REFERENCE

The creation and characterization of the deletion library described below is reported in the following manuscript:

Homann OR, Dea J, Noble SM, Johnson AD. <u>A phenotypic profile of the Candida albicans regulatory network</u>. PLoS Genet. 2009 Dec;5(12):e1000783. Epub 2009 Dec 24.

BACKGROUND STRAIN

The source strain (SN152) used to construct the deletion library was created by Suzanne Noble and has the following genotype:

 $arg4\Delta/arg4\Delta$ $leu2\Delta/leu2\Delta$ $his1\Delta/his1\Delta$ URA3/ $ura3\Delta$:: imm^{434} IRO1/ $iro1\Delta$:: imm^{434}

The construction of the source strain and the long-flanking-homology disruption technique used to create the deletion mutants are described in the following manuscript:

Noble, S.M. & Johnson, A.D. Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen Candida albicans. *Eukaryot Cell* **4**, 298-309 (2005).

KNOCKOUT STRAINS and CONTROL "WILD-TYPE" STRAIN

Note that the deletion strains all have a copy of *LEU2* and a copy of *HIS1* restored, but they remain arginine auxotrophs. A paired "wild-type" strain is also provided, which also contains a restored copy of *LEU2* and *HIS2* and also remains an arginine auxotroph.

PLATE LAYOUT AND STRAIN NOMENCLATURE

The library is provided in four 96-well plates, as diagrammed in the spreadsheet accompanying this document. Deletion strains were created for 165 different transcriptional regulators (TF001 through TF165), and in most cases two independently derived knockouts were created for each transcriptional regulator and designated 'X' and 'Y' (e.g. TF021-X and TF021-Y).

The plates are designated X1, X2, Y1, and Y2 and contain the following strains:

<u>Plate X1:</u> *TF001-X* through *TF096-X* <u>Plate X2:</u> *TF097-X* through *TF165-X* (and a "wild-type" strain in well H12) <u>Plate Y1:</u> *TF001-Y* through *TF096-Y* <u>Plate Y2:</u> *TF097-Y* through *TF147-Y* plus *TF164-Y* and *TF165-Y* (and a "wild-type" strain in well H12)

OLIGOS

The spreadsheet that accompanies this document contains the ORF names associated with each TF### designation, as well as the oligos used to create and verify the deletion mutants. The oligo names P1, P3, P4 and P6 are taken from the Noble and Johnson manuscript referenced above, and are the oligos used to create the left and right flanks of the fusion PCR, respectively.

The oligo named PV5 lies upstream of P1 and was used for flank diagnostic PCR in conjunction with an oligo corresponding to the *HIS* or *LEU* marker (again, as described in the Noble and Johnson manuscript). Similarly, the oligo named PV3 lies downstream of P6.

The oligos designated INT5 and INT3 are paired primers internal to the ORF being deleted, and were used to further verify that the ORF was absent in the deletion mutant (although a PCR with no result isn't the ideal diagnostic, all such tests were paired with the wild-type strain as positive control).

A few additional details:

- 1. Please note that all oligos were designed using Assembly 19 of the Candida albicans genome.
- 2. In some cases the 'X' and 'Y' isolates of a deletion mutant were constructed using different oligos.
- 3. In a few cases additional oligos were used to further validate the deletion. These are included in additional columns in the spreadsheet.
- 4. A small number of deletion mutants have oligo entries marked "[SN]" and only have INT3 and INT5 oligo information supplied. These mutants were created by Suzanne Noble and the oligos used to create the mutants will be provided in an upcoming publication.

SIGNATURE TAGGED MARKERS (STMs)

The deletion library incorporated STMs (as described in the Noble and Johnson paper referenced above) into the deletion mutants (from a set of 48). The spreadsheet indicates the STM number for each strain (note that the 'X' and 'Y' isolates sometimes have different markers), and the precise sequences of the STMs will be provided in a data supplement of an upcoming publication by Suzanne Noble. Please note that these STMs were not used in the Homann *et al.* publication, and their presence has not been thoroughly vetted.

ADDITIONAL COMMENTS

Please note that in some cases the independently-derived 'X' and 'Y' isolates exhibited subtle (and sometimes not-so-subtle) differences during phenotyping. This information is provided in the Data Set S2 of the Homann *et al.* manuscript, and <u>it is strongly recommended that you consult this Data Set whenever working with this library</u>.

Also note that in the few cases where only a single knockout isolate was obtained (*TF148-X* through *TF163-X*), these strains should be regarded with some suspicion since it was very difficult to obtain isolates (and thus the existing isolate may contain suppressor mutations or have other issues).