NON-INVASIVE PRENATAL DIAGNOSIS BY ANALYSIS OF CELL-FREE FETAL DNA FROM MATERNAL BLOOD

Lenka Veselovska

University of Cambridge

Cambridge Institute for Medical Research

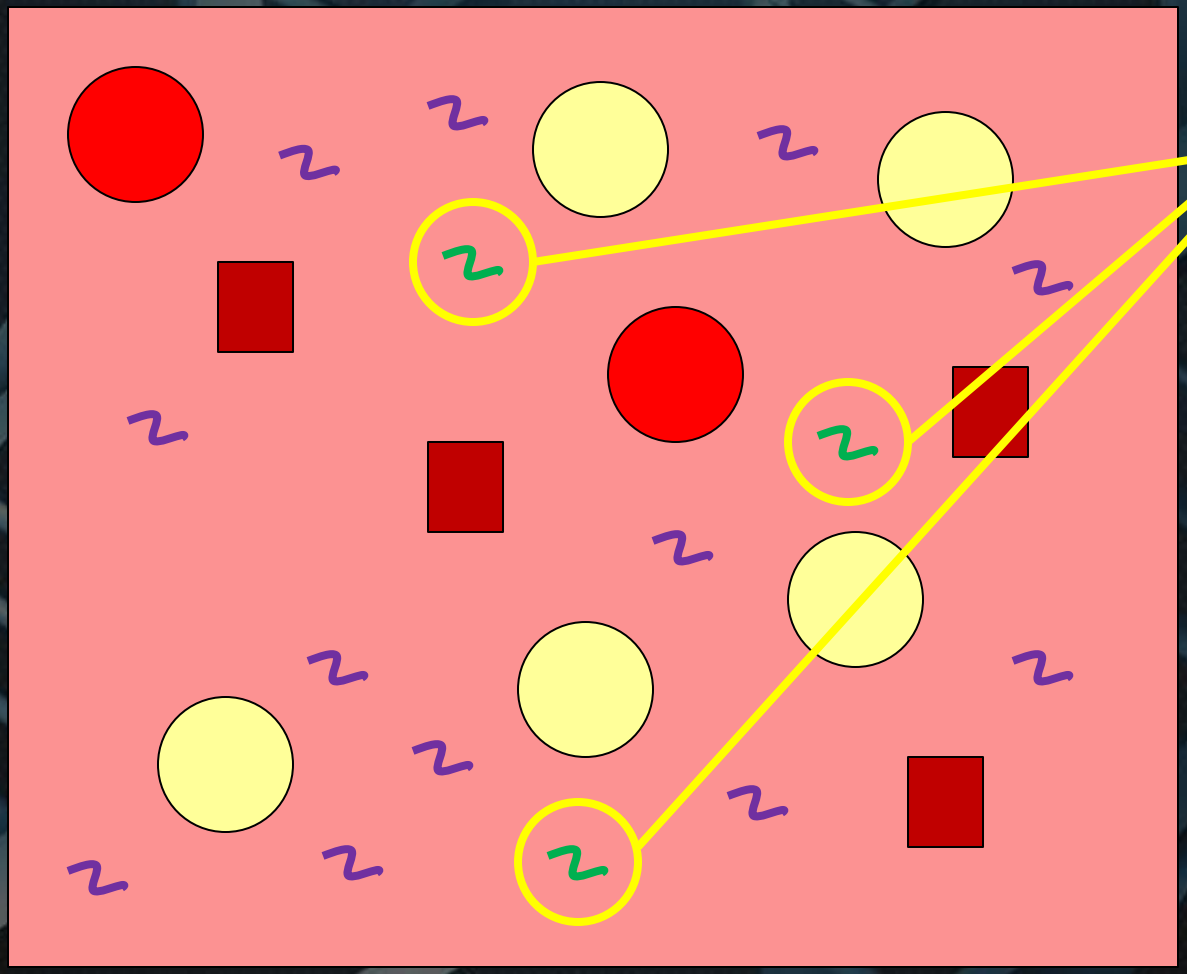
INVASIVE PRENATAL DIAGNOSIS
- amniocentesis
- chorionic villus sampling

risk of miscarriage 1%
from 11th week of pregnancy

NON-INVASIVE ALTERNATIVE ?

no risk of miscarriage
earlier

NON-INVASIVE PRENATAL DIAGNOSIS



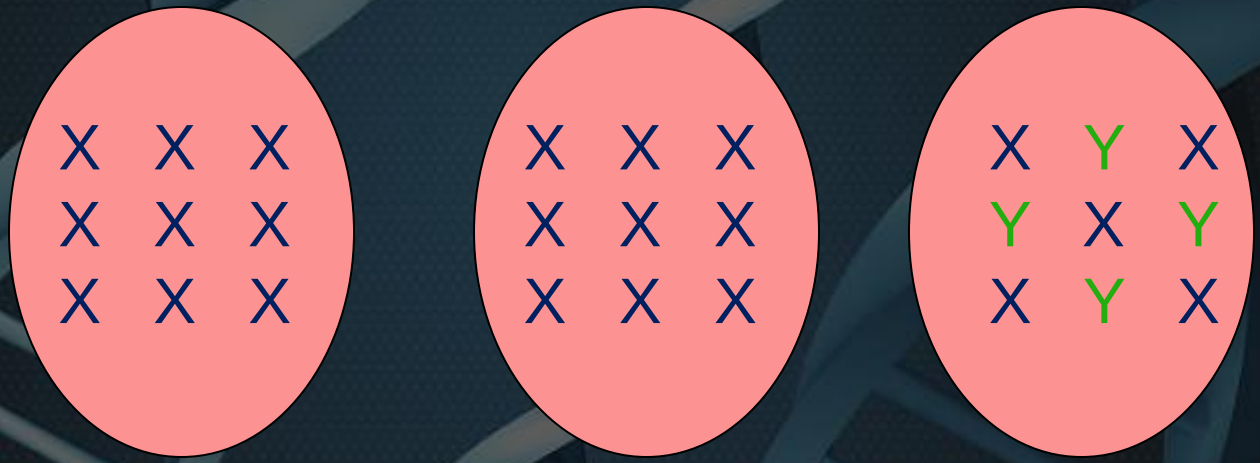
cell-free fetal DNA in maternal blood

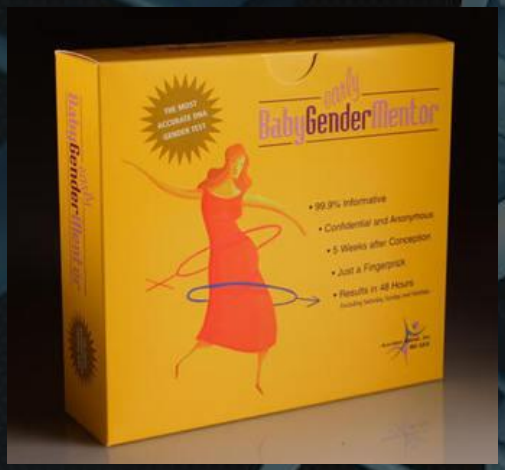
diagnosis from sample of maternal blood

(from 5th-7th week)

TYPES OF NON-INVASIVE PRENATAL DIAGNOSIS

1. **Sex determination**





2. **Diagnosis of trisomies (Down syndrome, ...)**

3. **Diagnosis of monogenic diseases**

TUBEROUS SCLEROSIS

- autosomal dominant disease with variable symptoms (mental retardation, epilepsy, autism, tumours)

- in top 10 diseases mostly required for prenatal diagnosis in the UK

- one parent affected

previous child affected

→ **prenatal diagnosis**

MATERNAL BLOOD

cell-free DNA

maternal

fetal

95%

5%

97.5%

2.5%

HOW IS THIS ALLELE DISTINGUISHED?

1. mutation

- \+ straightforward
- − many mutations
- − can fail

2. microsatellites

- \+ highly variable
- − problematic genotyping

3. SNPs

- \+ easy to distinguish
- − only 2 variants
- − allele frequencies not 0.5
- − only for AAxAB or BBxAB couples

HOW IS THIS ALLELE DISTINGUISHED?

GCAGTGCCGATATATATATATATCGTC
GCAGTGCCGATATATATCGTCCGTT

GCAGTGCCGATATATATATATATATATC
GCAGTGCCGATATATCGTCCGTTA

2. microsatellites

highly variable
problematic genotyping

3. SNPs

easy to distinguish
only 2 variants
allele frequencies not 0.5
only for AAxAB or BBxAB couples

HOW IS THIS ALLELE DISTINGUISHED?

GCAGTGCCGCGTCCGTTAGCAATG
GCAATGCCGCGTCCGTTAGCAATG

GCAATGCCGCGTCCGTTAGCAATG
GCAATGCCGCGTCCGTTAGCAATG

GCAGTGCCGCGTCCGTTAGCAATG
GCAGTGCCGCGTCCGTTAGCAATG

3. SNPs

easy to distinguish
only 2 variants
allele frequencies not 0.5
only for AAxAB or BBxAB couples

HOW IS THIS ALLELE DISTINGUISHED?

1. mutation

- allele-specific real-time PCR
- SNaPshot

2. microsatellites

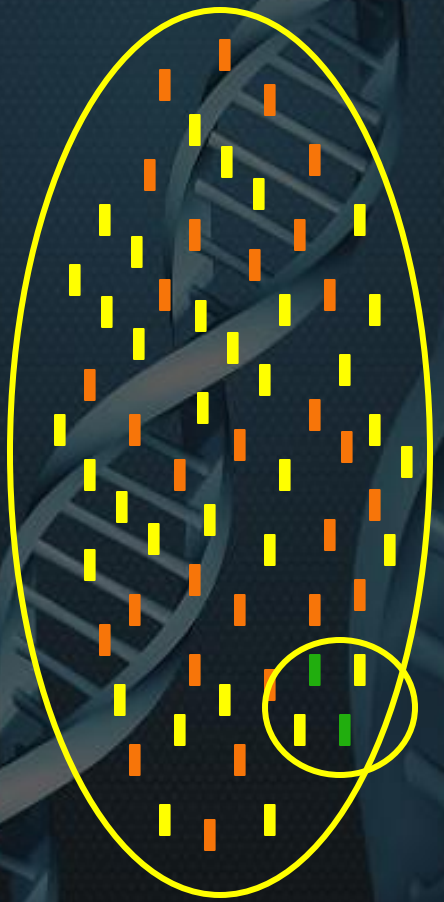
- genotyping

3. SNPs

- allele-specific real-time PCR
- SNaPshot

IS THE METHOD SENSITIVE ENOUGH TO DETECT PATERNAL ALLELE?

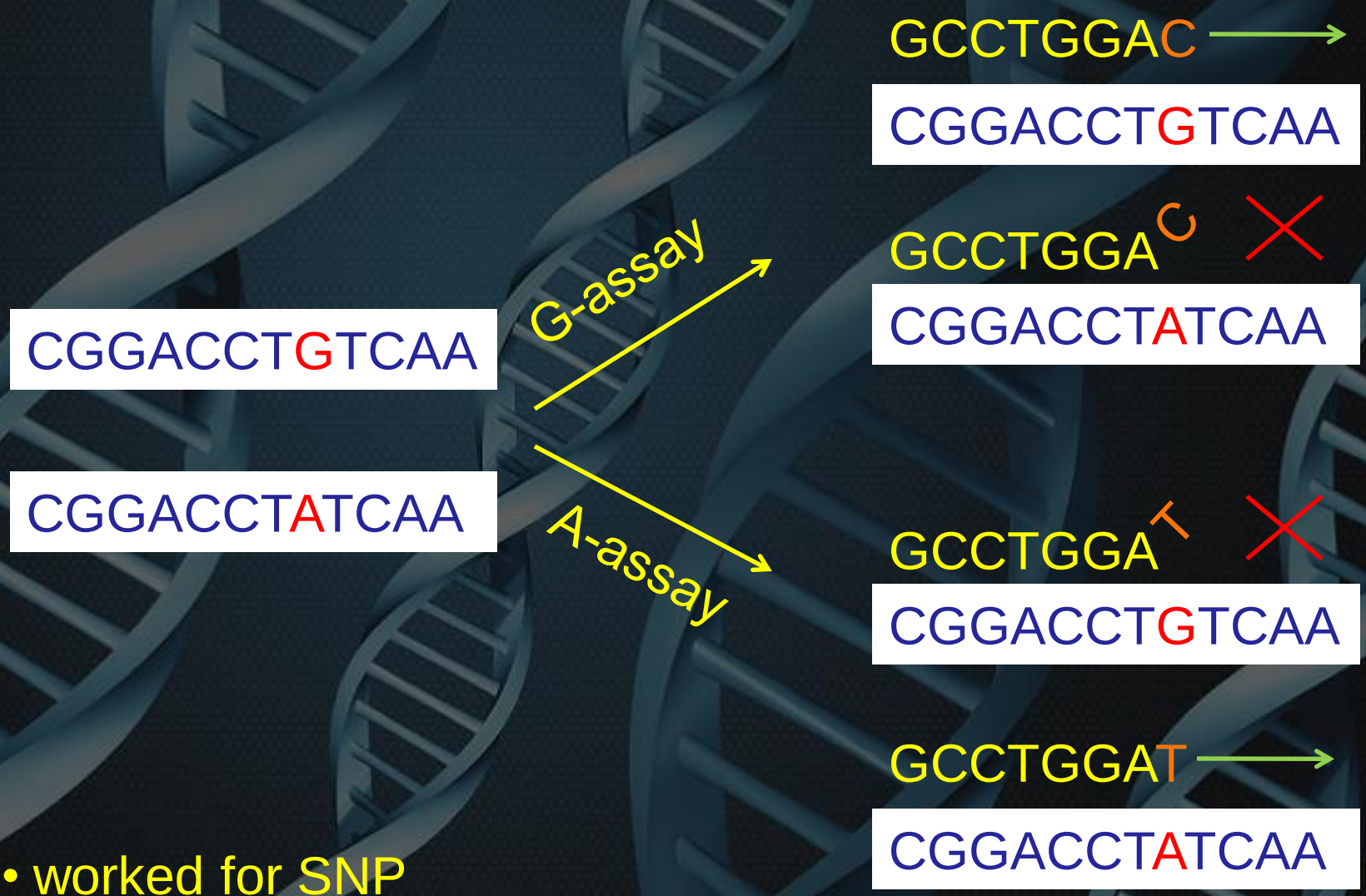
variable amount
(lower limit ~1ng per reaction)



artificial mixtures
of DNA samples:
- 99% major DNA
- 1% minor DNA
- total input 1ng

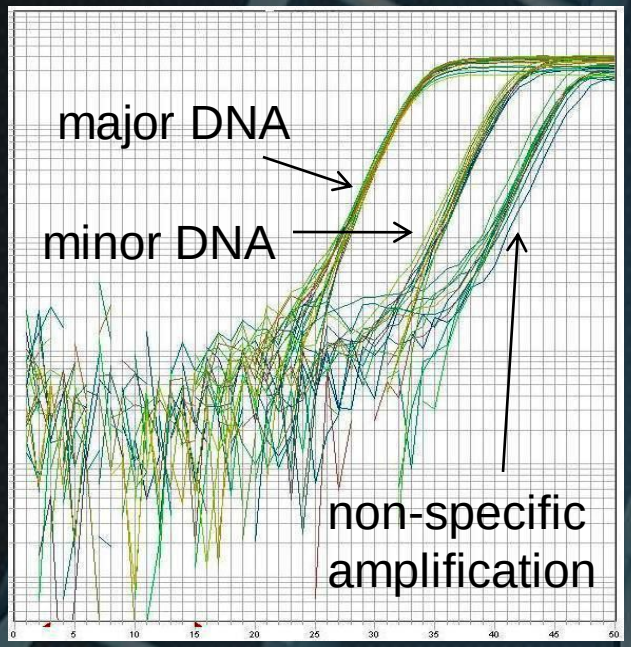
variable proportion
(lower limit ~1%)

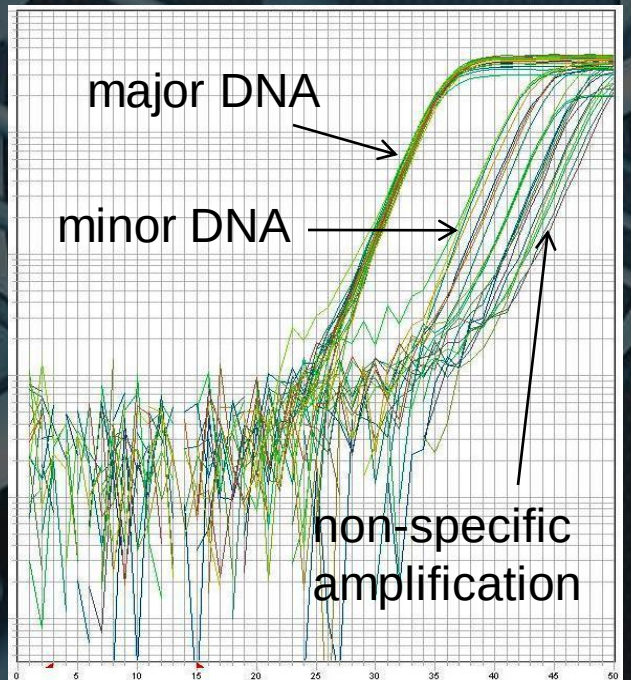
ALLELE-SPECIFIC REAL-TIME PCR



- worked for SNP
- did not work for mutation

ALLELE-SPECIFIC REAL-TIME PCR





1st experiment
- 5ng total DNA, 1% minor DNA
- minor DNA homozygous
- 46 samples
- 100% correctly genotyped (46)

2nd experiment
- 1ng total DNA, 1% minor DNA
- minor DNA homozygous
- 46 samples
- 95.7% correctly genotyped (44)

3rd experiment
- 1ng total DNA, 1% minor DNA
- minor DNA –majority heterozygous
- 46 samples
- 95.6% correctly genotyped (44)

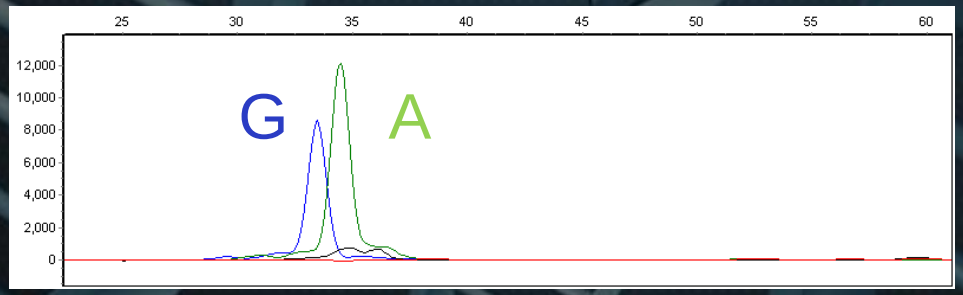
SNAPSHOT

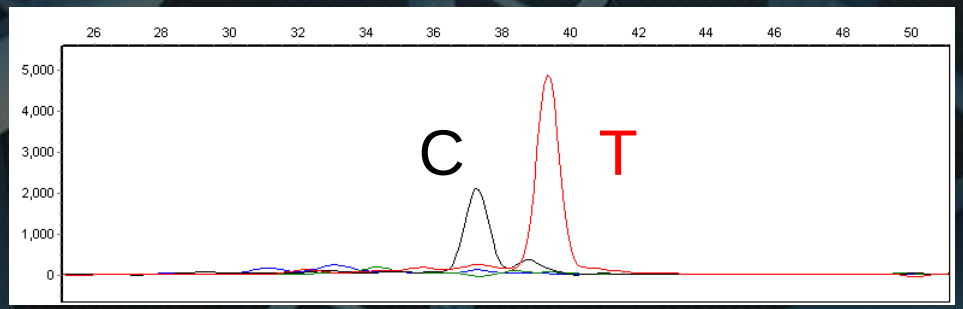
GCCTGGAC

CGGACCTGTCAA → CGGACCTGTCAA

GCCTGGAT

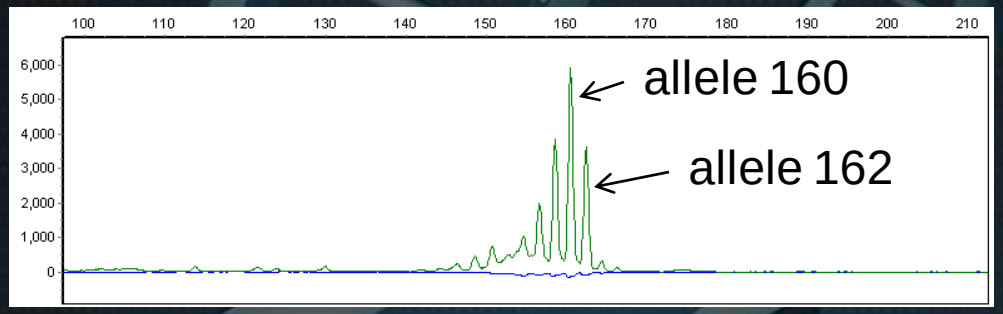
CGGACCTATCAA → CGGACCTATCAA



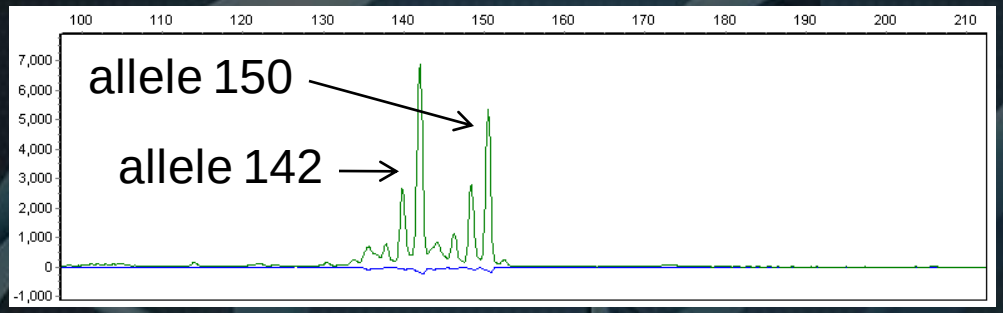


- 3 SNPs + mutation
- input DNA 10-50 ng
- minor DNA 50%

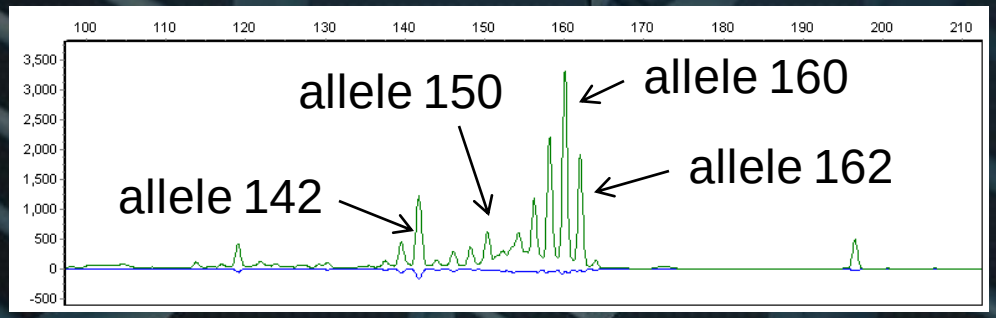
MICROSATELLITES GENOTYPING



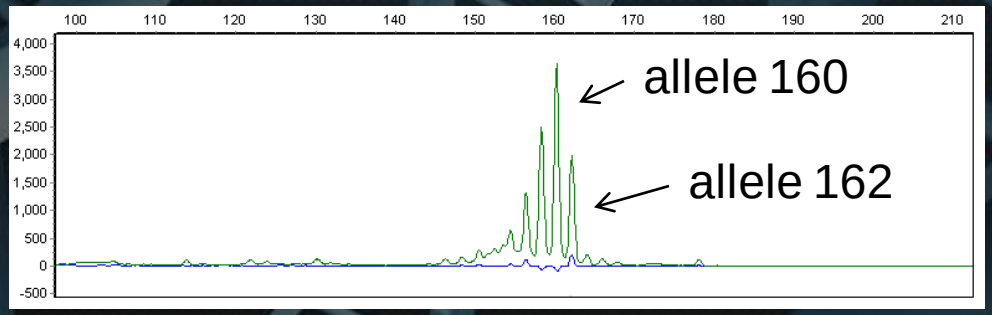
major DNA



minor DNA



- 1ng total DNA
- 5% minor DNA



- 1ng total DNA
- 1% minor DNA

SUMMARY

✗ **genotyping of microsatellites**

? **SNaPshot**

☺ **allele-specific real-time PCR**

How many SNPs do we need?

	Theory	Our data
‘best’ SNP	25%	
1 SNP	25%	30%
5 SNPs	59%	70%
all 67 SNPs	?	
haplotyped 31 SNPs	72%	
best 10 SNPs	70%	

+ back-up - single assay can fail

➡ allele-specific real-time PCR of 5/10 SNPs + mutation

FURTHER RESEARCH

- results from SNaPshot

- real samples of maternal plasma with cell-free fetal DNA – healthy individuals, patients with tuberous sclerosis

- optimalisation of extraction methods for cell-free DNA – how much DNA can we get from samples?

Similar strategy can be applied for other monogenic diseases