Swine that harbor heritable genetic modifications; induced or intended.

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NSRRC
National Swine Resource & Research Center

CGE
Somatic Cell Genome Editing

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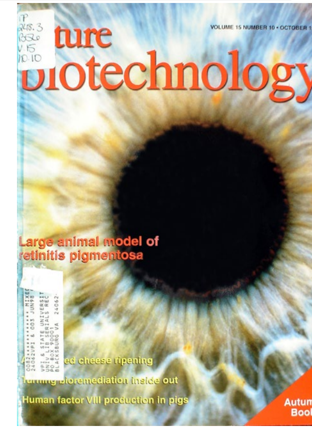
NSTC
National Swine Testing Center

CCGI
Collaborative Center for Genome Innovation

Speaker background:

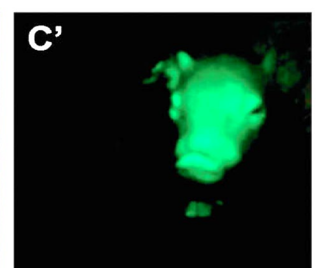


NCSU- Developed the first genetically engineered biomedical pig – retinitis pigmentosa.

USDA- Produced the first genetically engineered livestock to address a US Agricultural Problem - Mastitis

PPL Therapeutics/Revivicor- Gal-Safe pig and first three knockouts.

University of Missouri- many biomedical models, first ZFN & first CRISPR edited pigs.



Homologous Recombination:

Phelps CJ, Koike C, Vaught TD, Boone J, Wells KD, Chen SH, Ball S, Specht SM, Polejaeva IA, Monahan JA, Jobst PM, Sharma SB, Lamborn AE, Garst AS, Moore M, Demetris AJ, Rudert WA, Bottino R, Bertera S, Trucco M, Starzl TE, Dai Y, Ayares DL. Production of alpha 1,3-galactosyltransferase-deficient pigs. Science. 2003 Jan 17;299(5605):411-4. doi: 10.1126/science.1078942. Epub 2002 Dec 19. PubMed PMID: 12493821; PubMed Central PMCID: PMC3154759.

Mendicino M, Ramsoondar J, Phelps C, Vaught T, Ball S, LeRoith T, Monahan J, Chen S, Dandro A, Boone J, Jobst P, Vance A, Wertz N, Bergman Z, Sun XZ, Polejaeva I, Butler J, Dai Y, Ayares D, Wells K. Generation of antibody- and B cell-deficient pigs by targeted disruption of the J-region gene segment of the heavy chain locus. Transgenic Res. 2011 Jun;20(3):625-41. doi: 10.1007/s11248-010-9444-z.

Ramsoondar J, Mendicino M, Phelps C, Vaught T, Ball S, Monahan J, Chen S, Dandro A, Boone J, Jobst P, Vance A, Wertz N, Polejaeva I, Butler J, Dai Y, Ayares D, Wells K. Targeted disruption of the porcine immunoglobulin kappa light chain locus. Transgenic Res. 2011 Jun;20(3):643-53. doi: 10.1007/s11248-010-9445-y. Epub 2010 Sep 26. PubMed PMID: 20872247.

Lorson MA, Spate LD, Samuel MS, Murphy CN, Lorson CL, Prather RS, Wells KD. Disruption of the Survival Motor Neuron (SMN) gene in pigs using ssDNA. Transgenic Res. 2011 Dec;20(6):1293-304. doi: 10.1007/s11248-011-9496-8. Epub 2011 Feb 25. PubMed PMID: 21350916; PubMed Central PMCID: PMC4455718.

Wells KD, Bardot R, Whitworth KM, Trible BR, Fang Y, Mileham A, Kerrigan MA, Samuel MS, Prather RS, Rowland RRR. Replacement of Porcine CD163 Scavenger Receptor Cysteine-Rich Domain 5 with a CD163-Like Homolog Confers Resistance of Pigs to Genotype 1 but Not Genotype 2 Porcine Reproductive and Respiratory Syndrome Virus. J Virol. 2017 Jan 15;91(2). doi: 10.1128/JVI.01521-16.

Gene Editors and Viral Resistance:

Whitworth KM, Rowland RR, Ewen CL, Trible BR, Kerrigan MA, Cino-Ozuna AG, Samuel MS, Lightner JE, McLaren DG, Mileham AJ, Wells KD, Prather RS. Gene-edited pigs are protected from **porcine reproductive and respiratory syndrome virus**. Nat Biotechnol. 2016 Jan;34(1):20-2. doi: 10.1038/nbt.3434. Epub 2015 Dec 7. PubMed PMID: 26641533. **(PRRSV)**

Whitworth KM, Rowland RRR, Petrovan V, Sheahan M, Cino-Ozuna AG, Fang Y, Hesse R, Mileham A, Samuel MS, Wells KD, Prather RS. Resistance to **coronavirus infection** in amino peptidase N-deficient pigs. Transgenic Res. 2019 Feb;28(1):21-32. doi: 10.1007/s11248-018-0100-3. Epub 2018 Oct 12. PubMed PMID: 30315482; PubMed Central PMCID: PMC6353812. **(TGE)**

Stoian A, Rowland RRR, Petrovan V, Sheahan M, Samuel MS, Whitworth KM, Wells KD, Zhang J, Beaton B, Cigan M, Prather RS. The use of cells from ANPEP knockout pigs to evaluate the role of aminopeptidase N (APN) as a receptor for porcine deltacoronavirus **(PDCoV).** Virology. 2020 Feb;541:136-140. doi: 10.1016/j.virol.2019.12.007. Epub 2019 Dec 24. PMID: 32056711

Chen PR, Rowland RRR, Stoian AM, Petrovan V, Sheahan M, Ganta C, Cino-Ozuna G, Kim DY, Dunleavey JM, Whitworth KM, Samuel MS, Spate LD, Cecil RF, Benne JA, Yan X, Fang Y, Croix BS, Lechtenberg K, Wells KD, Prather RS. Disruption of anthrax toxin receptor 1 in pigs leads to a rare disease phenotype and protection from **senecavirus A** infection. Sci Rep. 2022 Mar 23;12(1):5009. doi: 10.1038/s41598-022-09123-x. PMID: 35322150

Kwon T, Artiaga BL, McDowell CD, Whitworth KM, Wells KD, Prather RS, Delhon G, Cigan M, White SN, Retallick J, Gaudreault NN, Morozov I, Richt JA. Gene editing of pigs to control **influenza A virus** infections. bioRxiv. 2024 Jan 16:2024.01.15.575771. doi: 10.1101/2024.01.15.575771. Preprint. PMID: 38293027

Important Parts First.

Problem:
NIH accepted responsibility for providing and continually updating guidelines for the use of recombinant DNA technologies. Asylomar conference, 1972.

NIH Focus:
Biomedical Research

Result:
1) Propagation of myths about the hazards of rDNA and 2) updates have been in context of laboratory use.

Need:
Agricultural principles need to be applied to rDNA technologies in authoritative guidance documents.

Correlate:
USDA needs to participate in the maintenance of guidelines for rDNA in food!

Important Parts First.

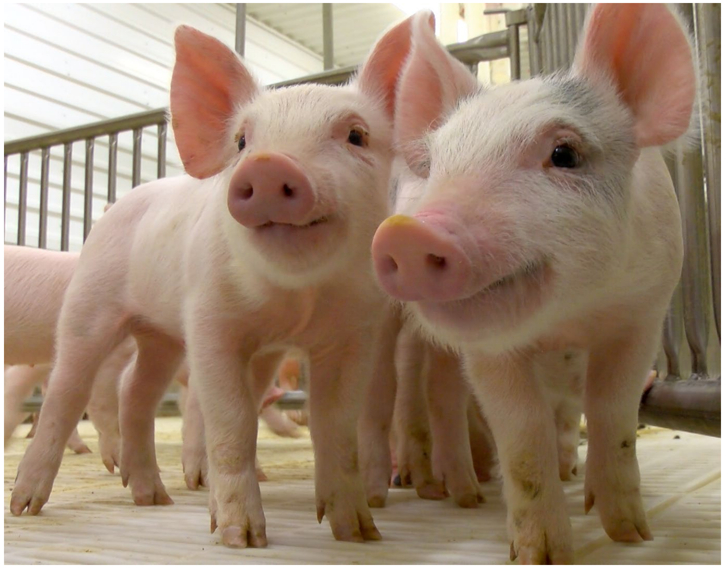
<u>Congressionally mandated question for this committee:</u>

What is the biological basis of a food safety concern for genetic variation- intentional, induced, or natural?

In other words,

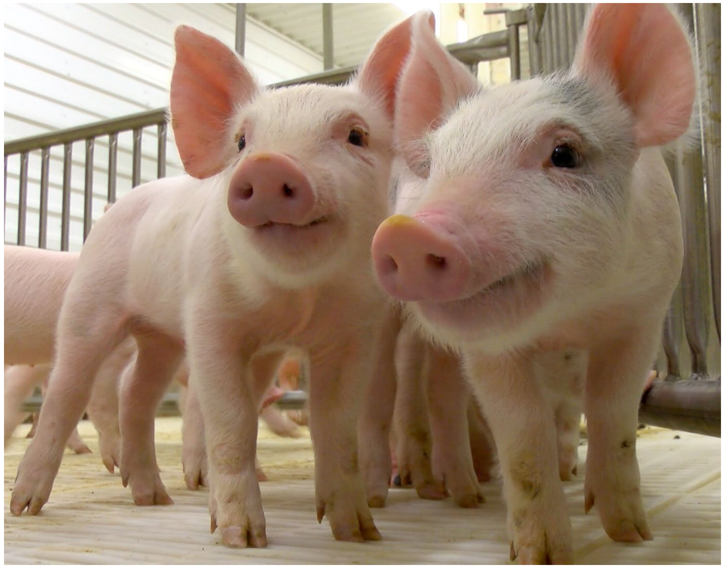
"What does science suggest is hazardous about heritable genetic information?"

What's coming?



What's coming?

If this committee focuses on standard regulatory concerns (unintended modifications, animal welfare, durability, technological strategy, etc.), the future of heritable genetic changes in swine will be limited to trivial edits, small deletions, and introgressions of naturally occurring variants.



What's coming?

Hopefully, everything!

Disease Resistance/ Resilience

Wells et al.,
Influenza- In addition to four knockout strategies (tmprss2 and 3 others), we have two new transgene-based strategies.
African Swine Fever Virus (ASFV)- Transgene strategy.

Others-
Classic Swine Fever Virus (CSFV) - Generation of pRSAD2 gene knock-in pig via CRISPR/Cas9 technology. Xie Z, Jiao H, Xiao H, Jiang Y, Liu Z, Qi C, Zhao D, Jiao S, Yu T, Tang X, Pang D, Ouyang H. Antiviral Res. 2020 Feb;174:104696. doi: 10.1016/j.antiviral.2019.104696. Epub 2019 Dec 17. PMID: 31862502 **(cisgenic)**

Diarrhea- (*Hsa* Lysozyme) -Lysozyme improves gut performance and protects against enterotoxigenic Escherichia coli infection in neonatal piglets. Huang G, Li X, Lu D, Liu S, Suo X, Li Q, Li N. Vet Res. 2018 Feb 20;49(1):20. doi: 10.1186/s13567-018-0511-4. PMID: 29463305 **(transgenic)**

Likely: **ANP32** novel variants, engineered variants, and knockouts.
CRISPR-mediated targeting of viruses (vDNA and vRNA). **[Off-target perceptions]**

Digestion – Wells et al.

- Abandoned cellulase (for now).

- Focused on polysaccharides that are NOT currently obvious targets for ethanol production.

Digestion - others

Phytase-Pigs expressing salivary phytase produce low-phosphorus manure. Golovan SP, Meidinger RG, Ajakaiye A, Cottrill M, Wiederkehr MZ, Barney DJ, Plante C, Pollard JW, Fan MZ, Hayes MA, Laursen J, Hjorth JP, Hacker RR, Phillips JP, Forsberg CW. Nat Biotechnol. 2001 Aug;19(8):741-5. doi: 10.1038/90788.

β-glucanase-Improvement of anti-nutritional effect resulting from β-glucanase specific expression in the parotid gland of transgenic pigs. Guan LZ, Cai JS, Zhao S, Sun YP, Wang JL, Jiang Y, Shu G, Jiang QY, Wu ZF, Xi QY, Zhang YL. Transgenic Res. 2017 Feb;26(1):1-11. doi: 10.1007/s11248-016-9984-y. Epub 2016 Dec 19. PMID: 27995503

Xylanase- Novel transgenic pigs with enhanced growth and reduced environmental impact. Zhang X, Li Z, Yang H, Liu D, Cai G, Li G, Mo J, Wang D, Zhong C, Wang H, Sun Y, Shi J, Zheng E, Meng F, Zhang M, He X, Zhou R, Zhang J, Huang M, Zhang R, Li N, Fan M, Yang J, Wu Z. Elife. 2018 May 22;7:e34286. doi: 10.7554/eLife.34286. PMID: 29784082

Pectinase- [Heterogenous expression and enzymatic property analysis of microbial-derived pectinases in pig PK15 cells].Mo JX, Wang HQ, Huang GY, Cai GY, Wu ZF, Zhang XW. Yi Chuan. 2019 Aug 20;41(8):736-745. doi: 10.16288/j.yczz.19-087. PMID: 31447424

Mycotoxins-The aflatoxin-detoxifizyme specific expression in the parotid gland of transgenic pigs. Lou AG, Cai JS, Zhang XM, Cui CD, Piao YS, Guan LZ. Transgenic Res. 2017 Oct;26(5):677-687. doi: 10.1007/s11248-017-0036-z. Epub 2017 Jul 26. PMID: 28748301

Intermediary Metabolism

<u>Wells et al.,</u>

Biosynthetic pathways for essential nutrients (mostly amino acids). We have developed a transgene strategy for simultaneous, coordinated expression of independent coding regions. This strategy can express multiple enzymes in the same cell, at the same time, at predetermined levels (tethered transcription).

Alternative metabolic pathways for more efficient utilization of excess nutrients (reduced carbon footprint/utilization of distiller's grains).

Intermediary Metabolism

<u>Others</u>

Piglet Thermoregulation- Reconstitution of UCP1 using CRISPR/Cas9 in the white adipose tissue of pigs decreases fat deposition and improves thermogenic capacity. Zheng Q, Lin J, Huang J, Zhang H, Zhang R, Zhang X, Cao C, Hambly C, Qin G, Yao J, Song R, Jia Q, Wang X, Li Y, Zhang N, Piao Z, Ye R, Speakman JR, Wang H, Zhou Q, Wang Y, Jin W, Zhao J. Proc Natl Acad Sci U S A. 2017 Nov 7;114(45):E9474-E9482. doi: 10.1073/pnas.1707853114. Epub 2017 Oct 23. PMID: 29078316

<u>Fatty acid profile-</u>

Linoleic Acid increase- Functional expression of a Delta12 fatty acid desaturase gene from spinach in transgenic pigs. Saeki K, Matsumoto K, Kinoshita M, Suzuki I, Tasaka Y, Kano K, Taguchi Y, Mikami K, Hirabayashi M, Kashiwazaki N, Hosoi Y, Murata N, Iritani A. Proc Natl Acad Sci U S A. 2004 Apr 27;101(17):6361-6. doi: 10.1073/pnas.0308111101. Epub 2004 Apr 5. PMID: 15067141

Omega 3 increase- Generation of cloned transgenic pigs rich in omega-3 fatty acids. Lai L, Kang JX, Li R, Wang J, Witt WT, Yong HY, Hao Y, Wax DM, Murphy CN, Rieke A, Samuel M, Linville ML, Korte SW, Evans RW, Starzl TE, Prather RS, Dai Y. Nat Biotechnol. 2006 Apr;24(4):435-6. doi: 10.1038/nbt1198. Epub 2006 Mar 26. PMID: 16565727

Mitotic Gene Drives

Creation of homozygosity as a dominant trait.

<u>Allelic conversion at fertilization- </u>produces novel, unevaluated genotypes every generation (cannot meet current durability standard imposed by FDA).

<u>Tissue specifically- </u>germline stable (may fit current "durability" standard better than above).

Mutator lines – no clear regulatory path

1) Produce animals with alterations in the rate of meiotic recombination resulting in reduced or increase genetic linkage.

2) Allele-specific or genome-wide increase in meiotic error. Rapid increase in random variation.

3) Unstable alleles- mostly novelty foods [variegated colors (Indian corn) or flavors (Asian Pear)].

Mutated lines – no clear regulatory path

Use of targeted, saturated mutagenesis and *in vitro* phenotyping to create novel alleles.

Alleles resulting from radiation and chemical mutagenesis are NOT regulated. Alleles resulting from editor-mediated mutagenesis are regulated.

What is the biological basis of this irrational inconsistency?

Other active projects

All genetically controlled phenotypes!

Nutrition:
Feed digestibility
Production of essentially nutrients
Mycotoxin degradation

Reproduction:
Litter size
Piglet thermoregulation

Lactation:
Milk production
Teat number
Antimicrobial milk
Sow stress

Growth:
Lean muscle accretion
Adipose deposition
Mature size
Growth rate

Meat quality
Tenderness
Flavor
Color

Disease resistance
Viral resistance
Bacterial resilience

Reminder

The only limits to these technologies are Imagination, Money, and Perceptions.

There are NO technical limits to genetic engineering in swine.