

Gene manipulation and targeting 4061



Potential Experimental Use of Genetically Engineered Mice

'Transgenic' Mice

1) Influence of a foreign gene on physiology

2) Use of a reporter gene

3) Disease models. eg. CF

4) 'Knockout' or 'Gene targeted' mice. Determine the role of a gene product during development

5) Conditional mutants

6) Precision engineering using Crispr/Cas9

Transgenic mice

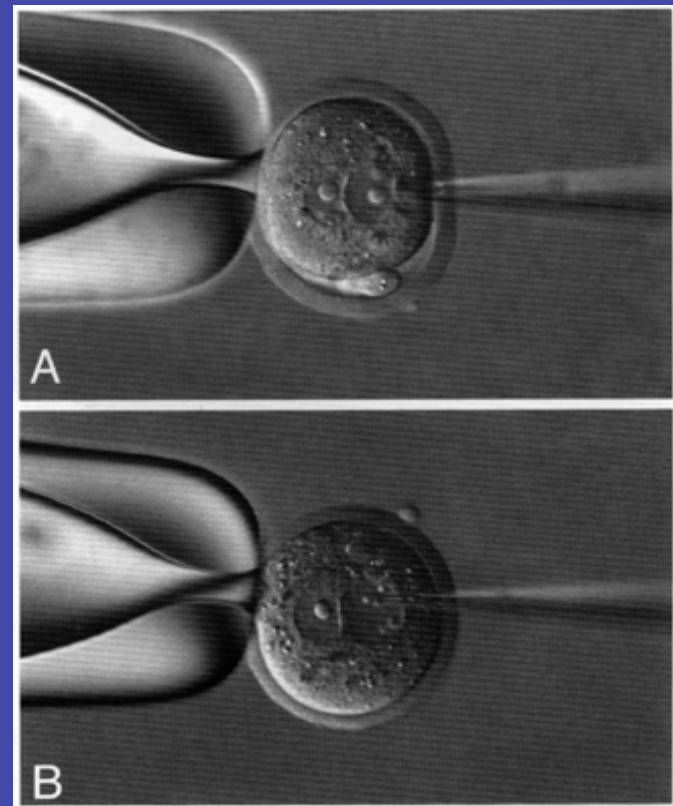
Transgene Linear Fragment of DNA



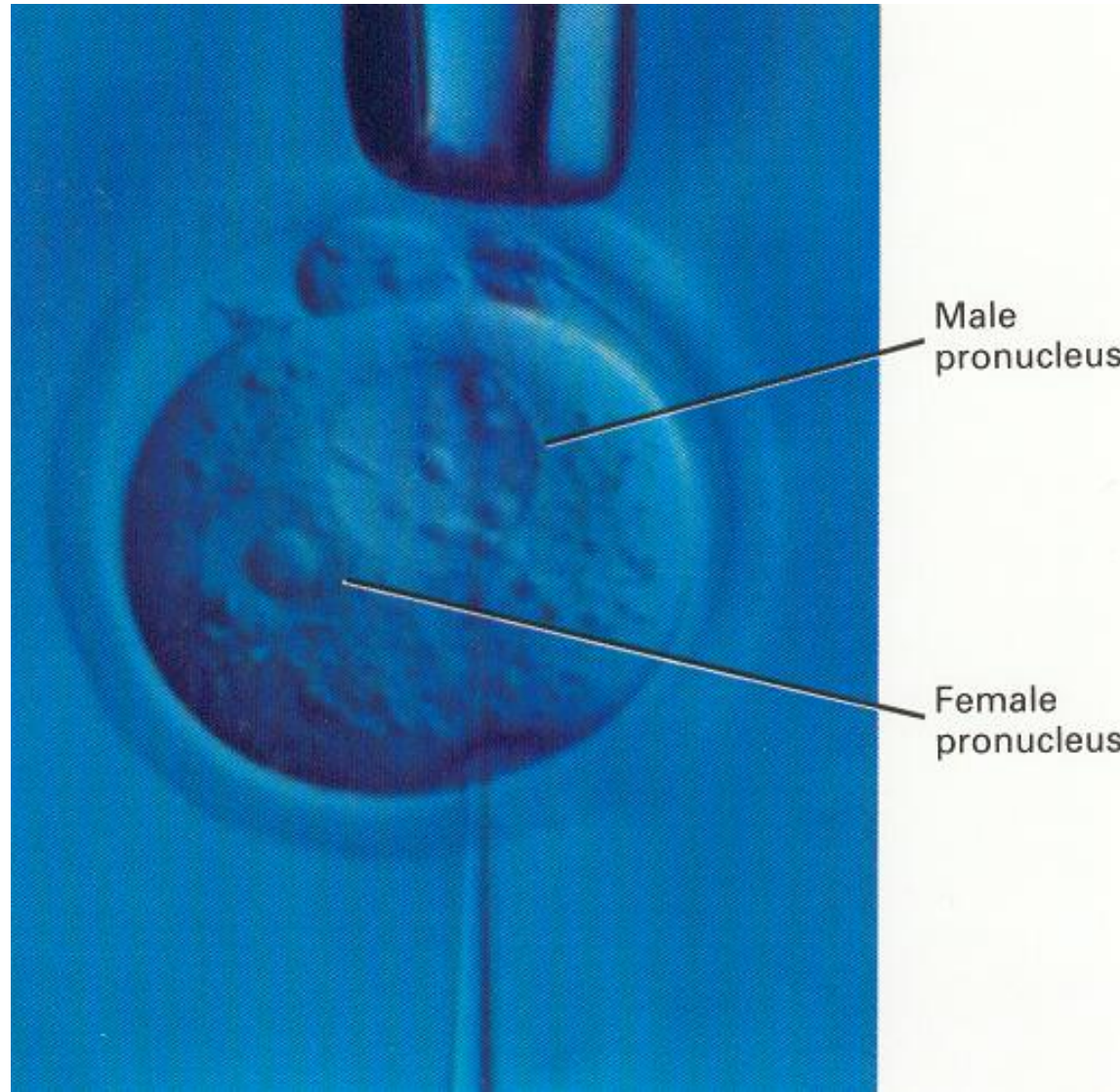
- tissue-specific
- regulatory elements
- chemically induced

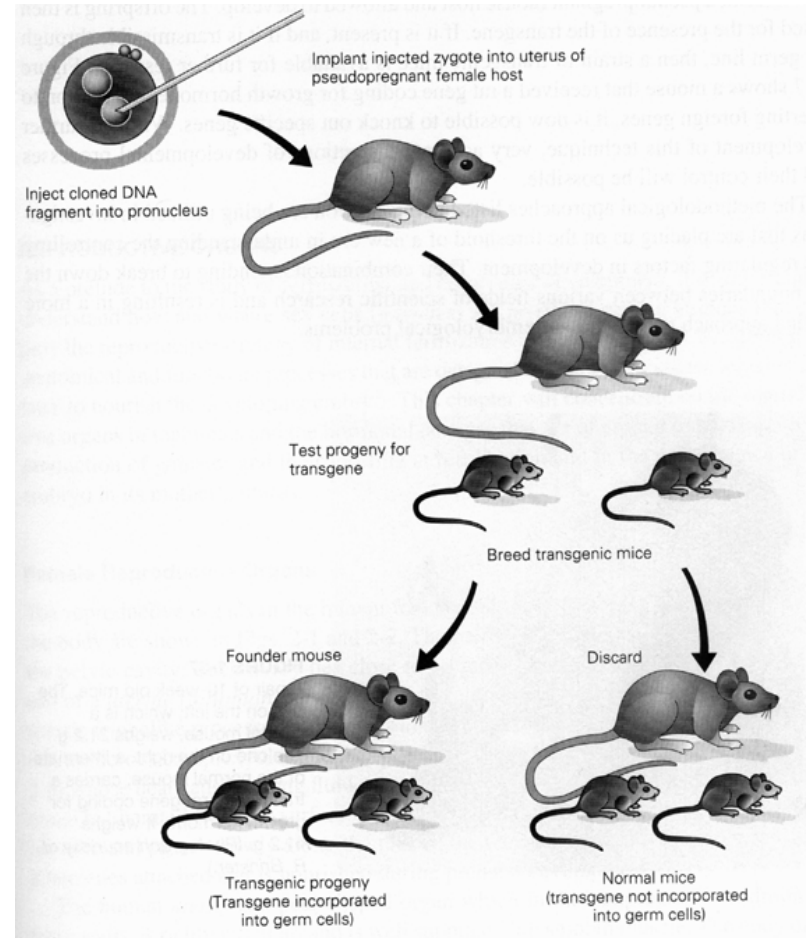
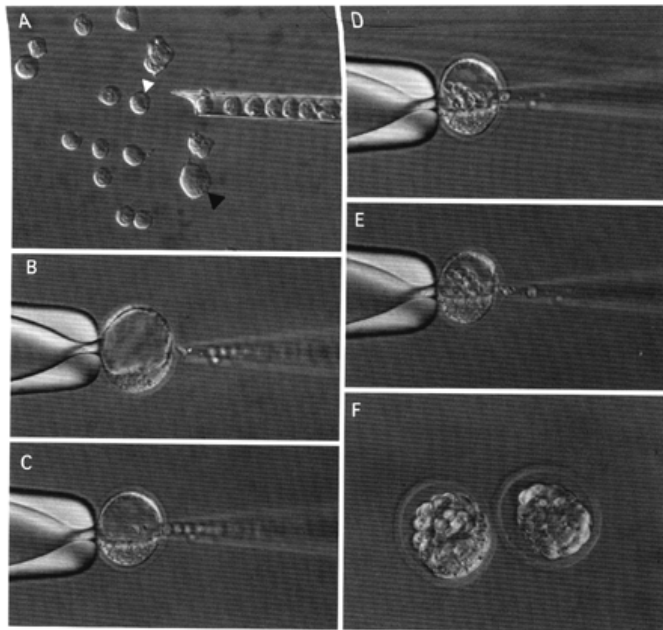
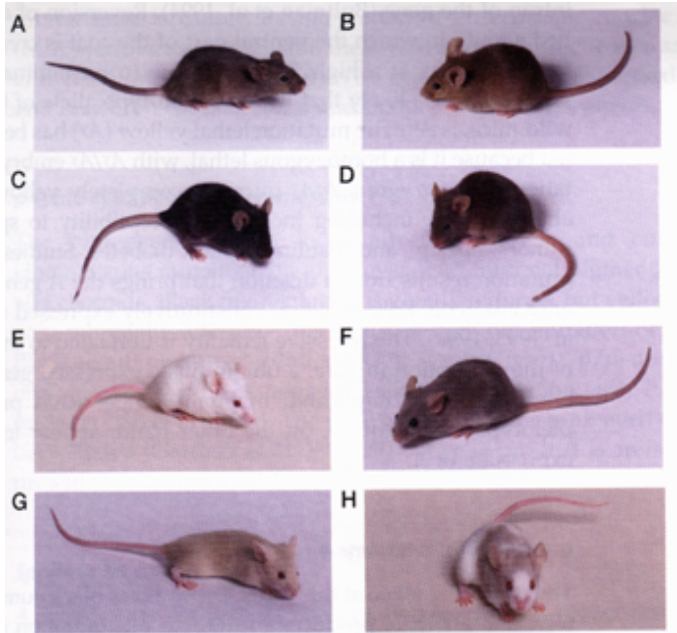
- ↓
- Anything with a start codon
 - Fluorescent proteins, enzymes (beta-galactosidase), Cre-recombinase

Pronuclear Injection into Zygotes



DNA can be directly injected into individual cells





Muscle Fibers

Myoblasts

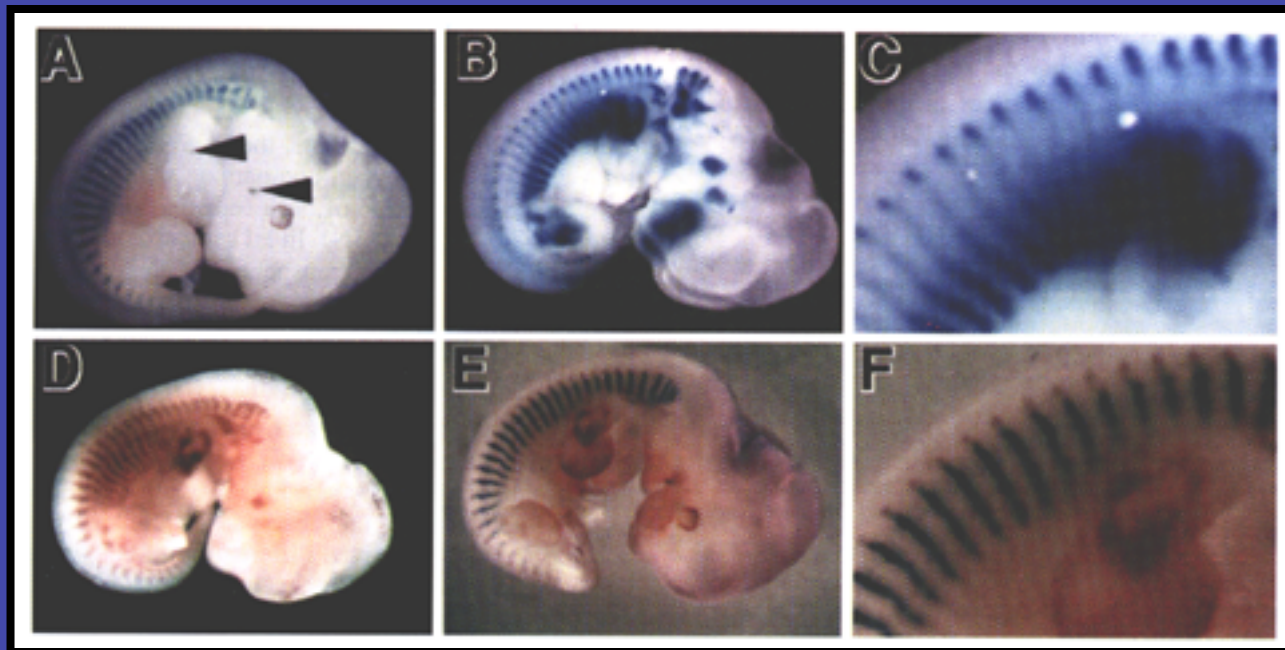
-20 kb Enhancer

-5 Kb Enhancer

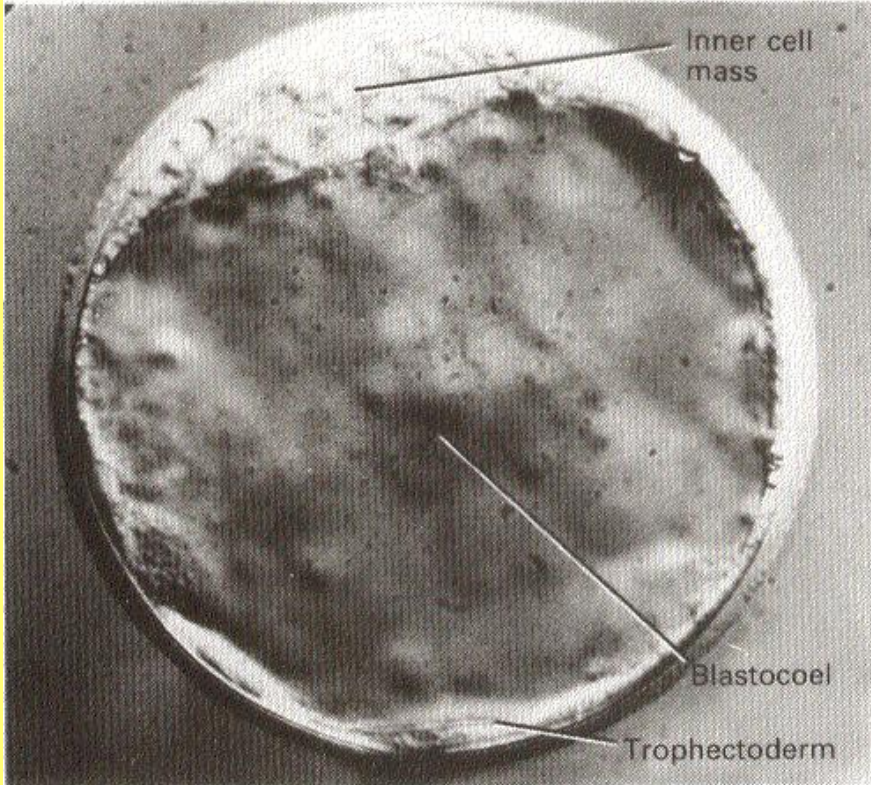
Beta-Galactosidase

Beta-Galactosidase

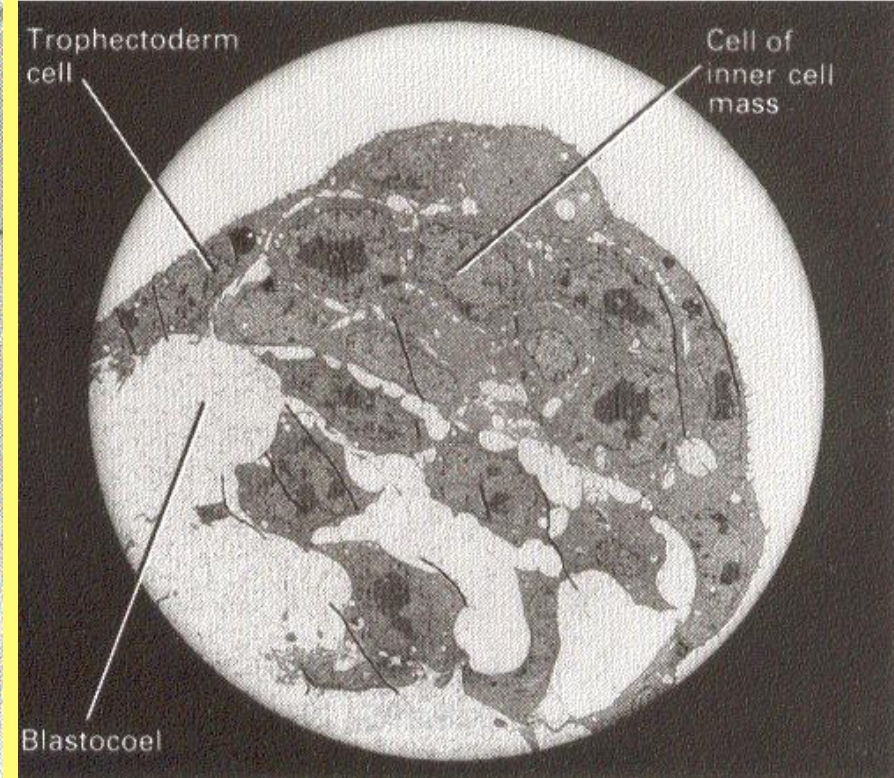
Beta-Galactosidase



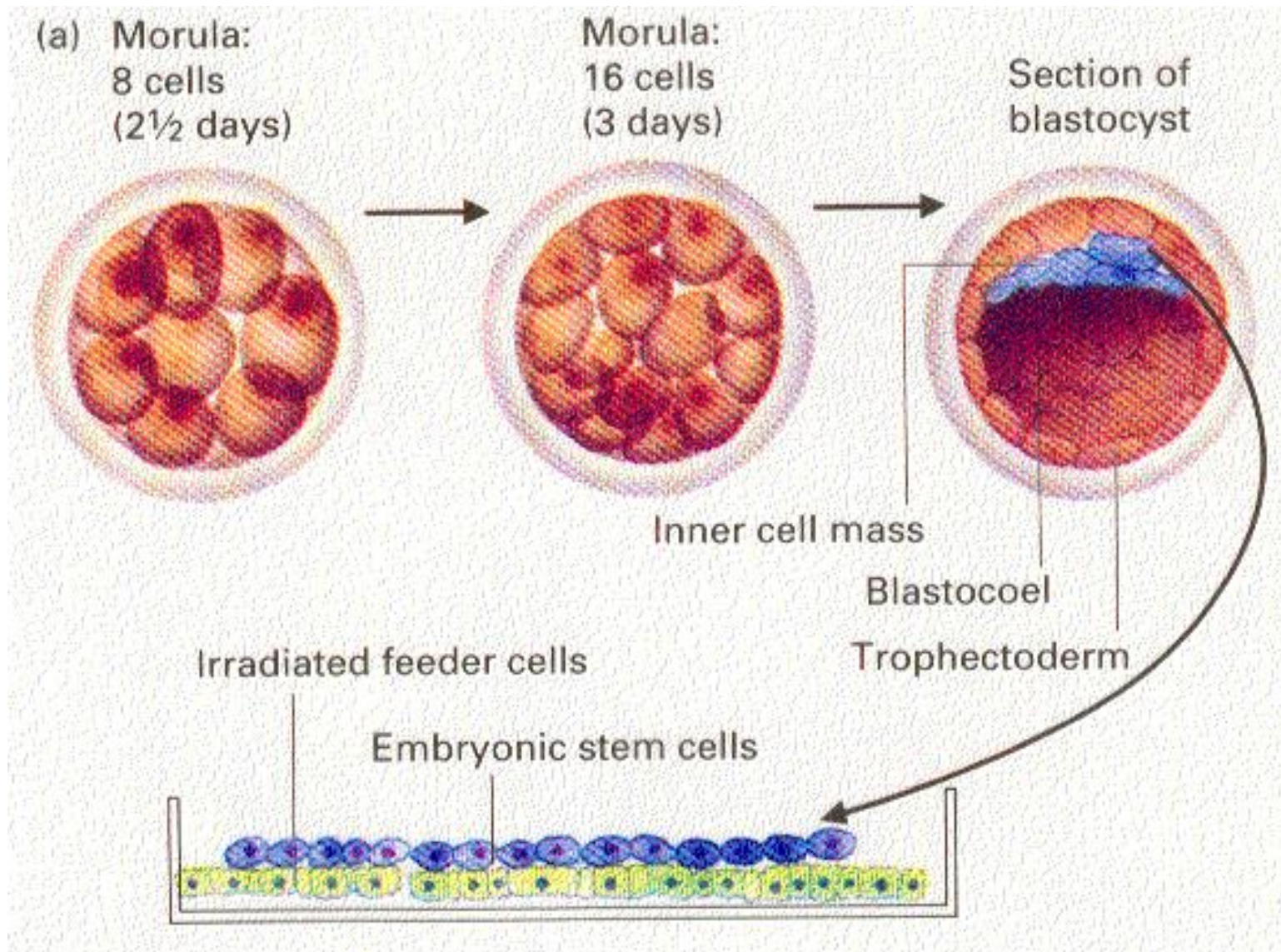
A light micrograph of a blastocyst



An electron micrograph of a section through a blastocyst



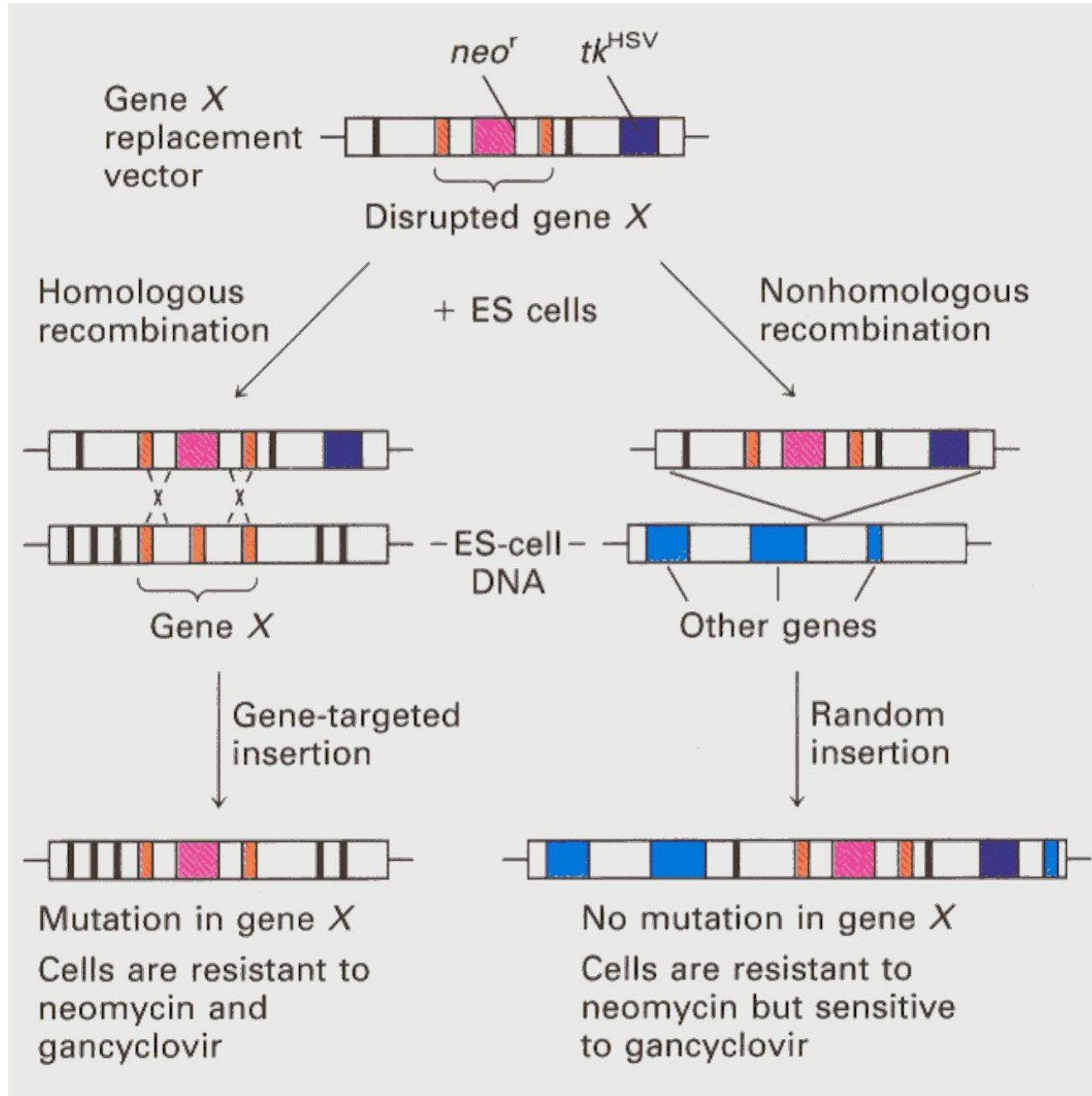
Preparation of embryonic stem (ES) cells



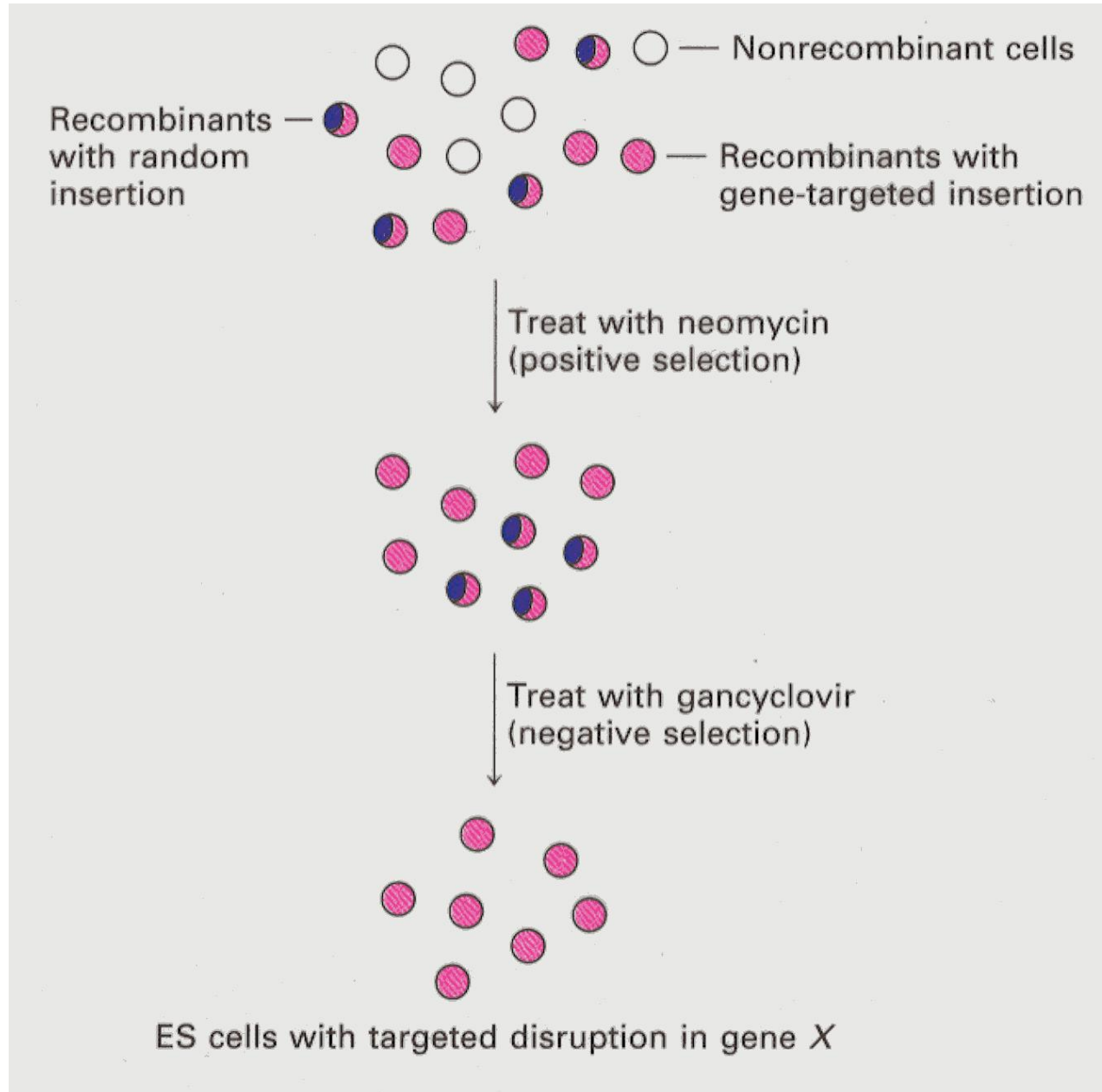
Formation of ES cells carrying a knockout mutation

■ $neo^r = (+)$
selection

■ $tk^{HSV} = (-)$
selection



Positive and negative selection of recombinant ES cells

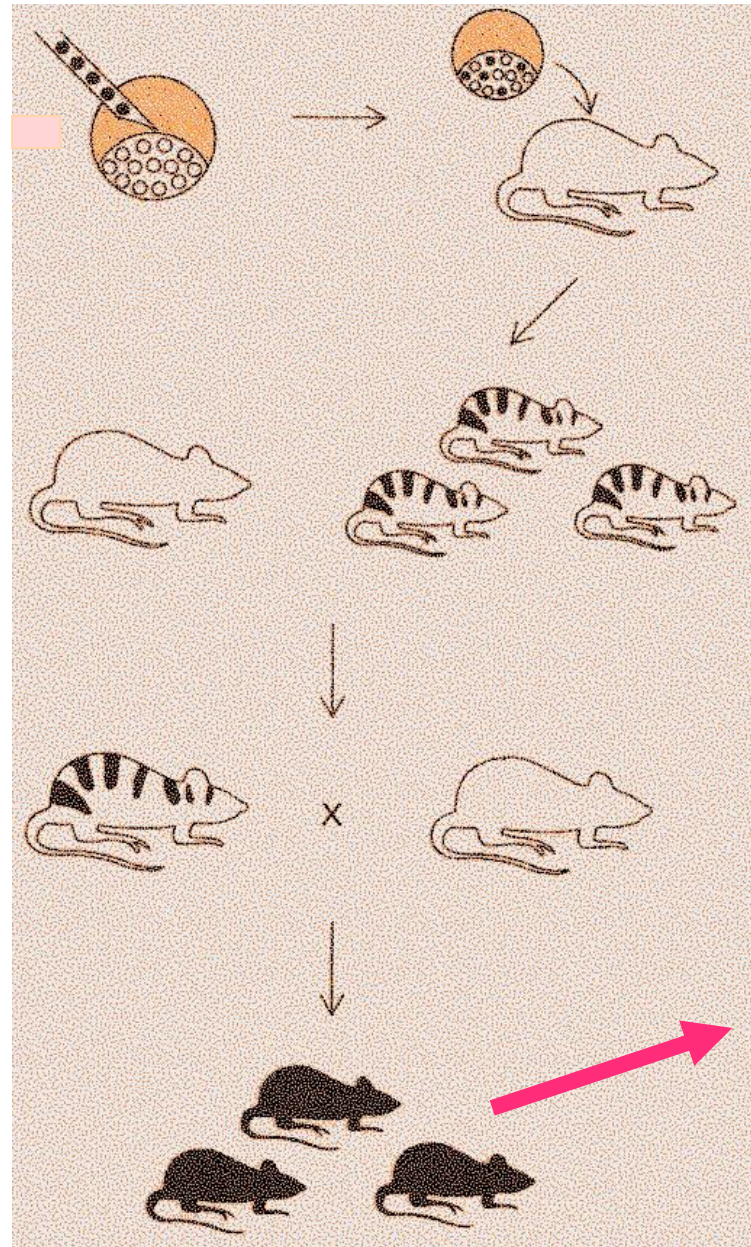


Procedure for gene targeted 'knockout' mice

ES cells into
blastocoel cavity of a
blastocyst

ES cells are
homozygous for a
marker trait (black
coat colour)

Population of mice that
are heterozygous for
disrupted gene x,
crossing of these mice
will result in
homozygotes



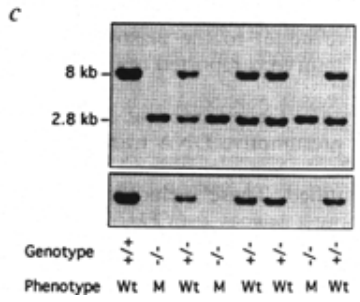
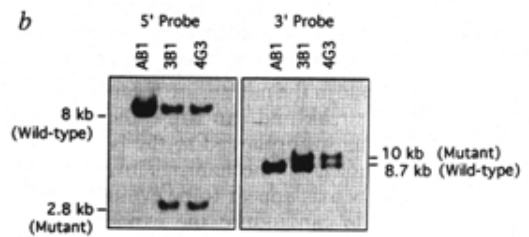
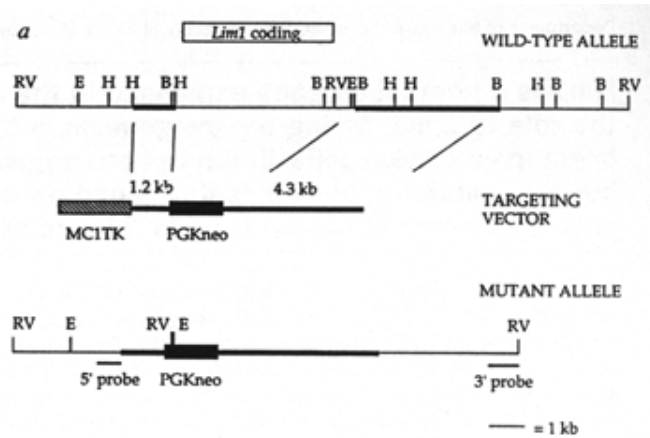
Transfer
pseudopregnant
mouse

Chimeric progeny

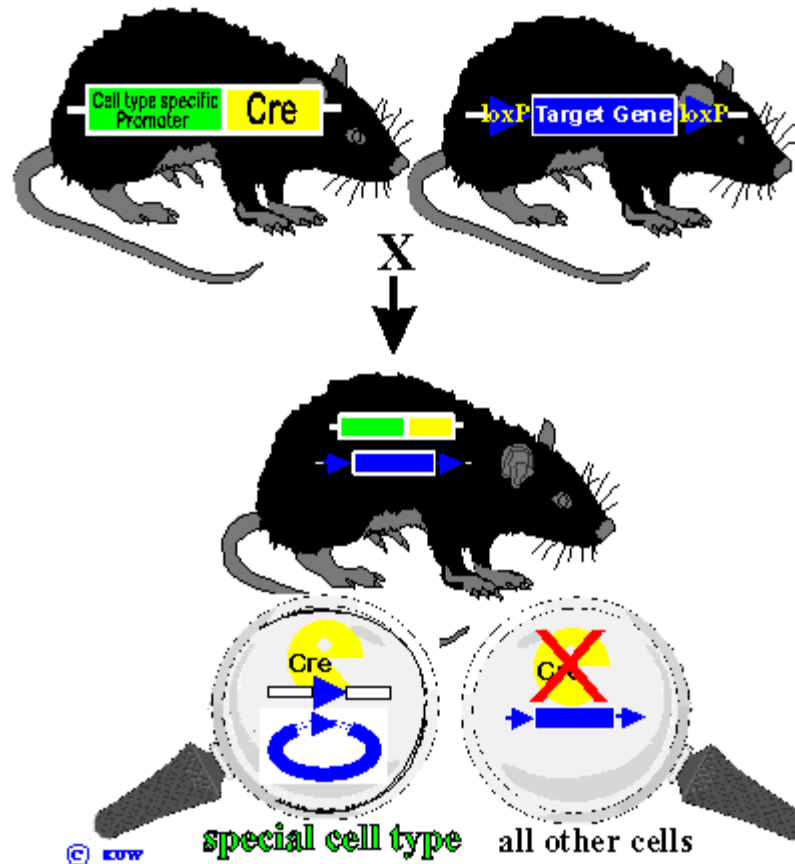
Mate chimeric mice to
homozygous white
mice

ANALYZE MICE??

Lim-1 Knock-Out and Obvious Phenotypes! Role In Head Development?



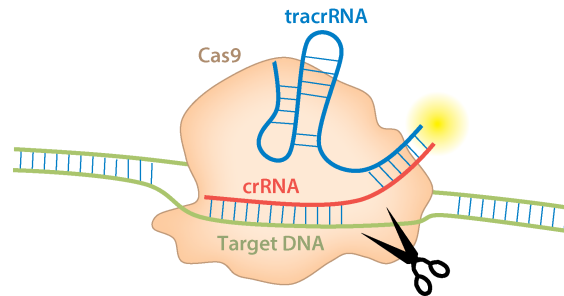
Cre loxP system



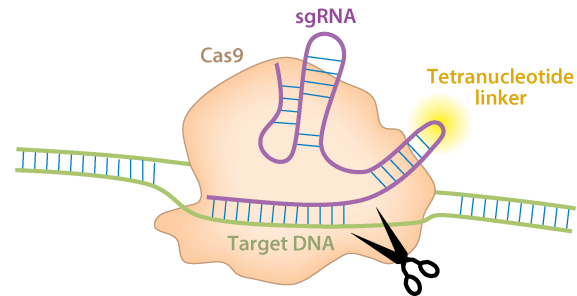
Crispr/Cas 9 : a genome editing game changer

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Type II system

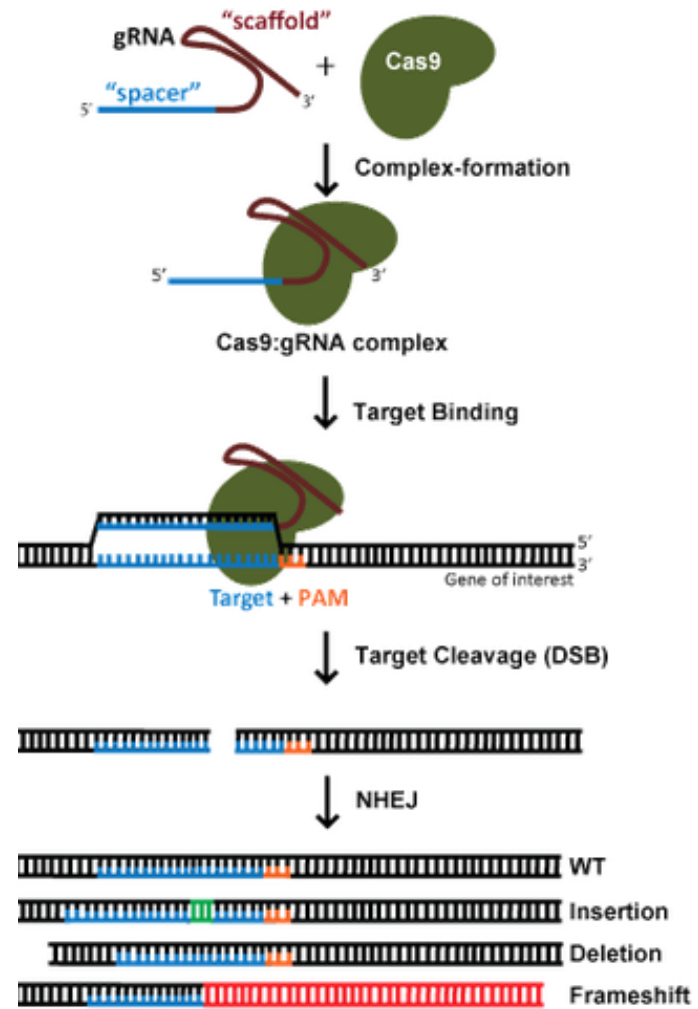
a Native type II CRISPR-Cas9 system

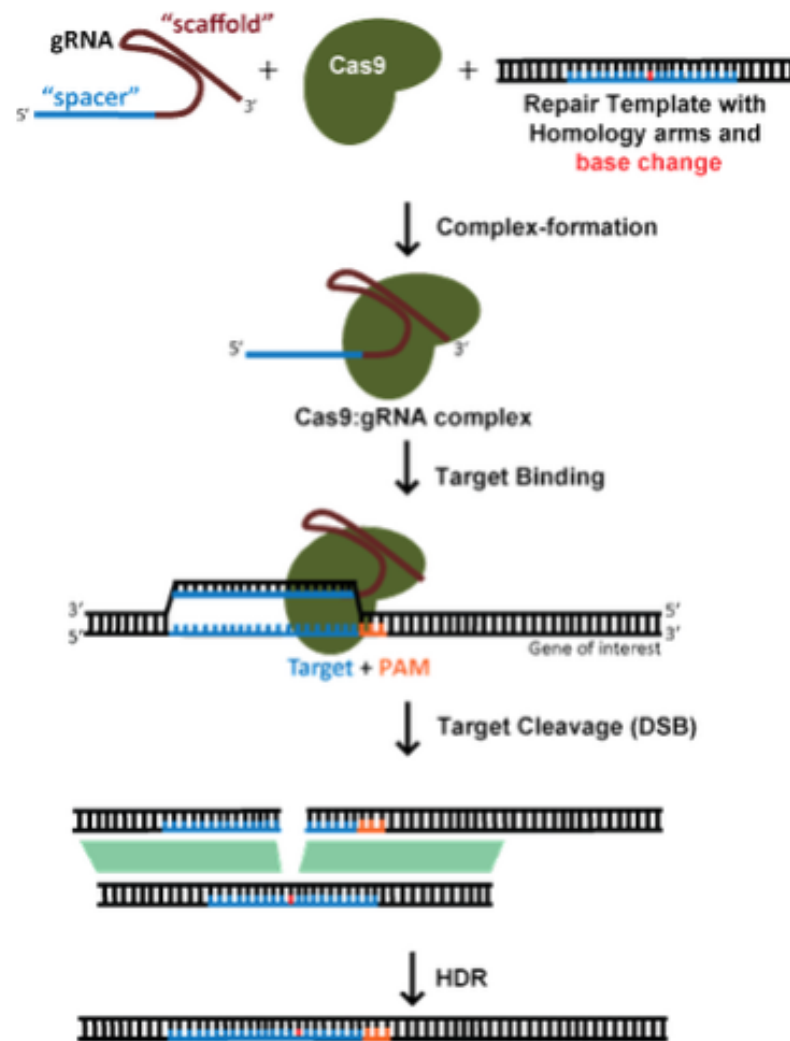


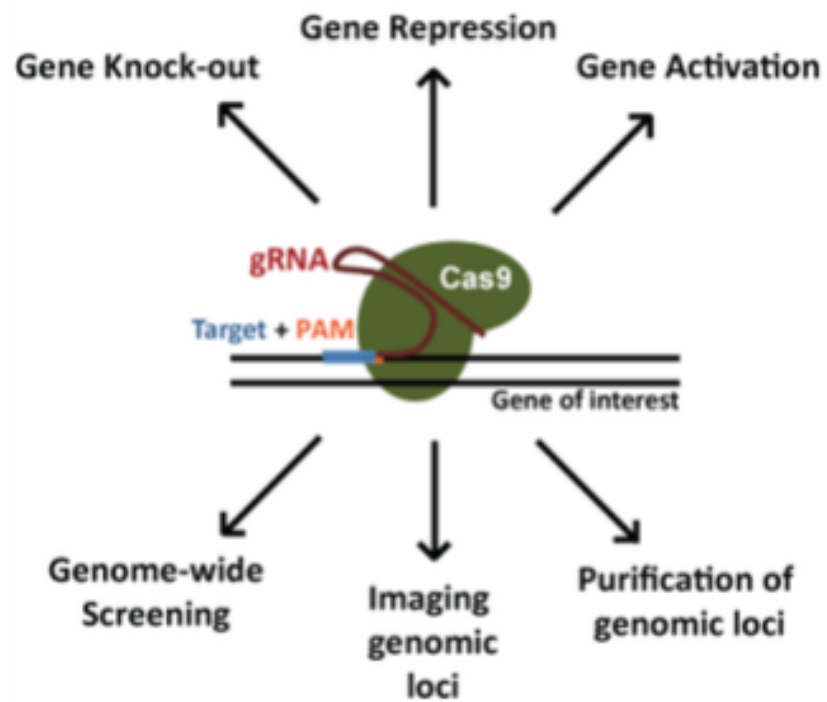
b Engineered CRISPR-Cas9 system with sgRNA



AR Hotta A, Yamanaka S. 2015.
Annu. Rev. Genet. 49:47–70





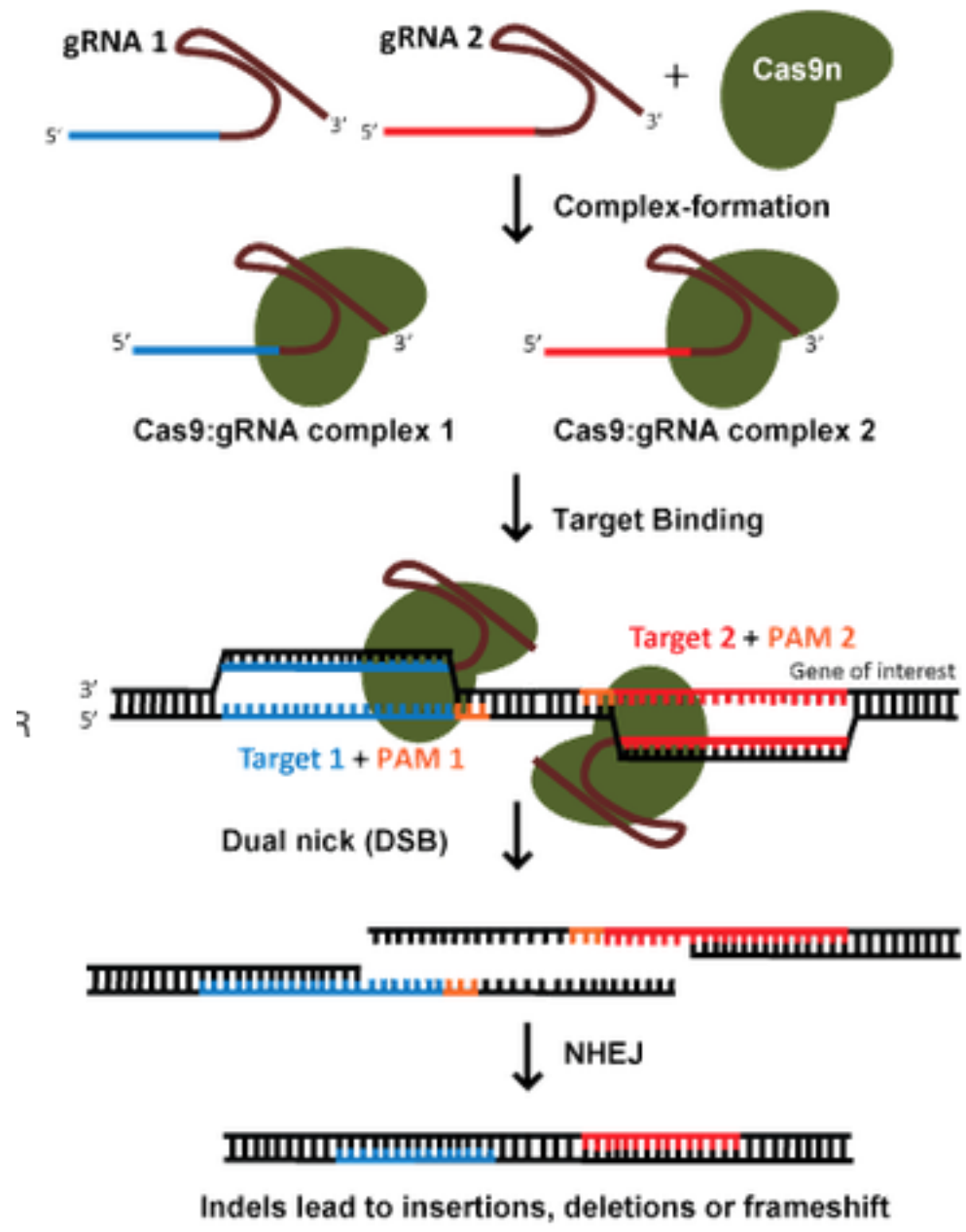


Summary

- **Transgenic and gene targeting techniques permit the analysis of gene expression and, to a limited extent, function during development**
- **More recent developments permit spatially and temporally regulated gene elimination/modification and ectopic expression**

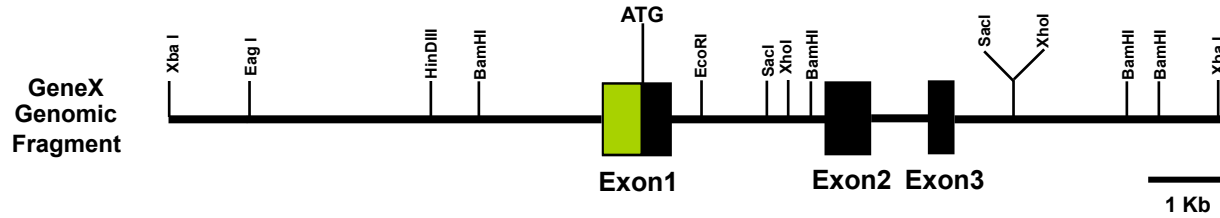
Crispr/Cas9 references

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- Engineered CRISPR-Cas9 nucleases with altered PAM specificities. Kleinstiver BP, Prew MS, Tsai SQ, Topkar VV, Nguyen NT, Zheng Z, Gonzales AP, Li Z, Peterson RT, Yeh JR, Aryee MJ, Joung JK. *Nature*. 2015 Jul 23;523(7561):481-5. doi: 10.1038/nature14592. [PubMed](#).
- CRISPR-Cas9-Mediated Genetic Screening in Mice with Haploid Embryonic Stem Cells Carrying a Guide RNA Library. Zhong C, Yin Q, Xie Z, Bai M, Dong R, Tang W, Xing YH, Zhang H, Yang S, Chen LL, Bartolomei MS, Ferguson-Smith A, Li D, Yang L, Wu Y, Li J. *Cell Stem Cell*. 2015 Aug 6;17(2):221-32. doi: 10.1016/j.stem.2015.06.005. [PubMed](#).
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- Increasing the efficiency of homology-directed repair for CRISPR-Cas9-induced precise gene editing in mammalian cells. Chu VT, Weber T, Wefers B, Wurst W, Sander S, Rajewsky K, Kühn R. *Nat Biotechnol*. 2015 May;33(5):543-8. doi: 10.1038/nbt.3198. [PubMed](#).
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- Genome engineering using CRISPR-Cas9 system. Cong L, Zhang F. *Methods Mol Biol*. 2015;1239:197-217. doi: 10.1007/978-1-4939-1862-1_10. [PubMed](#).

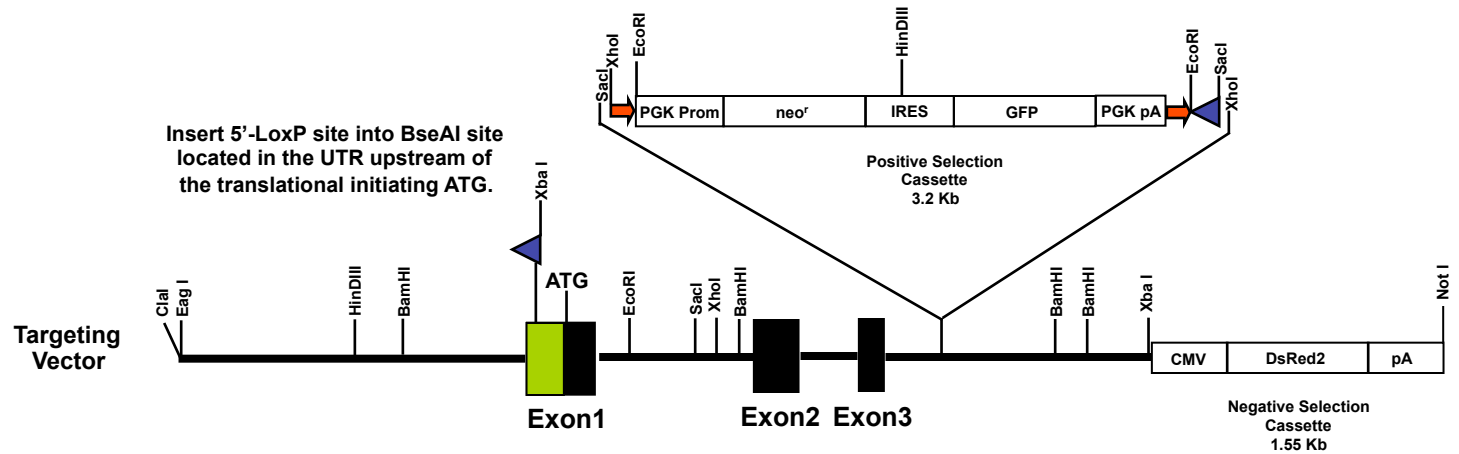


GeneX Gene Targeting

A

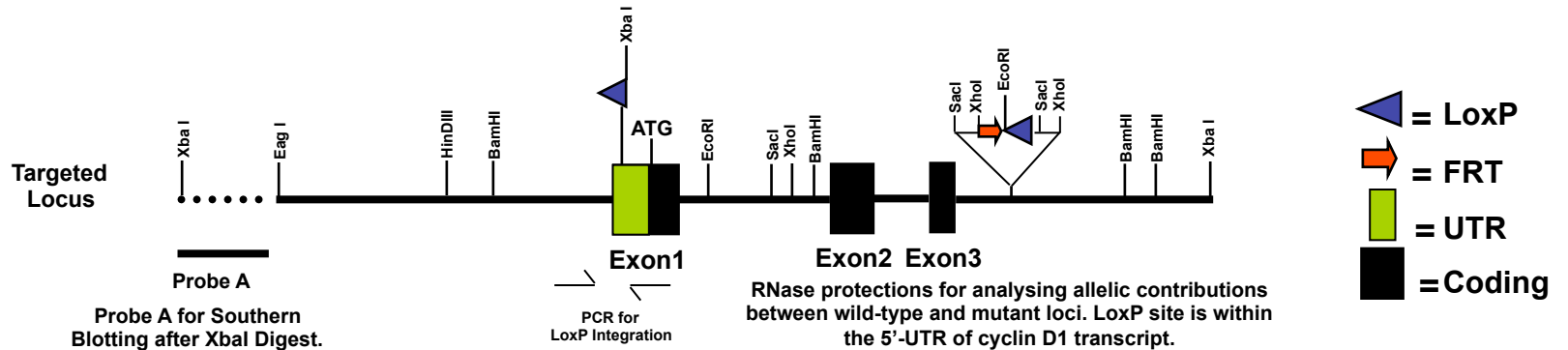


B

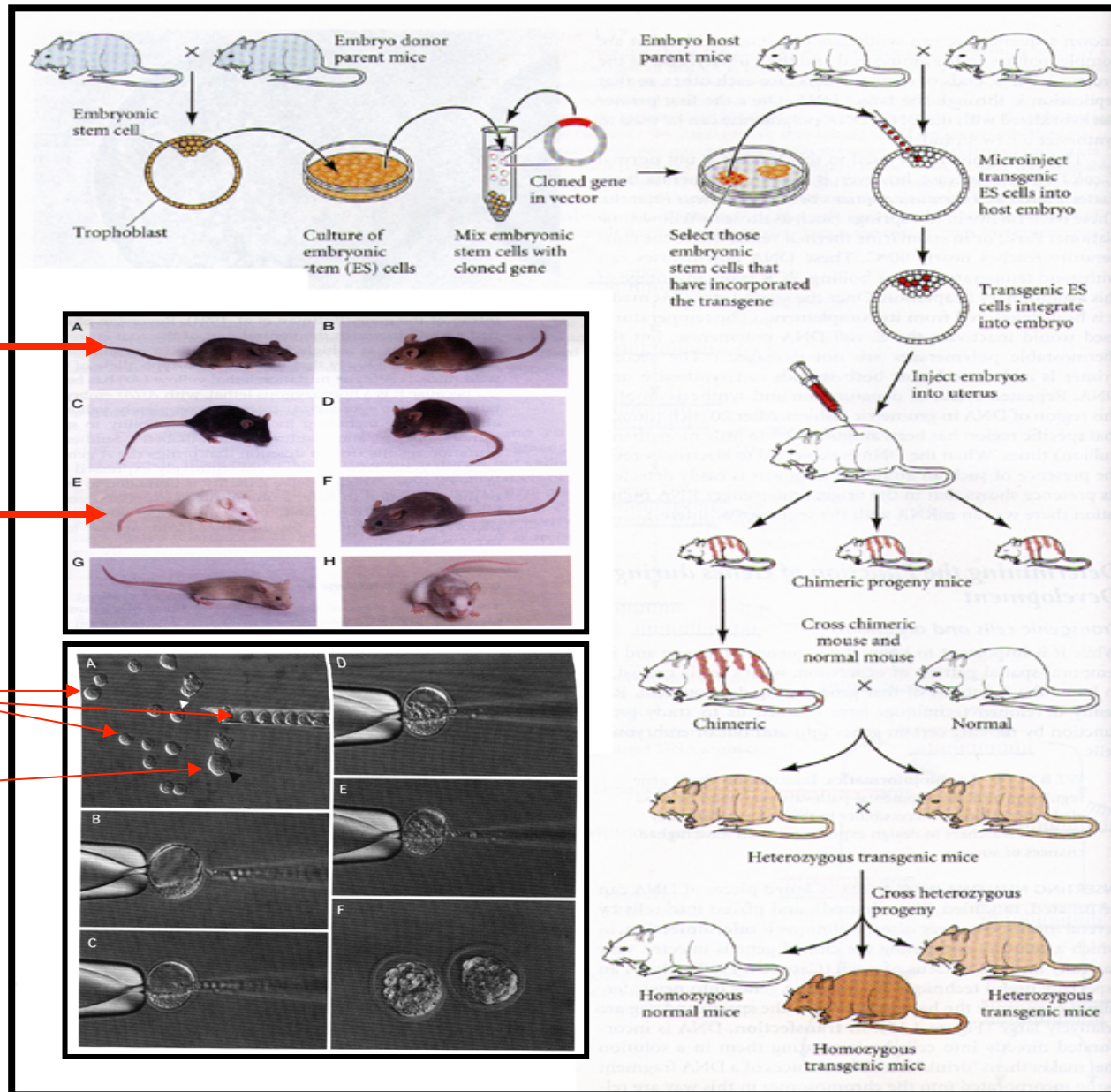


Selection by G418 and Green Only Fluorescence. Red Fluorescence indicates random integration. Removal of positive selection cassette by Flp recombinase expression. Identify by loss of GFP fluorescence. Adapt for FACS sorting and rapid identification of homologous recombinants.

C



Production of Gene Targeted Chimeric Mice

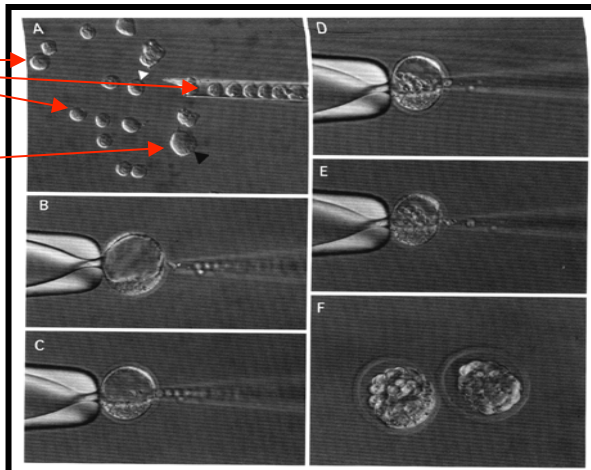
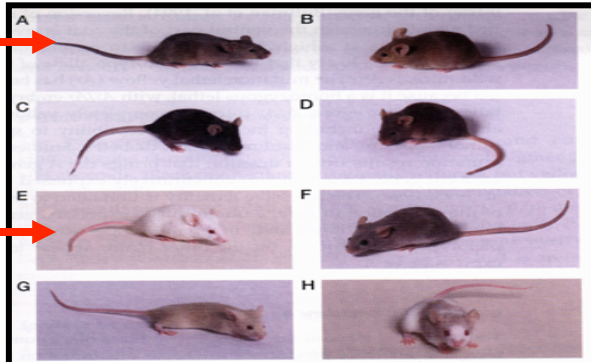


SV129
(Agouti)

Balb/C
(Albino)

ES Cell

Feeder Cell



More Advanced Techniques

Transgenic #1



Mammary Gland Specific

Transgenic #2



rtTA



**No Transcription without Tet in water.
No Tumors**

rtTA



**Transcription with Tet in water.
Tumors**

rtTA



**No Transcription without Tet in water.
Tumors Regress**