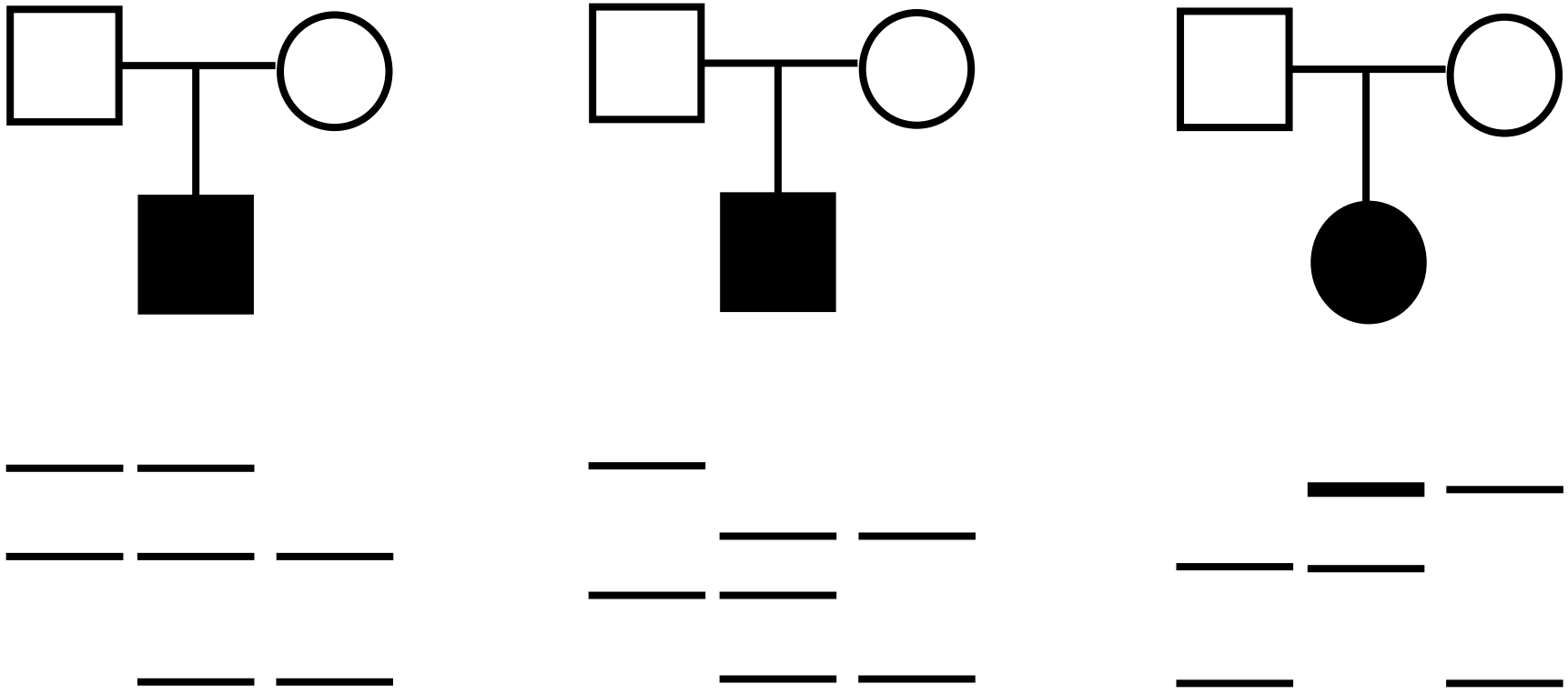
Molecular genetics IV

winter semester

6th week (Nov 5th – 9th, 2007)

Inst. of Biology and Medical Genetics, First Faculty of Medicine, Praha

Nondisjunction in Down syndrome

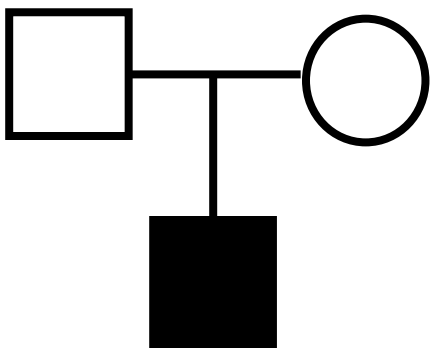


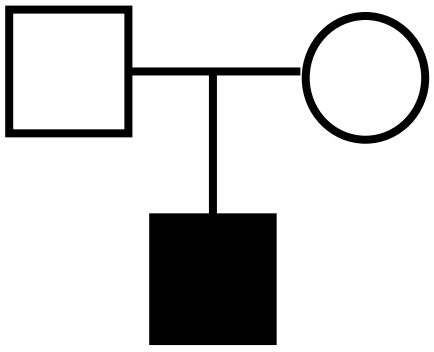
The pedigrees show families where children are affected with Down syndrome (simple trisomy). The results of DNA analysis are shown under pedigrees. Polymorphism of a tetranucleotide microsatellite on chromosome 21 was determined.

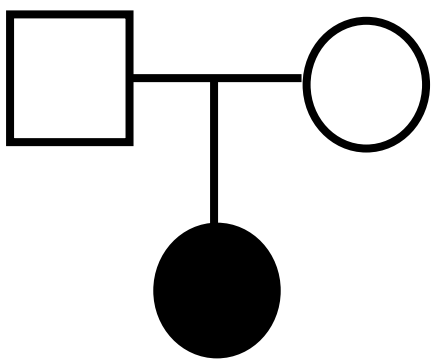
From which parent the affected child inherited an extra chromosome 21?

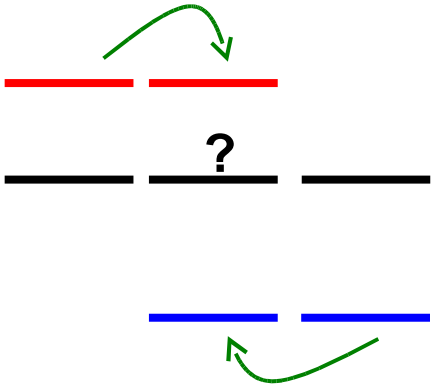
In which meiotic division the nondisjunction occurred?

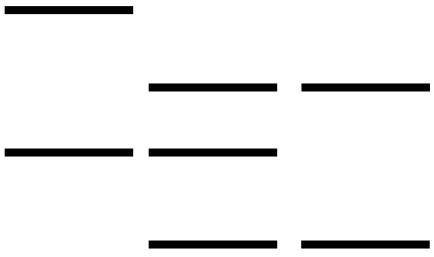
Nondisjunction in Down syndrome

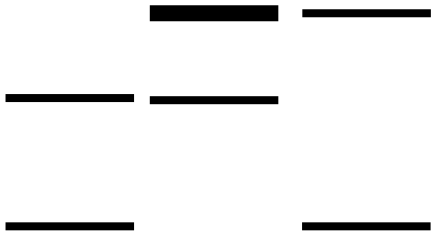






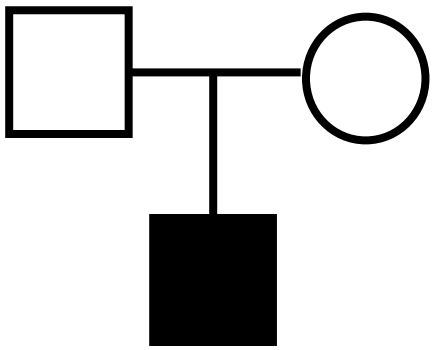


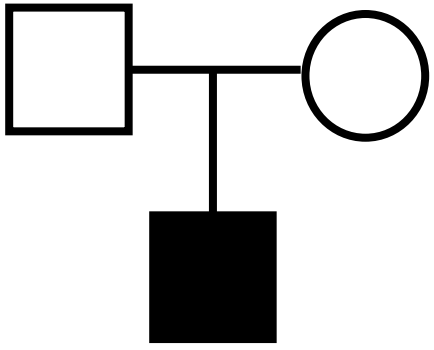


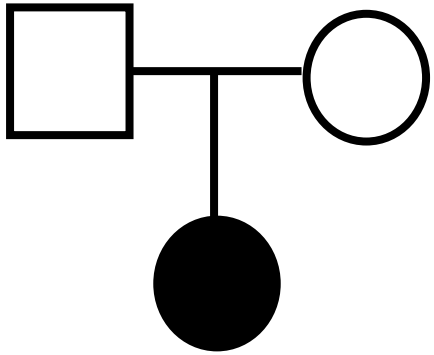


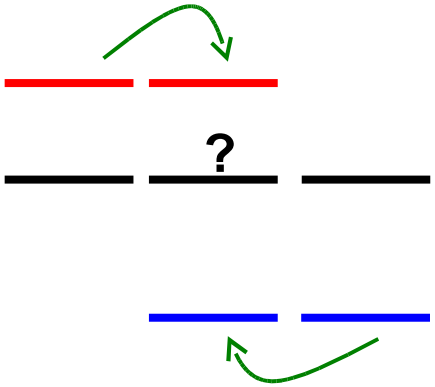
Meiosis I in mother or father

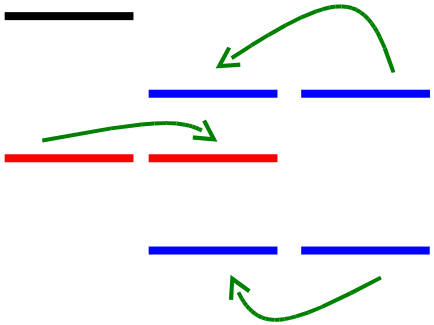
Nondisjunction in Down syndrome

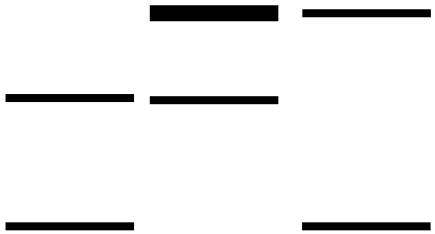












Meiosis I in mother or father

Meiosis I in mother

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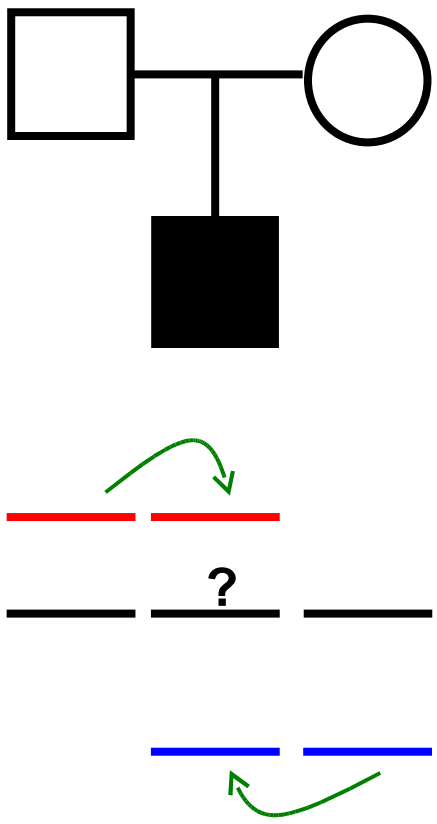
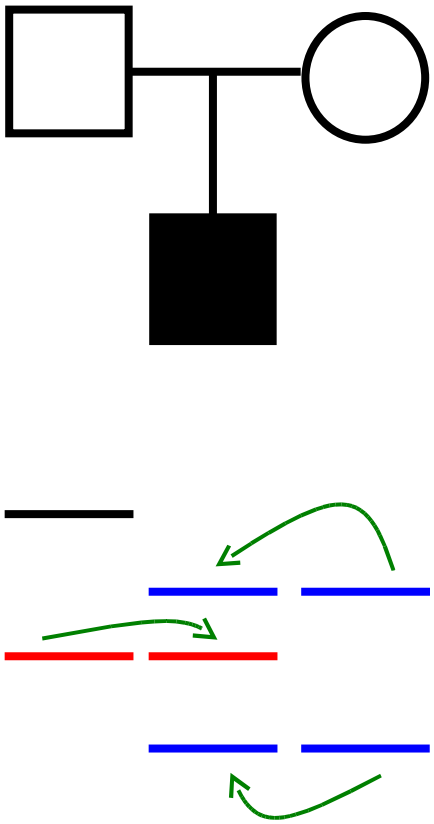
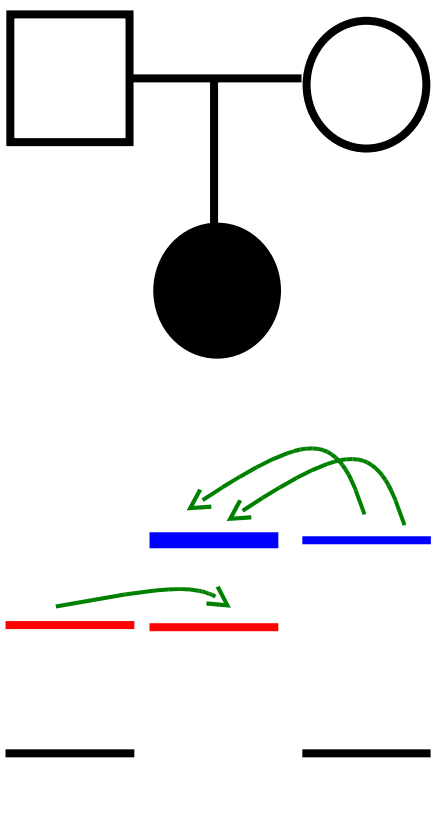


Meiosis I in mother or father



Meiosis I in mother



Meiosis II in mother

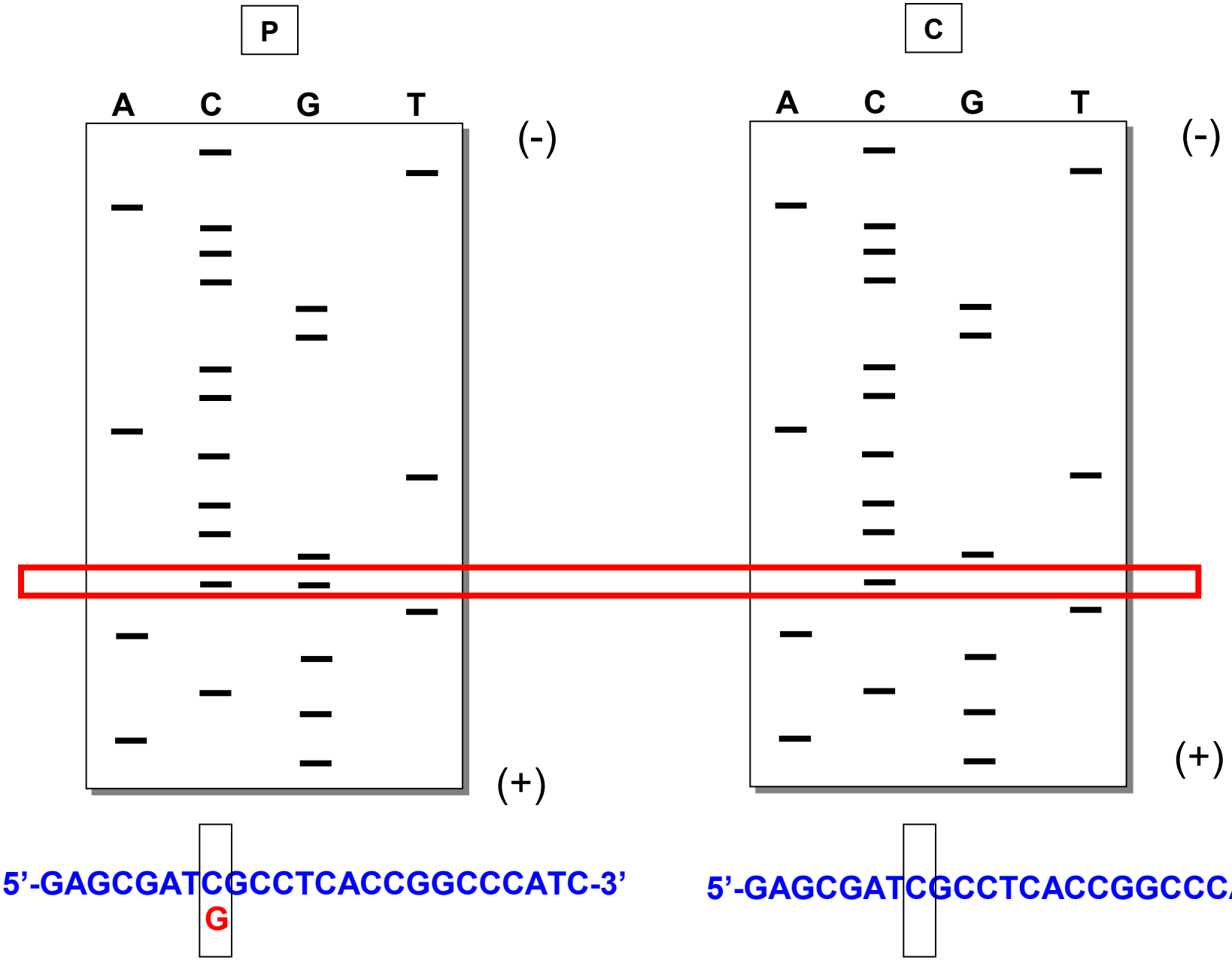
DNA sequencing

External files:

http://www.dnalc.org/ddnalc/resources/animations/sangerseq.exe

http://www.dnalc.org/ddnalc/resources/animations/cycseq.exe

FGFR2 mutation in Apert syndrome



FGFR2 mutation in Apert syndrome

5’-GAGCGATCGCCTCACCGGCCCATC-3’

5’-GAGCGATCGGCCTCACCGGCCCATC-3’

250

Glu Arg Ser Pro His Arg Pro Ile

…. …. …. GAG CGA TCG CCT CAC CGG CCC ATC… …. ….

250

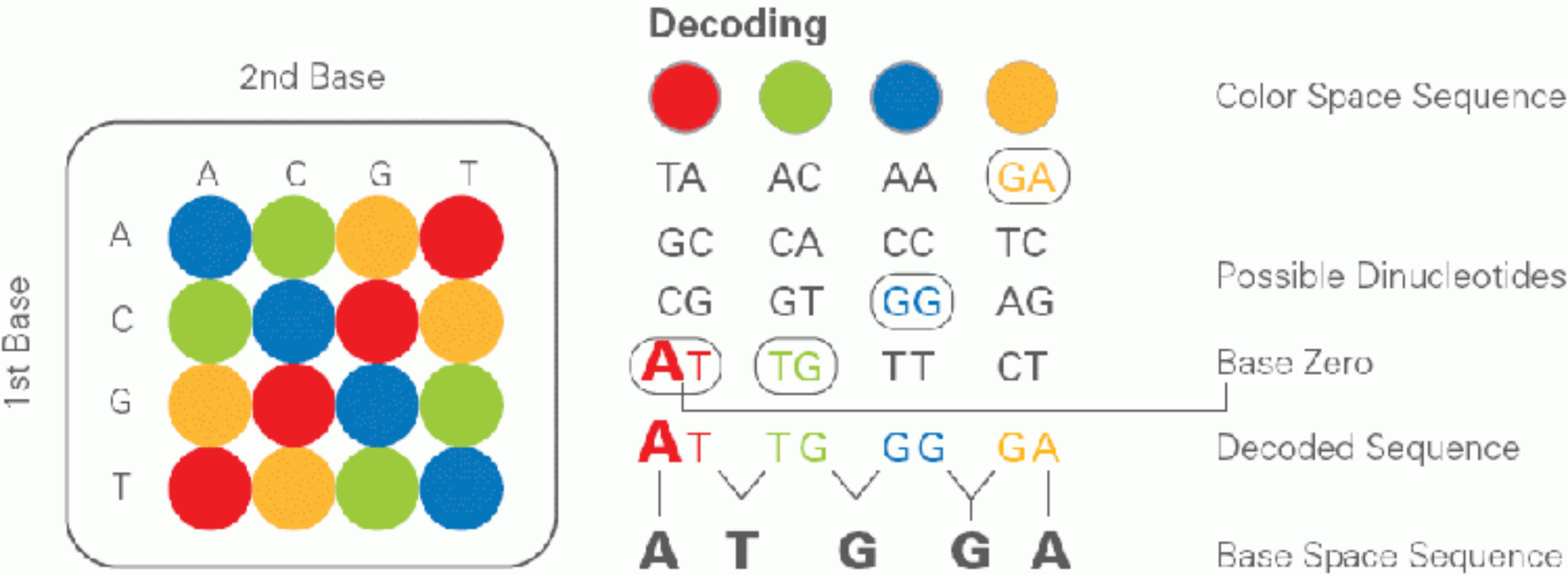
Glu Arg **Trp** Pro His Arg Pro Ile

…. …. …. GAG CGA TGG CCT CAC CGG CCC ATC… …. ….

Ser252Trp

S252W

„Next generation“ sequencing methods, an example: SOLiD



SOLiD: Rewrite the sequences from color space to the conventional format (first base is G). Which patient sequence represents genuine mutation and which is an error?

Control sequence

Patient 1

Patient 2

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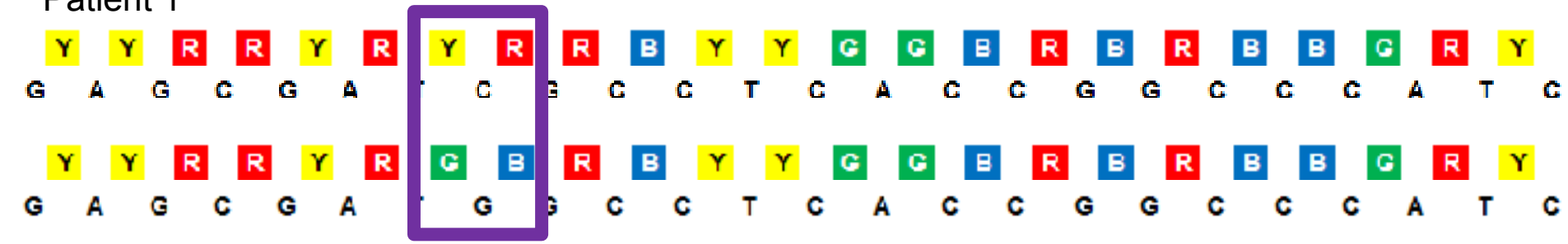
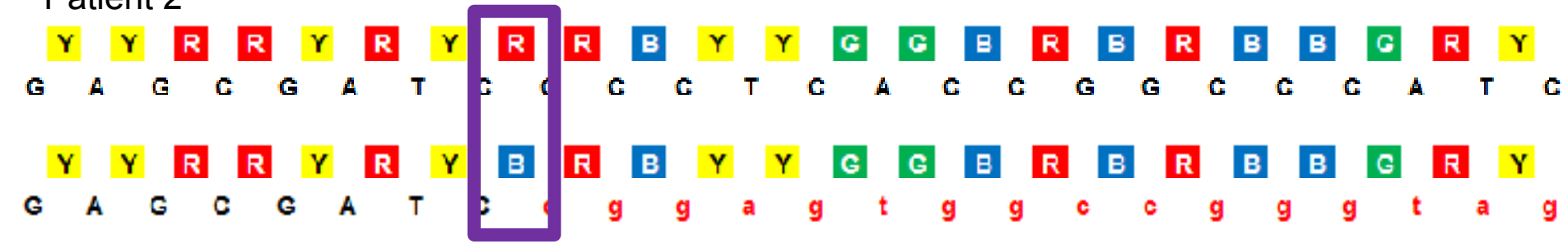
Control sequence

Patient 1



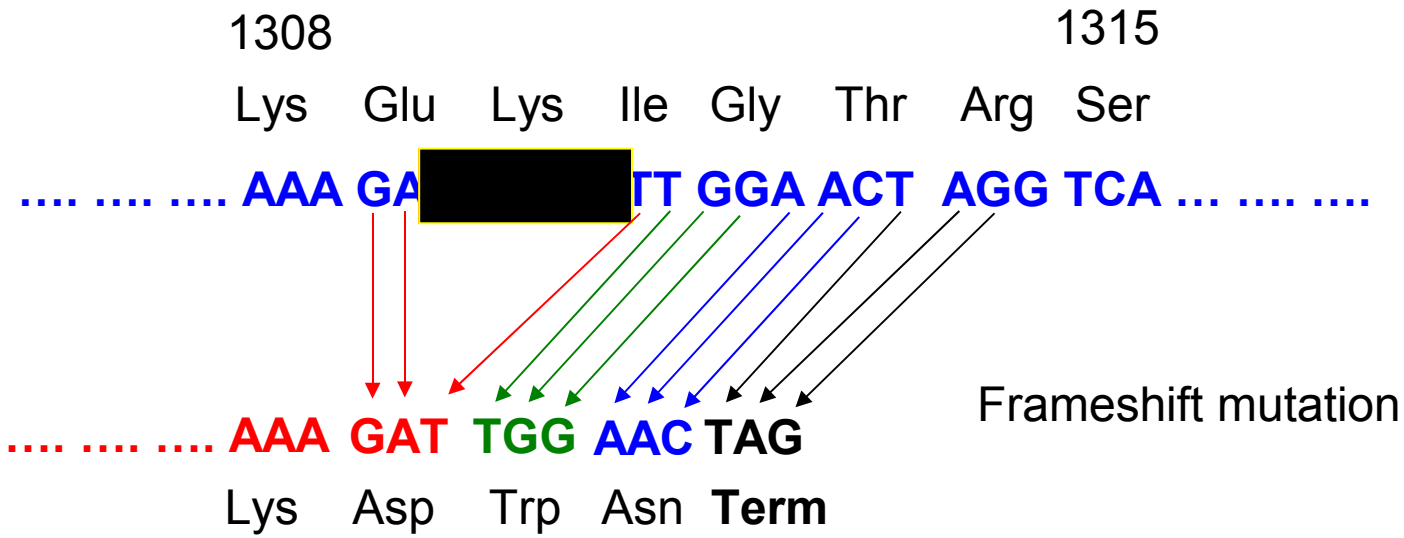
Patient 2



Patient 1 – mutation C -> G, patient 2 – assay error, recheck necessary

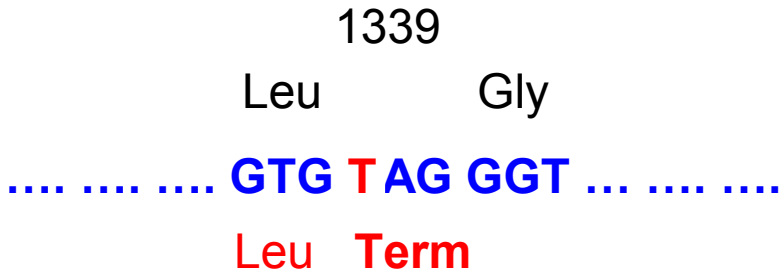
Task 5, p. 110

A.



Frameshift mutation

B.

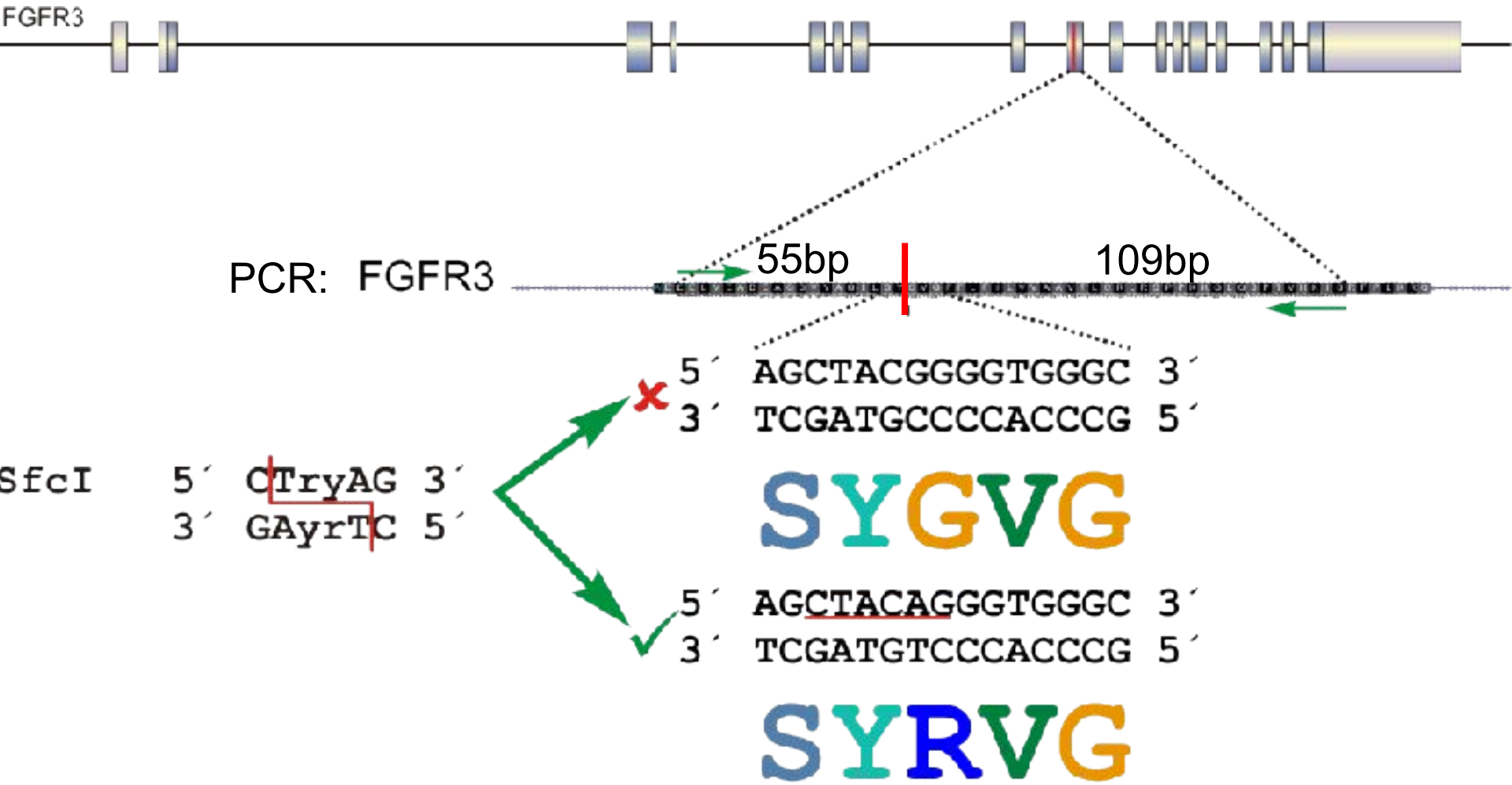


Nonsense mutation

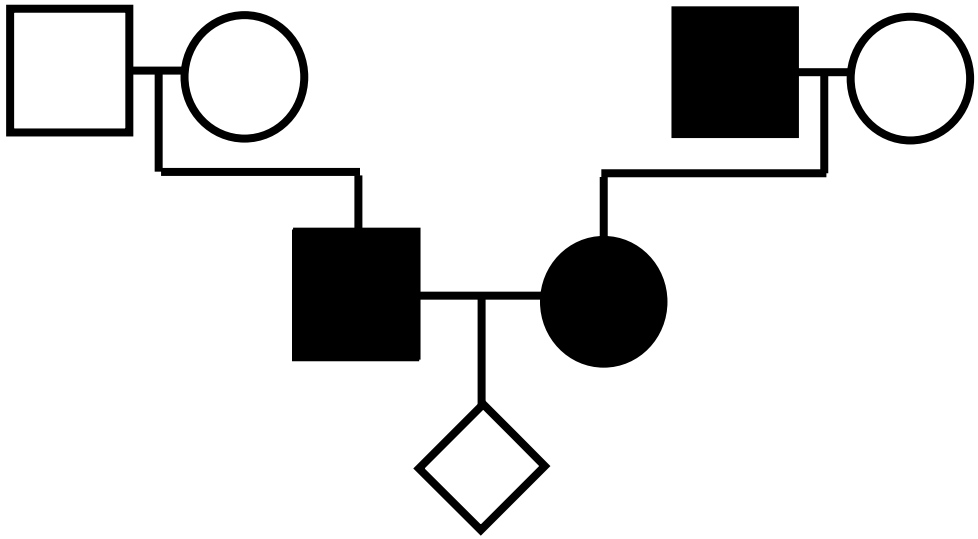
C.

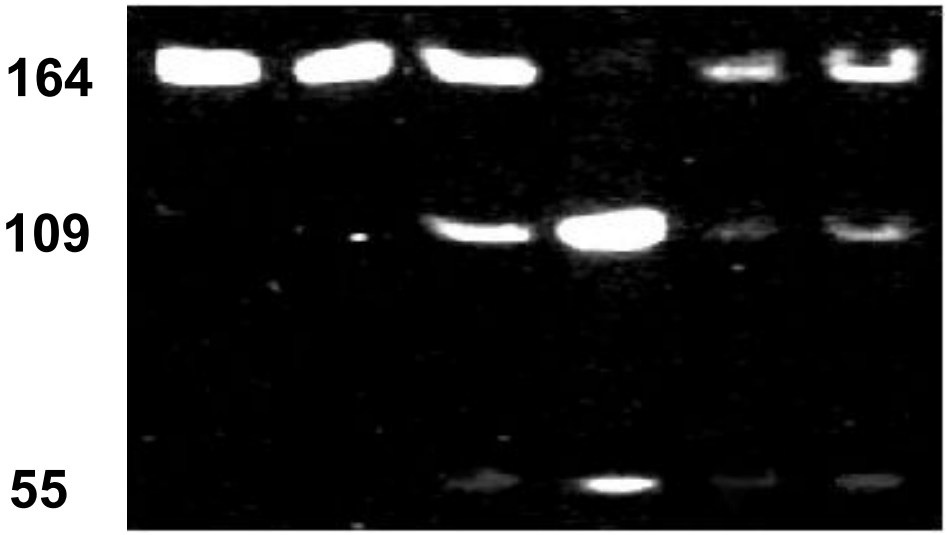
Missense mutation

Achondroplasia is the most common inhertited form of dwarfism. It was relealed, that the cause is a mutation in FGFR3 (fibroblast growth factor receptor 3. Approximately 95% of patients have a single mutation type – a transition 1138G>A, leading to substitution Gly380Arg in the protein. The mutation simultaneously creates a restriction site forSfcl



Task: evaluate the risk, that a couple, both suffering from achondroplasia, will have a child homozygous for the mutant allele (this genotype is invariably lethal after birth, and pregnancy may be life-threatening in some mothers with achondroplasia). :





Shiang et al., 1994

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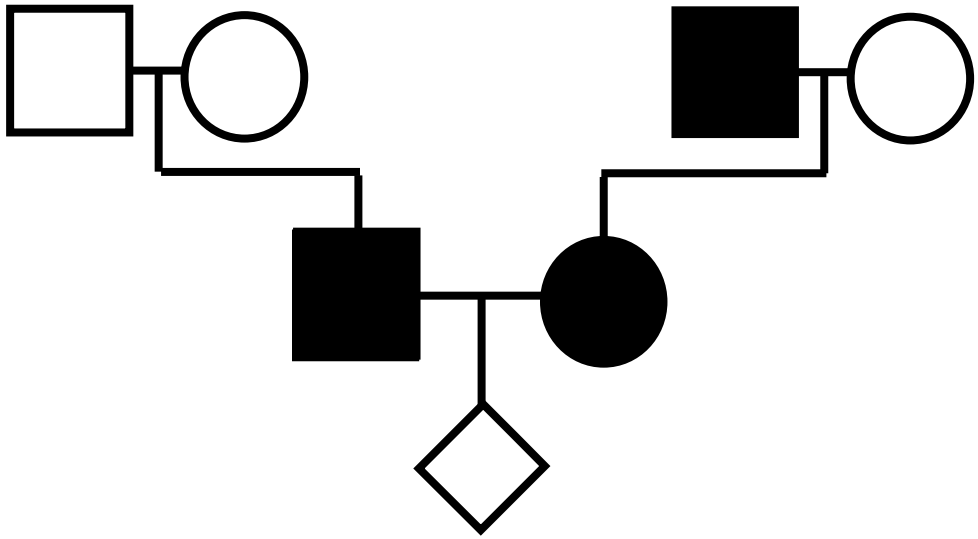
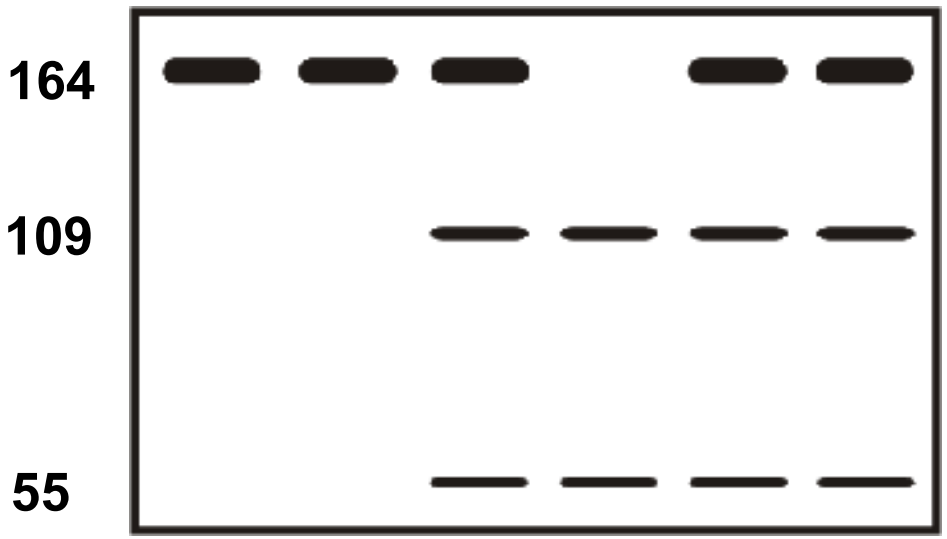
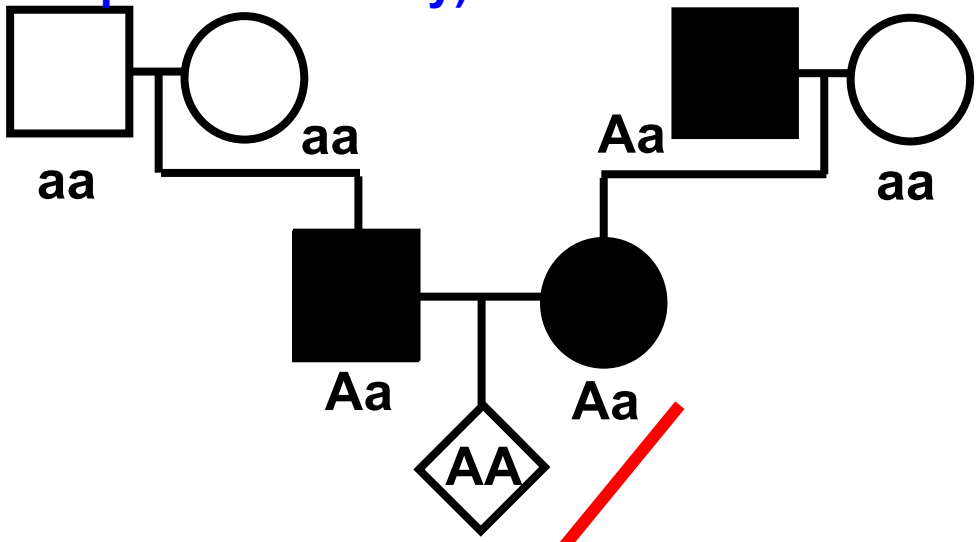
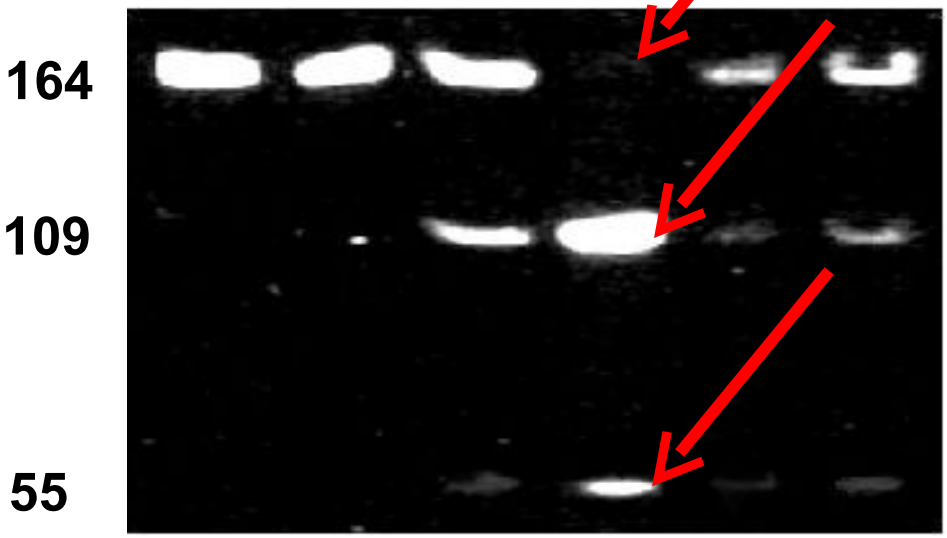




Shiang et al., 1994

Solution: both parents are heterozygous for G380R, as well as the father of the pregnant mother. DNA of the fetus contains only the allele with the restriction site, i.e. mutant allele, so the fetus is homozygous (AA) and pregnancy interruption is recommended. Note: in father´s family we see a new mutation arising (both parents healthy).





Shiang et al., 1994

Homework:

In few patients, achondroplasia is caused by a different mutation in FGFR3 gene, in the same codon, 1138G>C.

a) What is the resulting change in aminoacid?

b) Design a direct DNA diagnostic method to identify this mutation: further infromations on website of the Institute (click here)

Task 3, p. 113

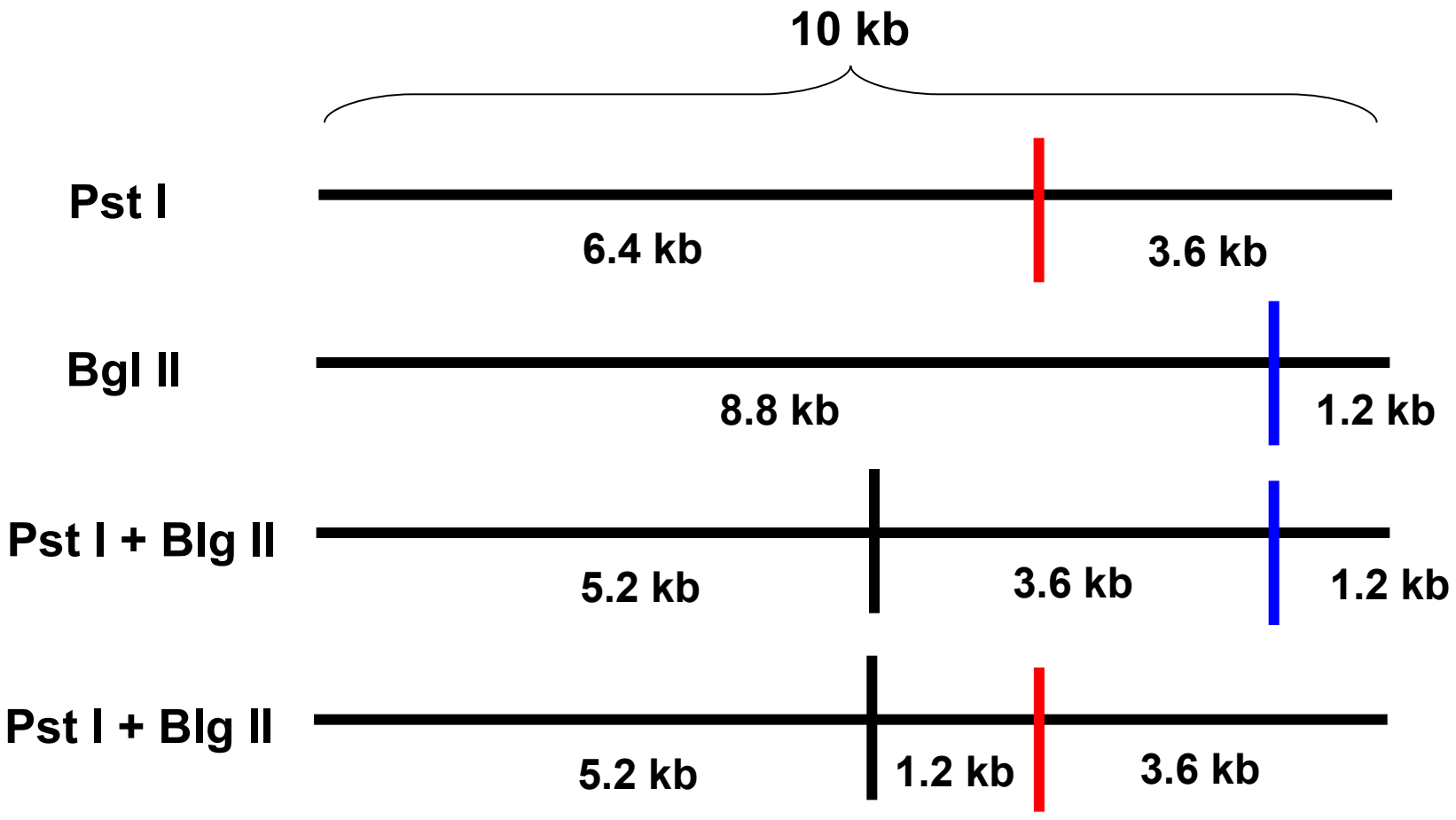
DNA sequence, 10kb long, contains one cleavage site for restriction endonuclease PstI and one for Bgl II. Three independent reactions were performed:

- Pst I only digest
- Bgl II only digest
- Double digest: both Pst I and Bgl II simultaneously

Following fragments were obtained:

digest	enzyme	fragment length(kb)
1.	Pst I	6.4 + 3.6
2.	Bgl II	8.8 + 1.2
3.	Pst I and Bgl II	5.2 + 3.6 + 1.2

Draw a restriction map of this sequence.


10 kb
Pst I
6.4 kb
3.6 kb
Bgl II
8.8 kb
1.2 kb
Pst I + Blg II
5.2 kb
3.6 kb
1.2 kb
Pst I + Blg II
5.2 kb
1.2 kb
3.6 kb
Pst I + Blg II
3.6 kb
5.2 kb
1.2 kb

Task 5, p. 131

Who, out of three suspects, is most probably guilty?

The examination is a Southern blot hybridized with a probe against high-frequency repetitive sequence (minisatellite type). Different length alleles of multiple loci merge in a individual-specific pattern (this method is little bit out of fashion now).