In compliance with the NIH *Guidelines for Research Involving Recombinant DNA Molecules*, this form registers your project with the Kenyon College Institutional Biosafety Committee (IBC) for the review process. Projects subject to IBC review include those that involve recombinant DNA or infectious agents.

* Recombinant DNA molecules are defined as either:
  1. molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate inside a living cell, or
  2. DNA molecules that result from the replication of those described in (1).

Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed in vivo as a biologically active polynucleotide or polypeptide product, it is exempt from the NIH Guidelines. Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the NIH Guidelines unless the transposon itself contains recombinant DNA.

* Infectious agents are defined as those biological agents, both pathogenic and non-pathogenic, known to infect human as well as selected animal agents that may pose theoretical risks if inoculated into humans.

Please refer to the IBC web site for guidelines and advice for completing this application.

*This application should be submitted via e-mail to:*

Kenyon College Institutional Biosafety Committee *at EHS@kenyon.edu*

|  |  |
| --- | --- |
| **P****rimary Investigator:** |  |
| **e-mail:** |  |
| **Office Location and Phone:** |  |
| **Lab Location and Phone:** |  |
| **Department:** |  |

**Brief Title of Project or Activity:**

**Brief description of project/activity in plain English:**

**In what room(s) do you plan to work with, store or dispose of recombinant DNA?** (Example: FSH 202 - sterile transfer of recombinant bacteria and cloning procedures; FSH 003, HIG 321, HIG 308 - autoclaving of wastes; FSH 203 - storage of recombinant cell lines)

**What vector(s) do you plan to use? Give the class, vector name, and vendor or other source.** (Examples: plasmid pGEMT-Easy [Promega] pCMV-Sport [Invitrogen], lambda phage, etc.)

**What gene(s) do you plan to use? Be sure to include the source organism.** (Examples: mouse androgen binding protein, cDNA library from zebrafish, *Arabidopsis thaliana,* small subunit of RUBISCO)

**What host organism(s) do you plan to use?** (Examples: *Escherichia coli* DH5; COS-7 cells (green monkey kidney); *Arabidopsis thaliana* plants.)

**Please provide a brief description of how you plan to use the recombinant genes.**

* **State whether this activity will be used in an instructional manner (for a class) and/or for research purposes.**
* **Also state the appropriate Biosafety Level for the project.**
* **Include a brief description of how accidents involving the recombinant DNA material and/or vector host organisms will be handled (e.g. applying bleach to the benchtop area of a small spill; autoclaving soil containing recombinant seeds).**

**Procedures should be in compliance with the NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES.**Sample description:

In order to study subcellular targeting of pyruvate decarboxylase PDC2 in tobacco, we will make a fusion between the cauliflower mosaic virus 35S promoter, the *Arabidopsis thaliana* pyruvate decarboxylase PDC2 coding sequence, and a blue-shifted green fluorescent protein gene. Initial cloning steps will be carried out in vector pBluescript using host *Escherichia coli* XL1-Blue (Stratagene), and selection on ampicillin. The gene fusion will be pasted into shuttle vector pGA748, and this construct will be electroporated into *Agrobacterium tumefaciens* LBA4404. *Agrobacterium* clones selected on kanamycin will be used for tobacco leaf disk transformation. The distribution of green fluorescent protein in transformed tobacco callus cells will be examined by fluorescence microscopy.

Biosafety level 1 is appropriate for these studies. Any spillage containing *E. coli* or *Agrobacteria* will be soaked into paper towels, which will be added to other accumulated biohazardous waste in labeled red bags and decontaminated *via* autoclaving; the surface(s) exposed to the spill will be treated with bleach.

# Safety assessment and signatures

**Please check all that apply:**

□ I have read and understood the precautions detailed in the NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES (NIH GUIDELINES), and I have identified the appropriate Biosafety Level for the proposed project. A description of the project-specific risks and precautions is attached.

□ I will report to the IBC any change in the class of vector used (such as switching from a plasmid vector to a viral vector).

□ I will report to the IBC any change in the source organism(s) used in this project.

□ I will report to the IBC any change in the host organism(s) used in this project.

Signature of PI Date:

**IBC Action:**

Project approved as presented.

Project approved with modifications.

Additional information requested.

Approval denied. Signature of IBC Chair \_ Date Signature of EHS Director \_ Date