Genetic screening: Isolation of yeast mutants

Introduction

In this lesson, we will describe the steps required to perform a genetic screen with an emphasis on processes specific for yeast. You will learn how to set up a strategy for a genetic screen and what the first steps are once you have isolated mutants in such a screen. Finally, we will discuss the strategies underlying a few relevant yeast genetic screens and discuss their implication for understanding eukaryotic cell biology.

General strategy to perform a genetic screen

In principle, all forward genetic screens follow a similar strategy shown in figure 3-1.

1 Selection of a biological process (phenotype)

2 Generation of a mutant population (mutagenesis)

3 Choosing a screening strategy (readout)

4 Identification of interesting mutants (screening vs selection)

5 Identification of the gene resulting in the mutant phenotype (mapping and cloning)

Figure 3-1 Work flow of a forward-genetic screen.

Mutagenesis

Since spontaneous mutations happen only at low frequency, the mutation rate has to be increased artificially in order to have a larger sample size of mutants to study in a genetic screen. One method of inducing mutations is by using chemical compounds, such as ethyl methanesulfonate (EMS). EMS produces mutations by alkylating guanines to produce 6-O-ethylguanine (see figure 3-2). This modified base is incorporated into the DNA during replication. In the next round of replication, the DNA polymerase places thymine instead of cytosine to pair with the 6-O-ethylguanine. After several rounds of replication, the original G:C base pair can become an A:T pair. Thus, EMS typically produces point mutations.

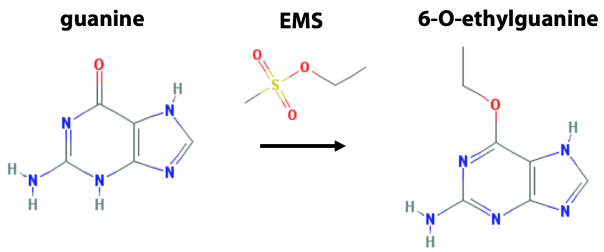

Figure 3-2 Mutagenesis by ethyl methanesulfonate (EMS). EMS alkylates guanines to produce 6-O-ethylguanine, typically causing transition mutations (from G:C to A:T).

Physical mutagenesis can be achieved by irradiation. For example, exposure to X-rays induces DNA double strand breaks, leading to chromosomal mutations such as deletions.

Steps on the way to isolate mutants

We will now briefly describe the steps and techniques involved in a hypothetical screen using yeast as a model (compare with figure 3-1). Let's imagine, we want to do a screen to identify genes involved in regulating cell growth (1. Phenotype). We decide to use EMS as a mutagen due to its power to induce point mutations (2. Mutagenesis). Then, we have to decide for a screening method (3. Readout). We could either measure the optical density of liquid cultures after a certain time point of growth or examine the size of yeast colonies using a microscope. Next, we have to identify the mutants for the desired phenotype (4. Screening versus selection), the most time consuming step in forward genetics. Here, one should consider whether one wants to do a screen or a selection. While screening requires examination of large numbers of mutants and finding a few among them with the desired phenotype, a selection establishes conditions in which only the mutants of interest survive. For example, in a screening procedure, one would screen for mutants that are sensitive to a specific drug, while in a selection experiment, one would select for mutants that are resistant to the drug and thus would survive. We have seen an example for a selection using drug resistance in the previous lesson where we discussed that mutations in the arginine transporter CAN1 cause resistance to the toxic arginine analog canavanine. Mutant cells are resistant to the drug because they cannot import it. Thus, mutants with impaired arginine uptake were isolated from yeast as canavanine-resistant strains simply by selection on canavanine-containing medium.

Both approaches, screening and selection, have their advantages and disadvantages: Selection saves a lot of time, because all the organisms that do not contain the desired mutation will not be examined; however, some mutations will not be recovered in a selection (for example, because they could survive, but grow too slowly). Screening is time-consuming, but it allows the examination of every single colony, such that a wide range of mutations with different phenotypes can be recovered.

Let's assume we performed a screen for cell growth, which worked well and we have identified several mutants that show abnormalities in cellular growth (small cell phenotype). Now, there are a few questions to answer regarding the nature of the mutants before we go on to map and identify the gene.

1. Is the phenotype caused by a single mutation or by several mutations in several genes? 2. Is the phenotype dominant or recessive? 3. How many genes have we identified all together?

All these questions can be answered easily in yeast due the fact that yeast cells are stable as haploid and diploid cells. To understand this analysis, we will quickly recall the life cycle of yeast and how this is used in genetic analyses.

One of the distinguishing features of the life cycle in ascomycetes, the group of fungi to which yeast belongs, is the formation of tetrads during meiosis. After the two haploid nuclei have fused (karyogamy) to form the diploid cell, this cell undergoes meiosis to form four haploid cells (spores) that are retained within an ascus (a kind of sac) and build a tetrad. This tetrad thus contains the four products of a single meiosis (figure 3-3).

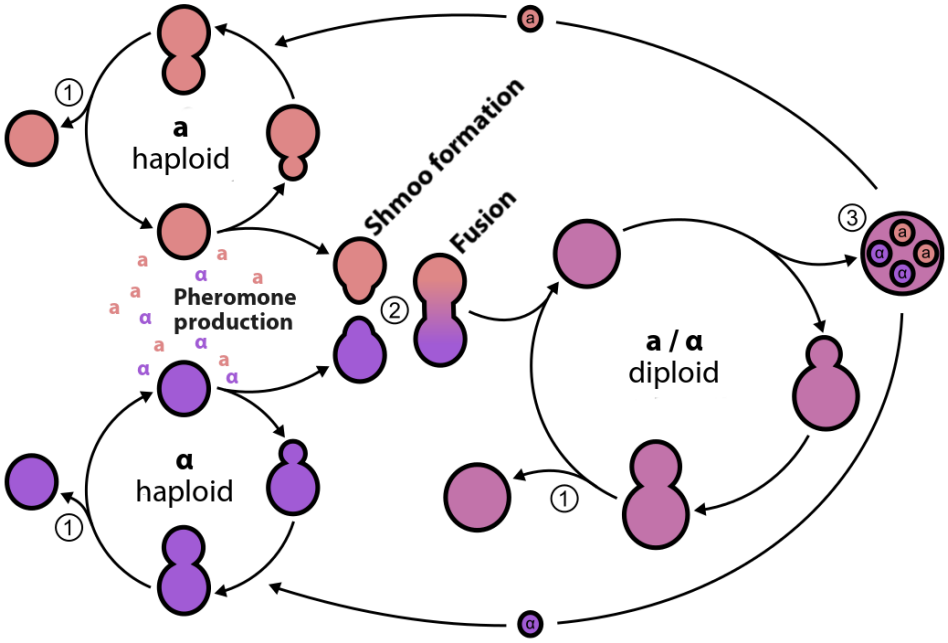

Figure 3-3 Yeast life cycle. Haploid cells can either divide asexually by forming buds (1) or fuse to produce a diploid cell with another haploid cell of the opposite mating type (2). Upon sensing pheromones of the other mating type, a cell forms a protrusion termed shmoo. After fusion, the resulting diploid cell can either divide asexually by forming buds (1) or undergo meiosis and produce four haploid spores (3) that are packaged together in an ascus. (adapted from Wikipedia: Mating in fungi)

The physical association of all products of a single meiosis within a tetrad allows a powerful genetic analysis of the progeny. The tetrads can be dissected in order to separate the four spores and each spore is placed onto a specific position on an agar plate (figure 3-4). The spores are then grown into colonies that can be tested phenotypically (e.g., checked if they show a mutant or wild type phenotype) or genotypically (e.g., sequenced).

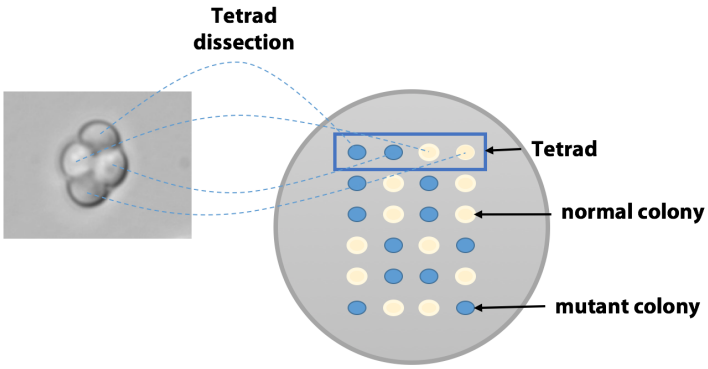

Figure 3-4 Tetrad analysis. When undergoing meiosis, yeast cells produce four haploid spores that are packaged together in an ascus and build a tetrad **(left)**. Tetrads can be dissected, i.e., separated under the microscope and transferred onto agar plates and grown to colonies **(right)**. The spores of each tetrad are placed such that the four colonies grow in one row.

The use of tetrad analysis in genetics

The tetrad analysis is the key tool in yeast genetics. It can be used to analyze inheritance patterns of mutations and thus allows making predictions about the nature of the mutation. It also allows mapping mutations onto the chromosomes (develop an idea of where the mutation is located in the genome) and to determine whether two mutations are linked (present nearby each other on the same chromosome).

The power of tetrad analysis comes from the fact that all four meiotic products are contained within a single ascus. Mendelian segregation patterns predict specific patterns of allele segregation in tetrads, and by analyzing the spores resulting from one tetrad we can determine whether our mutation segregates in the expected ratio (figure 3-5). This provides important information about the mutation and its phenotype. For example, if we know how many spores in an ascus show the mutant phenotype, we can deduce if the phenotype was inherited following the Mendelian rules or not. This information then tells us about the nature of the mutation underlying the phenotype – for example, phenotypes that are not inherited in a Mendelian fashion must either be caused by more than one gene, or may follow a non-Mendelian type of inheritance, because the responsible gene is not located in the nucleus.

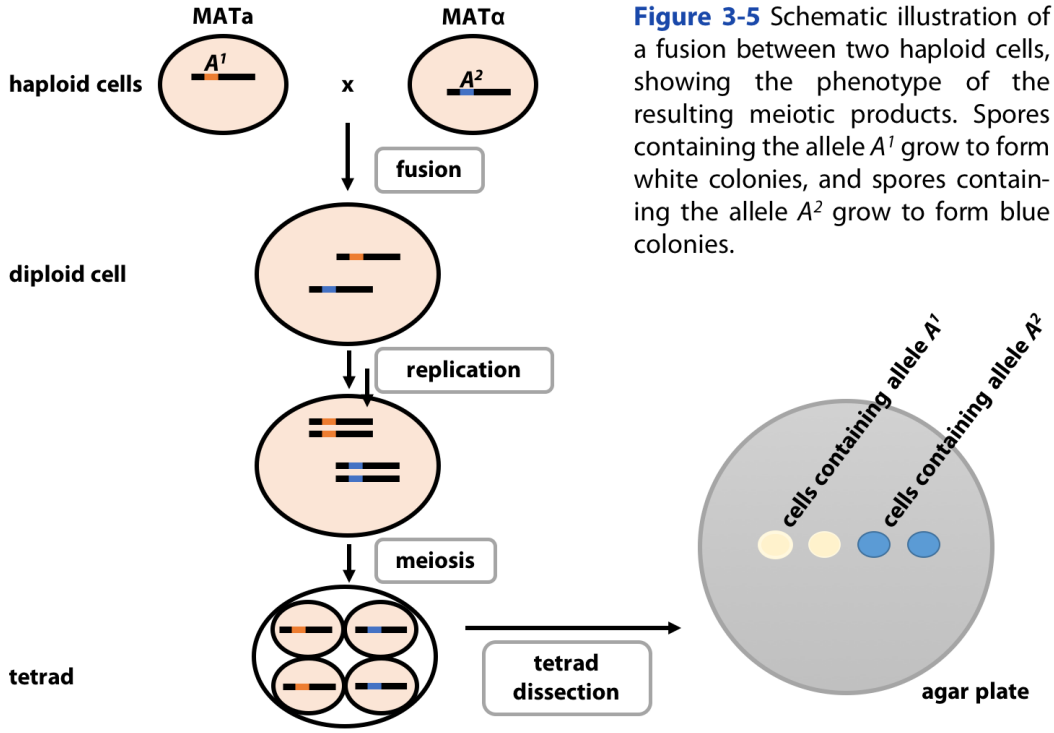

Figure 3-5 Schematic illustration of a fusion between two haploid cells, showing the phenotype of the resulting meiotic products. Spores containing the allele A^1 grow to form white colonies, and spores containing the allele A^2 grow to form blue colonies.

Further, tetrad analysis helps to localize the position of our mutation in the genome. If we follow how our mutation segregates relative to another known mutation (of which we know its genetic location), we can again see if the two genes are present on the same chromosome (then, they would be inherited together, because the two genes are segregated on the same chromosome during meiosis, and all spores would contain both genes, thus show the same phenotype) or whether they are located on two different chromosomes (then, they would be separated during meiosis, such that spores exist that show either one or the other phenotype). We will discuss this use of tetrad analysis in the next lesson on gene mapping.

Let's now look at how tetrad analysis can be used to analyze the mutations we have isolated in our hypothetical screen and answer the following question:

Is the phenotype we observe in a mutant caused by a single gene or by several genes?

Here, tetrad analysis can be used to test for single-gene segregation after crossing the mutant cell to a wild type strain. If a single gene is the cause, the mutant allele should segregate in a 2:2 manner, such that half of the resulting spores will carry the mutant gene and show the mutant phenotype, and the other half will carry the wild-type gene and show the wild-type phenotype. If the mutant phenotype does not segregate 2:2, the phenotype is not caused by a single gene.

Figure 3-6 shows a typical assay to test for single-gene segregation. Here, the haploid cells containing the mutant allele his1-1 cannot grow on plates lacking histidine or in other words: haploid cells with genotype his1-1 depend on the presence of histidine in the growth medium (histidine auxotrophy). Crosses between these cells and wild-type cells (carrying the allele HIS1) and subsequent tetrad analysis shows that the phenotype is determined by a single gene.

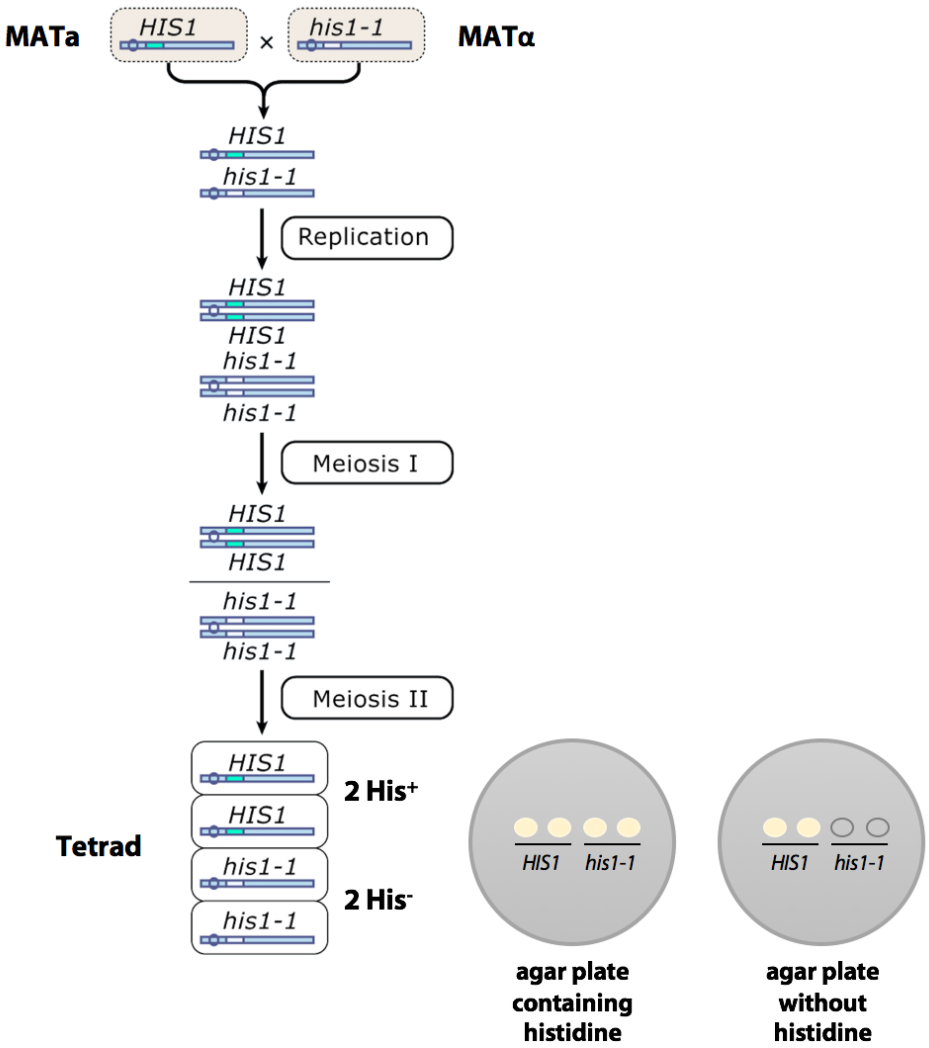

Figure 3-6 A mutation in a single gene will segregate in a Mendelian manner: two progeny will carry the wild-type gene and grow without histidine in the medium and two will carry the mutant gene and only grow in presence of histidine. (adapted from ergito.com)

We will learn later in this course how to interpret those cases where we do not observe a 2:2 segregation pattern (in lesson 5 and the lecture).

Is the mutant phenotype dominant or recessive?

Since we started our screen with haploid yeast cells, a mutation will result in a phenotype even if the mutation is recessive. In a screen, this provides a big advantage compared to diploid model organisms where another cross and a second generation are needed in order to recognize a recessive phenotype. However, after isolating a mutant in a screen, we still need to find out whether the mutation causes a dominant or recessive phenotype. Therefore, the mutant cells need to be crossed with wild-type cells. If the phenotype caused by the mutation is recessive, the diploid cells resulting from the cross show the wild-type phenotype; if the mutation results in a dominant phenotype, the diploid cells will show the mutant phenotype (figure 3-7).

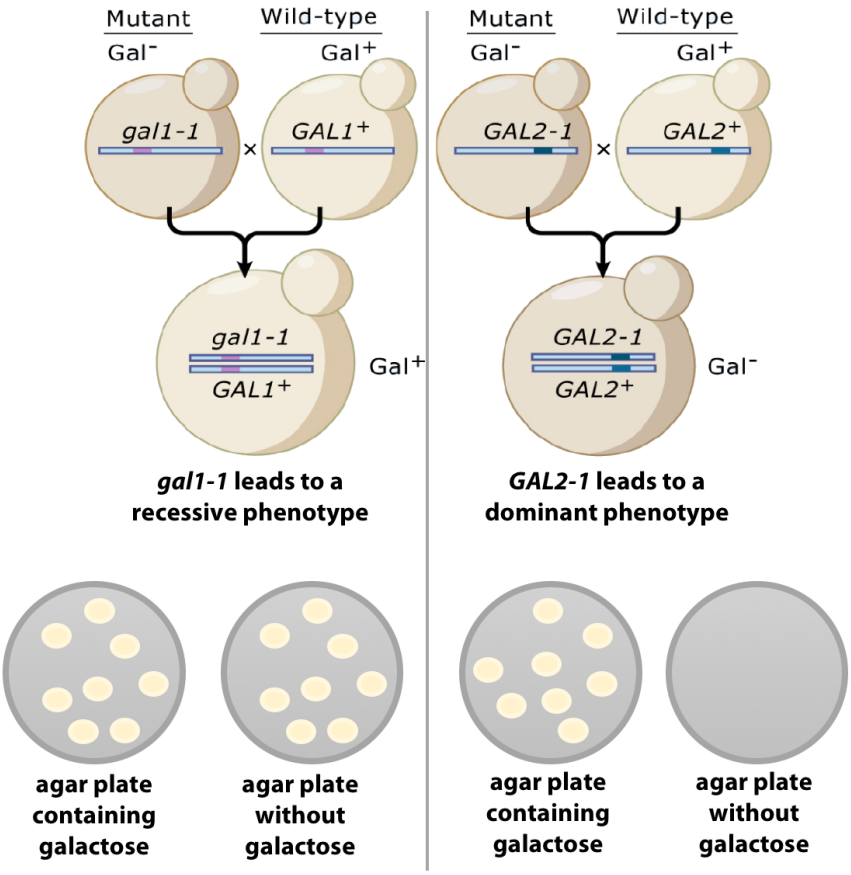

Figure 3-7 By crossing a mutant with a wild-type cell one can find out if a mutation leads to a recessive or dominant phenotype. Here, two different mutations that prevent growth without galactose are tested: *gal1-1* (left, wild type: *GAL1+*) and *GAL2-1* (right, wild type *GAL2⁺*). If the diploid shows the same phenotype as the wild type, the mutation is recessive (left); if it shows the mutant phenotype, the mutation is dominant (right). Diploid cells containing at least one copy of the *GAL2-1* gene, will not grow colonies on a plate lacking galactose. (adapted from ergito.com)

How many genes has the screen identified all together?

At the end of the screening phase, we would like to get an idea about the efficiency of the screen. Did we find all or nearly all possible mutants? Generally, one assumes that this point is reached if one identifies the same mutations over and over again. The screen is then called "saturated".

A straight-forward answer to this question can be retrieved by doing complementation tests. By crossing one mutant with another, we can determine whether one mutation can complement the

other one. If the mutants that were crossed contained a mutation in the same gene, both copies of the gene in the resulting diploid will be mutated and the diploid will therefore show the mutant phenotype. The mutations fail to complement each other. In contrast, if the resulting diploid cell shows the wild-type phenotype, the mutations complement each other and are likely in two different genes. This is because the mutation causing the phenotype in the haploid can be complemented for by the second wild-type allele of the gene in the diploid.

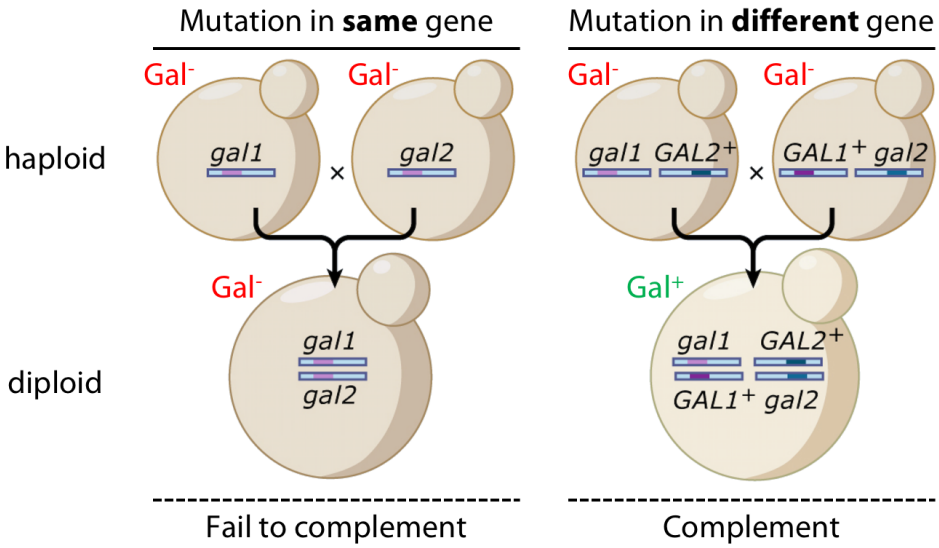

Figure 3-8 Complementation test in yeast. In order to test whether two mutants that were identified in a screen have recessive mutations in the same gene, they are crossed to produce a diploid cell. If the resulting diploid shows the mutant phenotype (Gal$^-$, cannot grow without galactose), the mutation is likely in the same gene (left). If the diploid shows the wild-type phenotype (Gal$^+$, can grow without galactose), complementation was successful and the mutation is likely in different genes (right). Here, *gal1* and *gal2* are the two identified mutant alleles causing galactose dependence. In the example on the right, *GAL1*$^+$ and *GAL2*$^+$ are the wild-type alleles encoding the dominant phenotype Gal$^+$. (adapted from ergito.com)

It is easy to perform complementation tests on many mutants to place them in complementation groups. A complementation group consists of mutations that do not complement each other. Thus, a complementation group is equivalent to a gene, and each mutation within a complementation group represents a mutant allele of the gene. Complementation therefore is a good initial test in determining the number of genes identified in a screen. However, complementation tests can only be performed with recessive phenotypes, since mutations leading to a dominant phenotype will be manifested even in a heterozygous cell, such that whether the other mutation is on the same or a different gene does not affect the phenotype.

How would the tetrads look like that arise when the diploid cells are sporulated after the complementation test shown in figure 3-8? For the scenario where the two mutations do not complement each other (left), all spores that result from the diploid would be *Gal*$^-$ (cannot grow without Galactose), because each haploid spore would contain either the mutant allele *gal1* or *gal2*. Both mutations are in the same gene that is responsible for the *Gal*$^-$ phenotype. However, if the two mutations do complement each other (right), the spores resulting from the diploid would be:

gal1 gal2
gal1 GAL2$^+$

GAL1$^+$ *gal2*
GAL1$^+$ *GAL2*$^+$

Thus, one spore (*GAL1*$^+$ *GAL2*$^+$) would be *Gal*$^+$ (thus behave like wild-type cells) and grow on medium without galactose, while the other 3 would not (thus behave like *Gal*$^-$ mutants), because the presence of a single mutant allele (either *gal1* or *gal2*) is sufficient to produce the phenotype. Thus, a ratio of segregation of 1:3 (wild type:mutant) tells us that the two mutations are on independent genes.

There are two special cases where complementation tests do not show the expected results. First, mutations in different genes may fail to complement each other if they act in the same pathway or are functionally connected. A case of non-allelic non-complementation has been observed for example for tubulin: mutations in alpha- (A) and beta-tubulin (B) can lead to non-allelic non-complementation, because A and B bind to each other in a dimer (A/B). Point mutations in both genes can lead to the formation in the same cells of the dimers A/b, a/B, A/B, and a/b, of which only one (A/B) is functional. If, as it has been observed, the complex a/b is unstable and the other complexes are stable, then the complexes A/b and a/B become predominant and the cells show the mutant phenotype despite being heterozygous diploid. In that case, tetrad dissection shows a ratio of spores with a 3:1 ratio (mutant phenotype:wild-type phenotype), despite the two mutations not-complementing each other.

Mutations in the same gene may complement each other if the second mutation can reverse the effect of the first mutation. For example, when a multimeric protein is formed from subunits produced by different mutant alleles of the same gene. The resulting hybrid protein may exhibit greater enzymatic activity than is found in either of the single mutant proteins. Although each of the mutant genes produces defective gene products, the defective products may associate to produce a product that has sufficient activity to promote function. This is particularly easy to understand in the case of a protein that assembles into a dimer, and where protein A is made of two functionally separable domains A' and A''. Therefore, one mutant protein may be defective in A' (written a'-A'') and the other in A'' (A'-a''). In each haploid strain, the dimer is in the form a'-A''/a'-A'' and A'-a''/A'-a'' and is non-functional. In the heterozygous diploid, half of the dimers formed are a'-A''/A'-a'' and are therefore functional. As a consequence, we observe complementation despite both mutations being in the same gene. Tetrad analysis would reveal that fact, because it leads to a 4:0 segregation, where all spores show a mutant phenotype, indicating that the two mutations are tightly linked (located on the same gene).

Therefore, it is important to back up the complementation assays with segregation tests to confirm that mutations that do not complement each other are indeed in the same gene or vice-versa that mutations that do complement each other are indeed in different genes. However, non-allelic non-complementation and allelic complementation are overall rare events.

At the end of these analyses, we would like to identify the gene causing the phenotype. To do so, we first need to know where the mutation is located (gene mapping) and eventually identify the mutation by sequencing. Then, we have to verify that the phenotype is actually caused by this mutation and not by any other one that is present somewhere on the chromosome. Further, we may want to evaluate if the mutation interacts with other mutations (epistasis analysis). Finally, we may want to clone the gene and do other functional tests. We will discuss the concepts underlying the mapping and identification of genes in the next part.