

Advances in Plant and Animal Genetics



Martina Newell-McGloughlin

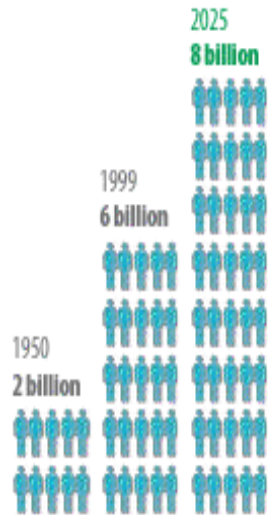
Research Division, Higher Education Sector

Adj Professor Plant Pathology, UC Davis

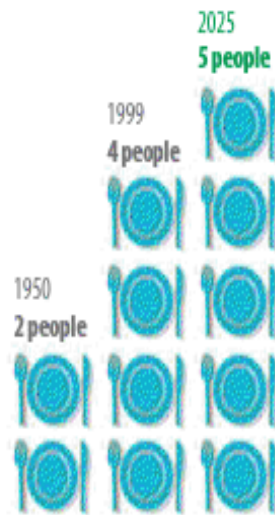
Global population, estimates and projections (billions)

7 more mouths to feed

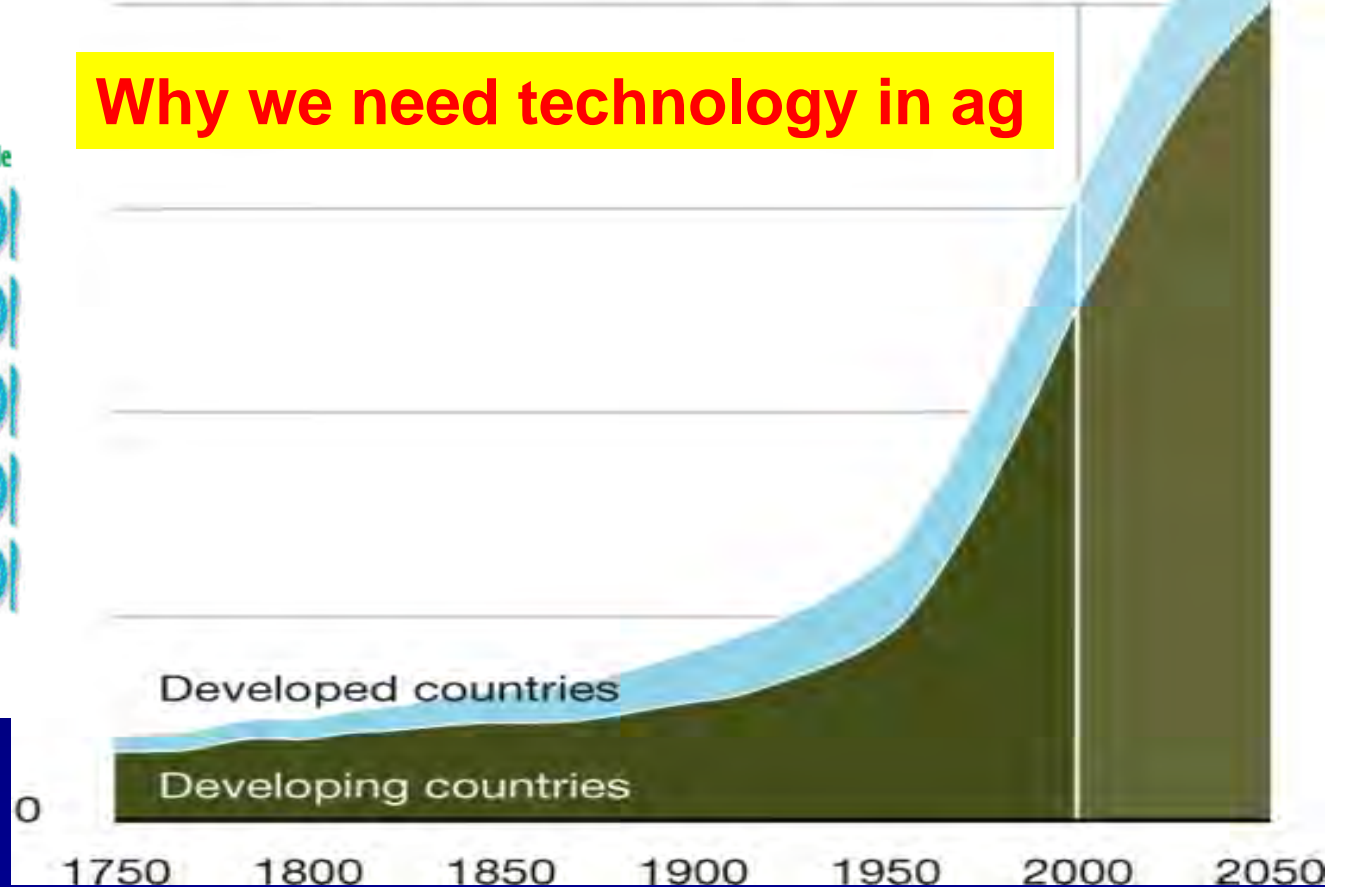
World Population



People fed per hectare



Why we need technology in ag



Source: FAO World Bank

Greatest Grand Challenge of our time!

Population
9 billion by 2050!
70-100% More Food Required

- Need to produce more on less under unprecedented conditions Changing Climate, diminishing arable land,
- High yielding, affordable, high quality food, feed, fuel, fiber
- Sustainable production: Less water, less fuel, less fertilizer, less pesticides, less degradation, less GHG output
- Major opportunity for Ireland

The Future for Irish Agriculture : Smart Plants Smart Livestock Smart Environments



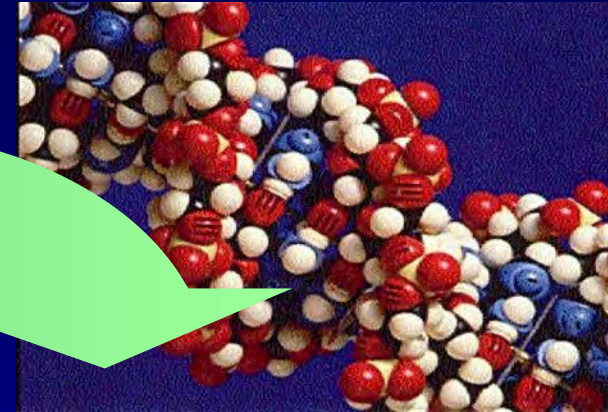
- **Smart Plants - Smart Livestock: “Systems” precision Breeding**
 - **Holistic Macro level – “omics” technologies**
 - **Reductive Micro level – trait modification**
- **Holistic/reductive approaches to identify, modify, introgress desirable traits into optimized genetic backgrounds**
- **Study /modify/ the expression/interaction of functional networks of genes, epigenes, the microbiome and their products’ to determine realtime response of plants and livestock to their environment**

Future Plant and Animal Breeding - A new Research-Paradigm



Phenotype
*Appearance and Traits
of an Organism*

Improved
Crops and
Livestock

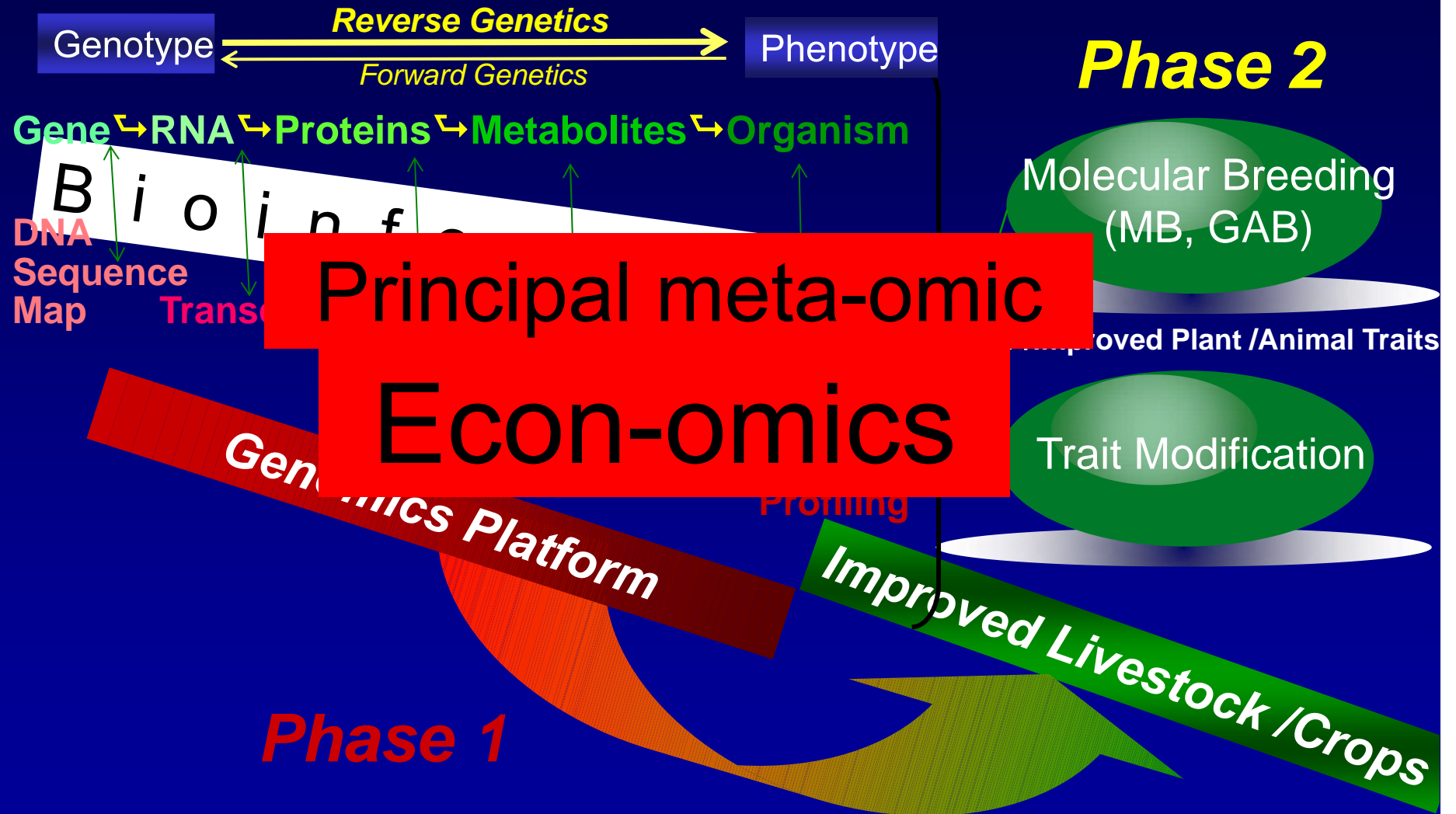


Genotype / Genes / seq
*(includes extra
nuclear/microbiome
genome) Inherited
Information
Defining an Organism*

Genomics

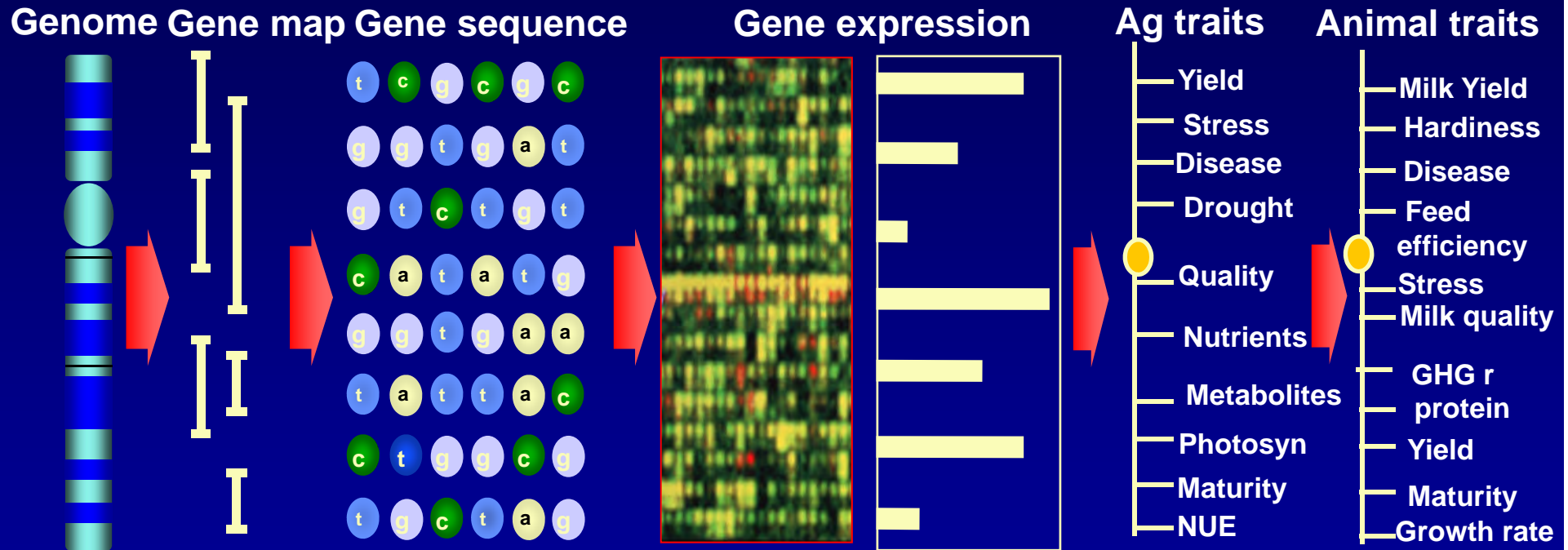
= the Totality of the Information
of all Genes and their Functions

From Omics to Improved Crops and Livestock



Genomics tools

Genotype select largest possible number of genes for traits of importance



Advances NGS, markers and “omic” analysis and in the associated information technology will accelerate the discovery and characterization of genes, QTLs, NCS, having potential utility.

SNPs representing thousands of individual genotyping indicators allow very high throughput analysis of genes and gene expression patterns.



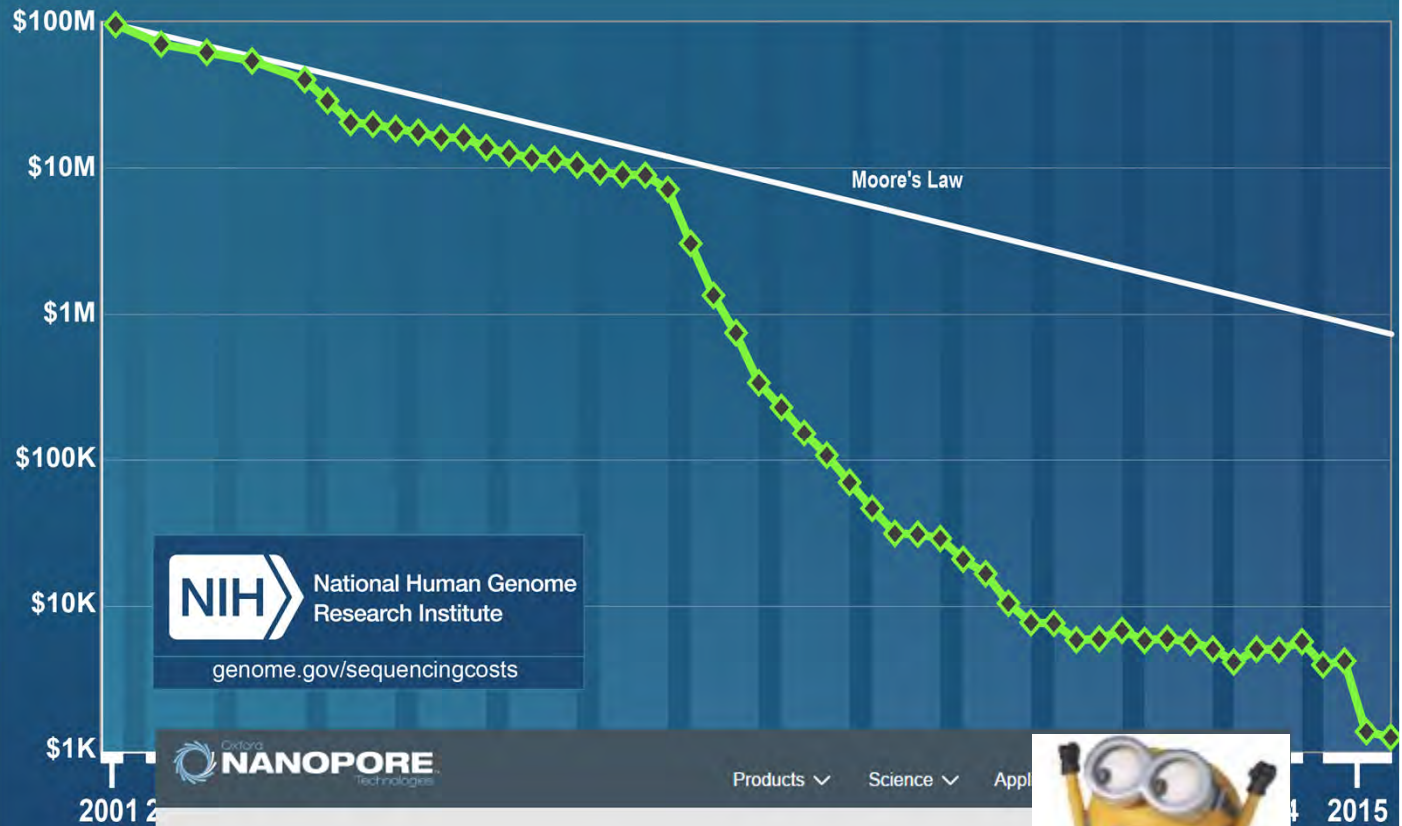
Table 2.1.1 Genome Sizes and Estimated Numbers of Genes

Organism	Haploid Genome Size (Mb)	Number of Genes	Genes per Mb
Bacteria			
<i>Haemophilus influenzae</i>	1.8	1,700	940
<i>Escherichia coli</i>	4.6	4,400	950
Archaea			
<i>Archaeoglobus fulgidus</i>	2.2	2,500	1,130
<i>Methanosarcina barkeri</i>	4.8	3,600	750
Eukaryotes			
<i>Saccharomyces cerevisiae</i> (yeast, a fungus)	12	6,300	525
<i>Caenorhabditis elegans</i> (nematode)	100	20,100	200
<i>Arabidopsis thaliana</i> (mustard family plant)	120	27,000	225
<i>Drosophila melanogaster</i> (fruit fly)	165	13,700	83
<i>Oryza sativa</i> (rice)	430	42,000	98
<i>Zea mays</i> (corn)	2,300	32,000	14
<i>Mus musculus</i> (house mouse)	2,600	22,000	11
<i>Ailuropoda melanoleuca</i> (giant panda)	2,400	21,000	9
<i>Homo sapiens</i> (human)	3,000	<21,000	7
<i>Fritillaria assyriaca</i> (lily family plant)	124,000	ND	ND

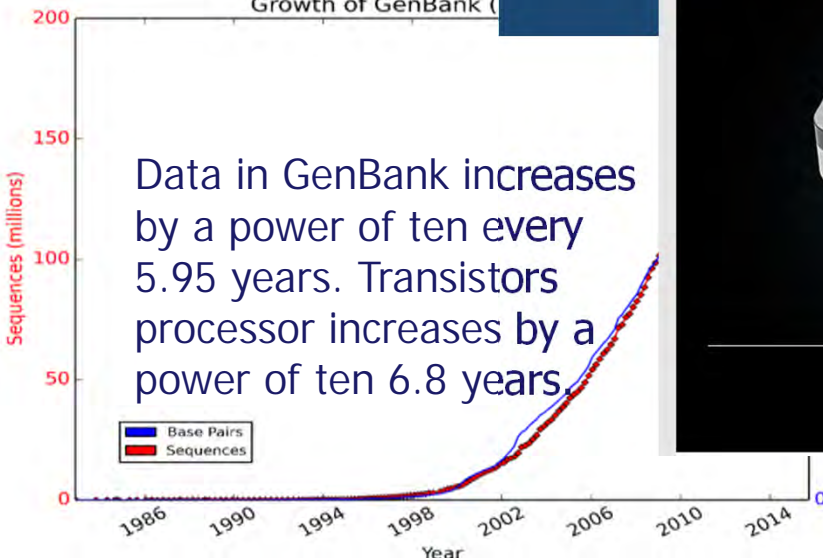
*Some values given here are likely to be revised as genome analysis continues. Mb = million base pairs. ND = not determined.

Bottleneck _
Phenomic gap –
correlation of
genotypes with
desirable trait

Data analysis, Co-
evolution between
statistical models,
sequencing and HT
phenotyping -
control for spurious
associations



Growth of GenBank (



Data in GenBank increases
by a power of ten every
5.95 years. Transistors
processor increases by a
power of ten 6.8 years.

OXFORD
NANOPORE
Technologies

Products Science Appl

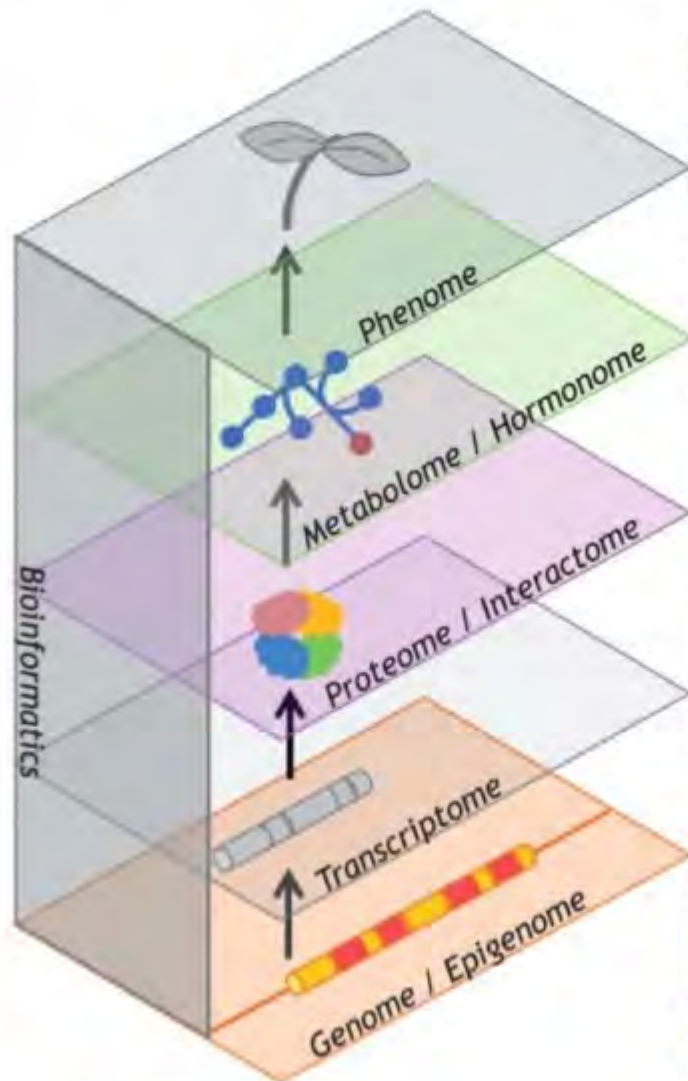
MinION
Portable, real-time biological analyses

MinION is a portable device for molecular analyses that is driven by nanopore technology. It is adaptable for the analysis of DNA, RNA, proteins or small molecules with a straightforward workflow. The MinION product specification is available here.

More about sequencing with MinION

Explore all publications > Start using MinION >

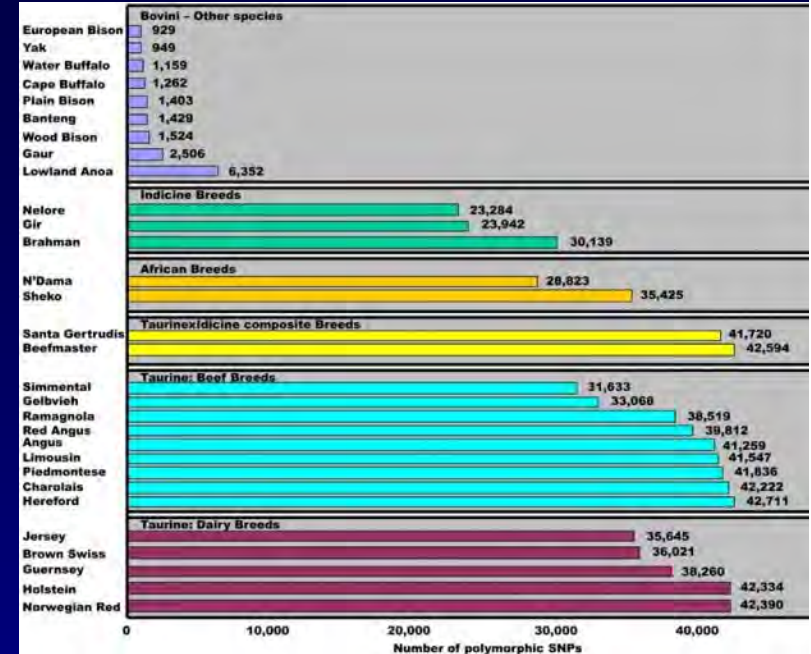
3. PLANT –OMICS SPACE



Omics instances	Resources
Integrated database	TAIR ¹
Mutant lines	FOX line ² , Ac/Ds tag line ³ , T-DNA tag line ⁴ , TILLING ⁵
Natural variations	NASC ⁶ , ABRC ⁷
Metabolic map	PMN AraCyc ⁸ , Reactome ⁹
Metabolome profiles	PRIME ¹⁰ , Golm Metabolome Database ¹¹
Hormonome profile	RiceFOX DB ²
Proteome / modificome profiles	RIPP-DB ¹² , PPDB ¹³ , PhosPhAt ¹⁴
Subcellular localization	PODB2 ¹⁵ , SUBAII ¹⁶ , NASC proteome database ¹⁷
Interactome maps	TAIR ¹ , AtPID ¹⁸ , Plant Interactome Database ¹⁹
Full-length cDNA clones, ESTs	RAFL clones ²⁰ , RARGE ²¹
Expression profiles	AtGenExpress ²² , Genevestigator ²³
Non-coding RNA profiles	Arabidopsis MPSS ²⁴
Co-expression network	ATTEDII ²⁵
Genome sequence, gene annotation	TAIR ¹
Re-sequencing	Arabidopsis 1001 genome project ²⁶
Focused gene family database (eg. Transcription factor)	RARTF ²⁷ , AGRIS ²⁸ , DATF ²⁹
DNA methylome	SIGnAL ³⁰
Chromatin epigenome	EPIC web site ³¹

The Farmer Scientist!

- Genomic selection has increased rate of genetic gain in dairy herd by 60%
- Custom SNP chip (Teagasc, ICBF, Weatherby's, Illumina) developed in 2013 – imputation tool
- Ireland will soon have the largest set of genotyped animals in the world 250,000/yr
- Now research to leverage this data – 250 trillion data points



IDB SNP CHIP
INTERNATIONAL DAIRY & BEEF
SNP CHIP

Designed in association with the Irish Cattle Breeding Federation (ICBF), Teagasc, Weatherby's and ICBF's Agricultural Research Service.

This custom chip is the very latest design catering for both Beef and Dairy. The chip consists of the Illumina LD (70) base content plus a further 10,000 IDB SNPs carefully selected to ensure very high imputation accuracy to HD & to convert to Microsatellite data for pedigree verification. This extra panel of SNPs provides the very latest data points for both Beef & Dairy breeds.

Both the chip and the Illumina BeadChip are recommended for pedigree panels as shown on the LD70.

The IDB also contains a comprehensive selection of genetic markers to screen for DNA markers & major genes.

For more details Contact: Weatherby's Ireland DNA Laboratory
+353(0)45875521 | WEATHERBYS Ireland | j@w@weatherbys.ie

- Marker Assisted Breeding
- Determine breeding value at birth
- Increase accuracy of selection
- Increase selection intensity
- Reduce generation interval
- Increase rate of genetic gain
- Specialty herds
- Personalized animal health

CHIP CONTENTS FOR DISEASES & TRAITS

Infected/resistant

1. Bovine Tuberculosis resistance
2. BVD
3. BVDV
4. BVDV2

Complexed diseases

1. Bovine Leishmaniasis
2. Bovine Leishmaniasis (Leishmania infantum)
3. Bovine Leishmaniasis (Leishmania tropica)
4. Bovine Leishmaniasis (Leishmania major)
5. Bovine Leishmaniasis (Leishmania braziliensis)
6. Bovine Leishmaniasis (Leishmania genovae)
7. Bovine Leishmaniasis (Leishmania shawi)
8. Bovine Leishmaniasis (Leishmania smithi)
9. Bovine Leishmaniasis (Leishmania sp.)
10. Bovine Leishmaniasis (Leishmania sp.)
11. Bovine Leishmaniasis (Leishmania sp.)
12. Bovine Leishmaniasis (Leishmania sp.)
13. Bovine Leishmaniasis (Leishmania sp.)
14. Bovine Leishmaniasis (Leishmania sp.)
15. Bovine Leishmaniasis (Leishmania sp.)
16. Bovine Leishmaniasis (Leishmania sp.)
17. Bovine Leishmaniasis (Leishmania sp.)
18. Bovine Leishmaniasis (Leishmania sp.)
19. Bovine Leishmaniasis (Leishmania sp.)
20. Bovine Leishmaniasis (Leishmania sp.)

Major genes

1. Bovine Growth Hormone
2. Bovine Growth Hormone Receptor
3. Bovine Growth Hormone Receptor 2
4. Bovine Growth Hormone Receptor 3
5. Bovine Growth Hormone Receptor 4
6. Bovine Growth Hormone Receptor 5
7. Bovine Growth Hormone Receptor 6
8. Bovine Growth Hormone Receptor 7
9. Bovine Growth Hormone Receptor 8
10. Bovine Growth Hormone Receptor 9
11. Bovine Growth Hormone Receptor 10
12. Bovine Growth Hormone Receptor 11
13. Bovine Growth Hormone Receptor 12
14. Bovine Growth Hormone Receptor 13
15. Bovine Growth Hormone Receptor 14
16. Bovine Growth Hormone Receptor 15
17. Bovine Growth Hormone Receptor 16
18. Bovine Growth Hormone Receptor 17
19. Bovine Growth Hormone Receptor 18
20. Bovine Growth Hormone Receptor 19

Illumina

illuminasnp | ICBF | Teagasc | SAC

Source Frank O'Mara

Sheep QTL/associations data summary

Release 28
(Dec 29, 2015)



(Dec 29, 2015)

Chicken QTL/associations data summary

As of Release
curated fr

PigQTLdb

Browse Search View Maps F A

Release 28
(Dec 29, 2015)

- 1. Numt As of Release were curated f
- 2. Numt 1. Number of
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- 7. Numt 6. Number of
- 8. Numt 7. Number of
- 9. Type: 8. Number of
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- against the

Pig QTL/associations data summary

Cattle QTL/associations data summary

(Dec 29, 2015)

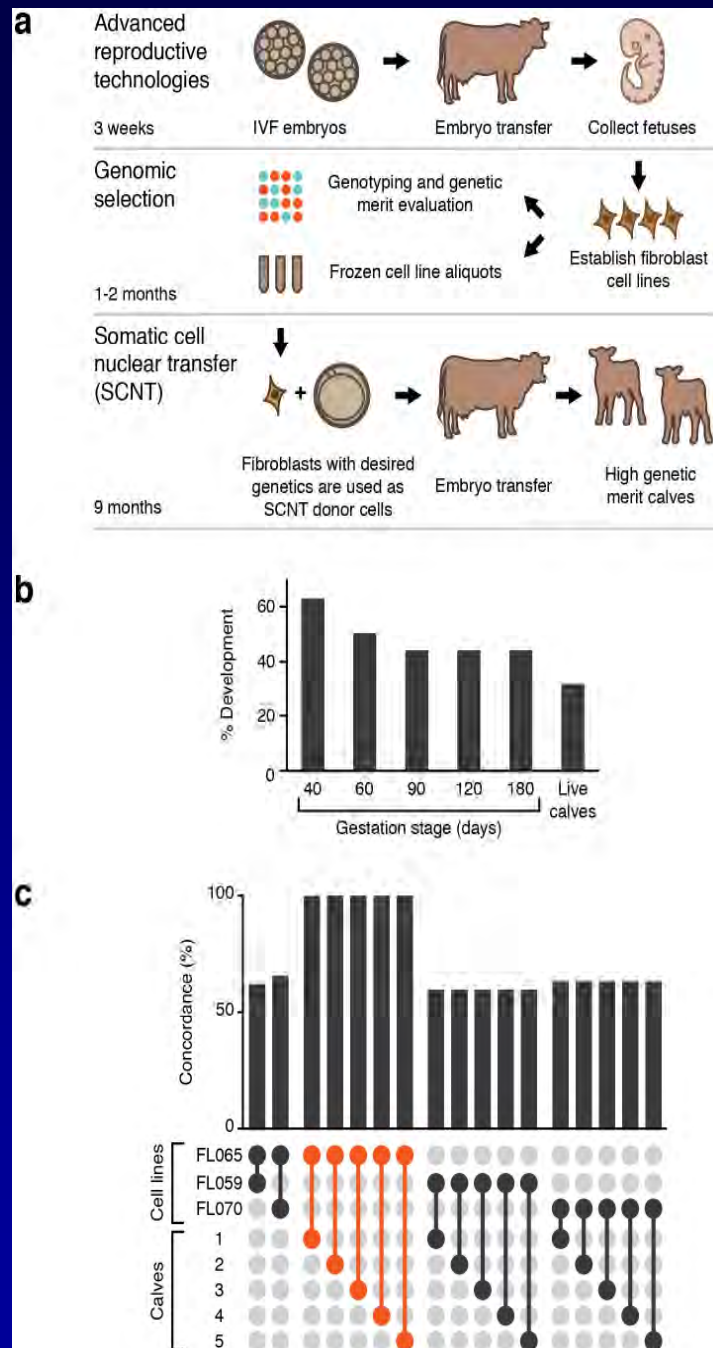
- 1. Numb
- 2. Numb As of Release 28, there have been **42,019** cattle QTLs released for public access on the Cattle QTLdb. These data were curated from **646** publications and represent **482** different cattle traits.
- 3. Numb
- 4. Numb 1. Number of QTL/associations by traits
- 5. Numb 2. Number of QTL/associations by trait types
- 6. Numb 3. Number of QTL/associations by trait classes
- 7. Numb 4. Number of QTL/associations by chromosomes
- 8. Numb 5. Number of QTL/associations by publishing years
- 9. Types 6. Number of QTL/associations by publishing journals
- against 7. Number of papers by publishing years
- 8. Number of QTL/associations in curation pipeline
- 9. Types of structural genome information aligned against the QTL/association maps

Top 15 QTL/associations

Traits	Number of QTL
Milk fat percentage	1,942
Inseminations per conception	1,662
Milk C14 index	1,545
Interval from first to last insemination	1,508
Milk protein percentage	1,298
Calving ease	1,148
Somatic cell score	1,066
Milk fat yield	1,010
Milk protein yield	989
Rear leg set	986
Net merit	979
Length of productive life	867
Stillbirth	792
Calving ease (maternal)	770
Udder depth	761
....



To speed up introgression of desirable traits

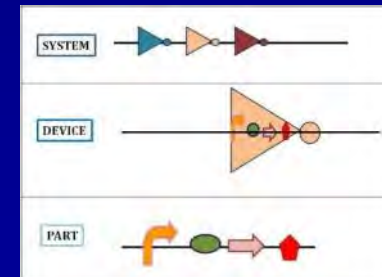


- GS leverages genomic readouts to provide estimates of breeding value early in the life of animals.
- Capacity limited by generation interval
- Reduces by 7 months
- Chance to produce multiple animals at the same or later time from banked, frozen fibroblast cell lines.
- Selection of high genetic merit at an early embryonic stage reduces 40% cost of purchasing and managing recipient females and maximizes value by selectively transferring embryos with the desired genotypes.
- We expect that the application of this method will substantially improve genetic gain

Kasinathan P, Wei H, Xiang T, et al. Acceleration of genetic gain in cattle by reduction of generation interval. *Scientific Reports*. 2015

Tools to select/create those desirable traits

- Next generation sequencing, RNAseq, GWAS, CNV (P+A)
- Functional/Comparative Genomics transcriptome analysis (P+A)
- SNaPshot high-information-content-fingerprinting (HICF) (P+A)
- “RNA family” RNA interference (RNAi, miRNA, shRNA) (P+A)
- TILLING – site directed mutagenizes (P)
- Genome Editing: GEENs, MENs, Transcription activator-like effector nucleases (TALEN), Zn Fingers, CRISPRs-RGENs (P+A)
- Excising/breeding out transgene (P+A)
- Traitup extra chromosomal (plastid) transient expression operon (P)
- Chemical genomics (P+A)
- Tunable Promoters & Transcription factors (Tfs), natural and synthetic (P+A)
- Target of rapamycin (TOR) kinase, a master regulator (P+A)
- Pentatricopeptide repeat (PPR) Dynamically switch endogenous gene -Track or relocate endogenous RNAs -Alter sequence/coding capacity of RNAs-
- Novel Maternal/paternal Haploid production (P) –
- Centromere engineering (CENH3) (Chan) (P)
- Epigenetic modification –RdDM, siRNA (P+A)
- Metagenomics (microbiome) (P+A)
- Network engineering (P+A)
- Mini-chromosomes Combinatorial multigene transformation (P+A)
- Synthetic Biology – synthesis assembly complete circuits (BioBricks) (P+A)
- Systems Biology (P+A)



• P=Plant, A=Animal

How to circumnavigate the “GMO” issues!

Or Why Ireland should be interested in next gen trait modification

Group 1:	Site specific mutagenesis	Zinc Finger Nuclease (ZFN)
<p>The diagram illustrates three methods of genetic modification:</p> <ul style="list-style-type: none"> Conventional breeding: Involves crossing an elite recipient (red tomato) with a donor (green tomato). This is followed by a backcrossing process (BC_n, n > 5) and selection to produce an elite variety with desirable traits. Genetic modification: <ul style="list-style-type: none"> Transgenesis: A gene from a different species (e.g., a green gene from a green tomato) is introduced into a red tomato genome. Cisgenesis (intragenesis): A gene from a related species (e.g., a green gene from a green tomato) is introduced into a red tomato genome. Genome editing: Uses CRISPR/Cas9 technology to make precise edits to the genome, resulting in GEC (Genome Edited Crop). 		
Group 2:	Cisgenesis and Intragenesis	Cisgenesis Intragenesis
Group 3:	Breeding with transgenic inducer line	RNA-dependent DNA methylation (RdDM)
Reverse breeding		
Accelerated early flowering		
Group 4:	Grafting	Non-GM scions on GM rootstocks
Group 5:	Agro-infiltration transient expression	Agro-infiltration ‘sensu stricto’ Autonomous replicating operon, floral dip
Group 6???:	Synthetic Genomics	Viable minimal genomes

EU Position ??

ODM	<p>Commercial development: oilseed rape (HT + other traits), maize (HT), flax, potato, tomato, tobacco, Petunia</p> <p>Additional info from literature: HT rice</p>
ZFN	<p>Commercial dev.: maize (HT), oilseed rape, tomato, tobacco, sugar beet, potato (starch quality), trees (lunber/paper), lettuce, Petunia, Agyranthemum, poplar</p>
MGN	<p>Commercial dev.: maize (HT) and tobacco</p>
TALEN	<p>Literature: HT tobacco, low sugar potatoes</p>
CRISPR/Cas9	<p>Literature/Commercial dev Wheat, barley, rice, sorghum, tomato, potato, brassica , sweet orange</p>
RdDM	<p>Commercial dev.: maize, oilseed rape</p> <p>Literature: maize (male sterility), potato (starch content), tomato (no ripening), Petunia (reduce pigmentation)</p>
REVERSE BREEDING	<p>Commercial dev.: tomato (taste), broccoli</p> <p>Literature: oilseed rape</p>
CISGENESIS-INTRAGENESIS	<p>Commercial dev.: potato (fung. res. and starch content), maize, oilseed rape, barley (less phytate)</p> <p>Literature: potato (sev. traits), apple (fung. res and red flesh), grapevine and melon (fung. res.), alfalfa (reduced lignine), poplar (wood properties)</p>
GRAFTING ON GM ROOTSTOCK	<p>Commercial dev.: grapevine (virus res.), apple and pear (root. ab.), citrus (dwarfing and fung. res.)</p> <p>Literature: apple, rose and walnut (root. ab.), watermelon, pea, potato and cucumber (virus res.)</p>



First to challenge and succeed(!) within EU in getting non-GM status

- CIBUS Rapid Trait Development System (RTDS™) “non-transgenic breeding technology”
- February 2015, authorities in Germany told Cibus that they would not consider products created by gene editing as GM, but as products of conventional plant breeding.
- Canola: sulfonylurea (SUT), Flax (glyphosate tolerant), Rice and Potato (Phytophthora R).

Genome Engineering

“Genome editing basically provides the variation you want, where you want it,” Bruce Whitelaw, Roslin Institute

- **Synthetic Nucleases**

- **Tools**

- Zinc Fingers

- Transcription activator-like effector nucleases (TALEN)

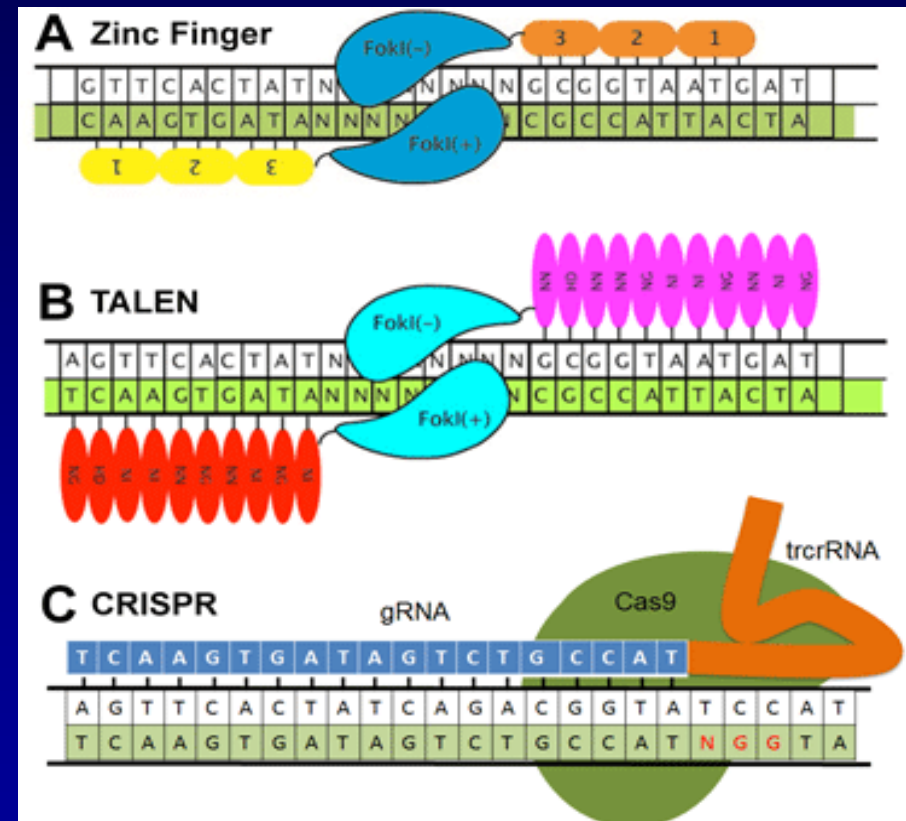
- CRISPR/Cas9 system

- **Applications**

- Single gene knockouts

- Subtle modification of gene functions

- Sequence-specific integration of foreign genes for gene stacking





Bespoke genome engineering

- Generating a Knock-out Using CRISPR/Cas9
- Enhancing Specificity with Cas9 Nickase
- Making Precise Modifications (insertions) Using Homology Directed Repair (HDR)
- Activation or Repression of Target s CRISPR/dCas9 CRISPRi
- Multiplex Genome Engineering with CRISPR/Cas9
- Genome-wide Screens Using CRISPR/Cas9
- CRISPR as a sensor
- Gene drive the creation "gene drive" that could eliminate pests or the diseases, pest R, MCR

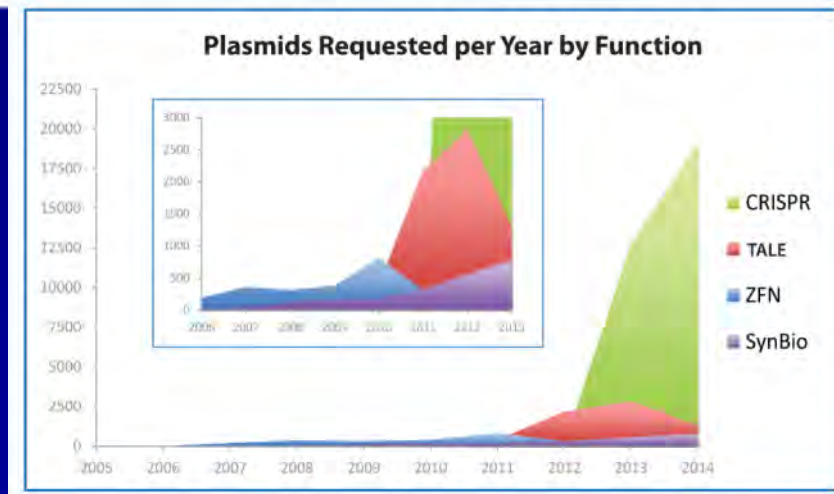
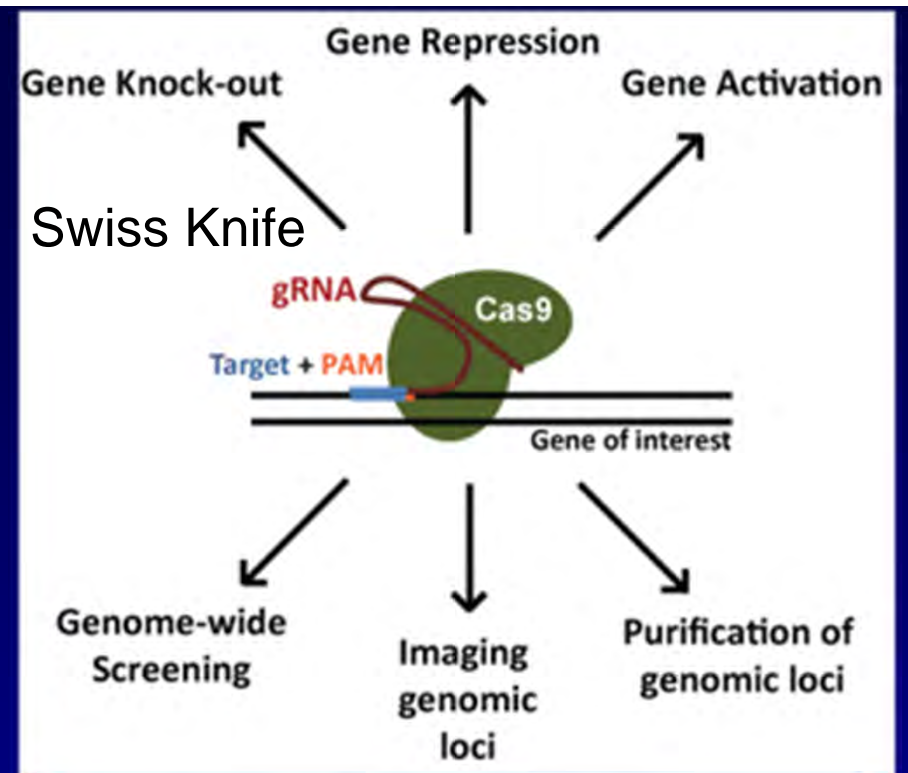


Table 1
CRISPR-Cas9 mediated NHEJ in transient tra

Species	Transformation method
Arabidopsis thaliana	PEG-protoplast transfection

Table 2
CRISPR-Cas9 mediated NHEJ in stable

Species	Transformation method
Arabidopsis thaliana	A gro-transform floral dip
A. thaliana	A gro-transform floral dip
A. thaliana	A gro-transform floral dip
A. thaliana	A gro-transform floral dip
A. thaliana	A gro-transform floral dip

Species	Transformation method
Arabidopsis thaliana	A gro-transform floral dip
A. thaliana	A gro-transform floral dip
A. thaliana	A gro-transform floral dip
A. thaliana	A gro-transform floral dip
A. thaliana	A gro-transform floral dip

46

Table 3
Homologous recombination

Species
Arabidopsis thaliana
A. thaliana
A. thaliana
A. thaliana
A. thaliana

Species
Arabidopsis thaliana
A. thaliana
A. thaliana
A. thaliana
A. thaliana

<u>Nicotiana glauca</u>
<u>Nicotiana glauca</u>
<u>Oryza sativa</u>
<u>O. sativa</u>

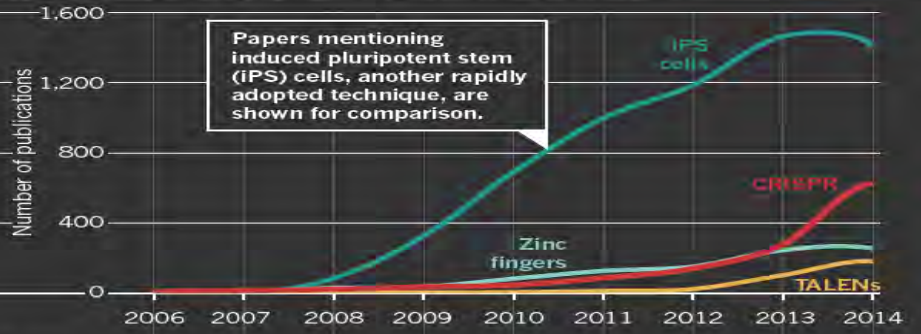
AtUBQ1, U6 = A. thaliana U6 promoter; CaM = calcium dependent enhancer; CaM = polymerase chain reaction repair pathway.

THE RISE OF CRISPR

DNA sequences called CRISPRs (clustered regularly interspaced short palindromic repeats) are part of a bacterial defence system. After researchers showed in 2012 that CRISPRs could be used to edit genomes, use of the tools quickly spread, as reflected by sharp rises in publications, patent applications and funding.

PUBLICATIONS

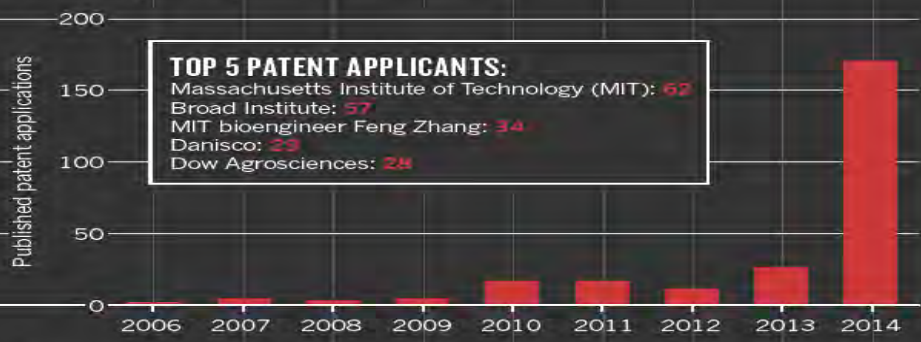
The number of papers about CRISPR has outstripped the numbers mentioning the gene-editing technologies known as TALENs and zinc fingers.



Papers mentioning induced pluripotent stem (iPS) cells, another rapidly adopted technique, are shown for comparison.

PATENTS

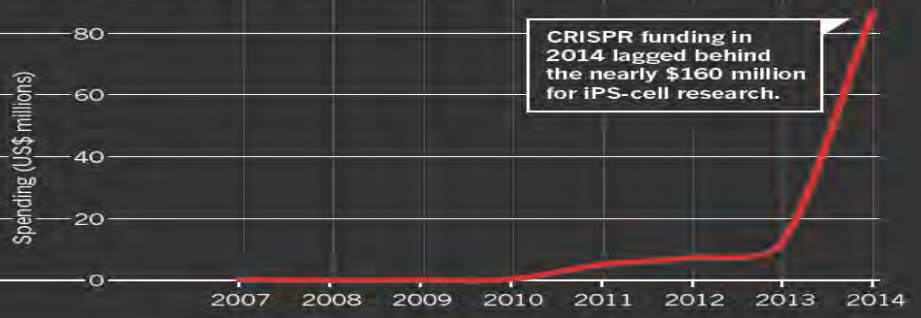
In 2014, worldwide patent applications that mention CRISPR leapt and a patent battle intensified.



TOP 5 PATENT APPLICANTS:
 Massachusetts Institute of Technology (MIT): 62
 Broad Institute: 57
 MIT bioengineer Feng Zhang: 34
 Danisco: 23
 Dow Agrosciences: 24

FUNDING

A sharp jump in US National Institutes of Health funding for projects involving CRISPR is a harbinger of future advances.



CRISPR funding in 2014 lagged behind the nearly \$160 million for iPS-cell research.

Multiplex (deletion)	Reference
Yes (230 bp)	Li et al. (2013)

Target/detection method	Multiplex (deletion)	Transmission to progeny	Reference
	Yes (230 bp)		Mao et al. (2013)
			Mao et al. (2013)
		Yes	Fausser et al. (2014)
		Yes	Fausser et al. (2014)
		Yes	Schimpl et al. (2014)

Template	HR frequency	detection method	Reference
	11.4%	GUS staining	Mao et al. (2013)
	n.d.	GUS staining	Fausser et al. (2014)
	n.d.	GUS staining	Fausser et al. (2014)
	18.8%	YFP fluorescence	Feng et al. (2013)
ds DNA, 673 bp	0.14%	PCR, phenotype, sequencing	Schimpl et al. (2014)
14 bp	10.7%	PCR + RE	Li et al., 2013
	7%	RE + PCR + RE	Shan et al. (2013)
	n.d.	GUS staining	Miao et al. (2013)

promoter of the 35S gene of the cauliflower mosaic virus with duplicative U3 promoter; ZmUbi = Z. mays ubiquitin 3 promoters; PCR repair pathway; SDSA = synthesis-dependent strand annealing

Nucleic Acids Research

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The most recent version of this article [gkt780] was published on 2013-10-31

Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice

Wenzhi Jiang¹, Huanbin Zhou², Honghao Bi², Michael Fromm³, Bing Yang² and Donald P. Weeks^{1,*}

Design → **Clone** → **Deliver** → **Screen**

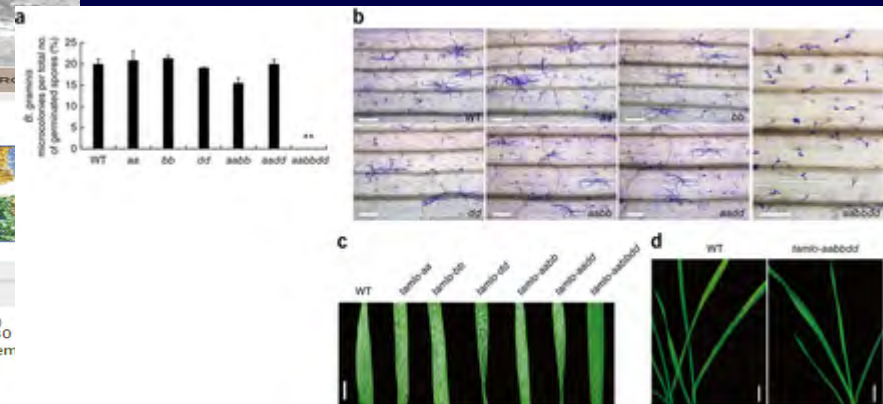
system in two dicot plant species, Arabidopsis and tobacco, and two monocot crop species, rice and sorghum. *Agrobacterium tumefaciens* was used for delivery of genes encoding Cas9, sgRNA and a non-functional

This Article
Nucl. Acids Res. (2013)
doi: 10.1093/nar/gkt780
First published online: September 2, 2013

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Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew

• Yanpeng Wang

Drought-resistant corn and wheat than can breed like a hybrid rather than self-pollinate. Increase wheat yield by 10-15%. CRISPR/Cas9 edited, like soybeans, rice, potatoes. *DuPont, Caribou Biosciences,*



Barley and brassica, off-target edits were sometimes found in a very closely related gene. *John Innes*

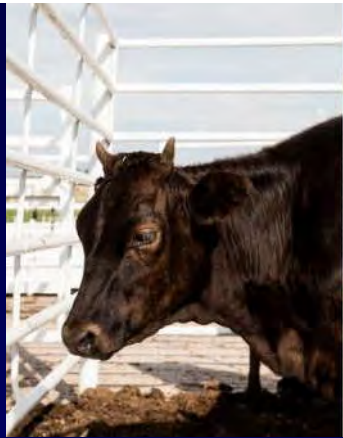
CRISPR Genome Analysis Tool

Welcome to the Iowa State University Crop Bioengineering Consortium's CRISPR Genome Analysis Tool.

This tool works in two steps:

1. Identify potential target sites for CRISPR gene editing in DNA sequences
2. Optionally, use identified target sequences from step 1 to search a genome of interest for potential off-target matches

Iowa State University



Roslin and Texas A&M
More meat: The genome of the Nelore bull on the right was edited to produce 30 percent more muscle fiber.

TALEN modified calves

“Recombinetics introduced Angus seq into skin cells from a horned Holstein bull. In total, 10 BP deleted 212 bp added from Angus.

Roslin Institute African swine fever warthog RELA gene only 3 /500 amino acids Mammalian interspecies substitution of immune modulatory alleles by genome editing
 Bruce A. Whitelaw et al

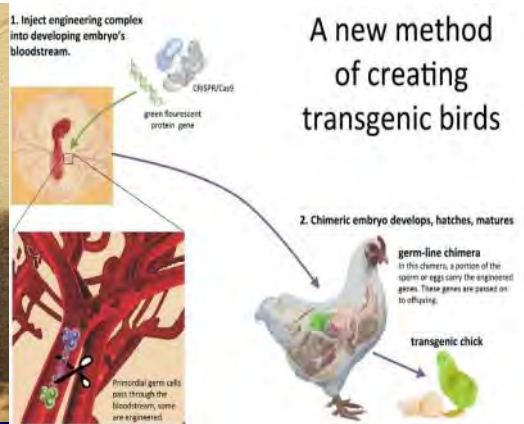


“.....an animal could be edited to have the very best genes its species can offer.”
 “The genome is information. And this is information technology. We have gone from being able to read the genome to being able to write it.” Scott Fahrenkrug

In combination with chicken primordial germ cell line with germ-line transmission capacity, ovalbumin gene knockout chickens by TALEN-mediated gene targeting.

Targeted gene knockout in chickens mediated by TALENs (2014)

Tae Sub Park^{a,b}, Hong Jo Lee^a, Ki Hyun Kim^a, Jin-Soo Kim^c, and Jae Yong Han



Chickens that only produce female offspring (for egg-laying) male for meat

CD163 is the receptor for entry of PRRSV into cells. Used CRISPR/Cas9 to generate CD163- Pigs displayed no symptoms to PRRSV. “Because CD163 was edited using CRISPR-Cas9, **the pigs challenged in this study do not contain any transgenes**” Wells 2015, MSU

Genome edited sheep and cattle
Chris Proudfoot et al, 2014

- Myostatin KOed gets you Schwartznegger phenotype



Using CRISPR Cas9 pig cells have been engineered to inactivate all 62 Porcine Endogenous Retrovirus in the pig genome, which eliminated infection from the pig to human cells in culture Church, *Genome-wide inactivation of porcine endogenous retroviruses (PERVs) Science, 2015*

Beef cattle that only produce males (for more efficient feed-to-meat conversion).

Edit the SRY gene, create visually-appearing males from genotypic XX females. Approach to contain transgenes through sterility, facilitate coexistence and acceptable cisgenic containment genetic engineering in animal agriculture. Van Eenennaam

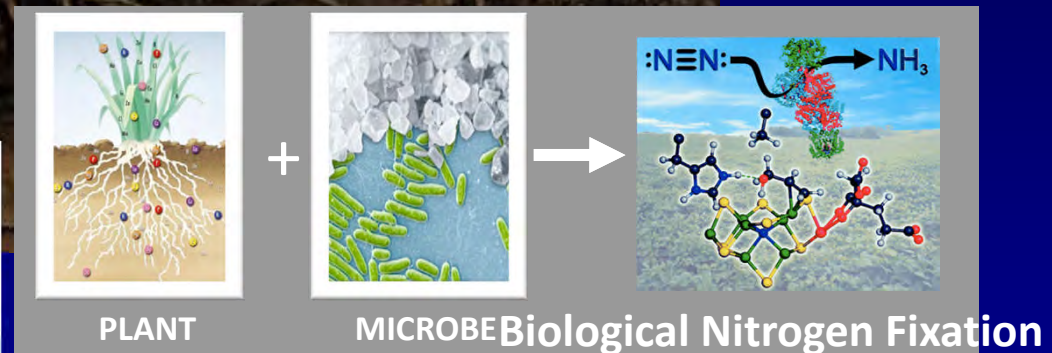
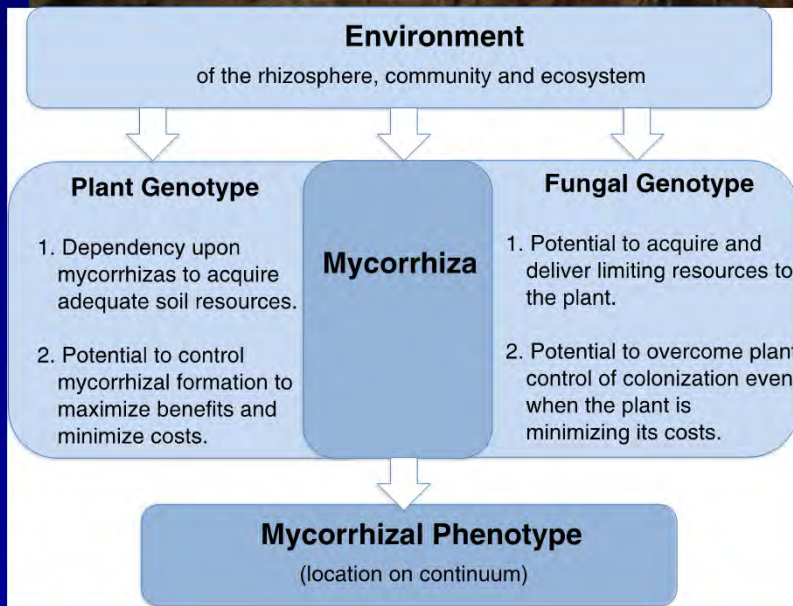


- A "precision"
- An "immunization" of the genome with specific sequences
- A "reversal" of the drive system, although downstream ecological changes might remain.
- A "repetitive" drive features multiple iterations to further establish or maintain new trait(s).
- Drive systems could destroy or modify plant, animal pests and target populations of invasive species, such as rats and kudzu.

Science. 2015 Apr 24;348(6233):442-4. doi: 10.1126/science.aaa5945. Epub 2015 Mar 19.

Genome editing. The mutagenic chain reaction: a method for converting heterozygous to homozygous mutations. [Gantz VM](#)¹, [Bier E](#)¹.

Plant Microbiome



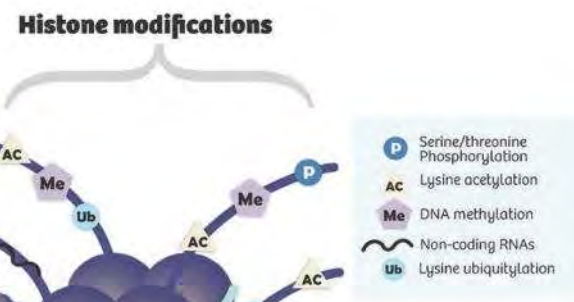
Hypothesis: indigenous landraces of corn grown in isolated regions of Mexico co-evolved diazotrophic microbiomes that contribute to plant performance due to nitrogen deficiency in the soil.

A

EPIGENETIC MARKS
 are impacted by the following processes:
 - Nutrients, pathogens
 - Environmental chemicals
 - Reproductive technologies
 - Maternal environment, etc.

B

COMPACT CHROMATIN
 Contain silent genes, modified DNA and histones. Genes are inaccessible to transcription factors and non-coding RNA



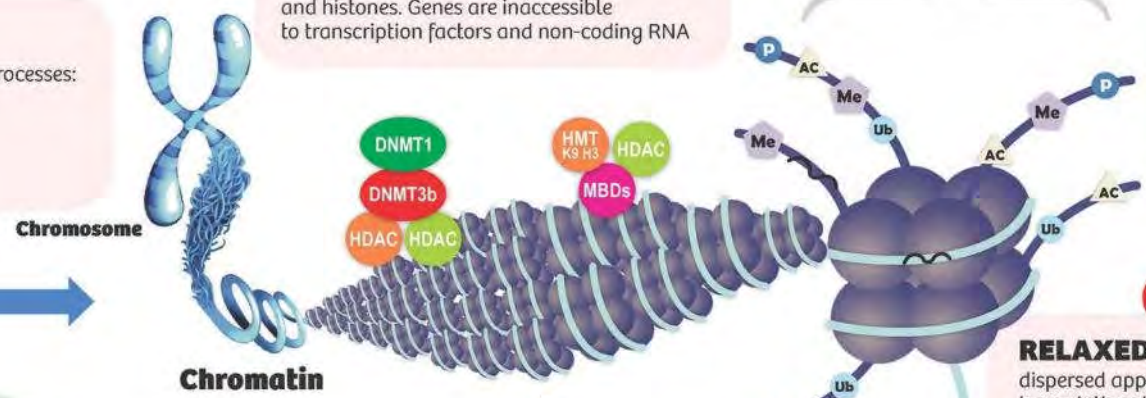
C

RELAXED CHROMATIN
 dispersed appearance and contain transcriptionally active genes rich in unmethylated DNA. Genes are accessible to transcription actors and non-coding RNAs

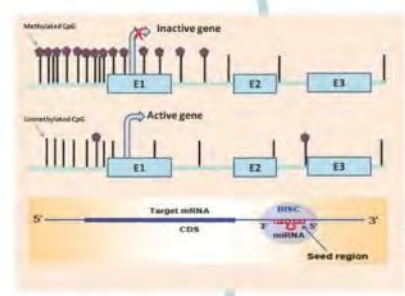


D

DIVERSE PHENOTYPES
 - Reproductive performance
 - Disease susceptibility/resistance
 - Increased/decreased productivity
 - Growth rate
 - Milk production
 - Lipogenesis



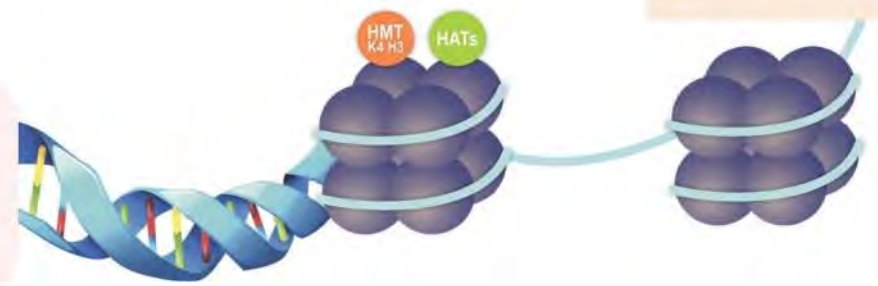
Deregulation of DNMT targets



Transcriptionally inactive gene, rich in methylated DNA

Transcriptionally active gene

Binding of miRNA to 3UTR of genes may repress gene expression





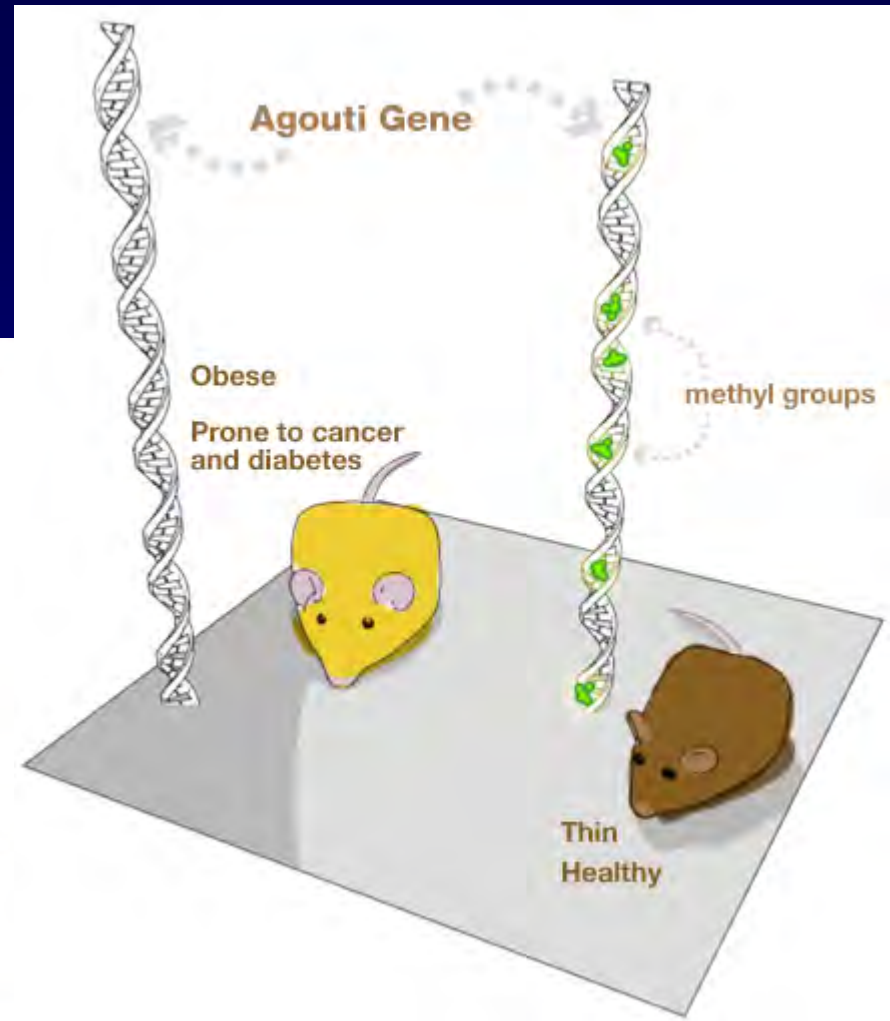
These Two Mice are Genetically Identical and the Same Age



While pregnant, both of their mothers were fed Bisphenol A (BPA) but **DIFFERENT DIETS**:

The mother of this mouse received a **normal mouse diet**

The mother of this mouse received a diet **supplemented** with choline, folic acid, betaine and vitamin B12

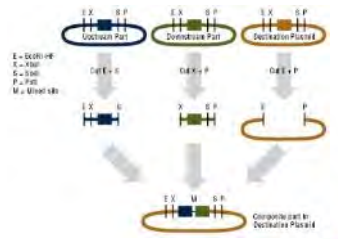


Future for Ireland

- Challenges: Technology, application, regulation, acceptance (by farmer & consumer), practicality and economics of implementation

Low Hanging Fruit (from practicality perspective)

- Systems biology modelling of biological systems and processes.
- Improved genome-wide analyses and computational tools will allow better understanding of complex regulatory and metabolic pathways.
- Systems approach for discovery and decoding pathways - modify with new tools
- Better genotyping/phenotyping - predictive biology of indicator traits (and better indicators that lends to automation), the harmonization of data from automated systems –standardization (ontologies), automation, high repeatability
- Very precise trait modifications using genome editing
- Tuning' expression with tunable promoters and TFs



Higher Fruit

- Synthetic biology systems - synthetic circuits and synthetic genomes
- Artificial chromosomes, operons large multigenic constructs
- synthetic inducible promoter (successful in microbes –phytosensing)
- Construct artificial biological systems and devices that exhibit predictable behaviors – automation – parts reuse



Most important focus orchestrating networks – subcellular-populations-organizations

