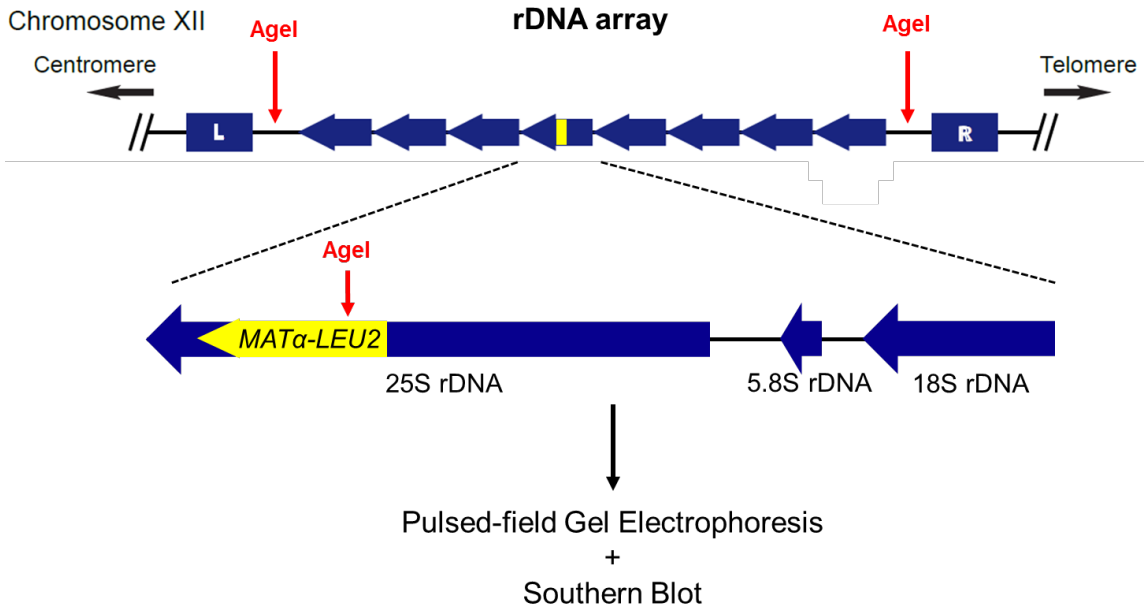
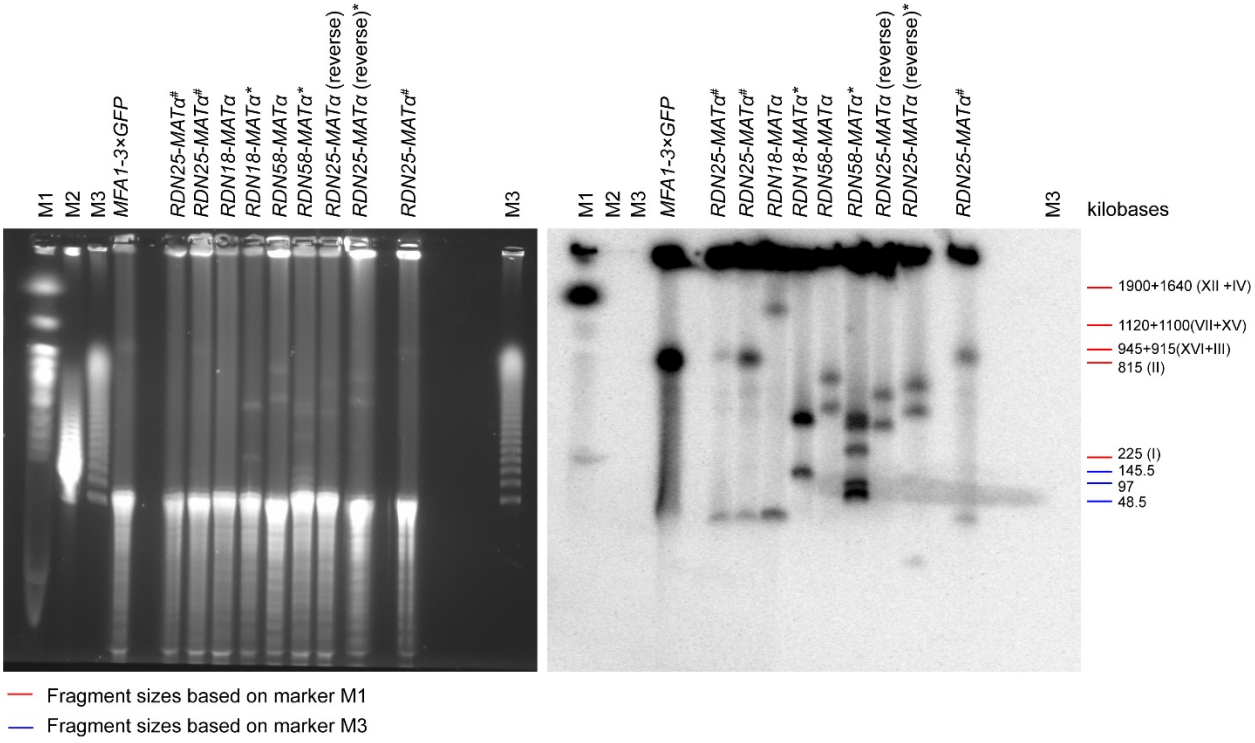
S2 Extended Methods. Determination of the position of the *MATα-LEU2* tagged repeat within the rDNA array

A) Schematic representation of the strategy to determine the position of the *MATα-LEU2* tagged repeat within the rDNA array. The *MATα-LEU2* construct contains a single AgeI restriction site, while the rDNA repeat contains none. Digestion of yeast chromosomes with AgeI in untagged strains will liberate intact rDNA arrays. In strains where *MATα-LEU2* has been integrated at the rDNA array, AgeI digestion is expected to produce 2 or more rDNA fragments depending on the number of *MATα-LEU2* units integrated into the rDNA array. The sizes of the rDNA fragments can be used to infer the relative position of the *MATα-LEU2* tagged rDNA repeat within the array. The number and size of rDNA fragments can be analyzed by pulsed-field gel electrophoresis (PFGE) followed by Southern blotting to probe for the rDNA fragments.



B) Determination of the position of the *MATα-LEU2* construct within the rDNA array in a subset of reporter strains used in this study. AgeI digested chromosomes from various reporter strains analyzed by pulsed-field gel electrophoresis, stained with GelRed (left), and Southern blot probed with a 25S rDNA fragment (right). M1 – Yeast chromosome PFG marker, M2 – Lambda PFG ladder (NEB), M3 – Lambda CHEF standard (BioRad). # - multiple biological replicates of *RDN25-MATα* strain. * - Isolates not used in this study. Fragment sizes (in kilobases) inferred from markers M1 and M3 are indicated. For M1 fragments, chromosomes that constitute the band are in parentheses.

RDN25-MATα has one *MATα-LEU2* unit inserted at one end of the array, ~5 repeats into the array. *RDN18-MATα* has one *MATα-LEU2* unit inserted at one end of the array, ~5 repeats into the array. *RDN58-MATα* has one *MATα-LEU2* unit inserted ~15 repeats into the array. *RDN25-MATα* (reverse) has one *MATα-LEU2* unit inserted in the middle of the array.



C) Methods for PFGE and Southern blotting

Preparation of cells, restriction enzyme digestion of chromosomes, and PFGE were carried out as described in (Ide & Kobayashi, 2010) with some modifications. Reporter strains were grown overnight in 5mL growth medium at 30°C. In the morning, cultures were diluted to OD600 ~0.1, and grown to OD600 ~0.3-0.5. ~8×10^7 cells were harvested and embedded in agarose plugs for PFGE. Restriction digestion of chromosomes with AgeI was carried out in the plugs at 37°C overnight. DNA fragments were separated on a 1% Pulsed-Field certified agarose (Bio-Rad) gel in 0.5x TBE using the CHEF-DR III Pulsed Field Electrophoresis System (Bio-Rad) at 6V/cm, with an included angle of 120°, and switch times 0.2-204s, for 15.2h at 14°C. Markers used were M1 - Yeast Chromosome PFG marker (NEB N0345), M2 - Lambda PFG Ladder (NEB N0341), and M3 – Lambda CHEF standard (BioRad 1703635). The gel was stained with 3x GelRed in 0.5x TBE, photographed, transferred on to a Amersham Hybond N+ membrane (GE Healthcare) and probed with a 25S rDNA fragment radiolabeled with α-^{32}P-dCTP. The blots were exposed to storage phosphor screens and imaged using a Typhoon 9400 imager (GE Healthcare).

References

Ide, S., & Kobayashi, T. (2010). Analysis of DNA replication in Saccharomyces cerevisiae by two-dimensional and pulsed-field gel electrophoresis. *Curr Protoc Cell Biol, Chapter 22*, Unit 22 14. doi:10.1002/0471143030.cb2214s49