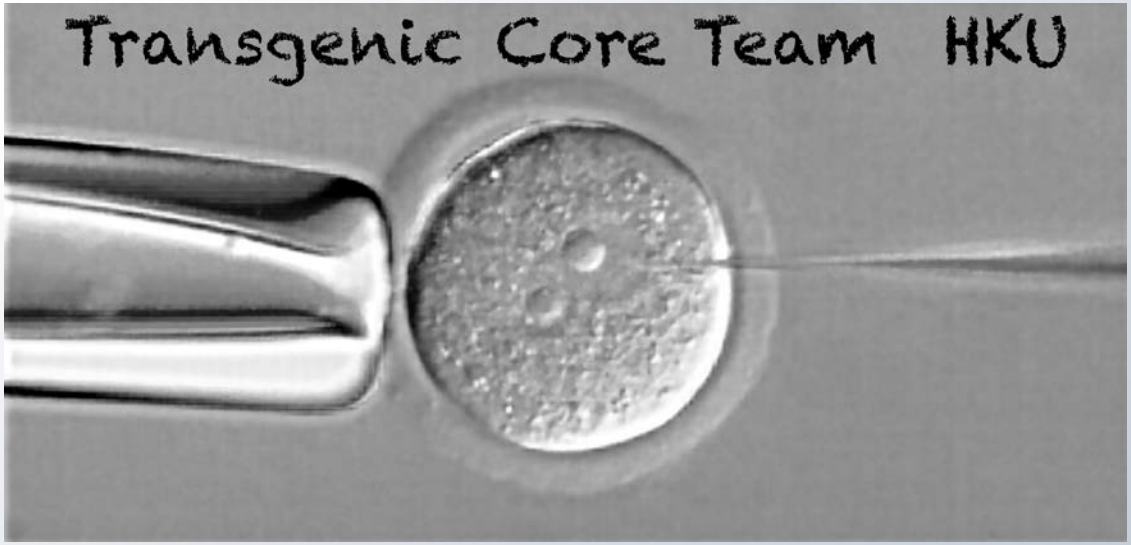
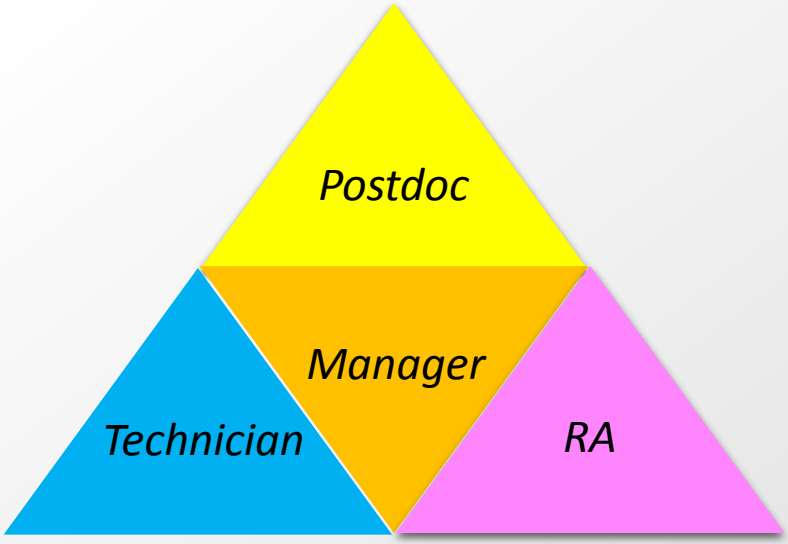
Management of a Mouse Colony



Keith Leung

Infrastructure

Generation and manipulation of transgenic, chimeric mice, cryopreservation ……



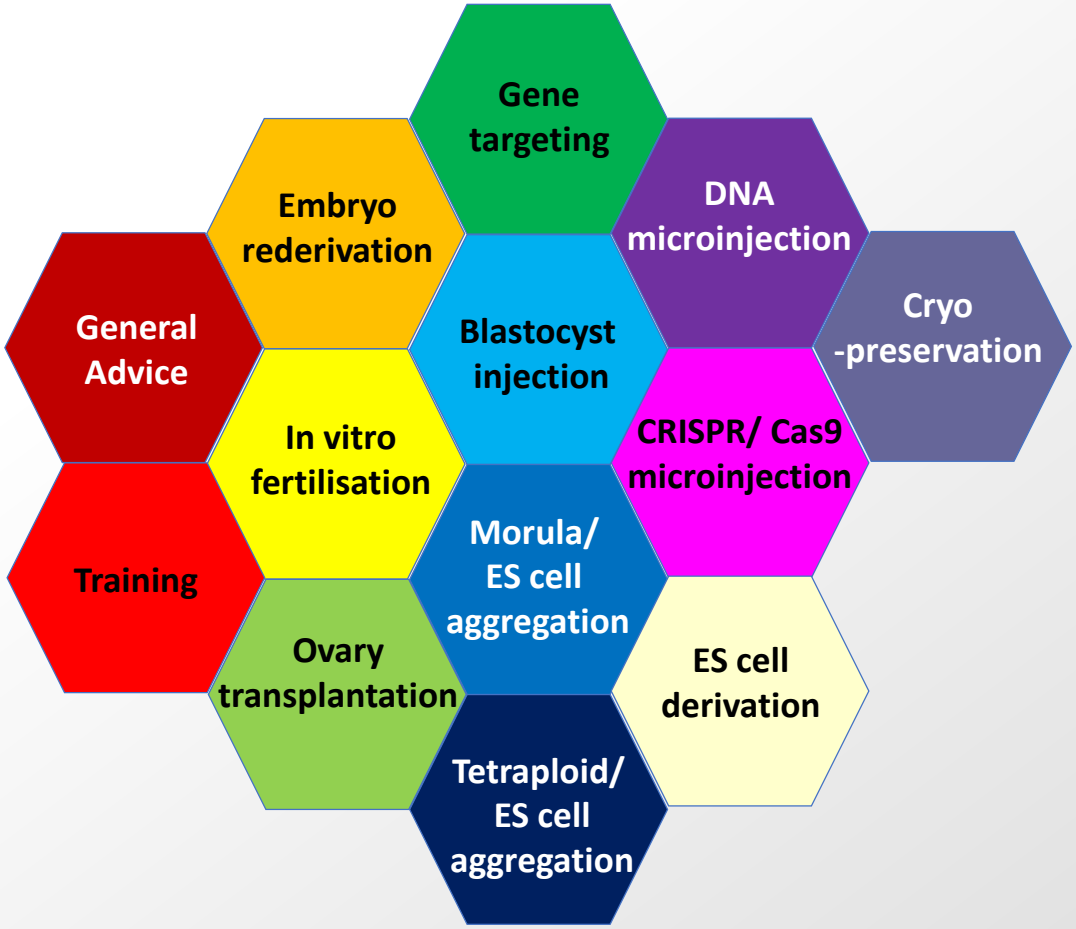
Gene Targeting

Maintenance of the mouse colony

Facility


Tissue culture room
Microinjection room
Mouse bank
Breeding area

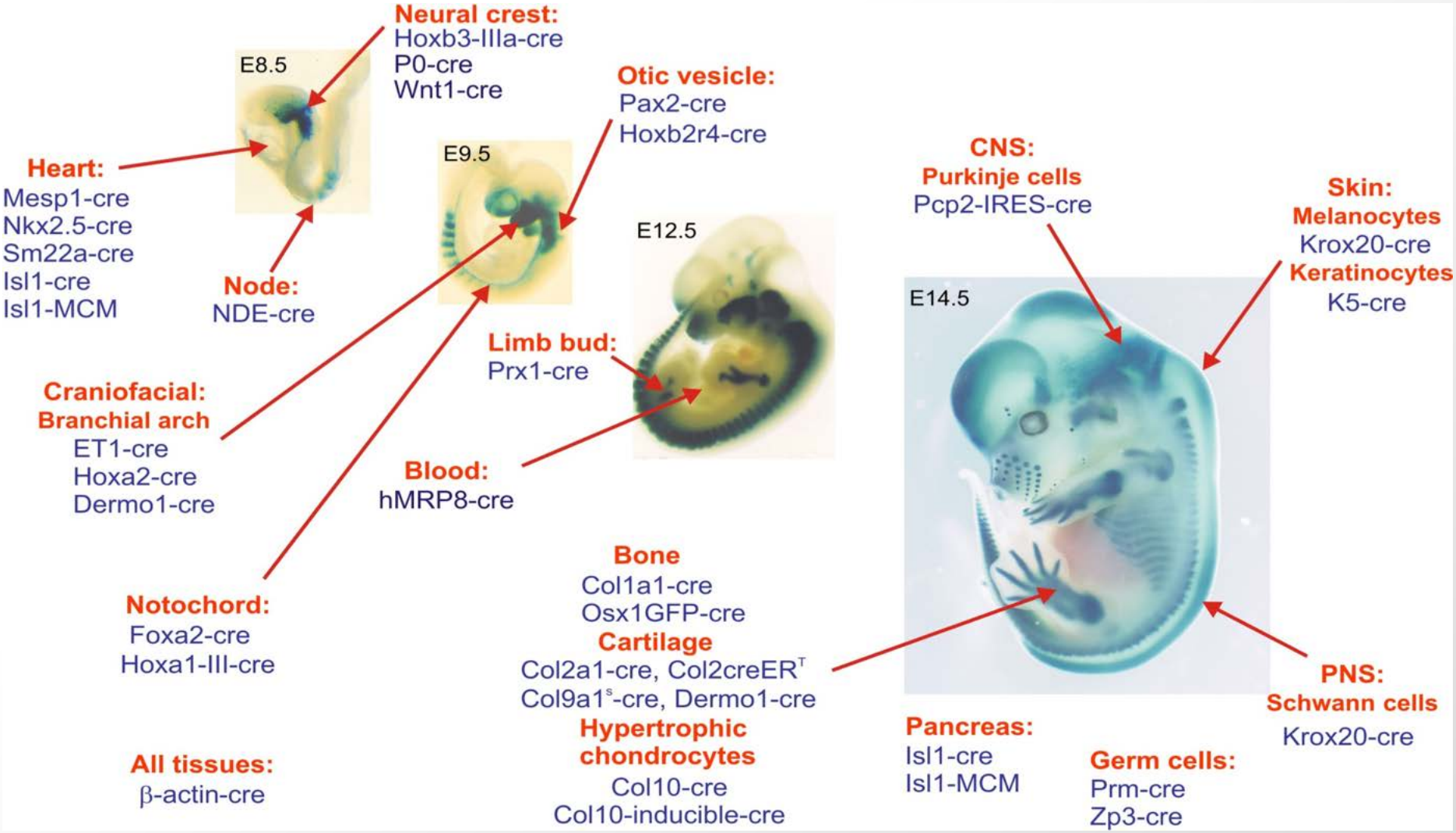
Capacity


Gene targeting
Embryo rederivation
DNA microinjection
General Advice
Blastocyst injection
Cryo -preservation
In vitro fertilisation
CRISPR/ Cas9 microinjection
Training
Morula/ ES cell aggregation
Ovary transplantation
ES cell derivation
Tetraploid/ ES cell aggregation

Rich Mouse Resources

Life-Act RFP	Confetti 4-color	Sox9 3'EGFP	H2B GFP	4-Oct GFP	TetO-H2B-GFP	R26 tdTomato	Rosa26 YFP	GBS-GFP
Noto GFP	GFP	R26 EGFP DTA	Z/EG	Sox9 CreERT2	Col10 BacCre	New Col10Cre	Osx:Cre	Lgr5 KI Cre
β-actin Cre (C57)	β-actin Cre (FVB)	R26 Cre ERT2	Sm22α Cre	Pax2 Cre	Wnt1 Cre	Prm Cre	Nde Cre	Prx1 Cre
Zp3 Cre (FVB)	Zp3 Cre (129 svj)	K5 Cre	Hoxb2 (r4) Cre	Col2a1 Cre	Col2Cre ERTM2	Ihh E95k (neoless)	Smo Flox	MT1-MMP Flox
Cdc42 flox	Gata3 Flox	Dicer flox	Smad1/5 flox	Smad4 flox	Furin flox	Sox2 flox	Sox10 flox	Prkci flox
β-cat ex3 flox	β-cat flox	Ptc flox	Hsp47 flox	Bmp2 flox	Ihh flox	Krox 20 flox	β1-Integ flox	Fn flox
Kif5b flox	Kindlin2 Flox	Ilk flox	Mgp KO	Col10a1 KO	Oasis	IIA 33 neoless	IIAICR	Smad 4 KO
IIA Neo 125	Bmpr1a KO	IIA Hnrnpa1	IIAKOCT	IIA129	Ppr null	Fgf21 KO	CV2 KO	Ihh KO
Rosa26 LacZ	Ptc LacZ	Axin2 LacZ	Topgal	61F08LacZ	Sm22α LacZ	Col2a1 LacZ	Nodal LacZ	Msx2 LacZ
80.3	Flp	Sm22α-Col10	TetONmyc	rtTA	rtTA*M2	rtTA (neo in)	Col1a1 TetO OSKM	Col1a1 TetO OSKM; TetO-H2B-GFP
MgpKO; Sm mgp	rtTA*M2; Col1a1 TetO OSKM	IIAKOCT; Hnrnpa1	Hoxb2> Sox2 fl	FLP> Smo Flox	R26tdTom> β-actin Cre	129svev wt	C57 wt	C3H

Cre Mouse Resources

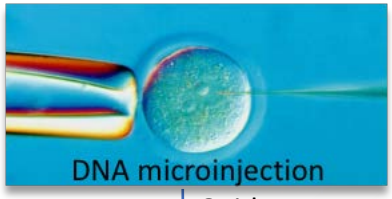


Mouse Mutants for the Manipulation of Signaling Pathways

Signaling pathways	Conditional Mutants	Other Mutants
Wnt	β-catenin Flox	Wnt1 cre, Topgal
BMP	Bmp1ar Flox, Smad1 Flox, Smad4 Flox, Smad5 Flox	Bmp1ar KO, Smad4 KO, BRE-LacZ, CV2 KO
HH	Ppr flox, Smo flox, Ihh Flox	Smo-Rosa26, Ihh KO, Ptc-lacZ
Fgf	Fgfr1 Flox	NIL
Nodal	NIL	Nodal-LacZ, NDE cre

Random Integration of Transgene

Tg



Oviduct

Pseudopregnant mice

Expression

Tg1 - Strong

Tg2 - Weak

Tg3 - No

Non-transgenic pups

Transgenic pups

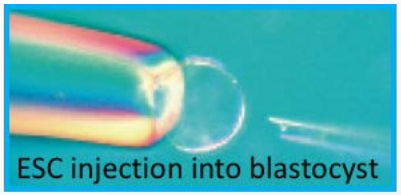
- Expression level and pattern are affected by the integrated sites and copy number

Gene Targeting

Tg + (gRNA + Cas9 + Puro) vector

Electroporation

Puro Selection



Uterus

Pseudopregnant mice

Chimera

X

Wild-type

Germ-line Transmitted

Non-germ-line Transmitted

CRISPR-Cas9-mediated Gene Targeting

Tg

+

Single-stranded guide RNA

+

Cas9 mRNA

Oviduct

Pseudopregnant mice

Tg

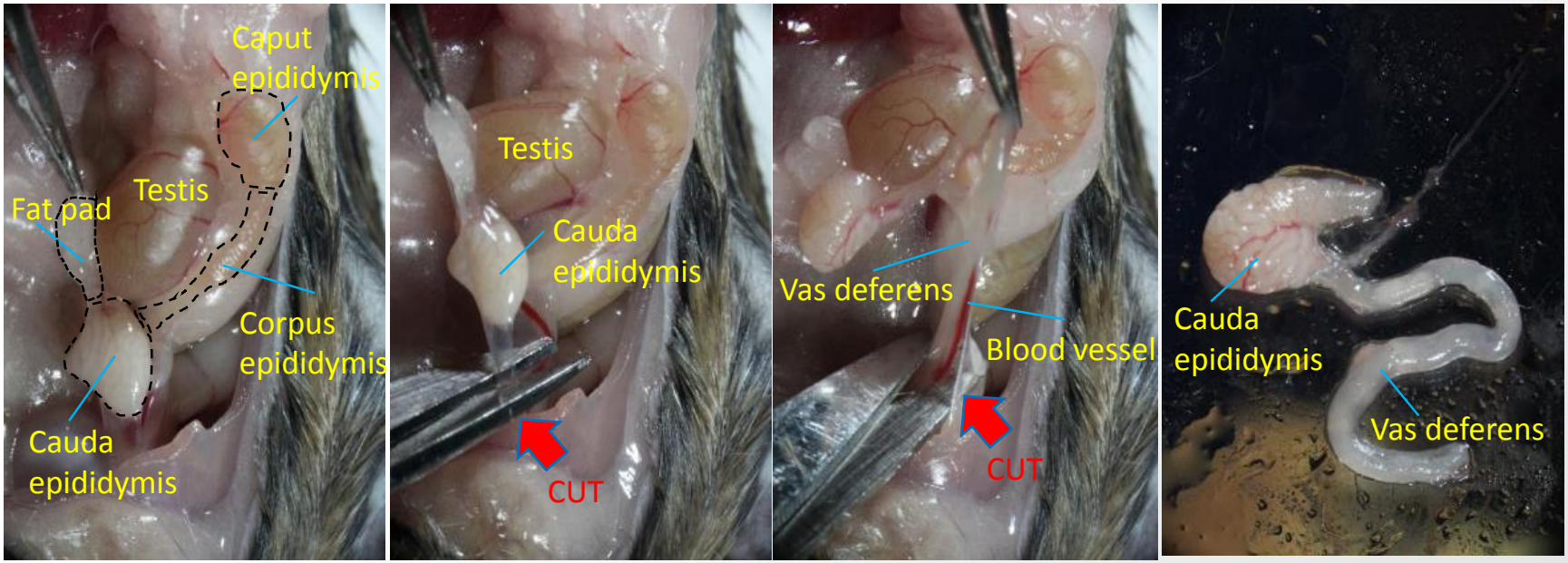
Tg

Tg

Non-transgenic pups

Transgene is knocked into the same targeted site with same expression level and pattern

Harvesting Cauda Epididymides for Transport in Lifor medium at 4°C



- Sperm is viable for 5 days.
- Ready for in vitro fertilization.
- Cheap: about HK$ 3,000 for transport (send out a Thermos flask with Lifor and return with cauda epididymides).
- Several strains can be sent together.
- Less paper work.
- Less technique demanding.

XYClone

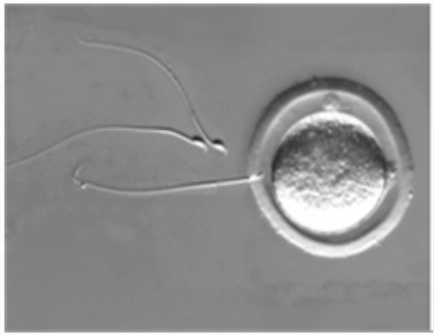
- Laser-assisted injection of ES cells into morula
  - ❑ Enhances germline transmission
- Inner Cell Mass Isolation – ES cell derivation
- Intracytoplasmic Sperm Injection
- Laser-assisted IVF
- Somatic Cell Nuclear Transfer

Maintenance of Mouse Colony

- Keep the colony young - less than 9 months old because their reproductive power declines with age.
- Keep the colony size to minimal to reduce running cost.
  - ❑ At least 4 cages to keep a mouse strain
  - ❑ $2,500 yearly cost to maintain them on shelf
- We had 4200 cages of mice in 2014, holding and husbandry cost was 2.5 million per year.
- Good genotyping protocol.
- Good database system: we use Google Spreadsheet – can be accessed anywhere and free of charge.
- Don’t keep many males together because only one of them is fertile and the others are sterile.
- Functional test of the transgenic mutants as their expression may change when the expressing copy is segregated.

Cryopreservation

- Save space and animal care resources.
  - ❑ $12,000 – cost to make a transgenic mouse strain
  - ❑ $120,000 – cost to make a gene targeting mouse strain
  - ❑ 6-12 months to develop a transgenic mouse strain
- As a backup in case of any catastrophic effects like MHV outbreaks and genetic drift – minimize impact to research by quick cryo-recovery.
- Cryopreserve embryos and sperm.
- Efficiency is increased by IVF: 2 males + 15 females can be used to produce 300-500 2-cell embryos.
- Sperm cryo is cheap and fast. It can be done with two stud breeders.



In vitro Fertilization (IVF)

- is a process by which an egg is fertilized by sperm outside the body.
- to rapidly expand mouse lines from a male that carry the desired genotype.
- to maintain or rescue strains with poor breeding efficiency.
- to produce age-matched mice or fetuses.
- cryopreservation at the two-cell stage (speed cryo).
- to produce two-cell embryos which are then cultured to morulae for aggregation.

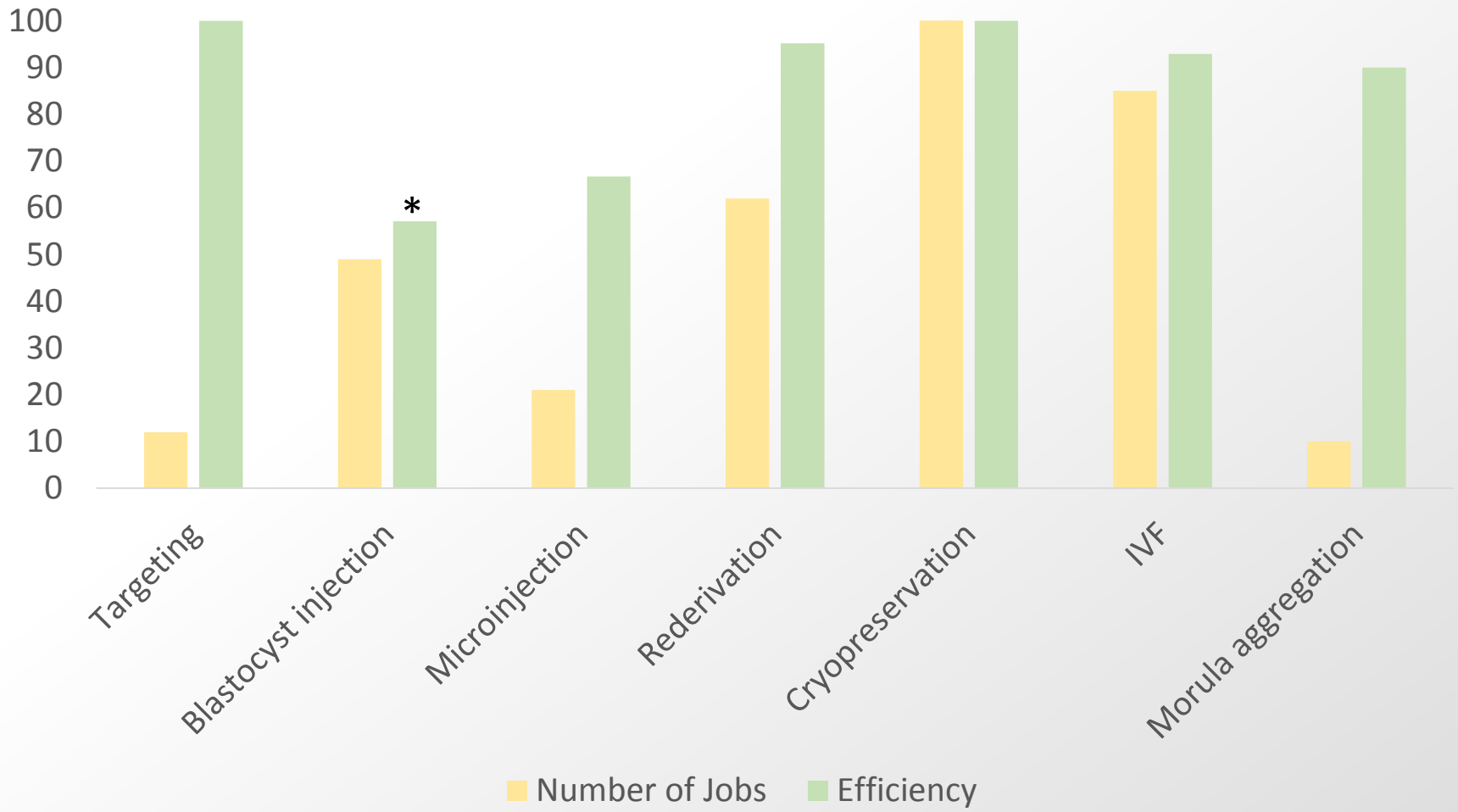
Output: 1999 to present

- generated 90 vectors for gene targeting and transgenic mice.
- generated 71 knockout / knock-in mice; 32 transgenic mouse lines.
- imported, rederived and established over 100 lines of transgenic /mutant mouse lines.
- 200 mouse lines were cryopreserved.
- provided training in the form of hands-on training to many students and investigators.
- supported in transgenic/knockout aspects for 50 RGC-funded projects, 3 RGC Group Research projects, the AoE programme, the AOSpine programme and the Theme-based Research Scheme.

TCT Has Been Providing Services for Many Researchers from the Faculty of Medicine of HKU and Other Institutes

	Principal investigators	Department /School
AoE	K Cheah, D Chan, KM Yao, MH Sham, JD Huang, Z Zhou	Biochem
	K Cheung	Orthopaedics and Traumacology
TRS	K Cheah, D Chan, KM Yao, JD Huang	Biochem /SBMS
	K Cheung	Orthopaedics and Traumacology
	QZ Lian	Medicine
Others	D Chan, M Cheung, MH Sham, JD Huang	Biochem /SBMS
	ZJ Zhou, B Gao, DY Jin	Biochem /SBMS
	S Chung	Anatomy /SBMS
	HF Tse, SL Ho, MY Mok, YL Lau	Medicine
	SY Yung, E Tse	Medicine
	CH Lui and P Tam	Surgery
	M Peiris and HL Yen	School of Public Health
	SY Leung	Pathology
	Y Wang	Pharmacology and Pharmacy
	SY Chan	Paediatrics and Adolescent Medicine
	KY Yuen	Microbiology
	V Leung	Orthopaedics and Traumacology
	KM Kwan	CUHK
	Peng Li	Guangzhou Institute of Biomedicine and Health

Efficiency of Jobs 2014-2017



- 57% of ES cell clones produced chimeras
- Germline transmission rate: 82%