



# GENES OUT, VIRUS IN

## Making designer mice

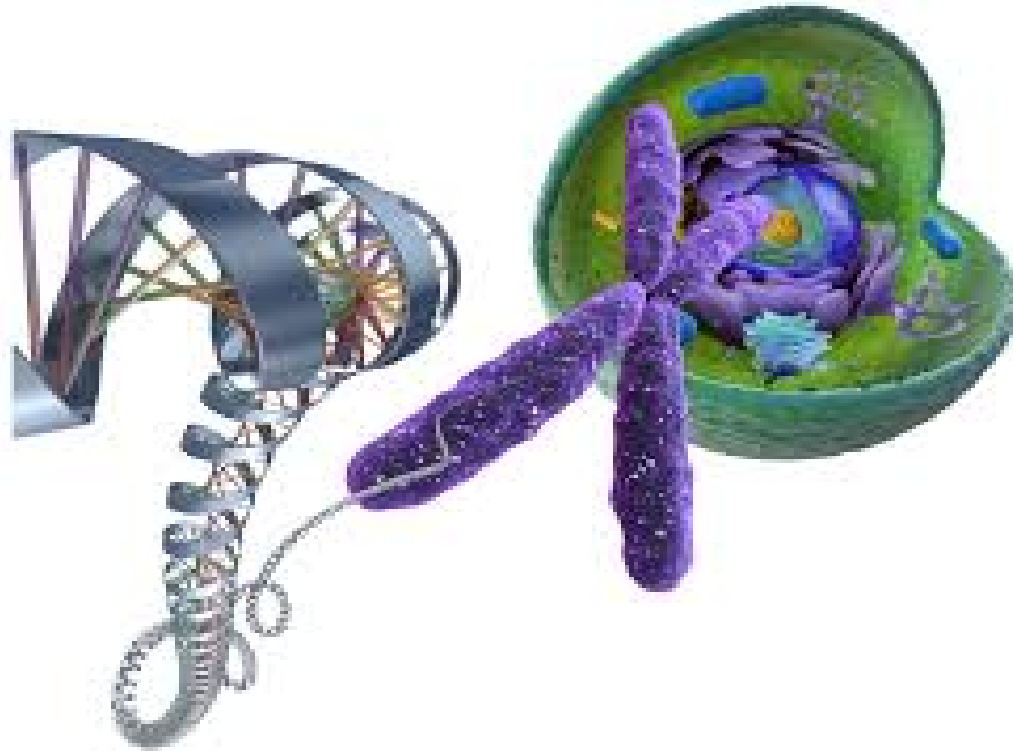
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**Department of Neuroscience**  
**Philadelphia, PA**

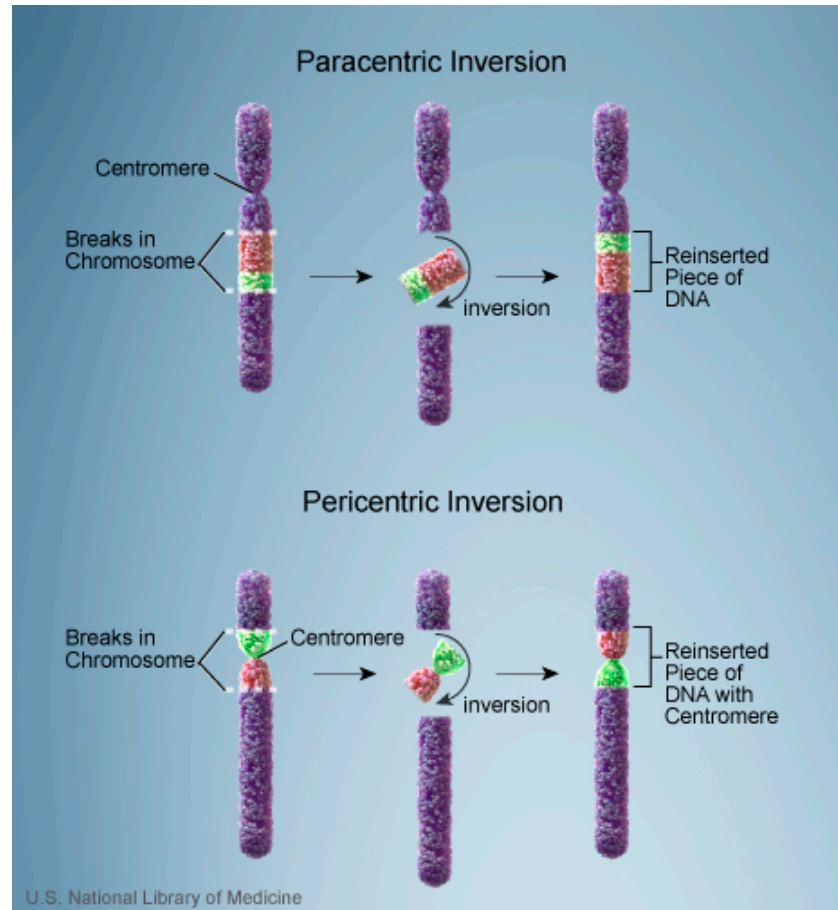
# GENES OUT





## Types of Gene Alterations

# Inversions



# Inversions

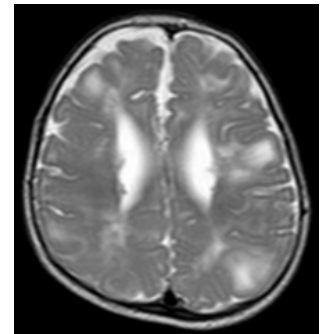
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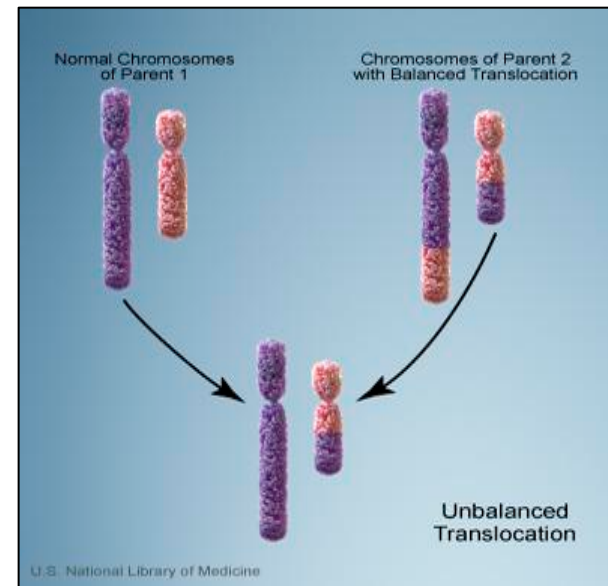
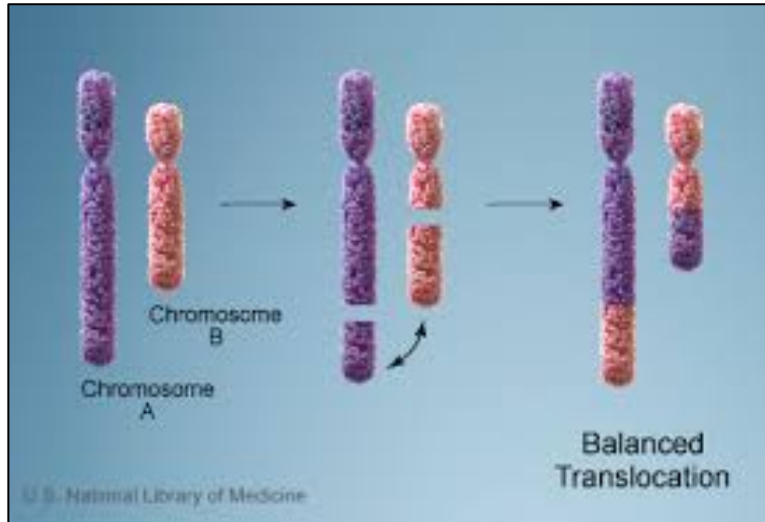


In humans, inversions most frequently occur in chromosome 9. Usually inversions are not deleterious. Make cause decreased fertility. Some genes on chromosome 9 that are disrupted cause disease.

tubersclerosis

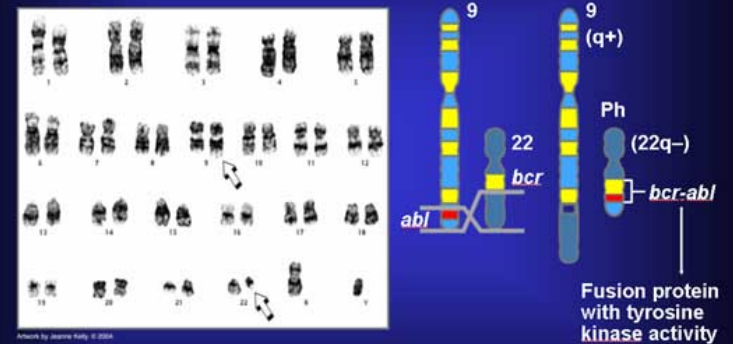
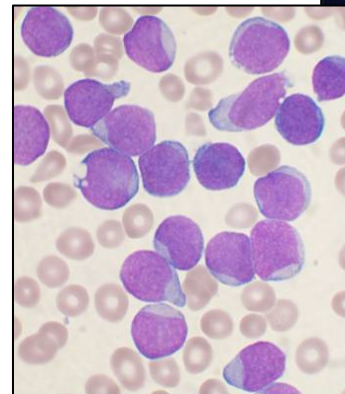


# Translocations

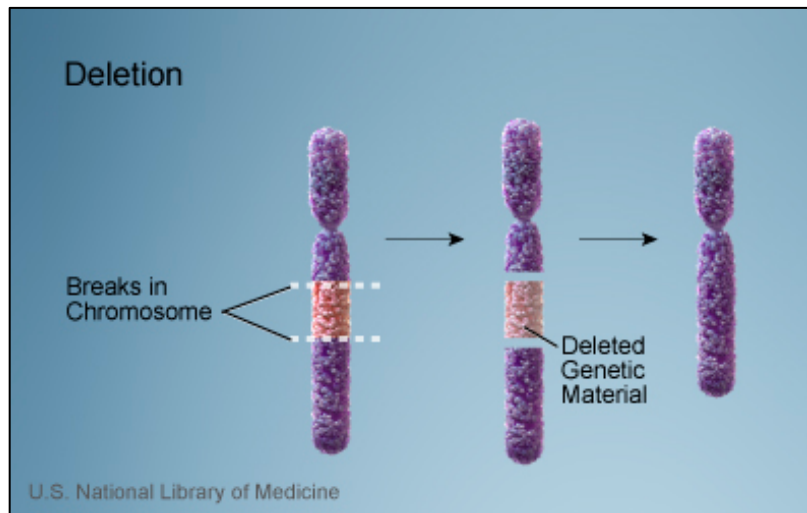


## Example: Translocation of *Bcr-Abl* Genes

Leukemia



# Deletions



**22q** DELETION  
Syndrome



# CRE-LOX Technology

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General Knockouts, Conditional  
Knockouts and Reporter Strains

# Cre-Lox requires two components

LoxP sites: specific 34 base pair sequence consisting of a core 8 bp sequence, where recombination takes place and two flanking 13 bp inverted repeats.

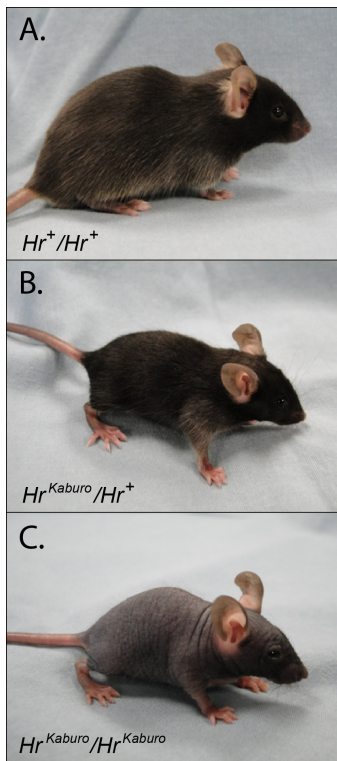


ATAACTTCGTATAGCATACATTATACGAAGTTAT

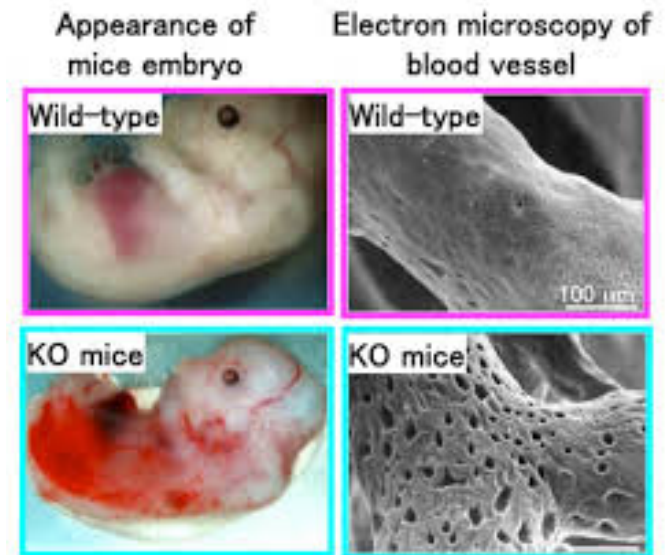
Cre-recombinase: enzyme that catalyzes recombination between two loxP sites

# Utility of Cre-Lox

- General Knockouts



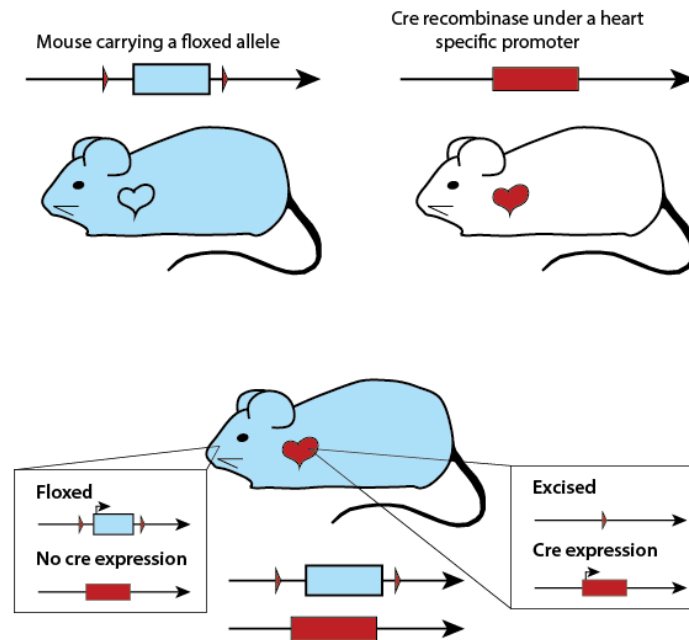
hairless



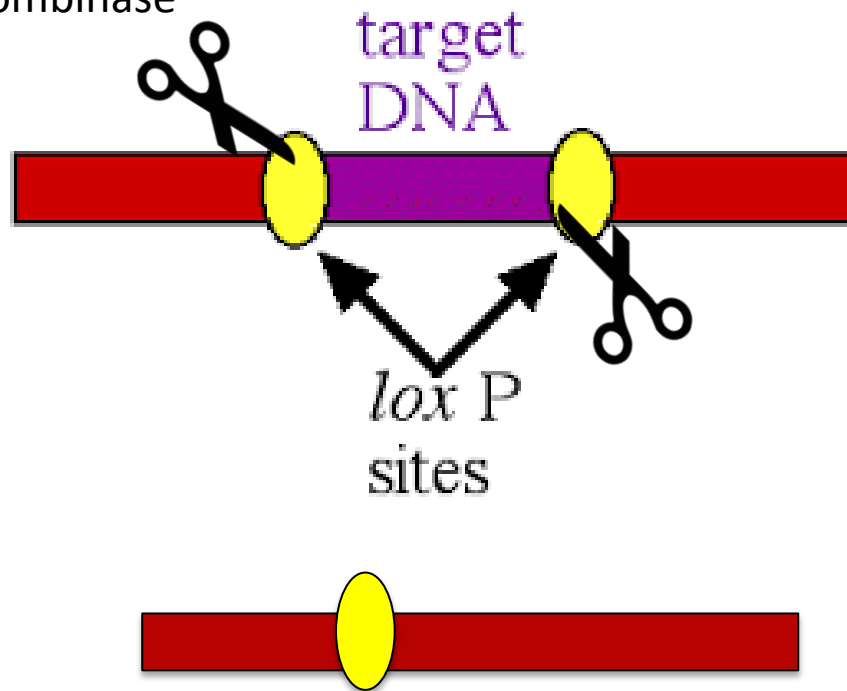
Embryonic lethal

# Utility of Cre-Lox

- Conditional Knockouts  
(cell type, stimulus induced or both)



Cre-recombinase



target  
DNA

*lox P*  
sites



# Utility of Cre-Lox

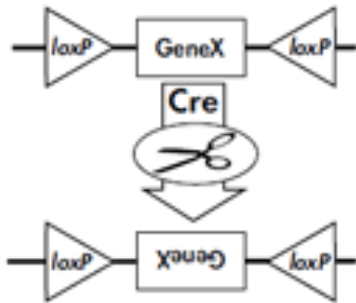
## Reporter Strains



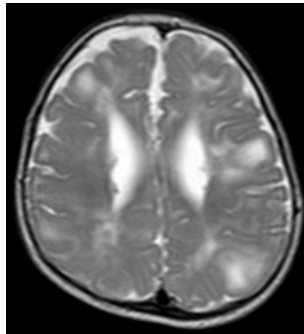
# Types of gene alterations

A

Inversion

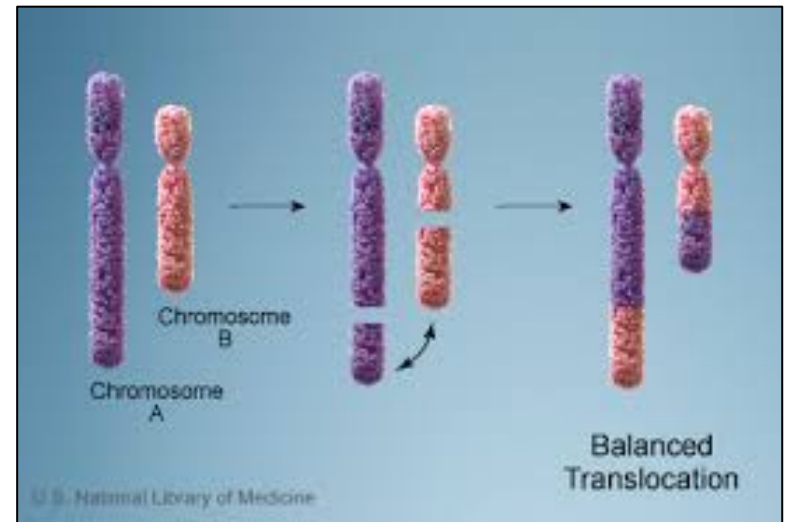


tubersclerosis



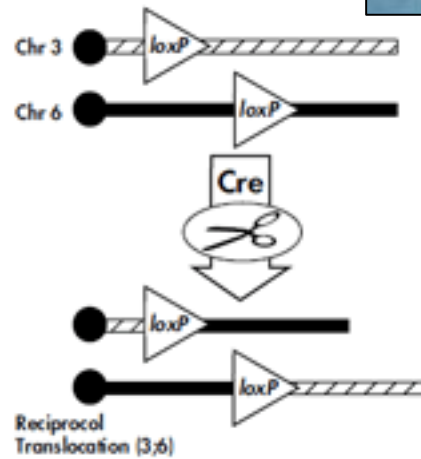
# Types of gene alterations

## Translocations



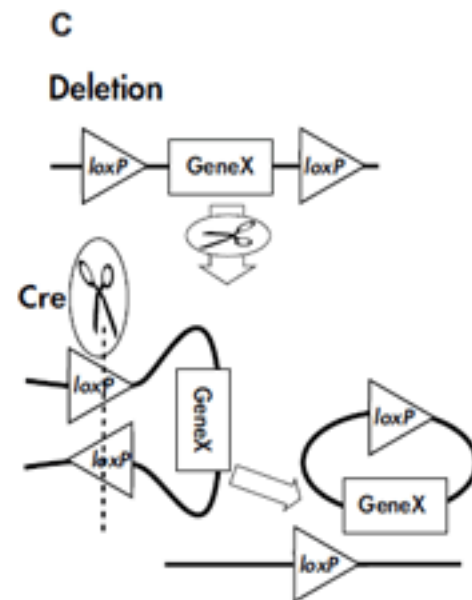
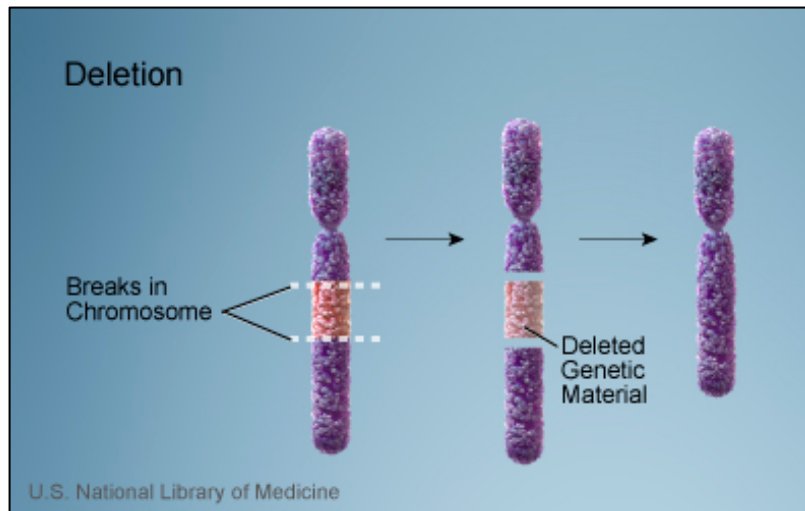
B

### Translocation

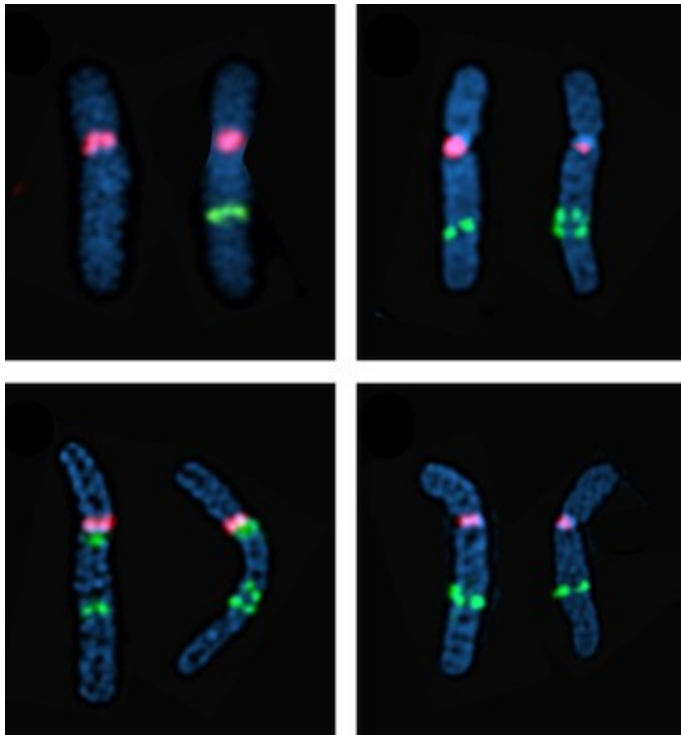




# Types of gene alterations

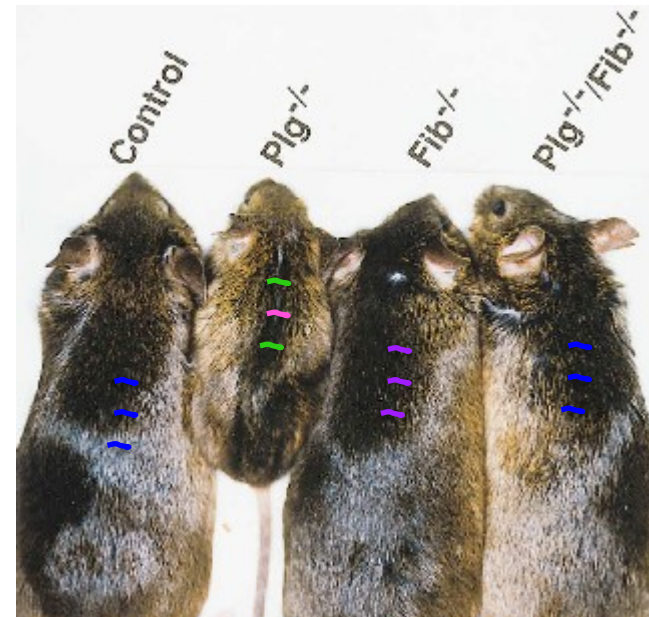
- Deletions: MOST COMMON



# Autism Research



 Hotspots for gene deletions that  
 may be associated with Autism



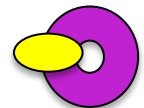
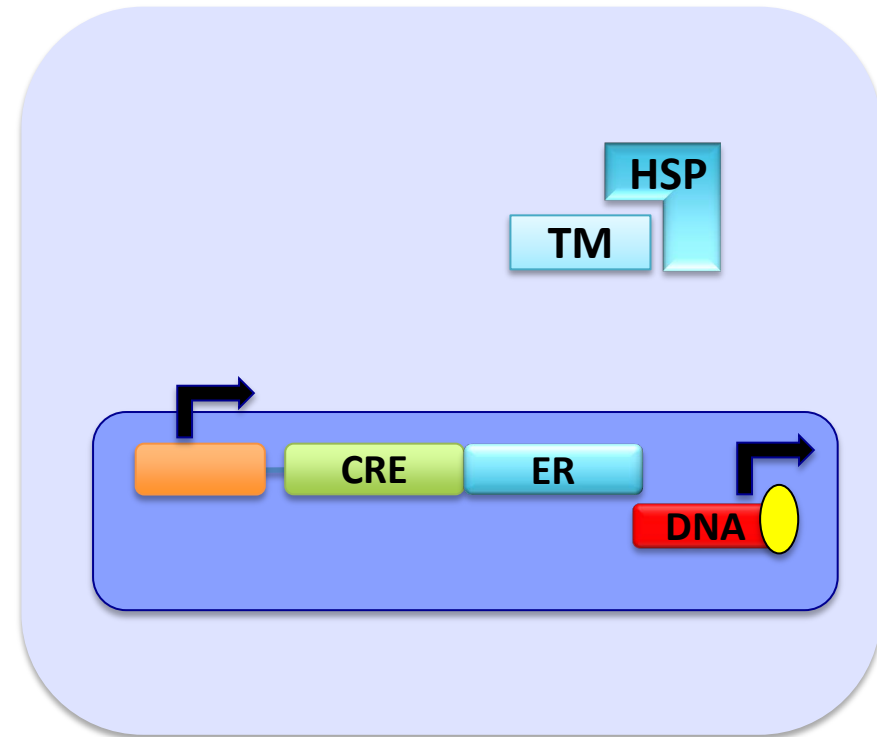
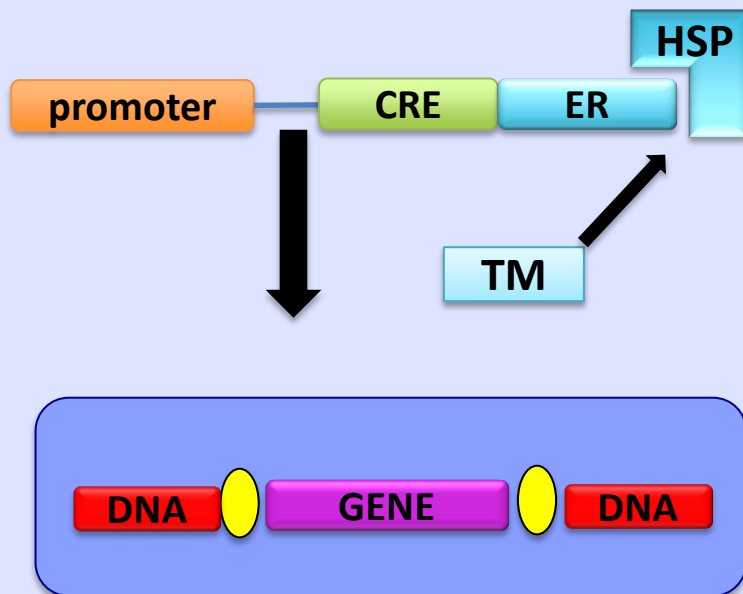
If loss of one gene is deleterious,  
knocking out a second gene may rescue phenotype

# Why do we need to use tamoxifen?

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- Cross of the floxed mouse with the cre mouse
  - Inducible and Conditional

# knockout of a particular gene in adult mice using tamoxifen



What are the hazards?

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# Tamoxifen

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Binds the Estrogen Receptor by competing with Estrogen.

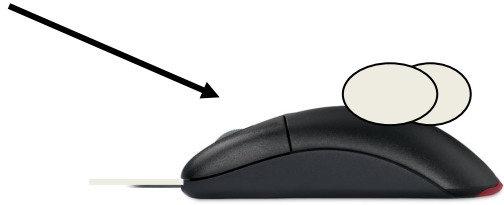
Tamoxifen is metabolized by the liver and two of the metabolites,

$\alpha$ -hydroxy-tamoxifen &

$\alpha$ -hydroxy-N-desmethyl-tamoxifen

have the potential to be hydroxylated and become highly toxic.

Tamoxifen



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Carcinogenic

Tetragenic

Genotoxic

Reproductive Toxin

# Window of risk = 72 h post injection

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Potential Routes of Exposure: aerosol, ingestion, injection, absorption through skin.

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## WHO IS AT RISK?

- Everyone who comes in contact with mice, bedding and feces
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## HOW TO MANAGE PROJECTS?

- Restricted access Trained personnel will change bedding/food /water the day of injection.
- Provide enhanced PPE for those in contact with mice.
- Yellow CARD with date of admin and all clear date.
- Cages will be taped with YELLOW chemo tape
- Will provide YELLOW bags for Chemo Waste: carcass disposal in yellow bags
- Place SIGN on DOOR

**DANGER**



## **HAZARDOUS DRUG USED IN ANIMALS**

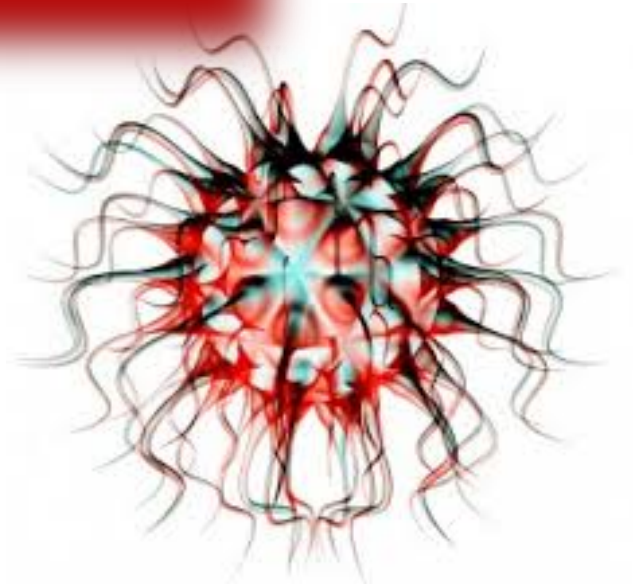
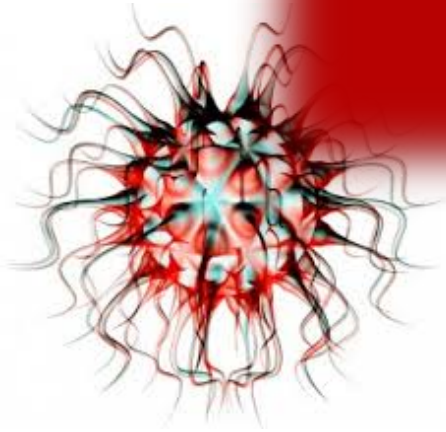
**NAME OF HAZARDOUS DRUG**     TAMOXIFEN    

**carcinogen, reproductive toxin, teratogen, may cause harm to breast-fed children, skin, eye and respiratory tract irritant, harmful if swallowed**

**Minimum PPE Requirements:** nitrile gloves, eye protection, face protection, skin protection, head cover, lab coat, shoe covers

**Additional PPE or Other Requirements:** Please refer to Manufacturer Safety Data Sheet

# VIRUS IN



# Effects of HIV on the CNS

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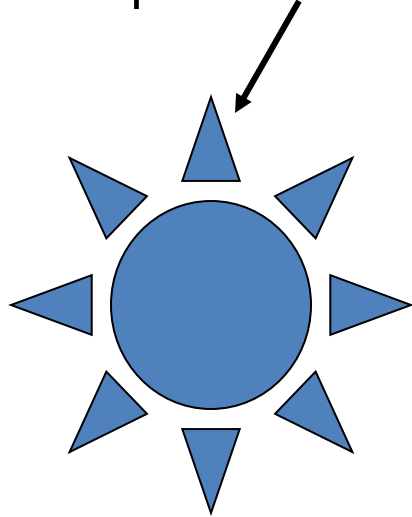
Pseudotyped HIV in the mouse  
model

# Why do we need to generate a pseudotype virus?

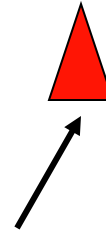
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- Biological barriers
- Cell receptors

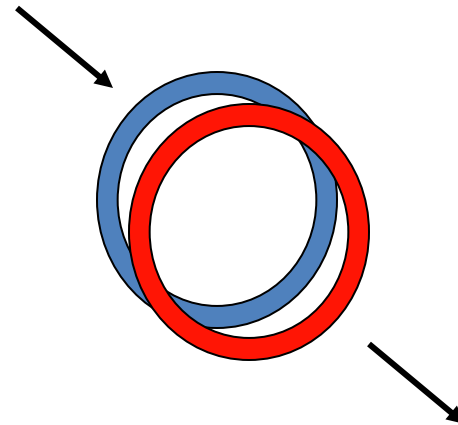
Human cell specific envelope proteins

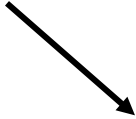
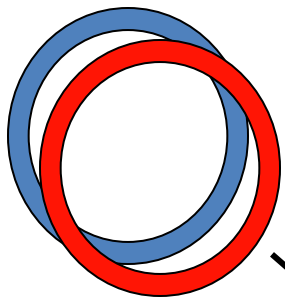


Mouse cell specific envelope protein  
(15ug plasmid DNA into 293T cells)

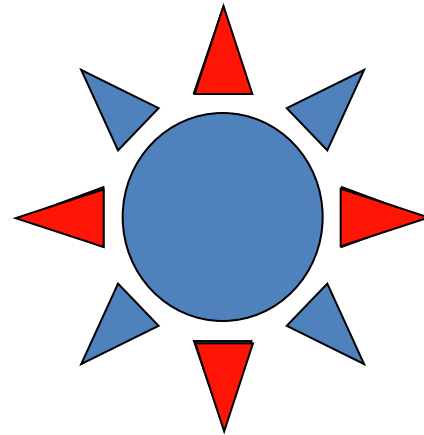
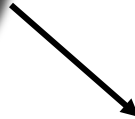
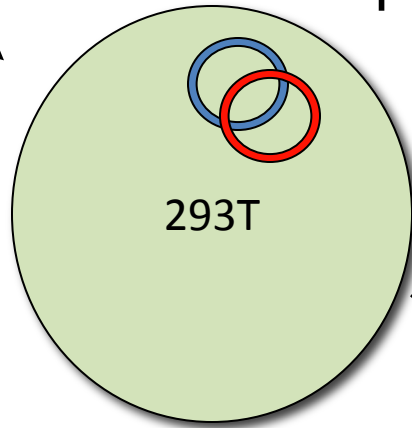


Plasmid vector driven HIV production  
via pYU2 or pYK-JRCSF  
(10ug plasmid DNA into  $1.5 \times 10^6$  293T cells)

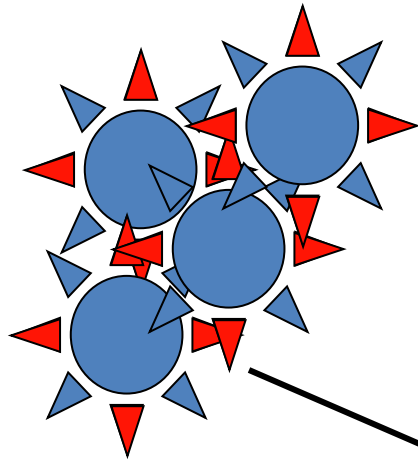




Cell permissive for transfection



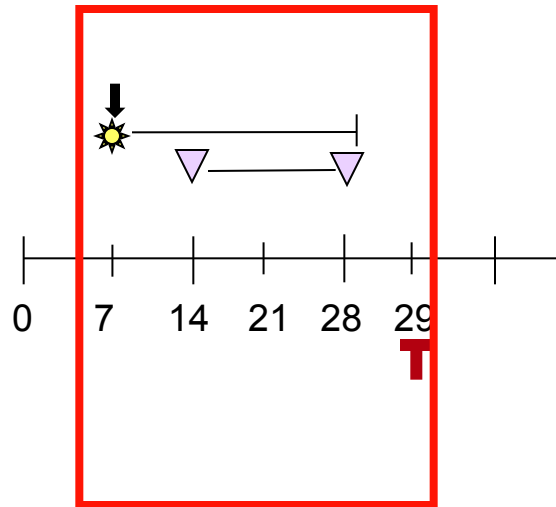
Pseudotype virus



Cell free supernatants with  $10^4$ - $10^5$  pg p24 in 0.1ml saline



Cocaine +  
Pseudotype virus



# Window of risk =22 days

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## WHO IS AT RISK?

- Everyone who comes in contact with mice

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## HOW TO MANAGE PROJECT?

- Restricted access
- Have trained personnel change bedding/food /water
- Provide enhanced PPE for those in contact with mice

**Safety First**

**OHAZARI**

