Video Article

# A Rapid Technique for the Visualization of Live Immobilized Yeast Cells

Karl Zawadzki[1], James Broach[1]

[1]Department of Molecular Biology, Princeton University

Correspondence to: Karl Zawadzki at kzawadzk@princeton.edu, James Broach at jbroach@princeton.edu

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## Abstract

We present here a simple, rapid, and extremely flexible technique for the immobilization and visualization of growing yeast cells by epifluorescence microscopy. The technique is equally suited for visualization of static yeast populations, or time courses experiments up to ten hours in length. My microscopy investigates epigenetic inheritance at the silent mating loci in S. cerevisiae. There are two silent mating loci, HML and HMR, which are normally not expressed as they are packaged in heterochromatin. In the sir1 mutant background silencing is weakened such that each locus can either be in the expressed or silenced epigenetic state, so in the population as a whole there is a mix of cells of different epigenetic states for both HML and HMR. My microscopy demonstrated that there is no relationship between the epigenetic state of HML and HMR in an individual cell. sir1 cells stochastically switch epigenetic states, establishing silencing at a previously expressed locus or expressing a previously silenced locus. My time course microscopy tracked individual sir1 cells and their offspring to score the frequency of each of the four possible epigenetic switches, and thus the stability of each of the epigenetic states in sir1 cells. See also Xu et al., Mol. Cell 2006.

## Video Link

The video component of this article can be found at http://www.jove.com/video/84/

## Protocol

1. Immobilized cells for time course epifluorescence microscopy. Please note that this protocol is only useful if the objective lenses of your microscope are below the microscope stage.

2. Place cells at desired concentration in liquid media on a long coverslip (roughly 24x50mm)

3. Cut an agar block of the desired size from a synthetic media plate (these have lower autofluorescence) and place over the cells. Place the side of the agar block not handled by tweezers in contact with the cells.

4. For shorter time courses (up to 3 hours), this set up is sufficient. If cells are moving when visualized on the microscope, decrease volume of cells or increase area of the agar block.

5. For longer time courses, place a drop of fresh liquid media on top of the agar block, and place a glass slide on top of the agar block. This slide reduces evaporation through the top of the agar block. If desired, the edges of the agar block in contact with the cover slip can be sealed with vaseline to further reduce moisture. I have used this set up for movies up to 9 hours long, with the cells dividing at wild type rates.

## Discussion

This is a rapid technique that immobilizes yeast cells while still allowing growth. We have followed yeast growth for up to ten hours, with the yeast displaying wild type division rates throughout the experiment. Note that this setup requires the objective lens to be below the microscope stage.

## Disclosures

The authors have nothing to disclose.

 

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## References

1. Xu E.Y., Zawadzki, K.A., and Broach, J.R. Single-cell observations reveal intermediate transcriptional silencing states. Mol. Cell 23, 219-229 (2006)

 