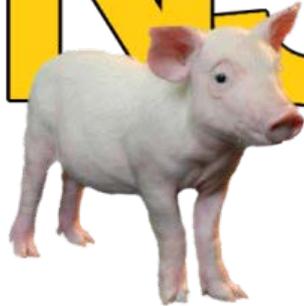


"The NSRRC and Genetic Engineering of Swine"

NSRRC



*National Swine Resource
& Research Center*

Randall S. Prather

Curators' Professor

University of Missouri-Columbia

Animal Reproductive Biology Group

Food for the 21st Century
University of Missouri - Columbia



College of
Agriculture
Food and
Natural
Resources



Animal Sciences

University of Missouri-Columbia

Swine are being used extensively as biomedical models of human health (Comparative Medicine)

- **Xenotransplantation, Organ Transplantation**
- **Ophthalmology**
- **Cystic Fibrosis**
- **Cardiovascular disease/Atherosclerosis**
- **Diabetes**
- **Muscular Dystrophy, Spinal Muscular Atrophy**
- **Pharmaceutical Production**
- **Cancer**
- **Cutaneous pharmacology/Wound repair/Dermatology**
- **Toxicology**
- **Lipoprotein metabolism**

Things that Count in a Biomedical Model

(1 of 2):

- **Availability Counts**
- **Phenotype Counts**
 - Mice don't always have the same phenotype as humans (Cystic Fibrosis)
- **Physiology Counts**
 - There are metabolic and hemodynamic differences between rodents and humans (Cardiovascular Disease)
 - Pig mammary gland can properly post-translationally modify the Vitamin K-dependent proteins of hemostasis (Pharmaceutical Production)

Things that Count in a Biomedical Model (2 of 2):

- **Size Counts**
 - Difficult to translate therapeutics (Spinal Muscular Atrophy)
 - Difficult to take blood flow measurements on rodents (Cardiovascular Studies)
 - Difficult to acquire enough tissue for proteomics from rodents
 - Organ size of pigs is similar to humans (Xenotransplantation)
 - Rodents are difficult on which to practice techniques (Osteogenesis Imperfecta)
- **Genome Counts**
 - Ability to genetically alter the genome
 - Availability of inbred strains
 - Sequenced genome
 - Genome similarity to humans
 - Phylogenetically the pig is 3X closer to the human than the mouse to human
 - Synteny of pig is closer to human than the mouse is to human
- **Acceptability as a Preclinical Model Counts**



**At the
University of Missouri
a genetic resource for the biomedical
community**

OD011140, ORIP, NIAID, NHLBI

NSRRC Function/Operation

- **Importation/Rederivation/Distribution/Repository**
- **Health Monitoring**
- **New Model Creation**
 - PI-driven
 - Steering Committee (bi-monthly)
- **Advisory Board**
- **Advertisement**
- **Workshops/Training**

Challenges/Opportunities

- **Challenges**
 - Gestation length (4 months)
 - Puberty (7-9 months)
 - Few inbred strains
- **Opportunities**
 - Large animal with physiologically relevant phenotype

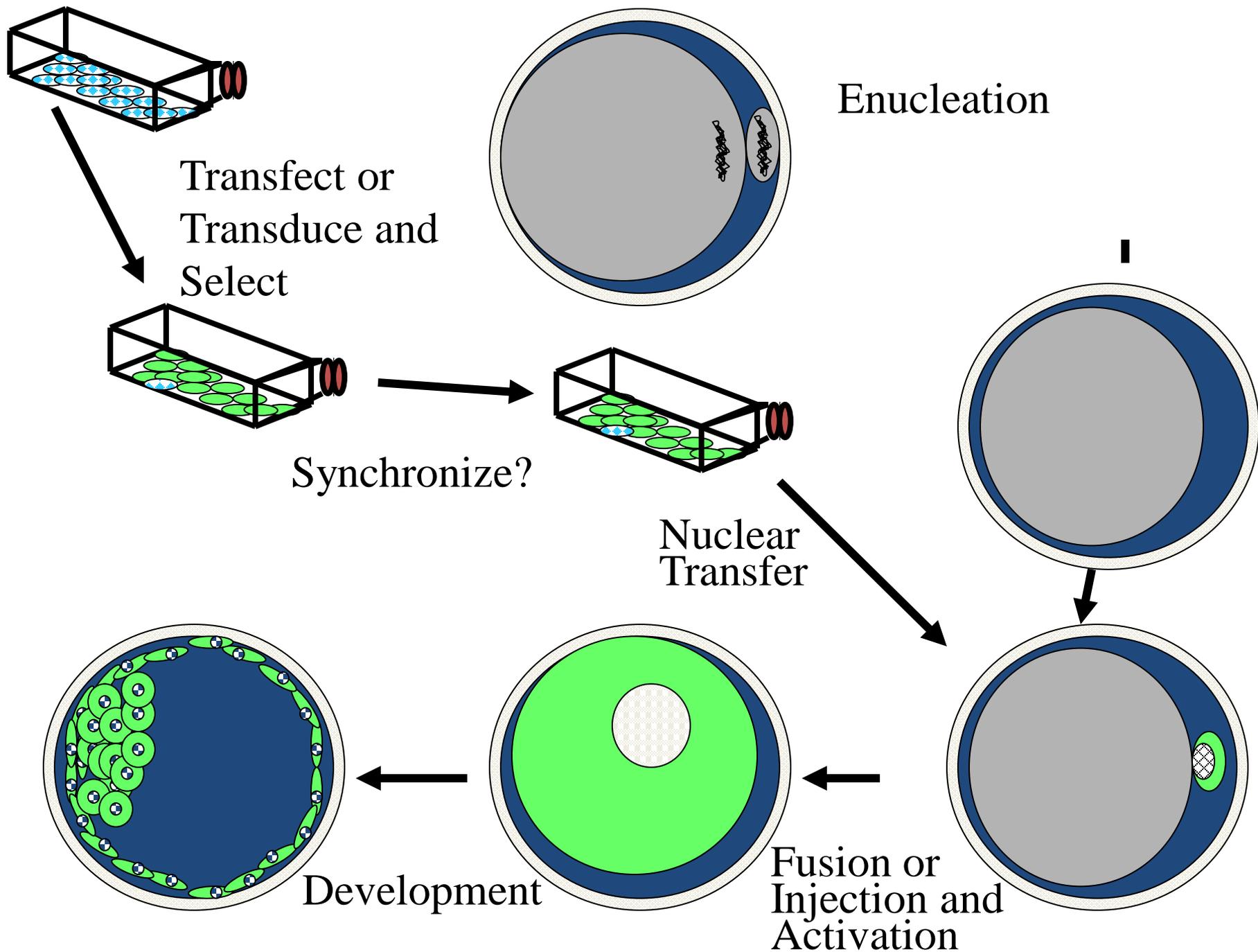
Swine Models

- **Some naturally occurring models are useful:**
 - **Ossabaw Island Pigs- Type II diabetes**
 - **Apo B- Hypercholesterolemic**
 - **Etc.**
- **Additional genetic modification can create models that are otherwise unavailable.**

Methods to Make Genetically Modified Pigs

- Pronuclear Injection.
- Sperm-Mediated Transfection.
- Oocyte Transduction.
- **Genetic Modification followed by Nuclear Transfer (Somatic Cell Nuclear Transfer (SCNT)).**
- Future-
 - Germ Cell Transplantation?
 - Stem Cell Technology (Chimeras or SCNT)?

**No Control
Over Insert**



Swine Gene Modifications @ MU:

- Xenotransplantation

ORGAN TRANSPLANTATION

To solve a deficiency of human organs for transplantation, scientists want to use organs from other species. MU researchers are working to prevent rejection of the donated organ by the body.

NORMAL

1 Normal **DNA** contains instructions for sugar molecules on the surface of each cell in the transplanted organ.

2 Human **anti-bodies** attach to the **sugar molecules**.

3 The anti-bodies kill cells in the organ causing hyper-acute rejection.

MANIPULATED

1 Researchers **alter the DNA** to disrupt the creation of sugar molecules on the surface of the cells.

2 Without the sugar molecules anti-bodies cannot connect to the cell.

3 So the organ is not rejected.

Swine Gene Modifications @ MU:

- Xenotransplantation
 - Hyperacute Rejection
 - [α1-3 Galactosyltransferase \(GGTA1\) KO](#): (Lai et al '02 Science)- Immerge Biotherapeutics US Patent # 7,547,816



Swine Gene Modifications @ MU:

- **Xenotransplantation**
 - Hyperacute Rejection
 - [GGTA1 KO](#): (Lai et al '02 Science)
 - [Cytidine Monophospho-N-Acetylneuraminic Acid Hydroxylase \(CMAH\) KO](#): (Lee et al unpublished)- Konkuk University, Korea

Born April 4, '12



Swine Gene Modifications @ MU:

- Xenotransplantation
 - Hyperacute Rejection
 - GGTA1 KO: (Lai et al '02 Science)
 - CMAH KO: (Lee et al unpublished)
 - hDAF on the GGTA1 KO background (Lai et al unpublished)

e.g. DAFney : Born July '04. hDAF and GGTA1 KO. Base genetics from Imutran Ltd.



Swine Gene Modifications @ MU:

- Xenotransplantation
 - Hyperacute Rejection
 - GGTA1 KO: (Lai et al '02 Science)
 - CMAH KO: (Lee et al unpublished)
 - hDAF on the GGTA1 KO background (Lai et al unpublished)
 - hDAF into GGTA1^{+/-}: (Beaton et al '11)



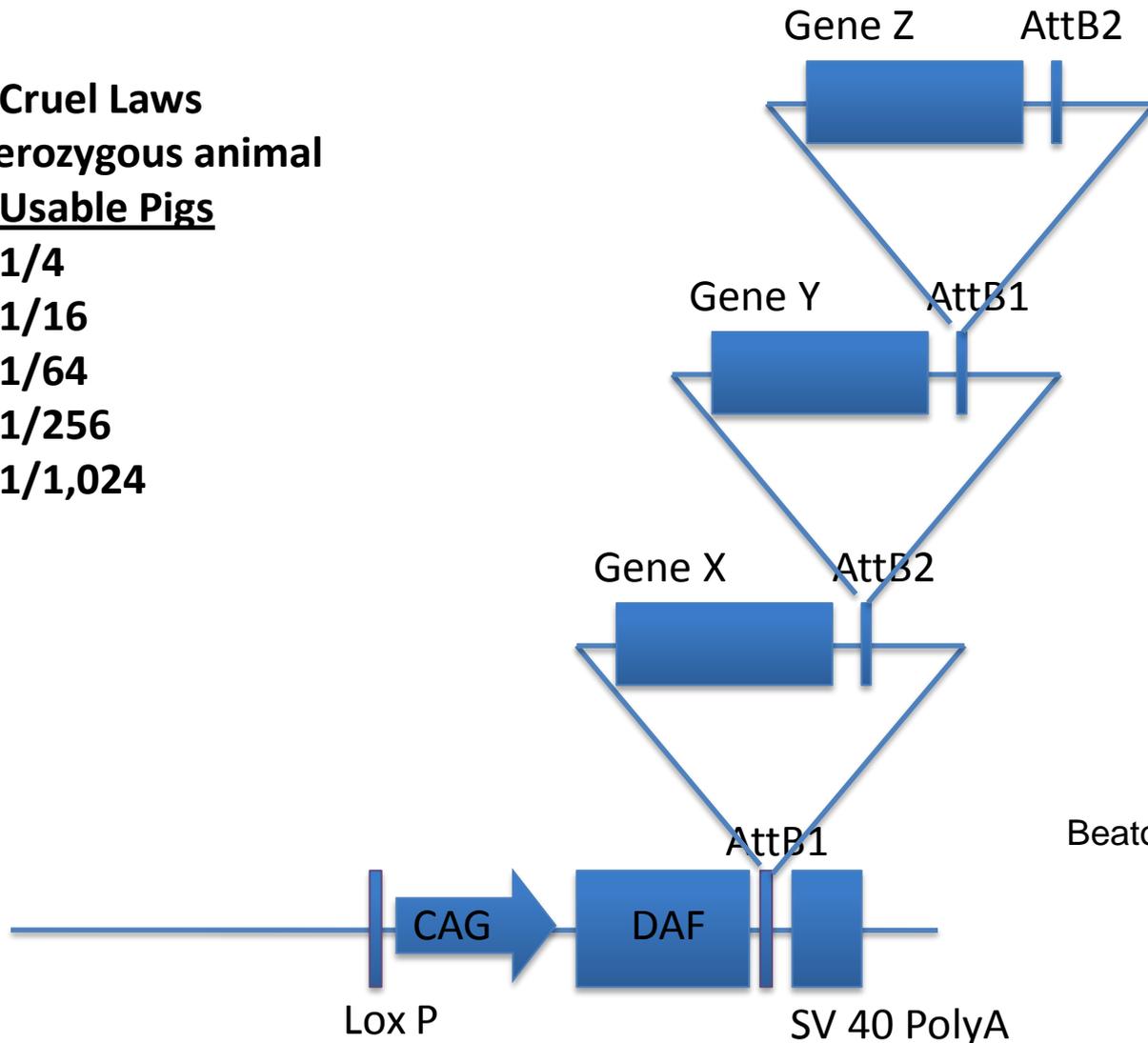
Born December 13, '10



Alternate use of only 2 phage integrase systems allows for unending insertion into the same locus

Mendel's Cruel Laws
Mate Heterozygous animal

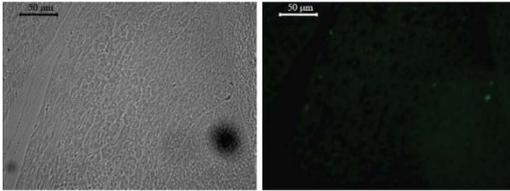
<u>Genes</u>	<u>Usable Pigs</u>
1	1/4
2	1/16
3	1/64
4	1/256
5	1/1,024



Beaton et al, unpublished

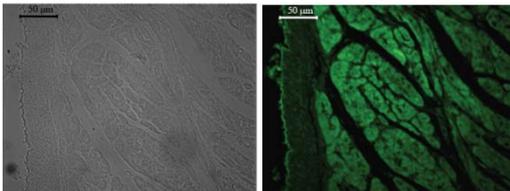
hCD55 Expression in heart, liver, lung, kidney and pancreas in control and hDAF into GGTA1.

Wildtype Animal 15-6

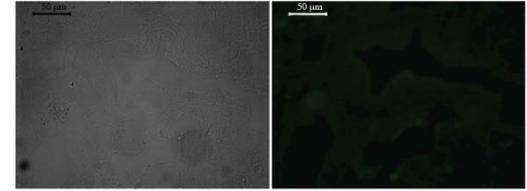


Heart

Transgenic Animal 177-4 (+/- GGTA-1; 1 copy hCD55)

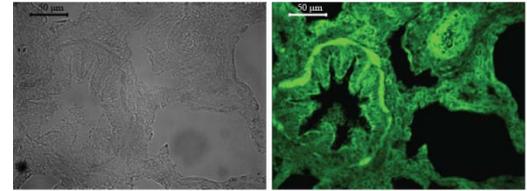


Wildtype Animal 15-6

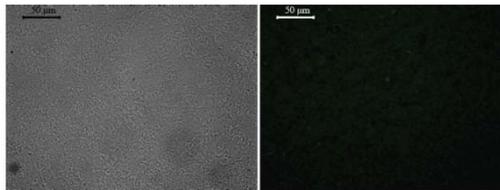


Lung

Transgenic Animal 177-4 (+/- GGTA-1; 1 copy hCD55)

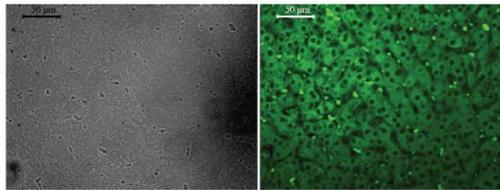


Wildtype Animal 15-6

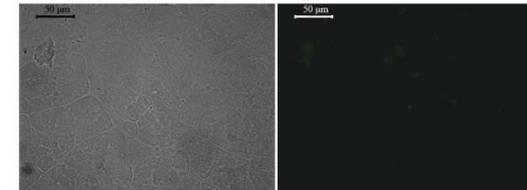


Liver

Transgenic Animal 177-3 (+/- GGTA-1; 1 copy hCD55)

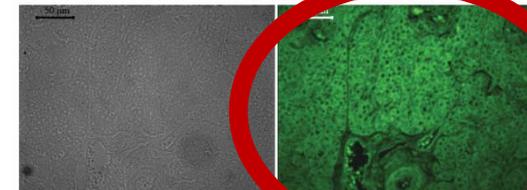


Wildtype Animal 15-6

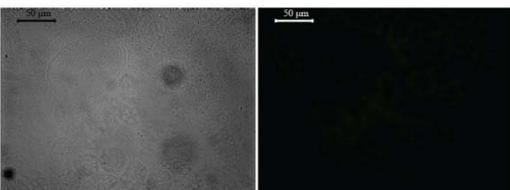


Pancreas

Transgenic Animal 177-4 (+/- GGTA-1; 1 copy hCD55)

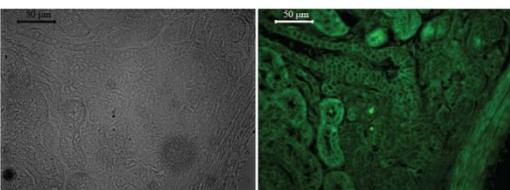


Wildtype Animal 15-6



Kidney

Transgenic Animal 177-4 (+/- GGTA-1; 1 copy hCD55)



Swine Gene Modifications @ MU:

- Xenotransplantation
 - Hyperacute Rejection
 - GGTA1 KO: (Lai et al '02 Science)
 - CMAH KO: (Lee et al unpublished)
 - hDAF on the GGTA1 KO background: (Lai et al unpublished)
 - hDAF into GGTA1^{+/-}: (Beaton et al '11)
 - Post-Hyperacute Rejection (Acute Vascular Rejection)
 - Cell Mediated Rejection
 - Non-Vascular Rejection (Neurodegenerative Disorders)
 - Porcine Endogenous Retroviruses

Xenotransplantation Combination

- GGTA1 KO with the following transgenes to deal with both Hyper- and Post Hyper-Acute Rejection:
 - CD55: decay accelerating factor; inhibits complement system
 - CD59: complement regulatory protein
 - ENTPD1: aka CD39, ectonucleoside triphosphate diphosphohydrolase 1; inhibits platelet aggregation
 - THBD: Thrombomodulin; anticoagulant

Dr. Simon Robson, Beth Israel Deaconess Medical Center.

Dr. Peter Cowan, St. Vincent's Hospital, Australia.

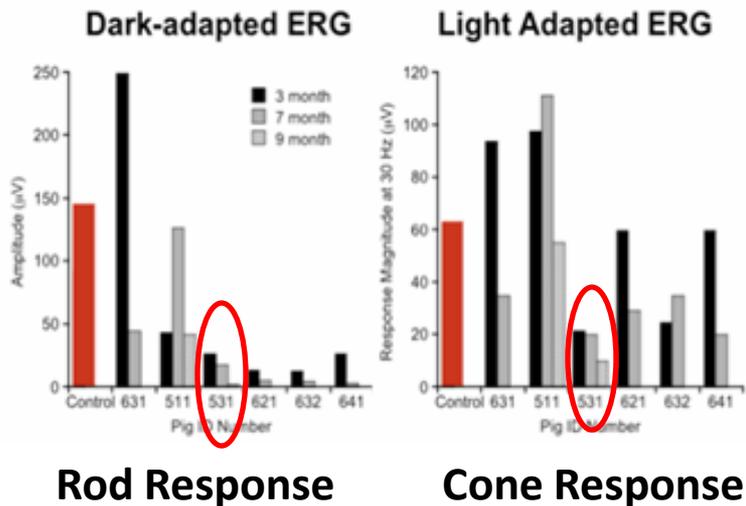
Born March 28, '11



Swine Gene Modifications @ MU:

- Models of Human Diseases
 - Retinitis Pigmentosa
 - Rhodopsin – P23H NIH SLA^{cc} background : (Ross et al '12, NSRRC: H. Kaplan, U. Louisville)

Born May 26, '08

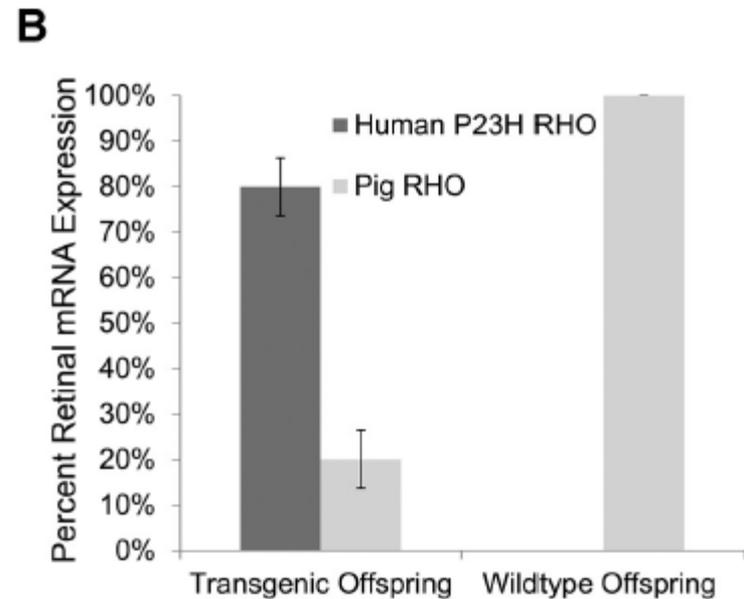
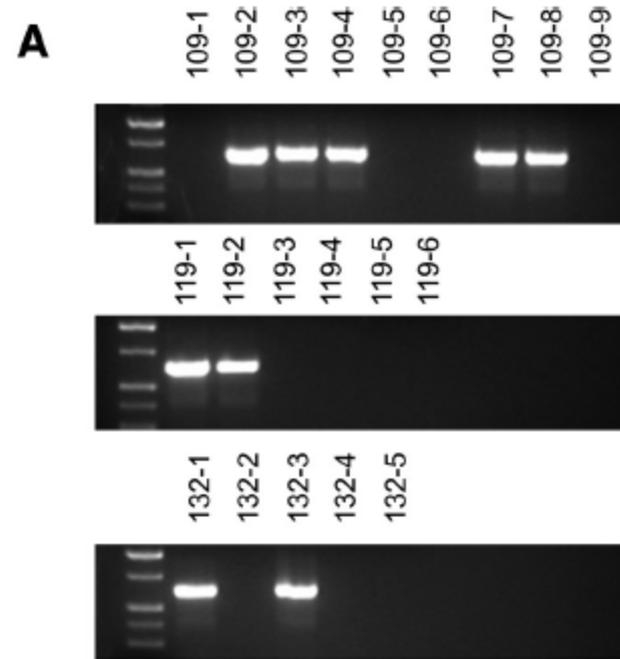


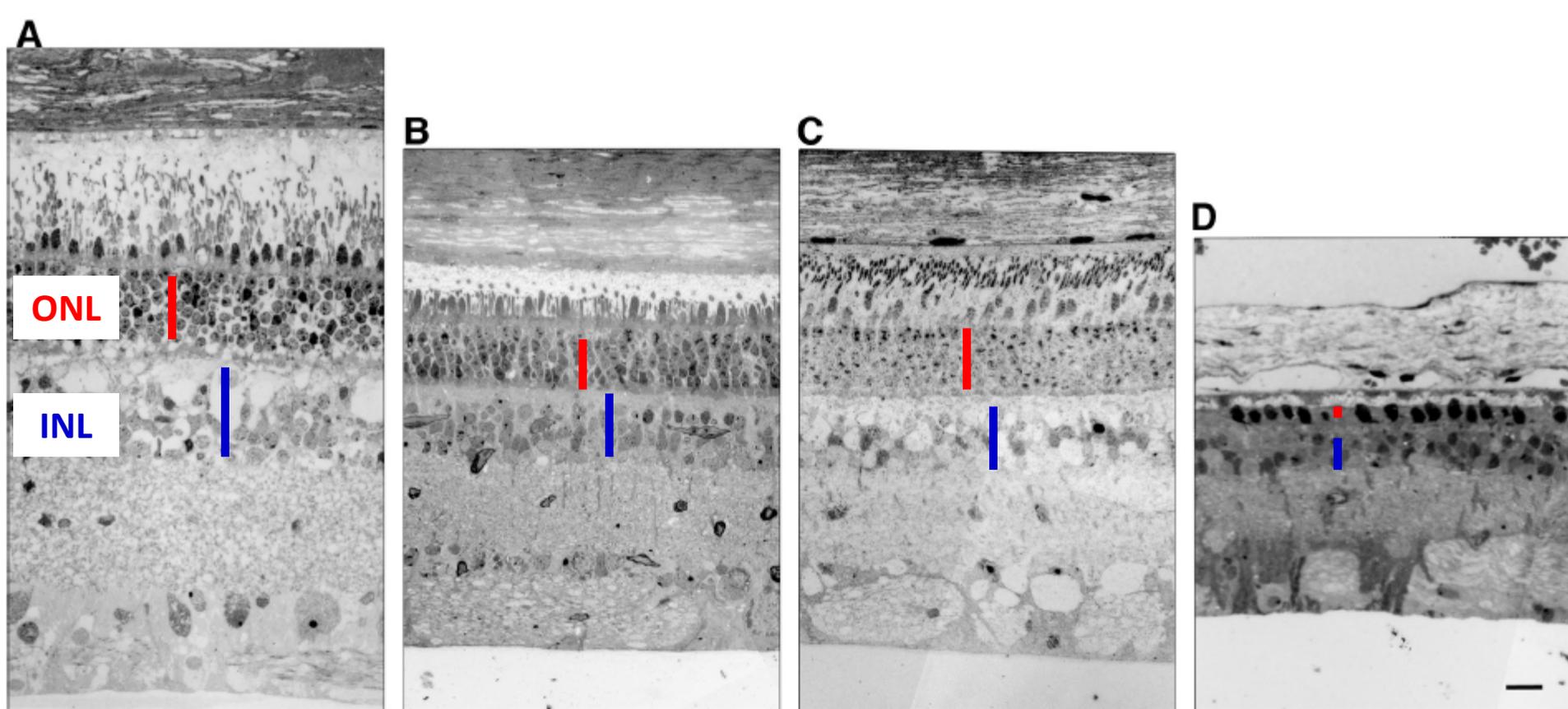
53-1 had a decrease in both a functional rod and cone response at 3, 7 and 9 months of age.



- Mendelian inheritance
9/20 offspring

- 80% of the colonies
represented P23H *RHO*





**Sow
Wild Type**

**51-1, 18 mo
Moderately Affected**

**63-1, 22 mo
Moderately Affected**

**63-2, 12 mo
Severely Affected**

63-2 has a single layer of cone nuclei in the outer nuclear layer (ONL) and a reduction of nuclei in the inner nuclear layer (INL)

51-1 and 63-1 have less photoreceptor degeneration although outer segments are shorter and the ONL thickness is reduced.

Cystic Fibrosis

- Michael J. Welsh, et al. University of Iowa
 - *PPG P01 NHLBI*
- Subcontract to the University of Missouri-Prather
- Caused by mutation of a single gene (cystic fibrosis transmembrane conductance regulator: CFTR).
- Pig lungs share many anatomical, histological, biochemical and physiologic features with human lungs. Pigs are an excellent model for abnormalities in disease, including pulmonary infections.
- Pig airway epithelia and submucosal gland function resemble humans and is dependent upon CFTR.
- Goal was to make a CFTR KO (exon 10) and a $\Delta F508$ pig.

Cystic Fibrosis

- **Defective Chloride ion transport:**
 - Intestine
 - Gallbladder
 - Pancreas
 - Bile duct
 - Liver
 - Male genital tract
 - Lung

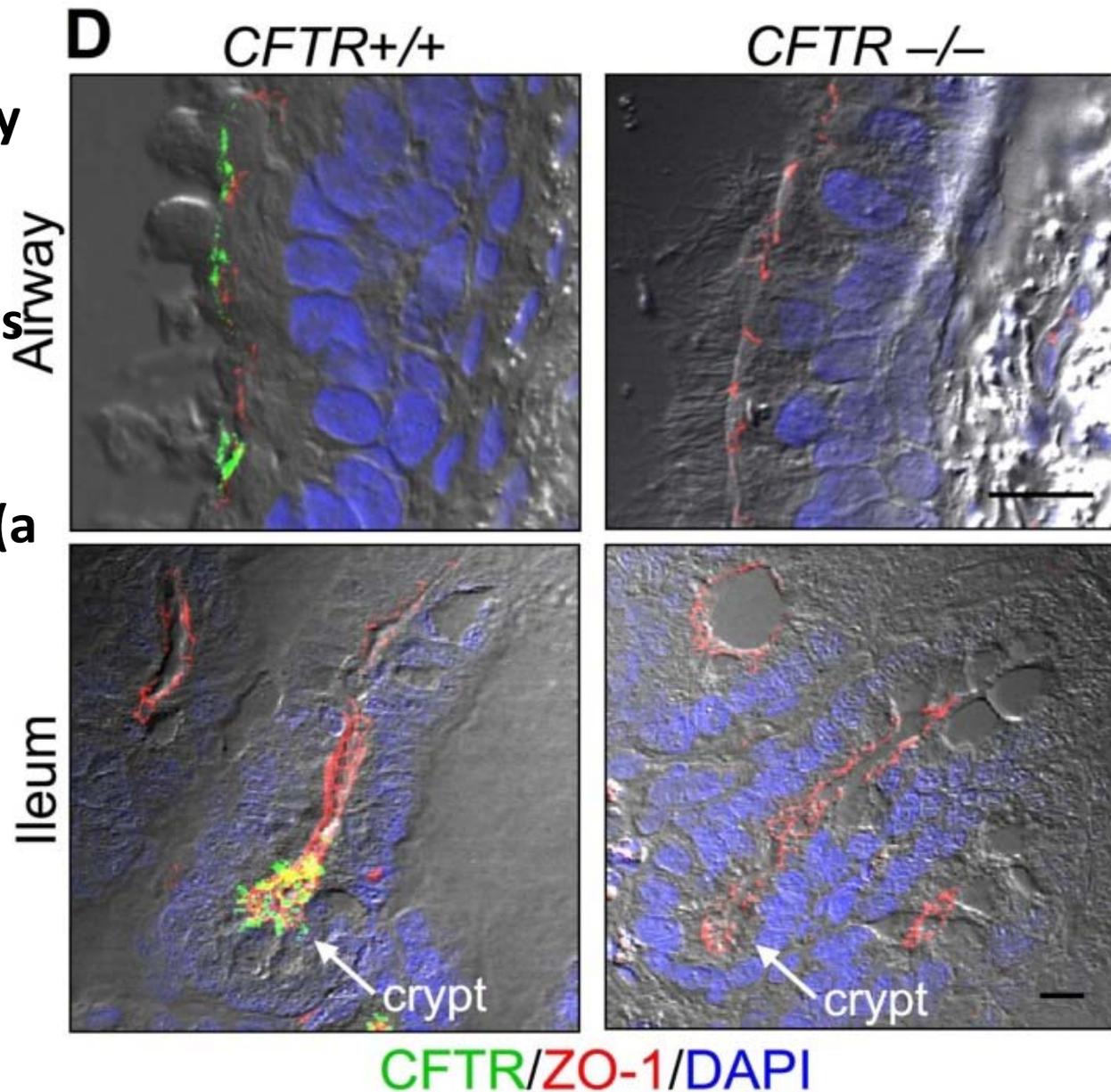
Cystic Fibrosis

- **Autosomal recessive**
 - Carrier rate of 5% in Caucasians
 - Named: *cystic fibrosis transmembrane conductance regulator (CFTR)*
 - 70% of the people with CF have a $\Delta F508$

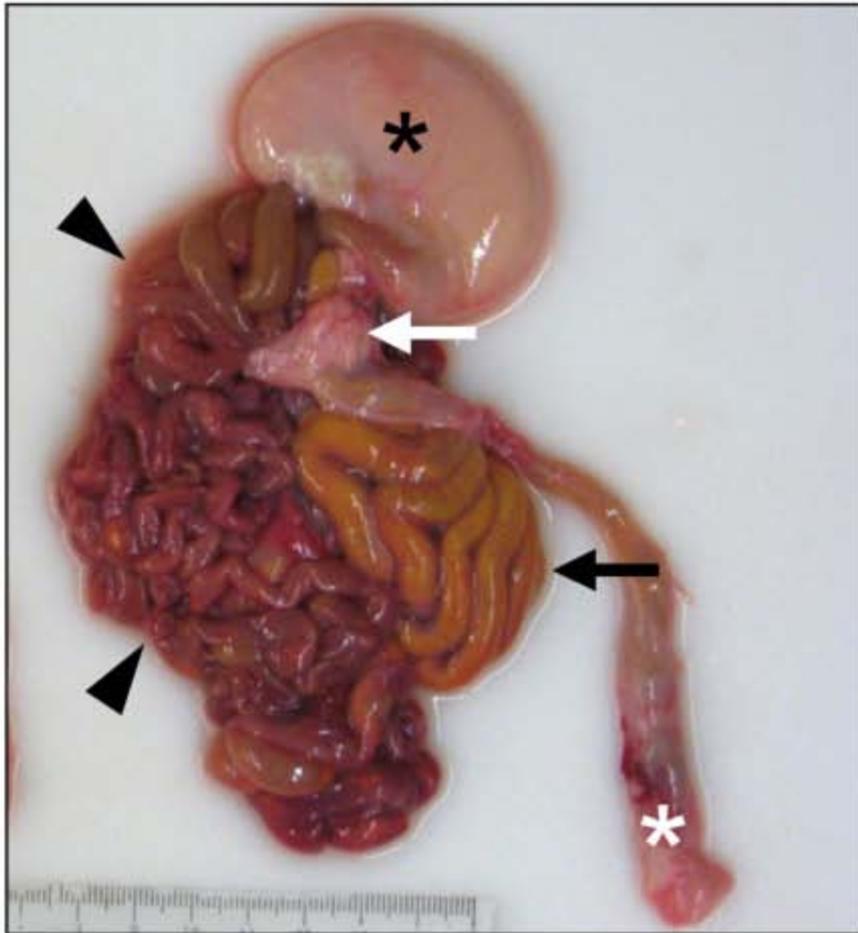
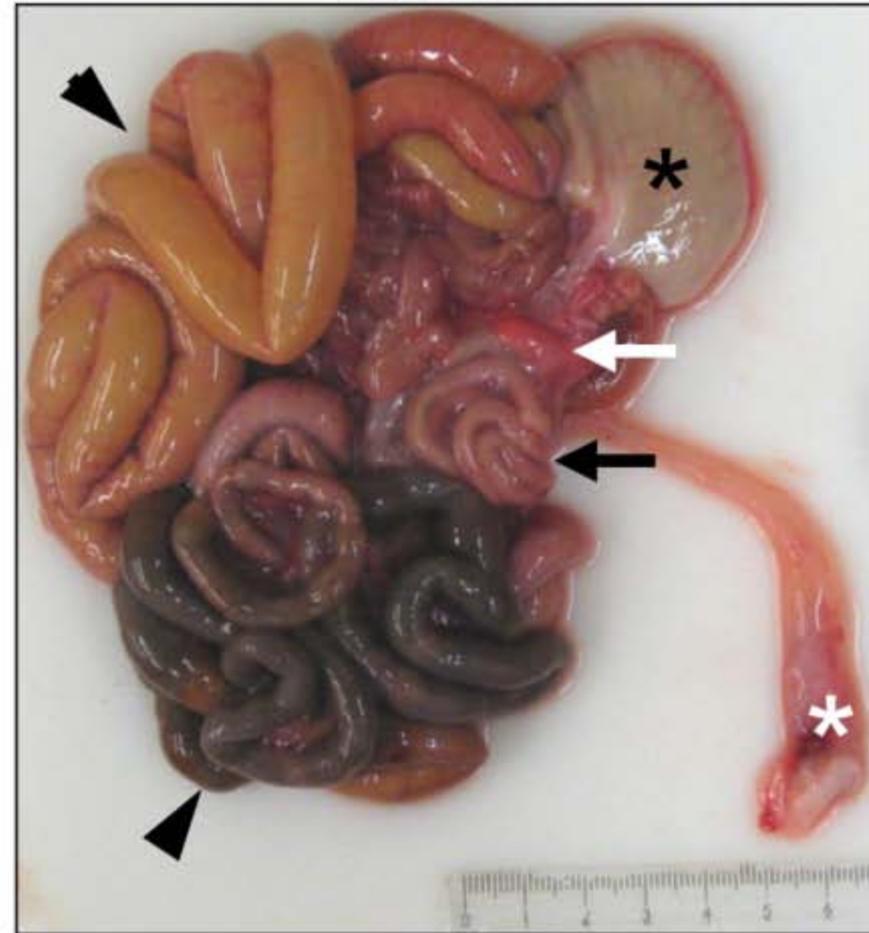
Cystic Fibrosis

- **CFTR KO Mice don't develop the characteristic abnormalities.**
 - Meconium ileus
 - Pancreatic destruction
 - Focal biliary cirrhosis
 - Lung disease
 - Occluded/absent vas deferens
- **Thus they are not a good model for studying CF in humans.**

Immuno-cytochemistry of CFTR in airway epithelia (top) and ileum (bottom). Figures are differential interference contrast with staining for ZO-1 (a component of tight junctions, red), CFTR (green), and nuclei (DAPI, blue). Bars, 10 μm .



C

CFTR^{+/+}CFTR^{-/-}

Gross appearance of gastrointestinal tract. Piglets were fed colostrum and milk-replacer for 30-40 h and then euthanized. Stomach (black *), small intestine (arrowheads), pancreas (white arrow), rectum (white *), and spiral colon (black arrow). Of 16 *CFTR*^{-/-} piglets, the obstruction occurred in small intestine in 7 and spiral colon in 9 piglets.

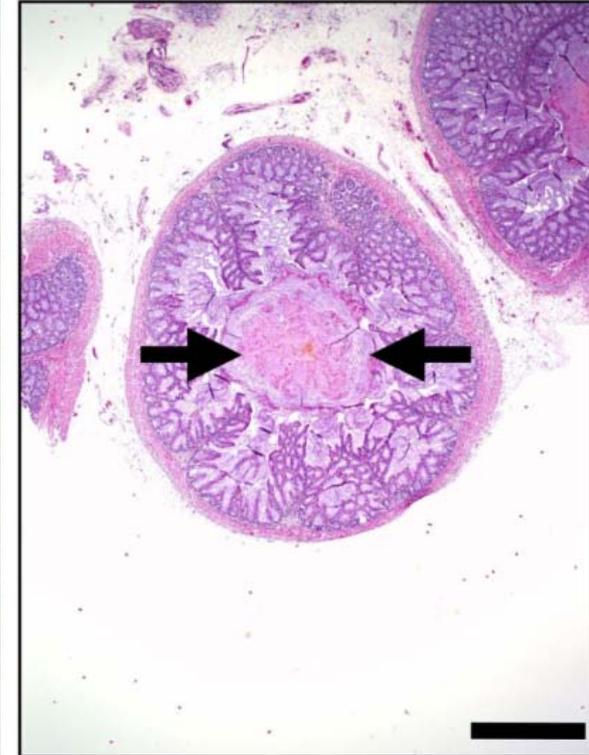
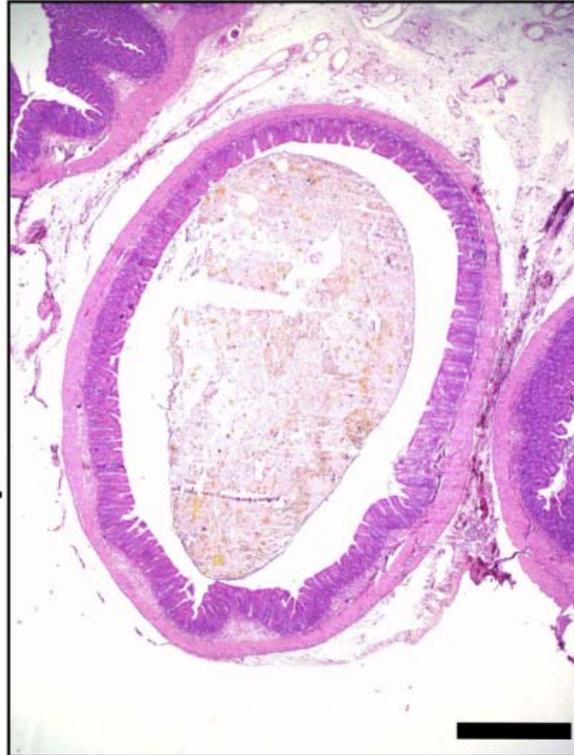
Microscopic appearance of the colon. H&E stain. Bars, 1 mm. Images are representative of severe meconium ileus occurring in 16 of 16 *CFTR*^{-/-} piglets.

E

CFTR^{+/+}

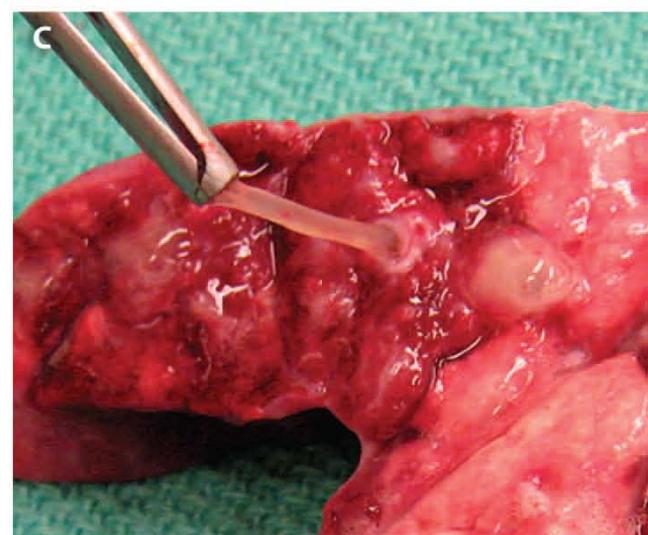
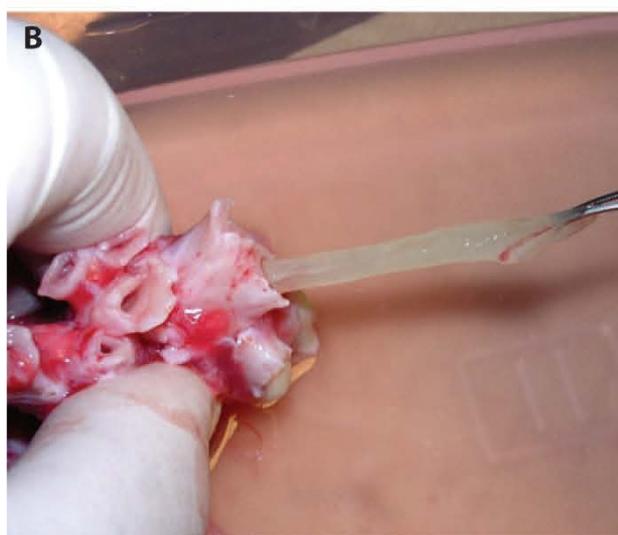
CFTR^{-/-}

Spiral Colon



Corrective Surgery

- **CFTR^{-/-} and Δ F508 CFTR piglets had an ileostomy or cecostomy.**
- **Piglets had pancreatic insufficiency.**
- **At 10 weeks of age – had something similar to distal intestinal obstruction syndrome.**
- **This was corrected.**



Matching mucus traits. (A and B) These airways, obtained during a lung transplantation performed on a 15-year-old CF patient, show two kinds of mucus obstructions observed in the removed CF lungs: a more common form of purulent, ivory, semi-liquid mucus [see arrows in (A)] and a less common form of translucent, resilient, elastic mucus, shown here being stretched and pulled from a bronchus [(A) and (B)]. Compare with (C), in which translucent mucus is being pulled from the airway of a CF pig described in Stoltz et al. and shown in their Fig. 3B, right panel. *J.J.Wine 2010 Science Translational Medicine April 29.*

Conclusions

- **In contrast to mice, Pigs develop the characteristic abnormalities associated with CF** (Rogers et al '08)
 - Defective chloride ion transport
 - Meconium ileus
 - Partial pancreatic destruction
 - Focal biliary cirrhosis
 - Congealed bile & duct blockage
 - Lung Disease
 - Develop a blocked vas deferens (Pierucci-Alves et al '11)
- **Bacterial infection comes before the inflammation** (Stoltz et al '10)
- **Reduced IGF-1 at birth may explain slightly smaller stature of humans with CF** (Rogan et al '10)
- ***K18-rtTA, K18-tTS, TRE-CFTR+* is being tested to rescue the gut phenotype of the *CFTR* ^{$\Delta F508/\Delta F508$}** (unpub.)

Swine Gene Modifications @ MU:

- Models of Human Diseases
 - Retinitis Pigmentosa
 - Rhodopsin – P23H NIH SLA^{cc} background : (Ross et al '12, NSRRC)
 - Cystic Fibrosis
 - CFTR -shRNA approach : (Unpublished- NSRRC S. Fahrenkrug, UM) – Gene was silenced.
 - CFTR - KO: (Rogers et al '08 J. Clinic. Invest., Science) M. Welsh, U. Iowa
 - CFTR- $\Delta F508$: (Rogers et al '08 J. Clinic. Invest.) M. Welsh, U. Iowa
 - Intestinal Tet-On CFTR on the CFTR $\Delta F508$ or CFTR $^{-/-}$ pigs: M. Welsh, U. Iowa
 - Cardiovascular Disease
 - FAT-1: Yifan Dai, U. Pitt, J.X. Kang, Harvard U. (Lai et al '06, Nature Biotech.)
 - Endothelial cell-specific reduction of nitric oxide: (Tie2-Catalase; Whyte et al '11)
 - Endothelial cell-specific increase of nitric oxide: (Tie2-eNOS; Samuel et al unpublished)



Tie2-Catalase
Born March
16, '09

Tie2-Catalase & Tie2-eNOS
are distributed by the



Tie2-eNOS
Born
November
2, '09



Swine Gene Modifications @ MU:

- Models of Human Diseases

- Muscular Dystrophy

- [Becker Muscular Dystrophy \(DMD^{+/-}\) TALEN-mediated \(deletion of exon 46\)](#): Jason Ross, Iowa State. born Feb 4, '12.



- Diabetes

- [ssGIP-hINS](#): (JDRF: Glucose-dependent insulinotropic polypeptide-human insulin) Tim Kieffer, University of British Columbia. Born June 1, '10.



- Spinal Muscular Atrophy

- [SMN^{+/-}](#): (Lorson et al '11)
 - [SMN^{+/-}; hSMN2⁺](#): (Lorson et al Unpublished). Born May 23, '11



- Cancer

- [Onco-Pig](#): (Cancer Model): Larry Schook, U. Illinois. Born May 21, '12
LoxP-Stop-LoxP, G12D KRAS, R167H p53



Swine Gene Modifications @ MU:

- Pharmaceuticals
 - **Human Coagulation Factor VIII + AAT + vWF**: (FVIII stability & hemophilic therapy): NSRRC, W. Velander, U. Neb. (Zhao et al '08).
 - **Human Coagulation Factor IX + AAT + PACE**: (Propeptide Cleavage Enzyme): NSRRC, W. Velander, U. Neb. (Zhao et al '11).



FVIII Born March 10, '08

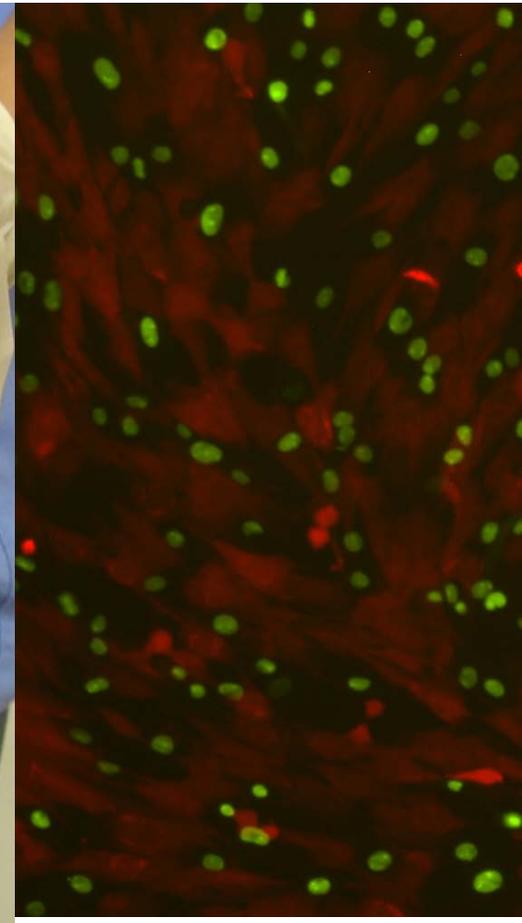


FIX Born September 28, '08



Swine Gene Modifications @ MU:

- Cell Tracking/Tools
 - eGFP: (Cabot et al '01; Park et al '01; Whitworth et al '09).
 - NLS-CAG-eGFP: (NSRRC Unpublished)
 - CAG-Tomato: (NSRRC Unpublished)



Minnesota Miniature pigs expressing Tomato (left) or NLS-eGFP (right) with UV excitation (left) or normal (right) exposure.

Born 1/17/2012

Swine Gene Modifications:

- Cell Tracking/Tools
 - eGFP: (Cabot et al '01; Park et al '01; Whitworth et al '09).
 - NLS-CAG-eGFP: (NSRRC Unpublished)
 - CAG-Tomato: (NSRRC Unpublished)
 - eGFP-Proteasome: (PSMA1) : (NSRRC) P. Sutovsky, MU (O’Gorman et al ‘10)
- Human/Pig Hybrid Organs -Suicide/Pro-drug System
 - Alpha fetoprotein promoter- cytosine deaminase: (Beschoner et al '03a) Ximerex, Inc.
 - Albumin promoter – thymidine kinase: (Beschoner et al '03b) Ximerex, Inc.

Born July 29, '02

Distributed by



Other Modifications Currently Underway at MU

- **FGF8 Conditional KO:** (Heart Development): Anne Moon, U. Utah
- **Mammary Tumor:** (NSRRC) Geoffrey Clark, U. Louisville
- **GUCA1A : Y99C and L151F, retinal degeneration:** (NSRRC) Maureen McCall, U. Louisville
- **ZP3 – Cre – LoxP/eGFP** Tool Pig: (NSRRC) K. Wells, MU
- **IFNRA1 KO, IFN γ KO, and RAG1 KO:** MU/Plum Island
- **CD163 KO:** (PRRSV resistance) MU/Plum Island
- **MSTN KO:** (Myostatin): Pfizer, K. Wells; A. Dilger, U. Illinois
- **RAG2 & IL2RG** TALEN KO: (Xenotransplantation) Kon-Kuk University, Korea

Conclusions

- **Swine can be useful for Comparative Medicine as in many cases they model the human condition.**
- **Many types of genetic modification can be completed in pigs that have applications in:**
 - **Medicine**
 - **Production Agriculture**
- **We are limited only by our imagination !!**

Current Projects/Grants–

- NIH U42 RR18877/OD011140 - National Swine Resource and Research Center (RSP, PI)
- NIH U42 RR18877/OD011140 – National Swine Resource and Research Center Administrative Supplement to clone pigs for xenotransplantation (RSP, PI)
- NIH R21 NS078299 - Large Animal Model of Spinal Muscular Atrophy (Monique Lorson, PI)
- NIH P01 HL51670 Iowa PPG –Gene Therapy for Cystic Fibrosis Lung Disease (Subcontract from Michael Welsh, University of Iowa)
- NIH U01 HL102288 Iowa- Iowa Phase II Clinical Trials of Novel Therapies for Lung Diseases. (Subcontract from L. Durairaj, University of Iowa)
- NIH R01 RR13438 - Differential Methylation Hybridization of Nuclear Transfer and Normal Embryos (RSP, PI)
- Korean Government via Kon-Kuk University – *GGTA1* and *CMAH* KO's. (RSP, PI)
- USDA ARS 58-1940-5-519 -Program for the Prevention of Animal Infectious Diseases and Advanced Technologies for Vaccines and Diagnostics (M. McIntosh, PI)
- Christopher Columbus Foundation – *CD163* KO. (RSP, PI)
- NIH R01 HD069979 – Induced pluripotent stem cells from swine... (R.M. Roberts, PI)

Current Lab Members

- **Dr. Clifton Murphy**
- **Dr. Kiho Lee**
- **Dr. Jiude Mao**
- **Dr. Kim Tessanne**
- **Dr. Eric Walters**
- **Dr. Kristin Whitworth**
- **Dr. Jeff Whyte**
- **Bethany Bauer**
- **Alana Brown**
- **Keith Giroux**
- **Melissa Samuel**
- **Lee Spate**
- **Armedia Stump**
- **Jennifer Teson**
- **Mingtao Zhao**
- **Carli Carter**
- **Alyssa Davis**
- **Lindsay Kelso**
- **Rebecca Mattucks**
- **Elaine Martin**
- **Jacob Miller**
- **Megan Pemberton**
- **Chris Perry**
- **Maren Ritterling**
- **F. Santibanez**
- **Jon Sarno**
- **Alyssa Thomas**
- **Destany Wilson**

Others on Campus

- **Dr. Jon Green**
- **Dr. Monique Lorson**
- **Dr. Peter Sutovsky**
- **Dr. Kevin Wells**
- **Ben Beaton**
- **Shasta Cernea**
- **Susan Cushing**
- **Tina Egen**
- **Chad O’Gorman**

