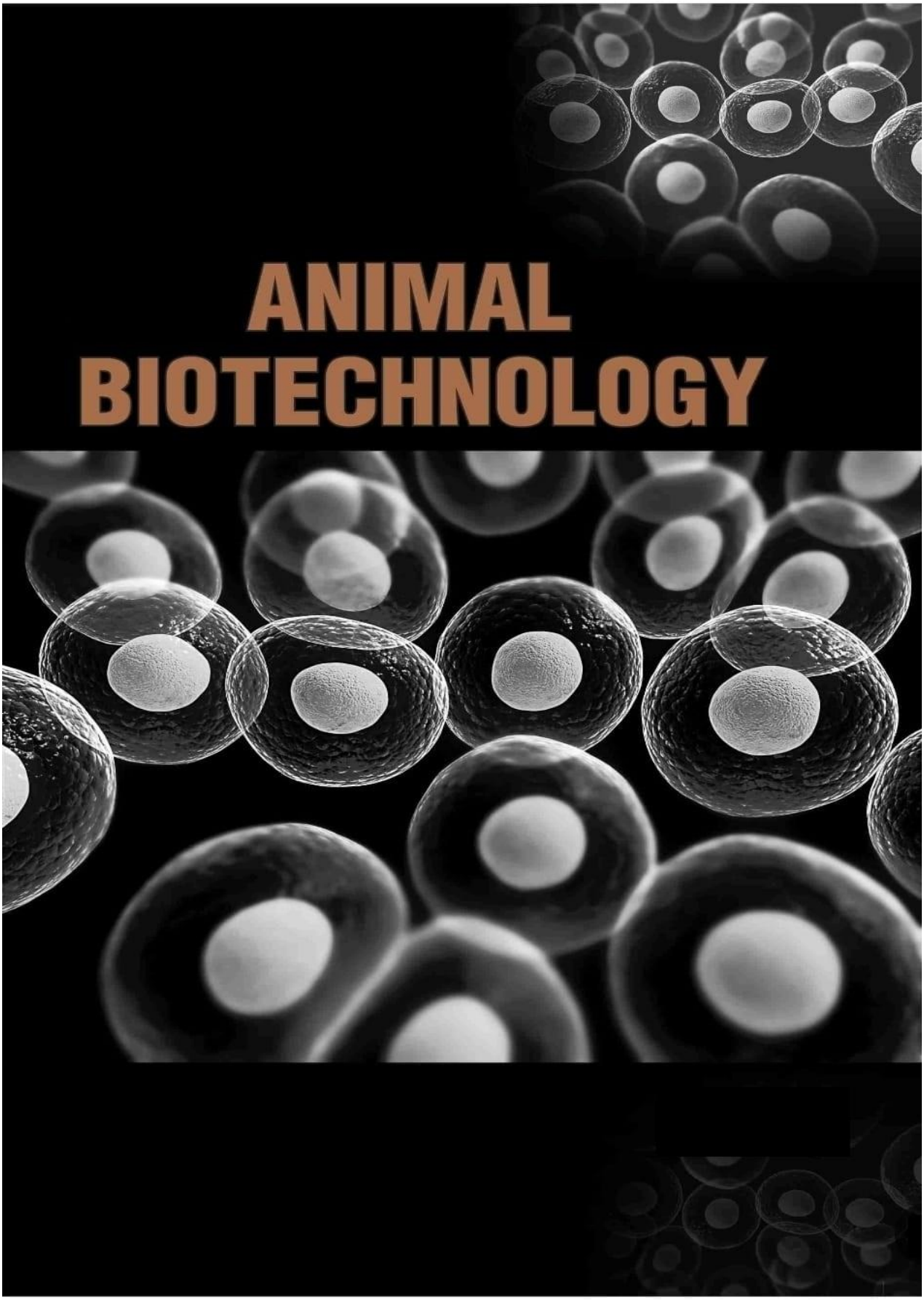

ANIMAL
BIOTECHNOLOGY

Animal Biotechnology

*How genetically engineer animal can be used ?

- Development of treatment
- Improved foods
- Use in research

-Scientist use These animal in research because we can't DO our Experiments in Human the good things there are some similarity physiology and biochemical properties between Human and animals.

Applications

- Polio vaccine (done by animal models , without it this vaccine can NOT be found) .

- Dialysis: (first done in animal before try it in human).
- Cataract : (cloudy area in the lens of eye).

*All these applications try on animal first , then on human

Most animals use in research : Rats & Mice

Other animals use in research : (zebra fish , fruit fly , nematode , dogs and chimpanzee).

•Dogs, monkeys, chimpanzees, cats make up less

than 1 percent of total number of research animals

For example : zebra fish use in embryonic & drug toxicity because it small and grow in large quantities and short generation time (3 month)

Casper fish : is mutant type of zebra fish generate from mating between two types of mutant zebra fish : one of parent lacked reflective pigment with another parent was lacked black pigment.

*Casper fish used to study cancer metastasis because it has transparent skin allow to see migration (movement) of cancer cells

*Zebra fish use to study embryogenesis it take (120) hour

• Gut, liver and kidneys developed in the first(48-72) hours

*Drug test :companies test the toxicity of drugs in human by test in animal because if it toxic in animal it will probably toxic in human if not it can now test in human.

*Drugs Show effect in animal quickly take around(5)days in zebra fish compare other animals

*Rats are use more than mice in research because they are closer to human to respond the drugs.

• more toxicological data has been collected so better

understood

*Rats have large size that will facilitates surgical and physiological Experimentation

SCIENTISTS USE other animals to. Study different drugs

*For example :dogs are used to study cardiovascular and lung drugs because are Similar to human (Have similar lungs and cardiovascular)

*Monkeys and chimpanzee: are use to study the HIV because they only animal that affect by HIV virus

propose HIV is transfer from Monkeys and chimpanzee to human

*HIV : Human immunodeficiency virus that cause Aids

*Aids :acquired immune deficiency syndrome.

*As we said the drugs before try on human it must try on animals

- The numbers of these animals used in experiments has

been declining for the past 20 years

TABLE 7.1 FOOD AND DRUG ADMINISTRATION REQUIRED TESTING PHASES FOR DRUG APPROVAL

FDA Phase testing involves the use of animals for pre-clinical testing before allowed in humans. If the new drug candidate has proven to be non-toxic and has benefit, then it can be awarded and Investigational New Drug (IND)status. If it is successful in the three phases of human testing it can receive a New Drug Application (NDA) and likely approval for marketing. The FDA continues evaluating the NDA for another 2.5 years, resulting a total of about 12 years for a successful drug approval.

	Preclinical Testing		Phase I	Phase II	Phase III		FDA		Phase IV
Years	3.5		1	2	3		2.5	12 total	
Tested on	**Animals in the lab**		20–80 healthy volunteers	100–300 patient volunteers	1,000–3,000 patient volunteers				
Purpose	Assess safety and biological activity	File IND at FDA	Determine safety and dosage	Evaluate effectiveness and look for side effects	Verify effectiveness, monitor adverse reactions from long-term use	File NDA at FDA	Review process/ approval		Additional testing after approval required by FDA
Success rate	5,000 compounds evaluated			5 enter trials			1 approved		

*FDA: food and drugs administration involved different stages to prove the drugs it take (12) years an Scientist d 1billion dollar.

*First : preclinical before test drugs on human

*It require (3.5) years to make sure the drugs is Safe and what drug exactly do.

*If it all things are Ok then the they will file ind

*FDA: the Scientist that will review the results

*Then Clinical test (Three phases)

Phase one drugs test on healthy volunteers it take (1) year

- And test on (20-80) volunteers if the drugs are safety the FDA will move to

phase 2 the drugs will test on patients volunteers test on (100-300) patients volunteers and it take 2 years if all things are Ok then FDA will move to phase(3) test on (1000-3000) patients volunteers it take 3 years if all things are Ok the FDA will take (2) years to review all previous stages if all things are Ok then the drugs are approved.

*After the drugs are approved the FDA the FDA will do additional test.

*So clinical test mean : test the drugs on the human

*There are four phases 3 before approved the drug and 1 phase after approved the drug.

*As we see the approve the one drug for example it take many time and a lot of money

7.1 Animals in Research

- Alternatives to Animal Models

1. Cell Culture

- Preliminary screen to check the toxicity of substances
- Can answer fundamental questions about biology

- Cannot provide information about potential impacts on entire living Organism

2. Computer Models

- Simulate specific molecular and chemical structures and their interactions
- Limited by programming and knowledge of how the physiological system works

7.2 Cloning

*In 1997 the first animal was clone called dolly

Dolly is sheep.

-Dolly born (cloned) after many failed attempts to do cloning

-Dolly was cloned by SCNT (somatic cell nuclear transfer)

-Scientists know the cloning in animal is more challenge than animal but they still try it in animal .

-One of the ways to try the clone is

Embryo Twining : very common in cattle industry

• The first successful experiment produced two health calves that were

identical twins

-In General Consider easy procedure

-Cloning Improve by create certain animal from one parent and from somatic cell from adult cell.

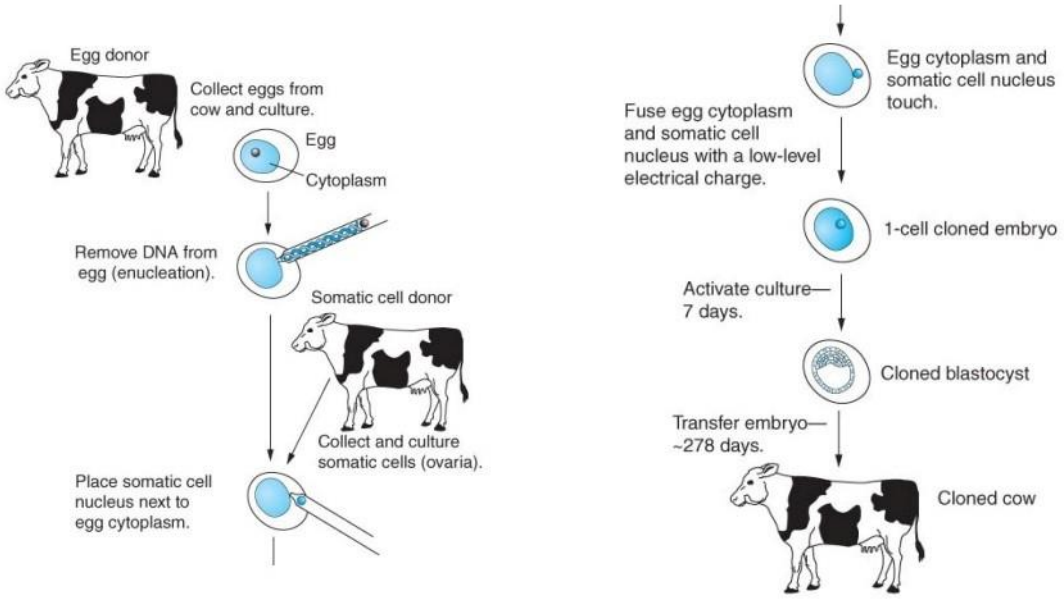
• Creating a Clone from an Adult

-First remove the nucleus from the egg the by process called enucleation then we max the e

-nucleated egg with nucleus from somatic cell take from adult or embryo then the egg

-cytoplasm and nucleus from somatic cell will fused and now we have cloned embryo.

-Genetic material of cloned animal will be identical to parent animal who was donate nucleus.



-We have some limitation for cloning

-We cant get clone from dead Organism

-The organism will not exactly identical phenotypically but identical Genotype

Low success rate

-The first clone cat called CC(Carbon copy).

-Some animal suffer medical conditions because if the donor cell old somatic cells this mean

-already have short telomeres.

-Telomeres : repetitive sequences found at ends of chromosomes consist of 6 nucleotides

-repeat 3000 time for example (TTAGGG) for each cell divide it will protect the genes from

-damage after each round of cell division.

-Embryonic cells have longer telomeres.

-When Scientists Try to clone Dolly they use old adult cells from adult donor telomeres are

-already short which mean when Dolly was cloned the cells of Dolly already have short telomeres in ends of chromosomes.

-The epigenetic program of Dolly is different than the naturally program.

-Epigenetic program : is chemical modification that occur at surface of histone or other chemical groups that effect the genes.

-During embryogenesis some genes acquire must be pre express because in donor nucleus

-These genes been silent by epigenetic program.

-Remember the cell that was used to clone Dolly was take from adult donor which mean this

-cell was undergo to genetic program and some genes that require to express in

embryogenesis are already express in the donor animal not in Dolly.

-Clone Macaque Monkey by SCNT

-This Monkey clone for research.

-Scientists use the same procedure that was done to clone Dolly but Scientist use additional steps.

-In this technique very important step was done to make sure the telomeres have there proper length they take the nucleus form fetal fibro blast somatic cell in term age is very young somatic cell which mean the telomeres inside the nucleus are proper length and fused it with Enucleated Oocyte and they activated the fusion between nucleus and egg with electric shock then injected M-RNA that encode demethylase enzyme.

kdm4d m-RNA : important to encode demethylase enzyme

Wait why we need demethylase enzyme?

-This enzyme will remove methyl group to allow any genes to express These genes are important to early stage of Development to be express.

-Addition of methyl group is one of modification the occur in early stage and this

modification is important to cells differentiation.

-As we know if we talk about diploid Organism The whole diploid organism is arise from

-divide the Zygote as we know the Zygote is divide by mitosis why the diploid organism has

different cells :bone cells blood cells hair cells skin cell why not all cells are in the same kind?

-These different cells result by cells differentiation.

-Lest back to Macaque cloning

-As we said the Scientists inject kdm4d and also they inject substance called trichostatin A.

Trichostatin A : substance inhabit de acetylation enzyme or it called histone de acetylation enzyme.

Why we need inhabit histone de acetylation?

-To allow to acetylation modification to take place

Histone acetylation: addition of an acetyl group to an amino acid in histone tails.

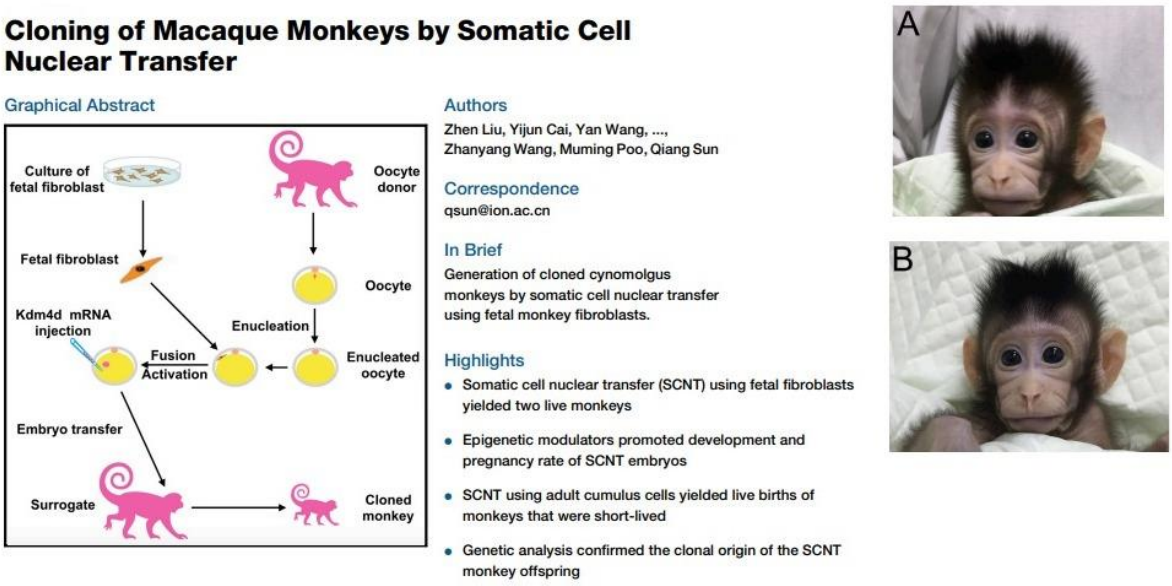
-Histone acetylation is important to allow to start expression.

-Histone de acetylation remove the acetyl group that cause the chromatin more Condense and no expression.

-So we inhabit this enzyme to allow the acetylation of histone tail that cause chromatin less condense and now the expression of early stage genes will start.

-We do all this things to allow the early stage genes to start express in adult cells and in nature These genes was masked.

-There are some limitation of These technique.



Transgenic animals

How I can introduce DNA or Gene in animal?

-We can use viruses

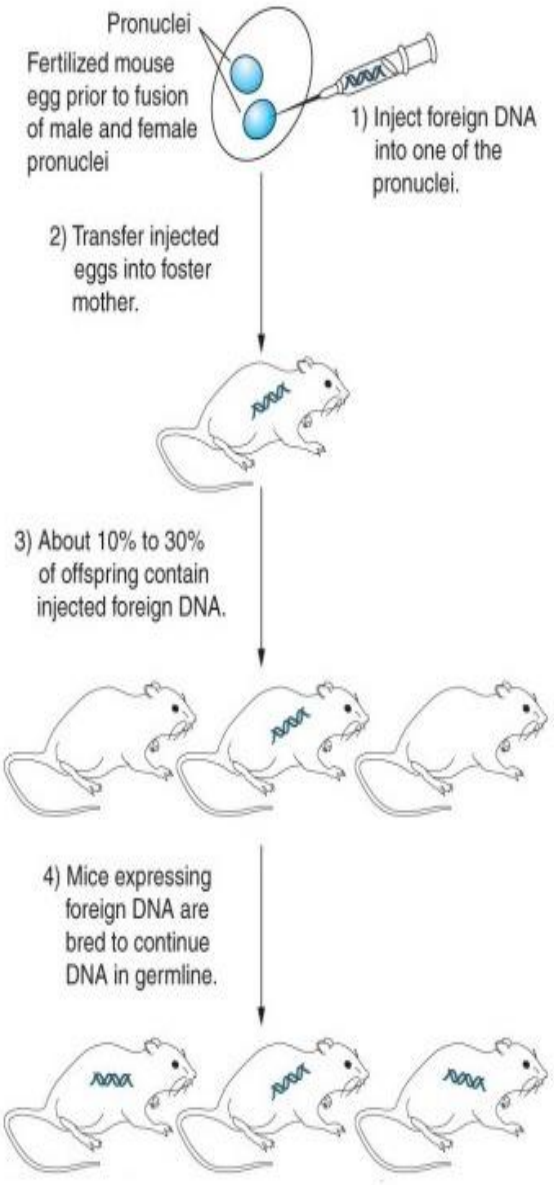
-To clone DNA or Gene we want introduce in animal and take the embryo from the animal then infect it with retro virus

-On of limitation is size of transgene (transfer genetic material).

-Second technique called pronuclear micro injection

-Transgene inject at early stage before the tow nuclei of (egg and sperm) fuse to gather.

-The efficient of this method is 10% - 30% that mean only 10% to 30% offspring get the foreign DNA.



-Some of dis advantage after inject The DNA the embryo might no developed and DIE

because injection is so harsh to embryo or the foreign DNA not integrated to chromosome or maybe DNA integrated in essential genes that causes damage of These genes and This mean the survival of embryo will affected.

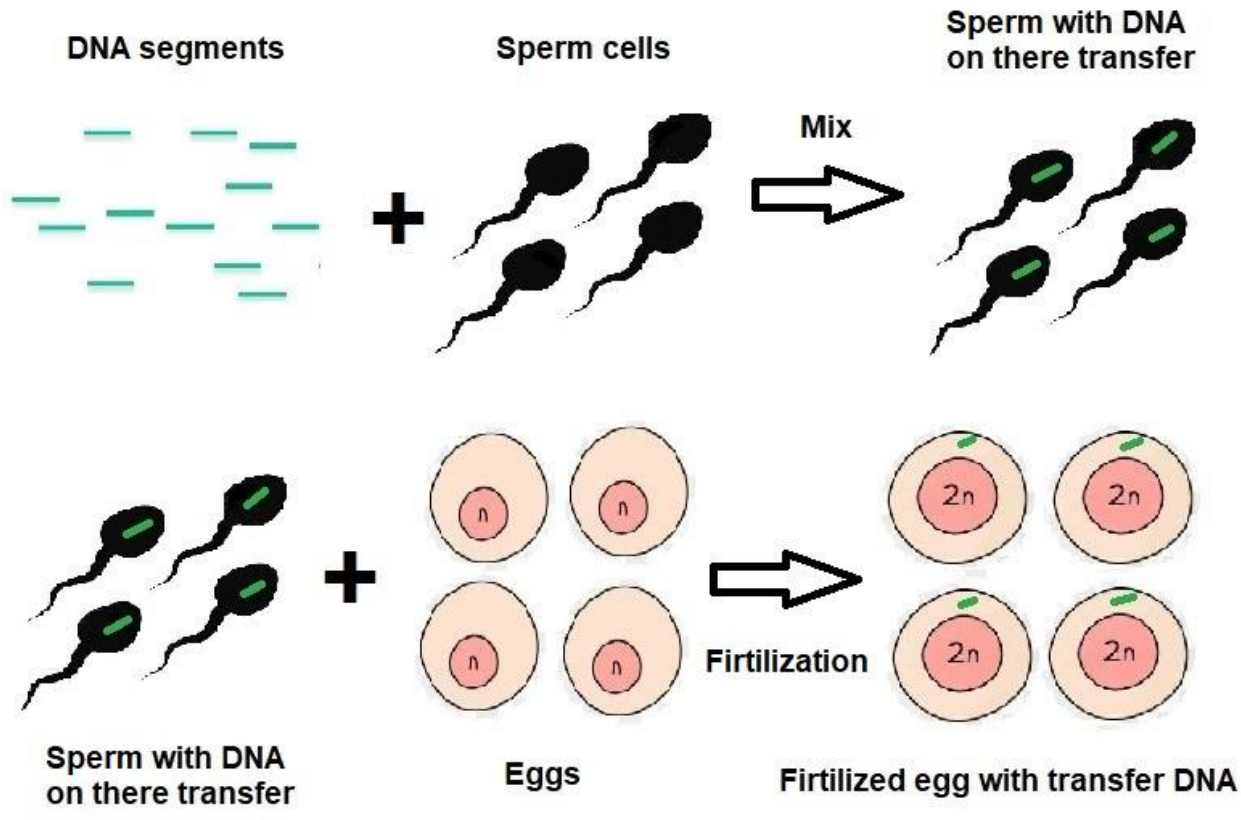
-Or it can integrated Correctly but not express.

-Virus could infect dividing and non dividing cells and they are less than damage happen to the cell unlike micro injection also the rate of integration of DNA (trans gene) into embryogenesis.

-Virus is higher than pronuclear micro injection in term of DNA integration.

-Another technique use to introduce DNA or Gene in animal is called Sperm mediated transfer.

-In this technique we max the sperm with DNA and DNA will attach to the surface of DNA because on the surface of sperm there are linker proteins the DNA will attach to These proteins and then we allow the sperms that contain the DNA on there surfaces to fertilize the eggs.



-There are many advantages of this technique : very simple low cost no need handle embryo.

-Also there are some disadvantages :random integration of transgene maybe cause problem for embryo

Another method called gene guns this technique use to introduce DNA or Gene in animals and plants.

-Embryonic stem cells method : this technique use to generate transgenic animals by done

what we called knock out.

-Scientist are try to introduce gene to Improve life stock for example

Herman – bull this animal carry human gene for lactoferrin

Lactoferrin : protein that important to bind iron.

-This mean the animal that have this gene that have more iron in the milk.

-Instead of taken iron supplements because some people have Disease called iron deficiency

-These people can take Milk from These animal Instead of supplements.

-Some transgenic animals produce anti-bodies These antibodies could be use for research or treatment or diagnosis.

But what is the procedure to make transgenic animal to produce anti bodies?

Lest talk about cow for example.

-Human gene encoding These antibodies use to replace bovine genes

-Immune genes : antibodies genes or the genes that produce anti bodies.

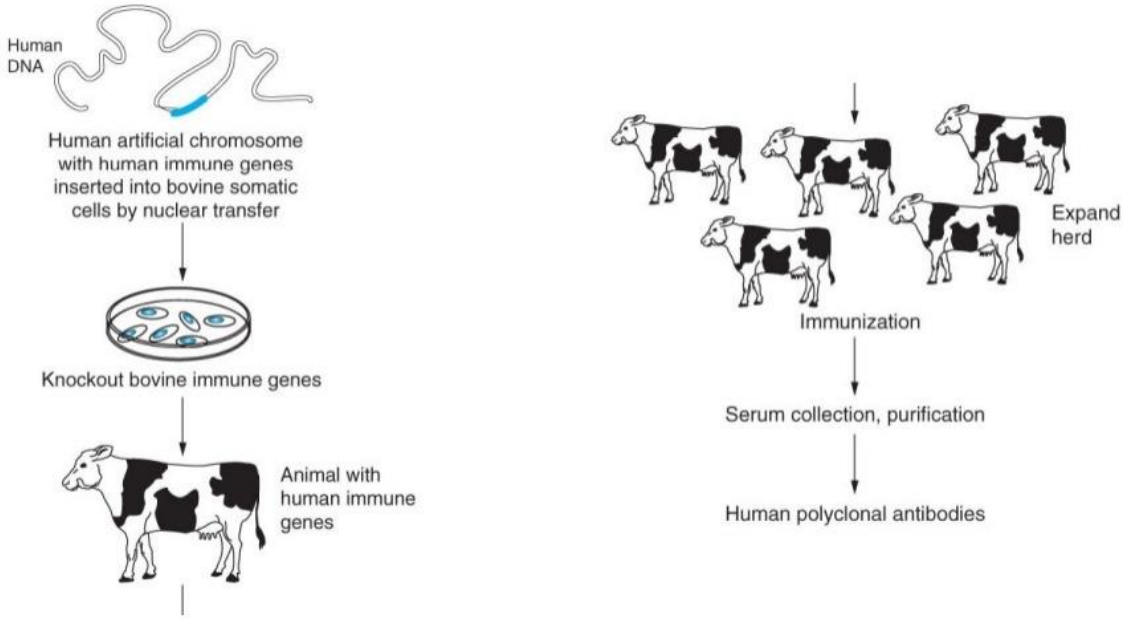
-Anti-bodies protect our Against viruses and Bacteria.

-Knock out bovine immune genes and knock in Human immune genes.

-Then we immunize cow by inject with human pathogens so then the cow will produce antibodies against Human pathogens then we collect the blood to get These anti-bodies and These anti bodies called Polyclonal

Polyclonal anti bodies :mixture of antibodies were secreted by different B cells.

-If we need use anti bodies to treatment the human it must be Human antibodies if we inject cow anti bodies to human bodies the body will not accept it and it might Consider as foreign Substance and it will stimulate the immune system.



Lysostaphin: enzyme that can degrade the cell wall of Staphylococcus aureus (type of Bacteria) to become easy to kill it.

-Spider web protein: use to make bio steel.

-Bio steel : is used in bulletproof vests.

-Atryn : protein needed to treat people suffer hereditary clotting Disease.

Atryn produce now by company called REVO.

-After patients take Atryn they can know manage clotting in blood.

• Transgenic goats developed by the company GTC have Atryn under control of a mammary specific promoter so can produce the anticlotting protein faster, more reliably and more cheaply than traditional pharmaceutical methods

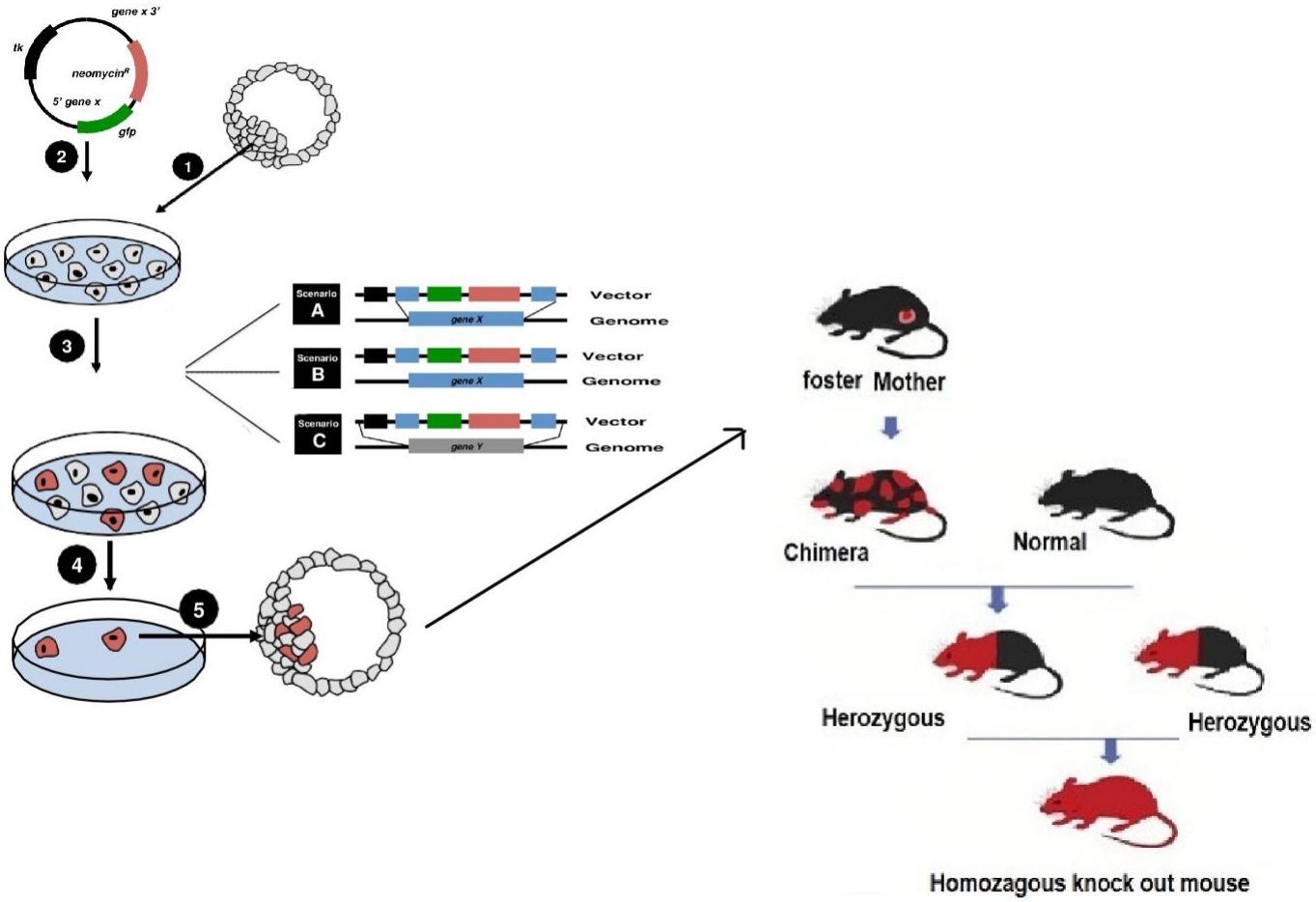
Generate animal Disease model

-If we have human Disease and this Disease cause by disrupt one gene or mutation in certain gene we cant do Experiment on the human so what we do is introduce same mutation or disrupt the same gene in some animal because we know that there are some Similarity between Human and animal that's mean there are some genes found in animal and in Human.

-So we can use animals as model organism to do our Experiments about some Diseases that affect the human.

-So we can use the animals to do Experiments about some Disease and test the drugs before test in human to treat certain Disease.

-Most common method to carry the knock out is embryonic stem cells method.



-First we take the steam cells from blastocyts stage

-Then grow These cells in cell culture after this step we do transfection.

-Transfection : transfer or introduce DNA into animal cells.

-This DNA will help

-This DNA will help in knock out process for certain gene.

-For example: we want to knock out gene X we have to prepare in which clone the gene in the plasmid

-The gene we want to clone it in plasmid is the same gene we want to disrupt it.

-But in the same plasmid we will clone Another gene called neomycin resistant gene this gene will clone it in the middle of Gene X.

-Also we have Another gene in the plasmid called TK Gene

-TK gene :encoding for thymdine kinase it's a marker gene.

-The DNA (plasmid) will introduce inside the ESC (Embryonic stem cells).

-Then what will happen is homology recombination that will lead to knock out.

-To allow to homology recombination take place we must design some segments before and after gene X in the plasmid.

-The segment we want to design it after and before the gene X in plasmid it must be identical to the segment that present before and after the gene X in stem cell to allow to homology recombination to happen.

-If the recombination is happen the neo R (neomycin resistant gene) will transfer form the middle of gene X in the plasmid to the middle of gene X in the stem cell which mean the gene X inside the ESC will disrupt.

-There are different probabilities

-The first is the correct recombination happen.

-The second is no recombination happen.

-The third is non specific recombination happen.

Next step is selection.

-We put the cells in selection media that contain neomycin (type of anti-Biotech) and aciclovir (anti – virus).

-Neomycin: allow to select cells with neo (R) gene which mean the cells that no Recombination take place will killed by neomycin.

-So the only the cells that Recombination take place will survive

-Which mean the cells that undergo to non specific Recombination will survive.

-TK gene will transfer to ESC if the Recombination is non specific.

-As we know the TK encode thymdine kinase.

-So any cell contain this gene will kill by aciclovir that will allow to select only the cells that undergo to specific Recombination.

-Now the cells is neo R / gene X –

-This mean this cell are have resistant gene to neomycin and disrupted gene X.

-Before Recombination happen These cells were neo R - / gene X

-Which mean these cells are sensitive to neomycin and have functional gene X.

-After we do selection we take These cells and back them to embryo.

-Now we have tow type of cells the original cells (neo R - / gene X)

And (neo R /gene X -).

-After all These steps we put the embryo inside foster mother.

-This mother will give birth offspring but These offspring have some cells (neo R - /gene X) and some cells (neo R/ gene X-) this called chimera.

-If we mate chimera with normal mouse it will produce heterozygous.

-To get homozygous mice for neo R /gene X - we mate tow heterozygous mice to gather.

-The result is homozygous knock out mouse for gene X.

-Knock down : gene remain intact in Genome but not express.

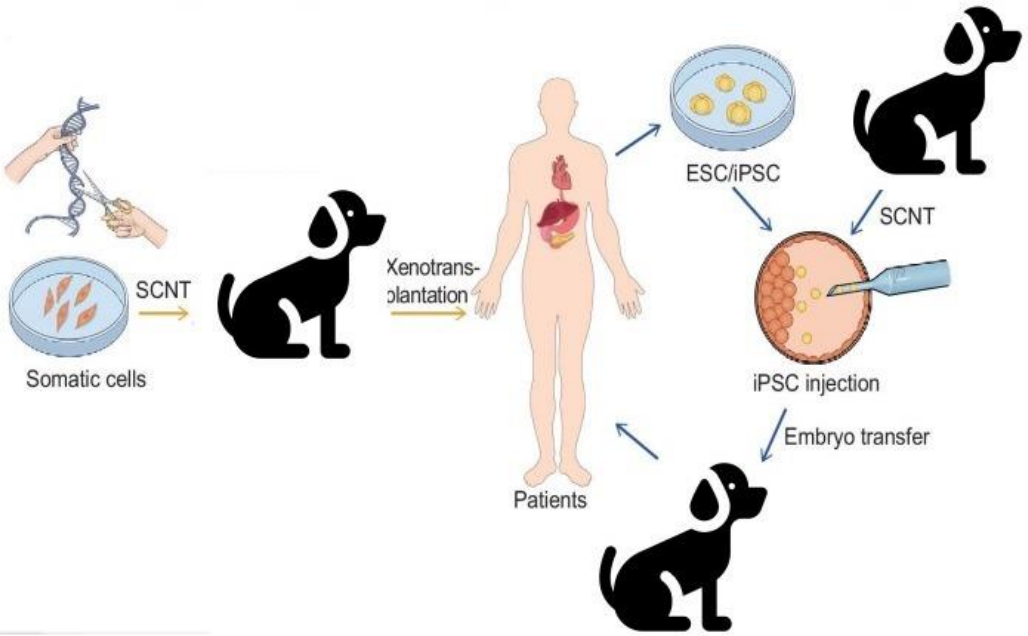
We can use RNA i or Crisper - Cas to do Knock down.

*Prepare Xenotransplantation: Prepare animal Organs to use it in human.

What we do is try to generate transgenic animal to use it as a Organ donor this could be done by edit certain genes to be compatible.

Or it could be done by ESC/ipcs IPCS induced pluripotent stem cells.

-We could take ipsc from human and inject in embryo of animal then transfer this embryo to foster mother then after this embryo is born we can use it as Organ donor.



Produce antibodies

*Monoclonal anti body: anti body that recognize bind to one epitope.

*Mono: mean one. Bind to epitope

*Epitope : sequence of polymers.

*Hybridoma: use to make anti bodies and These anti bodies could be use for treatment or for research or Diagnosis of certain Diseases.

What are the steps to do Hybridoma?

First inject animal by the antigen or the epitope

-The epitope will recognize by anti-bodies.

-The epitope could be protein from virus or Bacteria

-After inject The animal by epitopes the immune system of animal will produce anti-bodies against to These epitopes (antigen).

-Spleen cells contain(8) cells These B cells are responsible to produce anti bodies.

-Spleen cells is are mortal which mean it cant grow in cell culture These cell will DIE after

some cell divisions.

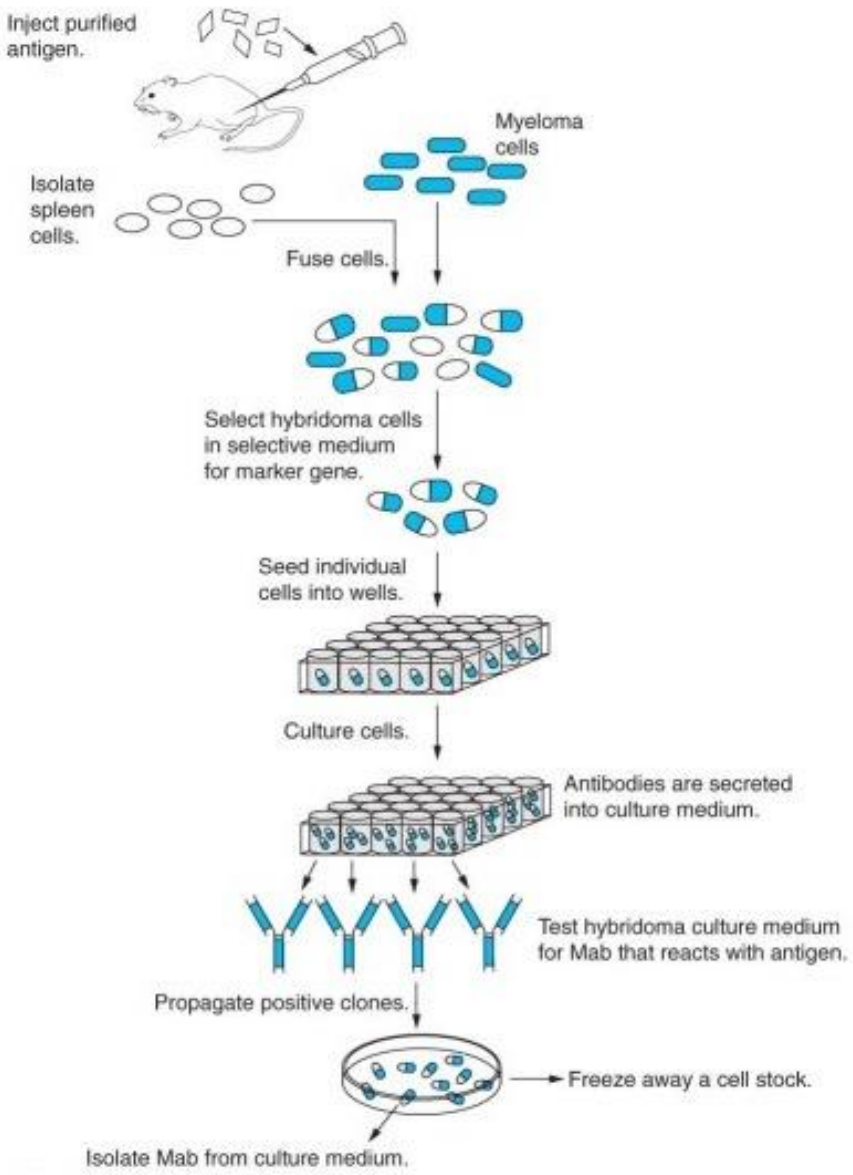
What we do are mix These cells with immortal called myloma.

-Myloma :cancer cells (cancer B cells)

-Myloma it can grow in cell culture but it cant produce antibodies.

-To inhance the fusion between Spleen cells and myloma cells we use PEG(poly Ethylen Glycol) or by some viruses.

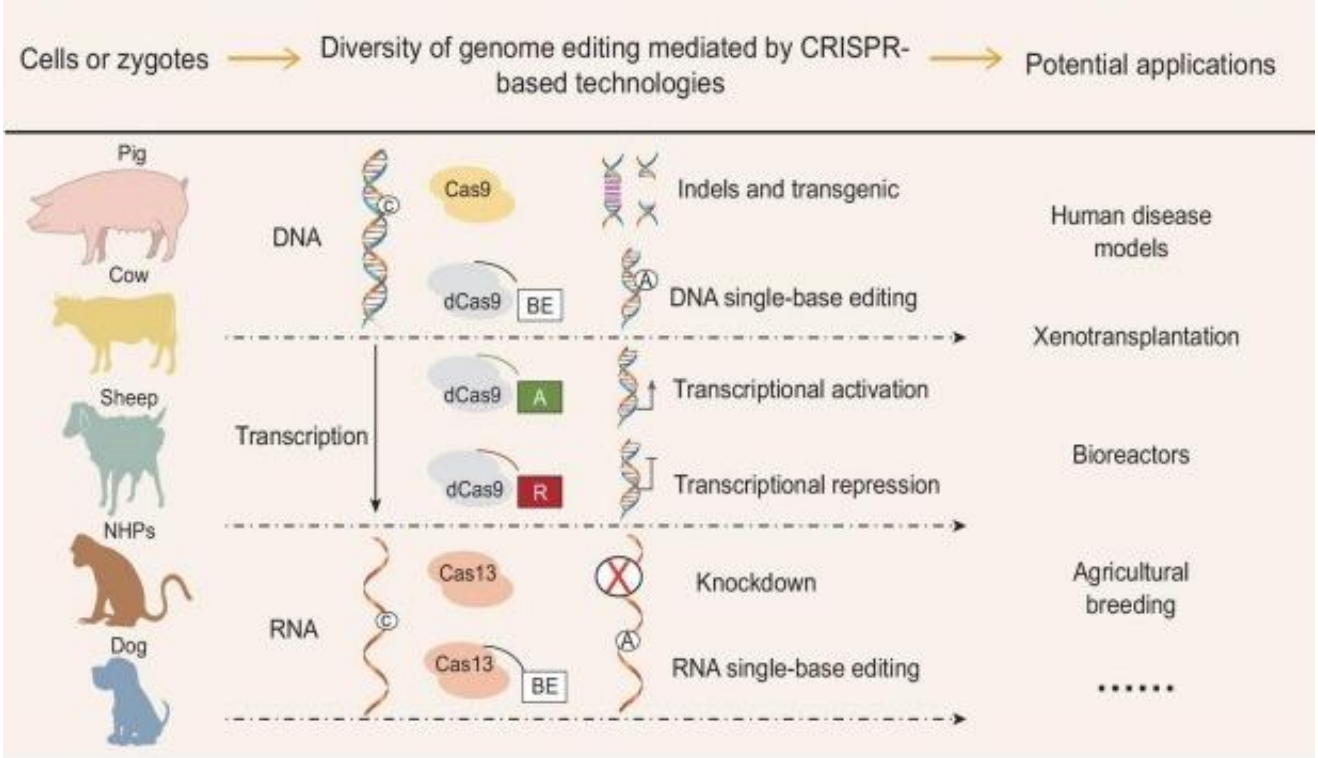
-The result is Hybridmoa cells These cells can divide and produce antibodies.



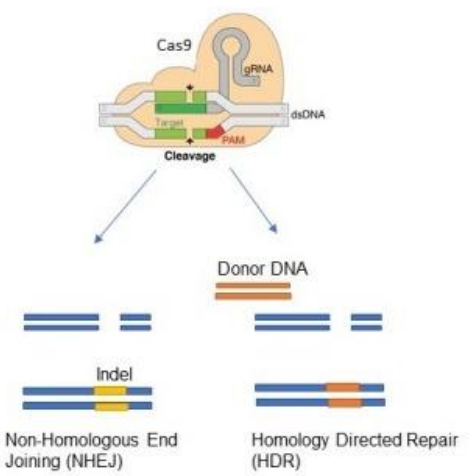
Mab : Monoclona anti-bodies

-CRISPR/Cas technology in animals

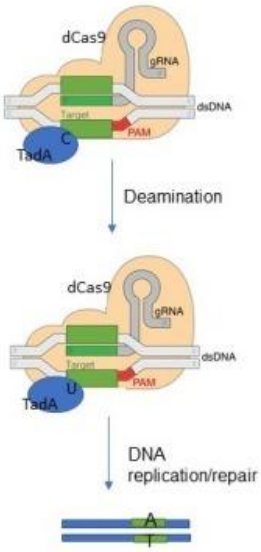
•Major strategies to recruit DNA- and RNA-targeting and modifying enzymes via the CRISPR/Cas systems



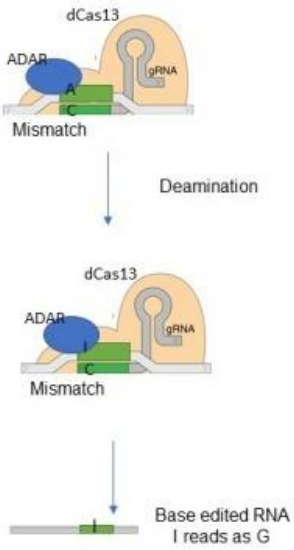
- Base-editing by CRISPR/Cas systems



GENE editing with CRISPR



DNA base editing



RNA base editing

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