Application to do Non-exempt Recombinant work
Crown College Institutional Biosafety Committee

1. Researcher Name(s) (including principle investigator/supervisor):

   Aeisha Thomas July 17,2025

Project Name: D2D Bacterial Work

Additional Staff handling material: NONE

2. Proposed Work (very brief description of the experiment, including goals):

The Design2Data project is based in the Siegel lab at UCDavis. It involves choosing point mutations of the protein beta glucosidase. The introduction of the mutations using Kunkel mutagenesis. Transformation of competent cells, plasmid prep and sequencing to check the mutation. Transformation of BLR or similar cells with mutant and wildtype plasmid to generate proteins which are then purified and studied. A plasmid with Green Fluorescent Protein (GFP) is also used in the second transformation and processed as a control for protein purification. The detailed process is provided in the lab manual which can be accessed at https://d2d.ucdavis.edu/d2d-lab-manual and includes a short explanation of Kunkel mutagenesis.

3. Relevant section from NIH guidelines:

   Section III-F-8

4. Organisms involved (e.g. "commercial, non-pathogenic *E. Coli*"):

a. Kunkel mutagenesis will be done with this plasmid provided by the Siegel lab and primers based on the point mutation. The beta glucosidase plasmid is pET29b+BlgB which can be viewed at https://benchling.com/s/seq-wAln5ZL6jXGsPs9hnB8B/edit
b. Competent *E. coli.* bacteria are obtained from commercial sources (e.g. NEB 5 alpha competent E. coli or Fisher DH5 alpha competent cells). These are transformed with Kunkel, wildtype or control (e.g. single stranded) plasmid DNA and selected using the antibiotic kanamycin. DNA sequencing is then done to check mutagenesis.
c. Plasmids with mutant or wildtype beta glucosidase or GFP protein sequence (provided by Siegel lab) are then used to transform competent cells from commercial sources (e.g. BL21(DE3) competent cells from Thermoscientific or BLR (D3) competence cells from Millipore Sigma). Selection with kanamycin continues in this step and induction of protein expression is done with isopropyl β-D-1-thiogalactopyranoside (IPTG). Proteins are purified and then studied (protein gel, enzyme, kinetic assay, etc.).

Annual Report on non-exempt work should be submitted at the end of each academic year (May-August) to the IBC chair, contact person, and Vice President of Academic Affairs. This report should include checks on containment.

5. Is this a renewal application? [X] Yes. [ ] No

If 'Yes,' please indicate any changes:

N/A

6. Containment Plans:

Bacteria are grown in culture or in plates. Liquid culture volumes rarely exceed 10 mls and typically are 2-5 mls. The organisms are maintained in incubators, on the bench, in the fridge or the freezer.

7. Waste Containment and Disposal Plans:

Waste is kept in sturdy containers or bags in sturdy containers until disposal. Bacteria will be autoclaved for at least 20 minutes at 121 degrees Celsius or treated with a 10% or higher bleach solution for at least 20 minutes or other disinfectant such as 70% ethanol (also possibly Lysol).

8. Biosafety Plan:

Biosafety Level 1. Gloves will be worn while working with the microorganisms. Eye protection will be used whenever there is any possibility of splash. Any spills will be cleaned with 10% bleach or appropriate disinfectant. Working surfaces are routinely cleaned with disinfectant such as 70% ethanol (also possibly Lysol).

IBC decision:

[X] Yes

[ ] Yes, with these suggestions that have been included in the update.

[ ] Yes, with these conditions

[ ] No

Approval date: Aug. 4, 2025

Expiration date (3 years post approval): Aug. 4, 2028

IBC Chair (Robert L. Evans III) signature: 

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