# PROTOCOL FOR ANIMAL USE AND CARE

**Handwritten forms are not accepted**

## Investigator Information

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<tr>
<th>Last Name:</th>
<th>First:</th>
<th>Middle:</th>
<th>Email:</th>
<th>Department:</th>
<th>Phone / Fax:</th>
<th>After hrs. #:</th>
</tr>
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</table>

## Contact Information

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<th>Middle:</th>
<th>Email:</th>
<th>Department:</th>
<th>Phone:</th>
<th>After hrs. #:</th>
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## Species Information

<table>
<thead>
<tr>
<th>Species (common names):</th>
<th>Number:</th>
<th>Source:</th>
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</thead>
<tbody>
<tr>
<td>Rhesus Monkeys</td>
<td>8/yr</td>
<td>CRPRC colony</td>
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<tr>
<td></td>
<td>(16 total)</td>
<td></td>
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</tbody>
</table>

## Project Title

Pulmonary Gene Therapy

## Overnight Housing Location

CRPRC  
Day use only:

## Animals to be Maintained

- [ X ] Vivarium  
- [ ] Investigator  

*(If investigator maintained, attach husbandry SOP's.)*

## Procedures

Animals will be treated with gene therapy agents through the inhalation of aerosols into the lung or placement directly into the airway through a bronchoscope. Transfer of genes and the effects of transfer will be evaluated by bronchoscopy, blood tests and/or at necropsy.

## Special Husbandry Requirements

No special requirements except that for their safety animals will need to be fasted prior to procedures.

## Other Instructions for Animal Care Staff

- [ X ] Call Investigator
- [ ] Call Investigator
- [ ] Clinician to treat
- [ ] Save for Investigator
- [ ] Terminate
- [ ] Bag for disposal
- [ X ] Necropsy
- [ X ] OK to use pesticides
- [ ] No Pesticides in animal area
- [ X ] Necropsy

## Hazardous Materials

### Infectious Agents

- [ X ] Yes  
- [ No ]

Agent(s): Recombinant DNA vectors + EGFP reporter inc. lenti, ad & AAV vectors

### Radioisotopes

- [ X ] Yes  
- [ No ]

Agent(s): 

### Chemical Carcinogens

- [ X ] Yes  
- [ No ]

Agent(s): 

### Toxic Chemicals

- [ X ] Yes  
- [ No ]

Agent(s):
Summary of Procedures:

a) Briefly describe the overall intent of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

The hypothesis is that the effectiveness of genes used as treatment agents for lung diseases will be determined by the specific vector given and specific cell types present in the airway. We will evaluate the distribution of gene therapy agents, including uniformity of distribution and also the safety of various vectors given by inhalation or direct instillation. Lastly, we will evaluate immunologic and histological responses to maximize information obtained from each treated animal.

b) Procedures employed in this project:

Please check the appropriate boxes if any of these procedures will be employed in your project:

- [ ] Monoclonal Antibody Production
- [ ] Polyclonal Antibody Production
- [ ] LD 50 or ID50 studies.
- [ ] catheters, blood collection, intubation
- [ ] Prolonged restraint. (8 hrs+)
- [ ] Fasting prior to a procedure.
- [ ] Food or water restriction
- [ ] Non-recovery surgical procedures
- [ ] Survival surgical procedures
- [ ] Multiple survival surgery
- [ ] Behavioral modification.
- [ ] Aversive conditioning.
- [ ] Special diets; food or water treatment.
- [ ] Induced illness, intoxication, or disease
- [ ] Death as an endpoint (see i below)
- [ ] Trapping, banding or marking wild animals

** If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)
Animals will be treated with gene therapy agents by 1) aerosol inhalation or by 2) direct bronchoscopic instillation. The consequences of these treatments will be evaluated by 3) bronchoscopy or at 4) necropsy.

Gene therapy agents will include Lenti-EGFP (third generation lentivirus vector carrying the enhanced green fluorescent protein reporter, EGFP) AAV-EGFP (the adeno-associated virus carrying EGFP) Ad EGFP (adenoviral vector) or non-viral liposomeDNA complexes, also carrying reporter genes such as EGFP or luciferase, or free plasmid DNA of reporter genes EGFP or luciferase. All vectors will be non-replicating. Expression of the reporter genes will be evaluated in a comprehensive fashion. Some of these reporters are expressed rapidly (3 days for Ad vectors, liposome DNA complexes and free DNA) and some may require weeks for maximal expression (AAV vectors) so animals will be euthanized 3 days to 8 weeks after treatment.

AGENT
Treatment By Bronchoscopic Instillation
Treatment by Inhalation
Bronchoscopy for Analysis
Necropsy (# of days after treatment)

Lenti-EGFP
4
No
Yes
28

AAV-EGFP
4
4
Yes
28

Ad-EGFP
2
No
Yes
5

Lipid EGFP Plasmid
2
No
Yes
14

1) Aerosol inhalation will be performed in deeply sedated animals via a standard veterinary face mask in spontaneously breathing animals or in animals intubated and breathing with positive pressure via a ventilator system attached to a liquid aerosol nebulizer. Treatment should be completed
in 60-90 min, with total time of the procedure approx. 2 hours. Depending on length of time and material being tested, some animals may need venous catheters placed to assist in monitoring and blood collection, as determined by the CRPRC veterinarian in attendance.

2) Bronchoscopy will be accomplished in sedated animals by the PI, a Pediatric Pulmonologist who routinely performs this procedure on infants and children. In sedated animals, the bronchoscope is passed into the lower airway, and test material (Vector, DNA or other gene therapy agent) is deposited in one or possibly two specific regions, the right lower lobe +/- the right middle lobe, maximum total volume 1.5 cc/kg.

3) Bronchoscopy will also be used in some animals to evaluate the response in the lung by both bronchoalveolar lavage (BAL) and bronchial biopsy (Bbx). For BAL, sterile saline (1.5 ml/kg) is instilled and recovered through the suction channel of the bronchoscope. For Bbx, very small biopsy forceps are passed into the airway under direct visualization and several (maximum of 5 total) small (1 mm) specimens of the airway mucosa are taken. Bbx will be taken from large lobar bronchi from both the left and right lung. Bronchoscopy will be performed at most twice on any animal (see above), and bronchial biopsy once.

4) Comprehensive analysis of the distribution of materials throughout the lung will be completed following necropsy: the distribution of reporter genes throughout the airway will be analyzed in detail. Lung tissues will be fixed, imaged, microdissected and analyzed by immunohistochemistry and PCR.

In brief, all studies will be relatively limited. Animals will be treated with gene therapy agents once and euthanized 5 to 28 days after treatment, depending on the vector. For those animals in which maximal expression of the reporter is expected to occur > 10 days after treatment, animals will undergo bronchoscopy for BAL (once) and/or Bbx (once) prior to necropsy.

d) Study Groups and Numbers: Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; you may add as many rows as you require. The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedures / Drugs</th>
<th>Number of Animals</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bronchoscopy with instillation of vector, bronchoscopy with lavage, blood draws</td>
<td>12 total</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Aerosol inhalation treatment with vector, bronchoscopy with lavage, blood draws</td>
<td>4 total</td>
<td>2</td>
</tr>
</tbody>
</table>
Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Little or no discomfort or stress</td>
</tr>
<tr>
<td></td>
<td>Examples: domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral.</td>
</tr>
<tr>
<td>2</td>
<td>Minor stress or pain of short duration</td>
</tr>
<tr>
<td></td>
<td>Examples: cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress</td>
</tr>
<tr>
<td>3</td>
<td>Moderate to severe distress</td>
</tr>
<tr>
<td></td>
<td>Examples: major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation</td>
</tr>
<tr>
<td>4</td>
<td>Severe pain near, at or above the pain tolerance threshold</td>
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<td></td>
<td>Examples: exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness.</td>
</tr>
</tbody>
</table>

Further descriptions of these categories are included in the instructions following this document.

e) Rationale for species and numbers: How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

1) Both vectors and routes of administration have been used in rodent studies; application to non-human primates is essential prior to further development for human use. The rhesus lung is developmentally and immunologically similar to the human, both in basic architecture (branching pattern, size) and cell types that populate the airway surface in the distal airway, the most important target region for gene therapy.

2) The lung from each animal will be comprehensively analyzed, hence small numbers will be used. Numbers of animals to be used was determined by comparison of expected results with previously completed studies with other vectors and routes of administration.

f) Surgery: If the project involves survival surgery, where will the surgery be conducted?

Building: no  Room: 

Who will be the surgeon?  

g) Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

<table>
<thead>
<tr>
<th>Species</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>When and how often will it be given?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus m.</td>
<td>Telazol</td>
<td>9 mg/k</td>
<td>IM</td>
<td>once each procedure</td>
</tr>
<tr>
<td></td>
<td>Ketamine</td>
<td>5-10mg/k</td>
<td>IM, IV</td>
<td>1-3 times/procedure</td>
</tr>
<tr>
<td></td>
<td>Lidocaine</td>
<td>3-7 mg/k</td>
<td>topical</td>
<td>once each procedure</td>
</tr>
<tr>
<td></td>
<td>Fentanyl</td>
<td>1-5ug/k</td>
<td>IV</td>
<td>hr or continuous drip</td>
</tr>
<tr>
<td></td>
<td>Propofol</td>
<td>6-12mg/kg</td>
<td>IV</td>
<td>hr or continuous drip</td>
</tr>
</tbody>
</table>

h) Neuromuscular blocking agents can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent? 

n/a
What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

i) Adverse effects:
Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

Fever, malaise and breathing discomfort could occur, but have not been seen in previous studies.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

Each animal demonstrating adverse effects will be evaluated by the veterinarian. Therapy may include administration of fluids or albuterol (to resolve breathing discomfort due to bronchospasm). If animals demonstrate more than minimal distress necropsy will be performed early.

Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.

Is death an endpoint in your experimental procedure? [ ] Yes [X] No
(Note: “Death as an endpoint” refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.

j) Literature search for alternatives and unnecessary duplication:
This section is specifically required by Federal law. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this study, or 2) there are alternative methodologies, but these are not appropriate for your particular study. “Alternative methodologies” refers to reduction, replacement, and refinement (the three R’s) of animal use, not just animal replacement. You must also show that the study is not unnecessarily duplicative of other studies.

What was the date on which you conducted this search? April 29, 2002

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

<table>
<thead>
<tr>
<th>Database Name</th>
<th>Years Covered</th>
<th>Keywords / Search Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrimateLit</td>
<td>1940–present</td>
<td>Lung, gene therapy or gene transfer</td>
</tr>
<tr>
<td>Swisstox.net</td>
<td></td>
<td>lung vector gene therapy</td>
</tr>
<tr>
<td>Scientific meetings</td>
<td>Yearly</td>
<td>Am. Soc Gene Therapy, Am Thoracic Society, Cystic Fibrosis Foundation</td>
</tr>
</tbody>
</table>
What were your findings with respect to alternative methodologies?

No alternatives.

Has this study been previously conducted?  [ ] Yes  [X] No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

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k) Disposition of animals: At what point in the study, if any, will the animals be euthanized?

3 days to 8 weeks after treatment with gene therapy vectors. Euthanasia is required for all animals treated with recombinant DNA for gene therapy studies for 1) Biological containment and 2) to evaluate the "spread" of the DNA into other tissues and organs.

l) Methods of euthanasia: Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>Overdose</td>
<td>Pentobarbital</td>
<td>60 mg/kg</td>
<td>IV</td>
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</tbody>
</table>

m) Surplus animals: What will you do with any animals not euthanized at the conclusion of the project?

Return to colony if not treated with gene therapy vectors.
n) Project Roster: Please provide the names of all the individuals who will work with animals on this project. This page will not be made available to the public. Give either the University Employee ID # or a valid UC Davis email address so that we can document training and occupational health compliance for regulatory agencies. Include all investigators, student employees, post-doctoral researchers, staff research associates, post-graduate researchers and laboratory assistants who will actually work with the animals. You don't need to include the staff of the vivarium in which your animals will be housed.

The principal investigator is responsible for keeping this roster current. If any staff is added or subtracted from this project, you must amend the protocol by sending the campus veterinarian a memo describing any changes.

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Middle Name</th>
<th>UC ID Number or SSN</th>
<th>Email Address</th>
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<tbody>
<tr>
<td>CRPRC staff</td>
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Occupational Health Program:
Supervisors must enroll their employees in the campus Occupational Health Program if the workers are at increased risk of illness or injury (such as allergy, physical injury, or infectious disease) because of their work. Enroll workers by having them complete an "Animal Contact History Form", available from Employee Health Services (phone 752-2330). For further information, visit our web site at [http://clueless.ucdavis.edu/health/](http://clueless.ucdavis.edu/health/) or read the UC Davis Policy & Procedure Manual 290-25.

Training:
Supervisors are responsible for insuring that their employees are adequate trained, both in the specifics of their job and in the requirements of the Federal Animal Welfare Act. EH&S offers free, basic wet labs in laboratory animal handling and techniques, and lecture format classes in the requirements of the Animal Welfare Act. To schedule a class for your unit, contact EH&S at 2-2364. Autotutorials are also available on the world wide web at [http://clueless.ucdavis.edu/](http://clueless.ucdavis.edu/).
Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator’s Statement:

I have read and agree to abide by the **UC Davis Policy and Procedure Manual section 290-30** (Animal Use and Care). This project will be conducted in accordance with the **ILAR Guide for the Care and Use of Laboratory Animals**, and the **UC Davis Animal Welfare Assurance** on file with the US Public Health Service. (These documents are available from the Campus Veterinarian and at [http://ehs.ucdavis.edu/](http://ehs.ucdavis.edu/)). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

I will advise the Animal Use and Care Administrative Advisory Committee in writing of any significant changes in the procedures or personnel involved in this project.

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**Principal Investigator**                  **Rank / Title**                  **Date**

---

**Committee Use Only Below**

**Conditions necessary for Committee Approval:**

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**Final Disposition of this protocol:**

[ ] Approved

[ ] Not Approved

[ ] Withdrawn by Investigator

**Date of Action:**  _____ / _____ / _____

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I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

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**Campus Veterinarian**                  **Date**
ANIMAL ROOM SAFETY INFORMATION

Complete this form if you will be using biohazards, radioisotopes, carcinogens, or toxic chemicals in the animal room.

PROTOCOL #________
EXPIRES: ________

RUA#: 
BUA#: 0563/0515
CCA#: 0547

Identity of Hazard: Viral vectors for gene therapy with EGFP reporter. Lentiviral, AAV and Adenoviral vectors.

Investigator Last Name: 
First Name: 
Department: 
Phone: 
Email: 
Fax: 

Provide a short description of the agent:

1) Lentiviral vector: The lentiviral vectors are self-inactivating and replication defective. Self-inactivating vectors enhance the safety of these vectors by further reducing the possibility of recombination. The only potential risk for infection would be if recombination were to occur between vectors of the packaging sequences, extremely unlikely. 2) AAV these are replication incompetent vectors also. 3) Adenoviral vectors are also replication defective and well characterized. 4) free DNA or Lipid/DNA is non-replicating.

This agent / material is hazardous for: [ ] Humans only  [ ] Animals only  [ X ] Humans and Animals  theoretical hazard only

For which Animal Species?  Monkeys

The agent can be spread by: [ X ] Blood  [ X ] Feces/urine  [ X ] Saliva/nasal droplets  [ ] Does not leave animal  [ X ] Other: Resp. secretions (theoretical)

Describe any human health risk associated with this agent:

No known risks: these vectors are replication incompetent, i.e. not truly "infectious" but they contain recombinant DNA and should be handled with care. These vectors have all functional viral genes removed. There are no known cases of accidental human infection or recombination to date. AAV is not known to cause any human disease. Animals will be treated with replication incompetent viral vectors, or plasmids alone. Some types of native Adenoviruses can cause human infections, including colds or pneumonia but types and amounts used for vectors in animals are not known to be hazardous to humans. Plasmid DNA or DNA plus lipid with the EGFP reporter is not know to present risk.

The precautions checked below apply to this experiment: ALL CRPRC Primate handling and housing conditions (BSL 2) apply  [ ] The researcher or his/her technicians are responsible for the feeding and care of these animals.

[ ] The following items must be assumed to be contaminated with hazardous material and must be handled only by the researcher or his/her technicians.

[ ] Cage  [ ] Stall  [ ] Water Bottle  [ ] Animal Carcasses

[ ] Bedding  [ ] Other:

[ ] Cages must be autoclaved before cleaning.

[ ] Label cages and remove label after decontamination.

[ ] Animal carcasses must be labeled and disposed of as follows:

[ ] Incineration  [ ] Bag and Autoclave  [ ] Biohazardous Waste Container

[ ] EH&S will pick-up (2-1493).

[ ] All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows

[ ] Incineration  [ ] Bag and Autoclave  [ ] Biohazardous Waste Container

[ ] EH&S will pick-up (2-1493).

Personal Protective Equipment Required: ALL CRPRC Primate handling protective attire must be used.

[ X ] The following personal protective equipment must be worn/used in the room:

[ X ] Lab Coat/Coveralls  [ X ] Shoe Covers/Booties

[ X ] Disposable Gloves  [ X ] Head Cover

[ X ] NIOSH Certified Dust Mask  [ ] Disinfectant footbath

[ ] Eye Protection/Face Shield  [ ]

[ ] Fitted Respirator  [ ] Type:

[ ] Other:  Describe:

[ ] Personal protective equipment must be removed before leaving the room.

[ ] Personal protective equipment must be discarded or decontaminated at the end of the project

[ ] Hands, arms, and face must be thoroughly washed upon leaving the room
Full shower, including washing of hair, must be taken upon leaving the room.

Decontaminate Room (Inform ARS area supervisor when cage and/or room can be returned to general use).

Provide any other information needed to safely work in this room: