Part I. Case examples showing contribution of genome editing

11:20-11:50 Genome Editing in Argentina: Initiatives and Prospective

Dr. Sergio Feingold, Coordinador Programa Nacional de Biotecnología, Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina

Genome Editing in Argentina: Initiatives and Prospective

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Abstract:

Selection of superior genotypes –plants or animals- has always relied in the existence of genetic diversity. Introduction of variability had been achieved by different techniques since the domestication of species. Natural and anthropic hybridization, both intraspecific and among related species, followed by natural or induced mutations by physical and chemical agents were and still are basic tools in plant breeding. However, the ability to produce viable offspring from interspecific crosses sets a barrier to the introduction of diversity. The discovery of mutagenic agents to increase the frequency of genetic variations, led to the idea that genetic variability could be infinite, but soon it was recognized that this random process often derived in deleterious or lethal events and the chance to impact traits with a polygenic inheritance was very odd. The advent of genetic engineering opened the possibility to overcome sexual incompatibility of any living organisms, even across different kingdoms. Unfortunately, this technique did not yield its full potential. The raise of unexpected –and negative- public awareness, the existence of recalcitrant genotypes or species to either be transformed or regenerated and the strict and often expensive de-regulation process to release improved developments, were probable the main factors that may explain this fact. Genome Editing constitutes a bigger leap in genetic modification and variability introduction. It has the potential of custom modification of specific genes to alter their expression (from knock-out to overexpression), replace alleles and introduce transgenes at specific sites in the genome. It can also reduce breeding times dramatically, and can produce a radical modification in breeding programs of clonal plants such as potato, cassava, sugarcane and grape among others.

Genome editing can be a technical challenge, especially if transient expression of the edition machinery is required. The absence of foreign sequences can allow the release of the improved species without special regulatory requirements as GMOs has to pass through today. The lack of selection markers, also poses a bottleneck that will imply significant effort in identifying the “edited” offspring. A full understanding of genes, their functions and regulations is a pre-requisite in identifying the target sequences and in which way the edition will change its sequence. Also, full genome sequences of good quality can minimize the

chance of occurrence of “off targets”. Moreover, the knowledge of naturally occurring allelic variants and their impact on the phenotype can lead allele replacement. At INTA - the main National Agricultural Research Institution of Argentina- initial attempts on the use of genome editing are focused on the knock-out of genes related to industrial and nutritional quality, water use efficiency and disease resistance in potato; the elimination of an allergenic protein in cow milk, and the edition of the bovine prion gene responsible for mad cow disease. These developments will be soon followed by others in wheat, alfalfa, maize and ornamental petunia. As a public institution from a developing country, a great concern is centered in the still unresolved intellectual property rights around Genome Editing and the freedom to operate with this technique. Strategically, it can represent a great opportunity for developing improved varieties of crops in traits of local interest and added value, and to enhance crop diversification out of herbicide resistant soybean.

Application of Genome Editing Techniques in Agriculture in and Outside of Japan – Current Situation and Future

"Genome Editing in Argentina: Initiatives and Prospective"

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National Biotechnology Program
National Institute of Agriculture and Farming
INTA

Tokio - July 10th, 2017

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Ministerio de Agroindustria
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History of Plant Breeding

10000- 8000AC

Plant domestication

First Sedentaries

Mass Selection

Mary Evans Picture Library/Grosvenor Prin

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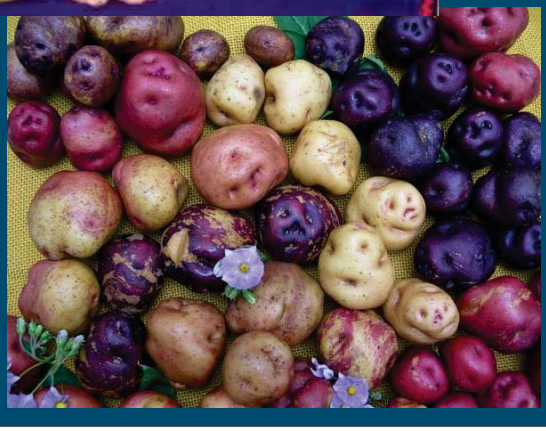
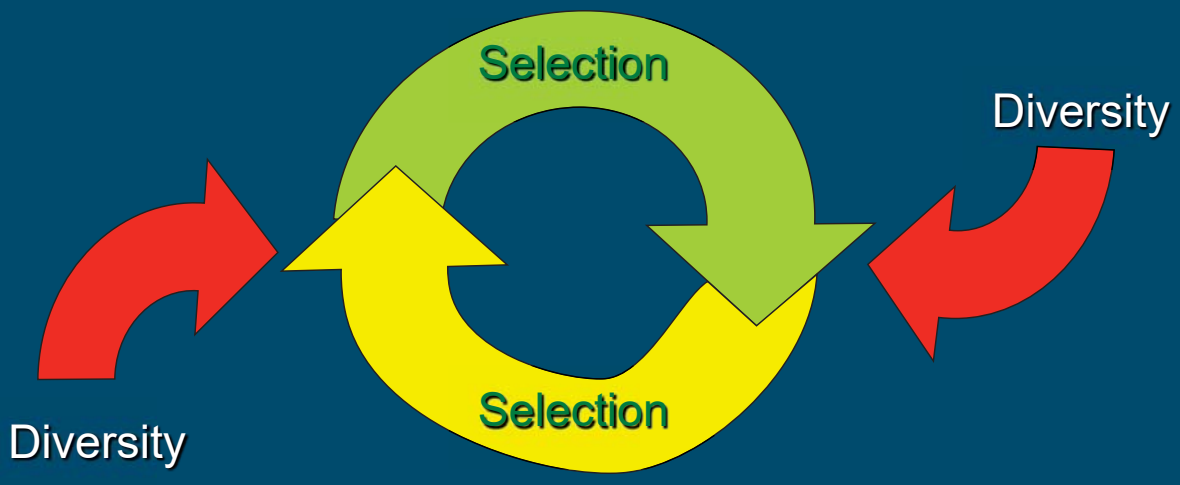
Mass Selection



History of Plant Breeding

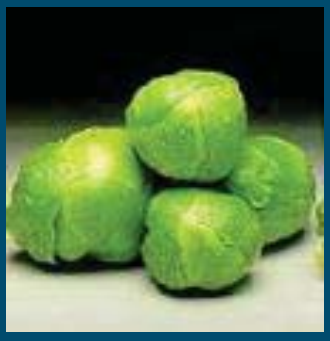
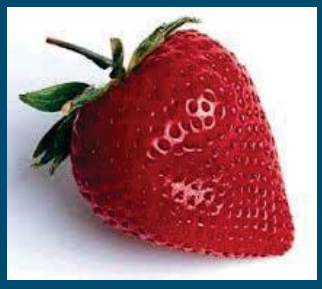


History of Plant Breeding

Re-introduction of variability

End of XIXth Century	First crosses • within-species • betwen related species
XXth Century	Mutation breeding: X-rays, γ-rays , EMS
	Genetic modified Organisms (GMOs): Transgenesis
XXIth Century	Genome Editing

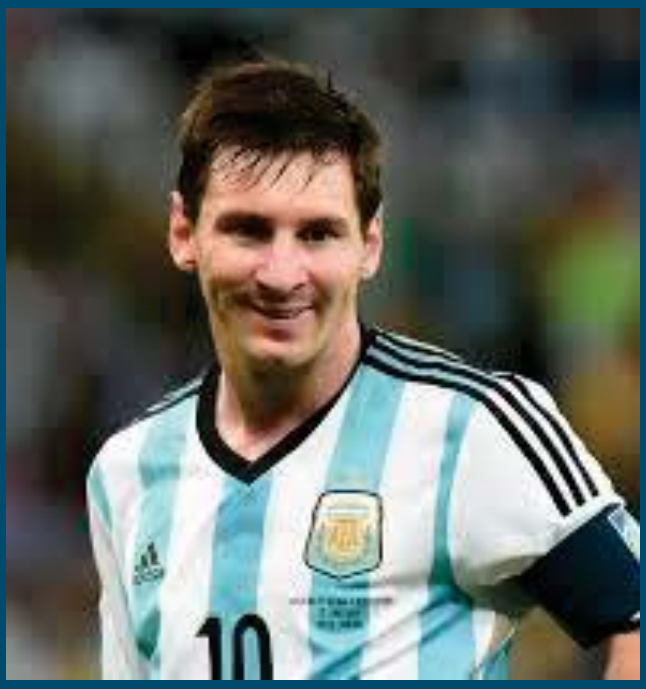
"Natural vs. Artificial"



"Natural vs. Artificial"

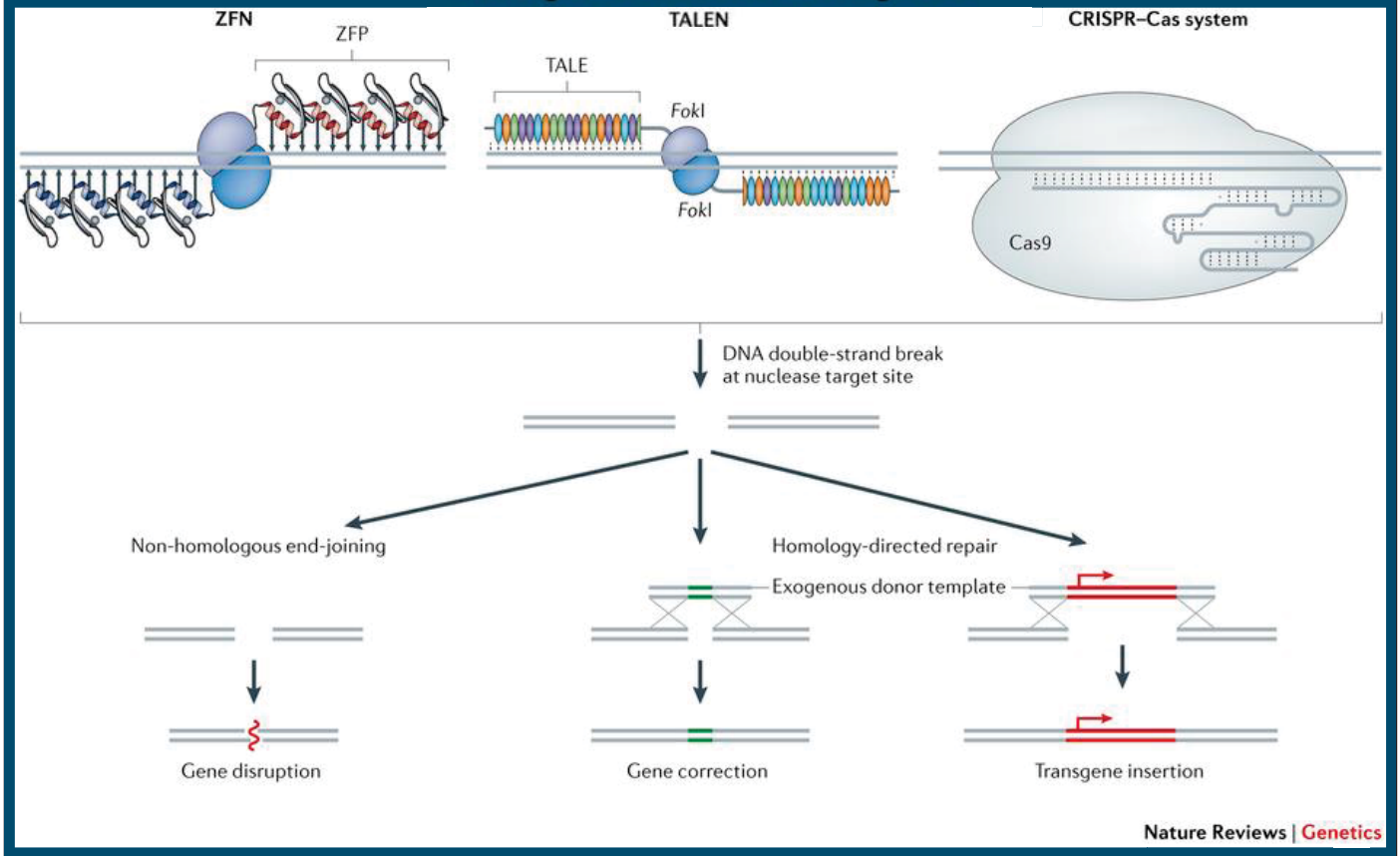
"Natural vs. Artificial"



Human Growth Hormone (somatotropin) treatment produced out of recombinant bacteria, instead of corpses

Genome Editing

Site/ gene directed mutagenesis



probabilistically a seq of 24 bp occurs only once in *A. t.*

Hao Yin et al., 2014

Considerations for Genome Editing Technology

- Gene, sequence, sense
- Genomes, Genes and natural variation (allele diversity)
- Constraints and bottlenecks
- self-fertilizing, hybrids and clones
- Regulatory affairs
- Intelectual Property Rights
- GMOs vs. Genome Edition
- Stakeholders Landscape

Which gene,

What sequence

In which sense

Genomes, genes and natural genetic diversity

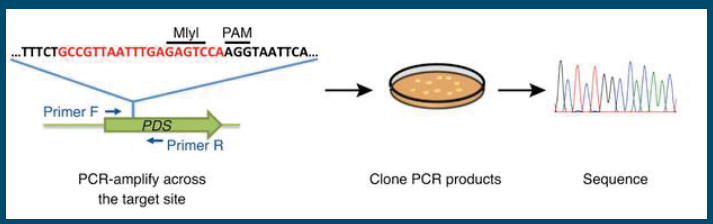
Constraints of Genome Editing

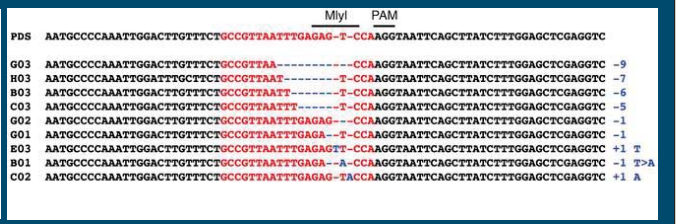
off-targets

selection method

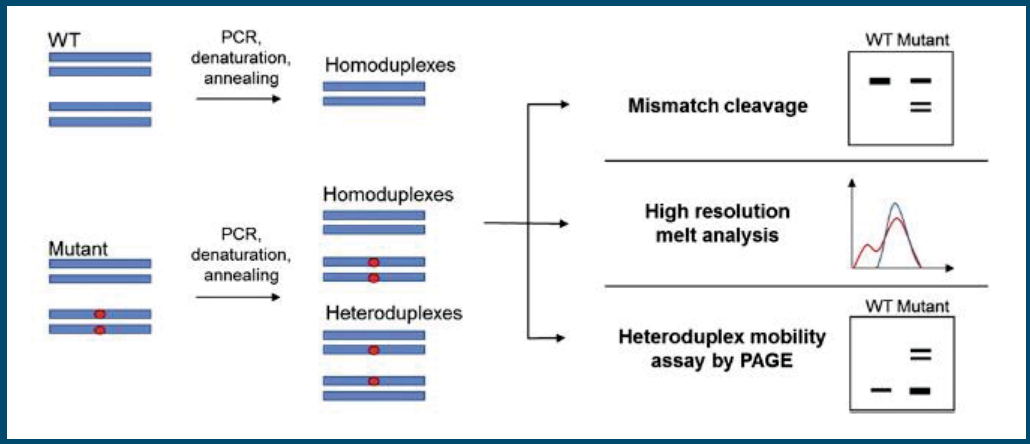
transient expression

Mutation Detection





Nekrasov *et al*., 2013



Zischewski *et al*., 2017

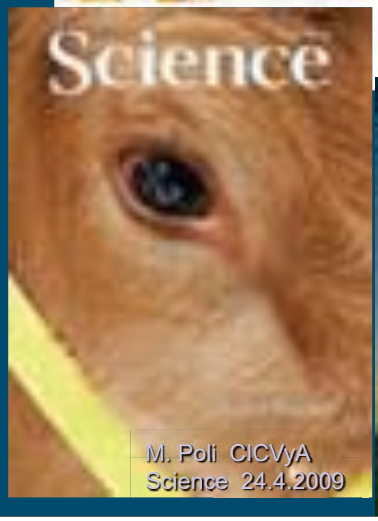
Whole Genome Sequencing: Some examples at INTA

Feingold, et al.
EEA Balcarce, 2011

Carrari et al.,
CICVyA, 2012

Pontaroli, EEA Balcarce, 2012

Debat et al., CIAP, 2014



Helguera et al.,
EEA MJ, 2014

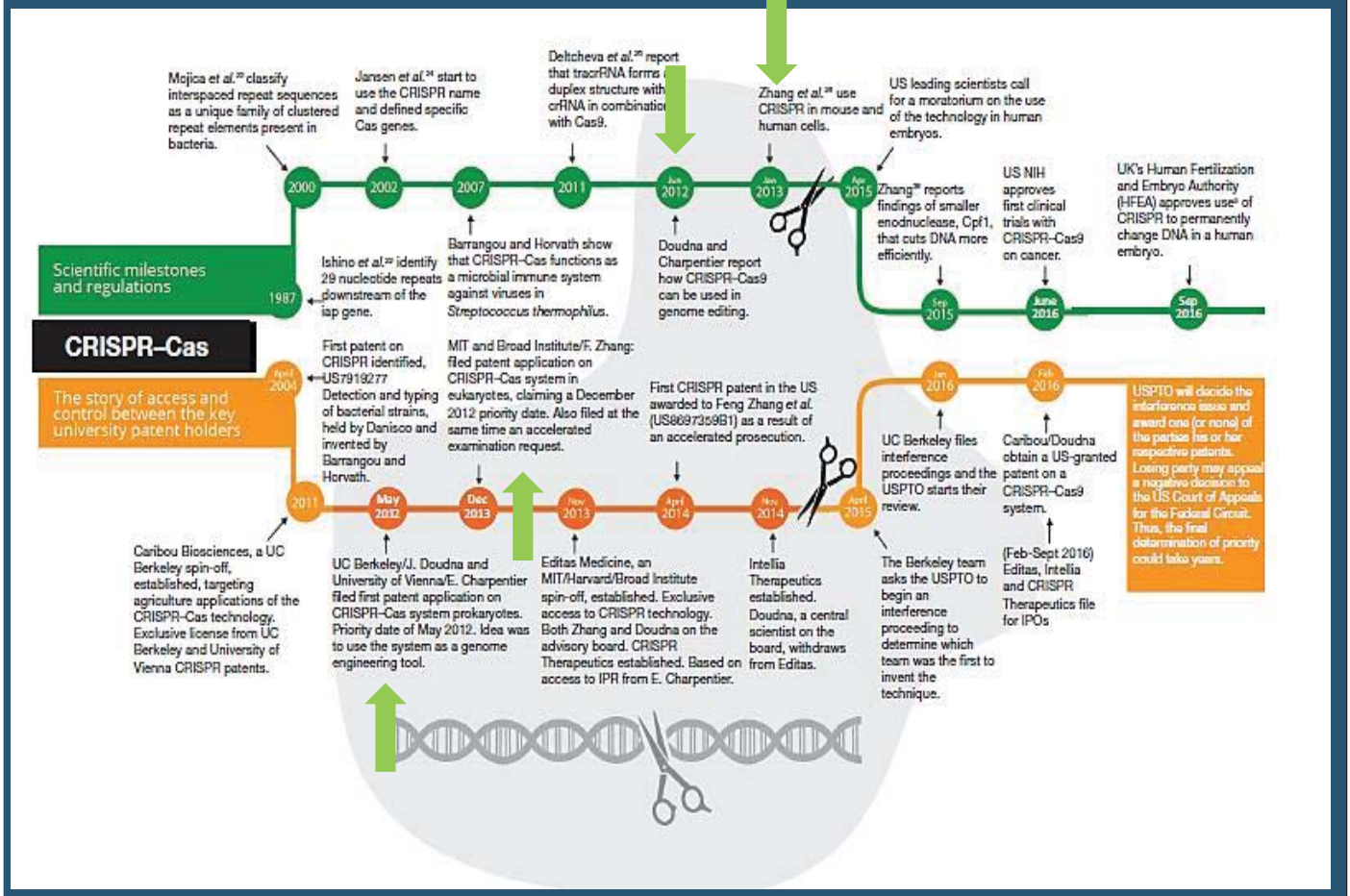
self-fertilizing,

hybrids

and clones

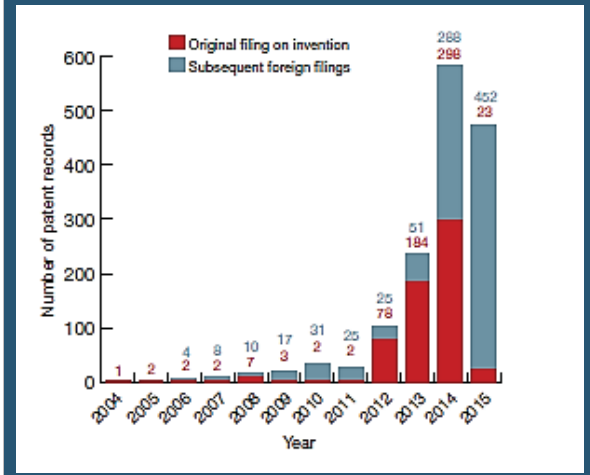
Regulatory affairs

Intelectual Property Rights

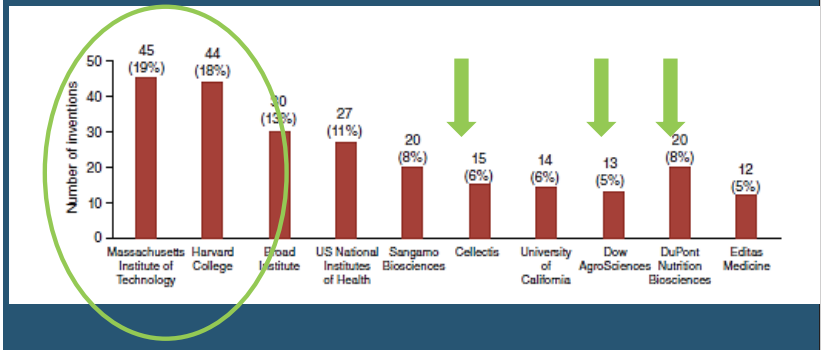


Egelie et al 2016

evolution of patents based on CRISPR/Cas9



Increase since 2012.
same pattern as publications.
Decay in 18 months lag



Public institutions with control over CRISPR/Cas applications in medicine

Private stakeholders emerging in agriculture and food production application

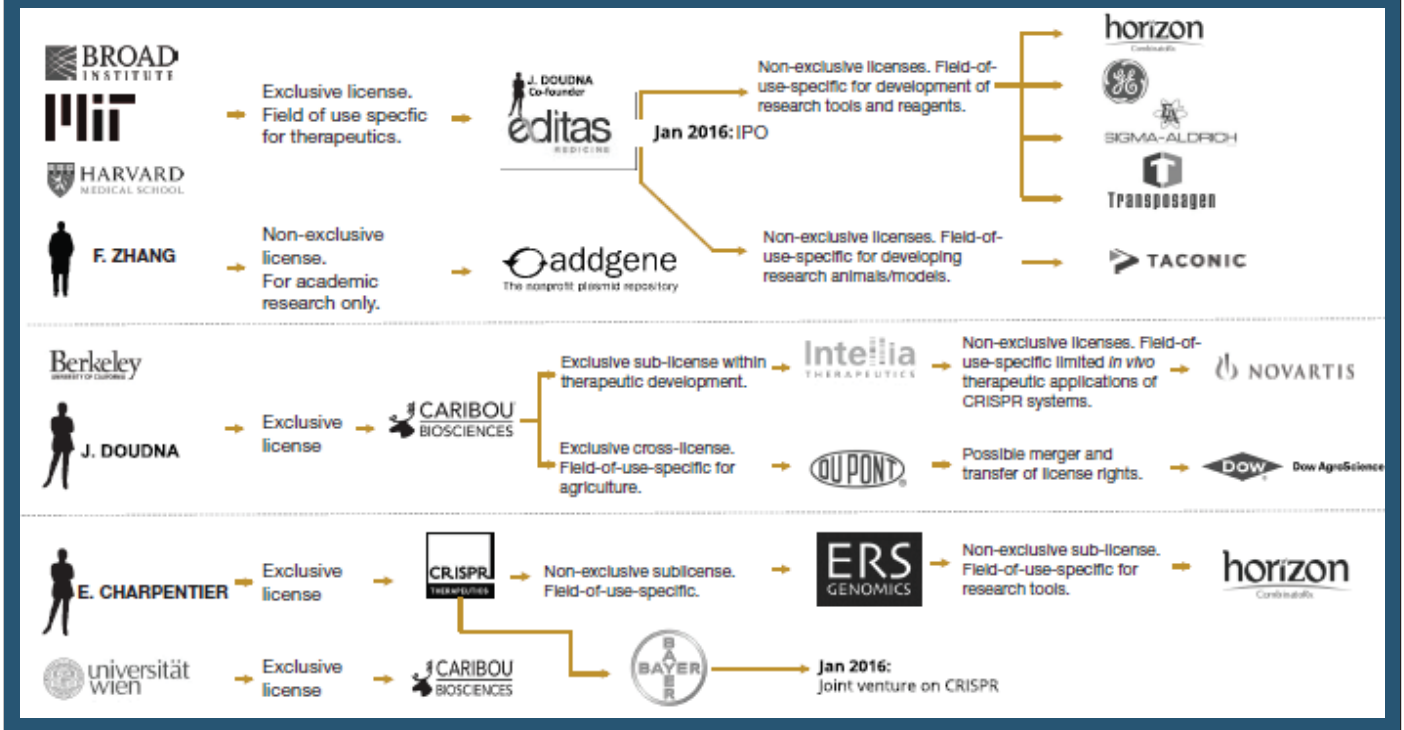
Egelie et al 2016

What is protected?

Technical categories	Detailed technical categories	Total inventions	MIT/Harvard/ Broad/Zhang group	Doudna/ Charpentier/UC Berkeley–Vienna group	Dow/ DuPont
CRISPR–Cas9 components	CRISPR RNA	139	14	4	6
	tracrRNA	63	11	0	0
	gRNA	212	38	7	3
	PAM	56	8	2	0
	Cas9 enzyme	121	25	0	0
Total		591			
CRISPR–Cas9 activity	RNA-Cas complex	54	6	0	4
	Spacer integration	10	1	0	3
	Cas cleavage	31	3	1	5
Total		95			
Vectors	Expression vectors	94	7	4	0
	Bacterial	12	0	0	2
	Viral	97	28	1	2
	Plasmid	132	27	2	7
Total		335			
Delivery	Liposome	30	10	0	1
	Nanoparticle	33	16	0	0
	Exosome	16	12	0	0
	Microvesicle	16	11	0	1
Total		95			
Application	Gene editing	78	19	2	1
	Gene therapy	105	23	3	1
	Drug discovery	10	4	0	0
	Diagnosis	79	11	0	0
	Regulating	70	6	3	3
	Targeting	167	24	3	5
Total		509			

Egelie et al 2016

Access and control of CRISPR/Cas9



Egelie et al 2016

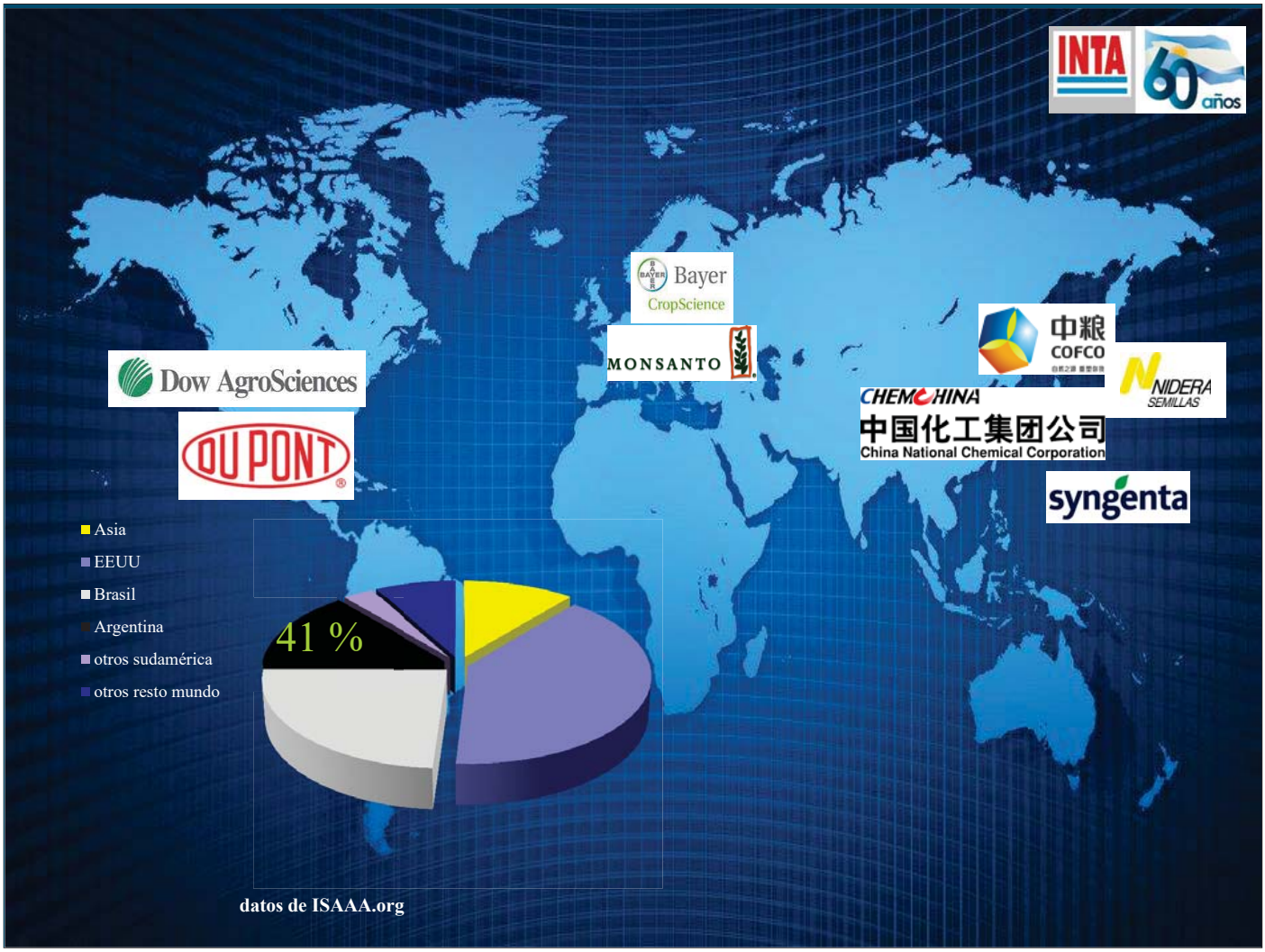
GMOs vs. Genome Editing

Stakeholders Landscape :

Big Players,

NARs,

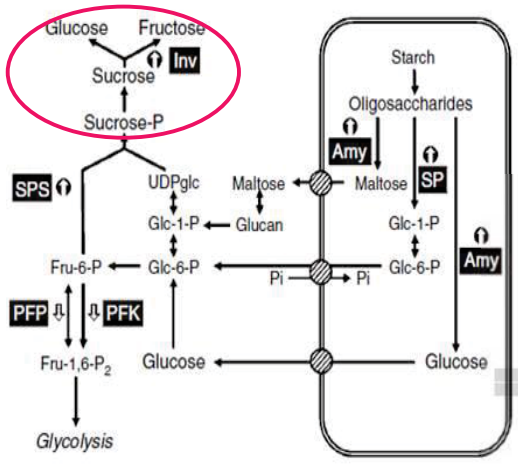
and small business

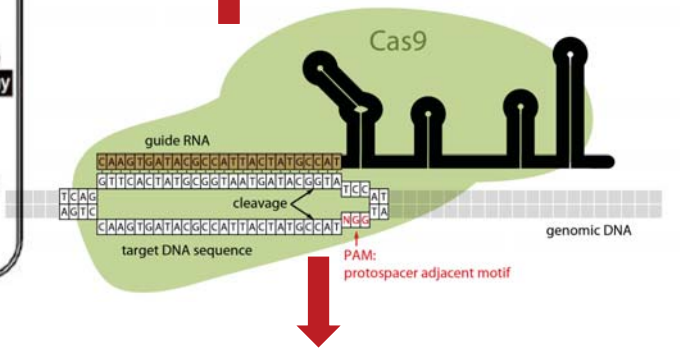


Some research in progress

Cold induced sweetening in potato

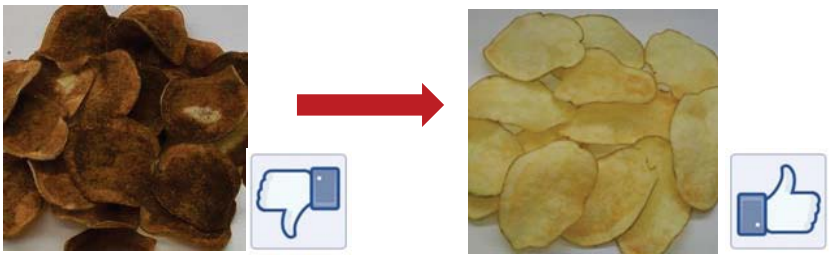
Vacuolar Invertase



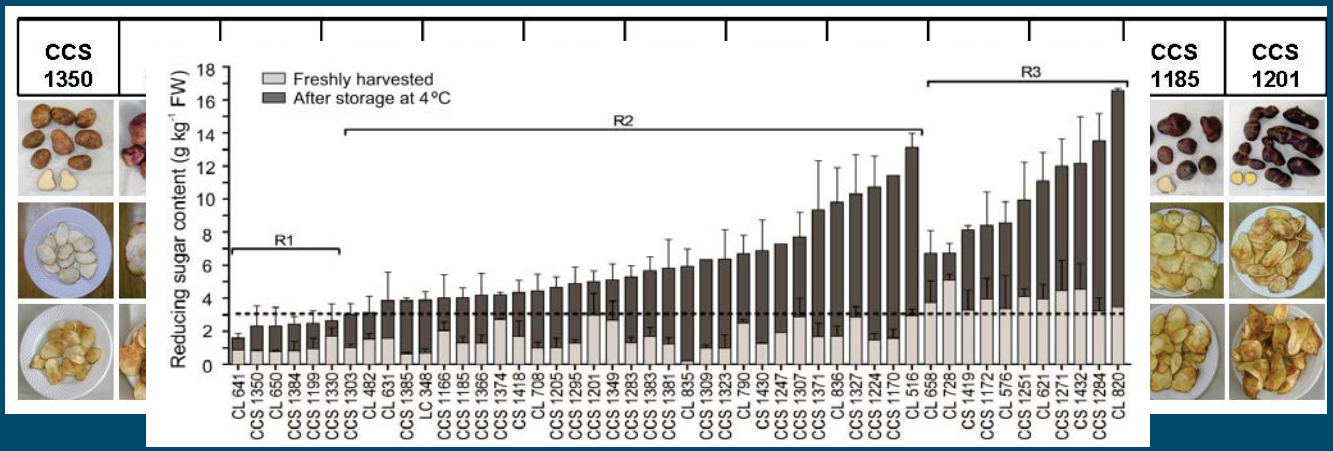


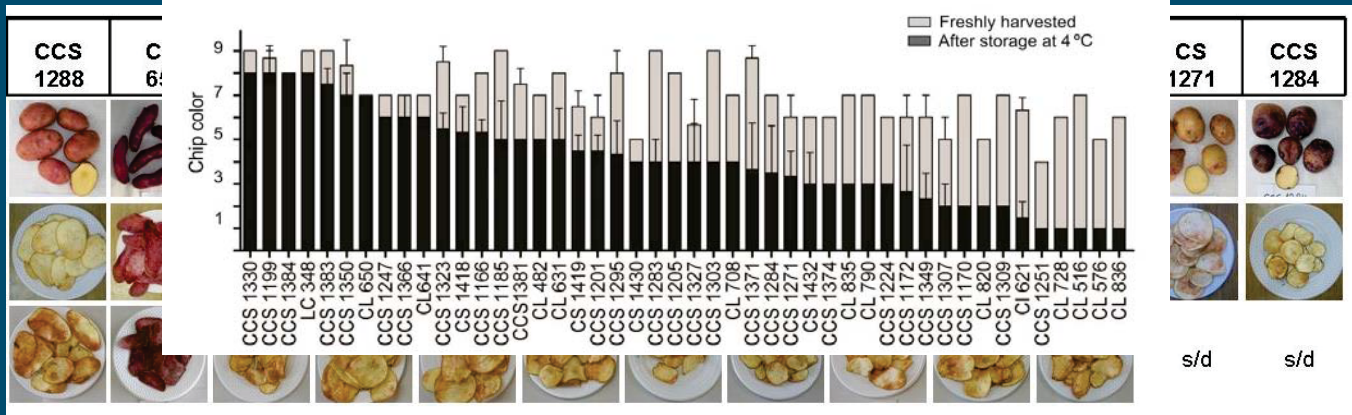
Adaptad from Malone *et al*., (2006).

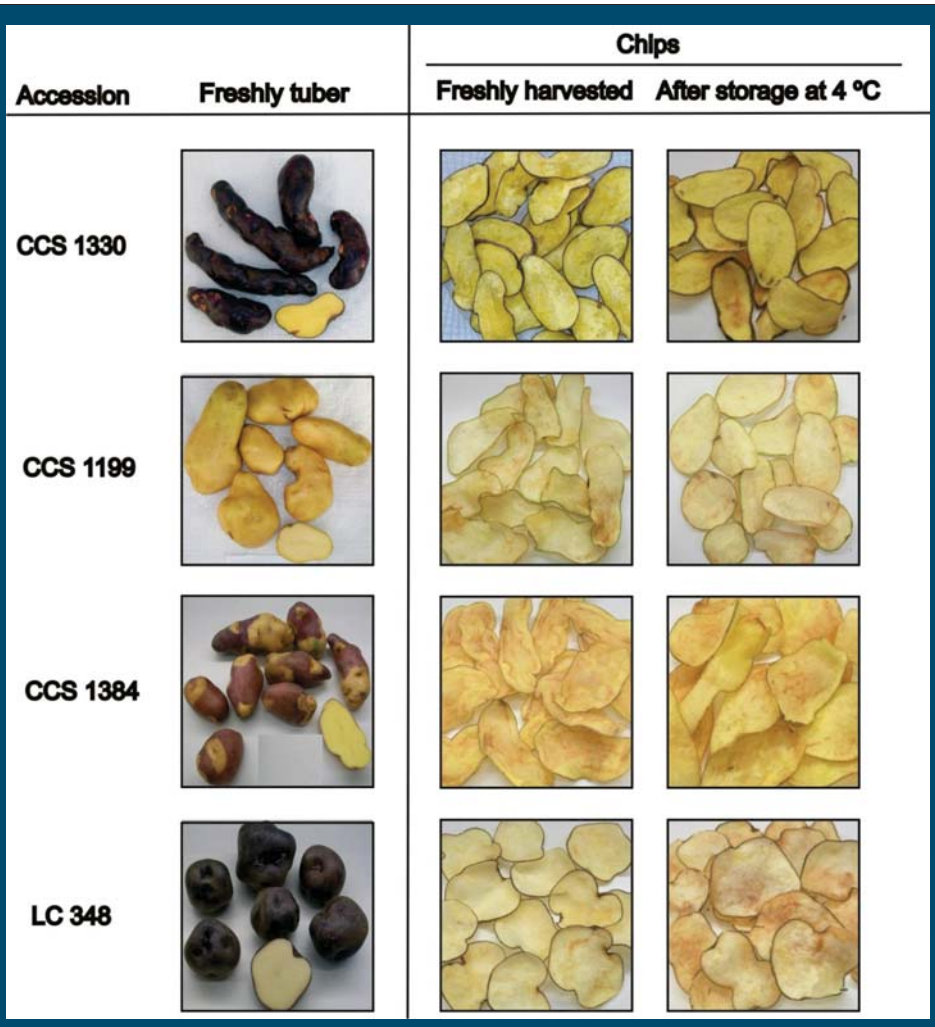
Gene Editing: knock-out of Inv-Vac



Phenotypic variation

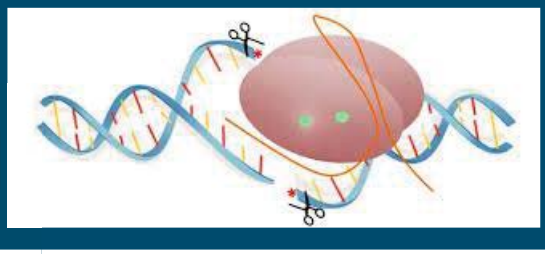


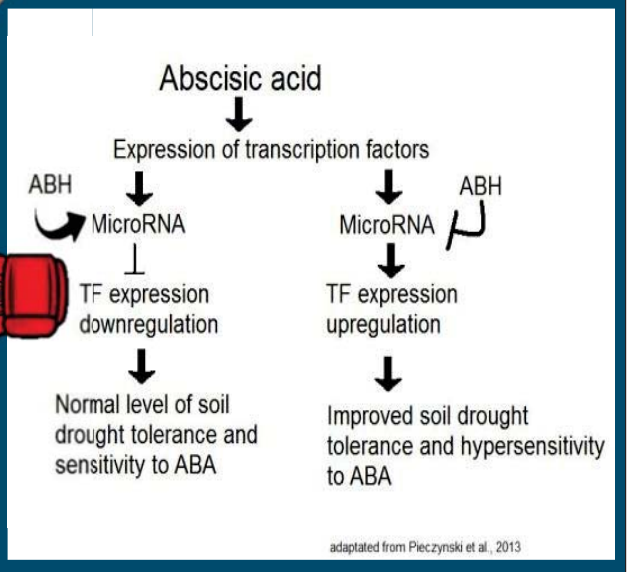




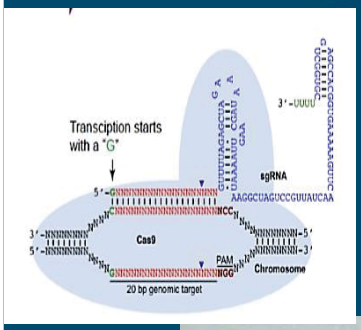
Improving water use efficiency in potato

Due to the climatic change the application of irrigation has increased in the last 15 years in order to maintain the potato production. This fact results in soil degradation by erosion and salinity



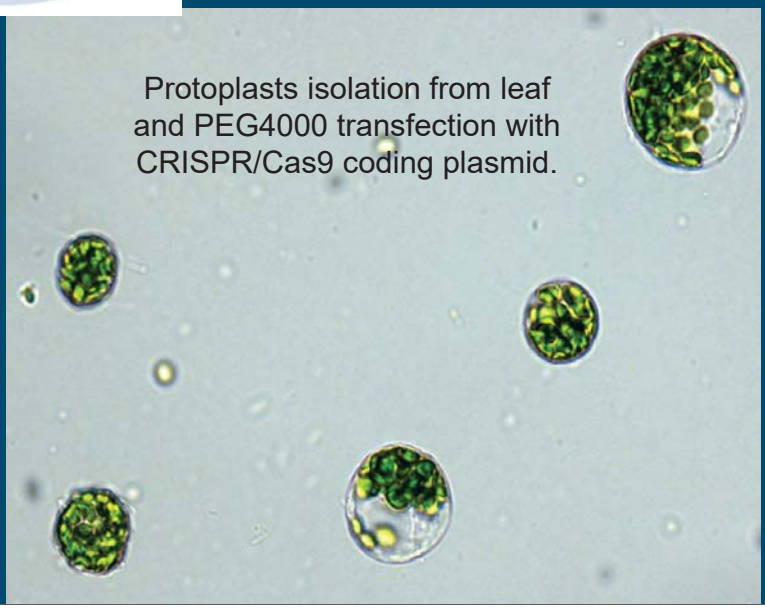


Transient expression in protoplast for potato genome editing with CRISPR/Cas9



Target site identification and sgRNA design.
Assembly of CRISPR/Cas9 coding plasmid.

In vitro potato plant

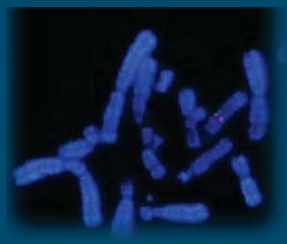


Protoplasts isolation from leaf and PEG4000 transfection with CRISPR/Cas9 coding plasmid.

Protoplasts regeneration and shoot genotyping

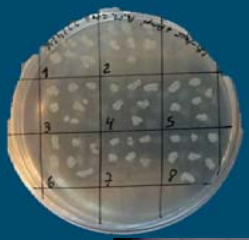
Edition of milk protein genes

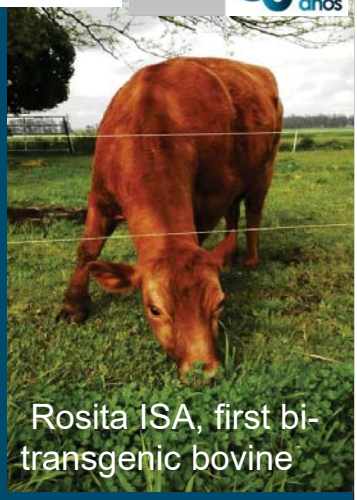
- Deficit (lysozime – Lactoferrin)
- Excess (Caseins – β-Lactoglobulin)



β-Lactoglobulin

- Milk allergy
- Diabetes
- Cancer





Rosita ISA, first bi-transgenic bovine

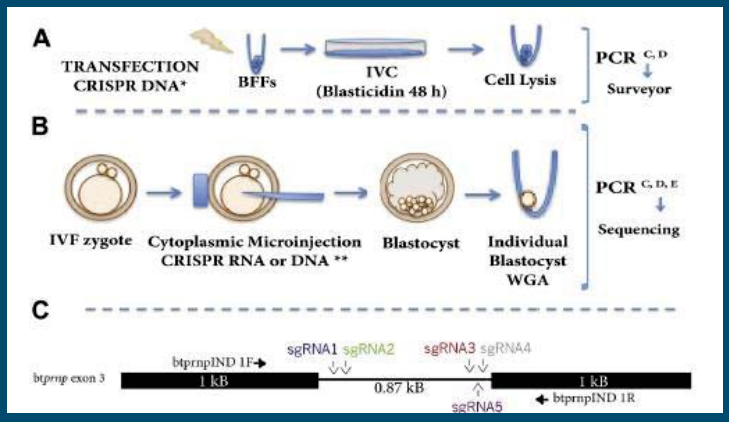
Gene edition (knock-out) of β-Lactoglobulin bovine gene using CRISPR/CAS9

- In vitro embryo production and microinjection

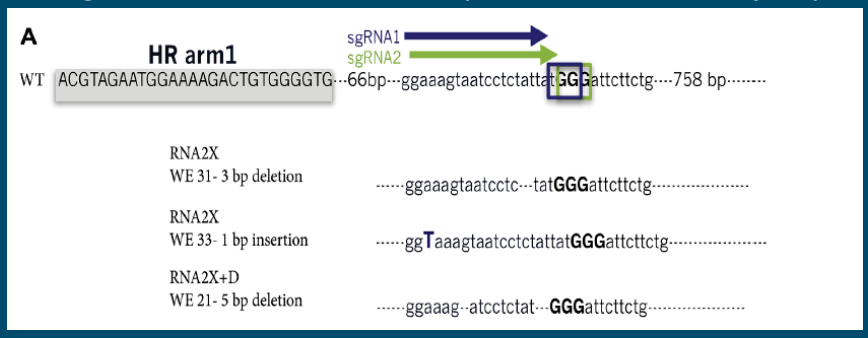
(Intracitoplasmic injection of mRNAg and mRNA CAS9 (transient expression) / 54% mutation efficiency

- Embryo transfer over 20 cows (waiting for pregnancy results)

Edition of the bovine prion gene responsible for mad cow disease



•PRNP gene- EXON 3 edited (cell lines + embryos) deletions + indels



Bevacqua *et al* 2016

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Wrap-up and Prospective

- powerful technique for agriculture and farming
- genome and natural diversity exploration/exploitation
- incremental breeding schemes for clonal crops
- potentially with no regulation requirments
- public awarness /ethical and environmental issues
- IPR dependant
- multiple stakeholders as actors

Thank you for you attention

Muchas Gracias

ありがとうございます！

INTA 60 años
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COI Disclosure Information

I have no financial relationships to disclose

Sergio E. Feingold