Advanced Cell Biology. Lecture 26

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Outline

Genomes

Analysis of DNA

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Genomes

Analysis of DNA

Three main branches of life

- Bacteria
- Archaea
- Eukaryotes

Ribosomal 5S RNA in three domains

Figure 9-25 Essential Cell Biology 3/e (© Garland Science 2010)

Human genome findings

- Only 25,000 genes
- Only 2% are protein-encoding or regulatory
- Only 1% are unique: 50 sites of human-accelerated changes

Pseudogenes

- 20,000 in human genome
- Closely resemble the functioning gene but are not expressed

Human genome content

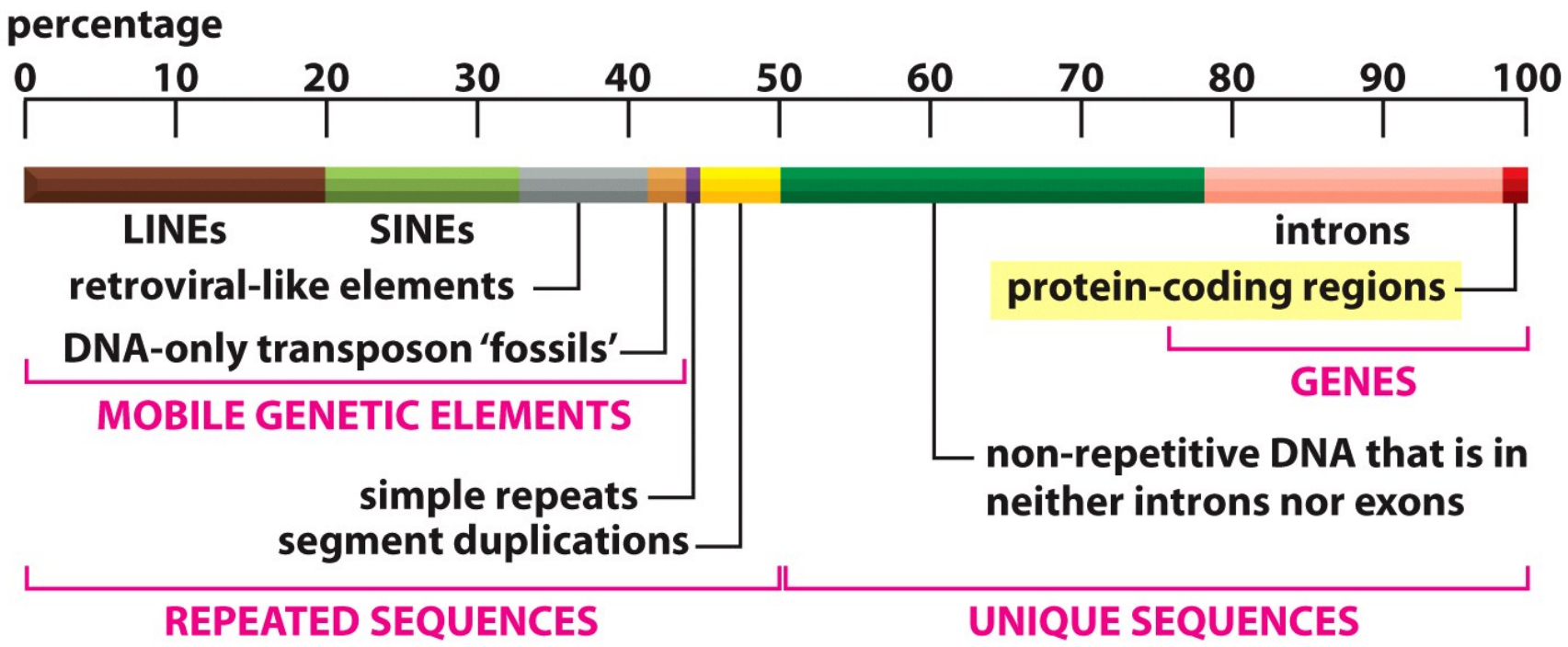


Figure 9-29 Essential Cell Biology 3/e (© Garland Science 2010)

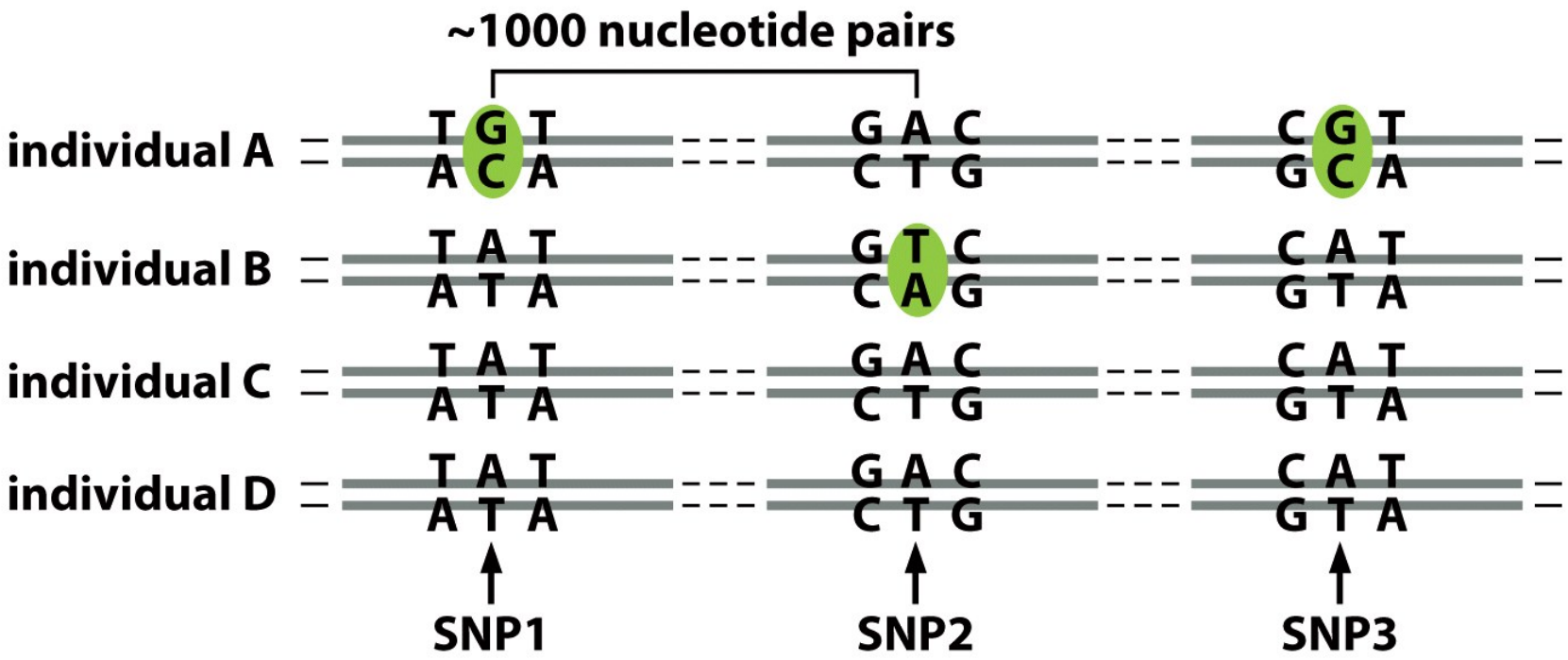
Genome annotation basics

- ORFs (open reading frames): more than 100 bp without stop codons, start with ATG and stops with stop codons
- Examination of ESTs: mRNAs are isolated, converted to cDNAs, then expressed sequence tags (ESTs) from them are sequenced and searched through a genome
- BLAST search through nucleotide databases reveals all similar sequences

SNPs

- Single nucleotide polymorphisms (SNPs) could be individual characters
- CA repeats and other repetitive sequences like microsatellites are freely mutable
- SNPs and CAs and microsats are basics for DNA fingerprinting

Single nucleotide polymorphisms (SNPs)



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Things which should be deciphered

- Gene content is much more similar than was previously thought
- Alternative splicing

Fly *Dscam* gene: ways of alternative splicing

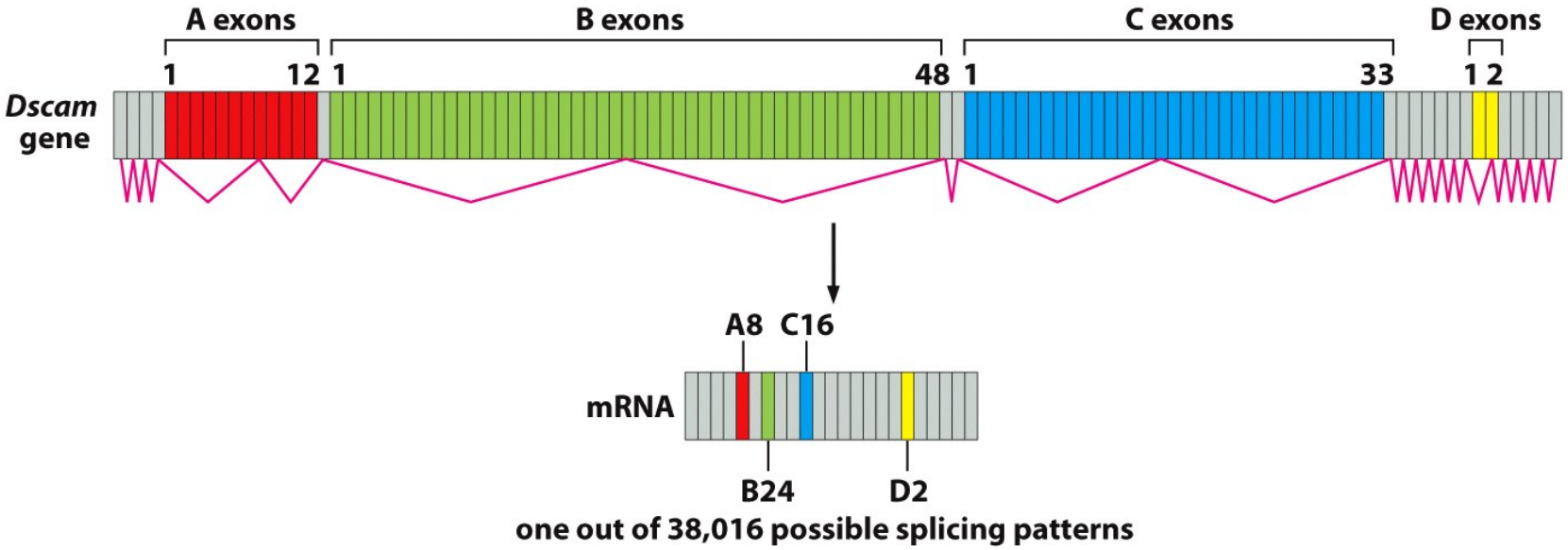


Figure 9-32 Essential Cell Biology 3/e (© Garland Science 2010)

Restriction nucleases

- Often site-specific
- Stable

Restrictases

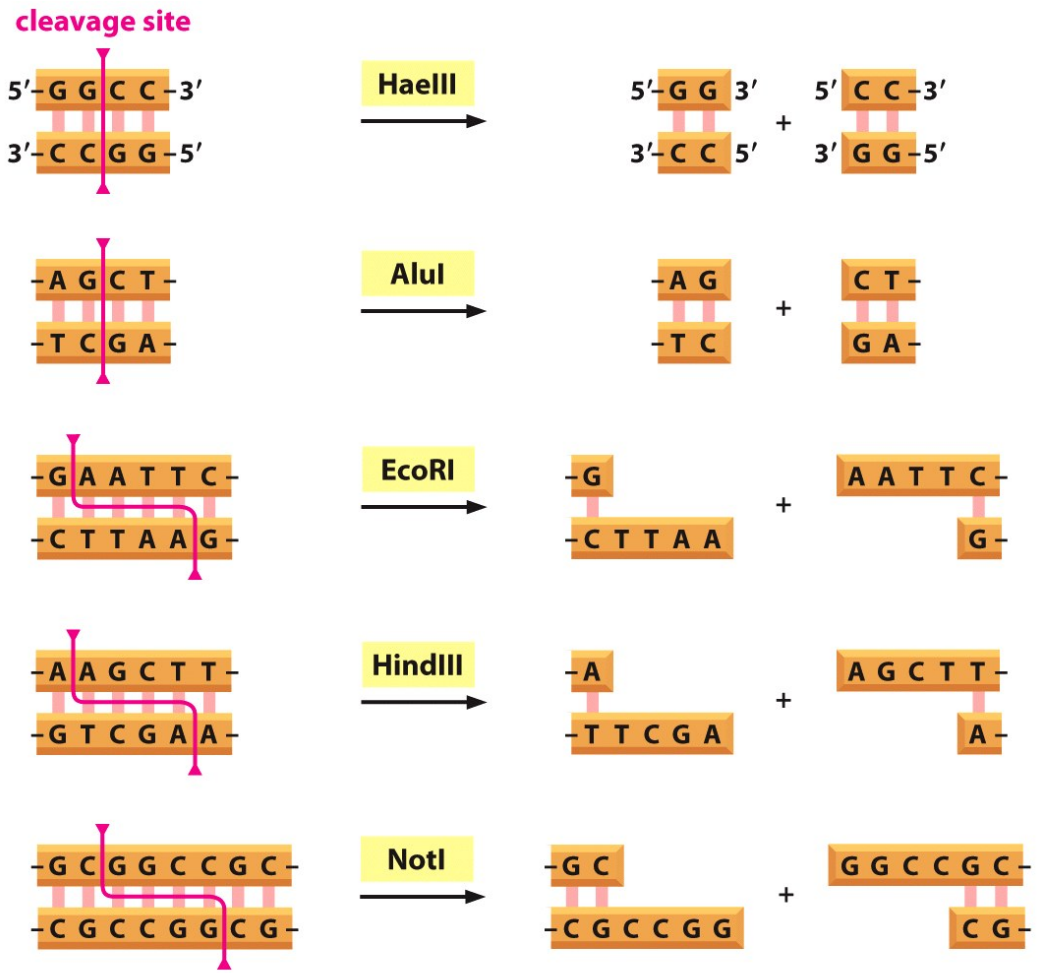
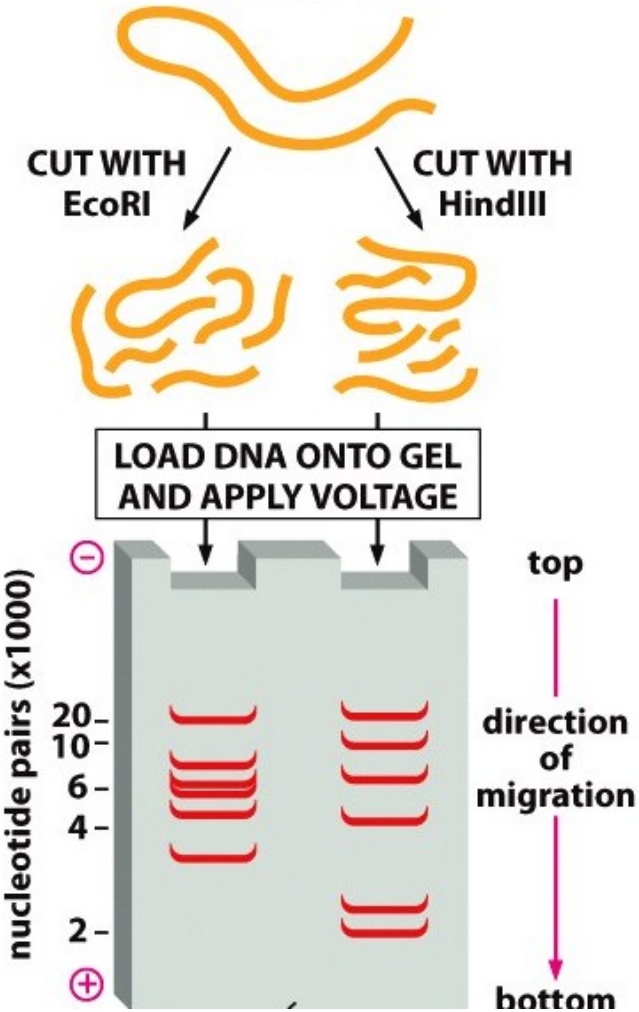


Figure 10-2 Essential Cell Biology 3/e (© Garland Science 2010)

Gel electrophoresis and RFLP

- DNA is normally negatively charged
- With two specific restrictases, we may achieve a specific gel images
- These are a restricted fragment length polymorphisms (RFLP)

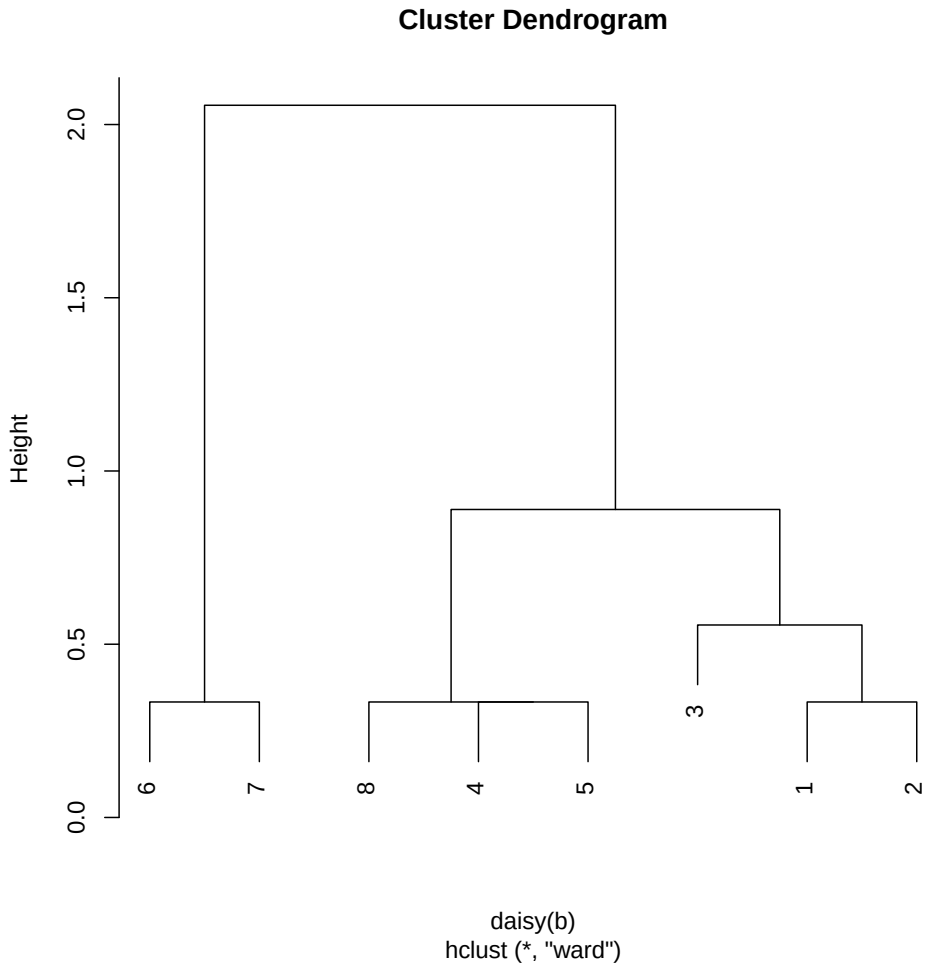
Restriction fragments in gel



Restriction fragments polymorphisms, RFLPs

Cluster analysis of RFLP

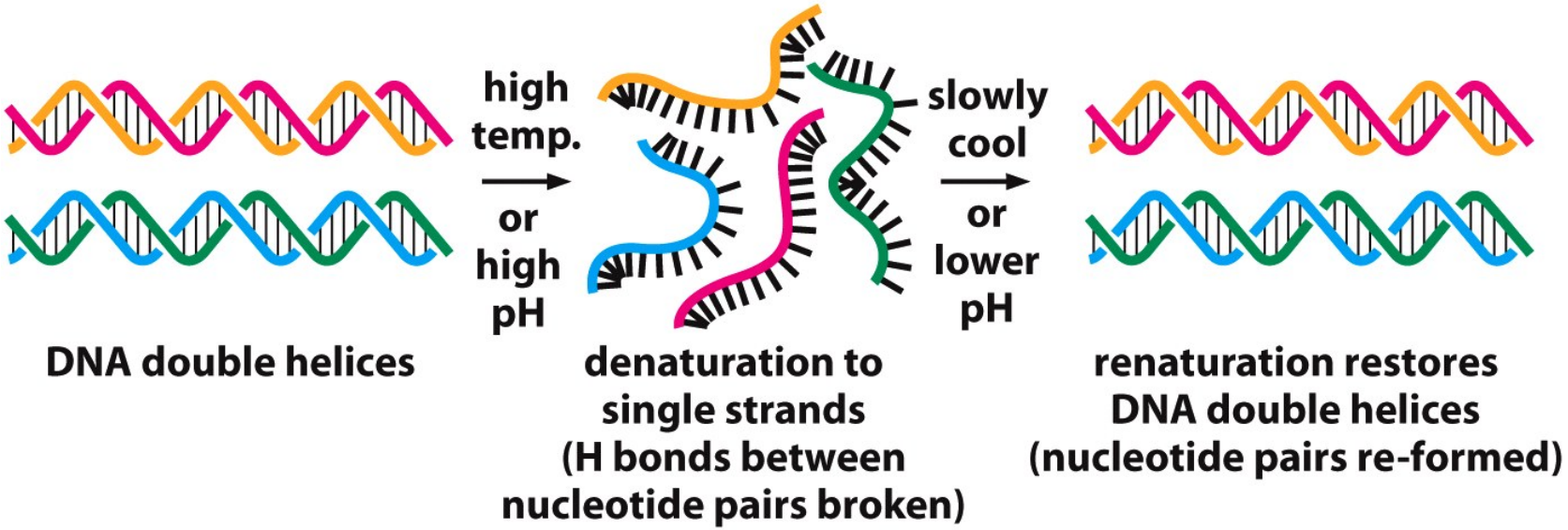
Cluster analysis of RFLP



DNA hybridization

- Denaturation, mixing and renaturation
- May provide a useful information itself: the percentage of hybridized DNA reflects similarity
- More advanced techniques like Southern blotting help to search specific DNA fragments

DNA hybridization



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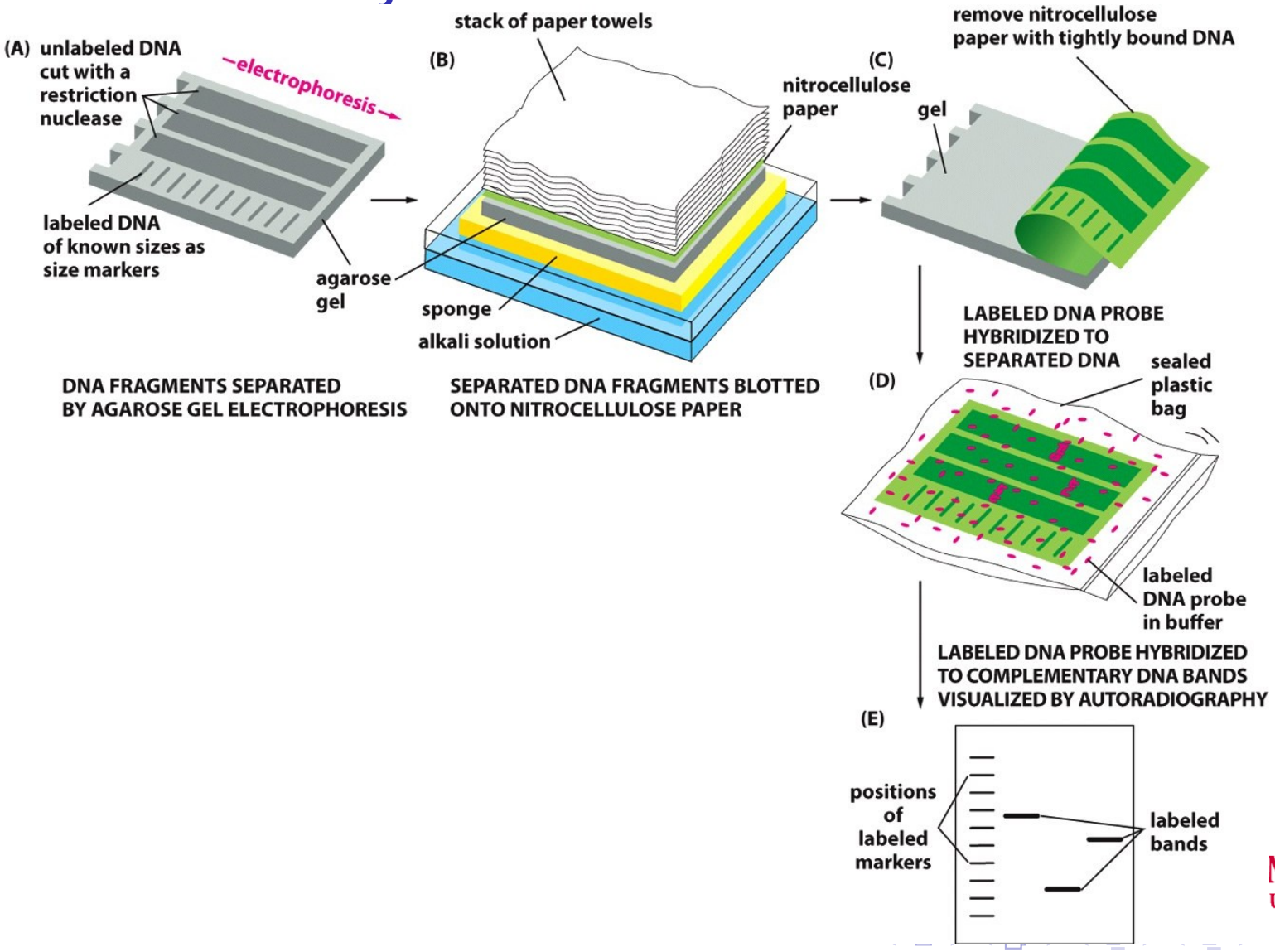
Southern blot analysis



Final question (1 point)

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What is a EST?

Summary

- Human genome project still needs to reveal translation regulators and alternative splicing
- DNA restriction and DNA hybridization are simple techniques which help to obtain information about DNA polymorphisms

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.
Chapters 9 and 10: pp. 313–333.