IBC Application Form
University of Pittsburgh - Institutional Biosafety Committee

Use this form as of 14 February 2011© for:

New research proposals involving the use of recombinant materials
Modifications to previously approved IBC protocols (see Qualifying Modifications, below)
Third year (Full renewal) continuing review of previously approved IBC protocols

To UPCI Lentiviral Facility USERS

Please see the following for our recommendations for submission or modification of your rDNA protocol to be able to obtain Lentivirus from the core facility.

Only those sections specific to Lentivirus use are filled out. The remainder of the protocol will be specific to your project.

This modification is only for those requesting lentivirus that is to be used under BSL-2 biosafety containment.

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Third year (Full renewal) continuing review of previously approved IBC protocols

1) **Submit the application electronically via email attachment to: rdna@pitt.edu**
2) **Submit the Investigator's NIH-style biosketch via email attachment**
3) **Fax a Signed copy of the Assurance page to the IBC Office -- FAX: 412 383-1769**

SECTION A.1. Investigator Assurance

a. I agree to conduct this research in accordance with the compliance policies of the IBC Office, University of Pittsburgh Institutional Biosafety Committee, including all requisite training of students, staff and other professionals participating in this research.

b. I have consulted **Section IV-B-7** of the *NIH Guidelines* describing the responsibilities of the Principal Investigator and hereby agree to comply fully with all provisions of the *NIH Guidelines*

c. I understand I am responsible for assuring that my research facilities are in compliance with local, state and federal environmental laws and regulations.

d. I understand that I am responsible for the proper conduct of any research by Co-Investigator(s) that are directly related to this protocol application

e. I understand that all changes in the research protocol (including changes in the source of DNA, host-vector systems, dosage ranges, laboratory room changes, etc.) or research participants must be reported to the IBC Office and all other university regulatory offices in connection with this protocol.

f. If funded by an extramural source, I assure that this application accurately reflects all procedures involving recombinant DNA as described in the grant proposal to the funding agency.

g. The information within this application is accurate to the best of my knowledge.

h. I understand that yearly reporting is required for continuing approved research.

i. I understand that all protocols must be resubmitted for committee review after a term of three years.

j. By the submission and acceptance of this signed document at the IBC Office I am in agreement with the statements a-i (above).

NOTE: The IBC Office and IBC in conjunction with the EHS Office reserve the right to conduct inspections of the research facilities at any time

Principal Investigator's name typed:

Principal Investigator's signature ______________________________________ November 12, 2013

Fax a Signed copy of *ONLY* this first page to the IBC Office -- FAX: 412 383-1769

It is the Principal Investigator's responsibility to ensure all personnel involved in this study are appropriately trained, and are provided the equipment necessary to perform at the designated biosafety containment level.

SECTION A.2. IBC Research Project Title	

Additional Information

Containment determination and issues to consider: In determining the appropriate containment for a project, it is important to consider factors that may raise concerns regarding the materials or agent(s) used.

Factors used to determine the level of containment include: virulence, pathogenicity, infectious dose, environmental stability/instability, route of spread/infection, communicability/pathogenicity, safety procedures/operations, quantity of agent(s), availability of vaccine or treatment and any gene product effects such as: toxicity, physiological activity, or allergenicity.[1]

> **Note that careful consideration should be given to the type of manipulation planned for some higher risk group agents. Any strain that is known to be more hazardous than the parent (wild-type) strain should be considered for handling at a higher containment level.[2]

Investigators should additionally consider any potential for unintended adverse events and/or potential for misuse of the research. Special consideration should be given to any experimental paradigms that: [3]

- ✓ Would demonstrate how to render a vaccine ineffective and thus potentially jeopardize public health
- ✓ Would confer resistance to therapeutically useful antibiotics or antiviral agents
- ✓ Would enhance the virulence of a pathogen or render a non-pathogen virulent
- ✓ Would increase transmissibility of a pathogen
- ✓ Would alter the host range of a pathogen
- ✓ Would enable the evasion of diagnostic/detection modalities
- ✓ Would enable the weaponization of a biological agent or toxin

[1,2]NIH Guideline for Research Involving Recombinant DNA Molecules (NIH Guidelines), II-A-3, 2002
[3]National Research Council, Biotechnology Research In An Age of Terrorism, National Academies Press, 2004

Note regarding inserted DNA: In some instances the investigator may not be able to determine in advance all of the inserted DNA segments (or transgenes) to be used in a particular vector system. In most instances, the addition of new inserts/transgenes will not alter the biosafety level and will not require IBC review, and all that is required is for the investigator to inform the IBC Office of the change. However, there are exceptions that necessitate submission of a modification for review by the committee, including:

- Inserting DNA with oncogenic potential into any lentiviral vector
- Manipulating genes from any HHS or USDA Select Agent or Toxin
- Manipulating genes from H5N1 avian, 1918 pandemic H1N1, or non-contemporaneous H2N2 influenza strains
- Inserting DNA that has the potential to increase the pathogenicity or virulence of a vector system
- Transferring a drug resistance trait that has the potential to compromise the use of the drug to control disease
- Transferring a herbicide or insecticide resistance trait into a crop plant
- Transgenic modification of a food animal

If in doubt, investigators are encouraged to contact the IBC Office for advise on whether modifications are required.

Qualifying Modifications: Typically minor modifications are handled at the IBC Office level and do not require IBC review. Please contact the IBC Office for help in determining the type of modification. Significant (qualifying) modifications require IBC review and will require the investigator to complete the entire form. These include such changes as:

- Addition of a new vector system not previously approved by the IBC for use by the investigator
- Addition of new *in vivo* work not previously approved by the IBC (previous approval was for *in vitro* work only)
- Change in Biosafety Level (either upgrade or downgrade)
- Significant change in DNA inserts as noted above (see “Note regarding inserted DNA”)
- Change to an investigator not having any current IBC registrations – IBCs must review the investigator’s background and experience

IBC Registration: All research involving recombinant DNA materials or procedures must be registered with the IBC Office. Registration includes research in which genetically-modified animals are bred in any University of Pittsburgh facility.

- Research qualifying for IBC Registration status will be provided a complete term of three years, which is renewable
- The IBC Office distributes renewal notices via email at 120, 60, 30-day, and two weeks prior to expiration of the registration
- Investigators are responsible for updating contact information so that renewal notices are received
- ***Have questions? Contact the IBC Office: 412-383-1768 or email rdna@pitt.edu***

Annual renewal reports: IBC approved protocols must be renewed annually at the IBC Office to maintain continuing IBC approval. Annual renewals with *no changes* may use the IBC Renewal Report form.

- The IBC Office distributes renewal notices via email at 120, 60, 30-day, and two weeks prior to expiration of the approval
- Investigators are responsible for updating contact information so that renewal notices are received
- ***Have questions? Contact the IBC Office: 412-383-1768 or email rdna@pitt.edu***

Termination: Protocols that are not renewed expire the day following the expiration date and are automatically terminated. A termination letter is distributed to the investigator and other compliance offices/divisions as appropriate.

- Terminated protocols may not be "re-started"
- A new application must be completed. Send all correspondence to rdna@pitt.edu

Co-Investigators: The *NIH Guidelines* states that the Principal Investigator is responsible for ensuring that the laboratory staff are appropriately trained (*Section IV-B-1-h*), is responsible for full compliance of the conduct of the rDNA research, and supervision of safety performance of laboratory staff (*Section IV-B-7*).
If Principal Investigators wish to indentify co-investigators with a specific IBC protocol, the **Co-Investigator Assurance** form must be completed and signed by each Co-Investigator, and submitted to the IBC Office along with their NIH style biosketch. Please note that correspondence from the IBC will be directed to the Principal Investigator as the recognized responsible individual for the research.

If the investigator is a post-doctoral fellow, graduate student, or equivalent, a mentor must be identified in Section **A4** (Alternate Contact) of the application and a **Mentor Agreement** must be completed and provided with the application.

Alternate Contacts: The Alternate Contact is a designated individual to whom IBC correspondence is copied with regards to the named investigator's IBC research protocols. The IBC Office cannot release protocol documents or other information unless named as an alternate or through written permission from the named investigator.

Application instructions: Do not type in the gray-shaded sections. Do not leave any blanks, unless instructed to do so. Incomplete applications will be returned. Send to: rdna@pitt.edu

SECTION A3.	**Principal Investigator –** ***The person responsible for the recombinant research***
Principal Investigator name	
Professional title/Job Title	
Degree	
Department/Division	
Office; room and building	
Office telephone	
Office facsimile	
Office street address	
Mailing zip code	
E-mail address	

SECTION A4.	**Alternate Contact –** ***The named alternate contact receives copies of IBC approval and final renewal reminders (2 weeks prior to protocol expiration)***
Alternate contact name	
Professional title/Job Title	
Degree	
Location; room and building	
Telephone	
E-mail address	

SECTION A5. Project Submission				
a. Is this a *modification* or a *3rd year renewal* of an existing approved IBC application? **(Mark "X" for Yes/No in un-shaded box)** • **If "NO" skip to the next section**	Yes		No	
b. Provide the IBC protocol number for this continuing review in the text box provided				
c. Have there been any changes in the location of the research facilities since the last IBC review interval? Did the lab relocate to another building or room? **(Mark "X" for Yes/No in un-shaded box)** • **If "YES", Section F must also be completed to reflect the new information**	Yes		No	
d. Have there been any reported injuries/exposures since the last IBC review interval? (Mark "X" for Yes/No in un-shaded box)	Yes		No	
e. If "YES" to "d" (above), were these changes reported to the Department of Environmental Health and Safety (EHS) or the IBC? **(Mark "X" for Yes/No in un-shaded box)**	Yes		No	

Date:	**Protocol Number:**	**Revision:**

SECTION B1. Declaration of Conflict of Interest				
Does the investigator or any other research personnel involved in this study (or in aggregate with his/her spouse, dependents, or other members of his/her household):				
a. possess an equity interest in the publicly-traded entity that either sponsors this research or owns the technology being evaluated that exceeds 5% ownership interest or a current value of $10,000? (Mark "X" for Yes/No in un-shaded box)	Yes		No	
b. possess any equity interest in the non-publicly-traded entity that either sponsors this research or owns the technology being evaluated? (Mark "X" for Yes/No in un-shaded box)	Yes		No	
c. receive salary, consulting fees, honoraria, royalties or other payments from the entity that either sponsors this research or owns the technology being evaluated that is expected to exceed $10,000 in any twelve-month period? (Mark "X" for Yes/No in un-shaded box)	Yes		No	
d. have rights to the intellectual property (IP) being evaluated as either the inventor of the IP for which a patent has been issued, or as the inventor of the IP that has been optioned or licensed to a company? (Mark "X" for Yes/No in un-shaded box)	Yes		No	
e. have a financial relationship with a Licensed Start-up Company (which is being monitored by the COI Committee) that has an option or license to utilize the technology being evaluated? (Mark "X" for Yes/No in un-shaded box)	Yes		No	
If any responses to questions in Section B1 are "yes", please attach a separate sheet providing the name of the person(s) with the potential conflict of interest, and describe the nature of the relationship indicated above. Please note that any COI attached information will be forwarded to the University's Conflict of Interest Committee for review.				

SECTION B2. Funding information Reference the *NIH Guidelines*: Sections: I-C-1-a, I-C-1-b				
a. Is this project NIH funded? (Mark "X" for Yes/No in un-shaded box)	Yes		No	
b. List *ALL* funding sources that are supporting this protocol: • **Provide all funding for this project if there is more than one funding source** • **Provide the funding start and end dates** • **Include internal (departmental) funding (e.g. "start up")** **Note: For internal funding insert "not applicable" for grant info.**	**Funding sources**		**Grant #s and active dates of the grant**	
c. Is funding administered through the University of Pittsburgh or UPMC? (Mark "X" for Yes/No in un-shaded box)	Yes		No	
d. If "NO" to the question directly above, name the institution responsible for administering the grant				

SECTION B3. Breeding/CrossBreeding Reference the *NIH Guidelines*: Sections: III-D-4-c(i)				
a. Does the project involve *on-site* breeding or crossbreeding of genetically-modified vertebrate animals)? (Mark "X" for Yes/No in the un-shaded box) • **If "NO" skip to the next section (C1)**	Yes		No	
b. Provide the *IACUC* protocol number and the *Biosafety Level* of the facilities	IACUC		BSL	
c. Does the project involve rodents (parental or offspring) that contain more than 50% of the genome of an exogenous eukaryotic virus from a single virus family? (Mark Yes or No in un-shaded box)	Yes		No	
d. Does the project involve rodents where a transgene is under the control of a gammaretroviral long-terminal repeat (LTR) and where the LTR is functional? (Mark Yes/No in un-shaded box)	Yes		No	
e. Does this project involve *any other* recombinant DNA materials or include any toxins included in *Appendix F*? (Mark "X" for Yes/No in un-shaded box) • **If "NO" to c, d, and e (above); the IBC breeding application is complete**	Yes		No	

	SECTION C1. Determination of use Indicate all that apply; mark the unshaded boxes		***NIH Guidelines* references (links)**
a.	**Using recombinant DNA/RNA (rDNA) molecules for detection purposes**		III-F
b.	**Creating or using genomic libraries**		III-E, III-F
c.	**Cloning and vector construction in bacteria and yeasts**		III-E, III-F
d.	**Expression of rDNA products in cultured cells**		III-E, III-F
e	**The use of human cells/cell lines or tissues (e.g. human blood, 293 cell lines, CSF)**	*	II-A-3; BBP
f.	**Using animal cells/cell lines or tissues (e.g. tissue culture research)**	*	II-A-3 Appendix C
g.	**Use of *human* stem cells (embryonic or adult) – If "yes" provide hSCRO identifier *or* the submission date for hSCRO review in the text box below**		hSCRO –Stem Cell Oversight
h.	**Using or cloning genes from, or into a risk group 2 or 3 agent (e.g. HSV, SIV)**	X	III-D-1 III-D-2
i.	**Administration of rDNA material into animals (e.g. transformed cells, vectors)**		III-D-4
j.	**Experiments involving whole plants in research - requires completion of IBC Form Attachment 2 with application**		III-D-5
k.	**Propagating culture volumes exceeding 10 liters at one time**		III-D-6
L.	**The use or manipulation of infectious viruses or replication-defective viruses or viral vector(s) with helper viruses**	X	III-E-1 III-D-3
m.	**Experiments involving influenza viruses**		III-D-7 **NEW**
n.	**Using or cloning of genes from, or into a risk group 4 or a Select Agent**		III-D-1-d
o.	**Administration of rDNA materials into humans - requires completion of IBC Form Attachment 1 with application, plus any additional documents**		III-C-1
p	**Using or cloning of toxin molecule genes (e.g. deliberate formation)**		III-B-1
Q*	**Transfer of a drug resistance trait into a risk group 2 or 3 agent**		III-A-1-a
R*	**Transfer of a drug resistance trait into a risk group 4 or a Select Agent** see also 42CFR73, 7CFR331, 9CFR121 for more information regarding Select Agents Regulations		III-A-1-a

NOTE for boxes ***Q* & ***R*** :
Per the *NIH Guidelines*: Section III-A-1-a. The deliberate transfer of a ***drug resistance trait*** to a microorganism, that is not known to acquire the trait naturally, when such a manipulation could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, is considered to be a ***Major Action*** and requires federal Recombinant Advisory Committee (RAC) review.

s.	**Other purpose for IBC review- Please state the purpose in text box below:**	

*** Use of animal or human cells lines must be checked depending on your specific project.**

SECTION C2. Biosafety Level Containment and Risk Group Information

Please reference the *NIH Guidelines*: Sections: II-A-1, IV-B-7-c, Appendix B and Appendix G

For Research proposed at BSL-2+ and above, a facilities inspection and Biosafety Operations Manual is REQUIRED for IBC approval; contact the Department of Environmental Health and Safety (EH&S) for additional information 412-624-9505 or safety@ehs.pitt.edu

1. Provide the risk groups (or class) of ALL material(s) used in this project by marking the un-shaded box(es)

Risk Group 1	Agents are *Not* associated with disease in healthy adult humans.	
Risk Group 2	Agents are associated with human disease that is rarely serious. There are often preventive or therapeutic interventions available.	X
Risk Group 3	Agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *MAY* be available.	X
Risk Group 4	Agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *NOT USUALLY* available.	

2. Indicate the biosafety level(s) at which work is performed for this project by marking the un-shaded box(es)

Note: more than one biosafety level may apply to your project

BSL-1	• Low risk agents (generally risk group 1), special containment equipment not required • Work is done on open bench tops • Standard microbiological practices are observed • Biohazard signs should be posted	
BSL-2	• Moderate risk agents (generally risk group 2), biosafety cabinets, restrictions to research areas • All BSL-1 containment and practices plus the following: • Laboratory access is restricted when experimental work is in progress • Personnel have specific training in handling of agents • Biological safety cabinets (BSC) or other physical containment devices are used for potential aerosol generation procedures • Biohazard signs must be posted • Specific PPE (personnel protective equipment) and entrance requirements	X
BSL-2+	• Moderate-High risk agents (generally risk groups 2 or 3), BSL-2 containment **with** BSL-3 practices • All BSL-2 containment and practices plus the following: • Laboratory access is restricted • Personnel have specific training in handling of agents; *Section H must be completed* • All procedures are performed in biological safety cabinets (BSC) • Biohazard signs must be posted • Written safety policies provided by the investigator defining laboratory procedures, waste disposal, disinfection and medical surveillance (*Biosafety Operations Manual*) • Centrifuge safety cups must be used	
BSL-3 Or Enhanced BSL-3	• High risk agents (generally risk group 3), BSL-3 containment facilities, and practices • All BSL-2 containment and practices plus the following: • Laboratory access is restricted • Personnel have specific training in handling of agents; *Section H must be completed* • All procedures are performed in biological safety cabinets (BSC) • Biohazard signs must be posted • Written safety policies provided by the investigator defining laboratory procedures, waste disposal, disinfection and medical surveillance • Centrifuge safety cups must be used • Specific facility design parameters must be followed, including requirements for location, ventilation, room integrity and security • Facility must be commissioned according to University of Pittsburgh BSL-3/ABSL-commissioning processes	

SECTION C3. Project summary

Describe the experimental procedures involving recombinant DNA materials. Use non-technical terminology to enable IBC community representatives to understand your project. Include in this summary the following information:

1) **Provide a 2-3 sentence abstract of the project that *specifically relates to the recombinant work***
2) **Describe the procedures and techniques to be used with the recombinant material in the project**
   For example, if the research involves a recombinant virus, bacteria, or other organism:
   - **Describe the vectors and the transgenes being used**
   - **Describe how the vectors are used in the research project**
3) **Summarize the use of all viruses, including lentiviral vectors**
4) **If an IACUC protocol will be associated with this IBC protocol, be sure to summarize how the recombinant work relates to the animal work**
5) **If using multiple biosafety levels, describe the procedures to be performed at each level**
   For example, work with infectious HIV-1 at BSL-2+; work with 3rd generation (4-plasmids) HIV-1 lentiviral vectors at BSL-2; vaccine study with a single HIV-1 gene at BSL-1

Helpful Hints: Call the IBC Office if you need assistance or have questions about the application: 412-383-1766

- ✓ **Do *not* copy from a grant application**
- ✓ **Limit the description to the rDNA relevant to the project**
- ✓ **Address any potential biosafety issues and how they will be minimized**
- ✓ **Use non-scientific language so that community members may understand the research proposed**

NOTE: The IBC Office reserves the right to return any proposals that do not meet the above conditions or require extensive clarification or corrections prior to committee review

Enter Project Summary here:

SECTION C4. Biosafety Risk Information ➢ *Note: Protocols using BSL-2 or higher must be registered with EH&S*	
1. Provide the date of your most recent laboratory inspection *or* corresponding EH&S Workbook number (contact EH&S @ 412-624-9505 for required information)	You must submit an EHS workbook.
2. Describe whether the agent(s) used in the course of this research may be infectious to humans	Yes, HIV based lentivirus can infect human cells
3. Describe the risks of accidental exposure to personnel or the environment	All needed precautions are taken under consideration for the work proposed in this project. Since we use lentiviruses, we operate under BSL-2 conditions as needed.
4. Describe whether there is any potential for airborne transmission of agent(s) used in this research	Accidental exposure is minimized by using gloves, wearing lab coats and masks when needed.
5. Describe any precautions to be taken by personnel including any personal protective equipment (PPE) used, engineering controls (e.g. biosafety cabinet), and/or routine monitoring (e.g. TB testing - if applicable to materials used)	For work with the lentiviruses, these experiments are performed in a BSL-2 approved cell culture lab by trained lab personnel in BS-2 cabinets following University of Pittsburgh approved SOPs for use of lentivirus expression systems.
6. Describe the method used for disposal of the agent(s); describe the specific methods of disposal or inactivation of the agent(s) or contaminated/infectious material(s)	Virus is disposed in bleach (10%) and the surfaces of the BS-2 cabinets are rinsed with (i) 10% bleach, (ii) water and (iii) 70% ethanol after use and the UV light in the cabinet is then illuminated for at least 15 minutes. **Solid Biological Waste:** Solid wastes, such as plastic culture plates, will be disposed in approved biological waste bags. Solid, plastic waste used, during or in contact with potentially infectious material will be disinfected with an appropriate disinfectant prior to disposal of the solid waste in approved biohazardous bags. All biological waste bags will be placed or contained in approved biological waste boxes (labeled with a Pitt biological waste label), and the boxes will be sealed with packing tape and placed in designated areas of the building for pickup. For the HCC, this is outside of the tissue culture rooms Lab 2.1 and Lab 2.11. **Liquid Biological Waste:** Liquid wastes (blood, virus stock, cell culture waste, etc.) will be carefully poured or collected into the appropriate disinfectant (bleach) to inactivate potentially infectious materials. Following sufficient contact time, the disinfected solution will be poured directly down the drain. This will be done carefully to avoid splashing and aerosol generation. The drain will be flushed with disinfectant of sufficient quantity to at least fill the trap. If needed, large volumes of liquid wastes will be autoclaved prior to disposal down the drain.

SECTION C5. Investigator Experience and Training Requirements				
1. Determination of the Principal Investigator's experience and background. **Does the investigator have experience with all vectors, viruses or recombinant materials described in this application?** **(Mark "X" for Yes/No in un-shaded box)** • **If "Yes", provide the number of years experience and any training relevant to the recombinant materials used** • **If "No", describe all training and experience relevant to the proposed work that will support IBC approval of research with the recombinant materials described**	Yes		No	
2. Determination of the investigator's experience and background. Please list all relevant University of Pittsburgh training taken, such as on-line modules, live sessions or seminars (for example, blood borne pathogens, laboratory safety, small/large animal training, etc.)				
NOTE: For all research applications at BSL-2+ or above, Section G is required to be completed. **All personnel listed in Section G of the application must be current with applicable Health and Safety training in order to obtain IBC Approval for rDNA research performed at BSL 2+ and higher**				

SECTION C6. Vectors, Hosts, and rDNA Agents Used in the Research ***Do not leave blanks*** If the question is not applicable to your research, indicate by stating "none" or "not applicable"				
➢ If desired, insert vector map(s) at the END of the application.				
1. List all plasmid backbones used (for example, pUC19, pGEM) • **If none are used, state "None"**				
2. List any oligonucleotides used to manipulate gene function (for example, siRNA) or as adjuvants (for example, CpG-containing DNA) either in cell culture or in vivo • **If no oligionucleotides are used, state "None"**				
3. List inserted DNA used; include the species from which the insert is derived and what gene product is expressed • **If no inserts are used, state "None" in the first box**	**Species**		**Gene product expressed**	
4. List any known oncogenes, or inserts from the question directly above that have oncogenic properties • **If none are used, state "None" and skip to question 6.**				
5. Describe the system used to express the oncogenes. Identify the use of any viral vectors with the expression of the oncogenes				
6. Is there any potential for increased virulence with insertion of DNA into the vector or organism? **(Mark "X" for Yes/No in un-shaded box)** • **If "Yes", explain details in the text box provided**	Yes		No	
7. Are toxins to be expressed and released as part of this research? **(Mark "X" for Yes/No in un-shaded box)** • **If "Yes", describe a) the toxic product(s) (LD_{50} of <100 µg/kg) that could be produced/released and b) the containment precautions to be used**	Yes		No	
8. List all bacterial and/or fungal agents used in the course of this research. • **Provide the specific strains of bacteria, for example, HB 101, DH5α, etc. when feasible.** • **Describe how the bacteria/fungi will be used in the research (for example: amplification of plasmids)** • **If none are used, state "None"**				
9. List all cell lines or eukaryotic cells *including* human cell lines (for example, CHO, COS, or HEK 293 cells) • **State the species of origin of each of the cell lines used** • **If no cells are used, state "None"**				
10. List other organisms (e.g., amoebas, nematodes, drosophilia) • **If none are used, state "None" and skip to the next section**				
11. If transporting or shipping genetically modified arthropods or insects, provide the authorized transport permit number and agency source(s) **Reference 7 CFR 340** • **If it does not apply, state "N/A" and go to the next section**				

SECTION C7. Biosafety Information; Use of Viruses/Viral Vectors *Do not leave blanks* If a question is not applicable to your research, indicate by stating "none" or "not applicable"				
1. Does the project involve the use of viruses or viral vectors? **(Mark "X" for Yes/No in un-shaded box)** • **If "No", skip to Section D**	Yes	X	No	
2. Does the project involve the use of Lentiviruses or Lentiviral vectors? **(Mark "X" for Yes/No in un-shaded box)** • **If "Yes", Sections C8 and C9 must be completed** • **If "No", Section C7 must be completed for non-lentiviral work**	Yes	X	No	
3. List viruses and/or viral vectors used in this research project: • **Specify the Virus Family and/or Subfamily (for example, Herpesvirus, Oncogenic Retrovirus, Adenovirus, Adeno-Associated virus, etc.)** • **State the species of origin for each virus or vector used**	Lentivirus			
4. List inserted DNA used; include the species (column A) from which the insert is derived and the gene product (column B) that is expressed • **If no inserts are used, state "None" in the first box**	**Species** This depends on your project. If using shRNA, indicate "shRNA".	**Gene Product** This depends on your project. If using shRNA, indicate the target of the shRNA.		
5. Is the virus or viral vector able to enter or infect human cells including cell lines, such as 293 cells? (Mark "X" for Yes/No in un-shaded box) • **If "No", skip to Question C7-7**	Yes	X	No	
6. Describe whether it is a productive or limited infection (is it an abortive infection? Is it a single-round infection?)	The lentiviral vectors produced by the UPCI LCF are replication defecetive.			
7. State whether the virus or viral vector could cause any other health effects such as seroconversion	None			
8. Is a helper virus used in this project? **(Mark "X" for Yes/No in un-shaded box)** • **If "Yes", describe the helper virus used in the text box provided**	Yes		No	X
9. Is the virus/viral vector replication-defective? **(Mark "X" for Yes/No in un-shaded box)** • **If "No", skip to Section D (*unless also using Lentivirus/Lentiviral vectors*)** • **If "Yes", A) describe the deletions rendering it defective in the text box provided and B) complete the question below**	Yes	X	No	
	See below			
10. Has the replication-defective vector been tested for replication competent virus? **(Mark "X" for Yes/No in un-shaded box)** • **If "Yes", provide details of the assay used** • **If "No", describe the likelihood of conversion to replication-competent virus?**	Yes		No	X
	Almost impossible to convert. No record of either of these systems ever producing replication competent virus.			

Question 9

Lentivirus supplied by the UPCI-LCF is prepared as follows:

The 3rd generation lentiviral system comprises four plasmids (the expression plasmid plus three packaging vectors: pMD2.g(VSVG), pRSV-REV and pMDLg/pRRE). This generation packaging system offers maximal biosafety, as described in: Dull et al "A third generation lentivirus vector with a conditional packaging system"(1998) J. Virol. 72, 8463-8471 and in Klages et al. "A stable cell line for the high-titer production of third generation lentiviral vectors". Mol. Ther. (2000) 2, 170-6.

SECTION C8. Lentivirus/Lentiviral Vectors; part 1 ***NOTE: If your work does not use lentiviruses or lentiviral vectors, skip to Section D.***				
***NOTE*: It is not required that vectors generated with 4-plasmid lentivirus systems (3rd generation vectors) be tested for replication-competent virus. However, 3-plasmid vector lentivirus systems (2nd generation vectors) must be shown to be free of replicating virus for approval at BSL-2 (for example, you must provide data or the results of a RCV assay). Please see the current IBC Policy on Biosafety Level Assignment for Lentivirus Vectors for details**				
1. List the specific virus or the strain and species of origin. (for example, HIV, human; BSL-2+; FIV, feline; BSL-2) For more information refer to: Lentiviral Resource information	**HIV-based lentiviruses for expression of cDNA and shRNA.**			
2. Is the lentivirus/lentiviral vector obtained from another source? (for example, a company or other investigator) (Mark "X" for Yes/No in un-shaded box) • **If "No", proceed to question 4**	Yes	**X**	No	
3. Provide the name of the source of the lentivirus or lentiviral vector (for example, the company name or investigator name and institution)	**UPCI-Lentiviral Core Facility (UPCI-LCF)**			
4. Are you using viral particles that have already been produced? (Mark "X" for Yes/No in un-shaded box)	Yes	**X**	No	
5. Are you generating or producing vector in your laboratory from a multi-component system? (for example, separate plasmids for packaging, envelope and gene transfer) (Mark "X" for Yes/No in un-shaded box) • **If "No", proceed to question 7 below**	Yes		No	**X**
6. Provide the number of plasmids involved (e.g. 2-, 3-, 4- or more separate plasmids)	**See below**			
7. For the lentivirus or lentiviral vector used, provide information describing the safety features of the system	**See below**			
8. For the lentivirus or lentiviral vector, please state the expected volume of vector to be produced or received	**0.1 ml** **0.5 ml** **5 ml** **25 ml** **75 ml** **(depending on order)**			
9. For the lentivirus or lentiviral vector used, please list the transgenes used in the virus • **If no transgenes are used or inserted, state "None"**	**This depends on the lentivirus ordered.**			

Questions 6 & 7

Lentivirus supplied by the UPCI-LCF is prepared as follows:

The 3rd generation lentiviral system comprises four plasmids (the expression plasmid plus three packaging vectors: pMD2.g(VSVG), pRSV-REV and pMDLg/pRRE). This generation packaging system offers maximal biosafety, as described in: Dull et al "A third generation lentivirus vector with a conditional packaging system"(1998) J. Virol. 72, 8463-8471 and in Klages et al. "A stable cell line for the high-titer production of third generation lentiviral vectors". Mol. Ther. (2000) 2, 170-6.

Lentiviral particles are generated by transfection of four plasmids (the control plasmid pLKO.1-puro-TurboGFP or the expression plasmid (ie., pLKO.1-puro-shRNA), plus pMD2.g(VSVG), pRSV-REV and pMDLg/pRRE) into 293-FT cells using FuGene 6 transfection reagent (Roche Diagnostic Corp, Indianapolis, IN). Culture media from transfected cells is collected 48 hours after transfection to isolate the viral particles, passed through 0.45 filters, used immediately or stored at -80˚C in single-use aliquots. Volume is always less than 100 ml.

Need Additional Information on Lentiviral Vectors?

IBC Policy on Biosafety Level Assignment for Lentivirus Vectors

EHS Lentivirus SOP: http://www.ehs.pitt.edu/assets/docs/lentivirus.pdf

SECTION C9. Lentivirus/Lentiviral Vectors; part 2

NOTE: If your work does not use lentiviruses or lentiviral vectors, skip to Section D.

***NOTE*: It is not required that vectors generated with 4-plasmid lentivirus systems (3rd generation vectors) be tested for replication-competent virus. However, 3-plasmid vector lentivirus systems (2nd generation vectors) must be shown to be free of replicating virus for approval at BSL-2 (for example, you must provide data or the results of a RCV assay). Please see the current IBC Recommended BSL Assignment for Lentivirus Vectors for details**

Question	Yes		No	
1. Do any of the transgenes listed above have oncogenic properties or the potential to increase pathogenicity of the vector? **(Mark "X" for Yes/No in un-shaded box)** • **If "Yes", provide details**	Yes		No	X
2. Is the lentivirus/lentiviral vector pseudotyped (for example, expressing a different envelope gene)? **(Mark "X" for Yes/No in un-shaded box)** • **If "Yes", does it increase the natural host range? Describe whether or how the pseudotyping alters the host and cell tropism**	Yes	X	No	
	VSV-G, can infect all cell types			
3. Is the lentivirus/lentiviral vector replication-defective? **(Mark "X" for Yes/No in un-shaded box)** • **If "No", skip to Section D** • **If "Yes", describe the deletions or bioengineering rendering it defective and complete the next question**	Yes	X	No	
	See below			
4. Has the replication-defective vector been tested for replication-competent virus (RCV)? **(Mark "X" for Yes/No in un-shaded box)** • **If "Yes", describe the assay used to test for RCV** • **If "No", describe the likelihood of conversion to replication-competent virus?**	Yes		No	X
	See below			

Questions 3 & 4

The 4-plasmid HIV lentiviral systems in use herein separates packaging and gene transfer functions onto multiple plasmids and lack viral accessory genes Vif, Vpu and Nef and the structural gene Env. These viruses are made to express the vesicular stomatitis virus G protein in place of viral Env to increase cell tropism. The vectors contain less than 2/3 of the HIV-1 genome.

The 4-plasmid HIV lentiviral system comprises only *gag* (coding for the virion main structural proteins), *pol* (responsible for the retrovirus-specific enzymes), and *rev* (which encodes a post-transcriptional regulator necessary for efficient gag and pol expression). A cDNA encoding rev is provided on a separate plasmid. This generation packaging system offers maximal biosafety.

4-plasmid lentiviral system

The 3rd generation lentiviral system comprises four plasmids. This generation packaging system offers maximal biosafety, as described in: Dull et al "A third generation lentivirus vector with a conditional packaging system"(1998) J. Virol. 72, 8463-8471 and in Klages et al. "A stable cell line for the high-titer production of third generation lentiviral vectors". Mol. Ther. (2000) 2, 170-6.

These 4-plasmid lentiviral system viruses and cells derived thereof are prepared and used at BSL-2.

Almost impossible to convert. No record of either of these systems ever producing replication competent virus.

The 4-plasmid HIV lentiviral system viruses and cells derived thereof are prepared and used at BSL-2. There is no likelihood of conversion.

SECTION D1. Animal Use Information; Part I

Reference *NIH Guidelines* II-A-1, Appendix B, III-E-3

NOTE: This section does NOT apply to in vitro studies using commercially available or established cell lines. **However, if you are obtaining cells or tissues from live animals under an IACUC protocol specific for this rDNA research, or you plan on administering rDNA materials to animals, you must complete this section!**

Question				
1. Does the work involve live (living) or intact animals? (Mark "X" for Yes/No in un-shaded box) • **If "No" skip to Section E**	Yes		No	
2. Has an Institutional Animal Care and Use Committee (IACUC) application been submitted for this recombinant research? (Mark "X" for Yes/No in un-shaded box) • **If "Yes" provide the IACUC protocol number to be linked to this rDNA project (provide the temporary number or the date of submission, if the IACUC number is not yet known)** • **NOTE: If you are not the named PI on the linked animal protocol application, provide the name of the investigator on the IACUC protocol** ***ATTENTION*: Recombinant DNA work described in an IACUC protocol must correspond to recombinant DNA research approval by the IBC**	Yes		No	

Need more information? Please visit the IACUC website for animal research review and approval requirements

Question				
3. Will animal tissues or cells be used *in vitro*? For example, do you plan to harvest tissues for culture or analysis? (Mark "X" for Yes/No in un-shaded box) • **If "Yes", explain**	Yes		No	
4. Will transgenic or gene-targeted animals be used? (Mark "X" for Yes/No in un-shaded box) • **If "Yes", explain**	Yes		No	
5. Will recombinant agents be administered to live or intact animals? For example: viral vectors, *transfected cells*, plasmids, or the *transplantation of genetically modified cells*, tissues or organs to live animal subjects. (Mark "X" for Yes/No in un-shaded box) • **If "No", skip to Section E (animal tissues are used only in culture)** • **If "Yes", continue to the next Section**	Yes		No	

SECTION D2. Animal Subjects Involvement; Part II Reference *NIH Guidelines* II-A-1, Appendix B, III-E-3 This section is to be completed only if there is administration of rDNA into live animal subjects				
1. What are the target cells/tissues/organs for the recombinant materials?				
2. List ALL recombinant materials to be administered to animals: ➢ **include both transformed or infected cells and any vectors (viral or non-viral)** ➢ **For cells, identify the vector(s) used to modify the cells prior to administration** ➢ **If in doubt, investigators are encouraged to contact the IBC Office for advice- 383-1768**				
3. Do you anticipate that work with the live animal subjects will be conducted at a different BSL than the *in vitro* or wet bench portions of the study? (Mark "X" for Yes/No in un-shaded box) • **If "Yes", explain the differing containment requirements in the text box:**	Yes		No	
4. Provide the animal species (and strain if applicable) receiving the recombinant DNA material; be sure to list each species to be used				
5. Describe the route of administration for each recombinant material used *in vivo* and per species as applicable				
6. Provide the concentration and volume for each recombinant material to be administered and per species as applicable				
NOTE TO ANIMAL USERS: For animal research involving the administration of rDNA agents/materials at BSL-2 or higher, the DLAR (Division of Laboratory Animal Resources) must be notified.				

SECTION E. Human Subjects Involvement Reference *NIH Guidelines* III-B, III-C				
Please visit the IRB website for additional information regarding human subjects research and human subjects protection				
1. Does work involve human subjects, unfixed human tissues or blood, or human cell lines that are obtained directly from human participants? **(Mark "X" for Yes/No in un-shaded box)** • **If "No" skip to the next Section (Section F)**	Yes		No	
2. Has an Institutional Review Board (IRB) application been submitted? **(Mark "X" for Yes/No in un-shaded box)** • **If "Yes" Provide the IRB protocol (preferred) or the IRB submission date** • **IRB Exemption information: http://www.irb.pitt.edu/Exempt/default.htm**	Yes		No	
3. Will human tissues or primary cells* be used *in vitro*? **(Mark "X" for Yes/No in un-shaded box)** • **If "Yes", describe the use of the tissues or cells in your research** ➢ *** Note that the term "*primary cells*" indicates that the cell cultures are *directly* derived from the subjects blood or tissues**	Yes		No	
4. Is this a gene transfer proposal (deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA into human subjects)? **(Mark "X" for Yes/No in un-shaded box)** • **If "Yes", Note that the supplemental form Attachment 1 must also be submitted for IBC review of a new human gene transfer proposal, in addition to other required documents** • **See also: Human Gene Transfer Information on the IBC website**	Yes		No	

What is Human Gene Transfer?

Human Gene Transfer is the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into human subjects

Examples:

- **Use as a marker in cells**
- **Production of a potentially therapeutic substance**
- **Replacement or compensation of defective genes**
- **To stimulate the immune system to fight disease**

SECTION F1. **Research Facilities Information; Part I** Facilities: Locations where the recombinant DNA research will be conducted				
1. Is any of the work proposed within the RBL (8th floor BST-3 Building)? (Mark "X" for Yes/No in un-shaded box)	Yes		No	

2. Provide facilities information for *all* other locations, including the facility used for work with animals or human subjects (clinical areas), as applicable to your described project.

- **Provide the procedures performed in each location, for example, cell transfections, propagation of plasmids, administration of viral vector into animals, animal housing, etc**
- **Provide the EHS approved biosafety level of the location- not the procedural biosafety level. If a specific site is not currently EHS approved, please provide an explanation as to why the site does not have approval (for example, a clinical site may be approved under the UPMC Dept of Infection Control)**

Location #1	Room number and building	
	Describe procedures for this location	
	Provide approved biosafety level	
Location #2	Room number and building	
	Describe procedures for this location	
	Provide approved biosafety level	
Location #3	Room number and building	
	Describe procedures for this location	
	Provide approved biosafety level	
Location #4	Room number and building	
	Describe procedures for this location	
	Provide approved biosafety level	

SECTION F2. **Research Facilities Information; Part II** Production and Distribution: distributing laboratories must have current university IBC approvals				
1. Does this research proposal involve the production and potential distribution from a CORE Facility* of recombinant materials to other research laboratories? (Mark "X" for Yes/No in un-shaded box) ➢ ***The term "Core Facility" indicates that a goal of the research protocol is to produce rDNA materials for use by others**	Yes		No	X
2. Does this research proposal use recombinant materials produced or obtained from an outside/external source? (Mark "X" for Yes/No in un-shaded box) • **If "No" skip to question F2-4, below**	Yes		No	X
3. A) List ALL recombinant materials obtained from outside or external sources for this research project, for example, materials obtained from companies, or *non-Pitt* researchers, *and* **B) provide the name of each source (institution and/or the individual) responsible for producing or providing the recombinant material(s).** ***Note: The IBC may request the investigator to provide additional information***				
4. Does the research proposal use recombinant materials produced or obtained from another University of Pittsburgh laboratory for this research project? (Mark "X" for Yes/No in un-shaded box) • **If "Yes", answer the following question**	Yes	X	No	
5. Provide the IBC registration number(s) and the name of the University of Pittsburgh investigator(s) providing recombinant materials for your research. ***Note: the IBC Office cannot provide this information; contact the investigator from whom you are obtaining the recombinant materials***	**R.W. Sobol; UPCI-LCF #009-08**			

SECTION G. Personnel and Training Information

This information is ONLY required for applications with a designated containment of BSL-2+ or higher at this time

- List all personnel working in the laboratory.
- All personnel must be current on all applicable Health and Safety training prior to IBC granting approval
- To expedite IBC approval of your research, please verify that all personnel listed below have current training certificates.
- Any questions regarding EH&S training programs and certification should be directed to EH&S at 412-624-9505

Last Name	First Name	E-mail address	Role on Project	University Identifier *

*** What is the University Identifier?**
The unique identification number located on the bottom right on the University of Pittsburgh identification card and is specific to individuals. If personnel do not have a University of Pittsburgh identification card, complete all the other requested information for the individual(s) and leave the last column blank or place N/A for "not applicable".

Important information regarding Facilities Inspections and Biosafety Operations Manuals

For laboratories intending to operate at BSL-2+ (BSL-2 enhanced) or higher, the IBC will NOT provide approval for the research until the following conditions are verified:

- A Biosafety Operations Manual must be reviewed and approved by the University Biosafety Officer (BSO), Biohazards Committee, and other authorized officials as required for the designated biosafety level
- Lab facilities must be inspected by the Department of Environmental Health and Safety (Pitt EH&S)

For questions, or to schedule an inspection appointment, contact the EH&S Office at **624-9505** or visit the EHS biological safety website

Section H: Additional Materials

Insert any additional materials below if desired. For example, restriction map(s), host-vector diagrams, data in support of a lower BSL, detailed preparatory information that does not fit into the provided space, etc. These materials will be reviewed by the IBC members and become part of the record for this research.

IBC Protocol

Attention Reviewers:

1) Please review the four questions in Section B1 of the above protocol, and inform the IBC Office staff immediately *if* you could answer "yes" to any of those questions,

- OR if you have an interest in the technology under development described in the research covered by the application (for example, the reviewer is named on a patent application on the technology),
- OR if you receive financial support from the named grant that supports this research

You must recuse yourself from reviewing this protocol if you have such a conflict of interest. For additional information regarding these concerns, please refer to the IBC Conflict of Interest Policy.

2) When preparing comments/concerns for the investigator to address, reference the Section and Question Number from the application. Word comments carefully; the IBC Office staff will send comments verbatim to the investigator for response. Use explanations and examples to assist the investigator in understanding your concern. It is appropriate to cite the *NIH Guidelines* or IBC Policies in support of your concern(s).

3) Keep in mind that all materials reviewed by committee members may be determined to be confidential, and as such, must be appropriately safeguarded.

4) Have concerns, questions about review? Call the IBC Office Staff! 412-383-1768

IBC reviewer evaluation, summary or concerns:	
Reviewer recommendation: (Mark an "X" in the blue-shaded box)	
	Approval; no revisions required. Current application is approvable
	Approval pending; minor revisions required A revised application is required for approval
	Additional information required; substantial revisions are needed or important information is missing from the application
	Defer to convened meeting; biosafety issues are identified which required full committee discussion

IBC reviewer evaluation, summary or concerns:	
Reviewer recommendation: (Mark an "X" in the blue-shaded box)	
	Approval; no revisions required. Current application is approvable
	Approval pending; minor revisions required A revised application is required for approval
	Additional information required; substantial revisions are needed or important information is missing from the application
	Defer to convened meeting; biosafety issues are identified which required full committee discussion