Generation of Anti-Müllerian Knockout in Chickens by CRISPR/Cas9 Genome Editing to Study Sex Development

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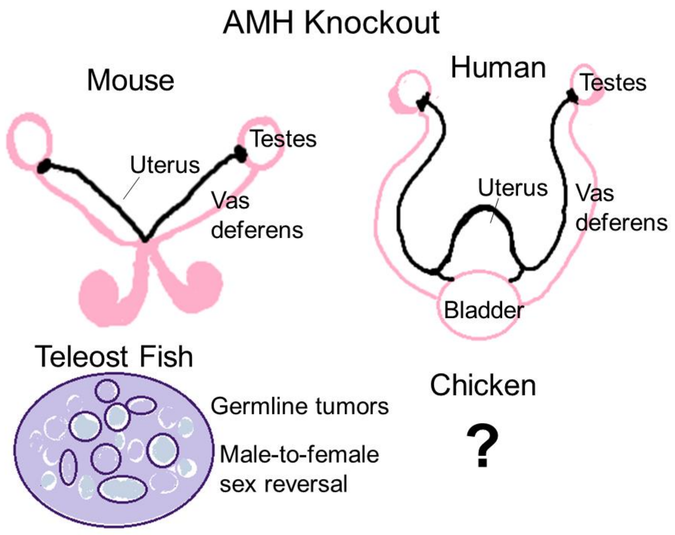
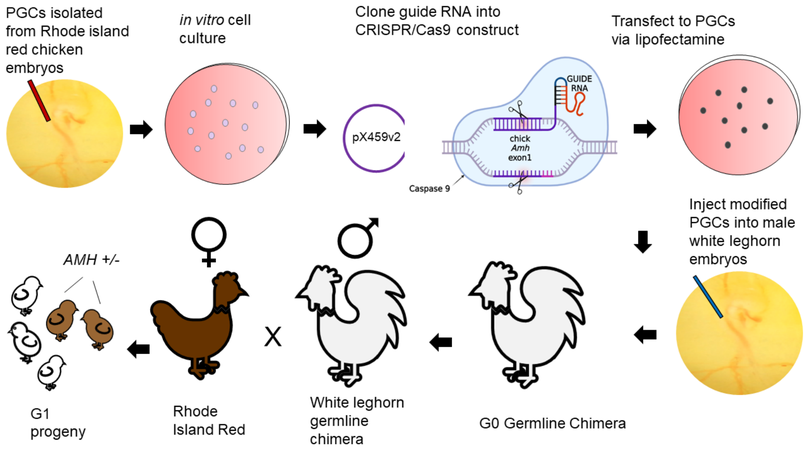
Introduction



Methods

CRISPR modification

- Plasmid DNA Isolation
- Cloning
  - Restriction Enzyme Digest and Ligation
- PGC culture
- Lipofectamine Transfection



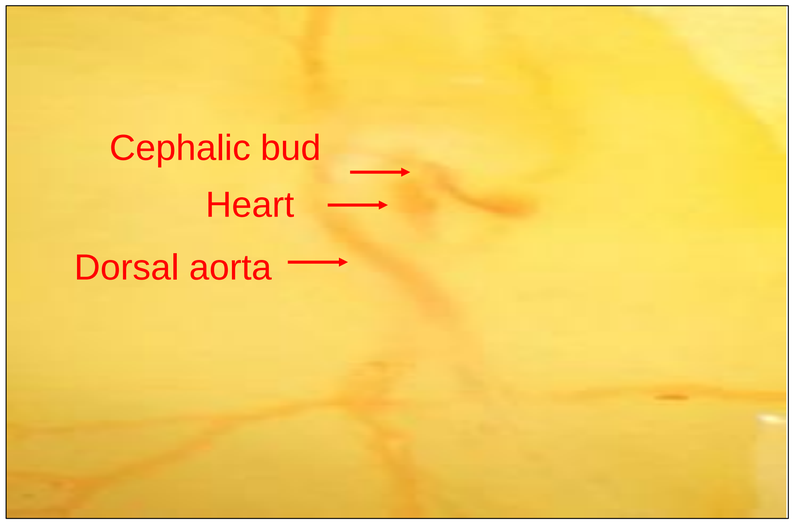


Figure 1. Fertilized chicken embryo. Circulating PGCs in blood are drawn from dorsal aorta. Modified PGCs are injected into dorsal aorta.

Results

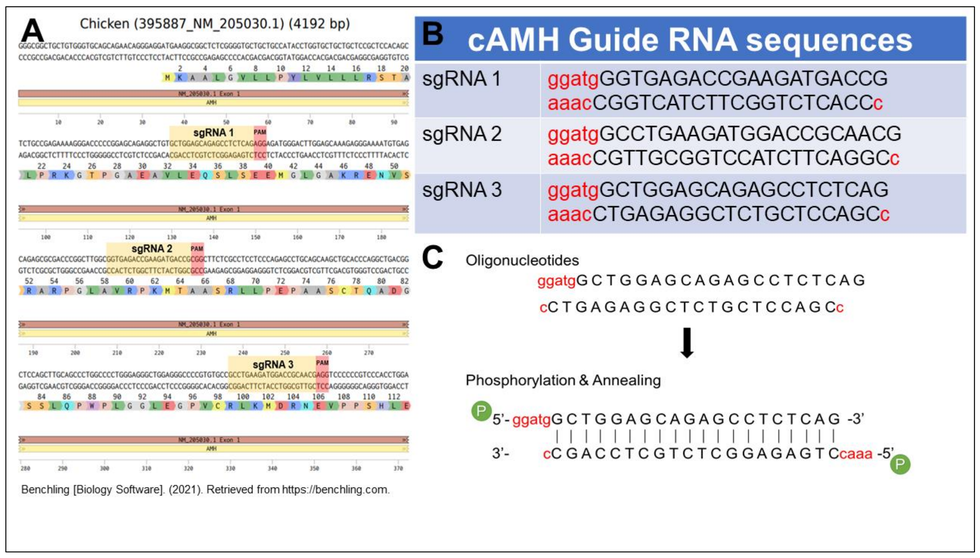


Figure 2. Exon 1 of chicken *AMH* gene. A) Three target sequences were identified in exon 1 of Chicken *AMH* using Benchling software. B) Guide RNA Oligonucleotide sequences. C) Oligonucleotides were annealed and phosphorylated to generate inserts to clone into pX459v2 plasmid.

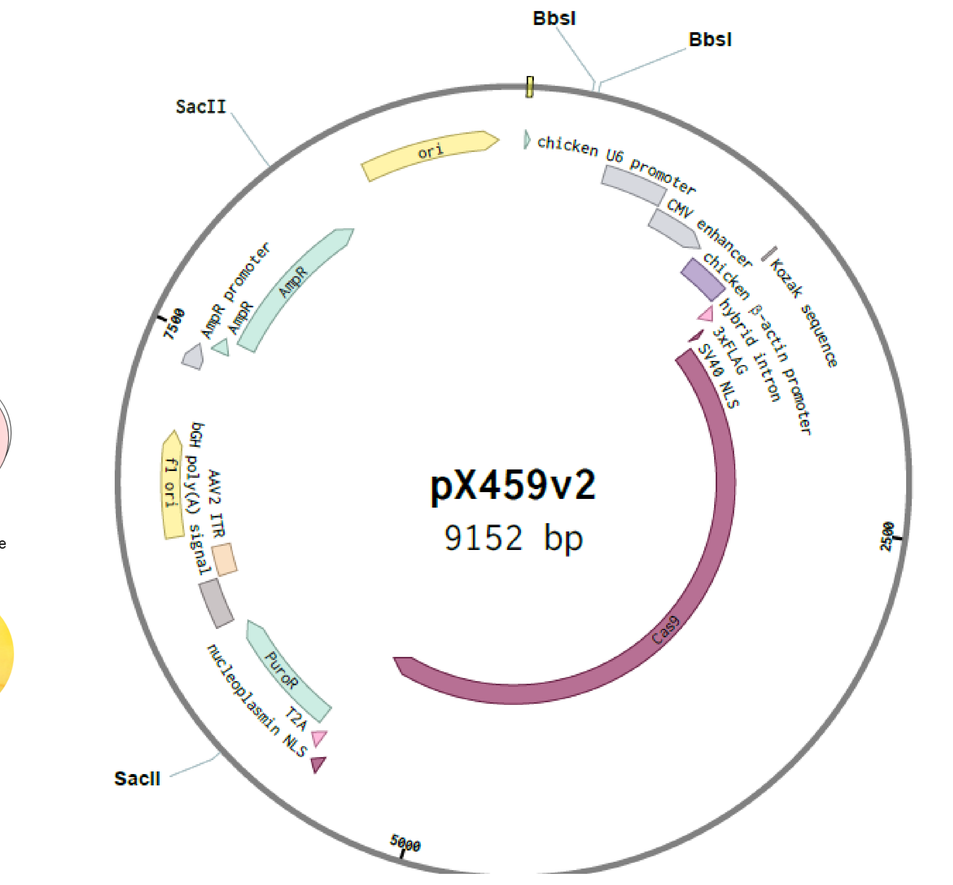


Fig. 3. pX459v2 plasmid vector. Modified by Dr. Jeff Barrow from pSpCas9(BB)-2A-Puro (PX459) V2.0 Feng Zhang (Addgene plasmid # 62988; http://n2t.net/addgene:62988; RRID: Addgene_62988). Construct contains U6 chicken promoter, Cas9 expression, puromycin and ampicillin selection. Oligonucleotides are cloned into vector at BbsI restriction site after restriction enzyme digest. Cas9 recognized PAM sequence and cuts to make an indel mutation. Frameshift mutation leads to *AMH* knockout.

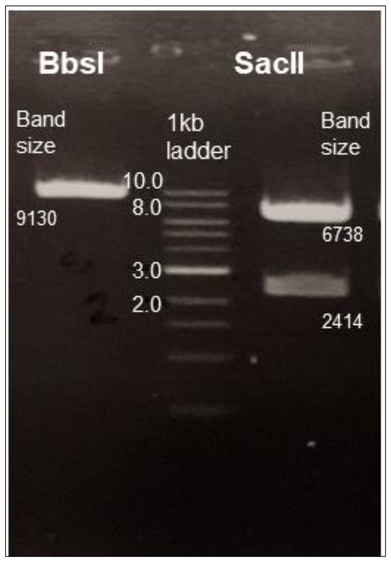


Figure 4. pX459 Digest with BbsI and Sac II

Conclusions

Molecular cloning is currently ongoing. Once cloning is complete, transfection of CRISPR/Cas9 constructs with guide RNA into PGCs will begin. The modified PGCs will be injected into male chicken embryos that will generate germline chimeras capable of transmitting the *AMH* null allele. White leghorn germline chimeras will be crossed with Rhode Island Red chickens to produce heterozygote progeny. These progeny will be crossed to each other to produce homozygous *AMH* knockout mutants.

The study will elucidate the role of AMH signaling in chicken sex development. Studying AMH signaling in chickens helps determine if AMH signaling is a conserved evolutionary mechanism across vertebrates. Phenotypes of the AMH knockout chicken model will be compared to phenotypes of other AMH knockout models.

References

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