Transgenic and Knock-Out mouse models in Cancer Research

Dr.G.MATHAN
Assistant Professor
Department of Biomedical Science
Bharathidasan University
Tiruchirappalli, Tamil Nadu

Transgenesis – Definitions

- Transgenic animal – one that carries a foreign gene that has been deliberately inserted into its genome.
- **First transgenic animal produced = "Founder Animal"**
- Chimeric animal – one that carries an altered gene introduced using manipulated embryonic stem (ES) cells. Some tissues are derived from cells of the recipient blastocyst; other tissues are derived from the injected ES cells.
- Knockout mutation – replacement of a gene segment by homologous recombination that normally results in a nonfunctional or "null" allele.

Organisms utilized as transgenic models

- Arabadopsis (plant)
- C. elegans (worm)
- Fruit flies
- Xenopus (frog)
- Zebrafish
- Mice
- Rats
- Pigs
- Sheep
- Goats
- Cows

Mouse Models of Human Disease

- Physiologically similar to humans.
- Large genetic reservoir of potential models has been generated through identification of >1000 spontaneous, radiation- or chemically-induced mutant loci.
- Recent technological advances have dramatically increased our ability to create mouse models of human disease.

    1. Development of high resolution genetic and physical linkage maps of the mouse genome – facilitates identification and cloning of mouse disease loci.

    2. Transgenic technologies that allow one to ectopically express or make germline mutations in virtually any gene in the mouse genome; i.e., transgenic mice, ES cell knockouts.

    3. Methods for analyzing complex genetic diseases.

Mouse Models of Human Disease (Continued)

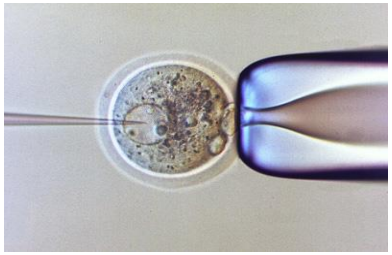
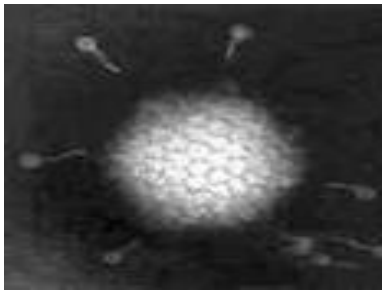
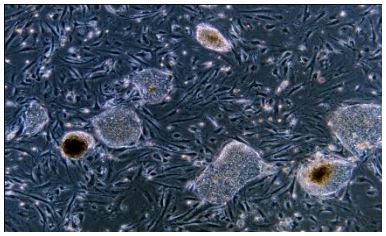
- >100 mouse models of human disease where the homologous gene has been shown to be mutated in both human and mouse.

  1. Mouse mutant phenotype very closely resembles the human disease phenotypes.

  2. Provide valuable resources to understand how the diseases develop and test ways to prevent or treat these diseases.

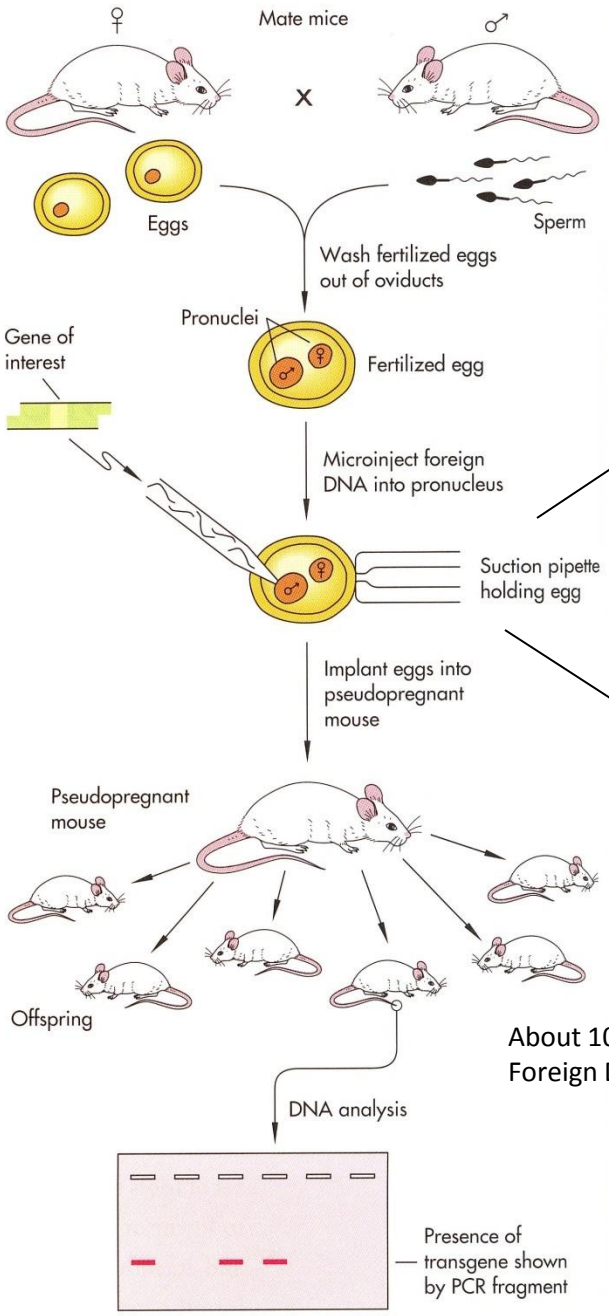
- Allow study of disease on uniform genetic background.

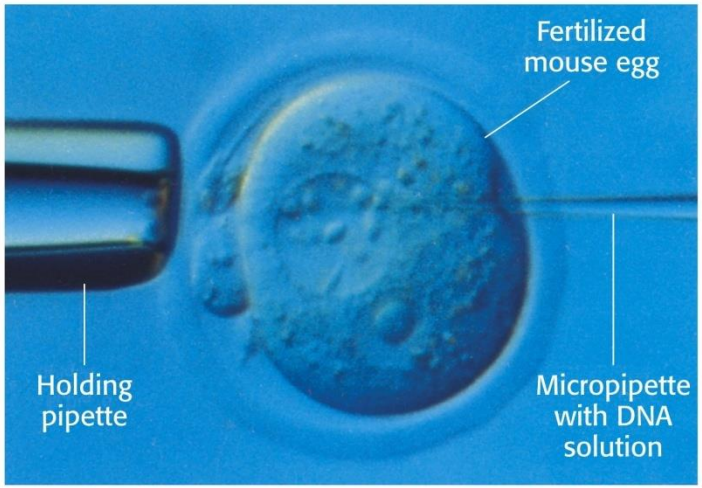
Mouse Transgenesis Methods

	pros	cons
Pronuclear microinjection 	Relatively simple and efficient Long transgenes possible Potentially all species	Random integration Multicopy insertions (Strain limitations)
Lentivral infection 	Very efficient Single copy insertions No technical equipment Works in many species	High embryo mortality 9.5 kb packaging limit Safety issues (?) Only random integration
ES based transgenesis 	Long transgenes possible Gene targeting possible Single copy insertions	Technically difficult Time consuming Species / Strain limitations

Microinjection

into the germ line -> transgenic animal



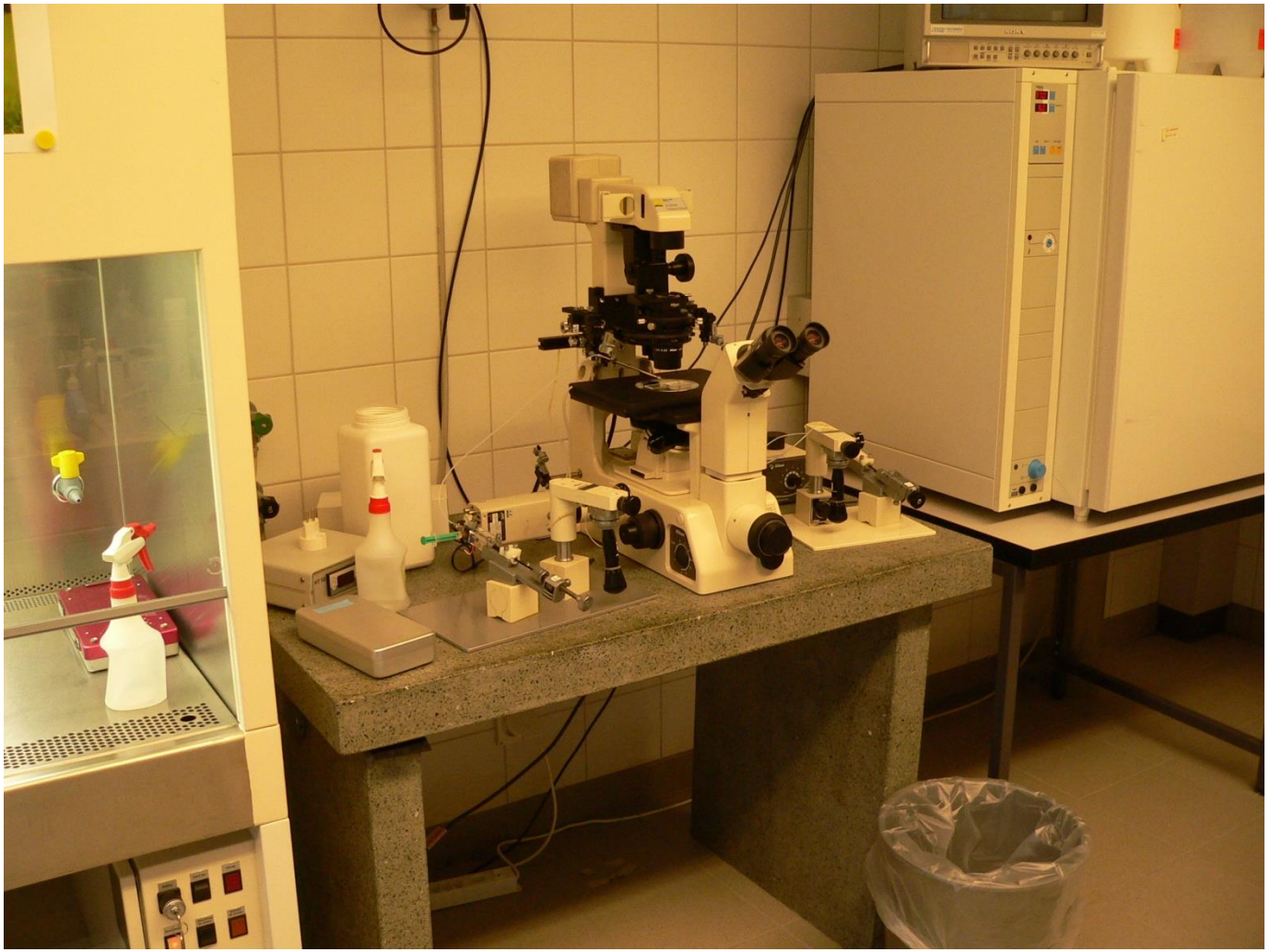


Gene injected into the male pronuclei

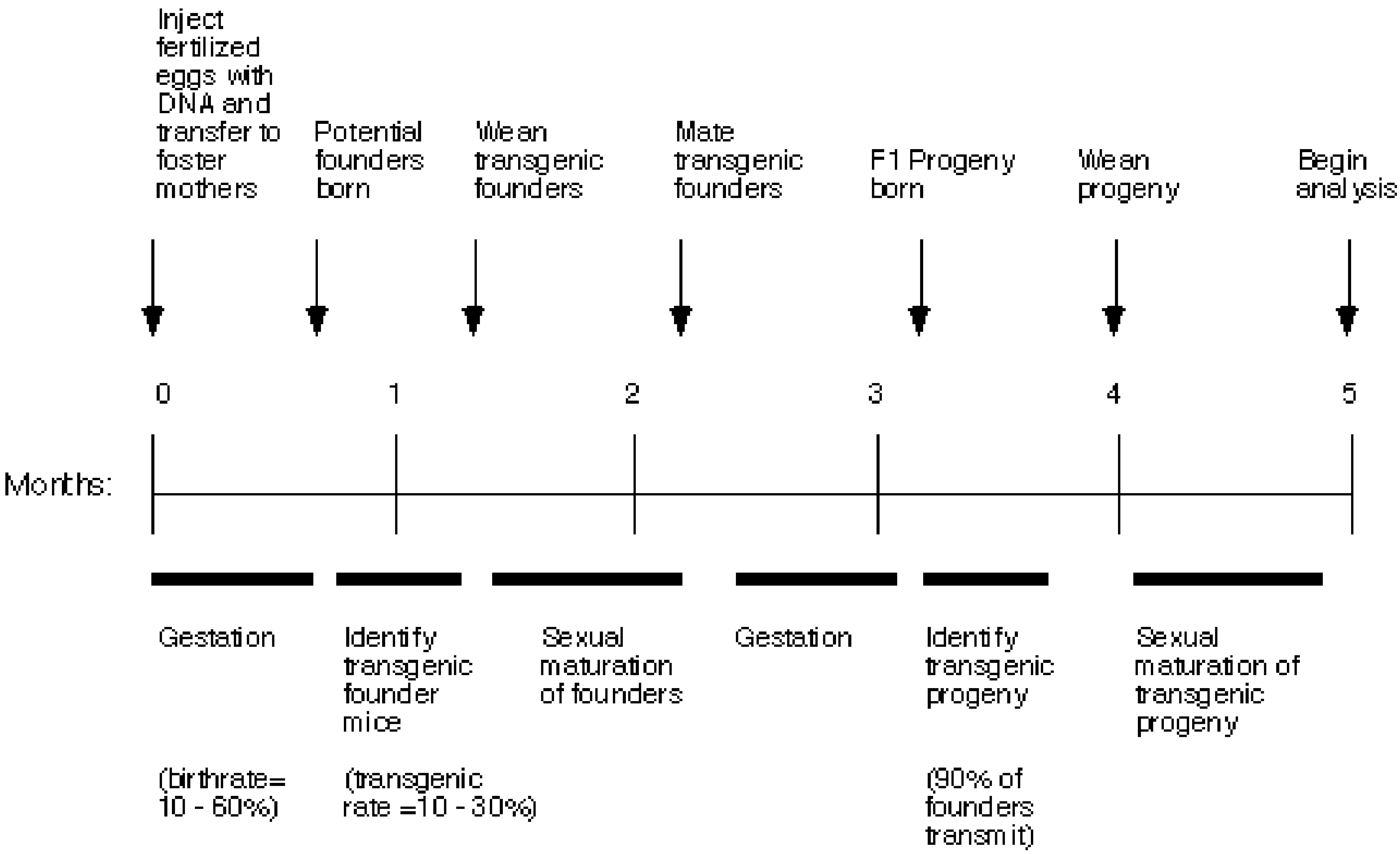
About 10%-30% of offspring contain the injected foreign DNA
Foreign DNA present equal amount in all tissues

Mice expressing foreign DNA are bread to continue DNA in germ line

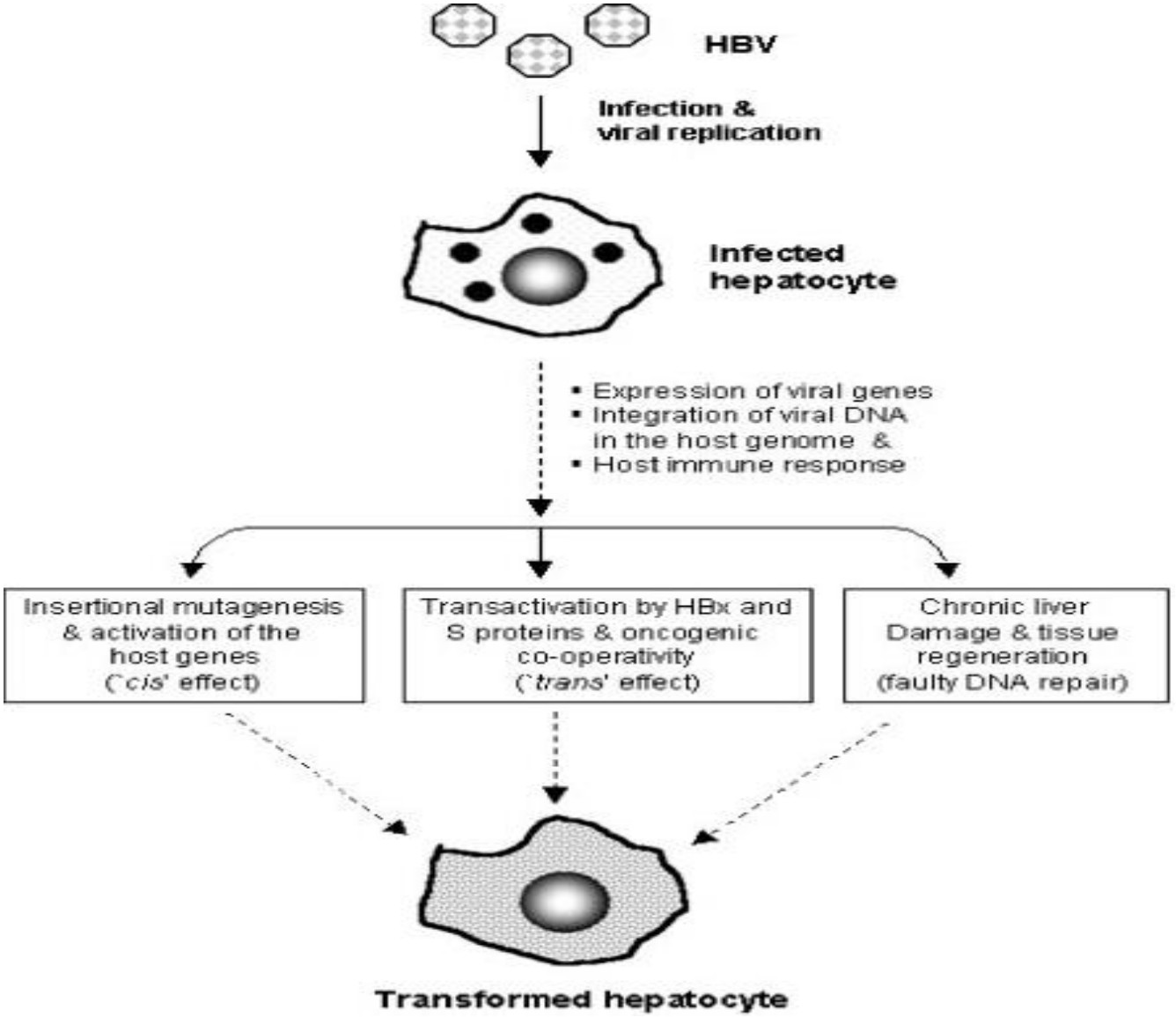
Microinjection station

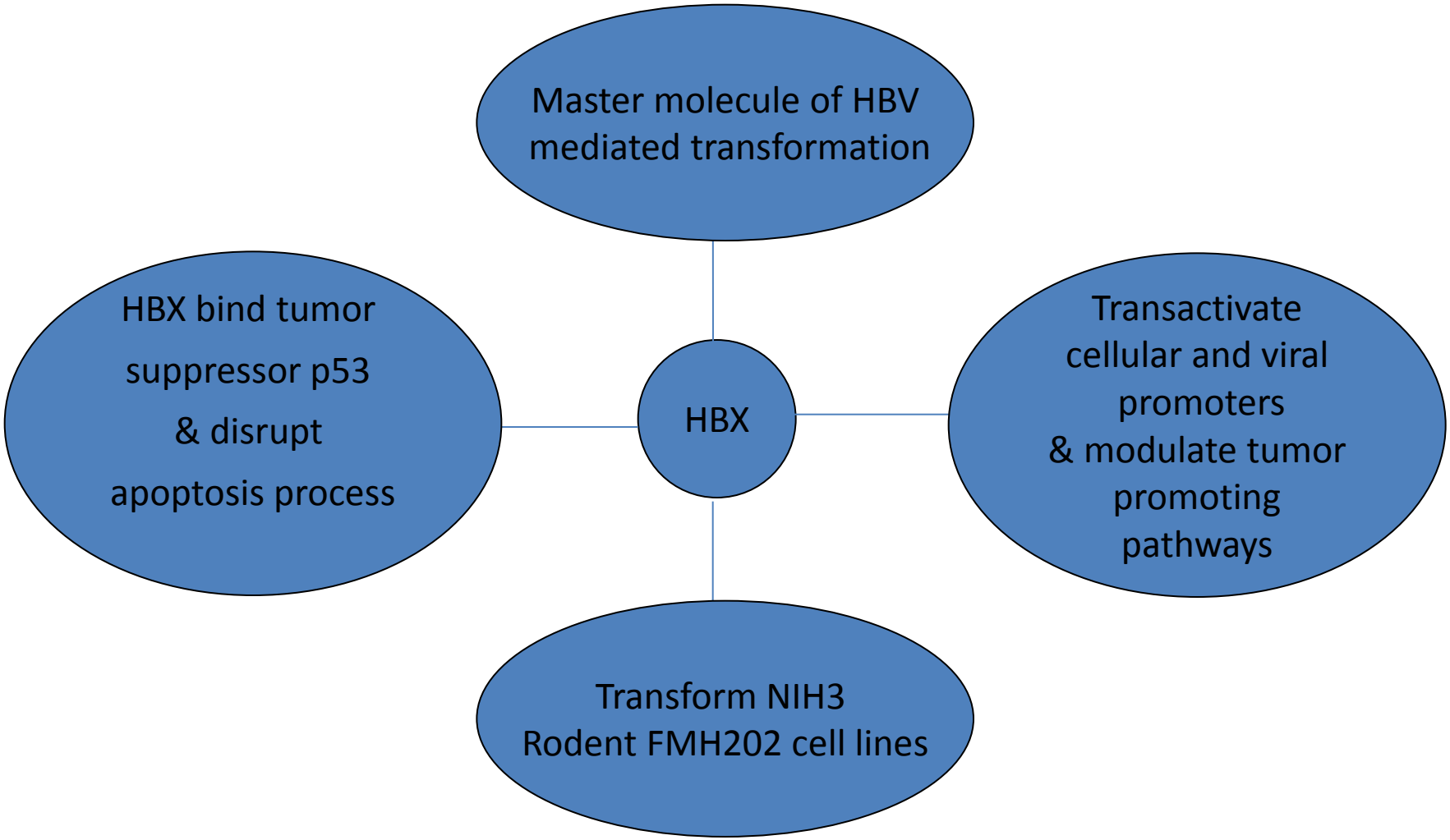


Timeline for Transgenic Mouse Analysis



Possible mechanisms for the HBV-associated HCC development




Master molecule of HBV mediated transformation
HBX bind tumor suppressor p53 & disrupt apoptosis process
HBX
Transactivate cellular and viral promoters & modulate tumor promoting pathways
Transform NIH3 Rodent FMH202 cell lines

X15myc-Transgenic construct

Regulatory elements in HBV genome

FIG. 1

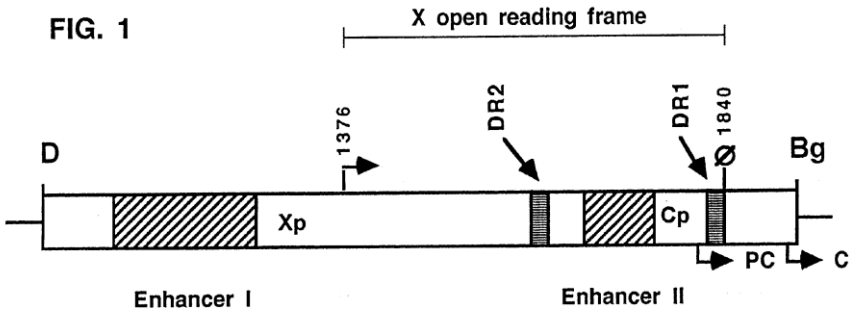
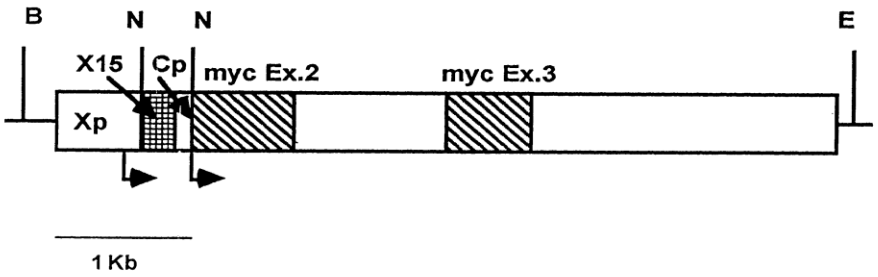


FIG. 2 X15-myc bicistronic construct



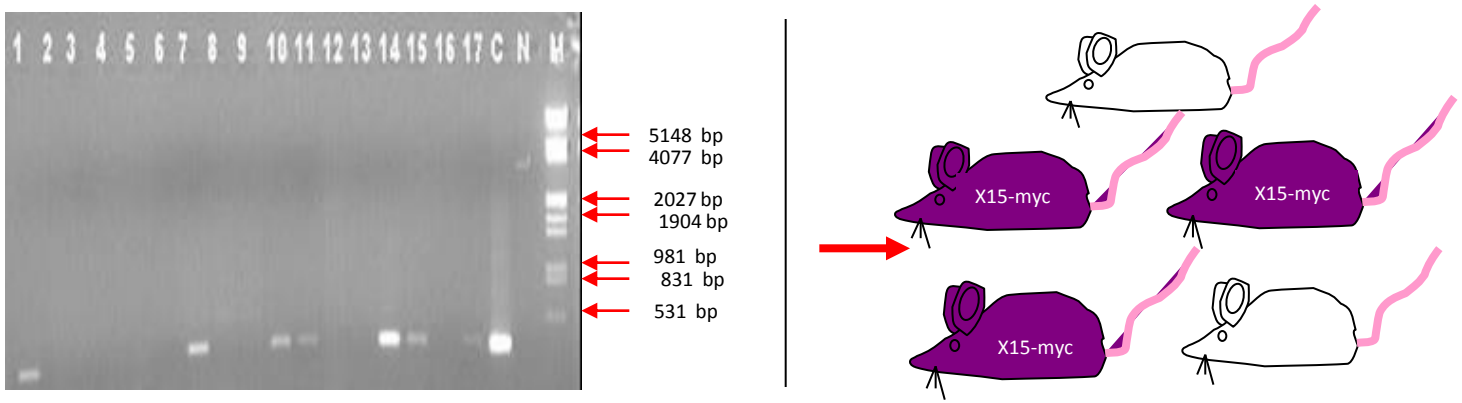
X15 region positioned to 5' to the murine c-myc gene, and is operatively linked to under the regulatory control of its natural promoter and enhancer I element.

C-myc gene is operatively linked to under the regulatory control of core promoter and enhancer II elements

This compact present construct facilitate the the core promoter and and enhancer II regions are embodied in the X gene sequence

5.7Kb EcoR I and Bam HI fragment

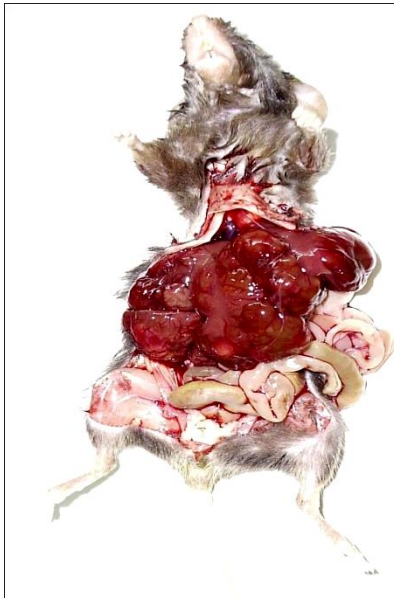
D-DraI, Bg-Bgl II, Xp-Natural X promoter, Cp-Core promoter, B-BamhH I, N-Nco I, E-EcoR I,



PCR analysis for X15-myc Transgenic positive animals

" C "- Positive control; " N" – Negative control; " M "- λ DNA Marker

X 15- myc Transgenic Mouse Model for HCC



➢Recombinant bicistronic construct (Singh *et al*, 2003) (Kumar *et al*, 2001) US patent no: 6274788 B1.

➢ X-15 myc transgenic mice appeared to be an ideal model to study the disease process and also screening drugs.

Transgene	expressed Promoters	used Mouse strain	Pathology	Reference
HBx and c-myc	Xp and Cp	C57BL/6 x SJL	HCC in the first half of animals' life span (3 to 5 months)	1. Singh M,et.al., 1998 2. Kumar V,et.al., 2001(us patent)

X15-myc transgenic mouse model

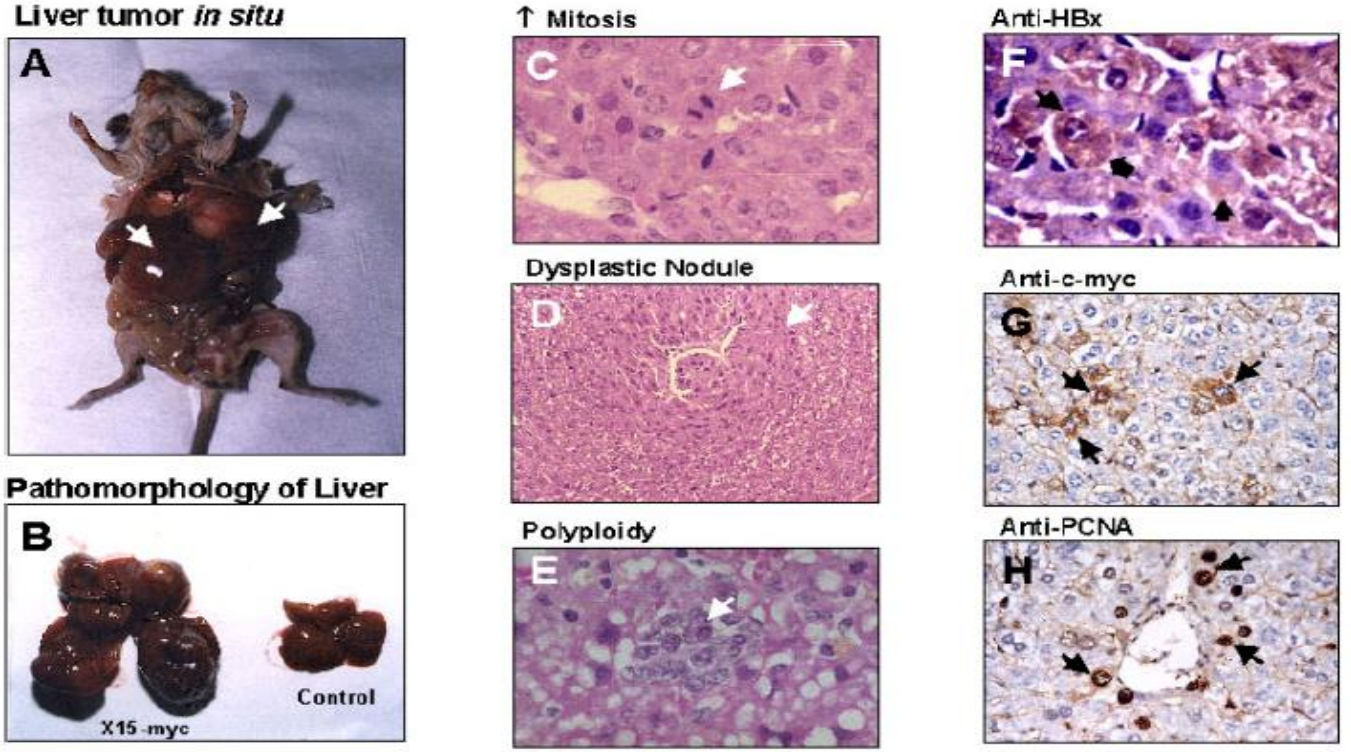


Figure 3. The X15-myc transgenic mouse model of HCC. Liver anatomy (A,B); histology of liver (C–E) and immuno-histochemistry for specific antigens (F–H)

- Most of the pathomorphological and microscopic changes were similar to those observed with the HCC patients (Lakhtakia *et al*, 2003)

Applications of transgenic mice

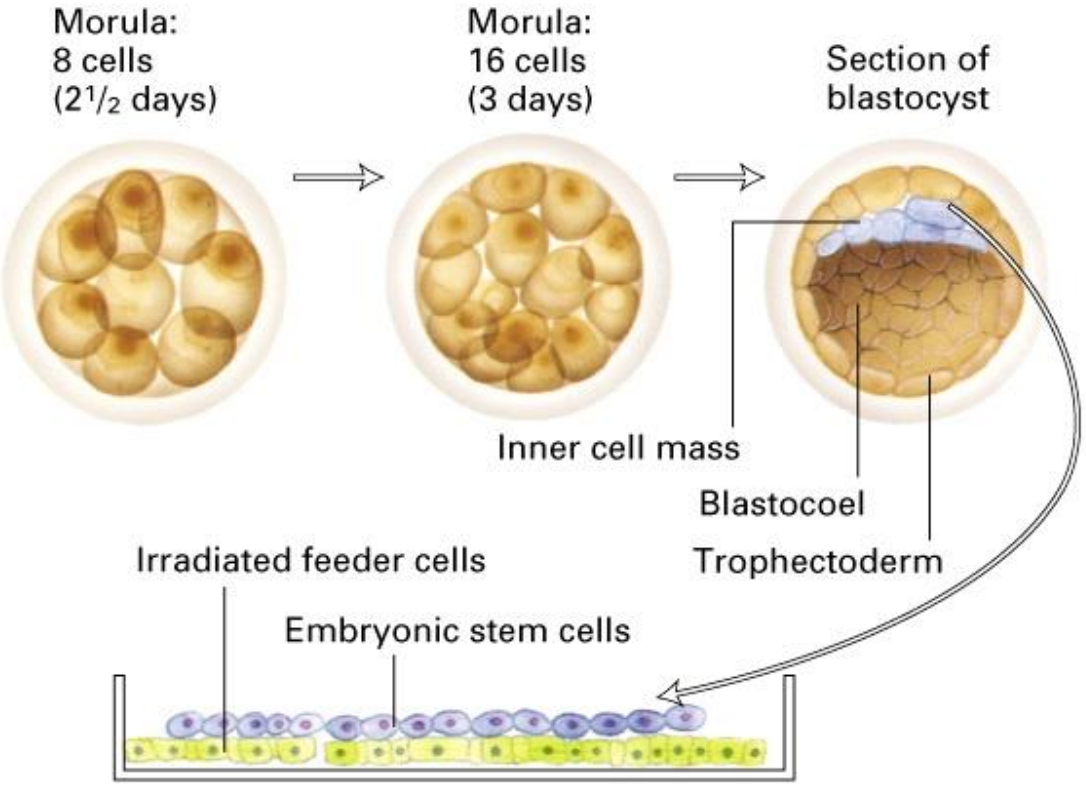
Transgenic mice are often generated to address the role a gene plays in a biological process at the level of the whole organism:

- To confirm the role of a gene mutation
- To help unravel the molecular mechanisms that control gene expression
- To help unravel the biochemical in vivo mechanisms and the origin of disease
- To develop an animal model to test therapeutic strategies

Gene Knockout (Gene Targeting)

- **Knock-out technology allows for the specific loss of a gene in mice**
- **Allows for the function of the KO'd gene to be deduced from the defects seen in the mice**
- **Can be used to mimick some disease**
- **Unlike traditional transgenics the trangene is targeted to a specific site in the DNA of the mouse**
- **Homologous recombination: recombination between the exogenous DNA and its homologous chromosomal site in the embryonic stem (ES) cells**

Embryonic Stem Cells



Mouse Knock-outs require embryonic stem (ES) cells

These are derived from the inner cell mass (ICM) of a blastocyst (the ICM is what will become the fetus)

ES cells are pluripotent meaning they can become all the different cell types found in an adult

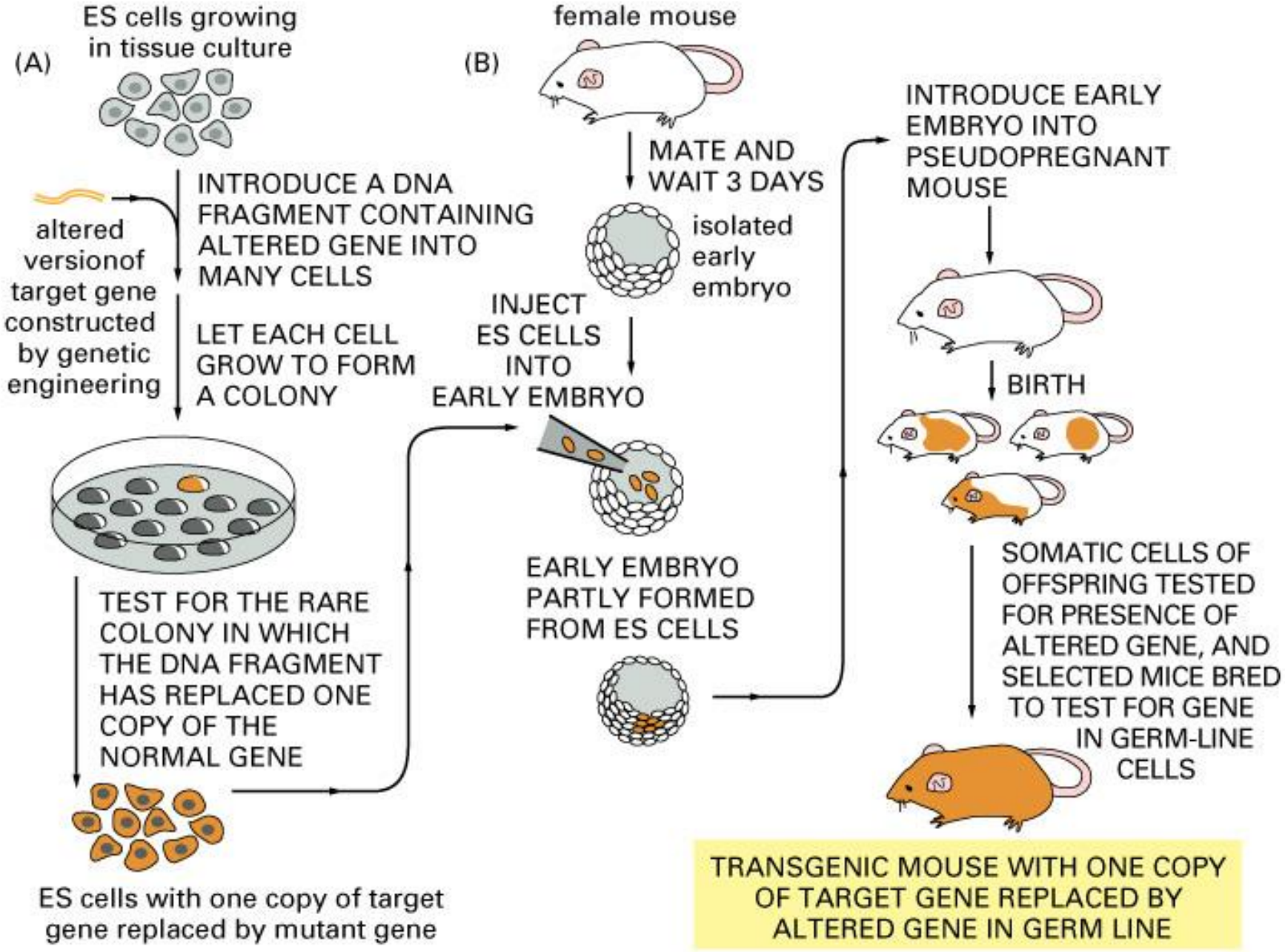


Figure 10-38 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Production of chimeric mice (overview)

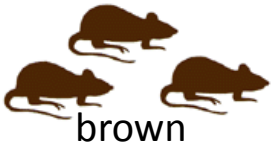
Inject genetically modified embryonic stem (ES) cells from brown mouse into black mouse blastocyst (embryo)

Implant into foster mother

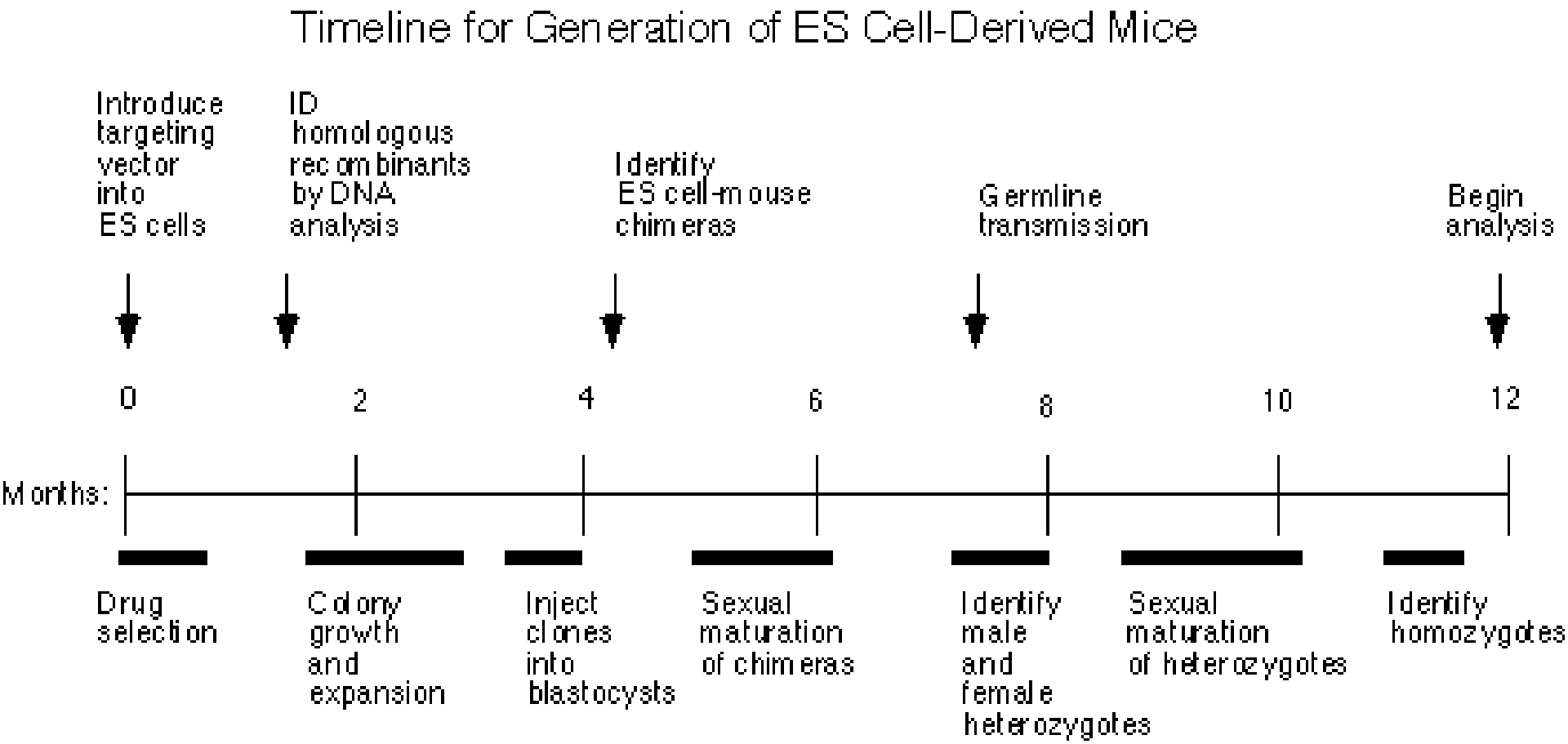
Brown (or mixed) coat color mice are chimeras

Look for chimeric progeny (coat color)

Breed for germline transmission



Check offspring for coat color


Timeline for Generation of ES Cell-Derived Mice
Introduce targeting vector into ES cells
ID homologous recombinants by DNA analysis
Identify ES cell-mouse chimeras
Germline transmission
Begin analysis
0
2
4
6
8
10
12
Months:
Drug selection
Colony growth and expansion
Inject clones into blastocysts
Sexual maturation of chimeras
Identify male and female heterozygotes
Sexual maturation of heterozygotes
Identify homozygotes

Limitations

- **Knockout technology is highly advantageous for both biomedical research and drug development.**

- **Developmental defects, many knockout mice die while they are still embryos before the researcher has a chance to use the model for experimentation.**

- **Even if a mouse survives, several mouse models have somewhat different phenotypic traits than their human counterparts. An example of this phenomenon is the p53 knockout.**

- **Gene p53 has been implicated in as many as half of all human cancers. However p53 knockout mice develop a completely different range of tumors than do humans. In particular, mice develop lymphomas and sarcomas, whereas humans tend to develop epithelial cell-derived cancers.**

- **Such differences exist it cannot be assumed that a particular gene will exhibit identical function in both mouse and human, and thus limits the utility of knockout mice as models of human disease.**

Different Knock-out approaches:

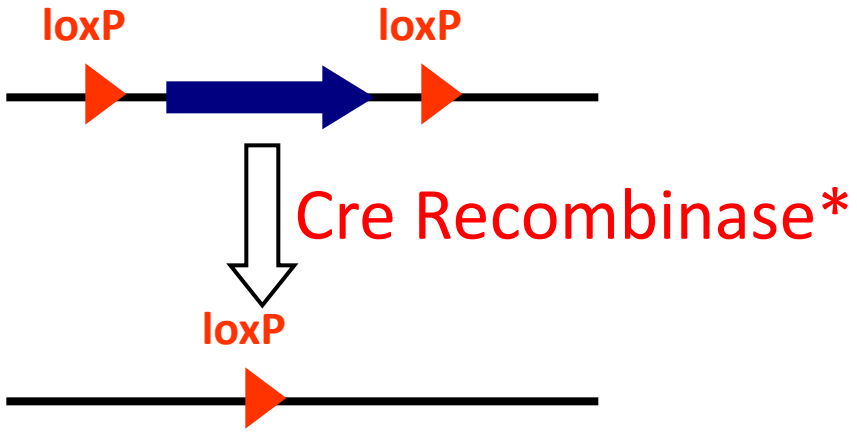
- conventional knock-out
- knock-in/replacement
- tissue-specific knock-out
- inducible knock-out:

  tissue-specific with temporal control

Conditional knock-outs

The Cre/loxP-Mediated Gene Deletion - Controlled

34 bp: 8-bp sequence flanked by 13-bp inverted repeats



Deletion of loxP-flanked sequences

Conditional knock-outs

inactivate a gene only in specific tissues
and at certain times during development and life.

Cre-lox technology

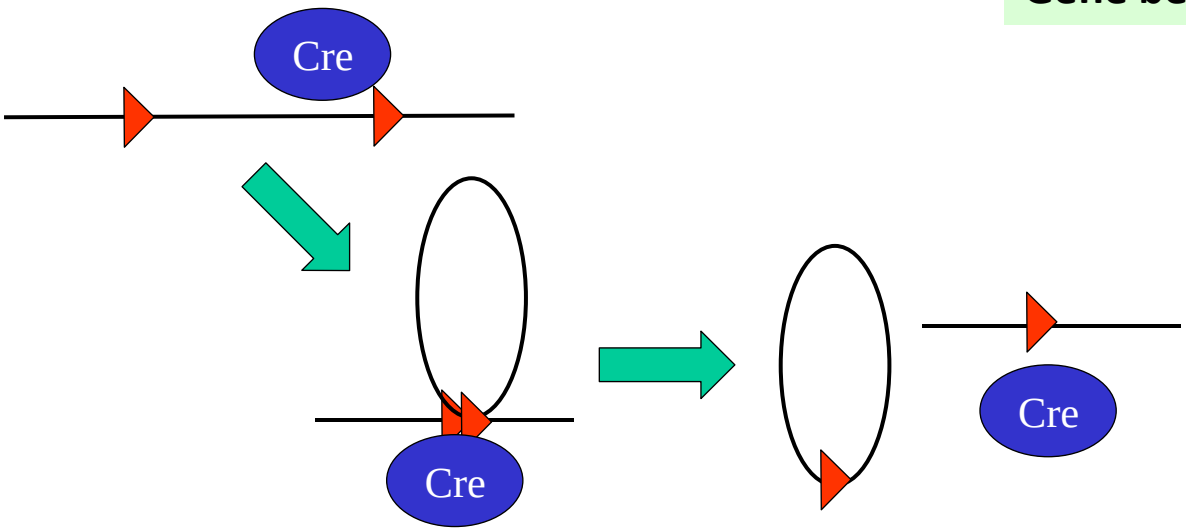
Your gene of interest
is **flanked by 34 bp *loxP* sites (floxed).**

Cre – a site-specific recombinase enzyme from the P1 phage.

Recognises a 34bp DNA sequence *loxP* =

If CRE recombinase expressed

Gene between loxP sites is removed



From Dr. J. Martin Collinson

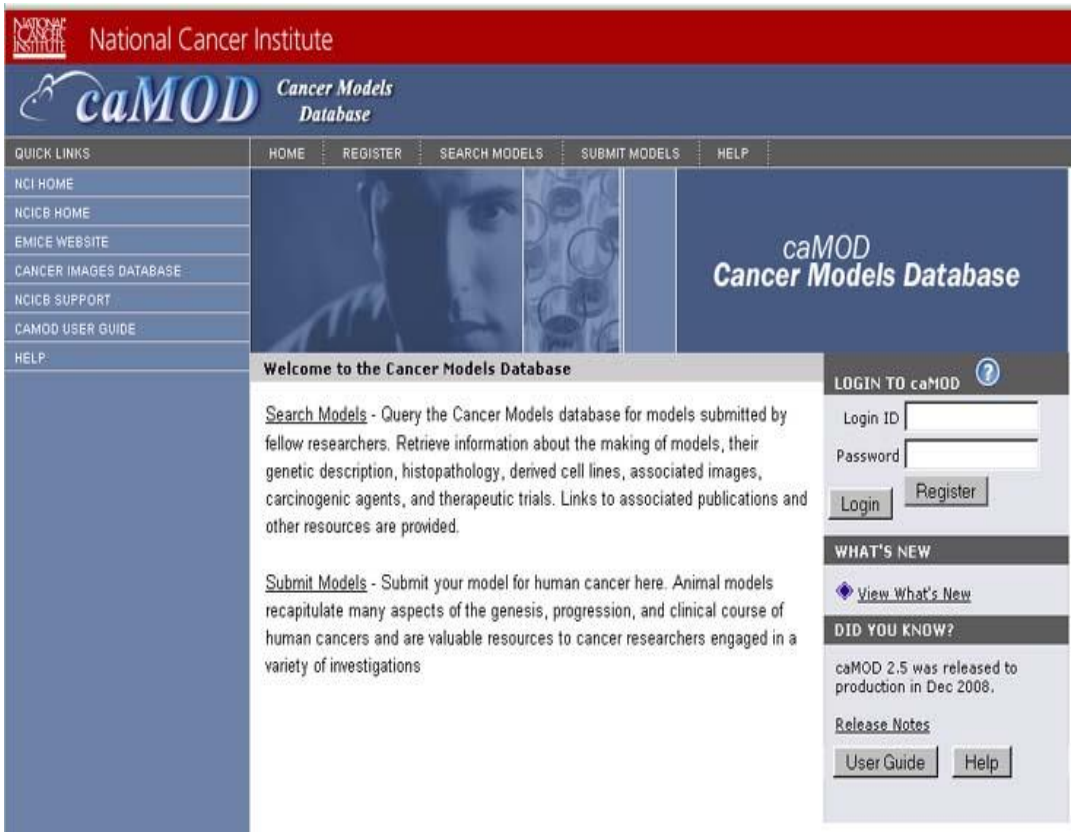
Comparison between Knock-outs and Transgenics

	Knock-out/in	Transgenic
Cells	ES cells	fertilized eggs
Homologous DNA	yes	no
Insertion site	targeted	random
Copy number	1	tandem repeats
	usu. Loss-of-function	usu. Gain-of-function
Reproducibility*	yes	no

* Using the same construct in the same strain background

caMOD
The Cancer Models Database

caMOD is a web-based resource that provides information about animal models for human cancer to the public research community



- **Submission**—Data extracted from literature by curators or submitted by scientists.
- **Search**– Customizes searches or predefined searches.
- **System Function Administration—** User management and review of models.

http://cancermodels.nci.nih.gov

Thanks for your Attention!!!

Acknowledgement

- The Presentation is being used for educational and non commercial purpose
- Thanks are due to all those original contributors and entities whose pictures used for making this presentation.