

PRENATAL DIAGNOSIS (PND) - DNA

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OVERVIEW

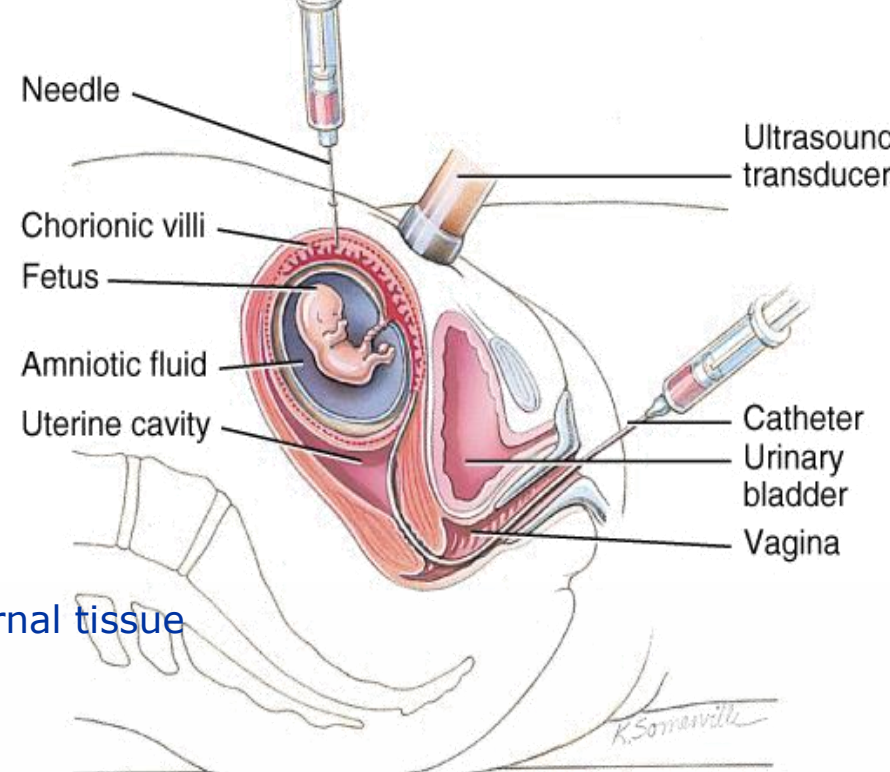
- Fetal material used for PND
- (molecular techniques => see previous classes)
- Maternal contamination of fetal sample

MATERIALS

- Chorionic villi
- Amniotic fluid cells (amniotic fluid)
- Cord blood
- (maternal blood \Rightarrow NIPT)

CHORIONIC VILLI

- Between 10 – 13 weeks of pregnancy
- Chorionic villi: selection of villi, discard maternal tissue
 - direct analysis (most samples); culture as backup material
 - culture (overgrowth with maternal cells!)



CHORIONIC VILLI

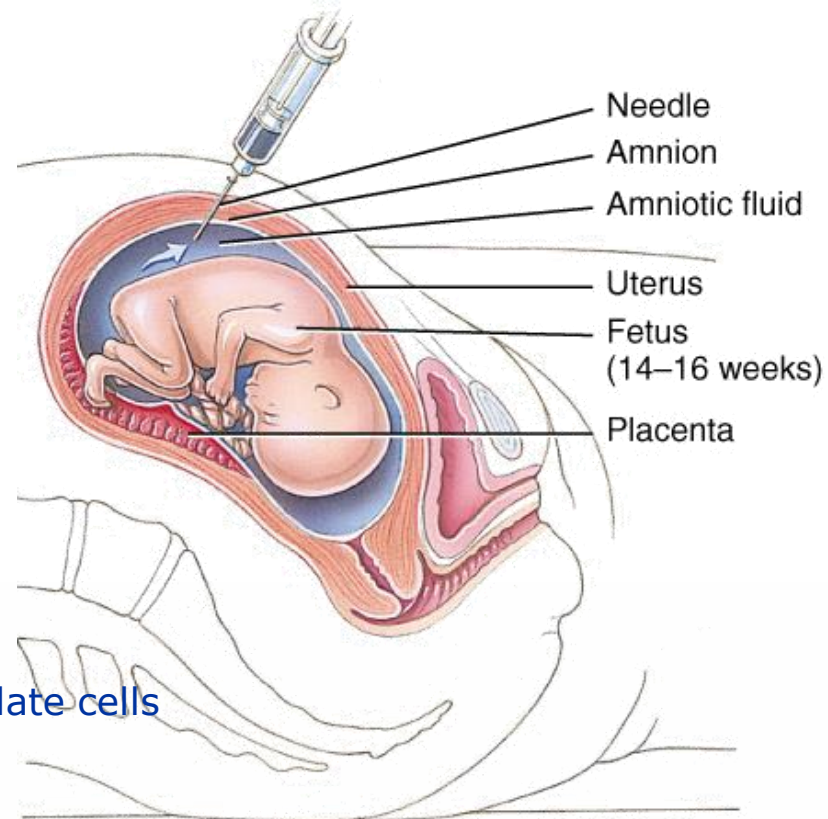
- DNA extraction and analysis (PCR amplification + further analysis)
- Protein extraction and analysis (enzyme assay, electrophoresis, ...)

AMNIOTIC FLUID CELLS

- Often midtrimester (> 14 weeks of pregnancy)
- direct for DNA analysis:
 - centrifugation of AF (typical 1-2 ml) to isolate cells
 - DNA extraction
 - PCR amplification + further analysis

-Requires set-up in advance!

-Not all tests are possible

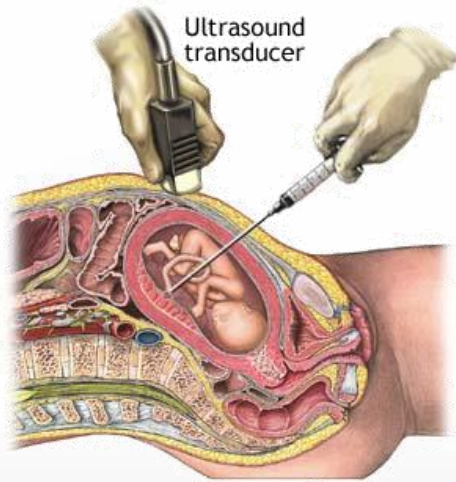


AMNIOTIC FLUID CELLS

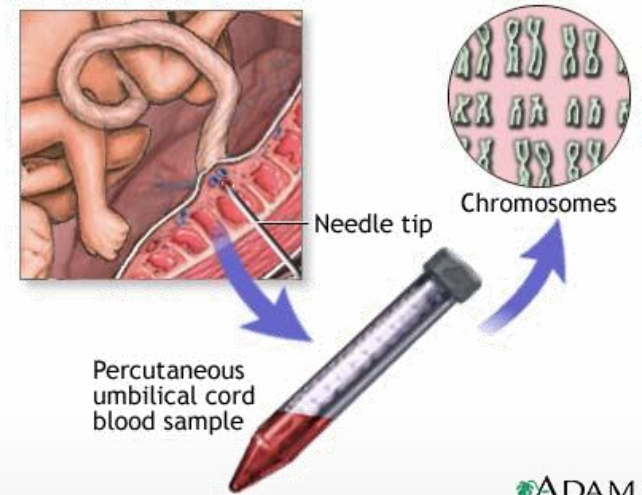
- cultured cells (most samples):
 - DNA extraction and analysis (PCR + further analysis)
 - Protein extraction and analysis (enzyme assay, electrophoresis, ...)
 - Back-up for direct analysis

CORD BLOOD

- 1-2 ml of blood:
 - White blood cells for DNA or biochemical analysis
 - Serum/plasma for biochemistry



ADAM.



ADAM.

CORD BLOOD

- White blood cells for DNA or biochemical analysis
- WBC per μl of blood:

16-19 weeks 4700 ± 800

20-27 weeks 4300 ± 900

Neonate 14100 ± 3000

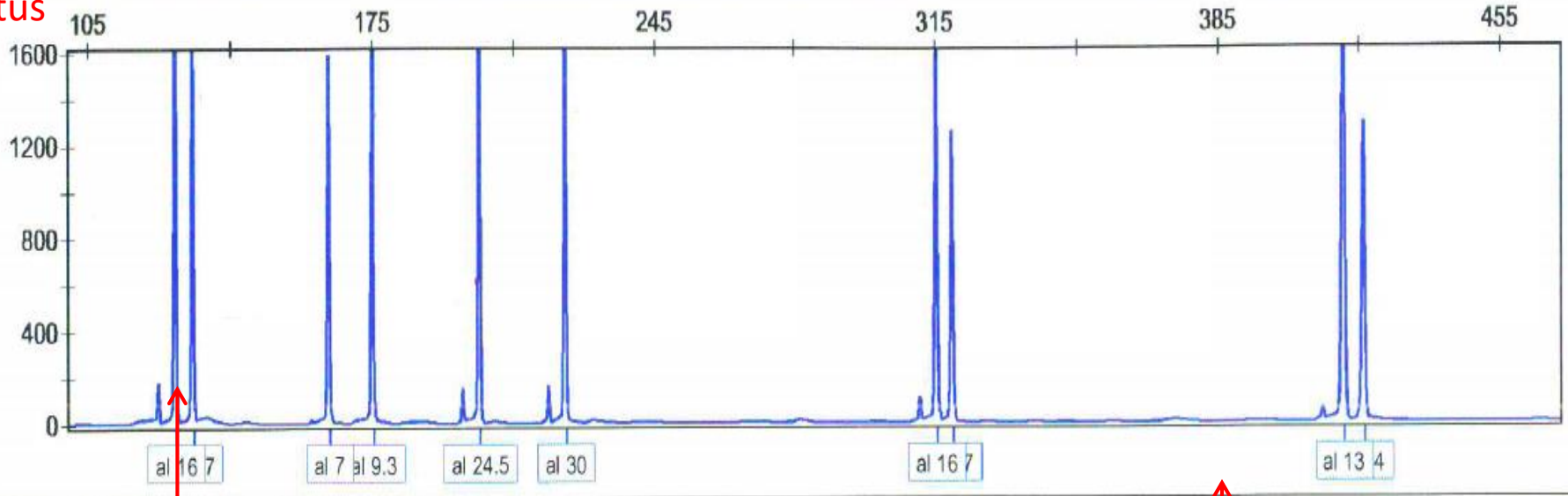
Adult 6000 ± 1400

MATERNAL CONTAMINATION

- Detection of maternal (cell) contamination (M(C)C)
- Fragment analysis (di-, tri-,...nucleotide repeats): home-made or commercial (PCR) kits
- Principle:
 - Fetus and mother have one common band
 - Informative** when:
 - Second maternal band does not coincide with band from the fetus (paternal)

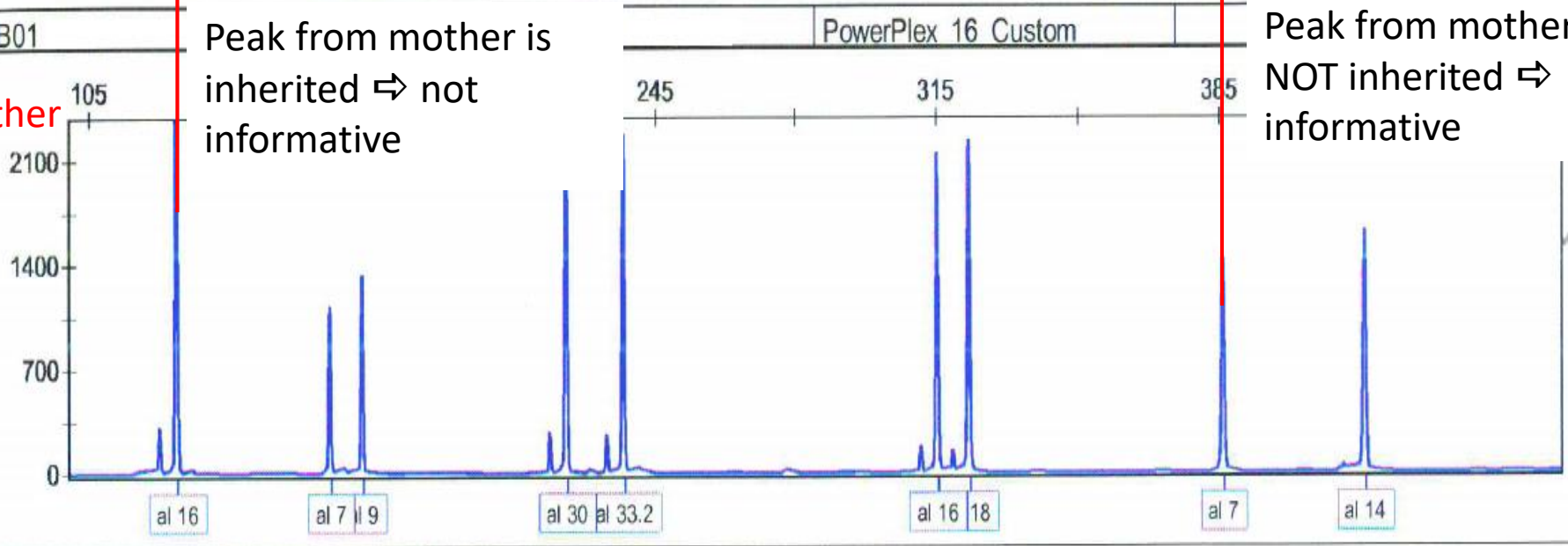
Sample File	Sample Name	Panel	SQI	OS	SQ
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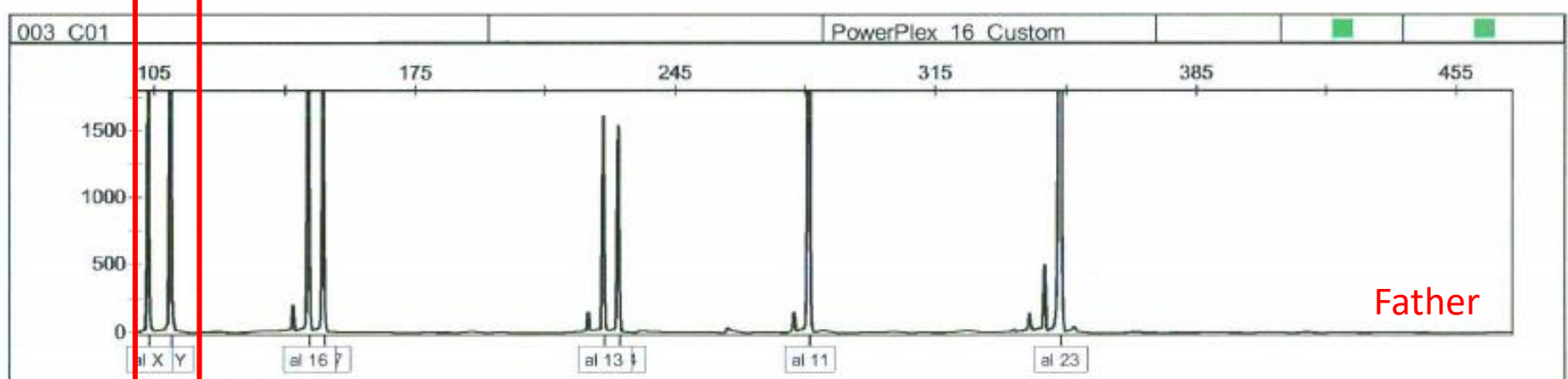
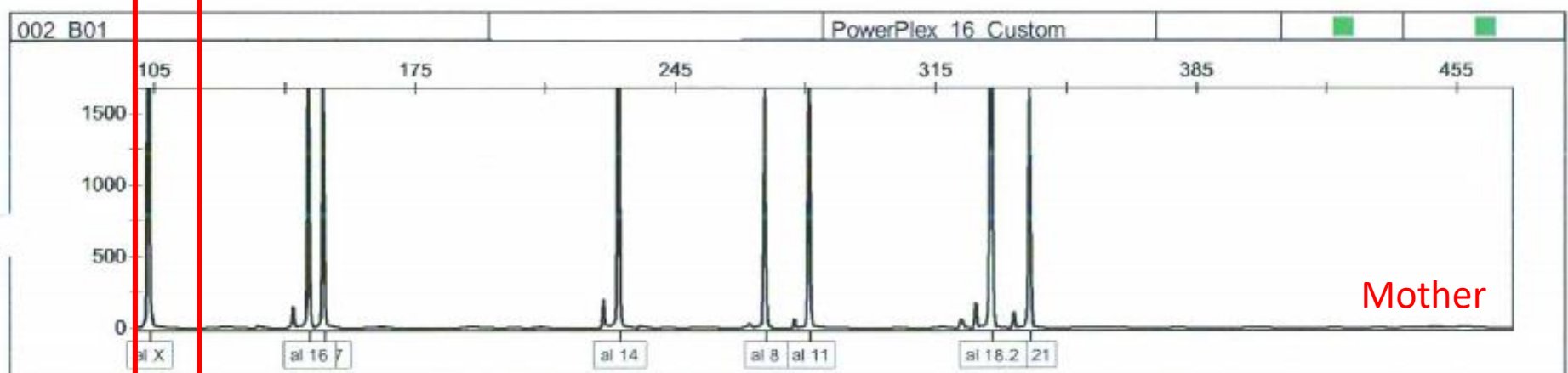
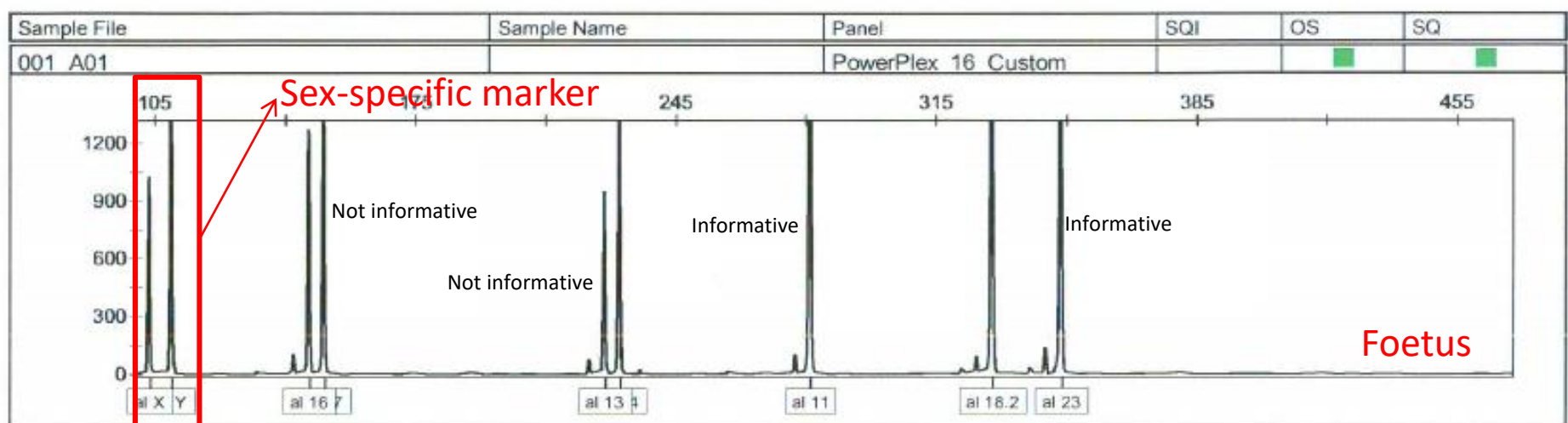
Foetus

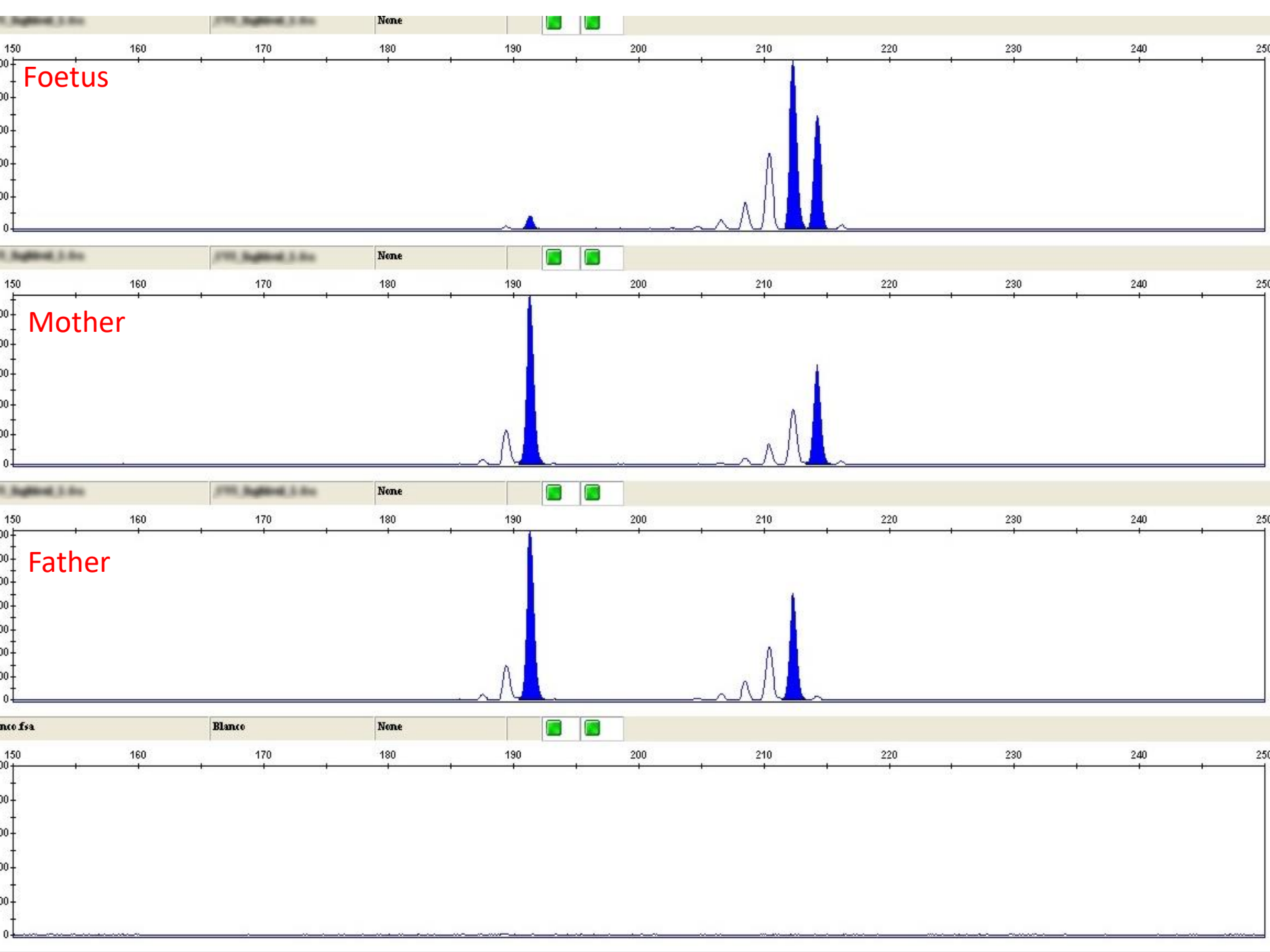


002 B01

Mother







MATERNAL CONTAMINATION

- Sensitivity determined by mixing experiments of 2 samples: detection limit around 10(-20)%

Major message: **AVOID** contamination

by careful selection of CV

- But: in many cases low level contamination has no major influence on the final result!

MATERNAL CONTAMINATION

[But: in many cases low level contamination has no major influence on the final result!]

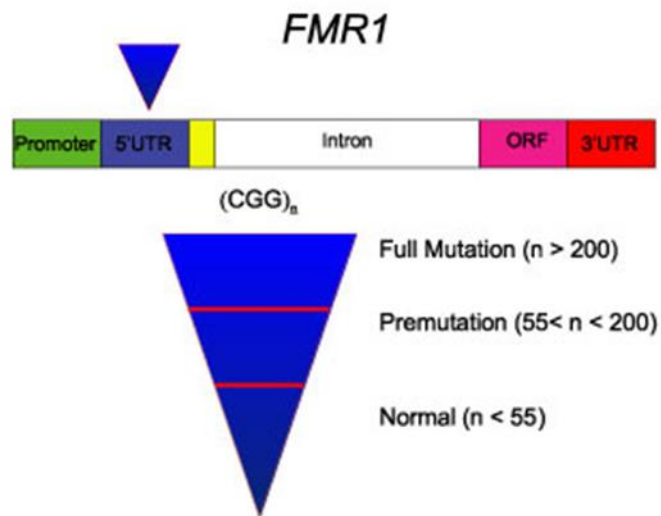
Exceptions !

Be careful! Selection (PCR) of normal or mutant allele by preferential amplification

Two examples: fragile X syndrome and incontinentia pigmenti.

MATERNAL CONTAMINATION

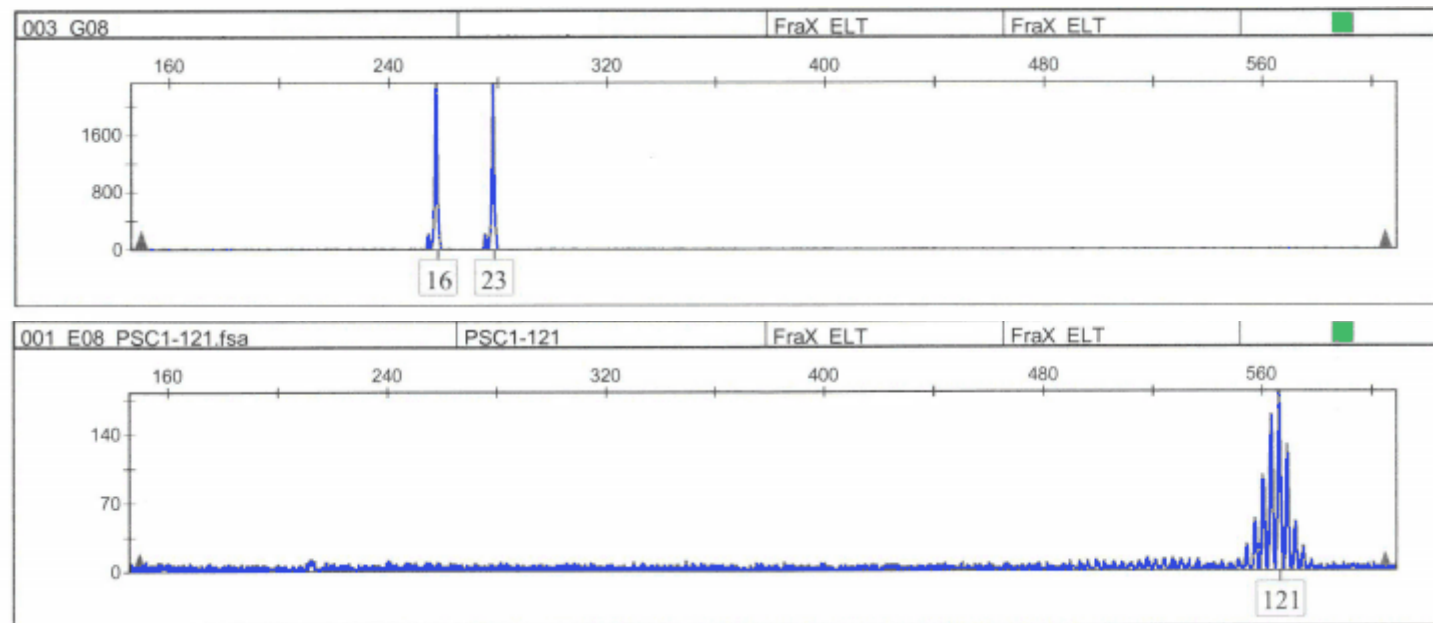
Fragile X syndrome (X chromosome)



MATERNAL CONTAMINATION

Fragile X syndrome (X chromosome)

-conventional PCR for CGG repeat (limit to # repeats that can be detected)



MATERNAL CONTAMINATION

Fragile X syndrome (X chromosome)

- Conventional PCR for CGG repeat (limit to # repeats that can be detected)
- Affected boy: no conventional PCR amplification expected
- Normal boy: PCR amplification of normal maternal allele

MATERNAL CONTAMINATION

Fragile X syndrome

-RESULT: No amplification.

PCR failure ? versus affected?

-RESULT: Normal maternal allele.

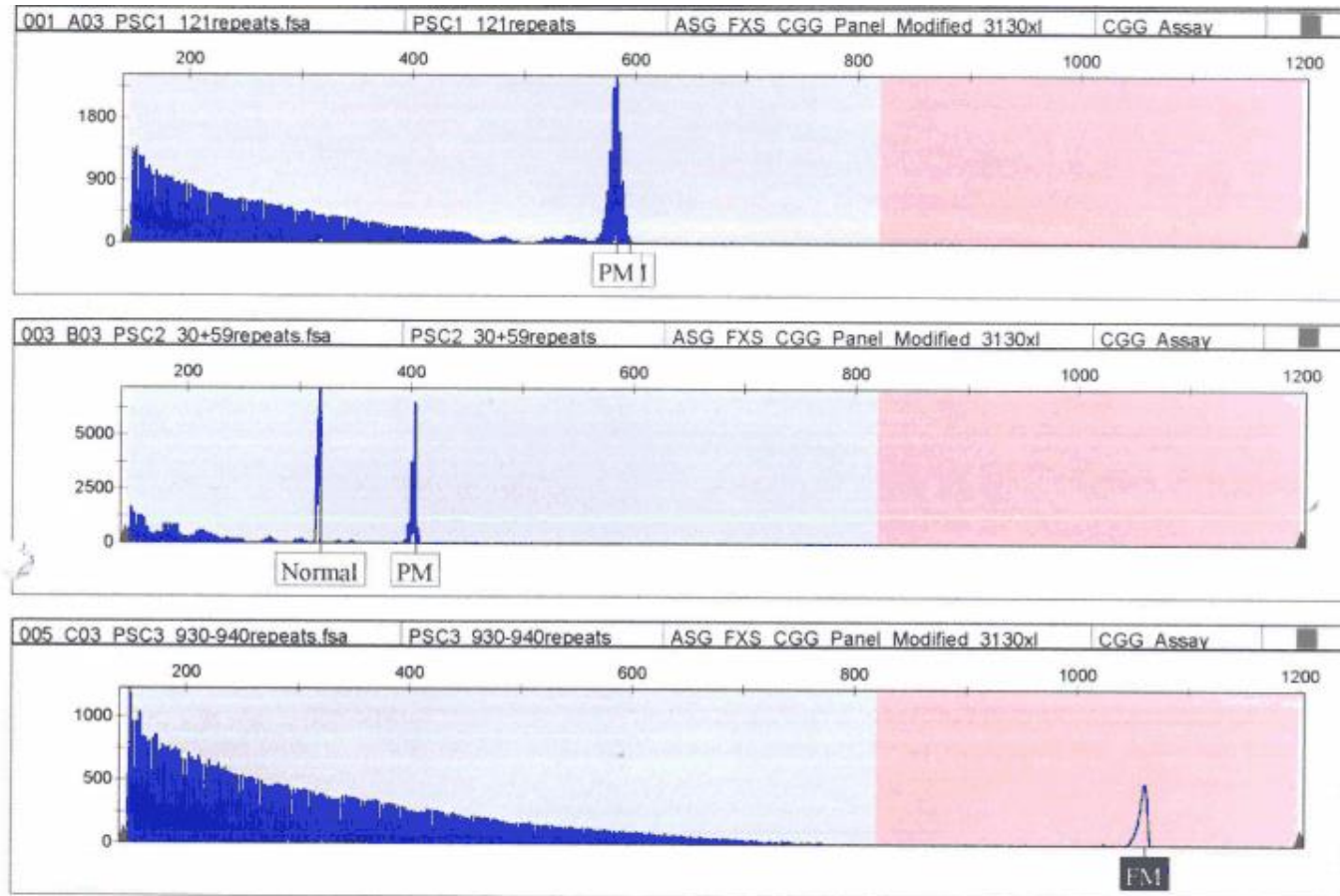
Normal ? versus maternal contamination?

-Affected fetus: sometimes mock normal maternal allele is seen, as if fetus is normal (no expansion)

-Additional analysis suggested: TP-PCR

MATERNAL CONTAMINATION

Fragile X syndrome



MATERNAL CONTAMINATION

Incontinentia pigmenti (IKBKG gene): X dominant

- lethal in boys



INHERITANCE

- X-linked dominant

GROWTH

Height

- Short stature

HEAD & NECK

Head

- Microcephaly

Eyes

- Microphthalmos
- Cataract
- Optic atrophy
- Strabismus
- Retinal vascular proliferation
- Retinal ischemia
- Retinal bleeding
- Retinal fibrosis
- Retinal detachment
- Uveitis
- Keratitis
- Foveal hypoplasia
- Foveal disorganization
- Extraretinal neovascularization

Teeth

- Hypodontia
- Delayed eruption
- Conical forms
- Accessory cusps

CHEST

Ribs Sternum Clavicles & Scapulae

- Extra ribs

Breasts

- Supernumerary nipple
- Nipple hypoplasia
- Breast hypoplasia
- Breast aplasia

SKELETAL

Spine

- Hemivertebrae
- Kyphoscoliosis

SKIN, NAILS, & HAIR

Skin

- STAGE 1 - skin erythema, vesicles, pustules
- Onset birth-newborn period
- Affects limbs and trunk
- Occurs in linear distribution
- STAGE 2 - Skin papules, verrucous lesions, hyperkeratosis
- Affects distal limb and scalp
- STAGE 3 - Skin hyperpigmentation
- Primarily affects trunk
- Follows Blaschko's lines
- Streaks and whorls
- Fades in adolescence
- STAGE 4 - skin pallor, atrophy, and scarring
- Most evident on lower legs

Nails

- Nail dystrophy
- Nail ridging
- Nail pitting
- Onychogrypsis
- Subungual keratotic tumors

Hair

- Atrophic, patchy alopecia (vertex)
- Wiry, coarse hair (childhood)
- Thin, sparse hair (childhood)

NEUROLOGIC

Central Nervous System

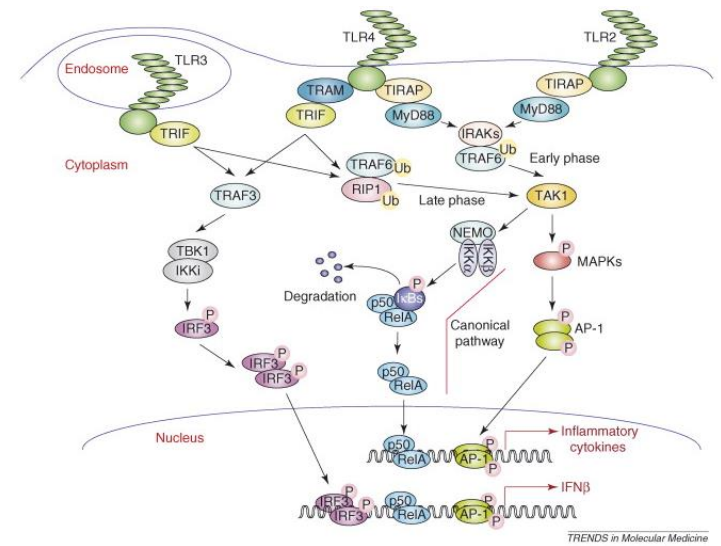
- Seizures
- Mental retardation
- Spasticity

HEMATOLOGY

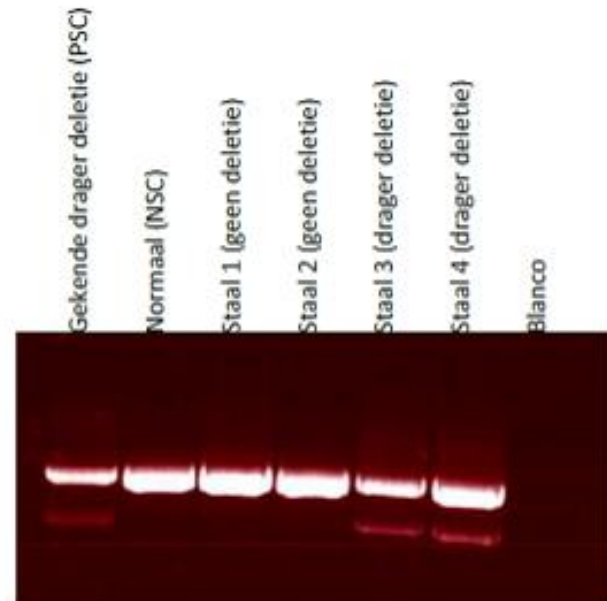
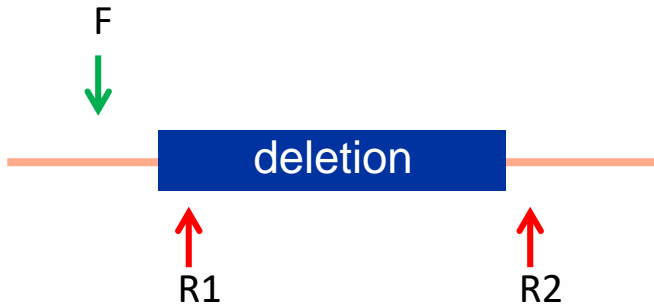
MATERNAL CONTAMINATION

Incontinentia pigmenti (IKBKKG gene): X dominant

- Most common alteration (90%) is a large deletion of exons 4-10 of the IKBKG gene: 9kb
- PCR amplification of the deleted and normal fragment

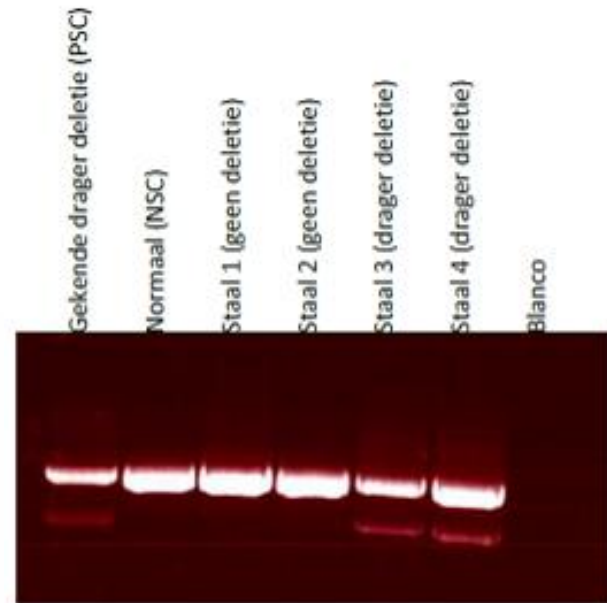
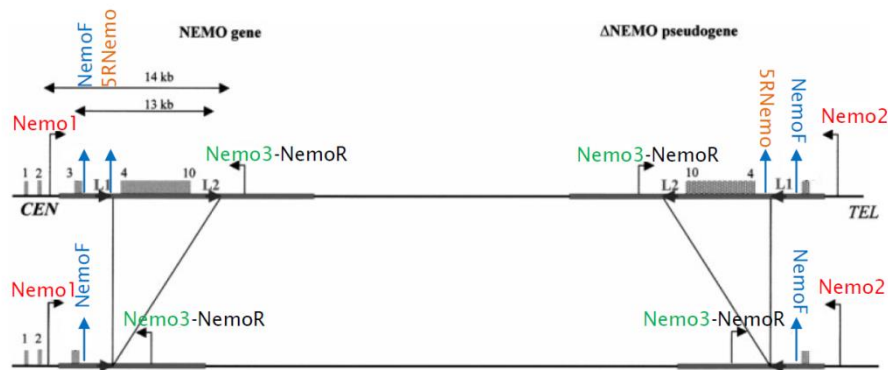
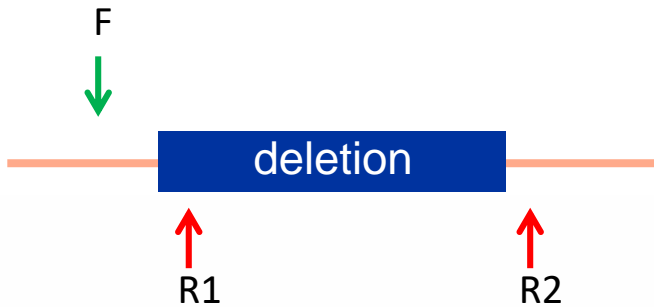


MATERNAL CONTAMINATION



Normal: only F – R1
Heterozygous: also F – R2

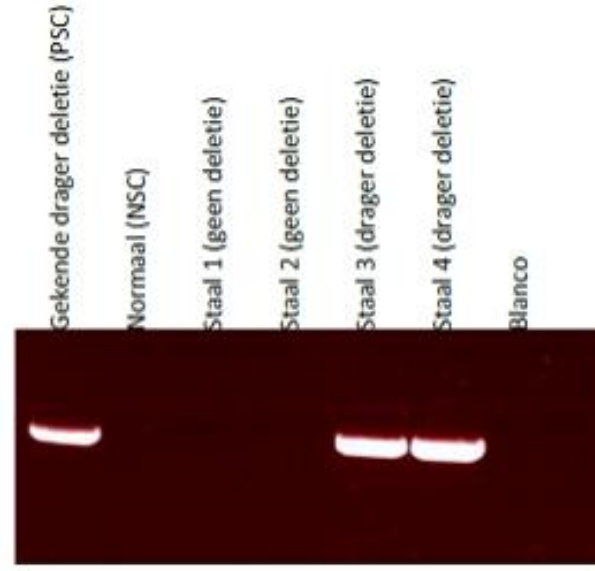
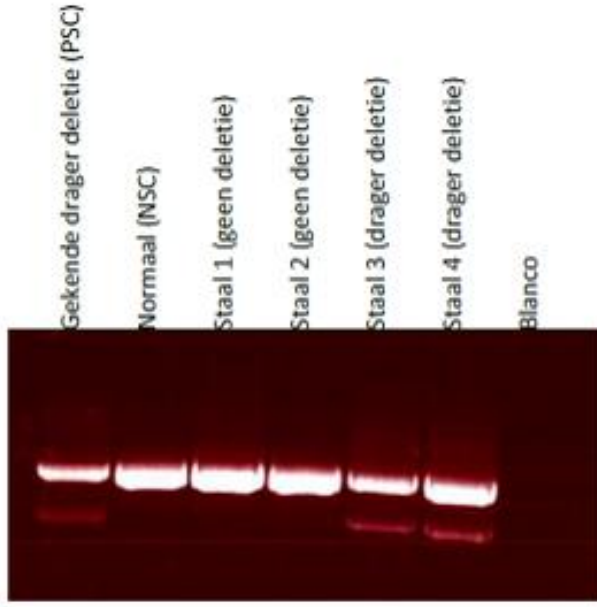
MATERNAL CONTAMINATION



4 copies!

Normal: only F – R1
Carrier: also F – R2

MATERNAL CONTAMINATION



MATERNAL CONTAMINATION

Incontinentia pigmenti (IKBKG gene): X dominant

- PCR amplification of the deleted and normal fragment
- 'Deleted fragment' can only be amplified in case of a deletion (normally too large)
- Contamination: mock 'amplification' of maternal deletion (when carrier)
- Additional analysis difficult/impossible due to pseudogene

=> preferred material to test = Amniotic fluid

