AG-260-04-02

STATEMENT OF WORK FOR SOLICITATION PURPOSES

1. BACKGROUND

Many NIA funded investigations use rodents to model aging processes and age-related diseases. Strict control of genetic purity, health of the animals including specific-pathogen-free status, and environmental variables is required to provide valid animal models that may be used to study the biological and behavioral processes in aging. Transgenic, knockout and mutant mice have added significantly to studies addressing specific questions or aspects of normal aging and age-related disease etiology. Sharing of genetically engineered and naturally occurring mutant mouse models between labs greatly enhances the research opportunities and generation of new information. This contract provides a mechanism to assume the cost of generating aging mice from the originators of the lines, distribute the mice to investigators, and provide for strict control of health, environment and genetic background of the mice.

2. OBJECTIVE(S)

The objective is to develop, maintain and distribute a standing colony of aged mutant mice of defined genotypes, including genetically engineered and spontaneously arising mutations, for use by investigators in studies of aging. The word 'mutant' is used here to refer to transgenic, knockout and spontaneously arising mutant genotypes. This colony is to be developed and maintained within controlled and defined barrier environments where animals are monitored and characterized for disease status and markers of genetic purity. Each line of mice will be characterized for the specific mutation and control animals will be provided for some lines where needed.

3. WORK TO BE PERFORMED

Independently, and not as an agent of the Government, the Contractor shall furnish all necessary services, qualified personnel, materials, facilities and equipment not otherwise provided by the Government as needed to perform the work described below.

Specifically, the Contractor shall develop, maintain and distribute a standing colony of aged mice of NIA-specified genotypes for use by investigators in studies of aging. The expected duration of this contract is nine years. During years 1 - 3, mouse lines will be acquired from the research community at the direction of the Project Officer. The lines will be re-derived, embryos cryopreserved, breeding colonies established, and offspring will be entered into the aging colony at levels specified by the Project Officer. It is anticipated that there will be 10-20 lines of mice acquired for this colony, approximately half in the first year and the rest in years 2 and 3. It is estimated that between 300 and 600 mice per year will be entered into the aging colony for each line.

During the first two years of entries into the aging colony for each line, animals shall be entered into the colony but few animals shall be removed from the colony except for the purposes of monitoring health and genetic purity. However, animals may be distributed from the colony at anytime at the direction of the Project Officer. As the aging colony for each new line of mice is established, animals shall continue to be entered into the colony and animals shall be distributed to investigators, such that young animals enter the colony at approximately the same rate as older animals leave the colony due to distribution and normal attrition. The final two year period of the contract, years 8 and 9, will serve as the colony close-out period. Animals shall be maintained and disbursed, but entry of animals shall cease at the end of year 7 or when entries begin in a renewal contract colony. Thus the population will decline over the final two years of the contract to a point of few animals at the end of year 9.

The total colony population at the end of each year of the nine year period should be approximately as follows:

YEAR 1	1,000	YEAR 4	12,000	YEAR 7	12,000
YEAR 2	5,000	YEAR 5	12,000	YEAR 8	7,000
YEAR 3	12,000	YEAR 6	12,000	YEAR 9	NEAR 0

The colony shall be maintained behind two independent barriers, specific pathogen- and parasite-free, for the entire contract period. The two barriers do not need to be geographically separated. Both barriers shall specifically be helicobacter-negative. The purpose of colony division is to insure the survival of at least one half of the colony in the event of pathologic breach of a barrier, genetic contamination, mechanical failure of environmental maintenance systems, or accidental disaster such as fire, or flood. Total independence of colony segments therefore means separation of buildings, power systems and back-up systems, environmental controls (heat, air conditioning, and air filtration) and breeding stock. Each barrier shall have an independent back-up generator and alarm system in case of loss of power. Each colony segment shall be maintained solely within its barrier facility without intermingling. More specifically, the Contractor shall perform the following:

I. Supply of the Mouse Genotype

A. The Contractor shall acquire the genotypes specified by the Project Officer and shall provide to the Project Officer documentation of the re-derivation and genetic history of each of these genotypes which serve as the progenitors for the mice in the aging colony. All animals obtained for this contract and produced under this contract, including all breeding mice and all mice entered into the aging colonies, shall be the property of the Government. Acquisition of lines for this contract colony does not confer any rights to the Contractor to commercialize the lines. The Contractor shall not commercialize any lines of mice acquired for or under this contract.

B. The Contractor shall collect tail tips from all mice acquired from the research community, shall isolate DNA from the tail tips, and shall genotype the mice for the specific mutation or genetic alteration as directed by the Project Officer. This testing is to confirm the identity of the acquired lines. The Contractor shall also perform genetic monitoring on these samples to confirm the genetic purity or identity of the background as appropriate. The specific monitoring plan for confirming genetic background shall be approved by the Project Officer and should include at least one marker per chromosome. Leftover DNA from these tests shall not be discarded without written authorization from the Contracting Officer. The results of this testing shall be reported in writing to the Project Officer within seven (7) calendar days of receipt of the results and re-derivation of the lines shall not proceed until the Project Officer has received these results and approved the re-derivation.

C. When the mice acquired from the research community are euthanized, the livers shall be collected, labeled with the strain name, source, date and any identification numbers, and stored at -80^{o}C as a genetic repository. This tissue shall not be discarded without written authorization from the Contracting Officer.

D. The Government may alter the number of entries of particular genotypes and change genotypes as research demands change during the performance period. Alterations in the number or genotype of mice entered require the specific written approval of the Contracting Officer. The numbers of breeding mice of each line to be maintained shall be specified by the Project Officer.

II. Environmental Requirements

A. Mating, isolation, and primary enclosures shall meet or exceed minimum floor space requirements per mouse, and population size per cage as stated in the Institute for Laboratory Animal Research (ILAR) "Guide for the Care and Use of Laboratory Animals" (http://www.nap.edu/books/0309053773/html/index.html). All primary enclosures and bedding type shall require the prior approval of the Project Officer. Mating cages shall be numbered consecutively.

B. The Contractor shall provide discrete production space within the barriers for the breeding and aging colonies. This space shall be defined within each unit, and the animals held therein until the scheduled removal or expiration of the animals. Facilities set aside for these colonies shall be provided with all equipment, materials, and supplies necessary to maintain these animals effectively within the barrier enclosures in a stable condition and environment. The barriers shall be equipped with environmental sensors and alarms and back-up generators that automatically take over in case of power failure. The Contractor shall be responsible for the cost to the government of replacing any animals lost due to mechanical failure, breach of the barriers, or genetic contamination.

C. No animal shall be medicated during any part of the breeding, rearing, or maintenance period except on specific instruction of the Project Officer.

D. The Contractor shall periodically submit members of this colony for evaluation by a qualified animal diagnostic center approved by the Project Officer (with shipping and laboratory fees being charged to the contract), as specified under 3.V.B. below.

E. The Contractor shall notify the Project Officer immediately if any abnormal condition(s) are detected in the colony. Upon direction by the Project Officer, the Contractor shall notify investigators whose research may be affected.

F. Upon obtaining pathologic or other evidence that a barrier has been breached, the Contractor shall immediately notify the Project Officer and the Contracting Officer by telephone. The Contracting Officer shall arrange a meeting of the Contractor's representative, the Project Officer, and the Contracting Officer as soon as practicable, but in all cases within seven (7) calendar days, to determine an appropriate course of action. Such action may range from medication to reestablishment of the colony in newly constituted barrier facilities. Final determination of the appropriate course of action will be made by the Project Officer. Potentially affected investigators shall be notified by written notice approved by the Project Officer.

G. The barrier systems or isolation rooms shall be constructed and operated to effectively exclude pathogens, ecto-, endo-parasites as well as vermin. Personnel shall follow the sanitary and/or sterile practices and techniques that have been established for the maintenance of barrier-sustained animals as described in the ILAR "Guide for the Care and Use of Laboratory Animals". Personnel assigned to the project shall have no contact with rodent colonies other than barrier-sustained animals colonies. Staff shall not at any time be permitted to enter the area assigned this project unless all necessary precautionary measures have been taken with respect to entry and exit from the barrier enclosure. Visitors other than the Project Officer or her designated representative shall not be permitted to enter the area assigned to this project.

    - Note: Floor plans for the containment of the colony should be submitted with the proposal.

H. The following items shall be controlled within the ranges specified: temperature (72-77^{o}F), humidity (40-55%), light cycles (12/12 hrs.), air circulation (0.25m/hr/animal) and filtration (HEPA), water chlorination (7-8 PPM at discharge end) and water acidification (pH 5.8-6.0). A maximum of 25% re-circulated air shall be allowed. The Project Officer shall be notified immediately if the ambient temperature remains outside the above noted temperature range for a period in excess of 4 hours or if there is any loss of animals due to breakdown of environmental controls.

    - Note: Description of the methods of control and monitoring of limits should accompany the proposal.

I. Current ILAR and Department of Health and Human Services (DHHS) (http://grants.nih.gov/grants/olaw/references/phspol.htm) guidelines for breeding, care and maintenance of laboratory mice shall apply where specifications have not been detailed.

III. Breeding Colony

The foundation animals for each breeding colony shall be re-derived by cesarean section or embryo transfer, shall be entered from isolators into the barrier system without contamination by pathogens or parasites, and shall be maintained as such. The Contractor shall provide discrete space within the rederivation area for the lines and shall not house other animals within the isolators other than this contract's mice and their foster mothers. Breeding and maintenance of commercial colonies of mice and rats is permissible within the barrier rooms housing the NIA colonies, but activities such as surgery or euthanasia are not permissible within the same rooms as the NIA colonies.

Colonies of mice to generate foster mothers shall be maintained in isolators; the strain(s) chosen for foster mothers shall be easily distinguishable from the lines being re-derived by both color and genetic markers.

Once entered into the barriers, mice shall be removed from the barriers only for shipment to investigators as directed by the Project Officer or the Project Officer's designated representative, for monitoring of genetic purity or colony health, or for other purposes as directed by the Project Officer. No other types of animals and no other types of activity other than that defined in the work-scope shall be permitted in the barrier rooms used to rear and maintain the mouse genotypes specified. Rearing and maintenance shall conform to the following requirements.

A. All mice of both breeding and aging colonies of all lines shall be considered Government owned property. Genotypes acquired for this contract colony shall not be commercialized in any way by the Contractor.

B. After re-derivation of each line, all foundation colony mice will be tested to confirm the genotype (mutation, transgene or knockout marker) and the correct genetic background. The Contractor shall provide a report on the genotype and genetic monitoring of the foundation colony to the Project Officer within seven (7) calendar days of receipt of the test results but no more than sixty (60) calendar days from the birth of the foundation stock.

C. After re-derivation of each line and after the genotyping and genetic monitoring have confirmed the correct genetic identity of the mice, a minimum of 500 embryos/line shall be cryopreserved. The viability of cryopreserved embryos shall be tested within one (1) month after cryopreservation is complete, by thawing and implanting embryos in foster mothers and determining the percent of embryos that

resulted in pups alive at weaning. The results of this test shall be reported to the Project Officer within fifteen (15) calendar days of completion of the test.

- Note: cryopreservation protocols should be included in the proposal.

D. Breeding and recording of pedigrees of animals shall conform to the standards established for laboratory mouse strains by the ILAR "Guide for the Care and Use of Laboratory Animals". Breeding protocols specific to a line of mice may be specified by the Project Officer at the time the line is acquired.

E. Unless otherwise specified by the Project Officer, litters larger than 8 shall have the smallest pups culled and euthanized within five (5) calendar days of birth to bring the litter size down to 8, and animals from litters smaller than 3 shall be excluded from introduction into the aging colony and shall be euthanized.

F. Records of re-derivation, health and genetic monitoring, breeding, environment, genotyping and colony monitoring shall be maintained by the Contractor for the entire performance period of the contract. These records shall be supplied to the investigators upon request by the Project Officer.

G. All cages of mice shall be permanently identified by the name of the line and include the identification number, sex, genotype and date of birth (DOB) of each weaned mouse in the cage. Age shall be denoted as month/day/year of birth with the understanding that the day of birth may be +/- one day. Recombination or consolidation of cages is prohibited.

H. All mice shall be permanently marked by clipping one toe on the hind foot at 5-7 days of age, with DNA extracted from the toe for use in genotyping (3.III.I. below). All mice shall be assigned a unique identification number at the time of marking, consisting of 2 letters indicating the line and a 5 digit number assigned sequentially for that line. The digit cut shall correspond to the last number of the unique identification number.

I. All mice shall be genotyped for the specific mutation or genetic alteration and the results of the genotyping shall be maintained and provided to the Project Officer upon request. In addition, a copy of the genotyping results shall be sent to recipients of the mice. Genotyping assays will vary depending on the line of mice and will be provided by the Project Officer at the time the line is acquired. Typical assays involve PCR analysis of the toe DNA lysate (III.H.) followed by agarose gel electrophoresis, but tail biopsy and Southern blot hybridization may be required for some lines.

J. Breeder mice shall be euthanized and replaced when they are 12-15 months old, or sooner if birth rates diminish sooner for a particular line.

- Note: the method and equipment used for euthanasia should be described in the proposal. Euthanasia protocols must conform to PHS policy as described in http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-062.html .

IV. Maintenance of the Aging Colony

A. Mice shall be weaned at approximately 3 to 4 weeks of age and shall be group housed in the aging colony with a maximum of 5 mice per cage. Generally, same sex littermates will be housed together or same sex non-littermates of the same line of mice and born within a week of each other may be housed together. Specific housing protocols may be specified by the Project Officer, such as for dwarf mutant mice that require wild-type mice in the cage in order to maintain body temperature. All offspring from the breeding colony shall be entered into the aging colony unless otherwise specified by the Project Officer.

B. The weanling mice shall be from barrier maintained parents and shall be maintained within the barriers until scheduled for removal (shipment or sacrifice) or natural expiration of the animal.

C. Weaned mice assigned to the aging colony shall receive a laboratory animal feed, the formulation of which is consistent with NIH31 diet with regard to ingredients, both in kind and amount. The kind of ingredient shall be absolute; the percentage of the kinds of ingredients may vary dependent on nutrient content provided the final formulation remains consistent with NIH31 diet. The type of cage bedding is also subject to review and approval by the Project Officer.

- Note: The laboratory animal feed formulation should be included with the proposal and is subject to review and approval by the Project Officer.

V. Colony Evaluation, Characterization, and Monitoring

A. All spontaneous deaths in the colony shall be reported by strain, sex, barrier and age in the semiannual progress reports and on the weekly census report. Necropsies shall not be performed on mice unless there is an unusual pattern of animal deaths or upon the direction of the Project Officer. Postmortem findings in such cases shall be tabulated, summarized, and provided to the Project Officer within sixty (60) calendar days of the date the animals were sent for evaluation. Primary and secondary lesions possibly attributable to cause of death are to be described. Differences in mortality between lines shall be identified and reported in the semiannual progress report.

B. Routine monitoring for disease and post mortem examinations is to be performed by either an independent laboratory approved by the Project Officer or the Contractor if Contractor plans, personnel and facilities are approved by the Project Officer. Numbers of each genotype, sex and barrier submitted for monitoring shall be determined by the Project Officer. Up to fifty (50) mice total shall be submitted for routine health monitoring each quarter unless otherwise specified by the Project Officer. Specimens from the colonies of mice used to generate foster mothers shall also be submitted for health monitoring each quarter.

C. Routine clinical and postmortem evaluation shall include:

   a. descriptive clinical condition of the animals including general condition, weight, and visible lesions and symptoms;

   b. gross pathologic observations of body tissues and organs;

   c. microbial, parasitological and serological evaluation necessary to monitor effectiveness of the barrier system and animal health;

   d. histopathology of major organs and systems.

      - Note: The specific microbial, parasitological, histopathological and serological assessments shall be detailed in the proposal and require approval by the Project Officer.

D. Mice shall be monitored for wounds and open lesions caused by fighting or excessive scratching and for visible tumors. Mice shall be euthanized when wounds or tumors are extreme enough to cause pain, wasting or decreased mobility in the animal, or when tumors reach 1 cm in diameter.

E. The Contractor shall conduct semiannual genetic monitoring testing to detect genetic contamination. Breeder mice from each line shall be tested with a panel of microsatellite markers that distinguish between different common strains, with a minimum of one marker per chromosome. For most lines, semi-annual testing of 4 breeder mice from each line will be sufficient but the Project Officer may direct otherwise. Procedures for genetic monitoring testing are subject to the prior written approval of the Project Officer.

VI. Shipment of Animals to Investigators

A. Animals from the aging mouse colony shall be distributed to investigators or laboratories only as specified by the Project Officer or the designated representative of the Project Officer. A copy of the most recent Health Evaluation Summary for the animals, prepared under V.B. above, and a copy of the genotyping results for the animals shall be sent with each order.

B. The aged mice shall be shipped in containers that are made of new materials that are non-reusable and non-returnable, the design and fabrication of which are approved by the Project Officer, with filters for ventilation openings of a type approved by the Project Officer; and so designed that stacking will not interfere with normal circulation of air through the container. Food and a source of liquid shall be included in the crates.

C. The number of mice per container shall not exceed twenty (20) animals. If animals from different cages are shipped in the same container, the container shall be sub-divided into compartments, each of which contains only cage-mates. In such cases, the boxes shall be clearly marked to indicate the animal id numbers in each compartment. Special provisions may be specified by the Project Officer as needed.

D. The Contractor shall report to the Project Officer or the designated representative of the Project Officer immediately if there are delays in the shipment of animals.

E. Shipment via dedicated, climate-controlled truck is the preferred method. The Contractor shall bear responsibility for the cost of animals and shipping charges for replacing animals or providing credits for animals that die during shipment or within 48 hours of delivery, or that are unacceptable to the consignee due to breached or crushed shipping containers. The Contractor is also responsible for the cost of replacing orders not filled correctly according to the instructions provided by the Project Officer or the representative designated by the Project Officer.

F. When shipment by dedicated, climate-controlled truck is not possible, air freight shall be used. The Contractor shall take all necessary precautions to assure expedient delivery of animals shipped by air freight, by advising the recipient of expected arrival time, mode of transport and carrier number, and any responsibility the consignee has for pick-up at the point of arrival. When the consignee is notified that they are required to meet animals at point of arrival, late or delayed pick up of animals shall constitute neglect by recipient and Contractor is relieved of all replacement or reimbursement obligations.

G. Title to mice shipped will be transferred to the consignee once animals are received and accepted. The consignee shall have the right to reject all mice considered to be unsatisfactory upon arrival at his facility if animals are obviously ill or moribund or if shipping criteria have been disregarded.

VII. Cost of Animals to Investigators

Mice produced under this contract shall be made available to investigators under conditions as stated herein or as otherwise specified by the Project Officer. The cost of each mouse to the investigators, a basic acquisition cost plus a maintenance charge determined by age of mouse, shall be determined by the Project Officer. Initial costs are expected to be $20.00 plus $5.00 per month of age, per mouse. These costs will be subject to annual adjustment. The consignee is also responsible for all shipping costs. Total cost (as charged to the consignee) of animals (acquisition, maintenance, crate and shipping) which are not useable when received by the consignee due to negligence on the part of the contractor or subcontractors, shall be borne by the Contractor and are not chargeable to the Government.

VIII. Reporting Requirements

A. Semiannual progress reports shall include any unusual developments or problems involving colony health, genetic purity, environment or environmental control, and any differences between barriers. The report shall include the results of routine genetic monitoring and all laboratory tests executed either by Contractor as a routine part of the protocol or results from tests that have direct implication for colony health or development. All deaths or sacrifices from the colony shall be accounted for by barrier, strain, sex and age of the animal sacrificed or expired. Differences in mortality between lines shall be identified and reported in the semiannual progress report. Unusual problems, with animal health or genotyping assays, shall be reported to the Project Officer immediately. A copy of the genotyping results from 3 litters of each line born within the reporting period shall be included in the report. Semiannual progress reports shall be submitted to the Project Officer within fifteen (15) calendar days of the end of each six (6) month period of the contract. The initial report shall be submitted for the first full six (6) months of the contract performance including any fractional part of the initial month. Thereafter, the reporting period shall consist of six (6) full calendar months. A semiannual report will not be required for the period when the final report is due.

B. A weekly census report shall be submitted to the Project Officer in accordance with a format approved by the Project Officer. Electronic transfer of the census in Excel spreadsheet format is the preferred means. The weekly census report is due to the Project Officer no later than one week (7 calendar days) from date of census. Access to a "real-time" electronic inventory would take the place of the weekly census report subject to approval by the Project Officer.

C. Complete quarterly health monitoring reports shall be submitted to the Project Officer no later than three (3) months from the date the animals were submitted for evaluation. Semiannual summaries of these reports shall be furnished as a part of (A.) above.

D. Results of the pre-re-derivation (3.I.B.) and post-re-derivation (3.III.B.) genotyping and genetic monitoring tests shall be reported in writing to the Project Officer within seven (7) calendar days of receipt of the results, and in the case of the post-re-derivation testing, not more than sixty (60) calendar days from birth of the foundation stock.

E. Results of the semiannual genetic monitoring for strain purity of the breeding colonies shall be reported to the Project Officer no later than sixty (60) calendar days from the date the animals were submitted for evaluation. Semiannual summaries of these reports shall be furnished as a part of (A.) above.

F. The results of the cryopreserved embryo viability test for each line shall be reported to the Project Officer within fifteen (15) calendar days of completion of the test.

G. Complete postmortem reports on individual animals chosen for additional health monitoring independent of the quarterly health monitoring shall be submitted to the Project Officer no later that sixty (60) calendar days from the date the animals were submitted for evaluation.

H. A final report shall be submitted to the Project Officer on or before the contract expiration date. The final report shall include a summation of the work performed and results obtained for the entire contract period of performance. This report shall be submitted on or before the contract expiration date.

IX. Proceeds From Sales

All proceeds from the sale of animals shall be used to offset the total estimated costs under the contract. Proceeds from the sale of animal will be shown as a separate line item on monthly invoices. Likewise, adjustments required (3.VI.E; 3.VII) will be a separate line item on the monthly invoice.