How gene editing has the potential to revolutionize the livestock industry

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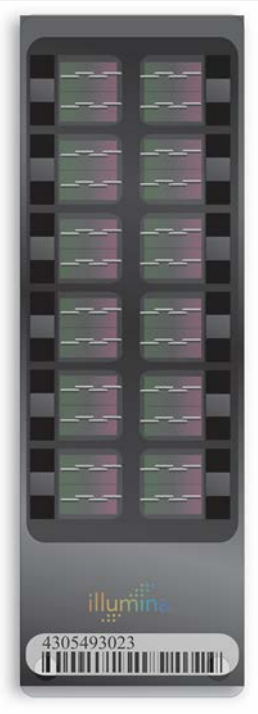
Genetic Selection

- Animal breeders have used selection on naturally occurring genetic variation to make great phenotypic progress
- Originally used the “black box” quantitative approach to select on based on ancestors phenotypic records (Best Linear Unbiased Prediction or BLUP)
- Transitioned to using few genetic markers for specific genes/traits (Marker Assisted Selection)
  - Based on candidate genes

Genetic Selection



- With sequencing of animal genomes came selection on larger numbers of genetic markers
- Genome Wide Association Studies using 60,000 markers at a time
  - Potential to find genetic markers without known function associated with traits of interest
- Genomic BLUP
- Individual animal genome sequencing
- Genotyping by sequencing

Traditional selection has its limitations

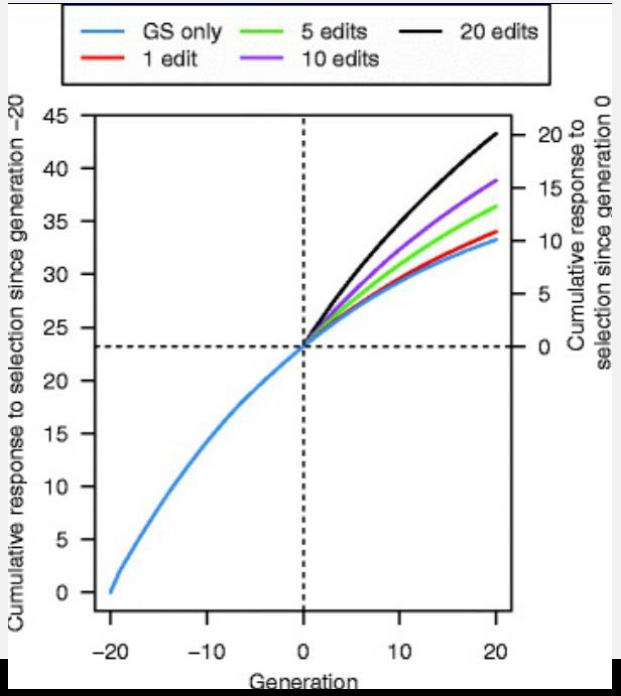
- Identification of new and desirable genetic variation is part chance and part experimental design
- Genetic Linkage
  - Selection for certain traits might inadvertently not carry on other favorable genetic variants
  - Harmful naturally occurring mutations may be carried into future generations due to linkage to positive variants

Dairy Example

- The Dairy Industry Lost $420 Million From a Flaw in a Single Bull
- Pawnee Farm Arlinda Chief born in 1962
  - Daughters were fantastic milkers
  - 16,000 daughters
  - 500,000 grand daughters
  - 2,000,000 great grand daughters
  - Had mutation that lead to spontaneous abortion in the womb
  - Crazy fact is that his genetic influence in milk production produced an extra **$30 Billion** in milk production

Gene Editing

- Gene editing will NOT replace traditional breeding programs
- Can accelerate genetic progress



Jenko,J et al. 2015. Potential of promotion of alleles by genome edition to improve quantitative traits in livestock breeding programs. Genetics Selection Evolution 47:1-14

EXTENSION

Use of gene editing to introduce a naturally-occurring polled allele into Holstein cattle vs selective breeding

Attribute	Polled Holstein through gene editing	Polled Holstein through introgression
Phenotype: No horns	YES	YES
Mutation uniquely detectable	NO	NO
Food safety concerns associated with phenotype	NO	NO
# generations taken to achieve polled >15/16 Holstein	ONE (FAST)	MANY (SLOW)
Linkage drag?	NO	YES
Improved animal welfare	YES	YES
Regulated?	Depends on definition of regulated article	NO
Likely to happen	Not if takes years and costs millions of dollars	NO

From Van Eenennaam, A.

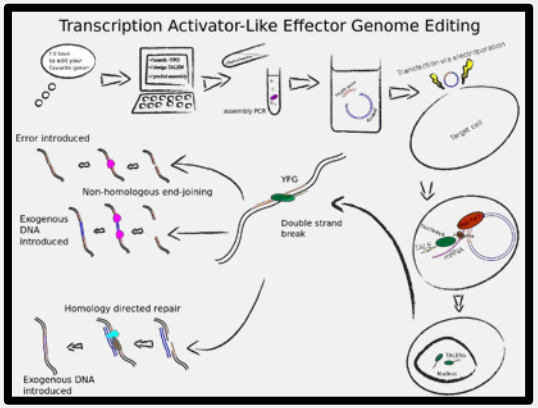
Genetically Modified Organism

- 1950's expose plants to radiation to induce mutations

- 1973 Boyer and Cohen inserted foreign DNA into bacteria
  - Began using *E. coli* modified with human gene to manufacture insulin for diabetics
- 1974 Jaenisch created first transgenic mouse
  - Spiked animal's genome with viruses containing DNA from other species

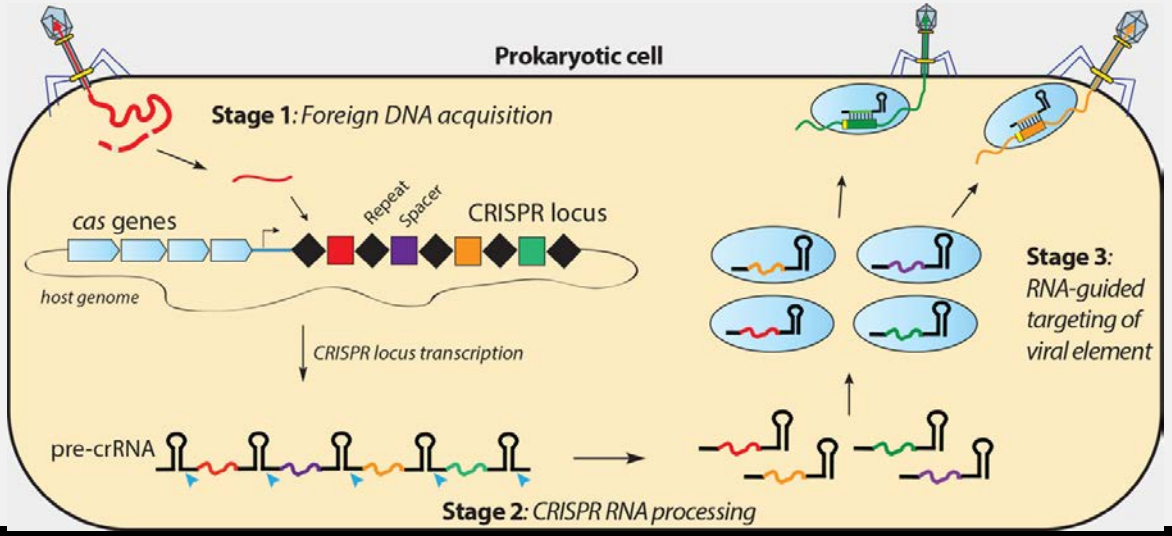
Genetically Modified Organism

- 1996 Zinc finger nucleases
  - One end recognizes DNA sequence and other end cuts
  - Cell naturally repairs those cuts
  - Difficult to use and doesn't always work
- 2010 TALENS
  - Transcription activator-like effector nucleases
  - Proteins that find and cut desired DNA sequence
  - Are large proteins that are cumbersome to work with and deliver into cells



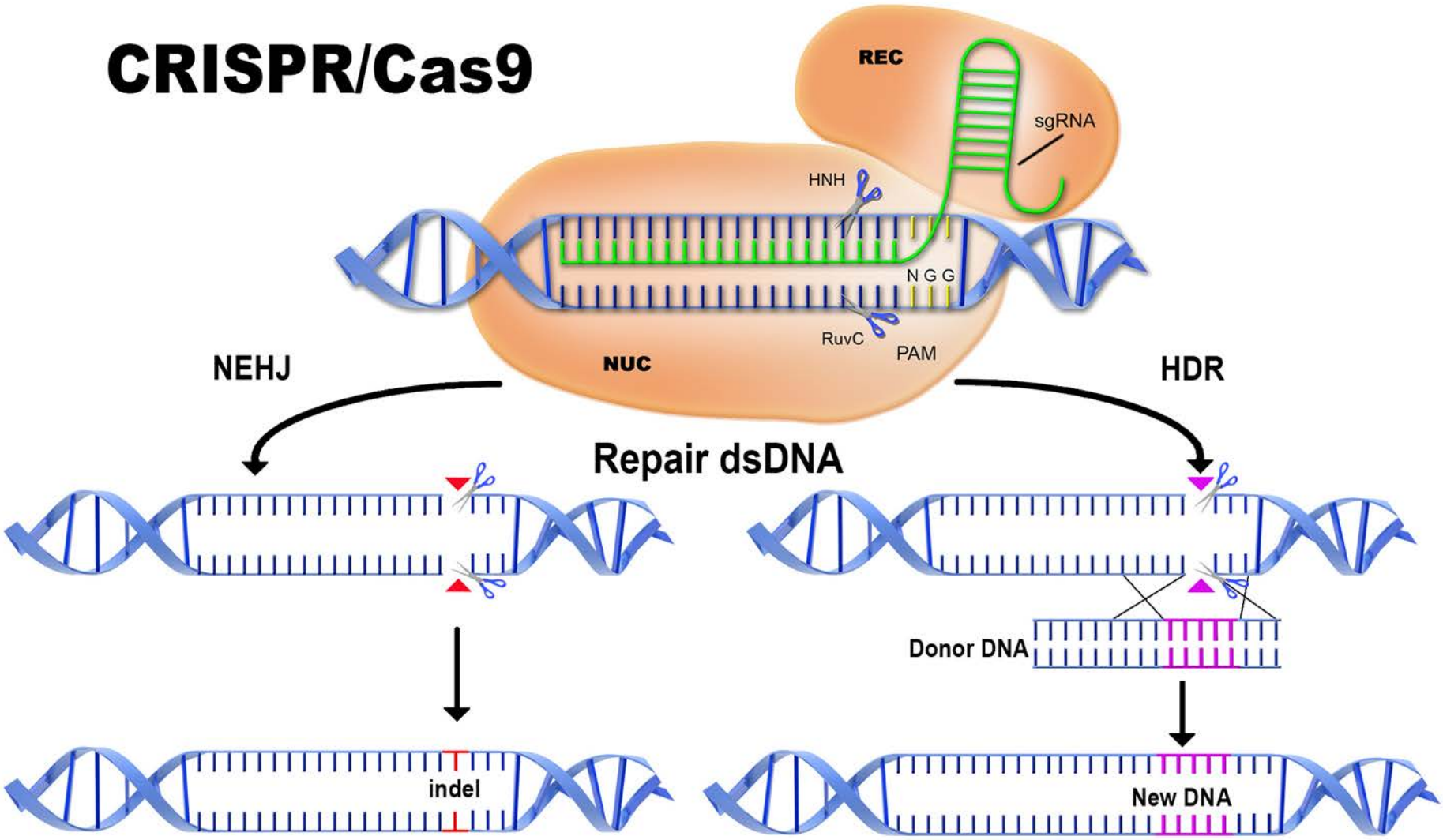
Genetically Modified Organism

- 2013 Doudna identified **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats and **C**RISPR-**as**sociated (Cas) genes
  - Naturally occurring in bacteria
  - System bacteria use to rid itself of invading viral DNA



CRISPR

- Using CRISPR/Cas9, researchers can create the break in DNA at specific locations using 20 base pair recognition sequence
- Once the DNA is “broken” at the region you are interested in, DNA repair mechanisms can be used to insert a new sequence
- CRISPR was not the first method to break DNA at specific sites
  - To date is the most specific because it uses RNA to target the region and not proteins
  - Can edit both chromosomes at one time


CRISPR/Cas9
REC
sgRNA
HNH
N G G
RuvC
PAM
NUC
NEHJ
HDR
Repair dsDNA
Donor DNA
indel
New DNA

CRISPR/Cas9

- Editing occurs at embryo stage for gene editing
- Rate of success isn't perfect
  - Early work for the Beagle, they edited 65 embryos, 27 puppies were born, two had the edit in both chromosomes
  - Improvements in both editing and embryo work have transpired
- Off-target edits
  - 20 base pair recognition sequence
  - Could identify multiple sites in genome with same sequence
  - Early work showed non-specific cuts (only 18 or 19 of 20 bp)
  - Drastic improvements in specificity have occurred recently
- Ethical decisions

Myostatin and muscle

Belgian Blue bull

"Bully" Whippet and "regular" Whippet
(yes, this is natural)

- Natural mutations in myostatin increase muscle (double-muscle)

Genome editing – alter MSTN gene of cattle, pigs, and Beagles

Horned vs Polled

Gene Edited Holsteins

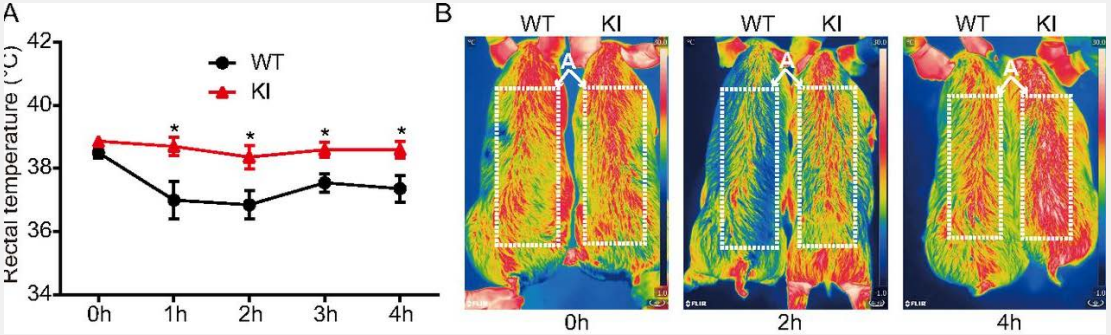
- Changed the gene for horns so the calves on the right were born polled (Recombinetics)

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

- PRRSv costs US swine industry $664 million per year
  - Reproductive failures and abortions
  - Loss of growth and feed efficiency
  - Medical treatment
- PRRSv is uncoated by CD163 thus allowing the virus into the cytoplasm of the pig
  - 2015 Prather developed pigs resistant to PRRS
    - Unfortunately not all subtypes
  - 2018 Roslin developed pigs resistant to all known PRRS subtypes
  - Show no adverse effects when maintained under standard husbandry conditions.

CRISPR Bacon

- Chinese scientist added back the function of the UCP1 gene responsible for brown adipose tissue thermoregulation
  - Modern pigs lost this function in evolution
  - Can burn fat to stay warm
  - Could drastically improve welfare of baby pigs and reduce carbon footprint by reducing supplemental heat



http://www.pnas.org/content/114/45/E9474 Zheng et al 2017

EXTENSION

Potential gene targets

- Any trait with single or few genes affecting trait
- Cattle
  - Cancer eye, tick resistance, heat tolerance, marbling, tenderness, fatty acid composition
  - Rid high genetic value lines of genetic defects
- Swine
  - PRRS, Circo, PEDv, African Swine Fever, marbling, tenderness, teat number, coat/hair color

- Note these are all traits where natural genetic variations already exist that affect these traits

Other Applications

- Scientists are editing pigs to remove the proteins that cause a human immune response
  - Use of pig organs for human transplant

- Possibilities in Humans
  - Otherwise non-viable embryos edited to fix a gene causing beta-thalassaemia (blood disease)
  - Now editing to eliminate HIV from infected cells and also make cells resistant
  - Used CRISPR to treat muscular dystrophy in mice
  - Imagine if we can use gene editing to remove FMD?

Is Not Simply Plug and Play

- Need quantitative and molecular genetics, biologist
  - Need to identify genes that affect traits
    - Not all edits will work – ie complete PRRS resistance
      - Need for testing
  - Even if found in other species or within a species, some gene edits will only work in specific genetic backgrounds
  - Unpublished myostatin research indicated
    - Knocking out whole myostatin gene still produced normal appearing animals
    - Some breeds/lines dies shortly after birth

Challenges

- Public fearful of unintended consequences even though they don't know what that could be
  - Off-target edits
    - Can be sequenced to confirm before release
  - Not traceable
    - Unless added "tags/barcodes"
    - Not possible with all edits -barcode for traceability would change protein if within exon
- McDonald's, Arby's, and Culver's all stated at 2018 NPIC they don't want gene edited product unless consumers ask for it
- Need to educate consumers

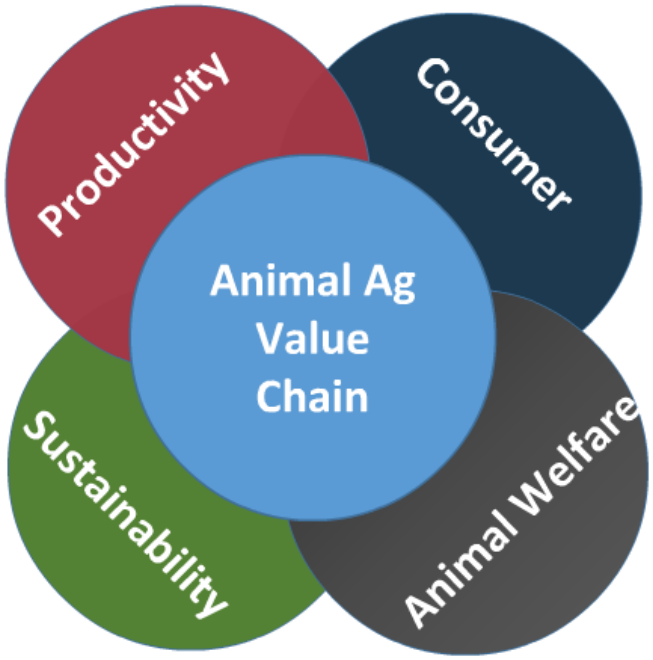
Traits Benefit Producers, Consumers, Animals & Planet

FARM PRODUCTIVITY

- Yield & Production cost
- Reduced input / Increased Output
- Animal Maintenance

SUSTAINABILITY

- Reduced use of antibiotics, vaccines and drugs.
- More efficient feed conversion
- Climate adaptation
- Genetic diversity



CONSUMER BENEFITS

- Palatability / Tenderness
- Product Health Profile
- Drug free/antibiotic free

ANIMAL WELFARE

- Humane production methods
- Precise gender selection
- Disease resistance
- Animal comfort

From Dr. Tad Sonstegard, CSO Acceligen

European Regulation

- European Court of Justice ruled July 25, 2018 that altering living things using gene editing counts as genetic engineering
  - Regulated as GMOs
  - Exempts older techniques used in the 1950's in which plants are exposed to radiation to induce random mutations in an organisms DNA. Deemed safe due to their long safety record.

If government regulation and the court of public opinion allows:

Gene-editing has the potential to greatly advance animal agriculture's charge to produce healthy productive animals to sustainably feed a growing world population.

Regulation in USA

- The Food and Drug Administration's Center for Veterinary Medicine evaluates GE animals under the new animal drug provisions of the Federal Food Drug and Cosmetic Act.
- The act defines drugs as "articles (other than food) intended to affect the structure or any function of the body of man or other animals." The rDNA construct in the resulting GE animal is thus a regulated article that meets the drug definition; the GE animal itself is not a drug.
- However
- The FDA defines "genetically engineered animals" as those animals modified by rDNA techniques, including the entire lineage of animals that contain the modification, and regulates based on the use of rDNA techniques. All GE animals are captured under these provisions regardless of their intended use.