

NEXT TRAITS – RUMINANTS



Dan Carlson – CSO – Recombinetics

Heritable Genetic Modification in Food Animals: Potentials and Concerns

February 27, 2024

Disclosures

What? Employee and Shareholder, Recombinetics/Acceligen

Why? Recombinetics is a for-profit company developing gene-edited livestock for commercial applications

Agenda

Question: What traits might be pursued within the next two years?

- Principles of trait selection
- Example of deploying natural variants to adapt cattle for dairy production in Africa
- Example of testing a novel variant for bovine Viral Diarrhea Virus
- Conclusions

Selecting Traits that Appeal to Producers and the Public



Trait/edit Selection Specific to Breed/Region



Objective = Improve Meat Quality in Tropical Environment



Heat adapted angus



Objective = improve Meat Quality in Tropical Environment



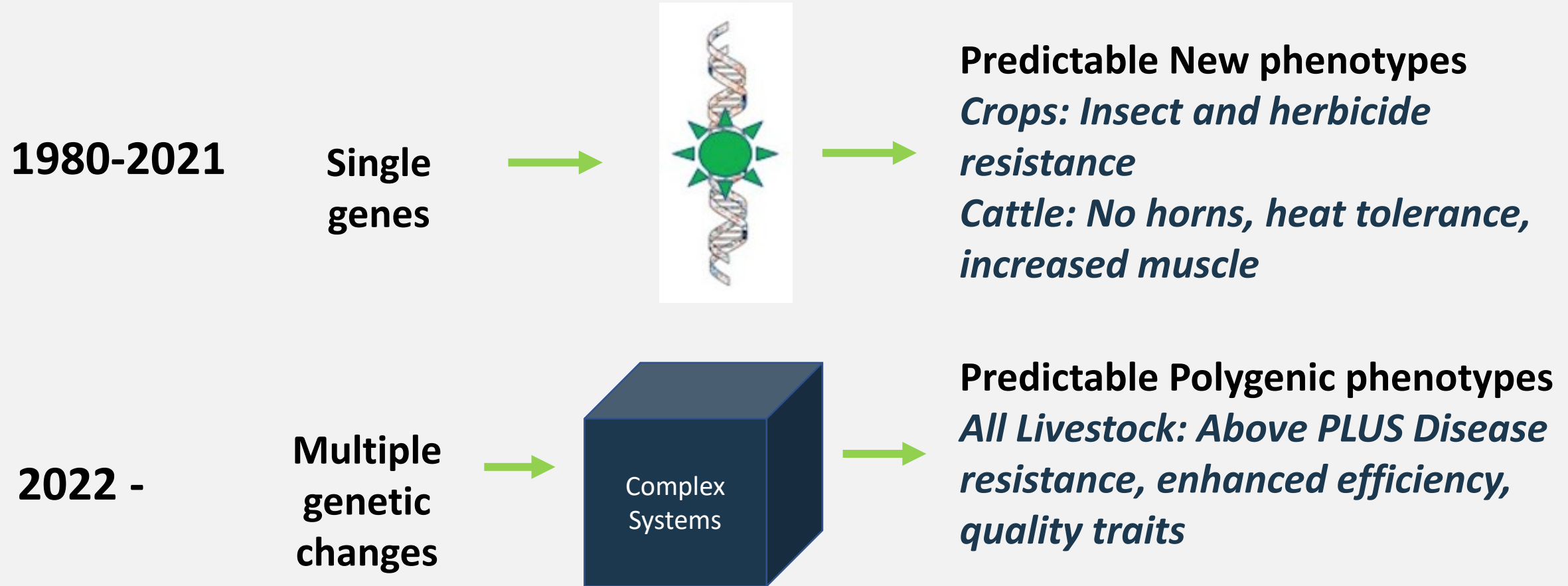
Nellore w/ quality trait

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How will Traits be Deployed?

More Applications will Leverage Multiplex Gene Editing





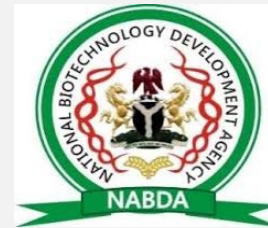
Example 1 – Multiplex of Native Alleles to Enhance African Dairy

Multiple gene targets, heat tolerance & disease resilience, native alleles

Precision Crossbreeding for African Dairy Production Systems

High-level Project Goals

- Generate dairy animals that contribute sustainable production gains for African dairy production systems.
- Deploy novel sequence variants delivered by multiplex gene editing
- Develop breeding systems to propagate gene-edited animals
- 2020-2026 time-frame



Project Duration
63 months

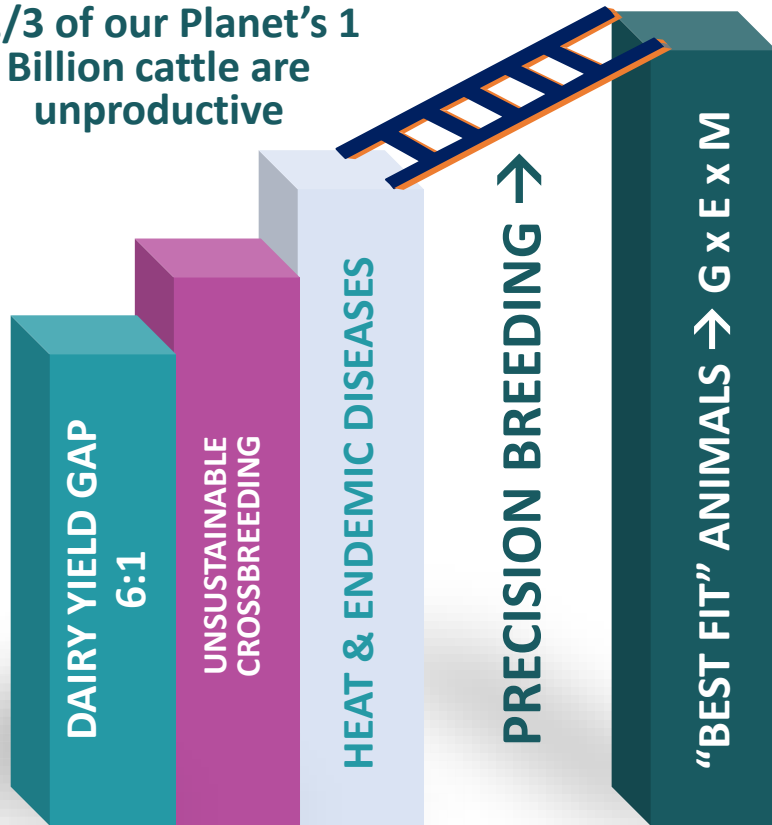
Project Inception
August 2020

Project Commitment
~\$3.7M

Commercialization
2025+

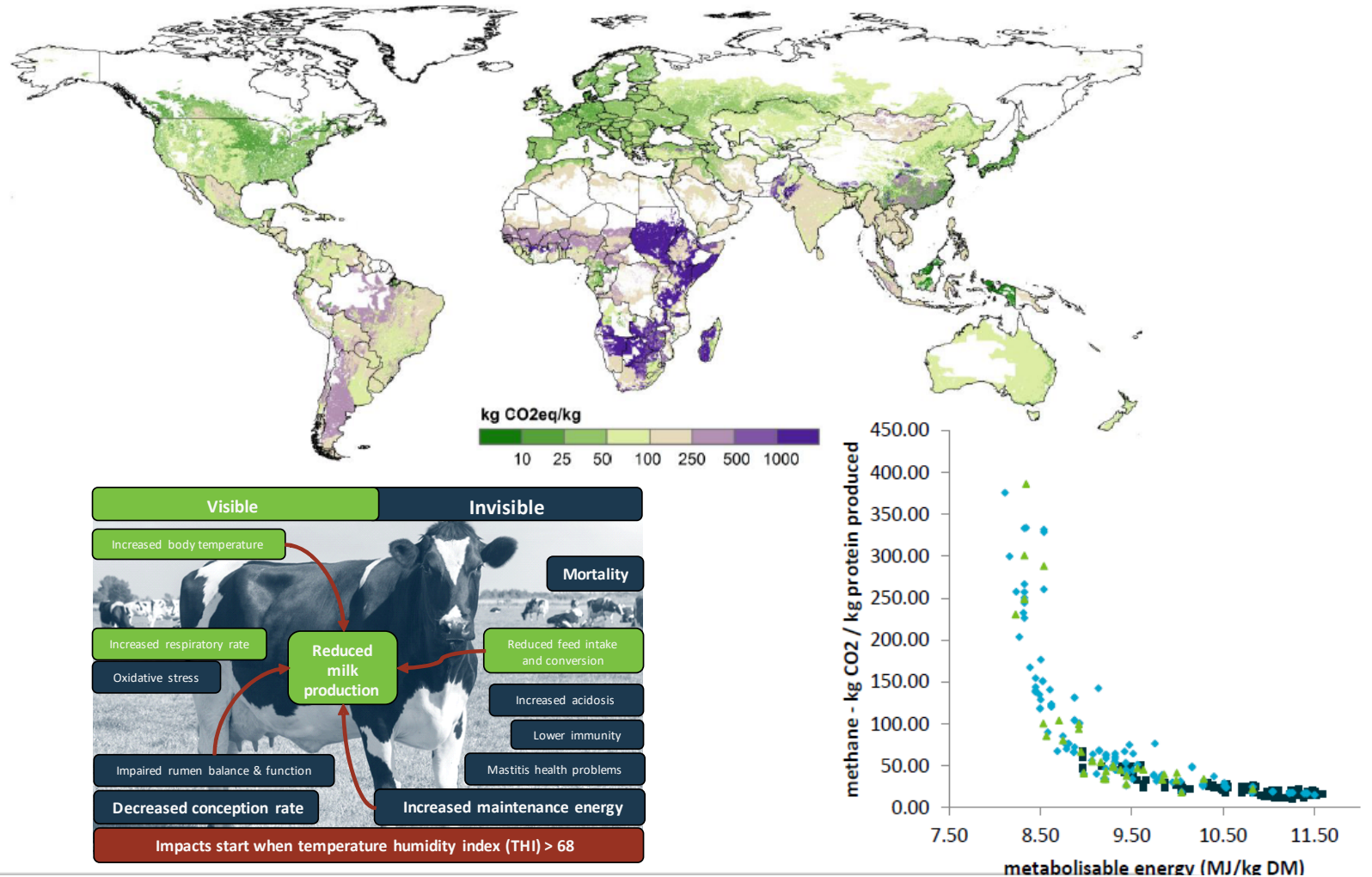
Global Carbon Efficiency Addressed by Gene-Editing

~2/3 of our Planet's 1 Billion cattle are unproductive



ACCELIGEN 2020

Global greenhouse gas efficiency per kilogram of animal protein produced



Thamani Dairy Rollout

Breed Selection

- Efficient production
- Regional adaptation

Edit Selection

- High regional impact
- Low prevalence in selected breeds

Gene Editing

- Selection of donor genetics/material
- Gene editing – Founder production

Validation and deployment

- Performance testing
- Breeding systems and extension

Breed Selection - baseline



GIR	category	Holstein
Avg. 7-9 liters per day	Milk Production	<u>Avg. 25-30 liters/day</u>
<u>Adapted to tropical conditions and known for resistance to ticks and rinderpest</u>	Other traits	Best suited for temperate climates

Breed Selection – production animals



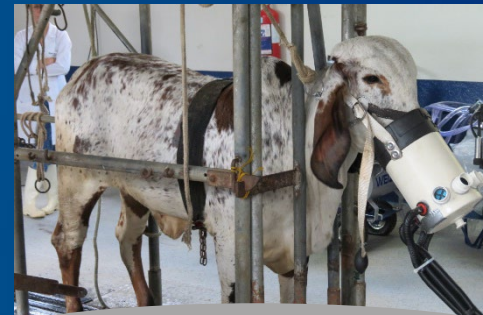
**Top milk producing Girolando cows from
BASA farms (Brazil)**

category	Girolando
Breed Origin	First developed in Brazil & 21 million cows are in milk production
Type	Variable sizes, horned with black and white spotting, 3/8 Gir & 5/8 Holstein
Milk Production	Avg. 10-13 liters/day
Other traits	More suited for tropical climates than Holstein, long-term selection for breeding goals in milk production & stability for adaptation

Edit Selction

Led by Eui-Soo Kim and Tad Sonstegard

Girlando higher milk yield and carbon footprint



Gir



Girolando

CH₄

CH₄

CH₄

CH₄

Carbon/kg milk

Girolando

- 1,000 kg more milk; but,
- **0.2 more kg carbon/kg milk**

Why? Holstein genes - not adapted to heat even after 20 yrs of breeding

Heat stress – convergent adaptations

Slick causing mutations come in many forms – all in the same gene
Nature found a way to adapt animals to the tropics



Breeds of Criollo cattle in the Caribbean Basin that transmit PRLR truncation mutations for the dominant SLICK trait



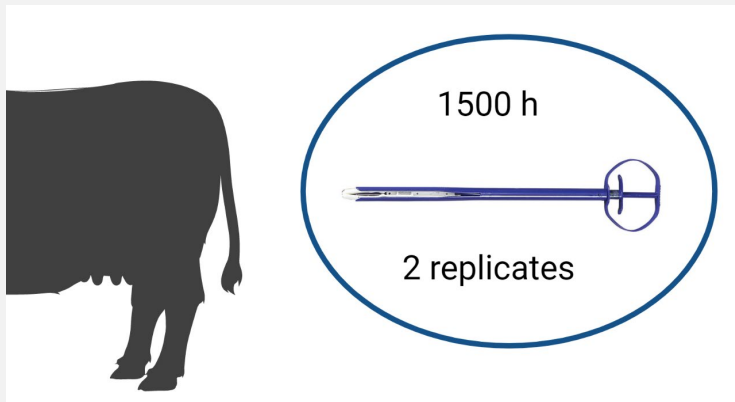
ANIMAL MOLECULAR
AND CELLULAR BIOLOGY



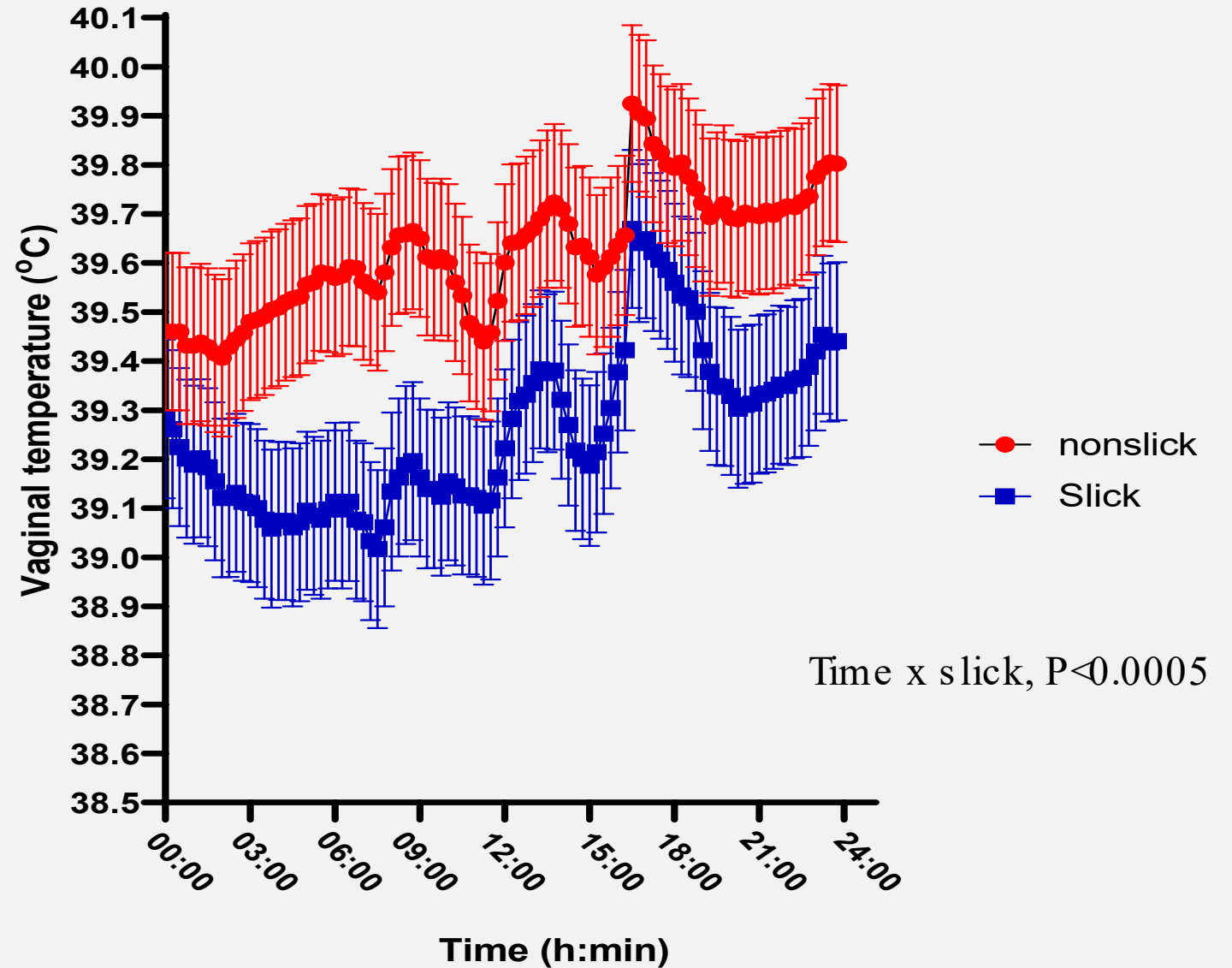
Consequences of gene editing of *PRLR* on thermotolerance, growth, and reproduction in cattle

Camila J. Cuellar¹, Thiago F. Amaral², P. Rodriguez-Villamil³, F. Ongaratto³, D.O-Martinez¹, J.D.A. Losano¹, E. Estrada-Cortés⁴, Q.A. Hoorn¹,
D.O. Rae⁵, Jeremy Block⁶, B.W. Daigneault¹, S. Dikmen⁷, Joao Bittar⁵, Daniel F. Carlson⁴, Tad Sonstegard⁴, and P. J. Hansen¹

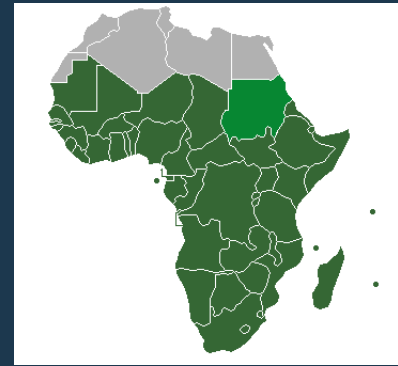
Differences in vaginal temperature during heat stress between slick and non-slick animals



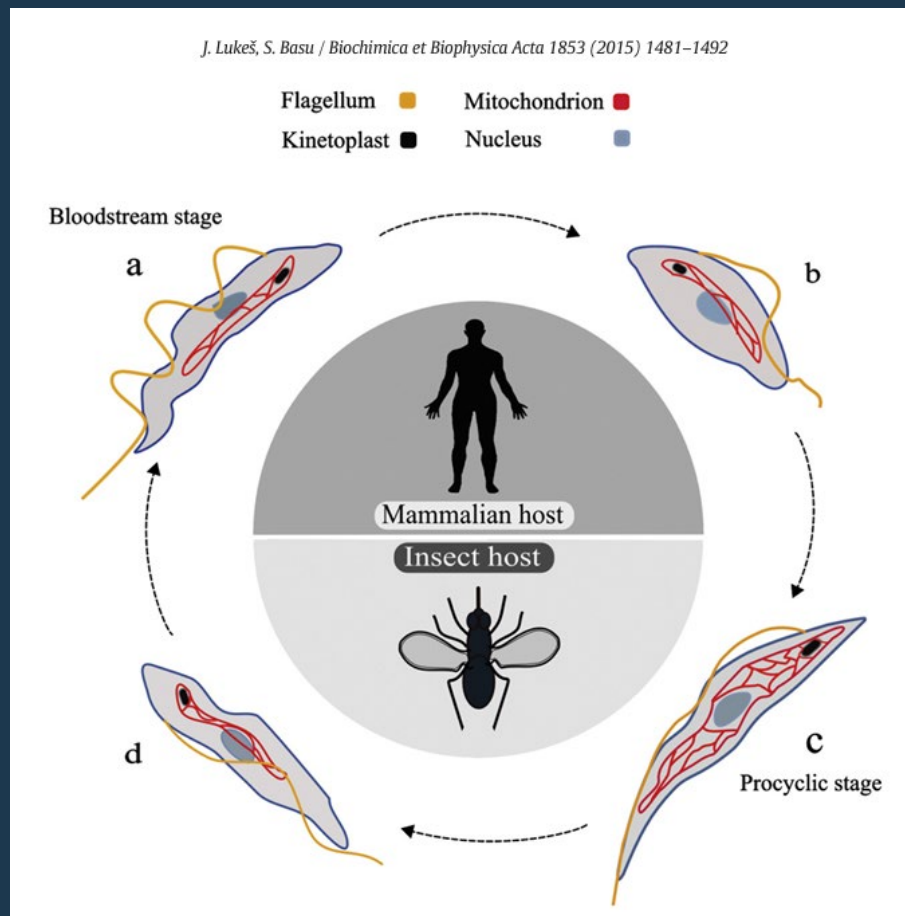
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Trypanosome problem



Heteroxenous life cycle = two obligate hosts



- Trypanosome infection is endemic in sub-Saharan Africa (SSA)
- ~37% of the continent is infested with tsetse fly - 65% of which could be used for livestock production
- *T. brucei* causes sleeping sickness in humans
- Responsible for ~50,000 deaths in humans each year

Trypanotolerant Quantitative Trait Loci (QTL)

➤ QTL of F2 between tolerant x susceptible cattle breeds (2011, Hanotte/ILRI)

- Tolerant breed: N'Dama, African bos taurus
- Susceptible breed: Boran, African bos indicus

➤ QTLs on BTA 2, 4, 7, 16, 27

- BTA2: **ARHGAP15**, innate immune cell (macrophage, neutrophil) and blood cell number
- BTA7: QTL between 10-20 Mb region using microsatellites, **FDX2** at 14.8 Mb (ARS12), ~10 functional candidate genes



N'Dama (**QQ**)



Boran (**qq**)

x

F1

Qt Qt Qt ...

F2

QQ(.25) Qq(.5) qq(.25)



Edit Decisions Consider Allele Frequency

Edit	Breed	Trait	Euro-cont	Holstein	LA-Cri	Zebu	LA-Gir
	(n)		112	28	316	178	490
Holstein	SLICK	Thermo	0	0	84	0	0
Gir	GHR	Milk	9	7	5	0	0
-	DGAT	Milk	55	nd	0	9	6
-	PLAG1	Stature	36	0	44	100	100
Both	DHRS4	Tryp	25	15	18	31	13
Both	FDX2	Tryp	1	0	7	20	0
Gir	ARhGAP15	Tryp		Low*		Low*	
Holstein	MARCO	<i>M. Bovis</i>	2	2	11	76	100

Ambitious Goals

16 genetically diverse founders with 4 edits each
Timeline < 2 years



Bull HA1



Bull HB2



Bull HC1



Bull HD1



Bull G1+



Bull G2+



Bull G3+



Bull G4+



Heifer HA3



Heifer HB3



Heifer HC4



Heifer HD4



Heifer GA+



Heifer GB+



Heifer GC+



Heifer GD+



Holstein

vs

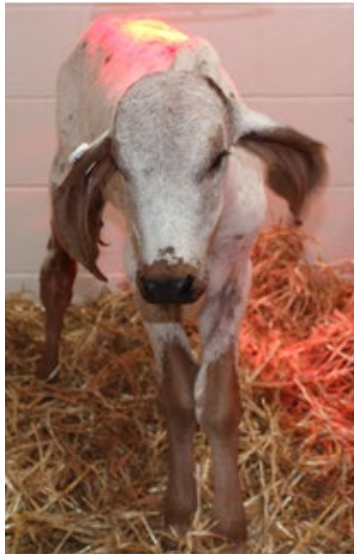
Gir

- 13 edited lines
- 10 live in herd
- 1 pregnancy

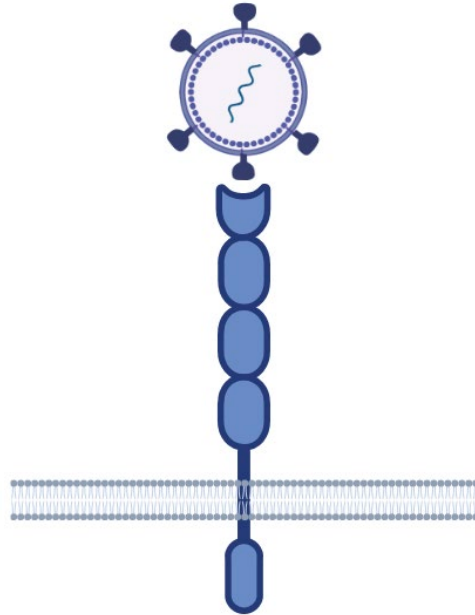
- 9 edited lines
- 1 live in herd
- 7 pregnancies

First Gene-Edited Calf with Reduced Genetic Susceptibility to Bovine Viral Diarrhea Virus

Drs. Aspen M. Workman, Micheal P. Heaton
USDA, ARS, US Meat Animal Research Center



CD46 is the cellular receptor for BVDV



Essential Functions:

Complement regulator

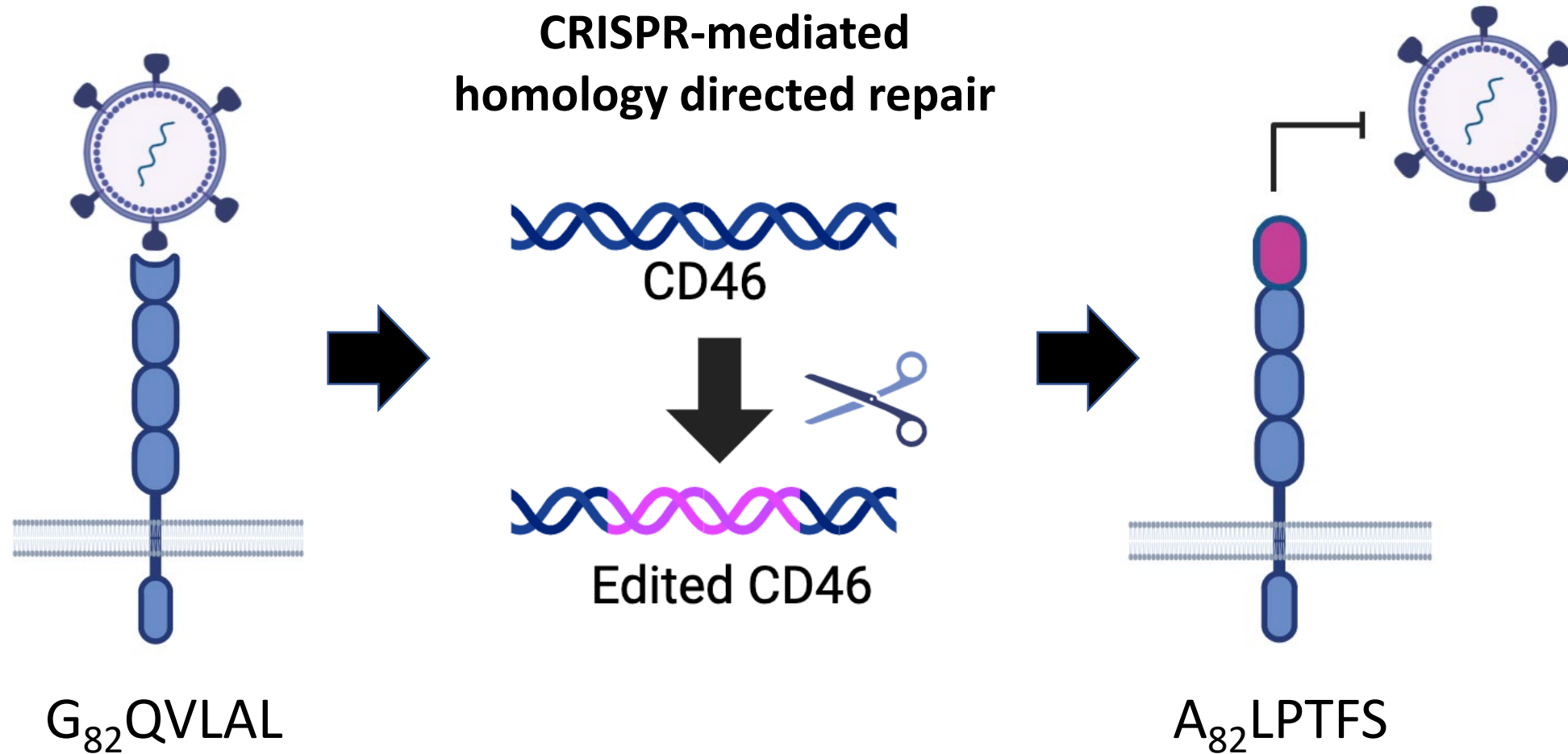
T-cell activation

Autophagy

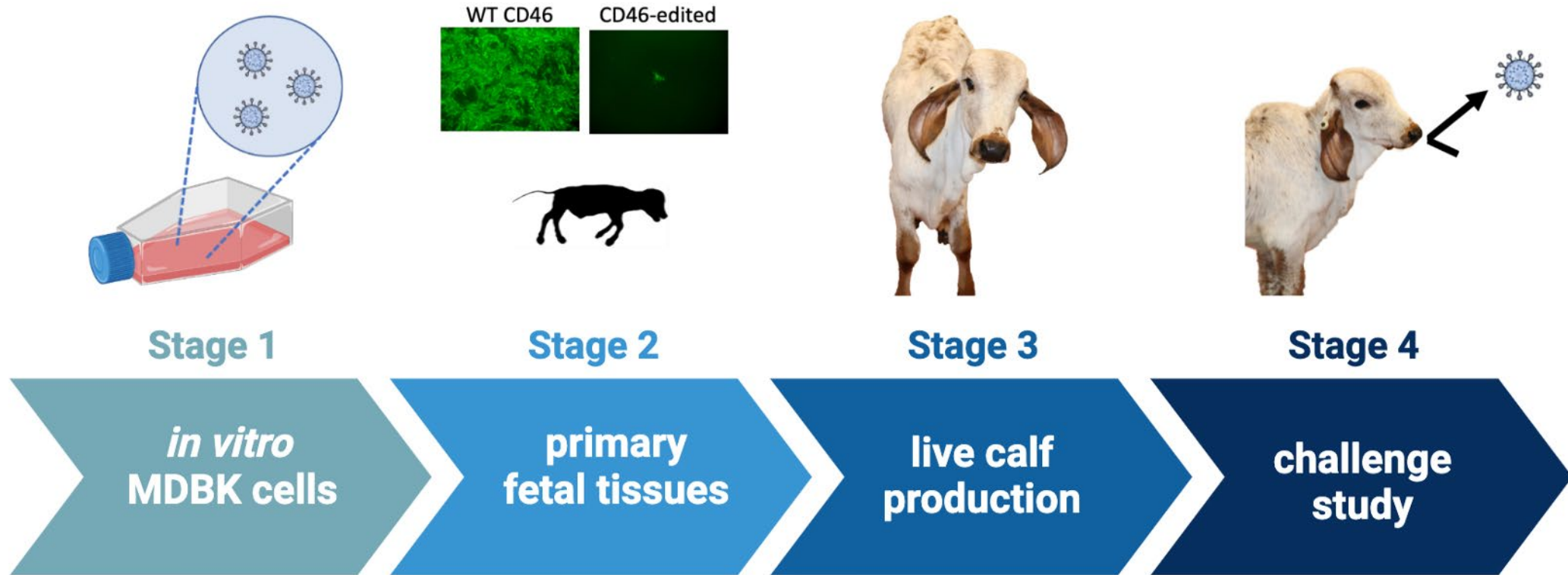
Reproduction

Knockout of *CD46* cannot be employed as a strategy for controlling BVDV infection cattle

Hypothesis



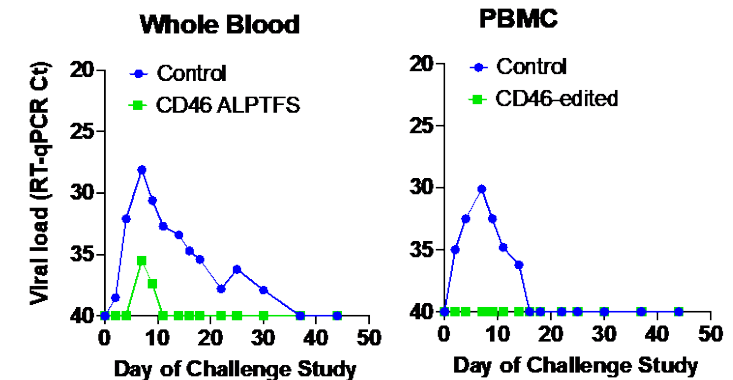
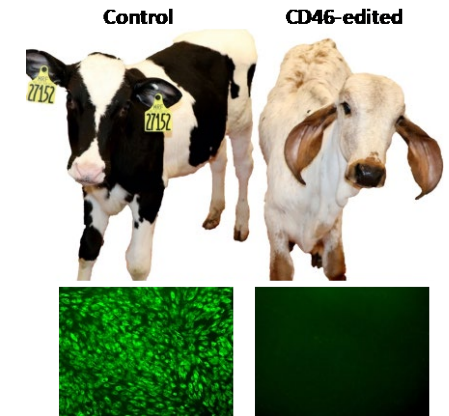
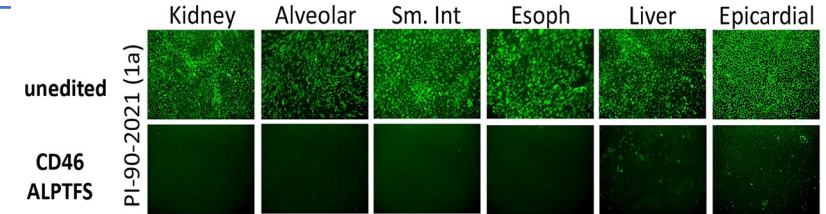
Experimental Approach



stepwise experiments to evaluate the impact of *CD46*-edits on BVDV susceptibility and calf health

BVDV Summary

- CD46-edited fetal kidney, lung, small intestine, esophagus, liver, and heart cells all had significantly reduced susceptibility to BVDV when infected *ex vivo*.
- Primary skin fibroblasts, lymphocytes, and monocytes from the live *CD46*-edited calf had dramatically reduced BVDV susceptibility.
- The strong reduction in both the duration and peak viral RNA load in the blood of the *CD46*-edited calf was consistent with reduced viral replication in tissues *in vivo*.



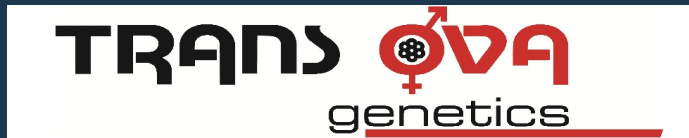
Conclusions

- What traits might be pursued within the next two years?
 - Predominantly naturally occurring variants
 - Regulatory advantages
 - More variants will be mined with impact on resilience, efficiency, and quality
- How will they be deployed?
 - Multiplexing to address more challenging objectives
 - Best chance to adapt productive genetics to new environments
 - Enables stacking of variants that are QTL or unproven candidates

Thank You

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<https://www.acceligen.com/>

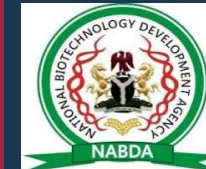


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Discussion topics

- Should native QTL be deployed without confirmation of impact on traits?
- What level of trait characterization is required for novel alleles when they are:
 - Informed by variation in other species?
 - A product of rational design?
 - A product of (*in vitro*) evolved gene function?