

Supplementary Figures and Tables for:

Prevalence and Dynamics of Ribosomal DNA Micro-heterogeneity Are Linked to Population History in Two Contrasting Yeast Species

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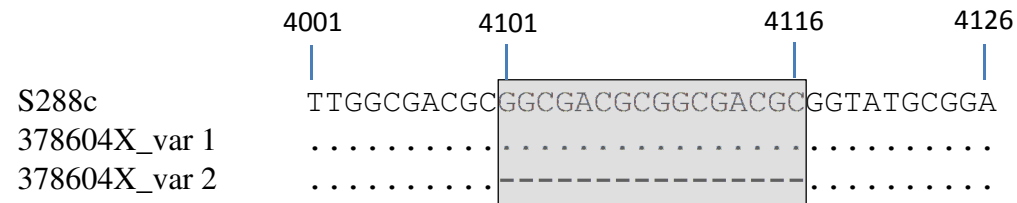
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Supplementary Figure S1: examples of partial insertions, deletions and complex variants

a) A Type 1 partial deletion in *S. cerevisiae*

Deletion variant identified in the *S. cerevisiae* IGS1 region (TURNIP alignment positions 4101 to 4116) of strain 378604X



Sequence Type	Tract length	Frequency
378604X_var 1 ^A	16	28.6% (14 reads)
378604X_var 2 ^B	0	71.4% (35 reads)

^A Identical in length to S288c (reference strain); ^B Dominant sequence variant

In *S. cerevisiae* strain 378604X, this 16 nucleotide tract has been deleted from approximately 70% of its rDNA units. Almost all of the strains are found to possess deletions in this area (see Supplementary Table S2). However, most are fixed deletions.

b) A Type 2 partial insertion in *S. paradoxus*

Variable length homopolymeric polyT tract found in the *S. paradoxus* IGS1 region (TURNIP alignment positions 3929 to 3937) of strain N-45



Sequence Type	Tract length	Frequency
N-45_var 1 ^A	9	6.1% (2 reads)
N-45_var 2	32	9.1% (3 reads)
N-45_var 3 ^B	33	84.8% (28 reads)

^A Identical in length to CBS 432 (reference strain); ^B Dominant sequence variant

In the reference strain, this homopolymeric tract comprises of 9 T residues. However, in both IFO 1804 and N-45 it appears to be variable in length. As we see above, in N-45 three different variants were detected, one identical in length to the reference strain, and two significantly longer (32 T's and 33 T's), with the longest length variant (33 T's) found in the majority of covering reads (28/33). In contrast, although significantly longer than the reference strain, this tract appears to be of fixed length in the other two Far Eastern strains (N-43, 23 T's; N-44, 26 T's).

c) Homopolymeric tracts

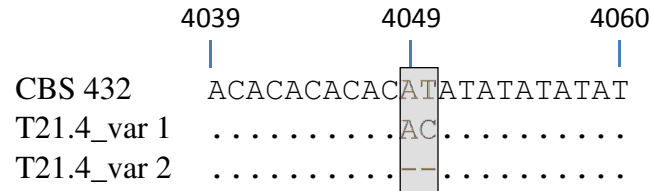
Location and size of the five largest IGS1 poly(dA).(dT) tracts in *S. cerevisiae* (S288c) and their equivalent counterparts in *S. paradoxus* (CBS 432)

Tract type:	Location		Length	
	S288c	CBS 432	S288c	CBS 432
polyT	3627-3642	3638-3653	16	16
polyA	3834-3841	3856-3861	8	6
polyT	3914-3935	3930-3938	22	9
polyT	4300-4316	Absent	17	0
polyA	4487-4515	4479-4495	29	17

In the *S. cerevisiae* reference strain S288c, these five tracts range from 8 to 29 residues in length. Some of these tract-specific deletions are fixed while others are partial variants. For example, in S288c we observe a 16 residue poly(dT) tract between base positions 3627 and 3642. In the soil strain DBVPG 1788, this same tract is shorter and only 13 residues in length, whereas in the beer spoilage strain NCYC 361, it exists in two variant forms, one identical in length to S288c (16 T residues) and present on the majority of covering reads, and a shorter variant (12 T residues) present on only six covering reads. These results indicate that not only can homopolymeric tracts vary in length between different rDNA arrays of the same species, but they can also vary in length between individual repeats of the same rDNA array (i.e. a Type 2 partial variant). In contrast in the *S. paradoxus* reference strain (CBS 432^T), there are only four equivalent poly(dA).poly(dT) tracts in the IGS1 region, and two of these are significantly shorter in length than their *S. cerevisiae* counterparts.

d) A complex mutation in *S. paradoxus*

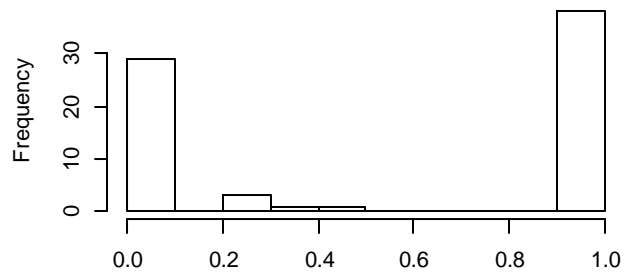
A SNP/DEL complex mutation at positions 4049 to 4050 was putatively identified in the European *S. paradoxus* strains T21.4 and Q59.1. A number of reads derived from each of the strains exhibit a ‘C’ nucleotide instead of a ‘T’ at position 4050 (adjacent to an ‘A’ at position 4049), with the remaining reads showing a deletion of positions 4049 and 4050. Crucially, a T→C SNP was identified at position 4050 of the closely related strain Y6.5. We therefore hypothesise that such a SNP was also harboured by T21.4 and Q59.1 (i.e. the polymorphism became fixed in an ancestor of T21.4, Q59.1 and Y6.5), with the deletion event occurring subsequently.



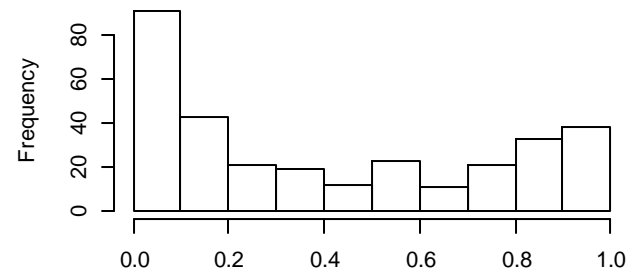
Sequence Type	Frequency
T21.4_var 1 ^A	34.9% (21 reads 4049 /23 reads 4050)
T21.4_var 2 ^B	65.1% (41 reads)

^A Identical in length to CBS 432 (reference strain); ^B Dominant sequence variant

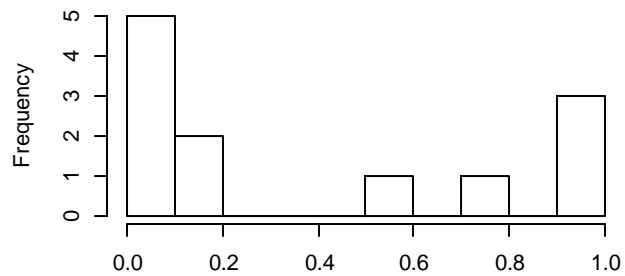
Supplementary Figure S2: Unit occupancy distributions



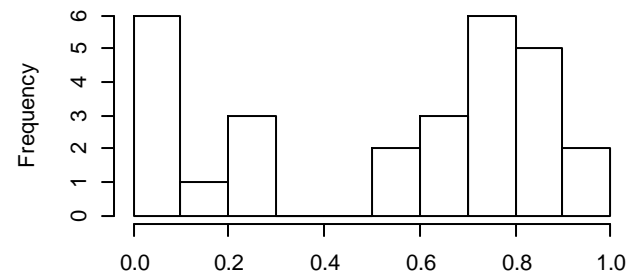
a) *S. paradoxus* pSNPs



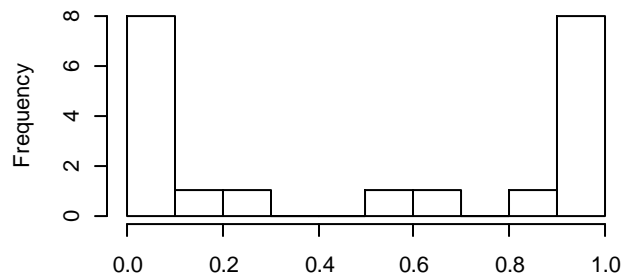
b) *S. cerevisiae* pSNPs



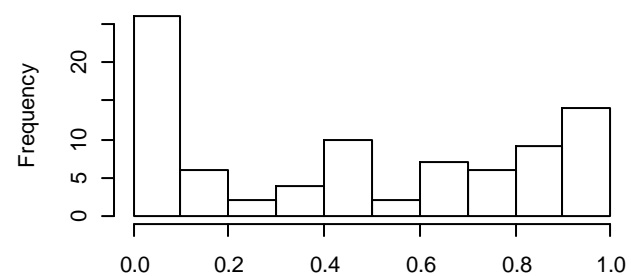
c) *S. paradoxus* pINSs



d) *S. cerevisiae* pINSs

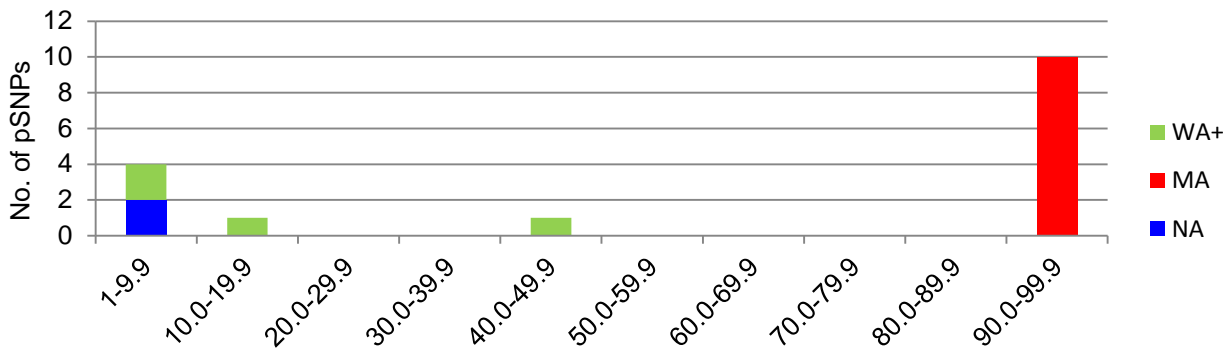


e) *S. paradoxus* pDELs

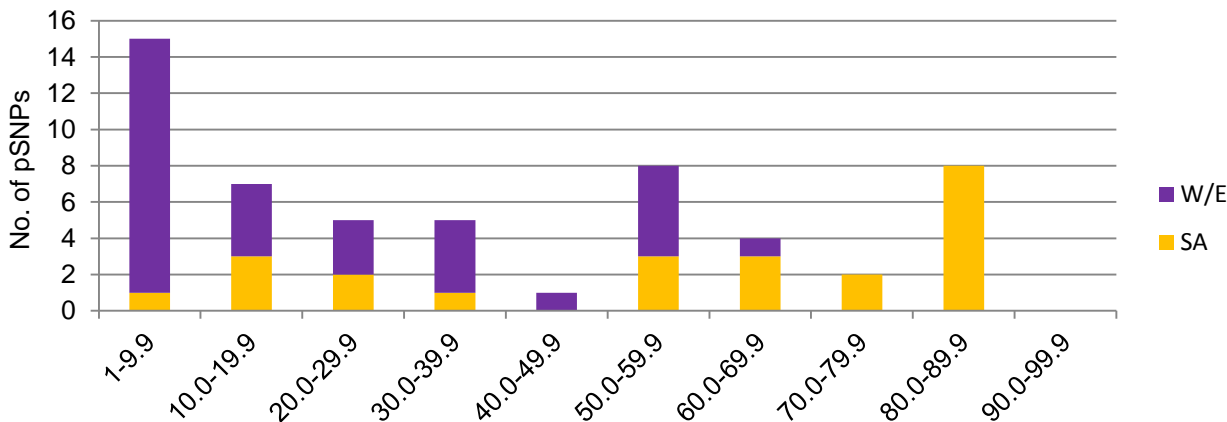


f) *S. cerevisiae* pDELs

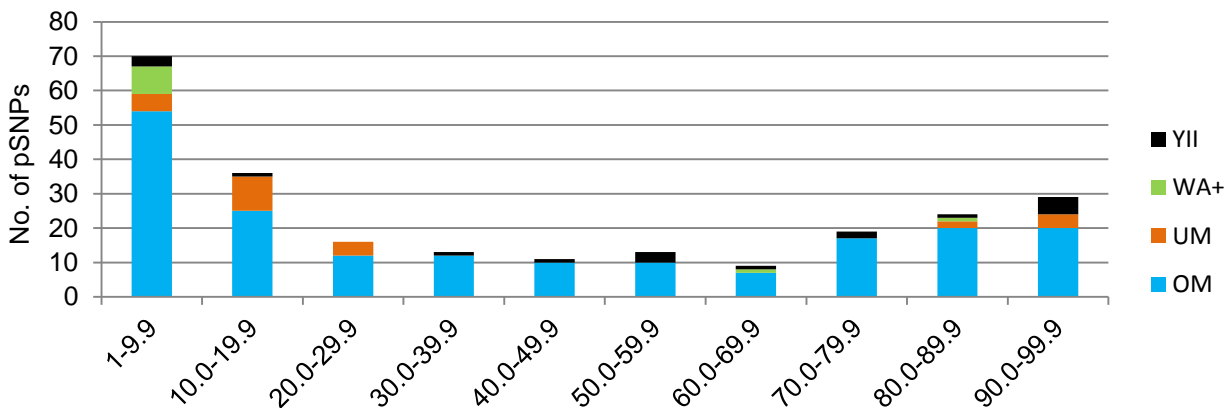
Supplementary Figure S3: Differing pSNP occupancy distributions between Structured clean, Structured mosaic and Mosaic strains of *Saccharomyces cerevisiae*



a) Percentage occupancies of 16 rDNA pSNPs in 7 Structured clean *Saccharomyces cerevisiae* strains

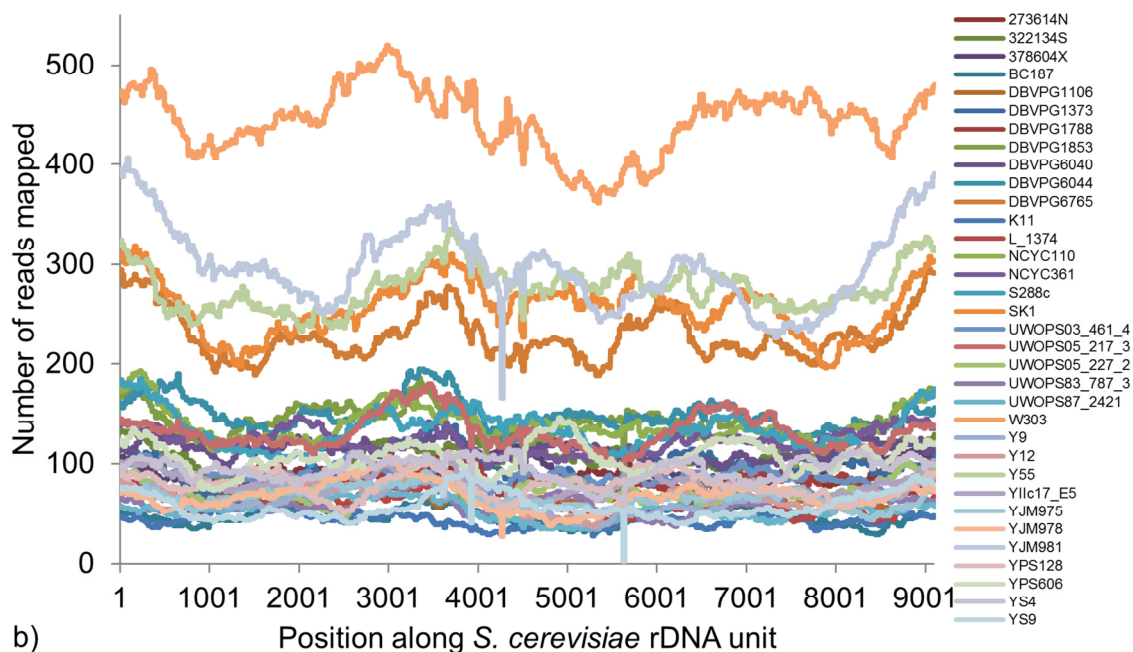
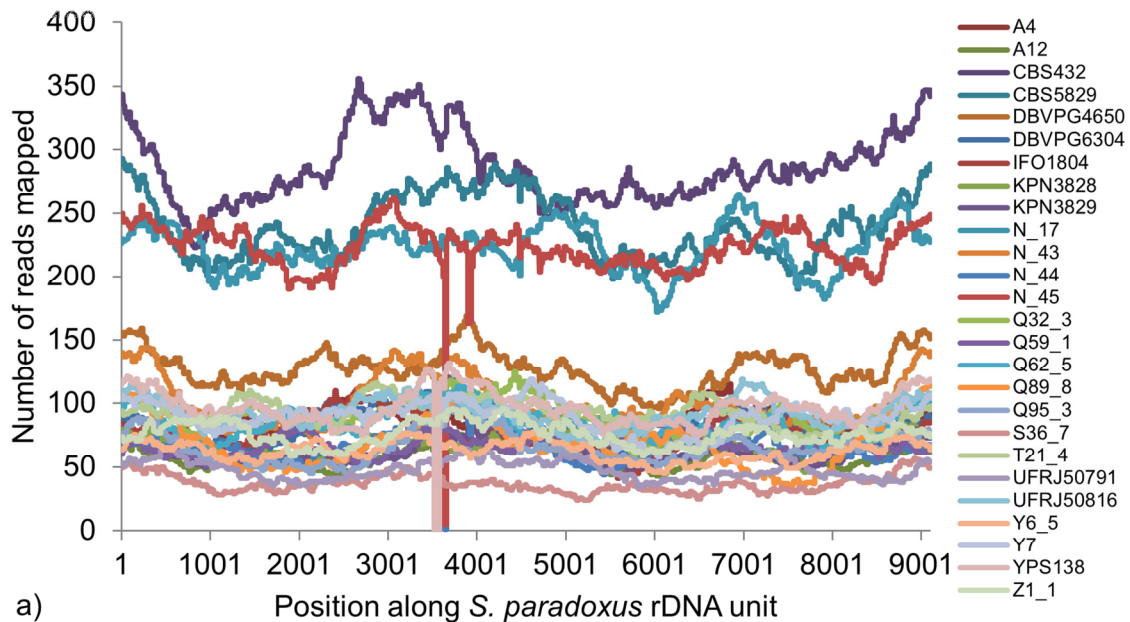


b) Percentage occupancies of 55 rDNA pSNPs in 12 Structured mosaic *Saccharomyces cerevisiae* strains



c) Percentage occupancies of 240 rDNA pSNPs in 15 Mosaic *Saccharomyces cerevisiae* strains

Supplementary Figure S4: Sequence read coverage



For the 26 *Saccharomyces paradoxus* strains, most coverage values were found to range from 40 to 100, though with four strains (CBS432, CBS5829, N-17 and N-45) ranging from 180 to 360. All six American strains and two Far Eastern strains (N-44 and N-45) were found to possess small (up to 20 bp) sections within the ETS2/IGS1 region that were either not covered or very poorly covered. The ETS2 and IGS1 regions have been shown to display high quantities of polymorphisms in *S. paradoxus* (see main text Fig. 3 and Table 4). When the 20 bp windows flanking these areas of poor/no coverage were examined in detail, it was discovered that all eight strains had SNPs, insertions and/or deletions on either side of these coverage anomalies. This implied that any reads spanning these areas were either too dissimilar to the reference consensus to pass the BLAST or multiple alignment filters within the read mapping procedure, or else carried large deletions. For example, all six American strains have consistently no coverage over one specific area (ETS2 region, positions 3520 to 3540, and 3560 to 3580), which would appear to be a feature of this group and its diversification from the species type strain, which derives from Europe. For the 34 *Saccharomyces cerevisiae* strains, the read coverage of most strains fell within the range 40 to 200, although five strains (W303, Y55, YJM981, SK1 and DBVPG6765) were found to possess a sequence read coverage of between 200 and 480. Two strains (the closely related YS4 and YS9) both exhibited only two or three mapped reads in a single small area, positions 5620-5639.

Supplementary Figure S5

```
#!/usr/bin/perl -w
```

```
#script to try blast against consensus sequences to remove contamination and reads from other areas of the genome
```

```
#v2, added scoring HSPs, only allowing those above min identity and % of original read length.
```

```
#v3 changed subs to be called from one, filter_strains. Added sub to remove all of the blast outfiles (as too many and too much space taken up),
```

```
#plus added sub to run formatdb on the filtered fasta output.
```

```
#used in James SA, West C, Davey RP, Dicks J and Roberts IN. Prevalence and Dynamics of Ribosomal DNA Micro-heterogeneity Are Linked to Population History in Two Contrasting Yeast Species.
```

```
use strict;
```

```
use warnings;
```

```
use Bio::SearchIO;
```

```
use Bio::Seq;
```

```
use Bio::SeqIO;
```

```
use Bio::Tools::Run::StandAloneBlast;
```

```
use Data::Dumper;
```

```
use File::Path;
```

```
use Cwd;
```

```
use File::Copy;
```

```
#variables
```

```
my $db_dir = "../database_files/";#location of blast database for consensus
```

```
my $strain_dir = "../strains/";#location of strains
```

```
my $db_name = "wrapped_Sc_consensus_sequence.fa";#consensus strain to compare to
```

```
my $strain_name;
```

```
my $strain_suffix = ".fasta";#file name suffix of strains
```

```
my $results_dir;
```

```
my @reads= ();
```

```
#specify names of strains to filter
```

```
my @strains
```

```
=("YJM789");#;"273614N","322134S","378604X","BC187","DBVPG1106","DBVPG1373","DBVPG1788","DBVPG1853","DBVPG6040","DBVPG6044","DBVPG6765","K11","L_1374","L_1528","NCYC110","NCYC361","S288c","SK1","UWOPS03_461_4","UWOPS05_217_3","UWOPS05_227_2","UWOPS83_787_3","UWOPS87_2421","W303","Y12","Y55","Y9","YIIc17_E5","YJM789","YJM975","YJM978","YJM981","YPS128","YPS606","YS2","YS4","YS9");
```

```
#;"A12","A4","CBS432","CBS5829","DBVPG4650","DBVPG6304","IFO1804","KPN3828","KPN3829","N_17","N_43","N_44","N_45","Q32_3","Q59_1","Q62_5","Q89_8","Q95_3","S36_7","T21_4","UFRJ50791","UFRJ50816","UWOPS91_917_1","Y6_5","Y7","YPS138","Z1_1"
```

```
my $min_read_len = 150;#min length of read
```

```
my $min_identity = 75;#min %identity of hit
```

```
my $min_percent = 75;#minimum % of original read involved
```

```
&filter_strains;
```

```
#run for all of the strains in the array
```

```
sub filter_strains
```

```
{
```

```
    foreach my $strain_name (@strains)
```

```
    {
```

```
        if(-e $strain_dir.$strain_name.$strain_suffix)#if the file exists
```

```

    {
        my $results_dir = "../results/$strain_name";

        if(!(-e $results_dir))
        {
            mkdir "../results/$strain_name";
        }

        &get_reads($strain_name);#read in sequence for that strain
        &blast_reads($results_dir);#blast against the consensus
        &rm_blast_dir($strain_name);#clear blast dir
        &format_fasta($strain_name);#make new filtered file into a blastdb
        @reads= ();#clear reads array
    }
}

#read in sequence file for a strain
sub get_reads
{
    my $the_strain = shift;
    my $seqio_obj = Bio::SeqIO->new(-file => $strain_dir.$the_strain.$strain_suffix, -format => "fasta" );

    while (my $seq_obj = $seqio_obj->next_seq)
    {
        print $seq_obj->id, "\n";

        #if read length is greater than minimum, store it
        if(length($seq_obj->seq)>=$min_read_len)
        {
            #store reads in an array
            push(@reads, $seq_obj);
        }
        else
        {
            print "Read".$seq_obj->id." not stored!!"
        }
    }

    my $rlen = @reads;
    print "There are $rlen reads in this strain\n";
}

#BLAST parameters and call blast (used in "blast_reads" sub)
sub get_blast {
    my $results_dir = shift;
    my $read_id = shift;
    my $input = shift;
    my $db = shift;
    my $scores = 2;

    #Inputs dna sequence string (any FASTA should work) and outputs record containing all BLAST alignments
    my $blast_outfile = "$results_dir/$read_id-blast.out";
    my @bparams = ('program' => 'blastn', 'database' => $db, 'outfile' => $blast_outfile);
    my $factory = Bio::Tools::Run::StandAloneBlast->new(@bparams);

```

```

#custom params
$factory->X(1); #x dropoff
$factory->G(3); #gap open pen
$factory->E(1); #gap extend pen
$factory->q(-1); #nucleotide mismatch pen
$factory->g("T"); #gapped alignment
$factory->e(1e-10); #evaluate
$factory->F("F"); #filters
$factory->a($scores); #CPUs/cores

my $blast_report = $factory->blastall($input);
if (!-e $blast_outfile) { die "Cannot open $blast_outfile! Cannot continue...\n"; }
else {
    my $blastObj = new Bio::SearchIO(-format => 'blast', -file => $blast_outfile);
    return $blastObj;
}
}

# blast each read from the strain using "get_blast" sub, writing to file all those which pass threshold of
#%id and length
sub blast_reads
{
    my $results_dir = shift;

    my $good = 0;

    #file to write to
    my $seqio_obj2 = Bio::SeqIO->new(-file => '>filtered.fasta', -format => 'fasta' );

    #for each read, blast
    for(my $i=0; $i<(@reads);$i++)
    {
        my $input = $reads[$i];
        my $read_id = $input->id;
        my $read_orig_len = length($input->seq);
        my $pass = 0;

        my $bl = get_blast($results_dir,$read_id, $input, $db_dir.$db_name);

        my $result = $bl->next_result();
        my @alignments = $result->hits;

        print "$i blast done\n";

        foreach my $alignment (@alignments)
        {
            my $hsp;
            while( my $h = $alignment->next_hsp())
            {
                if ($h->rank == 1) { $hsp = $h; }
                last;
            }

            if (defined $hsp)

```

```

        {
            #if hsp has > min percent identity and is > min size of read involved, set read to pass
            if ($hsp->percent_identity >= $min_identity && $hsp->length('query') >= (($read_orig_len/100)*
$min_percent) )
                {
                    $pass = 1;
                }
        }
    }#end of foreach

    if($pass==1)#if the read passes thresholds for %id and min size, write that seq to file
    {
        $good++;
        $seqio_obj2->write_seq($reads[$i]);
    }
}

#all reads done
print "$good reads passed\n";
}

#remove blast dir for the strain
sub rm_blast_dir
{
    my $strain_name = shift;
    my $dir = "../results/$strain_name";
    rmtree([$dir]);
    print "Blast results deleted for $strain_name \n";
}

#run formatdb on the fasta file
sub format_fasta
{
    my $strain_name = shift;
    #get the current working directory
    my $pwd = cwd();
    # print "$pwd - formatdb files to go in here\n";

    my $output_dir = "../output/$strain_name";

    if(!( -e $output_dir))
    {
        mkdir "../output/$strain_name";
        print "made output directory\n";
    }

    #copy output file into correct dir
    my $oldfile = "filtered.fasta";
    my $newlocation = "../output/$strain_name/$strain_name".'_filtered$strain_suffix';
    move($oldfile, $newlocation);

    #run formatdb
    system("formatdb", "-i",$newlocation,"-o","T", "-p","F");
}

```

Supplementary Table S1. *Saccharomyces paradoxus* and *Saccharomyces cerevisiae* strains

S. paradoxus strain	Source	Geographic location	Population
A4	Bark of <i>Quercus rubra</i>	Mont St-Hilaire, Quebec, Canada	American
A12	Soil beneath <i>Q. rubra</i>	Mont St-Hilaire, Quebec, Canada	American
CBS 432 ^{A,NT}	Bark of <i>Quercus</i> sp.	Moscow area, Russia	European
CBS 5829	Mor soil (pH 3.6)	Denmark	European
DBVPG 4650	Fossilized guano in a cavern	Marche, Italy	European
DBVPG 6304	<i>Drosophila pseudoobscura</i>	Yosemite, California, USA	American
IFO 1804	Bark of <i>Quercus</i> sp.	Japan	Far Eastern
KPN 3828	Bark of <i>Q. rubra</i>	Novosibirsk, Siberia, Russia	European
KPN 3829	Bark of <i>Q. rubra</i>	Novosibirsk, Siberia, Russia	European
N-17	Exudate of <i>Q. robur</i>	Tatarstan, Russia	European
N-43	Exudate of <i>Q. mongolica</i>	Vladivostok, Russia	Far Eastern
N-44	Exudate of <i>Q. mongolica</i>	Terney, Russia	Far Eastern
N-45	Exudate of <i>Q. mongolica</i>	Terney, Russia	Far Eastern
Q32.3	Bark of <i>Quercus</i> sp.	Windsor Great Park, UK	European
Q59.1	Bark of <i>Quercus</i> sp.	Windsor Great Park, UK	European
Q62.5	Bark of <i>Quercus</i> sp.	Windsor Great Park, UK	European
Q89.8	Bark of <i>Quercus</i> sp.	Windsor Great Park, UK	European
Q95.3	Bark of <i>Quercus</i> sp.	Windsor Great Park, UK	European
S36.7	Bark of <i>Quercus</i> sp.	Silwood Park, UK	European
T21.4	Bark of <i>Quercus</i> sp.	Silwood Park, UK	European
UFRJ 50791	<i>Drosophila</i> sp.	Catalao Point, Rio de Janeiro, Brazil	American
UFRJ 50816	<i>Drosophila</i> sp.	Tijuca Forest, Rio de Janeiro, Brazil	American
Y6.5	Bark of <i>Quercus</i> sp.	Silwood Park, UK	European
Y7.2	Bark of <i>Quercus</i> sp.	Silwood Park, UK	European
YPS138	Soil beneath <i>Q. velutina</i>	Pennsylvania, USA	American
Z1.1	Bark of <i>Quercus</i> sp.	Silwood Park, UK	European

^AReference strain; ^{NT}Neotype strain

S. cerevisiae strain	Source	Geographic location	Genome type^c	Lineage^c
27361N	Clinical isolate (fecal)	Royal Victoria Infirmary, Newcastle, UK	Mosaic	OM
322134S	Clinical isolate (Throat-sputum)	Royal Victoria Infirmary, Newcastle, UK	Mosaic	OM
378604X	Clinical isolate (Sputum)	Royal Victoria Infirmary, Newcastle, UK	Mosaic	OM
BC187	Barrel fermentation	Napa Valley, USA	Structured mosaic	W/E
DBVPG 1106	Grapes	Australia	Structured mosaic	W/E
DBVPG 1373	Soil	Netherlands	Structured mosaic	W/E
DBVPG 1788	Soil	Turku, Finland	Structured mosaic	W/E
DBVPG 1853	White Teff	Ethiopia	Mosaic	OM
DBVPG 6040	Fermenting fruit juice	Netherlands	Mosaic	OM
DBVPG 6044	Bili wine, from <i>Osbeckia grandiflora</i>	West Africa	Structured clean	WA+
DBVPG 6765	Unknown	Unknown	Structured mosaic	W/E
K11	Shochu sake strain	Japan	Structured mosaic	SA
L_1374	Fermentation from must Pais	Cauquenes, Chile	Structured mosaic	W/E
NCYC 110	Ginger beer from <i>Z. officinale</i>	West Africa	Structured clean	WA+
NCYC 361	Beer spoilage strain from wort	Ireland	Mosaic	OM
S288c ^{A,B}	Rotting fig	Merced, California, USA	Mosaic	OM
SK1 ^B	Soil	USA	Mosaic	WA+
UWOPS03-461-4	Nectar, Bertram palm	Telok Senangin, Malaysia	Structured clean	MA
UWOPS05-217-3	Nectar, Bertram palm	Telok Senangin, Malaysia	Structured clean	MA
UWOPS05-227-2	Stingless bee (<i>Trigona</i> sp.)	Telok Senangin, Malaysia	Structured clean	MA
UWOPS83-787-3	Fruit, <i>Opuntia stricta</i>	Great Inagua Island, Bahamas	Mosaic	UM
UWOPS87-2421	Cladode, <i>Opuntia megacantha</i>	Puhelu Road, Maui, Hawaii	Mosaic	UM
W303 ^B	Laboratory generated	NA	Mosaic	OM
Y12	Palm wine strain	Ivory Coast	Structured mosaic	SA
Y55 ^B	Grape	France	Mosaic	WA+
Y9	Ragi (similar to sake wine)	Indonesia	Structured mosaic	SA
YIIc17_E5	Wine	Sauternes, France	Mosaic	YII
YJM975	Vaginal isolate from patient with vaginitis	Ospedali Riuniti di Bergamo, Italy	Structured mosaic	W/E
YJM978	Vaginal isolate from patient with vaginitis	Ospedali Riuniti di Bergamo, Italy	Structured mosaic	W/E
YJM981	Vaginal isolate from patient with vaginitis	Ospedali Riuniti di Bergamo, Italy	Structured mosaic	W/E
YPS128	Soil beneath <i>Quercus alba</i>	Pennsylvania, USA	Structured clean	NA
YPS606	Bark of <i>Q. rubra</i>	Pennsylvania, USA	Structured clean	NA
YS4	Baker's strain	Netherlands	Mosaic	OM
YS9	Baker's strain	Singapore	Mosaic	OM

^AReference strain; ^BLaboratory strain

^CClassification according to West *et al.* (2014) [13]: MA [Malaysian], NA [North American], SA [Sake], W/E [Wine/European], WA+ [West African + other mosaics], YII [strain YIIc17-E5], UM [UWOPS mosaics] or OM [Other Mosaics]

Supplementary Table S2: SNPs and pSNPs in *Saccharomyces paradoxus* strains

Position	Region	No. of strains	SNPs	pSNPs	Strains
248	26S	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
543	26S	1	1	0	N-43
1174	26S	1	0	1	N-45
3456	ETS2	5	3	2	N-17 IFO1804 N-44 N-43 N-45
3517	ETS2	2	2	0	UFRJ50816 DBVPG6304
3547	ETS2	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3548	ETS2	5	5	0	Q62.5 IFO1804 N-44 N-43 N-45
3549	ETS2	4	4	0	IFO1804 N-44 N-43 N-45
3550	ETS2	4	4	0	A4 YPS138 UFRJ50791 DBVPG6304
3551	ETS2	4	3	1	IFO1804 N-44 N-43 N-45
3553	ETS2	2	2	0	A12 UFRJ50816
3554	ETS2	4	4	0	A4 YPS138 UFRJ50791 DBVPG6304
3555	ETS2	4	4	0	A4 YPS138 UFRJ50791 DBVPG6304
3556	ETS2	4	4	0	A4 YPS138 UFRJ50791 DBVPG6304
3557	ETS2	4	4	0	A4 YPS138 UFRJ50791 DBVPG6304
3558	ETS2	8	6	2	Q62.5 Q59.1 A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3559	ETS2	8	6	2	Q62.5 Q59.1 A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3586	ETS2	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3587	ETS2	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3595	ETS2	5	5	0	A12 A4 UFRJ50816 UFRJ50791 DBVPG6304
3599	ETS2	1	1	0	YPS138
3601	ETS2	1	1	0	YPS138
3603	ETS2	1	1	0	YPS138
3619	IGS1	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3631	IGS1	1	0	1	N-17
3635	IGS1	3	3	0	UFRJ50816 YPS138 DBVPG6304
3637	IGS1	2	2	0	A12 DBVPG6304
3638	IGS1	1	1	0	DBVPG6304
3643	IGS1	3	3	0	A12 A4 YPS138
3645	IGS1	1	0	1	DBVPG6304
3667	IGS1	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3668	IGS1	2	2	0	UFRJ50816 UFRJ50791
3691	IGS1	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3728	IGS1	5	3	2	N-17 IFO1804 N-44 N-43 N-45
3746	IGS1	5	3	2	N-17 IFO1804 N-44 N-43 N-45
3747	IGS1	5	3	2	N-17 IFO1804 N-44 N-43 N-45
3759	IGS1	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3760	IGS1	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3769	IGS1	2	2	0	UFRJ50816 UFRJ50791
3775	IGS1	11	9	2	N-17 IFO1804 N-44 N-43 N-45 A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3818	IGS1	5	3	2	N-17 IFO1804 N-44 N-43 N-45
3821	IGS1	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3824	IGS1	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3842	IGS1	5	3	2	N-17 IFO1804 N-44 N-43 N-45
3925	IGS1	1	1	0	DBVPG6304
3926	IGS1	1	1	0	DBVPG6304
3927	IGS1	1	1	0	DBVPG6304
3944	IGS1	1	1	0	Y7.2
3960	IGS1	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3967	IGS1	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3995	IGS1	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
4014	IGS1	3	3	0	UFRJ50816 UFRJ50791 DBVPG6304
4024	IGS1	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
4028	IGS1	1	0	1	N-17
4041	IGS1	1	0	1	N-43
4046	IGS1	3	3	0	A4 YPS138 DBVPG6304
4048	IGS1	6	6	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304

Supplementary Table S2: pSNP frequencies in *Saccharomyces paradoxus* strains

Position Region	pSNPs	Strains	Reference	Variants	Frequencies
1174 26S	1	N-45	192	2	0.010
3456 ETS2	2	N-17 N-45	182	3	0.016
			4	124	0.969
3551 ETS2	1	N-45	4	92	0.958
3558 ETS2	2	Q62.5 Q59.1	55	2	0.035
			34	2	0.056
3559 ETS2	2	Q62.5 Q59.1	55	2	0.035
			34	2	0.056
3631 IGS1	1	N-17	182	3	0.016
3645 IGS1	1	DBVPG6304	3	50	0.943
3728 IGS1	2	N-17 N-45	188	2	0.011
			4	85	0.955
3746 IGS1	2	N-17 N-45	183	2	0.011
			3	96	0.970
3747 IGS1	2	N-17 N-45	184	2	0.011
			3	96	0.970
3775 IGS1	2	N-17 N-45	180	2	0.011
			4	51	0.927
3818 IGS1	2	N-17 N-45	181	4	0.022
			3	78	0.963
3842 IGS1	2	N-17 N-45	183	4	0.021
			3	119	0.975
4028 IGS1	1	N-17	148	5	0.033
4041 IGS1	1	N-43	60	2	0.032
4050 IGS1	1	DBVPG4650	67	36	0.350
4052 IGS1	1	CBS5829	5	207	0.976
4054 IGS1	1	CBS5829	5	206	0.976
4056 IGS1	1	CBS5829	7	205	0.967
4067 IGS1	2	DBVPG4650 DBVPG6304	47	19	0.288
			64	2	0.030

4068 IGS1	1	DBVPG4650		47	19	0.288
4296 IGS1	2	Q59.1	DBVPG4650	48	2	0.040
				70	29	0.293
4378 IGS1	1	N-45		6	107	0.947
4379 IGS1	1	N-45		6	92	0.939
4380 IGS1	1	N-45		6	91	0.938
4385 IGS1	1	N-45		6	99	0.943
4454 IGS1	1	N-45		4	122	0.968
4655 IGS2	1	N-45		5	146	0.967
4679 IGS2	1	N-45		6	140	0.959
4684 IGS2	1	N-45		7	138	0.952
4735 IGS2	1	N-45		6	140	0.959
4792 IGS2	1	N-45		5	154	0.969
4879 IGS2	1	N-45		3	148	0.980
5011 IGS2	1	KPN3828		63	3	0.045
5126 IGS2	1	N-45		3	122	0.976
5325 IGS2	1	N-45		3	122	0.976
5334 IGS2	1	N-45		3	132	0.978
5347 IGS2	2	N-17	N-45	179	2	0.011
				4	124	0.969
5435 IGS2	1	N-45		4	124	0.969
5634 IGS2	1	N-45		2	97	0.980
5642 IGS2	1	N-45		2	92	0.979
5644 IGS2	1	N-45		3	92	0.968
5817 IGS2	1	N-45		147	3	0.020
5825 IGS2	1	N-45		14	128	0.901
5846 IGS2	1	N-45		2	136	0.986
6045 ETS1	3	Q59.1	N-17 N-45	56	3	0.051
				2	136	0.986
				175	2	0.011
6104 ETS1	1	Q59.1		31	24	0.436
6296 ETS1	2	N-17	N-45	162	2	0.012
				2	121	0.984

6321 ETS1	1	N-45		2	128	0.985
6436 ETS1	1	KPN3829		43	2	0.044
6510 ETS1	1	N-45		2	131	0.985
8377 ITS1	1	N-44		46	3	0.061
8528 ITS1	1	N-17		191	2	0.010
8532 ITS1	2	N-17	N-45	189	2	0.010
				4	125	0.969
8619 ITS1	1	N-17		206	2	0.010
8951 ITS2	1	N-17		187	3	0.016
	72					

Supplementary Table S2: INsS and pINsS in *Saccharomyces paradoxus* strains

Position	Region	No. of strains	INsS	pINsS	Type 1 pINsS	Type 2 pINsS	Strains
3515	ETS2	1	1	0	0	0	UFRJ50791
3517	ETS2	3	3	0	0	0	A12 A4 YPS138
3518	ETS2	1	1	0	0	0	DBVPG6304
3519	ETS2	2	2	0	0	0	A12 UFRJ50816
3547	ETS2	11	11	0	0	0	Q89.8 Q32.3 S36.7 Z1.1 T21.4 Q59.1 CBS432 DBVPG4650 CBS5829 KPN3828 KPN3829
3548	ETS2	3	3	0	0	0	Q95.3 Y7.2 Y6.5
3549	ETS2	1	1	0	0	0	Q62.5
3558	ETS2	4	3	1	0	1	IFO1804 N-44 N-43 N-45
3626	IGS1	1	1	0	0	0	A4
3627	IGS1	1	1	0	0	0	UFRJ50791
3631	IGS1	2	2	0	0	0	A4 UFRJ50791
3635	IGS1	3	3	0	0	0	A12 A4 UFRJ50791
3636	IGS1	2	2	0	0	0	UFRJ50816 YPS138
3637	IGS1	2	2	0	0	0	A4 UFRJ50791
3638	IGS1	3	1	2	2	0	Q95.3 T21.4 A12
3643	IGS1	1	1	0	0	0	UFRJ50791
3645	IGS1	1	0	1	1	0	UFRJ50816
3646	IGS1	1	0	1	1	0	Y6.5
3647	IGS1	1	0	1	1	0	DBVPG6304
3748	IGS1	4	3	1	1	0	CBS432 CBS5829 KPN3829 N-17
3754	IGS1	2	1	1	1	0	DBVPG4650 KPN3828
3761	IGS1	1	1	0	0	0	N-43
3855	IGS1	3	3	0	0	0	A12 UFRJ50791 DBVPG6304
3856	IGS1	2	2	0	0	0	A4 YPS138
3861	IGS1	1	1	0	0	0	YPS138
3910	IGS1	3	1	2	2	0	N-17 N-44 N-45
3915	IGS1	1	1	0	0	0	N-43
3925	IGS1	4	4	0	0	0	A12 A4 YPS138 UFRJ50791
3927	IGS1	1	1	0	0	0	A4
3929	IGS1	7	5	2	0	2	IFO1804 N-43 N-45 A12 UFRJ50816 UFRJ50791 DBVPG6304
3938	IGS1	2	2	0	0	0	N-44 YPS138
3969	IGS1	6	6	0	0	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
4011	IGS1	1	1	0	0	0	DBVPG6304
4033	IGS1	1	0	1	1	0	Q59.1
4041	IGS1	1	1	0	0	0	N-45
4050	IGS1	1	1	0	0	0	N-17
4052	IGS1	2	1	1	1	0	Q59.1 N-17
4071	IGS1	1	0	1	1	0	N-45
4252	IGS2	1	1	0	0	0	UFRJ50816
5642	IGS2	5	5	0	0	0	A12 A4 UFRJ50816 YPS138 DBVPG6304
5644	IGS2	1	1	0	0	0	IFO1804
		95	80	15	12	3	

Supplementary Table S2: pINS frequencies in *Saccharomyces paradoxus* strains

Position Region	Type 1 pINSs	Strains	Reference	Variants	Frequencies
3638 IGS1	2	Q95.3 T21.4	37	2	0.051
			57	12	0.174
3645 IGS1	1	UFRJ50816	10	40	0.800
3646 IGS1	1	Y6.5	14	15	0.517
3647 IGS1	1	DBVPG6304	4	50	0.926
3748 IGS1	1	N-17	181	5	0.027
3754 IGS1	1	DBVPG4650	85	19	0.183
3910 IGS1	2	N-17 N-45	184	2	0.011
			3	60	0.952
4033 IGS1	1	Q59.1	34	3	0.081
4052 IGS1	1	Q59.1	41	2	0.047
4071 IGS1	1	N-45	2	50	0.962
	12				

Supplementary Table S2: DELs and pDELs in *Saccharomyces paradoxus* strains

Start position	End position	Region	No. of strains	DELs	pDELs	Type 1 pDELs	Type 2 pDELs	Strains
3516	3517	ETS2	7	4	3	3	0	CBS432 DBVPG4650 KPN3828 N-17 IFO1804 N-44 N-43
3519	3519	ETS2	1	1	0	0	0	KPN3829
3521	3522	ETS2	2	1	1	1	0	N-43 N-45
3540	3544	ETS2	6	6	0	0	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
3554	3554	ETS2	2	2	0	0	0	A12 UFRJ50816
3554	3557	ETS2	1	0	1	1	0	N-17
3557	3557	ETS2	2	2	0	0	0	A12 UFRJ50816
3592	3596	ETS2	1	1	0	0	0	YPS138
3596	3603	ETS2	6	5	1	1	0	N-44 N-43 N-45 A12 UFRJ50816 DBVPG6304
3597	3603	ETS2	1	1	0	0	0	IFO1804
3597	3604	ETS2	1	1	0	0	0	A4
3598	3603	ETS2	1	1	0	0	0	UFRJ50791
3598	3606	ETS2	1	0	1	1	0	N-17
3604	3604	ETS2	1	1	0	0	0	YPS138
3624	3625	IGS1	3	3	0	0	0	A12 UFRJ50816 DBVPG6304
3624	3639	IGS1	2	1	1	1	0	N-44 N-45
3624	3640	IGS1	1	1	0	0	0	N-43
3624	3645	IGS1	1	1	0	0	0	IFO1804
3627	3628	IGS1	1	1	0	0	0	YPS138
3632	3641	IGS1	1	0	1	1	0	N-17
3634	3634	IGS1	1	1	0	0	0	UFRJ50791
3638	3641	IGS1	3	2	1	0	1	CBS432 KPN3828 KPN3829
3640	3641	IGS1	1	1	0	0	0	CBS5829
3751	3754	IGS1	6	6	0	0	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
4033	4034	IGS1	2	0	2	2	0	T21.4 Y6.5
4043	4050	IGS1	1	1	0	0	0	CBS432
4049	4050	IGS1	6	3	3	2	1	Q32.3 S36.7 Q95.3 Y7.2 Z1.1 Q62.5
4061	4063	IGS1	1	0	1	0	1	DBVPG6304
4063	4063	IGS1	4	4	0	0	0	A12 A4 UFRJ50816 UFRJ50791
4068	4068	IGS1	3	3	0	0	0	A12 UFRJ50816 UFRJ50791
4206	4215	IGS1	1	0	1	1	0	DBVPG4650
4474	4480	IGS1	1	1	0	0	0	UFRJ50816
4475	4480	IGS1	1	1	0	0	0	UFRJ50791
4478	4478	IGS1	4	1	3	3	0	Y6.5 DBVPG4650 KPN3828 KPN3829
4478	4480	IGS1	2	0	2	1	1	N-44 N-45
4478	4481	IGS1	2	2	0	0	0	IFO1804 N-43
4478	4482	IGS1	1	1	0	0	0	A4
4480	4482	IGS1	2	2	0	0	0	A12 DBVPG6304
4480	4483	IGS1	1	1	0	0	0	YPS138
4539	4539	5S	1	0	1	1	0	N-17
4710	4712	IGS2	6	6	0	0	0	A12 A4 UFRJ50816 YPS138 UFRJ50791 DBVPG6304
6330	6330	ETS1	1	0	1	1	0	N-45
8950	8950	ITS2	1	0	1	1	0	N-17
			94	69	25	21	4	

Supplementary Table S2: pDEL frequencies in *Saccharomyces paradoxus* strains

Start position	End position	Region	Type	1 pDELs	Strains	Reference	Variants	Frequencies
3516	3517	ETS2	3		CBS432 DBVPG4650 N-17	3	169	0.983
						59	18	0.234
						143	6	0.040
3521	3522	ETS2	1		N-45	2	95	0.979
3554	3557	ETS2	1		N-17	116	3	0.025
3596	3603	ETS2	1		N-45	4	98	0.961
3598	3606	ETS2	1		N-17	189	4	0.021
3624	3639	IGS1	1		N-45	4	130	0.970
3632	3641	IGS1	1		N-17	180	3	0.016
4033	4034	IGS1	2		T21.4 Y6.5	20	31	0.608
						6	27	0.818
4049	4050	IGS1	2		Y7.2 Q62.5	3	66	0.957
						2	54	0.964
4206	4215	IGS1	1		DBVPG4650	37	51	0.580
4478	4478	IGS1	3		Y6.5 DBVPG4650 KPN3829	44	4	0.083
						59	13	0.181
						3	35	0.921
4478	4480	IGS1	1		N-45	3	96	0.970
4539	4539	5S	1		N-17	178	2	0.011
6330	6330	ETS1	1		N-45	136	8	0.056
8950	8950	ITS2	1		N-17	189	2	0.010
			21					

Supplementary Table S2: Complex variants in *Saccharomyces paradoxus* strains

Strain	Start position	End position	Region	Details
T21.4	4049	4050	IGS1	Likely a fixed SNP at 4050 t --> c (as for Y6.5) followed by a pDEL of 4049-4050
Q59.1	4049	4050	IGS1	Likely a fixed SNP at 4050 t --> c (as for Y6.5) followed by a pDEL of 4049-4050
N_17	3547	3547	ETS2	Likely a fixed insertion followed by a pSNP
A4	4068	4068	IGS1	Likely a fixed SNP at 4068 t --> a followed by a pDEL
DBVPG6304	4068	4068	IGS1	Likely a fixed SNP at 4068 t --> a followed by a pDEL

Supplementary Table S2: All variants in *Saccharomyces paradoxus* strains

Strain	SNPs	pSNPs	INSs	pINSs	DELs	pDELs	CX	Total
Q89.8	0	0	1	0	0	0	0	1
Q32.3	0	0	1	0	0	1	0	2
S36.7	0	0	1	0	1	0	0	2
Q95.3	0	0	1	1	1	0	0	3
Y7.2	1	0	1	0	0	1	0	3
Z1.1	1	0	1	0	1	0	0	3
T21.4	0	0	1	1	0	1	1	4
Y6.5	1	0	1	1	0	2	0	5
Q62.5	2	2	1	0	0	1	0	6
Q59.1	0	5	1	2	0	0	1	9
CBS432	5	0	2	0	1	2	0	10
DBVPG4650	2	4	1	1	0	3	0	11
CBS5829	6	3	2	0	1	0	0	12
KPN3828	7	1	2	0	3	0	0	13
KPN3829	7	1	2	0	2	1	0	13
N-17	1	16	2	2	0	6	1	28
IFO1804	39	0	2	1	4	0	0	46
N-44	38	1	3	0	3	1	0	46
N-43	40	1	4	0	5	0	0	50
N-45	4	36	1	4	0	5	0	50
A12	84	0	9	0	10	0	0	103
A4	88	0	10	0	6	0	1	105
UFRJ50816	91	0	6	1	10	0	0	108
YPS138	95	0	8	0	7	0	0	110
UFRJ50791	95	0	10	0	8	0	0	113
DBVPG6304	97	2	6	1	6	1	1	114
	704	72	80	15	69	25	5	970

Supplementary Table S2: pSNP frequencies in *Saccharomyces cerevisiae* strains

Position	Region	pSNPs	Strains														Reference	Variants	Frequencies		
524	26S	2	DBVPG6040	NCYC361															81	10	0.110
679	26S	1	DBVPG6044																91	2	0.022
1112	26S	1	UWOPS87-2421																132	6	0.043
1426	26S	1	DBVPG1373																35	2	0.054
1813	26S	1	YS9																45	21	0.318
1852	26S	1	DBVPG6765																40	2	0.048
1887	26S	1	DBVPG1853																182	10	0.052
2017	26S	1	YJM981																89	24	0.212
2420	26S	1	DBVPG1373																200	5	0.024
2996	26S	1	YPS606																75	3	0.038
3012	26S	1	DBVPG6765																86	3	0.034
3096	26S	1	273614N																177	19	0.097
3154	26S	1	322134S																68	2	0.029
3177	26S	3	NCYC110	SK1	Y55														100	2	0.020
																			119	9	0.070
																			236	9	0.037
																			237	8	0.033
3266	26S	1	DBVPG1853																4	94	0.959
3430	ETS2	1	YS4																38	4	0.095
3494	ETS2	2	YS9	YS4															6	31	0.838
																			6	46	0.885
3517	ETS2	3	UWOPS87-2421	UWOPS05-217-3	UWOPS83-787-3														52	2	0.037
																			2	121	0.984
																			55	7	0.113
3538	ETS2	2	Y9	Y12															11	45	0.804
																			23	35	0.603
3555	ETS2	2	Y9	Y12															11	38	0.776
																			23	33	0.589
3590	ETS2	1	NCYC361																82	6	0.068
3599	ETS2	1	NCYC361																81	6	0.069
3609	IGS1	1	Y55																185	3	0.016
3610	IGS1	1	Y55																185	3	0.016
3611	IGS1	1	Y55																185	3	0.016
3612	IGS1	2	Y55	Yllc17_E5															185	3	0.016
																			44	2	0.043
3624	IGS1	1	Yllc17_E5																17	12	0.414
3659	IGS1	12	DBVPG1106	YJM975	YJM978	YJM981	BC187	DBVPG1373	273614N	378604X	DBVPG6040	Yllc17_E5	NCYC361	YS4					16	17	0.515
																			16	35	0.686
																			17	18	0.514
																			59	84	0.587
																			12	14	0.538
																			29	43	0.597
																			7	29	0.806
																			62	5	0.075
																			79	5	0.060
																			10	41	0.804
																			8	57	0.877
																			5	36	0.878
3678	IGS1	2	YS9	YS4															3	29	0.906
																			8	45	0.849
3687	IGS1	1	BC187																29	8	0.216
3697	IGS1	5	S288c	Y9	Y12	DBVPG6040	Yllc17_E5												108	17	0.136
																			41	6	0.128

Supplementary Table S2: pSNP frequencies in *Saccharomyces paradoxus* strains

												42	11	0.208
												90	7	0.072
												52	2	0.037
3713 IGS1	2	UWOPS87-2421	UWOPS83-787-3									47	2	0.041
												43	7	0.140
3769 IGS1	1	YS9										6	42	0.875
3813 IGS1	5	273614N	378604X	YIIC17_E5	NCYC361	DBVPG1853						6	32	0.842
												11	40	0.784
												27	12	0.308
												13	47	0.783
												83	26	0.239
3840 IGS1	1	S288c										101	8	0.073
3871 IGS1	1	BC187										23	2	0.080
3902 IGS1	5	S288c	378604X	DBVPG6040	YS9	NCYC361						102	10	0.089
												2	37	0.949
												57	2	0.034
												3	26	0.897
												5	53	0.914
3903 IGS1	2	YS9	YS4									18	11	0.379
												18	27	0.600
3967 IGS1	1	YS5										252	2	0.008
3974 IGS1	6	UWOPS87-2421	322134S	Y9	Y12	UWOPS83-787-3	NCYC361					2	23	0.920
												6	63	0.913
												5	28	0.848
												12	23	0.657
												5	24	0.828
												8	50	0.862
3989 IGS1	4	322134S	DBVPG6040	YS9	YS4							76	2	0.026
												79	2	0.025
												29	27	0.482
												32	43	0.573
4056 IGS1	1	YIIC17_E5										14	20	0.588
4058 IGS1	1	YIIC17_E5										14	20	0.588
4070 IGS1	1	YS4										35	8	0.186
4129 IGS1	1	YS4										28	2	0.067
4156 IGS1	6	322134S	273614N	378604X	YS9	NCYC361	YS4					4	69	0.945
												20	31	0.608
												14	46	0.767
												11	28	0.718
												8	51	0.864
												4	60	0.938
4166 IGS1	9	S288c	322134S	273614N	378604X	DBVPG6040	YS9	YIIC17_E5	NCYC361	YS4		94	9	0.087
												3	66	0.957
												20	28	0.583
												13	38	0.745
												47	20	0.299
												10	27	0.730
												15	18	0.545
												4	43	0.915
												3	54	0.947
4183 IGS1	2	Y9	Y12									3	24	0.889
												10	16	0.615
4203 IGS1	3	322134S	YS9	NCYC361								5	84	0.944
												10	38	0.792
												5	58	0.921
4215 IGS1	7	S288c	322134S	273614N	378604X	DBVPG6040	YIIC17_E5	NCYC361				66	10	0.132

													66	3	0.043
													18	25	0.581
													12	46	0.793
													48	19	0.284
													11	21	0.656
													40	4	0.091
4224 IGS1	2	YS9	YS4										21	10	0.323
													20	30	0.600
4241 IGS1	1	YS4											46	11	0.193
4248 IGS1	5	322134S	273614N	378604X	YS9	NCYC361							4	67	0.944
													36	2	0.053
													11	42	0.792
													8	34	0.810
													5	45	0.900
4270 IGS1	1	UWOPS83-787-3											29	8	0.216
4284 IGS1	1	UWOPS83-787-3											18	6	0.250
4305 IGS1	1	DBVPG6040											45	12	0.211
4307 IGS1	1	S288c											90	4	0.043
4365 IGS1	1	UWOPS83-787-3											30	9	0.231
4386 IGS1	1	UWOPS83-787-3											30	9	0.231
4401 IGS1	1	DBVPG1853											51	17	0.250
4418 IGS1	1	Y12											39	4	0.093
4431 IGS1	1	W303											189	8	0.041
4484 IGS1	10	L_1374	YJM975	YJM981	BC187	SK1	Y12	K11	YS9	NCYC361	YS4		30	4	0.118
													33	2	0.057
													177	4	0.022
													26	2	0.071
													161	6	0.036
													22	4	0.154
													16	2	0.111
													26	3	0.103
													51	3	0.056
													42	4	0.087
4495 IGS1	1	DBVPG1853											38	19	0.333
4496 IGS1	4	UWOPS05-217-3	YS9	YS4	DBVPG1853								3	71	0.959
													22	7	0.241
													31	16	0.340
													21	40	0.656
4498 IGS1	3	273614N	YS9	YS4									22	18	0.450
													22	7	0.241
													31	16	0.340
4499 IGS1	1	DBVPG1853											41	20	0.328
4508 IGS1	1	DBVPG1373											24	16	0.400
4652 IGS2	1	DBVPG6765											133	58	0.304
4657 IGS2	1	L_1374											42	10	0.192
4664 IGS2	2	322134S	378604X										89	3	0.033
													19	42	0.689
4701 IGS2	3	UWOPS05-227-2	UWOPS05-217-3	UWOPS83-787-3									3	51	0.944
													2	92	0.979
													38	8	0.174
4728 IGS2	1	DBVPG1853											38	40	0.513
4754 IGS2	3	S288c	378604X	DBVPG6040									105	8	0.071
													58	15	0.205
													70	14	0.167
4763 IGS2	2	273614N	378604X										26	29	0.527
													65	2	0.030

4769 IGS2	5	322134S	UWOPS05-227-2	378604X	YS9	UWOPS83-787-3						81	3	0.036
												3	57	0.950
												18	52	0.743
												2	29	0.935
												42	9	0.176
4786 IGS2	1	BC187										27	2	0.069
4809 IGS2	1	DBVPG6040										83	3	0.035
4854 IGS2	4	UWOPS05-227-2	DBVPG6040	UWOPS83-787-3	NCYC361							3	54	0.947
												91	2	0.022
												3	37	0.925
												68	6	0.081
4925 IGS2	2	UWOPS05-227-2	UWOPS83-787-3									3	53	0.946
												46	8	0.148
4928 IGS2	2	UWOPS05-227-2	UWOPS83-787-3									3	56	0.949
												46	7	0.132
4963 IGS2	1	DBVPG6040										92	3	0.032
5064 IGS2	3	UWOPS05-227-2	DBVPG6040	UWOPS83-787-3								2	40	0.952
												90	2	0.022
												39	7	0.152
5069 IGS2	6	S288c	273614N	378604X	DBVPG6040	YS9	NCYC361					110	7	0.060
												32	26	0.448
												13	40	0.755
												72	17	0.191
												3	25	0.893
												6	54	0.900
5131 IGS2	4	UWOPS05-227-2	DBVPG6040	Yllc17_E5	UWOPS83-787-3							3	44	0.936
												89	2	0.022
												2	43	0.956
												35	4	0.103
5142 IGS2	2	UWOPS83-787-3	NCYC361									8	35	0.814
												69	7	0.092
5143 IGS2	4	322134S	378604X	YS9	YS4							78	2	0.025
												14	39	0.736
												18	14	0.438
												19	25	0.568
5218 IGS2	2	DBVPG6040	UWOPS83-787-3									82	2	0.024
												32	5	0.135
5221 IGS2	1	NCYC361										62	6	0.088
5238 IGS2	9	S288c	273614N	Y9	Y12	378604X	DBVPG6040	YS9	NCYC361	YS4		92	6	0.061
												34	30	0.469
												17	8	0.320
												18	7	0.280
												55	2	0.035
												68	15	0.181
												22	14	0.389
												58	10	0.147
												31	6	0.162
5248 IGS2	6	S288c	273614N	378604X	DBVPG6040	YS9	NCYC361					91	8	0.081
												34	29	0.460
												11	36	0.766
												70	15	0.176
												4	32	0.889
												3	69	0.958
5260 IGS2	5	273614N	Y9	YS9	Yllc17_E5	YS4						34	29	0.460
												3	17	0.850
												18	22	0.550

									2	42	0.955
									32	7	0.179
5279 IGS2	2	YS9	YS4						4	28	0.875
									10	28	0.737
5301 IGS2	2	DBVPG6040	UWOPS83-787-3						84	2	0.023
									36	3	0.077
5329 IGS2	6	S288c	273614N	378604X	DBVPG6040	YS9	NCYC361		84	8	0.087
									32	27	0.458
									12	32	0.727
									69	15	0.179
									2	26	0.929
									3	60	0.952
5396 IGS2	2	DBVPG6040	UWOPS83-787-3						87	2	0.022
									42	2	0.045
5443 IGS2	1	DBVPG1853							108	12	0.100
5455 IGS2	1	NCYC361							13	56	0.812
5457 IGS2	1	BC187							27	2	0.069
5473 IGS2	1	YPS606							65	4	0.058
5554 IGS2	3	YJM975	YJM978	YJM981					32	5	0.135
									23	13	0.361
									103	53	0.340
5579 IGS2	1	S288c							69	8	0.104
5601 IGS2	3	W303	S288c	YIIC17_E5					276	3	0.011
									77	8	0.094
									44	3	0.064
5602 IGS2	2	DBVPG6040	NCYC361						51	11	0.177
									8	44	0.846
5659 IGS2	4	DBVPG6040	YS9	NCYC361	378604X				53	11	0.172
									2	32	0.941
									50	2	0.038
									8	39	0.830
5662 IGS2	2	Y9	Y12						3	16	0.842
									13	18	0.581
5667 IGS2	1	W303							273	3	0.011
5714 IGS2	1	DBVPG1853							59	31	0.344
5756 IGS2	3	YS9	YS4	DBVPG1853					26	11	0.297
									35	28	0.444
									33	70	0.680
5818 IGS2	1	UWOPS83-787-3							24	4	0.143
5846 IGS2	1	DBVPG1853							107	3	0.027
5850 IGS2	1	YIIC17_E5							4	55	0.932
5853 IGS2	1	NCYC361							76	7	0.084
5940 ETS1	1	DBVPG1373							70	12	0.146
5998 ETS1	1	378604X							9	49	0.845
6004 ETS1	2	322134S	NCYC361						3	51	0.944
									17	59	0.776
6089 ETS1	5	S288c	DBVPG6040	YIIC17_E5	UWOPS83-787	NCYC361			99	6	0.057
									55	12	0.179
									4	57	0.934
									2	23	0.920
									72	13	0.153
6295 ETS1	2	Y9	Y12						7	30	0.811
									12	29	0.707
6479 ETS1	1	DBVPG1373							85	2	0.023
6514 ETS1	3	378604X	YS9	YS4					8	25	0.758
									17	4	0.190

6521 ETS1	1	DBVPG1853						23	34	0.596
6540 ETS1	1	DBVPG1853						36	48	0.571
6549 ETS1	1	DBVPG1853						77	4	0.049
6595 18S	1	YJM975						36	46	0.561
7322 18S	5	273614N	378604X	YIIC17_E5	YS4	DBVPG1853		29	2	0.065
								65	2	0.030
								16	37	0.698
								2	37	0.949
								54	9	0.143
								72	16	0.182
7881 18S	1	BC187						30	2	0.063
8295 18S	2	Y9	Y12					5	28	0.848
								21	25	0.543
8462 ITS1	1	DBVPG1373						77	2	0.025
8505 ITS1	1	YIIC17_E5						49	12	0.197
8546 ITS1	1	DBVPG1853						87	21	0.194
8568 ITS1	4	NCYC110	DBVPG6044	SK1	Y55			42	37	0.468
								93	16	0.147
								28	147	0.840
								74	122	0.622
8652 ITS1	1	<u>DBVPG1853</u>						56	17	0.233
8686 ITS1	5	DBVPG6040	YIIC17_E5	UWOPS83-787-3	NCYC361	YS4		63	28	0.308
								15	37	0.712
								2	36	0.947
								80	9	0.101
								38	21	0.356
8692 ITS1	1	DBVPG6040						58	26	0.310
8702 ITS1	2	YS9	YS4					28	24	0.462
								50	17	0.254
8738 ITS1	2	DBVPG6040	YIIC17_E5					55	26	0.321
								15	40	0.727
8990 ITS2	1	K11						2	17	0.895
9026 ITS2	1	YJM978						31	11	0.262
9027 ITS2	1	YJM978						32	11	0.256

Supplementary Table S2: INs and pINs in *Saccharomyces cerevisiae* strains

Position Region	No. of strains	INs	pINs	Type 1 pINs	Type 2 pINs	Strains					
547 26S	2	0	2	2	0	UWOPS83-787-3	NCYC361				
3625 IGS1	1	0	1	0	1	<u>YS9</u>					
3626 IGS1	5	0	5	2	3	273614N	<u>378604X</u>	DBVPG6040	<u>Y9</u>	<u>Yllc17_E5</u>	
3629 IGS1	1	0	1	0	1	<u>DBVPG1853</u>					
3630 IGS1	3	1	2	1	1	NCYC110	BC187	<u>YS4</u>			
3631 IGS1	2	1	1	0	1	Y55	<u>Y12</u>				
3632 IGS1	1	1	0	0	0	DBVPG6044					
3702 IGS1	1	0	1	1	0	Yllc17_E5					
3901 IGS1	1	0	1	0	1	<u>DBVPG1853</u>					
3902 IGS1	1	0	1	0	1	<u>Y12</u>					
3903 IGS1	1	1	0	0	0	K11					
4049 IGS1	5	1	4	4	0	S288c	322134S	378604X	YS9	YS4	
4051 IGS1	1	1	0	0	0	K11					
4052 IGS1	1	1	0	0	0	Y12					
4054 IGS1	1	0	1	1	0	NCYC361					
4056 IGS1	2	1	1	1	0	Y9	DBVPG6040				
4059 IGS1	1	0	1	1	0	Yllc17_E5					
4312 IGS1	1	1	0	0	0	K11					
4315 IGS1	1	1	0	0	0	K11					
4319 IGS1	1	1	0	0	0	K11					
5622 IGS2	2	1	1	1	0	273614N	378604X				
5632 IGS2	7	2	5	3	2	NCYC110	DBVPG1373	Y9	Yllc17_E5	DBVPG1853	NCYC361 Y12
5643 IGS2	1	0	1	1	0	YS4					
5644 IGS2	1	0	1	1	0	DBVPG6044					
5653 IGS2	1	0	1	1	0	DBVPG1853					
5707 IGS2	1	1	0	0	0	273614N					
8413 ITS1	2	0	2	1	1	DBVPG1853	<u>NCYC361</u>				
8414 ITS1	1	0	1	0	1	<u>YS9</u>					
8652 ITS1	1	0	1	1	0	378604X					
8665 ITS1	1	0	1	1	0	YS4					
8982 ITS2	4	0	4	3	1	322134S	YS9	DBVPG1853	<u>YS4</u>		
9031 ITS2	2	0	2	2	0	Y9	Y12				
	57	15	42	28	14						

Supplementary Table S2: pINS frequencies in *Saccharomyces cerevisiae* strains

Position	Region	Type 1 pINSs	Strains			Reference	Variants	Frequencies	
547	26S	2	UWOPS83-787-3	NCYC361		26	9	0.257	
						91	2	0.022	
3626	IGS1	2	273614N	DBVPG6040		29	2	0.065	
						75	2	0.026	
3630	IGS1	1	BC187			19	8	0.296	
3702	IGS1	1	YIIC17_E5			24	28	0.538	
4049	IGS1	4	S288c	322134S	378604X	YS9	106	10	0.086
						6	39	0.867	
						14	28	0.667	
						9	27	0.750	
4054	IGS1	1	NCYC361			2	41	0.953	
4056	IGS1	1	DBVPG6040			5	36	0.878	
4059	IGS1	1	YIIC17_E5			14	20	0.588	
5622	IGS2	1	378604X			8	25	0.758	
5632	IGS2	3	DBVPG1373	Y9	YIIC17_E5	37	8	0.178	
						5	12	0.706	
						6	34	0.850	
5643	IGS2	1	YS4			43	4	0.085	
5644	IGS2	1	DBVPG6044			3	70	0.959	
5653	IGS2	1	DBVPG1853			85	28	0.248	
8413	ITS1	1	DBVPG1853			18	57	0.760	
8652	ITS1	1	378604X			16	33	0.673	
8665	ITS1	1	YS4			20	35	0.636	
8982	ITS2	3	322134S	YS9	DBVPG1853	77	3	0.038	
						7	34	0.829	
						30	71	0.703	
9031	ITS2	2	Y9	Y12		10	26	0.722	
						8	41	0.837	

Supplementary Table S2: pDEL frequencies in *Saccharomyces cerevisiae* strains

Start position	End position	Region	Type 1 pDELS	Strains	Reference	Variants	Frequencies
309	309	26S	1	DBVPG6765	242	2	0.008
3543	3543	ETS2	1	SK1	252	2	0.008
3578	3601	ETS2	1	DBVPG1853	45	75	0.625
3580	3603	ETS2	1	378604X	60	8	0.118
3592	3597	ETS2	1	NCYC361	79	6	0.071
3593	3599	ETS2	2	273614N	59	3	0.048
				UWOPS05-217-3	3	84	0.966
3595	3600	ETS2	1	Y9	5	39	0.886
3595	3608	ETS2	1	Yllc17_E5	28	22	0.440
3597	3605	ETS2	1	DBVPG6040	82	4	0.047
3600	3605	ETS2	1	NCYC361	79	8	0.092
3601	3607	ETS2	1	273614N	28	6	0.176
3626	3628	IGS1	1	DBVPG1373	40	40	0.500
3626	3629	IGS1	4	YJM975	30	25	0.455
				YJM978	23	21	0.477
				YJM981	105	79	0.429
				NCYC361	68	6	0.081
3627	3627	IGS1	1	273614N	3	28	0.903
3627	3628	IGS1	1	Yllc17_E5	17	13	0.433
3627	3629	IGS1	1	BC187	9	18	0.667
3833	3833	IGS1	4	378604X	9	43	0.827
				DBVPG6040	78	5	0.060
				NCYC361	7	46	0.868
				DBVPG1853	80	20	0.200
3837	3837	IGS1	1	S288c	113	9	0.074
3840	3840	IGS1	1	DBVPG6765	2	134	0.985
3841	3841	IGS1	1	S288c	99	8	0.075
3882	3888	IGS1	1	Y12	16	13	0.448
3893	3900	IGS1	1	YS9	17	17	0.500
3900	3900	IGS1	4	S288c	104	10	0.088
				378604X	2	37	0.949
				NCYC361	5	53	0.914
				DBVPG1853	15	64	0.810
3901	3901	IGS1	2	Y12	20	12	0.375
				DBVPG6040	57	2	0.034
3920	3921	IGS1	1	YS9	4	13	0.765
3920	3924	IGS1	1	S288c	117	10	0.079
4101	4116	IGS1	5	322134S	2	60	0.968
				378604X	14	35	0.714
				DBVPG6040	69	2	0.028
				YS9	10	31	0.756
				NCYC361	8	52	0.867
4109	4119	IGS1	1	S288c	98	8	0.075
4240	4259	IGS1	2	YJM975	34	5	0.128
				YJM978	17	17	0.500
4244	4259	IGS1	1	YJM981	112	45	0.287
4299	4305	IGS1	1	NCYC361	11	23	0.676
4300	4303	IGS1	1	DBVPG6040	43	13	0.232

4300	4306 IGS1	1	S288c								86	4	0.044
4486	4492 IGS1	1	378604X								2	13	0.867
4486	4495 IGS1	2	YJM981	K11							5	192	0.975
											5	14	0.737
4486	4497 IGS1	1	SK1								4	167	0.977
4486	4498 IGS1	1	YIIC17_E5								8	28	0.778
4498	4510 IGS1	1	S288c								79	4	0.048
4511	4512 IGS1	1	273614N								25	12	0.324
5221	5221 IGS2	1	UWOPS83-787-3								4	36	0.900
5222	5222 IGS2	1	NCYC361								61	6	0.090
5585	5585 IGS2	1	UWOPS05-217-3								2	51	0.962
5594	5599 IGS2	2	378604X	DBVPG6040							9	21	0.700
											53	10	0.159
5594	5602 IGS2	1	YS9								2	26	0.929
5605	5613 IGS2	1	378604X								10	31	0.756
5665	5665 IGS2	1	W303								277	3	0.011
5667	5667 IGS2	2	S288c	YIIC17_E5							73	6	0.076
											4	40	0.909
6520	6520 ETS1	1	DBVPG1853								36	48	0.571
6521	6521 ETS1	2	YS9	YS4							15	7	0.318
											24	32	0.571
6521	6524 ETS1	2	S288c	NCYC361							107	3	0.027
											31	29	0.483
6528	6531 ETS1	1	DBVPG6040								65	7	0.097
6534	6534 ETS1	1	DBVPG1853								27	58	0.682
6547	6549 ETS1	2	S288c	NCYC361							110	3	0.027
											8	49	0.860
6548	6550 ETS1	2	378604X	DBVPG6040							28	6	0.176
											65	7	0.097
8013	8013 18S	1	UWOPS83-787-3								55	2	0.035
9067	9067 ITS2	8	NCYC110	BC187	322134S	DBVPG6040	UWOPS05-217-3	YS9	NCYC361	DBVPG1853	2	86	0.977
											12	21	0.636
											87	3	0.033
											47	24	0.338
											2	67	0.971
											6	34	0.850
											2	67	0.971
											38	71	0.651

Supplementary Table S2: Complex variants in *Saccharomyces cerevisiae* strains

Strain	Start position	End position	Region	Details
Y9	3901	3904	IGS1	Possibly a pDEL + pINS, with sequence aca replaced by ta in 5 out of 27 reads

Supplementary Table S2: All variants in *Saccharomyces cerevisiae* strains

Strain	SNPs	pSNPs	INs	pINs	DEs	pDEs	CX	Total
W303	0	3	0	0	0	1	0	4
L_1374	6	2	0	0	7	1	0	16
DBVPG1106	7	1	0	0	9	1	0	18
DBVPG1788	8	0	0	0	11	0	0	19
YJM975	6	4	0	0	7	3	0	20
YJM978	6	4	0	0	7	3	0	20
YJM981	6	4	0	0	6	5	0	21
YPS128	14	0	0	0	10	0	0	24
S288c	0	14	0	1	0	10	0	25
BC187	7	7	0	1	5	5	0	25
DBVPG1373	8	7	0	1	5	4	0	25
YPS606	14	2	0	0	9	2	0	27
NCYC110	15	2	2	0	8	1	0	28
DBVPG6765	13	3	0	0	8	4	0	28
DBVPG6044	15	2	1	1	9	1	0	29
SK1	16	3	0	0	7	3	0	29
UWOPS87-2421	14	4	0	0	11	1	0	30
322134S	6	12	0	2	5	5	0	30
Y9	8	10	1	3	6	4	1	33
Y55	15	7	1	0	9	2	0	34
273614N	4	15	2	1	6	6	0	34
Y12	9	11	1	4	6	5	0	36
378604X	0	20	0	4	1	11	0	36
DBVPG6040	0	27	0	2	0	11	0	40
K11	23	2	5	0	9	2	0	41
YS9	1	27	0	4	1	8	0	41
YIIc17_E5	7	18	0	4	6	6	0	41
UWOPS05-227-2	24	7	0	0	11	0	0	42
UWOPS05-217-3	27	3	0	0	7	6	0	43
UWOPS83-787-3	8	21	0	1	6	7	0	43
UWOPS03-461-4	29	0	0	0	15	0	0	44
NCYC361	0	27	0	4	0	13	0	44
YS4	9	24	1	4	6	5	0	49
DBVPG1853	14	18	1	5	2	9	0	49
	339	311	15	42	215	145	1	1068