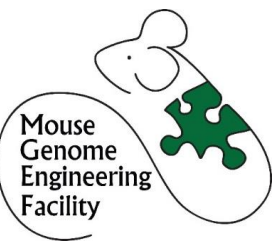


the Mouse Genome Engineering Facility: a National Resource for Cryo Archiving, Rederivation & Generation of Novel Mouse Models

*Shilpakumari BA, Saumya Mary Mathew, Jasper Chrysolite Paul, Adarsh M, Debajeet Das,
Latha Chukki, Aurelie [Lily] Jory*



contact: mgef@ncbs.res.in
www.ncbs.res.in/research-facilities/acrc



MGEF SERVICES provided to BLiSC and External Scientists:

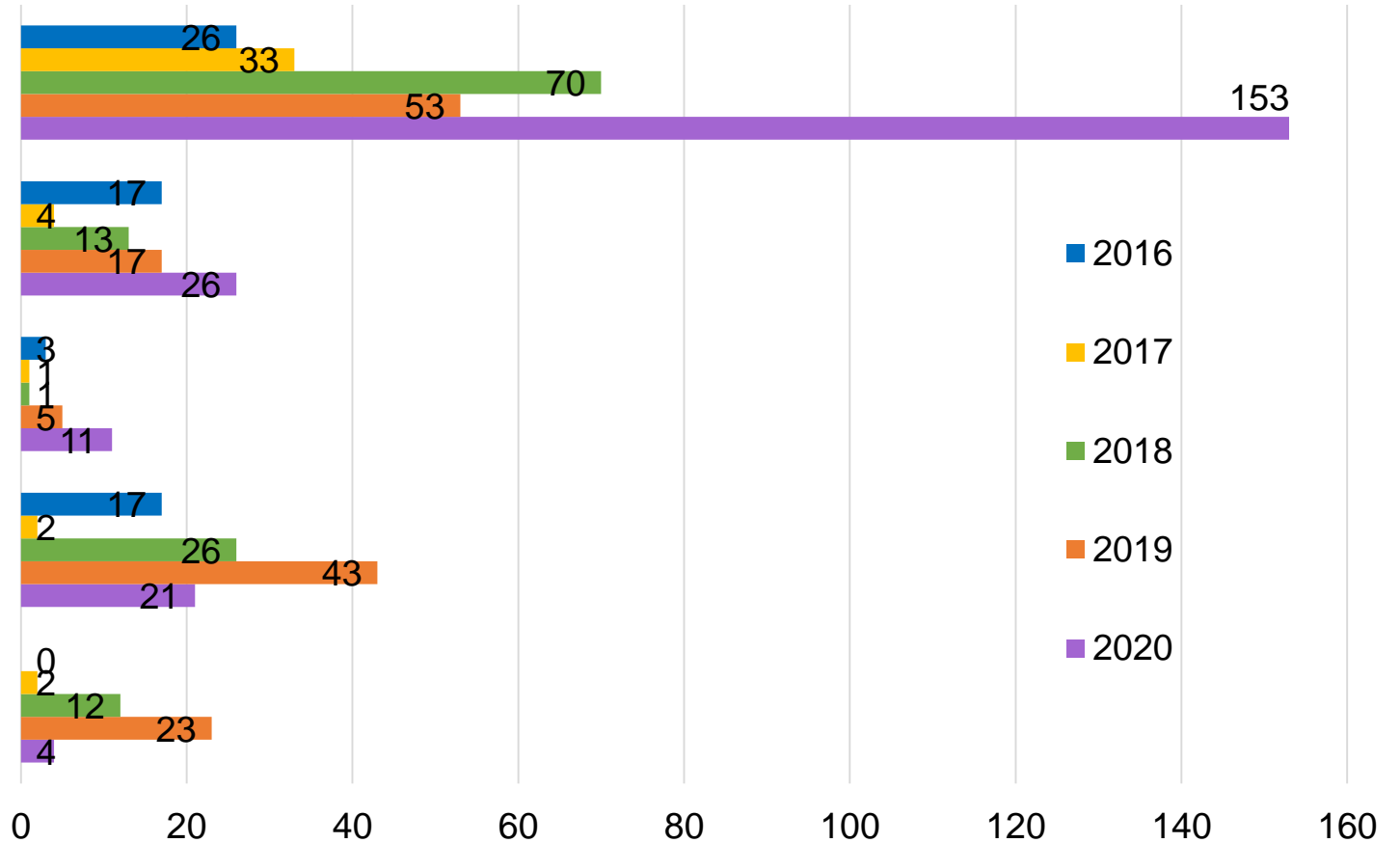
1) Mouse Sperm / Embryo cryopreservation & stock archiving services

2) Mouse Stock Cryo-recovery / IVF / rederivation services

3) Generation of New Mouse Models (Crispr/Cas; ...)

4) Number of external scientists & students having attended MGEF/ACRC hands-on Training programs

5) Providing WT mouse sperm & embryos for *inVItro* assays, MEFs, fertility R&D...



Number of projects catered per year

List of MGEF Clients :



क्षेत्रीय जैव प्रौद्योगिकी केन्द्र
Regional Centre
for Biotechnology



[SpOvum® Technologies Pvt. Ltd.](#)
Technology Enhanced to Create Lives



MANIPAL
ACADEMY of HIGHER EDUCATION
(Deemed to be University under Section 3 of the UGC Act, 1956)

ORIGIO



MOMSOON
Fertility & IVF Centre

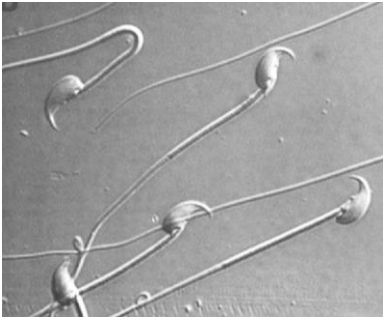


भाकृअनुप - राष्ट्रीय पशु पोषण एवं शरीर क्रिया विज्ञान संस्थान
ICAR - National Institute of Animal Nutrition and Physiology



MOUSE STOCK CRYO-BANKING SERVICES

Sperm Cryopreservation



10 X 10 μ l sperm cryostraws per male



Cryo-stocks archived in duplicate LN2 tanks



Quality Control



National Mouse Repository referencing over 280 Mouse stocks:

<https://www.ncbs.res.in/transgenic/>

Embryo Vitrification



30-60 embryos per cryovial

Animal Colony Management System (ACoMaS)

Home x Strain DB

Animal Strain view

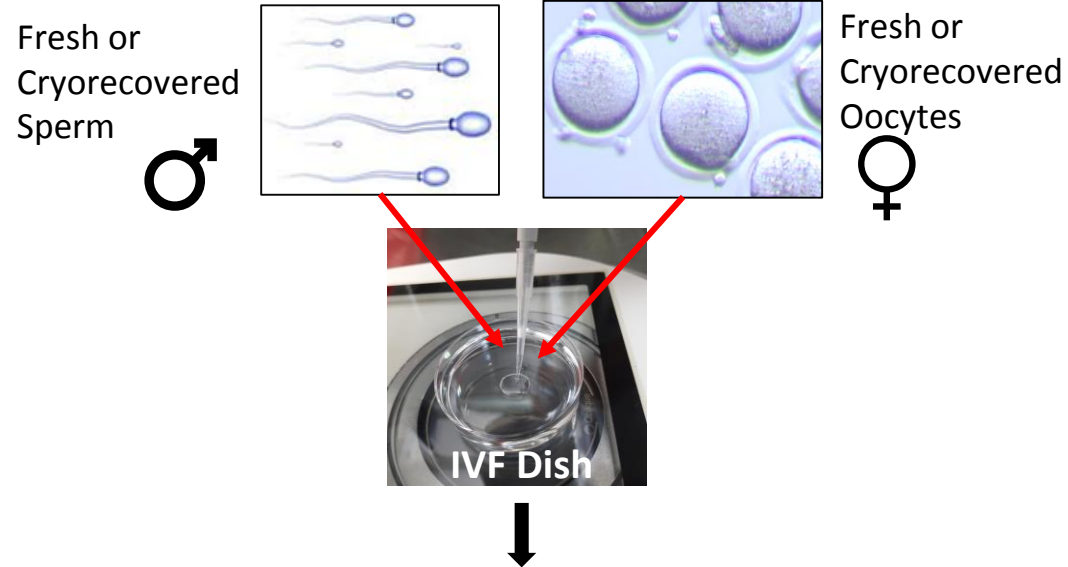
NCBS Stock ID: Full Scientific Strain Name: Original Stock ID:

Strain Type: species: Stock Status:

NCBS Stock ID	Strain ID	Full Scientific Strain Name	Repository Status	Stock Status	Sign
acrc 00001	129X1	129X1/SvJNcbs	Public	Cryo-S	M M
	ACE2 KO [B6NDQ]	B6N.DBA2-Ace2em2MgefBlsoc for the (g53-g55 47kb deletion); B6N.DBA2-Ace2em1MgefBlsoc for the Exon1 Deletion	Public	Live	M M
acrc 00011	Adipoq-CreER	B6 129Tg(Adipoq-cre(Esr1))1Evdv/JNcbs	Public	Live, Cryo-S	M M

IN VITRO FERTILIZATION

IVF Procedure:



Wash and culture O/N in HTF media and further Culture in KSOM up to Blastocyst stage



Oviduct Embryo Transfer in 0.5dpc foster female

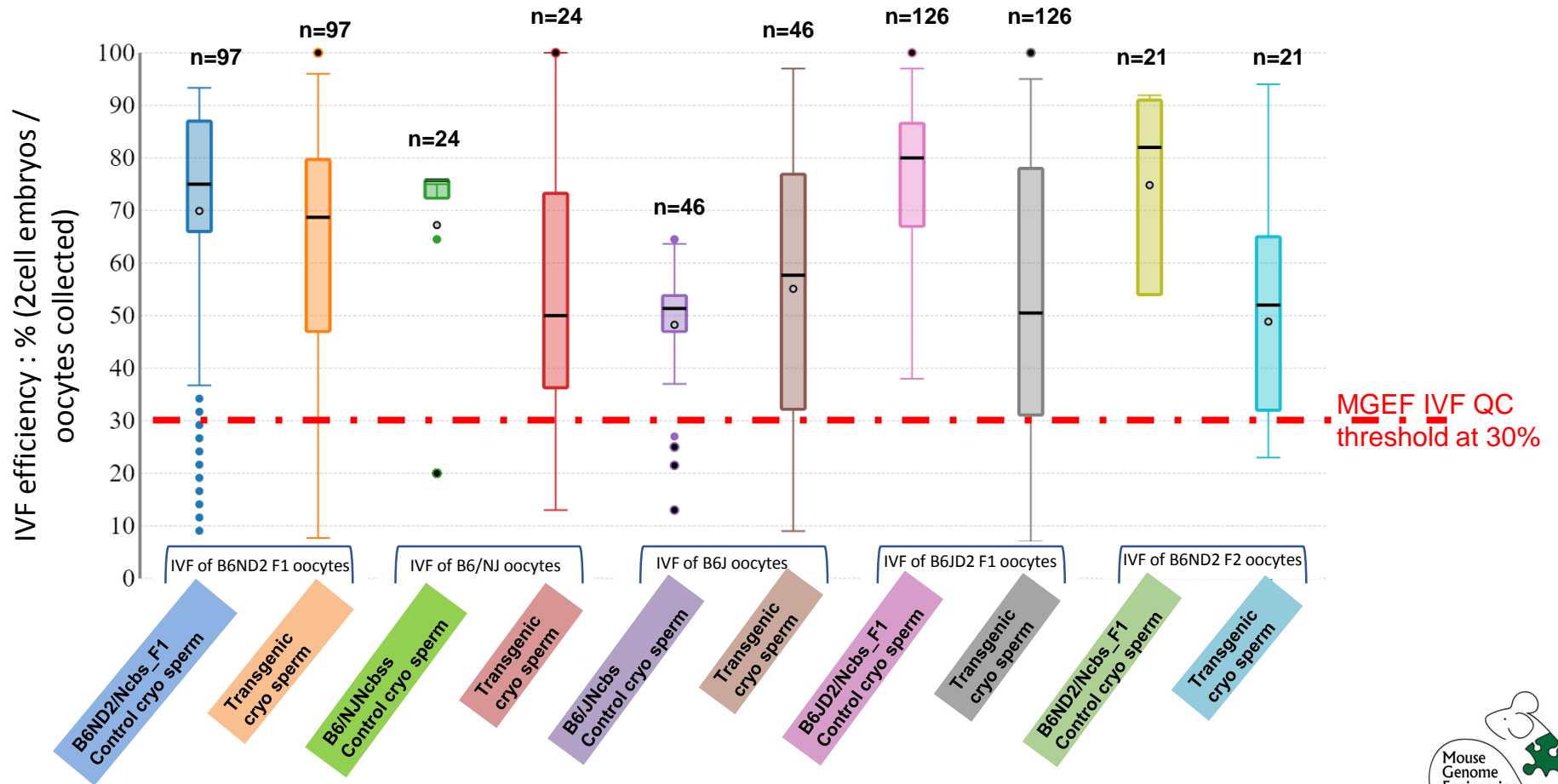
Uterine Embryo Transfer in 2.5 dpc foster female

➤ OR Embryo Vitrification

Applications

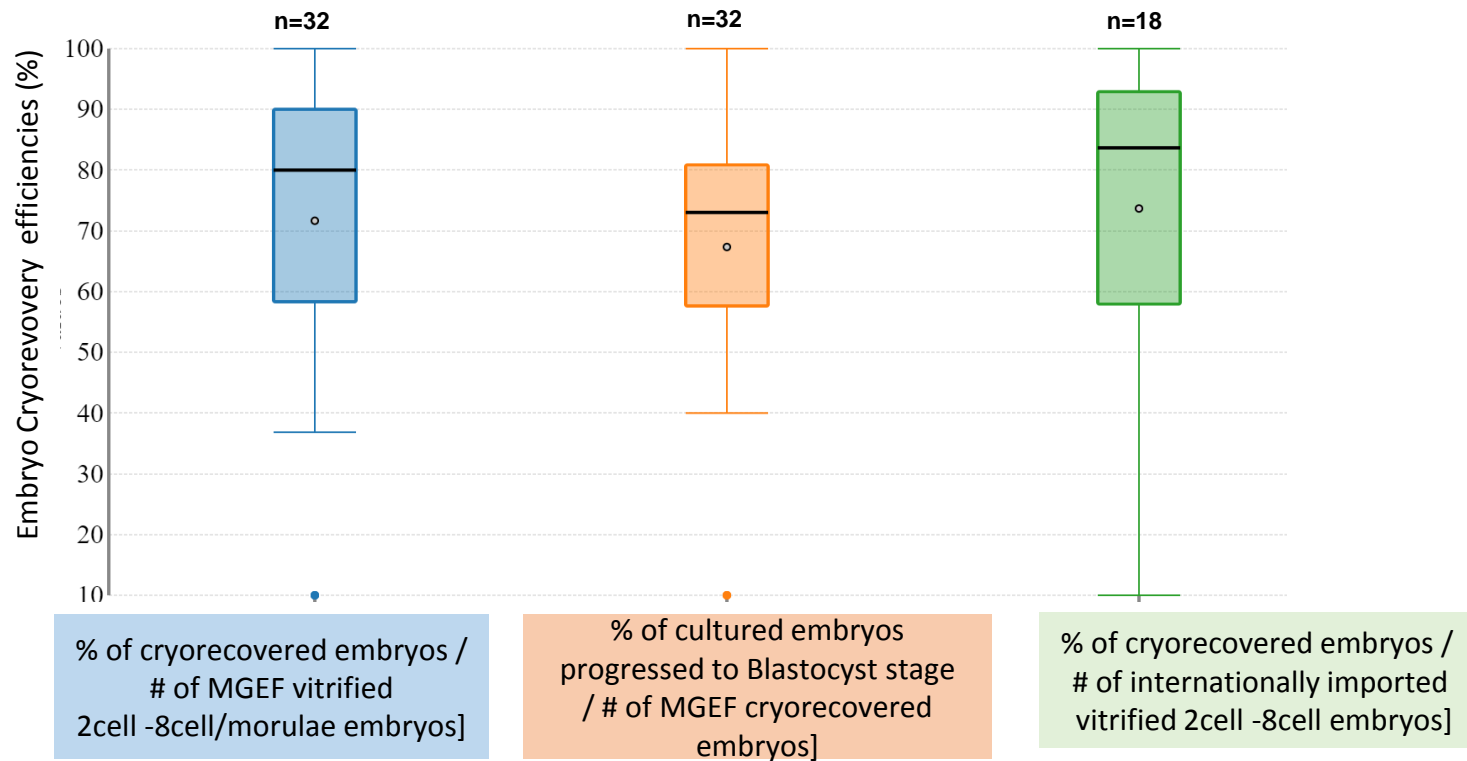
- Method used for quality control of cryopreserved sperm / sperm cryo-recovery
- Maintaining Genetic Quality of mouse stocks / genetic drift control.
- Rederivation to SPF quality stocks
- Producing large cohort of embryos
- Rescue of poor breeders
- Maintenance of stocks with pregnancy / embryo implantation deficiencies.

*inV*itro Fertilization Efficiency of cryorecovered sperm for Control and Genetically Engineered Mouse Strains



MOUSE embryo Vitrification

MGEF Embryo Cryorecovery Efficiencies



Mouse stock REDERIVATION via embryo transfer

Embryo collection options:

1) Via Natural mating



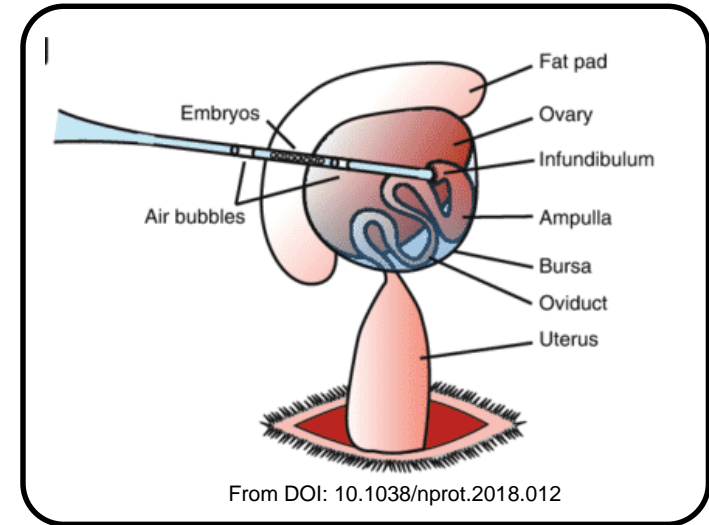
2) IVF generated embryos



3) Embryo Cryorecovery

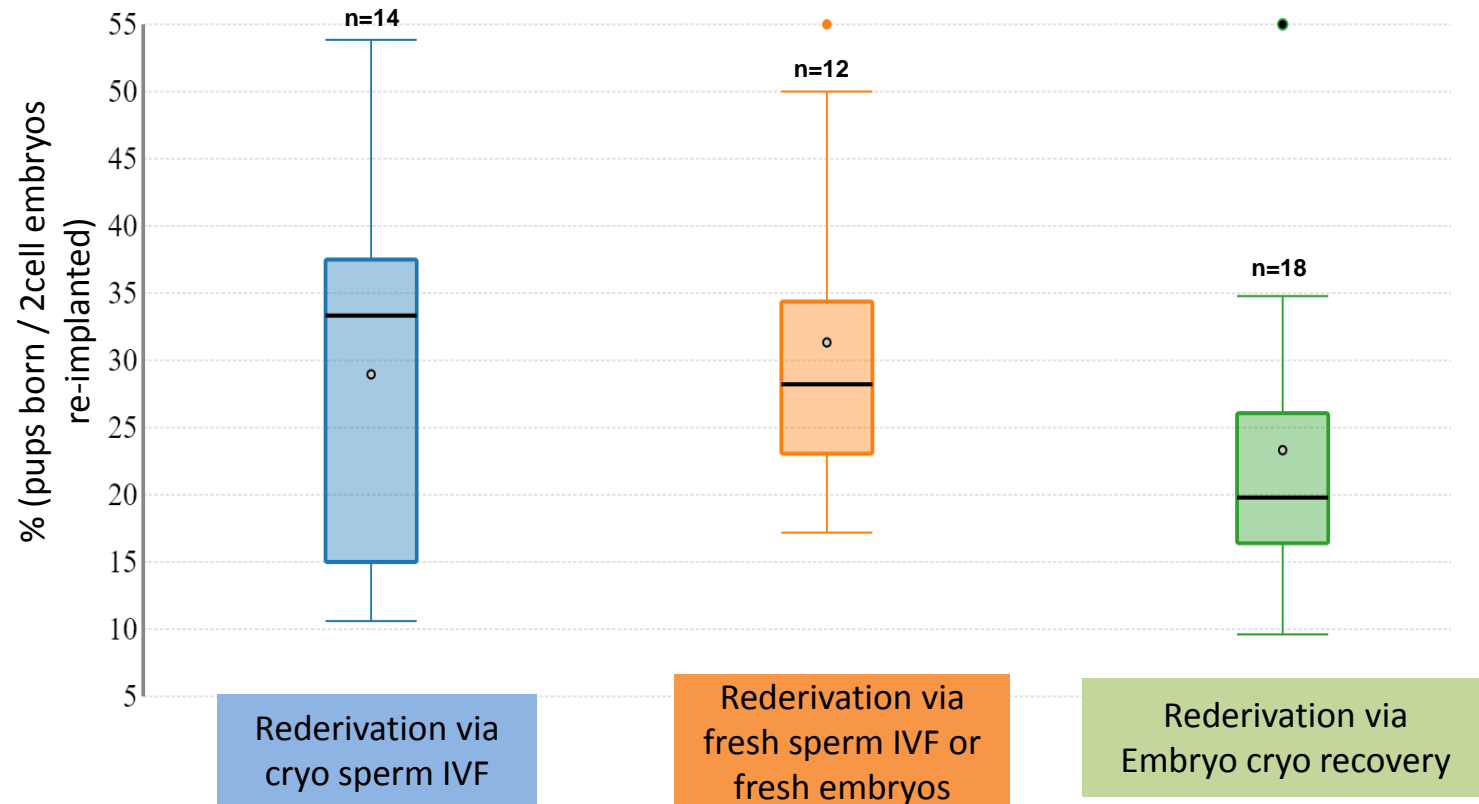


Surgical Oviduct Embryo Transfer of 2-8 cell embryos in Pseudo-pregnant females



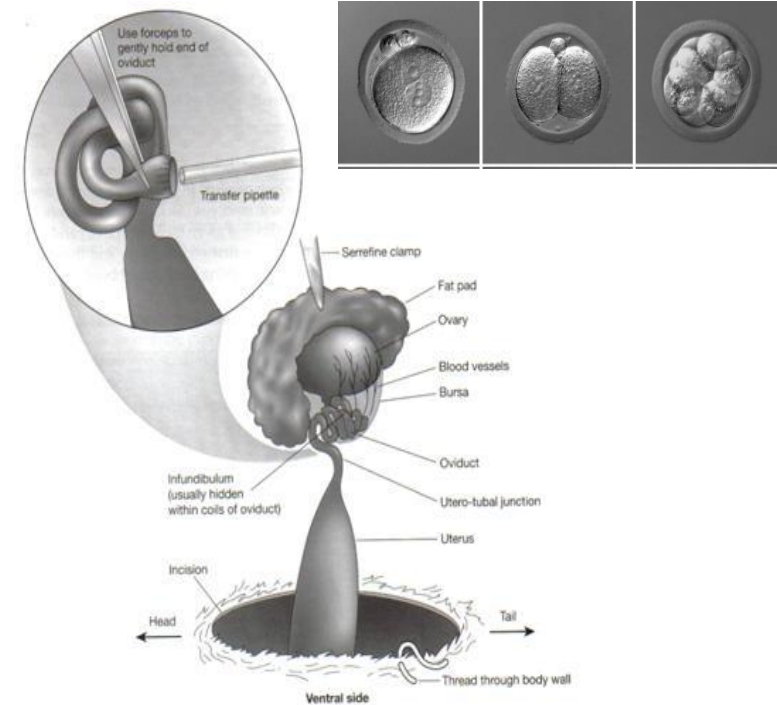
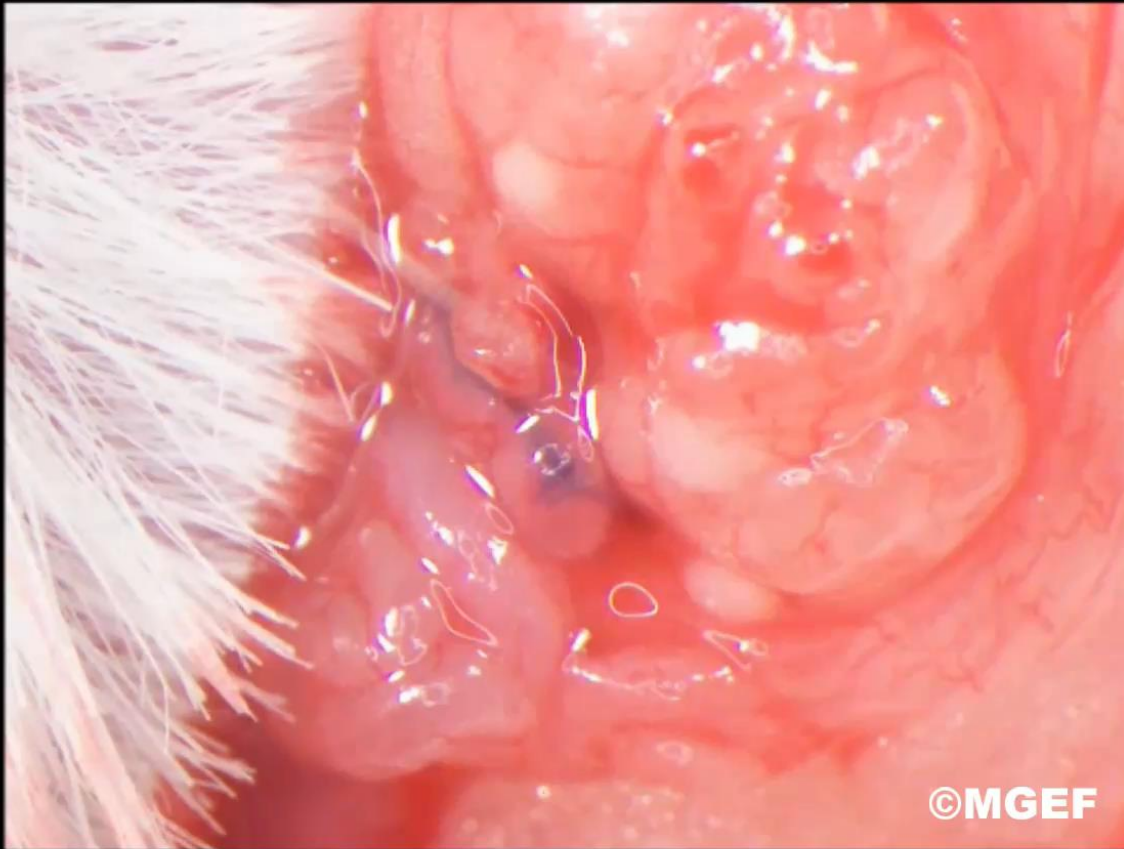
Health Monitoring to SPF status and genotyping of rederived pups

MGEF Rederivation Efficiencies



Mouse stock REDERIVATION via embryo transfer

2nd embryo transfer DEMO using blue dyed media



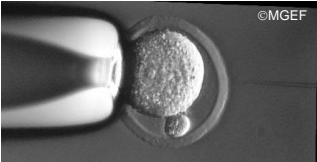
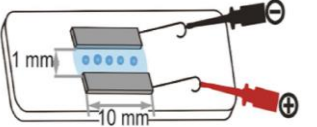

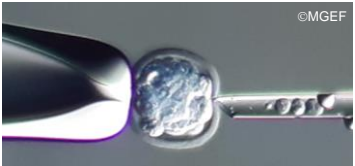
Generating novel mouse models

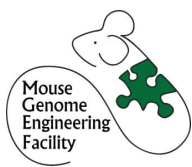


© Ravi Kumar Boyapati



Generating novel mouse models at MGEF (Tg, KO, cKO, KI, KD)

	Transgenics, Random insertions / overexpression	Targeted Knock-OUT	Targeted conditional Knock-OUT	Targeted Knock-IN / Humanization / gene replacement	Knock Down (shRNA,...)
 <p>Pronuclear or cytoplasmic microinjection in zygote</p>	<p>✓</p> <p>linearised DNA inserts up to 15Kb or BACs</p>	<p>✓</p> <ul style="list-style-type: none"> ➤ Crispr/Cas mediated Indels ➤ Crispr/Cas deletions 	<p>Possible with Crispr/Cas + small dsDNA or ssDNA inserts (50-150bp)</p>	<p>Possible with Crispr/Cas + small dsDNA or ssDNA inserts (50-150bp)</p>	<p>✓</p> <p>circular plasmids</p>
 <p>Zygote electroporation</p>	<p>✗</p>	<p>✓</p> <ul style="list-style-type: none"> ➤ Crispr/Cas mediated Indels ➤ Crispr/Cas deletions 	<p>✗</p>	<p>✗</p>	<p>✗</p>
 <p>2 cell micro-injection (2C-HR)-CRISPR</p>	<p>✗</p>	<p>✗</p>	<p>✓</p> <ul style="list-style-type: none"> ➤ Crispr/Cas-Streptavidin + HDR-Flox BIOT-inserts <5-6Kb 	<p>✓</p> <ul style="list-style-type: none"> ➤ Crispr/Cas-Streptavidin + HDR BIOT-inserts <5-6Kb 	<p>✗</p>
 <p>mESc microinjections in 8cell/16cell embryos</p>	<p>✗</p>	<p>✗</p>	<p>✓</p> <ul style="list-style-type: none"> ➤ mESc for Flox inserts >5 Kb 	<p>✓</p> <ul style="list-style-type: none"> ➤ mESc for inserts >5Kb 	<p>✗</p>



Generating novel mouse models at MGEF (Tg, KO, cKO, KI, KD)

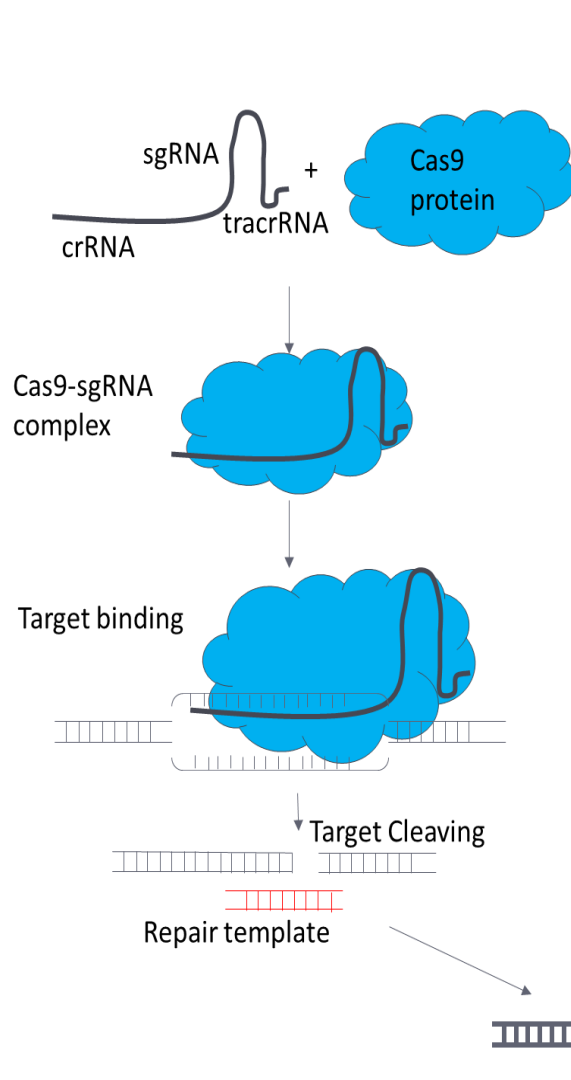
MGEF New Genetically Engineered Mouse Models:

- Successfully designed various models for NCBS, inStem, RGCB, University of Hyderabad, NII, RCB...
- Ongoing projects with IGIB, Syngene, RGCB, CCMB, NCBS...

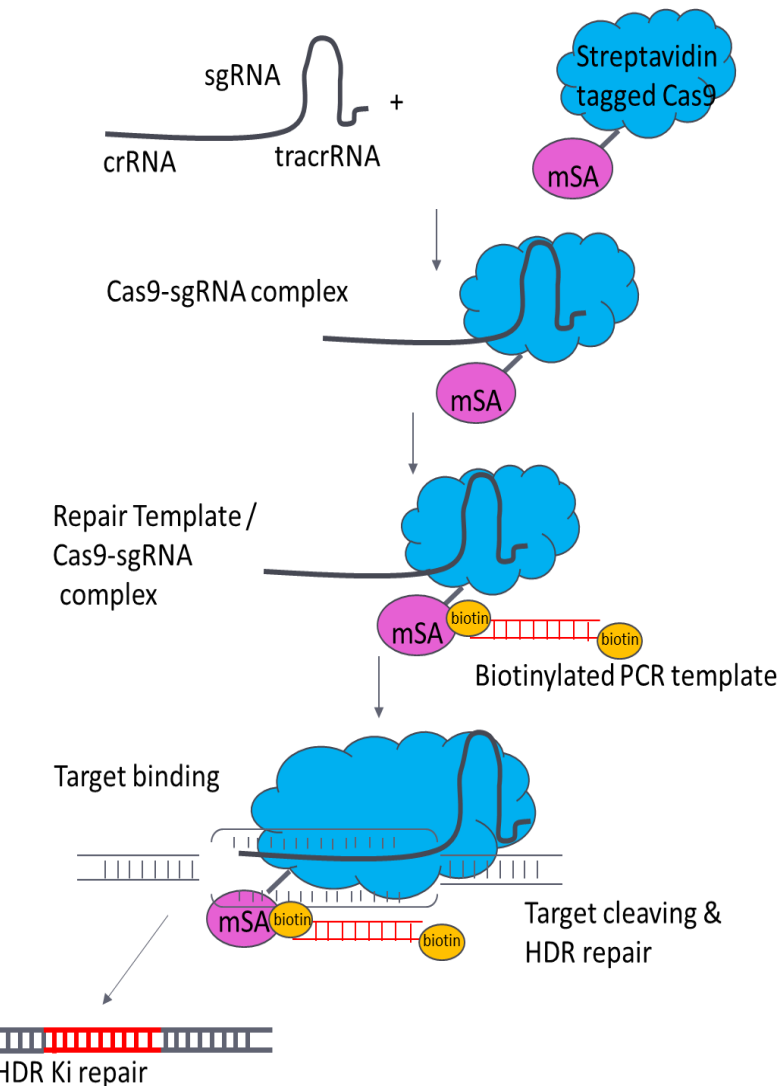
Project Type:	Reagents and concentration used	Micro-Injection or electroporation survival efficiency (% live 2cell embryos/ total embryos injected or electroporated)	Crispr - indel targeting efficiency	Crispr - double guide- correct deletion targeting efficiency	% of successfully generated transgenic /engineered desired live founders (% targeted/ total # live founders born)
Crispr/Cas single guide targeting - Pronuclear zygote microinjection	sgRNA [25ng/ul] + Cas9 or CPF1 protein [50ng/ul]	90% certified staff ~30-50% trainees	63%		
Crispr/Cas double guide – targeted deletion projects (500bp to 300Kb deletions via Pronuclear zygote microinjection)	sgRNA [25ng/ul total guides] + Cas9 or CPF1 protein [50ng/ul]	88%	69%	31%	31%
Crispr/Cas single/double guide + small Insert (<2Kb linear dsDNA) Pronuclear zygote microinjection	sgRNA [25ng/ul total guides] + Cas9 or CPF1 protein [50ng/ul] + 10ng/ul purified dsDNA insert	85%			8%
single or double guide mediated deletions via zygote electroporation	sgRNA [100ng/ul total guides] + Cas9 protein [200ng/ul]	96%	75%	40%	40%
2-cell [2C-HR- crispr] Knock-Ins microinjection with Biotylinated HDR insert 2-5Kb) Knock-Ins	sgRNA [50ng/ul total guides] + Cas9 mSA mRNA [100ng/ul] + 20ng/ul purified Biotylinated dsDNA insert	75%			16% (n=3 projects only so far)
Random transgenesis zygote pronuclear microinjections	dsDNA gel purified linear/digested 3Kb-8Kb Inserts [5-6ng/ul]	87%			20%

Generating KIs by direct embryo microinjections

A- classic CRISPR/Cas HDR mechanism



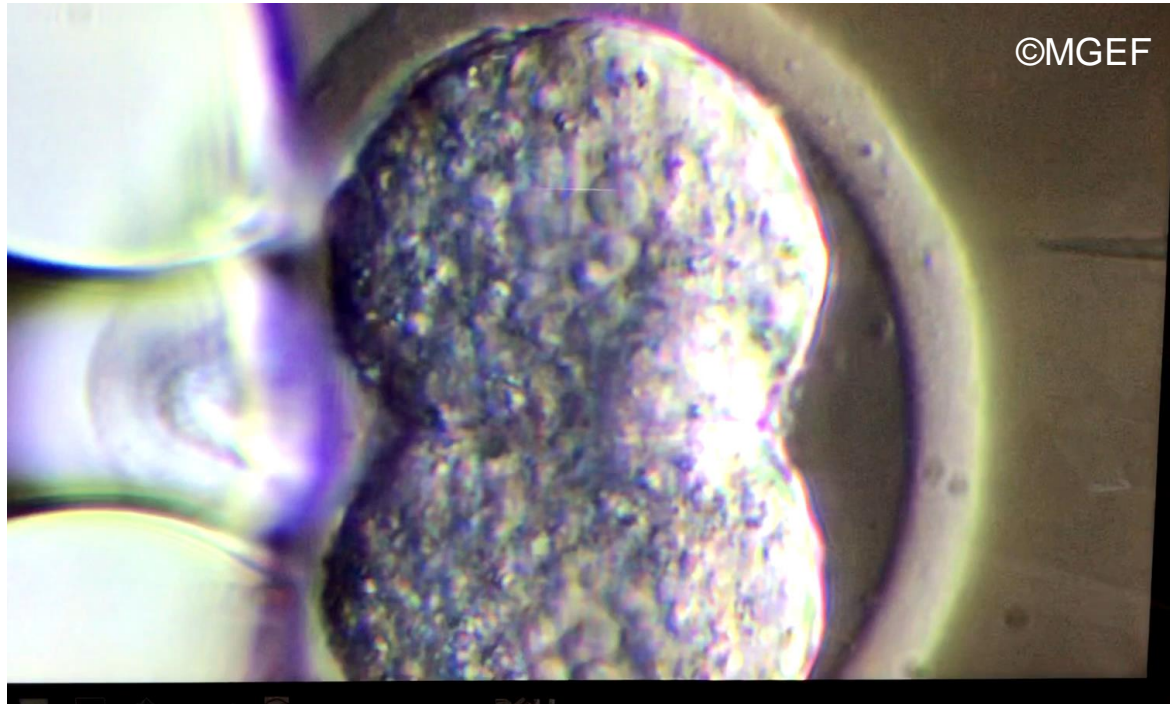
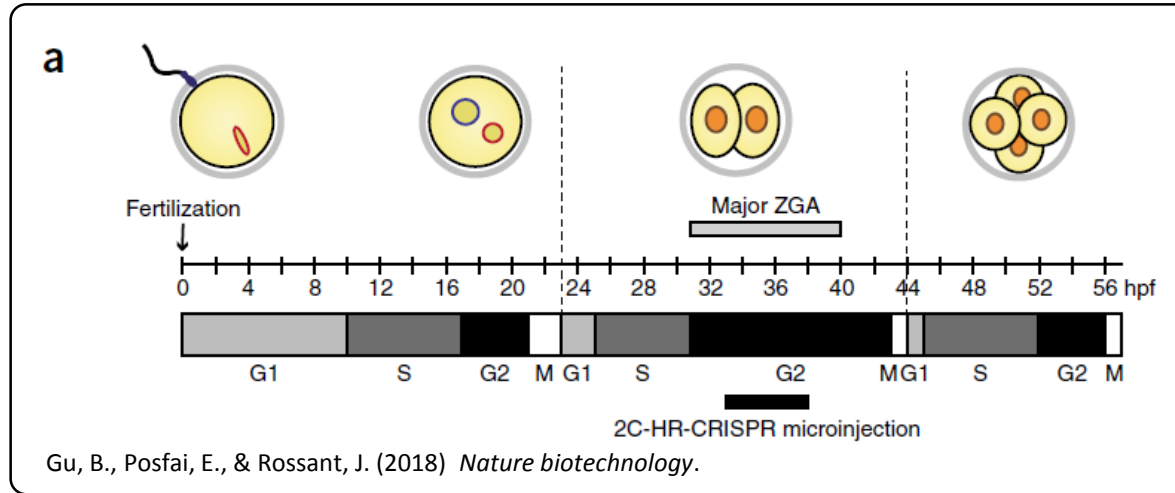
B- Enhanced CRISPR/Cas mediated HDR KI mechanism



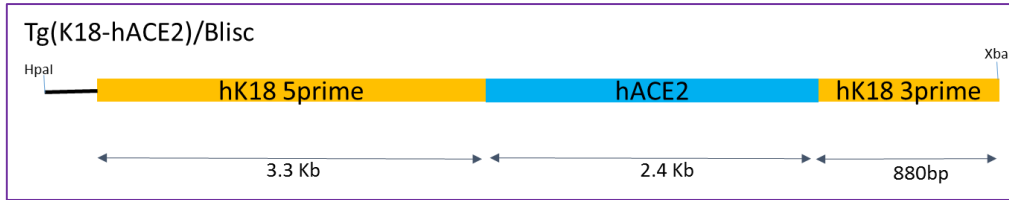
2 cell micro-injection
(2C-HR)-CRISPR

Adapted from: Gu, B., Posfai, E., & Rossant, J. (2018). Efficient generation of targeted large insertions by microinjection into two-cell-stage mouse embryos. *Nature biotechnology*.

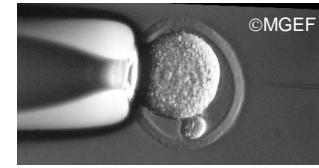
Generating KIs by direct embryo microinjections



1) – Tg humanized

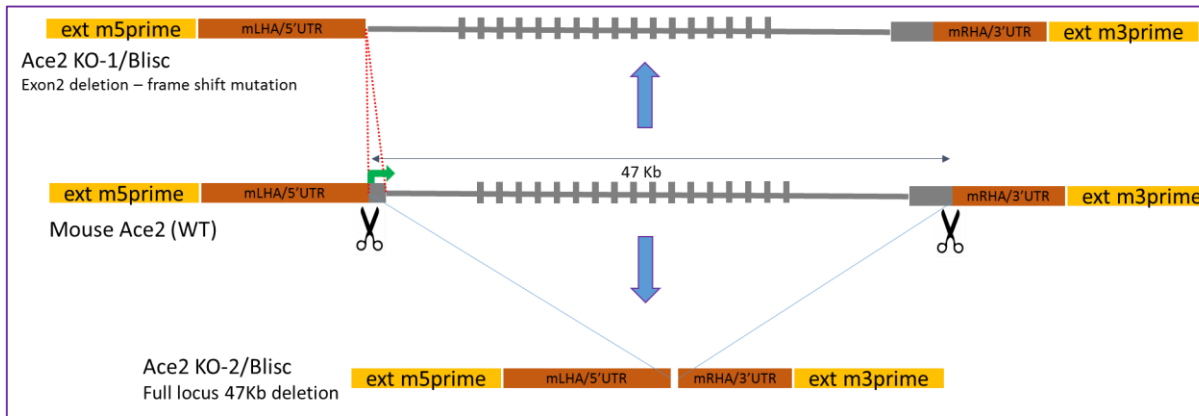


K18-hACE2 plasmid generously donated by Paul McCray - doi:10.1128/JVI.02012-06

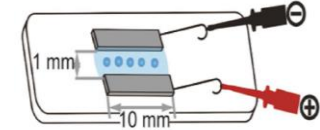


- Zygote pronuclear μ -injection
- 6ng/ μ l linearized 6.8kb insert

2) – KO-1

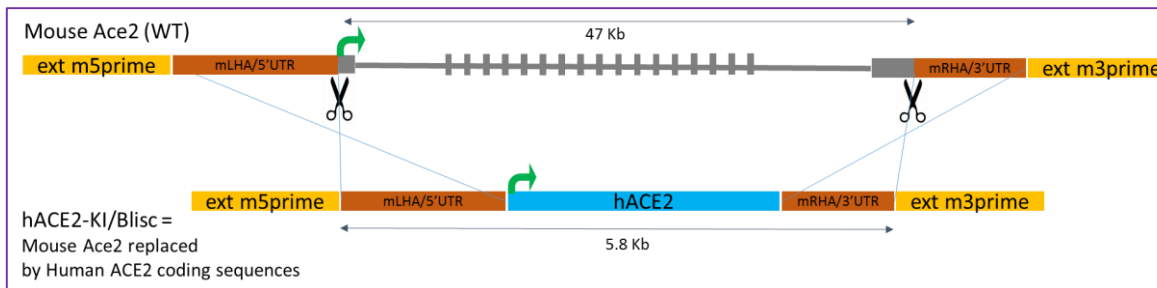


Pronuclear & cytoplasmic microinjection in zygote



Zygote electroporation

3) – KO-2



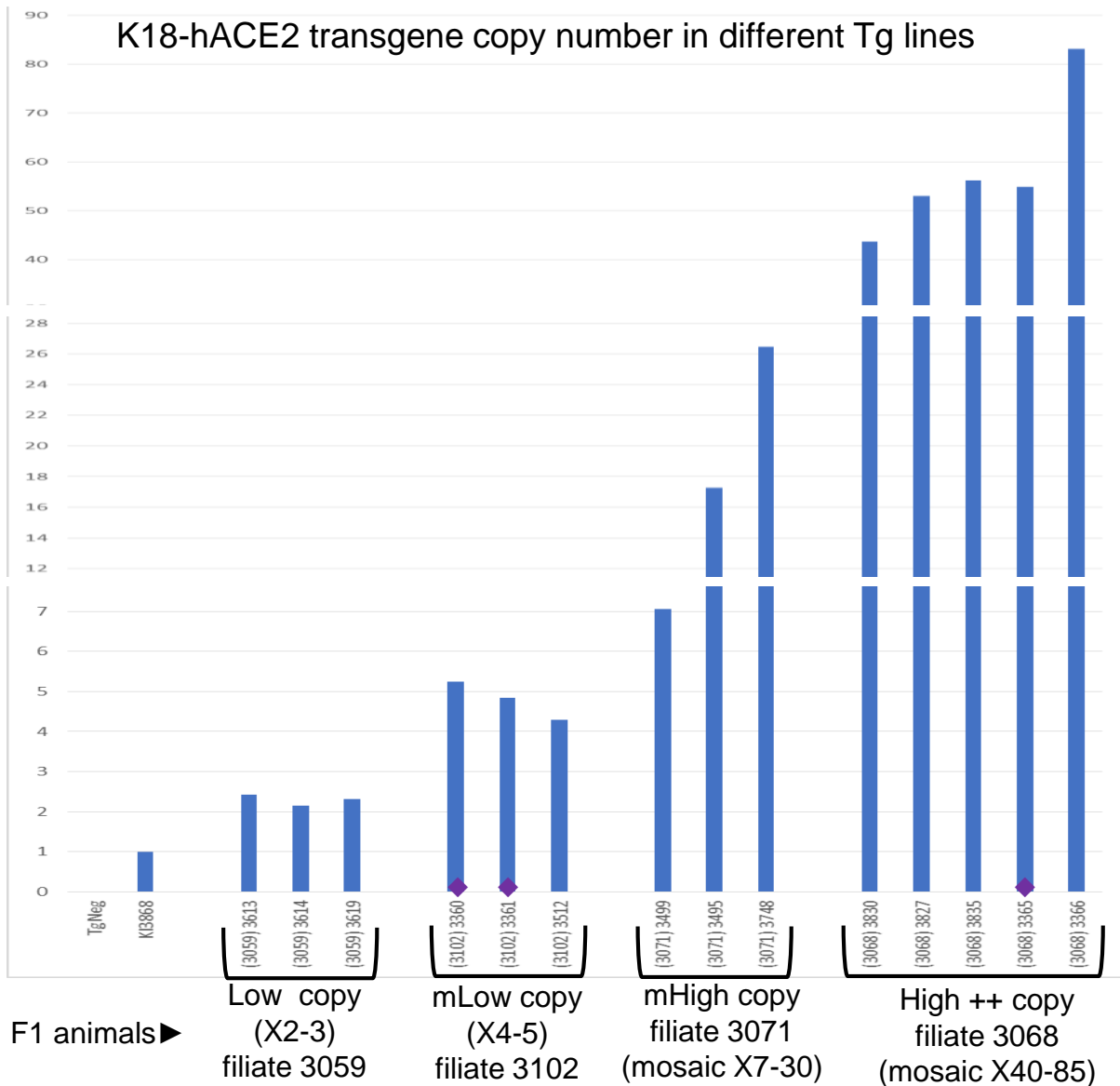
4) - KI humanized

- sgRNA [50ng/ μ l total guides] -> 2 guides
- + Cas9-mSA mRNA [75-100ng/ μ l]
- + 20ng/ μ l purified PCR-Biotynilated dsDNA insert



2 cell nuclear & cytoplasmic μ -injection (2C-HR)-CRISPR

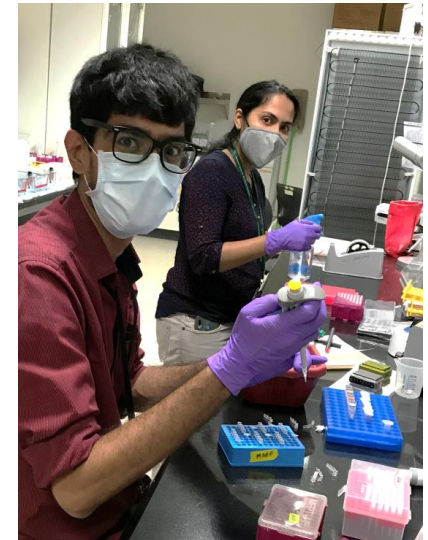
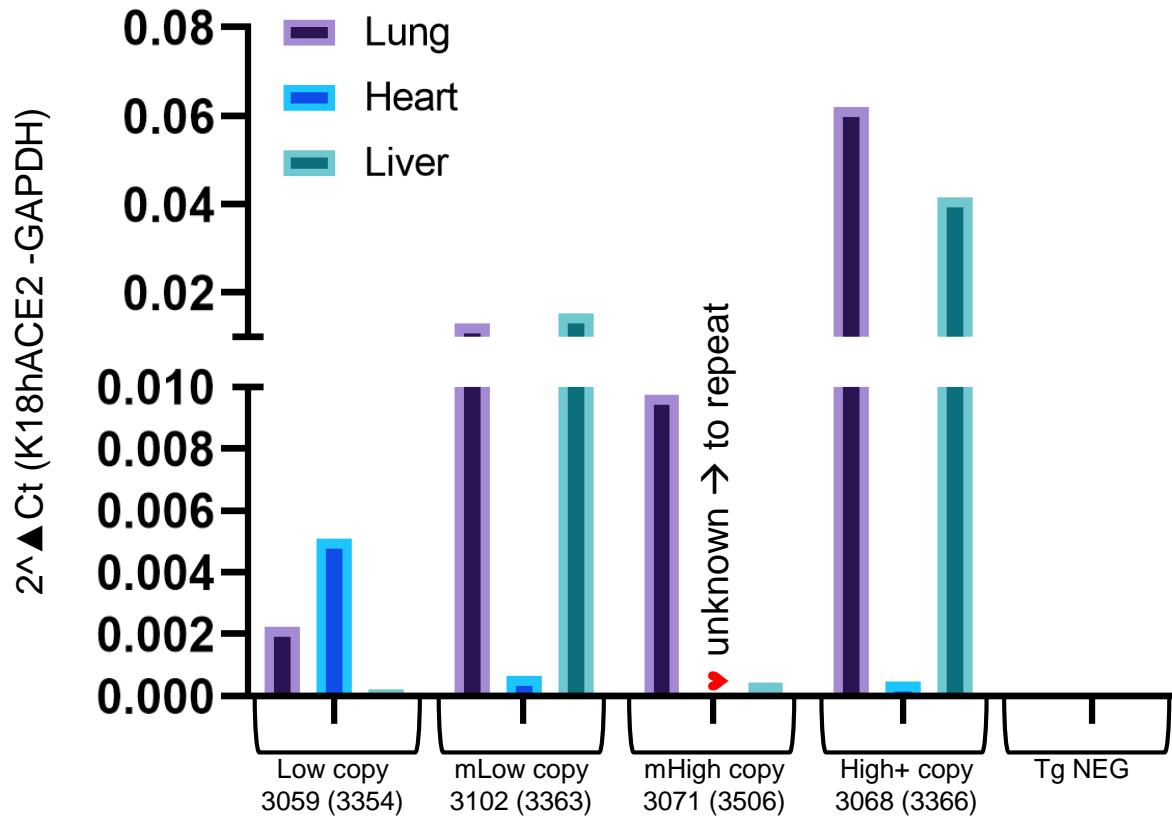
Fold change: ▲ Ct (tg:K18hACE2 / Kl:hACE2)



Divij Kinger & Saumya M Mathew

◆ = Males backcrossed & F2 animals available

Organ specific hACE2 relative mRNA levels



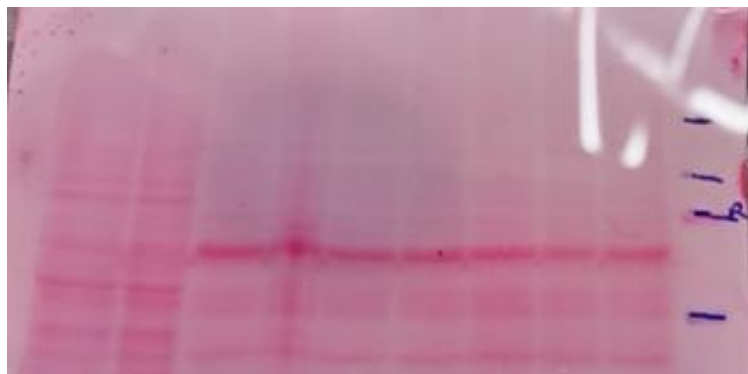
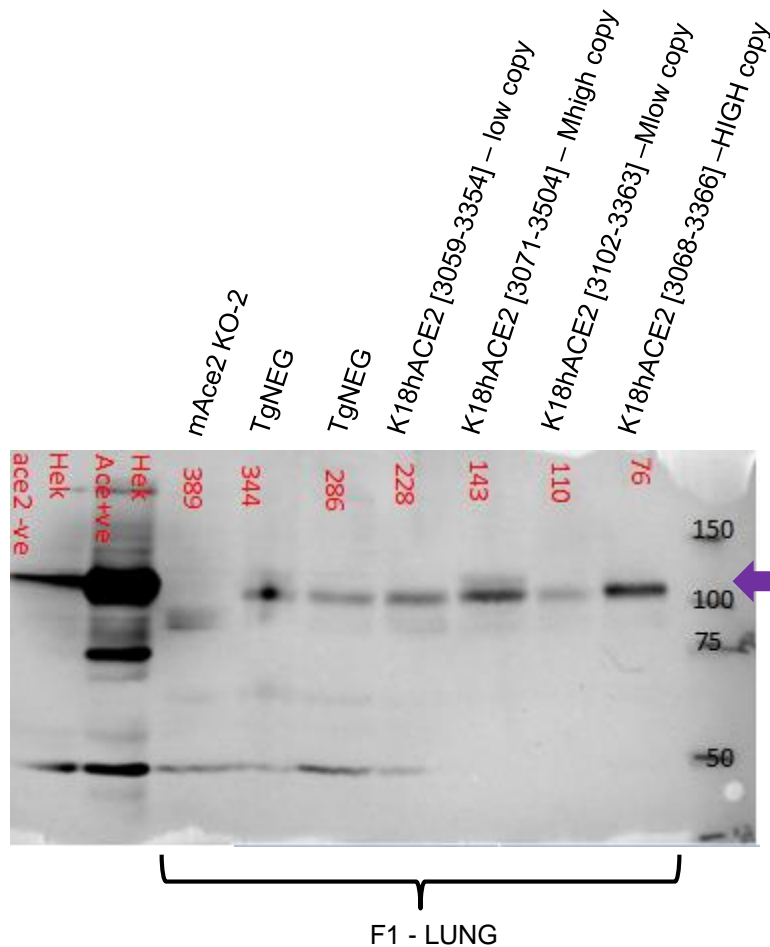
Divij Kinger

F1 animals from different Tg K18hACE2 filiates of different transgene copy numbers

ACE2 protein expression levels by Western Blot



Mona Hosny

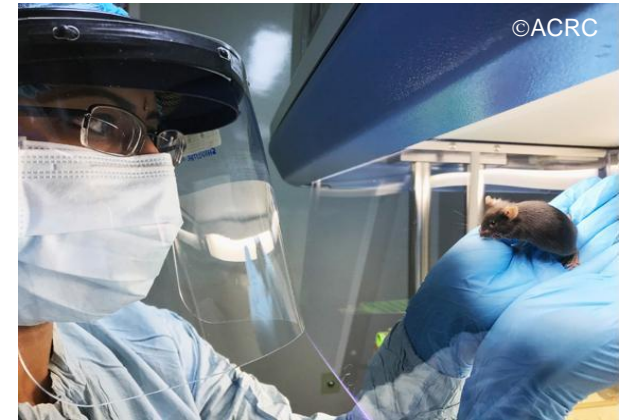


- 100 µg total protein / well
- 25 µl / well

➤ antibody NOT human specific as advertised → also recognizes mouse ACE2 protein

1) MGEF generated various [ACE2 animal Models for COVID19 research](#):

- Tg: K18-hACE2/Blisc mouse models – ready for distribution
- mACE2 KO/Blisc model – validated and ready for distribution
- hACE2 Ki/Blisc mouse model (in collaboration with TIGS) – in progress



2) MGEF standardized in-house production of ALL IVF and embryo culture medias

- Self-sufficient during lockdown / no delays in ordering (backorders), import and custom clearances
- important cost savings \$\$\$

3) Standardisation of 4C COLD transportation of Cauda/Sperm and embryos

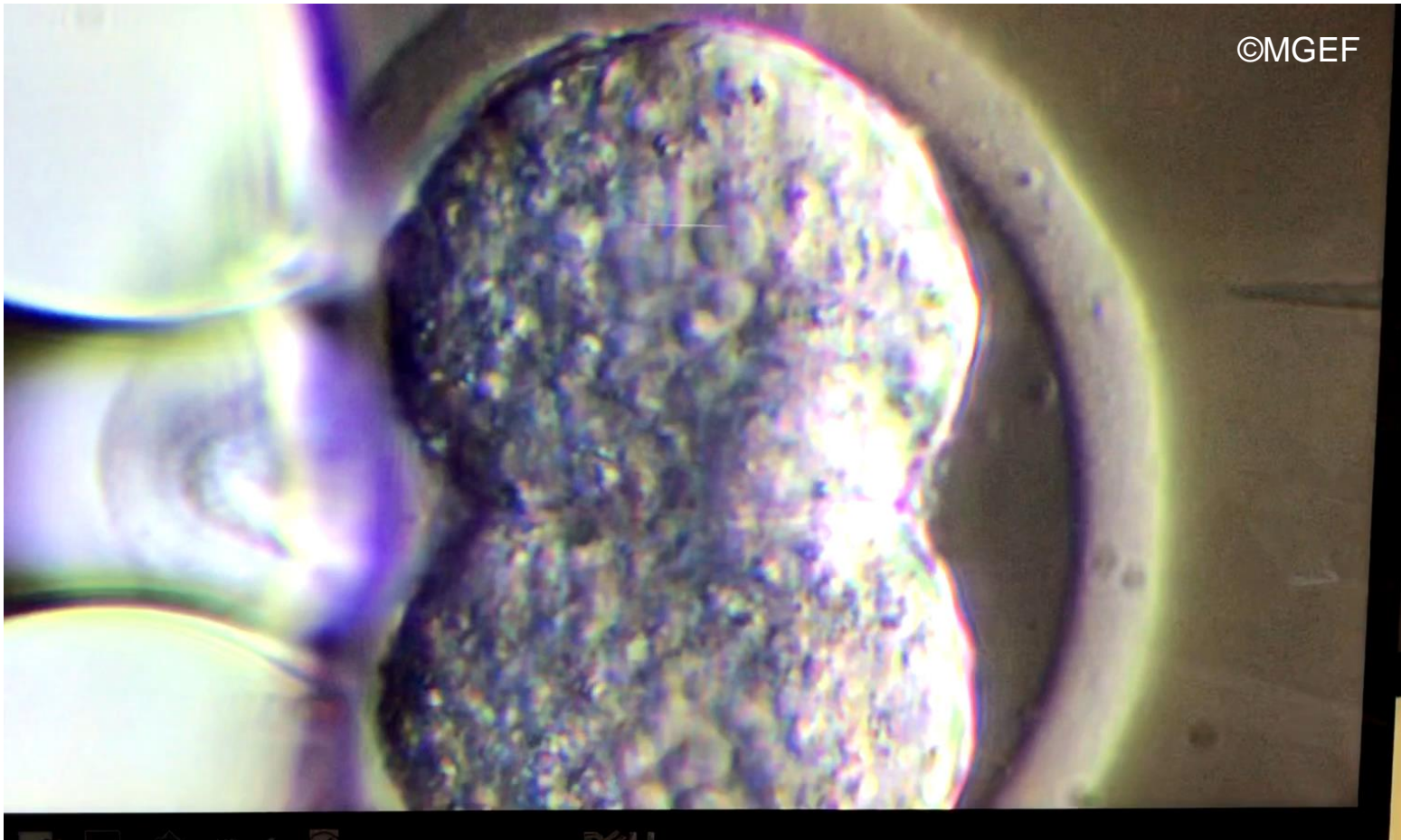
- Facilitate stock sharing within India
- important cost savings \$\$\$

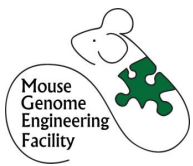
4) Use of Cryorecovered embryos for Microinjections

- Reducing animal usage
- maximizing the usage of unwanted embryo donors
- simplified logistics

Generating novel mouse models at MGEF (Tg, KO, KI, cKO, KD)

Using Cryo-recovered Embryos for nuclear microinjections → Crispr/Cas mediated KI and KOs





standardized in-house production of cryopreservation, vitrification, IVF and embryo culture medias

<u>MGEF home made Medias :</u>	Osmolarity value	Shelf Life	Storage Conditions	Quantity produced/Year
Human Tubal Fluid Media (HTF)	260-290 mOsm	3 Months	4 C	1600ml
Sperm pre-incubation Media (SPI)	260-280 mOsm	3 Months	4 C	800ml
Fertilization Media (HTF+GSH)	NA	3 Months	4 C	600ml
KSOM (for Embryo culture till Blastocyst)	256 mOsm (+/-5mOsm)	6 Months	-30 C	1600ml of 2X KSOM
PB-1 Media for Embryo vitrification (includes 1M DMSO, DAP213 and 1M Sucrose)	285-295 mOsm	3 Months	4 C	1600ml
Sperm Cryo Protective Agent (gCPA)	480-500 mOsm	3 Months	Room Temperature	200ml
M2 media (embryo handling)	265-280 mOsm	6 Months	-30 C	2-2.5 Liters

- MGEF produces cryo / IVF and embryo culture medias with same or higher efficiencies as of costly commercial medias.
- Better management of medias with short shelf lives and delicate storage conditions
- No delays in ordering (backorders), import and custom clearances
- Very significant cost savings

MGEF Workshops and Outreach

- Organizing 2-3 hands-on workshops / year
- ➔ online/video training
- Participating in numerous other training programs nationwide



1st MGEF mouse Microinjection & Crispr/Cas Workshop
October 21-25, 2019



2nd MGEF Mouse Cryobiology / IVF Workshop – August 16-21, 2018

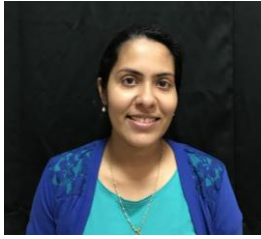




2020 – MGEF staff:



Shilpa



Saumya

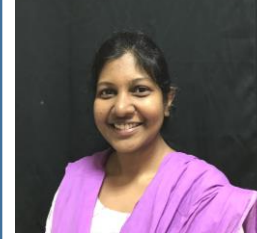


Latha



Lily

2020 – MGEF alumni:



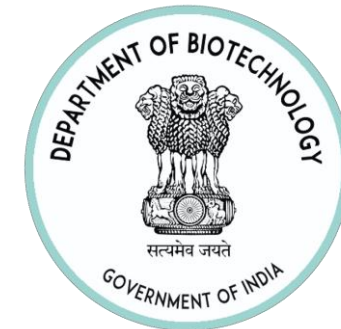
Jasper



Debajeet



Adarsh





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