Hypothetical Case Study

Confined release of Cattle resistant to *Trypanosoma brucei rhodesiense* in Kenya.

Yanina L. Petracca & Ruth Mbabazi

The case study is based on an hypothetical GM animal that has not been developed yet.

Risk Assessment Methodology

PROBLEM FORMULATION

- RA CONTEXT AND SCOPE
- HAZARD IDENTIFICATION

It combines:

a) stablishing the **RA context** and scope (**protection goals and endpoints**).

b) identification of any **novel characteristic** associated with the GMO that may **have adverse effects** on environment.

- **EXPOSURE ASSESSMENT**
- **CONSEQUENCES ASSESSMENT**

Evaluation of the **likelihood of adverse effects** being realized and the **consequences** if this happens.

RISK CHARACTERIZATION

Make a qualitative and/or quantitative **estimation of the overall risk** posed by the GMO based on the **likelihood and consequences** of the identified adverse effects being realized.

- **MANAGEMENT ASSESSMENT**
- **MONITORING ASSESSMENT**

Assessment of the risk management or monitoring strategies that may be employed to reduce and/or keep under control the level of potential adverse effects posed by the GMO.

RISK ASSESSMENT REPORT

FOCUSING ON CONFINEMENT

Why focusing on confinement?

- **To Prevent the regulated GM animal from:**
  - Entering the **food supply** (e.g., posing potential risk to human/other animals health, having negative impact on domestic and/or international trade).
  - Posing **risk to environment** (e.g., invasive animal, disease healthy carriers).

FOCUSING ON CONFINEMENT

Important issues to address in order to determine confinement measures required & assure them.

- ✓ Relevant **characteristics of the non-GMA** e.g. biological characteristics, potential for inbreeding or interbreeding with other wild or native populations, potential invasiveness, distribution area.

- ✓ Description of the **(intended) genetic modification** & **detection method,** e.g. Relevant characteristics of the genes and of other functional sequences that have been inserted or modified (focusing on confinement!).

- ✓ Description of the **potential receiving environment,** e.g. physical & biological characteristics, biotic interactions.

FOCUSING ON CONFINEMENT

Important issues to address in order to determine confinement measures required & assure them.

- ✓ **Phenotypic changes** in the GMA (intended- unintended) **comparing with the non-modified recipient** (focusing of confinement!).

a. **Phenotypic traits** relevant for developing a **fitness advantage** (increasing the survival or sexual component of fitness), alterations in susceptibility to pest and diseases, increased potential for scaping and dispersing out of the releasing site, becoming persistent or invasive.

b. **Potential adverse effects** of the incidental exposure of wild, feral animals or humans to the GMA, such as toxic or allergenic effects.

FOCUSING ON CONFINEMENT

Important issues to address in order to determine confinement measures required & assure them.

✓ Relevant information on **biosafety conditions & risk mitigation**:

a. Information on the **facilities**. e.g. **location** & its surroundings, **infrastructure**, size, **confinement conditions** (scale & level), location of the release, **other activities done within facilities**, climatic conditions.

b. Measures to **prevent incidental release of the GMA** or introduction of GM derived products into the food supply. e.g. Procedures for GMA **Transport & handling,** treatment and **disposal of GM** waste, **contingency plan in case of emergency** (accidental escape).

c. Experimental design & **activities involved**, tentative schedule, routine procedures, **rearing practices**.

FOCUSING ON CONFINEMENT

In this stage, we shall mainly focus on confinement measures.

1) **Problem formulation:** Define **risk hypothesis** and **pathway to risk**.

2) Ask **relevant questions** to be answered by the applicant (**nice to know vs. need to know**).

3) Determine if the **risk is acceptable**.

4) Define follow-up & **monitoring measures**.

Hypothetical Case Study

PRODUCT DESCRIPTION: GM cattle carrying Hamadryas baboon gene APOL1.

TRAIT: Resistance to *T. b. rhodesiense*- cattle & human pathogen.

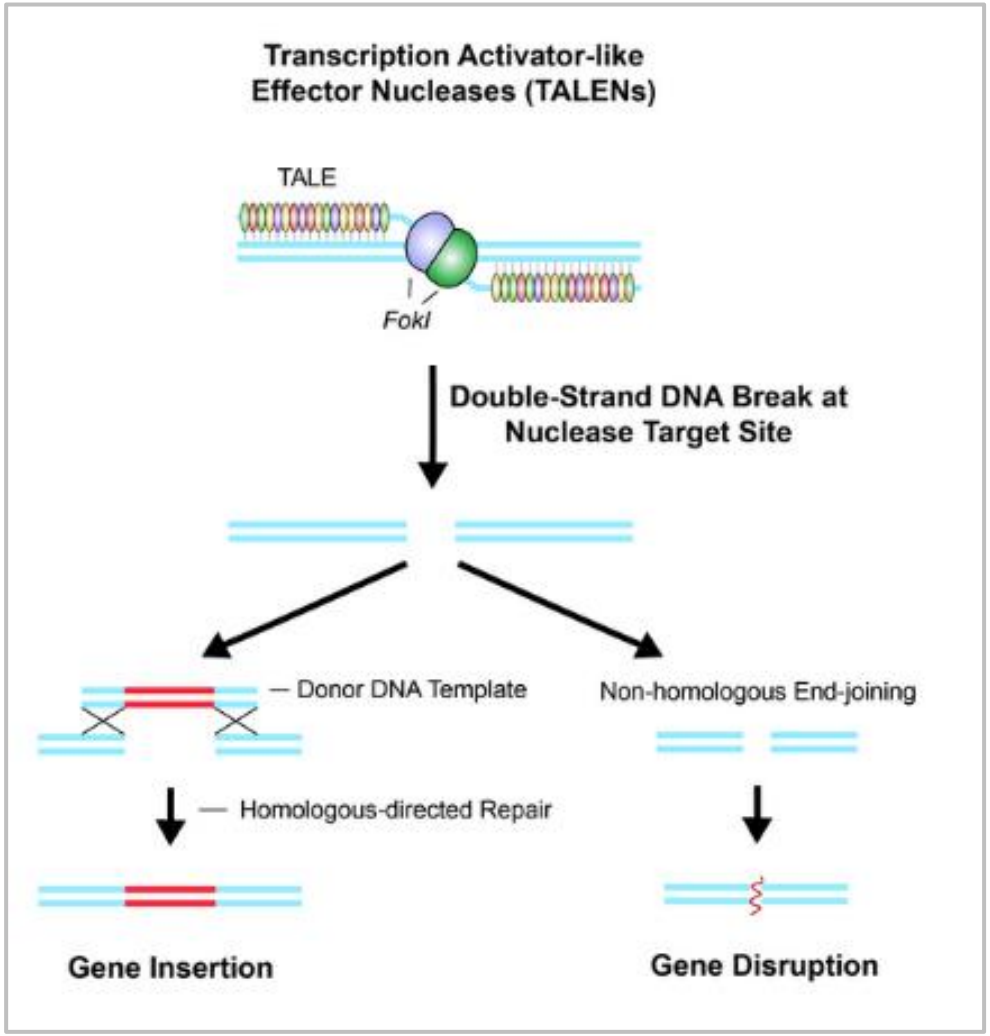
HOST ORGANISM: Boran Cattle (East Africa's most important cattle breed).

INTENDED USE (in the future): It is expected that GM cattle resistant to Trypanosomiasis will survive in African regions where trypanosome infection is endemic.

Hypothetical Case Study

METHODS OF INTRODUCTION OF TRAIT:

TALEN technology- Genome editing of BEFs by use of site-specific nucleases + Somatic cell nuclear transfer (Cloning).



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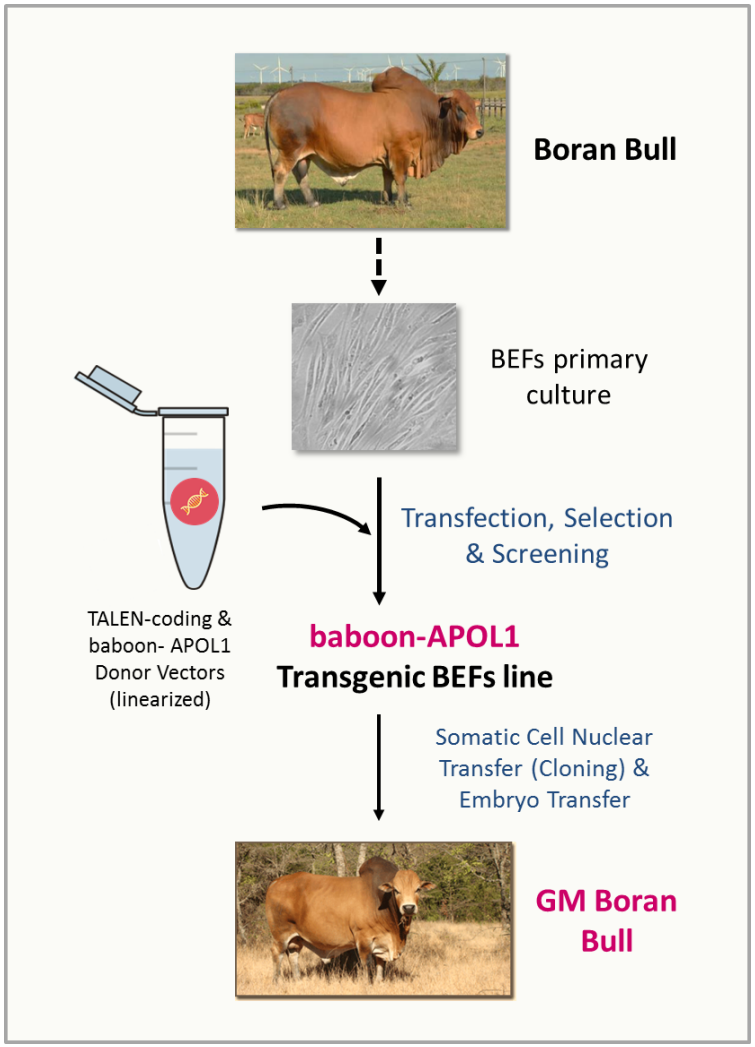
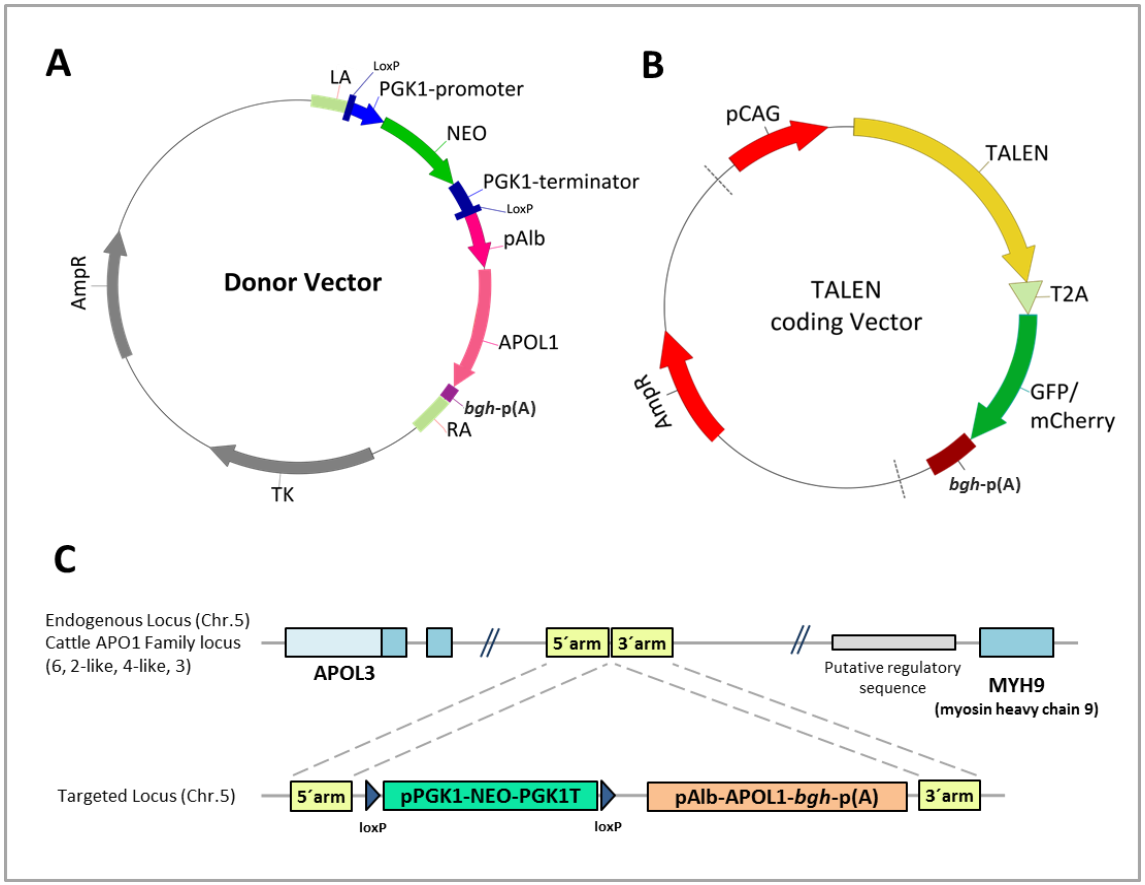


Hypothetical Case Study

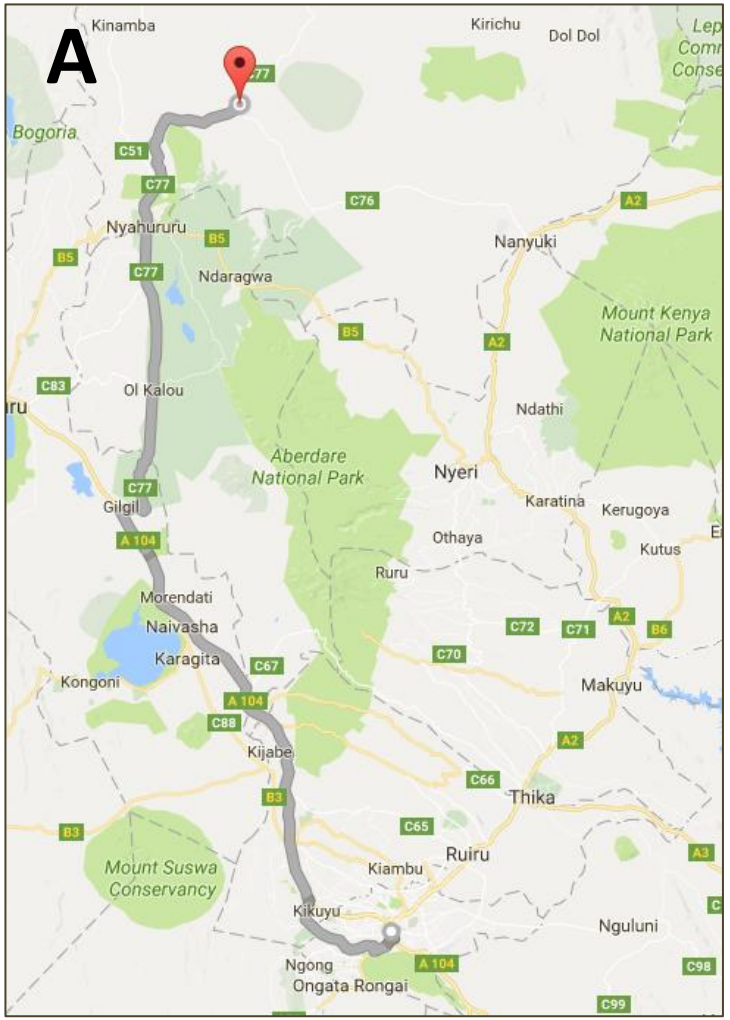
GENETIC MODIFICATION:

A pair of TALE nucleases and a donor vector with homologous arms were designed to insert the coding sequence of the Hamadryas baboon APOL1 gene into the cattle Apol family locus, in a 10-kb non-coding region between APOL3 and MHY9 endogenous genes.



Hypothetical Case Study

Advanced and fully equipped facilities will be required for conducting GM animals´ characterization studies. For that porpoise, GM young bulls will be transported from Nairobi to the National Institute of Livestock Research Beef Cattle facilities in Rift Valley region (south of the country).



Hypothetical Case Study

RELEASING SITE

- ✓ Animal facilities are located, at Rumuruti town, about 230 km (143 miles) north of Nairobi.
- ✓ Most of residents practice subsistence farming on rural farms. Farmers weekly sell their cattle at the busy livestock market in downtown Rumuruti.
- ✓ Cattle Beef facilities are 915 mts. (3,000 ft.) above sea level, in an area where floods, storms, earthquakes and other natural disasters are absent.

Hypothetical Case Study

RELEASING SITE

- ✓ Their perimeter is surrounded by 2-mt (6,6-foot) high chain link fence with barbed wire on top in order to prevent the unauthorized access to the site by people and wild or farm animals.

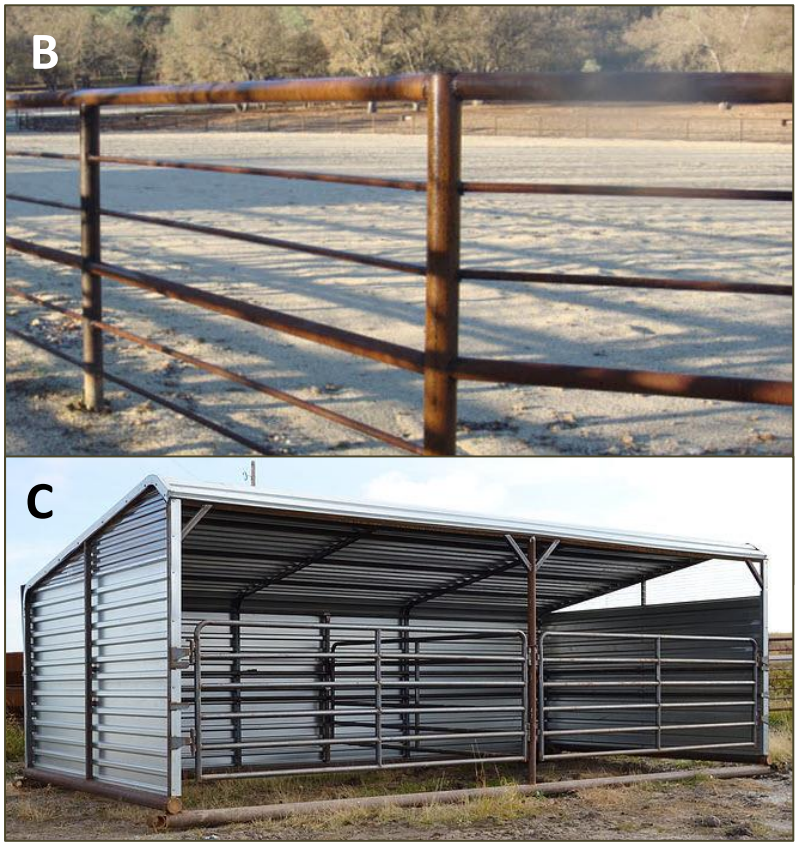
- ✓ There is also at least two person permanently stationed at the facilities to control the normal movement of authorized personnel.

Hypothetical Case Study

RELEASING SITE

- ✓ The GM animals will be kept in a 150 mt2 (1600 square feet) corral located 400 mts. (1300 feet) away from the facilities´ perimeter.

- ✓ It features an outdoor ring made of 1,5-mt (5-foot) pipe fencing that serves to house and handle the animals during the day and an electric fence to prevent GM bulls from escaping and eventually mate with cows or interact with other farm animals that are kept in nearby.



Hypothetical Case Study

RELEASING SITE

- ✓ Fences have two gates and only authorized personnel has access to the keys of the safety locks. In addition, a passive alarm system with motion- activated sensors will be activated at night for surveilling the corral´s exteriors.

- ✓ Within the corral there is a 3,7 x 7.3 mts. (12 x 24 feet) Cattle Shelter divided in two pens where the bulls can rest in the shade by day or be confined during the night.

- ✓ Despite the fact that initially the GM young bulls will be kept together, they would be separated in the future if they exhibit an aggressive behavior.

Hypothetical Case Study

RELEASING SITE

- ✓ Next to the GM bull´s corral, there are Biosafety level 2 (BSL-2) laboratories, recommended for research activities with infective stages of Trypanosomes.

- ✓ There is also a Large Animal BSL-2 facility located in a separate building provided with the special biocontainment features suitable for work involving laboratory animals infected with agents associated with human disease and that pose moderate hazards to personnel.

Hypothetical Case Study

HEALTH & WEALFARE

- ✓ The general rearing conditions of the GM bulls will be consistent with tropical livestock systems conditions.
- ✓ The health of young growing bulls will be assessed daily by animal care personnel.
- ✓ Animals will be tested for the most prevalent diseases (African trypanosomiasis, foot-and-mouth disease, lumpy skin disease and contagious bovine pleuropneumonia) periodically.
- ✓ A sanitation plan will be implemented to control infestation of animal facilities by vermin (e.g., flies, mosquitoes, lice, mites, ticks, etc.).

Hypothetical Case Study

HEALTH & WEALFARE

- ✓ GM animals will be identified with distinctive ear tags and a tattoo for quick visual and permanent identification respectively.
- ✓ If an animal is euthanized or dies the animal care personnel will give notice to the competent authority and its entire carcass will be disposed by incineration within the facility.
- ✓ Dead transgenic animals will be kept in a locked freezer, until promptly transportation for incineration has been arranged.
- ✓ The development of GM bulls has been comparable to the one of wild type Boran calves and no significant differences were detected in the biology of the animal.

Hypothetical Case Study

POTENCIAL RECEIVING ENVIRONMENT: Beef Cattle facilities and its surroundings, in Rumuruti (Rift Valley region, in the south of Kenya). In the future, regions of Kenya where cattle would be raised.

BIOTIC INTERACTIONS (in Kenya):

- Livestock-wildlife interactions, e.g., Cows are sometimes preyed on by large, wild carnivores (but human is its biggest consumer).
- African buffalo is also in Kenya – They do not successfully interbreed.
- No wild or feral cattle that can breed with domesticated cattle.
- Pest and diseases – Regions are infested by tsetse flies. Environment encourages the spread of diseases e.g. nagana, foot & mouth, etc.
- Large herds of poor animals are ruining pastureland and spreading diseases.

Hypothetical Case Study

PROPOSED ACTIVITIES

- ✓ Transport GM young bulls via ground routes from Nairobi to Rumuruti.
- ✓ Individual records will be regularly kept (e.g. birth date, body weight, semen collection dates, protocols it is assigned, dates of vaccination or medication, other veterinary data, its ultimate disposition, etc.).
- ✓ Monitor their health conditions and development with growth in order to identify any condition associated with the introduced transgene.
- ✓ Analyze blood baboon apoL-I levels.
- ✓ Assess the Trypanosomes killing capacity of their serum in vitro.

Hypothetical Case Study

PROPOSED ACTIVITIES

- ✓ Further analyze the mechanism for how baboon apoL-I protects against African trypanosomiasis.
- ✓ Carry out transmission experiments to determine if GM animals are truly resistant to infection or asymptomatic carriers that could still played host to the trypanosome threatening humans and other animals (disease reservoirs).
- ✓ Confirm the safety of meat and milk consumption of GM cattle.

Let’s work!!!

FOCUSING ON CONFINEMENT

In this stage, we shall mainly focus on confinement measures.

1) **Problem formulation**: Define **risk hypothesis** and **pathway to risk**

(Are facilities & confinement measures appropriate for conducting the trial? If not, why? What would be potential risks and how can they would be actualized?)

2) Ask **relevant questions** to be answered by the applicant (nice to know vs. need to know).

3) Determine if the **risk is acceptable**.

4) Define **mitigation & monitoring** measures.

FOCUSING ON APPROVAL

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Environmental Risk assessment- Problem Formulation.

1) **Problem formulation:** Define risk hypothesis and pathway to risk.

2) Ask **relevant questions** to be answered by the applicant (nice to know vs. need to know).

3) Determine **if the risk is acceptable**.

4) Define **monitoring & mitigation** measures.

FOCUSING ON APPROVAL

Genetic Modification Characterization

a. **Vector characterization-** Relevant characteristics of the genes and of other functional sequences that have been inserted or modified (e.g., description of the elements, donor organism- pathogenic characteristics).

b. **Method/ Strategy for producing the GMA** (which genetic engineering techniques were applied?).

FOCUSING ON APPROVAL

Genetic Modification Characterization

a. Molecular Analysis

- number of insertions,
- integrity of the insert (s), e.g. rearrangements, deletion/insertion, changes in base pairs,
- presence/absence of backbone sequences/ selection markers,
- genotypic stability,
- characterization of the insertion site, e.g. disruption of endogenous gene or regulatory sequence, deletions/insertions,
- pattern of inheritance.
- Relevant characteristics of the genes and of other functional sequences that have been inserted or modified

FOCUSING ON APPROVAL

Genetic Modification Characterization

b. **New Product(s) expressed** (intended or unintended) / Compositional Analysis (Food & Feed safety).

c. GM **detection method**.

FOCUSING ON APPROVAL

GM Animal Characterization

a. **Recipient species**

- **Phenotypic** characteristics, e.g. r**eproductive** characteristics,
- **Interaction with other organisms** in the potential recipient environment, e.g. potential for inbreeding or interbreeding with other wild or native populations, potential invasiveness, distribution area.

b. **New Product(s) expressed** (intended)

- **Mechanism of action**,
- Levels of **expression**,
- **Interaction** with other expressed or endogenous **products**.

FOCUSING ON APPROVAL

GM Animal Characterization

c. **Phenotype characterization** (phenotypic characteristic different from the non-recipient organism), e.g. Phenotypic traits relevant for **developing a fitness advantage** (increasing the survival or sexual component of fitness).

FOCUSING ON APPROVAL

Interaction of the GMA with the environment

a. Interaction with **other species**
    a. Successfully competing for resources? Invasiveness?
    b. Predatory or Parasitic?
    c. Toxic or Allergen Protein producer?
    d. Cross-breeding potential?
    e. Sanitary issues (susceptibility to disease, healthy carriers)?

b. **Management of potential undesired effects** (Mitigation & monitoring measures).