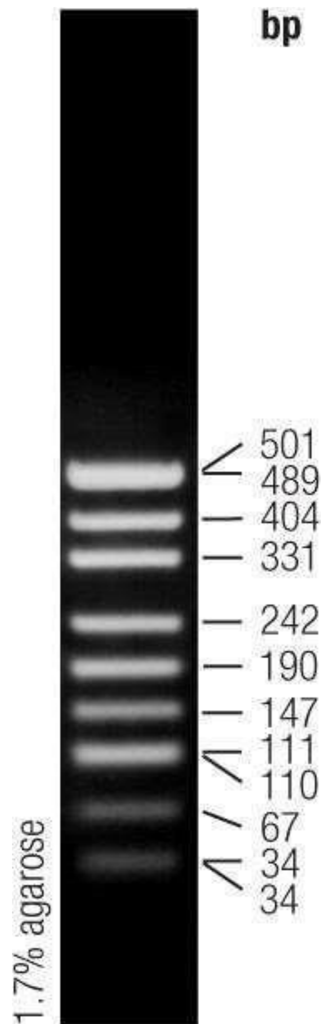
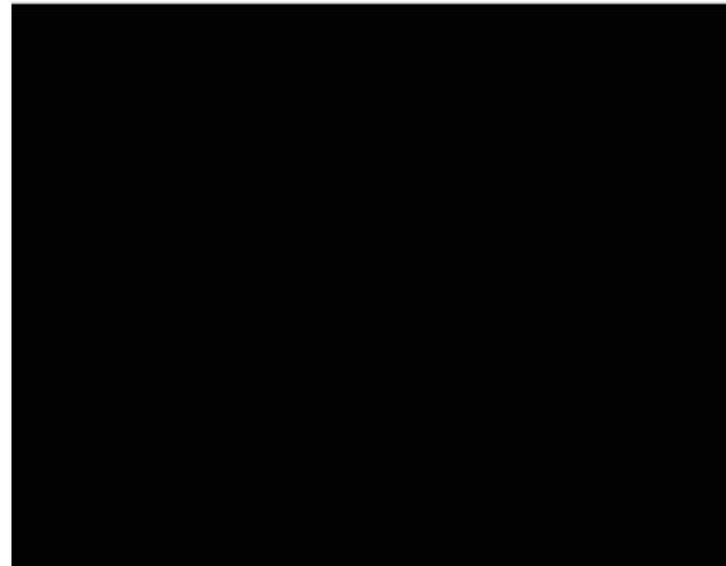
Marker of molecular weight for gel electrophoresis





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EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
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MŠMT
MINISTRY OF EDUCATION,
YOUTH AND SPORTS

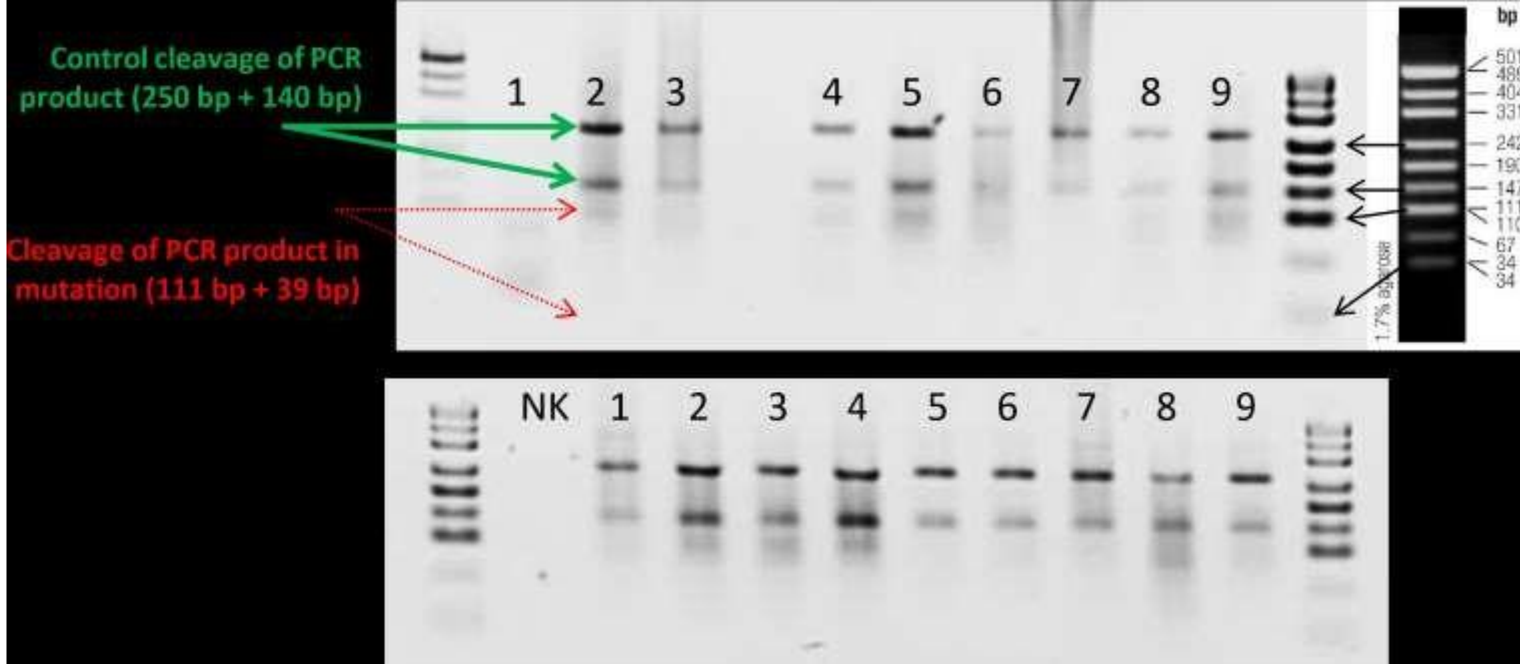
Evaluation of the results of CFTR gene analysis
Evaluation of the results of HFE gene analysis

Analysis: PCR with general primers followed by RFLP + gel electrophoresis

PCR: Amplification of HFE gene part with full length of 390 bp

RFLP: Restriction enzyme RsaI – cleavage site is created by mutation (mutation = cleavage) + additional control clevage site

- One cleavage of PCR product in control site (250 bp + 140 bp) = healthy homozygote
- Twice cleaved PCR product: in control site as well as in mutated site (250 bp + 111 bp + 39 bp) = homozygote with mutation
- Combination of both cases above (250 bp + 140 bp + 111 bp + 39 bp) = heterozygote



Hemochromatosis mutation H63D analysis (HFE gene)

Mutation H63D detection

Analysis: General PCR + subsequent analysis of PCR product using RFLP + gel electrophoresis

PCR: primers in PCR reaction bind **close** to the sequence of interest (place of possible mutation), but **not directly** on the sequence of interest subsequent analysis is needed RFLP: cleavage of PCR product by restriction endonuclease in the sequence of interest

Restriction enzyme BclI – cleavage site (TGATCA) is deleted by mutation (mutation = no cleavage)

 Full length PCR product (no cleavage, 208 bp) = homozygote with mutation

- Cleaved PCR product (138 bp + 70 bp) = healthy homozygote
- Full length PCR product combined with cleaved PCR product (208 bp + 138 bp + 70 bp) = heterozygote

Full length PCR product (208 bp)

homozygote with mutation | healthy homozygote | heterozygote

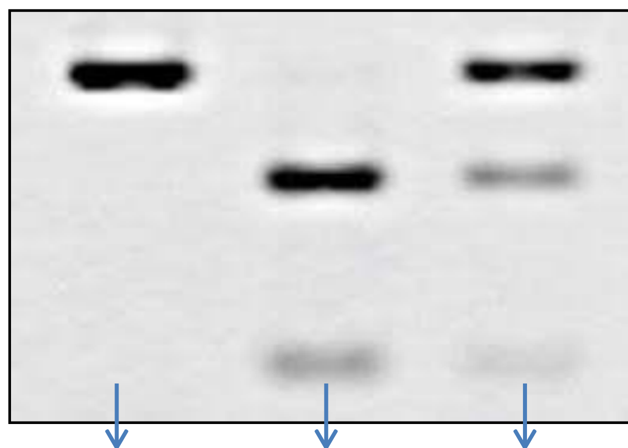
Evaluation of the results of HFE gene analysis

Analysis: PCR with general primers followed by RFLP + gel electrophoresis

PCR: Amplification of HFE gene part with full length of 208 bp

RFLP: Restriction enzyme BclI – cleavage site is deleted by mutation (mutation = no cleavage)

- Full length PCR product (no cleavage, 208 bp) = homozygote with mutation



Cleaved PCR product (first fragment: 138 bp)

Cleaved PCR product (second fragment: 70 bp)

- Cleaved PCR product (138 bp + 70 bp) = healthy homozygote

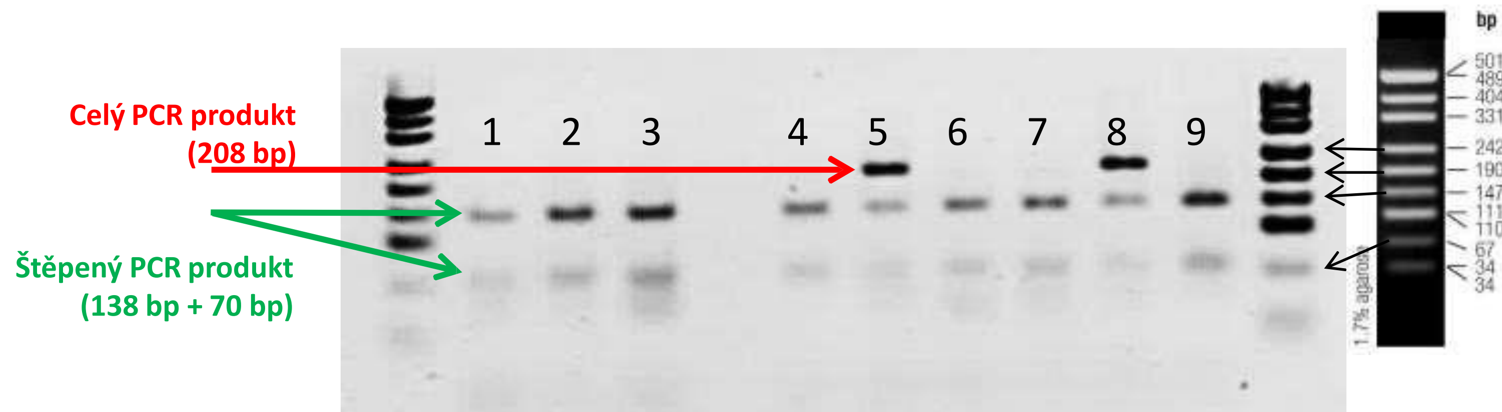
 Combination of full length PCR product and cleaved PCR product (208 bp + 138 bp + 70 bp)

Evaluation of the results of FV gene analysis

HemochromatosisLeiden **mutation** mutation **H63D** detection **analysis (HFE gene)**

Analysis: General PCR + subsequent analysis of PCR product using RFLP + gel electrophoresis

PCR: primers in PCR reaction bind **close** to the sequence of interest (place of possible mutation), but **not directly** on the sequence of interest subsequent analysis is needed RFLP:



= heterozygote

cleavage of PCR product by restriction endonuclease in the sequence of interest

Restriction enzyme MnlI – cleavage site (GGAG) is <u>deleted</u> by mutation (mutation = no cleavage) – one additional cleavage site (GGAG) is always presented in amplified

DNA part = normal sequence serving as a positive control of cleavage

PCR product (287 bp) cleaved once i.e. in control point only (157 bp + 130 bp) = homozygote with mutation PCR product (287 bp) cleaved twice i.e. in control point as well as in position without mutation (157 bp + 93 bp + 37 bp) = healthy homozygote

 PCR product (287 bp) cleaved in control point only (mutated alele, 157 bp + 130 bp) combined with PCR product (287 bp) cleaved in both control as well as nonmutated position (healthy alele) (157 bp + 93 bp + 37 bp) i.e. 157 bp + 130 bp + 93 bp + 37 bp = heterozygote

PCR product cleaved in control site (first fragment: 157 bp)

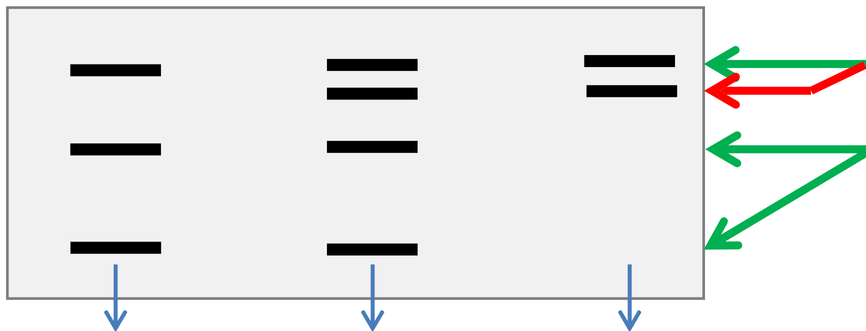
healthy heterozygote homozygote (second fragment: 130 bp)

PCR product cleaved in healthy sequence

(first fragment: 93 bp)

(second fragment: 37 bp)

homozygote with mutation



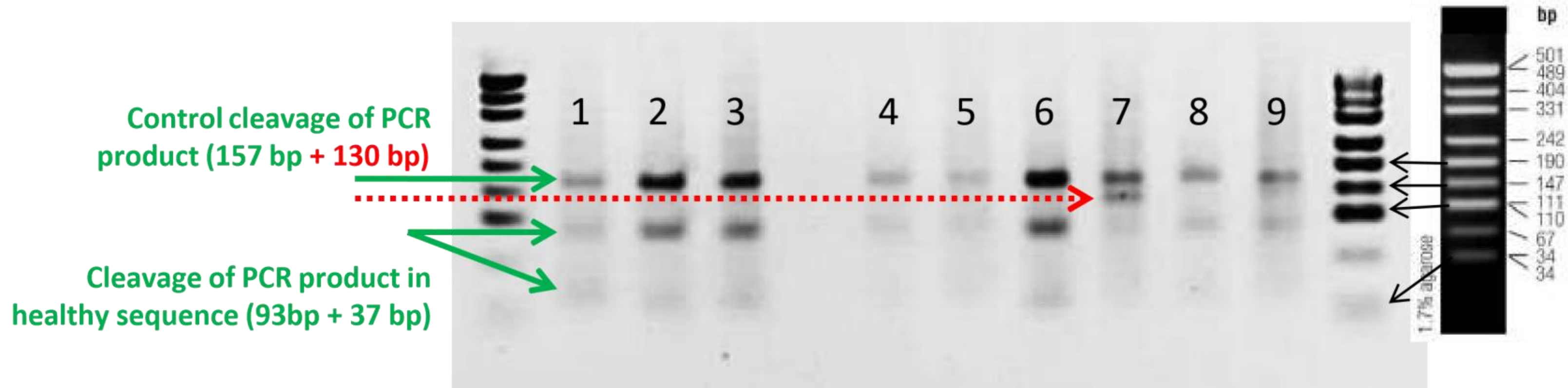
Analysis of Leiden mutation R506Q (FV gene)

Analysis: PCR with general primers followed by RFLP + gel electrophoresis

PCR: Amplification of FV gene part with full length of 287 bp

RFLP: Restriction enzyme MnlI – cleavage site is deleted by mutation (mutation = no cleavage) + additional control clevage site

 One cleavage of PCR product in control site (157 bp + 130 bp) = homozygote with mutation



 Twice cleaved PCR product: in control site as well as in non-mutated site (157 bp + 93 bp + 37 bp) = healthy homozygote

 Combination of both cases above (250 bp + 130 bp + 93 bp + 37 bp) = heterozygote

Autor: kolektiv autorů pod vedením prof. MUDr. Petra Zacha, CSc. z Ústavu Anatomie 3. LF UK