# 转基因动物及其应用

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# 提 纲

- 转基因动物的基本概念
- 转基因动物发展简史
- 转基因动物的应用
- 转基因动物相关伦理问题

# 转基因动物

- **转基因动物（transgenic animal）**
  ——基因组中整合有外源基因，该基因能够表达并通过生殖系遗传的一类动物。
  ——指基因组上携带设计的遗传修饰并产生可遗传性状的动物。

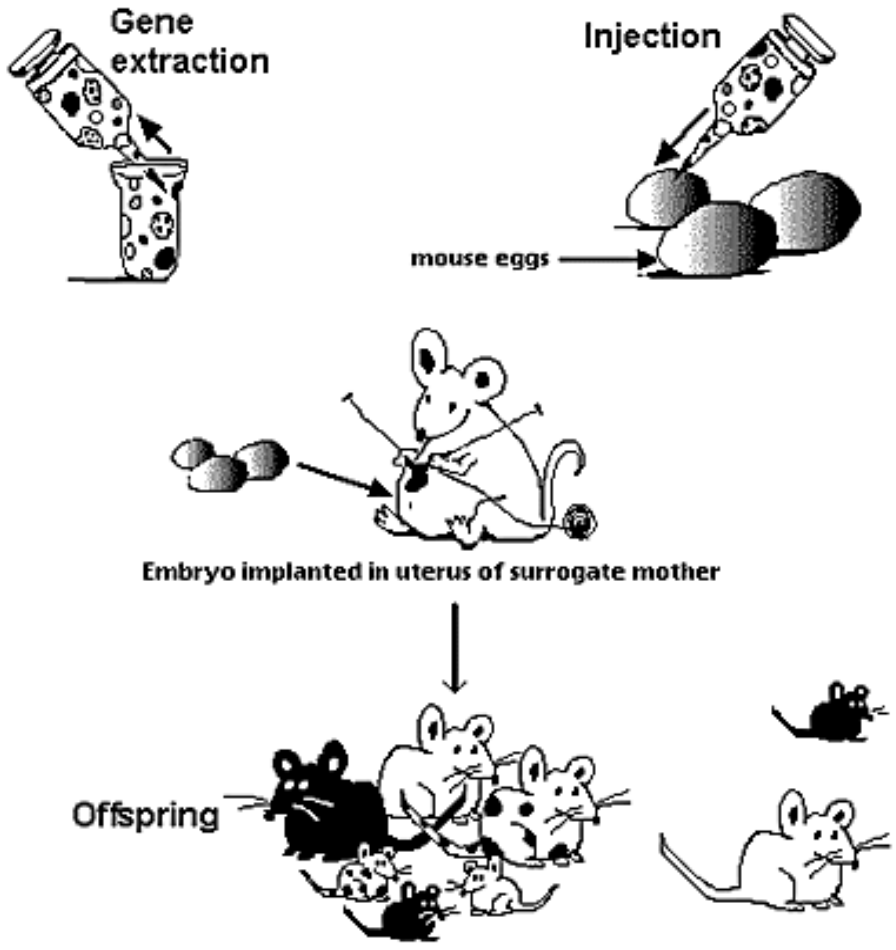
- **转基因（transgene）**
  整合入动物基因组的外源基因。

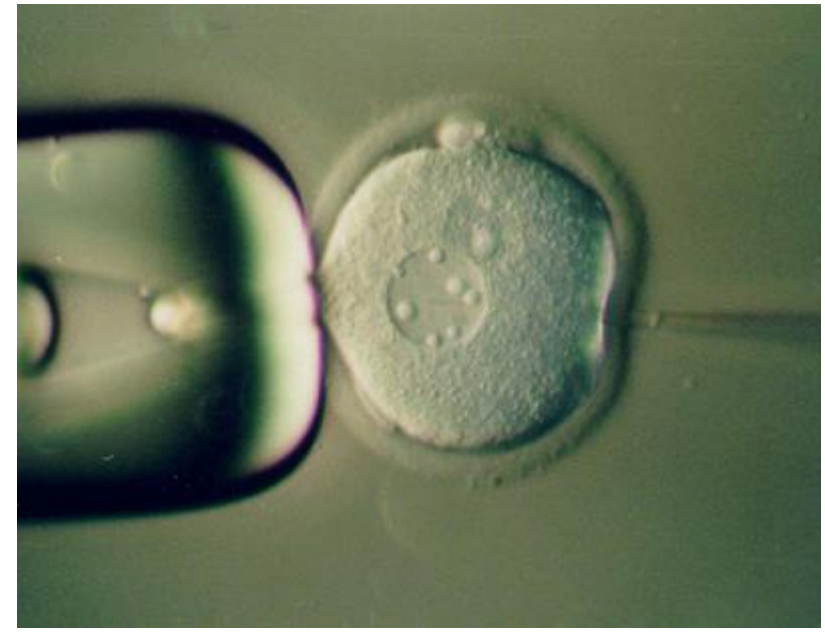
# 转基因动物发展史上的主要事件

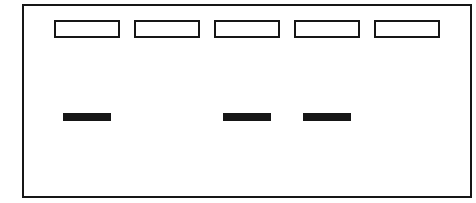
公元前12000，人类开始动植物的驯化和选育。

- 1974年，Jaenisch R等获得首例转基因小鼠。
- 1985年，Palmiter RD和Brinster RL等报道首例转基因家畜。
- 1987年，Smithies O和Capecchi MR等在小鼠ES细胞实现基因打靶。1989年，基因敲除小鼠诞生。
- 1997年，Wilmut I等报道首例体细胞克隆动物羊。
- 2013年， Jaenisch R等报道基因编辑小鼠。

# 通过受精卵显微注射研制转基因小鼠



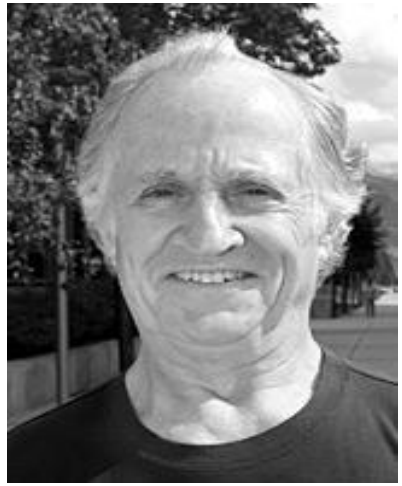


Presence of transgene shown by PCR fragment

# 小鼠基因打靶技术

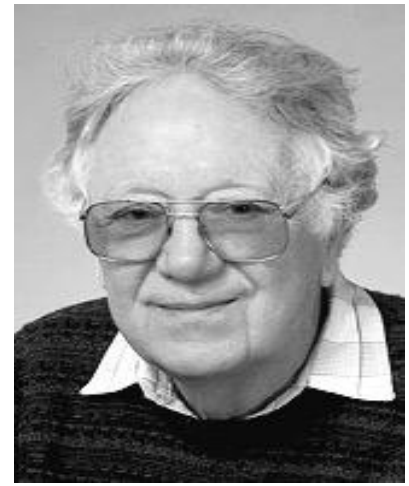
## (1987-至今)

— 基于同源重组和胚胎干细胞技术
— 开创遗传学新纪元
— 研究基因功能的“金标准”



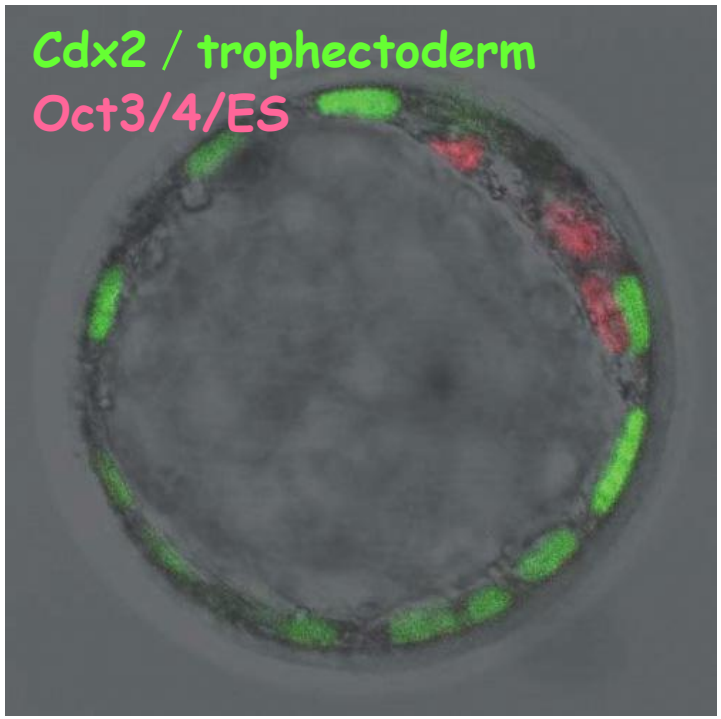
Mario Capecchi

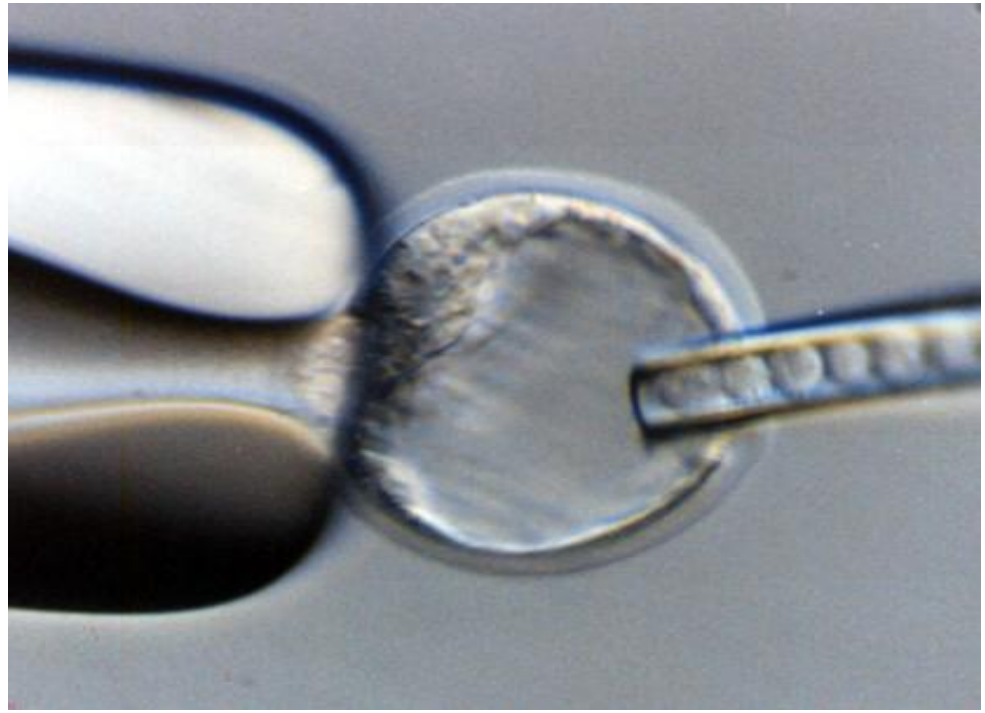
Martin Evans



Oliver Smithies

# 囊胚显微注射技术

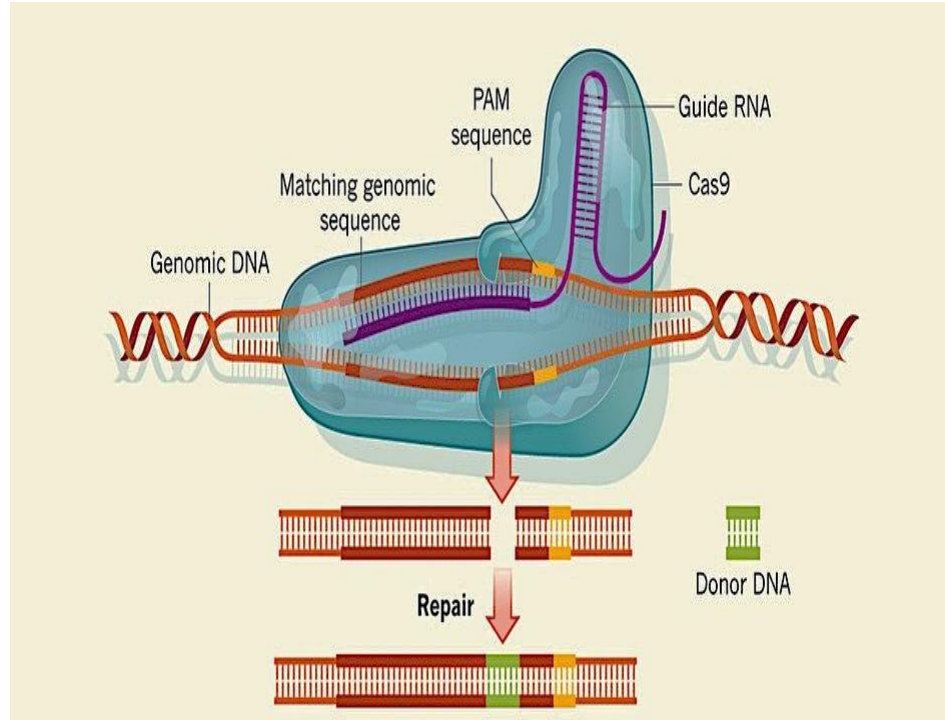


# 基于Cas9的基因编辑技术

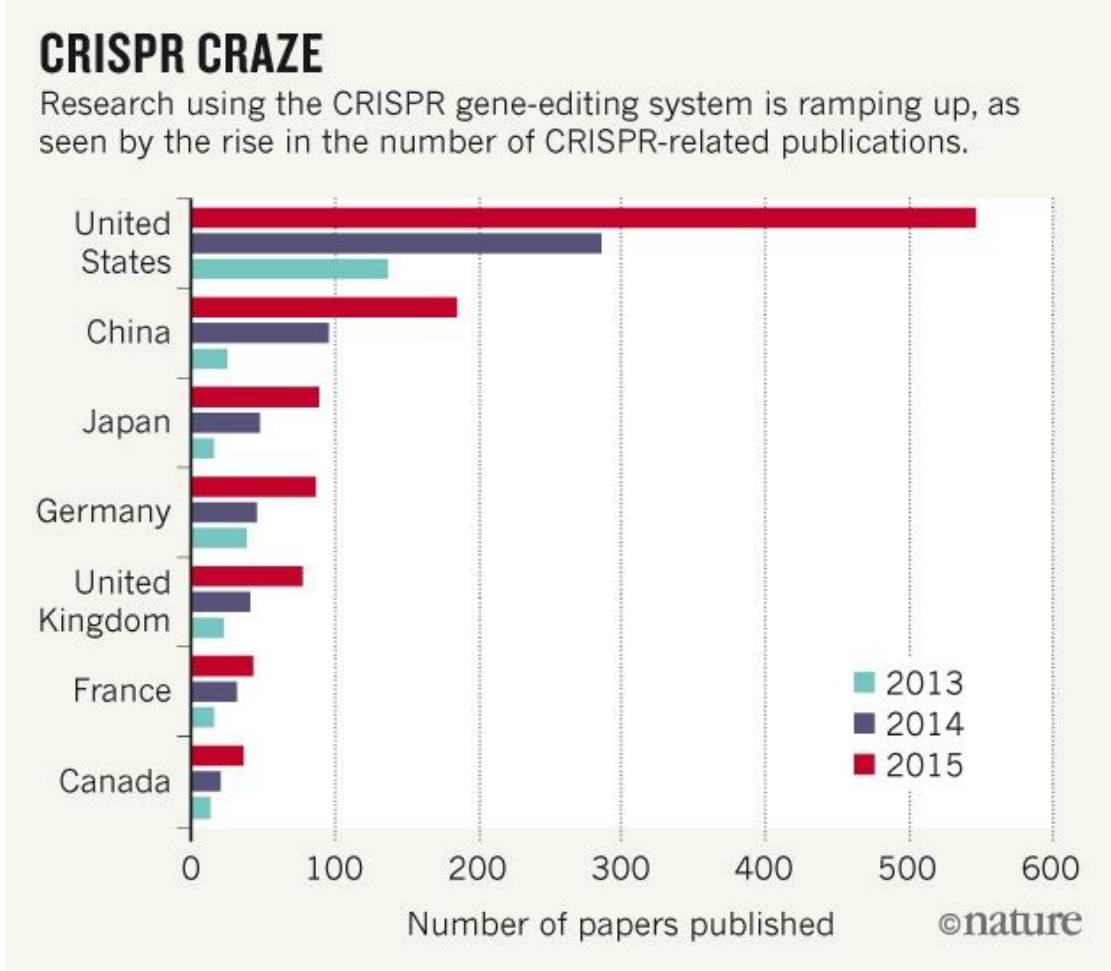
（2012-至今）

- 只需与靶序列互补的向导gRNA
- 可同时靶向多个基因
- 无基因序列、细胞类型和物种的限制
- 成本低廉、高效、操作简便

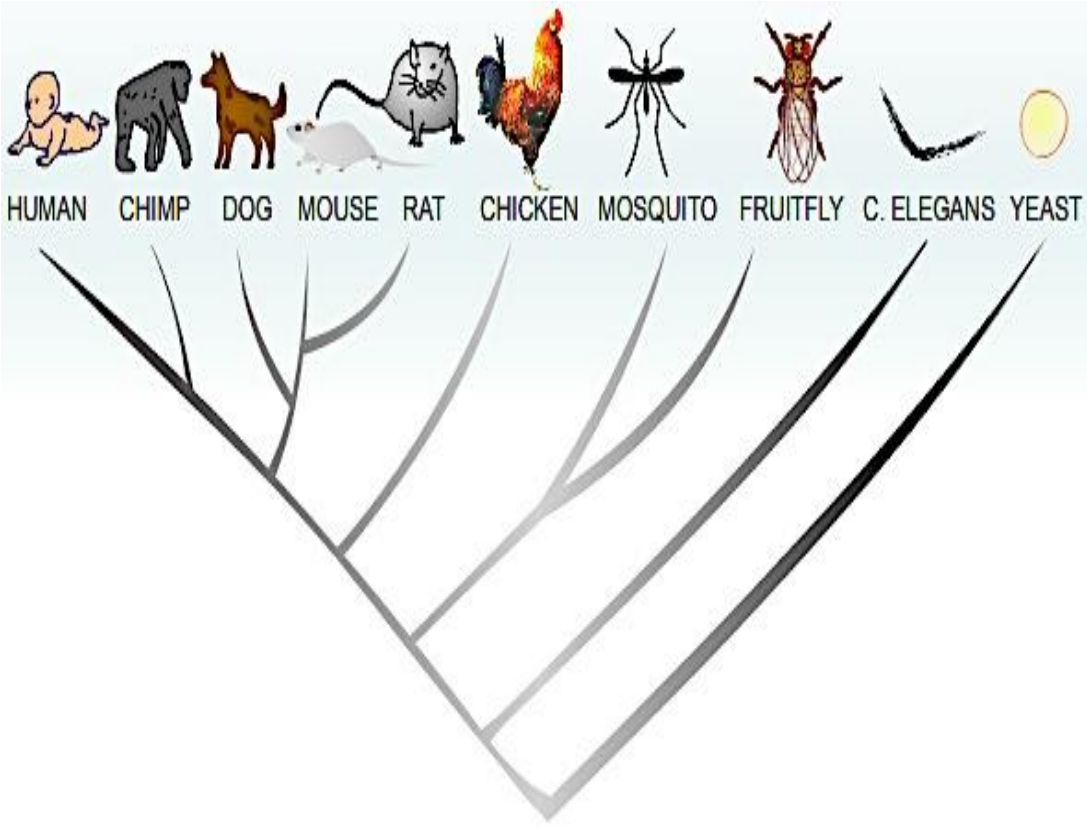


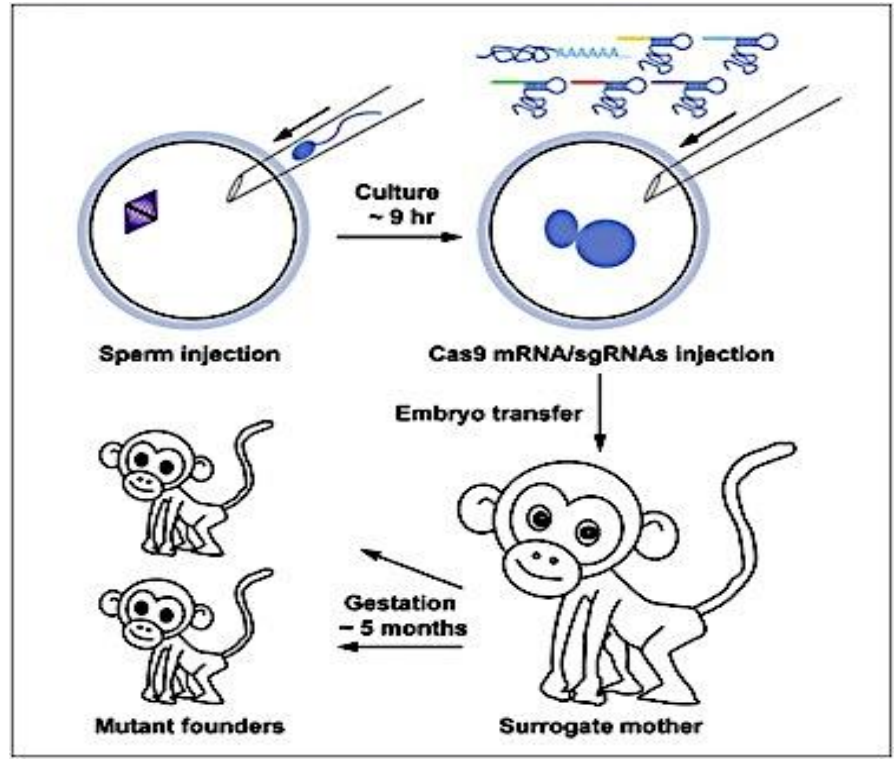
# Nature杂志2015年度科学事件

## —基于CRISPR/Cas9的基因编辑技术

# 多物种基因组定点编辑



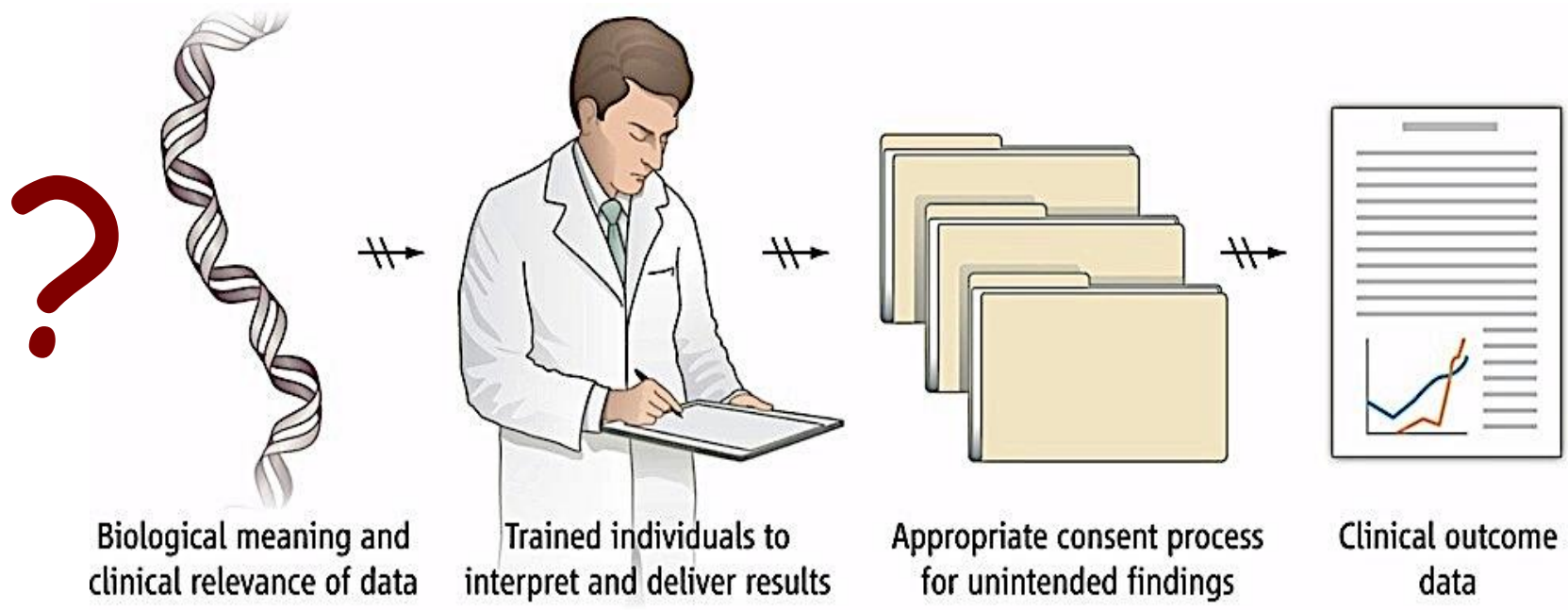




Niu Y. Cell, 2014

# 转基因动物的应用

- 研究基因组功能
- 研制人类疾病动物模型
- 改良动物生产性状

# 全基因组测序与精准医学间的鸿沟



ARTICLE 2011, 474(7351):337-342

doi:10.1038/nature10163

# A conditional knockout resource for the genome-wide study of mouse gene function

William C. Skarnes[1], Barry Rosen[1], Anthony P. West[1], Manousos Koutsourakis[1], Wendy Bushell[1], Vivek Iyer[1], Alejandro O. Mujica[1]†, Mark Thomas[1], Jennifer Harrow[1], Tony Cox[1], David Jackson[1], Jessica Severin[1]†, Patrick Biggs[1]†, Jun Fu[2], Michael Nefedov[3], Pieter J. de Jong[3], A. Francis Stewart[2] & Allan Bradley[1]

**Gene targeting in embryonic stem cells has become the principal technology for manipulation of the mouse genome, offering unrivalled accuracy in allele design and access to conditional mutagenesis. To bring these advantages to the wider research community, large-scale mouse knockout programmes are producing a permanent resource of targeted mutations in all protein-coding genes. Here we report the establishment of a high-throughput gene-targeting pipeline for the generation of reporter-tagged, conditional alleles. Computational allele design, 96-well modular vector construction and high-efficiency gene-targeting strategies have been combined to mutate genes on an unprecedented scale. So far, more than 12,000 vectors and 9,000 conditional targeted alleles have been produced in highly germline-competent C57BL/6N embryonic stem cells. High-throughput genome engineering highlighted by this study is broadly applicable to rat and human stem cells and provides a foundation for future genome-wide efforts aimed at deciphering the function of all genes encoded by the mammalian genome.**

Following the complete sequencing of the human and mouse genomes, the functional analysis of each of the twenty thousand or so protein-coding genes remains an important goal and a major technical challenge. Several genome-wide mutagenesis strategies have been applied in the mouse, including ethyl-nitrosourea (ENU) mutagenesis, transposon mutagenesis, gene trapping and gene targeting. Gene trapping in mouse embryonic stem (ES) cells[1,2] has been the most productive so far, providing hundreds of thousands of random insertional mutations in more than half of the protein-coding genes in the mouse[3–5]. Notably, these ES cell resources can be archived indefinitely and are easily distributed to the scientific community for the purpose of generating knockout mice. However, gene-trap alleles cannot be precisely engineered and the strategy favours genes expressed in mouse ES cells.

Given the limitations of gene trapping, it is clear that the generation of a complete set of gene knockouts in the mouse will require the application of gene-targeting technology in ES cells[6–8]. Gene targeting can be used to engineer virtually any alteration in the mammalian genome by homologous recombination in mouse ES cells, from point mutations to large chromosomal rearrangements[9,10]. Over the past 20 years, gene targeting has been used to elucidate the function of more than 5,000 mammalian genes. Scaling this technology to the remainder of the genome presents numerous technical challenges and requires the production of targeted ES cells on an unprecedented scale, beyond the scope of conventional methodologies.

The first targeting pipeline for ES cells was reported several years ago before the completion of the mouse genome sequence (Velocigene)[11]. Bacterial artificial chromosome (BAC)-based targeting vectors were constructed to replace the coding sequence of the target gene with a *lacZ* reporter and promoter-driven selection cassette. Oligonucleotides required for the construction of targeting vectors by recombineering were based on cDNA sequences surrounding the translation initiation and termination signals of each target gene, thus requiring no previous knowledge of the underlying genomic structure of the gene. In a single recombineering step, modified BAC clones were generated with high efficiency and used to target genes in ES cells. Correctly targeted events, which involved the deletion of up to 70-kilobases (kb) of genomic sequence, were identified using a novel high-throughput allele-counting assay. The deletion of large regions of genomic sequence, although effective for eliminating the function of the target gene, can have unintended consequences on the regulation of adjacent and distant transcriptional units[12,13].

To support and accelerate progress towards the genetic analysis of all mammalian genes, large-scale knockout consortia were established in 2006 with the goal of generating a complete resource of reporter-tagged null mutations in C57BL/6 mouse ES cells[14]. C57BL/6 is one of the best characterized inbred strains, is the reference strain for the mouse genome sequence and breed well in the laboratory. Thus, the study of mutant alleles in a pure C57BL/6 genetic background is considered to be ideal for large-scale phenotyping efforts that will follow. Highly germline-competent ES cell lines from the C57BL/6N substrain of mice have been established for this project[15–17]. A common web portal providing information and access to the resource has been established[18], with links to designated repositories for ordering vectors, ES cell clones and mice.

Here we describe a pipeline for the design and mass parallel construction of conditional targeting vectors by serial 96-well BAC recombineering and high-throughput gene targeting in C57BL/6 ES cells. Our pipeline is configured to create a number of useful resources en route to the generation of targeted ES cells (Supplementary Fig. 1). Ongoing large-scale production of targeted ES cell lines demonstrates rates of homologous recombination in C57BL/6 ES cells well above the historical average. Our pipeline forms the basis for the generation

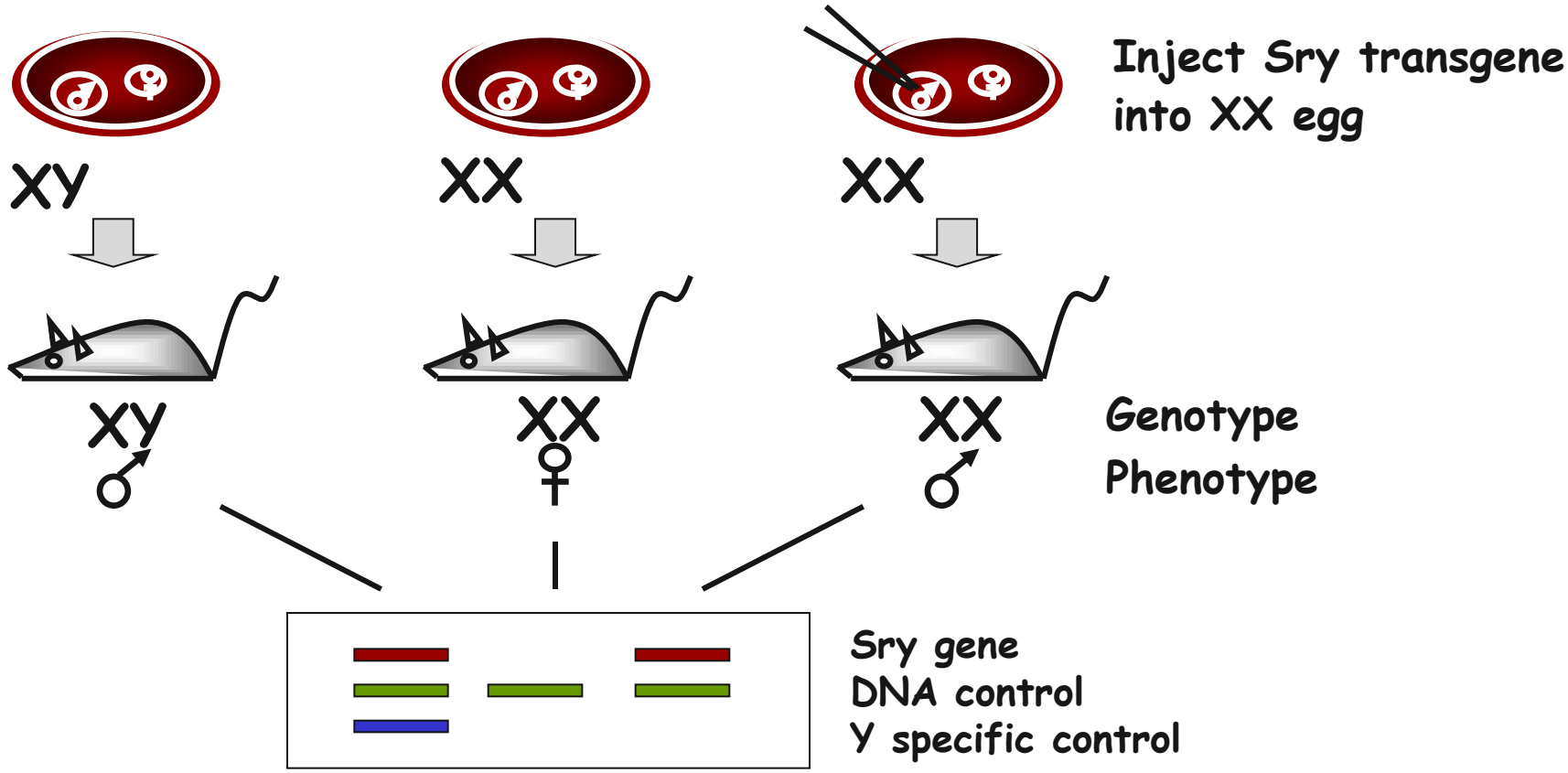
[1]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK. [2]Biotechnologisches Zentrum, TU Dresden, 01062 Dresden, Germany. [3]Children's Hospital Oakland Research Institute, Oakland, California 94609, USA. †Present addresses: Regeneron Pharmaceuticals, Inc., Tarrytown, New York, USA (A.O.M.); RIKEN Omics Science Center, Yokohama City, Japan (J.S.); Hopkirk Institute, Massey University, New Zealand (P.B.).





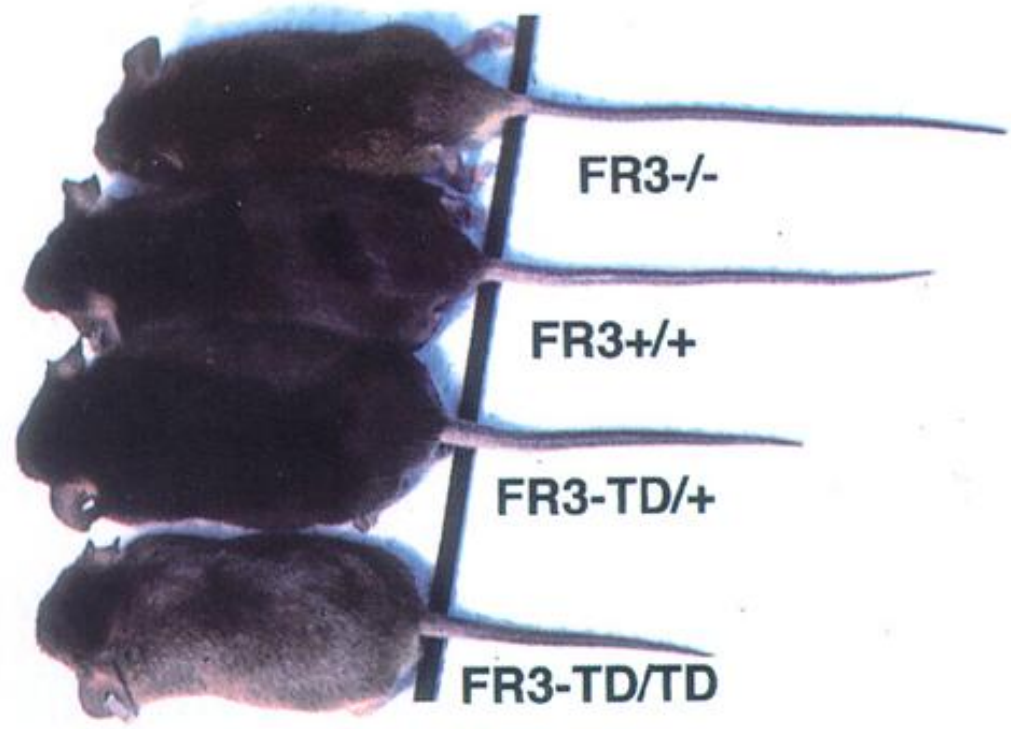
# 小鼠全基因组基因敲除计划

# 对特定基因生理功能的假设进行实验验证



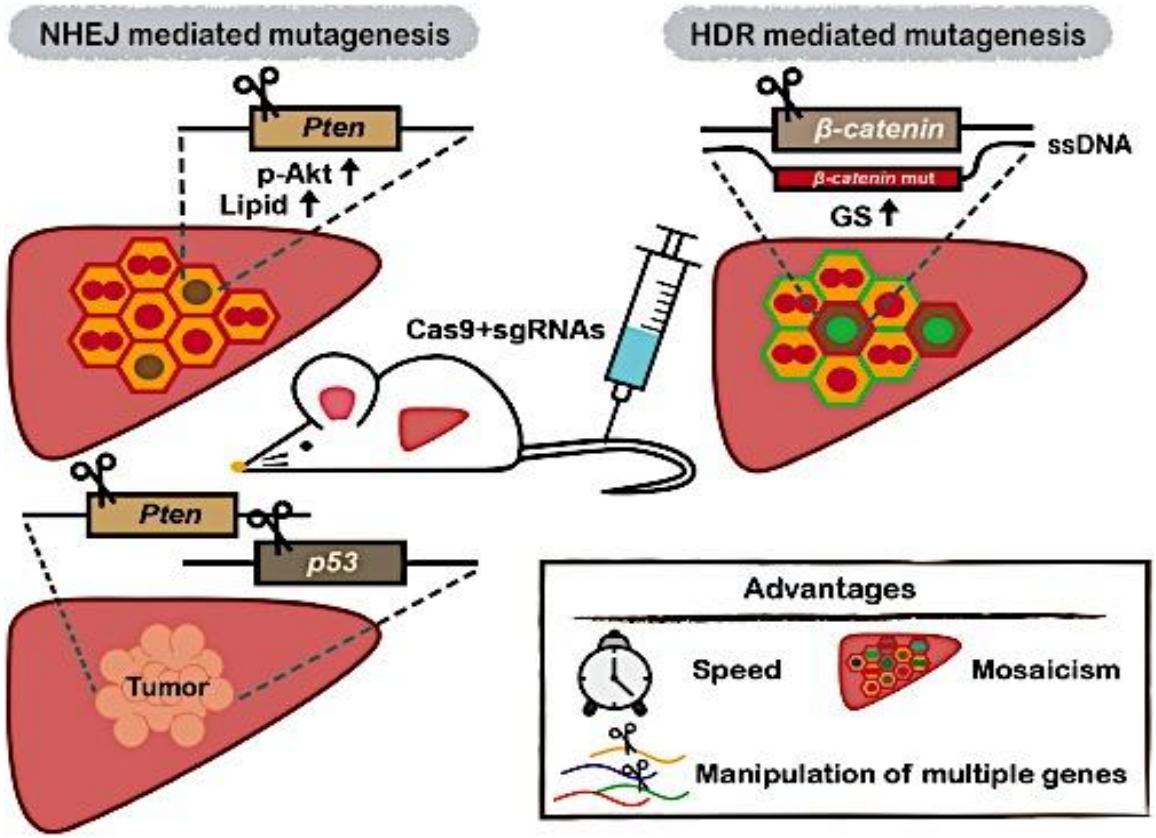

Sry基因决定雄性发育（Nature，1991）

# 建立基因突变和疾病间的因果关系



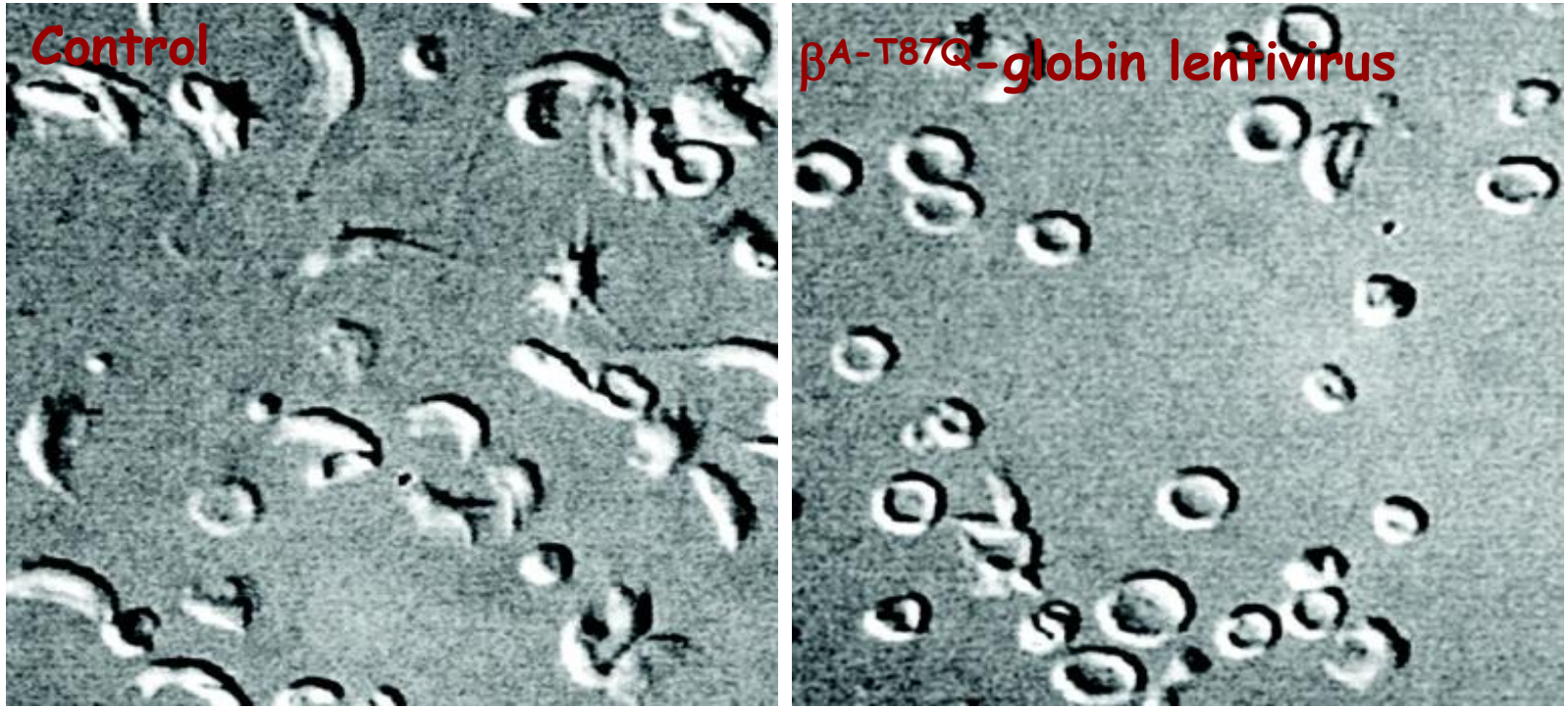

人类侏儒症小鼠模型（Cell, 1996; HMG; JCI, 1999）

# 建立基因突变和疾病间的因果关系



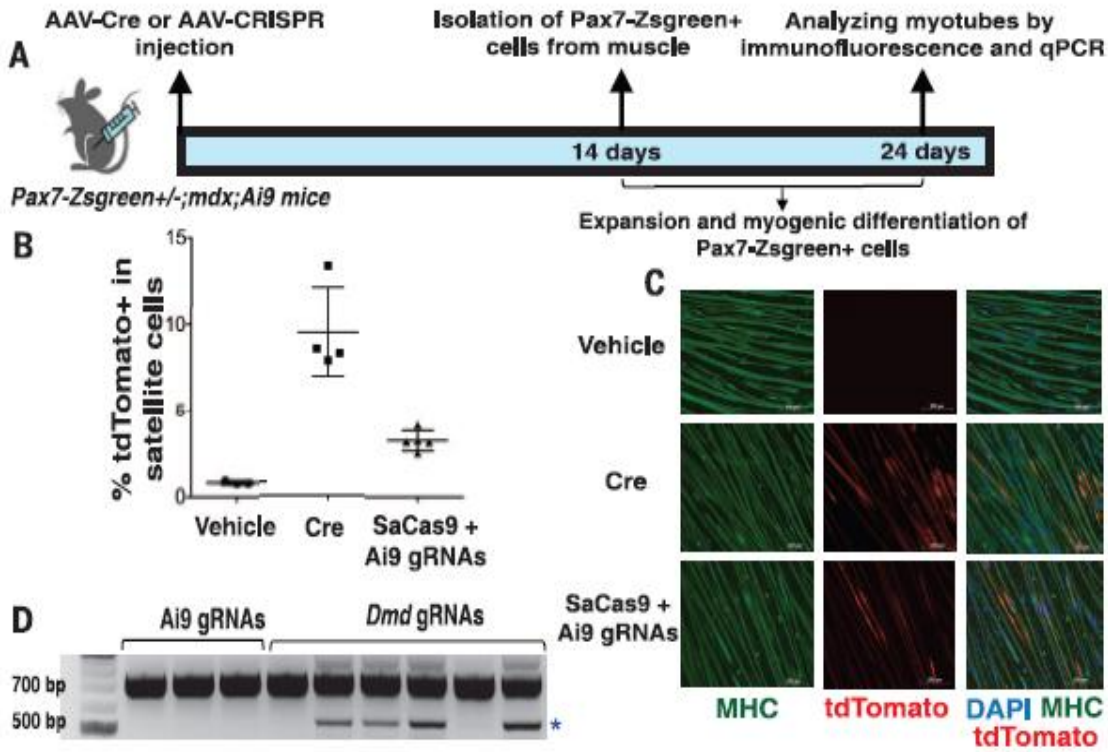

Cas9介导的体细胞基因编辑研制癌症模型（Nature，2014）

# 人类疾病动物模型用于治疗方案探索




基因治疗治愈转基因小鼠的镰刀型细胞贫血症（Science 2001）

# 利用体内基因编辑技术治疗器质性疾病




GENE EDITING

## In vivo gene editing in dystrophic mouse muscle and muscle stem cells

Mohammadsharif Tabebordbar,[1,2]* Kexian Zhu,[1,3]* Jason K. W. Cheng,[1] Wei Leong Chew,[2,4] Jeffrey J. Widrick,[5] Winston X. Yan,[6,7] Claire Maesner,[1] Elizabeth Y. Wu,[1]† Ru Xiao,[8] F. Ann Ran,[6,7] Le Cong,[6,7] Feng Zhang,[6,7] Luk H. Vandenberghe,[8] George M. Church,[4] Amy J. Wagers[1]‡

GENE EDITING

## In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy

Christopher E. Nelson,[1,2] Chady H. Hakim,[3] David G. Ousterout,[1,2] Pratiksha I. Thakore,[1,2] Eirik A. Moreb,[1,2] Ruth M. Castellanos Rivera,[4] Sarina Madhavan,[1,2] Xiufang Pan,[3] F. Ann Ran,[5,6] Winston X. Yan,[5,7,8] Aravind Asokan,[4] Feng Zhang,[5,9,10,11] Dongsheng Duan,[3,12] Charles A. Gersbach[1,2,13]*

GENE EDITING

## Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy

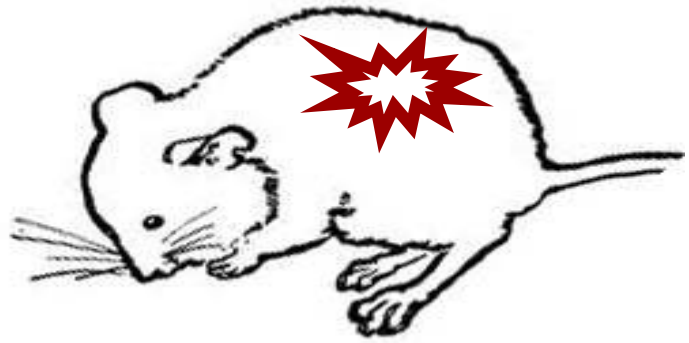
Chengzu Long,[1,2,3]* Leonela Amoasii,[1,2,3]* Alex A. Mireault,[1,2,3] John R. McAnally,[1,2,3] Hui Li,[1,2,3] Efrain Sanchez-Ortiz,[1,2,3] Samadrita Bhattacharyya,[1,2,3] John M. Shelton,[4] Rhonda Bassel-Duby,[1,2,3] Eric N. Olson[1,2,3]†

Science， 2016

# 人类疾病动物模型用于新药研发

Disease sample procurement and preparation → Disease gene discovery → Computational biology → Disease gene validation → Disease gene mechanism

Genetic Inactivation



Pharmacological Inactivation

Assay development and optimization → mAb lead humanization → Preclinical therapeutics (GEMMs) → Biomarker identification/ development → Clinical trials

# 改良动物生产性状

2015年11月19日，美国FDA批准全球首例转基因食品动物——转基因鲑鱼上市。FDA确定转基因鲑鱼在食用安全性、营养价值以及营养结构上与养殖的非转基因鲑鱼并无差别。

# 转基因动物的社会伦理问题

- 转基因技术的安全性问题
- 转基因动物产品的安全性问题
- 动物权益的保护

# 总　结

- 转基因动物相关研究是人类探索生命未知奥秘的利器，也将为精准医学提供理论基础和治疗策略。
- 加强对转基因动物相关研究的支持、监督、和相关伦理的系统研究将确保其造福人类。

# 致謝