Production of *COL6A3* Knock Down Pigs:

Use of a Dual CRISPR System and Zygote Cytoplasmic RNA Microinjection

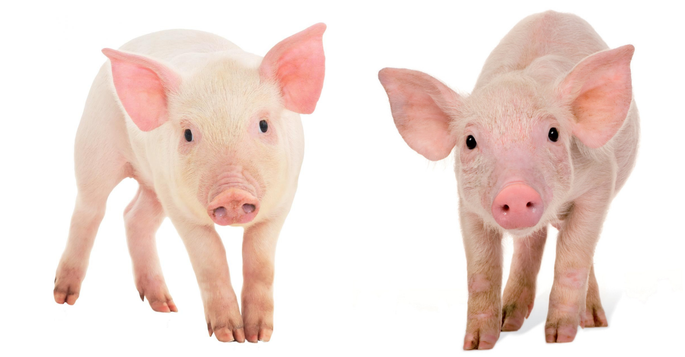
Brooke D. Pallas, Anna M. Spate, Kevin D. Wells
Department of Animal Science, University of Missouri, Columbia

Veterinary Research Scholars Program
University of Missouri

National Swine Resource and Research Center
University of Missouri

WHAT ARE WE DOING?

- The objective is to produce genetically modified pigs with a specific mutation (551 bp deletion, including exon 19) in the *COL6A3* gene, which induces a dominantly inherited muscular dystrophy phenotype
- The goal is to use the newly developed CRISPR gene editing system, in combination with G-Blocks™ to create RNA for direct microinjection into porcine zygotes

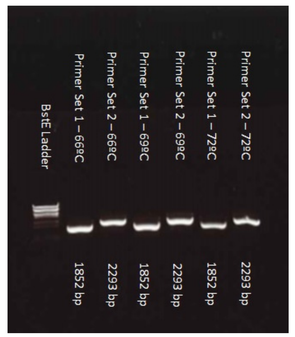


WHY DO IT?

National Swine Resource and Research Center

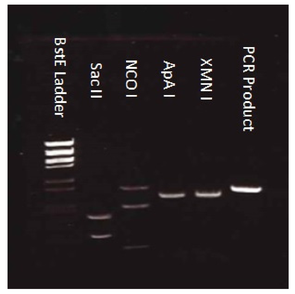
- Established in 2003, the NSRRC provides the infrastructure to allow biomedical investigators access to requesting specific genetically engineered swine models of human disease
- Due to similarities in the genetic makeup, anatomical size, and functional physiology between pigs and humans, pigs are often the only animal model that completely replicates the entire human condition
- An investigator at the National Institute of Health, requested a swine model for a unique mutation site that leads to muscular dystrophy in human infants
- Mice and rat models have failed to exhibit the proper phenotype, due to lack of adequate size and force exerted on the musculature

HOW DID WE DO IT?



Genotype Assay Creation

- 3 possible forward and reverse primers were evaluated at 3 temperatures to determine an optimal combination for amplifying the editing target region

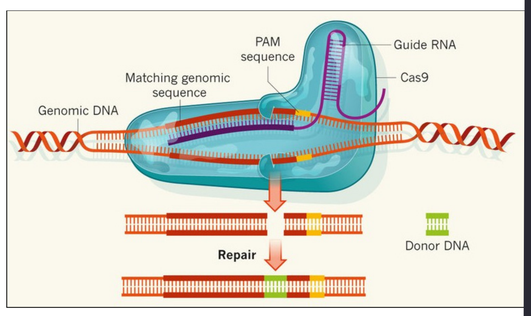


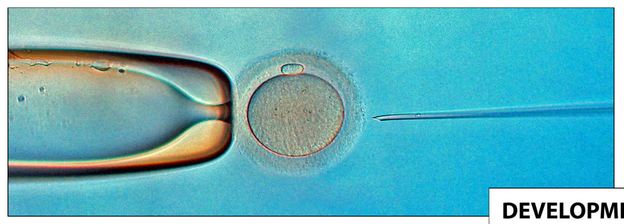
Assay Verification

- To ensure the amplified sequence is actually the desired sequence, restriction enzymes were used to digest the region into predictable sizes, based on unique enzyme cut sites

CRISPR / Cas9

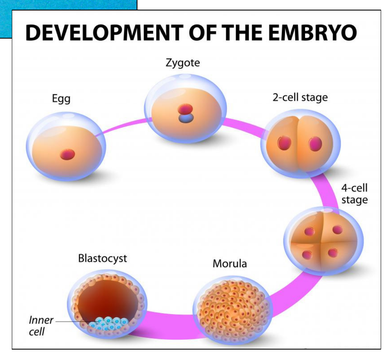
- A CRISPR was designed to cut at each end of the deletion region
- Obtained as DS DNA fragments (G-Blocks), converted to RNA for injection into zygote cytoplasm





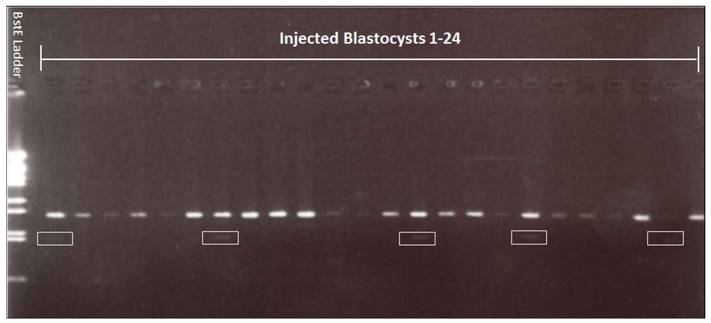
Microinjection

- The CRISPR RNA is injected into the cytoplasm of porcine zygotes
- The embryos are then cultured to blastocysts and prepared for embryo transfer to a surrogate female pig



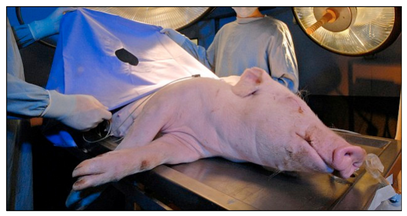
DID IT WORK?

- 140 zygotes were injected with RNA CRISPR constructs and Cas9 cassette
- 29 blastocysts resulted, after being cultured for 6-7 days
- 24 blastocysts were genotyped
- 5 blastocysts appear to be heterozygous for the mutation and all appear to be mosaics
- None appeared to be homozygous



NOW WHAT?

- A new set of zygotes was injected with the CRISPR constructs and Cas9 cassette



- The embryos were cultured to blastocyst stage and embryo transferred to 2 surrogate female pigs
- Any correctly modified piglets that result, will be hand raised and sent to the requesting investigator

PROJECT FUNDING

- *National Swine Resource and Research Center*
- *An IDEXX-BioResearch Endowment*
- *UM Veterinary Research Scholars Program*