

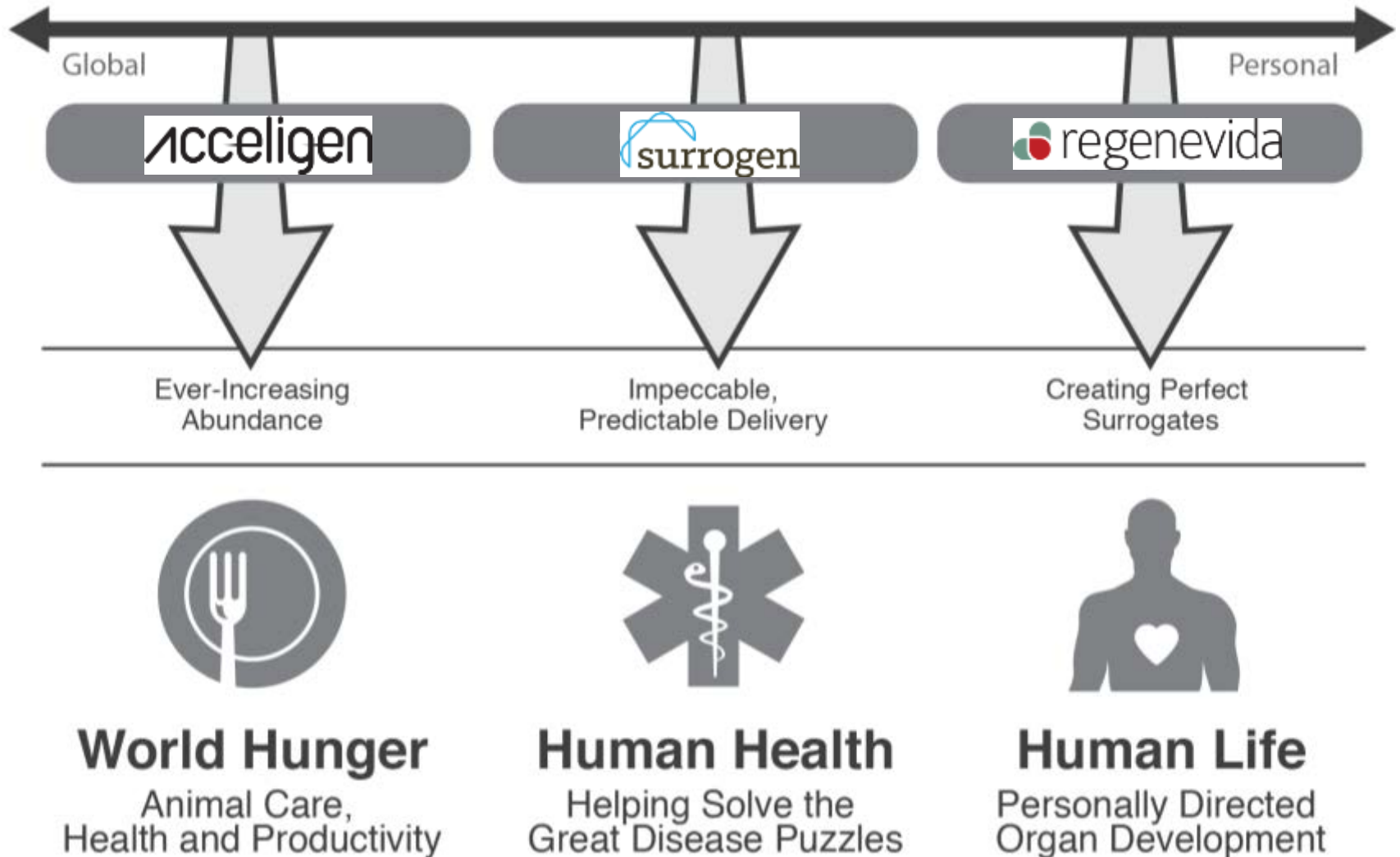


Gene Editing in Food Animals

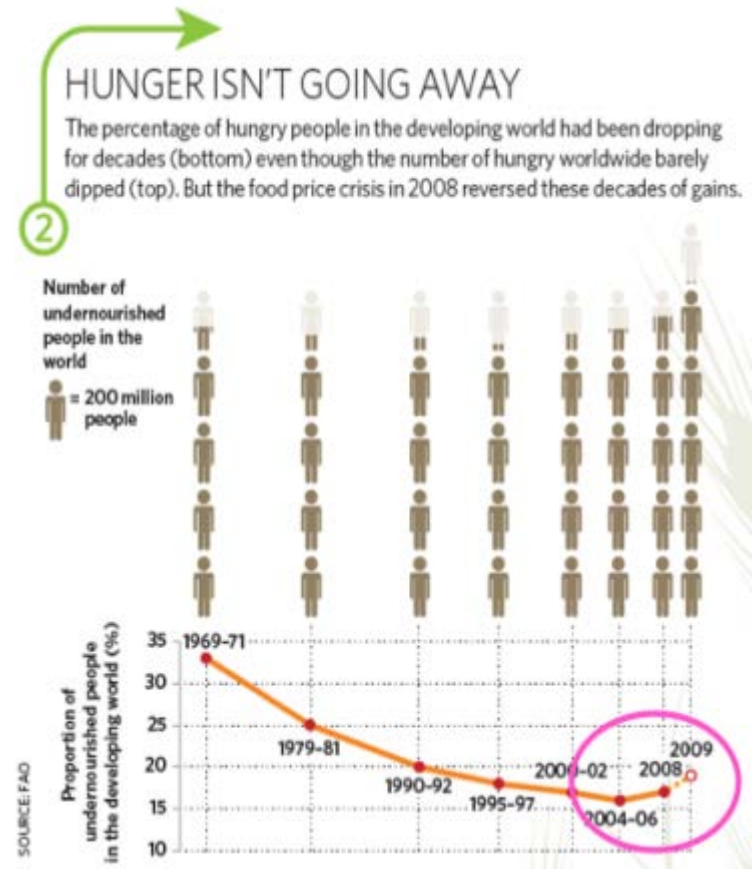
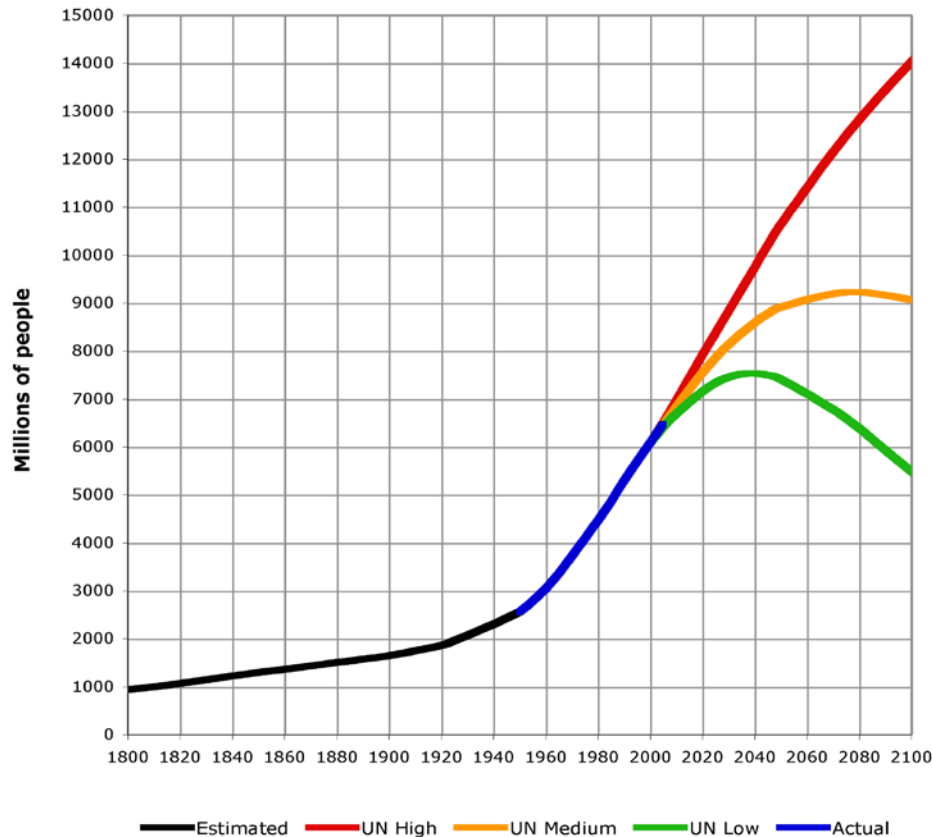
**Scott C. Fahrenkrug, Ph.D.
CEO Recombinetics, Inc.**



recombinetics



Agriculture: The Real “Hunger Game”



Population expected to grow to 10 billion by 2050

•Food production must double

70% of gains in food production must come from innovative agricultural technologies

A Growing Demand for Meat & Milk

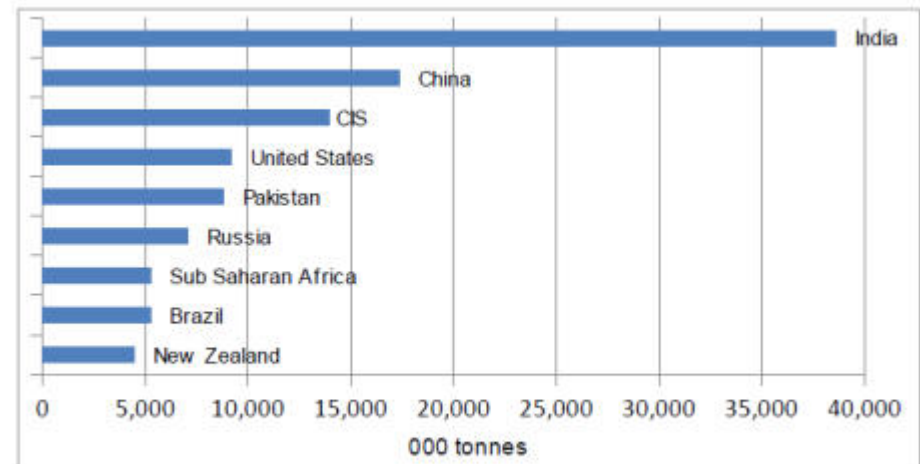
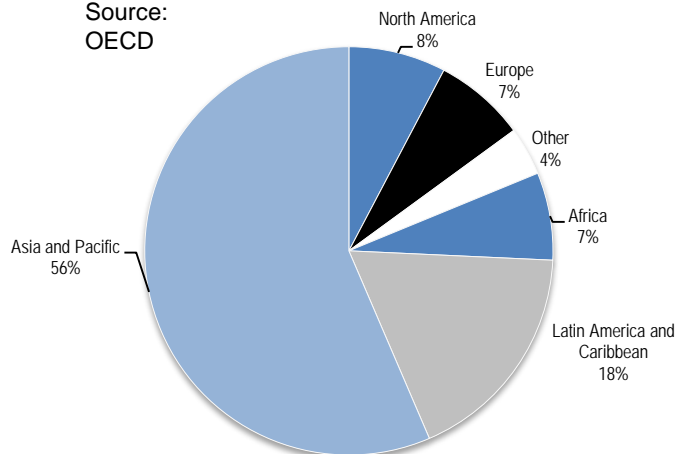


Projected growth in meat demand to 2020



Projected growth in milk production 2009 - 2018

Source:
OECD



Source: OECD



Traditional Selection

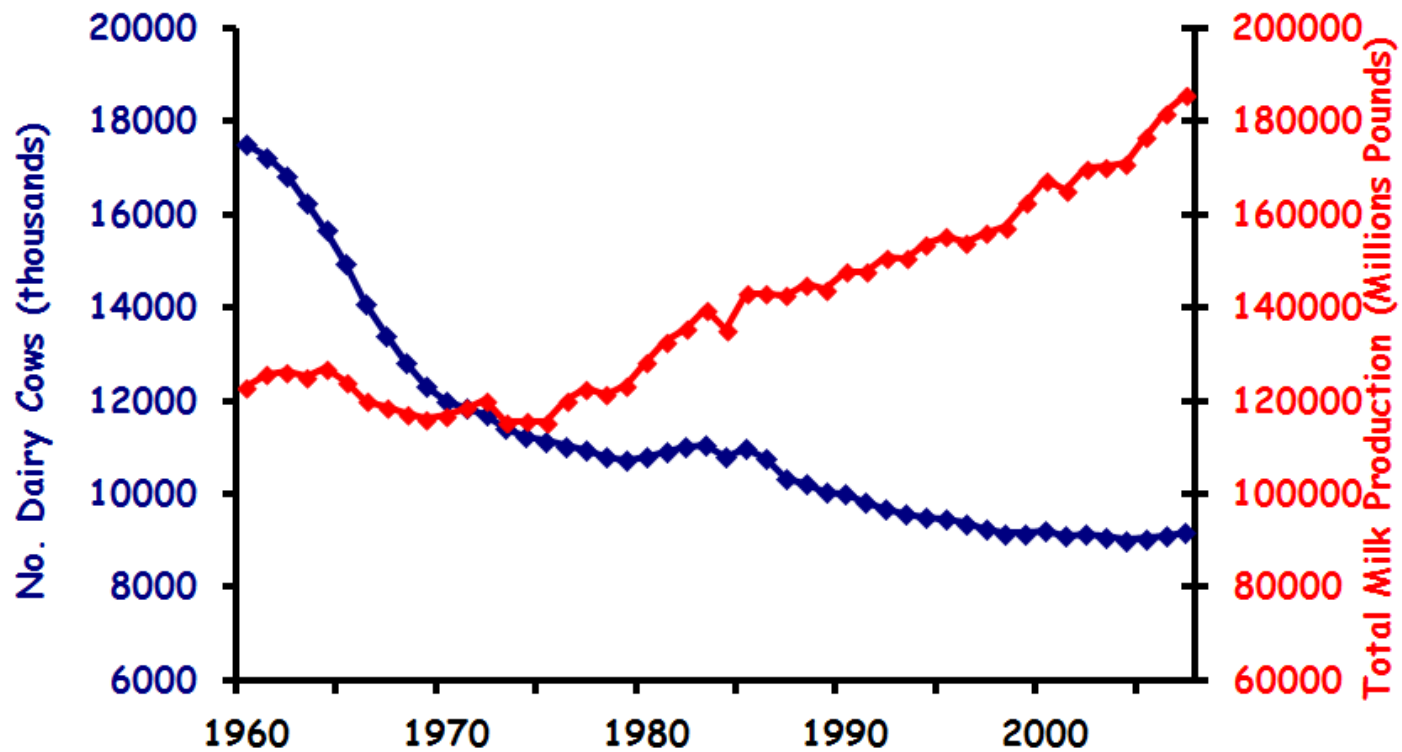


- Phenotypes have been collected for more than a century
- Estimate genetic merit for animals in a population
- Select superior animals as parents of future generations



Selection Works

In 2007 U.S. Produced 34% more milk with 48% fewer dairy cows than in 1960



Inbreeding Increases Frequency of Deleterious Mutations

Deleterious Fertility Alleles

Name	Chr	Loc	Freq	Earliest Known Ancestors	Gene
	BTA	Mbase	%		
HH1	5	62-68	4.5	Pawnee Farm Arlinda Chief	APAF1/stop gain
HH2	1	93-98	4.6	Willowholme Mark Anthony	ND
HH3	8	92-97	4.7	Glendell Arlinda Chief, Gray View Skyliner	SMC2/non-synonymous
JH1	15	11-16	23.4	Observer Chocolate Soldier	CWC15/stop gain
BH1	7	42-47	14.0	West Lawn Stretch Improver	

More mutations on the horizon as selection continues

VanRaden, P.M., et al. Reporting of haplotypes with recessive effects on fertility. Proc. Interbull Mtg., Stavanger, Norway, Aug. 26–28, 4 pp. 2011.

Crossbreeding to Introgress Superior Performance Also Dilutes Congenital Mutations



Bull of breed A (100%)



Cow of breed B (100%)



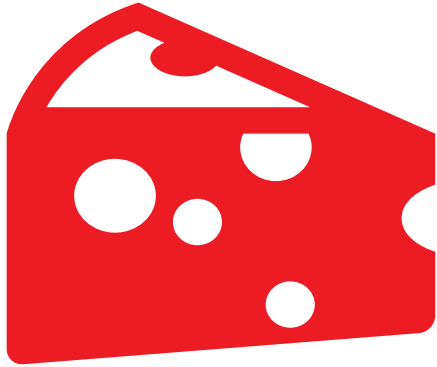
F1 crossbred progeny
50% A and 50% B

Two breed cross occurs where breed A and breed B are two purebreeds and the F1 progeny (AB) contains equal parts of the two breeds.

Crossbreeding Dilutes Traits of Local Adaptation



Non-Meiotic Allele Introgression



Sustainability

Reduced environmental impact

More efficient feed conversion

Enhanced reproduction

Animal Welfare

Humane production

Gender selection

Disease resilience

Disease gene repair

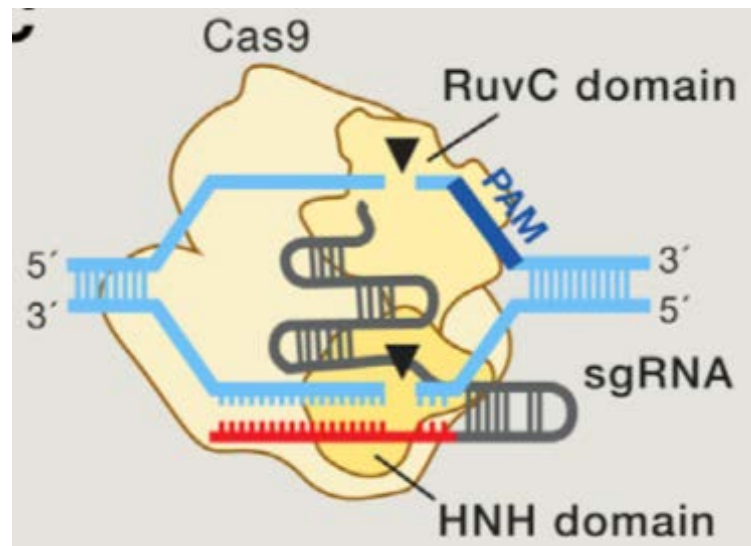
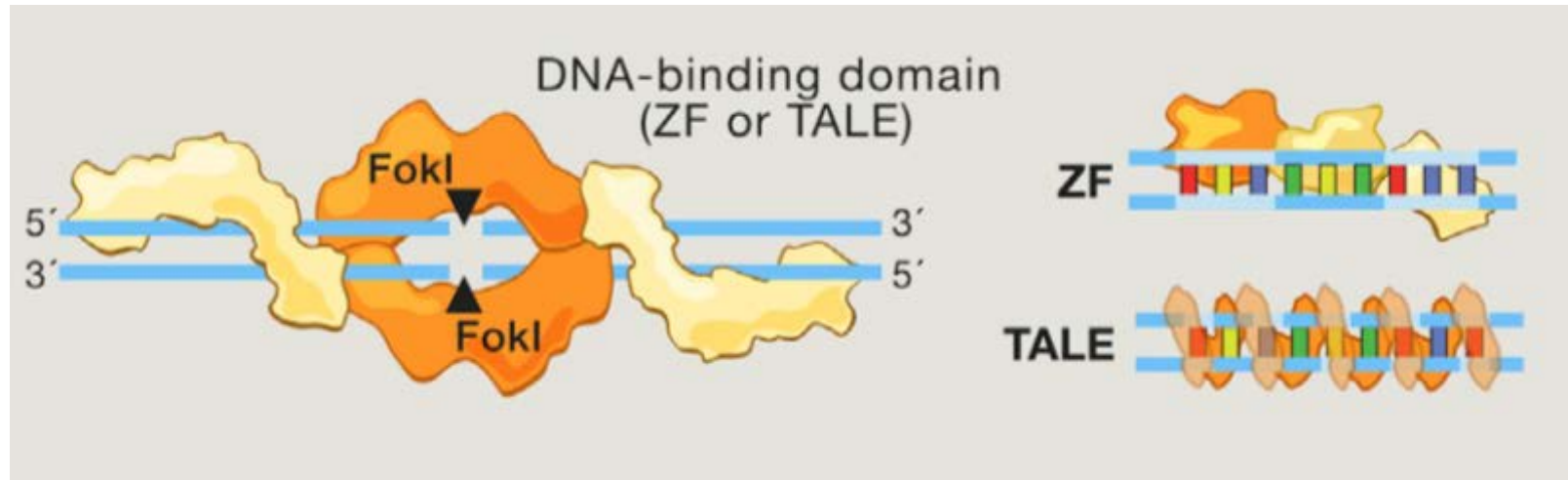
Product Quality

Food safety

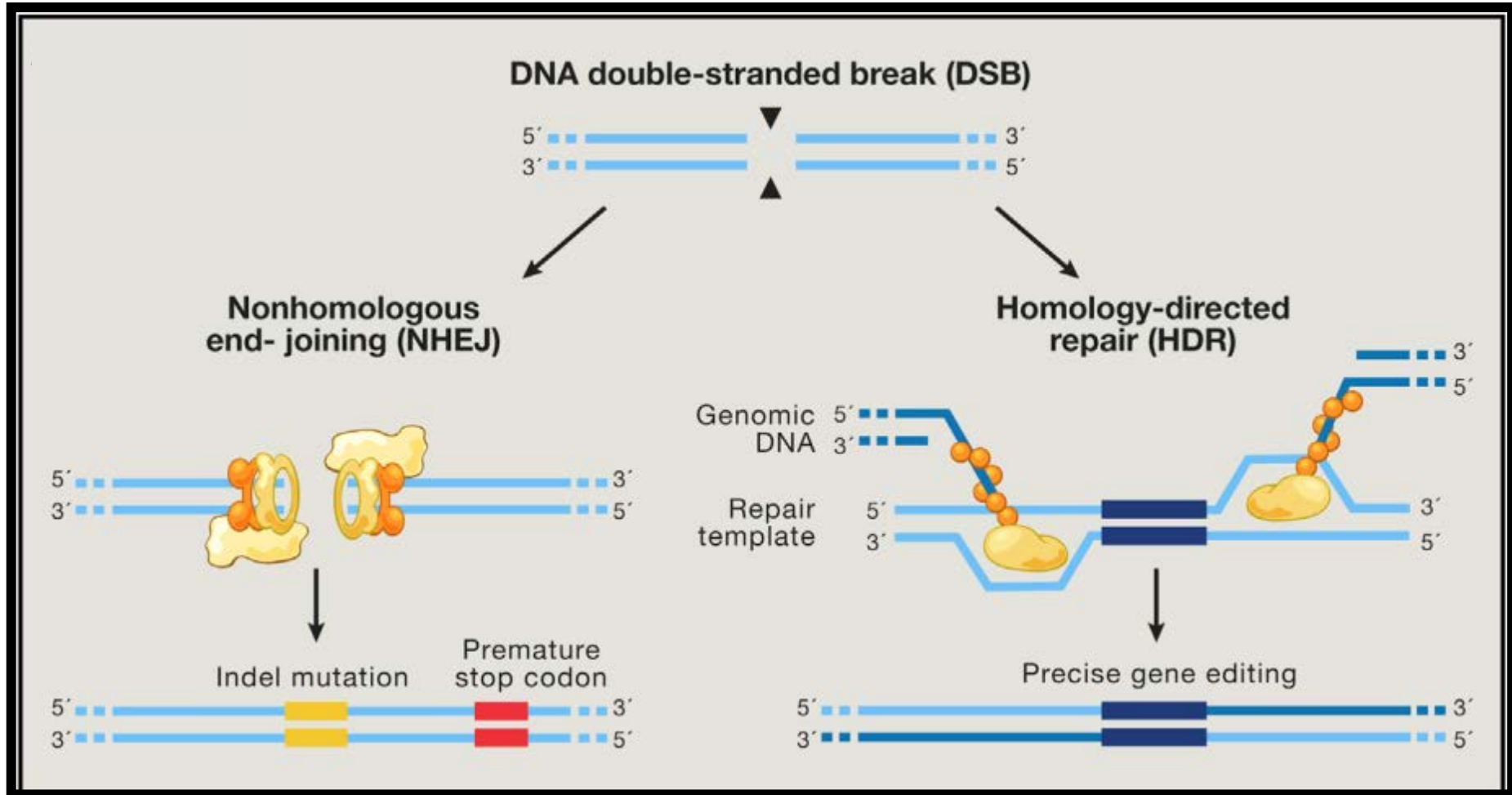
Healthfulness

Palatability

Tools for Enhancing Valuable Variation



Gene Editing Platforms



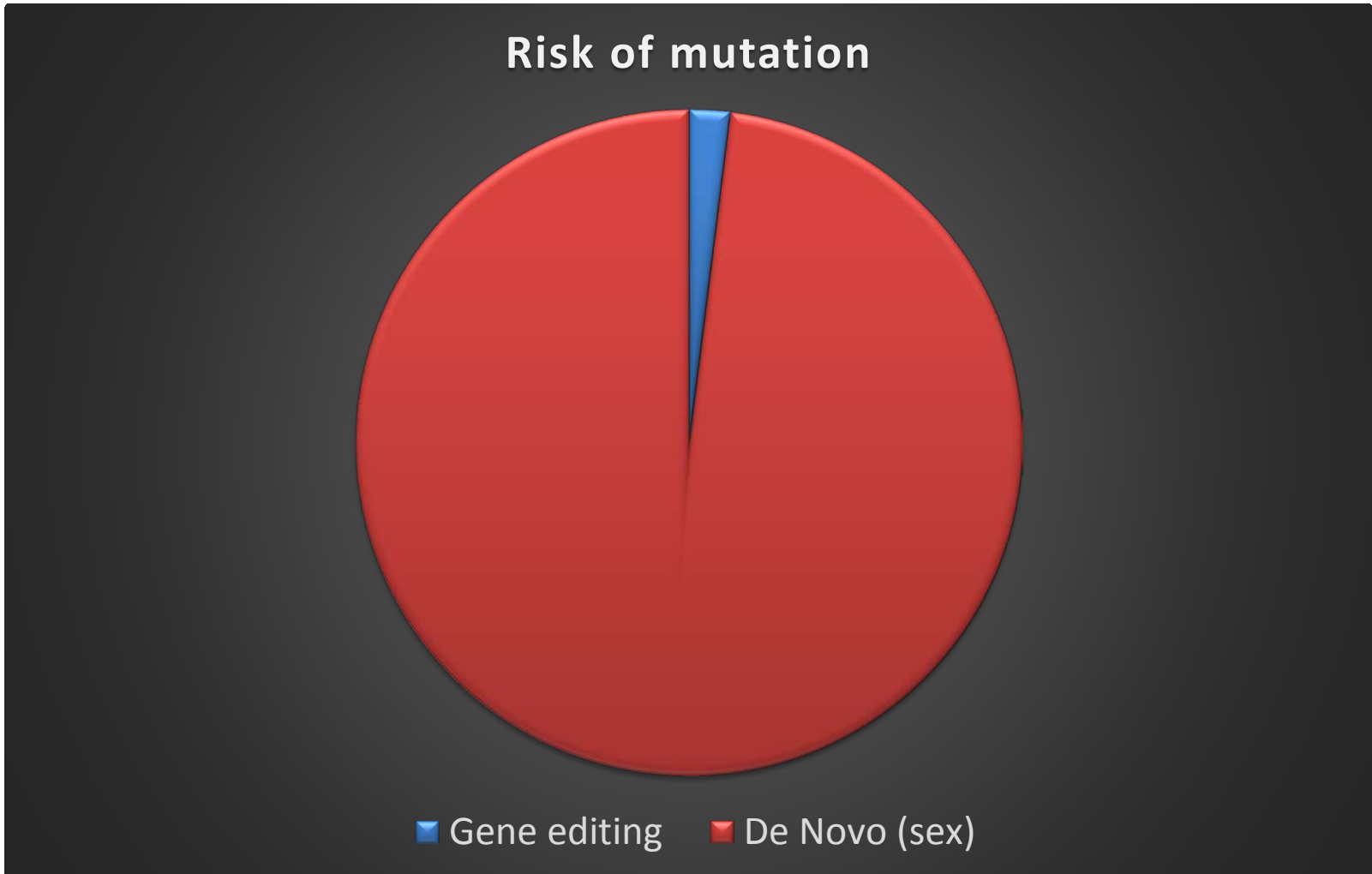
Gene Editing Platforms

Table 2 | **Comparison of three classes of programmable nucleases***

	ZFNs	TALENs	GENs
DNA targeting specificity determinant	Zinc-finger proteins	Transcription activator-like effectors	crRNA or sgRNA
Nuclease	FokI	FokI	Cas9
Success rate [†]	Low (~24%)	High (>99%)	High (~90%)
Average mutation rate [‡]	Low or variable (~10%)	High (~20%)	High (~20%)
Specificity-determining length of target site	18–36 bp	30–40 bp	22 bp (total length 23 bp)
Restriction in target site	G-rich	Start with T and end with A (owing to the heterodimer structure)	End with an NGG or NAG (lower activity) sequence (that is, PAM)
Design density	One per ~100 bp	At least one per base pair	One per 8 bp (NGG PAM) or 4 bp (NGG and NAG PAM)
Off-target effects	High	Low	Variable
Cytotoxicity	Variable to high	Low	Low
Size	~1 kb×2	~3 kb×2	4.2 kb (Cas9 from <i>Streptococcus pyogenes</i>) + 0.1 kb (sgRNA)

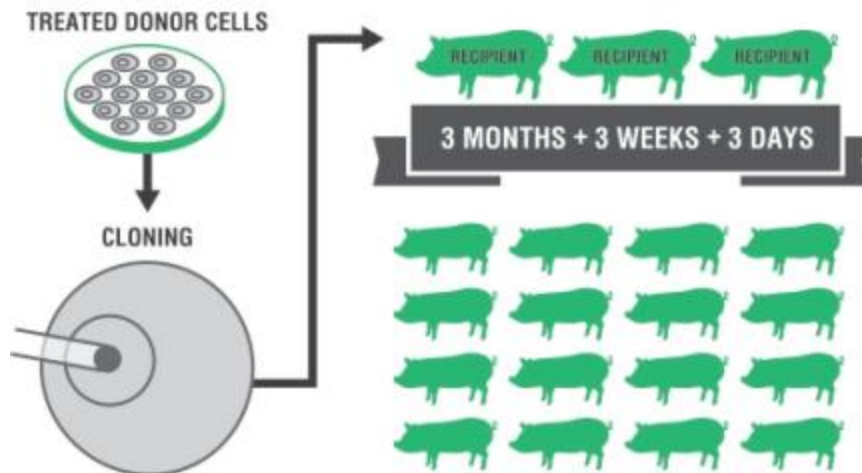
<10⁻⁴

Rates of De Novo mutation in germline

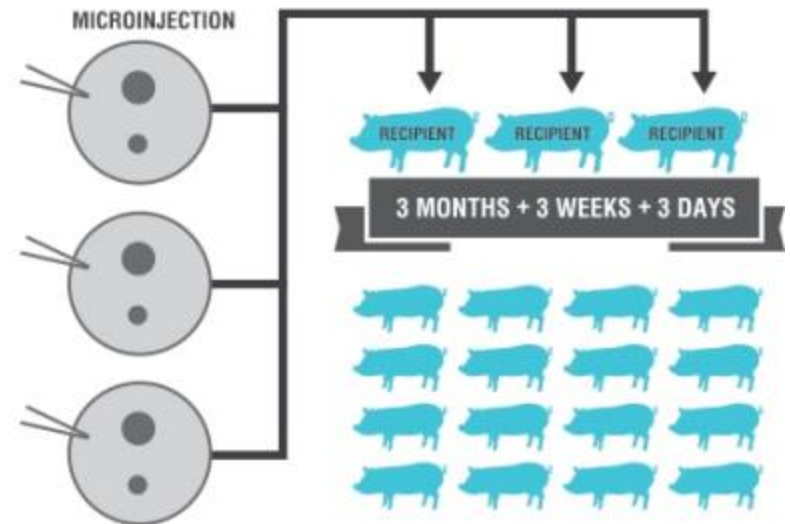


Deployment of Gene Editing in Livestock

CELL TARGETING



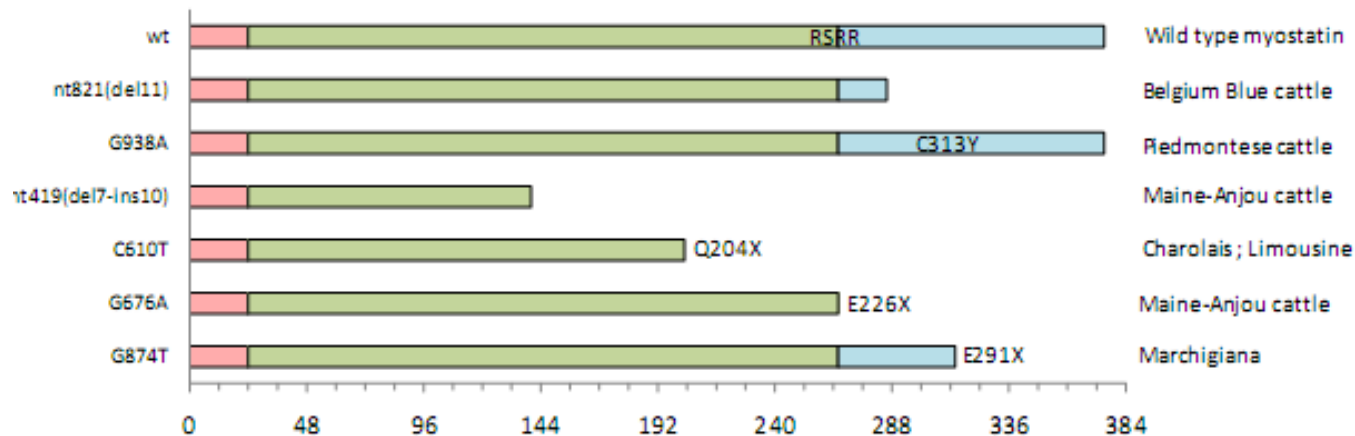
EMBRYO TARGETING



Double Muscling to Decrease Time-to-Market and Increase RPY



- At least 7 Spontaneous Mutations of Myostatin are known in cattle
- Results in 7% and 30% Increased RPY in European Breeds (+/mh; mh/mh)
- Mutation not found in some breeds
- Heterozygotes come to market weight on less feed/time.
- Can introduce by cross-breeding but changes genetics





- More Meat- Less Time, Same Feed
- Old Trait in a New Breed
- Centuries of Safe Consumption

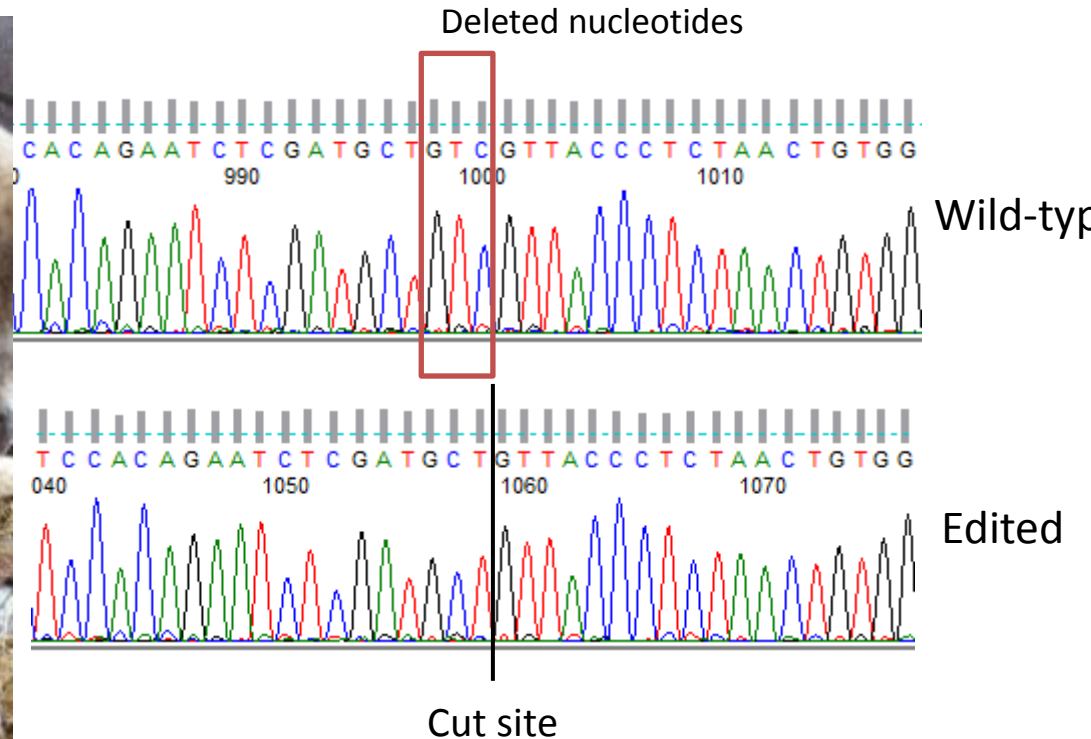
His	Ser	Thr	Glu	Ser	Arg	Cys	Cys	Arg	Tyr	Pro	Leu	Thr	Val	Asp
CACTC	CACAGAACT	CGATGCTGTC	GTTACCCTCT	AACTGTGGAI	WT									
GTGAG	GTGTCTTAGA	GCTACGACAG	CAATGGGAGA	TTGACACCTA										

His	Ser	Thr	Glu	Ser	Arg	Cys	Val	Val	Thr	Leu	***	Leu	Trp	Ile
CACTC	CACAGAACT	CGATGCGTCG	TTACCCTCTA	ACTGTGGATT	844Del1									
GTGAG	GTGTCTTAGA	GCTACGCAGC	AATGGGAGAT	TGACACCTAA										

282
Frame
Shift

Premature
Stop

Myostatin KO in sheep



- TGT-CGT-TAC (Cys-Arg-Tyr)

– Cut site 2

T**GT**-CGT-TAC

→

TGT-TAC (Cys-Tyr)

Deletion of arginine 283

African Swine Fever

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0022-538X/11/\$12.00 doi:10.1128/JVI.00331-11

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Species-Specific Variation in RELA Underlies Differences in NF- κ B Activity: a Potential Role in African Swine Fever Pathogenesis[∇]

Christopher J. Palgrave,^{1,2,†} Linzi Gilmour,^{2,‡} C. Stewart Lowden,³ Simon G. Lillico,²
Martha A. Mellencamp,^{4,§} and C. Bruce A. Whitelaw^{2,4}

Veterinary Pathology Unit, Division of Veterinary Clinical Sciences, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian EH25 9RG, United Kingdom¹; Division of Developmental Biology, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Roslin, Midlothian EH25 9RG, United Kingdom²; Veterinary Health Research Pty Ltd., Trevenna Rd., West Armadale, NSW 2350, Australia³; and Genus/PIC, 100 Bluegrass Commons Blvd., Hendersonville, Tennessee 37075⁴

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African swine fever virus (ASFV) is a highly infectious disease of domestic pigs, with virulent isolates causing a rapidly fatal hemorrhagic fever. In contrast, the porcine species endogenous to Africa tolerate infection. The ability of the virus to persist in one host while killing another genetically related host implies that disease severity may be, in part, modulated by host genetic variation. To complement transcription profiling approaches to identify the underlying genetic variation in the host response to ASFV, we have taken a candidate gene approach based on known signaling pathways that interact with the virus-encoded immunomodulatory protein A238L. We report the sequencing of these genes from different pig species and the identification and initial *in vitro* characterization of polymorphic variation in RELA (p65; v-rel reticulendotheliosis viral oncogene homolog A), the major component of the NF- κ B transcription factor. Warthog RELA and domestic pig RELA differ at three amino acids. Transient cell transfection assays indicate that this variation is reflected in reduced NF- κ B activity *in vitro* for warthog RELA but not for domestic pig RELA. Induction assays indicate that warthog RELA and domestic pig RELA are elevated essentially to the same extent. Finally, mutational studies indicate that the S531P site conveys the majority of the functional variation between warthog RELA and domestic pig RELA. We propose that the variation in RELA identified between the warthog and domestic pig has the potential to underlie the difference between tolerance and rapid death upon ASFV infection.



S531P

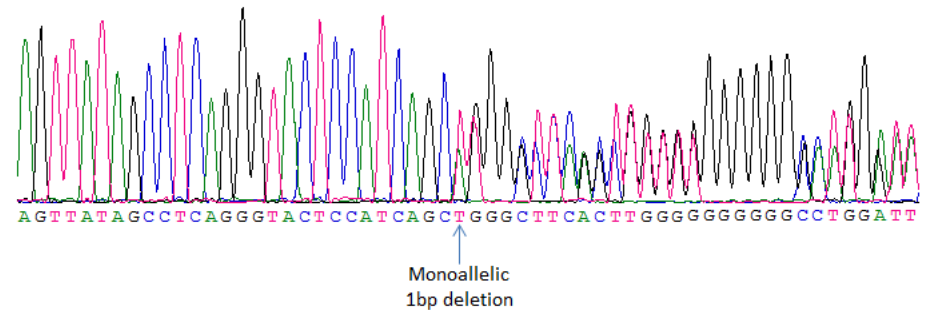


Truncation of RELA in pigs by embryo injection with TALENs



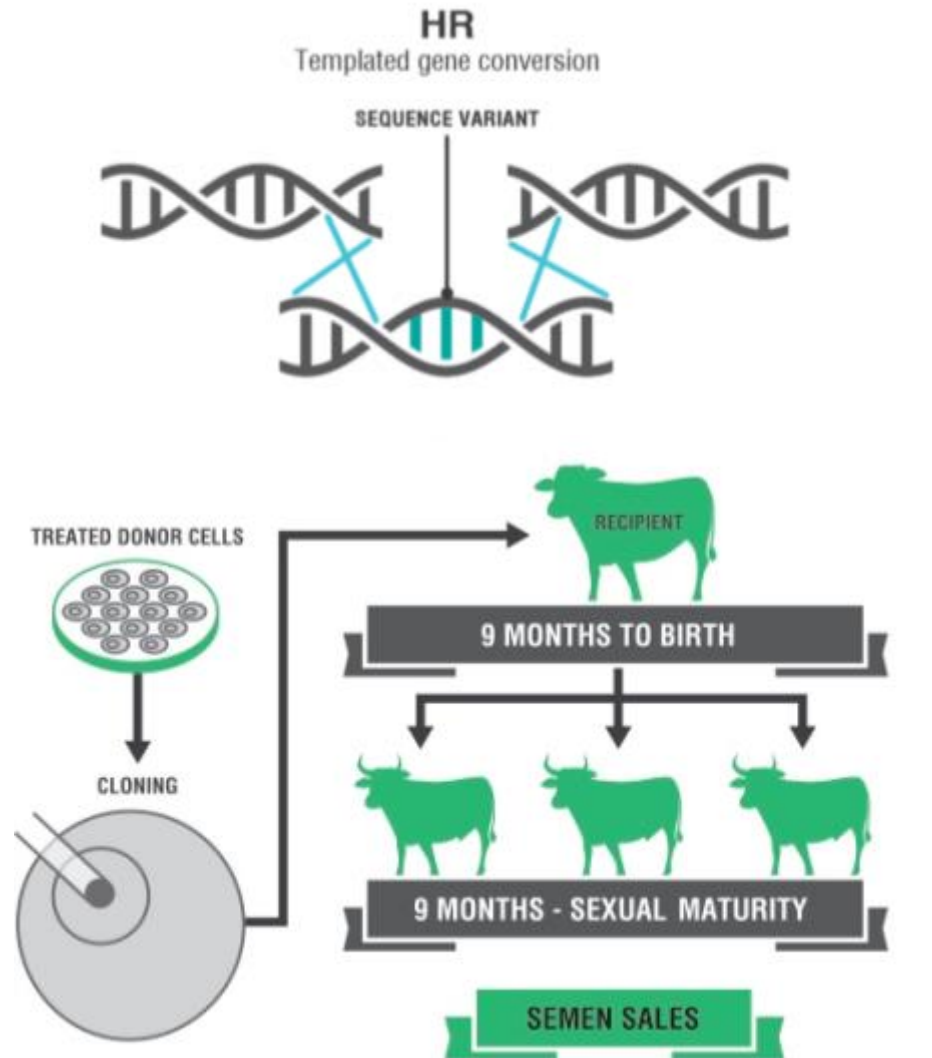
Parturition in 4 of 7 recipients
resulting in 39 piglets, 8 of which
carried editing events (21%).

Piglet 26



AGTTATAGCCTCAGGGTACTCCATCAGCATGGGCTCAGCTGTGTGGGGGGGCA (wt)
AGTTATAGCCTCAGGGTACTCCATCAGC-TGGGCTCAGCTGTGTGGGGGGGCA (1 del)

Livestock Gene Conversion with TALENs

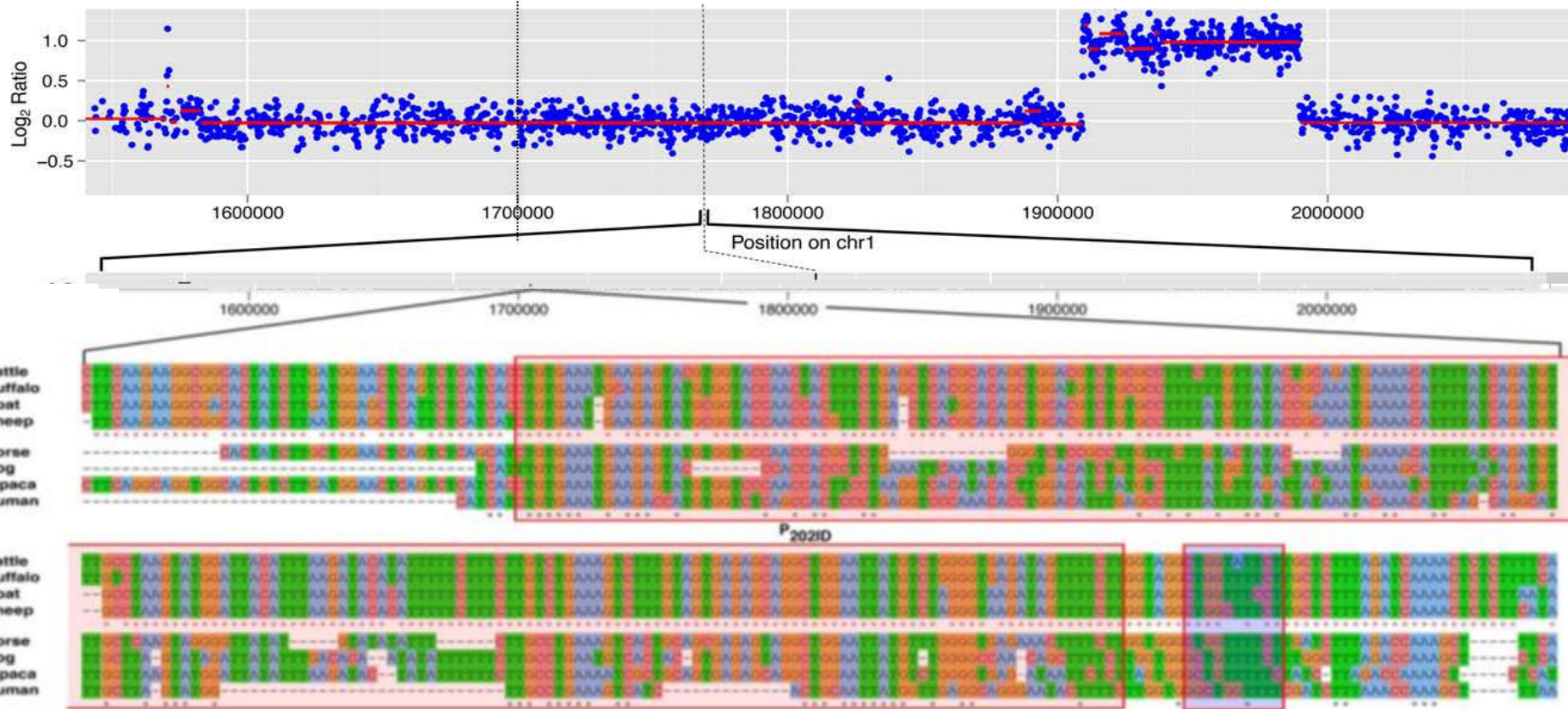




Lead U.S. Product: Genetic Dehorning of dairy cows

- Horns are hazardous to people & cattle
- Most U.S. dairy animals are dehorned (USD\$5-20 each)
- Polled at VERY low frequency in Dairy breeds (<1%)
- Breeding into Holstein would take >20 years & destroy dairy performance.
- Polled mitigates regulatory risk- NATURAL trait, NOT GMO
- Polled mitigates CONSUMER acceptance risk- STRONG market demand for animal welfare

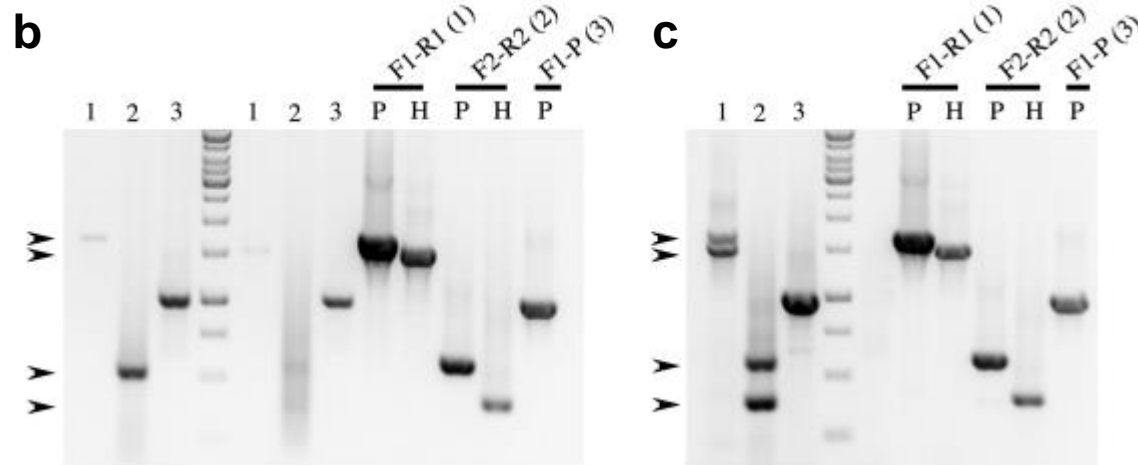
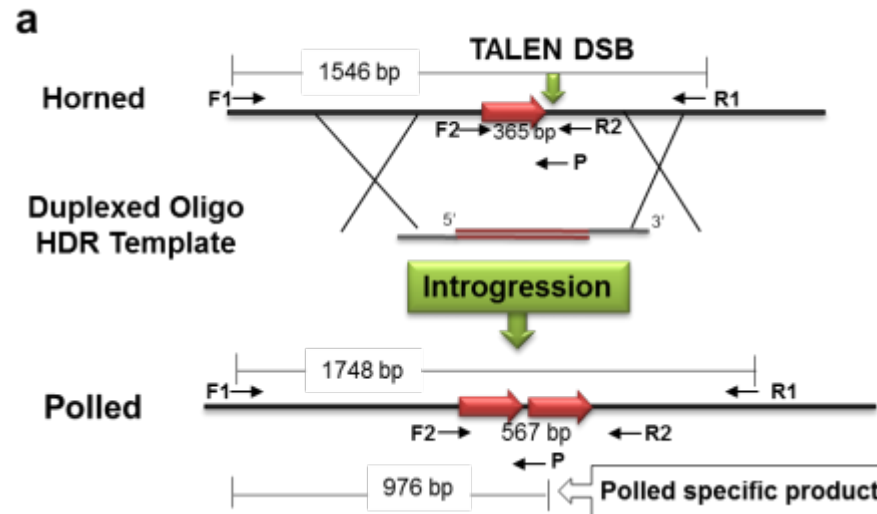
Polled is a Target for Introgression



P_C, Celtic origin (212 bp, 1,705,834–1,706,045 bp) is duplicated (and replaces a sequence of 10 bp (1,706,051–1,706,060 bp). Angus, Galloway, Fleckvieh, Gelbvieh and Murnau-Werdenfelser

P_F, Friesian origin. **P5ID** (replace 7 bp (cgcatca with ttctcagaatag; 1,649,163–1,649,169) and 80,128 bp duplication (1,909,352–1,989,480 bp **P_{80kbID}**, plus five point mutations at the positions (**G1654405A**, **C1655463T**, **T1671849G**, **T1680646C**, **C1768587A**))

Polled Allele Introgression into Holstein



Percentage of clones homozygous for introgression = 1-5%

Dairy Breeds Horned



Polled Beef Breeds NATURALLY no horns



Introgression

The polled allele (P)
is dominant to the
horned allele (h)



1. Crossbreeding.

Meiotic contamination



8+ generations of backcross
required to recover dairy
genetic merit

2. TALEN mediated gene conversion.

TALEN Cut



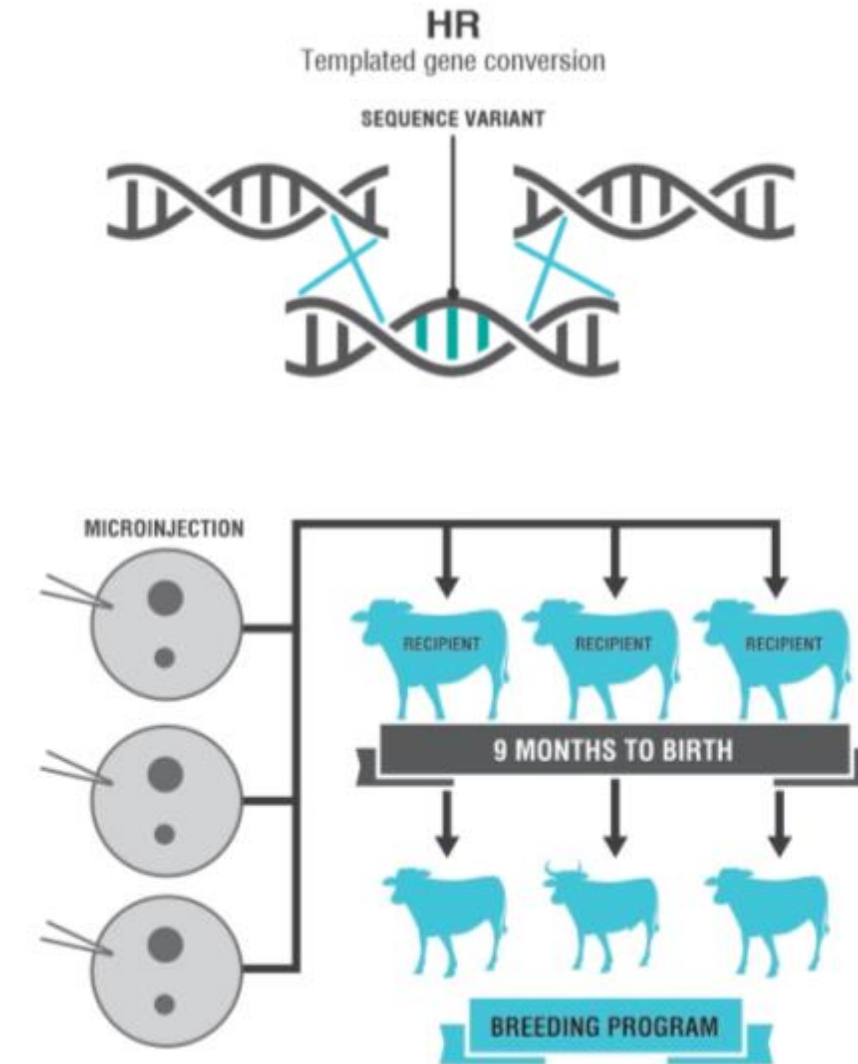
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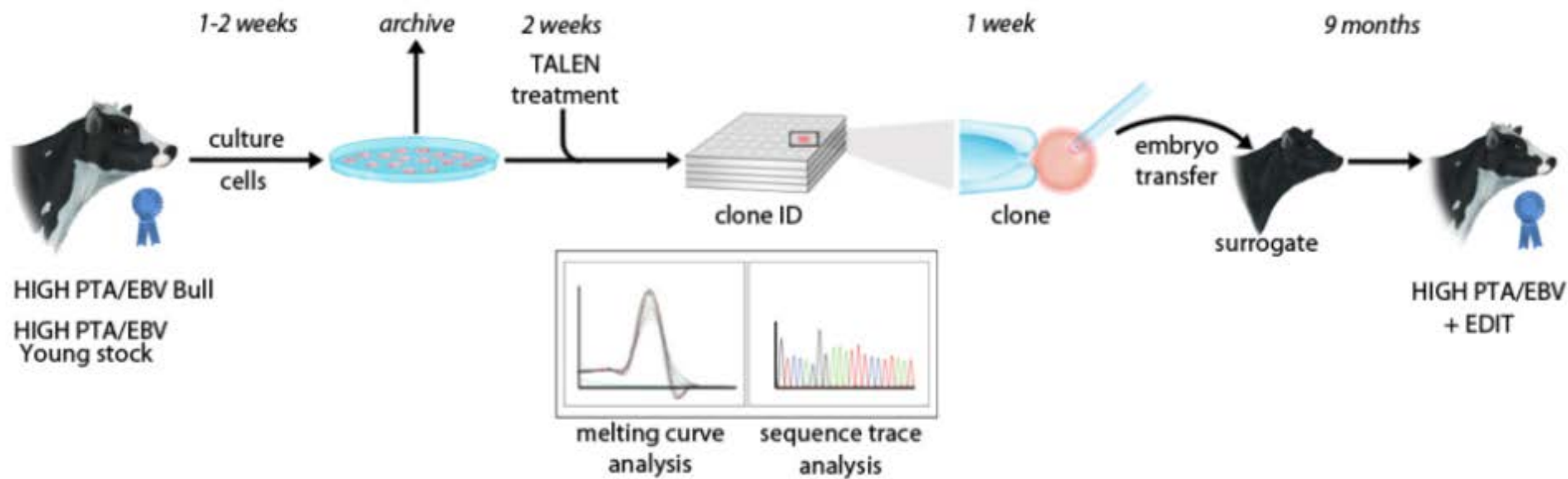


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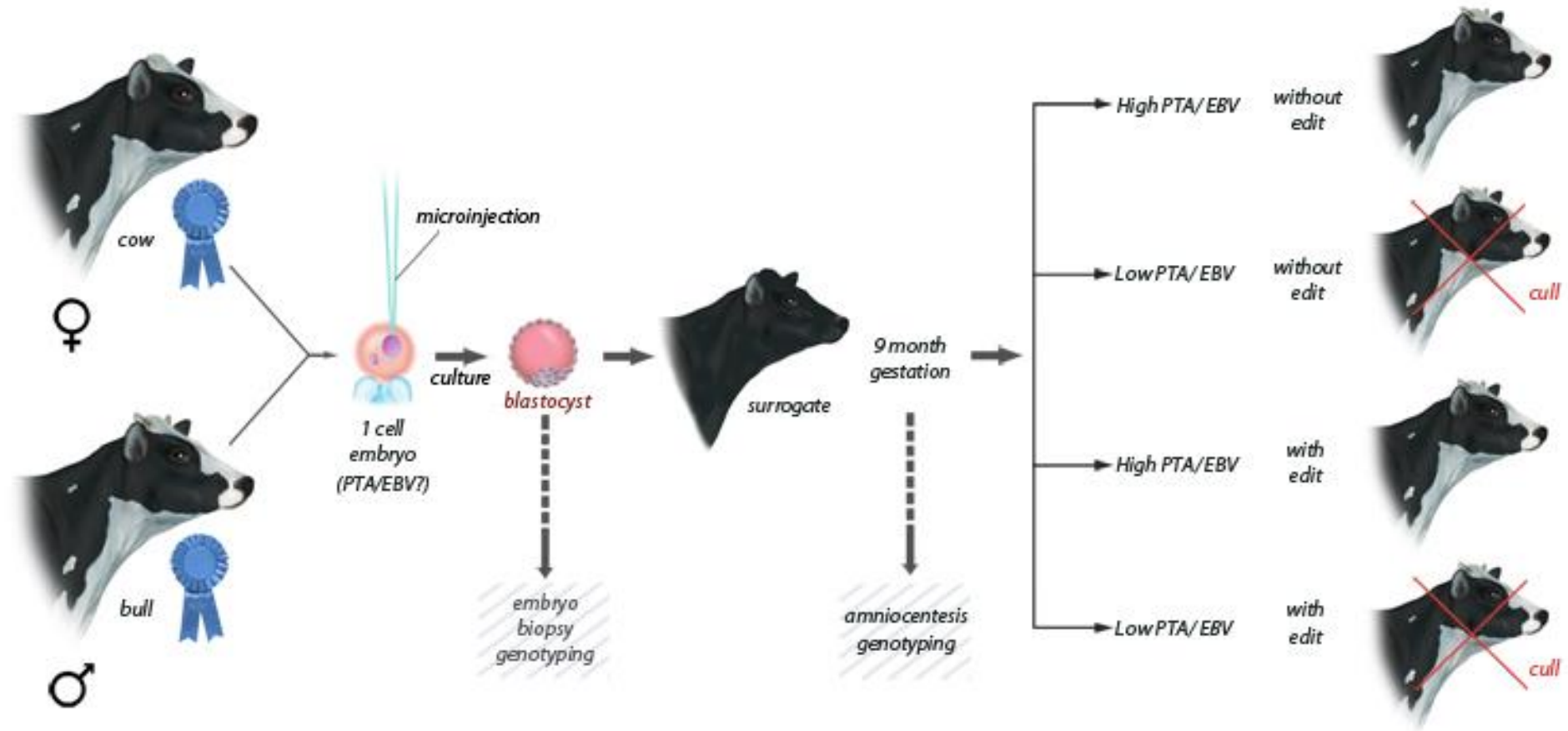


Livestock Gene Conversion with TALENs





Horizontal Allele Introgression



Gene Editing is Breeding

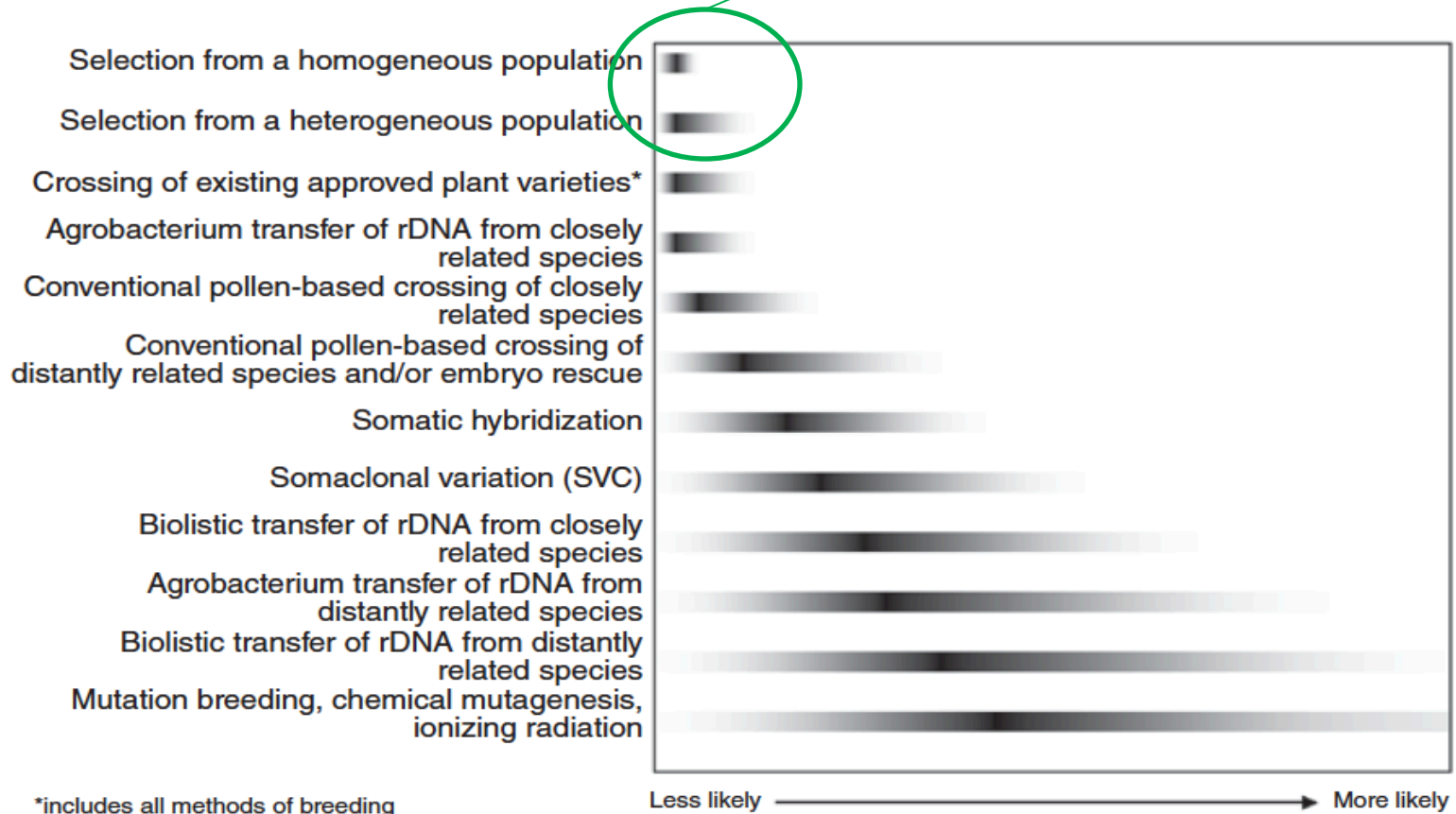


Figure 1 The NAS committee on the safety of genetically engineered food expressed the likelihood of unintended changes as a continuum with gene transfer more likely than all other modification techniques other than mutagenesis.