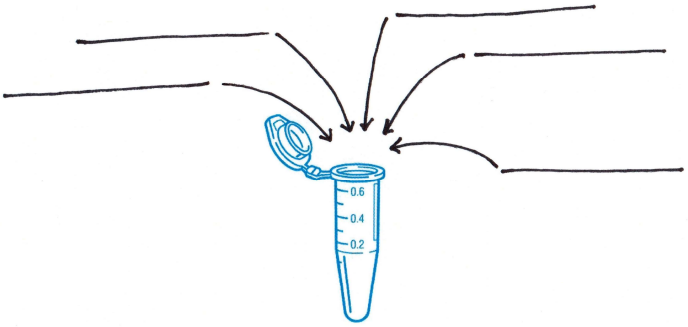
1. Fill in the individual basic components that should be added to a reaction mixture for correct PCR performance (excluding water, buffer and magnesium ions):



2. Determine **the size of PCR product** with following primers (in 5´-3´ orientation):

   Forward: **CGCATTCCTCATCCCAGTAT**
   Reverse: **AAGGACTTGGTGCAGAGTTCA**

   in a sequence (in 5´- 3´ orientation):

GGCACAGACAGAAGCTTGATGACTCTAAACCTAGTTTGTTCTCTGACCGCCTCAGTGATTTAG

GGCGCATTCCTCATCCCAGTATGAGAGTAGGTGTCCCGCCTCAGAACCCACGGCCCTCCCTGA

ACTCTGCACCAAGTCCTTTTAATCCACAAGGACAGAGTCAGATTACAG

   a. length:

   b. Methods of PCR product detection:

Worked out by: …… Group:…… Checked by:……

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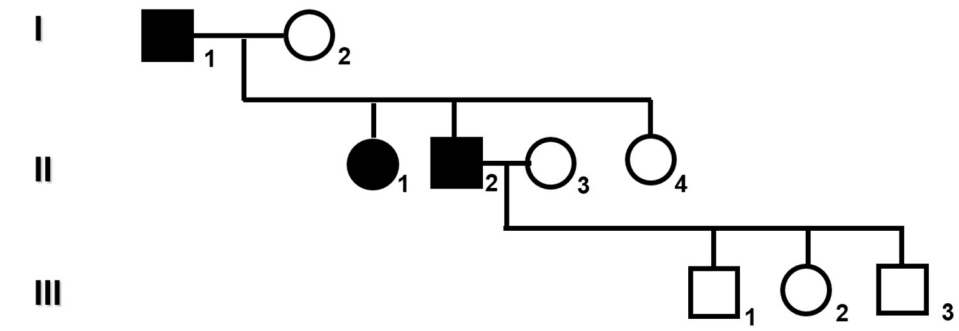
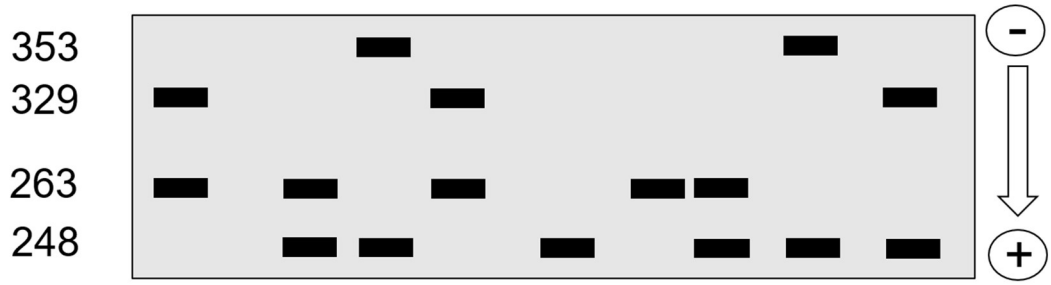
3. **Task 3, p. 129**: Direct DNA diagnostics in a family with Huntington chorea. Molecular cause of the disease is an excessively increased polyglutamine tract length in a protein called huntingtin. The triplet CAG that codes for glutamine is present in a tandem repetition even in a healthy person (repeat number 10-25). In affected individuals, the repeat number grows to 35 or more. Direct DNA diagnostics is based on **evaluation of PCR product size**. The product is 203 bp long, excepting the repeat region $(CAG)_n$. Onset of the disease is usually in late adulthood, therefore we can perform presymptomatic diagnostics in young individuals. **Who will be affected in the third generation?**

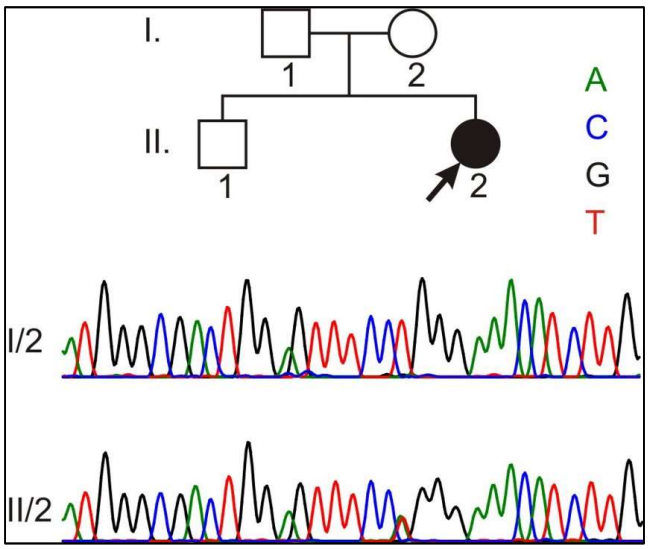




4. **Evaluation of Sanger sequencing electropherograms**. You obtained a printout of an automatized Sanger sequencing trace. Using publicly available databases, e.g. UCSC Genome Browser, OMIM and ClinVar, try to address following questions:
    a. Which **gene** is this sequence derived from?
    b. Using the OMIM database try to find out information about the gene, its protein product, and the pathology caused by mutations of this gene (**disease** or syndrome, **mode** of inheritance).
    c. The sequence contains a **variant** allele. Try to describe the allele (change on the DNA level, protein level, prediction of the mutated protein function). Compare your result with ClinVar database.

Electropherogram number	gene	Disease + mode of inheritance	DNA variant description

5. Inborn cataract (AD inheritance).



Reference sequence is ATG GGC GAC TGG AGT TTC CTG GGA AAC ATC TTG – in a 5´-3´ orientation (corresponding to I/2). :

a. Describe the mutation on DNA level
b. Describe the mutation on protein level (first nucleotide is also first position of the initiation codon of connexin 50).
c. Is the mutation pathogenic (chemical similarity and evolutionary conservation, use internet).
d. What is the risk of the disease for proband´s children? How is it possible that the parents are unaffected?

Answers:

a.

b.

c.

d.

6. Restriction digestion is a method using restriction endonucleases for obtaining some information about DNA sequence. Using a sequence fragment given below, fill in the complementary strand and find out restriction sites for following enzymes:

TaqI (T/CGA) AluI (AG/CT) MspI (C/CGG)

A G A A G C T T G A T G A C T C T A A A C C T A G T T T G T T C T C T G A C C G C C T C G A T

Decide whether the ends of the newly formed fragments will be blunt or sticky (cohesive):

TaqI	blunt	sticky
AluI	blunt	sticky
MspI	blunt	sticky

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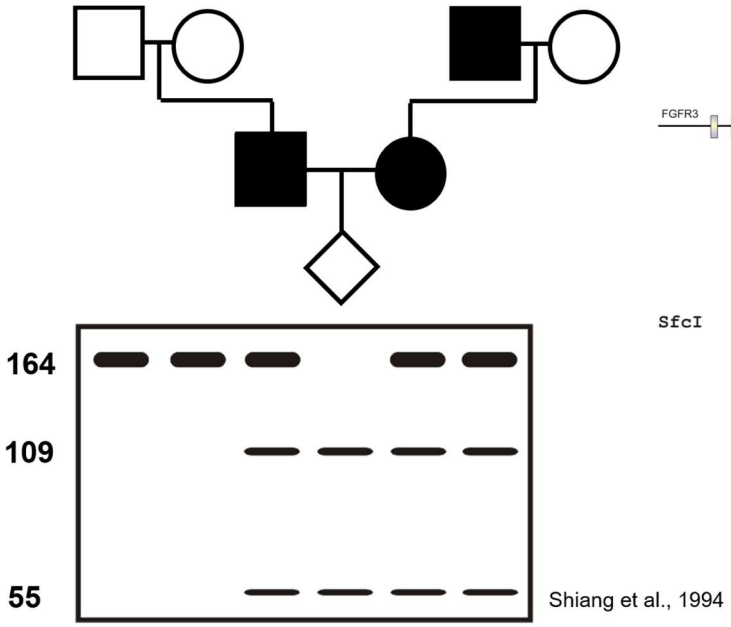
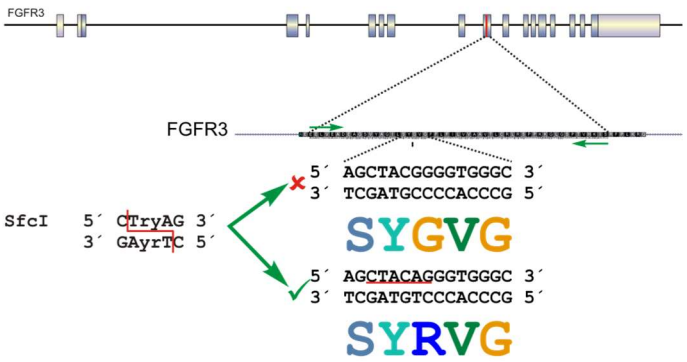
7. In a family with achondroplasia prenatal direct DNA diagnostics was performed focusing on the most common mutation **c.1138G>A** in *FGFR3* gene. The mutation also creates a new ScfI site. Below the pedigree there is a scheme of an electrophoretic gel containing amplified DNA fragments subsequently cleaved by restriction endonuclease SfcI.



Shiang et al., 1994



Genotype of the fetus:

Probable clinical presentation of the fetus: