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A simple, cheap, and robust protocol for the identification of mating type in *Saccharomyces cerevisiae*

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Abstract

Saccharomyces cerevisiae is an exceptional genetic system, with genetic crosses facilitated by its ability to be maintained in haploid and diploid forms. Such crosses are straightforward as long as the mating type and ploidy of the strains are known. Haploid *S. cerevisiae* cells are either *MAT***a** or *MAT*α mating type. Several techniques can be used to determine mating type (or ploidy), but are typically time-consuming, require specialized components, and/or the results are inconsistent and transient. Here we validated a simple, cheap and robust method to enable rapid identification of *S. cerevisiae* mating types. When cells of opposite mating type are mixed in liquid media, they creep up culture vessel sides, a phenotype that can easily be detected visually. In contrast, mixtures of cells of the same mating type or with a diploid strain(s) simply settle out. The method does not require specialized equipment, and is robust to different media, cell densities, temperatures and strains. It can be performed in 96-well plates, and the phenotype is observable for several days. The simplicity and robustness of this method makes it ideal for routine verification of *S. cerevisiae* mating type, and it could be used to screen for genes underlying the creeping phenotype.

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Introduction

The demonstration of transformation (Hinnen et al. 1978) and plasmid maintenance (Beggs 1978) in Brewer's yeast established *Saccharomyces cerevisiae* as a premier eukaryotic model for molecular genetics. This status is maintained today, aided by its ease of culturing, single cell growth habit, small genome and remarkably efficient homologous recombination, but also because it can be maintained in haploid and diploid forms, and undergo genetic crossing. Haploid *S. cerevisiae* cells are one of two mating types, *MAT***a** or *MAT*α, and mating type is determined by the identity of the DNA sequence at the *MAT* locus (Haber 2012). The *MAT*α allele encodes two genes that specify the α-specific phenotype, while the *MAT***a** allele encodes a transcriptional repressor that, in diploids, suppresses the default **a** phenotype. *S. cerevisiae* cells contain all the genetic information at silent *HML* and *HMR* mating type loci (located separately to the *MAT* locus) to be either *MAT***a** or *MAT*α, but only the allele at the *MAT* locus is expressed (Haber 2012). Nevertheless, wild-type *S. cerevisiae* are homothallic, meaning they contain the genetic machinery necessary to self-mate. This occurs through mating-type switching, where expression of an endonuclease encoded at the *HO* locus in mother cells results in homologous recombination-mediated swapping of the *MAT* allele to the opposite allele *via* the genetic material present at the silent *HML* or *HMR* locus (Haber 2012). In most laboratory *S. cerevisiae* strains, however, mating type is extremely stable and heritable due to deletion of the *HO* endonuclease gene, which abolishes mating type switching. The ability to maintain haploid yeast strains of stable mating types so that genetic crosses between different mating types can be performed has been pivotal to the success of *S. cerevisiae* as a model genetic organism.

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The ability to perform genetic crosses in *S. cerevisiae* relies on knowing the mating types of the haploid strains involved. While the mating type and ploidy of established laboratory strains are generally known, there are a number of scenarios where they need to be determined; for example, when dissecting ascospores to produce haploid strains, when mating haploid strains without the use of markers, or when mutating the *HO* locus in wild-type strains. Current methodologies for determining mating type and ploidy include using PCR with mating type locus primers that result in different amplification product sizes depending on which mating type allele is present (Bradbury et al. 2006). Flow cytometry/FACS can also be used to determine if a cell is haploid or diploid. Furthermore, crossing known *MAT***a** or *MAT*α strains with unknown strains by mixing the colonies, incubating overnight with shaking, and then looking for the mating phenotype by 'shmoo' formation under a microscope can be used to assay mating type. The shmoo phenotype results from morphological changes that are induced upon detection of the opposite mating type pheromone, and involves cells elongating to contact each other (Duntze et al. 1970; Sprague 1991). However, in our experience at least, shmoo formation is sometimes ephemeral and only observed in a small number of cells, thus this assay lacks robustness. Alternatively, the resultant mated mixture can be spread on selective plates to look for cells containing markers from both parents, but this is time-consuming and requires appropriate markers (Sherman 1991). Another method is the "halo" method that relies on inhibition of certain strains by pheromone production, but this requires specialized tester strains (Sprague 1991). Thus, these methods are time-consuming, require specific components, and/or produce inconsistent and transient results.

Here, we demonstrate a simple, cheap and robust method to enable the rapid identification of the mating type and ploidy of *S. cerevisiae* strains. The principle of the method is that when cells of opposite mating type are mixed, they undergo a change that can easily be detected

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visually in a culture tube. An unknown strain can be individually mixed with known tester strains of each mating type in a culture medium, left for ~18 hours (overnight), and then simply observed. When the cells are of opposite mating type, the cultures "creep" up the sides of the culture vessel, unlike cultures with a mixture of cells of the same mating type or cultures with a diploid strain, which simply settle out. Observing the pattern formed with the two tester strains allows the accurate distinction of both mating types as well as of diploid strains. This response is long-lasting (the difference is routinely observed for several days, making it much less transient than shmoo formation), it does not require the specialised equipment or reagents used in other assays, and it requires little hands-on time.

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Methods

Yeast strains and standard growth conditions

All strains used in this study are listed in **Supplementary Table 1**. Culture media used were YPD (1% yeast extract, 2% bacto-peptone and 2% glucose), YPGlycerol (1% yeast extract, 2% bacto-peptone and 2% glycerol) or YNB (0.45% yeast nitrogen base with ammonium sulphate, appropriate amino acids and bases, and 2% glucose). Strains were stored in 15% glycerol at -80˚C until use.

Mating type assay

Strains were revived from glycerol stocks on YPD medium then grown overnight in liquid YPD at 30˚C unless stated otherwise. Strains were diluted to OD_{600nm} 0.2 in culture medium and added in equal volumes to a tube (2 mL per strain for a test tube, 250 µL for an eppendorf tube, or 100 µL for a 96-well plate). Cultures were left with no shaking for approximately 18 hours, after which they were observed and photographed. Time-lapse photography was performed using a Nikon D850 camera with a 60 mm macro lens and polarising filter. Images were exposed at ISO200, f/16 for 10 seconds. Photographs were taken every minute from the start of the experiment to 20 hours.

Multiplex PCR to confirm mating type assay

Primers MAT-a 5'-CAATGATTAAAATAGCATAGTCGG-3', MAT-alpha 5'-CAGCACGGAATATGGGAC-3' and MAT-R 5'-GGTGCATTTGTCATCCGTC-3' (Bradbury et al. 2006), were used in a multiplex PCR reaction to amplify mating-type specific PCR products from 2 µL of mating culture in a final volume of 25 µL. PCR was performed

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with the KAPA2G Robust DNA polymerase in GC buffer (Custom Science). Cycling parameters were initial incubation of 10 mins at 94°C, then 35 cycles of 94°C for 25 seconds, 55°C for 25 seconds and 72°C for 90 seconds. The MAT**a** amplicon (466 bp) and MATα amplicon (489 bp) were visualized on a 2% agarose gel run at 135 V for 1 hour in sodium borate buffer (10 mM NaOH, 36 mM boric acid).

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Results and discussion

A quick and reliable assay for determination of mating type in *S. cerevisiae*

Routine observations in our laboratory showed that *S. cerevisiae* cells of different mating types settled in non-shaking cultures differently to cells of the same mating type. The basis of this "mating type phenotype" is that cultures of *S. cerevisiae* cells of opposite mating types consistently "creep" up the sides of the culture vessel when mixed and left overnight on the bench with no shaking (**Figure 1**). This phenotype is not observed in cultures with a mixture of cells of the same mating type (**a** or α) or where one or both types are diploid - these instead simply settle to the bottom of the vessel (**Figure 1**).

We decided to investigate this observation further under a variety of conditions to determine whether it could form a robust mating type assay. We found that the creeping phenotype is observed in large glass test tubes, plastic microfuge tubes, and round bottom microwell plates (**Figure 1**), demonstrating its scalability and flexibility, although curiously we did not observe the phenotype in flat-bottomed microwell plates. Time-lapse photography reveals that the formation of the creeping phenotype is visible from ~4 hours after the mixing of YPD cultures of **a** and α cells at room temperature, and intensifies between about 4 and 8 hours (**Figure 2**). A complete video of the formation of the creeping phenotype over a 20 hour duration is available online (**Supplementary Movie**). Moreover, the phenotype is visible for many days, thus allowing for easy identification of mating type for unknown *S. cerevisiae* cells.

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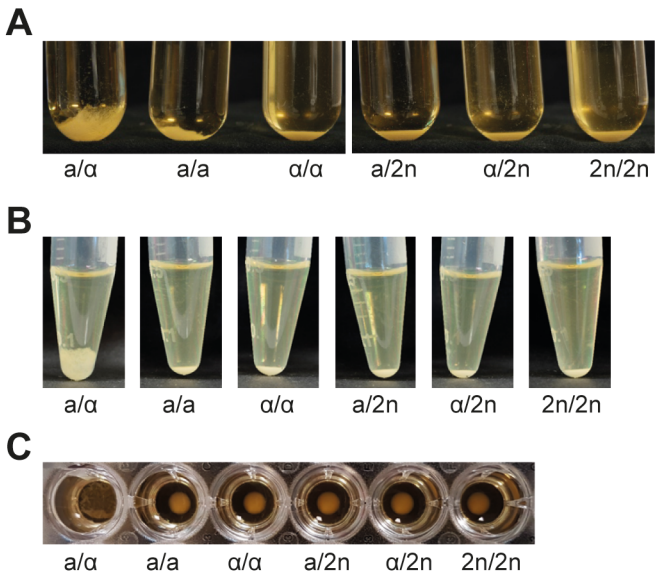

Figure 1: A 1:1 mixture of a/α cells reveals a creeping phonotype. YPD cultures (OD_{600nm} 0.2) were mixed in equal volumes of: 2 mL each in 20 mL glass tubes (**A**); 250 µL each in microcentrifuge tubes (**B**); and 100 µL each in a round-bottomed 96-well plate (**C**). Mixtures were allowed to settle on the bench for 18 hours and photographed. The creeping phenotype is only observed for the mixtures of opposite mating types (**a**/α). Mating type **a** (**a**), Mating type α (α), diploid cells (2n) are indicated below the tubes. Note that we did not observe the phenotype in flat-bottomed 96-well plates.

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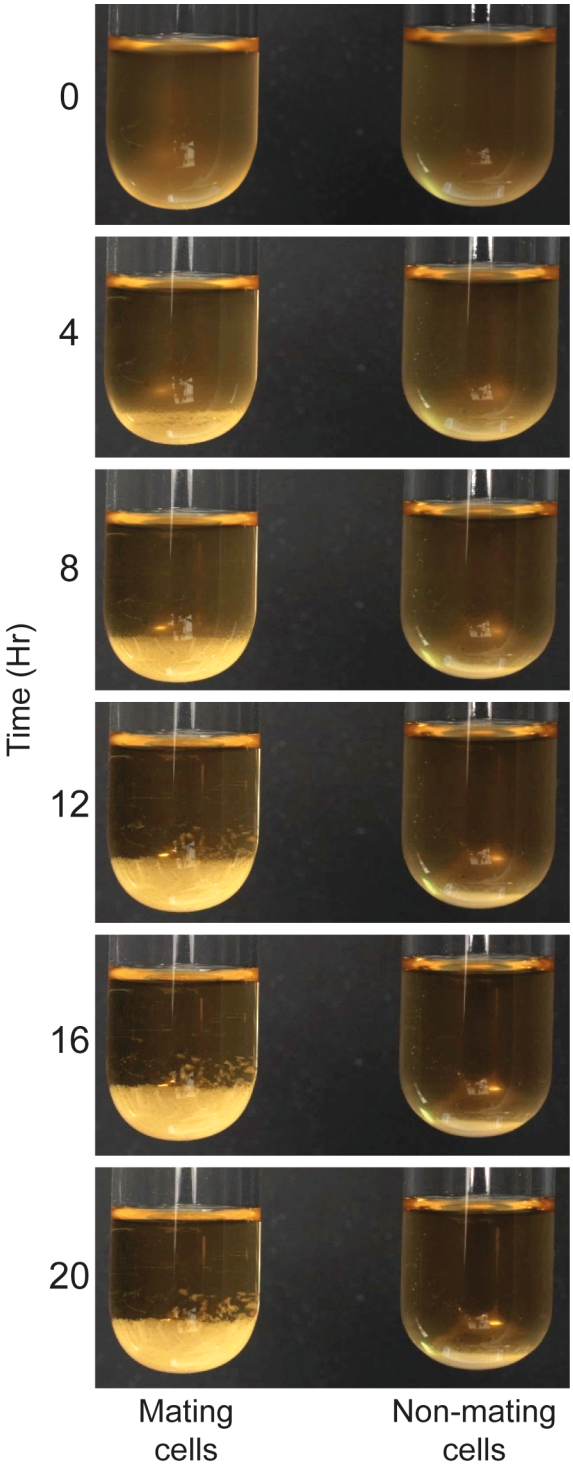

Figure 2: Time-lapse photography of the mating assay. The assay was set up as in **Figure 1A** and photographs were taken every minute for 20 hours (h). Views at 0, 4, 8, 12, 16 and 20 hours show the formation of the creeping phenotype compared to a tight pellet for non-mating cells. For a full video, see **Supplementary Movie**.

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Determination of conditions compatible with the mating type assay

We next wanted to further characterize how robust the mating type phenotype is under different conditions to assess its practicality as a mating type assay (**Figure 3**). We first tried different media, including rich and synthetic media, and water. The creeping phenotype forms reliably in both rich and synthetic media containing glucose as the carbon source, but is less obvious when glycerol is used instead (**Figure 3A**). The phenotype is not revealed when cells are diluted at OD_{600nm} 0.2 in water. Next we tested the effects of cell density and partner ratio on the phenotype for cells grown in glucose rich medium. Cell density can be varied from 0.2 to 1.8 (OD_{600nm}) without affecting the formation of the creeping phenotype (**Figure 3B**), while increasing the proportion of one partner over 60% impedes the creeping up the side of the vessel (**Figure 3C**). Finally, we find that the phenotype is obvious at temperatures in the optimal growth range for *S. cerevisiae* (24˚C and 30˚C) but is nevertheless still visible when the cultures are incubated at 37°C (**Figure 3D**). However, as for cells diluted in water, formation of the creeping phenotype does not occur at 4˚C (**Figure 3D**).

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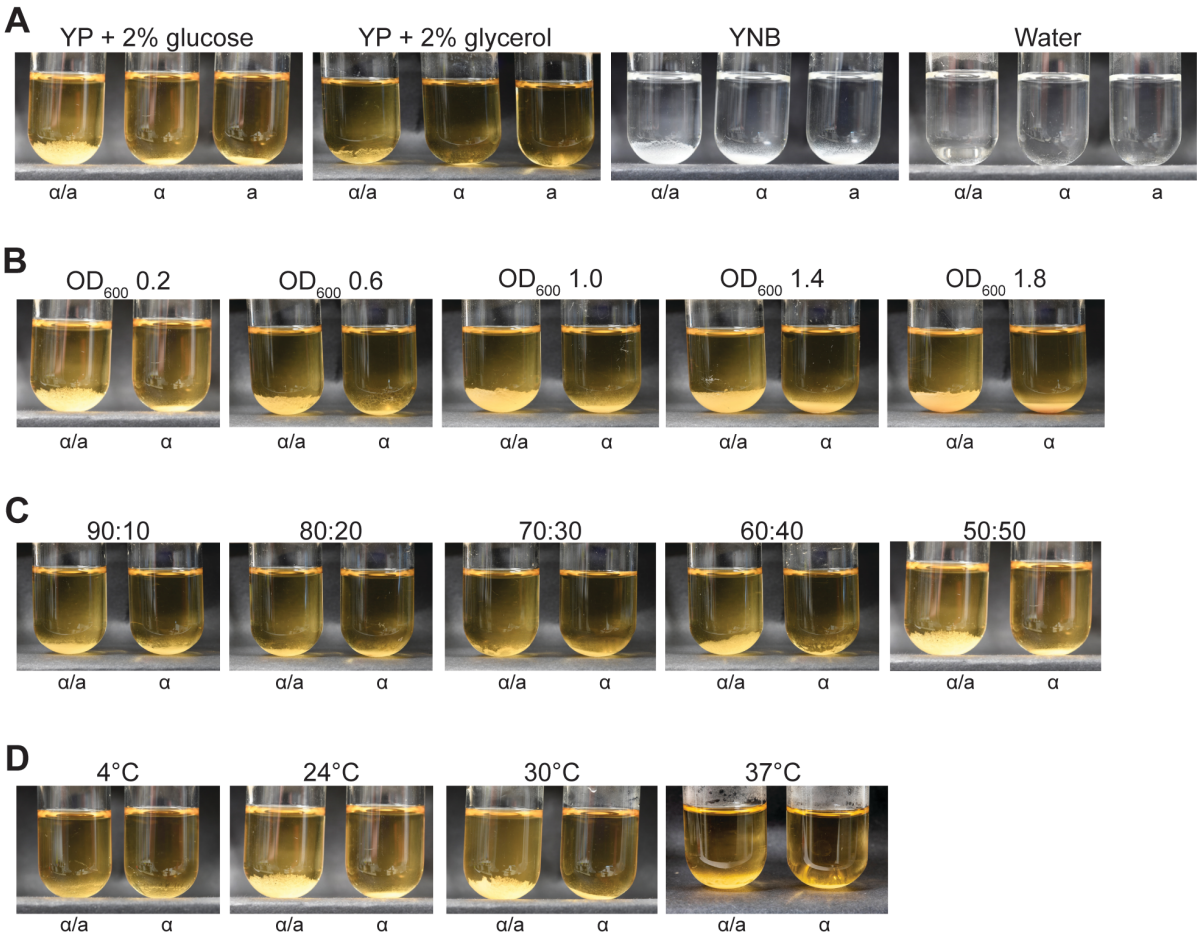

Figure 3: Establishing working parameters for the mating assay. Four parameters were varied: medium (**A**), cell density (**B**), partner ratio (**C**), and temperature (**D**). Strains were diluted to OD_{600} 0.2 in YPD, mixed equally (50:50) and incubated at 24°C unless otherwise indicated. All mixtures were photographed at 18 hours. (**A**) Overnight YPD cultures were diluted in the indicated media or water prior to the mating assay. Note that YNB and water appear this color because of medium composition, not black and white photography. (**B**) The same overnight YPD cultures from (**A**) were diluted to OD_{600nm} 0.2, 0.6, 1.0, 1.4, or 1.8 to perform the assay. **(C)** Opposite mating type cells were mixed at the different ratios indicated in a final volume of 4 mL. Ratios represent α:**a**. (**D***)* Mixtures were incubated at 4˚C, 24˚C (room temperature), 30˚C or 37˚C for 18 hours. Note that the first panel in (**A**) and (**B**), the last panel in (**C**) and the second panel in (**D**) are the same.

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Mating type determination of unknown *S. cerevisiae* strains

To assess how robust the assay is to strain type, we selected seven *S. cerevisiae* strains derived from different wild isolates (Cubillos et al. 2009), and carried out a blind test. Each strain was individually mixed with known tester **a** and α strains using standard conditions (YPD medium, OD_{600nm} 0.2, overnight incubation on the bench without shaking). The creeping phenotype is visible when unknown strains 1, 5, 6 and 7 are paired with tester **a** only, indicating that these unknown strains are haploid, and are of α mating type (**Figure 4A**). The opposite was observed for unknown strains 2, 3 and 4, with the creeping phenotype only visible when mixed with α, indicating they are haploid of **a** mating type. It is noteworthy that while results were obvious for strains 1 to 6 after overnight incubation (**Figure 4A**), additional incubation for one day was necessary to unambiguously visualize the creeping phenotype for unknown strain 7, which is slow growing (**Supplementary Figure 1**). To validate that the mating type determinations are reliable, we carried out multiplex PCR on these seven strains with primers previously designed to determine the presence of **a** or α allele within the active *MAT* locus (Bradbury et al. 2006). Each of the unknown strains gave the 489 bp **a** or 466 bp α band as expected based on the creeping phenotype assay (**Figure 4B**), which is consistent with the previous mating type characterization of these strains (**Supplementary Table 1**). Together, these results show that the assay can accurately determine mating type for a variety of *S. cerevisiae* strains under a variety of conditions.

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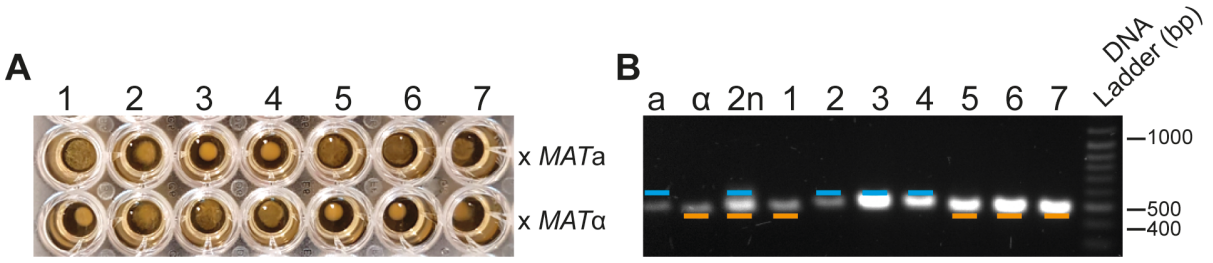

Figure 4: Determination of mating type of unknown *S. cerevisiae* strains. (A) Seven strains where mating type was blinded were mixed with *MAT***a** and *MAT*α tester strains using standard assay conditions (YPD medium, OD_{600nm} 0.2, 24°C, overnight incubation). The creeping phenotype is visible for strains 1, 5, 6 and 7 crossed with *MAT***a** tester but not with *MAT*α tester. The opposite result is visible for unknown strains 2, 3 and 4. Mixtures were photographed at 18 hours. (**B**) Mating assay results were confirmed by multiplex PCR carried out on cells from the seven unknown strains and tester **a**, α and 2n strains. Expected MAT**a** and MATα specific amplicons are 489 bp (blue line) and 466 bp (green line), respectively.

While we have characterized the creeping phenotype as a simple assay for *S. cerevisiae* mating type here, we have not attempted to ascertain the basis for the phenotype. The timing of the creeping phenotype, starting at ~4 hours, suggests it is a property of zygotes formed from the mating rather than mating reaction (Duntze et al. 1970; Sena et al. 1973), although we cannot exclude that it is a property of unmated haploids still responding to mating pheromone. The requirement for a growth medium, the poor performance on glycerol (which is not a favoured carbon source for *S. cerevisiae*), and the absence of the phenotype under 4˚C incubation all suggest that growth and/or metabolism is required for the assay to work, and these requirements are reminiscent of those reported for mating (Sena et al. 1973; Lipke et al. 1976; Fehrenbacher et al. 1978). However, we cannot fully rule out an osmotic effect. Furthermore, we do not know if these requirements are for the initial mating reaction or subsequent zygote activity, or both. Similarly, we do not know what the mechanistic basis of the creeping is, including whether it involves adhesion changes (such as those seen during mating (Betz et al. 1978; Fehrenbacher

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et al. 1978)) and/or changes in cell density. Future work targeted at the molecular basis of the phenotype is required to answer these questions.

Conclusions

Here, we describe a rapid, inexpensive and robust method to determine not only the mating type of an unknown *S. cerevisiae* strain, but also whether the strain is haploid or diploid. While we serendipitously discovered the creeping phenotype in our laboratory, anecdotal evidence suggests we are not the first to discover it, and we understand it is currently used as a mating type assay in some *S. cerevisiae* laboratories. Therefore, we focused on comprehensively evaluating and optimizing the phenotype so that it can be more widely utilized as a mating type assay by the yeast community. The assay is as simple as adding an "unknown" sample to known **a** and α strains, and observing the results after leaving for 18 hours. Thus, this assay is cheaper and more robust than currently utilised methods, with less hands-on time. So far all *S. cerevisiae* strains that we have tried in our laboratory are amenable to the assay, but it may not work with certain mutants, and we do not know whether it will work for other *Saccharomyces* species. Finally, the molecular basis for the creeping phenotype is unknown, but as the phenotype is easily screened in 96-well plate format, it is amenable to high-throughput genetic dissection, for example, using the yeast knockout collection (Giaever and Nislow 2014).

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