Advanced Cell Biology. Lecture 27

Alexey Shipunov

Minot State University

April 1st, 2011

Outline

Outline

Previous final question: the answer

What is a EST?

Previous final question: the answer

What is a EST?

- Expression Sequence Taq, small fragment of cDNA used to identify which parts of genome are actually expressed

Southern blot again

- Southern blot employs both electrophoresis (for DNA fragments separation) and DNA hybridization (for the discovery of given DNA)
- Points of hybridization are visualized using autoradiography

Southern blot: last steps

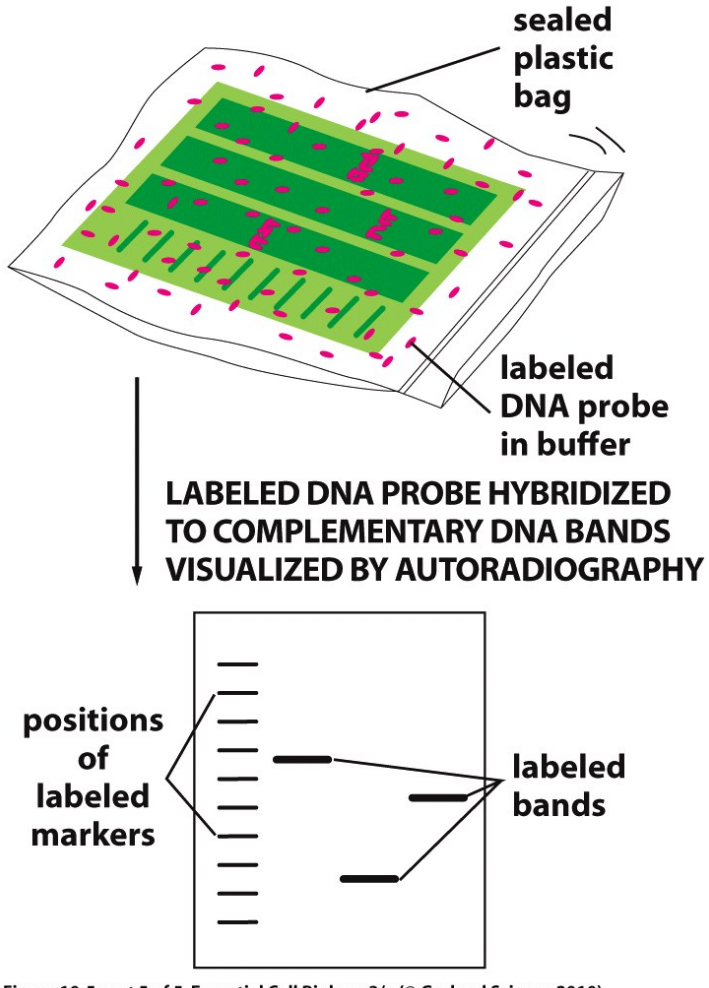


Figure 10-5 part 5 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

Recombinant molecules

- Restricted fragments may be re-assembled with ligase
- Staggered end needs to be completed with DNA polymerase first, and then molecules will me joined with ligase
- Ligase may insert “foreign” fragment between to others

Ligation after restriction

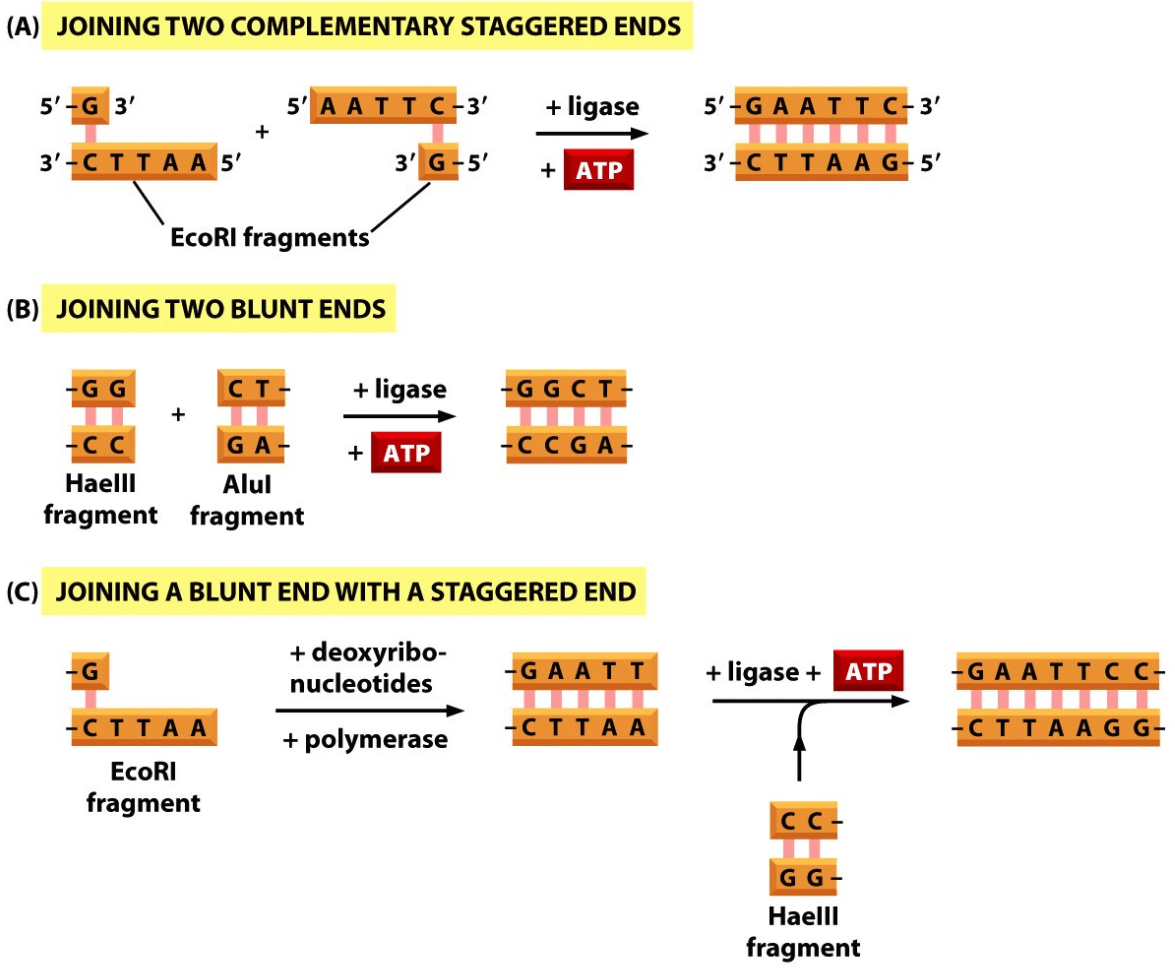


Figure 10-6 Essential Cell Biology 3/e (© Garland Science 2010)

Plasmid vectors

- The best way to transform bacterial cell is to use a plasmid
- Recombinant plasmids are easily taken into bacterial cells and then multiple with bacterial cell divisions

Recombinant plasmid

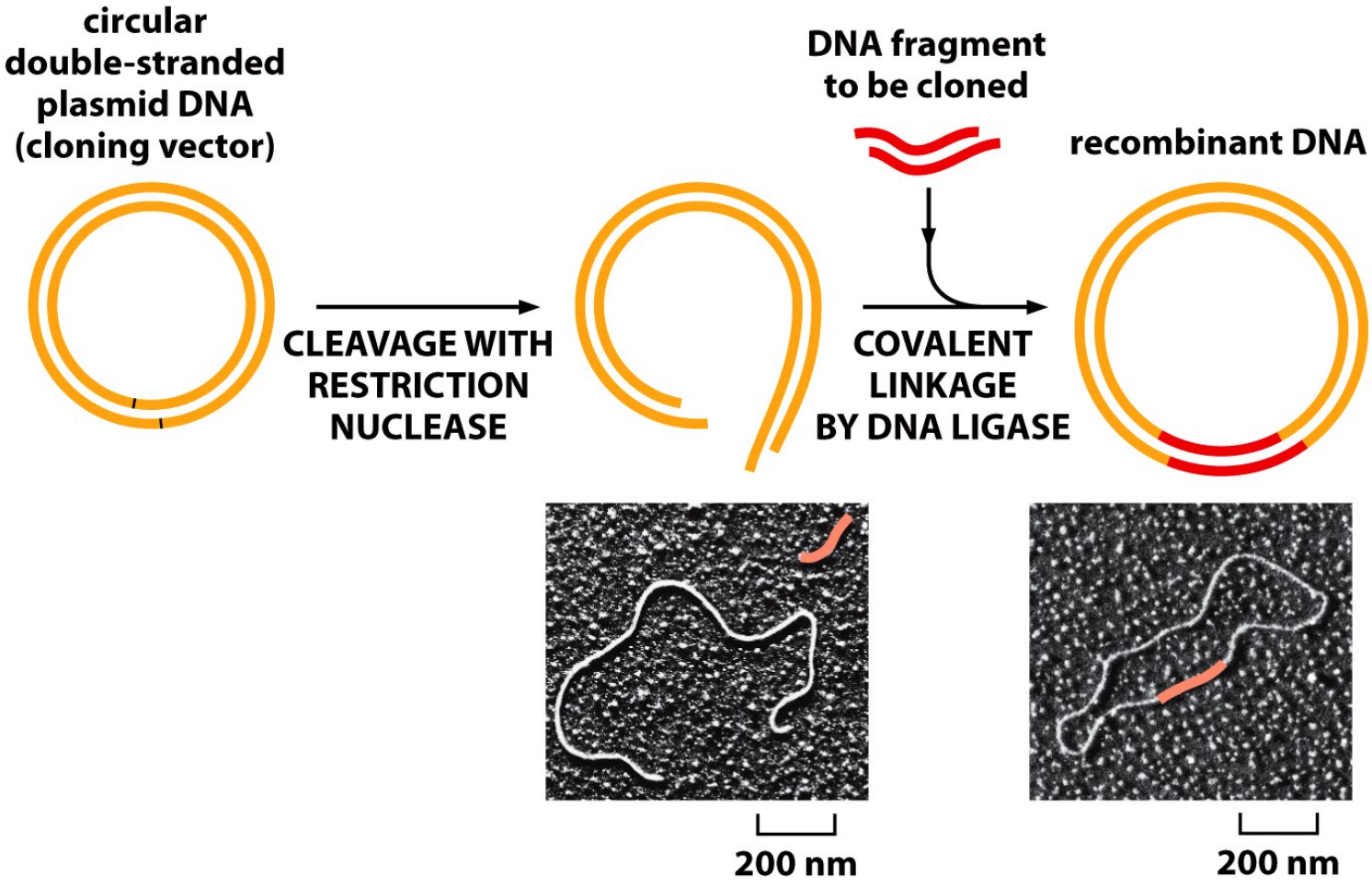


Figure 10-9 Essential Cell Biology 3/e (© Garland Science 2010)

DNA libraries

- DNA may be saved in plasmids and then re-identified with radioactive DNA labels
- Genomic libraries may use plasmids, or BACs (bacterial artifical chromosomes)

Creation of DNA library

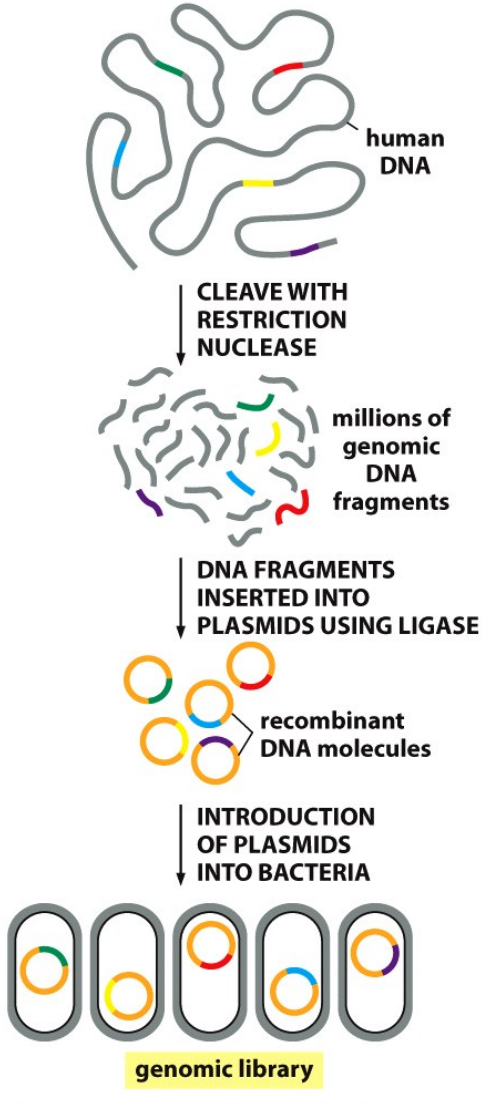


Figure 10-11 Essential Cell Biology 3/e (© Garland Science 2010)

cDNA libraries

- cDNA is a “reversed” DNA (DNA from mRNA)
- cDNA library could be different from genomic library

Genomic library vs. cDNA library

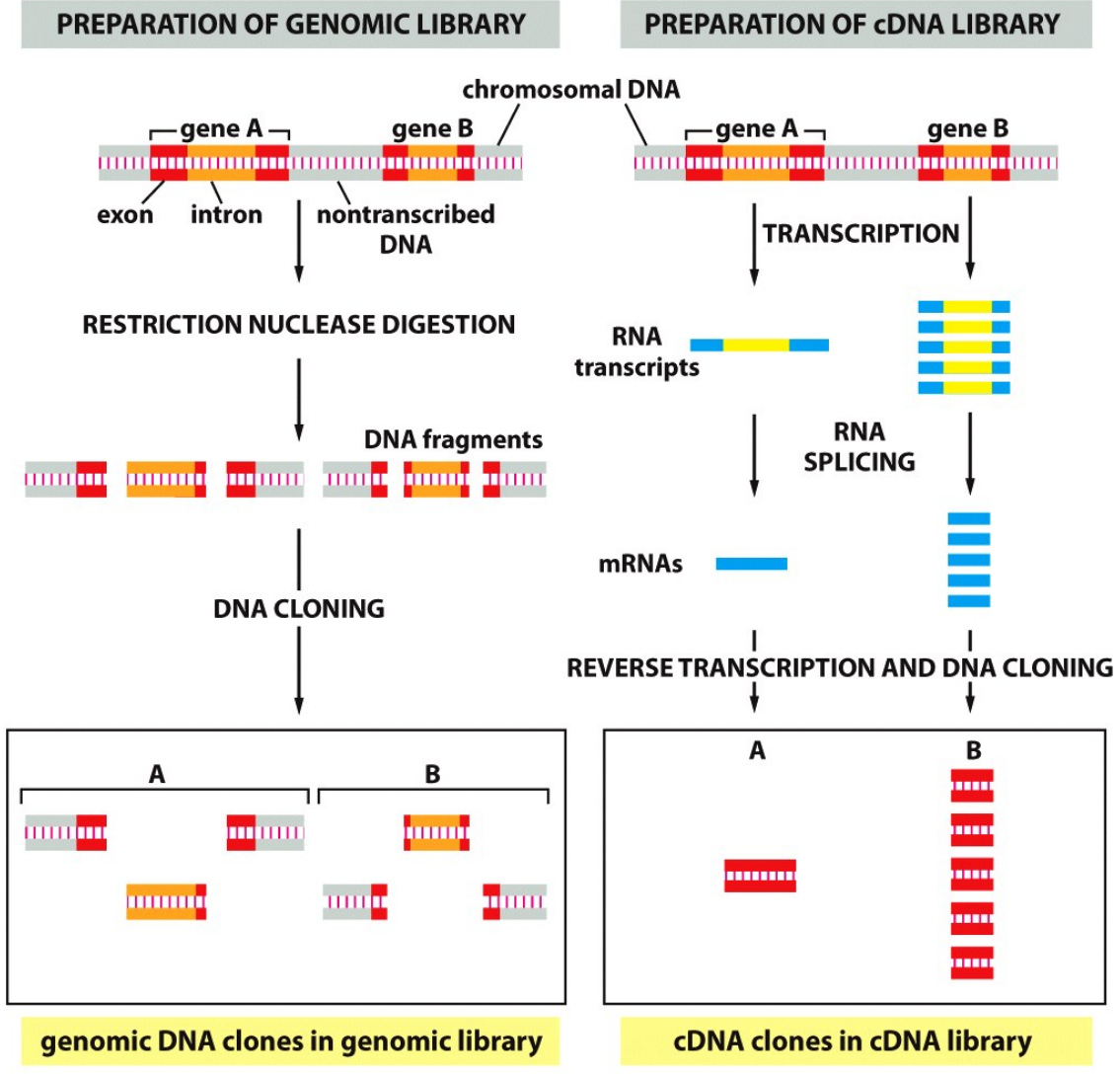


Figure 10-14 Essential Cell Biology 3/e (© Garland Science 2010)

PCR

- PCR is using temperature-specific polymerase, DNA hybridization and thermal annealing instead of helicase
- PCR is a chain reaction because the growth of desired DNA fragments is exponential
- PCR products are almost pure (contain short fragments because primers will eventually amplify short products) and may be used as clones of genomic DNA or cDNA
- Why we normally stop PCR after 25–35 cycles?

- PCR is mostly employed as a detection technique; human STR repeats are especially useful here

PCR

- PCR is using temperature-specific polymerase, DNA hybridization and thermal annealing instead of helicase
- PCR is a chain reaction because the growth of desired DNA fragments is exponential
- PCR products are almost pure (contain short fragments because primers will eventually amplify short products) and may be used as clones of genomic DNA or cDNA
- Why we normally stop PCR after 25–35 cycles? Because with more cycles, result becomes noisy
- PCR is mostly employed as a detection technique; human STR repeats are especially useful here

Small tandem repeats (STRs) for human identification

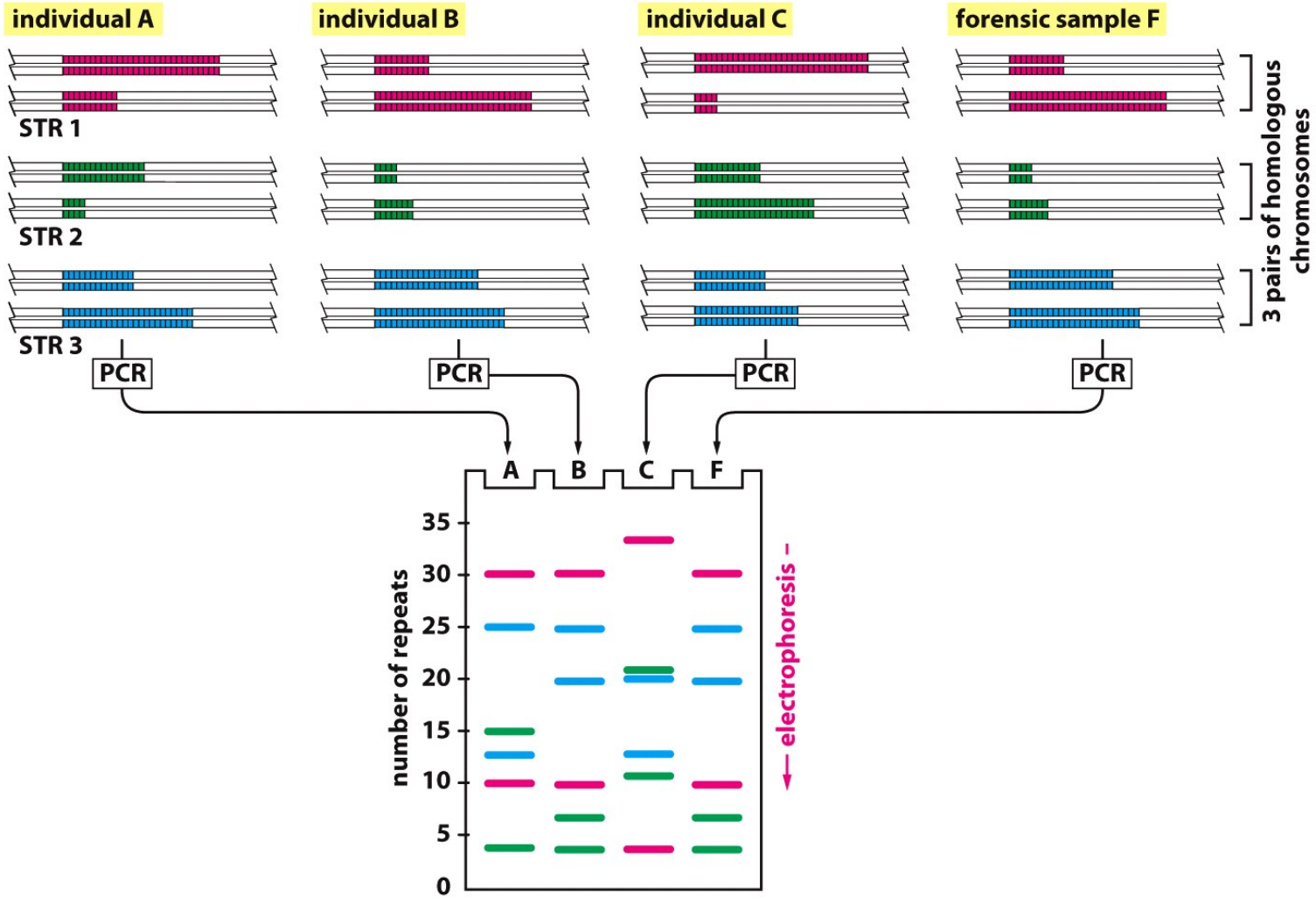


Figure 10-19b Essential Cell Biology 3/e (© Garland Science 2010)

PCR, first steps

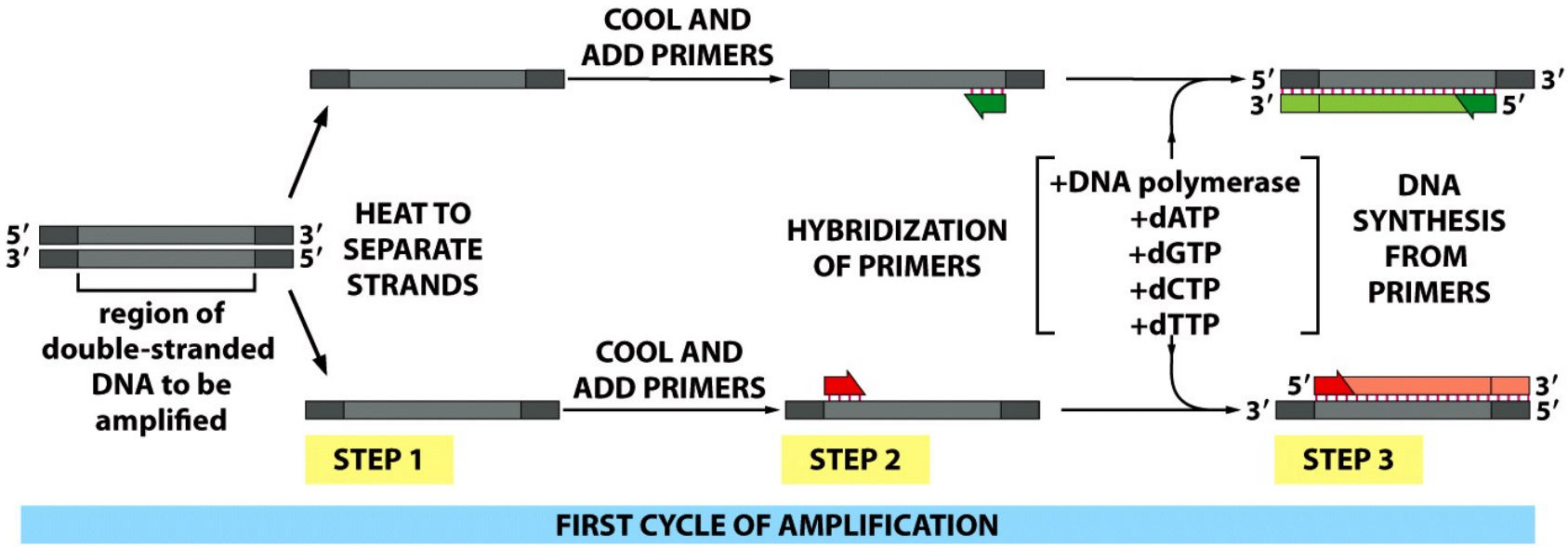


Figure 10-15 Essential Cell Biology 3/e (© Garland Science 2010)

PCR, several cycles

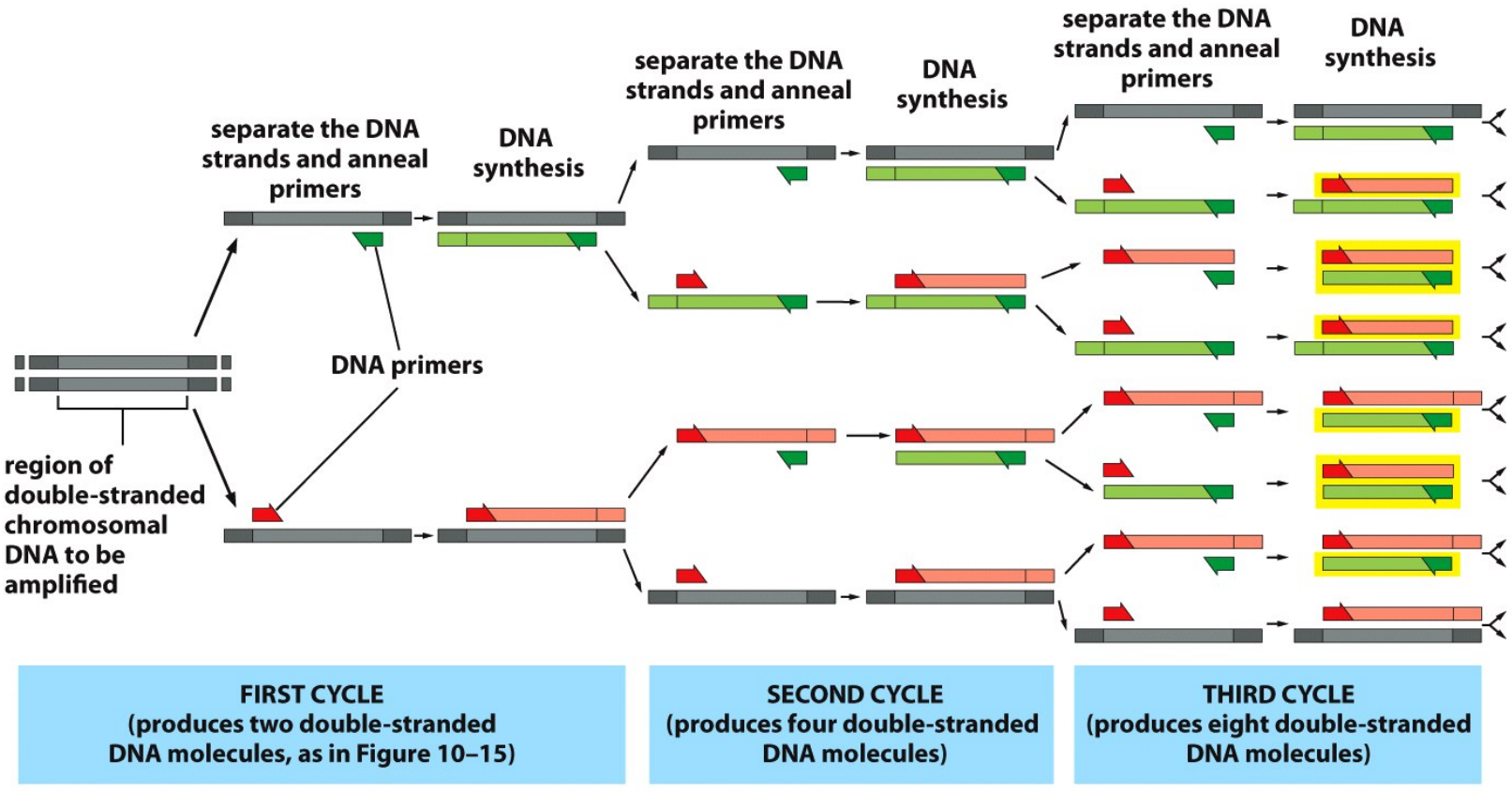


Figure 10-16 Essential Cell Biology 3/e (© Garland Science 2010)

PCR movie

DNA sequencing

- Employs specific way of polymerization used ddNTPs (dideoxy nucleotide triphosphares)
- ddNTPs stop the formation of DNA chain
- ddNTPs could be detected via fluorecsent of radioactive label
- Therefore, we will know length of fragment (because we will do electrophoresis) and which ddNTP is in there (because it is fluorescent or radioactive)
- Then we can calculate a location of specific nucleotide

Dideoxy nucleotide triphposphates (ddNTPs)

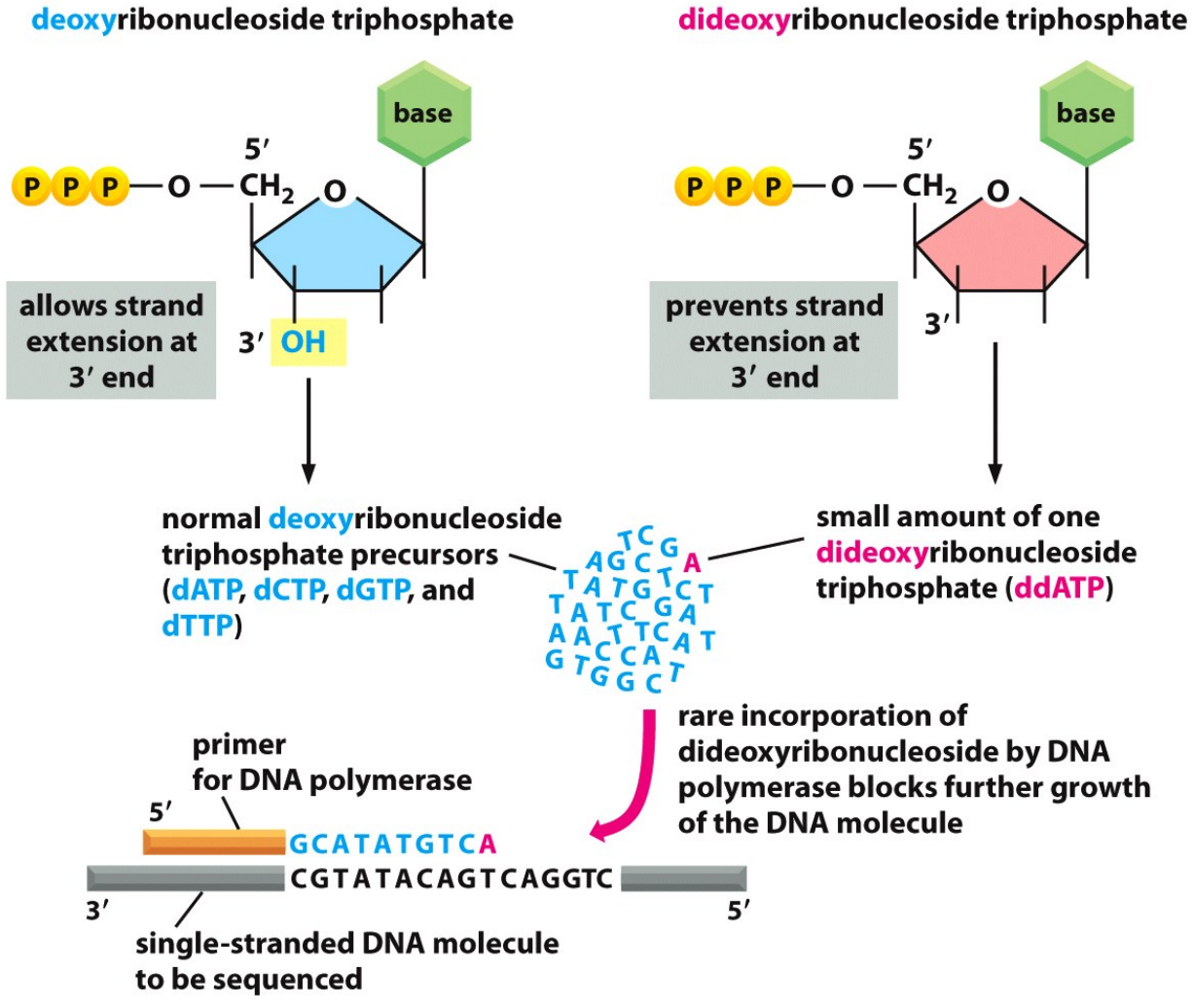
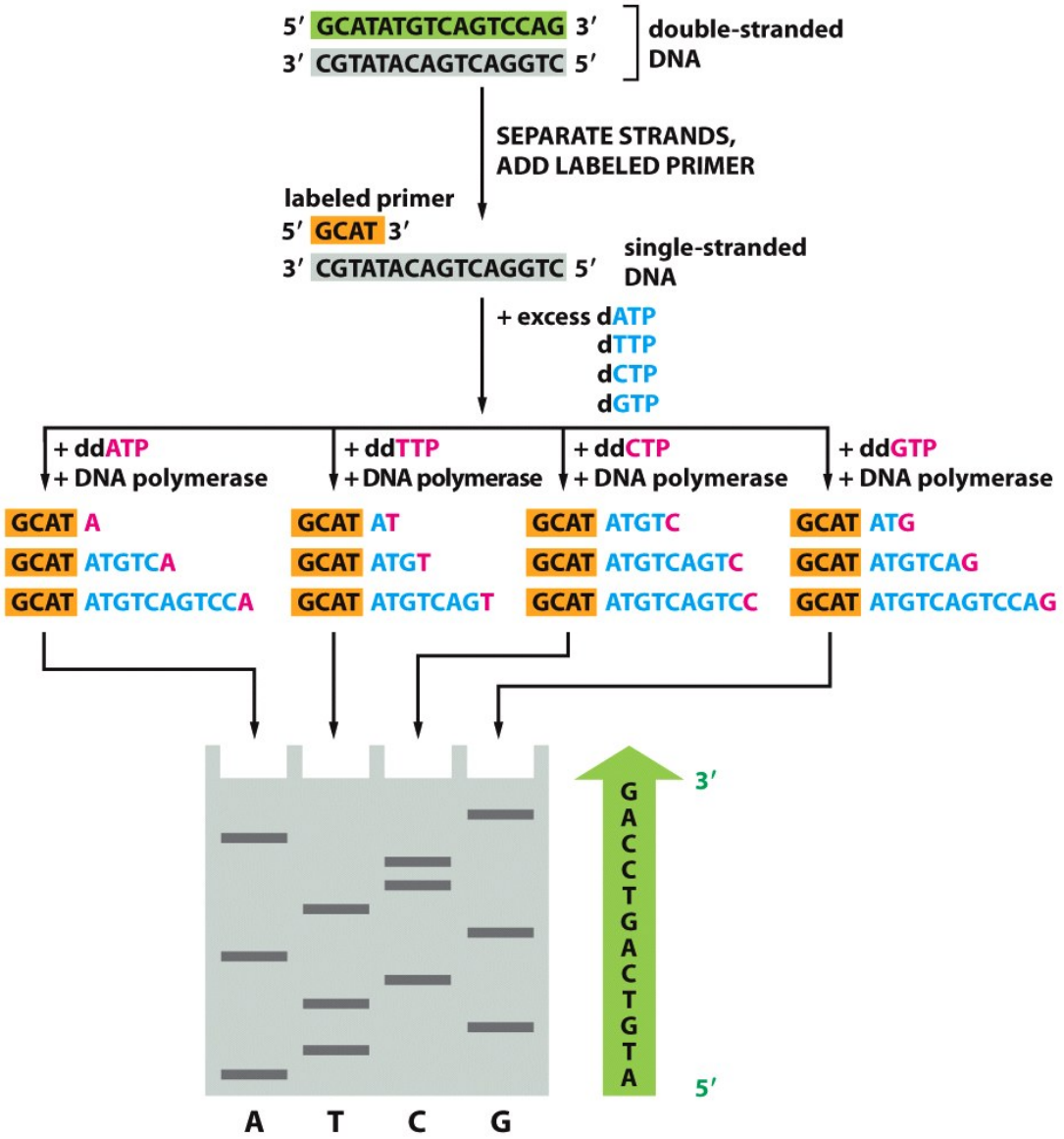


Figure 10-20 Essential Cell Biology 3/e (© Garland Science 2010)

dd-sequencing



Shotgun sequencing

- This is one of genome sequencing approaches
- DNA fragmentated then sequenced

Shotgun approach for the genome sequencing

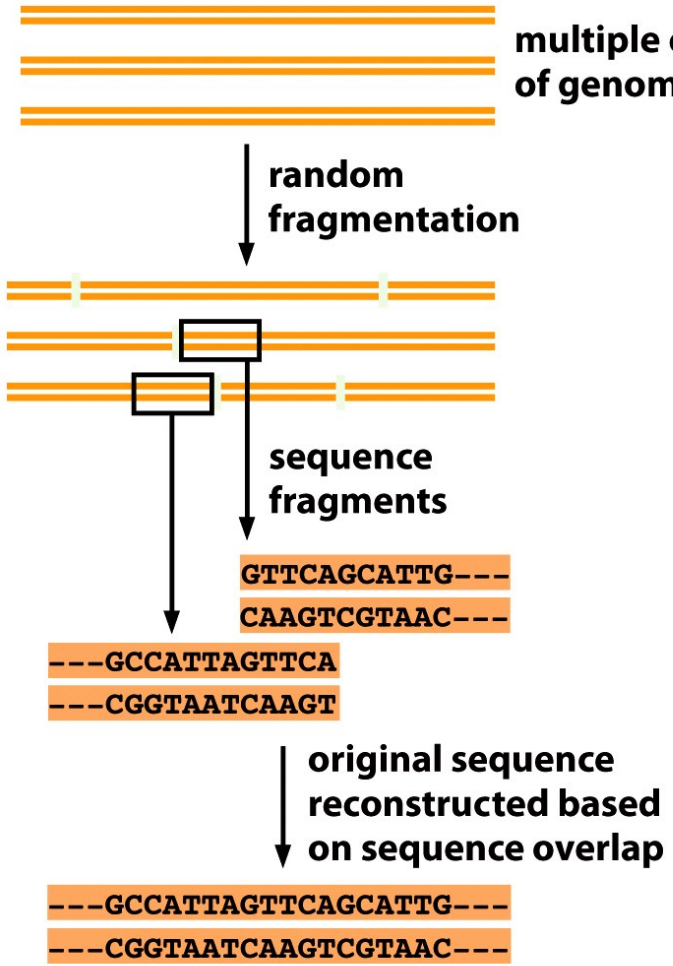


Figure 10-25 Essential Cell Biology 3/e (© Garland Science 2010)

Minot State UNIVERSITY

Final question (2 points)

Final question (2 points)

Why DNA sequencing employs ddNTPs?

Summary

- DNA cloning used recombinant DNA and plasmid carriers
- PCR is based on temperature-specific DNA polymerase and DNA hybridization
- DNA sequencing (dd method) is based on the use of ddNTPs and electrophoresis

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: pp. 332–349.