OMRF
Oklahoma Medical Research Foundation

PRESIDENT'S OFFICE

May 12, 2006

Mr. Edward H. Hammond
The Sushine Project
78 Linda Ave, #5A
Oakland, CA 94611

Via Federal Express

Dear Mr. Hammond:

Per my letter of May 11, enclosed are a full set of unredacted minutes of the Oklahoma Medical Research Foundation's Institutional Biosafety Committee meetings from May 1, 2003, to the present.

Sincerely,

Adam Cohen
Director, Legal & Public Affairs

enclosures

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OMRF Institutional Biosafety Committee (IBC)
Meeting Date: 28 June 2005

Present
Chair, Linda Thompson, V. Chair, Bart Frank, Chris Li, Phil Silverman, Bill Rodgers, Cheri Marcham, Bill Canfield, Don Ewert , and Kerry Humphrey (Admin Assistant)

Agenda

- Review of Standard Operating Procedures (SOP) for Handling *Bacillus anthracis* Stern Strain and Contaminated Materials
- Review of OMRF Policies for Registration of Recombinant DNA Experiments

I. Review of 'SOP for Handling *Bacillus anthracis* Stern Strain and Contaminated Materials'. A draft document provided by Chris Li was discussed and modified. Please see the attached revised SOP.

II. Review of OMRF Policies for Registration of Recombinant DNA Experiments

The relationship between the OMRF Recombinant DNA Committee (recDNAC) and the IBC was discussed. It was decided that future recombinant DNA protocols will be reviewed by the recDNAC as in the past to determine the appropriate Biosafety level and identify any potential concerns. Those protocols requiring approval prior to the initiation of experiments will be discussed and approved by the entire IBC at their next meeting.

Bart Frank and Don Ewert will develop a new web-based version of the recombinant DNA protocol form incorporating the comments of the IBC, with the intention of clarifying the criteria requiring registration and approval of recombinant DNA experiments with/by the recDNAC. When this is available, it will be e-mailed to committee members for their approval. Bart and Don will also draft a statement outlining OMRF's policies regarding recombinant DNA procedures and approvals. Please see attached policy statement.

The recDNAC will also circulate a copy of the current OMRF Grant Routing Sheet. Please see attachment and suggest improvements at our next meeting.

It was suggested that OMRF establish a policy that PIs who check PENDING for Recombinant DNA on the Grant Routing Sheets will be urged to submit their OMRF recDNA Registration Forms for recDNAC approval one month after their grant submission date (December 1, April 1, or August 1) to assure timely approval before the need to submit "Just in time" documents and/or the onset of funding.

Berva Wood will forward to Dr. Frank all copies of Grant Routing Sheets that have PENDING checked.

Minutes of the Biosafety Committee Meeting – June 22, 2004

The Biosafety Committee met on June 22, 2004 to discuss issues related to the pending grant: Molecular and Immunologic Analysis of the Pathobiology of Human Anthrax. Those in attendance were: Chip Morgan, Bart Frank, Ken Hensley, Chris Li, Bill Rodgers, and Linda Thompson. Also present were non-committee members Mike Bailey and Mark Coggeshall, the co-PI of the grant.

The overall organization of the application was presented. It consists of three main projects (James/Farris, Coggeshall, and Tang), three technical components (Harley, Metcalf, and Kurosawa), two pilot projects (Webb and White), two cores (flow cytometry/Kincade and microarrays/Centola), and an educational component (Coggeshall). There was also a brief discussion of the anthrax-related materials that will be used in the research described in this grant: purified recombinant toxins prepared by Jimmy Ballard, vegetative cultures, and spores in liquid suspension. Initially, the only anthrax strain used will be Sterne strain 34F2. It is an attenuated strain of *B. anthracis* and lacks the ability to form a capsule. It is 10^3-10^5 less virulent than other strains. However, it does produce as much toxin as more virulent strains. It is the same strain used to immunize military personnel. Sterne strain is not on the CDC list of select agents and is, therefore, exempt from the regulatory controls of select agents.

Reviews of individual components:

1. James/Farris – Ken Hensley, reviewer.
   Concerns and items needing clarification: Are these investigators using vegetative bacteria or only spores? How will the animal bedding be disposed/decontaminated? Exactly what precautions will be taken with blood samples from guinea pigs from an inhalation anthrax model? Since these latter experiments are several years into the future, the Committee agreed to approve the project except for these specific experiments. If these experiments are finally undertaken, the investigators must then come back to the Committee for approval.

2. Coggeshall – Bill Rodgers, reviewer.
   No concerns. Dr. Coggeshall agreed that he should do a test to determine if any live bacteria are present in commercial preparations of anthrax cell walls he will purchase.

3. Tang – Linda Thompson, reviewer.
   Items needing clarification: More details are needed for the animal experiments.

4. Harley – Linda Thompson, reviewer.
   No anthrax-related biohazard concerns. Only serum and DNA samples from military personnel will be utilized.

5. Metcalf – Linda Thompson, reviewer.
   Most of Metcalf's experiments will be conducted at OU and must be approved by OU. We are concerned only with the material he will give to OMRF investigators. This

includes human lung tissue. This poses a standard blood borne pathogen risk, but no anthrax-related risk.
Concern: Dr. Metcalf will also give Dr. Coggeshall protein extracts from anthrax-infected cells for evaluation of signaling pathways via western blots. It is unclear if these extracts pose a biohazard risk. Dr. Coggeshall will investigate whether anthrax spores are killed by boiling in standard SDS sample buffer.

6. Kurosawa – Bart Frank, reviewer.
   Items needing clarification: Will Dr. Kurosawa be obtaining anthrax toxins from Dr. Jimmy Ballard, or isolating them himself? Will anthrax spores be purchased or obtained from Dr. Ballard? Where will anthrax spores be stored and who will have access to keys? The autoclaved anthrax-contaminated material should be placed in an OMRF Biohazard Waste container for incineration, not placed in the dumpster. The method of safe transport of anthrax cultures from OMRF to OU for the baboon studies needs to be explained. A method should be described for the clean up of large spills of bacterial cultures and well as for the disposal of unneeded toxin.

7. Webb – Linda Thompson, reviewer.
   This project utilizes lethal toxin and human macrophages or lung tissue. Risk is minimal – no concerns.

8. White – Linda Thompson, reviewer.
   No concerns – this project utilizes only molecular biology and protein engineering.

9. Flow cytometry core – Linda Thompson, reviewer.
   No concerns – only fixed cells may be brought into the flow cytometry lab.

10. Microarray core – Linda Thompson, reviewer.
    No concerns – only purified RNA will be brought into the microarray core lab.

The Committee recommends the following general policies:

1. All *B. anthracis* should be grown in a single location. For now, that will be Shinichiro Kurasawa's lab. We recommend that an alternate site be designated, perhaps a room in the Main Building LARC. This room would need a shaking 37° incubator, centrifuge, BSL2 biosafety cabinet, and sink. The room should have card access and be accessible only to those personnel who have completed training regarding the safe growth of anthrax and how to prevent spore formation. The door of the room should have a Biohazard sign with *B. anthracis* listed as the hazardous agent.

2. The committee decided that it should not be necessary to maintain inventory records of anthrax cultures, spores, or toxins. Since the Sterne strain of anthrax is not on the CDC select agent list, neither the CDC nor the NIH requires such record keeping. Therefore, maintaining an inventory would place an unreasonable burden on the investigators.

3. There is already documentation of training of many personnel involved with this grant. When new personnel are trained, their names should be submitted to the Biosafety Committee along with documentation of training. Chris Li will develop an on-line exam to test whether proper training has occurred. People passing the exam and needing to work with vegetative cultures will then be issued card access to the room in which the bacteria will be grown.

4. Every attempt must be made to prevent the development of anthrax spores. This includes growing vegetative cultures under conditions where there are sufficient nutrients and decontaminating culture flasks immediately after use. The Committee agreed that liquid suspensions of anthrax spores pose much less threat than dry spore powder and can be safely handled in a BSL2 biosafety cabinet. The spore preparation OMRF investigators will use is the same material used to vaccinate cattle. It can be handled safely even in a ranch environment.

5. Chris Li will make an addition to OMRF's chemical hygiene plan that includes the proper procedures for cleaning up a spill of anthrax cultures. This information should be included in the training material for personnel joining this project.

Summary and Recommendation:

The Committee agreed that the protocols should all be approved contingent upon the clarification of issues outlined above.

RECOMBINANT DNA REGISTRATION FORM
Institutional Biosafety Committee (IBC)
Oklahoma Medical Research Foundation

Please complete this form to satisfy federal regulations specified in *the Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines).* (A copy of this document is available in the OMRF library.) Attach additional pages if needed. DO NOT ATTACH GRANT APPLICATIONS UNLESS REQUESTED.

A. 1. PRINCIPAL INVESTIGATOR: Kenneth Hensley

2. Department: Free Radical Biology & Aging Phone: 271-7569 Lab: 271-1412

3. Project Title: ~~Neuroinflammatory activation of glial cells by toxic damage to the mitochondrial~~ electron transport chain : Relevance to amyotrophic lateral sclerosis (ALS)

B. EXPERIMENTAL CATEGORY. Please check one (see *NIH Guidelines* Section III).

1. ___ Experiments requiring IBC approval, RAC review, and NIH approval before initiation.
2. ___ Experiments requiring NIH/ORDA and IBC approval before initiation.
3. ✓ Experiments requiring IBC approval before initiation.
4. ___ Experiment requiring IBC notification simultaneously with initiation.
5. ___ Exempt experiments.

C. PROJECT INFORMATION

1. Briefly describe the project including the sequences to be cloned and their function. If these sequences are from a eukaryotic virus, indicate if > 2/3 of the viral genome will be cloned, if the virus is replication defective, and describe any helper viruses that will be used.

Cytochrome C will be cloned into an expression vector containing a fluorescent label-binding tag sequence

2. Do these sequences encode molecules toxic to vertebrates at an LD50 ≤ 100 µg/kg of body weight? No If so, specify LD50 _____ (see *NIH Guidelines* Appendix F).

3. List vectors to be used (attach maps, if available): _____

Examples: pcDNA 3.1; pcDNA 3.1 D (Invitrogen)

4. List recipient hosts. Include plants and animals and if they will become transgenic. If humans are the intended recipients, complete this form and attach an addendum addressing issues in Appendix M of the *NIH Guidelines*). Cultured primary mouse astrocytes

5. List any product to be expressed and identify its function (if known). cytochrome C (electron transport chain protein)

6. Will greater than 10 liters of culture containing this recombinant DNA be grown at any one time? no

7. If applicable, review health and environmental hazards that may result from this work in an attached addendum. A few suggestions to help you formulate your answer are offered below. These are not meant to cover all circumstances.
   - If DNA sources are from class 2 or 3 infectious microorganisms, exotic animal or plant pathogens, or USDA-regulated articles, discuss safety aspects of the facility, containment practices, personnel practices, and staff training that will assist in the safe conduct of the study. Summarize pathogenic aspects of organisms (modes of transmission, etc). Include measures to prevent or minimize expression of pathogenic or infectious sequences.
   - If oncogenes of other regulatory sequences are transferred, will pathogenic virulence, toxicity, antibiotic resistance, or immune competence be affected?
   - Will the experiments require the release into the environment of organisms containing recombinant DNA molecules?

*** CHECK HERE IF ITEM 7 IS NOT APPLICABLE ✔

D. CONTAINMENT LEVELS

Based on the above information and the *NIH Guidelines* (Section III and Appendices G, I, K, P & Q), circle the appropriate containment levels for these experiments.

Physical Containment	Animal Biosafety Level
(BL1) BL2 BL3	BL1 BL2 BL3
GLSP BL1-LS BL2-LS BL3-LS	BL-1N BL-2N BL-3N

E. PERSONNEL

List all personnel who will conduct these experiments. Including visiting staff, students, and temporary help. For each person, check if they are currently training or will be trained by the PI to perform their duties.

Name	Trained	Will Train
Dr. Charles Stewart	✔	
Shenyun Mou	✔	
Quentin Pye	✔	

F. I am familiar with and agree to abide by the *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* and other specific NIH instructions pertaining to the proposed project. I understand that it is my responsibility that all laboratory personnel working on this project are trained and remain fully informed with regard to biosafety practices, techniques, and applicable emergency procedures. I also agree to comply with federal requirements pertaining to shipment of recombinant DNA molecules. The above information is accurate and complete.

PI Signature Kenneth Hensley Date March 9 2004

IBC Action:
Approved ✔ Disapproved ___ Pending ___

Comments: ___

MB Frank 3/11/04
IBC Chair or Member Date

RECOMBINANT DNA REGISTRATION FORM
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A. 1. PRINCIPAL INVESTIGATOR: Stephen Fields
2. Department: MCD Biology Phone: 271-7723 Lab: M125
3. Project Title: Role of a myosin V homologue in C. elegans neurons.

B. EXPERIMENTAL CATEGORY. Please check one (see *NIH Guidelines* Section III).
1. ___ Experiments requiring IBC approval, RAC review, and NIH approval before initiation.
2. ___ Experiments requiring NIH/ORDA and IBC approval before initiation.
3. ___ Experiments requiring IBC approval before initiation.
4. ___ Experiment requiring IBC notification simultaneously with initiation.
5. ✓ Exempt experiments.

C. PROJECT INFORMATION
1. Briefly describe the project including the sequences to be cloned and their function. If these sequences are from a eukaryotic virus, indicate if > 2/3 of the viral genome will be cloned, if the virus is replication defective, and describe any helper viruses that will be used.
We will characterize the function of HUM-2, a homologue to the vertebrate unconventional myosin V. Various portions of hum-2, as well as full-length transcripts, will be cloned into E. coli, yeast, and C. elegans vectors for the purpose of making transgenic C. elegans strains and detecting potential protein interactors with regions of HUM-2.

No pathogenic organisms or genes involved in pathogenesis will be used

2. Do these sequences encode molecules toxic to vertebrates at an LD50 ≤ 100 µg/kg of body weight? no If so, specify LD50 _____ (see *NIH Guidelines* Appendix F).

3. List vectors to be used (attach maps, if available):
pACT, pGBKT7, pGADT7, pCR2.1-TOPO, RM#641p, RM#647p

4. List recipient hosts. Include plants and animals and if they will become transgenic. If humans are the intended recipients, complete this form and attach an addendum addressing issues in Appendix M of the *NIH Guidelines*). E. coli (XL1BLUE) , S. cerevisiae (yeast), C. elegans
All non-pathogenic

5. List any product to be expressed and identify its function (if known).
Various portions of the HUM-2 protein fused to GFP will be expressed in nematodes. Possible functions of HUM-2 include actin-based motility of synaptic vesicles or other endosomally-derived vesicles.

6. Will greater than 10 liters of culture containing this recombinant DNA be grown at any one time? no

7. If applicable, review health and environmental hazards that may result from this work in an attached addendum. A few suggestions to help you formulate your answer are offered below. These are not meant to cover all circumstances.
   - If DNA sources are from class 2 or 3 infectious microorganisms, exotic animal or plant pathogens, or USDA-regulated articles, discuss safety aspects of the facility, containment practices, personnel practices, and staff training that will assist in the safe conduct of the study. Summarize pathogenic aspects of organisms (modes of transmission, etc). Include measures to prevent or minimize expression of pathogenic or infectious sequences.
   - If oncogenes of other regulatory sequences are transferred, will pathogenic virulence, toxicity, antibiotic resistance, or immune competence be affected?
   - Will the experiments require the release into the environment of organisms containing recombinant DNA molecules?

*** CHECK HERE IF ITEM 7 IS NOT APPLICABLE ✔

D. CONTAINMENT LEVELS

Based on the above information and the *NIH Guidelines* (Section III and Appendices G, I, K, P & Q), circle the appropriate containment levels for these experiments.

Physical Containment	Animal Biosafety Level
(BL1) BL2 BL3	BL1 BL2 BL3
GLSP BL1-LS BL2-LS BL3-LS	BL-1N BL-2N BL-3N

E. PERSONNEL

List all personnel who will conduct these experiments. Including visiting staff, students, and temporary help. For each person, check if they are currently training or will be trained by the PI to perform their duties.

Name	Trained	Will Train
Stephen Fields	✔	
Jamie Osborne	✔	

F. I am familiar with and agree to abide by the *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* and other specific NIH instructions pertaining to the proposed project. I understand that it is my responsibility that all laboratory personnel working on this project are trained and remain fully informed with regard to biosafety practices, techniques, and applicable emergency procedures. I also agree to comply with federal requirements pertaining to shipment of recombinant DNA molecules. The above information is accurate and complete.

PI Signature Stephen Fields Date 2/11/04

IBC Action: ✔
Approved ___ Disapproved ___ Pending ___

Comments: ___

MB Frank 2/13/04
IBC Chair or Member Date

RECOMBINANT DNA REGISTRATION FORM
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Oklahoma Medical Research Foundation

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A. 1. PRINCIPAL INVESTIGATOR: Michael N. Conrad
2. Department: MCDB Phone: 405-271-7668 Lab: _____
3. Project Title: Telomere Function in Meiosis

B. EXPERIMENTAL CATEGORY. Please check one (see *NIH Guidelines* Section III).
1. __ Experiments requiring IBC approval, RAC review, and NIH approval before initiation.
2. __ Experiments requiring NIH/ORDA and IBC approval before initiation.
3. __ Experiments requiring IBC approval before initiation.
4. __ Experiment requiring IBC notification simultaneously with initiation.
5. ✓ Exempt experiments.

C. PROJECT INFORMATION
1. Briefly describe the project including the sequences to be cloned and their function. If these sequences are from a eukaryotic virus, indicate if > 2/3 of the viral genome will be cloned, if the virus is replication defective, and describe any helper viruses that will be used.

The project involves cloning yeast genes from Saccharomyces cerevisiae in to E. coli in order to manipulate them and study effect on chromosome segregation in yeast.

No pathogenic organisms or genes involved in pathogenesis will be used.

2. Do these sequences encode molecules toxic to vertebrates at an LD50 ≤ 100 µg/kg of body weight? No If so, specify LD50 _____ (see *NIH Guidelines* Appendix F).

3. List vectors to be used (attach maps, if available): Standard yeast-E. coli shuttle vectors, e.g. pRS306, pRS316

4. List recipient hosts. Include plants and animals and if they will become transgenic. If humans are the intended recipients, complete this form and attach an addendum addressing issues in Appendix M of the *NIH Guidelines*). Nonpathogenic strains of S. cerevisiae and standard nonpathogenic E. coli strains such as DH5alpha

5. List any product to be expressed and identify its function (if known). n/a

6. Will greater than 10 liters of culture containing this recombinant DNA be grown at any one time? No

7. If applicable, review health and environmental hazards that may result from this work in an attached addendum. A few suggestions to help you formulate your answer are offered below. These are not meant to cover all circumstances.
   - If DNA sources are from class 2 or 3 infectious microorganisms, exotic animal or plant pathogens, or USDA-regulated articles, discuss safety aspects of the facility, containment practices, personnel practices, and staff training that will assist in the safe conduct of the study. Summarize pathogenic aspects of organisms (modes of transmission, etc). Include measures to prevent or minimize expression of pathogenic or infectious sequences.
   - If oncogenes of other regulatory sequences are transferred, will pathogenic virulence, toxicity, antibiotic resistance, or immune competence be affected?
   - Will the experiments require the release into the environment of organisms containing recombinant DNA molecules?

*** CHECK HERE IF ITEM 7 IS NOT APPLICABLE ✔

D. CONTAINMENT LEVELS

Based on the above information and the *NIH Guidelines* (Section III and Appendices G, I, K, P & Q), circle the appropriate containment levels for these experiments.

Physical Containment	Animal Biosafety Level
(BL1) BL2 BL3	BL1 BL2 BL3 N/A
GLSP BL1-LS BL2-LS BL3-LS	BL-1N BL-2N BL-3N

E. PERSONNEL

List all personnel who will conduct these experiments. Including visiting staff, students, and temporary help. For each person, check if they are currently training or will be trained by the PI to perform their duties.

Name	Trained	Will Train
Michael Conrad	✔	
To be appointed		✔

F. I am familiar with and agree to abide by the *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* and other specific NIH instructions pertaining to the proposed project. I understand that it is my responsibility that all laboratory personnel working on this project are trained and remain fully informed with regard to biosafety practices, techniques, and applicable emergency procedures. I also agree to comply with federal requirements pertaining to shipment of recombinant DNA molecules. The above information is accurate and complete.

PI Signature Michael R Conrad Date 2/11/2004

IBC Action: ✓ Approved ___ Disapproved ___ Pending ___

Comments: _____

MBBrac 2/13/04

IBC Chair or Member Date

OMRF Recombinant DNA Committee Minutes
March 31, 2005

The following projects were considered and the listed actions taken:

Project: Dynamics of Intracellular Pathogen:Host Interactions
PI: Dr. Margaret Clarke
Date of submission: 1/20/05
Review and Points of Discussion: L pneumophila expressing a GFP fluorescent protein to monitor infection will be used to infect Dictostelium, a non-pathogenic soil amoeba maintained in the lab. Dictostelium will contain cytoskeletal proteins fused to GFP.
Concerns: L pneumophila is a Risk Group 2 agent requiring BL2 containment. The organism is associated with human disease that is rarely serious and for which therapeutic interventions are readily available. Laboratory personnel are trained and use standard microbiological practices for safe handling. Physical facilities are in place for BL2 containment. Only small quantities of bacteria (1- 10 ml) will cultured. Conditions that may create aerosols will be avoided and work will be performed in a class II biological safety cabinet. Any materials that come into contact with either organism and the organisms themselves at the end of the experiment will be stored in biohazard bags and disinfected by autoclaving. No organism will be released into the environment. Biohazard signs are displayed on lab doors, access to the lab is restricted and doors are locked when the lab is not in use.
Outcome: The project meets criteria for BL2 containment
Approved

Project: Inhibitor Development for Activated Protein C
PI: Dr. Tim Mather
Date of submission: 1/25/05
Review and Points of Discussion: Recombinant and truncated forms of protein C will be expressed in the 293 cell line. The proteins will be purified and used in inhibitor design studies using an in vitro assay system.
Concerns: No health, environmental, LS issues.
Outcome: The project meets the criteria for BL1 containment.
Approved

Project: Telomere Function in Meiosis
PI: Dr. Micahel Conrad
Date of submission: 1/25/05
Review and Points of Discussion: Genes encoding particular structural proteins and enzymes in the yeast Saccharomyces cerevisiae (baker's yeast) into E coli to study the effect of chromosomal segregation un yeast.
Concerns: No pathogenic organisms or genes involved in pathogenesis will be used.
Outcome: The project is exempt from NIH Guidelines. Experiments will be conducted under BL1 conditions.
Approved

Project: Novel Methods for Human T Cell Development
PI: Michelle L Joachims (Thompson lab)
Date of submission: 2/28/05
Review and Points of Discussion: cDNA encoding the human telomerase genes and the human Notch ligands Delta-4 and Jagged-2 will be cloned into LZRS retroviral vector upstream of an IRES-driven GFP expression cassette. The vectors will be cloned into Phoenix-A (for human target cells) and Phoenix-E (mouse target cells) cells. Supernatants will be used to transducer human or murine stromal cell lines to produce transfected cell lines for use in cultures.Delta-4 and Jagged-2 induce Notch signaling telomerase, promote extension of telomere sequences, thus delaying the onset of cellular senescence.Murine OP9 stromal cell line will be transduced with the Notch ligand retroviruses. Thymic stromal cells from neonatal thymus will be transduced with the telomerase retrovirus to immortalize them and create a continuous cell line.
Concerns: The Phoenix cells contain MoLV packaging constructs (CMV-Env and RSV gag/pol. The system was developed in the Nolan lab at Stanford. The use of CMV and RSV minimize recombination events to generate viral sequences.
Outcome: The NIH Guidleines indicate retroviral vectors can be maintained at BL1 conditions. The PI will use BL2 level for all experiments. The PI was has agreed to confirm the cells lack helper virus.
Approved

Project: Beta Secretase Inhibition for Treating Alzheimer's Disease
PI: Dr. Jordan Tang
Date of submission: 2/28/05
Review and Points of Discussion: Genes for Memapsin2 and APP (constituents of amyloid beta) will be cloned the expression vectors pET11, pcDNA 3.1, pSECtag2, and pcDNA6. Recombinant constructs will be used to transfect HeLA, HEK293, CHO, and M17 (a human neuroblastoma cell line) to express these proteins. Structure-function relationships will be derived using candidate inhibitors of these proteins.
Concerns: No health, environmental, LS issues.
The project requires IBC notification at time of initiation.
Outcome: Approved

Project: GEM Domains in T Cell Signaling
PI: Dr. William Rodgers
Date of Submission: 3/16/05
Review & Points of Discussion: In this project, various constructs will be produced for membrane expression of green fluorescent protein and separately a domain of a protein tyrosine phosphatase using a replication defective lentiviral system. This expression system (ViraPower Lentiviral Expression System) is sold by Invitrogen and describe in detail in http://www.invitrogen.com/content/sfs/brochures/ViraPowerAnnounc.pdf Proteins from this recombinant DNA will be expressed in cell lines and primary T and B cells. BL2 containment is suggested for use by Invitrogen. All materials that come into contact with these viruses will be disinfected with 10% bleach including benchtops, centrifuges, and other work areas. Dr. Rodgers and his staff will test to assure that replication competent viruses are not produced.

Concerns: Risk of replication competent virus & human infection were considered minimal. This is yet another lentiviral expression system proposed for use here.
Outcome: The project meets criteria for BL2 containment and is consistent with practices required in the past by others at OMRF using lentiviral systems.
Approved

7:5 Lincoln NC
474 4622 460 454

RECOMBINANT DNA REGISTRATION FORM
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A. 1. PRINCIPAL INVESTIGATOR: Mark Coggeshall
2. Department: Immunobiology & Cancer Phone: 271-7209 Lab: 271-7883
3. Project Title: Phosphatidylinositol-3 kinase in B cell activation & development

B. EXPERIMENTAL CATEGORY. Please check one (see *NIH Guidelines* Section III).
1. ___ Experiments requiring IBC approval, RAC review, and NIH approval before initiation.
2. ___ Experiments requiring NIH/ORDA and IBC approval before initiation.
3. ___ Experiments requiring IBC approval before initiation.
4. ✔ Experiment requiring IBC notification simultaneously with initiation.
5. ___ Exempt experiments.

C. PROJECT INFORMATION
1. Briefly describe the project including the sequences to be cloned and their function. If these sequences are from a eukaryotic virus, indicate if > 2/3 of the viral genome will be cloned, if the virus is replication defective, and describe any helper viruses that will be used.

~~The project involves the investigation of signal transduction in B cell development and antigen receptor internalization. The sequences to be cloned will be mutants of signal transduction enzymes. None of the sequences are of viral origin. Some protocol involves~~ virus infections using virues that do not infect human cells.
type? mouse stem cell virus MSCV 2.2 / Clontech
Ecopak-293 packaging cells

2. Do these sequences encode molecules toxic to vertebrates at an LD50 ≤ 100 µg/kg of body weight? NO If so, specify LD50 _____ (see *NIH Guidelines* Appendix F).

3. List vectors to be used (attach maps, if available): pEF, pTracer

4. List recipient hosts. Include plants and animals and if they will become transgenic. If humans are the intended recipients, complete this form and attach an addendum addressing issues in Appendix M of the *NIH Guidelines*).

5. List any product to be expressed and identify its function (if known).

6. Will greater than 10 liters of culture containing this recombinant DNA be grown at any one time? NO

7. If applicable, review health and environmental hazards that may result from this work in an attached addendum. A few suggestions to help you formulate your answer are offered below. These are not meant to cover all circumstances.
   - If DNA sources are from class 2 or 3 infectious microorganisms, exotic animal or plant pathogens, or USDA-regulated articles, discuss safety aspects of the facility, containment practices, personnel practices, and staff training that will assist in the safe conduct of the study. Summarize pathogenic aspects of organisms (modes of transmission, etc). Include measures to prevent or minimize expression of pathogenic or infectious sequences.
   - If oncogenes of other regulatory sequences are transferred, will pathogenic virulence, toxicity, antibiotic resistance, or immune competence be affected?
   - Will the experiments require the release into the environment of organisms containing recombinant DNA molecules?

*** CHECK HERE IF ITEM 7 IS NOT APPLICABLE X

D. CONTAINMENT LEVELS

Based on the above information and the *NIH Guidelines* (Section III and Appendices G, I, K, P & Q), circle the appropriate containment levels for these experiments.

BL2 NOT BL-1 BT

Physical Containment	Animal Biosafety Level
~~BL1~~ (BL2) BL3	~~BL1~~ (BL2) BL3
GLSP BL1-LS BL2-LS BL3-LS	BL-1N BL-2N BL-3N

E. PERSONNEL

List all personnel who will conduct these experiments. Including visiting staff, students, and temporary help. For each person, check if they are currently training or will be trained by the PI to perform their duties.

Name	Trained	Will Train
Koji Nakamura	✓	
Jami Milam		✓
Vidya Vedham	✓	
Kaz Maeda	✓	
Midge Carey	✓	

F. I am familiar with and agree to abide by the *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* and other specific NIH instructions pertaining to the proposed project. I understand that it is my responsibility that all laboratory personnel working on this project are trained and remain fully informed with regard to biosafety practices, techniques, and applicable emergency procedures. I also agree to comply with federal requirements pertaining to shipment of recombinant DNA molecules. The above information is accurate and complete.

PI Signature _____ Date 10 Feb, '04

IBC Action: ✓

Approved ___ Disapproved ___ Pending ___

Comments: OK @ BL2, not BL-1 advised by email

B Frank 2/10/04

IBC Chair or Member Date

Clontech
- Products
- Online Catalog
- Product Families
- Custom Services
- Technologies
- New Products
- Ordering
- Sales Reps
- Special Offers
- Support
- User Manuals
- Vector Information
- Bioinformatics
- Citations
- Application Notes
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MSCV Retroviral Expression System

Product Quick Links:

Catalog Numbers Components References Licensing

- *Efficient gene expression in hematopoietic, ES and EC cells*
- *Choice of three selectable markers*
- *Complete retroviral system including PT67 packaging cell line*

The MSCV (Murine Stem Cell Virus) Retroviral Expression System contains vectors that are optimized for introducing target genes into pluripotent cell lines, including murine or human hematopoietic, embryonic stem (ES), and embryonal carcinoma (EC) cells. They can also be used effectively with any mammalian cell line (1–3). This highly efficient system is ideal for analyzing gene function in development, embryogenesis, or immune response, in both cell culture and transgenic assays.

Designed for difficult-to-infect cells

The MSCV System contains three vectors: pMSCVneo, pMSCVhyg, and pMSCVpuro. These vectors contain a specially designed long terminal repeat (LTR) from the murine stem cell PCMV virus that allows you to work with difficult-to-express cell lines. This LTR differs from the MoMuLV LTR used in other retroviral vectors by introducing several point mutations and a deletion that enhance transcriptional activation and prevent transcriptional suppression in ES and EC cells. As a result, the PCMV LTR drives high-level, constitutive expression of the target gene in stem cells or other mammalian cell lines. The MSCV System includes the BD RetroPack™ PT67 Packaging Cell Line, which produces high-titer virus able to infect a broad range of mammalian host cells.

TOP

Frank, PhD

om: Mark Coggeshall [coggeshallm@omrf.ouhsc.edu]
ent: Wednesday, February 11, 2004 10:22 AM
To: frank@omrf.ouhsc.edu

Bart:

It seems many of these viral infection schemes are based on the murine stem cell virus (MSCV) gag, pol and env genes. The packaging cell contains the gag, pol and env genes for the retrovirus. The retroviral vector (what we transfect into the packaging cell lines) provides the essential elements of virus production and the target gene and the GFP selectable marker.
The viral particles produced cannot replicate in other cells since there are missing genes.

The vector we use is pMIG. This vector was created (not by us) by placing GFP downstream of the pCITE1 IRES (a commercial vector from Novagen) and cloning it into the EcoRI and SalI sites of the MSCV 2.2 vector (described in a paper by Hawley et al. The abstract from this paper is below).

The envelope protein made by the packaging cell line determines the range of infectivity (tropism) of the viral particles produced. We use the Ecopack-293 cell line from Clontech, which is based on MSCV - i.e., it has the gag, pol and env from MSCV.

So the upshot is that the virus is replication-incompetent, infects only mouse cell lines, and is sold commercially by Clontech/BDBiosciences (http://www.bdbiosciences.com/clontech/retroviral/index.shtml)

I hope this clears the situation upšit certainly was educational for me.

Mark

Gene Ther. 1994 Mar;1(2):136-8. Versatile retroviral vectors for potential use in gene therapy. Hawley RG, Lieu FH, Fong AZ, Hawley TS.

A set of retroviral vectors is described whose capacity for high efficiency transduction of functional genes into undifferentiated murine embryonic and haematopoietic cells makes them ideally suited for preclinical studies with murine models. Multiple unique cloning sites permit insertion of genes into the vectors such that no selectable marker exists or either the neomycin phosphotransferase (neo) gene, the hygromycin B phosphotransferase (hph) gene or the puromycin N-acetyl transferase (pac) gene is included as a dominantly acting selectable marker. Because the sequences in the viral gag region shown to improve the encapsidation of viral RNA have been modified to prevent viral protein synthesis and all env sequences have been removed to eliminate helper virus production by homologous recombination with packaging DNA, these vectors might prove useful in human gene therapy protocols.

--

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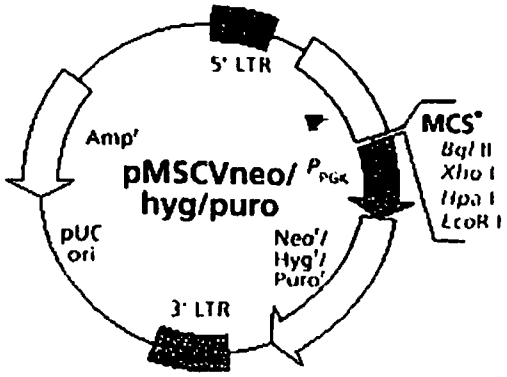


Figure 1. pMSCV Vectors map.

TOP

Product	Size	Cat.#	New Cat.#
MSCV Retroviral Expression System	each	K1062-1	634401

TOP

Components

pMSCVneo Vector
pMSCVhyg Vector
pMSCVpuro Vector
RetroPack PT67 Cell Line
MSCV Primers
Vector Information Packets
User Manual (PT3132-1)

TOP

References

1. Hawley, R. G., *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**:10297–10302.
2. Keller, G., *et al.* (1998) *Blood* **92**:877–887.
3. Hawley, R. G., et al. (1994) *Gene Ther.* **1**:136–138.

TOP

Related Products

Retroviral Systems

Licensing

Notice to Purchaser: MSCV Retroviral Expression System

Notice to Purchaser: Retroviral Products

OMRF Recombinant DNA Committee Minutes
June 30, 2005

The following projects were considered and the listed actions taken:

Project: Formation and Function of the Meiotic Bouquet
PI: Dr. Michael Dresser
Date of submission: 5/23/005
Review and Points of Discussion: Various genes cloned from baker's yeast *Saccharomyces cerevisiae* will be engineered in E coli using standard E coli – yeast shuttle vectors and replaced into yeast. No pathogenic organisms or genes involved in pathogenesis will be used.
Concerns: No health, environmental, LS issues.
Outcome: Experiments will be conducted under BL1 containment. The experiments are exempt per NIH Guidelines.
Approved

Project: Tyrosine Sulfation and Chemokines Receptor Function
PI: Dr. Kevin Moore
Date of submission: 5/27/05
Review and Points of Discussion: Chemokine receptors are7-transmembrane G-protein-coupled receptors involved in the regulation of trafficking of hematopoietic cells. Epitope-tagged versions of the murine chemokines receptors CCR1 through CCR10, CXCR2 through CXCR6, Scr1, CX3CR1, Darc, and C5L2/Gpr77 will be expressed to undertake biochemical analysis on recombinant receptors after transfection in the murine L1.2 pre-B cell line. Vectors to be used include pBluescript, pT3T7D-Pac, pCMV-SPORT6, SPORT1, and Uni-ZAP XR.
Concerns: No health, environmental, LS issues.
Outcome: Experiments will be conducted under BL1 containment.
Approved

OMRF Recombinant DNA Committee Minutes
December 22, 2005

The following projects were considered and the listed actions taken:

Project: TPST Inhibitos for Male Contraception
PI: Dr. Kevin Moore
Date of submission: 10/14/05
Review and Points of Discussion: The project will construct expression plasmids (pEE14.1 GS, a eukaryotic expression vector/Lonza Biologics) to produce soluble forms of human TPST-1 and -2 in the CHO cell line. TPST is tyrosyl protein sulfotransferase. These proteins are involved in post-translational sulfation of tyrosine residues in proteins. Trangenic mice will also be produced to drive tissue-specific expression of TPST under the controlof the mouse calmegin promoter in the FMV mouse strain.
Concerns: The PI clarified issues related to the expression vector where "GS" is a selectable marker and the proteins are expressed under control of an hCMV promoter. No health, environmental, LS issues.
Outcome: Experiments will be conducted under BL1 containment.
Approved for cell expression experiment. Approval for transgenic mouse experiments is contingent upon IACUC approval.

Project: Identification of Synoptic DAG Effectors and Regulators
PI: Dr. Kenneth G. Miller
Date of submission: 12/22/05
Review and Points of Discussion: The investigator seeks to continue his neuronal signal transduction studies in the nematode *C elegans* by screening for mutations that rescue paralysis of a mutant with low diacylglycerol (DAG) levels. Mutations will then be studied to identify the proteins they interact with. Genes or cDNA sequences will be cloned into pUC-based vectors with 3' UTR sequences for expression in *C elegans*.
Concerns: C elegans is a nonpathogenic round worm. There are no health, environmental, or LS issues.
Outcome: The experiments are exempt per NIH Guidelines. All experiments will be conducted under BL1 containment.
Approved

RECOMBINANT DNA REGISTRATION FORM
Institutional Biosafety Committee (IBC)
Oklahoma Medical Research Foundation

Pease complete this form to satisfy federal regulations specified in the *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. (A copy of this document is available in the OMRF library.) Attach additional pages if needed. DO NOT ATTACH GRANT APPLICATIONS UNLESS REQUESTED.

A. 1. PRINCIPAL INVESTIGATOR: A. Darise Farris, Ph.D.
2. Department: Arthritis and Immunology Phone: 17389 Lab: 17519
3. Project Title: B Cell Tolerance to Nuclear Antigen La

B. EXPERIMENTAL CATEGORY. Please check one (see *NIH Guidelines* Section III).
1. ___ Experiments requiring IBC approval, RAC review, and NIH approval before initiation.
2. ___ Experiments requiring NIH/ORDA and IBC approval before initiation.
3. X Experiments requiring IBC approval before initiation.
4. ___ Experiment requiring IBC notification simultaneously with initiation.
5. ___ Exempt experiments.

C. PROJECT INFORMATION

1. Briefly describe the project including the sequences to be cloned and their function. If these sequences are from a eukaryotic virus, indicate if >2/3 of the viral genome will be cloned, if the virus is replication defective, and describe any helper viruses that will be used.

I. Rearranged heavy (H) and light (L) chain immunoglobulin receptor genes from an anti-human La (hLa) specific B cell hybridoma will be cloned into the pGEM-11 and pSV2-LkCk vectors, respectively, containing Ig promoters, leader sequences and constant regions (see attached maps). For the reconstructed H-chain gene, the μ constant region from vector pSVGneoV$_{10}$CμM will be used. The reconstructed genes will be injected into the fertilized ova of FVB/N mice to produce (see atc

2. Do these sequences encode molecules toxic to vertebrates at an LD50 $\leq$ 100 μg/kg of body weight? No If so, specify LD50 _____ (see *NIH Guidelines* Appendix F).

3. List vectors to be used (attach maps, if available): 1) pGEM-11L$_H$, 2) pSV2-LkCk, 3) pSVGneoV$_{10}$CμM, 4) 3H9 H-chain targeting vector, and 5) Vk8RTV inpBlueScript (see attached list of references)

4. List recipient hosts. Include plants and animals and if they will become transgenic. If humans are the intended recipients, complete this form and attach an addendum addressing issues in Appendix M of the *NIH Guidelines*). Ig transgenic mice will be on the FVB/N backgrou then backcrossed to the A/T inbred background for study. Ig knock-in mice will be produced on the 129SV/EVXC57BL/6 background, then crossed to the A/J backgrou for further study.

5. List any product to be expressed and identify its function (if known). Immunoglobulin receptor with specificity for the La nuclear antigen.

Function: B cell recognition and signaling.

6. Will greater than 10 liters of culture containing this recombinant DNA be grown at any one time? No

7. If applicable, review health and environmental hazards that may result from this work in an attached addendum. A few suggestions to help you formulate your answer are offered below. These are not meant to cover all circumstances.
   - If DNA sources are from class 2 or 3 infectious microorganisms, exotic animal or plant pathogens, or USDA-regulated articles, discuss safety aspects of the facility, containment practices, personnel practices, and staff training that will assist in the safe conduct of the study. Summarize pathogenic aspects of organisms (modes of transmission, etc). Include measures to prevent or minimize expression of pathogenic or infectious sequences.
   - If oncogenes of other regulatory sequences are transferred, will pathogenic virulence, toxicity, antibiotic resistance, or immune competence be affected?
   - Will the experiments require the release into the environment of organisms containing recombinant DNA molecules?

*** CHECK HERE IF ITEM 7 IS NOT APPLICABLE X

D. CONTAINMENT LEVELS

Based on the above information and the *NIH Guidelines* (Section III and Appendices G, I, K, P & Q), circle the appropriate containment levels for these experiments.

Physical Containment	Animal Biosafety Level
(BL1) BL2 BL3	(BL1) BL2 BL3
GLSP BL1-LS BL2-LS BL3-LS	BL-1N BL-2N BL-3N

E. PERSONNEL

List all personnel who will conduct these experiments. Include visiting staff, students, and temporary help. For each person, check if they are currently trained or will be trained by the PI to perform their duties.

Name	Trained	Will Train
A. Darise Farris, Ph.D.	X	
Michael P. Bachmann, Ph.D.	X	
Britt Nakken, Ph.D.		X

F. I am familiar with and agree to abide by the *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* and other specific NIH instructions pertaining to the proposed project. I understand that it is my responsibility that all laboratory personnel working on this project are trained and remain fully informed with regard to biosafety practices, techniques, and applicable emergency procedures. I also agree to comply with federal requirements pertaining to shipment of recombinant DNA molecules. The above information is accurate and complete.

PI Signature A. Darise Farris Date 9/28/01

IBC Action:

Approved ✓ Disapproved ___ Pending ___

MB Frank 5/28/03

IBC Chair or Member Date

Comments:
Relocated 5/27-03 / more coming.

C. 1. Continued

I. **(continued)** Ig H- and L-chain transgenics. The mice will be studied separately and intercrossed to produce mice that possess a large proportion of B cells expressing an anti-hLa specific B cell receptor.

II. Using rearranged Ig receptor genes from another anti-hLa specific B cell hybridoma, mouse Ig gene region targeting vectors will be constructed. Existing targeting vectors (3H9 H-chain and Vk8RTV; see attached reference) will be used, where the rearranged variable region genes will be replaced with those of an anti-hLa specific B cell hybridoma. These will be used to produce homologous recombinants in mouse embryonic stem cells. Recombinant stem cells bearing the anti-La Ig H- and L-chain locus specific rearrangements will separately be injected into C57BL/6 blastocysts to produce chimeric mice. The chimeras will be bred to identify animals transmitting the rearrangements in the germline. The resulting H- and L-chain knock-in mice will be studied separately and intercrossed to produce mice that possess a large proportion of B cells expressing an anti-hLa specific B cell receptor.

Approaches I. and II. are not duplications but, rather, are methods selected based on the particular nature of the B cell receptors employed.

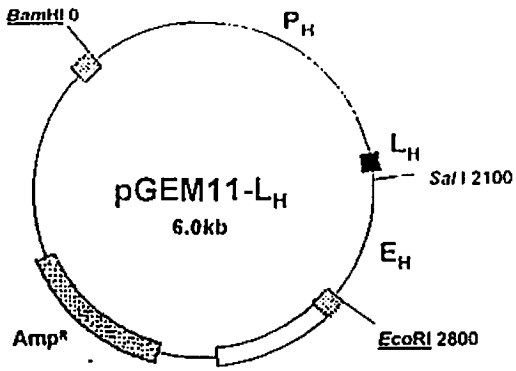
References

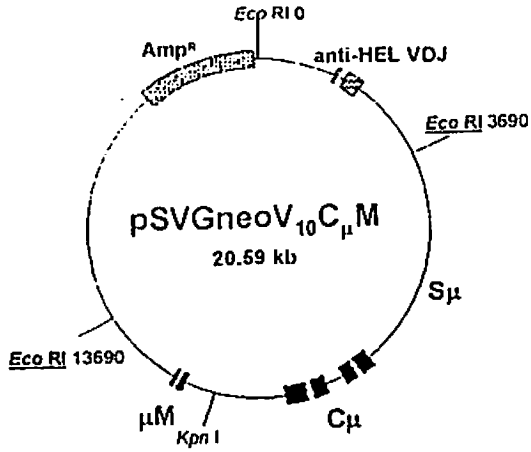
I. Gascoigne NR, Goodnow CC, et al. 1987. Secretion of a chimeric T-cell receptor-immunoglobulin protein. *Proc Natl Acad Sci U S A 4:2936-2940.* (Vectors 1 and 2)

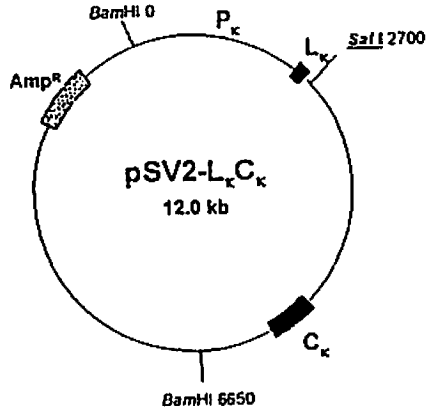
II. Goodnow CC, Crosbie J, et al. 1988. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. *Nature 334:676-682.* (Vector 3)

III. Chen C, Nagy Z, et al. 1995. Immunoglobulin heavy chain gene replacement: a mechanism of receptor editing. *Immunity 3(6): 747-55.* (Vector 4)

IV. Prak EL and Weigert M. 1995. Light chain replacement: a new model for antibody gene rearrangement. *J Exp Med 182(2):541-8.* (Vector 5)







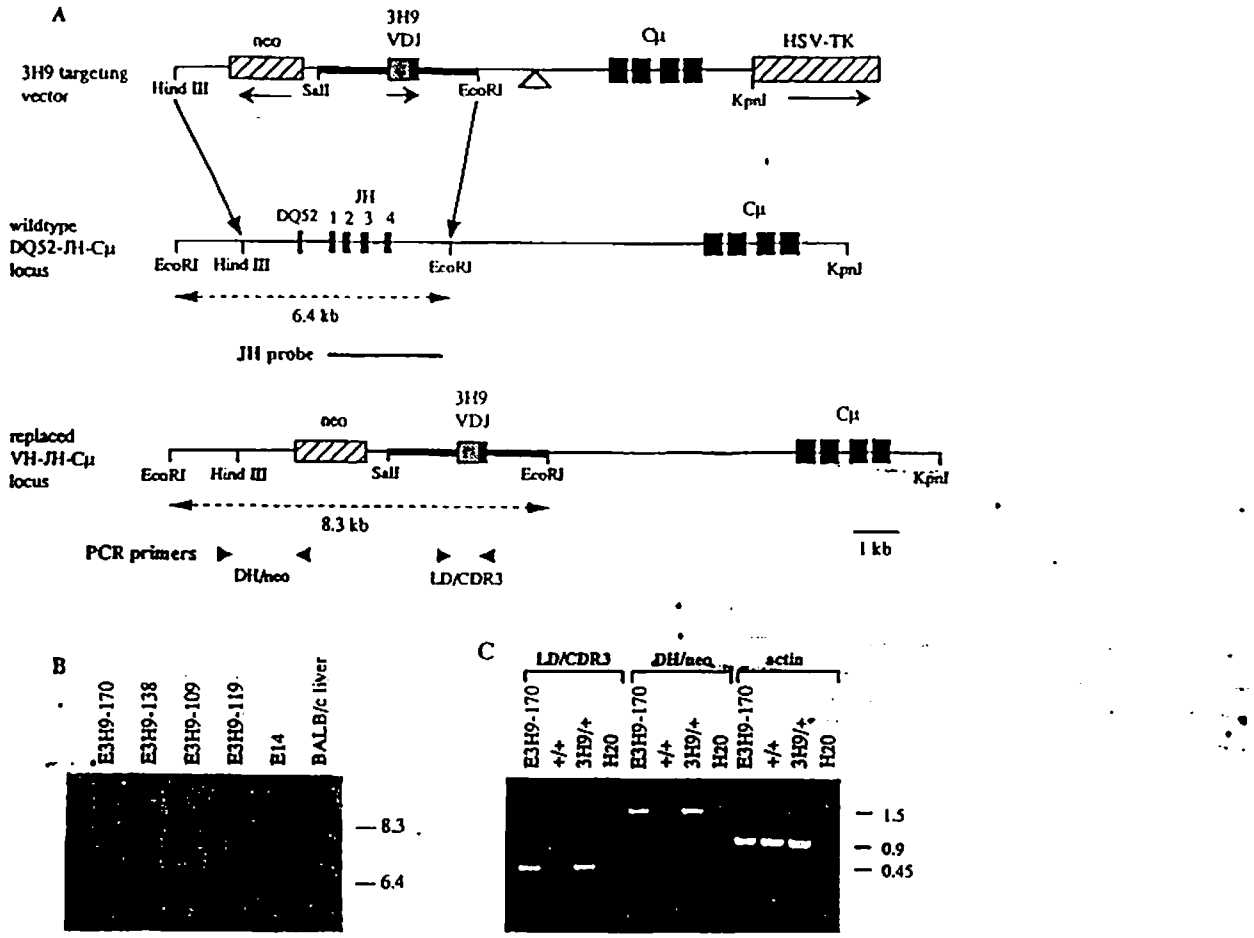


Figure 1. Site-Directed Replacement of the J_H Locus with 3H9 V_H Gene

(A) Structure of the 3H9 targeting construct, the germline IgH locus, and the targeted IgH–3H9 locus. The 3H9 sequence includes the core VDJ region (stippled box) and the surrounding regulatory sequences (thick line). The *neo*r and HSV–*tk* genes are shown as hatched boxes and the Cμ, DQ52, and J_H segments are represented by closed boxes. Transcriptional orientation of *neo*r, V_H3H9, and HSV–*tk* genes is indicated by arrows. The triangle indicates a 3 kb deletion in the Sμ region of the targeting construct. The germline and the replaced J_H loci are expected to give 6.4 kb and 8.3 kb EcoRI restriction fragments, respectively. The positions of the J_H probe and the PCR primers are shown.

(B) Southern blot analysis of the transfected ES clones that tested positive by D_H/*neo* PCR. The DNA was digested with EcoRI and hybridized with a J_H probe. The nontargeted alleles generate a 6.4 kb germline band as seen in the liver and the parental E14–1 cell DNA controls. The targeted alleles gave the expected 8.3 kb band. The 6.4 kb band is darker than the 8.3 kb band because the J_H probe hybridizes more strongly to the complete germline J_H sequence (present on the wild-type allele but truncated on the targeted allele).

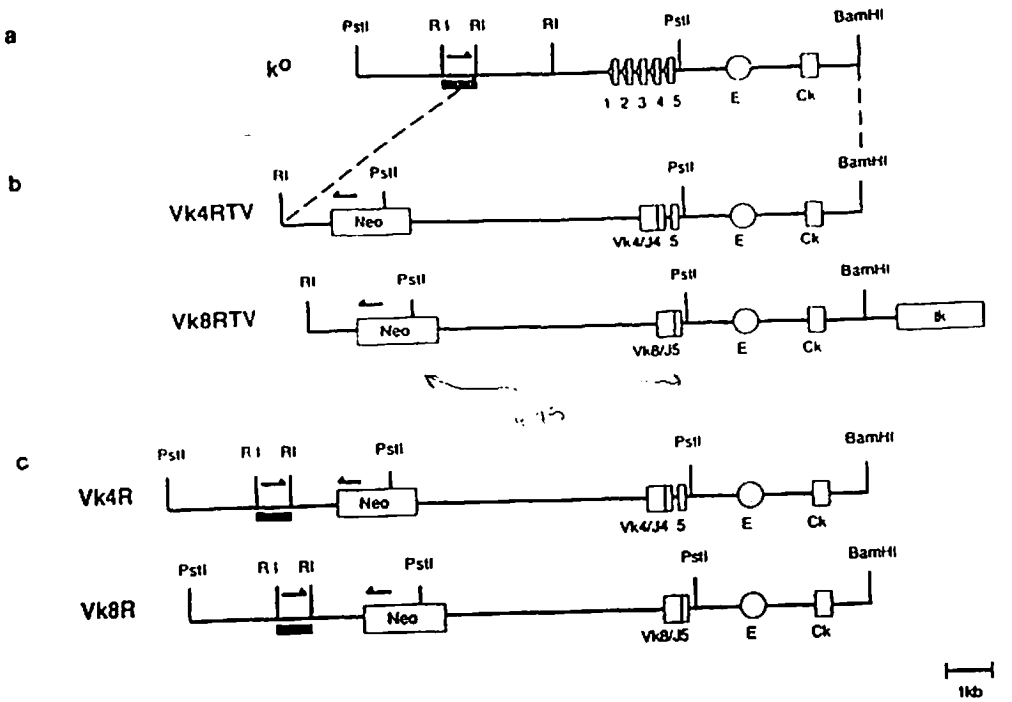
(C) PCR analysis of ES cell–derived offspring. The targeted ES cell clone, E3H9–170, which contributed to the germline chimeras, is used as a positive control and the water is used as a negative control. The tail DNA samples of a transmitted heterozygous mouse (3H9/+) and a wild-type littermate (+/+) were tested by three PCR assays. The LD/CDR3 PCR tests the presence of the 3H9 gene; the D_H/*neo* PCR confirms the correct insertion of the targeting construct; the actin PCR assures the quality and quantity of the DNA in each sample. The transmitted mouse is positive for all three PCRs, whereas the littermate only shows the actin band.

extent to which editing at the H chain locus contributes to B cell tolerance: it has upstream V_H genes, it has embedded heptamers, and it contributes unilaterally to the specificity for a self-antigen.

Results

Generation of Site-Directed Immunoglobulin H Chain Transgenic Mice

The 3H9 H chain targeting vector used to replace the J_H locus is shown in Figure 1A. The rearranged V_H region of the anti-DNA antibody, 3H9, was joined to the Cμ gene and then flanked by the positive and negative selection marker genes *neo* and *tk*. A 1.5 kb sequence upstream of DQ52 was added to provide the 5′ homology. The 3H9 targeting construct was introduced into the embryonic stem (ES) cells and drug-resistant colonies were screened for homologous recombination events by polymerase chain reaction (PCR) using the primers indicated in Figure 1A. Four PCR positive colonies were analyzed by Southern blot analysis and gave rise to the expected 8.3 kb band (Figure 1B). The targeted ES cells were injected into C57BL/6 blastocysts to generate chimeric mice. Progeny of chimeric mice were scored for germline transmission of the ES cell genotype by coat color. ES cell–derived offspring were tested for the presence of the sd-Ig by PCR of tail DNA (Figure 1C).



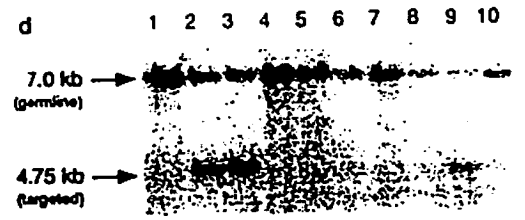


Figure 1. Targeted replacement of the Jκ region with functional Vκ-Jκ genes. Shown are the germline κ locus (*a*), replacement targeting vectors for Vκ4-Jκ4 and Vκ8-Jκ5 (*b*), and the resultant Vκ4 replaced (Vκ4R) or Vκ8 replaced (Vκ8R) κ loci (*c*). Dashed lines denote the borders of homology between the targeting vector and the germline locus. Arrowheads indicate the positions of PCR primers used to screen neomycin-resistant embryonic stem (ES) cell colonies for homologous recombination events. The upstream κ DNA probe, PKP6 (3), is denoted by a cross-hatched bar. PKP6 was used in Southern analysis (*d*) to confirm the genotype of targeted ES cells. Shown are genomic DNA samples from untransfected ES cells (lane *1*) and Vκ8R ES cells (lane *2*) and tail DNA samples from the offspring of germline chimeras (lanes *3–6*, offspring of a Vκ4R chimera; lanes *7–10*, offspring of a Vκ8R chimera). PstI digestion yields a 7.0-kb fragment in the wild-type germline κ locus (*a*) and a 4.75-kb fragment in the Vκ4R or Vκ8R loci.

type mice (Luning Prak, E., R. R. Hardy, and M. Weigert, manuscript in preparation).

Germline transmission was achieved in a C57Bl6 (ES cell)/ICR chimera for Vκ4R and in an E14.1 (ES cell)/C57Bl6 chimera for Vκ8R. Offspring in which the replaced κ locus was present were identified by Vκ4- or Vκ8-specific PCR assays of tail DNA (data not shown). Transmission of VκR was confirmed by Southern analysis (Fig. 1 *d*). PstI digestion of genomic DNA yields a 7.0-kb fragment in the wild-type germline κ locus and a 4.75-kb fragment in the Vκ4 or Vκ8 L chain replaced locus.

Analysis of L Chain Genotypes in VκR Hybridomas. To study the effect of Vκ4R and Vκ8R on the rearrangement of other L chain genes, LPS hybridomas were prepared from κ hemizygous Vκ4R/κ^0 and Vκ8R/κ^0 mice. The rearrangement status of κ and λ genes in individual IgM-secreting lines was tested using a series of PCR assays. First, each clone was tested for the presence of Vκ4R or Vκ8R DNA by PCR. Next, additional κ rearrangements on the targeted allele and on the wild-type κ allele (when they occurred) were identified using a series of PCR amplifications with forward Vκ primers and reverse Jκ primers (primer positions are shown in Fig. 2 *a*). The size of the amplification product in these assays is diagnostic of the Jκ segment used in the rearrangement (Fig. 2 *b–d*). For example, using Vs and Jκ5 primers (Fig. 2 *b*), rearrangement to Jκ1 gives a 1.6-kb product, whereas Jκ2 rearrangements are 1.2 kb, Jκ4 are 600 bp, and Jκ5 are 270 bp. Because Jκ1 rearrangements are not always discernible by Vκ + Jκ5 PCR, Vs and Jκ2 primers were used to verify Jκ1 rearrangements (Fig. 2 *c*). The L5 + Jκ5 PCR (Fig. 2 *d*) was used to type Jκ2 rearrangements on the untargeted κ allele (The Vs + Jκ5 PCR cannot be used for this purpose because the Jκ2 rearrangement of the fusion partner is amplified in all of the hybridomas).

The pattern of Jκ segment usage revealed by these assays will in nearly all cases reveal the rearrangement status at each κ allele, yielding a κ genotype for each hybridoma (κ genotypes are shown in Fig. 2 *a* and all observed genotypes are

OMRF Recombinant DNA Subcommittee Minutes

March 23, 2006
Massman Bldg, L2, OMRF
820 NE 15th Street, Oklahoma City, OK
2 pm CST

Committee members in attendance at today's meeting were Drs. Li, Frank and Silverman. A quorum of the committee was present. The meeting was open to any interested parties.

A brief discussion indicated a desire for more detailed minutes of IBC meetings. Additions will include date, place, time, and list members present.

The following projects were considered and the listed actions taken:

Project: Role of Monocyte Chemokine Receptor Tyrosine Sulfation in Atherosclerosis
PI: Dr. Andrew D. Westmuckett
Date of submission: 1/11/06
Review and Points of Discussion: Chemokines and their receptors are play critical roles in trafficking of blood cells. The PI seeks to clone N-terminal tagged mouse chemokine receptors CCRS, CX3CR1 and CXCR2 in the pcDNA 3.1 expression vector and transfect these constructs into L1.2 and HEK 293 cell lines. Permanent transfectants will be tested for functional response to CR ligands (JE, fractalkine and KC, respectively).
Concerns: There were no health, environmental, or LS concerns. Experiments will be conducted under BL1 containment.
Outcome: Approved

Project: Role of Myosin V in Neuronal Plasticity
PI: Dr. Stephen Fields
Date of submission: 2/15/06
Review and Points of Discussion: Possible functions of the *C elegans* myosin V protein in actin-based motility of synaptic vesicles and RNA will be investigated using truncated mutants of the protein fused to GFP. These will be cloned into yeast and E coli vectors for their analysis in a yeast two-hybrid system.
Concerns: Hosts include *E coli* DH5-alpha, *S cerevisiase* and *C elegans*. All are considered to be non-pathogenic. No pathogenic organsisms or genes involved in pathogenesis will be used.
Outcome: The experiments are exempt per NIH Guidelines. Experiments will be conducted under BL1 containment. There were no health, environmental, or LS concerns. Approved

Project: Telomere Involvement in Chromosome Segregation
PI: Dr. Michael Conrad
Date of submission: 2/16/06
Review and Points of Discussion: *Saccharomyces cerevisiae* genes involved in chromosome segregation will be cloned for use in two-hybrid assays and to construct

mutant alleles that will be reintroduced into yeast for further analysis. Yeast – *E coli* shuttle vectors will be used.
Concerns: There were no health, environmental, or LS concerns.
Outcome: The experiments are exempt per NIH Guidelines. Experiments will be conducted under BL1 containment.
Approved

Project: The Role of Slk19 in Mitotic Cell Cycle Progression
PI: Dr. Dean Dawson
Date of submission: 2/17/06
Review and Points of Discussion: The project will clone and various forms of the yeast genes SLk419 and CDC14 from *Saccharomyces cerevisiae* (baker's yeast). Conventional *E coli* – yeast shuttle vectors will be used.
Concerns: There were no health, environmental, or LS issues.
Outcome: The experiments are exempt per NIH Guidelines. Experiments will be conducted under BL1 containment.
Approved

Project: Sororin, Chromosome Cohesion and Cell Cycle Control
PI: Dr. Susannah Rankin
Date of submission: 2/17/06
Review and Points of Discussion: Frog and human genes encoding chromosomal structural and centromeric proteins will be cloned in expression standard *E coli* vectors to generate RNA and recombinant proteins in transient expressed cell lines.
Concerns: There were no health, environmental, or LS concerns.
Outcome: BL1 containment is appropriate for these experiments.
Approved

There being no more actions before the IBC, the meeting adjourned at 2:20 pm. All actions taken are believed to be consistent with OMRF Policies and NIH Guidelines.

OMRF Recombinant DNA Committee Minutes
October 26, 2004

The following two projects were considered by the OMRF Recombinant DNA IBC and the listed actions taken:

Project: Molecular Analysis of Human IgA for Mucosal Immunity
PI: Dr. White
Date: 6/23/04
Review: In this project, human IgA, J chain, polymeric Ig receptors, and CD89 will be cloned in baculovirus vectors and expressed in insect cell lines
Concerns: No health, environmental, LS issues.
The project requires only IBC notification at time of initiation.
The project meets criteria for BL1 containment.
Approved

Project: Chromosome Movement in Prometaphase
PI: Dr. Gorbsky
Date: 10/21/04
Review: In this project, conventional E. coli separately encoding cyclin-dependent kinase, Ndc80, Aurora B kinase, and Piol-like kinase will undergo in vitro mutagenesis, followed by transfection into human, pig, and frog cell lines. SiRNA will be used to repress protein production. Phenotypic changes will be determined.
Concerns: No health, environmental, LS issues.
The project requires only IBC notification at time of initiation.
The project meets criteria for BL1 containment.
Approved

RECOMBINANT DNA REGISTRATION FORM
Institutional Biosafety Committee (IBC)
Oklahoma Medical Research Foundation

Please complete this form to satisfy federal regulations specified in *the Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. (A copy of this document is available in the OMRF library.) Attach additional pages if needed. DO NOT ATTACH GRANT APPLICATIONS UNLESS REQUESTED.

A. 1. PRINCIPAL INVESTIGATOR: Gary J. Gorbsky

2. Department: MCDB Phone: 1-7660 Lab: 1-2037

3. Project Title: The Regulation of Cytokinesis in Vertebrate Cells

B. EXPERIMENTAL CATEGORY. Please check one (see *NIH Guidelines* Section III).

1. __ Experiments requiring IBC approval, RAC review, and NIH approval before initiation.
2. __ Experiments requiring NIH/ORDA and IBC approval before initiation.
3. __ Experiments requiring IBC approval before initiation.
4. ✓ Experiment requiring IBC notification simultaneously with initiation.
5. ✓ Exempt experiments.

C. PROJECT INFORMATION

1. Briefly describe the project including the sequences to be cloned and their function. If these sequences are from a eukaryotic virus, indicate if > 2/3 of the viral genome will be cloned, if the virus is replication defective, and describe any helper viruses that will be used.
Conventional E. coli plasmids encoding Cyclin-dependent kinase-1, a cell cycle regulatory enzyme will be sublect to in vitro mutagensis and then transfected into pig or frog tissue culture cells. Small inhibitory double stranded RNA (siRNA) molecules will be transfected into tissue culture cells (human, pig, frog) to repress the expression polo-like kinase. siRNA be chemically synthesized and purchased commercially.

2. Do these sequences encode molecules toxic to vertebrates at an LD50 ≤ 100 μg/kg of body weight? No If so, specify LD50 _____ (see *NIH Guidelines* Appendix F).

3. List vectors to be used (attach maps, if available): pcDNA3.1

4. List recipient hosts. Include plants and animals and if they will become transgenic. If humans are the intended recipients, complete this form and attach an addendum addressing issues in Appendix M of the *NIH Guidelines*). LLc-Pk cell line (porcine) Hela cell line (human) S3 cell line (frog)

5. List any product to be expressed and identify its function (if known). Cyclin-dependent kinase1 a cell cycle regulatory kinase that controls cell progression in mitosis.

6. Will greater than 10 liters of culture containing this recombinant DNA be grown at any one time? No

7. If applicable, review health and environmental hazards that may result from this work in an attached addendum. A few suggestions to help you formulate your answer are offered below. These are not meant to cover all circumstances.
   - If DNA sources are from class 2 or 3 infectious microorganisms, exotic animal or plant pathogens, or USDA-regulated articles, discuss safety aspects of the facility, containment practices, personnel practices, and staff training that will assist in the safe conduct of the study. Summarize pathogenic aspects of organisms (modes of transmission, etc). Include measures to prevent or minimize expression of pathogenic or infectious sequences.
   - If oncogenes of other regulatory sequences are transferred, will pathogenic virulence, toxicity, antibiotic resistance, or immune competence be affected?
   - Will the experiments require the release into the environment of organisms containing recombinant DNA molecules?

*** CHECK HERE IF ITEM 7 IS NOT APPLICABLE ✔

D. CONTAINMENT LEVELS

Based on the above information and the *NIH Guidelines* (Section III and Appendices G, I, K, P & Q), circle the appropriate containment levels for these experiments.

Physical Containment	Animal Biosafety Level
(BL1) BL2 BL3	BL1 BL2 BL3
GLSP BL1-LS BL2-LS BL3-LS	BL-1N BL-2N BL-3N

E. PERSONNEL

List all personnel who will conduct these experiments. Including visiting staff, students, and temporary help. For each person, check if they are currently training or will be trained by the PI to perform their duties.

Name	Trained	Will Train
Gary J. Gorbsky	✔	
John Daum	✔	
Tara Jones	✔	

F. I am familiar with and agree to abide by the *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* and other specific NIH instructions pertaining to the proposed project. I understand that it is my responsibility that all laboratory personnel working on this project are trained and remain fully informed with regard to biosafety practices, techniques, and applicable emergency procedures. I also agree to comply with federal requirements pertaining to shipment of recombinant DNA molecules. The above information is accurate and complete.

PI Signature Gary J. Gorbsky Date 2/11/04

IBC Action: Approved ✔ Disapproved ___ Pending ___

Comments: ___

MB Frank 2/13/04

IBC Chair or Member Date

Minutes of the Biosafety Committee Meeting – June 22, 2004

The Biosafety Committee met on June 22, 2004 to discuss issues related to the pending grant: Molecular and Immunologic Analysis of the Pathobiology of Human Anthrax. Those in attendance were: CONFIDENTIAL. Also present were non-committee members CONFIDENTIAL and CONFIDNETIAL, the co-PI of the grant.

The overall organization of the application was presented. It consists of three main projects (CONFIDENTIAL), three technical components (CONFIDENTIAL), two pilot projects (CONFIDENTIAL), two cores (flow cytometry/CONFIDENTIAL and microarrays/CONFIDENTIAL), and an educational component (CONFIDENTIAL). There was also a brief discussion of the anthrax-related materials that will be used in the research described in this grant: purified recombinant toxins prepared by CONFIDENTIAL, vegetative cultures, and spores in liquid suspension. Initially, the only anthrax strain used will be Sterne strain 34F2. It is an attenuated strain of *B. anthracis* and lacks the ability to form a capsule. It is 10^3-10^5 less virulent than other strains. However, it does produce as much toxin as more virulent strains. It is the same strain used to immunize military personnel. Sterne strain is not on the CDC list of select agents and is, therefore, exempt from the regulatory controls of select agents.

Reviews of individual components:

1. CONFIDENTIAL – CONFIDENTIAL, reviewer.
   Concerns and items needing clarification: Are these investigators using vegetative bacteria or only spores? How will the animal bedding be disposed/decontaminated? Exactly what precautions will be taken with blood samples from guinea pigs from an inhalation anthrax model? Since these latter experiments are several years into the future, the Committee agreed to approve the project except for these specific experiments. If these experiments are finally undertaken, the investigators must then come back to the Committee for approval.

2. CONFIDENTIAL – CONFIDENTIAL, reviewer.
   No concerns. CONFIDENTIAL agreed that he should do a test to determine if any live bacteria are present in commercial preparations of anthrax cell walls he will purchase.

3. CONFIDENTIAL – CONFIDENTIAL, reviewer.
   Items needing clarification: More details are needed for the animal experiments.

4. CONFIDENTIAL – CONFIDENTIAL, reviewer.
   No anthrax-related biohazard concerns. Only serum and DNA samples from military personnel will be utilized.

5. CONFIDENTIAL – CONFIDENTIAL, reviewer.
   Most of CONFIDENTIAL experiments will be conducted at OU and must be approved by OU. We are concerned only with the material he will give to OMRF investigators. This includes human lung tissue. This poses a standard blood borne pathogen risk, but no anthrax-related risk.

Concern: CONFIDENTIAL will also give Dr. CONFIDENTIAL protein extracts from anthrax-infected cells for evaluation of signaling pathways via western blots. It is unclear if these extracts pose a biohazard risk. CONFIDENTIAL will investigate whether anthrax spores are killed by boiling in standard SDS sample buffer.

6. CONFIDENTIAL – CONFIDENTIAL, reviewer.
   Items needing clarification: Will CONFIDENTIAL be obtaining anthrax toxins from CONFIDENTIAL, or isolating them himself? Will anthrax spores be purchased or obtained from CONFIDENTIAL? Where will anthrax spores be stored and who will have access to keys? The autoclaved anthrax-contaminated material should be placed in an OMRF Biohazard Waste container for incineration, not placed in the dumpster. The method of safe transport of anthrax cultures from OMRF to OU for the baboon studies needs to be explained. A method should be described for the clean up of large spills of bacterial cultures and well as for the disposal of unneeded toxin.

7. CONFIDENTIAL – CONFIDENTIAL, reviewer.
   This project utilizes lethal toxin and human macrophages or lung tissue. Risk is minimal – no concerns.

8. CONFIDENTIAL – CONFIDENTIAL, reviewer.
   No concerns – this project utilizes only molecular biology and protein engineering.

9. Flow cytometry core – CONFIDENTIAL, reviewer.
   No concerns – only fixed cells may be brought into the flow cytometry lab.

10. Microarray core – CONFIDENTIAL, reviewer.
    No concerns – only purified RNA will be brought into the microarray core lab.

The Committee recommends the following general policies:

1. All *B. anthracis* should be grown in a single location. For now, that will be CONFIDENTIAL lab. We recommend that an alternate site be designated, perhaps a room in the Main Building LARC. This room would need a shaking 37° incubator, centrifuge, BSL2 biosafety cabinet, and sink. The room should have card access and be accessible only to those personnel who have completed training regarding the safe growth of anthrax and how to prevent spore formation. The door of the room should have a Biohazard sign with *B. anthracis* listed as the hazardous agent.

2. The committee decided that it should not be necessary to maintain inventory records of anthrax cultures, spores, or toxins. Since the Sterne strain of anthrax is not on the CDC select agent list, neither the CDC nor the NIH requires such record keeping. Therefore, maintaining an inventory would place an unreasonable burden on the investigators.

3. There is already documentation of training of many personnel involved with this grant. When new personnel are trained, their names should be submitted to the Biosafety

Committee along with documentation of training. CONFIDENTIAL will develop an on-line exam to test whether proper training has occurred. People passing the exam and needing to work with vegetative cultures will then be issued card access to the room in which the bacteria will be grown.

4. Every attempt must be made to prevent the development of anthrax spores. This includes growing vegetative cultures under conditions where there are sufficient nutrients and decontaminating culture flasks immediately after use. The Committee agreed that liquid suspensions of anthrax spores pose much less threat than dry spore powder and can be safely handled in a BSL2 biosafety cabinet. The spore preparation OMRF investigators will use is the same material used to vaccinate cattle. It can be handled safely even in a ranch environment.

5. CONFIDENTIAL will make an addition to OMRF's chemical hygiene plan that includes the proper procedures for cleaning up a spill of anthrax cultures. This information should be included in the training material for personnel joining this project.

Summary and Recommendation:

The Committee agreed that the protocols should all be approved contingent upon the clarification of issues outlined above.

OMRF Institutional Biosafety Committee (IBC)
Meeting Date: 28 June 2005

Present
CONFIDENTIAL

Agenda

- Review of Standard Operating Procedures (SOP) for Handling *Bacillus anthracis* Stern Strain and Contaminated Materials
- Review of OMRF Policies for Registration of Recombinant DNA Experiments

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I. Review of 'SOP for Handling *Bacillus anthracis* Stern Strain and Contaminated Materials'. A draft document provided by CONFIDENTIAL was discussed and modified. Please see the attached revised SOP.

II. Review of OMRF Policies for Registration of Recombinant DNA Experiments

The relationship between the OMRF Recombinant DNA Committee (recDNAC) and the IBC was discussed. It was decided that future recombinant DNA protocols will be reviewed by the recDNAC as in the past to determine the appropriate Biosafety level and identify any potential concerns. Those protocols requiring approval prior to the initiation of experiments will be discussed and approved by the entire IBC at their next meeting.

CONFIDENTIAL and CONFIDENTIAL will develop a new web-based version of the recombinant DNA protocol form incorporating the comments of the IBC, with the intention of clarifying the criteria requiring registration and approval of recombinant DNA experiments with/by the recDNAC. When this is available, it will be e-mailed to committee members for their approval. CONFIDENTIAL and CONFIDENTIAL will also draft a statement outlining OMRF's policies regarding recombinant DNA procedures and approvals. Please see attached policy statement.

The recDNAC will also circulate a copy of the current OMRF Grant Routing Sheet. Please see attachment and suggest improvements at our next meeting.

It was suggested that OMRF establish a policy that PIs who check PENDING for Recombinant DNA on the Grant Routing Sheets will be urged to submit their OMRF recDNA Registration Forms for recDNAC approval one month after their grant submission date (December 1, April 1, or August 1) to assure timely approval before the need to submit "Just in time" documents and/or the onset of funding.

CONFIDENTIAL will forward to CONFIDENTIAL all copies of Grant Routing Sheets that have PENDING checked.
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OMRF Recombinant DNA Subcommittee Minutes

March 23, 2006
Massman Bldg, L2, OMRF
820 NE 15th Street, Oklahoma City, OK
2 pm CST

Committee members in attendance at today's meeting were Drs. Li, Frank and Silverman. A quorum of the committee was present. The meeting was open to any interested parties.

A brief discussion indicated a desire for more detailed minutes of IBC meetings. Additions will include date, place, time, and list members present.

The following projects were considered and the listed actions taken:

Project: Role of Monocyte Chemokine Receptor Tyrosine Sulfation in Atherosclerosis
PI: CONFIDENTIAL
Date of submission: 1/11/06
Review and Points of Discussion: Chemokines and their receptors are play critical roles in trafficking of blood cells. The PI seeks to clone N-terminal tagged mouse chemokine receptors CCRS, CX3CR1 and CXCR2 in the pcDNA 3.1 expression vector and transfect these constructs into L1.2 and HEK 293 cell lines. Permanent transfectants will be tested for functional response to CR ligands (JE, fractalkine and KC, respectively).
Concerns: There were no health, environmental, or LS concerns. Experiments will be conducted under BL1 containment.
Outcome: Approved

Project: Role of Myosin V in Neuronal Plasticity
PI: CONFIDENTIAL
Date of submission: 2/15/06
Review and Points of Discussion: Possible functions of the *C elegans* myosin V protein in actin-based motility of synaptic vesicles and RNA will be investigated using truncated mutants of the protein fused to GFP. These will be cloned into yeast and E coli vectors for their analysis in a yeast two-hybrid system.
Concerns: Hosts include *E coli* DH5-alpha, *S cerevisiase* and *C elegans*. All are considered to be non-pathogenic. No pathogenic organsisms or genes involved in pathogenesis will be used.
Outcome: The experiments are exempt per NIH Guidelines. Experiments will be conducted under BL1 containment. There were no health, environmental, or LS concerns. Approved

Project: Telomere Involvement in Chromosome Segregation
PI: CONFIDENTIAL
Date of submission: 2/16/06
Review and Points of Discussion: *Saccharomyces cerevisiae* genes involved in chromosome segregation will be cloned for use in two-hybrid assays and to construct

mutant alleles that will be reintroduced into yeast for further analysis. Yeast – *E coli* shuttle vectors will be used.
Concerns: There were no health, environmental, or LS concerns.
Outcome: The experiments are exempt per NIH Guidelines. Experiments will be conducted under BL1 containment.
Approved

Project: The Role of Slk19 in Mitotic Cell Cycle Progression
PI: CONFIDENTIAL
Date of submission: 2/17/06
Review and Points of Discussion: The project will clone and various forms of the yeast genes SLk419 and CDC14 from *Saccharomyces cerevisiae* (baker's yeast). Conventional *E coli* – yeast shuttle vectors will be used.
Concerns: There were no health, environmental, or LS issues.
Outcome: The experiments are exempt per NIH Guidelines. Experiments will be conducted under BL1 containment.
Approved

Project: Sororin, Chromosome Cohesion and Cell Cycle Control
PI: CONFIDENTIAL
Date of submission: 2/17/06
Review and Points of Discussion: Frog and human genes encoding chromosomal structural and centromeric proteins will be cloned in expression standard *E coli* vectors to generate RNA and recombinant proteins in transient expressed cell lines.
Concerns: There were no health, environmental, or LS concerns.
Outcome: BL1 containment is appropriate for these experiments.
Approved

There being no more actions before the IBC, the meeting adjourned at 2:20 pm. All actions taken are believed to be consistent with OMRF Policies and NIH Guidelines.

OMRF Recombinant DNA Committee Minutes
December 22, 2005

The following projects were considered and the listed actions taken:

Project: TPST Inhibitos for Male Contraception
PI: CONFIDENTIAL
Date of submission: 10/14/05
Review and Points of Discussion: The project will construct expression plasmids (pEE14.1 GS, a eukaryotic expression vector/Lonza Biologics) to produce soluble forms of human TPST-1 and -2 in the CHO cell line. TPST is tyrosyl protein sulfotransferase. These proteins are involved in post-translational sulfation of tyrosine residues in proteins. Trangenic mice will also be produced to drive tissue-specific expression of TPST under the controlof the mouse calmegin promoter in the FMV mouse strain.
Concerns: The PI clarified issues related to the expression vector where "GS" is a selectable marker and the proteins are expressed under control of an hCMV promoter. No health, environmental, LS issues.
Outcome: Experiments will be conducted under BL1 containment.
Approved for cell expression experiment. Approval for transgenic mouse experiments is contingent upon IACUC approval.

Project: Identification of Synoptic DAG Effectors and Regulators
PI: CONFIDENTIAL
Date of submission: 12/22/05
Review and Points of Discussion: The investigator seeks to continue his neuronal signal transduction studies in the nematode *C elegans* by screening for mutations that rescue paralysis of a mutant with low diacylglycerol (DAG) levels. Mutations will then be studied to identify the proteins they interact with. Genes or cDNA sequences will be cloned into pUC-based vectors with 3' UTR sequences for expression in *C elegans*.
Concerns: C elegans is a nonpathogenic round worm. There are no health, environmental, or LS issues.
Outcome: The experiments are exempt per NIH Guidelines. All experiments will be conducted under BL1 containment.
Approved

OMRF Recombinant DNA Committee Minutes
June 30, 2005

The following projects were considered and the listed actions taken:

Project: Formation and Function of the Meiotic Bouquet
PI: CONFIDENTIAL
Date of submission: 5/23/005
Review and Points of Discussion: Various genes cloned from baker's yeast *Saccharomyces cerevisiae* will be engineered in E coli using standard E coli – yeast shuttle vectors and replaced into yeast. No pathogenic organisms or genes involved in pathogenesis will be used.
Concerns: No health, environmental, LS issues.
Outcome: Experiments will be conducted under BL1 containment. The experiments are exempt per NIH Guidelines.
Approved

Project: Tyrosine Sulfation and Chemokines Receptor Function
PI: CONFIDENTIAL
Date of submission: 5/27/05
Review and Points of Discussion: Chemokine receptors are7-transmembrane G-protein-coupled receptors involved in the regulation of trafficking of hematopoietic cells. Epitope-tagged versions of the murine chemokines receptors CCR1 through CCR10, CXCR2 through CXCR6, Scr1, CX3CR1, Darc, and C5L2/Gpr77 will be expressed to undertake biochemical analysis on recombinant receptors after transfection in the murine L1.2 pre-B cell line. Vectors to be used include pBluescript, pT3T7D-Pac, pCMV-SPORT6, SPORT1, and Uni-ZAP XR.
Concerns: No health, environmental, LS issues.
Outcome: Experiments will be conducted under BL1 containment.
Approved

OMRF Recombinant DNA Committee Minutes
March 31, 2005

The following projects were considered and the listed actions taken:

Project: Dynamics of Intracellular Pathogen:Host Interactions
PI: CONFIDENTIAL
Date of submission: 1/20/05
Review and Points of Discussion: L pneumophila expressing a GFP fluorescent protein to monitor infection will be used to infect Dictostelium, a non-pathogenic soil amoeba maintained in the lab. Dictostelium will contain cytoskeletal proteins fused to GFP.
Concerns: L pneumophila is a Risk Group 2 agent requiring BL2 containment. The organism is associated with human disease that is rarely serious and for which therapeutic interventions are readily available. Laboratory personnel are trained and use standard microbiological practices for safe handling. Physical facilities are in place for BL2 containment. Only small quantities of bacteria (1- 10 ml) will cultured. Conditions that may create aerosols will be avoided and work will be performed in a class II biological safety cabinet. Any materials that come into contact with either organism and the organisms themselves at the end of the experiment will be stored in biohazard bags and disinfected by autoclaving. No organism will be released into the environment. Biohazard signs are displayed on lab doors, access to the lab is restricted and doors are locked when the lab is not in use.
Outcome: The project meets criteria for BL2 containment
Approved

Project: Inhibitor Development for Activated Protein C
PI: CONFIDENTIAL
Date of submission: 1/25/05
Review and Points of Discussion: Recombinant and truncated forms of protein C will be expressed in the 293 cell line. The proteins will be purified and used in inhibitor design studies using an in vitro assay system.
Concerns: No health, environmental, LS issues.
Outcome: The project meets the criteria for BL1 containment.
Approved

Project: Telomere Function in Meiosis
PI: CONFIDENTIAL Date of submission: 1/25/05
Review and Points of Discussion: Genes encoding particular structural proteins and enzymes in the yeast Saccharomyces cerevisiae (baker's yeast) into E coli to study the effect of chromosomal segregation un yeast.
Concerns: No pathogenic organisms or genes involved in pathogenesis will be used.
Outcome: The project is exempt from NIH Guidelines. Experiments will be conducted under BL1 conditions.
Approved

Project: Novel Methods for Human T Cell Development
PI: CONFIDENTIAL
Date of submission: 2/28/05
Review and Points of Discussion: cDNA encoding the human telomerase genes and the human Notch ligands Delta-4 and Jagged-2 will be cloned into LZRS retroviral vector upstream of an IRES-driven GFP expression cassette. The vectors will be cloned into Phoenix-A (for human target cells) and Phoenix-E (mouse target cells) cells. Supernatants will be used to transducer human or murine stromal cell lines to produce transfected cell lines for use in cultures.Delta-4 and Jagged-2 induce Notch signaling telomerase, promote extension of telomere sequences, thus delaying the onset of cellular senescence.Murine OP9 stromal cell line will be transduced with the Notch ligand retroviruses. Thymic stromal cells from neonatal thymus will be transduced with the telomerase retrovirus to immortalize them and create a continuous cell line.
Concerns: The Phoenix cells contain MoLV packaging constructs (CMV-Env and RSV gag/pol. The system was developed in the Nolan lab at Stanford. The use of CMV and RSV minimize recombination events to generate viral sequences.
Outcome: The NIH Guidleines indicate retroviral vectors can be maintained at BL1 conditions. The PI will use BL2 level for all experiments. The PI was has agreed to confirm the cells lack helper virus.
Approved

Project: Beta Secretase Inhibition for Treating Alzheimer's Disease
PI: CONFIDENTIAL
Date of submission: 2/28/05
Review and Points of Discussion: Genes for Memapsin2 and APP (constituents of amyloid beta) will be cloned the expression vectors pET11, pcDNA 3.1, pSECtag2, and pcDNA6. Recombinant constructs will be used to transfect HeLA, HEK293, CHO, and M17 (a human neuroblastoma cell line) to express these proteins. Structure-function relationships will be derived using candidate inhibitors of these proteins.
Concerns: No health, environmental, LS issues.
The project requires IBC notification at time of initiation.
Outcome: Approved

Project: GEM Domains in T Cell Signaling
PI: CONFIDENTIAL
Date of Submission: 3/16/05
Review & Points of Discussion: In this project, various constructs will be produced for membrane expression of green fluorescent protein and separately a domain of a protein tyrosine phosphatase using a replication defective lentiviral system. This expression system (ViraPower Lentiviral Expression System) is sold by Invitrogen and describe in detail in http://www.invitrogen.com/content/sfs/brochures/ViraPowerAnnounc.pdf
Proteins from this recombinant DNA will be expressed in cell lines and primary T and B cells. BL2 containment is suggested for use by Invitrogen. All materials that come into contact with these viruses will be disinfected with 10% bleach including benchtops, centrifuges, and other work areas. Dr. Rodgers and his staff will test to assure that replication competent viruses are not produced.

Concerns: Risk of replication competent virus & human infection were considered minimal. This is yet another lentiviral expression system proposed for use here.
Outcome: The project meets criteria for BL2 containment and is consistent with practices required in the past by others at OMRF using lentiviral systems.
Approved

OMRF Recombinant DNA Committee Minutes
October 26, 2004

The following two projects were considered by the OMRF Recombinant DNA IBC and the listed actions taken:

Project: Molecular Analysis of Human IgA for Mucosal Immunity
PI: CONFIDENTIAL
Date: 6/23/04
Review: In this project, human IgA, J chain, polymeric Ig receptors, and CD89 will be cloned in baculovirus vectors and expressed in insect cell lines
Concerns: No health, environmental, LS issues.
The project requires only IBC notification at time of initiation.
The project meets criteria for BL1 containment.
Approved

Project: Chromosome Movement in Prometaphase
PI: CONFIDENTIAL
Date: 10/21/04
Review: In this project, conventional E. coli separately encoding cyclin-dependent kinase, Ndc80, Aurora B kinase, and Piol-like kinase will undergo in vitro mutagenesis, followed by transfection into human, pig, and frog cell lines. SiRNA will be used to repress protein production. Phenotypic changes will be determined.
Concerns: No health, environmental, LS issues.
The project requires only IBC notification at time of initiation.
The project meets criteria for BL1 containment.
Approved

RECOMBINANT DNA REGISTRATION FORM
Institutional Biosafety Committee (IBC)
Oklahoma Medical Research Foundation

Pease complete this form to satisfy federal regulations specified in the *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. (A copy of this document is available in the OMRF library.) Attach additional pages if needed. DO NOT ATTACH GRANT APPLICATIONS UNLESS REQUESTED.

CONFIDENTIAL

A. 1. PRINCIPAL
2. Department: _____
3. Project Title: B Cell Tolerance to Nuclear Antigen La

B. EXPERIMENTAL CATEGORY. Please check one (see *NIH Guidelines* Section III).
1. ___ Experiments requiring IBC approval, RAC review, and NIH approval before initiation.
2. ___ Experiments requiring NIH/ORDA and IBC approval before initiation.
3. X Experiments requiring IBC approval before initiation.
4. ___ Experiment requiring IBC notification simultaneously with initiation.
5. ___ Exempt experiments.

C. PROJECT INFORMATION
1. Briefly describe the project including the sequences to be cloned and their function. If these sequences are from a eukaryotic virus, indicate if >2/3 of the viral genome will be cloned, if the virus is replication defective, and describe any helper viruses that will be used.

I. Rearranged heavy (H) and light (L) chain immunoglobulin receptor genes from an anti-human La (hLa) specific B cell hybridoma will be cloned into the pGEM-11 and pSV2-LkCk vectors, respectively, containing Ig promoters, leader sequences and constant regions (see attached maps). For the reconstructed H-chain gene, the μ constant region from vector pSVGneoV$_{10}$CμM will be used. The reconstructed genes will be injected into the fertilized ova of FVB/N mice to produce (see atc

2. Do these sequences encode molecules toxic to vertebrates at an LD50 $\leq$ 100 μg/kg of body weight? No If so, specify LD50 _____ (see *NIH Guidelines* Appendix F).

3. List vectors to be used (attach maps, if available): 1) pGEM-11L$_{11}$, 2) pSV2-LkCk, 3) pSVGneoV$_{10}$CμM, 4) 3H9 H-chain targeting vector, and 5) Vk8RTV inpBlueScript (see attached list of references)

4. List recipient hosts. Include plants and animals and if they will become transgenic. If humans are the intended recipients, complete this form and attach an addendum addressing issues in Appendix M of the *NIH Guidelines*). Ig transgenic mice will be on the FVB/N backgrou then backcrossed to the A/T inbred background for study. Ig knock-in mice will be produced on the 129SV/EVXC57BL/6 background, then crossed to the A/J backgrou for further study.

5. List any product to be expressed and identify its function (if known). Immunoglobulin receptor with specificity for the La nuclear antigen.

Function: B cell recognition and signaling.

6. Will greater than 10 liters of culture containing this recombinant DNA be grown at any one time? No

7. If applicable, review health and environmental hazards that may result from this work in an attached addendum. A few suggestions to help you formulate your answer are offered below. These are not meant to cover all circumstances.
   - If DNA sources are from class 2 or 3 infectious microorganisms, exotic animal or plant pathogens, or USDA-regulated articles, discuss safety aspects of the facility, containment practices, personnel practices, and staff training that will assist in the safe conduct of the study. Summarize pathogenic aspects of organisms (modes of transmission, etc). Include measures to prevent or minimize expression of pathogenic or infectious sequences.
   - If oncogenes of other regulatory sequences are transferred, will pathogenic virulence, toxicity, antibiotic resistance, or immune competence be affected?
   - Will the experiments require the release into the environment of organisms containing recombinant DNA molecules?

*** CHECK HERE IF ITEM 7 IS NOT APPLICABLE X

D. CONTAINMENT LEVELS

Based on the above information and the *NIH Guidelines* (Section III and Appendices G, I, K, P & Q), circle the appropriate containment levels for these experiments.

Physical Containment	Animal Biosafety Level
BL1 BL2 BL3	BL1 BL2 BL3
GLSP BL1-LS BL2-LS BL3-LS	BL-1N BL-2N BL-3N

E. PERSONNEL

List all personnel who will conduct these experiments. Include visiting staff, students, and temporary help. For each person, check if they are currently trained or will be trained by the PI to perform their duties. Will

CONFIDENTIAL

F. I am familiar with and agree to abide by the *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* and other specific NIH instructions pertaining to the proposed project. I understand that it is my responsibility that all laboratory personnel working on this project are trained and remain fully informed with regard to biosafety practices, techniques, and applicable emergency procedures. I also agree to comply with federal requirements pertaining to shipment of recombinant DNA molecules. The above information is accurate and complete.

/ /

CONFIDENTIAL

C. 1. Continued

I. (continued) Ig H- and L-chain transgenics. The mice will be studied separately and intercrossed to produce mice that possess a large proportion of B cells expressing an anti-hLa specific B cell receptor.

II. Using rearranged Ig receptor genes from another anti-hLa specific B cell hybridoma, mouse Ig gene region targeting vectors will be constructed. Existing targeting vectors (3H9 H-chain and Vk8RTV; see attached reference) will be used, where the rearranged variable region genes will be replaced with those of an anti-hLa specific B cell hybridoma. These will be used to produce homologous recombinants in mouse embryonic stem cells. Recombinant stem cells bearing the anti-La Ig H- and L-chain locus specific rearrangements will separately be injected into C57BL/6 blastocysts to produce chimeric mice. The chimeras will be bred to identify animals transmitting the rearrangements in the germline. The resulting H- and L-chain knock-in mice will be studied separately and intercrossed to produce mice that possess a large proportion of B cells expressing an anti-hLa specific B cell receptor.

Approaches I. and II. are not duplications but, rather, are methods selected based on the particular nature of the B cell receptors employed.

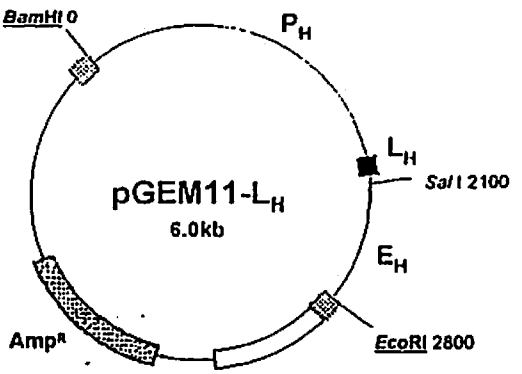
References

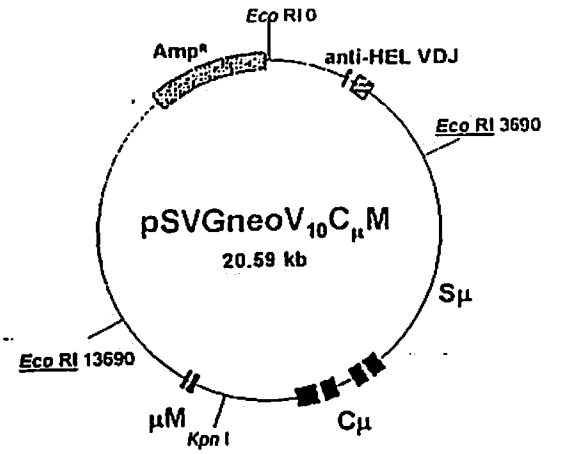
I. Gascoigne NR, Goodnow CC, et al. 1987. Secretion of a chimeric T-cell receptor-immunoglobulin protein. *Proc Natl Acad Sci U S A 4:2936-2940.* (Vectors 1 and 2)

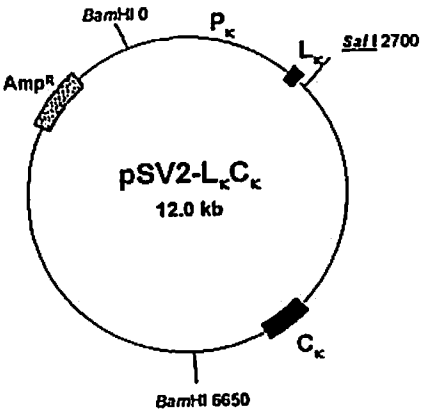
II. Goodnow CC, Crosbie J, et al. 1988. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. *Nature 334:676-682.* (Vector 3)

III. Chen C, Nagy Z, et al. 1995. Immunoglobulin heavy chain gene replacement: a mechanism of receptor editing. *Immunity 3(6): 747-55.* (Vector 4)

IV. Prak EL and Weigert M. 1995. Light chain replacement: a new model for antibody gene rearrangement. *J Exp Med 182(2):541-8.* (Vector 5)







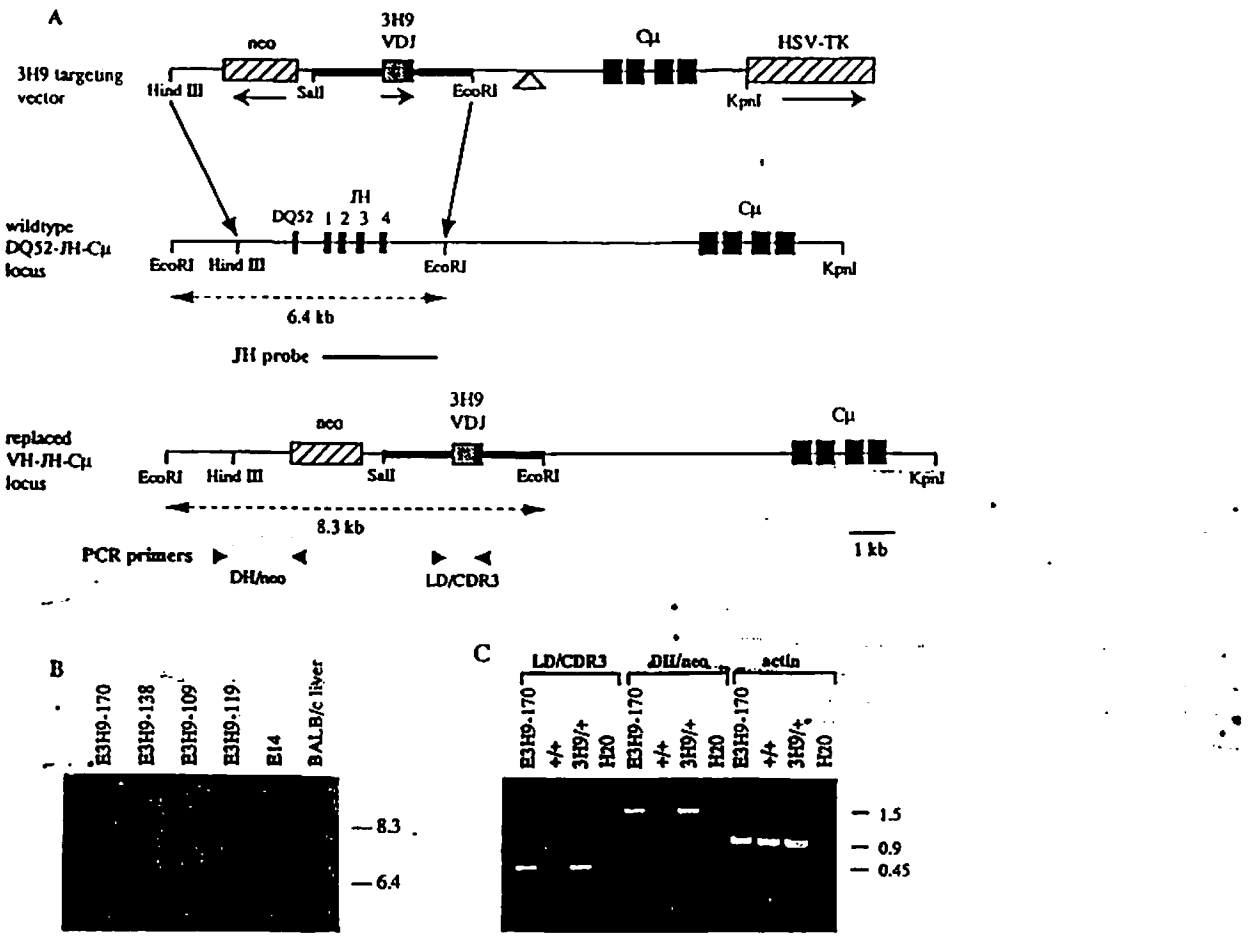


Figure 1. Site-Directed Replacement of the J_H Locus with 3H9 V_H Gene

(A) Structure of the 3H9 targeting construct, the germline IgH locus, and the targeted IgH–3H9 locus. The 3H9 sequence includes the core VDJ region (stippled box) and the surrounding regulatory sequences (thick line). The *neo*^{r} and HSV-*tk* genes are shown as hatched boxes and the Cμ, DQ52, and J_H segments are represented by closed boxes. Transcriptional orientation of *neo*^{r}, V_H3H9, and HSV-*tk* genes is indicated by arrows. The triangle indicates a 3 kb deletion in the Sμ region of the targeting construct. The germline and the replaced J_H loci are expected to give 6.4 kb and 8.3 kb EcoRI restriction fragments, respectively. The positions of the J_H probe and the PCR primers are shown.

(B) Southern blot analysis of the transfected ES clones that tested positive by D_H/*neo* PCR. The DNA was digested with EcoRI and hybridized with a J_H probe. The nontargeted alleles generate a 6.4 kb germline band as seen in the liver and the parental E14–1 cell DNA controls. The targeted alleles gave the expected 8.3 kb band. The 6.4 kb band is darker than the 8.3 kb band because the J_H probe hybridizes more strongly to the complete germline J_H sequence (present on the wild-type allele but truncated on the targeted allele).

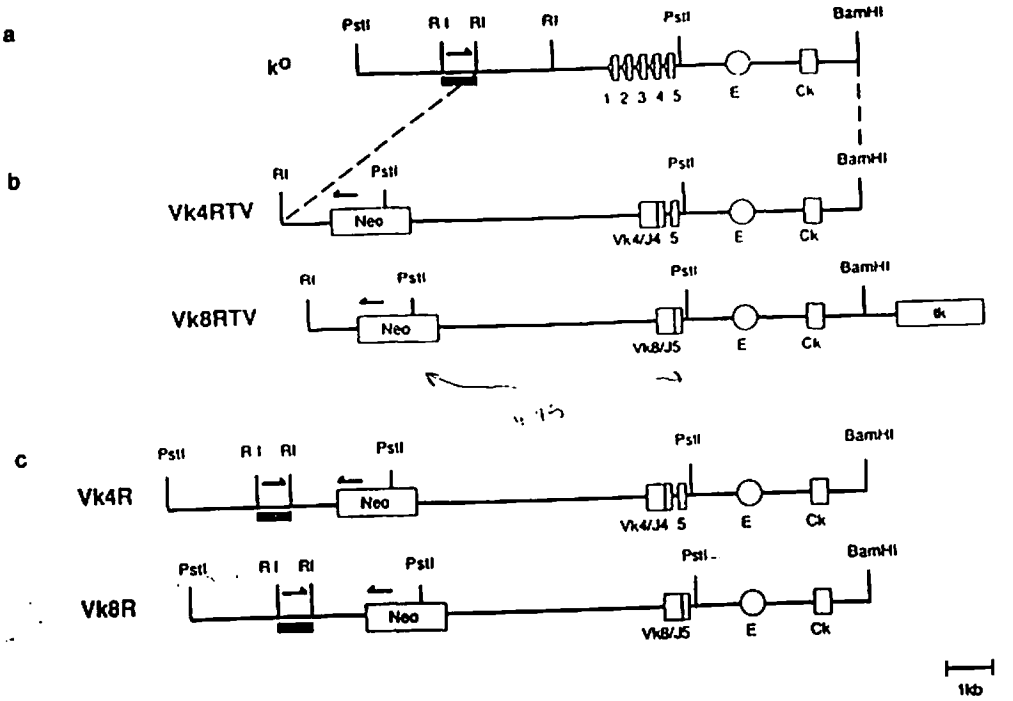
(C) PCR analysis of ES cell–derived offspring. The targeted ES cell clone, E3H9–170, which contributed to the germline chimeras, is used as a positive control and the water is used as a negative control. The tail DNA samples of a transmitted heterozygous mouse (3H9/+) and a wild-type littermate (+/+) were tested by three PCR assays. The LD/CDR3 PCR tests the presence of the 3H9 gene; the D_H/*neo* PCR confirms the correct insertion of the targeting construct; the actin PCR assures the quality and quantity of the DNA in each sample. The transmitted mouse is positive for all three PCRs, whereas the littermate only shows the actin band.

extent to which editing at the H chain locus contributes to B cell tolerance: it has upstream V_H genes, it has embedded heptamers, and it contributes unilaterally to the specificity for a self-antigen.

Results

Generation of Site-Directed Immunoglobulin H Chain Transgenic Mice

The 3H9 H chain targeting vector used to replace the J_H locus is shown in Figure 1A. The rearranged V_H region of the anti-DNA antibody, 3H9, was joined to the Cμ gene and then flanked by the positive and negative selection marker genes *neo* and *tk*. A 1.5 kb sequence upstream of DQ52 was added to provide the 5′ homology. The 3H9 targeting construct was introduced into the embryonic stem (ES) cells and drug-resistant colonies were screened for homologous recombination events by polymerase chain reaction (PCR) using the primers indicated in Figure 1A. Four PCR positive colonies were analyzed by Southern blot analysis and gave rise to the expected 8.3 kb band (Figure 1B). The targeted ES cells were injected into C57BL/6 blastocysts to generate chimeric mice. Progeny of chimeric mice were scored for germline transmission of the ES cell genotype by coat color. ES cell–derived offspring were tested for the presence of the sd-tg by PCR of tail DNA (Figure 1C).



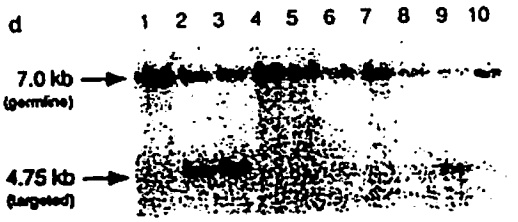


Figure 1. Targeted replacement of the Jκ region with functional Vκ-Jκ genes. Shown are the germline κ locus (*a*), replacement targeting vectors for Vκ4-Jκ4 and Vκ8-Jκ5 (*b*), and the resultant Vκ4 replaced (Vκ4R) or Vκ8 replaced (Vκ8R) κ loci (*c*). Dashed lines denote the borders of homology between the targeting vector and the germline locus. Arrowheads indicate the positions of PCR primers used to screen neomycin-resistant embryonic stem (ES) cell colonies for homologous recombination events. The upstream κ DNA probe, PKP6 (3), is denoted by a cross-hatched bar. PKP6 was used in Southern analysis (*d*) to confirm the genotype of targeted ES cells. Shown are genomic DNA samples from untransfected ES cells (lane *1*) and Vκ8R ES cells (lane *2*) and tail DNA samples from the offspring of germline chimeras (lanes *3–6*, offspring of a Vκ4R chimera; lanes *7–10*, offspring of a Vκ8R chimera). Pst1 digestion yields a 7.0-kb fragment in the wild-type germline κ locus (*a*) and a 4.75-kb fragment in the Vκ4R or Vκ8R loci.

type mice (Luning Prak, E., R. R. Hardy, and M. Weigert, manuscript in preparation).

Germline transmission was achieved in a C57Bl6 (ES cell)/ICR chimera for Vκ4R and in an E14.1 (ES cell)/C57Bl6 chimera for Vκ8R. Offspring in which the replaced κ locus was present were identified by Vκ4- or Vκ8-specific PCR assays of tail DNA (data not shown). Transmission of VκR was confirmed by Southern analysis (Fig. 1 *d*). Pst1 digestion of genomic DNA yields a 7.0-kb fragment in the wild-type germline κ locus and a 4.75-kb fragment in the Vκ4 or Vκ8 L chain replaced locus.

Analysis of L Chain Genotypes in VκR Hybridomas. To study the effect of Vκ4R and Vκ8R on the rearrangement of other L chain genes, LPS hybridomas were prepared from κ hemizygous Vκ4R/κ^0 and Vκ8R/κ^0 mice. The rearrangement status of κ and λ genes in individual IgM-secreting lines was tested using a series of PCR assays. First, each clone was tested for the presence of Vκ4R or Vκ8R DNA by PCR. Next, additional κ rearrangements on the targeted allele and on the wild-type κ allele (when they occurred) were identified using a series of PCR amplifications with forward Vκ primers and reverse Jκ primers (primer positions are shown in Fig. 2 *a*). The size of the amplification product in these assays is diagnostic of the Jκ segment used in the rearrangement (Fig. 2 *b–d*). For example, using Vs and Jκ5 primers (Fig. 2 *b*), rearrangement to Jκ1 gives a 1.6-kb product, whereas Jκ2 rearrangements are 1.2 kb, Jκ4 are 600 bp, and Jκ5 are 270 bp. Because Jκ1 rearrangements are not always discernible by Vκ + Jκ5 PCR, Vs and Jκ2 primers were used to verify Jκ1 rearrangements (Fig. 2 *c*). The L5 + Jκ5 PCR (Fig. 2 *d*) was used to type Jκ2 rearrangements on the untargeted κ allele. (The Vs + Jκ5 PCR cannot be used for this purpose because the Jκ2 rearrangement of the fusion partner is amplified in all of the hybridomas).

The pattern of Jκ segment usage revealed by these assays will in nearly all cases reveal the rearrangement status at each κ allele, yielding a κ genotype for each hybridoma (κ genotypes are shown in Fig. 2 *a* and all observed genotypes are

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CONFIDENTIAL

A. 1. PRINCIPAL
2. Department: _____
3. Project Title: Phosphatidylinositol-3 kinase in B cell activation & development

B. EXPERIMENTAL CATEGORY. Please check one (see *NIH Guidelines* Section III).
1. ___ Experiments requiring IBC approval, RAC review, and NIH approval before initiation.
2. ___ Experiments requiring NIH/ORDA and IBC approval before initiation.
3. ___ Experiments requiring IBC approval before initiation.
4. ✓ Experiment requiring IBC notification simultaneously with initiation.
5. ___ Exempt experiments.

C. PROJECT INFORMATION
1. Briefly describe the project including the sequences to be cloned and their function. If these sequences are from a eukaryotic virus, indicate if > 2/3 of the viral genome will be cloned, if the virus is replication defective, and describe any helper viruses that will be used.

~~The project involves the investigation of signal transduction in B cell development and antigen receptor internalization. The sequences to be cloned will be mutants of signal transduction enzymes. None of the sequences are of viral origin. Some protocol involves~~ virus infections using virues that do not infect human cells.
type?
mouse stem cell virus MSCV 2.2 / Clontech
Ecopak-293 packaging cells

2. Do these sequences encode molecules toxic to vertebrates at an LD50 ≤ 100 µg/kg of body weight? NO If so, specify LD50 _____ (see *NIH Guidelines* Appendix F).

3. List vectors to be used (attach maps, if available): pEF, pTracer

4. List recipient hosts. Include plants and animals and if they will become transgenic. If humans are the intended recipients, complete this form and attach an addendum addressing issues in Appendix M of the *NIH Guidelines*).

5. List any product to be expressed and identify its function (if known).

6. Will greater than 10 liters of culture containing this recombinant DNA be grown at any one time? ~~no~~

7. If applicable, review health and environmental hazards that may result from this work in an attached addendum. A few suggestions to help you formulate your answer are offered below. These are not meant to cover all circumstances.
   - If DNA sources are from class 2 or 3 infectious microorganisms, exotic animal or plant pathogens, or USDA-regulated articles, discuss safety aspects of the facility, containment practices, personnel practices, and staff training that will assist in the safe conduct of the study. Summarize pathogenic aspects of organisms (modes of transmission, etc). Include measures to prevent or minimize expression of pathogenic or infectious sequences.
   - If oncogenes of other regulatory sequences are transferred, will pathogenic virulence, toxicity, antibiotic resistance, or immune competence be affected?
   - Will the experiments require the release into the environment of organisms containing recombinant DNA molecules?

*** CHECK HERE IF ITEM 7 IS NOT APPLICABLE X

D. CONTAINMENT LEVELS

Based on the above information and the *NIH Guidelines* (Section III and Appendices G, I, K, P & Q), circle the appropriate containment levels for these experiments.

BL2 NOT BL-1 BL3

Physical Containment

~~BL1~~ BL2 BL3

GLSP BL1-LS BL2-LS BL3-LS

Animal Biosafety Level

~~BL1~~ BL2 BL3

BL-1N BL-2N BL-3N

E. PERSONNEL

List all personnel who will conduct these experiments. Including visiting staff, students, and temporary help. For each person, check if they are currently training or will be trained by the PI to perform their duties.

Will

CONFIDENTIAL

F. I am familiar with and agree to abide by the *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* and other specific NIH instructions pertaining to the proposed project. I understand that it is my responsibility that all laboratory personnel working on this project are trained and remain fully informed with regard to biosafety practices, techniques, and applicable emergency procedures. I also agree to comply with federal requirements pertaining to shipment of recombinant DNA molecules. The above information is accurate and complete.

Date 10 Feb. '04

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MSCV Retroviral Expression System

Product Quick Links:

Catalog Numbers Components References Licensing

- *Efficient gene expression in hematopoietic, ES and EC cells*
- *Choice of three selectable markers*
- *Complete retroviral system including PT67 packaging cell line*

The MSCV (Murine Stem Cell Virus) Retroviral Expression System contains vectors that are optimized for introducing target genes into pluripotent cell lines, including murine or human hematopoietic, embryonic stem (ES), and embryonal carcinoma (EC) cells. They can also be used effectively with any mammalian cell line (1–3). This highly efficient system is ideal for analyzing gene function in development, embryogenesis, or immune response, in both cell culture and transgenic assays.

Designed for difficult-to-infect cells

The MSCV System contains three vectors: pMSCVneo, pMSCVhyg, and pMSCVpuro. These vectors contain a specially designed long terminal repeat (LTR) from the murine stem cell PCMV virus that allows you to work with difficult-to-express cell lines. This LTR differs from the MoMuLV LTR used in other retroviral vectors by introducing several point mutations and a deletion that enhance transcriptional activation and prevent transcriptional suppression in ES and EC cells. As a result, the PCMV LTR drives high-level, constitutive expression of the target gene in stem cells or other mammalian cell lines. The MSCV System includes the BD RetroPack™ PT67 Packaging Cell Line, which produces high-titer virus able to infect a broad range of mammalian host cells.

TOP

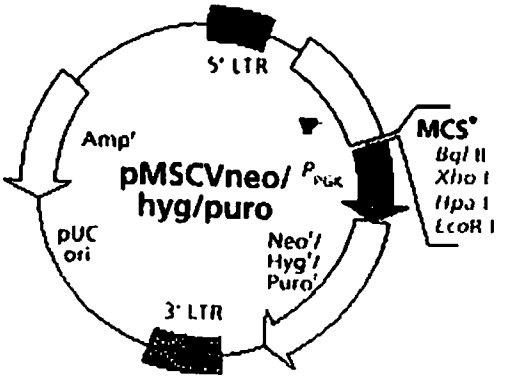

Figure 1. pMSCV Vectors map.

TOP

Product	Size	Cat.#	New Cat.#
MSCV Retroviral Expression System	each	K1062-1	634401

TOP

Components

pMSCVneo Vector
pMSCVhyg Vector
pMSCVpuro Vector
RetroPack PT67 Cell Line
MSCV Primers
Vector Information Packets
User Manual (PT3132-1)

TOP

References

1. Hawley, R. G., *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**:10297–10302.
2. Keller, G., *et al.* (1998) *Blood* **92**:877–887.
3. Hawley, R. G., et al. (1994) *Gene Ther.* **1**:136–138.

TOP

Related Products

Retroviral Systems

Licensing

Notice to Purchaser: MSCV Retroviral Expression System

Notice to Purchaser: Retroviral Products

RECOMBINANT DNA REGISTRATION FORM
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Oklahoma Medical Research Foundation

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CONFIDENTIAL

A. 1. PRINCIPAL I

2. Department: I

3. Project Title: The Regulation of Cytokinesis in Vertebrate Cells

B. EXPERIMENTAL CATEGORY. Please check one (see *NIH Guidelines* Section III).

1. __ Experiments requiring IBC approval, RAC review, and NIH approval before initiation.
2. __ Experiments requiring NIH/ORDA and IBC approval before initiation.
3. __ Experiments requiring IBC approval before initiation.
4. ✓ Experiment requiring IBC notification simultaneously with initiation.
5. ✓ Exempt experiments.

C. PROJECT INFORMATION

1. Briefly describe the project including the sequences to be cloned and their function. If these sequences are from a eukaryotic virus, indicate if > 2/3 of the viral genome will be cloned, if the virus is replication defective, and describe any helper viruses that will be used.
Conventional E. coli plasmids encoding Cyclin-dependent kinase-1, a cell cycle regulatory enzyme will be subject to in vitro mutagensis and then transfected into pig or frog tissue culture cells. Small inhibitory double stranded RNA (siRNA) molecules will be transfected into tissue culture cells (human, pig, frog) to repress the expression polo-like kinase. siRNA be chemically synthesized and purchased commercially.

2. Do these sequences encode molecules toxic to vertebrates at an LD50 ≤ 100 µg/kg of body weight? No If so, specify LD50 _____ (see *NIH Guidelines* Appendix F).

3. List vectors to be used (attach maps, if available): pcDNA3.1

4. List recipient hosts. Include plants and animals and if they will become transgenic. If humans are the intended recipients, complete this form and attach an addendum addressing issues in Appendix M of the *NIH Guidelines*). LLc-Pk cell line (porcine) Hela cell line (human) S3 cell line (frog)

5. List any product to be expressed and identify its function (if known). Cyclin-dependent kinase1 a cell cycle regulatory kinase that controls cell progression in mitosis.

6. Will greater than 10 liters of culture containing this recombinant DNA be grown at any one time? No

7. If applicable, review health and environmental hazards that may result from this work in an attached addendum. A few suggestions to help you formulate your answer are offered below. These are not meant to cover all circumstances.
   - If DNA sources are from class 2 or 3 infectious microorganisms, exotic animal or plant pathogens, or USDA-regulated articles, discuss safety aspects of the facility, containment practices, personnel practices, and staff training that will assist in the safe conduct of the study. Summarize pathogenic aspects of organisms (modes of transmission, etc). Include measures to prevent or minimize expression of pathogenic or infectious sequences.
   - If oncogenes of other regulatory sequences are transferred, will pathogenic virulence, toxicity, antibiotic resistance, or immune competence be affected?
   - Will the experiments require the release into the environment of organisms containing recombinant DNA molecules?

*** CHECK HERE IF ITEM 7 IS NOT APPLICABLE ✔

D. CONTAINMENT LEVELS

Based on the above information and the *NIH Guidelines* (Section III and Appendices G, I, K, P & Q), circle the appropriate containment levels for these experiments.

Physical Containment	Animal Biosafety Level
(BL1) BL2 BL3	BL1 BL2 BL3
GLSP BL1-LS BL2-LS BL3-LS	BL-1N BL-2N BL-3N

E. PERSONNEL

List all personnel who will conduct these experiments. Including visiting staff, students, and temporary help. For each person, check if they are currently training or will be trained by the PI to perform their duties.

Name	Will Train

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F. I am familiar with and agree to abide by the Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) and other specific NIH instructions pertaining to the proposed project. I understand that it is my responsibility that all laboratory personnel working on this project are trained and remain fully informed with regard to biosafety practices, techniques, and applicable emergency procedures. I also agree to comply with federal requirements pertaining to shipment of recombinant DNA moleeules. The above information is accurate and complete.

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CONFIDENTIAL

A. 1. PRINCIPAL I
2. Department: _____ _____ Lab: _____
3. Project Title: Telomere Function in Meiosis

B. EXPERIMENTAL CATEGORY. Please check one (see *NIH Guidelines* Section III).
1. ___ Experiments requiring IBC approval, RAC review, and NIH approval before initiation.
2. ___ Experiments requiring NIH/ORDA and IBC approval before initiation.
3. ___ Experiments requiring IBC approval before initiation.
4. ___ Experiment requiring IBC notification simultaneously with initiation.
5. ✓ Exempt experiments.

C. PROJECT INFORMATION
1. Briefly describe the project including the sequences to be cloned and their function. If these sequences are from a eukaryotic virus, indicate if > 2/3 of the viral genome will be cloned, if the virus is replication defective, and describe any helper viruses that will be used.

The project involves cloning yeast genes from Saccharomyces cerevisiae in to E. coli in order to manipulate them and study effect on chromosome segregation in yeast.

No pathogenic organisms or genes involved in pathogenesis will be used.

2. Do these sequences encode molecules toxic to vertebrates at an LD50 ≤ 100 µg/kg of body weight? No If so, specify LD50 _____ (see *NIH Guidelines* Appendix F).

3. List vectors to be used (attach maps, if available): Standard yeast-E. coli shuttle vectors, e.g. pRS306, pRS316

4. List recipient hosts. Include plants and animals and if they will become transgenic. If humans are the intended recipients, complete this form and attach an addendum addressing issues in Appendix M of the *NIH Guidelines*). Nonpathogenic strains of S. cerevisiae and standard nonpathogenic E. coli strains such as DH5alpha

5. List any product to be expressed and identify its function (if known). n/a

6. Will greater than 10 liters of culture containing this recombinant DNA be grown at any one time? No

7. If applicable, review health and environmental hazards that may result from this work in an attached addendum. A few suggestions to help you formulate your answer are offered below. These are not meant to cover all circumstances.
   - If DNA sources are from class 2 or 3 infectious microorganisms, exotic animal or plant pathogens, or USDA-regulated articles, discuss safety aspects of the facility, containment practices, personnel practices, and staff training that will assist in the safe conduct of the study. Summarize pathogenic aspects of organisms (modes of transmission, etc). Include measures to prevent or minimize expression of pathogenic or infectious sequences.
   - If oncogenes of other regulatory sequences are transferred, will pathogenic virulence, toxicity, antibiotic resistance, or immune competence be affected?
   - Will the experiments require the release into the environment of organisms containing recombinant DNA molecules?

*** CHECK HERE IF ITEM 7 IS NOT APPLICABLE ✔

D. CONTAINMENT LEVELS

Based on the above information and the *NIH Guidelines* (Section III and Appendices G, I, K, P & Q), circle the appropriate containment levels for these experiments.

Physical Containment	Animal Biosafety Level
(BL1) BL2 BL3	BL1 BL2 BL3 N/A
GLSP BL1-LS BL2-LS BL3-LS	BL-1N BL-2N BL-3N

E. PERSONNEL

List all personnel who will conduct these experiments. Including visiting staff, students, and temporary help. For each person, check if they are currently training or will be trained by the PI to perform their duties.

Name Will

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[redacted] *Molecules (NIH Guidelines)* and other specific NIH instructions pertaining to the proposed project. I understand that it is my responsibility that all laboratory personnel working on this project are trained and remain fully informed with regard to biosafety practices, techniques, and applicable emergency procedures. I also agree to comply with federal requirements pertaining to shipment of [redacted]. The above information is accurate and complete.

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CONFIDENTIAL

A. 1. PRINCIPAL I
2. Department: _____
3. Project Title: Role of a myosin V homologue in C. elegans neurons.

B. EXPERIMENTAL CATEGORY. Please check one (see *NIH Guidelines* Section III).
1. ___ Experiments requiring IBC approval, RAC review, and NIH approval before initiation.
2. ___ Experiments requiring NIH/ORDA and IBC approval before initiation.
3. ___ Experiments requiring IBC approval before initiation.
4. ___ Experiment requiring IBC notification simultaneously with initiation.
5. ✔ Exempt experiments.

C. PROJECT INFORMATION
1. Briefly describe the project including the sequences to be cloned and their function. If these sequences are from a eukaryotic virus, indicate if > 2/3 of the viral genome will be cloned, if the virus is replication defective, and describe any helper viruses that will be used.
We will characterize the function of HUM-2, a homologue to the vertebrate unconventional myosin V. Various portions of hum-2, as well as full-length transcripts, will be cloned into E. coli, yeast, and C. elegans vectors for the purpose of making transgenic C. elegans strains and detecting potential protein interactors with regions of HUM-2.

No pathogenic organisms or genes involved in pathogenesis will be used

2. Do these sequences encode molecules toxic to vertebrates at an LD50 ≤ 100 µg/kg of body weight? no If so, specify LD50 _____ (see *NIH Guidelines* Appendix F).

3. List vectors to be used (attach maps, if available): _____
pACT, pGBKT7, pGADT7, pCR2.1-TOPO, RM#641p, RM#647p

4. List recipient hosts. Include plants and animals and if they will become transgenic. If humans are the intended recipients, complete this form and attach an addendum addressing issues in Appendix M of the *NIH Guidelines*). E. coli (XL1BLUE), S. cerevisiae (yeast), C. elegans
All non-pathogenic

5. List any product to be expressed and identify its function (if known). _____
Various portions of the HUM-2 protein fused to GFP will be expressed in nematodes. Possible functions of HUM-2 include actin-based motility of synaptic vesicles or other endosomally-derived vesicles.

6. Will greater than 10 liters of culture containing this recombinant DNA be grown at any one time? no

7. If applicable, review health and environmental hazards that may result from this work in an attached addendum. A few suggestions to help you formulate your answer are offered below. These are not meant to cover all circumstances.
   - If DNA sources are from class 2 or 3 infectious microorganisms, exotic animal or plant pathogens, or USDA-regulated articles, discuss safety aspects of the facility, containment practices, personnel practices, and staff training that will assist in the safe conduct of the study. Summarize pathogenic aspects of organisms (modes of transmission, etc). Include measures to prevent or minimize expression of pathogenic or infectious sequences.
   - If oncogenes of other regulatory sequences are transferred, will pathogenic virulence, toxicity, antibiotic resistance, or immune competence be affected?
   - Will the experiments require the release into the environment of organisms containing recombinant DNA molecules?

*** CHECK HERE IF ITEM 7 IS NOT APPLICABLE ✔

D. CONTAINMENT LEVELS

Based on the above information and the *NIH Guidelines* (Section III and Appendices G, I, K, P & Q), circle the appropriate containment levels for these experiments.

Physical Containment	Animal Biosafety Level
(BL1) BL2 BL3	BL1 BL2 BL3
GLSP BL1-LS BL2-LS BL3-LS	BL-1N BL-2N BL-3N

E. PERSONNEL

List all personnel who will conduct these experiments. Including visiting staff, students, and temporary help. For each person, check if they are currently training or will be trained by the PI to perform their duties.

Name	Trained	Will Train

CONFIDENTIAL

F. I am familiar with and agree to abide by the *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* and other specific NIH instructions pertaining to the proposed project. I understand that it is my responsibility that all laboratory personnel working on this project are trained and remain fully informed with regard to biosafety practices, techniques, and applicable emergency procedures. I also agree to comply with federal requirements pertaining to shipment of recombinant DNA molecules. The above information is accurate and complete.

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Oklahoma Medical Research Foundation

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CONFIDENTIAL

A. 1. PRINCIPAL I

2. Department: Free Radical Biology & Aging Phone: [illegible] Lab: _____

3. Project Title: ~~Neuroinflammatory activation of glial cells by toxic damage to the mitochondrial~~ electron transport chain : Relevance to amyotrophic lateral sclerosis (ALS)

B. EXPERIMENTAL CATEGORY. Please check one (see *NIH Guidelines* Section III).

1. ___ Experiments requiring IBC approval, RAC review, and NIH approval before initiation.
2. ___ Experiments requiring NIH/ORDA and IBC approval before initiation.
3. ✔ Experiments requiring IBC approval before initiation.
4. ___ Experiment requiring IBC notification simultaneously with initiation.
5. ___ Exempt experiments.

C. PROJECT INFORMATION

1. Briefly describe the project including the sequences to be cloned and their function. If these sequences are from a eukaryotic virus, indicate if > 2/3 of the viral genome will be cloned, if the virus is replication defective, and describe any helper viruses that will be used.

Cytochrome C will be cloned into an expression vector containing a fluorescent label-binding tag sequence

2. Do these sequences encode molecules toxic to vertebrates at an LD50 ≤ 100 µg/kg of body weight? No If so, specify LD50 _____ (see *NIH Guidelines* Appendix F).

3. List vectors to be used (attach maps, if available): _____
Examples: pcDNA 3.1; pcDNA 3.1 D (Invitrogen)

4. List recipient hosts. Include plants and animals and if they will become transgenic. If humans are the intended recipients, complete this form and attach an addendum addressing issues in Appendix M of the *NIH Guidelines*). Cultured primary mouse astrocytes

5. List any product to be expressed and identify its function (if known). cytochrome C (electron transport chain protein)

6. Will greater than 10 liters of culture containing this recombinant DNA be grown at any one time? no

7. If applicable, review health and environmental hazards that may result from this work in an attached addendum. A few suggestions to help you formulate your answer are offered below. These are not meant to cover all circumstances.
   - If DNA sources are from class 2 or 3 infectious microorganisms, exotic animal or plant pathogens, or USDA-regulated articles, discuss safety aspects of the facility, containment practices, personnel practices, and staff training that will assist in the safe conduct of the study. Summarize pathogenic aspects of organisms (modes of transmission, etc). Include measures to prevent or minimize expression of pathogenic or infectious sequences.
   - If oncogenes of other regulatory sequences are transferred, will pathogenic virulence, toxicity, antibiotic resistance, or immune competence be affected?
   - Will the experiments require the release into the environment of organisms containing recombinant DNA molecules?

*** CHECK HERE IF ITEM 7 IS NOT APPLICABLE ✔

D. CONTAINMENT LEVELS

Based on the above information and the *NIH Guidelines* (Section III and Appendices G, I, K, P & Q), circle the appropriate containment levels for these experiments.

Physical Containment	Animal Biosafety Level
(BL1) BL2 BL3	BL1 BL2 BL3
GLSP BL1-LS BL2-LS BL3-LS	BL-1N BL-2N BL-3N

E. PERSONNEL

List all personnel who will conduct these experiments. Including visiting staff, students, and temporary help. For each person, check if they are currently training or will be trained by the PI to perform their duties.

Name	Trained	Will Train

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F. I am familiar with and agree to abide by the *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* and other specific NIH instructions pertaining to the proposed project. I understand that it is my responsibility that all laboratory personnel working on this project are trained and remain fully informed with regard to biosafety practices, techniques, and applicable emergency procedures. I also agree to comply with federal requirements pertaining to shipment of recombinant DNA molecules. The above information is accurate and complete.

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OMRF
Oklahoma Medical Research Foundation

PRESIDENT'S OFFICE

May 8, 2006

Mr. Edward H. Hammond
The Sushine Project
78 Linda Ave, #5A
Oakland, CA 94611

Via Federal Express - Overnight delivery

Dear Mr. Hammond:

Enclosed, as you have requested, are copies of all minutes of meetings from the Oklahoma Medical Research Foundation Institutional Biosafety Committee from May 1, 2003, to the present. The spaces marked "confidential" denote the removal of the names of principal investigators and committee members, but the minutes are otherwise complete.

In response to your email today, OMRF and the University of Oklahoma Health Sciences Center are legally distinct entities, with separate and distinct facilities and laboratories. As an independent recipient of NIH funding, OMRF is required to review and approve all recombinant DNA work being done by our scientists, all such work being done by other scientists at our facilities, and all such work being done by other scientists on grants that are funded through OMRF, and we adhere to those requirements.

Sincerely,

Adam Cohen
Director, Legal & Public Affairs

ABC/hb

enclosures

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