EXHIBIT C
DELTAGEN'S PHENOTYPIC ANALYSIS PROGRAM OUTLINE

(Pages Following)

A. 7 Week Clinical Findings and Pathology
   1. Procedures
      a) Physical Examination
      b) Necropsy
      c) Histopathology
      d) Histopathology images
      e) Densitometry/DEXA
      f) X-Ray
      g) Clinical Chemistry
      h) Hematology
      i) Home cage observations
   2. Cohort
      a) 3 KO males, 3 KO females, 2 WT males, 2 WT females
      b) 6 HETs are analyzed when phenotype is present in the KOs
      c) Age: 7 weeks

B. Behavior
   1. Procedures
      a) Open Field (anxiety, activity)
      b) Tail Suspension (depression)
      c) Hot Plate (analgesia)
      d) Tail flick (analgesia)
      e) Rotarod (motor coordination, learning)
      f) Metrazol (seizure susceptibility)
      g) Startle Response (auditory reflex)
      h) Pre-pulse Inhibition (schizophrenic-like processing)
   2. Cohort
      a) 10 KO males, 10 WT males
      b) Age: 10 weeks

C. Expression Analysis
   All targets have either lacZ, RT-PCR or TaqMan data. Some targets have combinations of lacZ, RT-PCR and TaqMan data.
   1. Lac-Z
   2. RT-PCR RT-PCR
   3. TaqMan

D. Developmental Lethality
   1. Identification of stage of juvenile, perinatal or embryonic lethality.
   2. HETS are analyzed in place of KOs for 7 week clinical findings and pathology, behavior and aging programs.

E. Fertility
   1. Cohort: 3 male KOs, 3 female KOs.
   2. Score the number of pups born and the number of pups weaned, up to 6 months in mating.

F. Aging
   1. Procedures
      a) Clinical chemistry: 49 day, 3 month, 6 month, 10 month
      b) Hematology: 49 day, 4 month, 7 month, 10 month
      c) Mouse Metrics (growth curves): 49 day, 4 month, 7 month, 10 month
      d) Home Cage Observations: acquired over the lifetime of the animal
      e) Physical Examination: 10 month

f) Necropsy and Histopathology: 10 month
g) Densitometry and X-ray: 10 month

2. Cohort
   a) 16 mice total (4 KO males, 4 KO females, 4 WT males, 4 WT females)
   b) 8 mice sent to necropsy (2 KO males, 2 KO females, 2 WT males, 2 WT females)

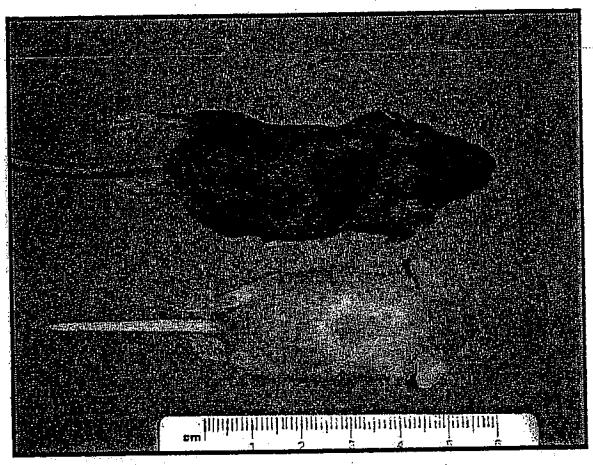
EXHIBIT D
DELTAGEN'S PHENOTYPIC ANALYSIS PROTOCOLS

(Pages Following)

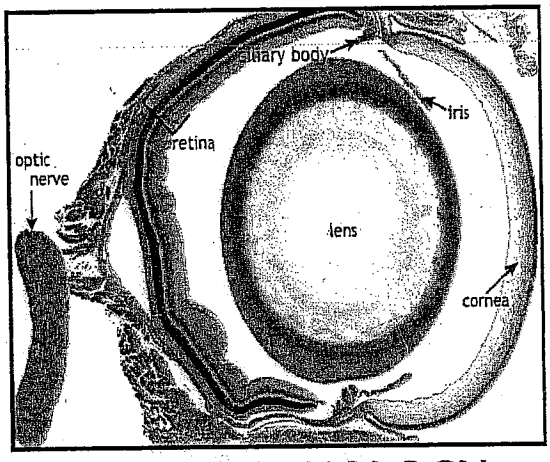
Deltagen

Gene Function to Drug Discovery™

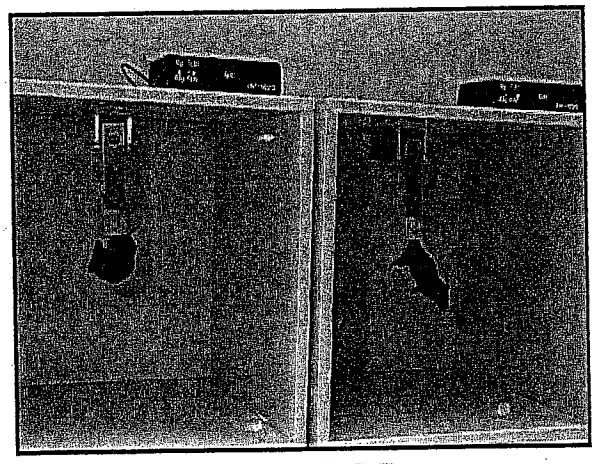
Phenotypic Analysis Protocols



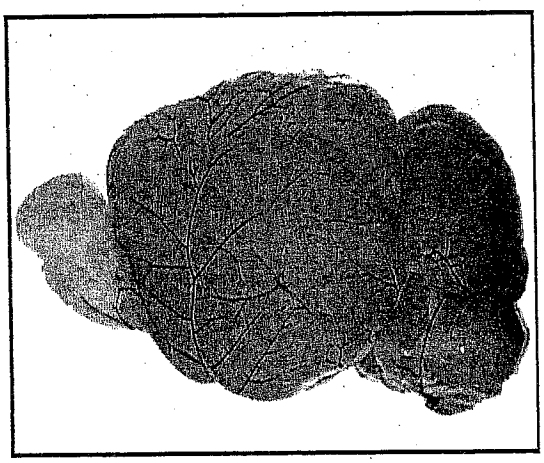
PHYSICAL EXAM



HISTOPATHOLOGY



BEHAVIOR



EXPRESSION

Section 1: Overview

Deltagen Mice

We use two sequences of matings to produce experimental animals:

1. In general, the first sequence is for the 49-day-old necropsy cohort and consists of an outcross then intercross.
2. The second sequence is for phenotypic programs other than necropsy and consists of an outcross, then backcross, then intercross (occasionally, this breeding also provides the necropsy mice).

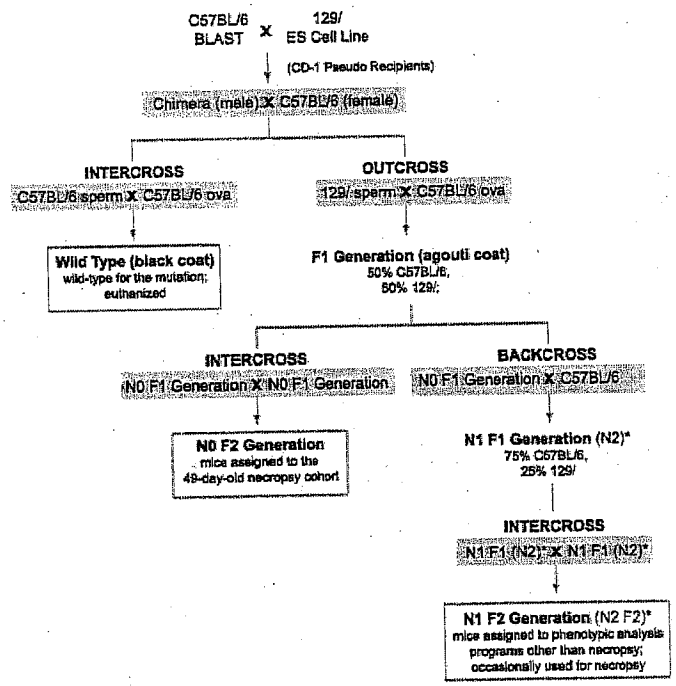
Blastocyst strain = C57BL/6
ES strain = 129
Male chimeras (producing C57BL/6 and 129 sperm) are mated to C57BL/6 females

Inbred offspring result from C57BL/6 sperm fertilizing the C57BL/6 ova. These pups are black and are sacrificed. Offspring that result from the outcross of the 129 sperm and the C57BL/6 ova are the F1 generation and have an agouti coat color. The agouti pups are genotyped.

F1 animals are 50% C57BL/6 and 50% 129. Male and Female F1 heterozygotes are then mated and produce the F2 generation, which are assigned to the 49-day-old necropsy cohort. (Deltagen refers to these mice as N0 generation).

The F1 heterozygous males are then backcrossed to C57BL/6 females. The resultant offspring are what Deltagen refers to as N1 F1s. Using conventional nomenclature, these would be referred to as N2s (the second generation back from the first cross).

N1 F1s are then genotyped and the carrier mice are identified and then mated to each other. The resultant offspring are what Deltagen refers to as N1 F2s. These offspring are then assigned to the other phenotypic analysis programs. In conventional nomenclature, these would be referred to as N2 F1s.



*N and F numbers in red [e.g., (N2 F2)] indicate corresponding conventional nomenclature.
Other N and F numbers refer to Deltagen's nomenclature.

Figure 1. Deltagen mating strategy

Deltagen Phenotypic Analysis Protocols

About Deltagen's Nomenclature
Both Deltagen nomenclature and the conventional nomenclature designate the same number of generations removed from the founder (three), but the Deltagen nomenclature does not describe the sequence of the type of crosses (outcross, backcross, intercross).

Backcross
Breeding back to a parental line (e.g., breed F1 or F2, etc., to a C57BL/6); offspring are N1 (in Deltagen notation, first backcross)

F#
Filial generation number; the term Filial in genetics refers to a generation or the sequence of generations following the parental (founder) generation.

F1 Generation
The first filial generation; refers to offspring produced by crossing two unrelated parental (founder) lines.

F2 Generation
The second filial generation; refers to offspring that result from mating brothers and sisters from the F1 generation. Twenty generations of brother-sister mating results in an inbred strain.

Intercross
Breeding siblings together (e.g., from the initial outcross); offspring are F2, F3, etc.

N#
Backcross generation number; designates a series of backcross events leading to a particular generation. Backcrossing refers to the mating of an offspring back to one of the parental (founder) lines.

N1 Generation
The progeny that results from the initial backcross of the F1 generation.

N2 Generation
The progeny of an N1 generation crossed to the same parental (founder) line (serial backcrosses).

Outcross
Breeding two different "founders" or "parental lines" together; N# does not apply.

Section 2: First Pass Phenotypic Analysis

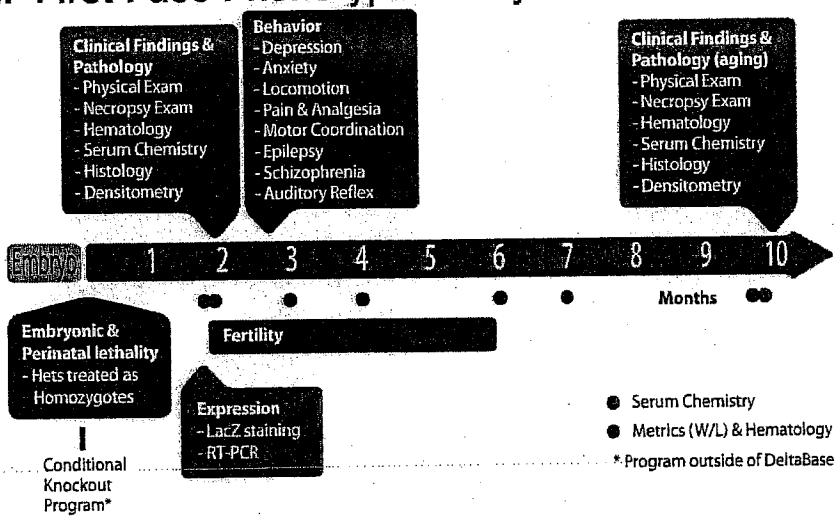


Figure 2. First Pass Phenotypic Analysis Timeline

A. Clinical Findings and Pathology

Physical Examination

Mice are first observed in their home cages for a number of general characteristics such as activity level, behavior toward siblings, posture, grooming, breathing pattern and sounds, and movement. General body condition and size are noted as well. Following a visual inspection of the mouse in the cage, the mouse is handled for a detailed, stepwise examination. The head is examined first, including eyes, ears, and nose, noting any discharge, malformations, or other abnormalities. Lymph nodes and glands of the head and neck are palpated. Skin, hair coat, axial and appendicular skeleton, and abdomen are also assessed. The limbs and torso are palpated for masses, malformations or other abnormalities. The anogenital region is examined for discharges, staining of hair, or other changes. If the mouse defecates during the examination, the feces are assessed for color and consistency. Abnormal behavior, movement, or physical changes may reveal defects in general health, growth, metabolism, motor reflexes, sensory systems, or development of the central nervous system.

Prior to necropsy, each mouse undergoes visual inspections of the following observables, the results of which are collected and recorded in DeltaBase:

Table 1. Physical exam observables

Physical Examination (46 observables)

Observable	Observable (cont'd)	Observable (cont'd)
mice in cage	head shape	forelimb number of digits - left
behavior	snout	forelimb number of amputated digits - left
locomotor	whiskers	hindlimb - right
urine exam	teeth color	hindlimb number of digits - right
feces exam	teeth length	hindlimb number of amputated digits - right
general	eye - left	hindlimb - left
respiration	eye - right	hindlimb number of digits - left
body shape	eye color - left	hindlimb number of amputated digits - left
lesions	eye color - right	claws
swelling - joints	ear - left	anus
lumps - masses	ear - right	mammary glands exam
coat color - back	limb shape	genitals - male
coat color - belly	forelimb - right	genitals - female
coat - fur	forelimb number of digits - right	tail
hair type	forelimb number of amputated digits - right	
skin appearance	forelimb - left	

Necropsy Exam

The necropsy, or postmortem examination, is performed following deep general anesthesia, cardiac puncture for terminal blood collection, and euthanasia. Body length and body weight are recorded for each mouse. The necropsy, which is done in a stepwise fashion, includes detailed examination of the whole mouse, the skinned carcass, skeleton, and all major organ systems. Significant lesions and abnormal findings in organs and tissues are noted during the examination. When an organ appears normal, the term "NSA" or "No Significant Abnormalities" is chosen. Terms such as mass, exudate, discoloration, and others are chosen as appropriate to describe changes in organs. Designated organs, from which extraneous fat and connective tissue have been removed, are weighed on a Metler 333 balance; the weight values are downloaded directly into the database. Organs are fixed in 10% neutral buffered formalin (see complete list below) and are then submitted to the histopathology laboratory for processing.

The following organs are weighed and values are expressed relative to body weight:

- Kidney
- Heart
- Thymus
- Liver
- Spleen

During necropsy, each mouse undergoes the following visual inspections, the results of which are collected and recorded in DeltaBase:

Table 2. Necropsy Observables. Note: White adipose tissue and brown adipose tissue have been added to the required observables.

Necropsy Exam (50 observables)

Observable
body weight
body length
skin exam
skinned mouse exam
lymph nodes exam
salivary glands exam
spleen exam
spleen weight
pancreas exam
liver exam
liver weight
gallbladder exam
penis exam
epididymis, seminal vesicle exam
vagina exam
uterus exam
testes exam
testes, epididymis weight
ovaries exam
urinary bladder exam
urine exam
stomach exam
duodenum exam
jejunum exam
ileum exam
cecum exam
colon exam

Observable (cont'd)
mesentery exam
kidneys exam
kidney weight
adrenal glands exam
thymus exam
thymus weight
heart exam
heart weight
lungs exam
trachea exam
esophagus exam
tongue exam
skeletal muscle exam
sciatic nerve exam
bone, sternum exam
bone, vertebral column exam
bone, stifle joint exam
bone, femur exam
bone, cranium exam
eyes exam
harderian glands exam
brain exam
bone marrow smear exam

Serum Chemistry

Blood samples are collected via a terminal cardiac puncture in a syringe. One hundred microliters of each whole blood sample are transferred into a tube pre-filled with EDTA (see Hematology protocol). The remainder of the blood sample is converted to serum by centrifugation in a serum tube with a gel separator. Each serum sample is then analyzed as described below. Non-terminal blood samples for the aging program are collected via retro-orbital venous puncture in capillary tubes. This procedure yields approximately 200μL of whole blood that is either transferred into a serum tube with a gel separator for serum chemistry analysis (see below), or into a tube pre-filled with EDTA for hematology analysis (see Hematology protocol).

Table 3. Serum chemistry analytes measured

Serum Chemistry

Electrolytes		**Liver Function (Enzymes)**	
Sodium	Na	Alkaline Phosphatase	ALP
Potassium	K	Alanine Aminotransferase	ALT
Chloride	Cl	Aspartate Transferase	AST
Bicarbonate	Bicarb	Lactate Dehydrogenase	LD
Renal Function Tests		**Liver Function (Other)**	
Blood Urea Nitrogen	BUN	Protein, Total	T Prot
Creatinine	Creat	Albumin	Alb
Osmolality	Osm	Globulin	Glob
		Bilirubin, Total	Bil T
Inorganic Ions			
Calcium	Ca		
Phosphorus	Phos	**Lipid Profile**	
		Cholesterol	Chol
Other		High density Lipoproteins	HDL
Glucose	Glu	Low density Lipoproteins	LDL
Creatine Kinase	CK	Triglycerides	TG

Summary of Assays Performed on Serum Samples.

The parameters measured with the amount of serum required for each test are indicated. Serum data are collected on a Hitachi 912 Automatic Analyzer using Boehringer Mannheim Corporation reagents. The type of assay is briefly stated below.

Reference Intervals

Reference intervals, determined according to NCCLS C28-A guidelines, are presented in the database in the report entitled "Phenotypic Ranges – Serum Chemistry", partitioned by age bin and gender.

Notes

Random glucose measurements are obtained. Hemolysis may affect the determinations of alkaline phosphatase, creatine kinase, glucose, phosphorus, potassium, and total protein, with smaller effects on calcium and sodium. Platelet lysis may affect potassium determinations. Determinations of aspartate aminotransferase and creatine kinase may be affected by release from muscle, such as can result from handling. Incorporation of data for HDL, LDL, and triglyceride determinations were begun in March 2000. Blank space(s) may be intentionally left when values are below the lower limit of detection for a test and cannot be discretely represented; a comment is made in the summary instead (e.g., Creat < 0.1 or T Bili < 0.1).

Table 4. Serum Chemistry Abbreviations and Methods

Analyte (Abbreviation)	Units	Method
Alanine Aminotransferase (ALT)	IU/L	α-Ketoglutarate and L-Ala (Modified IFCC)
Albumin (Alb)	g/dL	Bromocresol Green
Alkaline Phosphatase (ALP)	IU/L	*p*-Nitrophenolphosphate Substrate, AMP Buffer
Aspartate Transferase (AST)	IU/L	α-Ketoglutarate and L-Asp (Modified IFCC)
Bicarbonate (Bicarb)	mg/dL	Phosphoenolpyruvate Carboxylase
Bilirubin, Total (Bil T)	mg/dL	Dichlorophenyl Diazonium
Blood Urea Nitrogen (BUN)	mg/dL	Urease-glutamtate dehydrogenase, Kinetic
Calcium (Ca)	mg/dL	*o*-Cresolphthalein Complexone
Chloride (Cl)	mmol/L	Ion-Selective Electrode, Indirect
Cholesterol (Chol)	mg/dL	Cholesterol Oxidase
Creatine Kinase (CK)	IU/L	Creatine Phosphate and ADP Substrates
Creatinine (Creat)	mg/dL	Jaffe, Alkaline Picrate
Globulin (Glob)	g/dL	Calculated (= T Prot – Alb)
Glucose (Glu)	mg/dL	Hexokinase
High Density Lipoproteins (HDL)	mg/dL	Homogeneous Enzymatic Colorimetric Test
Lactate dehydrogenase (LD)	IU/L	Lactate and NAD in Tris (modified Gay et al)
Low Density Lipoproteins (LDL)	mg/dL	Homogeneous Enzymatic Colorimetric Test
Osmolality (Osm)	mOsm/Kg	Calculated (= 1.86Na + Glu/18 + BUN/2.8)
Phosphorus (Phos)	mg/dL	Ammonium Phosphomolybdate Colorimetric Test
Potassium (K)	mmol/L	Ion-Selective Electrode, Indirect
Protein, Total (T Prot)	g/dL	Modified Biuret Reaction (Weichselbaum)
Sodium (Na)	mmol/L	Ion-Selective Electrode, Indirect
Triglycerides	mg/dL	Enzymatic Colorimetric Test (modified Wahlefeld)

Hematology

The mice proceed from the general appearance procedure to the necropsy program. Blood samples are collected via a terminal cardiac puncture in a syringe. One hundred microliters of each whole blood sample are transferred into tubes pre-filled with EDTA. Approximately 25μL of the blood is placed onto a glass slide to prepare a peripheral blood smear. The blood smears are later stained with Wright's Stain that differentially stains white blood cell nuclei, granules and cytoplasm, and allows the identification of different cell types. The slides are analyzed microscopically by counting and noting each cell type in a total of 100 white blood cells. The percentage of each of the cell types counted is then calculated. Red blood cell morphology is also evaluated. The rest of the EDTA-anticoagulated whole blood sample is used to perform the hematology tests described below. The remainder of the blood in the syringe is converted to serum for chemistry analysis (see Serum Chemistry protocol). Blood samples for the aging program are collected via retro-orbital venous puncture in capillary tubes. This procedure yields approximately 200μL of whole blood that is either transferred into a tube pre-filled with EDTA for hematology analysis, as described below, or into a serum tube for serum chemistry analysis (see Serum Chemistry protocol).

Blood Analysis Program
Whole blood is analyzed using a Cell-Dyn 3700 Hematology Analyzer. This instrument has been calibrated for mouse blood parameters. Numerical values are given for each data point.

Table 5. Hematology Parameters

Hematology

A) The overall white, red, and platelet counts

Measurement	Abbreviation	Units
white blood cells	WBC	* 10^3/microliter
red blood cell morphology	RBC Morph	
red blood cells	RBC	* 10^6/microliter
hemoglobin	HGB	grams/deciliter
hematocrit	HCT	percent
mean corpuscular volume	MCV	femtoliter
mean corpuscular hemoglobin	MCH	picograms
mean corpuscular hemoglobin concentration	MCHC	grams/deciliter
platelets	plt	estimate or * 10^3/microliter

B) Differential Cell Count

Measurement	Abbreviation	Units
neutrophils	Neut	percent
lymphocytes	Lymphocytes	percent
monocytes	Monocytes	percent
eosinophils	EOS	percent
basophils	BASO	percent
absolute neutrophils	Abs Neut	* 10^3/microliter
absolute lymphocytes	Abs Lymph	* 10^3/microliter
absolute monocytes	Abs Mono	* 10^3/microliter
absolute eosinophils	Abs EOS	* 10^3/microliter
absolute basophils	Abs BASO	* 10^3/microliter

Histology

Harvested organs are fixed in 10% neutral buffered formalin for a minimum of 48 hours at room temperature. Tissues are trimmed and samples taken to include the major features of each organ. If any abnormalities are noted at necropsy or at the time of sampling, additional sample(s), if necessary, will be taken to include the abnormality so that it is available for microscopic analysis. Tissues are grouped together in tissue processing cassettes. Bones and calcified tissues are decalcified with a formic acid or EDTA-based solution prior to processing.

The infiltration of the tissues by paraffin is performed using an automated tissue processor. Steps in the cycle include dehydration through a graded series of ethanols, clearing using xylene or xylene substitute and infiltration with paraffin. Tissues are embedded in paraffin blocks with a standard orientation of specified tissues within each block. Sections are cut from each block at a thickness of 3-5μm and mounted onto glass slides. After drying, the slides are stained with hematoxylin and eosin (H&E) and a glass coverslip is mounted over the sections.

Bone Marrow
Sections of sternum and femur that contain marrow are collected. The samples are analyzed for cellularity, myeloid, erythroid, and megakaryocytic maturation series, and for normal development. The presence of mature lymphocytes, myeloid to erythroid (M:E) ratios and neoplastic or other abnormal cells are also evaluated.

Images
Predetermined images from representative wild-type and homozygous mutant mice for each target, regardless of the presence or absence of lesions, are included in DeltaBase. For targets with phenotypic alterations, images of individual lesions from the affected tissues are also recorded in DeltaBase.

Table 6. Histology Tissues and Images

Organ System	Organs/Tissues
Cardiovascular System	Aorta Heart - overview Heart - atrium Heart - ventricle
Digestive System	Salivary gland Esophagus Liver/Gallbladder Liver -central vein Liver -portal triad Gallbladder Pancreas - overview Pancreas - islets of Langerhans Stomach (junction and glandular) Stomach - forestomach Stomach - glandular Intestine, small - duodenum Intestine, small - jejunum Intestine, small - ileum Intestine, large - cecum Intestine, large - colon Intestine, large - rectum Tongue
Endocrine System	Adrenal gland - overview Adrenal gland - medulla Adrenal gland - cortex Pituitary gland Thyroid gland
Immune System	Lymph node - overview Lymph node - medulla Lymph node - cortex Spleen - overview Spleen - white pulp Spleen - red pulp Thymus - overview Thymus - medulla Thymus - cortex
Hematopoietic System	Bone Marrow (Sternal)
Integumentary System	Clitoral gland (if present) Harderian gland Mammary gland Preputial gland Skin

Densitometer

Deltagen uses the PIXImus™ densitometer. PIXImus utilizes Dual Energy X-ray absorptiometry (DEXA or DXA) technology. An x-ray source exposes the entire mouse to a beam of both high and low energy x-rays. The ratio of attenuation of the high and low energies allows the separation of bone from soft tissue, and, from within the tissue samples, lean and fat.

Equipment Vendor:
GE Lunar
726 Heartland Trail
Madison, WI 53717-1915 USA
Phone: (800) 445-8627
Fax: (608) 826-7102
www.gemedicalsystems.com

Procedure

A daily quality control procedure is performed each day before image acquisition is initiated. A phantom provided by GE Lunar specifically for the PIXImus is used for the procedure.
Each mouse is processed on the PIXImus densitometer following euthanasia. Specific information for each mouse, including identification number, date of birth, body length and body weight, is entered into the PIXImus program. The mouse is placed on a specimen tray, which is coated with an adhesive to prevent accidental repositioning prior to image acquisition. The subject's head is oriented to the left, the body is aligned so the spinal column is straight, and the tail is wrapped around the subject toward the head. The legs are always positioned slightly away from the body.
The specimen tray with the positioned mouse is then placed into the imaging area. Using the PIXImus program, the head of the mouse is excluded from the analysis region.

Acquisition of the image and body composition analysis takes approximately five minutes. The PIXImus program automatically displays the image and analysis results after a measurement is completed. If the position of the mouse in the image is not satisfactory, the mouse can be repositioned and the measurement repeated. After measurement, the mouse is transferred to a prosector for a postmortem examination.

Data Viewing and Data Analysis
Two images are displayed, one with outlines defining bone and soft tissue, and the other as a plain radiograph. Within the outlined image, the red rectangular outline defines the region of interest (ROI), which in most cases includes the whole mouse, with the exception of the head. The green oval area encircling the head defines a region excluded from analysis. The analysis results associated with the image are displayed as Bone (BMD or bone mineral density in g/cm2, BMC or bone mineral content in grams, and Area in cm2) and Tissue (Lean in grams, Fat in grams, Total in grams, and % Fat). This data is also presented in text format adjacent to the images, with the addition of tissue area (soft tissue) in g/cm2.

Evaluations of densitometric data included Bone Mineral Density (BMD presented as g/cm2), Bone Mineral Content (BMC in g), bone and tissue area, total tissue mass, and fat as a percent of body soft tissue (presented as fat %).

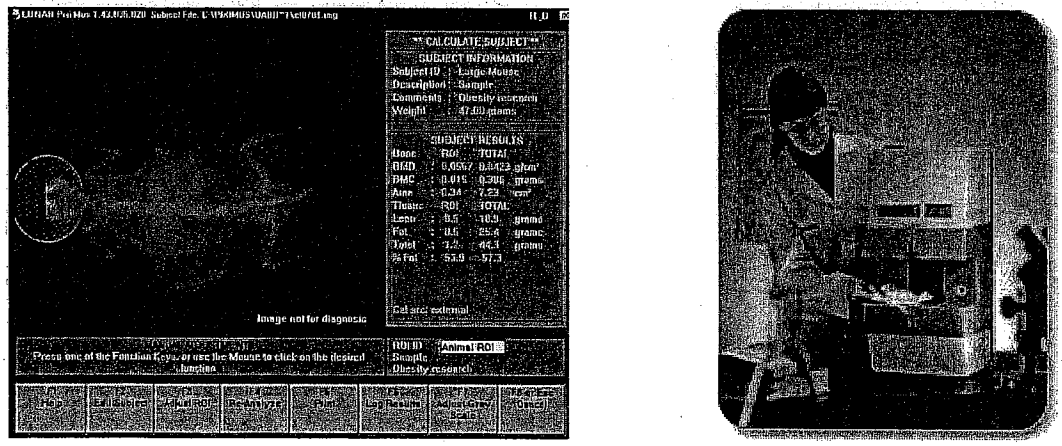


Figure 3. PIXImus and sample image

B. Embryonic and Perinatal Lethality

Embryonic Development

Procedure

If homozygous mutant mice are not identified at weaning (3-4 weeks old), animals are assessed for lethality linked with the introduced mutation. This evaluation includes embryonic, perinatal or juvenile death.

Perinatal Death

Newborn mice are genotyped 24-48 hours after birth and monitored closely for any signs of stress. Dead/dying pups are recorded and grossly inspected and if possible, genotyped. In the case of perinatal death, late gestation embryos (~E19.5) or newborn pups are analyzed, genotyped and subject to further characterization.

Embryonic Death

If there is no evidence of perinatal or juvenile lethality, heterozygous mutant mice are set up for timed pregnancies. Routinely, E10.5 embryos are analyzed for gross abnormalities and genotyped. Depending on these findings, earlier (routinely >E8.5) or later embryonic stages are characterized to identify the approximate time of death. If no homozygous mutant progeny are detected, blastocysts (E3.5) are isolated, genotyped directly or grown for 6 days in culture and then genotyped.

Any gross abnormalities which may be linked to the introduced mutation are described.

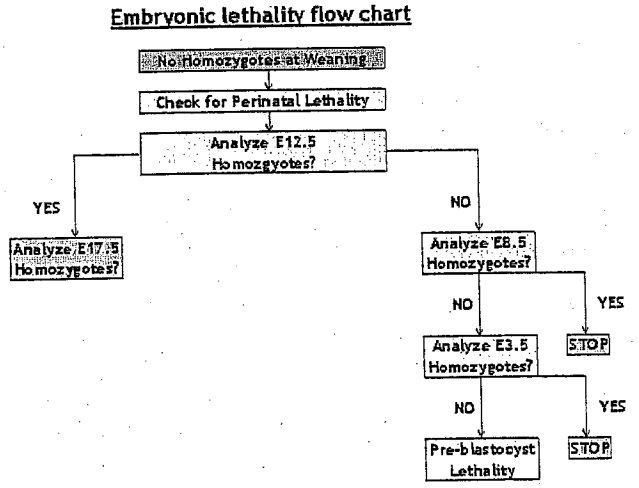


Figure 4. Embryonic Lethality flow chart

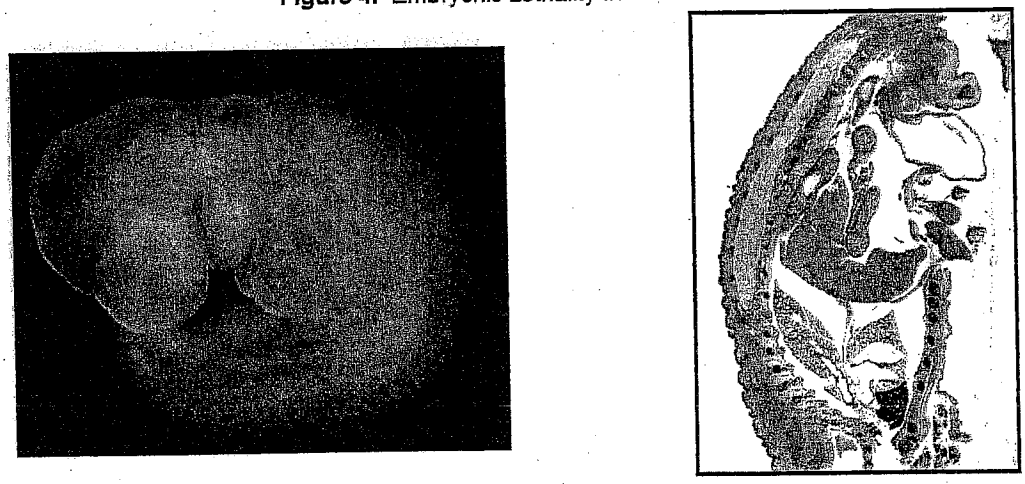


Figure 5. Sample Embryonic/Perinatal Lethality Images.

Fertility

Rationale

The reproductive traits of male and female homozygous mutant mice are tested to identify potential defects in spermatogenesis, oogenesis, maternal ability to support pre- or post-embryonic development, or mammary gland defects and ability of the female knockout mice to nurse their pups.

Standard procedures

Two to three homozygous mutant mice of each gender are set up in a fertility mating with either a wild-type C57BL/6 mate or a homozygous mutant mouse of the opposite gender at seven to eight weeks of age. The numbers of pups born from one to three litters are recorded at birth. Three weeks later, the live pups are counted and weaned.

Males and females are separated after they have produced two litters or at six months (26 weeks) of age, whichever comes first.

C. Behavior

Behavioral Procedures Overview

General points

All behavioral tests are performed on the same group of adult males 10-12 weeks (n=7-10 per group for WT and KO). The mice are run through the behavioral programs in the following order: open field test, tail suspension test, rotarod test, hot plate test, metrazol test. There is a 1-2 day rest period between the open field and tail suspension tests. Females are not studied due to variability caused by the estrous cycle. Males are group-housed, with no access to females post-weaning. Wild-type and homozygous mutant mice are not segregated by cage. For targets where adult male knockout mice cannot be obtained (i.e., early lethality), heterozygous males of the same age are substituted.

Background white noise is played throughout testing. Mice are allowed to habituate to the behavior room environment for 30 minutes prior to experimentation unless otherwise indicated. Chambers are sanitized with a 0.25% bleach solution before each test and between animals. At the end of each experiment, animals are returned to fresh cages. The experimenters change gloves before handling mice from different cages.

The background strains 129, C57BL/6 and the F1 hybrid (129 x C57BL/6) mice have been tested to establish baseline measurements. The standard tests are performed on the offspring of intercrossed F1 mice (F2N0 mice or F2N1 mice).

Reference

Crawley JN, Paylor, R. A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. Horm Behav 1997;31:197-211.

Open Field

Background

Testing in the open field is designed to examine overall locomotion and anxiety levels in mice.

Overall locomotion

Increases or decreases in total distance traveled over the test time are an indication of hyperactivity or hypoactivity, respectively.

Anxiety

The open field provides a novel environment that creates an approach-avoidance conflict situation in which the animal desires to explore, yet instinctively seeks to protect itself. The chamber is lighted in the center and has no places to hide other than the corners. A normal mouse typically spends more time in the corners and around the periphery than it does in the center. Normal mice, however, will venture into the central regions as they explore the chamber. Anxious mice spend most of their time in the corners, with almost no exploration of the center, whereas bold mice travel more, and show less preference for the periphery versus the central regions of the chamber.

Equipment

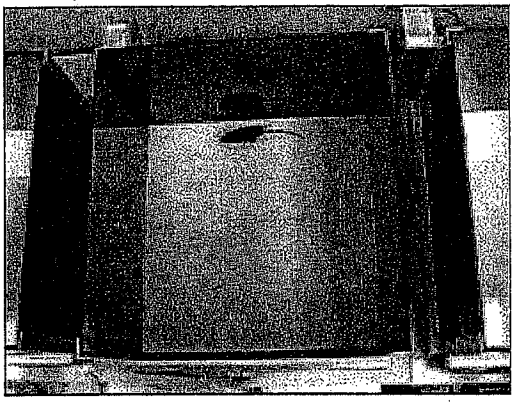


Figure 6: Open Field testing unit

Equipment Vendor
MED Associates, Inc.,
Post Office Box 319,
St. Albans, Vermont 05478
Phone (802) 527-2343 Fax (802) 527-5095,
www.med-associates.com

Open Field Test Environment (ENV-515): This open field test environment is constructed with opaque PVC,17" x 17" x 12"H (43.2 x 43.2 x 30.5 cm). The movement of animals is monitored by an array of 16 infrared detectors. The computer controller allows the operation of 8 independent chambers simultaneously.

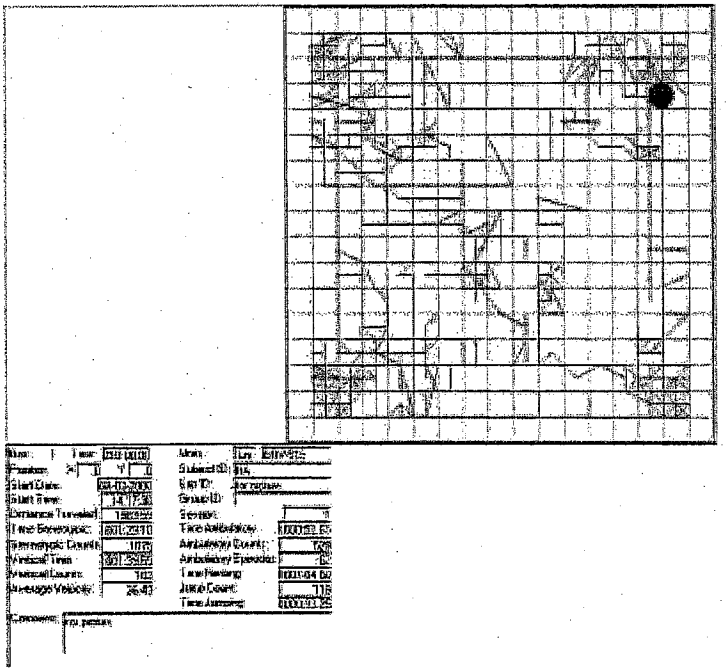


Figure 7. Sample computer output for the Open Field Test

Experimental Procedure

Mice are screened for behavioral abnormalities by evaluating the response to a novel environment using an open field test. Seven to ten adult wild-type and homozygous males are used in each experiment.

Animals are group housed prior to testing. Each animal is placed gently in the center of its assigned chamber. Tests are for 10 minutes, with the experimenter out of the animals' sight. Activity of individual mice is recorded for the 10-minute test session and monitored by photobeam breaks in the x-, y- and z-axes.

Measurements taken include total distance traveled, percent of session time spent in the central region of the test apparatus, and average velocity during the ambulatory episodes. Increases or decreases in total distance traveled over the test time may indicate hyperactivity or hypoactivity, respectively. Alterations in the regional distribution of movement may indicate anxiety phenotypes i.e., increased anxiety if there is a decrease in the time spent in the central region.

References

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Kash, S. F., L. H. Tecott, C. Hodge, and S. Baekkeskov . 1999. Increased anxiety and altered responses to anxiolytics in mice deficient in the 65-kDa isoform of glutamic acid decarboxylase. Proc Natl Acad Sci U S A 96:1698-1703.
Steiner, H., S. Fuchs, and D. Accili. 1997. D3 dopamine receptor-deficient mouse: evidence for reduced anxiety. Physiol Behav 63:137-41.
Stenzel-Poore, M. P., S. C. Heinrichs, S. Rivest, G. F. Koob, and W. W. Vale. 1994. Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. J Neurosci 14:2579-84.

Tail Suspension

Background

The tail suspension test is a single-trial test that measures a mouse's propensity towards depression. This method for testing antidepressants in mice was reported in Psychopharmacology 1985(85): 367 - 370 and is widely used for a range of compounds including SSRI's, benzodiazepines, typical and atypical antipsychotics. It is believed that a depressive state can be elicited in laboratory animals by continuously subjecting them to aversive situations over which they have no control. It is reported that a condition of "learned helplessness" is eventually reached.

Mice are suspended on a metal hanger by the tail in an acoustically and visually isolated setting. Total immobility time during the six-minute test period is determined using a computer algorithm based upon measuring the force exerted by the mouse on the metal hanger. An increase in immobility time for mutant mice compared to wild-type mice may indicate increased "depression." Animals that cease struggling sooner may be more prone to "depression". Studies have shown that the administration of antidepressants prior to testing increases the amount of time that animals struggle.

Equipment

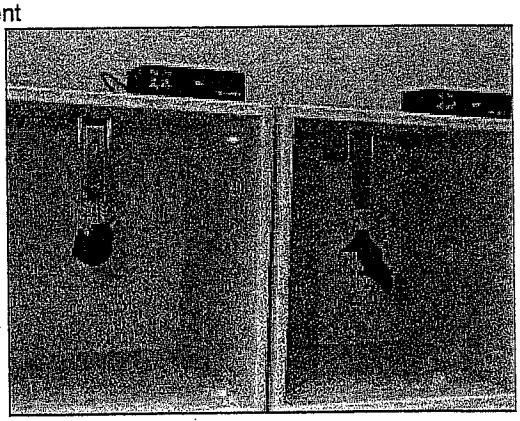

Figure 8. Tail Suspension testing unit

The Tail Suspension System automatically measures the duration and number of immobility episodes for mice and provides a measure of the energy expended by each animal (area under the curve). Total immobility time is calculated and reported in DeltaBase.

Equipment Vendor:
MED Associates, Inc.,
Post Office Box 319,
St. Albans, Vermont 05478
Phone (802) 527-2343 Fax (802) 527-5095,
www.med-associates.com

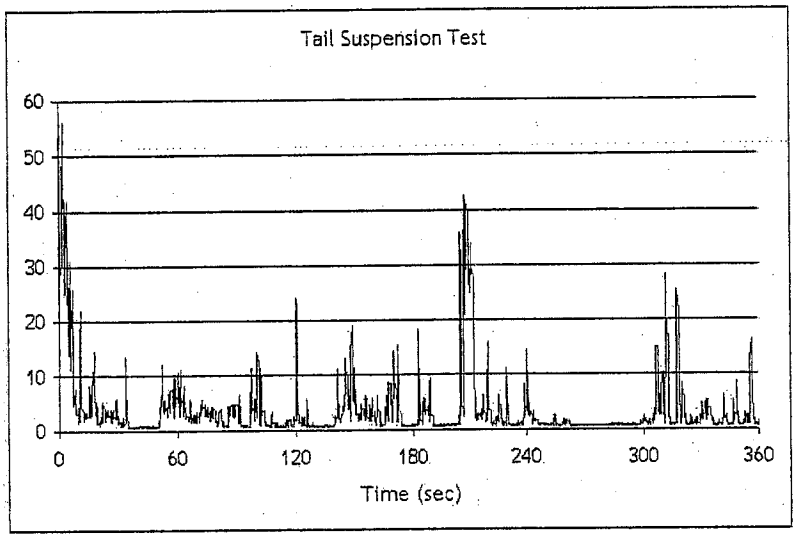

Figure 9. Sample computer output for Tail Suspension test

Procedure

The tail suspension test is performed to screen for phenotypes involving depression (either increased or decreased). Seven to ten adult wild-type and homozygous males are used. Animals are group housed prior to testing.

Each animal is suspended by the tail from the metal hanger of the tail suspension apparatus such that the end of the hanger is 1/8 of an inch or less from the base of the tail. Tests are 6 minutes in duration and the experimenter is out of the animals' sight. These data are analyzed to determine the total time immobile during the test period.

References

Ferrari, F., M. Cassinadri, P. L. Tartoni, and A. Tampieri. 1991. Effects of B-HT 920 in the tail-suspension test. Pharmacol Res 24:75-81.

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Heisler, L. K., H. M. Chu, T. J. Brennan, J. A. Danao, P. Bajwa, L. H. Parsons, and L. H. Tecott. 1998. Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. Proc Natl Acad Sci U S A 95:15049-54.

Nomura, S., H. Okada, R. Naruse, and K. Yamaoka. 1991. The tail suspension test for screening antidepressant drugs: comparison of movement in ICR and NMRI mice. Jpn J Psychiatry Neurol 45:113-4.

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Steru, L., R. Chermat, B. Thierry, and P. Simon. 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85:367-70.

Steru, L., R. Chermat, B. Thierry, J. A. Mico, A. Lenegre, M. Steru, P. Simon, and R. D. Porsolt. 1987. The automated Tail Suspension Test: a computerized device which differentiates psychotropic drugs. Prog Neuropsychopharmacol Biol Psychiatry 11:659-71.

Thierry, B., L. Steru, P. Simon, and R. D. Porsolt. 1986. The tail suspension test: ethical considerations. Psychopharmacology 90:284-5.

Rotarod

Background

The Rotarod test is designed to measure coordination and balance in mice. Animals are placed on a smooth rod that acts as a rotating treadmill. The rotarod rotates slowly at first then progressively increases in speed until it reaches a speed of 60 revolutions per minute. The mice must continually reposition themselves in order to keep from falling. Mice are motivated to stay on the rod and avoid falling. The animals are tested over three trials. Those mice that are generally able to stay on the rod the longest may have better coordination and balance than those that fall off early.

Equipment

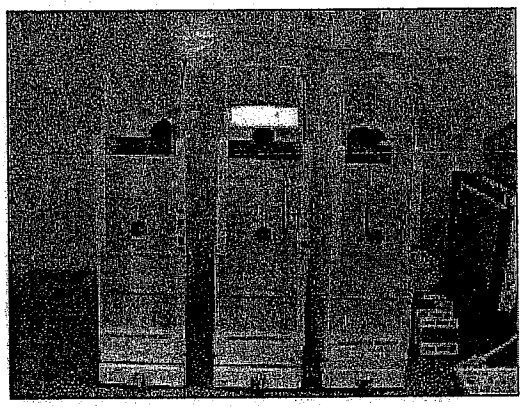

Figure 10. Accelerated Rotarod Testing Unit

The unit consists of an analyzer with 4 ports to connect up to 4 test chambers. Each test chamber is individually controlled. The analyzer is connected to a computer's serial COM port, and interfaced via Windows3.1 software. Light beams are used for sensing an animal's fall. Each test chamber records the time when the animal falls off the rotating rod (70 mm in diameter), and the speed of rod at the time of fall. Test chambers are fully enclosed so the animals are not able to jump out or see neighboring subjects.

Equipment Vendor:
AccuScan Instruments, Inc.,
5090 Trabue Road,
Columbus, OH 43228
Phone (800) 822-1344, Fax (614) 878-3560

Procedure

The Accelerating Rotarod is used to screen for motor coordination, balance and ataxia phenotypes. Seven to ten adult wild-type and homozygous males are used.

Mice are allowed to move about on their wire-cage top for 30 seconds prior to testing to ensure awareness. Mice are placed on the stationary rod, facing away from the experimenter. The "speed profile" programs the rotarod to reach 60 rpm after six minutes. Most mice fall from the rotarod between 30 and 90 seconds after the test is begun. A photobeam is broken when the animal falls, which stops the test clock for that chamber. The animals are tested over three trials with a 20-minute rest period between trials, after which the mice are returned to fresh cages. The data are analyzed to determine the average speed of the rotating rod at the fall time over the three trials. A decrease in the speed of the rotating rod at the time of fall compared to wild-types may indicate decreased motor coordination.

References

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Boyce, S., J. K. Webb, E. Carlson, N. M. Rupniak, R. G. Hill, and J. E. Martin. 1999. Onset and progression of motor deficits in motor neuron degeneration (mnd) mice are unaltered by the glycine/NMDA receptor antagonist L-701,324 or the MAO-B inhibitor R(-)-deprenyl. Exp Neurol 155:49-58

Liu, Y., A. Hoffmann, A. Grinberg, H. Westphal, M. P. McDonald, K. M. Miller, J. N. Crawley, K. Sandhoff, K. Suzuki, and R. L. Proia. 1997. Mouse model of GM2 activator deficiency manifests cerebellar pathology and motor impairment. Proc Natl Acad Sci U S A 94:8138-43.
Rozas, G., E. Lopez-Martin, M. J. Guerra, and J. L. Labandeira-Garcia. 1998. The overall rod performance test in the MPTP-treated-mouse model of Parkinsonism. J Neurosci Methods 83:165-75.

Hot Plate

Background

The hot plate analgesia test is designed to indicate an animal's sensitivity to a painful stimulus. The mouse is placed on a 55.5º C hot plate, and his latency to pick up and lick or fan a hindpaw is recorded (up to a 60 second maximum). Alterations in latency to hindpaw licking may indicate changes in pain thresholds.

Equipment

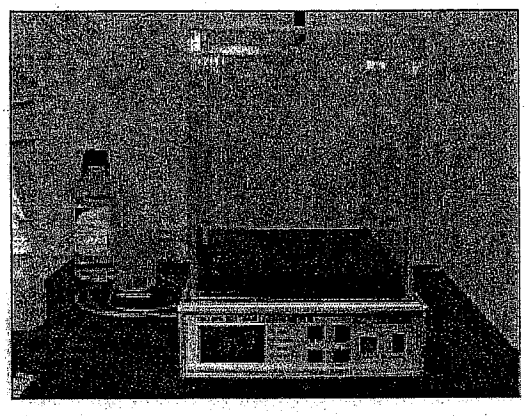

Figure 11. Hot Plate Analgesia Meter

The Hot Plate Analgesia Meter utilizes a metal plate which can be heated up to 60 degrees C. A built-in thermostat maintains the plate's temperature and a front panel thermometer displays the current temperature.

Equipment Vendor:
Columbus Instruments,
950 North Hague Ave.,
Columbus, OH 43204
Phone (614) 276-0861 , Fax (614) 276-0529,
www.colinst.com

Procedure

Seven to ten adult wild-type and homozygous males are tested. The hot plate is preheated to 55.5°C. Mice are placed on the hot plate surface one at a time, and the operator starts a built-in timer. The operator stops the timer at the instant the animal lifts its paw from the plate, reacting to the discomfort. The front panel timer displays the number of seconds it took the animal to react. Animal reaction time is a measurement of the animal's resistance to pain. The time points to hindpaw licking or fanning, up to a 60-second maximum, is recorded. Once the behavior has been observed, the animal is immediately removed from the hot plate to prevent discomfort or injury. The data are analyzed to determine the latency to hindpaw licking.

References

Clark, J.D. and B.L. Tempel. Hyperalgesia in mice lacking the Kv1.1 potassium channel gene. Neurosci Lett. 1988. 251:121-124.
Konig, M., A. M. Zimmer, H. Steiner, P. V. Holmes, J. N. Crawley, M. J. Brownstein, and A. Zimmer. 1996. Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin. Nature 383:535-8.
Sora, I., N. Takahashi, M. Funada, H. Ujike, R. S. Revay, D. M. Donovan, L. L. Miner, and G. R. Uhl. 1997. Opiate receptor knockout mice define mu receptor rolesin endogenous nociceptive responses and morphine-induced analgesia. Proc Natl Acad Sci USA.

Startle Response Test

Sound Response Profile

Mice are tested in a San Diego Instruments SR-LAB sound response chamber. Each mouse is exposed to 9 stimulus types that are repeated in pseudo-random order ten times during the course of the entire 25 minute test. The stimulus types in decibels are:

p80
p90
p100
p110
p120
pp80p120
pp90p120
pp100p120

where p=40 msec pulse, pp=20 msec prepulse

The length of time between a prepulse and a pulse is 100 msec (onset to onset).

The mean Vmax of the ten repetitions for each trial type is computed for each mouse.

Prepulse Inhibition

The % prepulse inhibition (PPI) compared to p120 alone is computed for each mouse at three prepulse levels from the mean Vmax values and this is presented in a chart. This is computed by determining the mean "p120", "pp80p120", "pp90p120", and "pp100p120" value for each mouse and then producing the ratios of % inhibition.

Example ((p120-pp80p120)/p120) X 100)

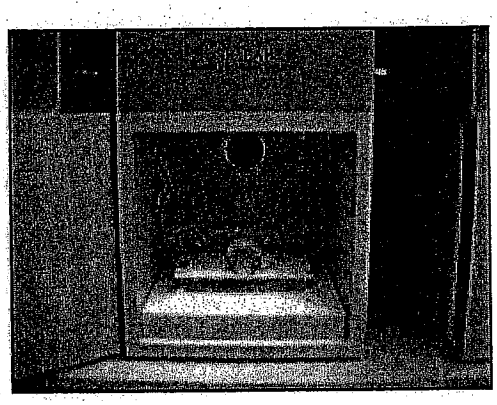

Figure 12. PPI testing unit

Pentylenetetrazole (Metrazol) Testing

Background

To screen for phenotypes involving changes in seizure susceptibility, the Metrazol Test is used. A 5mg/ml solution of Metrazol is infused through the tail vein of the mouse at a constant rate of 0.375ml/min. The infusion will cause all mice to experience seizures. Those mice that enter the seizure stage the quickest are thought to be more prone to seizures in general.

Four clear physiological stages are recorded during the course of the experiment. They are: first twitch, tonic-clonic seizure, tonic extension and death (respiratory arrest). Decreased latency to the seizure phases is suggestive of an overall imbalance in excitatory or inhibitory neurotransmitter levels.

Procedure

To screen for phenotypes involving epilepsy, the Metrazol test is used. Seven to ten adult wild-type and homozygote males will be used. A fresh solution of 5mg/ml pentylenetetrazole in 0.9% NaCl is prepared prior to testing. Mice are weighed and loosely held in a restrainer. After exposure to a heat lamp to dilate the tail vein, mice are continuously infused with the pentylenetetrazole solution using a syringe pump set at a constant flow rate. The following stages are recorded: first twitch (sometimes accompanied by a squeak), beginning of the tonic/clonic seizure, tonic extension and survival time. The dose required for each phase is determined and the latency to each phase is determined between genotypes. Alterations in any stage may indicate an overall imbalance in excitatory or inhibitory neurotransmitter levels.

References

Kash, S. F., R. S. Johnson, L. H. Tecott, J. L. Noebels, R. D. Mayfield, D. Hanahan, and S. Baekkeskov. 1997. Epilepsy in mice deficient in the 65-kDa isoform of glutamic acid decarboxylase. Proc Natl Acad Sci U S A 94:14060-5.

Tecott, L.H., L. M. Sun, S. F. Akana, A. M. Strack, D. H. Lowenstein, M. F. Dallman and D. Julius. 1995. Eating disroder and epilepsy in mice lacking 5-HT2c serotonin receptors. Nature. 374:542-6.

D. Expression Analysis

Expression Analysis Overview

Deltagen conducts gene expression analysis on all targets using either the knocked-in lacZ as a reporter gene, RT-PCR or TaqMan expression analysis. In the case of lacZ expression assays, no signals may be detected due to insertional silencing or insertional mutations.

LacZ Reporter Gene Expression Analysis

In general, tissues from 7-12 week old heterozygous mutant mice are analyzed for lacZ expression. Organs from heterozygous mutant mice are frozen, sectioned (10 μm), stained and analyzed for lacZ expression using X-Gal as a substrate for beta-galactosidase, followed by a Nuclear Fast Red counterstaining.

In addition, wholemount staining is performed for brain. The dissected brain is cut longitudinally, fixed and stained using X-Gal as the substrate for beta-galactosidase. The reaction is stopped by washing the brain in PBS and then fixed in PBS-buffered formaldehyde.

Wild-type control tissues are also stained for lacZ expression to reveal any background or signals due to endogenous beta-galactosidase activity. The following tissues can show staining in the wild-type control sections and are therefore not suitable for X-gal staining: small and large intestines, stomach, vas deferens and epididymis. It has been previously reported that these organs contain high levels of endogenous beta-galactosidase activity.

Tissues Analyzed for lacZ Expression:
Brain, sciatic nerve, eyes, Harderian glands, thymus, spleen, lymph nodes, bone marrow smear, aorta, heart, lung, liver, gallbladder, pancreas, kidney, urinary bladder, trachea, larynx, esophagus, thyroid gland, pituitary gland, adrenal glands, salivary glands, tongue, skeletal muscle, skin and reproductive system.

RT-PCR Expression Analysis

Total RNA was isolated from the organs/tissues listed below from adult C57Bl/6 wild type mice. RNA is DNaseI treated and reverse transcribed using random primers. The resulting cDNA is checked for the absence of genomic contamination using primers specific to non-transcribed genomic mouse DNA . cDNAs are assayed for both, quality and concentration by RT-PCR for the ubiquitously expressed gene hypoxanthine-guanine phosphoribosyltransferase (HPRT) or beta-actin.

List of tissues analyzed by RT-PCR:
Brain, cortex, subcortical region, cerebellum, brainstem, olfactory bulb, spinal cord eyes, heart, lung, liver, pancreas, kidneys, spleen, thymus, lymph nodes, bone marrow, skin, gallbladder, urinary bladder, pituitary gland, adrenal gland, salivary gland, skeletal muscle, tongue, stomach, small intestine, large intestine, cecum, testis, epididymis, seminal vesicle, coagulating gland, prostate gland, ovaries, uterus and white fat.

Primers and Conditions

Primers and conditions for genomic contamination check:

5' Primer: oligo 18945 TGATGTGTAACCTAACCGCC
3' Primer: oligo 18948 GCAAGAGGACTCAGAGAGGT

Band size of cDNA: no band
Band size of genomic DNA: 600 bp

Cycle conditions for PE9700:

96ºC (8 sec), 55 ºC (10 sec), 72 ºC (60 sec) x 30; 72ºC (10 min) x 1

Primers and conditions for house-keeping genes:

2a) HPRT
5' Primer: oligo 6363 TTCTGGGCCTCGGCCTTTGA
3' Primer: oligo 6325 GCTGGTGAAAAGGACCTCT
Band size of cDNA: 575 bp
Band size of genomic DNA: ~1400 bp
Cycle conditions for PE9700:
96ºC (8 sec), 55 ºC (10 sec), 72 ºC (60 sec) x 30; 72ºC (10 min) x 1

2b) Beta actin
5' Primer: oligo 40870 CACAGCTTCTTTGCAGCTCCTT
3' Primer: oligo 40871 ATGCCGGAGCCGTTGTC
Band size of cDNA: 101 bp
Band size of genomic DNA: none

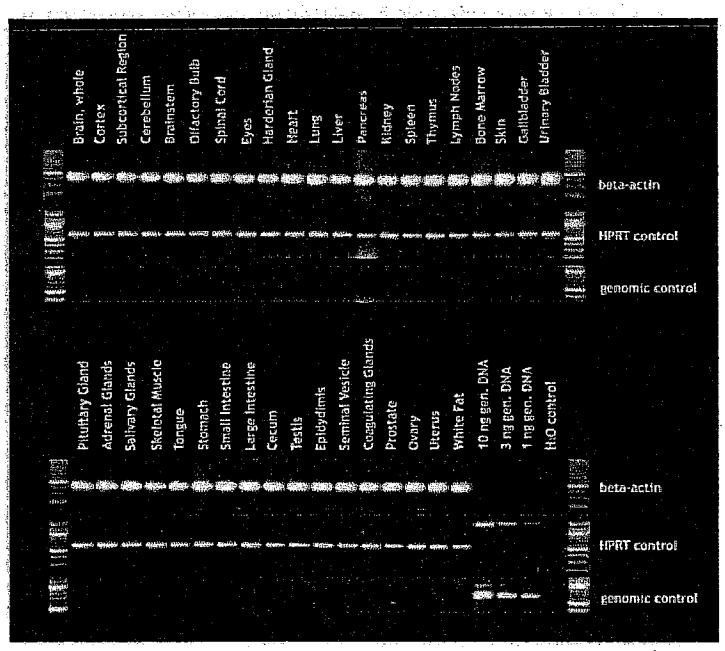

Figure 13. RT-PCR Controls

Use of TaqMan assay to determine relative gene expression

Total RNA is isolated from tissues using standard methods. Before cDNA synthesis, the total RNA is subject to a DNAsel treatment to remove all traces of genomic DNA. cDNA is then synthesized using random primers and standard methods.

To create a relative normalization of the cDNA synthesized, RT-PCRs are performed on a standard endogenous gene (e.g. Transferrin Receptor) using the Taqman approach. The normalized cDNA is then assayed for the Target gene sequence. Primer-probe combinations are chosen to be 3' of the Gene Trap insertion event. Serial dilutions of cDNA, each in quadruplet, are assayed for each sample. From this the primer -probe efficiency can be ascertained for each gene under test.

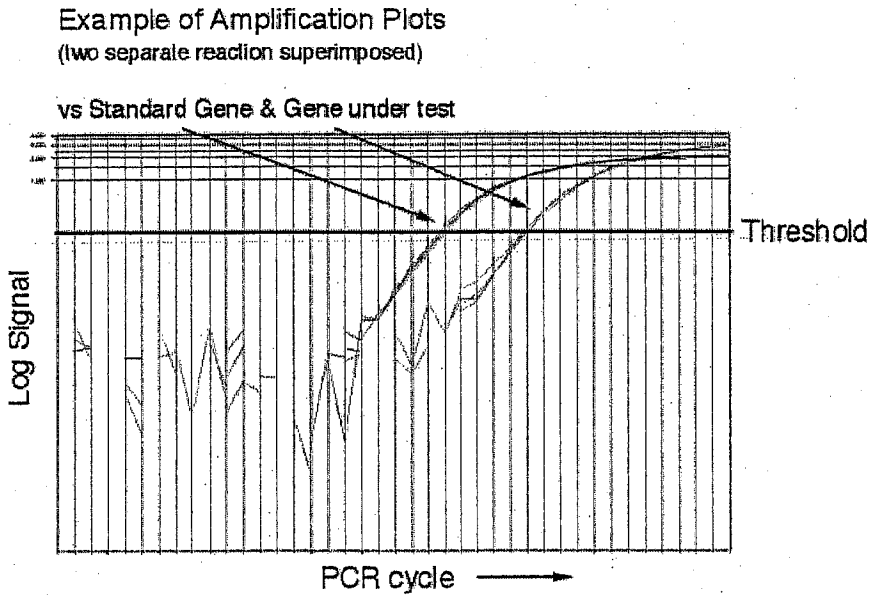

Figure 14. RT-PCR expression was assayed using a using a Taqman approach with a ABI 7900 machine and SDS software.

The Ct value is derived from where the Threshold intercepts the log phase of the PCR reaction. This value is directly proportional to the initial copy number of the target sequence. The Ct is then plotted vs. the Log10 of the relative cDNA concentration. From the slope, the PCR efficiency can be calculated. The intercept point is equivalent to the initial abundance of the target sequence.

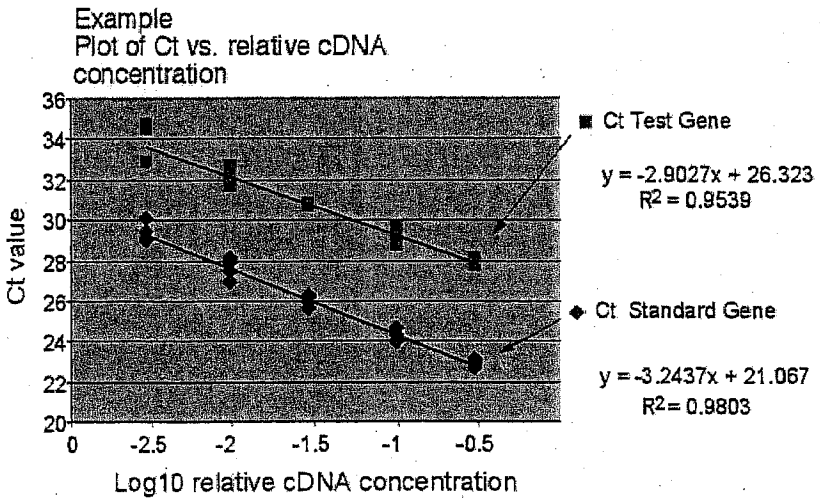

Figure 15. Plot of Ct vs. relative cDNA concentration

EXHIBIT E
DELTAGEN'S PHENOTYPIC DATA SET CONTENT

Physical Exam

1. General Behavior
2. Locomotion
3. Urine exam
4. Feces exam
5. General appearance
6. Respiration
7. Body shape
8. Lesions
9. Joints
10. Lumps – masses
11. Coat color – back
12. Coat color – belly
13. Coat – fur
14. Hair type
15. Skin appearance
16. Head shape
17. Snout
18. Whiskers
19. Teeth color
20. Teeth length
21. Eye – left
22. Eye – right
23. Eye color – left
24. Eye color – right
25. Ear – left
26. Ear – right
27. Limb shape
28. Forelimb – left
29. Forelimb # of digits – left
30. Forelimb # of amputated digits - left
31. Forelimb – right
32. Forelimb # of digits – right
33. Forelimb # of amputated digits – right
34. Hindlimb – left
35. Hindlimb # of digits – left
36. Hindlimb # of amputated digits – left
37. Hindlimb – right
38. Hindlimb # of digits – right
39. Hindlimb # of amputated digits – right
40. Claws
41. Anus
42. Mammary glands exam
43. Genitals – male
44. Genitals – female
45. Tail

Necropsy Exam

1. Body weight
2. Body length
3. Skin exam
4. Skinned mouse exam
5. Lymph nodes exam
6. Thymus exam
7. Thymus weight
8. Spleen exam
9. Spleen weight
10. Pancreas exam
11. Liver exam
12. Liver weight
13. Gallbladder exam
14. Epididymis, seminal vesicle exam
15. Vagina exam
16. Uterus exam
17. Testes exam
19. Ovaries exam
20. Urinary bladder exam
21. Urine exam
22. Stomach exam
23. Duodenum exam
24. Cecum exam
25. Colon exam
26. Mesentery exam
27. Kidneys exam
28. Kidney weight
29. Adrenal glands exam
30. Heart exam
31. Heart weight
32. Lungs exam
33. Sciatic nerve exam
34. Bone, sternum exam
43. Bone, vertebral column exam
35. Bone, knee joint exam
36. Bone, femur exam
37. Skeletal muscle exam
38. Eyes exam

Serum Chemistry

Bilirubin, Total
Blood Urea Nitrogen
Cholesterol
Creatinine
Globulin
Glucose
High Density Lipoproteins
Low Density Lipoproteins
Protein, Total
Triglycerides

Hematology

Red blood cells

Hemoglobin
Packed cell volume
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Red blood cell morphology
Platelets
White blood cells
Segmented neutrophils
Lymphocytes
Monocytes
Eosinophils
Basophils

Histopathology (Tissues examined list)

Cardiovascular System
- Aorta
- Heart

Digestive System
- Salivary gland
- Esophagus
- Liver -
- Gallbladder
- Pancreas
- Stomach
- Intestine, small
- Intestine, large
- Tongue

Endocrine System
- Adrenal gland
- Pituitary gland
- Thyroid gland

Hematolymphoid System
- Lymph node
- Spleen
- Thymus
- Bone Marrow

Integumentary System
- Skin
- Mammary gland (if present)
- Harderian gland

Musculoskeletal System
- Skeletal muscle
- Bone – knee joint
- Bone - femoral shaft
- Bone - vertebra
- Bone - sternum

Nervous System

Sciatic nerve
Spinal cord
Brain-cerebellum
Brain-cerebrum
Brain-brainstem

Reproductive System
Female reproductive organs - ovary
Female reproductive organs - uterus
Female reproductive organs - cervix
Male reproductive organs - testis
Male reproductive organs - epididymis
Male reproductive organs - seminal vesicle

Respiratory System
Lung
Trachea

Special Sense Organs
Eye - optic nerve
Eye - retina
Eye - cornea
Eye - ciliary body

Urinary System
Kidney
Urinary bladder

Expression analysis

LacZ, RT-PCR and/or Taqman gene expression analysis in numerous tissues/organs, including testis, brain, spinal cord, sciatic nerve, eye, Harderian glands, thymus, spleen, lymph nodes, bone marrow, aorta, heart, lung, liver, gall bladder, pancreas, kidney, urinary bladder, trachea, larynx, esophagus, thyroid gland, pituitary gland, adrenal glands, salivary glands, tongue, skeletal muscle, skin and female reproductive systems.

Additional phenotypic data are available for certain of the knockout mouse lines, including:

"Aging" data (see Exhibit E for a detailed description)
Behavioral data
Fertility data
Densitometry data

EXHIBIT F
DELTAGEN'S "AGING" PHENOTYPIC DATA OUTLINE

The typical cohort analyzed was 8 wild-type and 8 homozygotes, split between gender. For early-stage lethality targets, heterozygotes were analyzed in place of homozygotes.

1) Weight and length measurements were taken at the following time points: 49 day, 90 day, 180 day and 300 day. The observables were weight and length.

2) Serum Chemistry was performed on animals at the following time points: 90 day, 180 day and 300 day. The observables were as follows:

Serum Chemistry (49 d, 90 d, 180 d, 300 d)
Bilirubin, Total
Blood Urea Nitrogen
Cholesterol
Creatinine
Globulin
Glucose
High Density Lipoproteins
Low Density Lipoproteins
Protein, Total
Triglycerides

3) Hematology was performed on animals at the following time points: 49 day, 110 day, 200 day and 300 day. The observables were as follows:

Hematology (49 d, 110 d, 200 d, 300 d)
Red blood cells
Hemoglobin
Packed cell volume
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Red blood cell morphology
Platelets
White blood cells
Segmented neutrophils
Lymphocytes
Monocytes
Eosinophils
Basophils

4) At 300 days, half of the cohort was necropsied, and the following data were collected:

Weight/Length (as above)
Serum Chemistry (as above)
Hematology (as above)
Physical Exam (see below)
Necropsy Exam (see below)
Histopathology (see below)

If a phenotype was present at the 300-day timepoint, histopathology images were taken of the appropriate tissues and wild-type controls.

Physical Exam (300 d)

1. General Behavior
2. Locomotion
3. Urine exam
4. Feces exam
5. General appearance
6. Respiration
7. Body shape
8. Lesions
9. Joints
10. Lumps – masses
11. Coat color – back
12. Coat color – belly
13. Coat – fur
14. Hair type
15. Skin appearance
16. Head shape
17. Snout
18. Whiskers
19. Teeth color
20. Teeth length
21. Eye – left
22. Eye – right
23. Eye color – left
24. Eye color – right
25. Ear – left
26. Ear – right
27. Limb shape
28. Forelimb – left
29. Forelimb # of digits – left
30. Forelimb # of amputated digits - left
31. Forelimb – right
32. Forelimb # of digits – right
33. Forelimb # of amputated digits – right
34. Hindlimb – left
35. Hindlimb # of digits – left
36. Hindlimb # of amputated digits – left
37. Hindlimb – right
38. Hindlimb # of digits – right
39. Hindlimb # of amputated digits – right
40. Claws
41. Anus
42. Mammary glands exam
43. Genitals – male
44. Genitals – female
45. Tail

Necropsy Exam (300 d)

1. Body weight
2. Body length
3. Skin exam
4. Skinned mouse exam
5. Lymph nodes exam
6. Thymus exam
7. Thymus weight
8. Spleen exam
9. Spleen weight
10. Pancreas exam
11. Liver exam
12. Liver weight
13. Gallbladder exam
14. Epididymis, seminal vesicle exam
15. Vagina exam
16. Uterus exam
17. Testes exam
19. Ovaries exam
20. Urinary bladder exam
21. Urine exam
22. Stomach exam
23. Duodenum exam
24. Cecum exam
25. Colon exam
26. Mesentery exam
27. Kidneys exam
28. Kidney weight
29. Adrenal glands exam
30. Heart exam
31. Heart weight
32. Lungs exam
33. Sciatic nerve exam
34. Bone, sternum exam
43. Bone, vertebral column exam
35. Bone, knee joint exam
36. Bone, femur exam
37. Skeletal muscle exam
38. Eyes exam

Histopathology (Tissues examined list; 300 d)

Cardiovascular System
- Aorta
- Heart

Digestive System
- Salivary gland
- Esophagus
- Liver -
- Gallbladder
- Pancreas
- Stomach
- Intestine, small

- Intestine, large
- Tongue

Endocrine System
- Adrenal gland
- Pituitary gland
- Thyroid gland

Hematolymphoid System
- Lymph node
- Spleen
- Thymus
- Bone Marrow

Integumentary System
- Skin
- Mammary gland (if present)
- Harderian gland

Musculoskeletal System
- Skeletal muscle
- Bone – knee joint
- Bone - femoral shaft
- Bone - vertebra
- Bone - sternum

Nervous System
- Sciatic nerve
- Spinal cord
- Brain-cerebellum
- Brain-cerebrum
- Brain-brainstem

Reproductive System
- Female reproductive organs - ovary
- Female reproductive organs - uterus
- Female reproductive organs - cervix
- Male reproductive organs - testis
- Male reproductive organs - epididymis
- Male reproductive organs - seminal vesicle

Respiratory System
- Lung
- Trachea

Special Sense Organs
- Eye - optic nerve
- Eye - retina
- Eye - cornea
- Eye - ciliary body

Urinary System
- Kidney
- Urinary bladder