

Nitrogen starvation Reveals the Mitotic Potential of Mutants in the S/MAPK Pathways

Rostyslav Makarenko^{1,2¶}, Claire Denis^{1¶}, Stefania Francesconi^{1¶}, Serge Gangloff¹,
and Benoît Arcangioli^{1*}

¹ Genome Dynamics Unit, Institut Pasteur, UMR3525 CNRS, Paris 75015, France

² Sorbonne Université, École Doctorale 515, F-75005 Paris, France

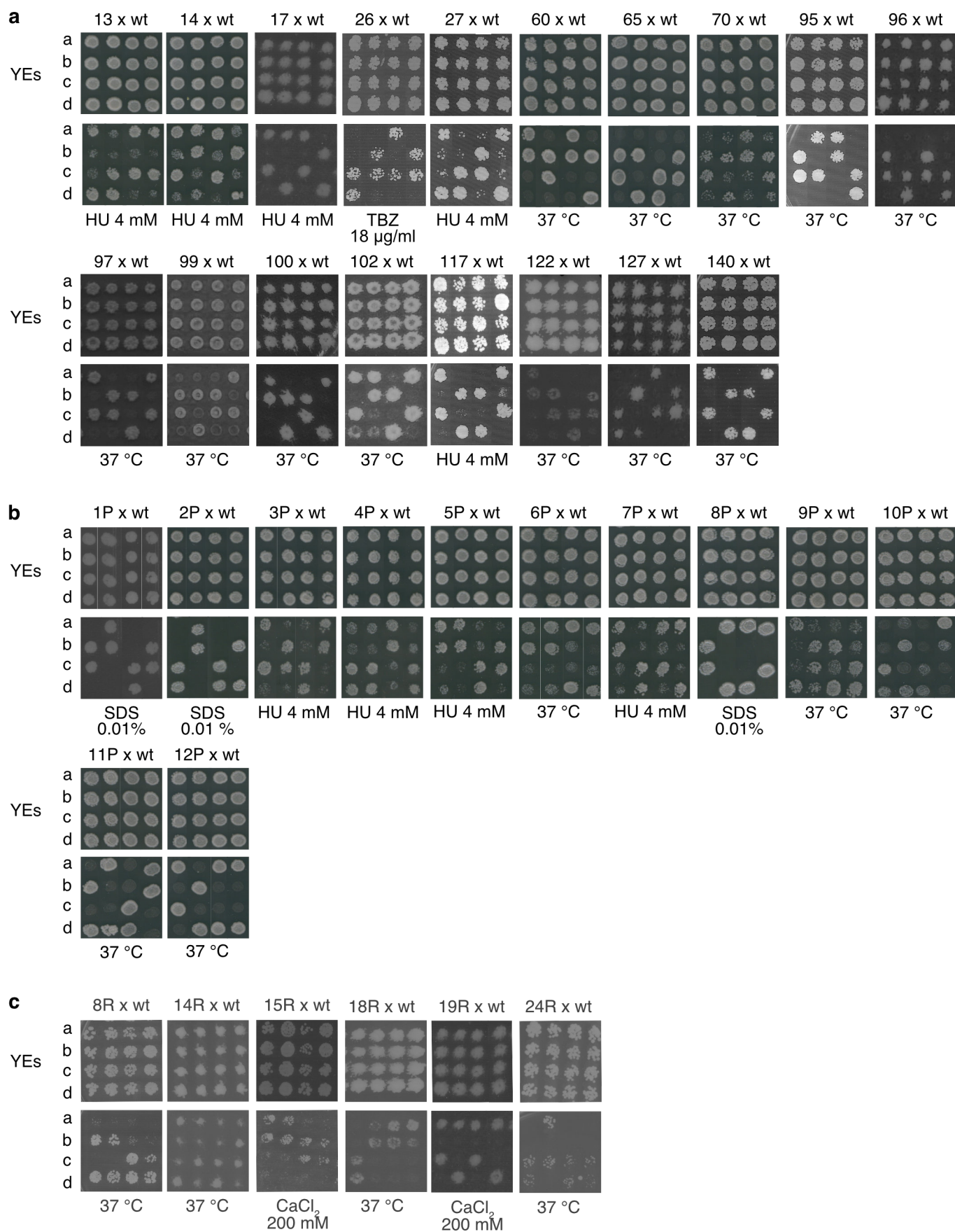
* Corresponding author

E-mail: benoit.arcangioli@pasteur.fr

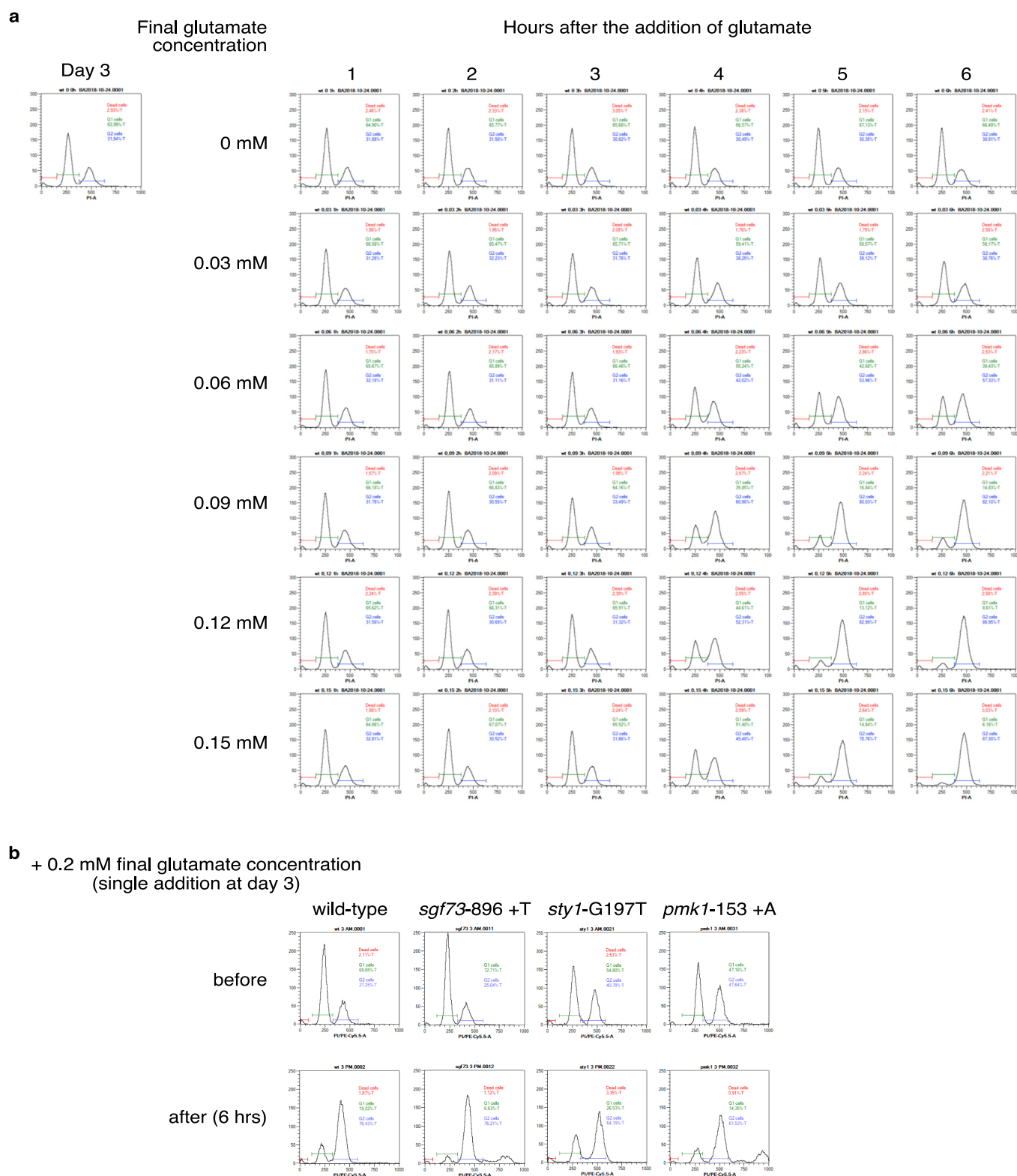
Running Title: S/MAPK mutants increase the fitness of quiescence

Key Words: Quiescence, Mutations, S/MAPK, RpoS, Adaptation, Kin selection

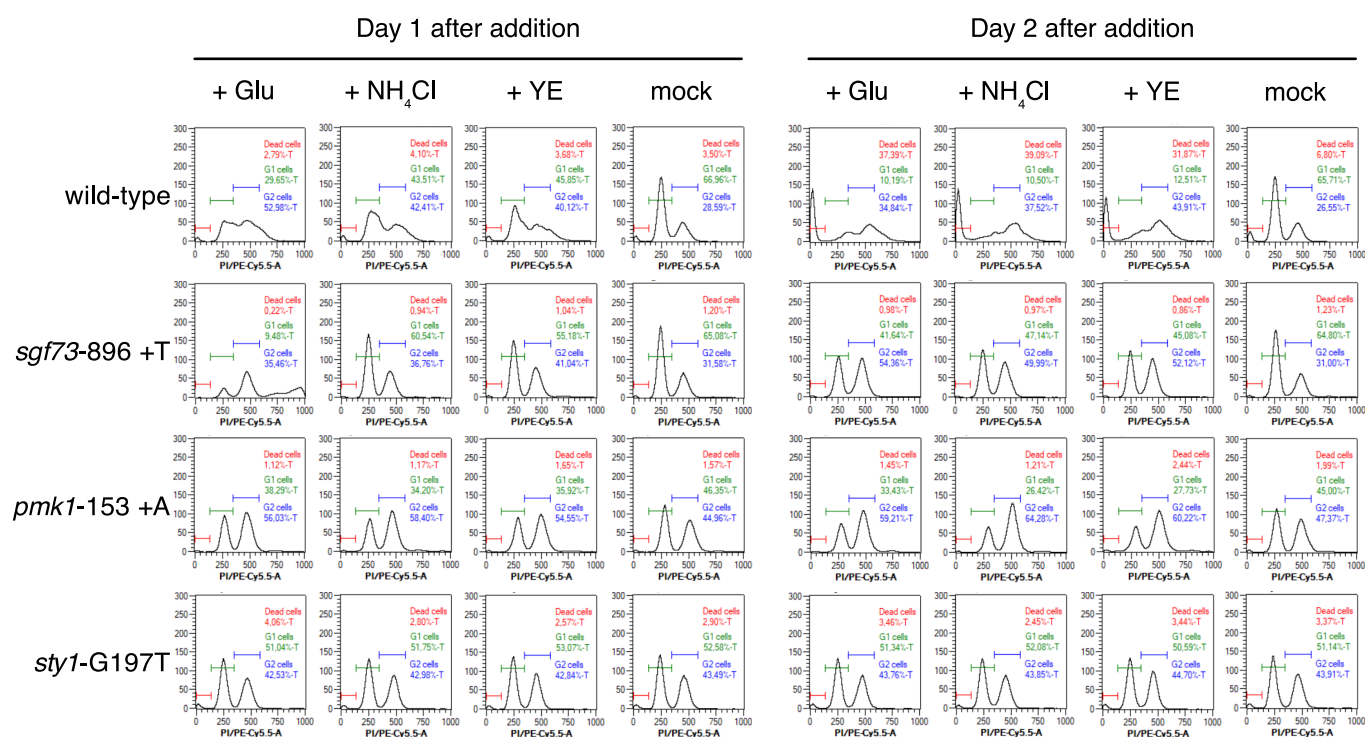
¶These authors contributed equally to this work



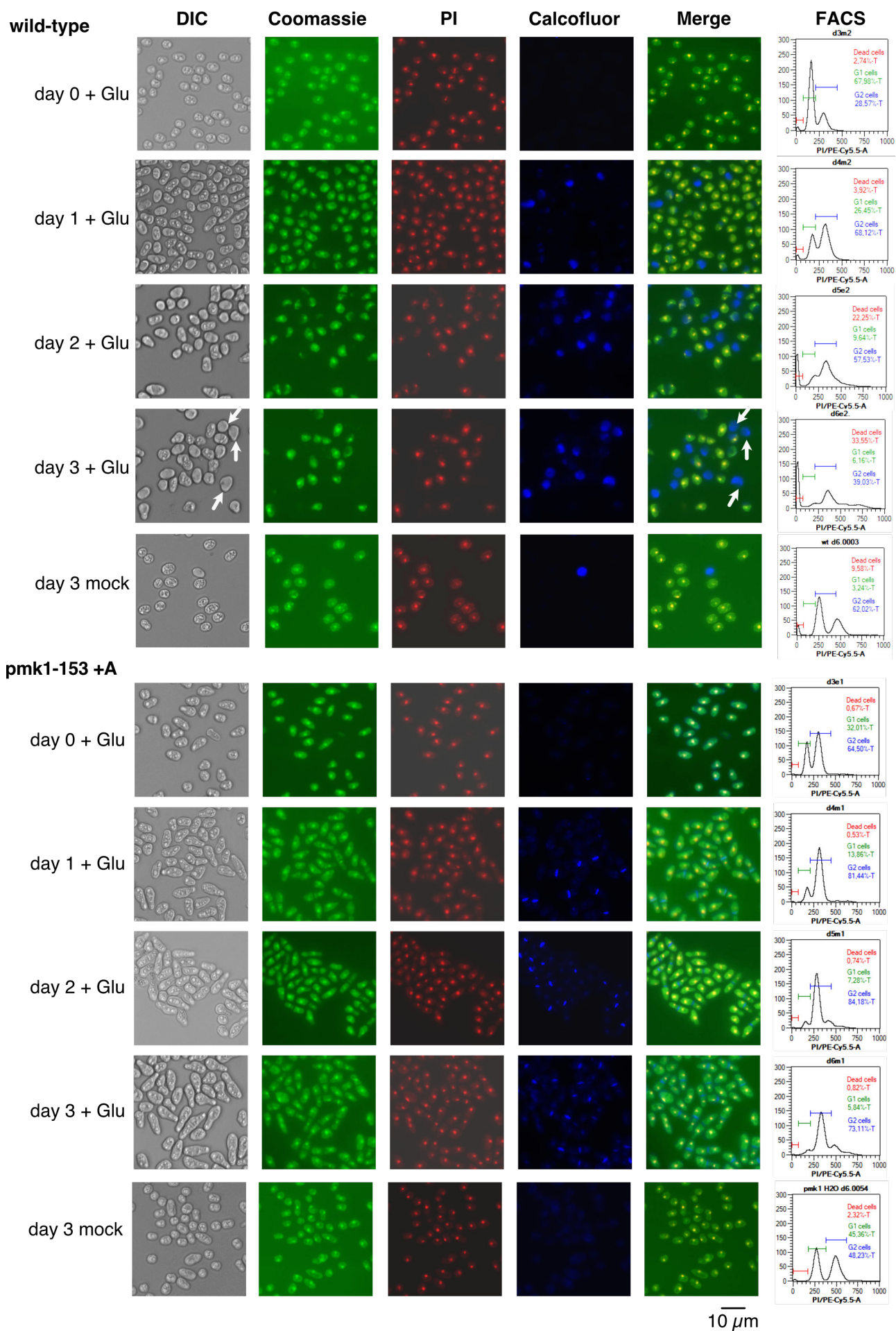
Supplementary Figure 1. Mendelian segregation of the mutations causing a sensitivity phenotype. The mutants from the 30 initially identified clones and the 6 identified later in the 36 randomly picked clones (Supplementary Table 3) were crossed with a PΔ17 prototrophic strain, sporulated and dissected. Replica plating or spotting was performed on the media containing the various drugs at the given concentration. Four representative tetrads are shown for a sensitivity phenotype for each strain.



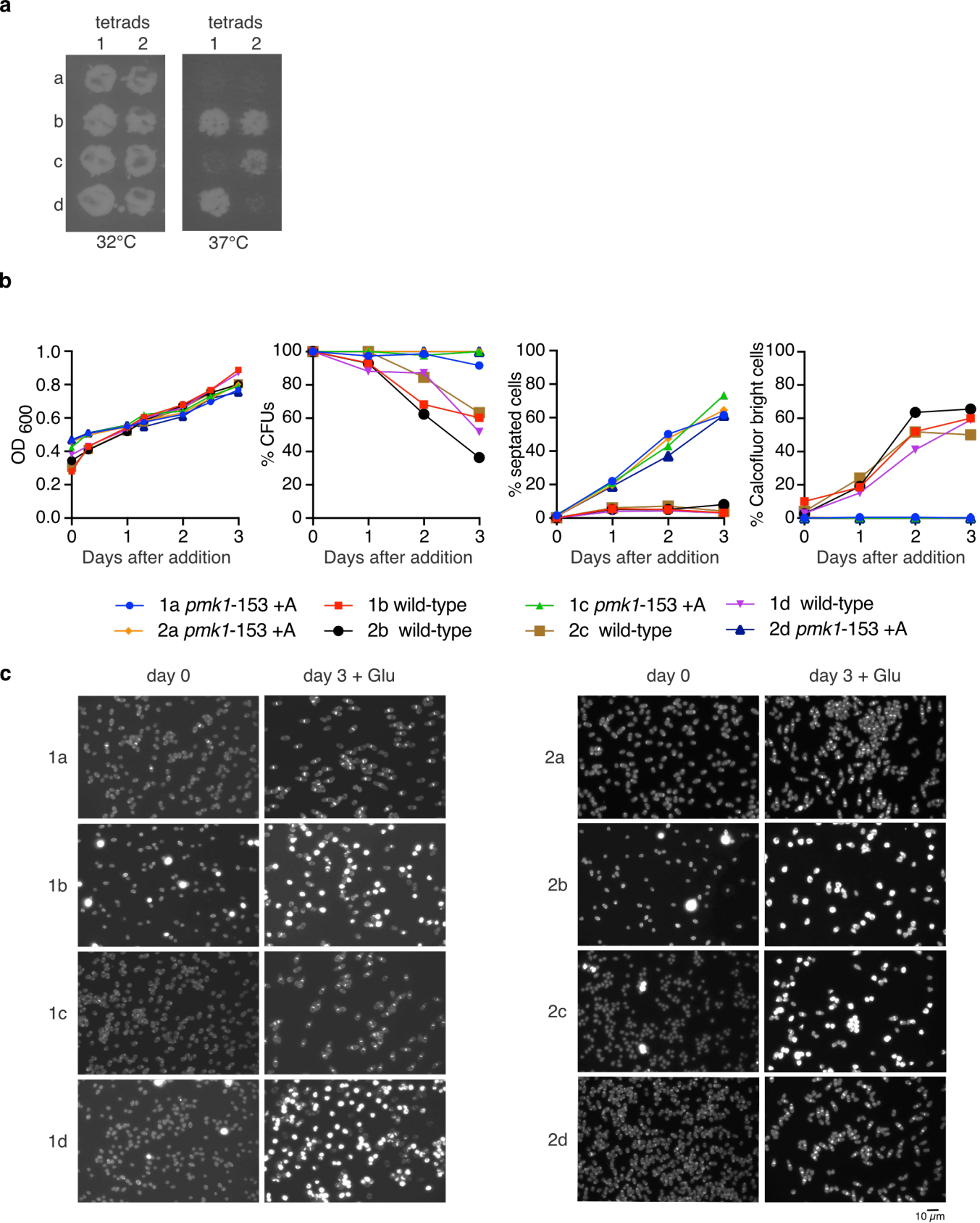
Supplementary Figure 2. Determination of the amount of nitrogen necessary to trigger a physiological response. (a) Effect of glutamate concentration on the cell cycle of a three-day-old quiescent culture of a wild-type strain. FACS analysis of the DNA content was followed for six hours after the addition of glutamate at the indicated final concentration. (b) Effect of glutamate addition at a final concentration of 0.2 mM on the cell cycle of three-day-old quiescent cultures of S/MAPK mutants. FACS analysis of the DNA content of strains determined before and six hours after the addition.



Supplementary Figure 3. DNA content at days 1 and 2 after the first addition. This figure extends Figure 6. Effect of the addition of various nitrogen sources to three-day-old quiescent cultures (day 0) twice a day (mornings and evenings) determined by FACS analysis in the morning. Glutamate (5g/L), ammonium chloride (1.6 g/L) or yeast extract (5 g/L) was added at 1/1,500 of the volume of the culture at each addition. DNA content analysis of 10,000 ethanol fixed cells was determined by FACS.



Supplementary Figure 4. Cellular response to the repeated addition of traces of glutamate to three-day-old quiescent cultures. Day 0 is before the first addition. Cells at the indicated time points were heat fixed at 60 °C on a glass slide and stained with a solution containing equal volumes of 0.05% Coomassie Brilliant Blue G in PBS (proteins staining), 0.1 µg/mL of propidium iodide (PI) (nucleic acids staining) and 25 µg/mL of Calcofluor white (staining of the septum in cells that have passed mitosis). Images were taken using a Zeiss Axioplan 2 Imaging M epifluorescence microscope equipped with an AxioCam MRm digital camera and Zen 2 software. When provided with traces of glutamate, only the wild-type culture accumulates cells with a smooth appearance under differential-interference-contrast (DIC) microscopy that stain brightly with Calcofluor. These cells are negative for both Coomassie and propidium iodide staining as shown in the merged images. White arrows point to some of these cells. The accumulation of this population correlates with the fraction of propidium negative cells observed by FACS analysis. Unlike wild-type, all the *pmk1*-153 +A mutant cells are positive for the three dyes and Calcofluor specifically stains the septum of binucleated cells that have passed mitosis. The presented micrographs are representative of the situation observed in the field of 20 pictures each from 2 independent experiments.



Supplementary Figure 5. Scavenging behavior of the *pmk1* mutant crossed into the 972 background. The PΔ17 *pmk1*-153 +A mutant was crossed with the 972 h⁻ strain and two full tetrads were analyzed after supplementing traces of glutamate to three-day-old cultures (day 0) as described in Figure 6. (a) The thermosensitive phenotype conferred by the *pmk1*-153 +A allele segregates 2:2 in the spores of both tetrads used in this experiment. (b) Cell mass increase (OD_{600nm}), colony forming units, % of septated cells and % of Calcofluor white bright cells were determined for 3 days following the first addition of glutamate. A single determination for each spore is presented (c) Images taken with a Zeiss Axioplan 2 Imaging M epifluorescence microscope on days 0 and 3 after the first supplement of glutamate of fixed cells stained with Calcofluor white (25 μg/mL) that highlights septum formation in cells that have passed mitosis or cells devoid of nucleic acids and proteins. The presented micrographs are representative of the situation observed in the field of 20 pictures from each spore.

Supplementary Table 1: Differences between PB1623 and 972 h- established on 243 sequenced genomes - Publicly available at the NCBI BioProject database (accession no: PRJNA413662)

SNVs						
#CHROM	POS	ID	REF	ALT	QUAL	
II	731642	.	G	T	3548.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Gat/Tat D110Y SPBC119.08.1:exon:1 transcript:SPBC119.08.1 4 1)
I	5115257	.	C	G	2997.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE gaG/gaC E386D SPAC29A4.16.1:exon:1 transcript:SPAC29A4.16.1 1 1)
II	1048712	.	G	A	2737.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE gCc/gTc A240V SPBC337.10c.1:exon:1 transcript:SPBC337.10c.1 1 1),SYNONYMOUS_CODING(LOW SILENT cgG/cgA R96 SPNCRNA.1407.1:exon:1 transcript:SPNCRNA.1407.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS)
II	1238931	.	G	A	2532.0	EFF=INTRON(MODIFIER SPBC651.01c.1:exon:1 transcript:SPBC651.01c.1 2 1)
II	1987098	.	C	A	2502.0	EFF=SPLICE_SITE_REGION(LOW SPBC1A4.06c.1:exon:1 transcript:SPBC1A4.06c.1 4 1),SYNONYMOUS_CODING(LOW SILENT ctG/ctT L376 SPBC1A4.06c.1:exon:1 transcript:SPBC1A4.06c.1 4 1),UTR_3_PRIME(MODIFIER 427 SPBC1A4.05.1:exon:1 transcript:SPBC1A4.05.1 2 1)
I	477739	.	T	C	2305.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Att/Gtt I304V SPAC24H6.06.1:exon:1 transcript:SPAC24H6.06.1 2 1)
II	879033	.	T	A	2303.0	EFF=SYNONYMOUS_CODING(LOW SILENT gtT/gtA V510 SPBC713.06.1:exon:1 transcript:SPBC713.06.1 1 1),UTR_3_PRIME(MODIFIER 886 SPBC713.07c.1:exon:1 transcript:SPBC713.07c.1 1 1)
I	1999192	.	G	C	2296.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Cgg/Ggg R34G SPAC767.01c.1:exon:1 transcript:SPAC767.01c.1 2 1),NON_SYNONYMOUS_CODING(MODERATE MISSENSE Gta/Cta V20L SPNCRNA.785.1:exon:1 transcript:SPNCRNA.785.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS)
II	1849777	.	G	A	2255.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE aCc/aTc T1496 SPBC28E12.06c.1:exon:1 transcript:SPBC28E12.06c.1 1 1),UTR_3_PRIME(MODIFIER 3611 SPBC28E12.05.1:exon:1 transcript:SPBC28E12.05.1 1 1)
I	3074001	.	C	T	2232.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE aCc/aTc T275 SPAC24C9.14.1:exon:1 transcript:SPAC24C9.14.1 1 1)
I	5115148	.	G	C	2210.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Ctg/Gtg L423V SPAC29A4.16.1:exon:1 transcript:SPAC29A4.16.1 1 1)
I	3583106	.	G	A	2205.0	EFF=INTERGENIC(MODIFIER 1)
II	923543	.	C	A	2159.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE gaC/gaA D273E SPBC646.02.1:exon:1 transcript:SPBC646.02.1 1 1)
I	527156	.	C	A	2153.0	EFF=STOP_GAINED(HIGH NONSENSE taC/taA Y172) SPNCRNA.659.1:exon:1 transcript:SPNCRNA.659.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
III	1765401	.	G	T	2059.0	EFF=INTERGENIC(MODIFIER 1)
I	3119299	.	G	A	2044.0	EFF=UTR_3_PRIME(MODIFIER 80 SPAC688.07c.1:exon:1 transcript:SPAC688.07c.1 1 1)
I	3111508	.	A	G	2034.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE gAa/gGa E375G SPAC589.12.1:exon:1 transcript:SPAC589.12.1 2 1)
II	685728	.	G	A	2014.0	EFF=INTERGENIC(MODIFIER 1)
II	4122326	.	T	C	2009.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Tat/Cat Y478H SPNCRNA.1671.1:exon:1 transcript:SPNCRNA.1671.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE),NON_SYNONYMOUS_CODING(MODERATE MISSENSE atA/atG 101M SPBC56F2.03.1:exon:1 transcript:SPBC56F2.03.1 1 1)
I	3179238	.	G	C	2006.0	EFF=STOP_LOST(HIGH MISSENSE taG/taC *436Y SPNCRNA.893.1:exon:1 transcript:SPNCRNA.893.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
I	2097159	.	C	T	1946.0	EFF=SYNONYMOUS_CODING(LOW SILENT aaG/aaA K209 SPAC25A8.03c.1:exon:1 transcript:SPAC25A8.03c.1 1 1)
II	4538164	.	A	T	1945.0	EFF=INTERGENIC(MODIFIER 1)
III	748812	.	A	T	1929.0	EFF=NON_SYNONYMOUS_START(LOW MISSENSE Atg/Ttg M1L SPCC14G10.04.1:exon:1 transcript:SPCC14G10.04.1 1 1)
III	2128341	.	C	T	1911.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE gGc/gAc G370D SPCC126.07c.1:exon:1 transcript:SPCC126.07c.1 1 1)
I	4919603	.	A	G	1862.0	EFF=INTERGENIC(MODIFIER 1)
II	438465	.	G	A	1858.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Cgt/Tgt R105C SPBC582.10c.1:exon:1 transcript:SPBC582.10c.1 1 1),NON_SYNONYMOUS_CODING(MODERATE MISSENSE cGt/cAt R207H SPNCRNA.1357.1:exon:1 transcript:SPNCRNA.1357.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
II	1422317	.	T	A	1852.0	EFF=SYNONYMOUS_CODING(LOW SILENT ctA/ctT L531 SPBC691.02c.1:exon:1 transcript:SPBC691.02c.1 1 1)
II	700113	.	A	T	1848.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE ttT/ttA F565L SPNCRNA.1381.1:exon:1 transcript:SPNCRNA.1381.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
III	1374938	.	A	T	1813.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE tAv/tTt Y256F SPCC1281.01.1:exon:1 transcript:SPCC1281.01.1 1 1)
I	759334	.	G	T	1786.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE aCt/aAt T406N SPAC4G8.03c.1:exon:1 transcript:SPAC4G8.03c.1 1 1)
III	679772	.	T	C	1770.0	EFF=UTR_5_PRIME(MODIFIER 590 SPCC16C4.09.1:exon:1 transcript:SPCC16C4.09.1 1 1)
II	943554	.	C	A	1749.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE aCa/aAa T130K SPBC646.11.1:exon:1 transcript:SPBC646.11.1 4 1),UTR_3_PRIME(MODIFIER 1548 SPBC646.12c.1:exon:1 transcript:SPBC646.12c.1 1 1)
I	862964	.	G	T	1737.0	EFF=INTRON(MODIFIER SPAC23G3.02c.1:exon:1 transcript:SPAC23G3.02c.1 1 1)
II	1159678	.	G	C	1726.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Cgc/Ggc R391G SPNCRNA.1417.1:exon:1 transcript:SPNCRNA.1417.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE),NON_SYNONYMOUS_CODING(MODERATE MISSENSE cGc/cCc R362P SPBC409.11.1:exon:1 transcript:SPBC409.11.1 1 1)
I	1783458	.	C	T	1706.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE aGa/aAa R543K SPAC17A5.15c.1:exon:1 transcript:SPAC17A5.15c.1 1 1)
I	5400080	.	C	T	1704.0	EFF=SYNONYMOUS_CODING(LOW SILENT caC/caT H281 SPAC3G6.11.1:exon:1 transcript:SPAC3G6.11.1 3 1)
I	4375421	.	A	T	1697.0	EFF=UTR_5_PRIME(MODIFIER 78 SPAC1527.01.1:exon:1 transcript:SPAC1527.01.1 1 1)
III	1862803	.	A	G	1629.0	EFF=INTERGENIC(MODIFIER 1)
II	2228775	.	T	G	1617.0	EFF=INTRON(MODIFIER SPBC14C8.14c.1:exon:1 transcript:SPBC14C8.14c.1 1 1),UTR_3_PRIME(MODIFIER 2632 SPBC14C8.13.1:exon:1 transcript:SPBC14C8.13.1 1 1)
II	1784826	.	G	A	1611.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE aCt/aTt T392 SPBC18H10.08c.1 4 1),UTR_3_PRIME(MODIFIER 820 SPBC18H10.07.1:exon:1 transcript:SPBC18H10.07.1 2 1)
II	1672859	.	C	T	1610.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Cgg/Tgg R760W SPBC19C2.01.1:exon:1 transcript:SPBC19C2.01.1 1 1),NON_SYNONYMOUS_CODING(MODERATE MISSENSE Gac/Aac D794N SPNCRNA.1451.1:exon:1 transcript:SPNCRNA.1451.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS)
I	523603	.	G	A	1607.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Gcg/Acg A120T SPAC227.15.1:exon:1 transcript:SPAC227.15.1 1 1)
II	1962419	.	T	A	1590.0	EFF=UTR_5_PRIME(MODIFIER 602 SPBC1E8.03c.1:exon:1 transcript:SPBC1E8.03c.1 1 1)
II	2228777	.	G	T	1572.0	EFF=INTRON(MODIFIER SPBC14C8.14c.1:exon:1 transcript:SPBC14C8.14c.1 1 1),UTR_3_PRIME(MODIFIER 2634 SPBC14C8.13.1:exon:1 transcript:SPBC14C8.13.1 1 1)
II	635763	.	G	A	1562.0	EFF=UTR_5_PRIME(MODIFIER 313 SPBC115.03.1:five_prime_UTR:1 transcript:SPBC115.03.1 1 1)
II	1673002	.	G	C	1558.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE aCg/aGg T746R SPNCRNA.1451.1:exon:1 transcript:SPNCRNA.1451.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS),SYNONYMOUS_CODING(LOW SILENT acG/acC T807 SPBC19C2.01.1:exon:1 transcript:SPBC19C2.01.1 1 1)
II	1962439	.	A	G	1556.0	EFF=UTR_5_PRIME(MODIFIER 622 SPBC1E8.03c.1:exon:1 transcript:SPBC1E8.03c.1 1 1)
I	3630676	.	T	A	1536.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE agA/agT R222S SPAC1142.03c.1:exon:1 transcript:SPAC1142.03c.1 1 1)
II	1159694	.	A	T	1520.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE gaT/gaA D385E SPNCRNA.1417.1:exon:1 transcript:SPNCRNA.1417.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE),SYNONYMOUS_CODING(LOW SILENT ccA/ccT P367 SPBC409.11.1:exon:1 transcript:SPBC409.11.1 1 1)
II	276539	.	A	T	1496.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE gaA/gaT E211D SPBC800.11.1:exon:1 transcript:SPBC800.11.1 1 1)
I	523883	.	G	A	1495.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE aGc/aAc S213N SPAC227.15.1:exon:1 transcript:SPAC227.15.1 1 1)

SNVs							
#CHROM	POS	ID	REF	ALT	QUAL		
II	953201	.	A	G	1495.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE tTt/tCt F328S SPBC646.14c.1:exon:1 transcript:SPBC646.14c.1 3 1)	
III	963343	.	A	G	1487.0	EFF=SYNONYMOUS_CODING(LOW SILENT taT/taC Y210 SPCC1742.01.1:exon:1 transcript:SPCC1742.01.1 1 1)	
II	699961	.	A	T	1475.0	EFF=INTERGENIC(MODIFIER 1)	
II	3006137	.	A	T	1413.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Tct/Act S482T SPBC15D4.01c.1:exon:1 transcript:SPBC15D4.01c.1 3 1),UTR_3_PRIME(MODIFIER 747 SPBC2D10.20.1:exon:1 transcript:SPBC2D10.20.1 6 1)	
II	2676336	.	C	T	1400.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Gat/Aat D8N SPBC3E7.08c.1:exon:1 transcript:SPBC3E7.08c.1 1 1)	
II	2901509	.	G	T	1386.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Cat/Aat H367N SPNCRNA.1562.1:exon:1 transcript:SPNCRNA.1562.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE),NON_SYNONYMOUS_CODING(MODERATE MISSENSE atG/atT M89I SPNCRNA.411.1:exon:1 transcript:SPNCRNA.411.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS),NON_SYNONYMOUS_CODING(MODERATE MISSENSE tGt/tTt C134F SPNCRNA.1561.1:exon:1 transcript:SPNCRNA.1561.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS)	
II	266718	.	A	C	1384.0	EFF=SYNONYMOUS_CODING(LOW SILENT cgA/cgC R403 SPBC800.08.1:exon:1 transcript:SPBC800.08.1 2 1),UTR_5_PRIME(MODIFIER 1904 SPBC800.07c.1:exon:1 transcript:SPBC800.07c.1 1 1)	
III	1246664	.	G	T	1382.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Gtc/Ttc V468F SPCC645.07.1:exon:1 transcript:SPCC645.07.1 1 1)	
II	1962458	.	T	A	1358.0	EFF=UTR_5_PRIME(MODIFIER 641 SPBC1E8.03c.1:exon:1 transcript:SPBC1E8.03c.1 1 1)	
II	2689713	.	C	T	1355.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Gtc/Atc V212 SPBC3E7.16c.1:exon:1 transcript:SPBC3E7.16c.1 1 1)	
I	1263527	.	A	G	1329.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Aca/Gca T26A SPNCRNA.730.1:exon:1 transcript:SPNCRNA.730.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS)	
II	700028	.	A	T	1317.0	EFF=SYNONYMOUS_CODING(LOW SILENT Tc/Ac ?594 SPNCRNA.1381.1:exon:1 transcript:SPNCRNA.1381.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)	
II	266780	.	T	A	1270.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE tTc/tAc F424Y SPBC800.08.1:exon:1 transcript:SPBC800.08.1 2 1),UTR_5_PRIME(MODIFIER 1966 SPBC800.07c.1:exon:1 transcript:SPBC800.07c.1 1 1)	
I	3204845	.	G	A	1265.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE cGa/cAa R162Q SPAC1486.06.1:five_prime_UTR:1 transcript:SPAC1486.06.1 2 1)	
II	700024	.	T	A	1241.0	EFF=INTERGENIC(MODIFIER 1)	
II	967094	.	A	G	1225.0	EFF=SYNONYMOUS_CODING(LOW SILENT gaA/gaG E128 SPBP35G2.02.1:exon:1 transcript:SPBP35G2.02.1 1 1)	
I	2104097	.	C	A	1214.0	EFF=UTR_3_PRIME(MODIFIER 100 SPAC631.01c.1:exon:1 transcript:SPAC631.01c.1 3 1),UTR_3_PRIME(MODIFIER 143 SPAC25A8.02.1:exon:1 transcript:SPAC25A8.02.1 1 1)	
II	699952	.	G	T	1202.0	EFF=INTERGENIC(MODIFIER 1)	
III	227730	.	A	T	1184.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE gTt/gAt V224D SPCC548.06c.1:exon:1 transcript:SPCC548.06c.1 1 1)	
II	869806	.	G	C	1181.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Cat/Gat H42D SPBC713.02c.1:exon:1 transcript:SPBC713.02c.1 3 1)	
II	699951	.	T	G	1179.0	EFF=INTERGENIC(MODIFIER 1)	
I	2104091	.	G	T	1171.0	EFF=UTR_3_PRIME(MODIFIER 106 SPAC631.01c.1:exon:1 transcript:SPAC631.01c.1 3 1),UTR_3_PRIME(MODIFIER 137 SPAC25A8.02.1:exon:1 transcript:SPAC25A8.02.1 1 1)	
II	2444543	.	T	C	1160.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE gTt/gCt V72A SPBC12C2.13c.1:exon:1 transcript:SPBC12C2.13c.1 1 1),SYNONYMOUS_STOP(LOW SILENT taA/taG *96 SPNCRNA.1514.1:exon:1 transcript:SPNCRNA.1514.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)	
II	700020	.	C	T	1124.0	EFF=INTERGENIC(MODIFIER 1)	
II	700019	.	T	A	1123.0	EFF=INTERGENIC(MODIFIER 1)	
II	2806053	.	G	A	1120.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Gga/AgA G24R SPNCRNA.1550.1:exon:1 transcript:SPNCRNA.1550.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)	
II	700021	.	A	C	1117.0	EFF=INTERGENIC(MODIFIER 1)	
II	2007715	.	A	G	1099.0	EFF=INTERGENIC(MODIFIER 1)	
II	962880	.	C	G	1095.0	EFF=UTR_5_PRIME(MODIFIER 365 SPBP35G2.16c.1:exon:1 transcript:SPBP35G2.16c.1 1 1)	
III	1772337	.	G	A	1046.0	EFF=SYNONYMOUS_CODING(LOW SILENT agG/agA R241 SPCC1442.03.1:exon:1 transcript:SPCC1442.03.1 2 1)	
III	1260881	.	G	A	1011.0	EFF=UTR_3_PRIME(MODIFIER 82 SPCC645.13.1:five_prime_UTR:1 transcript:SPCC645.13.1 2 1)	
III	1260882	.	G	A	999.0	EFF=UTR_3_PRIME(MODIFIER 83 SPCC645.13.1:five_prime_UTR:1 transcript:SPCC645.13.1 2 1)	
II	2044929	.	A	T	995.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Aac/Tac N290Y SPNCRNA.1483.1:exon:1 transcript:SPNCRNA.1483.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS)	
II	2436580	.	C	G	987.0	EFF=SYNONYMOUS_CODING(LOW SILENT tcC/tcG S315 SPBC21D10.06c.1:exon:1 transcript:SPBC21D10.06c.1 1 1)	
I	2506044	.	T	A	933.0	EFF=INTERGENIC(MODIFIER 1)	
II	1697124	.	C	T	904.0	EFF=UTR_3_PRIME(MODIFIER 283 SPBC19C2.10.1:exon:1 transcript:SPBC19C2.10.1 2 1)	
I	943745	.	T	C	890.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE atA/atG I709M SPAC1687.22c.1:exon:1 transcript:SPAC1687.22c.1 2 1)	
II	1716326	.	G	T	824.0	EFF=INTERGENIC(MODIFIER 1)	
II	4400666	.	A	C	824.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE Tgg/Ggg W728G SPBC1289.10c.1:exon:1 transcript:SPBC1289.10c.1 1 1)	
II	1683324	.	T	G	817.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE gTa/gGa V155G SPBC19C2.05.1:exon:1 transcript:SPBC19C2.05.1 1 1)	
II	2437627	.	C	T	795.0	EFF=SYNONYMOUS_CODING(LOW SILENT gtC/gtT V664 SPBC21D10.06c.1:exon:1 transcript:SPBC21D10.06c.1 1 1)	
III	284657	.	A	G	787.0	EFF=UTR_5_PRIME(MODIFIER 186 SPCC553.10.1:exon:1 transcript:SPCC553.10.1 1 1)	
III	1168328	.	T	C	771.0	EFF=INTERGENIC(MODIFIER 1)	
II	4538155	.	T	G	736.0	EFF=INTERGENIC(MODIFIER 1)	
II	1678693	.	T	A	671.0	EFF=INTERGENIC(MODIFIER 1)	
II	1716318	.	A	G	670.0	EFF=INTERGENIC(MODIFIER 1)	
I	4442714	.	G	A	666.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE aCt/aTt T69 SPNCRNA.988.1:exon:1 transcript:SPNCRNA.988.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)	
II	1716320	.	T	G	665.0	EFF=INTERGENIC(MODIFIER 1)	
I	2605764	.	T	A	650.0	EFF=UTR_5_PRIME(MODIFIER 421 SPAC823.14.1:exon:1 transcript:SPAC823.14.1 1 1)	
II	689794	.	T	C	630.0	EFF=SYNONYMOUS_CODING(LOW SILENT tcT/tcC S693 SPBPJ4664.02.1:exon:1 transcript:SPBPJ4664.02.1 1 1)	
I	2747078	.	A	G	624.0	EFF=INTRON(MODIFIER SPAC6F6.08c.1:exon:1 transcript:SPAC6F6.08c.1 3 1)	
II	4532088	.	G	T	595.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE gGt/gTt G1735V SPBCPT2R1.08c.1:exon:1 transcript:SPBCPT2R1.08c.1 1 1)	
II	1155903	.	T	A	589.0	EFF=INTERGENIC(MODIFIER 1)	
II	689487	.	A	C	547.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE tAt/tCt Y591S SPBPJ4664.02.1:exon:1 transcript:SPBPJ4664.02.1 1 1)	
II	1553976	.	A	T	544.0	EFF=STOP_LOST(HIGH MISSENSE taA/taT *156Y SPNCRNA.1442.1:exon:1 transcript:SPNCRNA.1442.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)	

SNVs						
#CHROM	POS	ID	REF	ALT	QUAL	
I	3548913	.	T	A	507.0	EFF=INTERGENIC(MODIFIER 1)
II	689485	.	A	T	480.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE agA/agT R590S) SPBPJ4664.02.1:exon:1 transcript:SPBPJ4664.02.1 1 1)
II	4538038	.	A	G	471.0	EFF=INTERGENIC(MODIFIER 1)
II	4537844	.	T	C	463.0	SF=0,1
II	4538052	.	A	G	461.0	EFF=INTERGENIC(MODIFIER 1)
I	3538450	.	A	T	440.0	EFF=UTR_3_PRIME(MODIFIER 191 SPAC1B1.01.1:exon:1 transcript:SPAC1B1.01.1 2 1)
III	720001	.	C	T	361.0	EFF=INTERGENIC(MODIFIER 1)
I	3115841	.	C	A	345.0	EFF=UTR_3_PRIME(MODIFIER 226 SPAC688.04c.1:exon:1 transcript:SPAC688.04c.1 1 1)
I	1664907	.	T	A	317.0	EFF=INTERGENIC(MODIFIER 1)
I	3992214	.	C	A	288.0	EFF=NON_SYNONYMOUS_CODING(MODERATE MISSENSE aaC/aaA N291K) SPNCRNA.954.1:exon:1 transcript:SPNCRNA.954.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE),NON_SYNONYMOUS_CODING(MODERATE MISSENSE tgG/tgT W77C) SPAPB15E9.02c.1:exon:1 transcript:SPAPB15E9.02c.1 1 1)
I	1465627	.	G	A	251.0	EFF=INTERGENIC(MODIFIER 1)
I	2245490	.	G	C	205.0	EFF=INTERGENIC(MODIFIER 1)

Indels						
#CHROM	POS	REF		ALT	QUAL	
I	10859	GTT		G	0.0	EFF=INTERGENIC(MODIFIER 1)
I	19694	T		TA	184.97	EFF=INTERGENIC(MODIFIER 1)
I	101871	A		AG	1276.97	EFF=FRAME_SHIFT(HIGH gcc/gcCc A558A?) SPAC1F8.07c.1:exon:1 transcript:SPAC1F8.07c.1 1 1),SPLICE_SITE_REGION(LOW SPAC1F8.07c.1:exon:1 transcript:SPAC1F8.07c.1 1 1)
I		C		CT	2397.97	EFF=NON_SYNONYMOUS_CODING(MODERATE cgtgacNN/c79.793NN RD?1886??? SPAC212.11.1:exon:1 transcript:SPAC212.11.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
I	470378	AC		A	1989.97	EFF=UTR_3_PRIME(MODIFIER 204 SPAC24H6.09.1:exon:1 transcript:SPAC24H6.09.1 2 1)
I	470390	AC		A	1870.97	EFF=UTR_3_PRIME(MODIFIER 192 SPAC24H6.09.1:exon:1 transcript:SPAC24H6.09.1 2 1)
I	482737	A		AG	2752.97	EFF=UTR_3_PRIME(MODIFIER 709 SPAC24H6.04.1:exon:1 transcript:SPAC24H6.04.1 1 1)
I	526170	G		GA	1908.97	EFF=UTR_3_PRIME(MODIFIER 304 SPAC227.15.1:exon:1 transcript:SPAC227.15.1 1 1)
I	554527	A		AT	326.97	EFF=INTERGENIC(MODIFIER 1)
I	616208	G		GT	2061.97	EFF=FRAME_SHIFT(HIGH aca/aAca T214N?) SPAC1F3.02c.1:exon:1 transcript:SPAC1F3.02c.1 1 1)
I	660056	TG		T	764.97	EFF=UTR_5_PRIME(MODIFIER 1225 SPAC1D4.11c.1:exon:1 transcript:SPAC1D4.11c.1 1 1)
I	670032	C		CA	2032.97	EFF=INTERGENIC(MODIFIER 1)
I	682993	TC		T	1689.97	EFF=FRAME_SHIFT(HIGH -/-41 SPAC22F3.11c.1:exon:1 transcript:SPAC22F3.11c.1 1 1)
I	862962	C		CT	3637.97	EFF=INTRON(MODIFIER SPAC23G3.02c.1:exon:1 transcript:SPAC23G3.02c.1 1 1)
I	959770	AT		A	1278.97	EFF=INTERGENIC(MODIFIER 1)
I	960025	G		GT	96.97	EFF=UTR_5_PRIME(MODIFIER 35 SPAC222.09.1:exon:1 transcript:SPAC222.09.1 1 1)
I	995016	GT		G	1016.97	EFF=FRAME_SHIFT(HIGH -/-377 SPNCRNA.703.1:exon:1 transcript:SPNCRNA.703.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE),UTR_3_PRIME(MODIFIER 459 SPAC821.08c.1:exon:1 transcript:SPAC821.08c.1 1 1)
I	1048635	G		GA	223.97	EFF=FRAME_SHIFT(HIGH ttc/ttTc F100F?) SPNCRNA.708.1:exon:1 transcript:SPNCRNA.708.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS)
I	1274803	T		TA	1552.97	EFF=INTERGENIC(MODIFIER 1)
I	1424708	CA		C	2861.97	EFF=FRAME_SHIFT(HIGH -/-232 SPAC3A12.04c.1:exon:1 transcript:SPAC3A12.04c.1 4 1)
I	1465713	CT		C	1183.97	EFF=INTERGENIC(MODIFIER 1)
I	1509277	C		CT	0.0	EFF=INTERGENIC(MODIFIER 1)
I	1625092	T		TC	1942.97	EFF=FRAME_SHIFT(HIGH gag/gGag E1560G?) SPAP27G11.10c.1:exon:1 transcript:SPAP27G11.10c.1 2 1)
I	1936294	G		GA	1534.97	EFF=INTRON(MODIFIER SPAC3H1.04c.1:exon:1 transcript:SPAC3H1.04c.1 1 1)
I	1952313	A		AT	1606.97	EFF=FRAME_SHIFT(HIGH ata/aAta 428N?) SPNCRNA.777.1:exon:1 transcript:SPNCRNA.777.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
I	2139856	G		GT	86.97	EFF=INTERGENIC(MODIFIER 1)
I	2154491	T		TA	2841.97	EFF=FRAME_SHIFT(HIGH cta/ctTa L7L?) SPNCRNA.800.1:exon:1 transcript:SPNCRNA.800.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
I	2288240	CG		C	2592.97	EFF=UTR_3_PRIME(MODIFIER 52 SPAC4G9.19.1:exon:1 transcript:SPAC4G9.19.1 1 1)
I	2290217	C		CTAGTA	2266.97	EFF=INTERGENIC(MODIFIER 1)
I	2343703	G		GA	2152.97	EFF=FRAME_SHIFT(HIGH act/actT T2T?) SPAC17G8.01c.1:exon:1 transcript:SPAC17G8.01c.1 2 1),SPLICE_SITE_REGION(LOW SPAC17G8.01c.1:exon:1 transcript:SPAC17G8.01c.1 2 1)
I	2421576	AC		A	2625.97	EFF=UTR_3_PRIME(MODIFIER 214 SPAC6B12.07c.1:exon:1 transcript:SPAC6B12.07c.1 1 1)

Indels					
#CHROM	POS	REF	ALT	QUAL	
I	2499149	AT	A	1046.97	EFF=FRAME_SHIFT(HIGH -/- -39 SPAC23H3.04.1:exon:1 transcript:SPAC23H3.04.1 1 1)
I	2507462	T	TA	2421.97	EFF=UTR_5_PRIME(MODIFIER 157 SPAC23H3.08c.1:exon:1 transcript:SPAC23H3.08c.1 1 1)
I	2510060	T	TA	1587.97	EFF=INTERGENIC(MODIFIER 1)
I	2588021	C	CA	2266.97	EFF=FRAME_SHIFT(HIGH aca/acAa T80T?) SPAC823.04.1:exon:1 transcript:SPAC823.04.1 2 1)
I	2588066	C	CA	1487.97	EFF=FRAME_SHIFT(HIGH aaa/Aaaa K95K?) SPAC823.04.1:exon:1 transcript:SPAC823.04.1 3 1)
I	2594263	C	CT	2198.97	EFF=INTERGENIC(MODIFIER 1)
I	2594324	T	TA	2841.97	EFF=INTERGENIC(MODIFIER 1)
I	2594351	T	TA	2442.97	EFF=INTERGENIC(MODIFIER 1)
I	2594391	A	AT	2474.97	EFF=INTERGENIC(MODIFIER 1)
I	2605860	GT	G	2631.97	EFF=UTR_5_PRIME(MODIFIER 324 SPAC823.14.1:exon:1 transcript:SPAC823.14.1 1 1)
I	2607669	A	AT	2206.97	EFF=FRAME_SHIFT(HIGH aat/aaAt N116K?) SPNCRNA.841.1:exon:1 transcript:SPNCRNA.841.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE),INTRAGENIC(MODIFIER ppa1 1),UTR_5_PRIME(MODIFIER 433 SPAC823.15.1:five_prime_UTR:1 transcript:SPAC823.15.1 1 1)
I	2683902	TC	T	1992.97	EFF=FRAME_SHIFT(HIGH -/- -456 SPNCRNA.844.1:exon:1 transcript:SPNCRNA.844.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS)
I	2751236	TA	T	121.97	EFF=INTERGENIC(MODIFIER 1)
I	2759871	T	TC	1827.97	EFF=FRAME_SHIFT(HIGH tct/tCct S361S?) SPNCRNA.851.1:exon:1 transcript:SPNCRNA.851.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS)
I	2949974	GAT	G	438.97	EFF=UTR_3_PRIME(MODIFIER 247 SPAPB24D3.01.1:exon:1 transcript:SPAPB24D3.01.1 1 1)
I	2958937	GAA	G	417.97	EFF=FRAME_SHIFT(HIGH -/- -712 SPNCRNA.865.1:exon:1 transcript:SPNCRNA.865.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE),UTR_5_PRIME(MODIFIER 12 SPAPB24D3.07c.1:exon:1 transcript:SPAPB24D3.07c.1 1 1)
I	3125118	A	AT	2804.97	EFF=FRAME_SHIFT(HIGH ata/aTta 639I?) SPAC688.08.1:exon:1 transcript:SPAC688.08.1 2 1)
I	3178494	C	CT	2238.97	EFF=FRAME_SHIFT(HIGH ttc/Ttc F189F?) SPNCRNA.893.1:exon:1 transcript:SPNCRNA.893.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
I	3197528	A	AG	1041.97	EFF=FRAME_SHIFT(HIGH agg/aGgg R167R?) SPAC1486.05.1:exon:1 transcript:SPAC1486.05.1 2 1),SPlice_SITE_REGION(LOW) SPAC1486.05.1:exon:1 transcript:SPAC1486.05.1 2 1)
I	3318900	T	TA	1042.97	EFF=UTR_5_PRIME(MODIFIER 269 SPAPB1E7.11c.1:five_prime_UTR:1 transcript:SPAPB1E7.11c.1 1 1)
I	3450130	GT	G	1672.97	EFF=FRAME_SHIFT(HIGH -/- -755 SPAC3A11.09.1:exon:1 transcript:SPAC3A11.09.1 2 1)
I	3460318	T	TC	1709.97	EFF=FRAME_SHIFT(HIGH gga/ggGa G365G?) SPAC3A11.06.1:exon:1 transcript:SPAC3A11.06.1 6 1)
I	3538413	A	AT	1452.97	EFF=UTR_3_PRIME(MODIFIER 155 SPAC1B1.01.1:exon:1 transcript:SPAC1B1.01.1 2 1)
I	3538428	G	GT	1340.97	EFF=UTR_3_PRIME(MODIFIER 170 SPAC1B1.01.1:exon:1 transcript:SPAC1B1.01.1 2 1)
I	3538436	T	TA	1193.97	EFF=UTR_3_PRIME(MODIFIER 178 SPAC1B1.01.1:exon:1 transcript:SPAC1B1.01.1 2 1)
I	3538449	T	TTA	1076.97	EFF=UTR_3_PRIME(MODIFIER 191 SPAC1B1.01.1:exon:1 transcript:SPAC1B1.01.1 2 1)
I	3538456	A	AAT	1124.97	EFF=UTR_3_PRIME(MODIFIER 198 SPAC1B1.01.1:exon:1 transcript:SPAC1B1.01.1 2 1)
I	3548899	AAAAAAAAAAGAAT	A	552.97	EFF=INTERGENIC(MODIFIER 1)
I	3576756	T	TTCTTCTC	741.97	EFF=INTERGENIC(MODIFIER 1)
I	3576929	C	CT	221.97	EFF=UTR_5_PRIME(MODIFIER 179 SPAC17A2.11.1:exon:1 transcript:SPAC17A2.11.1 1 1)
I	3578638	AT	A	0.0	EFF=FRAME_SHIFT(HIGH -/- -897 SPNCRNA.918.1:exon:1 transcript:SPNCRNA.918.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE),UTR_3_PRIME(MODIFIER 877 SPAC17A2.11.1:exon:1 transcript:SPAC17A2.11.1 1 1)
I	3665610	G	GC	2126.97	EFF=INTERGENIC(MODIFIER 1)
I	3730369	A	AT	1534.97	EFF=INTRON(MODIFIER) SPAC15E1.08.1:exon:1 transcript:SPAC15E1.08.1 2 1)
I	3855790	GT	G	2414.97	EFF=FRAME_SHIFT(HIGH -/- -668 SPAC1071.01c.1:exon:1 transcript:SPAC1071.01c.1 1 1)
I	3940025	G	GT	544.97	EFF=FRAME_SHIFT(HIGH aac/aaAc N104K?) SPNCRNA.949.1:exon:1 transcript:SPNCRNA.949.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE),UTR_5_PRIME(MODIFIER 237 SPAC2F3.09.1:exon:1 transcript:SPAC2F3.09.1 1 1)
I	4055052	T	TA	99.97	EFF=INTRON(MODIFIER) SPAC19G12.07c.1:exon:1 transcript:SPAC19G12.07c.1 2 1)
I	4261340	C	CT	461.97	EFF=INTERGENIC(MODIFIER 1)
I	4276890	G	GT	0.0	EFF=UTR_3_PRIME(MODIFIER 105 SPAC2C4.09.1:exon:1 transcript:SPAC2C4.09.1 3 1)
I	4304169	G	GA	1140.97	EFF=INTERGENIC(MODIFIER 1)
I	4374342	AG	A	2563.97	EFF=INTRON(MODIFIER) SPAC23D3.14c.1:exon:1 transcript:SPAC23D3.14c.1 2 1)
I	4407494	T	TG	2501.97	EFF=FRAME_SHIFT(HIGH cgc/cgC R590R?) SPAC29E6.03c.1:exon:1 transcript:SPAC29E6.03c.1 2 1),UTR_3_PRIME(MODIFIER 1559 SPAC29E6.02.1:exon:1 transcript:SPAC29E6.02.1 5 1)
I	4410191	CG	C	2553.97	EFF=FRAME_SHIFT(HIGH -/- -140 SPAC29E6.04.1:exon:1 transcript:SPAC29E6.04.1 1 1),SPlice_SITE_REGION(LOW) SPAC29E6.04.1:exon:1 transcript:SPAC29E6.04.1 1 1),UTR_3_PRIME(MODIFIER 413 SPAC29E6.05c.1:exon:1 transcript:SPAC29E6.05c.1 2 1)
I	4431856	G	GA	2287.97	EFF=INTERGENIC(MODIFIER 1)
I	4431868	C	CA	2151.97	EFF=INTRAGENIC(MODIFIER) sfp1 1),UTR_3_PRIME(MODIFIER 435 SPAC16.05c.1:exon:1 transcript:SPAC16.05c.1 1 1)
I	4455149	G	GA	88.97	EFF=UTR_5_PRIME(MODIFIER 79 SPAC9E9.09c.1:exon:1 transcript:SPAC9E9.09c.1 1 1)
I	4721875	G	GA	929.97	EFF=FRAME_SHIFT(HIGH gaa/gAaa E208E?) SPNCRNA.1018.1:exon:1 transcript:SPNCRNA.1018.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS),UTR_3_PRIME(MODIFIER 472 SPAC1834.13.1:exon:1 transcript:SPAC1834.13.1 1 1)
I	4764253	G	GA	110.97	EFF=INTERGENIC(MODIFIER 1)
I	5055030	GA	G	1321.97	EFF=INTERGENIC(MODIFIER 1)
I	5142627	A	AG	2501.97	EFF=FRAME_SHIFT(HIGH aag/aaGg K74K?) SPAC29A4.03c.1:exon:1 transcript:SPAC29A4.03c.1 1 1),SPlice_SITE_REGION(LOW) SPAC29A4.03c.1:exon:1 transcript:SPAC29A4.03c.1 1 1)
I	5148414	TA	T	1655.97	EFF=INTERGENIC(MODIFIER 1)
I	5173087	C	CT	409.97	EFF=FRAME_SHIFT(HIGH agc/aAgc S132K?) SPNCRNA.1055.1:exon:1 transcript:SPNCRNA.1055.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
I	5368262	TC	T	1709.97	EFF=FRAME_SHIFT(HIGH -/- -156 SPAC4D7.09.1:exon:1 transcript:SPAC4D7.09.1 3 1)
I	5368273	G	GT	1731.97	EFF=FRAME_SHIFT(HIGH ggv/ggTt G159G?) SPAC4D7.09.1:exon:1 transcript:SPAC4D7.09.1 3 1)
I	5413497	A	AT	801.97	EFF=INTERGENIC(MODIFIER 1)
I	5426622	C	CT	78.97	EFF=FRAME_SHIFT(HIGH aag/aaAg K120K?) SPNCRNA.1082.1:exon:1 transcript:SPNCRNA.1082.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS)
II	146079	TC	T	1745.97	EFF=FRAME_SHIFT(HIGH -/- -910 SPNCRNA.1316.1:exon:1 transcript:SPNCRNA.1316.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)

Indels					
#CHROM	POS	REF	ALT	QUAL	
II	295849	CT	C	908.97	EFF=FRAME_SHIFT(HIGH -/- -374 SPNCRNA.1336.1:exon:1 transcript:SPNCRNA.1336.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS)
II	528051	A	ATGTGTGTGTGTG	0.0	EFF=CODON_INSERTION(MODERATE ata/aCACACACACAta 85THH SPNCRNA.134.1:exon:1 transcript:SPNCRNA.134.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE),CODON_INSERTION(MODERATE atg/aTGTGTGTGTGTGtg M337MCVCV SPNCRNA.133.1:exon:1 transcript:SPNCRNA.133.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE),UTR_3_PRIME(MODIFIER 1410 SPBC1685.13.1:exon:1 transcript:SPBC1685.13.1 1 1)
II	602525	TA	T	332.97	EFF=INTERGENIC(MODIFIER 1)
II	603279	AT	A	1399.97	EFF=FRAME_SHIFT(HIGH -/- -114 SPNCRNA.317.1:exon:1 transcript:SPNCRNA.317.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
II	603305	AC	A	1719.97	EFF=FRAME_SHIFT(HIGH -/- -123 SPNCRNA.317.1:exon:1 transcript:SPNCRNA.317.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
II	700009	TA	T	1913.97	EFF=INTERGENIC(MODIFIER 1)
II	888599	AC	A	2379.97	EFF=UTR_5_PRIME(MODIFIER 58 SPBC713.11c.1:exon:1 transcript:SPBC713.11c.1 1 1)
II	903106	G	GT	2313.97	EFF=UTR_3_PRIME(MODIFIER 49 SPBC216.04c.1:exon:1 transcript:SPBC216.04c.1 1 1)
II	1079843	T	TA	81.97	EFF=INTERGENIC(MODIFIER 1)
II	1146497	A	AT	0.0	EFF=UTR_5_PRIME(MODIFIER 714 wis1:exon:1 transcript:wis1 1 1)
II	1163154	AT	A	2778.97	EFF=UTR_5_PRIME(MODIFIER 73 SPBC409.12c.1:exon:1 transcript:SPBC409.12c.1 1 1)
II	1308618	GT	G	988.97	EFF=INTERGENIC(MODIFIER 1)
II	1555919	A	ATTTATT	705.97	EFF=CODON_INSERTION(MODERATE ttt/TTTATTttt F360F F SPNCRNA.1443.1:exon:1 transcript:SPNCRNA.1443.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS)
II	1641053	T	TCTAGTGTCACTA	526.97	EFF=CODON_INSERTION(MODERATE tct/tCTAGTGTCACTact S314SSVST SPNCRNA.373.1:exon:1 transcript:SPNCRNA.373.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
II	1678205	TA	T	236.97	EFF=UTR_5_PRIME(MODIFIER 630 SPBC19C2.04c.1:exon:1 transcript:SPBC19C2.04c.1 1 1)
II	1716287	TG	T	352.97	EFF=INTERGENIC(MODIFIER 1)
II	1747580	TA	T	990.97	EFF=UTR_5_PRIME(MODIFIER 155 SPBC1D7.03.1:exon:1 transcript:SPBC1D7.03.1 1 1)
II	1816760	T	TC	0.0	EFF=UTR_5_PRIME(MODIFIER 12 SPBC9B6.02c.1:exon:1 transcript:SPBC9B6.02c.1 1 1)
II	1818220	CTCTCT	C	3007.97	EFF=UTR_5_PRIME(MODIFIER 52 SPBC9B6.03.1:exon:1 transcript:SPBC9B6.03.1 1 1)
II	1861420	TG	T	2919.97	EFF=INTERGENIC(MODIFIER 1)
II	1867795	A	AT	240.97	EFF=INTERGENIC(MODIFIER 1)
II	1873779	T	TA	1500.97	EFF=UTR_5_PRIME(MODIFIER 679 SPBC3H7.05c.1:exon:1 transcript:SPBC3H7.05c.1 1 1)
II	1877294	A	AT	309.97	EFF=FRAME_SHIFT(HIGH ttt/Ttt F1F?) SPNCRNA.1472.1:exon:1 transcript:SPNCRNA.1472.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS)
II	1941966	A	AT	778.97	EFF=FRAME_SHIFT(HIGH ctc/cAtc L142H?) SPNCRNA.1479.1:exon:1 transcript:SPNCRNA.1479.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
II	1941982	A	AT	732.97	EFF=FRAME_SHIFT(HIGH ctc/ctcA L136L?) SPNCRNA.1479.1:exon:1 transcript:SPNCRNA.1479.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
II	1948953	GA	G	548.97	EFF=INTRON(MODIFIER SPBC16E9.16c.1:exon:1 transcript:SPBC16E9.16c.1 2 1),SPLICE_SITE_ACCEPTOR(HIGH SPBC16E9.16c.1:exon:1 transcript:SPBC16E9.16c.1 3 1),SPLICE_SITE_DONOR(HIGH SPBC16E9.16c.1:exon:1 transcript:SPBC16E9.16c.1 2 1)
II	1950050	A	AG	769.97	EFF=INTRON(MODIFIER SPBC16E9.16c.1:exon:1 transcript:SPBC16E9.16c.1 1 1),SPLICE_SITE_ACCEPTOR(HIGH SPBC16E9.16c.1:exon:1 transcript:SPBC16E9.16c.1 2 1),SPLICE_SITE_DONOR(HIGH SPBC16E9.16c.1:exon:1 transcript:SPBC16E9.16c.1 1 1)
II	1955495	T	TA	0.0	EFF=FRAME_SHIFT(HIGH tta/ttAa L910L?) SPNCRNA.1481.1:exon:1 transcript:SPNCRNA.1481.1 1 1 WARNING_TRANSCRIPT_INCOMPLETE)
II	1960392	A	AG	2908.97	EFF=FRAME_SHIFT(HIGH gaa/gaaC E475E?) SPBC1E8.03c.1:exon:1 transcript:SPBC1E8.03c.1 1 1),INTRON(MODIFIER SPBC1E8.02.1:exon:1 transcript:SPBC1E8.02.1 1 1)
II	1970829	C	CATCT	3040.97	EFF=UTR_5_PRIME(MODIFIER 84 SPBC1E8.05.1:exon:1 transcript:SPBC1E8.05.1 1 1)
II	1987101	CG	C	2882.97	EFF=FRAME_SHIFT(HIGH -/- -376 SPBC1A4.06c.1:exon:1 transcript:SPBC1A4.06c.1 3 1),SPLICE_SITE_REGION(LOW SPBC1A4.06c.1:exon:1 transcript:SPBC1A4.06c.1 3 1),UTR_3_PRIME(MODIFIER 431 SPBC1A4.05.1:exon:1 transcript:SPBC1A4.05.1 2 1)
II	1987117	TG	T	2754.97	EFF=FRAME_SHIFT(HIGH -/- -370 SPBC1A4.06c.1:exon:1 transcript:SPBC1A4.06c.1 3 1),UTR_3_PRIME(MODIFIER 447 SPBC1A4.05.1:exon:1 transcript:SPBC1A4.05.1 2 1)
II	2049891	AT	A	1705.97	EFF=FRAME_SHIFT(HIGH -/- -555 SPBC29A3.06.1:exon:1 transcript:SPBC29A3.06.1 1 1),SPLICE_SITE_REGION(LOW SPBC29A3.06.1:exon:1 transcript:SPBC29A3.06.1 1 1)
II	2053516	G	GC	2418.97	EFF=FRAME_SHIFT(HIGH gcc/gCcc A162A?) SPBC29A3.08.1:exon:1 transcript:SPBC29A3.08.1 1 1),SPLICE_SITE_REGION(LOW SPBC29A3.08.1:exon:1 transcript:SPBC29A3.08.1 1 1)
II	2108180	T	TA	3930.97	EFF=FRAME_SHIFT(HIGH tgg/tggT W202W?) SPBC23G7.06c.1:exon:1 transcript:SPBC23G7.06c.1 1 1),SPLICE_SITE_REGION(LOW SPBC23G7.06c.1:exon:1 transcript:SPBC23G7.06c.1 1 1)
II	2147726	G	GT	1700.97	EFF=UTR_3_PRIME(MODIFIER 29 SPBC1711.07.1:exon:1 transcript:SPBC1711.07.1 1 1)
II	2187407	G	GA	1813.97	EFF=INTERGENIC(MODIFIER 1)
II	2199987	ATAACC	A	1214.97	EFF=INTERGENIC(MODIFIER 1)
II	2219928	A	AT	2544.97	EFF=FRAME_SHIFT(HIGH cat/caAt H131Q?) SPBC14C8.09c.1:exon:1 transcript:SPBC14C8.09c.1 2 1),UTR_5_PRIME(MODIFIER 834 SPBC14C8.10.1.five_prime_UTR:1 transcript:SPBC14C8.10.1 2 1)
II	2293297	GA	G	2079.97	EFF=UTR_3_PRIME(MODIFIER 95 SPBC16H5.04.1:exon:1 transcript:SPBC16H5.04.1 3 1)
II	2322353	G	GA	1110.97	EFF=UTR_3_PRIME(MODIFIER 22 SPBC24C6.02.1:exon:1 transcript:SPBC24C6.02.1 2 1)
II	2592423	T	TA	449.97	EFF=INTRON(MODIFIER SPBC2G5.07c.1:exon:1 transcript:SPBC2G5.07c.1 3 1)
II	2630607	C	CA	0.0	EFF=UTR_3_PRIME(MODIFIER 153 SPBC6B1.02.1:exon:1 transcript:SPBC6B1.02.1 1 1)
II	2709414	G	GC	1997.97	EFF=FRAME_SHIFT(HIGH gct/gCct A447A?) SPBC4F6.10.1:exon:1 transcript:SPBC4F6.10.1 1 1),SPLICE_SITE_REGION(LOW SPBC4F6.10.1:exon:1 transcript:SPBC4F6.10.1 1 1)
II	2713912	C	CT	383.97	EFF=UTR_3_PRIME(MODIFIER 29 SPBC4F6.12.1:exon:1 transcript:SPBC4F6.12.1 2 1)
II	2755338	CAT	C	309.97	EFF=INTERGENIC(MODIFIER 1)
II	2788933	C	CG	3400.97	EFF=UTR_3_PRIME(MODIFIER 137 SPBC32F12.03c.1:exon:1 transcript:SPBC32F12.03c.1 1 1)
II	2798040	CT	C	2394.97	EFF=FRAME_SHIFT(HIGH -/- -156 SPBC32F12.08c.1:exon:1 transcript:SPBC32F12.08c.1 1 1)
II	2798514	AG	A	1714.97	EFF=UTR_5_PRIME(MODIFIER 8 SPBC32F12.08c.1:exon:1 transcript:SPBC32F12.08c.1 1 1)
II	2811495	CA	C	3589.97	EFF=INTRON(MODIFIER SPBC32F12.12c.1:exon:1 transcript:SPBC32F12.12c.1 2 1)
II	2938956	C	CT	103.97	EFF=INTERGENIC(MODIFIER 1)
II	3040332	C	CG	2719.97	EFF=INTRON(MODIFIER SPBC13E7.01.1:exon:1 transcript:SPBC13E7.01.1 1 1),SPLICE_SITE_ACCEPTOR(HIGH SPBC13E7.01.1:exon:1 transcript:SPBC13E7.01.1 2 1),SPLICE_SITE_DONOR(HIGH SPBC13E7.01.1:exon:1 transcript:SPBC13E7.01.1 1 1)
II	3058544	TG	T	165.97	EFF=UTR_3_PRIME(MODIFIER 2089 SPBC13E7.09.1:exon:1 transcript:SPBC13E7.09.1 4 1),UTR_5_PRIME(MODIFIER 89 SPBC13E7.10c.1:exon:1 transcript:SPBC13E7.10c.1 1 1)
II	3619003	A	AG	1997.97	EFF=FRAME_SHIFT(HIGH agt/aGgt S240R?) SPBC16D10.10.1:exon:1 transcript:SPBC16D10.10.1 5 1)
II	3838101	CA	C	92.97	EFF=INTERGENIC(MODIFIER 1)

Indels					
#CHROM	POS	REF	ALT	QUAL	
II	3860185	CTAGTA	C	1620.97	EFF=INTERGENIC(MODIFIER 1)
II	4254960	GA	G	1694.97	EFF=UTR_3_PRIME(MODIFIER 521 SPBC1652.01.1:exon:1 transcript:SPBC1652.01.1 1 1)
II	4308414	C	CA	85.97	EFF=INTRON(MODIFIER SPBC543.05c.1:exon:1 transcript:SPBC543.05c.1 4 1)
II	4537387	AAATAAGAG	A	2328.97	SF=0,1
III	138018	C	CA	554.97	EFF=UTR_3_PRIME(MODIFIER 77 SPCC330.14c.1:exon:1 transcript:SPCC330.14c.1 1 1)
III	140890	C	CT	1105.97	EFF=UTR_5_PRIME(MODIFIER 698 SPCC320.14.1:exon:1 transcript:SPCC320.14.1 1 1)
III	221285	TTGCATTTCCTATCCCTCCAAA	T	1103.97	EFF=CODON_DELETION(MODERATE) ttggagggataggaaatgca/- FGGIGNA329- SPCC548.03c.1:exon:1 transcript:SPCC548.03c.1 6 1)
III	719955	AC	A	979.97	EFF=INTERGENIC(MODIFIER 1)
III	720683	A	AT	40.97	EFF=FRAME_SHIFT(HIGH aaa/aaaA K295K? SPNCRNA.1154.1:exon:1 transcript:SPNCRNA.1154.1 1 1 WARNING_TRANSCRIPT_MULTIPLE_STOP_CODONS)
III	750204	CG	C	0.0	EFF=FRAME_SHIFT(HIGH -/- 312 SPCC14G10.04.1:exon:1 transcript:SPCC14G10.04.1 4 1)
III	867865	CT	C	237.97	EFF=UTR_5_PRIME(MODIFIER 130 SPCC16A11.01.1:exon:1 transcript:SPCC16A11.01.1 1 1)
III	903387	A	AAAT	2041.97	EFF=UTR_3_PRIME(MODIFIER 162 SPCC24B10.02c.1:exon:1 transcript:SPCC24B10.02c.1 1 1),UTR_3_PRIME(MODIFIER 251 SPCC16A11.17.1:exon:1 transcript:SPCC16A11.17.1 1 1)
III	1016311	G	GC	2368.97	EFF=INTERGENIC(MODIFIER 1)
III	1079346	A	AG	163.97	EFF=INTERGENIC(MODIFIER 1)
III	1168405	TA	T	567.97	EFF=INTERGENIC(MODIFIER 1)
III	1663670	A	AT	0.0	EFF=FRAME_SHIFT(HIGH gta/gAta V317D? SPCC1902.02.1:exon:1 transcript:SPCC1902.02.1 3 1)
III	1663677	GCTCCTTGGCGATTTC	G	0.0	EFF=CODON_DELETION(MODERATE) gaaatcgccaaggag/- EIAKE310- SPCC1902.02.1:exon:1 transcript:SPCC1902.02.1 3 1)
III	1663694	ACTG	A	0.0	EFF=CODON_CHANGE_PLUS_CODON_DELETION(MODERATE) ccagtc/ctc PV308L SPCC1902.02.1:exon:1 transcript:SPCC1902.02.1 3 1)
III	1685487	G	GA	2207.97	EFF=INTERGENIC(MODIFIER 1)
III	1756400	TA	T	3541.97	EFF=INTRON(MODIFIER SPCC1450.12.1:exon:1 transcript:SPCC1450.12.1 4 1)
III	1774235	T	TGATC	6019.97	EFF=FRAME_SHIFT(HIGH aag/aagGATC K398KD? SPCC1442.04c.1:exon:1 transcript:SPCC1442.04c.1 1 1),SPLICE_SITE_REGION(LOW) SPCC1442.04c.1:exon:1 transcript:SPCC1442.04c.1 1 1)
III	1929127	AG	A	152.97	EFF=INTERGENIC(MODIFIER 1)
III	2122889	T	TA	1612.97	EFF=FRAME_SHIFT(HIGH tta/ttTa L299F? SPCC126.04c.1:exon:1 transcript:SPCC126.04c.1 2 1)
III	2352293	A	AG	0.0	EFF=UTR_5_PRIME(MODIFIER 88 SPCC70.03c.1:exon:1 transcript:SPCC70.03c.1 1 1)

Supplementary Table 1. Differences between PB1623 and 972 h- established on 243 sequenced genomes. SNVs and indels that are shared among the 243 strains previously sequenced and polymorphic to the 972 h- reference strain are listed. The predicted effect of the polymorphisms was obtained with the snpEff algorithm ¹. The sequencing data are publicly available at the NCBI BioProject database (accession no: PRJNA413662).

Supplementary Table 2: Phenotypic characterization of the 150 long-term survivors.

Clone #	Sorbitol 2 M	KCl 0.8 M	SDS 0.01%	TBZ 14 µg/mL	HU 4 mM	18 °C	37 °C	CaCl2 0.3 M	Strain name	S/MAPK mutation
Wild±type									PB1623	
1	+	+	+	+	±	+	+			
2	+	+	+	+	+	+	+			
3	+	+	+	+	±	+	+			
4	+	+	+	+	±	+	+			
5	+	+	+	+	±	+	+			
6	+	+	+	+	+	+	+			
7									1P	<i>pmc1-T326A</i>
8	+	+	+	+	±	+	+			
9	+	+	+	+	±	+	+			
10	+	+	+	+	±	+	+			
11	+	+	+	+	±	+	+			
12	+	+	+	+	+	+	±			
13								ND		
14								ND		
15	+	+	+	±	+	+	+			
16	+	+	+	+	+	+	+			
17								ND		
18	+	+	+	+	±	+	+			
19	+	+	+	+	±	+	+			
20	+	+	+	+	±	+	+			
21	+	+	+	+	±	+	+			
22	+	+	+	+	±	+	+			
23	+	+	+	±	+	+	+			
24									2P	<i>pmc1-2906 -C</i>
25	+	+	+	+	±	+	+			
26								ND		
27								ND		
28	+	+	+	+	+	+	+			
29	+	+	+	+	±	+	+			
30	+	+	+	+	±	+	+			
31	+	+	+	+	±	+	+			
32	+	+	+	+	±	+	+			
33	+	+	+	+	±	+	+			
34	+	+	+	+	±	+	+			
35									3P	<i>sgf73-896 +T</i>
36	+	+	+	+	±	+	+			
37	+	+	+	+	+	+	+			
38	+	+	+	+	±	+	+			
39									4P	<i>sgf73-896 +T</i>
40	+	+	+	+	+	+	±			
41	+	+	+	+	±	+	+			
42	+	+	+	+	+	+	+			
43	+	+	+	+	±	+	+			
44									5P	<i>sgf73-896 +T</i>
45	+	+	+	+	±	+	+			
46	+	+	+	+	±	+	+			
47	+	+	+	+	+	+	+			
48									6P	<i>sty1-C74G</i>
49	+	+	+	±	±	+	+			
50	+	+	+	+	±	+	+			
51	+	+	+	+	±	+	+			
52	+	+	+	+	±	+	+			
53	+	+	+	+	+	+	+			
54	+	+	+	+	±	+	+			

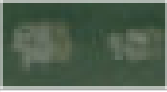
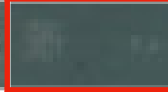
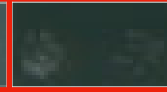
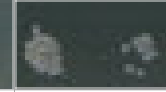
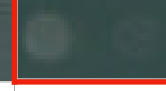
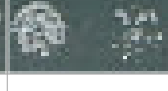
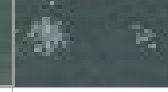
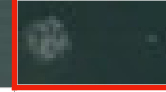
Strong phenotype

Sensitivity

Mild sensitivity

ND: Not Determined

Clone #	Sorbitol 2 M	KCl 0.8 M	SDS 0.01%	TBZ 14 µg/mL	HU 4 mM	18 °C	37 °C	CaCl2 0.3 M	Strain name	S/MAPK mutation
55									7P	<i>sgf73-896 +T</i>
56	+	+	+	+	±	+	+			
57	+	+	+	+	±	+	+			
58	+	+	+	+	+	+	+			
59	+	+	+	+	+	+	+			
60								ND		
61	+	+	+	+	+	+	+			
62	+	+	+	+	+	+	+			
63	+	+	+	+	+	+	+			
64	+	+	+	+	+	+	+			
65								ND		
66	+	+	+	+	+	+	+			
67	+	+	+	+	±	+	+			
68	+	+	+	+	+	+	+			
69								—	8P	<i>pmc1-C382T</i>
70								ND		
71								+	9P	<i>win1-1086 -G</i>
72	+	+	+	+	+	+	+			
73	+	+	+	+	+	+	+			
74	+	+	+	+	+	+	+			
75	+	+	+	+	+	+	+			
76	+	+	+	+	+	+	+			
77	+	+	+	+	+	+	+			
78	+	+	+	+	+	+	+			
79	+	+	+	+	+	+	+			
80	+	+	+	+	+	+	+			
81	+	+	+	+	+	+	+			
82	+	+	+	+	+	+	+			
83	+	+	+	+	+	+	+			
84	+	+	+	+	+	+	+			
85	+	+	+	+	+	+	+			
86	+	+	+	+	+	+	+			
87	+	+	+	+	+	+	+			
88	+	+	+	+	+	+	+			
89	+	+	+	+	+	+	+			
90	+	+	+	+	+	+	+			
91	+	+	+	+	+	+	+			
92	+	+	+	+	+	+	+			
93								ND	10P	<i>cut1 + qcr6</i>
94	+	+	+	+	+	+	+			
95								ND		
96								ND		
97								ND		
98								ND	11P	SPCC14G10.04
99								ND		
100								ND		
101	+	+	+	+	+	+	+			
102								ND		
103	+	+	+	+	+	+	+			
104	+	+	+	+	+	+	+			
105	+	+	+	+	+	+	+			
106								ND	12P	<i>meu23</i>
107	+	+	+	+	+	+	+			
108	+	+	+	+	+	+	+			

Clone #	Sorbitol 2 M	KCl 0.8 M	SDS 0.01%	TBZ 14 µg/mL	HU 4 mM	18 °C	37 °C	CaCl2 0.3 M	Strain name	S/MAPK mutation
109	+	+	+	+	+	+	+			
110	+	+	+	+	+	+	+			
111	+	+	+	+	+	+	+			
112	+	+	+	+	+	+	+			
113	+	+	+	+	+	+	±			
114	+	+	+	+	+	+	+			
115	+	+	+	+	+	+	+			
116	+	+	+	+	+	+	+			
117								ND		
118	+	+	+	+	+	+	+			
119	+	+	+	+	+	+	+			
120	+	+	+	+	+	+	+			
121	+	+	+	+	+	+	+			
122								ND		
123	+	+	+	+	+	+	+			
124	+	+	+	+	+	+	+			
125	+	+	+	+	+	+	+			
126	+	+	+	+	+	+	+			
127								ND		
128	+	+	+	+	+	+	±			
129	+	+	+	+	+	+	+			
130	+	+	+	+	+	+	+			
131	+	+	+	+	+	+	+			
132	+	+	+	+	+	+	+			
133	+	+	+	+	+	+	+			
134	+	+	+	+	+	+	+			
135	+	+	+	+	+	+	+			
136	+	+	+	+	+	+	+			
137	+	±	+	+	+	+	±			
138	+	+	+	+	+	+	+			
139	+	+	+	+	+	+	+			
140								ND		
141	+	+	+	+	+	+	+			
142	+	+	+	+	+	+	±			
143	+	+	+	+	+	+	+			
144	+	+	+	+	+	+	+			
145	+	+	+	+	+	+	±			
146	+	+	+	+	+	+	+			
147	+	+	+	+	+	+	+			
148	+	+	+	+	+	+	+			
149	+	+	+	+	+	+	+			
150	+	+	+	+	+	+	+			

Supplementary Table 2. Phenotypic characterization of the 150 long-term survivors. The strains with a confirmed phenotype of sensitivity are highlighted in gray and drops of five-fold serial dilutions for all the conditions tested are shown. The twelve P strains selected for their phenotype to perform WGS and the genes of the S/MAPK pathways found mutated in these strains are also indicated.

Supplementary Table 3: Mutations found after 3 months of quiescence in the 48 genomes that were sequenced.							
Strain	Chromosome	Position	Reference	Alternative	Mutation type	Gene	Allele
1R	II	945943	A	T	SNP	<i>gap1</i>	<i>gap1</i> - T1460A
2R	-	-	-	-	-	-	
3R	II	1532631	A	G	SNP	SPBC83.12	
4R	I	616209	G	GT	Insertion	<i>mkh1</i>	<i>mkh1</i> -640+A
	II	1048712	G	A	SNP	<i>dre2</i> (predicted)	<i>dre2</i> -C719T
5R	I	1591589	T	A	SNP	<i>thi4</i>	<i>thi4</i> -T1490A
6R	-	-	-	-	-	-	
7R	-	-	-	-	-	-	
8R	I	207717	C	A	SNP	<i>styl</i>	<i>styl</i> -G197T
	III	175767	A	ATACATCTAA	Insertion	SPCC1235.01	
9R	-	-	-	-	-	-	
10R	I	2714004	TAACCCACAA	T	Deletion	<i>pmc1</i>	<i>pmc1</i> -2920 -9 bp
11R	-	-	-	-	-	-	
12R	III	1493950	A	G	SNP	<i>pfl4</i>	<i>pfl4</i> -T818C
13R	-	-	-	-	-	-	
14R	I	255219	A	G	SNP	SPNCRNA.630 (predicted)	
	II	693417	-36bp		Deletion	SPBPJ4664.02	
	I	613911	CTATGCTTAGA	C	Deletion	<i>mkh1</i>	<i>mkh1</i> -2929 -10 bp
15R	II	4313731	T	A	SNP	<i>pek1</i>	<i>pek1</i> - T969A
	III	903386	GAAA	G	Deletion	<i>mcm4</i>	
16R	-	-	-	-	-	-	
17R	-	-	-	-	-	-	
18R	II	731112	T	TA	Insertion	<i>pmk1</i>	<i>pmk1</i> -153+A
19R	I	2715686	C	A	SNP	<i>pmc1</i>	<i>pmc1</i> -G1246T
	II	3860184	TCTAGT	T	Deletion	Intergenic	
20R	III	1493950	A	G	SNP	<i>pfl4</i>	<i>pfl4</i> -T818C
21R	II	1134749	G	T	SNP	<i>tif452</i>	<i>tif452</i> -G251T
22R	I	616209	G	GT	Insertion	<i>mkh1</i>	<i>mkh1</i> -640+A
	I	2245479	T	TCCC	Insertion	Intergenic	
23R	II	53302	G	C	SNP	Intergenic	
	I	760579		-AT	Deletion	Intergenic	
	I	801095		-AAGTATATAT	Deletion	Intergenic	
24R	I	5090834	T	TCTACTACATCCTC	Insertion	<i>win1</i>	<i>win1</i> -394 +13 bp
25R	-	-	-	-	-	-	
26R	II	1146497	C	CA	Insertion	<i>wis1</i>	- 713 +A

27R	-	-	-	-	-	-	
28R	II	4538083	GA	G	Deletion	Intergenic	
29R	I	5091713	A	ATATCCTTCACGTCGTTC	Insertion	<i>win1</i>	<i>win1</i> -1273 + 17bp
30R	I	614355	C	T	SNP	<i>mkl1</i>	<i>mkl1</i> -G2494A
	II	1649054	A	C	SNP	<i>rec6</i>	<i>rec6</i> -T533G
31R	-	-	-	-	-	-	
32R	II	1146497	C	CA	Insertion	<i>wis1</i>	- 713 +A
33R	II	4255819	T	G	SNP	SPBC1652.02-antisense-1 (predicted)	
	I	2714492	C	CAATATTATCACCAGTAAC	Insertion	<i>pmc1</i>	<i>pmc1</i> -2441+18bp
34R	II	731112	T	TA	Insertion	<i>pmk1</i>	<i>pmk1</i> -153+A
35R	II	1049917	T	TGTAA	Insertion	<i>dre2</i> (predicted)	
36R	II	1665445	C	CTTTAT	Insertion	intergenic	
1P	I	2716606	A	T	SNP	<i>pmc1</i>	<i>pmc1</i> -T326A
2P	I	2714026	AG	A	Deletion	<i>pmc1</i>	<i>pmc1</i> -2906-C
3P	III	2122890	T	TA	Insertion	<i>sgf73</i>	<i>sgf73</i> -896+T
4P	III	2122890	T	TA	Insertion	<i>sgf73</i>	<i>sgf73</i> -896+T
	I	1544016	T	A	SNP	<i>pabp</i>	<i>pabp</i> -T1152A
5P	III	2122890	T	TA	Insertion	<i>sgf73</i>	<i>sgf73</i> -896+T
6P	I	207840	G	C	SNP	<i>styl</i>	<i>styl</i> -G74C
	I	2499161	TG	T	Deletion	SPAC23H3.04	
	II	2437855	A	C	SNP	<i>map4</i>	<i>map4</i> -A2220C
	III	1493950	A	G	SNP	<i>pfl4</i>	<i>pfl4</i> -T818C
7P	III	2122890	T	TA	Insertion	<i>sgf73</i>	<i>sgf73</i> -896+T
8P	I	2716550	G	A	SNP	<i>pmc1</i>	<i>pmc1</i> -C382T
9P	I	5091526	TG	T	Deletion	<i>win1</i>	<i>win1</i> -1086-G
10P	III	648242	T	C	SNP	<i>cut1</i>	<i>cut1</i> -T4313C
	II	4344813	A	ATTTTCTTC	Insertion	<i>qcr6</i>	<i>qcr6</i> -T325+6 bp
11P	III	748812	A	T	SNP	SPCC14G10.04	
12P	III	748812	A	T	SNP	SPCC14G10.04	
	III	99099	A	AACTATGCAAGGGACCATGAAC	Insertion	<i>meu23</i>	<i>meu23</i> -T387 +21bp
	III	2433240	A	G	SNP	SPCC569.02c-antisense-1 (predicted)	
	III	2433242	T	A	SNP	SPCC569.02c-antisense-1 (predicted)	
	III	2433279	A	G		SPCC569.02c-antisense-1 (predicted)	
	III	2433281	A	C		SPCC569.02c-antisense-1 (predicted)	

Supplementary Table 3. Mutations found after 3 months of quiescence in the 48 genomes that were sequenced. In green are highlighted the genes belonging to the S/MAPK pathways.

Supplementary Table 4: Distribution of the mutants in the S/MAPK pathway among 48 strains sequenced after three months of quiescence.				
genes	P strains	R strains	function	human homologue
<i>win1</i>	1	2	SAPKKK	MAP3K4
<i>wis1</i>	-	2	SAPKK	MAP2K1
<i>sty1</i>	1	1	SAPK	p38
<i>mkh1</i>	-	4	MAPKKK	MEKK1
<i>pek1</i>	-	1	MAPKK	MEK2
<i>pmk1</i>	-	2	MAPK	ERK
<i>pmc1</i>	3	3	Calcium efflux	PMCA
<i>sgf73</i>	4	-	SAGA subunit	ATXN7
<i>tif452</i>	-	1	CAP binding	eIF4E

Supplementary Table 4. Distribution of the mutants in the S/MAPK pathways among 48 strains sequenced after three months of quiescence. The P strains were picked for their phenotype while the R strains were picked randomly.

Supplementary Table 5. Mutations identified in the targeted resequencing experiment									
Sample	Mutation type	Gene	cDNA	Frequency	Ref	VarAllele	Mutation	time in quiescence	P-value < 0.05
culture_0	Insertion	<i>sgf73</i>	896	0.9 %	T	+A	+T	2_months	9.69E-23
culture_0	Insertion	<i>win1</i>	394	0.2 %	T	+CTACTACATCCTC	+CTACTACATCCTC	2_months	2.96E-02
culture_0	Insertion	<i>win1</i>	1273	0.3 %	A	+TATCCTTCACGTCGTTTC	+TATCCTTCACGTCGTTTC	2_months	2.44E-02
culture_0	Insertion	<i>mkh1</i>	640	13.0 %	G	+T	+A	2_months	0.00E+00
culture_0	SNP	<i>mkh1</i>	959	1.4 %	A	G	T>C	2_months	1.13E-43
culture_0	SNP	<i>mkh1</i>	1885	0.2 %	A	G	T>C	2_months	4.80E-02
culture_0	SNP	<i>mkh1</i>	2914	0.4 %	A	G	T>C	2_months	3.57E-03
culture_0	SNP	<i>mkh1</i>	3351	0.4 %	T	A	A>T	2_months	1.32E-14
culture_0	Deletion	<i>pmk1</i>	254	0.7 %	A	-T	-T	2_months	1.06E-02
culture_0	SNP	<i>tif452</i>	251	0.2 %	G	T	G>T	2_months	3.25E-05
total = 17.7%									
culture_0	Insertion	<i>sgf73</i>	896	4.3 %	T	+A	+T	3_months	2.12E-169
culture_0	Insertion	<i>win1</i>	394	1.5 %	T	+CTACTACATCCTC	+CTACTACATCCTC	3_months	5.40E-80
culture_0	Insertion	<i>win1</i>	549	0.3 %	T	+GCACAGAGGAC	+GCACAGAGGAC	3_months	1.66E-05
culture_0	Deletion	<i>win1</i>	756	0.2 %	C	-AG	-AG	3_months	2.20E-02
culture_0	Insertion	<i>win1</i>	1158	0.3 %	G	+TATGGTTGATGTGC	+TATGGTTGATGTGC	3_months	4.60E-02
culture_0	Insertion	<i>win1</i>	1273	1.5 %	A	+TATCCTTCACGTCGTTTC	+TATCCTTCACGTCGTTTC	3_months	6.51E-13
culture_0	Deletion	<i>win1</i>	1573	0.7 %	C	-A	-A	3_months	2.01E-10
culture_0	SNP	<i>win1</i>	2348	0.2 %	C	A	C>A	3_months	1.72E-03
culture_0	SNP	<i>win1</i>	2556	0.2 %	T	G	T>G	3_months	2.89E-02
culture_0	Insertion	<i>win1</i>	3010	0.9 %	T	+A	+A	3_months	7.55E-65
culture_0	SNP	<i>sty1</i>	74	0.6 %	G	C	C>G	3_months	1.12E-11
culture_0	SNP	<i>sty1</i>	197	0.5 %	C	A	G>T	3_months	6.15E-12
culture_0	Insertion	<i>mkh1</i>	640	9.0 %	G	+T	+A	3_months	6.41E-291
culture_0	SNP	<i>mkh1</i>	959	0.9 %	A	G	T>C	3_months	2.07E-29
culture_0	Insertion	<i>pmk1</i>	1091	0.3 %	C	+CAACAAACCCAACAGTCAACATCCCG	+CAACAAACCCAACAGTCAACATCCCG	3_months	2.80E-05
culture_0	SNP	<i>tif452</i>	251	0.7 %	G	T	G>T	3_months	2.11E-40
total = 22.1%									
culture_1	Insertion	<i>win1</i>	1273	0.3 %	A	+TATCCTTCACGTCGTTTC	+TATCCTTCACGTCGTTTC	2_months	1.73E-02
culture_1	Insertion	<i>win1</i>	3010	0.9 %	T	+A	+A	2_months	3.13E-79
culture_1	Insertion	<i>sty1</i>	216	0.4 %	C	+TTTAATAGT	+ACTATTAA	2_months	5.70E-13
culture_1	SNP	<i>sty1</i>	647	1.1 %	C	G	G>C	2_months	5.47E-18
culture_1	SNP	<i>mkh1</i>	639	1.9 %	T	C	A>G	2_months	7.29E-63
culture_1	Insertion	<i>mkh1</i>	640	21.3 %	G	+T	+A	2_months	0.00E+00
culture_1	SNP	<i>mkh1</i>	2378	0.8 %	G	C	C>G	2_months	1.97E-36
culture_1	SNP	<i>mkh1</i>	3138	0.3 %	T	G	A>C	2_months	8.15E-08
culture_1	Deletion	<i>mkh1</i>	3236	1.6 %	A	-GCATTTCACAGTAAAGCAT	-ATGCTTTACTGTGAATGC	2_months	1.34E-102
culture_1	SNP	<i>pmk1</i>	497	1.2 %	G	T	G>T	2_months	1.13E-27
total = 29.8%									
culture_1	SNP	<i>win1</i>	2666	1.2 %	C	T	C>T	3_months	3.02E-59
culture_1	Insertion	<i>win1</i>	3010	1.2 %	T	+A	+A	3_months	2.62E-56
culture_1	Insertion	<i>sty1</i>	216	1.8 %	C	+TTTAATAGT	+ACTATTAA	3_months	6.60E-52
culture_1	Insertion	<i>mkh1</i>	640	10.2 %	G	+T	+A	3_months	4.73E-234
culture_1	Deletion	<i>mkh1</i>	3236	0.2 %	A	-GCATTTCACAGTAAAGCAT	-ATGCTTTACTGTGAATGC	3_months	1.91E-03
culture_1	SNP	<i>pmk1</i>	577	6.1 %	T	G	T>G	3_months	5.94E-146
total = 20.7%									
culture_2	Insertion	<i>sgf73</i>	896	9.6 %	T	+A	+T	2_months	0.00E+00
culture_2	Insertion	<i>win1</i>	393	0.6 %	A	+TCTACTACATCCT	+TCTACTACATCCT	2_months	7.32E-19
culture_2	SNP	<i>win1</i>	811	0.8 %	C	A	C>A	2_months	5.50E-38
culture_2	Deletion	<i>win1</i>	2339	0.8 %	T	-ACGTA	-ACGTA	2_months	6.53E-18
culture_2	SNP	<i>win1</i>	2379	0.3 %	T	G	T>G	2_months	5.01E-10
culture_2	SNP	<i>sty1</i>	188	3.7 %	C	G	G>C	2_months	1.30E-146
culture_2	SNP	<i>mkh1</i>	250	4.7 %	C	A	G>T	2_months	2.41E-227
culture_2	Insertion	<i>mkh1</i>	640	27.0 %	G	+T	+A	2_months	0.00E+00
culture_2	Insertion	<i>mkh1</i>	2517	1.2 %	A	+TACCCTTACCATAAG	+CTATGGTAAAGGTA	2_months	2.19E-34
culture_2	SNP	<i>mkh1</i>	3145	0.2 %	C	A	G>T	2_months	1.06E-02
culture_2	Deletion	<i>pmk1</i>	251	1.7 %	T	-A	-A	2_months	2.26E-24
culture_2	SNP	<i>pmk1</i>	592	2.5 %	G	A	G>A	2_months	3.35E-100
culture_2	Deletion	<i>pmk1</i>	1097	10.4 %	A	-ACCCAACAGTCAACAT	-ACCCAACAGTCAACAT	2_months	2.58E-317
culture_2	Insertion	<i>pmc1</i>	2441	1.2 %	G	+CAGTAAACATATTATCAC	+GTGATAATATTGTTACTG	2_months	8.66E-12
total = 64.7%									
culture_2	Insertion	<i>sgf73</i>	896	43.5 %	T	+A	+T	3_months	0.00E+00
culture_2	Deletion	<i>win1</i>	2339	0.2 %	T	-ACGTA	-ACGTA	3_months	1.75E-04
culture_2	SNP	<i>sty1</i>	188	0.7 %	C	G	G>C	3_months	5.97E-27
culture_2	Insertion	<i>mkh1</i>	640	5.3 %	G	+T	+A	3_months	1.39E-199
culture_2	SNP	<i>pmk1</i>	592	2.7 %	G	A	G>A	3_months	2.86E-100
total = 52.4%									
culture_3	Insertion	<i>win1</i>	383	0.5 %	G	+ATACTAGTAAT	+ATACTAGTAAT	2_months	1.12E-15
culture_3	Insertion	<i>win1</i>	394	1.6 %	T	+CTACTACATC	+CTACTACATC	2_months	1.15E-77
culture_3	Deletion	<i>win1</i>	504	1.7 %	C	-A	-A	2_months	1.00E-162
culture_3	Deletion	<i>win1</i>	556	0.6 %	A	-G	-G	2_months	2.48E-19
culture_3	Insertion	<i>win1</i>	1273	0.6 %	A	+TATCCTTCACGTCGTTTC	+TATCCTTCACGTCGTTTC	2_months	8.84E-07
culture_3	SNP	<i>win1</i>	1859	0.6 %	C	T	C>T	2_months	2.15E-18
culture_3	Deletion	<i>win1</i>	2363	3.8 %	G	-TTGATTTCCCCTAA	-TTGATTTCCCCTAA	2_months	0.00E+00
culture_3	Deletion	<i>win1</i>	2545	0.7 %	C	-CGTT	-CGTT	2_months	3.27E-26
culture_3	Insertion	<i>win1</i>	2768	0.7 %	A	+ATCTAAAATTAAGATTAAAGCTGGAATTAAAGTCCAATGAATTCAACAATTG	+ATCTAAAATTAAGATTAAAGCTGGAATTAAAGTCCAATGAATTCAACAATTG	2_months	2.24E-25
culture_3	Deletion	<i>win1</i>	3680	3.5 %	C	-A	-A	2_months	1.28E-90
culture_3	SNP	<i>win1</i>	4179	0.2 %	G	T	G>T	2_months	1.29E-04
culture_3	Deletion	<i>mkh1</i>	236	1.2 %	A	-T	-A	2_months	3.22E-58
culture_3	Insertion	<i>mkh1</i>	640	15.8 %	G	+T	+A	2_months	0.00E+00
culture_3	Complex	<i>mkh1</i>	1884	0.2 %	G	A	CT>TG	2_months	4.53E-04
culture_3	Insertion	<i>mkh1</i>	2254	0.7 %	G	+A	+T	2_months	1.15E-03
culture_3	SNP	<i>mkh1</i>	3124	0.2 %	C	A	G>T	2_months	2.59E-02
culture_3	SNP	<i>mkh1</i>	3137	0.5 %	A	C	T>G	2_months	4.67E-16
culture_3	Deletion	<i>pmk1</i>	435	0.7 %	T	-TA	-TA	2_months	1.49E-13
culture_3	Insertion	<i>pmk1</i>	902	0.2 %	A	+GACGTATTTCGGTT	+GACGTATTTCGGTT	2_months	5.64E-03
total = 34.0%									
culture_3	Insertion	<i>win1</i>	383	0.2 %	G	+ATACTAGTAAT	+ATACTAGTAAT	3_months	1.40E-07
culture_3	Insertion	<i>win1</i>	394	1.1 %	T	+CTACTACATC	+CTACTACATC	3_months	2.14E-93
culture_3	Deletion	<i>win1</i>	504	1.1 %	C	-A	-A	3_months	1.14E-153
culture_3	Deletion	<i>win1</i>	556	0.7 %	A	-G	-G	3_months	3.91E-42
culture_3	Insertion	<i>win1</i>	1273	0.2 %	A	+TATCCTTCACGTCGTTTC	+TATCCTTCACGTCGTTTC	3_months	1.78E-02
culture_3	SNP	<i>win1</i>	1859	0.8 %	C	T	C>T	3_months	5.76E-57
culture_3	Deletion	<i>win1</i>	2363	3.8 %	G	-TTGATTTCCCCTAA	-TTGATTTCCCCTAA	3_months	0.00E+00
culture_3	Deletion	<i>win1</i>	2545	0.9 %	C	-CGTT	-CGTT	3_months	1.55E-60
culture_3	Insertion	<i>win1</i>	2768	0.6 %	A	+ATCTAAAATTAAGATTAAAGCTGGAATTAAAGTCCAATGAATTCAACAATTG	+ATCTAAAATTAAGATTAAAGCTGGAATTAAAGTCCAATGAATTCAACAATTG	3_months	4.17E-36
culture_3	Deletion	<i>win1</i>	3680	4.4 %	C	-A	-A	3_months	2.17E-146
culture_3	Deletion	<i>mkh1</i>	236	1.4 %	A	-T	-A	3_months	1.43E-105
culture_3	Insertion	<i>mkh1</i>	640	21.5 %	G	+T	+A	3_months	0.00E+00
culture_3	Insertion	<i>mkh1</i>	2593	0.2 %	C	+GGCCATTAAATAGTTTGTGGTATTCCAACCTTGCTTTACT	+AGTAAAGCAAGTTGAAATACCACAACCTATTATATGGCC	3_months	2.01E-03
culture_3	Deletion	<i>mkh1</i>	3340	1.2 %	C	-T	-A	3_months	6.81E-89
culture_3	Deletion	<i>pmk1</i>	435	0.3 %	T	-TA	-TA	3_months	4.45E-06
culture_3	SNP	<i>pmc1</i>	280	0.2 %	C	A	G>T	3_months	1.79E-03
total = 38.6%									
subculture_1	Complex	<i>win1</i>	357	1.2 %	A	-TCGTGCT*T>A*T>A*C>A	-TCGTGCT*T>A*T>A*C>A	2_months	6.00E-213
subculture_1	Insertion	<i>win1</i>	394	3.4 %	T	+CTACTACATCCTC	+CTACTACATCCTC	2_months	0.00E+00
subculture_1	Insertion	<i>win1</i>	397	0.2 %	A	+CTACATCCTC	+CTACATCCTC	2_months	6.41E-13
subculture_1	Deletion	<i>win1</i>	506	0.2 %	A	-AT	-AT	2_months	2.90E-18
subculture_1	SNP	<i>win1</i>	602	0.2 %	C	G	C>G	2_months	3.77E-06
subculture_1	Insertion	<i>win1</i>	1273	0.3 %	A	+TATCCTTCACGTCGTTTC	+TATCCTTCACGTCGTTTC	2_months	6.94E-04
subculture_1	SNP	<i>win1</i>	2478	1.8 %	T	A	T>A	2_months	0.00E+00
subculture_1	Insertion	<i>win1</i>	3010	2.5 %	T	+A	+A	2_months	0.00E+00
subculture_1	Deletion	<i>win1</i>	3305	0.5 %	G	-AGAA	-AGAA	2_months	1.55E-72
subculture_1	Insertion	<i>win1</i>	3314	0.3 %	C	+TA	+A	2_months	1.62E-35
subculture_1	SNP	<i>win1</i>	3319	0.3 %	A	G	A>G	2_months	4.13E-31
subculture_1	Insertion	<i>win1</i>	3544	0.2 %	C	+GTATCGTATTATG	+GTATCGTATTATG	2_months	9.61E-13
subculture_1	Deletion	<i>win1</i>	3752	0.3 %	C	-TT	-TT	2_months	5.54E-06
subculture_1	SNP	<i>win1</i>	4195	0.3 %	G	T	G>T	2_months	2.85E-31
subculture_1	SNP	<i>wis1</i>	868	1.6 %	C	G	G>C	2_months	0.00E+00
subculture_1	SNP	<i>wis1</i>	1108	3.4 %	A	C	T>G	2_months	0.00E+00
subculture_1	SNP	<i>wis1</i>	1493	0.3 %	T	C	A>G	2_months	4.84E-14
subculture_1	Insertion	<i>wis1</i>	1740	1.0 %	T	+A	+T	2_months	1.94E-57
subculture_1	SNP	<i>sty1</i>	562	0.2 %	A	G	T>C	2_months	5.44E-06
subculture_1	Insertion	<i>mkh1</i>	640	0.6 %	G	+T	+A	2_months	2.15E-57
subculture_1	SNP	<i>tif452</i>	390	4.3 %	G	T	G>T	2_months	0.00E+00
total = 23.1%									
subculture_2	Insertion	<i>win1</i>	394	2.4 %	T	+CTACTACATCCTC	+CTACTACATCCTC	2_months	0.00E+00
subculture_2	SNP	<i>win1</i>	1100	1.0 %	G	A	G>A	2_months	4.16E-206
subculture_2	Insertion	<i>win1</i>	1273	0.6 %	A	+TATCCTTCACGTCGTTTC	+TATCCTTCACGTCGTTTC	2_months	2.96E-32
subculture_2	SNP	<i>win1</i>	2519	0.4 %	C	A	C>A	2_months	1.50E-57
subculture_2	Deletion	<i>win1</i>	3489	1.1 %	T	-C	-C	2_months	1.62E-131
subculture_2	Deletion	<i>win1</i>	3833	0.2 %	G	-AACAGTT	-AACAGTT	2_months	2.25E-09
subculture_2	SNP	<i>wis1</i>	1380	4.6 %	A	T	T>A	2_months	0.00E+00

subculture_2	SNP	<i>wis1</i>	1511	1.0%	A	C	T>G	2_months	5,00E-286
subculture_2	SNP	<i>styl</i>	553	12.5%	T	A	A>T	2_months	0,00E+00
total = 23.8%									
subculture_3	Deletion	<i>win1</i>	292	0.6%	A	-CAGCCTC	-CAGCCTC	2_months	1,59E-79
subculture_3	Insertion	<i>win1</i>	394	0.4%	T	+CTACTACATCCTC	+CTACTACATCCTC	2_months	8,93E-42
subculture_3	Deletion	<i>win1</i>	1007	0.2%	C	-GAICT	-GAICT	2_months	1,32E-07
subculture_3	Insertion	<i>win1</i>	1273	1.8%	A	+TATCCTTCACGTCGTTTC	+TATCCTTCACGTCGTTTC	2_months	2,71E-84
subculture_3	Deletion	<i>win1</i>	1365	0.8%	A	-TG	-TG	2_months	1,12E-31
subculture_3	Deletion	<i>win1</i>	1846	0.8%	A	-T	-T	2_months	7,47E-185
subculture_3	Deletion	<i>win1</i>	1877	1.6%	A	-T	-T	2_months	0,00E+00
subculture_3	Insertion	<i>win1</i>	2179	1.8%	C	+TTCCGAAAATTGATGAG	+TTCCGAAAATTGATGAG	2_months	2,01E-31
subculture_3	Insertion	<i>win1</i>	3010	2.8%	T	+A	+A	2_months	0,00E+00
subculture_3	Insertion	<i>win1</i>	3105	0.7%	G	+T	+T	2_months	7,29E-41
subculture_3	Insertion	<i>win1</i>	3716	0.6%	G	+T	+T	2_months	2,92E-27
subculture_3	Insertion	<i>win1</i>	3880	0.6%	A	+T	+T	2_months	4,28E-24
subculture_3	SNP	<i>win1</i>	4177	0.5%	C	T	C>T	2_months	3,36E-91
subculture_3	SNP	<i>wis1</i>	944	9.2%	A	C	T>G	2_months	0,00E+00
subculture_3	Complex	<i>wis1</i>	1281	8.5%	GG	AA	AA	2_months	0,00E+00
subculture_3	SNP	<i>wis1</i>	1329	0.4%	T	A	A>T	2_months	1,91E-24
subculture_3	Insertion	<i>wis1</i>	1740	6.2%	T	+A	+T	2_months	0,00E+00
subculture_3	SNP	<i>styl</i>	1	1.0%	T	C	A>G	2_months	5,77E-103
subculture_3	SNP	<i>styl</i>	481	3.5%	C	A	G>T	2_months	0,00E+00
subculture_3	SNP	<i>styl</i>	535	1.4%	A	G	T>C	2_months	0,00E+00
subculture_3	Insertion	<i>mkh1</i>	640	1.0%	G	+T	+A	2_months	1,76E-144
subculture_3	SNP	<i>mkh1</i>	1885	0.2%	A	G	T>C	2_months	8,16E-08
subculture_3	Deletion	<i>pmc1</i>	1296	0.1%	T	-TCTG	-CAGA	2_months	2,32E-02
total = 43.4%									
subculture_4	Deletion	<i>win1</i>	241	0.2%	C	-AAGGCGAGCGCCAAAGAGGACTTATTTTCAGAAGCTTTCAGAATGGCTG	-AAGGCGAGCGCCAAAGAGGACTTATTTTCAGAAGCTTTCAGAATGGCTG	2_months	8,39E-19
subculture_4	Insertion	<i>win1</i>	394	0.5%	T	+CTACTACATCCTC	+CTACTACATCCTC	2_months	4,48E-52
subculture_4	Deletion	<i>win1</i>	2982	2.4%	C	-AT	-AT	2_months	3,69E-312
subculture_4	Insertion	<i>win1</i>	3010	0.8%	T	+A	+A	2_months	3,73E-83
subculture_4	Deletion	<i>win1</i>	3045	1.2%	G	-A	-A	2_months	1,68E-129
subculture_4	Deletion	<i>win1</i>	3050	0.8%	T	-G	-G	2_months	1,17E-71
subculture_4	Deletion	<i>win1</i>	3097	0.2%	T	-GC	-GC	2_months	5,36E-04
subculture_4	Deletion	<i>win1</i>	3352	3.6%	G	-ATTCTGA	-ATTCTGA	2_months	0,00E+00
subculture_4	Deletion	<i>win1</i>	3374	0.6%	C	-TTATAGGTAGCGGTTCT	-TTATAGGTAGCGGTTCT	2_months	2,16E-77
subculture_4	Insertion	<i>win1</i>	3507	0.5%	A	+AATGTTAGTACTTTGAACCTTTTGATCATCCT	+AATGTTAGTACTTTGAACCTTTTGATCATCCT	2_months	5,87E-53
subculture_4	Deletion	<i>win1</i>	3617	0.5%	C	-TTTTCGAAATTCTACG	-TTTTCGAAATTCTACG	2_months	7,73E-52
subculture_4	Deletion	<i>win1</i>	3618	0.2%	T	-TTTCGAA	-TTTCGAA	2_months	1,13E-16
subculture_4	Deletion	<i>win1</i>	3682	0.8%	A	-T	-T	2_months	5,36E-49
subculture_4	Deletion	<i>win1</i>	3817	0.3%	T	-TC	-TC	2_months	1,06E-09
subculture_4	SNP	<i>wis1</i>	1588	1.9%	A	C	C	2_months	0,00E+00
subculture_4	Insertion	<i>wis1</i>	1740	1.2%	T	+A	+A	2_months	2,56E-167
subculture_4	SNP	<i>styl</i>	35	1.0%	G	C	C	2_months	3,24E-122
subculture_4	Insertion	<i>mkh1</i>	640	1.6%	G	+T	+T	2_months	7,16E-247
subculture_4	Insertion	<i>mkh1</i>	2396	0.8%	G	+C	+C	2_months	2,87E-157
subculture_4	Insertion	<i>mkh1</i>	2846	0.2%	T	+TTAGA	+TTAGA	2_months	4,00E-02
subculture_4	SNP	<i>mkh1</i>	3152	0.2%	T	C	C	2_months	1,93E-06
total = 19.5%									
subculture_5	Deletion	<i>win1</i>	314	0.7%	A	-C	-C	2_months	5,12E-95
subculture_5	Deletion	<i>win1</i>	370	0.2%	C	-GTAA	-GTAA	2_months	2,78E-08
subculture_5	Insertion	<i>win1</i>	394	4.0%	T	+CTACTACATCCTC	+CTACTACATCCTC	2_months	0,00E+00
subculture_5	Deletion	<i>win1</i>	942	0.7%	C	-A	-A	2_months	6,42E-94
subculture_5	Deletion	<i>win1</i>	1024	2.0%	G	-C	-C	2_months	0,00E+00
subculture_5	SNP	<i>win1</i>	1129	0.4%	A	T	A>T	2_months	1,33E-45
subculture_5	SNP	<i>win1</i>	1251	0.2%	A	G	A>G	2_months	1,47E-02
subculture_5	Deletion	<i>win1</i>	1258	0.9%	T	-A	-A	2_months	3,32E-54
subculture_5	Deletion	<i>win1</i>	1265	2.4%	G	-AACGT	-AACGT	2_months	2,37E-189
subculture_5	Insertion	<i>win1</i>	1273	0.8%	A	+TATCCTTCACGTCGTTTC	+TATCCTTCACGTCGTTTC	2_months	1,43E-37
subculture_5	Deletion	<i>win1</i>	2376	0.2%	A	-TATACT	-TATACT	2_months	8,56E-05
subculture_5	Insertion	<i>win1</i>	3010	1.8%	T	+A	+A	2_months	0,00E+00
subculture_5	Deletion	<i>win1</i>	3142	0.2%	C	-CTTGTTTCGAAATAATCCGACCGAAGTCGTTTAT	-CTTGTTTCGAAATAATCCGACCGAAGTCGTTTAT	2_months	3,77E-03
subculture_5	Deletion	<i>win1</i>	3407	0.5%	C	-GT	-GT	2_months	6,46E-59
subculture_5	Deletion	<i>win1</i>	3626	0.3%	T	-TTCTACGTTAC	-TTCTACGTTAC	2_months	4,45E-26
subculture_5	Insertion	<i>win1</i>	3922	0.3%	A	+GGAACACCTACGTATAT	+GGAACACCTACGTATAT	2_months	1,44E-09
subculture_5	SNP	<i>win1</i>	4177	0.9%	C	T	C>T	2_months	1,02E-204
subculture_5	SNP	<i>wis1</i>	1088	1.0%	A	C	T>G	2_months	5,31E-116
subculture_5	SNP	<i>wis1</i>	1182	5.2%	A	C	T>G	2_months	0,00E+00
subculture_5	SNP	<i>wis1</i>	1427	0.7%	C	G	G>C	2_months	5,14E-50
subculture_5	SNP	<i>wis1</i>	1430	0.2%	C	T	G>A	2_months	2,63E-02
subculture_5	SNP	<i>styl</i>	545	13.2%	G	A	C>T	2_months	0,00E+00
subculture_5	SNP	<i>styl</i>	558	1.4%	T	G	A>C	2_months	0,00E+00
subculture_5	SNP	<i>styl</i>	646	0.7%	C	T	G>A	2_months	9,02E-130
subculture_5	SNP	<i>styl</i>	659	10.2%	A	T	T>A	2_months	0,00E+00
subculture_5	Insertion	<i>mkh1</i>	640	1.6%	G	+T	+A	2_months	0,00E+00
subculture_5	Deletion	<i>mkh1</i>	3302	0.2%	G	-TTGAATTCT	-AGAATTCAA	2_months	2,16E-06
total = 50.9%									
subculture_6	Deletion	<i>win1</i>	292	0.8%	A	-CAGCCTC	-CAGCCTC	2_months	8,24E-121
subculture_6	Insertion	<i>win1</i>	394	4.5%	T	+CTACTACATCCTC	+CTACTACATCCTC	2_months	0,00E+00
subculture_6	Insertion	<i>win1</i>	397	1.4%	A	+CTACATCCTC	+CTACATCCTC	2_months	5,06E-294
subculture_6	Deletion	<i>win1</i>	666	0.6%	G	-A	-A	2_months	7,66E-43
subculture_6	Deletion	<i>win1</i>	972	0.7%	A	-TATGTTGACTTCAGTTC	-TATGTTGACTTCAGTTC	2_months	8,61E-99
subculture_6	Deletion	<i>win1</i>	1253	0.5%	C	-TTGA	-TTGA	2_months	8,32E-13
subculture_6	Insertion	<i>win1</i>	1273	0.4%	A	+TATCCTTCACGTCGTTTC	+TATCCTTCACGTCGTTTC	2_months	5,44E-06
subculture_6	Deletion	<i>win1</i>	1644	0.6%	A	-T	-T	2_months	5,80E-08
subculture_6	Deletion	<i>win1</i>	1681	0.8%	C	-A	-A	2_months	6,91E-152
subculture_6	SNP	<i>win1</i>	1749	0.9%	C	T	C>T	2_months	5,12E-184
subculture_6	Deletion	<i>win1</i>	1905	1.0%	T	-C	-C	2_months	8,92E-195
subculture_6	Insertion	<i>win1</i>	2313	4.8%	C	+G	+G	2_months	0,00E+00
subculture_6	Insertion	<i>win1</i>	2512	0.7%	G	+GA	+GA	2_months	1,57E-68
subculture_6	SNP	<i>win1</i>	2762	2.2%	C	T	C>T	2_months	0,00E+00
subculture_6	Insertion	<i>win1</i>	3010	1.1%	T	+A	+A	2_months	2,47E-254
subculture_6	Insertion	<i>win1</i>	3105	1.2%	G	+TA	+TA	2_months	4,04E-96
subculture_6	Deletion	<i>win1</i>	3401	1.2%	G	-T	-T	2_months	4,04E-182
subculture_6	Insertion	<i>win1</i>	3507	1.2%	A	+AATGTTAGTACTTGAACCTTTTGATCATCCT	+AATGTTAGTACTTGAACCTTTTGATCATCCT	2_months	1,95E-173
subculture_6	SNP	<i>win1</i>	3546	1.2%	C	A	C>A	2_months	7,68E-165
subculture_6	Deletion	<i>win1</i>	3551	1.1%	T	-A	-A	2_months	5,04E-156
subculture_6	Deletion	<i>win1</i>	3682	1.3%	A	-T	-T	2_months	4,67E-78
subculture_6	Deletion	<i>win1</i>	3783	0.4%	C	-AGAT	-AGAT	2_months	8,43E-12
subculture_6	Complex	<i>win1</i>	3784	1.0%	T	T>C*-C	-T>C*-C	2_months	1,50E-50
subculture_6	Deletion	<i>win1</i>	3816	1.3%	C	-T	-T	2_months	4,48E-71
subculture_6	SNP	<i>win1</i>	4177	0.4%	C	T	C>T	2_months	1,27E-48
subculture_6	Insertion	<i>wis1</i>	1740	1.2%	T	+A	+T	2_months	4,31E-108
subculture_6	SNP	<i>styl</i>	535	4.8%	A	G	T>C	2_months	0,00E+00
subculture_6	Insertion	<i>mkh1</i>	640	2.2%	G	+T	+A	2_months	0,00E+00
subculture_6	SNP	<i>tif452</i>	260	1.0%	T	C	T>C	2_months	1,75E-295
subculture_6	SNP	<i>tif452</i>	383	0.5%	C	G	C>G	2_months	5,97E-28
total = 41.0%									
total number of mutations from independent cultures and subcultures = 195									

Supplementary Table 5. Mutations identified in the targeted resequencing experiment. The actual presence of some mutations in the population at two and three months was confirmed by Sanger sequencing (red alleles): one hundred and twenty cells per frozen pool from the two and three-month-old cultures were plated out and scored for their sensitivity to temperature, calcium, HU and SDS. The DNA of the sensitive colonies was prepared and used to amplify and sequence by Sanger the relevant target genes.

Supplementary Table 6: No increase in cell number (cells/ml) is observed after addition of traces of glutamate, ammonium chloride or yeast extract to 3-day-old cultures of the indicated strains.

Strains	day 3	day 6			
		Glu	NH ₄ Cl	YE	Mock
wild-type	3.8E+06	4.6E+06	4.4E+06	4.6E+06	4.0E+06
<i>sgf73-896</i> +T	4.0E+06	4.7E+06	4.3E+06	4.4E+06	4.0E+06
<i>pmk1-153</i> +A	3.9E+06	4.0E+06	4.0E+06	4.0E+06	3.8E+06
<i>sty1-G197T</i>	4.1E+06	4.4E+06	4.0E+06	4.2E+06	4.3E+06

Supplementary Table 6. Determination of an increase in cell number following addition of traces of nitrogen. No increase in cell number (cells/mL) is observed after addition of traces of glutamate, ammonium chloride or yeast extract to three-day-old cultures of the indicated strains. The number of cells was determined with a Beckman Coulter Z1 Particle Counter readout of a 1/100 dilution.

Supplementary Table 7. Primers Used in this Study

Primer name	Gene targeted	Primer Sequence 5' > 3'
OL1451	<i>sgf73</i>	AAAGTCGAAAATGCGTGG
OL1452	<i>sgf73</i>	TGTTTTGCTTGCAGCTTGT
OL1453	<i>sgf73</i>	TTCTTTTGCAGTTTCGAGG
OL1454	<i>sgf73</i>	TAAATCGACAGCTAAGGG
OL1455	<i>sgf73</i>	TTCTTTTCATGATTCCCAG
OL1456	<i>sgf73</i>	AAACTCGGTTCTTATCAA
OL1073	<i>win1</i>	TAGTTGCATTGACTTCGCT
OL1074	<i>win1</i>	TGGCCTTCTGTGATTTTCTT
OL1075	<i>win1</i>	CATCGTCGCTCTTTGACT
OL1076	<i>win1</i>	CTGTGCATATAATCGTCTTCCT
OL1077	<i>win1</i>	CTTCTTCCTCTTCCATTCC
OL1078	<i>win1</i>	CGAGTCCTCCTCATCATAA
OL1079	<i>win1</i>	CTTCTCTAAGCATTTCGTC
OL1080	<i>win1</i>	TAGCACATCAACCATAACC
OL1081	<i>win1</i>	GTGGATGGAAATATGGTGT
OL1082	<i>win1</i>	TGGTCCGTTTGAAATTTGAG
OL1083	<i>win1</i>	GGTTGGTAATGAGGAAATGG
OL1084	<i>win1</i>	TTGTTGAATGATATGGACGG
OL1085	<i>win1</i>	CGTCTTCTTTCTTTTCCGT
OL1086	<i>win1</i>	ATATTTACCAACCAGCG
OL1087	<i>win1</i>	CCACATCGAACACGGATT
OL1088	<i>win1</i>	CGCATATGGAGGTACACTTT
OL1089	<i>win1</i>	CGAACATGAAGGAGCATAC
OL1090	<i>win1</i>	CACATCAAGCAATGACAAAG
OL1091	<i>win1</i>	CTCACTTGTTGATTCTGC
OL1092	<i>win1</i>	CCACACATTTTCCAACCT
OL1093	<i>win1</i>	CAGTTGGGTTGGATTTCAT
OL1094	<i>win1</i>	CCTGTTCTCCTTTGTCGT
OL1095	<i>win1</i>	AAAGTAATAGAAGGGACCGA
OL1096	<i>win1</i>	CCTCAATTCTACCGTAAC
OL1097	<i>win1</i>	GAAGTGCATCGTAAAAAG
OL1098	<i>win1</i>	AATATCCATAGCACCAACC
OL1099	<i>win1</i>	GCAGCATCTAGACCAAAA
OL1100	<i>win1</i>	TTCGACTACGGATGAGTT
OL1101	<i>win1</i>	CGTTGTTTGTCTTCTGATCCC
OL1102	<i>win1</i>	TCTCTTCCTAGCGCCGTA
OL1509	<i>wis1</i>	AACAGAGCAGGCAATTAGA
OL1510	<i>wis1</i>	ACATGGCTCCTGAAAGAA

OL1511	<i>wisl</i>	GATAAGCTCCTAAAGCCA
OL1512	<i>wisl</i>	TGGTGCCTTTTTGTGGA
OL1513	<i>wisl</i>	ACTCCTTCGTCCTTGATACC
OL1514	<i>wisl</i>	CTAAACATTCCCTCCACCC
OL1515	<i>wisl</i>	GAAAATGTGGAAAAAGGGG
OL1516	<i>wisl</i>	ACAGGAGAAATTGAAGGC
OL1517	<i>wisl</i>	TCCGGCATTGATTTTGATCT
OL1518	<i>wisl</i>	CTTCTCCAAATAATCAACCC
OL1041	<i>styl</i>	CATAACATACCCCGAGAACA
OL1042	<i>styl</i>	CCGAGACCACGTTAACCA
OL1043	<i>styl</i>	ATTACTTCCATAGGCGGC
OL1044	<i>styl</i>	ACTACTTACATCGCGACC
OL1045	<i>styl</i>	TGGGTTTCAGATCACGGT
OL1046	<i>styl</i>	CCTTGCGCCTCGTTTGTT
OL1047	<i>styl</i>	AACAGCGACATTCATTCCA
OL1048	<i>styl</i>	ACATACCTCCTTACCACAAC
OL1329	<i>mkhl</i>	CGAGGAAACGCTAAGTAA
OL1330	<i>mkhl</i>	GCAAACCTGTCCATGCAA
OL1331	<i>mkhl</i>	TTCCAACACTACACATCC
OL1332	<i>mkhl</i>	TAGTGCAATATCTGGGTTTC
OL1333	<i>mkhl</i>	AATTCGCGAAACATCGAC
OL1334	<i>mkhl</i>	AAATACGACATAACGCGAG
OL1335	<i>mkhl</i>	TACAATCCTAGAGCCCCAAA
OL1336	<i>mkhl</i>	TCGCCTAAAACTCCTGAA
OL1337	<i>mkhl</i>	CCCAGTTACCAGAATTGA
OL1338	<i>mkhl</i>	CCGGACATAACGAAGCTA
OL1339	<i>mkhl</i>	GCTTCCACACTTTCCTTT
OL1340	<i>mkhl</i>	GACGTTTTTCGAGGGTTTG
OL1341	<i>mkhl</i>	GCCATTTCTCCTTTAGCAC
OL1342	<i>mkhl</i>	GGCGGGGCTCAATACATA
OL1343	<i>mkhl</i>	CACTATCCATACCCGATTCT
OL1344	<i>mkhl</i>	CAAATTCGAACCCATTCCA
OL1345	<i>mkhl</i>	TACCCACTCACCTTGATCC
OL1346	<i>mkhl</i>	CTCTGGTTTCCGCTCTTC
OL1347	<i>mkhl</i>	CTTTTGAGGGACGAATTTTG
OL1348	<i>mkhl</i>	GTTCTGGGTATATGGCTG
OL1049	<i>pek1</i>	GGTTGTTTGTGCGAGG
OL1050	<i>pek1</i>	TATCCCGGCTCTCCTTTC
OL1051	<i>pek1</i>	TGGTGTTTTCTAGCGAGT
OL1052	<i>pek1</i>	TTTGTATATCGGTCGAG
OL1053	<i>pek1</i>	GGAATATTGCGGAGCAGG

OL1054	<i>pek1</i>	AGGTAAAAGGGGAGGTGG
OL1055	<i>pek1</i>	GGTTTAACATTGATGGAGG
OL1056	<i>pek1</i>	AACAAGCGAACTGGAAGA
OL1057	<i>pek1</i>	AGAGTTCCTTCGTCAAGT
OL1058	<i>pek1</i>	AAGCTAGCAAGGCGTAAA
OL1059	<i>pmk1</i>	TTTTTCCACATCCCTTTTGCC
OL1060	<i>pmk1</i>	AATCAATCACAGGAAGCCG
OL1061	<i>pmk1</i>	GAGAGAGATAAAACTGCTGA
OL1062	<i>pmk1</i>	TCGCGTAACATACCAACT
OL1063	<i>pmk1</i>	TGCATTTACGATCTCGAC
OL1064	<i>pmk1</i>	TGATAGCTGCTGAACGAA
OL1065	<i>pmk1</i>	TGGTTTATGACGGAGTATG
OL1066	<i>pmk1</i>	GAAATACGTCTGTTGGGG
OL1067	<i>pmk1</i>	ACAGGAGTATGTTCGAAG
OL1068	<i>pmk1</i>	AGGTTTGAGAGGATGAAG
OL1069	<i>pmk1</i>	CTCATCCAACAAACCCAAC
OL1070	<i>pmk1</i>	GCAAGAAAAGGGAGCACA
OL1201	<i>pmk1</i>	CTTGCATTACGATCTCGAC
OL1202	<i>pmk1</i>	ACACTCCAAACATCAATACC
OL1173	<i>tif452</i>	AACCCAATTACGACCACC
OL1174	<i>tif452</i>	CCCCAAAACCTCTTCCACT
OL1175	<i>tif452</i>	AAAAACACACCCCCCTTCA
OL1176	<i>tif452</i>	CACCAGTAACCTCTTTCCCA
OL1177	<i>tif452</i>	ACTGCTGTGATATCGACT
OL1178	<i>tif452</i>	TCGTAAATGAACACCCCT
OL1293	<i>pmc1</i>	GGCGATTTCACACGAGAA
OL1294	<i>pmc1</i>	GAACCCACATCTCCAAC
OL1295	<i>pmc1</i>	GAGTTGTTGAGTAGGTGG
OL1296	<i>pmc1</i>	CGGAGATTGGATAACAAG
OL1297	<i>pmc1</i>	GCACCTCCGAAAAAACA
OL1298	<i>pmc1</i>	TCGACTGACCAAAGCTCT
OL1299	<i>pmc1</i>	TCTCAGGTTTCCTCTTTAACAC
OL1300	<i>pmc1</i>	TTGCACGCTCATCTCCTT
OL1301	<i>pmc1</i>	CTTTTTTTAGTGCAGGCG
OL1302	<i>pmc1</i>	TCCCAAAGATATTCCCACCA
OL1303	<i>pmc1</i>	GTCAAACACACGATAGCAT
OL1304	<i>pmc1</i>	ACTTCGGGACTTAACTCT
OL1305	<i>pmc1</i>	CCTTGGGGTGTGAGAATTA
OL1306	<i>pmc1</i>	AGAACAAGGGTCAAGAGT
OL1307	<i>pmc1</i>	AGCAAAAGCCAAAGCCAA
OL1308	<i>pmc1</i>	GAAAGAACATCGCCGGAC

OL1309	<i>pmcI</i>	AATTAACACCGACAGCAG
OL1310	<i>pmcI</i>	GTGTCGAATGGGTGGAAG
OL1311	<i>pmcI</i>	CAGTCATTCACACCACCCA
OL1312	<i>pmcI</i>	ATTTCTACTCACGAACCTCA
OL1313	<i>pmcI</i>	CTCATCTGCAATTTATCGGC
OL1314	<i>pmcI</i>	TTCCTCGGTGTTCTTCT
OL1315	<i>pmcI</i>	CCACTTCGAGAGCTATTT
OL1316	<i>pmcI</i>	TCTCCTCTTCATCTTCTCTT

SUPPLEMENTARY METHODS

Whole Genome and Targeted Sequencing Analysis

Library construction was performed using Illumina TrueSeq DNA PCR-Free Prep Kit following the manufacturer's instructions. Paired end sequencing was achieved with either Illumina HiSeq 2500 (250 nucleotide-long reads at Novogene Bioinformatics Technology Company LTD) or an Illumina MiSeq System (300 nucleotide-long reads at the Pasteur Institute) technology following the manufacturer's instructions. The preprocessing step was performed with fqCleanER (v.5.01) which includes the trimming of short and aberrant reads with AlienTrimmer ², correcting sequencing mistakes with Musket ³, and merging the overlapping reads using Flash ⁴. The remaining reads were aligned to the *S. pombe* ASM294v2.23 (strain 972 h-) reference genome with Burrows-Wheeler Aligner (v.0.7.5a.) ⁵. The resulting reads were sorted and indexed with Samtools (v.0.1.19) and duplicated reads were marked with Picard tools (v.1.96). Indel realignment, removing the duplicates, variant filtering and calling was performed according to the GATK best practices using GATK version 2.7-2 ⁶. The output .vcf file was annotated using snpEff (v.3.5) ¹. In the targeted resequencing experiments, the aligned reads were sorted and indexed, further processed with Samtools (v.1.3) and converted to the .mpileup format. We used VarScan ⁷ to call the variants. According to the experimental design that we have developed, we only consider variants from the positions covered at least 5,000 times exhibiting an allele frequency above 0.1%. To distinguish mutations from sequencing errors, we have applied several filters. First, based on the comparison of the distribution of the reference and alternative variants on the

reads, VarScan calculates a p-value based on the Fisher's exact test. Second, we discarded the variants with a base quality below 35 and exhibiting more than a 7% difference in quality with the reference allele. Third, we retained the alleles for which mutations are present in equimolar proportions on the paired reads (within a 20% range). In addition, in the six-subculture experiment in which two independent PCR reactions were performed and sequenced, we have considered as true positive variants only those that were found in both batches. Using this approach, we are able to detect both SNVs and indels present at a 0.1% frequency, and short indels (± 1 nucleotides) at frequency of 0.5%.

REFERENCES

1. Cingolani, P. *et al.* A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. *Fly* **6**, 80–92 (2014).
2. Criscuolo, A. & Brisse, S. AlienTrimmer: A tool to quickly and accurately trim off multiple short contaminant sequences from high-throughput sequencing reads. *Genomics* **102**, 500–506 (2013).
3. Liu, Y., Schröder, J. & Schmidt, B. Muskiet: a multistage k-mer spectrum-based error corrector for Illumina sequence data. *Bioinformatics* **29**, 308–315 (2012).
4. Magoc, T. & Salzberg, S. L. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**, 2957–2963 (2011).
5. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
6. McKenna, A. *et al.* The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* **20**, 1297–1303 (2010).

7. Koboldt, D. C. *et al.* VarScan: variant detection in massively parallel sequencing of individual and pooled samples. *Bioinformatics* **25**, 2283–2285 (2009).