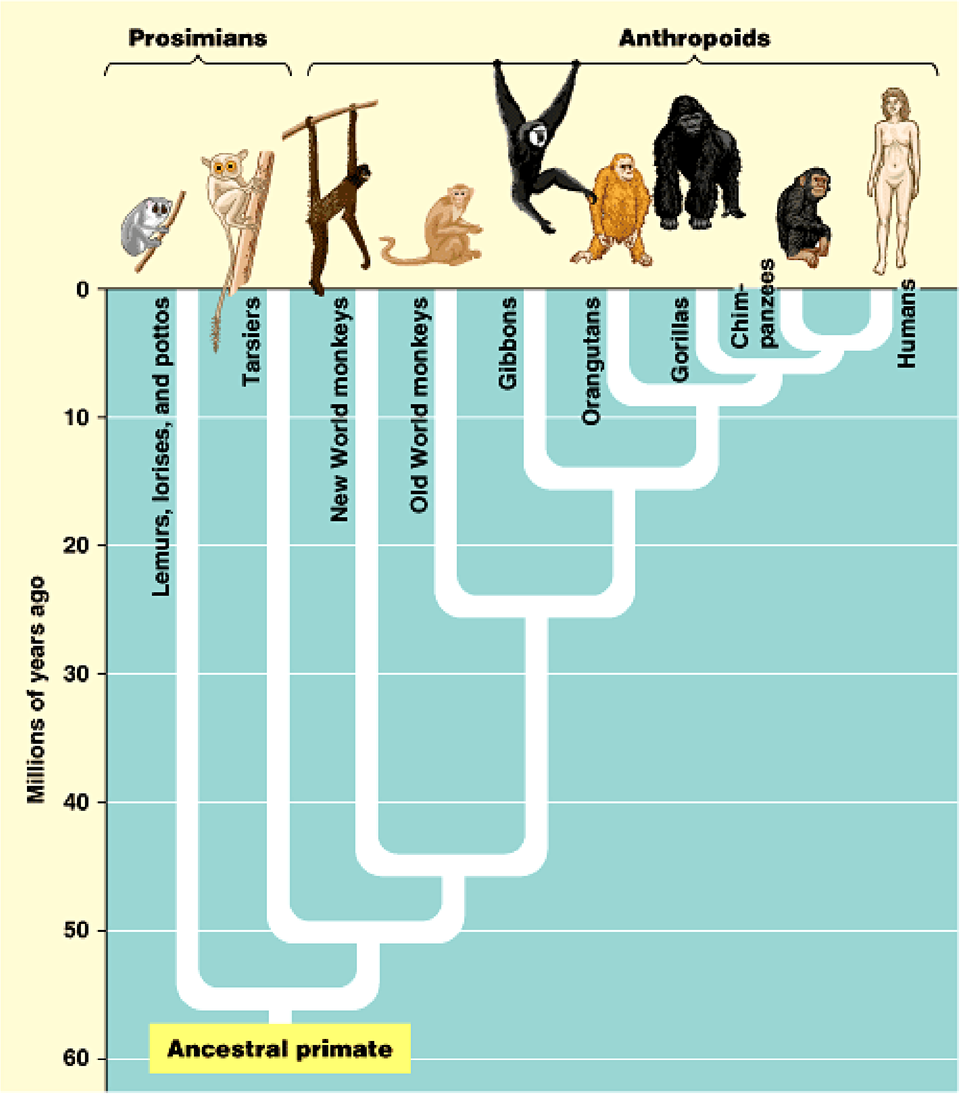
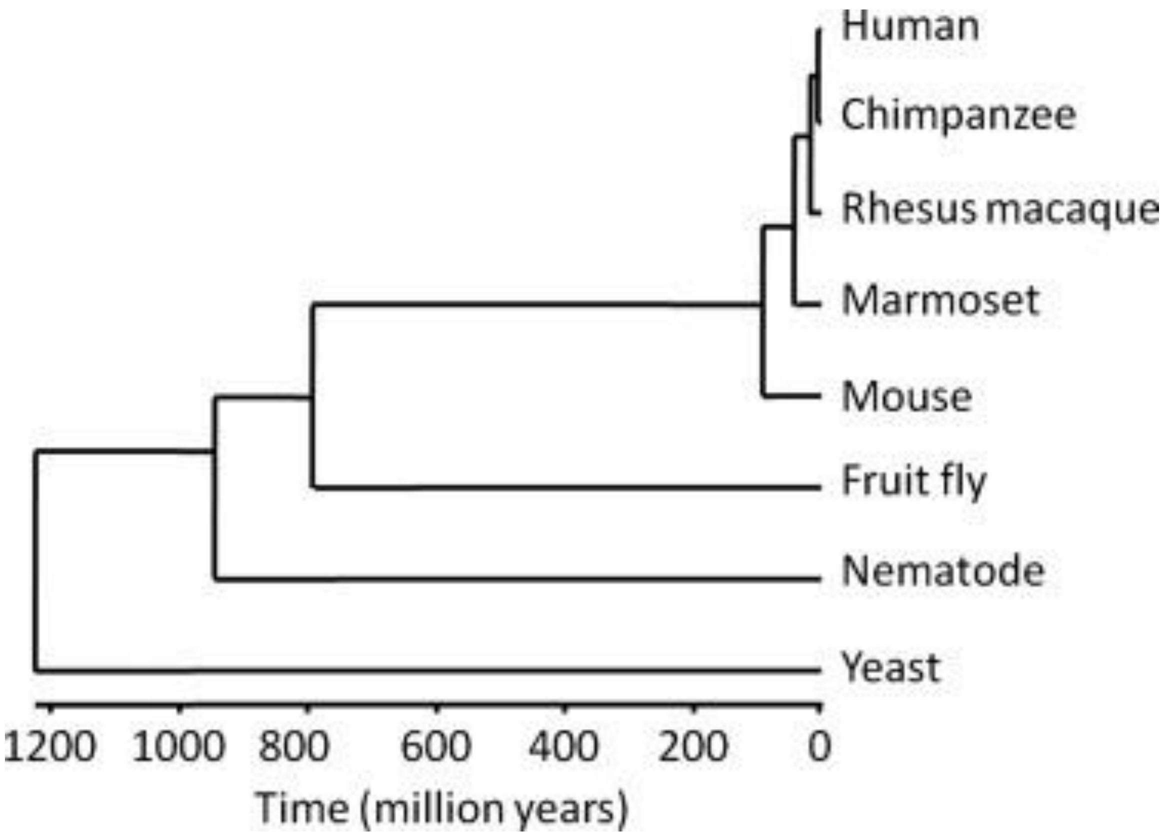
Non-Human Primate Models: Advancement, Challenges and Opportunities

Jiang-An Yin

2020-03-03

Primate





Mammal, hands, handlike feet, forward-facing eyes, varied locomotion, complex/flexible behaviour(especially social)

Research fields using NHP

- neurological research: that involves advanced brain responses which can be tracked in various ways
- safety testing for novel medicines and new batches of vaccines
- defence studies and studies that may benefit wild animals

Table 3.4 Classification of non-human primate publications from UK researchers[67]

	Number of UK research publications						
Field of research	**1995**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**
Neuroscience	**31**	**31**	**42**	**38**	**34**	**26**	**41**
Basic neuroscience, brain structure and function	11	18	23	18	18	15	26
Applied Neuroscience:							
Parkinson's Disease	9	6	9	12	7	5	7
Vision	11	2	8	8	6	5	6
Alzheimer's Disease		2	1				1
Stroke		2	1		3	1	
Addiction		1					
Infectious disease	**11**	**2**	**8**	**4**	**4**	**5**	**5**
AIDS	10	2	7	2	2	4	4
Other	1		1	2	2	1	1
Other							
Reproduction	6	7	9	3	2	2	3
Behavioural / Welfare studies	3	3	3	2	7	13	5
Xenotransplantation		2	3	1		1	
Anatomy – basic and applied	4	1	2	4	4	1	
Pharmaceutical R&D	6	1	2		1		
Gene Therapy							1
Total	**61**	**47**	**69**	**52**	**52**	**48**	**56**

From Pathogens and Immunity - Vol 2, No 3

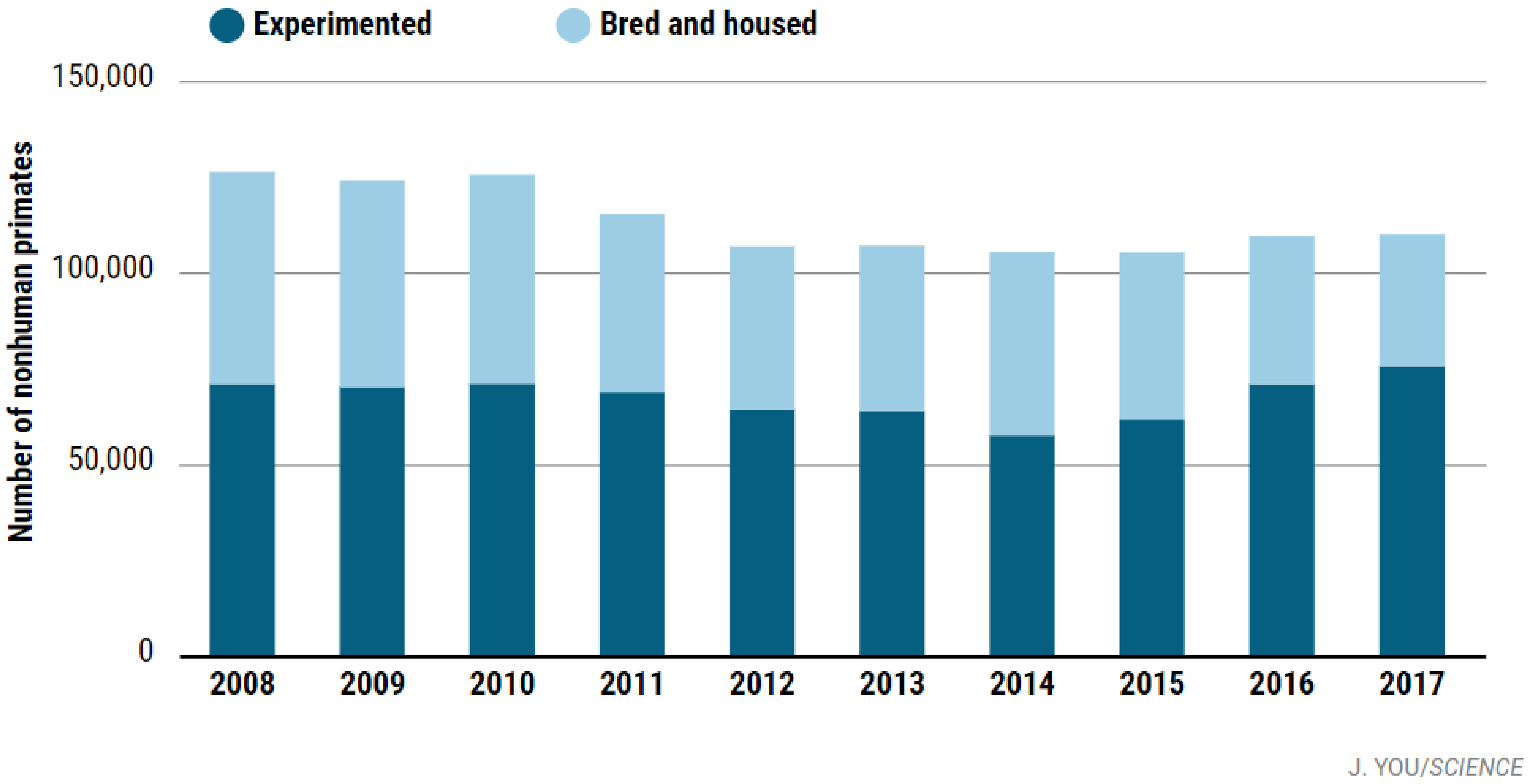
Lab Animals by Species

Rodents Monkeys All Others

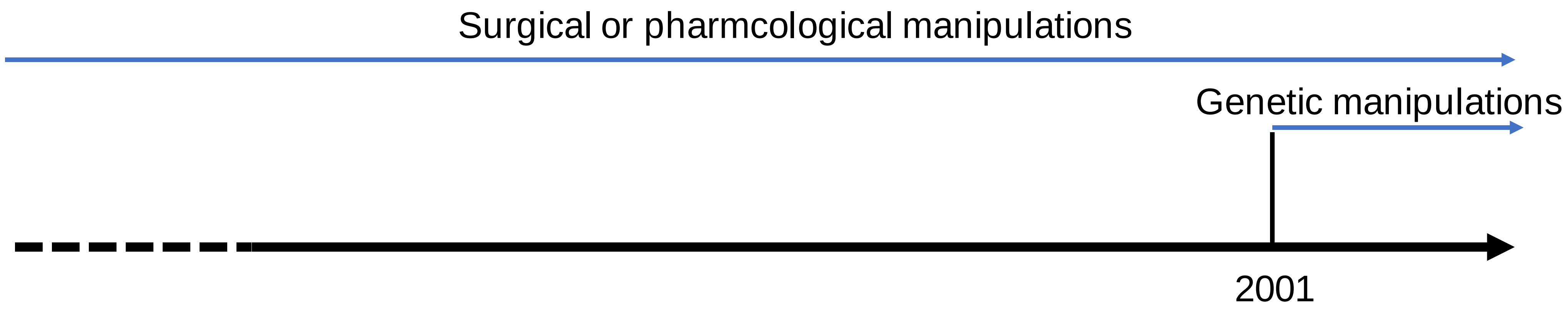
The number of nonhuman primates used in research is less than 1%. But its impact on human health is enormous.

From Pathogens and Immunity - Vol 2, No 3

The total number of monkeys in research labs



Researches using NHPs



- Components of blood and plasma discovered.
- Ability to diagnose and treat typhoid fever.
- Modern anesthesia.
- Mumps virus discovered.
- Treatment of rheumatoid arthritis.
- Discovery of the Rh factor, blood-typing knowledge critical for safe blood transfusions.
- Development of polio vaccine.
- Development of antipsychotic medication chlorpromazine and its tranquilizing derivatives.
- Cancer chemotherapy.
- Development of yellow fever vaccine.

From Pathogens and Immunity - Vol 2, No 3

Genetic modified NHPs

Transgenic Monkeys Produced by Retroviral Gene Transfer into Mature Oocytes

A. W. S. Chan, K. Y. Chong, C. Martinovich, C. Simerly, G. Schatten*

Transgenic rhesus monkeys carrying the green fluorescent protein (GFP) gene were produced by injecting pseudotyped replication-defective retroviral vector into the perivitelline space of 224 mature rhesus oocytes, later fertilized by intracytoplasmic sperm injection. Of the three males born from 20 embryo transfers, one was transgenic when accessible tissues were assayed for transgene DNA and messenger RNA. All tissues that were studied from a fraternal set of twins, miscarried at 73 days, carried the transgene, as confirmed by Southern analyses, and the GFP transgene reporter was detected by both direct and indirect fluorescence imaging.

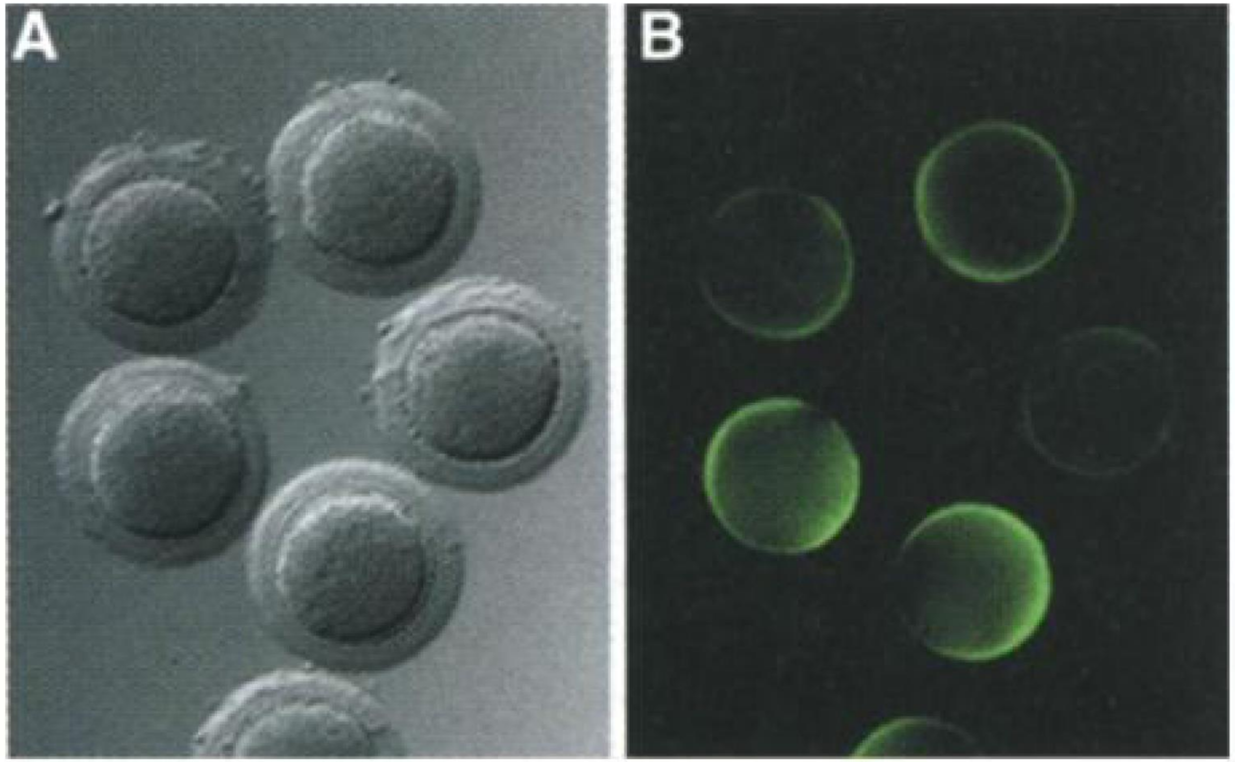


Fig. 1. Injection of VSV-G pseudotyped retroviral vector, enclosing the GFP gene and protein, into the perivitelline space of mature rhesus oocytes. (**A**) Transmitted light and (**B**) epifluorescence imaging of GFP carried within the vector particles. Magnification: ×100.

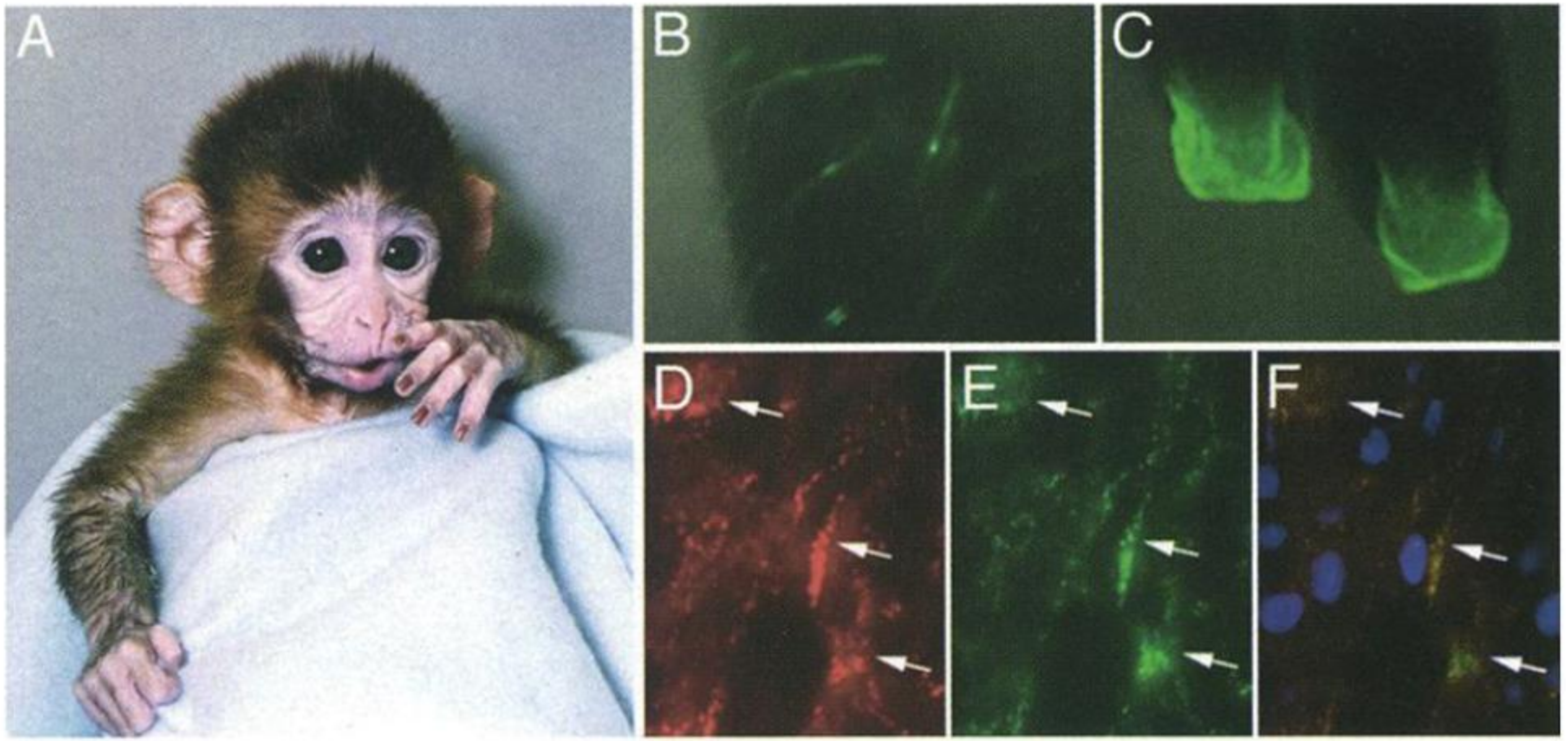


Fig. 2. (**A**) Transgenic rhesus male with inserted DNA ("ANDi"). GFP expression was observed in hair shafts (**B**) and toenails (**C**) by direct epifluorescent examination in the male stillborn but not in the accessible tissues from ANDi. Immunostaining and epifluorescent examination of placental frozen sections from the male stillborn demonstrates the presence of the GFP protein. (**D**) Anti-GFP detection in placenta by rhodamine (red) immunofluorescent microscopy. (**E**) GFP detection by fluorescein (green) epifluorescence of the same section demonstrates the direct expression of the transgene. (**F**) Overlay of the green (E) and red (D) images demonstrates colocalization of direct GFP fluorescence with anti-GFP imaging. Blue, Hoechst 33342 DNA staining. Magnification in (D) through (F): $\times 400$.

Table 1. Transgenesis efficiency in rhesus embryos, fetuses, and offspring.

Construct	VSV-G pseudotype		Overall
	LNCEGFP	LNEFEGFP	
Eggs injected with vector	157	67	224
Eggs then injected with sperm	157	65	222
Fertilization rate	108 (69%)	58 (89%)	166 (75%)
Embryonic development of fertilized eggs	85 (79%)	41 (71%)	126 (76%)
Embryos transferred (two/surrogate)	22	18	40
Number of surrogates	11	9	20
Pregnancies/surrogate	1* (9%)	4 (44%)	5 (25%)
Fetal losses	2 (100%)	1 (25%)	3 (50%)
Births	0	3	3
Transgenic	2 of 2	1 of 4	3 of 6
Transgenic birth/embryos transferred	0	1 (5.5%)	1 (2.5%)
Transgenic birth/pregnancies	0	1 (25%)	1 (20%)

*Twin pregnancy.

Cell

Resource

Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos

Yuyu Niu,[1,5,7] Bin Shen,[2,7] Yiqiang Cui,[3,7] Yongchang Chen,[1,5,7] Jianying Wang,[2] Lei Wang,[3] Yu Kang,[1,5] Xiaoyang Zhao,[4] Wei Si,[1,5] Wei Li,[4] Andy Peng Xiang,[6] Jiankui Zhou,[2] Xuejiang Guo,[3] Ye Bi,[3] Chenyang Si,[1,5] Bian Hu,[2] Guoying Dong,[3] Hong Wang,[1,5] Zuomin Zhou,[3] Tianqing Li,[1,5] Tao Tan,[1,5] Xiuqiong Pu,[1,5] Fang Wang,[1,5] Shaohui Ji,[1,5] Qi Zhou,[4] Xingxu Huang,[2,*] Weizhi Ji,[1,5,*] and Jiahao Sha[3,*]

[1]Yunnan Key Laboratory of Primate Biomedical Research, Kunming 650500, China

[2]MOE Key Laboratory of Model Animal for Disease Study, Model Animal Research Center of Nanjing University, National Resource Center for Mutant Mice, Nanjing 210061, China

[3]State Key Laboratory of Reproductive Medicine, Department of Histology and Embryology, Nanjing Medical University, Nanjing 210029, China

[4]State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China

[5]Kunming Biomed International and National Engineering Research Center of Biomedicine and Animal Science, Kunming 650500, China

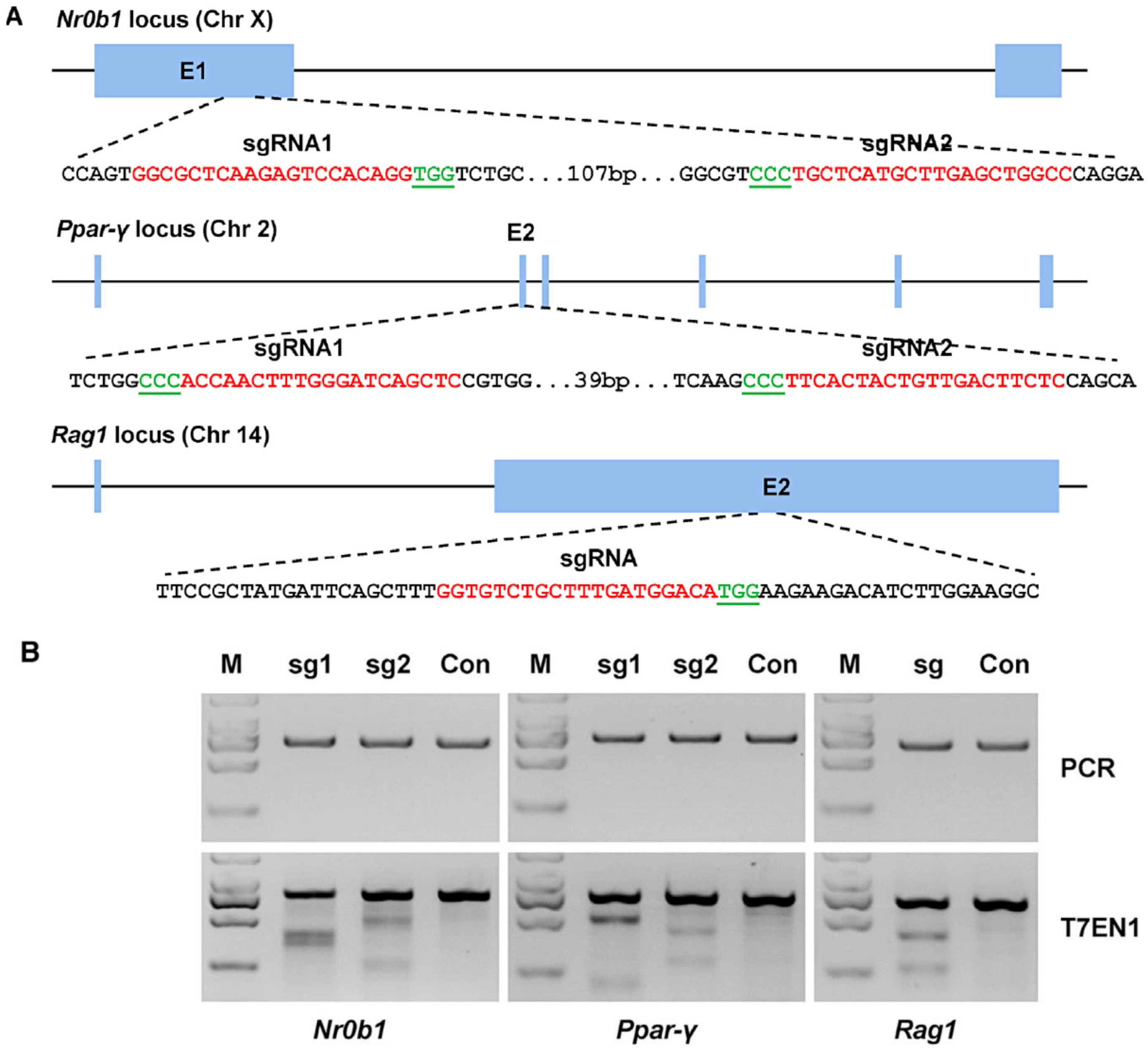
[6]Center for Stem Cell Biology and Tissue Engineering, Key Laboratory for Stem Cells and Tissue Engineering, Sun Yat-Sen University, Guangzhou 510080, China

[7]These authors contributed equally to this work

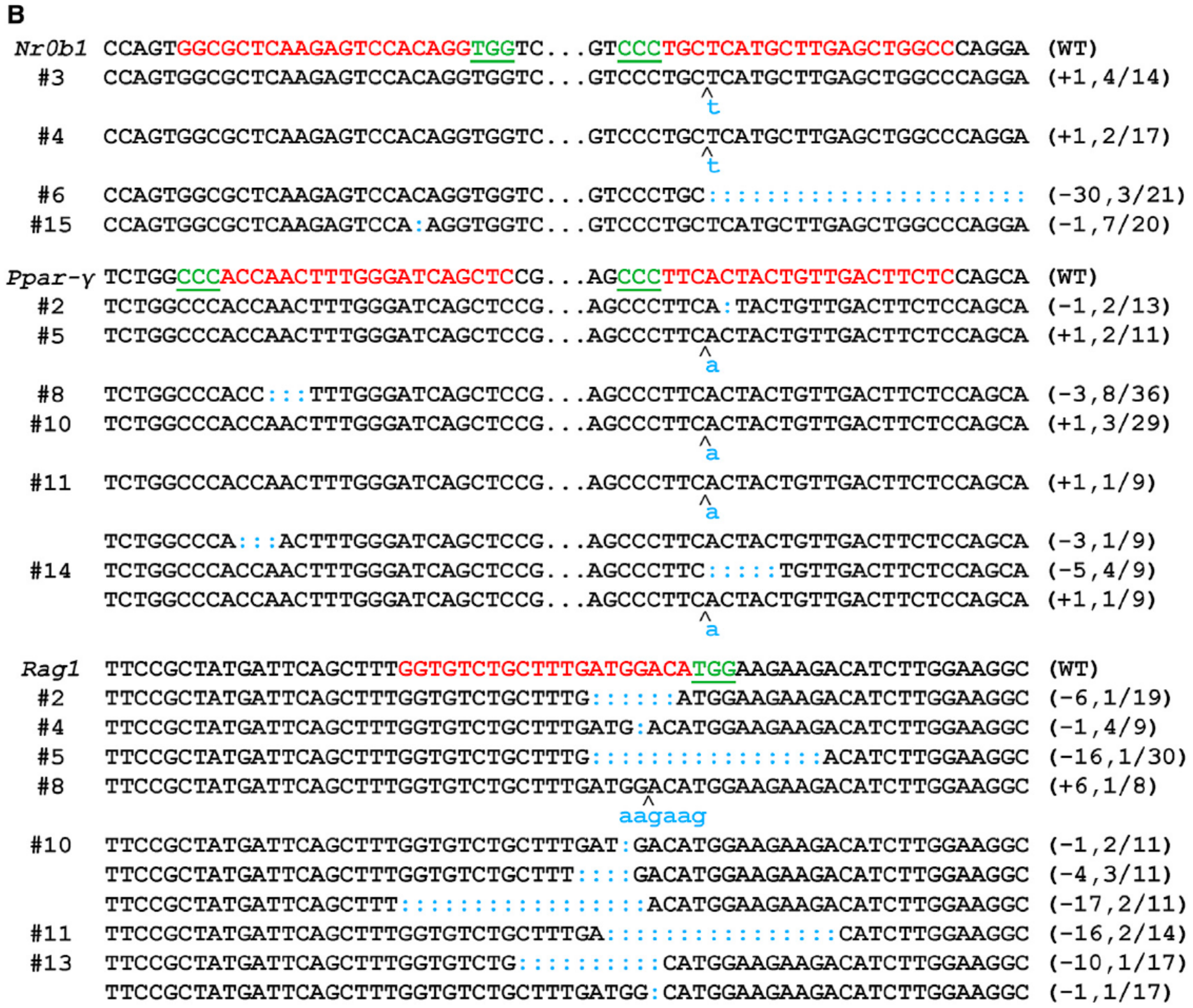
*Correspondence: shajh@njmu.edu.cn (J.S.), wji@kbimed.com (W.J.), xingxuhuang@mail.nju.edu.cn (X.H.)

http://dx.doi.org/10.1016/j.cell.2014.01.027

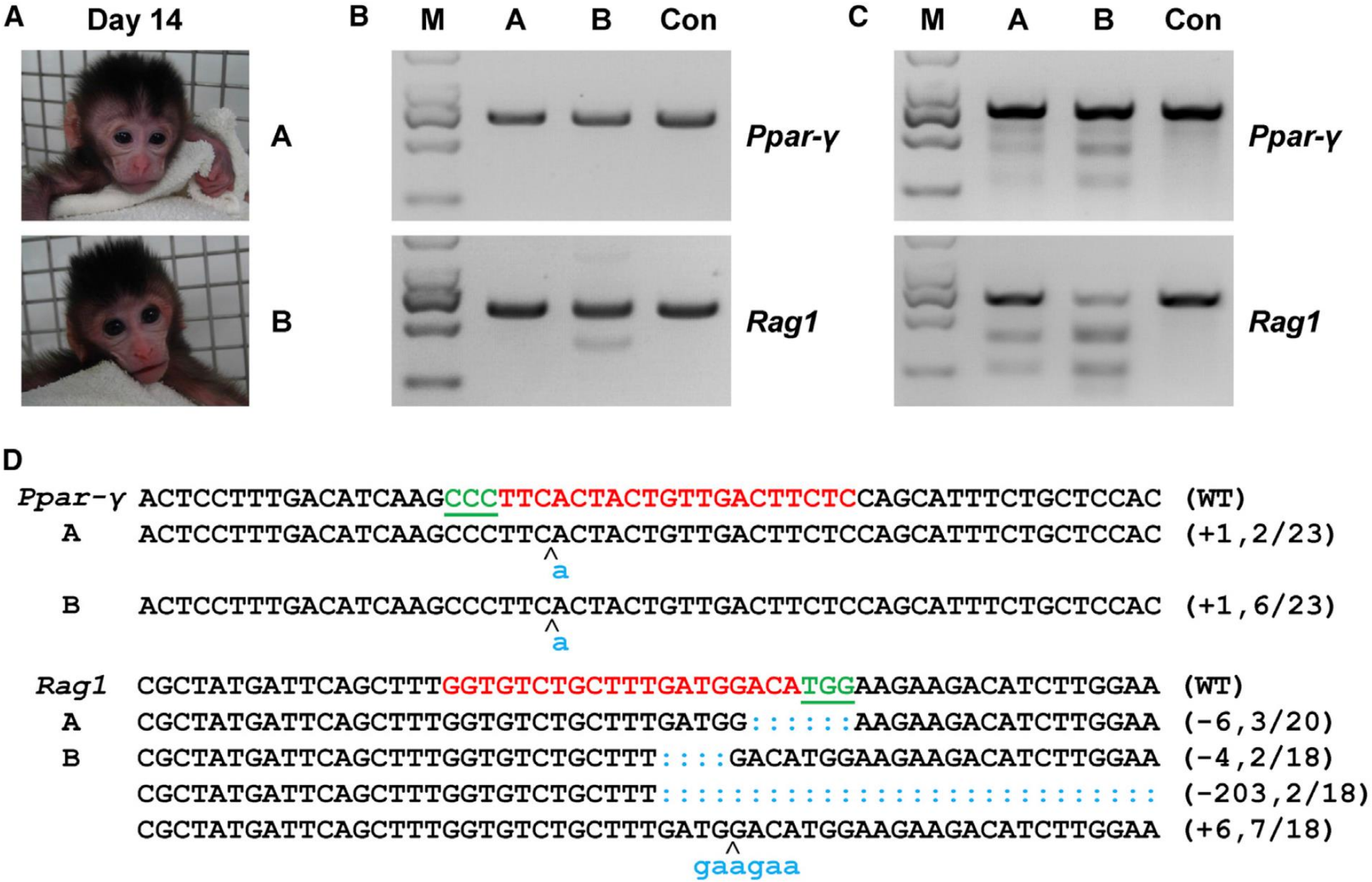
sgRNA design and validation in COS-7 cells



sgRNA:Cas9-Mediated Modifications of Nr0b1, Ppar-g, and Rag1 in Cultured Embryos



sgRNA:Cas9-Mediated Modifications of Ppar-g and Rag1 in Founder Cynomolgus Monkeys



Cas9-Mediated Modifications of Nr0b1, Ppar-g, and Rag1 in Ear and Placenta of Founders

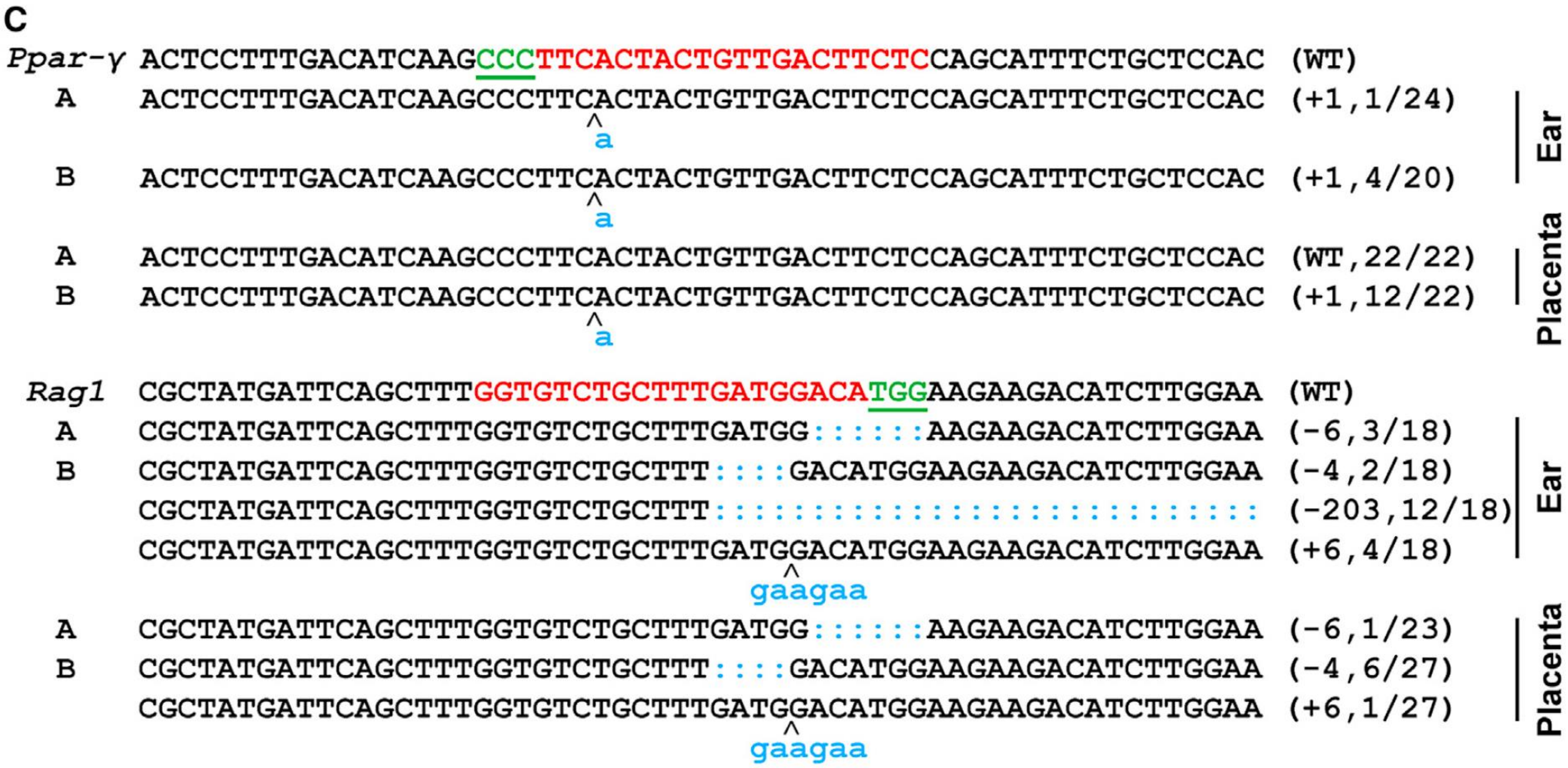


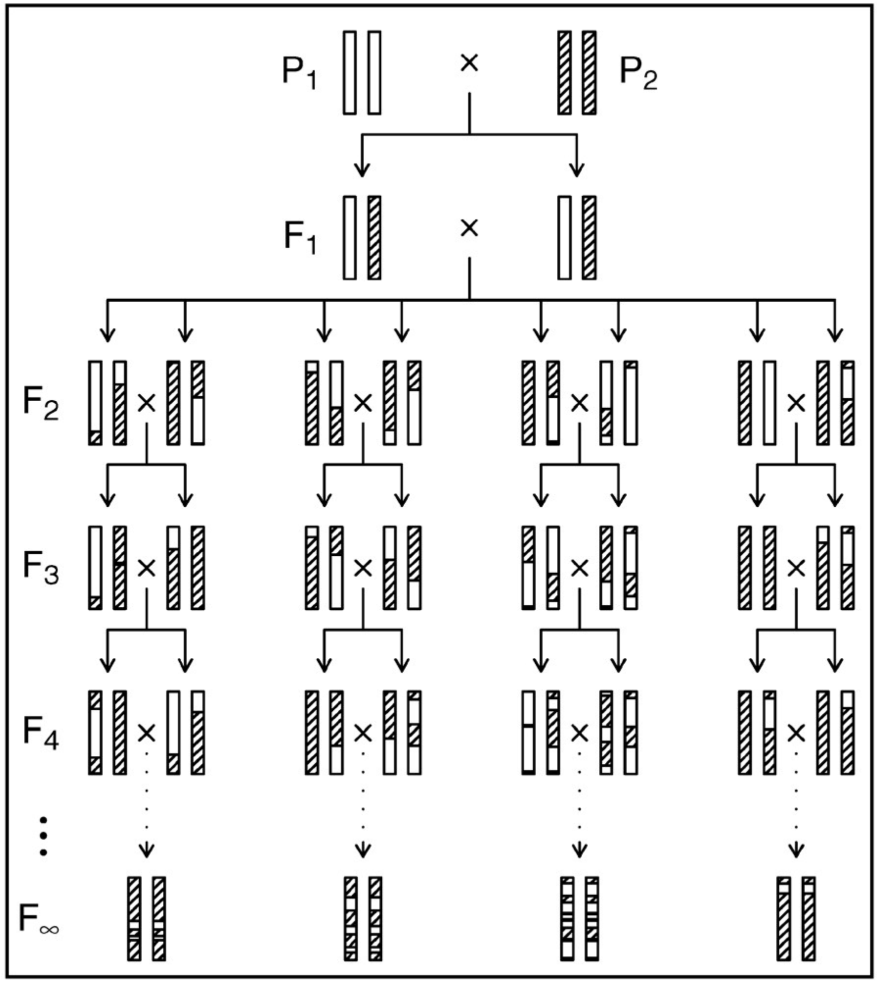
Table 1. Summary of Embryo Microinjection of Cas9 mRNA and sgRNAs

MII Oocyte	Injected Embryos	Embryos for ET	Pregnancies /Surrogates	Single Pregnancy	Multiple Pregnancy	Fetuses
198	186	83	34.5% (10/29)	4[a]	3 twins, 3 triplets	19

[a]One miscarried 36 days after embryo transfer.

Genetic uniformity of NHPs

Inbreeding?



Cloning?

Cloning of monkeys via somatic nuclear transfer fails in the last 20 years

BIOLOGY OF REPRODUCTION **66,** 1367–1373 (2002)

Rhesus Monkey Embryos Produced by Nuclear Transfer from Embryonic Blastomeres or Somatic Cells[1]

Research article Development and disease 2475

Human Reproduction Vol.22, No.8 pp. 2232–2242, 2007 doi:10.1093/humrep/dem136
Advance Access publication on June 11, 2007

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Reprogramming following somatic cell nuclear transfer in primates is dependent upon nuclear remodeling

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S.M. Mitalipov[1,5], Q. Zhou[2], J.A. Byrne[1], W.Z. Ji[3], R.B. Norgren[4] and D.P. Wolf[1]

[1]Division of Reproductive Sciences, Oregon National Primate Research Center, Oregon Health and Science University, 505 NW 185th Avenue, Beaverton, OR 97006, USA; [2]Institute of Zoology, Chinese Academy of Sciences, Beijing, China; [3]Kunming Institute of Zoology, Kunming Primate Research Center, Chinese Academy of Sciences, Kunming, Yunnan, China; [4]Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, NE, USA

Int. J. Dev. Biol. 54: 1671-1678 (2010)
doi: 10.1387/ijdb.103196ms

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Cloning of non-human primates: the road "less traveled by"

MICHELLE L. SPARMAN[1], MASAHITO TACHIBANA[1] and SHOUKHRAT M. MITALIPOV*[,1,2,3]

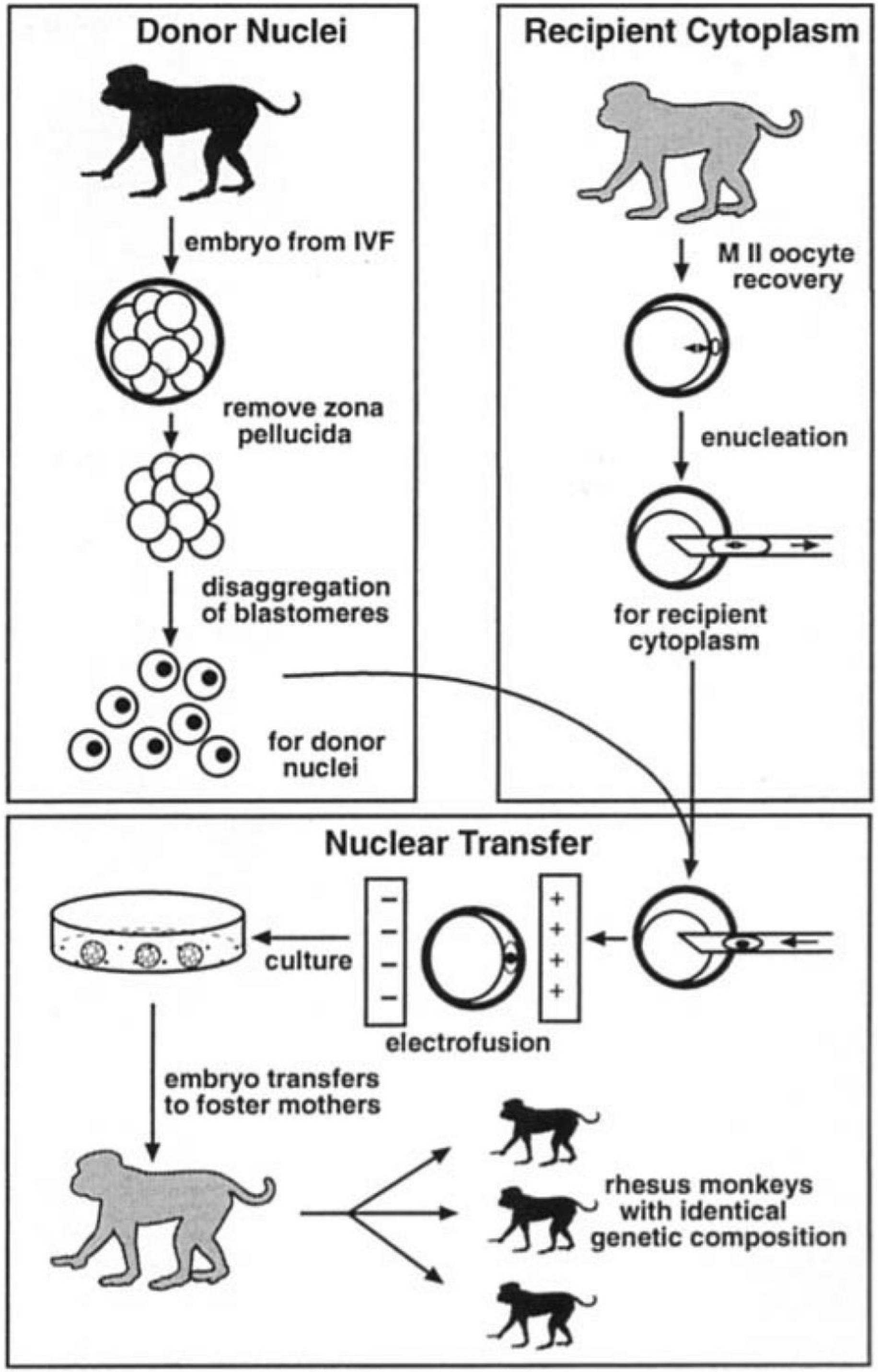
[1]Oregon National Primate Research Center, [2]Oregon Stem Cell Center and [3]Departments of Obstetrics & Gynecology and Molecular & Medical Genetics, Oregon Health & Science University, Beaverton, OR, USA

BIOLOGY OF REPRODUCTION **57,** 454–459 (1997)

Rhesus Monkeys Produced by Nuclear Transfer[1]

Li Meng,[3] John J. Ely,[6] Richard L. Stouffer,[3,4] and Don P. Wolf[2,3,4,5]

Division of Reproductive Sciences,[3] Oregon Regional Primate Research Center, Beaverton, Oregon 97006–3499
Departments of Physiology and Pharmacology[4] and Obstetrics and Gynecology,[5] Oregon Health Sciences University, Portland, Oregon 97201–5164
Department of Biology,[6] Trinity University, San Antonio, Texas 78212



Resource

Cell

Cloning of Macaque Monkeys by Somatic Cell Nuclear Transfer

Zhen Liu,[1] Yijun Cai,[1] Yan Wang,[1] Yanhong Nie,[1] Chenchen Zhang,[1] Yuting Xu,[1] Xiaotong Zhang,[1] Yong Lu,[1] Zhanyang Wang,[1] Muming Poo,[1] and Qiang Sun[1,2,*]

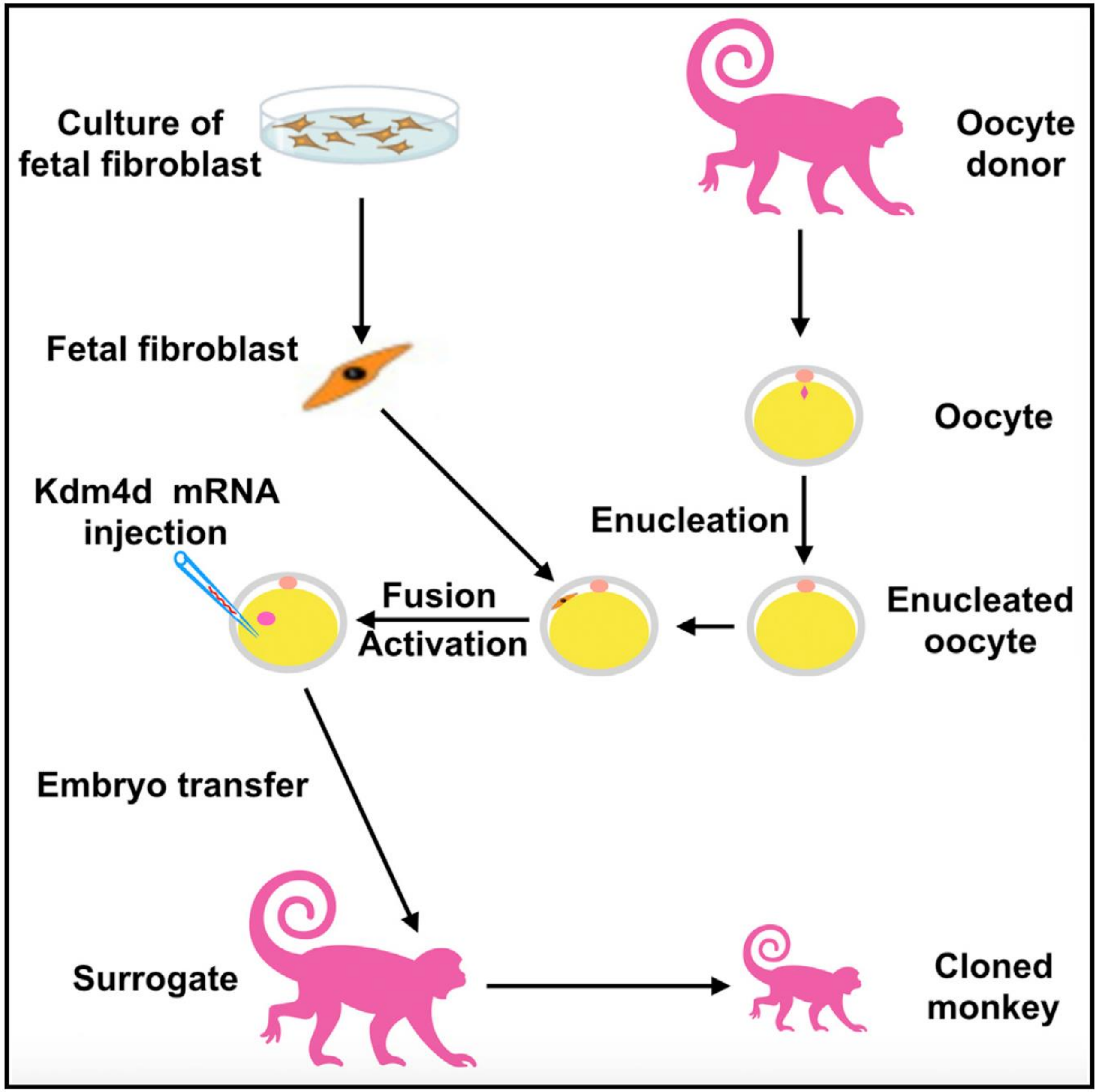
[1]Institute of Neuroscience, CAS Center for Excellence in Brain Science and Intelligence Technology, State Key Laboratory of Neuroscience, CAS Key Laboratory of Primate Neurobiology, Chinese Academy of Sciences, Shanghai, China

[2]Lead Contact

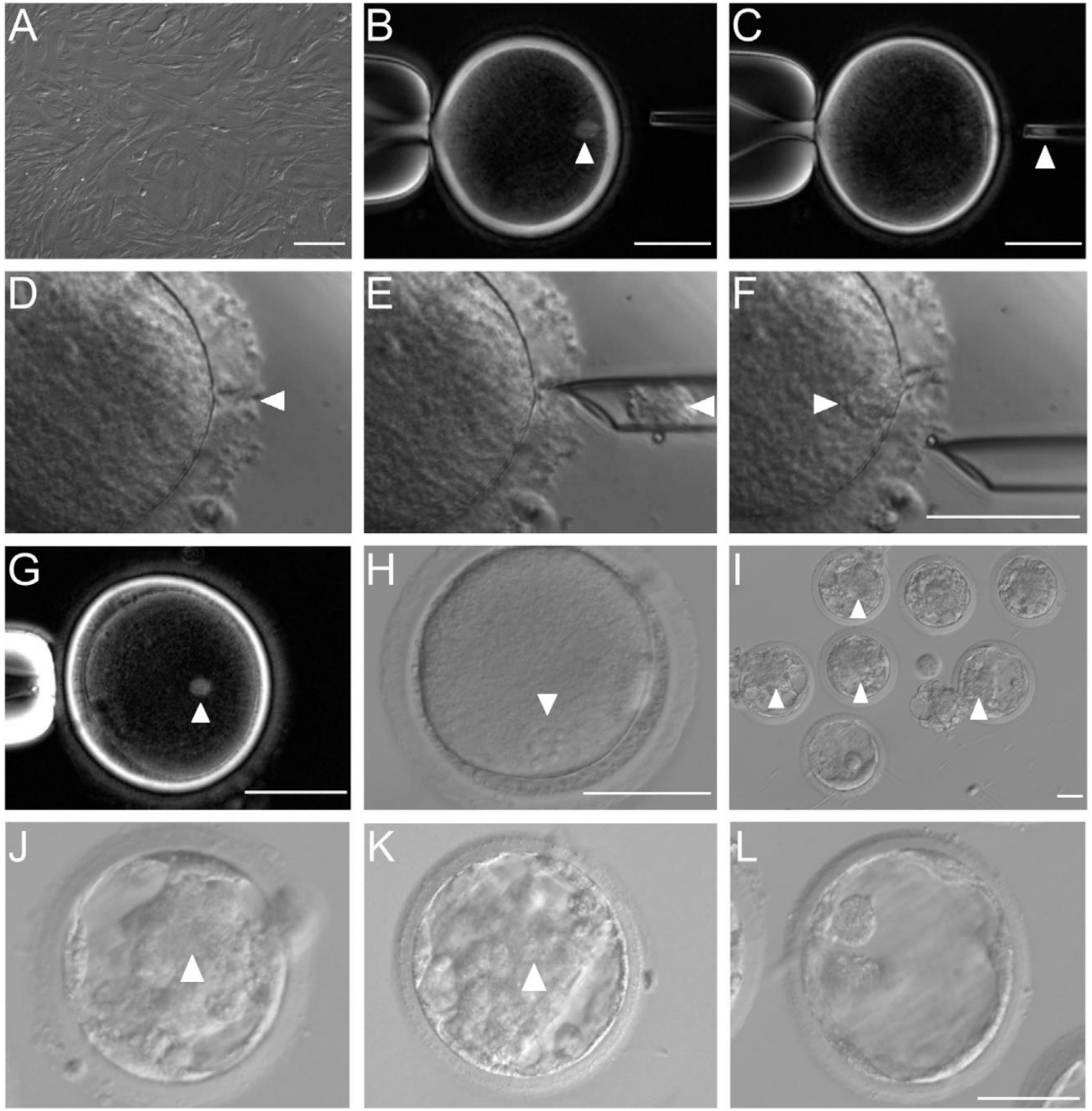
*Correspondence: qsun@ion.ac.cn

https://doi.org/10.1016/j.cell.2018.01.020

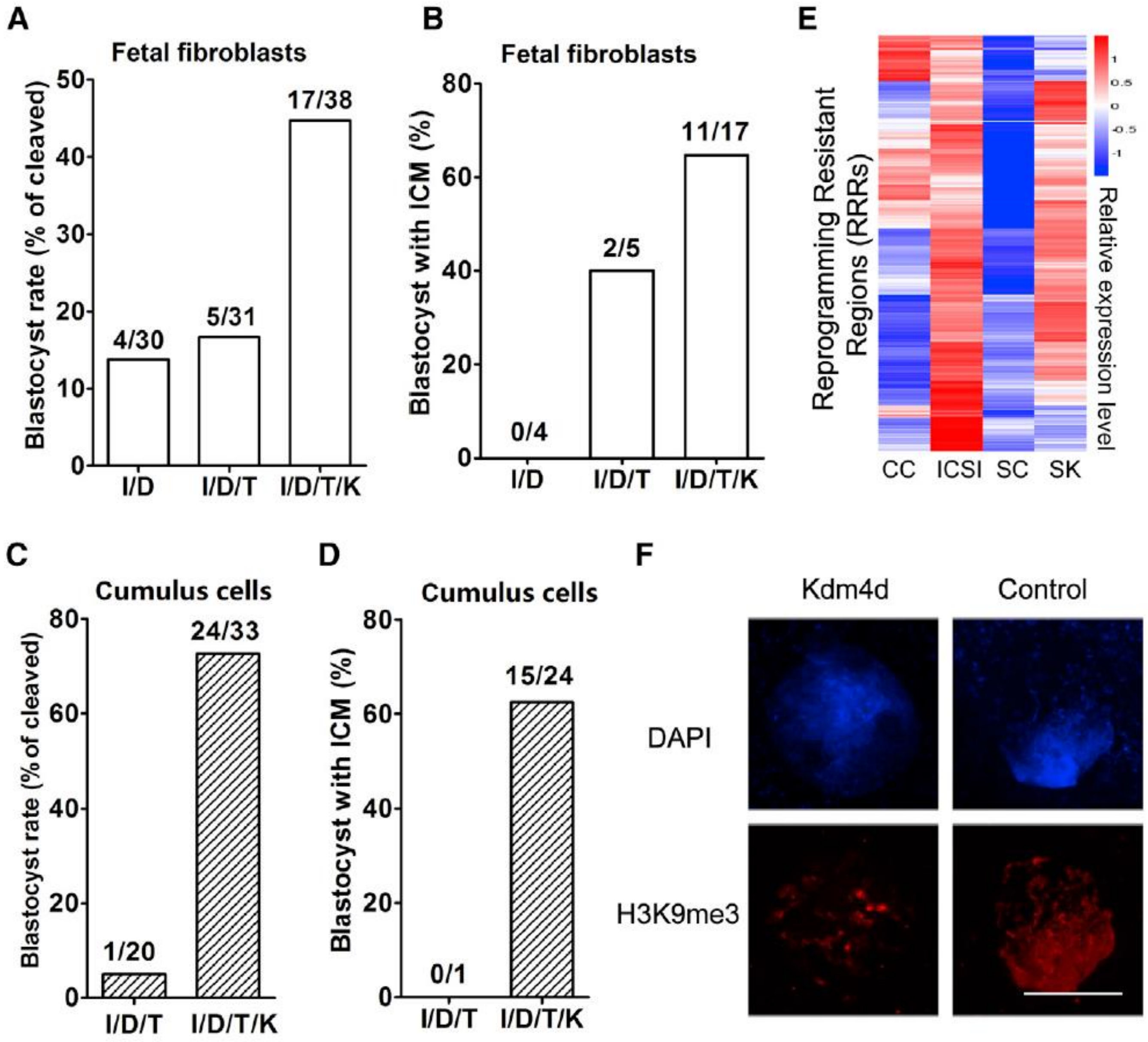
Cloning strategy



Procedure for Monkey SCNT Using Fetal Monkey Fibroblasts

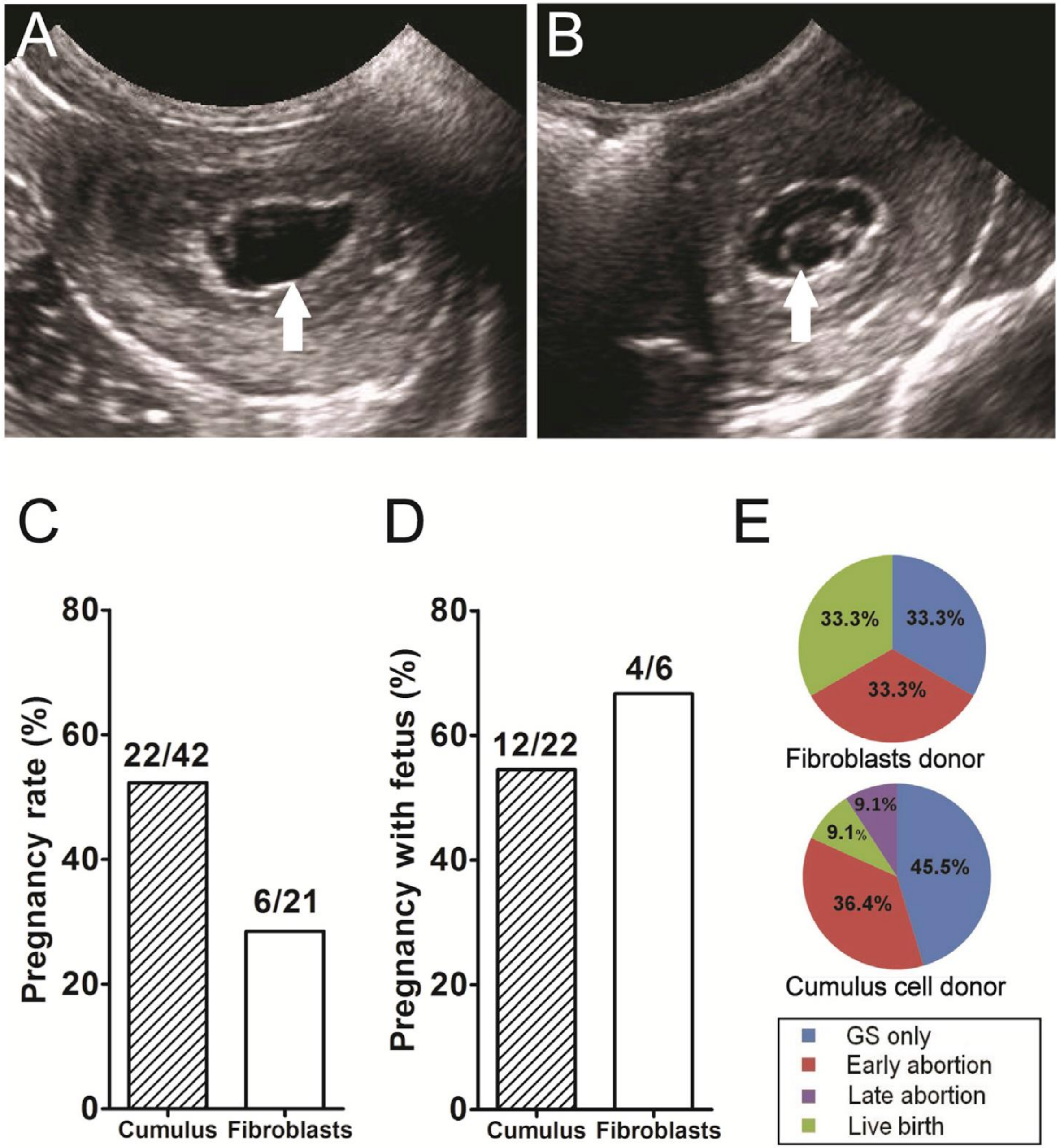


Blastocyst Development of SCNT Monkey Embryos

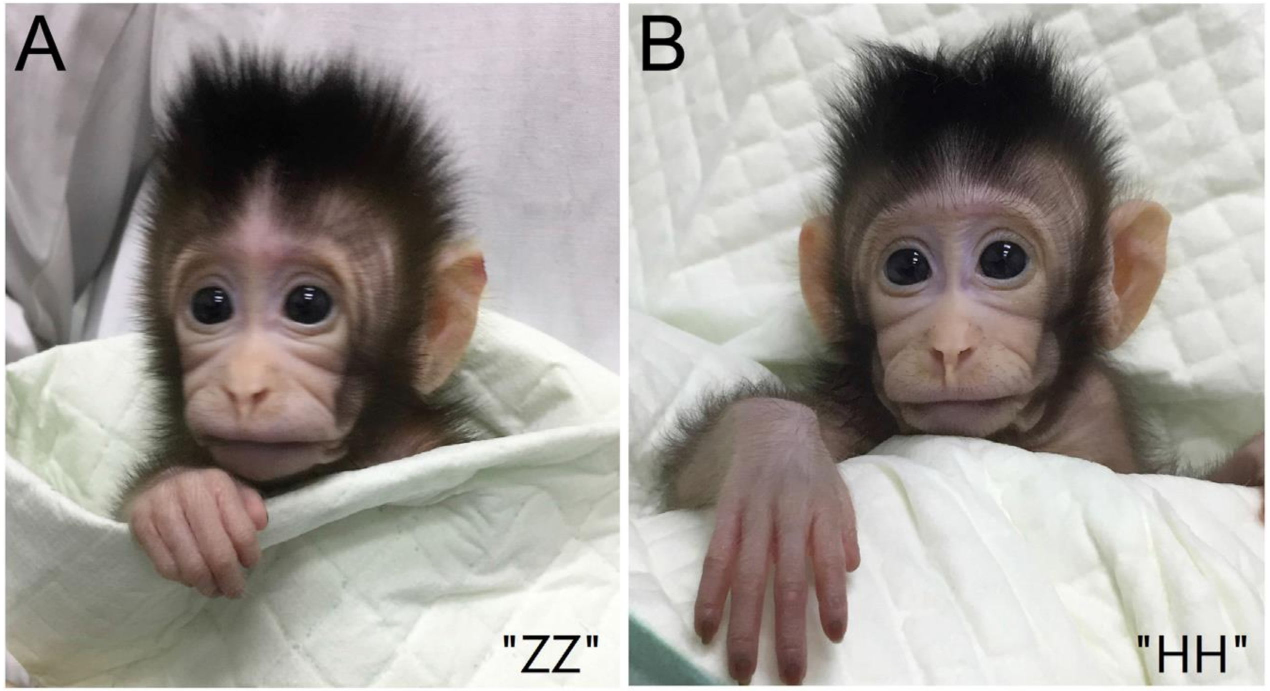


I: ionomycin
D: 6-dimethylaminopurine
T: TSA(histone deacetylase inhibitor trichostatin A)
K: Kdm4d mRNA (histone demethylase Kdm4d)

Pregnancy and Fetal Development of SCNT Embryos

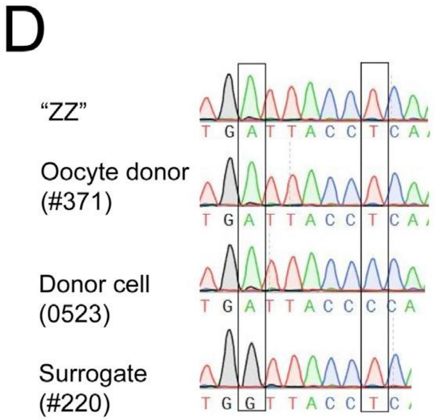


Analysis of Cloned Cynomolgus Monkeys



C

Monkey/Donor cell	D6S2741	D16S409	D18S72
Cloned monkey "ZZ"	250/277	135/148	200/217
Cloned monkey "HH"	250/277	135/148	200/217
Fibroblast donor cell (0523)	250/277	135/148	200/217
Oocyte donor for "ZZ"(#371)	274/278	255/255	202/223
Surrogate for "ZZ"(#220)	268/268	254/256	202/208
Oocyte donor for "HH"(#380)	271/271	133/148	198/204
Surrogate for "HH"(#516)	262/262	131/131	207/210



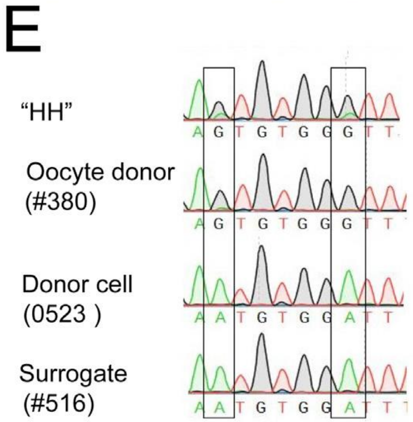


Table 1. Statistics on the Development of SCNT Embryos

Donor cells	Oocytes	SCNT embryos	Embryos transferred	Surrogates	Pregnancies	Live birth	Survived offspring
Fetal fibroblasts	127	109	79	21	6	2	2
Cumulus cells	290	192	181	42	22	2	0

RESEARCH ARTICLE

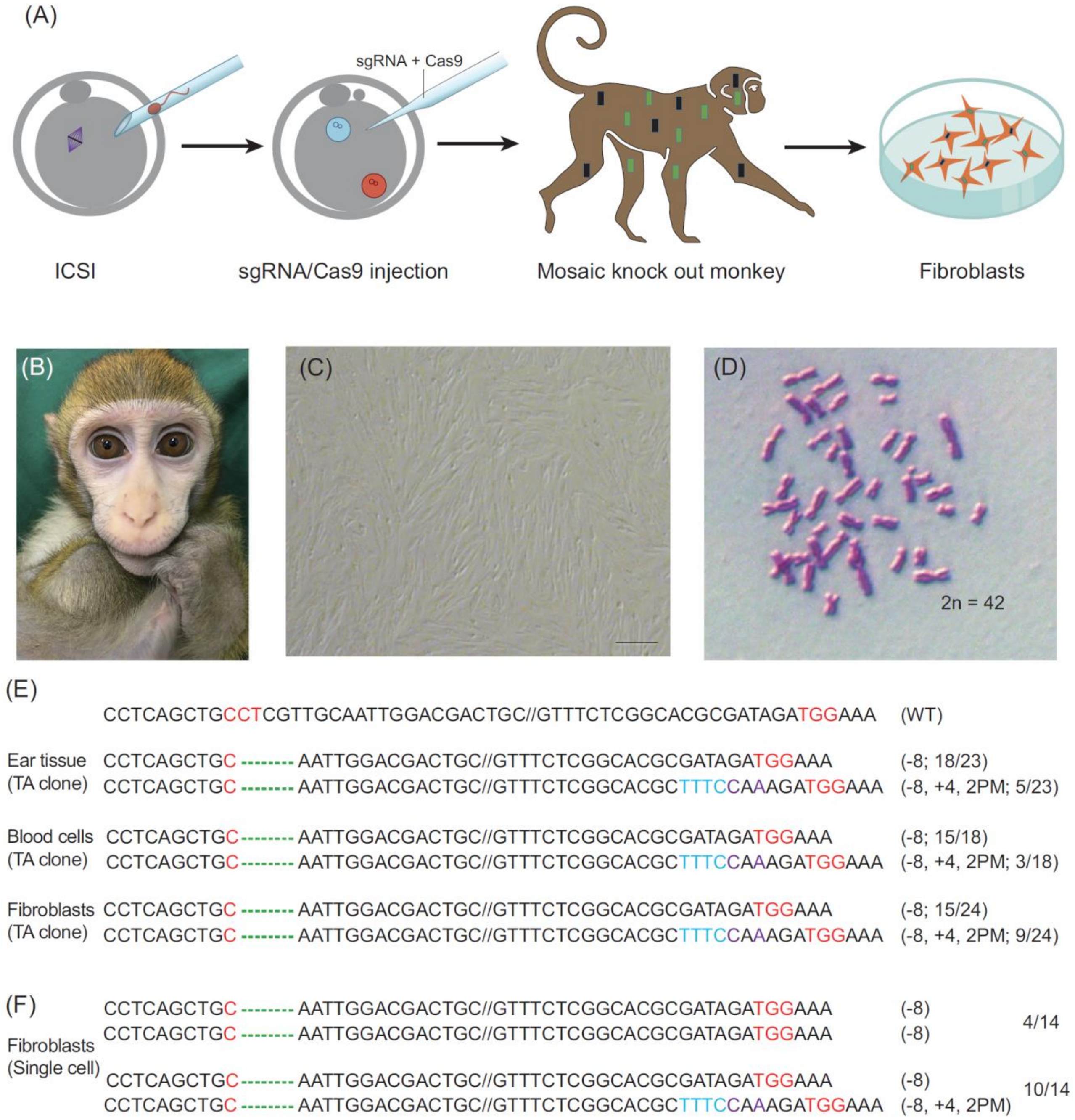
National Science Review
6: 101–108, 2019
doi: 10.1093/nsr/nwz003
Advance access publication 24 January 2019

NEUROSCIENCE

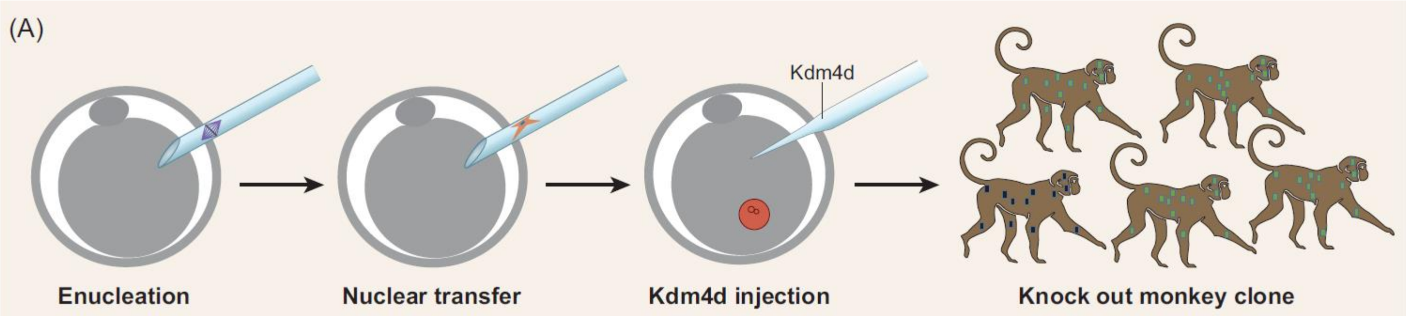
Cloning of a gene-edited macaque monkey by somatic cell nuclear transfer

Zhen Liu[1,2,*,†], Yijun Cai[1,2,†], Zhaodi Liao[1,2,†], Yuting Xu[1,2], Yan Wang[1,2], Zhanyang Wang[1,2], Xiaoyu Jiang[1,2], Yuzhuo Li[1,2], Yong Lu[1,2], Yanhong Nie[1,2], Xiaotong Zhang[1,2], Chunyang Li[1,2], Xinyan Bian[1,2], Mu-ming Poo[1,2], Hung-Chun Chang[1,2,*] and Qiang Sun[1,2,*]

Preparation of fibroblasts from a *BMAL1*-edited monkey



Generation of monkey offspring by SCNT using fibroblasts from a BMAL1 knockout monkey



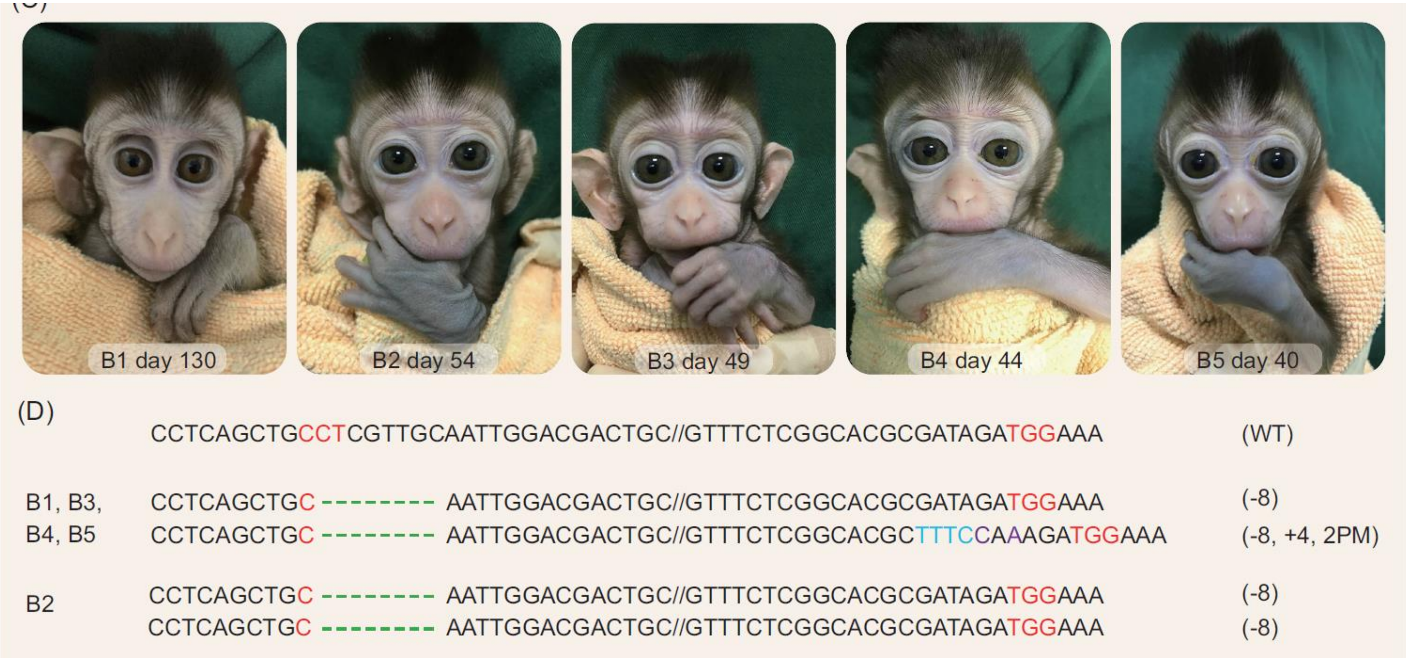


Table 1. Statistics for the development of the SCNT embryos.

Cell passage	Embryos transferred	Surrogates	Pregnancies	Live birth (Number)
2	118	23	7	1 (B1)
3	148	30	4	1 (B2)
4	59	12	5	3 (B3, B4 and B5)
Total	325	65	16	5

Opportunities

Disease modelling

Drug development

JARROD BAILEY, 2005

TABLE 13.1 Commonly Used Nonhuman Primate Models of Human Diseases

Disease/condition	Commonly used nonhuman primate models	References
Type 2 diabetes mellitus	Cynomolgus macaque	[3–5]
	Rhesus monkey	[6,7]
	African green monkey	[8,9]
Atherosclerosis	Cynomolgus macaque	[3,16–19]
Metabolic disease	Cynomolgus macaque	[20,21]
	Rhesus monkey	[22–28]
	Pigtail macaque	[24]
Aging	Rhesus monkey	[29–32]
Sarcopenia	Rhesus monkey	[33,34]
Osteoporosis	Rhesus monkey	[35–38]
	Cynomolgus macaque	[38,39]
Cognitive decline	Rhesus monkey	[40–44]
	Cynomolgus macaque	[45]
Menopause/perimenopause	Rhesus monkey	[46–48]
	Cynomolgus monkey	[47,49–52]
Endometriosis	Rhesus monkey	[53,54]
	Cynomolgus monkey	[53]
Simian immunodeficiency virus	Rhesus monkey	[55–61]
	Cynomolgus monkey	[56,58,59,61,62]
	Pigtail macaque	[55,59,61,63]
Radiation	Rhesus monkey	[64–69]
Malaria	Rhesus monkey	[70–81]
	Cynomolgus macaque	[70]
	Japanese macaque	[70,82,83]

Challenges

Low successful rate

High cost

Ethics

About 60% of primate species are threatened with extinction. Common threats include deforestation, forest fragmentation, monkey drives, and primate hunting for use in medicines, as pets, and for food. Large-scale tropical forest clearing for agriculture most threatens primates.

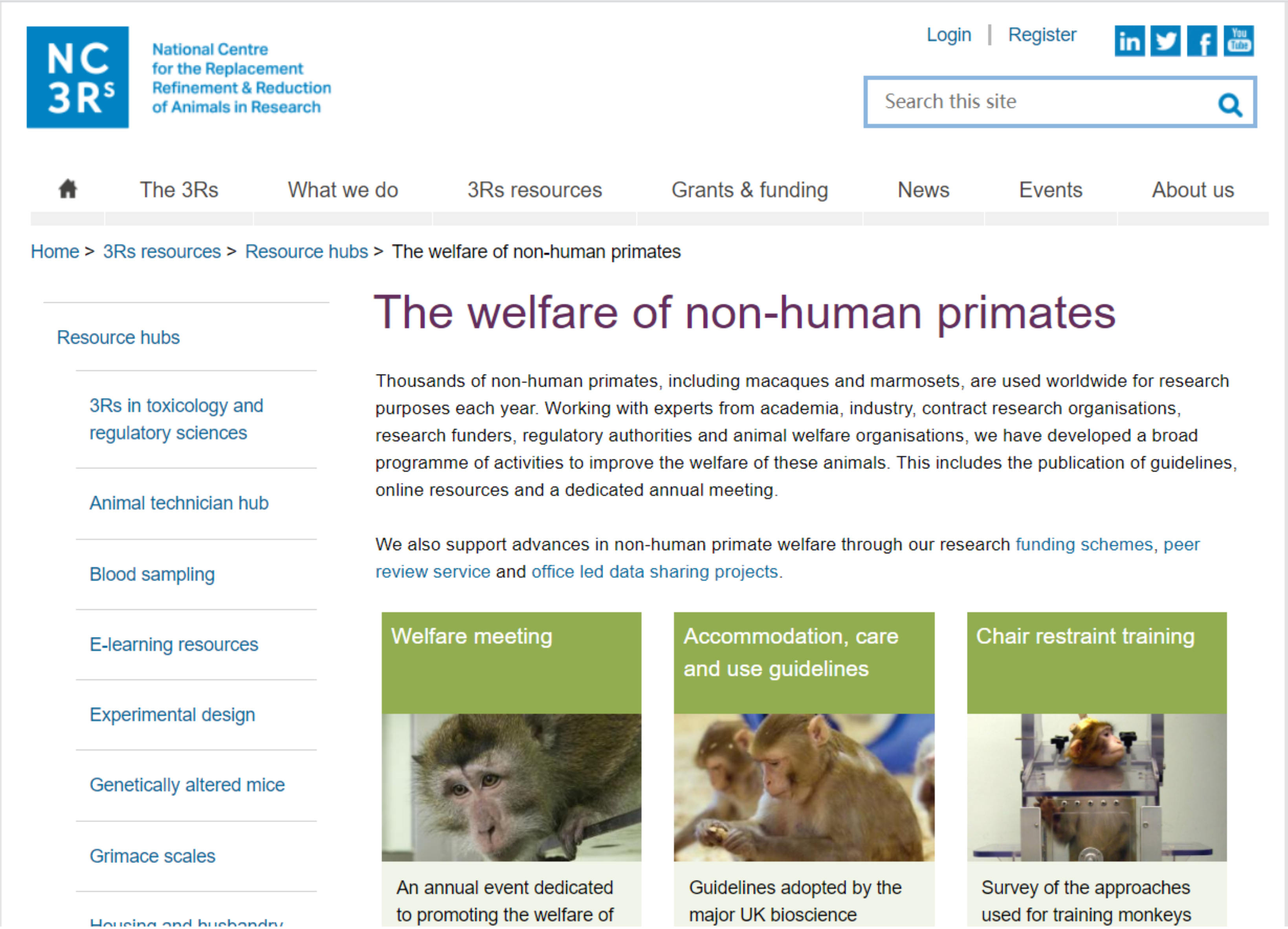
3R and welfare of the NHPs

3R

Replacement

Reduction

Refinement



Thanks for your attention!