

## 7.1 Animals in Research

- Zebrafish are about 3 cm in length, so can house large numbers in small spaces → small , grow in large quantities
- About 3 months between generations
- Average of 200 progeny per female
  - useful to study different stages of
- Embryogenesis is completed in about 120 hours
  - Gut, liver and kidneys developed in the first 48-72 hours
  - their eggs are transparent & can't be easily studied on microscopes
- Can test drugs for toxicity or adverse effects in 5 days
  - useful for

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## 7.1 Animals in Research

- Rats **superior to mice for early drug toxicity tests**
  - larger size from mice, so we can see their organs & tissue more easily
  - closer to us in term of their physiology & biochemistry, responses to drugs similar to us
    - have more human like responses to drugs
    - larger size facilitates surgical and physiological experimentation
    - more toxicological data has been collected so better understood

## 7.1 Animals in Research

- Cats, dogs, and primates are used in specific instances when their particular biology is important to research
  - The lung and cardiovascular systems of dogs are similar to those of humans
  - Monkeys and chimpanzees are the only known animals that share humans' vulnerability to HIV
- The numbers of these animals used in experiments has been declining for the past 20 years

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\* It takes 12 years on average  
cost to prove a drug & on average it costs 1 billion dollar to develop a drug  
\* drug testing very long expensive process

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 an 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 in 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	File NDA at FDA	Review process/approval
Success rate	5,000 compounds evaluated		5 enter trials	make sure it's safe	1 approved	Additional testing after approval required by FDA

Source: [www.fda.gov/cder/handbook/develop.htm](http://www.fda.gov/cder/handbook/develop.htm)

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## 7.1 Animals in Research

### • Alternatives to Animal Models

#### 1. Cell Culture

- Preliminary screen <sup>early</sup> 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
  - develop to try to simulate how the drug behave or <sup>© 2013 Pearson Education, Inc.</sup> be mutabilize based on their molecular & chemical structure once inside our bodies

## 7.2 Cloning

fusing adult cell with an egg lacking nucleus

- In 1997, the first animal was cloned from the nucleus of another adult animal
  - Dolly the sheep
- Created controversy over the possibility of human cloning

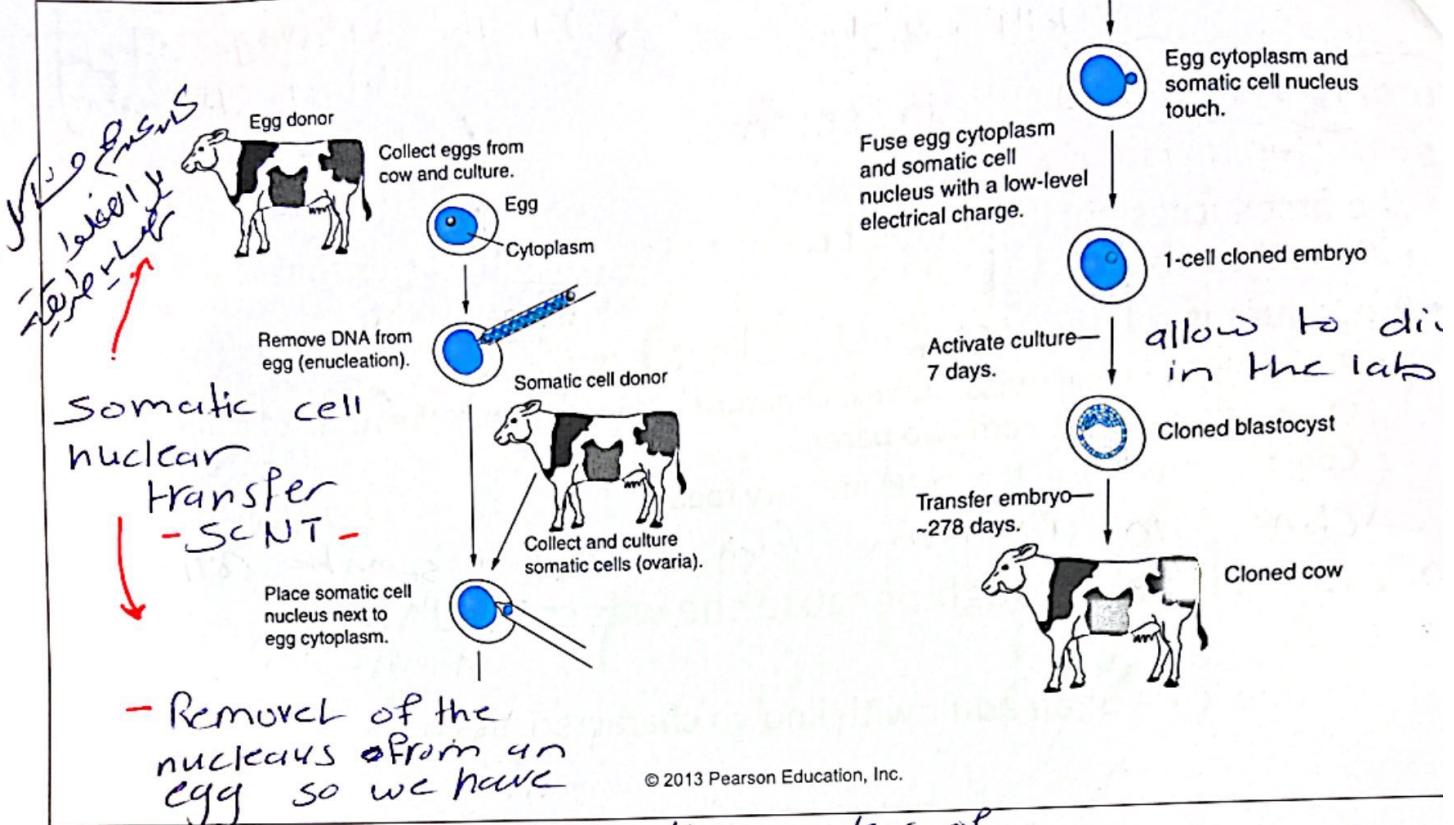
## 1.2 Cloning

### • Embryo Twinning

→ splitting embryos in half

- First step toward cloning
- The first successful experiment produced two health calves that were identical twins
- Procedure is relatively easy, but has limited applications
  - Results in identical twins, but exact nature of those twins is the result of a mix of genetic material from two parents
  - Common practice in the cattle industry today

- Clone an organism from a diploid somatic cell
- Dolly was a breakthrough because she was created from an adult cell
    - Exact duplicate of an adult with known characteristics



innucleate egg, then the nucleus of a somatic cell donor - diploid adult cell - will donate 2n nucleus that will be added to innucleate egg. Now we have the zygote that will contain only genetic material of the donor. allows to divide in the lab

## 7.2 Cloning

- The Limits to Cloning
    - Donor cell must come from a living organism
    - Clones are not exactly identical because of environmental factors
      - Shaped by experiences and environments
    - Present success rate is quite low
      - Dolly was result of 277 efforts
      - Carbon Copy (Cc) was only success out of 87 implanted clone embryos
    - Clones may be old before their time
      - Shortened telomeres
- Yours truly*
- 4pt ✓*



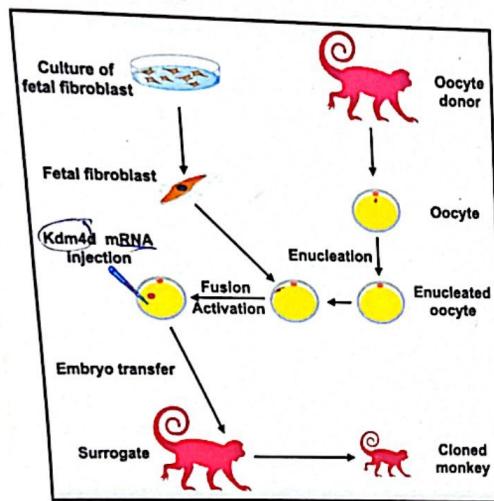
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They also injected into the fused cell an mRNA & it code demethylase enzyme & treating with a chemical called trichostatin  $\alpha$  (inhibit enzyme histone deacetylase)

Cell

# Cloning of Macaque Monkeys by Somatic Cell Nuclear Transfer

## Graphical Abstract



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## In Brief

Generation of cloned cynomolgus monkeys by somatic cell nuclear transfer using fetal monkey fibroblasts.

## Highlights

- Somatic cell nuclear transfer (SCNT) using fetal fibroblasts yielded two live monkeys
  - Epigenetic modulators promoted development and pregnancy rate of SCNT embryos
  - SCNT using adult cumulus cells yielded live births of monkeys that were short-lived
  - Genetic analysis confirmed the clonal origin of the SCNT monkey offspring



تقریباً عَنْهُ SCNT میں اسے عین عالیٰ سطح پہنچ لیتاً افروز کو donor nucleus میں ملے جائے۔

oocyte donor oocyte اخذ و می کنند Pat 2 fibroblast

~~♂~~ fused nucleus of female  
fused nucleus from the donor into  
innucleated egg then they made

longer sie  
telomērs  
 $\rightarrow \approx 1\%$

## 7.3 Transgenic Animals

#### • Introducing New Genetic Material into A cell

### 7.3 Transgenic

#### • Introducing New Genetic Material into Animals

done  
by

##### ① • Retrovirus-mediated transgenics

- Infecting mouse embryos with retroviruses before the embryos are implanted
- Size of transgene (transferred genetic material) is limited

some viruses could be material loaded whatever genetic we want to deliver &

this viruses could be used to

infect embryos before it's implanted

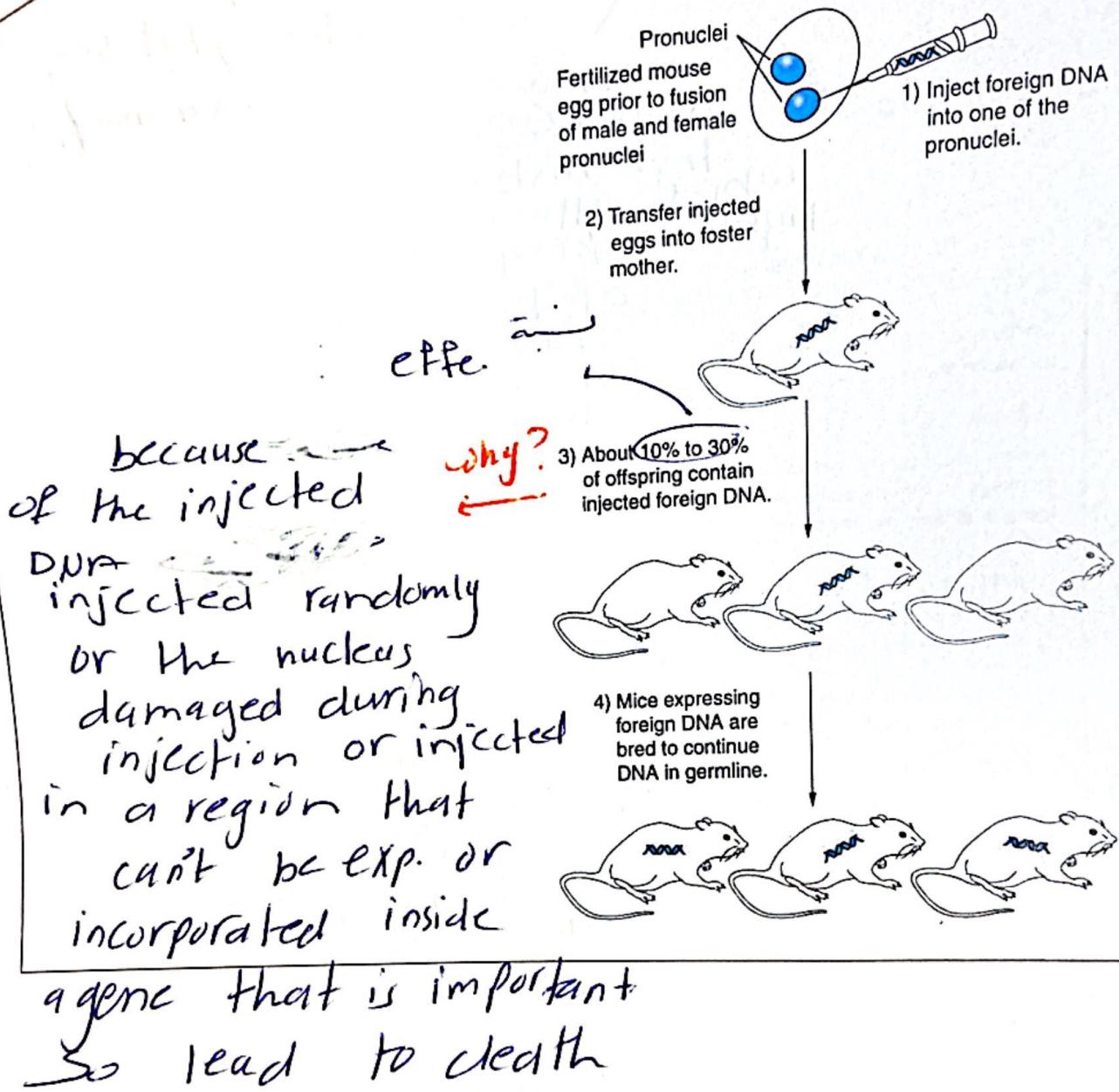
genetic material that could be introduced will be limited because these viruses can't accommodate large fragment

##### ② • Pronuclear microinjection

- Introduces the transgene DNA at the earliest possible stage of development of the zygote
- DNA is injected directly into nucleus of egg or sperm

before they are fused together

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## 7.3 Transgenic Animals

- Introducing New Genetic Material into An removing embryonic stem - cell in t

## 7.3 Transgenic Animals

- Introducing New Genetic Material into Animals

(3) • Embryonic stem cell method  
removing embryonic stem cell in the lab from the embryo & mix it with PMA & bring it back

- Embryonic stem cells are mixed with DNA and will absorb the DNA

(4) • Sperm-mediated transfer  
on the surface of sperm there is proteins called linker protein

- Uses "linker proteins" to attach DNA to sperm cells

(5) • Gene guns can also be used on animal cells  
- lower eff. technique  
~~DNA jets to sperm use is~~

## 7.3 Transgenic Animals

- ② • Gene transfer can produce healthier foods
- Tweaking the genes responsible for cholesterol production could result in healthier, lower-cholesterol eggs
  - Herman, a bull produced by transgenics nuclear transfer carries the human gene for lactoferrin which increases the availability of iron in milk

↳ protein that  
can bind iron

the daughter of herman will  
be a cow that will produce a milk  
rich in lactoferrin → milk rich  
in iron

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Scientist transfert a gene called lysostaphin  
into the genome of cattle/cows, this enzyme  
can attack the cell wall *S. aureus* & kill it which  
decrease the chance that the cows  
will have mastitis

### 3 Transgenic Animals

Animals can be engineered with genes that make them resistant  
to diseases

- The U.S. Department of Agriculture in Maryland created transgenic dairy cows that could resist mastitis done by
  - Highly contagious condition caused by the bacterium *S. aureus*
- The transgenic cattle have a gene that produces lysostaphin which kills *S. aureus*
  - Preliminary data show some resistance, though may not be fully protected

as it's  
production  
of  
milk

Some animals carry  
out naturally pathogens  
like chicken  
 salmonella

to farm animals  
food poisoning<sup>e</sup>

## 7.3 Transgenic Animals

### • Transgenic Animals as Bioreactors

- Biosteel may be used to strengthen bulletproof vests and suture silk
  - Spiderweb protein, one of the strongest givers on earth has been transferred to goats where it is expressed in their milk
  - A human gene, Atryn, is needed by patients with a hereditary clotting deficiency

scientist have successfully produced biosteel

a spiderweb protein  
in the milk of goats

anti-thrombin → Thrombin → clotting Factor

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

(Atryn) A company called GTC successfully introduced human gene encoding a protein called antithrombin, & this gene was cloned into the cells of the goat under the control of a mammary specific promoter. So Mammary tissue will express human gene &

### 3 Transgenic Animals

#### knockouts: A Special Case of Transgenics

- Mice that have been genetically engineered so that a specific gene is disrupted for research or to make disease animal model
  - DNA is modified and added to the embryonic stem cells, where it recombines with the existing gene on a chromosome
    - Called homologous recombination
  - Modified ES cells are introduced into normal embryo and embryo is implanted into a mother

A special case of  
Transgenic is knockout  
removing disrupting  
a gene in an  
animal

This  
could be  
done by the  
help of  
embryonic  
stem  
cell

## .3 Transgenic Animals

### Knockouts: A Special Case of Transgenesis

- The mouse pup is a **chimera** – some cells are from knockouts
- Two generations of breeding are required to get pure knockouts

Knock-in animals – have a human gene in their genome with their own counterpart

→ An animal gene is replaced with the same gene from another animal

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## \* Research animal Model

→ like mouse, to study certain mechanism --

## \* Disease animal Model

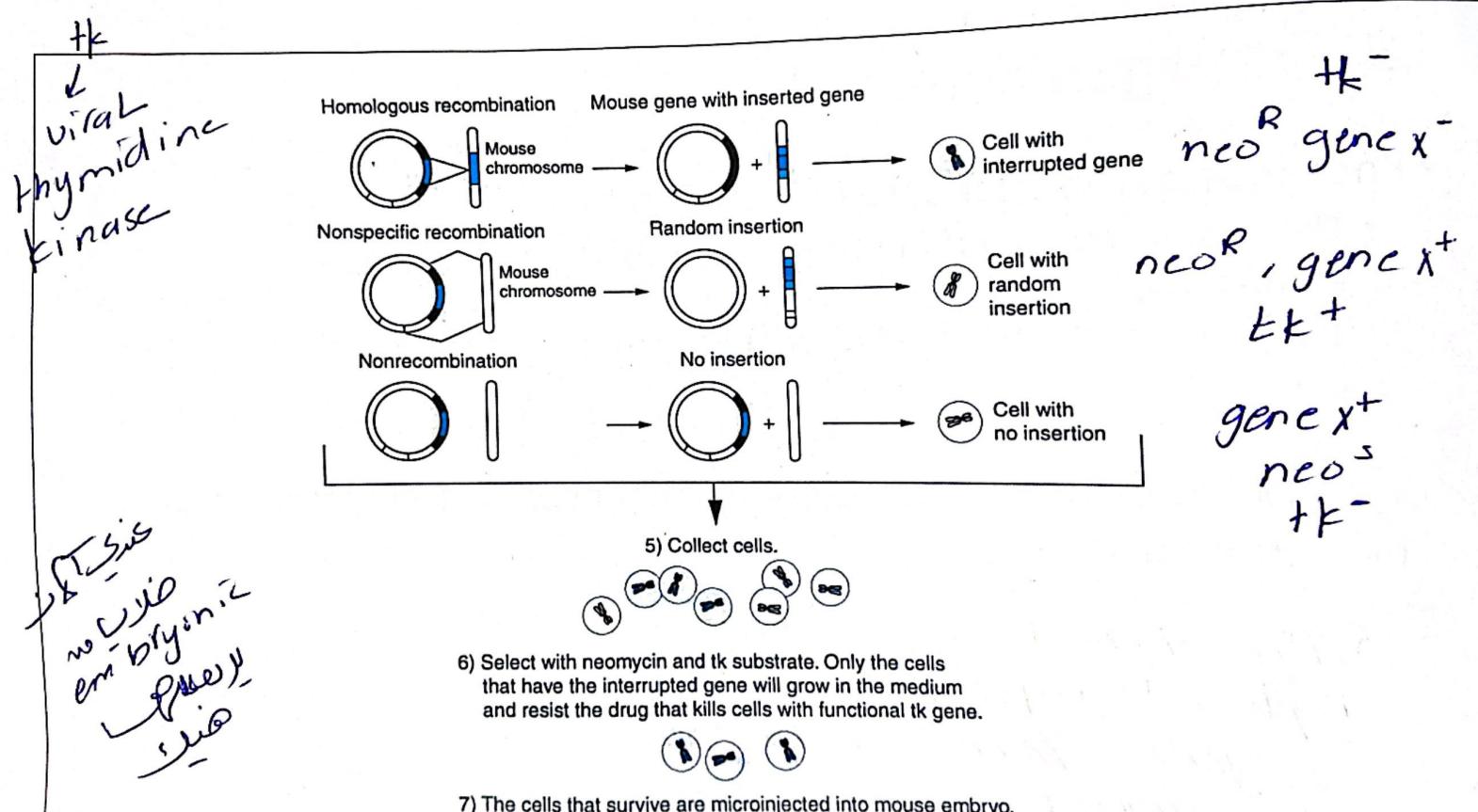
- we have zoonotic diseases

these are disease could be share, transfer from animals to human

• First thing is to do testing on cells in the tissue culture, Then if it's not toxic to cells they will try it on animals for the purpose of that is to assess safety & biological activity

Then → If it's safe & active the company developed the drug will file IND to FDA to give them approve to test it on humans

\* human tests go through 3 phases



\* Generating a knockout begins by obtaining an embryo at blastocyst stage

this embryo will contain an inner cell mass that will contain embryonic stem cell & this embryonic stem cell will give rise to all cells of the mice

The first stage is to take some of these embryonic stem cells & grow them in the lab in tissue culture, then we have to design a plasmid & this plasmid will allow us to disrupt the gene that we want to remove

Note → the plasmid should have homologous sequencing that is identical to the sequencing within gene X

here in this example region - a - from gene X will be cloned & region - b - & between them we could add a marker gene neomycin

& in the plasmid there a gene called thymidine kinase

so this plasmid could be introduced to embryonic stem cell by electroporation & once it's inside the nucleus

We will have homologous Recombination

will allow the exchange of the gene X with  $\text{neo}^R$

Ex: Lysyl + His -

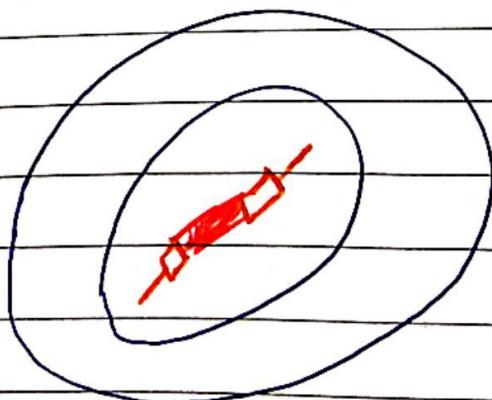
homologous  $\rightarrow$   $\text{Lysyl} \rightarrow \text{His}$

crossover  $\text{neo}^S$  is a homo. 'a'  
" " " " b " " b'

So the embryonic stem cell after homologous recombination will look like this

before homologous recombination  
it was  $\text{neo}^S$  & gene X<sup>+</sup>

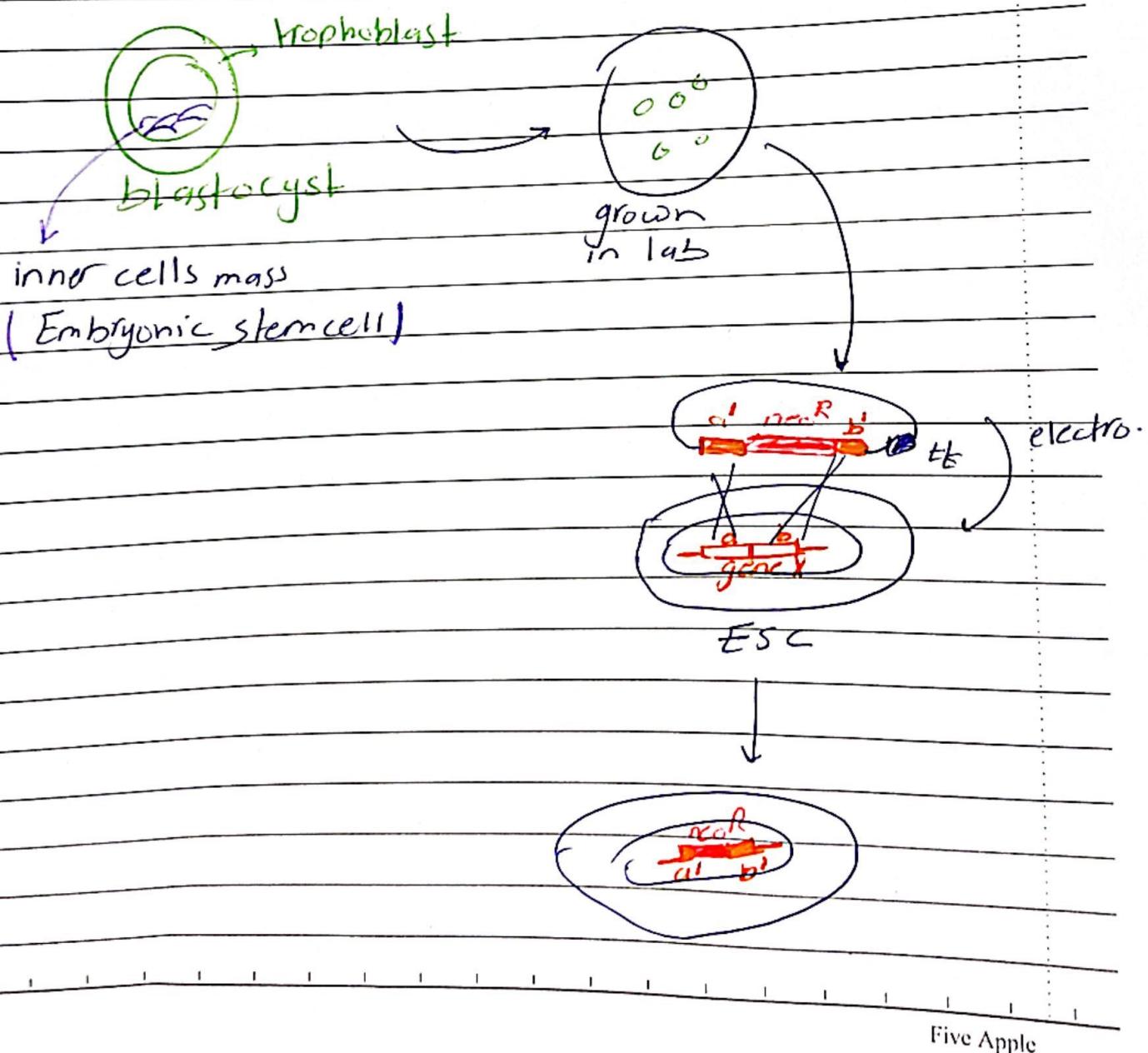
& after successful homologous recombination  
 $\text{neo}^R$  & gene X<sup>-</sup>



~~is called Recombination module -~~  
~~Nonrecombination~~

~~neo<sup>R</sup> LoxP loxP gene insertion~~

~~Donspecific Recombination module -~~  
~~cells with random insertion~~



Five Apple

~~using lipid selection, since lipid~~  
~~contains lipid~~  
neomycin & tk substrate (acyclovir)

↓  
"ganciclovir"

Analogue

& if it's incorporated

the DNA replication

will be stopped because

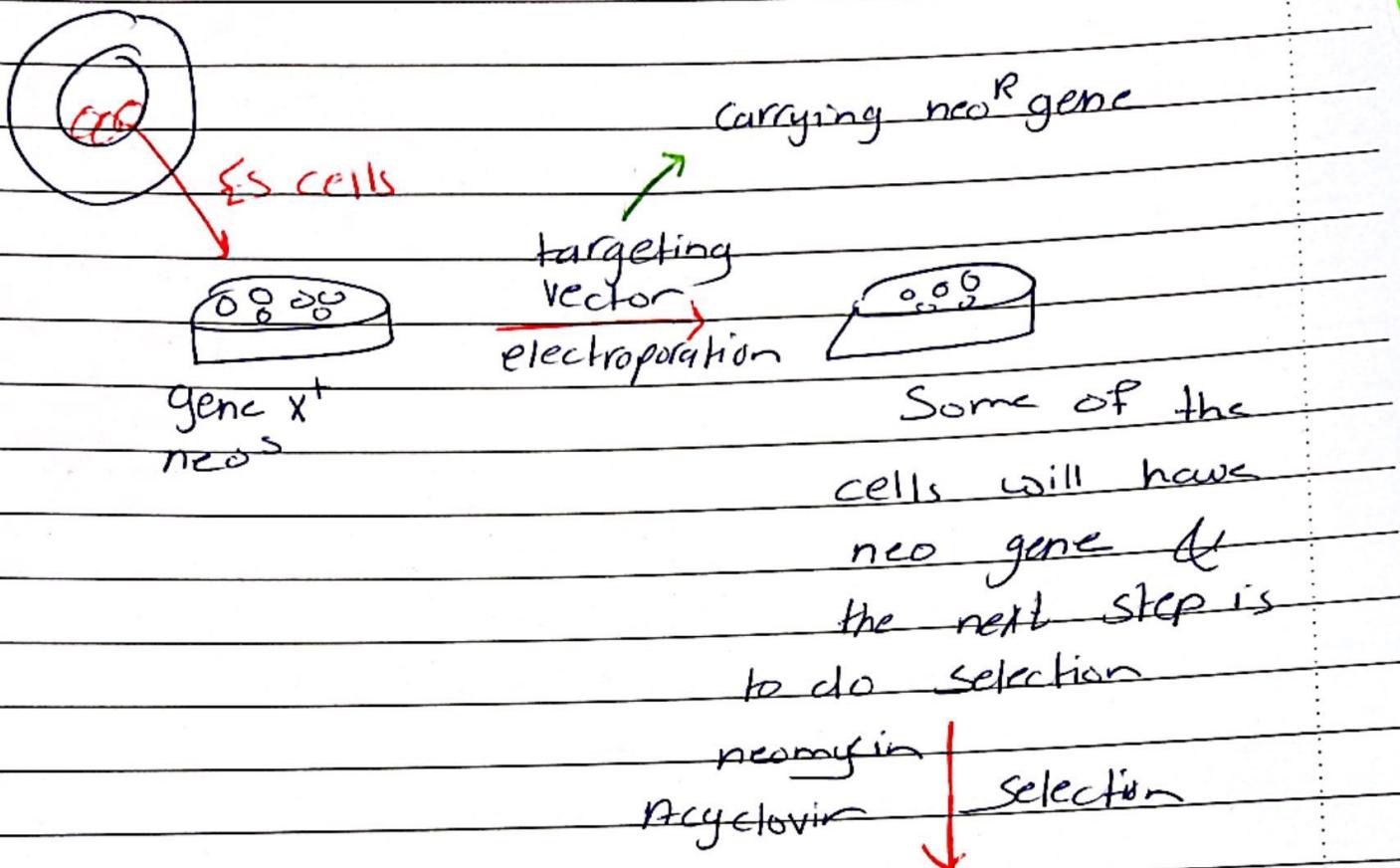
DNA poly doesn't recognize  
this "ganciclovir - False nucleotide -"

- neomycin will kill nonrecombination
- tk substrate will kill nonspecific recombination

- After selection you bring back the cells to inner cell mass

& now we have in the blastocyst  
two types of cells

gene  $x^+$       gene  $x^-$   
 $neo^S$        $neo^R$



Some of the cells will be brought back to ES cells & then we take the embryo & put it inside a foster mother.

Only the cells that have the  $\text{neo}$  gene inside them will survive.

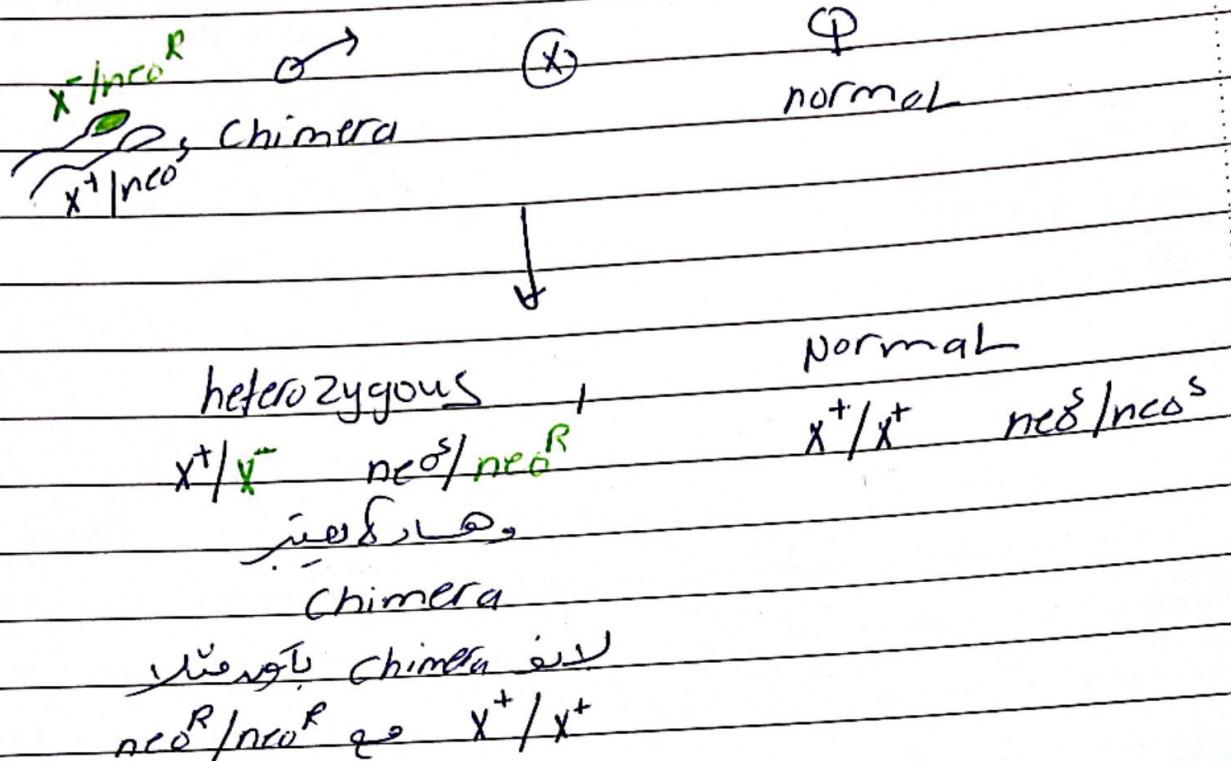
$\text{genex}^-$ ,  $\text{neo}^R$

Offspring will be born.

Will have some of their cells  $\text{neo}$  gene & some aren't - chimera mouse.

Normal mouse  $\Rightarrow$  Chimera mouse.

Chimera mouse  $\Rightarrow$  Embryo.



## وَكُنْتَ بِخَالِدٍ نَّوِي

$X^-X^-$

homozygous/knock out mouse

\* knot down

gene remain intact but  
not expressed

## RPAi , CRisPR-cas

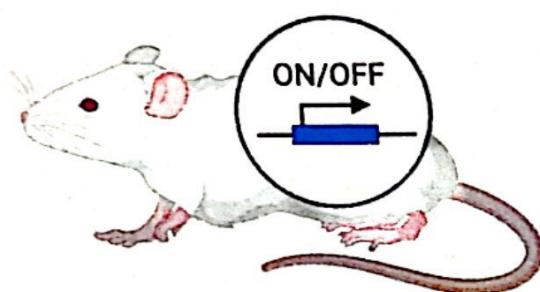
~~new out new checkout? in  
return~~

# Genetic and Genomic Variations (Mouse Model)

Control exp. of

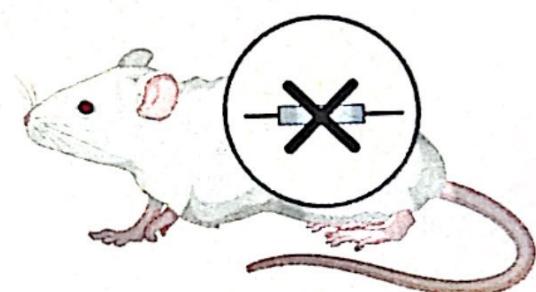
## Inducible and conditional

Temporal or spatial control of gene expression



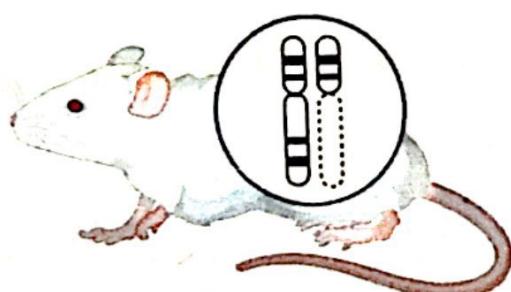
## Knockout

Functionally delete a gene



## Chromosome engineered

A chromosome region is duplicated or deleted

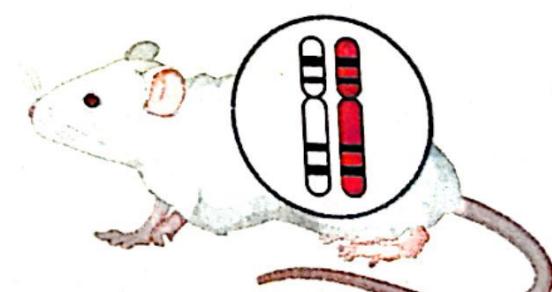


delete  
some  
region

biorender

## Transchromosomal model

Human chromosome added to the mouse genome



introduce  
whole  
chromosome

