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Learning Upper Level Molecular Biology Using the Yeast Two-Hybrid (Y2h) System

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Abstract

A challenge with upper level biology labs is that experiments cannot normally be done in a 3-hour window. It can also be a challenge to stay motivated when doing labs with predetermined outcomes. Third, a “one size fits all” laboratory can be boring for those of us with previous experience. Finally, having to share a small lab space with many classmates simultaneously makes working efficiently difficult. An unexpectedly large enrollment in our molecular biology lab course provided our professor with an opportunity to use a class project to address the above concerns. One of our professors has a collection of clones from an initial Y2H screen for proteins that interact with a myosin protein. Each of us was assigned 3 clones in yeast that we characterized. This included plasmid isolation from yeast, transformation of E. coli, plasmid isolation from E. coli, DNA quantitation, restriction analysis, DNA sequencing, and BLAST analysis to identify and characterize our clones. We could not begin our research until we had verbally demonstrated understanding of the Y2H system to the instructor. We needed our lab notebook signed before each step in the process, which we could then do at any time the lab was open. Results were presented in a formal lab report. This approach allowed us to take ownership of our projects, troubleshoot problems, and learn techniques on our own. It also staggered the use of lab space and facilities, allowing more of us to work on our own time, while less experienced students could use the regular time block with more instructor availability. This lab was a positive learning experience; we got a better feel for the nature of scientific research, gained independence in the lab and were more invested in obtaining results.

Keywords

biology instruction, Dordt College, molecular biology, laboratory experiments, Y2H clones

Disciplines

Biology | Higher Education

Comments

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Abstract:

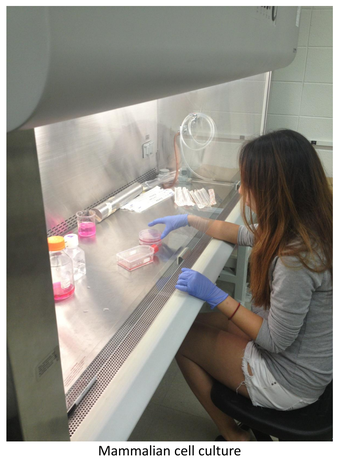
A challenge with upper level biology labs is that experiments cannot normally be done in a 3-hour window. It can also be a challenge to stay motivated when doing labs with predetermined outcomes. Third, a "one size fits all" laboratory can be boring for those of us with previous experience. Finally, having to share a small lab space with many classmates simultaneously makes working efficiently difficult. An unexpectedly large enrollment in our molecular biology lab course provided our professor with an opportunity to use a class project to address the above concerns. One of our professors has a collection of clones from an initial Y2H screen for proteins that interact with a myosin protein. Each of us was assigned 3 clones in yeast that we characterized. This included plasmid isolation from yeast, transformation of *E. coli*, plasmid isolation from *E. coli,* DNA quantitation, restriction analysis, DNA sequencing, and BLAST analysis to identify and characterize our clones. We could not begin our research until we had verbally demonstrated understanding of the Y2H system to the instructor. We needed our lab notebook signed before each step in the process, which we could then do at any time the lab was open. Results were presented in a formal lab report. This approach allowed us to take ownership of our projects, troubleshoot problems, and learn techniques on our own. It also staggered the use of lab space and facilities, allowing more of us to work on our own time, while less experienced students could use the regular time block with more instructor availability. This lab was a positive learning experience; we got a better feel for the nature of scientific research, gained independence in the lab and were more invested in obtaining results.

Challenges for upper level labs:

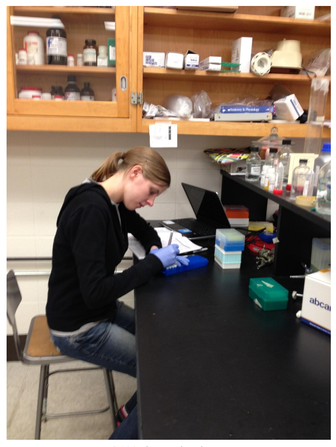
- Techniques can be difficult to master at first
- Experiments do not fit into a weekly 3-hour block
- Expensive, limited equipment cannot be used by everyone simultaneously
- Following protocols alone is not a helpful way to learn
- Want to do original research that fits into a semester

Learning benefits:

- Understanding several molecular biology techniques
- Took ownership of our projects
- Able to repeat and troubleshoot procedures if they didn't work the first time
- Became more independent in the lab
- Experience writing lab results into a scientific format



Mammalian cell culture

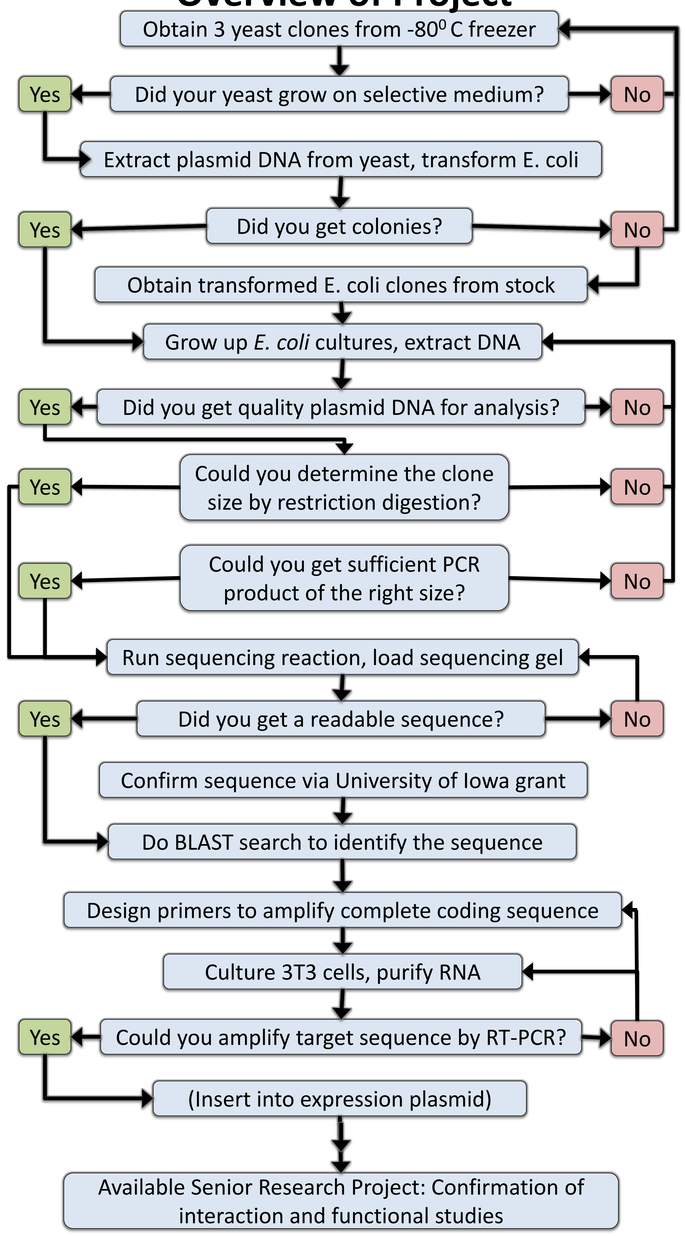


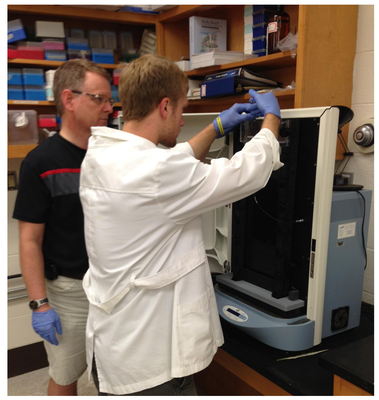
DNA Quantitation

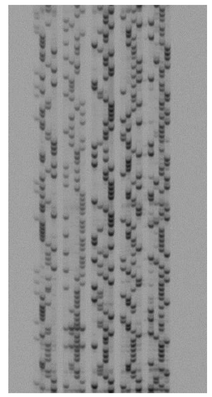
Yeast Two Hybrid Screen:

- Identifies proteins that bind *in vivo* to a protein of interest (myosin Va)
- Yeast colonies contain plasmids encoding this protein
- Plasmid DNA needs to be extracted and characterized

Overview of Project







DNA sequencing with the LI-COR® 4300 DNA Analysis System, using nonradioactive IRDye® labeled primers. Images were analyzed using e-Seq™ software and sequences were identified using BLAST

Other benefits:

- The lab was continuously open to give us more flexibility
- We could begin when we demonstrated understanding of the project, allowing us to work at our own pace
- The professor checked each procedure in our lab notebooks and was available for assistance when needed
- Able to help each other troubleshoot

Results of the project:

- Numerous candidate proteins were identified
- Potential for continuation of the project
- One set of clones was further characterized in a senior research project

Remaining challenges:

- Troubleshooting is time-consuming and frustrating
- Repeated failure dampens motivation
- Sharing resources risks contamination
- Very challenging for less independent students
- If this same research oriented, hands-on model is used for other upper level labs, multiple projects in one semester may overwhelm students

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