

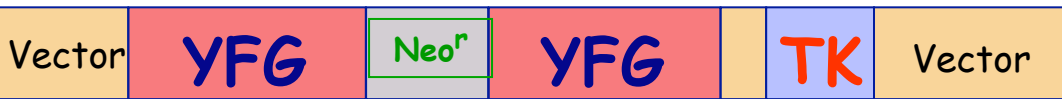
MCDB 4650 Class 20

Developmental genetics in mice, continued

Learning goals:

- Distinguish between mice generated using the Cre-Lox system and knock-out or knock-in mice, and explain the advantages of the Cre-Lox technology.
- Solve problems and design experiments using the knock-out, knock-in, and Cre-Lox technologies.

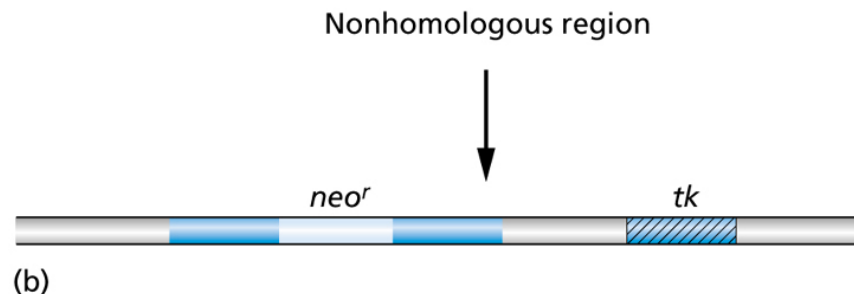
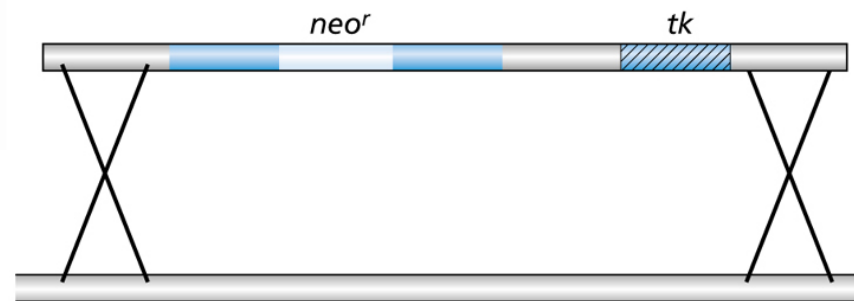
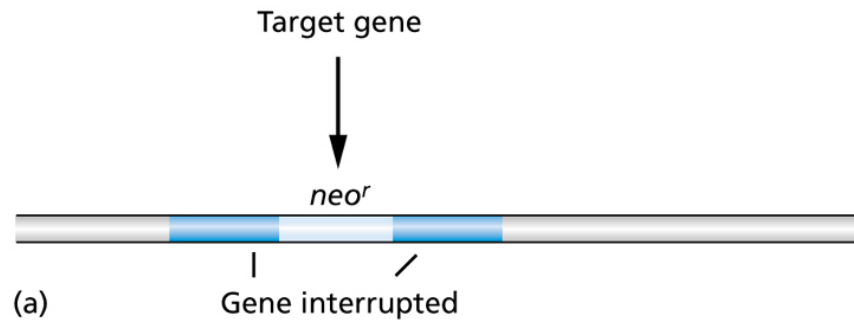
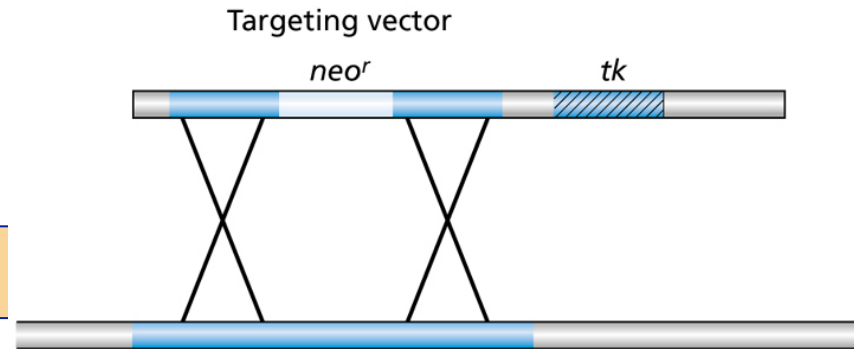
- Homologous recombination is rare, and thus requires selection to ensure it's happened



HR



Non-homologous recombination
(ie, random insertion)



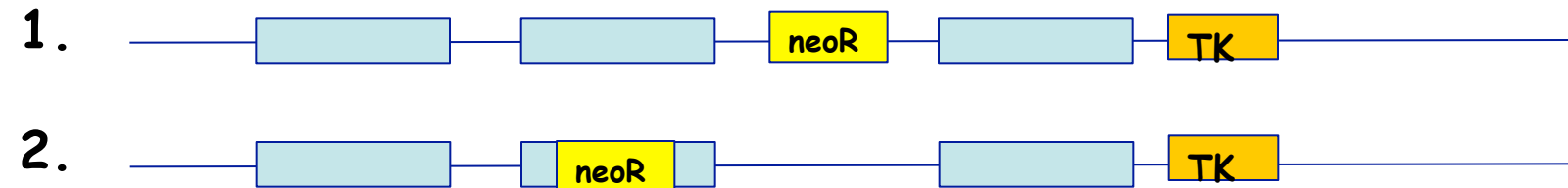
If HR is successful with the constructs below (the ES cell incorporates the construct shown), Assume exon 2 is required for normal function of the protein

- Cells with construct 1 will survive in the presence of neomycin
- Cells with construct 2 will survive in the presence of neomycin
- Cells with either of these constructs will survive in the presence of neomycin

Normal gene: 3 exons

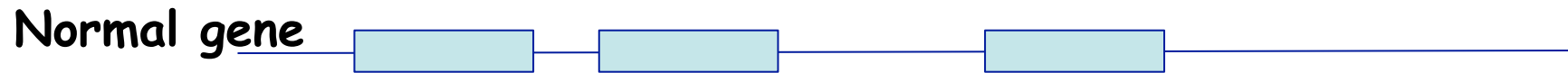


Engineered constructs:

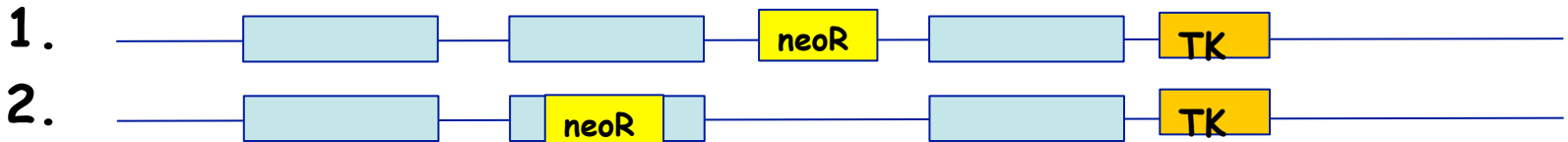


If you manage to make transgenic mice (using homologous recombination) with one of the engineered constructs below:

- Mice who are homozygous for construct 1 will have a knockout phenotype for this gene
- Mice who are homozygous for construct 2 will have a knockout phenotype for this gene
- Mice homozygous for either of these constructs will have a knockout phenotype for this gene
- Neither lines of mice homozygous for these constructs would have a knockout phenotype



Engineered constructs:



Goal of homologous recombination for generating Knock-Out mice

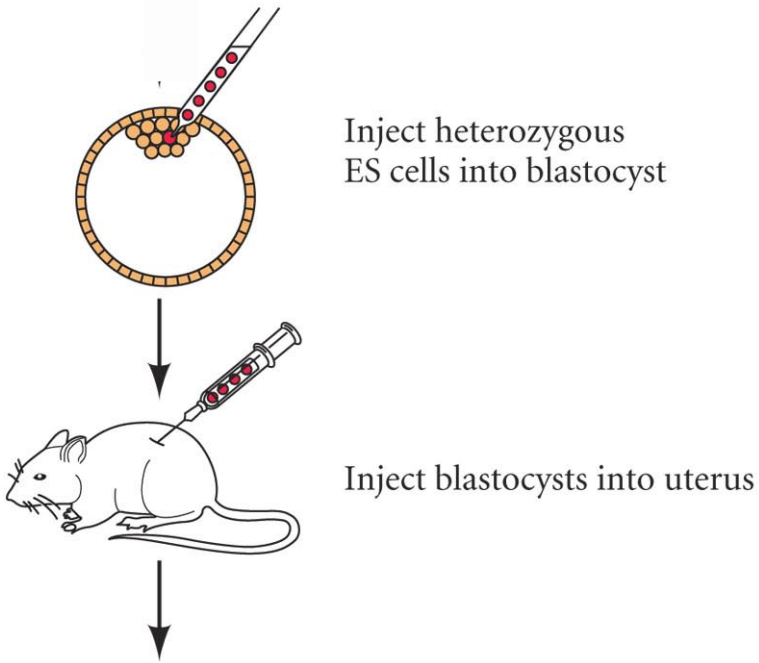
Remove function of a gene

Can remove a whole exon, many exons, or just interrupt one exon.

Either way, one incorporates the neoR gene (with its own translational start site, etc) for the positive selection

We have the ES cells (from a homozygous black mouse) that have undergone homologous recombination at the gene of interest

Next step: inject into blastocysts obtained from a mated white mouse and reimplant them into pseudopregnant white females (surrogate moms).



Inject heterozygous
ES cells into blastocyst

Inject blastocysts into uterus

**When the female gives birth to offspring
(all embryos are those that were
injected with ES cells):**

- a. All will be variegated (both black and white patches of coat color)
- b. All will be white
- c. 50% white and 50% variegated
- d. There could be variegated, all white and all black offspring

Hint: Think about those ES cells that you put into the early embryo. What will they become?

Usually, they are variegated, but if the skin cells of the mouse are made by only ES cells or only host cells, a mouse could be all black or all white.

Now you take a chimeric mouse (variegated color) and mate it to a male wild type, white mouse.

What will the mouse pups from this cross look like?
(black is a dominant marker only; the “black” gene is not on the same chromosome as your gene of interest)

- a. All Black
- b. All White
- c. All variegated
- d. Some white and some black
- e. 50% white and 50% black



The phenotype (and obviously, the genotype) of a mouse's progeny depends on the genotype of the germ line cells of its mother!

A chimeric mouse could have different genotype of germ line cells than genotype of hair cells, for example

You mate a variegated (chimeric) female mouse to a white male, and examine their offspring (F1) for coat color and the presence of the knocked out YFG. If you assume that the chimeric mouse has a germline composed only of ES derived cells, what are the possible genotypes of her oocytes with regard to the black marker and YFG?

- a. Blck - and YFG -
- b. Blck + and YFG +
- c. Blck + and YFG -
- d. Blck + and YFG- or Blck + and YFG+
- e. Blck+ and YFG- or Blck- and YFG+

You mate a variegated (chimeric) female mouse to a white male, and examine their offspring (F1) for coat color and the presence of the knocked out *YFG*. Which of the following statements is true? (black coat color is dominant, and the *black* gene is on a different chromosome from *YFG*).

- a. All the black offspring are homozygous for the transgene (*YFG*⁻/*YFG*⁻)
- b. Some of the black offspring are homozygous for the transgene (*YFG*⁻/*YFG*⁻)
- c. All of the black offspring are heterozygous for the transgene (*YFG*⁻/*YFG*⁺)
- d. All of the black offspring are homozygous normal (*YFG*⁺/*YFG*⁺)
- e. Some of the black offspring are heterozygous for the transgene (*YFG*⁻/*YFG*⁺)

Summary

Inject heterozygous ES cells into blastocyst

ES cells are from a black mouse, and the black coat color allele is dominant

Inject blastocysts into uterus

Formation of chimeric mice

Gametes: black and transgene
OR black and wt
OR white and wt

Breed chimeric to wild-type

Mate heterozygous $BMP7^{+}/BMP7^{-}$

Wild-type
 $BMP7^{+}/BMP7^{+}$

Heterozygote
 $BMP7^{+}/BMP7^{-}$

Heterozygote
 $BMP7^{+}/BMP7^{-}$

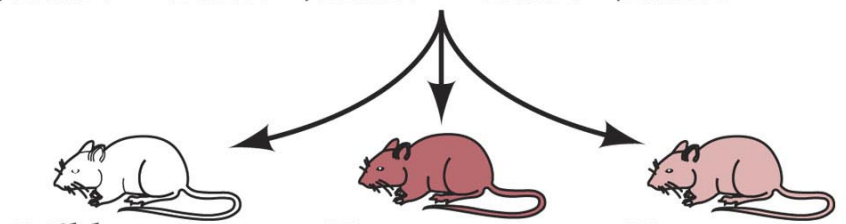
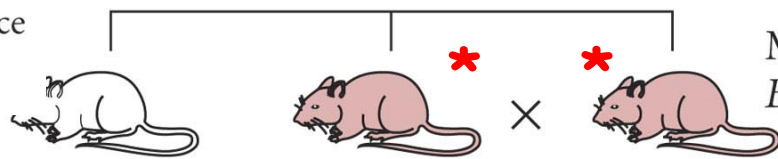
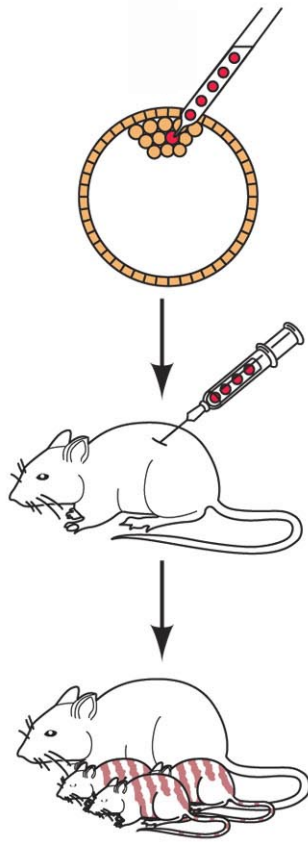
Wild-type
 $BMP7^{+}/BMP7^{+}$

Homozygote
 $BMP7^{-}/BMP7^{-}$

Heterozygote
 $BMP7^{+}/BMP7^{-}$

*** Check here to make sure black mouse actually carries transgene**

Finally, phenotype of homozygous progeny tells you the function of the gene



Example: phenotype of BMP7 Knock out mice

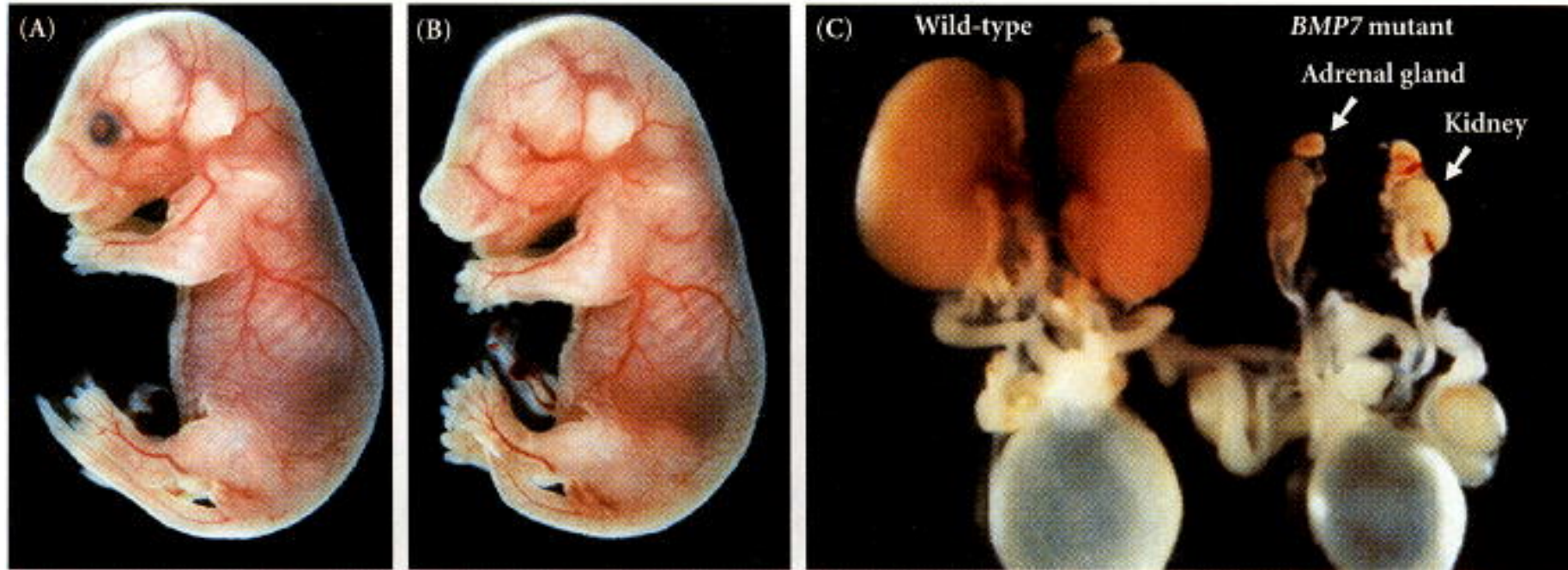


Figure 4.21. Morphological analysis of BMP7 knockout mice. A wild-type (A) and a homozygous BMP7-deficient mouse (B) at day 17 of their 21-day gestation. The BMP7-deficient mouse lacks eyes. The kidneys of these mice at day 19 of gestation are shown in (C). The kidney of the BMP7-deficient mouse is severely atrophied. Microscopic sections revealed the death of the cells that would otherwise have formed the nephrons. (From Dudley et al. 1995; photographs courtesy of E. Robertson.)

Other applications

- **Knock-Ins:** replace a gene or part of a gene with another gene

Engineer a mutation, a replacement, whatever you want in gene of interest

Include neoR and TK

Transfect into ES cells

Select for ES cells that have undergone HR

Proceed as before

What if a null mutation is embryonic or perinatally lethal?

How could you determine the function of the gene in the adult from the knockout phenotype?

How could you determine the function of the gene in a particular tissue?

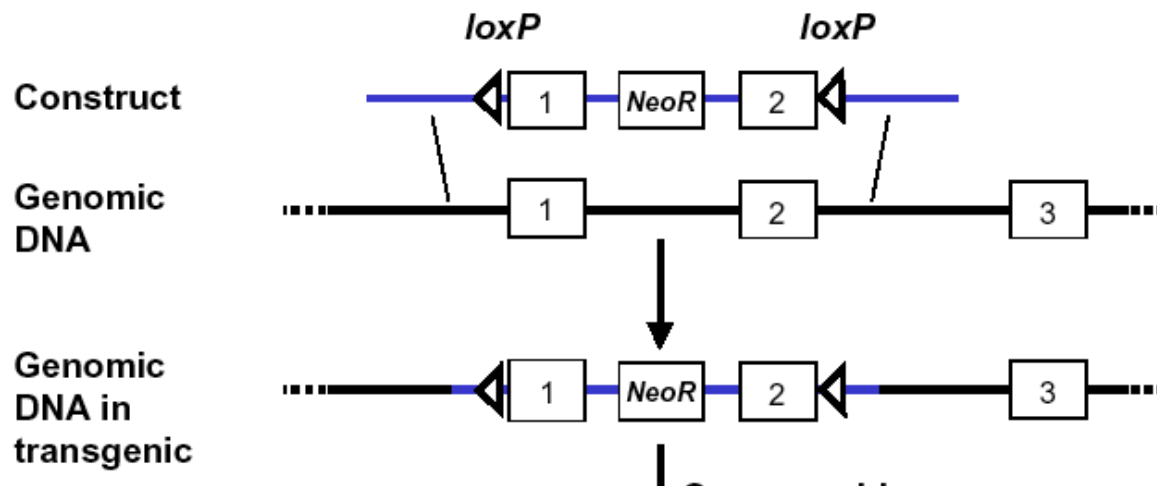
Conditional knock-outs: remove the function of a gene, but only in a certain place or at a certain time

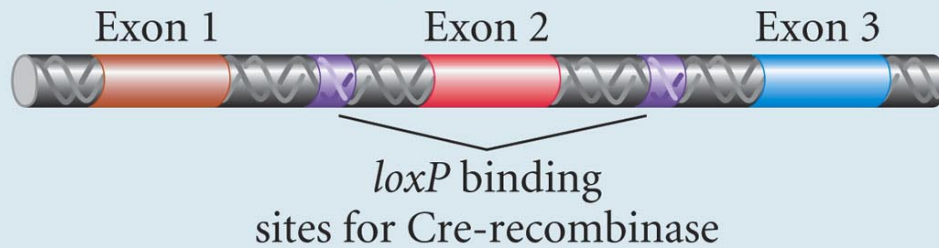
It is possible to do this in the mouse using *Cre/lox* technology...

3. Tissue Specific Knockouts: generate a transgenic mouse where the gene of interest is knocked out **only in specific tissues**
"Cre-lox"

Cre: a protein from bacteriophage that acts as a "recombinase", recognizing 34-bp tandem repeat sequences called *lox*

Engineer DNA of interest to be surrounded by the *lox* sites



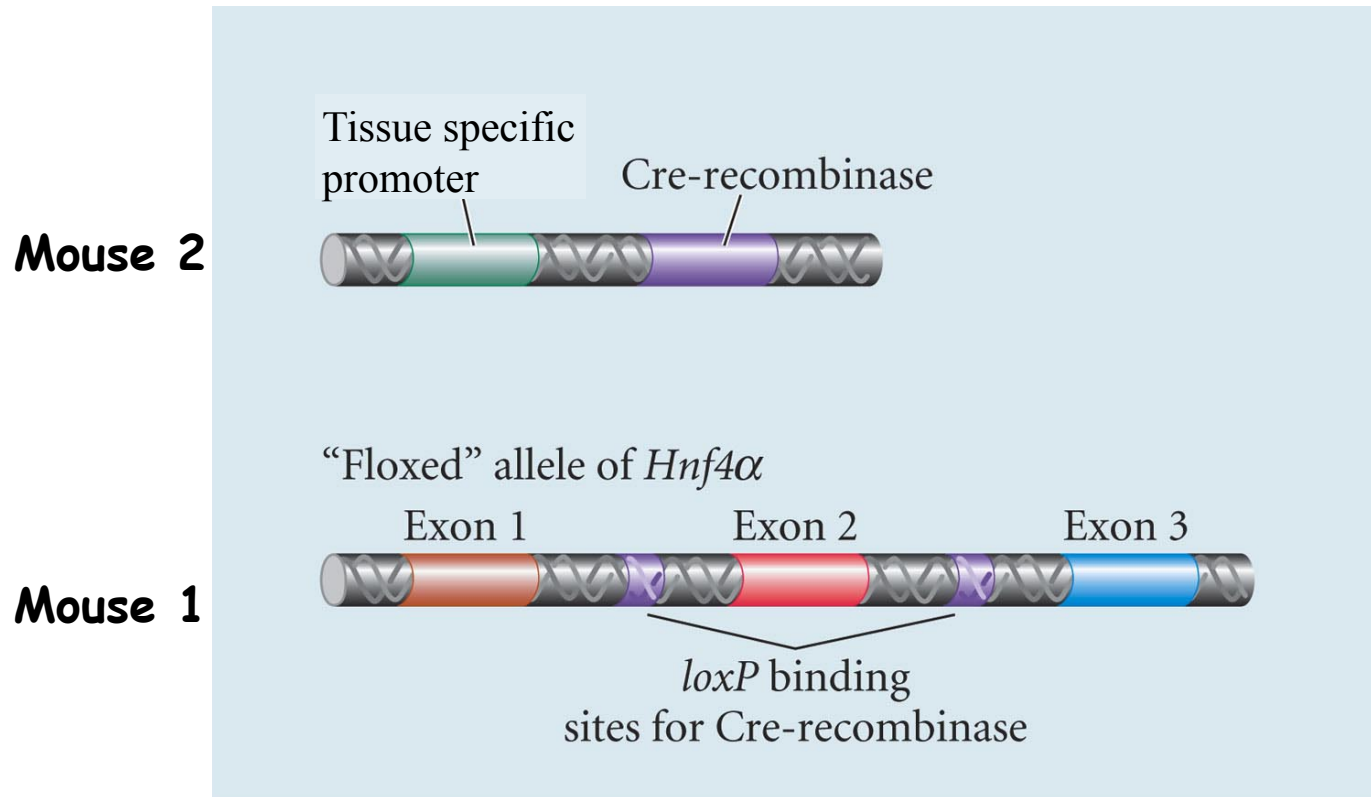


If you have a mouse that is homozygous for the engineered gene, in which loxP sites surround the piece of DNA you plan to remove, **Will this mouse have a defective phenotype?**

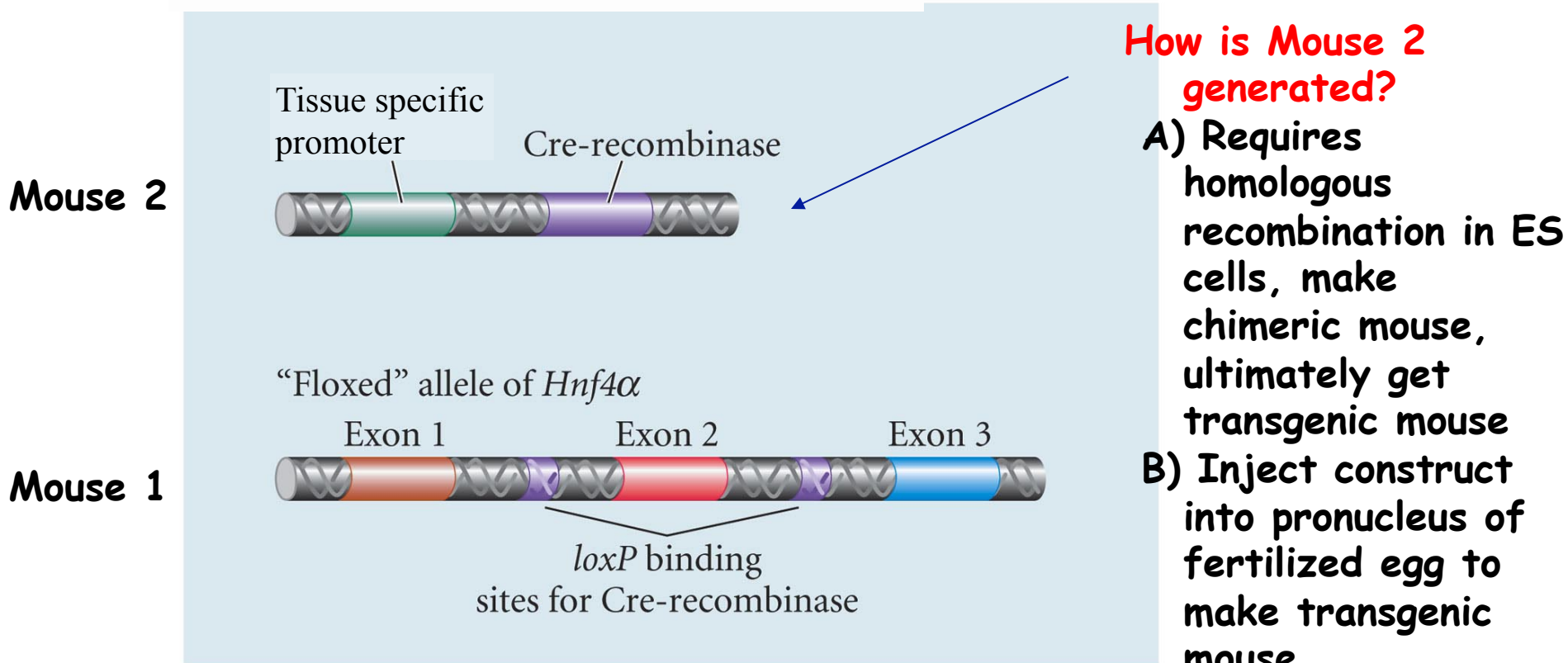
a. Yes

b. No

- Now, you generate a second mouse
- This mouse is transgenic for the Cre recombinase, driven by whatever promoter you choose



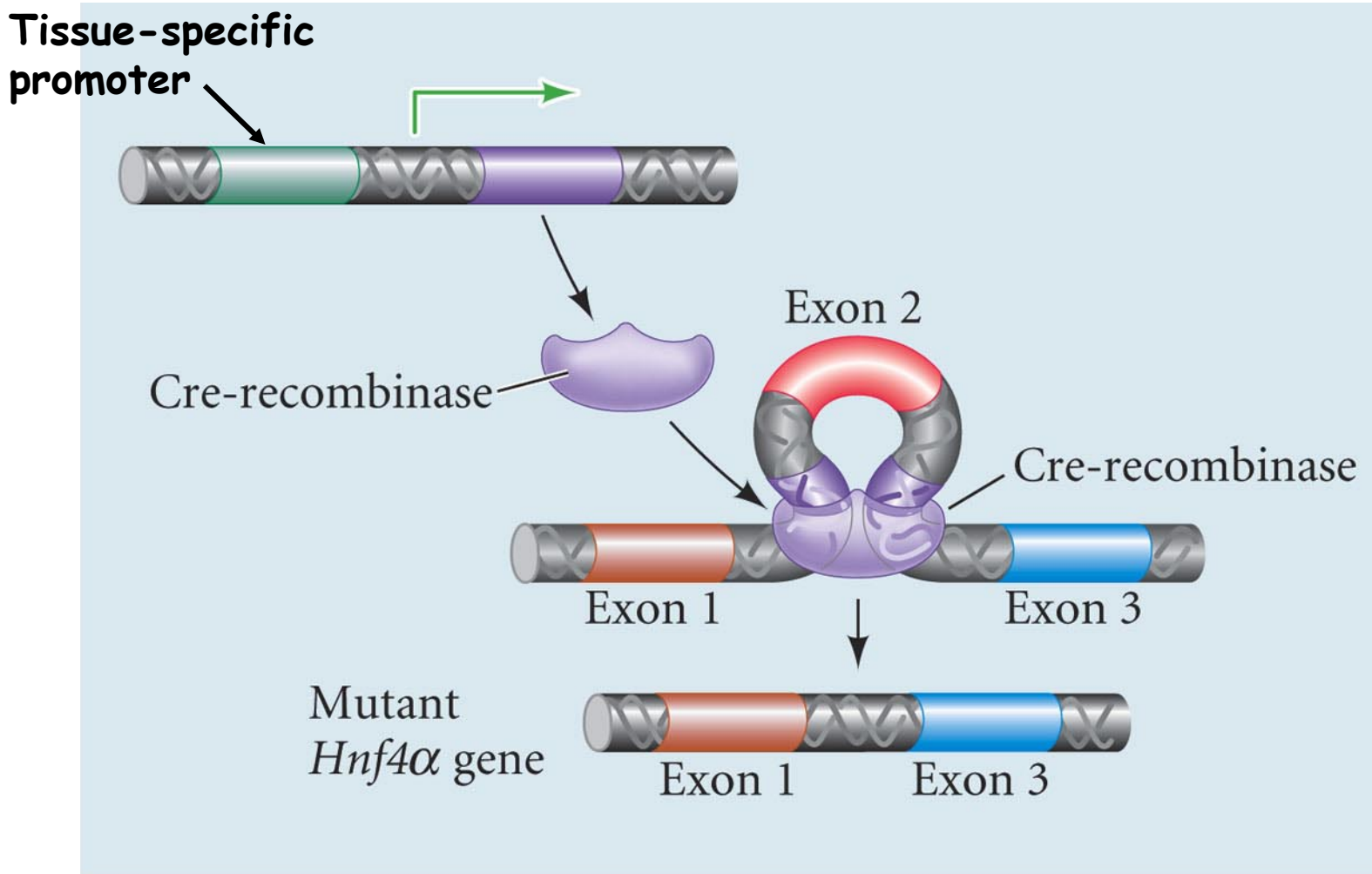
Mouse 2 is transgenic for the Cre recombinase, driven by whatever promoter you choose



DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 5.14 (Part 1) Sinauer Associates, Inc. © 2003 All rights reserved.

Now, mate the mice together

In cells where Cre protein is generated, it induces recombination between the loxP sites, removing whatever piece of DNA they were flanking



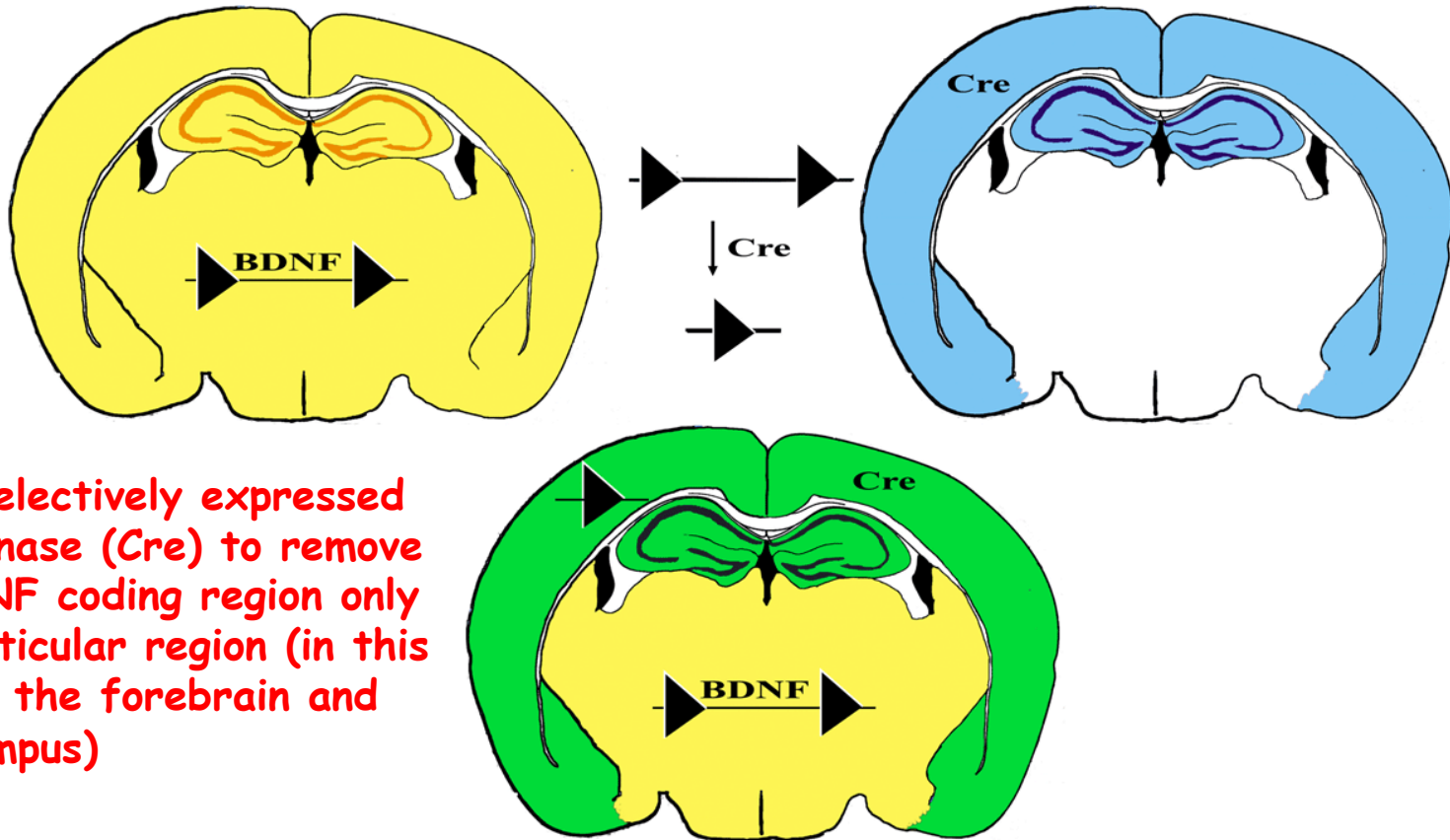
Generating models of Huntington's Chorea

The huntingtin gene has been proposed to be an activator of cortical **BDNF** transcription

- BDNF knockout mice die shortly after birth.
- But BDNFs are expressed in adulthood, particularly in the brain.
- By preferentially knocking them out in the brain, scientists could study the role of BDNF in particular areas

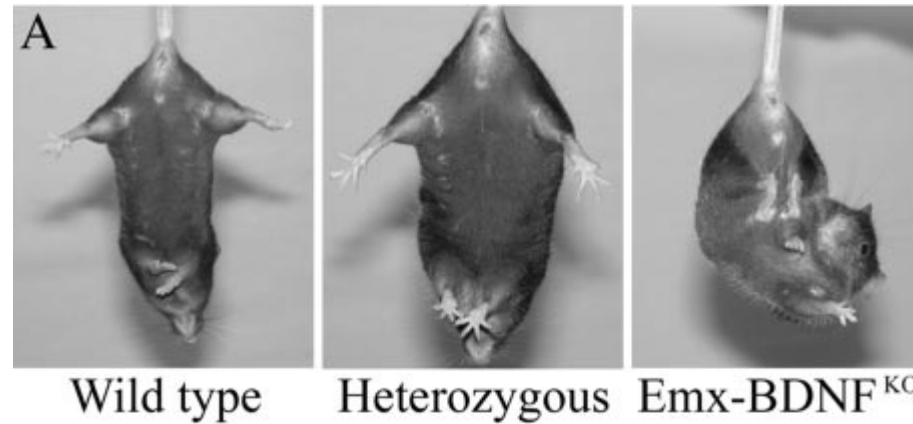
Jones lab, MCDB

Cre-lox Strategy for Tissue-Specific Knockouts



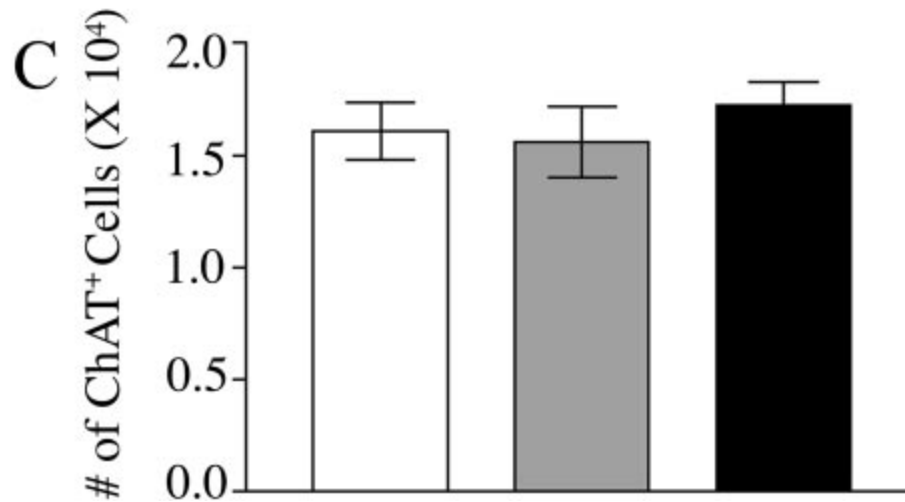
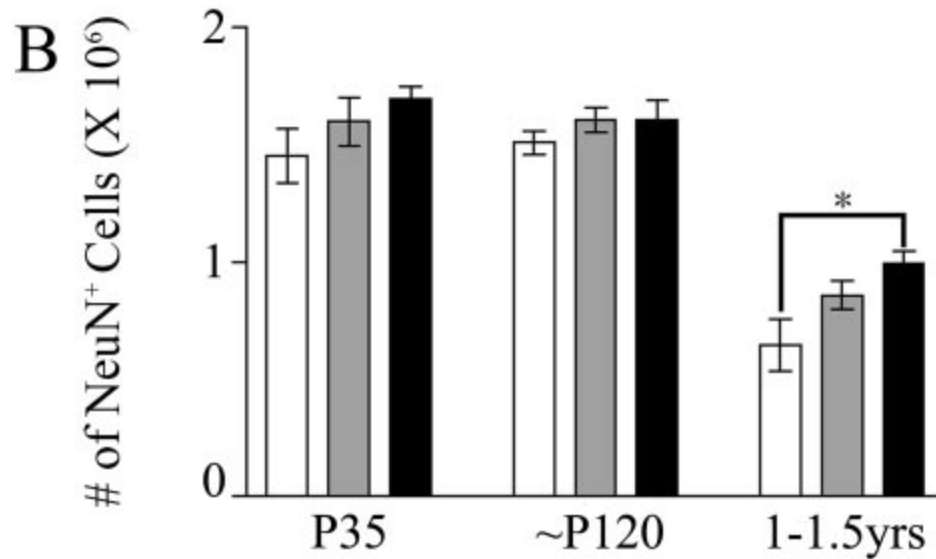
Use a selectively expressed recombinase (Cre) to remove the BDNF coding region only in a particular region (in this case, in the forebrain and hippocampus)

BDNF conditional knockouts have a “Huntington’s phenotype”



Mice without BDNF in cortex show clasping and writhing phenotype similar to that seen in other Huntington’s models

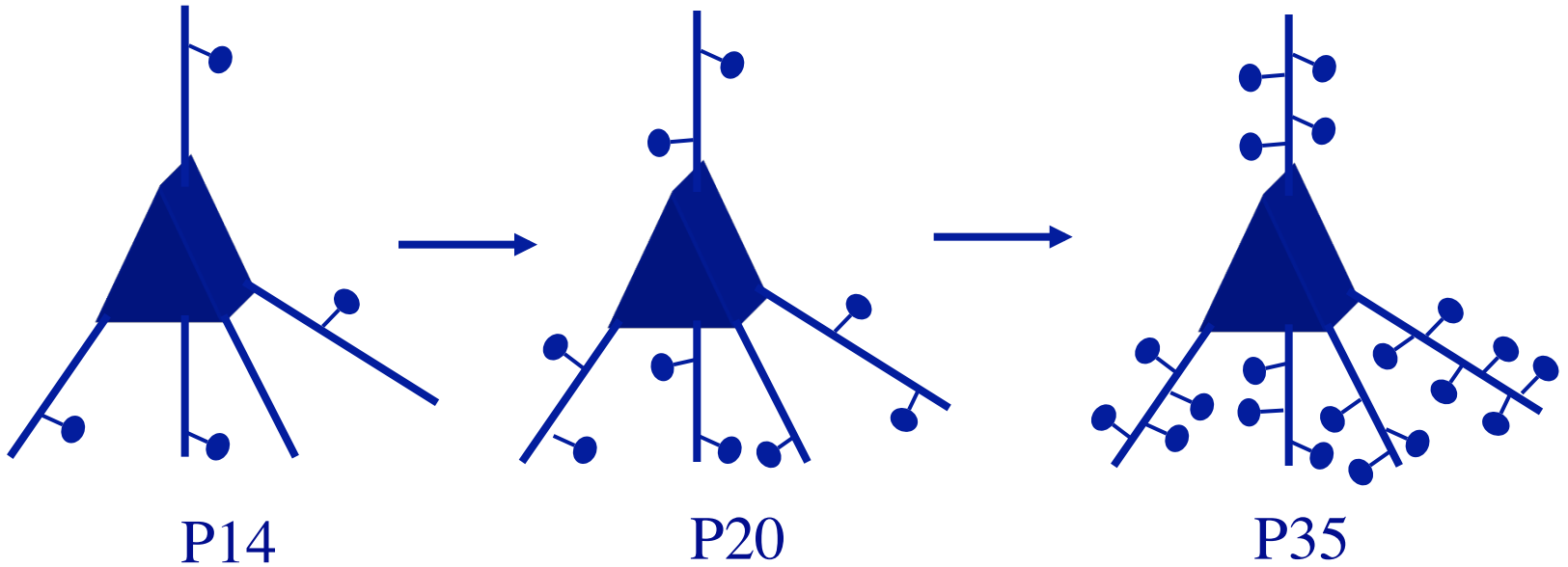
BDNF is required for long-term survival of striatal neurons



(control)

BDNF Functions in Postnatal Dendrite Maintenance

Wild-
Type



BDNF
Mutant

