8-2-2013

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

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Recommended Citation
Wubben, Brandon; Buyert, Kim; De Nooy, Sam; Wilson, Nick; Jelsma, Tony N.; and Eppinga, Robbin, 'Learning Upper Level Molecular Biology Using the Yeast Two-Hybrid (Y2h) System' (2013). Faculty Work: Comprehensive List. Paper 186.
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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
Poster presented at the 5th Annual FUTURE in Biomedicine Symposium held on the campus of the University of Iowa in Iowa City, Iowa, August 2, 2013.

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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.

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

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
- Obtain 3 yeast clones from -80°C freezer
- Did your yeast grow on selective medium? No
- Extract plasmid DNA from yeast, transform E. coli
- Did you get colonies? No
- Obtain transformed E. coli clones from stock
- Grow up E. coli cultures, extract DNA
- Did you get quality plasmid DNA for analysis? No
- Could you determine the clone size by restriction digestion? No
- Could you get sufficient PCR product of the right size? No
- Run sequencing reaction, load sequencing gel
- Did you get a readable sequence? No
- Confirm sequence via University of Iowa grant
- Do BLAST search to identify the sequence
- Design primers to amplify complete coding sequence
- Culture 3T3 cells, purify RNA
- Could you amplify target sequence by RT-PCR? No
- (Insert into expression plasmid)
- Available Senior Research Project: Confirmation of interaction and functional studies

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

Acknowledgments:
- Jim Lin, University of Iowa, for Y2H clones
- “Better Futures for Iowa” grant for further sequencing on follow-up research
- Li-COR® for Genomics Education Matching Fund grant toward cost of DNA Analyzer
- Andreas Center for Research and Scholarship, Dordt College, to support follow-up research