

Instructor Tips and Notes for “Keys of Corn” project

This project is in its second year of development and is constantly being changed and updated. The materials provided are based largely on the 2007 fall semester. Below I provide some notes and tips in regards to the different experiments.

Week 1: Database Searching

1: There are no wet-lab experiments this week - students will learn about databases and database mining. They will search specifically for a full length cDNA clone for a novel transcription factor (TF) that will clone later in the semester. Note that updated GRASSISUS web database for Grass Transcription Factors will be available August 1st 2008 (www.GRASSISUS.org). For maize we avail of the cDNA collections deposited at The Arizona Genomics Institute (AGI) (www.genome.arizona.edu) - for other species one has to seek other locations.

2: Use the pre-lab lecture to carefully explain what the semester-long overall project is about explain they are doing something unique. Many students are used to labs being very routine and predictable. Many find the open-endedness or unknown outcome of their experiments somewhat frustrating. Here I emphasize that if they ever want to contribute new information to society they must be ready to approach the unknown.

3: If an instructor decides to participate in the Keys of Corn project and clone maize transcription factor genes, then please double check that you are cloning genes already cloned and placed in the GRASSSIUS database. We welcome the deposition of clone sin the GRASSSIUS collection where they will be made available to other researchers.

Week 2 Plasmid Isolation.

1: We obtained clones from the Arizona Genomics Institute for students to work with and will supply them for this lab.

2: Students generally don't think about the details of the techniques so use lecture to explain what is happening during a plasmid isolation and the care and attention needed in each step. Notes have pictures of actual lab equipment to help prepare them. This is usually a busy lab but we use a rapid plasmid isolation protocol (Eppendorf kit) so it goes a lot more quickly than the traditional methods.

3: Use codes to simplify labeling of tubes. Keep all plasmid samples and give to technician who will send them out for DNA sequencing and primer design.

Week 3 Open reading Frame Amplification using PCR

1: Students will amplify the coding region of the TF that they isolated two weeks ago. We supply the primers for each plasmid set. Student pairs will each work to isolate a different TF gene - uniqueness of work tends to generate interest and care.

2: Run PCR products from last week's experiment and analyze results using online database. Notes talk about traditional cloning and introduce new Gateway cloning technology that they will use in class.

3: Important to stress sterile technique (for *E. coli* transformation step) in lecture - have TAs demo the correct process at start of lab.

Week 4 Cloning into Entry Vector and Transformation of *E. coli*

1: Ligation and transformation of PCR products from last week. Again use labels that instructor provides. Keep all plates and in research lab. We usually isolate and sequence the entry clones in the research lab over the Thanksgiving break. Students will analyze these for PCR errors in last week and set up destination recombination reaction if time permits.

Week 5 Checking of Entry Clones

1: As stated above, we usually isolate the entry plasmids but depending on course schedule, students could also do this. Plasmids are sequenced at a sequencing facility and then the students learn to use software (Vector NTI® ContigExpress) to check that no errors occurred during PCR.

Week 6 Destination Clones

1: Students create destination clones and again transform *E. coli* - the recombination reaction is very short.

2: Students also perform the last computer assignment has them analyze their cloned TFs in a number of ways. This analysis is to be included in their final report and makes their reports unique.