

**A short overview on the current state of animal biotechnology
as well as
regulatory considerations for genome edited animals**

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Animals

- Buffalo
- Goat
- Sheep
- Poultry



Genes

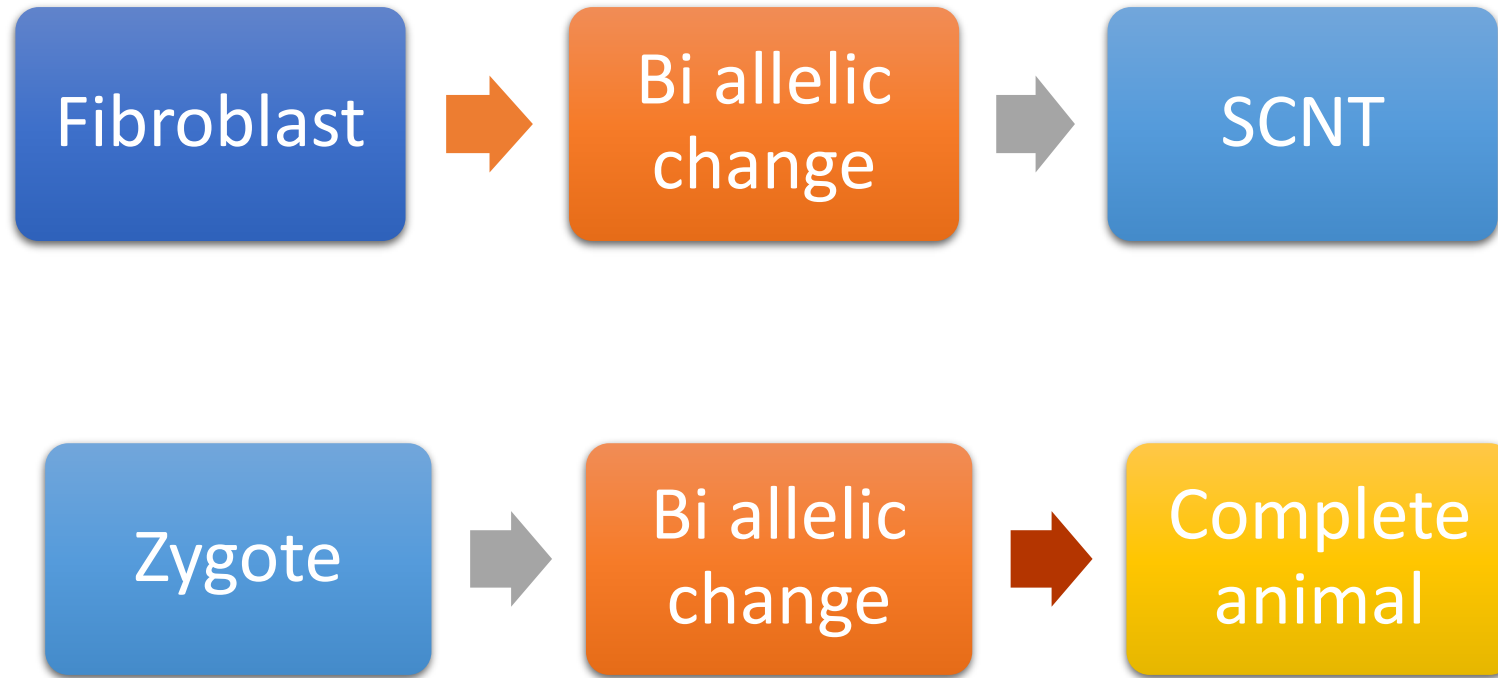
- Gene Path way
- QTL
- BMPR1B
- myostatin



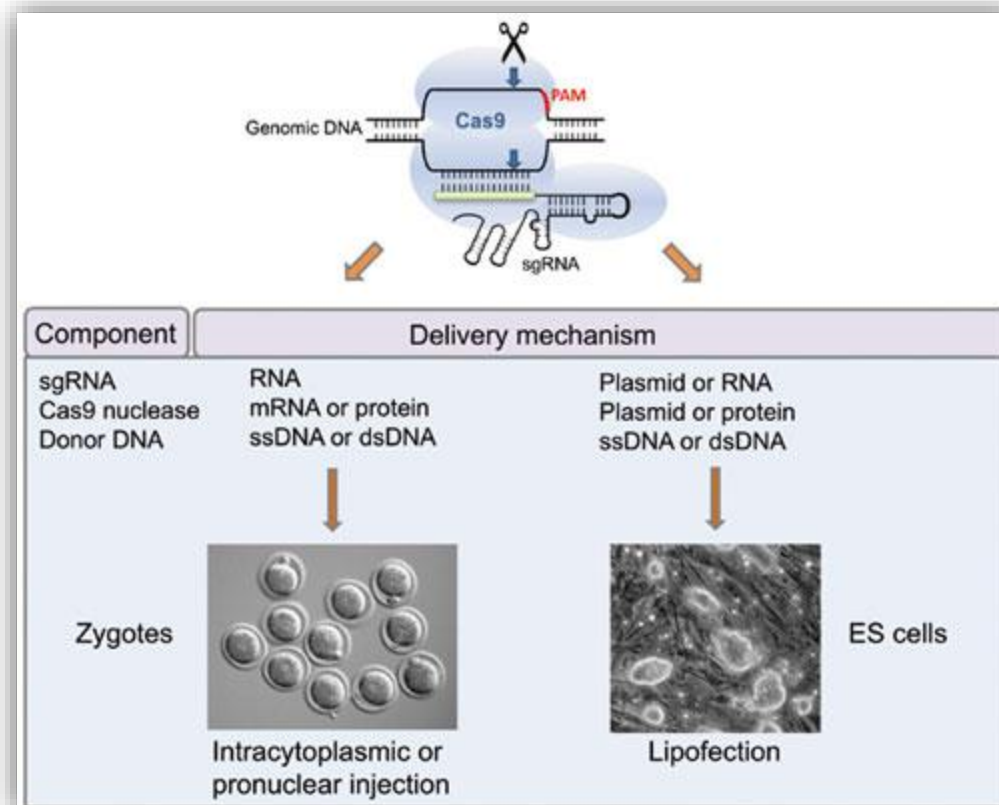
Stages

- Cell culture
- Animal cloning

How to make Genetically Modified Animal/ Transgenic animal



How it works in Animal Cell



Issues in animal cell:

1. Diploid cells (Mono / bi allelic)
2. Screening process
3. Cell line / complete animal
4. Somatic cells / Stem cells
5. Zygote / single cell

Use of the CRISPR/Cas9 system for genome editing in the mouse: to target any given sequence of genomic DNA in the germ line of the mouse using zygotes or mouse embryonic stem cells (ES cells).

Some Examples

- **The thymidine kinase (TK) knocked-out Vero Cell line developed through CRISPR-Cas9 technique**
- **CRISPR-Cas9 edited BHK -21 host cell line for enhanced multiplication of Foot and mouth disease virus**
- **The role of immune response gene(s) against Peste des Petits ruminants virus using CRISPR/Cas9 a**
- **Functional role of EGR1 in Prostaglandin F2 alpha induced luteal regression applying CRISPR in corpus luteum**
- **CRISPR/Cas9 assisted gene targeting efficiently inhibits bovine herpesvirus-1 replication**
- **Double muscle knock out livestock using CRISPR cas**
- **Gene Knock out Chicken by Genome Editing with CRISPR/Cas for augmentation of productivity in poultry**

Important Reproductive Technologies in Farm animal

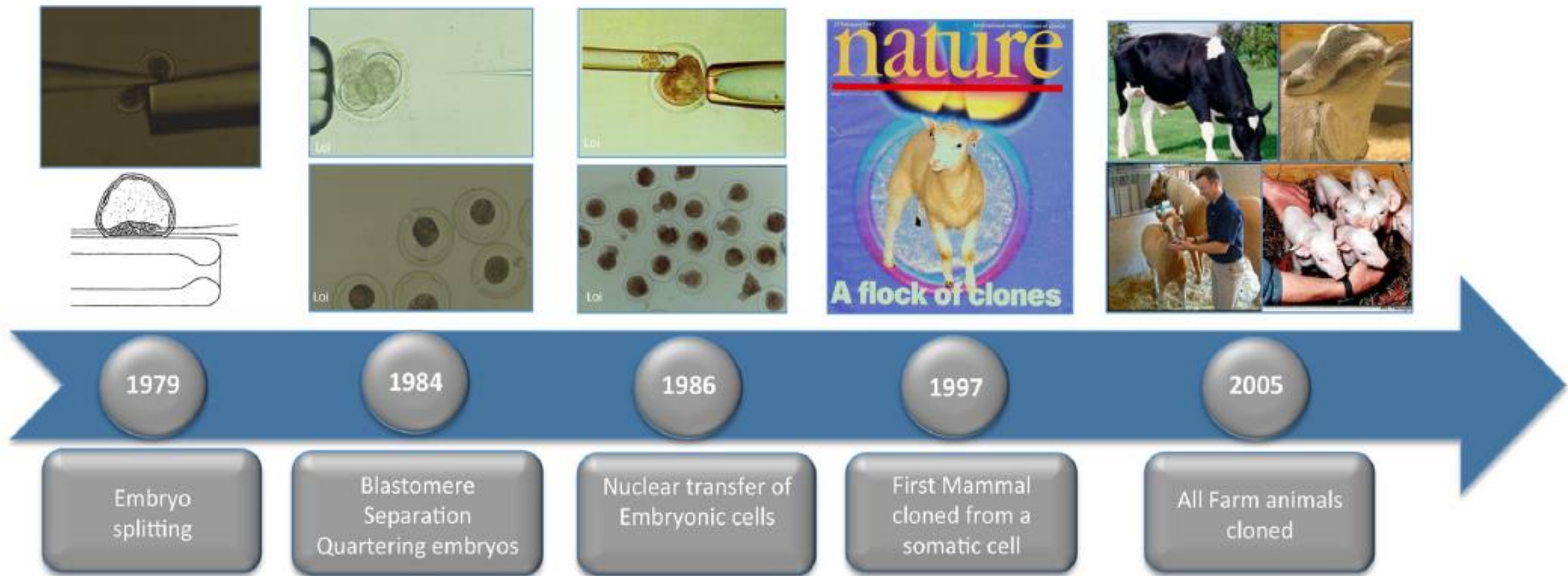


Fig. 1 Timeline of the development of reproductive biotechnologies in farm animals

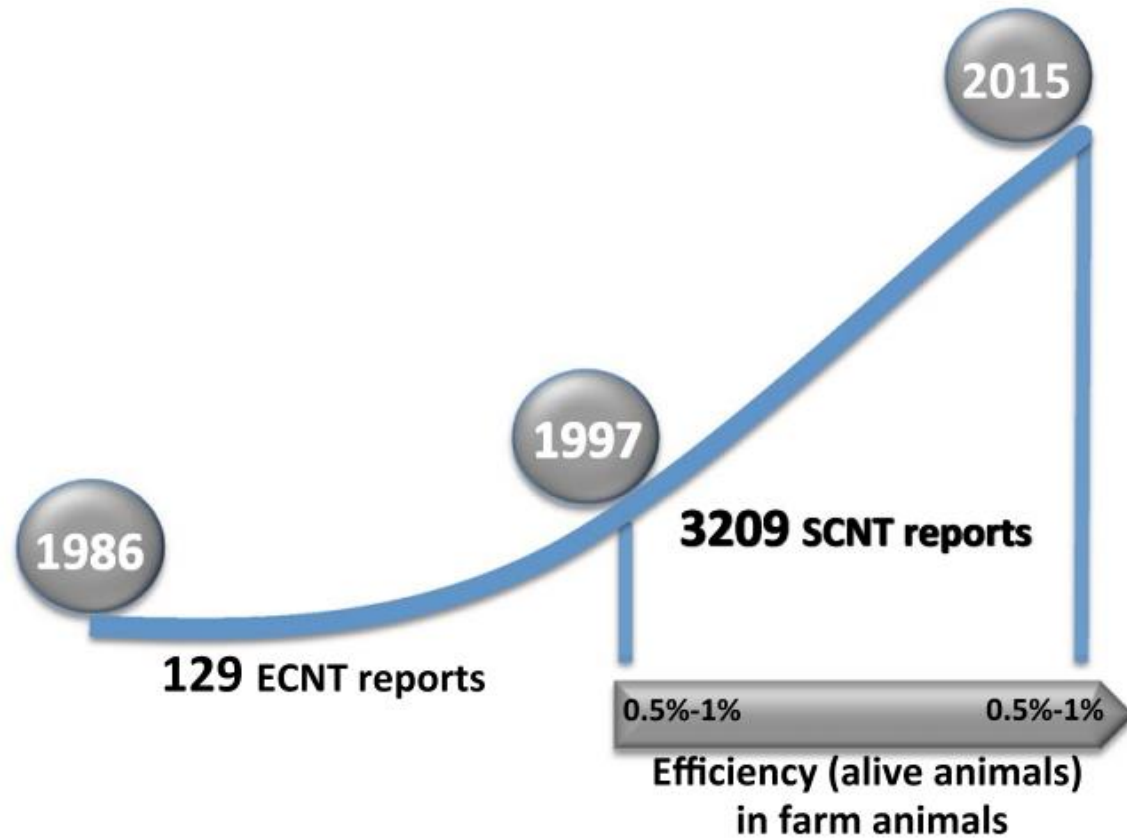


Fig. 3 Trend in publications on cloning using embryonic cells nuclear transfer (ECNT) and later somatic cell nuclear transfer (SCNT). Overall efficiency since the production of the first cloned sheep Dolly

Water Buffalo (*Bubalus bubalis*)

Chro no= 50

**India, Nepal, Bhutan,
Cambodia and
Thailand**

Meat animal



Milk

Meat

Cloning in Buffalo (*Bubalus bubalis*)



< 5% of calves, the rate of live offspring obtained from cloned blastocysts in buffalo






Figure 4. Donor bull and its cloned. Cloned bull has no physical abnormalities on the body, including face. White tail-switch color mark is identical to its donor bull (indicated by arrow).

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Successful cloning of a superior buffalo bull

Naresh L. Selokar ¹, Pappi Sharma¹, Monika Saini^{1,4}, Suman Sheoran¹, Rasika Rajendran¹, Dharmendra Kumar ¹, Rakesh Kumar Sharma¹, Rajender K. Motiani^{3,5}, Pradeep Kumar ¹, A Jerome¹, Sudhir Khanna² & Prem Singh Yadav¹

QTL: Sheep and Goat

Bone morphogenetic protein receptor type 1B (BMPR1B; also known as FecB) was the first major gene for prolificacy identified in sheep, and it plays a vital role in the control of follicular growth and development (Davis et al. 2006; Reader et al. 2012).

The FecBB mutation (A746G, p.Q249R) in BMPR1B has been reported to be highly associated with increased ovulation rates and litter size in sheep (McNatty et al. 2001), including several Chinese indigenous breeds (Chu et al. 2007; Tian et al. 2009).

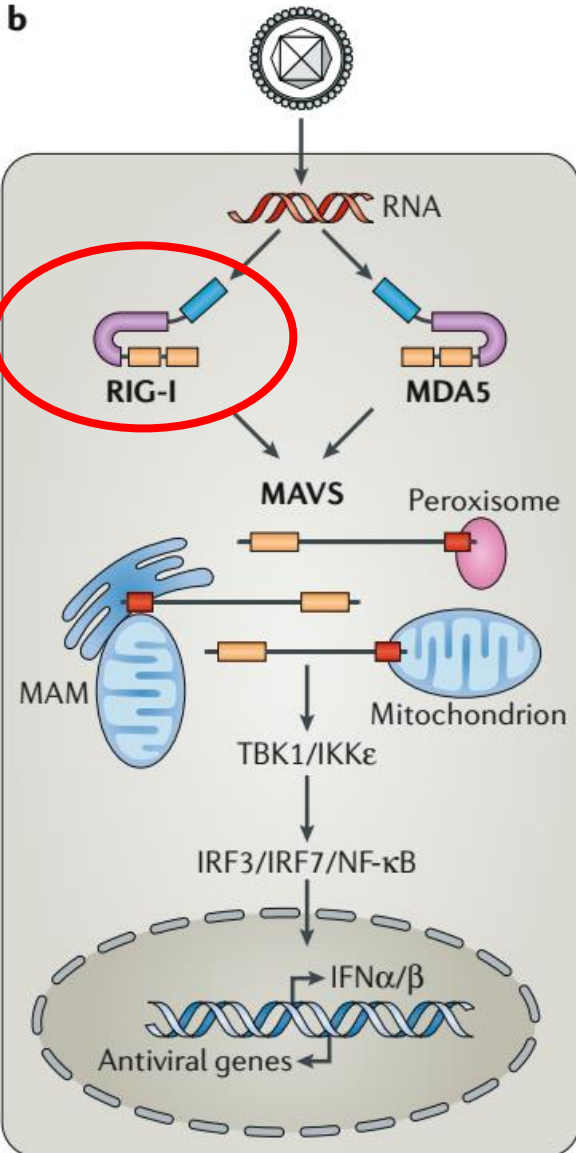


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Modulation of granulosa cell function via CRISPR-Cas fuelled editing of BMPR-IB gene in goats (*Capra hircus*)

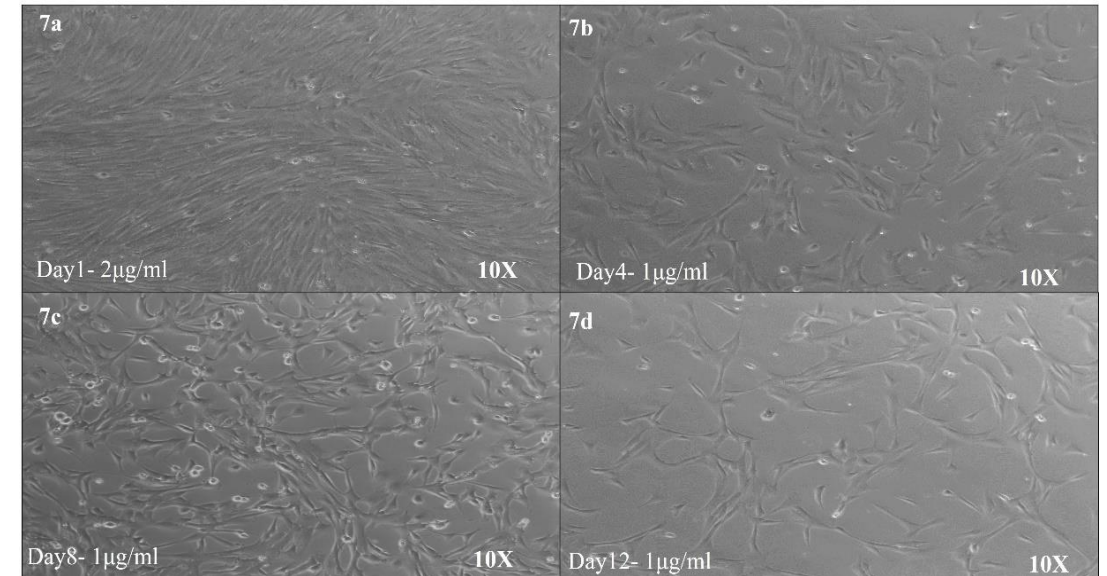
Sai Kumar¹, Meeti Punetha¹, Bosco Jose¹, Jaya Bharati¹, Shivani Khanna¹, Arvind Sonwane², Jonathan A. Green³, Kristin Whitworth³ & Mihir Sarkar¹✉

Disease Resistance : Antiviral Immunity : Goat / Buffalo primary cell culture



- Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) are key sensors of virus infection
- RLR activation; this can lead to an effective antiviral response
- RLR biology could be translated into new therapeutics

**Induce Antiviral Immunity
by using small molecule**



Myostatin (Mstn)

Myostatin is a cytokine produced by muscle which auto regulates the **total muscle mass** by inhibiting mesenchymal stem cell proliferation and differentiation.



Belgian Blue



Piedmontese

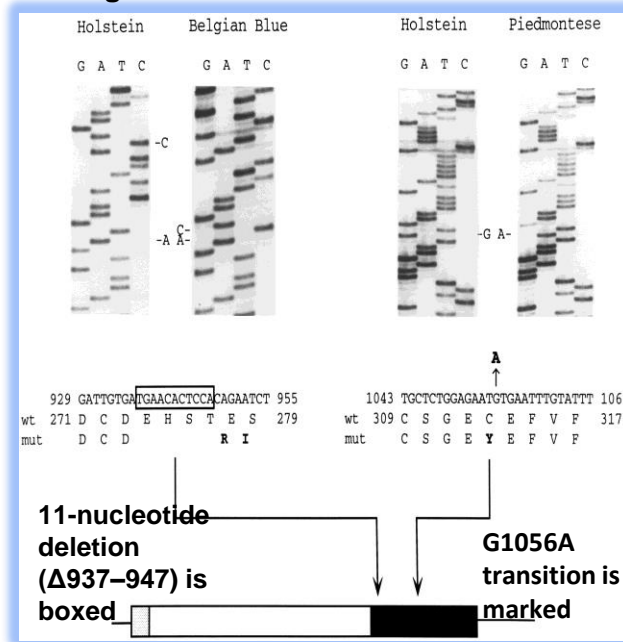
Chromosome 5

Occasionally the myostatin gene is defective in **cattle, dogs, sheep and humans**

Some breeds of beef cattle (e.g. Belgian blues) and racing dogs (whippets) have been **selectively bred** for defective myostatin regulation.

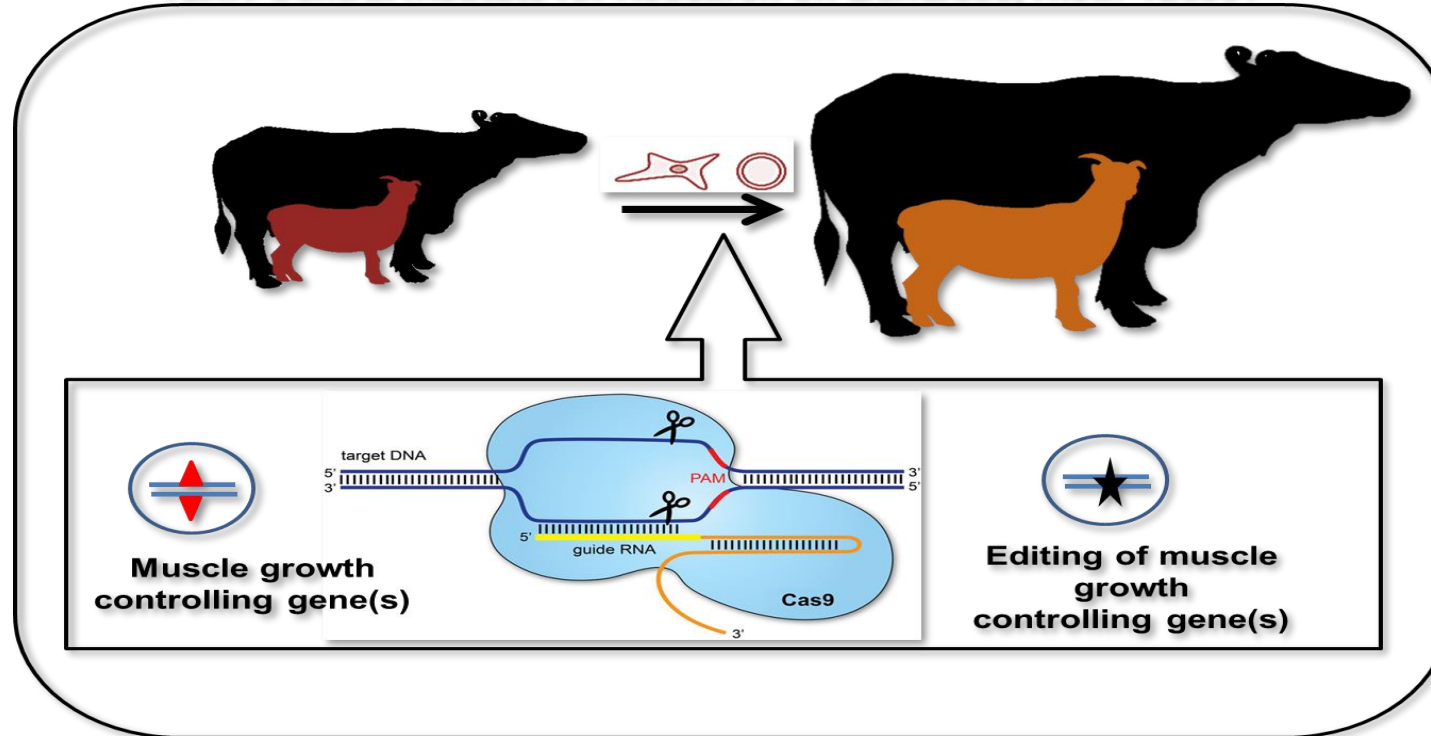
Myostatin-null animals have increased bone density and **double the normal muscle mass**, heterozygote's may show enhanced athletic performance.

SDN 1



Production of double-musced mass farm animals using CRISPR

Lead Centre:
ICAR-Central Institute for Research on Buffaloes, Hisar-125001



SDN 1

Partners:

1. ICAR- Central Institute for Research on Goats, Makhdum, Uttar Pradesh-281122
2. ICAR-National Dairy Research Institute, Karnal, Haryana-132001
3. Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir



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Thanks for your attention