Administrative Use Only
IBC Registration Number [redacted]
Biosafety Level BL-2+
IBC Approval Required

UNIVERSITY OF KENTUCKY **BIOSAFETY REGISTRATION FORM**

PART 1: ADMINISTRATIVE INFORMATION

Principal Investigator: [redacted]	Date: 10 October 2007
Position: Assistant Professor	Phone Number: 323-[redacted]
Department: [redacted]	Office Address: [redacted]
Lab Address: [redacted]	E-mail Address: [redacted]@uky.edu
Speed Sort Code: [redacted]	FAX Number: 257-[redacted]
Title of Research Project: [redacted]	

Does your research involve:

Place an "X" before your answer

1. ☒ **Yes** ☐ **No** Infectious Agents. If yes, complete and submit Parts 1-5 and Attachment A.
2. ☒ **Yes** ☐ **No** Recombinant DNA Molecules or Organisms. If yes, complete and submit Parts 1-5 and Attachment R.
3. ☐ **Yes** ☒ **No** Only human blood, or human body fluids, not involving culture of infectious agents, or not simply diagnostic, clinical testing. If yes, complete and submit Parts 1-5 and Attachment BBP.
4. ☒ **Yes** ☐ **No** Animal or human tissue cultures, cell lines, or non-human primate clinical specimens. If yes, complete and submit Parts 1-5 and Attachment TC. Additionally, complete Attachment BBP for human tissue cultures and cell lines.
5. ☐ **Yes** ☒ **No** Human Gene Transfer Experiment/Clinical Trial. Complete and submit Parts 1-5 and Attachment G.
6. ☐ **Yes** ☒ **No** Recombinant DNA used with plants or the use of plant pathogens. If yes, complete and submit Parts 1-5P, Attachment P, and Attachment AP.

OTHER INSTITUTIONAL REVIEWS/APPROVALS

1. Institutional Animal Care and Use Committee (IACUC)

Use of animals:
☐ Yes ☒ No Approval received: ☐ Yes ☐ No ☐ Pending
If yes, date of approval IACUC Approval Number:

2. Institutional Review Board (IRB)

Use of human subjects:
Yes ☐ No Approval received: ☒ Yes ☐ No ☐ Pending
If yes, date of approval (1) May 2005 Approval Number: [redacted]
(2) October 2007 [redacted]

3. Radiation Safety Committee (RSC)

Use of radiological materials:
☐ Yes ☒ No Approval received: ☐ Yes ☐ No ☐ Pending
If yes, date of approval Approval Number:

PART 2: RESEARCH PROTOCOL DESCRIPTION

Section A. Lay Summary

In terms that are understandable to a non-scientist (i.e. avoiding technical jargon and the use of unexplained acronyms):

- Provide a very brief introduction to your research project.
- Simply and briefly state the specific goal(s) of your experiment(s).
- Describe, very briefly, the experimental approach(es).
- Identify potential biohazards and associated risks.

Introduction:
Lung cancer is the leading killer of all cancers and Kentucky has the highest rate of incidence and mortality from this dreaded disease. Although lung cancer is the most preventable cancer, those who do not choose to stop smoking will likely become the population that most needs the best available treatment for lung cancer. It is important to acknowledge that great strides have been made in treatment options that extend the lifespan of those afflicted. However, five-year survival rates are very low. We propose that survival rates can be increased if second line therapeutic options are assessed when a patient is diagnosed with relapsed or recurrent lung cancer. Thus, if an individual fails the standard of care front line treatment, a reasoned choice can then be made that gives the patient the best opportunity at increased survival time and lessens the potential toxic effects of treatment.

Specific Aims:
1. To develop and analyze genomic predictors of response to targeted therapeutics in non-small cell lung cancer
2. To develop genomic predictors of activated signaling cascades in non-small cell lung cancers
3. To interrogate the signaling molecules activated in non-small cell lung cancers

Experimental Approaches:
Specific Aim 1: Human lung cancer cells will be propagated in the laboratory, tested for drug sensitivity, and RNA will be extracted from each cell line. The RNA will be prepared for DNA microarray analysis. The gene expression data will be mathematically processed to develop a gene expression predictor of response to each drug of interest. Subsequently, the gene expression signature of response will be tested on microarray data derived from biopsy samples from patients with recurrent or relapsed lung cancer in need of new treatment.

Specific Aim 2: Human bronchial epithelial cells will be infected with adenoviral vectors expressing oncogenes specifically activated in lung cancers. RNA will be harvested from the infected cells following an overnight incubation and processed for microarray analysis. The gene expression data derived from the microarray experiments will be mathematically processed to develop gene expression predictors of activation of specific oncogenes.

Specific Aim 3: Data from SA2 will be used to determine interesting proteins to characterize in lung cancer. The underlying goal is to identify new targets for lung cancer therapeutics. Lentiviral vectors will be utilized to knockdown protein levels of the proteins of interest by shRNA methods.

Potential Biohazards/Risks:
Human cell lines and tissues may certainly pose a risk for transmission of infectious agents. Adenoviral vectors do not integrate in host genome but can infect a wide variety of cells. High titer stocks may pose a risk of pinkeye. Lentiviral vectors may also be a concern due to integration in HIV-positive individuals. Chemicals used to generate the RNA from cells can also present a risk of toxic exposure if kit directions are not followed.

Section B. Scientific Summary

The Institutional Biosafety Committee evaluates research protocols to ensure that they incorporate steps to minimize potential biohazard exposures and that biohazardous materials are disposed of in an appropriate manner. The IBC does not review the scientific merit of the proposed work. Protocols involving recombinant DNA work must also follow specific guidelines. Therefore, for each of the protocols that will be used in your project, you must:

- Briefly, describe the manipulations to be performed (e.g. cloning target gene into shuttle vector, protein purification, transfection of cell lines, use of microbial agents, use of cell lines and strains, etc.). You should provide sufficient information that the IBC members can envision the experimental procedure. Do not include details that are irrelevant to the safety issues. However, concentrations or infectivity of microbes and their environmental stability is important. For this reason, you should not simply paste lab protocols or methods sections from grant applications or published papers. An example is available at http://ehs.uky.edu/biosafety/forms/sample_reg_form.rtf.
- Identify any potential biological risks, even if exposure is unlikely.
- Describe the steps that will be taken to minimize those risks.
- Describe disposal procedures.

Cell Culture:
All cells will be maintained under BSL II conditions in COP 423. Subculture, infections, transfections or other manipulations with live cells will be performed in a biosafety cabinet (BSC). Cells will be grown in a humidified incubator (5-7% CO_2). Most of the cell lines are human lung cancer lines purchased from ATCC. We do have one cell line generated here by Yannelli and colleagues for which we do not know pathogen status. We also use BEAS2b cells which are bronchial epithelial cells transformed with SV40 T antigen.
The BSC is equipped with a germicidal lamp that will be used with air circulation for 15 minutes prior to the first use of the day and 5 minutes post each use. Each morning following germicidal lamp activation, the floor of the hood will be wiped with 1:10 dil. bleach. The floor of the BSC will be wiped with 70% ethanol following each experiment. Media and cells will be aspirated into a vacuum trap containing a final 1:10 dilution of bleach. All used cell culture plates, flasks and pipettes will be contained in bins marked “Biohazard” in autoclavable bags. Trash will be autoclaved as needed under a ‘dry’ cycle (one hour, 121C, 15lbs pressure) and disposed of in regular waste.

Human Tissue Procurement:
Both IRB protocols involve collection of human lung cancer tissue, either from frozen stocks in the UK Biospecimen Core or from patient biopsy. In either case, the tubes containing tissue will be opened in the BSC and tissue will be dissected and placed in a new tube containing the RNA extraction reagent (Trizol, Invitrogen). At that time the solubilized tissue will be further processed on the benchtop. Human tumor tissue will be handling by personnel wearing gloves, eye protection and lab coats. The surface of the biosafety hood will be wiped down with 1:10 dil. bleach following use with human tissue to prevent the risk of transmission of infectious agents. Remaining tissue will be flash frozen in LN2 or disposed of in Biohazard waste and subsequently autoclaved.

Generation of recombinant adenoviral vectors:
The AdEasy system (QBiogene) will be used to generate replication-defective adenoviral particles encoding proteins of interest according the manufacturer’s protocol. Generation of the plasmid constructs (pShuttleCMV or AdTrack) will take place in [redacted]. Transfection of AdEasy clones into HEK293 cells will take place in [redacted] under BSL 2+ procedures. Viruses will be propagated in HEK293 cells and purified by cesium chloride gradients and PD10 columns. Purified virus, in phosphate buffered saline (PBS) and 10% glycerol, will be stored at -20C in [redacted].
Specific proteins of interest are outlined in attachment R: Dusp4, EGFR and K-ras. EGFR and K-ras are known oncogenes while DUSP4 is predicted to harbor tumor suppressor activity. Each will be cloned into either pShuttleCMV or Ad Track (generates a GFP ‘tag’) and then recombined into AdEasy for propogation of adenoviral particles in HEK293 cells. Because adenoviral vectors provide for transient expression of the proteins of interest, are replication deficient in normal human cells, and do not integrate into the cellular genome, the potential for causing to harm to personnel is greatly reduced. That said, experiments involving these viruses will be short-term infections (16-24h) of normal human bronchial epithelial cells for the purpose of extracting RNA or protein for analysis. We will follow the procedures listed below to protect personnel carrying out these experiments.

To express proteins of interest, cells will be infected with the virus of interest at 50-100 ffu/cell (multiplicity of infection). Following infections and collection of infected cells, all surfaces of the BSC will be wiped down with 1:10 diluted bleach and the incubator will be wiped down with 70% ethanol.
Personnel will wear gloves, eye protection and lab coats when performing adenoviral experiments.
The greatest risk noted here is the preparation of high titer stocks because 20-30 150mm dishes will be infected with virus (1-2L). The ultracentrifuge in [redacted] will also be disinfected with 1:10 bleach following centrifugation of the cesium chloride gradients.
All cell culture materials used for virus preparation or utilization will be autoclaved immediately following use to minimize exposure, even in Biohazard bins, because [redacted] is a multi-user facility.

Use of lentiviral vectors in cultured cells:
Lentiviral vectors expressing shRNA are commercially available (Sigma) as virus particles. These are 2nd and 3rd generation vectors. Also, because we do not plan to make virus particles on our own, the risk is minimized for laboratory personnel. We will utilize BL-2+ procedures for these procedures.
In knockdown experiments, cell lines of interest will be transduced with 5 x10^{6} particles (this will likely need to be titrated for each experiment) and cultured under puromycin selection to isolate stable transformants. Insertion of the viral DNA into the host genome will be confirmed by polymerase chain reaction (PCR) with commercially-available primers. Suppression of expression of the protein of interest will be confirmed by immunoblot of the cell lysates. Positive clones will be propagated and frozen for use in future experiments. Used tissue culture materials will be disposed of in Biohazard containers and immediately autoclaved.

PART 3: PERSONNEL AND FACILITIES

The list of personnel should include **all those**, starting with the principal investigator, who will physically handle the biohazardous agents or recombinant DNA molecules and are conceivably at risk from research procedures involving the use of these biological materials. Approval of the proposed experiment is given only for the identified personnel listed below. The Biosafety Officer must be notified if any new personnel are added. The PI is responsible for insuring that all personnel receive appropriate training, and any required vaccinations prior to the initiation of work.

Complete and submit a "modification of protocol" form when personnel change.

SECTION A: NAMES OF PERSONNEL HANDLING AGENTS

(Fill this out for **EACH** person working on this project or handling the biological agents or recombinant DNA molecules, starting with the PI.)

NAME: [redacted]
TITLE or POSITION: Asst Prof
RELEVANT EDUCATION/EXPERIENCE/CREDENTIALS: PhD
E-MAIL ADDRESS: [redacted]@uky.edu

Training:
- ☒Yes ☐No Blood-Borne Pathogen (Must be updated annually), Date Completed: 10/10/07
- ☒Yes ☐No Lab Specific Safety Training, Date Completed: 10/17/2004
- ☒Yes ☐No Hazardous Materials Training, Date Completed: 10/27/2004
- ☐Yes ☒No DOT/ IATA Shipping Training, Date Completed: _____
- ☒Yes ☐No Biological Safety Training, Date Completed: 10/10/07

If yes in any area, keep documentation of training in laboratory's safety manual for audit purposes.

NAME: [redacted]
TITLE or POSITION: Graduate Student
RELEVANT EDUCATION/EXPERIENCE/CREDENTIALS: [redacted]D
E-MAIL ADDRESS: [redacted]@email.uky.edu

Training:
- ☒Yes ☐No Blood-Borne Pathogen (Must be updated annually), Date Completed: 10/9/07
- ☒Yes ☐No Lab Specific Safety Training, Date Completed: 11/05
- ☒Yes ☐No Hazardous Materials Training, Date Completed: 09/13/2004
- ☐Yes ☒No DOT/ IATA Shipping Training, Date Completed: _____
- ☒Yes ☐No Biological Safety Training, Date Completed: 10/10/07

If yes in any area, keep documentation of training in laboratory's safety manual for audit purposes.

NAME: [redacted]
TITLE or POSITION: Graduate Student
RELEVANT EDUCATION/EXPERIENCE/CREDENTIALS: BS
E-MAIL ADDRESS: [redacted]@email.uky.edu

Training:
- ☒Yes ☐No Blood-Borne Pathogen (Must be updated annually), Date Completed: 10/9/07
- ☒Yes ☐No Lab Specific Safety Training, Date Completed: 8/14/2005
- ☒Yes ☐No Hazardous Materials Training, Date Completed: 8/15/05
- ☐Yes ☒No DOT/ IATA Shipping Training, Date Completed: _____
- ☒Yes ☐No Biological Safety Training, Date Completed: 7/26/07

If yes in any area, keep documentation of training in laboratory's safety manual for audit purposes.

NAME: [redacted]
TITLE or POSITION: [redacted] Student
RELEVANT EDUCATION/EXPERIENCE/CREDENTIALS: BS
E-MAIL ADDRESS: [redacted]@uky.edu

Training:
- ☒Yes ☐No Blood-Borne Pathogen (Must be updated annually), Date Completed: 10/9/07
- ☒Yes ☐No Lab Specific Safety Training, Date Completed: 5/18/2005
- ☒Yes ☐No Hazardous Materials Training, Date Completed: 8/28/2006
- ☐Yes ☒No DOT/ IATA Shipping Training, Date Completed: _____
- ☒Yes ☐No Biological Safety Training, Date Completed: 10/07/07

If yes in any area, keep documentation of training in laboratory's safety manual for audit purposes.

SECTION B: LOCATION OF EXPERIMENTS
Complete and submit a "modification of protocol" form when rooms change.
Approval of the proposed experiment is given only for the locations listed below

Primary Laboratory:

Building: [redacted] Room: [redacted] E-Bar # for room: [redacted]
Room locked: 24 hours/day ☐Yes ☒ No Only when no one is in the lab ☒Yes ☐ No

Infectious Agents **used** here ☒Yes ☐ No

Infectious Agents **stored** here ☒Yes ☐ No ☒ Short-term (≤ 1 month) ☒Long-term (> 1 month)

rDNA **used** here? ☒Yes ☐ No

rDNA **stored** here? ☒Yes ☐ No ☒ Short-term (≤ 1 month) ☒ Long-term (>1 month)

Refrigerator Locked? ☐Yes ☒ No Freezer Locked? ☐ Yes ☒ No

WHAT PROCEDURES ARE PERFORMED IN THIS ROOM? RNA and DNA isolation, cloning, other molecular biology techniques (protein purification, DNA gels, western analysis, etc.)

Cell Culture room is located inside of a locked/secured laboratory? ☐ Yes ☐ No ☒ N/A

Estimate the biosafety level of these procedures: ☒ BL-1 ☐ BL-2 ☐ BL-2+ ☐ BL-3 ☐ BL-4

Will any work be performed off campus? ☐Yes ☒No

If yes, list procedures and location(s):_____

Secondary Laboratory: ☐ N/A

Building: [redacted] Room: [redacted] E-Bar # for room: [redacted]
Room locked: 24 hours/day ☐Yes ☒ No Only when no one is in the lab ☒Yes ☐ No

Infectious Agents **used** here ☒Yes ☐ No

Infectious Agents **stored** here ☐Yes ☒ No ☐ Short-term (≤ 1 month) ☐Long-term (> 1 month)

rDNA **used** here? ☒Yes ☐ No

rDNA **stored** here? ☐Yes ☒ No ☐ Short-term (≤ 1 month) ☐ Long-term (>1 month)

Refrigerator Locked? ☐Yes ☐ No Freezer Locked? ☐ Yes ☐ No

WHAT PROCEDURES ARE PERFORMED IN THIS ROOM? Cell culture, adeno- and lenti-viral infections

Cell Culture room is located inside of a locked/secured laboratory? ☒ Yes ☐ No ☐ N/A

Estimate the biosafety level of these procedures: ☐ BL-1 ☒ BL-2 ☒ BL-2+ ☐ BL-3 ☐ BL-4

Will any work be performed off campus? ☐Yes ☒ No

If yes, list procedures and location(s):_____

PART 4: BIOSAFETY ISSUES

SECTION A: PHYSICAL CONTAINMENT EQUIPMENT

☐ Please check here if you are **NOT** using a biological safety cabinet (BSC).

Indicate the location of any **biosafety cabinets** used in the experiments. Provide the classification of biosafety cabinet and date of certification. This information should be on the front of the equipment. For information on biological safety cabinets: http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm

Building: [redacted] **Room:** [redacted] **Serial Number or UK Property Tag Barcode Number:** [redacted]

Biosafety Cabinet Class: ☐ I ☒ II ☐ III **Type:** ☐ A1 ☒ A2/B3 ☐ B1 ☐ B2

Manufacturer: Baker **Model:** Steriguard **Certification Expiration Date:** 6-2008

Certified by (company name): Agape **Certifier's Phone number:** (800) 829-0293

What procedures will be done in the BSC? ☐ **N/A**
☐ Transfer microbial cultures
☒ Transfer tissue culture
☐ PCR
☐ Elisa
☒ Vacuum line/suction
☒ Homogenization
☒ Sonication
☒ Inoculations of culture media
☐ Inoculation of animals
☐ Manipulation of animals (surgery, necropsy, specimen collection, etc.)
☐ Other procedures with the potential to produce aerosols describe: _____

Will you be using hazardous or volatile chemicals (e.g. ether, paraformaldehyde) in the BSC?
☐ YES ☒ NO If yes, provide chemical names, CAS #, and quantity**:** _____

Will you be using radioactive material in the BSC? ☐YES ☒NO
If yes, provide the name of isotopes, chemical form, and radioactivity (e.g. mCi's): _____

☐ Please check here if you are **NOT** using a centrifuge.

Indicate the following for each centrifuge used with biohazardous materials:
☒ Floor model
☒ Bench (top-sealed rotor)

Building: [redacted] **Room:** [redacted] **Serial Number and/or UK Property Tag Number:** R[redacted]

Manufacturer: Eppendorf (bench) **Model:** 5415D (tag #A653446)
Ultracentrifuge (floor)- **Manufacturer**: Beckman **Model**: XL90 ([redacted])

SECTION B: BIOSAFETY AND HANDLING CONSIDERATIONS

1. Will the experiment involve a potential for amplifying the risk of exposure?
(Check all that apply)

☒ Aerosol generating procedures (centrifugation, vortexing or mixing, sonic disruption, homogenization, grinding, opening containers with different internal pressures, inoculating animals intranasally, harvesting infected tissues from animals or embryonate eggs, etc.)
☐ Use of transgenic animals
☐ Use of transgenic plants
☒ Use of sharps
☐ Use of helper virus
☒ Use of oncogenes
☒ High concentrations
☐ N/A
☐ Other Explain: _____

2. Amount of infectious agents: ☐ **N/A**
Concentration of agents used (cfu, pfu, etc. per ml): 50-100 ffu/ml adenovirus and 10^6 lentiviral particles

Volume of culture to be generated:
☒ <1.0ml
☐ 1.0ml – 50.0ml
☐ 50.0 ml – 1 liter
☒ 1 – 10 liters
☐ >10 liters

3. Routes of Exposure:
Based on proposed laboratory procedures, indicate the most likely routes of exposure for laboratory personnel to biohazardous material (check all that apply)
☐ Ingestion
☒ Inhalation
☒ Mucous membrane
☒ Parenteral inoculation
☒ Skin contact
☒ Eyes
☐ N/A
☐ Other, describe: _____

Briefly explain any of the boxes that have been checked and describe the procedures the lab will use to minimize the potential for exposure based on the routes of exposure indicated above (e.g., engineering controls, personal protective equipment and safety procedures that will be used)*:*
Procedures involving the use of adeno- or lenti- viral expression systems will be carried out in a BSC under BL-2+ conditions to minimize exposure by inhalation or by the eyes. Lab coats and gloves will be worn by personnel involved in these experiments to limit contact with skin or mucous membranes. Sharps will be placed in appropriately marked containers. No eating or drinking is allowed in these areas. All BSC surfaces will be wiped with 1:10 bleach at the end of the experiment.

SECTION C: BIOHAZARDOUS WASTE

Which of the following types of waste will you generate in your laboratory experiment?
☐ Recognizable human/animal fluid blood
☒ Sharps waste (e.g. needles)
☒ Unfixed human tissues
☒ Pathogenic microbiological cultures
☐ Infected animal carcasses
☒ Broken glass contaminated with biohazardous materials
☐ N/A
☐ Other, please indicate:_____

Describe the procedures that you will use to dispose of biohazardous waste. Indicate if any biohazardous waste will require disposal in a red bag lined bin. Please describe any additional requirements that must be taken to safely containerize biohazardous waste.

Pipettes will be placed in a rigid container (to prevent puncture wounds to lab personnel and housekeeping staff), bagged in an orange autoclave bag and autoclaved under 'dry cycle' for one hour (15 lbs pressure, > 121C) prior to disposal.
Cell culture materials (i.e. flasks, closed culture plates, etc…) and other non-sharp items will be disposed of in orange autoclave bags and sterilized as described above. Pipette tips, serological pipets will be collected in autoclave bag lined tray or upright, rigid container within the BSC. Upon completion of work the autoclave bag will be closed, sprayed with 1:10 dil. bleach, removed from cabinet and added to the biohazard waste (described above) and autoclaved.
Vacuum flasks are equipped with an inline filter and secondary catch vessel. The 4L flask will be emptied at the end of every day and 50ml of bleach will be added to the flask. Prior to emptying the following day additional bleach will be added if the volume exceeds 500ml in the flask in order to achieve a 1:10dilution of bleach in the flask prior to emptying down the sink. The hood work surface will be cleaned with 70% ethanol before and 70% ethanol after normal use and following virus or human tissue work, the surface will be decontaminated with 1:10 bleach.
Human tissue will be present in quantities less than 100mg. In most cases, no residual tissue will remain following the procedure. Therefore, any consumables that are used to prepare the tissue will be placed in the orange autoclave bags and sterilized as described above.

SECTION D: TRANSPORT

Is transport of animals/agents/plants needed? ☒Yes ☐ No ☐ N/A

If yes, complete the following:

Animals: If animals are transported to and from the lab from the Animal Care Facility, please describe below the method of transport and the precautions taken to minimize the potential for an accidental release or exposure.
NOTE: Animals must be completely enclosed so as to not be visible to the public.

Agents: If agents are transported to and from the lab (e.g., from the field, to another lab, to storage area, to flow cytometry, tissue culture room, animal care facilities, greenhouse), describe below the method of transport and the precautions taken to minimize the potential for an accidental release or exposure. Information on transport containers can be found at http://ehs.uky.edu/biosafety/sec-containment.html.

Human tissue will be transported from the Biospecimen Core, frozen, in an approved liquid nitrogen transport container.
Human tissue from biopsy will be transported from Radiology at Chandler Hospital in Nunc, screw-cap tubes, on dry ice in a covered secondary container.
Frozen virus preparations, in Nunc screw-cap tubes, placed in a zip-lock bag will be transported to/from [redacted] on ice in a secondary (Styrofoam) container.

Plants: If transgenic plants are transported from one facility to another (e.g. from growth chamber to greenhouse, etc), describe below the method of transport and the precautions taken to minimize the potential for an accidental release or exposure.

SECTION E: BIOHAZARD COMMUNICATION

Has all laboratory equipment that will contain biohazardous material been labeled with the Universal Biohazard Symbol? ☒ Yes ☐ No ☐N/A
Refrigerator(s) ☒Yes ☐ No ☐N/A
Incubator(s) ☒ Yes ☐ No ☐N/A
Freezer(s) ☒ Yes ☐ No ☐N/A
Centrifuge(s) ☒ Yes ☐ No ☐N/A

Biological safety cabinet(s) ☒ Yes ☐ No ☐N/A
Does the outside door have a biohazard sign? ☒ Yes ☐No
Does the laboratory door have the principal investigator's name and contact numbers, including an after-hours number in case of an emergency or questions about the laboratory? ☒ Yes ☐ No
If you need labels, contact the Biosafety Officer, Marcia Finucane mfinu2@email.uky.edu

SECTION F: DISINFECTANTS

All laboratory areas/growth rooms/growth chambers/greenhouses must have adequate disinfectants on hand for clean up of work areas and spills. Please check which of the following disinfectants that you will use in your facility. If efficacy data is not on file with the Biological Safety Officer, please provide with your registration forms. A discussion of disinfectants is available at http://ehs.uky.edu/biosafety/disinfectants.html.

Class of Disinfectant	Percent concentration	Exposure time		
		10 min.	20 min.	30 min.
Chlorine Type: bleach (1:10 dil.)	0.5% Na Hypochlorite	☐	☐	☒
Alcohol ☐ Isopropyl ☒ Ethyl	70	☒	☐	☐
Quaternary Ammonium Compounds Name: ____	____	☐	☐	☐
Phenolic Compounds Name: ____	____	☐	☐	☐
Iodophor Compounds Name: Wescodyne	1%	☒	☐	☐
Glutaraldehyde	____	☐	☐	☐
Other: spill kit Versa-Clean (Fisher)	manufacturer recs 3%	☒	☐	☐

SECTION G: LABORATORY SPILL PROCEDURE

Use the template at http://ehs.uky.edu/hmm/spill.html to describe your spill/exposure protocol for the particular safety issues of your laboratory work. Include: Where to go; who to contact; Worker's Care information. Copies of the spill procedure must be kept in the Laboratory Safety Manual. You may attach your spill procedure separately or copy and paste it here.

1. Notify people in immediate area of spill.
2. Don appropriate PPE (lab coat , gloves, eye protection).
3. Cover spill with paper towel absorbant diapers. (Spill kit is underneath [redacted] sink)
4. If needed for biological material spill, pour 1:10 dil. bleach on paper towels and wait 30 minutes before removing. If not, use spill kit.
5. Use additional absorbant material to clean spill, working from outside to the center.
6. If biological, clean spill area again with fresh paper towels and 1:10 dil. bleach.
7. Place all materials in appropriately bag for disposal-autoclavable biohazard bag if biological.
8. Notify PI ([redacted]) that spill has been contained.

For the research agents or recombinant molecules to be used in this protocol, describe in detail any additional biosafety precautions and containment methods that will be employed. _____

See spill protocol above.
Briefly, human or other biological spills will be contained, absorbed and autoclaved for 60 minutes at 15 lbs pressure, >121C.

PART 5: PRINCIPAL INVESTIGATOR'S STATEMENT

I attest that the information contained in the attached registration, dated 10/10/07, is accurate and complete. I agree to comply with all requirements pertaining to the use, handling, storage and disposal of biohazardous agents and recombinant DNA molecules. I also agree to follow the current **NIH *Guidelines for the Use of Recombinant DNA Molecules*** and the CDC recommendations from the CDC/NIH handbook, ***Biosafety in Microbiological and Biomedical Laboratories***. I shall not initiate or modify any recombinant DNA research until I have registered with the IBC, received IBC approval, and received notification by the Biological Safety Officer (BSO) that work may start. I will ensure that, prior to the initiation of the experimental work, all laboratory workers under my supervision receive training in and adhere to:

Good microbiological and general laboratory practice
Emergency procedures for exposure and spills
The safe operation of laboratory equipment
The hazards and symptoms of exposure relevant to the biological materials used within the laboratory
Written protocols for all laboratory procedures that they have access to

I will select and provide appropriate personal protective equipment to all laboratory workers as necessary for the procedures required in the experiment. If use of a biosafety cabinet is required while working with biological materials, then I will ensure that it is certified ***annually*** and maintained properly. Any vaccinations or medical surveillance requirements as determined by the IBC will also be met prior to the initiation of experimental work. I will comply with shipping requirements for recombinant DNA molecules, infectious agents, and biohazardous materials.

I will correct work errors and conditions that may result in the release of recombinant DNA materials.

I will notify the IBC through the Biosafety Officer in the event of any of the following as soon as possible and within 30 days:

1. Any accident that results in inoculation, ingestion, and inhalation of biohazardous agents or recombinant DNA or any incident causing serious exposure of personnel or danger of environmental contamination.
2. Any problem pertaining to the operation of biological and physical containment safety equipment such as a biosafety cabinet or facility failure such as a power outage which may compromise building engineering controls and subsequently, the safety of the workers in the lab.
3. Any problems with biologic containment (e.g., purity, genotypic or phenotypic characteristics).
4. All significant problems with and violations of the NIH Guidelines and all significant research-related accidents and illnesses, including adverse events in human gene transfer trials.
5. The experimental work has been completed and/or I am leaving University of Kentucky. In either instance, a close-out inspection will need to be conducted by the Biosafety Officer. I will contact the Biosafety Officer at 257-1049 to arrange for the inspection.

I acknowledge that the IBC registration or approval granted by this registration is not transferable to any other University of Kentucky faculty member. I also acknowledge that I will not transfer biological materials that I have received approval to use to any other University of Kentucky faculty member without the consent and approval of the IBC (who will verify that the receiving faculty member has appropriate IBC approval to use the agents).

Principal Investigator's Name (printed) **Principal Investigator's Signature** **Date**

ATTACHMENT A: USE OF INFECTIOUS AGENTS

Infectious agent is used broadly to apply to microorganisms that infect humans, animals, plants, or insects.

Use a separate sheet for each infectious agent to be used or created.

1. **Name of the agent & source of the agent** (where the agent was acquired, e.g.; Dr. Smith from Harvard, commercially acquired, ATCC product number). Mission shRNA Lentiviral particles (Sigma, St Louis)
2. **Risk level**
   Please check the appropriate Risk Group and Containment Level (refer to CDC/NIH Biosafety in Microbiological and Biomedical laboratories, http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm).

   Risk Group ☐RG1 ☒RG2 ☐RG3 ☐RG4

   Containment Level ☐BSL1 ☒BSL2 ☐BSL3 ☐BSL4

3. What is route of Infectious dose for humans? Ingestion, parenteral, mucous membranes
4. Symptoms of exposure: none
5. Disease resulting from exposure to this agent: none
6. Medical surveillance required? ☒ No ☐ Yes If yes, please describe: _____

   All laboratory personnel involved with an exposure incident must notify the PI immediately and the Biosafety Officer (during normal work hours). Vaccinations must be offered to at-risk personnel whenever available. The PI must verify documentation for the vaccination or signed declination.

   Is a vaccination available for the organism to be used? ☒No ☐ Yes If yes, please indicate: _____

   Have all at-risk personnel been offered the vaccination? ☒ N/A ☐ Yes ☐ No
   If no, explain: _____

   Have all at-risk personnel been advised of the risks and benefits of the vaccine? ☒ N/A ☐Yes ☐No If no, consult with an occupational health physician: _____

   Have at risk personnel who refuse vaccination signed a waiver? ☒N/A ☐ Yes ☐ No

PLEASE KEEP IN LABORATORY SAFETY MANUAL FOR AUDIT PURPOSES.

ATTACHMENT A: USE OF INFECTIOUS AGENTS

Infectious agent is used broadly to apply to microorganisms that infect humans, animals, plants, or insects.

Use a separate sheet for each infectious agent to be used or created.

7. **Name of the agent & source of the agent** (where the agent was acquired, e.g.; Dr. Smith from Harvard, commercially acquired, ATCC product number). Adenovirus, type 5; replication deficient – vectors purchased fron Q-Biogene, CA.
8. **Risk level**
   Please check the appropriate Risk Group and Containment Level (refer to CDC/NIH Biosafety in Microbiological and Biomedical laboratories, http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm).

   Risk Group ☐RG1 ☒RG2 ☐RG3 ☐RG4

   Containment Level ☐BSL1 ☒BSL2 ☐BSL3 ☐BSL4

9. What is route of Infectious dose for humans? Inhalation, mucous membranes and parenteral
10. Symptoms of exposure: cold-like symptoms, conjunctivitis, pneumonia, corneal inflammation
11. Disease resulting from exposure to this agent: none
12. Medical surveillance required? ☒ No ☐ Yes If yes, please describe: _____

    All laboratory personnel involved with an exposure incident must notify the PI immediately and the Biosafety Officer (during normal work hours). Vaccinations must be offered to at-risk personnel whenever available. The PI must verify documentation for the vaccination or signed declination.

    Is a vaccination available for the organism to be used? ☒No ☐ Yes If yes, please indicate: _____

    Have all at-risk personnel been offered the vaccination? ☒ N/A ☐ Yes ☐ No
    If no, explain: _____

    Have all at-risk personnel been advised of the risks and benefits of the vaccine? ☒ N/A ☐Yes ☐No If no, consult with an occupational health physician: _____

    Have at risk personnel who refuse vaccination signed a waiver? ☒N/A ☐ Yes ☐ No

PLEASE KEEP IN LABORATORY SAFETY MANUAL FOR AUDIT PURPOSES.

PI Name: [redacted]

ATTACHMENT R: RECOMBINANT DNA EXPERIMENTS

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

Describe rDNA construct(s) below.

Include a description, in molecular terms (e.g. promoter[s], Orbs, selectable markers), of the rDNA construct and provide a map if available. It is **not** necessary to provide details about every construct; categorical descriptions that are useful in assessing risks are acceptable. Describe the method of transfer or transfection. **Describe measures taken to prevent or minimize expression of pathogenic/infectious sequences**. Please avoid or explain acronyms. Refer to the NIH Guidelines for additional information regarding information requested in this form. (http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html)

Gene Source (Genus, species, strain)	**Gene Name (Explain Acronyms)**	**Gene Category (See below)**	**Use of Construct (Expression in cell culture, animals, etc.)**
H. sapiens	Dusp4	Enzymatic protein	Expression in cell culture
H. sapiens	K-ras	oncogene	Expression in cell culture
H. sapiens	EGFR	oncogene	Expression in cell culture
_____	_____	_____	_____

Example Gene Categories:

Structural, Enzymatic proteins, Metabolic enzymes, Cell growth/Housekeeping, Cell cycle/Cell division, DNA replication, Membrane proteins, Tracking genes (GFP, Luciferase), Toxins, Regulatory genes, Oncogenes

Do the proposed experiments transfer a drug resistance trait to microorganisms, not known to acquire the trait naturally, that could compromise the use of the drug to control disease in humans, veterinary medicine, or agriculture? (Section III-A-1-a, V-B) ☒No ☐Yes Name the drug: _____

Do the proposed experiments involve the cloning of toxin molecules? (Section III-B-1) ☒No ☐Yes Name the toxin: _____

Do the proposed experiments involve the deliberate transfer of recombinant material to human research participants? (Section III-C-1) ☒No ☐Yes If yes, complete Attachment G.

Do the proposed experiments utilize Risk Group 2, 3, or 4 agents as a host-vector system or as a source of genetic material for nonpathogenic prokaryotic or lower eukaryotic host vector systems? (Section III-D-1, D-2) ☒No ☐Yes Name the agent: _____

Do the proposed experiments involve the use of infectious or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems? (Section III-D-3) ☒No ☐Yes Name the viruses: _____

Do the proposed experiments involve the transfer of recombinant material to whole animals? (Section III-D-4) ☒ No ☐Yes

Will recombinant material representing greater than two-thirds of a eukaryotic viral genome be transferred to whole animals? (Section III-D-4-a) ☒No ☐Yes

Will any of the proposed experiments generate transgenic animals? (Section III-D-4-b, 4-c, III-E-3) ☒No ☐ Yes Genus, species of transgenic animal: _____

Will transgenic rodents be purchased or transferred for this research? (Section III-F, Appendix C-VI) ☒No ☐ Yes

Do the proposed experiments involve the genetic engineering of whole plants? (Section III-D-5) ☒No ☐Yes If yes, complete plant specific registration forms.

Vector Description(s)

Attach a construct map; if this is a commonly-used commercial vector, no map is necessary. If viral vector, indicate what viral sequences are being deleted from the wild-type vector (e.g. replication competent vector rendered replication defective), what foreign viral sequences are being inserted and the specific location for insertion.

Vector Backbone (Plasmid, Phage, Virus)	Vector Technical Name	Vector Source	Gene Transfer Method	Host	Stable or Transient Expression
virus	pShuttle-CMV	Q-Biogene	Electroporation	E. Coli	both
virus	pAdTrack	Q-biogene	Electroporation	E.Coli	both

Description of deleted viral sequences for replication defective vectors: (1) E1 and E3 of adenoviral (Ad5) genome
(2) For lentiviral shRNA vector, I extracted this from the Sigma website:
The MISSION TRC lentiviral particles are replication incompetent. The particles are made using features of 2nd and 3nd generation lentiviral packaging systems. Genes for replication and structural proteins are absent in the packaged viral genome since these genes are supplied by other plasmids in the packaging cells. The viral genome contains only the region between the 5' and 3' LTRs of pLKO.1. In addition, the lentiviral vector contains a self-inactivating 3' LTR that chromosome.

Description and location of inserted foreign viral sequences: (1) human Dusp4, K-ras, and EGFR into the pAdTrack/pShuttleCMV vector and (2) shRNAs toward genes of interest in the lentiviral vectors.

Risk Attenuation (Replication-defective, Helper-dependent, Disarmed, K-12 derivative, Restricted to prokaryotic expression): both adenoviral and lentiviral vectors are replication defective

Will DNA integrate into the host genome? ☐No ☒Yes

What is the probability of generating replication competent viruses? Minimal; however staff has been advised that use of lentivirus in HIV positive individuals raises the risk of generating replication competent retroviruses.

Packaging Cell Line(s) for Production of Virus Particles

Name of Cell Line(s) and/or Helper Plasmids (co-transfection): (1)HEK 293 containing E1 and E3 for propagation of adenoviral vectors (2) lentiviral vectors will not be propagated in the laboratory

Source(s): ATCC

Source of envelope glycoprotein if ☐Retrovirus or ☒Lentivirus: plasmid

Characterization with respect to host range if Retro- or Lentivirus (ecotrophic, amphotrophic): amphotrophic

ATTACHMENT TC
Complete this form for work with animal or human organ, tissue, or cell cultures (OTCC).
For work with plant cell culture, complete Attachment P.

Technical Name of OTCC	OTCC Origin (Genus, species, tissue type)	Source of Material	Passage: Primary Immortal	Will the material contain recombinant nucleic acids upon acquisition? Yes or No If yes, describe	Is the material known to be infected with a pathogen? Yes or No If yes, describe	If infected, is shedding expected?
293 HEK	Human Embryonic Kidney	ATCC	Immortal	No	No	N/A
Beas2b	human bronchial epithelial cells	ATCC	Immortal	No	Yes, Immortalized by modified adenovirus expressing SV40 T antigen	N/A
SAEC	small airway epithelial cells	Promocell	primary	No	No	N/A
HCC827	lung cancer cell line	[redacted], MDAnderson, Texas	Immortal	No	No	N/A
PC9	lung cancer cell line	[redacted] HL Moffitt Cancer Center, FLA	Immortal	No	No	N/A
H3255	lung cancer cell line	[redacted], NCI, Bethesda, MD	Immortal	No	No	N/A
H1650	lung cancer cell line	ATCC	Immortal	No	No	N/A
H1975	lung cancer cell line	ATCC	Immortal	No	No	N/A

ATTACHMENT TC
Complete this form for work with animal or human organ, tissue, or cell cultures (OTCC).
For work with plant cell culture, complete Attachment P.

Technical Name of OTCC	OTCC Origin (Genus, species, tissue type)	Source of Material	Passage: Primary Immortal	Will the material contain recombinant nucleic acids upon acquisition? Yes or No If yes, describe	Is the material known to be infected with a pathogen? Yes or No If yes, describe	If infected, is shedding expected?
H820	lung cancer cell line	[redacted], UTSW, Texas	Immortal	No	No	N/A
H358	lung cancer cell line	ATCC	Immortal	No	N/A	N/A
H431	epidermoid cancer cell line	ATCC	Immortal	No	N/A	N/A
A549	lung cancer cell line	ATCC	Immortal	No	N/A	N/A
H460	large cell lung cancer	[redacted], UK	Immortal	No	N/A	N/A
H2122	lung cancer cell line	[redacted], UTSW, Texas	Immortal	No	No	N/A
H322	lung cancer cell line	[redacted] U Colorado, Denver, CO	Immortal	No	No	N/A
UKY29	lung cancer cell line	[redacted], UK	Immortal	No	N/A? not tested to my knowledge	N/A

ATTACHMENT TC

Complete this form for work with animal or human organ, tissue, or cell cultures (OTCC).
For work with plant cell culture, complete Attachment P.

Technical Name of OTCC	OTCC Origin (Genus, species, tissue type)	Source of Material	Passage: Primary Immortal	Will the material contain recombinant nucleic acids upon acquisition? Yes or No If yes, describe	Is the material known to be infected with a pathogen? Yes or No If yes, describe	If infected, is shedding expected?
adenocarcinoma	Human lung cancer	UK Biospecimen Core or biopsy	primary	No	N/A	N/A

All human tissue/cell culture work is expected to be conducted under BSL2 containment and procedures. Note: ATCC does not screen cell lines for human pathogens. Staff doing this work is covered under the OSHA blood borne pathogen regulations (other potentially infectious material, OPIM) which require:

1. **Blood Borne Pathogen training (http://ehs.uky.edu/classes/bloodborne/bptrain.html) updated annually**
2. **Exposure control plan (a completed Attachment BBP of this form meets this requirement)**
3. **Hepatitis B vaccination offered at employer's expense**
   If vaccination is declined, a signed declination form must be kept in your safety manual