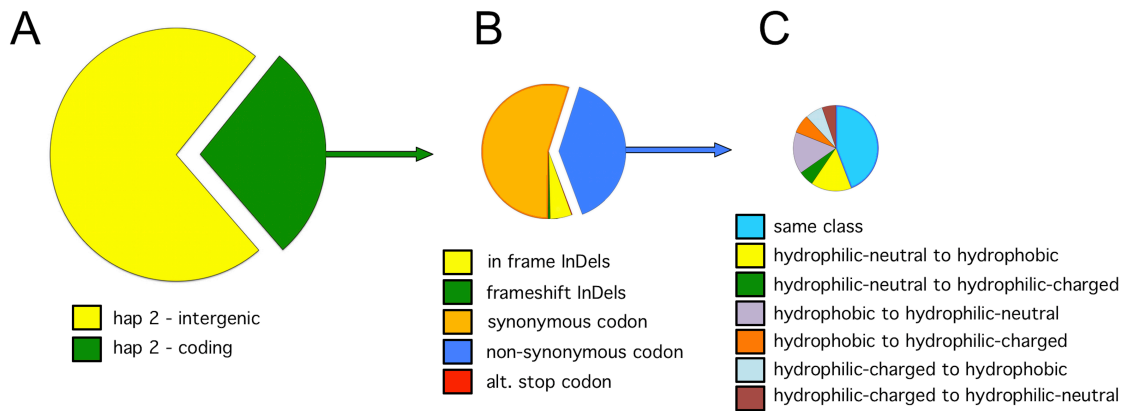
(B) Haplotype scoring by analytical gel electrophoresis. DNA from CC-1009 and CC-1010 was subjected to PCR amplification with all 82 primer pairs, and scored by analytical gel electrophoresis. In each panel, the haplotype 1 specific product is on the left, and the haplotype 2 product is on the right. 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.



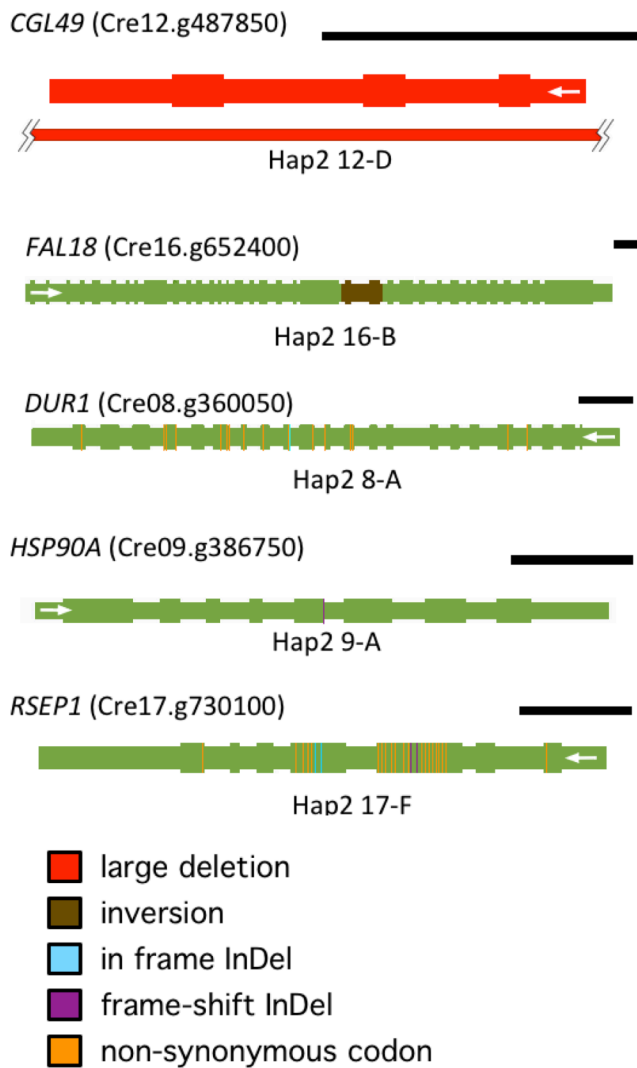
Supplemental Figure 7. Predicted Effects of Haplotype 2 Variants on Gene Models

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.

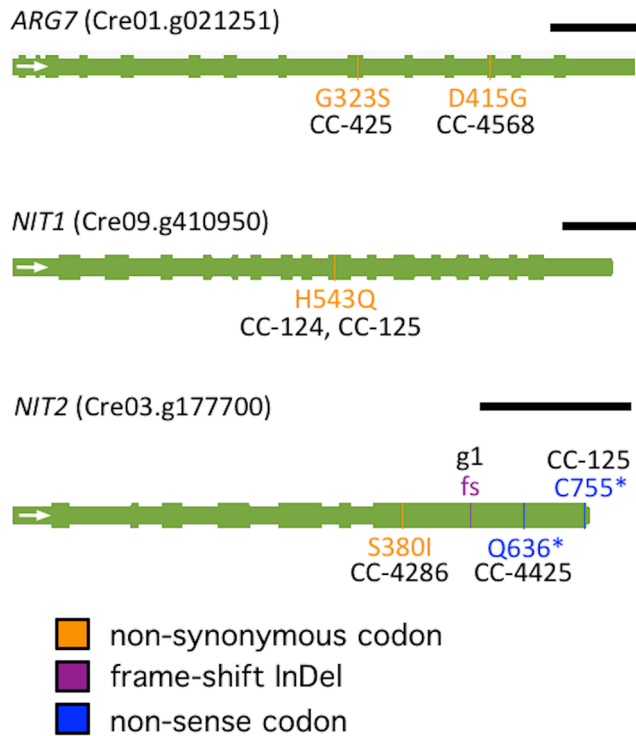
(A) All haplotype 2 variants. 164,606 out of 592,554 haplotype 2 small variants were localized within coding regions.

(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.

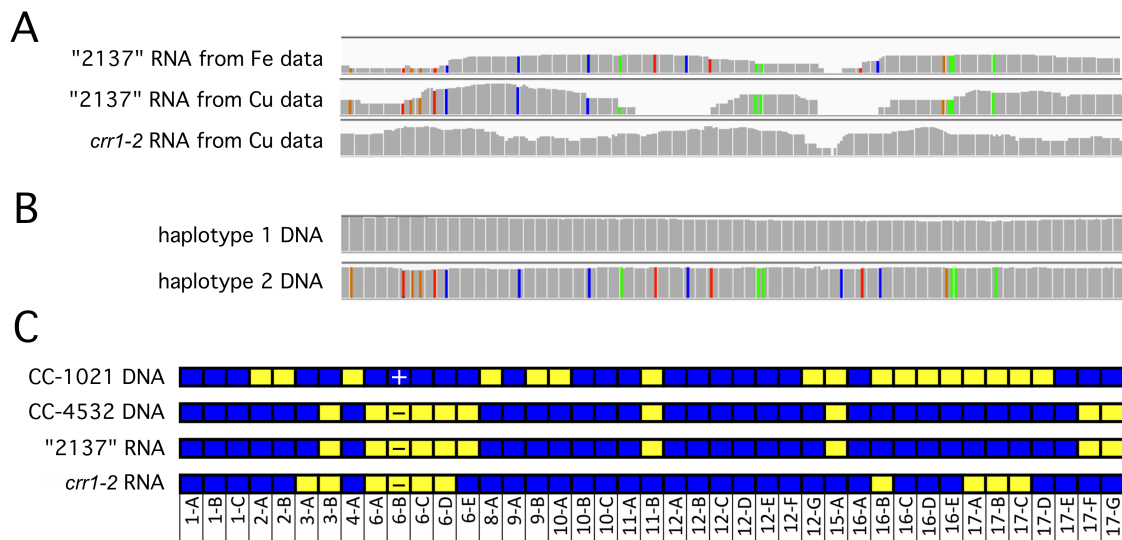


Supplemental Figure 8. Variants in Genes Attributable to Haplotype 2
The predicted effects of haplotype 2 variants on gene models are color coded according to the legend, and a black scale bar representing 1 kbp of DNA is included for each.



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.

Gene ID	Gene Name	Gene Description	Haplotype 2 Variant
Cre01.g009650	BUG25	Basal body protein and putative AP2 domain transcription factor	9112 bp deletion removes all of exon 1 and part of exon 2 in BUG25. 1 other gene is completely removed 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 nonsynonymous codons.
Cre01.g012800	FAP230	Flagellar Associated Protein	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	Dihydroliipoamide dehydrogenase	1 in frame InDel, 21 non-synonymous codons
Cre01.g044100	AMYB3	Beta-amylase	10 non-synonymous codons
Cre02.g113850	PBGD2 / HEM3	Porphobilinogen deaminase	2 frame shift InDels, 2 non-synonymous codons
Cre02.g118950	PRPS17	Plastid ribosomal protein S17	1 in frame InDel
Cre06.g252200	TOC34	Translocon at the outer envelope membrane of chloroplasts. 75 kD	14 non-synonymous codons
Cre06.g255450	MAT3	Retinoblastoma protein	6 non-synonymous codons
Cre06.g260450	LCI20	2-oxoglutarate/malate translocator	1 in frame InDel
Cre08.g360050	DUR1	Urea carboxylase/allophanate hydrolase	1 in frame InDel, 16 non-synonymous codons
Cre08.g360100	DUR2	Allophanate hydrolase	7 non-synonymous codons
Cre09.g386750	HSP90A	Heat shock protein 90A	1 frame shift
Cre09.g394200	FAP102	Flagellar Associated Protein	Insertion in 5th exon of FAP102.
Cre09.g395950	AOX1	Alternative oxidase	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	ACK1	Acetate kinase	9 non-synonymous codons
Cre09.g399400	FAP199 / TGL15	Triacylglycerol lipase	265 bp deletion removes most of exon 1.
Cre09.g403900	FAP294	Flagellar Associated Protein	12,661 bp deletion removes FAP294 and 2 other 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	CipD 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 / ARL11	ARF/SAR superfamily small monomeric GTP binding protein	27,736 bp deletion removes all of CGL49 and 3 other genes, and partially removes 1 other gene.
Cre12.g488500	ARC6	unannotated in Phytozome10	12 non-synonymous codons
Cre12.g490700	MIN1	Mini-eyespot protein	1 in frame InDel, 3 non-synonymous codons
Cre12.g495100	PSR1	Phosphorus starvation response protein, Myb-like transcriptional regulator	1 in frame InDel, 4 non-synonymous codons
Cre12.g547100	CGL2 / SMM43	S-adenosyl-L-methionine-dependent methyltransferase	23,595 bp deletion removes all of CGL2 and 4 other genes, and part of 1 other gene.
Cre12.g554250	LPB1	Low Photochemical Bleaching protein	2 in frame InDels, 7 non-synonymous codons
Cre12.g554300	NSS6	Sodium:solute symporter	5 in frame InDels, 24 non-synonymous codons
Cre16.g652400	FAL18	Similar to Flagellar Associated Protein FAP183	A 579 bp inversion reverses the orientation of exon 22. A second 762 bp inversion reverses the orientation of exon 23.
Cre16.g654150	FAP63	Flagellar Associated Protein	17,916 bp deletion removes 5' UTR of FAP63 and completely removes 4 other genes.
Cre17.g698850	ISA2	Isoamylase-type starch debranching enzyme	19,265 bp inversion disrupts ISA2 and 1 other gene. 2 other genes in reverse orientation. Also, 1 in frame InDel, 9 non-synonymous codons.
Cre17.g699500	TTLL9 / FAP267 / TTL8	Tubulin tyrosine ligase	2412 bp inversion disrupts TTLL9.
Cre17.g713200	OMT2	Chloroplast oxoglutarate-malate translocator	17,076 bp deletion removes part of OMT2 and completely removes 2 other genes.
Cre17.g720400	HMA1	Heavy metal transporting ATPase	5 in frame InDels, 24 non-synonymous codons
Cre17.g728900	FSA1 / TAL3	Transaldolase	22,593 bp deletion completely removes FSA1 and 1 other gene, and partially removes 2 other genes.
Cre17.g730100	RSEP1 / RSE1	Intramembrane metalloprotease	2 frame shifts, 2 in frame InDels, 21 non-synonymous codons
Cre17.g739650	MFT1 / MAE1	MATE efflux family protein	4 in frame InDels, 28 non-synonymous codons

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

Gene ID	Gene Name	GO Identifiers	GO Names
Cre01.g053450	CYA1	GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, signaling, single organism signaling
Cre02.g100500	CYG22	GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, signaling, single organism signaling
Cre02.g103200		GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, signaling, single organism signaling
Cre04.g217450		GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, signaling, single organism signaling
Cre05.g237800	CYG64	GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, signaling, single organism signaling
Cre06.g290200	CYG39	GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, signaling, single organism signaling
Cre07.g318551	CYA8	GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, signaling, single organism signaling
Cre07.g342350		GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, signaling, single organism signaling
Cre08.g362100		GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, signaling, single organism signaling
Cre08.g373200		GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, signaling, single organism signaling
Cre09.g386900		GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, signaling, single organism signaling
Cre10.g429750		GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, signaling, single organism signaling
Cre11.g467651		GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, signaling, single organism signaling
Cre13.g567800		GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, signaling, single organism signaling
Cre13.g605100		GO:0007154, GO:0007165, GO:0023052, GO:0044700	cell communication, signal transduction, 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
<i>pcc1-1</i>	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