Review: Regulation of gene expression

- Gene X encodes a microRNA that is an exact complement to a section of mRNA for the gene that produces the protein *c-Myc*
  - What would likely happen to the level of *c-Myc* protein if a transcriptional activator bound to an *enhancer* for Gene X?
  - What would likely happen to the level of *c-Myc* protein if there was a *mutation inside of Gene X* that changed a nucleotide from an A to a C?
  - What would likely happen to the level of *c-Myc* protein if there was a *mutation in the DNA coding sequence for the c-Myc gene* at a "wobble base" 3rd codon position that changed the nucleotide without affecting the amino acid sequence?

Review: Regulation of gene expression

- Gene X encodes a microRNA that is an exact complement to a section of mRNA for the gene that produces the protein *c-Myc*.
  - What would likely happen to the level of *c-Myc* protein if a transcriptional activator bound to an enhancer for Gene X?
    - The level of *c-Myc* protein would likely be lowered, because Gene X would be transcribed at high levels into the non-coding miRNA that would selectively target the mRNA that is translated into *c-Myc* for destruction by the RISC complex before translation could occur.
  - What would likely happen to the level of *c-Myc* protein if there was a mutation inside of Gene X that changed a nucleotide from an A to a C?
    - The level of *c-Myc* protein would likely be increased, because this mutation in Gene X would lead to incomplete complementarity between the miRNA and the target mRNA for *c-Myc*, thereby allowing *c-Myc* to be translated into protein as normal.
  - What would likely happen to the level of *c-Myc* protein if there was a mutation in the DNA coding sequence for the *c-Myc* gene at a "wobble base" 3rd codon position that changed the nucleotide without affecting the amino acid sequence?
    - The level of *c-Myc* protein would likely be increased, because this mutation in the *c-Myc* gene would likewise lead to incomplete complementarity with the miRNA and would not affect translation into *c-Myc* protein.

Analyzing the Structure and Function of Genes

Chapter 10

BIOL 366

Dr. Matthew Ellis

Learning Objectives for Chapter 10

By the end of this module, you should be able to:

- Understand experimental methods for expressing, amplifying, and identifying specific DNA sequences (e.g., restriction enzymes, cloning, PCR, sequencing)
- Describe experimental techniques researchers use to investigate gene regulation and gene function (e.g., reporter genes, RNAi, CRISPR, mutant organisms)
- Evaluate scientific findings using your knowledge of experimental methods used to study gene regulation and gene function (Group Activity #2)

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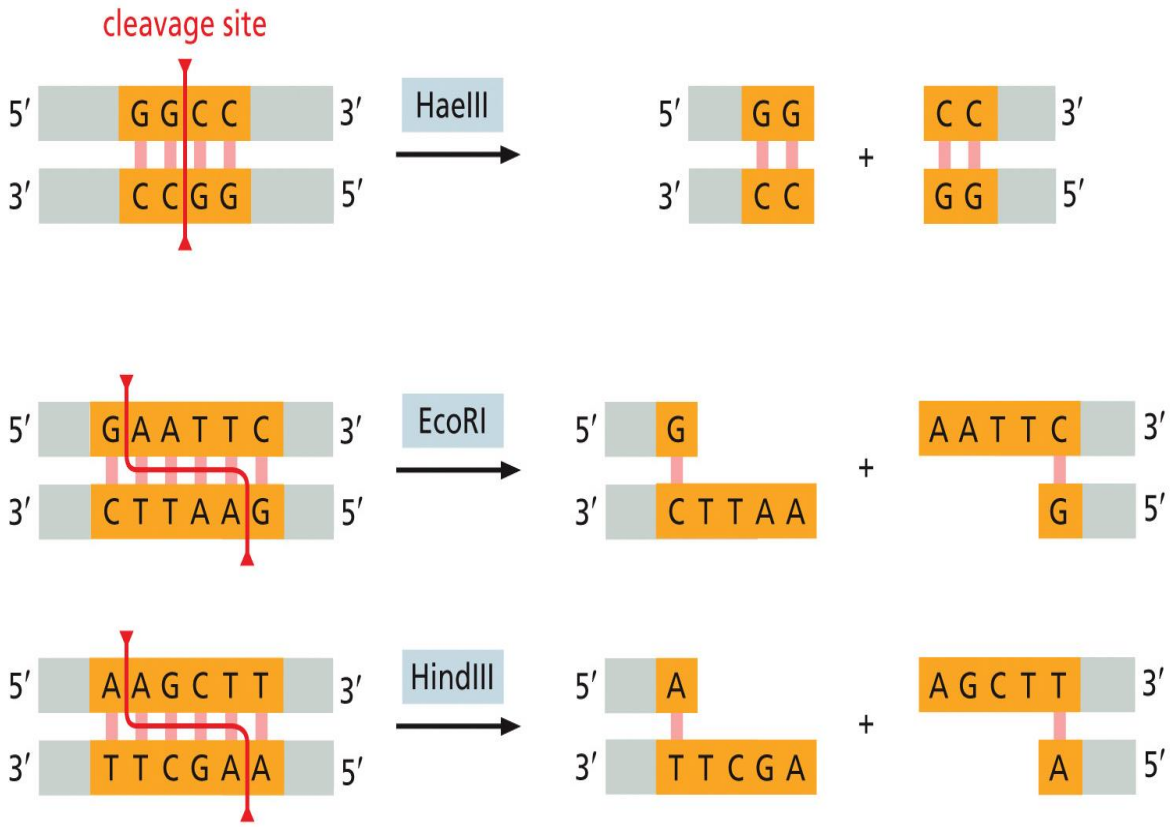
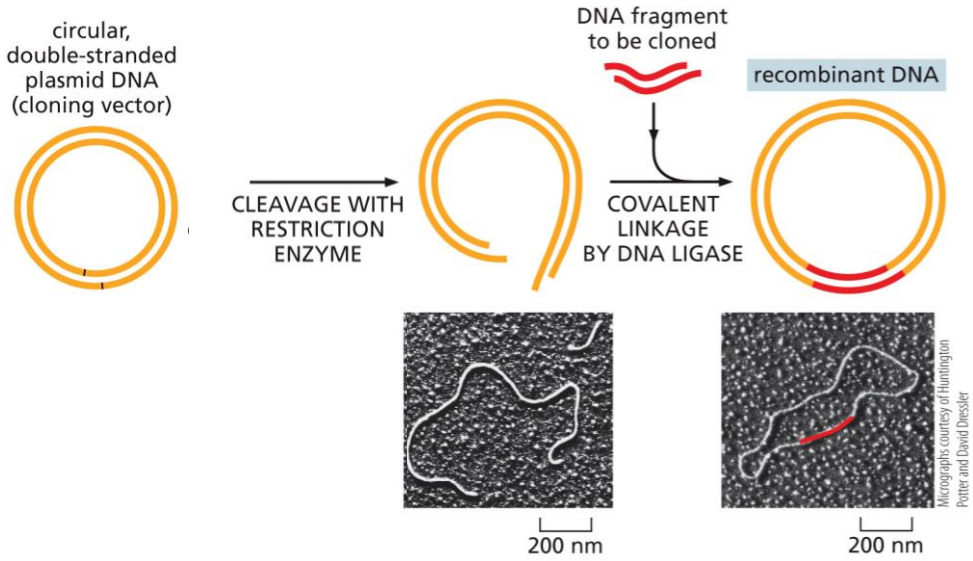
Restriction enzymes cut DNA molecules at specific sites

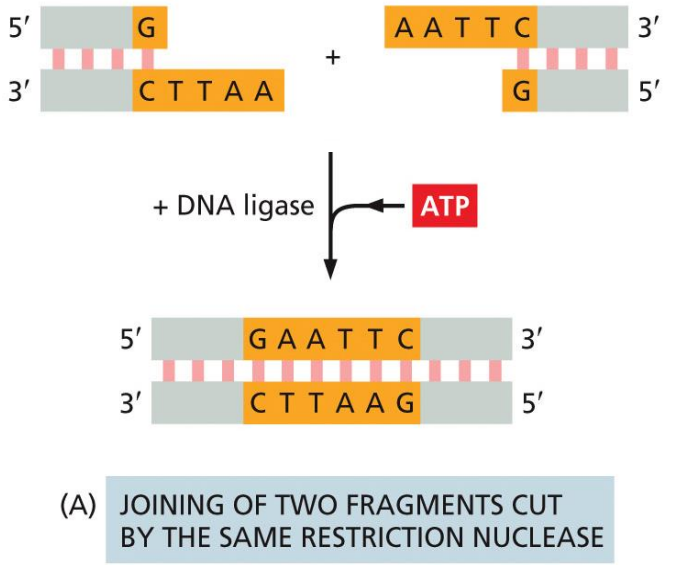
- **Restriction enzymes** are nucleases that cleave both strands of DNA at specific palindromic nucleotide sequences (~4-8 nucleotides)
- Some enzymes cut straight across the double helix and leave two blunt-ended DNA molecules (ex. HaeIII)
- Other enzymes cut in a staggered manner creating short, single-stranded overhangs "*sticky ends*" (ex. EcoRI and HindIII)
  - These can be leveraged by researchers to introduce novel DNA sequences through exposed complementary base pairing



Cloning is the process of inserting a gene or sequence of interest into a plasmid

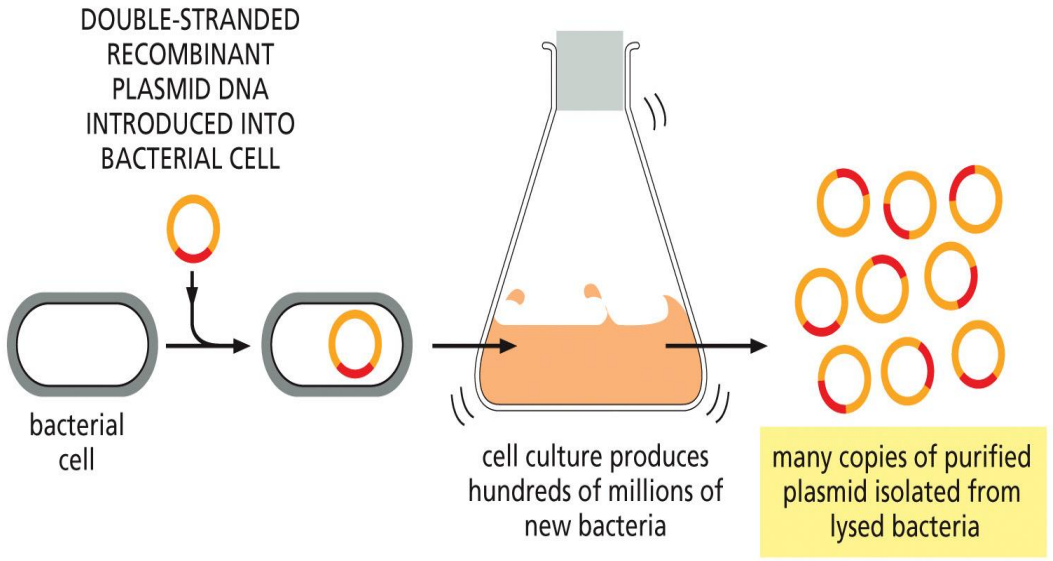
- A DNA fragment can be inserted into a **plasmid**: small circular DNA molecules with their own replication of origin and restriction enzyme cleavage sites
  - Target site cleavage is performed by a restriction enzyme, leaving exposed "sticky ends"
  - DNA insert is the mixed with the cleaved plasmid
    - The staggered ends base-pair, and DNA ligase (utilizing ATP) seals the nicks in the DNA to produce a *recombinant* DNA molecule



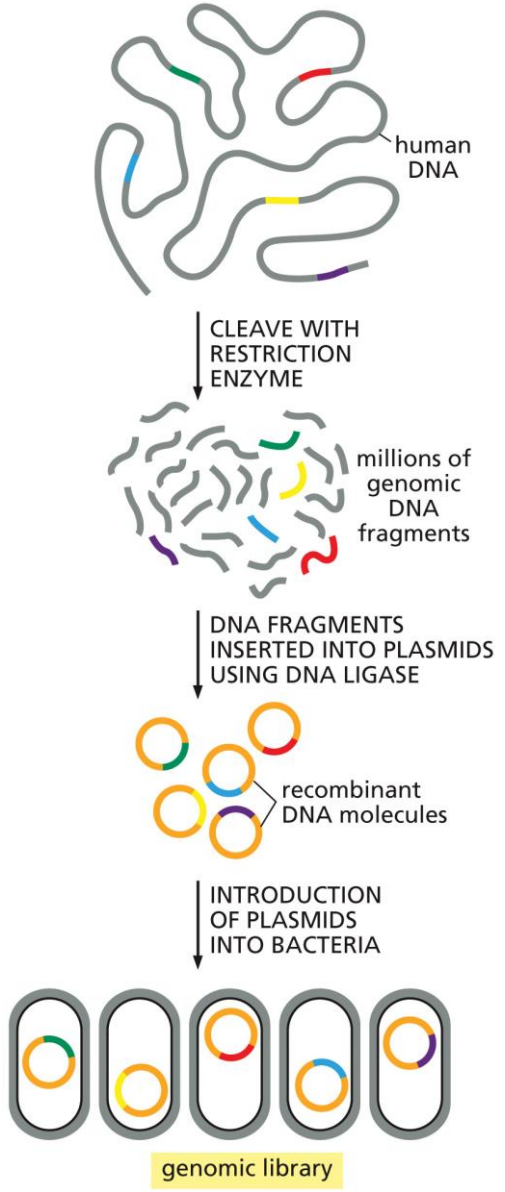


We can then expand our recombinant DNA fragment leveraging bacterial DNA replication machinery

- Bacterial cells are able to take up the plasmid DNA in a process known as *transformation* and then make many copies of the plasmid as they divide



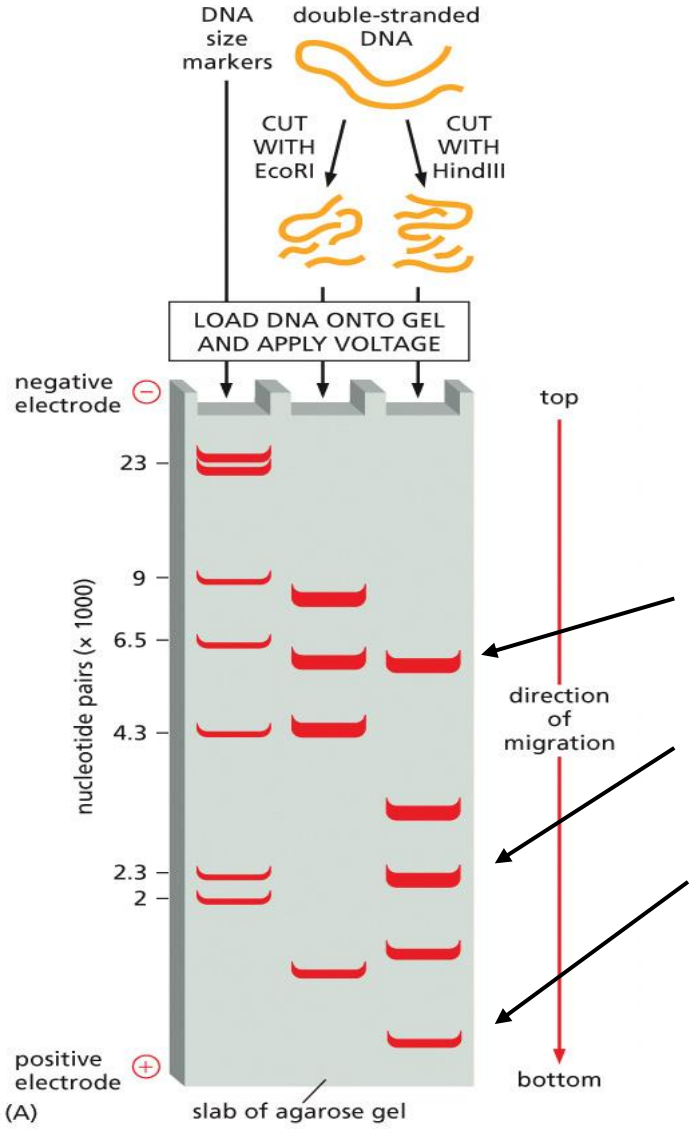
- Cloning can also be used for *whole genomes*
  - Millions of DNA fragments generated, with separate fragments inserted into each plasmid
  - Results in a genomic library which can be *sequenced* to determine DNA nucleotide order
  - Can also use mRNA to generate a *cDNA library* with reverse transcriptase to study only transcribed genes



We can check whether our restriction enzyme cut the DNA successfully through gel electrophoresis

- After using our restriction enzymes, we can load a mixture of DNA fragments into a gel and apply voltage
  - Sizes of the fragments can be estimated by comparing them against a DNA ladder with fragments of known sizes

- For DNA gels, we do not need to add SDS like we did for western blotting, why is this true?

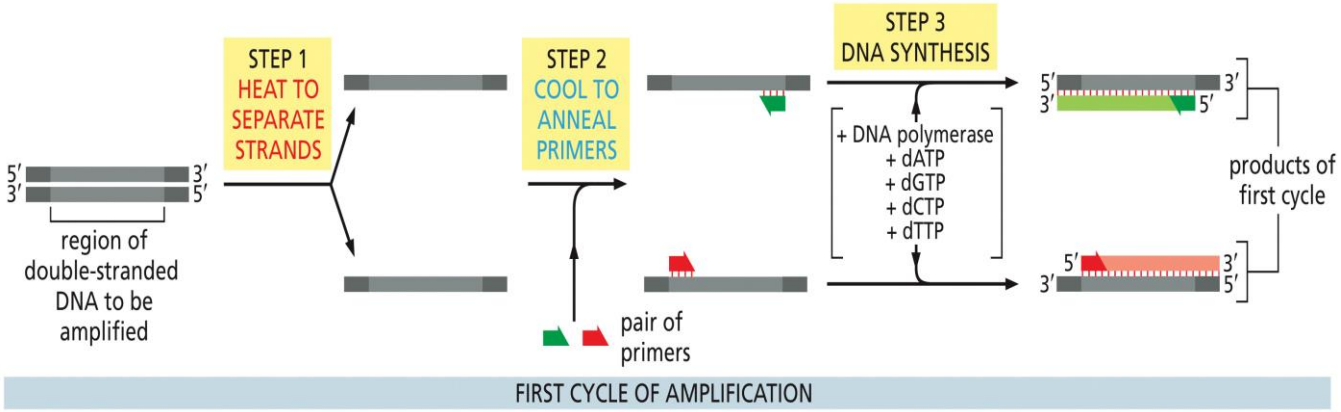


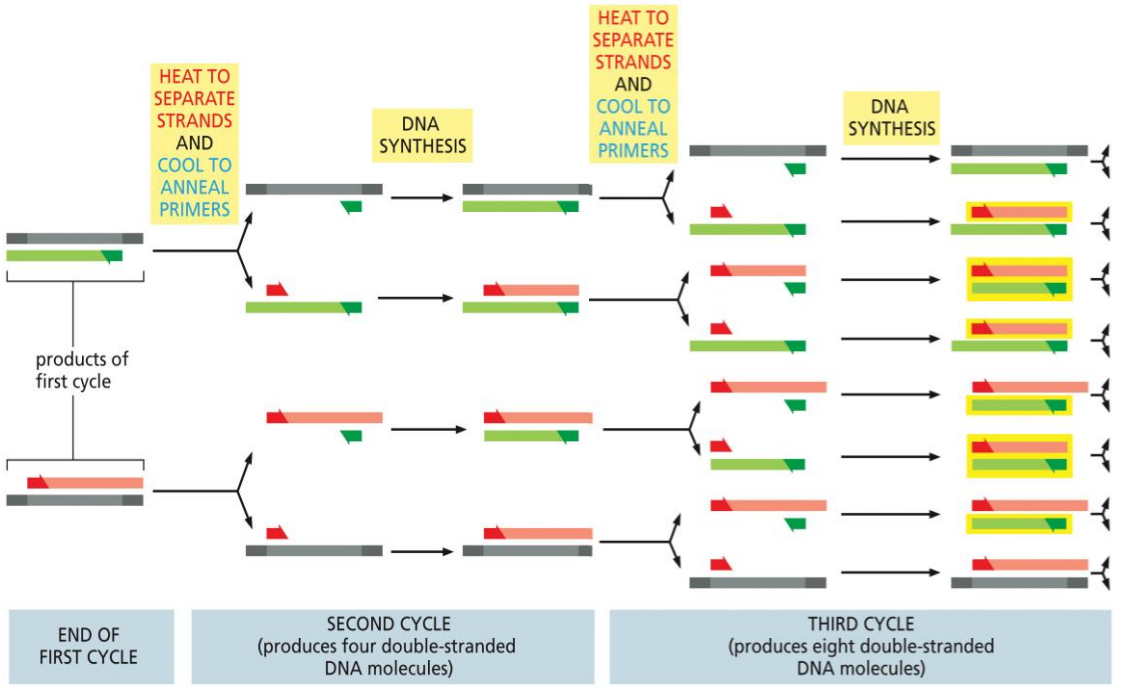
Gel shows various fragments of different sizes indicating successful digestion of the DNA. We can compare this to a *map* of the plasmid to see if the sizes are as expected

A Polymerase Chain Reaction (PCR) can be used to amplify a specific DNA Sequence

- PCR utilizes specific DNA primers and DNA polymerase to amplify a target sequence in an exponential manner

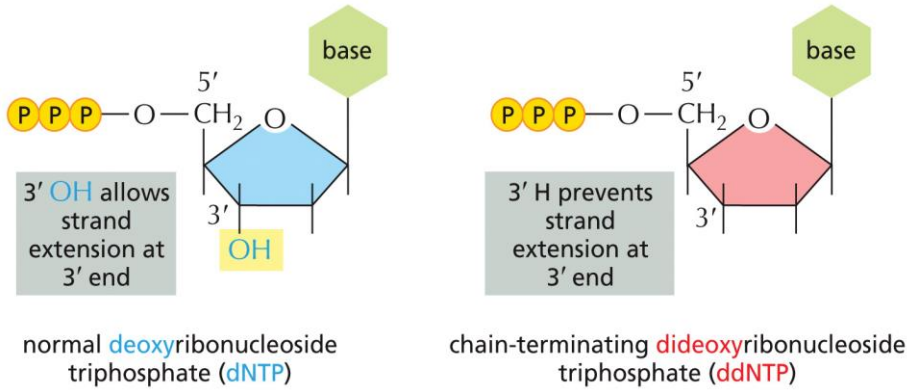
- The DNA Primer serves both to guide to the specific nucleotide sequence within DNA and to provide a 3’ OH for DNA polymerase to expand from (**Ch. 5**)

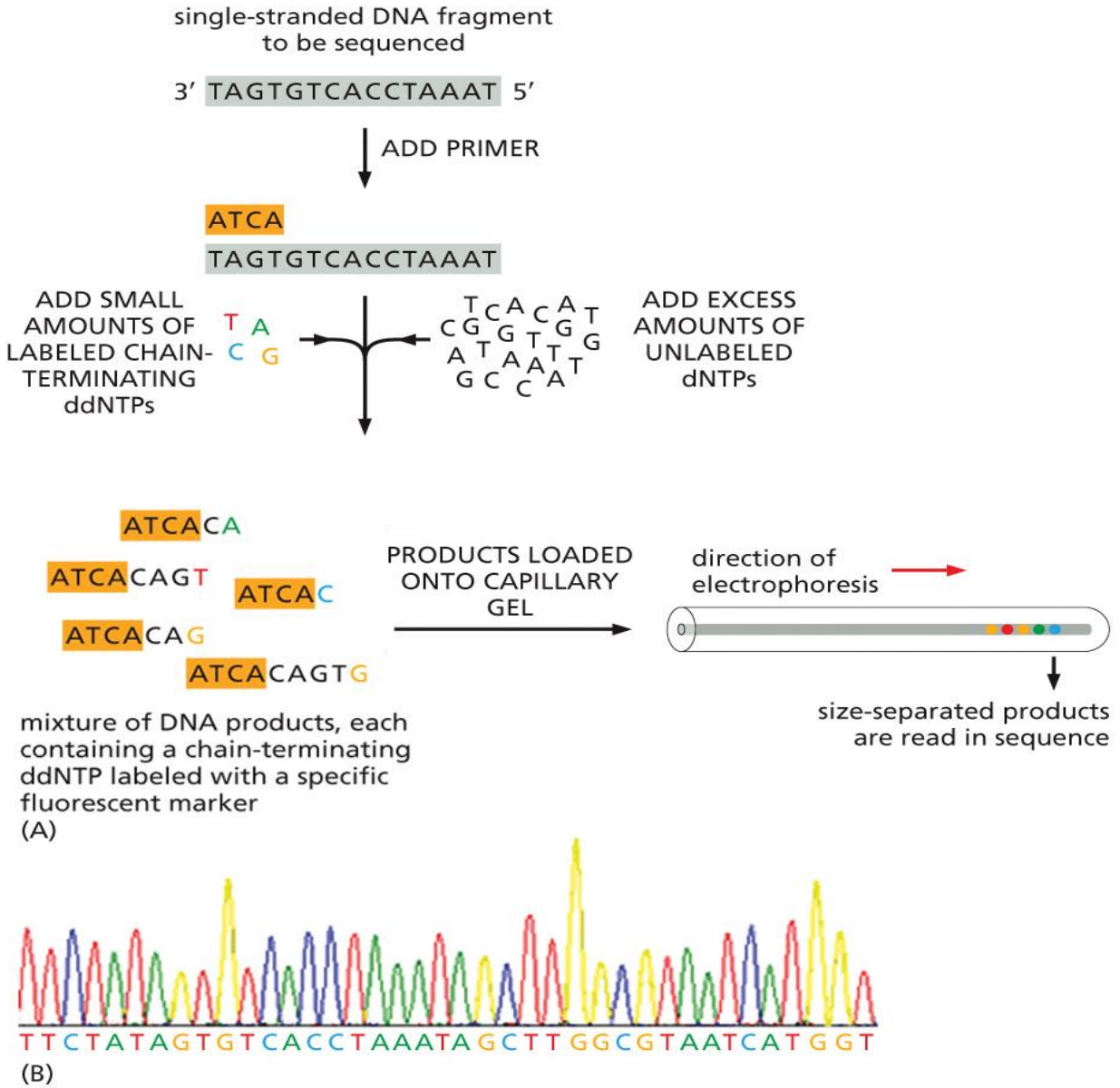




Sequencing is used to define the precise nucleotide sequence of DNA from start to finish

- Dideoxy sequencing (*Sanger sequencing*) uses special **chain terminating** dideoxynucleoside triphosphates (ddNTPs) tagged with a different colored fluorescent dye to produce DNA copies that terminate at different points in the sequence
- Reaction products are separated by electrophoresis in a capillary gel and read by a camera and computer to generate the final sequence





Squarecap Q#1-3

Learning Objectives for Chapter 10

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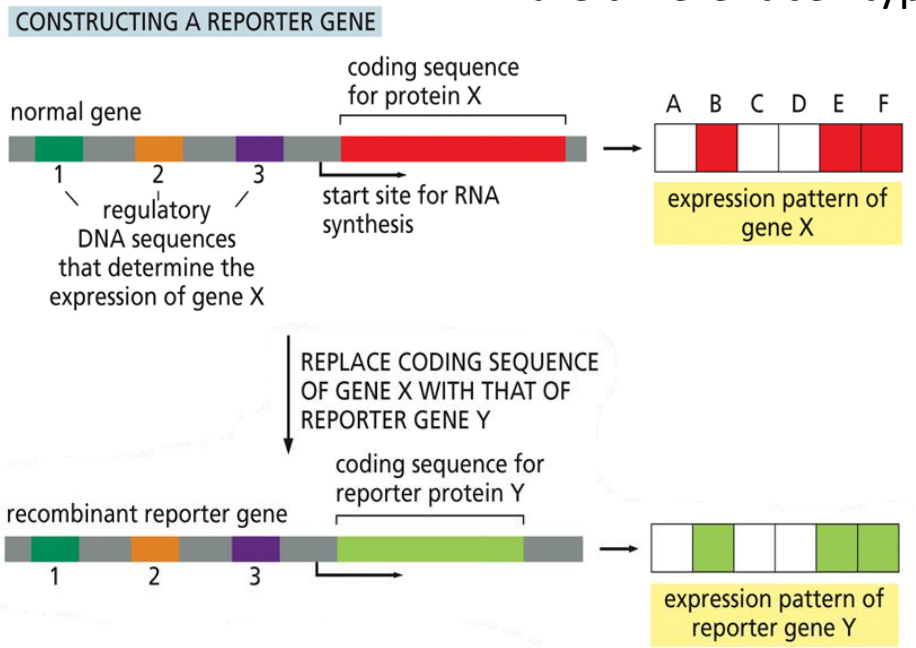
Reporter genes allow specific proteins to be tracked in living cells

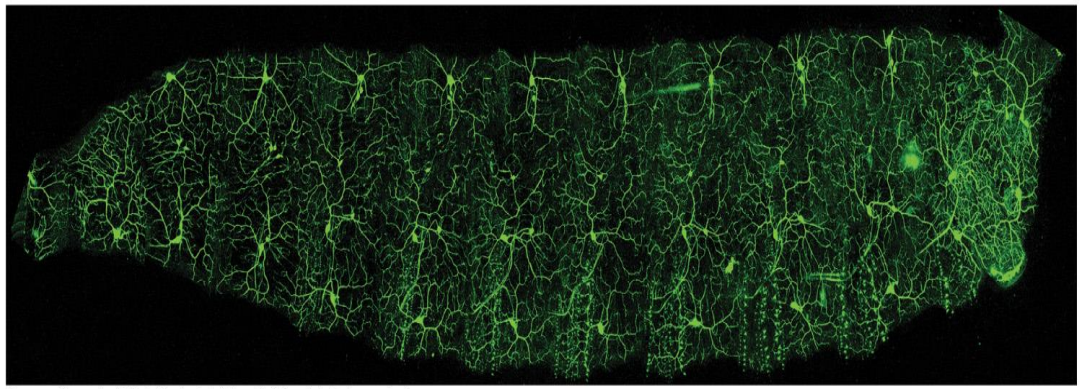
- A reporter gene uses regulatory DNA sequences of genes of interest to drive expression of a protein that can be monitored based on fluorescence (e.g., GFP) or enzymatic activity (e.g., β-galactosidase)

- The reporter mimics expression of the gene of interest to produce reporter protein at the same place, same time, and in the same amounts as the normal protein
  - This helps us learn about expression patterns of genes across differing cell types

- **Example**: Neuronal specific expression of GFP

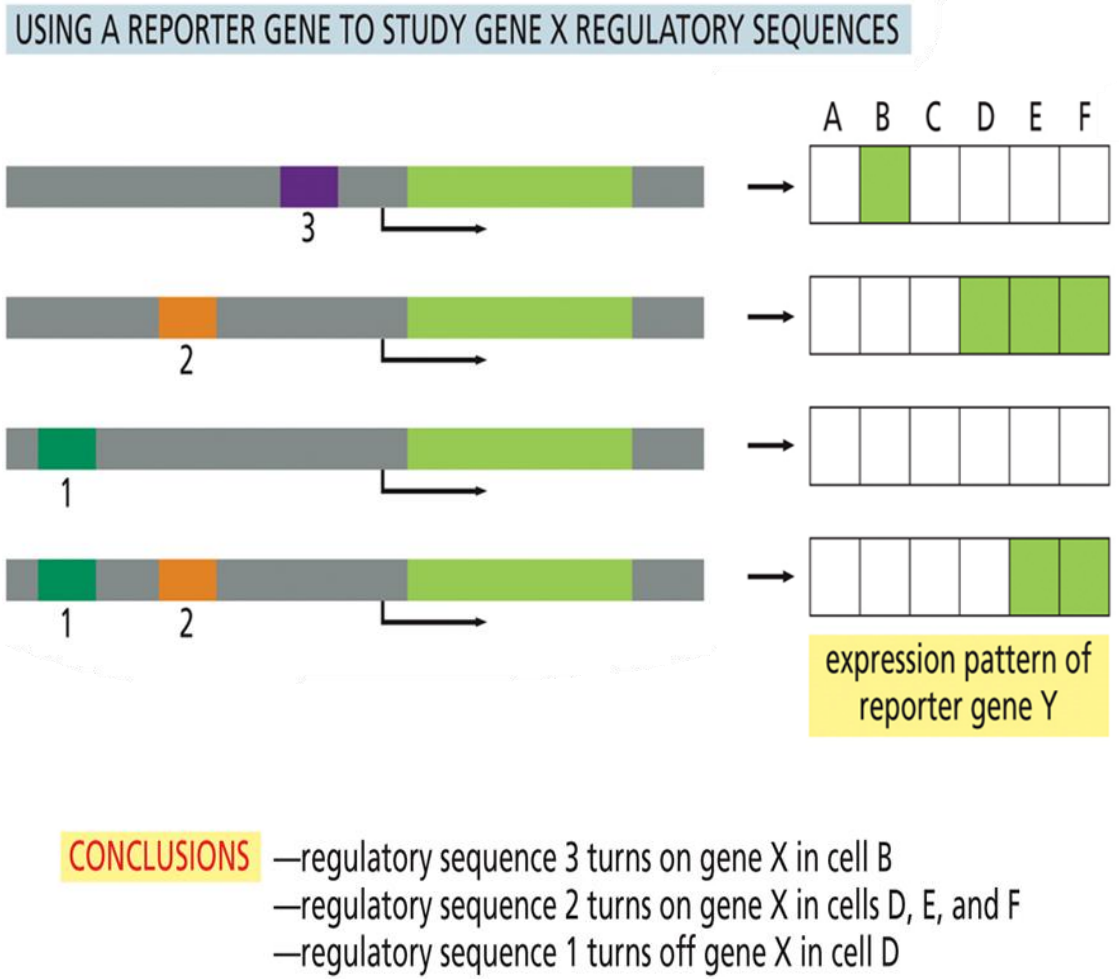
A-F are different cell types





Reporter genes allow the study of regulatory sequences that control gene expression

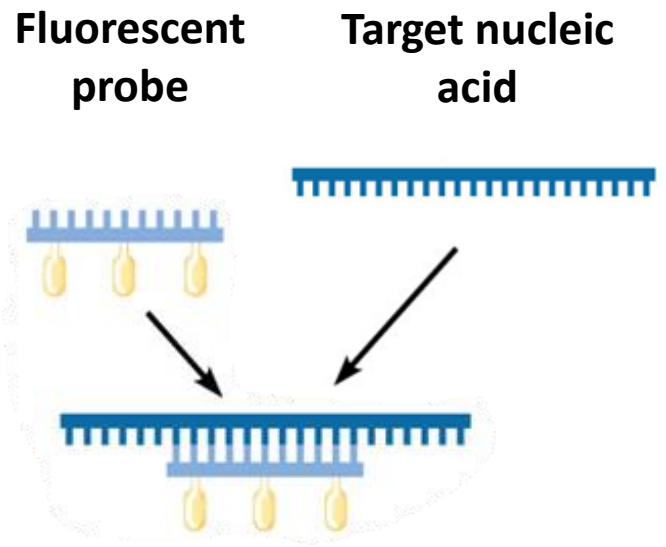
- We can generate different reporter genes with various combinations of the regulatory regions associated waith gene X (here sequences 1, 2, and 3)
- Then observe differences in reporter gene Y expression with different regulatory regions across different cell types (A-F)
- What are some conclusions you can draw about how these regulatory sequences control the expression pattern of gene X in this example?



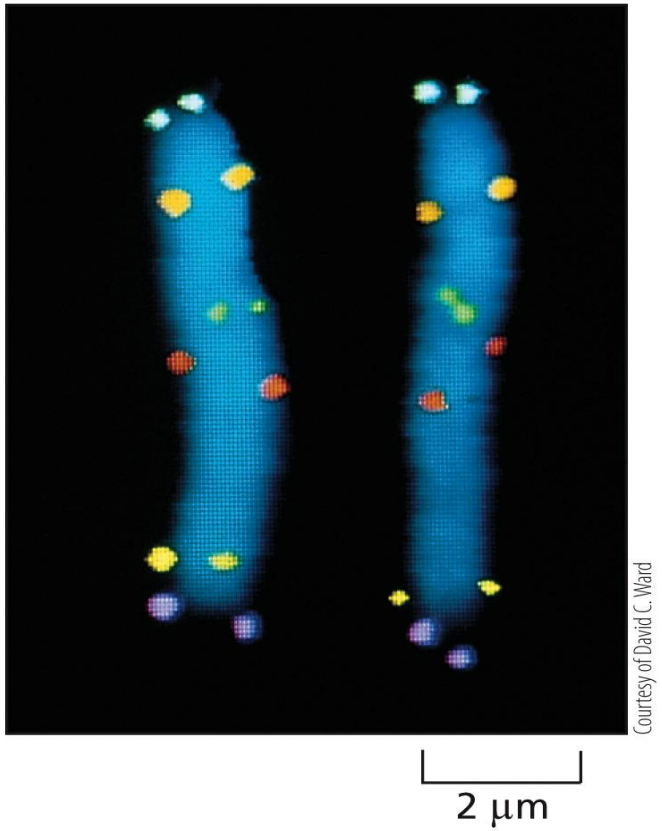
Researchers also use *in situ* hybridization to detect the presence of a nucleic acid sequence inside of a cell

- A single stranded DNA or RNA fluorescently labeled probe is used to bind a particular RNA or DNA sequence in fixed cells or tissues

- This is a useful technique for determining gene location on isolated chromosomes as well as in analyzing expression patterns of genes in developing tissues
  - Helps determine at what stage are these genes expressed and at what location



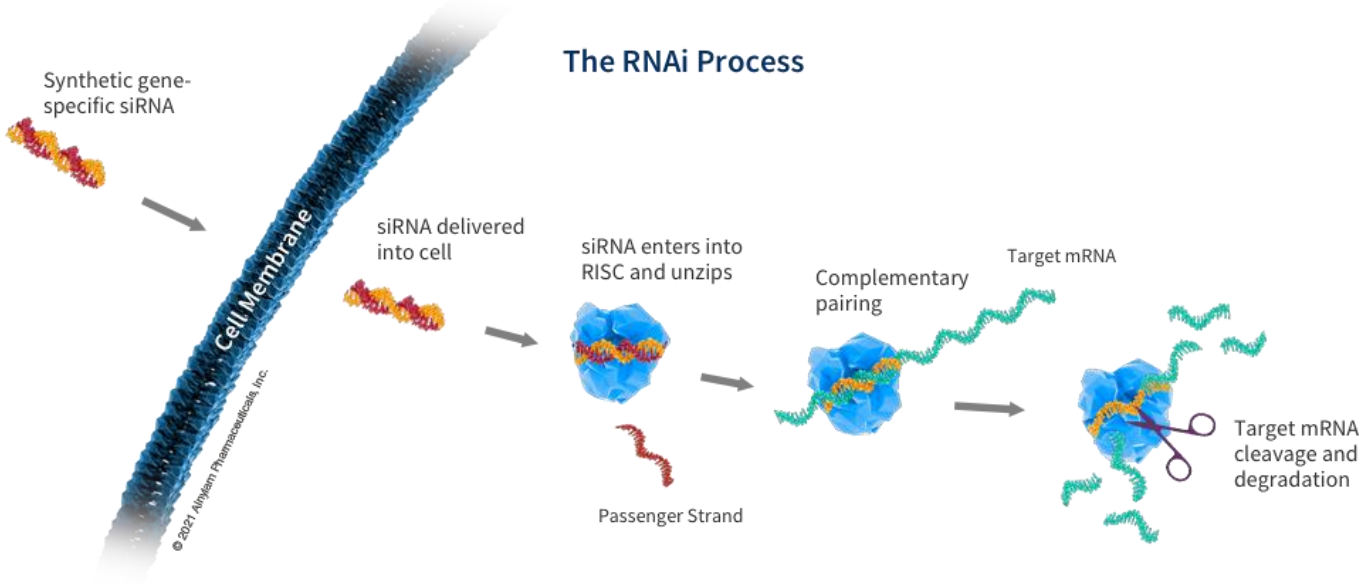
***In situ* hybridization in set of homologous chromosomes**



RNA interference (RNAi) leverages the microRNA endogenous mechanism to artificially control gene expression

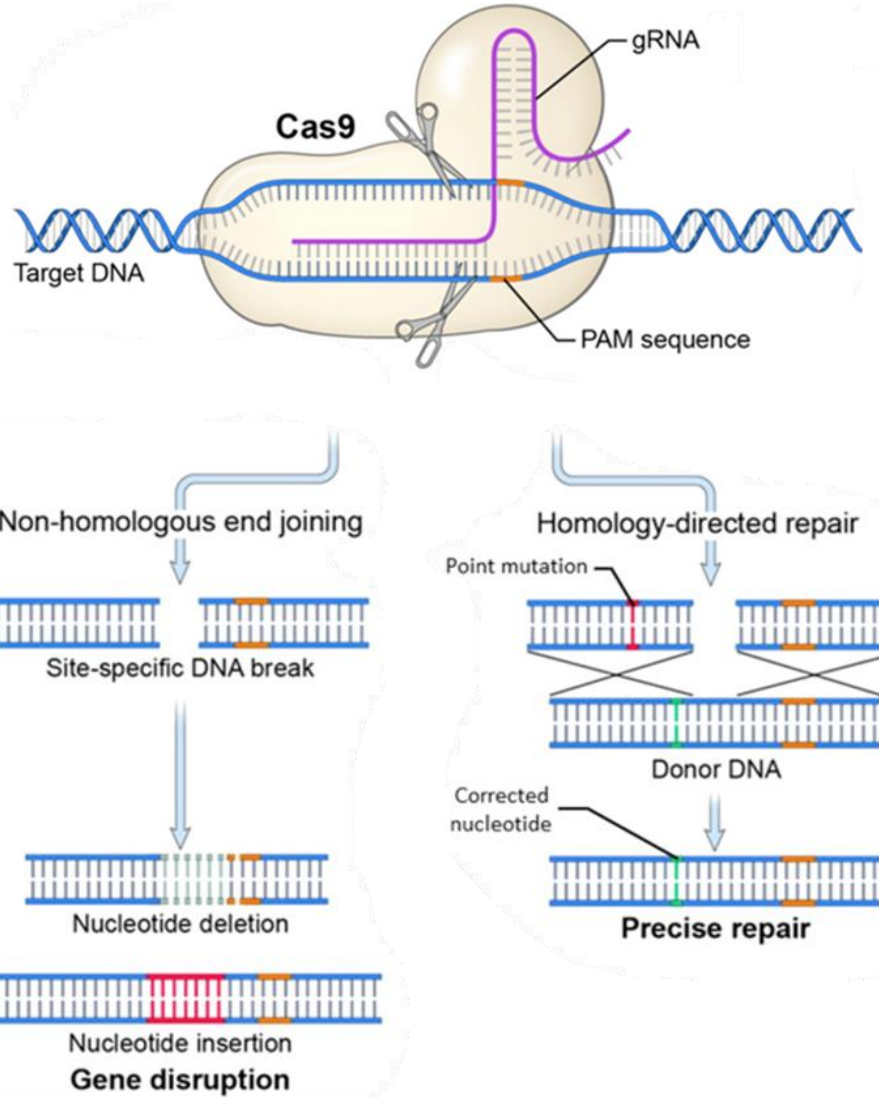
- *Recall:* miRNAs base pair to target mRNAs leading to their degradation (**Ch. 8**)

- RNAi uses synthetically designed small interfering RNAs (**siRNAs**) to inhibit the activity of specific target genes by degrading their mRNA
  - Addition of siRNA molecules through a process known as *transfection* can thus facilitate genetic "knockdown," meaning less of the protein will ultimately be produced in the cell

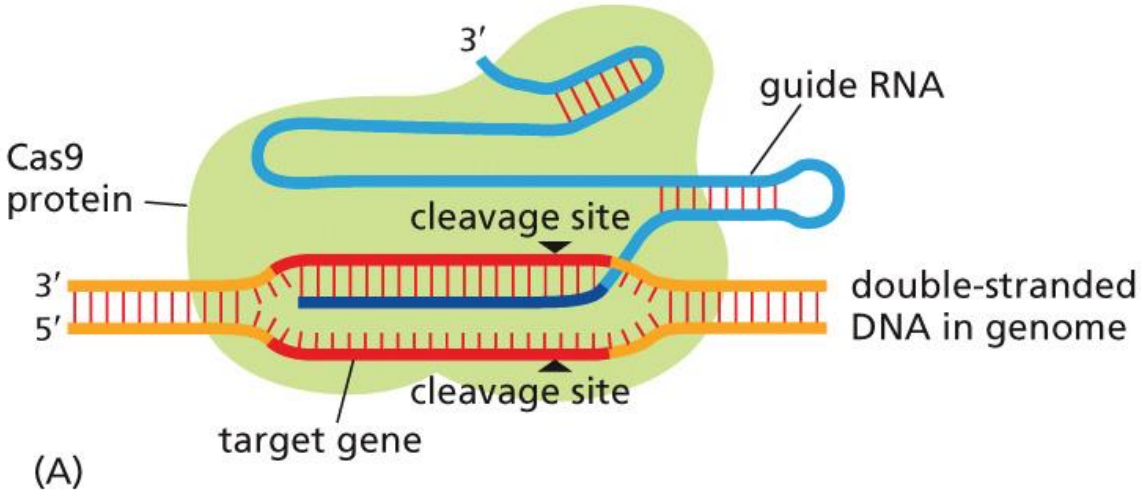


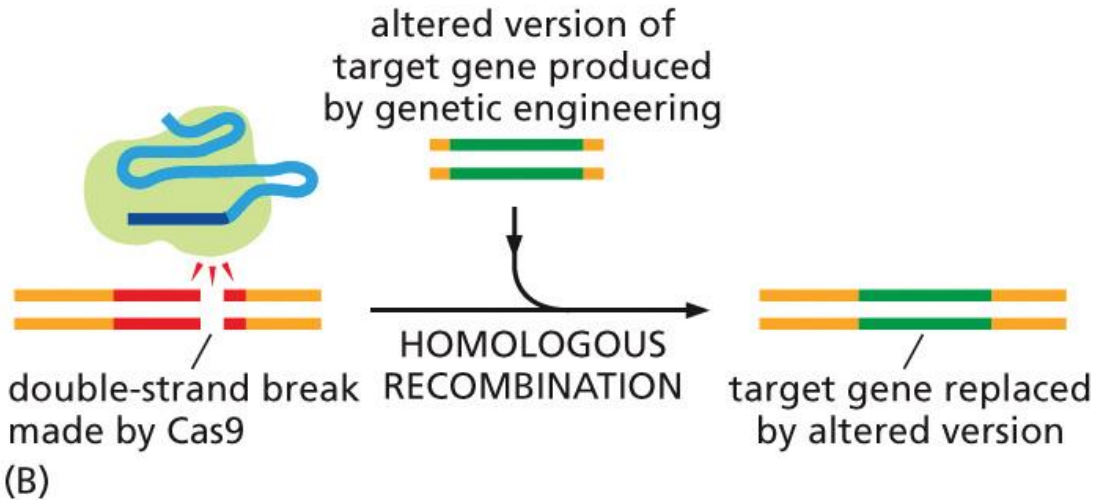
CRISPR/Cas9 can be used to selectively modify target DNA sequences

- *Recall:* The role of small noncoding RNAs in bacteria to selectively protect against repeat viral infection (**Ch. 8**)
- A guide RNA (gRNA) is used as a probe to direct the Cas9 nuclease to a specific site, leading to a double strand DNA break
- As we learned in **Ch. 6**, double strand breaks can either be repaired through non-homologous end joining (NHEJ) or homology-directed repair (homologous recombination; HR)
  - NHEJ often leads to insertion, deletion, or frameshift at the target site, likely affecting normal gene expression
  - HR leverages a donor DNA template to selectively replace the DNA sequence as desired (gene editing)



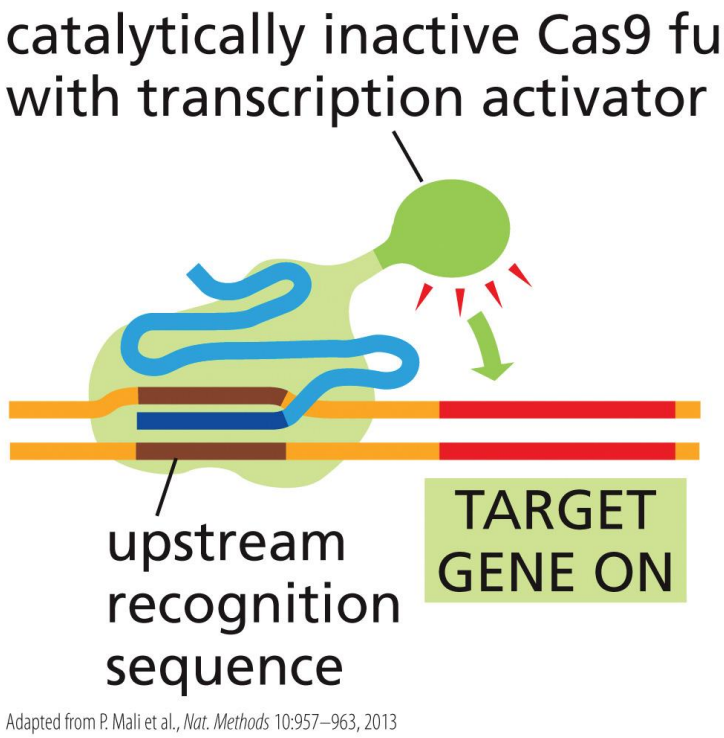
Genetic editing using CRISPR/Cas9

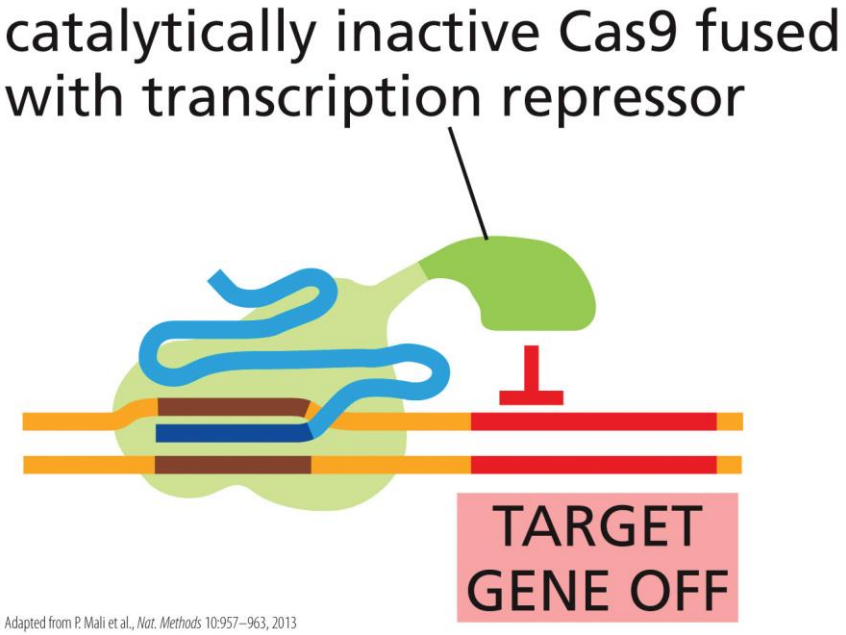




Modified Cas9 proteins can be used to study gene function

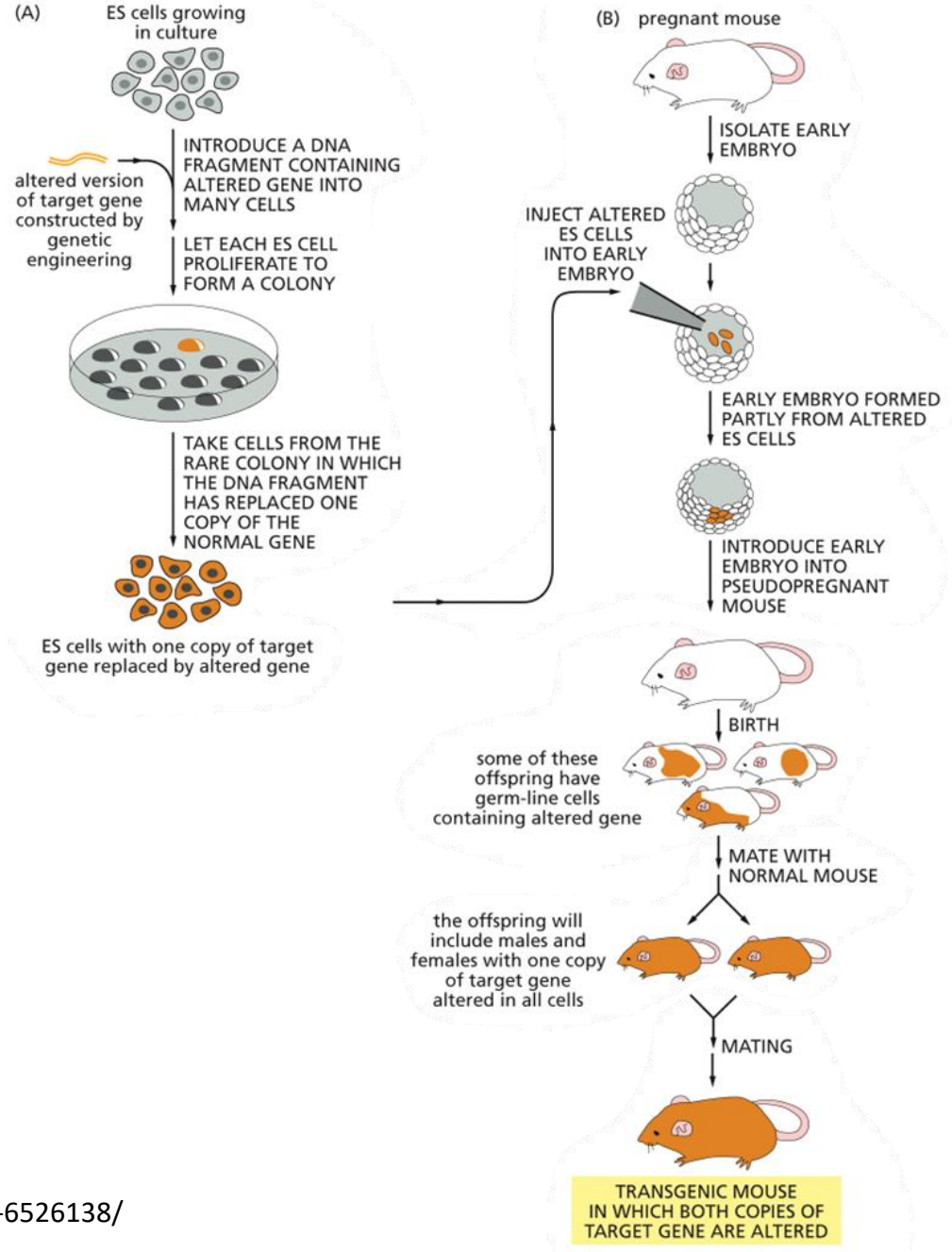
- A mutant form of Cas9 that can no longer cleave DNA can be fused to a transcriptional regulator to change the expression of a gene. Directed to specific site by guide RNA
  - Can be used to activate a normally dormant gene
  - Can be used to turn off an actively expressed gene





Transgenic animals can help to reveal the function of a gene *in vivo*

- Transgenic organisms have altered genomes where a *transgene* (altered gene) is expressed in embryonic stem (ES) cells for generating whole organism expression
  - *Recall:* When modified cells are injected into an early embryo these cells become part of the organism – both somatic and germ-line cells will have the mutation (**Ch. 9**)
  - Chimeric offspring are produced where some cells have the transgene and some cells don't (orange` patches represent cells with transgene)
  - By mating these chimeric animals with normal mice you can identify offspring with the mutation *in their gametes* (offspring will be fully orange – all cells contain transgene)
  - Crossing the fully orange females with fully orange males will create homozygous transgenic animals

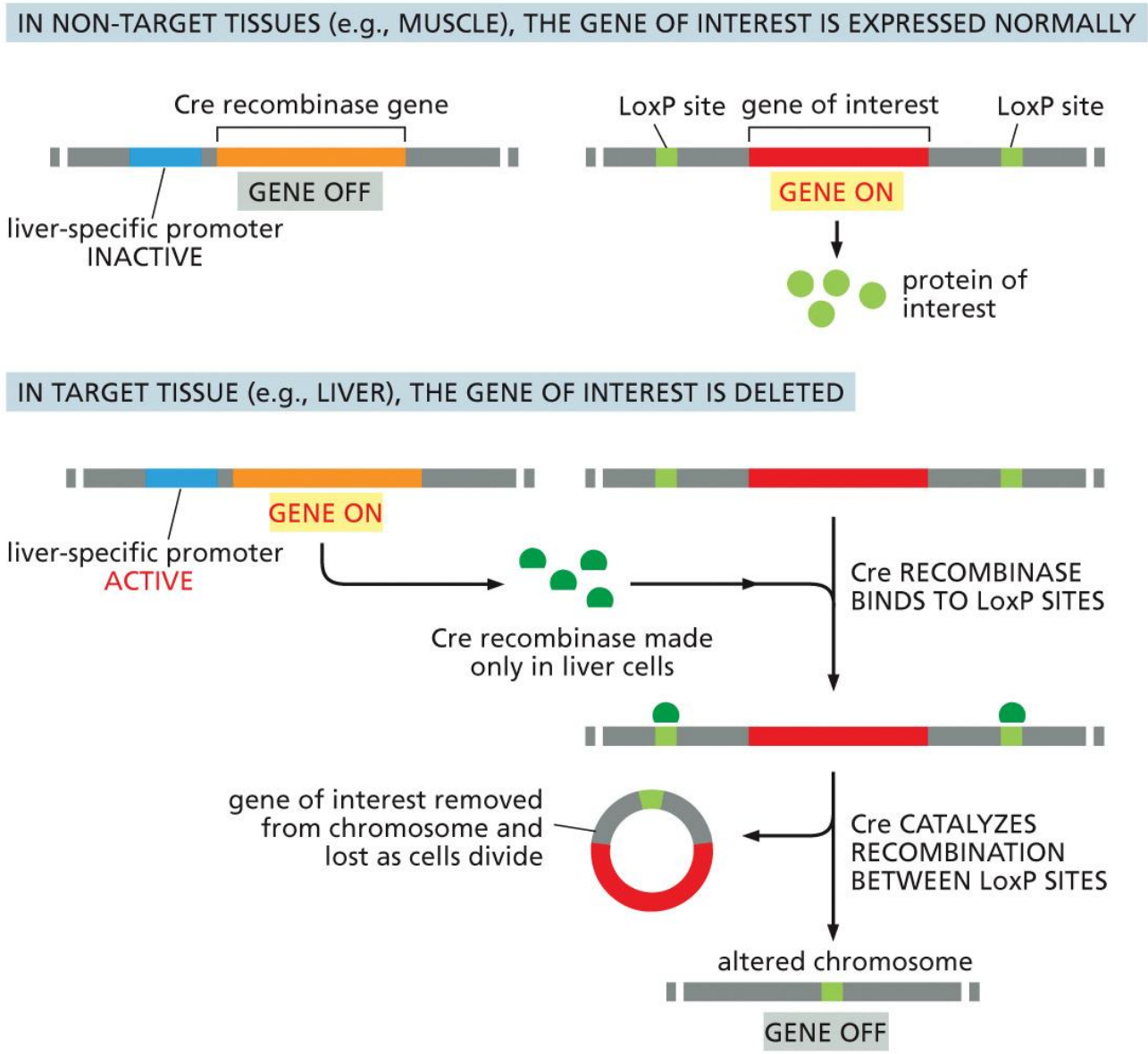


For more info about this technology

https://www.nature.com/scitable/topicpage/scientists-can-analyze-gene-function-by-deleting-6526138/

In conditional knockout mice the gene of interest can be disabled in specific tissues

- Transgenic mice are generated that have two transgenes:
  - One encodes a recombinase (Cre) under control of a tissue specific promoter
  - Other is the gene of interest flanked by nucleotide sequences (LoxP sites) that are recognized by Cre recombinase (similar to mobile genetic elements **Ch. 9**)
- In non-target tissues there is no recombinase, so the gene is expressed normally
- In target tissue the recombinase is produced and will bind to LoxP sites to catalyze excision of the gene, preventing expression



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Reflection/Feedback

Reminder that Ch. 7-10 Check Your Understanding quizzes due tomorrow 10/9 by midnight!

Group Activity #2 on Thursday

https://forms.gle/vbdZXee89Dh9diY78