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„Systematic identification of chromosomal copy number interactions in yeast“

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1 Introduction

1.1 Missegregation, Aneuploidy and CIN

1.1.1 Chromosome missegregation and aneuploidy

During mitosis, every cell has to distribute each of its two sister chromatids equally between the two evolving cells. Various mechanisms should ensure the accurate segregation of all chromosomes. [1]

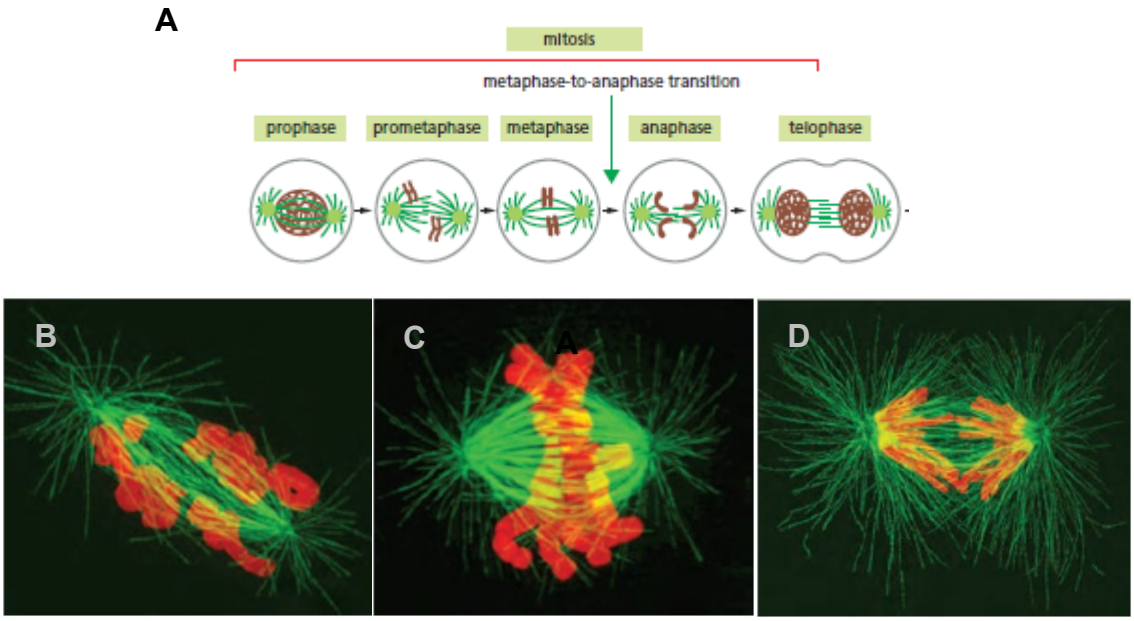


Figure 1 (adapted from Alberts et al. 2015[2]): Example for sister chromatids that are bound correctly to the microtubules of the mitotic spindle and can be segregated during mitosis. (A) Schematic figure of the different stages during mitosis. Chromosomes depicted in red, the microtubules are colored in green. **(B)** Fluorescence micrograph of the mitotic spindle (green) and chromosomes (red) in prometaphase, when the kinetochores (yellow) get in touch with the microtubules (green) for the first time. **(C)** Fluorescence micrograph showing the alignment of the chromosomes at the spindle equator during metaphase. **(D)** Fluorescence micrograph after transition to anaphase, the sister chromatids are pulled correctly towards opposite spindle poles.

In metaphase the microtubules of the mitotic spindle bind to the kinetochores on the centromeric region of the chromosomes. As long as checkpoint mechanisms are intact, cell cycle progression to anaphase is blocked until the kinetochore of every sister chromatid is bound correctly by microtubules (Figure 1).[2] A defect in this mechanism can be one cause of missegregation and subsequent aneuploidy. Aneuploidy is a state where cells have an abnormal karyotype, which means an

aberrant copy number of at least one chromosome (Figure 2 [3]).[1] In a healthy eukaryotic organism, aneuploidy usually occurs at a rate of 1 in 10^5 cell divisions.[4] Frequently these cells then die or are outcompeted by euploid cells, due to slower proliferation rates after chromosome missegregation.[5] When it comes to the topic of aneuploidy, chromosomal instability (CIN) is a recurring term, as cells with CIN develop aneuploidy at high rates.[1] In addition, aneuploidy itself can be a driver for genomic instability. [6]

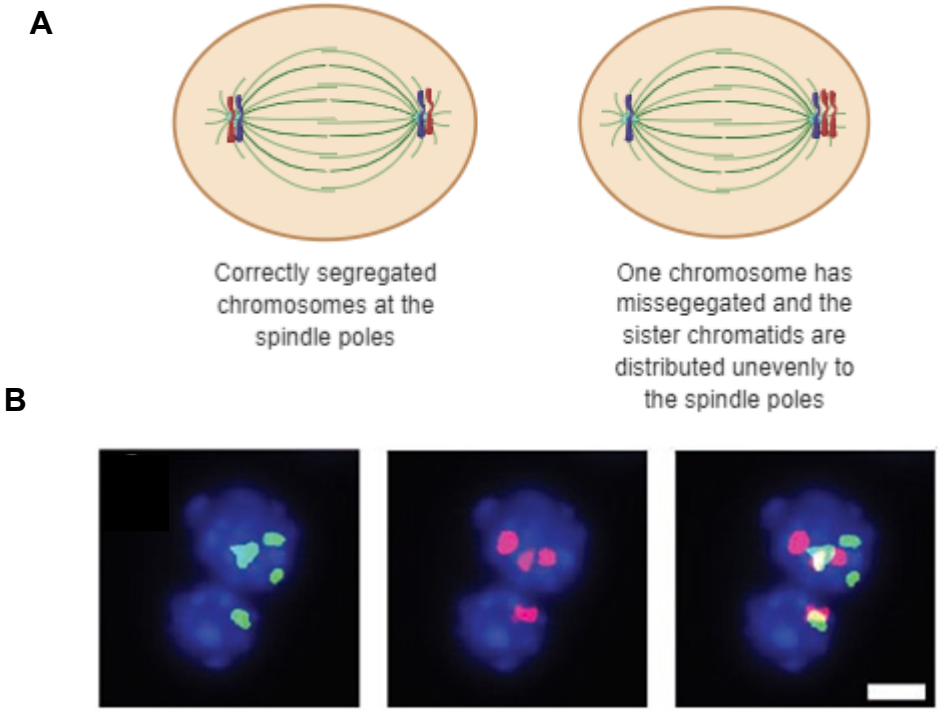


Figure 2: Chromosome missegregation can lead to aneuploidy and abnormal karyotypes. (A) Simplified schematic picture of two haploid cells. In one cell chromosomes have segregated correctly during anaphase (left) and in the other cell (right) one of the two depicted chromosomes (red) has missegregated and distribution to the spindle poles is uneven. The chromosome colored in blue has segregated correctly in both cells. Microtubules and the mitotic spindle are represented in green. **(B)** Fluorescence picture adapted from Yang et al. 2003, showing neuronal progenitor cells blocked in cytokinesis. Missegregated chromosomes have been marked fluorescently in green (Chromosome 4) and red (Chromosome X). [3]

1.1.2 Consequences of aneuploidy

Aneuploidy usually leads to stress in the cell, which normally results in defects in proliferation, as well as changes in gene dosage, which lead to genomic instability and aberrant phenotypes.[1,7] Some studies showed that the unfavorable effects on cells after missegregation are due to the resulting protein imbalances the cell has to cope with.[8] Protein turnover is an important mechanism to cope with sudden higher amount of some proteins or protein subunits.[9] However, as the quality-control system

for proteins in aneuploid cells is often impaired, or simply overwhelmed by the additional protein load, protein aggregates can evolve and lead to proteotoxic stress in the cell.[10]

In the 1960s, the first aneuploid budding yeast strains (Saccharomyces cerevisiae) were created.[11] Aneuploidy can also occur in yeast naturally, for example as a reaction to exogenous stress.[12] Experiments up to now showed that the gain of most chromosomes is in general tolerated well by yeast cells (Figure 3). Nevertheless, yeast cells suffer from proliferation defects and impaired protein homeostasis after chromosomal missegregation. [13]

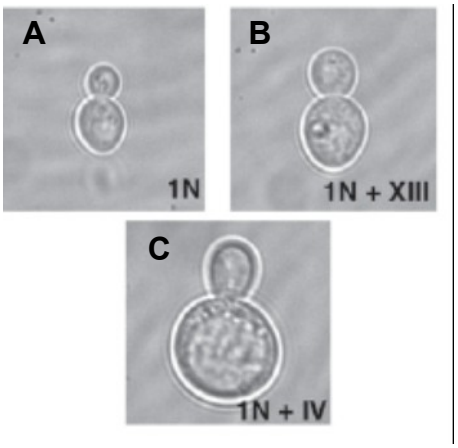


Figure 3[13]: Microscopy pictures of euploid and aneuploid budding yeast cells. (A) Picture of haploid budding yeast (Saccharomyces cerevisiae) with euploid chromosome number; **(B)** an extra copy of chromosome 8; **(C)** and an extra copy of chromosome 4.

Beach et al. 2017 has studied the effects of single aneuploidies in specific chromosomes extensively in budding yeast. Disomy was induced in 15 specific chromosomes in haploid cells, whereas in diploid cells the effects of monosomy and trisomy of the same 15 chromosomes on cellular fitness were investigated. (Figure 4 A).[7] Cellular fitness of single cells was determined by integrating a lac operator near the centromere of the target chromosome. Through the expression of a GFP-LacI fusion protein, the missegregated chromosome could be detected by fluorescence microscopy (Figure 4 B[7]).[14] In order to determine the fitness of the aneuploid cells, colony size was compared to a euploid wild-type (WT).

The loss of a chromosome seems to have in general a more severe effect in yeast than the gain of a chromosome. Only six monosomic chromosomes (Chromosomes 1, 3, 5, 6, 8, and 9) are viable to a certain percentage (Figure 4 C). [15]

The other main observation in these experiments was that the phenotype of aneuploid cells correlates directly with the size of their missegregated chromosome. The number of active genes (open reading frames = ORF) on an aneuploid chromosome seems to have a great impact on the cells capability to cope with that state, which has already been shown in other studies in different eukaryotic organisms (Figure 4 C).[5,7] This theory is supported by the fact that Trisomy 21, where the affected chromosome 21 is the smallest in the human cell, is the only viable autosomal trisomy in humans. The chromosomes 13 and 18, which are, in respect to the number of active genes, the next smallest human chromosomes, lead to death within the first few months of life. The same tendency could also be observed in embryonic mouse models. [15]

It is still not entirely clear why a larger aneuploid chromosome has a stronger negative effect on cell proliferation than a smaller aneuploid chromosome. There is one study which claims that the aneuploid phenotype is mainly caused by the change in copy number of many genes, which all do not cause a phenotype when gained or lost on their own. This study implies that the amplification or reduction of specific dosage sensitive genes only plays a minor role in the severity of the aneuploid phenotype.[16] However, another study shows that already the change of single gene copy numbers, like of some transcription factors for example, can have a great impact on the biological system of an organism.[17] The greater likelihood to encounter more of such dosage sensitive genes on a larger chromosome could explain the observed phenotypic trend correlating with the number of ORFs.

Consequences of aneuploidy are not only CIN. They also include higher rates of genetic alterations, such as DNA double strand breaks for example.[1]
Taken together, these findings suggest that chromosome missegregation can lead to an aberrant karyotype, which can contribute to different types of genetic instability and mutations, as well as to impaired protein homeostasis in a cell. Moreover, it appears to have a strong influence on the resulting cell phenotype which chromosomes were gained or lost.

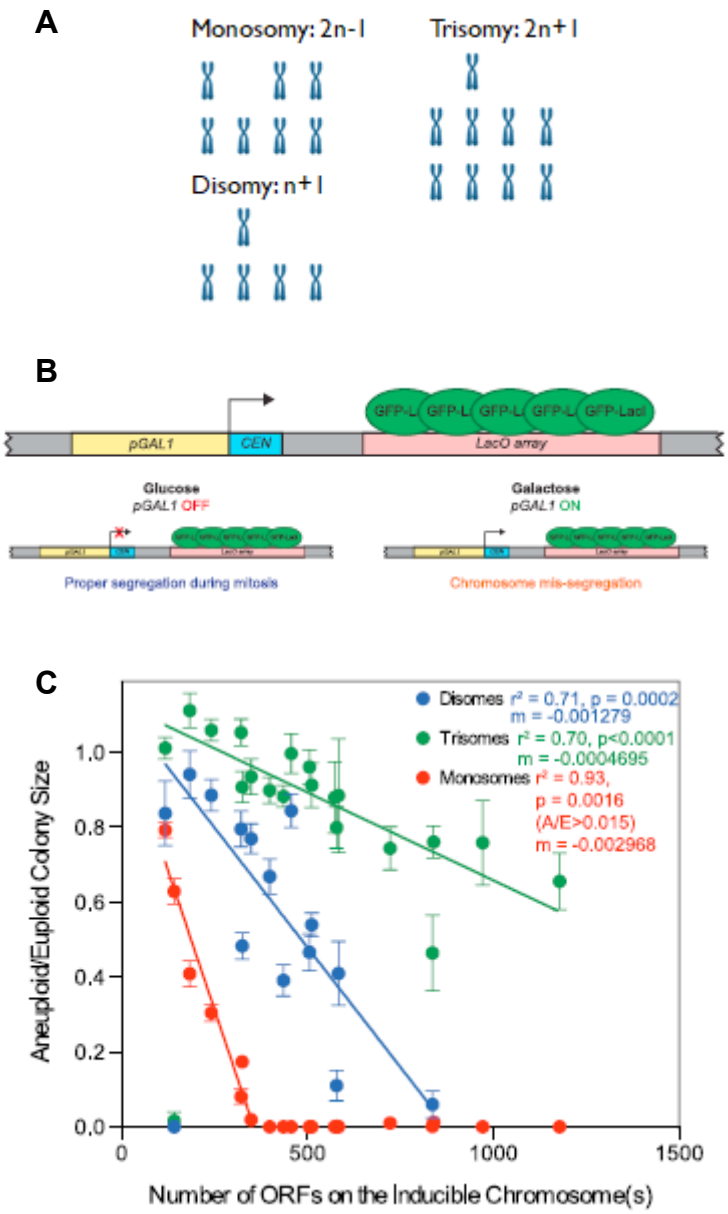


Figure 4: Cellular fitness is strongly dependent on the size of the aneuploid chromosome. (A) Schematic description of the genotypes of monosomy and trisomy in diploid cells, and disomy in haploid cells. Chromosomes are depicted in blue. **(B)** Selection system used by Beach et al. 2017 to follow the fate of a single chromosome after the induction of missegregation.[7] **(C)** Graph from Beach et al 2017 showing cellular fitness of aneuploid cells after the gain of a single chromosome (disomies in haploids and trisomies in diploids) or the loss of a single chromosome (monosomies in diploids) relative to the number of genes for each chromosome. Colony sizes are compared to wild-type (Percentage of Wt growth). The error bars show the standard deviation.[7]

1.2 Aneuploidy in Cancer

1.2.1 The aneuploidy paradox

The first discovery of aneuploidy was already at the beginning of the 20th century by Theodor Boveri. He found out that an unequal number of chromosome copies is lethal for sea urchin eggs. Theodor Boveri was also the first scientist to assume a

connection between aneuploidy and cancer.[18] Chromosome missegregation occurs at high rates in cancer cells.[19] In solid tumors, aneuploidy occurs at a frequency of 90%.[20] In addition, cancers which are aneuploid or have high rates of CIN, have also been reported to have a worse prognosis than those that have a normal karyotype.[21,22] The discovery of this connection raised an important question. It had been observed frequently that aneuploidy leads to a reduced cellular fitness in both yeast and noncancerous human cells. Therefore it was inexplicable why most solid tumors were shown to be aneuploid, and aneuploidy apparently provided a proliferation advantage in these tumors. This phenomenon is called the “aneuploidy paradox”.[1,5,12]

Although the rates of aneuploidy and chromosome missegregation can vary from cancer cell to cancer cell, it has been observed in several studies that the karyotypes in cancers follow a specific pattern (Figure 5 A/B).[12;23]

Figure 5[23]: Cancer cells develop aberrant karyotypes frequently. (A) Pictures for comparison of a normal karyotype **(B)** and an abnormal cancer karyotype (bottom) derived from a colorectal cancer cell line. Karyotypes were obtained by SKY analysis; single-dye chromosome painting was done for confirmation. Images show karyotypes after completed metaphase of the cells.

There are numerous indications shown in humans, as well as in mouse experiments, that aneuploidy is a relatively late event occurring in cancer. Some studies even observed that aneuploidy seems to be antiproliferative in some cancers.[24,25] On the other hand, there were also some experiments describing an early-onset of unusual

karyotypes during some cancer developments.[24] These findings suggest that the selective advantage observed in tumor cells might derive from specific karyotypes they develop.

1.3 Complex aneuploidy in yeast

1.3.1 Highly missegregating yeast cells develop specific karyotypic patterns

In the Campbell lab, haploid yeast strains were engineered to develop high rates of CIN. Interestingly, after adaptation to CIN, specific patterns of karyotypes could be observed in these missegregated yeast strains (Figure 6 A). It was noticed, that the cells tend to develop aneuploidy in the chromosomes 1, 2, 3, 8 and 10 more frequently than in others. In addition, as can be seen in Figure 6 A, chromosome 8 and 10 were, although both aneuploid in a large number of samples, rarely disomic within the same adapted cell. All chromosomal combinations showing positive or negative correlation with a significance of $P<0.001$ after random missegregation were then tested by specific combinations of chromosomal gains in haploid strains. The strains with chromosomal combinations that showed the highest positive correlation after adaptation to random missegregation showed also the highest cellular fitness compared to other chromosome combinations when these two chromosomes were induced specifically to co-missegregate. The inverse relationship could be observed with chromosomes showing negative correlation (Figure 6 B). These results suggest that the development of complex karyotypes is influenced by positive and negative genetic interactions between whole chromosomes. These genetic interactions are called chromosome copy number interactions (CCNIs).[26]

In Figure 6 C/D, the cellular fitness after the simultaneous gain of chromosomes 8 and 10 can be observed. Although strains which only gain chromosome 8 or chromosome 10 alone show moderate colony sizes, the cellular fitness is impaired severely when both chromosomal gains are combined within one strain. This is an indication for a negative genetic interaction between aneuploidy of chromosomes 8 and 10. [26]

From these findings we conclude that CCNIs between aneuploid chromosomes seem to have an important impact on the fitness of cells. The important questions that remain are therefore how many of these interactions exist between aneuploid chromosomes, and what the genes causing these interactions are.

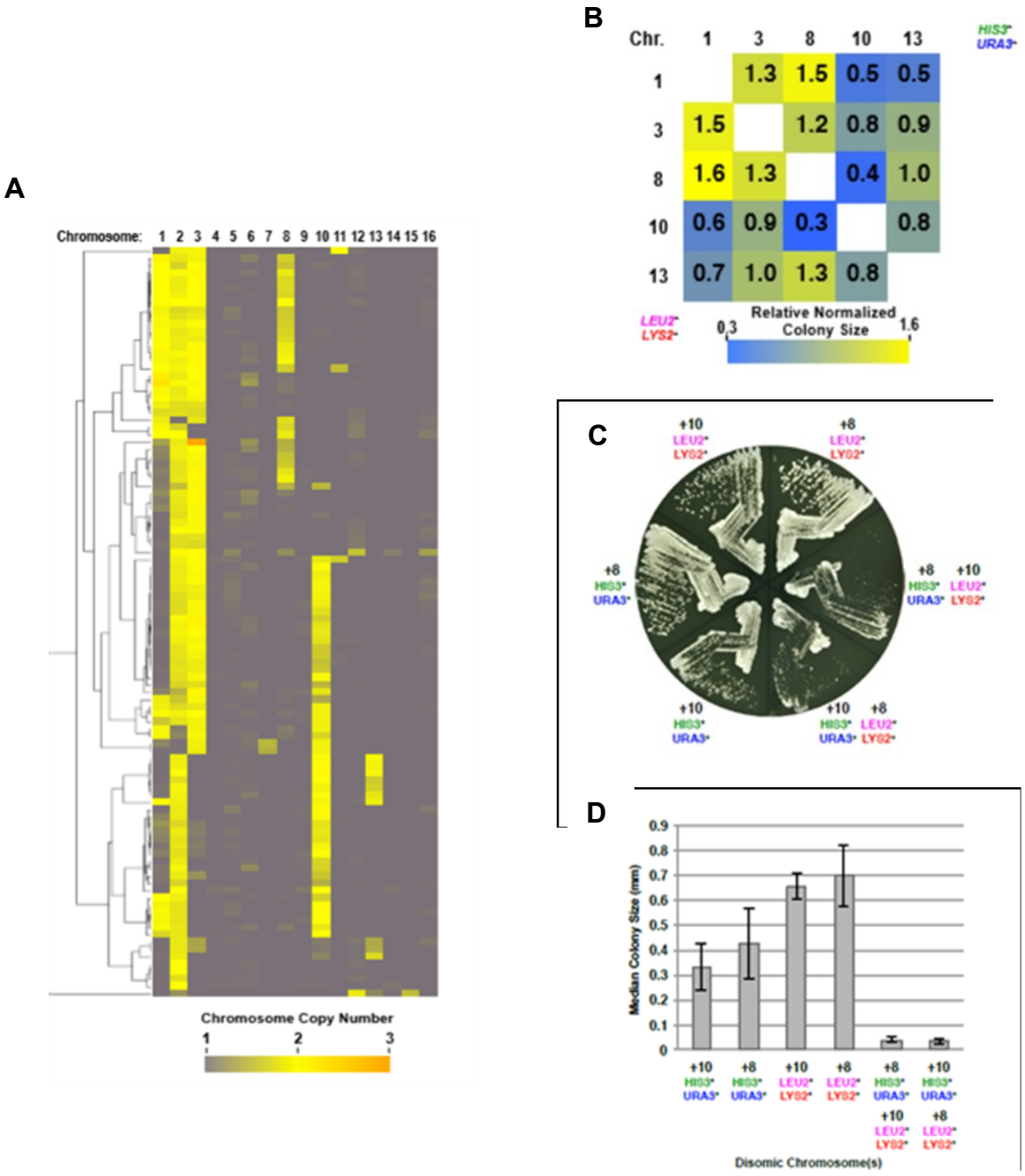


Figure 6[26]**: Haploid yeast strains develop specific karyotypes when CIN is induced in high rates. Chromosome copy number interactions largely influence karyotypic patterns.** **(A)** Cluster of occurring aneuploidies in 102 different CIN adapted haploid yeast strains. Chromosome copy number was measured by whole genome sequencing (Color scale on bottom of picture). **(B)** Relative colony sizes after induction of the gain of two specific chromosomes within the same haploid strain. **(C)** An example of a negative genetic interaction after the Gain/Gain of chromosome 8 and 10. **(D)** Graph showing the colony sizes measured in (C). The areas of the colonies were measured with Image J and the median size was calculated.

1.3.2 There is a positive genetic interaction between tubulin genes in yeast

Apart from the negative genetic interaction between chromosomes 8 and 10, only one other genetic interaction between different chromosomes has been published so far.

Microtubules in budding yeast consist of two subunits, α- and β-tubulin. In higher organisms α- and β-tubulin are encoded by a number of genes, whereas in yeast only two genes (TUB 1 and TUB 3) encode α-tubulin, and only one gene (TUB2) encodes β-tubulin. In the study of Katz et al 1990, it was observed that yeast cells tolerate an excess number of α-tubulin copies, but are unable to cope with an excess of β-tubulin.[27] In addition, it was demonstrated in further experiments by Ander et al 2009, that the induction of an extra copy of chromosome 6 in a haploid yeast strain, which harbors the TUB2 gene, is lethal for the cell. However, when a plasmid containing an excess of the TUB1 gene, which is normally present on chromosome 13, was added before the missegregation of chromosome 6 was induced, the strains viability increased substantially (Figure 7).[8] This is an example of toxicity following an imbalance of different protein complex components, which can be rescued by the restoration of protein balance.[27;8] It therefore suggests a positive genetic interaction between chromosomes 6 and 13.

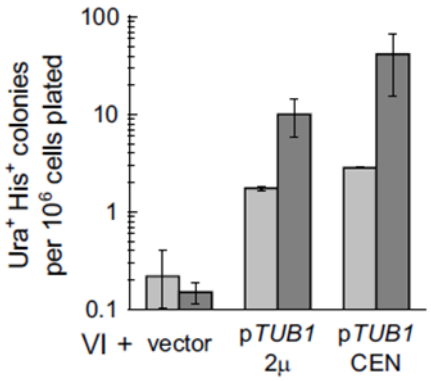


Figure 7[8]: The lethality of an extra copy of chromosome 6 can be rescued by an excess of TUB1. Viability measurement of haploid yeast cells after an extra copy of chromosome 6 was induced, with or without an excess of TUB1 which is usually encoded by chromosome 13. pTUB1_2μ = TUB1 carried on high-copy 2-micron plasmid; pTUB1_CEN = TUB 1 gene carried on low copy CEN plasmid.

1.4 The topic of this project: Systematic identification of chromosomal copy number interactions in yeast

In this project, I wanted to systematically investigate copy number interactions between different chromosomal aneuploidies and identify their underlying genes. Therefore, the simultaneous loss or gain of two different chromosomes was induced specifically in yeast strains (Saccharomyces cerevisiae) and different aneuploid karyotypes were created. The cellular fitness of the aneuploid colonies could be examined and compared to each other or to a WT.

A high-throughput system for induction and selection of aneuploid strains first had to be developed. The results from Beach et al. 2017 on single aneuploidies could thereby be used as a guideline for verification of our system.[7] Through the combination of different chromosome aneuploidies, the presence of positive or negative CCNIs could then be indicated by the severity of the phenotype.

To sum up, the goal of this thesis was to discover the frequency of chromosome copy number interactions in yeast and if these occur in equal numbers between chromosome gains and chromosome losses. In addition, I wanted to determine the genes that cause these specific interactions and find out if there are other, nonspecific, genetic interactions in aneuploid cells that have an impact on cellular fitness. This can provide a better insight into the nature of aneuploidy. We hope that these investigations will be an important step for understanding the behavior of aneuploidy and complex karyotypes in human cancer cells in the future.

2 Methodology

2.1 Single Spore Purification

Single Spore purification was performed in order to receive a- and alpha-type cells which could then be mated to establish a diploid Loss/Loss collection, and also to obtain the haploid Gain/Gain collection from a diploid collection. Yeast cells were transferred from Yeast Extract Peptone Adenine Dextrose (YPAD) plates onto Sporulation (SPM) plates (11.5 List of Media). After 2 days on SPM, cells were checked under the microscope for tetrads and random spore purification was performed. A clump of cells was dissolved in 10 µl of zymolase (Zymoresearch; 1mg/ml in sorbitol; see 11.4 List of Reagents and Equipment) in order to digest the ascus. After 10 minutes at 30°C, 500 µl of sterile H_2O was added and spun down at 3,000 revolutions per minute (rpm) for 1 minute in a table centrifuge (Eppendorf). The supernatant was taken off and the pellet resuspended in 100 µl H_2O. The tubes were vortexed evenly, so that the cells could stick to the tube wall. After 5 washing steps with 1ml H_2O per each step, 1 ml of 0.01% NP-40 was added. The tubes were vortexed and the cells sonicated on ice for 1 minute (Sonicator (Bandelin); Mode: 5x10%, Power: 20%). After vortexing for 1 minute, 5 µl of the cell suspension was put on a fresh selection plate and streaked out for single colonies.

2.2 Mating type test

This test was done after the Single Spore Purification to find out which colonies that had grown out of the single spores were Mat-a or Mat-alpha. Therefore an YPAD plate was covered with 80 µl of a cell-H_2O suspension containing haploid control cells, with a known mating type (CCY80 (=Mat-a) and CCY81 (=Mat-alpha); 11.1 List of Yeast strains). Single colonies gained from Single Spore Purification were mixed with 2 µl H_2O per colony and transferred on that plate. Colonies that were Mat-a would mate with the Mat-alpha CCY81, whereas colonies that were Mat-alpha would mate with the Mat-a CCY80. In order to find out which colonies had mated with which of the known types, all plates were stamped on Synthetic Minimal (SM) plates. Only cells that had mated could survive on these plates. Thereby the mating type could be clearly determined.

2.3 Polymerase chain reaction (PCR) to amplify required DNA for yeast transformation

Ingredients for 1 PCR reaction:

- 36 µl H_2O
- 10 µl HF Reaction Buffer (Thermo Fischer)
- 1 µl dNTPs (New England BioLabs Inc. (NEB))
- 1 µl of 10 mM forward and reverse primer (Microsynth; 11.2 List of Primer)
- 1 µl of the DNA
- 0.5 µl of the Phusion HF DNA polymerase (Thermo Fisher)

After mixing gently the PCR program “CC INTEGR” was run (0

PCR programs). In order to check if the PCR was successful, gel electrophoresis was performed (2.8).

2.4 High Efficiency Lithium Acetate (LiAc) yeast transformation

Transformations were done in order to mate haploid strains for Loss/Loss selection, to insert a conditional centromere for chromosomes 10, 12 and 16 while remaking these strains for testing, as well as for TUB2 deletion. For the TUB2 deletion a hygromycin resistance gene was inserted instead at the same time (11.3 List of Plasmids). 700 µl of overnight cultures in YPAD were diluted in 50 ml fresh YPAD medium in 250 ml Erlenmeyer flasks. After shaking for about 5 hours at 30°C the whole medium-cell suspension was transferred into 50 ml conical tubes and spinned down for 5 min at 1,000 rpm and 4°C. The pellet was then dissolved in 25 ml H_2O. Meanwhile salmon sperm DNA (ss-DNA) was put for 5 minutes on 95°C and afterwards put quickly on ice. The ss-DNA was added to promote the uptake of the required DNA into the yeast cell. After the previous centrifugation was done, H_2O was poured off and the pellet was resuspended in 1 ml of 100 mM lithium acetate (LiAc; Merck). The suspension was transferred to a 1.5 ml tube and was centrifuged at 14,000 rpm for 30 sec. The supernatant was discarded and the pellet again resuspended in 400 µl of 100 mM LiAc. After vortexing, 50 µl of the cell suspension were transferred to fresh 1.5 ml microcentrifuge tubes and again pelleted for 30 sec at 14,000 rpm. The supernatant was discarded and the following components added to the pellet in an exact order: 240 µl 50% polyethylene glycol (PEG), 36 µl 1M LiAc, 10 µl ss-DNA, 10 µl of the PCR product that should be transformed into the cell and 74 µl H_2O. The sample was vortexed for 1 min and put for 40 min into a water bath at 42°C for heat shock. After this step centrifugation was done for 30 sec at 8 000 rpm and the supernatant poured off again. The pellet was dissolved in 100 µl SOS and put on a selection plate to see if required gene was successfully integrated.

2.5 Rapid Yeast Genomic Preparation

0.2 ml of Buffer A (11.6 List of Buffers) was mixed with glass beads (Sigma Aldrich). A bunch of yeast cells were added and the complete cell suspension was suspended with 0.2 ml of Phenol-Chloroform. After 10 min of vortexing at 4°C, 0.2 ml of Elution buffer (EB) was added. Centrifugation was performed for 5 min at 14,000 rpm and

the transparent DNA phase could be taken off and immediately used or stored at 4°C. After numerous unsuccessful purification attempts with this protocol, DNA purification was solely done with the Wizard Genomic DNA Purification Kit. This protocol is explained in the next subchapter.

2.6 DNA Purification with Wizard Genomic DNA Purification Kit

After several unsuccessful DNA purifications with the Rapid Yeast Genomic Prep protocol, purification was performed according mainly to the protocol of the Wizard Genomic Purification Kit by Promega:

A clump of yeast cells was dissolved in 298 µl of ethylenediaminetetraacetic acid (EDTA) with a concentration of 50mM. 2µl of 21mg/ml lyticase (Sigma Aldrich) were added and mixed gently. The cell suspension was incubated for about 35 minutes at 37°C. After centrifugation at around 14 000 rpm for 2 minutes the supernatant was discarded and the pellet dissolved in 300µl Nuclei Lysis Solution and 100µl Protein Precipitation Solution from the Purification Kit. After vortexing, the cells were incubated on ice for 5 minutes. The samples were then centrifuged for 3 min at around 14,000 rpm and the supernatant was transferred to a fresh tube containing 300µl of isopropanol. After inverting the tubes multiple times, a centrifugation was performed for 2 min at around 14,000 rpm. The supernatant was discarded and a washing step with 70% ethanol was performed. After centrifugation at 14,000 rpm for another 2 min, the ethanol was aspirated and the pellet air-dried. 50 µl of DNA Rehydration solution were added and after incubation for 15 min on room temperature, 1.5 µl RNase were given to the cell suspension. After 15 min of incubation at 37°C, the DNA was purified and ready to use or to be frozen down at -20°C.

2.7 PCR for verification of DNA integration after High Efficiency LiAc yeast transformation

The following Master Mix was prepared and could be used for 20 samples (20 PCR tubes):

- 50 µl HF Buffer
- 0.5 µl forward and reverse Primer (100 mM concentration)

- 5 µl dNTP
- 185 µl H_2O
- 10 µl DNA Polymerase

For each PCR tube (VWR), 12 µl of this Master Mix were joined with 2 µl DNA sample. The PCR program CCY Phusio was used.

2.8 Agarose Gel electrophoresis

PCR products were examined by using gel electrophoresis with a 0.8% agarose gel. The used dye is PeqGreen (PeqLab), a 1kb Plus DNA ladder was used to compare sample size. The gel was run with 90 Volt for 30 to 35 minutes (time depended on fragment size). The resulting bands could be examined with the Gel Doc^{TM} XR^{+} (Bio Rad) and compared to the bands of the DNA ladder and eventually to positive and negative controls.

2.9 Establishing a conditional centromere and selecting for aneuploidy

In order to induce missegregation in a target chromosome, a plasmid containing a conditional centromere; PGAL1-CEN3; with a galactose promoter (GAL-1) and an amino acid gene as a selection marker was integrated into the target chromosome to replace the original centromere. This method was also used before by Anders et al. 2009. [8]

The promoter GAL1 is involved in galactose metabolism. This leads to an approximately 1,000-fold induction of transcription of this gene when galactose is supplemented in the medium, which impairs the centromere function and subsequently leads to missegregation. [28]

For selection of the aneuploid yeast cells, different markers are used for different aneuploid conditions. As the experimental design of the induction, as well as the selection for aneuploidy differed between the Loss/Loss, Gain/Loss and Gain/Gain strains, the methodological description of these was split up in the following sections.

2.10 Induction of missegregation and selection for monosomy in the Loss/Loss collection

For a Loss/Loss event, two different chromosomes within a diploid strain were induced to missegregate and become monosomic, which means that only one copy of each target chromosome should be left in the cell after successful missegregation.

2.10.1 Establishment of a Loss/Loss selection

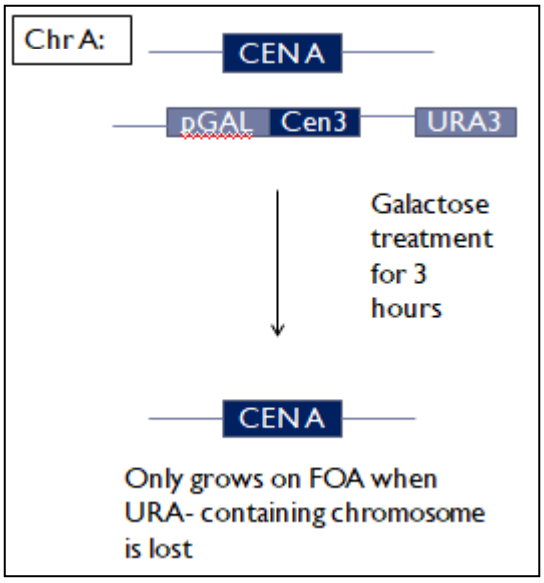


Figure 8: Scheme of the induction and selection for a loss event in a diploid yeast strain. The conditional centromere contains a GAL-promoter that leads to the disruption of normal segregation fidelity when galactose is added to the medium. Furthermore the conditional centromere for a Loss event contains an active URA3 gene. For growth on the FOA selection plates the cell has to lose the chromosome that contains the URA3 gene. Otherwise FOA can be converted to a compound which is toxic for the cell by a protein encoded by the URA3 gene. [29]

The loss of a chromosome could only be tested in diploid yeast cells, as haploid strains are not viable after losing one of their chromosomes.[7] For inducing a loss event of a specific chromosome, the conditional centromere containing a GAL-1 promoter was used. The selection system for loss differs from the one in Gain/Gain strains. The conditional centromere solely contained a URA3 gene (pGAL-CEN3 URA3). When the target chromosome was lost, the ability to produce uracil was lost. Colonies were then selected on FOA plates. These plates contain 5-fluoroorotic acid (FOA), which can be converted to a compound toxic for the cell by the OMP decarboxylase, which is encoded by the URA3 gene.[29] Cells were therefore only able to grow on the selection plates, when they had successfully lost the chromosome containing the URA 3 gene (Figure 8). The same system could be applied for losing

two chromosomes simultaneously. It is important to notice, that in the Loss/Loss collection, the chromosomal losses were not combined in both ways when haploid strains were mated. This means for example the loss of chromosome 1 (from a haploid Mat-a strain) and 8 (from a haploid Mat-alpha strain) was induced simultaneously, but not the loss of chromosome 8 (from a haploid Mat-a strain) and chromosome 1 (from a haploid Mat-alpha strains), as the selection for both chromosome losses always was the same, unlike the selection for Gain/Gain events. The induction experiment of Gain/Gain will be described in detail in 2.11.

2.10.2 Galactose-induced missegregation of Loss/Loss strains in test tubes with subsequent serial dilution

In order to be able to measure cellular fitness of missegregated Loss/Loss strains more preciously this method was used for chromosomal combinations that were suspected to show positive or negative genetic interactions in the High Throughput results (0). As in the induction in 96-well format, overnights with 2ml of 2%-YPAD were prepared of every sample. The next day, 40 µl of every sample were diluted in 2ml of 2%- Yeast Extract Peptone Adenine Raffinose medium (YPAR). After 4 hours, 1ml of every sample was transferred into a 2ml Eppendorfer tube and spinned down at 14,680 rpm for 30 sec. The supernatant was discarded, and the pellet was dispersed in 1ml Yeast Extract Peptone Adenine Raffinose medium (YPAGR 1% raffinose and 1% galactose) and transferred to a fresh test tube. Both tubes, containing the 2%-YPAR and the 1%/1%-YPAGR medium were put back at 30°C. After another 3 hours of shaking, dilution series in 1x Phosphate buffered saline (1x PBS; dilutions: 1; 1:10; 1:100; 1:1000; 1:10,000) were made. 4 µl of each dilution were put on selection plates by using a multichannel pipette. An outline of this method is also depicted by a flow chart in the results (Figure 10).

2.10.3 Galactose-induced missegregation of Loss/Loss in 96-well format

This method for induction of missegregation was used in order to test the entire Loss/Loss collection in High Throughput. The consistency of this method was tested by comparing our results to the published results for Single Loss growth before the entire Loss/Loss collection was tested.[30] Overnights in 2 ml of 2%-YPAD liquid culture were made. The next day, 4 µl per each sample were put into 150 µl 1%-

YPAR. After 4 hours, 50 µl of YPA medium containing 4% galactose were pipetted into each sample, except for the ones used as a negative raffinose control. After 3 hours induction time with galactose the samples were put on selection plates by either using the robot by Singer Rotor instruments or with 4 µl by using a multichannel pipette. When using the robot, usually duplicates of each galactose-induced sample were made. An outline of this method is also depicted by a flow chart in the results (Figure 12).

2.11 Induction of missegregation and selection for aneuploidy in the Gain/Gain collection

For the selection of Single Gain-events, the plasmid contained the conditional centromere plus HIS3 and an internal fragment of ura3. This was then integrated into the URA3 gene (PGAL1-CEN3 ura3::HIS3), therefore disrupting function of this gene. Alternatively, the centromere of the chromosome is replaced by a conditional centromere containing LEU2, which disrupts the function of LYS2 (PGAL1-CEN3 lys2::LEU2). The same method can also be applied for Gain/Gain events, where both possible centromeric constructs are integrated in the different target chromosomes to be gained. Excision and loss of the HIS3 or LEU2 gene, which is also called pop-out event, happens spontaneously in a frequency of approximately 1:1,000 and restores the function of the disrupted genes. Cells that have for example gained an extra copy of the required chromosome through missegregation, and restored the function of their URA gene through an excision event of HIS3 in one of the two chromosome copies, can therefore be selected on plates lacking histidine and uracil. The selection with LYS/LEU follows the same mechanism. [8] Cells are able to grow on –All 4 selection medium, which is –LYS/-LEU/-HIS/-URA, if they have gained another copy of each of the two required chromosomes and successfully popped out the disrupting genes in one copy per each chromosome (Figure 9).

For the haploid Gain/Gain collection single spore purification of an already existing diploid Gain/Gain collection was performed. Only the collection containing solely alpha-type strains has been analyzed up to now. Chromosomal combinations were tested in both ways, which means for example the gain of chromosomes 1(PGAL1-CEN3 ura3::HIS3) and 8 (PGAL1-CEN3 lys2::LEU2), as well as the gain of

chromosomes 8 (PGAL1-CEN3 ura3::HIS3) and 1(PGAL1-CEN3 lys2::LEU2) were analyzed.

After growing for 3 hours in a medium containing galactose, chromosomes containing the conditional centromere should have failed to separate correctly during mitosis. [8] Usually chromosome missegregation leads both sister chromatids to stay in the mother cell. [30] The induction experiment for Gain/Gain strains is described in detail in 2.11.1.

2.11.1 Analysis of the cellular fitness of Gain/Gain strains in high-throughput

As it was not possible to use the same High-Throughput system for the induction of the gain of chromosomes as for the losses (2.10.3), a different system was invented here. Overnights in 2ml YPAD medium were made and 2 times 100µl per each strain were suspended in 2 test tubes containing 2ml YPAR (2% raffinose) each. After 3 hours of shaking at 30°C, one of the two samples per each strain was spun down for 3 min at 7,000 rpm and the resulting pellet was resuspended in 2ml of YPAGR (1% raffinose, 1% galactose). Galactose samples and their raffinose controls were shaken for another 3 hours at 30°C. Dilution series on –LYS/-LEU plates in 1xPBS (1; 1:10; 1:100; 1:1,000; 1:10,000) were made. After approximately 2 days multiple colonies were picked and patched on –All 4 plates. This was only done if a clear difference between the galactose sample and the raffinose control could be observed. After another 2 days, 3 single colonies could be picked per each strain from the patches and patched on a fresh –All 4 plate. After sufficient growth on this plate, a clump of cells per each sample was frozen in 200 µl of 50% glycerol in 96-well format.

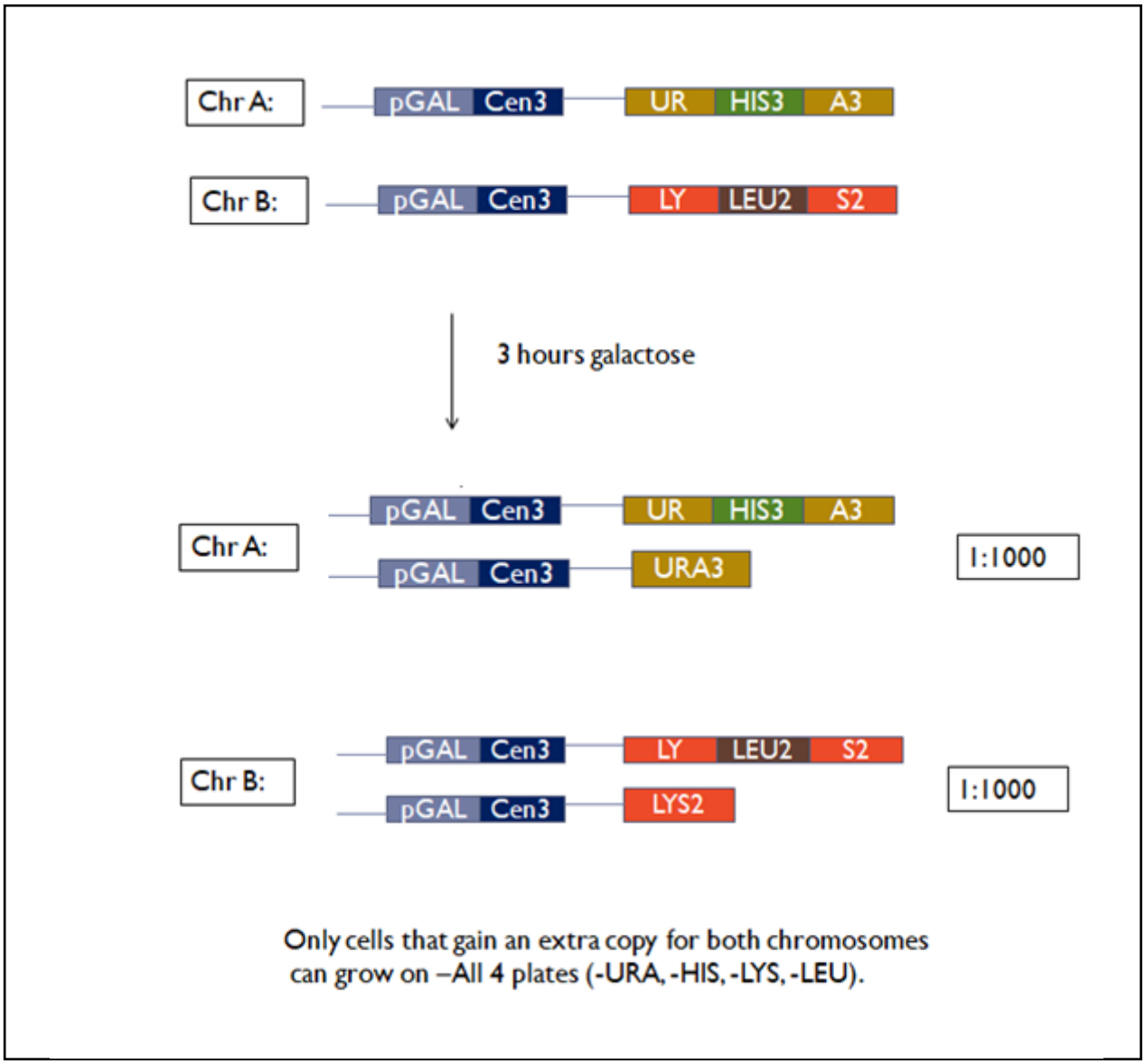


Figure 9: Scheme of the induction of and selection for a Gain/Gain event in a haploid yeast strain. The conditional centromere on the chromosomes that should gain an extra copy contains a GAL-promoter that leads to the disruption of normal segregation fidelity when galactose is added to the medium. Furthermore a conditional centromere for a Gain/Gain event contains either a URA3 gene disrupted by an intact HIS3 gene or a LYS2 gene disrupted by an intact LEU2 gene. For growth on the – All 4 selection plates two events have to take place within one cell. First, the cell needs to gain an extra copy per each of the two chromosomes that are required, second in one of these two copies obtained by the gain event, a pop-out of the disrupting gene (HIS or LEU) that then leads to the restoration of the activity of the before disrupted genes (URA or LYS) has to take place. This happens usually with a frequency of 1:1000

For analysis of cellular fitness, the samples from the frozen plates could be applied to a fresh –All 4 plate by a 96-well replica plater (V&P Scientific Inc.) and overnights were done in 96 well format in 200 µl of YPAD. The overnight samples were then diluted 1:10 in 1xPBS, the robot from Singer instruments transferred these to –All4, and integrated density could be measured with Image J after 25-26 hours. A schematic outline of these experiments can be found in Figure 21.

2.12 Induction for missegregation and selection for aneuploidy in Gain/Loss strains

In Gain/Loss strains, the centromere of the chromosome to be gained was replaced with PGAL1-CEN3 lys2::LEU2, the conditional centromere of the chromosome to be lost contained the URA gene for selection, as explained in the selection for Loss/Loss.

2.12.1 Galactose-induced missegregation of Gain/Loss in test tubes with subsequent serial dilution

Unlike the inductions of the other missegregation events, for Gain/Loss it was found out to be most effective when galactose treatment was done for 7 hours. The 7 hours induction is performed equally to the 3 hours induction in test tubes for Loss strains in all other steps and only used for higher efficiency of Gain/Loss events.

Dilution series were then done on FOA selection plates, and after 2-3 days colonies were patched on FOA –LYS/-LEU plates. Single colonies could then be patched further on fresh FOA –LYS/-LEU double selection plates and after another 2 days qPCR could be done to confirm aneuploidy.

2.13 Quantitative Polymerase Chain Reaction (qPCR)

Single colonies of the samples were taken from the induction plate and streaked out on a fresh selection plate. After approximately 2 days a clump of cells was taken for each sample and dissolved in 25 µl of 0.02 M sodium hydroxide (NaOH). The sample was then prepared for qPCR with the PCR program CC100. After centrifugation for 5 min in the minicentrifuge (Thermo Fisher Scientific), 1 µl of DNA per sample was taken and mixed with 7 µl H_2O and 10 µl qPCR Mastermix (New England BioLabs Inc.). For every sample the quantity of each chromosome was determined with

primers for both the left and the right chromosome arm. Each primer pair was diluted with H_2O at the rate of 30:4:4 (H_2O: forward primer: reverse primer). 2 µl of the Primer-Mix was given to each DNA-Mix explained above. qPCR was done in 96-well plates (Eppendorf) and always the same qPCR program was used. Before the program was started, the plate was spun down shortly (Megafuge 1.0 R, Thermo Fisher Scientific). Chromosomal copy numbers were calculated from the Ct (threshold cycle) values measured by the qPCR machine. For calculations Microsoft Excel was used.

2.14 Statistical analysis

2.14.1 Quantification of cellular fitness

Cellular fitness after induction of missegregation was measured by using Image J (National Institute of Health). This was usually done 60-72 hours after missegregation for strains in 96-well format. The raw integrated density was measured for the same area size of every sample. In this case, the density of the background was subtracted from the value for each sample. The average value of the samples per each strain could be calculated and densities could then be compared between the strains or normalized to a WT strain. The areas of single colonies were also quantified with Image J and compared between the different conditional strains, or to a WT strain. In this case no background subtraction was done.
No normalization to a WT was done in Gain strains.

For all calculations Microsoft Excel was used. GraphPad Prism 8 was used for creating the figures; flow charts were created with BioRender.

2.14.2 Normalization of cellular fitness

The cellular fitness of loss strains was first normalized to a WT strain. For single colony measurement the actual area of each colony per strain was measured in mm, and the average size calculated.
After measurement of the integrated density of Loss/Loss in high-throughput, an internal normalization was done by multiplying the average growth of both chromosomes lost in one strain (predicted internal normalization). This was then subtracted from the actual internal normalization, which is the density of a single

strain divided by the average density of all chromosomes. Values that were smaller than predicted from the average were a hint for a negative genetic interaction between 2 chromosomes; strains with a higher value than the predicted average suggested a positive genetic interaction between 2 strains.

In the Gain/Gain strains in 96-well format, the integrated density was measured and the average for each strain divided by the total average of the integrated density for each plate.

3 Results

Beach et al. published the effects of specific aneuploidies on the cellular fitness of budding yeast in 2017. There aneuploidy was selected for by targeting the required chromosome with GFP and then following its fate under the light microscope. This is a very precise, but also time-consuming system. Since the topic of this thesis was to investigate chromosomal copy number interactions, the missegregation of two different chromosomes within one cell had to be induced. Therefore a system for testing different chromosomal combinations of aneuploidies in high-throughput was required. In order to design such a high-throughput system and verify its consistency, the published results on single aneuploidies by Beach et al. were hereby used as a guideline.[7]

3.1 Inducing the loss of single chromosomes

3.1.1 Monosomy is not stable in every chromosome

In our selection system, the selection for loss is simpler than that for the gain of a chromosome. This is the reason why we began this project with investigating the effects of chromosome loss. Before inducing the simultaneous loss of two chromosomes, I started by inducing the loss of single yeast chromosomes specifically. The cellular fitness after a single loss could then be normalized to a WT strain and compared to the published results for monosomy. After the induction with galactose, dilution series of the samples and the negative raffinose controls were put on FOA-containing medium. Only cells that have lost the required chromosome successfully should be able to grow on this selection medium. Colony areas could be measured with Image J.

It could be observed that the synthetic complete medium (SC) seemed to provide results most similar to the ones published by Beach et al (Supplemental Experiments; Figure 23; Figure 24). In order to measure cellular fitness that is representative for the entire strain after chromosome loss, multiple colonies were picked from the FOA selection plate and streaked out for singles on SC medium (Figure 10).

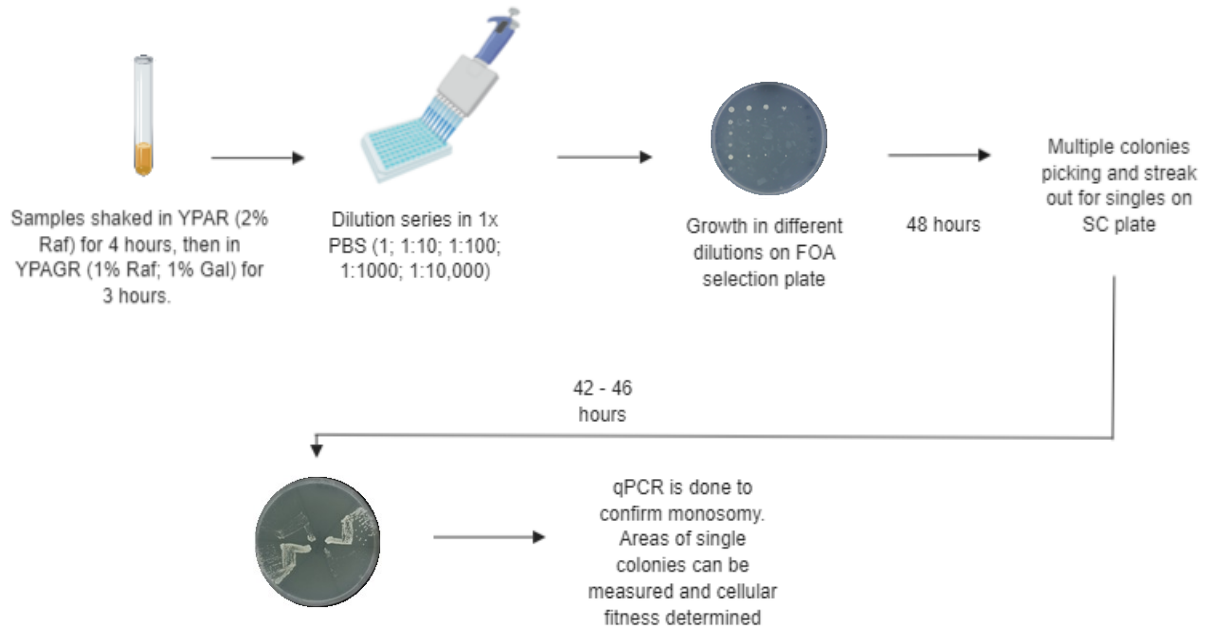


Figure 10: The streak-out of multiple colonies from the FOA selection medium onto a SC medium is the method of choice for determining cellular fitness. Scheme showing the experimental design that was used in order to induce single chromosome loss and then determine single colony growth.

A qPCR was done in order to confirm the required loss of one copy of the target chromosome. (Figure 11 A).[7] When single colonies had grown on the SC plate, which was about 42-46 hours after the transfer from the FOA selection, colony size measurement and qPCR were done. The qPCR showed that not all chromosomal losses are stable. Up to this time point, some strains that have a highly selective disadvantage caused by the loss of their specific chromosome seem to find a way to avoid this destructive fate. They then either dispose of their URA3 gene; that otherwise makes it impossible for them to grow on FOA; without losing a chromosome, or, what is more likely, manage to gain their lost chromosome back and thereby recover their growth potency. The growth of these samples resembled the growth of their raffinose control on the FOA selection medium. Therefore, all Single Loss strains (SL) that showed equal growth as their raffinose control and were disomic in qPCR; which are the strains after the single loss of chromosome 2 (SL 2), SL 10, SL 13, SL 14 and SL 15; were determined to have a cellular fitness of 0% (Figure 11 B/C). SL 12 was the only chromosome loss that had grown significantly more than its raffinose control, but appeared to be disomic in qPCR. One reason for the unexpected high cellular fitness could be that, the loss of specific genes on chromosome 12 increases CIN to such a high rate that most cells are able to

missegregate again immediately after the initial chromosome loss and thereby retain disomy. However, it has to be noted that Beach et al. claimed to have observed a growth of only 0.1 % of SL12 compared to the WT (Figure 11 C). As we do not know why chromosome 12 seems to be able to gain its lost copy back at such a high frequency, this chromosome was excluded from further investigations.

For some of the chromosome losses there was no growth visible on the dilution plates (SL 4, SL 7). These could then not be transferred further on SC plates, and therefore not be tested by qPCR. These chromosomes were determined to have a growth of 0% as well (Figure 11 B/C).

All other SL were shown to have remained monosomic in qPCR. The strains with SL 3, SL 11 and SL 16 showed increased cellular fitness compared to Beach et al., whereas SL 6 seems to have a clearly reduced cellular fitness. This could be due to differences in strain background. However, it is important to note that the single loss of chromosome 3 shows by far the highest standard deviation (Figure 11 B/C).[7] We first assumed that this frequently observed high variability of SL3 growth might have been caused by the presence of the mating type locus on this chromosome.[31] After the loss of one chromosome copy, the resulting strains could theoretically mate again and thereby become tetraploid. This theory could be discounted by an experiment that is described closer in 8 Supplemental Experiments (Figure 25).

3.1.2 The relationship between cellular fitness after missegregation and the size of the lost chromosome is not linear

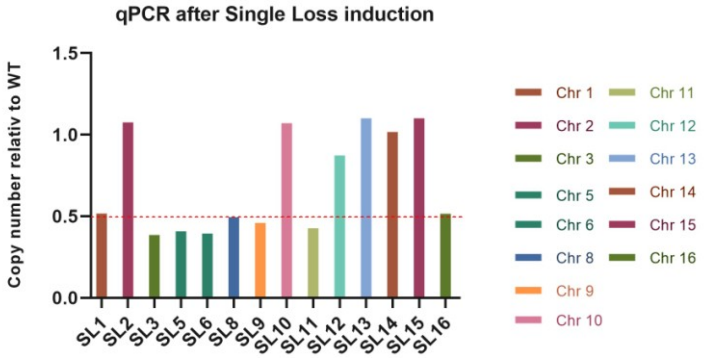
As it can be observed in Figure 11 B, the overall growth trend of our monosomic strains is very similar to the one from Beach et al. It supports the theory that cellular fitness after missegregation is largely influenced by the size of the aneuploid chromosome. In addition, the relationship between the chromosome size and the cellular fitness after missegregation is clearly not linear, as a linear function would not meet the y-axis at a growth of 100% when applied to the data. Therefore it can be hypothesized that the fitnesses following chromosome loss are also influenced by genetic interactions within a chromosome.

As the goal was to reveal genetic interactions between simultaneous chromosome losses, it was necessary to find a suitable statistical method to be able to identify and

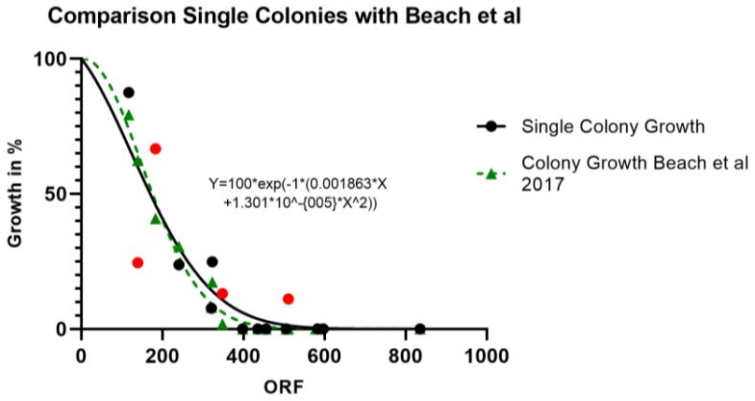
clearly distinguish these interactions from expected cellular fitness patterns. Therefore I was looking for an equation that could be fit best to the SL results. The first attempt here was to use a product equation. This equation was published to be less biased by extreme values than other equations used frequently to identify genetic interactions. Colony size after a chromosome loss with 200 ORF is about 50% compared to a WT strain. This means, that if there would be a multiplicative relationship, the colony size with 400 ORF should be around 25%. In fact, after the loss of a chromosome with this size the cells show only little to no growth. The resulting product curve showed a fit of 0.685 when applied to our results. This illustrates that the relationship between chromosome size and the cellular fitness after missegregation of the specific chromosome does not fit ideally to the initially predicted product relationship. An additive relationship would predict the observed transition from 50% growth with 200 ORF to a growth of 0% with 400 ORF. This possible definition was however excluded because it was shown to be easily biased by extreme values.[32]

We also fit the data using the linear quadratic equation, which shows an R square of 0.754. In addition, the predicted cellular fitness from this equation of about 40% after the loss of 200 ORF and 6% with 400 lost ORF is very similar to the observed values. This equation is normally used in radiology, to depict the relationship of cell survival and radioactive dosage. The radioactive force leads to cell death directly (linear) and indirectly through the accumulation of other reactions caused by the radioactivity (quadratic).[33] As the relationship between chromosome size and cellular fitness after loss is not completely linear, we hypothesized that fitness is also influenced by genetic interactions within a chromosome, which could be represented by a quadratic function. In conclusion, there seem to be parallels between the relationship of radioactivity and cell survival and the relationship of chromosome missegregation and resulting cellular fitness and therefore this equation seems to be very suitable for our results.

A



B



C

	Single colony sizes SL	SD	Beach et al 2017	ORF
	Growth in %	in %	Growth in %	
Chr1	87.5	7.5	79.3	117
Chr2	0.0	0.0	0.0	456
Chr 3	66.7	24.2	40.9	183
Chr4	0.0	0.0	0.1	836
Chr5	24.9	10.4	17.4	323
Chr6	24.5	0.9	62.4	139
Chr7	0.0	0.0	0.0	583
Chr8	7.7	2.5	8.1	321
Chr9	23.8	4.0	30.5	241
Chr10	0.0	0.0	0.0	398
Chr11	13.1	3.3	1.9	348
Chr13	0.0	0.0	0.0	505
Chr14	0.0	0.0	0.1	435
Chr15	0.0	0.0		597
Chr16	11.1	1.6	0.0	511

Figure 11: Monosomy is not stable in all chromosome losses. The relationship between chromosome size and cellular fitness after loss is nonlinear. (A) qPCR after single loss induction of every chromosome. Numbers are normalized to a diploid WT and a diploid chromosome within every sample (Chromosome 9 or 14). 0.5 = 1 chromosome copy number; 1 = 2 chromosome copy numbers. Chromosomes above the applied line (in red) were considered not to be monosomic. Chromosome 4 and 7 are missing from the calculations because these are the only chromosome losses showing no growth on the FOA selection plate. **(B)** Graph showing average growth of SL colonies compared to the growth for monosomy determined by Beach et al. SL that did not grow on FOA (SL 4, 7) or showed identical growth as their negative raffinose controls (SL 2, 10, 13, 14, 15) were considered to have a growth of 0%. SL 12 was excluded. Growth of all other SL was determined by the average colony area of each SL strain measured by Image J in 2 independent experiments. Values were then normalized to a WT strain. Curves were created with a linear quadratic function. Formula shows equation for the single colony growth of our SL strains. Colony sizes differing for more than 10% compared to the numbers of Beach et al were marked in red. Cellular fitness is compared to the size of the lost chromosome (ORF= open reading frames).[7] **(C)** Table depicting average single colony size for every SL normalized to a WT strain. Numbers were calculated from 2 independent experiments. Colony sizes differing for more than 10% compared to the numbers of Beach et al were marked in red. [7]

3.1.3 Cellular fitness after the Single Loss in high-throughput shows similarity in growth trend compared to previous results

Our target was to investigate genetic interactions occurring in diploid yeast cells after the loss of two specific chromosomes simultaneously. Therefore a more high-throughput method than the experimental design of the dilution series had to be developed. This method then first had to be verified through comparison of the high-throughput results to the previous ones for the SL. For the purpose of testing every single combination of two different chromosomes in high-throughput, a robot from Singer Instruments working in a 96-well format was used to transfer the samples on the selection plate (Figure 12 A/B). After three days, the integrated density of every sample spot was measured by Image J and again normalized to a diploid WT strain.

The high-throughput results for monosomy growth showed largely the same trend compared to our previous results with single colony streak-out (Figure 13 A/B). However, the cell growth measured in high-throughput was higher for most chromosome losses than the colony sizes that were observed in the previous experimental setup. More than 10% gain compared to the cellular fitness of single colony streak-out was observed in SL 2, SL 3, SL 4, SL 5, SL 6, SL 8, SL 9 and SL 13. Viability of the high-throughput samples was not determined by comparison to their raffinose controls and no qPCR was done. The unexpected growth rate for the SL 2 and SL 13, which were shown to be disomic in qPCR in the previous experiments, is likely the result of regaining the lost chromosome (Figure 11). As for SL 3, we still cannot explain the remarkable variability in cellular fitness. For the SL 4 it can only be assumed that this strain also managed to gain back its lost chromosome, as this one did show little to no growth in the dilution series. For SL 5, SL 6, SL 8 and SL 9 the reason for a difference in cellular fitness could simply be the lower precision of the high-throughput method compared to the single colony steak-out. The relationship in growth between those strains stays basically the same as it was observed after the single colony streak-out.

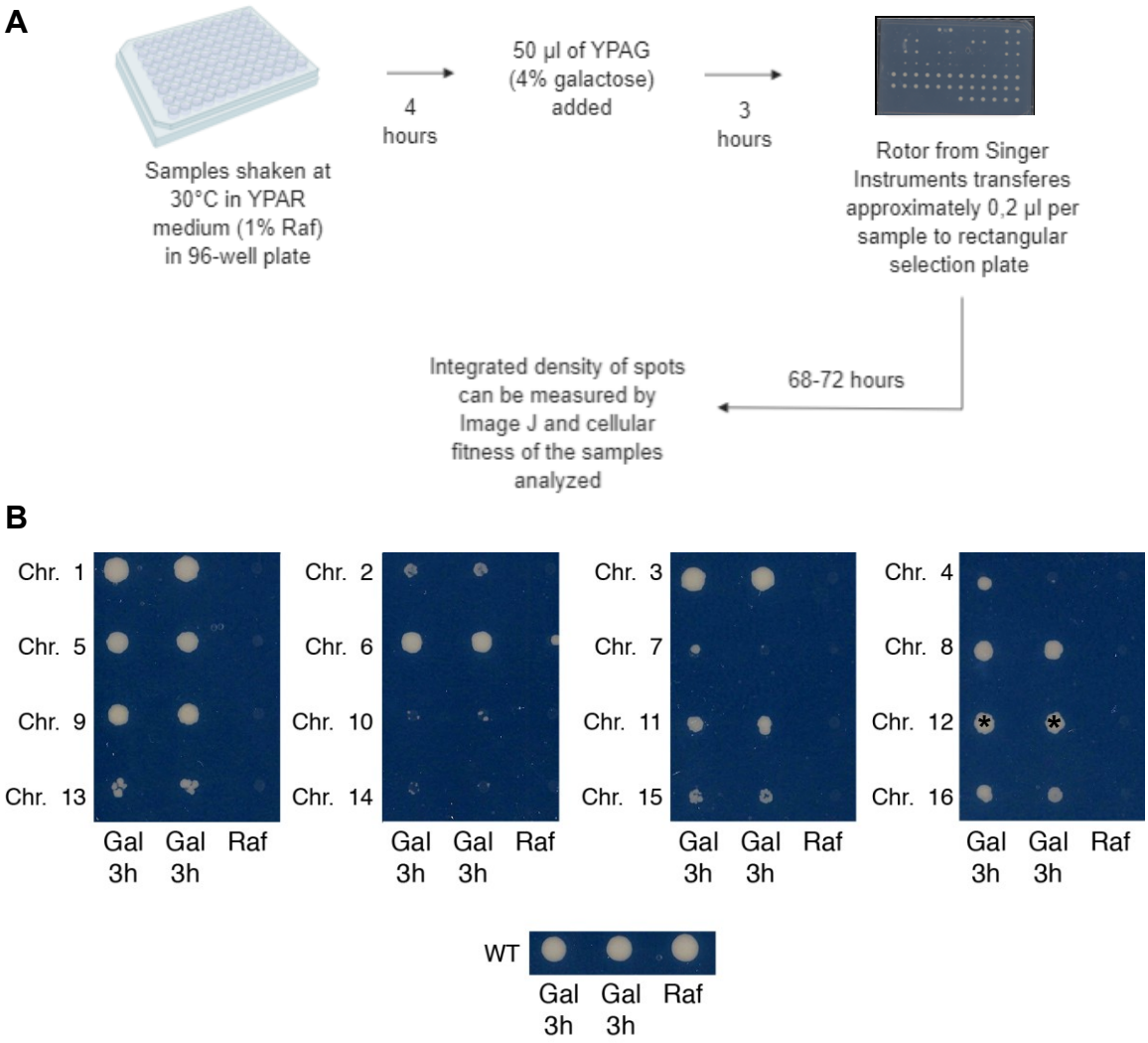
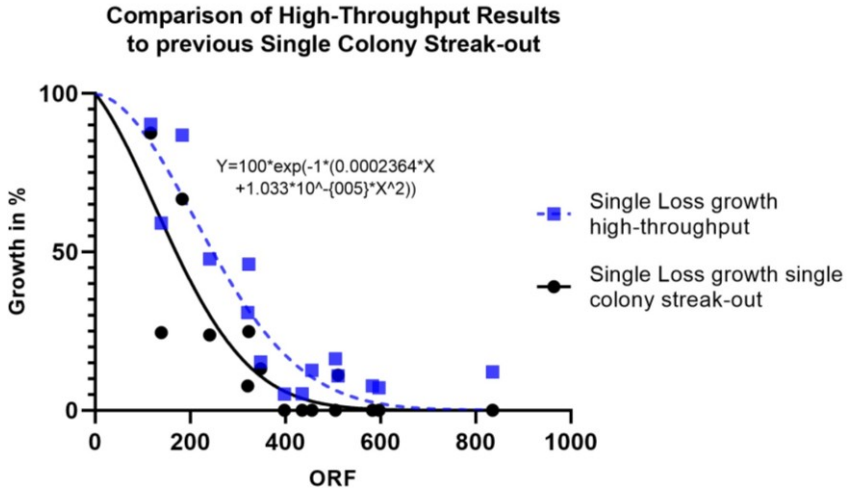


Figure 12: The loss of a single chromosome in diploid yeast strains can be induced in high-throughput. (A) Schematic description of the experimental setup for inducing chromosome loss in high-throughput. **(B)** Example for a plate containing SL spots 3 days on a FOA selection plate after induction of missegregation with galactose (Gal). Duplicates for each strain were induced. Every 3rd column is a negative control grown only in raffinose (Raf). Measured growth was normalized to a homozygous URA-deleted strain (WT). The galactose samples of Chr 12 are marked with asterisks, because these samples were excluded from the measurements (Figure 11).

Although the high-throughput induction and measurement does not seem to be as precise as the previous method, the fact that the overall growth trend for SL is very similar between both experimental designs, verifies the new system to be suitable for the first observation of growth trends in chromosome loss combinations (Figure 13 A).

A



B

	Average high throughput Growth in %	Single colony streak-out Growth in %	ORF
Chr1	90.3	87.5	117
Chr2	12.6	0.0	456
Chr3	86.9	66.7	183
Chr4	12.1	0.0	836
Chr5	46.1	24.9	323
Chr6	59.1	24.5	139
Chr7	7.7	0.0	583
Chr8	30.9	7.7	321
Chr9	47.8	23.8	241
Chr10	5.1	0.0	398
Chr11	15.3	13.1	348
Chr13	16.3	0.0	505
Chr14	5.2	0.0	435
Chr15	7.1	0.0	597
Chr16	10.9	11.1	511

Figure 13: An increased cellular fitness can be seen for most chromosome losses in high-throughput, but in general the cellular fitness follows the overall growth trend observed in the previous experimental setup. (A) Graph showing the comparison of single cell growth after the SL of one specific chromosome measured through single colony areas and the growth of multiple colonies after the loss of a single chromosome in 96-well format illustrated in Figure 12. The growth was measured through integrated density with Image J. The growth after chromosome loss was compared to a diploid WT strain to determine the cellular fitness, as was done after the streak-out for single colonies. A linear quadratic survival curve was used to depict the growth trend relative to the size of the lost chromosome (ORF). Formula shown in graph is the equation for the High-throughput results. **(B)** Table depicting the growth in percentages shown in the graph above. The red marks show where the results in growth after chromosome loss differ more than 10% from the results of single colony size measurements.

3.2 Chromosomal copy number interactions investigated through the simultaneous loss of two chromosomes in diploid yeast

3.2.1 Reproducible cellular fitness can be determined in an entire Loss/Loss collection in high-throughput

A collection of simultaneous losses of two different chromosomes in all possible combinations was established. The induction of missegregation and the subsequent plating on the FOA selection plate was performed in three independent experiments

in the same high-throughput system tested with the single losses (Figure 12). After the measurement of cellular fitness of all chromosome loss combinations, an internal normalization was done (Figure 14). Lethal SL are highlighted in red in the cluster.
As we wanted to investigate which chromosome combinations can lead to a selective advantage or disadvantage for the cell, this cluster showed first interesting hints for some interactions. From these results two different categories of interactions could be hypothesized. One category represents interactions where a lethal chromosome loss seems to be rescued by another loss. This could be an indication for a strong positive genetic interaction between these chromosome combinations. The other category describes the simultaneous loss of two viable chromosomes that seem to cause either a positive or negative effect on cell growth due to positive or negative genetic interactions between these.

Chr	1	2	3	4	5	6	7	8	9	10	11	13	14	15	16
1		-0.39	-2.05	-0.30	0.86	1.84	1.39	0.09	2.47	1.83	-0.32	-0.55	-0.04	-0.31	2.16
2	-0.39		-0.31	0.18	-0.19	0.09	0.03	0.16	0.08	0.14	0.21	0.16	0.25	0.34	0.41
3	-2.05	-0.31		-0.30	1.10	1.39	1.25	-0.26	1.55	-1.77	0.23	-0.35	0.02	0.07	-0.55
4	-0.30	0.18	-0.30		-0.31	-0.39	-0.05	0.10	-0.53	-0.31	0.06	0.04	0.39	0.20	0.00
5	0.86	-0.19	1.10	-0.31		0.17	-0.86	-0.63	-1.67	1.62	-0.29	-0.37	0.15	0.03	-0.63
6	1.84	0.09	1.39	-0.39	0.17		-1.06	-0.77	-1.73	0.66	-0.47	3.10	-0.29	-0.41	-0.47
7	1.39	0.03	1.25	-0.05	-0.86	-1.06		-0.46	-0.98	0.81	-0.08	-0.23	-0.30	-0.11	-0.22
8	0.09	0.16	-0.26	0.10	-0.63	-0.77	-0.46		-0.48	0.46	0.01	-0.19	-0.06	-0.11	-0.20
9	2.47	0.08	1.55	-0.53	-1.67	-1.73	-0.98	-0.48		0.84	-0.45	-0.78	-0.34	-0.11	-0.34
10	1.83	0.14	-1.77	-0.31	1.62	0.66	0.81	0.46	0.84		-0.31	-0.47	-0.08	-0.02	-0.29
11	-0.32	0.21	0.23	0.06	-0.29	-0.47	-0.08	0.01	-0.45	-0.31		0.12	0.29	0.38	0.20
13	-0.55	0.16	-0.35	0.04	-0.37	3.10	-0.23	-0.19	-0.78	-0.47	0.12		0.02	-0.11	-0.41
14	-0.04	0.25	0.02	0.39	0.15	-0.29	-0.30	-0.06	-0.34	-0.08	0.29	0.02		-0.09	-0.72
15	-0.31	0.34	0.07	0.20	0.03	-0.41	-0.11	-0.11	-0.11	-0.02	0.38	-0.11	-0.09		-0.58
16	2.16	0.41	-0.55	0.00	-0.63	-0.47	-0.22	-0.20	-0.34	-0.29	0.20	-0.41	-0.72	-0.58	

Figure 14: High-throughput analysis of all possible Loss/Loss combinations provides a first hint on potential genetic interactions between chromosomes. Cellular fitness after the induced simultaneous loss of two different chromosomes in 96-well format. Cluster shows the average of an internal normalization from three independent experiments with all possible chromosome combinations. A color scheme was applied. Values higher than +1 are suspected positive genetic interactions (yellow), Values lower than -1 are suspected negative genetic interactions (blue). Chromosomes for which the single loss is lethal for the cell are highlighted in red. (Figure 11) Combinations picked for further investigations because of suspected positive genetic interactions that rescue a lethal chromosome loss are outlined in green.

3.2.2 The lethality of a single chromosome loss can rarely be rescued by the loss of a second chromosome

For the majority of lethal SL chromosomes that indicated to be rescued by the loss of another chromosome, only the combinations with one or two other chromosomes seemed to be beneficial. Therefore it was considered suspicious that for chromosome 10 many combinations seemed to provide the escape from lethality. The chromosome combinations chosen for further investigations because of especially high values are outlined in green (Figure 14).
In order define if clear genetic interactions were present, the more precise dilution series and a qPCR were done with the suspected combinations. For the combinations of the Loss/Loss of chromosomes 6 and 13 (LL 6x13), LL 1x7, and LL 3x7, clear benefits in cellular fitness compared to the lethal SL chromosome could be observed after the combinations with the second chromosome loss (Figure 15 A). These combinations could also be verified to have remained monosomic by qPCR (Figure 15 B). Therefore these loss combinations provided a strong hint on possible positive chromosome copy number interactions, which seemed to provide a selective advantage compared to other strains that had lost one of the monosomic lethal chromosomes.

For LL 1x10, qPCR results showed that the higher growth observed in the dilution series was due to recovered disomy of the lethal SL chromosome (Figure 15 A/B). This combination could therefore not be considered to have a positive genetic interaction. A theory why exactly this one stood out the most in our cluster could be that the simultaneous loss of chromosome 1 and 10 still shows a weak positive genetic interaction. This strain then has a higher chance to gain back the lost lethal chromosome before cell death than all the other combinations with chromosome 10, and therefore a higher probability to retain some of its ability to grow. This theory could be possibly tested in future experiments.

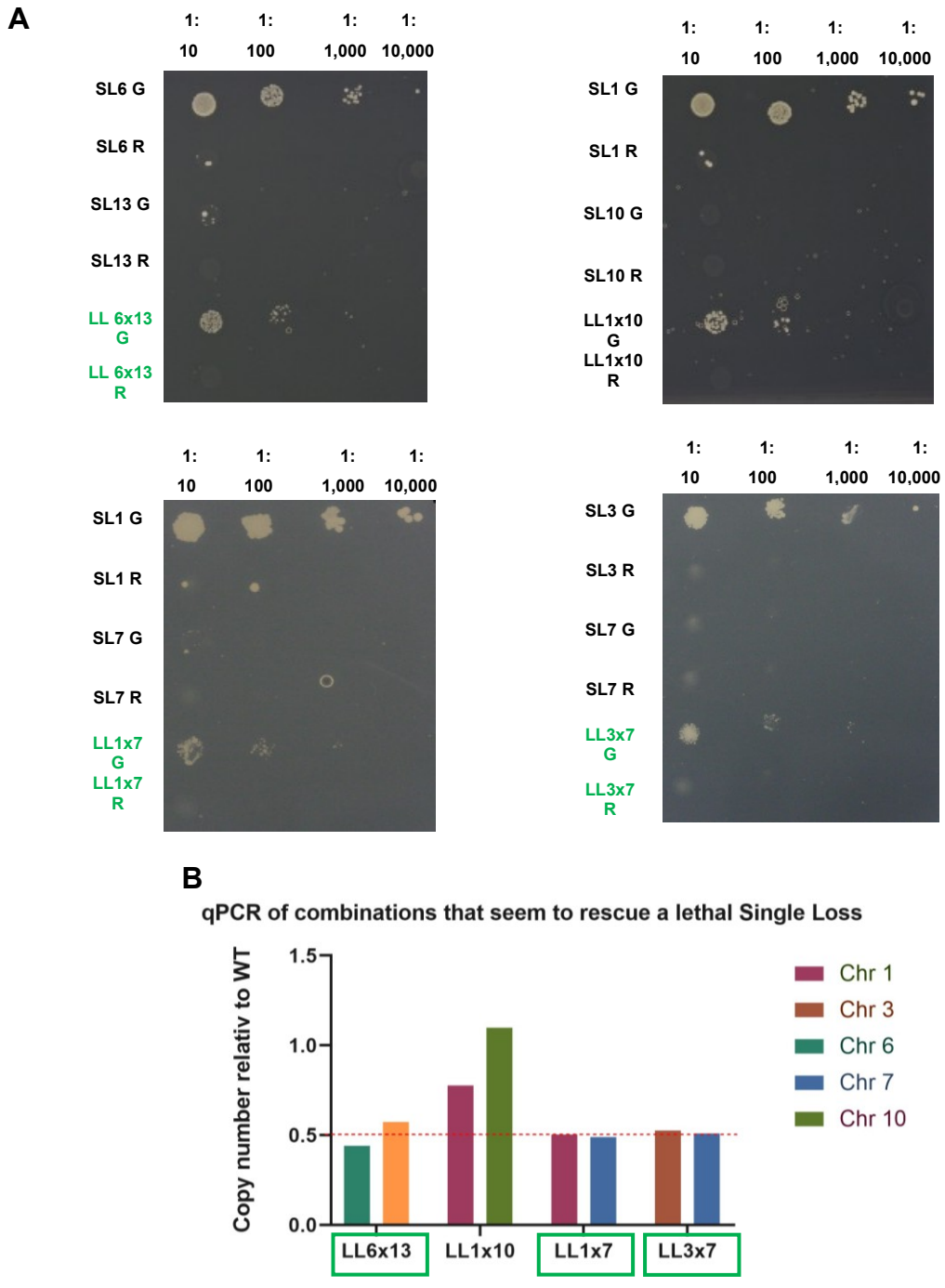


Figure 15: The lethality of a chromosome single loss can be occasionally rescued by the loss of a second chromosome. (A) Dilution series on FOA of suspected positive genetic interactions that rescue the lethal loss of a single chromosome outlined in Figure 14. The level of dilution in 1xPBS is written on top; lost chromosomes are written on the left of the pictures. G= Galactose treatment; R= Negative raffinose control; SL= Single Loss; LL = Loss/Loss. All pictures were taken after about 66 h, except for LL 1x7 (about 90 h). For this combination clear positive effect on cellular fitness can only be seen after a longer time on the selection plate. All LL samples shown to be monosomic in qPCR (B) are written in green letters. **(B)** qPCR of all suspected positive genetic interactions of lethal SL chromosomes. Chromosome copy numbers were normalized to a diploid WT and a disomic chromosome within each strain (chromosome 14). 0.5 = 1 chromosome copies; 1 = 2 chromosome copies. The red line indicates expected value for a monosomic chromosome. The average of both chromosome arms was calculated for every sample. Strains that could be confirmed to be monosomic for both required chromosomes are outlined in green.

3.2.3 Positive and negative genetic interactions between chromosome losses that are viable

Genetic interactions between chromosomes that do not lead to cell death when monosomic could also be observed in the high-throughput results of LL. A minimized version of the cluster from Figure 14 that only shows combinations of these viable monosomic chromosomes is shown in Figure 16. Combinations of some of these chromosomes seemed to indicate positive or negative genetic interactions through higher or lower cellular fitness than expected after internal normalization. For the combination of LL 1x3 a strong negative genetic interaction is implied, and for the combination of LL 1x16 a strong positive genetic interaction. Interestingly, for chromosome 9, genetic interactions with most other viable monosomic chromosomes could be observed.

For the combination of chromosomes 1x16, there was no difference in growth between the SL 16 strain and the strain with the simultaneous loss of chromosome 1 in the dilution series. qPCR showed monosomy for both chromosomes in LL 1x16, but solely monosomy for chromosome 16 in the SL16 strain. This excludes the possibility that the, compared to the size of the lost chromosome, unexpected cellular fitness of SL 16, was due to a general loss of chromosome 1 in monosomic chromosome 16 samples. It seems that the combination with the chromosome loss that leads to the least severe phenotype, which is chromosome 1, does have the least negative impact on the fitness of this strain and therefore makes it possible for LL 1x16 to be still barely alive. No positive genetic interaction between these chromosomes can be concluded.

As the other chromosome combinations, LL 1x3, LL 1x9, LL 3x9, LL 5x9 and LL 6x9 seemed promising to discover chromosome copy number interactions after the dilution series, these were picked for further investigations.

Chr	1	3	5	6	8	9	11	16
1		-2.05	0.86	1.84	0.09	2.47	-0.32	2.16
3			1.10	1.39	-0.26	1.55	0.23	-0.55
5				0.17	-0.63	-1.67	-0.29	-0.63
6					-0.77	-1.73	-0.47	-0.47
8						-0.48	0.01	-0.2021
9							-0.45	-0.3393
11								0.1993
16								

Figure 16: Chromosomes that do not lead to cell death as a SL can show positive or negative genetic interactions when combined with the loss of a second chromosome. Minimized cluster from Figure 14, only showing chromosomes for which SL is usually viable for the cell (Figure 11). Combinations that suggest a positive or negative genetic interaction between chromosomes and were picked for further investigations are outlined in green (suspected positive genetic interaction) or red (suspected negative genetic interaction).

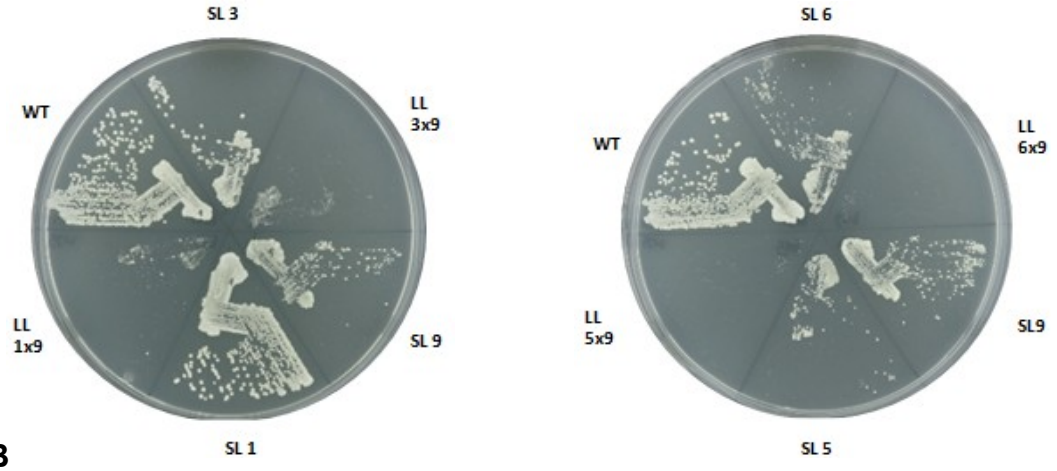
3.2.4 Clear genetic interactions cannot be stated for all suspected chromosome combinations

For all combinations that seemed to rescue a lethal SL due to a positive genetic interaction, as well as those combinations between viable SL chromosomes that seemed to show positive and negative genetic interactions, it was decided to use the initial method of single colony streak-out (Figure 10). Thereby cellular fitness could be determined more precisely. This could then confirm if a clear genetic interaction for these combinations was truly present or not. Examples for single colony streak-outs on SC plates can be observed in Figure 17 A.

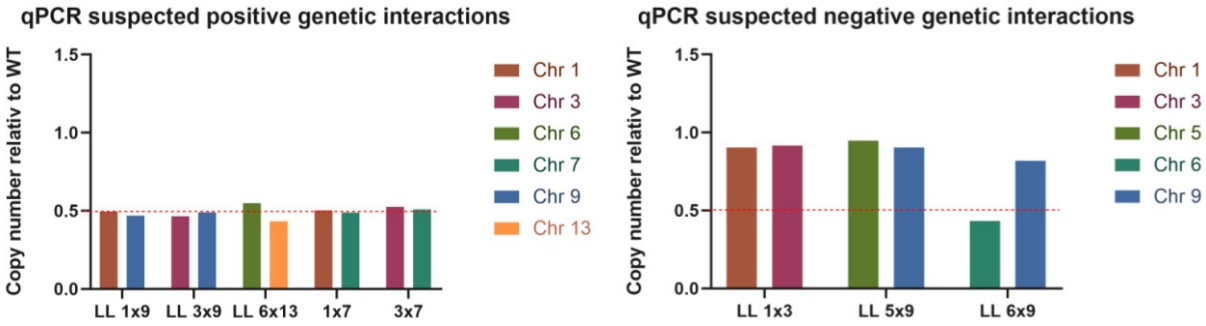
All combinations with suspected negative genetic interactions showed little to no growth on the selection plates. The negligible growth that could be observed of these induced strains was identical to the growth of their raffinose controls. Therefore for these strains a cellular fitness of 0% was stated. In addition, all suspected negative combinations appeared to have at least one of their two target chromosomes shown to be disomic by qPCR (Figure 17 B). All suspected positive genetic interactions showed significantly more growth in dilution series than their raffinose controls. After confirmation of monosomy by qPCR (Figure 17 B), the average colony areas of the suspected LL strains were normalized to WT. The results for suspected positive and negative genetic interactions could then be compared to the cellular fitness equation of the SL strains and interpreted (Figure 17 C/D). However, only the combinations of

LL 6x13, 1x7 and 3x7 seem to have a clear specific positive genetic interaction, whereas LL 1x3 is the only one showing clearly a negative genetic interaction when compared to the SL growth trend.

A



B



C

	Loss/Loss (Growth in %)	Sum of ORF
LL 1x9	9.3	358
LL 3x9	7.0	424
LL 6x13	10.8	644
LL 1x7	11.7	700
LL 3x7	9.3	766
LL 1x3	0.0	300
LL 5x9	0.0	564
LL 6x9	0.0	380

D

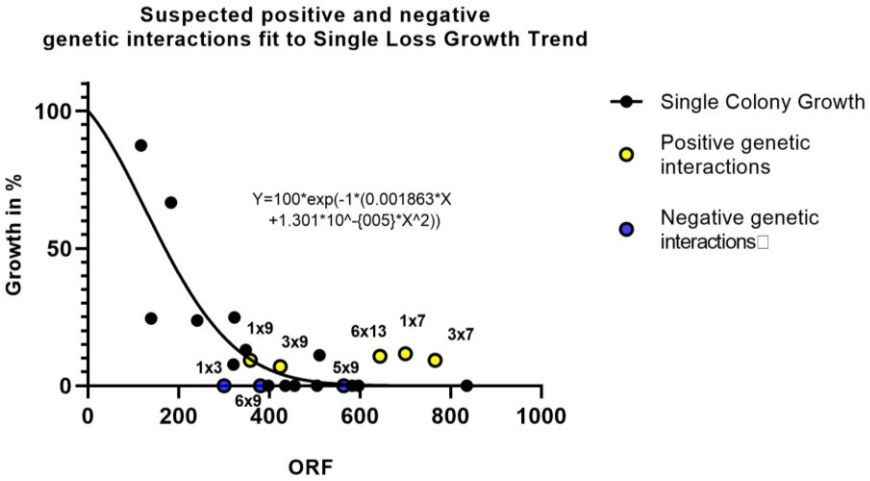


Figure 17: Quantification of Loss/Loss growth rates demonstrates positive and negative genetic interactions between aneuploid chromosomes. (A) Example of a streak-out for single colonies on SC for suspected positive genetic interactions (left) and suspected negative genetic interactions (right) after about 44 h. After confirmation of monosomy by qPCR, areas of the single colonies can be measured by Image J and compared to WT. **(B)** qPCR of strains streaked out from dilution series on FOA partly shown in Figure 17 A. The red line marks the value were chromosomes are monosomic. 0.5 = 1 copy number; 1.0= 2 copy numbers. The average of the left and right arm for each chromosome copy number is shown. **(C)** Table showing calculations of percentage growth measured through the area of single colonies with Image J and compared to a WT. The average area was calculated for every strain. Strains with chromosomes that showed equal growth as their negative raffinose controls appeared to be disomic in qPCR and were set to a growth of 0%. Suspected positive genetic interactions are marked in yellow, negative genetic interactions are marked in blue. **(D)** Calculated colony sizes in % from (C) fit to the SL growth curve. Suspected positive genetic interactions marked in yellow, suspected negative genetic interactions marked in blue. Number of ORF was calculated as the sum of the ORF of both chromosomes lost upon induction. Formula shows the linear quadratic equation of the SL fitness.

3.2.5 General genetic interactions mask specific genetic interactions in most Loss/Loss events

Colony sizes for LL were also compared directly to the sizes of their SL chromosomes, as well as to the expected values calculated by the product of the SL growth, and the expected value by the sum of lost ORF calculated with the linear quadratic equation (Figure 18). As it can be examined in the graphs, the measurement of a genetic interaction depends largely on the equation used. Nevertheless, the combinations of chromosomes 6x13, 1x7, 3x7, and 1x3 remain the only strongly indicated interactions.

A very strong general genetic effect, correlating mainly with the number of ORF on the missegregated chromosomes, can be observed in the growth trend of aneuploid strains. Especially in SL strains, this effect that we call the general genetic interactions, leads to cell death after most chromosome losses. Therefore, usually every combination of two simultaneous chromosome losses puts the number of active genes that are lost into a range already lethal for the cell. This makes it extremely difficult to measure specific positive genetic interactions, unless these are strong enough to overcome these general genetic interactions and prevent cell death. In addition, because of the highly detrimental effect of the general genetic interactions after the loss of two chromosomes, it is almost impossible to measure a clear specific negative genetic interaction. Therefore, we believe that general genetic interactions mask the existence of many more specific genetic interactions between chromosome losses.

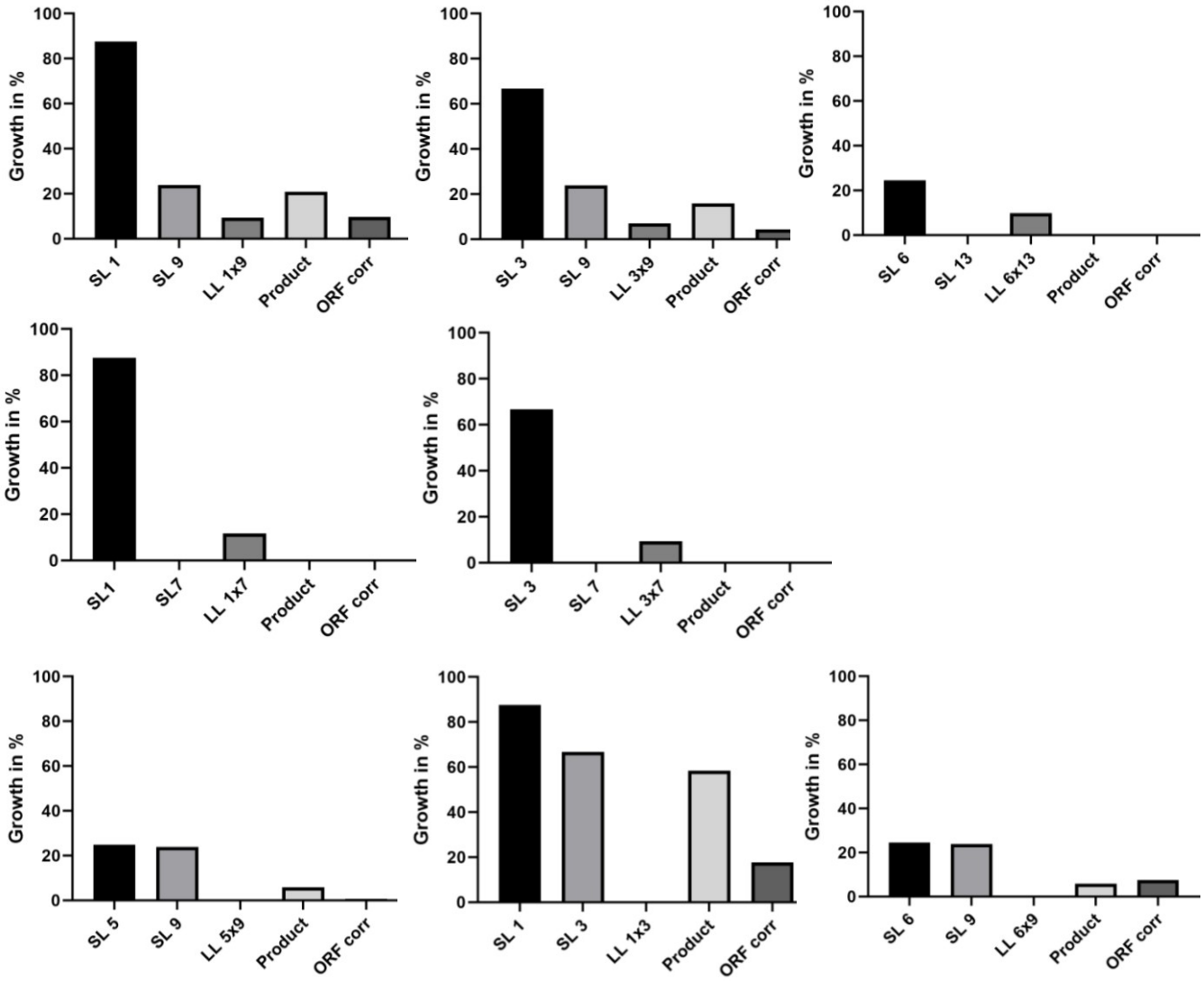


Figure 18: The appearance of a genetic interaction is largely dependent on the used equation to measure the presence of this interaction. Growth in % of Loss/Loss strains compared to their Single Loss strains, the expected growth calculated by the product of the SL, and the expected growth calculated by the sum of ORF of both chromosomes. Growth was calculated as the mean of single colony areas, measured by Image J, and then compared to a WT strain. Monosomy was verified by qPCR.

3.2.6 Lethality of Single Loss 13 can be rescued by TUB2-deletion

Next I wanted to investigate the genetic basis of the observed chromosome copy number interactions. A connection between chromosome 6 and 13 has already been discovered in the past. There, experiments showed that the gain of the single chromosome 6, which is harboring the TUB2 gene, is lethal to the yeast cell, due to an imbalance in protein amount compared to TUB1 and TUB3, which are on chromosome 13. As Anders et al. 2009 were able to show, the cells could be rescued by adding a plasmid containing an excess of the haploinsufficient gene TUB1 before

missegregation of chromosome 6 was induced.[8,27,34] Therefore, it could be expected that the detrimental effect after the loss of chromosome 13 is at least partially due to imbalance in this protein stoichiometry. I tested the loss of chromosome 13 by deleting a single copy of TUB2 before inducing. Whereas SL 13 is only viable after retaining disomy for chromosome 13, LL 6x13 monosomic for chromosomes 6 and 13, as well as SL 13 *tub2*Δ monosomic for chromosome 13, exhibit growth (Figure 19) It also stands out in Figure 19 D that the SL13 *tub2*Δ strain shows colony sizes about double as large compared to the LL 6x13 strain. This can be easily explained, as the loss of two chromosomes within a strain is expected to cause more stress and therefore less cellular fitness than the loss of only one chromosome.

This outcome demonstrated that the rescue of SL chromosome 13 by the simultaneous loss of chromosome 6 is due to the restoration of equal amounts of the tubulin protein subunits.

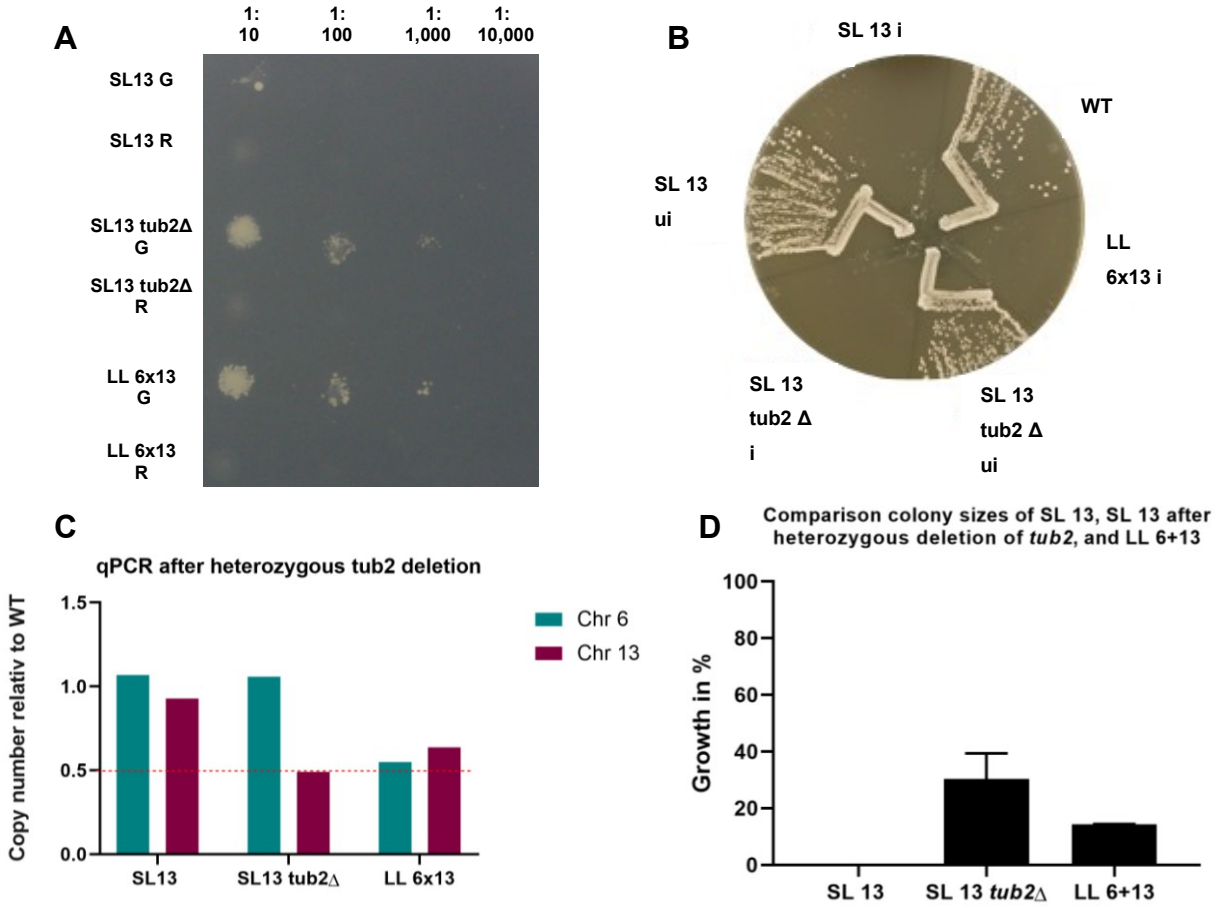


Figure 19: The positive genetic interaction between chromosomes 6 and 13 is based on the stoichiometry of the tubulin genes. (A) Dilution series on FOA after about 72 h. G = galactose sample; R = raffinose control. The grade of dilution in 1xPBS is depicted on top of the plate. **(B)** SC plate about 44 h after the streak-out for single colonies. i= induced strains; ui = uninduced strains. **(C)** qPCR results to confirm chromosomal copy numbers. 0.5 = 1 copy number; 1.0 = 2 copy numbers. Numbers were normalized to a WT and to the disomic chromosome 14. The average of both chromosome arms was calculated. qPCR results are from a single induction experiment. **(D)** Colony size of TUB2-deleted SL13 compared to LL6x13, induced SL13 and the uninduced strains. Percentage of growth was calculated by normalizing colony sizes to a WT strain (100%). Colony sizes were measured through average colony area by Image J. Numbers are from 2 independent experiments, pictures were taken after 44-48h on SC. Error bars depict the standard deviation (n=2). SL13 showed basically no growth in dilution series (0%) and was disomic for chromosome 13 according to the qPCR results shown in (C).

3.2.7 The positive genetic interactions observed between Loss/Loss 1 and 7 and Loss/Loss 3 and 7 could be based on interactions with the MLC1 gene on chromosome 7

One gene probably causing the interactions between LL 1x7 and LL 3x7 is a gene encoding for a myosin light chain of the protein myo2p, which is called MLC1. This gene is located on chromosome 7. The main function of the MLC1 protein is to drive bud formation during cytokinesis. Importantly MLC1 is the only essential haploinsuffcient gene in yeast. This means, that a diploid strain with only one copy of

MLC1 is not viable, which suggests that a loss of chromosome 7 could only be not lethal for the cell if the loss of MLC1 is compensated by the accompanied loss of another gene. Therefore it was searched for known MLC1 interaction partners on chromosomes 1 and 3. For identification of these genes the yeast genome database (SGD) was used. Thereby it could be assessed that the MYO4 gene on chromosome 1 would be an interesting candidate for a possible genetic interaction. Both the MLC1 and the MYO4 protein are part of the Myosin class V complex MYO4 variant. In addition, it was published that an additional copy of MLC1 is able to rescue toxicity of MYO4 overexpression. Though there have been no publications that MYO4 is able to rescue the haploinsufficiency of MLC1, Stevens and Davis have shown in 1998 that depleting one copy of the MYO2 gene is enough to rescue the lethal effect of the loss of one MLC1 copy. As MYO2 and MYO4 encode for very similar proteins, which both serve the same function to localize MLC1 to the bud cortex during cytokinesis, this highly suggests that a positive genetic interaction with MYO4 could be the cause of the SL7 rescue by the simultaneous loss of chromosome 1. [34–38]

We identified two genes on chromosome 3 that could possibly be the cause of the chromosome 7 loss by the loss of chromosome 3. One of these is SRO9, which is a protein that shuttles between the cytoplasm and the nucleus and plays a role in protein transcription and translation. Moreover SRO9 is another haploinsufficient gene that results in growth defects compared to WT when one copy is deleted. This protein shows physical interaction with MLC1. [34,39,40]

Another possible gene that could cause the positive genetic interaction between those two chromosomes could be KRR1. This protein takes part in ribosomal assembly, and is haploinsufficient and lethal after complete deletion. MLC1 and KRR1 also interact physically. [34,41] Future experiments are necessary to test these genes as the sources of the observed interactions.

For the negative genetic interaction observed between chromosomes 1 and 3 no potential explanation could be found up to now. However, as these chromosomes both take part in the before discussed positive genetic interactions, it is possible that the underlying genes for this negative genetic interactions are identical with the genes interacting positively with chromosome 7.

3.2.8 Predicted growth rates based on haploinsufficiencies on a chromosome partially correlate with the size of that chromosome

The positive genetic interaction between LL 6x13 was based on haploinsufficient genes balanced by the loss of another gene, and we suspect that the positive interactions observed in LL 1x7 and LL 3x7 could also be based on haploinsufficient genes. We then wanted to know if the loss of one copy of the haploinsufficient genes on a chromosome through missegregation could be partly the cause of the detrimental phenotype that can be observed in the SL. Therefore a list of haploinsufficient genes and their effect on cellular fitness was examined and the product of cellular fitness calculated for each chromosome.[34] As the essential haploinsufficient gene MLC1 was missing from that list, this one was added to the calculations and the product of chromosome 7 therefore 0%.[35]

The product of the cellular fitness for haploinsufficiencies generally correlates with the number of ORF on a chromosome, and therefore resembles the measured SL growth trend (Figure 20). This is most conspicuous in the chromosomes with less severe phenotypes (Chromosomes 1 and 3). Intriguingly, both the measured and predicted growth rates for chromosome 5 were far higher than for chromosome 8, despite chromosomes 5 and 8 both have a size of about 320 ORF. These could be hints that general genetic interactions depend to some part on haploinsufficient genes. Therefore the possible haploinsufficient background of strong positive specific genetic interactions between aneuploid chromosomes should be kept in mind for the identification of further chromosome copy number interactions in the future.

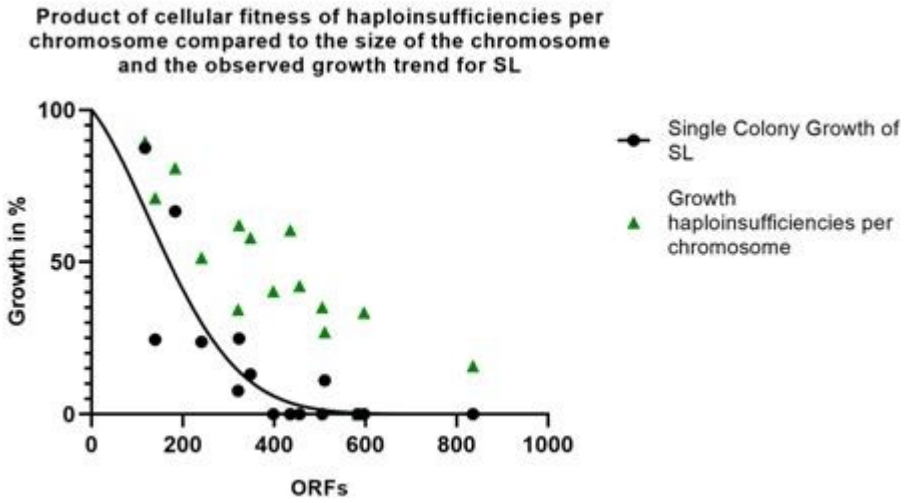


Figure 20: The cellular fitness product in haploinsufficiencies is related to the size of the chromosome harboring these genes and correlates partly with the observed growth trend in SL. Cellular fitness after deletion of one copy of a haploinsufficient gene was determined by Deutschbauer et al. 2005. [34] The product of cellular fitness of these genes was calculated for every chromosome and set into relation with the size of each chromosome. The only essential haploinsufficient gene MLC1 was added to the product of chromosome 7.

3.3 Diploid Single Gain strains do not show strong phenotypes for most chromosomes

As the gain of a single chromosome generally leads to less severe detrimental effects than single chromosome losses, we would expect to see a higher number of potential genetic interactions in the combinations of simultaneous chromosome gains. After the characterization of the Loss/Loss collection, I aimed to establish a high-throughput method by which the simultaneous gain of two chromosomes within one cell could be induced. First, diploid Single Gain (SG) collections were established. However, we noticed that the published trend regarding cellular fitness compared to size of the aneuploid chromosome was not reproduced for the SG strains. The outcomes suggested that the phenotypes of the gain strains seem generally not strong enough to recognize clear differences between most chromosomes with our technique. There it is important to point out that chromosome trisomy in diploid strains does not show a phenotype as severe as the phenotype for disomy in haploid strains.[7] Only for chromosome 6, which should not be viable after the gain of another copy and therefore have the most severe phenotype, the least cellular fitness could continuously be observed. As our goal was to induce the gain of two different chromosomes within a cell in order to observe chromosomal copy number

interactions, it was therefore decided to immediately continue with haploid Gain/Gain strains, for which a stronger effect and therefore clearer phenotypes were expected.

3.4 Induction of haploid Gain/Gain strains

3.4.1 Haploid Gain/ Gain strains can be analyzed in high-throughput when they are frozen in 96-well format after the induction

At first a haploid collection with every possible combination of two chromosome gains needed to be established. The selection for Gain/Gain (GG) requires the recombination-based excision of genes in 2 different chromosomes. Induced samples were only selected for one chromosome gain at first. This allows the cell to have more time for the second recombination event, and through this more cells should be able to survive on the selection medium. As the growth on -LYS/-LEU medium appeared to be usually a bit higher, this medium was chosen for the first selection. These samples were then finally selected for the URA3, HIS3, LYS2 and the LEU2 genes on a -All4 medium. For every chromosome gain combination, two different haploid strains were created, which contained the two different selection markers in one way and inversely. Thereby consistency in cellular fitness between the selection markers could be tested.

As the selection for the gains is continuous and therefore in general more stable than that for the losses the induced strains could be frozen in 96-well format. The complete experimental setup is shown in the flow chart in Figure 21. Reliability of the new haploid GG collection, as wells as consistency of the high-throughput method were confirmed by comparing to previous measurements of cellular fitnesses in our lab (8 Supplemental Experiments, Figure 26, Figure 27).

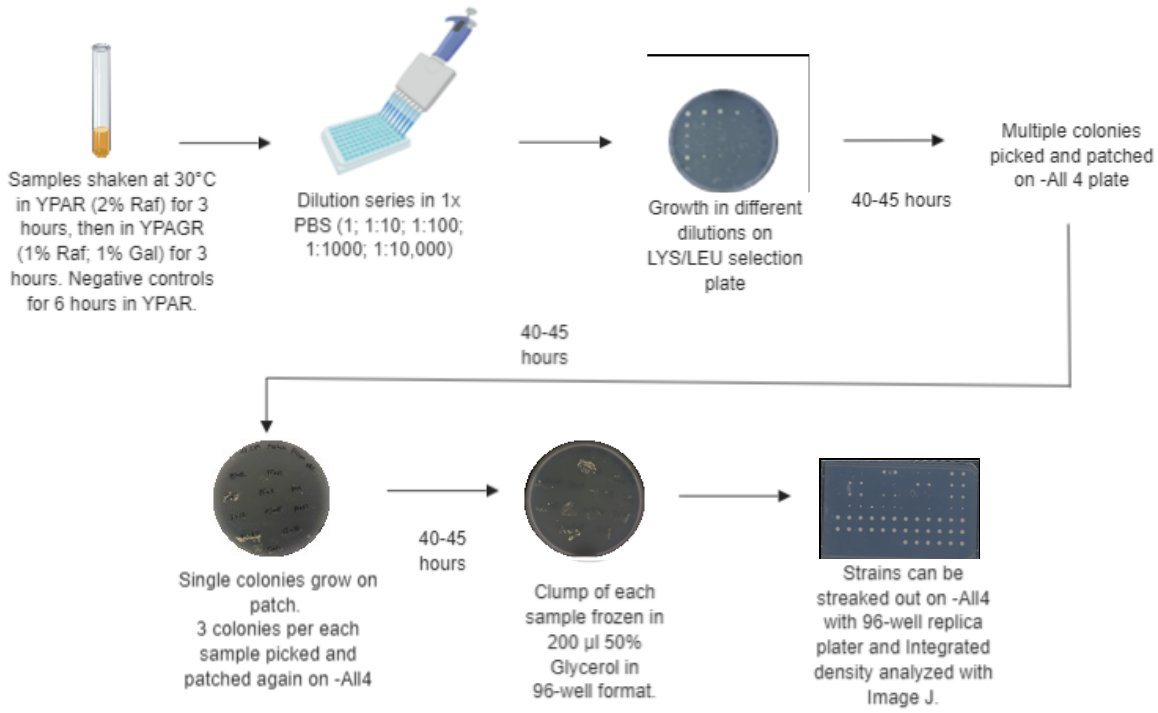


Figure 21: Haploid Gain/Gain strains can be analyzed High-throughput. Scheme of the method of choice for inducing haploid GG and analysis in high-throughput.

Based on the results of the LL strains, it is difficult to measure clear specific genetic interactions in samples where the cellular fitness is largely diminished by a general genetic effect (Figure 17, Figure 18). In addition, we would not expect to see a lot obvious genetic interactions between chromosomes that both show a very high cellular fitness as SG, especially not positive genetic interactions. As the goal at this point was to see as many indications for genetic interactions between chromosome gains as possible, it was therefore decided for the beginning to only induce GG in chromosomes that were published to lead to intermediate cell growth as a SG.[7] These are chromosomes 5, 7, 10, 12, 13, 14, 15, and 16.

If possible, 3 patches derived from single colonies were frozen per strain. If the raffinose control had grown as much as the galactose sample on the dilution series, those samples were not added to the double-disomic collection. For some strains no colonies could be frozen, mainly due to no growth on the first –All 4 selection plate. If this was observed for the selection in both ways of a chromosome combination, these were noted as being potentially synthetic lethal. Especially for all combinations with chromosome 15 it was difficult to obtain induced samples. Only the combination between chromosome 14 and chromosome 15 gain grew on –All4 and could be frozen. Therefore chromosome 15 is not included in the following analysis. All other

strains that did not grow after induction are listed in the Supplemental Experiments (8.2.3).

All strains that had been frozen were plated with the robot two times independently. An example for a plate containing the already induced GG strains can be examined in Figure 22 A. qPCR was done for six of these combinations. These were showing diverse growth between the different samples or, in case of the combination of chromosome 5 and 10, displaying conspicuously high growth and therefore wanted to be checked. Samples shown to be monosomic in qPCR were excluded from the calculations (Figure 22 B). Combinations that showed variability between the reversed selection markers are outlined in red (Figure 22 C). In Figure 22 D, the measured cellular fitnesses for chromosome combinations with the two selection markers alternately on both chromosomes are plotted against each other. This shows as in the cluster, that for most chromosome combinations the numbers between the reversed selections seem to be consistent. The general growth trend was as expected, cellular fitness is influenced strongly by the size of the gained chromosomes. Based on this, I identified a possible strong negative genetic interaction between the chromosomes 10 and 12 (Figure 22 E). However, weaker genetic interactions will be more accurately identified after the complete collection has been tested.

In conclusion, it can be said that the new system that was developed for high-throughput analysis of haploid GG shows reproducible results and already indicates for one strong negative genetic interaction. Therefore it seems promising to reveal more positive or negative genetic interactions between haploid GG strains in the future.

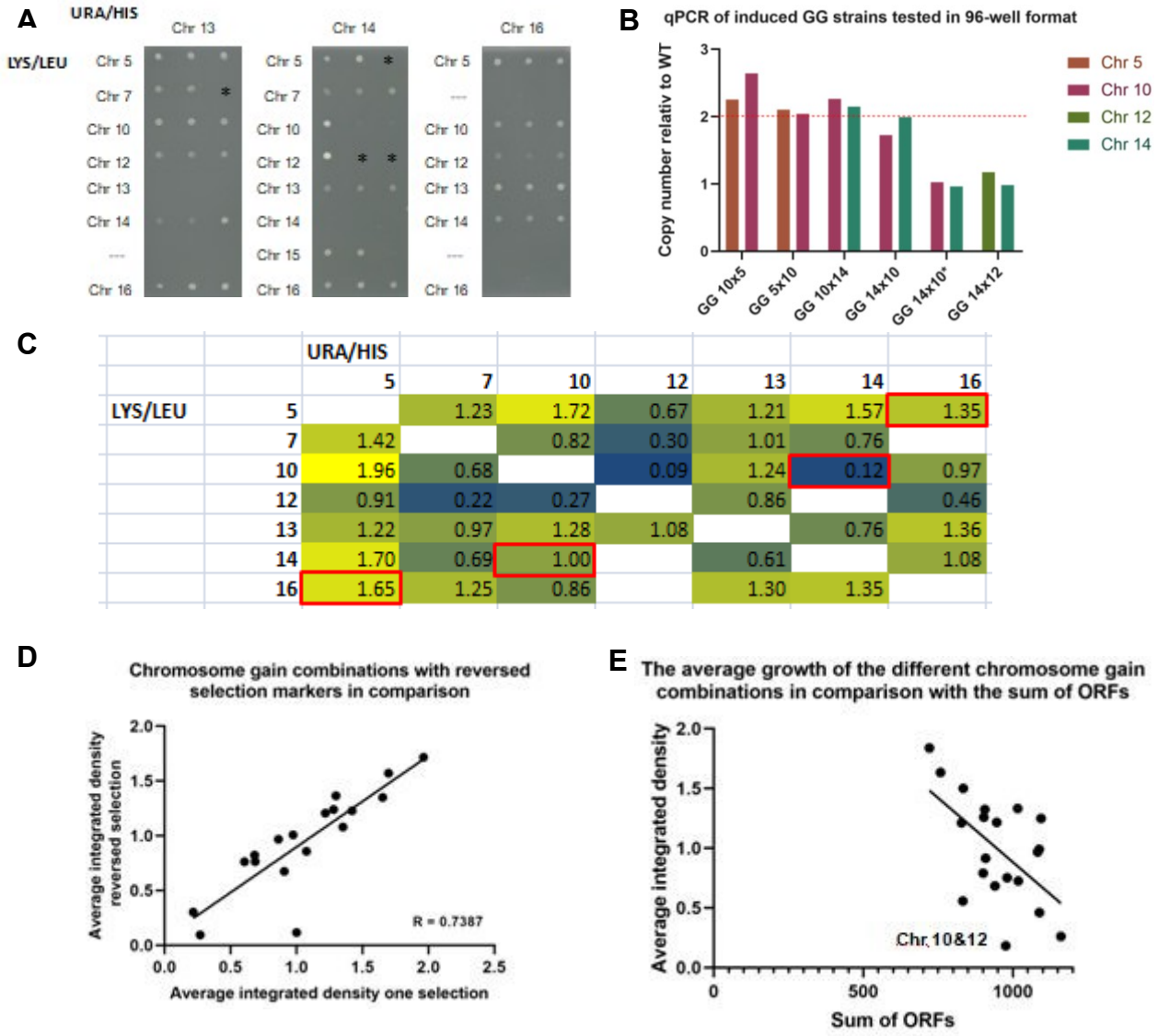


Figure 22: Analysis of haploid GG in high-throughput leads to reproducible numbers and indications of genetic interactions. (A) Example for –All4 selection plate with already induced GG, 26 h after plating by the robot. Spots marked by an asterisk are empty, for these strains it was not possible to freeze 3 colonies because of rare growth on the selection plates after the induction. **(B)** qPCR of GG strains that showed very diverse growth between the 3 independent samples. GG 5x10 and GG 10x5 have been tested due to very large growth and not because of inconsistencies. Copy numbers were normalized to a haploid WT and compared to the monosomic chromosome 9. The red line indicates the expected value for disomy.GG 14x10 = spots with only little growth in (A), GG 14x10* = large spot in (A). **(C)** Cluster showing the average values of already induced GG plated by the robot in 2 independent experiments. Measurements were done after 25-26 h. Raw values for integrated intensity measured by Image J were reduced by the background density, the average of the 3 samples per strain was calculated and divided by the average intensity measured on one plate. No internal normalization was done. A color scale was applied: Yellow = high values; Green = intermediate values; Blue = low levels. Red frames outline inconsistency higher than a difference of 0.3 of integrated density between the reversed selection markers. **(D)** Chromosomal combinations with reverse selection markers plotted against each other. **(E)** Graph depicting the growth trend for the average of each chromosomal combination set into relationship to the sum of ORFs. Chromosomes 10 and 12 are labeled because of possible hint on negative genetic interaction.

4 Discussion

4.1 Chromosome loss in diploid yeast strains

When inducing the simultaneous loss of 2 chromosomes, a number of different benefits and downsides can be encountered in these experiments. First of all, the FOA selection system is in general a very reliable system to analyze cellular fitness after single colony streak-out, as well as in high-throughput. On the other hand, monosomy of many chromosomes is not very stable. Already two days after the induction, growth due to the regaining of a lost chromosome could be observed frequently (Figure 11 A). The comparison between the galactose sample and the raffinose control on the dilution series thereby provided a good tool to identify lethal loss chromosomes that only grew because they became disomic again. These usually had about the same growth as their negative raffinose controls. An exception from these observations was the loss of chromosome 12, which showed much higher growth than its raffinose control after retaining disomy. It therefore had to be excluded from further investigations.

4.2 Specific genetic interactions are often masked by the stronger general genetic interactions after chromosome loss

For a correct interpretation of the LL results it is important to differentiate between two different types of genetic interactions that we observed in our results, the general and the specific genetic interactions. First, as it can be examined in Figure 11, the synergy between the size of a chromosome and the cellular fitness of the strain after this chromosome was lost is not linear. This suggests that not only the number of ORF is substantial, but that there are some genetic interactions within chromosomes as well that partly determine the cellular fitness of an aneuploid cell. We termed these interactions the general genetic interactions. We found that the relationship between chromosome size and the aneuploid phenotype is best fit with a linear quadratic equation (Figure 11 B). How strong these general interactions are can also be observed in Figure 11 B. Whereas cells that lose a chromosome with about 200 ORF still have a cellular fitness about 50% of WT, the increase to a loss of 400 ORF leads, with some exceptions, to the death of the cell.

In addition, when it comes to multiple chromosome aneuploidies, there is another factor that influences the phenotype of the cell. This is what we call chromosome copy number interactions, which are genetic interactions between specific genes on different chromosomes. These can either have a beneficial or a detrimental phenotypic effect. The topic of this thesis was to investigate the frequency and genetic causes of these chromosome copy number interactions. Four such specific genetic interactions were identified between chromosome losses. We would expect a lot more of these genetic interactions to exist, but apparently the general effect we have on the cell's fitness after the chromosome losses leads to a detrimental effect so strong, that it masks most of the weaker specific genetic interactions. Especially the discovery of negative genetic interactions is impossible starting from a certain amount of lost ORF, as this means the death for basically every combination. A positive genetic interaction can only be identified if it is strong enough to partly overcome the general genetic effects.

However, I was able to discover three apparently very strong positive genetic interactions between LL 6x13, LL 1x7 and LL 3x7. In addition, a strong negative genetic interaction is indicated between the two chromosomes with the least severe phenotypes as SL, chromosomes 1 and 3 (Figure 17).

4.3 The observed positive genetic interactions between chromosome losses are suspected to be based on the rescue of haploinsufficient genes

The simultaneous loss of chromosome 6 was found to be able to rescue the lethality of SL13. By deleting the TUB2 gene on chromosome 6 of the SL13 strain, before missegregation of chromosome 13 was induced, it was possible to restore the growth of this strain (Figure 19). This demonstrated that the observed positive genetic interaction between LL 6x13 is based on the rebalanced stoichiometry of the haploinsufficient TUB1 and TUB3 genes on chromosome 13.

The positive genetic interactions between LL 1x7 and LL 3x7 also appear to be very strong, as they are able to overcome the general genetic interactions on the chromosome losses. For these interactions, it is suspected that the loss of the MLC1 gene on chromosome 7, which is the only known essential haploinsufficient gene in

yeast, might be balanced by the simultaneous loss of interacting genes on chromosomes 1 and 3.

As haploinsufficient genes seem to have a great impact on the cellular fitness after chromosome loss, we wondered if haploinsufficient genes in general could be the main basis of the observed SL growth trend. By calculating the product of cellular fitness effects of haploinsufficiencies for every chromosome, a growth trend correlating with the size of the chromosome could be examined similar to the growth trend in chromosome aneuploidies.[34] Although this can be basically caused by the higher chance of a large chromosome to contain more haploinsufficient genes, the fact that the chromosome loss growth rate partially correlated with the predicted growth based on haploinsufficient genes should be kept in mind in future investigations.

For the negative genetic interaction observed between chromosomes 1 and 3, no potential genetic background could be found up to now.

4.4 Chromosome gain in haploid yeast strains

As the gain of a single chromosome does not have effects as severe on cellular fitness as the loss of a chromosome, it was expected to discover more genetic interactions in haploid GG strains.[7] The gain of a specific chromosome also shows different advantages as well as disadvantages in terms of the selection and analysis. In these strains, the risk that chromosomes regain their initial copy number was not as present as in the losses, because of a continuous selection on –All 4 plates. However, strains that were able to grow on the selection plates, although they had not gained another copy of the required chromosomes, could still be observed occasionally. Unfortunately we do not know how these cells manage to grow on selection medium with monosomic chromosomes. One downside when selecting for a gain event is also the recombination-based excision that only happens at a frequency of about 1:1,000; which made it impossible to use a high-throughput method as simple as for chromosome losses. The main issue there was to transfer enough cells to the plate to be able to see growth of the samples. After numerous unsuccessful attempts, a system to investigate on different chromosomal combinations in a short time could finally be developed.

4.5 First results for Gain/Gains are largely reproducible and show first hints on possible genetic interactions

As it was expected to observe the most specific genetic interactions in chromosome combinations that show intermediate cellular fitness as SG, investigations were started with these. Initially chromosome 15 was planned to be included in the experiments. However, it was soon noticed that it was extremely difficult to freeze combinations with this chromosome, as these strains usually showed no growth at all after they had been applied to the - All 4 selection plate. In addition, some other chromosomal combinations could not been frozen down. These combinations are suspected to be synthetically lethal and listed in chapter 8.2.3.

The observed cellular fitness for the induced samples is largely reproducible, based on the strains with reversed selection markers. No internal normalization was done, as not enough combinations have been measured up to now. Nevertheless, it can be stated that the cellular fitness of the strains correlates strongly with the sum of the ORFs of the gained chromosomes (Figure 22 E). However, the observation of this correlation plot already suggests one possible chromosome copy number interaction. There seems to be a strong negative genetic interaction between the gain of the chromosomes 10 and 12. The number of tested combinations is though still too small for reliable interpretation of weaker genetic interactions.

4.6 Relevance of chromosome copy number interactions and future ideas for this project

In this thesis, specific aneuploidies between different chromosome combinations were induced and the cellular fitness of these yeast strains was determined. This was shown to be an effective tool to investigate chromosome copy number interactions and their underlying specific genes.

Specific aneuploid karyotypes occur frequently in tumours. Chromosome copy number interactions in these specific karyotypes are suspected to partly provide the selective advantage observed in aneuploid cancer cells.[26] Therefore, the identification of the basis of this phenomenon can contribute to understanding the behaviour of these cells, as well as provide new ideas for more effective treatment of cancer in the future. Further steps in this project would be to induce the entire GG

collection; which will then reveal how frequent chromosome copy number interactions occur, identify the genetic background of observed interactions, as well as search for human homologues of the discovered genes to test these findings also in human cells.

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6 Abstract

Aneuploidy is occurring frequently in solid tumours. Although it has been shown in numerous studies that the state of having an abnormal number of chromosomal copies has usually a detrimental effect on cells of different organisms, cancer cells seem to gain a selective advantage through their aneuploid state. This is believed to be partly due to chromosomal copy number interactions, which is the topic of this thesis. In our lab, we are using budding yeast (Saccharomyces cerevisiae) as a model organism to investigate the underlying genetic background of selective growth advantage through the induction of specific chromosomal gains and losses. One positive genetic interaction between chromosomes 6 and 13 has already been published. This interaction is based on the stoichiometry between the subunits of the protein tubulin. In addition a negative genetic interaction between the chromosomes 8 and 10 has been discovered previously in our lab. However, the genetic background of this interaction is still unknown. Apart from these findings, no results on chromosomal copy number interactions have been published so far.

Nevertheless, Beach et al. 2017 investigated the effect of induced aneuploidies of specific chromosomes on cellular fitness. There it could be observed that the cellular fitness after a specific chromosome loss or gain correlates strongly with the size of the missegregated chromosome. This relationship is not completely linear, which suggests that there are also interactions within a chromosome, which have influence on the cellular fitness.

The goal of this thesis was to investigate interactions between different aneuploid chromosomes. Therefore the missegregation of two different chromosomes within a cell was induced by using a conditional centromere. The effects of the simultaneous loss or gain of two chromosomes; or the gain of one chromosome whereas another chromosome is lost; on cellular fitness could then be observed. Hence possible underlying genetic interactions between specific chromosomes could be indicated.

In order to investigate the simultaneous loss of two different chromosomes in every possible combination, a High-throughput system was developed. Cellular fitness of these strains could be measured and then internally normalized. After further investigations on thereby illustrated possible genetic interactions, four different chromosome combinations could be concluded to show a strong indication on a genetic interaction. The combinations of chromosomes 6x13, 1x7 and 3x7 indicated

strong positive genetic interactions, whereas the combination of chromosomes 1x3 suggested a strong negative genetic interaction. For the Loss/Loss 6x13 strains it could be proofed that the observed interaction is caused by the subunits of the tubulin protein, as already described in literature likewise for the gain of these two genes. For the combinations with chromosome 7, it is assumed that these may be based on interactions with the MLC1 gene on this chromosome. MLC1 encodes a myosin light chain protein, which is the only essential haploinsufficient protein in yeast that has been shown up to now. Therefore it is very likely that the observed interactions are connected to this gene.

As the gain of a chromosome is in general not as detrimental for the cell as a loss, it was expected to see more possible genetic interactions after the simultaneous gain of two chromosomes. Therefore a different High-throughput system was developed in order to establish haploid yeast strains with two disomic chromosomes. Up to now, experiments on only a few combinations of chromosomes that lead to intermediate colony growth as a single disomy have been done. The first results here seem promising that this system will make it possible to discover more genetic interactions with these strains.

To sum up, our investigations on chromosome copy number interactions seem feasible to discover the underlying genetic background of selective advantage or disadvantage of an aneuploid yeast cell. Thereby identified genes could then be used to detect the genetic basis of the observed growth advantage in aneuploid human cancer cells. This can be an important step for understanding part of the behaviour of aneuploid tumours.

Keywords: Aneuploidy, cancer, chromosome copy number interactions, yeast

7 Deutsches Abstract

Aneuploidie kommt häufig in soliden Tumoren vor. Obwohl in mehreren Studien gezeigt werden konnte, dass eine anormale Anzahl an Chromosomenkopien generell einen destruktiven Einfluss auf die Zellen verschiedener Organismen hat, scheinen Krebszellen durch ihren aneuploiden Status einen selektiven Vorteil zu gewinnen. Es wird vermutet, dass dieses Phänomen teilweise aufgrund von Interaktionen zwischen unterschiedlichen Chromosomenkopien auftritt, welche das Thema dieser Masterarbeit sind. In unserem Labor wird Bäckerhefe (Saccharomyces cerevisiae) als Modellorganismus verwendet, um den genetischen Hintergrund des selektiven Wachstumsvorteils durch Gewinn oder Verlust von spezifischen Chromosomen zu untersuchen. Eine positive genetische Interaktion zwischen den Chromosomen 6 und 13 ist bereits aus der Literatur bekannt. Diese basiert auf der Stöchiometrie zwischen den Untereinheiten des Proteins Tubulin. Eine negative genetische Interaktion zwischen den Chromosomen 8 und 10 wurde in unserem Labor entdeckt, wobei der genetische Hintergrund dieser Interaktion noch unklar ist. Abgesehen von diesen Erkenntnissen, wurden bis jetzt noch keine Ergebnisse über Interaktionen der Kopien verschiedener Chromosomen publiziert.

Beach et al. 2017 hingegen untersuchte den Effekt von induzierter Aneuploidie in jeweils einem bestimmten Chromosom auf die zelluläre Fitness. In dieser Studie konnte beobachtet werden, dass die zelluläre Fitness nach dem Gewinn oder Verlust eines spezifischen Chromosoms stark mit der Größe dieses fehlerhaft segregierten Chromosoms korreliert. Dieser Zusammenhang ist allerdings nicht vollständig linear, was vermuten lässt, dass es Interaktionen innerhalb eines Chromosoms gibt, welche ebenfalls Einfluss auf die zelluläre Fitness haben.

Das Ziel dieser Masterarbeit war, die Interaktionen zwischen verschiedenen aneuploiden Chromosomen zu untersuchen. Dafür wurde die fehlerhafte Teilung von zwei verschiedenen Chromosomen innerhalb einer Zelle, durch Verwendung eines kontrollierbaren Centromers, induziert. Der Effekt von dem gleichzeitigen Verlust oder Gewinn von zwei Chromosomen, oder der des gleichzeitige Gewinns von einem Chromosom während ein anderes verloren wird, auf die zelluläre Fitness konnte beobachtet werden. Daraus konnten mögliche zugrundeliegende genetische Interaktionen zwischen bestimmten Chromosomen vermutet werden.

Um den gleichzeitigen Verlust von zwei verschieden Chromosomen in jeder möglichen Kombination untersuchen zu können, wurde ein „High-throughput“ System entwickelt. Die zelluläre Fitness dieser Stämme konnte dann bestimmt und normalisiert werden. Nach weiteren Untersuchen von dadurch aufgezeigten möglichen genetischen Interaktionen, konnte geschlussfolgert werden, dass vier verschiedene Kombinationen von Chromosomen auf eine starke genetische Interaktion hinweisen. Die Kombinationen von den Chromosomen 6x13, 1x7 und 3x7 lassen eine starke positive genetische Interaktion vermuten, wohingegen die Kombination von den Chromosomen 1x3 auf eine starke negative Interaktion hinweisen lassen. Für den gleichzeitigen Verlust von Chromosom 6 und 13 konnte bewiesen werden, dass die beobachtete Interaktion von den Untereinheiten des Proteins Tubulin verursacht wird, so wie es bereits in der Literatur für den Gewinn beider Gene beschrieben wurde. Die Kombinationen mit Chromosom 7 basieren möglicherweise auf Interaktionen des MLC1 Gens auf diesem Chromosom. Das MLC1 Gen codiert für ein Myosin-leichte-Ketten Protein, welches das einzige essentielle haploinsuffiziente Protein in Hefe ist, das bis jetzt gefunden wurde. Deshalb ist es sehr wahrscheinlich, dass die beobachteten Interaktionen mit diesem Gen zusammenhängen.

Da der Gewinn eines Chromosoms im Allgemeinen nicht so destruktiv ist wie der Verlust eines Chromosoms, wurde erwartet mehr mögliche genetische Interaktionen nach dem gleichzeitigen Gewinn von zwei Chromosomen sehen zu können. Deshalb wurde noch ein „High-throughput“ System entwickelt um haploide Stämme mit Disomie von zwei Chromosomen zu etablieren. Bisher wurden Experimente nur an ein paar Kombinationen durchgeführt, mit Chromosomen die bei alleiniger Disomie mittleres Wachstum zeigten verglichen mit dem Wild typ zeigen. Die ersten Ergebnisse hierbei wirken vielversprechend, dass es möglich sein wird noch weitere genetische Interaktionen mit diesen Stämmen zu entdecken.

Zusammenfassend kann gesagt werden, dass unsere Untersuchungen zu den Interaktionen verschiedener Chromosomenkopien praktikabel scheinen, den zugrundeliegenden genetischen Hintergrund von selektiven Vor- und Nachteilen aneuploider Hefezellen zu ermitteln. Die hierbei entdeckten Gene könnten in Zukunft verwendet werden um die genetische Basis von aneuploiden humanen Krebszellen

zu identifizieren. Dies kann ein wichtiger Schritt sein, um das Verhalten von aneuploiden Tumoren teilweise zu verstehen.

Schlüsselwörter: Krebs, Aneuploidie, Interaktionen von Chromosomenkopien, Hefe

8 Supplemental Experiments

8.1 Induction of Loss events

8.1.1 The fate of an individual colony after missegregation is not representative for the entire population

Initially, a single colony for each sample was picked about 2 days after the induction and dilution series on FOA. These were then streaked out on YPAD, and measurements of colony areas could be done with Image J (Figure 23 A). However, it was early noticed that the streak-out for single colonies did not lead to reproducible numbers in all chromosome losses. Especially for chromosome 3 it seemed like cellular fitness had reduced significantly after the streak-out on YPAD (Figure 23 B).

We tested two different possibilities that could be the cause of this phenomenon. On the one hand, this lack of reproducibility for the losses of different chromosomes could be due to some media-specific preference developed by the cells after one copy of a specific chromosome was lost. On the other hand, the picking of only one individual colony from the selection plate for a streak-out on the following plate could lead to outcomes not representative for the entire population due to heterogeneity after increased chromosomal instability.

In order to investigate this issue, the growth after missegregation was tested on different media. In this experiment no single colony streak-out was done. Multiple colonies were picked concurrently from the FOA selection plate and suspended in 1xPBS. Dilution series were made with the mixed colonies; these were then transferred to the different plates to be tested (Figure 24 A). The second dilution series was done in this experiment in order to test the exact same colonies on every media, whereas with picking and direct streak-out of a bunch of individual colonies on every plate heterogeneity could have caused a problem again.

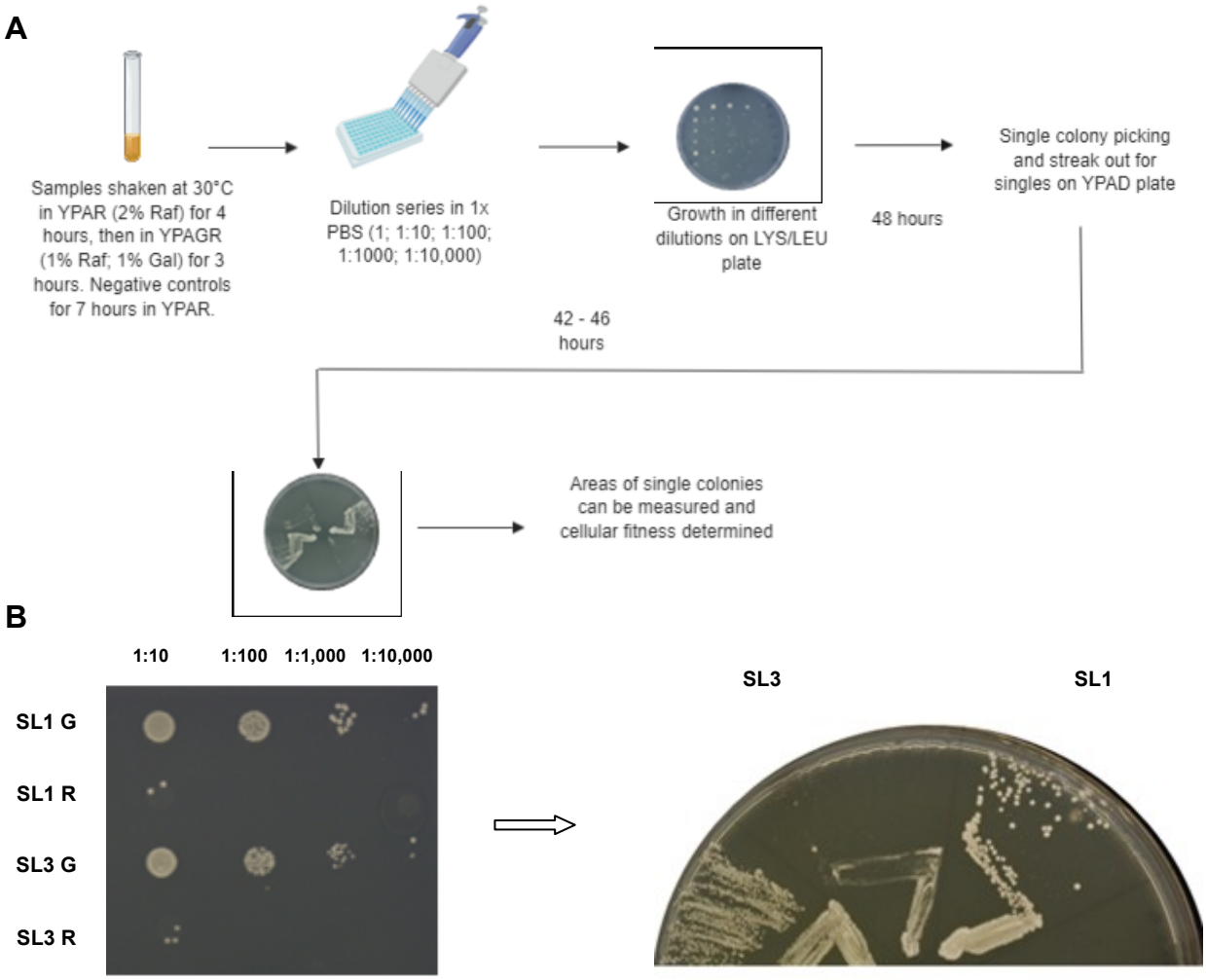


Figure 23: Streak-out for single colonies with the initial method does not lead to reproducible results of cellular fitness. (A) Scheme of experimental design for measuring single colony sizes after missegregation. **(B)** A FOA plate (left) with SL Chromosome 1 (SL 1) and SL Chromosome 3 (SL 3) in different dilutions after about 48 hours (G= galactose treatment; R= raffinose control). The arrow points to the YPAD plate (right) where a SL 1 and a SL 3 colony (galactose treatment) were picked from the previous FOA plate and streaked out for single colonies. Picture of the YPAD plate was taken after about 44h.

From the media that were tested, the results on synthetic complete (SC) seemed to resemble to the ones published by Beach et al. the most. However, different media ingredients seem not to be the main issue for receiving unstable growth numbers (Figure 24 B/C). The picking of multiple colonies instead of a single colony seemed to have restored the reproducibility in growth rates of all samples. We concluded therefore, that in some cases single colonies can show heterogeneity and have an individual fate after the SL of one chromosome.

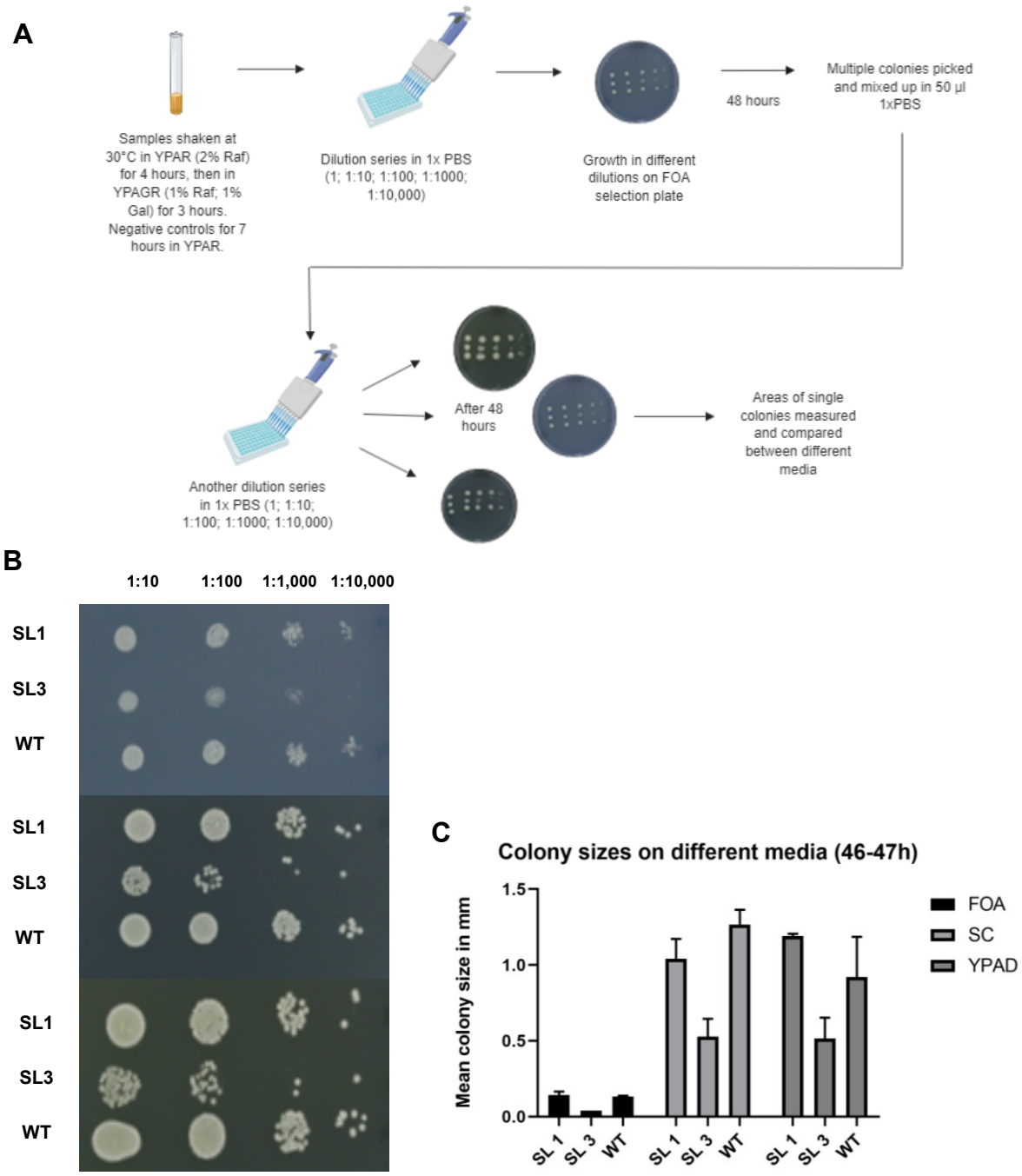


Figure 24: Colony growth after missegregation is not influenced by plate ingredients, but heterogeneity between different colonies within an aneuploid population can lead to irreproducibility with single colony picking. (A) Scheme of the experimental design to investigate the roles of plate ingredients and single vs. multiple colony picking on cellular fitness outcomes after missegregation. (B) Dilution series of SL1, SL3 and a WT on a FOA plate (top), a SC (synthetic complete) plate (middle) and an YPAD plate (bottom) after about 46-47h. These are pictures from the second dilution series after the first selection on FOA (see (A)). (C) Colony sizes from the average colony growth on the different plates shown in (B) in 2 independent experiments. The area of single colonies in the 3 highest dilutions (1:100; 1:1,000; 1:10,000) was measured with Image J and the mean colony size per each sample calculated. Error bars show the standard deviation between the 2 experiments.

8.1.2 Variable cellular fitness after the SL of chromosome 3 is not due to tetraploidy

On the right arm of chromosome 3 lies the MAT-locus, which determines the mating type in a haploid yeast cell. In a diploid cell, one of the two copies of chromosomes 3

carries a Mat-a allele, whereas the other copy of chromosome 3 carries the allele of a Mat-alpha type.[31] As in our SL 3 cells, one Mat-locus is lost with losing a copy of chromosome 3, these cells could be theoretically free to mate again with each other, and thereby creating a tetraploid cell containing two copies of chromosome 3. This could then not be detected by qPCR, as the ratio between chromosome 3 and a correctly segregated control chromosome would still be 1:2. To investigate this theory, Fluorescence-activated Cell Sorting was performed after the induction of missegregation, with the cells grown for two days on SC after FOA selection. By comparing the SL 3 strain to a haploid and a diploid WT strain, these experiments showed that these cells remained diploid after chromosome 3 missegregation, Although cells were not synchronized and therefore in different states of cell division, the similarity in DNA content between SL 3 and the diploid WT can be clearly observed (Figure 25). This theory could therefore not explain the variability in growth that was observed in SL 3.

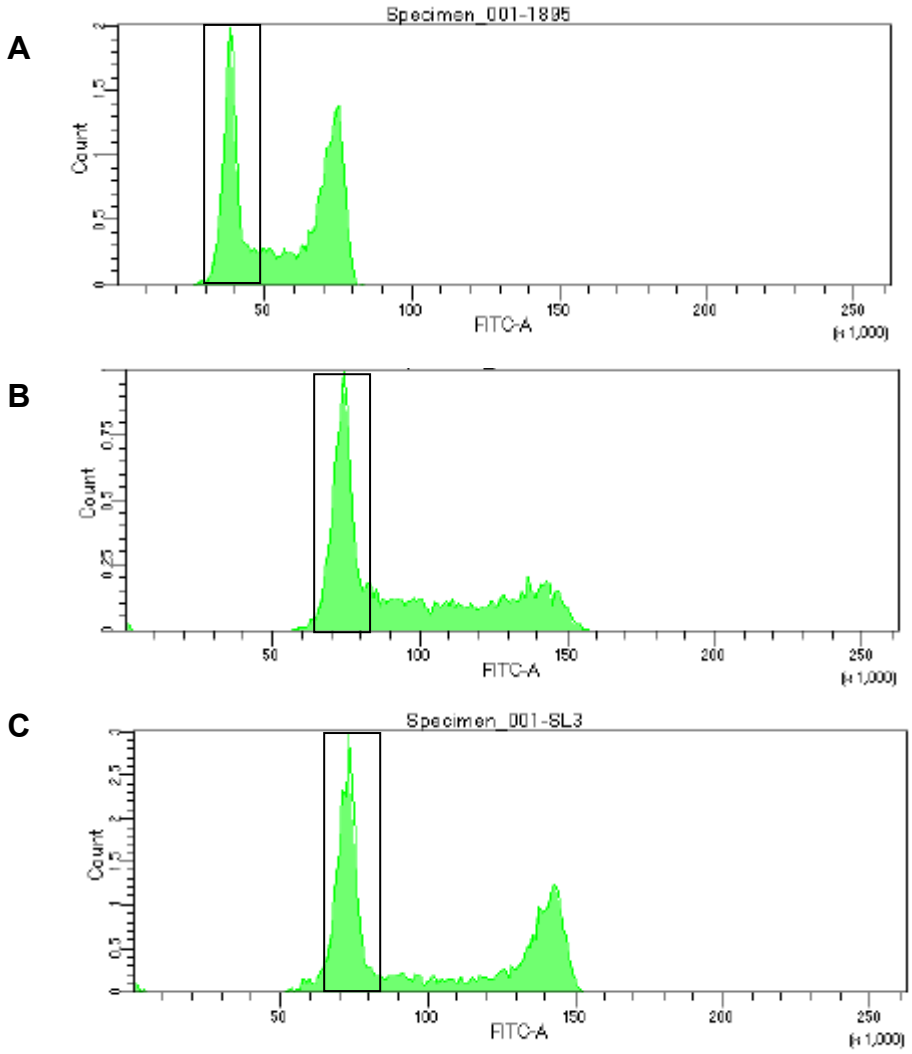


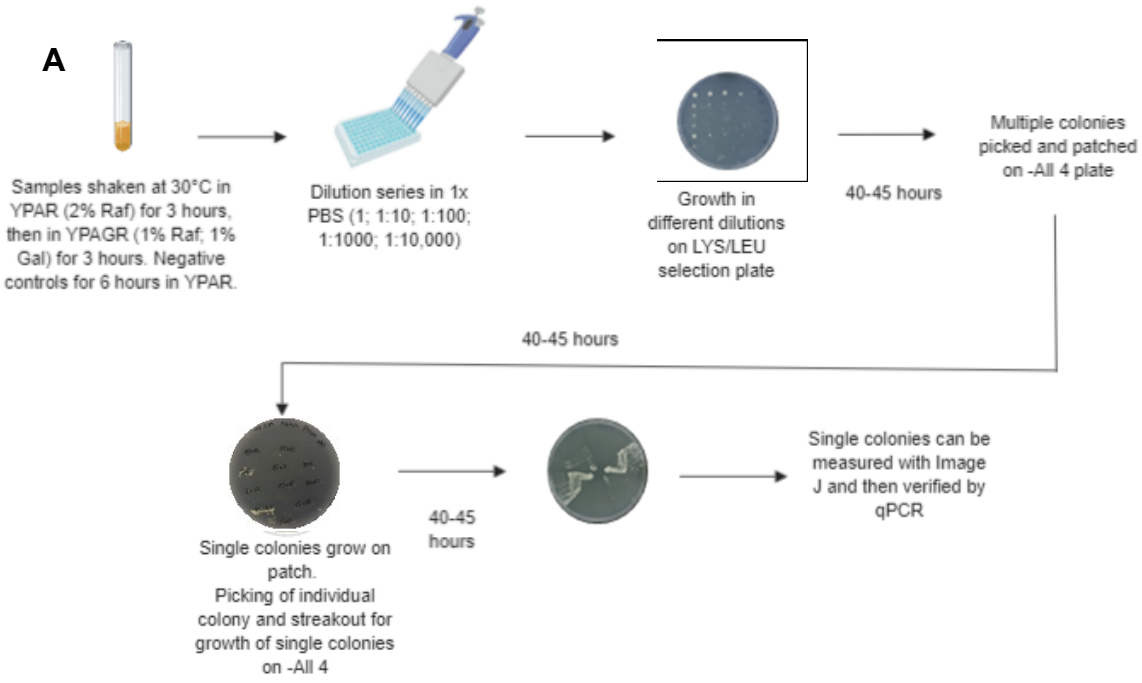
Figure 25: The SL 3 strain does not show tetraploidy after induction of missegregation. (A) FACS measurements for haploid control strain. Cells were not synchronized before doing FACS. **(B)** FACS measurements for diploid control strain. Cells were not synchronized before doing FACS. **(C)** FACS measurements for SL 3 strain after induction of missegregation. Preparations for analysis were performed 4 days after induction, after 2 days on FOA selection and another 2 days on SC. Monosomy for chromosome 3 was confirmed by qPCR. Cells were not synchronized before doing FACS.

8.2 Induction of Gain events

8.2.1 Previously published growth trends from our lab can be reproduced with the new haploid Gain/Gain collection

Before the development of a high-throughput method for GG induction, I wanted to test the reliability of the established haploid Gain/Gain (GG) collection. Therefore at first only chromosome combinations already previously quantified in our lab were induced. The experimental setup can be observed in Figure 26 A.

For some individual chromosome combinations the comparison between the two different haploid GG strains does not fit perfectly. These were outlined by a red frame in the cluster of the new collection (Figure 26 B/C) . The overall trend between the two clusters can be stated to be similar. Thereby the consistency of the new haploid GG collection could be confirmed and it could be continued to test for possible high-throughput methods.[26]



B

LYS/LEU \ URA/HIS	1	3	8	10	13
3			1.4	0.7	
8	1.8	1.7		0.3	1.0
10		0.8			

C

LYS/LEU \ URA/HIS	1	3	8	10	13
3			1.6	0.8	
8	1.2	1.2		0.7	0.9
10		0.5			

Figure 26: Previously observed cellular fitnesses from the lab can be reproduced with new haploid Gain/Gain collection predominately. (A) Scheme of experimental setup to induced the simultaneous gain of two different chromosomes in the new haploid collection. **(B)** Results derived previously in our lab for different haploid GG strains. For these samples the average growth of individual colonies has been calculated and the numbers have been divided by the total average of the numbers for all samples. Strains were not compared to a WT. Induced strains were tested by qPCR. A color scale was applied: Yellow = high values; Green = intermediate values; Blue = low levels. **(C)** Results of induced disomies from the new Gain/Gain collection for the same chromosomal combinations as shown in (B) with induction experiment shown in (A). Single colony sizes were measured by Image J and the average size for each sample calculated. Numbers were then normalized to the average growth of the samples. No normalization to a WT was done. Induced strains shown here were tested by qPCR. Missing combinations did either not grow or were exclude by qPCR. A color scale was applied: Yellow = high values; Green = intermediate values; Blue = low levels. Red frames indicate a difference from the previous results in the lab higher than 0.3.

8.2.2 High-throughput analysis of haploid GG can be verified by comparison to previous results on haploid GG published from our lab

In order to test the developed high-throughput system, again some chromosome combinations for which cellular fitness had already been published by our lab were induced and measured with this new method (Figure 21). The thereby obtained

numbers showed more similarity to the published results than the values in the previous experimental design (Figure 26 B/C; Figure 27A/B). In addition, there seems to be high reproducibility between the cellular fitness of the new haploid GG collection in high-throughput and the measurements with single colony streak-out. The same chromosome combinations differed for a value higher than 0.3 from the published results in high throughput as in the previous experiment in Figure 26.[26] This outcome verified the new system for the high-throughput analysis of haploid GG strains.

A

	URA/HIS				
LYS/LEU	1	3	8	10	13
3			1.4	0.7	
8	1.8	1.7		0.3	1.0
10		0.8			

B

	URA/HIS				
LYS/LEU	1	3	8	10	13
3			1.3	0.6	
8	1.3	1.3		0.7	0.8
10		0.7			

Figure 27: Analysis in high-throughput shows similarity to the previously published combinations. (A) Average of colony sizes for each strain published previously from our lab. Numbers were divided by the total average of all values. A color scale was applied: Yellow = high values; Green = intermediate values; Blue = low levels. [26] **(B)** Analysis of cellular fitness in high-throughput of strains from the GG collection with chromosome combinations that have been previously tested in our lab (shown in A). Numbers were divided by the total average of all values. A color scale was applied: Yellow = high values; Green = intermediate values; Blue = low levels. Values that differ more than 0.3 from the previous established results (A) are outlined by a red frame.

8.2.3 Haploid GG strains that could not be frozen due to no growth after induction

Strain	**Number of colonies frozen**	**Description**
GG 13x7	2	This strain had to be induced multiple times to obtain colonies on the –All4 plate that could then be frozen. Finally only 2 small colonies could be picked. The strain GG 7x13 had to be induced multiple times as well to observe growing colonies.

GG 12x14	None	No growth on -All 4 selection plates. This may suggest a very strong negative genetic interaction between these chromosomes. The strain has to be checked for reliability.
GG 14x12	1	Only one colony of this strain could be frozen down, this one was then later shown by qPCR to be monosomic for both chromosomes. This may suggest a very strong negative genetic interaction between these chromosomes. The strain has to be checked for reliability.
GG 16x7	None	This strain shows no growth on –LYS/-LEU plates. The strain GG 7x16, with the reversed selection markers shows intermediate growth in the quantification. This strain should therefore be checked by qPCR.

8.3 Induction of Gain/Loss events

8.3.1 The gain of one chromosome and the loss of another chromosome can be induced simultaneously within the same diploid yeast cell

Another investigation in this thesis was if the gain of one chromosome and the loss of another chromosome could be induced within the same diploid cell. As after an induction time of 3 hours most strains did not show any growth, an induction with 7 hours was tried. Thereby most induced Gain/Loss (GL) strains were able to grow on the FOA/-LYS/-LEU selection medium. However, as it can be examined in one example of the GL of chromosomes 1 and 13 (Figure 28), after a treatment with galactose for 7 hours the cells seem to be at risk to gain more than only one copy of the required chromosome. Therefore an induction time longer than 3 hours, but less than 7 hours might be most suitable for GL. Nevertheless, this outcome can be seen as a proof that the induction of the simultaneous gain of one specific chromosome and the loss of another specific chromosome is possible within one organism. Strains observed in qPCR to have missegregated as required were frozen down for future investigations.

Results on these aneuploid cells have already been published by our lab.[26]

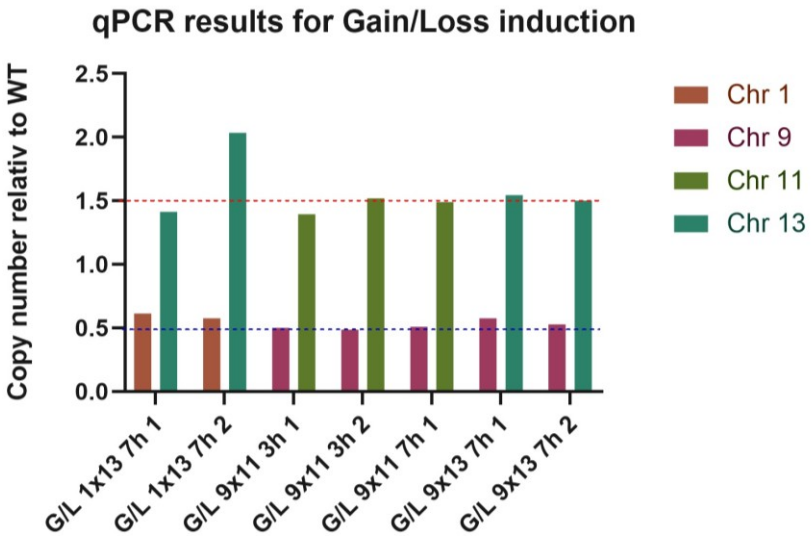


Figure 28: The gain of one chromosome and the loss of another chromosome can be successfully induced within one cell. qPCR showing results for a successful GL induction. The 1st number in strain description is always the lost chromosome; the 2nd number cites the gained chromosome. 3h = 3 hours induction with galactose; 7h = 7 hours induction with galactose. For GL 9x11 7h the duplicate is missing because of no growth on selection plate. Chromosome copy numbers are set relative to a diploid WT strain: 0.5 = 1 copy; 1 = 2 copies; 1.5 = 3 copies. The blue line indicates the supposed value after a chromosome loss. The red line indicates the supposed value after a chromosome gain.

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10 List of Abbreviations

Abbreviation	Meaning
CaCl2	Calcium chloride
CCY	Yeast strains
CCNI	Chromosome copy number interaction
CEN	Centromere
CIN	Chromosomal instability
Ct	Threshold cycle
EB	Elution buffer
EDTA	Ethylenediaminetetraacetic acid
FACS	Fluorescence-activated Cell Sorting
FOA	5-Fluoroorotic Acid
G	Galactose sample
pGAL	Galactose promoter
GFP	Green fluorescence protein
GG	Gain/Gain
GL	Gain/Loss
HIS	Gene encoding histidin
LEU	Gene encoding leucin
LL	Loss/Loss
LiAc	Lithium acetate
LYS	Gene encoding lysin
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NEB	New England BioLabs Inc.
NP-40	4-Nonylphenyl-Polyethylene glycol
OD	Optical density
ORF	Open reading frames
PBS	Phophate buffered saline
PEG	Polyethylene glycol
PBS	Phosphate buffered saline
PEG	Polyethylenglycol

PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction (alias Real time polymerase chain reaction)
R	Raffinose sample
Rpm	Revolutions per minute
pGAL	Galactose inducible promoter
SDS	Sodium dodecyl sulphate
SGD	Saccharomyces genome database
SL	Single Loss
SM	Synthetic Minimal Medium
SPM	Sporulation Medium
ss-DNA	Salmon sperm DNA
TAE	Tris-Acetate EDTA
TCGA	The Cancer Genome Atlas
URA	Gene encoding uracil
WT	Wild-type
YPAD	Yeast Extract Peptone Adenine Dextrose Medium
YPAGR	Yeast Extract Peptone Adenine Raffinose medium
YPAR	Yeast Extract Peptone Adenine Raffinose medium

11 Supplemental Material

11.1 List of Yeast strains

STRAIN	GENOTYPE	BACKGROUND	Mating type	DESCRIPTION
CCY1894	his3D1; leu2D0; lys2D0; ura3D2	S288C	a	WT strain for qPCR or mating type test
CCY1895	his3D1; leu2D0; lys2D0; ura3D2	S288C	alpha	WT strain for qPCR or mating type test
CCY80	thr4		a	a-type-tester for use with minimal plates. From D. Norris 1989 (only alpha-types will mate with this strain)
CCY81	thr4		alpha	alpha-type-tester for use with minimal plates. From D. Norris 1989 (only a-types will mate with this strain)
CCY801	trp1D63; ura3-52; his3D200	S288C	alpha	Positive control for alpha-type-testing
CCY802	trp1D63; ura3-52; his3D200	S288C	a	Positive control for a-type-testing
CCY803	trp1D63; ura3-52; his3D200	S288C	a/alpha	Negative control for mating type test
CCY1914	his3D1/his3D1; leu2D0/leu2D0; lys2D0/lys2D0; ura3D2/ura3D2	S288C	a/alpha	WT strain for comparison of colony sizes or qPCR normalization
CCY2645	leu2D0; lys2D0; ura3D2; pGalCEN1::LYS2::pCC644::LEU2 his3D1::His3	S288C	a/alpha	WT for comparison of cellular fitness and normalization in qPCR
CCY2646	As CCY2645 but conditional centromere on chromosome 2	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2647	As CCY2645 but conditional centromere on chromosome 3	S288C	a/alpha	Induction of the Single Gain of specific chromosome

CCY2648	As CCY2645 but conditional centromere on chromosome 4	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2649	As CCY2645 but conditional centromere on chromosome 5	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2681	As CCY2645 but conditional centromere on chromosome 6	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2650	As CCY2645 but conditional centromere on chromosome 7	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2651	As CCY2645 but conditional centromere on chromosome 8	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2652	As CCY2645 but conditional centromere on chromosome 9	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2653	As CCY2645 but conditional centromere on chromosome 10	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2655	As CCY2645 but conditional centromere on chromosome 11	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2656	As CCY2645 but conditional centromere on chromosome 12	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2657	As CCY2645 but conditional centromere on chromosome 13	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2658	As CCY2645 but conditional centromere on chromosome 14	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2659	As CCY2645 but conditional centromere on chromosome 15	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2660	As CCY2645 but conditional centromere on chromosome 16	S288C	a/alpha	Induction of the Single Gain of specific chromosome

CCY2729	his3D1; lys2D0; ura3D2; pGalCEN1::URA3::pCC631::HIS3; leu2D0::LEU2	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2730	As CCY2729 with conditional centromere on chromosome 2	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2731	As CCY2729 with conditional centromere on chromosome 3	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2732	As CCY2729 with conditional centromere on chromosome 4	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2733	As CCY2729 with conditional centromere on chromosome 5	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2734	As CCY2729 with conditional centromere on chromosome 6	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2735	As CCY2729 with conditional centromere on chromosome 7	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2736	As CCY2729 with conditional centromere on chromosome 8	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2737	As CCY2729 with conditional centromere on chromosome 9	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2738	As CCY2729 with conditional centromere on chromosome 10	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2739	As CCY2729 with conditional centromere on chromosome 11	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2740	As CCY2729 with conditional centromere on chromosome 12	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2741	As CCY2729 with conditional centromere on chromosome 13	S288C	a/alpha	Induction of the Single Gain of specific chromosome

CCY2742	As CCY2729 with conditional centromere on chromosome 14	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2743	As CCY2729 with conditional centromere on chromosome 15	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2744	As CCY2729 with conditional centromere on chromosome 16	S288C	a/alpha	Induction of the Single Gain of specific chromosome
CCY2090	his3D1/his3D1; leu2D0/leu2D0; lys2D0/lys2D0; ura3D2/ura3D2; pGalCEN1::URA3	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY1910	As CCY2090 with conditional centromere on chromosome 2	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY1911	As CCY2090 with conditional centromere on chromosome 3	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY2236	As CCY2090 with conditional centromere on chromosome 4	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY2238	As CCY2090 with conditional centromere on chromosome 5	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY2276	As CCY2090 with conditional centromere on chromosome 6	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY2274	As CCY2090 with conditional centromere on chromosome 7	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY1875	As CCY2090 with conditional centromere on chromosome 8	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY2272	As CCY2090 with conditional centromere on chromosome 9	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY1877	As CCY2090 with conditional	S288C	a/alpha	Induction of the Single

	centromere on chromosome 10			Loss of a specific chromosome
CCY2270	As CCY2090 with conditional centromere on chromosome 11	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY2280	As CCY2090 with conditional centromere on chromosome 12	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY2092	As CCY2090 with conditional centromere on chromosome 13	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY2278	As CCY2090 with conditional centromere on chromosome 14	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY2282	As CCY2090 with conditional centromere on chromosome 15	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY2268	As CCY2090 with conditional centromere on chromosome 16	S288C	a/alpha	Induction of the Single Loss of a specific chromosome
CCY2313	his3D1/his3D1; leu2D0/leu2D0; lys2D0/lys2D0; ura3D2/ura3D2; pGalCEN4::LYS2	S288C	a/alpha	Homozygous URA-deficient strain for growth comparison with Single Losses in 96-well format
CCY3146	his3D1/his3D1; leu2D0/leu2D0; lys2D0/lys2D0; ura3D2/ura3D2; pGal-CEN13::URA3; TUB2D0; ::HYGRO	S288C	a/alpha	deletion of 1 copy of TUB2 on Chr 6, insertion of hygromycin resistence gene

11.2 List of Primer

NAME	Sequence	Purpose	length	Tm
OCC919	ACAGCTTCTAAACGTTCCGTGTGC	ChrI Left arm (Forward)	25	72
OCC920	GCGGTGTGTGGATGATGGTTTCAT	ChrI Left arm (Reverse)	25	72
OCC921	GCACTTGATCCATGTAGCCATACTCG	ChrI Right arm (Forward)	27	78

OCC922	TTCGGGTGACCCTTATGGCATTCT	ChrI Right arm (Reverse)	25	72
OCC923	TTTCAGGATCACGAGCGCCATCTA	ChrII Left arm (Forward)	25	72
OCC924	CGGCAAGTGTCTCACTGTTGCATT	ChrII Left arm (Reverse)	25	72
OCC1008	TGGCTAAACATGCAGCCACACATA	ChrII Right arm (Forward)	24	70
OCC1009	TTCAAAATACCCAACGGGCAGCTG	ChrII Right arm (Reverse)	24	72
OCC927	TTGTTTCTGTCCTTGCCACAGCTC	ChrIII Left arm (Forward)	25	72
OCC928	AGCGCCTTTACCTCAACCTACCAT	ChrIII Left arm (Reverse)	25	72
OCC929	ATCCAGCCCGCACAAATGAATACC	ChrIII Right arm (Forward)	25	72
OCC930	AGAATGGAACACTCCTCACCACGA	ChrIII Right arm (Reverse)	25	72
OCC931	AGCCCTAGTTGCAGATCATCGTGT	ChrIV Left arm (Forward)	25	72
OCC932	AGAATATACGGCAACAGTGCCCGA	ChrIV Left arm (Reverse)	25	72
OCC933	GGCCAACAAATCTTGTACCTCGCT	ChrIV Right arm (Forward)	25	72
OCC934	GTTACCGAAGAAGGCCACCAATCT	ChrIV Right arm (Reverse)	25	72
OCC935	TCCGCCGGCAACTGTAACTGTAAA	ChrV Left arm (Forward)	25	72
OCC936	ATAGTAACCAACGAGAGCGCGCAA	ChrV Left arm (Reverse)	25	72
OCC1465	CAAGCCACTGTTGGCGTTTCAACT	ChrV Right arm (Forward)	25	72
OCC1466	TTTATGTGCGGCTTTGTCAGCAGG	ChrV Right arm (Reverse)	25	72
OCC937	GCGCTTATGTAAGGTTCCTGTATGGT	ChrVI Left arm (Forward)	27	76
OCC938	AGTGCGGATTCATTTCCAAGCAGC	ChrVI Left arm (Reverse)	25	72
OCC1467	TTAACCTTGGCGTTTCAGCATCCG	ChrVI Right arm (Forward)	25	72
OCC1468	TGA TCTTCCGCCGA TTGGTGTTCA	ChrVI Right arm (Reverse)	27	72
OCC941	TGTGCGTCTTCCCTAAAGCAGCTA	ChrVII Left arm (Forward)	25	72
OCC942	GCATTGGATGCGATGAGATGGCAA	ChrVII Left arm (Reverse)	25	72
OCC943	TTACGAGCCTTTCAGACCTGCGTA	ChrVII Right arm (Forward)	25	72
OCC944	GTGAAATACGGCCGCTAAGCATCT	ChrVII Right arm (Reverse)	25	72
OCC1002	AATGGGAGTGATCCGCTCAGTTCT	ChrVIII Left arm (Forward)	24	72
OCC1003	GAATCTCTGCAGCAAGAGCGTAGG	ChrVIII Left arm (Reverse)	24	74
OCC1004	TCATTGCAATAACAGAAAGGCCGG	ChrVIII Right arm (Forward)	24	70
OCC1005	GGGAAAAGTCCGCCGGAGATAATT	ChrVIII Right arm (Reverse)	24	72

OCC949	AAAGTTGGCGCTGGGTACTTTGAG	ChrIX Left arm (Forward)	25	72
OCC950	AGAACTGATGGCATTTGATGGCCG	ChrIX Left arm (Reverse)	25	72
OCC1469	TCTGTAGCAGAAAGAGTCTCCCGA	ChrIX Right arm (Forward)	25	72
OCC1470	GGTACTCTGTGGTTTGCCCTTTGT	ChrIX Right arm (Reverse)	25	72
OCC951	ATTTACCGGTTAGTGTCAGCGCCA	ChrX Left arm (Forward)	25	72
OCC952	CGACAGAGTAGTTTATGCCGAGGGTT	ChrX Left arm (Reverse)	27	78
OCC953	AGGCGAGTACCCTTAGCATTTCCT	ChrX Right arm (Forward)	25	72
OCC954	ACGAGGCAAGTGTAGGTCCTTTGT	ChrX Right arm (Reverse)	25	72
OCC955	AGCTGGTGATGAGCCAAATGTCGT	ChrXI Left arm (Forward)	25	72
OCC956	TTTAGAGCAAGCGCCTTTGTGAGC	ChrXI Left arm (Reverse)	25	72
OCC1471	TAGGCTTCCGGAACCACACAAGAT	ChrXI Right arm (Forward)	25	72
OCC1472	AGAGGCAGCTTCCCTTCTGA TTCT	ChrXI Right arm (Reverse)	26	72
OCC957	TGGAGATGAAGGGTTGTCGTTGGT	ChrXII Left arm (Forward)	25	72
OCC958	ACGTGTAGCGTTTCTGCTGGTCTT	ChrXII Left arm (Reverse)	25	72
OCC1473	ATGGCAGGCAGGTGAATGAGATGA	ChrXII Right arm (Forward)	25	72
OCC1474	AGAGTAGACCATGGGACGTCGTTT	ChrXII Right arm (Reverse)	25	72
OCC959	AACCGTCTTTCGAGCAGTTGAAGG	ChrXIII Left arm (Forward)	25	72
OCC960	ACAACAGCGGGAACTAAGTGCAGA	ChrXIII Left arm (Reverse)	25	72
OCC1475	TCACTCTTCCAATGGGCACCTGTA	ChrXIII Right arm (Forward)	25	72
OCC1476	TGGGATGATAACCTGTCGCTTCCT	ChrXIII Right arm (Reverse)	25	72
OCC961	GGGATTAACAATACGGTAAAGGGACG	ChrXIV Left arm (Forward)	27	76
OCC962	CAACCACTGTCAGCACAAACTCCT	ChrXIV Left arm (Reverse)	25	72
OCC963	TCGCTCAGAACATCAGCGAGAGTT	ChrXIV Right arm (Forward)	25	72
OCC964	GTTTCTGCGAAGGCCCTTTGTTCT	ChrXIV Right arm (Reverse)	25	72
OCC965	ATTTAGGCTGCACGGCTCAGTTCT	ChrXV Left arm (Forward)	25	72
OCC966	CTAGGTTCACTGCTTTGGCACACA	ChrXV Left arm (Reverse)	25	72
OCC1477	GTTCACGGTTTCCCAGA TTCGTGT	ChrXV Right arm (Forward)	26	72
OCC1478	ATTTCCTGAAGTGTCTGTCGTGCG	ChrXV Right arm (Reverse)	25	72
OCC967	AAGAGCCTTGAACTTCTCGGGTGA	ChrXVI Left arm (Forward)	25	72

OCC968	TGATGTTCCTCTCGTTTGGCACTC	ChrXVI Left arm (Reverse)	25	72
OCC1479	ACATGTGGAGCATAGCAGGCTCTT	ChrXVI Right arm (Forward)	25	72
OCC1480	ATTACCTCTTTCCCACAACCGGCA	ChrXVI Right arm (Reverse)	25	72
OCC1614	TTCTATTACTCTTGGCCTCCT	amplifies HIS3 gene (188 bp upstream of HIS3) fw	60	21
OCC1615	CAGCTTTAAATAATCGGTGTCACTA	amplifies HIS3 gene (inkl STOP) rv	68	25
OCC1616	TCCTCAACATAACGAGAACAC	amplifies LEU2 gene (321 bp upstream of LEU2) fw	60	21
OCC1617	ACAAATATCATAAAAAAAGAGAATCTTTTTAAG	amplifies LEU2 gene (inkl STOP) rv	78	33
OCC206	ACCTATCACCACAACTAACT	downstream of HIS3 for integration checking	56	20
OCC1253	GCAAGGCGATTAAGTTGGCCAATAGGTGGTTAGCAATCG	Upstream of LEU2 to check for integration fw	116	39
OCC1654	CCCTCCTCCTTGTCAATATT	226 bp downstream of LEU2 rv	58	20
OCC1139	TATTATACTCGTTCTCAACAACTCTACTAATTATTAAATAGGATTATATTAGAGTCCCCTTCCGCTTATAGTACAG	Amplify GalCen plasmid to replace CEN10 F	200	76
OCC1140	ATGTTAAAAATGGTGACTGTATCTACGTATCTATAAAAAAAGGTTAACTACCGGACGAGTACAACACCCGATCCT	Amplify GalCen plasmid to replace CEN10 R	206	75
OCC1141	GTTTAGTTGTTGTGGATGC	Check replacement of CEN10 F	54	19
OCC1142	GGCTTACCTTATCTATGC	Check replacement of CEN10 R	52	18
OCC1408	TTATAAGGAGAAAACTTGTAGTACGAGGTTAACATAAGAAAGAAAGAGAATGATGCCCCTTCCGCTTATAGTACAG	Amplify GalCen plasmid to replace CEN12 F	208	76
OCC1409	TGAAGTACACACCCGCGTAAAGAGTTTTTACCCCGAAAACAAATTTTTATGCTTGCGAGTACAACACCCGATCCT	Amplify GalCen plasmid to replace CEN12 R	214	75
OCC1410	GCGTCTTGCCGTTAACAA	CEN12 Check F	54	18
OCC1411	TTATCTTCTGCGCCTTTCC	CEN12 Check R	56	19
OCC1416	AATTACAGGTTATATTAAAGAGATTATCTTGACTGATATAAAATTTCTTATCATGCCC	Amplify GalCen plasmid to	198	76

	CTTCCGCTTATAGTACAG	replace CEN16 F		
OCC1417	AAACTGCTATTTAGCCGCTTTGCCGATTTCGCTTTAGAACCGCTACCATGGTGTGCGAGTACAACACCCGATCCT	Amplify GalCen plasmid to replace CEN16 R	224	75
OCC1418	CTGATCCAGAAAAGGCAAG	CEN16 Check F	56	19
OCC1419	GAATGCTTGACAAGCCAG	CEN16 Check R	54	18
OCC1949	GCTACTACAACTACAAAAGCAAAATCTCCACAAAGTAATATACGTACGCTGCAGGTCGAC	TUB2 S1 F	170	60
OCC1950	TTATTTTGCTCCAAGTGCTTCAATCCTAGAGAAGAAGAAAGATCGATGAATTCGAGCTCG	TUB2 S2 R	168	60
OCC1951	GATATACGTGTACAGTGACCT	to check deletion of TUB2 and integration of Hygromycin instead (upstream of TUB2) F	60	21
OCC1952	TAGCTCGGAAGGTTAAAGGT	to check deletion of TUB2 and integration of Hygromycin instead (downstream of TUB2) R	58	20
OCC282	AAACATAACGATCTTTGTAGA	To check Hygromycin integration pFA6-hphNT insert sequencing	54	21

11.3 List of Plasmids

Plasmid	**Purpose**
pCC239	Deletion of TUB2 gene on chromosome 6 and integration of hygromycin resistance
pCC632	Conditional centromere pGALCEN3 URA3 for Single Loss

11.4 List of Reagents and Equipment

Name	**Company**	**Notes**
Agarose	GERBU	0.8% in 1x Tris-Acetate-EDTA (TAE)

BD LSRFortessa TM	BD Biosciences	
BioRender	BioRender	
Canon EOS DIGITAL REBEL XSi	Canon	
Centrifuge 5424	Eppendorf	
Centrifuge 5804 R	Eppendorf	
Cover glass	VWR	
Cuvettes	SARSTEDT AG & Co. KG	
Deoxynucleotide (dNTP) Solution Mix	New England BioLabs Inc. (NEB)	
EDTA	AppliChem	
EZ plate™ beads	Sunrise Science Product	
Fisherbrand TM Standard Minicentrifuge	Thermo Fisher Scientific	
Glass Beads, acid washed	Sigma Aldrich	
Gel Doc™ XR+	BIO RAD	
GraphPad Prism 8	GraphPad Software	
Image J	National Institute of Health	
Lithium Acetate dihydrate	Merck	Use in 1 M and 100 mM concentration
Luna Universal qPCR Master Mix	New England BioLabs Inc.	Reaction in 1x concentration
Lyticase from Arthrobacter Luteus	Sigma Aldrich	Dissolve in H_2O to 20mg/ml
Mastercycler 5333 m IN SITU	Eppendorf	
Mastercycler realplex^2 epgradient S	Eppendorf	
Mastercycler realplex^4 epgradient S	Eppendorf	

Megafuge 1.0 R	Thermo Fischer Scientific	
Microplate 96 well, PS, F-BOTTOM, CLEAR	Greiner bio-one	
Microsoft Word 2007	Microsoft	
Microsoft Excel 2007	Microsoft	
Nonidet® P40	AppliChem	
Orange G	AppliChem	
PCR 8-tube strips, with separate domed cap strips	VWR	
PeqGreen	PeqLab	20,000 times in H_2O
Polyethylenglycol 4,000 (PEG 4,000)	AppliChem	Use 50% concentration in H_2O
Primers	Microsynth	
Proteinase K	Sigma Aldrich	20 mg/ml
Quick-Load® Purple 1 kb DNA Ladder	NEB	Use 0.5-1.0 µg per gel lane
Phusion High-Fidelity DNA Polymerase	Thermo Fisher	Dilute 1:20 before usage
RePads ™ 96 Long	Singer Instruments	
Replica plater 96-well format	V&P Scientific Inc.	
RNAse A	Sigma Aldrich	Use concentration of 5 mg/ml
ROTOR HDA	Singer Instruments	
Röhre 15 ml, 120x17 mm, PP	SARSTEDT AG & Co. KG	
Röhre 50 ml, 114x28 mm, PP	SARSTEDT AG & Co. KG	
Saccharomyces Genome Database (SGD)	Stanford University	
Sodium Citrate Dihydrate	Sigma Aldrich	Use in 50 nM concentration With pH 7
Sonicator Sonopuls	Bandelin	

Sytox Green	Invitrogen	Use concentration of 5 mM
Tube 13 ml, 100x16mm, PP	SARSTEDT AG & Co. KG	
Twin.tec real-time PCR Plate 96, skirted	Eppendorf	
T100^{TM} Thermal Cycler	BIO RAD	
U-2000 Spectrophotometer	HITACHI	
VWR Microscope Slides Ground Edges	VWR	
Wizard Genomic DNA Purification Kit	Promega	
1.5 ml Micro tubes	SARSTEDT AG & Co. KG	
2.0 ml Micro tubes	SARSTEDT AG & Co. KG	
5X Phusion HF Reaction Buffer	Thermo Fisher	

11.5 List of Media

Medium	**Contents**
Synthetic Minimal Medium (SM)	H2O; 1.7 g/l Bacto-yeast nitrogen base without amino acids and without ammonium sulfate; 5 g/l Ammonium sulfate; 20 g/l Bacto Agar; 20 g/l Glucose pH set to 5.5-6.0 with KOH
Sporulation medium (SPM)	H2O; 20 g/l Bacto Agar; 20 g/l KAC
Yeast Extract Peptone Adenine Dextrose medium (YPAD)	H2O; 10 g/l yeast extract; 20 g/l Bacto peptone; 20 g/l Agar; 20 g/l Glucose; 55 mg/l Adenine
5-Fluoroorotic acid medium (FOA)	H2O; Amino acid mix (20 mg/l Ade, 160 mg/l Arg, 80 mg/l Asp, 80 mg/l Glu, 80 mg/l Gly, 80 mg/l His, 100 mg/ l Ile, 160 mg/l Leu, 80 mg/l Lys, 80 mg/l Met, 20 mg/l Phe, 320 mg/l Ser, 160 mg/l Thr, 80 mg/l Trp, 80 mg/l Tyr, 160 mg/l Val, 48 mg/l Ura); 1g/l FOA ; 20 g/l Agar; 1.7 g/l Yeast Nitrogen Base without amino acid and without (NH4)2SO4; 5 g/l Ammonium Sulfate; 20 g/l Glucose; pH under 4.0

FOA-like synthetic complete (FOA-like SC)	See 5-Fluoroorotic acid medium (FOA) Without 1 g/l FOA
-LEU/-HIS/-URA/-LYS (-All 4) medium	H2O, 1.7 g/l Bacto-yeast nitrogen base without amino acids and without ammonium sulfate; 5 g/l Ammonium sulfate; 20 g/l Agar, 55 mg/l Tyrosine, 55 mg/l Adenine; 20 g/l Glucose; Amino Acid Dropout solution (0.1 g/l Arg, 1.5 g/l Ile, 0.1 g/l Met, 0.25 g/ Phe, 2 g/l Ser, 1 g/l Thr, 0.3 g/l Trp, 0.7 g/l Val)
–LEU medium	See –All 4 medium + 55 mg/l Uracil ; + 0.1 g/l His and 0.2 g/l Lys in Amino Acid Dropout solution
–HIS medium	See –All 4 medium + 55 mg/l Uracil ; + 0.4 g/l Leu and 0.2 g/l Lys in Amino Acid Dropout solution
–URA medium	See –All 4 medium + 0.1 g/l His, 0.4 g/l Leu and 0.2 g/l Lys in Amino Acid Dropout solution
–LYS medium	See –All 4 medium + 55 mg/l Uracil ; 0.1 g/l His and 0.4 g/l Leu in Amino Acid Dropout solution
-TRP-like synthetic complete (-TRP-like SC)	H2O; 1.7 g/l Bacto-yeast nitrogen base without amino acids and without ammonium sulfate; 5 g/l Ammonium sulfate; 11 g/l Casamino acids; 20 g/l Agar; 55 mg/l Adenine; 55 mg/l Tyrosine; 75 mg/l Uracil; 50 mg/l Leucine; 50 mg/l Tryptophan pH set to 6.0
YPAR Yeast Extract Peptone Adenine Raffinose medium	See YPAD + 20 g/l Raffinose instead of Glucose
YPAGR Yeast Extract Peptone Adenine Galactose/Raffinose medium	See YPAD + 10 g/l Raffinose and 10 g/l Galactose instead of Glucose
SOS medium	H2O; 300 ml/l YPAD (see YPAD); 6mM CaCl2
Hygromycin medium	See YPAD

	+ 0.3 g/l Hygromycin
–URA Glutamate medium	See –All 4 medium + 0.1 g/l His, 0.4 g/l Leu and 0.2 g/l Lys in Amino Acid Dropout solution + 1 g/ Glutamate instead of Ammonium sulfate

11.6 List of Buffers

Buffer	Ingredients
Buffer A (100 ml)	2% Triton X-100 1% Sodium Dodecyl Sulfate(SDS) 100 mM Sodium Chloride (NaCl) 10 mM Tris (pH = 8.0) 1 mM EDTA (pH = 8.0)
Phenol-Chloroform	Phenol : Chloroform : Isoamyl alcohol = 25 : 24 : 1
1x Phosphate-buffered saline (PBS)	137 mM NaCl 2.7 mM KCl 10 mM Na_2HPO_4 1.8 mM KH_2PO_4
Elution Buffer (EB)	10 mM Tris (pH= 8.8)
Phusion storage buffer	20 mM Tris-HCl (pH 7.4 at 25 °C) 0.1 mM EDTA 1 mM DTT 100 mM KCl Stabilizers 200 µg/mL BSA 50% glycerol

11.7 PCR programs

CCINTEGR

STEP	TEMPERATURE	TIME (min)	
1	95°C	03:00	
2	95°C	00:30	
3	54°C	00:30	
4	72°C	03:00	
5	-	-	GO TO 2 REP 7
6	95°C	00:30	
7	57°C	00:30	
8	72°C	03:00	
9	-	-	GO TO 6 REP 8
10	95°C	00:30	
11	59°C	00:30	
12	72°C	03:00	
13	-	-	GO TO 10 REP 8
14	95°C	00:30	
15	61°C	00:30	
16	72°C	03:00	
17	-	-	GO TO 14 REP 9
18	72°C	05:00	
∞	10°C	-	HOLD

CCPHUSIO

STEP	TEMPERATURE	TIME (min)	
1	95°C	03:00	
2	95°C	00:30	
3	52°C	00:30	
4	72°C	02:00-03:00 (depends on sample size)	
5	-	-	GO TO 2 REP 33
6	72°C		
7	12°C	-	HOLD

CC100

STEP	TEMPERATURE	TIME (min)	
1	99°C	10:00	
∞	4°C	-	HOLD

qPCR program

STEP	TEMPERATURE	TIME (min)	
1	95°C	05:00	
2	95°C	00:15	
3	60°C	01:00	MEASUREMENT GO TO 2 REP 39

4	95°C	00:15	
5	60°C	00:15	
6	TEMP INCREASE TO 95°C over TIME	20:00	MEASUREMENT during TEMP INCREASE
7	95°C	00:15	END