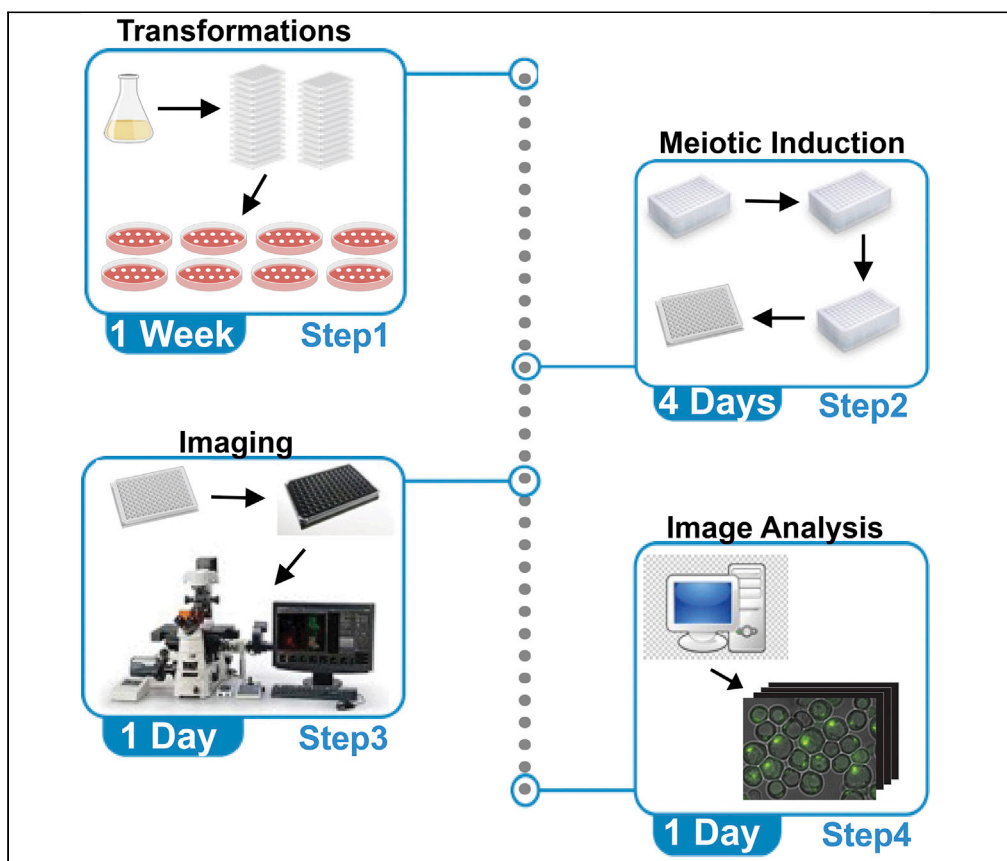# STAR Protocols

Protocol

High-throughput genetic screening of meiotic commitment using fluorescence microscopy in *Saccharomyces cerevisiae*

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Highlights
Step-by-step protocol to identify budding yeast genes involved in meiotic commitment

Protocol for high-throughput yeast transformations in 96-well plates

Protocol for meiotic induction in 96-well plates

Description of how to analyze meiotic cells using fluorescence microscopy

Simple genetic screens in budding yeast have identified many conserved meiotic regulators. However, the identification of genes involved in specific steps of meiosis may require a more complex genetic screen that allows visualization of meiosis. Here, we describe a high-throughput protocol using fluorescence microscopy to systematically screen an overexpression library to identify genes involved in meiotic commitment. We also explain how this protocol can be adapted for identifying proteins that function at different stages of meiosis.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

STAR Protocols

Protocol

High-throughput genetic screening of meiotic commitment using fluorescence microscopy in *Saccharomyces cerevisiae*

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SUMMARY

Simple genetic screens in budding yeast have identified many conserved meiotic regulators. However, the identification of genes involved in specific steps of meiosis may require a more complex genetic screen that allows visualization of meiosis. Here, we describe a high-throughput protocol using fluorescence microscopy to systematically screen an overexpression library to identify genes involved in meiotic commitment. We also explain how this protocol can be adapted for identifying proteins that function at different stages of meiosis.
For complete details on the use and execution of this protocol, please refer to Gavade et al. (2022).

BEFORE YOU BEGIN

Budding yeast provide a unique opportunity to study meiotic commitment, which is defined as the point in which cells no longer need the meiosis-inducing signal for continuation and completion of meiosis (Winter, 2012). Cells enter meiosis when starved, but if nutrients are added back before the cells reach a defined meiotic commitment point in prometaphase I, they can exit meiosis and undergo mitosis through a process called return-to-growth (Tsuchiya et al., 2014). Once past the commitment point, nutrient-rich medium addition does not disrupt meiosis and cells will finish meiosis and form spores. Little is known about how cells commit to meiosis or transition back to mitosis prior to the commitment point.

To identify genes involved in meiotic commitment, we looked for rescue of a mutant that disrupted meiotic commitment. We had previously found that high levels of the middle meiosis transcription factor Ndt80 were important for meiotic commitment (Tsuchiya et al., 2014). Ndt80 increases its own transcription by binding elements within its promoter (Chu and Herskowitz, 1998; Hepworth et al., 1998; Winter, 2012). Mutations in the *NDT80* promoter that disrupted Ndt80 binding sites resulted in low levels of *NDT80* expression and a failure in meiotic commitment; in the presence of nutrient-rich medium, cells exited meiosis and formed a bud (Tsuchiya et al., 2014). We refer to these cells as "low Ndt80 cells". We hypothesized that the low Ndt80 cells were defective in meiotic commitment due to low levels of one or more Ndt80 target genes (Gavade et al., 2022). To identify other regulators of meiotic commitment, we asked if the uncommitted phenotype of the low Ndt80 strain could be rescued by overexpression. In this protocol, we describe the high-throughput systematic genetic screen that we performed to identify genes involved in meiotic commitment. We detail how this screen can be modified to identify genes involved in various processes throughout meiosis and during the specialized cell cycle return-to-growth.

Prior to initiating the protocol, the features of the starting strain for the screen should be carefully considered. To study genes involved in progression through meiosis, meiotic commitment, and return-to-growth, we typically use cells that express three fluorescently-tagged proteins to monitor different stages of meiosis: Zip1-GFP, which marks the synaptonemal complex in prophase I, Spc42-mCherry, a component of spindle pole bodies, and GFP-Tub1, to monitor the spindle morphology at the different stages of meiosis (Carminati and Stearns, 1997; Donaldson and Kilmartin, 1996; Gihana et al., 2018; Scherthan et al., 2007; Tsuchiya et al., 2011; Tsuchiya and Lacefield, 2013). Although both Zip1 and Tub1 are tagged with GFP, we can easily distinguish between the markers due to differences in temporal regulation and morphology. Zip1 is assembled and disassembled in prophase I, whereas the spindle assembles as cells enter prometaphase I (Carminati and Stearns, 1997; Sym et al., 1993). For our screen, we fluorescently-tagged these proteins in the low Ndt80 strain to identify genes involved in meiotic commitment.

Additionally, the plasmids from of the Yeast Genomic Tiling Collection need to be isolated from the bacteria by miniprep. We used the Zyppy™-96 Plasmid Miniprep Kit, but other miniprep protocols could also be used. We did not pool the bacteria or the plasmids because we wanted to perform a systematic screen and transform each plasmid into the yeast so that we could score for the rescue of meiotic commitment upon the introduction of each plasmid within the collection.

> **Note:** This protocol is optimized for the W303 *S. cerevisiae* strain background. The protocol will need to be adjusted for other strain backgrounds. For standardization, the appropriate incubation timings in the SCD, SCA, and KAc steps will need to be determined. We advise running a small pilot experiment prior to performing the screen to ensure that the protocol allows efficient sporulation in 96-well plates for the chosen strain background. We do not recommend using inefficient sporulating strain backgrounds.

> **Note:** If the starting strain forms petites at a high frequency, we recommend first growing the strain on a rich media plate containing glycerol (YPG) to eliminate the petite cells that cannot enter meiosis. Furthermore, a control transformation should be performed ahead of time to ensure that the frequency of petite transformation is low.

> **Note:** To get overexpression of the yeast genes for our screen, we chose to use the Yeast Tiling Collection, which encompasses 92% of the yeast genome on a series of 2μ plasmids (Gavade et al., 2022; Jones et al., 2008). We found that a large fraction of the cells maintained the plasmid in sporulation medium. Other overexpression libraries can also be used with this protocol.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Bacto Peptone	BD Biosciences	Cat#211820
Bacto Yeast Extract	BD Biosciences	Cat#212720
Yeast Nitrogen Bases without amino acids	Difco	Cat#291920
Glucose/Dextrose	Fisher Bioreagents	Cat#D16-10
Bacto Agar	Difco	Cat#214010
Potassium Acetate	Fisher Bioreagents	Cat#P171-500
Adenine	Sigma-Aldrich	Cat#A8626
Alanine	Sigma-Aldrich	Cat#A7627
Arginine	Sigma-Aldrich	Cat#A5006
Asparagine	Sigma-Aldrich	Cat#A0884

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Aspartic acid	Sigma-Aldrich	Cat#A9256
Cysteine	Sigma-Aldrich	Cat#168149
Glutamine	Sigma-Aldrich	Cat#G3126
Glutamic acid	Sigma-Aldrich	Cat#G1251
Glycine	Sigma-Aldrich	Cat#G8898
Histidine	Sigma-Aldrich	Cat#H8000
Isoleucine	Sigma-Aldrich	Cat#I2752
Lysine	Sigma-Aldrich	Cat#L5501
Methionine	Sigma-Aldrich	Cat#M9625
Myoinositol	Sigma-Aldrich	Cat#I5125
Phenylalanine	Sigma-Aldrich	Cat#P2126
Proline	Sigma-Aldrich	Cat#P0380
Serine	Sigma-Aldrich	Cat#S4500
Threonine	Sigma-Aldrich	Cat#T8625
Tryptophan	Sigma-Aldrich	Cat#T0254
Tyrosine	Sigma-Aldrich	Cat#T3754
Uracil	Sigma-Aldrich	Cat#U0750
Valine	Sigma-Aldrich	Cat#V0500
Leucine	Sigma-Aldrich	Cat#L8000
4-Aminobenzoic acid	Sigma-Aldrich	Cat#A9878
Lithium acetate dihydrate	Sigma-Aldrich	Cat#L6883
Salmon sperm DNA	Sigma-Aldrich	Cat#D1626
PEG (MW 3350)	Sigma-Aldrich	Cat#1546547
Paraformaldehyde	Sigma-Aldrich	Cat#158127
Agarose	Bio-Rad	Cat#1613102
Potassium chloride	Sigma-Aldrich	Cat#P3911
Sodium Chloride	EMD	Cat#7647-14-5
Di-sodium hydrogen phosphate anhydrous	Sigma-Aldrich	Cat#1065590500
Potassium phosphate monobasic	Sigma-Aldrich	Cat#P5655
Tris base	Sigma-Aldrich	Cat#T1503
EDTA	Sigma-Aldrich	Cat#E9884
Sodium hydroxide (0.1 N)	Thermo Scientific	Cat#124190010
Experimental models: Organisms/strains		
MATa/α, P_{TUB1}-GFP-TUB1:URA3/+, ZIP1-GFP/+, SPC42-mCherry:kanMX/+, ndt80::kanMX/ ndt80:: kanMX, $P_{NDT80\text{-}mse1\Delta, \ mse1\Delta}$NDT80:HIS3/ $P_{NDT80\text{-}mse1\Delta, \ mse1\Delta}$NDT80:HIS3, ADE2/ADE2, TRP1/TRP1	Lacefield Lab	LY4273
Recombinant DNA		
Yeast Genome Tiling Collection	Jones et al. (2008)	
2μ plasmid with LEU2	Gavade et al. (2022)	pLB227
2μ plasmid with NDT80, LEU2	Gavade et al. (2022)	pLB225
Software and algorithms		
NIS Elements Viewer v4.20.00 (Build972) LO, 32 bit	Nikon	https://www.nikoninstruments.com/Products/Software/NIS-Elements-Advanced-Research/NISElements-Viewer
FIJI (ImageJ)	National Institutes of Health (Public Domain)	https://imagej.nih.gov/ij
Other		
Zyppy™-96 Plasmid Miniprep Kit	Zymo Research	Cat#D4043
96-well blocks	Zymo Research	Cat#P1001-10
96-well round bottom plates	Sigma-Aldrich	Cat#CLS3799
Reagent reservoirs	Thermo Fisher Scientific	Cat#95128095
Air permeable sealing cover	Zymo Research	Cat#C2011-8
10 cm petri dish	Fisher Scientific	Cat#S33580A
Micro stir bars	Fisher Scientific	Cat#14-511-82

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
1 mL 8-channel pipette	Rainin	Cat#17014496
200 μL 8-channel pipette	Rainin	Cat#17013805
20 μL 8-channel pipette	Rainin	Cat#17013803
96-well glass bottom plate for imaging	Cellvis	Cat#P96-1.5H-N
96-well flat bottom plates	Sigma-Aldrich	Cat#CLS3997
Nikon immersion oil	Nikon	Cat#MXA22168
Centrifuge for 96-well plate: Allegra 25R	Beckman Coulter	Cat#369434
Baffled flasks, 125 mL	DWK Life Sciences	Cat#25630125
Baffled flasks, 250 mL	DWK Life Sciences	Cat#25630250
Filter bottles, 0.2 μm, 500 mL	Nalgene	Cat#569-0020
Filter bottles, 0.2 μm, 1 L	Nalgene	Cat#567-0020
Filter bottles, 0.45 μm, 1 L	Nalgene	Cat#167-0045
Filter bottles, 0.45 μm, 115 mL	Nalgene	Cat#124-0045
Refrigerated Benchtop Centrifuge	VWR	Cat#10830-768
Microscopy system: Nikon TiE inverted microscope with a SNAPHQ2 CCD camera (Photometrics) and a 60× oil objective (PlanAPO VC, 1.4NA) with filters for mCherry and GFP.	Nikon	

MATERIALS AND EQUIPMENT

YPD liquid medium

Reagent	Final concentration	Amount
Bacto peptone	2% (w/v)	20 g
Bacto yeast extract	1% (w/v)	10 g
Dextrose	2% (w/v)	20 g
ddH$_2$O		Up to 1 L

Autoclave media at 15 psi for 20 min. Add 10 mls of 1% Tryptophan and 10 mls of 1% adenine after autoclaving. Store at room temperature for up to 1 year.

Lithium acetate solution

Reagent	Final concentration	Amount
Lithium acetate dihydrate	1 M	10.2 g
ddH$_2$O		Up to 100 mL

Sterilize by autoclaving at 15 psi for 20 min or with filtration. Store at room temperature for up to 1 year.

Tris Cl (pH 7.5)

Reagent	Final concentration	Amount
Tris base	1 M	6.05 g
ddH$_2$O		Up to 50 mL

Adjust pH to 7.5. Sterilize by autoclaving at 15 psi for 20 min or with filtration. Store at room temperature for up to 1 year.

EDTA (pH8.0)

Reagent	Final concentration	Amount
EDTA	0.5 M	18.61 g
ddH$_2$O		Up to 100 mL

Adjust pH to 8.0 with Sodium hydroxide. Sterilize by autoclaving at 15 psi for 20 min or with filtration. Store at room temperature for up to 1 year.

Tris EDTA (TE buffer)

Reagent	Final concentration	Amount
TrisCl (pH7.5)	10 mM	1 mL of a 1 M stock
EDTA (pH8.0)	1 mM	0.2 mL of a 0.5 M stock
ddH$_2$O		Up to 100 mL

Store at room temperature for up to 1 year.

Single stranded carrier DNA (2 mg/mL)

Reagent	Final concentration	Amount
Salmon sperm DNA	2 mg/mL	200 mg
Sterile Tris EDTA		Up to 100 mL

Dissolving the salmon sperm DNA takes several hours with stirring. Make 1 mL aliquots and store at −20°C for up to 1 year.

50% PEG

Reagent	Final concentration	Amount
PEG (MW 3350)	50% (w/v)	50 g
ddH$_2$O		Up to 100 mL

Dissolving PEG in ddH$_2$O takes several hours with stirring. Filter sterilize with a 0.45 μM filter. Alternatively, this solution can also be autoclaved at 15 psi for 20 min. Store at room temperature for up to 1 year.

Note: Tightly close the lid of the PEG bottle. Otherwise, the solution could evaporate and cause an increased PEG concentration and a lower transformation efficiency.

1% Tryptophan

Reagent	Final concentration	Amount
Tryptophan	1% (w/v)	1 g
ddH$_2$O		Up to 100 mL

Stir until it goes into solution. Sterilize by filtration and store covered in foil at 4°C for up to 1 year.

1% Adenine

Reagent	Final concentration	Amount
Adenine	1% (w/v)	1 g
ddH$_2$O		Up to 100 mL

Stir until it goes into solution, which may require low heating. Sterilize by filtration and store at room temperature for up to 1 year.

Amino acid mix (-leu)

Reagent	Final concentration	Amount
Adenine	4.7% (w/total)	2 g
Arginine	4.7% (w/total)	2 g
Asparagine	4.7% (w/total)	2 g
Aspartic acid	4.7% (w/total)	2 g
Cysteine	4.7% (w/total)	2 g
Glutamine	4.7% (w/total)	2 g
Glutamic acid	4.7% (w/total)	2 g
Glycine	4.7% (w/total)	2 g

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Reagent	Final concentration	Amount
Histidine	4.7% (w/total)	2 g
Isoleucine	4.7% (w/total)	2 g
Lysine	4.7% (w/total)	2 g
Methionine	4.7% (w/total)	2 g
Myoinositol	4.7% (w/total)	2 g
Phenylalanine	4.7% (w/total)	2 g
Proline	4.7% (w/total)	2 g
Serine	4.7% (w/total)	2 g
Threonine	4.7% (w/total)	2 g
Tryptophan	4.7% (w/total)	2 g
Tyrosine	4.7% (w/total)	2 g
Uracil	4.7% (w/total)	2 g
Valine	4.7% (w/total)	2 g
4-Aminobenzoic acid	4.7% (w/total)	1 g

Mix the amino acids well and store at room temperature in a dark container or wrapped in foil for up to 1 year.

SCD -leu plates

Reagent	Final concentration	Amount
Yeast Nitrogen Bases without amino acids	0.67% (w/v)	6.7 g
Amino acid dropout mix (-leu)	0.2% (w/v)	2 g
Dextrose	2% (w/v)	20 g
Bacto Agar	2% (w/v)	20 g
ddH$_2$O		Up to 1 L

Autoclave at 15 psi for 20 min. Add 10 mls of 1% Tryptophan and 10 mls of 1% adenine after autoclaving. Pour into petri dishes. Leave plates at room temperature for 1–2 days to solidify. Then store at 4°C wrapped and upside down for up to two months.

> *Note:* Depending on the autoclave and timing, adding the dextrose prior to autoclaving can sometimes affect the gelling of the agar plates or can carmelize the sugar. The dextrose can also be made into a 40% filter sterilized stock and then added after autoclaving.

SCD -leu liquid media

Reagent	Final concentration	Amount
Yeast Nitrogen Bases without amino acids	0.67% (w/v)	6.7 g
Amino acid dropout mix (-leu)	0.2% (w/v)	2 g
Dextrose	2% (w/v)	20 g
ddH$_2$O		Up to 1 L (after adding Trp and Leu)

Autoclave media at 15 psi for 20 min. Add 10 mls of 1% Tryptophan and 10 mls of 1% adenine after autoclaving. Store at room temperature for up to 3 months.

SCA -leu liquid media

Reagent	Final concentration	Amount
Yeast Nitrogen Bases without amino acids	0.67% (w/v)	6.7 g
Amino acid dropout mix (-leu)	0.2% (w/v)	2 g
Potassium acetate	2% (w/v)	20 g
ddH$_2$O		Up to 1 L

Autoclave media at 15 psi for 20 min. Add 10 mls of 1% Tryptophan and 10 mls of 1% adenine after autoclaving. Store at room temperature for up to 3 months.

Sporulation medium (SPM medium)

Reagent	Final concentration	Amount
Potassium acetate	1% (w/v)	10 g
ddH$_2$O		Up to 1 L

Autoclave media at 15 psi for 20 min. Add 10 mls of 1% Tryptophan and 10 mls of 1% adenine after autoclaving. Store at room temperature for up to 3 months.

2× SCD -leu liquid media

Reagent	Final concentration	Amount
Yeast Nitrogen Bases without amino acids	1.34% (w/v)	6.7 g
Amino acid dropout mix (-leu)	0.4% (w/v)	2 g
Dextrose	4% (w/v)	20 g
ddH$_2$O		Up to 500 mL

Autoclave media at 15 psi for 20 min. Add 5 mls of 1% Tryptophan and 5 mls of 1% adenine after autoclaving. Store at room temperature for up to 3 months.

Phosphate buffered saline (PBS) pH7.4

Reagent	Final concentration	Amount
NaCl	137 mM	8 g
KCl	2.7 mM	0.2 g
Na$_2$HPO$_4$	8 mM	1.14 g
KH$_2$PO$_4$	1.5 mM	0.2 g
ddH$_2$O		Up to 1 L

Adjust pH to 7.4. Filter sterilize with a 0.2 μM filter. Store at room temperature for up to 1 year.

Paraformaldehyde solution

Reagent	Final concentration	Amount
Paraformaldehyde (PFA)	4% (w/v)	4 g
Phosphate buffered saline (PBS)		Up to 100 mL

Dissolve PFA in PBS by stirring. Filter sterilize the solution with a 0.2 μM filter. Store at 4°C for up to 1 week.

Agarose pad

Reagent	Final concentration	Amount
Agarose	1.2% (w/v)	0.6 g
Phosphate buffered saline (PBS)		Up to 50 mL

Heat the mixture in a microwave to dissolve the agarose in PBS. Watch carefully so that it does not boil over. Let the agarose cool for one minute. Cut the bottom of a pipet tip to more easily aspirate the agarose and then immediately add 150 μL of the molten agarose to each well of a flat bottom sterile 96-well plate at room temperature.

STEP-BY-STEP METHOD DETAILS
Overall method summary

⏱ Timing: 1 week

We describe a systematic high-copy suppression screen using fluorescence microscopy to identify regulators of meiosis and meiotic commitment. First, a yeast strain is transformed in 96-well plates with individual plasmids from the Yeast Genomic Tiling Collection, which consists of 1588 2μ plasmids encompassing 92% of the yeast genome (Gavade et al., 2022; Gietz, 2014; Jones et al., 2008; Tsuchiya et al., 2014). Second, meiosis is induced in 96-well plates. For screens for meiotic

commitment, nutrient-rich medium is added after cells have passed the meiotic commitment point. The cells are incubated for several hours to allow cells to either complete meiosis or return to mitosis. Finally, the cells are fixed, imaged, and scored for the phenotype of interest.

High-throughput transformation of the overexpression library

This step details the transformation of individual plasmids from the overexpression library into yeast in a 96-well dish format.

> *Note:* Use of the Yeast Genomic Tiling Collection will require the transformation of 1588 plasmids in approximately 17 96-well plates. This protocol is written for transformation of one plate but should be scaled to the number of plates that the operator feels comfortable undertaking so that the screen can be performed faster.

> *Note:* Sterile technique is required throughout the protocol. All glassware should be autoclaved and kept sealed prior to use. All reagents and media should either be autoclaved or filtered (as noted).

1. Inoculate a 20 mL culture of YPD with a colony of your yeast strain of interest and let grow for 12 h at 30°C with agitation.

> *Note:* We prefer to use 125 mL glass culture flasks with baffles for 20 mL cultures and 250 mL glass culture flasks with baffles for 50 mL cultures to allow efficient culture aeration. Ensure that the incubation has a constant source of agitation. We use a platform shaker set to 200 RPM.

2. Dilute 2.5×10^8 cells in 50 mL of pre-warmed YPD.
   a. Incubate cells at 30°C for 4 h with constant agitation.
3. Boil the salmon sperm DNA for 5 min and then put on ice.
4. Prepare 35 µL of the transformation mix for each well, add 15 µL of 1 M Lithium acetate and 20 µL of the boiled salmon sperm DNA. Keep this mix on ice until use.

> *Note:* Since 35 µL of transformation mix is added to each well, calculate the amount of mix required for adding to each well of the 96-well plate and prepare a master mix of the transformation mix.

5. Transfer 200 µL of the cell suspension into each well of a 96-well round bottom plate.

> *Note:* The use of a multichannel pipette and sterile reagent reservoir can speed up the allocation of media/reagents into each well of a 96-well plate.

6. Centrifuge the 96-well plate at 1,300 g for 10 min.
7. Aspirate the supernatant using a multi-channel pipette.
8. Add 5 µL of miniprep DNA from the Yeast Genomic Tiling Collection to each well (100–400 ng per µL).
9. Add 35 µL of the transformation mix (15 µL of 1 M Lithium acetate + 20 µL of 2 mg/mL salmon sperm DNA) to each well.
10. Add 100 µL of 50% PEG to each well using a multi-channel pipette.

> *Note:* 50% PEG is viscous and requires slow pipetting to ensure accurate volumes. Check your pipette tips for the correct amount of fluid prior to adding to the wells.

> *Note:* Only use the PEG at molecular weight 3350. Other molecular weights do not allow efficient transformation.
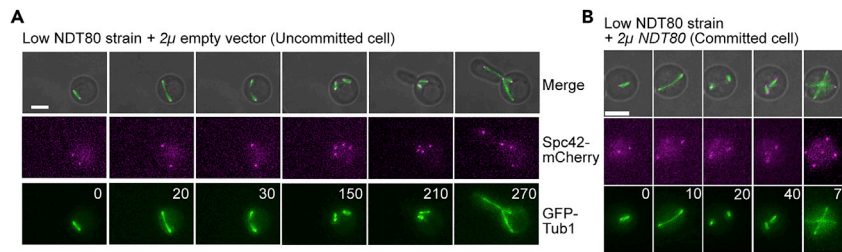
Figure 1. Controls used for our experiment

(A) As a negative control, the low Ndt80 strain with an empty vector plasmid is unable to stay committed to meiosis with the addition of nutrient-rich medium (t=0). The cell shown undergoes meiosis I and then buds and forms two spindles.

(B) As a positive control, the low Ndt80 strain with a plasmid containing *NDT80* will stay committed to meiosis upon addition of nutrient-rich medium (t=0). The cell shown completes meiosis, even in the presence of nutrient-rich media. Scale bar 5 μm.

11. Cover the 96-well plate and shake for 10 min at 75 rpm.

    Note: This step will ensure mixing of contents in each well.

12. Incubate at 42°C for 2 h.
13. Centrifuge the plate at 1,300 g for 10 min.
14. Aspirate the supernatant.
15. Resuspend the cell pellet in 10 μL of sterile water by slowly pipetting up and down.
16. Spot 5 μL of cells on SCD-leu plates.
    a. Incubate the plates at 30°C for 2–4 days.

    Note: We used 100 mm petri plates and spotted 48 wells (6 columns × 8 rows) on 1 plate. One 96-well plate was spotted onto 2 SCD-leu plates. Alternatively, rectangular petri plates can be used to spot all 96-wells onto 1 plate.

    Note: You should have confluent growth of the spots after the incubation.

    ⧖ Pause point: You can store the spotted SCD-leu plates at 4°C for 1–2 weeks after step 13. Plates should be stored upside down, wrapped with parafilm. However, if a strain other than W303 is used, the strain should be checked that it remains a stable diploid during this time and does not go petite.

    △ CRITICAL: A positive and negative control should be added to the genetic screen. As a negative control, we transformed cells with a 2μ plasmid that did not have additional yeast genes. As a positive control, we added a 2μ plasmid with *NDT80,* which will rescue meiotic commitment in the low Ndt80 strain (Figures 1A and 1B).

Inducing meiosis in cells in 96-well plates

⏱ Timing: 4 days

This step describes the culturing of yeast cells to initiate meiosis prior to imaging.

17. Add 1 mL of SCD-leu to each well of a sterile 96-well block.

    Note: The medium and the block should be pre-warmed to 30°C.

18. Using a pipette tip, scrape the cells from a single spot on the SCD-leu plates and swirl the tip into the appropriate well of the 96-well block from step 17.

   Note: To reduce errors, the cells on the spots should be picked up from the pipette tips of a multichannel pipette by carefully lifting a portion of the colony and inoculating it in the SCD-leu containing block.

19. Cover with an air permeable seal. Incubate the 96-well block at 30°C for 24 h.

   Note: The block can be kept sitting without agitation.

20. In an empty sterile block, add a 2 mm × 5 mm sterile magnetic stir bar to each well of a sterile 96-well block.

   Note: The 2 mm × 5 mm magnetic stir bars fit within the 96-well blocks and agitates the cells on the shaker. Alternatively, glass beads can be used instead of the magnetic stir bars to agitate the yeast within each well.

21. Add 1 mL of SCA-leu to each well in the block.

   Note: The medium and the block should be pre-warmed to 30°C.

22. Transfer 40 μL of SCD-leu grown cells from step 17 into each well of the 96-well block.
   a. Cover the block with a sterile air permeable cover.
   b. Incubate the block at 30°C for 15 h on a shaker set at 200 RPM.

   Note: Alternatively, you can use a stir plate to agitate the cells for facilitating proper aeration.

   Note: We taped the blocks onto a shaker. However, 96-well plate clamps can also be used to clamp the plates on the shaker.

23. Spin the 96-well block at 1,300 g for 1 min.
24. Aspirate the supernatant using a multi-channel pipette.
25. Wash the cells twice with 1 mL of sterile water, by adding the water, spinning down the cells and aspirating after each wash.
26. Resuspend the cells in 1 mL of SPM by pipetting up and down.
27. Seal the block with a sterile air permeable cover.
28. Incubate the cells at 25°C on a shaker set at 200 RPM until the cells reach the stage of meiosis that you are interested in observing.

   Note: The time will vary depending on the stage of meiosis that you are studying. Furthermore, both the time and temperature will vary depending on the yeast strain that you are using.

29. For analysis of meiotic commitment or return-to-growth, spin the 96-well block at 1,300 g for 1 min. Discard the supernatant and resuspend the cells in 1 mL of 2×SCD -leu media.
   a. Place the block on the magnetic stir plate at 25°C and stir at a low speed for 8 h.

   Note: This step is only for studying meiotic commitment or return-to-growth. SCD medium should not be added to cells if studying the normal process of meiosis.

   Note: Alternatively, plates can be placed on a shaker at low speed.

30. Centrifuge the block at 1,300 g for 1 min.

31. Aspirate the supernatant, removing 950 µL of media.
    a. Resuspend the cells in the remaining 50 µL of media.
32. Transfer the cells to a 96-well round bottom plate.
33. To fix the cells, add 150 µL of freshly prepared 4% paraformaldehyde.

    Note: Paraformaldehyde is toxic and gloves should be worn when handling.

    Note: Fixation times can vary depending on the fluorescent markers. We fixed the cells for 10 min.

34. Centrifuge the 96-well plate from step 33 at 1,300 g for 1 min.
35. Discard the paraformaldehyde and resuspend the cells in 150 µL of sterile PBS.

    Note: The paraformaldehyde needs to be collected in a hazardous waste container for proper disposal.

36. Centrifuge the plate at 1,300 g for 1 min.
37. Discard the supernatant and resuspend the cells in 150 µL of sterile PBS.

    ⅠⅠ **Pause point:** The plates can be stored at 4°C for 2–3 weeks. Wrap the plates with an aluminum foil to decrease the loss of the fluorescence signal.

Imaging cells in 96-well plates

⏱ **Timing: 1–5 days depending on the number of plates to image**

This step outlines the imaging of cells from each well of a 96-well glass-bottom plate.

38. Add 5 µL of fixed cells to each well of a 96-well imaging plate.

    Note: The 96-well plates should be specific for imaging, such as those with a cover glass bottom. We used Cellvis plates (see key resources table).

    Note: It is important to spread cells into a monolayer so that individual cells can be easily imaged. We spread out the cells by adding a premade agar pad to the top of the cells. We heat 1.2% agarose in 1× PBS (pH 7.4) in the microwave until the agarose goes into solution. We then add 150 µL of the molten agarose into each well of a clean flat-bottom 96-well plate. Once the agarose is solidified, this plate is inverted and placed over the 96-well plate with the cells, such that the agar pad can transfer to the wells with the cells. The plates are centrifuged at 100 g for 1 minute to transfer the agarose pad from the top plate to over the cells.

39. Image cells on a fluorescence microscope.
    a. Scan through each well and select at least 10 fields per well for imaging.

    Note: For the imaging, using brightfield or DIC, identify fields in which the cells form a monolayer and are on the same focal plane.

    b. Image using the appropriate excitation wavelengths for the fluorescent tags within the strains. Take at least five z-steps for each wavelength (1.2 µm). Also include a brightfield or DIC image.

    ⚠ CRITICAL: Exposure time must be optimized for your imaging system and tagged proteins of interest to ensure adequate signal and to avoid photobleaching. These parameters should be identified prior to the high-throughput imaging.

Note: This protocol is written for use of an inverted fluorescence microscope to image cells at the bottom of the wells of a glass-bottom 96-well plate.

Note: We used a Nikon Eclipse Ti inverted fluorescence microscope and a 60× oil objective (PlanAPO CB, 1.4NA) with filters for mCherry and GFP. We moved to each well and found 10 fields within each well to image with 5 z-stacks of both mCherry and GFP. It took us approximately 4 h to image each plate. However, use of an automated high-throughput screening fluorescence microscope can save time during image collection.

Analysis

⏱ Timing: 1–5 days depending on the number of plates imaged

This step describes how to analyze the cells after imaging.

40. Analyze the acquired images for the phenotype of interest using a software system compatible with the images taken on the microscope (like Nikon NIS Elements or ImageJ).

    Note: For our analysis we used Nikon NIS Elements. We opened each image set and made a maximum projection image from the z-stacks, overlaying GFP and mCherry fluorescence. We used the DIC image to ensure that we were analyzing cells in a monolayer and to see whether cells had budded.

    Note: In our screen, we were looking for cells that stayed committed to meiosis. Therefore, we scanned 10-fields per-well of the 96-well plates and counted the number of cells that had remained in meiosis, and those that had formed buds.

EXPECTED OUTCOMES

Execution of this protocol will yield a series of images of cells in each well. The images are then analyzed for whether the cells have suppressed the meiotic phenotype of interest. In our screen, we analyzed for rescue of meiotic commitment in the low Ndt80 strain. Normally, the low Ndt80 strain arrests in metaphase II and after nutrient addition, the cells will exit meiosis and undergo return-to-growth. Our screen asked for whether overexpression of genes could prevent cells from undergoing return-to-growth. Cells with plasmids that rescued the defect in meiotic commitment did not bud and either remained in meiosis or completed meiosis, which we could score with the fluorescently-tagged proteins GFP-Tub1 and Spc42-mCherry (Figure 2A). We scored for return-to-growth through the presence of budded cells in the brightfield images, which were also present in the negative control (the 2μ with no additional yeast genes; Figure 2B).

After performing the initial screen, the positive hits should be screened again and re-analyzed for the phenotype of interest. In our screen, we pursued only the positive hits that remained positive in the secondary screen. The Yeast Tiling Collection plasmids have several genes on each plasmid. Therefore, the plasmid needs subcloned to only contain one gene with its regulatory regions. The plasmids should then be transformed into the original starting strain and screened for the rescue of the phenotype of interest.

LIMITATIONS

This screen is designed to identify high-copy suppressors, and requires that the positive hits have a clear phenotype in a large fraction of the cells. Our screen for rescue of meiotic commitment had an easily observable phenotype of cells that either budded or remained in meiosis (unbudded with
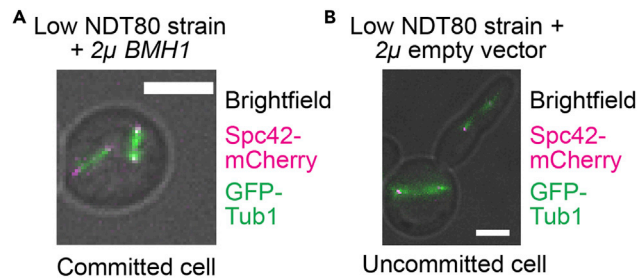
Figure 2. Expected results

(A) Positive hits in the screen will have cells that remain committed to meiosis with the addition of nutrient-rich medium in metaphase II. For example, low Ndt80 cells with a 2μ plasmid with *BMH1* stay committed to meiosis and arrest at metaphase II upon addition of nutrient-rich medium.

(B) Negative hits in the screen will have cells that fail to commit to meiosis and will undergo bud formation with the addition of nutrient-rich medium, such as this cell with an empty vector. Scale bar 5 μm.

meiotic spindles). Choosing useful fluorescently tagged proteins is important for the analysis of the phenotypes.

TROUBLESHOOTING

Problem 1

Transformation is not efficient (step-by-step method details: high-throughput transformation of the overexpression library).

Potential solution

Parameters of the transformation may need adjusted to the strain. These parameters include the time of growth in YPD, time at 42°C, and speed of spinning cells down.

Cells may need an additional growth step in SC after the heat shock at 42°C to allow recovery from the heat shock and to accumulate leucine prior to spotting on SC-leu plates.

Reagents such as the ssDNA and 50% PEG solution may need replaced.

Problem 2

Cells are dying after shaking (step-by-step method details: inducing meiosis in cells in 96-well plates).

Potential solution

The shaking speed may need adjusted. Shaking too vigorously with the magnetic stirrer or glass beads may cause the cells to die. Not enough shaking can also cause problems with meiotic induction.

Problem 3

Cells are not entering meiosis efficiently (step-by-step method details: inducing meiosis in cells in 96-well plates).

Potential solution

The cells need aeration to enter meiosis. Be sure to use air permeable covers and adjust the shaking speed.

Cells that are auxotrophic for amino acid production may need those amino acids supplemented in the SPM.

Problem 4

A monolayer of cells is not present during imaging (step-by-step method details: imaging cells in 96-well plates).

Potential solution

Decrease the density of the cell suspension for the imaging.

Problem 5

The fluorescent signal from the fluorescently tagged protein is insufficient (step-by-step method details: imaging cells in 96-well plates)).

Potential solution

Optimize the parameters for imaging (exposure time and percent exposure) for the protein of interest.

Use an alternate fluorophore. We typically use fluorescently-tag proteins with GFP- based fluorescent proteins due to their brightness and photostability.

Utilize a microscope or camera with higher sensitivity.

Pick a more abundant protein to fluorescently tag and image.

Included multiple copies of the fluorescent protein (e.g., 2×GFP, 3×GFP).

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Soni Lacefield (sonil@indiana.edu).

Materials availability

The budding yeast strains used in this protocol will be made available upon request without any restriction.

Data and code availability

This paper does not report original code.

The screen data obtained from this protocol are reported in (Gavade et al., 2022).

Any additional information required for working with this protocol will be provided by the lead contact upon request.

AUTHOR CONTRIBUTIONS

S.L. and J.N.G. collected, analyzed all the data, and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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