



# 生物育种专业·基因编辑技术课程

## 第七章：神话再临—技术改变世界

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QQ: 564737724 Tel:17792639752 2024.11.14

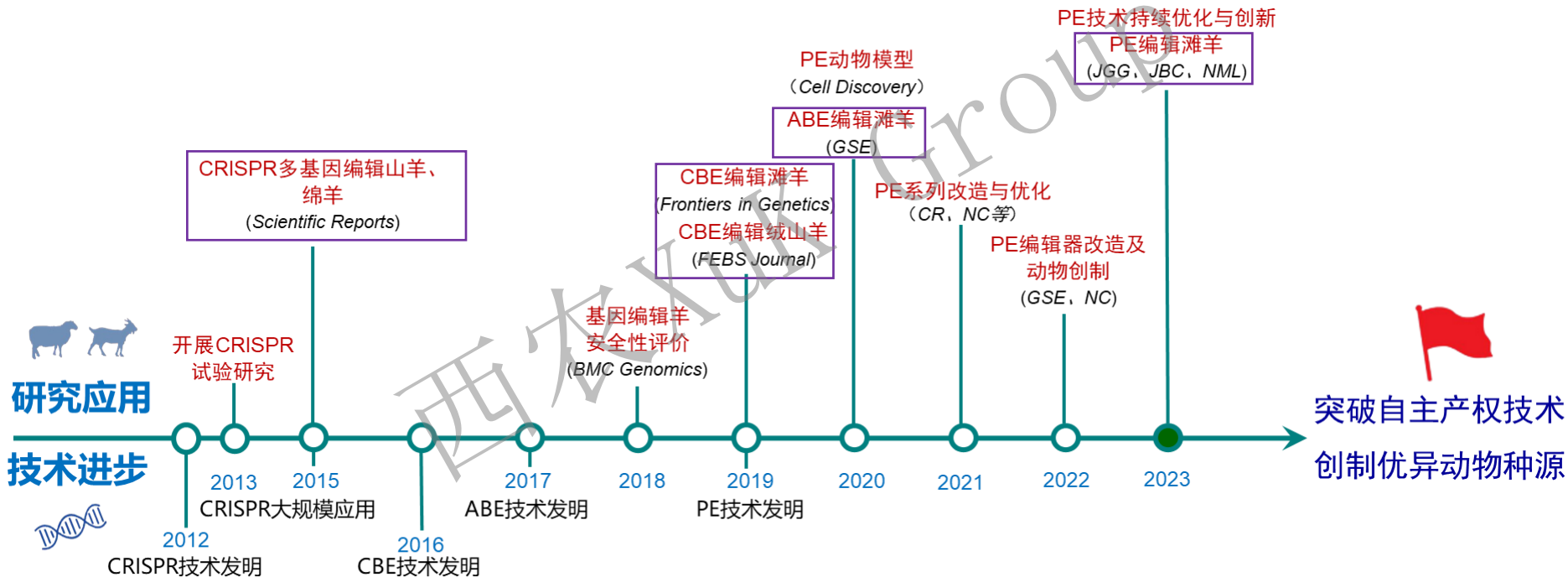


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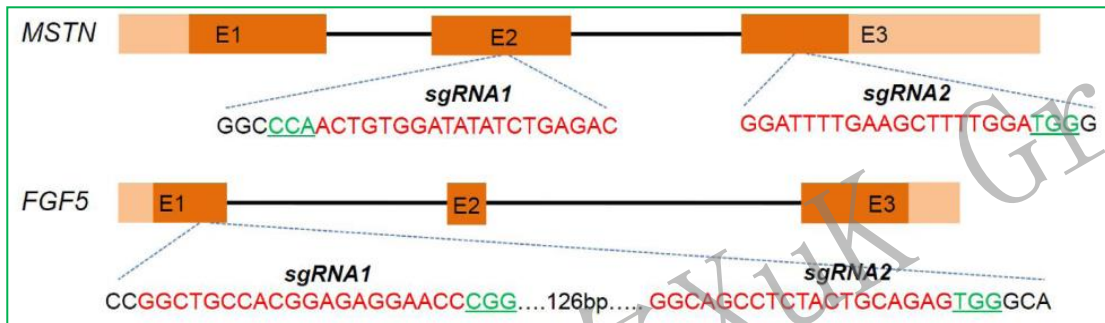


# 羊分子设计种应用

## 基因编辑技术发展促进动物育种（以本团队基因编辑羊为例）：



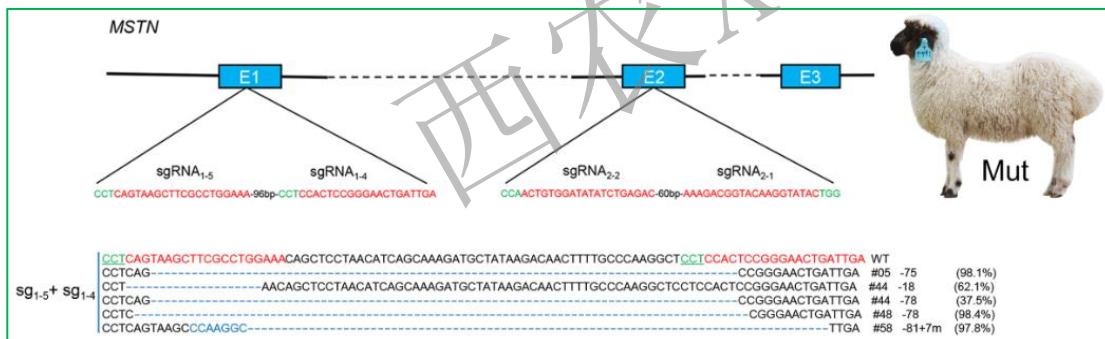
# ◆ 案例1-CRISPR/Cas9基因编辑绒山羊和滩羊 (NHEJ: 移码突变敲除)



Generation of gene-modified goats targeting MSTN and FGF5 via zygote injection of CRISPR/Cas9 system

Xiaolong Wang<sup>1</sup>, Honghao Yu<sup>2,3</sup>, Anmin Lei<sup>4</sup>, Jiankui Zhou<sup>5,6</sup>, Wenxian Zeng<sup>1</sup>, Haijing Zhu<sup>2,3</sup>, Zhiming Dong<sup>6</sup>, Yiyuan Niu<sup>1</sup>, Bingbo Shi<sup>1</sup>, Bei Cai<sup>1</sup>, Jinwang Liu<sup>2,3</sup>, Shuai Huang<sup>2,3</sup>, Hailong Yan<sup>1,2,3</sup>, Xiao Zhao<sup>4</sup>, Guangxian Zhou<sup>1</sup>, Xiaoling He<sup>1</sup>, Xiaoxu Chen<sup>1</sup>, Yuxin Yang<sup>1</sup>, Yu Jiang<sup>1</sup>, Lei Shi<sup>2,3</sup>, Xiue Tian<sup>1</sup>, Yongjun Wang<sup>1</sup>, Baohua Ma<sup>4</sup>, Xingxu Huang<sup>5,6</sup>, Lei Qu<sup>2,3</sup>, Yulin Chen<sup>1</sup>

Wang X, et al. *Sci Rep.* 2015.



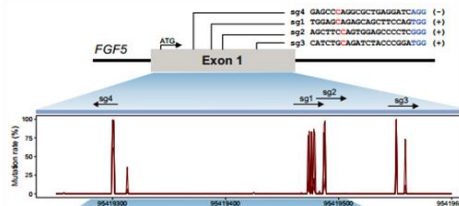
Multiplex gene editing via CRISPR/Cas9 exhibits desirable muscle hypertrophy without detectable off-target effects in sheep

Xiaolong Wang<sup>1</sup>, Yiyuan Niu<sup>1</sup>, Jiankui Zhou<sup>2,3</sup>, Honghao Yu<sup>4</sup>, Qifang Kou<sup>5</sup>, Anmin Lei<sup>6</sup>, Xiao Zhao<sup>6</sup>, Hailong Yan<sup>1,4</sup>, Bei Cai<sup>1</sup>, Qiaoyan Shen<sup>6</sup>, Shiwei Zhou<sup>1</sup>, Haijing Zhu<sup>4</sup>, Guangxian Zhou<sup>1</sup>, Wenzhi Niu<sup>5</sup>, Jinlian Hua<sup>6</sup>, Yu Jiang<sup>1</sup>, Xingxu Huang<sup>2,3</sup>, Baohua Ma<sup>6</sup>, Yulin Chen<sup>1</sup>

Wang X, et al. *Sci Rep.* 2016.

# ◆ 案例2-CBE基因编辑绒山羊 (CBE-STOP: 提前终止敲除)

- 绒山羊FGF5基因引入终止密码子
- 编辑羊绒毛长度增加



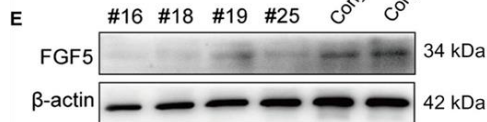
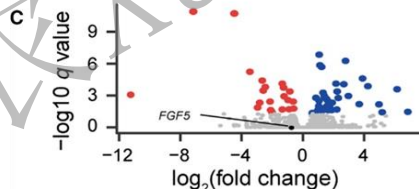
**C**

```

WT CCTGATCCTCAGCGCCTGGG...TGGAGCAAAGCAGCTTCCAGTGGAGCCCTC  Fr
#3 CCTGATCCTCAGCGCCTAAG...TGGAGCAGAGCAGCTTCCAGTGGAGCCCTC  9
CCTGATCCTCAGCGCCTATG...TGGAGCAGAGCAGCTTTAGTGGAGCCCTC  5
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#25 CCTGATCCTCAGCGCCTAAG...TGGAGCAGAGCAGCTTCCAGTGGAGCCCTC  3
    
```

**A**

Fiber traits	Mutant	Control	SD	P value
Guard fiber length (D30)	6.25	5.63	0.29	0.02
Guard fiber length (D60)	8.88	7.63	0.25	0.04
Cashmere length (D30)	nd	nd	-	-
Cashmere length (D60)	3.05	2.48	0.13	0.04



✓ 通过CBE编辑:

C/G→T/A

✓ 提前引入终止密码

TAA/TAG/TGA

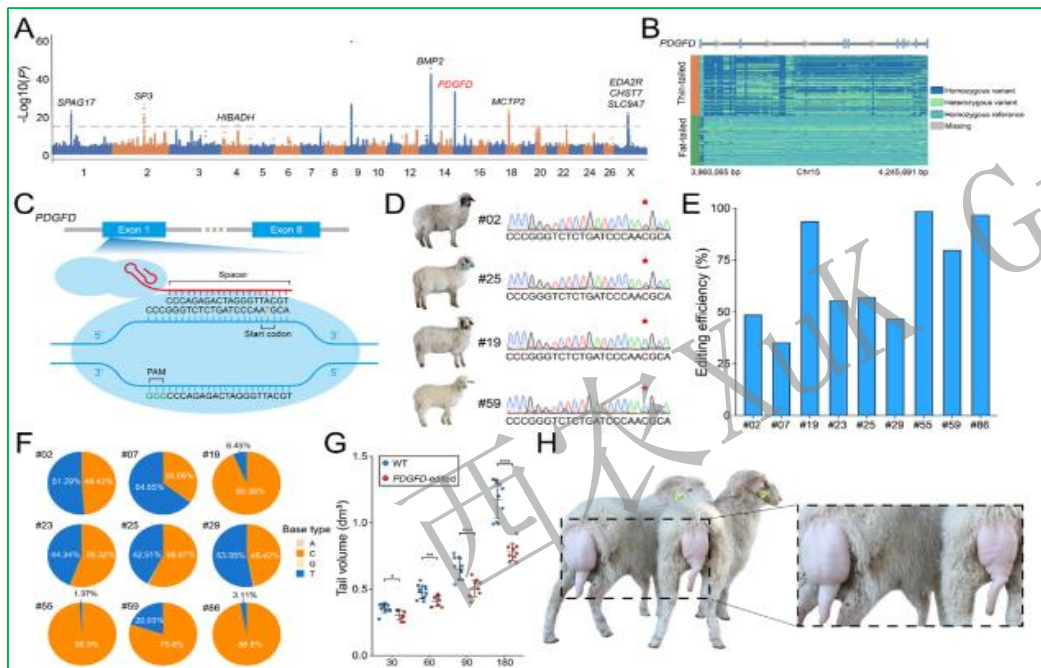
✓ 实现基因的提前终止敲除

Base pair editing in goat: nonsense codon introgression into FGF5 results in longer hair

Guanwei Li<sup>1</sup>, Shiwei Zhou<sup>1</sup>, Chao Li<sup>1</sup>, Bei Cai<sup>1</sup>, Honghao Yu<sup>2</sup>, Baohua Ma<sup>3</sup>, Yu Huang<sup>1</sup>, Yige Ding<sup>1</sup>, Yao Liu<sup>1</sup>, Qiang Ding<sup>1</sup>, Chong He<sup>4</sup>, Jiankui Zhou<sup>5</sup>, Ying Wang<sup>1</sup>, Guangxian Zhou<sup>1</sup>, Yan Li<sup>1</sup>, Yuan Yan<sup>3</sup>, Jinlian Hua<sup>3</sup>, Bjoern Petersen<sup>6</sup>, Yu Jiang<sup>1</sup>, Tad Sonstegard<sup>7</sup>, Xingxu Huang<sup>5</sup>, Yulin Chen<sup>1</sup>, Xiaolong Wang<sup>1</sup>

Li G, et al. **FEBS J.** 2019.

## ◆ 案例3-ABE基因编辑滩羊 (ABE-STOP: 起始沉默敲除)



- ✓ 通过ABE编辑:  
A/T→G/C
- ✓ 破坏翻译起始密码  
ATG→ACG/GTG/GCG
- ✓ 实现基因的**起始沉默敲除**

ABE-induced PDGFD start codon silencing unveils new insights into the genetic architecture of sheep fat tails

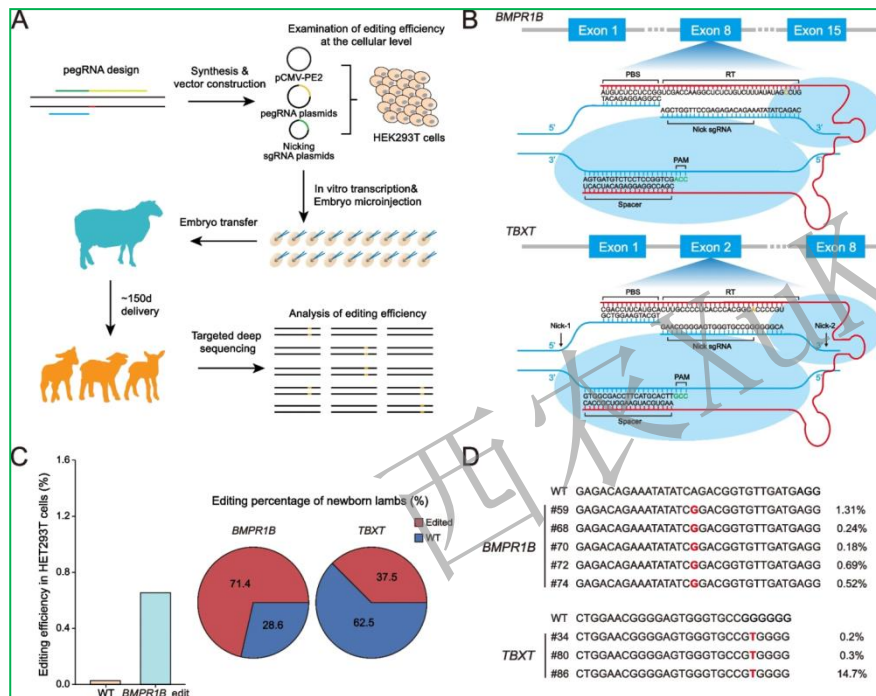
Peter Kalds <sup>1</sup>, Shuhong Huang <sup>2</sup>, Shiwei Zhou <sup>3</sup>, Siyuan Xi <sup>4</sup>, Yumeng Fang <sup>4</sup>, Yawei Gao <sup>4</sup>, Kexin Sun <sup>2</sup>, Chao Li <sup>2</sup>, Bei Cai <sup>5</sup>, Yao Liu <sup>2</sup>, Yige Ding <sup>2</sup>, Qifang Kou <sup>6</sup>, Tad Sonstegard <sup>7</sup>, Björn Petersen <sup>8</sup>, Stephen Kemp <sup>9</sup>, Baohua Ma <sup>10</sup>, Jian-Lin Han <sup>11</sup>, Yulin Chen <sup>12</sup>, Xiaolong Wang <sup>13</sup>

- PDGFD是影响绵羊尾型大小的关键基因，利用ABE-STOP技术敲除，绵羊尾巴显著变小

Kalds P et al. **J Genet Genomics**. 2023.



# 案例4-PE基因编辑滩羊 (点编辑)



- ✓ 双羔基因 *FecB<sup>B</sup>*, 尾型基因 *TBXT*
- ✓ 精准的点编辑
- ✓ 首次创制了PE基因编辑滩羊
- ✓ 但效率仍有待提升

Generation of sheep with defined *FecB<sup>B</sup>* and *TBXT* mutations and porcine blastocysts with *KCNJ5<sup>G151R/+</sup>* mutation using prime editing

Shiwei Zhou <sup># 1 2</sup>, Laura Johanna Lenk <sup># 3</sup>, Yawei Gao <sup># 2</sup>, Yuhui Wang <sup>2</sup>, Xiaoe Zhao <sup>1</sup>, Menghao Pan <sup>1</sup>, Shuhong Huang <sup>2</sup>, Kexin Sun <sup>2</sup>, Peter Kalds <sup>2 4</sup>, Qi Luo <sup>2</sup>, Simon Lillico <sup>5</sup>, Tad Sonstegard <sup>6</sup>, Ute I Scholl <sup>7</sup>, Baohua Ma <sup>1</sup>, Bjoern Petersen <sup>8</sup>, Yulin Chen <sup>9 10</sup>, Xiaolong Wang <sup>11 12</sup>

Zhou S et al. **BMC Genomics**. 2023



潘登科博士

# 打造未来器官工厂 异种移植改变未来

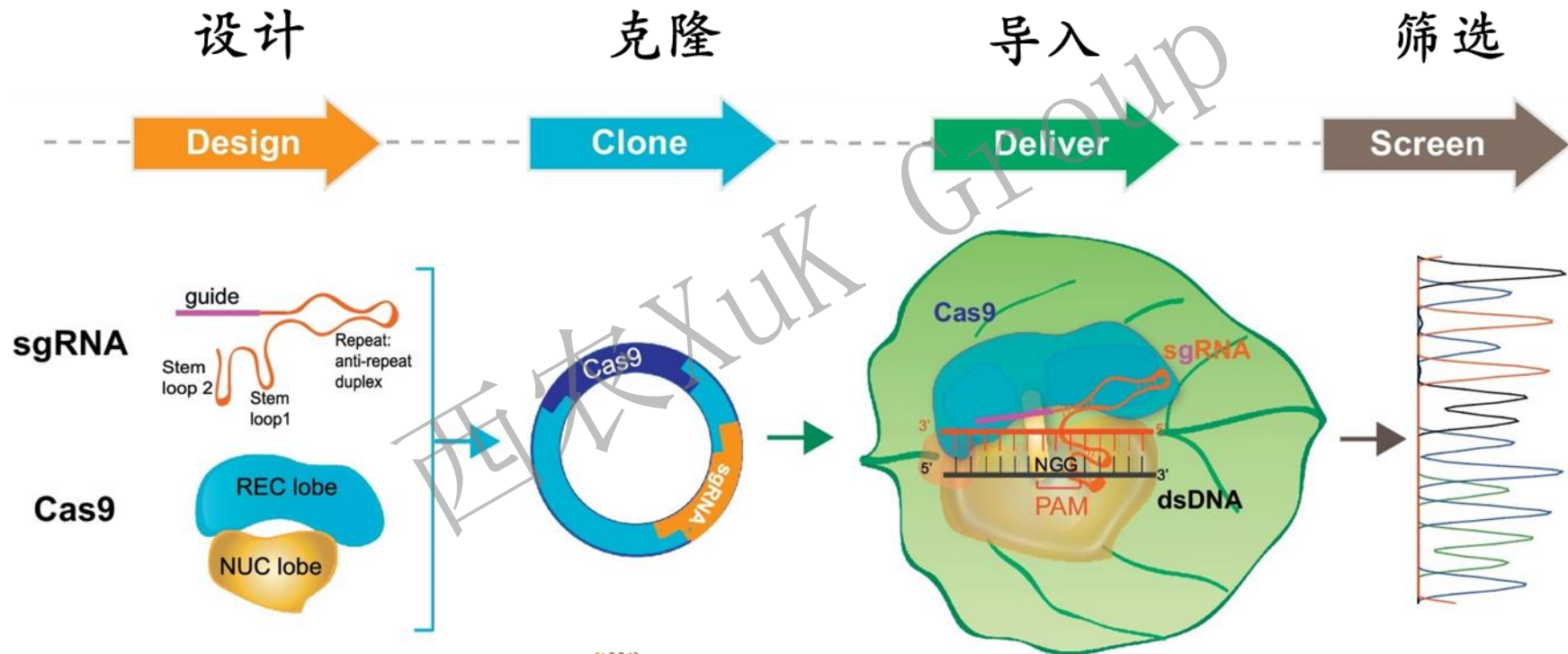
我们致力于异种器官生物医用组织等生物产品的研发



杨璐茵团队最新研究：有望用于临床异种器官移植的“猪3.0” ...

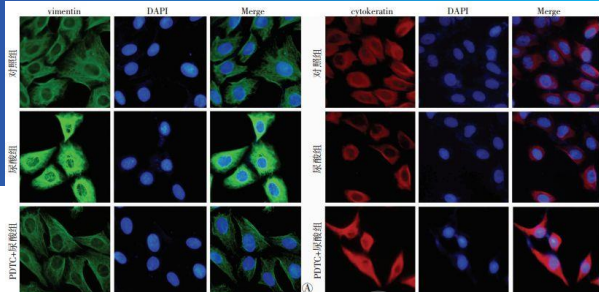
## 异种器官移植用猪

# 基因编辑的基本步骤





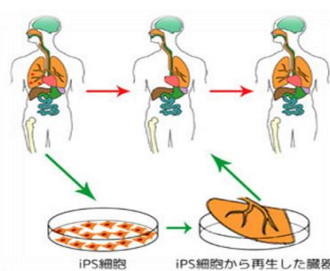
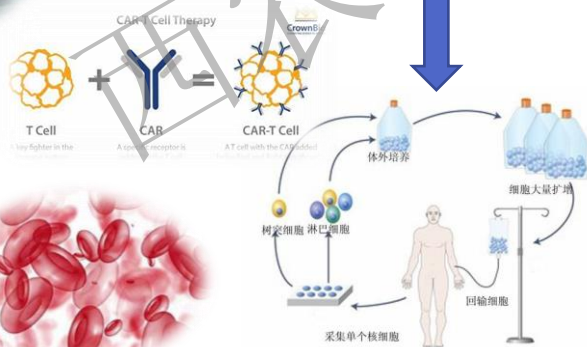
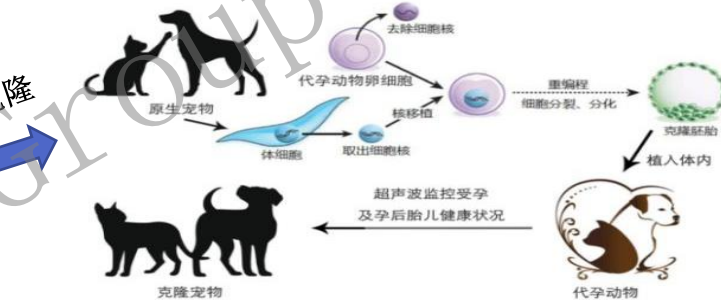
# 离体(体)细胞基因编辑



功能研究

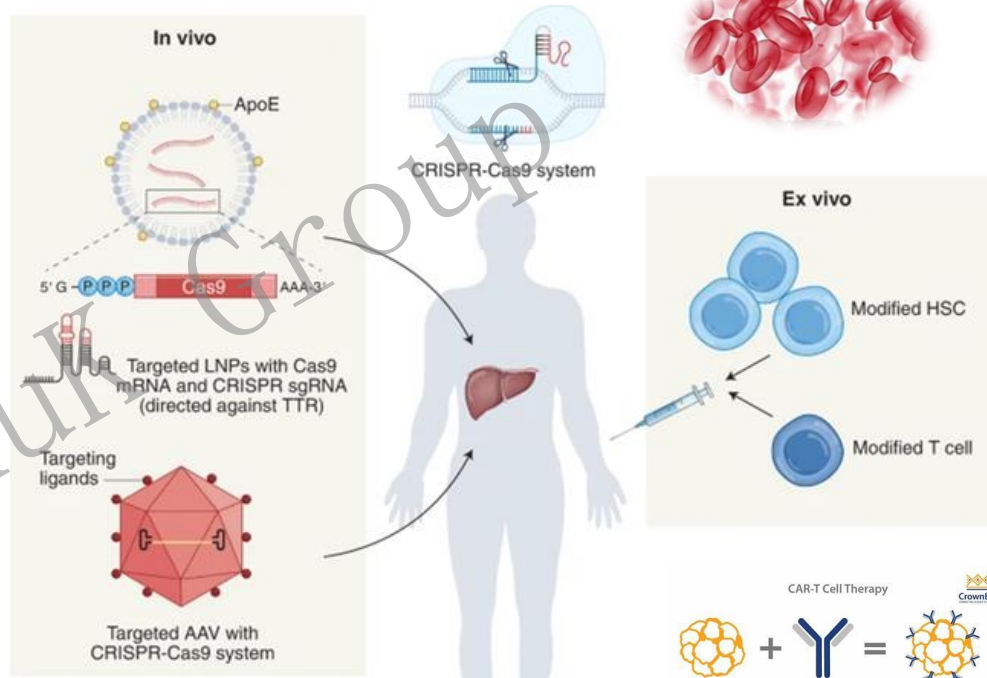
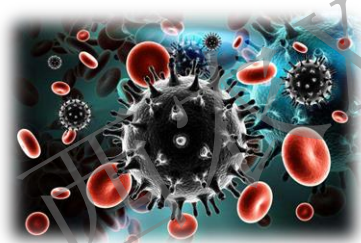
体细胞克隆

iPS编辑



体细胞治疗

# 活体体细胞编辑

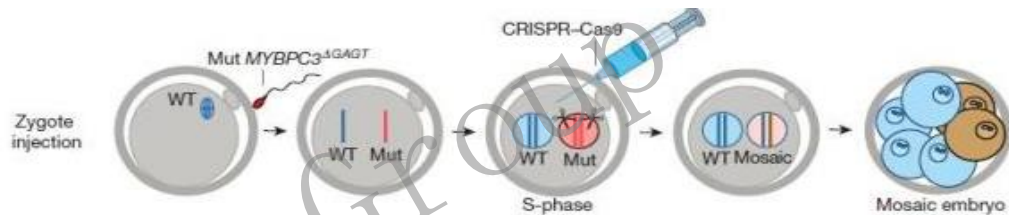
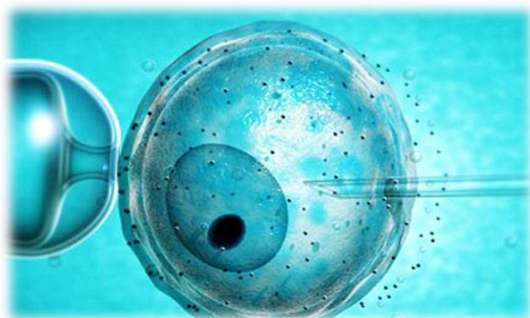


关键：基因编辑工具的活体精准靶向



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# 胚胎基因编辑



嵌合/杂合



西北农林科技大学



# 拓展思考

分子水平编辑? → 基因编辑动物?



基因敲除 (KO)

原核注射 → 基因编辑胚 → 胚胎移植



基因敲入 (KI)

体细胞编辑 → 核移植 → 胚胎移植

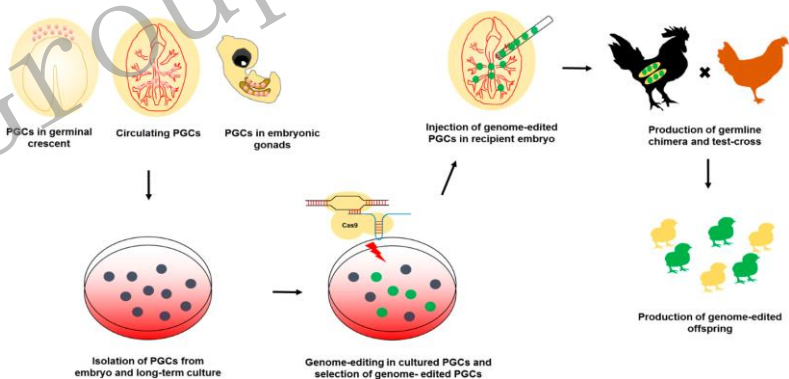
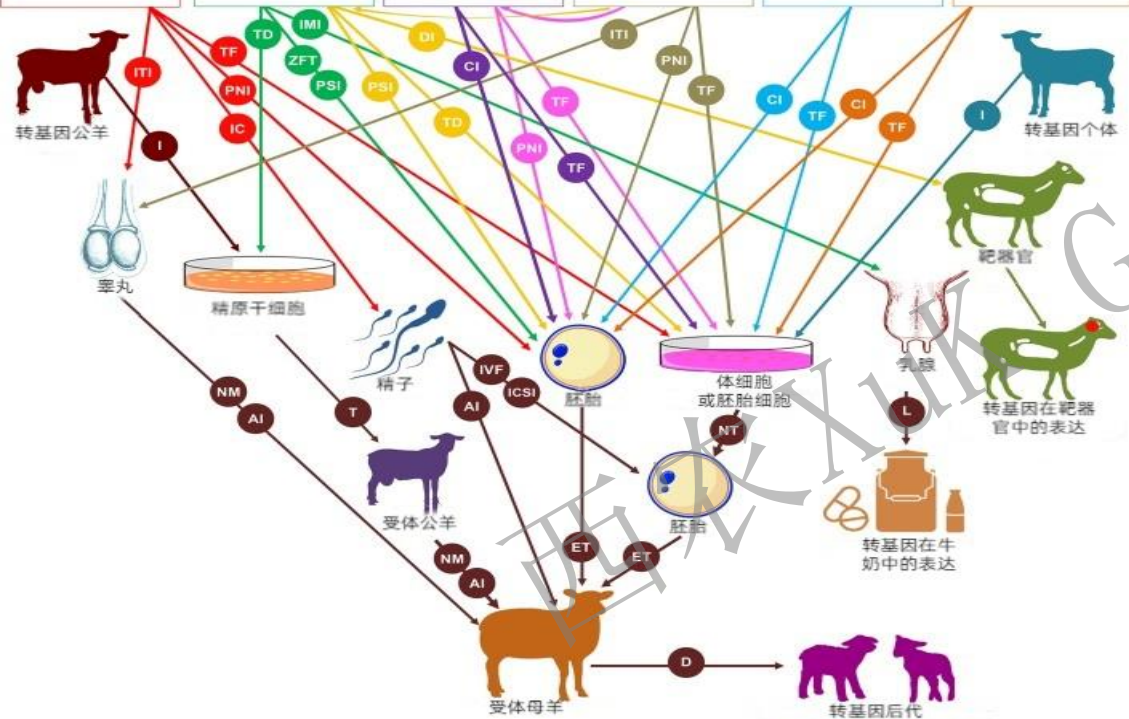


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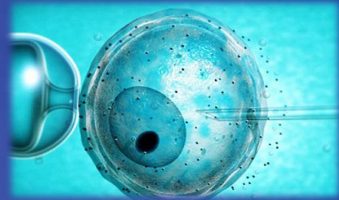
# 基因编辑技术与动物分子设计育种

方法	技术流程	适用范围	技术弊端
<p>受精卵显微注射 (难度低)</p>		<p>多种动物 基因敲除 基因敲入? 碱基编辑</p>	<p>嵌合体比例较高、 大片段整合效率低</p>
<p>体细胞克隆技术 (难度中等偏上)</p>		<p>多种动物; 基因敲除; 碱基编辑; 大片段整合等</p>	<p>体细胞克隆效率低</p>
<p>多能干细胞技术 (难度高)</p>		<p>主要为小鼠, 猪、牛进展较快,</p>	<p>大动物胚胎干细胞生殖系嵌合困难、 配子诱导分化技术不成熟</p>



PNI. 原核注射; NT. 核移植; CI. 细胞质注射; PSI. 卵黄周间隙注射; ZFT. 无带转导; TF. 转染; TD. 转导; IC. 孵育;  
 DI. 直接注射; IMI. 乳内注射; IVF. 体外受精; ICSI. 细胞质内精子注射; AI. 人工授精; NM. 自然交配; ET. 胚胎移植;  
 L. 哺乳; D. 分娩; I. 分离; T. 移植

# 基因编辑婴儿： 伦理之殇



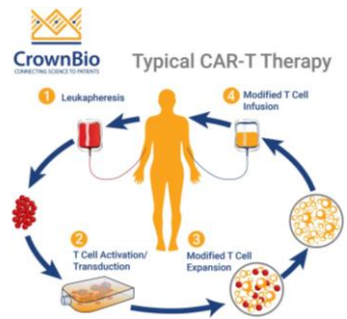
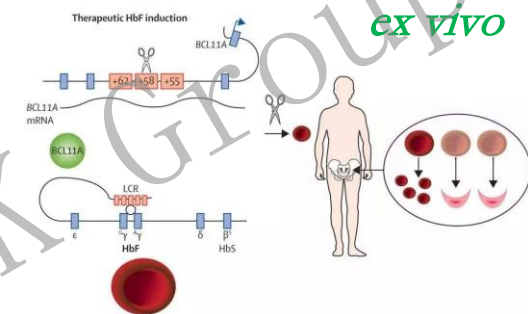


# 生殖细胞编辑：预防性改良

# 体细胞编辑：挽救性治疗



VS



*in vivo*



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