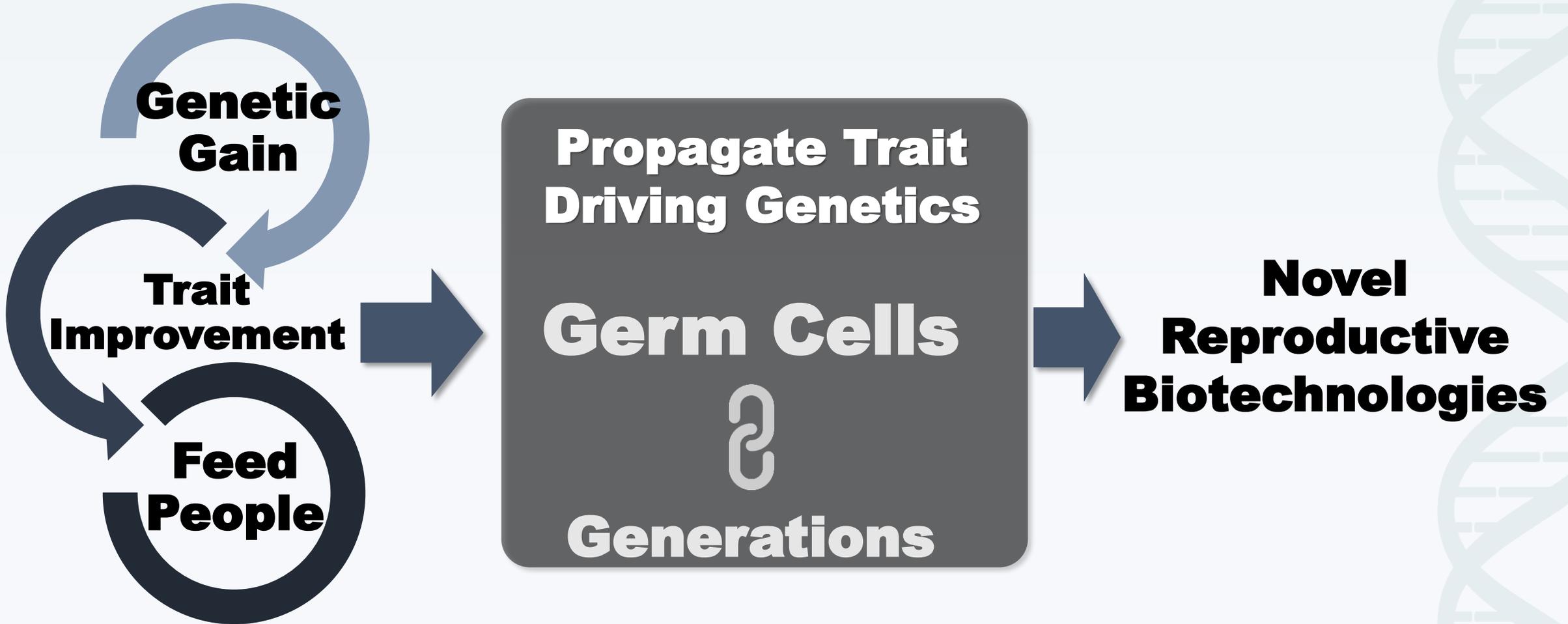


Genome Editing for Reproductive Capacity in Livestock

Jon M. Oatley, PhD
Washington State University



Guiding Principle



A black and white micrograph showing a filamentous fungus. The image features several long, thin, wavy hyphae that branch out. Attached to these hyphae are numerous dark, elongated, spindle-shaped spores. The background is a light, uniform gray, providing a clear contrast for the dark structures of the fungus.

**Widespread Dissemination of Elite
or Gene Edited Genetics**

Foundation of Spermatogenesis

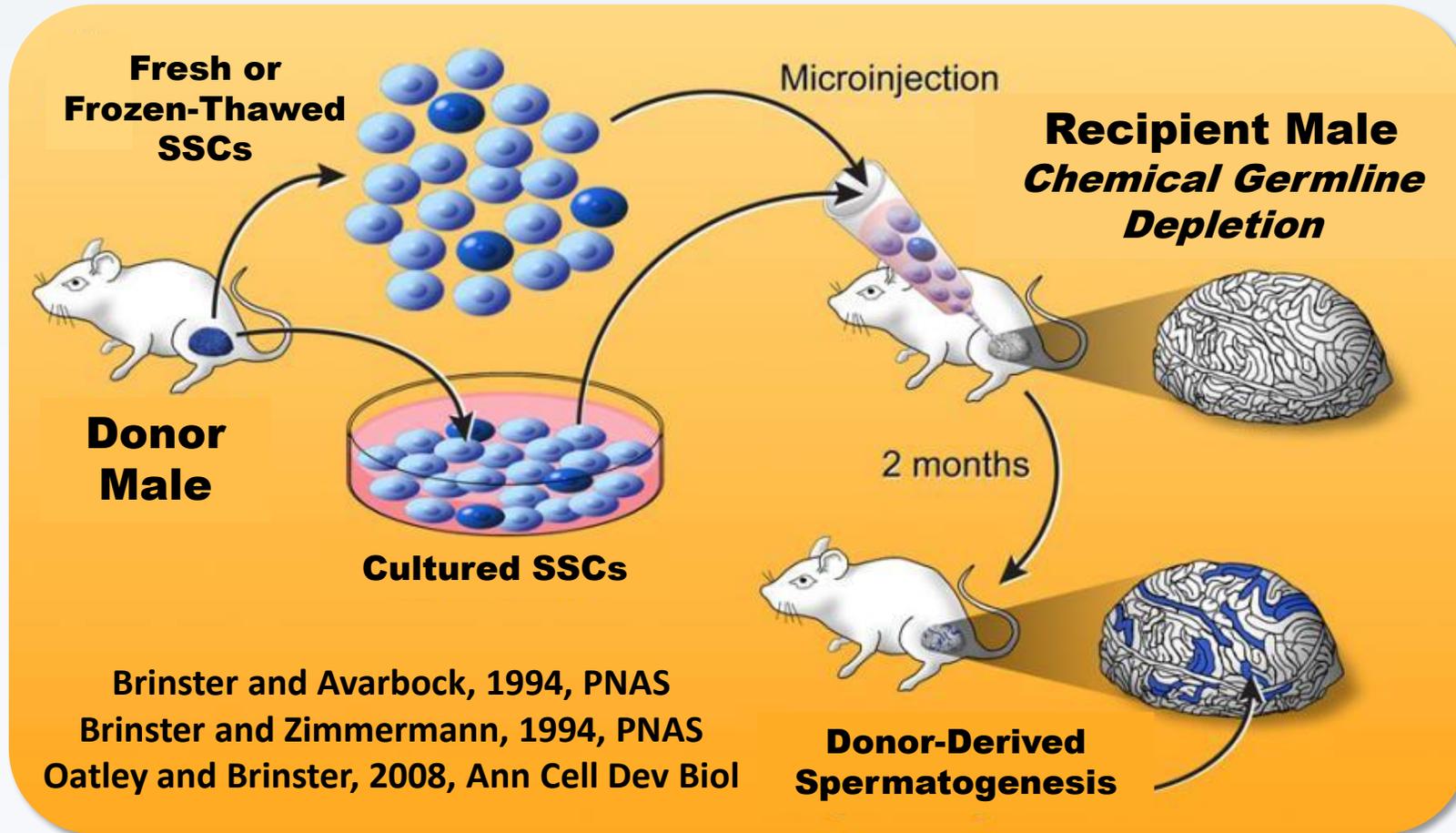
Spermatogenic Stem Cells

(Primordial Germ Cells – Prospermatogonia – Spermatogonia)

***Isolation, Culture, Modification,
Transplantation***



Spermatogonial Stem Cell (SSC) Transplantation



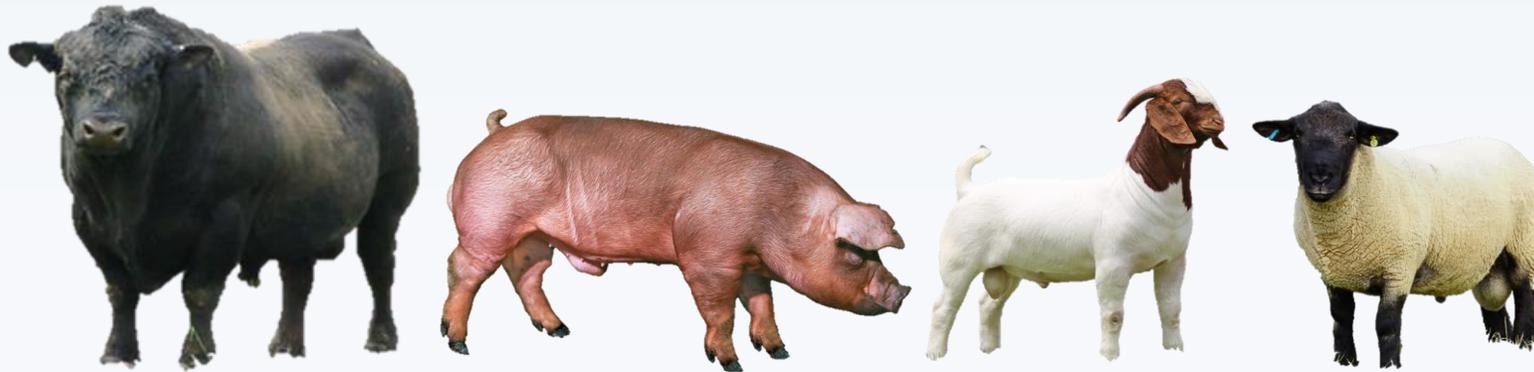


20+ Year Grand Goal
**Translate Spermatogenic Stem
Cell Methodologies from Mice to
Livestock**



CRISPR-Cas9 Application: Surrogate Sires

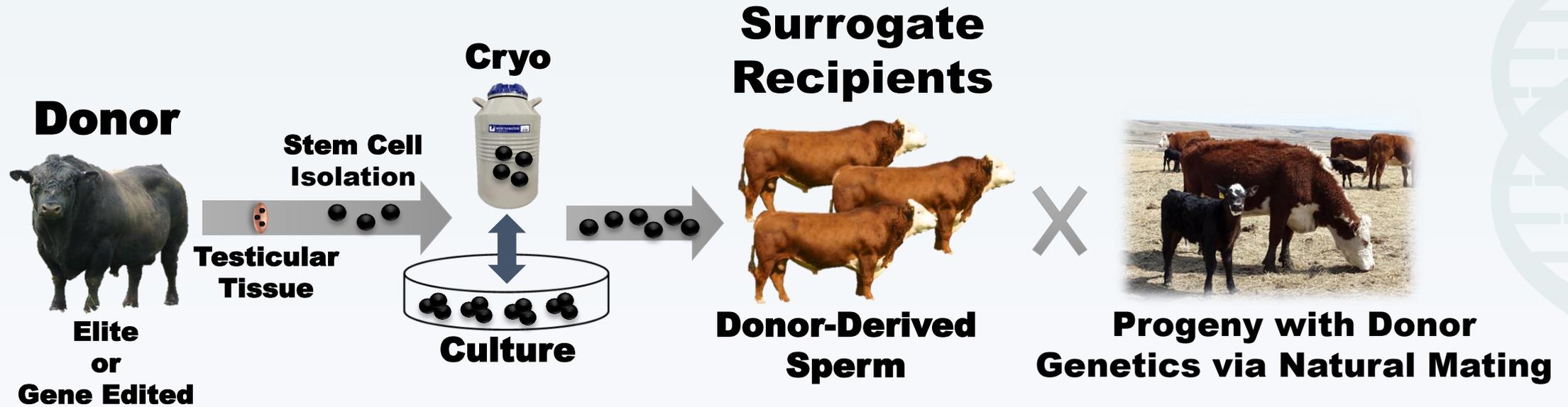
Giasseti et al., 2019, Ann Rev Anim Biosci



Breeding Tool for Efficient Propagation of Elite & Gene Edited Genetics



Overarching Concept



Key Aspects

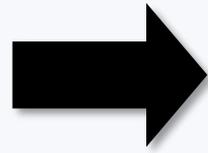
***In Vitro* Expansion of Donor Stem Cells**

Germline Ablated Recipient Males

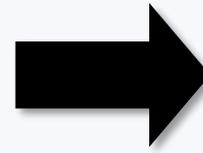


How to generate male livestock that don't make their own sperm but still have functional testes?

**Gene Editing
(CRISPR-Cas9)**



**Key
Genes**

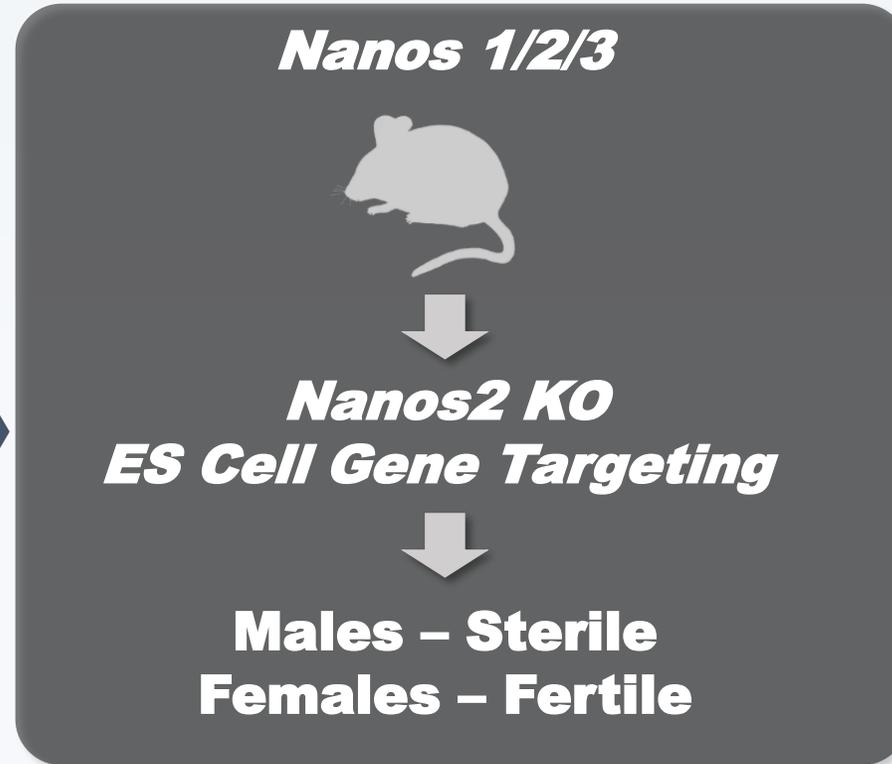
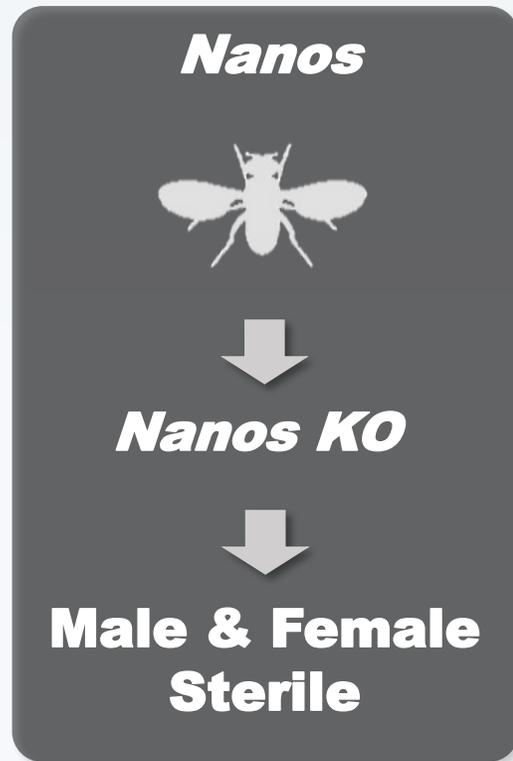


**Male ~~Germ~~
Cell ~~Survival~~**

Homozygosity = Male Sterile, Female Fertile
Heterozygosity = Male Fertile, Female Fertile



Our Target: *NANOS2*



Effect of *NANOS2* KO in other species?

Can *NANOS2* KO males become fertile?



Proof-of-Concept Pipeline

Generate
Nanos2 KO
CRISPR-Cas9

Phenotype:
Germline
Ablation?

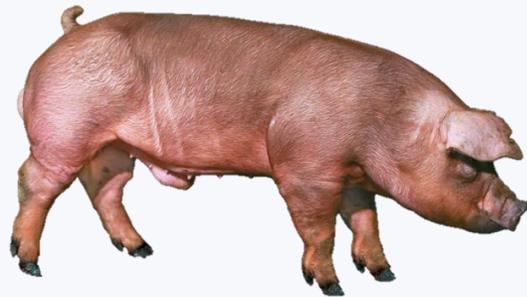
Transplant
Donor SSCs

Donor-Derived
Sperm?

Refine for
Fertility

← Ciccarelli et al., 2020, Proc. Natl. Acad. Sci. →

→ Unpublished

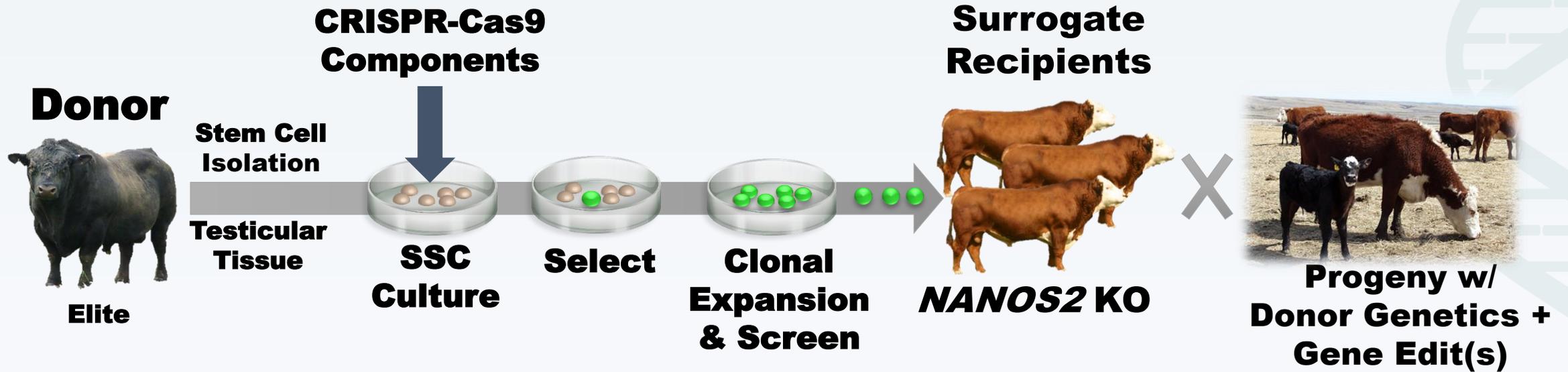


Next Frontier

Direct Germline Gene Editing



Overarching Concept



Key Aspects

Exponential Growth of Actual SSCs

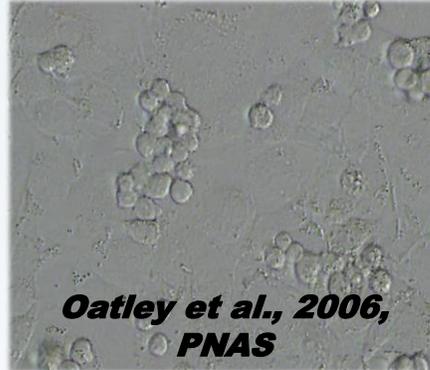
Efficient Delivery of Gene Editing Components

Clonal Expansion



Bonafide SSC Culture

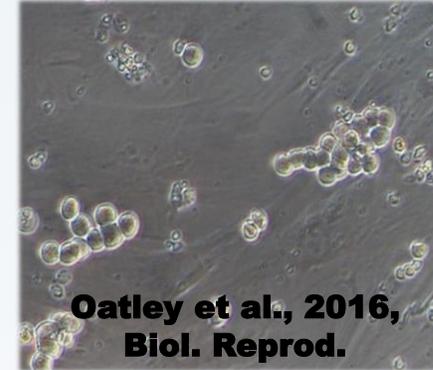
Mouse



Pig



Cattle



Sheep



POC Pipeline

Cell Isolation



Condition Optimization



Exponential Expansion



Biomarker Expression*



Sperm Generation via Transplantation



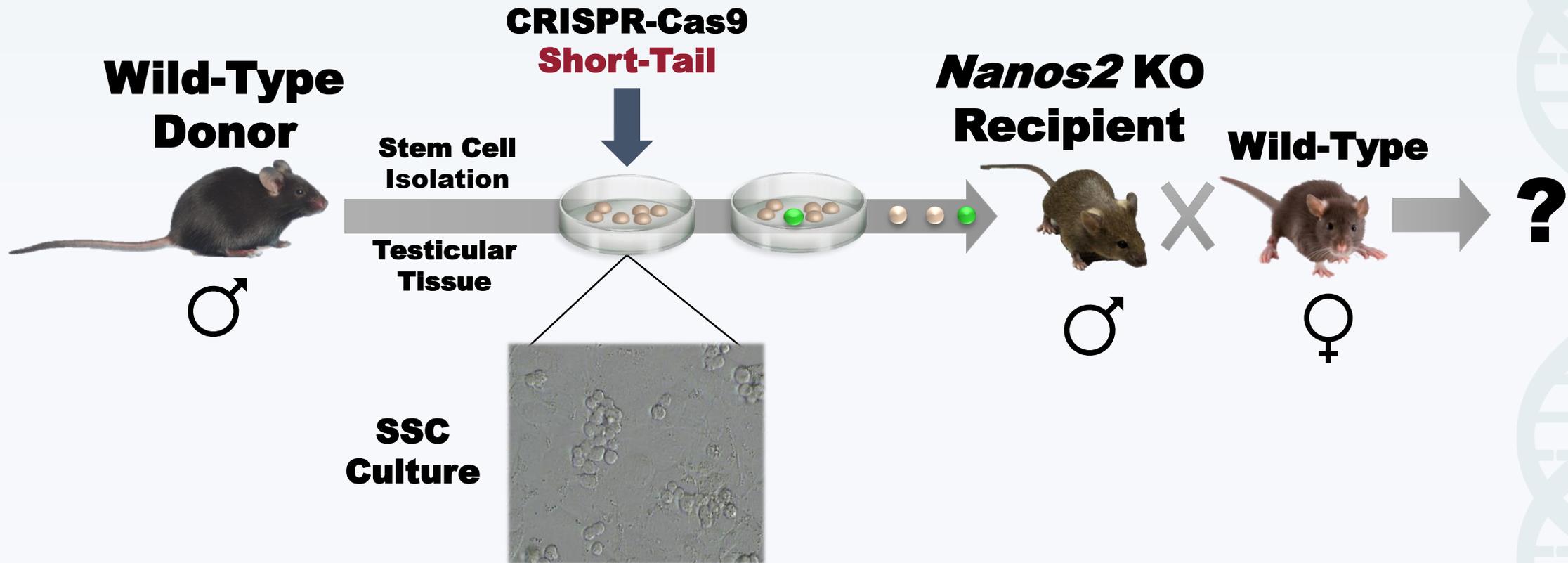
In Development

In Development



Direct Germline Gene Editing POC in Mice

Can we derive offspring with an engineered trait?



Direct Germline Edited Offspring



$\Delta/+$

$\Delta/+$

$\Delta/+$

$+/+$

Genotype



Implications

Direct Germline Gene Editing + Surrogate Sires

- **Empowers Efficient Generation of Gene Edited Livestock Germplasm**
- **Enables Characterization of Intended & Unintended DNA Editing Prior to Generating Animals**
- **Provides Simpler Dissemination of Edited Genetics into Production Populations**



Challenges & Barriers

Direct Germline Gene Editing + Surrogate Sires

- **Scalability & Customization** for *NANOS2* Knockout Sires
- **Optimization & Refinement** of SSC Culture Conditions & SSC Transplantation
- **Affordability & Harmonization** of Global Regulatory Frameworks for Integration into Production Systems



THANK YOU

joatley@wsu.edu

