

## Synchronization of *Saccharomyces cerevisiae* Cells in G1 Phase of the Cell Cycle

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**[Abstract]** The baker's yeast, *Saccharomyces cerevisiae* is a widely used model organism in molecular biology because of the high homology it shares with human cells in many basic cellular processes such as DNA replication, repair, recombination, transcription, and because of the ease its genome can be manipulated. Other advantages of working with yeast are its fast production rate which is comparable to bacteria's, and its cheap maintenance.

To examine certain phenomena, for example whether a mutation affects the passage through a cell cycle phase, it can be necessary to work with a yeast culture, in which all the cells are in the same phase of the cell cycle. Yeasts can be arrested and kept in different phases of the cell cycle. Here we describe the method that allows synchronizing and keeping yeast cells in the G1 phase of the cell cycle with the mating pheromone,  $\alpha$ -factor. Only MAT $\alpha$  cells can be synchronized with  $\alpha$ -factor which is produced by MAT $\alpha$  cells. It is highly recommended to use a MAT $\alpha$  *bar1* deletion strain. The *BAR1* gene encodes for an extracellular protease that inactivates  $\alpha$ -factor by cleaving it (MacKay *et al.*, 1988). To counteract the Bar1 protease activity when using *BAR1* cells, 100-1,000 times more  $\alpha$ -factor is needed as compared to *bar1* deletion cells ( $\alpha$ -factor is quite expensive!), and still the synchrony will be transient. In contrast, *bar1* deletion cells can be kept in G1 phase with  $\alpha$ -factor for several hours, and the degree of synchronization is usually higher than using a *BAR1* strain. Moreover, *bar1* deletion cells can be synchronized even at high cell density, whereas *BAR1* cells, due to the activity of the secreted Bar1 protease, only at low cell density.

### **Materials and Reagents**

1. MAT $\alpha$  *bar1* deletion strain (can be engineered in the lab, or purchased from stock repositories like EUROSCARF)
2. Yeast extract
3. Peptone
4. D-glucose
5. 95% ethanol

6. Yeast extract-peptone-dextrose (YPD) media (see Recipes) (or other media needed for the strain in use)
7.  $\alpha$ -factor ( $\alpha$ -mating factor acetate salt) (Sigma-Aldrich, catalog number: T6901-5MG) (see Recipes)
8. Pronase (protease, from streptomyces griseus) (Sigma-Aldrich, catalog number: P6911-5G) (see Recipes)

### **Equipment**

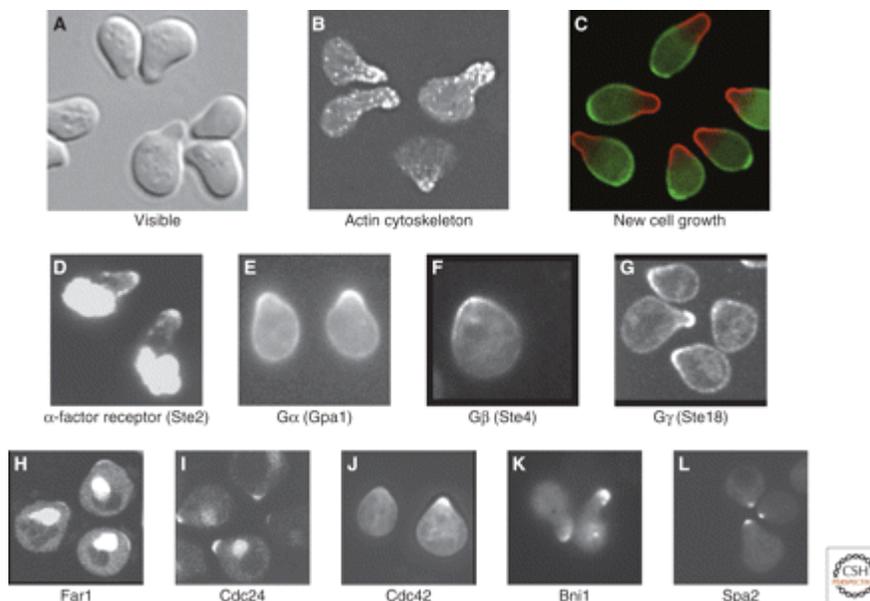
1. 30 °C incubator-shaker (180-200 rounds per minute)
2. Microscope
3. Glass slide
4. Coverslips
5. Centrifuge
6. Spectrophotometer
7. Vortex
8. Culture tube
9. Culture flask

### **Procedure**

1. Grow up a small overnight starter culture inoculated from one single colony from a YPD plate in YPD in a culture tube (180 rpm, 30 °C). Usually 1/10<sup>th</sup> of the final working culture is enough for the starter.
2. From the overnight culture inoculate into fresh media to get OD<sub>600</sub>:0.2 in a culture flask or tube (this is your working culture with its working volume).
3. Grow the culture to get the required amount of cells, but do not go higher than OD<sub>600</sub>:0.8 because at very high density even *bar1* deletion cells do not synchronize. Keep in mind that during synchronization the density of the culture will increase since cells that already passed G1 will complete the cycle until they reach G1 of the next cycle.
4. Spin down the cells and discard the media (3 min with 3,000 rpm at room temperature).
5. Wash the cell pellet with at least 10 cell pellet volumes of water.
6. Spin down the cells and discard the water (3 min, 3,000 rpm RT).
7. Resuspend the cells in working volume of fresh YPD containing 50 ng/ml  $\alpha$ -factor.
8. Grow the cells.

9. After 60, 90, 120 min check the cells under the microscope with 40x magnification. Before checking vortex the samples thoroughly to disturb clumps. G1 arrested cells have typical pear shape. Usually between 90 and 120 min ~100% synchrony can be observed.
10. To release the cells from the arrest, spin down the cells and wash them with water.
11. Repeat washing.
12. Resuspend the cells in the working volume of fresh YPD containing 50 µg/ml pronase. It is important to add pronase because even a remaining small amount of  $\alpha$ -factor can inhibit release.
13. After adding pronase cells will enter the cell cycle. Depending on your goal, you can let them cycle, treat them with different agents, or collect them at any desired time points. Pronase does not affect cell growth.

This experiment is easy to reproduce once you can recognize arrested cells (Figure 1). If your strain has a selectable marker, you can carry out the experiment using the appropriate media instead of YPD.



**Figure 1. Yeast cells treated with  $\alpha$ -factor.** (Source: Arkowitz, R. A. (2009). [Chemical gradients and chemotropism in yeast](#). *Cold Spring Harb Perspect Biol*; doi: 10.1101/cshperspect.a001958)

## Recipes

1. YPD liquid
  - 1% yeast extract
  - 2% peptone

- 2% D-glucose
- 2.  $\alpha$ -factor
  - 1 mg/ml stock solution in EtOH
  - Stored at -20 °C
- 3. Pronase
  - 40 mg/ml stock solution in sterile distilled water
  - Stored at -20 °C

### **Acknowledgments**

This protocol is a modified form of the one published by Amberg *et al.* (2005). We used this protocol in our work (Daraba *et al.*, 2014). Funding support: Wellcome Trust, 070247/Z/03/A.

### **References**

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2. MacKay, V. L., Welch, S. K., Insley, M. Y., Manney, T. R., Holly, J., Saari, G. C. and Parker, M. L. (1988). [The \*Saccharomyces cerevisiae\* BAR1 gene encodes an exported protein with homology to pepsin](#). *Proc Natl Acad Sci U S A* 85(1): 55-59.