LABORATORY WORKSHOP IN BIOLOGY EDUCATION

This is a project-oriented course where prepared materials will be used sequentially in experimental protocols. You will learn different molecular biology techniques involving DNA and protein analyses. Your success in learning to master and teach these techniques requires your **full participation**. Lectures and handouts explaining the scientific basis for each procedure accompany this course. An oral presentation, a written final exam as well as your participation in the workshop will determine your grade.

Students are required to keep a detailed notebook for recording the purpose, results and interpretation of each experimental protocol.

Week #1:
Basic Techniques
pGLO Plasmid Transformation, Purification

In these experiments you will transform the *Escherichia coli* strain HB101 with a recombinant plasmid called pGLO. This plasmid construct contains a cloned gene expressing the green fluorescent protein (GFP) under the control of the ARA C promoter.

1. Laboratory basics & calculations; preparation of LB, LB-AMP, LB-AMP-ARA plates and E.coli starter plate.
2. Transformation (DNA uptake by bacterial cells) will be performed with the recombinant plasmid pGLO containing a cloned green fluorescent protein (GFP). These experiments involved plasmid uptake by the *Escherichia coli* strain HB101.
3. The efficiency of transformation (plasmid DNA uptake by bacterial cells) will be calculated from your results.
4. Successful transformants will be identified by fluorescent: Plasmid DNA will be purified from transformants grown overnight in liquid cultures.
5. Minipreps of pGLO plasmid

Week #2:
Restriction Enzyme Analysis, DNA Fingerprinting
Amplification of GFP Gene by PCR

1. Purified plasmid from positive transformants is analyzed by endonuclease restriction enzyme and agarose gel electrophoresis.
2. DNA fingerprinting: prepare restrictions and analyze results by agarose gel electrophoresis; stain gels.
3. GMO PCR using GMO specific primers designed to amplify genetically modified foods
4. GFP PCR: pGLO plasmid will be subjected to PCR using primers designed to amplify GFP.
5. ELISA

Week #3:
Insertion of amplified GFP fragments into PCR cloning vectors (pGEM) & Analyses for cloned PCR fragments & Review; SDS-PAGE

1. PCR products will be purified and analyzed for expected fragment size by agarose gel electrophoresis and quantified.
2. GFP cloning: Amplified fragment corresponding to GFP gene will be inserted into a pGEM plasmid cloning vector.
3. Transformation will be performed with the recombinant pGEM plasmids.
4. Transformants containing cloned inserts will be detected by loss of expression of the enzyme, β-galactosidase (blue white screening).
5. Plasmid will be purified from positive transformants and subjected to restriction analysis for cloned inserts of expected sizes The cloned GFP insert will be analyzed by restriction endonuclease treatments of plasmid DNA preparations and analyzed by agarose gel electrophoresis.
6. GFP will be detected in column fractions by SDS-polyacrylamide electrophoresis (SDS-PAGE).
7. Lecture on additional teaching materials, such as, web-based teaching sites, vendor sources for reagents, supplies and teaching resources (e.g., CD-ROMs & literature).

Week #4: Oral Presentations and Final Exam

1. Oral presentations: Each group will prepare a project-oriented lesson plan for use in the high school classroom, using teaching materials, such as, transparencies and hand-outs. The lesson plan should focus on project goals, the laboratory protocols employed and their relationship to the appropriate lecture material. Presentations should also include a discussion of laboratory results and their application to current trends in biomedical research.
2. Written Final Exam

DAILY SCHEDULE

1st Week --- Transformation and DNA Miniprep

July 05 (Tues.) --- Prepare 10X TBE. Instructor/Class demo of starter plates*:*
LB-AMP and LB-AMP-ARA. Conduct Transformation on Plates.

July 06 (Wed.) --- Analyze results of transformation; overnight culture inoculation for DNA Miniprep

July 07 (Thurs.) --- DNA Miniprep,

July 08 (Fri.) --- Restriction, Agarose gel electrophoresis, Stain the gel

2nd Week --- Restriction & Agarose Gel Electrophoresis
DNA fingerprinting and PCR

July 11 (Mon.) --- Fingerprinting (Restriction and Agarose gel electrophoresis)

July 12 (Tues.) --- PCR using GMO primers for genetically modified foods

July 13 (Wed.) --- PCR using GFP primers for GFP; ELISA

July 14 (Thurs.) ---Analyze PCR products by running 2% agarose gel
Purify the **GFP** PCR product by QIAquick Spin Column

3rd Week ---Cloning and Analyses of Cloned GFP

July 18 (Mon.) --- Cloning (Ligation, Transformation, Detection)
----Overnight culture of pGLO bacteria for GFP Isolation

July 19 (Tues.)--- Analyze the plates;
---Lyse pGLO cells, store overnight in -80C

July 20 (Wed.)--- GFP Column Chromatography SDS PAGE electrophoresis
---Prepare 6 overnight cultures for DNA miniprep

July 21 (Thurs.) ---DNA Miniprep, Restriction, Agarose gel analysis

4th Week --- Presentation & Final Exam

July 25 (Mon.) ---- Presentation

July 26 (Tues.) ----- Presentation

July 28 (Thurs.) ---- Final Exam