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Food safety, GMOs and animal cloning

Modul no. 2: Conservation and Sustainable Use of Animal Genetic Resources

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- Food safety
  - - chemical
    - mutagenity
  - microbiological
    - DNA test of alimentary pathogens
- GMO
  - ověřování zdravotní nezávadnosti
  - Alergen
  - detection and labelling of GM foods
- cloned animals and safety

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GMO diagnostics in food

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a) qualitative: evidence of the presence of GMOs
- transgenic DNA: PCR
- transgenic protein: immunochemical - ELISA

b) Quantitative (determination of quantity):

real-time PCR: accurate determination of the amount of transgenic DNA in a food sample (due to labelling > 0.9%), used by comparing the sample with a series of standards of known GM fraction content

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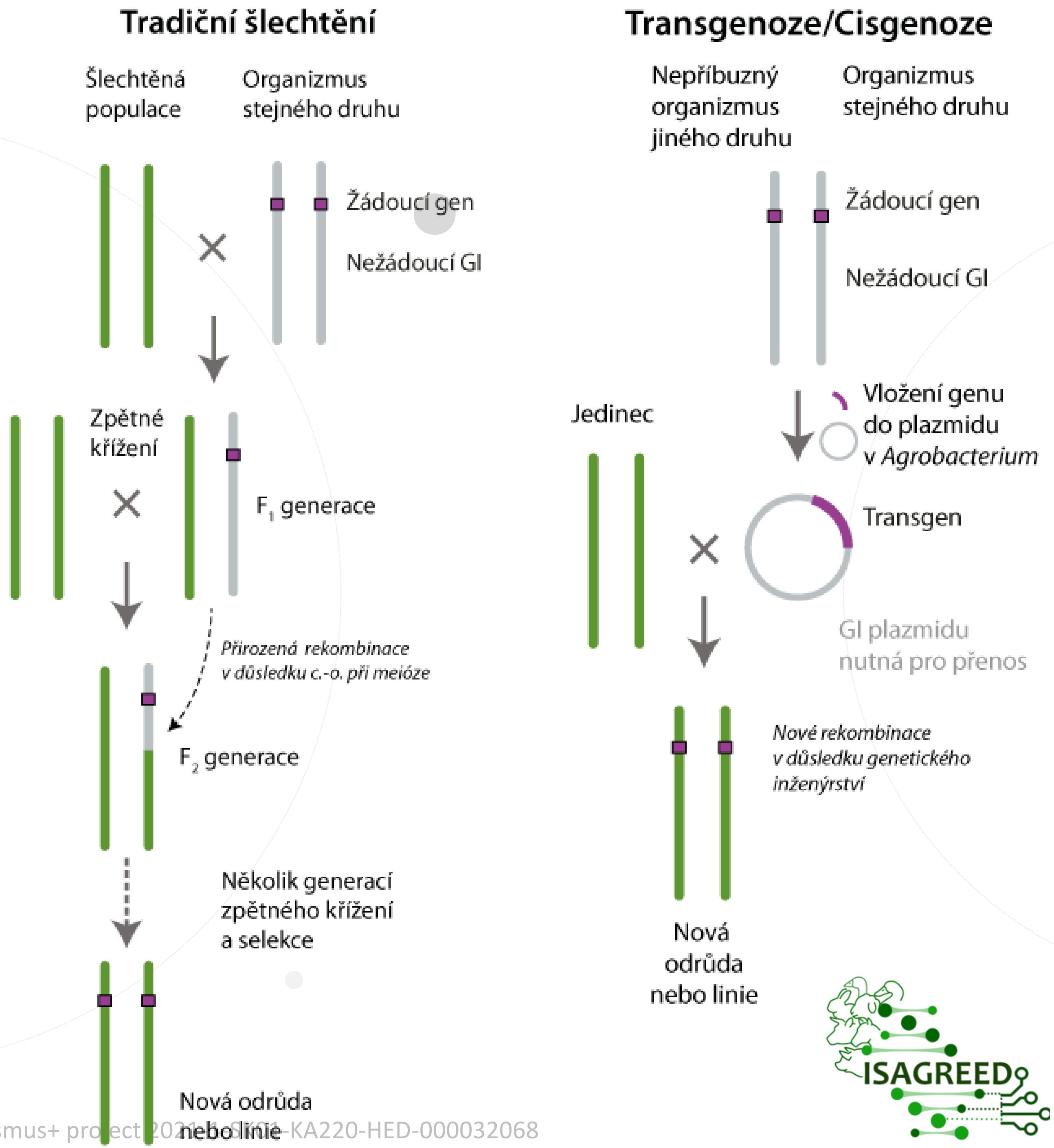
• Breeding and transgenesis have one goal…



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Genetic modifications - targeted interventions in the GI

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- accidental effects of mutagens or ionising radiation (creation of wheat varieties, rape varieties, etc.) **are not considered** genetic modification

Genetic modifications:

- Change in gene activity
- Change in "site of action"
- Replacement of a gene with another variant
- Gene knockout
- Introduction of foreign genes - transgenesis
  - plants into which a gene for herbicide resistance or a gene for insecticide production has been introduced with the help of *Agrobacterium tumefaciens* - e.g. Bt-maize.

- regulation resulting from the Act on GMO management (Act No. 78/2004 Coll.)

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Genetic modifications

- **Synonym for recombinant DNA techniques** (the impact of EU legislation)
- direct and targeted interference with the organism's hereditary material (DNA)
  - **transgenesis** -> recombination of DNA between species
    - **Introduction of individual genes into the genome by genetic engineering methods**
- **A genetically modified organism (GM organism, GMO)** is an organism (excluding humans) whose genetic material has been deliberately altered in a way that cannot be achieved by natural recombination.
- Genetically modified organism (GMO)
  - microorganism (GMM)
  - plants (GMR)
  - animals

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GM problems

- Low efficiency of insertion of advertisements.
- Inclusion of advertisements and their copies is still random.
- The product may form at low or high concentrations because we do not yet know and cannot control the regulation of structural gene expression
- Incorporation of foreign DNA is often unstable and may disappear in a sequence of generations
- Gene manipulation is still costly and the goal is achieved with great uncertainty
- ...

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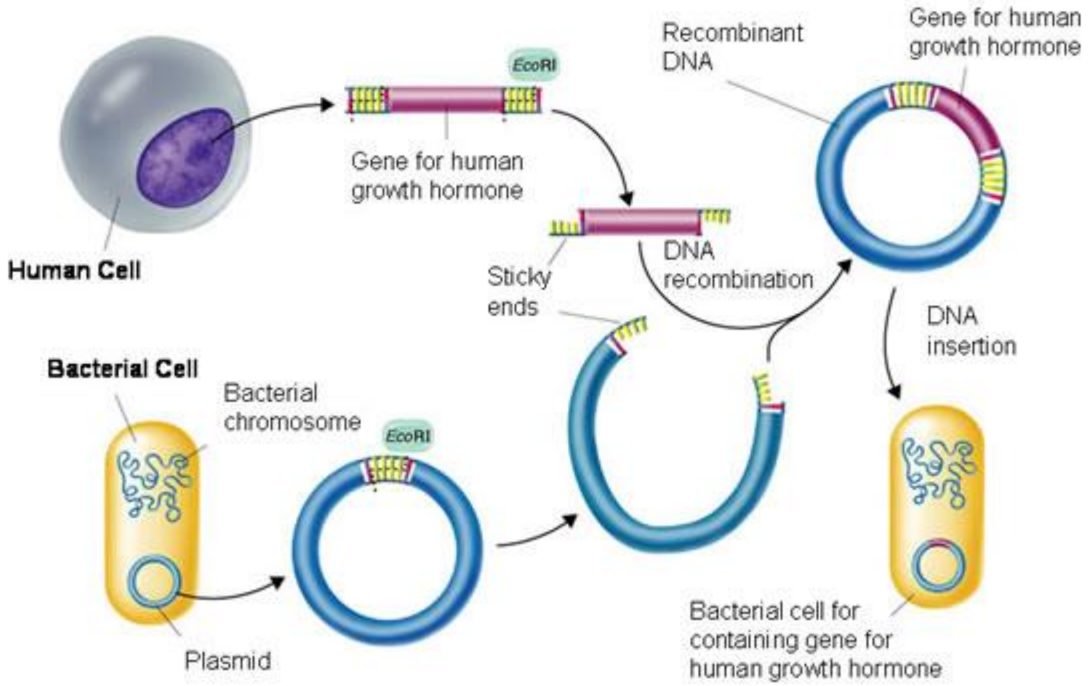
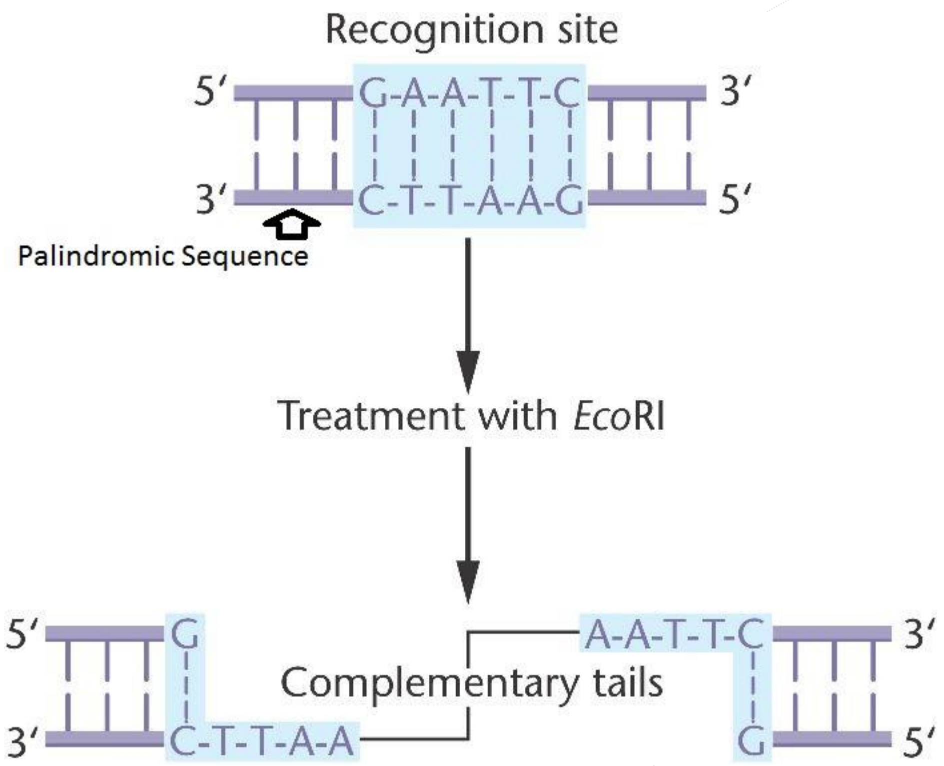
Recombinant DNA Technology Process (Genetic Engineering)





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The importance of transgenic livestock

(a) increase production and quality

(b) the production of new and better food e.g. the lactase gene in cattle will reduce the lactose content of milk; replacing allergenic proteins in milk with human proteins

c) production of high quality recombinant proteins (pharmaceuticals, etc.) or new materials in industry ('living bioreactors')

Note: bacteria cannot form some biologically active proteins (eukaryotic modifications are missing)

(d) resistance to disease and adverse effects

e.g. viral proteins produced by animals occupy cell receptors and viruses cannot penetrate

e.g. transfer of a gene for a freeze protection protein into the salmon genome

e) creation of animal models for human disease research, xenotransplantation

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Transfection methods - biological methods

- **lipofection** (via lipid micelles - encapsulate NA into liposomes -> into the cell nucleus)
- **transfection** with plasmid vectors
- transduction (viruses) of
  - **adenovirus** (dsDNA)
  - **retrovirus** (8 – 10 kb insertion, only proliferate cells)
  - **lentivirus** (infikují a integrují svůj genom do nedělících se buněk – neurony, makrofágy, svalové buňky, jaterní buňky)

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Physical methods

- **Microinjection (the insertion of DNA into a fertilized egg, often a primordial egg or embryonic stem cells)**
  - Simple, easy technique, foreign genes are expressed efficiently
  - Cannot be used later in development, low success rate, random incorporation
- **use of embryonic stem (ES) cells** mediated gene transfer (pluripotent blastocyst cells with in vitro inserted DNA -> into foreign embryo -> into uterus of surrogate mother (Capecchi, 1994) -> chimera born.
- Gene gun (biolistic transfection - DNA coated particles are "injected" into cells)
- Electroporation (el. pulses -> pores in the cell membrane
- Heat shock
- Magnet Assisted Transfection (MATra) – DNA is attached to magnetic nanoparticles and enters the cell in a strong magnetic field
- **All methods have low efficiency (max. 5%)**

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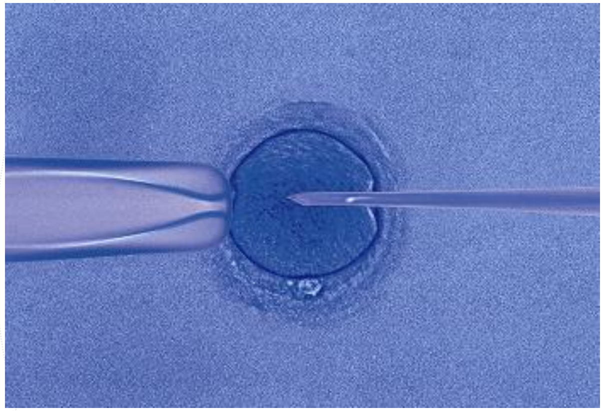
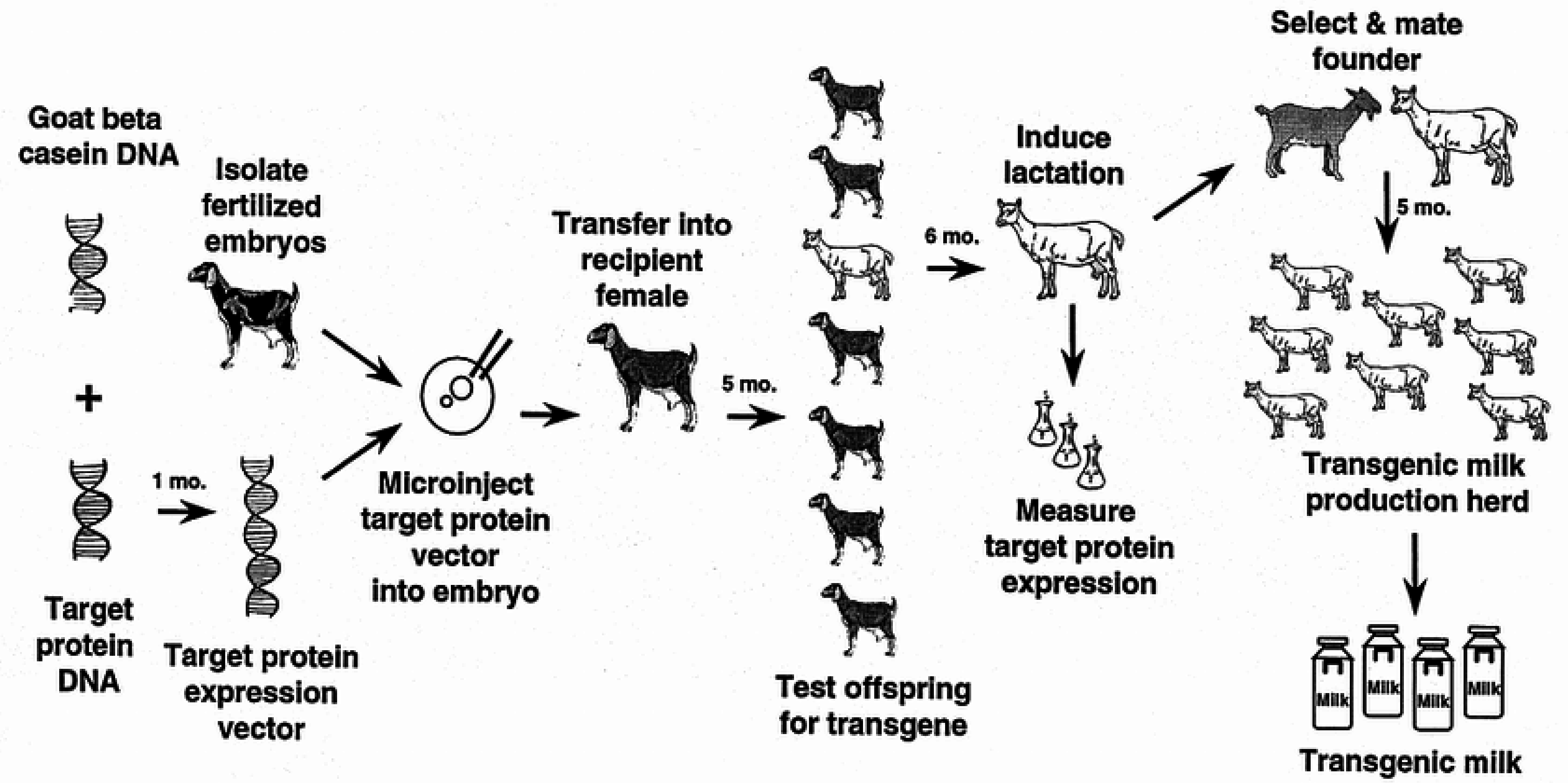
Example: microinjection techniques -> transgenic milk





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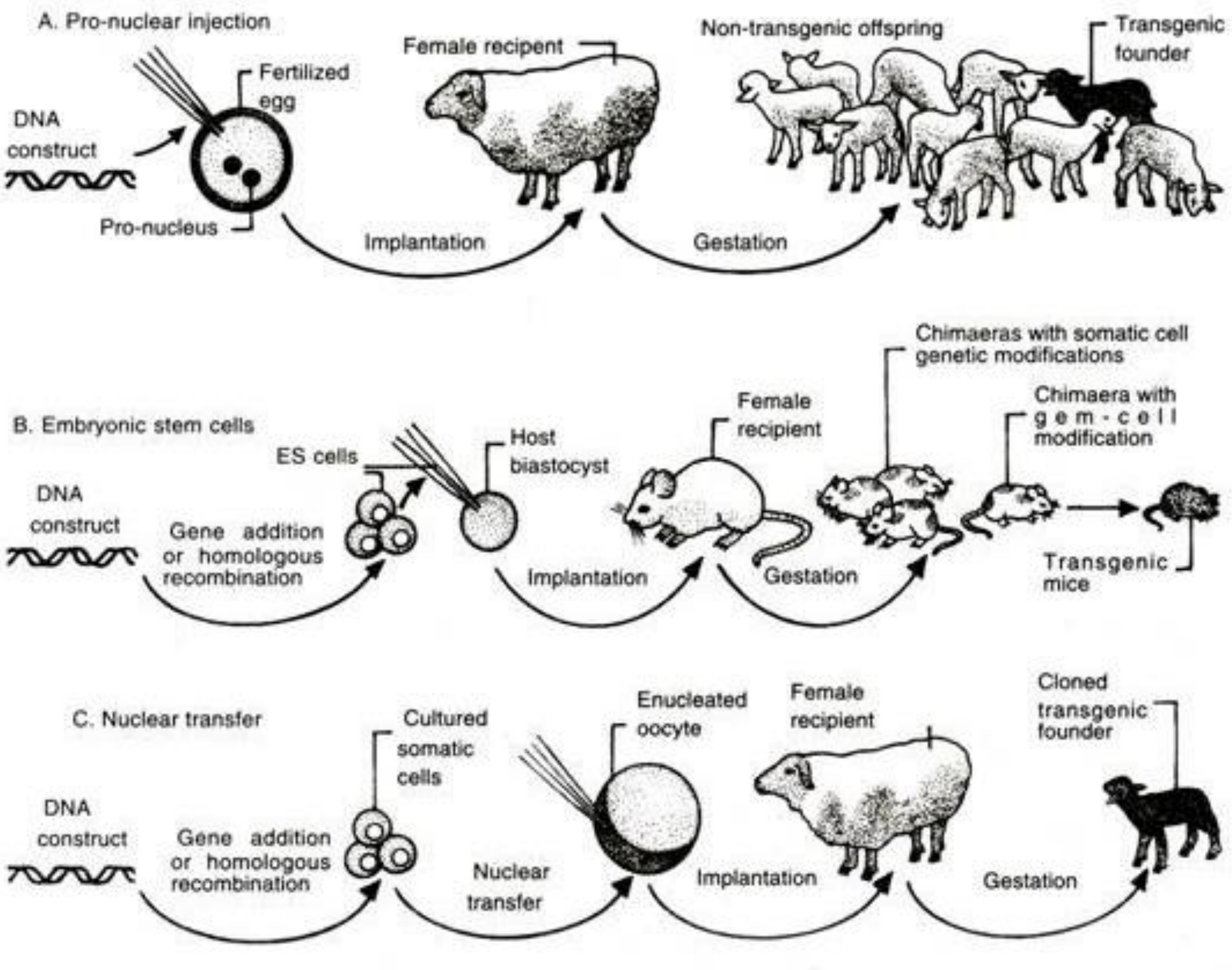

Fig. 18.2. Various approaches of producing transgenic animals using microinjection.

CRISPR-edited gene knockout in livestock: applications in agriculture

Species	*Gene*	Purpose of manipulation	Approach	Mosaicism (%)	References
Sheep	*ASIP*	Coat color pattern	MI	2/5 (40.0%)	Zhang X. et al. (2017)
	FGF5	Wool growth	MI	(6.3–100%)	Hu et al. (2017), Li W. R. et al. (2017), Zhang R. et al. (2020)
	MSTN, ASIP, and BCO2	Economically important traits	MI	2/2 (100%)	Wang X. et al. (2016b)
	MSTN	Meat production	MI or SCNT	(0–100%)	Deng et al. (2014); Crispo et al. (2015), Zhang Y. et al. (2019); Yi et al. (2020)
Goat	*BLG*	Milk quality	MI	3/4 (75.0%)	Zhou et al. (2017)
	MSTN and FGF5	Meat and cashmere production	MI	5/10 (50.0%)	Wang X. et al. (2015a)
	MSTN	Meat production	MI or SCNT	(0–100%)	Ni et al. (2014); Guo et al. (2016), He et al. (2018); Zhang Y. et al. (2019)
	NANOS2	Surrogate sires for genetic dissemination	SCNT	N/A	Ciccarelli et al. (2020)
	EDAR	Cashmere yield	SCNT	N/A	Hao et al. (2018)
Pig	*IGF2 regulatory element*	Meat production	MI (nCas9)	6/6 (100%)	Xiang et al. (2018)
	NANOS2	Surrogate sires for genetic dissemination	MI	6/18 (33.3%)	Park et al. (2017)
	ANPEP	Viral resistance	MI	1/9 (11.1%)	Whitworth et al. (2019)
	CD163	Resistance to PRRS virus	MI, EP, or SCNT	No	Whitworth et al. (2014); Yang et al. (2018), Tanihara et al. (2019)
	IRX3	Reduced fat content in Bama minipigs	SCNT	N/A	Zhu et al. (2020)
	NANOS2	Surrogate sires for genetic dissemination	SCNT	N/A	Ciccarelli et al. (2020)
	MSTN	Meat production	SCNT	N/A	Wang K. et al. (2015), Wang K. et al. (2017), Li R. et al. (2020)
	CD163 and pAPN	Viral resistance	SCNT	N/A	Xu et al. (2020)
	FBXO40	Meat production	SCNT	N/A	Zou et al. (2018)
Cattle	*NANOS2*	Surrogate sires for genetic dissemination	MI	1/3 (33.3%)	Ciccarelli et al. (2020)

SCNT, somatic cell nuclear transfer; MI, zygote microinjection; EP, zygote electroporation; nCas9, Cas9 nickase; N/A, not applicable.

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Species	Gene	Purpose of manipulation	Type of KI	Approach	SCNT or MI	KI Animals produced	Mosaicism (%)	References
		Agriculture: improvements in						
Sheep	*SOCS2*	Reproductive traits	Point mutation	Crispr/Cas9 BE	MI	3/4 (25%)	3/3 (100%)	Zhou et al. (2019)
	BMPR1B	Reproductive traits	Point mutation	Crispr/Cas9	MI	5/21 (23.8%)	Not stated	Zhou et al. (2018)
Goat	*Tβ4*	CCR5-targeted KI, cashmere yield	Gene insertion	Crispr/Cas9	SCNT	1	N/A	Li X. et al. (2019)
	FGF5	Cashmere yield	Point mutation	Crispr/Cas9 BE	MI	5/5 (100%)	5/5 (100%	Li G. et al. (2019)
	GDF9	Reproductive traits	Point mutation	Crispr/Cas9	MI	4/17 (23.5%)	2/4 (50.0%)	Niu et al. (2018)
	FAT-1	Disease resistance	Gene insertion	Crispr/Cas9	SCNT	1 from 8 pregnancies	N/A	Zhang J. et al. (2018)
Cattle	*Pc*	Generation of a polled genotype	Gene insertion	Crispr/Cas12a	SCNT	1, died on D1 after birth	N/A	Schuster et al. (2020)
	NRAMP1	Tuberculosis resistance	Gene insertion	Crispr/Cas9n	SCNT	9	N/A	Gao et al. (2017)
	IARS	Correction of IARS syndrome	Gene insertion	Crispr/Cas9	SCNT	5 viable fetuses	N/A	Ikeda et al. (2017)
Pig	*PBD-2*	Disease-resistant pigs	Gene insertion	Crispr/Cas9	SCNT	5 pigs	N/A	Huang et al. (2020)
	MSTN	Meat production	Gene insertion	Crispr/Cas9	SCNT	2 pigs	N/A	Zou Y.-L. et al. (2019)
	UCP1	Reproduction traits	Gene insertion	Crispr/Cas9	SCNT	12 piglets	N/A	Zheng et al. (2017)
	MSTN	Meat production	Point mutation	Crispr/Cas9	SCNT	1 stillborn piglet	N/A	Wang K. et al. (2016)
	MSTN	MSTN-KO without selectable marker	Gene insertion	Crispr/Cas9	SCNT	2 piglets	No	Bi et al. (2016)
	RSAD2	Generation of pigs with viral resistance	Gene insertion	Crispr/Cas9	SCNT	1 pig	No	Xie et al. (2020)
		Biomedical applications:						
Sheep	*ALPL*	Model of hypophosphatasia	Point mutation	Crispr/Cas9	MI	6/9 (66.6%)	No	Williams et al. (2018)
	PPT1	Infantile neuronal ceroid lipofuscinoses	Point mutation	Crispr/Cas9	MI	6/24 (25.0%)	Not stated	Eaton et al. (2019)
	tGFP	Rosa26-targeted KI	Gene insertion	Crispr/Cas9	MI	1/8 (12.5%)	Not stated	Wu et al. (2016)
	OTOF	Hearing loss phenotype	Point mutation	Crispr/Cas9	MI	8/73 (11.0%)	2/8 (25.0%)	Menchaca et al. (2020b)
Cattle	*CMAH*	Xenotransplantation	Point mutation	Crispr/Cas12a	SCNT	2	N/A	Perota et al. (2019)
Pig	*hF9*	Gene therapy for hemophilia B pigs	Gene insertion	Crispr/Cas9	SCNT	5 pigs	N/A	Chen et al. (2020)
	BgEgXyAp	Salivary gland as bioreactor	Gene insertion	Crispr/Cas9	SCNT	4 piglets (1/4 alive)	N/A	Li G. et al. (2020)
	hIAPP	Type 2 diabetic miniature pig model	Gene insertion	Crispr/Cas9	SCNT	24	N/A	Zou X. et al. (2019)
	SNCA	Parkinson's disease model	Gene insertion	Crispr/Cas9	SCNT	8 piglets	N/A	Zhu et al. (2018)
	HTT	Huntingtin KI model	Gene insertion	Crispr/Cas9	SCNT	6 piglets	N/A	Yan et al. (2018)
	GGTA1	Xenotransplantation	Gene insertion	*Fok*I-dCas9	SCNT	2 piglets	N/A	Nottle et al. (2017)
	tdTomato	porcine Oct4 reporter system	Gene insertion	Crispr/Cas9	SCNT	2 piglets	N/A	Lai et al. (2016)
	hALB	Tg animals as bioreactors	Gene insertion	Crispr/Cas9	MI	16/16 (100%)	1/16 (6.3%)	Peng et al. (2015)
	GFP	H11-targeted KI	Gene insertion	Crispr/Cas9	SCNT	1 piglet	N/A	Ruan et al. (2015)

SCNT, somatic cell nuclear transfer; MI, zygote microinjection; BE, base editing; N/A, not applicable.

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Example: pharmaceutical production

Domestic chicken

sebelipase alpha (Kanuma, fa alexion Pharmaceuticals)

Treatment of Wolman syndrome (lysosomal lipase deficiency),

Approved USA, EU, Japan

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Example: GM food

Salmon: GH of marine salmon (Chinook, King salmon) + strong promoter (metallothionein) to river (Atlantic, Atlantic) salmon

11x growth (US approved 2015, in approval since 1995, 2016 Canada, 2021 Brazil), AquAdvantageTM

Current variant grows to the same size, but earlier (faster growth)

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Ethics of transgenic technology

- Is the new product acceptable
- Animals may suffer due to expression of transgenes inducing tumours or neurodegenerative diseases
- Side effects due to modifying genes
- Humans may benefit from transgenic animals - transgenic animals themselves do not
- Foreign genes affect the host and there are many threats to ecological balance and species diversity (Miao, 2013)

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Cloning - generating genetically identical offspring

- Cloning techniques in mammals:
  - microsurgical embryo bisection,
  - isolation and proliferation or aggregation of single blastomeres
  - nuclear transfer!!!

- animal cloning
  - reproductive (animals)
  - therapeutic (potential in human)

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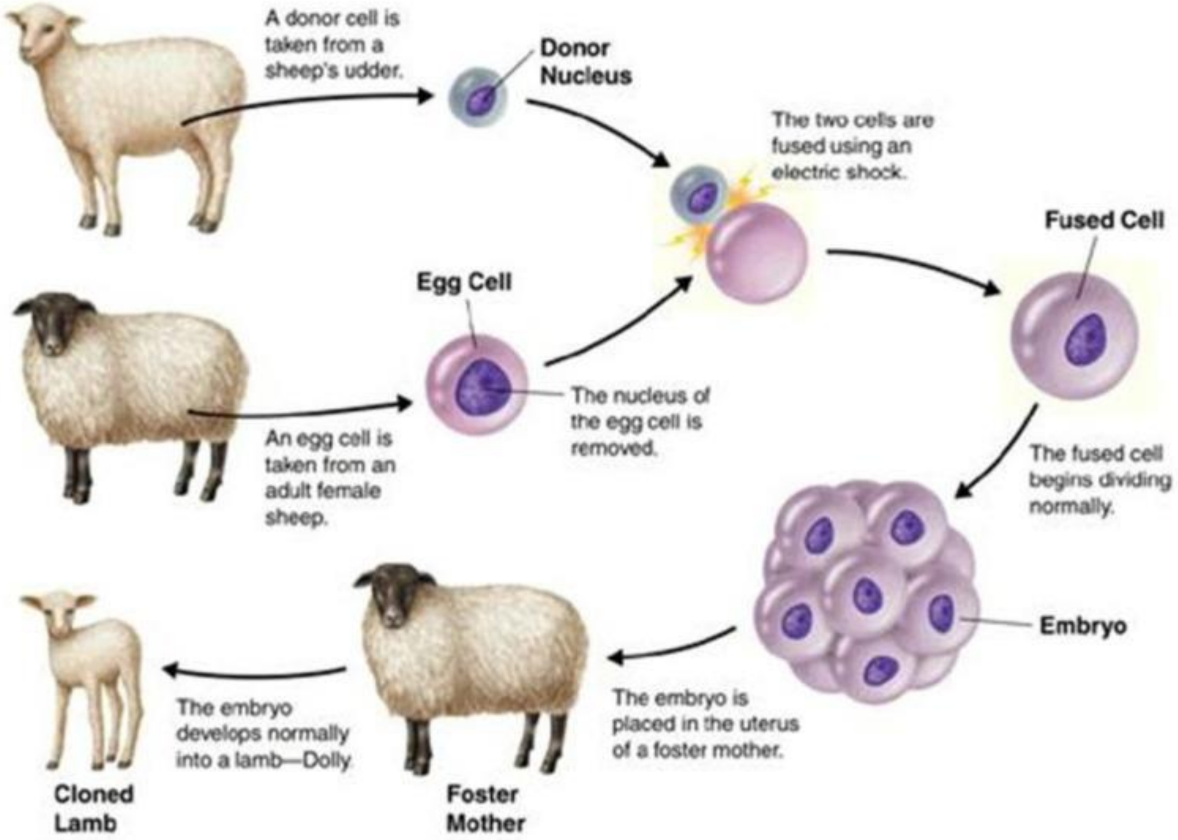
Cloning - the nuclear transfer method in Dolly the sheep

- 1996: Ian Wilmut and Dolly the Sheep



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Problems associated with cloning

- Small percentage of jamming
- Developmental defects - early mortality, stillbirths, early deaths after birth, short lifespan, obesity, malformations of various organs, poor immunity - "large offspring syndrome"
- mammals, intrauterine development
- Not accepted by breeders - horses (not included in studbooks,...)
- Legislative problems
- Ethical problems
- Food products from cloned animals ("cloned meat") - according to the FDA it is safe to consume meat from cloned animals - but economically highly inefficient (so far)
- European ESFA has also declared safety of animal products from clones, the problem is with the welfare of the recipients and the clones themselves

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Examples of cloned animals

- mouse, rat
- most large livestock (sheep, goat, pig, horse)
- domestic hen
- fish (carp, salmon, etc.)
- rhesus macaque
- pets (dog, cat, etc.)

Adolfo Cambiaso with six clones of the mare Cuartetera, which he rode during the horse polo at the 2016 Palermo Open in Argentina (idnes.cz).

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	References
Mammals	
Mice (*Mus musculus*)	Gordon et al. (1980), Joyner and Sedivy (2000)
Rats (*Rattus rattus*)	Hamra et al. (2002), Kato et al. (2004), Hirabayashi et al. (2005), Agca et al. (2008)
Rabbits (*Oryctolagus cuniculus*)	Fan and Watanabe (2003)
Sheep (*Ovis aries*)	McCreath et al. (2000), Denning and Priddle (2003), Wheeler (2003)
Pigs (*Sus domestica*)	Lai et al. (2002), Houdebine (2009), Kragh et al. (2009)
Cattle (*Bos taurus*)	Donovan et al. (2005), Richt et al. (2007), Houdebine (2009)
Goats (*Capra hircus*)	Wheeler (2003), Houdebine (2009)
Dogs (*Canis familiaris*)	Hong et al. (2009)
Marmosets (*Callithrix jacchus*)	Sasaki et al. (2009)
Rhesus monkeys (*Macaca mulatta*)	Yang et al. (2008)
Birds	
Chickens (*Gallus gallus*)	Mozdziak and Petitte (2004)
Japanese quail (*Coturnix japonica*)	Huss et al. (2008)
Amphibians	
Frogs (*Xenopus laevis* and *Xenopus tropicalis*)	Macha et al. (1997), Sinzelle et al. (2006), Ishibashi et al. (2008)
Fish	
Zebra fish (*Danio rerio*)	Zelenin et al. (1991), Davidson et al. (2003), Huang et al. (2008)
Goldfish (*Carassius auratus*)	Houdebine and Chourrout (1991), Wang et al. (1995)
Nile tilapia (*Oreochromis niloticus*)	Martinez et al. (2000), Maclean et al. (2002), Hrytsenko et al. (2009)
Carp (*Cyprinus carpio*)	Yoshizaki et al. (1991)
Channel catfish (*Ictalurus punctatus*)	Dunham et al. (2002)
Atlantic salmon (*Salmo salar*)	Sin et al. (2000), Houdebine (1997)
Invertebrates	
Arthropod fruit fly (*Drosophila melanogaster*)	Rubin and Spradling (1982), Fujioka et al. (2000)
Nematode (*Caenorhabditis elegans*)	Fire (1986), Mello et al. (1991)
Mollusk Japanese abalone (*Haliotis diversicolor suportexta*)	Tsai et al. (1997)
Mollusk Eastern oyster (*Crassostrea virginica*)	Cadoret et al. (1997)
Mollusk dwarf surfclam (*Mulinia lateralis*)	Lu et al. (1996)

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Therapeutic cloning

- realistically the main importance of studying mammalian cloning
- the potential for treating otherwise untreatable diseases
- replacement of damaged cells with cells of the body's own, possibly with corrected genetic information (treatment of genetic diseases)
- the use of embryonic stem cells (ESCs)
- the future of biomedicine

GI somatic cells - enucleated oocyte - pluripotent cells (EC) - controlled differentiation - somatic cell returned to patient

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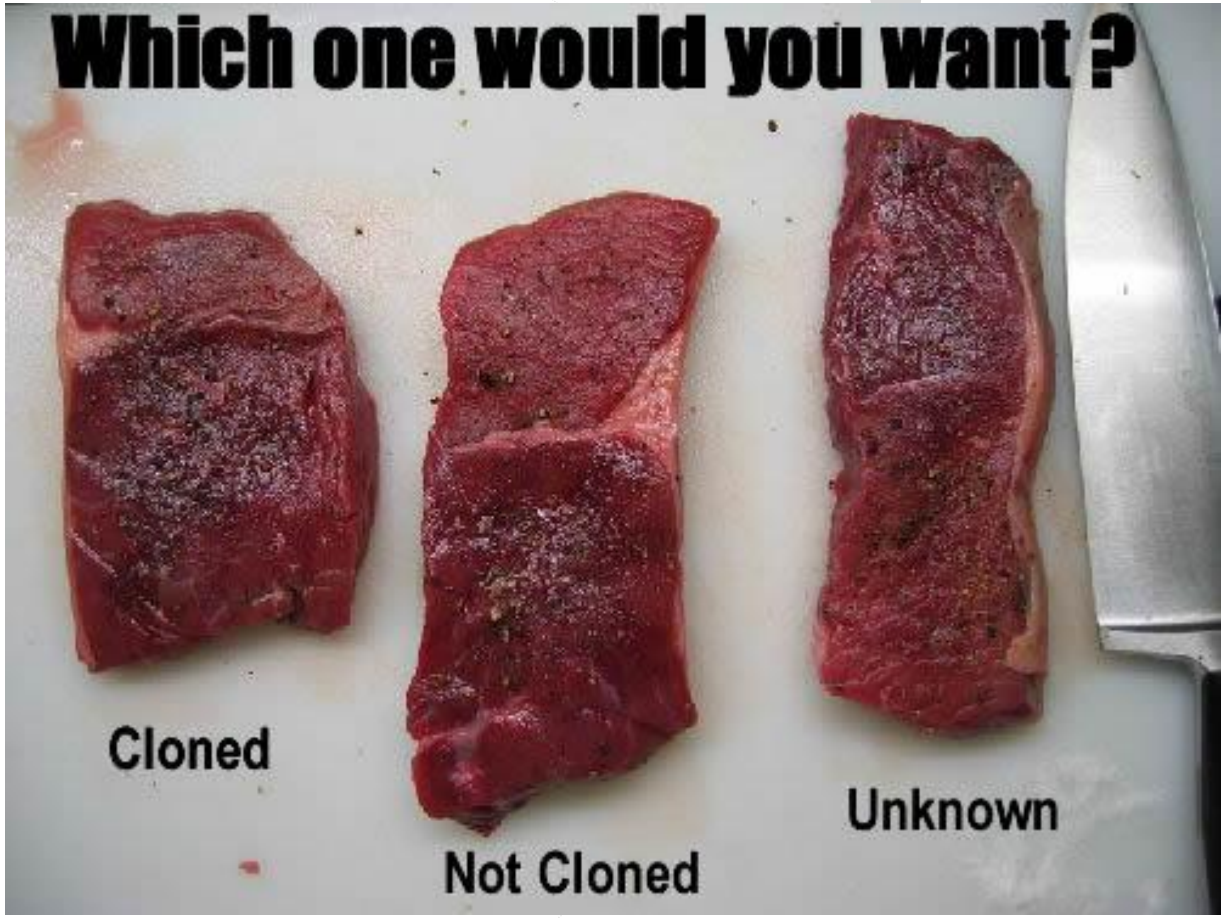

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Thank you for your attention!

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