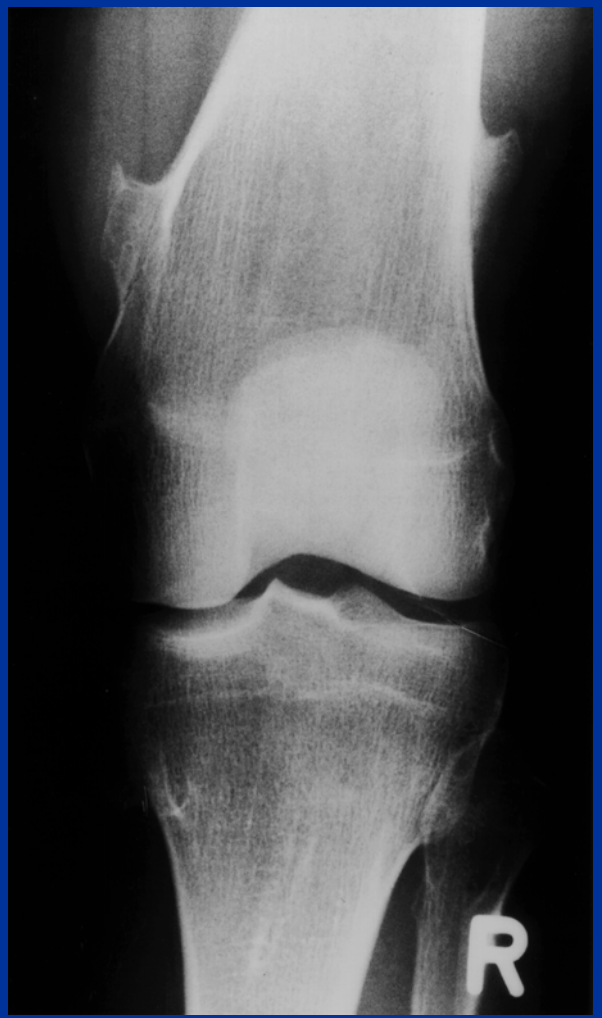
MHE: Mutation screening strategies

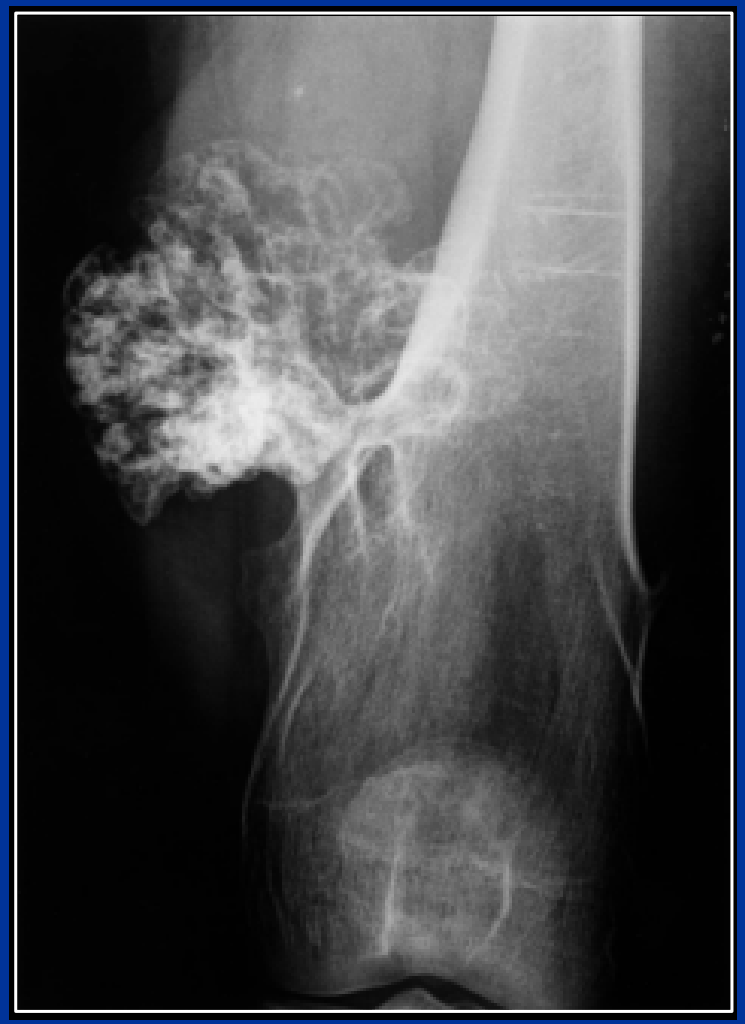
WIM WUYTS, PhD

Department of Medical Genetics
University of Antwerp-Belgium

CMMG
CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

Hereditary multiple exostoses/osteochondromas



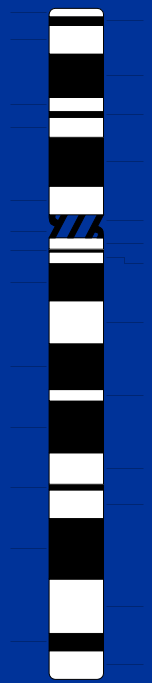


CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

Genetic aspects of MHE

- genetic heterogeneous

EXT1



8q24.1

Chromosome 8

EXT2

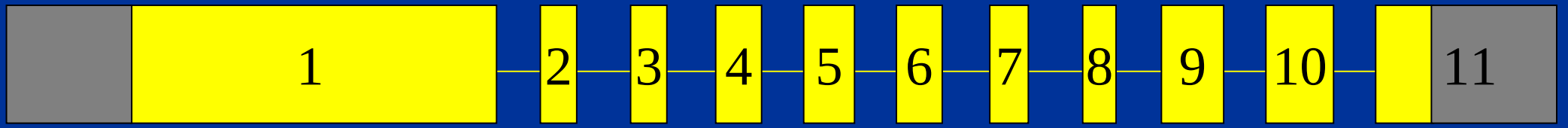
11p11-p12

Chromosome 11

?

CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

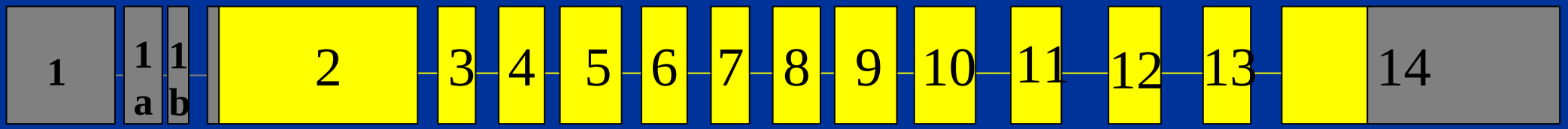
EXT1 gene



- 312 kb
- 746 amino acids
- 2238 coding bases

CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

EXT2 gene



- 150 kb
- 718 amino acids
- 2154 coding bases

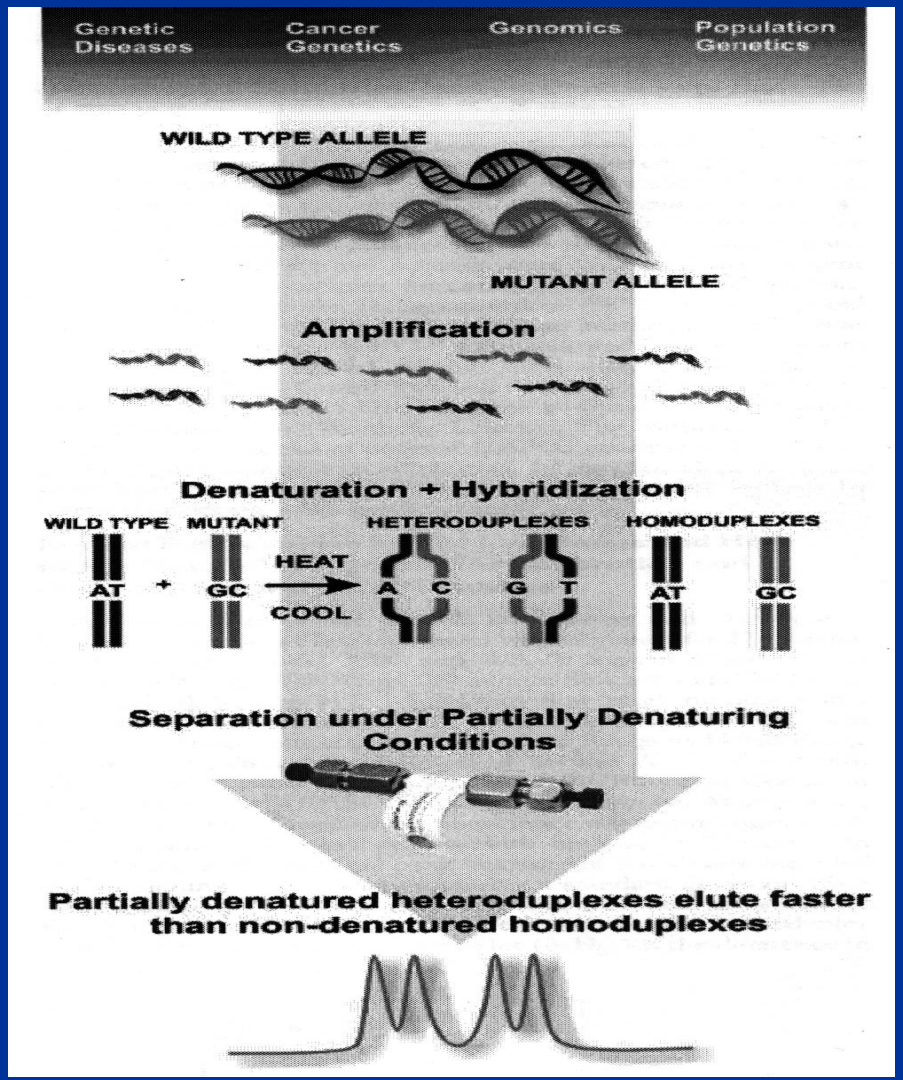
CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

Initial EXT mutation studies

- SSCP
  - EXT1 40-45%
  - EXT2 25-30%
  - no mutation 30%
- Sequencing
  - EXT1 50-60%
  - EXT2 30-35%
  - no mutation 15-20%

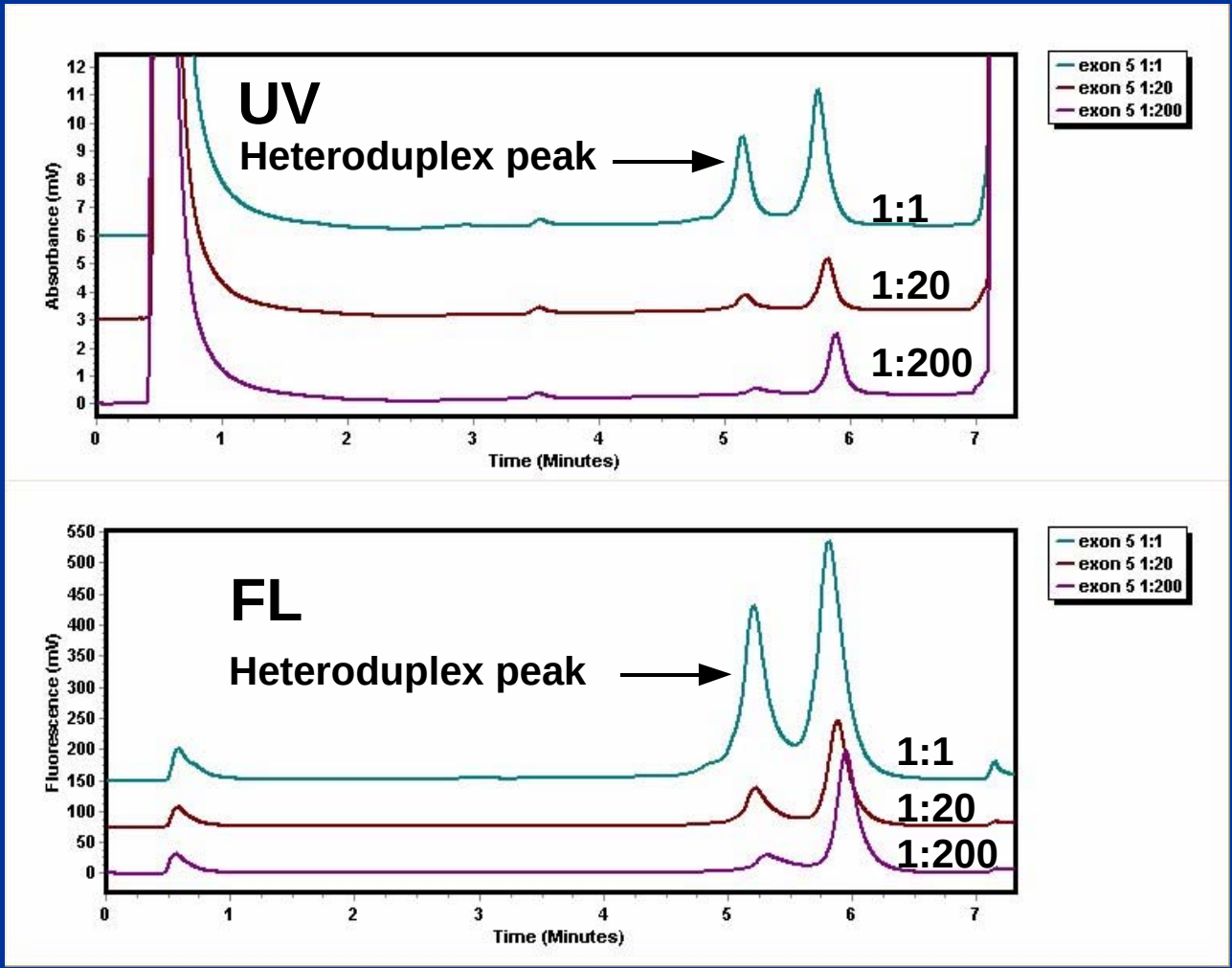
CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

DHPLC



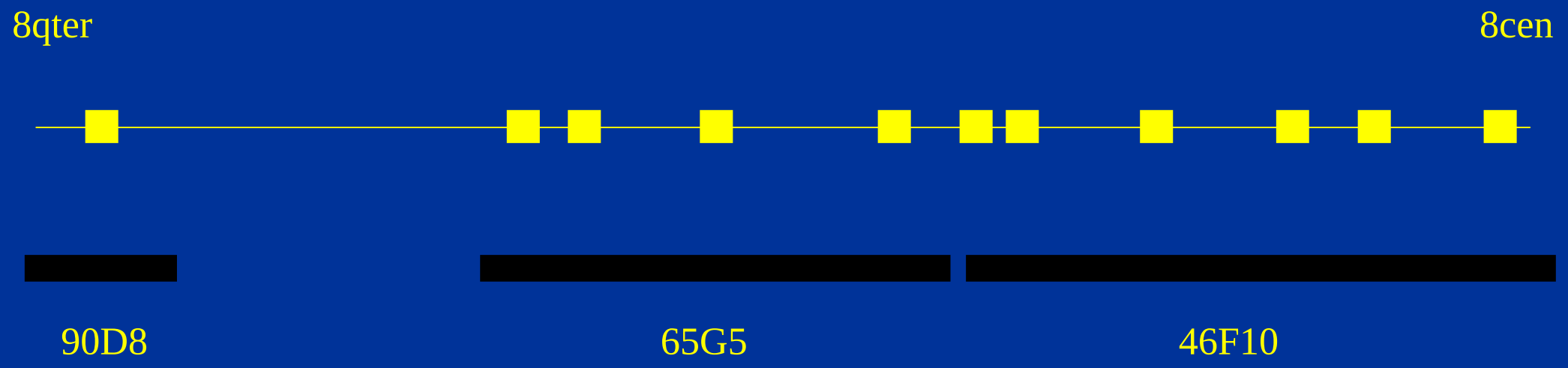
CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

DHPLC



CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

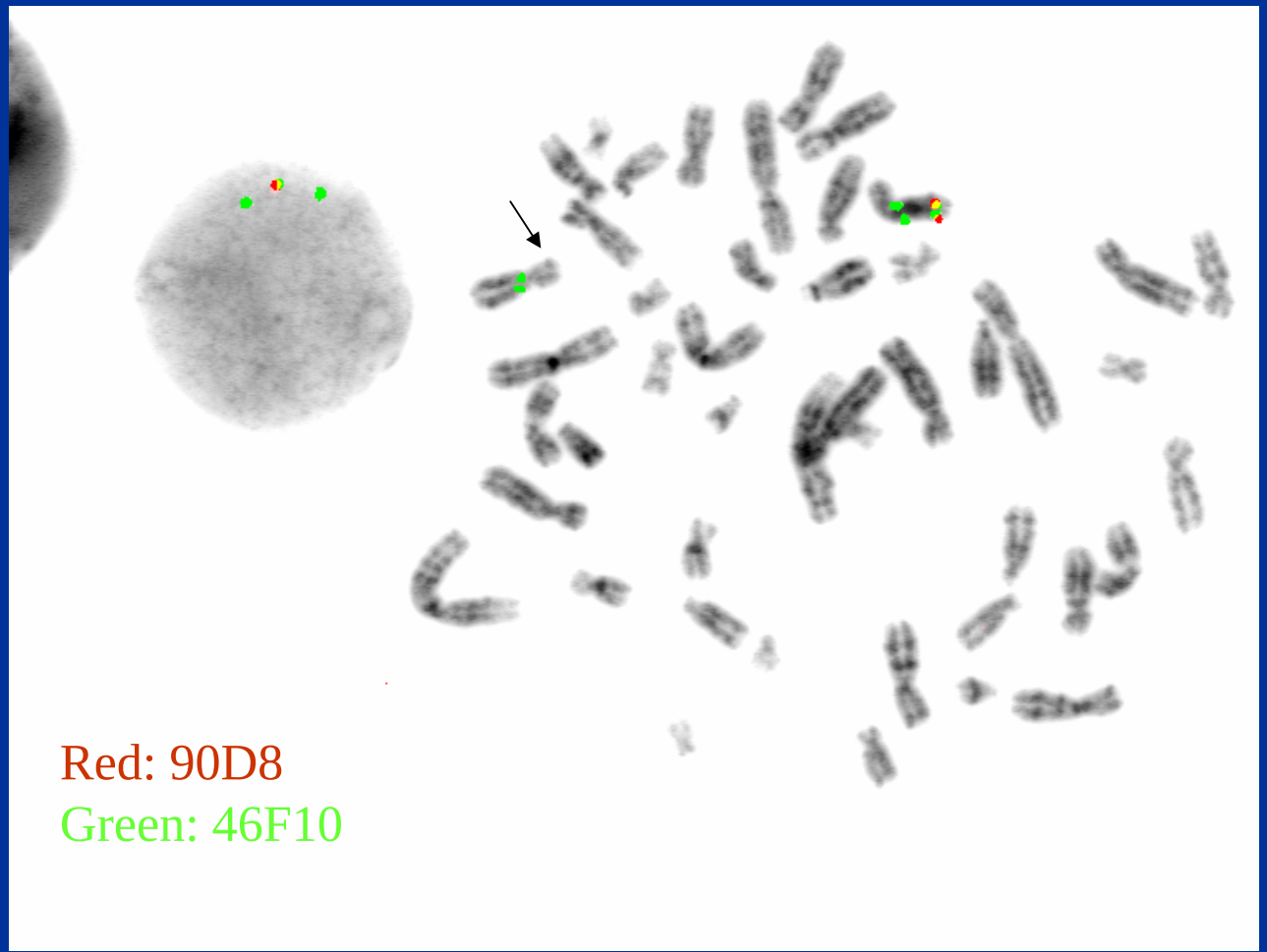
FISH analysis EXT1



Two-colour FISH

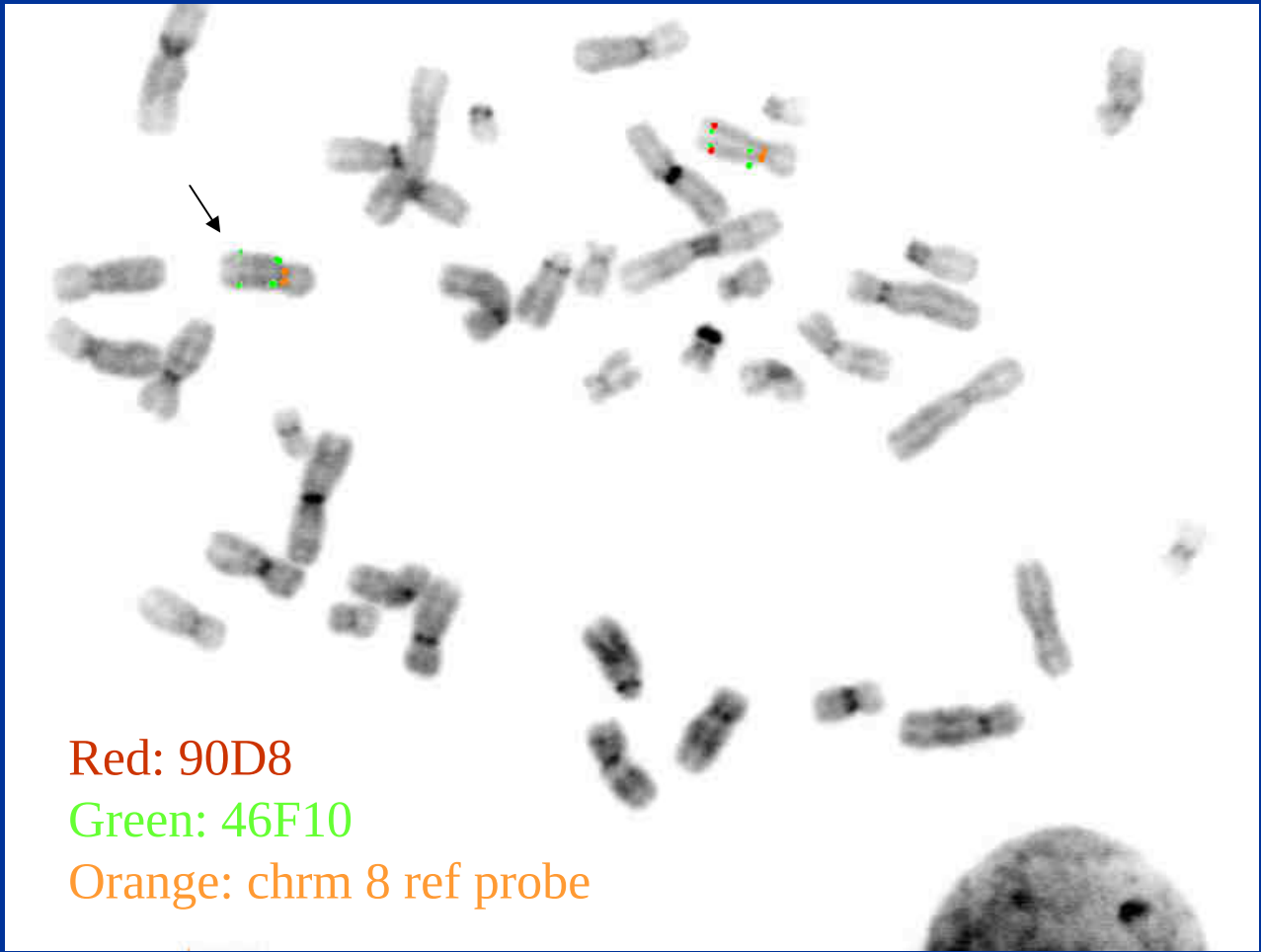
CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

EXT1 deletions



CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

EXT1 deletions



CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

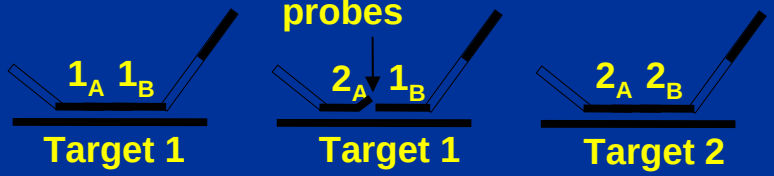
MLPA

Probe design

Synthetic oligonucleotide M13 derived oligonucleotide

Multiplex hybridisation and ligation

No ligation of mismatched probes



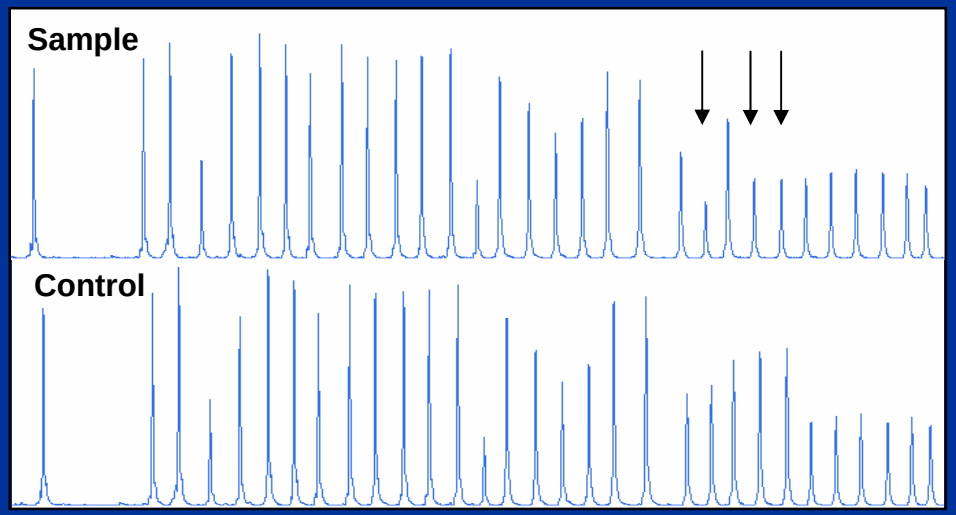
PCR with universal primers X and Y

No exponential amplification of non ligated probes

Fragment Analysis

CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

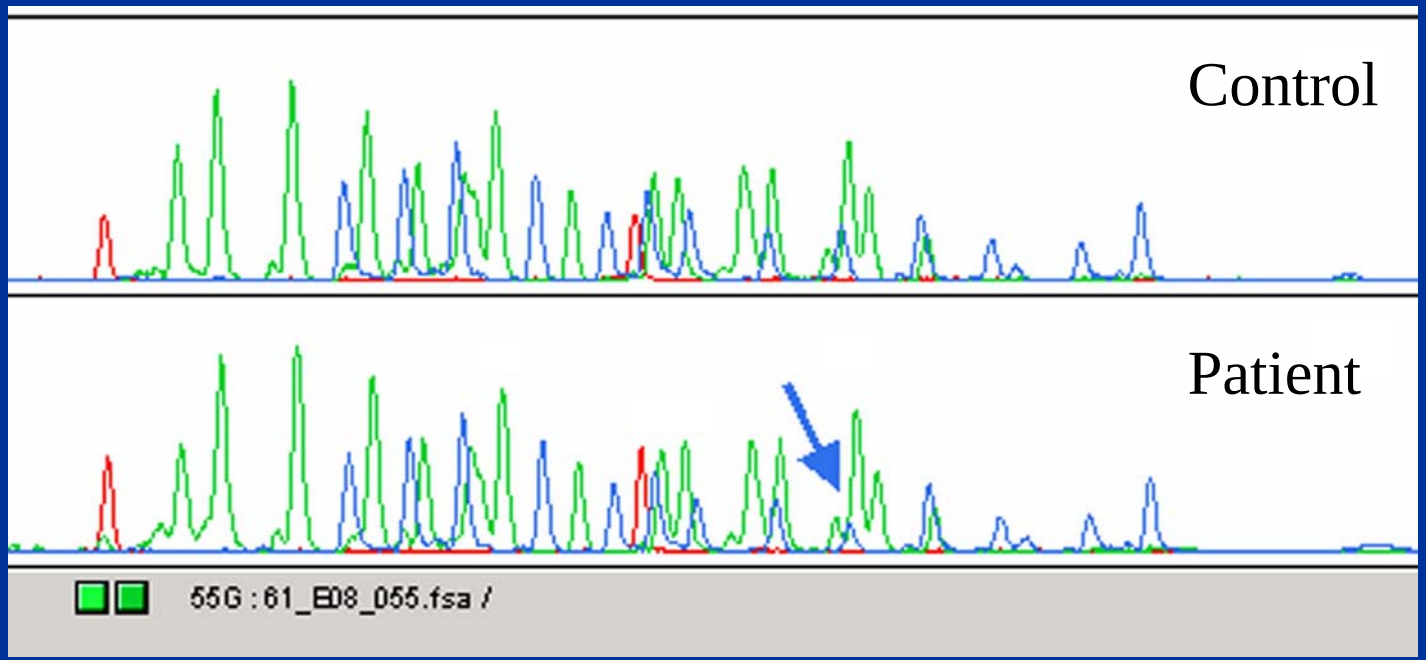
MLPA



- Amplification products are identified and quantified by capillary electrophoresis
- Copy numbers of target sequences are determined by comparison to a control sample.
- Peak areas are normalized by dividing each peak area by the combined peak areas of all peaks in that lane.

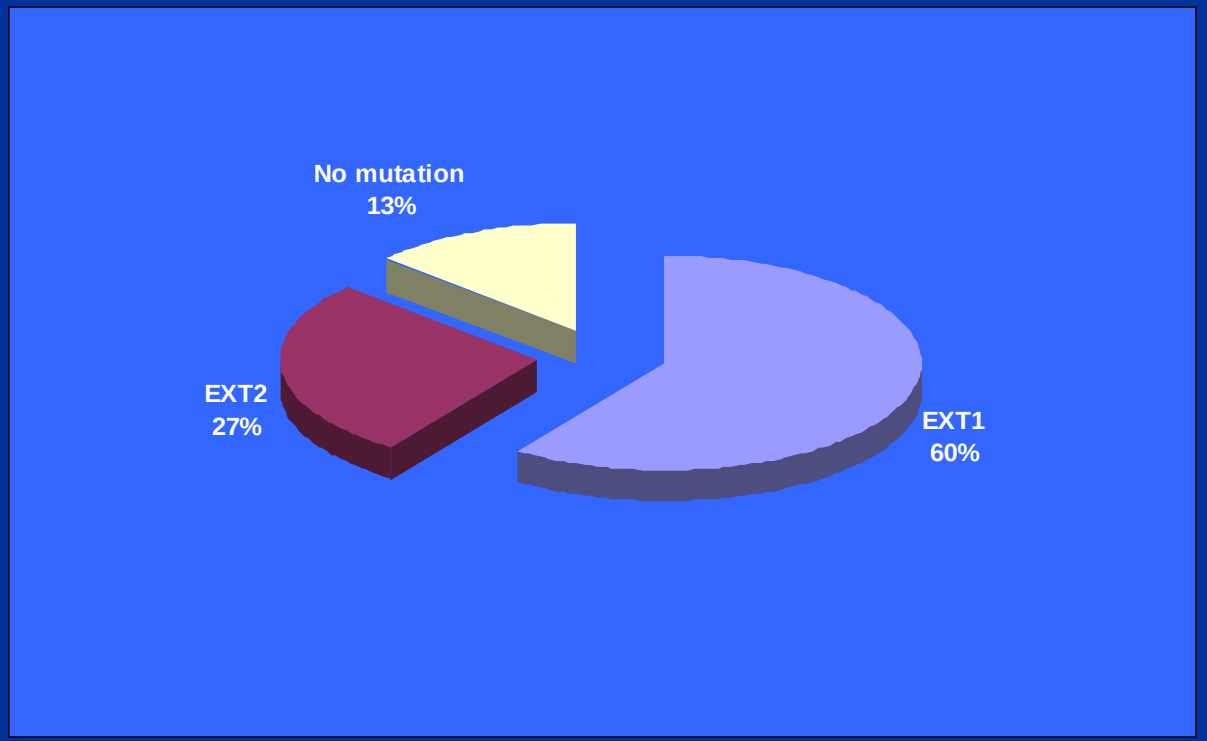
CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

MLPA EXT1 and EXT2



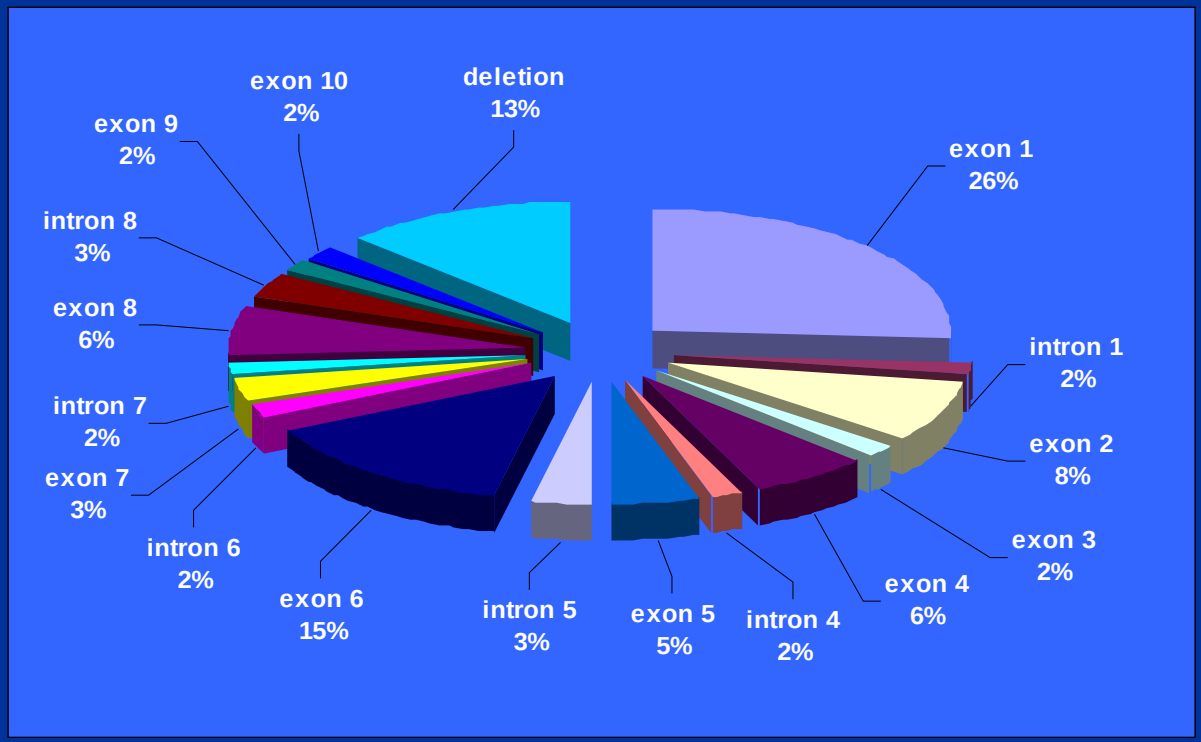
CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

Genetic screening for MHE



CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

EXT1 mutation distribution



CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

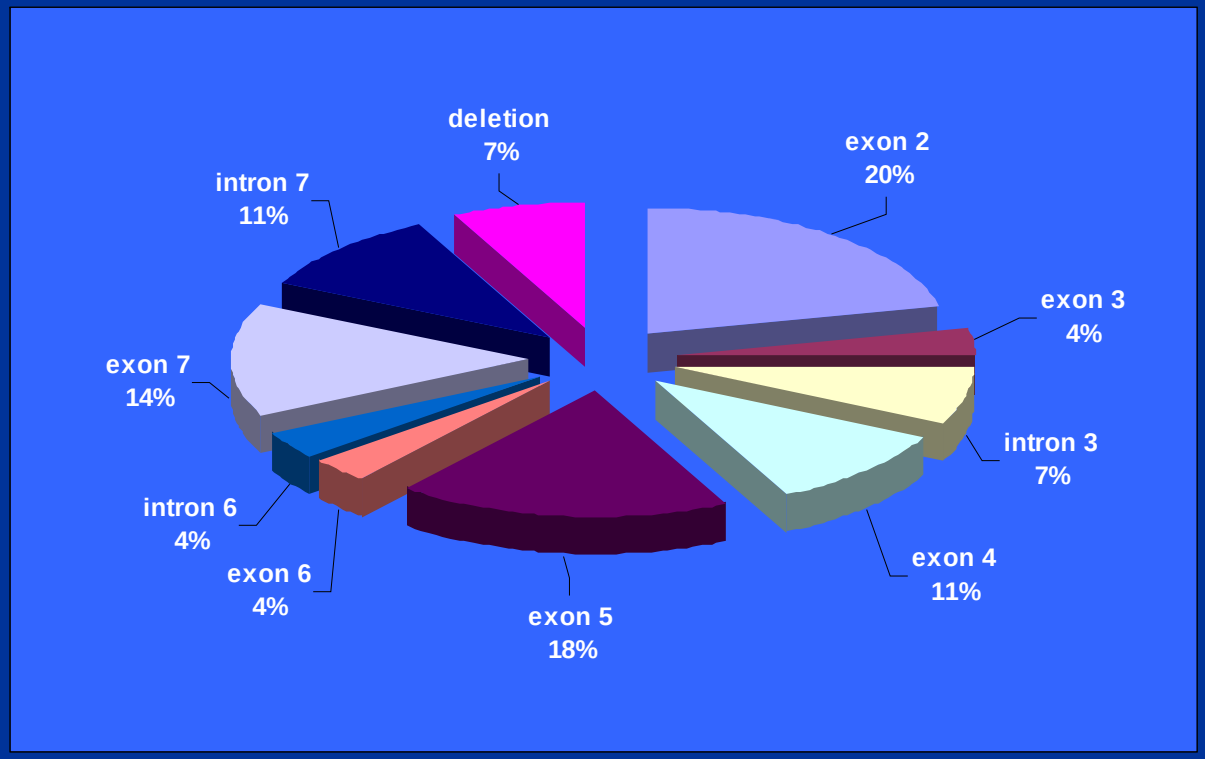
EXT1 mutation spectrum

- Frameshift mutations 34%
- Nonsense mutations 29%
- Splice site mutations 18%
- (partial) deletions 13%
- Missense mutations 6% (Arg280, Arg340, Gly339)

- private mutations – 1469delT

CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

EXT2 mutation distribution



CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

EXT2 mutation spectrum

- Splice site mutations 35%
- Nonsense mutations 28%
- Frameshift mutations 20%
- Missense mutations 10% (Asp227)
- (partial) deletions 7%

CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

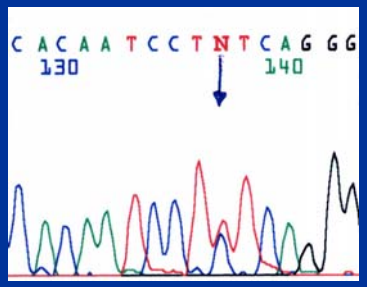
Research testing for MHE

- linkage analysis
- Non coding exons
- Promotor region
- Analysis of osteochondroma material
- RNA studies

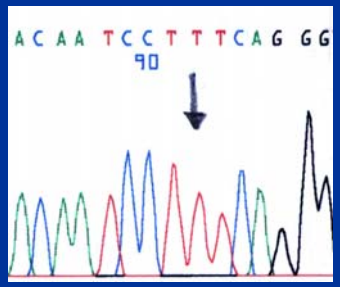
CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

RNA studies

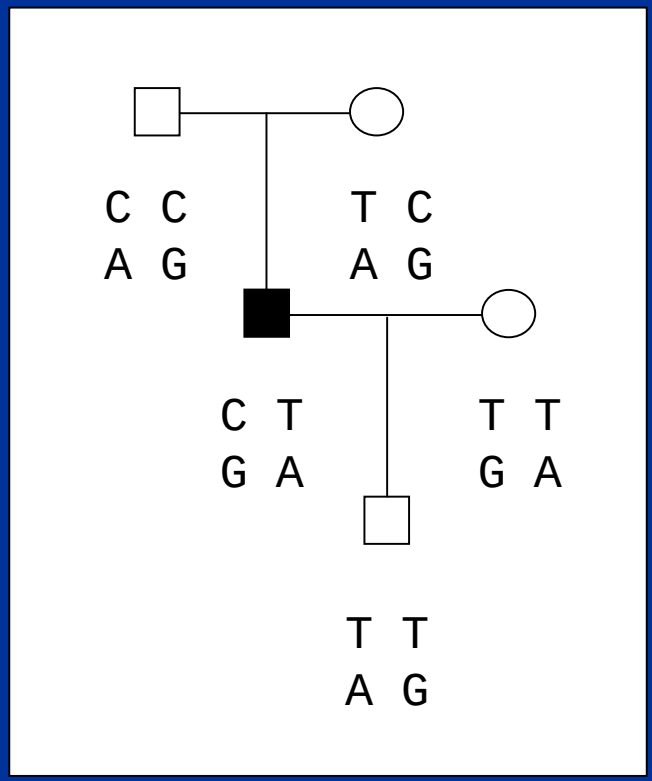
DNA



RNA



1065 C>T (Cys355Cys)
(exon 3)



1065 C>T (Cys355Cys)
1761 A>G (Glu587Glu)

CMMG
CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

Conclusion

- Analysis of EXT1 and EXT2
- Clinical Testing

  sequencing al coding exons

  faster, more expensive, quality label, counselling
- Research testing

  extended analysis

  no costs, no personal results, no counseling

CMG
CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN

Acknowledgements

- Department of Medical Genetics-University of Antwerp (Belgium)

Wim Wuyts, PhD
Wim Van Hul, PhD
Lieve Vits
Remco Radersma
Nele De Groof

- Patients and Families

CMG
CENTRUM MEDISCHE GENETICA
UNIVERSITEIT ANTWERPEN