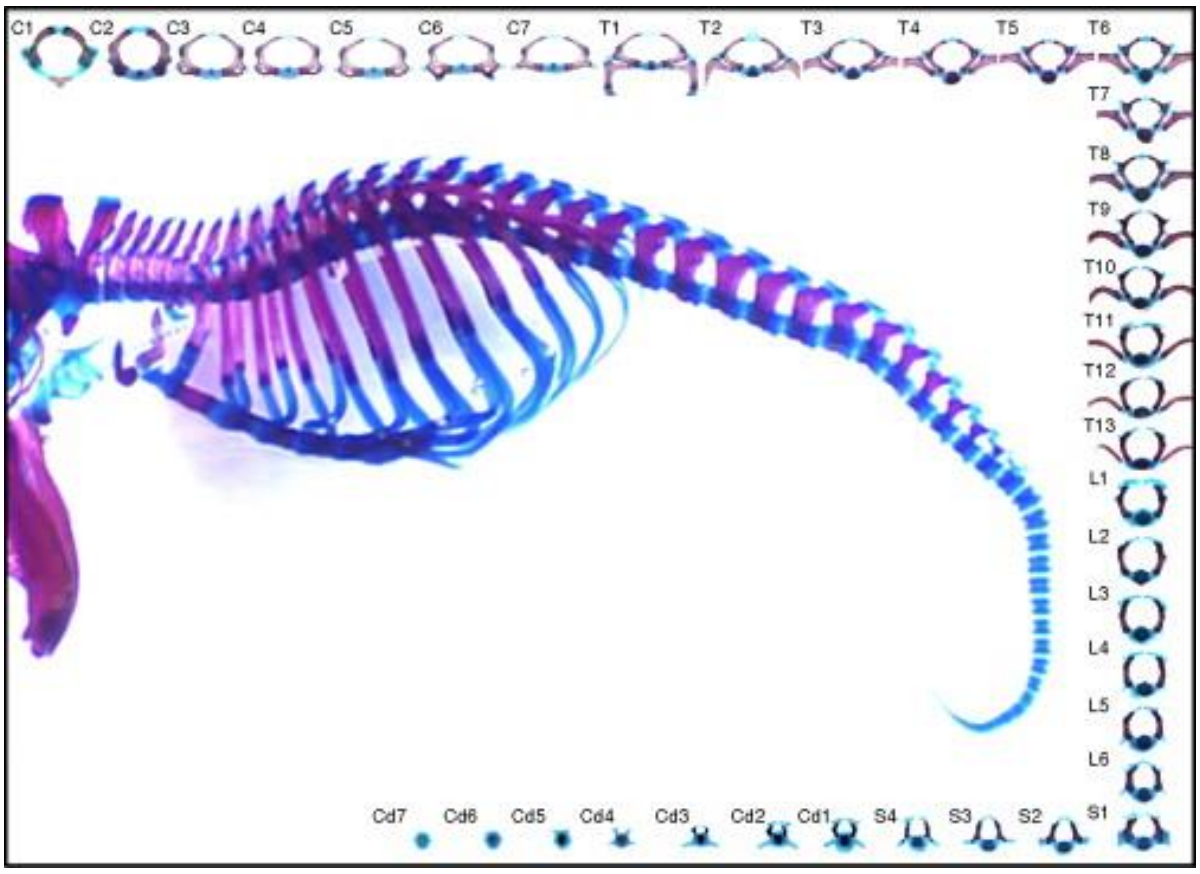
IN SILICO PRACTICAL – MOUSE VERTERBRAL COLUMN

HOX GENES AND PATTERN FORMATION OF THE VERTERBRAL COLUMN IN MOUSE EMBRYOS

You are provided with data from experiments in which genes of the *Hox10* and *Hox11* paralogous groups were functionally inactivated in mouse embryos. There are three *Hox10* paralogous genes – *Hoxa10*, *Hoxc10,* and *Hoxd10* and three *Hoxd11* paralogous genes- *Hoxa11, Hoxc11* and *Hoxd11* (see Box5E in textbook). Triple knockouts of *Hox10* paralogs were made by mating triple heterozygous males and females. Triple knockouts of *Hox11* paralogs were made by *in vitro* fertilization because both male and female triple heterozygous animals are infertile. Photographs are provided of the skeletons of the vertebral columns of 18.5 day triple mutant mouse embryos stained with Alcian blue (to show cartilage) and Alizarin red (to show ossified bone) (see also Fig. 3.1 in textbook) and of sample vertebrae which have been deconstructed from the stained vertebral columns. You are also provided with data from experiments in which genes of the *Hox5*, *Hox6* and *Hox9* paralogous groups were functionally activated (see Box5E in textbook). To help you identify the pattern of vertebrae in the mutant embryos, you are provided with photographs of the vertebral column and individual vertebrae from normal (control) embryos.

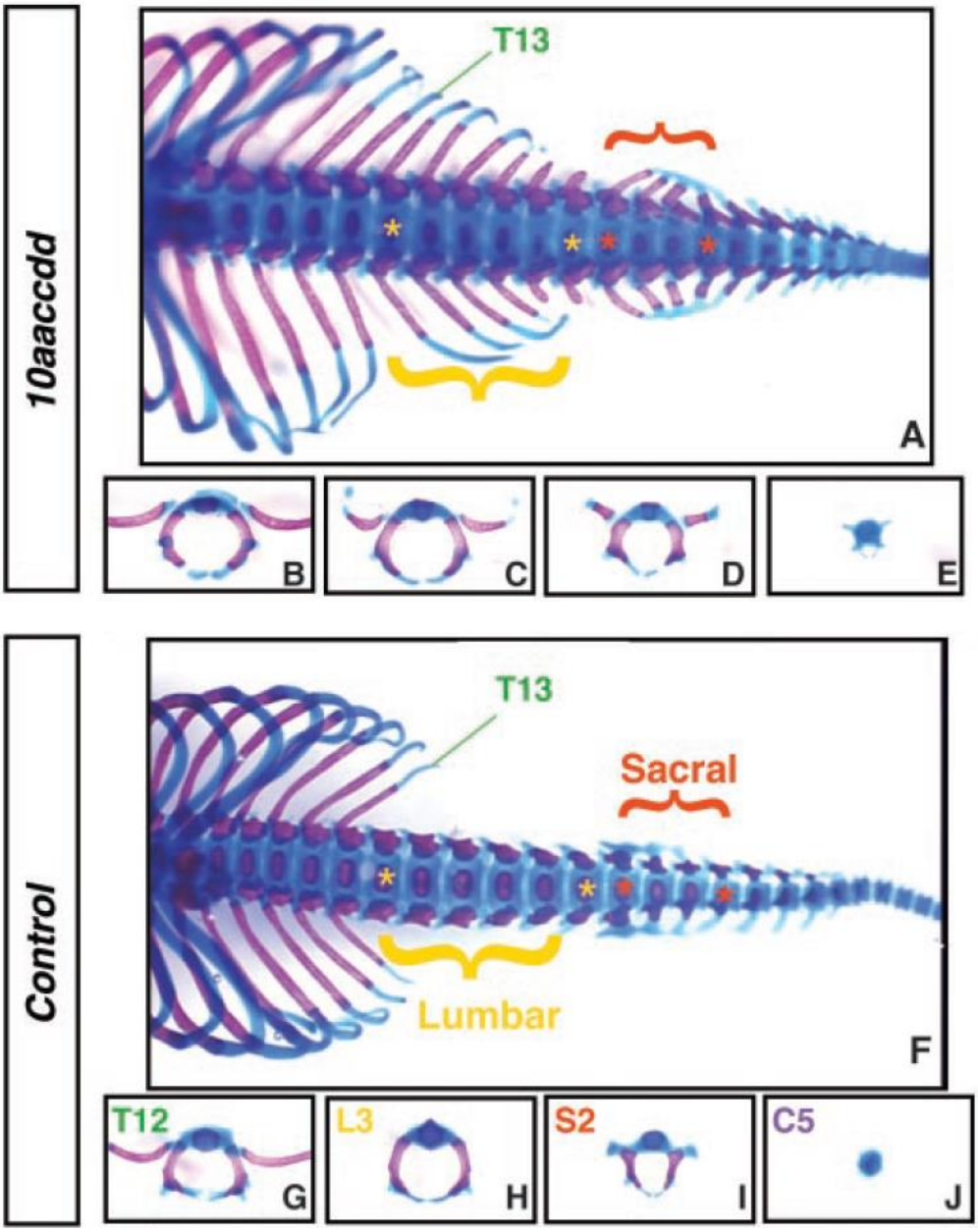
MOUSE VERTEBRAL COLUMN



The axial formula in mice is 7 cervical (C), 13 thoracic (T), 6 lumbar (L), 4 sacral (S) and numerous (slightly variable numbers of) caudal (Cd) vertebrae. Mice with *Hox5*, *Hox6*, *Hox9*, *Hox10* or *Hox11* paralogous mutations show drastic alterations of the axial formula.

EXPERIMENT 1

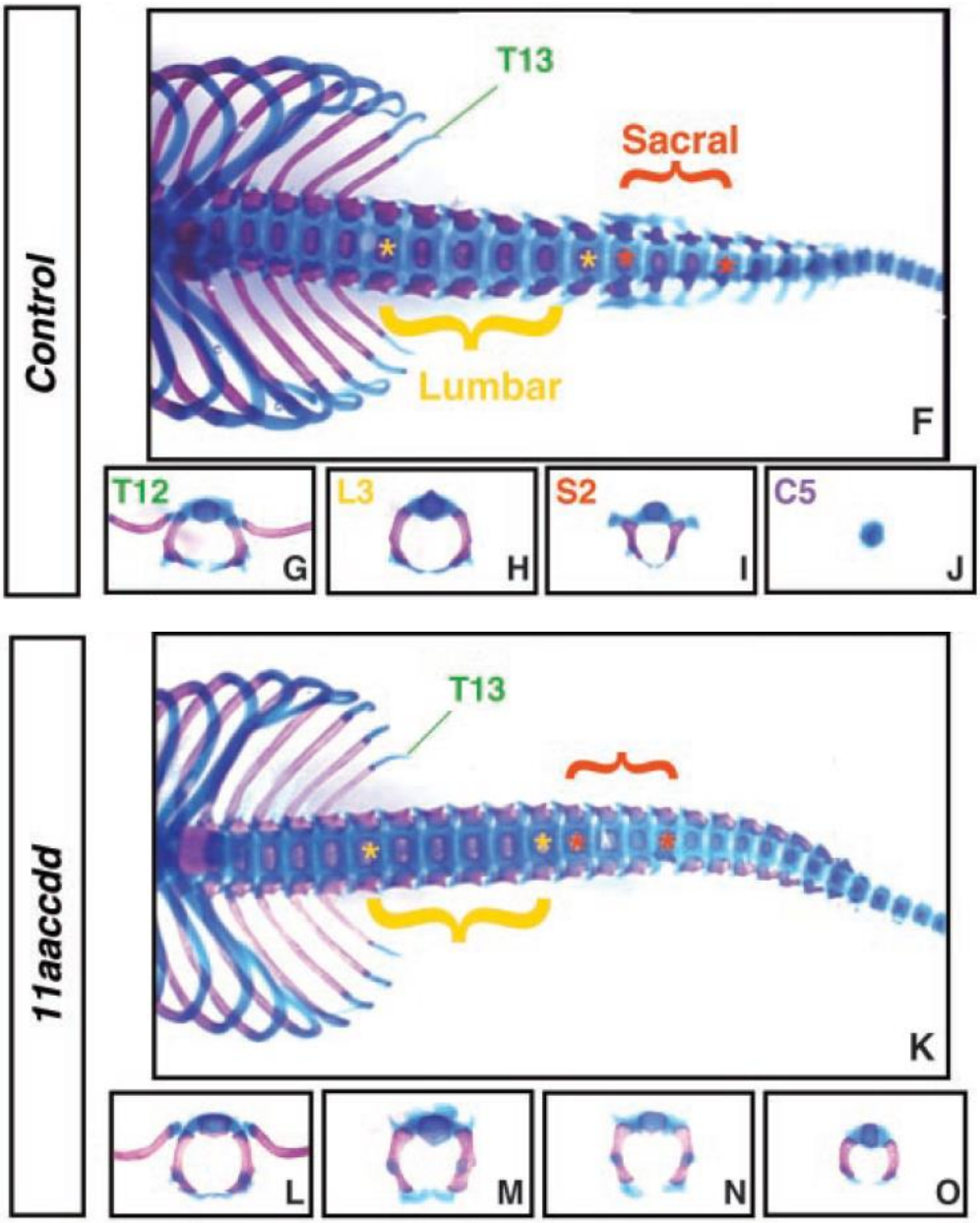
Triple knock-out mouse embryos in which *Hoxa10*, *Hoxc10* and *Hoxd10* were all functionally inactivated were generated. The vertebral columns of a control and a triple mutant mouse embryo are shown together with sample vertebrae which have been deconstructed from the vertebral columns. The 13th thoracic vertebra/rib is labelled in green, the lumbar region is labelled in yellow and the sacral region in orange in each case to aid in orientation.



Panels B, C, D and E show the vertebrae at the same positions in the vertebral column as the vertebrae of the control embryo shown in G, H, I and J respectively.

EXPERIMENT 2

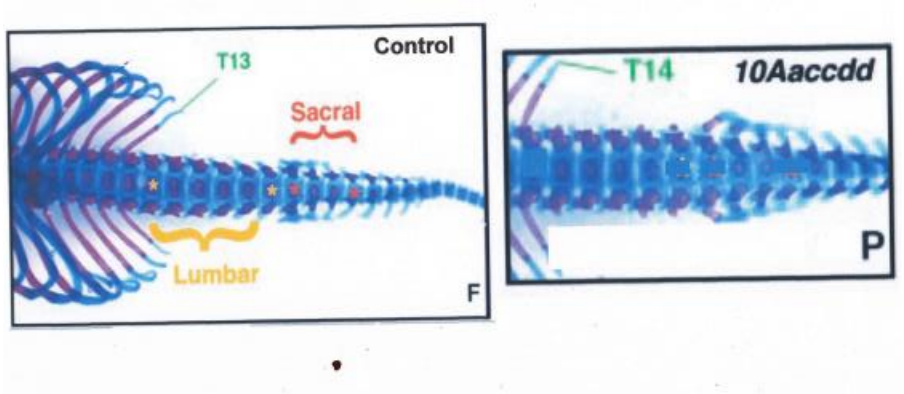
Triple knockout mouse embryos in which *Hoxa11*, *Hoxc11*, *Hoxd11* were all functionally inactivated were generated. The vertebral columns of a control and of a mutant mouse embryo are shown, including deconstructed vertebrae. The 13th thoracic vertebra/rib is labelled in green, the lumbar region is labelled in yellow and the sacral region in orange in each case to aid in orientation.



Panels L, M, N and O show the vertebrae at the same positions in the vertebral column as vertebrae in G, H, I and J respectively.

EXPERIMENT 3

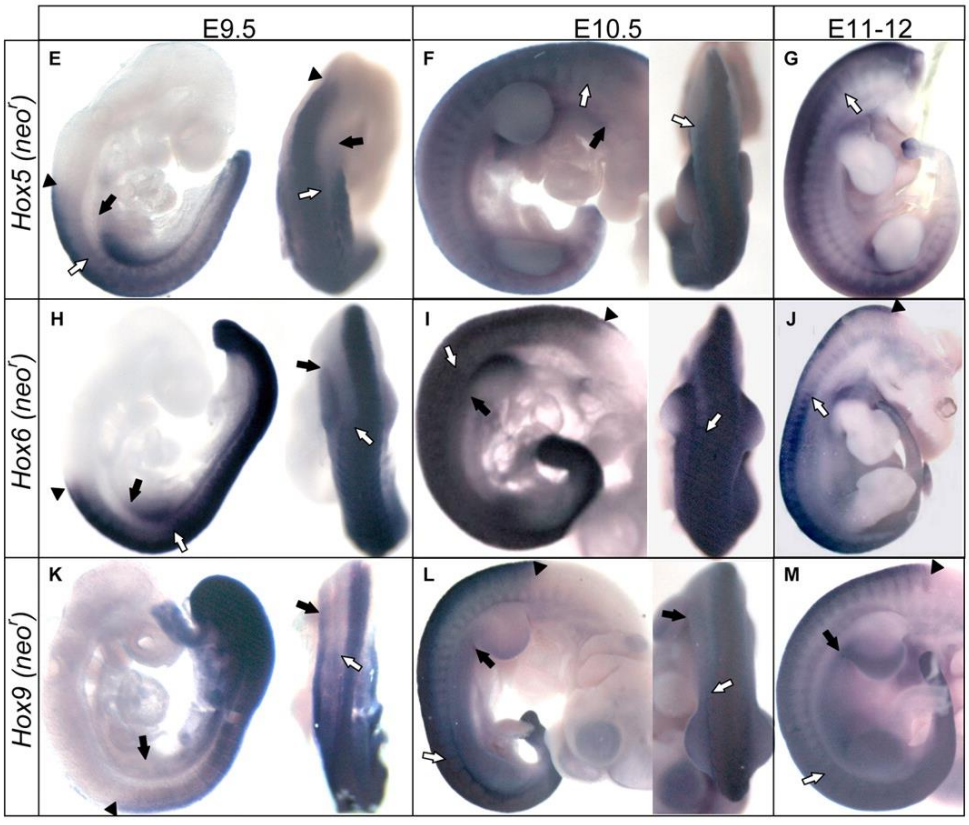
Knockout mouse embryos with one wild-type *Hox10* allele were generated (*Hox10Aaccdd*). The vertebral column of one of these mutants is shown alongside the vertebral column of a control mouse (*Hox10AACCDD*).



EXPERIMENT 4

Triple knockout embryos for *Hox5* and *Hox6* paralogs and quadruple knockout embryos for *Hox9* paralogs were generated. You are provided with photographs of the vertebral columns and rib cages of the mutant mouse embryos and also images of whole mounts of mouse embryos at E9.5, E10.5 and E11- 12 on which *in situ* hybridisation has been carried out to show the distribution of transcripts of *Hox5*, *Hox6* and *Hox9* paralogous genes (you should check which genes make up each of these paralogous groups using the diagram of Box5E in the textbook).

HOX EXPRESSION IN MOUSE EMBRYOS FROM E9.5- E12:



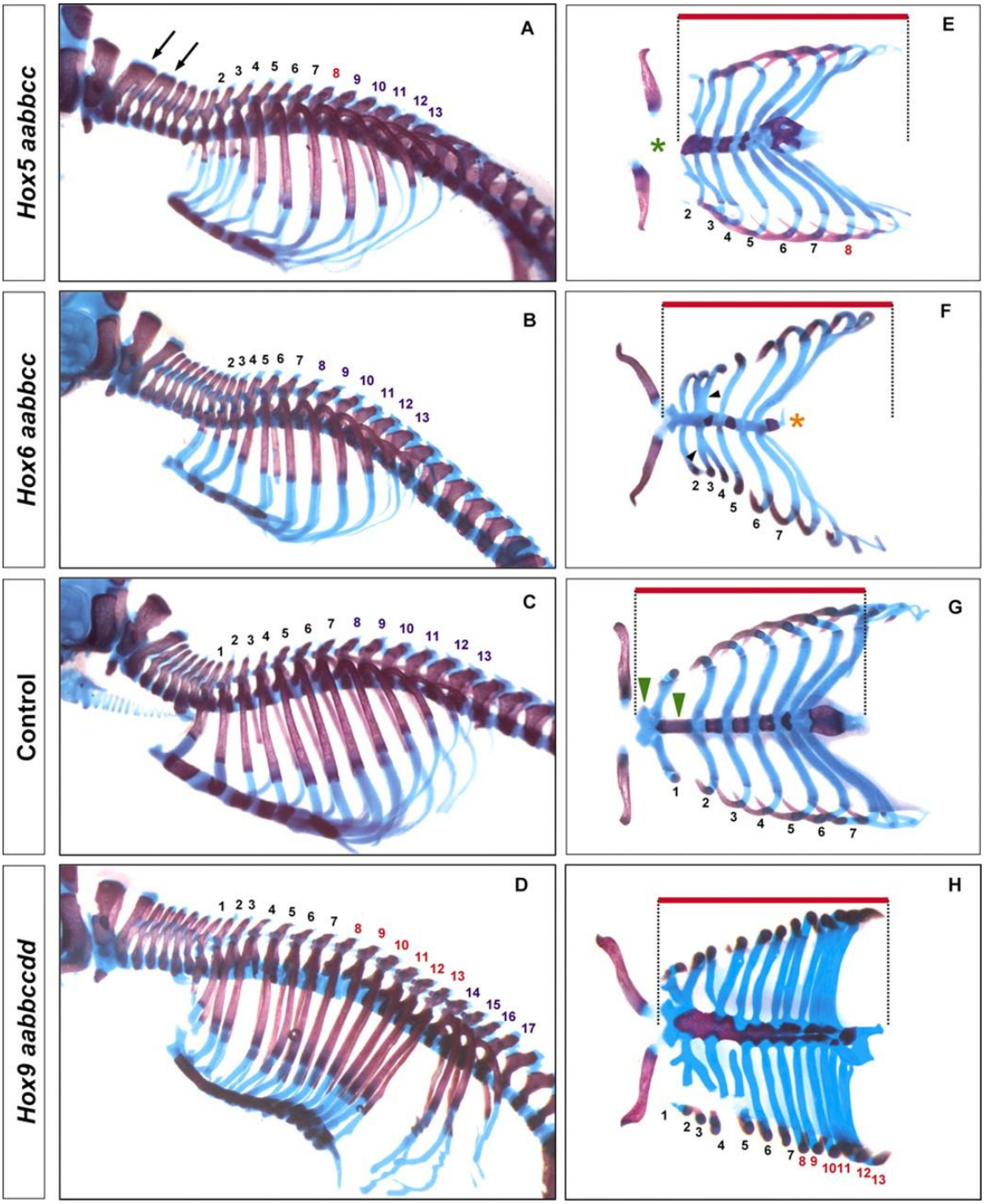
White arrow – somite anterior expression limit

Black arrow – lateral plate anterior expression limit

Black arrowhead – neural tube anterior expression limit.

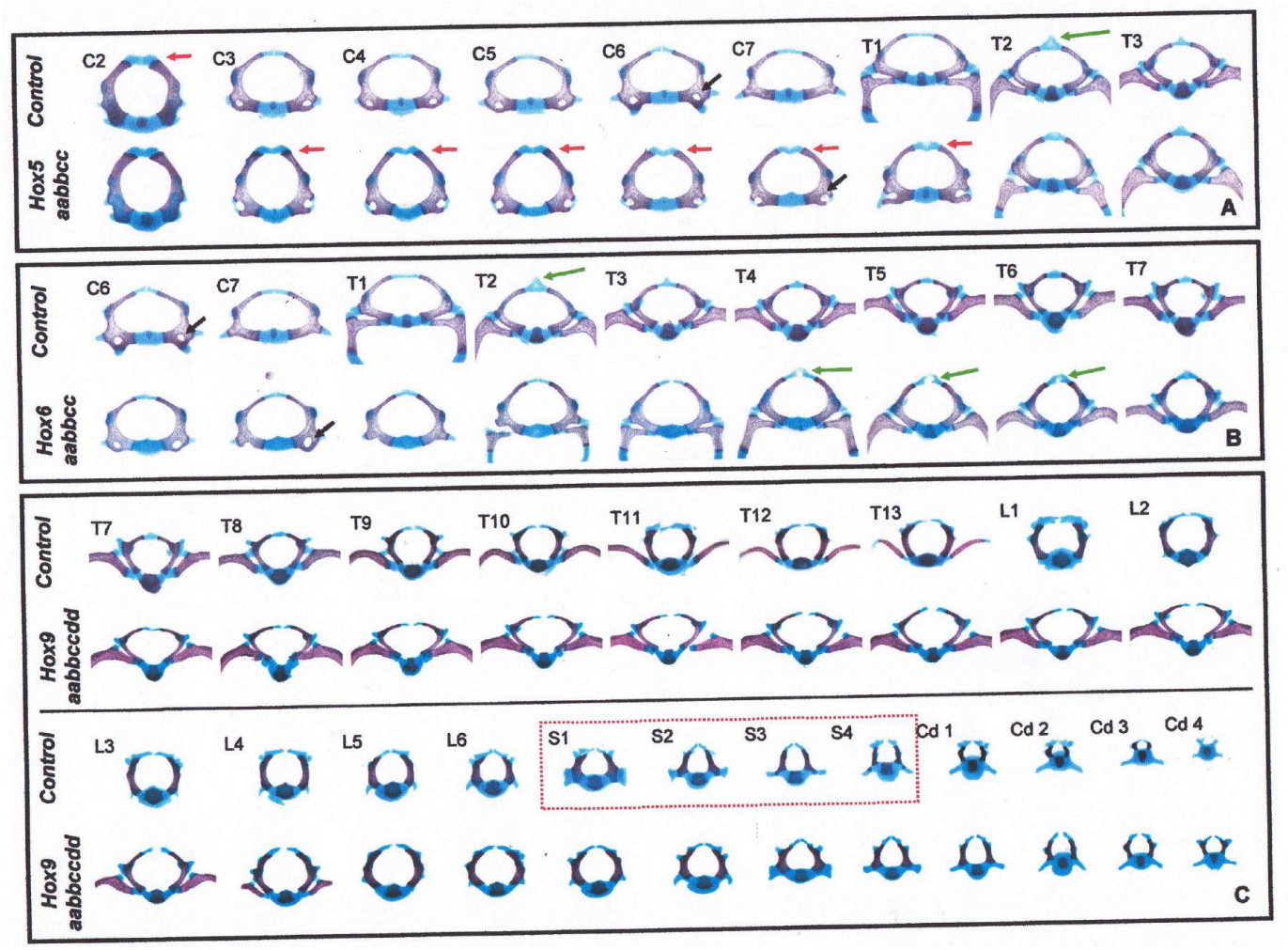
In situ hybridization (using a probe that detects the *Neo*^{r} cassette inserted into the gene loci) is used to show the expression boundaries of the *Hox5*, *Hox6* and *Hox9* paralogous groups at E9.5, E10.5 and E11- 12.

VERTEBRAL COLUMNS AND RIB CAGES FROM EMBRYOS THAT ARE *HOX5* TRIPLE MUTANT, *HOX6* TRIPLE MUTANT OR *HOX9* QUADRUPLE MUTANT TOGETHER WITH THE SAME REGIONS FROM NORMAL (CONTROL) EMBRYOS:



Black numbers indicate ribs that normally connect with the sternum as seen in the control. Green arrowheads in G denote wild-type manubrium and first sternabra. Red and blue numbers indicate abnormal ribs and other abnormalities are indicated by the green and orange asterisks.

VERTEBRAE DECONSTRUCTED FROM *HOX5*, *HOX6* AND *HOX9* PARALOGOUS MUTANT MOUSE EMBRYOS:



In the control embryos, the red arrow indicates a morphological feature of the second cervical vertebra, the black arrow a feature of the seventh cervical vertebra and the green arrow a feature of the second thoracic vertebra; the box encloses the four sacral vertebrae. The arrows in the mutant embryos indicate the same features as in the control embryos.

EXERCISE

1) Work out the steps that would have been carried out to generate the triple heterozygous mice that were mated to produce embryos for experiment 1 using the technique of homologous recombination in ES cells (see section 3.10 in textbook)- this was the only technique available until very recently. How could such mice now be generated using Cas9-CrispR technology and what advantages does this technology have?

2) Using the notation Hox10Aa;Hox10Cc;Hox10Dd to denote triple heterozygous mice work out the genotypes of the offspring that will be generated from mating triple heterozygous male and female mice for Experiment 1.

3) Genotyping results from a *Hox10* representative litter are provided below, with the seven embryos listed which are the result of a cross between *Hox10* triple heterozygous parents. Identify the triple mutants (if any) using the provided genotyping information.

	GENOTYPE		
Embryo	**A10**	**C10**	**D10**
A	Aa	CC	Dd
B	Aa	Cc	DD
C	Aa	CC	DD
D	AA	Cc	dd
E	Aa	cc	Dd
F	aa	cc	dd
G	AA	cc	Dd

4) *Hox11* triple knockout were made using *in vitro* fertilization because both male and female triple heterozygous animals are infertile.
   a) What are the odds of getting a mutant embryo in a Hox10AaCcDd x Hox10AaCcDd cross? Remember that *Hox10* triple heterozygotes are fertile.
   b) Considering that female mice tend to have 6- 10 embryos per litter (depends on the strain), are traditional triple or quadruple heterozygote matings the easiest way to get a large number of mutant mice for analysis? If not, what might be a better way?

5) Identify the sequence of vertebrae in the vertebral columns of the mutant mouse embryos in experiments 1 and 2. You will need to decide the criteria for distinguishing the vertebrae in different regions of the column using the skeletons of the normal mouse embryos and then count the number of vertebrae in each region.

6) Compare the patterns of the vertebral column of the mutant mouse embryos with that of the normal mouse embryo.
   a) What region of the vertebral column is affected when each of the Hox paralogous group's (*Hox5, Hox6, Hox9, Hox10* and *Hox11*) function is completely eliminated?
   b) How are these regions related to the patterns of *Hox5* transcripts in E9.5, E10.5 and E11- 12 day mouse embryos?
   c) What phenotype would you predict when both *Hox10* and *Hox11* function is completely eliminated?

7) Compare the vertebral columns of the mutant mouse embryos in experiments 1 and 3.
   a) How do these differ and what are the conclusions for the way in which *Hox10* paralogous genes function?

ACKNOWLEDGEMENTS AND REFERENCES

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Wellik, D.: **Hox Genes and Verterbrate Axial Pattern**. *Current Topics in Developmental Biology* 2009, **88**: 257-278.

Wellick, D. and Capecchi, M. R.: **Hox10 and Hox11 genes are required to globally pattern the mammalian skeleton**. *Science* 2003, **301**: 363-367.