UNIVERSITÄTS FREIBURG KLINIKUM

Mitochondrial Heteroplasmy

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Outline

- Characterization of length and sequence heteroplasmy
- Heteroplasmic sites
- Problems
- Heteroplasmy in single cells and triplets

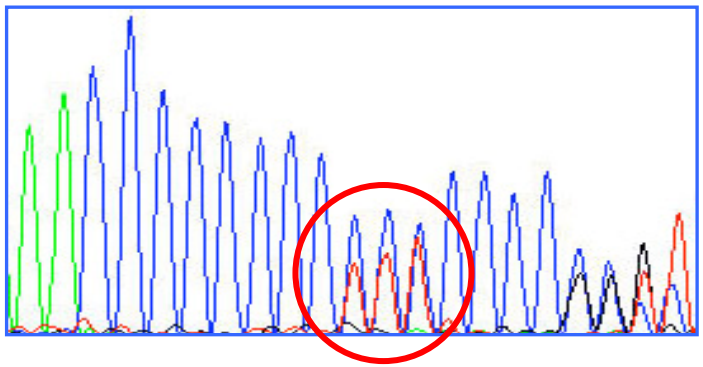
Definition

- *Mitochondrial heteroplasmy:*

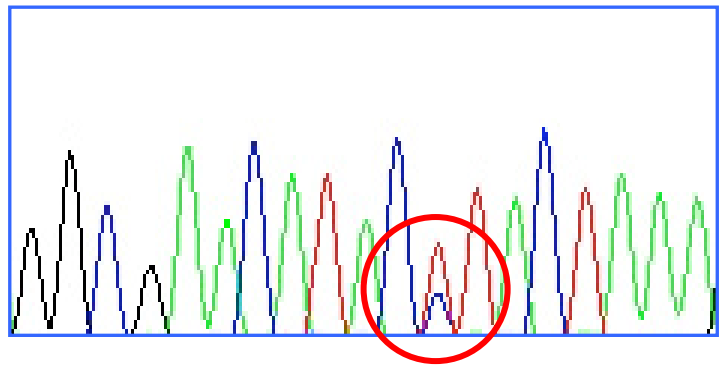
  presence of a mixture of more than one type of mtDNA within an individual

Differentiation of

length and sequence heteroplasmy



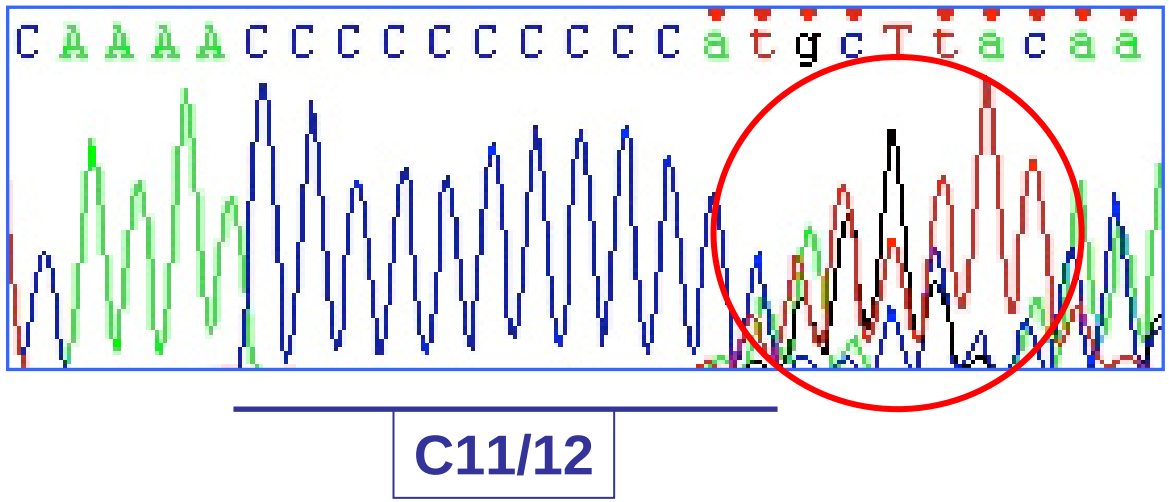
length heteroplasmy



sequence heteroplasmy

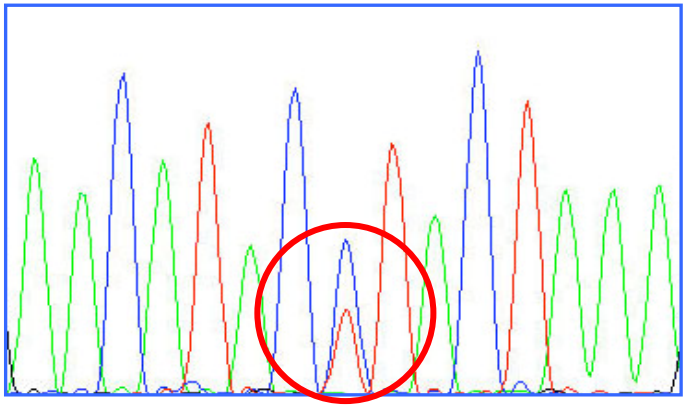
Length heteroplasmy

- Variants differ in the number of bases
- Elevated sequence background



Sequence heteroplasmy

- Variants differ in the type of bases
- Superimposition of usually two different bases at one position



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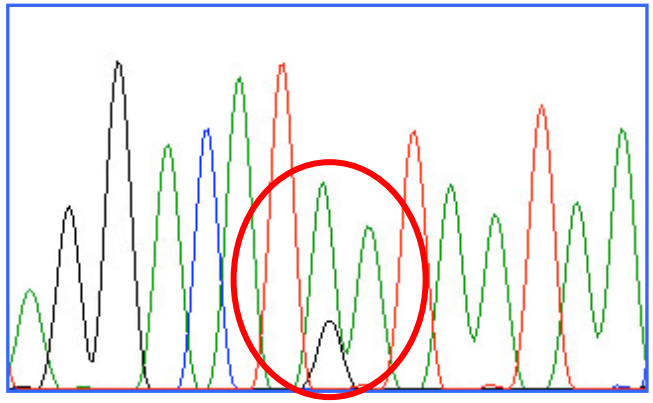
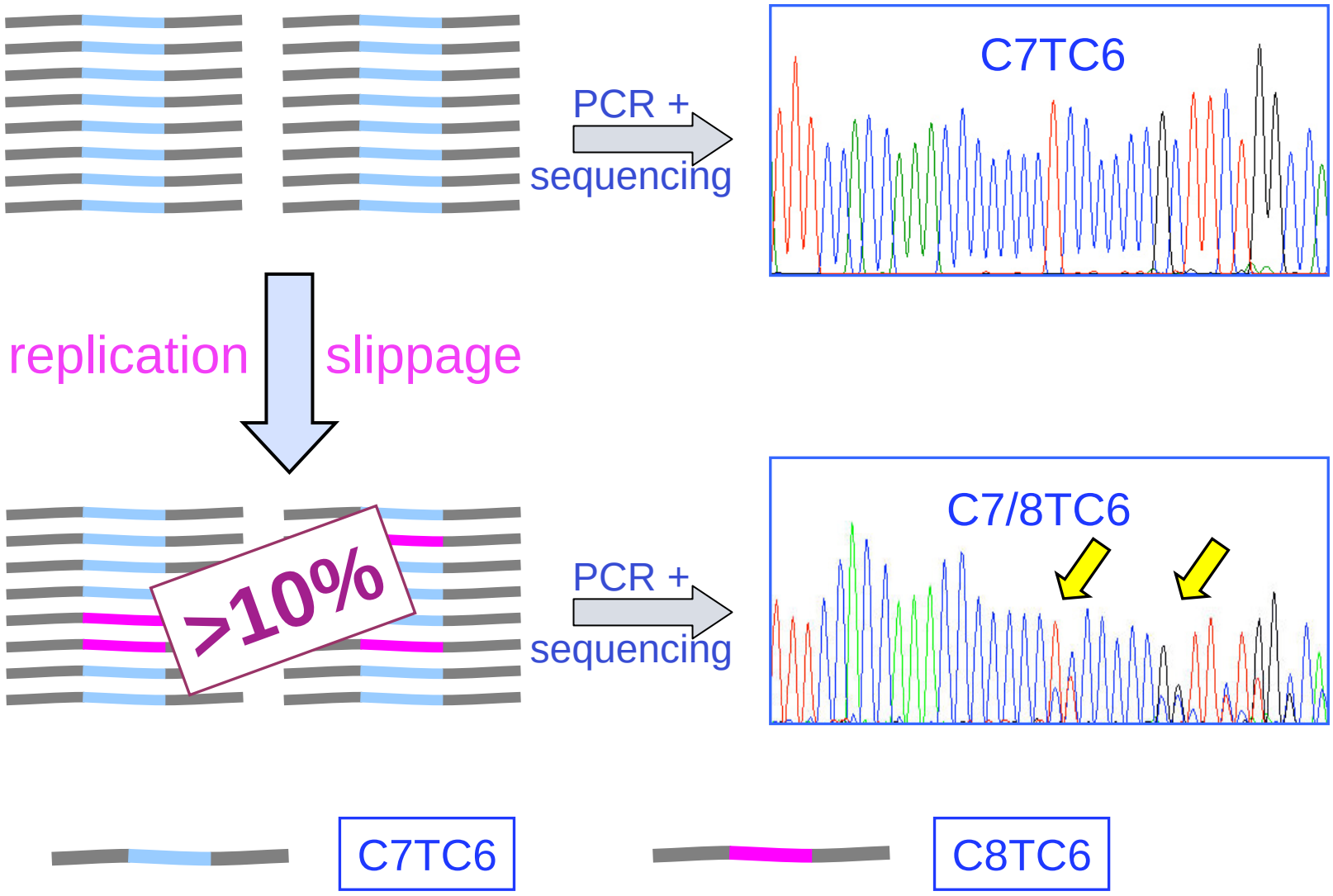
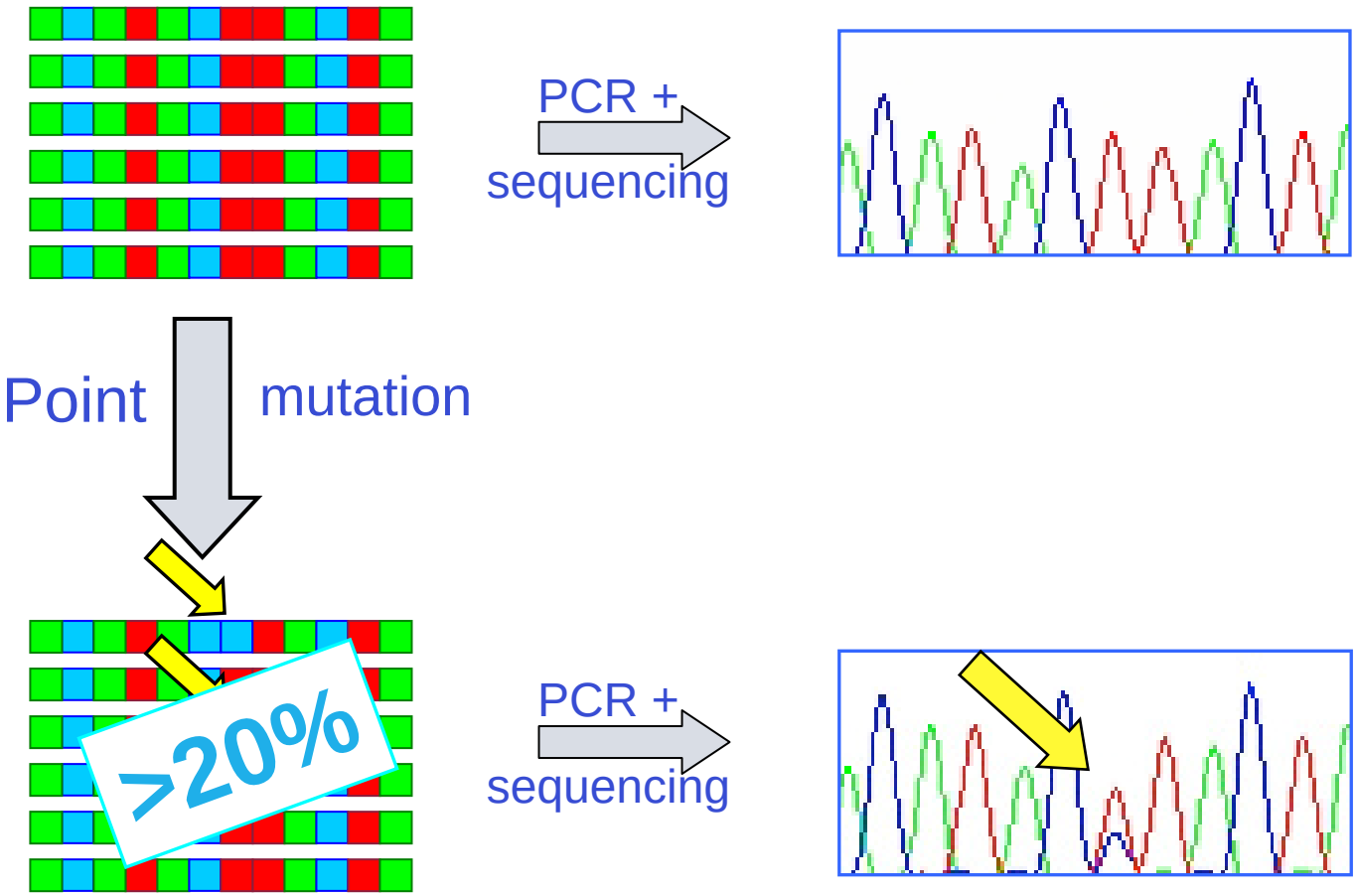


234R

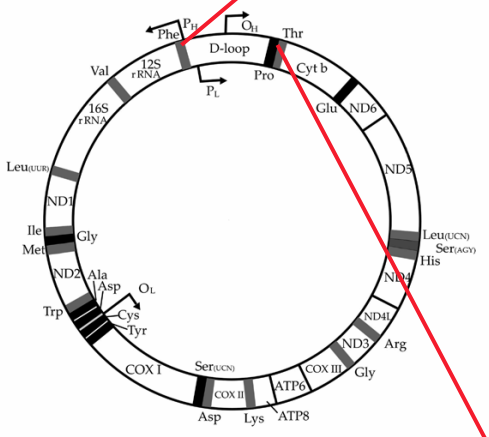
Length heteroplasmy - Mechanism



Sequence heteroplasmy - Mechanism



Length heteroplasmy - Sites

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16101  TTACTGCCAG CCACCATGAA TATTGTACGG TA                  GACCAC
16151  CTGTAGTACA TAAAAACCCA ATCCACATCA AA                  TGCTTA
16201  CAAGCAAGTA CAGCAATCAA CCCTCAACTA TC                  CAACTC
16251  CAAAGCCACC CCTCACCCAC TAGGATACCA ACAAACCTAC CCACCCTTAA
16301  CAGTACATAG TACATAAAGC CATTTACCGT ACATAGCACA TTACAGTCAA
16351  ATCCCTTCTC GTCCCCATGG ATGACCCCCC TCAGATAGGG GTCCCTTGAC
16401  CACCATCCTC CGTGAAATCA ATATCCCGCA CAAGAGTGCT ACTCTCCTCG
16451  CTCCGGGCCC ATAACACTTG GGGGTAGCTA AAGTGAACTG TATCCGACAT
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16551  TAAATAAGAC ATCACGATG
00001  GATCACAGGT CTATCACCCT ATTAACCACT CACGGGAGCT CTCCATGCAT
00051  TTGGTATTTT CGTCTGGGGG GTATGCACGC GATAGCATTG CGAGACGCTG
 101   GAGCCGGAGC ACCCTATGTC GCAGTATCTG TCTTTGATTC CTGCCTCATC
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 201   AAGTGTGTTA ATTAATTAAT GCTTGTAGGA CATAATAATA ACAATTGAAT
 251   GT                  TCCA CACAGACATC ATAACAAAAA ATTTCCACCA
 301   AA                  CTTC TGGCCACAGC ACTTAAACAC ATCTCTGCCA
 351   AA                  AACC CTAACACCAG CCTAACCAGA TTTCAAATTT
 401   TATCTTTTGG CGGT                  AACAGT CACCCCCCAA CTAACACATT
 451   ATTTTCCCCT CCCA                  ACTAAT CTCATCAATA CAACCCCCGC
 501   CCATCCTACC CAGC                  CGCTGC TAACCCCATA CCCCGAACCA
 551   ACCAAACCCC AAAGACAC                  ACA
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C5TC4

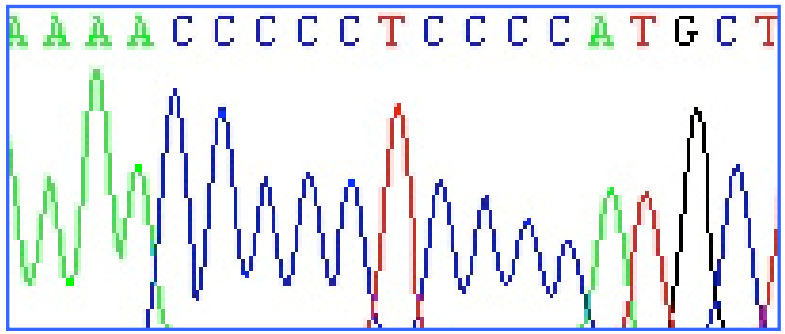
C7TC5

$(AC)_5$

C6

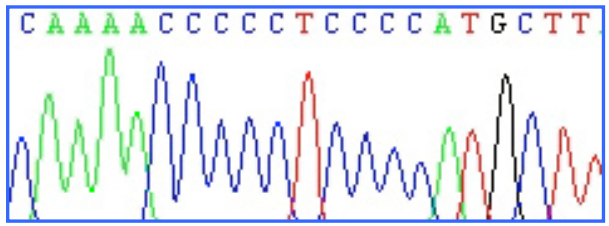
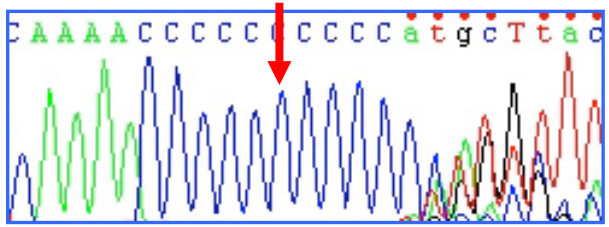
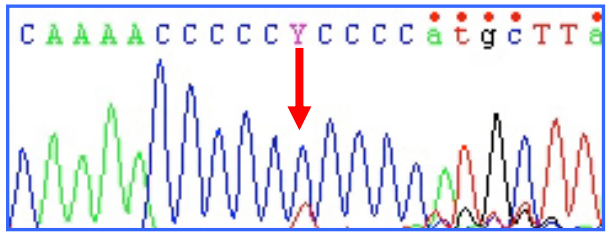
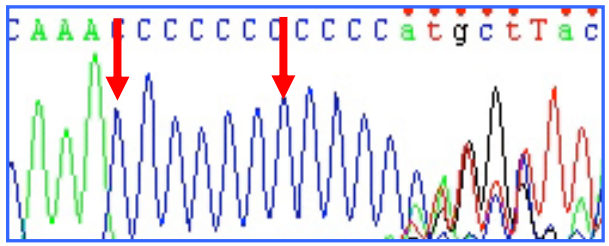
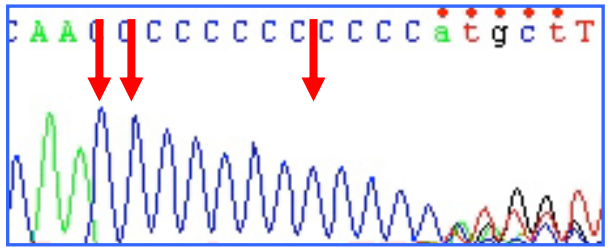
Positions 16184-16193

C5TC4

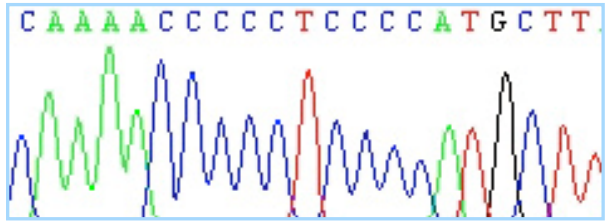


according to rCRS

Bendall & Sykes 1995, Am J Hum Genet 57, 248ff

according to rCRS		**AAAACCCCCTCCCCA**
T16189C		**AAAACCCCCCCCCCCN**
T16189Y		**AAAACCCCCYCCCCA**
A16183C; T16189C		**AAACCCCCCCCCCCN**
A16182C; A16183C; T16189C		**AACCCCCCCCCCCCN**

according to rCRS



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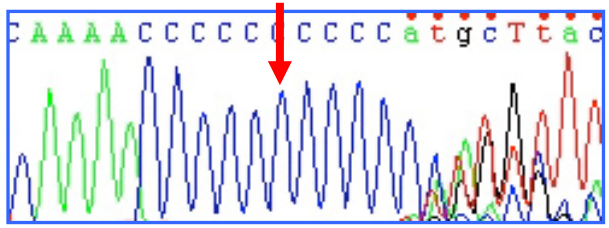

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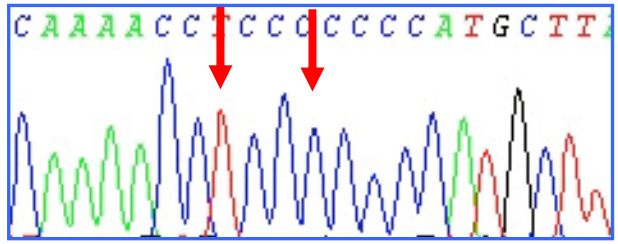

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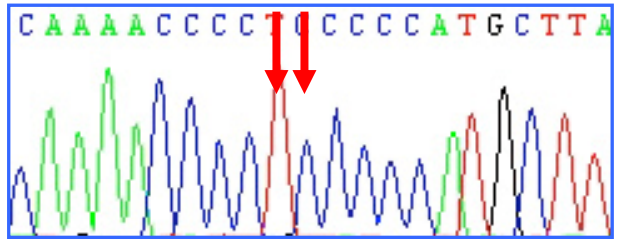
C16188T; T16189C



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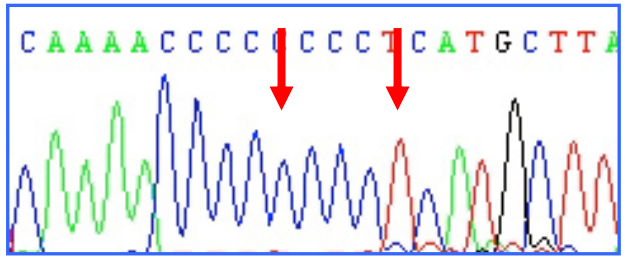
T16189C; C16193T



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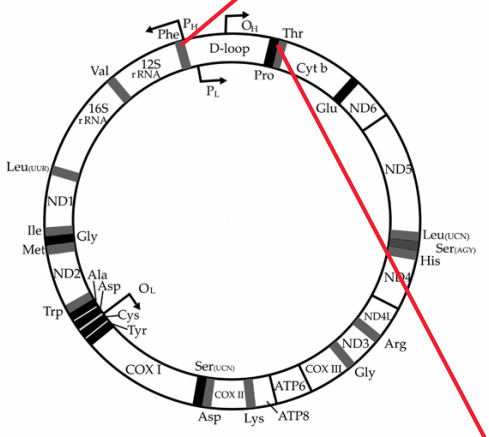
Length heteroplasmy - Sites

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16101  TTACTGCCAG CCACCATGAA TATTGTACGG TA          GACCAC
16151  CTGTAGTACA TAAAAACCCA ATCCACATCA AA          TGCTTA
16201  CAAGCAAGTA CAGCAATCAA CCCTCAACTA TC          CAACTC
16251  CAAAGCCACC CCTCACCCAC TAGGATACCA ACAAACCTAC CCACCCTTAA
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 401   TATCTTTTGG CGGT          TTTTAACAGT CACCCCCAA CTAACACATT
 451   ATTTTCCCCT CCCA          ACTAAT CTCATCAATA CAACCCCCGC
 501   CCATCCTACC CAGC          CGCTGC TAACCCCATA CCCCGAACCA
 551   ACCAAACCCC AAAGACAC          ACA
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C5TC4

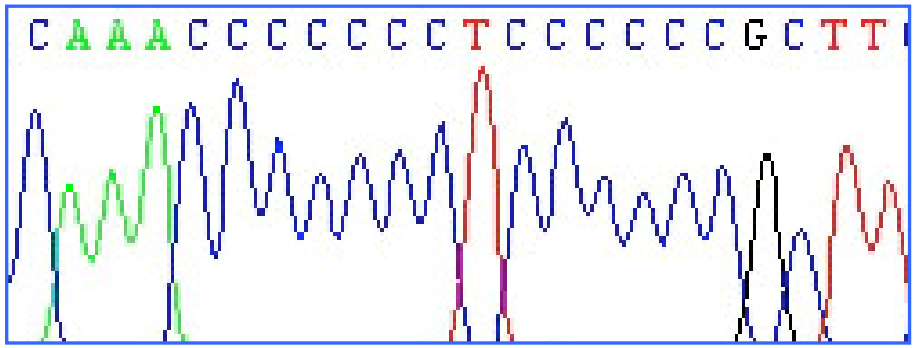
C7TC5

$(AC)_5$

C6

Positions 303-315

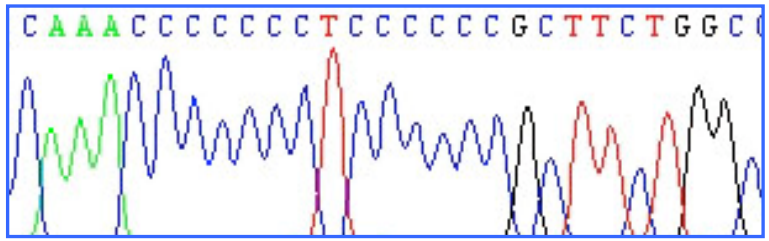
C7TC6



according to rCRS

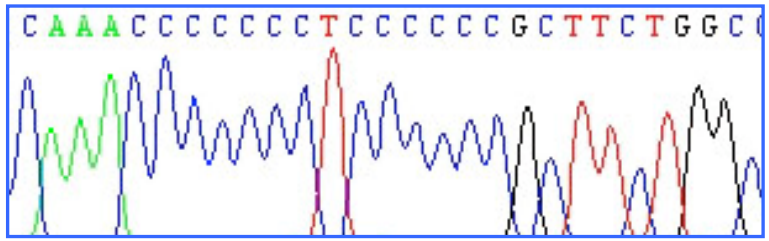
Greenberg et al. 1983, Gene 21, 33ff

Length heteroplasmy: 303-315



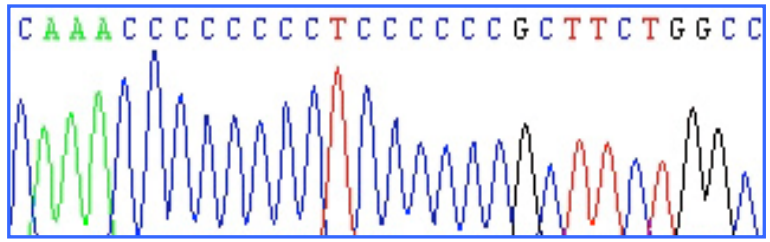
according to rCRS

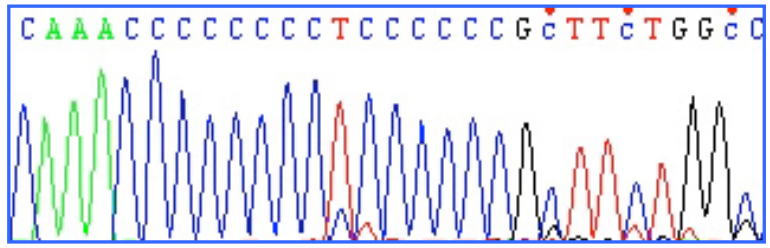
Length heteroplasmy: 303-315

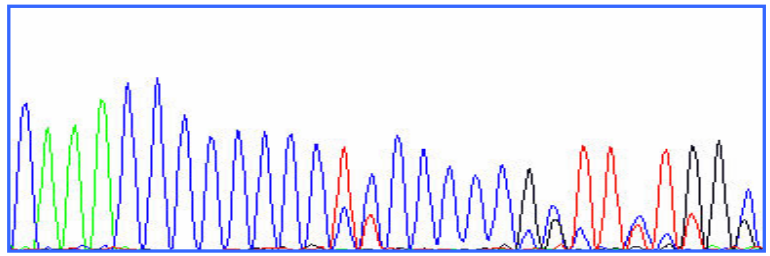


according to rCRS

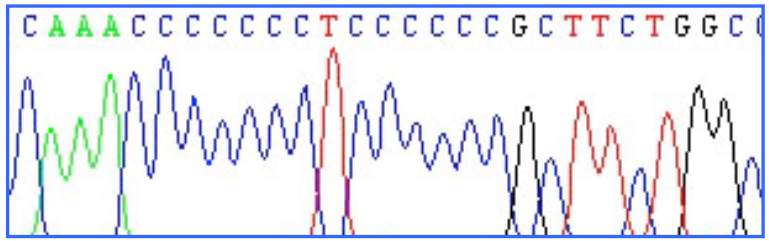
309.1C







Length heteroplasmy: 303-315



according to rCRS

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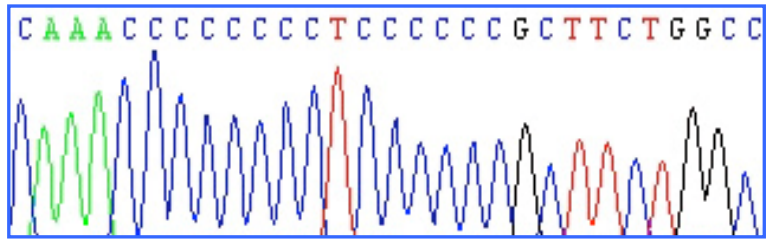
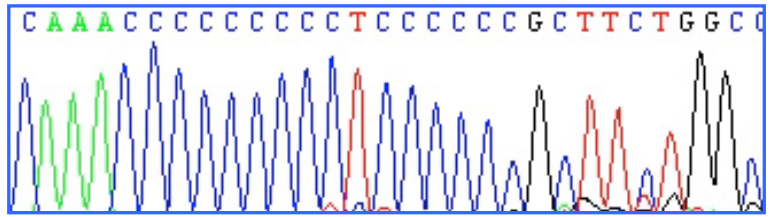
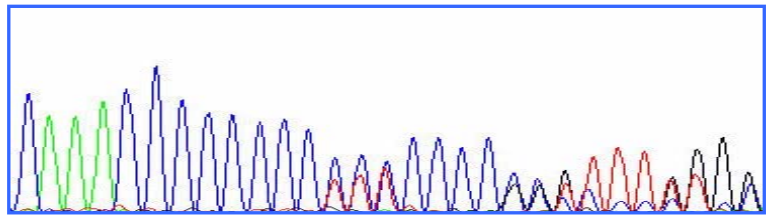
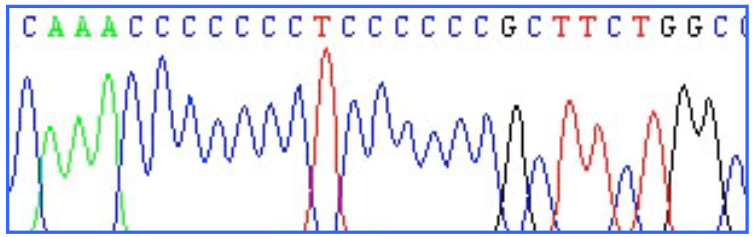


309.2C



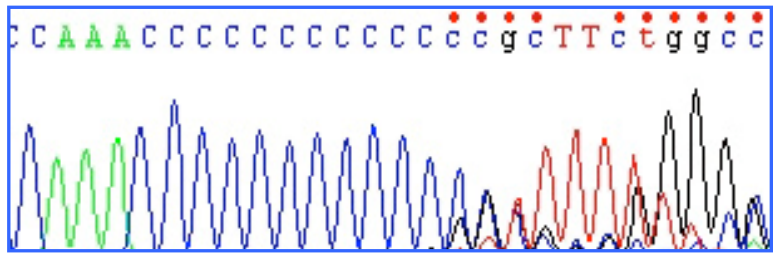


Length heteroplasmy: 303-315

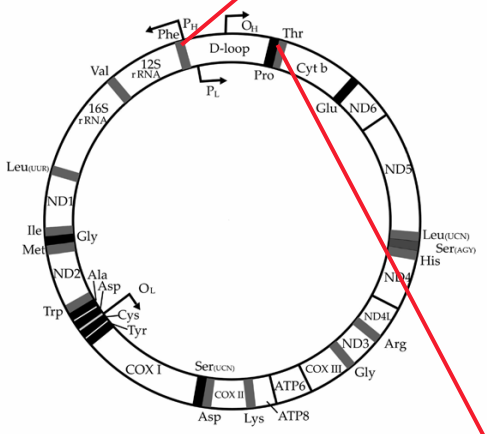


according to rCRS

T310C



Length heteroplasmy - Sites

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16201  CAAGCAAGTA CAGCAATCAA CCCTCAACTA TC[illegible]CAACTC
16251  CAAAGCCACC CCTCACCCAC TAGGATACCA ACAAACCTAC CCACCCTTAA
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 251   GT[illegible]TTCCA CACAGACATC ATAACAAAAA ATTTCCACCA
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C5TC4

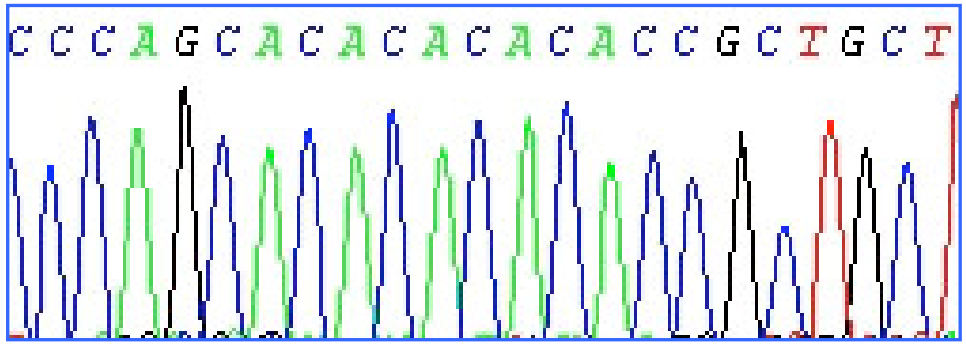
C7TC5

$(AC)_5$

C6

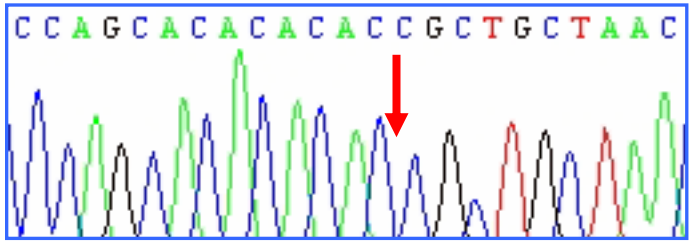
Positions 515-524

$(AC)_5$

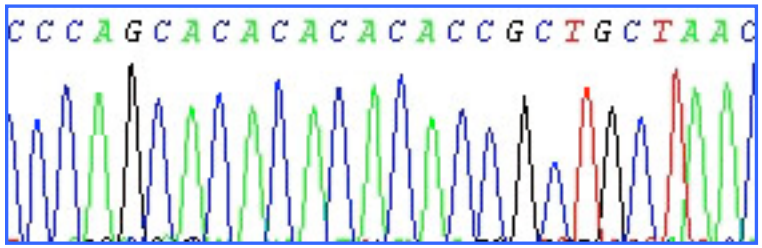


according to rCRS

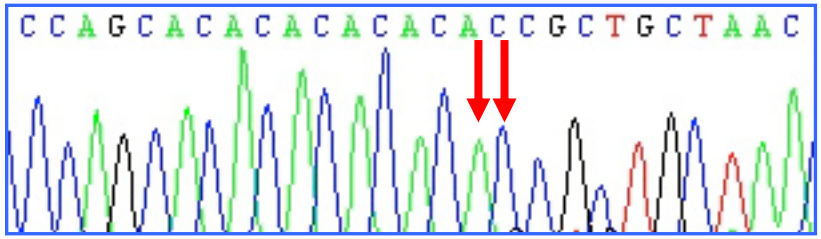
Length heteroplasmy: AC-repeat



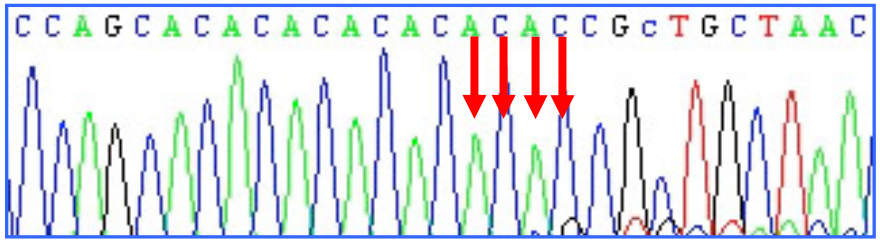
523 del; 524 del



according to rCRS

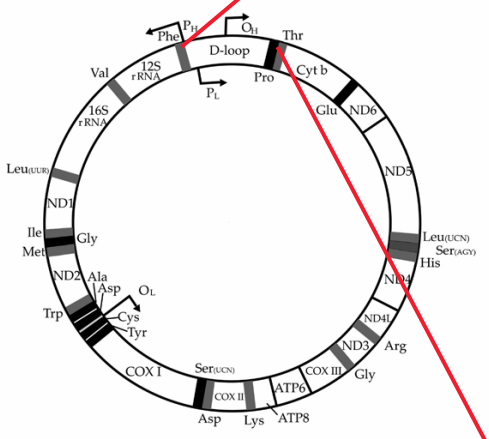


524.1 A; 524.2 C



524.1 A; 524.2 C
524.3 A; 524.4 C

Sequence heteroplasmy - Sites

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16151  CTGTAGTACA TAAAAACCCA ATCCACATCA AA                ATGCTTA
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16251  CAAAGCCACC CCTCACCCAC TAGGATACCA ACAAACCTAC CCACCCTTAA
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  101  GAGCCGGAGC ACCCTATGTC GCAGTATCTG TCTTTGATTC CTGCCTCATC
  151  CTATTATTTA TCGCACCTAC GTTCAATATT ACAGGCGAAC ATACTTACTA
  201  AAGTGTGTTA ATTAATTAAT GCTTGTAGGA CATAATAATA ACAATTGAAT
  251  GTCTGCACAG CCACTTTCCA CACAGACATC ATAACAAAAA ATTTCCACCA
  301  AA                CTTC TGGCCACAGC ACTTAAACAC ATCTCTGCCA
  351  AA                AACC CTAACACCAG CCTAACCAGA TTTCAAATTT
  401  TATCTTTTGG CGGTATGCAC TTTTAACAGT CACCCCCCAA CTAACACATT
  451  ATTTTCCCCT CCCA                TACTAAT CTCATCAATA CAACCCCCGC
  501  CCATCCTACC CAGC                CCGCTGC TAACCCCATA CCCCGAACCA
  551  ACCAAACCCC AAAGACAC                ACA
```

C5TC4

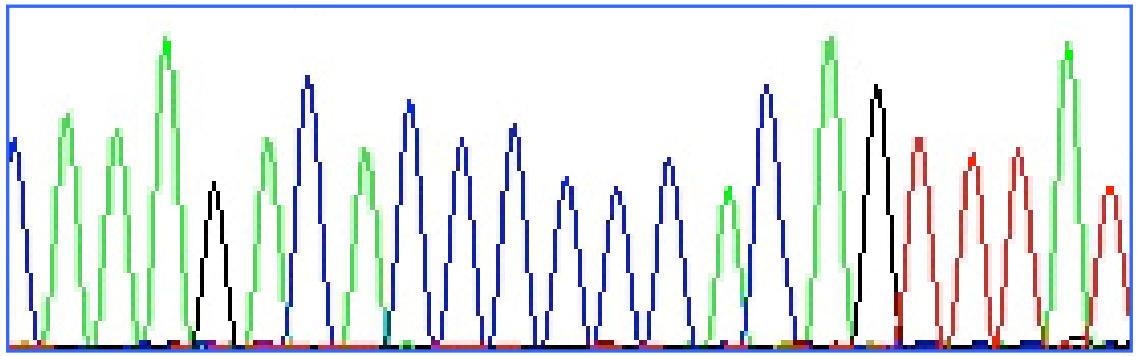
C7TC5

$(AC)_5$

C6

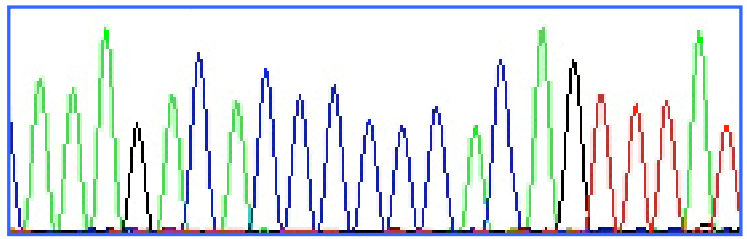
Positions 568-573

$(C)_6$

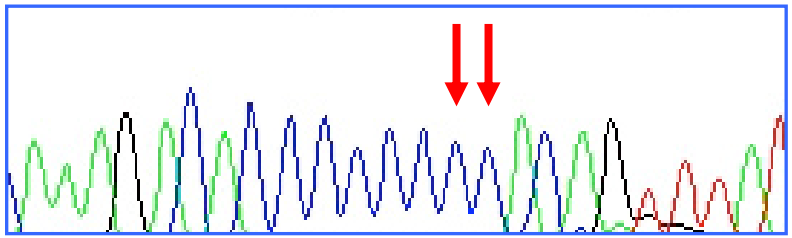


according to rCRS

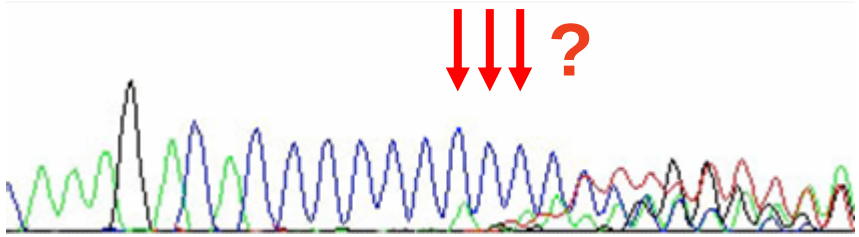
Length heteroplasmy: 568-576



according to rCRS

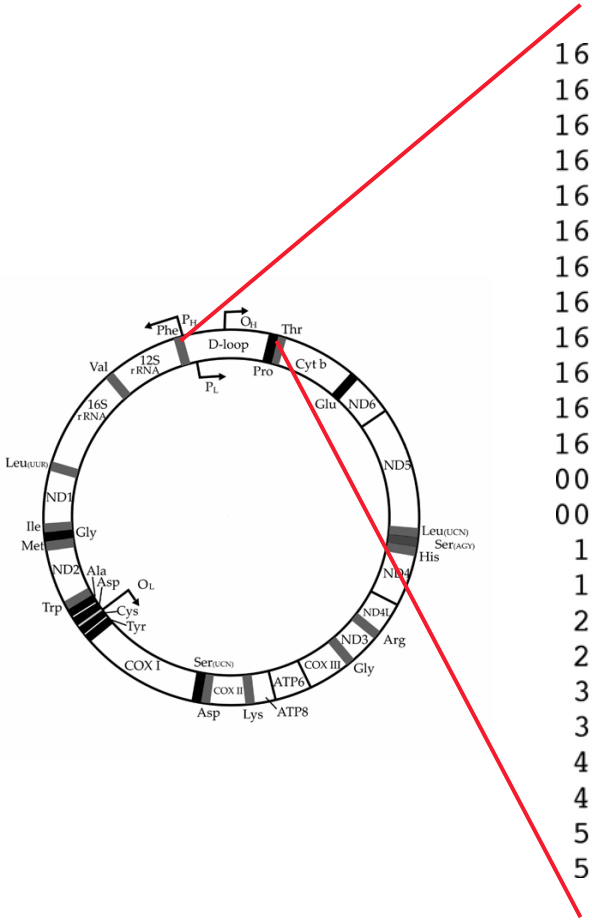


576.1 C; 576.2 C



576.1 C; 576.2 C
576.3 C; ?

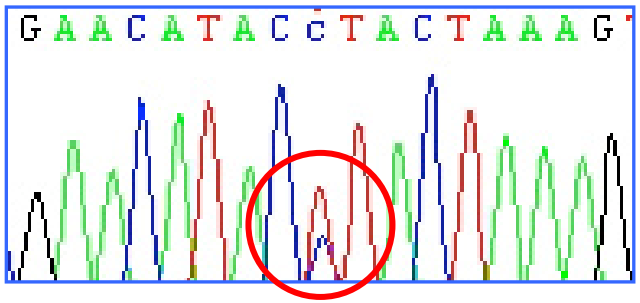
Sequence heteroplasmy - Sites



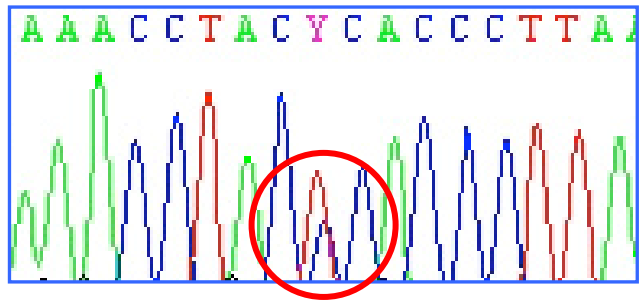

```
16024                                    TTCTTTC ATGGGGAAGC A     GGGT
16051  ACCACCCAAG TATTGACTCA CCCATCA       ACCGCTATGT A      TACA
16101  TTACTGCCAG CCACCATGAA TATTGTA                        CCAC
16151  CTGTAGTACA TAAAAACCCA ATCCACA                        TGCTTA
16201  CAAG   TA CAGCAATCAA CCCTCAACTA                      GCAACTC
16251  CAAA   CC    CACCCAC TAGGATACCA ACAAACCTAC CCACCCTTAA
16301  CAGT   AC    ATAAAGC CATTTACCGT ACATAGCACA TTACAGTCAA
16351  ATCCCTTCTC    CCCATGG ATGACCCCCC TCAGATAGGG GTCCCTTGAC
16401  CACCATCCTC CGTGAAATCA ATATCCCCCA GAAGAGTCCT ACTCTCCTCG
16451  CTCC                                                 ACAT
16501  CTGC                                                 CCCT
16551  TAAA
00001  GATC                                                 GCAT
00051  TTGC                                                    I
 101     GAGC ACCCTATGTC GCAGTATCTG TCTTTGA     TG
 151     TTTA TCGCACCTAC GTTCAATATT ACAGGCG     TA
 201     A ATTAATTAAT GCTTGTAGGA CATAATA       CA
 251  G  G CCACTTTCCA CACAGACATC ATAACAAAAA ATTTCCACCA
 301  AACCCCCCCT CCCCCGCTTC TGGCCACAGC ACTTAAACAC ATCTCTGCCA
 351  AACCCCAAAA ACAAAGAACC CTAACACCAG CCTAACCAGA TTTCAAATTT
 401  TATCTTTTGG CGGTATGCAC TTTTAACAGT CACCCCCCAA CTAACACATT
 451  ATTTTCCCCT CCCACTCCCA TACTACTAAT CTCATCAATA CAACCCCCGC
 501  CCATCCTACC CAGCACACAC ACACCGCTGC TAACCCCATA CCCCGAACCA
 551  ACCAAACCCC AAAGACACCC CCCACA
```

G 207 A

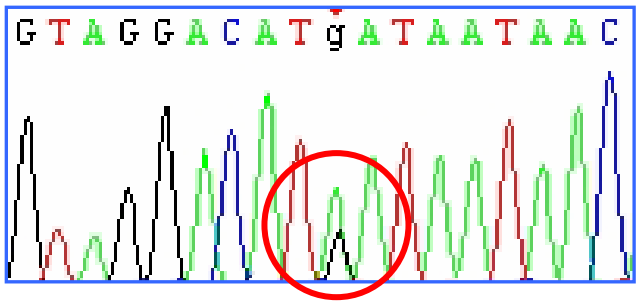
Sequence heteroplasmy - Sites



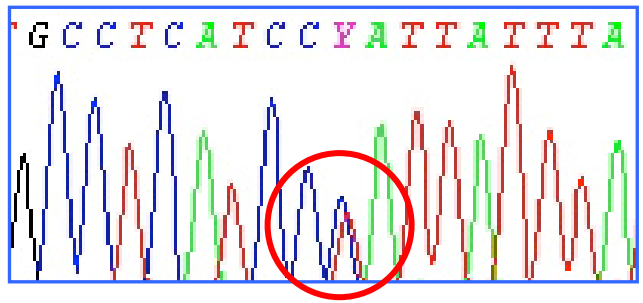
195Y



16291Y



234R



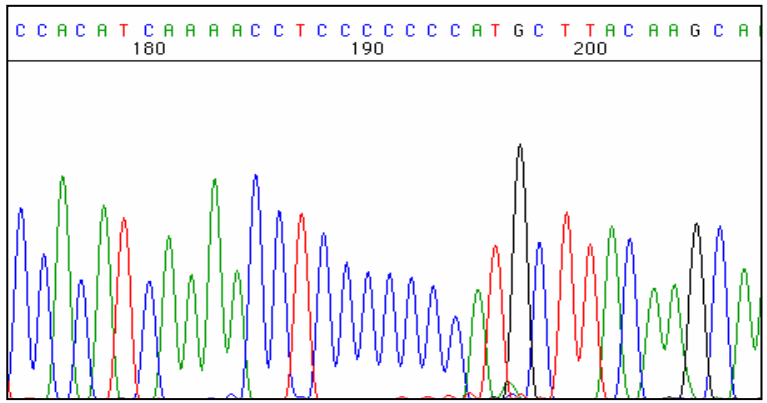
152Y

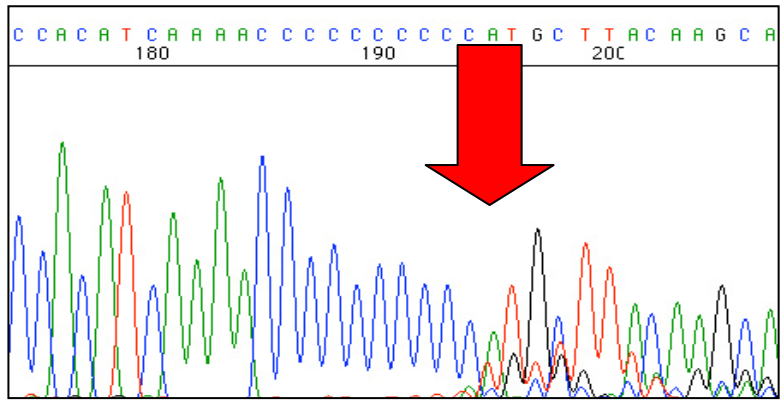
UNIVERSITÄTS KLINIKUM FREIBURG

Problems

Detection of length heteroplasmy

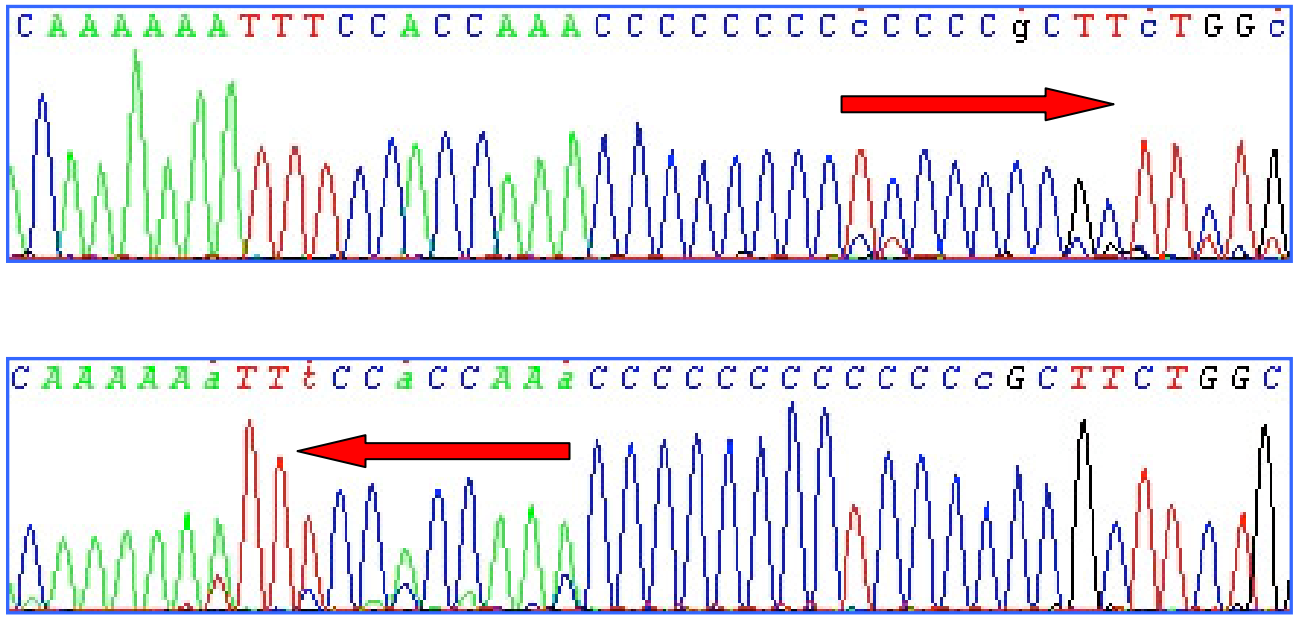
- „Blurred sequence“ following length heteroplasmy





Detection of length heteroplasmy

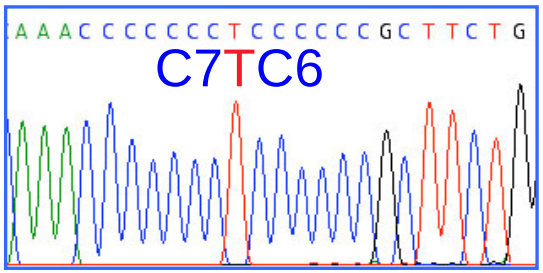
- „Blurred sequence“ **preceding** length heteroplasmy



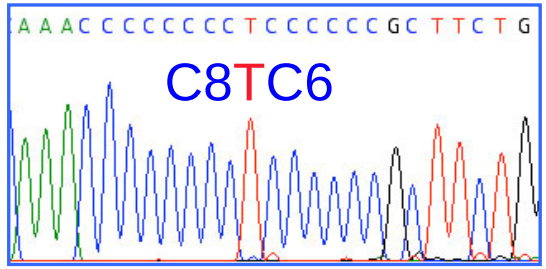
Detection of length heteroplasmy

- When do we have to report heteroplasmy?

homoplasmic sample

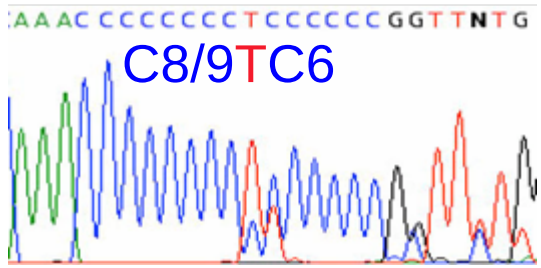


weak heteroplasmic sample



C-insertion at pos. 309.1

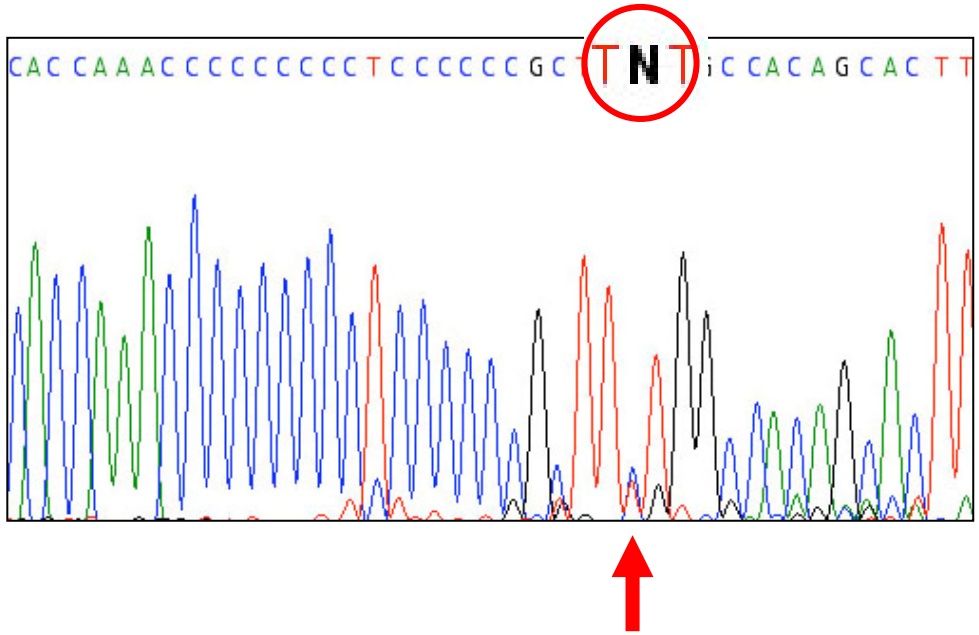
strong heteroplasmic sample



C-insertion(s) at pos. 309.1/.2

Detection of length heteroplasmy

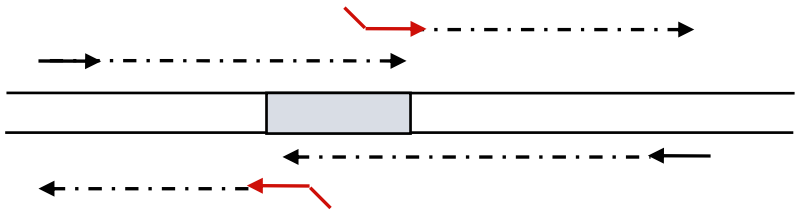
- Phantom mutations due to software

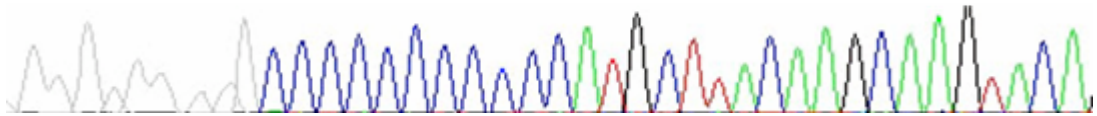


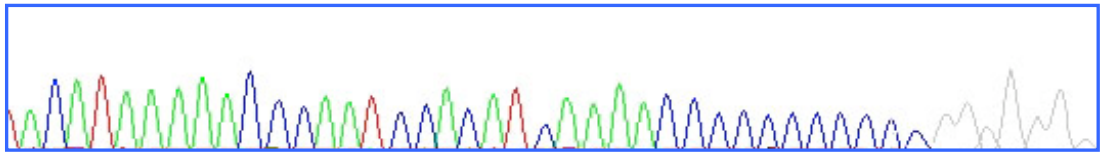
Brandstätter et al. 2005, Electrophoresis 26, 3414ff

Sequencing downstream length heteroplasmy

- Use of internal /"junction" primers



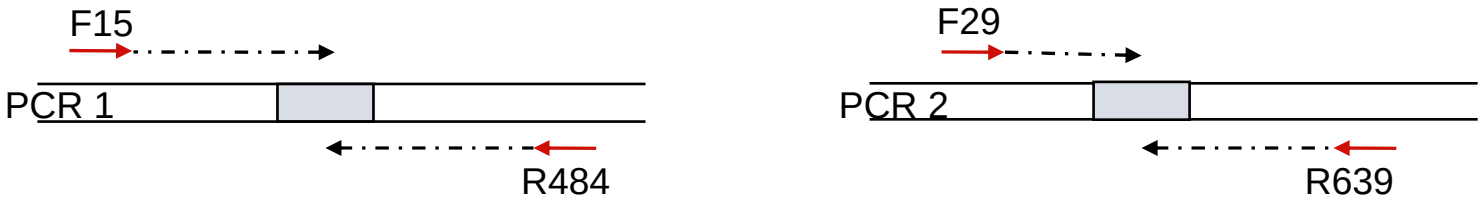


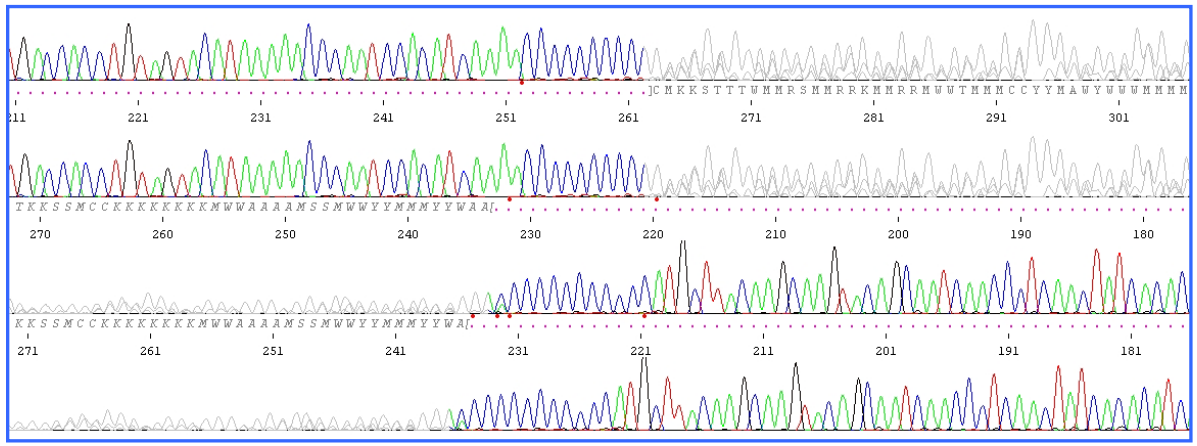


Szibor & Michael 1999, Int J Legal Med 112, 348ff / Rasmussen et al. 2002, For Sci Int 129, 209ff

Sequencing downstream length heteroplasmy

- double reactions from the same strand but from two PCR products

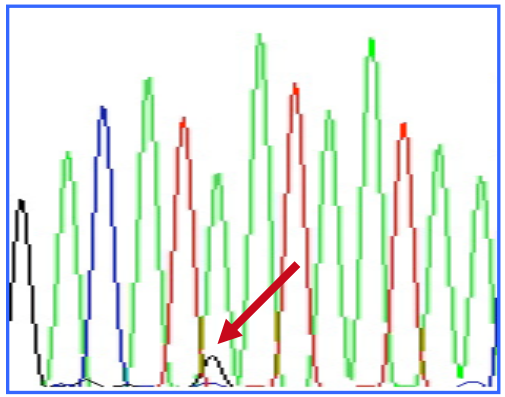


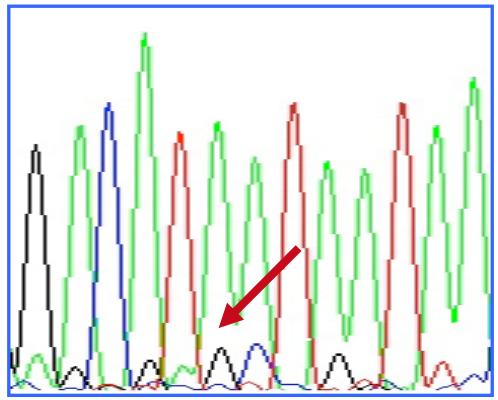


Parson & Bandelt 2007, For Sci Int Gen 1,13ff

Detection of sequence heteroplasmy

- with high sequence background



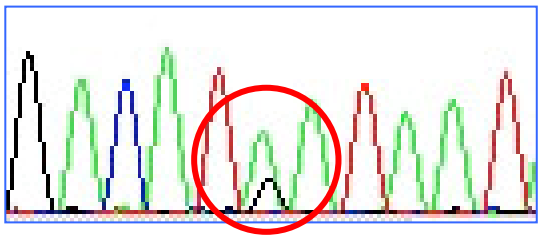


high sequence background

Brandstätter & Parson 2003, Int J Legal Med 117, 180ff

Detection of sequence heteroplasmy

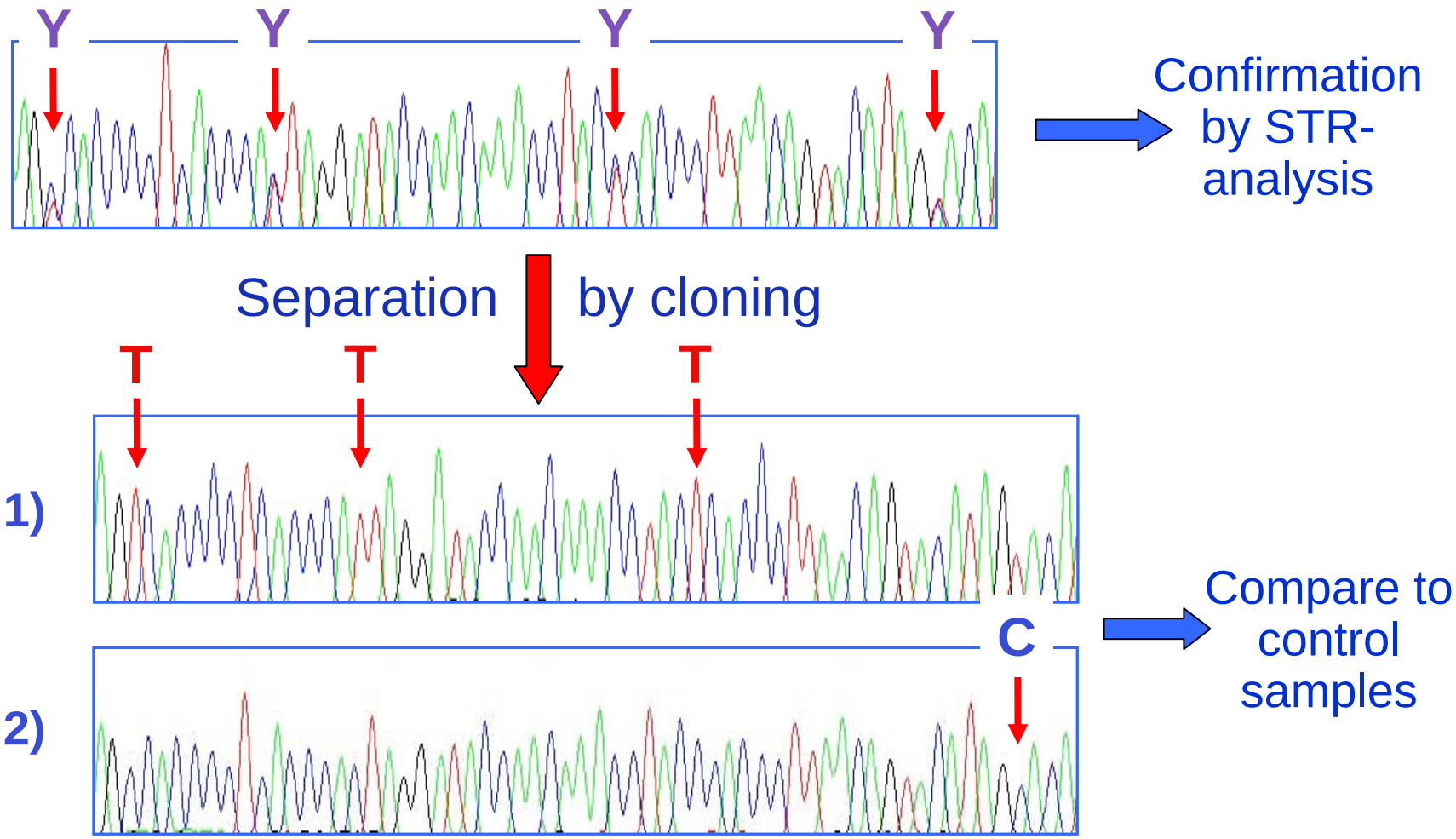
- using different primers

primer	L29	L16450
		
proportion	*~ 2:1*	*~ 6:1*

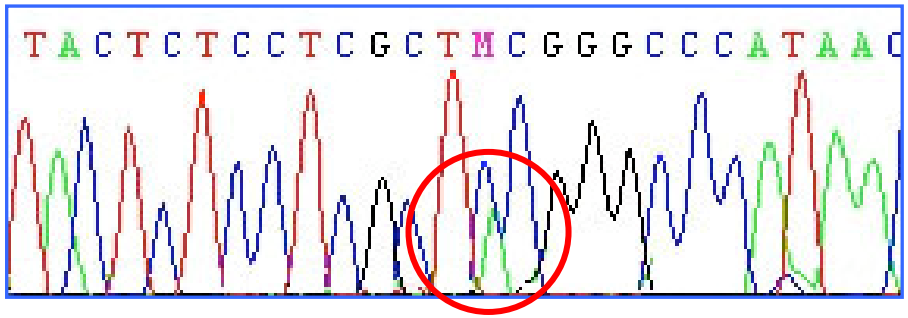
position 234R

Brandstätter & Parson 2003, Int J Legal Med 117, 180ff

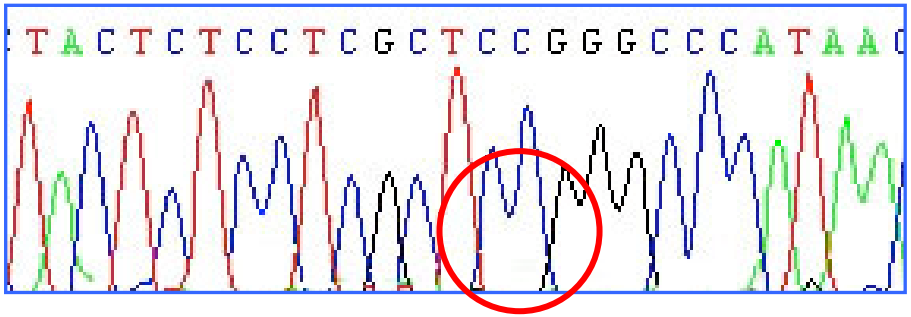
Differentiation of heteroplasmy from mixed stains or contaminations



Differentiation of heteroplasmy from polymerase errors

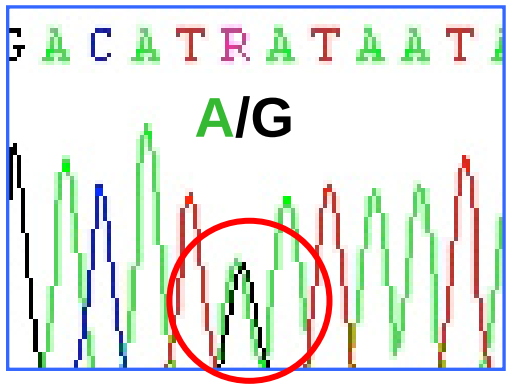
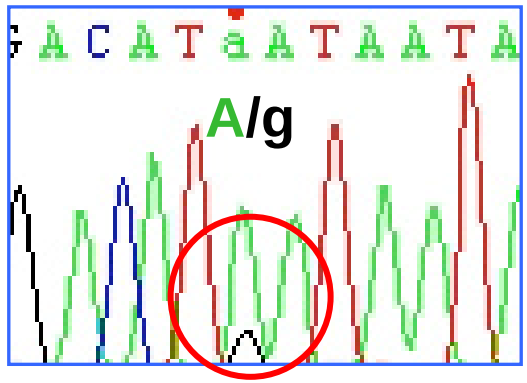


Analysis of two PCR products



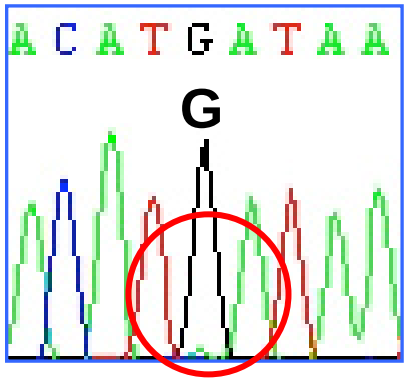
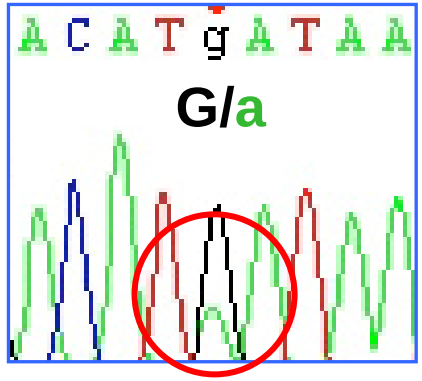
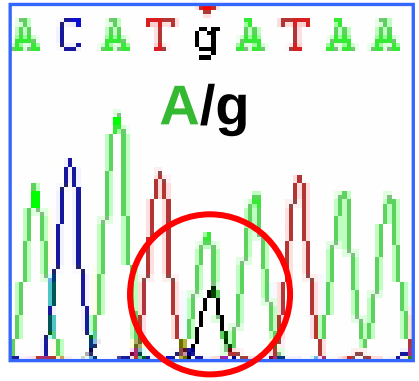
Parson & Bandelt 2007, For Sci Int Gen 1.13ff

Heteroplasmy in different tissues of an individual

tissue	*buccal cells*	*blood*
		
proportion	*1:1*	*4:1*

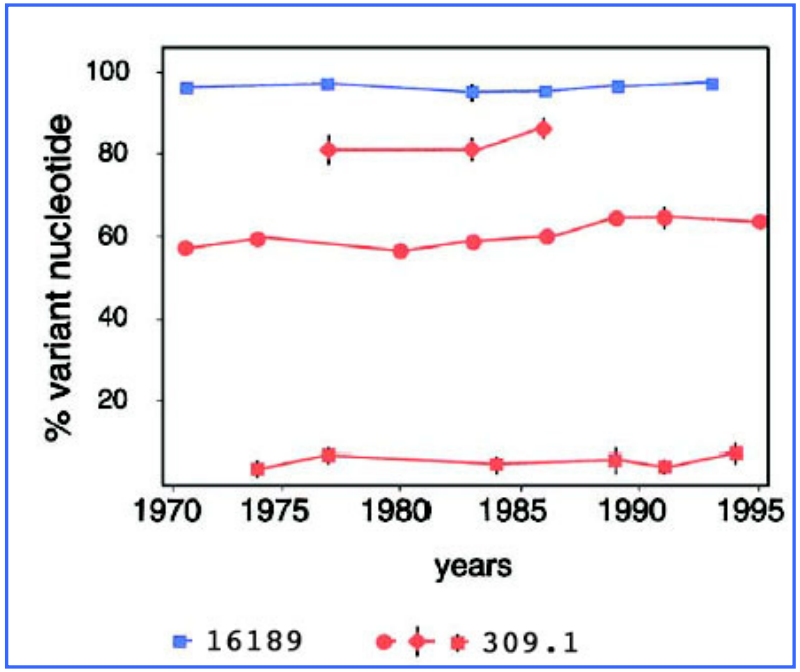
position 234R

Heteroplasmy in different hairs of an individual

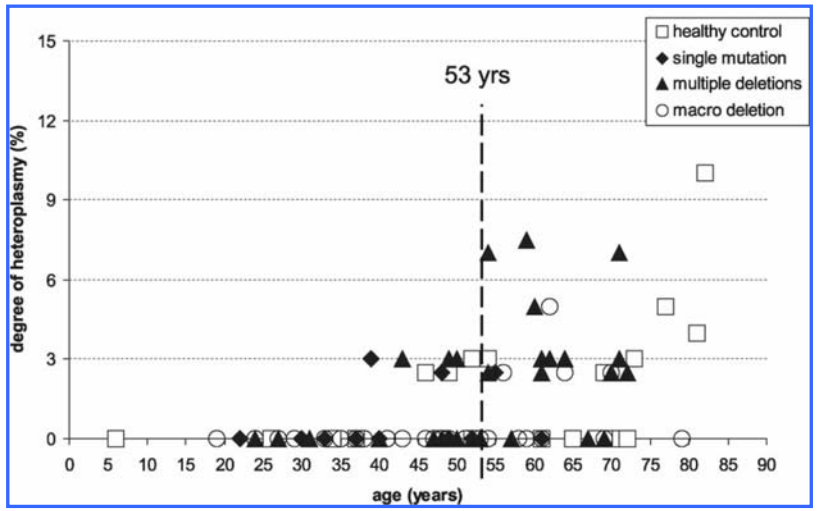
tissue	*hair 1*	*hair 2*	*hair 3*
			
proportion	*15:1*	*3:1*	*~1:2*

position 234R

Heteroplasmy in the course of life

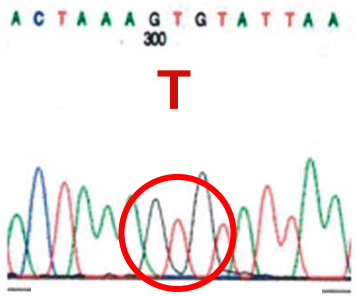


Lagerström-Fermér et al. 2001, Am J Hum Genet 68, 1299ff

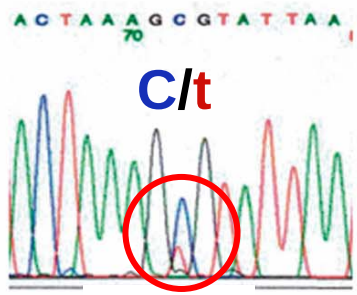


Del Bo et al. 2002, J Neurol Sci 202, 85ff

Sequence heteroplasmy in the maternal pedigree

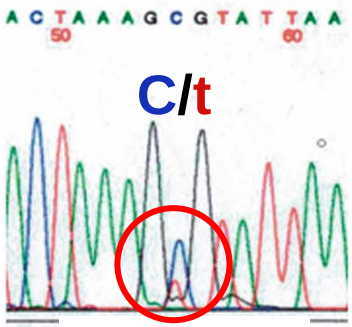


Grandmother

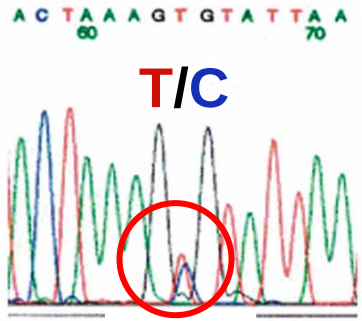


Mother

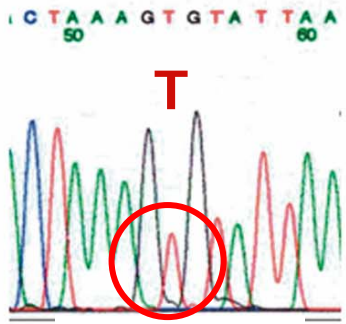
position 204Y



Daugther 1



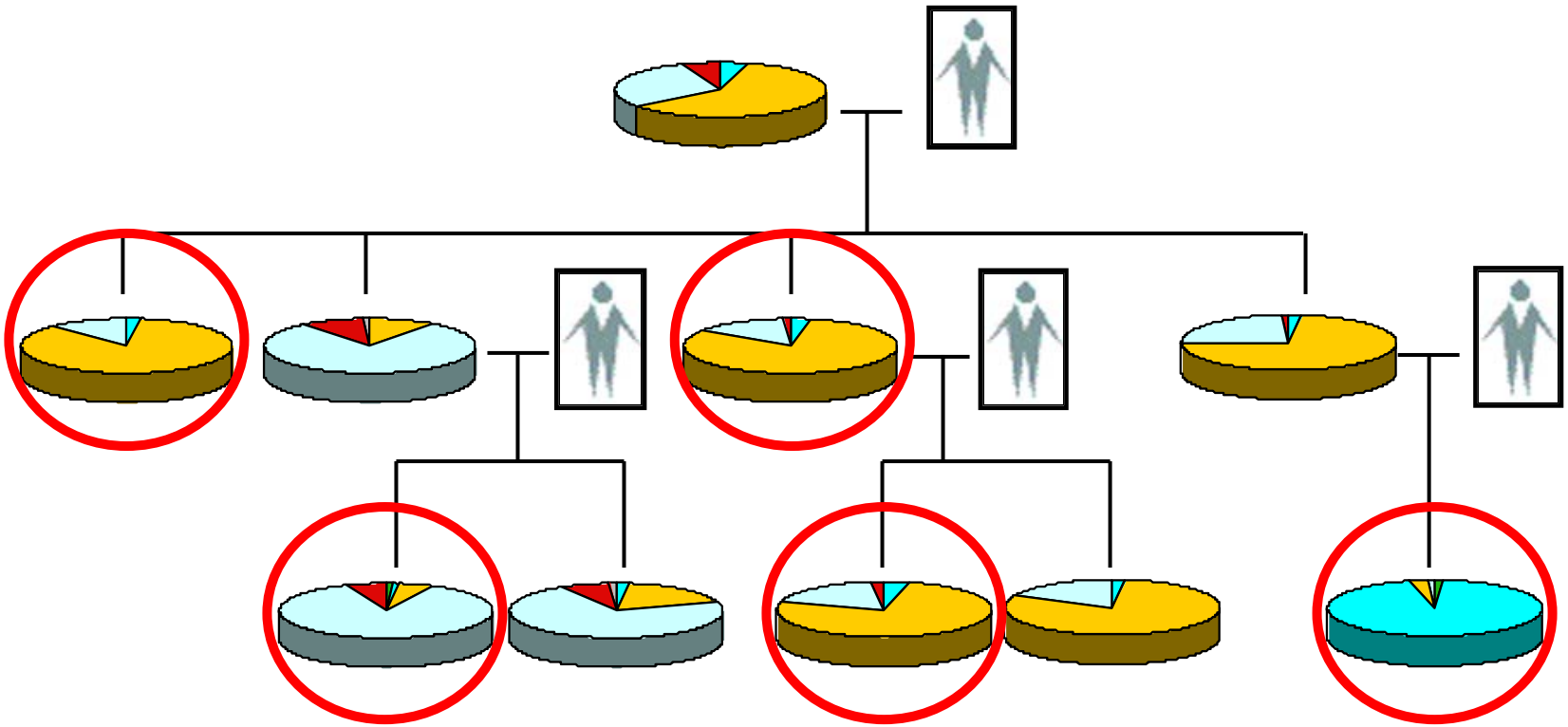
Son 1



Daugther 2

Lo et al. 2005, Ann NY Acad Sci 1042, 130ff

Length heteroplasmy in the maternal pedigree

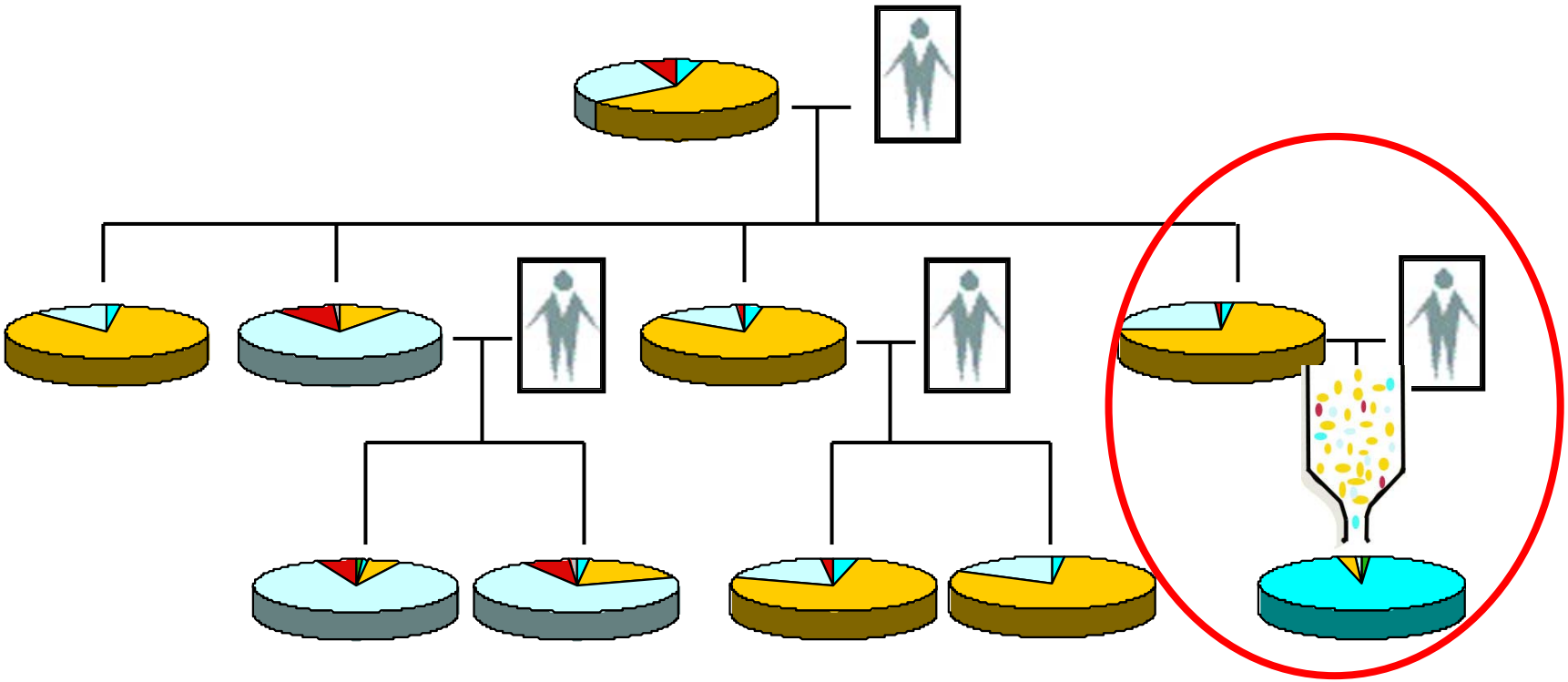


number of C-residues between positions 303-309:

6 7 8 9 10 11

Lutz et al. (2000) Int J Legal Med 113, 155ff

Length heteroplasmy in the maternal pedigree

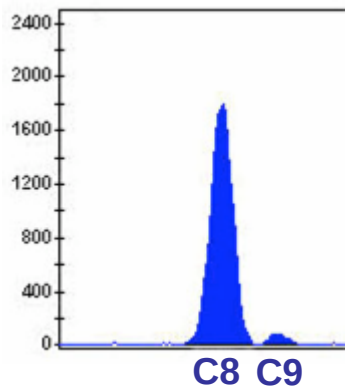


number of C-residues between positions 303-309:

6 7 8 9 10 11

Lutz et al. (2000) Int J Legal Med 113, 155ff

Length heteroplasmy in triplets

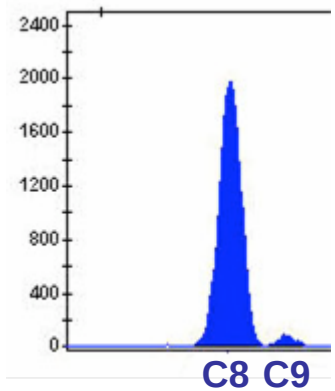


Sibling 1

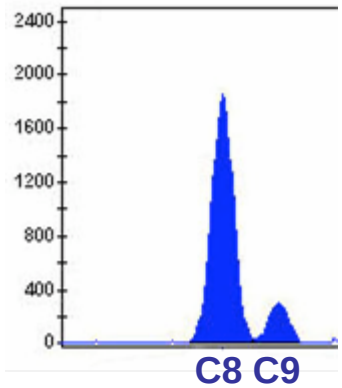
Monozygous

Non-monozygous

Non-monozygous



Sibling 2

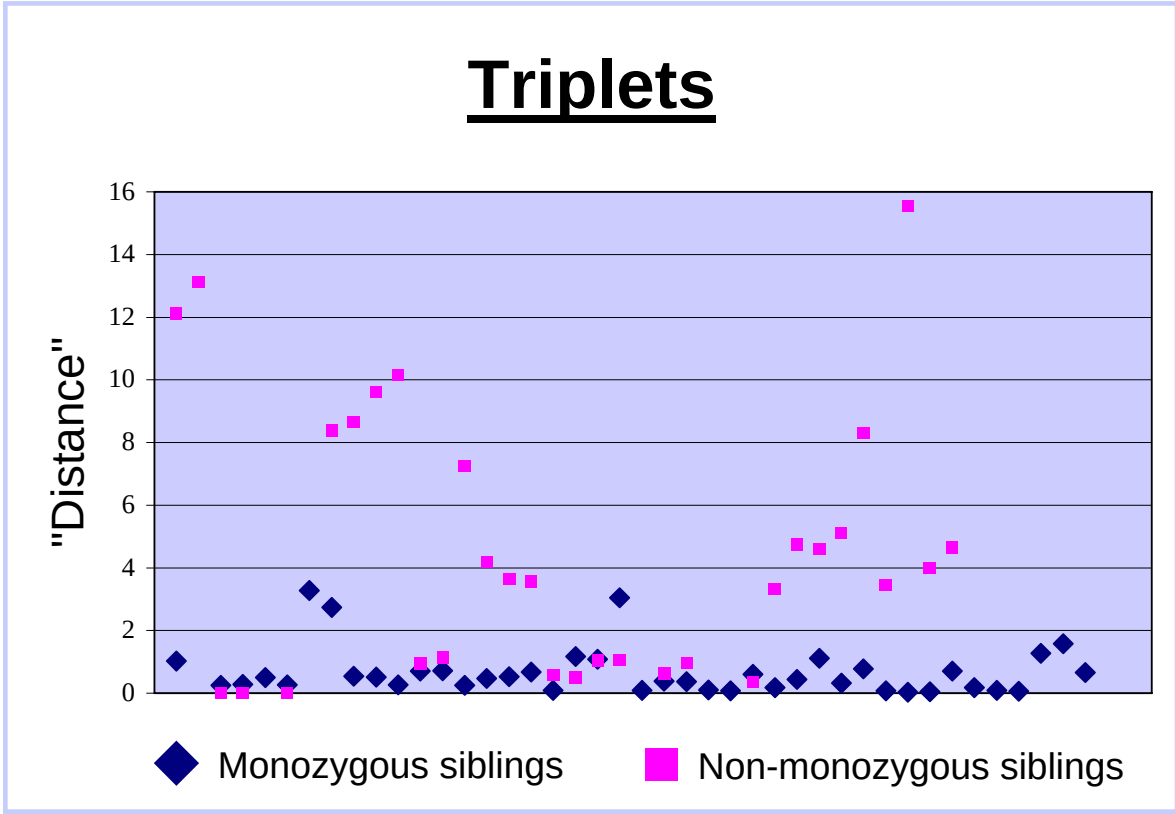


Sibling 3

RFLP-Analysis

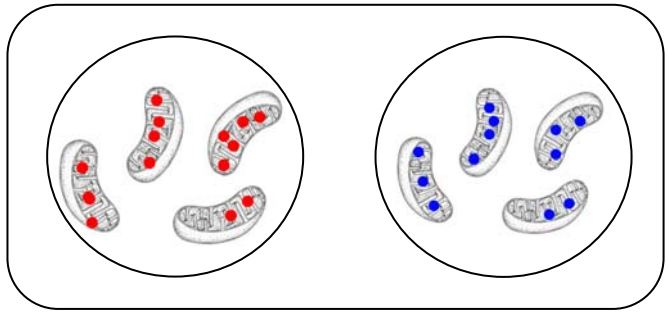
Lutz-Bonengel et al. (2004) Int J Legal Med 118, 274ff

Length heteroplasmy in triplets

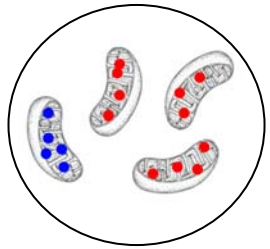


Level of heteroplasmy?

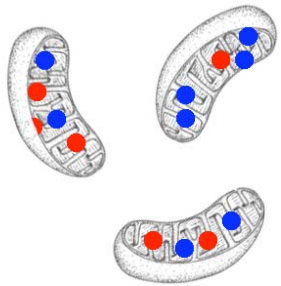
- Tissue



- Cell

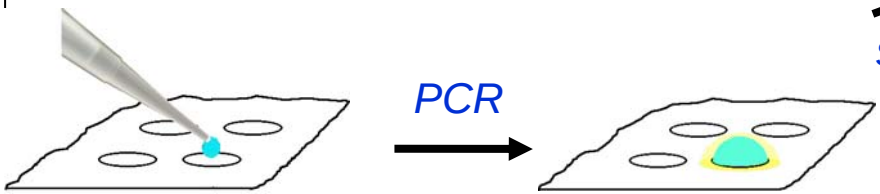


- Mitochondria



Sequence heteroplasmy within single cells

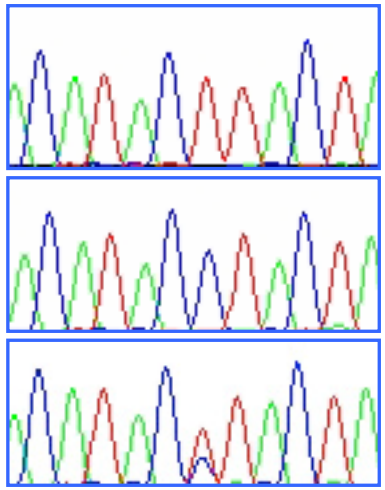
Results of single cells



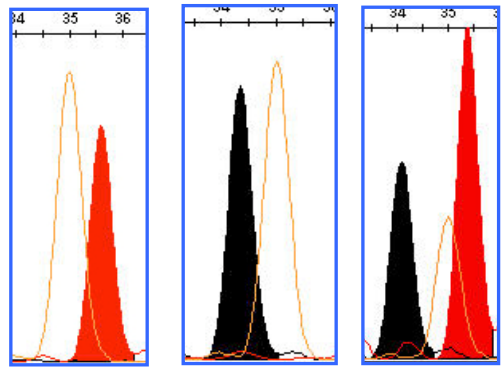
Single cells

PCR

sequencing



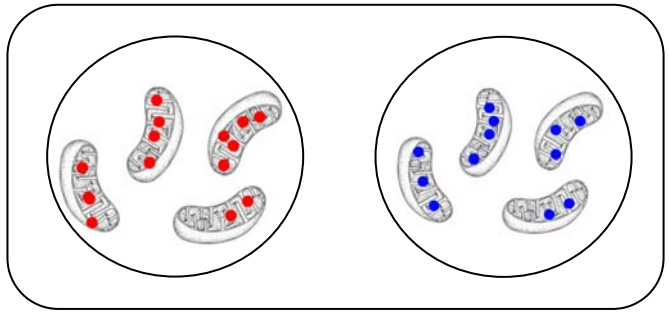
minisequencing



Lutz-Bonengel et al. Int. J. Legal Med. (in press)

Level of sequence heteroplasmy

- Tissue
- Cell
- Mitochondria



96%

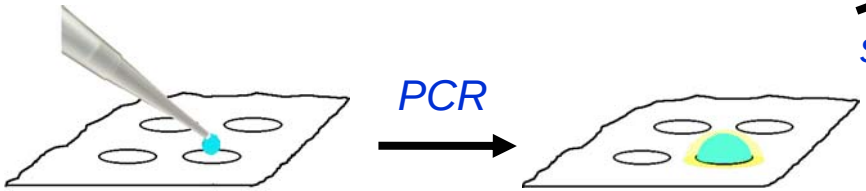
4%

?

Lutz-Bonengel et al. Int. J. Legal Med. (submitted)

Length heteroplasmy within single cells

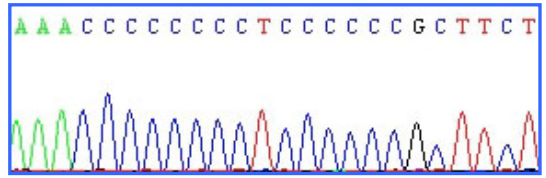
Results of single cells



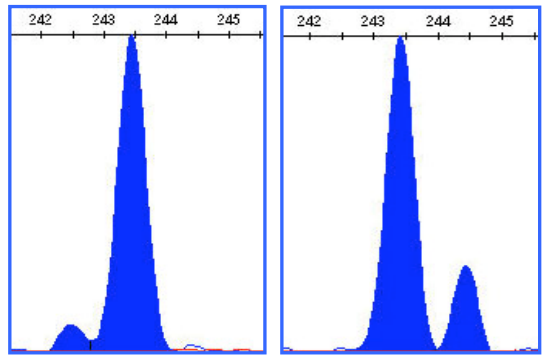
Single cells

PCR

sequencing



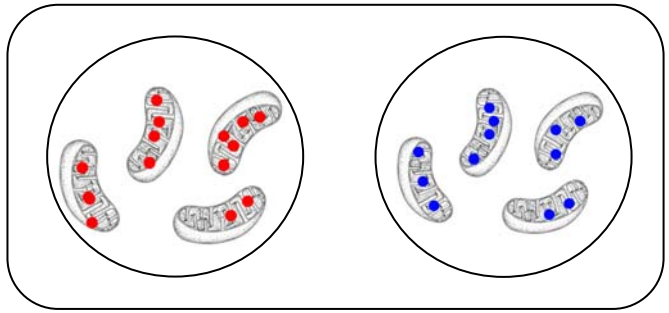
RFLP

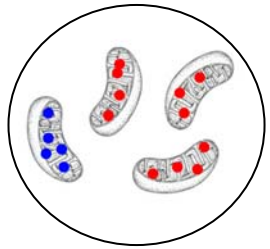


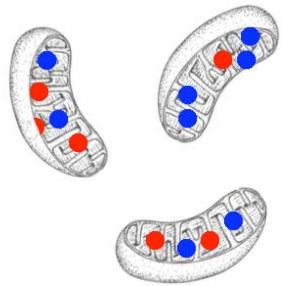
C7 C8 **C8 C9**

Level of length heteroplasmy

- Tissue
- Cell
- Mitochondria







Conclusions

- Agreement: when and how do we have to report heteroplasmy?
- Pitfalls: be aware of possible errors of interpretation
- Detection depends on quality and sensitivity of the used method
- No exclusions in casework because of heteroplasmic sites
- Increased discrimination power by corresponding results

Thank you for your attention !

Questions?
Comments?
Volunteers?