Molecular genetics II

winter semester

4th week (October 27th – 31st, 2008)

Institute of Biology and Medical Genetics 1st F .M. and VFN, Praha

DNA diagnostics

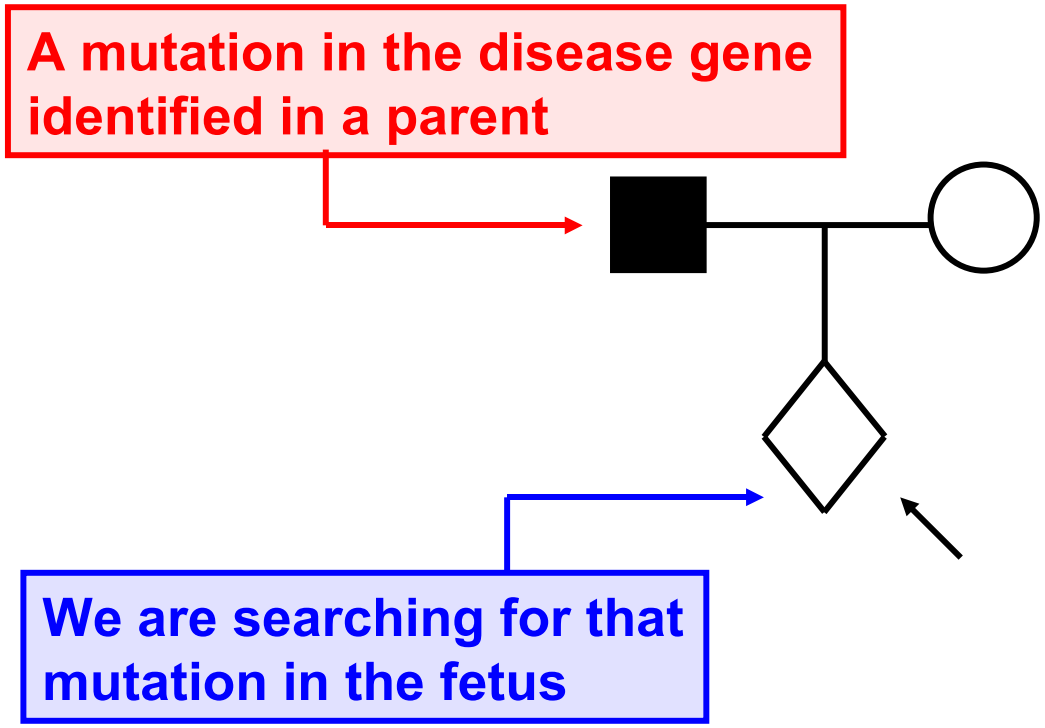
- prenatal
- presymptomatic
- Clinical diagnosis confirmation
- Carrier detection

According to the targeting in the genome:

Direct DNA diagnostics	–	Determination of the allele (mutation) responsible for the disease (syndrome)
Indirect DNA diagnostics	–	Determination of an allele (marker) that shows genetic linkage to the disease locus

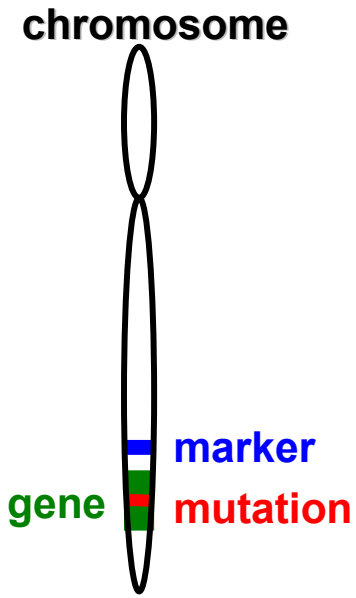
Direct DNA diagnostics

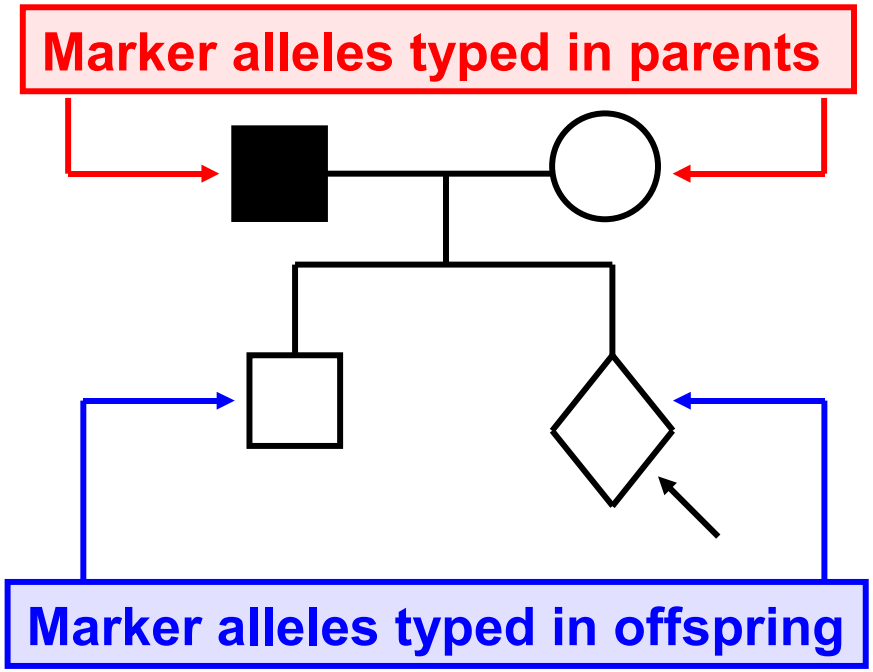
- We are searching for an allele (variant, mutation) that is responsible for the disease phenotype in the family
- For example, in AD disease, prenatal diagnosis:



Indirect DNA diagnostics

- It is not necessary to know, which mutation in the gene is responsible for the disease, given we know, where the gene is localized on a chromosome
- We use genetic linkage between known marker that is placed along the chromosome in vicinity to the disease gene (known or unknown) with unknown causative mutation in the gene. Which marker allele cosegregates with the disease? Has the person in question got this allele?





Several markers are usually assayed

Indirect DNA diagnostics

- It is not necessary to know, which mutation in the gene is responsible for the disease, given we know, where the gene is localized on a chromosome
- We use genetic linkage between known marker that is placed along the chromosome in vicinity to the disease gene (known or unknown) with unknown causative mutation in the gene.
  Which marker allele cosegregates with the disease? Has the person in question got this allele?

MAIN PITFALL of indirect DNA diagnostics: In different individuals, different marker alleles are linked to the mutated disease allele.

Vice versa, the same marker allele may be once linked to the disease allele, once to the standard allele.

Therefore, the marker may be not informative, or may be only partially infomative

A minor difficulty – a possible recombination between the marker and the gene. However, the probability is small and can be estimated with reasonable accuracy.

Variability on DNA level

- In the population, certain piece of DNA seauence exists in several forms (alleles)
- The variable site may be situated in a gene (in exons – coding sequence, in introns – noncoding sequence) or outside genes (i .e. always noncoding sequence)

Sorting according the allele frequency:

polymorfism – The population frequency of the less common allele is > 1%

„mutation“, „rare allele“ – Population frequency of such allele is < 1%

Sorting according the functional impact:

functional variant – The allele influences the phenotype (at least in some combination (recessive alleles)

silent variant – The allele has no functional consequence

Variability on DNA level

Types (that can be assayed):

SNPs – Single nucleotide polymorphisms

tandem repeats – Microsatellites, also dubbed STRs = short tandem repeats; minisatellites

Structural polymorfisms – Insertions, deletions, inversions (ranging from 1 bp to chromosome)

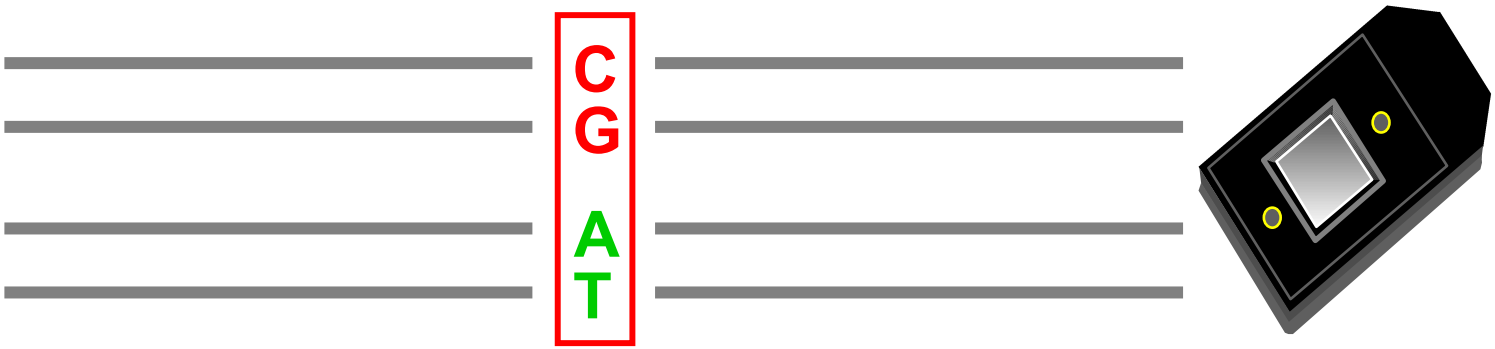
Duplications, „low copy repeats“ – Actually a kind of tandem repeats, but differing by detection techniques and functional significance; e.g. *FCGR3B* and glomerulonephritis risk, *RhD* a *RhCE* , red and green opsins on chromosome X, etc.

SNPs – single nucleotide polymorphisms

SNP

C / A

TAGCCATCGGTA**N**GTACTCAATGATCAGCT



99.9% DNA sequence is identical between any two chromosomes (individuals). Of the remaining 0.1% difference, more than 80% is represented by SNPs. It is technically feasible now to type 1 000 000 SNPs in a single DNA sample, which should expedite identification of the alleles responsible for a wide range of common diseases.

Methods for SNP detection

Mikroarrays (en masse), RFLP (one by one; Southern blot, PCR-RFLP)

Many non-RFLP methods (SSCP, allele-specific primers, …….)

A „special“ subset of SNPs:

RFLP = restriction fragment length polymorphism

Restriction endonucleases are bacterial enzymes that cleave DNA at a specific sequence.
They protect bacteria against bacteriophage infection (or perhaps have no function)

MaeII

TAGCCATCGGTA**C**GTACTCAATGATCA
ATCGGTAGCCA**T**GCATGAGTTACTAGT

TAGCCATCGGTA**A**GTACTCAATGATCA
ATCGGTAGCCAT**T**CATGAGTTACTAGT

Nomenclature of the restriction endonucleases

Eco RI

Three-letter organism abbreviation

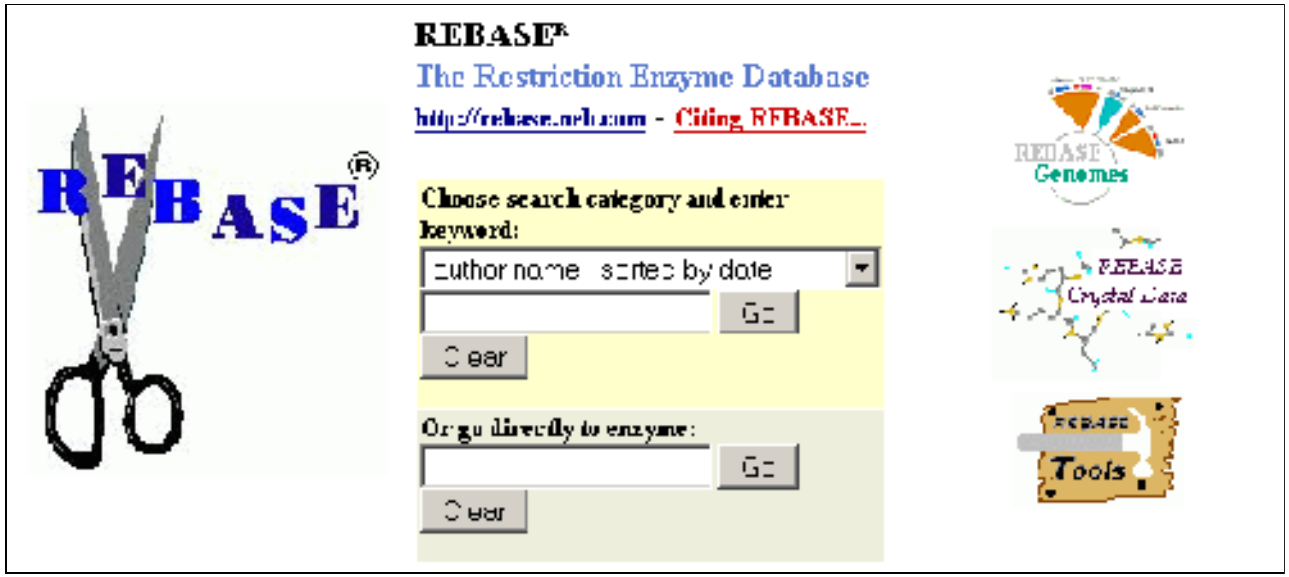
„Escherichia coli“

More detailed data on the origin

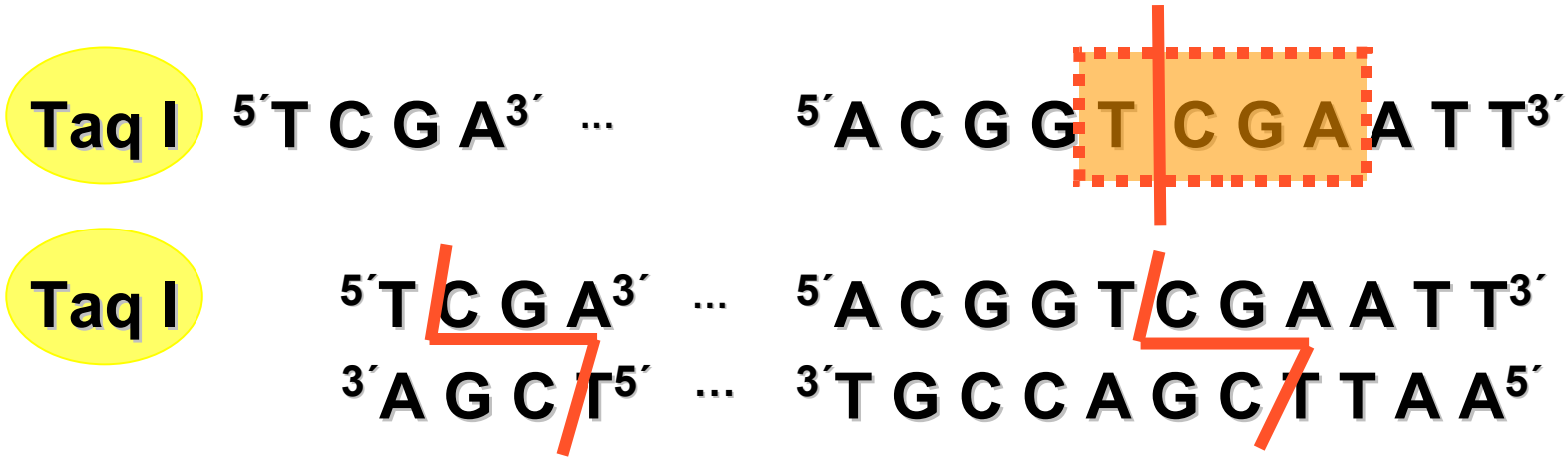
R = enzyme from strain RY13,

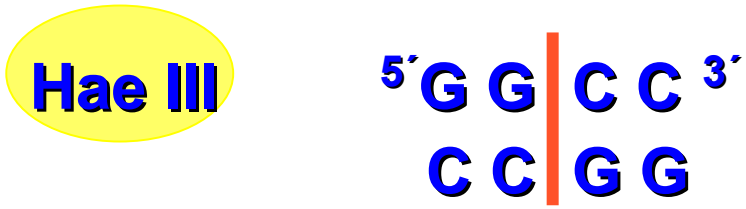
determined by R-plasmid, number I

http://rebase.neb.com

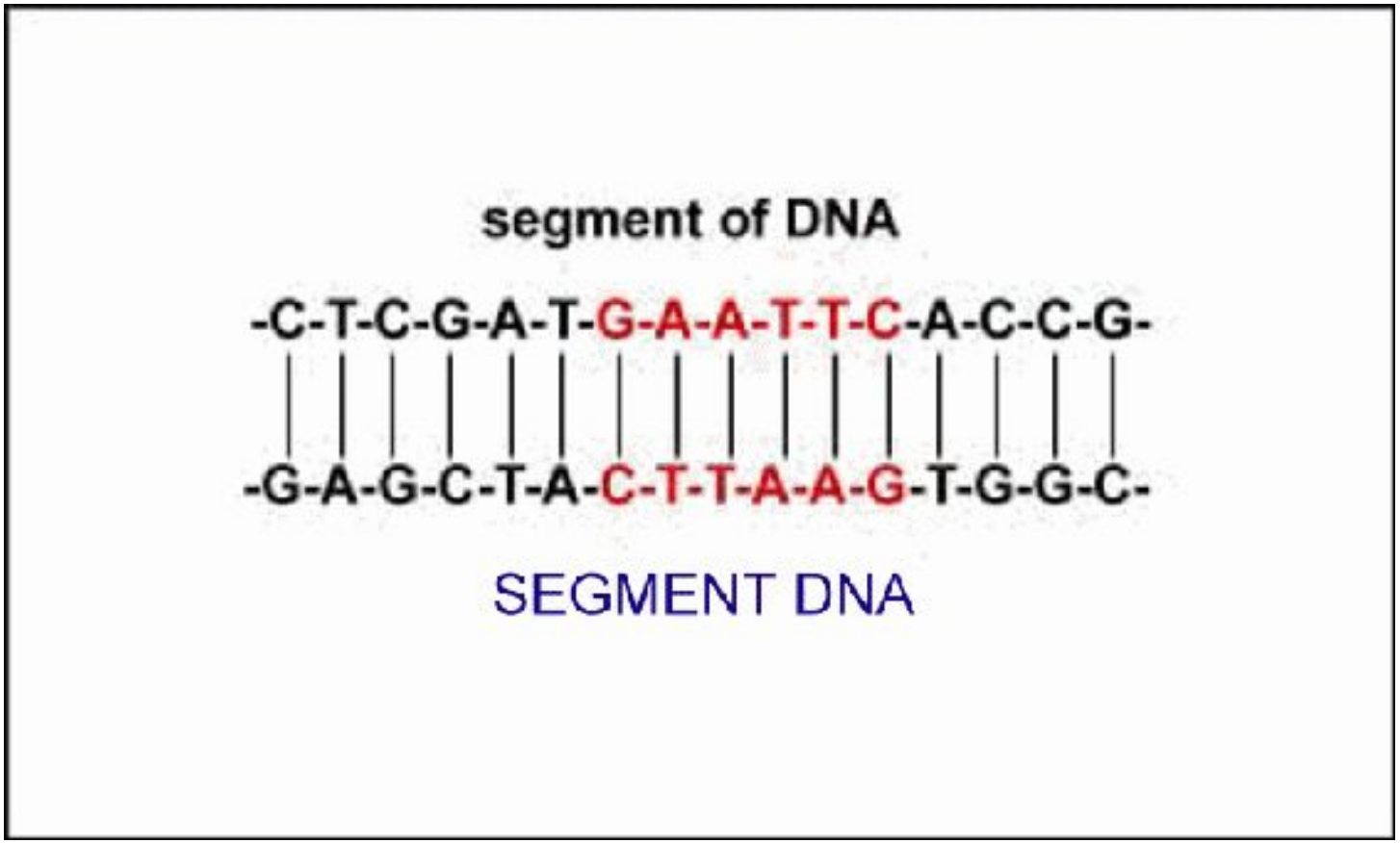


Each restriction endonuclease recognizes and cleaves a specific DNA sequence – restriction site





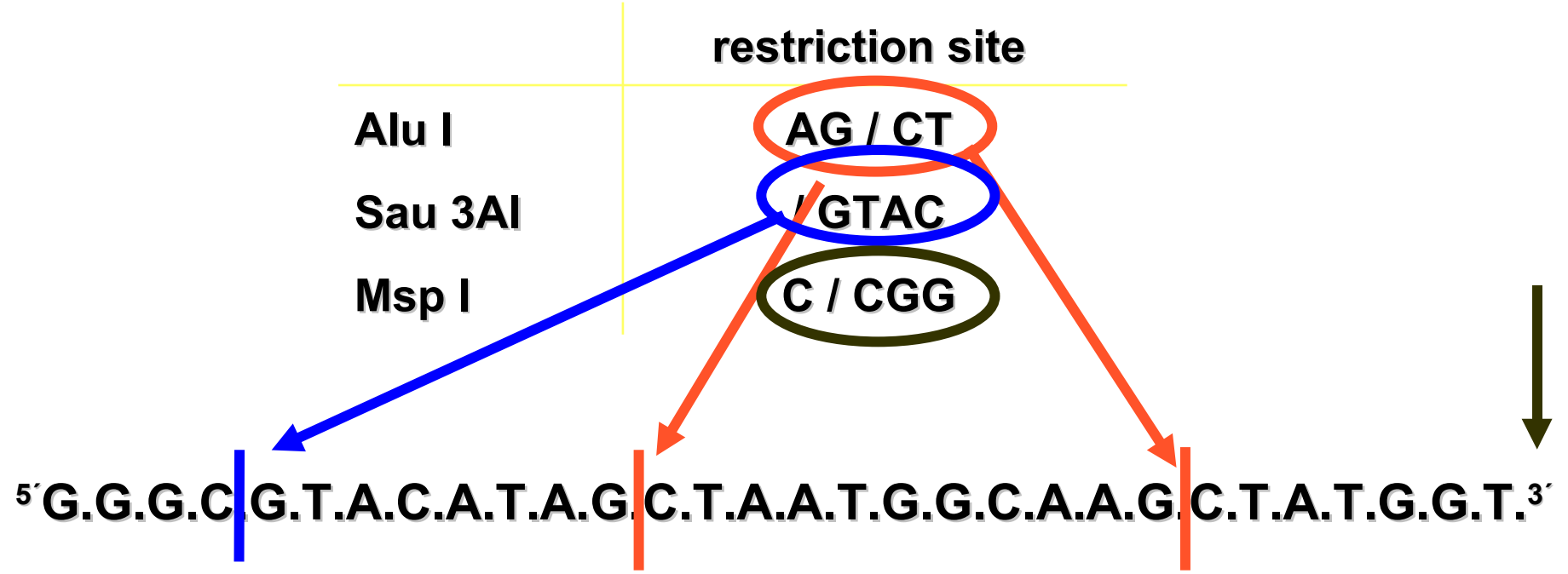
Cleavage of dsDNA by restriction endonuclease EcoRI



Identification of the restriction sites

Task 1, p. 112

Find the restriction sites for the given restriction endonucleases:



Identification of the restriction sites

Task 1, p. 112

Find the restriction sites for the given restriction endonucleases:

	restriction site	
Alu I	AG / CT	2x
Sau 3AI	/ GTAC	1x
Msp I	C / CGG	0

5´G.G.G.C|G.T.A.C.A.T.A.G|C.T.A.A.T.G.G.C.A.A.G|C.T.A.T.G.G.T.3´

Identification of the restriction sites

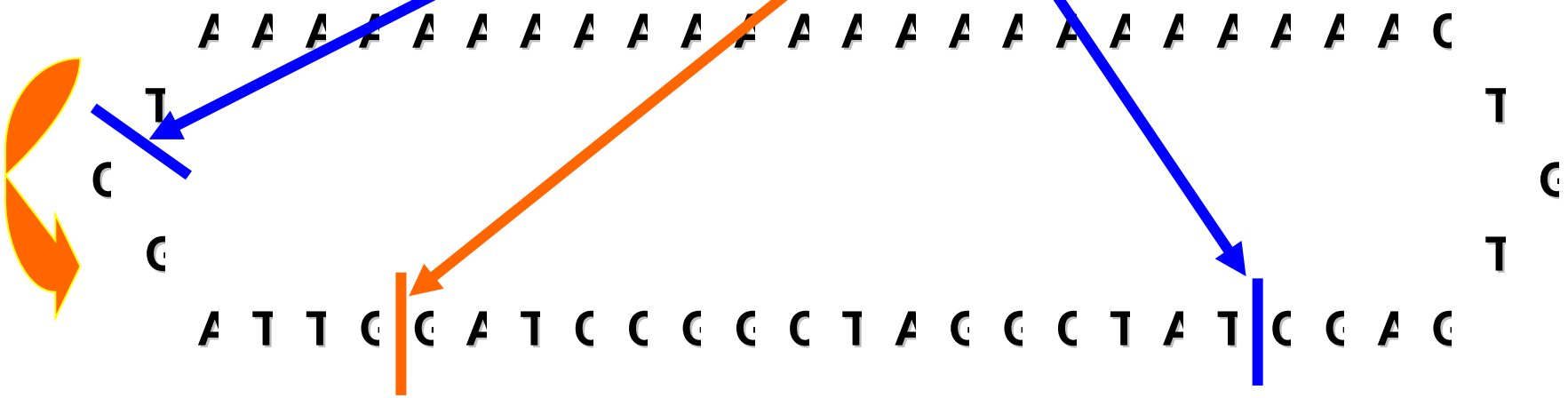
Task 2, p. 112

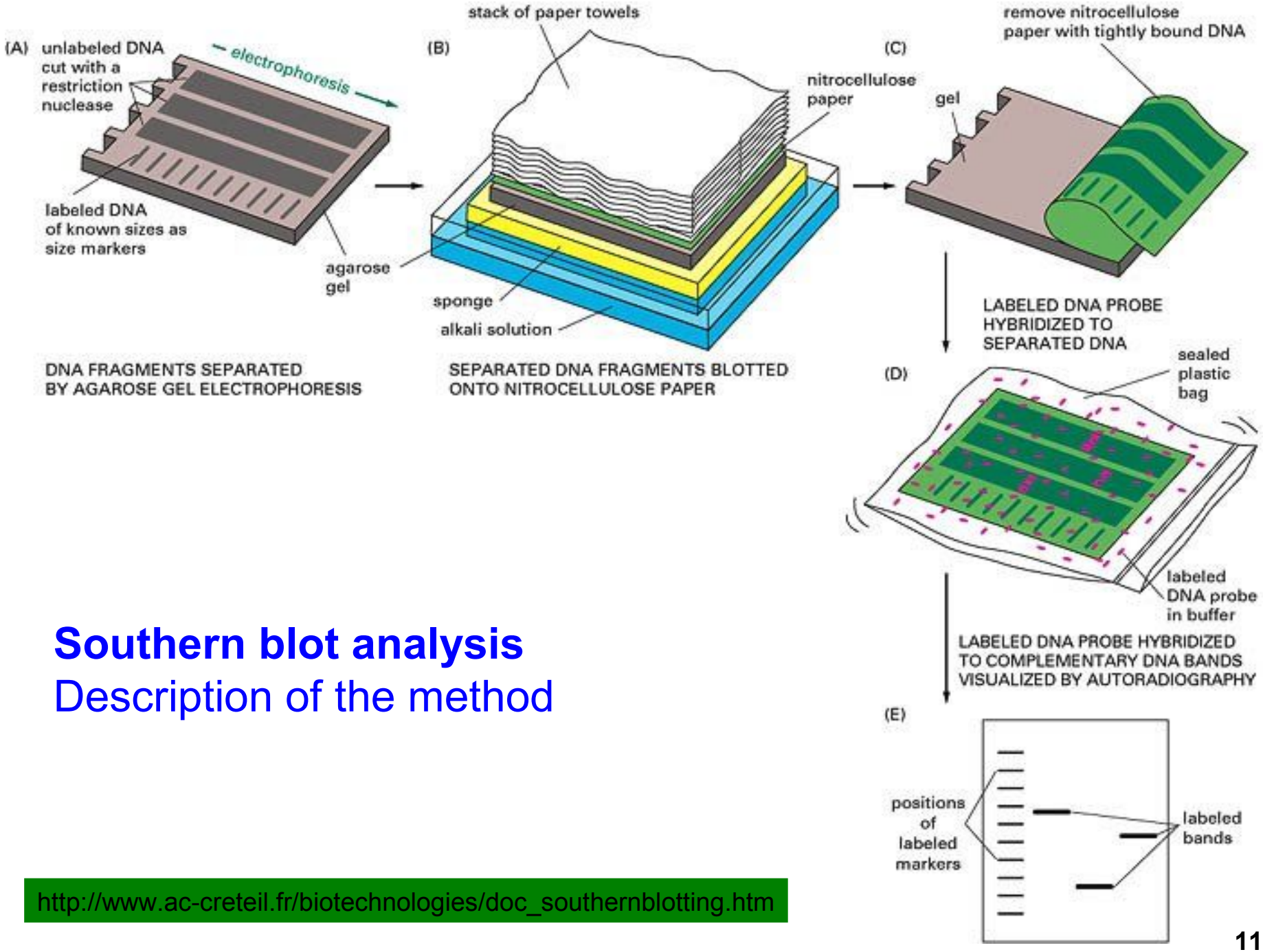
Which of the given enzymes can be used:
a) for cutting the given circular DNA to linearize it?
b) for cutting out a fragment containing a poly-A sequence?

	restriction site
Taq I	T / CGA
Pvu I	CGAT / CG
Bam HI	G / GATCC

2 sites - excision

1 site - linearization





Southern blot analysis

Description of the method

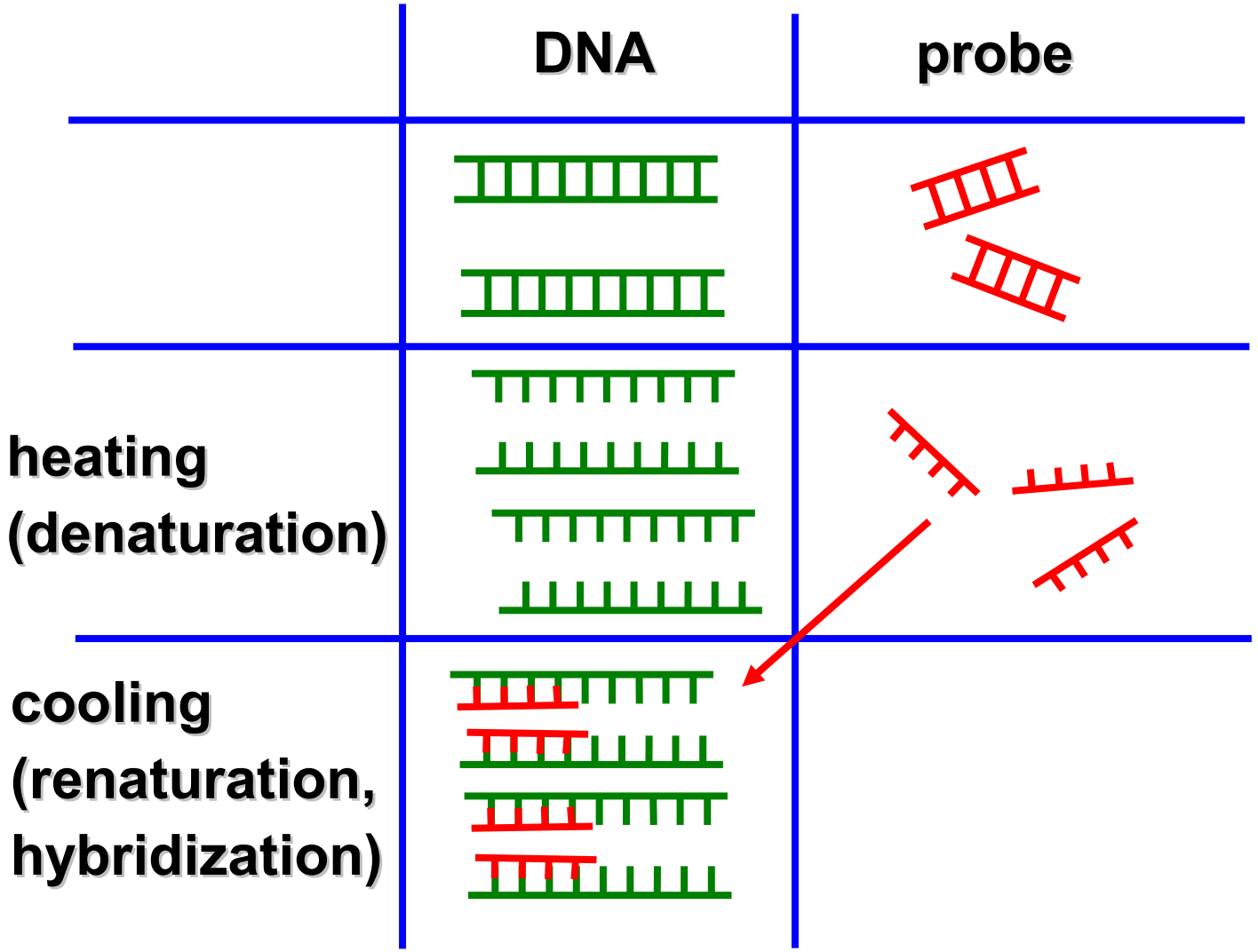
http://www.ac-creteil.fr/biotechnologies/doc_southernblotting.htm

Southern blot – animation

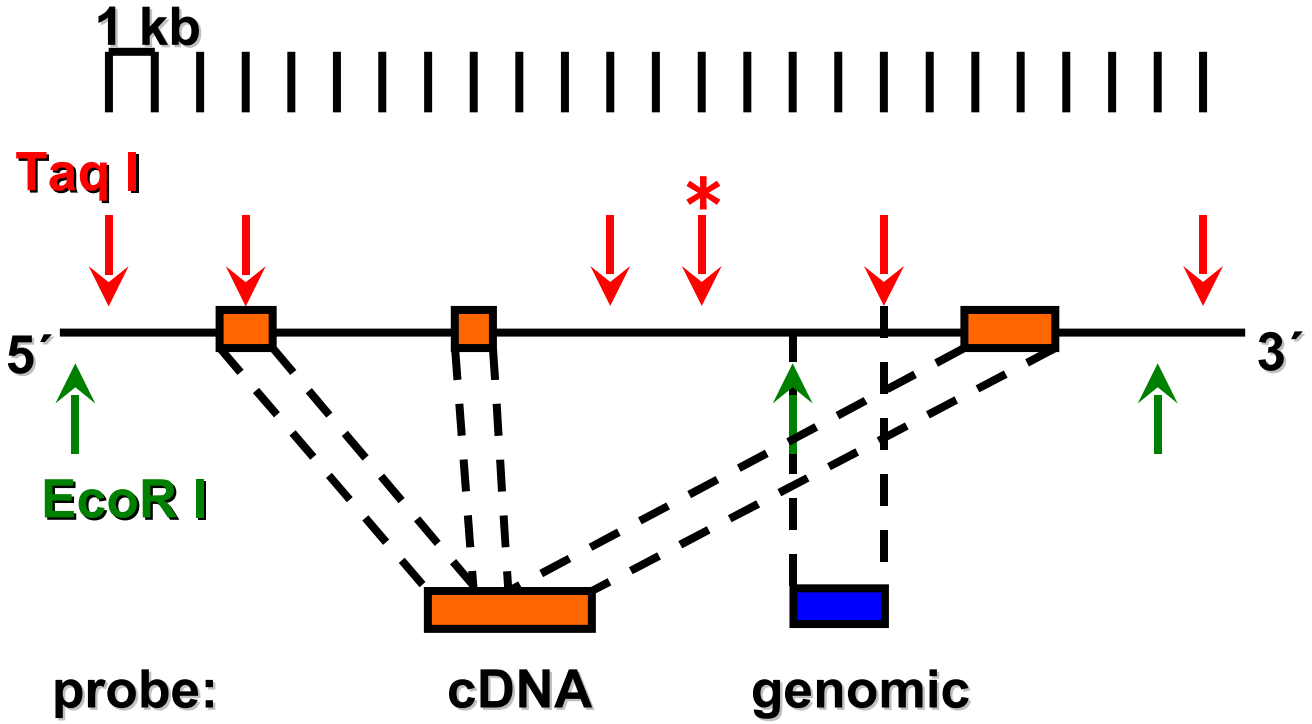
external file

http://www.ac-creteil.fr/biotechnologies/doc_englishbiomol.htm

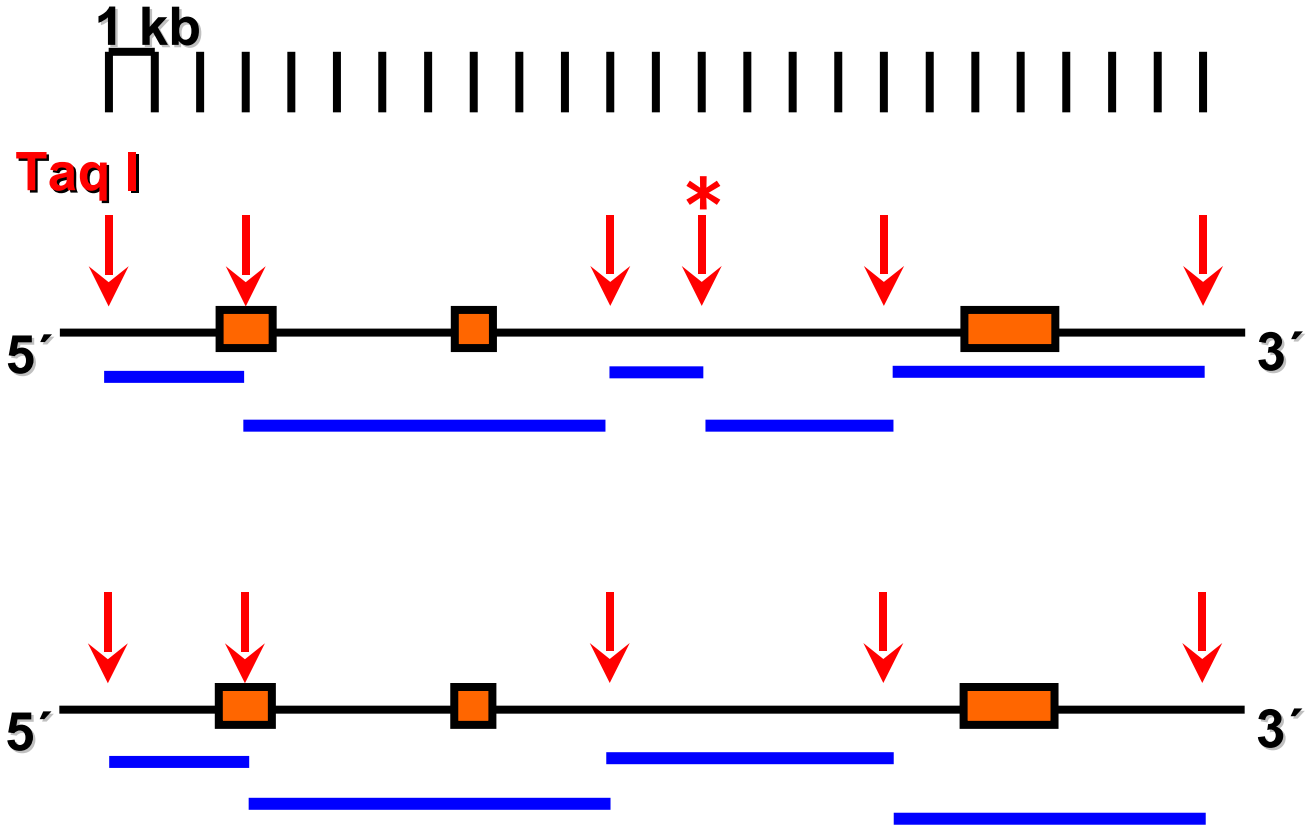
Southern blot – probe hybridization



Task 4, p. 114 Gene G



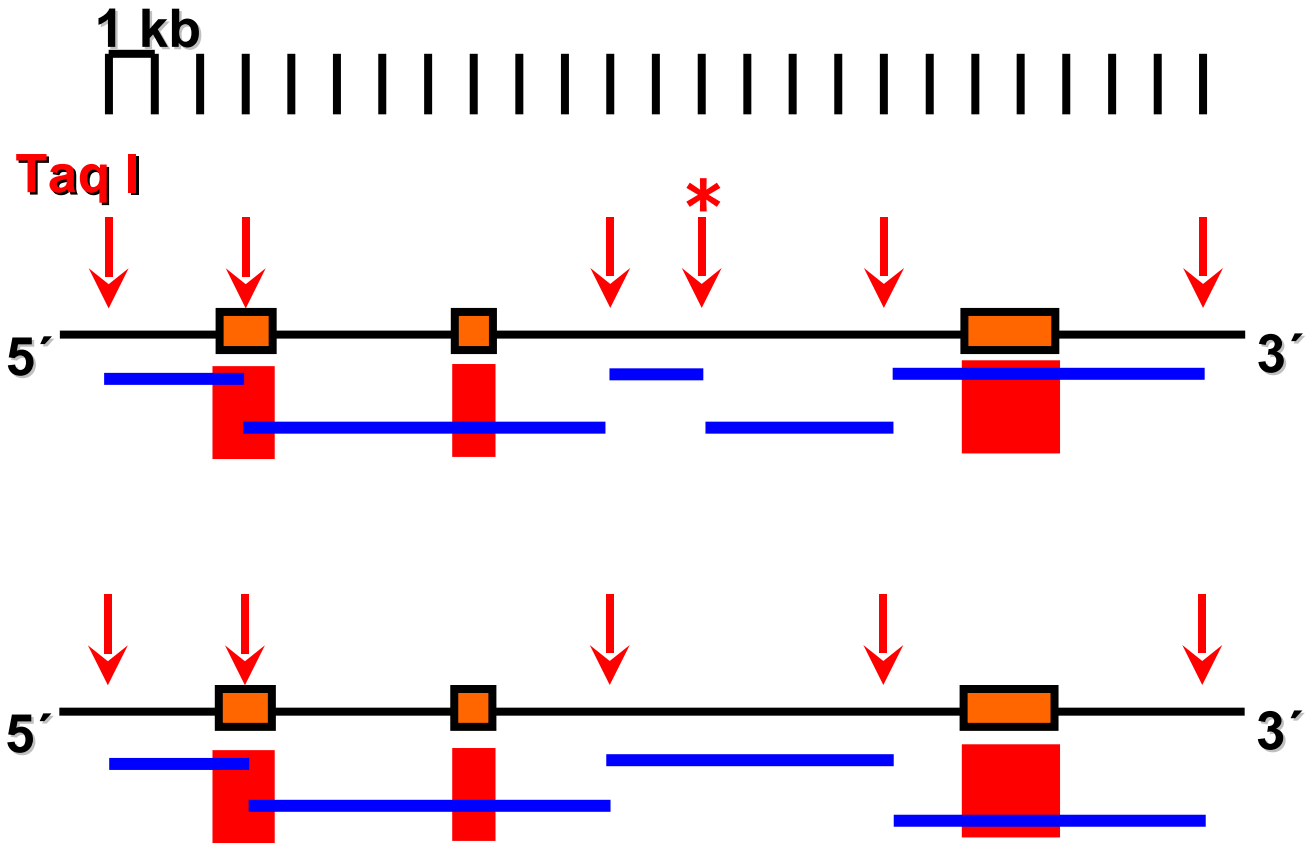
Task 4, p. 114 Gene G, cleavage



Results:

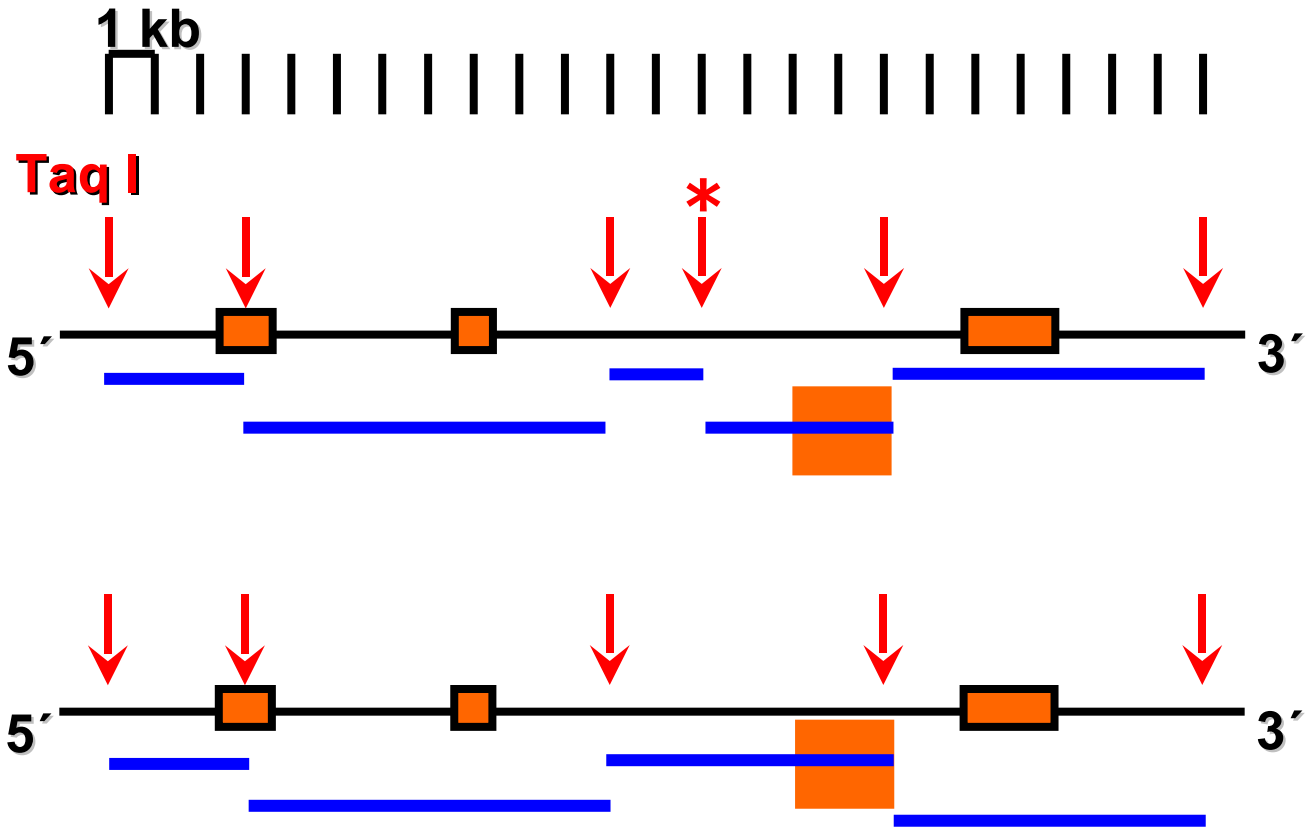
a) 4 or 5 fragments: 3kb, 8kb, 6kb/2kb+4kb, 7kb

Task 4, p. 114 Gene G, Southern blot with a cDNA probe



Results: b) 3 fragments: 3kb, 8kb, 7kb c) no

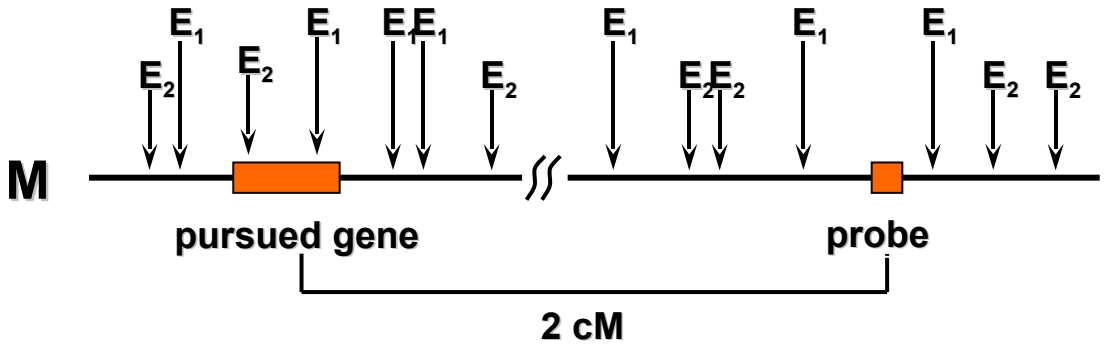
Task 4, p. 114 Gene G, Southern blot with a genomic probe

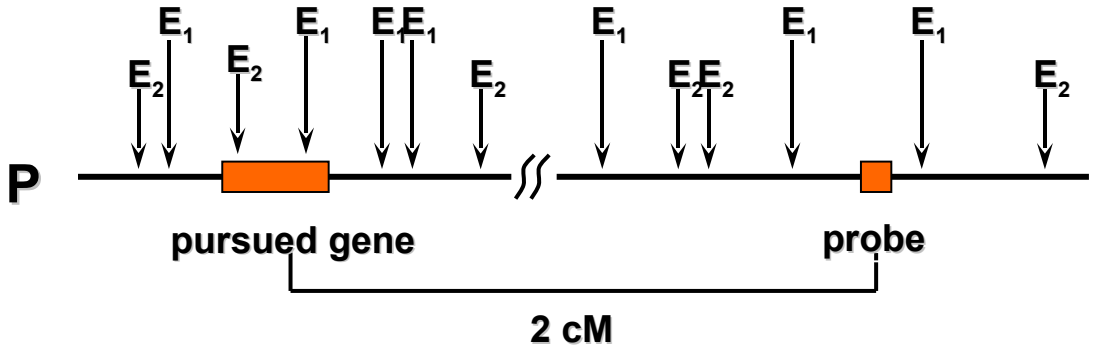


Results:

d) Yes 4kb (allele +) 6kb (allele -)

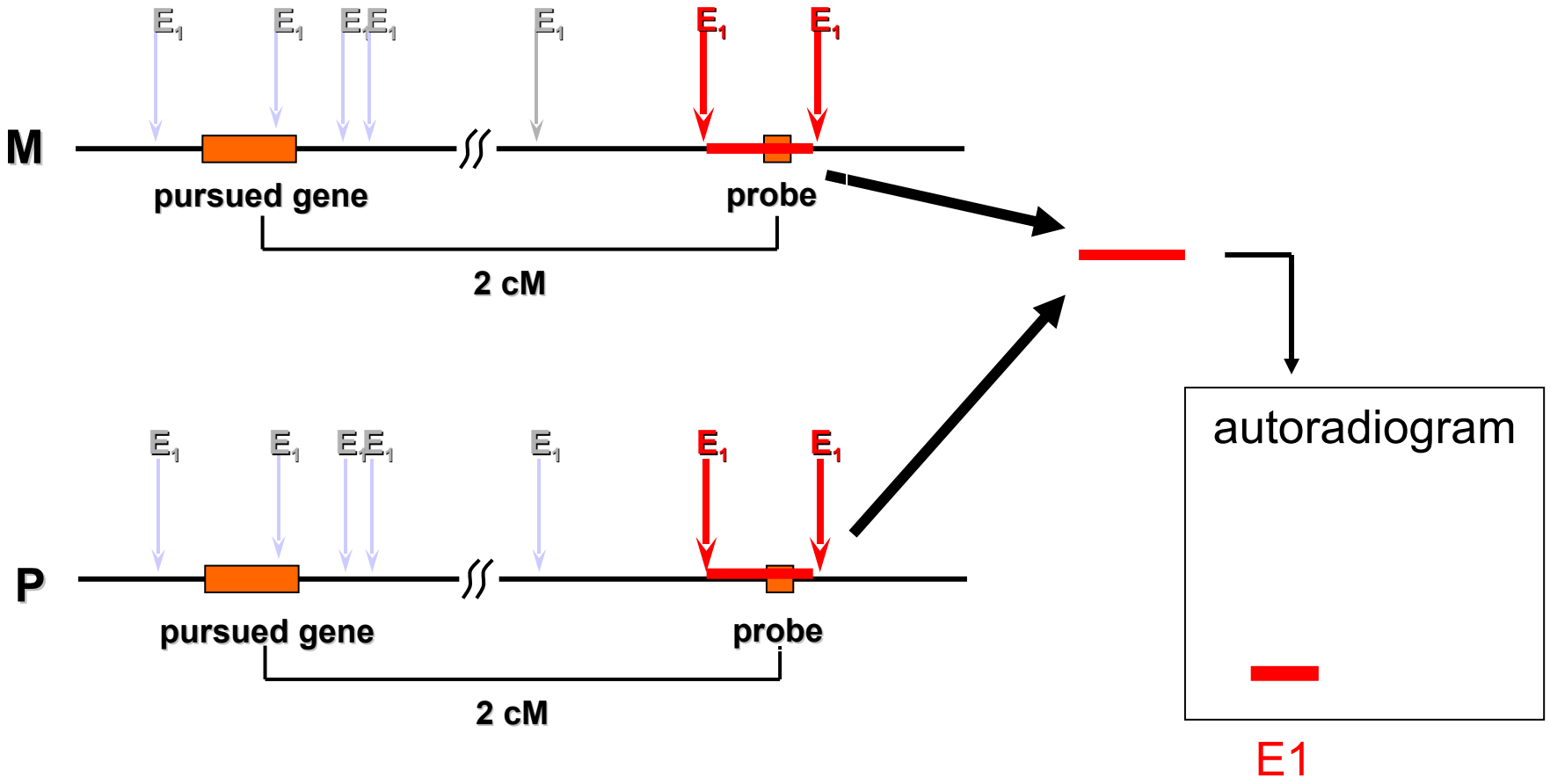
Extragenic probe





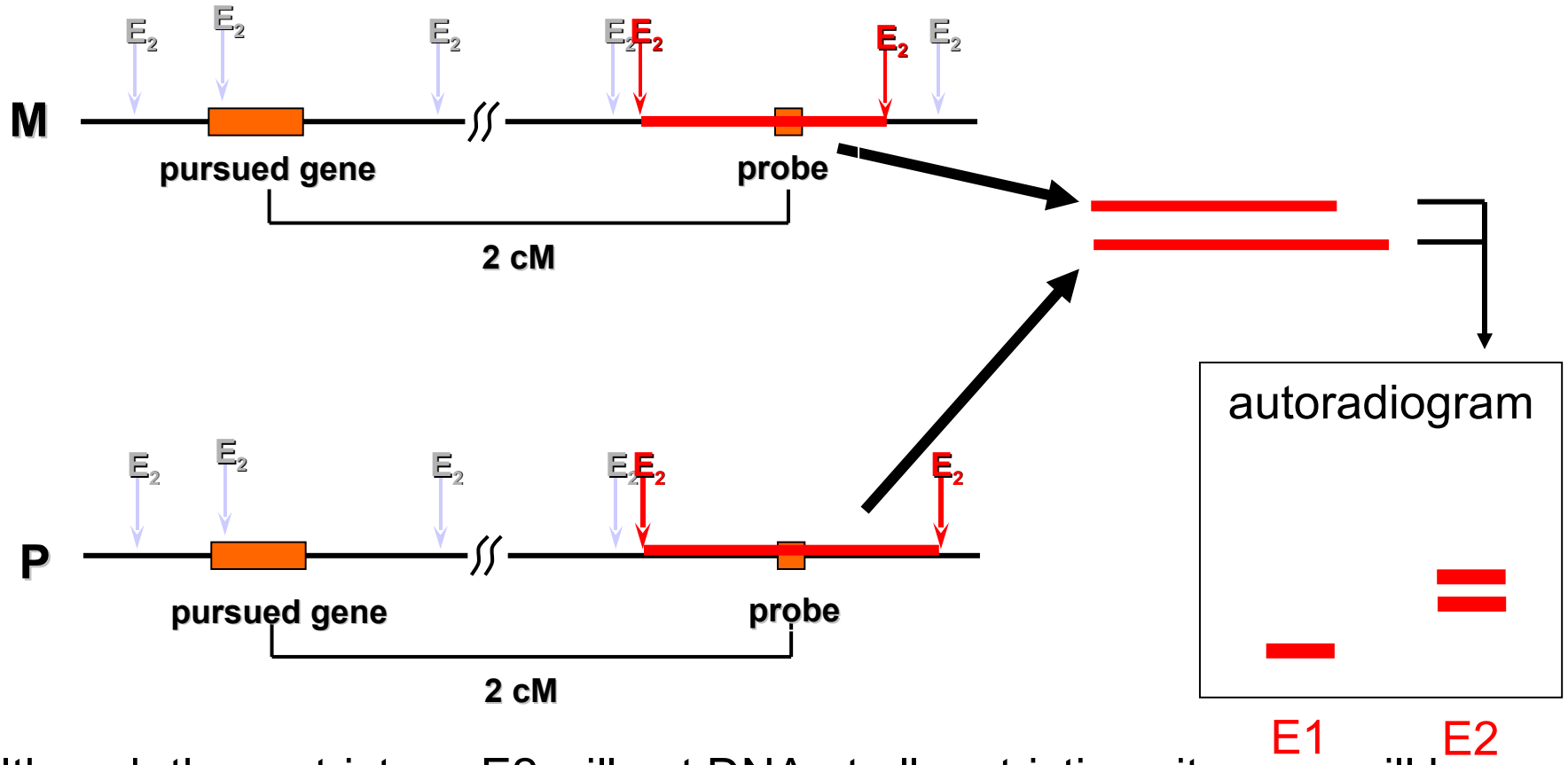
E1 – restriction endonuclease 1
E2 – restriction endonuclease 2
M – fragment of maternal chromosome
P – fragment of paternal chromosome
↓ - restriction site (restriction endonuclease cleaves DNA here)
2cM – linkage map distance between gene and extragene probe

Extragenic probe – cutting with E1 enzyme



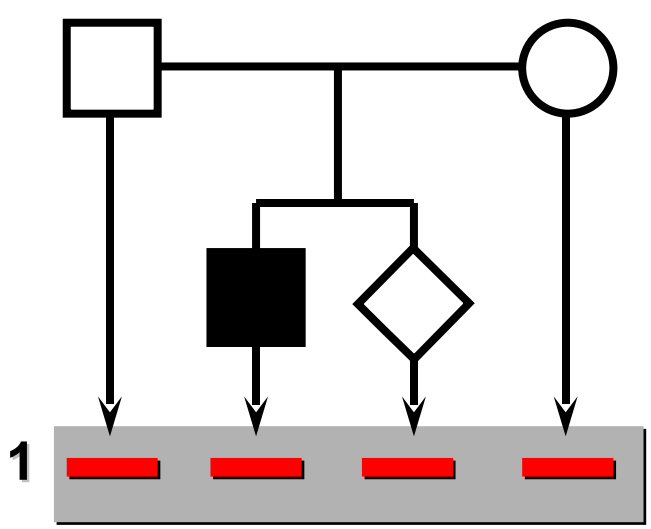
Although restriction endonuclease E1 will cut DNA at all restriction sites, we will be able to detect on the autoradiogram only fragments labeled by the hybridized probe. These are of the same length on both chromosomes, the individual is thus a **homozygote in restriction fragment length.**

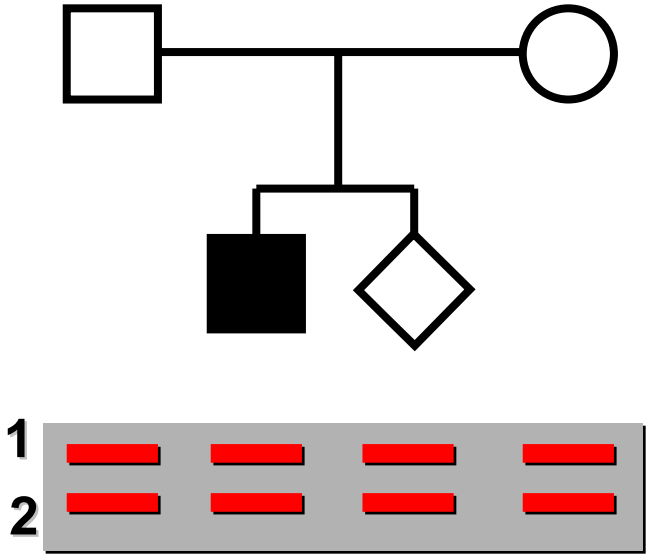
Extragenic probe – cutting with E2 enzyme



Although the restrictase E2 will cut DNA at all restriction sites, we will be able to detect only the fragments labeled by the hybridized probe. The fragments are of different length on maternal and paternal chromosomes, the individual is thus a

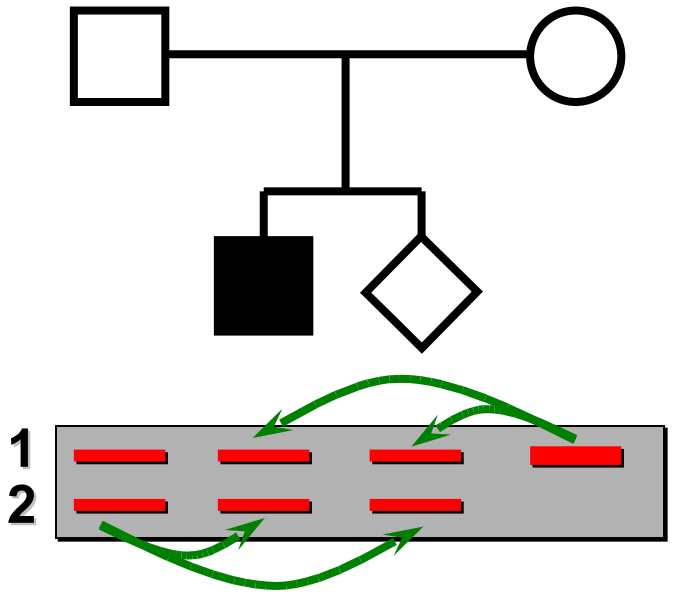
heterozygote for the length of restriction fragments.

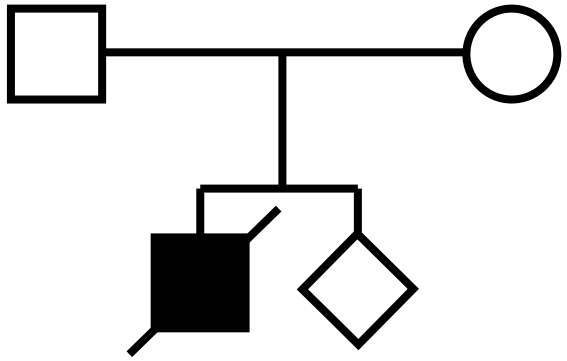




The family is noninformative. All family members are homozygotes in length of the restriction fragments. (the parents are heterozygotes in the gene of interest). However, we do not know, which allele of the gene was transmitted with allele 1 of the RFLP.

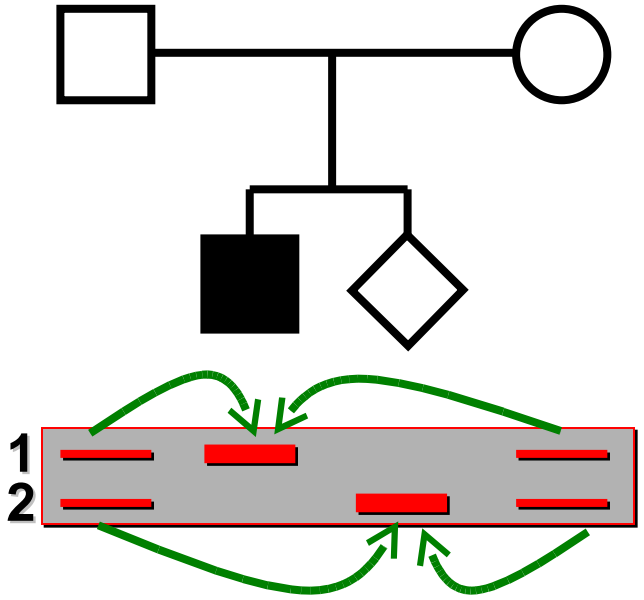
This family is only partially informative. It is not possible to distinguish the origin of RFLP fragment with mutant allele between mother and father. However, the second child is a homozygote (AA or aa)



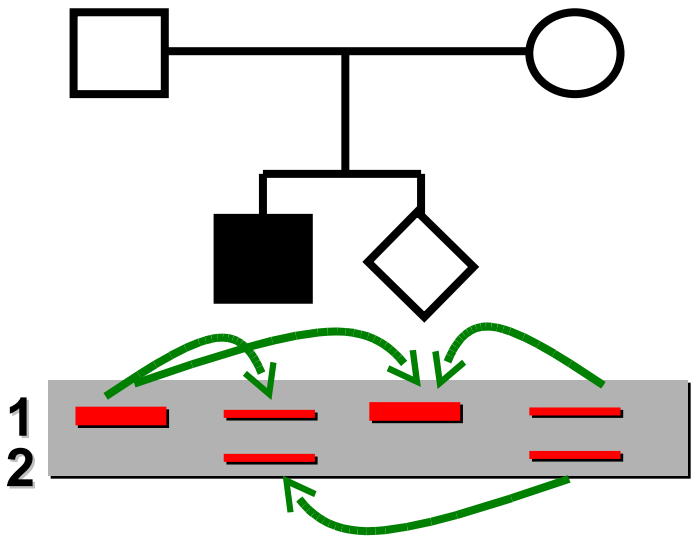


It is clear that father´s RFLP fragment 2 indicates the presence of the mutant allele. Mother can only pass fragment 1 either with mutant or normal allele. There is 50% probability that the second child will be affected and 50% that he/she will be a carrier.

The RFLP DNA analysis will not be successful in this case, because DNA of the dead son is not available. Risk for next child is Mendelian 25%.

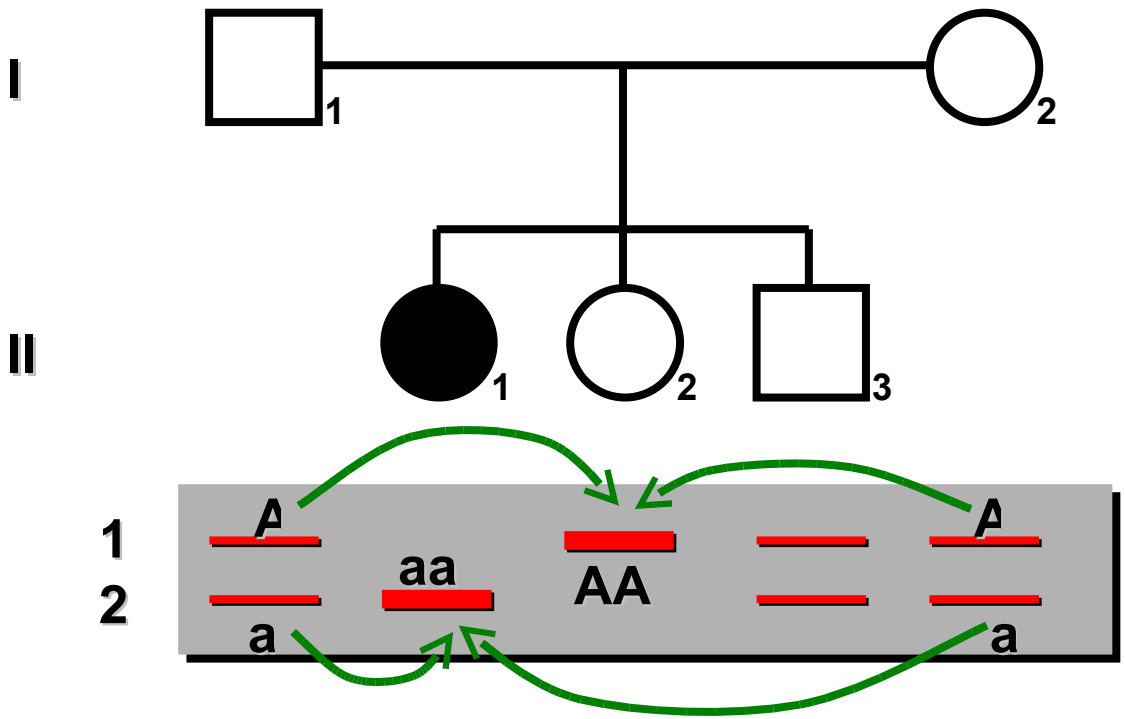


The son with AR disease is the homozygote in the RFLP (1,1). It is evident that mutant allele cosegregate with the fragments 1 of both parents. The second child is homozygote for fragment 2 (2,2) and therefore he is most likely dominant homozygote (completely normal).



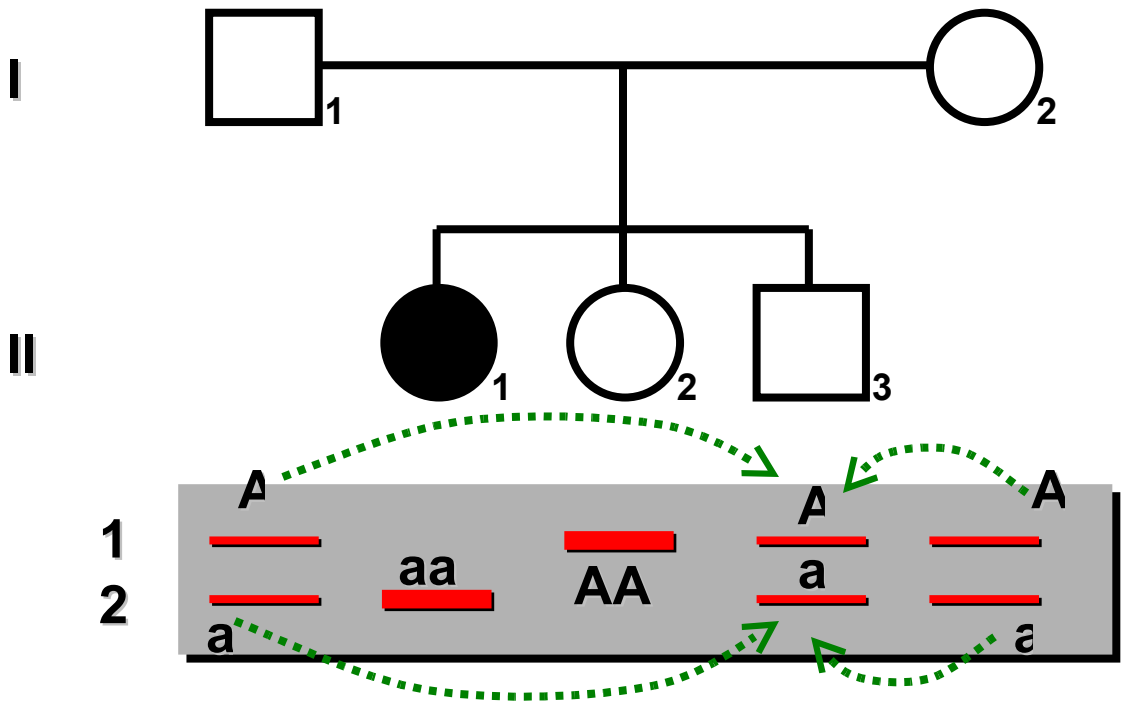
The affected son (1,2) has inherited fragment 1 from his father (1,1) and fragment 2 from mother (1,2) fragment 2, which is linked to the mutant allele. The second child (1,1) has maternal fragment 1 with the normal allele and fragment 1 from father. We don´t know if this fragment carries mutant or normal allele. So the prognosis is 50% carrier, 50% healthy homozygote

Task 1, p. 120



Are children II/2 and II/3 heterozygotes for the mutant allele?
AR disease, intragene probe (complete linkage)

Task 1, p. 120

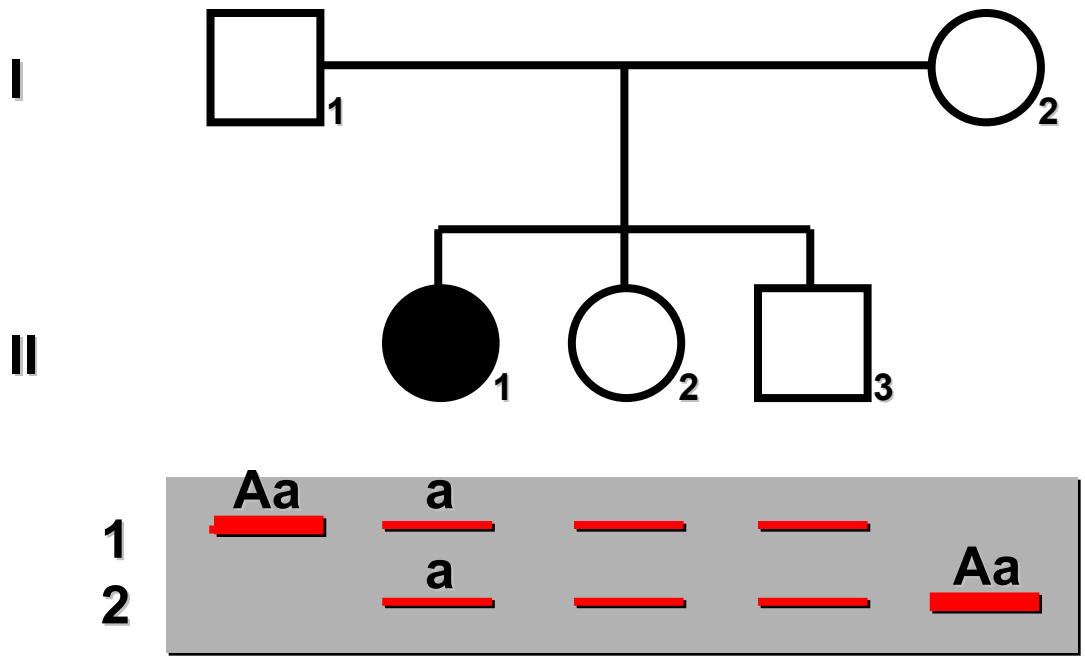


Are children II/2 and II/3 heterozygotes for the mutant allele?
AR disease, intragene probe (complete linkage)

II/2 no

II/3 yes

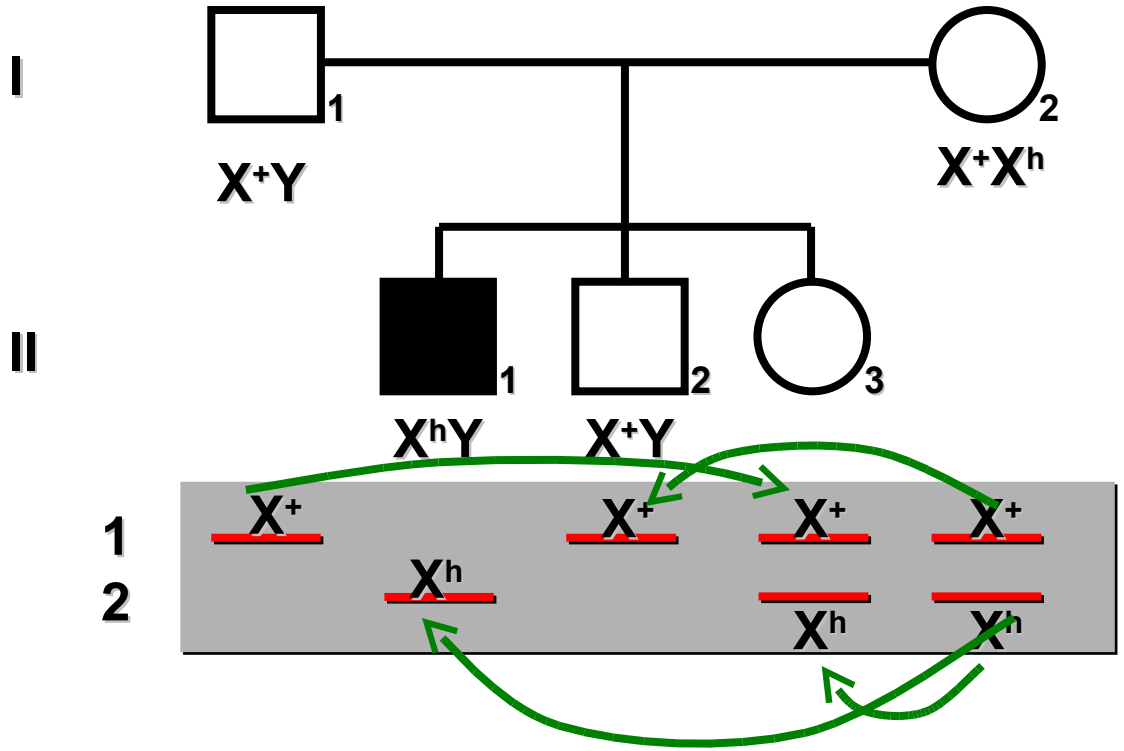
Task 2, p. 120



Are children II/2 and II/3 heterozygotes for the mutant allele?
AR disease, intragene probe (complete linkage)

II/2 AA or Aa
II/3 AA or Aa
Heterozygosity of II/2 and II/3 is undeterminable - RFLP analysis is unsuccessful.

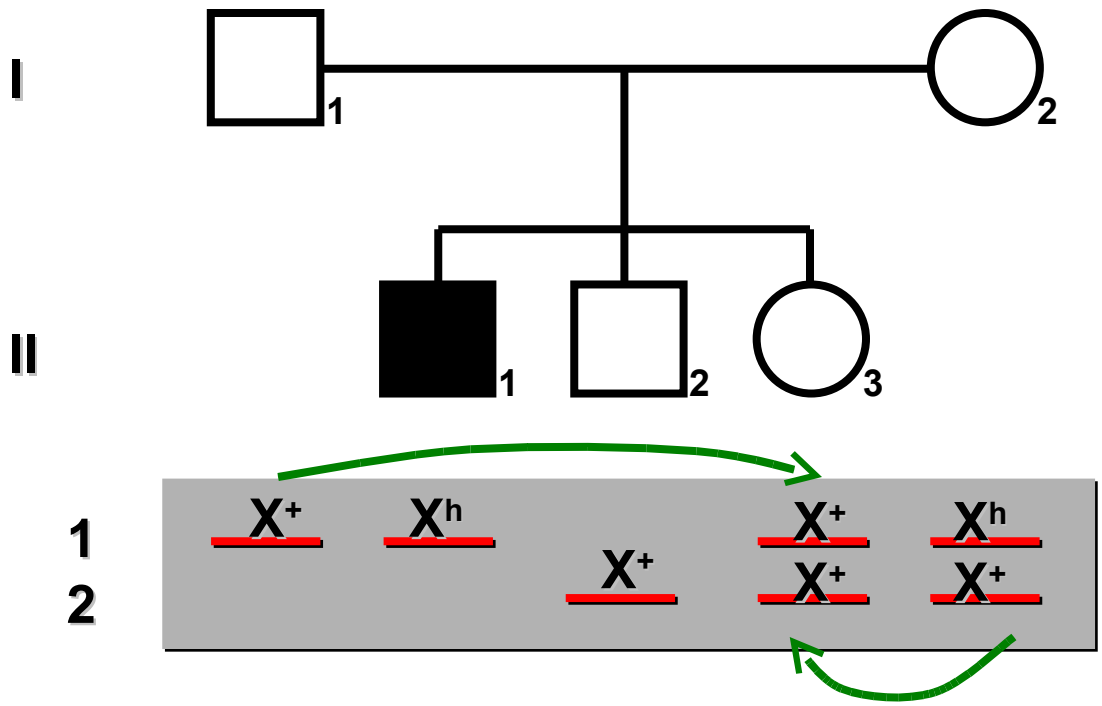
Task 3, p. 121



Is daughter II/3 heterozygote for the mutant allele of haemophilia?
X-linked recessive disease, intragene probe (complete linkage)

II/3 yes

Task 4, p. 121



Is daughter II/3 heterozygote for the mutant allele of haemophilia?
X-linked recessive disease, intragene probe

II/3 no