Molecular approach for traceability of animals and animal products

ERASMUS + PROJECT : AG-LAB

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Peter DOVC

University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Ljubljana, Slovenia

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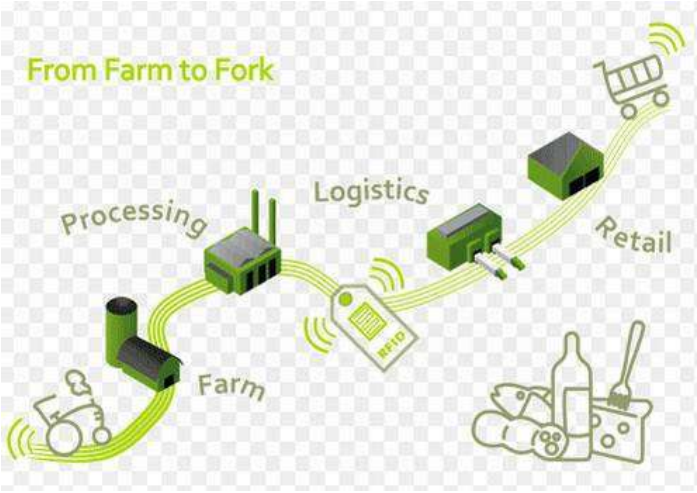
Traceability is required by the consumers

- Consumers increasingly insist on a comprehensive and integrated food safety policy (the so-called ‘farm to table’ policy), which has consequences both for producers and for control authorities.
- Traceability is widely recognised to be the basis of any modern food safety control system integrating both animal health and food hygiene components.
- Traceability is defined as the ability to trace the history, application, or location of an entity by means of recorded identifications.
- Traceback systems have been implemented for the purposes of animal health, as a part of surveillance, to provide the information required to prevent uncontrolled spreading of disease.



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Importance of the traceability of animals and animal products in epidemiology

- Traceability of animals and animal products has become a priority for governments, due to consumer safety demands.
- Animal identification and registration is the basis for different traceability and traceback systems.
- The importance of a traceability systems for food-borne risk assessment and management is recognized by comprehensive and integrated animal health and food safety policy.

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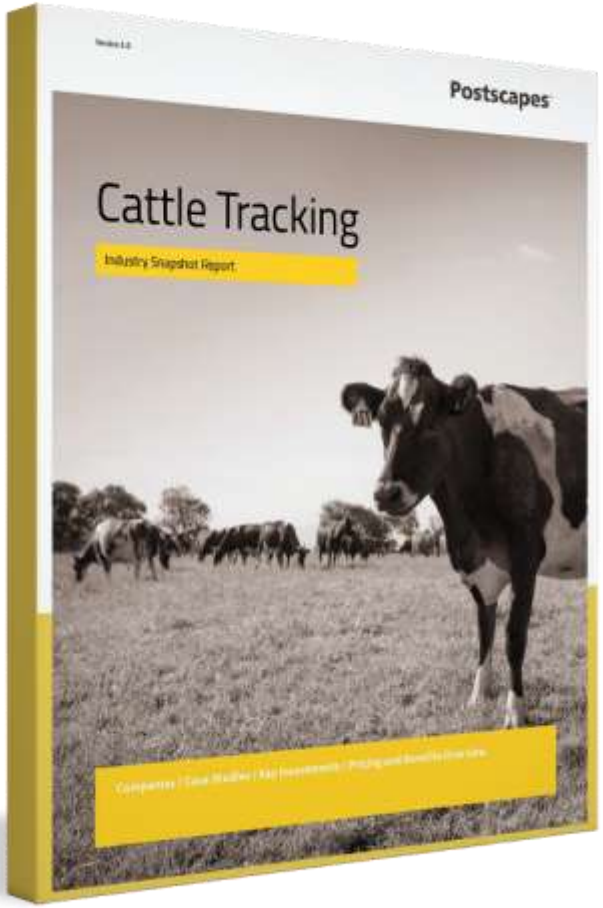
Zoonotic aspect and food safety

- The primary goal of an animal traceback system is to provide information on the source of infection or prohibited additives so that preventive and control measures can be applied to avoid the introduction of the contaminant.

- These systems should allow identification of sources of infection and prevent uncontrolled spreading of infections in animal production chain.



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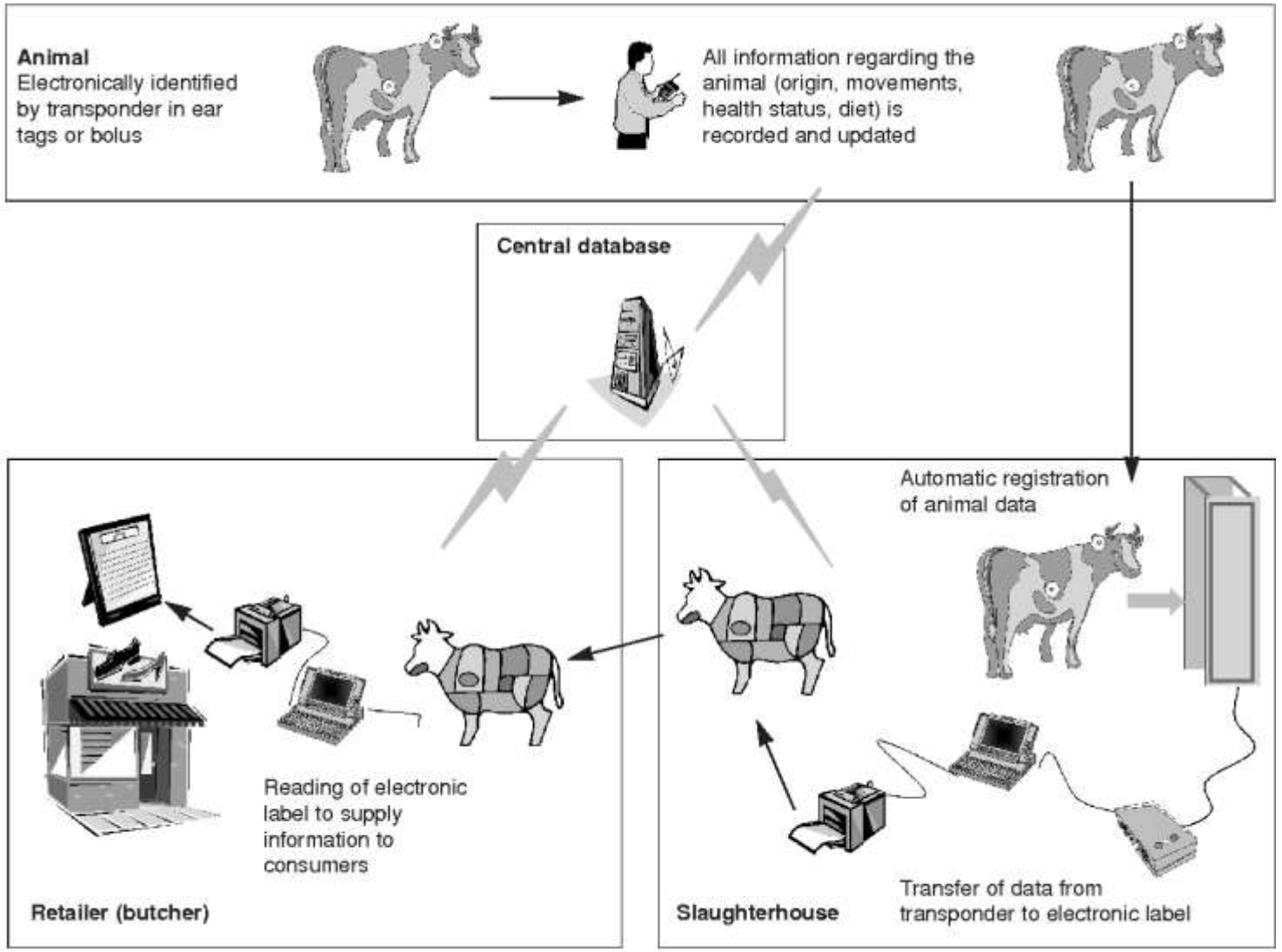
IT supported systems allow the basic level of traceability



- The IT supported systems allow information flow from the farm to the slaughterhouse and can be submitted to the consumer.
- There is a lack of physical link between products and data.

Detection of contaminants, including GMO

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Buskirk et al., 2013 JAFSCD

Some systems have been developed and adapted to small and medium size production units (low invetment, small quantities, special products, disappearing from the market).

Extension of the system through QR code, which can be read from the consumer, which can enter the system, the chance for frauds and mistakes is significantly reduced.

Infection events in animal production

Example of avian Mycoplasmas

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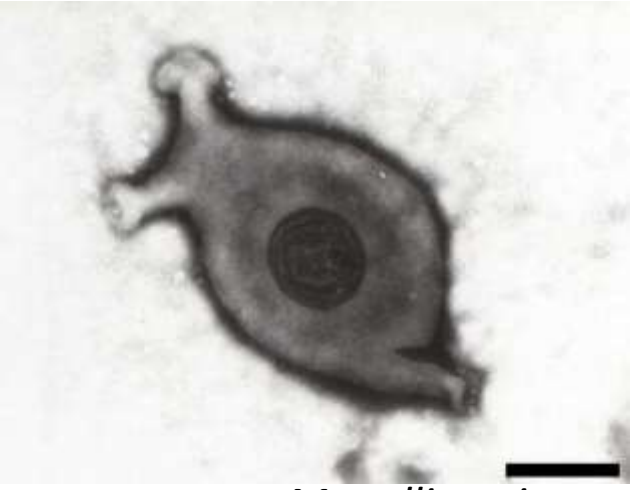
- Avian Mycoplasmas are one of the most fraquent infections of poultry in commercial farm. Infections are often subclinical and difficult to detect, however, they reduce productiveness of infected animals and can develop to acute infections when the immune response of birds is compromised.
- Therfore a special attantion has to be paid to infected breeding animals which are often the origin of subclinical infections in production flocks.
- Genetic discrimination of Mycoplasma strains is an important tool for tracing back the source of infection.



M. gallisepticum

Traceability of animal products - background

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- The most important arguments for asuring traceability of animal products are:
  - Food safety issues
  - Meat, milk, milk products with defined geographic origin / breed
  - Special breed related products (ham, cheese, premium quality meat)
  - Detection of mixed raw products (milk, meat from different species in meat products)
- Traceability can protect economically more vulnerable production chains from unfair competition
- Traceability can play important role in protection of local animal resources

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Architecture of the system

- Traceability of animals and animal products can be based on specific genetic traits which are characteristic for a certain breed, population or species. The system often relays on private, diagnostic alleles.

- The other systems are based on the genetic survey of the population, where all breeding animals are included in tissue sample colection, and genetic analysis can be performed upon request.

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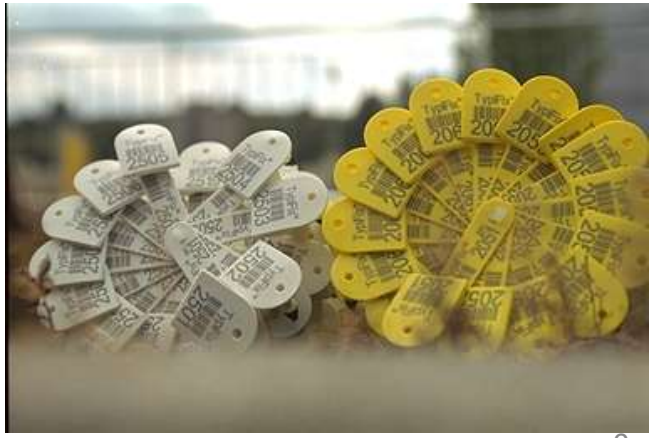
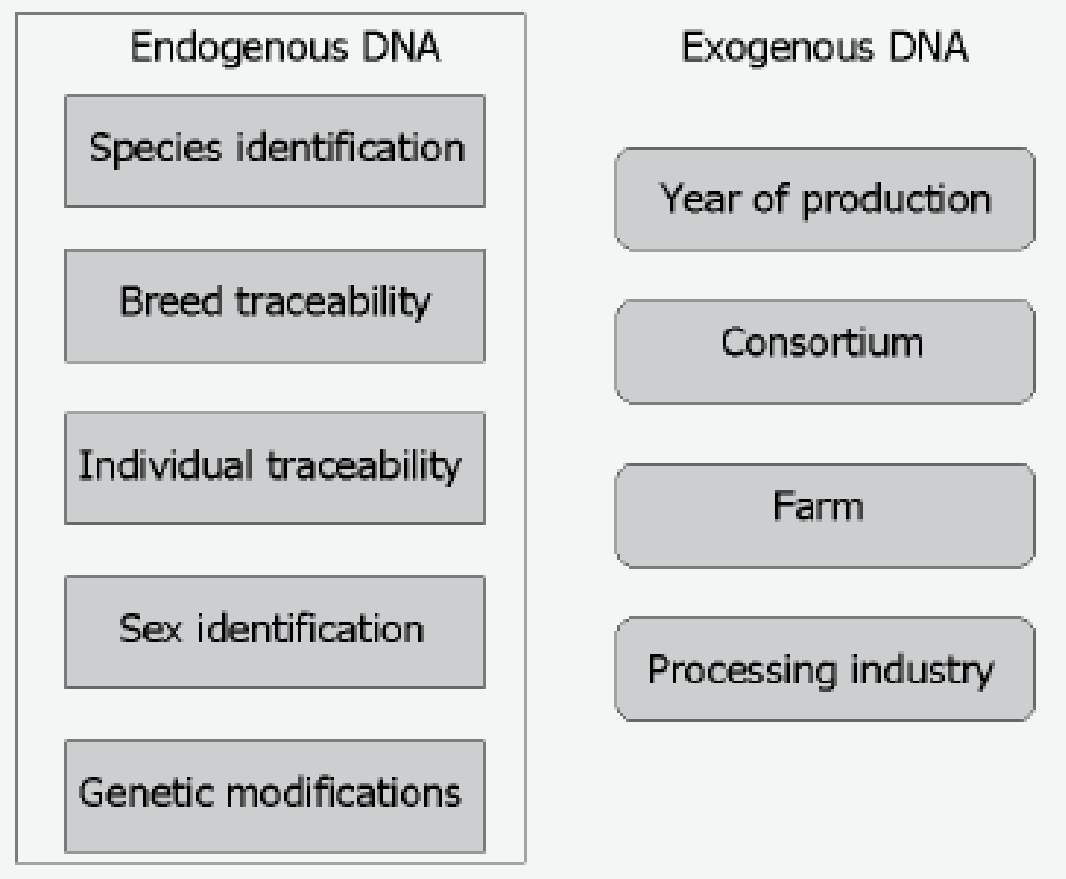


The authentications and traceability systems include information from endogenous and exogenous DNA



- The classical approaches are normally based on the analysis of endogeous DNA (often mtDNA, DNA barcoding).
- Newer methods which are applied to detect contaminations and intentional and non-intentional contaminations are often targeted to exogenous DNA.

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Arguments for different levels of identification

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- Species
  - Authentication of prodessed products (mtDNA, DNA barcoding)
- Breed
  - Products from specific breeds (PDO, PGD, TSG: Parmigiano Regiano: e at Extension MC1R locus)
- Individual
  - MS and SNP, sequencing: higher costs, therefore focused on parent animals
- Sex
  - Mainly in meat production (cattle, pig), AMELX, AMELY together with parentage testing.
- Genetic modifications
  - GMO animals (AquAdvantage salmon)

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The colour coat genes can identify the breed

- Example of Cinta senese breed and mutation in the KIT locus (g.43597545C<T).

- This marker can be used for breed authentication, however it is not general marker for all belted pig breeds.

Fontanesi et al., 2017

Dovc et al., 2018

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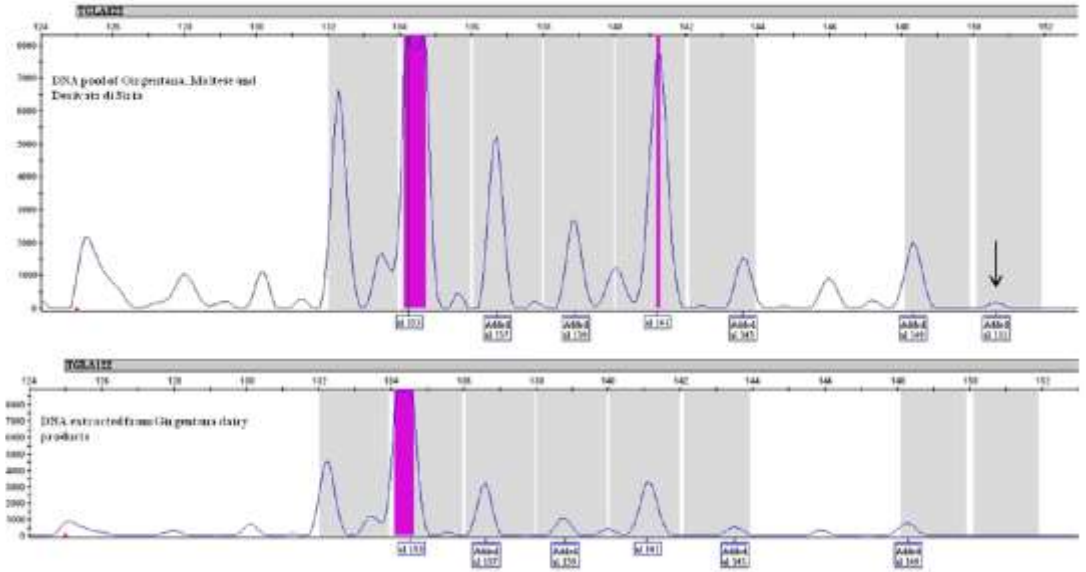
Girgentana goat: example

- Set of 20 STR loci
- Differentiation of Girgentana milk products
- Prevent contamination with milk from Maltese and Derivata si Siria breeds.
- For milk product analysis the use of three loci is promissing in detection of Girgentana products.



Sardina et al., 2015

Genetic traceability: a tool for the promotion of local alpine products

Genetic traceability allows **different identification levels**:

- individual
- breed
- species.

Individual traceability allows the identification of the animal from which a product has been obtained. DNA purified from food is analyzed using microsatellites or SNP molecular markers. Microsatellite analysis enables production of a unique genetic profile for each animal, the so called DNA fingerprinting. For this kind of studies in cattle the list of 30 microsatellites proposed by FAO and ISAG is sufficient.

Breed traceability aims to guarantee the origin of mono-breed food products. Some examples of these traditional Italian productions are the Spressa cheese from milk of Rendena cattle breed, the Parmigiano Reggiano obtained only by Reggiana cattle milk, the Pecorino cheese from Massese sheep and the Fiorentina steak from Chianina cattle. In this case, the ideal molecular marker should be “breed specific”. These markers are rather difficult to find.

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STR genetic profilling is efficient at individual level

STR markers have been used also to set up an **individual traceability system for Alpine Grey and Burlina meat**. The probability to find two individuals sharing the same genetic profile using set of only 11 microsatellites was 1 over 12 million.

A **field trial** was performed to verify the efficacy of the traceability system. Blood samples were collected from Burlina animals during their life in the farm before slaughter. Later, muscle samples were collected from the same animals in the slaughter-house. Both samples were submitted to DNA analysis with microsatellites markers. A **comparison between genetic profiles** of samples collected in farms and at the slaughter-house confirmed that the two samples came from the same individual.

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DNA metabarcoding for identification of animal meat

- The presented DNA metabarcoding method allows the identification and differentiation of 21 meat species, including 15 mammalian and 6 poultry species commonly consumed in Austria. The method should make it possible to detect and discriminate meat species of interest down to a proportion of 0.1%. To be applicable for verifying the authenticity of meat products in routine analysis, we aimed to develop a method that is as time- and cost-effective as possible. We therefore selected a targeted sequencing approach instead of wholegenome sequencing. In addition, due to the high coverage, targeted sequencing is particularly suitable to detect sequences of low abundance. For high throughput analysis, the method should allow sequencing of 96 samples in parallel.
- We selected the mitochondrial 16S rDNA gene because previous studies have demonstrated its suitability for species discrimination. To check if the 16S rDNA barcode region, recently found to be applicable

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DNA barcoding allows identification of species

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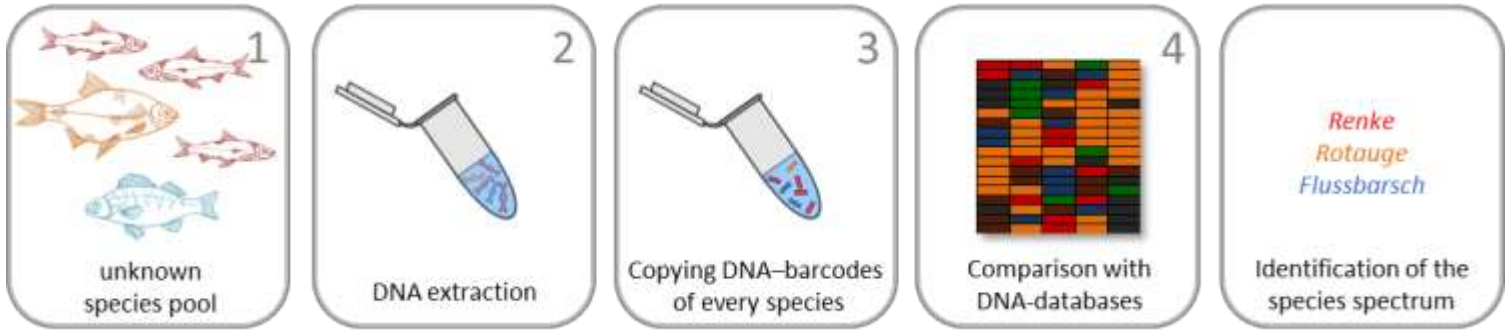
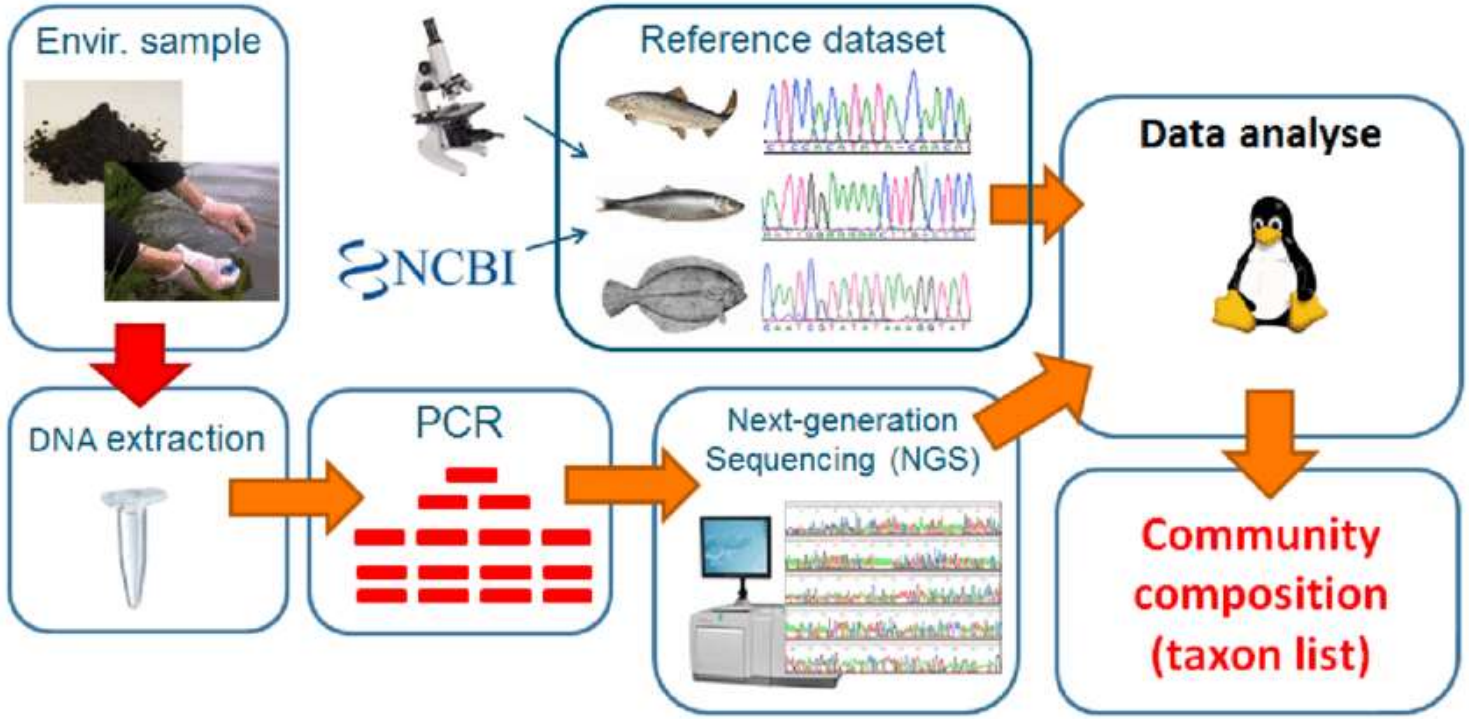


DNA barcoding has potential to detect common characteristics of the species (sometimes even of the breed.

In association with NGS strategies the power of he method can be tested on very complex materials (environmental samples)



Example of DNA metabarcoding:

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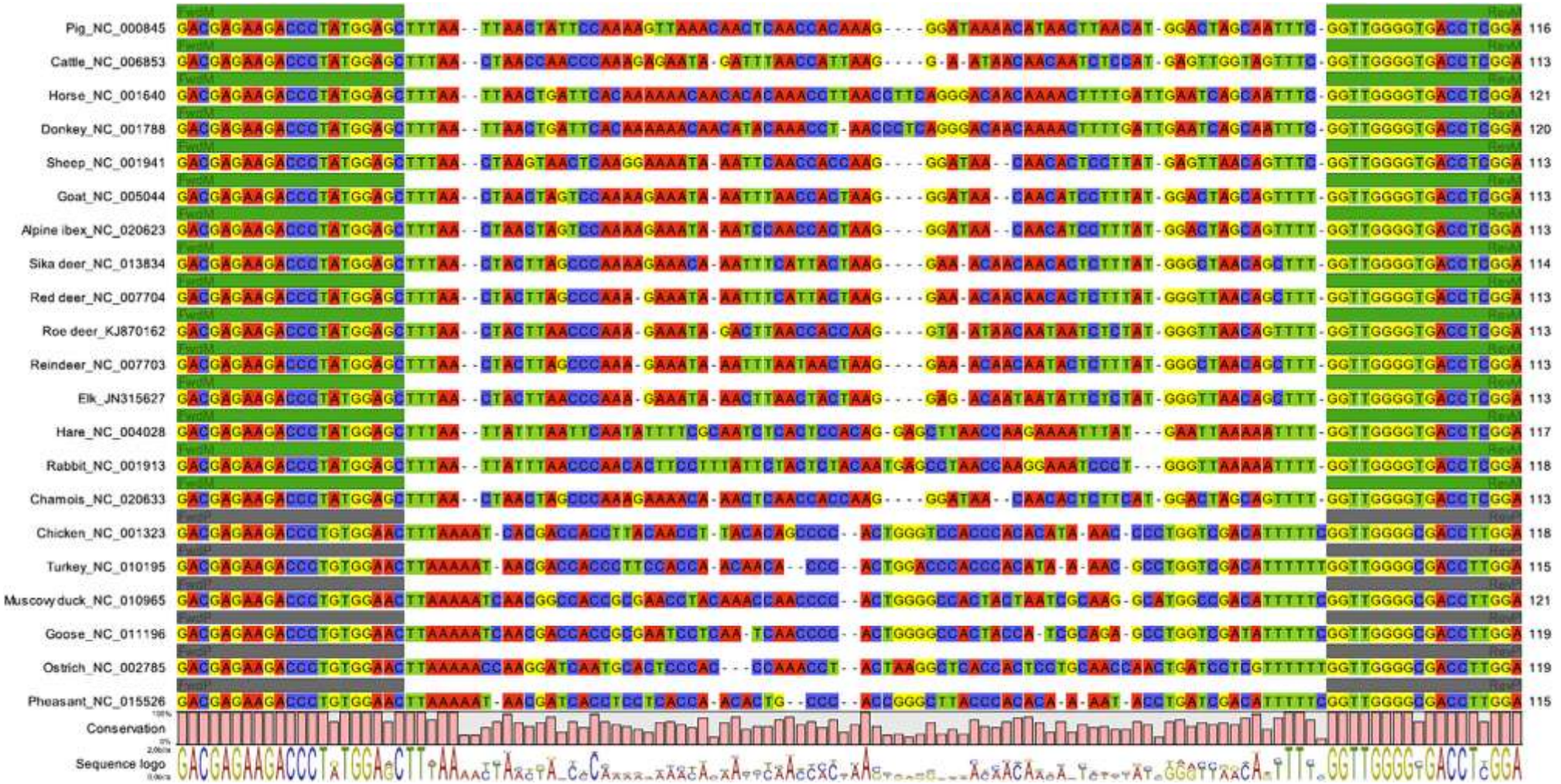

Dobrovolny et al., 2018

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Efficacy of the DNA meta barcoding approach

21 species were differentiated (pig, cattle, horse, donkey, sheep, goat, alpine ibex, sika deer, red deer, roe deer, reindeer, elk, hare, rabbit, chamois, chicken, turkey, Muscovy duck, goose, ostrich and pheasant), using DNA meta barcoding. DNA sequences of the mitochondrial 16S rDNA gene were used as target

Differences in only two bases were found for the following pairs of closely related species: goat and alpine ibex, horse and donkey as well as sika deer and red deer.

Differences between mammalian and poultry species were not only found in the variable part of the barcode region but also in the conserved regions, serving as binding sites for the primers.Therefore mammalian and poultry primer pairs were used.

Preservation of animal genetic resources

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- Identification and traceability of individual animals is especially important in small breeds where pedigree mistakes and lack of suitable mating combinations seriously compromise the afficacy of preservation plans.

Monitoring of small populations based on individual genotypic data is an important tool for preservation of small populations with less competitive position on the market.

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Development of new SNP arrays

- Specific breed SNP arrays combine important genotypic information for a certain breed, relevant health risks and enough information to be used for individual identificatopn. The lower price of these SNP arrays makes them suitable tool also for traceability purposes.

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Trade marks, brands…

- Introduction of breed or population specific trade marks requires traceability of animals and products.

- This is an important issue for smaller breeds with developed high value products competing in higher price categories.

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Thanks for your attention!