Which phenotypic traits for third millennium ?

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Traditional use of data

- **Farm management and extension services:**

  - Primary motivation to register traits

  - Challenge to balance high milk yield and reproduction, health,…

  - Need for appropriate and timely available information

- **Genetics / breeding:**

  - Complex breeding goals - EBVs for 1 - 43 functional traits in 23 countries (Stock et al, 2012);

  - Shifting focus of interest from production traits (milk yield, milk composition; weight gain) to functional traits, i.e. traits influencing production or production conditions

- **Other use:**

- Public: parameters for monitoring food safety, disease surveillance, animal welfare,..)

- Industrial: traceability and authentication; food chain self control, food quality etc

Consumers

Food safety : Consumer acceptance and confidence
Increasing concerns about risks connected to the use of antibiotics – resistances in humans (Healthy animals=Healthy people)

Food quality: Nutritional value, probiotics, nutraceutical etc.

Animal well-being: Scientific report on the effects of farming systems on dairy cow welfare and disease (EFSA-Q-2006-113, 2009): less drugs and vet interventions (Healthy animals=Healthy people)

Policy makers

Legal circumstances: EU comprehensive Animal Health Law in elaboration

- presently about 40 different Directives and Regulations [Pavon 2013]

- disease prevention (awareness, registration, traceability,..)

- disease control and eradication

- Intra-EU-movements and entry into EU of animals and animal products

- Antimicrobial resistant pathogens

Regulations on national level

Environmental circumstances

- World human population is expected to reach 9,6 billion people in 2050 [http://www.un.org]

- Expected increase in demand for animal products and pressure on resources (land, water, energy,..)

- Need to reduce environmental footprint of cattle emissions [Livestock's long shadow FAO 2006]

Trend in PVS

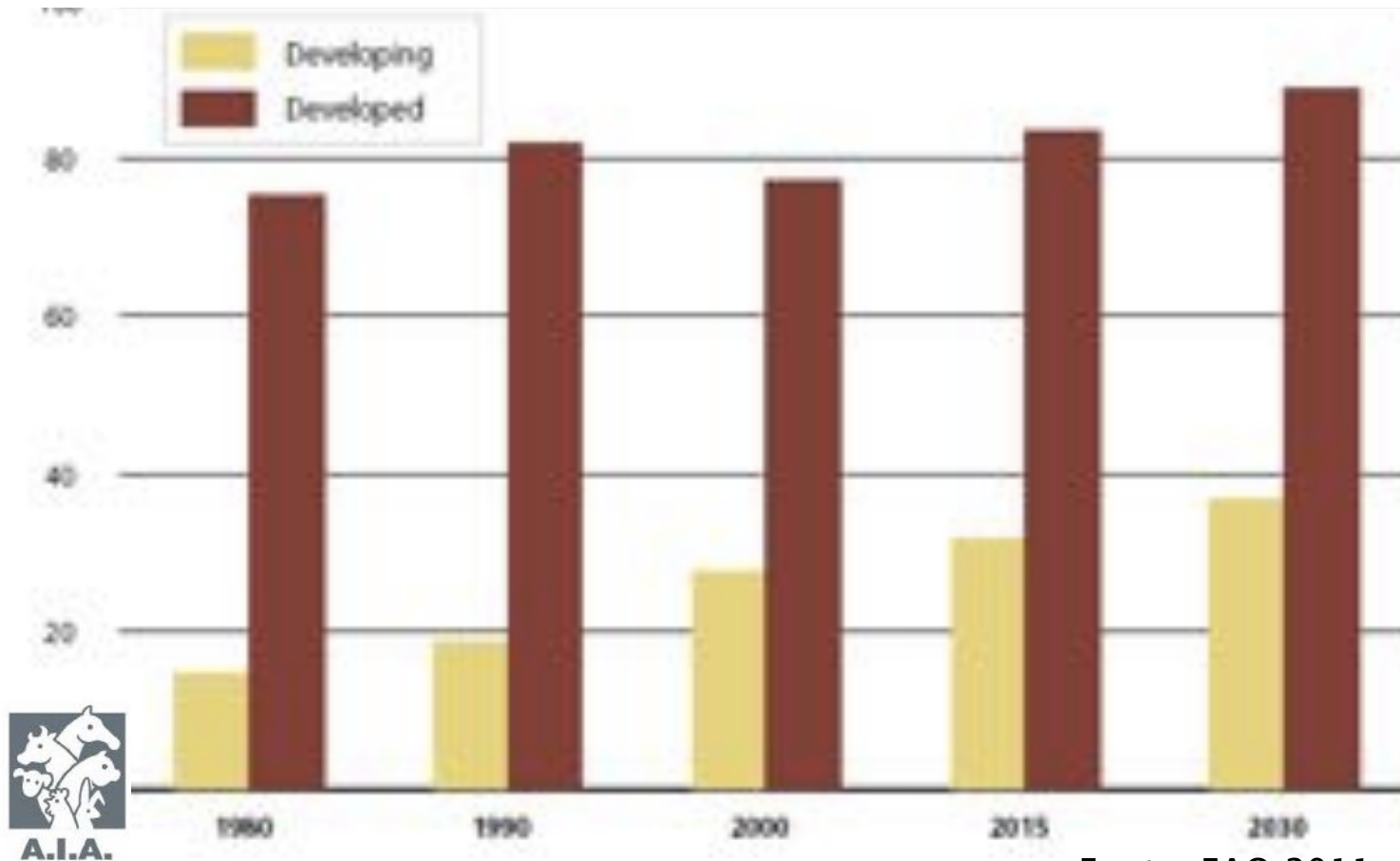|	1962	1970	1980	1990	2000	2003
Kg/procapite/year						
Cereals	132	145	159	170	161	156
Tubers	18	19	17	14	15	15
Roots	70	73	63	53	61	61
Beef	10	11	14	19	27	29
Milk	28	29	34	38	45	48

Animal products
1993 – 2020

Fonte: FAO 2011

Needs of farmer

- Essential for further developments and motivation to record traits

- Surveys in different countries (Rössler et al. 2013; Gene2Farm (2013), Steininger et al. 2013, Amer et al. 2013,..)
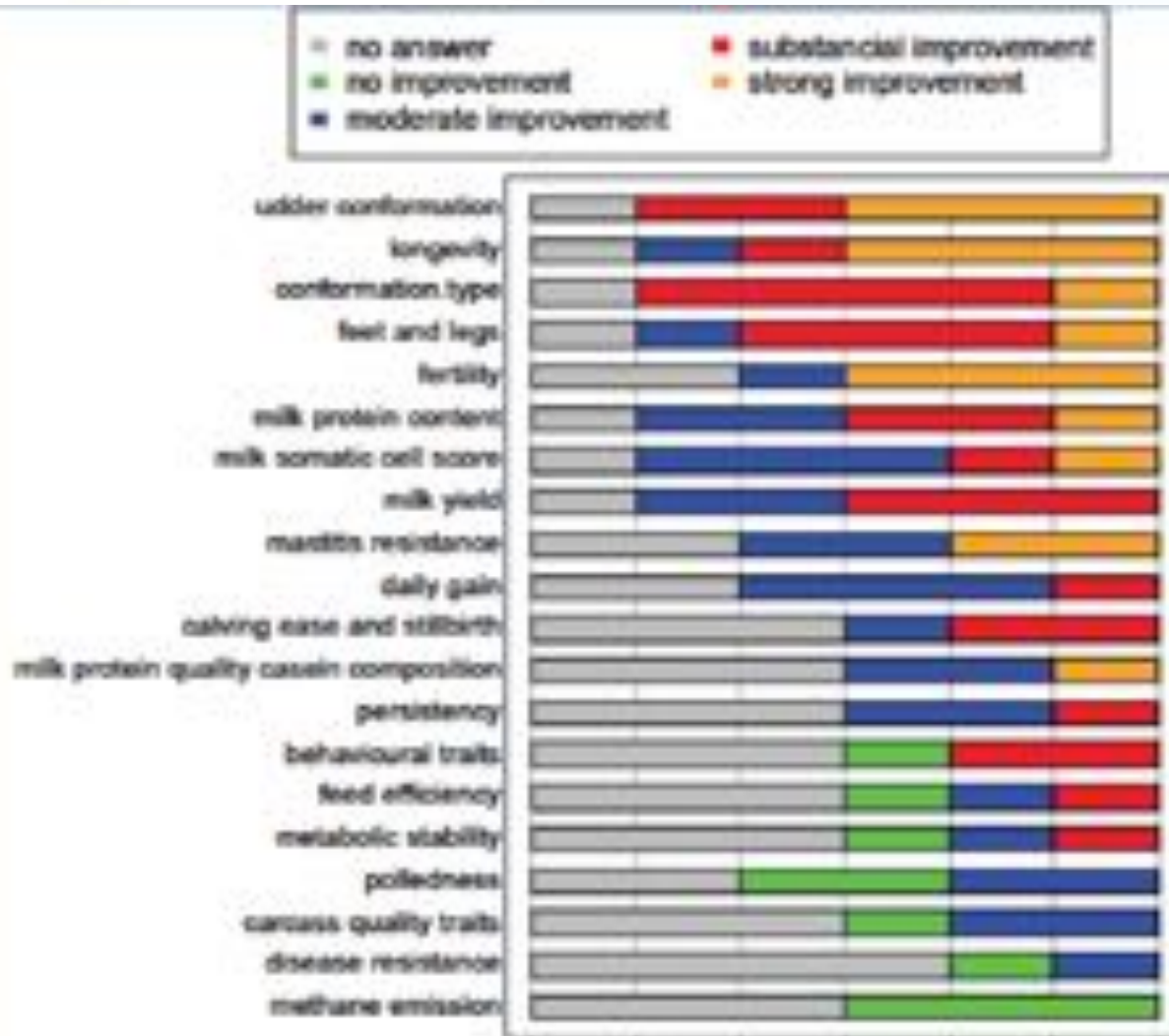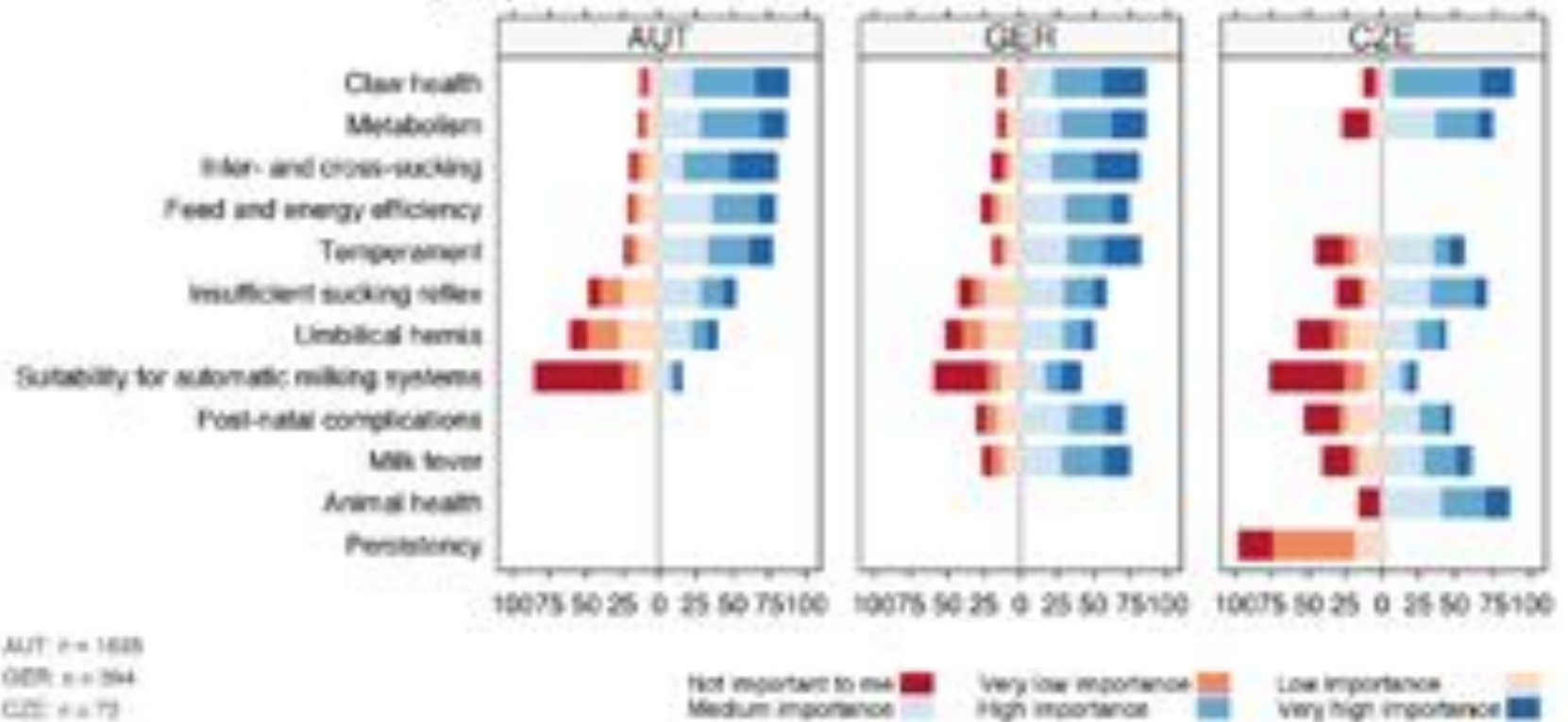
Courtesy:
Gene2Farm project

New traits

(Comparison between states - Fleckvieh, 2012)

[Steininger et al. 2013]

New selection methods

- Cattle selection has been long oriented towards production, with poor or no attention to sustainability;

- Traditional selection on production deteriorated most functional traits;

- Cattle selection have to prioritize sustainable production with its three pillars, economic, societal and environmental.

Emergence Genomic selection

- In GS, animals are evaluated from their DNA information trough markers covering the genome

- GS is already routinely applied in dairy cattle and will be soon extended to beef (already in use in few breeds as Limousine)

- It is a unique opportunity to   select for new traits considered as unachievable until recently
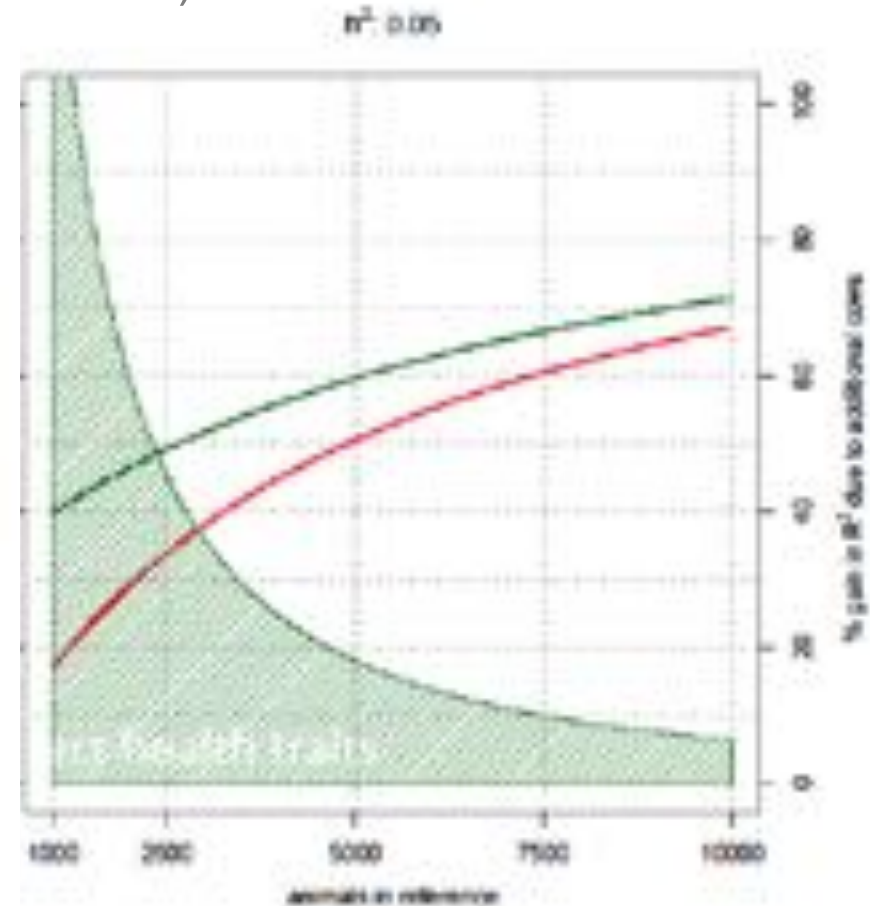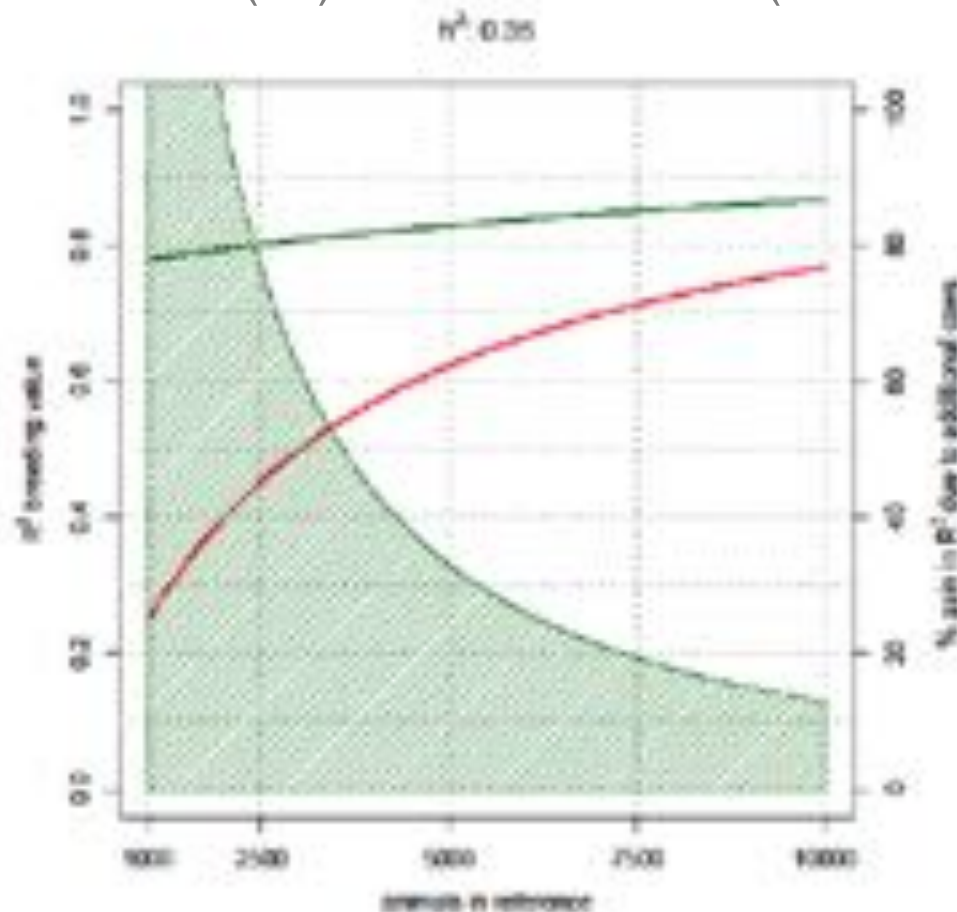
- Disconnection between performance recording (in a reference population) and selection (of candidates without performance)

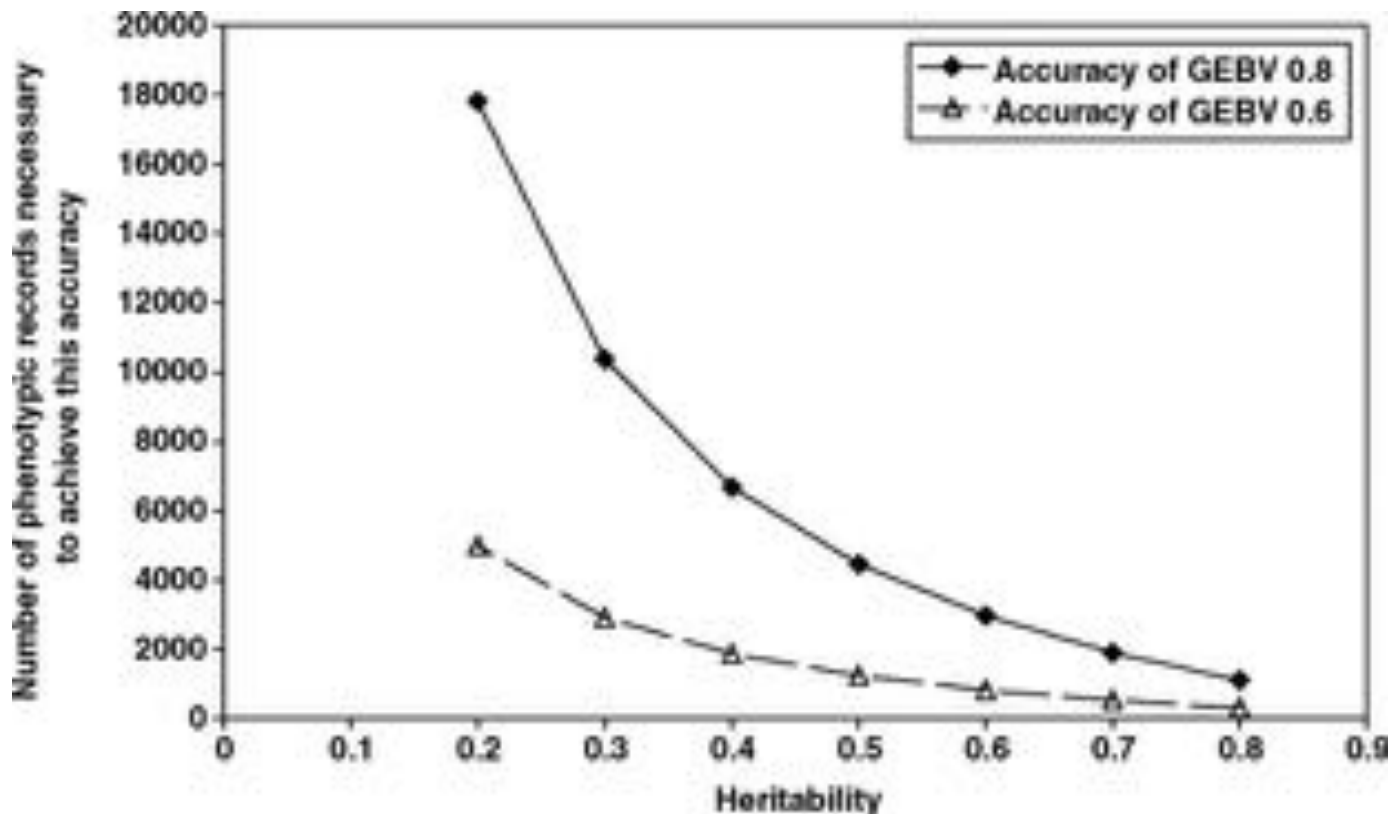- Boost Genetic trends

- Increased female genetic index accuracy

Impact of genotyping 25,000 cows additionally to bulls on reliability (R^2) for different traits (h^2=0.35; h^2=0.05)

[Daetwyler et al. 2010; Schwarzenbacher, 2012]

- Still high number of phenotypes required for low h^2 traits

[Hayes et al 2009]

Which "new" traits?

- Already known traits selected with low efficiency, such as fertility

- Already selected traits measured more accurately with new recording systems

- Measured for extension services but not yet selected traits, e.g. metabolic diseases

- Truly new traits as milk components (milk coagulation aptitude)

Health traits

- Direct or indirect measures of health traits → Direct ones more effective in breeding [Heringstad 2007; Egger-Danner, 2012]

- Directly observed measures of health from vet diagnosis [e.g. mastitis, retained placenta, puerperal disease, metritis, cystic ovaries ….]

- Systems of recording are presently being established in several countries (Italy included)

Udder health

- Improved SCC definitions (e.g. prolonged elevated SCC; cronical,)

- Improve diagnoses (clinical or subclinical mastitis)

- Accurate indicators traits (electrical conductivity, MIR etc)

- Pathogen information

  - Important for effective programs on prevention and prudent use of antimicrobials [Staph. Aureus; Str Uberis, Str.agalactiae

  - Potential for use in genetics [Sorensen et al. 2009; De Vliegher et al. 2012, Haugaard et al. 2012]

Metabolism

- Improved diagnoses (e.g. Ketosis; Acidosis, milk fever, etc) either by on farm quick test and by routine chip lab analyses (BHB)

- Improve accuracy of Milk composition based indicator traits (e.g. fat/ protein ratio measured by MIR)

- Other indicator traits (rumen activity, body weigh changes, body temperature etc)

- Clinical and subclinical diagnoses

Dehorning

- In modern cattle husbandry removing horns at an early age has become an accepted management practice

- Very painful practice

- Breeding polled cattle may constitute a non-invasive option to replace the common practice by means of genetic selection.

- A "pooled" gene = hornless gene, dominant, without any deleterious effect on the animals [mapped on BTA 1]

- In breeds without known pooled animals, a careful screening of pooled animals should be organized

Standardization of health traits

- Heterogeneity of recording schemes with a broad range of number of traits with 1 to > 900 documentation options (ICAR FTWG 2012)

- Already existing several health data recording systems with different approaches and world wide distributed experience with little exchange, sparse interdisciplinary collaboration

- Definition of data types i.e. **indirect health** data (health indicators correlated with health/disease like somatic cell score) **and direct health data** (disease diagnoses or observations of disease like sole ulcer, clinical signs / findings indicative of disease like lameness)

- Sources of data: veterinarians producers / farmers; expert groups (e.g. claw trimmers); technicians etc

Further issues

- Particular sensitivity of health data i.e. Legal framework for working with health data national requirements & data privacy standards

- Central role of health for animal welfare and consumer protection

- Type of information to be stored in the health database

- Data ownership & authority to permit transfer and use of data

- Institutions authorized to administer database & analyze data

- Access rights of (original) data & results from data analyses

ICAR guidelines for Recording, Evaluation and Genetic Improvement of Health Traits (approved in 2012; www.ICAR.org)

- In connection with obligatory base information, i.e. animal-ID, date and place/herd, data source: type of health incident (standardized code for recording)

- Requirements for reference health key: – clear trait definitions

– Compatibility with existing recording systems – broad usability across user groups

Coding of diagnoses: an international cooperation effort [IDF, ICAR, …]

Goal: comparability of results across vet and countries

	Comprehensive key of diagnoses	Reduced key of diagnoses	Simple key of diagnoses
Nr. of diag.	> 600	60-100	10-15
Source	veterinarian	veterinarian	producer
Recording	electronic submission (vet)	vet, perform-ance record., producer	producer
Example	ICAR-guideline: mastitis catarrhalis acute and subacuta, mastitis parenchymatosa acuta and subacuta, ...	E.g. AUT: acute mastitis chronical mastitis;	mastitis

Health traits key: database structure

Multiple levels hierarchical structure : category / disease group -> sub-category -> individual items with low to high specificity

category / disease group

Code	Technical term	No. of items
1.	Organ diseases	539
2.	Reproduction disorders in females	116
3.	Reproduction disorders in males	44
4.	Infectious disease and other microbe-related diseases *	103
5.	Parasitoses (parasite infestations)	42
6.	Metabolic diseases and deficiencies	73
7.	Poisoning	35
8.	Behavioral disorders and general findings	14
9.	Health-related information not representing diagnoses	45

* except local infections of udder and cows

sub-category

Code	Technical term	No. of items
2.	Reproduction disorders in females	116
2.01.	Diseases of the female reproductive system	23
2.02.	Pregnancy disorders	17
2.03.	Diseases related to calving	24
2.04.	Disorders in the postpartal period	16
2.05.	Female infertility	30

Individual items with low to high specificity

Code	Technical term
2.05.	Female infertility
2.05.00.	Control of oestrus cycle with no abnormality detected
2.05.01.	Uterine infertility
2.05.02.	Ovarial infertility
2.05.02.01.	Disturbance of the ovarial reproductive cycle
2.05.02.02.	Nymphomania
2.05.02.03.	Virilism
2.05.02.04.	Ovarial cysts
2.05.02.04.01.	Follicular cystic ovary disease
2.05.02.04.02.	Luteal cystic ovary disease
2.05.02.04.03.	Cystic ovarian degeneration
2.05.02.05.	Corpus luteum persistens (persisting corpus luteum)
2.05.02.06.	Atrophy of the ovaries
2.05.02.07.	Dystrophy of the ovaries
2.05.03.	Examination due to infertility
2.05.04.	Treatment due to infertility
2.99.	Other disturbances of female fertility

Claw horn disease

- Claw health is key for feeding and milking, significant issue affecting the overall productivity

- **Locomotion Scoring** Locomotion scoring uses a simple 1 to 5 scale to assess the severity of dairy cattle lameness. It is based on the observation of cows standing and walking (gait), with special emphasis on their back posture. Intuitive and easy to learn and implement.

1 normal cow
2 mildy lame
3 moderately lame
4 lame
5 severely lame

Many different pathologies under lameness:

Dermatitis;   Heel horn erosion; ID hyperplasia, Corkscrew claws, Sole hemorrhage, Sole ulcer, With Line Disease etc.

What is efficiency?

- Output per input unit on farm level
- Output per input unit on individual animals
- Output per input unit on milk/beef production per animal

RFI (Residual Feed Intake): This is defined as the difference between actual feed intake and that predicted on the basis of the animal's gain and maintenance requirements for its body weight. Correlations with other traits need to be understood (e.g. RFI and fertility may be negatively correlated; Pryce et al. 2013)

R-ADG (Residual Average Daily Gain): This is defined as the difference between actual weight gain and the gain predicted on the basis of dry matter intake, maintenance of body weight and fat cover.

Adj. DMI (Adjusted Dry Matter Intake): This is de- fined as the feed dry matter intake adjusted by multiplying the contemporary group's average mid-point metabolic weight, and then dividing by the individual's mid-point metabolic weight.

Method	More Desirable	Less Desirable	Difference
Raw F:G – Raw Feed Conversion: usually on dry matter basis (lbs feed/lb of gain)	Lower values Example: 4.5 lbs	Higher values Example 7.5 lbs	Example: 3.0 lbs of feed
Adj. F:G – Adjusted Feed Conversion: usually on dry matter basis (lbs feed/lb of gain)	Lower values Example: 4.5 lbs	Higher values Example: 6.5 lbs	Example: 2 lbs of dry matter
RFI – Residual Feed Intake: usually on dry matter basis	Negative values Example: -1.7	Positive values Example: +1.5	Example: 3.2 lbs of feed
R-ADG – Residual Average Daily Gain: usually on lbs gained per day	Positive values Example: +0.86	Negative values Example: −.63	Example: 1.49 lbs of average daily gain
Adj. DMI – Adjusted Dry Matter Intake: should be on dry matter basis	Negative values Example: -0.9	Positive values Example: +0.8	Example: 1.7 lbs of feed

Product quality

- Nutraceutical
- Probiotics
- Food industries and cheese-makers requests (e.g. milk coagulation aptitude)
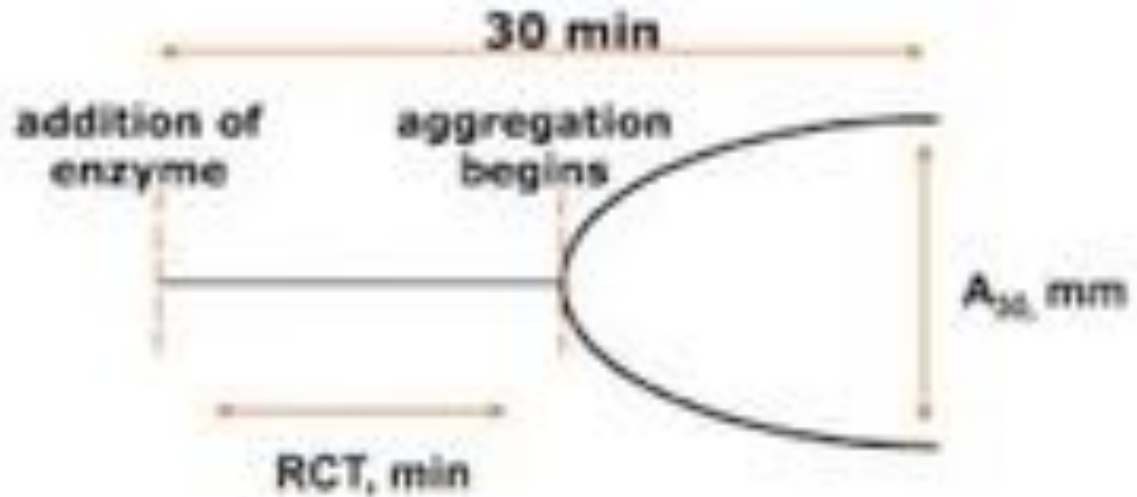
Standardization of MIR data

- Traditionally limited to fat, protein, SCC, Urea

- New methods based on Mid Infra Red (MIR) spectrometry very promising for:

                  Novel Milk composition traits (fatty acid components, coagulation, etc

                  Fertility (pregnancy)

                  Metabolic disease (BHB)

                  Feed efficiency (Urea)

                  ……

- Differences between responses of
    different MIR spectrometers

- Changes in response of a
    spectrometer over time

- Comparability of spectra from
    different brand (Foss, Bentley,.),
    machines inside brands – stability
    over time

- Repeatability of results across
    different breeds, production
    circumstances

- Spectra standardization is a complex issue
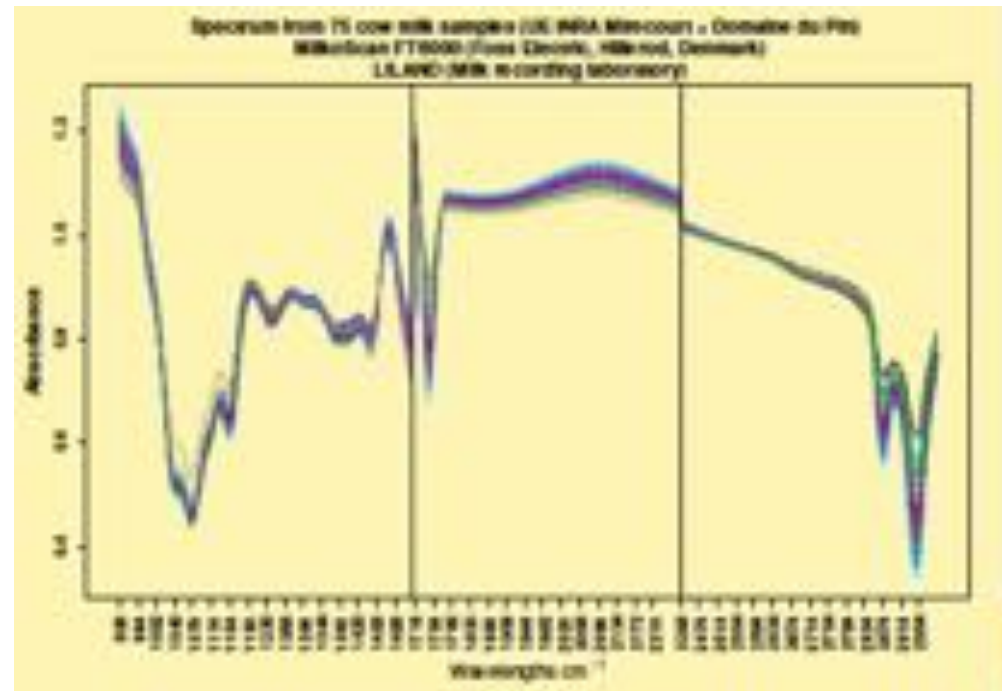
PIECE-WISE DIRECT STANDARDIZATION (PDS) [Wang et al. 2008]

This method transfers the MIR spectra from the instrument on which they were collected to the instrument on which the calibration model was developed (master).
PDS is based on the fact that the spectral information contained in a certain wavelength on the master instrument is highly correlated to the spectra of neighbor wavelengths on the local instrument.
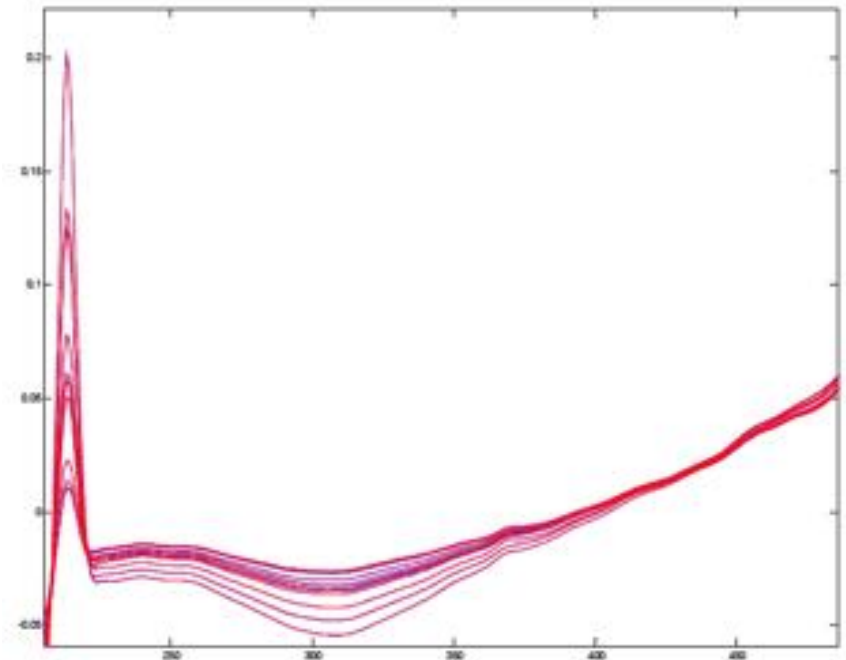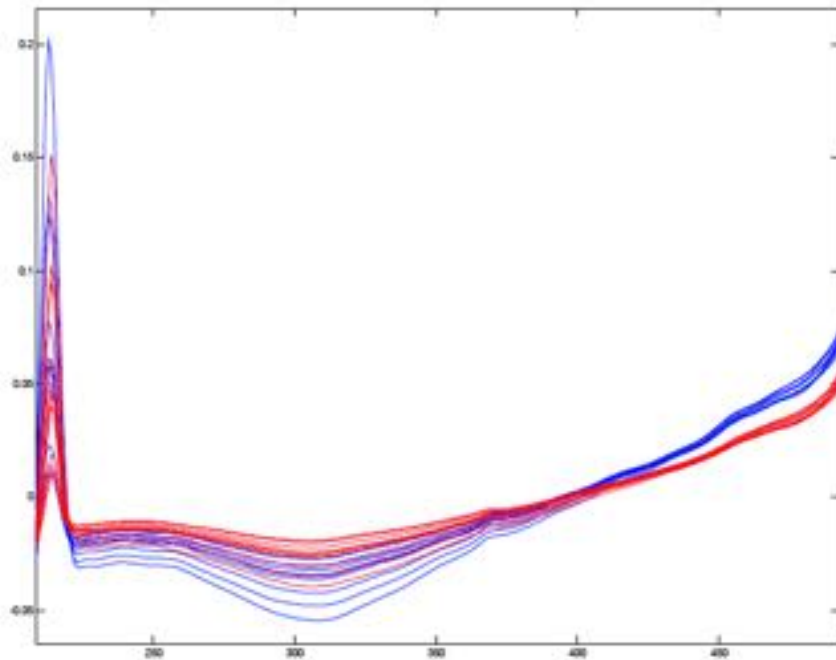
Before and after calibration

Once new traits prediction equations are available or updated, they can be applied on an historical basis (assuming machine-dependent calibration)

The **SEUROP** carcass classification – Assessment of conformation & fat grades by experts
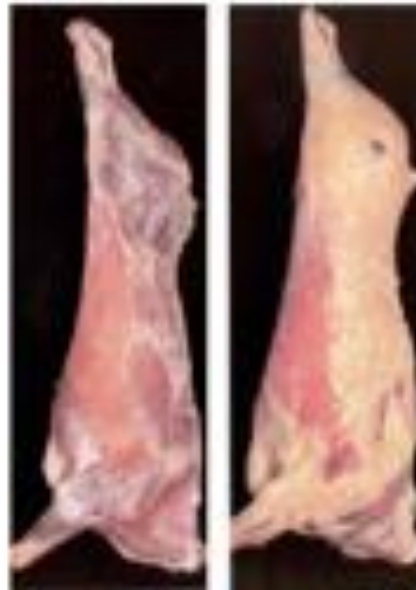
Current selection tool for carcass quality

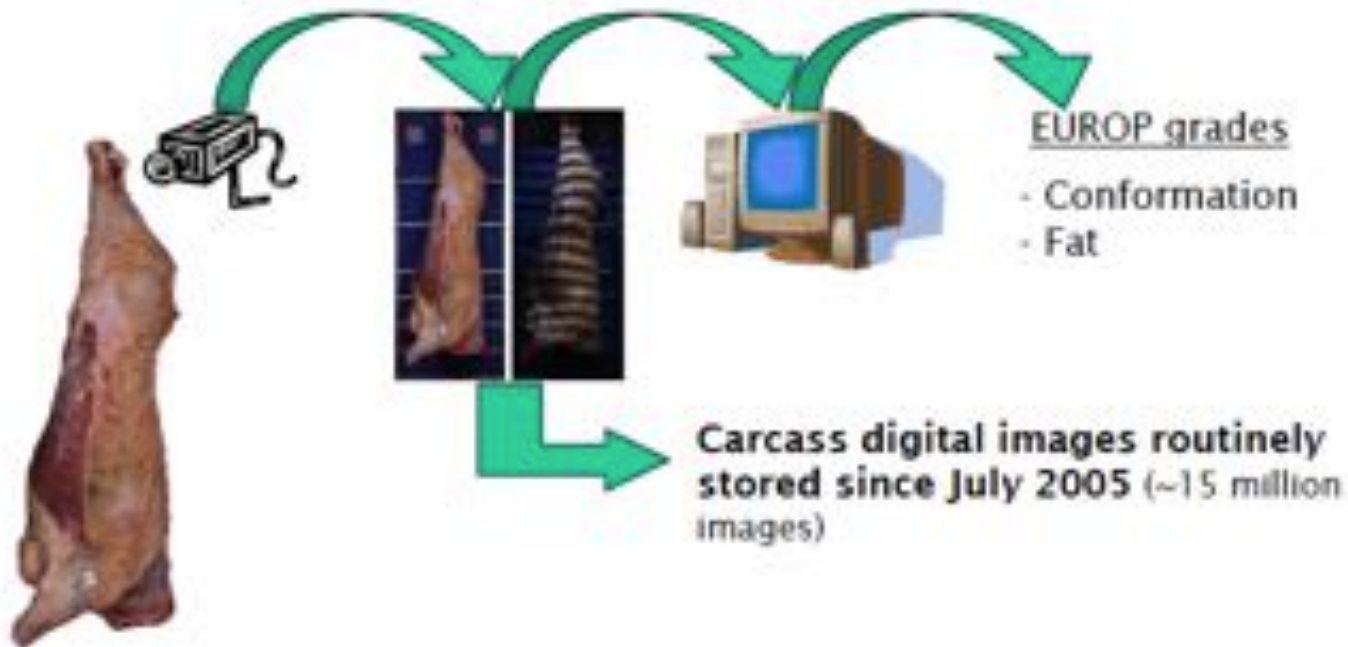EEC Regulations no 1208/81 ; 2930/81 ; 1026/91

Digital grading of the carcass

EUROP grades
- Conformation
- Fat

Carcass digital images routinely stored since July 2005 (~15 million images)

Building prediction equations using multivariate analysis

428 variables (contour, length, surface etc) on more than 500 heifers and steers

Lower value cuts

Medium value cuts

High value cuts

Very high value cuts

=
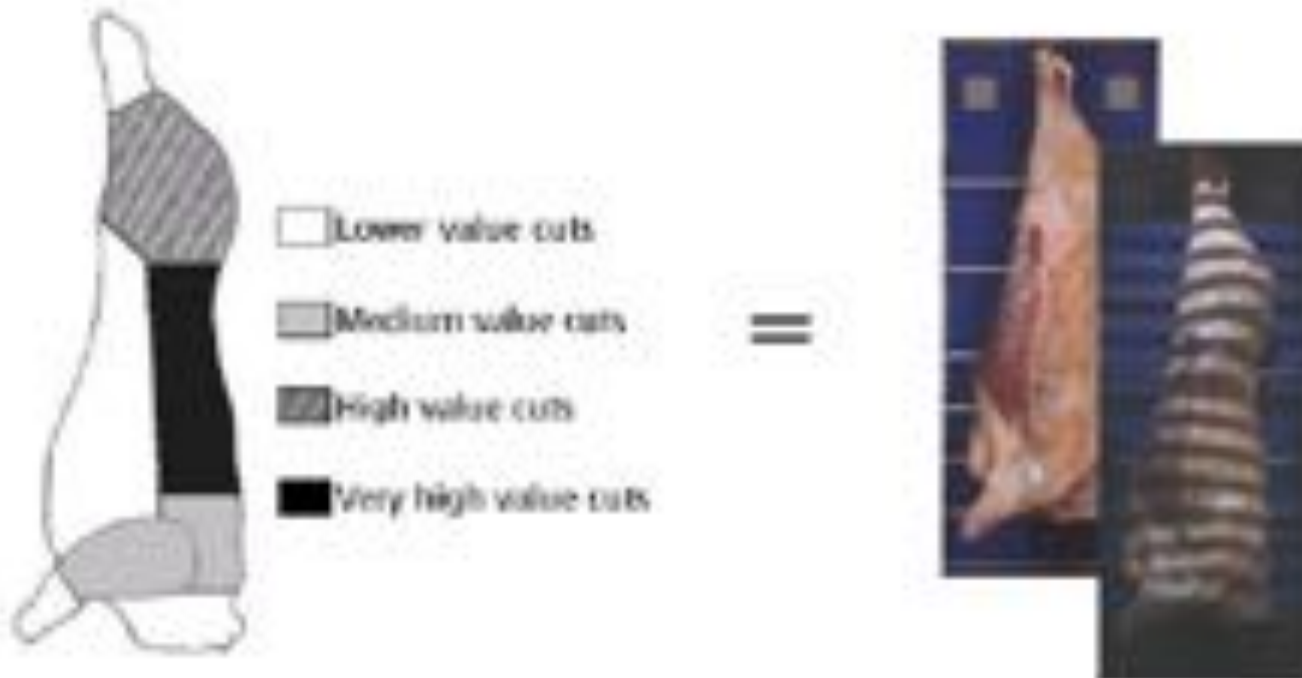
	STEER	HEIFER
Total meat	0.97	0.84
Total fat	0.77	n/a
Total bone	0.81	n/a
Lower Value Cuts	0.92	0.65
Medium Value Cuts	0.86	0.70
High Value Cuts	0.93	0.85
Very High Value Cuts	0.84	0.72

[Pabiou et al 2012]

Added values: new phenotype obtained by applying prediction equations to the digital images historically stored

Precision livestock farming

- Utilization of electronic devices and systems [ID, feeding concentrate, yield sensors, pedometers etc.]

- Automatic Milking Systems

- Integrated systems (ID, Yield, Data collection, sampling)

- In line sensors

- Software for data management in farm

THE PRECISION LIVESTOCK INITIATIVE : OPTIMISING LIVESTOCK PRODUCTION WITH PRECISION TECHNOLOGY

http://www.farmingfutures.org.uk/blog/precision-livestock-initiative-optimising-livestock-production-precision-technology

Automation and communication Challenges:

- Nearly no integration of already existing data

- Standardization of traits accross techniques

- Calibration of reference

- Carry over (that a milk sample from cow B also contains some milk from cow A that was milked just before B (Lovendahl et el. 2013))

- Communication on-farm between different technologies

- Communication between on-farm and central data base

- Still research needed;

[Rutten et al. 2013]

The risk for farmers: drowning in data but starving for information

Wrapping up

- Circumstances of production will require emphasis on new traits like health, welfare, efficiency,..

- Advances in technology enable "better" trait definitions

- Automation offers possibilities for indicator traits

- Standardization of traits and communication between data sources are challenges

- Reliable phenotypes for new traits needed.

- International cooperation needed

Time for a new model for livestock phenotyping ??

- Large scale phenotyping for common traits useful for farm management [milk yield, milk quality, reproduction etc.]

- Nucleus top herds in which collect complex or expensive genotypes to be translated in genetic improrvement
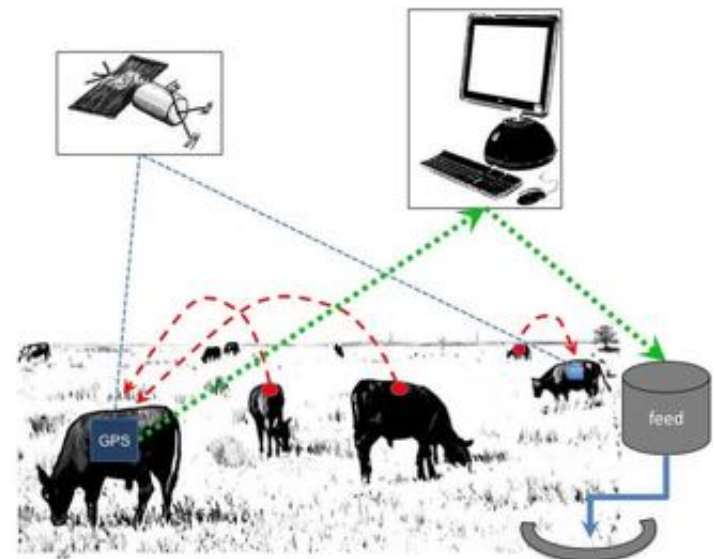
Most data used in the lecture are publicly available thanks to:

D. Boichard   (INRA)

C. Egger-Danne (ZuchtData)

K.F. Stock (Vit)

P. Nielsen (Danish Cattle Federation)

J.A. Fernandez Pierna (CRA_W)

T. Pabiou (ICBF

E. Trevisi (Unicatt-PC)

Please visit: www.ICAR.org

A bit of History

How it works

Potential applications

 Some pitfalls
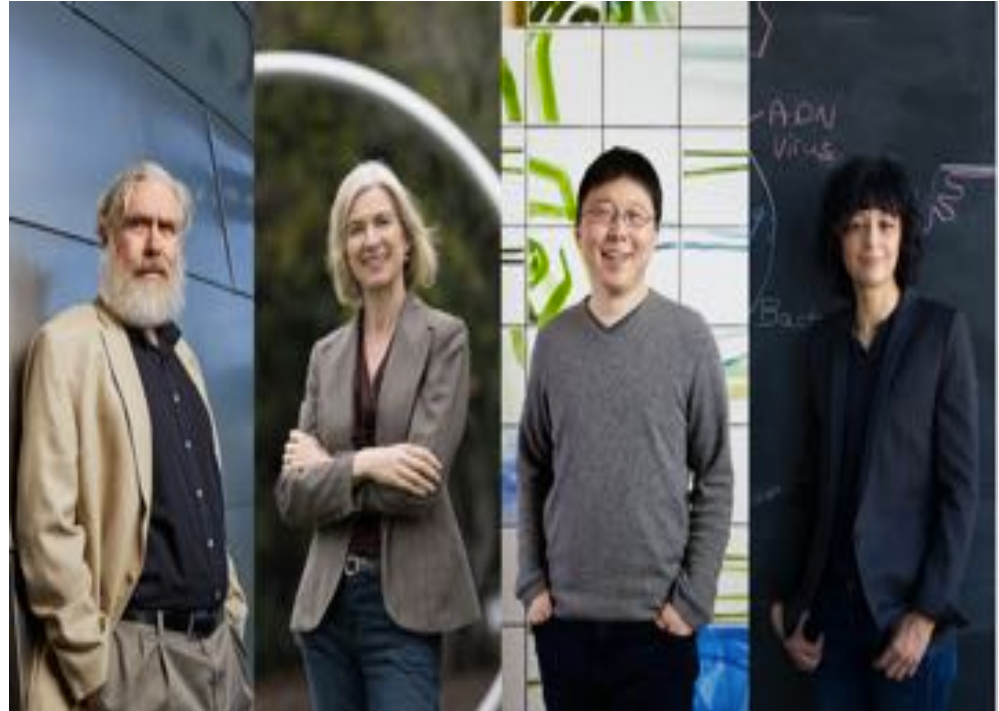
Univ Berkeley California,   and its rival, the Broad Institute of MIT and Harvard in Cambridge, Massachusetts,

1979
Gene replacement
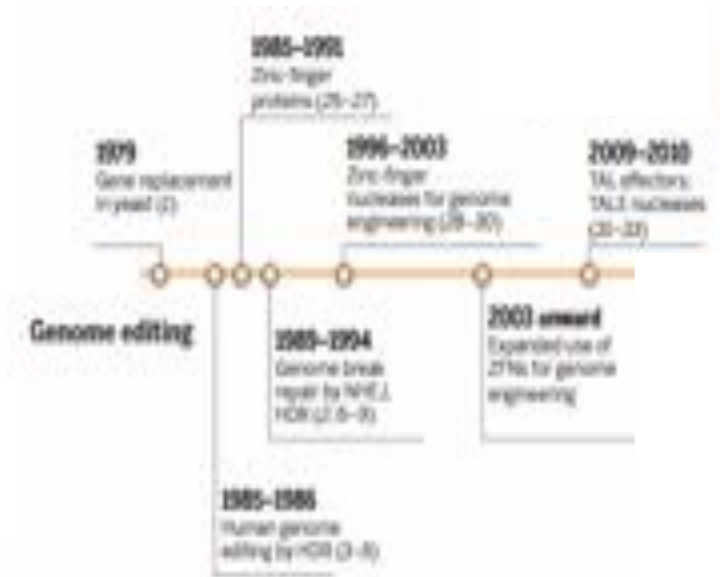in yeast (2)

Genome editing

Many of the earliest approaches to what has been referred to as genome editing relied on the **principle of site-specific recognition of DNA sequences.**
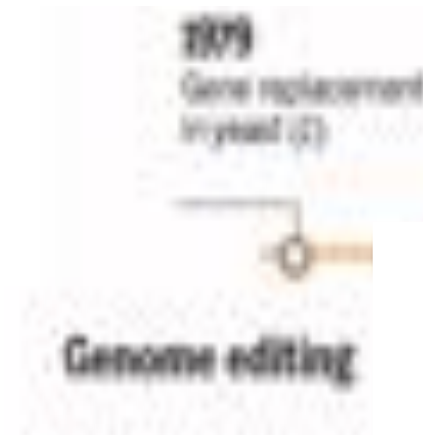
S. Scherer, R. W. Davis, Replacement of chromosome
segments with altered DNA sequences constructed in vitro.
Proc. Natl. Acad. Sci. U.S.A. 76, 4951–4955 (1979).

Thus, the field was primed **for the first reports of transcription activator–like (TAL) effectors, which occur naturally in bacteria that infect plants, enabling rapid creation of FokI coupled** versions that could be used similarly to ZFNs for site-directed genome editing. Such TAL effector nucleases (TALENs) were easier than ZFNs to produce and validate, generating widespread excitement about the possibility of facile genome editing that would be fast and inexpensive. But difficulties of protein design, synthesis, and validation remained a barrier to widespread adoption of these engineered nucleases for routine use.

CRISPRs were later detected in numerous bacteria and archaea, and predictions were made about their possible roles in DNA repair or gene regulation. (Mojica et al., 2000)

Jansent et al.(2002) further studied the short repetitive DNA sequences in prokaryotes. The authors named the family as the **clustered regularly interspaced short palindromic repeats (CRISPR**). Meanwhile, they identified four CRISPR-associated (Cas) genes, suggesting that CRISPR/Cas system might play an important role in biological processes

A key insight came **in 2005 with the observation that many spacer sequences within CRISPRs derive from plasmid and viral origins.**
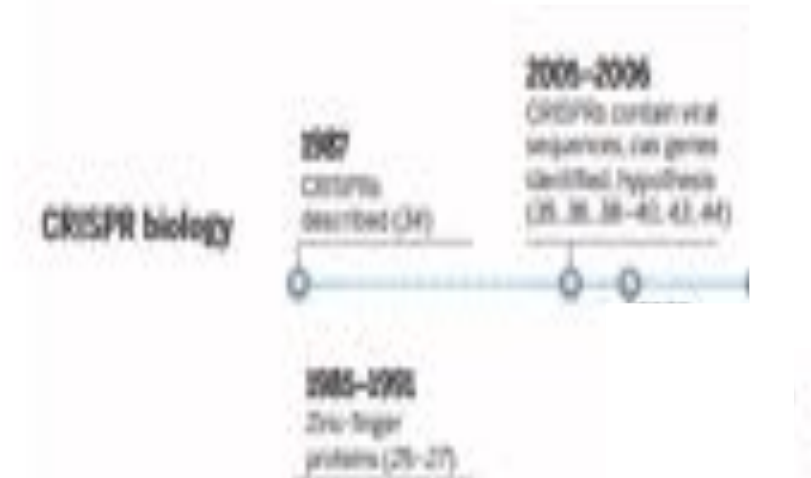
Garneau et al.(2010) reveled **that spacer sequences guided Cas9 to cleave target DNA.**

Deltcheva et al.(2011) found that **a duplex structure was formed by tracrRNA and crRNA and associated with Cas9.**

Jinek et al.(2012) demonstrated that **Cas9 was an RNA guided endonuclease.**

Cong et al.(2013) first used **CRISPR/Cas9 in eukaryotic cells and successfully achieved efficient and specific genome editing**
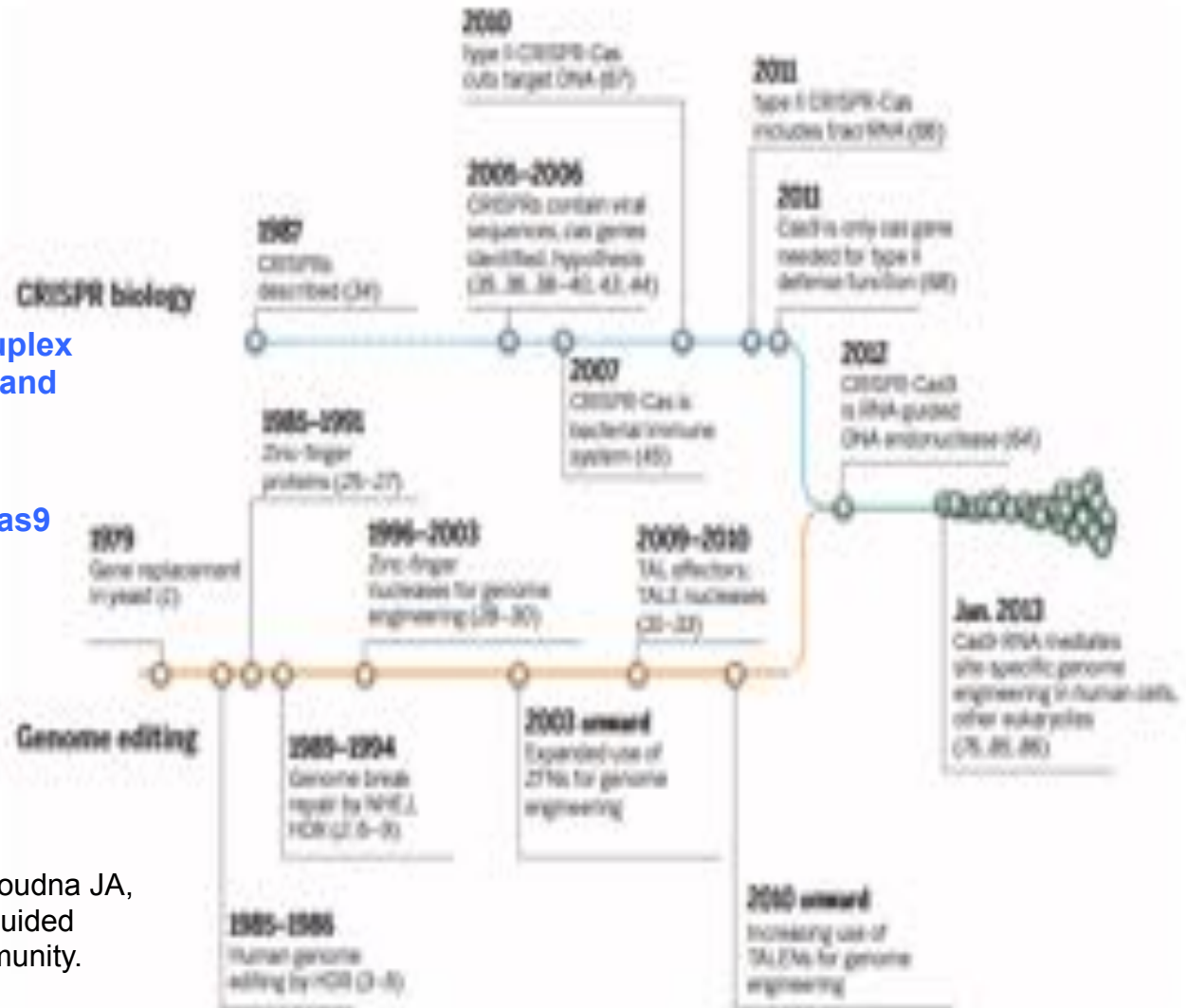
Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E.A programmable dual-RNA-guided DNAendonuclease in adaptive bacterial immunity. Science. 2012;337(6096): 816–21.

Different strategies for introducing blunt double-stranded DNA breaks into genomic loci, which become substrates for endogenous cellular DNA repair machinery that catalyze nonhomologous end joining (NHEJ)or homology-directed repair (HDR).
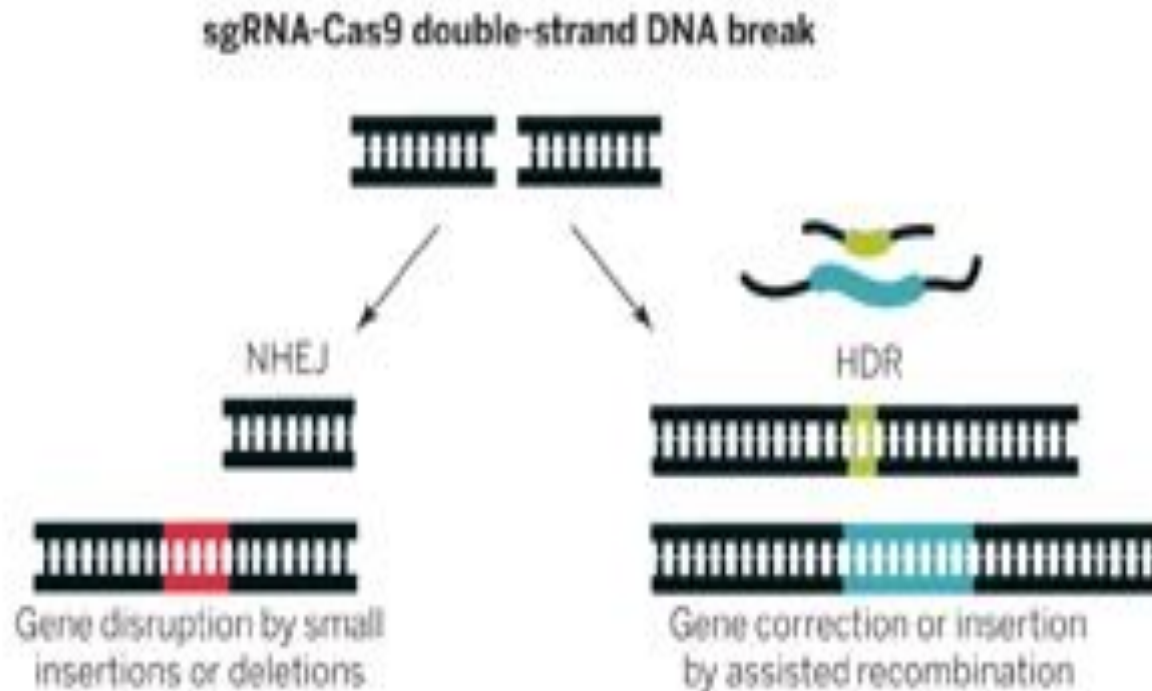
Genetic modifications in primate embryos.

CRISPR was carried out in one-cell embryos to successfully generate modified cynomolgus monkeys. This is the closest of the animal models in similarity to humans and can give evidence   for how the system might behave in human embryos.

> …..By coinjection of Cas9 mRNA and sgRNAs into one-cell-stage embryos, we successfully achieve precise gene targeting in cynomolgus monkeys. We also show that this system enables simultaneous disruption of two target genes (Ppar-g and Rag1) in one step, and no offtarget mutagenesis was detected by comprehensive analysis. Thus, coinjection of one-cell-stage embryos with Cas9 mRNA and sgRNAs is an efficient and reliable approach for gene-modified cynomolgus monkey generation.

This could be used **to potentially prevent non-complex hereditary diseases, such as single gene defects**. However, without full predictability of the off-target effects, germ line gene editing remains ethically and scientifically unsafe.

Niu Y, Shen B, Cui Y, et al. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one cell embryos. Cell. 2014;156(4):836–43.
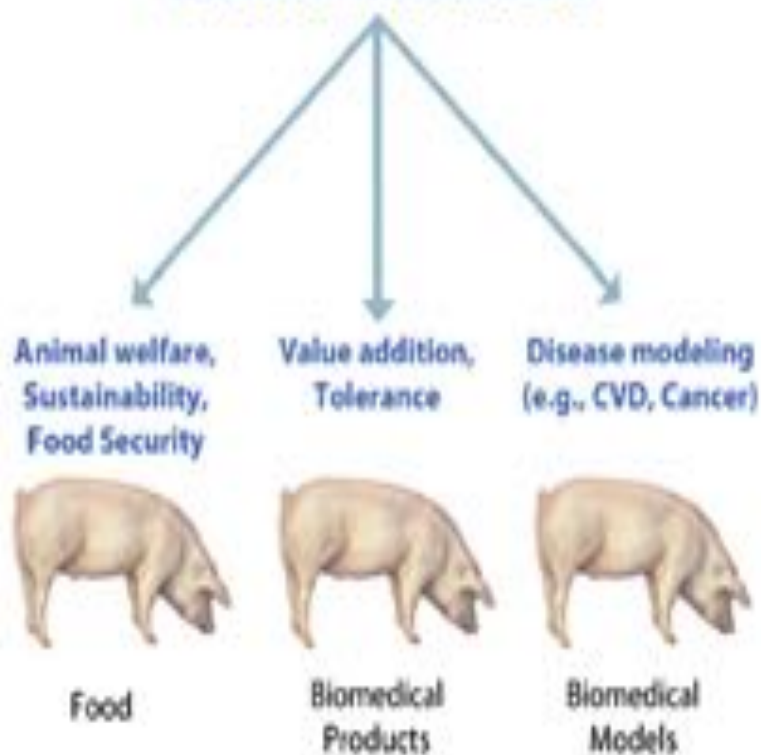
Slick gene

The SLICK hair locus derived from Senepol cattle confers thermotolerance to intensively managed lactating Holstein cows

S. Dikmen,* F. A. Khan,†[1] H. J. Huson,‡[2] T. S. Sonstegard,‡ J. I. Moss,† G. E. Dahl,† and P. J. Hansen†[3]

*University of Uludag, Faculty of Veterinary Medicine, Department of Animal Science, Bursa 16059, Turkey
†University of Florida, Department of Animal Sciences, Gainesville 32611-0910
‡USDA, Agricultural Research Service, Animal Genomics and Improvement Laboratory, Beltsville, MD 20705-2350

Gene dominante localizzato sul BTA 20, identificato in razza Caraibiche (Senepol) e introgresso in Holstein attraverso meticciamento

In conclusion, Holsteins with slick hair have superior thermoregulatory ability compared with non-slick animals and experience a less drastic depression in milk yield during the summer.

A. Introgression

Dairy Breeds
Horned

Polled Beef Breeds
NATURALLY no horns

Introgression

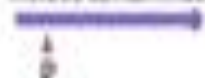The polled allele (P) is dominant to the horned allele (h)

Meat Yield

Meat Quality

Milk Yield

Milk Quality

Merit

Merit

B. Crossbreeding.
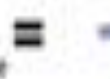
Meiotic contamination

=

8+ generations of backcross required to recover dairy genetic merit

C. TALEN mediated gene conversion.

TALEN Cut

± Red Angus template

=

+

Beneficial Allele Introgressed
Genetic Merit preserved

Thank you for the attention