# Advanced Cell Biology. Lecture 28

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April 4th, 2011

# Outline

Questions and answers

Analysis of DNA

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Analysis of DNA

# Previous final question: the answer

Why DNA sequencing employs ddNTPs?

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Why DNA sequencing employs ddNTPs?

- To stop elongation of DNA chain on given length AND
- To label nucleotides with specific radioactivity or flourescence

# Assembling a genome

- Programmatic re-assembly may easily go wrong especially if there are similar places in genome
- The best way is to create BAC libraries and then use “restriction signatures” to locate these fragments in the genome

# Using BACs and restriction signatures for assembling a genome

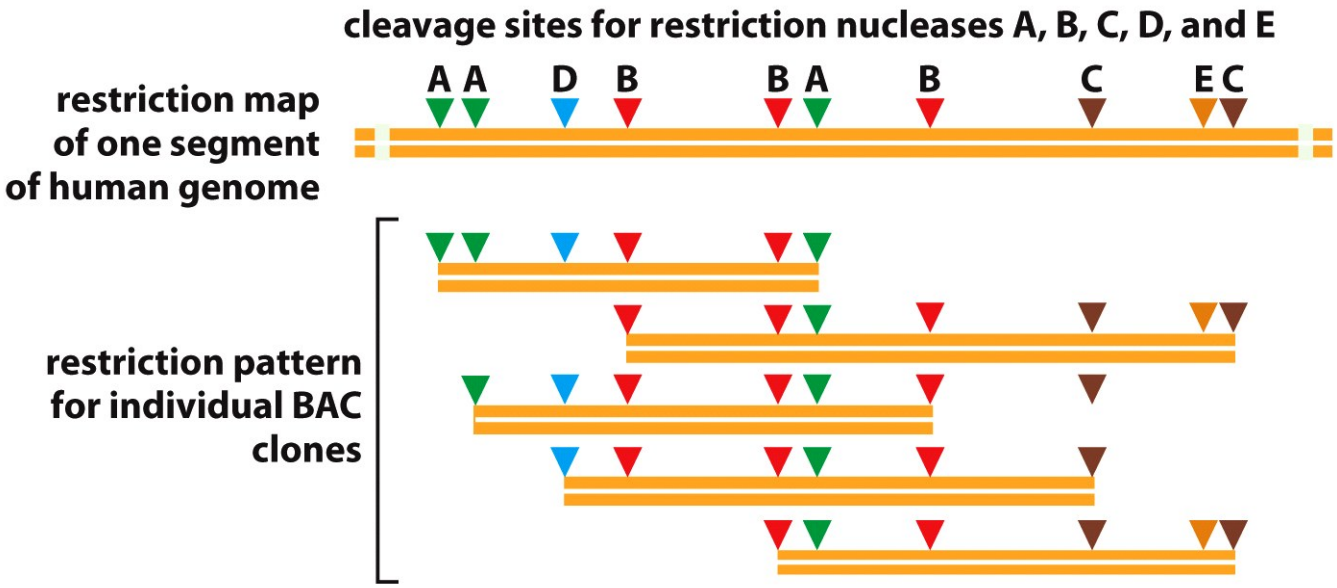
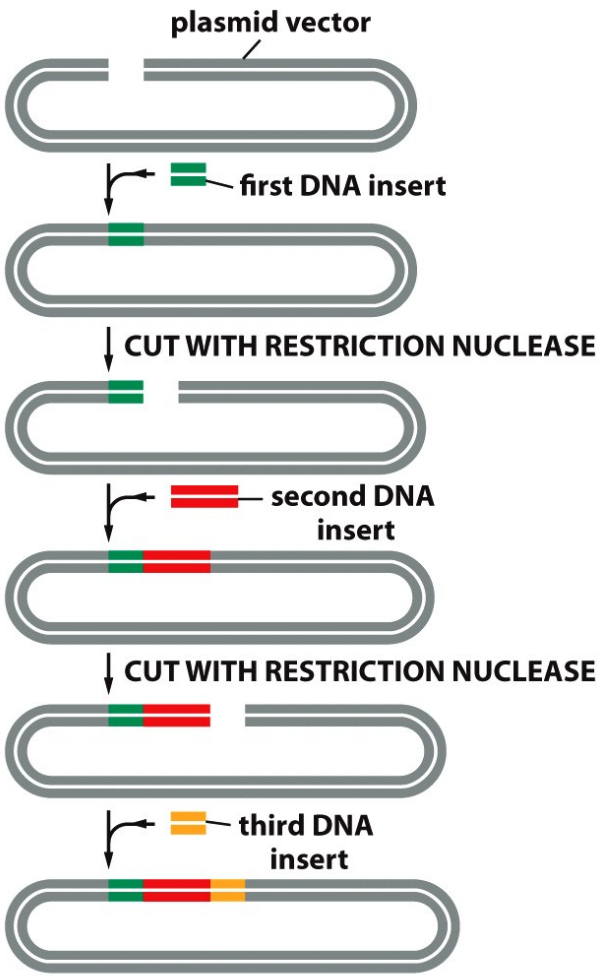



Figure 10-27 Essential Cell Biology 3/e (© Garland Science 2010)

# New DNA

- With multiple (serial) coning, it is possible to create new DNA sequences
- That may result in hybrid proteins or artificial mutations

# Making new DNA

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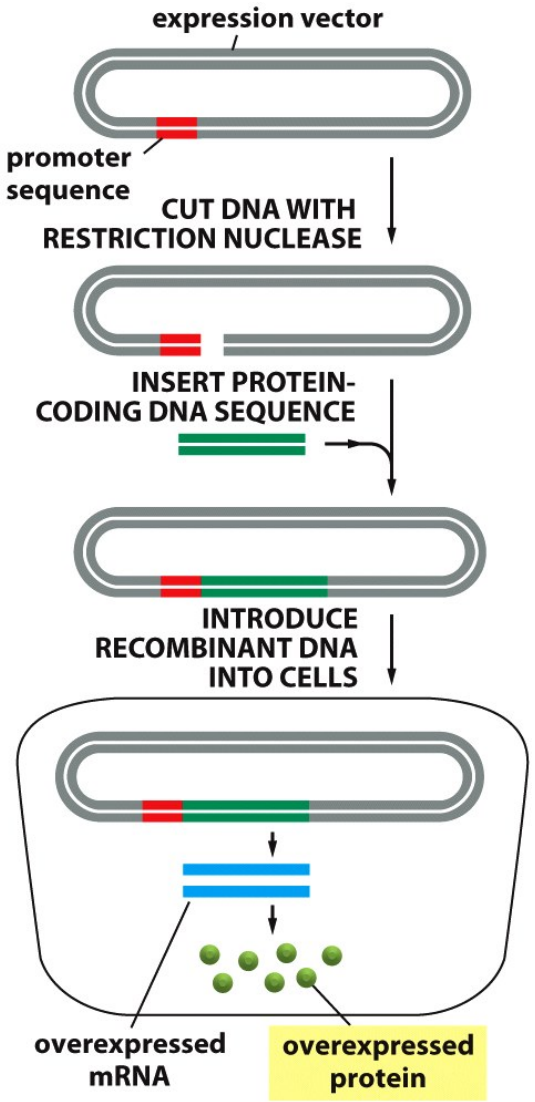
# Protein production

- Special expression vectors may include not only genes in strict sense, but also promoters and other regulatory pieces
- In this way, we may achieve gene over-expression
- Commercial proteins are now producing from bacteria with over-expressing DNA insertions

# Protein overxepression

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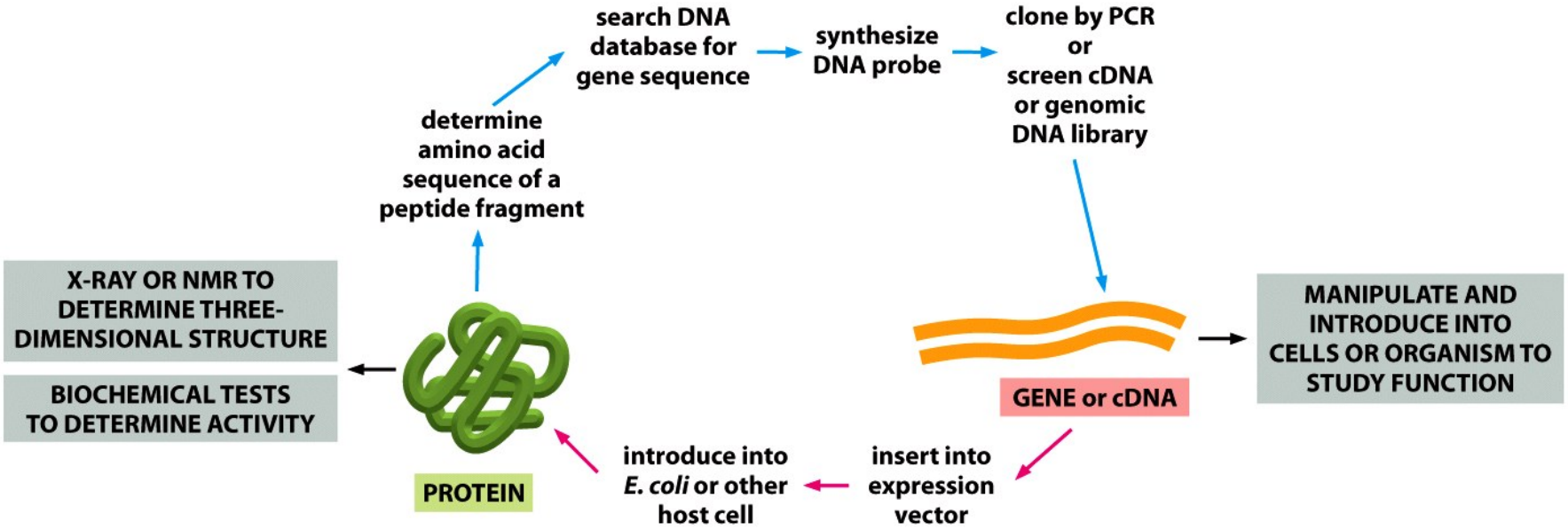
# DNA $\longrightarrow$ protein?

- With DNA database search, it is easy to find DNA which may produce a given protein
- Then we may to clone this DNA and use it for protein production

# How to “reverse” a Central Dogma

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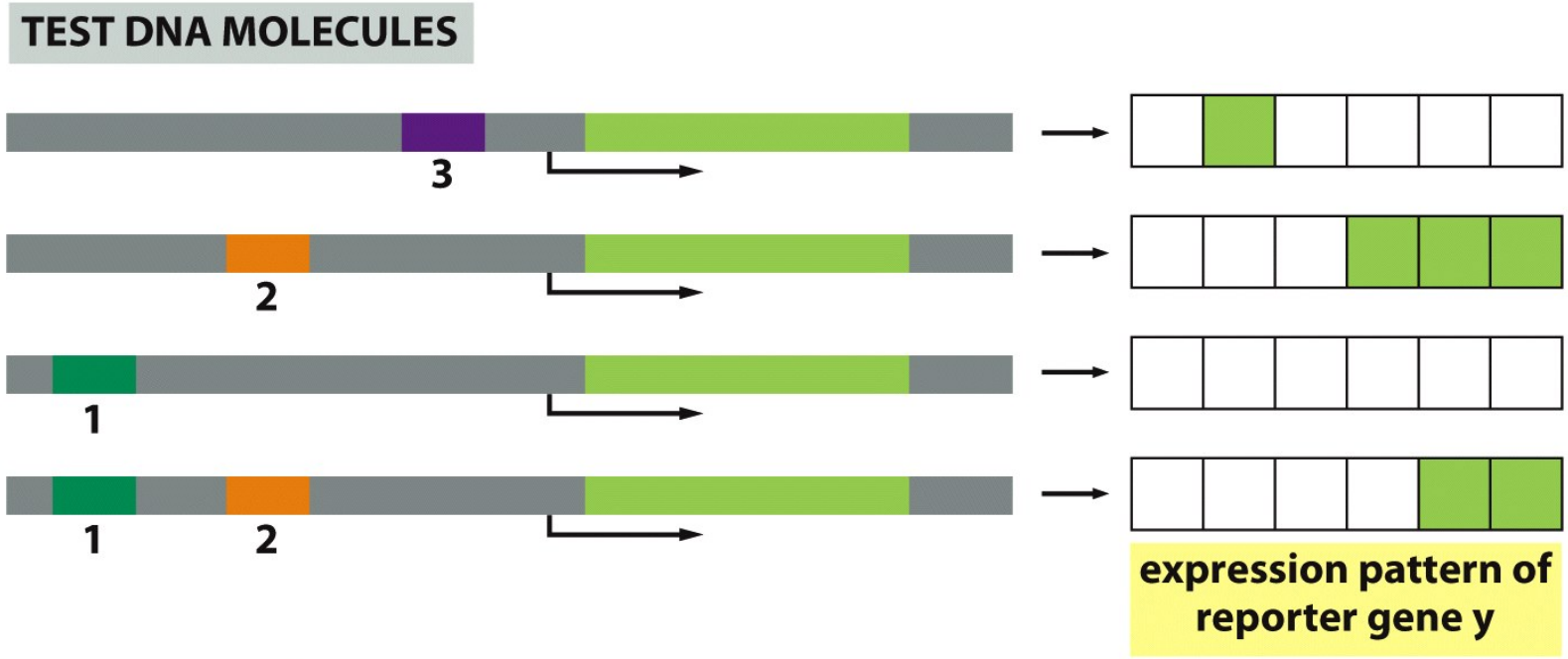
# Reporter genes

- If we combine different regulatory sequences and easily traced gene (reporter gene), we may spot different ways of transcription regulation
- It is especially useful with cascades of regulatory sequences (like *Eve* gene from fly)

# Reporter gene



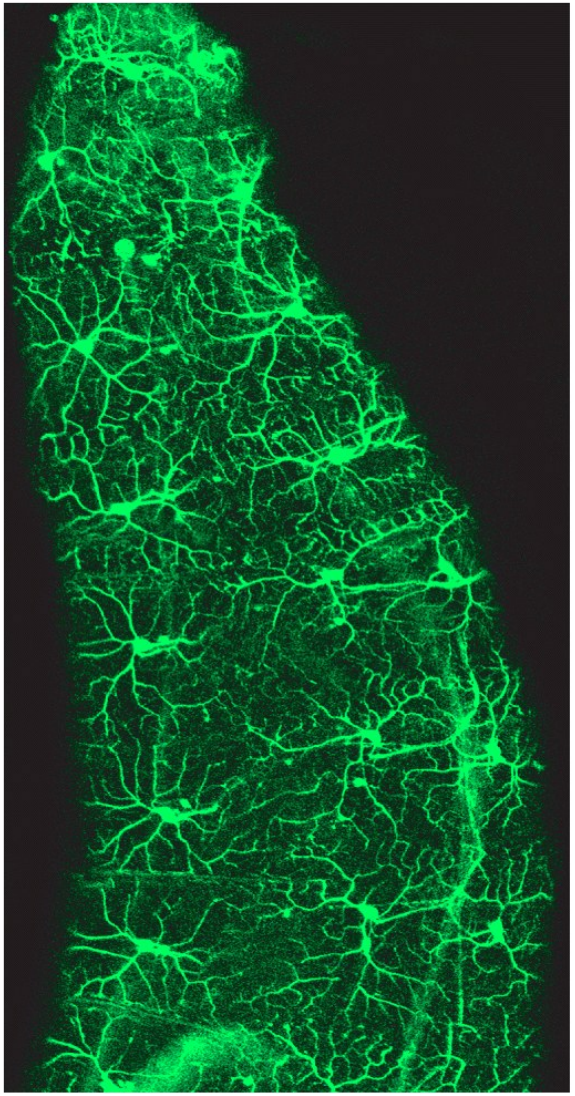

**CONCLUSIONS**
—regulatory sequence 3 turns on gene X in cell B
—regulatory sequence 2 turns on gene X in cells D, E, and F
—regulatory sequence 1 turns off gene X in cell D

Figure 10-29b Essential Cell Biology 3/e (© Garland Science 2010)

# *In situ* hybridization

- Green fluorescent protein may be fused with protein of interest
- If GFP gene is fused with promoter of interest, we may visualize gene expression
- With other fluorescent or radioactive DNA probes, it is possible to visualize genes in chromosomes etc.

# *In situ* DNA hybridization specific to neurons



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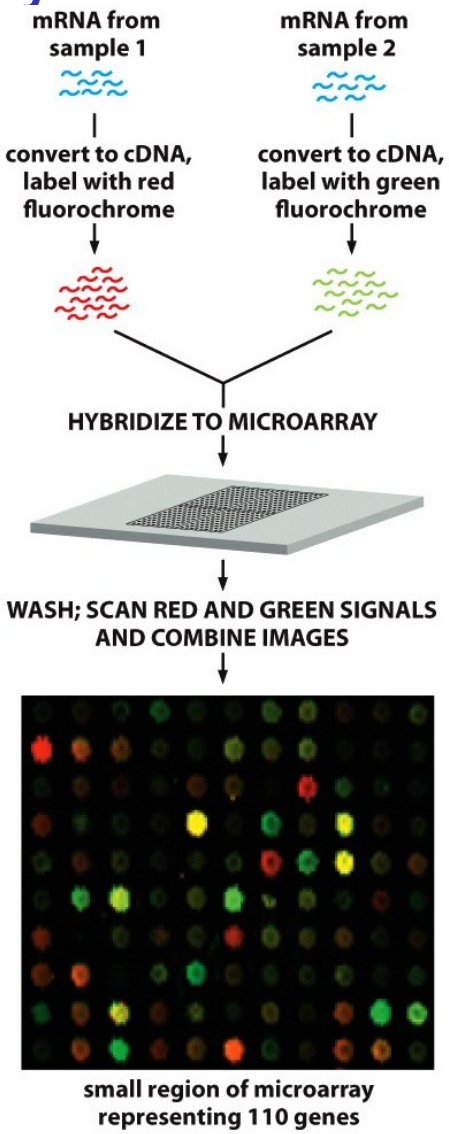
# Microarray

- Microarray is a way to simultaneously visualize thousands of genes
- We may, for example, hybridize cDNA from mRNA of cancer and non-cancer cells and understand which mRNAs have been involved in cancer activity

# Example of microarray



# Site-directed mutagenesis

- Modified primer sequences could initiate the synthesis of modified amplicons
- As a result, we may create proteins with replaced amino acids

# Site-directed mutagenesis HOWTO

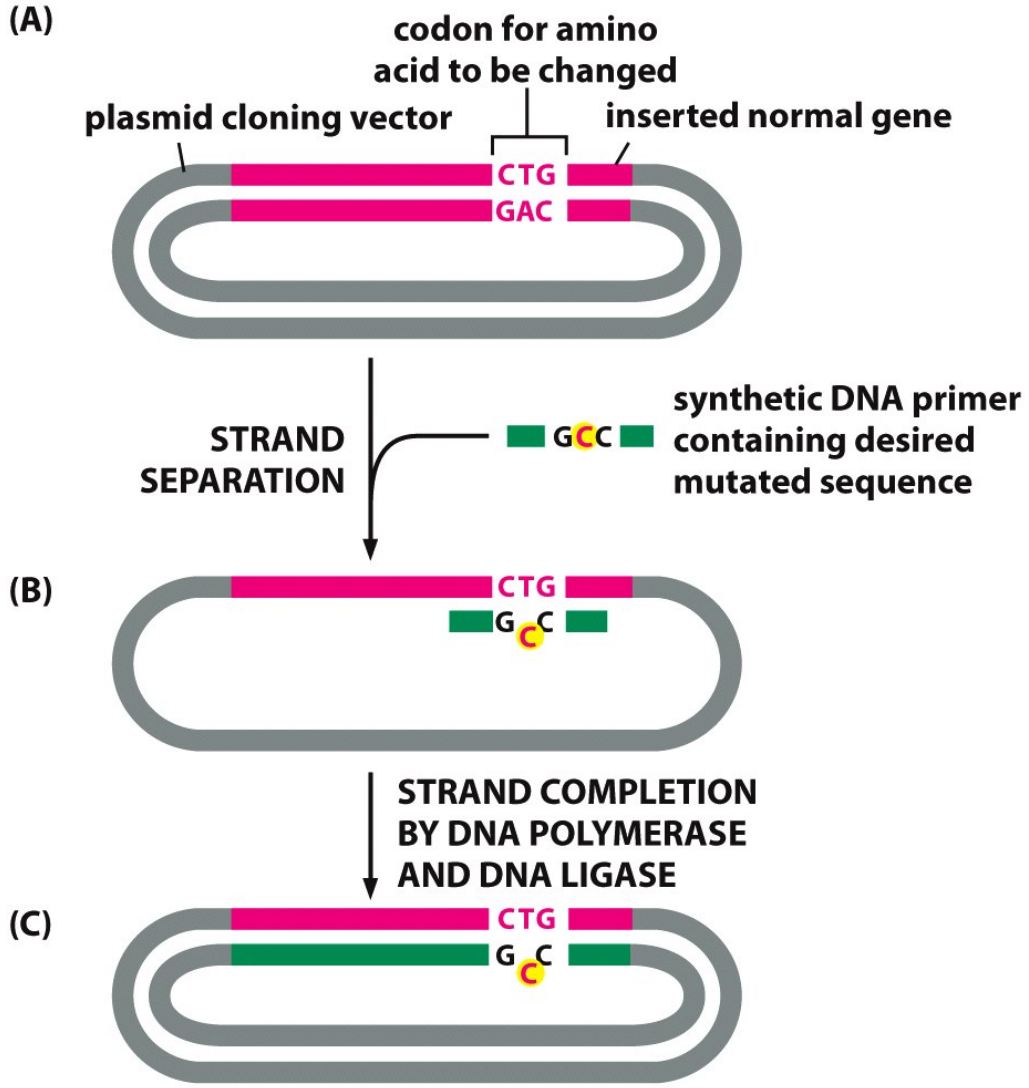
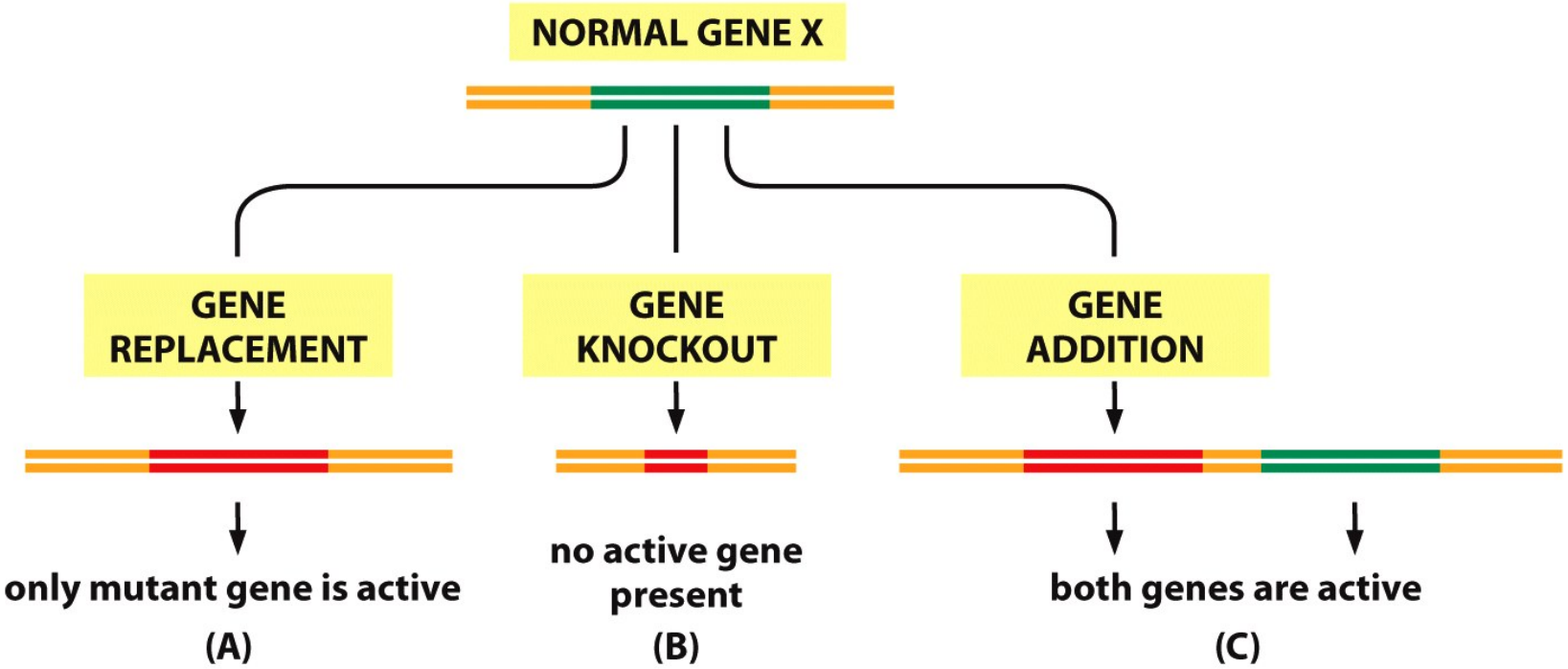



Figure 10-34 part 1 of 2 Essential Cell Biology 3/e (© Garland Science 2010)

# GMOs and others

- Gene replacement: recombination between mutant and normal DNA
- Gene knockout: same, but recombinant gene will not work
- Gene addition leads to transgenic organisms (GMOs)

# Ways of gene alteration

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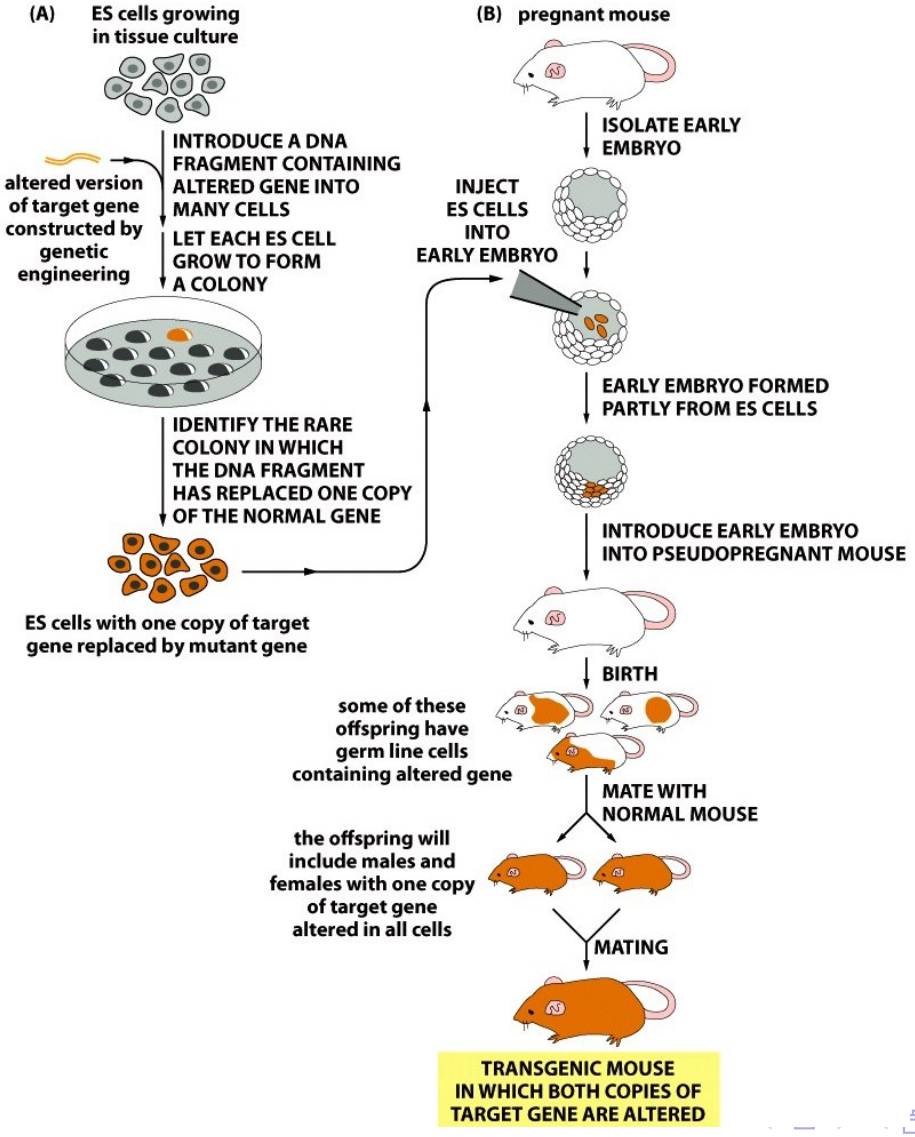
# Transgenic mice



# Wild (left) and *Xpd* knockout mouse (right)

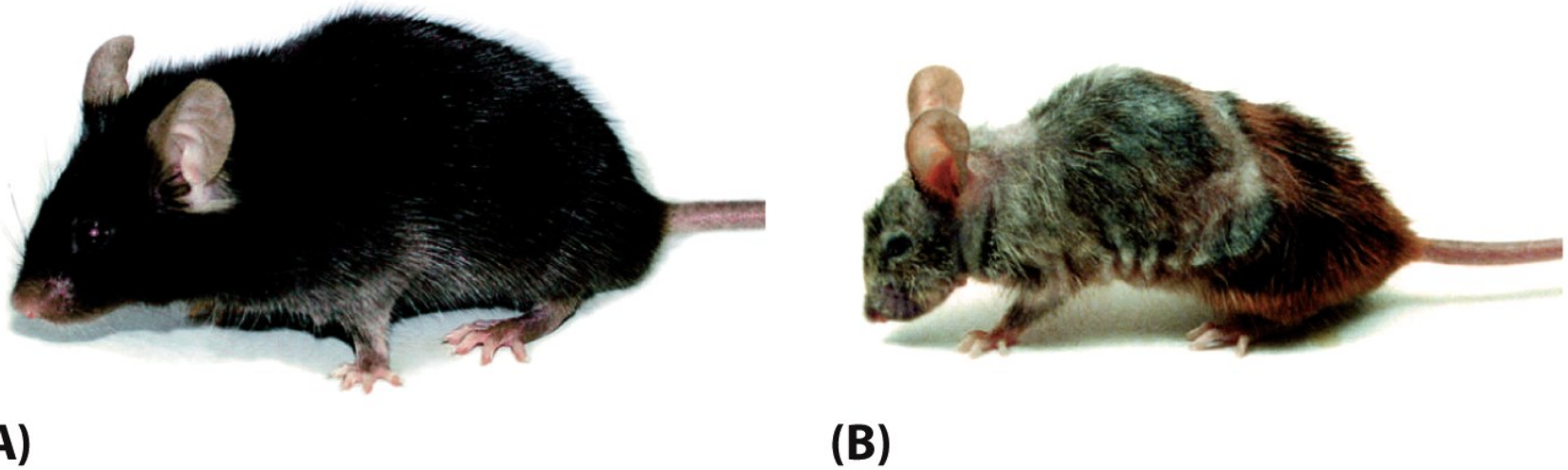



Figure 10-37 Essential Cell Biology 3/e (© Garland Science 2010)

# RNA interference

- If we introduce dsRNA of gene which exists in nucleus, we may de-activate its translation through RNA interference
- That is an efficient way to test gene function

# Final question (3 points)

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How to make a transgenic organism (GMO)? Shortly describe a technique.

# Summary

- Genome assembly employs both programmatic methods and creation of DNA BAC libraries
- *In situ* hybridization elucidates regions of gene expression
- Recombinant DNA makes possible to create new organisms

# For Further Reading

A. Shipunov.
*Advanced Cell Biology* [Electronic resource].
2011—onwards.
Mode of access: http://ashipunov.info/shipunov/school/biol_250.

B. Alberts et al.
*Essential Cell Biology*. 3rd edition.
Garland Science, 2009.
*Chapter 10*.