Biotechnology Lab-BIEN155

Recombinant Fluorescence Protein Production and FRET Assay

Week 1. (Sept.22nd-23rd) Take Online Mandatory Safety Training Course: Instruction. Bring the transcript to the first lab.

Go to: http://www.ehs.ucr.edu/training/online/

Select "Laboratory Safety Orientation"

Fill up the registration form "Training Record registration: Laboratory Safety Orientation" and Submit.

Follow the link to take the course.

Get training transcript: go to http://ucrlearning.ucr.edu and log into your account. "Transcript" tab located on the lower right column.

Week 2. (Sept.26th-Sept.30th) Trasfect pET 28(b)-CyPet-SUMO1 and pET 28(b)-Ypet-Ubc9, respectively, into DH5a by eletroporation.

TA Discussions: Molecular cloning;

Week 3. (Oct.3rd-7th) Purify plasmid DNA from bacterial cells by mini-prep, validate the genes by restriction enzyme digestion, and submit for sequencing.

TA Discussions: DNA sequencing and NCBI Bioinformatics Softwares;

Week 4. (Oct.10th-14th) Verify and compare the sequencing result of clones with sequences from NCBI database.

TA Discussions: Protein expression systems in bacterial in general.

Week 5. (Oct.17th-21st) (Report due)Trasfect pET 28(b)-CyPet-SUMO1 and pET 28(b)-Ypet-Ubc9, respectively,into protein expression bacterial strain $Bl_{21}(DE_3)$.

TA Discussion: His-tagged or other tagged protein purification.

Week 6. (Oct.24th- Oct.28th) CyPet-SUMO1 and Ypet-Ubc9 protein expression.

TA Discussions: Fermentor discussion.

Week 7. (Oct.31st- Nov.4th) CyPet-SUMO1 and Ypet-Ubc9 protein purification.

TA Discussions: Protein purity checking methods., Quantitative protein and gel electrophoresis.

Week 8. (Nov.7th-11th) (Report due) Protein concentration measurements and gel electrophoresis/Coomassie staining.

TA Discussions: Fluorescence Detection and Fluorescence Energy Transfer.

Week 9. (Nov.14th-18th) Fluorescence protein identification by imaging and spectroscopy, and Fluorescence Energy Transfer Assay.

TA Discussions: Presentation of research results.

Week 10. (Nov.21st-25th) Thanksgiving. No lab.

Week11. (Nov.28th-Dec.2nd) (Report due) Presentations.

Textbook

Sambrook J and Russell D, Molecular Cloning: A Laboratory Manual. CHSL Press, 2001

Walsh G..: Proteins Biochemistry and Biotechnology. John Wiley & Sons, Inc., 2002