Chapter 2

The Chromosome Transmission Fidelity Assay for Measuring Chromosome Loss in Yeast

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Abstract

The budding yeast *Saccharomyces cerevisiae* has served as an excellent model system for studying highly conserved biological pathways including pathways involved in genome transmission and maintenance. The Chromosome Transmission Fidelity (CTF) colony color assay was developed to assess chromosome instability (CIN) in yeast, by monitoring the loss or gain during cell division of an artificial chromosome fragment carrying a visual marker. The CTF assay monitors changes in chromosome number, allowing the detection of mutants that exhibit increased rates of chromosome nondisjunction or chromosome loss. In this article, we describe the *SUP11*-marker-based CTF assay system, and the methodologies for both qualitative analysis of mutants affecting chromosome transmission, and quantitative analysis for determining the types and rates of errors in chromosome transmission using half-sector analysis.

Key words Chromosome instability, Genome instability, Aneuploidy, Whole chromosomal loss, Chromosome transmission fidelity, Marker loss

1 Introduction

Chromosome replication and segregation during the mitotic cell cycle relies on the correct execution of a complex series of events, with functional determinants that act in *cis* (DNA sequence domains) and in *trans* (gene products). As these processes are essential to cell viability, functional characterization of mutants that are defective in chromosome transmission in higher eukaryotes has been challenging. In the budding yeast *Saccharomyces cerevisiae*, DNA replication and chromosome segregation occur with extremely high fidelity, as errors in chromosome transmission happen very infrequently with rates of chromosome mis-segregation, for example, occurring on the order of once per 10^5 cell divisions [1, 2]. However, as this rate of chromosome transmission fidelity in wild-type strains is much greater than the fidelity necessary for cell viability, it is possible to perform genetic analysis with mutants that

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reduce chromosome transmission fidelity over a several order of magnitude range [3, 4].

Taking advantage of the findings that yeast mutants with altered chromosome transmission fidelity remain viable [5] and can tolerate aneuploidy for many individual chromosomes [6], assays were developed for monitoring changes in chromosome ploidy [7, 8]. The two visual chromosome transmission fidelity (CTF) assays, *ade3-2p* and *SUP11*, can be used to detect and analyze mutants involved in chromosome transmission. This chapter will only describe the use of the *SUP11* system for qualitative and quantitative analyses of CTF. The general principles, however, apply directly to the *ade3-2p* system.

Both CTF assays make use of mutations in the purine biosynthesis pathway [9] to facilitate the colony color assay that detects chromosome loss. Wild-type yeast cells are colorless and produce white colonies. Mutations in either the *ADE1* or the *ADE2* genes required for purine biosynthesis cause the accumulation of an intermediate that generates a red hue. The degree of redness in yeast colonies harboring these mutations depends on accumulation of biosynthetic intermediates, which leads to the formation of a red pigment [3]. In the *SUP11* system cells contain an ochre mutation in the *ADE2* gene (*ade2*oc) and are red. This mutation is suppressed by the introduction of a chromosome fragment (CF) that carries the ochre-suppressing form of a tRNA gene, *SUP11* [10–12].

The standard CF used is a 125-kilobase fragment with the short arm containing a selectable marker that can be used during initial transformation and a colony color marker (*SUP11*), which is used in the visual colony assay. The structure and genotype of the CF makes it ideal for monitoring CTF [12, 13]. As the color marker is embedded in pBR322 that bears no homology to yeast DNA on the CF short arm, it will not be lost by mitotic recombination between the CF and endogenous chromosomes [4]. The presence of the CF results in disomy for less than 1% of the yeast genome with no effects on either cell growth or endogenous chromosome fidelity; in wild-type cells, the loss rate of the CF is ~2 in 10^4 cell divisions [12, 13].

In the CTF assay, a homozygous *ade2*-ochre diploid cell with zero copies of the CF produces no functional Ade2 and thus accumulates sufficient pigment to generate red colonies. A cell with only one copy of the CF will produce small amounts of Ade2, and will only suppress the ochre mutation partially, thus will generate pink colonies. A cell containing two or more copies of the CF that will be colorless will generate white colonies, because enough *ADE2* gene product will be made to prevent the accumulation of the intermediates that produce the red color. Thus, diploid cells with zero, one, and two or more copies of CF will generate red, pink, and white cell lineages respectively and the degree of red

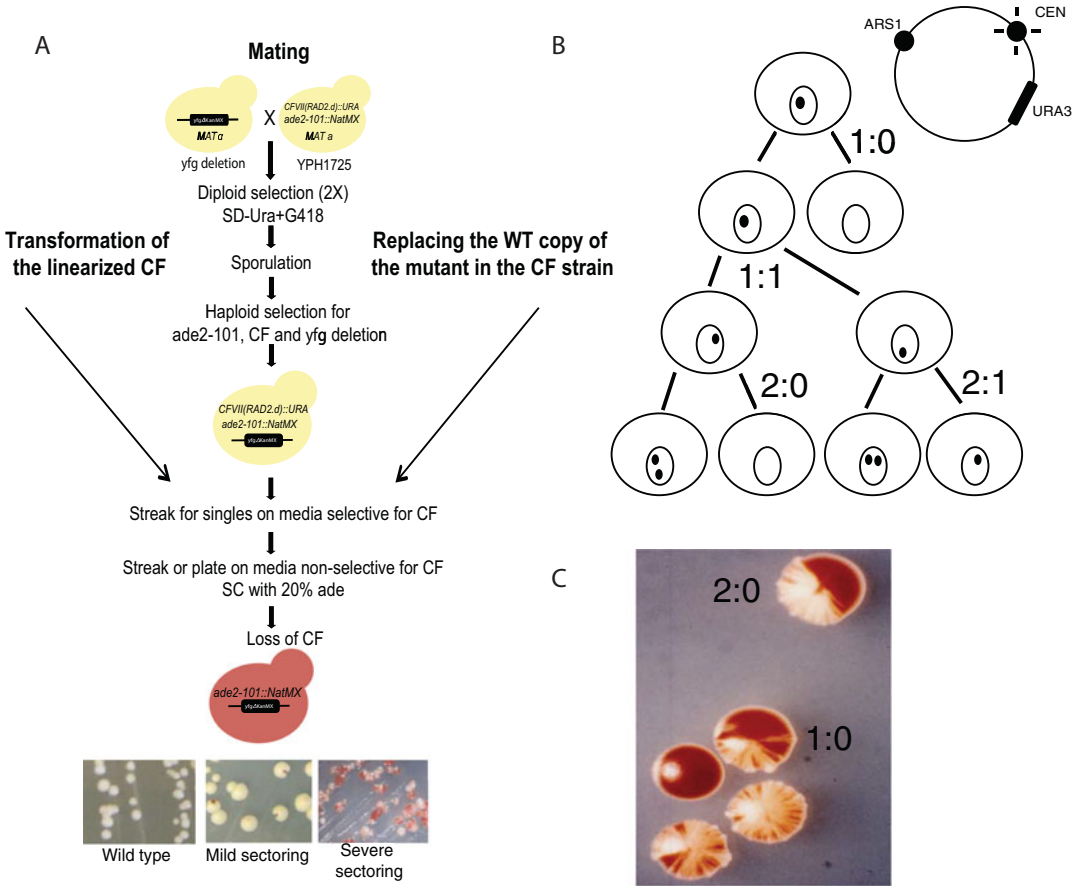

Fig. 1 The qualitative CTF assay and the half-sectoring assay. (**a**) Detailed steps involved in generating a CTF strain. For more details on generating a strain by transforming the CF or by introducing the mutant allele to the CF strain, refer to [4]. A starter strain (YPH1725) containing the *ade2-101* mutation and the *SUP11* (*CFVII (RAD2.d)::URA3*) is mated to a deletion strain of YFG and diploids are selected by plating onto appropriate selective media. Following sporulation, a haploid strain is generated where *yfgΔ KAN* is combined with the *ade2-101::NAT* mutation and the CF. Cells are then streaked onto media with the selection for the CF and after 48 h, are either streaked or plated on low adenine media plates. After growing 7–10 days at 25 °C, plates are placed at 4 °C for 2–3 days before scoring. Representative images from qualitative CTF phenotypes are shown at the bottom. (**b**) Segregation properties of the CF adapted from [3]. The CF includes an ARS element, a CEN element, and a selectable marker in yeast (URA). When the CTF strains are placed on nonselective media the pattern of sectoring in each colony reflects the inheritance of the CF. In about 99% of cell divisions, the CF replicates once and partitions equally to daughter cells (1→ 1:1 segregation). Aberrant CF transmission events (1→1:0, 1→2:0, and 1→2:1) are depicted here. Sample images are also included. (**c**) Samples from an output plate that shows *red*, *pink*, and *white* diploid colonies with 2:0 and 1:0 patterns of CF segregation

sectoring in colonies reflects the frequency of mitotic chromosome loss. In haploid cells, one copy of the CF will cause cells to appear white and a haploid cell that has lost the CF will appear red (Fig. 1a and c). The CTF assay can therefore distinguish between chromosome loss (1:0 segregation) and nondisjunction (2:0 segregation)

in diploid cells (Fig. 1b), which makes it an appropriate method for determining the types of aberrant transmissions that lead to cells with altered chromosome ploidy [7].

The qualitative CTF assay can be easily applied to large collections of loss-of-function (nonessential genes), reduction-of-function (essential genes) mutants and overexpression (essential and nonessential) mutants [14–16], whereas the half-sector analysis for determining the types and rates of chromosome aberrations is more labor intensive. It is also possible to assess CTF rates using fluctuation analysis [13]; however, this chapter will be limited to qualitative CTF and the half-sectoring assay.

To test the effects of a specific genetic perturbation, the mutation will need to be introduced into the CTF background using one of the following methods. Mutations to be tested can be introduced directly into CTF strain backgrounds (YPH1725 and YPH1726, *see* Subheading 2.3) using standard techniques. Alternatively, the CF can be generated de novo by transforming mutant strains of interest with the appropriate linearized plasmid [4]. Finally, strains can also be generated using mating and sporulation (Fig. 1a). Following strain generation using one of the methods above, cells are placed on low adenine containing agar media to allow accumulation of intermediates that give rise to red pigment (Fig. 1a). As wild-type cells will generate white colonies, changes in colony sectoring will reflect changes in the rates of chromosome loss and nondisjunction. The protocols described here primarily focus on genomically integrated loss-of-function or reduction-of-function alleles; however, the CTF assay can be conducted in strains with plasmids in the presence of alternate media for inducible expression.

2 Materials

2.1 Lab Ware

1. Disposable 10 cm plastic petri dishes.
2. Autoclaved toothpicks or sticks.
3. Sterile 1.5 mL microcentrifuge tubes.

2.2 Media Preparation

1. Synthetic Complete Plates with Limiting Adenine (SC+20% ade): 1.7 g/L yeast nitrogen base, 5 g/L ammonium sulfate, 20 g/L dextrose, 20 g/L agar (*see* **Notes 1** and **2**).
2. Prepare SC broth, 4% agar, and 20% dextrose in three separate autoclavable flasks/bottles as indicated above. Use at least one flask that will allow the desired final volume of media. Also place a stir bar in this flask as it will aid in the mixing process.
3. Autoclave each mixture.

4. Place the largest container on a stir plate and add the agar and the dextrose.
5. To this add 100 mL/3 L of solution containing the following supplements: adenine, 0.015%; uracil, 0.06%; L-lysine, 0.06%; L-histidine, 0.09%; L-trypsine, 0.09%; L-leucine, 0.06%.
6. Pour ~25 mL of media into 10 cm diameter plastic petri dishes after the media has cooled to a temperature of approximately 55 °C.
7. Allow agar mixture to cool and solidify before using.
8. Unused SC+1/5ade plates can be stored at 4 °C for several months.
9. Synthetic Complete agar plates (SC-URA): 1.7 g/L yeast nitrogen base, 5 g/L ammonium sulfate, 2 g/L of—Uracil dropout mix, 20 g/L dextrose, 20 g/L agar (*see* **Notes 3** and **4**).
   (a) Prepare SC broth, 4% agar, and 20% dextrose in three separate autoclavable flasks/bottles as indicated above. Use at least one flask that will allow the desired final volume of media. Also place a stir bar in this flask, as it will aid in the mixing process.
   (b) Autoclave each mixture.
   (c) Place the largest container on a stir plate and add the agar and the dextrose.
   (d) Pour ~25 mL of media into 10 cm diameter plastic petri dishes after the media has cooled to a temperature of approximately 55 °C.
   (e) Allow agar mixture to cool and solidify before using.
   (f) Unused SC-URA plates can be stored at 4 °C for several months.

2.3 Yeast Strains

1. The CTF starter strains are YPH1725 or YPH1726, which are *MAT***a** and *MAT*alpha strains with the *ade2-101* ochre mutation marked with NatMX and the CF marked with *URA3* [17]. The NatMX marker allows you to select for the presence of the *ade2-101* marker; therefore, the strains can be used in high-throughput screens. These strains can be used to generate final strains with genes of interest (Fig. 1a and [4]).
2. Positive control for high levels of CTF. These can be found in previously published work from the Hieter lab [12, 16–18]. The *CTF19* mutant strain used in [18] serves well as a control for the half-sectoring assay.
3. Negative control for CTF. Either YPH1725 or YPH1726 without perturbation serves as a good control.

3 Methods

3.1 Qualitative CTF Assay

1. Day 1. On a SC-URA plate, using a toothpick or stick, streak the CTF strains with *YFG* perturbation to achieve single colonies. Include the CTF starter strain and the *CTF19* mutant strains as controls (*see* **Note 5**).
2. Day 3. Pick two single colonies for each strain with a wooden stick and dilute in 1 mL of H_2O. Dilute the cells 1:1000 in a second microcentrifuge tube and plate 100 μL into SC plates with 20% of the standard adenine concentration to obtain 100–250 colonies per plate (*see* **Note 6**).

   Alternatively, two single colonies for each strain can be streaked onto SC plates with 20% of the standard adenine concentration to achieve single colonies (*see* **Note 7**).
3. Day 3. Incubate plates at 25 °C for 6–7 days (*see* **Note 8**).
4. Day 9–10. Incubate plates at 4 °C for additional 5–7 days to enhance the development of red pigment (*see* **Note 9**).
5. Day 14–17. Score the number of sectored colonies on the plates. The CTF starter strain should have almost no sectored colonies, whereas the *CTF19* mutant will have many sectored colonies giving a relative indication of chromosome instability (*see* **Note 10**).

3.2 Half-Sector Assay

1. Day 1. On a SC-URA plate, using a toothpick or stick, streak out the CTF strains with *YFG* perturbation to achieve single colonies. Include the CTF starter strain and the *CTF19* mutant strains as controls (*see* **Note 5**).
2. Day 3. Pick 9–12 single colonies for each strain with a wooden stick and dilute in 1 mL of H_2O. Dilute the cells 1:1000 in a second microcentrifuge tube and plate 100 μL into SC plates with 20% of the standard adenine concentration to obtain 100–250 colonies per plate. Plate 100 μL into SC plates with 20% of the standard adenine concentration to obtain 100–250 colonies per plate (*see* **Note 7**).
3. Day 3. Incubate plates at 25 °C for 6–7 days (*see* **Note 8**).
4. Day 9–10. Incubate plates at 4 °C for additional 5–7 days to enhance the development of red pigment (*see* **Note 9**).
5. Day 14–17. Score the number of "half-sectored" colonies on the plates to calculate the rate of chromosome loss relative to wild-type cells. Chromosome loss (1:0 segregation, Fig. 1b) will result in pink/red half-sectored colonies, whereas nondisjunction (2:0 segregation) will result in white/red half-sectored colonies (Fig. 1b). Note that colonies with greater than 1/2 red:white sectoring (e.g., ¾ red and ¼ white) are scored as half-sectored colonies; this rule was empirically

determined by comparisons to rates determined using fluctuation analysis.

6. The CTF rate is a ratio of "half-sectored" colonies to total CF containing colonies. Colonies that are completely red should be discounted, as these represent cells that did not have the CF when they were plated. For the *CTF19* mutant strain, the rate of chromosome loss should be ~100 times greater than wild-type and the rate of nondisjunction should be 60-fold higher than wild-type [18] (*see* **Notes 10** and **11**).

4 Notes

1. For all solid media (>1 L), autoclave at 121 °C for at least 30 min on a liquid cycle. Mix well after adding the solutions together.
2. Standard protocol includes 1/5 the concentration of adenine; however, it may be necessary to adjust this concentration as the red colony phenotype may vary when using different adenine stock solutions or strain backgrounds.
3. If a drug needs to be included to maintain a plasmid, ammonium sulfate should be substituted with 1 g/L monosodium glutamate.
4. To make the—Uracil dropout mix amino acids as follows. 6 g of each of serine, arginine, glycine, glutamic acid, alanine, histidine, glutamine, threonine, asparagine, phenylalanine, methionine, valine, isoleucine, proline, tryptophan, tyrosine, aspartic acid, lysine and cysteine, 12 g of leucine and 1.5 g of adenine hemisulfate.
5. It is important to maintain selection (URA) for the CF until you are ready to begin the CTF experiment, as the loss rate of the CF is higher than for native chromosomes.
6. It is assumed a single colony contains approximately 10^6 cells; therefore, 100 μL from the diluted micocentrifuge tube represents approximately 100 cells. For slow growing strains it may be necessary to plate more than 100 μL to obtain 100–250 cells. In the first pass it is advisable to plate at least two different volumes to get a final cell number of 100–250 cells/plate.
7. While streaking for singles is acceptable it is much harder to control for cell numbers using this method. It is advisable to score at least 100 single colonies during the first pass of a CTF experiment.
8. CTF starter strains can grow at any desired temperature; however, we have observed that slower growth at 25 °C enhances the accumulation of red pigment compared to growth at 30 °C.

9. This extended incubation at 4 °C is especially beneficial for detecting mild CTF phenotypes as it further enhances the red pigment development.
10. With a mis-segregation of 10^{-4} approximately 30,000–100,000 single colonies will need to be counted to generate the rate of chromosome mis-segregation events for wild-type cells. Depending on the degree of the CTF phenotype it may be necessary to score at least 3000 colonies per gene tested. A rule of thumb is to plate sufficient numbers of colonies so that you score >10 independent half-sectored colonies. Colonies with greater than 1/2 red:white sectoring (e.g., ¾ red and ¼ white are scored as half-sectored) are designated as half sectored colonies.
11. CTF with other media and plasmids: The CTF assay can also be used to test the effect of plasmids or plasmid borne genes. The conditions and specific media changes will vary depending on the plasmid system. In our experience it is important to: (a) maintain selection of the plasmid throughout growth on plates, but it is not necessary on the final SC+1/5 ade plate. (b) For galactose inductions in our experience it is better to incorporate two rounds of inductions each lasting 48 h of growth.

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