Transgenic Construct Submission Form

PI: _____

Construct name (short w/ no unusual characters): _____

Contact person: _____

Contact person info:

phone #1: _____ phone #2: _____

FAX: _____ pager: _____

Email:_____

Date Submited:_____

SERVICE DESCRIPTION: Transgenes will be injected into the pronuclei of fertilized, viable murine oocytes isolated from a set of 10-15 females (depending on strain requested for each session). Mouse strains available for injection are C57BL/6J (inbred), FVB/N (inbred) or C57Bl/6xCBA (hybrid). Other strains can be used for an increased fee. DNA from each resulting mouse will be screened by PCR to detect transgenic founder animals. To date, we have averaged 10% transgenic founder animals per live births, with significant variation between constructs. Before injection, you will need to provide some information about the transgene, a genotyping assay protocol, and some administrative details. Contact Mia Wallace after completing the form. Mia is at 314.747.4554, or mia@wustl.edu. We will arrange a meeting with you to turn over the DNA and PCR primers, and to review the construct information. All facility services are performed in the order received and kept confidential.

Please provide the information requested below. If you have any special circumstances or requirements (e.g. a detrimental phenotype or requirement for a different mouse strain), contact us to make arrangements. We are happy to provide specialized services.

Which strain of mice for injections?

FVB/N (inbred) _____
C57Bl/6xCBA (hybrid) _____
C57Bl6/J (inbred) _____

BACKGROUND: A brief description of the scientific rationale for producing the construct comprehensible to those outside the immediate field, including the expected sites of expression and phenotype.

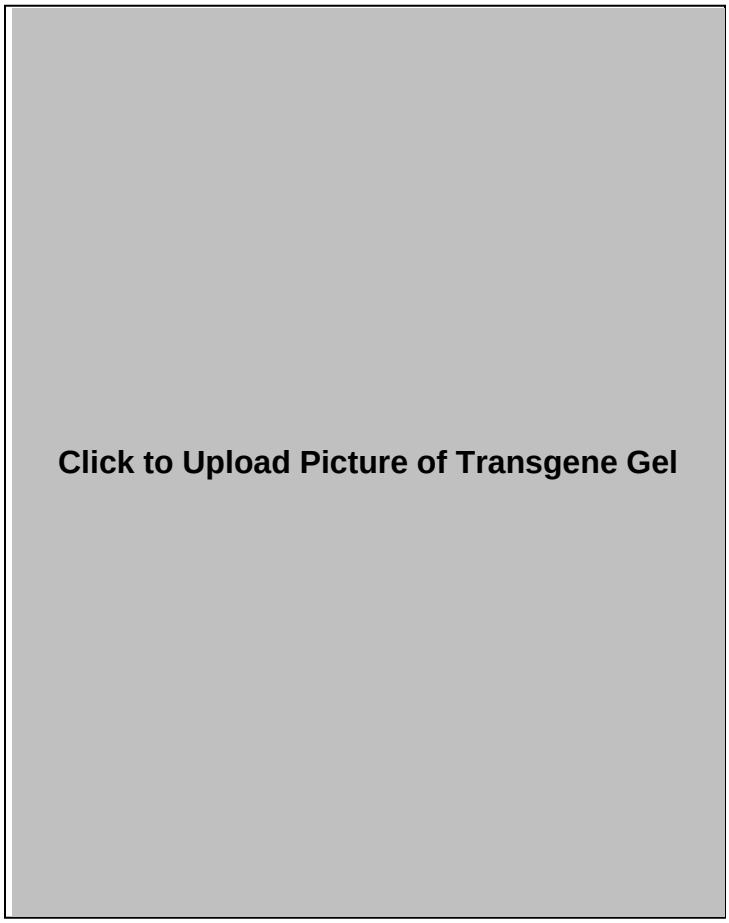
GENE STRUCTURE: Diagram the transgene construct and label salient features, including the promoter, reporter, stop and start sites for transcription, stop and start sites for translation, intron/exon boundaries, and size of each sequence.

CONSTRUCT PREPARATION PROTOCOL: The construct should be prepared for injection using the following protocol: http://mgc.wustl.edu/Protocols.aspx#xgene If you prefer a different method, please contact Mia in advance. BAC constructs should be supplied in high salt buffer. Please contact Mia in advance of all BAC submissions for information on construct preparation. The construct should be freed from vector sequences with a restriction digest resulting in fragments that are readily separated by agarose gel electrophoresis. Digest enough material to yield 1µg of construct after purification (yields are typically 25-50%). Please submit your DNA sample in TE or injection buffer (10mM TRIS, pH=7.4, 0.1mM EDTA). We will need at least 500ng of DNA for injection, and this should be provided in a tube labeled with the construct name and DNA concentration. The construct name should be short, alphanumeric, and not contain Greek or other unusual characters, subscripts or superscripts.

Elute the DNA from the QIAEX resin with 10mM Tris, pH=8.0 (buffer EB in the kit). Repeat the elution and combine the two eluants. Precipitate the DNA with 0.7 volumes of isopropanol. Incubate on ice for 30 min. Centrifuge at 4˚C for 30 min, 8000rpm.

Carefully remove the supernatant with a pipet and wash the DNA with an equal volume of cold 70% ETOH, air dry for 15 – 30 minutes until pellet is clear (check every 5 minutes – DO NOT OVERDRY), redissolve in TE or injection buffer (10mM TRIS, pH=7.4, 0.1mM EDT). Add buffer, let sit RT 15 min, pipet with barrier tip several times, solubilize overnight at 4°C.

Use 1µl of the construct solution to determine the concentration of the DNA with a fluorometer or low-volume spectrophotometer. Run 200ng of the construct on an agarose gel with appropriate size markers and visualize with ethidium bromide staining. A single, sharp band of the appropriate size should be evident. Attach an original picture of the gel to this report. We will need at least 500ng of DNA for injection, and this should be provided in a tube labeled with the construct name and DNA concentration. The construct name **should be short, alphanumeric, and not contain Greek or other unusual characters, subscripts or superscripts**. A fluorometer is available in Mia's laboratory.



GENOTYPING: Before injecting the transgene, you will need a set of PCR primers suitable for genotyping the resulting animals. We use a PCR genotyping protocol that works for all transgenes [Stratman and Simon *(Transgenic Res. 12*, 521-522 (2003)]. This protocol requires primers that are 30 nucleotides in length, and we have proven primer sets for many transgenes (please see Services / PCR page). If your transgene contains any of these sequences, you do not need to provide PCR primers. If your transgene does not contain these sequences, you should design primers as indicated on the Protocols page. All primers are 30-mers with ~50% GC content and produce an amplimer of 100-400 base pairs. We will pick the primers if you provide us with the transgene sequence. If you provide your own primers you we will need at least 50µL of a 100µM solution for each 30-mer. Please indicate either in-house primers or transgene specific primer sequences below.

☐ Proven MGC Primer Set (please see Primer Pairs): _____

☐ Primers Provided (please give primer names): _____ amplimer size

#1 _____

Sequence: _____

#2 _____

Sequence: _____

BILLING INFORMATION

PI: _____

Department/Division & Dept. #_____ `

PI signature: _____

Bill to fund (number)*: _____

Accounting contact (name):_____

* Investigators who expect to receive a subsidy from dedicated Core grants, please check the appropriate box below and fill out the required additional forms. The additional forms for Digestive Diseases Research Core Center (DDRCC), Diabetes Research Center (DRC), the WashU Center for Musculoskeletal Research (MRC) investigators can be found on the respective websites. Approval of the project by the Core Director is required for subsidy.

Subsidy cannot be guaranteed without approval BEFORE the service is performed.

DDRCC	DRC	MRC
☐	☐	☐

ANIMAL TRANSFER: We will automatically transfer the transgenic founder animals to you at weaning age unless you instruct us otherwise. Before injection, we will need an Animal Studies Committee protocol number for your project, and a location to transfer the mice. The ASC protocol need only be for the analysis of the animals - the Core Facility has ASC approval for the procedures used to generate the animals. At the time of transfer, we will notify you by email to expect the animals in your barrier room. Please allow approximately 2 weeks after weaning for animals to be moved; we will not transfer any mice until their health screen has come back negative for pathogens.

ASC number_____

Transfer animals to building: _____ Room #: _____

Signature:_____