MOLECULAR GENETICS I

Stomatology

Task 1, p. 107

Carry out replication of the following double-stranded DNA molecule:

5´ T T A A C G C G A T G G T C T 3´

3´ A A T T G C G C T A C C A G A 5´

Replication:

Result:

5´ T T A A C G C G A T G G T C T 3´
3´ A A T T G C G C T A C C A G A 5´

5´ T T A A C G C G A T G G T C T 3´
3´ A A T T G C G C T A C C A G A 5´

Task 2, p. 108

To the „coding“ strand of DNA of the following sequence:

5´ T T A A C G C G A T G G T C T 3´

form: a) „noncoding“ strand of DNA

b) mRNA

c) polypeptide

Solution:

a) noncoding strand of DNA

3´ A A T T G C G C T A C C A G A 5´

b) mRNA

5´ UUA ACG CGA UGG UCU 3´

c) polypeptide

Leu – Thr – Arg – Trp – Ser

Task 3, p. 109

Carry out translation of the fictive circular mRNA.

a)

met 9lys asn glu 9lys

b)

$(\text{met 9phe tyr val 8phe leu cys 9phe})_{\infty}$

c)

(met 9pro his ala 9pro cys 9 pro)∞

d)

(met 9gly asp 10gly trp 9gly)∞

RESTRICTION ENDONUCLEASES

ECo RI – Escherichia coli – enzyme I

Taq I – $^{5'}$T C G A$^{3'}$ ... $^{5'}$A C G G | T C G A A T T$^{3'}$

Taq I –

$^{5'}$T | C G A$^{3'}$ ... $^{5'}$A C G G T | C G A A T T$^{3'}$

$^{3'}$A G C | T$^{5'}$ ... $^{3'}$T G C C A G C | T T A A$^{5'}$

Hae II – $^{5'}$G G | C C $^{3'}$

C C | G G

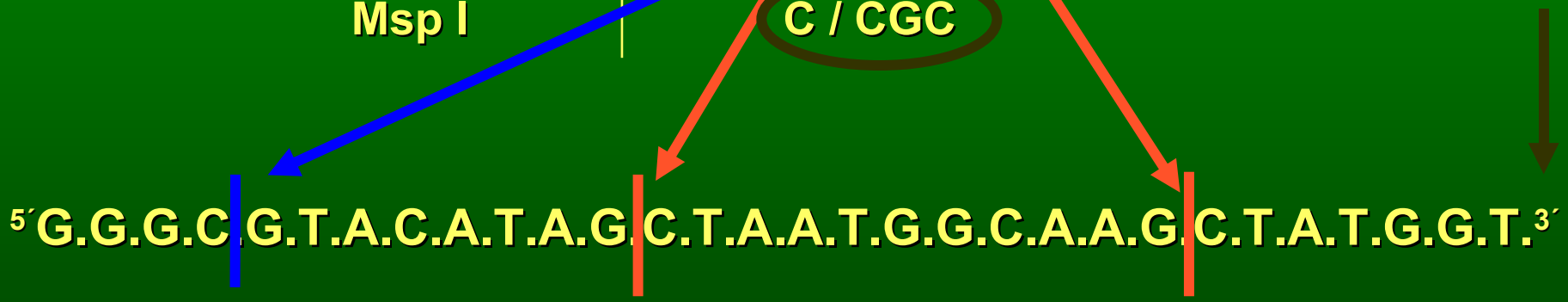
Bam HI – G | G A T C C

Identification of the restriction sites

Task 1, p. 112

Look for the restriction sites recognised by the enzymes:

enzyme	restriction site
Alu I	AG / CT
Sau 3A	/ GTAC
Msp I	C / CGC



Identification of the restriction sites

Task 1, p. 112

Look for the restriction sites recognised by the enzymes:

enzyme	restriction site	
Alu I	AG / CT	2x
Sau 3A	/ GTAC	1x
Msp I	C / CGC	0

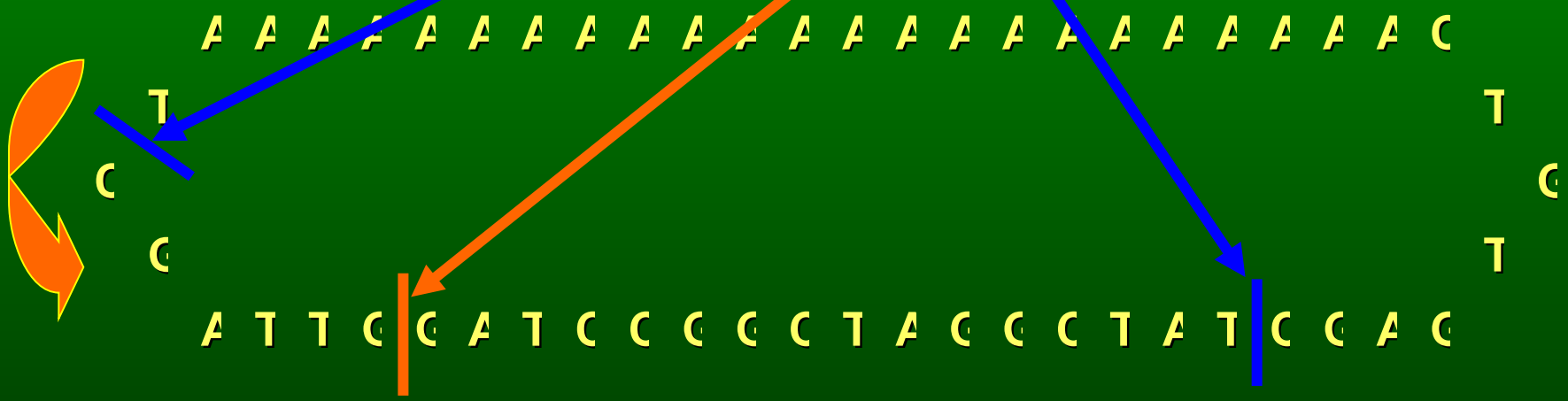
Identification of the restriction sites
Task 2, p. 112

Which of the given enzymes can be used:

a) for cutting a given circular DNA to linearize it?

b) for cutting out a fragment containing a poly-A sequence?

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



Identification of the restriction sites
Task 2, p. 112

Which of the given enzymes can be used:

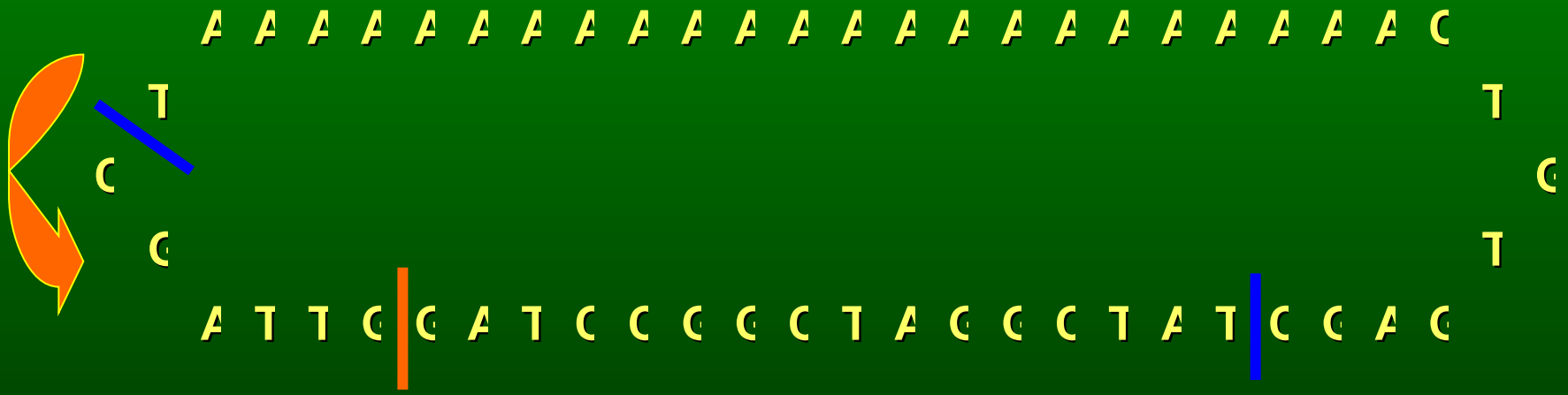
a) for cutting a given circular DNA to linearize it?

b) for cutting out a fragment containing a poly-A sequence?

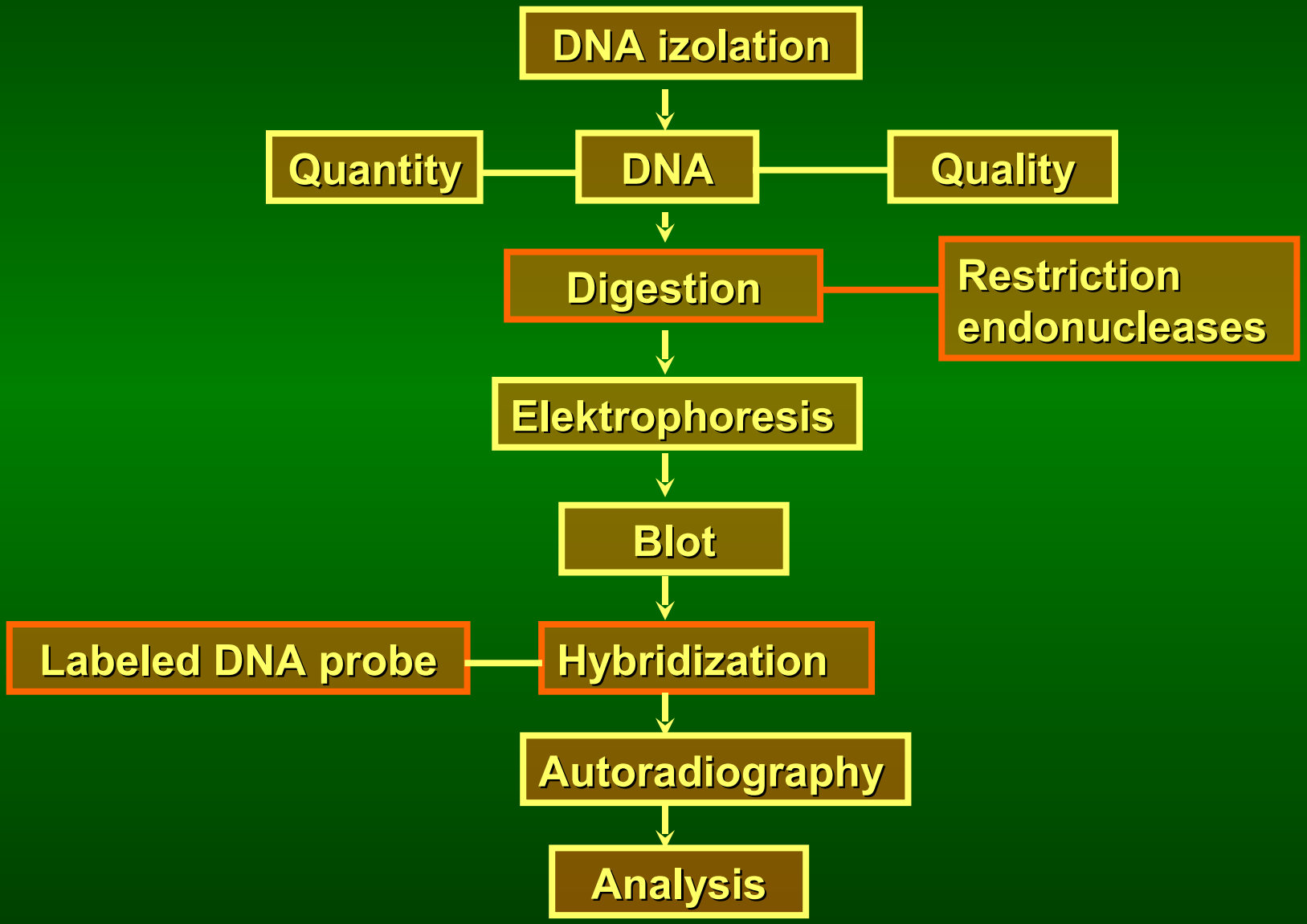
enzyme	restriction site
Taq I	T / CGA
Pvu I	CGAT / CG
Bam HI	G / GATCC

2 sites – cutting out

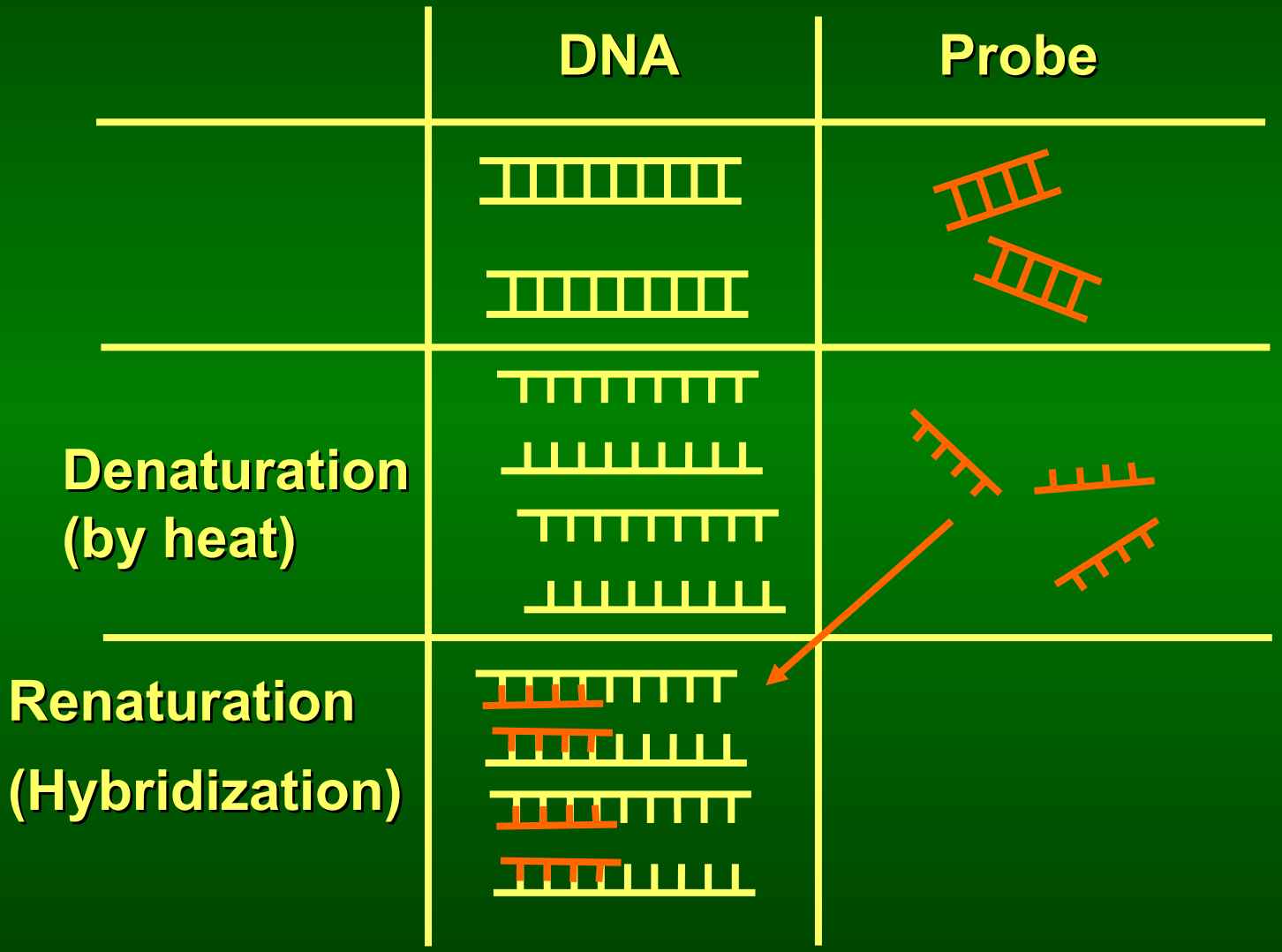
1 site - linearization



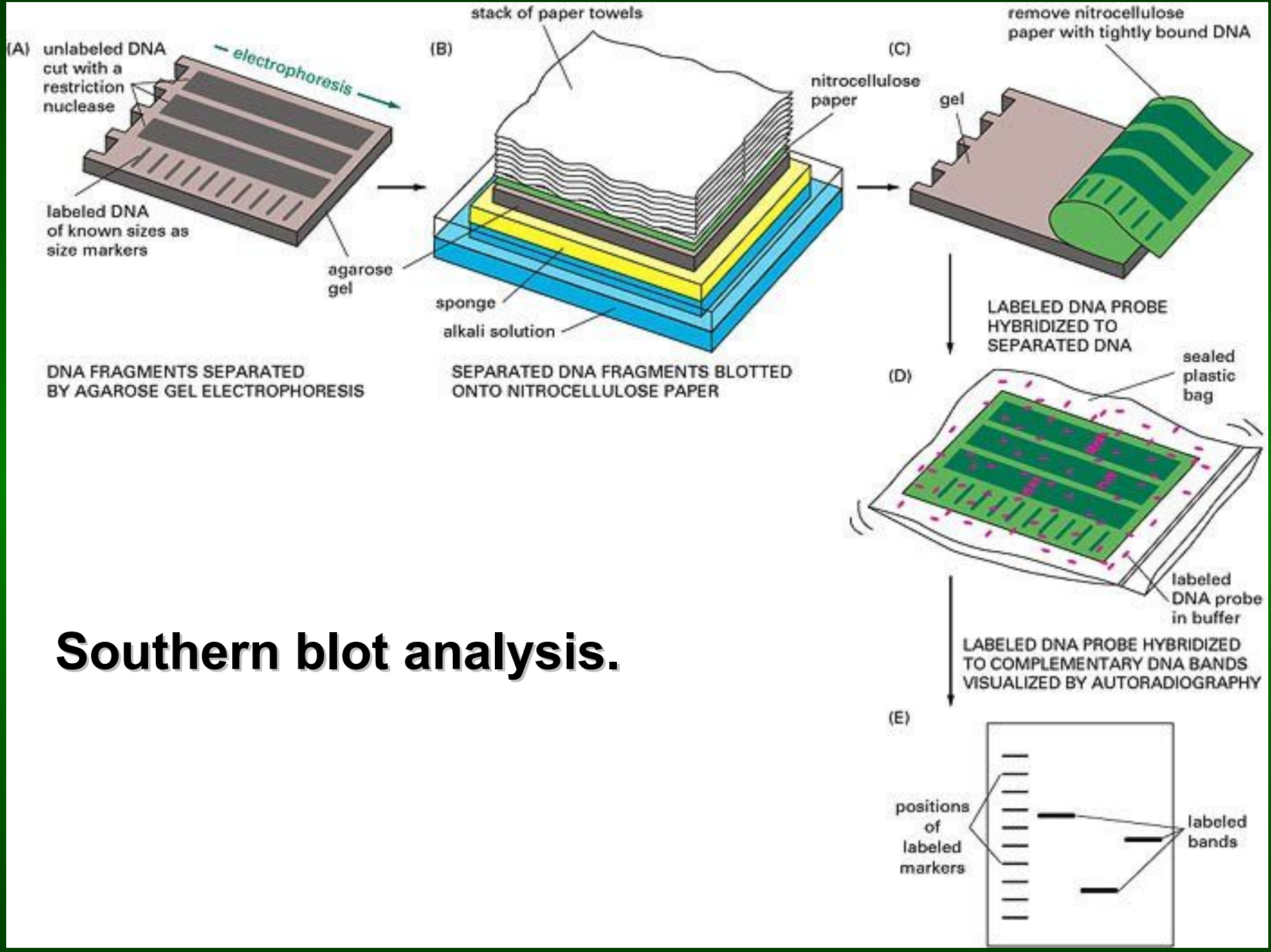
Southern blot analysis.



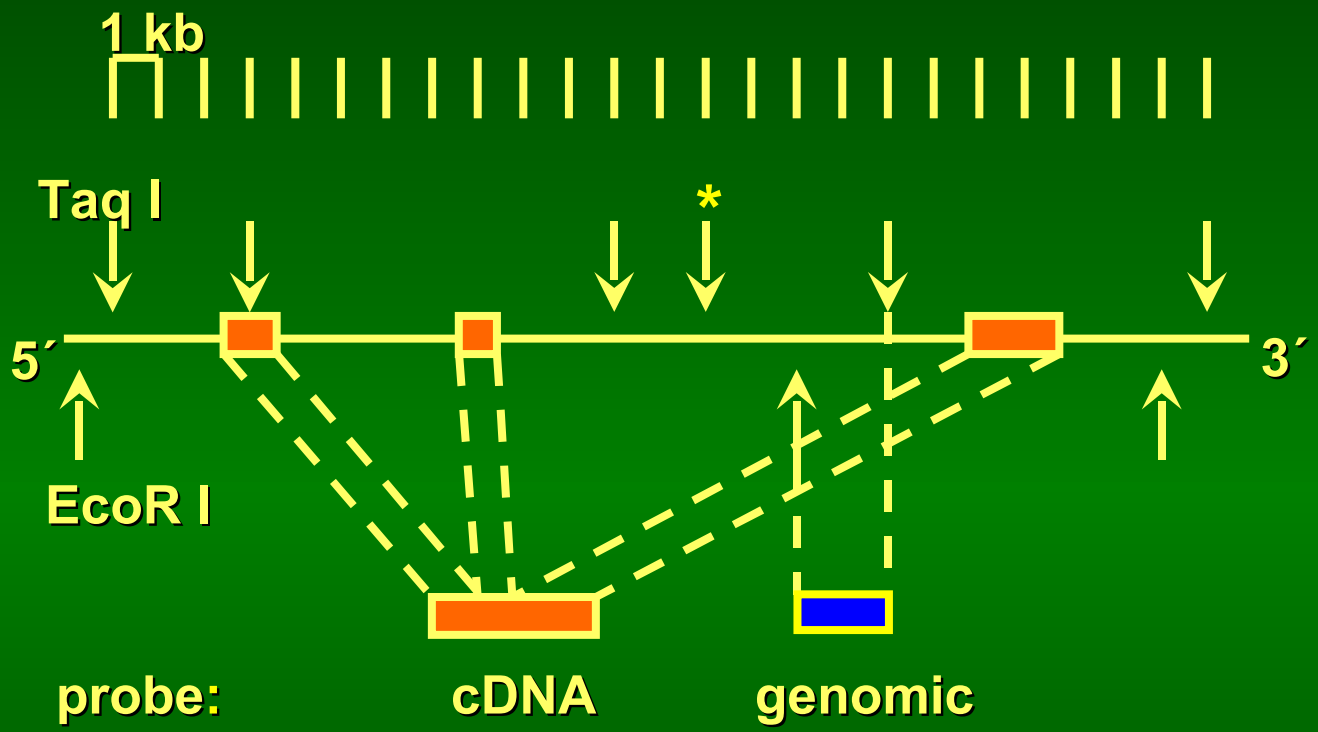
Southern blot analysis. Hybridization.



Southern blot analysis.



Task 4, p. 114 **Gene G**



Results:

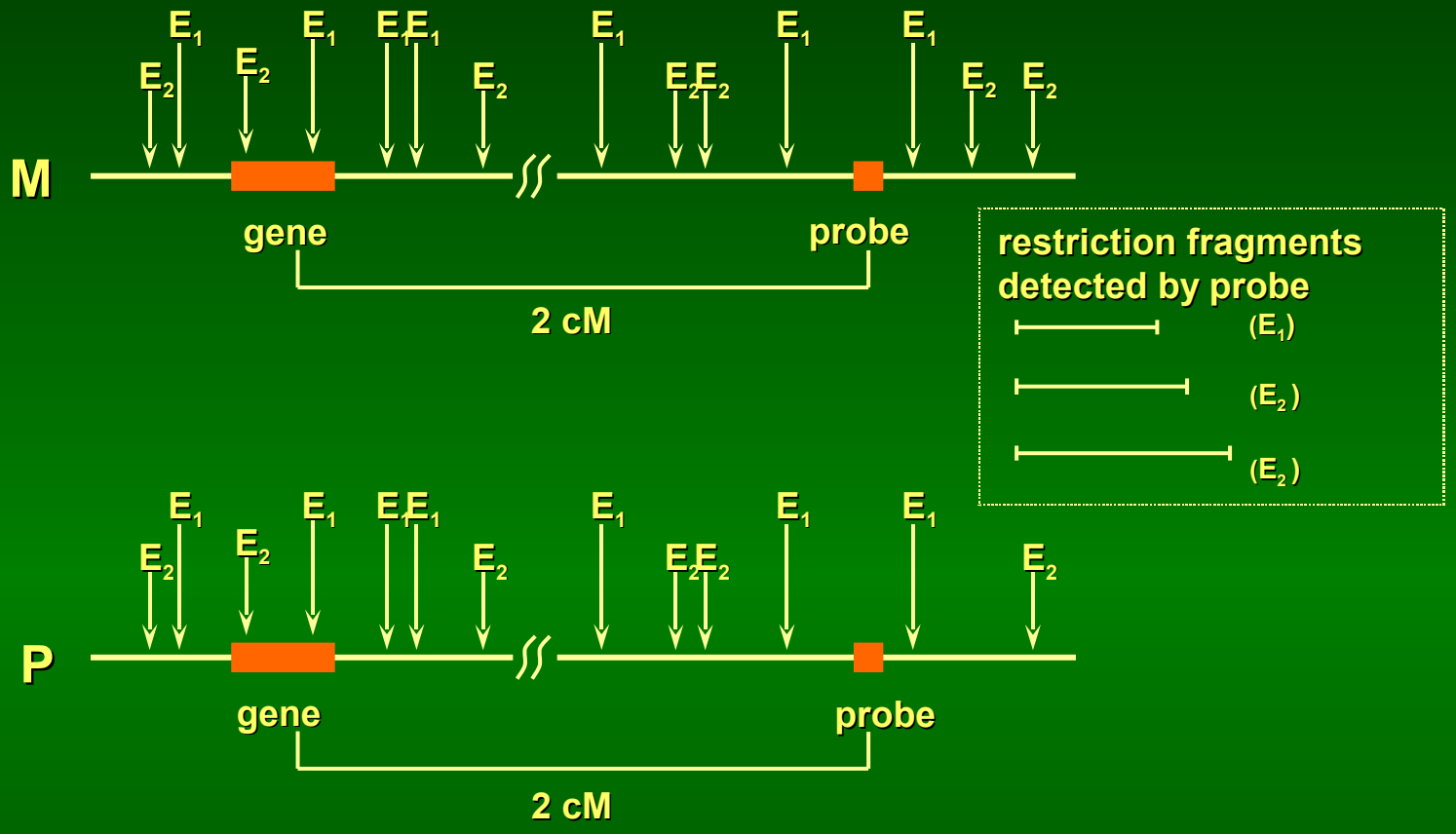
a) to 4 or 5 fragments:
3kb, 8kb, 6kb/2kb+4kb, 7kb

b) 3 fragments: 3kb, 8kb, 7kb

c) no

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

Extragene probe



E_1 – restriction endonuclease 1

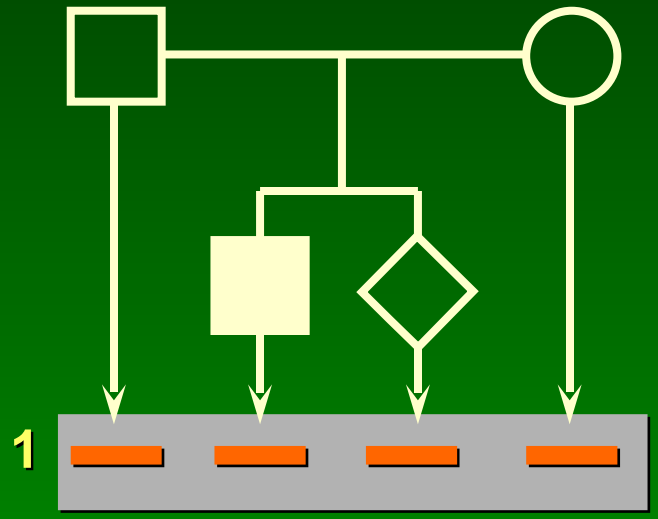
E_2 – restriction endonuclease 2

M – fragment originated from maternal chromosome

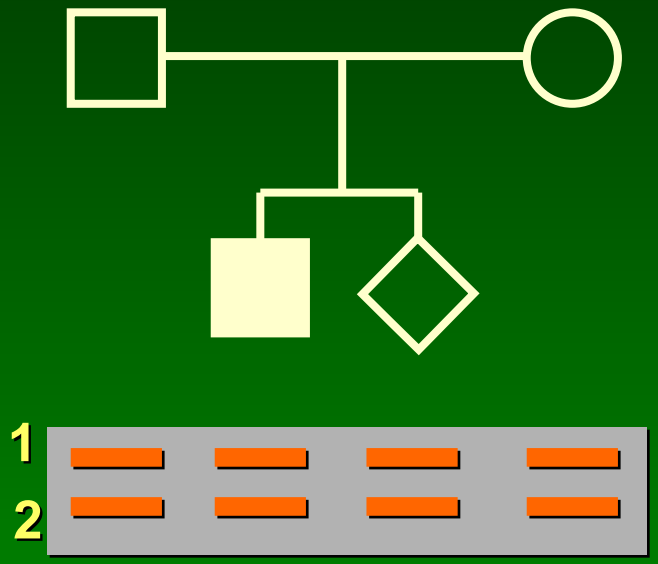
P – fragment originated from paternal chromosome

↓ - specific recognition sequence where restriction enzyme cuts DNA

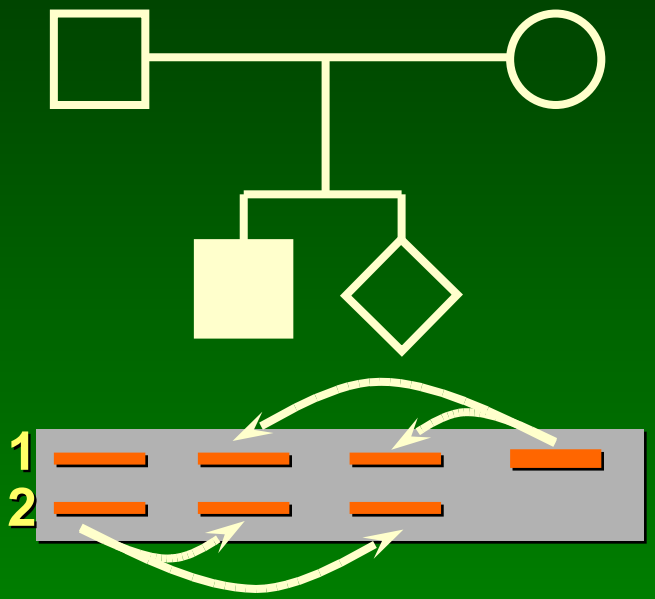
2cM – map distance between gene and extragene probe/site of restriction

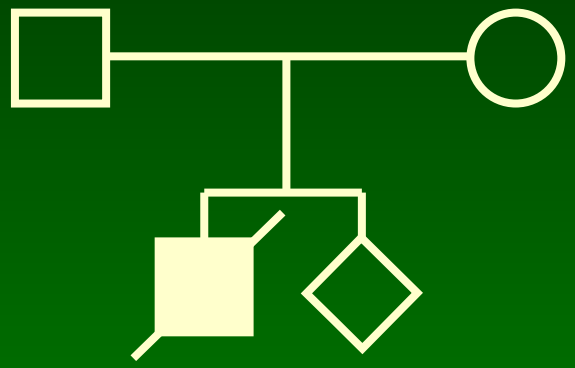


The parent are heterozygotes as fare as the gene of interest is concerned, but all family members are homozygotes in the length of the restriction fragments. So the family is noninformative for a given restriction enzyme and RFLP pattern.



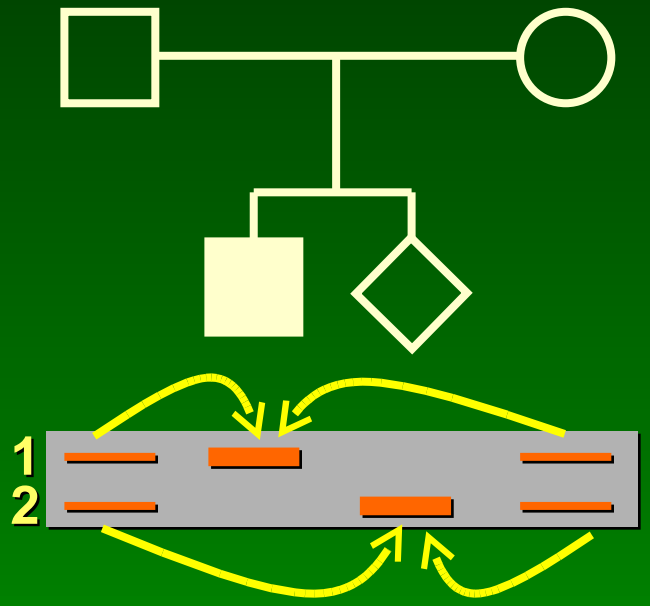
The family is partially informative. It is not possible to distinguish the origin of RFLP fragment with mutant allele between mother and father. However the second child must be either aa or AA homozygote.



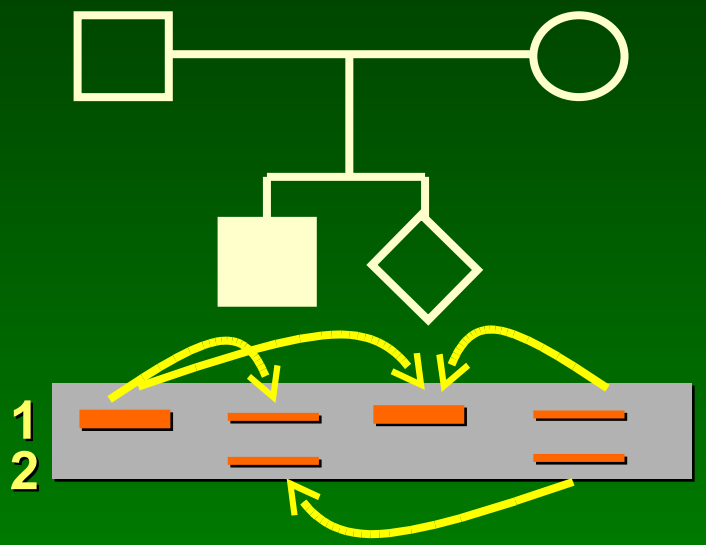


It is clear that father´s RFLP fragment 2 indicates the presence of the mutant allele. The mother can only pass fragment 1 either with mutant or normal allele. There is 50% probability that the second child will be affected.

The RFLP DNA analysis will not be successful because DNA of the dead son lacks. Risk for next child is Mendelian 25%.

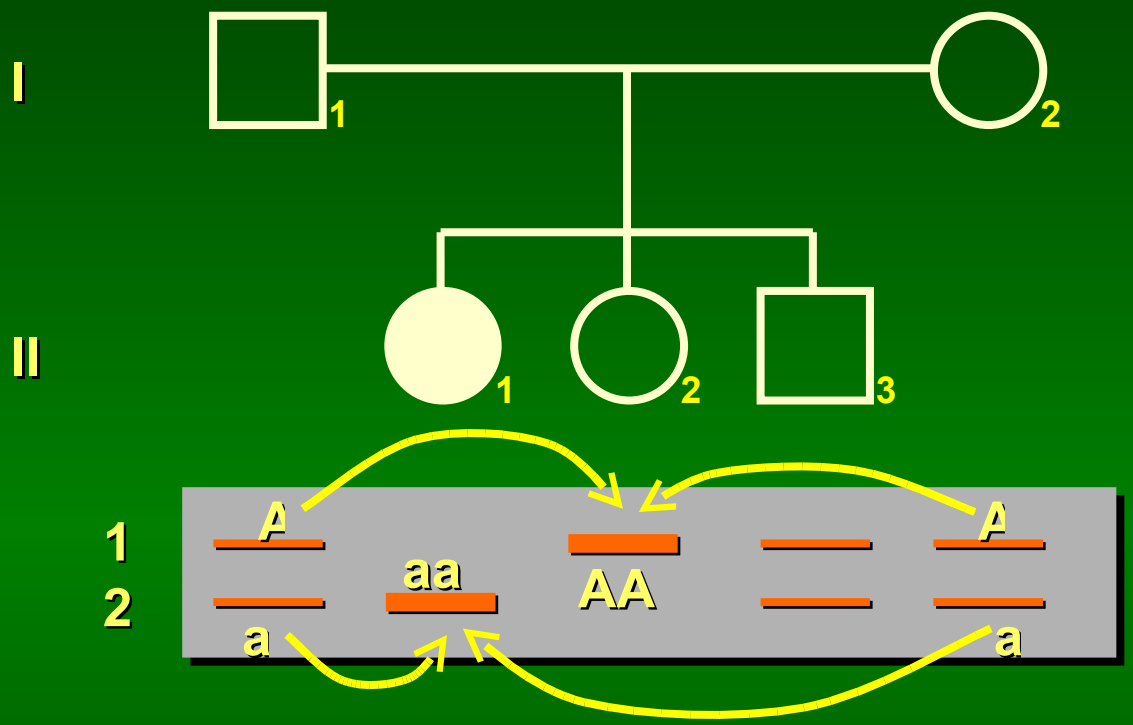


The son with AR disease is the homozygote in the RFLP (1,1). It is evident that mutant allele cosegregate with the fragments 1 of both parents. The second child has both fragments 2 (2,2) and therefore it is most likely that he is completely normal.



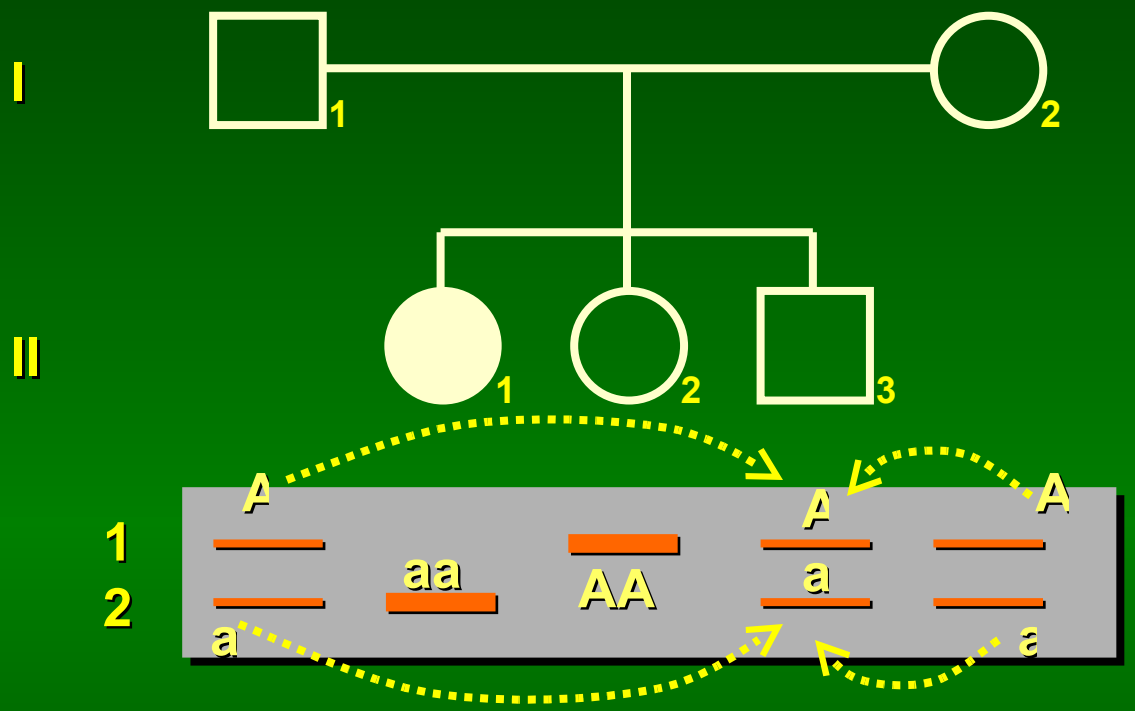
The affected son (1,2) has inherited the fragment 1 from his father (1,1) and from the mother fragment 2; both with the mutant allele. The second child (1,1) has inherited maternal fragment 1 with the normal allele and fragment 1 from father. We don´t know if this fragment carries mutant or normal allele.

Task 1, p. 120



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

Task 1, p. 120

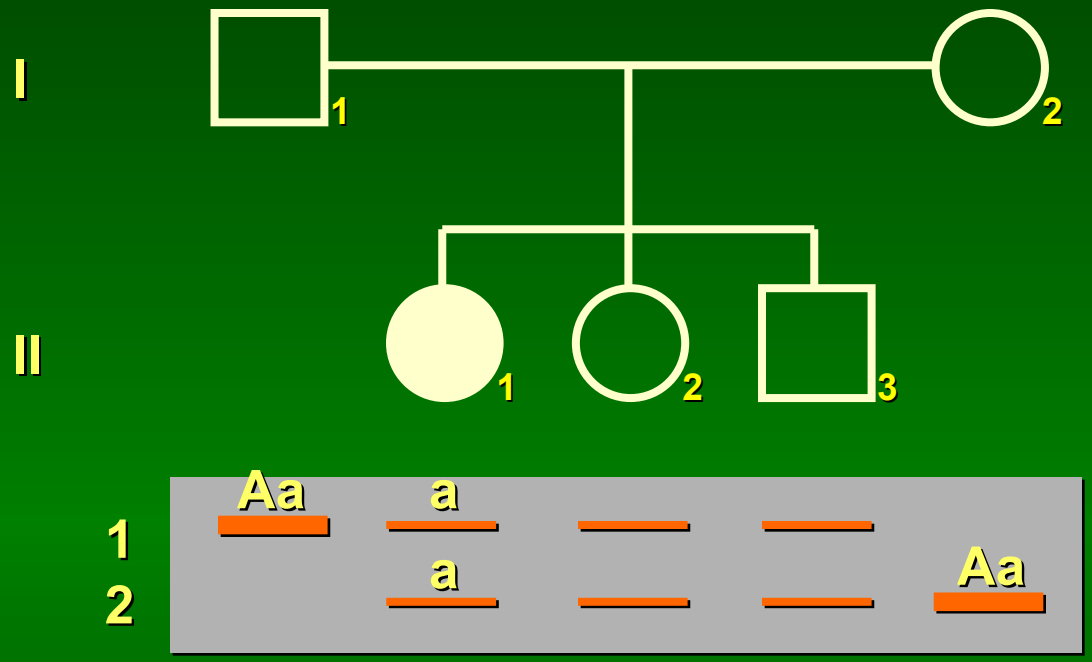


Are children II/2 and II/3 heterozygotes for the mutant allele?
AR disease, intragene probe

II/2 no

II/3 yes

Task 2, p. 120



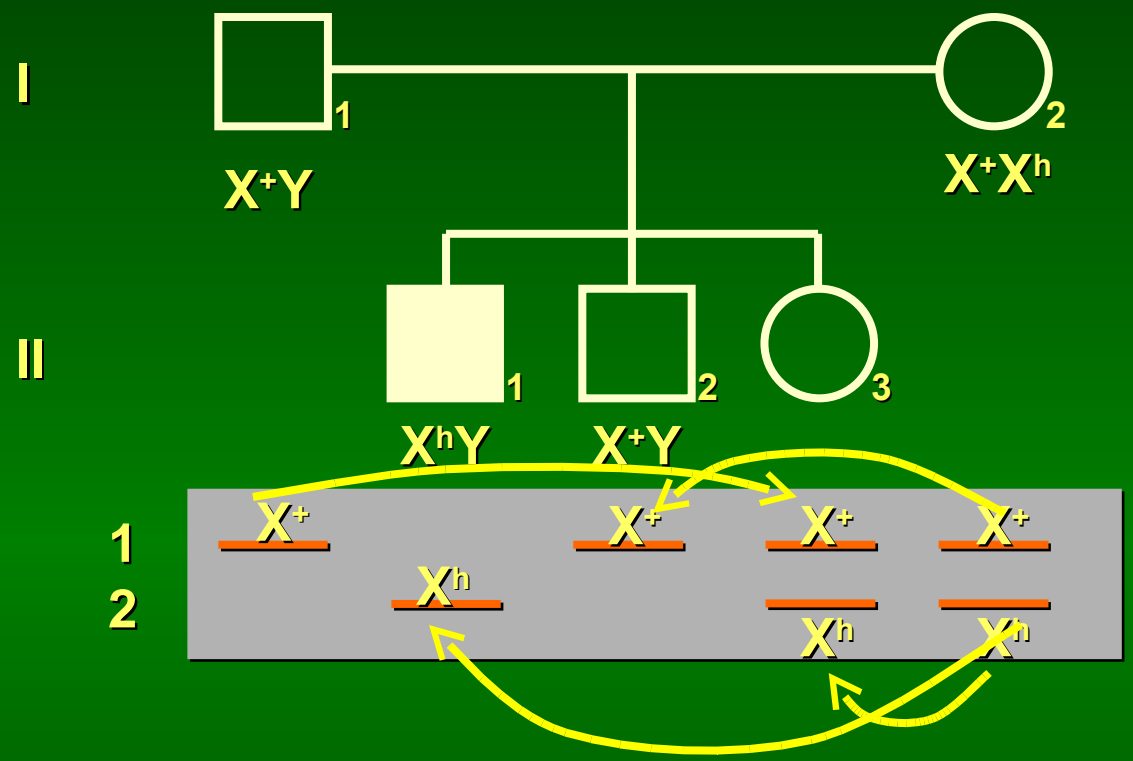
Are children II/2 and II/3 heterozygotes for the mutant allele?
AR disease, intragene probe

II/2 AA or Aa

II/3 AA or Aa

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

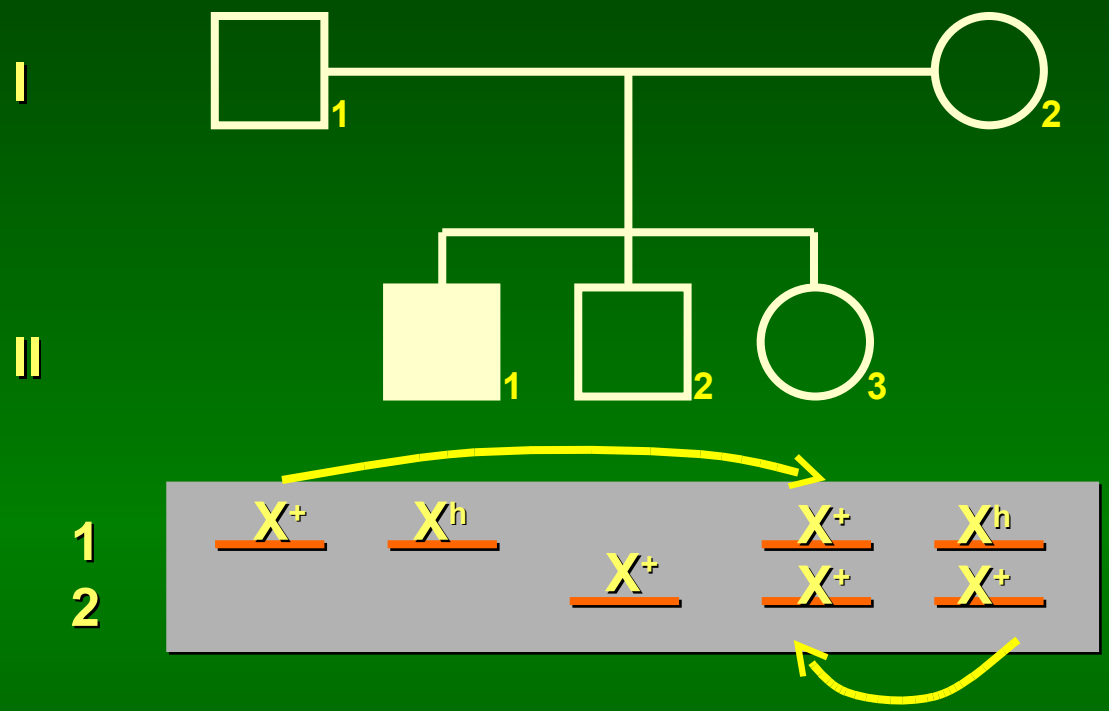
Task 3, p. 121



Is daughter II/3 heterozygote for the mutant allele of haemophilia?
GR disease, intragene probe

II/3 yes

Task 4, p. 121



Is daughter II/3 heterozygote for the mutant allele of haemophilia?
GR disease, intragene probe

II/3 no