

PCR and Sanger Sequencing

Quick overview of splicing

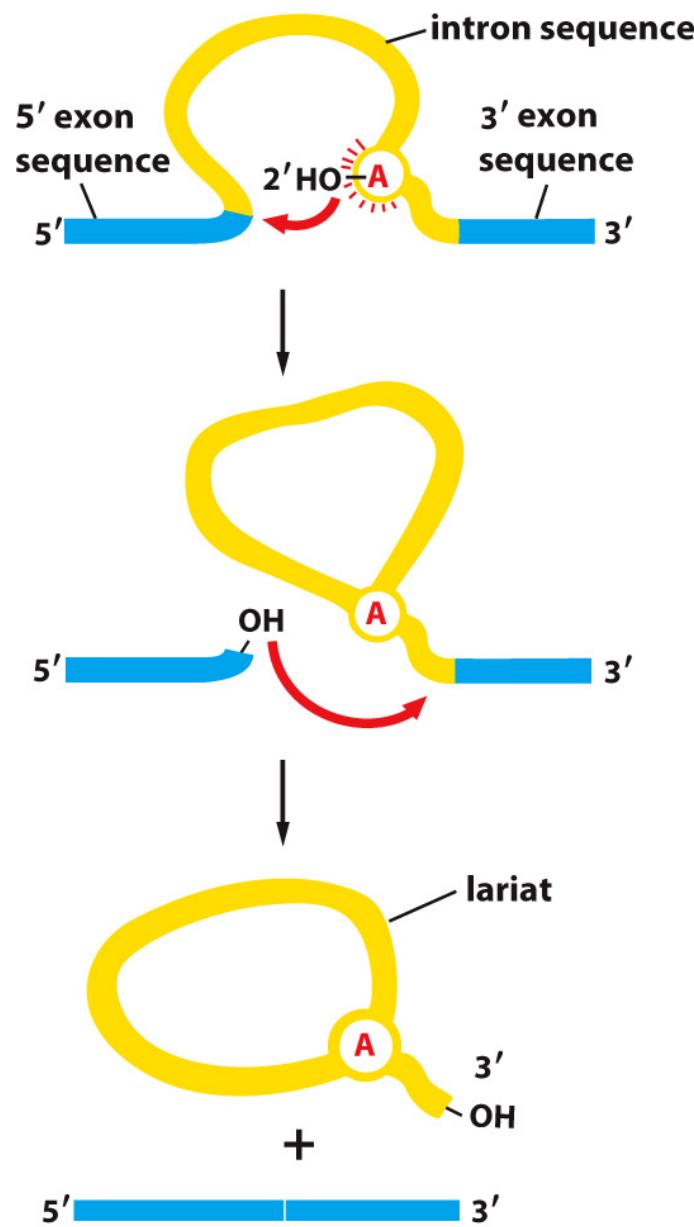


Figure 6-25a Molecular Biology of the Cell 6e (© Garland Science 2015)

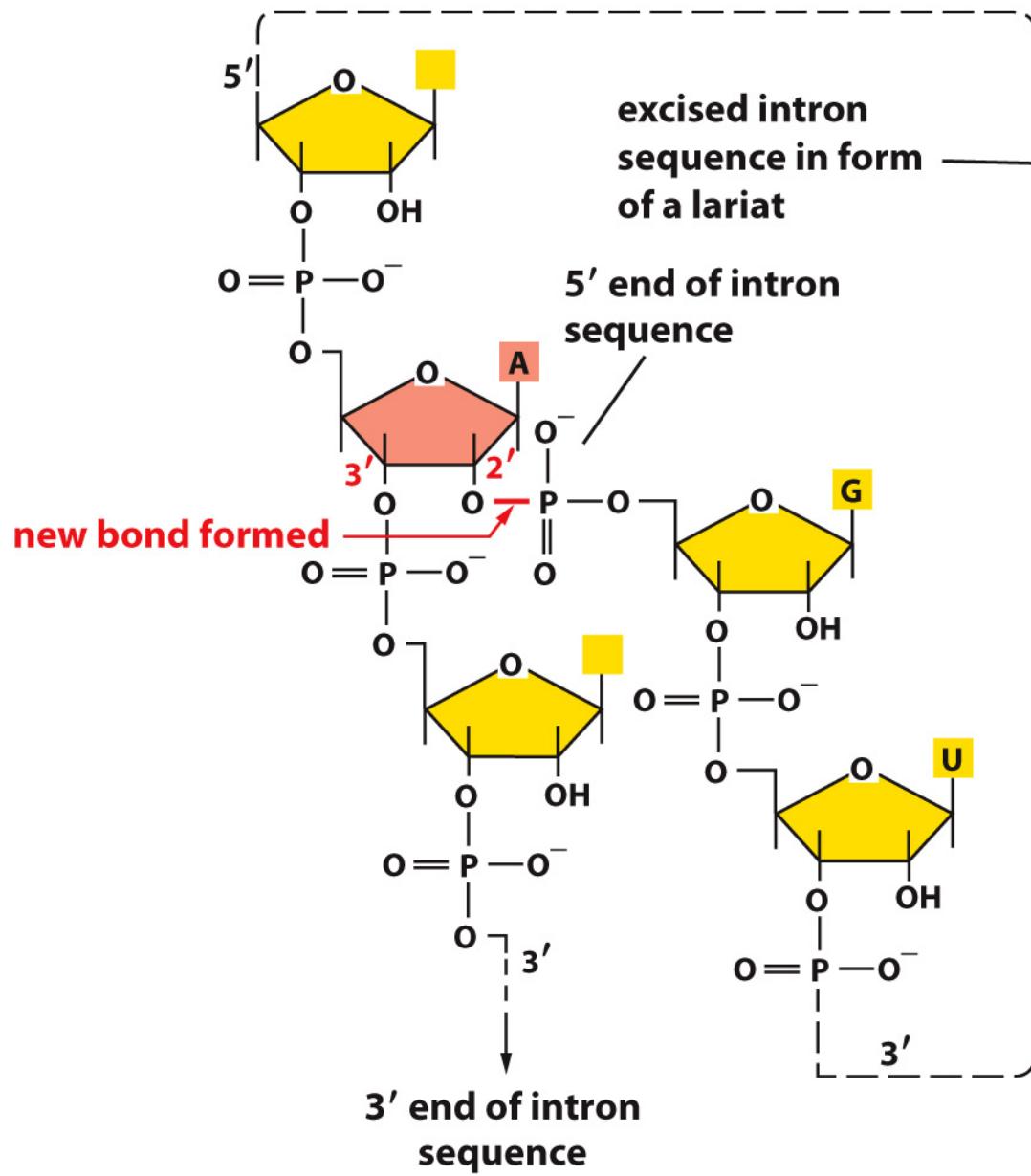


Figure 6-25b Molecular Biology of the Cell 6e (© Garland Science 2015)

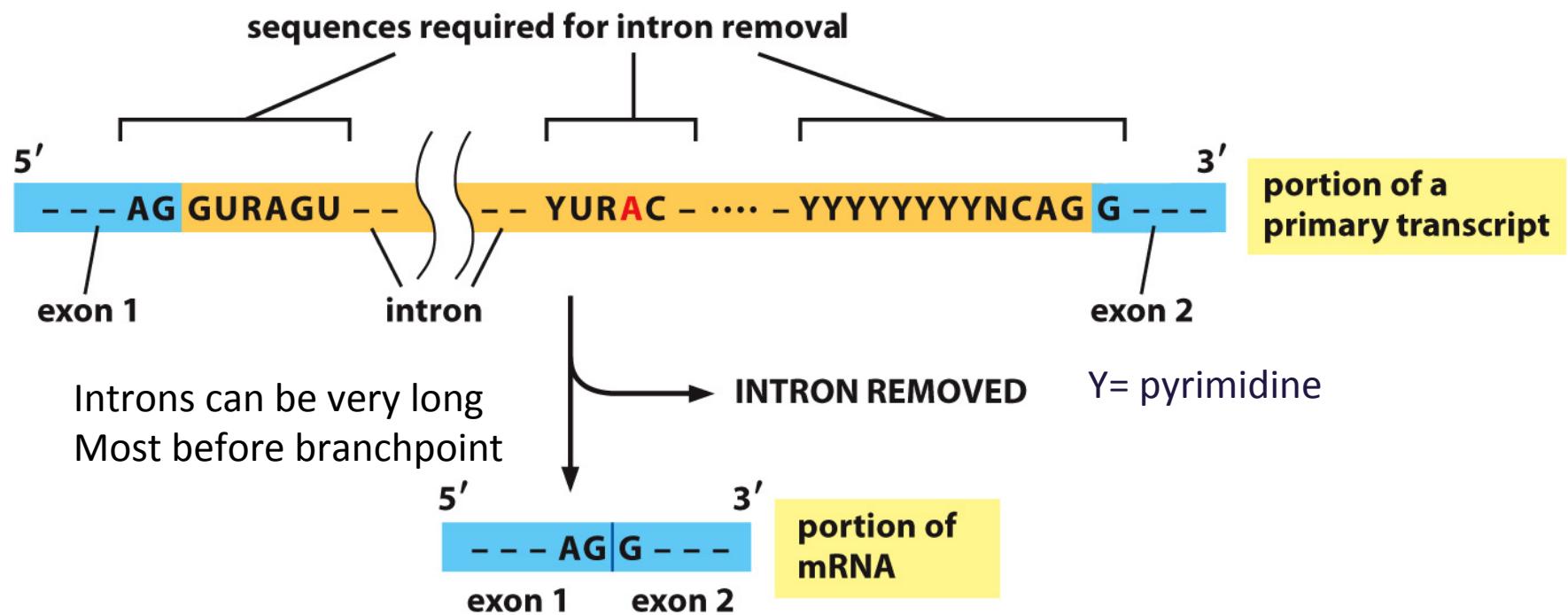
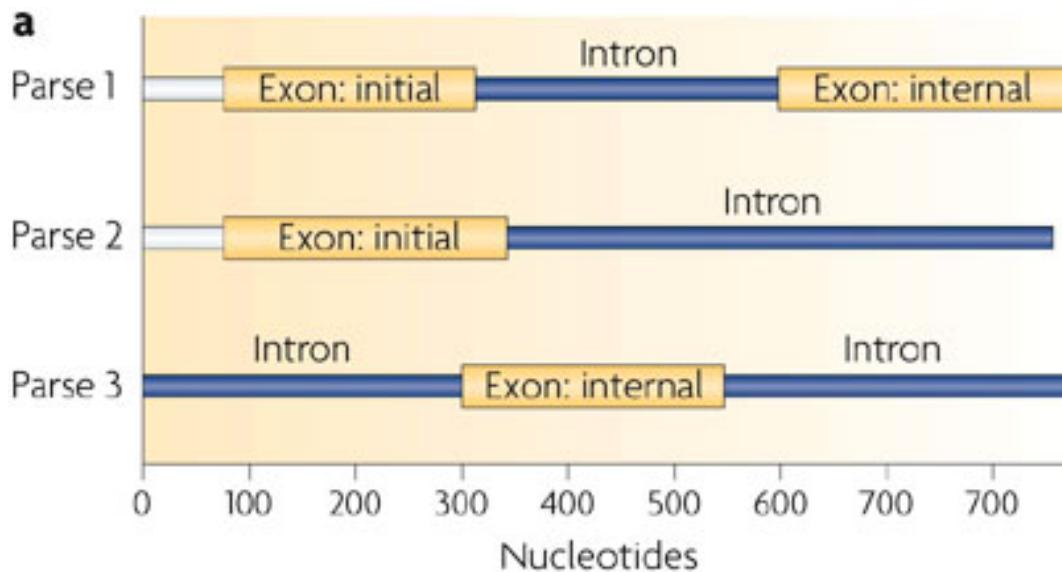


Figure 6-27 Molecular Biology of the Cell 6e (© Garland Science 2015)

Automatically start genome annotation

1. What structures can we find in genome?
2. How would you go about annotating genome in as automated a way as possible?
3. How would you confirm automated annotations?



Part of genome annotation



Graphical representation of a WMM model for intron starts

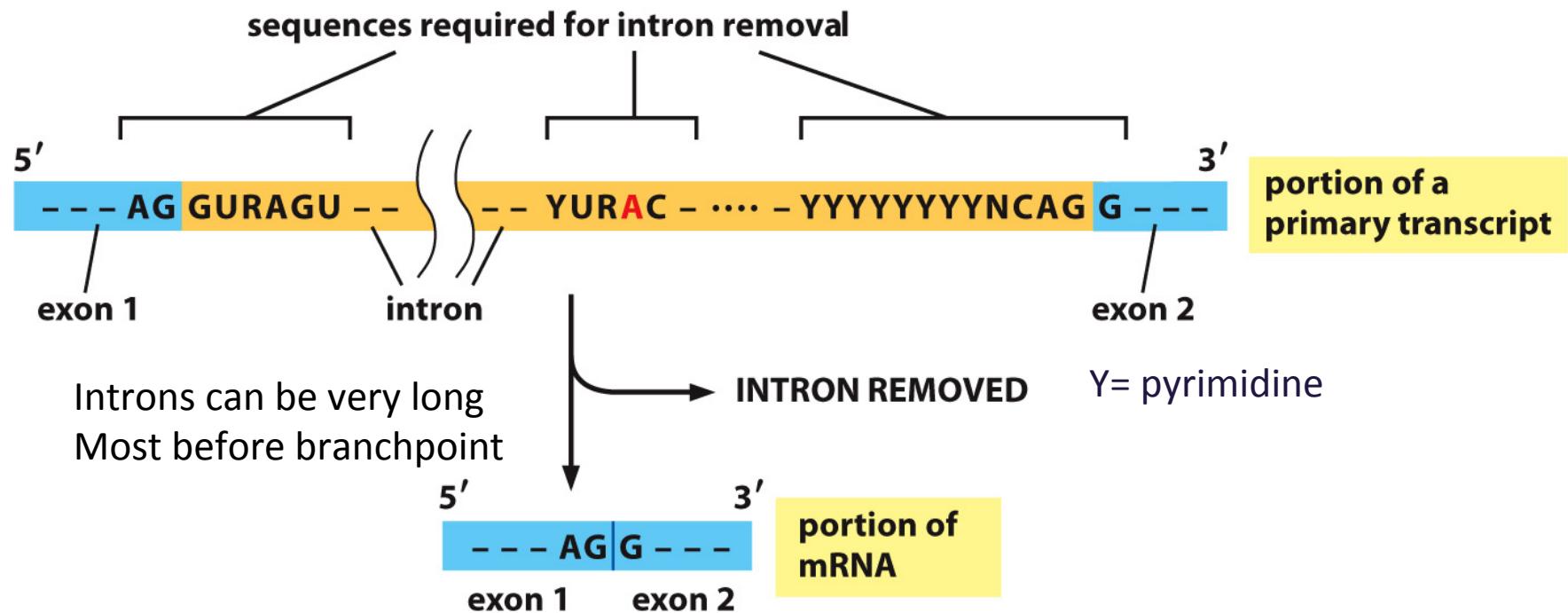
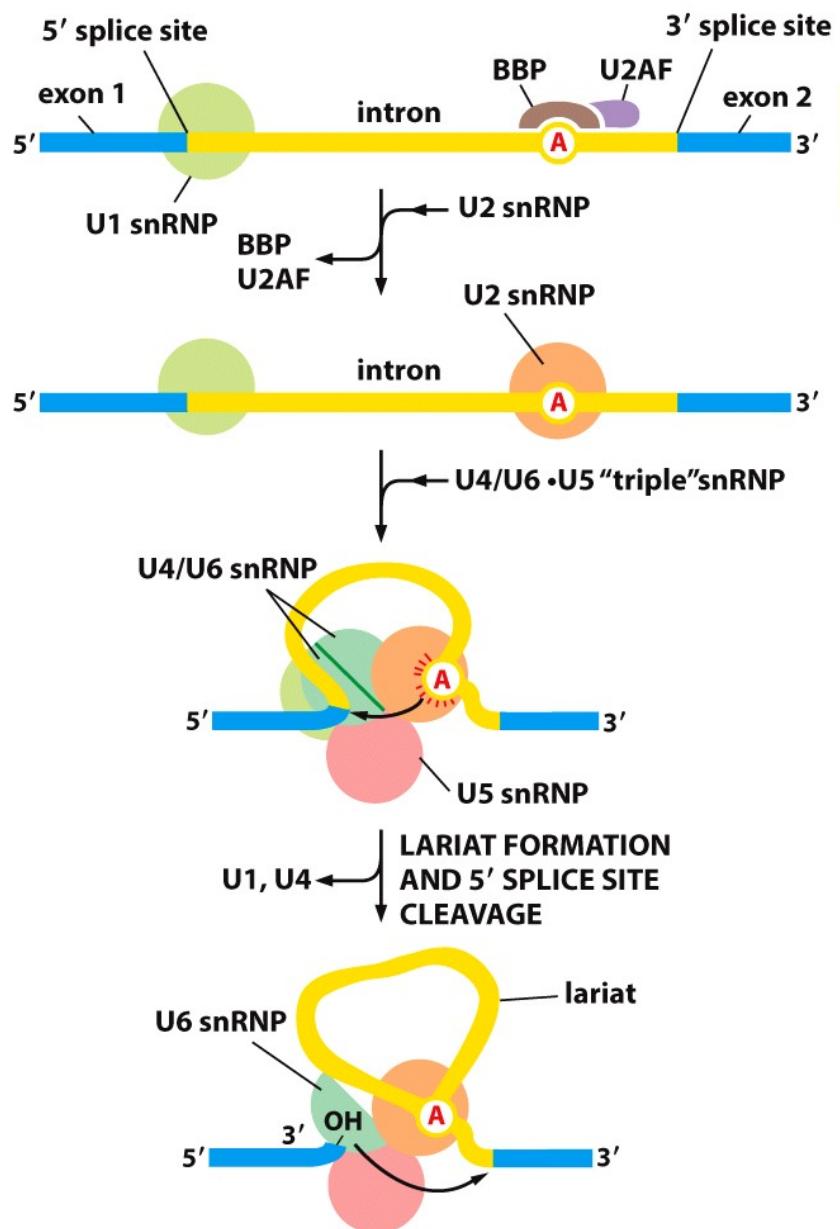


Figure 6-27 Molecular Biology of the Cell 6e (© Garland Science 2015)

Minimal requirements in exon, most signals in intron. Why?



Spliceosome
assembles to
catalyze reaction,
but reaction is
carried out by
RNA

Figure 6-28 (part 1 of 2) Molecular Biology of the Cell 6e (© Garland Science 2015)

Use of ATP to select splice sites helps to limit partial splicing and commits to a specific splice pattern

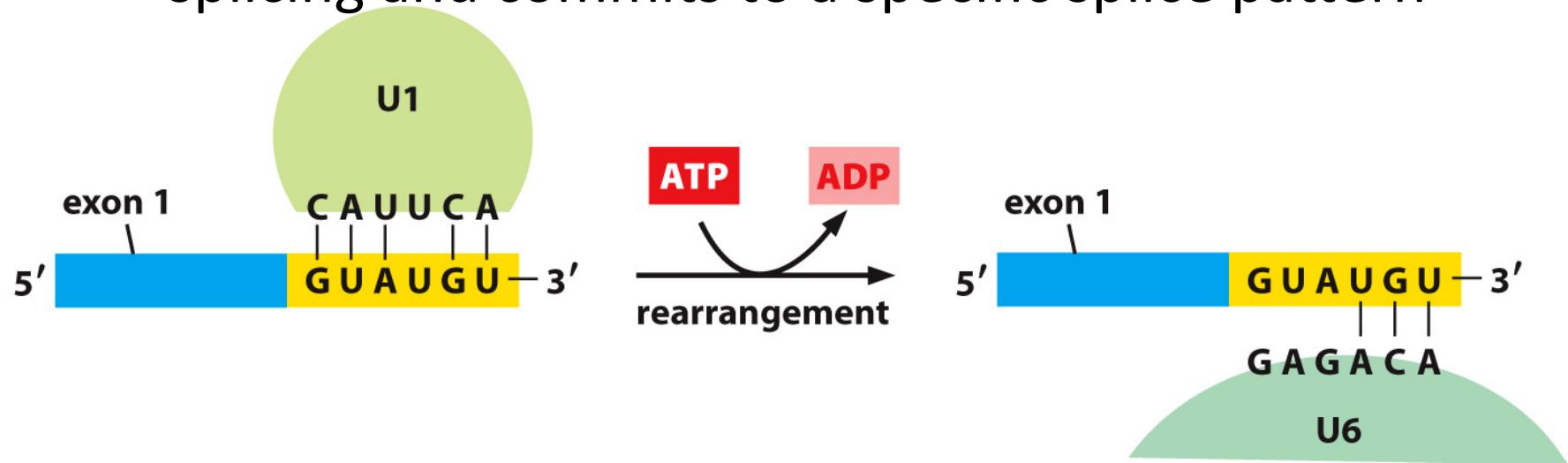


Figure 6-29 Molecular Biology of the Cell 6e (© Garland Science 2015)

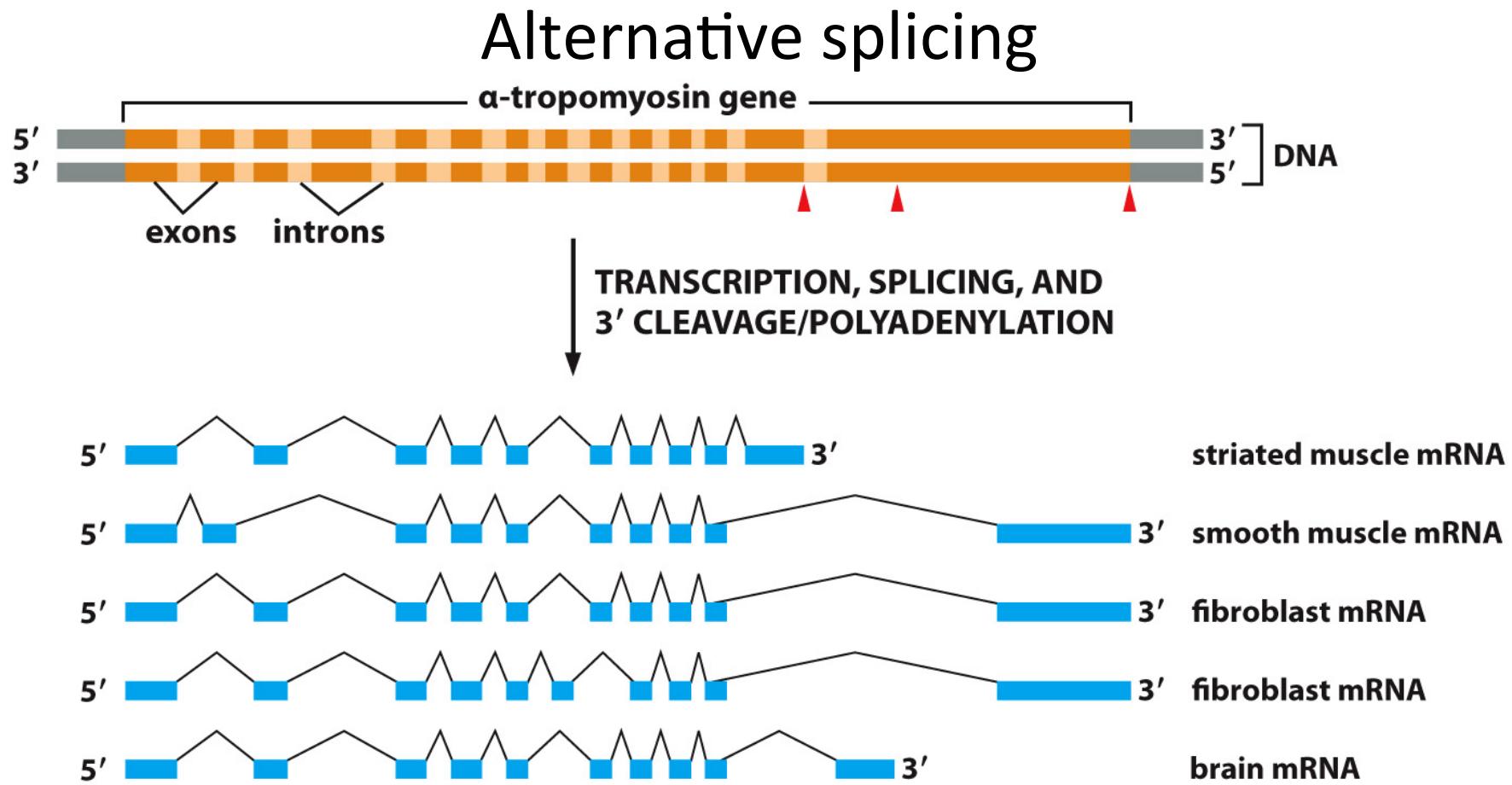


Figure 6-26 Molecular Biology of the Cell 6e (© Garland Science 2015)

What does alternative splicing allow eukaryotes to do?

What is alternative and what is a mistake depends on context

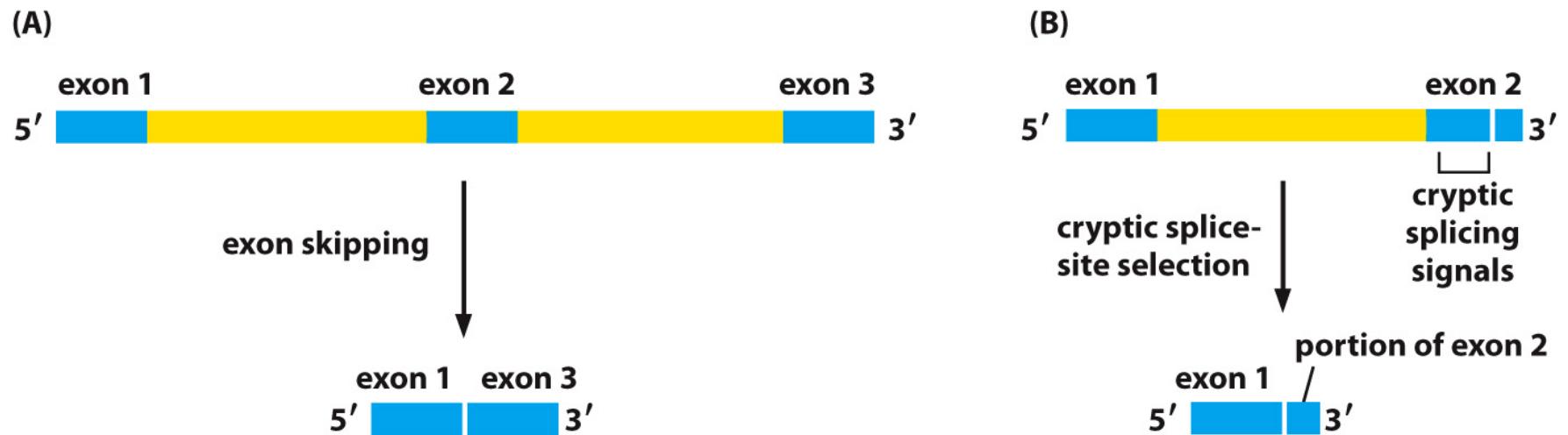
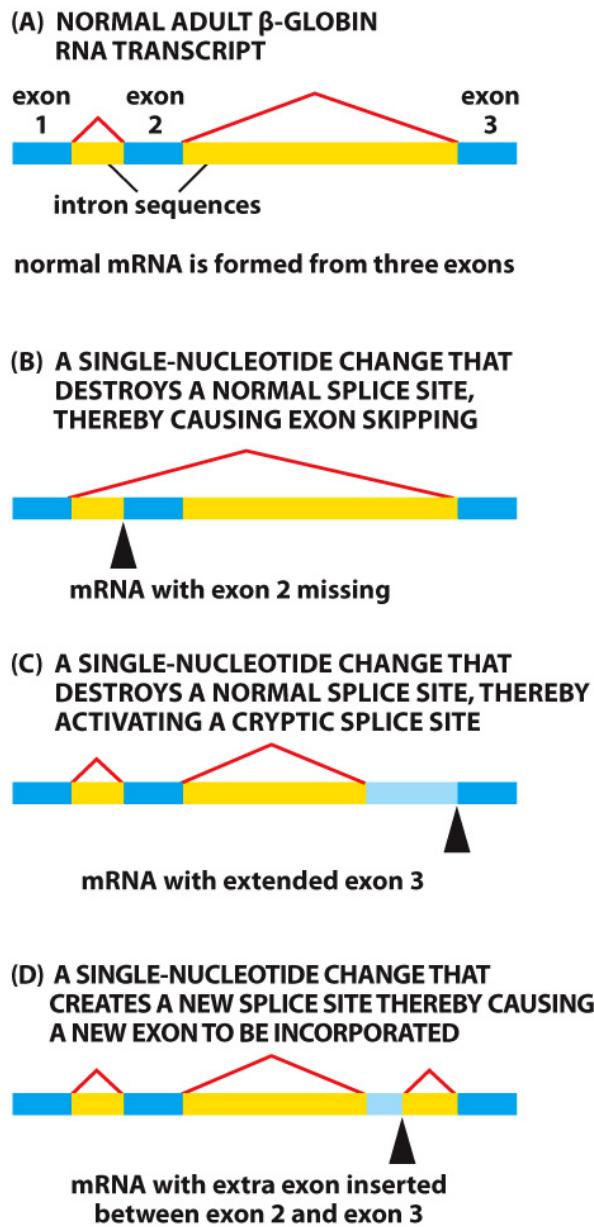


Figure 6-30 Molecular Biology of the Cell 6e (© Garland Science 2015)

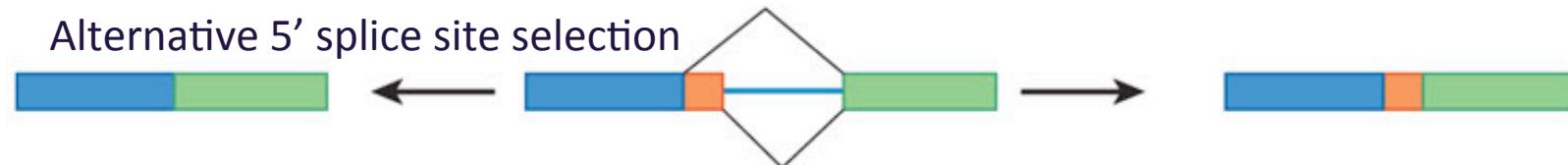
What kinds of mutations will affect splicing?



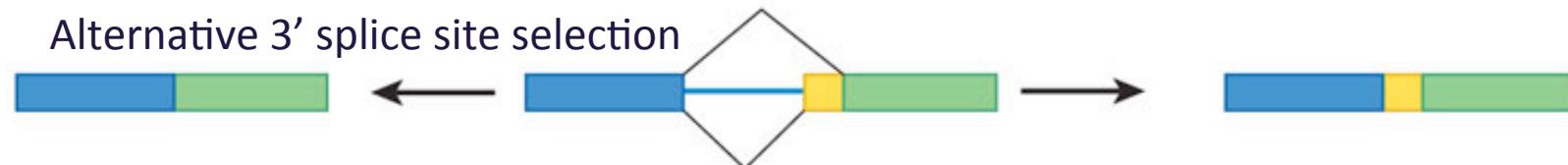
Evolution of splicing of a gene by small mutations – can evolve a protein quickly

Figure 6-33 Molecular Biology of the Cell 6e (© Garland Science 2015)

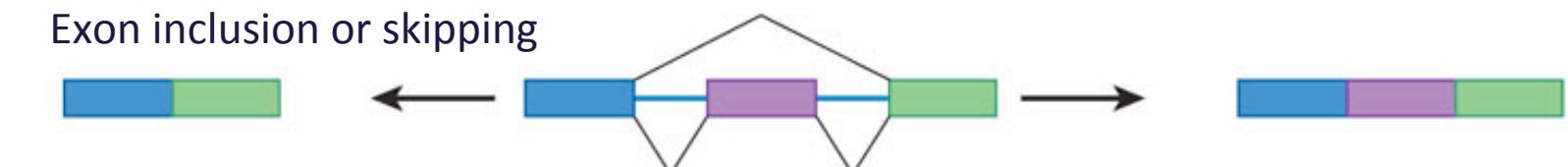
a Alternative 5' splice site selection



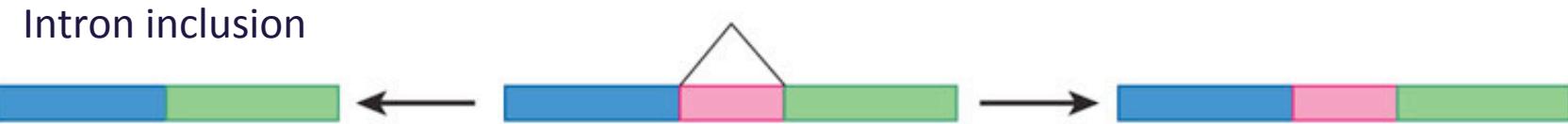
b Alternative 3' splice site selection



c Exon inclusion or skipping



d Intron inclusion



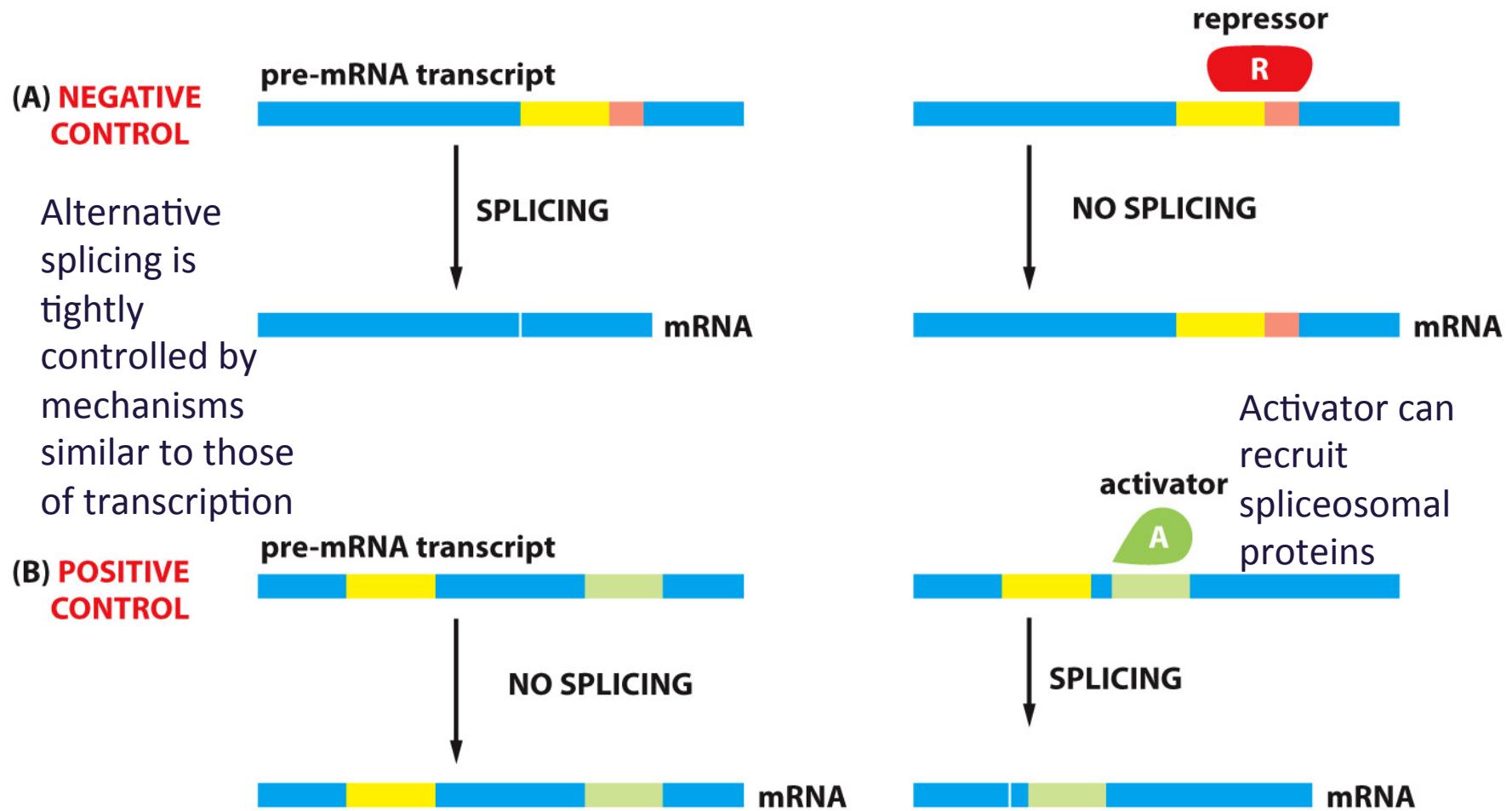


Figure 7-58 Molecular Biology of the Cell 6e (© Garland Science 2015)

Does this remind you of anything?

Which parts of this regulator system are cis-acting factors? Which trans-acting factors?

Nonsense Mediated Decay

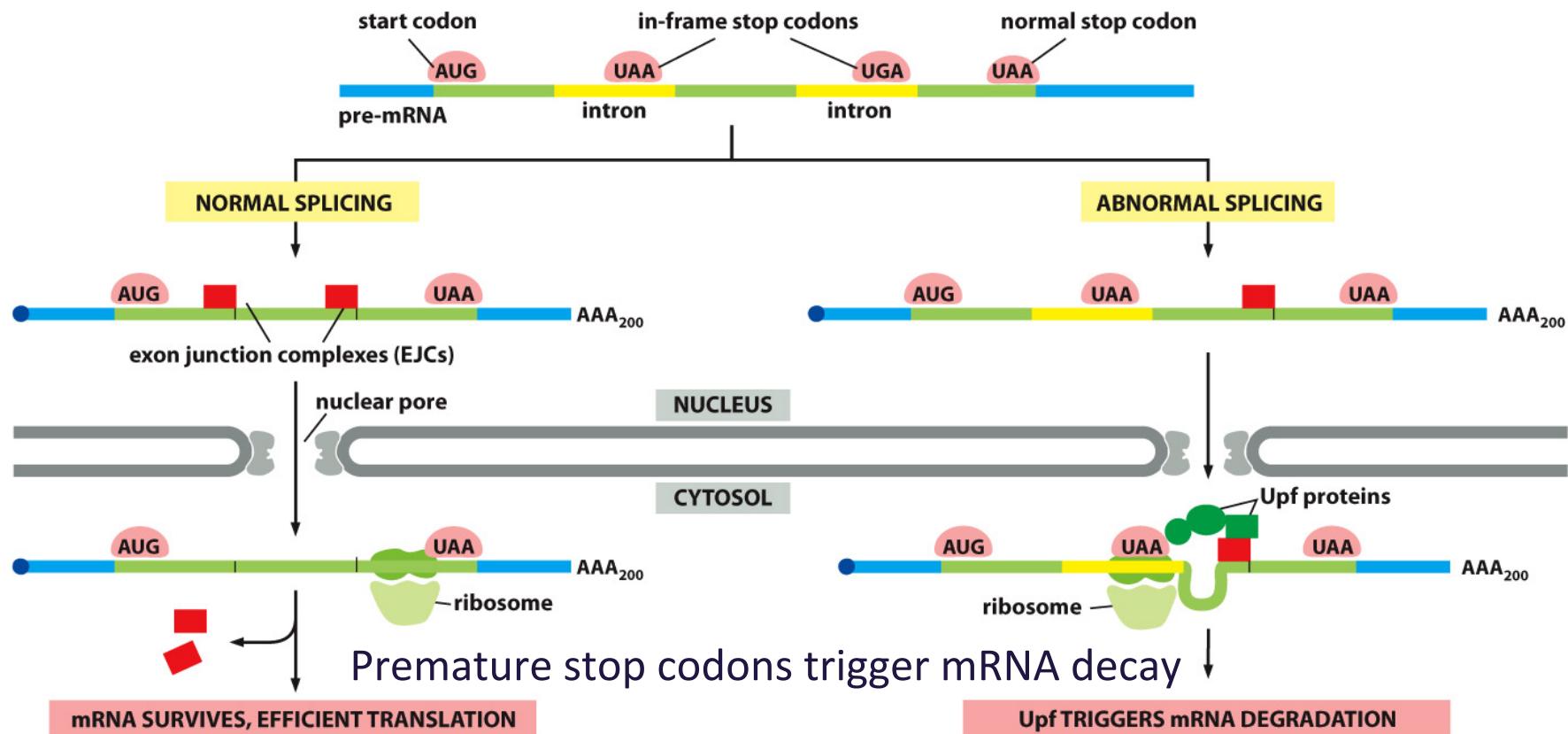
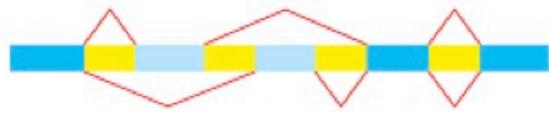
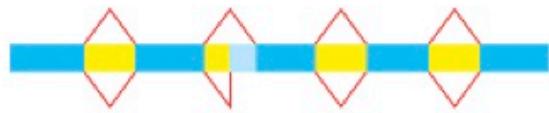
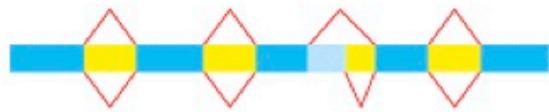
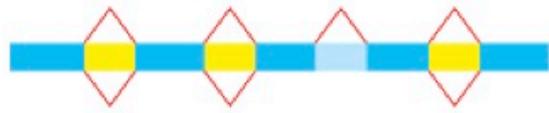
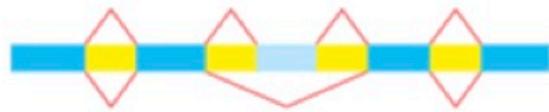
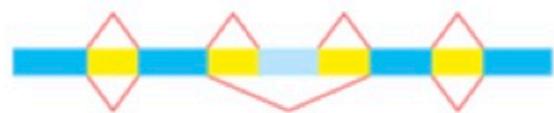


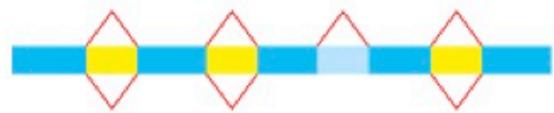
Figure 6-76 Molecular Biology of the Cell 6e (© Garland Science 2015)

Splicing exercise

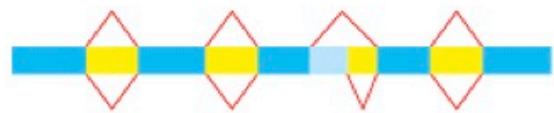




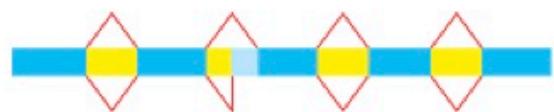
Exon skipping



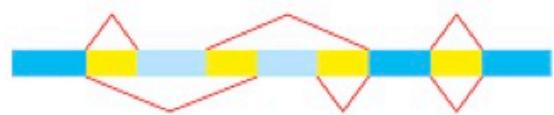
Intron inclusion



Alternative 3' splice site



Alternative 5' splice site



Cassette exon/mutually exclusive exons

Questions?

PCR

- Polymerase Chain Reaction
- Invented in 1983
- DNA polymerase from
Thermus aquaticus
 - 2.2×10^5 error rate



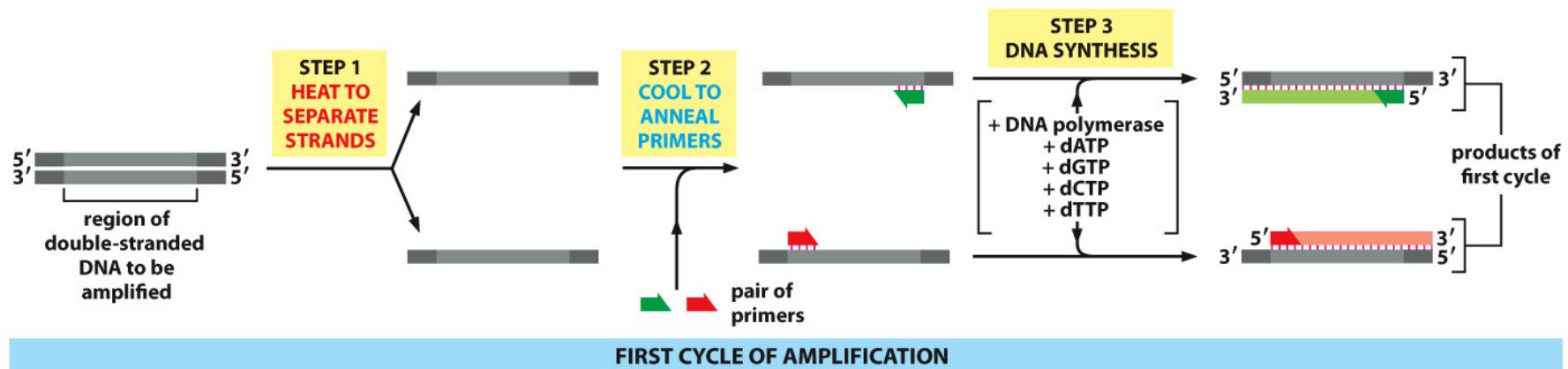


Figure 8-35 Molecular Biology of the Cell 6e (© Garland Science 2015)

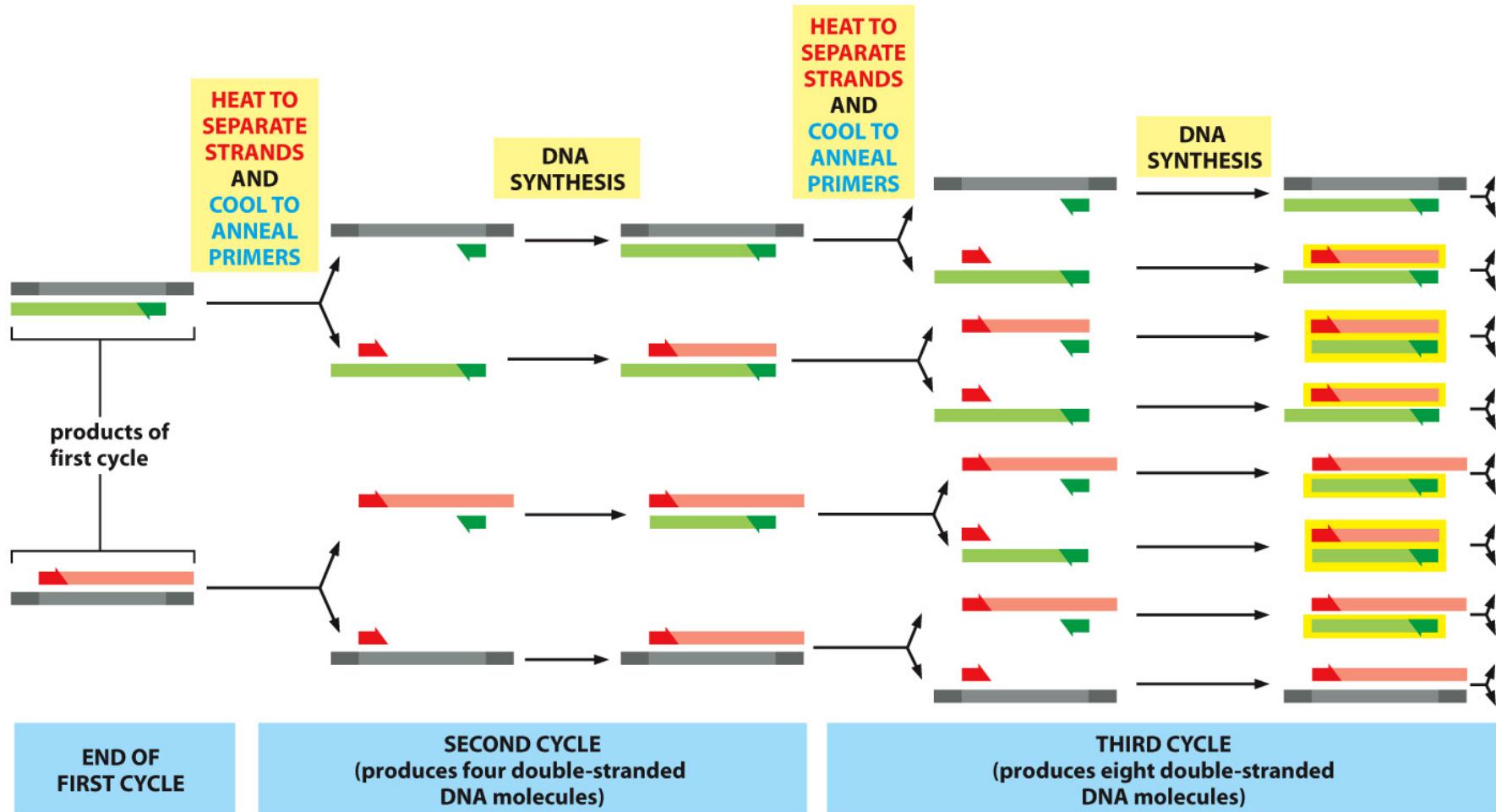


Figure 8-36 Molecular Biology of the Cell 6e (© Garland Science 2015)

What limits PCR?

What limits PCR?

Rate limiting

- Enzymes – need one enzyme per replicating DNA

Number limiting

- Primers – when you run out of primers can't make more DNAs

Let's perform paper PCR

5' ATGCGGTCA~~G~~TATGGGCTCAGTCGTTACGGGACATTGACAGTCAGCTCAAACGATCGC 3'
3' TACGCCAGTCATA~~CC~~GTGT~~C~~A~~G~~CAAATGCCCTGTAACTGT~~C~~AGTCGAAG~~TTT~~GCTAGCG 5'

Primers:

5' ATGCGTCAGT 3' Might add an extra base to each –
5' GCGATCGTT 3' usually want primers to end in G/C

5' **ATGCGGTCA~~G~~T**ATGGGCTCAGTCGTTACGGGACATTGACAGTCAGCTCAAACGATCGC 3'
3' TACGCCAGTCATA~~CC~~GTGT~~C~~A~~G~~CAAATGCCCTGTAACTGT~~C~~AGTCGAAG~~TTT~~GCTAGCG 5'
5' ATGCGGTCA~~G~~TATGGGCTCAGTCGTTACGGGACATTGACAGTCAGCTCAAACGATCGC 3'
3' TACGCCAGTCATA~~CC~~GTGT~~C~~A~~G~~CAAATGCCCTGTAACTGT~~C~~AGTCGAAG**TTT**GCTAGCG 5'

5' ATGCGGTCA GTATGGGCTCAGTCGTTACGGGACATTGACAGTCAGCTCAAACGATCGC 3'
3' TACGCCAGTCATAACCCGTGTCAGCAAATGCCCTGTAACTGTCAGTCGAAGTTGCTAGCG 5'
5' ATGCGGTCA GTATGGGCTCAGTCGTTACGGGACATTGACAGTCAGCTCAAACGATCGC 3'
3' TACGCCAGTCATAACCCGTGTCAGCAAATGCCCTGTAACTGTCAGTCGAAGTTGCTAGCG 5'
5' ATGCGGTCA GTATGGGCTCAGTCGTTACGGGACATTGACAGTCAGCTCAAACGATCGC 3'
3' TACGCCAGTCATAACCCGTGTCAGCAAATGCCCTGTAACTGTCAGTCGAAGTTGCTAGCG 5'
5' ATGCGGTCA GTATGGGCTCAGTCGTTACGGGACATTGACAGTCAGCTCAAACGATCGC 3'
3' TACGCCAGTCATAACCCGTGTCAGCAAATGCCCTGTAACTGTCAGTCGAAGTTGCTAGCG 5'

$$2^{10} = 1024$$

Polymerase Chain Reaction (PCR) overview

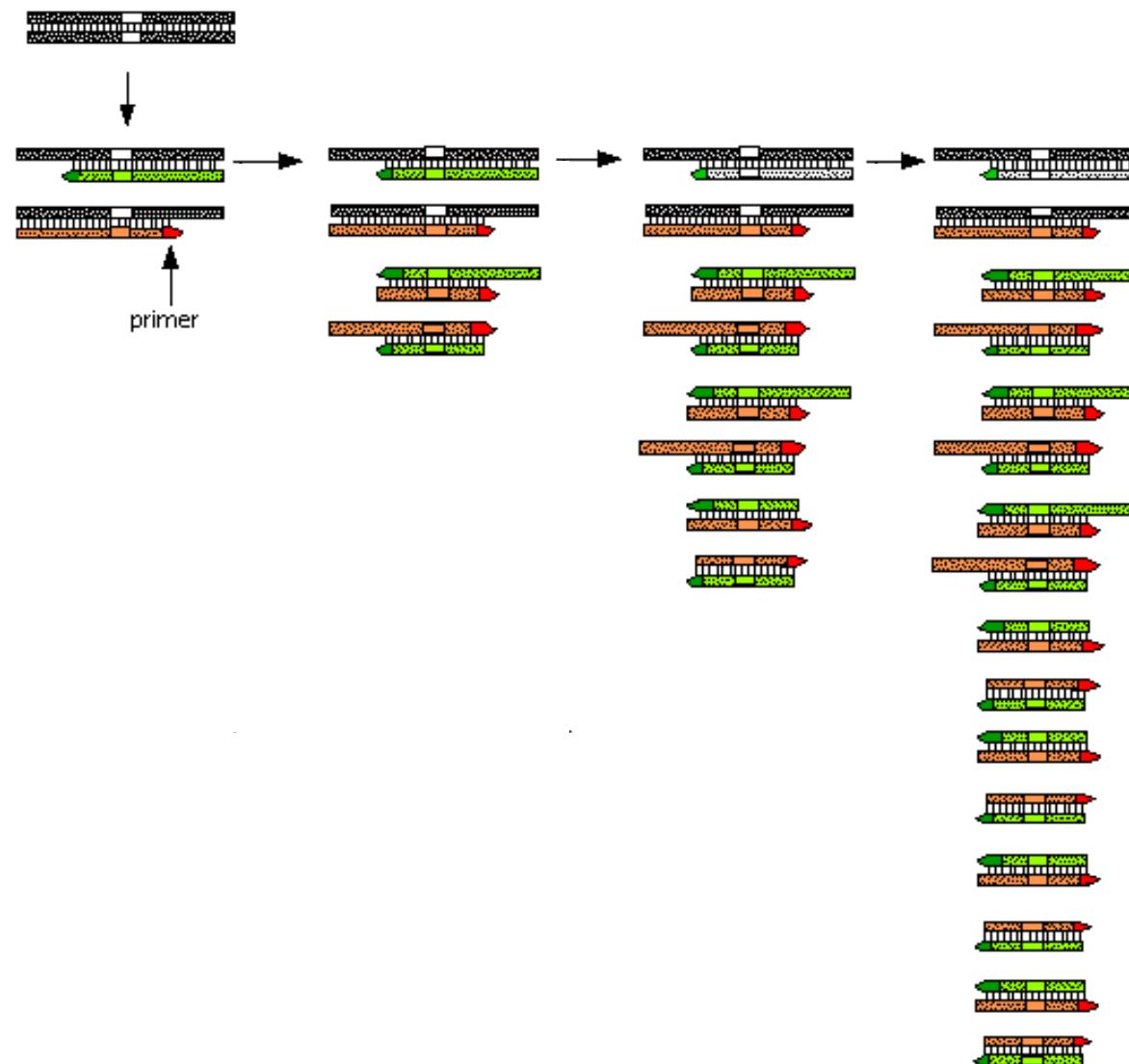
Starting DNA



Final DNA



Polymerase Chain Reaction (PCR) overview



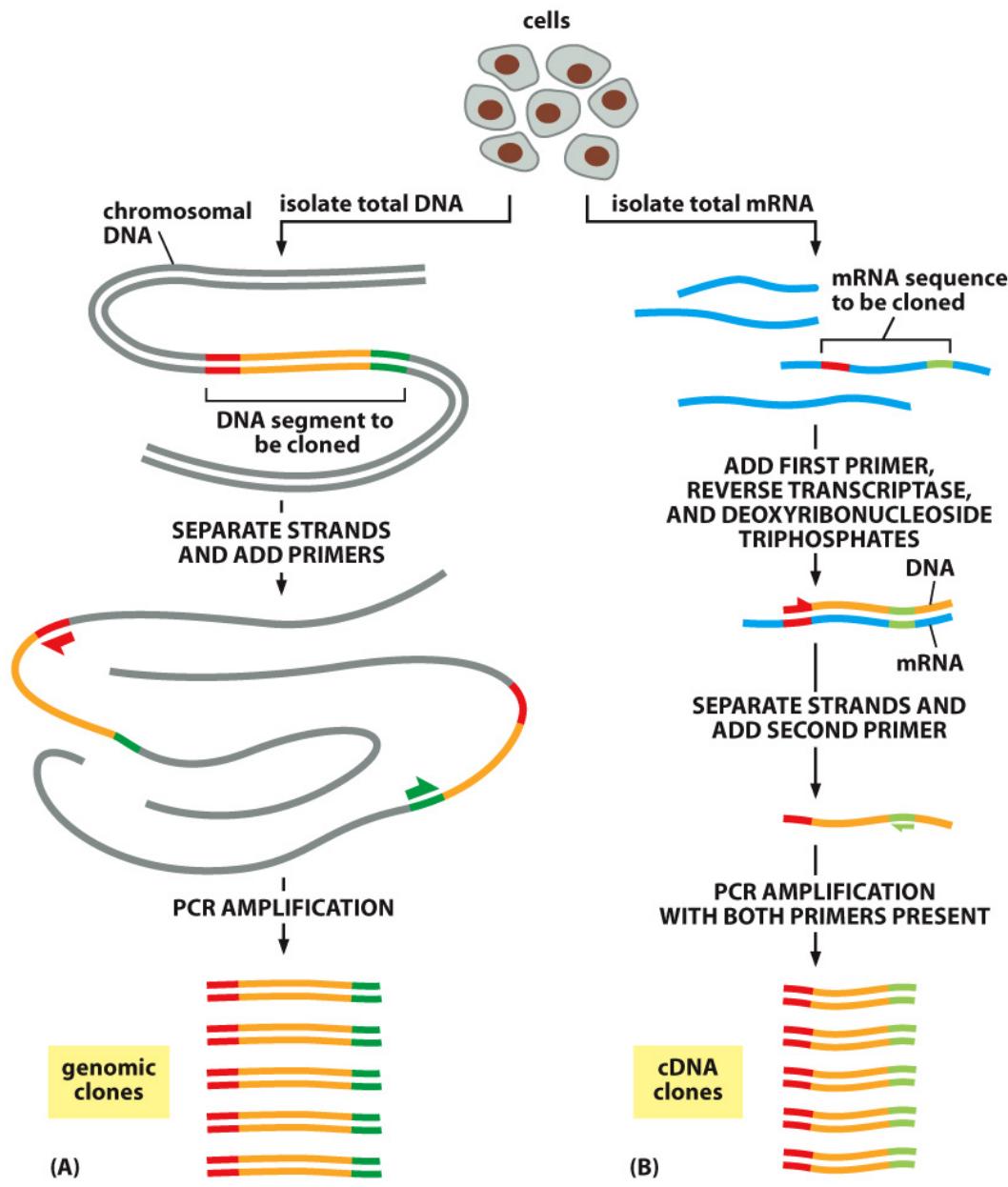
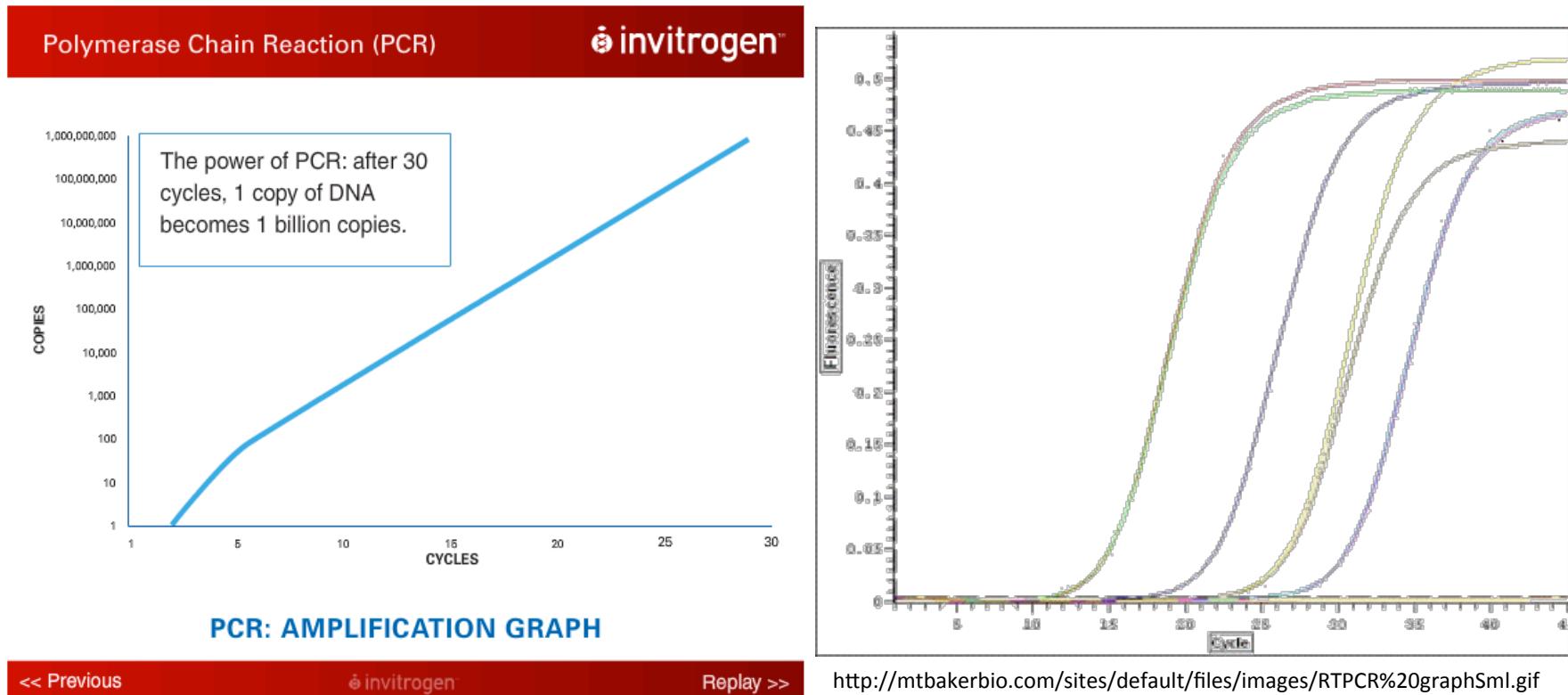


Figure 8-37 Molecular Biology of the Cell 6e (© Garland Science 2015)

PCR over time



Example use of PCR

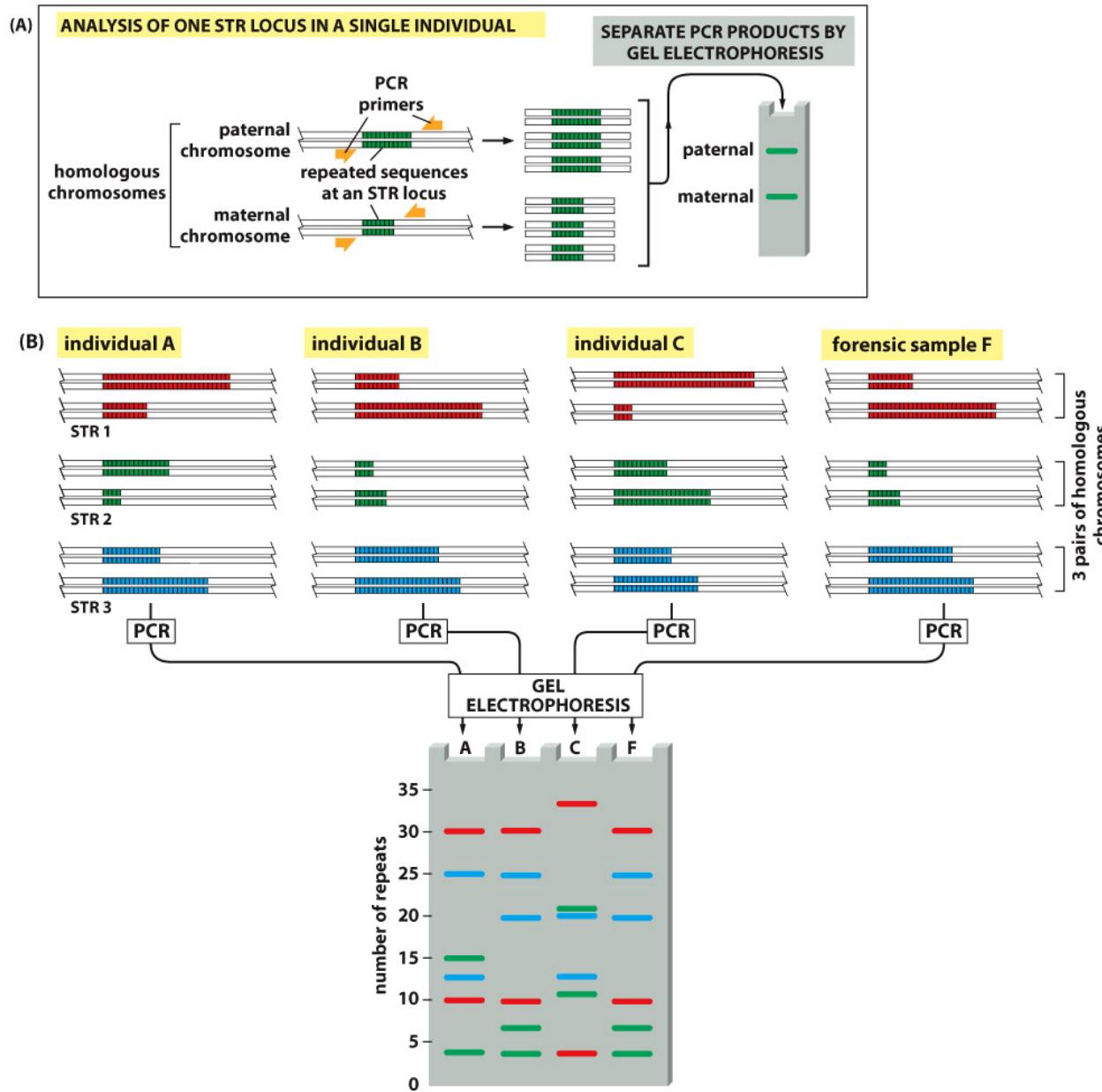


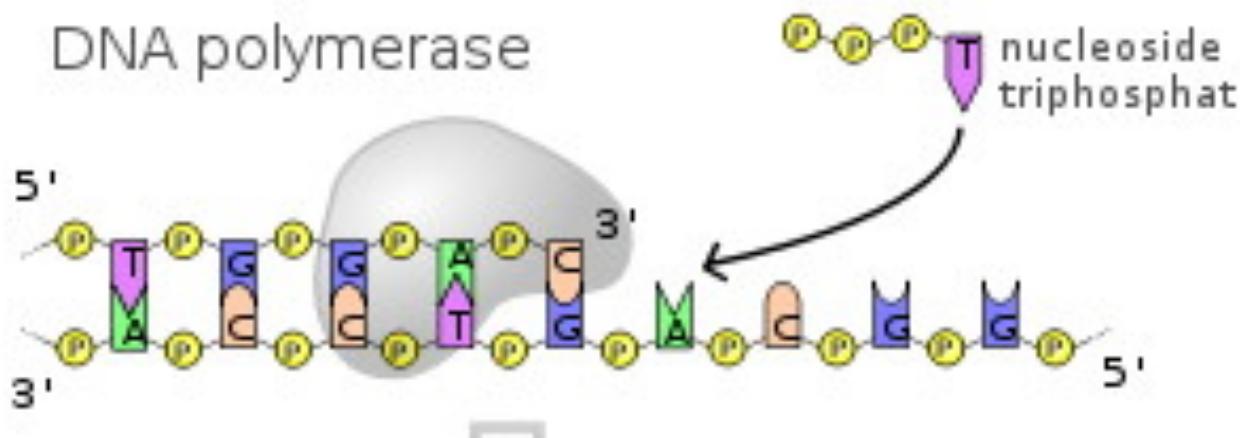
Figure 8-39 Molecular Biology of the Cell 6e (© Garland Science 2015)

Questions?

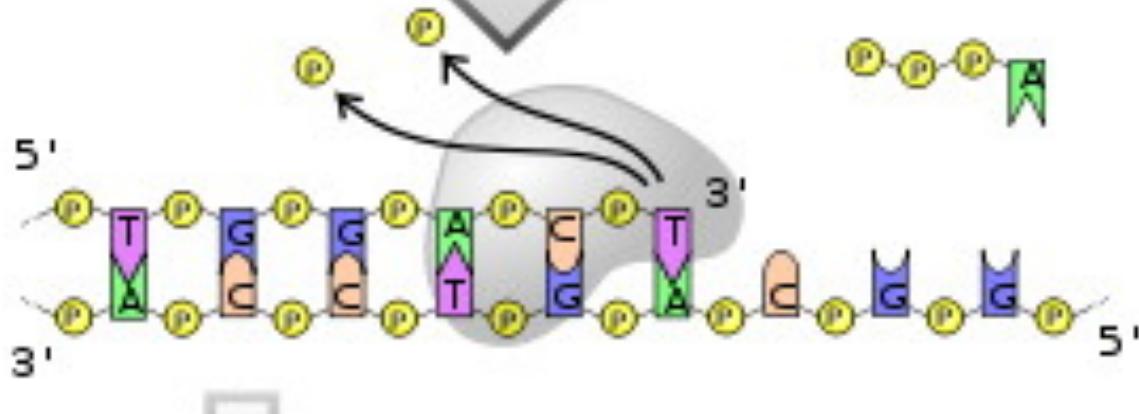
Sanger Sequencing

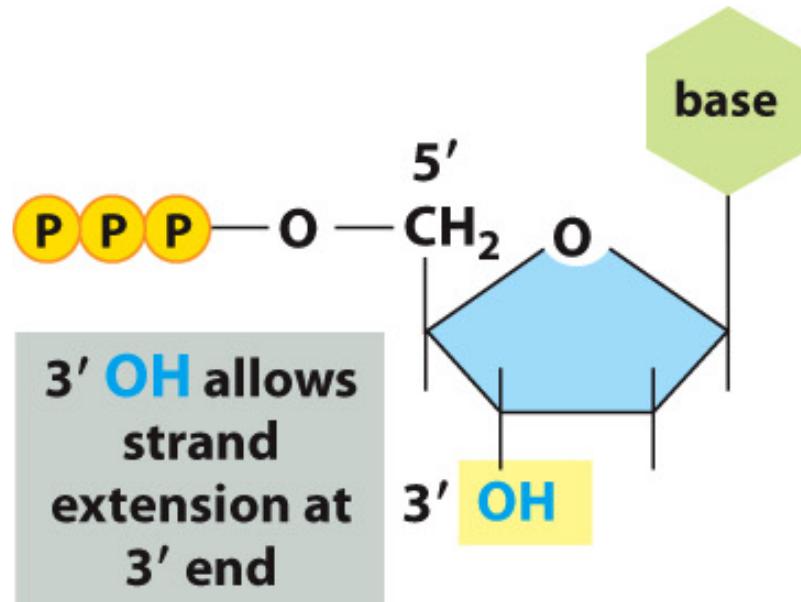
Sequencing by Synthesis

DNA polymerase

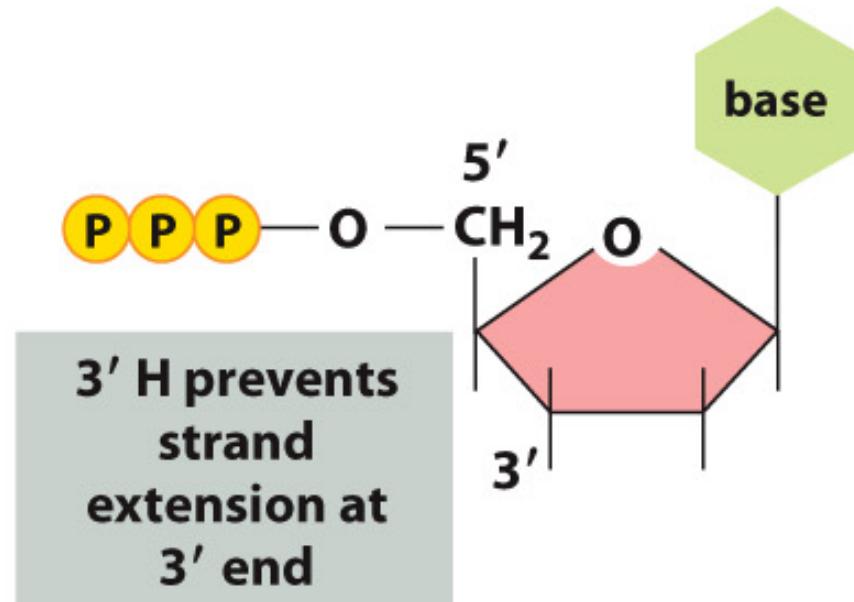


Extension



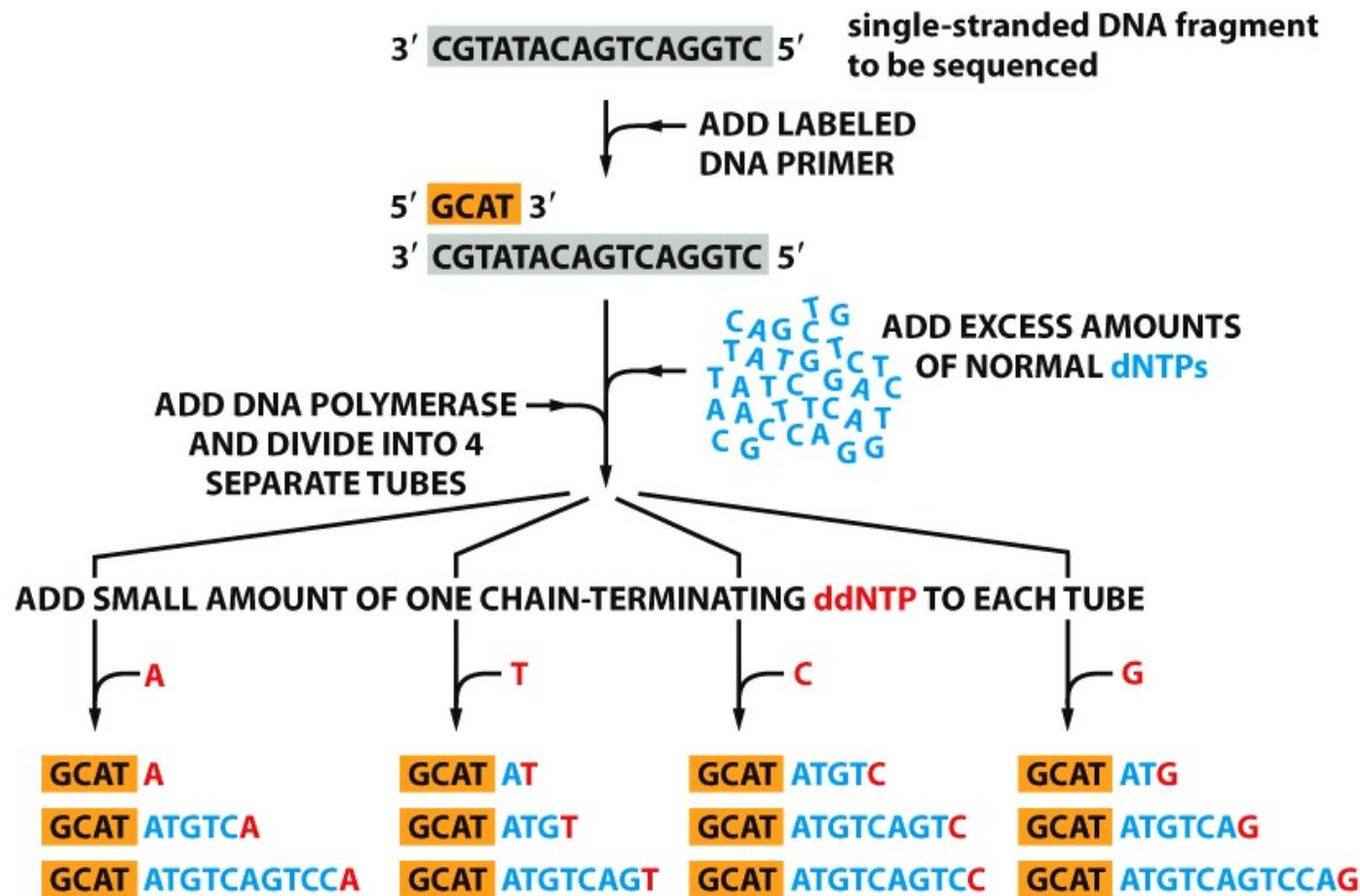


normal deoxyribonucleoside triphosphate (dNTP)



chain-terminating dideoxyribonucleoside triphosphate (ddNTP)

Following growing DNA strand with ddNTPs



Paper Sequencing

Paper sequencing

3' ATGCGGTCA~~G~~TATGGGCTCAG 5'
5' *TAC 3'

ddATP	ddTTP	ddCTP	ddGTP
*TACGCCA	*TACGCCAGT	*TACGC	*TACG
*TACGCCAGTCA	*TACGCCAGT	*TACGCC	*TACGCCAG
*TACGCCAGTCA	*TACGCCAGTCA	*TACGCCAGTC	*TACGCCAGTCA
TA	T	*TACGCCAGTCA	TACCCG
*TACGCCAGTCA	*TACGCCAGTCA	TAC	*TACGCCAGTCA
TACCCGA	TACCCGAGT	*TACGCCAGTCA	TACCCGAG
		TACC	*TACGCCAGTCA
		*TACGCCAGTCA	TACCCGAG
		TACCC	
		*TACGCCAGTCA	
		TACCCGAGTC	

Now that we have all these strands of DNA whose final base we know, what do we do with them?

Gel Electrophoresis



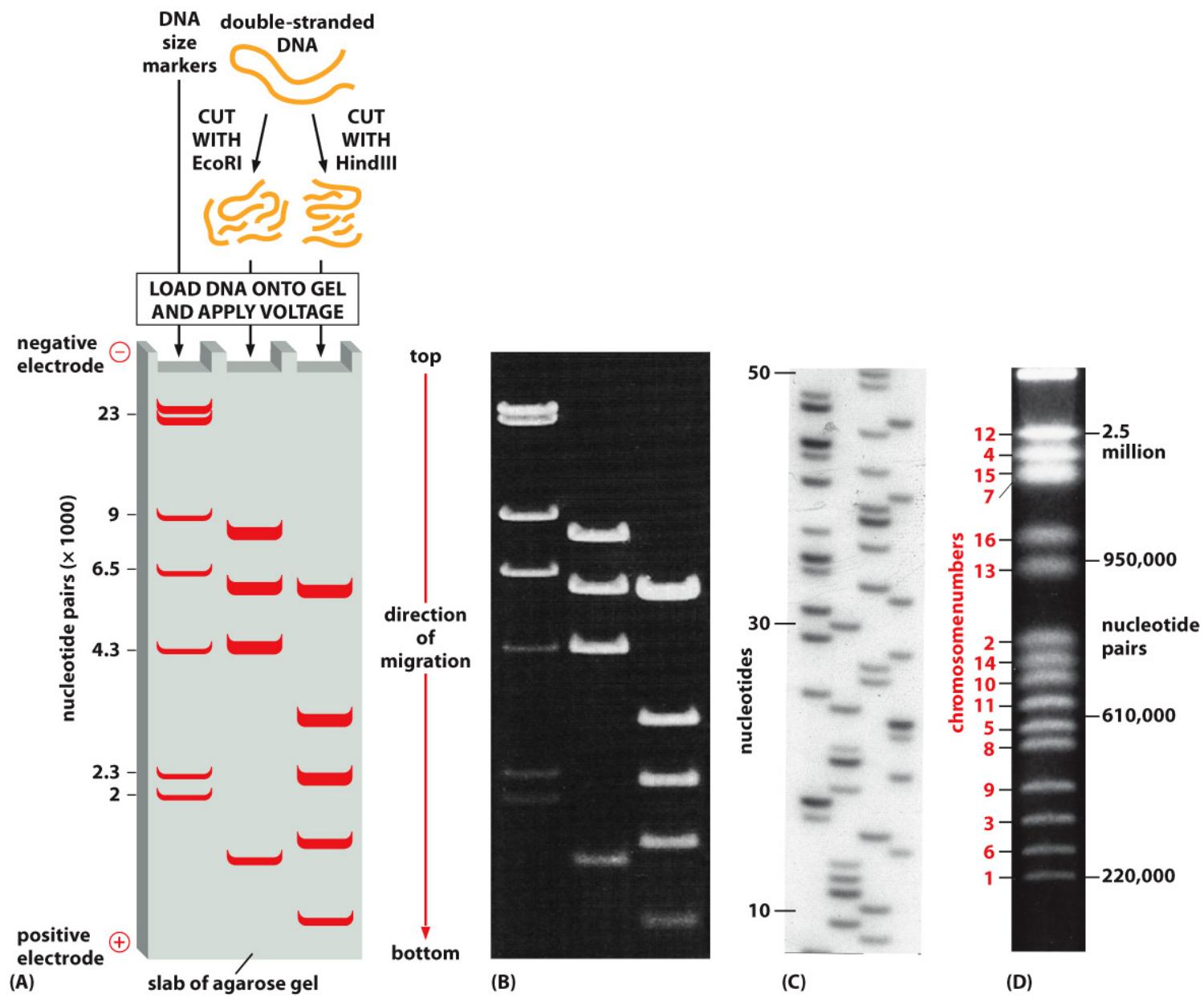
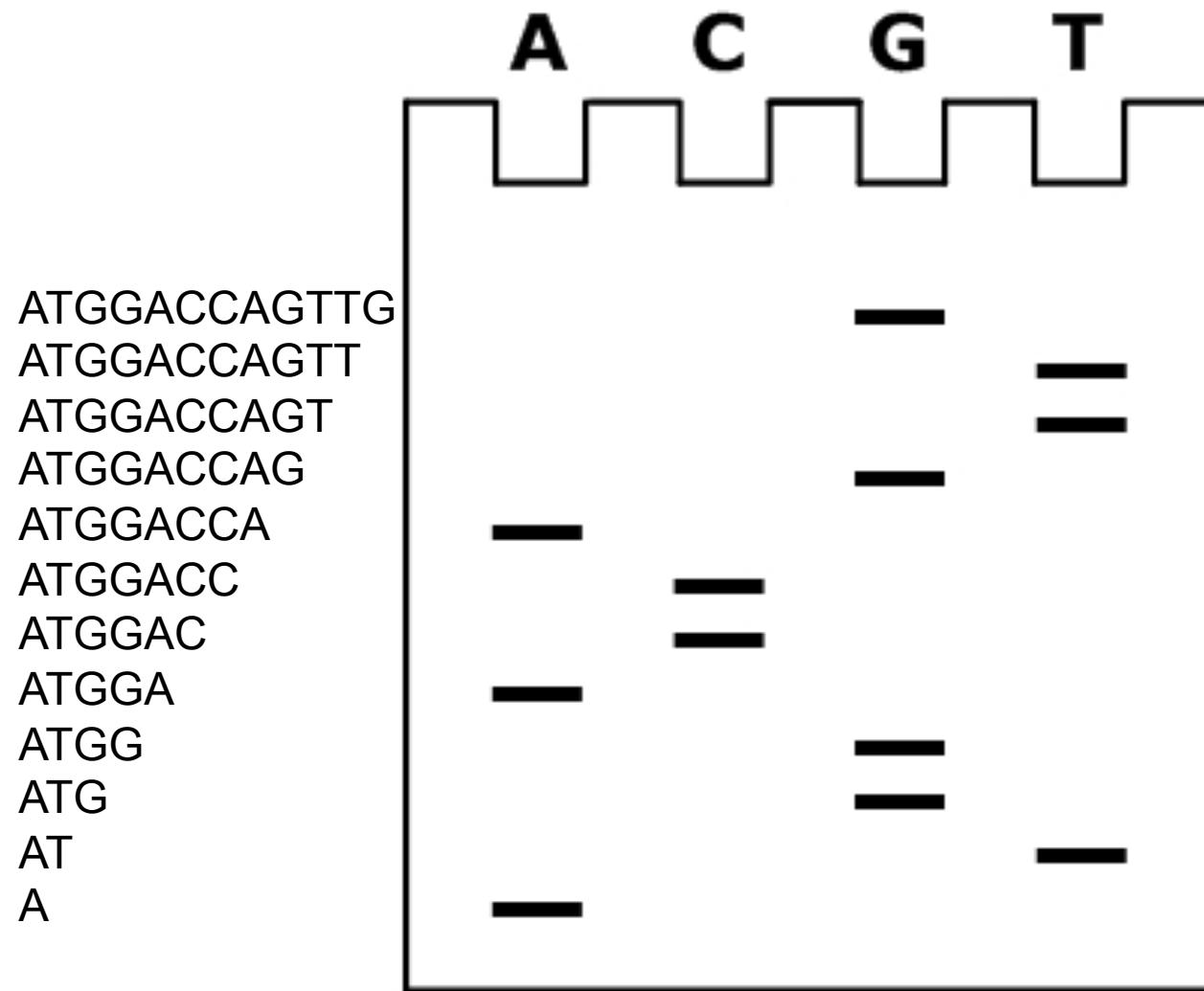
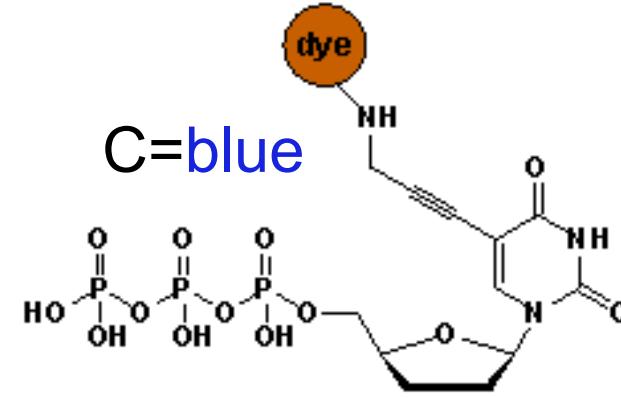
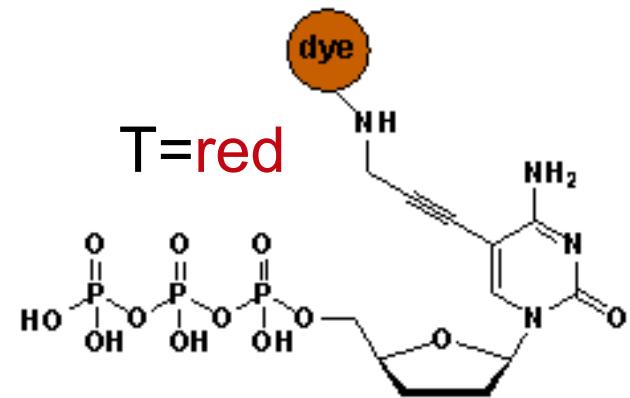
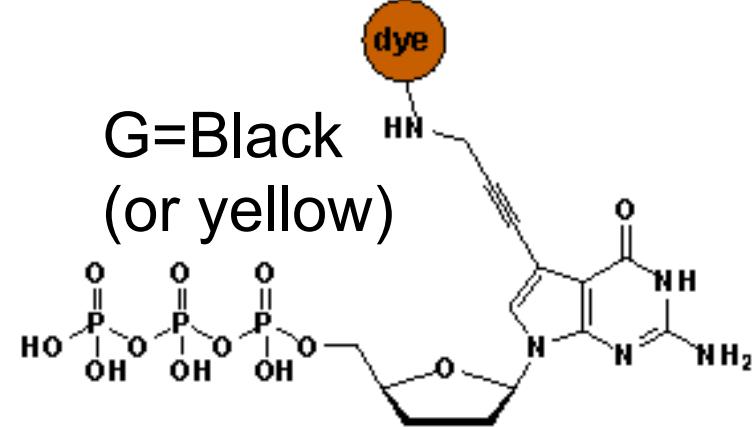
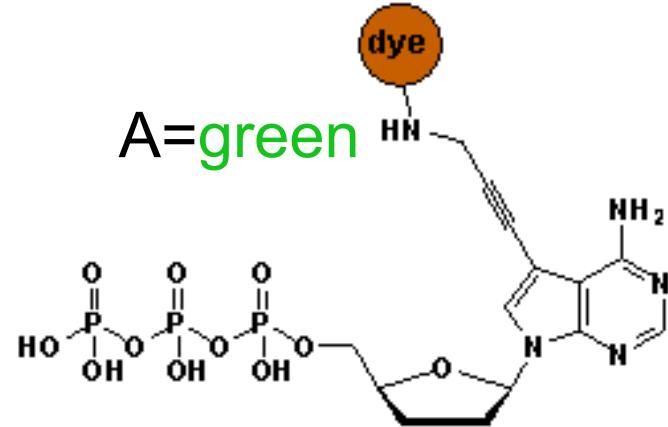


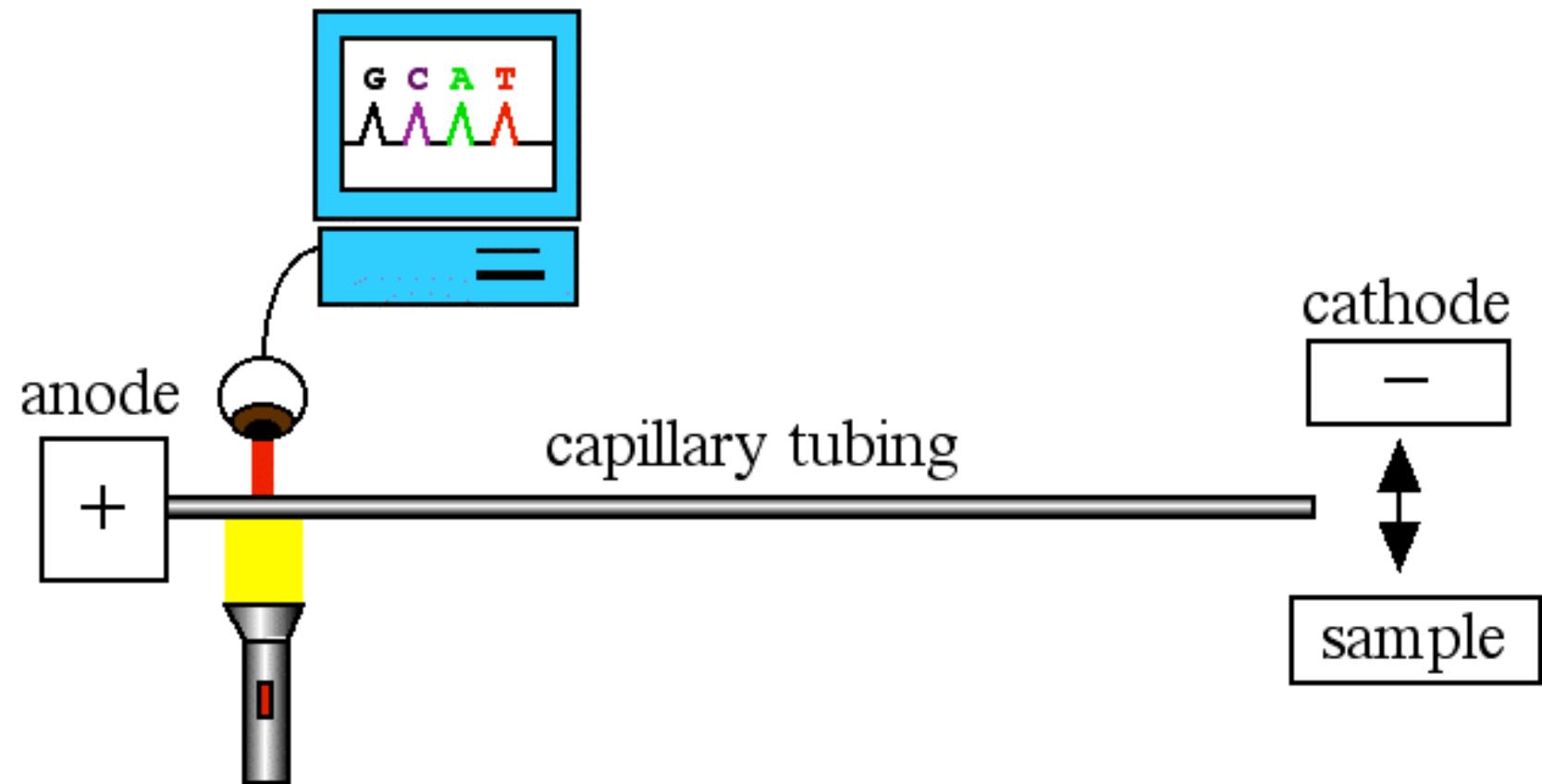
Figure 8-25 Molecular Biology of the Cell 6e (© Garland Science 2015)

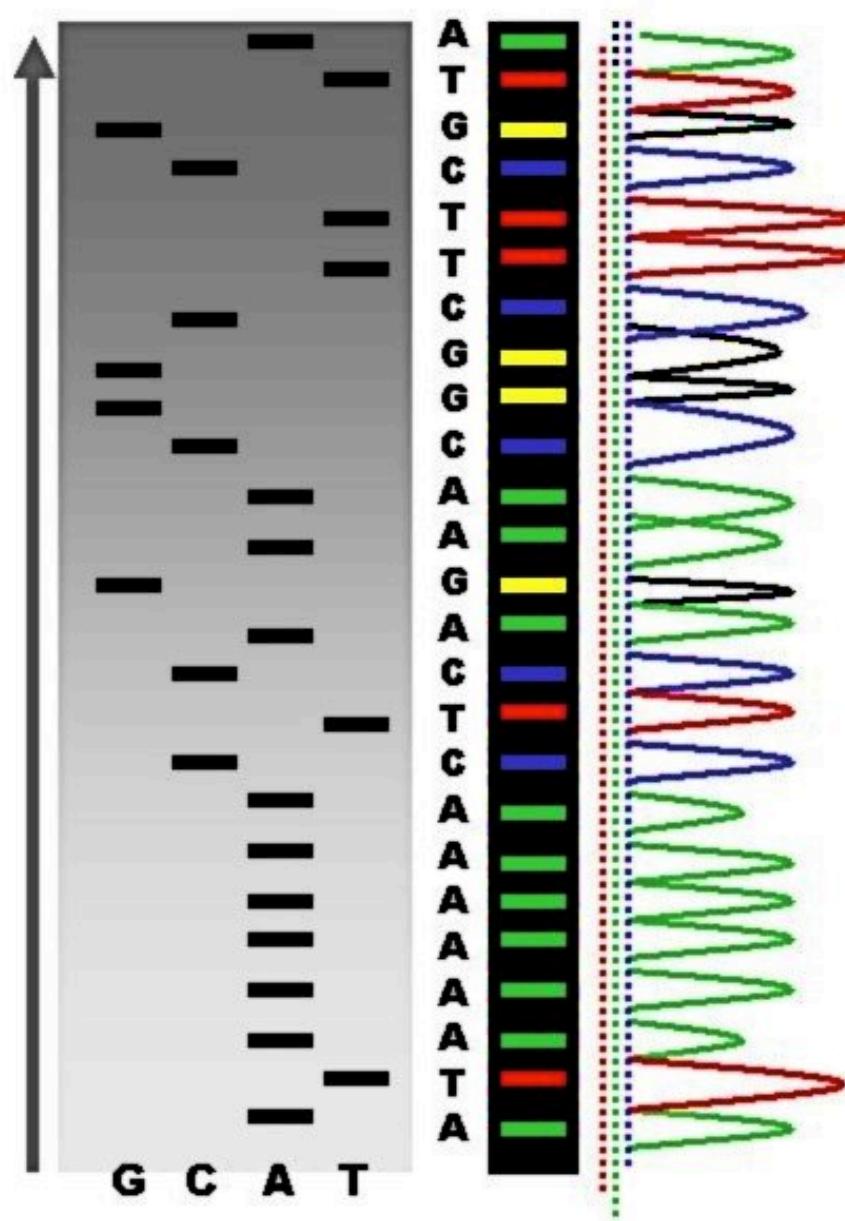
Paper Electrophoresis



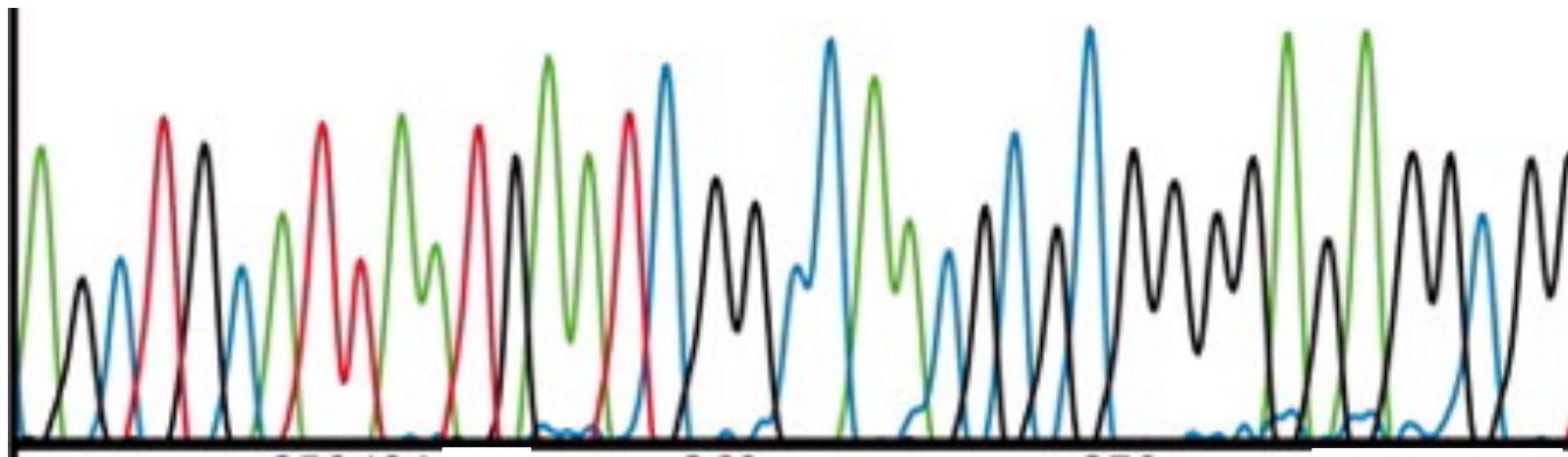


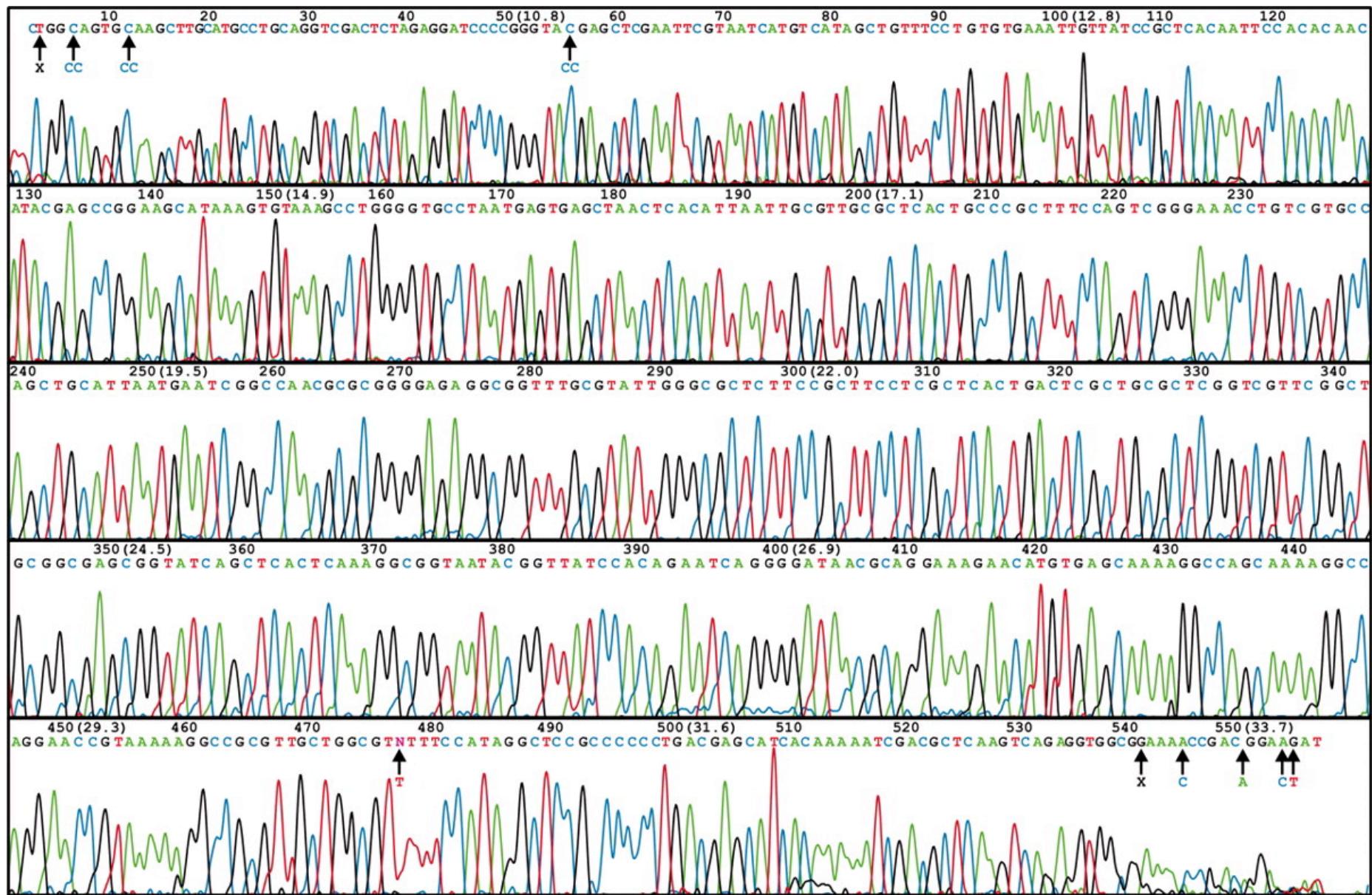






Identify the sequence in the chromatograph on
your paper
(below too – since I don't have a color printer)





HGP and Celera



ABI 3730x
(Sanger)

Pros and Cons of SS

- Polymerase errors average out
- Long sequences (~900 bp)
- Can only do 1 sequence at a time
- Need a lot of DNA to start with
- Expensive: 2¢/base

To solve these cons what do we need?

- Cheaper
- Multiplex different samples
- Smaller starting amount
- How might you do this?
 - What do you need to be able to do?

Design a Sequencer

- In groups of 2-3, discuss the things you would need to do to be able to sequence DNA in a multiplexed way.
- Write out a list. Write your names on it and give to me on your way out.
(participation points)

Questions?