Efficient Mating-Type Switching in *Candida glabrata* Induces Cell Death

Stéphanie Boisnard¹,²*, Youfang Zhou Li¹,², Sylvie Arnaise¹, Gregory Sequeira¹, Xavier Raffoux², Adela Enache-Angoulvant¹,³, Monique Bolotin-Fukuhara¹,², Cécile Fairhead¹,²

¹ Institut de Génétique et Microbiologie, Université Paris-Sud, UMR8621 CNRS, F-91405, Orsay, CEDEX, France, ² Génétique Quantitative et Évolution–Le Moulon, INRA–Université Paris-Sud–CNRS–AgroParisTech, Batiment 400, UFR des Sciences, F 91405, Orsay, CEDEX, France, ³ Hôpital de Bicêtre, Le Kremlin Bicêtre, APHP, France

* stephanie.boisnard@u-psud.fr

Abstract

*Candida glabrata* is an apparently asexual haploid yeast that is phylogenetically closer to *Saccharomyces cerevisiae* than to *Candida albicans*. Its genome contains three MAT-like cassettes, *MAT*, which encodes either *MATa* or *MATα* information in different strains, and the additional loci, *HML* and *HMR*. The genome also contains an *HO* gene homolog, but this yeast has never been shown to switch mating-types spontaneously, as *S. cerevisiae* does. We have recently sequenced the genomes of the five species that, together with *C. glabrata*, make up the *Nakaseomyces* clade. All contain *MAT*-like cassettes and an *HO* gene homolog. In this work, we express the *HO* gene of all *Nakaseomyces* and of *S. cerevisiae* in *C. glabrata*. All can induce mating-type switching, but, despite the larger phylogenetic distance, the most efficient endonuclease is the one from *S. cerevisiae*. Efficient mating-type switching in *C. glabrata* is accompanied by a high cell mortality, and sometimes results in conversion of the additional cassette *HML*. Mortality probably results from the cutting of the *HO* recognition sites that are present, in *HML* and possibly *HMR*, contrary to what happens naturally in *S. cerevisiae*. This has implications in the life-cycle of *C. glabrata*, as we show that efficient *MAT* switching is lethal for most cells, induces chromosomal rearrangements in survivors, and that the endogenous *HO* is probably rarely active indeed.

Introduction

Sexual reproduction in fungi takes diverse forms [1], some species preferring out-breeding and others inbreeding, either because spores resulting from meiosis conjugate inside the ascus, before germination, or because they mate with daughter cells, through homothallism [2]. Homothallism is formally defined by the fact that a single spore isolated after meiosis is able to undergo a full sexual cycle, without needing to meet a spore from the opposite mating-type. This is in opposition to heterothallism, where conjugation of cells from strains of opposite mating-types is required. Different underlying mechanisms for homothallism exist [1,3,4],
especially in higher fungi, but the best studied such mechanism in yeasts, is the mating-type interconversion mechanism from the model yeast Saccharomyces cerevisiae [5]. It is interesting to note that the haploid Schizosaccharomyces pombe, the other model yeast favored by scientists, also switches mating-types very efficiently. The two species use different initiation events, but undergo equivalent gene conversion events, in an example of convergent evolution [6,7]. Both mechanisms involve potentially dangerous chromosomal breaks, in pathways which are, by necessity, finely regulated. It is notable that the HO gene, which encodes the endonuclease central to the process in S. cerevisiae, has, indeed, a large regulatory region upstream [8]. Several levels of regulation control the expression of HO: it is only expressed in haploid cells, only in late G1 and only in mother cells [8,9].

In S. cerevisiae, cellular mating-type is determined by the MAT locus, which encodes transcription factors responsible for sexual identity [10–13]. Three types of sexual identity exist; mating-competent haploid MATa and MATalpha cells, according to the sequence present at the MAT locus, and meiosis-competent diploid cells which contain active copies of both types. Cells also contain transcriptionally silent copies of both mating-types [14,15] which are bordered by identical sequence segments that drive homologous recombination [16–18]. Mating-type interconversion in wild-type homothallic haploid cells relies on two components: the MAT-like loci and the Ho endonuclease (for review [5]). After the daughter cell has budded, the mother cell’s active MAT locus is cut by the Ho endonuclease at its recognition site, at the junction between the Y and Z1 segments of the locus [19–21]. This double strand break is repaired by homologous recombination using the HMLalpha or the HMRa cassette as a template for repair (for review [5,22]), resulting in a switch of mating-types. Switching is highly efficient, with 90% cells switching to the opposite sexual type [3,23,24], through the control by the sexual identity of the cell, MATalpha cells repressing the Recombination Enhancer present between the MAT and the HML loci, leading to repair with HMR [5,25,26]. In this process, the MAT locus is the only one to be cut, HMLalpha and HMRa being protected from the Ho cleavage by silencing processes involving the Sir proteins [27–29].

Asexual species abound in the fungal kingdom, and it has been noted that fungal pathogens of humans usually display an apparent lack of sexuality. The reasons for this include the absence of cells of one mating-type in an infectious population of a heterothallic species, such as in the case of Aspergillus fumigatus [30], or modification of mating pathways to yield atypical, rare, conjugation events between diploids in addition to conjugation events between even rarer haploids, such as in the case of C. albicans [31–33].

C. glabrata, the 2nd cause of invasive candidiasis after C. albicans [34], has never been shown to mate, but haploid cells of both mating-types are regularly isolated [35], and the genome contains both the triplicated MAT-like loci and an HO gene homolog [36,37]. We have previously shown that C. glabrata MATalpha cells express the MATa specific gene, MATa1, through transcriptional “leakage” of HMRa, and that both C. glabrata MATa and MATalpha cells are insensitive to their specific mating pheromones, while S. cerevisiae’s cells are sensitive to the same pheromones [38].

C. glabrata belongs to the Nakaseomyces genus, which includes five other species, two described recently as pathogens, Candida (Nakaseomyces) nivariensis [39] and Candida (Nakaseomyces) brasarensis [40]; and three species isolated in the environment: Kluyveromyces (Nakaseomyces) delphensis, Kluyveromyces (Nakaseomyces) bacillisporus, and Candida (Nakaseomyces) castellii [41]. This group of yeasts is closer to Saccharomyces cerevisiae than to Candida albicans, and therefore follows the universal nuclear genetic code. All Nakaseomyces species are haploid, except for N. bacillisporus, which is diploid [41,42]. We have recently sequenced the genomes of these five species, and compared them to C. glabrata [42]. Contrary to S. cerevisiae, where the three loci are on chromosome III, in C. glabrata, HMLalpha and
Mating-Type Switching in Candida glabrata

MAT are on chromosome II, and HMRα is on chromosome V [43]. This configuration is common to four species in the Nakaseomyces, while one, N. delphensis, has the three loci on the same chromosome like S. cerevisiae. As for the final species, N. bacillisporus, the sequence in its present state does not contain the HMR locus.

C. glabrata has an HO gene homolog, where known domains for nuclear localization, site recognition and endonuclease activity are conserved [44]. This is also true in all Nakaseomyces [42]. Mating-type switching has been suggested by PCR experiments in C. glabrata [45], but no living cell where spontaneous switching has occurred and been followed experimentally has been isolated, such as is possible in S. cerevisiae, where HO haploid cells switch every generation, and pedigrees of cells can be performed to follow this. Recently, induction of HO expression inducing mating-type switching in C. glabrata has been reported, using a constitutive promoter and the endogenous gene, resulting in switching of a MATα cell to a MATα cell [46]. Populations of C. glabrata contain strains of both mating-types with some collections exhibiting bias [35,47], and it has been reported that some strains exhibit “abnormal” cassette configurations, i.e., differing from the canonical HMLα and HMRα configuration [36]. This is also known in S. cerevisiae [48,49].

It has been proposed that triplicated MAT-like cassettes originated before the HO gene, and that these were used to allow rare mating-type switching events, and that the HO gene, a “selfish” self-transposing gene, was then “domesticated” [50–52]. Indeed, K. lactis switches mating-types inefficiently through mechanisms independent of Ho but dependent on triplicated cassettes [53,54]. The HO gene is part of the family of Homing Endonuclease Genes (HEGs), selfish genetic elements that can propagate through populations. HO is closely related to VDEI, the intein in S. cerevisiae’s genome [55]. Many HEGs propagate as self-splicing introns in organelle genomes; the intron encodes an endonuclease that can cut its site in a genome without the intron, upon mating of an intron-containing and an intron-less strain. The cut is then repaired by homologous recombination (HR), using the intron-containing gene as template, resulting in intron propagation [56]. Ho is an intein whose sole known activity in the cell is to cut its recognition site at MAT and that is encoded by a free-standing gene, dissociated from its own recognition site on chromosome III. Endonucleases of the Ho family have rather low activity, recognize large sites but tolerate degenerate bases within the site, as shown by mutagenesis of the Ho and I-Sce I recognition sites [20,21,57].

We have previously published the composition of MAT-like cassettes and comparison of the HO genes in the Nakaseomyces [42]. We now report on heterologous expression of HO from different Nakaseomyces species and from S. cerevisiae in C. glabrata, and the resulting switching events in both directions, with abnormal structures resulting from gene conversion events. We observe that a high frequency of switching is associated with cell mortality.

Materials and Methods

Strains, cultures and transformation

Strains used for in vivo experiments and for amplification of HO genes are listed in Table 1. Yeast strains are grown in broth or on plates at 28°C, in YDP (non-selective, 1% Yeast Extract, 1% Peptone, 2% glucose), Synthetic Complete medium lacking uracil (SC-Ura, 0.67% Yeast Nitrogen Base without amino acids, 2% glucose, supplemented with all amino acids and adenine) for HO induction experiments. For selection of transformants and maintenance in repressive conditions, strains are grown in SC-Ura added with 2mM each of methionine and cysteine. Stability of plasmids in C. glabrata and S. cerevisiae was confirmed by comparing the number of colonies on SC-Ura and SC+Ura. Transformation of S. cerevisiae and C. glabrata was done according to the lithium acetate transformation protocol from Gietz et al [58].
It must be noted that the *S. cerevisiae* strain used for HO induction has a mutant endogenous HO gene; while the *C. glabrata* strains still possess their endogenous HO gene.

Cloning of HO genes into expression plasmids for *C. glabrata*

The different HO genes were amplified by PCR from genomic DNA on type strains for the *Nakaseomyces* and on an HO+ strain in the case of *S. cerevisiae* (Tables 1 and 2). *Spe*I/*Sal*I

| Species          | Strain | Genotype                  | Reference                  |
|------------------|--------|---------------------------|----------------------------|
| *S. cerevisiae*  | J5     | HMLalpha, MATalpha, HMRalpha, HO, leu2, his4, thr4, lys2 | Kindly donated to us by Amar Klar |
| *S. cerevisiae*  | W303-1B | ho, MATa                  | ATCC 200060                |
| *S. cerevisiae*  | FY69   | ho, MATa, leu2Δ1          | [59]                       |
| *S. cerevisiae*  | FYC2-7B | ho, MATalpha, leu2Δ1      | [60]                       |
| *S. cerevisiae*  | FY1679-18D | ho, ura3-52, his3Δ200  | [60]                       |
| *S. cerevisiae*  | FY73   | ho, ura3-52, his3Δ200     | [59]                       |
| *N. delphensis*  | CBS2170 | Type strain, 1n, actively switching * | [42]                       |
| *N. bacillisporus* | CBS7720 | Type strain, 2n | [42]                       |
| *C. castelli*    | CBS4332 | Type strain, 1n, probably switching * | [42]                       |
| *C. bracarensis* | CBS10154 | Type strain, 1n, MATalpha | [42]                       |
| *C. nivariensis* | CBS9983  | Type strain, 1n, MATalpha | [42]                       |
| *C. glabrata*    | CBS138  | Type strain, 1n, MATalpha | [42]                       |
| *C. glabrata*    | BG2     | 1n, MATa                  | [61]                       |
| *C. glabrata*    | BG87    | MATa, NeoR:ura3, his3Δ     | [62]                       |
| *C. glabrata*    | HM100   | MATalpha, URA3Δ::KANMX    | [38]                       |

* our unpublished results

doi:10.1371/journal.pone.0140990.t001

| Primers     | Species name          | Primer sequence (5’-3’) |
|-------------|-----------------------|-------------------------|
| RX1-KLDE-F  | *N. delphensis*       | GCATACTAGTATGTTTGACATTAACACAAC |
| RX2-KLDE-R  | *N. delphensis*       | CGATGTCGACCTAATTTTATCATAGCACGCC |
| RX3-CANI-F  | *C. nivariensis*      | GCATACTAGTATGTTGAGATAAATACACAAC |
| RX4-CANI-R  | *C. nivariensis*      | CGATGTCGACCTAGTTCAGCATAAAC |
| RX5-CAFA-F  | *C. castelli*         | GCATACTAGTATGTTGAGAACAACTCA |
| RX6-CAFA-R  | *C. castelli*         | CGATGTCGACCTACATGCTTCTAATGCAA |
| RX7-CABR-F  | *C. bracarensis*      | GCATACTAGTATGTTGAGAACAACTCA |
| RX8-CABR-R  | *C. bracarensis*      | CGATGTCGACCTAATGCAAAGTACCTAGG |
| RX9-KLBA-F  | *N. bacillisporus*    | GCATACTAGTATGTTGAGAACAACTCA |
| RX10-KLBA-R | *N. bacillisporus*    | CGATGTCGACCTATATAGACAGATGAAT |
| RX11-CAGL-F | *C. glabrata*         | GCATACTAGTATGTTGAGAACAACTCA |
| RX12-CAGL-R | *C. glabrata*         | CGATGTCGACCTACATGCTTCTAATGCAA |
| RX13-SACE-F | *S. cerevisiae*       | GCATACTAGTATGTTGAGAACAACTCA |
| RX14-SACE-R | *S. cerevisiae*       | CGATGTCGACCTACATGCTTCTAATGCAA |
| A1 probe F  | *C. glabrata*         | CCAATACAGAGATCTACGCA |
| A1 probe R  | *C. glabrata*         | GATCTCTTGAGGCTATCTTG |
| ALPH1A probe F | *C. glabrata*      | ACTGGAAACACTGACTATGAA |
| ALPH1A probe R | *C. glabrata*      | CTGAGAGAATGAGGAGAG |

doi:10.1371/journal.pone.0140990.t002
sites were added to primers used for HO gene amplification. Appropriate PCR fragments were cloned at the SpeI/SalI sites into the pYR32 plasmid. The pYR32 plasmid, kindly provided by B. Cormack [63], is a replicative plasmid for C. glabrata, and was also used for transformation of S. cerevisiae. Absence of mutation in all the HO genes (amplified and cloned) was verified by sequencing.

PCR, Southern Blot and Sequencing

To test mating-type switching, we performed PCR on colonies before and after induction, using specific primers, which can discriminate the mating-type at the MAT locus, by amplifying the upstream part of the locus (Tables 3 and 4). In some cases, we also typed HML and HMR (Tables 3 and 4, and see results). Cells from a fresh colony were incubated in 5 μL of NaOH 20 mM 5 min at 95°C in the PCR tubes before placing at 4°C, adding the buffer, primers, dNTPs and Taq polymerase and proceeding with the PCR amplification.

For Southern blots, genomic DNAs were prepared using the Qiagen genomic DNA kit, according to manufacturer’s instructions. 5 μg DNA was subjected to enzymatic digestions by EcoRV or HindIII/PstI. Gels (0.8% agarose in 0.5x TBE) were transferred on positively charged Nylon membranes from Roche, using an Appligene vacuum blotter. Membranes underwent pre-hybridization for 4 hrs and overnight hybridization at 65°C in Church’s buffer [64], with a DIG-labeled PCR probe, and were submitted to high stringency washes at 65°C in 0.1% SDS, 1X and then 0.1X SSC buffer. DIG labeling and detection was done using a Roche kit, according to manufacturer’s instructions. Primers used for probe PCR amplifications are given in Table 2.

Table 3. Location and sequence of primers used for determining mating-type information at MAT, HML, and HMR.

| Name  | Primers                          | Localisation          |
|-------|----------------------------------|-----------------------|
| GS01  | TACCAAGAAGCAAGAGCCCA             | Upstream of MAT       |
| GS02  | TCTTGCGTAGTGAGACCTC              | Downstream of MAT     |
| GS06  | GACAGGAACATCTAAGCGAT             | Upstream of HMR       |
| GS07  | GTGATGATTACTGGGTGGA              | Downstream of HMR     |
| GS08  | GCTGTAGGGGGAAAATAAG              | Upstream of HML       |
| GS09  | GATCAGTTCTGATGAGAAAC             | Downstream of HML     |
| RX15  | GCTGATCGAGGGTAGACAT              | Upstream of MAT       |
| RX16  | CTCTACCGAGCAGGCAAAGCCAAG        | Inside Ya (MAT, HML, HMR) |
| RX17  | TTCAACGCCTAAAATTGC              | Inside Yalpha (MAT, HML, HMR) |

doi:10.1371/journal.pone.0140990.t003

Table 4. Combination of primers used and size of expected products.

| Primer pairs | Specific locus       | Amplification length expected |
|--------------|---------------------|-------------------------------|
| RX15/RX16    | Upstream MATa fragment | 778bp                        |
| RX15/RX17    | Upstream MATalpha fragment | 1161bp                      |
| GS08/RX16    | Upstream HMLa fragment | 1042bp                      |
| GS08/RX17    | Upstream HMLalpha fragment | 1425bp                    |
| GS06/RX16    | Upstream HMRa fragment | 1060bp                      |
| GS06/RX17    | Upstream HMRalpha fragment | 1443bp                   |
| GS01/GS02    | Whole MAT locus      | MATa 2315bp, MATalpha 2500bp |
| GS08/GS09    | Whole HML locus      | HMLa 2297bp, HMLalpha 2482bp |
| GS06/GS07    | Whole HMR locus      | HMRa 2297bp, HMRalpha 2482bp |

doi:10.1371/journal.pone.0140990.t004
For the three cassettes \textit{MAT}, \textit{HML} and \textit{HMR}, the whole locus was amplified using primers indicated on Tables 3 and 4, and purified PCR fragments were sequenced by Beckman Coulter Inc.

**Induction of \textit{HO} expression and quantification of the efficiency of mating-type switching**

Experimental procedure is shown on Fig 1. Transformants with the different \textit{HO} plasmids were streaked onto repressive medium and directly tested by PCR (see above) to check that they had not switched before induction. Cells from a starter overnight culture in repressive conditions (SC-URA +2mM Met/Cys) were then counted, and around 200 cells were usually plated on inductive (SC-URA) and on repressive medium as control, and incubated at 28°C, until colonies appeared (one to two days). Determination of mating-type was done on individual colonies (a minimum of 14 colonies were tested per transformant plated, and at least 2 transformants were tested per experiment), by PCR amplification of both \textit{MATa} and \textit{MATalpha}.

At this step, we obtain "negative" colonies (those that had not switched and yielded a PCR product only with primers amplifying the original mating-type), and "mixed" colonies (colonies yielding a PCR product with both pairs of primers, in which some cells have switched, and others have maintained their original mating-type).

At least two "mixed" colonies were then restreaked on repressive medium, cells allowed to grown into individual colonies, and the PCR experiment performed again on at least 20 colonies per mixed colony. Colonies become "pure" as to their mating-type at this stage (i.e., no switching back and forth between mating-types occurs and all cells respond identically to the PCR determination, yielding a single positive response, either \textit{MATa} or \textit{MATalpha}).
percentage of "pure" switched colonies out of the number of colonies sub-cloned was then used as an estimate of the efficiency of mating-type switching (see results).

In the case of the expression of the \( HO \) gene from \( S. \) cerevisiae, we observed a high cellular mortality, so we decided to analyze this phenomenon more precisely: we plated dilutions from \( 10^6 \) to \( 10^2 \) cells on inductive medium and on repressive medium and compared the number of colonies growing. This was done on three and five independent transformants for strains BG87 and HM100 respectively.

For the induction of \( HO \) expression in BG87s, (BG87 switched from \( MATa \) to \( MATalpha \)) a colony which had lost the original plasmid was isolated by growing the strain on SC+URA, and transformed anew with the plasmid expressing the \( HO \) gene from \( S. \) cerevisiae (see Results).

## Results

### Mating-type switching in a \( C. \) glabrata \( MATa \) strain

\( HO \) is particularly well-conserved in the \( Nakaseomyces \) [42], and we cloned all \( HO \) genes from the \( Nakaseomyces \) and from \( S. \) cerevisiae into a \( URA3 \) selectable plasmid that allows controlled expression of the cloned gene under the \( MET3 \) promoter [63]. Expression is repressed in conditions of high methionine and cysteine concentrations, and induced in the absence of these two amino acids in the medium (see Materials and Methods).

We first tested the expression of all \( HO \) genes in \( C. \) glabrata, using the \( MATa \) strain BG87, isogenic to the BG2 strain, commonly used in laboratories [38,62] (Table 1). Colonies were tested by specific PCR in order to discriminate the mating-type (specific primers and corresponding size expected are given in Tables 3 and 4). Results obtained after induction are shown in Table 5 (experiment A and 1 to 7). All \( HO \) genes, from the \( Nakaseomyces \) and from \( S. \) cerevisiae, are able to induce mating-type switching, i.e., induction of \( HO \) expression yields “mixed colonies”, as evidenced by the obtainment of both \( MATa \)- and \( MATalpha \)-specific amplifications (see Materials and Methods). Fig 2 shows such PCR amplification in colonies after expression of the \( HO \) gene from \( S. \) cerevisiae; i.e., most of the colonies tested present both \( MATa \)- and \( MATalpha \)-specific amplifications.

### Table 5. \( MAT \) switching in \( C. \) glabrata and in \( S. \) cerevisiae.

| Experiment | Strain | MAT | \( HO \) gene | Observation of switch | Pure switched cells | Molecular analysis of switch |
|------------|--------|-----|---------------|----------------------|---------------------|----------------------------|
| C. glabrata | A      | BG87 a | Without \( HO \) gene | - | Absent | ND |
| 1          | BG87 a | S. cerevisiae + | Frequent | Normal |
| 2          | BG87 a | C. glabrata + | Rare | Normal |
| 3          | BG87 a | C. bracarensis + | Rare | Normal |
| 4          | BG87 a | C. nivariensis + | Rare | ND |
| 5          | BG87 a | C. castellii + | Absent | ND |
| 6          | BG87 a | N. bacillisporus + | Rare | Normal |
| 7          | BG87 a | N. delphensis + | Rare | Normal |
| 8          | HM100 alpha | S. cerevisiae + | Frequent | Normal and Triple « a » strains |
| 1-b        | BG87s alpha | S. cerevisiae + | Frequent | Normal and Triple « a » strains |
| S. cerevisiae | 21 FY1679-18D a | S. cerevisiae + | Frequent | ND |
| 22 FY1679-18D a | C. glabrata - | Absent | ND |

\( HO \) genes from different species (column 5) were expressed in strains of \( C. \) glabrata and \( S. \) cerevisiae (column 3). BG87s (experiment 1-b) corresponds to the BG87 strain switched to \( MATalpha \). Switching events are detected by specific PCR (column 6); (+) indicates the presence of the opposite mating-type. After having isolated cells with pure genotypes (see materials and methods), estimation of the number of pure switched cells is referred in this table as absent, rare or frequent (column 7). Molecular analysis was performed by sequencing the \( MAT, HML, HMR \) loci (column 8). ND means Not Determined.

doi:10.1371/journal.pone.0140990.t005
From these mixed MATa/MATalpha colonies, we isolated pure switched cells (see Materials and Methods, and Fig 1). When S. cerevisiae’s HO gene is expressed, more than 80% of the isolated cells present the opposite sexual type (“frequent” in experiment 1, Table 5) whereas less than 10% of the isolated cells had switched when the different Nakaseomyces HO gene were expressed (“rare” in experiments 2, 3, 4, 6, and 7, Table 5). In the case of N. castelli’s HO, we could not isolate pure switched cells from a mixed colony exhibiting both MATa/MATalpha amplification (“absent” in experiment 5, Table 5).

As a control (experiment A, Table 5), we performed the same experiment with the pYR32 plasmid which does not contain an HO gene and we never observed mixed MATa/MATalpha colonies, but only non-switched parental colonies.

Southern blot analysis (Fig 3) of pure switched strains shows that the structure of the cassettes is as expected after mating-type switching i.e., HMLalpha, MATalpha, HMRa. The MAT locus from several pure switched clones (originating from expression of both S. cerevisiae HO and some Nakaseomyces HOs) was sequenced. This confirmed that the C. glabrata MAT locus undergoes switching from MATa to MATalpha. Sequence analyses show that the molecular structure of MAT locus is normal, suggesting a correct double strand break at the specific MATa HO cutting site and normal repair by homologous recombination with HMLalpha. Sequencing also confirmed that the switched clones obtained, truly originated from the BG87 strain, since several polymorphic sites at the MAT locus (discriminating the two C. glabrata background strains HM100 and BG87) were detected (Fig 4). HMLalpha and HMRa sequencing have shown that these loci were normal.
Since switching is observed at a high frequency only with *S. cerevisiae's* HO, and that all other give switching rates similar to *C. glabrata*'s HO, we focused on *S. cerevisiae* HO gene expression for further analyses.

**Mating-type switching in *C. glabrata MATalpha* strains**

Contrary to *S. cerevisiae* where the three cassettes *HML*, *HMR* and *MAT* are on the same chromosome (ChIII), in *C. glabrata*, the *HMR* locus is located on a different chromosome from the *HML* and *MAT* loci. In *S. cerevisiae*, it is known that the relocation of *HMR* or *HML* lowers the efficiency of repair of the Ho cut [65,66]. We wanted to know whether switching occurred in the same way in both directions in *C. glabrata*. Our first experiments described above involved repair of the *MAT* locus with the *HML* locus on the same chromosome. In a *MATalpha* strain, such as HM100, switching involves repair with *HMRa* on a distinct chromosome. Switching from *MATalpha* to *MATa* has been performed previously, using the HO gene from *C. glabrata* expressed from a constitutive promoter [46], but was not examined in detail.
Therefore, expression of the *HO* gene from *S. cerevisiae* was induced in the MAT alpha strain, HM100, isogenic to the sequenced strain, CBS138 (experiment 8, Table 5). Like previously, colonies grown on inductive medium were tested by specific PCR at the MAT locus (Fig 5). The expression of *S. cerevisiae’s* HO allows the obtainment of several pure switched subclones. Sequence analyses of these clones show that the *C. glabrata* MAT locus can switch from MAT alpha to MAT a, and suggest again a correct double strand break at the MAT alpha Ho cutting site and normal repair with HMR a as donor.
Switching from MATalpha to MATa reveals unexpected cutting at HML in C. glabrata

Sequencing of HML and HMR loci showed that half of the clones display a normal structure of these loci (Fig 6A); but others present switching at HML, i.e., HMLalpha has been switched to HMLa, leading to a “triple a” genotype: HMLa, MATa, HMRa (Fig 6B). Normal and “triple a” genotypes were confirmed by sequencing and by Southern blot experiments (data not shown). The finding of these “triple a” strains is evidence of cutting at the HML locus by S. cerevisiae’s Ho endonuclease.

Since the original MATa and MATalpha strains at our disposal were non isogenic, we decided to use a switched BG87s MATalpha strain to test whether “triple a” strains could be obtained in a different strain background (experiment 1-b, Table 5). We isolated two MATa BG87s clones, upon S. cerevisiae’s HO expression, confirming that the MATalpha switched locus can switch back to MATa. Sequencing shows that the first clone displays a normal structure at the MATa, HMLalpha, and HMRa loci, but that the second one exhibits a “triple a” structure: MATa, HMLa, HMRa, similar to the one obtained in the HM100 strain (data not shown).
The HML locus can act first as a recipient and then as a donor during mating-type switching

Sequence comparison showed the presence of a polymorphic site inside the X region between the three loci, \( \text{MAT}, \text{HML} \) and \( \text{HMR} \) in the HM100 strain (Fig 6). The \( \text{MAT} \) and \( \text{HMR} \) loci possess a “C” whereas the HML locus presents an “A” at the same position (+1269 from the Ho cut site). In \( S. \text{cerevisiae} \), we know that copying of \( \text{MAT} \) information during switching, includes
not only the Y region but extends in the X region with variable lengths [67]. We took advantage of this polymorphic site to understand the chronological steps of the establishment of the “triple a” strains. Sequencing of one “triple a” strain shows that the MAT locus contains an “A” at the polymorphic site. This result proves that the MAT locus has been cut and necessarily repaired using HML as a template after it has switched itself, using HMRa as template. This is unexpected because it suggests that HML is preferentially cut over MAT.

Switching-induced cell death in C. glabrata

Comparison of colony numbers between inductive and repressive conditions shows that S. cerevisiae HO gene expression induces a very strong lethality in C. glabrata cells (Table 6). This lethality is not observed during the Nakasomyces HO gene expression. We observed the same rate of lethality between the two strains BG87 MATa and BG87s MATalpha (experiments 1 and 1-b respectively, Table 6) suggesting that the switching direction (MATa versus MATalpha) does not influence cell death. We noticed that, in the HM100 strain background, lethality is ten times higher (Table 6, experiment 8).

The high lethality observed could be due to cutting of an Ho site outside of the triplicated cassettes, which could not be repaired by homologous recombination. We unsuccessfully searched the entire genome in silico, for additional Ho sites, even though we cannot exclude the presence of degenerate sites. Since we observe unexpected switching of HML (triple “a” genotype, see above), we hypothesize that the high lethality is linked to high efficiency of cutting at the Ho sites, including the HML site which can be cut in C. glabrata (see discussion).

S. cerevisiae and C. glabrata HO gene expression in S. cerevisiae

We were intrigued by the strong lethality observed in C. glabrata cells, when the HO gene from S. cerevisiae was expressed. Such lethality has not been reported, to our knowledge, when HO is expressed from a galactose-inducible promoter in a wild-type strain of S. cerevisiae. We therefore wanted to test whether our construction, with the HO gene under the control of the MET3

| Experiment | Strain     | MAT | HO gene          | Survival |
|------------|------------|-----|------------------|----------|
| C. glabrata| A          | BG87| a                | Without HO gene | 100 %   |
|            | 1          | BG87| a                | S. cerevisiae  | 0.1 % * |
|            | 2          | BG87| a                | C. glabrata    | 100 %   |
|            | 3          | BG87| a                | C. bracarensis | 100 %   |
|            | 4          | BG87| a                | C. nivariensis | 100 %   |
|            | 5          | BG87| a                | C. castellii   | 100 %   |
|            | 6          | BG87| a                | N. bacillisporus| 100 %   |
|            | 7          | BG87| a                | N. delphensis  | 100 %   |
|            | 8          | HM100| alpha           | S. cerevisiae  | 0.01 % *|
| S. cerevisiae| 1-b     | BG87s| alpha          | S. cerevisiae  | 0.1 % *|
|            | 21         | FY1679-18D| a           | S. cerevisiae  | 100 %   |
|            | 22         | FY1679-18D| a           | C. glabrata    | 100 %   |

In the case of lethality (*), dilutions from 10⁶ to 10² cells were plated on inductive medium and on repressive medium, and numbers of colonies compared. For experiments 1 and 1-b, number of cells counted, is between approximately 4 and 300 clones for dilutions ranging from 10³ to 10⁵ cells per plate. For experiment 8, we counted between 1 and 50 colonies for dilution ranging from 10⁴ up to 10⁶ cells per plate. This was done on three and five independent transformants for strains BG87 and HM100 respectively.

doi:10.1371/journal.pone.0140990.t006
promoter from C. glabrata could induce lethality in S. cerevisiae, taking advantage of the fact that ARS sequences can function as origins in both species [68].

A MATa ho S. cerevisiae strain (FY1679-18D) was transformed with the two constructs used previously, carrying either the C. glabrata or S. cerevisiae HO gene (experiments 21 and 22, Tables 5 and 6). Several independent transformants underwent the same treatment as C. glabrata cells, i.e. they were grown in broth in repressive conditions, and plated on either inductive or repressive medium at various concentrations. We confirmed the stability of the plasmid in S. cerevisiae by comparing the number of colonies on medium with or without uracil. In S. cerevisiae, switching events were searched for, by a mating assay with a complementing strain possessing the same MAT type, so that mating is possible only if switching has occurred. We observed that expression of S. cerevisiae’s HO leads to switching of the MATa locus of S. cerevisiae, whereas no switch is observed when the C. glabrata HO gene is expressed (experiments 21 and 22, Table 5).

No lethality is observed upon expression of either HO genes in S. cerevisiae (experiments 21 and 22, Table 6). Thus, the strong lethality observed in C. glabrata is not due to a toxic effect of our S. cerevisiae HO construct, but seems to be specific to C. glabrata cells.

**Discussion**

In S. cerevisiae, mating-type switching is a complex system relying on the existence of: 1) three cassettes (donors and recipient), 2) the presence of the Ho endonuclease 3) repair mechanisms, 4) silencing mechanisms at HMLalpha and HMRa. These silencing mechanisms allow the switching system to work with the MAT locus as the sole recipient and HMLalpha and HMRa as donors.

C. glabrata has never been shown to mate nor to switch mating-types naturally. Its genome has retained the three cassettes and the HO gene, but misses the SIR1 gene, involved in silencing of HML and HMR in S. cerevisiae. In this work, we address the functionality of different Ho proteins and of the switching system in C. glabrata by expressing all HO genes from the Nakaseomyces and S. cerevisiae. Our induction experiments are done on solid medium, so that the HO genes are expressed continuously. Studies in S. cerevisiae were performed in the same way (for review, [5]) and allowed the dissection of the MAT-switching mechanisms. This is very different from the natural situation in which HO gene expression is tightly regulated, and could lead to continuous cleavage during cell growth on plates. Nonetheless, in our experiments, we obtained a very reproducible proportion of switched colonies. This seems to show that the Ho proteins do not cut repeatedly and do not induce switching back and forth between MATa and MATalpha in our conditions.

HO genes from the Nakaseomyces and S. cerevisiae allow MAT switching in a MATa C. glabrata strain (BG87). We observe that the Nakaseomyces’ Hos are poorly efficient, even those of the two mating-competent species of the clade, N. delphensis and N. bacillisporus. Unexpectedly, S. cerevisiae’s Ho is the most efficient to induce MAT switching in C. glabrata and we thus focused the rest of our work on this endonuclease.

S. cerevisiae’s Ho is efficient in both directions and in both genetic backgrounds (strains BG87 and HM100). Thus, the localization of HMR on another chromosome from MAT does not prevent its role as donor during double-strand break repair in C. glabrata. In addition, inducing the switch in the two backgrounds (BG87 and HM100) allows us to obtain MATa and MATalpha isogenic strains, which could be useful for mating assays.

In addition to its high efficiency of switch, S. cerevisiae’s Ho induces a very high cell lethality in C. glabrata, which is not the case for the Nakaseomyces HO genes. Moreover, this strong lethality seems to be specific to C. glabrata since 100% of S. cerevisiae’s cells survive during S.
cerevisiae’s HO gene expression in the same conditions. Thus the lethality is not due to any construct toxicity or HO gene expression conditions. The same survival rate is observed in both sexual types BG87 (MATα) and BG87s (MATα), so that using the HMR locus as template does not seem to be involved in the observed lethality.

Switching in a MATα strain results in numerous clones which have also switched at HML, proof that Ho also cuts at this locus. On the contrary, we do not observe switching at HMR in our conditions. We cannot exclude that HMR is atypically cleaved by Ho, like HML, and that this leads to repair without switch, so that we cannot detect it. The fact that we observe cutting at HML and not at HMR is unexpected since HMR is transcriptionally leaky, contrary to HML [38]. Therefore, accessibility to transcription factors and accessibility to cleavage by Ho do not necessarily go hand in hand. In S. cerevisiae, extensive studies have revealed that HML and HMR are silenced by several proteins (mainly Sir proteins) and usually protected from Ho cleavage (for review, [5]). In sir- mutants, the two HML and HMR loci are unsilenced and can act as recipients and thus, switch [27–29]. Furthermore, cleavage at HML has been previously reported at a very low rate during constitutive HO expression in wild-type cells while none was observed at HMR [69], in accordance to what we observed in C. glabrata.

Natural variants carrying opposite configuration at the HML locus have been described previously in C. glabrata [36]. We screened 100 different strains of our C. glabrata collection [35] by typing the Ya or Yalpha information at the three loci MAT, HMR and HML by specific PCR amplifications (data not shown). We found 10% of the strains revealing rearrangements, with at least one strain harboring the HMLα genotype. These data confirm that cuts outside of MAT can occur in natural conditions.

Our results show, that in a wild-type strain of C. glabrata, both MAT and HML are cut at high levels by Ho during switching. We believe that this “illegal” cleavage is the result of a silencing system which is less efficient than S. cerevisiae’s, possibly due to the absence of SIR1 and that this may be the cause of the mortality. C. glabrata does not follow the rule of “one recipient for two opposite donors”, essential for the proper functioning of the switching system in S. cerevisiae. The high mortality associated to efficient switching, explains perhaps, why C. glabrata is not observed to switch regularly. Our work, relying on deregulation of the genes, shows that the low efficiency of the endogenous system is probably not due to a weak endogenous promoter, but may be linked to the properties of the protein itself, such as stability, nuclear localization and/or cleavage activity. Nonetheless, the fact that the genome of C. glabrata has retained the elements necessary for switching as well as the genes involved in mating, point to the possibility that undiscovered conditions may induce switching and/or mating.

Further experiments are under way to understand the correlation between switching and cell death in C. glabrata. Especially, mutations in the Ho recognition sites should allow us to understand better the switching system and the rules for cleavage of recipients and for donor preference in this pathogen.

Author Contributions
Conceived and designed the experiments: SB CF YZL. Performed the experiments: SB SA XR GS YZL. Analyzed the data: SB SA XR GS CF YZL. Contributed reagents/materials/analysis tools: MBF AEA. Wrote the paper: SB CF.

References
1. Ni M, Feretzaki M, Sun S, Wang X, Heitman J. Sex in fungi. Annu Rev Genet. 2011; 45: 405–430. doi: 10.1146/annurev-genet-110410-132536 PMID: 21942368
2. Murphy HA, Zeyl CW. Yeast sex: surprisingly high rates of outcrossing between asci. PloS One. 2010; 5: e10461. doi: 10.1371/journal.pone.0010461 PMID: 20463964
3. Hicks JB, Strathern JN, Herskowitz I. Interconversion of Yeast Mating Types III. Action of the Homothallism (HO) Gene in Cells Homozygous for the Mating Type Locus. Genetics. 1977; 85: 395–405. PMID: 17248736

4. Oshima Y, Takano I. Mating types in Saccharomyces: their convertibility and homothallism. Genetics. 1971; 67: 327–335. PMID: 5111359

5. Haber JE. Mating-type genes and MAT switching in Saccharomyces cerevisiae. Genetics. 2012; 191: 33–64. doi: 10.1534/genetics.111.134577 PMID: 22555442

6. Roach KC, Feretzkzi M, Sun S, Heitman J. Unisexual reproduction. Adv Genet. 2014; 85: 255–305. doi: 10.1016/B978-0-12-800271-1.00005-6 PMID: 24880737

7. Klar AJ. Lessons learned from studies of fission yeast mating-type switching and silencing. Annu Rev Genet. 2007; 41: 213–236. PMID: 17614787

8. Haber JE, Mascioli DW, Rogers DT. Illegal transposition of mating-type genes in yeast. Cell. 1980; 20: 519–528. PMID: 6993011
28. Klar AJ, Hicks JB, Strathern JN. Irregular transpositions of mating-type genes in yeast. Cold Spring Harbor Symp Quant Biol. 1981; 45 Pt 2: 983–990. PMID: 6266771

29. Miyazaki T, Bressan DA, Shinohara M, Haber JE, Shinohara A. In vivo assembly and disassembly of Rad51 and Rad52 complexes during double-strand break repair. EMBO J. 2004; 23: 939–949. PMID: 14765116

30. O’Gorman CM, Fuller HT, Dyer PS. Discovery of a sexual cycle in the opportunistic fungal pathogen Aspergillus fumigatus. Nature. 2009; 457: 471–474. doi: 10.1038/nature07528 PMID: 19043401

31. Hull CM, Raisner RM, Johnson AD. Evidence for mating of the “asexual” yeast Candida albicans in a mammalian host. Science. 2000; 289: 307–310. PMID: 10894780

32. Magee BB, Magee PT. Induction of mating in Candida albicans by construction of MTLα and MTLα strains. Science. 2000; 289: 310–313. PMID: 10894781

33. Hickman MA, Zeng G, Forche A, Hirakawa MP, Abbey D, Harrison BD, et al. The “obligate diploid” Candida albicans forms mating-competent haploids. Nature. 2013; 494: 55–59. doi: 10.1038/nature11865 PMID: 23364695

34. Pfaffer MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007; 20: 133–163. PMID: 17223626

35. Brisse S, Pannier C, Angoulvant A, de Meeus T, Diancourt L, Faure O, et al. Uneven distribution of mating types among genotypes of Candida glabrata isolates from clinical samples. Eukaryot Cell. 2009; 8: 287–295. doi: 10.1128/EC.0015-08 PMID: 19151326

36. Srikantha T, Lachke SA, Soli DR. Three mating type-like loci in Candida glabrata. Eukaryot Cell. 2003; 2: 328–340. PMID: 12684382

37. Wong S, Fares MA, Zimmermann W, Butler G, Wolfe KH. Evidence from comparative genomics for a complete sexual cycle in the “asexual” pathogenic yeast Candida glabrata. Genome Biol. 2003; 4: R10. PMID: 12620120

38. Muller H, Hennequin C, Gallaud J, Dujon B, Fairhead C. The asexual yeast Candida glabrata maintains distinct a and alpha haploid mating types. Eukaryot Cell. 2008; 7: 848–858. doi: 10.1128/EC.00456-07 PMID: 18375614

39. Alcoba-Flórez J, Méndez-Alvarez S, Cano J, Guarro J, Pérez-Roth E, del Pilar Arévalo M. Phenotypic and molecular characterization of Candida nivariensis sp. nov., a possible new opportunistic fungus. J Clin Microbiol. 2005; 43: 4107–4111. PMID: 16081957

40. Correia A, Sampaio P, James S, Pais C. Candida bracarensis sp. nov., a novel anamorphic yeast species phenotypically similar to Candida glabrata. Int J Syst Evol Microbiol. 2006; 56: 313–317. PMID: 16403904

41. Kurtzman CP. Phylogenetic circumscription of Saccharomyces, Kluyveromyces and other members of the Saccharomycetaceae, and the proposal of the new genera Lachancea, Nakaseomyces, Naumovia, Vanderwaltozyma and Zygotorulaspora. FEMS Yeast Res. 2003; 4: 233–240. PMID: 12684382

42. Gabaldón T, Martin T, Marceau-Houben M, Durrens P, Bolotin-Fukuhara M, Lespinet O, et al. Comparative genomics of emerging pathogens in the Candida glabrata clade. BMC Genomics. 2013; 14: 623. doi: 10.1186/1471-2164-14-623 PMID: 24034898

43. Dujon B, Sherman D, Fischer G, Durrens P, Casaregola S, Lafontaine I, et al. Genome evolution in yeasts. Nature. 2004; 430: 35. doi: 10.1038/nature01511

44. Muller H, Hennéquin C, Dujon B, Fairhead C. Ascomycetes: the Candida MAT Locus: Comparing MAT genotypes and molecular characterization of Candida nivariensis sp. nov., a possible new opportunistic fungus. J Clin Microbiol. 2005; 43: 4107–4111. PMID: 16081957

45. Gorman CM, Fuller HT, Dyer PS. Discovery of a sexual cycle in the opportunistic fungal pathogen Candida glabrata. Genome Biol. 2003; 4: R10. doi: 10.1186/1471-2164-14-623 PMID: 24034898

46. Alcoba-Flórez J, Méndez-Alvarez S, Cano J, Guarro J, Pérez-Roth E, del Pilar Arévalo M. Phenotypic and molecular characterization of Candida nivariensis sp. nov., a possible new opportunistic fungus. J Clin Microbiol. 2005; 43: 4107–4111. PMID: 16081957

47. Correia A, Sampaio P, James S, Pais C. Candida bracarensis sp. nov., a novel anamorphic yeast species phenotypically similar to Candida glabrata. Int J Syst Evol Microbiol. 2006; 56: 313–317. PMID: 16403904

48. Kurtzman CP. Phylogenetic circumscription of Saccharomyces, Kluyveromyces and other members of the Saccharomycetaceae, and the proposal of the new genera Lachancea, Nakaseomyces, Naumovia, Vanderwaltozyma and Zygotorulaspora. FEMS Yeast Res. 2003; 4: 233–245. PMID: 14654427

49. Gabaldón T, Martin T, Marceau-Houben M, Durrens P, Bolotin-Fukuhara M, Lespinet O, et al. Comparative genomics of emerging pathogens in the Candida glabrata clade. BMC Genomics. 2013; 14: 623. doi: 10.1186/1471-2164-14-623 PMID: 24034898

50. Dujon B, Sherman D, Fischer G, Durrens P, Casaregola S, Lafontaine I, et al. Genome evolution in yeasts. Nature. 2004; 430: 35–44. PMID: 15228992

51. Muller H, Hennéquin C, Dujon B, Fairhead C. Ascomycetes: the Candida MAT Locus: Comparing MAT genotypes and molecular characterization of Candida nivariensis sp. nov., a possible new opportunistic fungus. J Clin Microbiol. 2005; 43: 4107–4111. PMID: 16081957

52. Butler G, Kenny C, Fagan A, Kurtischko C, Gaillardin C, Wolfe KH. Evolution of the MAT locus and its Ho endonuclease in yeast species. Proc Natl Acad Sci U S A. 2004; 101: 1632–1637. PMID: 14745027

53. Erdeske HK, Wickner RB. The [URE3] prion in Candida. Eukaryot Cell. 2013; 12: 551–558. doi: 10.1128/EC.00015-13 PMID: 23397567

54. Lin C-Y, Chen Y-C, Lo H-J, Chen K-W, Li S-Y. Assessment of Candida glabrata strain relatedness by pulsed-field gel electrophoresis and multilocus sequence typing. J Clin Microbiol. 2007; 45: 2452–2459. PMID: 17553975

55. Naumov GI, Tolstokorov II. [Discovery of an unstable homothallic strain of Saccharomyces cerevisiae var. ellipsodeus]. Nauchnye Dokl Vysshe Obrazovatel’nye Instityuty Pushchino Materialy Nauchno-Issledovatel’skikh Tsentrov. 1971; 9: 92–94.

56. Tolstokorov II, Naumov GI. [Comparative genetics of yeasts. XI. A genetic study of autodiplidization in natural homothallic strains of Saccharomyces]. Nauchnye Dokl Vysshe Obrazovatel’nye Instityuty Pushchino Materialy Nauchno-Issledovatel’skikh Tsentrov. 1973; 117: 111–115.

57. Koufopanou V, Burt A. Degeneration and domestication of a selfish gene in yeast: molecular evolution versus site-directed mutagenesis. Mol Biol Evol. 2005; 22: 1535–1538. PMID: 15843599
51. Okuda Y, Sasaki D, Nogami S, Kaneko Y, Ohya Y, Anraku Y. Occurrence, horizontal transfer and degeneration of VDE intein family in Saccharomycete yeasts. Yeast Chichester Engl. 2003; 20: 563–573.

52. Keeling PJ, Roger AJ. The selfish pursuit of sex. Nature. 1995; 375: 283. PMID: 7753189

53. Barsoum E, Martínez P, Aström SU. Alpha3, a transposable element that promotes host sexual reproduction. Genes Dev. 2010; 24: 33–44. doi: 10.1101/gad.557310 PMID: 20008928

54. Rajaei N, Chiruvella KK, Lin F, Aström SU. Domesticated transposase Kat1 and its fossil imprints induce sexual differentiation in yeast. Proc Natl Acad Sci U S A. 2014; 111: 15491–15496. doi: 10.1073/pnas.140627111 PMID: 25313032

55. Bakhrat A, Jurica MS, Stoddard BL, Raveh D. Homology modeling and mutational analysis of Ho endonuclease of yeast. Genetics. 2004; 166: 721–728. PMID: 15020462

56. Chevalier BS, Stoddard BL. Homing endonucleases: structural and functional insight into the catalysts of intron/intein mobility. Nucleic Acids Res. 2001; 29: 3757–3774. PMID: 11557808

57. Monteihiel C, Perrin A, Thierry A, Colleaux L, Dujon B. Purification and characterization of the in vitro activity of I-Sce I, a novel and highly specific endonuclease encoded by a group I intron. Nucleic Acids Res. 1990; 18: 1407–1413. PMID: 2183191

58. Gietz RD, Schiestl RH, Willems AR, Woods RA. Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. Yeast Chichester Engl. 1995; 11: 355–360.

59. Winston F, Dollard C, Ricupero-Hovasse SL. Construction of a set of convenient Saccharomyces cerevisiae strains that are isogenic to S288C. Yeast Chichester Engl. 1995; 11: 53–55.

60. Thierry A, Fairhead C, Dujon B. The complete sequence of the 8.2 kb segment left of MAT on chromosome III reveals five ORFs, including a gene for a yeast ribokinase. Yeast Chichester Engl. 1990; 6: 521–534.

61. Fidel PL, Cutright JL, Tait L, Sobel JD. A murine model of Candida glabrata vaginitis. J Infect Dis. 1996; 173: 425–431. PMID: 8568305

62. Cormack BP, Falkow S. Efficient homologous and illegitimate recombination in the opportunistic yeast pathogen Candida glabrata. Genetics. 1999; 151: 979–987. PMID: 10049916

63. Zordan RE, Ren Y, Pan S-J, Rotondo G, De Las Peñas A, Iluore J, et al. Expression plasmids for use in Candida glabrata. G3 Bethesda Md. 2013; 3: 1675–1686.

64. Church GM, Gilbert W. Genomic sequencing. Proc Natl Acad Sci U S A. 1984; 81: 1991–1995. PMID: 6326095

65. Weiler KS, Broach JR. Donor locus selection during Saccharomyces cerevisiae mating type interconversion responds to distant regulatory signals. Genetics. 1992; 132: 929–942. PMID: 1459444

66. Wu X, Wu C, Haber JE. Rules of donor preference in saccharomyces mating-type gene switching revealed by a competition assay involving two types of recombination. Genetics. 1997; 147: 399–407. PMID: 9335581

67. McGill C, Shafer B, Strathern J. Coconversion of flanking sequences with homothallic switching. Cell. 1989; 57: 459–467. PMID: 2541914

68. Mehr RK, Thorvaldsen JL, Macreadie IG, Winge DR. Cloning system for Candida glabrata using elements from the metallothionein-IIa-encoding gene that confer autonomous replication. Gene. 1992; 113: 119–124. PMID: 1563627

69. Connolly B, White CI, Haber JE. Physical monitoring of mating type switching in Saccharomyces cerevisiae. Mol Cell Biol. 1988; 8: 2342–2349. PMID: 2841579