**SUPPLEMENTARY DATA**

**Supplementary Table 1:** primers used for qPCR

| Primer name | Sequence | Origin (*C. lusitaniae*, Broad Institute)[a)] | Reference |
|---|---|---|---|
| MFS7-R | GGAATGTAAGGCATGGCAGT | supercont_1.2 (1458215-1459123) | (1) |
| MFS7-F | ATGGTTTTCTCGCCAATGTC | supercont_1.2 (1458215-1459123) | (1) |
| ABC12-R | GTGCTTCAACCACCACCTTT | supercont_1.3 (1264183-1268415) | (1) |
| ABC12-F | TGATTGAGTCTGCGGACAAG | supercont_1.3 (1264183-1268415) | (1) |
| ABC15-F | GTGCTAAGGCGTTTTTCGAG | supercont_1.3 (1682175-1688644) | This study |
| ABC15-R | TGAGATGCACGCCAGTAGTC | supercont_1.3 (1682175-1688644) | This study |
| ABC9-F | TCCATTGCAGAGGCTTCTTT | supercont_1.2 (1950802-1957241) | This study |
| ABC9-R | GTACGCATCCTGGGAACACT | supercont_1.2 (1950802-1957241) | This study |
| ACT1-F | GTATCGCTGAGCGTATGCAA | supercont_1.4 (88657-89739) | This study |
| ACT1-R | GATGGATGGTCCAGACTCGT | supercont_1.4 (88657-89739) | This study |
| ERG11-F | AACACCAGCTACGTGGTTCC | supercont_1.6 (218130-219707) | This study |
| ERG11-R | AGGCAAACTGCTCTCCGATA | supercont_1.6 (218130-219707) | This study |

[a)] :Numbering gives the coordinates of the gene of interest according to supercontig data

**Supplementary Table 2**: primers used for CRISP-Cas9 system

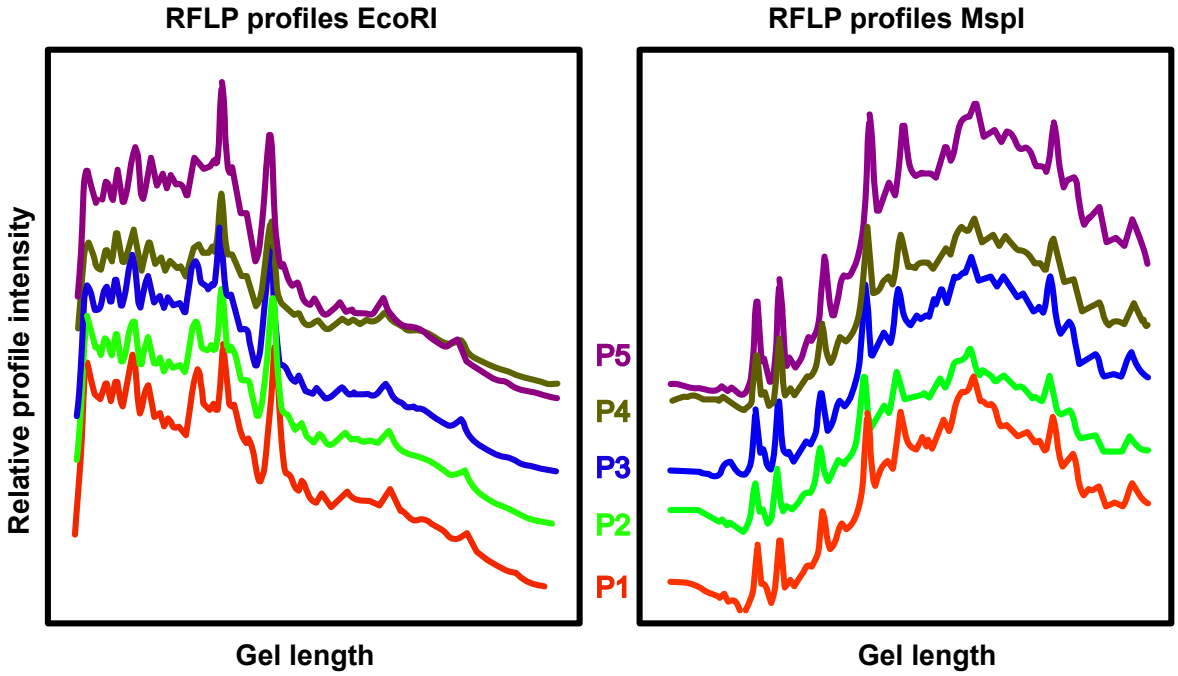
| Primer Name | Sequence | Purpose |
|---|---|---|
| FKS_crisp_R[a)] | GTTGATAACGGACTAGCCTTATTTTAACTTG CTATTTCTAGCTCTAAAACAATATTCAGAAT CGTACTACGATCATTTATCTTTCACTGCGGA GAAGTTTCGAACGCCGAAACATGCGCA | Generation of guide-pMEL10 sequence by complementary assembly of primers |
| FKS_crisp_F | TGCGCATGTTTCGGCGTTCGAAACTTCTCCG CAGTGAAAGATAAATGATCGTAGTACGATT CTGAATATTGTTTTAGAGCTAGAAATAGCA AGTTAAAATAAGGCTAGTCCGTTATCAAC | |
| FKS left arm_S636Y [b)] | GATAGATGGATGTCCTATTTAGTTTGGGTTA CTGTTTTTGCTGCCAAATATTC*AGAATA**T**TAC TACTTTTTAG* | Generation of repair fragment for S636Y by overlapping PCR |
| FKS right arm_S636Y | CATTGCAGTGGTGGACAAAATTCTAATTGG ATCTCTCAAAGATAAAAC*TAAAAAGTAGTAA**T** ATTCT* | |
| FKS left arm | GATAGATGGATGTCCTATTTAGTTTGGGTTA CTGTTTTTGCTGCCAAATATTCA*GAATCGTAC TACTTTTTAG* | Generation of repair fragment for S643Y by overlapping PCR |
| FKS right arm_S643Y | CATTGCAGTGGTGGACAAAATTCTAATTGG ATCTCTCAAA**T**ATAAAAC*TAAAAAGTAGTAC GATTCT* | |
| FKS right arm S643P | CATTGCAGTGGTGGACAAAATTCTAATTGG ATCTCTCAAAG**G**TAAAACTAAAAAGTAGTA CGATTCT | Generation of repair fragment for S643P by overlapping PCR using primer FKS left arm |
| FKS1verif left | TCTACAAGGCGTTATGTTGCAT | *FKS1* verification primers |
| FKS1verif right | CAATCTTGGGTTGCACTTTACA | |
| p426 CRISPR rv | GATCATTTATCTTTCACTGCGGAGAAG | Inverse PCR with pMEL10 |
| p426 CRISPR fw | GTTTTAGAGCTAGAAATAGCAAGTTAAAAT AAGGCTAGTC | |

a) The 20 nt sequence of the *FKS1* guide is underlined

b) Sequences in italics are overlapping sequences; Bold characters indicate mutated bases

**Supplementary Table 3 :** *S. cerevisiae* strain descriptions

| Strain number | Parent | Genotype | Reference |
|---|---|---|---|
| IMX581 | CEN.PK113-5D | *MAT*a *ura3-52 can1*Δ*::cas9-natNT2 TRP1 LEU2 HIS3 FKS1* | (2) |
| DSY4762 | IMX581 | *MAT*a *ura3-52 can1*Δ*::cas9-natNT2 TRP1 LEU2 HIS3 FKS1*$^{S636Y}$ | This study |
| DSY4763 | IMX581 | *MAT*a *ura3-52 can1*Δ*::cas9-natNT2 TRP1 LEU2 HIS3 FKS1*$^{S643P}$ | This study |
| DSY4764 | IMX581 | *MAT*a *ura3-52 can1*Δ*::cas9-natNT2 TRP1 LEU2 HIS3 FKS1*$^{S643Y}$ | This study |




**Supplementary Figure 1** : Profile plots of RFLP analysis. Gel electrophoresis pictures of Fig.2. were analysed with the ImageJ (version 1.49P) and profile plots were generated. The X-axis represents relative densities while Y-axis represents length of profiles.

**References**


(1) **Reboutier D, Piednoel M, Boisnard S, Conti A, Chevalier V, Florent M, Gibot-Leclerc S, Da Silva B, Chastin C, Fallague K, Favel A, Noel T, Ruprich-Robert G, Chapeland-Leclerc F, Papon N.** 2009. Combination of different molecular mechanisms leading to fluconazole resistance in a Candida lusitaniae clinical isolate. Diagn Microbiol Infect Dis **63:**188-193.

(2) **Mans R, van Rossum HM, Wijsman M, Backx A, Kuijpers NG, van den Broek M, Daran-Lapujade P, Pronk JT, van Maris AJ, Daran JM.** 2015. CRISPR/Cas9: a molecular Swiss army knife for simultaneous introduction of multiple genetic modifications in Saccharomyces cerevisiae. FEMS Yeast Res **15:**fov004-fov004.