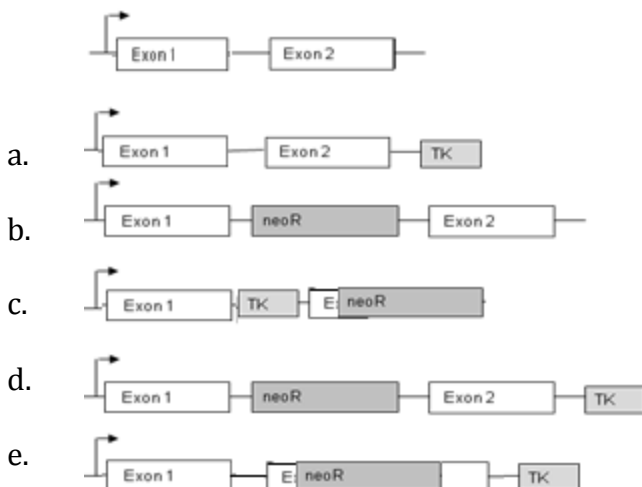


### MCDB 4650 Problem set 9

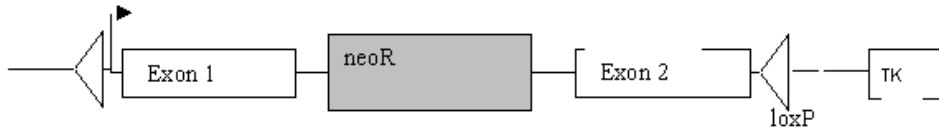
- (1) In mice and humans, embryos separate into a trophoctoderm and an inner cell mass by the 16 cell stage. The trophoctoderm cells will allow the embryo to adhere to the uterine wall, as well as later forming the chorion (part of the placenta). Interestingly, labeling experiments of cells in the trophoctoderm of the 16-cell stage embryo demonstrate that they occasionally end up as part of the embryo, while labeling at the 64-cell stage shows that the trophoctoderm cells never become part of the embryo. Explain why the trophoctoderm cells can still become part of the inner cell mass at the 16-cell stage.
- The gene Oct-4 is thought to be involved in maintenance of the stem-cell nature of the inner cell mass cells. What experiment could you do to determine if Oct4 is downregulated in the trophoblast cells?
- (1) Which of the following is/are true? Injection of a cloned mouse transgene into one pronucleus of a fertilized mouse egg is most likely to result in
  - integration by recombination of that piece of DNA at its homologous chromosomal sites.
  - random integration of the DNA into any chromosome.
  - self-recombination of the DNA into a large extrachromosomal array that does not integrate.
  - presence of that DNA in every cell in the body of the resulting mouse as long as the transgene has been integrated.
  - a chimeric mouse, with that DNA present in some cells but not in others.
- (1) In muscle development, there are two genes, MyoD and Myf 5 that are both expressed in developing muscle fibers, but at slightly different times. If you wanted to generate an animal that expressed Myf5 only, but at both times of development, which technique would you use, pronuclear injection or homologous recombination? Explain briefly.
- (.5) Imagine you take two mouse morula stage embryos, one from a black strain of mouse (*black+/+*) and one from a white mouse (*black/-*), and combine the cells into a single blastocyst that is then reimplanted in a foster mother for development. The mouse that is born from this chimeric embryo has a variegated coat with black and white patches. If this mouse is mated to another mouse from the white strain, the percentage of black progeny mice
  - will be 25%.
  - will be 50%.
  - could be anywhere from 0% to 50%.
  - could be anywhere from 0% to 100%.
- (1.5) To better study the neurodegenerative disease Huntington's Chorea, you want to make a mouse model for the disease. To do so, you would like to create a mouse that has the same mutation in the *huntingtin* gene as that found most commonly in humans. You have cloned the homolog of the *huntingtin* gene in mouse. Possible

steps you might take in carrying out the experiments that would answer your question are listed below in random order, and not all of them are appropriate. What would be an appropriate sequence of steps for experiments that would give you the desired information? List them by number.

- 1) Create a linear DNA construct that includes the *huntingtin* gene with the mutation commonly seen in humans.
  - 2) Create a linear DNA construct that includes the *huntingtin* gene with the mutation commonly seen in humans, a neoR gene inserted into one of its introns, and a TK gene on one end.
  - 3) Mate chimeric mice (generated from ES cells) to a white mouse, obtain F1 progeny.
  - 4) Test the black F1 progeny to see if they contain the transgene before conducting experiments, since they may not carry the transgene.
  - 5) Create a linear DNA construct that includes the *huntingtin* gene with the mutation commonly seen in humans and a neoR gene inserted into one of its exons, and a TK gene on one end.
  - 6) Inject a linear DNA construct that includes the *huntingtin* gene with the mutation commonly seen in humans directly into the pronucleus of a fertilized mouse egg.
  - 7) Inject ES cells transformed with an already made DNA construct (one of the other steps) into blastocyst-stage embryos obtained from a mated white mouse, and implant them into a white foster mother.
  - 8) After obtaining embryos from various procedures above, mate together offspring that contain the different DNA constructs you have introduced; assay the F1 progeny.
  - 9) Introduce linear construct of DNA into cultured ES cells from a black mouse by electroporation and select for colonies that are resistant to neomycin and gancyclovir.
7. (2) You would like to generate a knockout mouse. The normal gene has 2 exons and one intron, as shown below. Which of the constructs shown below should you introduce into an ES cell line from black mice to begin this process? EXPLAIN.



8. (2)



You are interested in studying the role of a gene in heart development. The normal gene has 2 exons and one intron, is normally expressed in all skeletal and heart muscle cells, and is required for their development. Mice die at an early stage without its expression. You generate the construct shown, with a neoR gene inserted into an intron of a cloned copy of the mouse gene, and LoxP sites inserted into the flanking 5' and 3' sequences of this gene, and the TK sequence at the end. You generate (by homologous recombination) a transgenic mouse that carries this construct instead of the normal gene.

How will you generate a mouse that is missing your gene of interest only in the heart so that you can study its role in heart development?