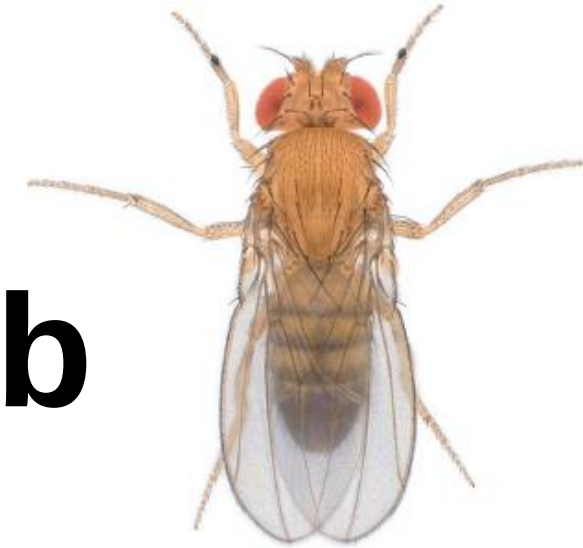
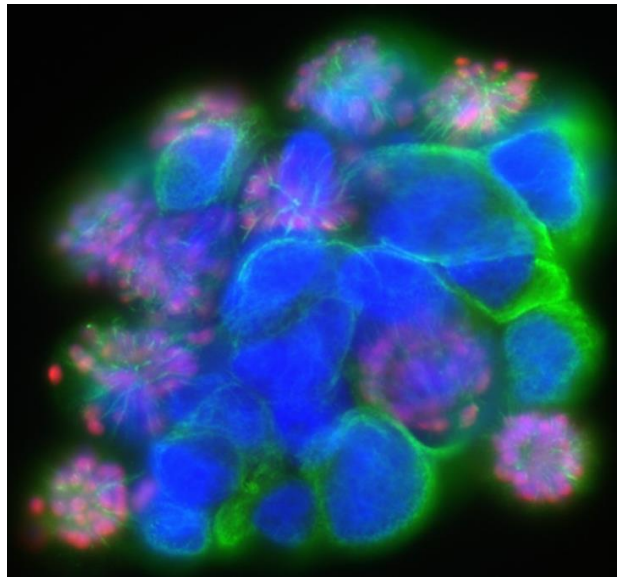
How to Model Disease in the Lab



Andrew B. Gladden, PhD
Associate Professor
Department of Pathology & Laboratory Medicine
University of North Carolina at Chapel Hill

Introduction to Pathology of Disease
October 5, 2021



Goals for today's class!

- Understand the intrinsic cellular response used in genetic engineering.
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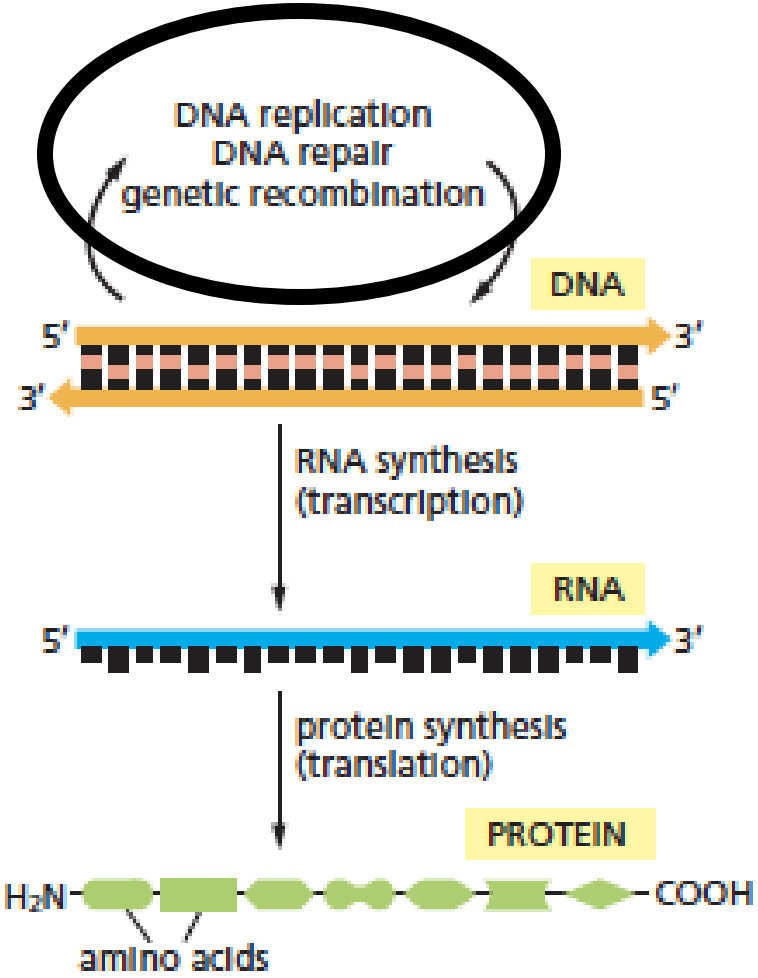
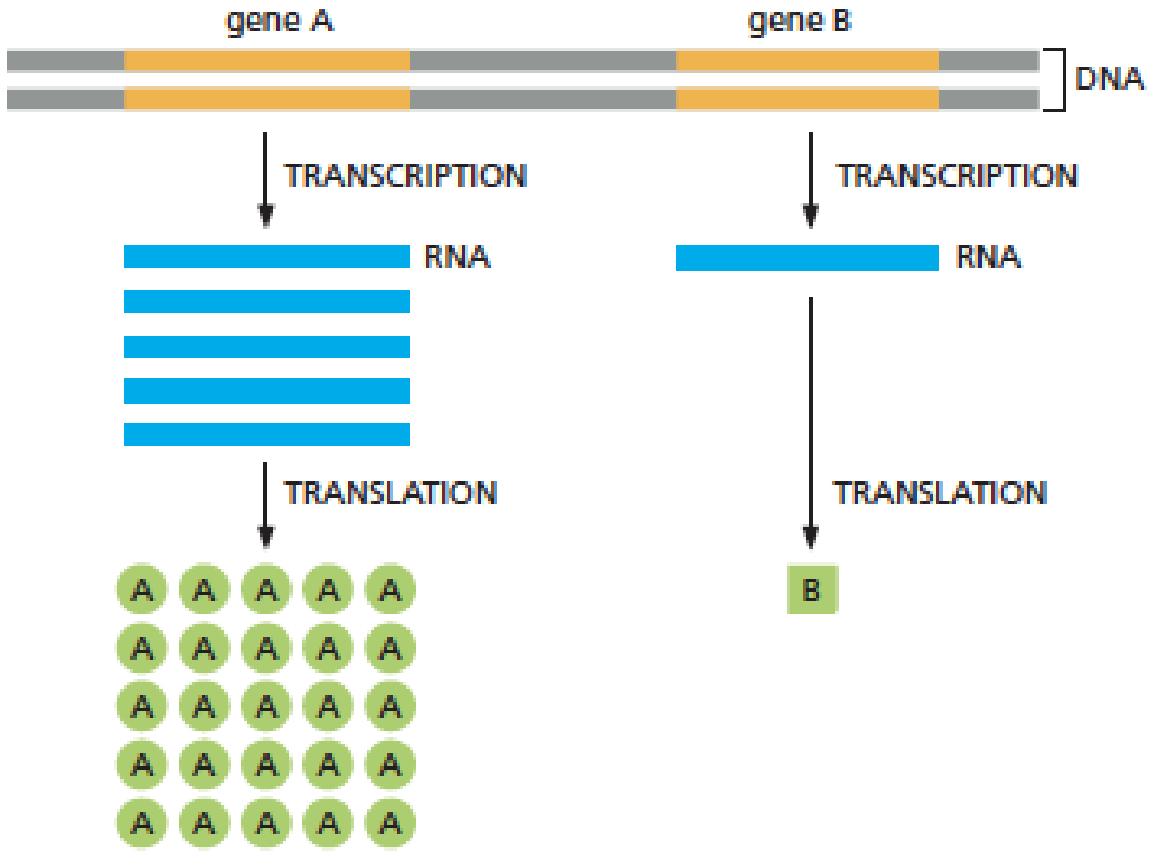
How Does One Gene Make a Large Amount of Protein?





Molecular Biology of the Cell, Taylor & Francis 2014

Poll Question 1: Yes or No

Poll Question 2: Yes or No

NHEJ = Non-homologous End Joining

HR = Homologous Recobination

Repair pathway	NHEJ	HR	alt-NHEJ/ MMEJ	SSA	ICL repair	SSB repair	BER	TLS	NER	MMR
Source of DNA damage	IR, radiomimetics, Topo II inhibitors	X-linking agents, replication inhibitors, antimetabolites, Topo I inhibitors			X-linking agents	IR, ROS, radiomimetics Topo I inhibitors H_2O_2, alkylating agents	Alkylating agents	UV, alkylating agents	Alkylating agents, X-linkers	DNA Pol proofreading errors
Damage sensors	Ku70/Ku80	MRN	PARP	MRN	FA core complex (FANCA, B, C, E, F, G, L and M)	PARP	DNA glycosylases, APE1	PCNA	XPC DDB2 CSA	MSH2, MSH3, MSH6, MLH1, PMS2
Signaling/ mediator proteins	DNAPK	ATM, ATR, MK2, CtIP, BRCA1/BARD1, BRCA2, PALB2, RPA		CtIP	FANCD1 [BRCA2] D2, I J [BRIP1] N [PALB2] O [RAD51C] P [SLX4]			RAD6 RAD18	XPA, XPF RPA	
Effector proteins	XRCC4 XLF LIG4 APLF Artemis PAXX WRN	RAD51 MUS81/EME1 SLX1/SLX4 RTEL1 BLM TOPOIII POLQ PARI RECQL5 FANCJ, BLM	XRCC1 LIG3, LIG1 CtIP POLQ	RAD52, others?	Shared with HR, TLS, and NER	XRCC1 PNKP POLβ FEN1, TDP1 Aprataxin, LIG1, LIG3A	As for SSB repair	REV1, POLH POLI, POLK	XPG ERCC1 POLE POLD1 LIG1, LIG3	EXO1 POLD LIG1

Brown et al.,
Cancer Discovery 7, p20 (2017)

Recombination repair DNA breaks by retrieving sequence information from undamaged DNA

Remember:
DNA is double
Stranded (red bars).

Each mammalian gene
2 alleles (sisters) in
the genome.

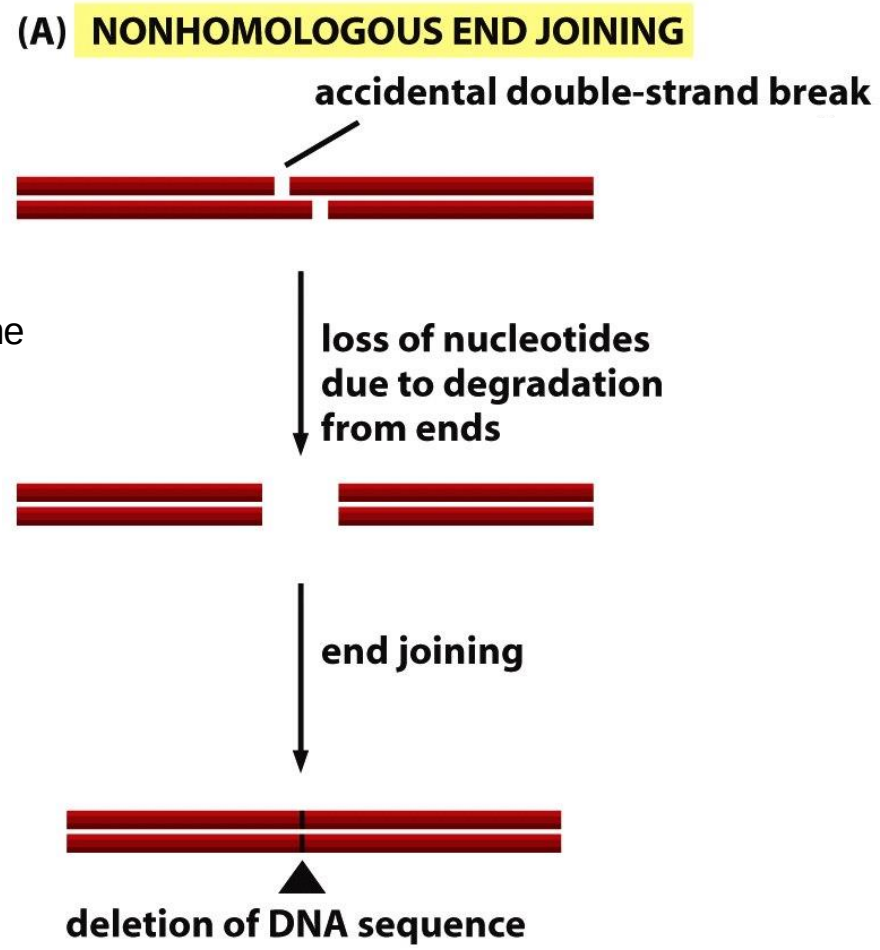


Figure 5-51 Molecular Biology of the Cell 5/e (© Garland Science 2008)

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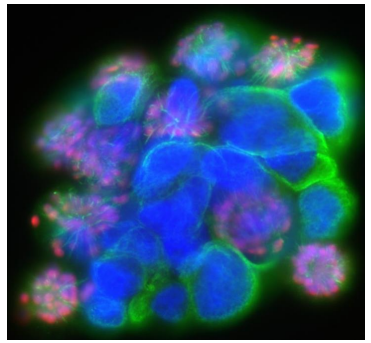
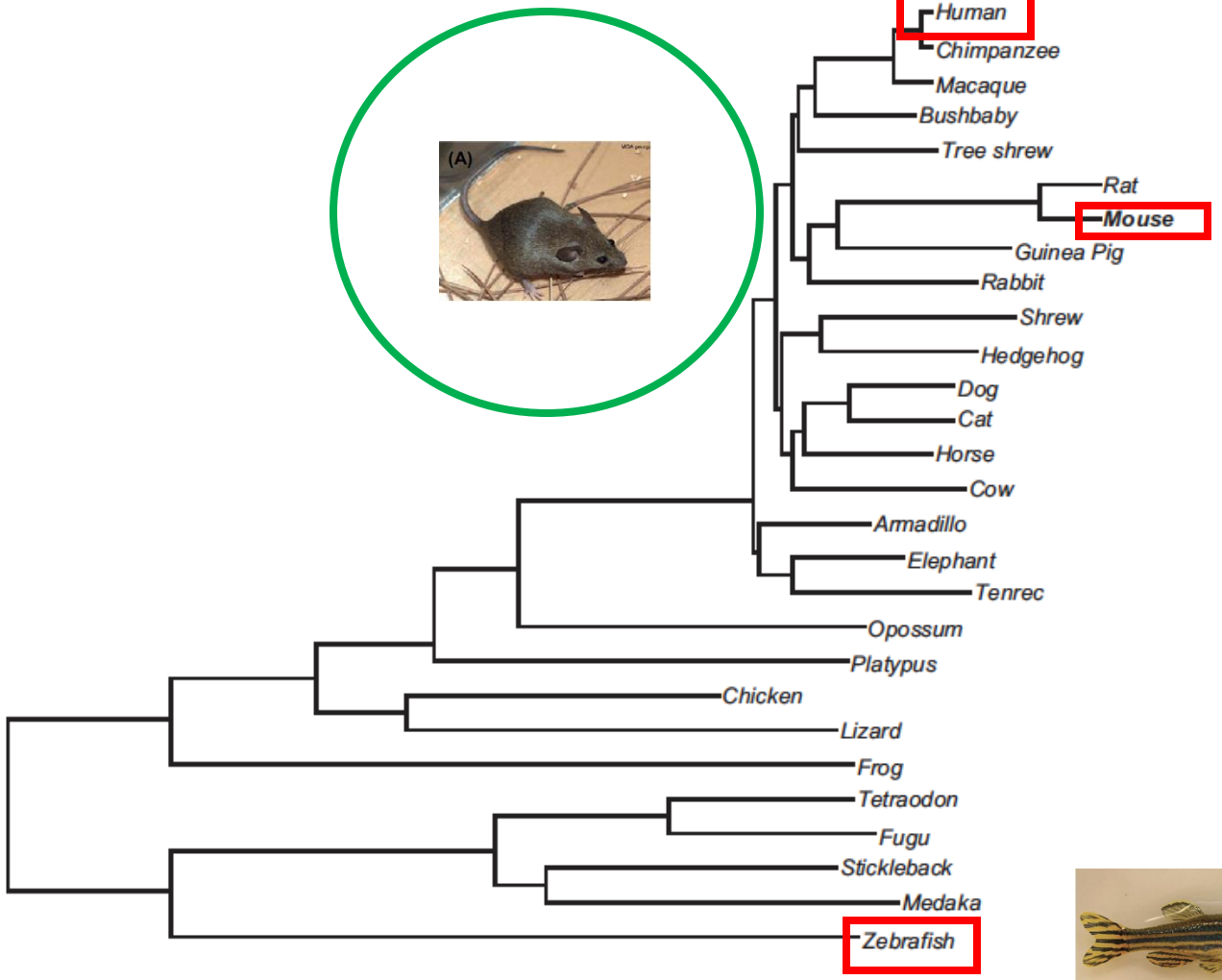
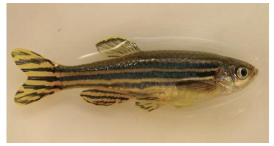
Are we all just a bag of cells?





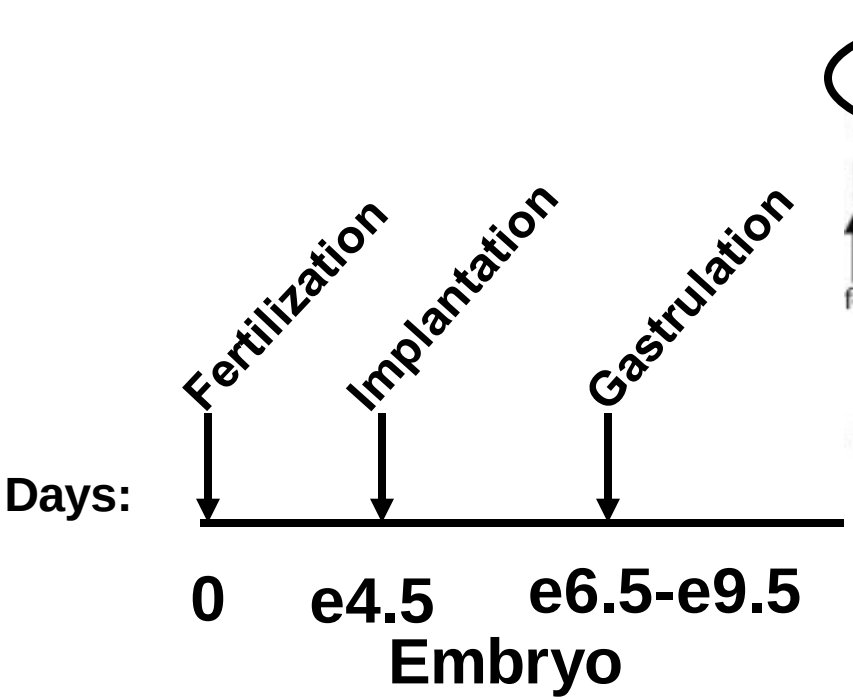
Way down the tree!

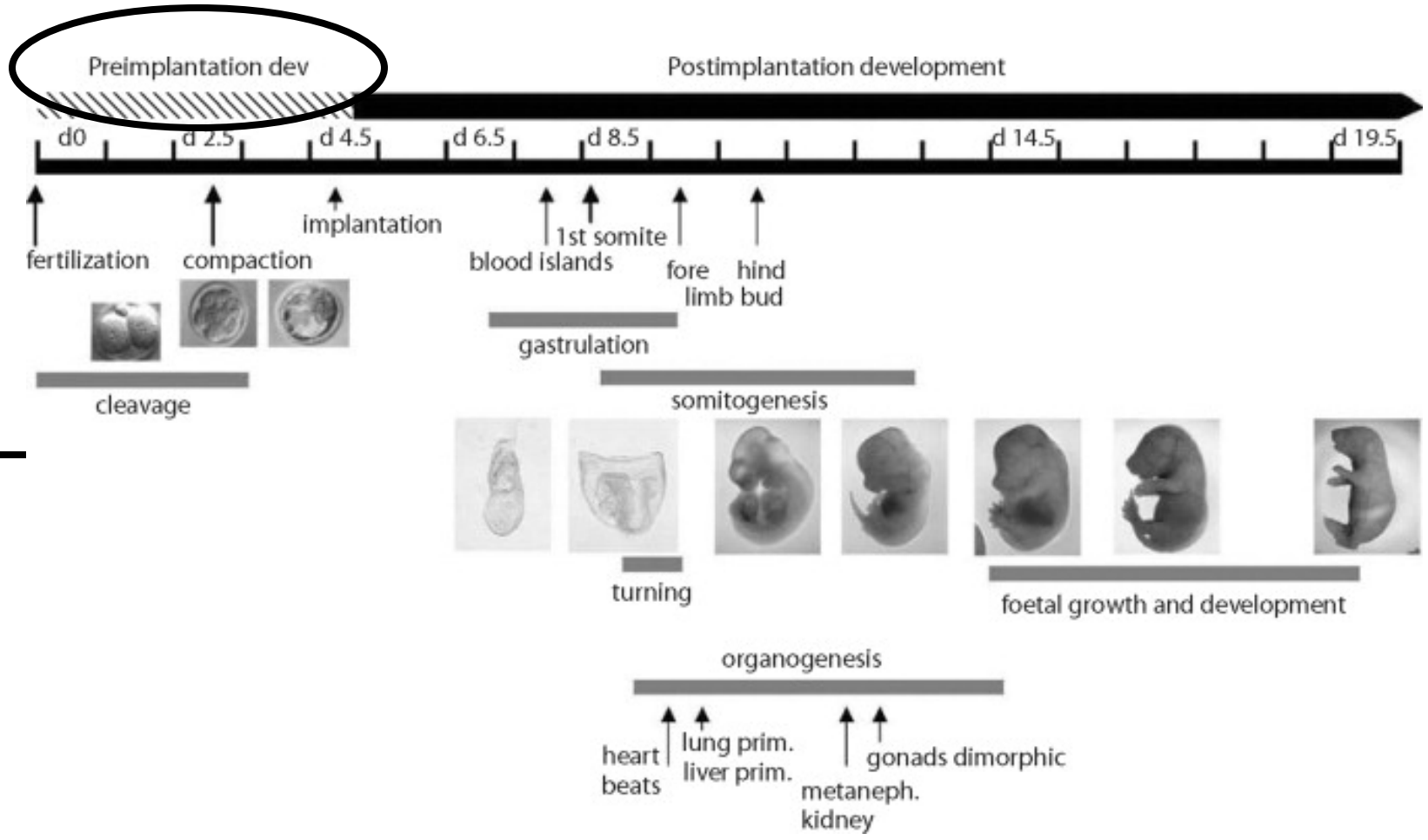
Drosophila



From: *The Laboratory Mouse 2012*

The Lab Mouse Developmental Timeline

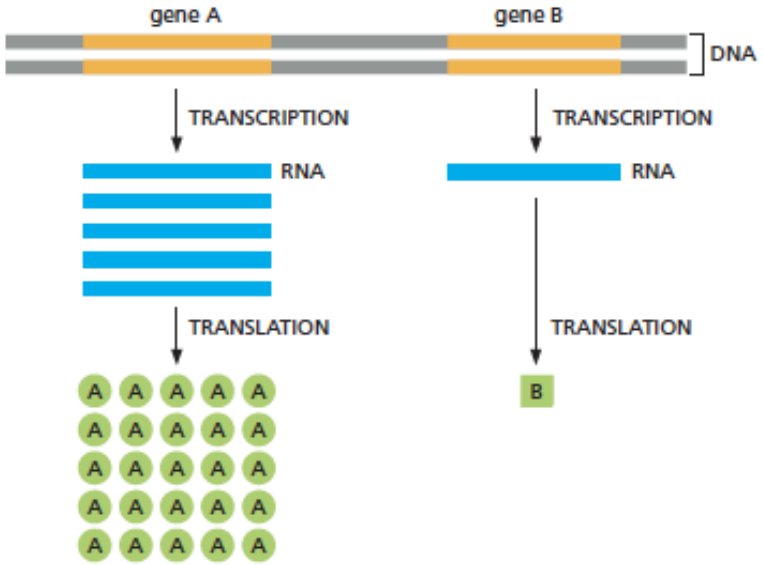


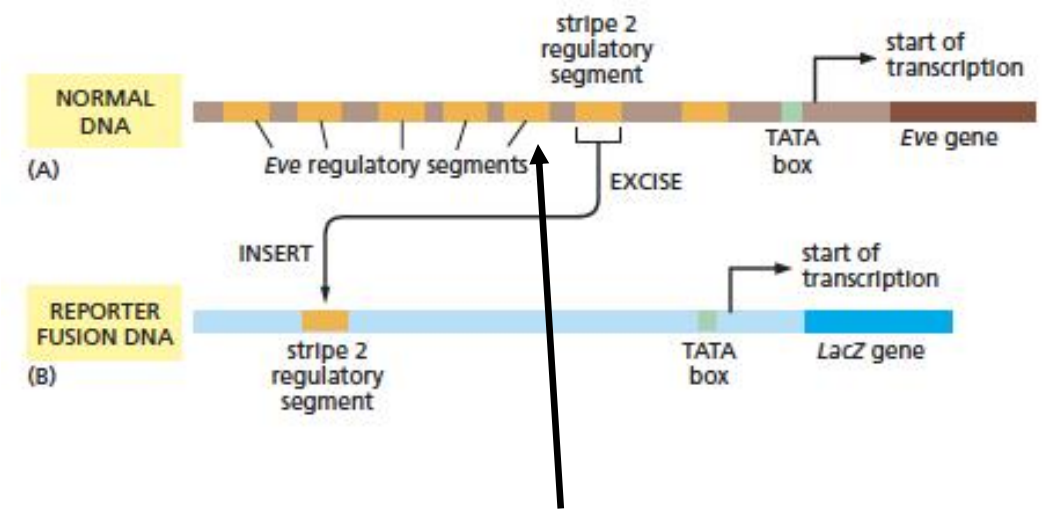


The Mouse Genetic Toolbox

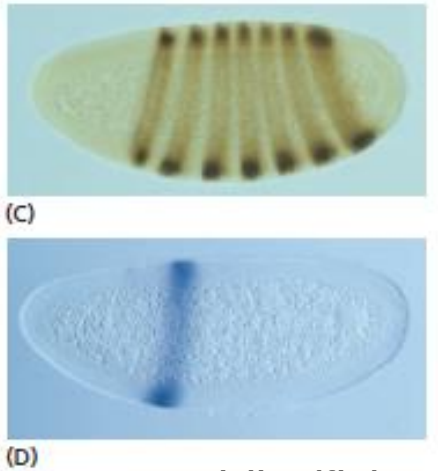
Transgenic Mice

Relatively fast way to generate mice.
Random integration, does not target endogenous gene (generally)
Can be used to overexpress genes or genes that make enzymes in specific tissues.





Regulatory Segment = Promoter
DNA Sequences that promote transcription



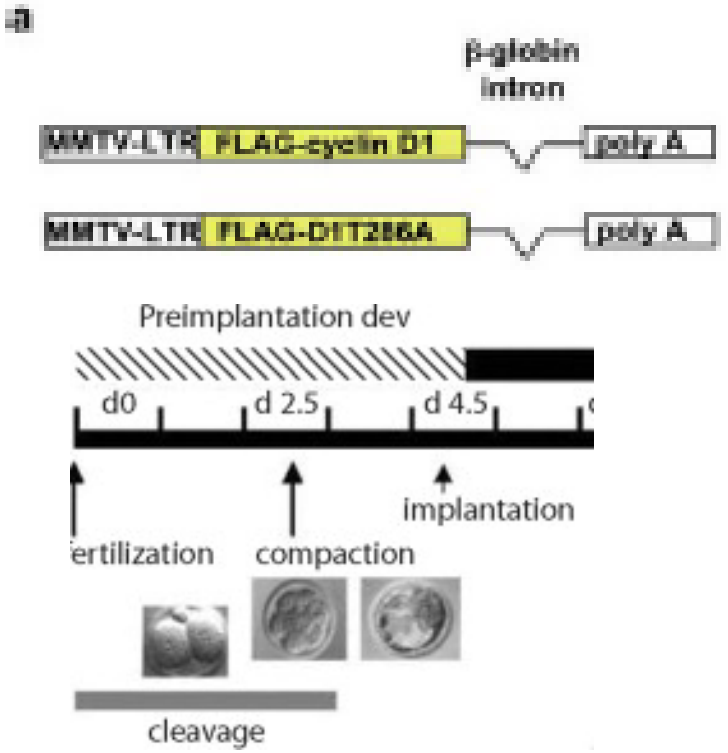
Drosophila (fly)
Embryo

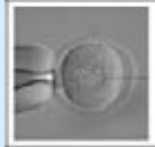
Molecular Biology of the Cell,
Taylor & Francis 2014

The Mouse Genetic Toolbox

Transgenic Mice

Relatively fast way to generate mice.
Random integration, does not target endogenous gene (generally)
Can be used to overexpress genes, examples: Eu-Myc (Lymphoma)
Can be used to drive expression of enzymes utilized in site-specific mutagenesis, examples: MMTV-Cre, Wnt7a-Cre, B-actin-Flp.



	Sperm Oocyte	• SMGT/ICSI-mediated gene transfer
	Zygote	• pronuclear DNA injection • transposon-mediated gene transfer • sub-zonal injection of lentiviral vectors
	2-cell / 4-cell stage	
	Morula	• ES cell injection and aggregation • co-culture with lentiviral vectors
	Blastula	• ES cell injection

ES cell, embryonic stem cell; ICSI, intracytoplasmic sperm injection; SMGT, sperm-mediated gene transfer.

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How Can You as a Researcher Hijack the Cells Intrinsic Responses to Disrupt a Gene?

Remember:
DNA is double Stranded (red bars).

Each mammalian gene
2 alleles (sisters) in the genome.

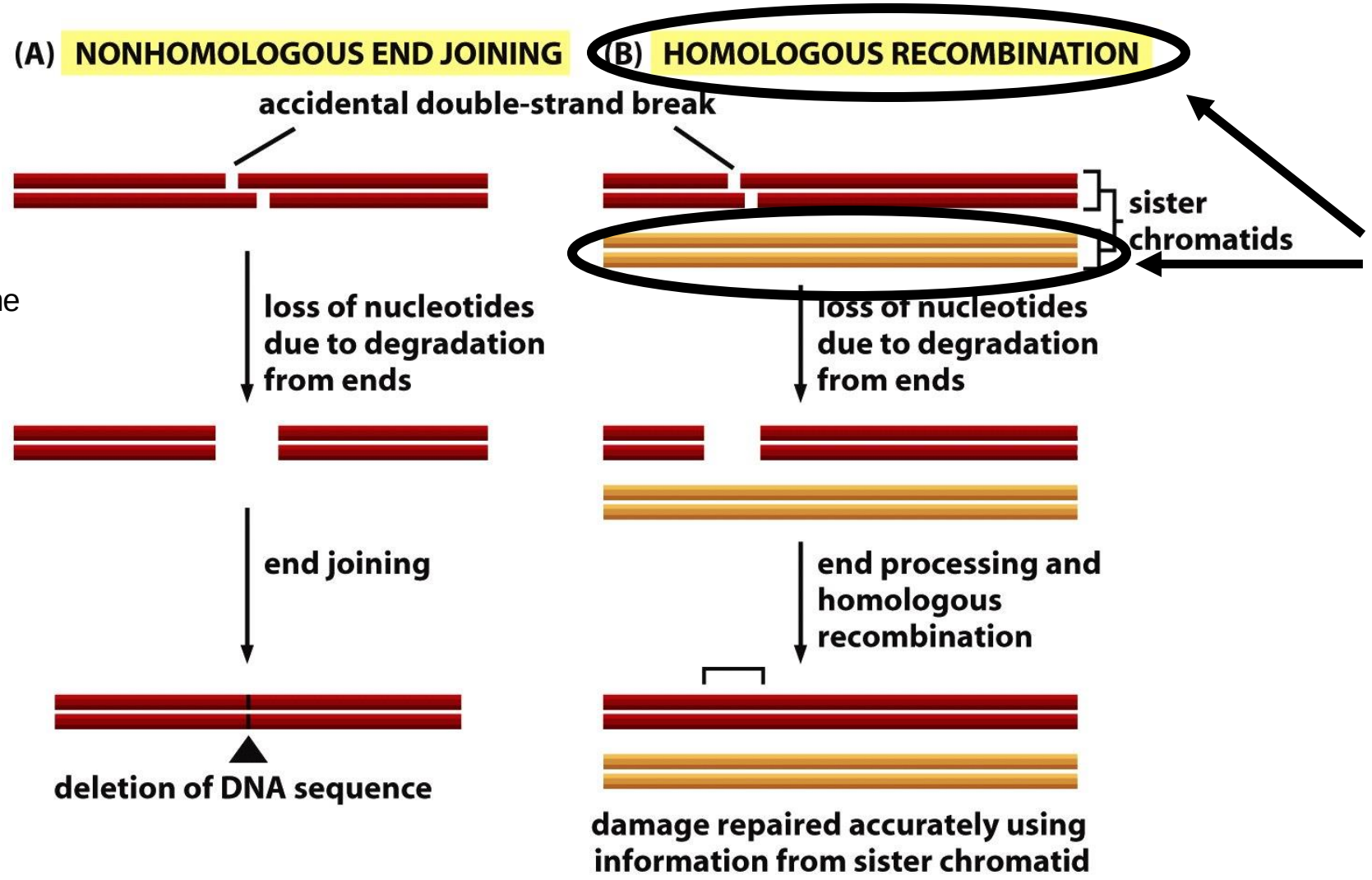
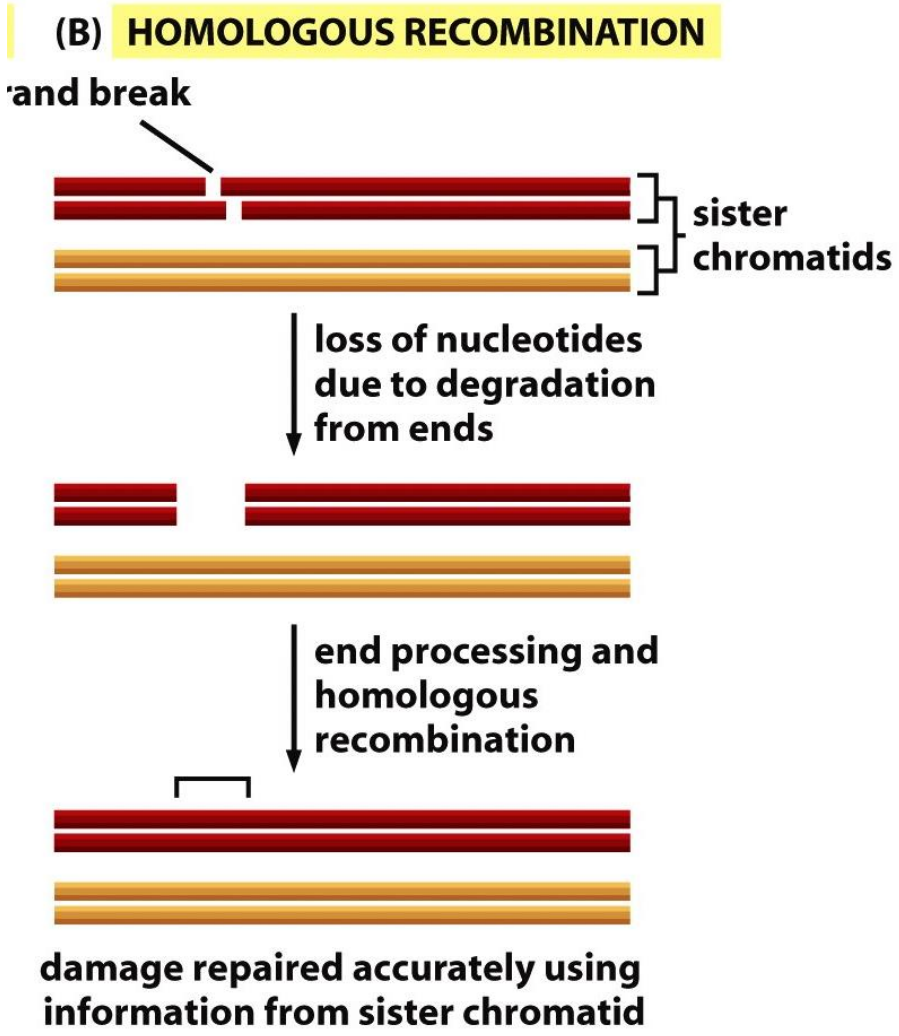
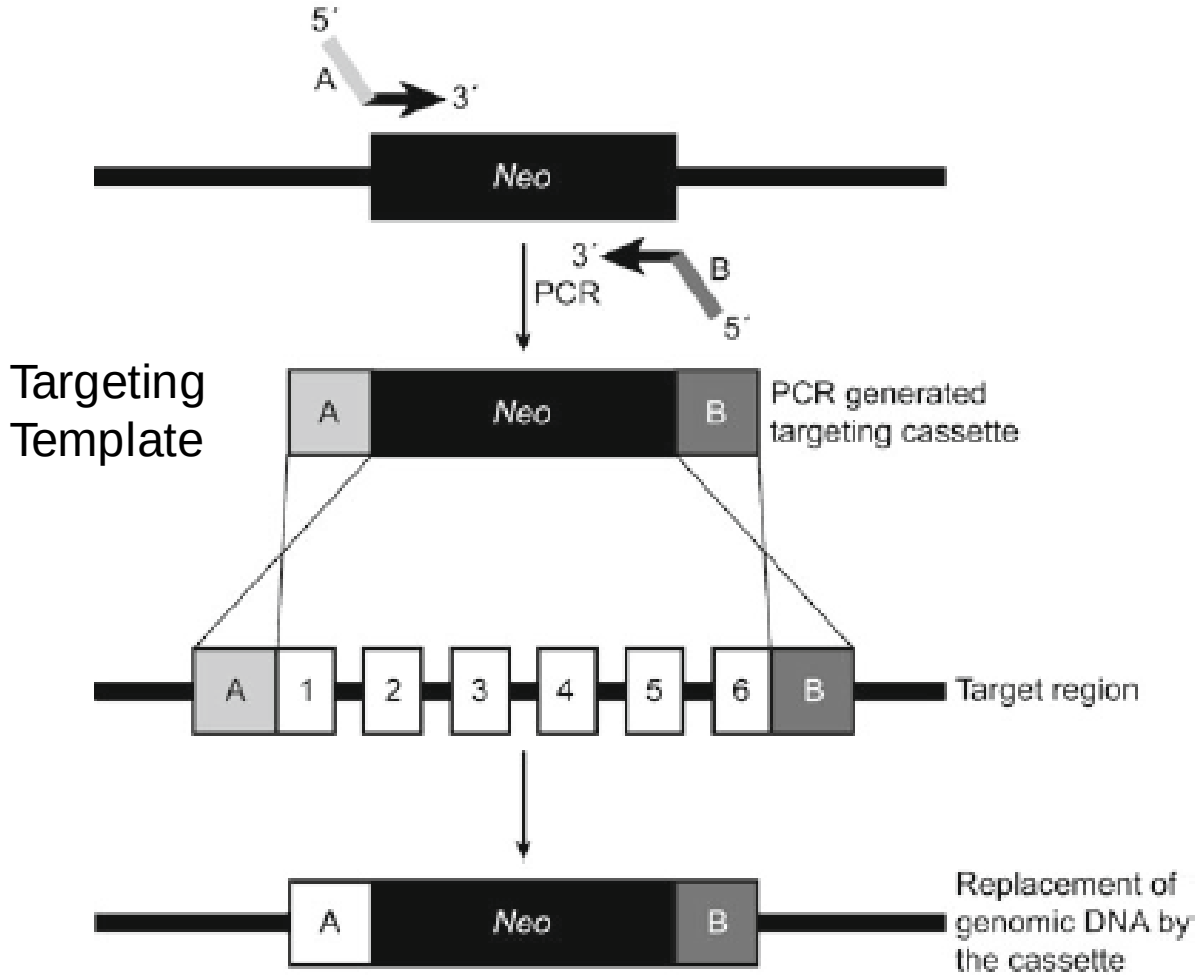


Figure 5-51 Molecular Biology of the Cell 5/e (© Garland Science 2008)

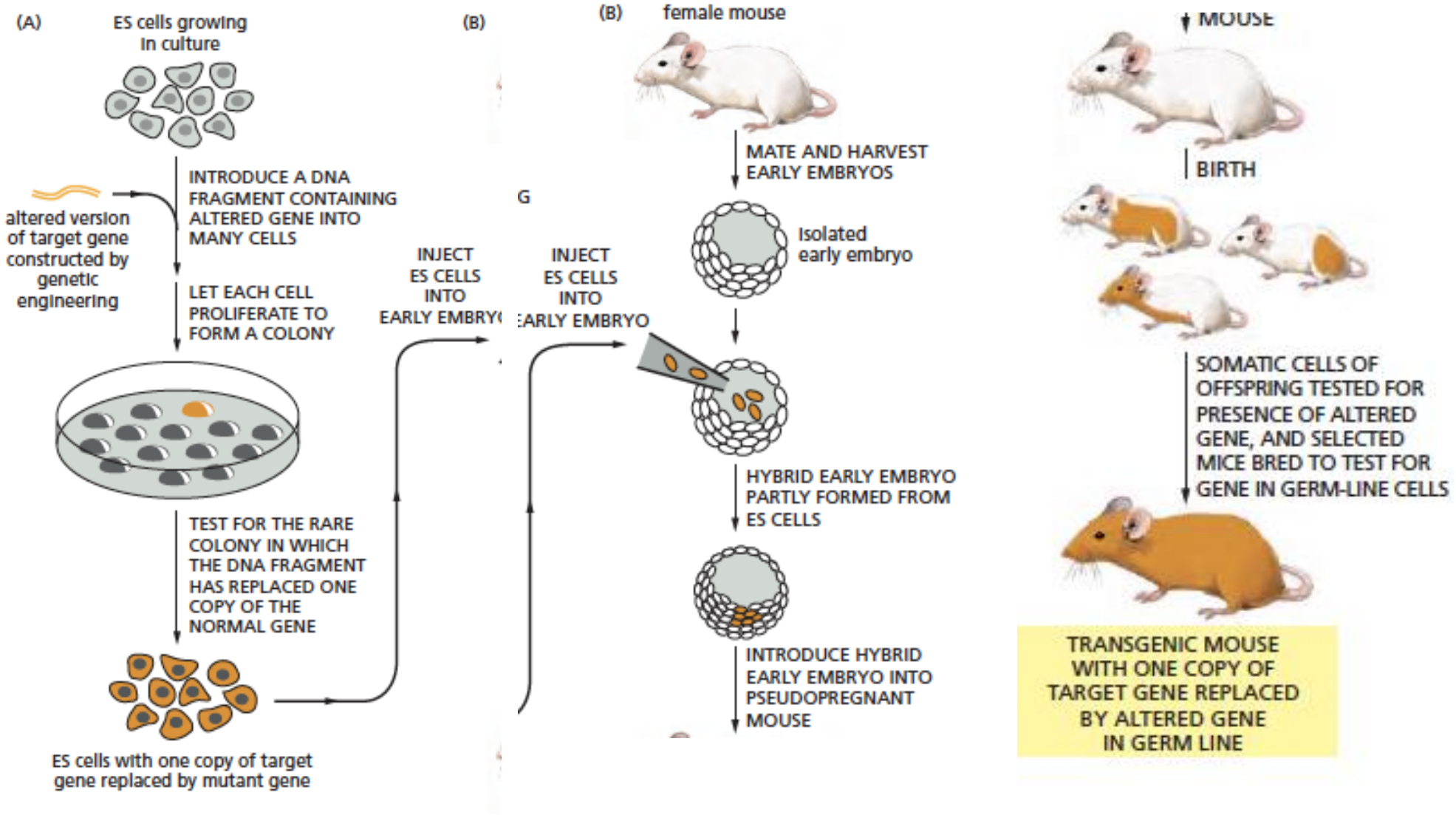
What if you substitute the template for repair?

How Can You as a Researcher Hijack the Cells Intrinsic Responses to Disrupt a Gene?





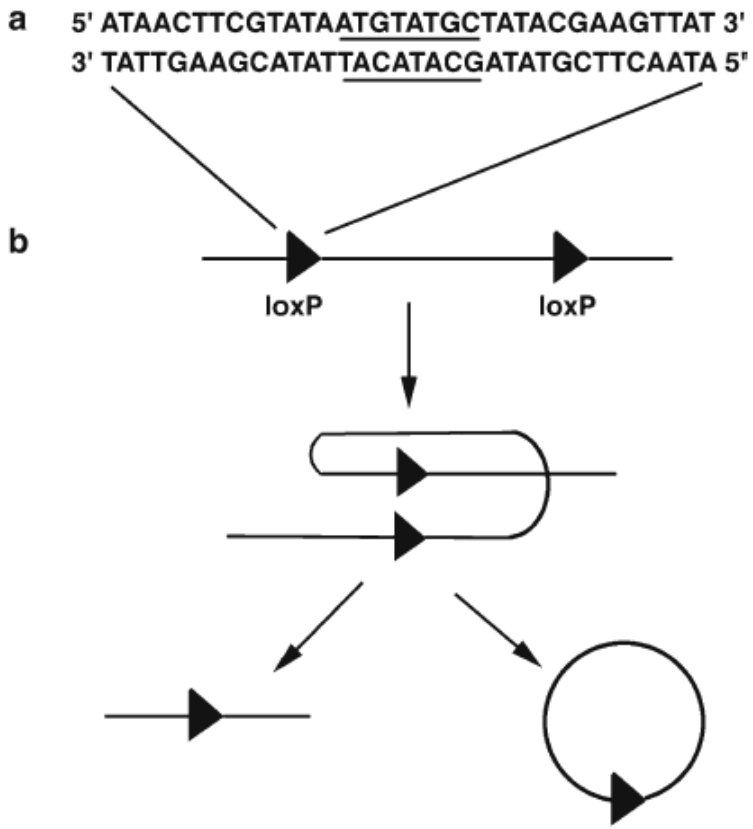
How Do You Get Your Targeting Template Into a Mouse?



The Mouse Genetic Toolbox

Homologous Recombineering in Mice Using Cre

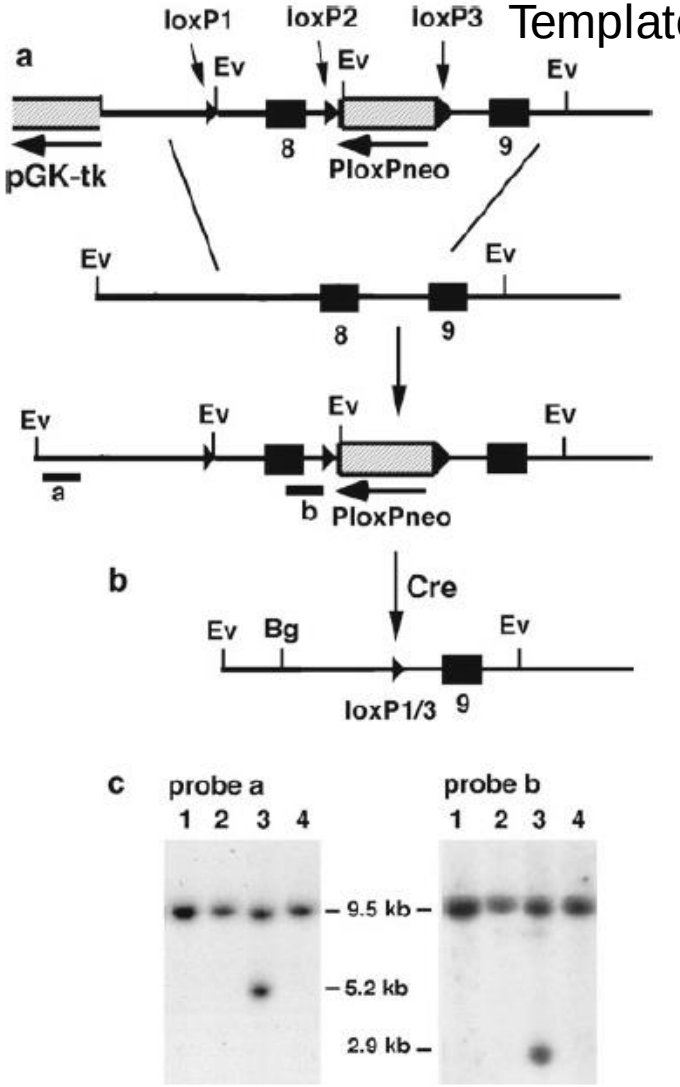
Not a fast may to generate mice includes multiple crosses.
Integration into the endogenous locus and uses endogenous promoter.
Can be used to knockout one or both alleles of a gene.
Can be used to knockin an altered allele (mutant, tagged etc.)



LoxP site contains internal 8bp non-palindromic sequence surrounded by 13bp inverted repeat.

**Cre is an enzyme, specifically, a DNA recombinase that identifies LoxP sites.

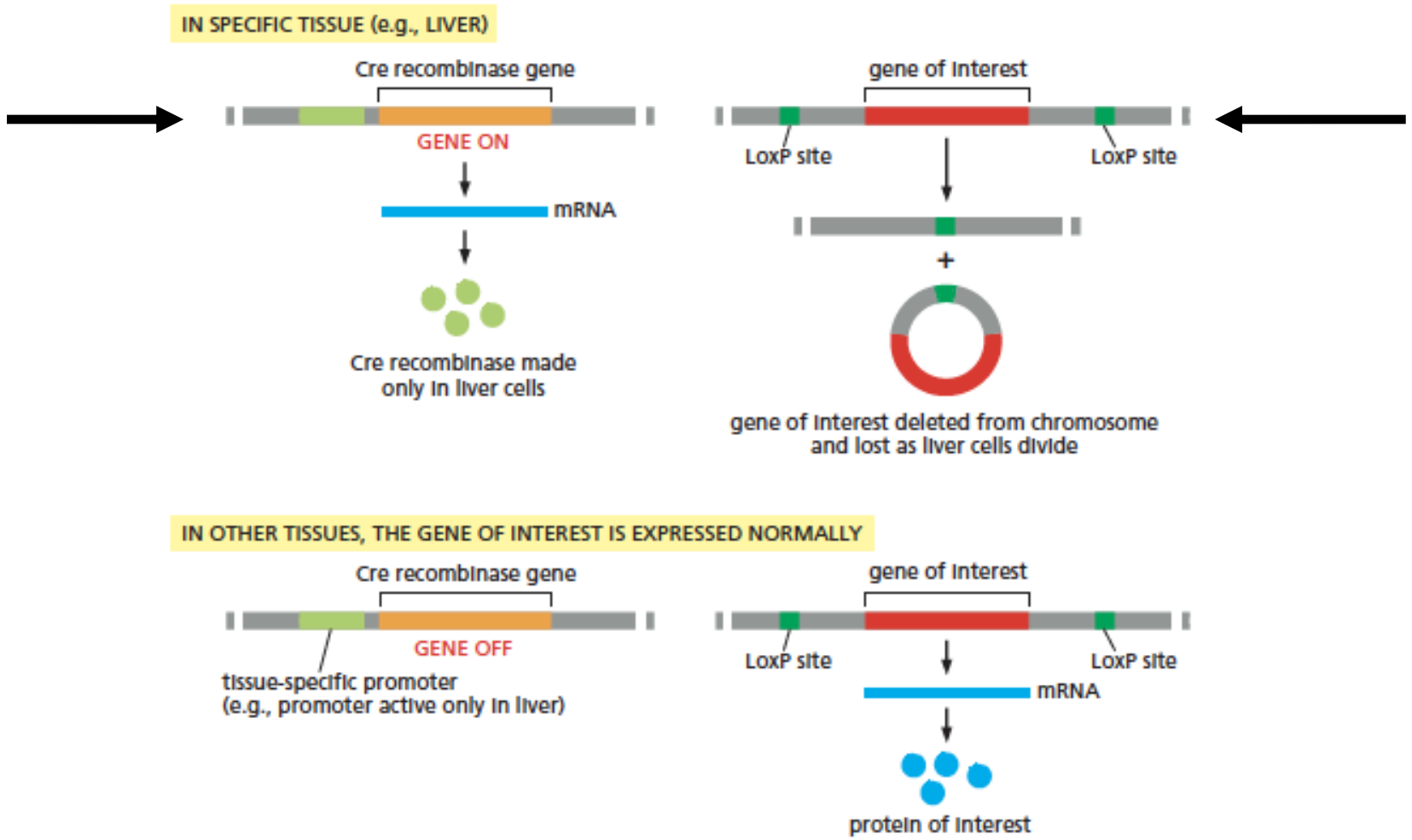
Targeting Template



The Mouse Genetic Toolbox

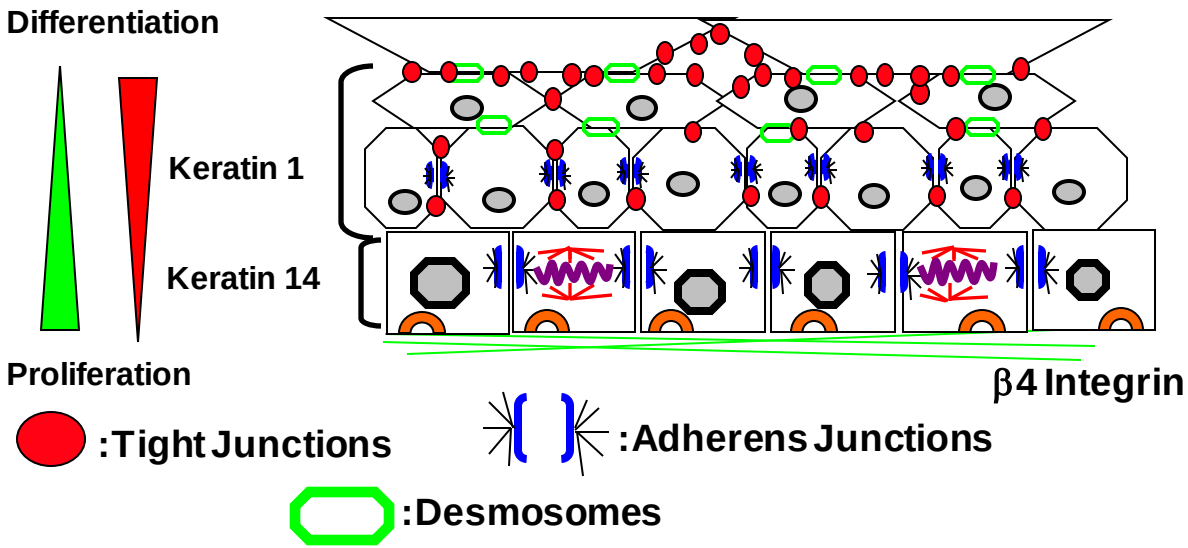
Homologous Recombineering in Mice Using Cre

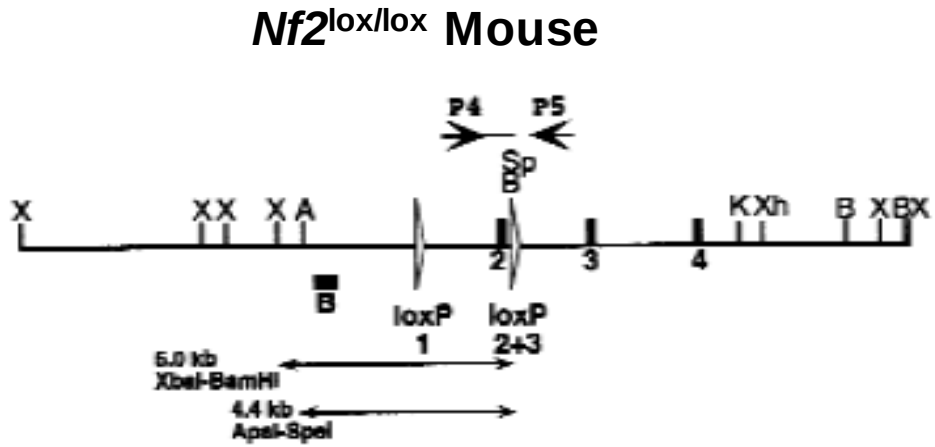
Transgene Overexpressing Cre



Targeted Endogenous Gene

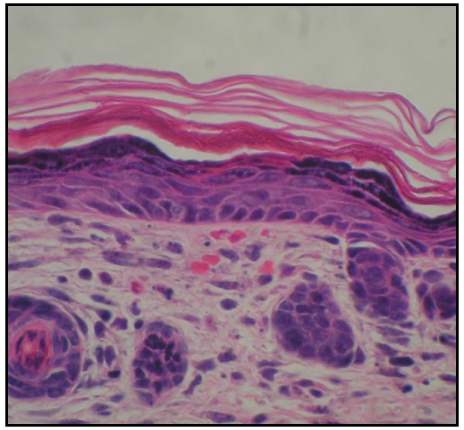
Using a Conditional Nf2 knockout to Study Skin Biology



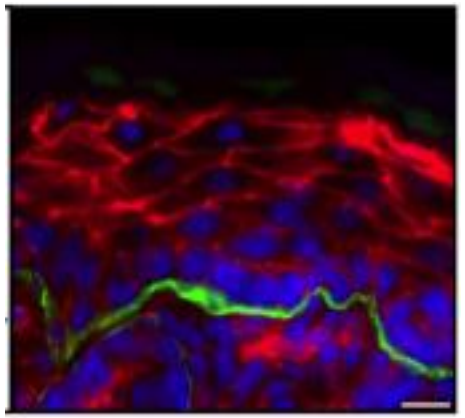


Giovannini et al. 2000

wild-type

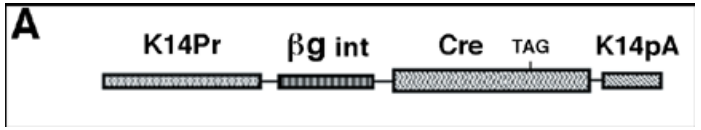


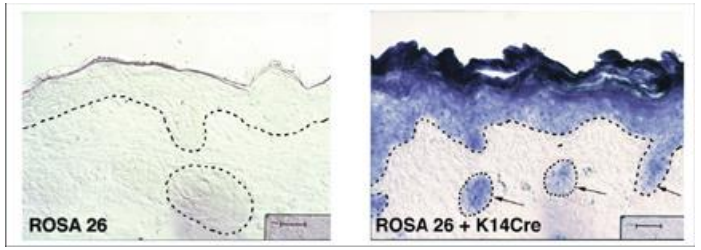
wild-type



Red: Actin **Green: β4-Integrin**

Keratin 14-Cre Mouse

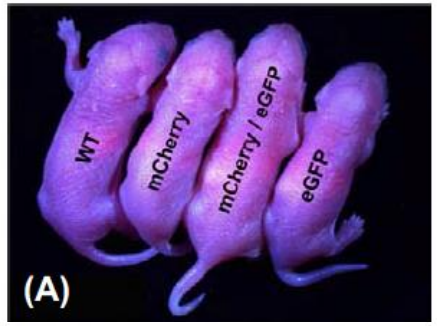




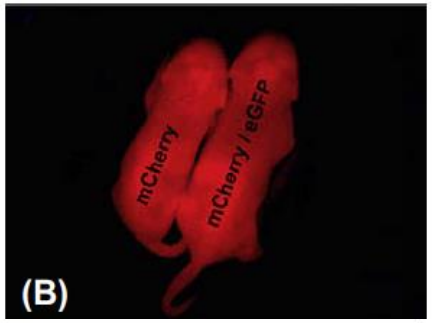
Vasioukhin et al. 1999

The Mouse Genetic Toolbox

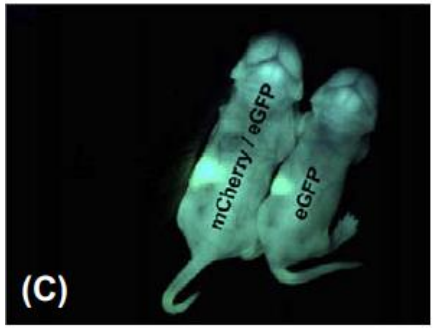
Homologous Recombineering in Mice



Brightfield



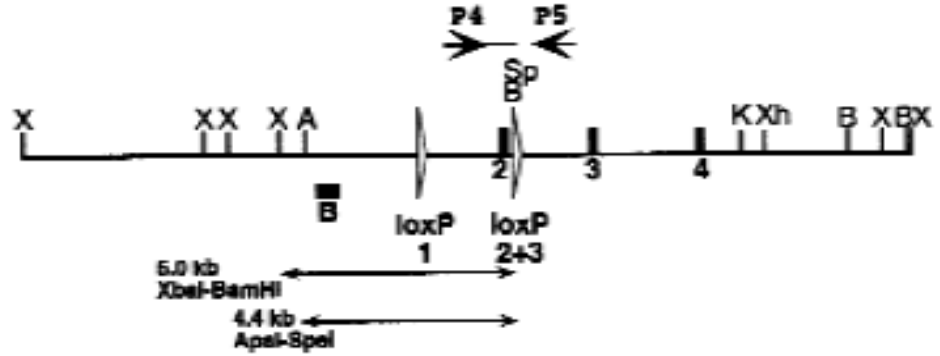
Cy3 Filter



eGFP Filter

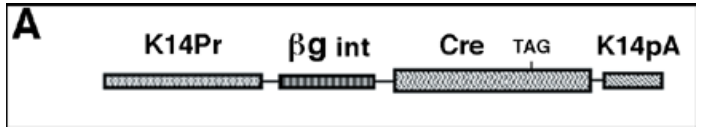
So nc working in your model,
but yc ate the rate of Cre activity
in live d you suggest doing this?

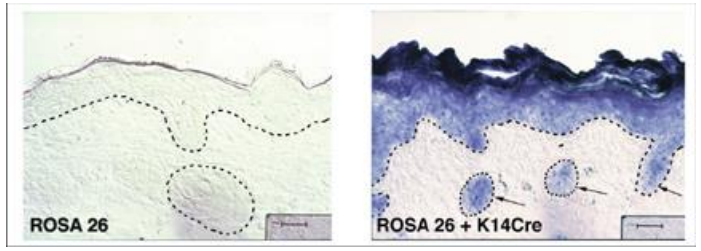
Nf2$^{lox/lox}$ Mouse



Giovannini et al. 2000

Keratin 14-Cre Mouse





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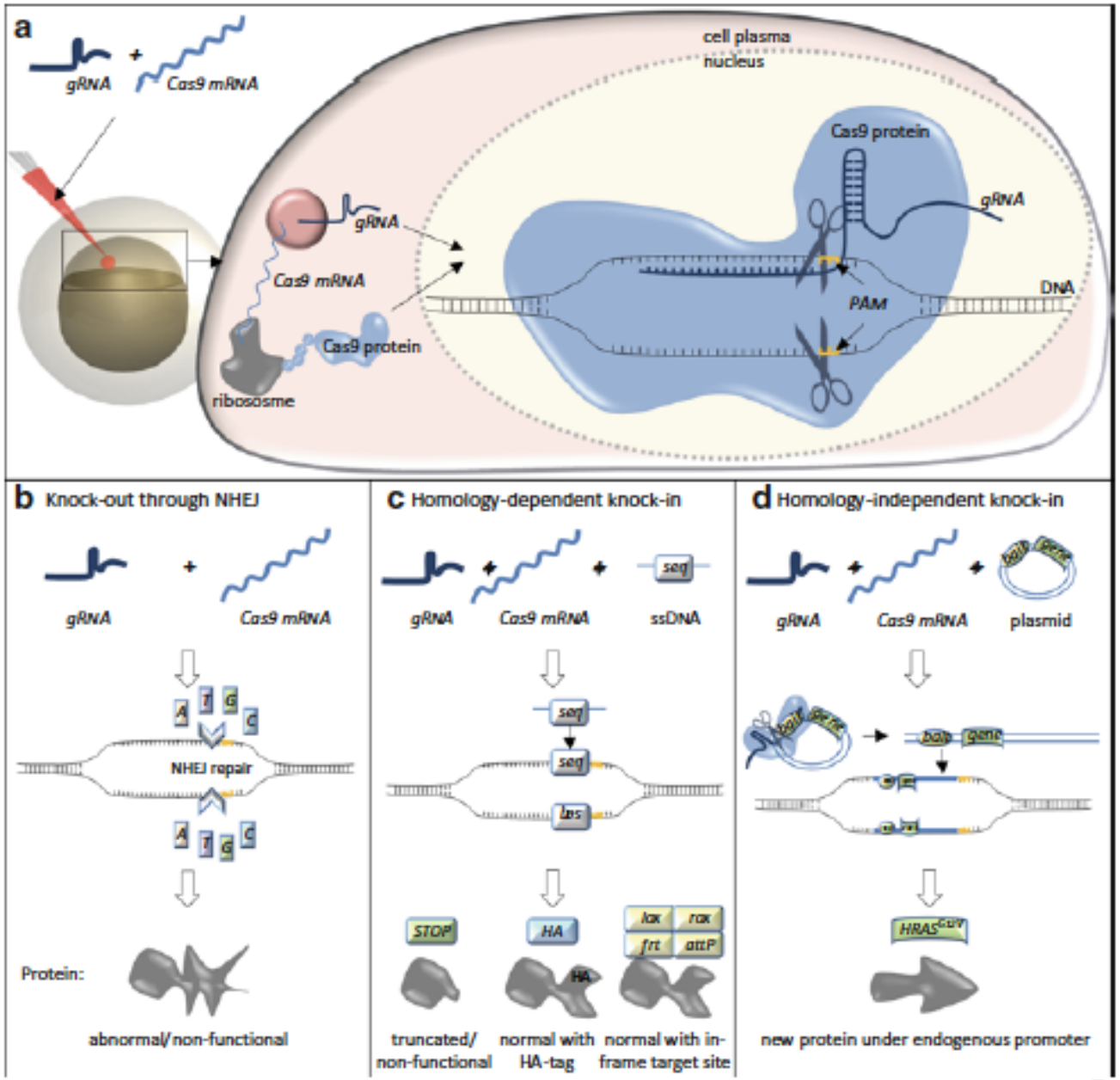
CRISPR/CAS9 the New Flavor on the Block

Cas9 is an enzyme that causes double strand breaks.

Why is it so popular?
Fast!
Multiple species.
Easier to generate guide RNA.
But……



From: *Cancer and Zebrafish 2016*

Recombination repair DNA breaks by retrieving sequence information from undamaged DNA

Rate of NHEJ
In CRISPR.
30 to 70%

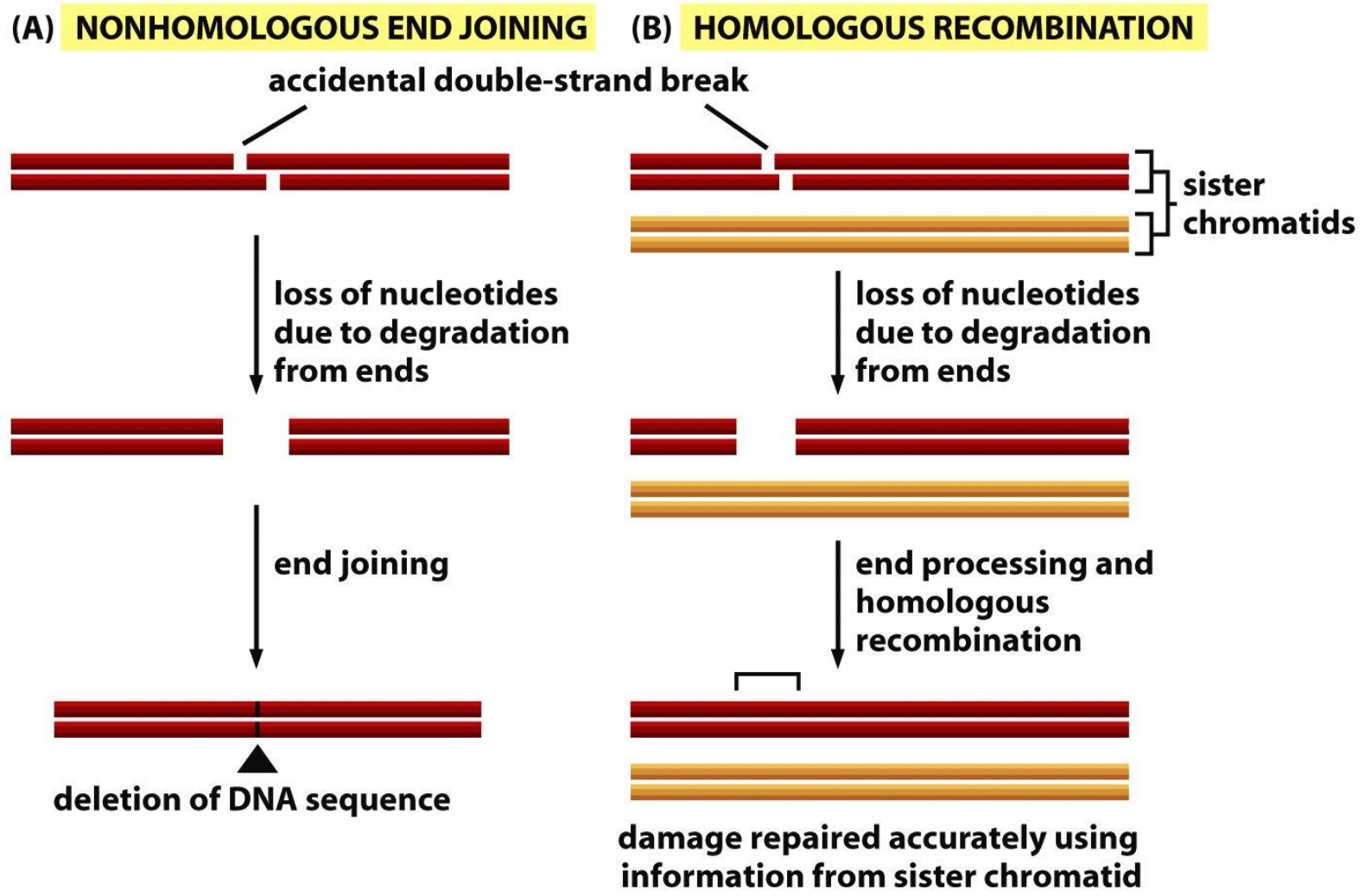


Figure 5-51 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Rate of HR
In CRISPR.
Less than 1%

CRISPR/CAS9 the New Flavor on the Block

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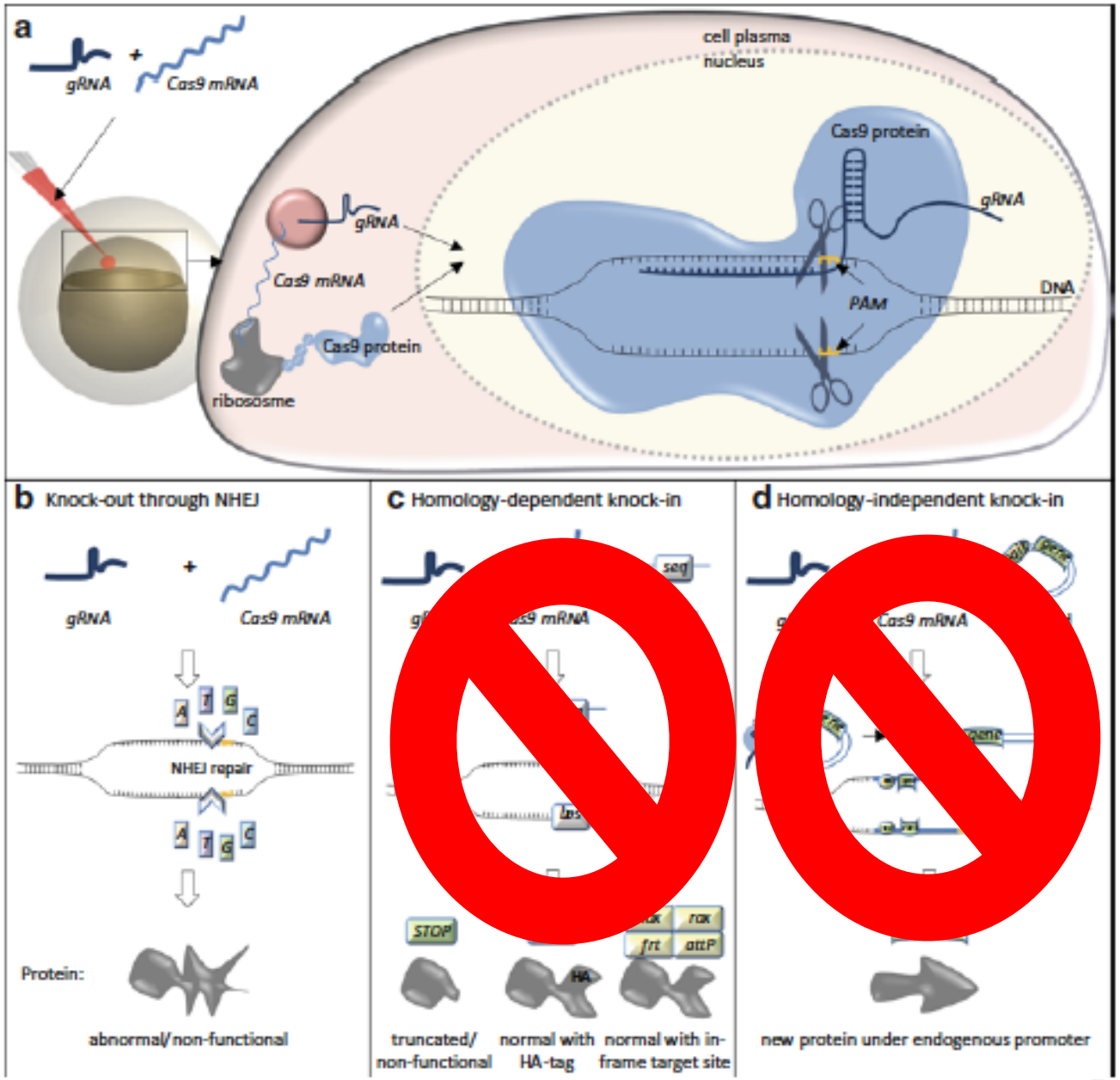

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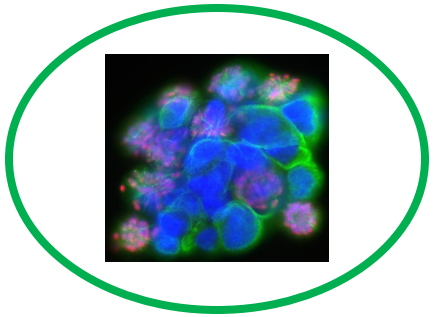
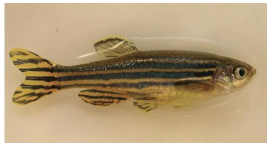
What about the bag of cells?



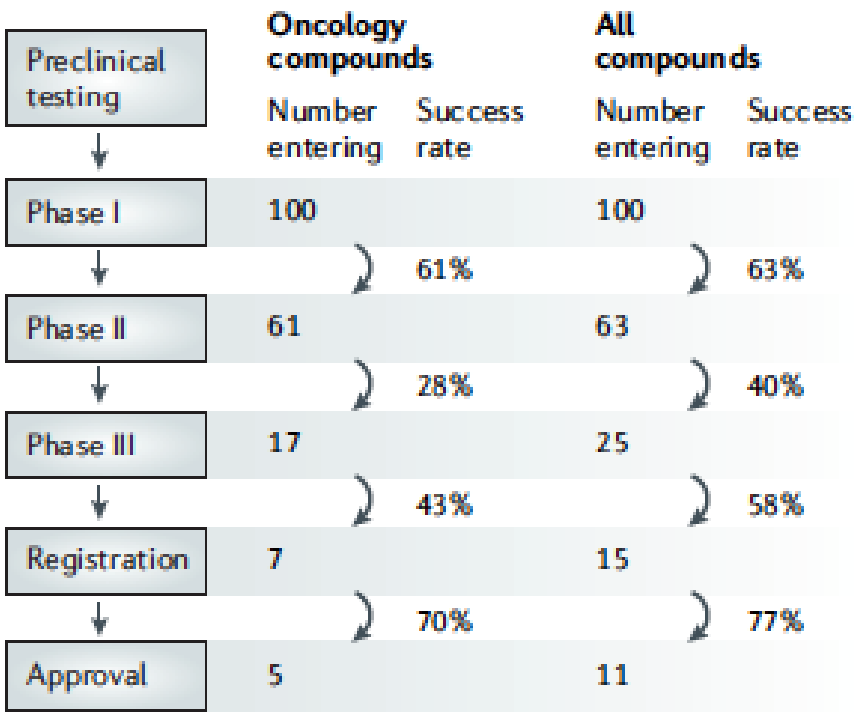
Way down the tree!

Drosophila



From: *The Laboratory Mouse 2012*

Clinical Testing Compound Attrition Rates

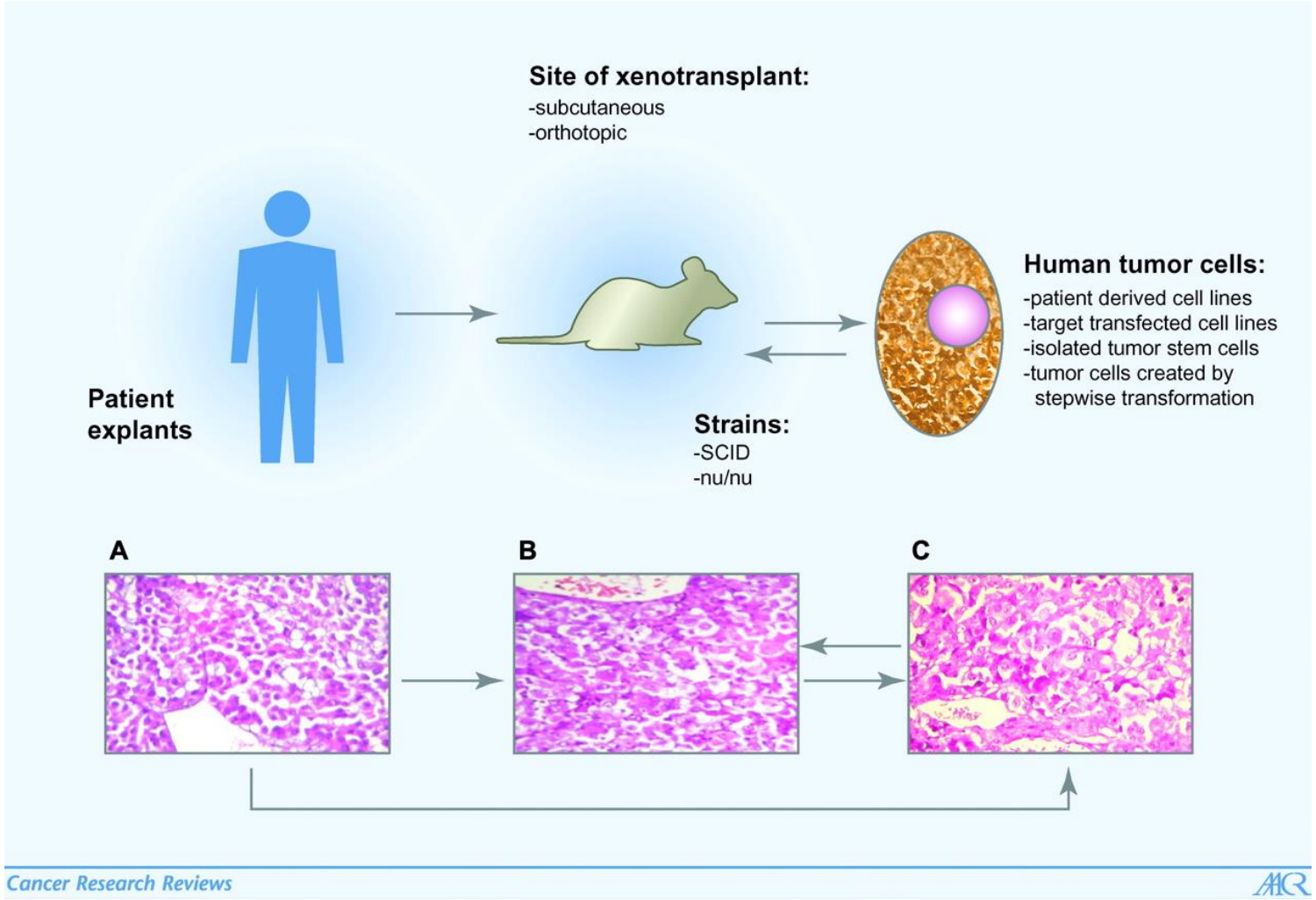


	Oncology compounds		All compounds	
	Number entering	Success rate	Number entering	Success rate
Preclinical testing				
↓				
Phase I	100		100	
		61%		63%
Phase II	61		63	
		28%		40%
Phase III	17		25	
		43%		58%
Registration	7		15	
		70%		77%
Approval	5		11	

Rates from ten large pharmaceutical companies In the US and Europe from 1991-2000.
Phase II, human efficacy assessment, is also the most expensive.

Sharpless and DePinho, 2006

How do we get a better view of potential efficacy?

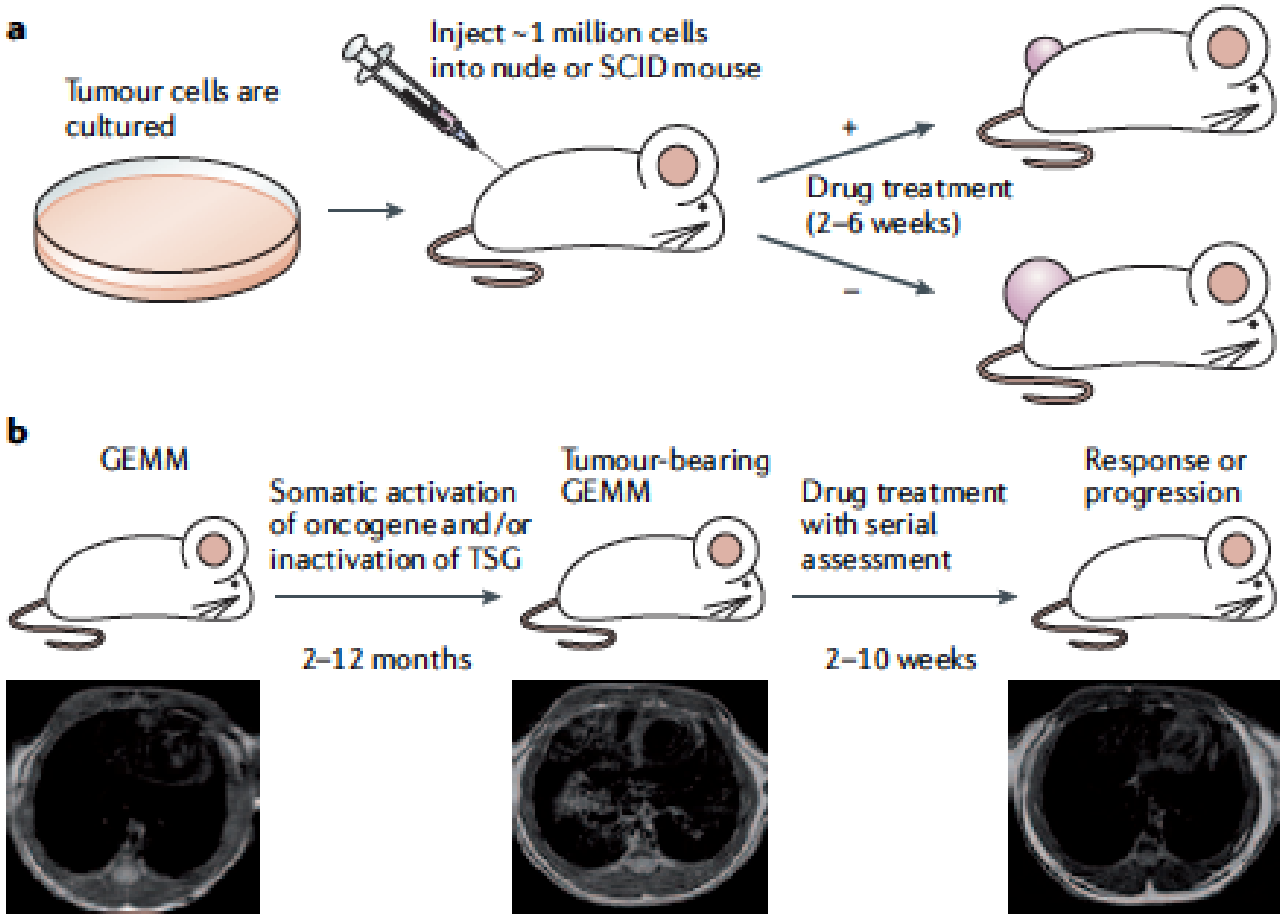


Sausville and Burger, 2006

Nude mice: *Foxn1*^{nu} homozygous no CD4 or CD8 T cells.

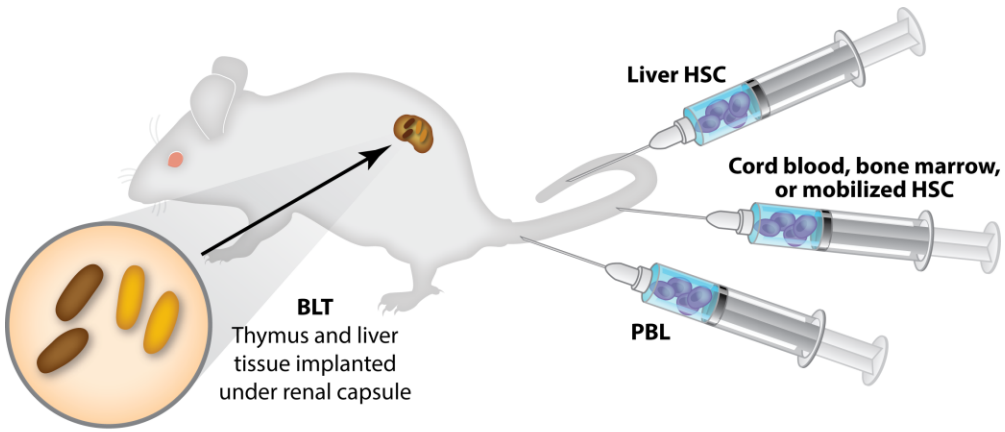
SCID mice: *Prkdc*^{scid} homozygous no T or B cells.

How Fast Can we Assess Drug Treatment?



Sharpless and DePinho, 2006

The Future of Humanized Mice



Walsh NC, et al. 2017.
Annu. Rev. Pathol. Mech. Dis. 12:187–215

Table 1 (*Continued*)

Pathogen	Model	Infection route	Major findings	Reference
Varicella-zoster virus	CB17-*scid* mice with fetal human thymus/liver, sensory neurons, or skin transplants	IP	Human-specific pathogen that causes chickenpox; when reactivated in older individuals, causes shingles. Humanized mice have been used to study viral replication in human grafts and how the virus establishes latency.	Reviewed in 152
Human T cell leukemia virus	NOG mice	Engraftment of $CD133^+$ human stem cells	Productive infection for 4–5 months, rapid expansion of $CD4^+$ T cells, and HTLV-1–specific immune responses were observed.	145
Nipah virus	NSG mice	Intragraft inoculation	Human lung xenograft model that was successfully infected with Nipah virus, which replicated to high titers in the engrafted lung tissues.	153
Chlamydia	NSG BLT mice	Transcervically into the uterus	UV-killed chlamydia complexed with synthetic adjuvant particles induced a protective immune response. Vaccinated mice had $CD4^+$ T cells producing IFNγ and decreased bacterial burdens 4 days post-rechallenge.	154

Abbreviations: BLT, bone marrow/liver/thymus; BRG, BALB/c-$Rag2^{null}$ $IL2rg^{null}$; HSC, hematopoietic stem cell; NK, natural killer; NOG, NODShi.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}; NSG, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}; PBSC, peripheral blood stem cell; UV, ultraviolet.

Walsh et al., 2017

Goals for today's class!

- Understand the intrinsic cellular response used in genetic engineering.
- Understand why the mouse is used as a disease model in the lab.
- Learn how to overexpress a gene in a specific mouse tissue.
- Identify how to disrupt an endogenous gene in mice or cells.
- Become familiar with how human cells or tissue are studied in the lab.
- Learn what type of mice are used to grow human tissue.
- #1 GOAL!! Participate in class, ask questions as we move through different topics!