

Undergraduates Study Gene Expression in *S. cerevisiae* Using a Student-Optimized Protocol

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Karen Bernd is an Associate Professor of Biology at Davidson College. Her laboratory studies protein targeting and localization. Her Cell Biology course extends this interest into student-designed studies of signaling cascades and changes of protein localization during the

mating reaction of *Saccharomyces cerevisiae*. Davidson College, Biology Department, Box 7118, Davidson, NC 28035. Tel (704) 894-2889, Fax (704) 892-2512, E-mail [kabernd@davidson.edu](mailto:kabernd@ davidson.edu) Web page <http://www.bio.davidson.edu/Biology/kabernd/BerndCV/Lab/Berndhm.htm>



Victoria Statler graduated from Davidson College in 2003 and presently attends the University of Louisville Medical School.

Courses in Davidson College's Biology Department rely on investigative laboratories to teach biology through research experiences. My Cell Biology lab studies extracellular and intracellular signaling using *S. cerevisiae* mating as a model. Students characterize mating mutants using a variety of procedures, including expression of genes involved in the mating reaction cascade. Since this is a teaching lab and the students have varying research experience, an RNA isolation method that did not use caustic chemicals (phenol) was preferred. Previous RNA isolation methods did not produce reliably high yields for the students and did not fit conveniently into the lab session.

Last year Victoria Statler (now at University of Louisville Medical School) worked to optimize an RNA isolation that was safe and reliable for undergraduates, gave high RNA yields, and fit into a standard three-hour laboratory session. To determine the protocol that would work best in student hands, she used EPICENTRE's MasterPure™ RNA Purification Kit with several combinations of cell-wall disruption steps (lysis with

lyticase, bead beating, and freeze/thaw cycles). In the fall of 2003 my Cell Biology students used the optimized protocol, including bead beating and the MasterPure Kit, to study a collection of novel *S. cerevisiae* mating mutants.

EPICENTRE now offers the MasterPure™ Yeast RNA Purification Kit that releases RNA, which is largely free of DNA and protein, in about 40 minutes, without mechanical force or caustic reagents.

I have a collection of MATa *S. cerevisiae* mating mutants, isolated through UV mutagenesis. Working in seven groups, the nineteen Cell Biology students characterized the mating defect strains from this collection using morphology, complementation and gene expression studies. The gene expression studies relied on total RNA isolated from the mating mutant strains using Victoria's optimized protocol. Spectrophotometry was used to evaluate the purity and quantity of RNA. These experiments were done before the release of the MasterPure Yeast RNA Purification Kit and demonstrate the versatility of EPICENTRE products, a must when teaching budgets require that you get as much out of every reagent as possible.

Conclusion

On their first attempt, all seven lab groups isolated high purity samples (by OD 260/280 ratios). Samples were also evaluated by running 1 µg on 0.8% agarose, TAE gels containing EtBr and visualized with a Bio-Rad Fluor-S® Multilmager. This group of students, who had never used a UV spectrophotometer before, let alone isolate RNA, isolated fourteen samples of

high quality RNA with yields of 79.75 µg to 239.8 µg per prep. As a bonus, the entire isolation, including getting settled, the prep, RNA quantification, and cleanup were completed within a standard three-hour laboratory session. The students and I were proud of their yields. We found they had plenty of RNA to perform any of the additional experiments that they designed. In fact, I still have RNA to use for follow-up experiments in my research lab.

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