Institute of Biochemistry and Cell Biology
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Chinese Academy of Sciences

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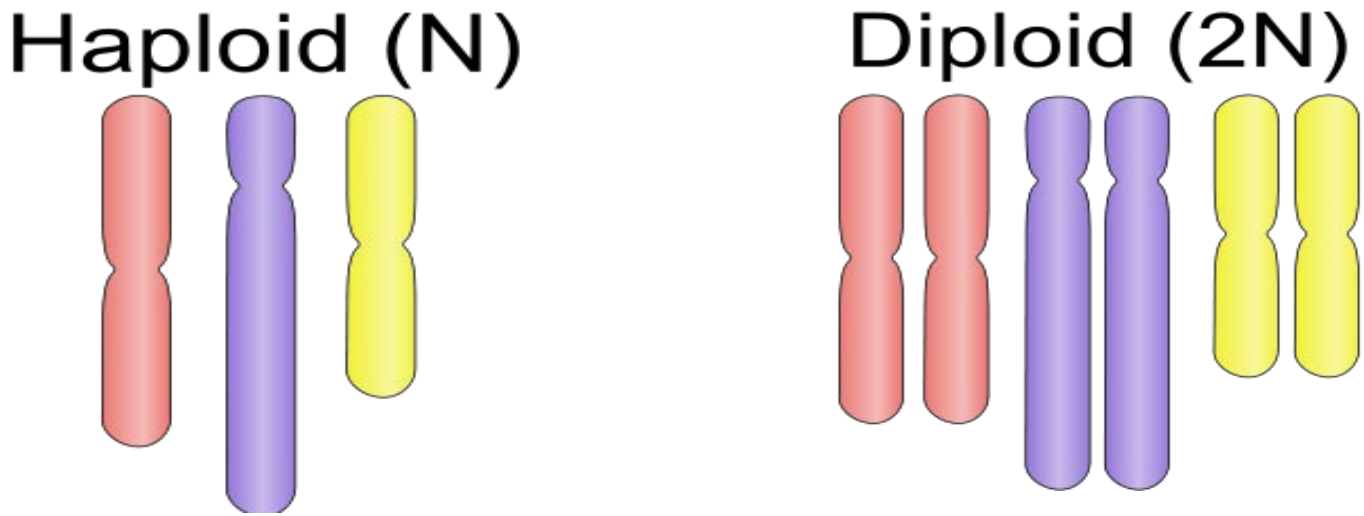
When Haploid Cells Met CRISPR-Cas9

Jinsong Li

jsli@sibcb.ac.cn

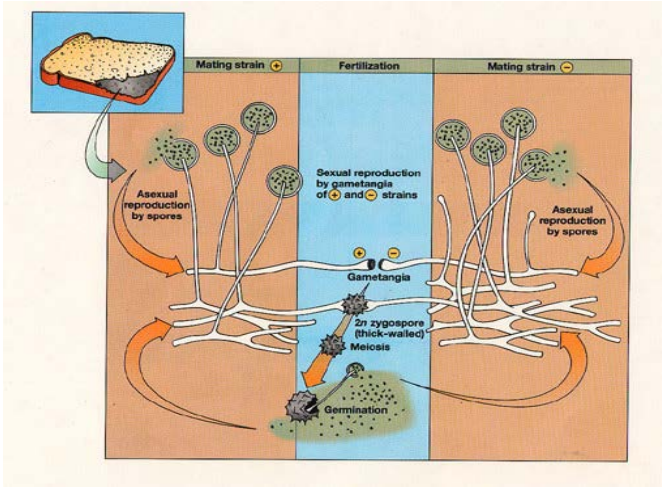
Shanghai Institute of Biochemistry and Cell Biology (SIBCB), CAS

- **Haploid cell is a powerful tool for genetic studies.**

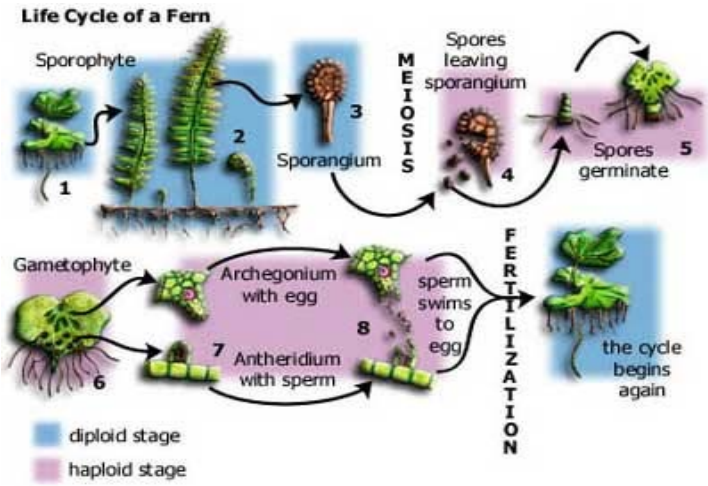


Haploid cells have been largely utilized for mutational screens on a genome-wide scale, as a recessive mutation should result in an unambiguous phenotype due to the absence of a second gene copy.

- **Haploid cells exist in lower organisms.**



Fungi



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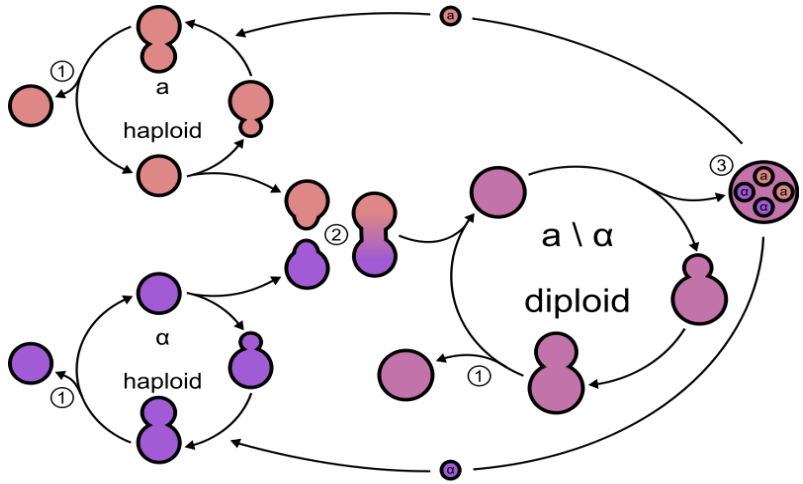
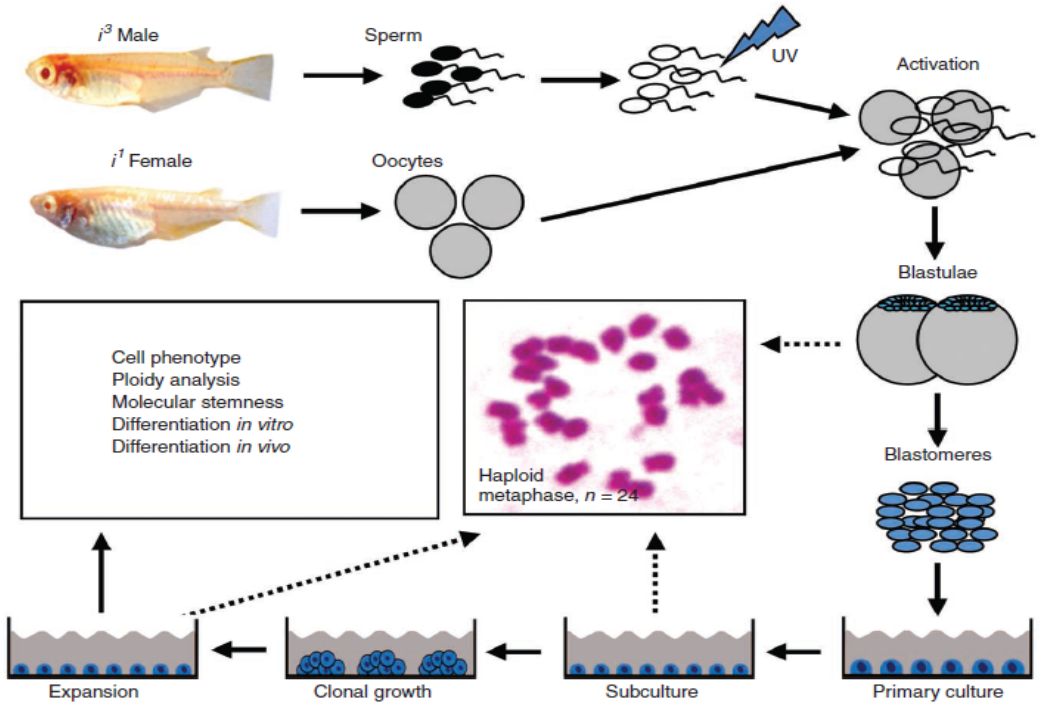
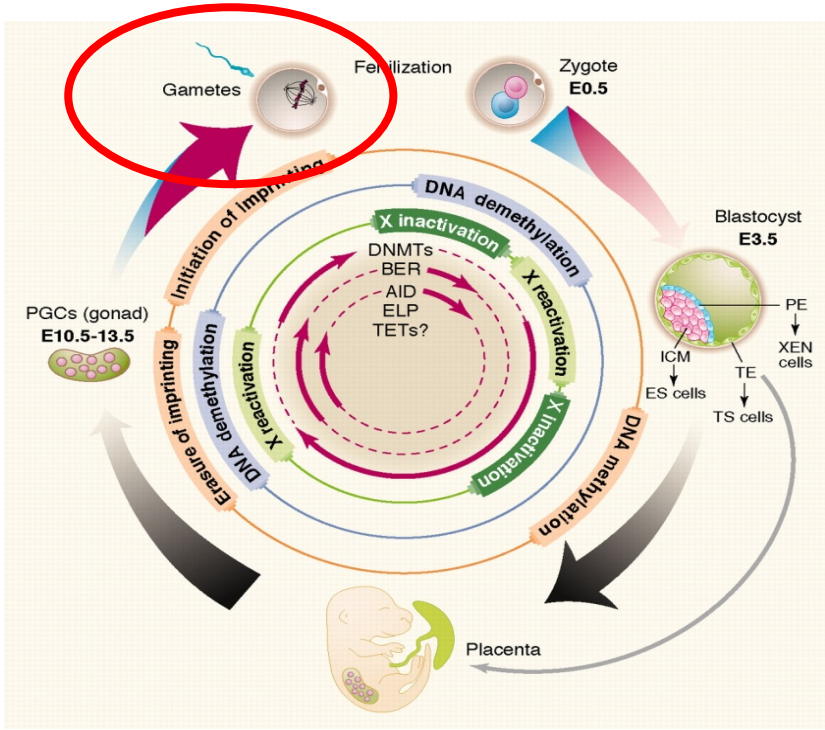


Life cycle of yeast

Yeast has been used as an important tool for genetic studies.

Question: how to derive haploid cells from other organisms?





Medaka Fish Haploid ESCs

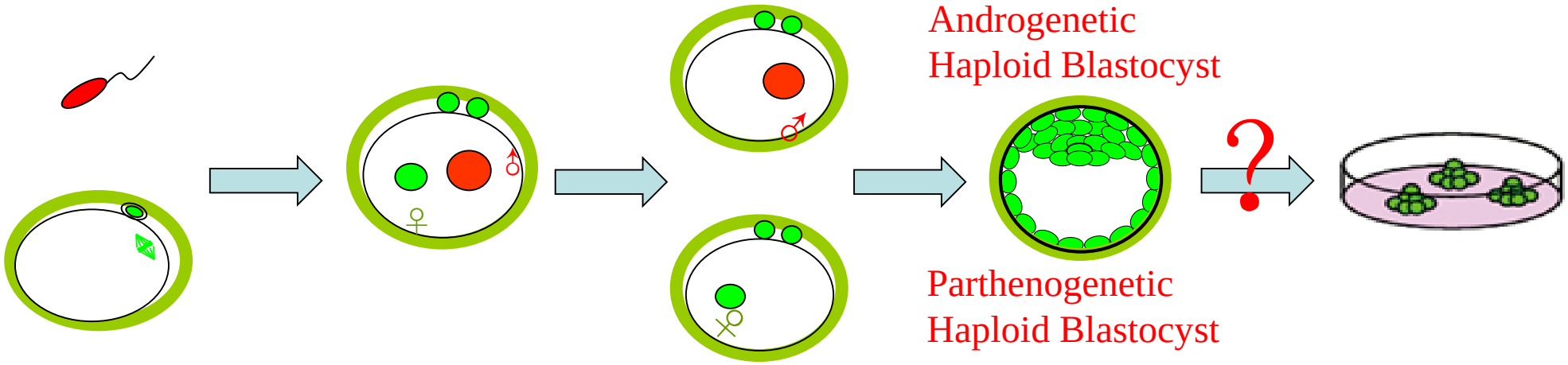
Meisheng et al., *Science* 2009

Meisheng et al., *Nat Protoc* 2010

Gametes are haploid cells in mammalian organisms

Feng et al., *Science* 2010

- **Question: can haploid cells be derived from mammalian gametes?**



Kaufman, *J Cell Sci* 1973 Kaufman & Gardner, *J Embryol Exp Morphol* 1974 Tarkowski & Rossant, *Nature* 1976

Kaufman, *J Embryol Exp Morphol* 1978

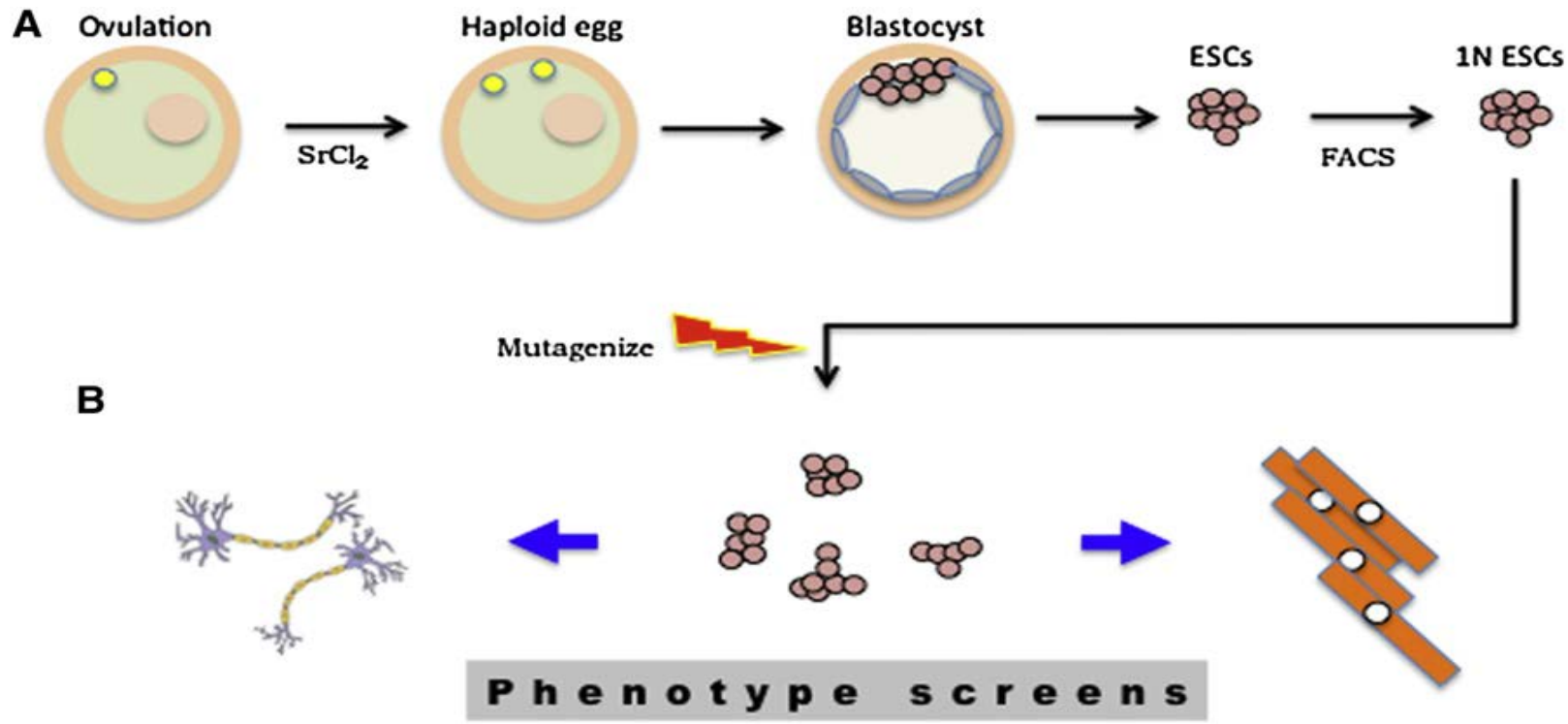
ESCs derived from haploid embryos are diploid

Table 2. *Haploid-derived pluripotent cell lines*

	Strain of origin	Lines established	Modal chromosome number
1.	129 SvE	HD1	40
2.	..	HD2	40
3.	(C57BL × CBA)F_1	HD3	40
4.	..	HD4	40

Evans & Kaufman, *Nature* 1981 Kaufman, *J Embryol Exp Morphol* 1983

- **Generation of mouse haploid ESCs from parthenogeneitc embryos.**



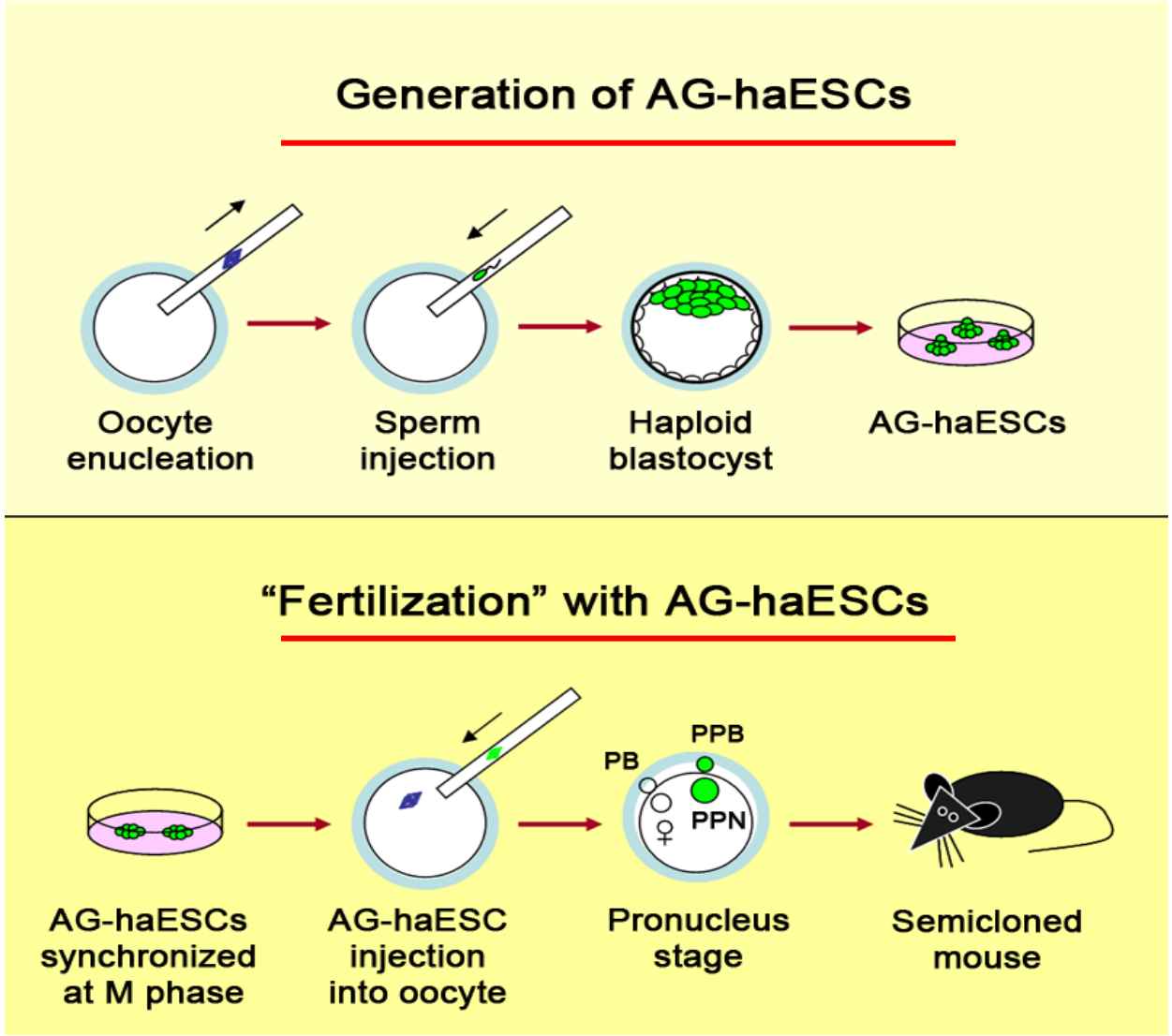
Repeated sorting is essential to establishing and maintaining haploid ESCs.

Leeb & Wutz, *Nature* 2011

Elling et al., *Cell Stem Cell* 2011

Schimenti, *Cell Stem Cell* 2011

Generation of culturable “spermatid cells”



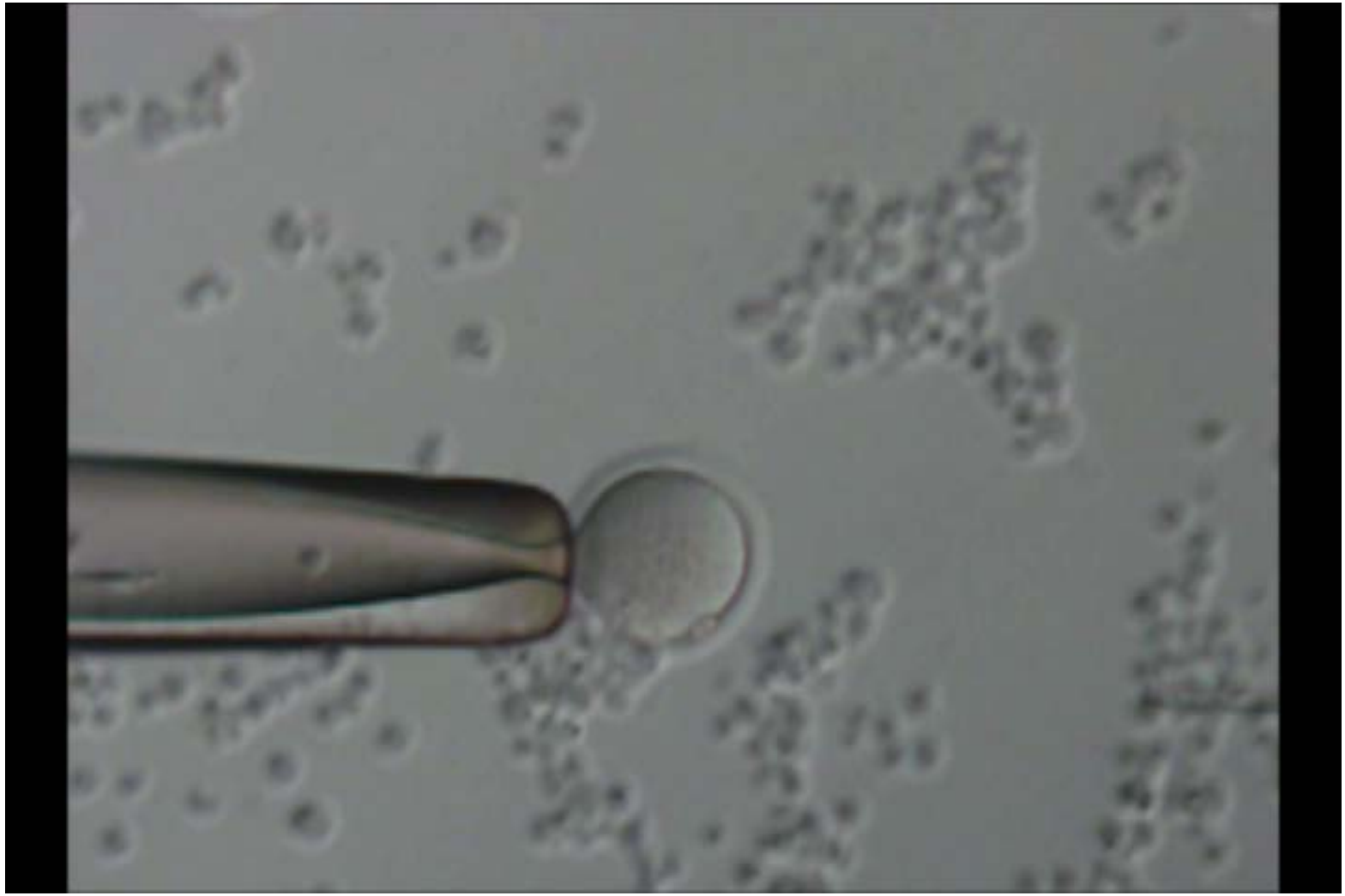
Androgenetic haploid ESCs (AG-haESCs)

Semi-cloned mice (SC mice)

Yang et al., Cell, 2012

In Collaboration with Dr. Guoliang Xu’s lab

Intracytoplasmic AG-haESC injection (ICAHCI) technology

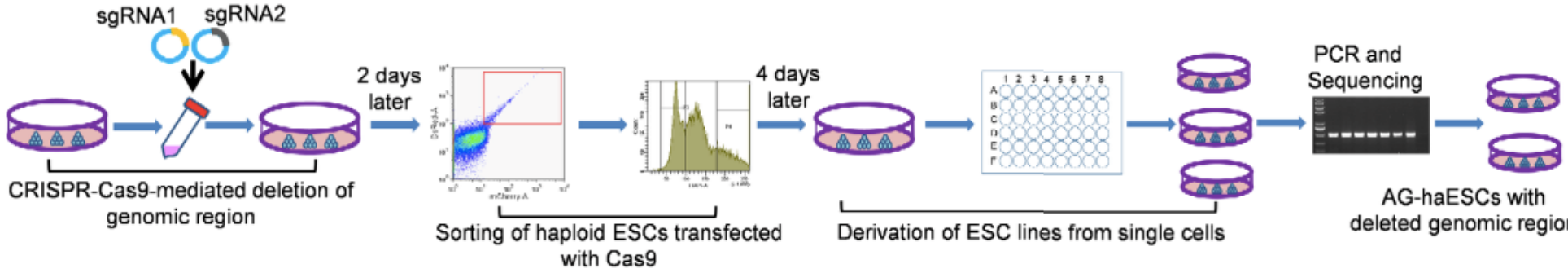


However, AG-haESCs can not be used as a tool to quickly make gene-modified mice due to the low efficiency of SC mice generation.

Question：

How to stabilize the “fertilization” ability of AG-haESCs?

- **AG-haESCs carrying *H19*-DMR and *IG*-DMR deletions can efficiently produce SC pups.**



58 DKO-AG-haESC lines

ICAHCI analysis

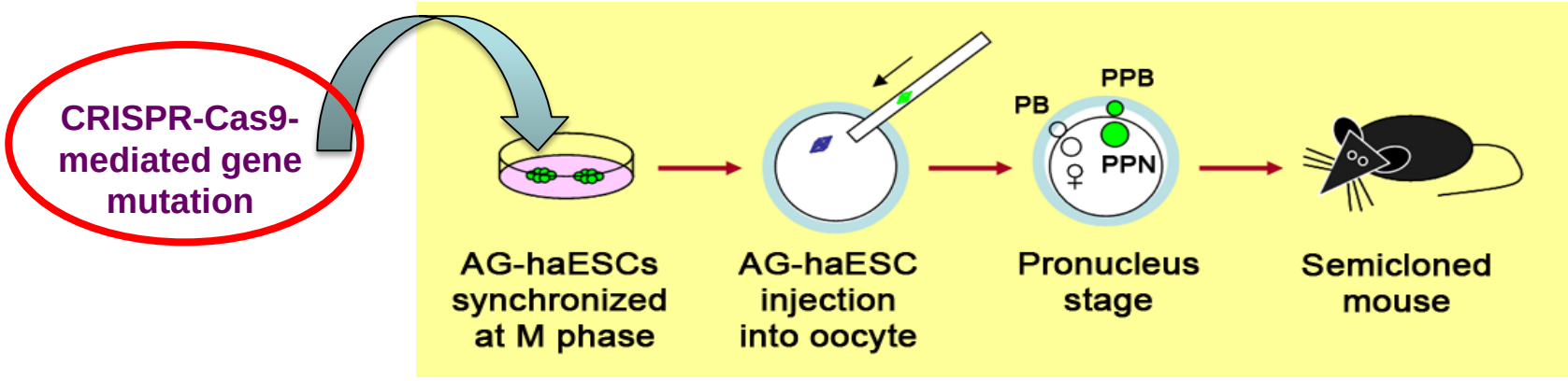
Haploid ESC Line	Passage Number	No. of Embryos Transferred	No. of Growth-Retarded Pups (% of Transferred Embryos)	No. of Normal Pups (% of Transferred Embryos)
H19$^{\Delta DMR}$-*IG*$^{\Delta DMR}$-AGH cells	p19-p33	939	4 (0.4)	210 (22.4)

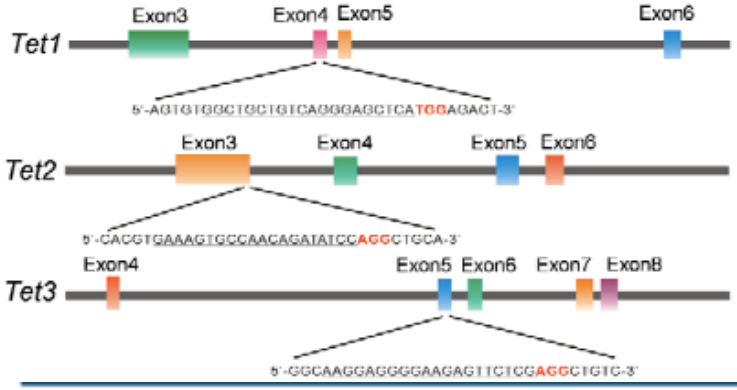
WT | 2% | 2%

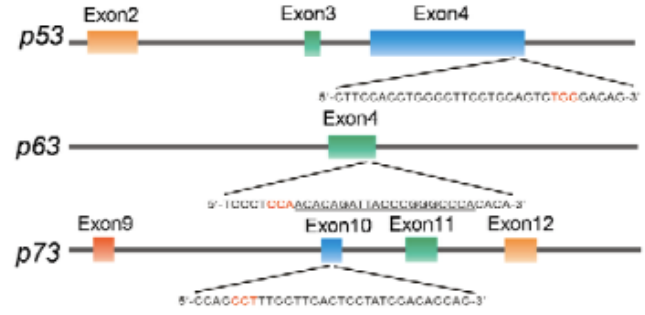
Question：

How to use DKO-AG-haESCs?

Application 1: multiple gene mutations in DKO-AG-haESCs.

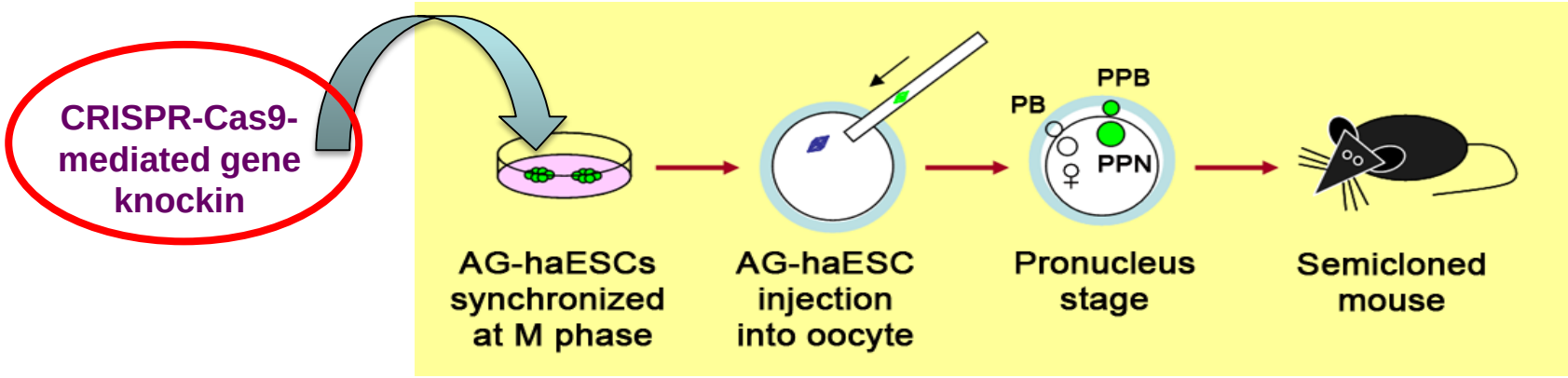


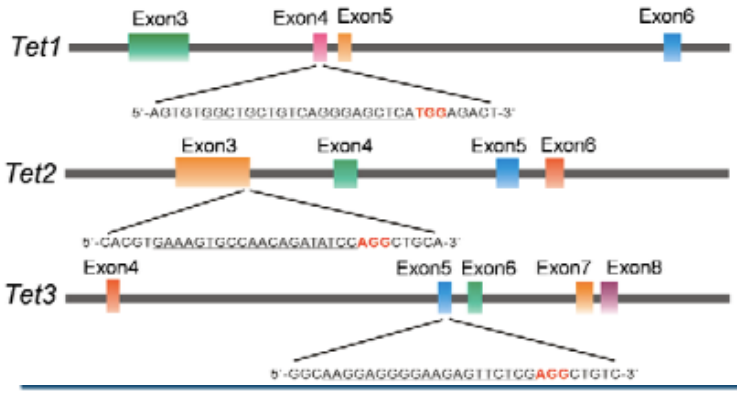


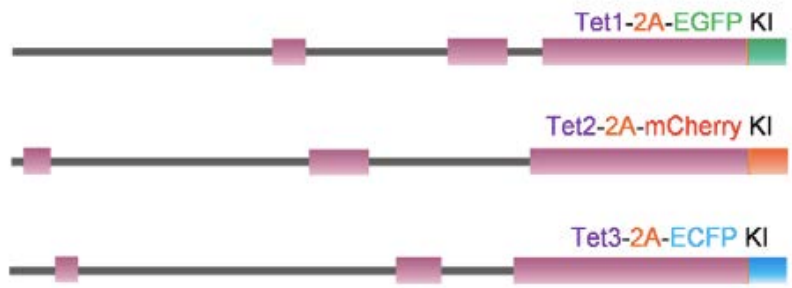


Donor Cell Type	Haploid ESC Line	Passage Number	No. of Embryos Transferred	No. of Growth-Retarded Pups (% of Transferred Embryos)	No. of Normal Pups (% of Transferred Embryos)
DKO-AG-haESCs carrying *Tet 1*, *2*, and *3* triple mutations	Tet-TKO-DAH cells	p35-p37	407	4 (1.0)	59 (14.5)
DKO-AG-haESCs carrying *p53*, *63*, and *73* triple mutations	p53-TKO-DAH cells	p41-p46	660	2 (0.3)	111 (16.7)

Application 2: multiple gene knockins in DKO-AG-haESCs.







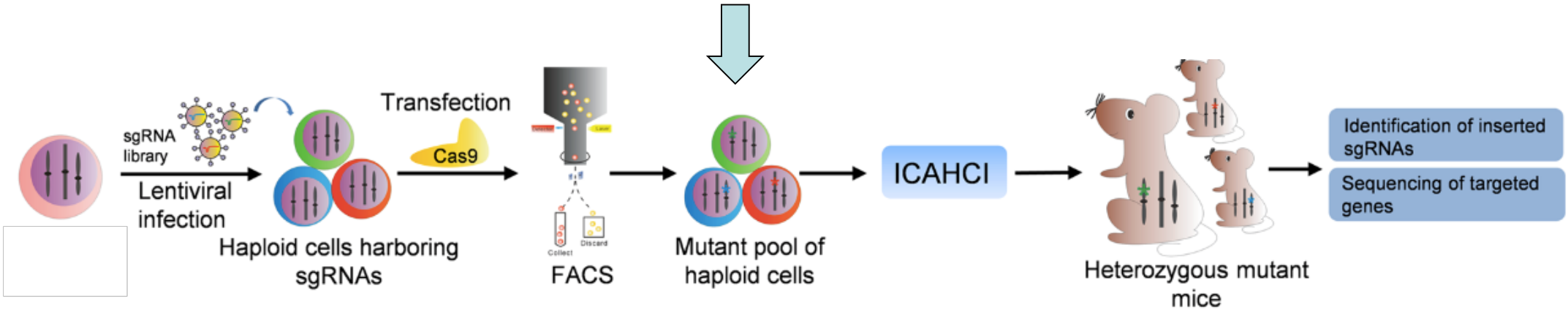
Donor Cell Type	Haploid ESC Line	Passage Number	No. of Embryos Transferred	No. of Growth-Retarded Pups (% of Transferred Embryos)	No. of Normal Pups (% of Transferred Embryos)
DKO-AG-haESCs carrying *Tet1*, *2*, and *3* Knockin	Tet-TKI-DAH-1	p47-p51	874	6 (0.7)	151 (17.3)

- **Application 3: one-step generation heterozygous mutant mice by DKO-AG-haESCs carrying CRISPR-Cas9 library.**

Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library

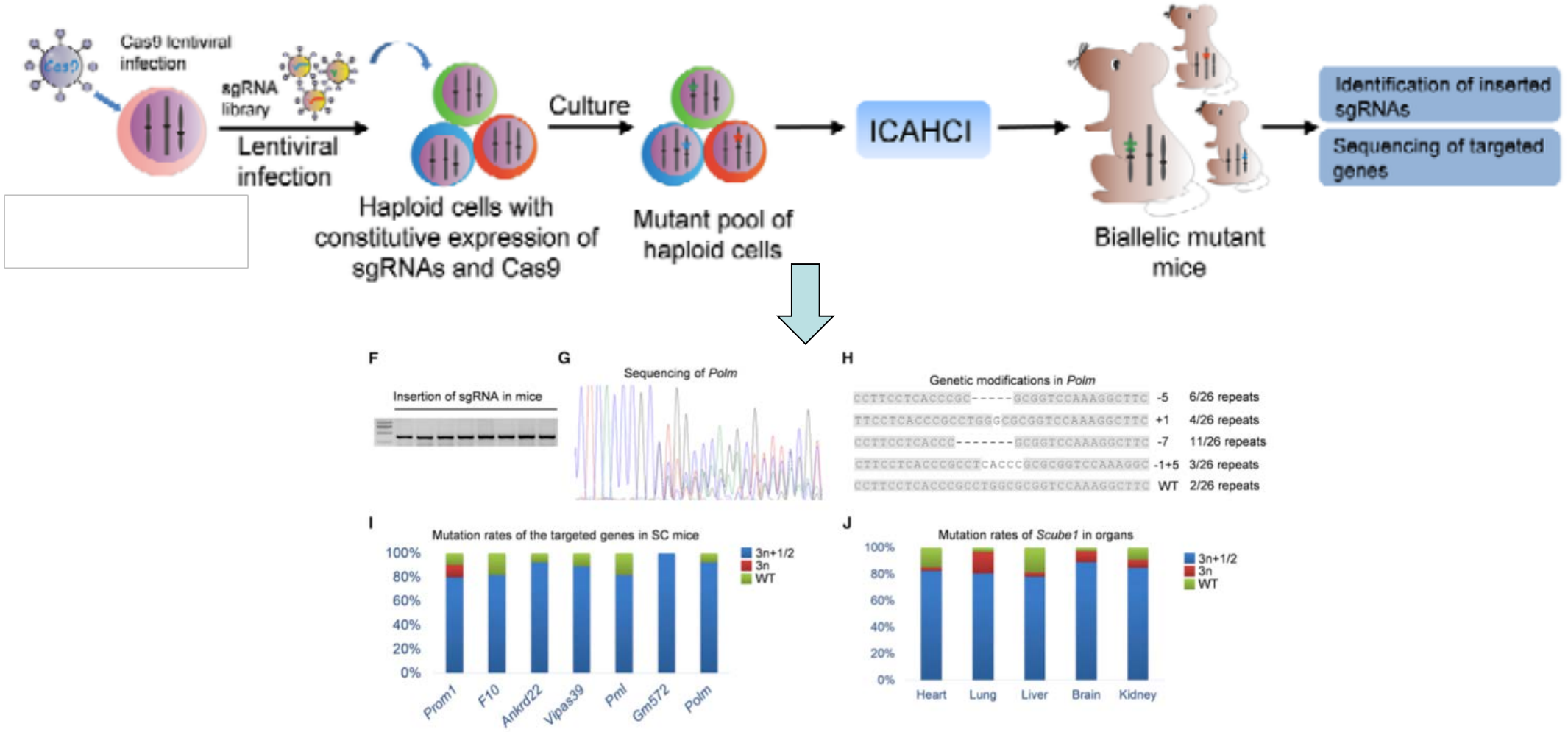
Koike-Yusa et al., *Nat Biotechnol* 2013

Hiroko Koike-Yusa[1,2], Yilong Li[1,2], E-Pien Tan[1], Martin Del Castillo Velasco-Herrera[1] & Kosuke Yusa[1]



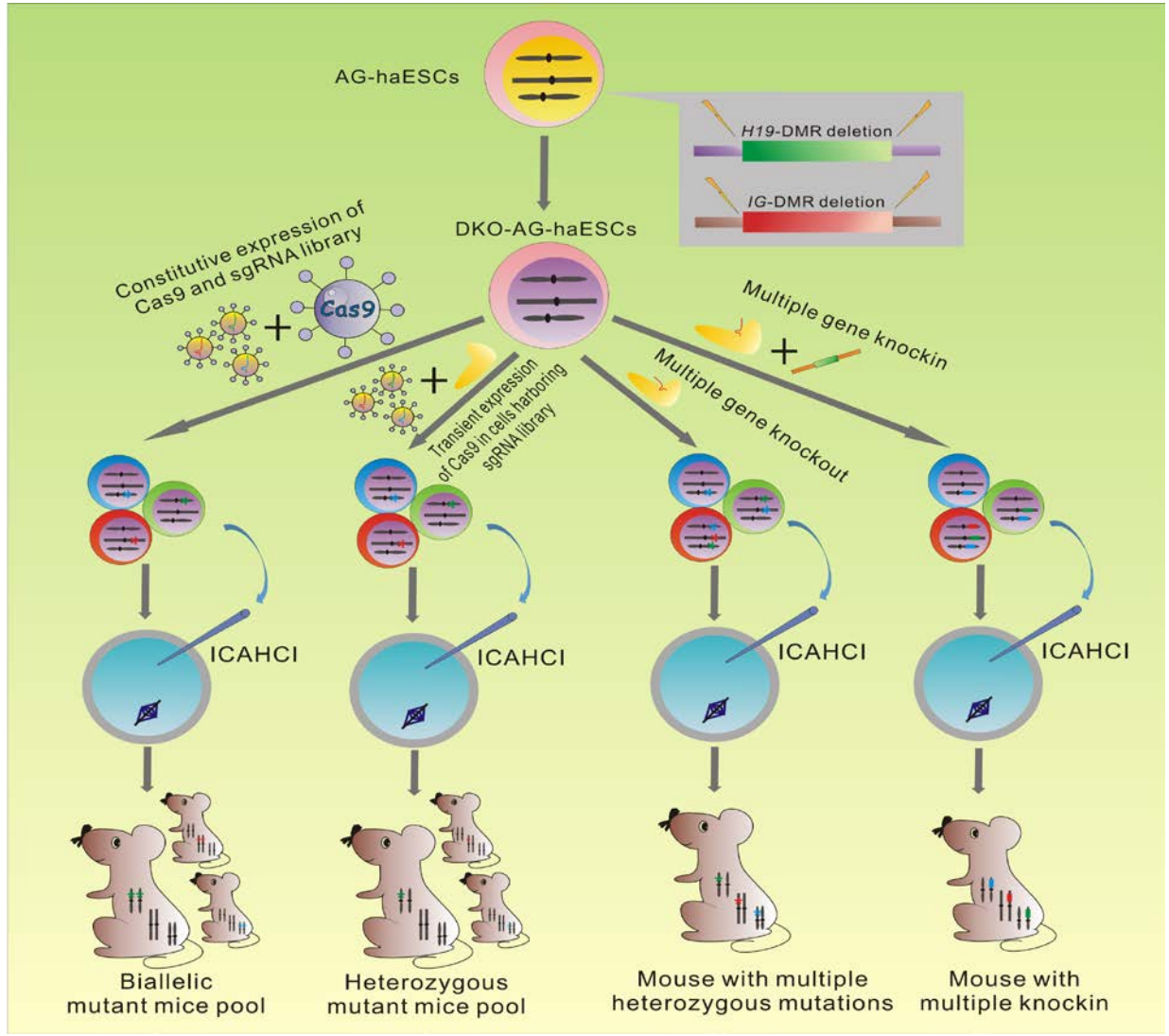
Mice carrying "barcode"

- **Application 4: one-step generation biallelic mutant mice by DKO-AG-haESCs carrying CRISPR-Cas9 library.**



Strategies	No. of Embryos Transferred	No. of SC Pups (% of Transferred Embryos)	No. of SC Pups without sgRNA	No. of SC Pups Carrying sgRNA ($n \geq 2$)	No. of SC Pups Carrying One sgRNA	No. of SC Pups with Biallelic Mutation	No. of SC Pups with Mo… lic Mutati…
lenti-Cas9+lenti-sgRNA	1,453	272 (18.7)	22	13	237	83	60

A new genetic tool enabling genetic screening in mice



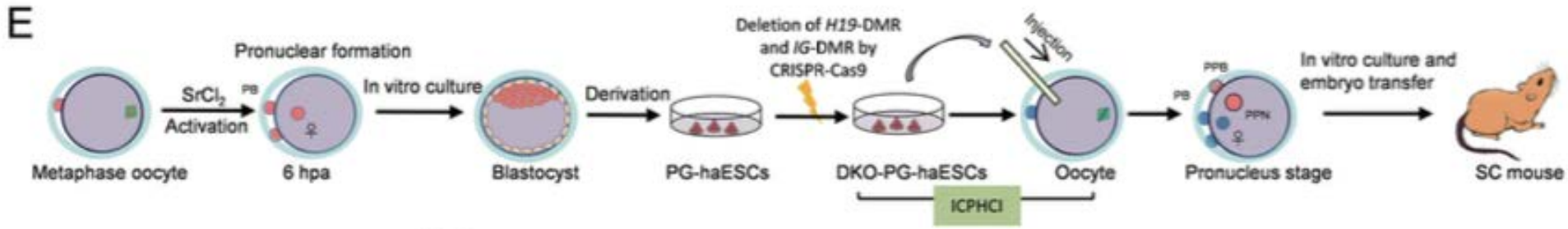
Zhong et al., Cell Stem Cell, 2015

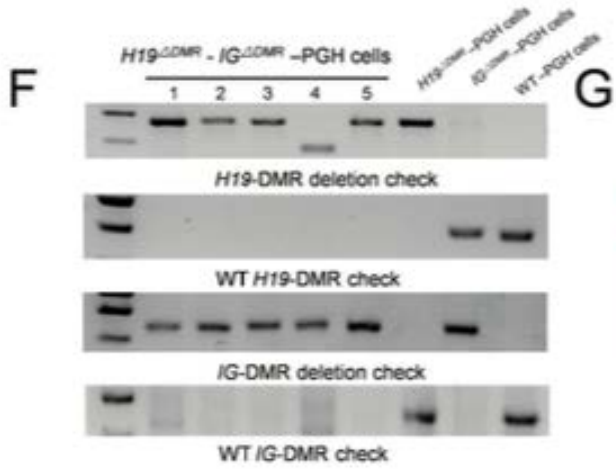
In Collaboration with Dr. Li Yang's lab

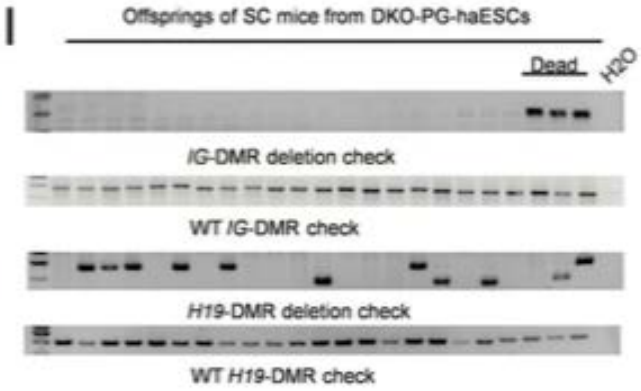
Question：

Can oocyte-derived haploid cells be used as a sperm replacement?

- **Oocyte-originated haploid cells carrying *H19*-DMR and *IG*-DMR deletions can efficiently produce SC pups.**

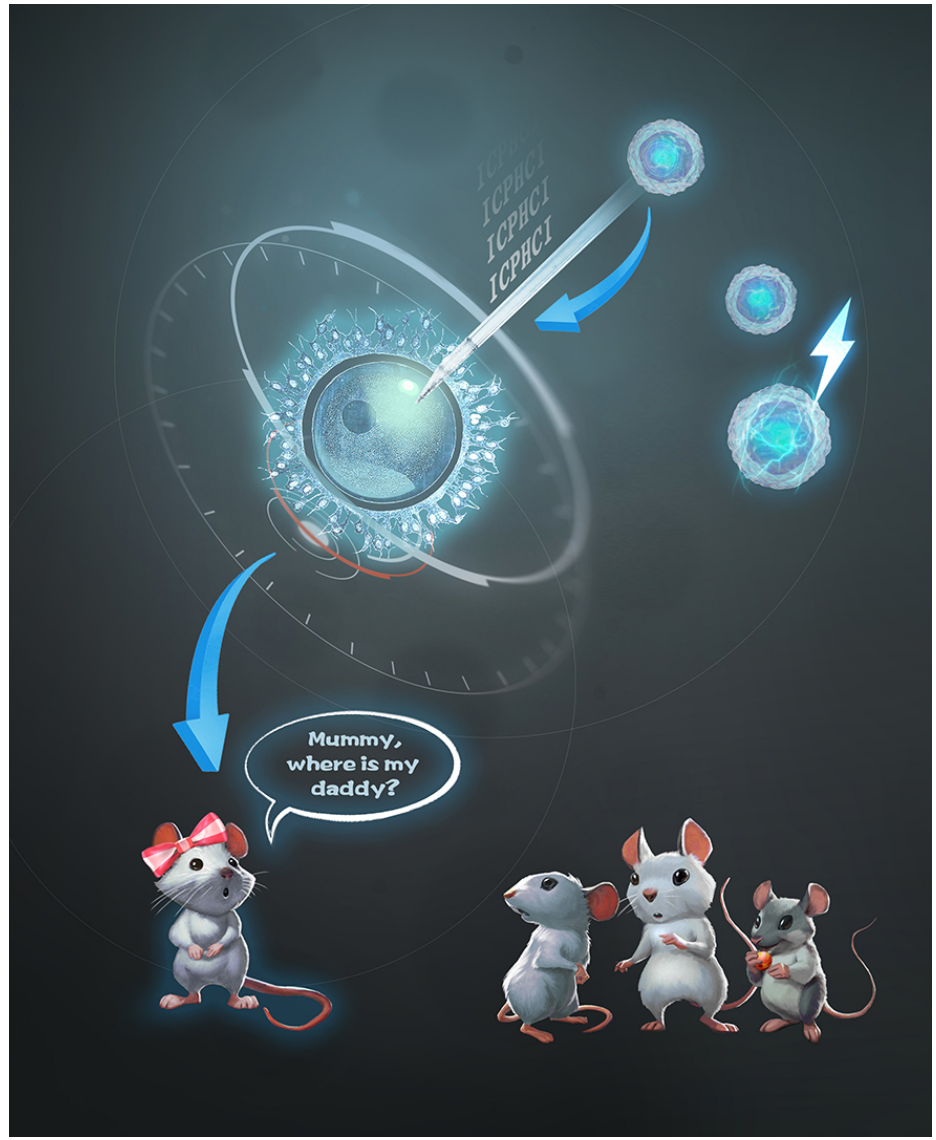






Donor cells	Passage number	No. of embryos transferred	No. of growth-retarded pups (% of transferred embryos)	No. of normal pups (% of transferred embryos)
DKO-PG-haESCs	p12-p39	1019	3 (0.3)	158 (15.5)

Generation of “spermatid-like” haploid cells from oocytes

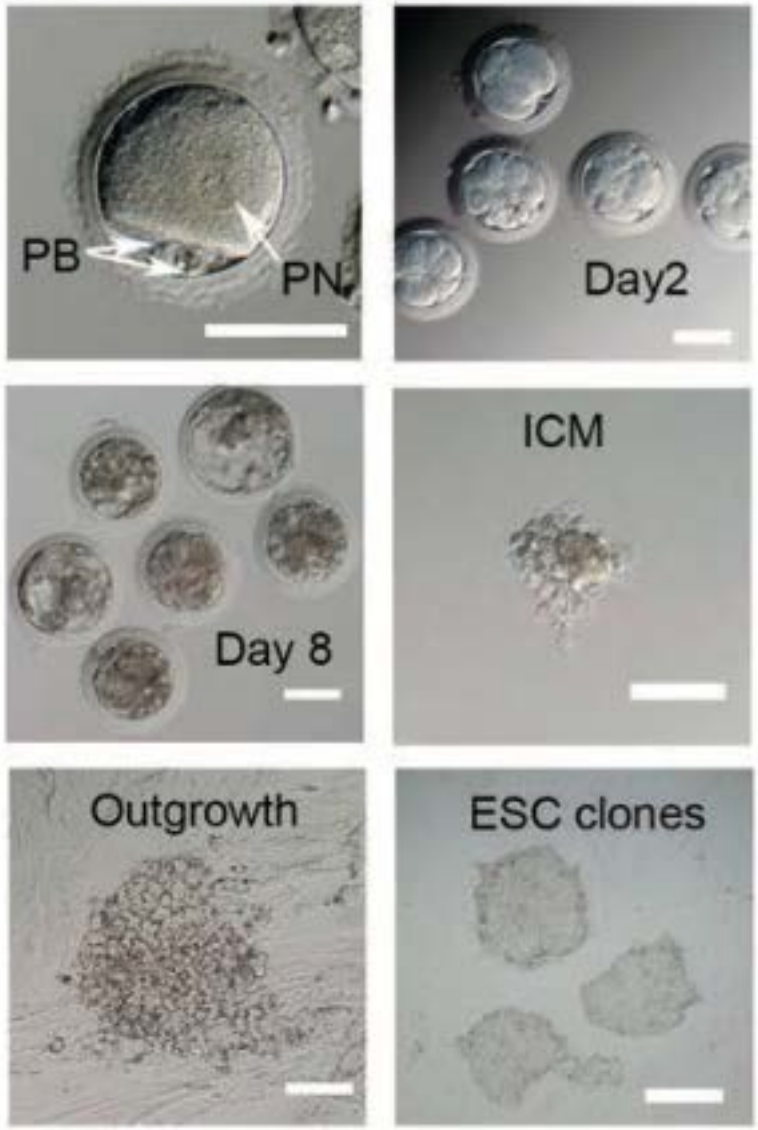


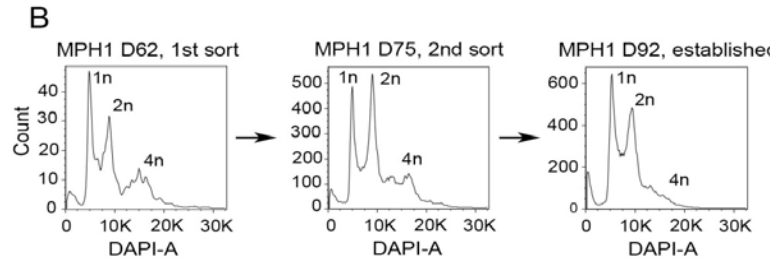
Zhong et al. Cell Res (2015) Nov 17th

Question：

Can haploid ESCs be generated from non-human primates?

Diploid and haploid ESC lines from *Macaca Fascicularis.*





MPH1

MPH 2

Treatment	Blastocyst	Established Lines	Established Lines with haploid cells
Ionomycin/ CHX	70	10	2
ICSI	23	4	/

Generation of haploid cells from primates

Yang et al., Cell Res, **2013** **In Collaboration with Dr. Qiang Sun and Dr. Ying Jin's lab**

Following questions：

- Can monkey androgenetic haploid ESCs be generated?
- Can human haploid ESCs be generated?
- How to stably maintain haploidy in vitro?
- What is the underlying mechanisms of high-efficient generation of SC mice by DKO-AG-haESCs?
- ……

Acknowledgments

Lab members

Hui Yang, ION

Yuxuan Wu

Collaborators:

Guoliang Xu, SIBCB

Qiang Sun, ION

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