



Era of Re-writing the (Livestock) Genome

Bruce Whitelaw

Genus Chair of Animal Biotechnology
The Roslin Institute

3rd International Workshop on Animal Biotechnology Regulation, Charlottesville, 26th June 2017



THE UNIVERSITY *of* EDINBURGH





Era of re-writing the genome

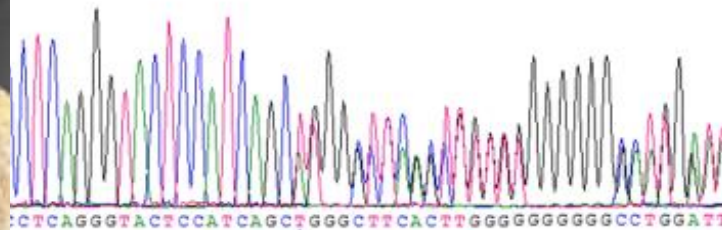


Genetic Engineering (of livestock):

Transgeneis (GM) - since 1985

Genome Editing - rewrite the genome

“Pig 26”



Monoallelic
1bp deletion

CCTCAGGGTACTCCATCA **CAT** GGCTCAGCTGTGTGGGGGGGCA (wt)

CCTCAGGGTACTCCATCA **C**-T GGCTCAGCTGTGTGGGGGGGCA (1 del)

Genetic Engineering (of livestock):

Transgeneis (GM) - since 1985

Genome Editing - rewrite the genome

Can we use these tools differently?

>> yes and no

Yes – editing allows allele swapping
(which is difficult with GM)



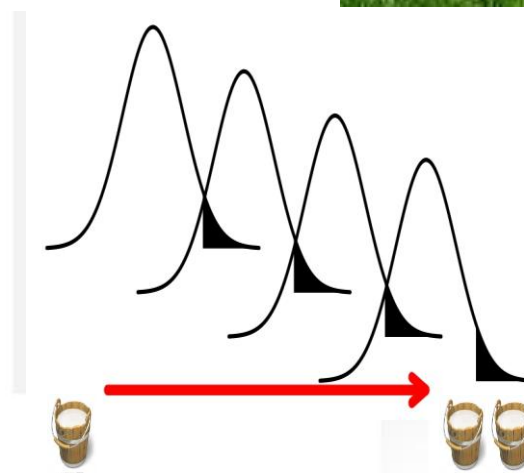
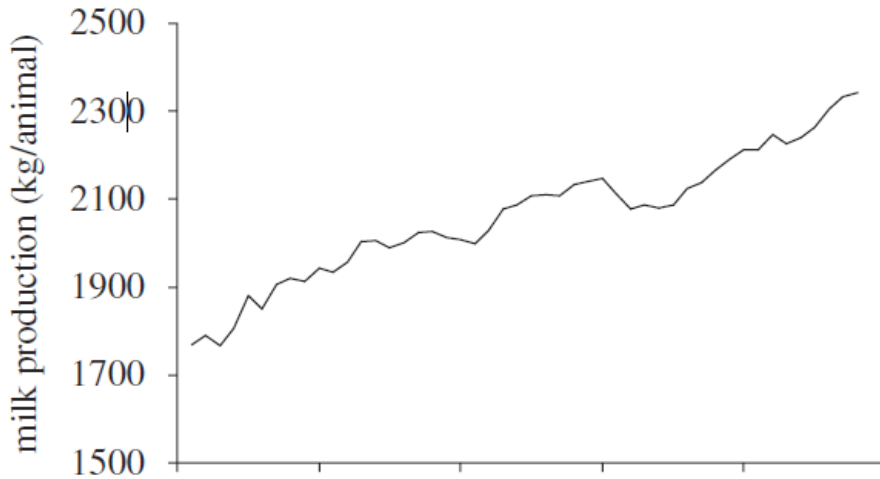
No – both allow gene addition / deletion

So - what can we do?

- accelerate genetics
- by-pass genetics



Livestock Breeding: we are good at it



Accelerate Genetics:

- increasing fixation rate for low frequency variation




- eliminating deleterious alleles

Pawnee Farm Arlinda Chief



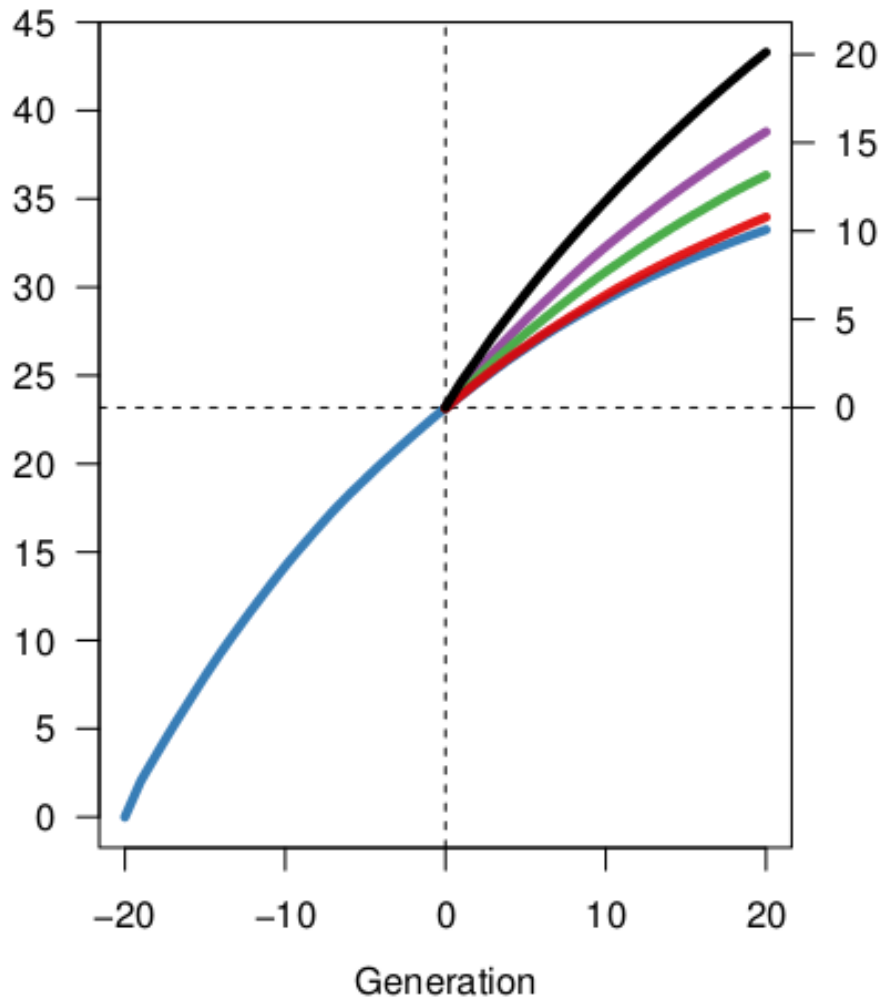
- multiple SNPs at the same time

Standard selection traits: reproductive performance, maternal ability, growth rate, feed efficiency, longevity, carcass merit / milk production

Promotion of Alleles by Genome Editing (PAGE)

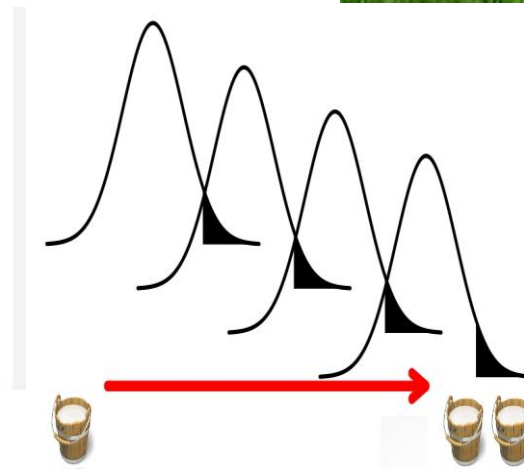
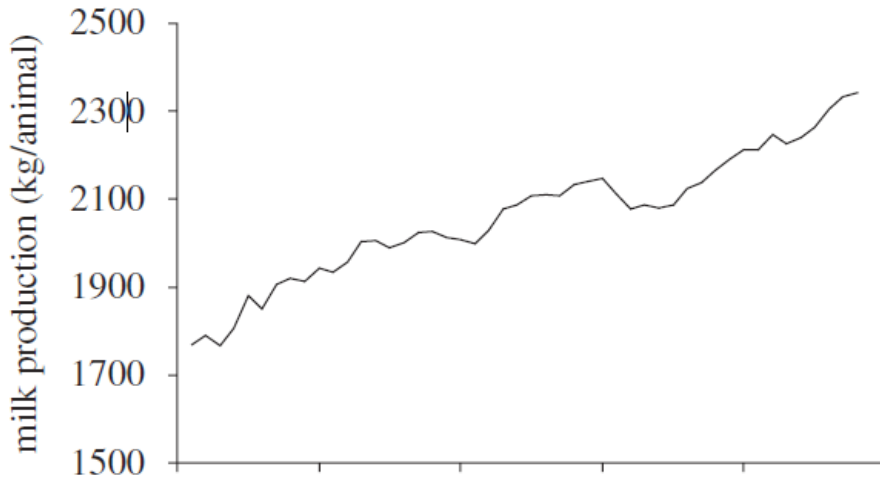
- weakness of GS with perfect accuracy is that alleles do not segregate independently
- with PAGE alleles behave as though they segregate independently (offers precision)
- genomic selection decoupled selection from phenotyping ... genome editing decouples gain from selection

Promotion of Alleles by Genome Editing (PAGE)



20 edits
10 edits
5 edits
1 edit
No editing

Livestock Breeding: use what already there



By-pass genetics:

- **introduce novel variation (e.g. Nanos2, CD163)**
- **“capturing” rare breed alleles or from different species (e.g. RELA)**
- **disease ‘resistance’**
- **single sex offspring**
- **adaption to stress (e.g. temperature)**
- **welfare traits**

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

Kristin M Whitworth, Raymond R R Rowland, Catherine L Ewen, Benjamin R Tribble, Maureen A Kerrigan, Ada G Cino-Ozuna, Melissa S Samuel, Jonathan E Lightner, David G McLaren, Alan J Mileham, Kevin D Wells & Randall S Prather

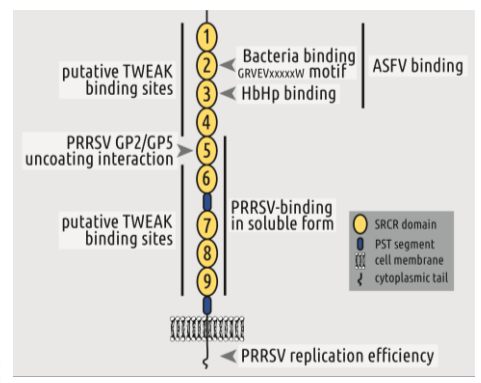
Affiliations | Corresponding author

Nature Biotechnology 34, 20–22 (2016) | doi:10.1038/nbt.3434
Published online 07 December 2015

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
Jim Reecy & Jack Dekkers – Iowa State University

Strong Industry Participation
PHGC Breeding Companies
Fast Genetics, Genesus, Choice Genetics
PIC/Genus, TOPIGS, PigGen Canada



RESEARCH ARTICLE

Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function

Christine Burkard¹, Simon G. Lillico¹, Elizabeth Reid², Ben Jackson², Alan J. Mileham³, Tahar Ait-Ali¹, C. Bruce A. Whitelaw¹, Alan L. Archibald^{1*}

¹ The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, United Kingdom, ² The Pirbright Institute, Ash Road, Pirbright, Woking, United Kingdom, ³ Genus pb, DeForest, Wisconsin, United States of America



SCIENTIFIC REPORTS

OPEN **Mammalian interspecies substitution of immune modulatory alleles by genome editing**

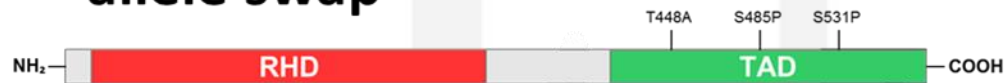
Received: 20 October 2015
 Accepted: 27 January 2016
 Published: 22 February 2016

Simon G. Lillico¹, Chris Proudfoot¹, Tim J. King¹, Wenfang Tan¹, Lei Zhang¹, Rachel Mardjuki², David E. Paschon³, Edward J. Rebar³, Fyodor D. Urmov³, Alan J. Mileham³, David G. McLaren¹ & C. Bruce A. Whitelaw¹

We describe a fundamentally novel feat of animal genetic engineering: the precise and efficient substitution of an agronomic haplotype into a domesticated species. Zinc finger nuclease in-embryo editing of the RELA locus generated live born domestic pigs with the warthog RELA orthologue, associated with resilience to African Swine Fever. The ability to efficiently achieve interspecies allele introgression in one generation opens unprecedented opportunities for agriculture and basic research.



allele swap



SCIENTIFIC REPORTS

OPEN

Generation of germline ablated male pigs by CRISPR/Cas9 editing of the *NANOS2* gene

Received: 21 October 2016
Accepted: 02 December 2016
Published: 10 January 2017

Ki-Eun Park^{1,2,3,*}, Amy V. Kaucher^{4,*}, Anne Powell², Muhammad Salman Waqas⁴, Shelley E.S. Sandmaier^{1,2}, Melissa J. Oatley⁴, Chi-Hun Park^{1,2}, Ahmed Tibary⁴, David M. Donovan², Le Ann Blomberg², Simon G. Lilloco⁵, C. Bruce A. Whitelaw⁵, Alan Mileham⁶, Bhanu P. Telugu^{1,2,3} & Jon M. Oatley⁴

Genome editing tools have revolutionized the generation of genetically modified animals including livestock. In particular, the domestic pig is a proven model of human physiology and an agriculturally important species. In this study, we utilized the CRISPR/Cas9 system to edit the *NANOS2* gene in pig embryos to generate offspring with mono-allelic and bi-allelic mutations. We found that *NANOS2* knockout pigs phenocopy knockout mice with male specific germline ablation but other aspects of testicular development are normal. Moreover, male pigs with one intact *NANOS2* allele and female knockout pigs are fertile. From an agriculture perspective, *NANOS2* knockout male pigs are expected to serve as an ideal surrogate for transplantation of donor spermatogonial stem cells to expand the availability of gametes from genetically desirable sires.

Likely next steps for genome editing in livestock:

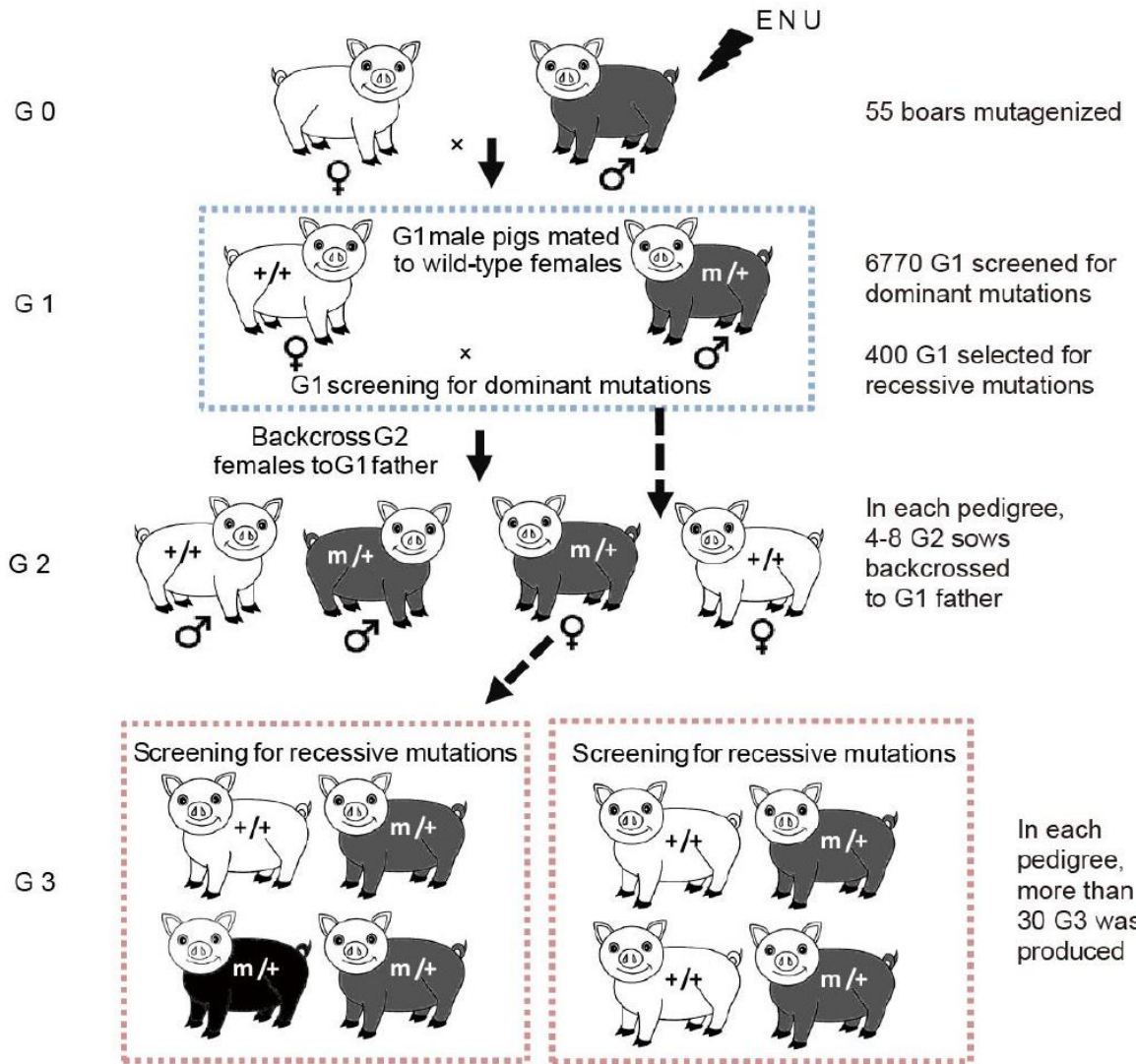
Short term = focus on disease traits

Short to medium term = surrogate sires

Medium term = fix up genetic load and deleterious mutations

Long term = PAGE for quantitative traits

Hai et al eLife (2017)





Simon Lillico
Chris Proudfoot
Vrushali Patil
Wenfang (Spring) Tan
Claire Neil
Maeve Ballantyne
Sarah Fletcher
Du (Dina) Yue
Akshay Joshi
Gus McFarlane

Christine Burkard
Alan Archibald

John Hickey
Gregor Gorjanc
Janez Jenko
Serap Gonen

