Rat Resource and Research Center

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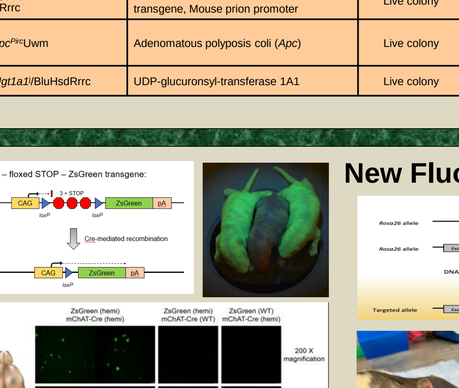
ABSTRACT

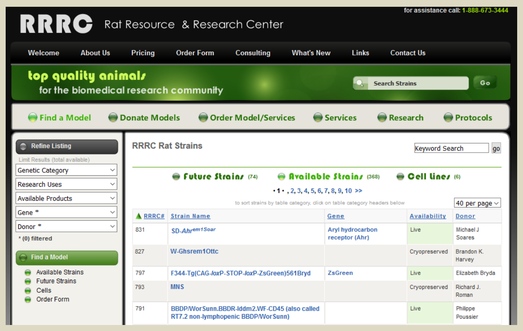
The NIH-funded Rat Resource and Research Center (RRRC) serves as a centralized repository for maintaining/distributing rat models and providing rat-related services to the biomedical community. Currently, the RRRC has close to 600 rat lines and all are archived by cryopreservation to ensure against future loss. The RRRC distributes live animals, cryopreserved sperm/embryos and rat embryonic stem (ES) cell lines. Quality control measures for all materials include extensive genetic validation and health monitoring. The RRRC has expertise in rat reproductive biology, colony management, health monitoring, genetic assay development/optimization, isolation of germline competent ES cell lines from transgenic rats and can partner as consultants/collaborators. Fee-for-service capabilities include a wide variety of genetic analyses, strain rederivation and cryopreservation, isolation of rat tissues, custom breeding/colony management, microbiota analysis and characterization of genetically engineered rats. The RRRC can make genetically engineered rat models from start to finish using a variety of state-of-the-art technologies including genome editing (*e.g.,* CRISPR/Cas9) as well as traditional methods such as random transgenesis and modified embryonic stem cell microinjection into blastocysts. Our website (www.rrrc.us) allows user-friendly navigation. Current research efforts include generation and characterization of a variety of new rat models, optimizing genetic engineering techniques in rats, and improvements to rat sperm cryopreservation and rat *in vitro* fertilization. The University of Missouri is home to the NIH-funded MU Mutant Mouse Resource and Research Center (MMRRC), the National Swine Resource and Research Center (NSRRC), the MU Animal Modeling Core and the MU Metagenomics Center. Together, these highly collaborative groups provide a variety of animal model-related services across species to facilitate biomedical research.

MODELS

Flowchart of RRRC Operations



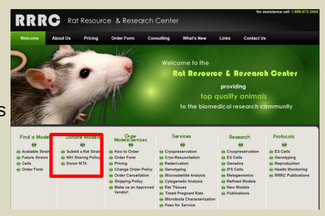
Search For a Model of Interest



RRRC Website

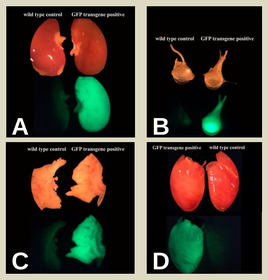
Donate Models

- Easy on-line application process
- Easy MTA process
- Ensures preservation of model
- Satisfies NIH and publisher sharing policies
- Relieves burden on individual investigator associated with sharing model



Examples of Available Rat Models

Strain name	Gene	Availability	RRRC#
SD-Tg (EGFP) 2BalRrrc	Enhanced green fluorescent protein transgene, Ubiquitin C promoter, known insertion on Chr. 14	Live colony	65
Lewis-Tg(CAG-EGFP)Ys	Enhanced green fluorescent protein transgene, chicken β-actin promoter	Live colony	296
F344-Tg (EGFP) F455/Rrrc	Enhanced green fluorescent protein transgene, Ubiquitin C promoter, known insertion on Chr. 5	Live colony	307
DA-Tg (CAG-lacZ) 19Jmsk	LacZ transgene, chicken β-actin promoter	Cryopreserved	294
Wistar-Tg(Alb-DsRed2)42Jmsk	DsRed2 transgene, Albumin promoter	Cryopreserved	260
Long Evans-Tg(TH-Cre)3.1Deis	Cre Recombinase transgene, Mouse tyrosine hydroxylase promoter	Live colony	659
Wistar-Tg(CAG-Ncre)81Jmsk	Cre Recombinase transgene, chicken β-actin promoter	Live colony	301
F344-Tg(CAG-hACE2)057Bryd	Human *ACE2* gene, chicken β-actin promoter	Live colony	946
F344-Tg(Prp-APP;Prp-PS1)19Rrrc	*APP* & *PS1* with common human mutations transgene, Mouse prion promoter	Live colony	699
F344-*Apc*^{Pirc}Uwm	Adenomatous polyposis coli (*Apc*)	Live colony	782
Gunn-*Ugt1a1*^{j}/BluHsdRrrc	UDP-glucuronsyl-transferase 1A1	Live colony	341

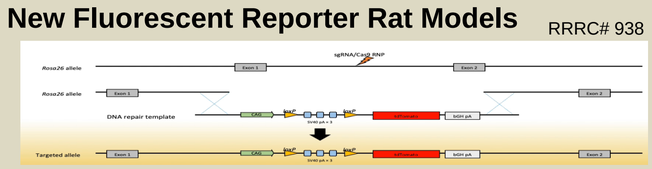


Example of phenotypic analysis for GFP strains. Pictures of organs from SD-Tg(GFP)2BalRrrc (RRRC:0065) animals and wild type controls. Upper images for each panel are under bright light, bottom images for each panel are under fluorescent light. **Panel A:** kidney; **Panel B:** eye; **Panel C:** lung; **Panel D:** heart.

New Fluorescent Reporter Rat Models

RRRC# 938

RRRC# 797



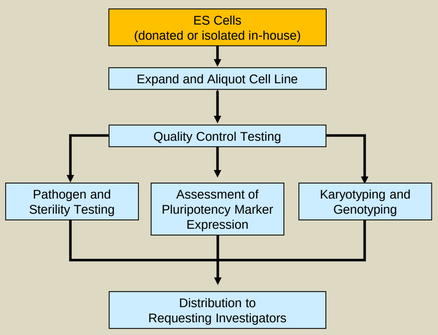
Bryda *et al*. (2019) Scientific Reports 9:13330.

RAT EMBRYONIC STEM CELLS

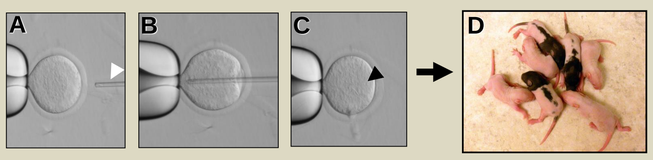
Available Rat Embryonic Stem Cell Lines

ES Cell Line	Genetic Background	Sex	Germline Competent ?	RRRC#
SD-Tg(GFP)2BalRrrc-ES1Rrrc	SD-Tg(GFP)2BalRrrc (RRRC:65)	male	yes	561
DAc2	Dark Agouti (DA)	female	yes	467
DAc8	Dark Agouti (DA)	male	yes	464
DAc11	Dark Agouti (DA)	male	?	465
F6	Fischer 344 (F344)	female	?	466
F344-Tg(UBC-EGFP)F455Rrrc-ES4011Rrrc	F344-Tg(EGFP)F455/Rrrc (RRRC:307)	male	yes	654

Flowchart of ES Cell Lab Operations



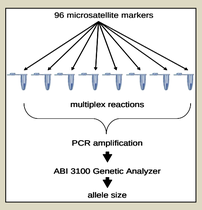
INTRACYTOPLASMIC SPERM INJECTION (ICSI)



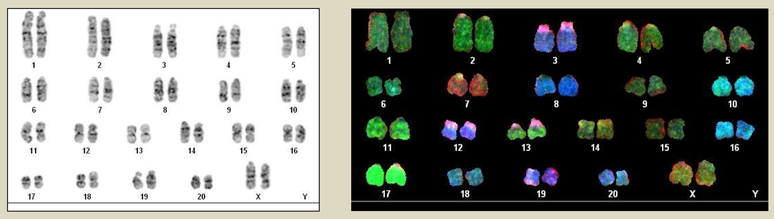
Generation of pups by ICSI. **Panels A-C:** Micro- injection of Sprague Dawley (SD) oocyte with sperm head from Long Evans (LE). White arrow in **Panel A and C** indicates sperm head. **Panel D:** Live pups. Pigmented pups derived from successful ICSI have characteristic hood of LE rats. The non-pigmented pups are from SD control embryos co-transferred during the embryo transfer step of the procedure.

www.rrrc.us

GENETIC TESTING: MICROSATELLITE ANALYSIS AND KARYOTYPING

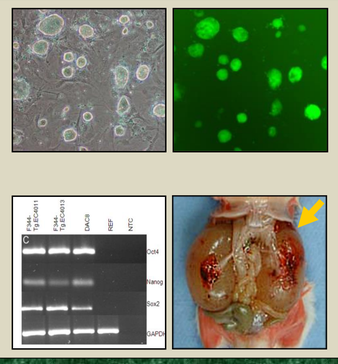


Overview of multiplex microsatellite analysis. Rat microsatellite markers (~ 20 cM intervals) are used for cost-effective genetic monitoring and speed congenic analysis.

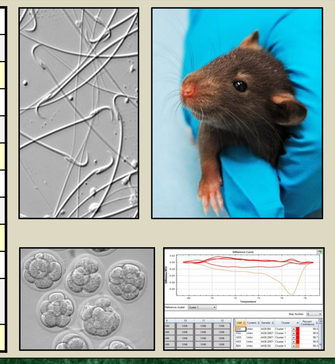


Chromosome analysis of ES cell line DAc2 (RRRC:0467). **A.** Karyotype analysis with Giemsa staining (G-banding). **B.** Spectral Karyotype (SKY) analysis showing a 42, XY karyotype.

AVAILABLE SERVICES

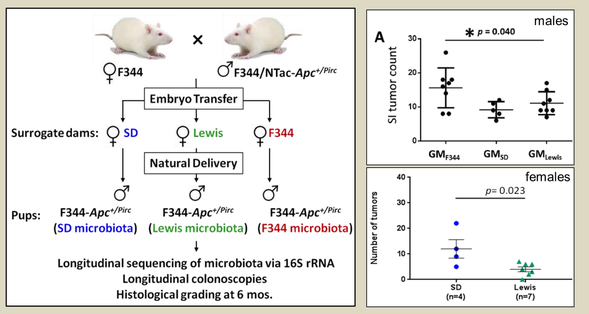


Spermatozoa cryopreservation
Cryo-storage (sperm or embryos)
Cryo-resuscitation via ICSI or embryo transfer
Strain rederivation
Genotyping analysis
Marker-assisted speed congenics
Time pregnant rats
Specimen collection from live rats (tissues, organs, etc.)
Colony management
Microbiota characterization
Consultation: rat reproductive biology, colony management, gamete and embryo cryopreservation, rat genetics
Research collaborations



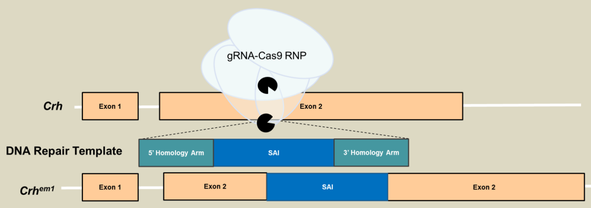
RESEARCH

Effect of Rederivation on Phenotype



Isogenic *Apc*$^{+/Pirc}$ rats rederived using surrogate dams carrying different gut microbiota (GM) had different disease severity. **A.** Animals with GM_{Lewis} had fewer tumors than rats harboring GM_{SD} or GM_{F344}. P values denote results of one-way ANOVA (males) or t-test (females) and asterisks indicate $p \leq 0.05$. Ericsson, AC *et al.* (2015) Oncotarget. 6:33689-33704.

Genome Editing Delivery Approaches



DNA repair template. A DNA construct containing a Short Artificial Intron (SAI) flanked by a 100 bp 5' homology arm and 99 bp 3' homology arm was designed for integration into the rat *Crh* gene. Successful integration of the SAI into exon 2 of the *Crh* gene generates the knock-in allele, *Crh*^{em1}. Figure created with BioRender.com.

Table 1. Embryo development after genetic manipulation

Treatment	# of SD zygotes	Survival after 24 hours[1] (%± SEM)*	Cleavage[2] (% ± SEM)	Development to 4-cell[3] (% ± SEM)
Culture only	111	109 (98 ± 1)[a]	109 (100 ± 0)	99 (91 ± 4)
PNI	175	101 (58 ± 2)[b]	92 (89 ± 4)	68 (67 ± 3)
EP	106	106 (100 ± 0)[a]	105 (99 ± 1)	78 (74 ± 8)
AAV+EP	130	124 (95 ± 2)[a]	118 (95 ± 4)	72 (64 ± 15)

[1] Number of zygotes that survived manipulation
[2] Number of embryos that survived manipulation that went on to the 2-cell stage
[3] Number of embryos that survived manipulation that went on to the 4-cell stage
* Different letters denote statistical difference ($p < 0.05$)
SEM=Standard Error of Mean.

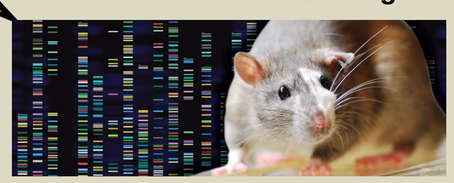
Table 2. Knock-in rate

Treatment	# Blastocysts analyzed	# Blastocysts with knock-in (%)
PNI	18	12 (67)
EP	36	0 (0)
AAV+EP	35	22 (63)

CONCLUSIONS: 1) PNI decreases embryo survivability but not development, 2) EP and AAV+EP do not decrease embryo survival or development, and 3) using a 400 bp DNA repair template, knock-in rates were similar with PNI and AAV+EP while the template failed to be inserted into the genome with EP only.

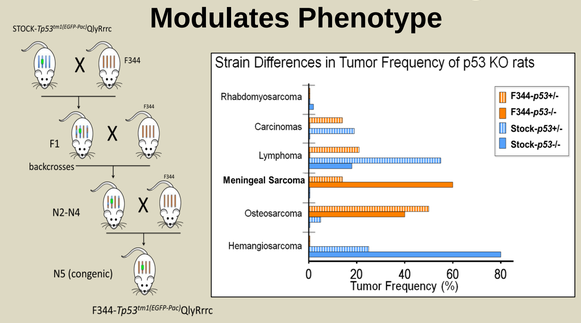
New

MU Rat Testing Center for Somatic Genome Editing



https://rtc.missouri.edu

Model Refinement: Genetic Background Modulates Phenotype



A microsatellite marker-assisted speed congenic approach was used to move a p53 knock allele from a DA;SD mixed genetic background to F344. The change of genetic background resulted in a shift in the type of tumors seen. This underscores the importance of genetic background on phenotype. The new rat strain is a novel model for pediatric bone cancer (Hansen, SA *et al*. (2016) Dis Mod & Mech 9:1139-1146.

Rat *In Vitro* Fertilization

GOAL: Investigate the feasibility of performing *in vitro* fertilization (IVF) for rat model cryo-resuscitation using two transgenic and two knock-in lines on Sprague Dawley (SD) and Long Evans (LE) genetic backgrounds. Sperm were frozen and *in vitro* fertilization was performed as described previously using thawed sperm (Nakagata *et al*. (2020) Scientific Reports 10:93; Takeo *et al.* (2022) Lab Animal 51:256-274).

Table 3. Embryo development following *in vitro* fertilization using thawed frozen rat sperm

Male stock	Genetic modification	# of Oocytes	Cleavage (% ± SEM)*	Blastocysts (% ± SEM)**	Hatched blastocysts (% ± SEM)***
SD	Transgenic	79	69 (87.3 ± 3.9)	36 (52.2 ± 13.4)	19 (52.8 ± 19.7)
LE	Transgenic	77	46 (59.7 ± 5.7)	15 (32.6 ± 13.3)	7 (46.7 ± 15.8)
LE	Knock in	129	81 (62.8 ± 2.6)	17 (20.98 ± 14.5)	4 (23.5 ± 3.8)
LE	Knock in	66	35 (58.3 ± 6.0)	19 (54.3 ± 13.1)	11 (57.9 ± 23.2)

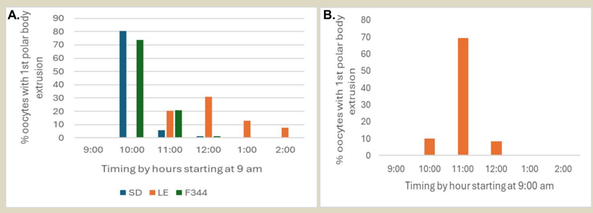
Combined data for 3 replicate experiments
*Ratio of cleaved embryos to oocytes; **ratio of blastocysts to cleaved embryos; ***ratio of hatched blastocysts to blastocysts
SEM = standard error of the mean

Reduced sperm incubation time and shortened sperm-oocyte co-culture time to 6 hours to fit into a conventional laboratory 9-hour workday.

Table 4. Embryo development with modified IVF protocol

Stock	Male #	Oocytes	Cleavage (%)*	Blastocysts (%)**	Hatched blastocysts (%)***
SD	SD-1	34	2 (5.9)	0 (0.0)	n/a
SD	SD-2	35	32 (91.4)	28 (87.5)	13 (46.4)
SD	SD-3	32	27 (84.4)	23 (85.2)	12 (52.2)
SD	SD-4	30	24 (80.0)	8 (33.3)	3 (37.5)
LE	LE-2	56	26 (46.4)	11 (42.3)	5 (45.5)
LE	LE-3	63	35 (55.6)	11 (31.4)	6 (54.5)

Data from 1 experiment
*Ratio of cleaved embryos to oocytes; **ratio of blastocysts to cleaved embryos; ***ratio of hatched blastocysts to blastocysts
n/a = not applicable



Five-week-old rats were superovulated by intraperitoneal (IP) injection of PMSG at 300 IU/kg. 55-57 hours (h) later, 300 IU/kg hCG was given IP. Oocytes were collected between 8:00-9:00 am the next morning. After removal of cumulus cells by hyaluronidase, oocytes were cultured in groups of 30 oocytes/drop in 50 µl KSOM-R drops. The oocytes were examined hourly for the extrusion of the polar body from 9:00 am to 2:00 pm. **A.** Timing of 1st polar body extrusion in oocytes from superovulated SD, LE and F344 rats under a superovulation protocol of 10:00 am PMSG administration and 5 pm hCG administration 55 h later. **B.** Timing of 1st polar body extrusion in oocytes from superovulated LE under a superovulation protocol of 8:00 am PMSG injection and 5:00 pm hCG injection 57 h later.

OTHER RESOURCE CENTERS AT UNIVERSITY OF MISSOURI

MMRRC Mutant Mouse Resource & Research Centers
www.mmrrc.org
Functions
- Importation
- Rederivation
- Cryopreservation
- Distribution
- Research

Metagenomics Center University of Missouri
mumc.missouri.edu
Functions
- DNA Extraction
- 16S rRNA Sequencing
- Informatics Analysis
- Consultation

NSRRC National Swine Resource & Research Center
nsrrc.missouri.edu
Functions
- Model Creation
- Rederivation
- Cryopreservation
- Distribution
- Research

Animal Modeling Core University of Missouri
research.missouri.edu/animal-modeling
Functions
- Model Creation
- Construct Design
- Colony Management
- Genotyping
- Research Collaboration

Acknowledgments: This work is supported by NIH grant 2P40 OD011062 and P40 OD011062-22S1 to ECB.