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

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

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

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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.	EX 1. 2. 3. 4. 5.	PERIMENTAL CATEGORY. Please check one (see NIH Guidelines Section III).  Experiments requiring IBC approval, RAC review, and NIH approval before initiation.  Experiments requiring NIH/ORDA and IBC approval before initiation.  Experiments requiring IBC approval before initiation.  Experiment requiring IBC notification simultaneously with initiation.  Exempt experiments.
C.	PRO 1.	OJECT INFORMATION  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 $\leq$ 100 $\mu$ g/kg of body weight? no If so, specify LD50 (see NIH Guidelines Appendix F).
	3.	List vectors to be used (attach maps, if available):
	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. coll (XL1BLUE), S. cerevislae (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.

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<b>7.</b>	<ul> <li>If applicable, review health and environmental haz attached addendum. A few suggestions to help you for These are not meant to cover all circumstances.</li> <li>If DNA sources are from class 2 or 3 infection pathogens, or USDA-regulated articles, discuss practices, personnel practices, and staff training study. Summarize pathogenic aspects of organic measures to prevent or minimize expression of personnel procession of personnel procession.</li> <li>If oncogenes of other regulatory sequences a toxicity, antibiotic resistance, or immune competer.</li> <li>Will the experiments require the release into recombinant DNA molecules?</li> </ul>	ormulate your answer are offer us microorganisms, exotic ani- safety aspects of the facility, that will assist in the safe co- sms (modes of transmission, eathogenic or infectious sequence re transferred, will pathogenic ence be affected? the environment of organism	mal or plant containment onduct of the etc). Include ces. ic virulence,
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#### 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.

Α.	1.	PRINCIPAL INVESTIGATOR: Michael N. Conrad
	2.	Department: MCDB Phone: 405-271-7668 Lab:
	3.	Project Title: Telomere Function in Meiosis
B.	2. 3. 4.	PERIMENTAL CATEGORY. Please check one (see NIH Guidelines Section III).  Experiments requiring IBC approval, RAC review, and NIH approval before initiation.  Experiments requiring NIH/ORDA and IBC approval before initiation.  Experiments requiring IBC approval before initiation.  Experiment requiring IBC notification simultaneously with initiation.  Exempt experiments.
C.		OJECT INFORMATION  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 (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

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			Will greater than 10 liters of culture containing this recombinant I		, 0
		U.	time? No	ona de grown at	any one
			<ul> <li>If applicable, review health and environmental hazards that may reattached addendum. A few suggestions to help you formulate your and These are not meant to cover all circumstances.</li> <li>If DNA sources are from class 2 or 3 infectious microorganism pathogens, or USDA-regulated articles, discuss safety aspects of practices, personnel practices, and staff training that will assist study. Summarize pathogenic aspects of organisms (modes of the measures to prevent or minimize expression of pathogenic or infection.</li> <li>If oncogenes of other regulatory sequences are transferred, we toxicity, antibiotic resistance, or immune competence be affected.</li> <li>Will the experiments require the release into the environment recombinant DNA molecules?</li> </ul>	ns, exotic animal of the facility, con in the safe conduransmission, etc). ctious sequences. will pathogenic v?	or plant stainment set of the Include
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#### 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.

SOURCE: IBC Archive | The Sunshine Project - FOI Fund | www.sunshine-project.org

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

# 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 (MIH Guidelines). (A copy of this document is available in the OMRF library.) Attach additional pages if needed. DO NOT ATTACH GRANT APPLICATIONS UNLESS REQUESTED.

5. List any product to be expressed and identify its function (if known). Appendix M of the WIH Guidelines). \_ are the intended recipients, complete this form and attach an addressing issues in List recipient hosts. Include plants and animals and if they will become transgenic. If humans PEE. pTracer List vectors to be used (attach maps, if available): weight? NO If so, specify LD50 (see MIH Guidelines Appendix F). Do these sequences encode molecules toxic to vertebrates at an LD50 \times 100 \mathbb{12}/kg of body Virus infections using virues that do not infect human cells.

More standard most 2.2 2.2 / Chat.

Euget. 29 part - 510 cells. transduction enzymes. None of the sequences are of viral origin. Some protocol involves antigen receptor internalization. The sequences to be cloned will be mutants of signal— <del>-The project involves the investigation of signal transduction in B cell development and-</del> the virus is replication defective, and describe any helper viruses that will be used. sequences are from a eukaryotic virus, indicate if > 2/3 of the viral genome will be cloned, if Briefly describe the project including the sequences to be cloned and their function. If these C. PROJECT INFORMATION Exempt experiments. Experiment requiring IBC notification simultaneously with initiation. ٦, Experiments requiring IBC approval before initiation. Experiments requiring MIH/ORDA and IBC approval before initiation. Experiments requiring IBC approval, RAC review, and NIH approval before initiation. EXPERIMENTAL CATEGORY. Please check one (see NIH Guidelines Section III). Project Title: Phosphatidylinositol-3 kinase in B cell activation & development Lab: 271-7883 Department: Immunobiology & Cancer Phone: 271-7209 PRINCIPAL INVESTIGATOR: Mark Coggeshall library.) Attach additional pages if needed. DO NOT ATTACH GRANT APPLICATIONS UNLESS

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	IBC Archive   The Sunshine Project - FOI Fund   www	v.sunshine-	projet
	6. Will greater than 10 liters of culture containing this recombinant time?	DNA be grown	n at any one
	<ul> <li>7. If applicable, review health and environmental hazards that may reattached addendum. A few suggestions to help you formulate your a These are not meant to cover all circumstances.</li> <li>If DNA sources are from class 2 or 3 infectious microorganist pathogens, or USDA-regulated articles, discuss safety aspects practices, personnel practices, and staff training that will assist study. Summarize pathogenic aspects of organisms (modes of measures to prevent or minimize expression of pathogenic or infection.</li> <li>If oncogenes of other regulatory sequences are transferred, toxicity, antibiotic resistance, or immune competence be affected.</li> <li>Will the experiments require the release into the environment recombinant DNA molecules?</li> <li>**** CHECK HERE IF ITEM 7 IS NOT APPLICABLE</li> </ul>	mswer are offer of the facility, in the safe co transmission, e ectious sequence will pathogenial?	mal or plant containment onduct of the etc). Include ces. ic virulence,
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### MSCV Retroviral Expression

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## System

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 hematopoletic, 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

http://www.omrf.ouhsc.edu/OMRF/Research/14/CoggeshallM.asp

frank@omf.ouhsc.edu

Wednesday, February 11, 2004 10:22 AM

Mark Coggeshall [coggeshallm@omr.ouhsc.edu]

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Frank, PhD

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402-211-1209; FAX 405-271-8568
                                                                          Oklahoma City, OK
                                                                           825 NE 134h. St.
                                                                     Immunobiology & Cancer
                                                       Oklahoma Medical Research Foundation
                                                                         K. Mark Coggeshall
                                                    useful in human gene therapy protocols.
      production by homologous recombination with packaging DNA, these vectors might prove
       protein synthesis and all env sequences have been removed to eliminate helper virus
       shown to improve the encapsidation of viral RNA have been modified to prevent viral
   as a dominantly acting selectable marker. Because the sequences in the viral gag region
phosphotransferase (hph) gene or the puromycin M-acetyl transferase (pac) gene is included
      marker exists or either the neomycin phosphotransferase (neo) gene, the hygromycin B
   unique cloning sites permit insertion of genes into the vectors such that no selectable
      cells makes them ideally suited for preclinical studies with murine models. Multiple
transduction of functional genes into undifferentiated murine embryonic and haematopoietic
         A set of retroviral vectors is described whose capacity for high efficiency
                                          therapy. Hawley RG, Lieu FH, Fong AZ, Hawley TS.
    Gene Ther. 1994 Mar;1(2):136-8. Versatile retroviral vectors for potential use in gene
                                                                                       Wark
                  I hope this clears the situation upšit certainly was educational for me.
                            (http://www.bdbiosciences.com/clontech/retroviral/index.shtml)
                                                                     Clontech/BDBiosciences
       replication-incompetent, infects only mouse cell lines, and is sold commercially by
                                                  so the upshot is that the virus is
                                                     it has the gag, pol and env from MSCV.
 produced. We use the Ecopack-293 cell line from Clontech, which is based on MSCV - i.e.,
  packaging cell line determines the range of infectivity (tropism) of the viral particles
                                                    The envelope protein made by the
                         a paper by Hawley et al. The abstract from this paper is below).
Novagen) and cloning it into the EcoRI and SalI sites of the MSCV 2.2 vector (described in
                                   downstream of the pCITE1 IRES (a commercial vector from
      This vector was created (not by us) by placing GFP
                                                          The vector we use is pMIG.
      The viral particles produced cannot replicate in other cells since there are missing
                                                                         selectable marker.
       provides the essential elements of virus production and the target gene and the GFP
  The retroviral vector (what we transfect into the packaging cell lines)
                                                                           the retrovirus.
The packaging cell contains the gag, pol and env genes for
                                                            (MSCV) gag, pol and env genes.
                                                                                      Virus
   It seems many of these viral infection schemes are based on the murine stem cell
                                                                                      Bart:
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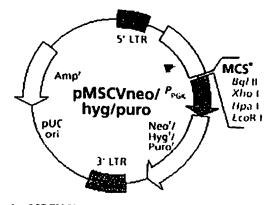


Figure 1. pMSCV Vectors map.

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Product

Size Cat.# New Cat.#

MSCV Retroviral Expression System each K1062-1 634401

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#### 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.

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#### **Related Products**

**Retroviral Systems** 

#### Licensing

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#### 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 are 7-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

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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.

Α.	1.	PRINCIPAL INVESTIGATOR: A. Darise Farris, Ph.D.
	2.	Department: Arthritis and Immunology Phone: 17389 Lab: 17519
		Project Title: B Cell Tolerance to Nuclear Antigen La
B.	1. 2. 3. 4.	<ul> <li>XPERIMENTAL CATEGORY. Please check one (see NIH Guidelines Section III).</li> <li>Experiments requiring IBC approval, RAC review, and NIH approval before initiation.</li> <li>Experiments requiring NIH/ORDA and IBC approval before initiation.</li> <li>Experiments requiring IBC approval before initiation.</li> <li>Experiment requiring IBC notification simultaneously with initiation.</li> <li>Exempt experiments.</li> </ul>
C	DD	OJECT INFORMATION
C.		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 $\mu$ constant region from vector pSVGneoV <sub>10</sub> C $\mu$ M will be used. The reconstructed
		genes will be injected into the fertilized ova of FVB/N mice to produce (see ato
	2.	Do these sequences encode molecules toxic to vertebrates at an LD50 $\leq$ 100 $\mu$ g/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 <sub>H</sub> , 2) pSV2-LkCk, 3) pSVGneoV <sub>10</sub> C <sub>M</sub> M, 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 background.
		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 background
		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.

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SOUR	CE: Wiff greath than Thomas I something of the combination of time? No.	inshinge or oi	ect org
	<ul> <li>7. If applicable, review health and environmental hazards that may resulated addendum. A few suggestions to help you formulate your an These are not meant to cover all circumstances.         <ul> <li>If DNA sources are from class 2 or 3 infectious microorganisms, pathogens, or USDA-regulated articles, discuss safety aspects of the practices, personnel practices, and staff training that will assist in study. Summarize pathogenic aspects of organisms (modes of trainmeasures to prevent or minimize expression of pathogenic or infect.</li> <li>If oncogenes of other regulatory sequences are transferred, will toxicity, antibiotic resistance, or immune competence be affected?</li> <li>Will the experiments require the release into the environment of recombinant DNA molecules?</li> </ul> </li> <li>**** CHECK HERE IF ITEM 7 IS NOT APPLICABLE X</li> </ul>	swer are offerd , exotic animal the facility, con the safe condustrission, etc). ious sequences I pathogenic v	ed below l or plan ntainmen uct of the Include irulence
D.	CONTAINMENT LEVELS  Based on the above information and the NIH Guidelines (Section III and A Q), circle the appropriate containment levels for these experiments.  Physical Containment BL1 BL2 BL3 BL1 BL2 BL3 BL-1N BL-2N BL-	evel	, K, P &
E.	PERSONNEL List all personnel who will conduct these experiments. Include visiting sta rary help. For each person, check if they are currently trained or will perform their duties.  Name  A. Darise Farris, Ph.D.  Michael P. Bachmann, Ph.D.  Britt Nakken, Ph.D.	ff, students, and be trained by  Trained  X X	nd tempo the PI to Will Train
F.	I am familiar with and agree to abide by the Guidelines for Research Invo Molecules (NIH Guidelines) and other specific NIH instructions pertaining I understand that it is my responsibility that all laboratory personnel wor trained and remain fully informed with regard to biosafety practices, tec emergency procedures. I also agree to comply with federal requirements precombinant DNA molecules. The above information is accurate and comp	to the propose rking on this p thniques, and a pertaining to sholete.	d project roject ar policable
	PI Signature / Januar / Januar .	Date <u>4/28</u>	101
	IBC Action: Comments: Like A S/27	las la Are	the second
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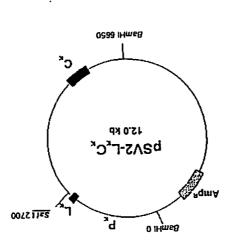
#### C. 1. Continued

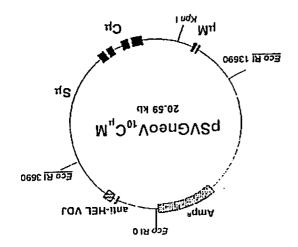
- I. (continued) Ig H- and L-chain transgenics. The mice will be studied separately and intercrossed to produce mice that possess a large proportion of B cells expressing an anti-hLa specific B cell receptor.
- II. Using rearranged Ig receptor genes from another anti-hLa specific B cell hybridoma, mouse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

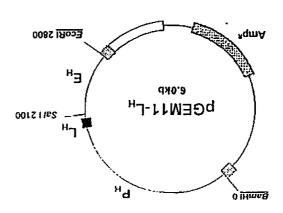
Approaches I. and II. are not duplications but, rather, are methods selected based on the particular nature of the B cell receptors employed.

#### References

- I. Gascoigne NR, Goodnow CC, et al. 1987. Secretion of a chimeric T-cell receptorimmunoglobulin protein. *Proc Natl Acad Sci USA* 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)







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#### SOURCE: IBC Archive | The Sunshine Project - FOI Fund | www.sunshine-project.org

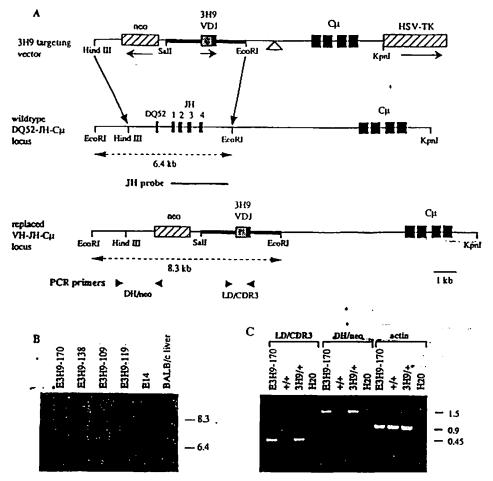


Figure 1. Site-Directed Replacement of the Jn Locus with 3H9 Vn Gene

(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 neor and HSV-tk genes are shown as hatched boxes and the Cµ, DC52, and J<sub>n</sub> segments are represented by closed boxes. Transcriptional orientation of neor, V<sub>n</sub>3H9, 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<sub>n</sub> loci are expected to give 6.4 kb and 8.3 kb EcoRI restriction fragments, respectively. The positions of the J<sub>n</sub> probe and the PCR primers are shown.

(B) Southern blot analysis of the transfected ES clones that tested positive by Dulneo PCR. The DNA was digested with EcoRI and hybridized with a Ju 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 Ju probe hybridizes more strongly to the complete germline Ju 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  $D_N$ -line 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  $J_{\text{H}}$  locus is shown in Figure 1A. The rearranged  $V_{\text{H}}$  region of the anti-DNA antibody, 3H9, was joined to the  $C\mu$  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 talf DNA (Figure 1C).

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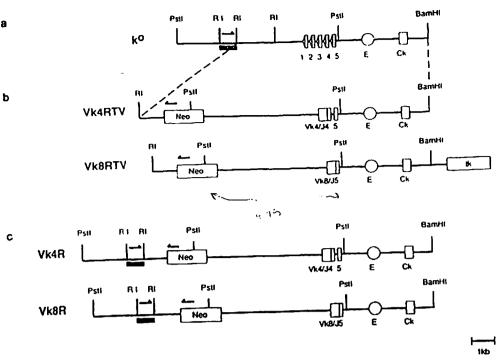




Figure 1. Targeted replacement of the JK region with functional VK-JK genes. Shown are the germline & locus (a), replacement targeting vectors for VK4-JK4 and VK8-JK5 (b), and the resultant VK4 replaced (VK4R) or VK8 replaced (VK8R) K loci (c). Dashed lines denote the borders of homology between the 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 & 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 V&R ES cells (lane 2) and tail DNA samples from the offspring of germline chimeras (lanes 3-6, offspring of a VKIR chimera; lanes 7-10, offspring of a VKBR chimera). Pst1 digestion yields a 7.0-kb fragment in the wild-type germline a locus (a) and a 4.75-kb fragment in the VK4R or VK8R 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 VK4R and in an E14.1 (ES cell)/C57B16 chimera for VK8R. Offspring in which the replaced K locus was present were identified by VK4- or VK8-specific PCR assays of tail DNA (data not shown). Transmission of VKR was confirmed by Southern analysis (Fig. 1 d). Pst1 digestion of genomic DNA yields a 7.0-kb fragment in the wild-type germline k locus and a 4.75-kb fragment in the Vk4 or Vk8 L chain replaced locus.

Analysis of L Chain Genotypes in VKR Hybridomas. To study the effect of VK4R and VK8R on the rearrangement of other L chain genes, LPS hybridomas were prepared from  $\kappa$  hemizygous VK4R/K0 and VK8R/K0 mice. The rearrangement status of  $\kappa$  and  $\lambda$  genes in individual IgM-secreting lines was tested using a series of PCR assays. First, each clone was tested for the presence of VK4R or VK8R DNA by PCR. Next, additional  $\kappa$  rearrangements on the targeted allele and on the wild-type  $\kappa$  allele (when they occurred) were identified using a series of PCR amplifications with forward V k primers and reverse Jk primers (primer positions are shown in Fig. 2 a). The size of the amplification product in these assays is diagnostic of the JK segment used in the rearrangement (Fig. 2 b-d). For example, using Vs and JK5 primers (Fig. 2 b), rearrangement to Jk1 gives a 1.6-kb product, whereas Jk2 rearrangements are 1.2 kb, Jk4 are 600 bp, and Jk5 are 270 bp Because Jr 1 rearrangements are not always discernible by Va + JK5 PCR, Vs and JK2 primers were used to verify JK1 rear rangements (Fig. 2 c). The L5 + Jx5 PCR (Fig. 2 d) wa used to type JK2 rearrangements on the untargeted K allele (The Vs + JK5 PCR cannot be used for this purpose becaus the JK2 rearrangement of the fusion partner is amplified in all of the hybridomas).

The pattern of Jk segment usage revealed by these assay will in nearly all cases reveal the rearrangement status at eacκ allele, yielding 2 κ genotype for each hybridom2 (κ genç types are shown in Fig. 2 a and all observed genotypes as

# OMRF Recombinant DNA Subcommittee Minutes March 23, 2006 Massman Bldg, L2, OMRF 820 NE 15<sup>th</sup> 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 colishuttle 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

SOURCE: IBC Archive | The Sunshine Project - FOI Fund | www.sunshine-project.org

# 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.

Α.	l.	PRINCIPAL INVESTIGATOR: Gary J. Gorb	sky			
	2.		Phone:	1-7660	Lab:	1-2037
	3.	Project Title: The Regulation of Cytokinesis in V	ertebrate	Cells		<del></del>
B.	1. 2. 3. 4.	Experiments requiring NIH/ORDA and II	C reviev BC approre initia	v, and NIH app oval before ini tion.	roval be tiation.	
C.	PRO 1.	ROJECT INFORMATION  Briefly describe the project including the sec sequences are from a eukaryotic virus, indicathe virus is replication defective, and describe Conventional E. coli plasmids encoding Cyclienzyme will be subject to in vitro mutagensis culture cells. Small inhibitory double strande into tissue culture cells (human, pig, frog) to be chemically synthesized and purchased company of the color o	ate if > any helin-deper and the d RNA (repress	2/3 of the viral per viruses that ndent kinase-1, n transfected in the expression	l genome will be a cell cy nto pig o ules will l	e will be cloned, if used. ycle regulatory r frog tissue be transfected
	2.	Do these sequences encode molecules toxic weight? No If so, specify LD50				
	3.	List vectors to be used (attach maps, if availab	ble): <u>pc</u>	DNA3.1		
	4.	List recipient hosts. Include plants and animare the intended recipients, complete this fo Appendix M of the NIH Guidelines). LLc-Pk (frog)	rm and	attach an adde	endum a	ddressing issues in
	5.	. List any product to be expressed and identify a cell cycle regulatory kinase that controls cell pro	its funct	ion (if known). i In mitosis.	Cyclin-c	dependent kinase1

	6.	Will greater than 10 liters of culture containing this recombinant D time? No	ONA be grow	n at any one
	***	<ul> <li>If applicable, review health and environmental hazards that may reattached addendum. A few suggestions to help you formulate your an These are not meant to cover all circumstances.</li> <li>If DNA sources are from class 2 or 3 infectious microorganism pathogens, or USDA-regulated articles, discuss safety aspects of practices, personnel practices, and staff training that will assist study. Summarize pathogenic aspects of organisms (modes of the measures to prevent or minimize expression of pathogenic or infection of the province of the conference of other regulatory sequences are transferred, we toxicity, antibiotic resistance, or immune competence be affected?</li> <li>Will the experiments require the release into the environment recombinant DNA molecules?</li> <li>CHECK HERE IF ITEM 7 IS NOT APPLICABLE</li> </ul>	ns, exotic ani f the facility, in the safe co ransmission, o ctious sequen vill pathogen	imal or plant containment onduct of the etc). Include ces. ic virulence,
D.	Bas	NTAINMENT LEVELS  sed on the above information and the NIH Guidelines (Section III and circle the appropriate containment levels for these experiments.  Physical Containment BL1 BL2 BL3 BL1 BL2 BL3 GLSP BL1-LS BL2-LS BL3-LS BL-1N BL-2N BL	evel	G, I, K, P &
E.	List temperf perf	all personnel who will conduct these experiments. Including viscorary help. For each person, check if they are currently training or viscorn their duties.  Name  Ty J. Gerbsky  an Daum  Ta Jones		
F.	Mod und train eme	familiar with and agree to abide by the Guidelines for Research Intecules (NIH Guidelines) and other specific NIH instructions pertaining erstand that it is my responsibility that all laboratory personnel water and remain fully informed with regard to biosafety practices, the regency procedures. I also agree to comply with federal requirements ombinant DNA molecules. The above information is accurate and complications.	g to the propos orking on thi techniques, as s pertaining to	sed project. I is project are and applicable
TD		tion: Comments:		
		ed Disapproved Pending		
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ना	C Ch	air or Member Date		

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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.
- 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.
- 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.

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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.
- 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 online 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:**

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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
- 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.

# OMRF Recombinant DNA Subcommittee Minutes March 23, 2006 Massman Bldg, L2, OMRF 820 NE 15<sup>th</sup> 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 colishuttle 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.

## 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 are 7-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.

## 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.

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.

## 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
Α.	2	PRINCIPAL Department: Project Title: B Cell Tolerance to Nuclear Antigen La
В.	1. 2. 3. 4.	PERIMENTAL CATEGORY. Please check one (see NIH Guidelines Section III).  Experiments requiring IBC approval, RAC review, and NIH approval before initiation.  Experiments requiring NIH/ORDA and IBC approval before initiation.  Experiments requiring IBC approval before initiation.  Experiment requiring IBC notification simultaneously with initiation.  Exempt experiments.
C.	PR	OJECT INFORMATION  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-ll and pSV2-LkCk vectors, respectively, containing Ig promoters, leader sequences and constant regions (see attached maps). For the reconstructed H-chain gene, the $\mu$ constant region from vector pSVGneoV C 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 $\mu$ g/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 <sub>H</sub> , 2) pSV2-LkCk, 3) pSVGneoV <sub>10</sub> C <sub>L</sub> M, 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 backgrouthen 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 backgrouf 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
BL1 BL2 BL3
GLSP BL1-LS BL2-LS BL3-LS

Animal Biosafety Level BLI BL2 BL3 BL-IN BL-2N BL-3N

1

#### 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

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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. Lalso agree to comply with federal requirements pertaining to shipment of

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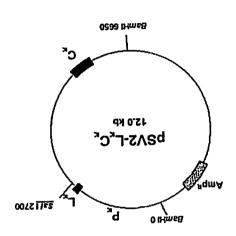
#### C. 1. Continued

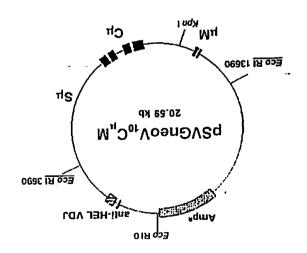
- I. (continued) Ig H- and L-chain transgenics. The mice will be studied separately and intercrossed to produce mice that possess a large proportion of B cells expressing an anti-hLa specific B cell receptor.
- II. Using rearranged Ig receptor genes from another anti-hLa specific B cell hybridoma, mouse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

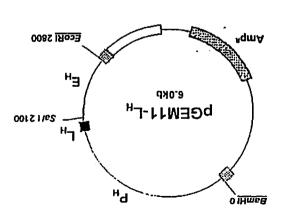
Approaches I. and II. are not duplications but, rather, are methods selected based on the particular nature of the B cell receptors employed.

#### 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)







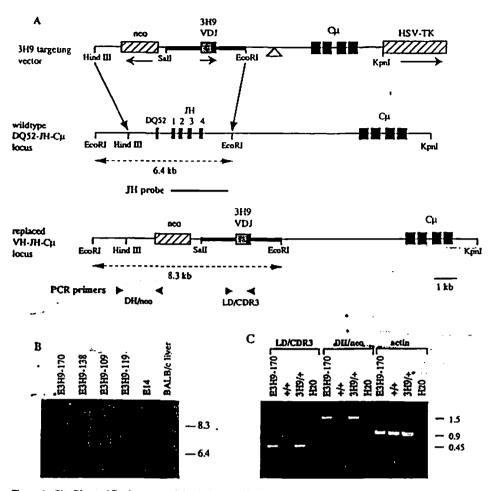


Figure 1. Site-Directed Replacement of the  $J_{\text{N}}$  Locus with 3H9  $V_{\text{N}}$  Gene

(A) Structure of the 3H9 targeting construct, the germline tgH locus, and the targeted tgH-3H9 locus. The 3H9 sequence includes the core VDJ region (stippled box) and the surrounding regulatory sequences (thick line). The neo' and HSV-tk genes are shown as hatched boxes and the Cµ, DC52, and J<sub>n</sub> segments are represented by closed boxes. Transcriptional orientation of neo', V<sub>n</sub>3H9, 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<sub>n</sub> loci are expected to give 6.4 kb and 8.3 kb EcoRI restriction fragments, respectively. The positions of the J<sub>n</sub> probe and the PCR primers are shown.

(B) Southern blot analysis of the transfected ES clones that tested positive by D<sub>M</sub>neo PCR. The DNA was digested with EccRI and hybridized with a J<sub>M</sub> 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<sub>M</sub> probe hybridizes more strongly to the complete germline J<sub>M</sub> 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<sub>e</sub>/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.

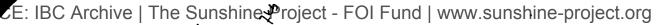
extent to which editing at the H chain locus contributes to B cell tolerance: it has upstream  $V_H$  genes, it has embedded heptamers, and it contributes unilaterally to the specificity for a self-antigen.

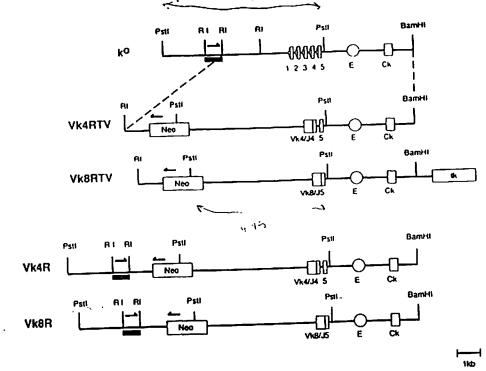
#### Results

#### 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  $C_H$  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 C578L/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 tall DNA (Figure 1C).





7 8 9 7.0 kb 4.75 kb

b

C

Figure 1. Targeted replacement of the J $\kappa$  region with functional  $V\kappa$ -J $\kappa$ genes. Shown are the germline x locus (a), replacement targeting vectors for VK4-JK4 and VK8-JK5 (b), and the resultant VK4 replaced (VK4R) or Va8 replaced (Va8R) a 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 & 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 VKBR ES cells (lane 2) and tail DNA samples from the offspring of germline chimeras (lanes 3-6, offspring of a VK4R chimera; lanes 7-10, offspring of a VeBR chimera). Pst1 digestion yields a 7.0-kb fragment in the wild-type germline a locus (a) and a 4.75-kb fragment in the Vx4R or Vx8R 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 VK4R and in an E14.1 (ES cell)/C57Bl6 chimera for Vk8R. Offspring in which the replaced k locus was present were identified by Vx4- or Vx8-specific PCR assays of tail DNA (data not shown). Transmission of VKR was confirmed by Southern analysis (Fig. 1 d). Pst1 digestion of genomic DNA yields a 7.0-kb fragment in the wild-type germline k locus and a 4.75-kb fragment in the Vk4 or Vk8 L chain replaced locus.

Analysis of L Chain Genotypes in VKR Hybridomas. To study the effect of VK4R and VK8R on the rearrangement of other L chain genes, LPS hybridomas were prepared from k hemizygous VK4R/K0 and VK8R/K0 mice. The rearrangement status of  $\kappa$  and  $\lambda$  genes in individual IgM-secreting lines was tested using a series of PCR assays. First, each clone was tested for the presence of VK4R or VK8R DNA by PCR. Next, additional  $\kappa$  rearrangements on the targeted allele and on the wild-type  $\kappa$  allele (when they occurred) were identified using a series of PCR amplifications with forward V k primers and reverse Jk primers (primer positions are shown in Fig. 2 a). The size of the amplification product in these assays is diagnostic of the JK segment used in the rearrangement (Fig. 2 b-d). For example, using Vs and JK5 primers (Fig. 2 b), rearrangement to JK1 gives a 1.6-kb product, whereas JK2 rearrangements are 1.2 kb, Jr4 are 600 bp, and Jr5 are 270 bp. Because JK1 rearrangements are not always discernible by VK + JK5 PCR, Vs and JK2 primers were used to verify JK1 rearrangements (Fig. 2 c). The L5 + JK5 PCR (Fig. 2 d) was used to type JKZ rearrangements on the untargeted K allele. (The Vs + Jr5 PCR cannot be used for this purpose because the JK2 rearrangement of the fusion partner is amplified in all of the hybridomas).

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

are the intended recipients, complete this form and attach an addendum addressing issues in List recipient hosts. Include plants and animals and if they will become transgenic. If humans

## Institutional Biosafety Committee (IBC) RECOMBINANT DNA REGISTRATION FORM 474 YERS 460 454

DEF. pTracer List vectors to be used (attach maps, if available): weight? NO If so, specify LD50 (see NIH Guidelines Appendix F). Do these sequences encode molecules toxic to vertebrates at an LD50 \times 100 \mathbb{\mathbb{Igkg}} of body Virus infections using virues that do not infect human cells.

More standard most 2.2 / Mat.

Euspal. 123 pooles in cells. transduction enzymes. None of the sequences are of viral origin. Some protocol involves antigen receptor internalization. The sequences to be cloned will be mutants of signal The project involves the investigation of signal transduction in B cell development and the virus is replication defective, and describe any helper viruses that will be used. sequences are from a cukaryotic virus, indicate if > 2/3 of the viral genome will be cloned, if Briefly describe the project including the sequences to be cloned and their function. If these C. PROJECT INFORMATION Exempt experiments. Experiment requiring IBC notification simultaneously with initiation. Experiments requiring IBC approval before initiation. Experiments requiring MH/ORDA and IBC approval before initiation. Experiments requiring IBC approval, RAC review, and MIH approval before initiation. B. EXPERIMENTAL CATEGORY. Please check one (see NIH Guidelines Section III). Project Title: Phosphatidylinositol-3 kinase in B cell activation & development Department: CONFIDENTIAL A. I. PRINCIPAL library.) Attach additional pages if needed. DO NOT ATTACH GRANT APPLICATIONS UNLESS ing Recombinant DNA Molecules (NIH Guidelines). (A copy of this document is available in the OMRF Please complete this form to satisfy federal regulations specified in the Guidelines for Research Involv-Oklahoma Medical Research Foundation

SOURCE: IBC Archive | The Sunshine Project - FOI Fund | www.sunshine-project.org

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

# CONFIDENTIAL

10 Feb. '04

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

# CONFIDENTIAL

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

**PERSONNEL** 

BLIN BL2N BL3N BEI BITS BITS Animal Biosafety Level

CICSP BLI-LS BL2-LS BL3-LS

BER BIS BI3 Physical Containment

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

D. CONTAINMENT LEVELS

\*\*\* CHECK HERE IF ITEM 7 IS NOT APPLICABLE  $\Delta$ 

recombinant DNA molecules? Will the experiments require the release into the environment of organisms containing toxicity, antibiotic resistance, or immune competence be affected?

If oncogenes of other regulatory sequences are transferred, will pathogenic virulence, measures to prevent or minimize expression of pathogenic or infectious sequences.

study. Summarize pathogenic aspects of organisms (modes of transmission, etc). Include practices, personnel practices, and staff training that will assist in the safe conduct of the pathogens, or USDA-regulated articles, discuss safety aspects of the facility, containment If DNA sources are from class 2 or 3 infectious microorganisms, exotic animal or plant

These are not meant to cover all circumstances.

attached addendum. A few suggestions to help you formulate your answer are offered below. If applicable, review health and environmental hazards that may result from this work in an

Will greater than 10 liters of culture containing this recombinant DNA be grown at any on

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## MSCV Retroviral Expression System

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

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Licensing

Retroviral Systems

Related Products

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3. Hawley, R. G., et al. (1994) Gene Ther. 1:136-138. 2. Keller, G., et al. (1998) Blood 92:877-887. .20501-76201:**56** ASU 1. Hawley, R. G., et al. (1996) Proc. Natl. Acad. Sci.

References

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

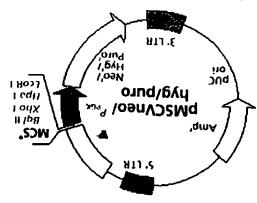
#### Components

dD1

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

401

### Figure 1. pMSCV Vectors map.



## 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. 2.	PRINCIPAL I Department: I
	3.	Project Title: The Regulation of Cytokinesis in Vertebrate Cells
В,	٠.	
C.	PR	OJECT INFORMATION  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.

	oires oldesilage H	L
10 liters of culture containing this recombinant DNA be grown at any one	time? No	

These are not meant to cover all circumstances.

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 & Physical Containment levels for these experiments.

Animal Biosafety Level

Animal Biosafety Level BL-1N BL-2N BL-3N BL-1N BL-2N BL-3N

L - ...: -- T

GESP BLI-LS BL2-LS BL3-LS
(BL) BL2 BL3

GESP BE

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.

AmeN

CONFIDENTIAL

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

## 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.

Α.		PRINCIPAL   Department: Project Title: Telomere Function in Meiosis
	2. · 3. · 4.	PERIMENTAL CATEGORY. Please check one (see NIH Guidelines Section III).  Experiments requiring IBC approval, RAC review, and NIH approval before initiation.  Experiments requiring NIH/ORDA and IBC approval before initiation.  Experiments requiring IBC approval before initiation.  Experiment requiring IBC notification simultaneously with initiation.  Exempt experiments.
C.	PR 1.	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 $\leq$ 100 $\mu$ g/kg of body weight? No (see NIH Guidelines Appendix F).
	3.	List vectors to be used (attach maps, if available): Standard veast-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. <u>If applicable</u>, 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

BLP 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.

Name

CONFIDENTIAL

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

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A.	1.	PRINCIPAL: CUNFIDENTIAL
	2. 3.	Department: Project Title: Role of a myosin V homologue in C. elegans neurons.
В.	1. 2. 3. 4.	PERIMENTAL CATEGORY. Please check one (see NIH Guidelines Section III).  Experiments requiring IBC approval, RAC review, and NIH approval before initiation.  Experiments requiring NIH/ORDA and IBC approval before initiation.  Experiments requiring IBC approval before initiation.
C.	PR 1.	OJECT INFORMATION  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, it 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):
	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. cerevislae (yeast), C. elegans All non-pathogenic
	5.	List any product to be expressed and identify its function (if known)
		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. <u>If applicable</u>, 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
BLD 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.

Townsed Toni

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

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		OOM IDEMINE
A.		PRINCIPAL I
В.	2.	Department: Free Radical Biology & Agiling Friends.
	EX 1. 2. 3.	Project Title: Neuroinflammatory activation of glial cells by toxic damage to the mitochondrial electron transport chain: Relevance to amyotrophic lateral sclerosis (ALS)  KPERIMENTAL CATEGORY. Please check one (see NIH Guidelines Section III).  Experiments requiring IBC approval, RAC review, and NIH approval before initiation.  Experiments requiring NIH/ORDA and IBC approval before initiation.
	4.	Experiment requiring IBC notification simultaneously with initiation.
	5.	Exempt experiments.
C.	PR 1.	OJECT INFORMATION  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 (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. <u>If applicable</u>, 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
(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.

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F. I am familiar with and agree to abide by the Guiaetines 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.



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