A scheme to induce and selectively maintain specific N+1 disomes

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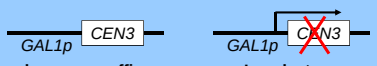
INTRODUCTION

We wish to create a controlled system in the yeast *Saccharomyces cerevisiae* to study aneuploidy. Aneuploidy is the condition of having abnormal numbers of chromosomes that can arise through a non-disjunction event during cell division. In continuous cultures of yeast in both industry and the lab, aneuploidy is a condition thought to arise when it provides some sort of growth advantage (1). Our aim is to develop a method by which strains of N+1 aneuploid yeast for each of the sixteen yeast chromosomes can be made and studied. Our scheme uses two genetic tools:

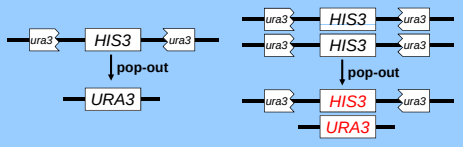
CONDITIONAL CENTROMERE. This centromere (*GAL-CEN*) can be temporarily disabled when cells are grown in galactose (2).



In glucose or raffinose: centromere functional — **In galactose: non-disjunction**

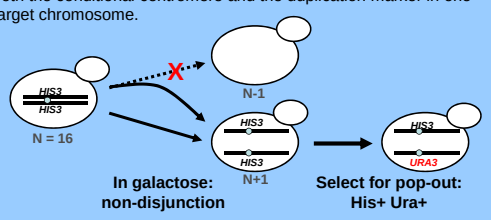
Transcription at the *GAL1* promoter prevents kinetochore assembly and microtubule attachment at *CEN3*. Non-disjunction of the chromosome generates N+1 aneuploid daughter cells after one round of cell division; inviable N-1 daughters should also form. In glucose, the promoter is repressed, microtubules attach to *CEN3* and accurate cell division continues.

DUPLICATION MARKER. This marker is a set of genes that can exist in two, mutually exclusive states. Only when it has duplicated can it exist in both states simultaneously (ie, ref. 3).



HIS3 is integrated into the middle of *URA3*, disrupting its function. *HIS3* can excise or "pop out" by homologous recombination excision, restoring the function of *URA3*. Cells that contain two copies of the duplication marker, such as N+1 aneuploids, should form colonies when *HIS3* and *URA3* are selected for simultaneously, whereas their parents should die.

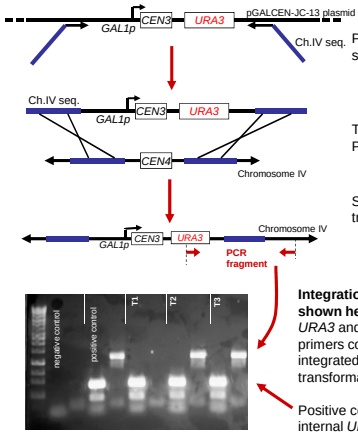
INDUCTION-SELECTION SCHEME. Haploid cells will contain both the conditional centromere and the duplication marker in one target chromosome.



With a system to study yeast aneuploidy, the following questions could be addressed: 1) What are the phenotypic effects of aneuploidy on *S. cerevisiae*? 2) Does an aneuploid condition result in altered gene expression in yeast and do genome-wide regulatory systems control expression in yeast? 3) Is aneuploidy a mechanism of gene and/or genome evolution?

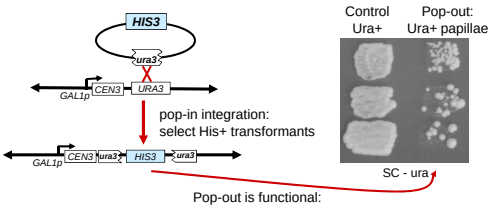
EXPERIMENTAL DESIGN AND RESULTS

Strain construction I: conditional centromere replaced *CEN* locus in target chromosome (*III, IV,* and *VI*)



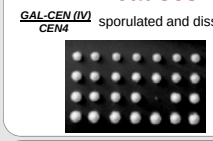
High-fidelity PCR (Phusion, Finnzymes) was used. Diploid strains (S288C background) were transformed. Centromeres *CEN3, CEN4,* and *CEN6* were targeted and replaced, twice independently.

Strain construction II: *HIS3* is integrated into *URA3* to form duplication marker



Pop-in plasmid (internal *ura3* fragment cloned into pRS303) was linearized with *Stu*I prior to transformation. Shown are strains containing the duplication marker at *CEN4*.

Insertion of conditional centromere causes no growth defects

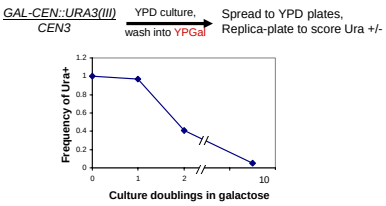


Tetrad dissection of strains hemizygous for the conditional centromere (*GAL-CEN*) produced spore clones that were indistinguishable from their wild-type counterparts in glucose.

The *ura3::HIS3* marker segregated 2:2 as a *CEN*-linked marker in these tetrads.

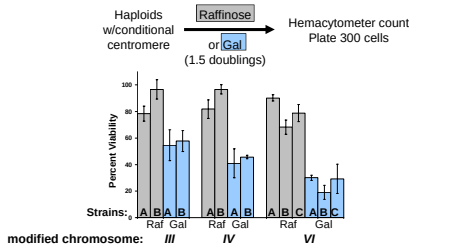
Galactose induces non-disjunction

In diploids: Marked conditional centromere is lost



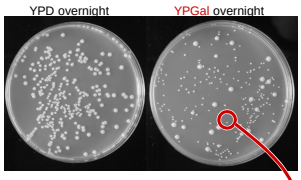
If every cell in the culture undergoes non-disjunction, we would expect 50% to lose *URA3* and become Ura-. This occurred between one and two culture doublings. Residual glucose repression of the *GAL1* promoter may contribute to this delay in centromere inactivation. Subsequent experiments use raffinose to avoid glucose repression.

In haploids: Inviable cells are produced (N-1?)

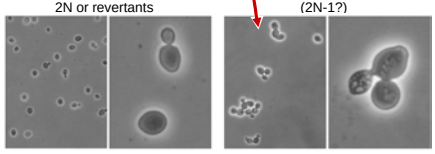


If all cells undergo non-disjunction, 50% inviability would be expected after one cell division. Each modified chromosome (*III*, *IV*, and *VI*) produced the expected inviability. Chromosome *VI* non-disjunction led to more than 50% inviability. Perhaps the corresponding N+1 viability is low.

Non-disjunction of chromosome *IV* causes abnormal 2N-1 phenotypes

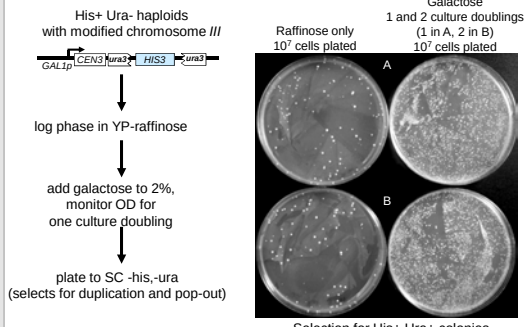


- All small colonies were Ura-
- Small colony phenotype was unstable (revertant frequency upon replating was 10-64%, median was 30%)
- Small colonies had an abnormal bud-neck phenotype
- Revertant large colonies had normal appearance, but remained Ura-



We hypothesize that the small Ura- colonies are 2N-1 for chromosome *IV*. If true, then the rapid reversion to normal growth and morphology may represent endoreduplication of the remaining chromosome *IV* as described (4).

Galactose increases frequency of His+ Ura+ colonies in haploids



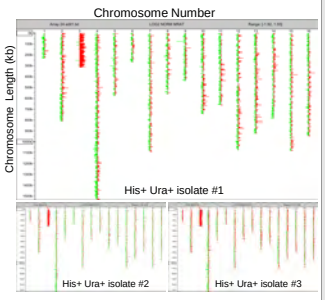
- His+Ura+ colonies in raffinose-only cultures represent spontaneous duplication and recombination events.
- Galactose treatment should increase His+Ura+ frequency by inducing duplication of the target chromosome, as shown above for a conditional centromere on *III*.
- Galactose treatment did not increase His+Ura+ frequency in haploid strains containing modified chromosome *IV* or *VI*. Diploid strains did exhibit a Gal-specific increase in His+Ura+.

Log-phase cultures were split and galactose was added to one half. The OD of the cultures doubled in about 3 hrs. Plates were incubated 3 days at 30 C.

Microarray-based CGH confirms that His+ Ura+ colonies are N+1

Red = His+Ura+ cells
Green = haploid parent

- Three His+Ura+ colonies were picked from the Gal-induced cells above.
- Comparative genome hybridization (CGH) arrays measured **chromosome *III*** copy number at **1.68X**, **1.67X**, and **1.80X** relative to the rest of the genome.
- One spontaneous His+Ura+ strain (not gal-induced) showed no aneuploidy (not shown).



DNA was isolated from each strain, labelled with Cy5 and co-hybridized to whole-genome ORF arrays. Cy3-labeled DNA from the haploid parent was the reference. Arrays were scanned, data were acquired with ScanAlyze (5), filtered to remove weak signals and plotted here with Caryoscope (6).

SUMMARY

- Strains were constructed to contain a **conditional centromere** and **duplication marker** in chromosome *III*, *IV*, or *VI*.
- Centromere replacement caused no growth defect.
- The conditional centromeres function properly: galactose causes chromosome loss or duplication.
- 2N-1 aneuploids missing chromosome *IV* grow slowly, exhibit an abnormal bud-neck morphology, and revert frequently.
- Microarrays indicate that His+ Ura+ colonies selected after galactose induction were N+1 for chromosome *III*.

DISCUSSION

►Induction of chromosome *III* disomy indicates our scheme is theoretically possible for any chromosome.

►Conditional centromere also allows induction of 2N-1 aneuploids.

- The abnormal bud-neck phenotype in diploids monosomic for chromosome *IV* suggests that at least one gene (or genes) on *IV* is haploinsufficient for this phenotype.
- Although the morphological defect and slow growth are linked, it is not clear if the bud neck defect is the sole cause of slow growth.

►Strains disomic for chromosome *IV* or *VI* appear difficult to isolate.

- The procedure to induce non-disjunction in haploids should produce many N+1 cells. However, the His(+)Ura(+) cells containing the duplicated marker fail to reveal an increase in chromosome *IV* copy number by microarray. Either these N+1(*IV*) cells have low viability, or they exhibit chromosome instability, duplicating the marker genes but not the whole chromosome upon selection.
- Literature discussing yeast aneuploidy indicates that chromosome *IV* disomes can only be maintained under strict selection conditions (7). Chromosome *VI* disome isolation is rare in cytoduction experiments (8).
- Chromosome *IV* is the largest chromosome in the genome. Chromosome *VI* contains genes known to have dosage effects on phenotype (eg, *ACT1* and *TUB2*).

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