Published in final edited form as:

Methods Mol Biol. 2017 ; 1551: 131–146. doi:10.1007/978-1-4939-6750-6_7.

Haplotyping a Non-meiotic Diploid Fungal Pathogen Using Induced Aneuploidies and SNP/CGH Microarray Analysis

Judith Berman and Anja Forche

Abstract

The generation of haplotype information has recently become very attractive due to its utility for identifying mutations associated with human disease and for the development of personalized medicine. Haplotype information also is crucial for studying recombination mechanisms and genetic diversity, and for analyzing allele-specific gene expression. Classic haplotyping methods require the analysis of hundreds of meiotic progeny. To facilitate haplotyping in the non-meiotic human fungal pathogen *Candida albicans*, we exploited trisomic heterozygous chromosomes generated via the *UAUI* selection strategy. Using this system, we obtained phasing information from allelic biases, detected by SNP/CGH microarray analysis. This strategy has the potential to be applicable to other diploid, asexual *Candida* species that are important causes of human disease.

Keywords

Haplotyping; Phased genome; *Candida albicans*; Non-meiotic organism; Aneuploidy; *UAUI* cassette; Heterozygosity; SNP/CGH microarrays; Ymap

1 Introduction

Haplotyping, in general terms, is the process by which the phased haploid sequence is determined from unordered (unphased) genotype data. Knowing the specific haplotype carried by a given individual is essential for understanding the phenotypic consequences of gene regulation, genetic diversity, recombination mechanisms, and for identifying mutations associated with monogenic and multigenic human diseases [1]. To give two examples, haplotype information facilitates the detection of loss of heterozygosity (LOH) in cancer cells, and enables identification of the alleles carried at the major histocompatibility complex locus, important for treating autoimmune disorders and organ transplantation [2]. The classical genetic approach to produce haplotype maps, or “hapmaps,” involves genotyping hundreds of meiotic progeny, and comparing recombinant and paternal genotypes. Current technologies, such as whole genome sequencing, rarely resolve diploid sequence data into their respective haplotypes, and haplotypes are most often inferred using statistical approaches [1].

Non-meiotic organisms pose a special challenge for haplotyping methods, as they do not normally produce recombinant offspring and can have highly divergent homologs. *C. albicans*, an opportunistic fungal pathogen of humans and the major cause of fungal infections in the clinic, is such a highly heterozygous ameiotic diploid [3]. Despite no evidence of meiosis, the *C. albicans* genome rapidly adapts to in vitro and in vivo stresses, including antifungal drugs [4–8]. To better understand this rapid adaptation, we constructed a hapmap for the standard laboratory strain SC5314 [9], which facilitated both linkage mapping and the analysis of recombination mechanisms. To generate this hapmap, we exploited the ameiotic nature of *C. albicans* and the ability to generate trisomic individuals with two copies of one homologous chromosome and a single copy of the other homolog. Thus, by determining allelic ratios along complete chromosomes, we were able to assign SNPs to differentiate homologs of the homozygous and/or aneuploid chromosome.

Initially, we used allelic ratios of a series of isolates from two different strain backgrounds (laboratory strain SC5314 and clinical isolate T118) that were either aneuploid or homozygous for whole chromosomes [allelic ratios of SNPs (1:2 or 2:1)] to assign alleles to haplotypes [5, 10]. This allowed us to generate hapmaps for a limited set of SNP markers (150) [9, 11]. Next, we used tiling SNP/CGH arrays to analyze ~39,000 additional SNPs for the SC5314 strain background [12]. Muzzey et al. followed up with massively parallel sequencing of the same set of strains derived from SC5314 to construct whole chromosome hapmaps for all the SNPs in this particular strain background [13]. Markers on Chr3R are homozygous in the original laboratory strain and were designated “allele a” in earlier studies. Unfortunately, Muzzey et al. analysis renamed the alleles on Chr3R such that all are named “allele b,” which then became the allele assignments in the CGD database [14].

Since only a limited number of strains homozygous or triploid for whole chromosomes were available, we tested a *UAUI* transformation strategy to artificially induce whole chromosome trisomy of specific chromosomes. We investigated the *UAUI* approach, originally developed to delete both copies of a desired gene during a single round of transformation, as it was already known to result in trisomic alleles when targeting essential genes [15]. To produce gene knockouts, a *UAUI* cassette consisting of a central functional gene (*ARG4*) flanked by two partial and overlapping fragments of the *URA3* gene is used; homologous recombination of the overlapping regions brings together the *URA3* fragments, resulting in a complete and functional copy of *URA3* [15, 16] (Fig. 1a, b). To delete both copies of a given gene (Your Favorite Gene 1, *TFG1*), a *C. albicans* strain lacking *ARG4* and *URA3* is transformed with a custom *UAUI* construct carrying sequences homologous to those flanking the open reading frame (ORF) of *TFG1*.

Homologous recombination between these sequences on the construct and those on the chromosome will yield transformants where the *TFG1* coding sequence has been replaced with the *UAUI* construct and can be detected because they are prototrophic for arginine (Arg⁺) (Fig. 1b). To obtain knockouts of both copies of *TFG1*, Arg⁺ transformants are grown on nonselective media to allow for two sequential rounds of recombination (Fig. 1c). The first recombination event results in the deletion of the second remaining *TFG1* allele by homologous recombination, resulting in loss of heterozygosity of the locus such that both copies contain the *UAUI* cassette. This recombination event can occur via a double

crossover that directly replaces one allele for the other, but more frequently involves a single crossover between the centromere and the *TFGI* locus followed by the segregation of the two *UAUI* alleles into the same daughter cell. The second recombination event results in a functional *URA3* allele by direct repeat recombination between the two *URA3* fragments. These cells will be both Ura⁺ and Arg⁺, as retention of the original *UAUI* copy on the other allele maintains the Arg⁺ phenotype, and can be easily identified by selection on medium lacking arginine and uridine (Fig. 1c).

Interestingly, the results for essential genes differed: repeated efforts to delete these genes did not eliminate the wild-type copy of *TFGI*, despite the presence of both the complete *UAUI* and the recombined *URA3* cassettes. Diagnostic PCR of these strains always amplified all three possible alleles for the *TFGI* locus, the wild-type allele, the *UAUI* cassette, and the functional *URA3* gene. In many of these cases, the transformants were trisomic for the chromosome carrying the essential *TFGI* (*TFGI*_{ess}) locus, with all known SNPs on the chromosome having a 1:2 allele ratio bias. Accordingly, by targeting the essential gene *ORC1* on chromosome 1 with *UAUI*, we were able to confirm whole chromosome trisomy in 43 % of the Arg⁺ Ura⁺ transformants (four of seven transformants analyzed by SNP microarray and aCGH) [9].

The following protocols will enable the reader to target a specific chromosome of interest in *C. albicans* by using the *UAUI* strategy to replace an essential gene on this chromosome. Once transformants tri-allelic for the targeted chromosome are obtained, the phased genotype can be determined by inferring the ratio of DNA amounts for each allele, using commercially available custom SNP/CGH arrays. Alternatively, the genotypes can be analyzed by deep sequencing, or by cost-effective reduced representation sequencing methods such as double-digest restriction site associated DNA sequencing (ddRADseq), and allele ratios for SNPs can be determined from the sequence data [12, 17–21].

One limitation of this method is that it is only applicable to strains that are auxotrophic for arginine and uridine. This limitation can be potentially circumvented by modifying the transformation protocol to use two drug resistance markers, such as nourseothricin (*NAT1*) and hygromycin B (*HTG*) (e.g., *NA-HTG-AT*). This strategy should then be adaptable not only to other strains of *C. albicans*, but also to other diploid *Candida* species such as *C. dubliniensis* and *C. parapsilosis*, thereby facilitating the analysis of genome structure and recombination mechanism in these species.

2 Materials

2.1 Custom-Ordered: Agilent 8× 60 K SNP/CGH Arrays for *C. albicans* (Amadid 039364)

Probes were 19–33 nt long with melting points (T_m) near 55 °C [22] and the SNP at the middle position. Initially, probes were designed for 43,658 SNP loci and 28,563 non-SNP loci (including regions that do not have high frequencies of SNPs). Probes were eliminated if they had more than one significant alignment (*e*-value, 0.001) with the reference genome using BLAST analysis [23]. The acceptable T_m range was reduced to optimize the number of probes to the Agilent 8× 60 K microarray format (41,616 SNP allele probes (two probes per SNP locus, representing the two SNP alleles) plus 20,363 non-SNP (CGH) probes (one

per locus). Quality control of the final array design resulted in a usable SNP allele probe count of 39,222. A total of 19,016 SNP loci (97 % of usable SNP loci) were informative and were mapped to one of the two homologs (see Table S1 for SNP probes) [12].

2.2 Equipment

1. Shaking incubator.
2. Eppendorf centrifuge 5430R (or similar).
3. Table top centrifuge (for 50 mL conical tubes).
4. Thermocycler machine for PCR reactions.
5. Digital water bath.
6. Gel electrophoresis setup.
7. Microscope (×40 magnification).
8. Plastic nitrogen purge desiccator cabinet for microarray slide storage.
9. Agilent hybridization oven with rotator rack and rotator rack conversion rod.
10. Agilent hybridization chamber.
11. Slide staining dish (×4).
12. Magnetic stir plate (×3).
13. Agilent microarray scanner.

2.3 Growth Media

1. Yeast extract Peptone Dextrose medium (YPD) for strain recovery from stock: 1 % yeast extract, 2 % bacto peptone, 2 % dextrose, 1.5 % agar (omit for broth), 5 mL uridine (20 mg/L), 995 mL deionized water, autoclave for 30 min.
2. Minimal medium (MIN) (see **Note**¹): 0.67 % yeast nitrogen base without amino acid (with ammonium sulfate), 2 % dextrose, 1.5 % agar (omit for broth), 1 L deionized water, autoclave for 20 min.
3. MIN + Uridine (MIN + Uri): 0.67 % yeast nitrogen base without amino acid (with ammonium sulfate), 2 % dextrose, 1.5 % agar (omit for broth), 5 mL uridine (20 mg/L), 995 mL deionized water, autoclave for 20 min.

2.4 Buffers

1. TENTS buffer: 100 mM NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA pH 8.0, 2 % triton X, 1 % SDS.
2. 10× TE buffer: 5 mL 1 M Tris, pH 7.5, 1 mL 0.5 M EDTA pH 8.0, 44 mL deionized water, filter sterilize.

¹Instead of MIN medium, a semidefined dropout medium (SDC) can be used [24] (for selection of Arg⁺, Ura⁻ transformants plate on SDC-Arg, when screening for Arg⁺Ura⁺ transformants plate on SDC-Arg-Uri).

3. TELiAc buffer (*see Note* ²): 8 vol deionized water, 1 vol 10× TE buffer, 1 vol 1 M LiAc.
4. 50 % Polyethyleneglycol (PEG) (*see Note* ³): to a 150 mL glass beaker add a magnetic stir bar, 50 g polyethylene glycol MW 3350, 40 mL deionized water, seal with parafilm, let stir overnight, raise volume to 100 mL, filter sterilize.
5. PLATE mix: 8 vol 50 % PEG, 1 vol 1 M LiAc, 1 vol 10× TE buffer.
6. Agilent 10× aCGH blocking agent.
7. Agilent 2× hybridization buffer.
8. Agilent OligoCGH wash buffer 2.
9. Agilent stabilization and drying solution.
10. 10× TBE gel electrophoresis buffer (1 L): 108 g Tris base, 55 g Boric acid, 40 mL 0.5 M EDTA pH 8.0, raise volume to 1 L with deionized water.

2.5 Chemicals

1. Phenol/Chloroform/Isoamylalcohol (25:24:1).
2. Isopropanol.
3. 3 M Na Acetate, pH 5.5.
4. Ethanol.
5. Ethidium bromide solution.
6. Acetonitrile (caution: extremely flammable).
7. Agarose (Seakem).

2.6 Molecular Biology

1. dNTP mix (for PCR).
2. Phusion Taq Polymerase.
3. Oligonucleotides YFG_{ess}-pBME101-F (70 nt of 5' YFG_{ess}-GTTTTCCCAGTCACGACGTT) and YFG_{ess}-pBME101-R (70 nt of 3' YFP_{ess}-TGTGGAATTGTGAGCGGATA) [15] for amplification of *UAU1* transformation cassette (Fig. 1a).
4. Oligonucleotides for diagnostic PCR (Fig. 2a, b) (*see Note* ⁴).
5. Plasmid carrying *UAU1* cassette (pBME101) [15].
6. 10 mg/mL sheared salmon sperm DNA.

²TELiAc buffer can be prepared as stock, and filter-sterilized. It can be stored for several months.

³Following the protocol for making PEG is very important, fresh stock should be prepared every month. Using older PEG will decrease transformation efficiency.

⁴Primers for diagnostic PCR need to be designed for each transformation. Primer 3 software is freely available online (<http://bioinfo.ut.ee/primer3/>).

7. *Hae*III restriction enzyme and buffer.
8. Invitrogen Bioprime kit.
9. dNTP mix (1.2 mM dATP, dGTP, dCTP, 0.9 mM dTTP) (for SNP/CGH array).
10. Cy3 and Cy5 Fluorolink monofunctional dyes.

2.6.1 Kits

1. Qiagen MinElute PCR purification kit.
2. Agilent Oligo aCGH/ChIP-Chip hybridization kit.

2.6.2 Disposables

1. Sterile, powder-free nitrile gloves.
2. Glass beads (for gDNA extraction).
3. Sterile 1.5 mL microfuge tubes.
4. Sterile 1.5 mL Eppendorf Safe-lock tubes.
5. Sterile 0.2 mL PCR tubes.
6. Sterile pipet tips.
7. Microcon 30 KDa centrifugal filters (Millipore).
8. Array gasket backings for Agilent slides.

2.6.3 Other

1. *C. albicans* strains (stocks in 50 % glycerol stored at -80°C).
2. Glass beads for plating.
3. Magnetic stir bars (4 $\times$).
4. BlueGnome microarray analysis software (Cambridge).

3 Methods

3.1 Amplification and Purification of UAU1 Transformation Cassette

1. For each transformation, prepare eight 50 μL PCR reactions as follows (*see Note 5*):
 - 32 μL deionized water.
 - 10 μL GC buffer.
 - 2 μL dNTPs (2.5 mM each).
 - 2 μL YFG_{ess}-pBME101-F (10 μM).

⁵Eight 50 μL PCR reactions are needed for high transformation efficiency.

2 μ L YFG_{ess}-pBME101-R (10 μ M).

4.5 μ L of 25 mM MgCl₂.

0.5 μ L Phusion Taq Polymerase (two units).

2 μ L of plasmid pBME101 (1:100 dilution of a quick plasmid prep).

2. Run PCR under the following conditions (see **Note** ⁶):

98 °C—30 s

34 cycles of:

98 °C—10 s

55 °C—30 s

72—2 min

72—10 min

15-hold

3. Run 5 μ L of each PCR reaction on a 1 % agarose gel to check for amplification of *UAU1* cassette (4.15 kb).
4. Pool all eight PCR reactions into a 1.5 mL Eppendorf tube, add 1 mL cold 95 % ethanol and 50 μ L 3.5 M Na Acetate pH 5.5, precipitate DNA at –20 °C for at least 20 min.
5. Centrifuge sample for 20 min at 13,000 rpm in Eppendorf centrifuge (M5430R), remove ethanol, air dry pellet, and resuspend DNA in 30 μ L deionized water. Store at –20 °C until use.

3.2 Lithium Acetate Transformation

1. Streak out *C. albicans* strains to be transformed onto YPD medium and incubate at 30 °C for 2 days.
2. Inoculate a single colony into 2 mL YPD and grow overnight at 30 °C with shaking.
3. Add 600 μ L overnight culture to 50 mL YPD (Erlenmeyer flask).
4. Incubate 4–5 h (OD₆₀₀ should be between 0.5 and 0.8) at 30 °C with shaking.
5. Check cells for active budding by microscopy. Transformation efficiency of actively budding cells is better because the cell walls of growing buds are not as rigid as stationary phase (non-budding) cells and budding cells are also more metabolically active.
6. Transfer entire culture to 50 mL conical tubes, pellet cells at 1200 rpm for 5 min, and discard medium.

⁶All PCRs in this chapter may need some optimization based on brand of polymerase, brand of PCR thermocycler machine, and primers used.

7. Resuspend pellet in 5 mL deionized water, spin for 5 min at 1200 rpm, and discard water.
8. Resuspend cells in 500 μ L TELiAc, centrifuge for 2 min at 1200 rpm in microfuge.
9. Remove TELiAc with pipette and resuspend cells in 250 μ L TELiAc (total volume including pellet $\sim$ 300 μ L).
10. To one 1.5 microfuge tube, add in this order: 5 μ L sheared salmon sperm DNA and 150 μ L cells (negative control).
11. To another microfuge tube add 5 μ L carrier DNA, entire 30 μ L of *UAU1* transformation cassette and 150 μ L cells, incubate for 30 min at room temperature (RT).
12. Add 700 μ L PLATE mix (*see Note* ⁷), invert to mix, and incubate overnight at RT.
13. Heat shock transformation reactions at 42 °C for 1 h in water bath.
14. Centrifuge cells for 3 min at 3000 rpm in microfuge.
15. Remove supernatant with pipette and resuspend in 150 μ L deionized water.
16. Plate entire mixture on MIN + Uri and incubate for up to 3 days at 30 °C.
17. Patch-streak Arg⁺ transformants onto YPD and grow at 30 °C for 2 days.
18. Replica-plate onto MIN plates to select transformants that underwent recombination (Arg⁺Ura⁺) (*see Fig. 1c*).

3.3 gDNA Extraction for Arg⁺Ura⁺ Transformants

1. Streak out Arg⁺Ura⁺ transformants for single colonies onto MIN plates (to maintain selection) and incubate plates at 30 °C for 2 days.
2. Transfer a single colony to 2 mL MIN broth and incubate overnight at 30 °C with shaking.
3. Transfer 1.0 mL *C. albicans* culture to 1.5 mL Eppendorf Safelock tube (*see Note* ⁸), spin down at max speed in Eppendorf centrifuge for 6 s, and discard liquid.
4. Add 1 mL deionized water, vortex tube until pellet is resuspended, spin down at max speed for 6 s, and discard water.
5. Add 500 μ L TENTS buffer (*see Subheading 2*), 200 μ L glass beads (use 0.2 mL scoop for measuring), and 500 μ L Phenol/Chloroform/Isoamylalcohol (25:24:1) (UNDER THE HOOD).
6. Close tubes WELL, turn tubes upside down (very carefully) to check for leaking.

⁷PLATE mix should be made fresh every time; TELiAc can be made as a stock and used for a couple of months.

⁸To avoid leaking of tubes use Safe-lock Eppendorf tubes for gDNA extractions.

7. Put tubes in tube adaptor of vortex shaker and shake for 20 min.
8. Spin tubes at max speed for 10 min, remove upper phase, and transfer to new, labeled 1.5 mL tube.
9. Add 1 mL ice cold isopropanol and 50 μ L 3.5 M Na acetate, mix gently by inversion.
10. Put tubes in a freezer for at least 20 min to allow for maximal DNA precipitation.
11. Spin tubes for 20 min at max speed, discard liquid (careful not to lose pellet).
12. Add 500 μ L ice cold 70 % ethanol to remove any residual salt (make sure you rinse the walls of tubes); remove ethanol with pipette.
13. Either air dry or speed vac dry the pellets.
14. Add 60 μ L TE buffer (with 20 μ g/mL RNase), vortex well, and incubate for 45 min at 37 °C.

3.4 Diagnostic PCR to Test for Presence of Potential Trisomy

To confirm the presence of three alleles at the *TFGess* locus in Arg⁺Ura⁺ transformants:

1. *URA3* and *TFGess* are of similar length—One-step diagnostic PCR (Fig. 2a, c):

32 μ L deionized water.

10 μ L GC buffer.

2 μ L dNTPs (2.5 mM each).

2 μ L YFGess-pBME101-F (10 μ M).

2 μ L YFGess-pBME101-R (10 μ M).

4.5 μ L of 25 mM MgCl₂.

0.5 μ L Phusion Taq Polymerase (two units).

2 μ L gDNA from transformant (30 ng/ μ L).

Run PCR under the following conditions (see **Note 6**):

98 °C—30 s

34 cycles of:

98 °C—10 s

55 °C—30 s

72—2 min

72—10 min

15-hold

Run out 10 μ L of each PCR reaction on a 1 % agarose gel to check for amplification of the correct-size products.

2. *URA3* and *YFGess* are of different length—Left-flank and Right-flank multiplex diagnostic PCRs (Fig. 2b, c):

Left-flank

32 μ L deionized water.

10 μ L GC buffer.

2 μ L dNTPs (2.5 mM each).

1.5 μ L UAU1-diagnostic-F (10 μ M).

0.5 μ L diagnostic-URA3-R (10 μ M).

0.5 μ L diagnostic-ARG4-R (10 μ M).

0.5 μ L diagnostic-YFGess-R (10 μ M).

4.5 μ L of 25 mM $MgCl_2$.

0.5 μ L Phusion Taq Polymerase (two units).

2 μ L gDNA from transformant (30 ng/ μ L).

Right-flank

32 μ L deionized water.

10 μ L GC buffer.

2 μ L dNTPs (2.5 mM each).

1.5 μ L UAU1-diagnostic-R (10 μ M).

0.5 μ L diagnostic-URA3-F (10 μ M).

0.5 μ L diagnostic-ARG4-F (10 μ M).

0.5 μ L diagnostic-YFGess-F (10 μ M).

4.5 μ L of 25 mM $MgCl_2$.

0.5 μ L Phusion Taq Polymerase (two units).

2 μ L gDNA from transformant (30 ng/ μ L).

Run PCRs under the following conditions (*see Note 6*):

98 °C—30 s

34 cycles of:

98 °C—10 s

55 °C—30 s

72—2 min

72—10 min

15-hold

3.5 SNP/CGH Arrays (See Note ⁹)

3.5.1 gDNA Restriction Enzyme Digests

1. Per sample (pipette into 1.5 mL Eppendorf tubes):
3 µg gDNA.
3 µL *Hae*III restriction enzyme.
6 µL 10× M restriction enzyme buffer
2. Add deionized water to total volume of 60 µL.
3. Vortex well, spin down tubes briefly, and incubate at 37 °C for at least 4 h and up to overnight (~16 h).

3.5.2 Purification of Restriction Digests

1. Add 60 µL DNA to a Qiagen minElute spin column. Add 120 µL NT1 (Binding) buffer, spin at max speed for 1 min, and discard flow-through.
2. Add 700 µL NT3 (Wash) buffer, spin at max speed for 1 min, and discard flow-through; repeat.
3. Spin one last time at max speed for 1 min and transfer column to a new 1.5 mL tube.
4. Add 10 µL AE (Elution) buffer, incubate for 1 min at RT, and spin at max speed for 1 min; repeat.
5. Transfer flow through to the top of the column (~20 µL), incubate for 1 min at RT, and spin at max speed for 1 min.

3.5.3 Labeling Reaction

1. Per sample, add 20 µL purified DNA and 20 µL random primer.
2. Incubate sample at 95 °C for 5 min and keep on ice for 5 min.
3. Per sample, add 5 µL dNTP mix (1.2 mM dATP, dGTP, dCTP, 0.9 mM dTTP), 1.5 µL Cy-dye (Cy3 for sample, Cy5 for diploid control strain), and 1 µL Klenow enzyme.
4. Incubate at 37 °C for 2 h.

3.5.4 Wash and Concentrate Probes

1. Combine corresponding Cy3- and Cy5-labeled samples into a Microcon 30 KDa centrifugal filter and add 450 µL deionized water.
2. Spin at 12,000 rpm in microfuge for 8 min. There should be 20–50 µL left in the filter. Discard flow through and add 450 µL deionized water.

⁹Each SNP/CGH hybridization requires the preparation of the experimental strain and of the diploid control strain. The control strain is typically labeled with Cy5 fluorolink dye and the experimental strain with Cy3 fluorolink dye.

3. Spin at 12,000 rpm in microfuge for 5 min in batches until there is approximately 20 μ L left in the filter. The concentrate should be purple in color (mix of Cy3 and Cy5 dyes).
4. Discard flow through. Invert Microcon 30 KDa centrifugal filter into new 1.5 mL tubes and spin at max speed for 2 min to collect the concentrate.

3.5.5 Hybridization

1. To a 0.2 mL PCR tube, add 20 μ L labeled concentrated DNA, 5 μ L 10 $\times$ blocking buffer, and 25 μ L 2 $\times$ hybridization buffer.
2. Incubate at 95 $^{\circ}$ C for 3 min followed by 30 min at 65 $^{\circ}$ C in PCR machine.
3. Set the hybridization oven to 65 $^{\circ}$ C. Place the gasket slide into the hybridization chamber, gasket side up.
4. Add 45 μ L of each sample to CENTER of each gasket. LABEL SAMPLE LOCATIONS ON SLIDE and SLIDE ID#.
5. Place array slide onto gasket slide with AGILENT labeled slide facing gasket slide.
6. Assemble the rest of the hybridization chamber (Fig. 3).
7. Check to see that any bubbles in gaskets are able to move.
8. Secure hybridization chambers into hybridization oven and start rotation, incubate at 65 $^{\circ}$ C for 24 h.

3.5.6 Slide Washing

1. Prepare five wash dishes:
 - a. Wash Buffer 1, RT and glass container—to pry gasket slide from array slide.
 - b. Wash Buffer 1, RT, glass container with stir bar.
 - c. Wash Buffer 2, 37 $^{\circ}$ C, glass container with stir bar.
 - d. Acetonitrile, RT, glass container with stir bar—FUME HOOD.
 - e. Stabilization and drying solution, RT, glass container with stir bar—FUME HOOD.
2. Remove array slide from oven and disassemble hybridization chamber.
3. Place joined slides into (a) Wash Buffer 1, using a razor blade, pry gasket slide from array slide at the barcode labeled end. Place array in slide rack.
4. Wash in (b) Wash Buffer 1 for 5 min, with stirring.
5. Wash in (c) Wash Buffer 2 for 1 min, with stirring.
6. Wash in (d) Acetonitrile for 1 min, with stirring in FUME HOOD.
7. Wash in (e) Stabilization and drying solution for 30 s, with stirring.

8. SLOWLY (5–10 s) remove slide rack.
9. Store slides in dry and dark slide box under nitrogen until scanned.

3.5.7 Scanning, Data Analysis, and Data Visualization

1. Place slide with AGILENT side up in scanner chamber. Parameters: Profile: AgilentHD_CGH (61 × 21.6 mm). Resolution: 2 µm TIFF 16-bit.
RPM/GPMT: 100 %.
XDR: None.
2. Analyze images using “BlueFuse for Microarrays” (BlueGnome, Cambridge).
3. Normalize data using Block Lowess method (analyze at two levels of stringency [one with no data excluded; the second, data excluded when “Quality less than 1; Confidence less than 0.4; PON Ch1 or Ch2 less than 0.6”]) (see Note ¹⁰).
4. Create Ymap account at <http://lovelace.cs.umn.edu/Ymap/> (see Note ¹¹).
5. Click on “Manage Dataset” tab.
6. Click on “Install New Dataset.”
7. Fill in “Dataset Name,” “Ploidy of experiment” (default is 2.0), and “Baseline Ploidy” (default is 2.0).
8. Under “Data type” select “SnpCgh microarray.”
9. Click on “Create New Dataset.”
10. Click on “Reload” button.
11. Click on “Add: SnpCgh array data” to upload your data file.
12. Data download and processing will start automatically.
13. When processing is done, click on “Visualize Datasets” tab to obtain graphic representation of your data (for example, see Fig. 2d).
14. Data can be saved in multiple formats (e.g., jpg, png, tif).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We like to thank Mathura A. Thevandavakkam for critical reading of the manuscript. A.F. is supported by a grant from the NIAID 2 R15 AI090633. J.B. is supported by the People Programme (Marie Curie Actions) of the European Union’s Seventh Framework Programme (FP7/2007-2013) REA grant agreement number 303635; by an European Research Council Advanced Award, number 340087, RAPLODAPT, by grants from the Israel Science foundation (340/13), and by the National Institute of Allergy and Infectious Disease (R01AI075096 and R01AI0624273).

¹⁰For more detailed description of how to process scanned microarray images, contact the authors.

¹¹Ymap has been optimized to work on Macintosh computers using Safari browser.

References

1. Xu J. Extracting haplotypes from diploid organisms. *Curr Issues Mol Biol*. 2006; 8:113–122. [PubMed: 16875417]
2. Glusman G, Cox H, Roach J. Whole-genome haplotyping approaches and genomic medicine. *Genome Med*. 2014; 6(9):73. [PubMed: 25473435]
3. Bennett RJ, Forche A, Berman J. Rapid mechanisms for generating genome diversity: whole ploidy shifts, aneuploidy, and loss of heterozygosity. *Cold Spring Harb Perspect Med*. 2015; 4(10):a019604.
4. Forche A, Abbey D, Pisithkul T, Weinzierl MA, Ringstrom T, et al. Stress alters rates and types of loss of heterozygosity in *Candida albicans*. *mBio*. 2011; 2:e00129–11. [PubMed: 21791579]
5. Forche A, Magee PT, Selmecki A, Berman J, May G. Evolution in *Candida albicans* populations during single passage through a mouse host. *Genetics*. 2009; 182:799–811. [PubMed: 19414562]
6. Bouchonville K, Forche A, Tang KE, Selmecki A, Berman J. Aneuploid chromosomes are highly unstable during DNA transformation of *Candida albicans*. *Eukaryot Cell*. 2009; 8:1554–1566. [PubMed: 19700634]
7. Selmecki AM, Gerami-Nejad M, Paulsen C, Forche A, Berman J. An isochromosome confers drug resistance in vivo by amplification of two genes, *ERG11* and *TAC1*. *Mol Microbiol*. 2008; 68:624–641. [PubMed: 18363649]
8. Gerstein AC, Berman J. Shift and adapt: the costs and benefits of karyotype variations. *Curr Opin Microbiol*. 2015; 26:130–136. [PubMed: 26321163]
9. Legrand M, Forche A, Selmecki A, Chan C, Kirkpatrick D, Berman J. Haplotype mapping of a diploid non-meiotic organism using existing and induced aneuploidies. *PLoS Genet*. 2008; 4:e1. [PubMed: 18179283]
10. Forche A, Alby K, Schaefer D, Johnson A, Berman J, Bennett R. The parasexual cycle in *Candida albicans* provides an alternative pathway to meiosis for the formation of recombinant strains. *PLoS Biol*. 2008; 6:e110. [PubMed: 18462019]
11. Cowen LE, Sanglard D, Calabrese D, Sirjusingh C, Anderson JB, Kohn LM. Evolution of drug resistance in experimental populations of *Candida albicans*. *J Bacteriol*. 2000; 182:1515–1522. [PubMed: 10692355]
12. Abbey D, Hickman M, Gresham D, Berman J. High-resolution SNP/CGH microarrays reveal the accumulation of loss of heterozygosity in commonly used *Candida albicans* strains. *G3 (Bethesda)*. 2011; 1:523–530. [PubMed: 22384363]
13. Muzzey D, Schwartz K, Weissman J, Sherlock G. Assembly of a phased diploid *Candida albicans* genome facilitates allele-specific measurements and provides a simple model for repeat and indel structure. *Genome Biol*. 2013; 14:R97. [PubMed: 24025428]
14. Arnaud M, Costanzo M, Skrzypek M, Binkley G, Lane C, et al. The *Candida* Genome Database (CGD), a community resource for *Candida albicans* gene and protein information. *Nucleic Acids Res*. 2005; 33:D358–D363. [PubMed: 15608216]
15. Enloe B, Diamond A, Mitchell AP. A single-transformation gene function test in diploid *Candida albicans*. *J Bacteriol*. 2000; 182:5730–5736. [PubMed: 11004171]
16. Nobile C, Mitchell AP. Large-scale gene disruption using the *UAU1* cassette. *Methods Mol Biol*. 2009; 499:175–194. [PubMed: 19152049]
17. Abbey D, Funt J, Lurie-Weinberger M, Thompson D, Regev A, et al. YMAP: a pipeline for visualization of copy number variation and loss of heterozygosity in eukaryotic pathogens. *Genome Med*. 2014; 6:100. [PubMed: 25505934]
18. Sirr A, Cromie GA, Jeffery EW, Gilbert TL, Ludlow CL, et al. Allelic variation, aneuploidy, and nongenetic mechanisms suppress a monogenic trait in yeast. *Genetics*. 2015; 199:247–262. [PubMed: 25398792]
19. Tan Z, Hays M, Cromie GA, Jeffery EW, Scott AC, et al. Aneuploidy underlies a multicellular phenotypic switch. *Proc Natl Acad Sci U S A*. 2013; 110:12367–12372. [PubMed: 23812752]
20. Ludlow CL, Scott AC, Cromie GA, Jeffery EW, Sirr A, et al. High-throughput tetrad analysis. *Nat Methods*. 2013; 10(7):671–675. [PubMed: 23666411]

21. Hickman MA, Paulson C, Dudley A, Berman J. Parasexual ploidy reduction drives population heterogeneity through random and transient aneuploidy in *Candida albicans*. *Genetics*. 2015; 200:781–794. [PubMed: 25991822]
22. Gresham D, Curry B, Ward A, Gordon DB, Brizuela L, et al. Optimized detection of sequence variation in heterozygous genomes using DNA microarrays with isothermal-melting probes. *Proc Natl Acad Sci U S A*. 2010; 107:1482–1487. [PubMed: 20080586]
23. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res*. 1997; 25:3389–3402. [PubMed: 9254694]
24. Rose MD. Isolation of genes by complementation in yeast. *Methods Enzymol*. 1987; 152:481–504. [PubMed: 2821357]
25. Berger MF, Bulyk ML. Universal protein-binding microarrays for the comprehensive characterization of the DNA-binding specificities of transcription factors. *Nat Protoc*. 2009; 4:393–411. [PubMed: 19265799]
26. Painter HJ, Altenhofen LM, Kafsack BF, Llinas M. Whole-genome analysis of *Plasmodium spp.* Utilizing a new agilent technologies DNA microarray platform. *Methods Mol Biol*. 2013; 923:213–219. [PubMed: 22990780]

Fig. 1. (a). Amplification of the *UAU1* cassette from plasmid pBME101, (b). Transformation of *UAU1* cassette into the *Candida albicans* genome. Adapted from [15]. (c). Two consecutive recombination events result in transformants that are Arg⁺ and Ura⁺ with the wild type allele deleted or, especially in the case of essential genes, with the wild type allele present on a third copy

Fig. 2.

Options for diagnostic PCR include (a). One-step PCR when *URA3* and *YFGess* are of sufficiently different sizes to be distinguished by gel electrophoresis. (b). Left-flank and Right-flank multiplex PCRs when *URA3* and *YFGess* are indistinguishable by gel electrophoresis. (c). Expected result of diagnostic PCR for both options (a and b) for a trisomic locus. (d). Illustrative image generated with YMAP for a strain exhibiting Chr1 trisomy and major regions of homozygosity on Chrs R, 2, 3, 5 and 7. White = homozygous in parental strain SC5314; grey = heterozygous; cyan = homozygous for 'a' homolog; magenta = homozygous for 'b' homolog; purple = bba allelic ratio (for trisomic chromosome 1 in this diagram)

Fig. 3.
Assembly of hybridization chamber for SNP/CGH arrays. Adapted from [25, 26]