**Duke Transgenic Mouse Core**

**Service Request Form (FY2024)**

**(Rodent Genetic Engineering Services Team (RGEST))**

**RGEST is a part of the DCI Rodent Cancer Models Shared Resource**

Create a code name for this project using ONLY 6 Letters and/or Numbers:

\_\_\_\_\_ \_\_\_\_\_ \_\_\_\_\_ \_\_\_\_\_ \_\_\_\_\_ \_\_\_\_\_

(Select ONLY a maximum 6 number/letter designation—This will be the “project code” with which we will refer to your project in our data base.

We will label all of your reagents, cells, cages of mice with the MAXIMUM 6 letter/number code—otherwise we have to write too much on small tubes.)

Date:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**PI of project for our yearly reports to the DCI:**

P.I. Name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Email:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Phone# :\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Duke Fund Code to pay for RGEST Services:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

If paying for services using a SOM/RNI Voucher, list complete reference details.

Identifying numbers:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Are you a current Duke Cancer Institute Member? Yes \_\_\_\_\_\_\_\_\_No\_\_\_\_\_\_\_\_\_\_

Cancer Institute Members receive priority service

Program Designation in DCI:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Is this a collaboration of a non-DCI member with a DCI Member? Yes\_\_\_\_\_\_\_\_No\_\_\_\_\_\_\_\_\_

DCI Collaborator’s Name;\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

(*DCI Member must use their fund code pay for all services to validate collaboration*)

If Collaboration with DCI Member list fund code to pay for RGEST services: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**WHO is the project leader in the lab that we should contact about questions and details of this work?:**

**Project Leader’s Name**:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Email:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Phone Number:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Department:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Building & Room#:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Should the PI above be copied on communication?: Yes\_\_\_\_\_\_\_ No\_\_\_\_\_\_\_\_\_

**Mouse Caretaker**: name and email of relevant laboratory person who oversees mouse space and should be alerted when mice are produced and transferred.

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Information Related to Mouse Transfer:**

**PLEASE NOTE : The Transgenic Core cannot transfer mice to MSRB3**

PI Name on Approved Animal Protocol:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

DUIACUC Protocol #:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Expiration Date:\_\_\_\_\_\_\_\_\_\_\_

Mouse Housing Building:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Room #:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Other:\_\_\_\_\_\_\_

Duke Fund Code to pay for new cage cards and transfer of mice by DLAR. This should be a code that is

already in the **DLAR Granite Billing System** (please verify this with your business manager)

(Labs cannot use voucher codes to pay for animal housing:):\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

List the email addresses of all people to be copied on the animal transfer paperwork that goes to DLAR:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**SAFETY:**

All projects involving rDNA & Transgenic Mice must be registered with the Duke

Institutional Biosafety Committee <http://www.safety.duke.edu/BioSafety/rDNA.htm>

PI responsibility

**PI Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

IBC Registration #:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Model Request (Check One):**

FY2023 SOM approved service rates

Transgenic Mouse via Gene Targeting Service in ES cells (Knockin, Knockout, cKI, cKO)

Please complete Section 1

Transgenic Mouse via CRISPR/Cas *in vivo* Genome Editing (RNA Electroporation/ Injection of embryos)

Please complete Section 2

Transgenic Mouse via Pronuclear Injection (Client provided DNA transgene)

Please complete Section 3

Transgenic Mouse made with ES cells provided by PI Lab (such as repository clones)

Please complete Section 1

**\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*Section 1\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\***

**ES Cell/Gene-Targeting Services**

**Please indicate by check boxes which services you are requesting**

**(Full package check off all 4 service lines)**

**HITI-Based Targeting Vector ……………………………………….…………………………$4,297**

Includes: Core generated Sequence verified targeting vector and activator plasmids (Knockout, knockin, cKO, cKI model;) plus validated PCR assay and MOCK control DNA for screening targeted ES cell clones.

**ES Cell Transfection of targeting vector ……………………………..……………………$4,456**

We have the best results with G4 ES cells which are a 129/B6N hybrid ES line that we have obtained by MTA from Mount Sinai Hospital in Toronto. Homozygous 129 ES cells that are: 129S6/SvEvTac can be used for transfection but this must be discussed before the targeting construct is made.

Which Cell Line is preferred?:  G4 hybrid line\_\_\_\_\_\_\_  129\_\_\_\_\_\_\_

**ES Cell Validation of Putative Targeted Clones by Core…………………………………………….$1,422**

Up to 18 PCR screened positive ES cell clones will be processed and validated using PCR and sequencing.

**DNA Preparation** only from ES clones by core for PI Lab to validate …..$**594**

**Morulae Microinjection of Positive ES Cell clones to create chimeras ………………..$4,721**

G4 ES cells of positive clones, confirmed by the contracting lab or by the RGEST, will be microinjected into ICR morulae to produce chimeric mice. Blastocysts from C57BL/6J can also be injected for 129 ES cell lines. Blastocyst injection is a less efficient method to produce chimeric mice.

Special Trouble Shooting Assistance:

Labs are asked to confirm mutations in all of the chimeras from tail samples, and after breeding, in the offspring. If a lab encounters PCR difficulty, and needs our Core to help in troubleshooting, the service fee is $335. The Core does not offer a standard genotyping service. Contact the Breeding Core to explore this option.

**PI Provided: IF you are submitting the ES cells for microinjection, list the details of acquisition and culture here:**

**For *de novo* targeting:**

What is the endogenous gene name and species:\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Accession Number of mRNA Primary Transcript:\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Briefly describe your mutation:

Please paste the exon or specific genomic region below that will be modified:

**PI Provided Construct: prior to submitting your targeting vector for ES cell targeting**, please attach a MAP of your planned alteration.

**Show the following on your map:**

\_\_\_\_Screening primer start sites \_\_\_\_\_lox p & frt sites \_\_\_\_\_selection genes: Neo and DTA or TK

What is the total length of homology of the targeting construct to endogenous site?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**MAP PLEASE—include all relevant information, especially positive & negative selection regions and primers sites:**

**For Targeting Constructs**

**LIST of Samples Submitted to RGEST:**

Targeting Construct:

Label on tube:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Concentration:\_\_\_\_\_\_\_\_\_\_Volume:\_\_\_\_\_\_\_\_\_\_\_\_

**HITI ROSA OTHER**

Is this targeting construct linearized????\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_YES

Traditional Constructs should be **Linearized!!** Submit at least 70 ug @ 1 ug/ul using high quality purification techniques

PCR Screening Primers (submit at least 300 UL of each at 10 UM only) PLEASE USE RGEST PCR Program & Cocktail Formulation. Takara.

If you are forced to change this formulation, be sure to include enough primers to screen 3 X 96 well PCR plates with some loss calculated for reservoir & multichannel pipettor:

5’ screening primer name on tube:\_\_\_\_\_\_\_\_\_\_\_\_ volume:\_\_\_\_\_\_\_\_

5’ screening primer name on tube:\_\_\_\_\_\_\_\_\_\_\_\_ volume:\_\_\_\_\_\_\_\_

5’ Expected product size:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Time of extension based on 5’ product size\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

3’ primer name on tube:\_\_\_\_\_\_\_\_\_\_\_\_ volume:\_\_\_\_\_\_\_\_\_

3’ primer name on tube:\_\_\_\_\_\_\_\_\_\_\_\_ volume:\_\_\_\_\_\_\_\_\_

3’ Expected Product size:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Time of extension based on 3’ product size:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Mock Construct as positive control for PCR Screening:

Dual 5’/3’ Mock: Label\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Concentration;\_\_\_\_\_\_\_\_\_\_\_ Volume:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

5’ Screening: Label on tube:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Concentration:\_\_\_\_\_\_\_\_\_\_\_\_\_ Volume:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

3” Screening: Label on tube:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Concentration:\_\_\_\_\_\_\_\_\_\_\_\_\_ Volume:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Have you attached a photograph of your PCR titration detection test: \_\_\_\_\_\_\_\_\_\_\_\_YES/NO\_\_\_\_\_\_\_\_

Did you use the Standard RGEST protocol using TAKARA for PCR detection or were you forced to use another program & reagents? Clonetech Takara LA TAQ #RR002M (Be sure to use this product version!)

Standard PCR Cocktail Takara per sample (Reagents come with kit) : H2O 14.025 UL; 10X LA PCR Buffer; 2.25 UL; dNTP MIX: 3.6 UL, F Primer 10UM: 0.45 UL; R Primer 10UM: 0.45 UL; LA Taq Polymerase: 0.225 UL

(DNA 2 UL) total 23 UL

STANDARD TAKARA Program: step #1 94C 1 min; #2 98C 10 Sec; #3 60C 15 Sec; #4 68C 1min/1 kb adjust accordingly!! # 5 go to #2 39 X; # 6 72C 10 min; # 7 4C 10 min

If your answer is NO:  
  
 Explain & include PCR program, cocktail formulation & source of reagents:

*Include an original photograph of your validated PCR screen showing* ***Serial Dilution*** *of the mock construct from 1 ng/UL to at least 1 fg/UL. PCR must follow all of our RGEST conditions for dilution into genomic DNA, PCR cocktail formulation and PCR machine programming. For consultation regarding this requirement please contact gary.kucera@duke.edu*

What is your neo promoter?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ (we will feed your ES cells G418 at 250 g/mL—be sure you have not used a mutated pgk neo promoter or all of your cells will die.)

Do you have Neo/DTA selection? Yes \_\_\_\_\_\_\_\_No\_\_\_\_\_\_\_\_\_ (this is our preferred system; we will use G418 only)

Have you used a Neo/TK strategy?: Yes\_\_\_\_\_\_\_\_\_No\_\_\_\_\_\_\_\_ ( if you used TK we need to feed your cells Ganciclovir)

Do we need to add Ganciclovir for selection in cell culture? Yes\_\_\_\_\_\_\_\_\_\_No\_\_\_\_\_\_\_\_\_\_\_

Does your targeting involve another selection method? Yes\_\_\_\_\_\_\_\_\_\_\_\_No\_\_\_\_\_\_\_\_\_\_\_\_

Will we make mice directly from your clones upon confirmation? Yes\_\_\_\_\_\_\_\_\_ No\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

We expect you to check ALL clones we give back to you as completely as possible before we make mice. Please send us a **written** confirmation of clones that can be used to inject. The more clones confirmed the greater your chance of germline transmission. We inject several confirmed clones. We assume that the project leader has confirmed all experimental details with the PI when the confirmation list is sent to us. Copy the PI on your email confirmation to us.

Date of Email confirmation of positive ES clones from PI or RGEST: \_\_\_\_\_\_\_\_\_\_\_\_\_\_yes\_\_\_\_\_\_\_\_\_\_\_\_\_\_no

**\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*Section 2\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\***

**CRISPR/Cas9 Reagents and In-Vivo Genome Editing Services**

**Please indicate by check boxes below which services you are requesting**

*Note: Knockins of single or multiple point mutations using CRISPR technology requires validation of founders by RGEST for additional fees (details below). Knockout, knockin of ORFs or large deletions may be validated by the PI lab.*

**Indicate which mouse strain you request for electroporation:**

**C57BL/6J-- preferred (JAX Stock# 000664)**

**ICR/Hsd Envigo**

**F1 (B6SJLF1/J (JAX Stock# 100012) very limited males in the core; females shipped to order**

**PI mutant strain or other stock strain, housed in your facility or elsewhere, like breeding core**

(PI lab must superovulate & provide plugged females to RGEST , ask for superovulation instructions)

If your mice are house in the breeding core, your lab must coordinate contact with staff there

Mouse background strain:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_mutation:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

“Official designation”:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Person who is coordinating superovulation & email:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Guide RNA design, validation, production………………………………………..………….………………$3,766**

Core identifies up to three sgRNAs per genome target. Synthesizes sgRNAs and validates activity in-vitro. Designs ssODN repair for point mutations. 200 bp.

Alternate service: PRODUCTION ONLY if PI Laboratory does all design work **(PI designed mutation) CRISPR sg RNA Production and screening (IVT)…………$1512**

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**Embryo Electroporation of CRISPR Reagents (or microinjection)……….……………………………..$4,456**

Validated sgRNA (RGEST provided reagent only) + repair/donor oligo (if required) + cas9 protein.

**REQUIRED Analysis:**

PLEASE NOTE: All KI models (point mutation, inserted sequence, etc.) require the TG Core to perform Founder Analysis.

**Founder Analysis, Initial……………………………………………………………………..$1,252**

Genomic DNA preparation and sequence analysis of targeted locus to identify Indels. Founder Analysis by RGEST is a REQUIRED step for any KI project.

**Founder analysis, Advanced, Allelic Subcloning…………..…………………………..$2,440**

Subclone and sequence individual targeted alleles from up to 6 founders to identify/verify mutation

**Details about mutation to be created:**

mRNA Accession Number associated with this gene:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Specific mutation being requested:

Please provide sequence of the exon(s) you wish to modify:

**Do you expect any lethal effects from this mutation or possible associated mutations that may occur?**

**NO\_\_\_\_\_\_\_\_\_\_\_\_\_**

**YES\_\_\_\_\_\_\_\_\_\_\_ Explain:**

**\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*Section 3\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\***

Pronuclear Injection of Client provided DNA for Transgenic Mouse Production (PI Lab)

**DNA Microinjection to Produce Transgenic Mice……………………………. $4084**

We provide NO Guarantees that founders will be produced.

One cell mouse embryos are microinjected with a transgene construct made by the PI lab,

Limited number of F1 males, females must be ordered. Embryos tend to be less fragile. B6J can be microinjected.

Endogenous Gene or brief description of your mutation:

**Which mouse strain? Check which strain:**

**B6SJLF1/J  C57BL/6J  ICR/Hsd  Other strain, house in PI lab**

PI mutant mice: name of strain\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ mouse background\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ PI needs to superovulate & provide plugged females to our lab; please meet to discuss.

The QUALITY of your DNA preparation is the ultimate factor that impacts success. Poorly prepared DNA fails to produce transgenic mice. Prepare your construct following approved methods for DNA microinjection into mouse embryos.

**BAC DNA Constructs have unique requirements & different buffers for success. Check with us to ensure success before you submit your construct.**

All **regular transgenic** constructs must have vector removed & be suspended in excellent quality TE or specially prepared microinjection buffer for transgenic mice.

**For regular constructs (not BAC Constructs), submit 6 separate tubes of DNA construct at about 50 ng/UL with 10 UL in each tube.**

**Label each tube with: Unique Project Name (from first page on this form), date, volume, concentration.**

Length of construct:\_**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**Volume:\_**\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Measured concentration:\_**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Did you use the Nanodrop machine to quantitate?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

What method did you use to purify your transgene fragment:

Has the promoter been used in other transgenic studies?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Do you expect any lethal effects of construct expression?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Where do you expect expression?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Have you tested expression of your construct in another system, such as cells?\_\_\_\_\_\_\_\_\_\_\_\_\_

Submit a well labeled **photo** of your construct to show it is cleanly purified in one band.

Submit a **MAP** of your construct with significant regions labeled.

Can you detect 1 copy of your construct in mouse tail DNA?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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**Statement of Understanding**

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**ES Cell/Gene Targeting Services**

* DUIACUC Protocol Number is required prior to initiating animal work.
* The RGEST does not guarantee chimera formation or germline transmission of client-provided ES cells, including cells from gene trap or knockout consortium, or gene targeting vectors from KOMP/EUCOMM.
* The RGEST does not guarantee positive transgenic mice and/or germline transmission of pronuclear injected transgenes or electroporated embryos.
* Subsequent rounds of injection or electroporation will incur additional charges at the original rate.

**CRISPR/Cas Genome Editing Services**

* IBC Registration number is required prior to initiating molecular biology work
* CRISPR/Cas system for genome editing is a new and rapidly changing technology, and has not yet been fully characterized. The RGEST will seek to provide services based on the latest “best practices” at the time. The client must acknowledge and accept that results may not meet expectations.
* If no CRISPR effects (i.e. Indels) are identified (by *CRISPR Founder screening,*) done by the PI lab RGEST will analyze the tail DNA. If Indels are detected by this screening , the PI lab will pay for this service if they have not already. If no indels are detected, re-electroporation will be done at no cost. If indels are detected, but desired point mutations are not present (as determined by *Allelic cloning*), subsequent rounds of electroporation will incur additional charges at the original rate.

**All publications resulting from animals and cells generated using the RGEST Services must acknowledge the DCI Grant CCSG grant (P30) and link the publication in MyNCBI.**

**The RGEST reports yearly to NCI and is reviewed at site visits based on publications.**

**By contracting for services with RGEST, the PI also agrees to abide by the Model Organism Policy of the Duke Cancer Institute (see attached) including rules on distributing Animals, MGI nomenclature & NIH repository & database.**

**Animals produced using G4 ES cells are bound by an MTA signed by Duke University with Mount Sinai Hospital.**

Collaborations of non-DCI members with a DCI member are validated with the DCI member paying for services on their grant codes.

In a brief paragraph or two, describe how your project will significantly contribute to or impact CANCER RESEARCH. (Attach on a separate page.)

Essay Attached?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Yes \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_NO

Remember, RGEST is funded by NCI & is reviewed yearly before publications result.

**I have read and understand the Statement of Understanding written above.**

**Signature of PI:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Date:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

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**Duke Cancer Institute**

**Plan for Sharing Model Organisms and Related Resources.** The Duke Cancer Center’s plan to share materials and our management of intellectual property will adhere to the NIH Gran Policy on Sharing of Unique Research Resources including the Sharing of Biomedical Research Resources Principles and Guidelines for Recipients of NIH Grants and Contracts issued in December, 1999 http://www.nih.gov/policy/rt\_guide\_final.html. All ‘model organism’ generated by the Duke Comprehensive Cancer Institute will be distributed freely or deposited into a repository/stock center making them available to the broader research community, either before or immediately after publication. If the Duke Cancer Institute assumes responsibility for distributing any newly generated model organisms, we will fill requests in a timely fashion. In addition, we will provide relevant protocols and published genetic and phenotypic data upon request. Material transfers will be made with no more restrictive terms than in the simple Letter Agreement (SLA) or the Uniform Biological Materials Transfer Agreement (UBMTA) and without reach through requirements. Should any intellectual property arise which requires a patent, we will ensure that the technology (materials and data) remains widely available to the research community in accordance with the NIH Principles and Guidelines document. The Duke Cancer Institute following the characterization and peer-reviewed publication of the transgenic mouse strain generated, mice will be freely distributed to investigators at academic institutions wanting mice for non-commercial research. Individual requests for shipment of mice generated by this program project funding to AAALAC

(Association for Assessment and Accreditation of Laboratory Animal Care International) accredited institutions will be honored. The recipient investigators would provide written assurance and evidence that the animals will be used solely in accord with their local IACAC review; that animals will not be further distributed by the recipient without consent of our institution; that animals will not be used for commercial purposes. Requests for mice from for-profit corporations to use the mice commercially will be negotiated by our institution’s technology transfer office. All licensing shall be subject to distribution pursuant to Duke’s policies and procedures on royalty income. The technology transfer office will report any invention disclosure submitted to them to the appropriate Federal Agency. In addition, all of the transgenic mice generated will be deposited in an NIH supported mouse repository and database. NIH supported repositories cryopreserve embryos or sperm and distribute the frozen embryos or mice to biomedical researchers. For the mice generated, we will use standard nomenclature and receive approval from the Mouse Genome Informatic (MGI) nomenclature committee (<http://www.infomatics.jax.org/mgihome>.) To facilitate sharing and distribution of the transgenic/knockout mice and associated resources developed under this grant, mice will be maintained in a specific pathogen free facility. This facility will maintain the mice free of the following micro-organisms and pathogens (e.g., pinworms, mouse hepatitis virus (MHV), Sendai virus, mycoplasma, mites, etc.) Should the transgenic/knockout mice become infected with any of these micro-organisms, the mice will be rederived through embryo transfer.

“Other Resources” generated with funds from this grant will include DNA constructs, etc. These resources, as available, would also be freely distributed upon request to qualified academic investigators for non-commercial research.