Application of new biotechnologies for improvements in swine nutrition and pork production efficiency

创新生物科技对猪营养和猪肉生产效率改进的评估

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The Need for Biotechnologies in Swine Production
养猪生产中对生物技术的需求

As the world’s population and living standards increase, demands for high-quality pork protein must also increase substantially in the face of reducing resources.
随着世界人口的不断增长和生活水平的提高，在现有资源不断减少的同时，人们对优质猪肉蛋白质的需求随之也会大幅度增加。

This requires not only improved diets, but also biotechnology-based breeding to generate swine with desired production traits for feed efficiency and rapid lean tissue growth.
这不仅需要改善日粮，还需要基于生物技术的育种手段，以培育出具有目标性状（饲料效率和瘦肉组织快速生长）的猪。

I. Basic Concepts of Biotechnologies
生物技术的基本概念

Chromosome and Gene in the Cell
细胞中的染色体和基因

Father
父本

Mother
母本

Gene基因
(a segment of DNA)
(DNA片段)

Gene基因
(DNA= deoxyribonucleic acid)
(DNA=脱氧核糖核酸)

A pair of chromosomes 一对染色体
(carriers of DNA molecules and associated proteins)
(DNA分子和相关蛋白的载体)

The domestic pig has 19 pairs of chromosomes. 家猪有19对染色体

Role of genes in growth, development, lactation, reproduction, and health
基因在猪的生长、发育、泌乳、繁殖和健康中发挥的作用

Paternal Chromosome 父系染色体 →  ← Maternal Chromosome 母系染色体

Genes (Segment of DNA) 基因（DNA片段）

↓ Transcription 转录

mRNA

Energy, AAs and minerals 能量、氨基酸和矿物质 → ↓ Translation 翻译

Protein 蛋白质

↓

Metabolism and Physiological Responses 代谢和生理反应

↓

Growth, Development, Lactation, Reproduction, Immunity, and Survival 生长、发育、泌乳、繁殖、免疫和存活

Environmental factors (e.g., nutrition, ambient temperature, toxins, immunological stimuli
环境因素
（如营养、环境温度、毒素、免疫刺激 ）

Biotechnologies: Techniques Used in Cell Biology
生物技术: 细胞生物学中使用的技术

Biotechnology can be classified as：生物技术可以归类为:

--- Cloning of animals with identical genetic composition
---克隆具有相同遗传组成的动物

Cloning helps to conserve species and breeds, particularly those with excellent biologic and economic traits.
克隆有助于保护物种和品种，特别是那些具有良好生物和经济特性的物种和品种。

--- Genetic engineering (via recombinant DNA technology and gene editing) to produce transgenic animals or microorganisms.
---基因工程（通过重组DNA技术和基因编辑）产生转基因动物或微生物。

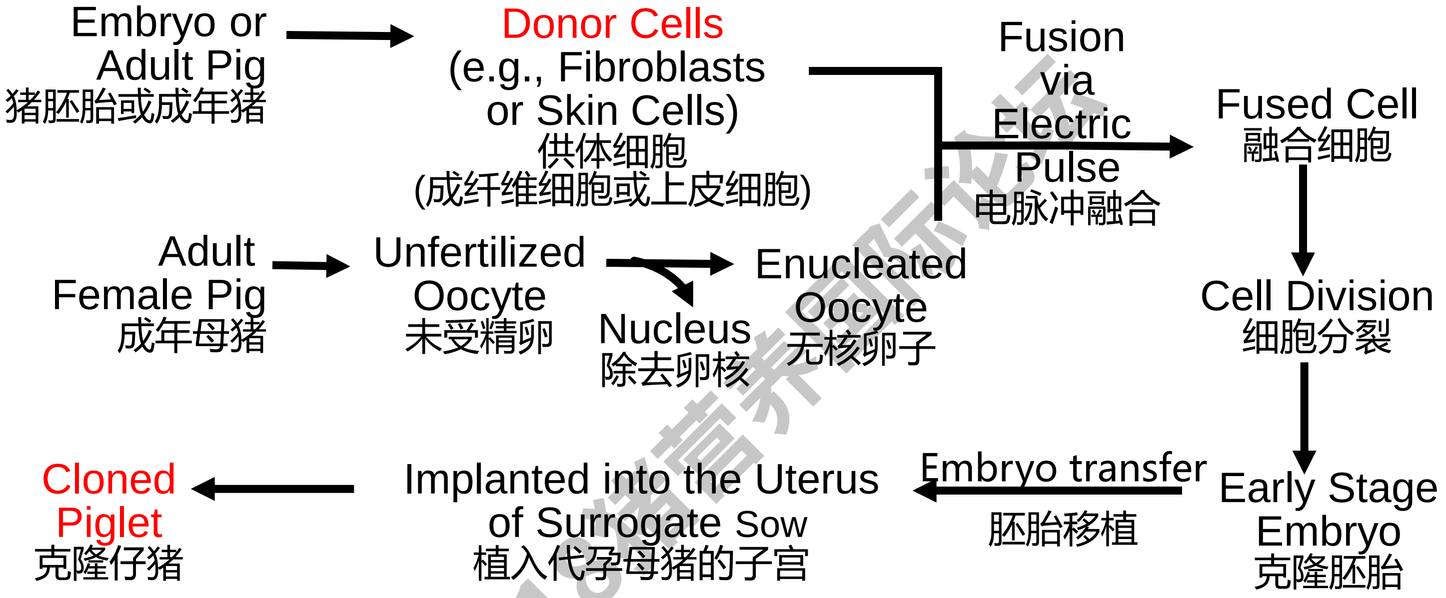
Recombinant DNA technology combines genetic materials from multiple sources into single cells to generate proteins, and is the basis for gene (genome) editing
重组DNA技术将来自多个来源的遗传物质结合到单个细胞中以产生蛋白质，且是基因（基因组）编辑的基础

DNA 1 + DNA 2 ------> New DNA

II. Cloning of Animals:
动物克隆:

Production of Genetically Identical Offspring
产生基因相同的后代

Cloning of Pigs from Embryonic or Adult Donor Cells
通过胚胎或成年供体细胞克隆猪



Embryonic cell nuclear transfer (ECNT): Donor cells (e.g., fibroblasts) are from early stage embryos,

ECNT 方法:供体细胞（例如成纤维细胞）来自早期胚胎

SCNT: Donor cells (e.g., skin cells) are from fetuses, young or mature animals. SCNT 方法:供体细胞（例如皮肤细胞）来自胎儿、幼龄或成年动物。

Advantages and Disadvantages of Animal Cloning
动物克隆的利弊

Advantages 优势:

Conserve breeds or species (particularly those that are endangered) 保护动物品种或物种（特别是濒危物种）

Allow castrated male animals to pass good genetic traits to offspring. 可以将阉割雄性动物的良好遗传特性传递给后代。

Disadvantages 劣势:

Technically difficult and costly procedure 操作难度大, 且成本高昂

Very low efficiency to produce offspring 繁殖后代的效率很低

Poor health and a low survival rate of offspring
健康状况不佳, 并且后代的存活率很低

Production of Cloned Livestock动物克隆的产生

Examples 实例:

1.After 276 attempts, Scottish animal scientists succeeded at producing a lamb called Dolly from the udder cells of a 6-year-old sheep in 1996. (Wilmut et al. 1997. *Nature* 385:810–813)

苏格兰动物科学家经过276次尝试后,在1996年成功地从6岁母羊乳房细胞培育出一只名叫多莉的羔羊 (Wilmut et al. 1997. *Nature* 385:810–813)

2. Onishi et al. (2000) transferred 110 cloned embryos to four surrogate sows, resulting in the birth of one apparently normal female piglet. *(Science 289:1188-1190)*

Onishi等人（2000年）将110个克隆胚胎转入到四头代孕母猪，培育出一个正常的雌性仔猪 *(Science 289:1188-1190)*

Note: Genetically identical animals may not be phenotypically identical, mainly because epigenetic factors and environmental factors influence gene expression in cells.

注意: 基因相同的动物表型不一定相同。这主要是因为表观遗传因素和环境因素也会影响细胞的基因表达。

Agricultural and Biomedical Implications of Animal Cloning
动物克隆的农业和生物医学意义

Animal agriculture: The main use of animal cloning is to maintain or increase genetic diversity in the population.
畜牧业: 克隆的主要用途是维持或增加种群的遗传多样性。

The United States FDA (2018) published an article stating that “meat and milk from clones of cattle, swine (pigs), and goats, and the offspring of clones from any species traditionally consumed as food, are as safe to eat as food from conventionally bred animals”.
2018年, 美国食品和药物管理局发表文章，表明“克隆牛、猪、羊所生产的肉和奶以及传统上用于食用的任何物种的克隆后代，与传统育种动物生产的动物产品一样都可以安全地食用” [FDA Veterinarian Newsletter XXIII(VI)]

Biomedicine: Pig cloning provide special organs for transplantation into human patients with certain diseases
生物医学: 猪克隆为某些疾病的人类患者提供特殊器官移植

(Prather RS et al. 2013. Annu. Rev Anim Biosci 1:203-19)

III. Recombinant DNA Technology:
重组DNA技术:

--- Formation of a new DNA molecule through laboratory methods from genetic materials from two or more sources

---通过实验室方法从两种或多种来源的遗传物质形成新的DNA分子

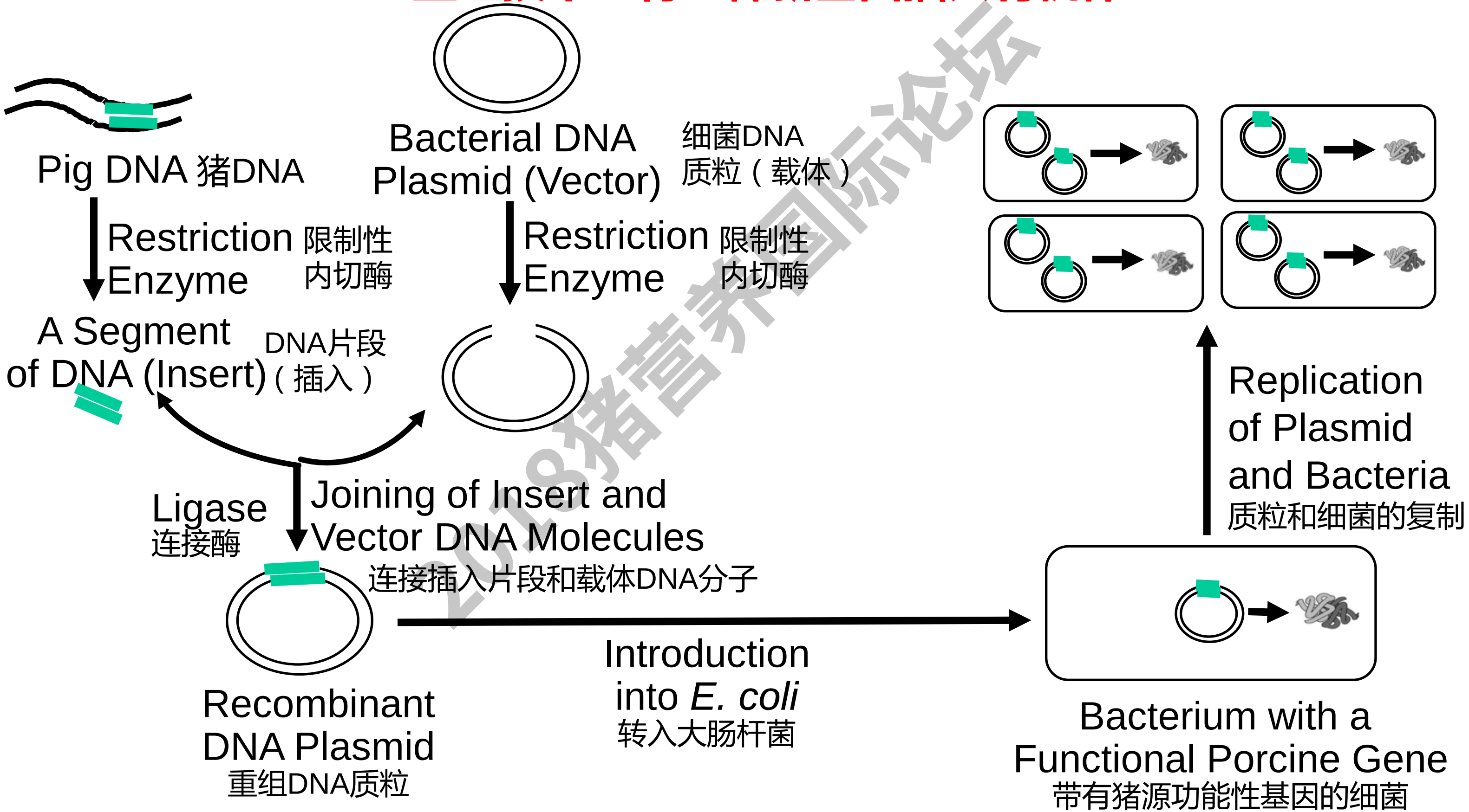
--- In both animal cells and bacteria

--- 在动物细胞和细菌中

--- The foundation of bacterial or animal transgenesis

--- 细菌或动物转基因的基础

Recombinant DNA Technology – Insert a New Gene Into an Organism
DNA重组技术—将一种新基因插入有机体



Recombinant DNA Technology: Bacteria
重组DNA技术: 细菌

Modify the bacterial genomes to 将细菌基因组修改为:

(1)Produce proteins (including interferon tau, hormones and feed enzymes), peptides, vaccines, feed-grade amino acids, fatty acids, and vitamins by bacteria, such *E. coli* . 通过细菌（如大肠杆菌）产生蛋白质（包括干扰素tau、激素和饲料酶）、肽类、疫苗、饲料级氨基酸、脂肪酸和维生素。

(2) Eliminate bacterial resistance to antibiotics by producing enzymes to remove the mediating molecules;通过产生酶去除介导分子来消除细菌对抗生素的抗性

(3) Manufacture vaccines 生产疫苗

Example: Prevent infection by African swine fever virus [viruses are neutralized by specific antibodies (proteins)]
例如: 防止感染非洲猪瘟病毒 [病毒被特异性抗体(蛋白质)中和]

(Brown and Bevins. 2018. Front Vet Sci 5:11)

Use of recombinant DNA technology in producing proteins, vaccines, amino acids and vitamins by bacteria
利用重组DNA技术通过细菌生产蛋白质、疫苗、氨基酸和维生素

Product 产品	**Function 功能**
Growth hormone 生长激素	Enhances lean tissue growth 促进瘦肉组织生长
Human insulin 人胰岛素	Regulates metabolism; treats diabetes 调节新陈代谢；治疗糖尿病
Vaccines 疫苗	Prevents bacterial and viral diseases 预防细菌和病毒性疾病
Antibodies 抗体	Controls viruses (e.g., **African swine fever virus**) 控制病毒（例如非洲猪瘟病毒）
Phytases 植酸酶	Hydrolyzes phytate in plants; increases the digestion of minerals and proteins in diets 水解植物中的植酸盐，促进日粮中矿物质和蛋白质的消化
Carbohydrases 糖化酶	Hydrolyzes carbohydrates in diets 水解日粮中的碳水化合物
Feed enzymes 饲用酶制剂	Hydrolyzes dietary carbohydrates and proteins in diets 水解日粮中碳水化合物和蛋白
Antimicrobials 抗菌剂	Kill pathogenic bacteria; enhances animal growth 杀灭致病菌；促进动物生长
Amino acids 氨基酸	Enhances animal growth and feed efficiency 促进动物生长和饲料效率
Vitamins 维生素	Enhances animal growth and feed efficiency 促进动物生长和饲料效率
Enzymes to ferment 酶制剂（发酵工艺）	Digests complex carbohydrates and proteins; produces small peptides and amino acids 消化复杂碳水化合物和蛋白质；产生小肽和氨基酸
Enzymes to degrade AMP 酶制剂（降低细菌耐药性）	Eliminate bacterial resistance to AMR mediators antibiotics消除细菌对AMR介质抗生素的耐药性

AMP = Antimicrobial resistance. AMP=抗菌素耐药性。

--- Advantages of Recombinant DNA Technology in Bacteria
--- 重组DNA技术在细菌中的优势

The costs are low, and yields are high. 成本低，产量高

Benefits are enormous for swine nutrition and production. 对猪营养和生产的好处是巨大的

For example, the availability of feed-grade amino acids can substantially reduce the content of protein in diet, thereby decreasing the excretion of nitrogen into the environment.
例如:饲料级氨基酸的供应可以显着降低日粮中蛋白质的含量，从而减少氮排放。

A reduction in dietary protein content by a 1% unit (e.g., from 16% to 15% crude protein) can decrease the excretion of total nitrogen (in urine plus feces) from growing pigs by 8.5%. 日粮蛋白质水平降低1%（例如，从16%至15%）可以使生长猪的总氮（尿液和粪便）排放总量减少8.5%。

--- Significance of Recombinant DNA Technology for Animals
--- 重组DNA技术对动物的意义

Production of transgenic animals as new breeds 生产转基因动物作为新品种

IV. Traditional Animal Transgenesis:
传统的动物转基因技术：

Production of Genetically Modified Offspring
生产转基因后代

Transgenic Animals 转基因动物

A transgenic animal is an animal that carries a foreign gene deliberately inserted into its genome.
转基因动物是一种携带外来基因的动物。

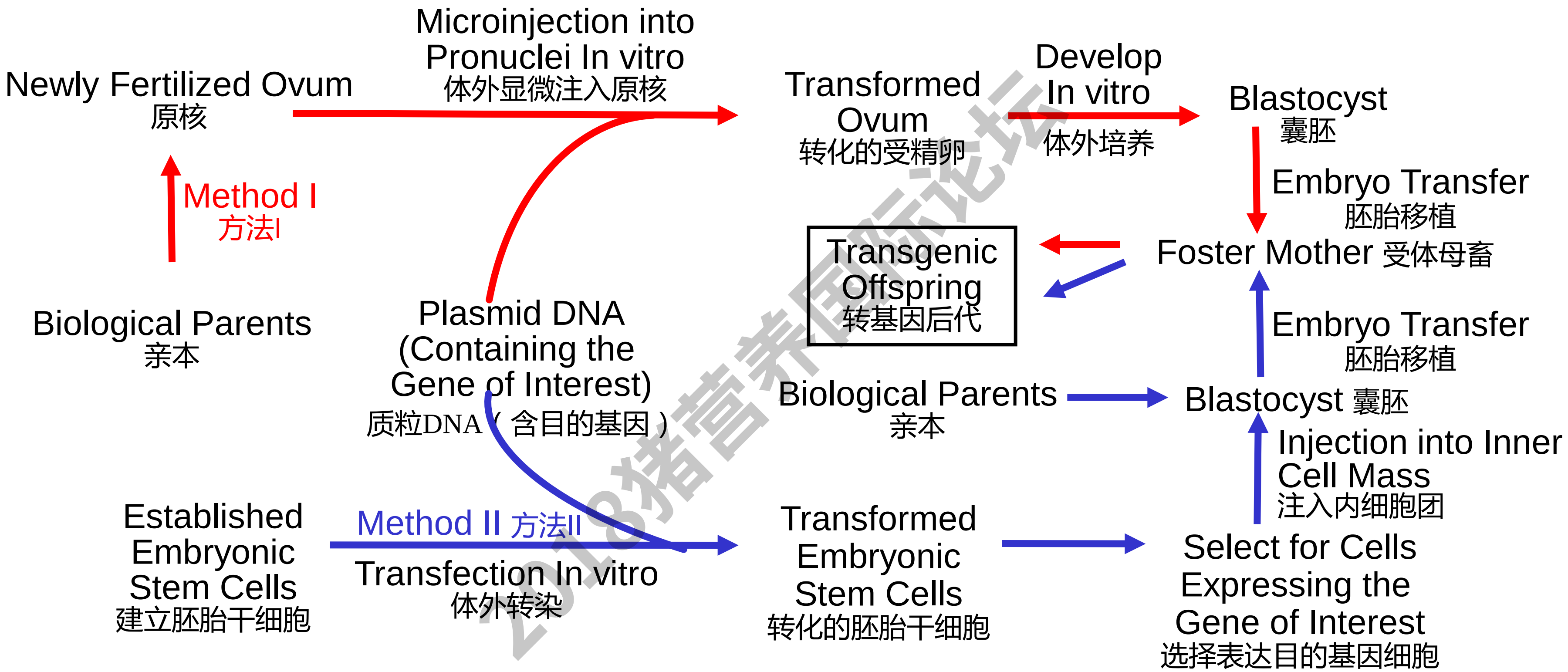
The foreign gene is constructed in vitro using the recombinant DNA technology.
使用重组DNA技术在体外构建外源基因。

Method: Germline transgenesis
方法: 种系转基因

Non-germline transgenesis
非种系转基因

Methods for the Production of Transgenic Animals

生产转基因动物的方法



Method I: Inject the recombinant DNA into the pronucleus of a fertilized ovum

方法 I: 将重组DNA注射到受精卵的原核中

Method II: Inject embryonic stem cells (containing the recombinant DNA) into a blastocyst

方法 II: 将含有重组DNA的胚胎干细胞注射到囊胚中

Advantages of Animal Transgenesis
转基因动物的优势

Animal agriculture 畜牧业:

Introduce a foreign gene into the germ line of an animal to establish a desirable trait and breed a new line of livestock.
将外源基因导入动物生殖系中以构建理想性状和培育新的牲畜品种

Complement the traditional breeding techniques to improve 补充传统育种技术以改善：

(1)the digestion, absorption and utilization of dietary nutrients;
膳食营养素的消化，吸收和利用；
(2)resistance to metabolic and infectious diseases; 对代谢和传染病的抵抗力;
(3)adaptation to the living environment. 适应生活环境的能力。

Biomedical application 生物医学应用:

Xenotransplantation of organs without the α-1,3-galactosyl-transferase gene that causes organ rejection response
没有导致器官排斥反应的α-1,3-半乳糖基转移酶基因的器官的异种移植

Production of transgenic pigs with important traits
生产具有重要生产性状的转基因猪

Gene 基因		Production trait 生产性状	
Bovine growth hormone (Tissues)	牛生长激素（组织）	Increases lean tissue growth and feed efficiency; reduces whole-body fat content and blood cholesterol concentration	增加瘦肉组织生长和饲料效率；减少全身脂肪含量和血液中胆固醇含量
Spinach Δ^{12} FAD (Adipose tissue)	菠菜 Δ^{12}脂肪酸去饱和酶（组织）	Desaturates oleic acid (18:1, ω9) at C12 to produce linoleic acid (18:2, ω6) in animals	在动物中，C12位去饱和油酸（18:1，ω9）产生亚油酸（18:2，ω6）
C. elegans FAD (Adipose tissue)	秀丽隐杆线虫脂肪酸去饱和酶（脂肪组织）	Desaturates linoleic acid (18:2, ω6) to produce ω3 polyunsaturated fatty acids in animals	在动物中，去饱和亚油酸（18:2，ω6）产生ω3多不饱和脂肪酸
Microbial phytase (Salivary gland)	微生物植酸酶（唾液腺）	Hydrolyzes phytate in plant-source ingredients; increases utilization of dietary phosphate	水解植物源成分中的植酸盐； 增加膳食中磷酸盐的利用率

FAD, fatty acid desaturase
FAD,脂肪酸去饱和酶

Disadvantages of Traditional Animal Transgenesis
传统动物转基因方法的弊端

--- Very low efficiency 效率很低
--- High rates of prenatal and pre-weaning mortalities 产前和断奶前的死亡率很高

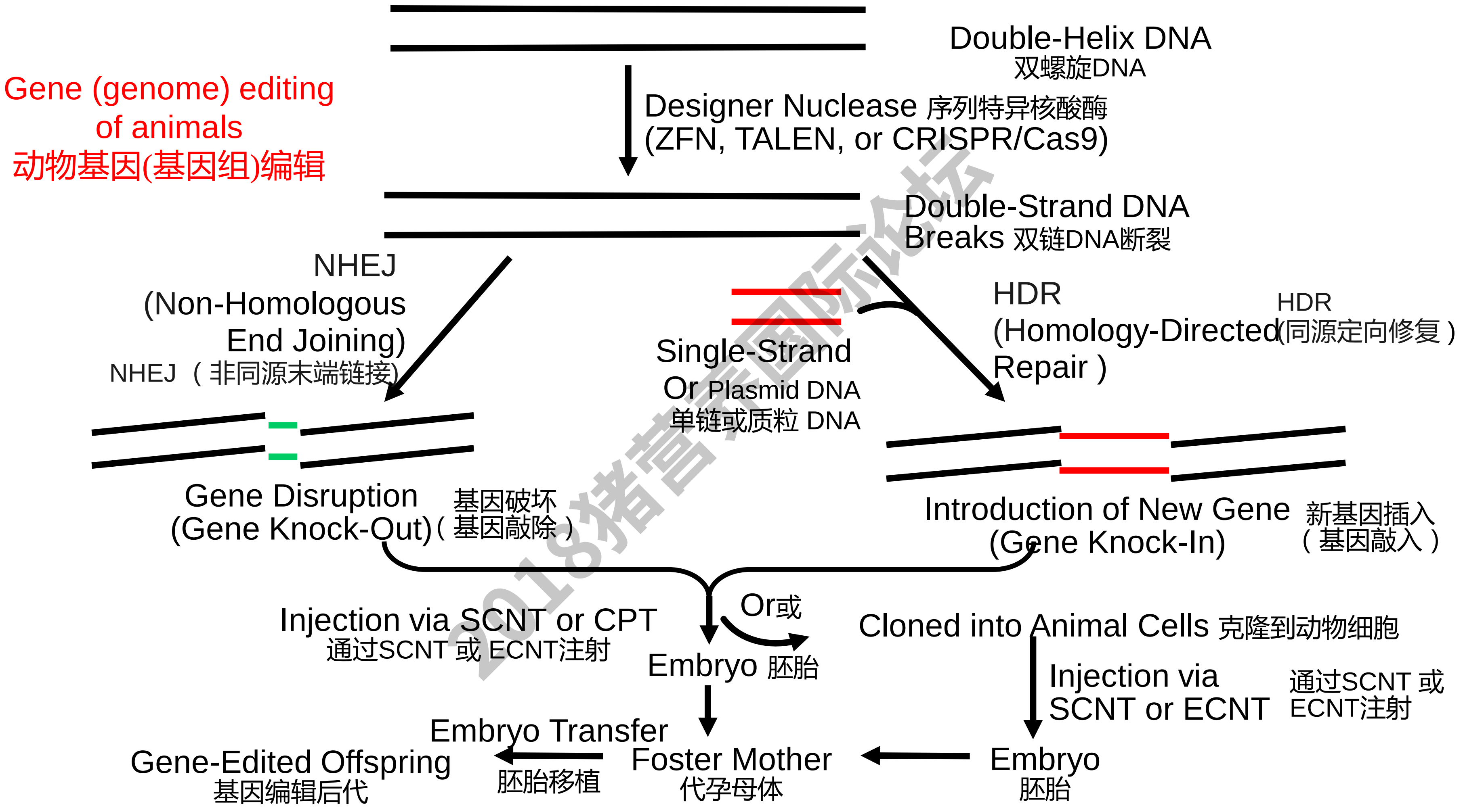
Example 实例:

Zhang et al. (2018) reported that after 4008 reconstructed embryos were transferred to 16 recipient sows, only 33 live piglets were born, with the efficiency of embryonic development to term being $< 1\%$.
Zhang(2018)等将4008个重建胚胎移植至16只受体母猪中，仅有33头活仔猪出生，胚胎发育效率不足1%。

Among the 33 live-born piglets, 25 of them were positive for a transgene, with 20 piglets having the intact transgene expression cassette. Disappointingly, only 9 transgenic pigs survived to weaning.
在33只活产仔猪中，25只转基因猪呈阳性，其中20只仔猪具有完整的基因表达盒。令人失望的是至断奶时仅有9只转基因猪存活

V. Gene (Genome) Editing in Animals
动物中基因（基因组）编辑

An improved new method for producing genetically modified animals
一种生产转基因动物的新方法


Gene (genome) editing
of animals
动物基因(基因组)编辑
Double-Helix DNA
双螺旋DNA
Designer Nuclease 序列特异核酸酶
(ZFN, TALEN, or CRISPR/Cas9)
Double-Strand DNA
Breaks 双链DNA断裂
NHEJ
(Non-Homologous
End Joining)
NHEJ（非同源末端链接)
Single-Strand
Or Plasmid DNA
单链或质粒 DNA
HDR
(Homology-Directed
Repair)
HDR
(同源定向修复）
Gene Disruption
(Gene Knock-Out)
基因破坏
（基因敲除）
Introduction of New Gene
(Gene Knock-In)
新基因插入
（基因敲入）
Injection via SCNT or CPT
通过SCNT 或 ECNT注射
Or或
Cloned into Animal Cells 克隆到动物细胞
Embryo 胚胎
Injection via
SCNT or ECNT
通过SCNT 或
ECNT注射
Embryo Transfer
胚胎移植
Gene-Edited Offspring
基因编辑后代
Foster Mother
代孕母体
Embryo
胚胎

CRISPR/Cas9: clustered regularly interspaced short palindromic repeats)-associated nuclease-9
CRISPR/Cas9 (成簇的、规律间隔的短回文重复序列相关的核酸酶-9)

Has rapidly gained momentum as the favored gene editor.
作为受欢迎的基因编辑器已迅速发展。

Originally found in 2007 in bacteria and archaea to defend themselves against invading viruses (bacteriophages).
最初于2007年在细菌和古细菌中发现，以防御入侵病毒（噬菌体）。

In response to a viral infection, the bacterial CRISPR/Cas9 is guided by a short RNA fragment known as a guide RNA to snip off a piece of viral DNA, creating a double-strand break in its target loci.
作为对病毒感染的反应，细菌CRISPR/Cas9是由一段短的RNA片段引导的，这段RNA片段被称为引导RNA，用来切断病毒的DNA片段，在其目标位点形成双链断裂。

Milestones for Gene Editing in Swine
生产基因编辑猪的里程碑

Gene 基因	Gene Editor基因编辑器	Year 年份
PPARγ	ZFN	2011
LDLR	TALEN	2012
RELA	ZFN	2013
RELA	TALEN	2013
APC	TALEN	2013
Myostatin	TALEN	2016
vWF	CRISPR/Cas9	2014
CD163	CRISPR/Cas9	2014
OTR	CRISPR/Cas9	2016
PERVs	CRISPR/Cas9	2017
UCP1	CRISPR/Cas9	2017
PRRSV-1	CRISPR/Cas9	2018

Advantages and Disadvantages of Gene Editing
基因（基因组）编辑的利弊

Advantages 优势:

--- Provides a more precise, more specific, more predictable and more rapid solution to producing new breeds of pigs.
---为生产新品种的猪提供更精确，更具体，更可预测和更快速的解决方案

--- Requires fewer steps and has a higher efficiency than the previous methods of animal transgenesis.
---与先前的动物转基因方法相比，需要的步骤更少且具有更高的效率。

Disadvantages 劣势:

--- Challenging and expensive technique
---挑战和昂贵的技术

--- Suboptimal efficiency in producing transgenic pigs
--- 生产转基因猪的效率欠佳

Production of transgenic pigs using CRISPR/Cas9
使用 CRISPR/Cas9 技术生产转基因猪

Gene 基因		Production trait 生产性状	
PERVs (Tissues)	猪内源性逆转录病毒 (组织)	Remove all porcine endogenous retroviruses from pigs to produce organs for transplantation into humans	去除猪内所有内源性逆转录病毒,产生猪器官移植到人类
Myostatin (Skeletal muscle)	肌肉生长抑制素（骨骼肌）	Increases the number of skeletal muscle fiber, skeletal muscle mass, protein deposition, and gain:feed ratio (feed efficiency)	增加骨骼肌纤维数量、骨骼肌质量、蛋白质沉积和增益：饲料的比率（饲料效率）
Uncoupling protein 1 (Tissues)	解偶联蛋白-1（组织）	Increases nonshivering thermogenesis and piglet survival; decreases the accretion of white adipose tissue; increases carcass lean tissue content	增加非颤抖性产热和仔猪存活率；减少白色脂肪组织的沉积；增加胴体瘦肉组织含量
CD163 (Tissues)	CD163基因（组织）	Resistant to porcine reproductive and respiratory syndrome virus (PRRSV, "blue ear disease")	抗猪繁殖与呼吸综合症病毒（PRRSV，“蓝耳病”）

PERVs = porcine endogenous retroviruses
PERVs =猪内源性逆转录病毒

VI. Gene (Genome) Editing in Bacteria
细菌中基因（基因组）编辑

Understanding Antibiotic Resistance in Animals and Humans
了解动物和人类的抗生素耐药性

Use of Antibiotics in Medicine and Animal Production
抗生素在医学和动物生产中的应用

Since the discovery of penicillin in 1928, antibiotics have been used to treat bacterial infections in humans and animals.
自从1928年发现青霉素以来，抗生素已被用于治疗人类和动物的细菌感染。

Since the 1950s, sub-therapeutic levels of antibiotics have been included in conventional diets to improve the growth performance of swine and poultry.
19世纪60年代以来，人们开始在传统日粮内添加亚治疗剂量的抗生素用以提高猪和禽类的生产性能。

However, due to the development and spread of the resistance of bacteria to antibiotics, feed antibiotics have been banned in many countries (e.g., the European union) and are being phased out in some major swine-producing nations (e.g., the U.S. and China).
然而，由于耐药菌的产生和传播，许多国家已经禁止饲用抗生素的使用（如：欧盟各国）；并且一些主要的猪肉输出大国，如美国和中国等国家已经在逐步淘汰饲用抗生素。

Resistance of Bacteria to Antibiotics In Animals and Humans
细菌对动物用和人用抗生素的耐药性

Some bacteria are resistant to one class of antibiotics, and the others are resistant to multiple antibiotics, therefore posing a serious global health concern. 一些耐药菌仅耐受一类抗生素，但有些耐药菌可耐受多种抗生素，因此导致了严重的全球健康问题

For ensuring the optimal efficacy of antibiotics in treating bacterial infections in animals and humans, there is increasing concern worldwide over *antimicrobial resistance* (AMR). 为了确保抗生素在治疗人类和动物的细菌感染上发挥最好效果，全世界对于抗生素耐药性（antimicrobial resistance, AMR）的担忧日益加剧

AMR can be defined as the ability of bacteria to resist the effects of an *antimicrobial* (e.g., antibiotics). 抗生素耐药性被定义为细菌能耐受抗菌药物（如：抗生素）的能力

Mechanisms Responsible for the Development of Antimicrobial Resistance in bacteria
细菌耐药性发展的机制

Bacteria 细菌

↓ Natural Acquisition 自然习得

New Antibiotic-Resistant Genes 新抗生素耐药基因

↓ Gene Expression 基因表达

Enzymes (e.g., Extended-Spectrum β-Lactamase) 酶（如：超广谱β-内酰胺酶）

Bacteria 细菌 →

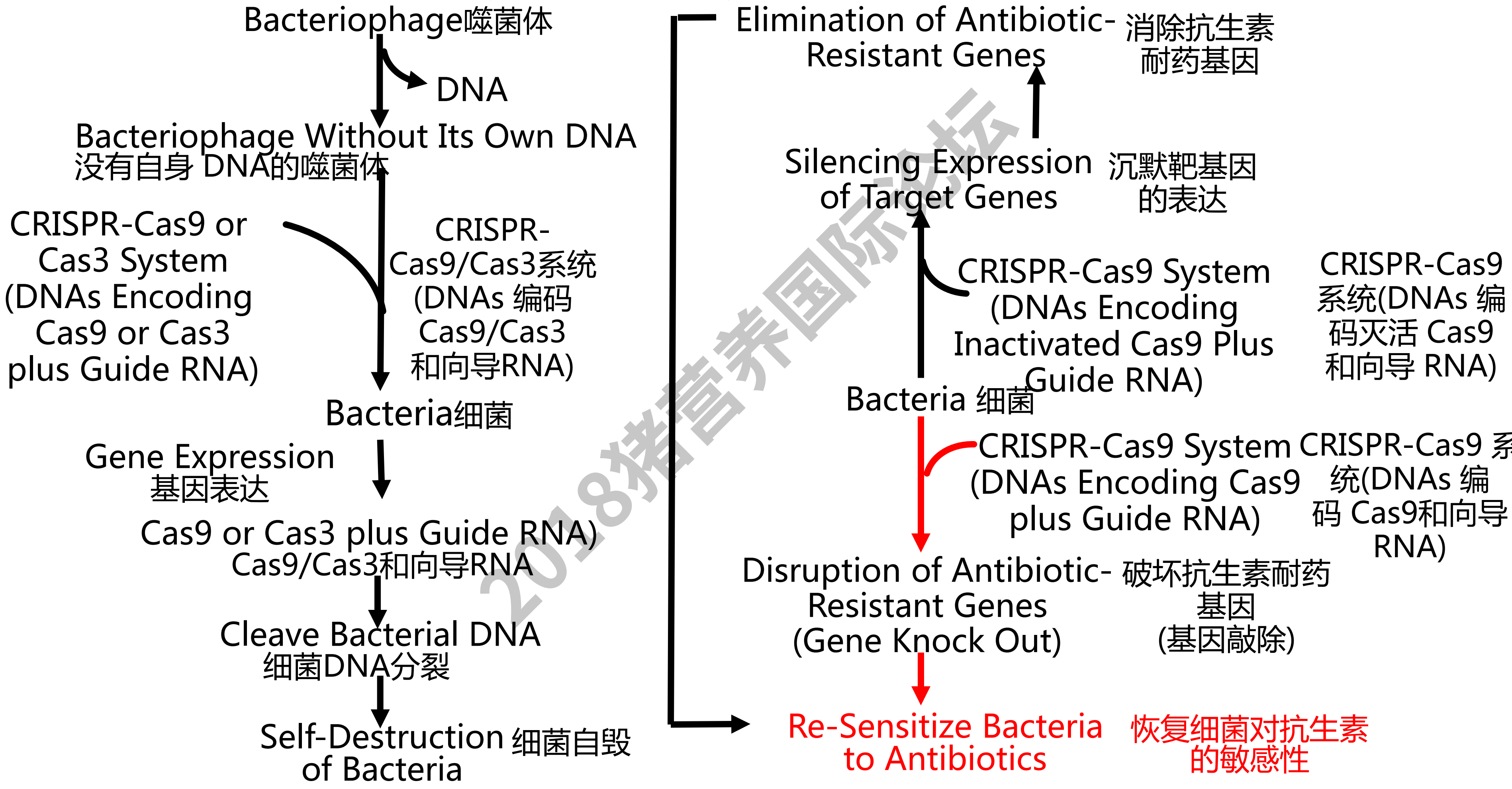
Other Organisms 其他生物体 →

Antibiotics (e.g., Penicillin) 抗生素（如：青霉素） → Inactiive Degradation Products (e.g., β-Ring being Open) 非活性降解产物（如：β环打开）

Bacteria 细菌

→ Resistance of Bacteria to Antibiotics 细菌对抗生素产生耐药性

Utilization of the CRISPR System As a New Alternative to Antibiotics
利用CRISPR系统作为替代抗生素的新选择



Utilization of the CRISPR-Cas9 system as new alternatives to antibiotics
利用CRISPR系统作为抗生素替代的新选择

System 系统	Vector 载体	Antibiotic-Resistant Bacteria 抗生素耐药菌
CRISPR-Cas9	Bacteriophages 噬菌体	*Clostridium difficile* 梭菌
CRISPR-Cas3	Bacteriophages 噬菌体	Gram-positive and negative bacteria 革兰氏阳性菌和阴性菌
CRISPR-Cas9	Plasmids 质粒	*Escherichia coli* 大肠杆菌
CRISPR-Cas9	CRISPRi	Staphylococci aureus plasmids and other Gram-positive bacteria 金黄色葡萄球菌质粒和其他革兰氏阳性菌

CRISPRi, CRISPR interference method
CRISPRi，CRISPR干扰方法

The CRISPR-Cas9 technologies, which involve bacteriophages or plasmids, hold promise for:
包含噬菌体或质粒在内的CRISPR-Cas9技术:

--- killing bacteria; and 杀灭细菌; 和

--- removing enzymes from bacteria, including antimicrobial-resistant bacteria, in the gastrointestinal tract of animals.清除动物胃肠道内的细菌(包括抗生素耐药细菌)

Conclusion 结论

To meet demands for high-quality meat protein and reduce carbon footprints and waste excretion, there has been revolutionary progress in animal biotechnology over the past 35 years to produce:

为满足优质肉蛋白的需求,同时减少碳排放和废物排泄,在过去的35年里，动物生物技术取得了革命性的进步, 生产了:

--- recombinant protein (including enzymes);
--- 重组蛋白质（包括酶);
--- organic nutrients (including amino acids and vitamins);
--- 有机营养物质（包括氨基酸和维生素);
--- clones of swine;
--- 克隆猪;
--- transgenic pigs for both agriculture and biomedicine;
--- 用于农业和生物医学的转基因猪;
--- transgenic bacteria that are not resistant to antibiotics
--- 不耐抗生素的转基因细菌

Conclusion 结论

In recent years, gene (genome) editing technologies based on ZFN, TALEN and CRISPR/Cas9 as editors have become available to delete, insert, or modify the genome of animals and bacteria at the specific sites of DNA sequences.

近年来，基于锌指核酸酶（ZFN）、TALEN和CRISPR/Cas9的基因（基因组）编辑技术已经可以用于在DNA序列的特定位点上删除、插入，或修饰动物和细菌的基因组

The biotechnology holds promise in conserving the diverse breeds of swine, augmenting feed efficiency and pork production, and developing alternatives to antibiotics.

生物技术在保护猪的品种多样性、提高饲料效率和猪肉产量，以及在未来开发抗生素替代品方面均具有广阔前景

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