Lecture 8: Molecular Markers and mapping

DNA polymorphism: the basis of molecular markers

Methods of detection and application (diagnosis, finger-printing)

Molecular mapping

Read 69-78 (ie. 2.6-2.7)

Fig. 2.22-2.27

Table 2.4

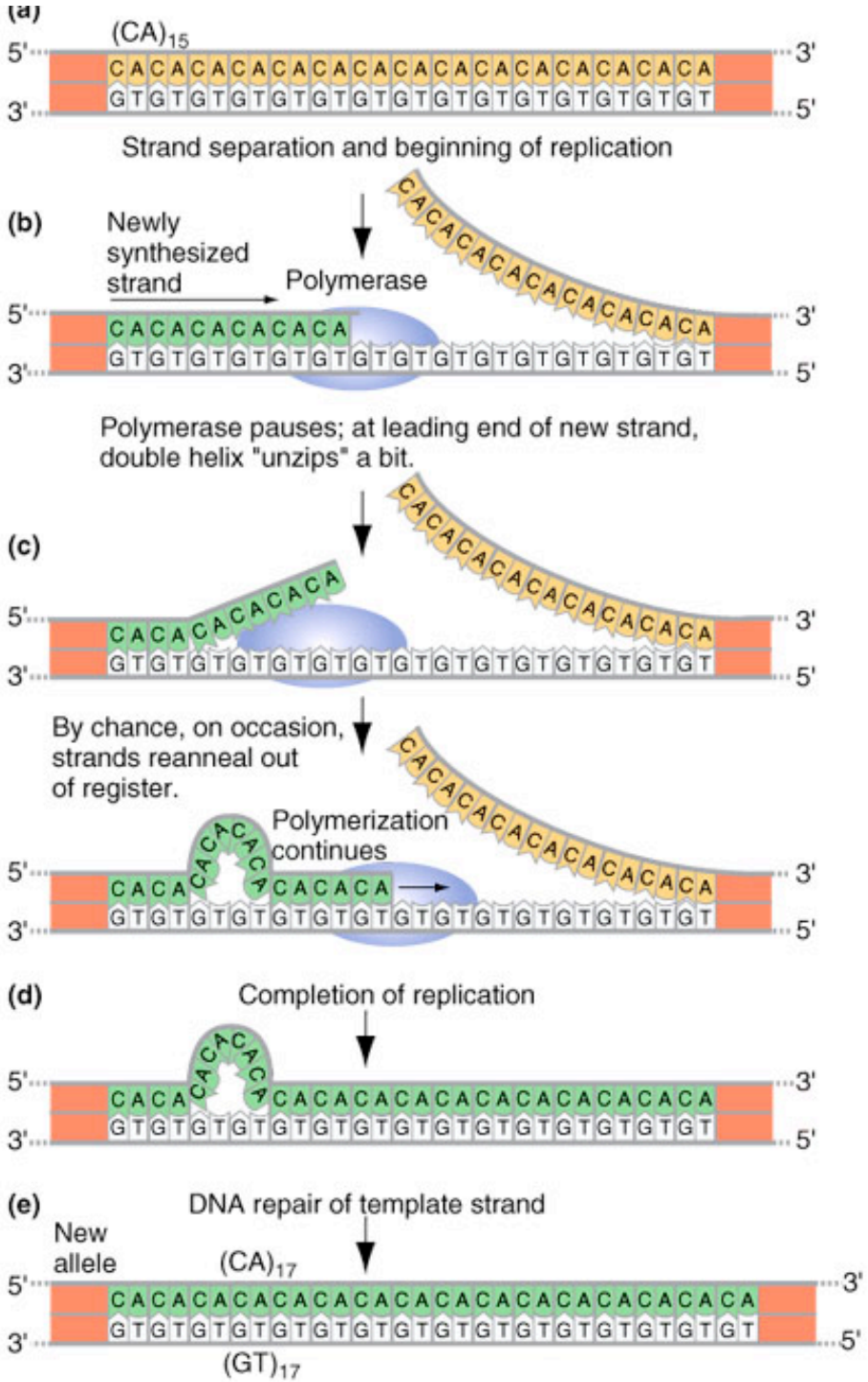
Four classes of DNA polymorphisms

TABLE 11.1 Classes of DNA Polymorphisms

Class	Size of Locus	Number of Alleles	Number of Loci in Population	Rate of Mutation	Use	Method of Detection
SNP	Single base pair	2	100 million	10^{-9}	Linkage mapping	PCR followed by ASO hybridization or primer extension
Microsatellite	30–300 bp	2–10	200,000	10^{-3}	Linkage mapping	PCR and gel electrophoresis
Multilocus Minisatellite	1–20 kb	2–10	30,000	10^{-3}	DNA fingerprinting	Southern blot and hybridization
Small Changes in DNA Content (deletions and duplications)	1–100 bp	2	N/A	$<10^{-9}$	Linkage mapping	PCR and gel electrophoresis

Single nucleotide polymorphism (SNP)

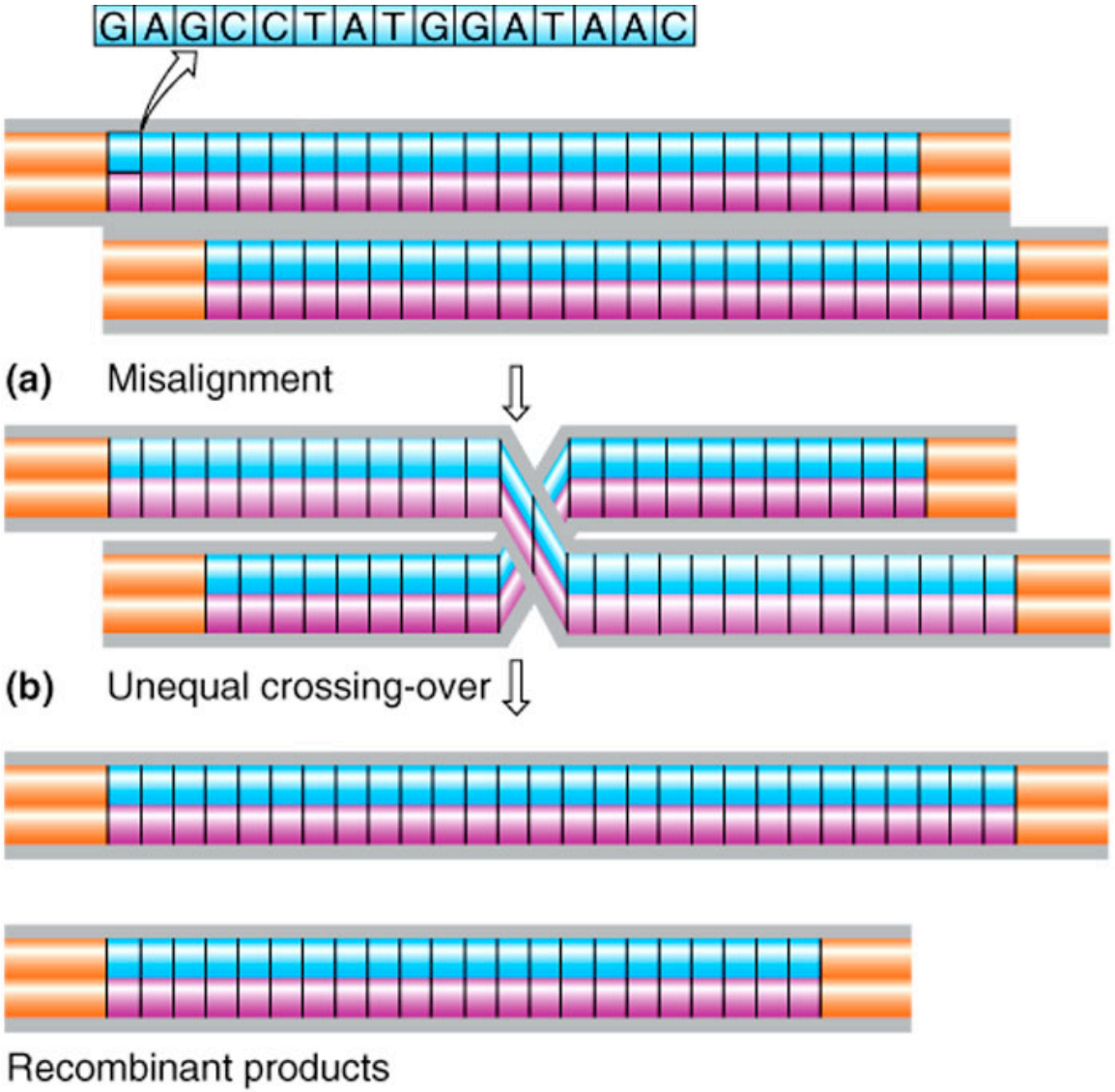
- Single base-pair substitutions
- Arise by mutagenic chemicals or mistakes in replication
- Biallelic – only two alleles
- Ratio of alleles ranges from 1:100 to 50:50
- 2001 – over 5 million human SNPs identified
- Most occur at anonymous loci
- Mutation rate of 1×10^{-9} per locus per generation
- Very few are thus new mutation in the species
- Useful as DNA markers



Microsatellites

- 1 every 30,000 bp
- Repeated units 2 – 5 bp in length
- Mutate by replication error
- Mutation rate of 10^{-3} per locus per gamete
- Useful as highly polymorphic DNA markers

Minisatellites

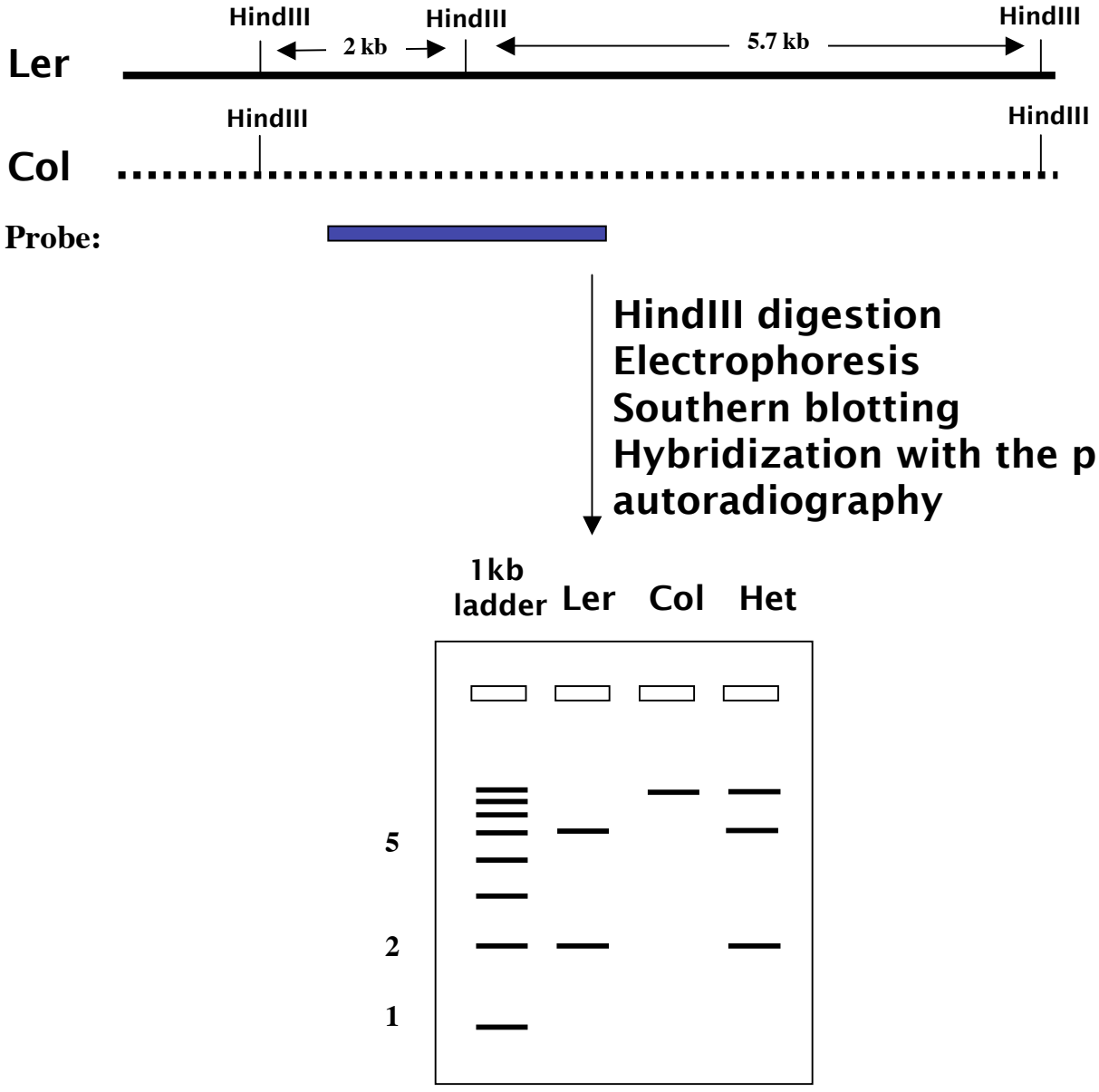


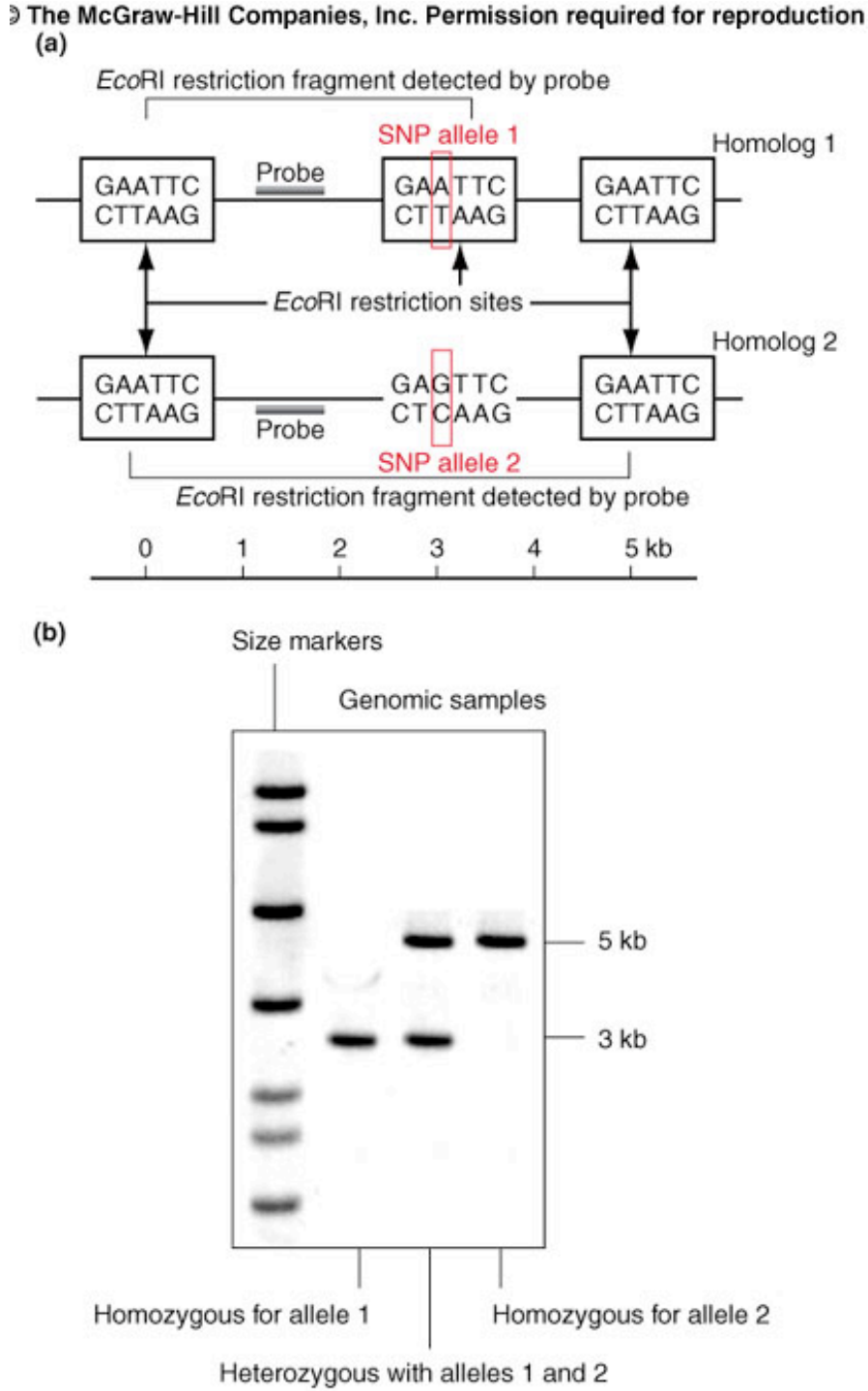
- Repeating units 20-100 bp long
- Total length of 0.5 – 20 kb
- 1 per 100,000 bp, or about 30,000 in whole genome

Deletions, duplications, and insertions

- Expand or contract the length of nonrepetitive DNA
- Small deletions and duplications arise by unequal crossing over
- Small insertions can also be caused by transposable elements
- Much less common than other polymorphisms

RFLP: Restriction Fragment Length Polymorphism

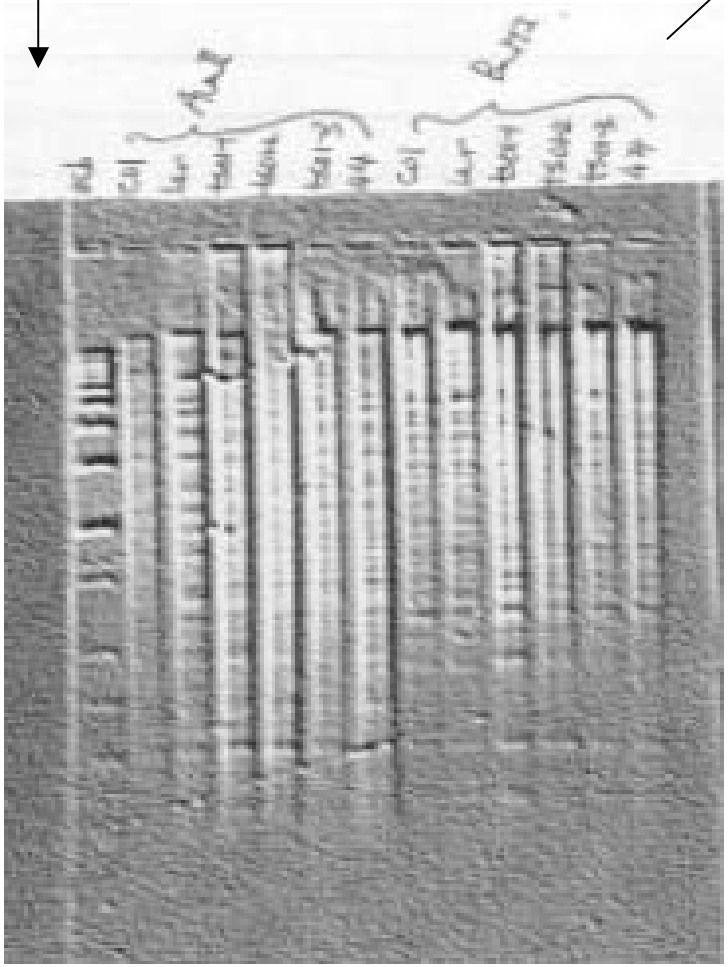




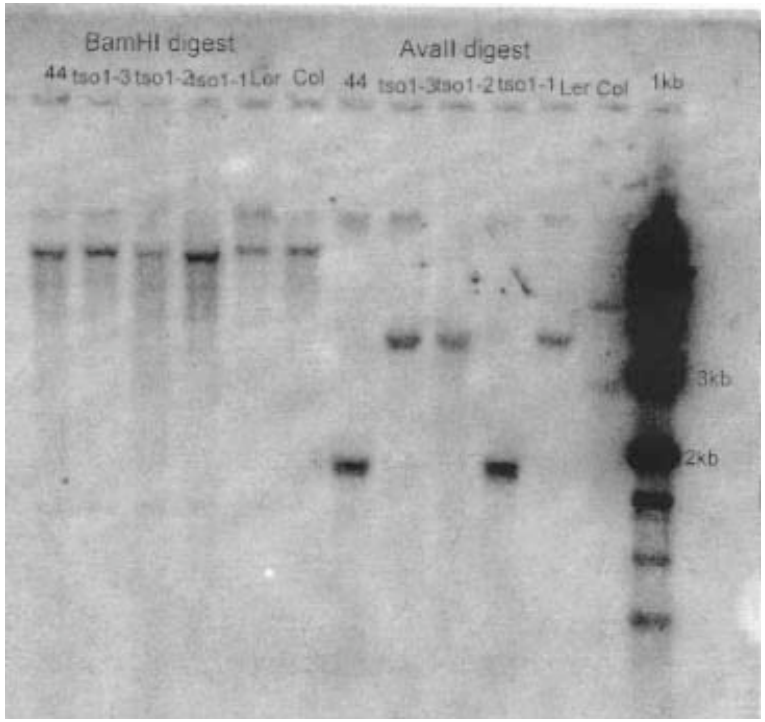
- SNP detection using southern blots
  - Restriction fragment length polymorphisms (RFLPs) are size changes in fragments due to the loss or gain of a restriction site

Example of RFLP

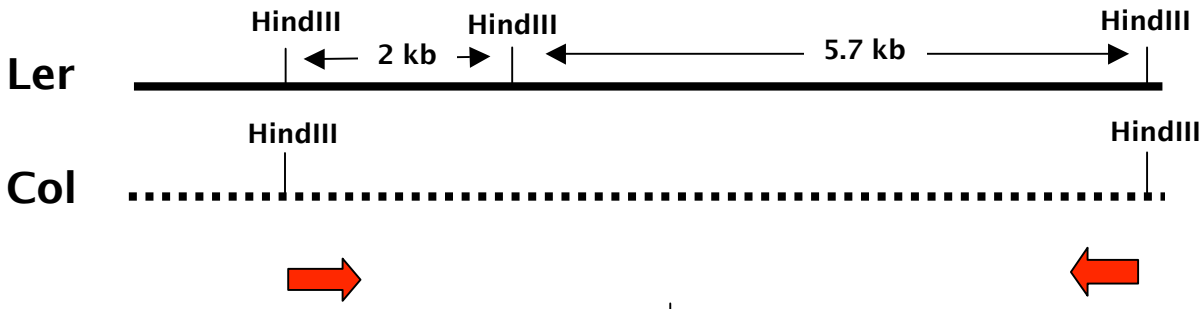
Restriction digestion
GE
Ethedium bromide stain



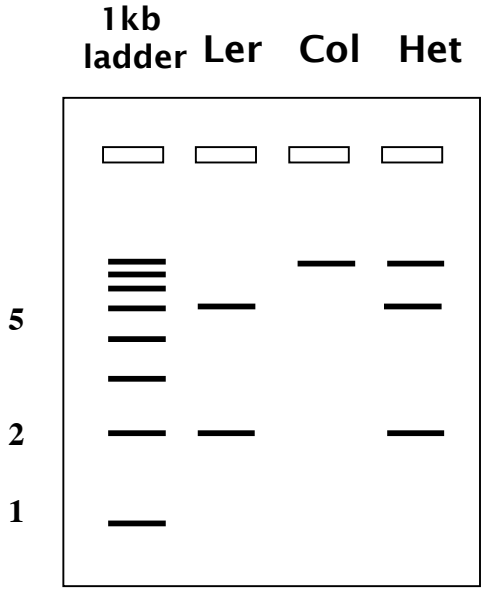
Blotting
Hybridization
X-ray radiography

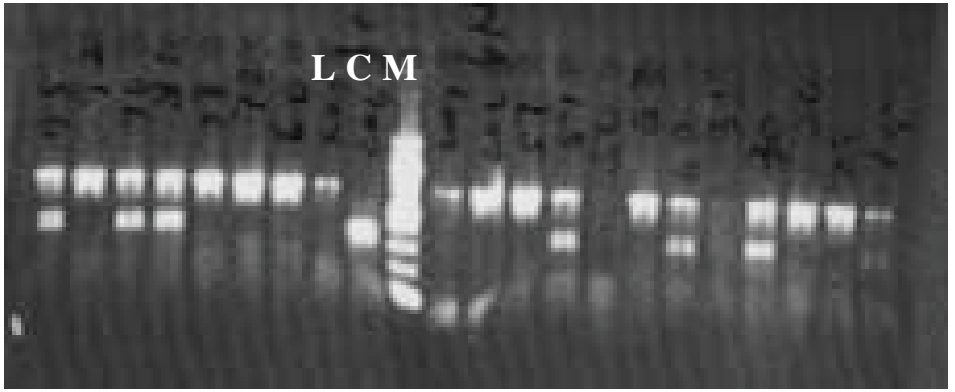


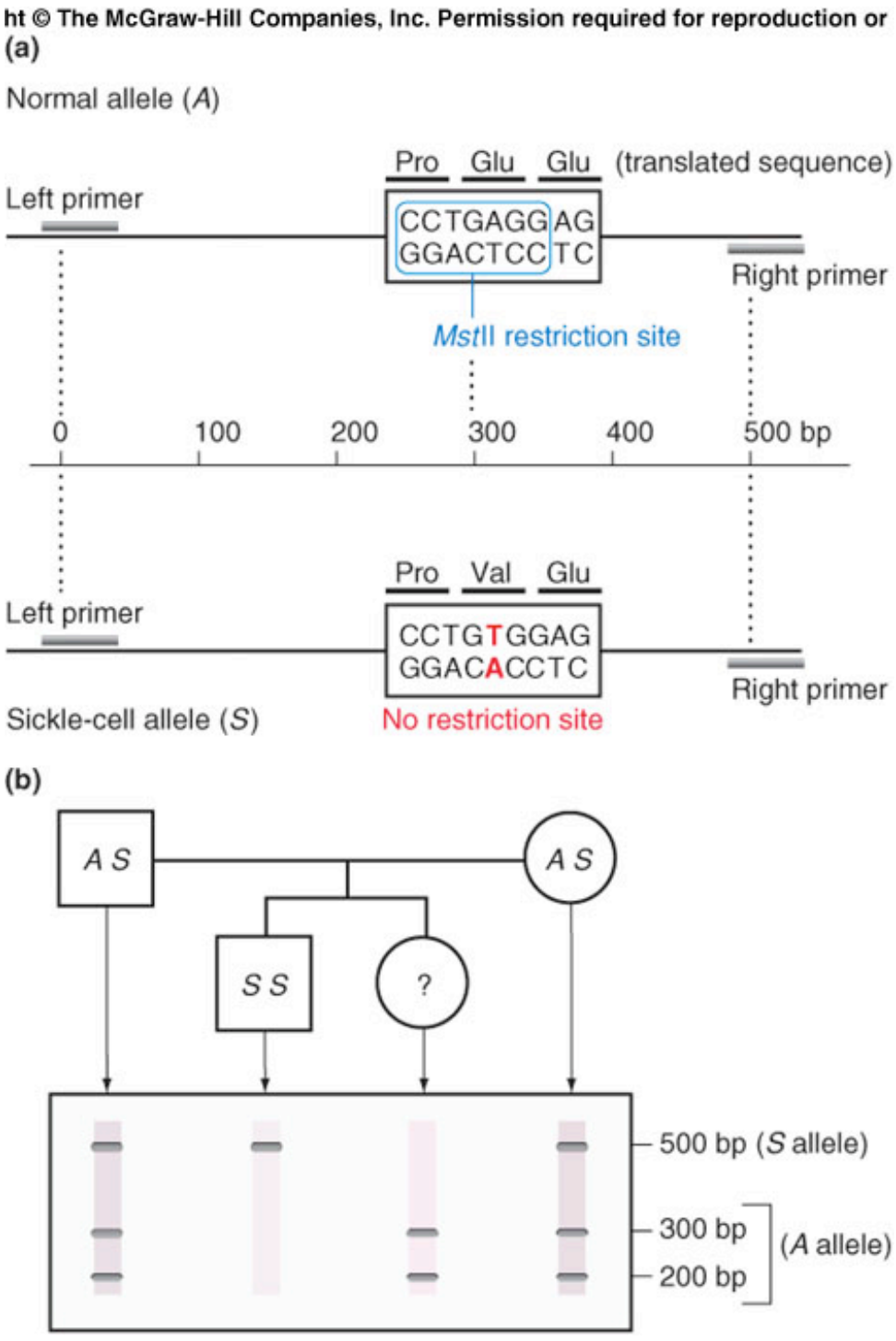
CAPS (Cleaved Amplified Polymorphic Sequences)
ie. RFLP detection by PCR



PCR
HindIII digestion
GE





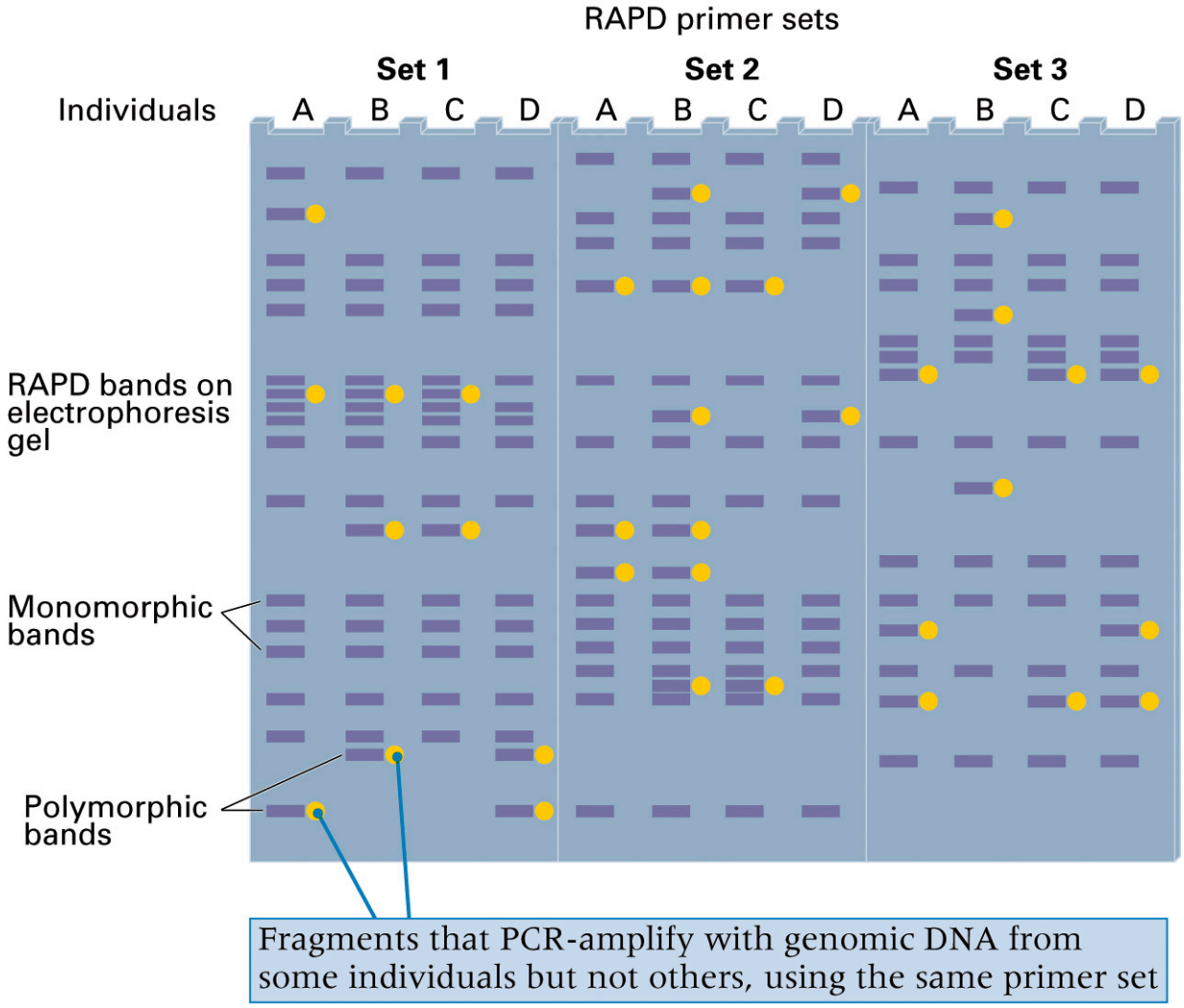


CAPS

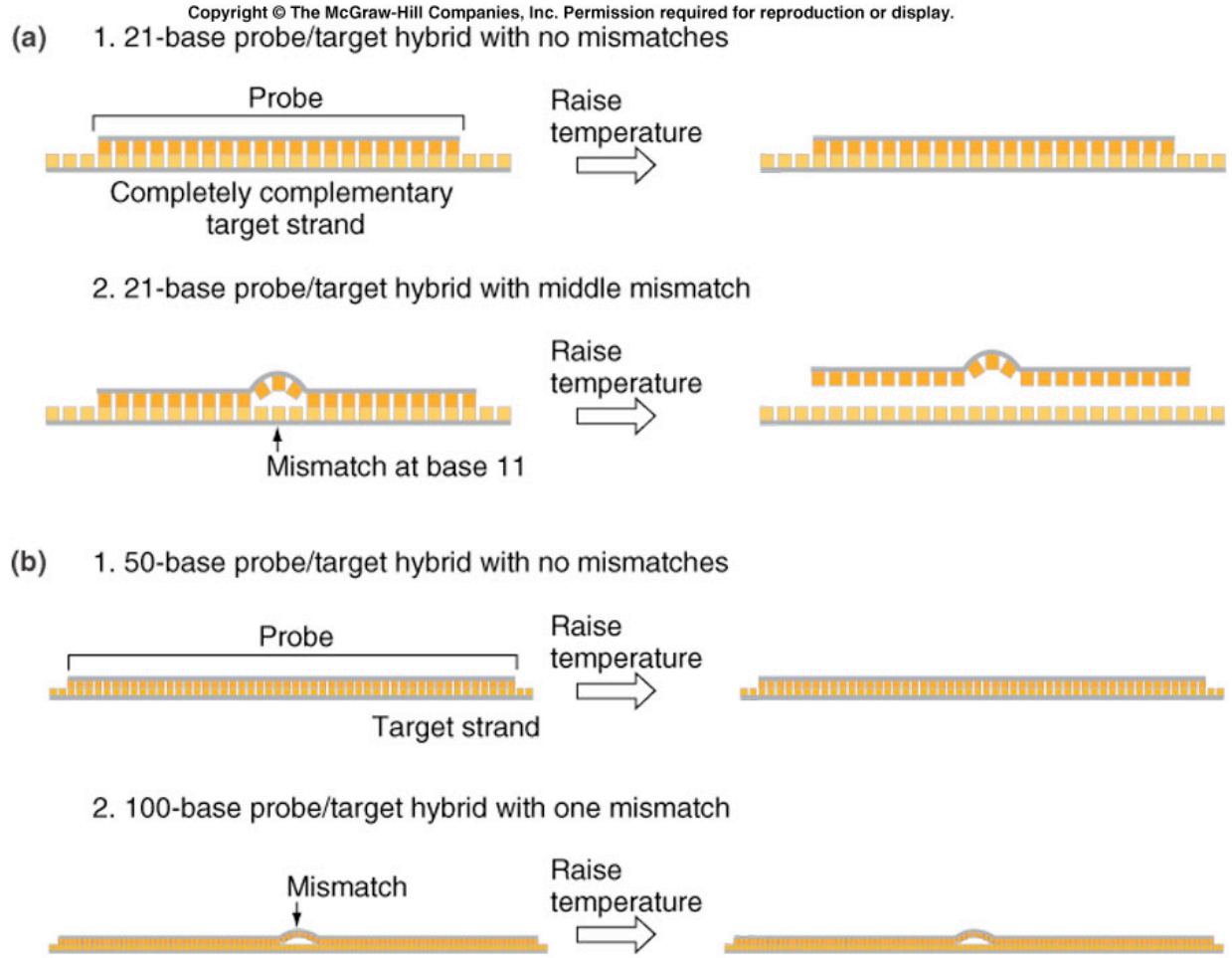
- Must have sequence on either side of polymorphism
  - Amplify fragment
  - Expose to restriction enzyme
  - Gel electrophoresis
- e.g., sickle-cell genotyping with a PCR based protocol

Randomly amplified polymorphic DNA (RAPD)

PCR is done with a single short primer that hybridizes to many places in a genome. Occasionally, 2 primers hybridize to complementary strands near each other generating a PCR product. Different organisms of a species have slightly different DNA sequences which yield mostly the same PCR products, but a few PCR products are gained or lost.

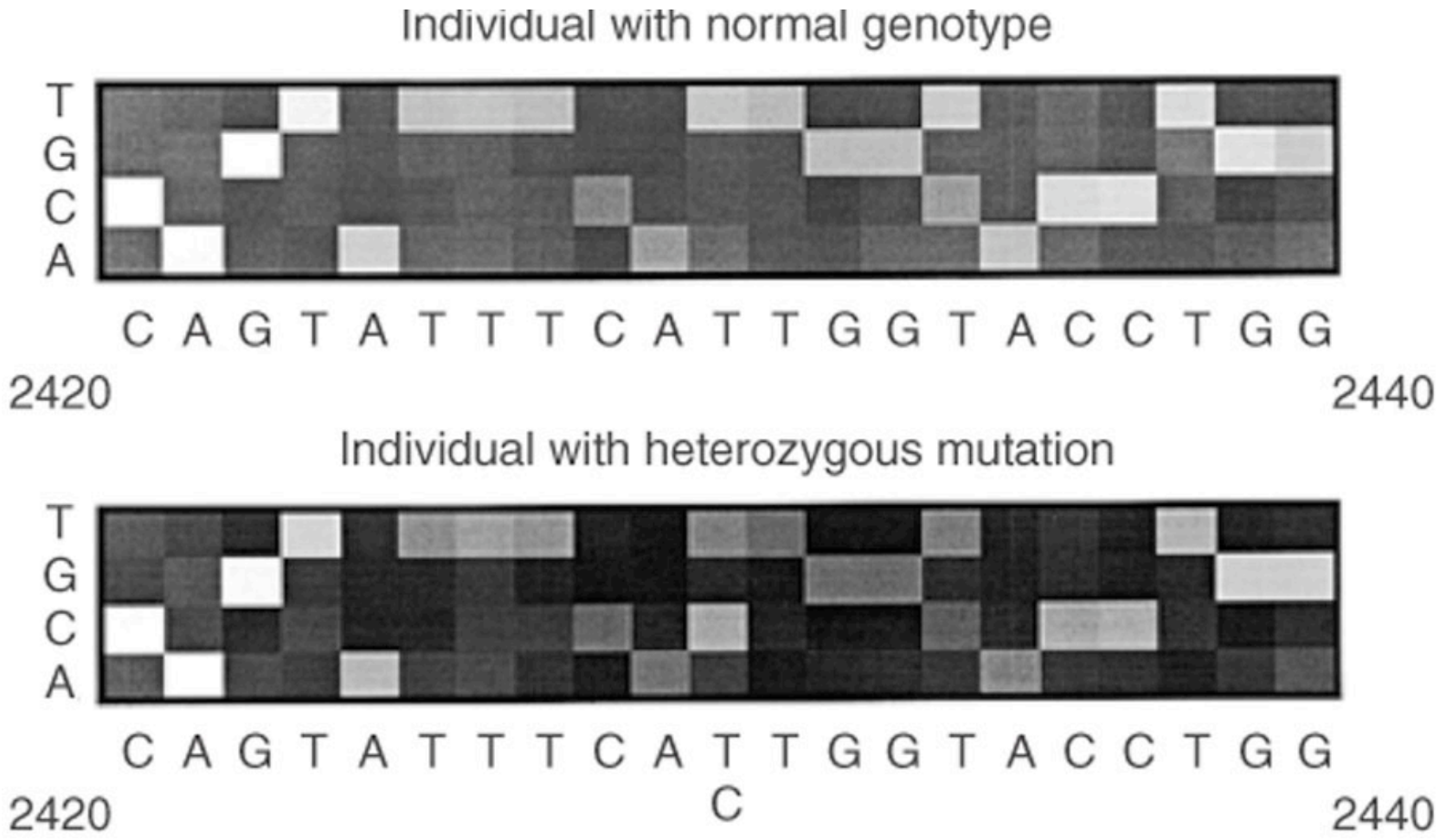


SNP detection by ASO



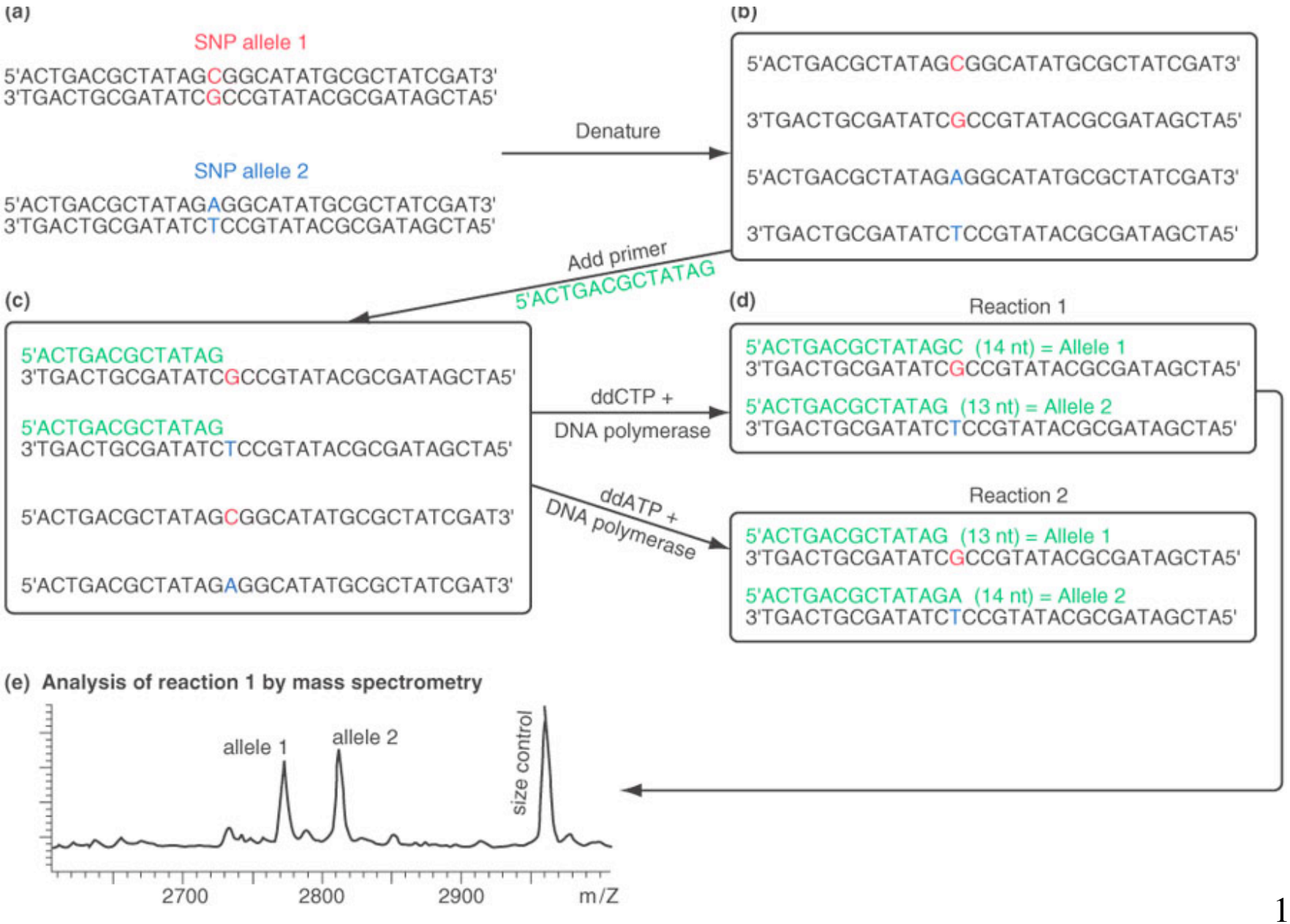
- Very short probes (<21 bp) specific which hybridize to one allele or other
- Such probes are allele-specific oligonucleotides (ASOs)

Large-scale ASO analysis with microarrays



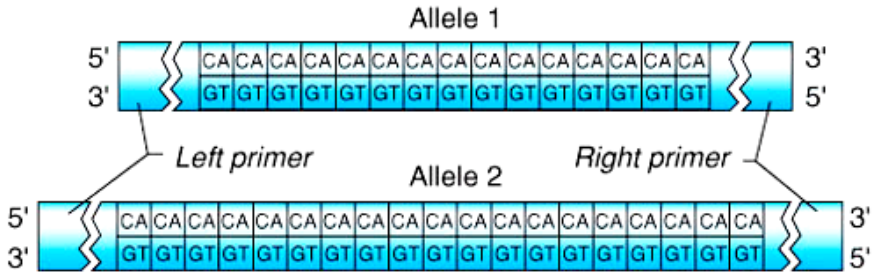
Each column contains an ASO differing only at the nucleotide position under analysis

Primer extension to detect SNPs

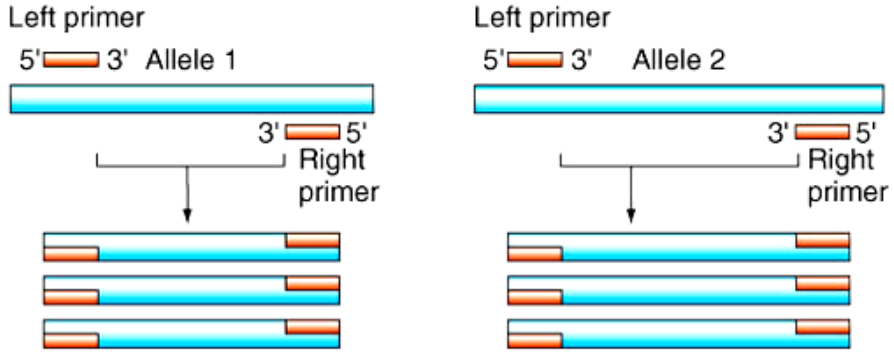


Detecting microsatellite markers

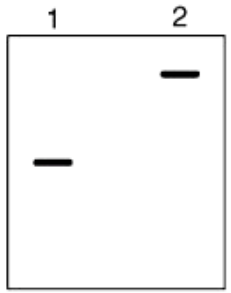
(a) Determine sequences flanking microsatellites.



(b) Amplify alleles by PCR.



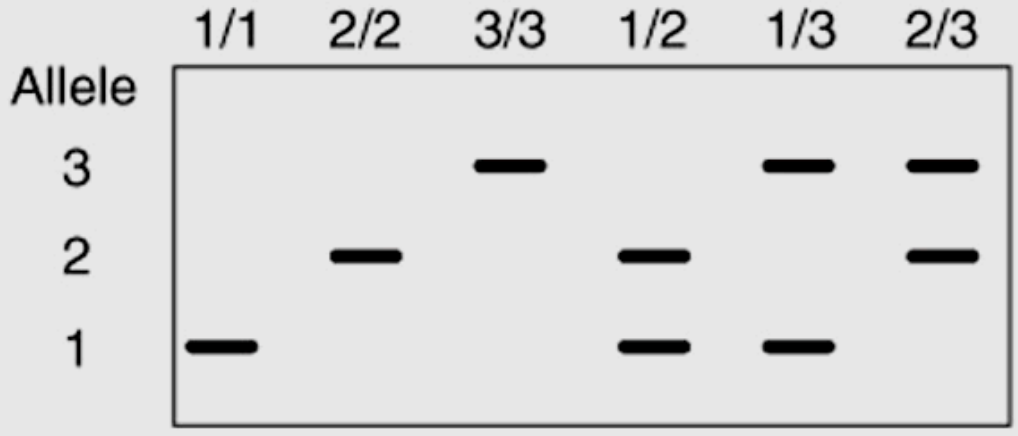
(c) Analyze PCR products by gel electrophoresis and staining.

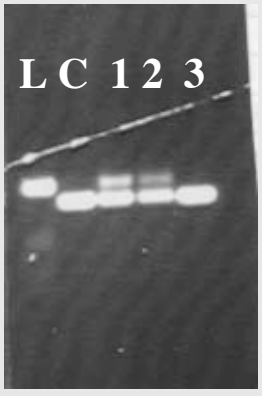


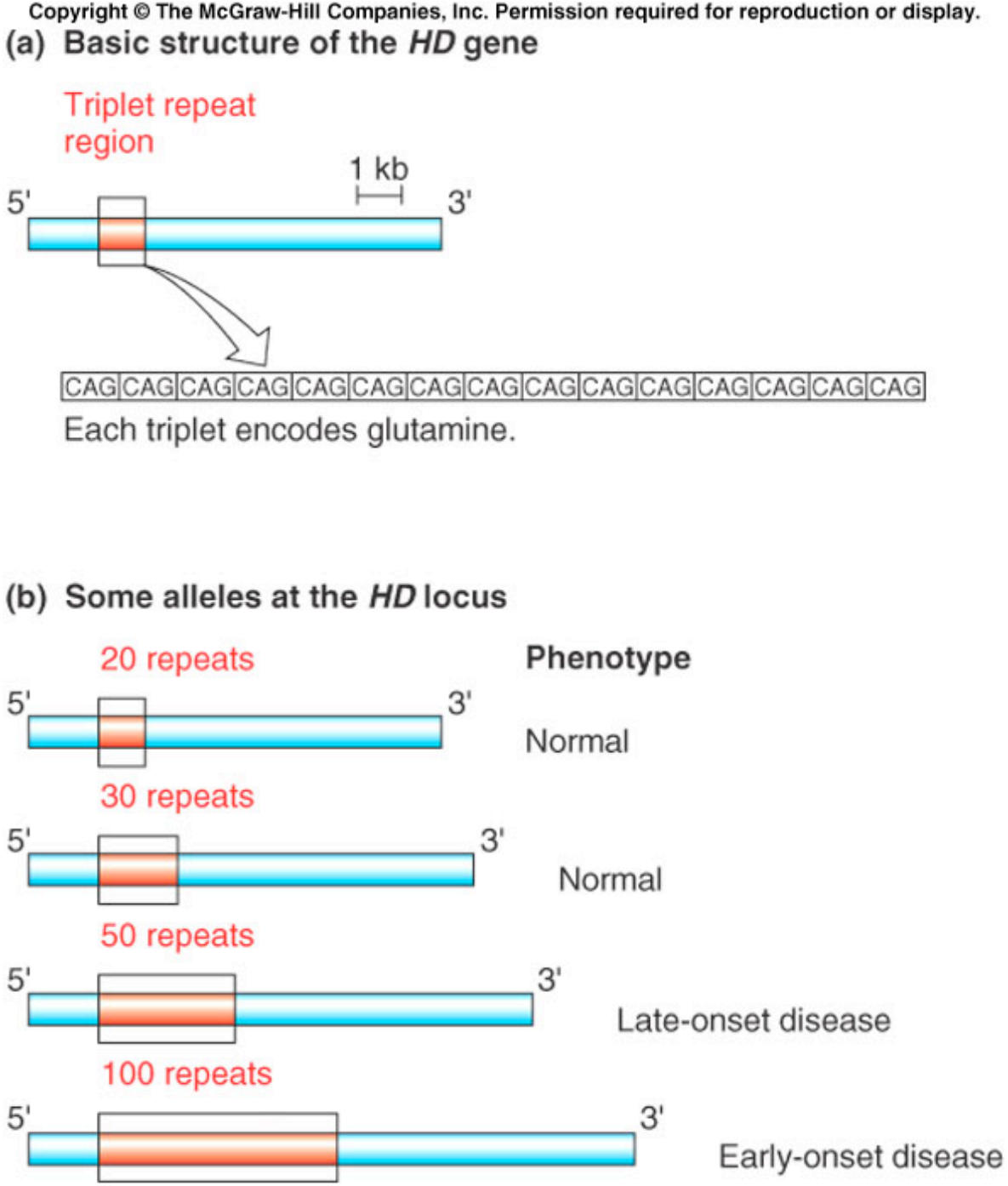
Microsatellite allele detection by PCR

(d) Example of population with three alleles.

Six diploid genotypes are present in this population.



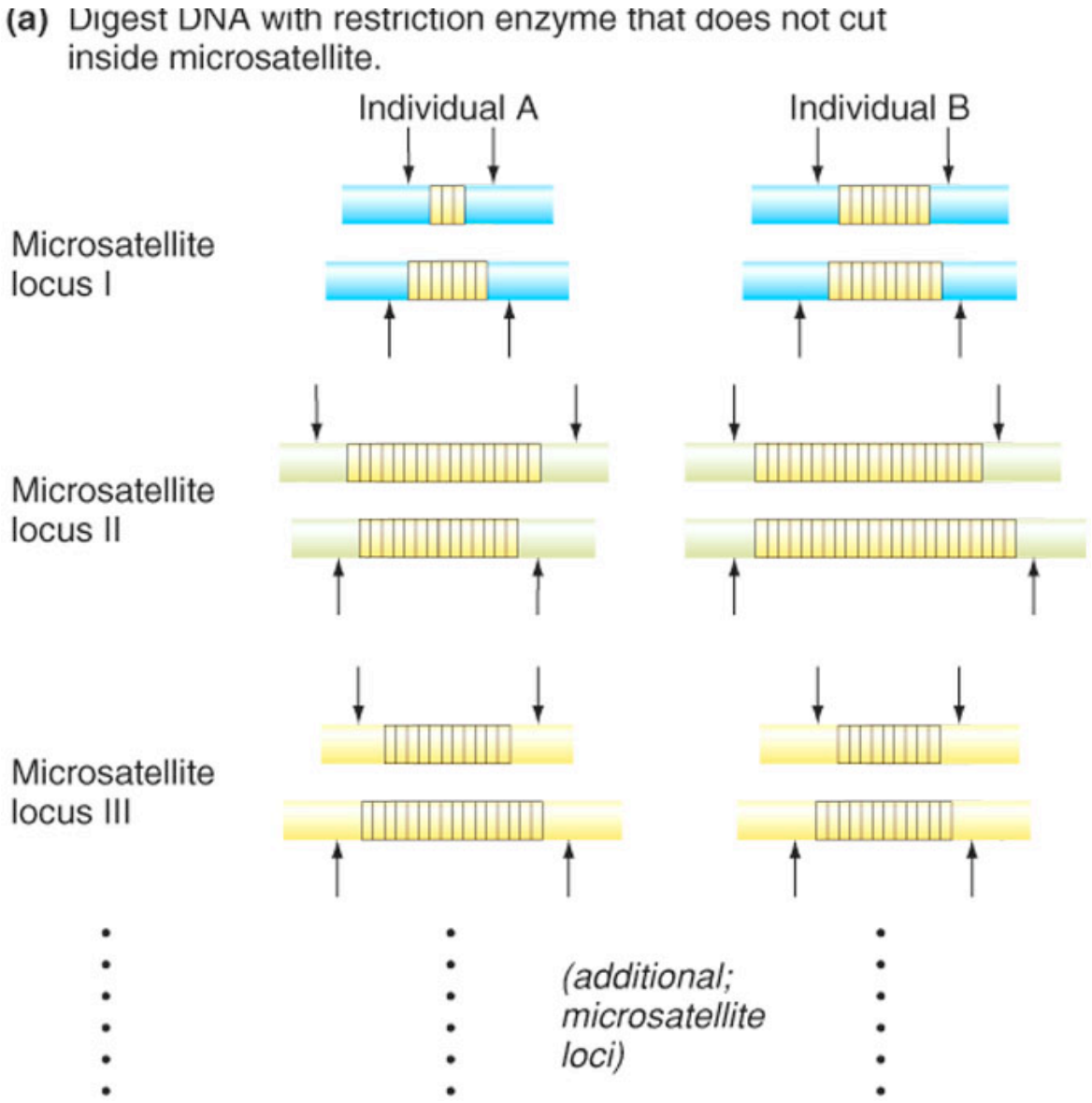




Huntington's disease is an example of a microsatellite triplet repeat in a coding region

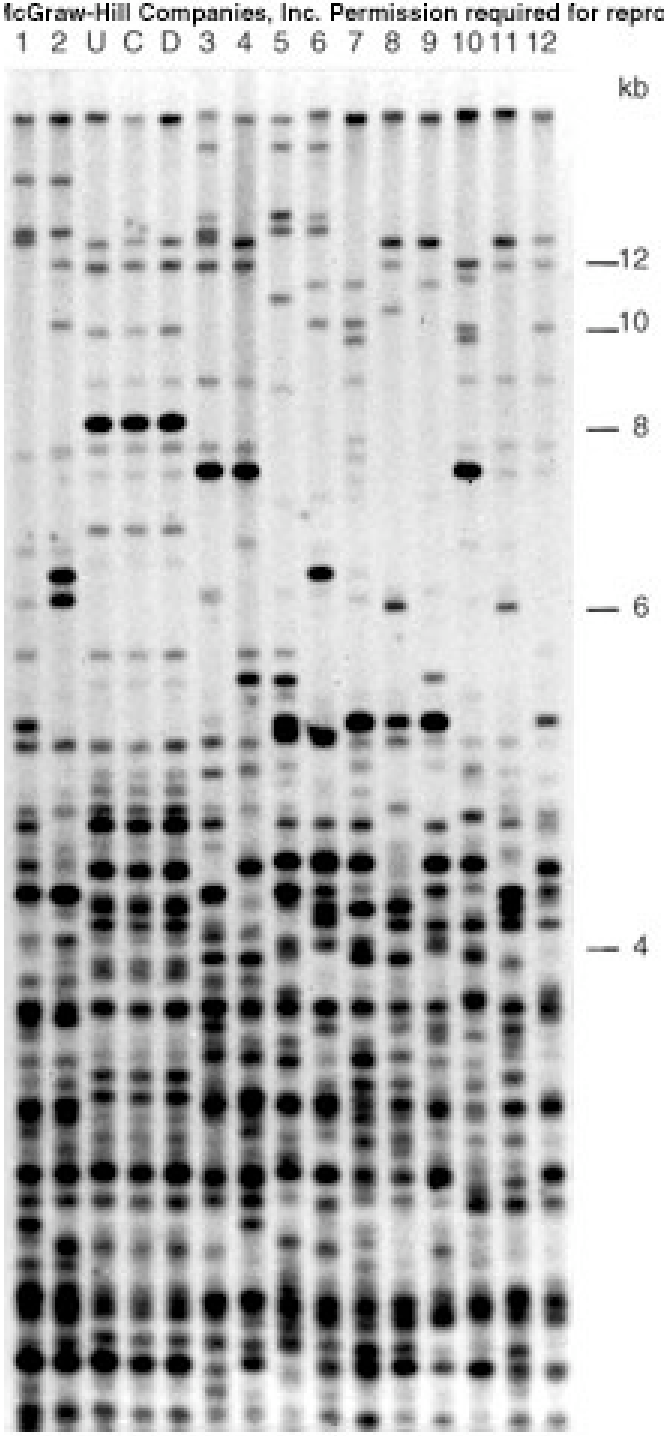
Minisatellite detection and DNA fingerprinting

- 1985 – Alec Jeffreys made two key findings
  - Each minisatellite locus is highly polymorphic
  - Most minisatellites occur at multiple sites around the genome
  - DNA fingerprint – pattern of simultaneous genotypes at a group of unlinked loci
  - Use restriction enzymes and southern blots to detect length differences at minisatellite loci
  - Most useful minisatellites have 10 – 20 sites around genome and can be analyzed on one gel



- Minisatellite analysis

Fig. 11.14



Dolly the sheep is a clone of adult udder cell

- DNA fingerprints can identify individuals and determine parentage
- E.g., DNA fingerprints confirmed Dolly the sheep was cloned from an adult udder cell
- Donor udder (U), cell culture from udder (C), Dolly's blood cell DNA (D), and control sheep 1-12