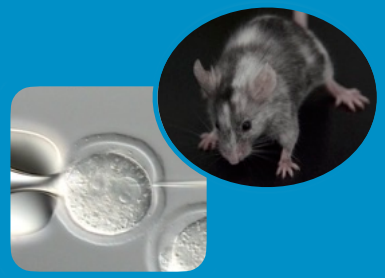
Memorial Sloan Kettering Cancer Center

Mouse Genetics (2)

GSK Experimental Biology Course

2025-09-12



Yasuhide Furuta
Developmental Biology Program
Mouse Genetics Core Facility (MGCF)
@ SKI, MSKCC

Our (Core) Perspective of GEM (vs. genetically altered or mutant)

Mice that carry designed genome alterations introduced into the germ line or germ line-competent lineages.

- **Transgenic** - exogenously introduced genetic elements, typically non-targeted manners
- **Knock-out** - targeted on a specific gene, locus or genomic region, loss-of function
- **Knock-in** - targeted, specific genetic alteration (point mutation, exogenous gene, reporter etc.

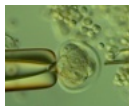
This lecture focuses on discussing:

- technical and procedural aspects of the GEM generation processes
- practical considerations of the generation and development of GEM models
- current trends and prospects of complex GEM models

Strategies and Approaches : different tools and flavors

ZYGOTES

One step



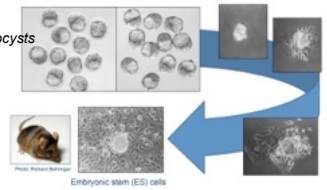
ES CELLS

Single cell = Individual animal

Embryo Manipulation & Transgenesis

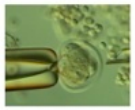
- **Zygotes**
  transgenesis and *genome editing*
- **Embryonic Stem Cells (ESC)**
  gene targeting (KO/KI) and chimera (ESC) mice

Blastocysts



Strategies and Approaches : different tools and flavors

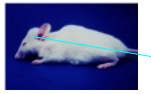
ZYGOTES

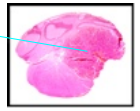


ES CELLS

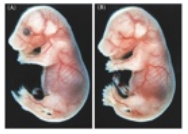
Transgenic Mice

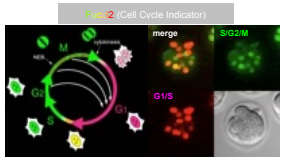

(Palmiter, Brinster, 1982)


(Brinster et al., 1984)



Gene Targeting


(Dudley *et al.*, 1995)



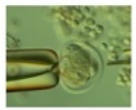
(Abe *et al.*, 2013)

Generation of GEM models

MGCF, MSKCC

Strategies and Approaches : different tools and flavors

ZYGOTES



ES CELLS

REQUIRE:

Production Colony
- embryo donor mice
- vas males & surrogate females

Reproductive Biology
- embryo collection, manipulation
- reproductive surgery

Infrastructure
- embryo collection, manipulation
- microscope, manipulator

Timeline

GEM: 3 mo - 2 yr's

Experiments: mo's - yr's

Paper: ∞ yr's

one gene at a time

↓

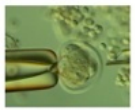
multiple genes simultaneously

Generation of GEM models

MGCF, MSKCC

Strategies and Approaches : different tools and flavors

ZYGOTES



ES CELLS

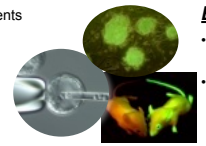
Allele	Time Line Risk	Cost
simple compact	one-step could go fast but unpredictable	may be lower
complex large	through germ line could take longer more QC-able	tend to be higher

Generation and Establishment of GEM models

MGCF, MSKCC

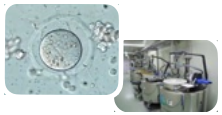
Expediting Genetically Engineered Mouse Studies

Molecular Biology & Embryonic Stem Cells
- Genome modification donors: design and construction
- Consolidating genome editing components
- Assistance in genotyping
- ES cell culture and ES cell derivation
- **Gene targeting in ES cells**



Embryo Manipulation & Transgenesis
- **Zygote micromanipulation**
  transgenics and *genome editing*
- **ES cell injection**
  "chimera" mice

Reproductive Biology: *Facilitating the Use of GEM Lines*
- Germ line transmission of genetic modifications
- Strain archiving by cryopreservation
- Re-establishment and expansion of mouse lines
  - cryo-recovery and assisted reproduction (IVF)

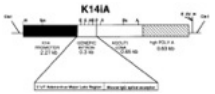


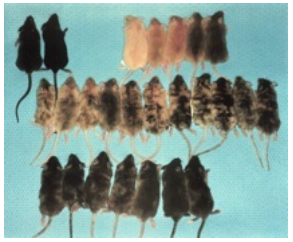
Generation and Establishment of GEM models

MGCF, MSKCC

Transgenic Mice (classical random transgenesis)

(Can be via PN injection, viral, ESC.)





(Kucera *et al.*, 1996)

- Copy number ≠ expression level
- Some integrations do not express
- Ectopic expression
- Novel expression patterns
  --- Position effects (variegation)
- Variation within a mouse line

Generation and Establishment of GEM models

MGCF, MSKCC

Expediting Genetically Engineered Mouse Studies
Beyond Conventional Mouse Genetics

- One target gene at a time —> ***in vivo manipulation of multiple targets*** simultaneously
- ***Genome editing technologies*** : improving the CRISPR/Cas system for efficient generation of large genetic modifications in mouse zygotes
- ***ES cell technologies***: application of ES cell technology to generate complex genotype animal models without genetic crosses

Timeline

GEM:	3 mo - 2 yrs
Experiments:	mo's - yr's
Paper:	∞ yrs

Generation and Establishment of GEM models

MGCF, MSKCC

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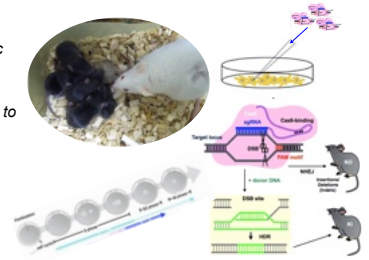
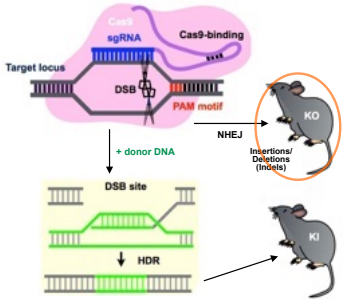


Genome Editing in Zygotes - One-Step GEM

MGCF, MSKCC

Towards efficient genome modification by CRISPR/Cas in mouse zygotes



(LARGE, RIKEN)

Genome Editing in Zygotes - One-Step GEM

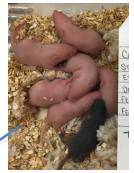
MGCF, MSKCC

Towards efficient genome modification by CRISPR/Cas in mouse zygotes

Improving the base-line genome editing efficiency by CRISPR/Cas in zygotes

Cas9 *activity comparison on the Tyr locus*

Cas9	# embryos transferred	# founders	# albinos	% albino founders
E	18	0	n/a	n/a
P	72	23	1	4.3
I	83	24	2	8.3
D	52	7	6	85.7



(MGCF, MSKCC)

Genome Editing in Zygotes - One-Step GEM

Towards efficient genome modification by CRISPR/Cas in mouse zygotes

Improving the base-line genome editing efficiency by CRISPR/Cas in zygotes

gRNA *comparison on the ROSA26 locus*

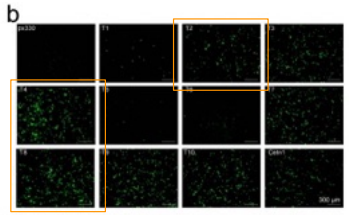


Table 1
CRISPR/Cas9 Mediated ROSA26 Gene Targeting by Cytoplasmic Injection

Donor vector	sgRNA	Treatment	Embryos Injected[a]	Transferred[a] (% b/a)	Pups born[a] (% c/b)	Genotyped[a] (% d/c)	Founders w/Indels[a] (% e/d)	Integration[a] (%)	w/ Desired KI alleles (%)
TRE-LTV	T2	–	160	155 (96.9)	40 (25.8)	39 (97.5)	36 (92.3)	0	0
			641	406 (92.1)	121(29.8)	121 (100)	ND	2/121 (1.7)	0
		CB (5 μg/mL)	137	109 (79.6)	28 (25.7)	26 (92.8)	16 (61.5)	1/26 (3.8)	1/26 (3.8)
			496	405 (81.7)	71 (15.6)	71 (100)	ND	3/71 (4.2)	2/71 (2.8)
								Total:	3/97 (3.1)
Tet3G-LTV2	T4	–	161	115 (71.4)	25 (21.7)	25 (100)	25 (100)	1/25 (4.0)	0
		CB (5 μg/mL)	277	197 (71.1)	40 (20.3)	39 (97.5)	39 (100)	3/39 (7.7)	1/39 (2.6)
Tet3G-STV2	T8	–	163	152 (93.3)	9 (5.9)	7 (77.8)	7 (100)	0	0
		CB (5 μg/mL)	145	131 (90.3)	25 (19.1)	22 (88.0)	13 (59.1)	2/22 (9.1)	1/22 (4.5)
TRP1-ERCreER-LTV2	T8	CB (5 μg/mL)	166	143 (86.1)	21 (14.7)	21 (100)	21 (100)	1/21 (4.8)	1/21 (4.8)
		CD (1 μg/mL)	193	171 (88.6)	30 (17.5)	30 (100)	ND	2/30 (6.7)	0
		CD (5 μg/mL)	156	147 (94.2)	19 (12.9)	19 (100)	16 (84.2)	1/19 (5.3)	1/19 (5.3)

FIG. 1. Validation of sgRNA efficacy for DNA DSBs in cultured cells. (a) Ten sgRNAs targeting *ROSA26* intron 1 (T1–T10) were designed.

cell lines ≠ zygotes

(Nakao *et al*., genesis 2016)

Genome Editing in Zygotes - One-Step GEM

Towards efficient genome modification by CRISPR/Cas in mouse zygotes

Improving the base-line genome editing efficiency by CRISPR/Cas in zygotes

gRNA *selection* (CRISPR design tools)

On-Going Efforts

Towards efficient genome modification by CRISPR/Cas in mouse zygotes

Zygote electroporation

100 embryos in 10 min.
- Faster
- Easier

(Hashimoto & Takemoto., 2015, 2016)

100 embryos in 1 hr.
- Technically involved

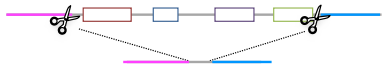
Tyr editing on C57BL/6	# embryos manipulated	# embryos transferred	# pups (# born)	# 100% albino pups
Injection Cas9	120	84	16 (20)	11 (68.8%)
Electroporation				
Cas9	187	79	27 (28)	27 (100%)
Cas9n	146	69	11 (11)	9 (81.8%)

(MGCF, MSKCC)

Genome Editing in Zygotes - One-Step GEM

Towards efficient genome modification by CRISPR/Cas in mouse zygotes

Large Deletions



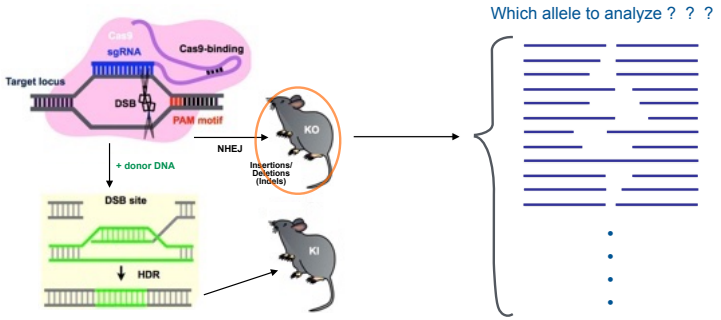
Gene/Locus	Deletion Size	# Embryos Transferred	# F0 Pups	# Desired Allele
A	300 bp	219	41	7 (17%)
B	2.2 kb	255	44	7 (16%)
C	740 bp	395	49	27 (55%)
C	561 bp	251	47	16 (34%)
D	340 kb	387	48	14 (29%)
M	> 1.5 mb	198	26	2 (7.7%)

(MGCF, MSKCC)

Genome Editing in Zygotes - One-Step GEM

MGCF, MSKCC

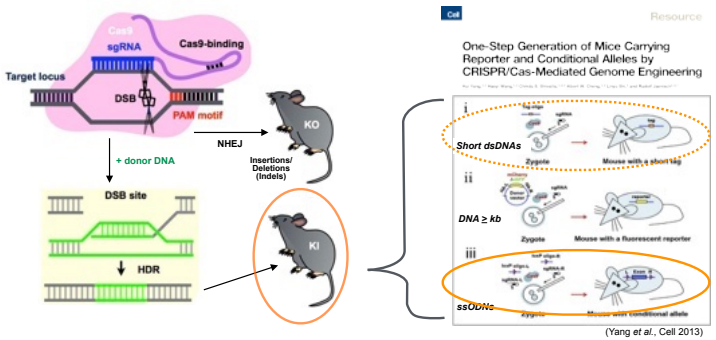
Towards efficient genome modification by CRISPR/Cas in mouse zygotes



Genome Editing in Zygotes - One-Step GEM

MGCF, MSKCC

Towards efficient genome modification by CRISPR/Cas in mouse zygotes



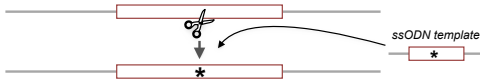
(Yang *et al.*, Cell 2013)

Genome Editing in Zygotes - One-Step GEM

MGCF, MSKCC

Towards efficient genome modification by CRISPR/Cas in mouse zygotes

Small Targeted Insertions



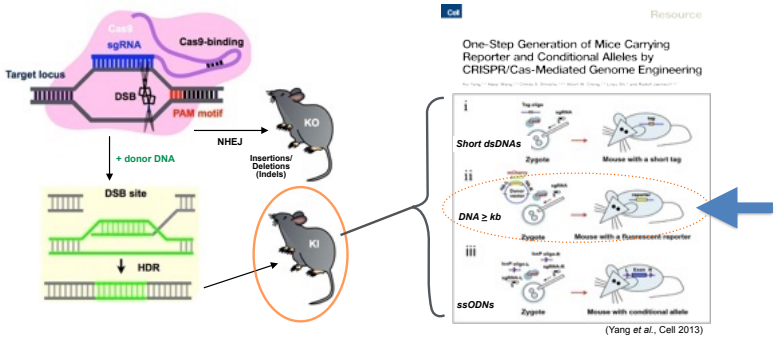
(MGCF, MSKCC)

Gene/Locus	Editing Type	# Embryos Transferred	# F0 Pups	# Desired Allele
E (Ex.28)	2-base sub.	66	22	4 (18%)
E (Ex.28)	2-base sub.	83	29	1 (3%)
F (Ex.2/3)	1 aa sub. / 1 aa sub.	461	16	1 (6.3%)
G	*loxP* <12 kb> *loxP*	482	102	4 (3.6%)
H	*loxP* <340 kb> *loxP*	387	48	1 (2%)
H	*loxP* <1 kb> *loxP*	334	24	3 (12.5%)

Genome Editing in Zygotes - One-Step GEM

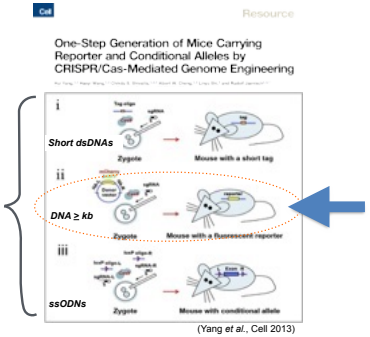
MGCF, MSKCC

Towards efficient genome modification by CRISPR/Cas in mouse zygotes



(Yang *et al.*, Cell 2013)

Targeted insertion of large DNA elements by CRISPR/Cas in mouse zygotes

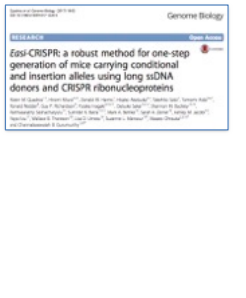


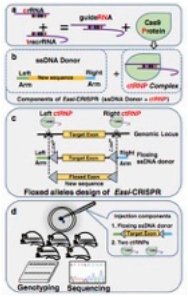
(Yang *et al.*, Cell 2013)

Targeted insertion of large DNA elements by CRISPR/Cas in mouse zygotes

Large Targeted Insertions

(long single strand (lss) DNA donors (cargo ≤ 1.5 kb))

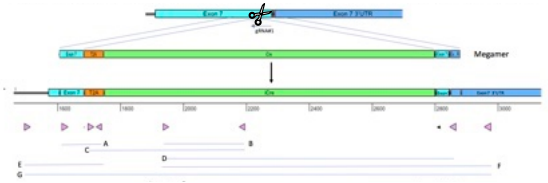




Targeted insertion of large DNA elements by CRISPR/Cas in mouse zygotes

Large Targeted Insertions

(lssDNA donors)



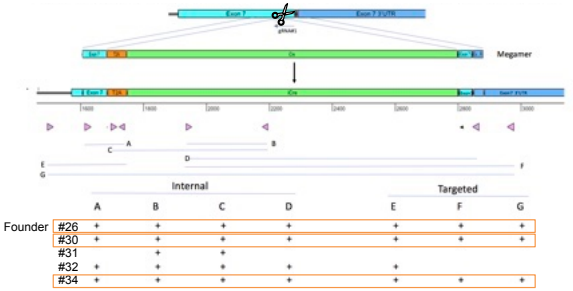
Locus	KI Cargo Size	# Embryos Transferred	# F0 Pups	# Desired Allele
L	1.1 kb	411	54	3 (5.6%)

(MGCF, MSKCC)

Targeted insertion of large DNA elements by CRISPR/Cas in mouse zygotes

Large Targeted Insertions

(lssDNA donors)

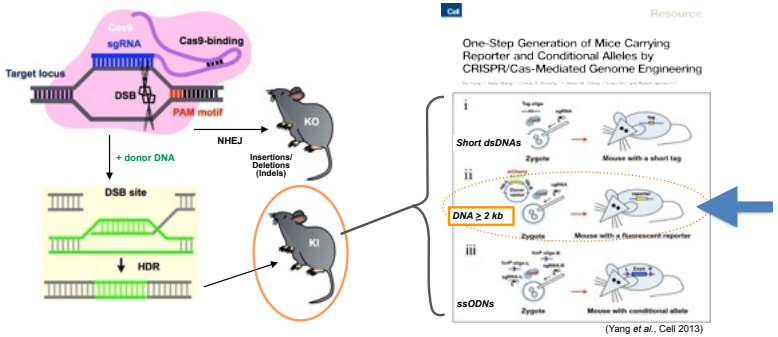


(MGCF, MSKCC)

Genome Editing in Zygotes - One-Step GEM

MGCF, MSKCC

Towards efficient genome modification by CRISPR/Cas in mouse zygotes



(Yang *et al.*, Cell 2013)

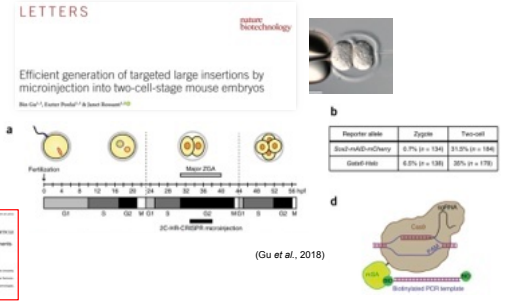
Genome Editing in Zygotes - One-Step GEM

MGCF, MSKCC

Towards efficient genome modification by CRISPR/Cas in mouse zygotes

Large Targeted Insertions

(dsDNA donors (cargo ≥ 1.5 kb))



(Gu *et al.*, 2018)

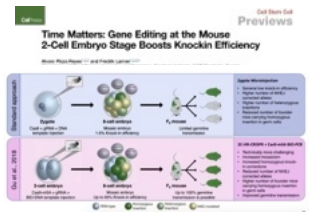
Genome Editing in Zygotes - One-Step GEM

MGCF, MSKCC

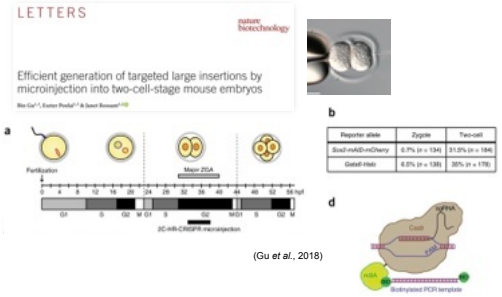
Towards efficient genome modification by CRISPR/Cas in mouse zygotes

Large Targeted Insertions

(dsDNA donors (cargo ≥ 1.5 kb))



- Technically less conventional
- Mosaicism



(Gu *et al.*, 2018)

Genome Editing in Zygotes - One-Step GEM

MGCF, MSKCC

Towards efficient genome modification by CRISPR/Cas in mouse zygotes

Large Targeted Insertions

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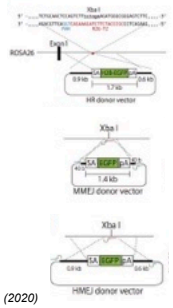
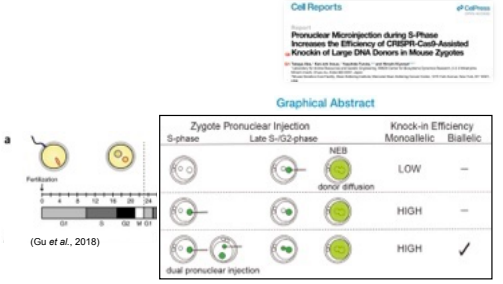


HR

MMEJ (PItCh)

HMEJ

Abe et al. (2020)



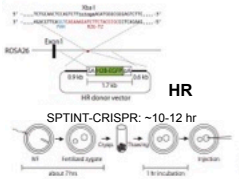
Moving Forward

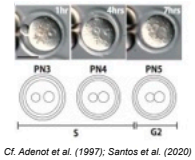
MGCF, MSKCC

Towards efficient genome modification by CRISPR/Cas in mouse zygotes

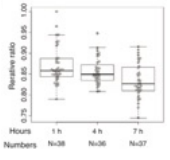
Large Targeted Insertions
(dsDNA donors (cargo ≥ 1.5 kb))

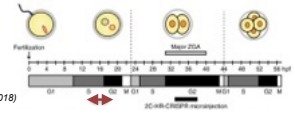
Abe et al. (2020)





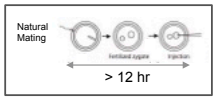
Cf. Adenot et al. (1997); Santos et al. (2020)





Gu et al. (2018)

Conventional Zygot Injection Schedule



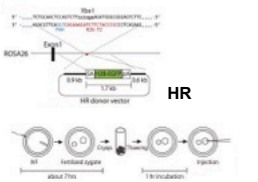
Genome Editing in Zygotes - One-Step GEM

MGCF, MSKCC

Towards efficient genome modification by CRISPR/Cas in mouse zygotes

Large Targeted Insertions
(dsDNA donors (cargo ≥ 1.5 kb))

Abe et al.(2020)



Ex.	Time	Injected	Transferred	E14.5 emb. (%*)	GFP	KI (%**)	KI/Emb.
#1	1h	232	227	50 (21.6%)	29	27 (93.1%)	54%
	1h	117	116	30 (25.6%)	17	17 (100%)	56.7%
#2	4h	116	116	40 (34.4%)	29	28 (96.6%)	70%
	7h	106	106	23 (21.7%)	0	0	0
	1h	110	110	39 (35.5%)	25	24 (96.0%)	61.5%
#3	4h	115	115	31 (27.0%)	17	17 (100%)	54.8%
	7h	103	103	38 (36.9%)	3	1 (33%)	2.6%

Gu et al. (2018)

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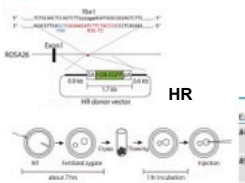


S-Phase Pronuclear Injection for Targeting with Large DNA by CRISPR
(SPRINT-CRISPR: 14 independent loci)

Efficiency range: 2.7%-89.3%

Ex.	Chromosome	Genetic Modification	Time	No. of Zygotes Injected	No. of Zygotes Transferred	No. of E14.5 Embryos Recovered	KI	KI/emb.	KI/zyg.
#4	Chr5 (Actb)	CDB0093E	1	114	114	23	4	17.4	3.5
			7	103	103	16	0	0	0
#5	Chr2	CDB0104E	1	116	115	41	11	26.8	9.5
			7	117	117	24	4	16.7	3.4
#6	Chr17	CDB0121E	1	115	112	26	23	88.5	20.0
			7	111	111	18	7	38.9	6.3
#7	Chr12	CDB0123E	1	117	117	16	12	75.0	10.3
			7	115	115	18	6	33.3	5.2
#8	Chr8 (Cicf.)	Gu et al., 2018	1	118	117	42	32	76.2	27.1
			7	115	115	42	16	38.1	13.9
#9	Chr2 (Cdk9)	Gu et al., 2018	1	119	117	28	25	89.3	21.0
			7	117	117	19	12	63.2	10.3

Generation and Establishment of GEM models

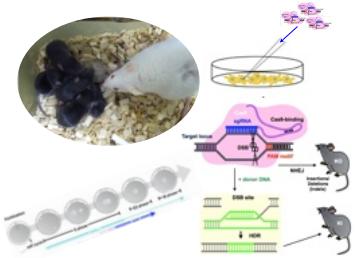
MGCF, MSKCC

Expediting Genetically Engineered Mouse Studies
Beyond Conventional Mouse Genetics

- One target gene at a time
- ***One-step genome engineering in zygotes:***

  "We can do everything we want quickly and cost-effectively."
- ***ES cell technologies:***

	mES cells	CRISPR (in zygotes)				
	conventional gene targeting	indel (small deletion/insertion)	deletion	precise editing (SNP)	conditional KO (LoxP, FRT)	KI (reporter)
% cost savings	baseline	81%	68%	59%	38%	41%
avg. timelines	12 months	6.5 months	7 months	7 months	8 months	8 months



Lui et al., EMBO Rep 2017

Generation and Establishment of GEM models

MGCF, MSKCC

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Beyond Conventional Mouse Genetics

- One target gene at a time
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"Is the ESC platform *obsolete*?"

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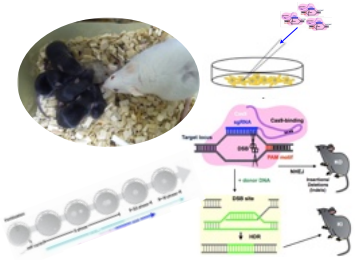


Generation and Establishment of GEM models

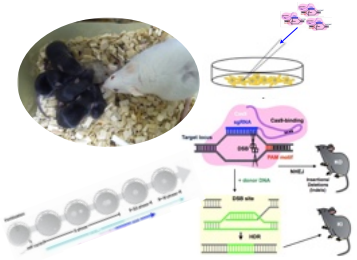
MGCF, MSKCC

Expediting Genetically Engineered Mouse Studies
Beyond Conventional Mouse Genetics

- One target gene at a time —> **<u>in vivo simultaneous manipulation of multiple targets</u>**
- ***One-step genome engineering in zygotes:***

"Is the ESC platform *obsolete*?"

- ***ES cell technologies:*** ***application of the ES cell platform to generate complex-genotype animal models without extensive genetic crosses***



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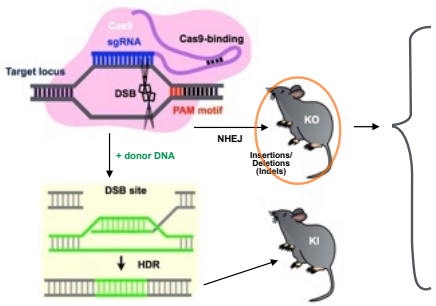
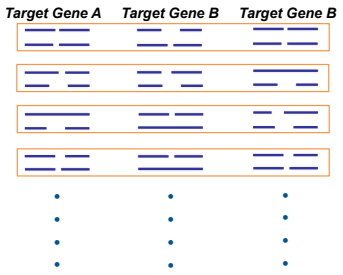
Towards efficient genome modification by CRISPR/Cas in mouse zygotes



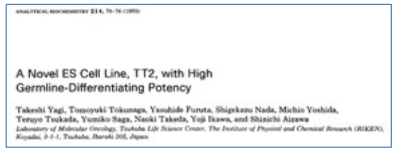
Which mice to analyze ? ? ?



Generation and Establishment of Complex GEMMs

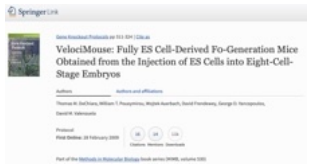
MGCF, MSKCC

Improving the efficiency of ES cell-derived genetically modified mice

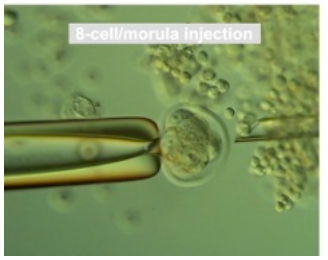


A Novel ES Cell Line, TT2, with High Germline-Differentiating Potency

Takeshi Yagi, Tomoyuki Tokunaga, Yasuhide Furuta, Shigekazu Nada, Michio Yoshida, Teruyo Tsukada, Yumiko Saga, Naoki Takeda, Yoji Ikawa, and Shinichi Aizawa



VelociMouse: Fully ES Cell-Derived F0-Generation Mice Obtained from the Injection of ES Cells into Eight-Cell-Stage Embryos



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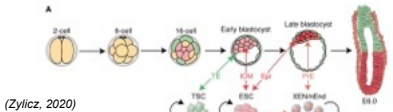
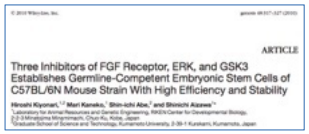


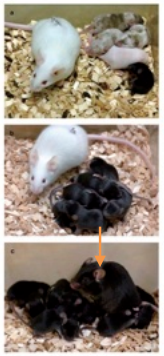
(Zylicz, 2020)

(Morgani et al., 2017)



ARTICLE

Three Inhibitors of FGF Receptor, ERK, and GSK3 Establishes Germline-Competent Embryonic Stem Cells of C57BL/6N Mouse Strain With High Efficiency and Stability



B6-KSR

B6-3i

100% GLT

(LARGE, RIKEN)

Generation and Establishment of Complex GEMMs

MGCF, MSKCC

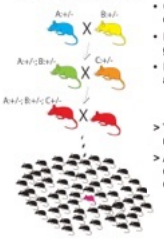
Generation of mice carrying complex genetic alleles and genotypes

CRISPR/Cas-mediated multi-gene locus targeting : cheating genetics

HK3i ES cell line
(C57BL/6N background)

Kiyonari *et al.*, 2010

Conventinal Genetic Crosses



- Genetic redundancy often causes significant challenges in genetic studies.
- Extensive crosses are required to obtain rare-occurring genotypes.
- Embryonic lethal mutants are even harder to obtain and anlyze.

> The CRISPR/Cas9 sustem may be used to mutate multiple genes.

> A robost embryonic stem cell system may allow for direct analyses of F0 embryos as a stable source of complex-genetype mutants.

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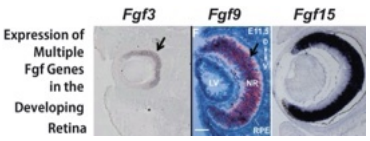
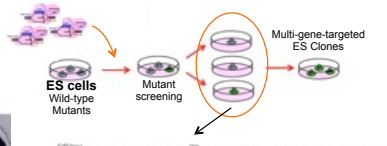
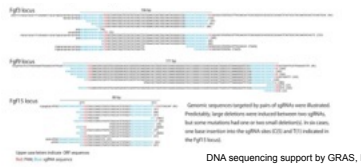


- Either of single KOs: no drastic eye phenotypes
- Genetic linkage: *Fgf3-Fgf4-Fgf15* within a 60-kb interval





DNA sequencing support by GRAS, RIKEN BDR

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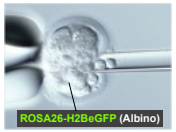
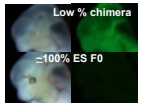
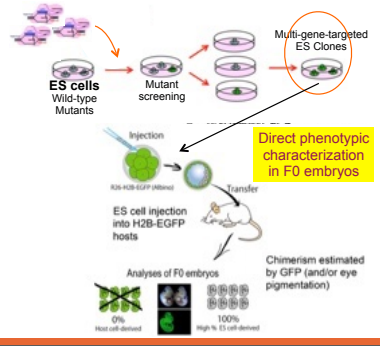


Chimera



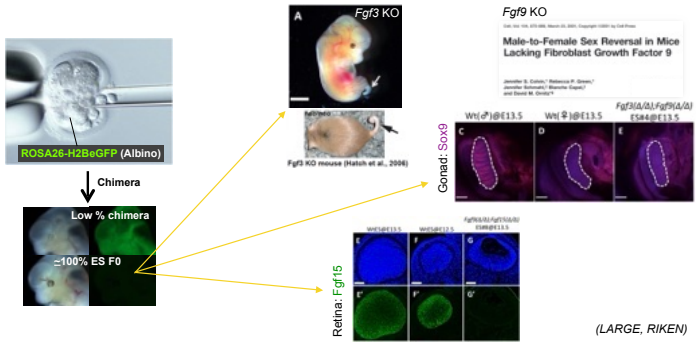


Generation and Establishment of Complex GEMMs

MGCF, MSKCC

Generation of mice carrying complex genetic alleles and genotypes

CRISPR/Cas-mediated multi-gene locus targeting : *Fgf3;Fgf9;Fgf15* Tri-KO



(LARGE, RIKEN)

Generation and Establishment of Complex GEMMs

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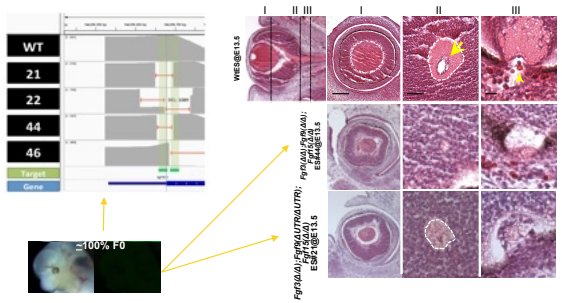


(LARGE, RIKEN)

Cheating Genetics via ES Cells

MGCF, MSKCC

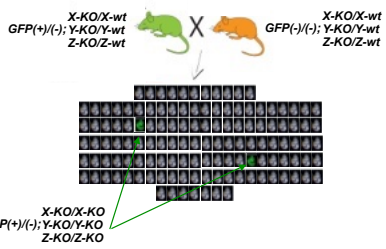
Generation of mice carrying complex genetic alleles and genotypes

Live imaging mutant embryos

R26-PHA7-EGFP
(Shioi et al. 2018)

Direct phenotypic characterization in an F0 embryo?

Conventional Genetic Cosses



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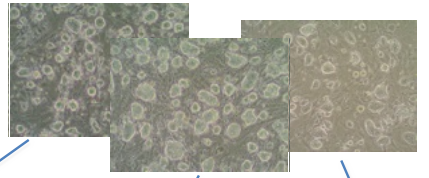

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Cheating Genetics via ES Cells

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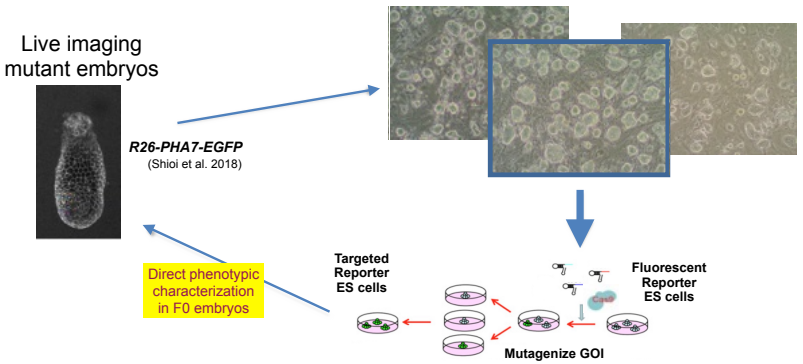
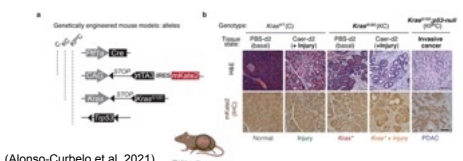


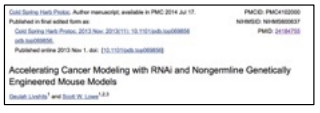
Generation and Establishment of Complex GEMMs

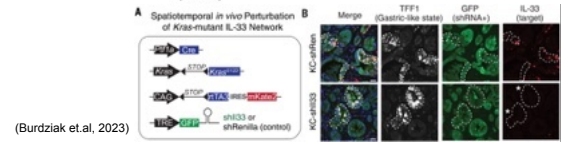
Generation of mice carrying complex genetic alleles and genotypes

Combining multiple alleles in ES cells : cheating genetics - ESC-GEM Models



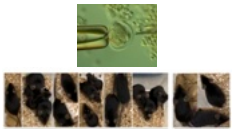
(Alonso-Curbelo et.al, 2021)





(Burdziak et.al, 2023)

GEMM ES cell 8-cell-stage injection



ES Cell Gene Targeting Revisited

Generation of mice carrying complex genetic alleles and genotypes

CRISPR-assisted targeted KI in ES cells

Locus/ allele	Method	# total clones screened	# targeted	# mono-allelic	# bi-allelic
Gxp	HR	198	1(/3) (0.5%)	1	0
R26-Gla	HR	194	6 (3.1%)	6	0
R26-Glb	CR/HR	98	20 (20.4%)	11	9
Ka7-dTag	CR/HR	96	13 (13.5%)	3	10
Iki-FKBP	CR/HR	48	31 (64.6%)	29	2
Vcg	CR/HR	42	40 (95.2%)	30	10
Afc	CR/HR	26	16 (61.5%)	12	4

ES Cell Gene Targeting Revisited

Generation of mice carrying complex genetic alleles and genotypes

CRISPR-assisted targeted KI in ES cells

Locus/ allele	Method	# total clones screened	# targeted	# mono-allelic	# bi-allelic
Gxp	HR	198	1(/3) (0.5%)	1	0

Afc	CR/HR	26	16 (61.5%)	12	4

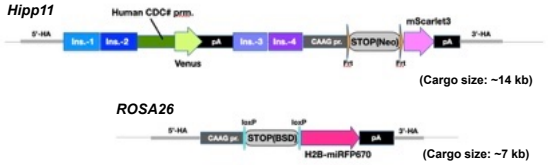
Generation and Establishment of Complex GEMMs

MGCF, MSKCC

Generation of mice carrying complex genetic alleles and genotypes

CRISPR-assisted dual targeted KI in ES cells

Locus/ allele	Method	# total clones screened	# targeted	# dual-Targeted	# 100% ESC coat
Hipp11-V•S	CR/HR	96	68 (70.8%)	21 (73.1%)	2/5
R26-RFP	CR/HR		24 (25%)		

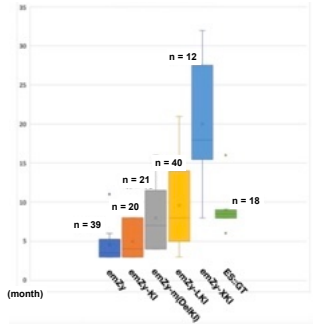


Generation of GEM models

MGCF, MSKCC

Strategies and Approaches : different tools and flavors

	Allele	Time Line Risk	Cost
ZYGOTES	simple compact	one-step could go fast but unpredictable	may be lower
ES CELLS	complex large	through germ line could take longer more QC-able	tend to be higher?



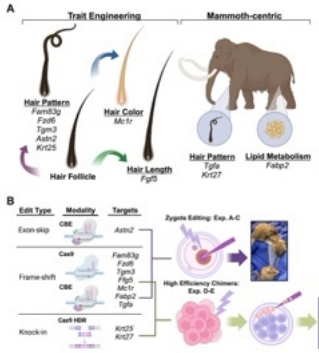
Where are GEMMs going?

MGCF, MSKCC

"DE-EXTINCTION" ?

Mar. 2025

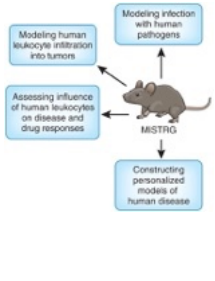
science.org



Where are GEMMs going?

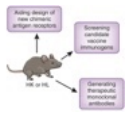
MGCF, MSKCC

Humanization

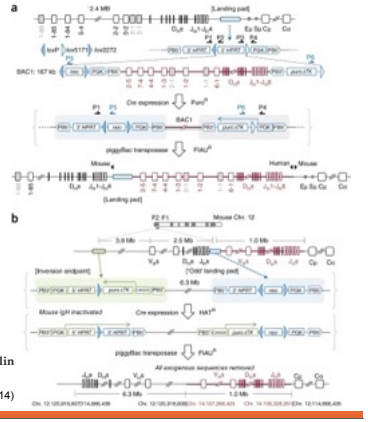


Rongvaux et al., *Nat Biotech* (2014)

M-CSF hu/hu
IL-3 hu/hu
BAC-hSIRPa
TPO hu/hu
Rag2 ko/ko
Il2rg ko/ko



Spits, Nat Biotech (2014)



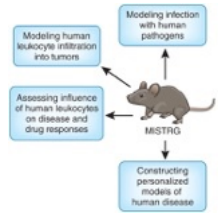
Complete humanization of the mouse immunoglobulin loci enables efficient therapeutic antibody discovery

Lee et al., *Nat Biotech* (2014)

Where are GEMMs going?

MGCF, MSKCC

Humanization



Rongvaux et al., *Nat Biotech* (2014)

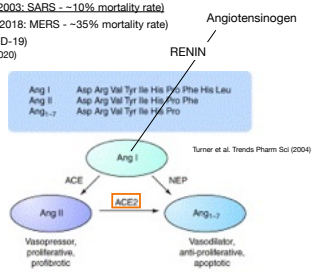
M-CSF hu/hu
IL-3 hu/hu
BAC-hSIRPa
TPO hu/hu
Rag2 ko/ko
Il2rg ko/ko

Spits, Nat Biotech (2014)

COVID Mouse Models

- SARS-CoV (2002-2003: SARS - ~10% mortality rate)
- MERS-CoV (2012-2018: MERS - ~35% mortality rate)
- SARS-CoV2 (COVID-19)

Zhu et al. NEJM (2020)



ACE2: angiotensin converting enzyme 2 as a functional receptor for SARS-CoV

(Li et al. Nature 2004; Li et al. EMBO J 2005)

Where are GEMMs going?

MGCF, MSKCC

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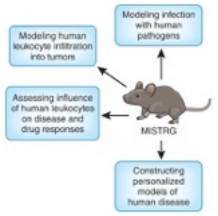

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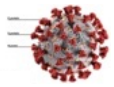
COVID Mouse Models

SARS-CoV



hACE2

~~mAce2~~

GE (humanized) ACE2 model

Genetic modification of the host receptor: hACE2 transgenic mice

- CMV enhancer/chick beta-actin promoter (Tseng et al. 2007)
- cytokeratin (K18) promoter (McCray et al. 2007; Netland et al. 2008)
- mAce2 promoter (Yang et al. 2007)
  - positive correlations between hACE2 expression and disease severity
  - airway epithelial pathologies
  - high viral load in the brain
  - neurological-related mortality: encephalitis

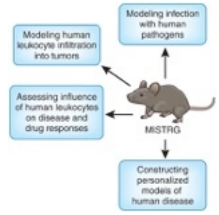
ACE2: angiotensin converting enzyme 2 as a functional receptor for SARS-CoV

(Li et al. Nature 2004; Li et al. EMBO J 2005)

Where are GEMMs going?

MGCF, MSKCC

Humanization



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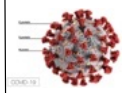
Spits, Nat Biotech (2014)

COVID Mouse Models

SARS-CoV2



hACE2

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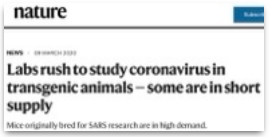
GE (humanized) ACE2 model


nature

Labs rush to study coronavirus in transgenic animals – some are in short supply

Mice originally bred for SARS research are in high demand.

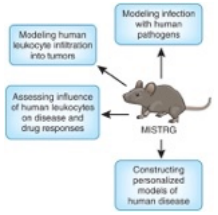

Science

Mice, hamsters, ferrets, monkeys. Which lab animals can help defeat the new coronavirus?

Where are GEMMs going?

MGCF, MSKCC

Humanization



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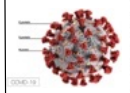
COVID Mouse Models

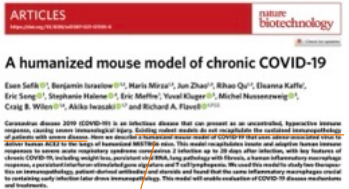
SARS-CoV2



hACE2

~~mAce2~~

GE (humanized) ACE2 model


ARTICLES

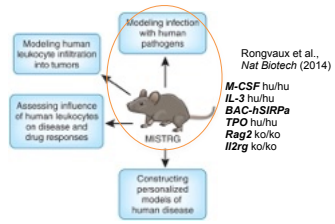
nature biotechnology

A humanized mouse model of chronic COVID-19

"Exisiting rodent models do not recapitulate the sustained immunopathology of patients with severe (COVID)."

Where are GEMMs going?

Humanization



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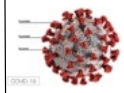
COVID Mouse Models

SARS-CoV2

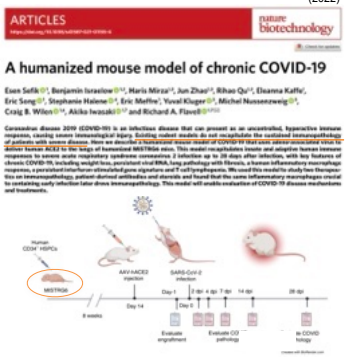


hACE2

~~mAce2~~

GE (humanized) ACE2 model

(2022)



Where are GEMMs going?

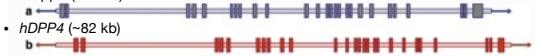
Humanization

COVID Mouse Models

MERS-CoV model development: engineering hDPP4 mice - KI approaches

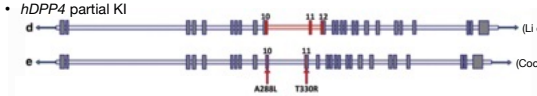
- m*Dpp4* (~72 kb)

- *hDPP4* (~82 kb)



- *hDPP4* CDS KI (Coleman et al. 2017; Pascal et al. 2015)

- *hDPP4* partial KI



(Li et al., 2017)

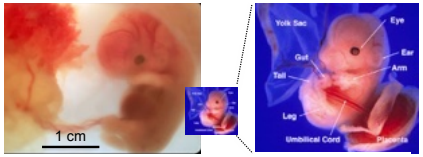
(Cockrell et al., 2016) <— CRIPR/Cas9

- high viral replication, but no clinical signs of disease
- Adoptive evolution (15 passages) required: maMERS-CoV to achieve lethality
- no detectable virus in the brain
- maMERS-CoV respiratory disease and lethality prevented by spike-directed vaccine and antibody

Where are GEMMs going?

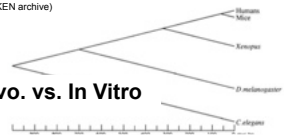
Eutherian mammal – close similarity to humans

Physiology, development, cell biology, ….

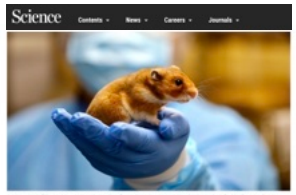


Human embryo (8 wks)
(RIKEN archive)

Mouse embryo (12 days)



In vivo. vs. In Vitro



Mice ≃ ≠ Humans

Got GEMMs? < furutay@mskcc.org / TRANSGENICS@mskcc.org >