GENETICS

Supporting Information
http://www.genetics.org/cgi/content/full/genetics.109.104497/DC1

Polymorphisms in Multiple Genes Contribute to the Spontaneous Mitochondrial Genome Instability of *Saccharomyces cerevisiae* S288C Strains

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DOI: 10.1534/genetics.109.104497

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Supporting Materials & Methods

Plasmid construction details: pLND44-4 was created by cloning into pRS306 (SIKORSKI and HIETER 1989) a fusion PCR product amplified from the mix of a left and a right PCR reaction as templates. The left PCR reaction used primers XhoI_*MIP1*_F (GTCTCG*CTCGAG*CCCGTAATATGGTCGAAGGA) and *MIP1*_661Thr_R (AACCACG**t**ATTTTCCACAGCTCTTCTAGTGATT), and S288C genomic DNA as template. The right PCR reaction used primers *MIP1*_661Thr_F (AATCACTAGAAGAGCTGTGGAAAAT**a**CGTGGTT) and EagI_SCD5_R (CGAGAC*CGGCCG*GATGGCCTCTTTTCTGCTTG), and S288C genomic DNA as template. The fusion PCR product was digested with *Xho*I and *Eag*I (italicized and underlined in XhoI_*MIP1*_F and EagI_SCD5_R) and was ligated into similarly digested pRS306. The *MIP1*_661Thr_R and *MIP1*_661Thr_F primers are responsible for introducing one nucleotide difference (bold lower case) from the S288C genome. This change replaces the GCG codon for alanine at the 661st position of *MIP1* in S288C with a threonine codon (**a**CG in the *MIP1*_661Thr_F primer). Sequence verification of pLND44-4 revealed that the cloned insert contains one additional nucleotide change 987 base pairs downstream of the intended single nucleotide change at the 661st amino acid of *MIP1*. This mutation was most likely introduced by the PCR amplification. During the pop-in/pop-out strategy for *MIP1* allele replacement with pLND44-4, we made sure that the pop-out recombination excised this secondary mutation out of the genomic DNA.

pRS306-*MKT1*-D30G was also created by cloning into pRS306 a fusion PCR product. The left PCR reaction used primers mkt1-1-SpeI (GATC*ACTAGT*ACCACCAAAACAGCTCATCAA) and mkt1-D30G-1 (CATAATGGTTGACGTCTATAcC**t**AGGGTACAATTATTCAG), and S288C genomic DNA as template. The right PCR reaction used primers mkt1-D30G-2 (CTGAATAATTGTACCCTaG**g**TATAGACGTCAACCATTATG) and mkt1-2-XhoI (GATC*CTCGAG*ATCAAACAGCTGAGGAACTGGA), and S288C genomic DNA as template. The fusion PCR product was digested with *Spe*I and *Xho*I (italicized and underlined) and was ligated into similarly digested pRS306. The mkt1-D30G-1 and mkt1-D30G-2 primers are responsible for introducing two nucleotide differences (lower case letters) from the S288C genome. One nucleotide change (bold lower case) changes the G**A**T codon for aspartate at the 30th amino acid position of *MKT1* in S288C into a glycine codon (G**g**T in the mkt1-D30G-2 primer). The other nucleotide change (non-bold lower case) changes the wobble position of the CTG codon for leucine at the 29th amino acid position of *MKT1* into another leucine codon (CTa in the mkt1-D30G-2 primer). While preserving the amino acid sequence of the protein, this second nucleotide change introduces a new *Avr*II restriction site (boxed) in this *MKT1-30G* allele to simplify genotyping. The insert in pRS306-*MKT1*-D30G was sequence verified and contains only these two additional nucleotide changes compared to the S288C sequence of *MKT1*.

Allelic replacement details: The *SAL1* and *CAT5* allelic replacements were executed by two sequential transformations as described in detail elsewhere (GRAY *et al.* 2004). Briefly, the first transformation integrates the *URA3* marker into the targeted locus (*sal1-1* or *CAT5-91I*). The second transformation replaces *URA3* with a PCR product, which in our case was amplified from RM11-1a genomic DNA.

For the replacement of the *sal1-1* allele, the PCR product was generated with primers *SAL1*_F2 (TTTACCTATTCAACGAAGATGTCG) and *SAL1*_R2 (AGCTGATGGAAACTGCTGGA). The RM11-1a sequence between these two primers differs from the S288C sequence only in the stretch 5'-GGTGGGCTC-3' for codons 401 through 403 in RM11-1a versus 5'-GGGGGGGGTC-3' in S288C (Figure 6A). Thus, in addition to reverting the frameshift mutation in S288C, our allele replacement also introduces a different codon for the glycine amino acid in the 401st position of Sal1p (GGT in RM11 versus GGG in S288C).

For the replacement of the *CAT5-91I* allele, the PCR product was generated with primers *CAT5*_F (GCAGAGGCTTTTCCGTCTTA) and i*CAT5*_R (TTTATCAAACCGTTTTCCTTTCA). The RM11-1a sequence between these two primers differs from the S288C sequence at only three nucleotides. Only one is a non-synonymous SNP that changes the amino acid at the 91st position of the Cat1p (Figure 6C). The other two are silent SNPs that do not change the amino acid sequence of the protein. Interestingly, these two silent SNPs cluster very closely to the 91st amino acid position – one alters the 85th codon for lysine (AAA in S288C versus AAG in RM11-a, the other alters the 89th codon for lysine (AAG in S288C versus AAA in RM11-1a). Therefore, our *CAT5* allele replacement changes the only amino acid difference between the S288C and RM11-1a Cat5p, namely the one at the 91st position (Figure 6C).

Colony counting by ImageJ: In order to count *petite* and *grande* colonies with ImageJ, YEPDG plates were scanned as a transparency with an Epson Expression 1680 scanner and SF Launcher v.2.1.0 software. The scan resolution was 300 dots per inch without a filter to generate a 16→8 bit greyscale tiff file, which was manipulated further in ImageJ. Using the "MultiThresholder" plug-in filter in ImageJ, the tiff files were converted to black and white, binary images. The "Watershed" function was run consecutively twice to maximize the separation of merged colonies. The "Analyze Particles" function was then used to count the colonies. The use of BD Falcon, 150x15mm plates (catalog number 35 1058) allowed us to apply the "Analyze Particles" function to the entire area of a plate because these plates do not contain additional edges on the bottom. The settings for the "Analyze Particles" function were: size (in pixels) between 5 and infinity; circularity between 0.60 and 1.00; excluding colonies that lay on the edges of the analyzed area. The "Analyze Particles" function returns data on the size of each colony and the total number of colonies. To count the number of *petite* colonies, the distribution of colony size was generated and a cut-off pixel size that readily distinguished

between small and large colonies was determined. That cut-off was used to count the number of *petite* colonies. In optimizing this protocol, we determined that the correlation between manually versus ImageJ counted plates with a broad range of median *petite* frequencies was extremely high ($R^2 = 0.9980$, data not shown).

OD doubling time measurements: OD doubling times were measured at 660nm in 96-well plates using a Powerwave XS plate reader (BioTek, Winooski, VT) using a modification of the protocol in (TOUSSAINT and CONCONI 2006; VEATCH *et al.* 2009). To follow the growth rate of the same culture for a long period of time, a series of seven four-fold dilutions of each original culture were made in final volumes of 100µl. The plates were grown at 30° with high, continuous shaking for 48 hours with absorbance reads every 10 minutes. OD doubling times of individual wells were calculated for values of the path-length-unadjusted absorbance between 0.0625 and 0.25. The OD doubling time was calculated by linear regression of the $\log_2$ transformed ODs using all data points within this specified range. Each strain was analyzed in biological triplicate.

Literature Cited for Supporting Materials & Methods

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SIKORSKI, R. S., and P. HIETER, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics **122:** 19-27.

TOUSSAINT, M., and A. CONCONI, 2006 High-throughput and sensitive assay to measure yeast cell growth: a bench protocol for testing genotoxic agents. Nat Protoc **1:** 1922-1928.

VEATCH, J. R., M. A. MCMURRAY, Z. W. NELSON and D. E. GOTTSCHLING, 2009 Mitochondrial dysfunction leads to nuclear genome instability: A link through iron-sulfur clusters. Cell**:** in press.

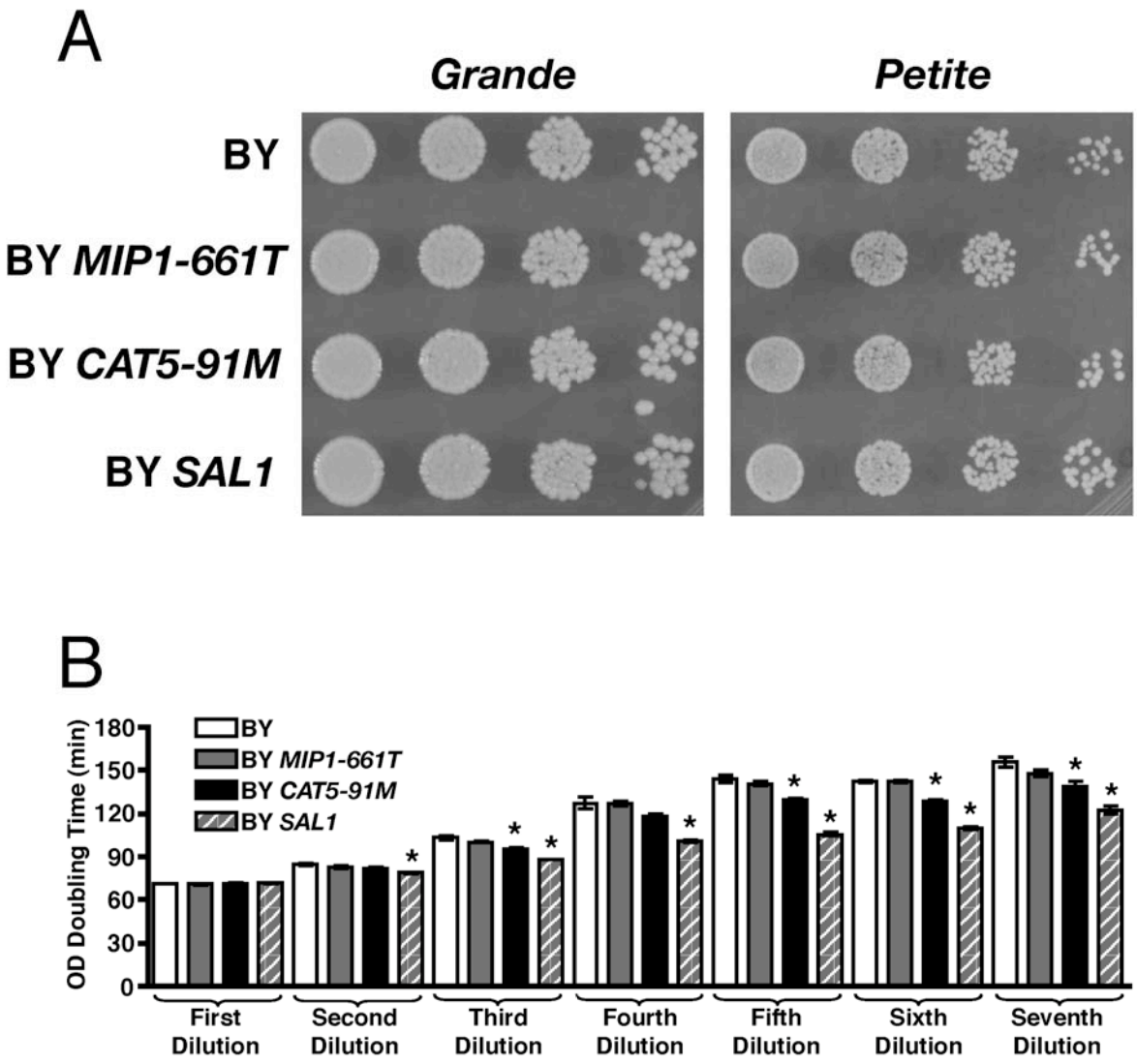

FIGURE S1.—The BY alleles of *SAL1*, *CAT5*, and *MIP1* do not affect growth of *petite*s. (A) Serial dilutions of *grande* (left) and *petite* (right) cells (see Materials & Methods) of the strains UCC8356, UCC8357, UCC8358, and UCC8360 were applied to YEPD plates. (B) Cultures of *petite* cells from the strains in (A) were generated as described in the Materials & Methods. Seven four-fold serial dilutions of each culture were analyzed in a Powerwave XS plate reader as described in Supporting Materials & Methods. Each bar represents the mean of three biological replicates for that strain. The error bars represent one standard error of the mean. The asterisks denote a statistically significant decrease (p-value < 0.05) in OD doubling time of the strain compared to the BY reference strain (UCC8356) in that particular dilution.

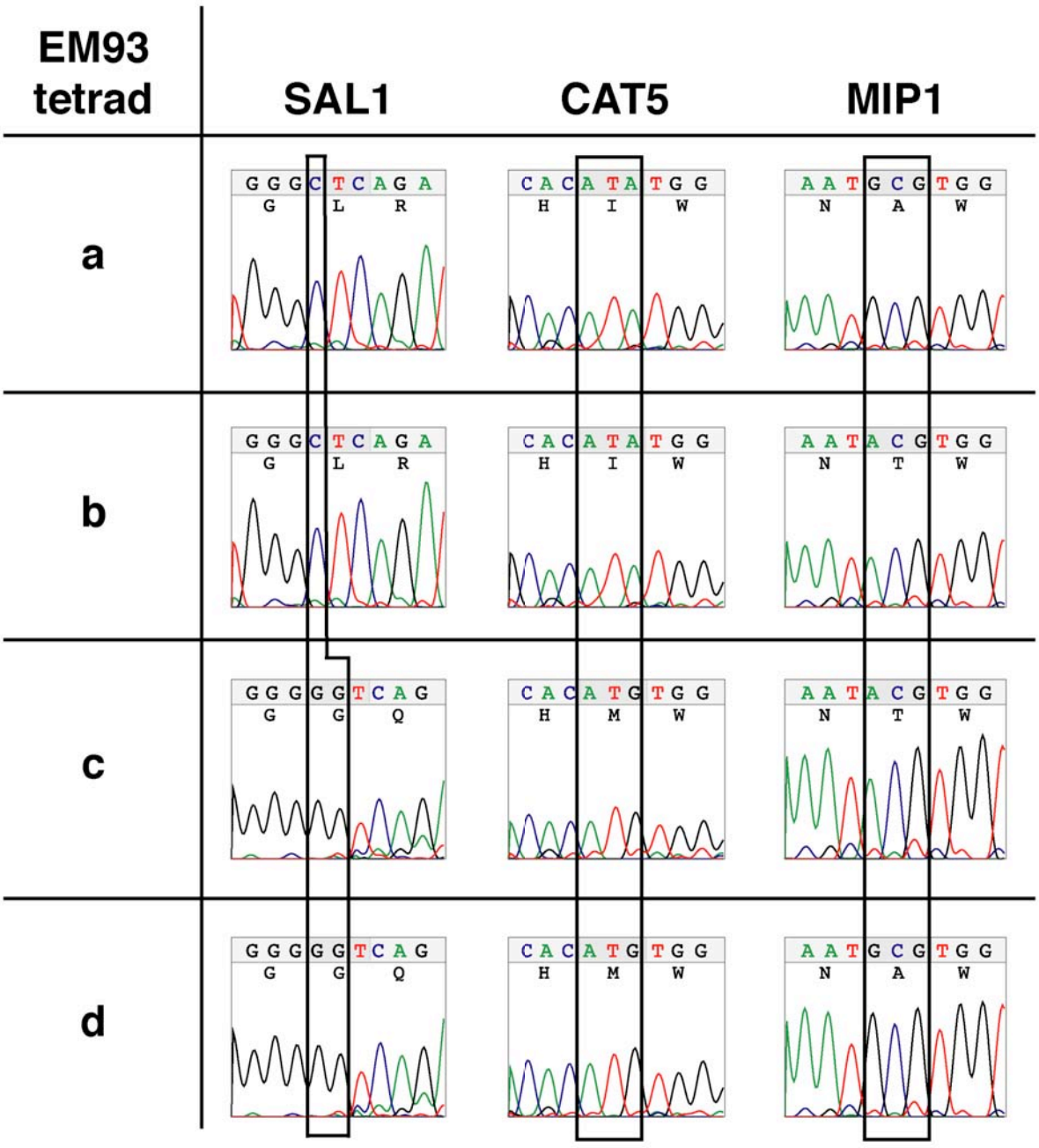

FIGURE S2.—EM93 is the source of the BY/S288C *sal1-1*, *CAT5-91I*, and *MIP1-661A* alleles. The diploid EM93 strain was sporulated. Each spore of a tetrad was sequenced for a region encompassing the relevant polymorphisms in the *SAL1*, *CAT5*, and *MIP1* genes. Each trace picture shows the relevant DNA (top) and protein (underneath) sequence. The black box in the *SAL1* column highlights the heterozygosity of the *sal1-1* frameshift mutation (present in spores c & d). The amino acid differences in *CAT5* and *MIP1* are also boxed in black.

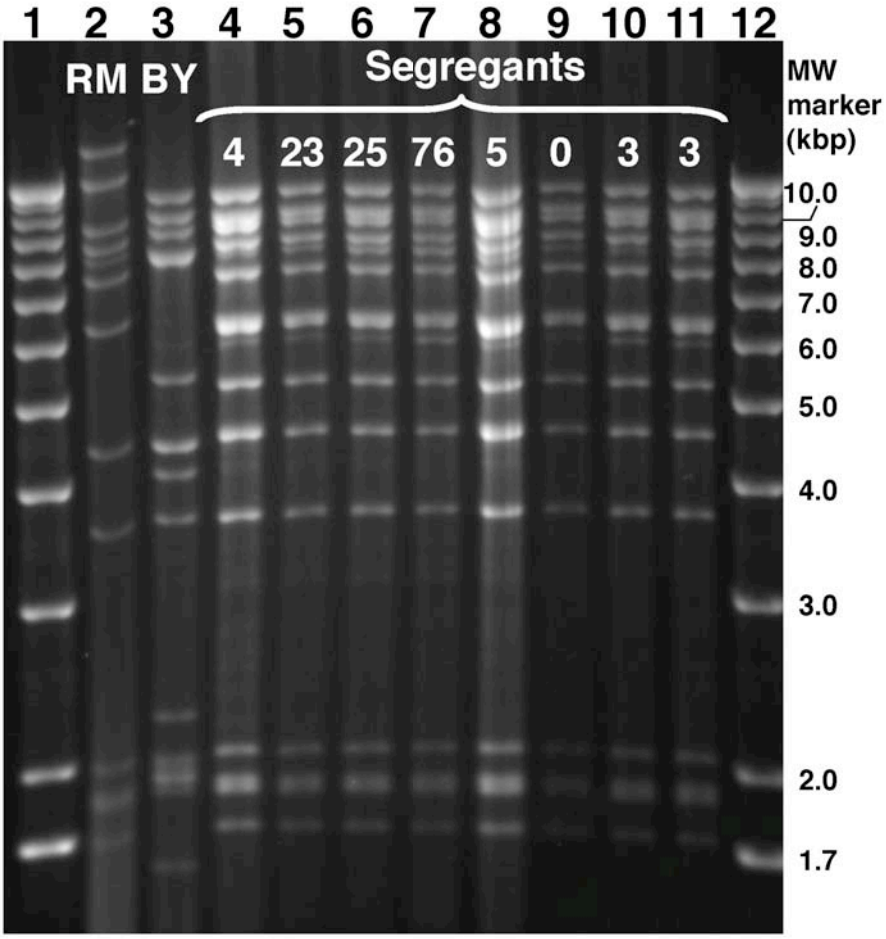

FIGURE S3.—Segregants of the BY x RM cross all have identical mtDNA, which is a recombinant version of the BY and RM mtDNAs. mtDNA was isolated from the two parents – BY4716 & RM11-1a, as well as from 8 segregants from the BY x RM cross whose median *petite* frequencies varied within a large range (indicated as a white number at the top of the lane). The mtDNA was digested with *Eco*RV and run out on a 0.6% agarose gel. Lanes 1 and 12 contain DNA size markers. Lane 2 and 3 contain the digested mtDNA of the RM11-1a and BY4716 parent, respectively. Lanes 4 through 11 contain the digested mtDNA of the 8 segregants.

TABLE S1

PCR and sequencing primers

Primer name	Sequence	Comments
MKT1_F2	TGGTGGAAAATCTGGAAAGC	Use with MKT1_R to PCR amplify and sequence the *MKT1-30G* or *MKT1-30D* allele
MKT1_R	TTCCATTGTGTCCAGCCTCT	See comments for MKT1_F2
SAL1_F	GGCTTTTTATGTTGGGAACG	Use with SAL1_R to PCR amplify and sequence the *sal1-1* or wild-type allele of *SAL1*
SAL1_R	GCATATGTTCCTTGGGCTTG	See comments for SAL1_F
CAT5_F2	CCTCCAAACCAATTCAAGGA	Use with iCAT5_R2 to PCR amplify the *CAT5* genomic locus. The PCR product is to be sequenced with CAT5_F to distinguish between the *CAT5-91M* and *CAT5-91I* allele
iCAT5_R2	TGAACCCATACCCCATTTACA	See comments for CAT5_F2
CAT5_F	GCAGAGGCTTTTCCGTCTTA	Use to sequence the PCR product generated with CAT5-F2 and iCAT5_R2
MIP1_F0	GGTGTTCCTGCAAAGTGTCA	Use with MIP1_R to sequence the *MIP1-661T* or *MIP1-661A* allele
MIP1_R	TTTGAGCAGTCTTCGTGTGC	See comments for MIP1_F0
COX1_A1_F	GGTATGGCAGGAACAGCAAT	Use with COX1_A2_R to check for the absence of intron aI1 in *COX1* in RM
COX1_A2_R	AGAAAATCATTAATACAGCATGACC AACTACTAAAA	See notes for COX1_A1_F
COX1_A5a_F	TGCTATGGCTTCAATTGGATT	Use with COX1_A6_R to check for the absence of introns aI5a, aI5b and aI5c in *COX1* in RM
COX1_A6_R	ATTTCATCCTGCGAAAGCAT	See notes to COX1_A5a_F
21S_rRNA_F	AGGTGTGAACCCCTCTTCG	Use with 21S_rRNA_R to check for the absence of the r1 (omega) intron in RM
21S_rRNA_R	CCATGGGTTGATTCATTATGG	See notes for 21S_rRNA_F
COX1_aI1_F	GCACAGGCAGTGTGAAAAAG	Use with COX1_aI1_R to confirm presence of intron aI1 in *COX1* in BY
COX1_aI1_R	TCTAAAACCATGTGAATGTGTTGA	See notes for COX1_aI1_F
COX1_aI5a_F	AAATCCCTTTAGCAAGGATAAAAA	Use with COX1_aI5a_R to confirm presence of intron aI5a in *COX1* in BY
COX1_aI5a_R	TCCACCTTTTACAAATGAACCA	See notes for COX1_aI5a_F
COX1_aI5b_F	GGCCCCCGAAACTAAAGATA	Use with COX1_aI5b_R to confirm presence of intron aI5b in *COX1* in BY
COX1_aI5b_R	CGGGCCGGACTAAAATATAA	See notes for COX1_aI5b_F
COX1_aI5c_F	TGCTCAACGAAAGTGAATCAA	Use with COX1_aI5c_R to confirm presence of intron aI5c in *COX1* in BY
COX1_aI5c_R	ACAAGTTTTCCCCCGGTAAG	See notes for COX1_aI5c_F
omega_F	ATTTACCCCCTTGTCCCATT	Use with omega_R to confirm presence of r1(omega) intron in BY
omega_R	CCAATACCTGCTTCAAATTGTTC	See notes for omega_F