Never Stop Improving

Genetic Trends in Selecting for Disease Resistance to PRRS

Pinoy Pork Challenge 2018

October 4, 2018

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PRRS is a VERY Costly Disease!!!

- Focus Areas to Prevent or Minimize the Impact
  1. Border control
  2. Biosecurity
  3. Management
  4. Vaccines
  5. Treatment
  6. Maintaining high health vs stable
  7. **Genetics**

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Geneticists Have Focused in 3 Areas

1. Selection for "Robust" pigs in a commercial environment
2. Selection for PRRS "Resilient" pigs
3. Selection for PRRS "Resistant" pigs

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Resistance, Tolerance, or Resilience?

Resistance

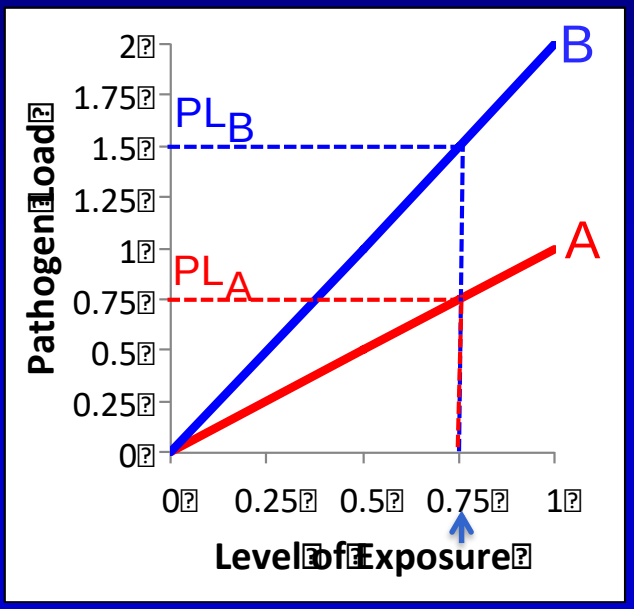
Ability to prevent infection or limit replication

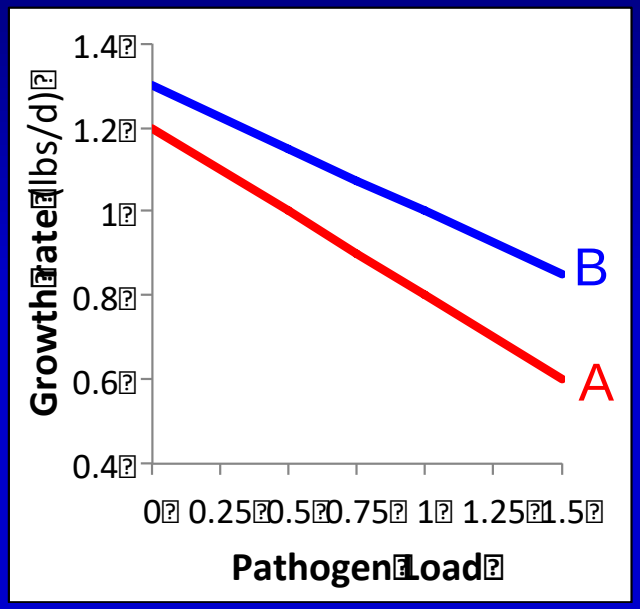
Tolerance

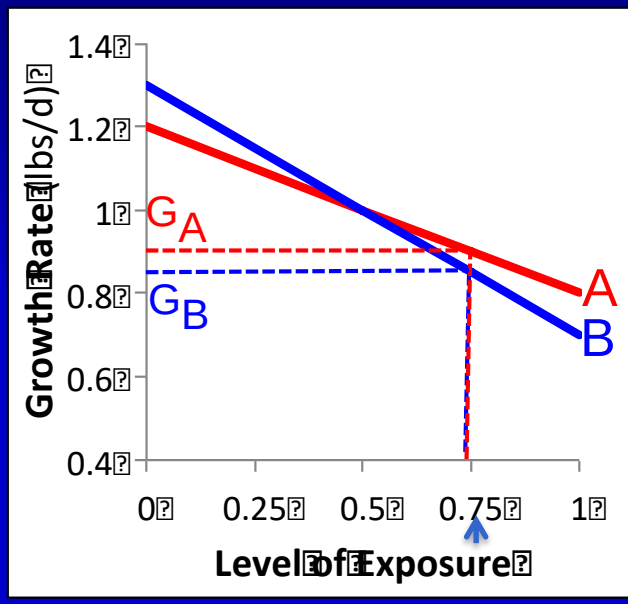
Ability to maintain performance as pathogen load increases

Resilience/ Robustness

Ability to maintain performance as pathogen exposure increases







Selection for "Robust" Pigs in a Commercial Environment

Black Box Method

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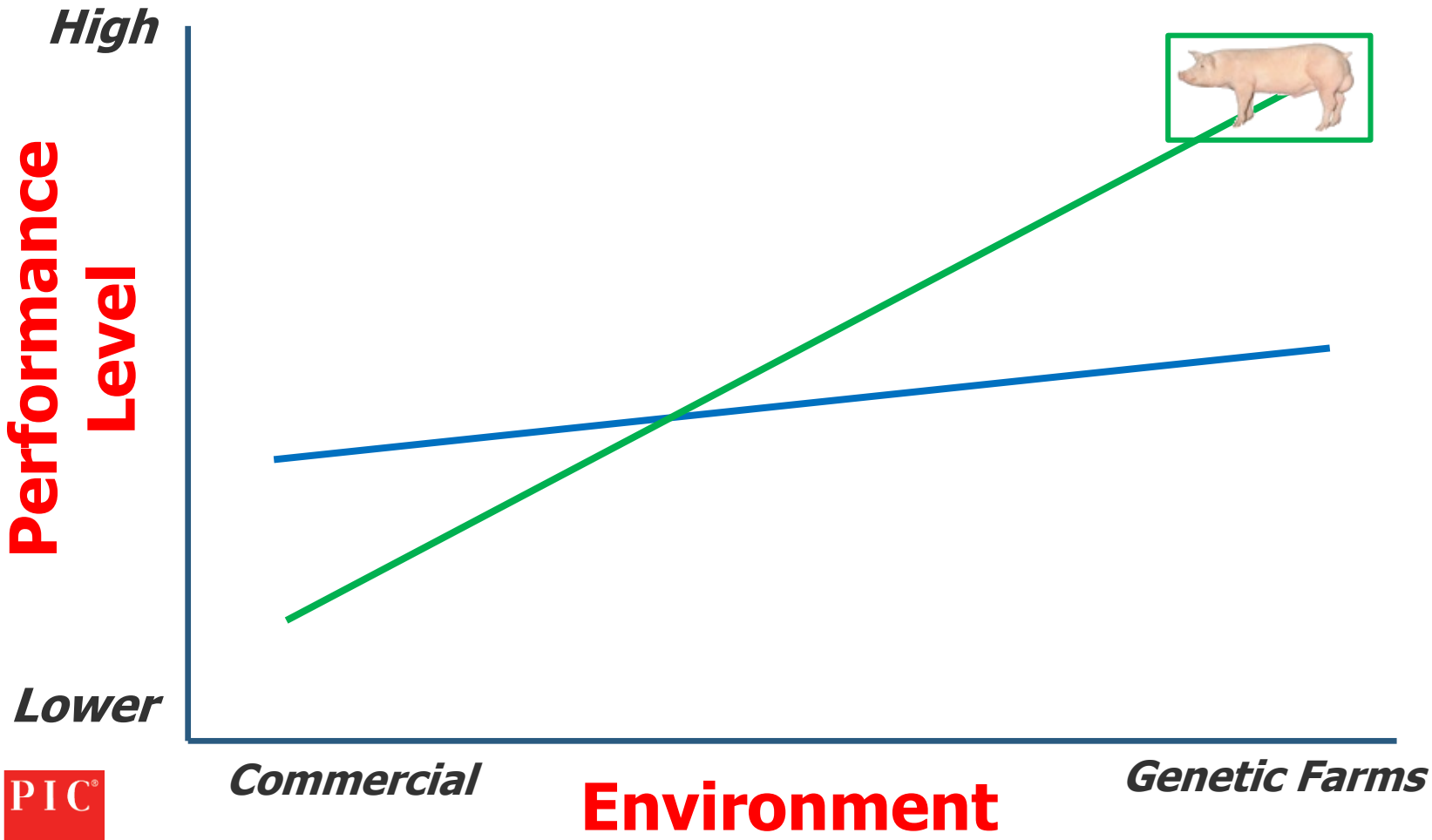
The Best Genetics in the Genetic Farms are not Always the Best Genetics in the Commercial Farms



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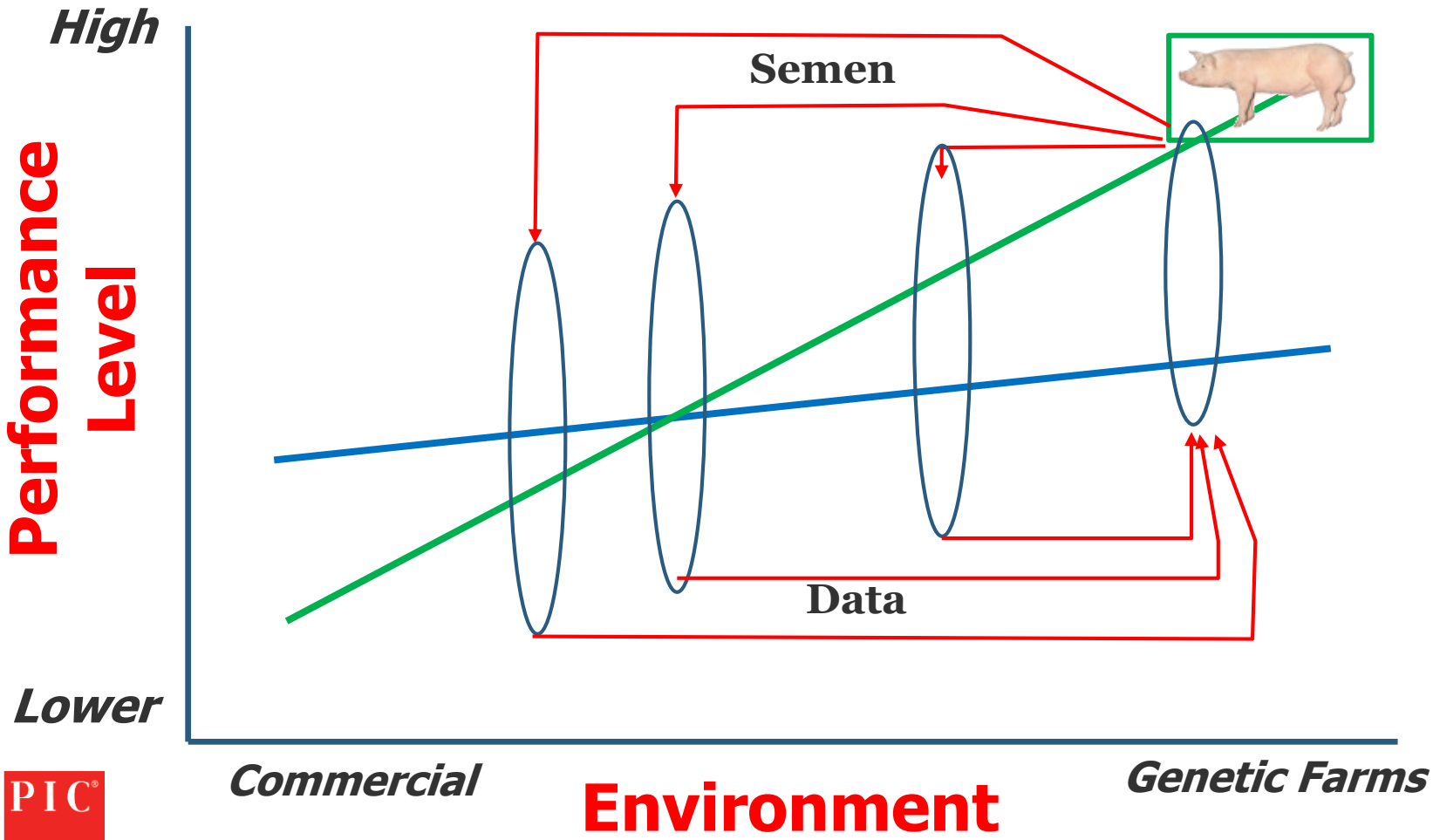
The Best Genetics in the Genetic Farms are not Always the Best Genetics in the Commercial Farms



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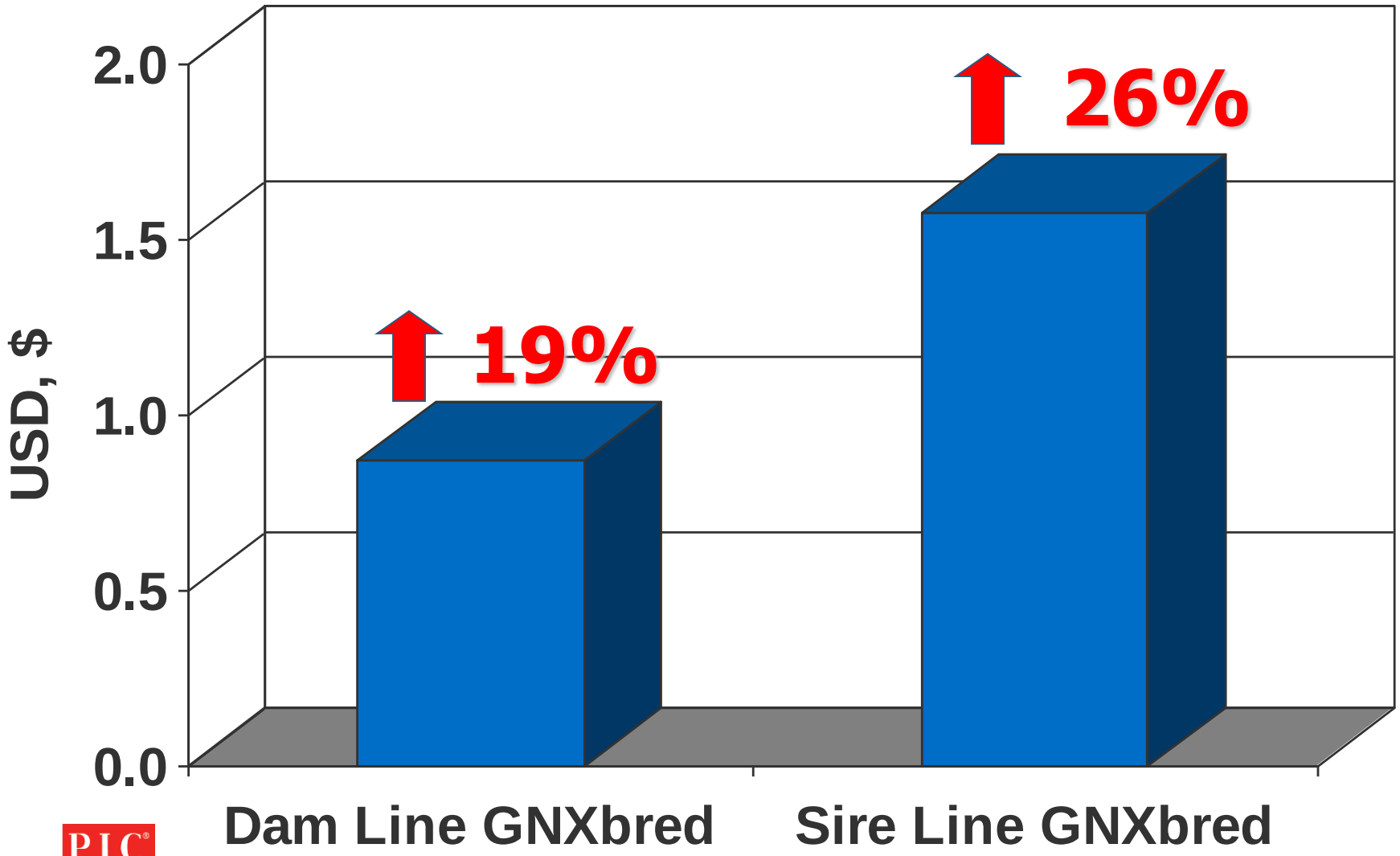
Benefit of Commercial Test/Pig after 5 Yrs



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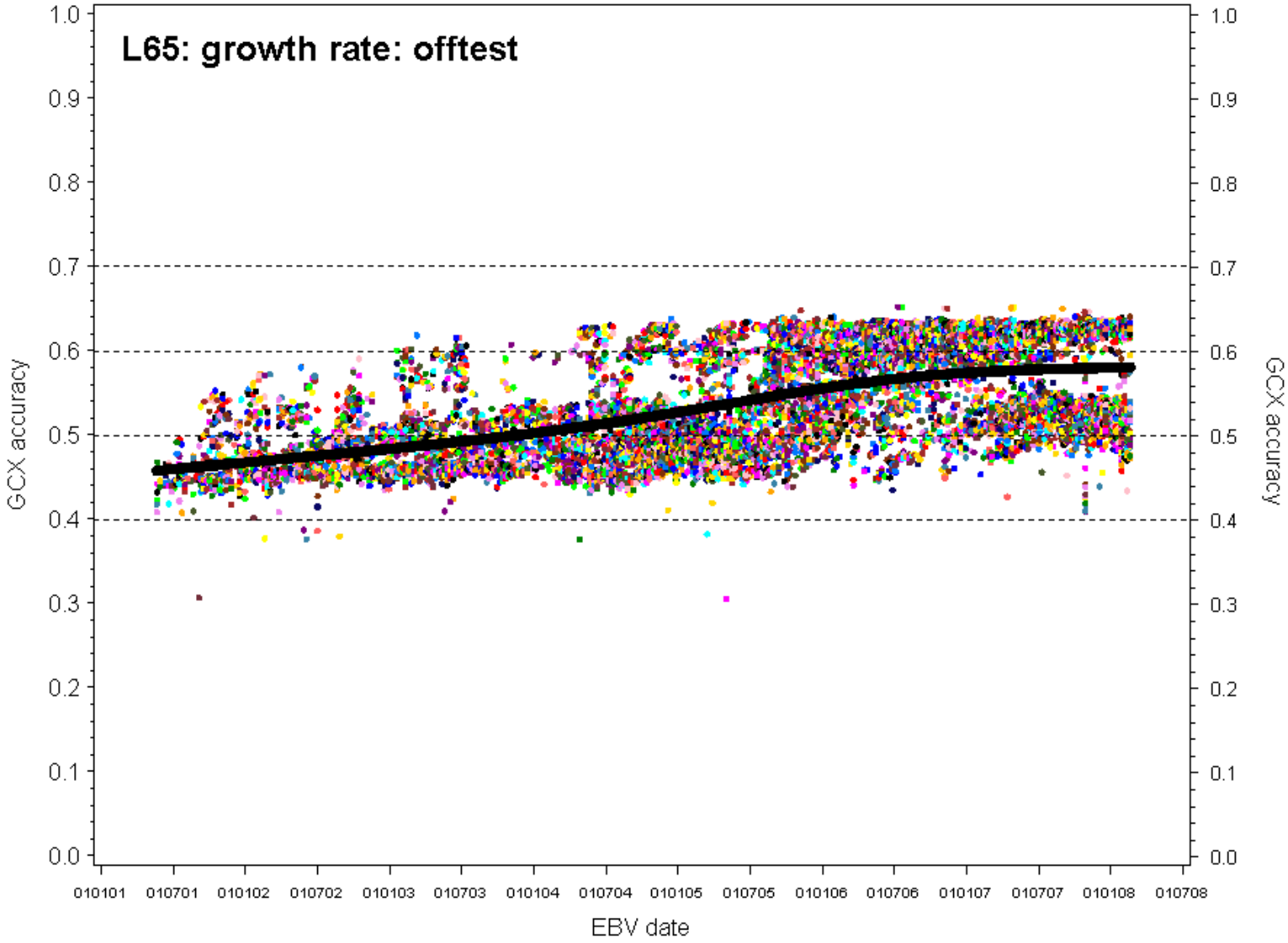
Beneficial for Growth Rate



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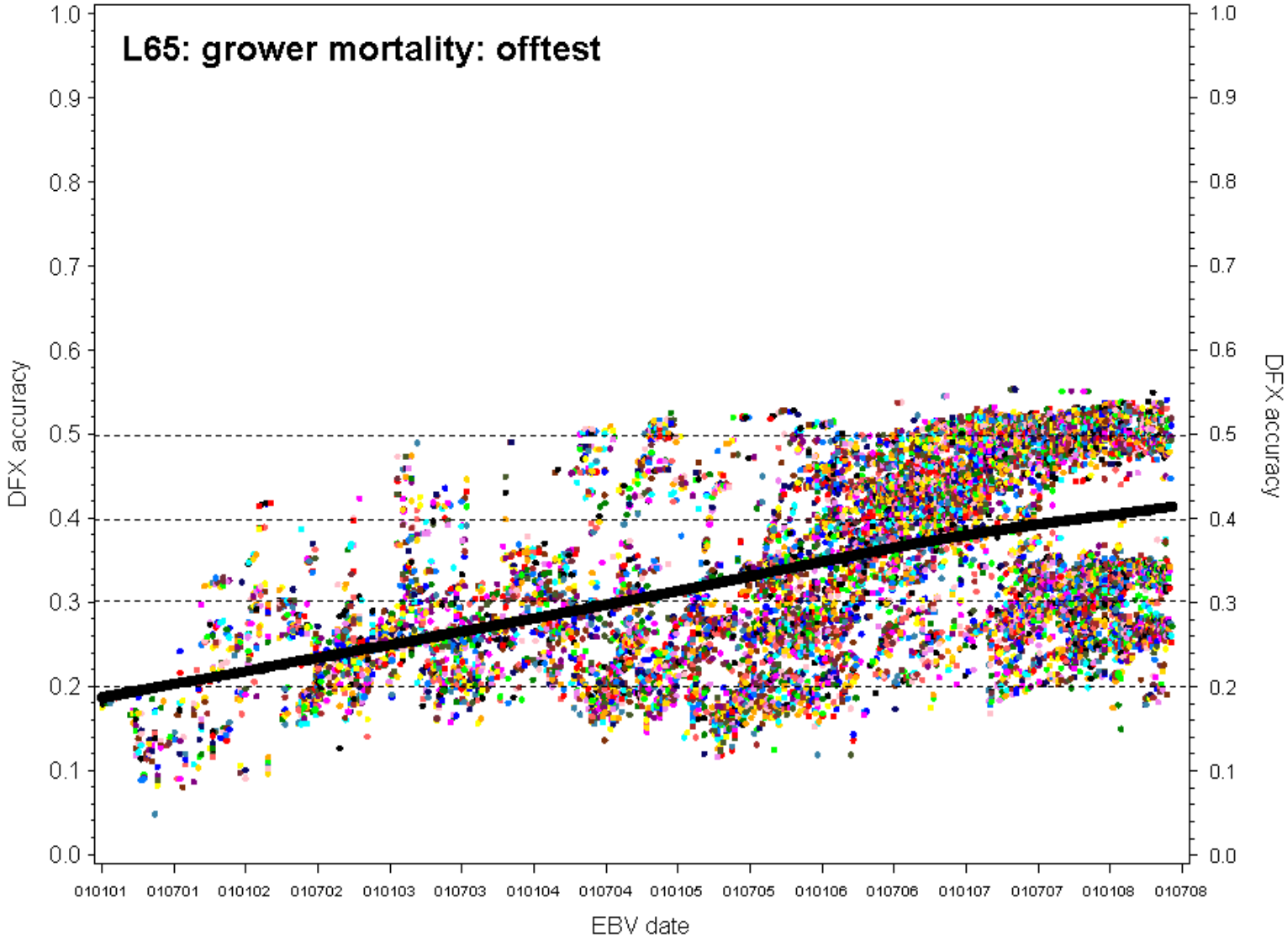
Extremely Beneficial for Wean to Finish Mortality



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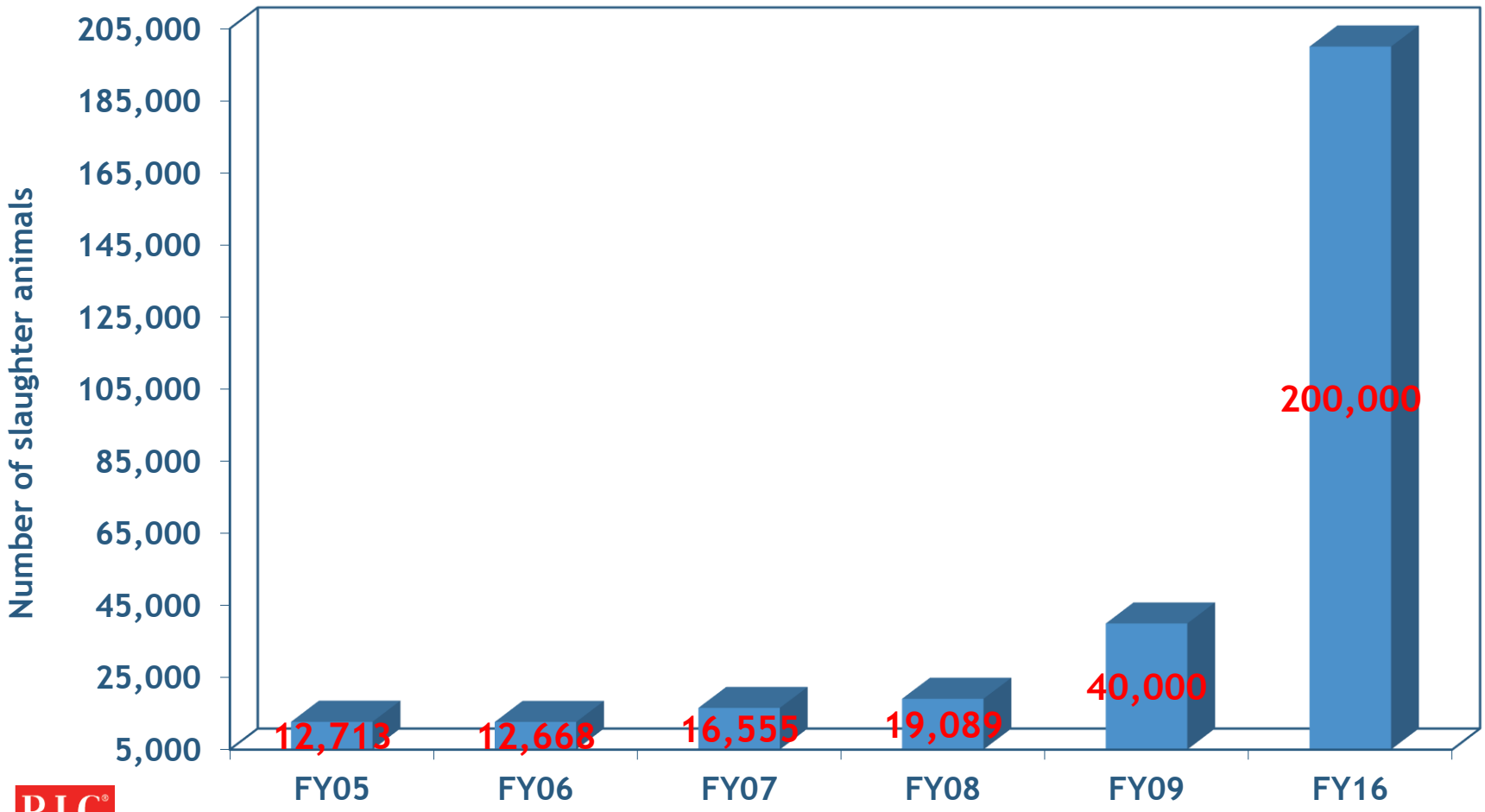
Number of Slaughter Pigs in the Genetic Evaluation



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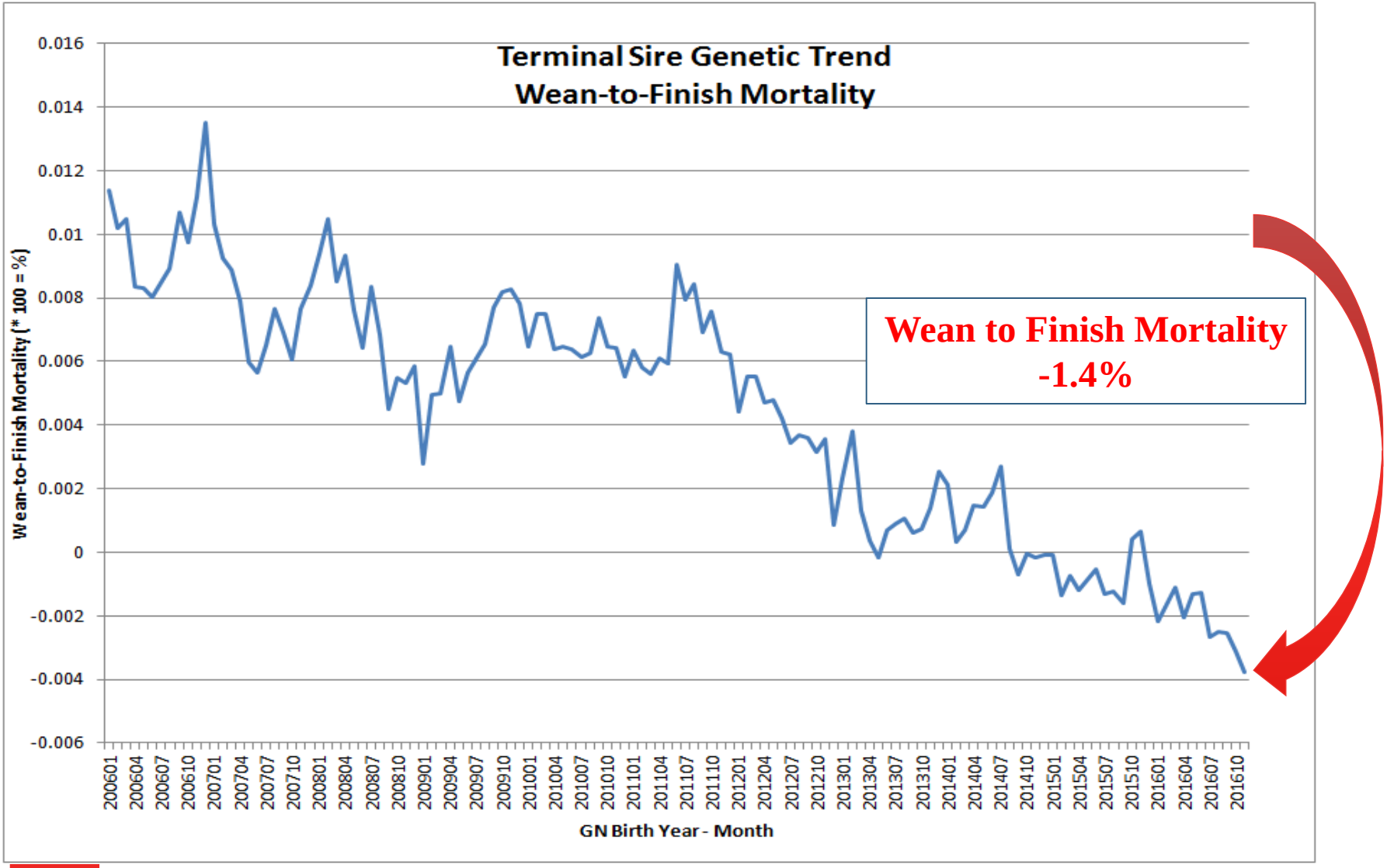
Significant Improvement has been Achieved



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Selection for PRRS “Resilient” Pigs

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2007

PHGC
PRRS Host Genetics Consortium

Objective

Use genomics to identify genes / genomic regions associated with resistance / susceptibility to PRRS virus infection

Led by

Joan Lunney – USDA – ARS Beltsville
Bob Rowland – Kansas State University
Jack Dekkers – Iowa State University

Strong Industry Participation

PHGC Breeding Companies

Fast Genetics, Genesus, Choice Genetics

PIC/Genus, TOPIGS, PigGen Canada

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USDA
United States Department of Agriculture
National Institute of Food and Agriculture

Evolution of PRRS Host Genetics Research

1. Experimental infection of nursery pigs with NVSL

2. Experimental infection of nursery pigs with KS06

Trial Number	n	Breed			PRRSv Isolate
1-3	530	LW	x	LR	NVSL
4	195	Duroc	x	LW/LR	
5	184	Duroc	x	LR/LW	
6	123	LR	x	LR	
7	194	Pietran	x	LW/LR	
8	188	Duroc	x	LW/LR	
15	184	LR	x	LW	
10	176	LR	x	LW	KS06
11	176	LW	x	LR	
12	174	LR	x	LW	
14	180	Duroc	x	LR/LW	

1. Experimental co-infection of nursery pigs: PRRS + PCV2 (incl. PRRS vaccination)

1. Field trials

2. Natural Challenge Model

➔ Disease Resilience

Nursery Pig Challenge Model

PHGC
PRRS Host Genetics Consortium

R.R.R. Rowland et al., Kansas State University

Groups of ~200 commercial crossbred nursery pigs infected with specific PRRS virus isolate

Acclimation

Day post infection: -7, 0, 4, 7, 11, 14, 21, 28, 35, 42

- Birth
- -7: Serum, Antibiotics
- 0: Weight, Serum, Inoculation
- 4: Serum
- 7: Weight, Serum
- 11: Serum
- 14: Weight, Serum
- 21: Weight, Serum
- 28: Weight, Serum
- 35: Weight, Serum
- 42: Weight, Serum, Slaughter, Ear for DNA

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Host Response Phenotypes

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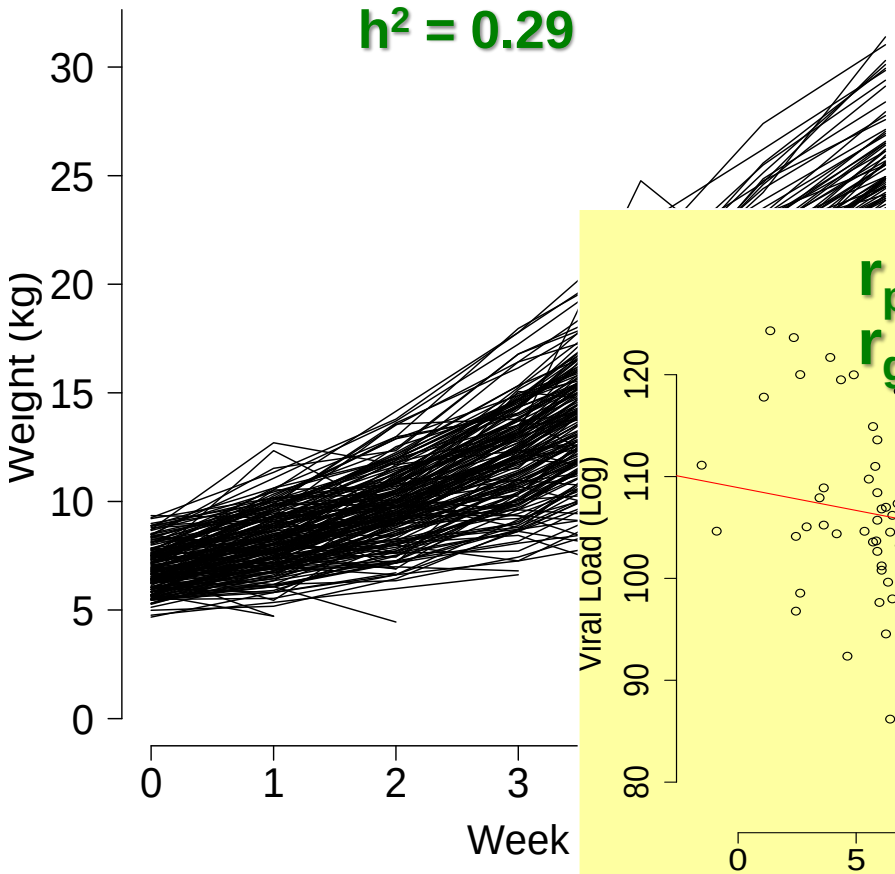
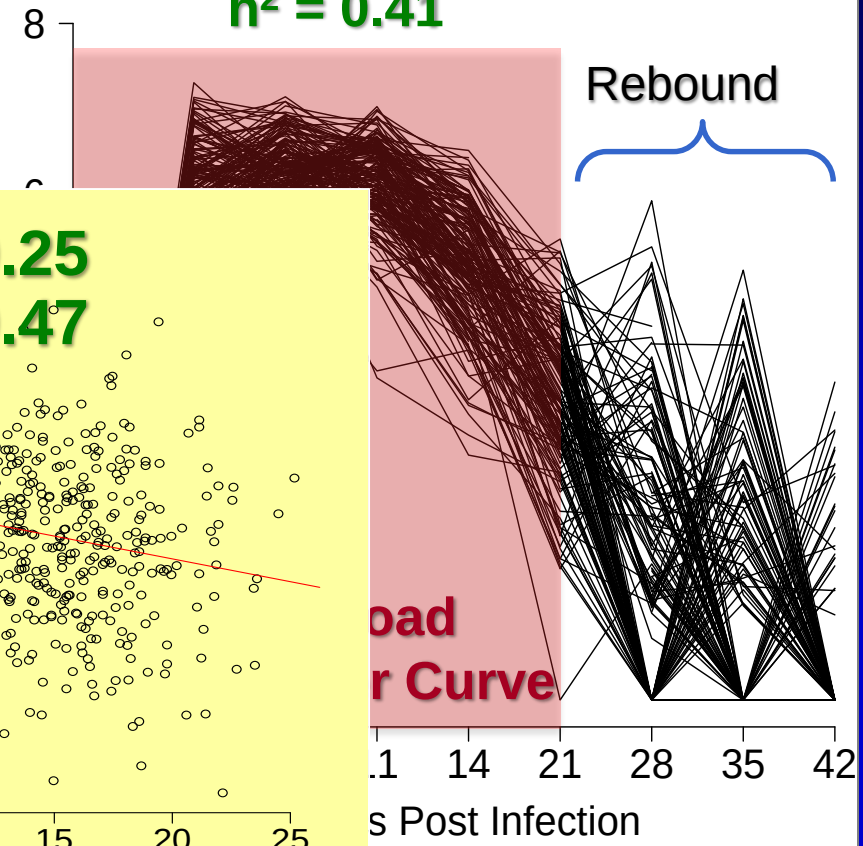
Body weight

$h^2 = 0.29$

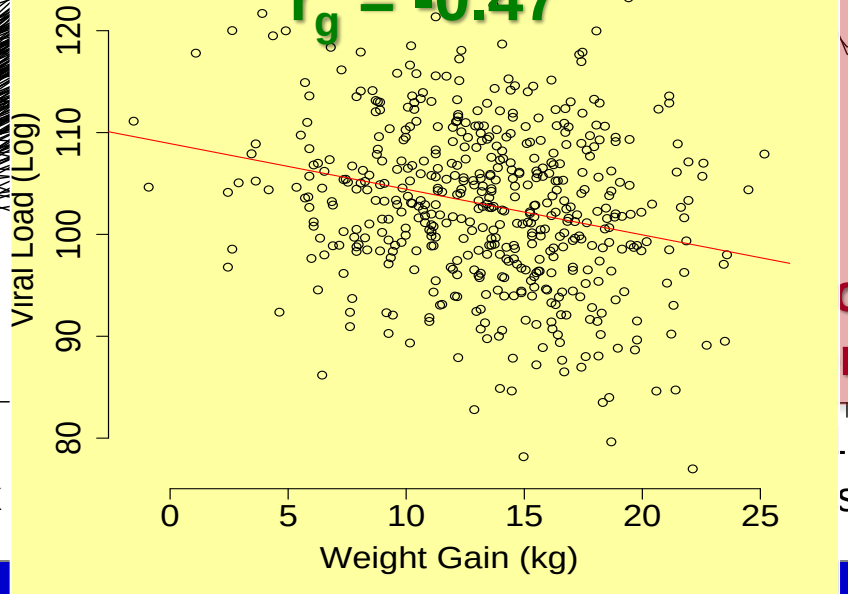


Log(viremia)

$h^2 = 0.41$



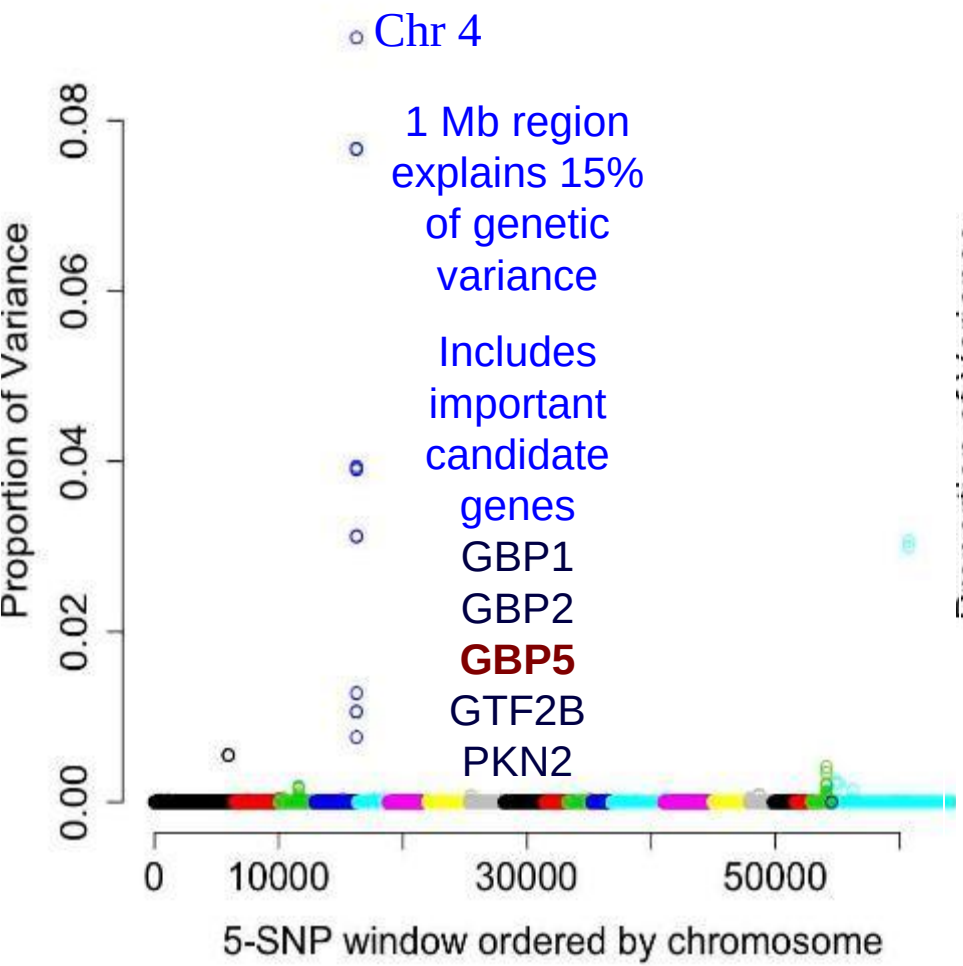
$r_p = -0.25$
$r_g = -0.47$



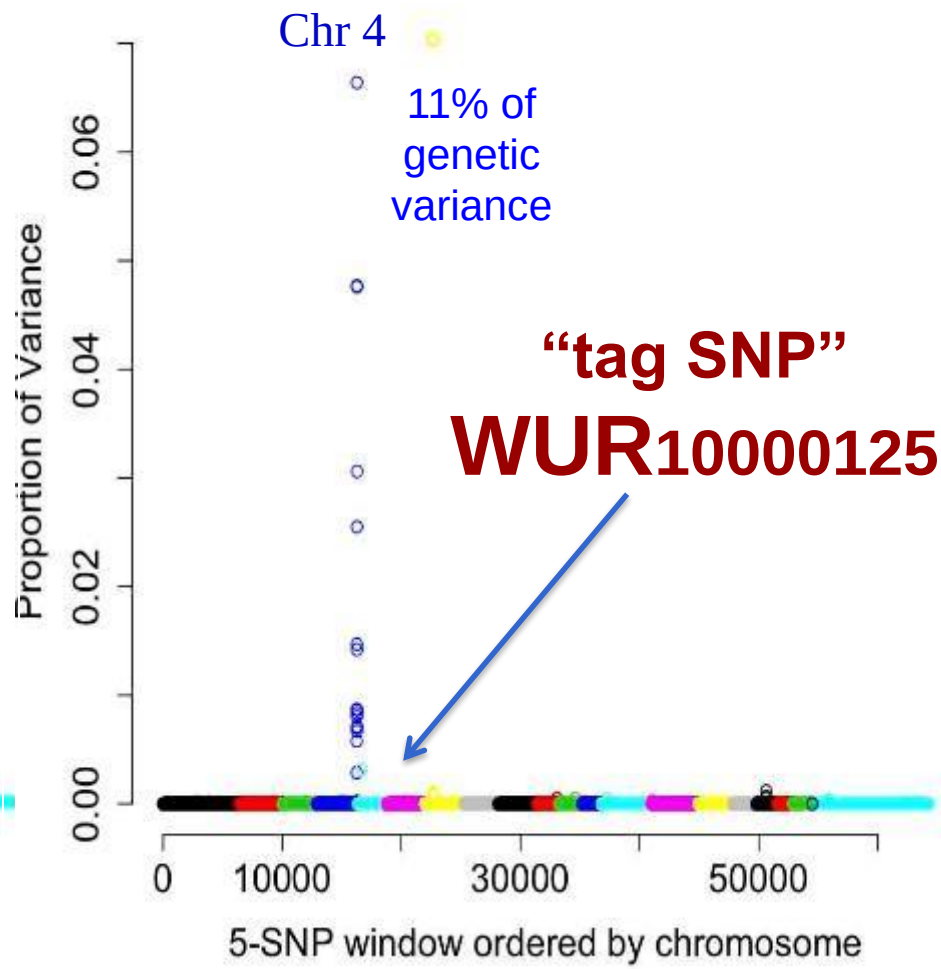
Genome-wide Association Studies
Trials 1-8 NVSL infections

Boddicker et al.
JAS 2012, GSE 2014a,b

Viral Load



Weight Gain



Effects of a Major Gene on SSC4 NVSL

Boddicker et al. 2012, 2014a,b

Trial Number	n	Breed			PRRSv Isolate
1-3	530	LW	x	LR	NVSL
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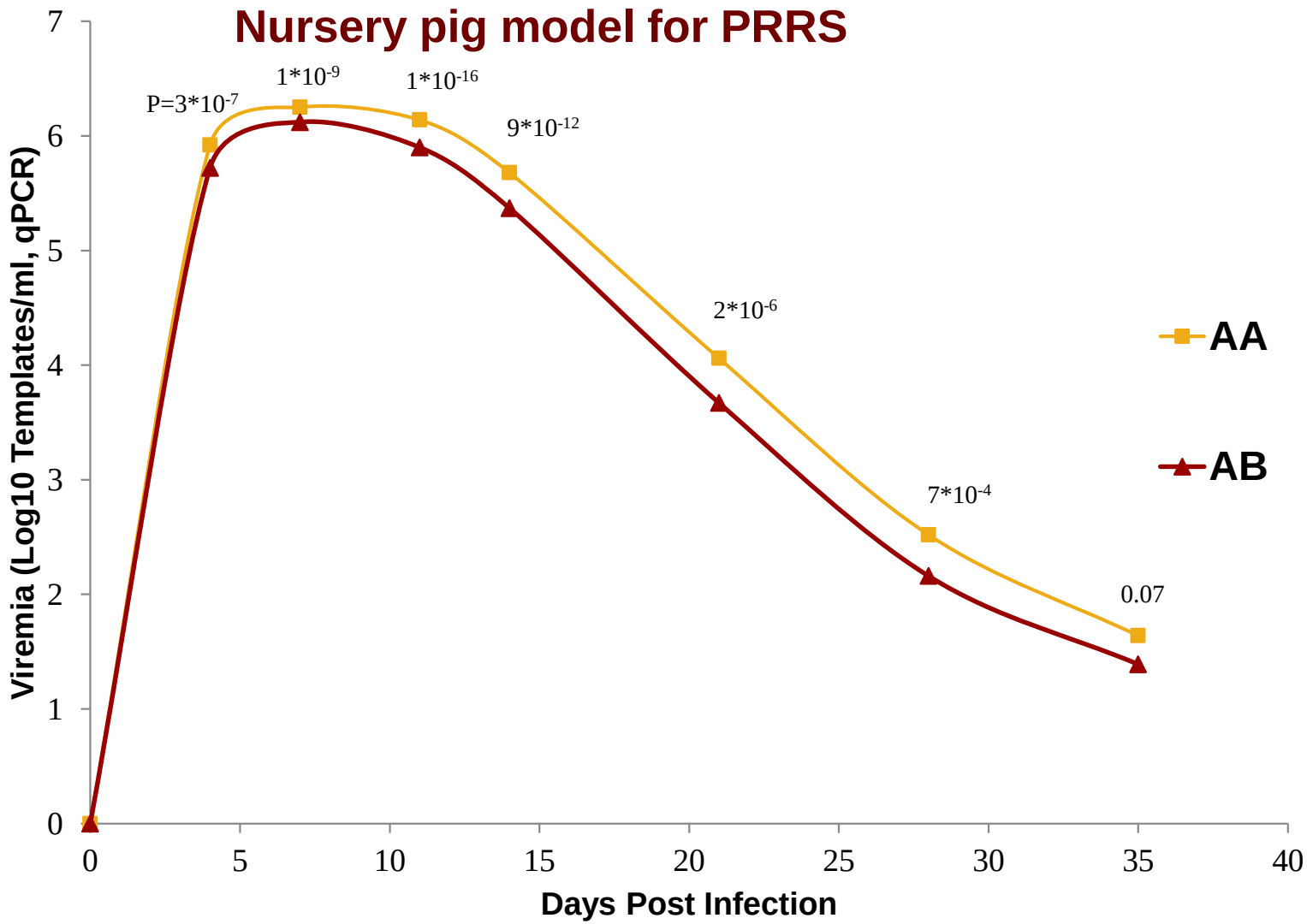

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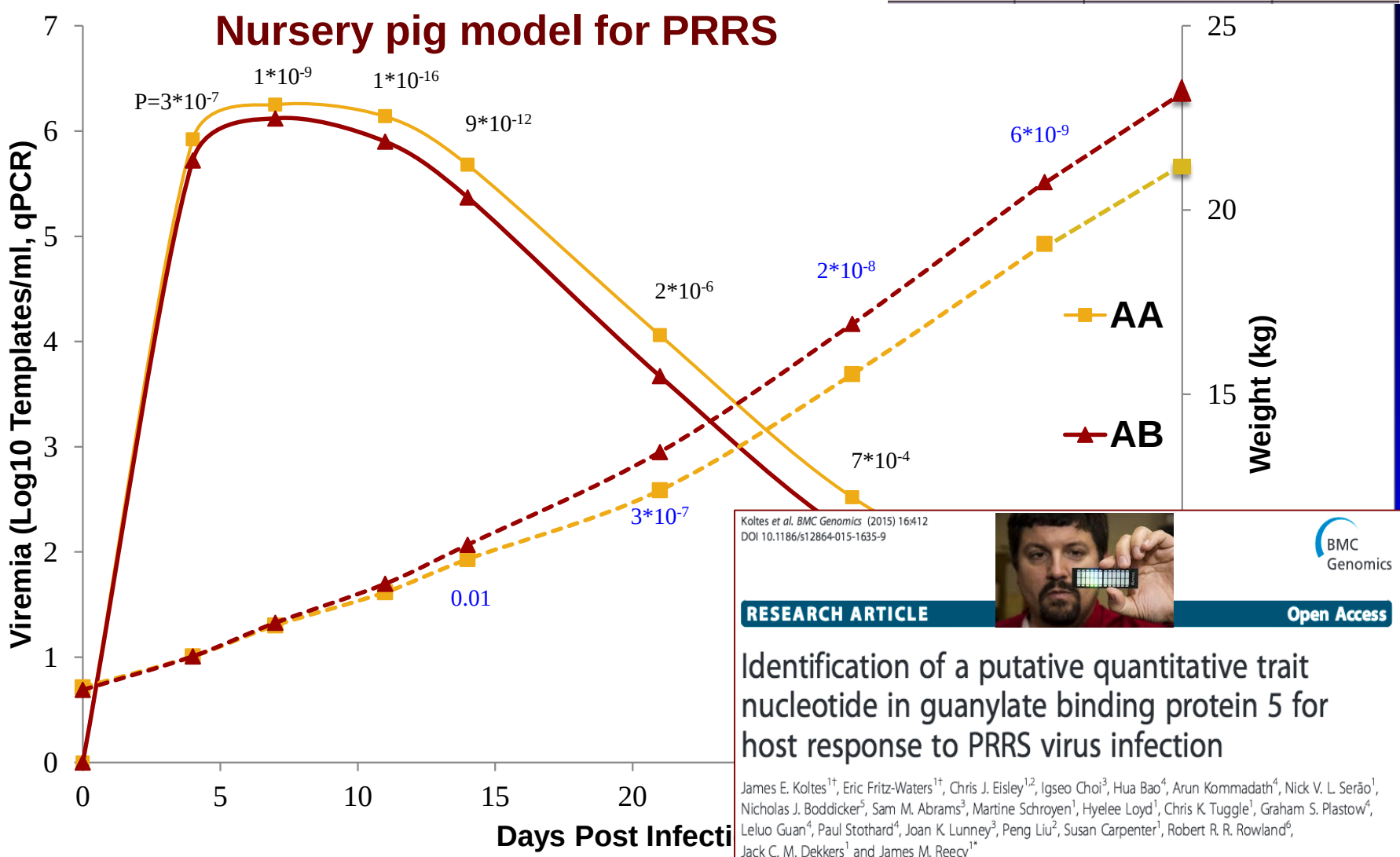
8	188	Duroc	x	LW/LR	
15	184	LR	x	LW	



Koltes et al. BMC Genomics (2015) 16:412
DOI 10.1186/s12864-015-1635-9

BMC Genomics

RESEARCH ARTICLE Open Access

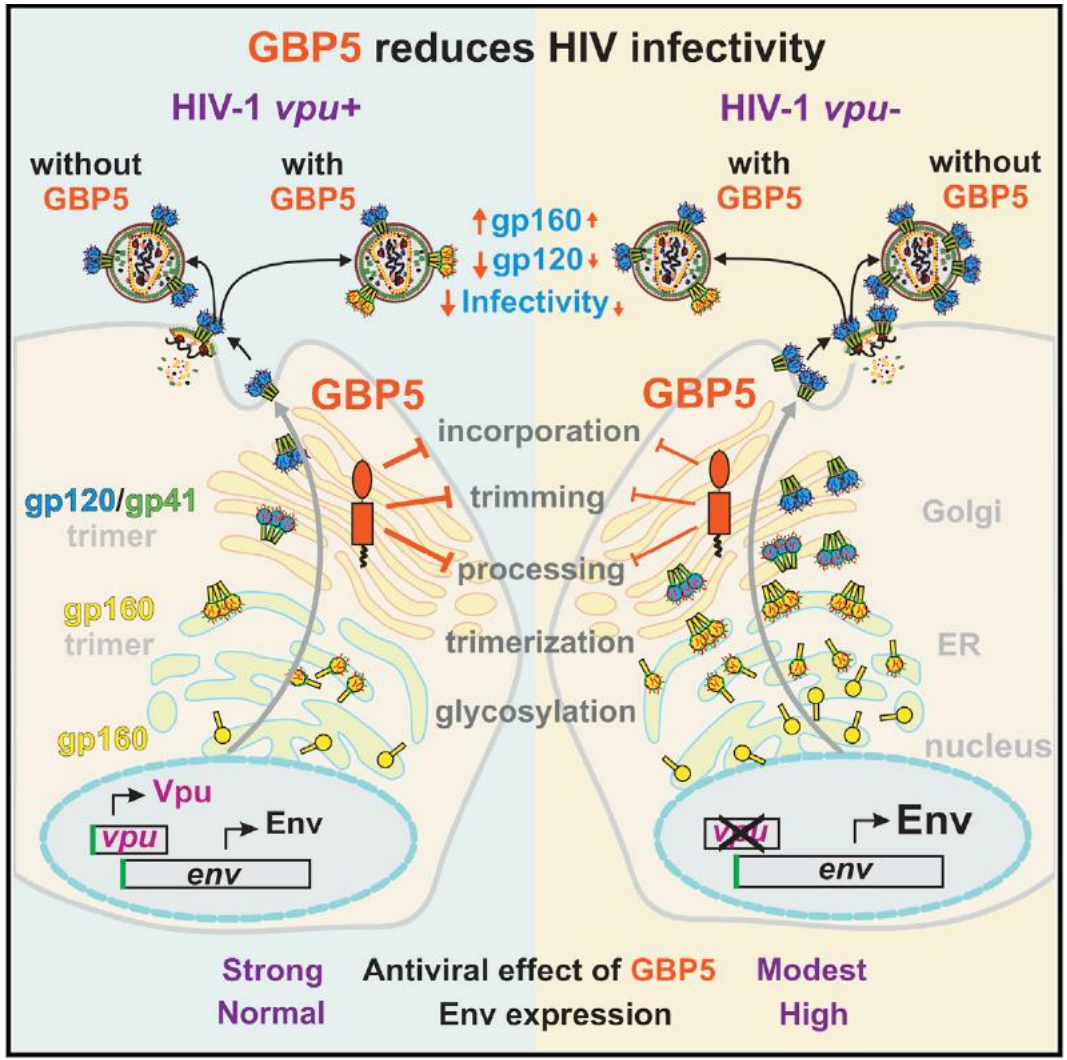
Identification of a putative quantitative trait nucleotide in guanylate binding protein 5 for host response to PRRS virus infection

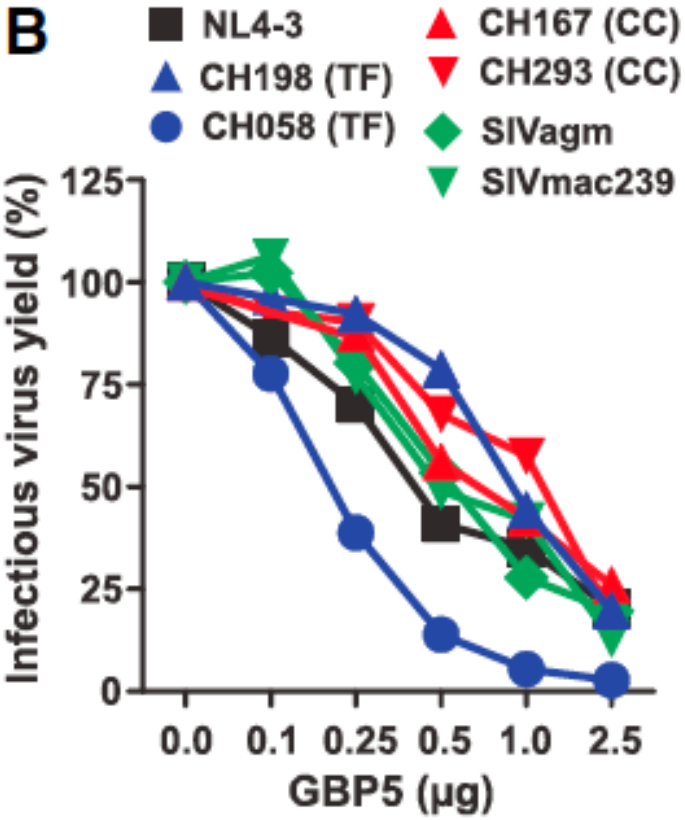
James E. Koltes[1†], Eric Fritz-Waters[1†], Chris J. Eisley[1,2], Igseo Choi[3], Hua Bao[4], Arun Kommadath[4], Nick V. L. Serão[1], Nicholas J. Boddicker[5], Sam M. Abrams[3], Martine Schroyen[1], Hyelee Loyd[1], Chris K. Tuggle[1], Graham S. Plastow[4], Leluo Guan[4], Paul Stothard[4], Joan K. Lunney[3], Peng Liu[2], Susan Carpenter[1], Robert R. R. Rowland[6], Jack C. M. Dekkers[1] and James M. Reecy[1*]

Guanylate Binding Protein (GBP) 5 Is an Interferon-Inducible Inhibitor of HIV-1 Infectivity

2016. Cell Host & Microbe 19, 504–514

Christian Krapp,[1,6] Dominik Hotter,[1,6] Ali Gawanbacht,[1] Paul J. McLaren,[2] Silvia F. Kluge,[1] Christina M. Stürzel,[1] Katharina Mack,[1] Elisabeth Reith,[1] Susanne Engelhart,[1] Angela Ciuffi,[3] Veit Hornung,[4] Daniel Sauter,[1] Amalio Telenti,[5] and Frank Kirchhoff[1,*]





Effects of a Major Gene on SSC4 NVSL / KS06

Hess et al. JAS 2015

Trial Number	n	Breed			PRRSv Isolate
1-3	530	LW	x	LR	NVSL
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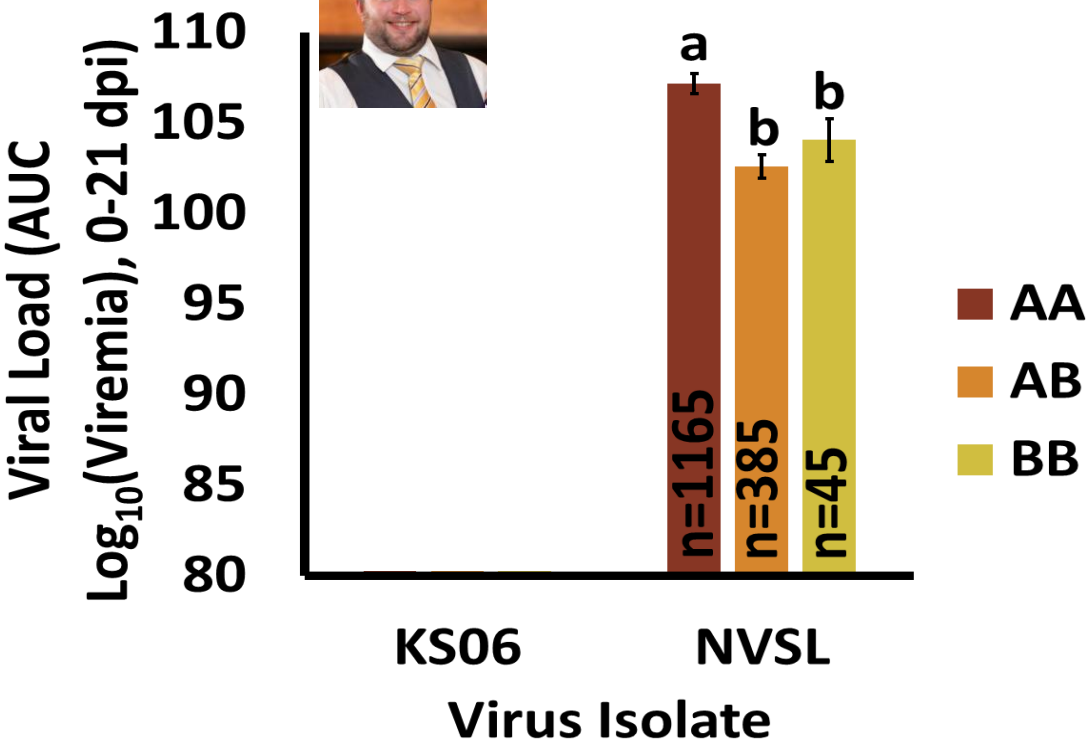
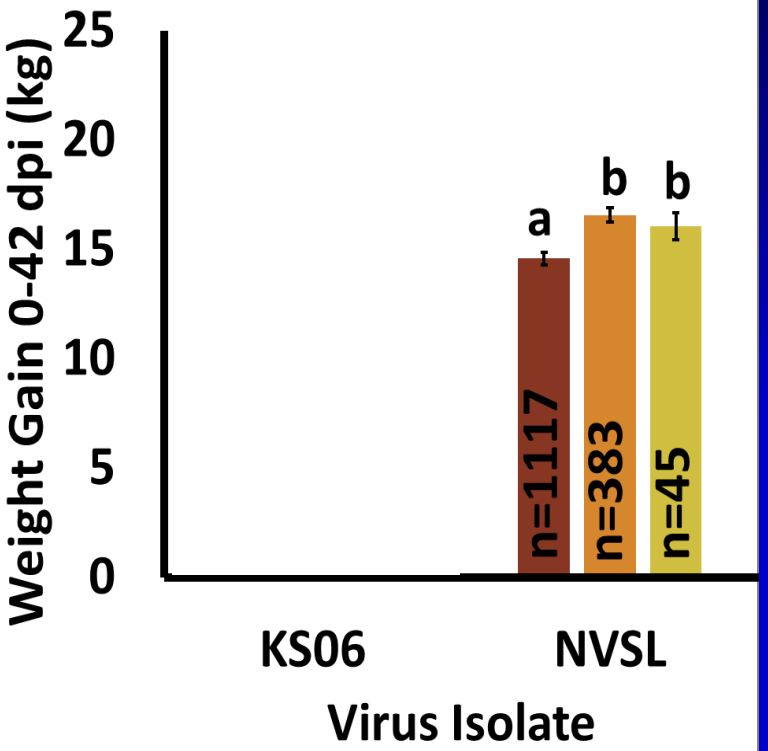
7	194	Pietran	x	LW/LR	
8	188	Duroc	x	LW/LR	
15	184	LR	x	LW	
10	176	LR	x	LW	KS06
11	176	LW	x	LR	
12	174	LR	x	LW	
14	180	Duroc	x	LR/LW	

VIRAL LOAD



WEIGHT GAIN



PHGC
PRRS Host Genetics Consortium

Application of Genomics to Improve Swine Health and Welfare
GenomeCanada

Host Response is affected by many genes

Trial Number	*n*	Breed			PRRSv Isolate
1-3	530	LW	x	LR	NVSL
4	195	Duroc	x	LW/LR	
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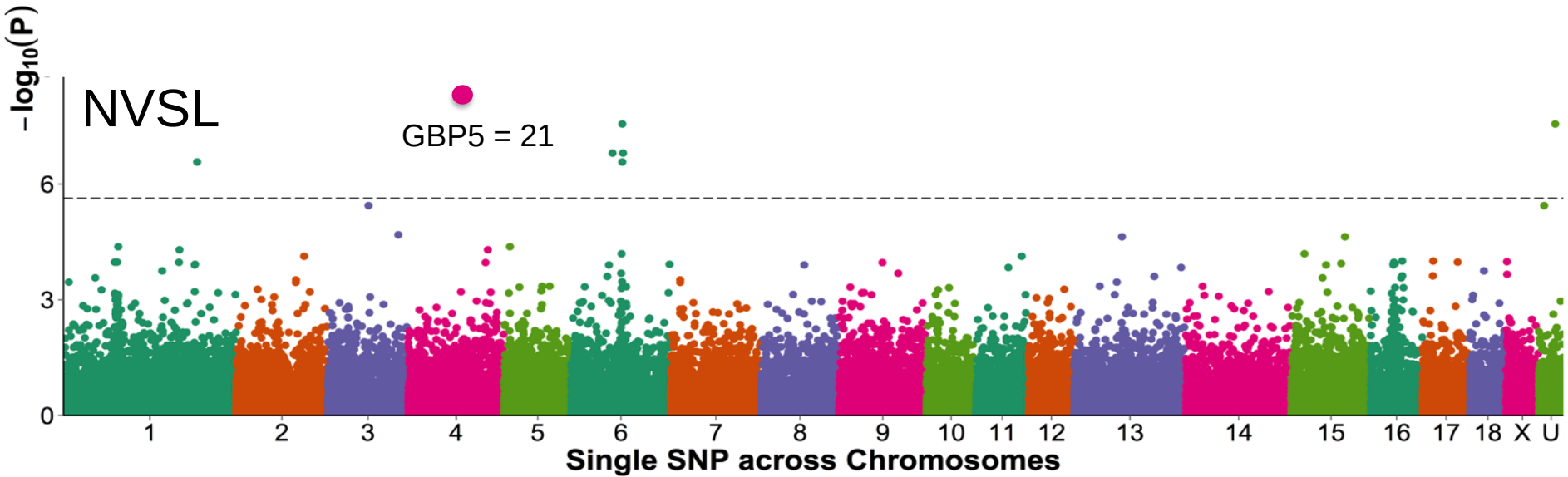
7	194	Pietran	x	LW/LR	
8	188	Duroc	x	LW/LR	
15	184	LR	x	LW	

Waide et al. 2016



Selection has Begun

Topigs Norsvin

TOPIGS NORSVIN IMPLEMENTS PRRS RESISTANCE IN BREEDING VALUE ESTIMATION

Topigs Norsvin has recently implemented selection for increased natural resistance to PRRS by using the WUR SNP in breeding value estimation. The WUR SNP is a genetic marker for a major gene associated with natural resistance to PRRS.

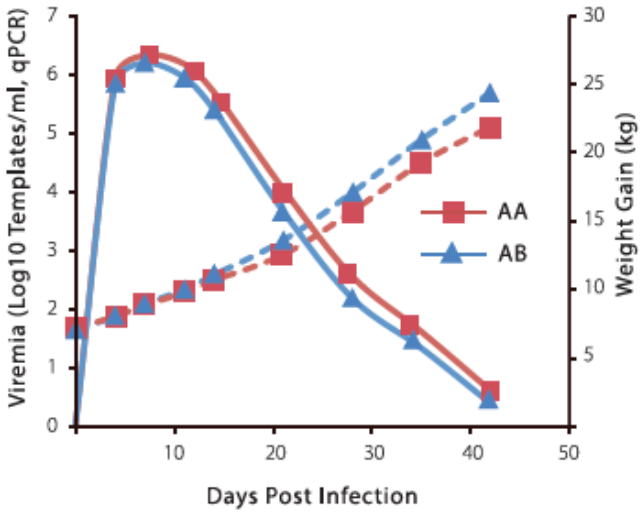
Several research trials have demonstrated that pigs with the

Selection has Begun

swine genetics companies.

One component of the project is large-scale disease challenges, in which pigs are infected with economically important diseases including Porcine Reproductive and Respiratory Syndrome Virus (PRRS) and Porcine Circovirus Type 2 (PCV2).

EFFECT OF GENOTYPE ON VIREMIA AND WEIGHT GAIN IN PIGS INFECTED WITH PRRS



Boddicker et al. 2012. Evidence for a Major QTL Associated with Host Response to Porcine Reproductive and Respiratory Syndrome Virus Challenge. Journal of Animal Science, 90:1733-1746.

Genesus is currently evaluating these regions in other populations and also their effect on other traits in the genetic improvement program.

Herds that break with PRRS possess a wealth of data on disease challenges in the field. Genesus has collected and analyzed genomic and phenotypic litter size information from an outbreak herd to identify important genomic markers or genes to incorporate into the genetic improvement program in order to produce pigs that can withstand disease challenges.

Conclusions

- **Host response to PRRS and other infectious disease has a sizeable genetic component**
- **Genomics provides opportunities to select pigs for increased host response to disease**
- **Marker assisted selection based on GBP5 genotype can be used to improve response…**
  - Not just to PRRSV infection, also co-infection with PCV2b
  - And perhaps other pathogens?
  - No negative effect on performance without major disease
- **Genetic improvement for disease resistance needs to focus on resilience to a range of diseases**

Selection for PRRS "Resistant" Pigs

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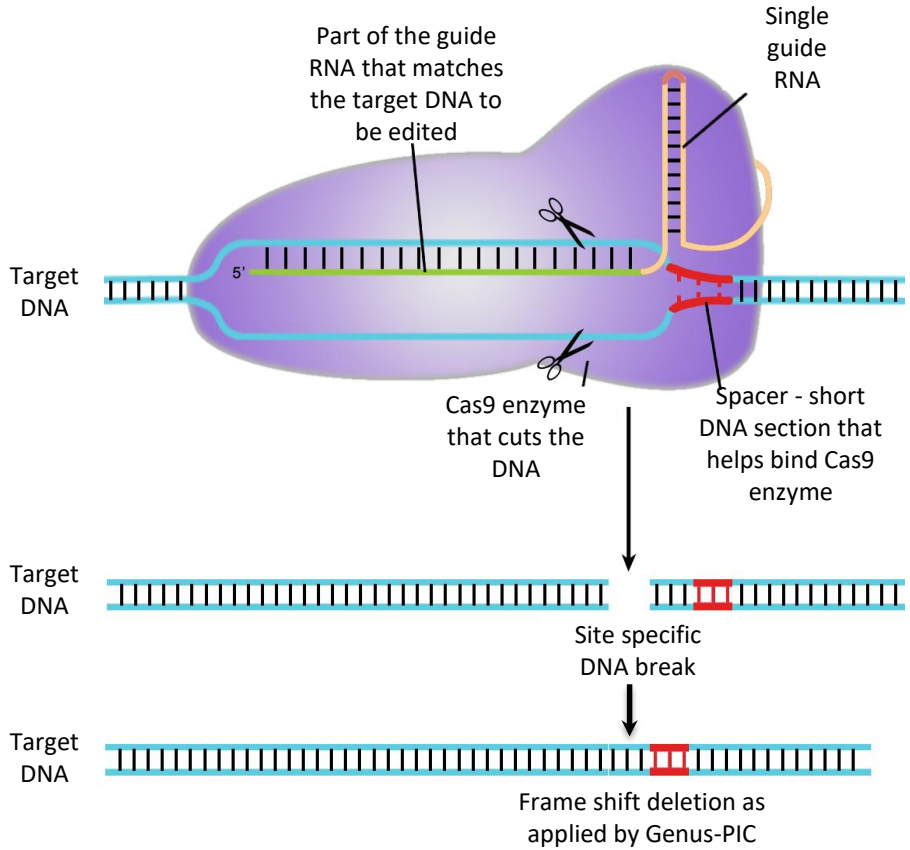
What is Gene Editing?

- Gene editing makes *precise*, *intentional* and *beneficial* changes in the genetic material of plants and animals used in food production, which can improve their health and sustainability. **This often mirrors changes that could occur in nature or through traditional breeding.**
- Gene editing helps farmers keep pace with the growing demand for more and better food, while using less water, land, nutrients and other resources.

CRISPR-Cas9 allows precise deactivation of part of the genome

Overview of gene editing with CRISPR/Cas9 as applied by Genus-PIC



Steps involved in gene editing with CRIPSR/Cas9

Step 1: Create a genetic sequence, called, guide RNA, that matches the piece of DNA to be edited

Step 2: Add guide RNA to a cell along with Cas9enzyme, which will act like a pair of scissors that cuts DNA

Step 3: The guide RNA binds to the target DNA sequence and Cas9 makes a cut in the target DNA

Step 4: Host cell factors repair the cut

Depending on the application, CRISPR-Cas9 enables deletions and/or addition of sequences to the target DNA. Genus-PIC focuses on removing sequences for PRRSv

Recap: Genus-PIC announcement in December 2015

Article Nature Biotech – Dec 2015

CORRESPONDENCE

Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus

To the Editor:
Porcine reproductive and respiratory syndrome (PRRS) is the most economically important disease of swine in North America, Europe and Asia, costing producers in North America more than $600 million annually[1]. The disease syndrome was first recognized in the United States in 1987 and described in 1989 (ref. 2). The causative agent, porcine reproductive and respiratory syndrome virus (PRRSV), was subsequently isolated and characterized in Europe in 1991 (ref. 3). Vaccines have been unable to control the disease. It has been suggested that disease syndrome and porcine circovirus–associated disease, and can establish a lifelong subclinical infection[6]. In 2006, a more severe form of the disease, called highly pathogenic PRRS, decimated pig populations throughout China[7]. Although genetic selection for natural resistance is an option, success to date has been limited, possibly due to the genetic diversity of the virus[8].

It had been proposed that PRRSV infects alveolar macrophages using the surface protein SIGLEC1 (CD169) as the primary viral receptor[9]. In this proposed model, after binding to CD169 and being taken homologous recombination and somatic cell nuclear transfer) were infected with PRRSV and compared with infected wild-type pigs, no difference in virus replication was found[8]. To test the role of CD163 in infection, we previously created 45 live-born piglets with insertions ranging from 1 bp to 2 kb, deletions from 11 bp to 1.7 kb, as well as a partial domain swap in *CD163* using CRISPR-Cas9 technology[5].

One founder male and one founder female, both of whom had mutations in exon 7 of *CD163*, were bred to produce offspring (**Supplementary Methods**). The founder

Genus press release – Dec 2015

Genus PIC

FOR IMMEDIATE RELEASE **8 December 2015**

Genus plc
('Genus' or the 'Company')

Genus tackles major pig disease with breakthrough technology

Genus (LSE: GNS), a global pioneer in animal genetics, announces the development of the first pigs resistant to Porcine Reproductive and Respiratory Syndrome Virus ("PRRSv"), through a long-standing collaboration with the University of Missouri.

The production of PRRSv resistant pigs is a significant breakthrough in combating this devastating porcine disease, and Genus is excited to be progressing the development of this technology under an exclusive global license from the University of Missouri.

December 2015 Announcement

- Genus-PIC and University of Missouri (MU) have developed the first pigs showing resistance to PRRSv
- By editing genes, MU enabled pigs to breed without producing the protein needed to spread PRRSv
- This new technology improves animal well-being, protects farmers, helps nourish society
- Genus-PIC has the global exclusive rights to the gene editing target
- Commercial availability of disease resistant pigs depends on validation of the technology, international regulatory approvals, market acceptance
- This technology is disruptive and the impact cannot be achieved through non-edited breeding stock

Source: Nature Biotechnology; Genus website

Backup: PRRSv-resistance demonstrated in MU research

- Genus-PIC and University of Missouri (MU) developed pigs showing PRRSv-resistance
- Pigs were created with minor nucleotide edits to CD163 gene
- No new or foreign DNA has been inserted into the pigs
- Findings have been confirmed by several other research institutes across the globe

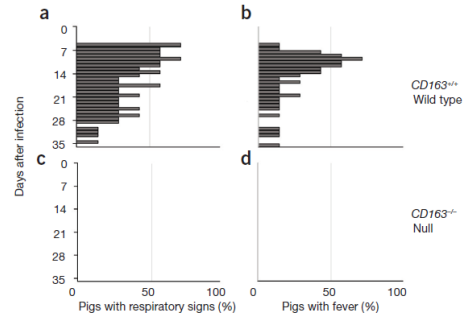


Figure 1 Clinical signs during acute PRRSV infection. (**a**–**d**) Results shown are compiled daily assessments for the presence of respiratory signs and fever for *CD163*$^{+/+}$ ($n = 7$) and *CD163*$^{-/-}$ ($n = 3$) pigs. The percentage of pigs with respiratory signs (**a**,**c**). The percentage of pigs with a fever (**b**,**d**). Fever was considered positive if it was ≥104 °F (normal body temperature, 101.6–103.6 °F). Respiratory scores ranged from 0: normal, to 1: mild dyspnea and/or tachypnea when stressed (when handled), 2: mild dyspnea and/or tachypnea when at rest, 3: moderate dyspnea and/or tachypnea when stressed (when handled), 4: moderate dyspnea and/or tachypnea when at rest, 5: severe dyspnea and/or tachypnea when stressed (when handled), 6: severe dyspnea and/or tachypnea when at rest. The percentage of piglets that had a fever or any sign of respiratory stress (a score of ≥1) at the various days of the challenge are shown. Note that the CD163$^{-/-}$ piglets displayed no signs of either respiratory stress or fever.

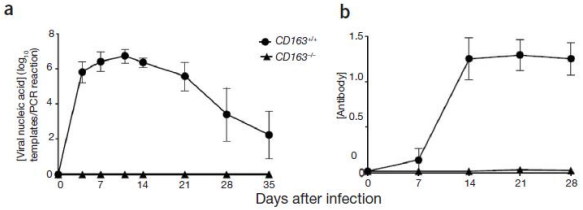


Figure 3 PRRSV-specific nucleic acid and antibody. (**a**,**b**) Mean and s.d. of PRRSV nucleic concentrations (**a**) and antibody (**b**) in serum from *CD163*$^{+/+}$ ($n = 7$) and *CD163*$^{-/-}$ ($n = 3$) pigs (one replication) are shown. Sample to positive ratio = the median fluorescent intensity (MFI) of the sample divided by the MFI of the positive control.

Genus-PIC has continued to develop the technology

Activities since announcement

Technology	• *MU findings confirmed in academic studies* • *Edit optimized for efficacy & regulatory approval* • *First pigs from edited embryos born*
Regulatory	• *Engaging with FDA for US regulatory approval* • *Initiating contact with international regulators* • *Collaborating with BIO*
Market acceptance	• *Set up of CFI's Gene Editing Coalition* • *Supporting public debate, e.g. CRISPRCon* • *Ongoing industry & consumer research*

PRRSv program – significant progress made

nature biotechnology

Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus

US and European patents granted[2]

First elite edited pigs born

US-FDA INAD granted[1]

Optimized Caribou reagents deployed for editing

December 2015	2016	2017	2018

- First PRRSv resistant pigs produced
- Genus exclusive global license

Genus Caribou exclusive collaboration

CARIBOU BIOSCIENCES

Renovate Bio formed to conduct elite editing

RENOVATE Biosciences

1. INAD refers to Investigational New Animal Drug application with the US FDA for the investigational PRRSv program ; 2. '475 patent granted to the University of Missouri; and patent '790 patent granted to the University of Edinburgh

PRRSv program – path forward and key milestones

Workstream

Animal Amplification & Evaluation

- First breedings of elite edited pigs
- ~100 Homozygous edited pure line animals off test → Gateway #1
- ~100 Homozygous Camborough™ parent sows farrowed → Gateway #2
- ~1000 terminal pigs evaluated

2018 | 2019 | 2020 | 2021 | 2022+

Disease Testing

- Cell line disease testing of Genus edit
- Live animal disease challenge of Genus edit
- Additional animal disease challenge of Genus edit

Regulatory Submissions

- First US FDA submission

Geneticists Have Made Significant Progress in the Improvement of Response to PRRS

1. Selection for "Robust" pigs in a commercial environment has reduced wean to finish mortality by 1.4%

2. Selection for PRRS "Resilient" pigs – A DNA marker was discovered that explains 15% of the variation in viral load – selection for the favorable allele is being evaluated or has begun

3. Selection for PRRS "Resistant" pigs – Resistant pigs have been created and will be going through the US FDA regulatory process

PIC®

Thank You!!!

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