NIPT: Non-invasive prenatal testing

Presentation by Ben Caljon –
Adapted by Ann Van Den Bogaert & Annelies Fieuw

Universitair Ziekenhuis Brussel

Overview

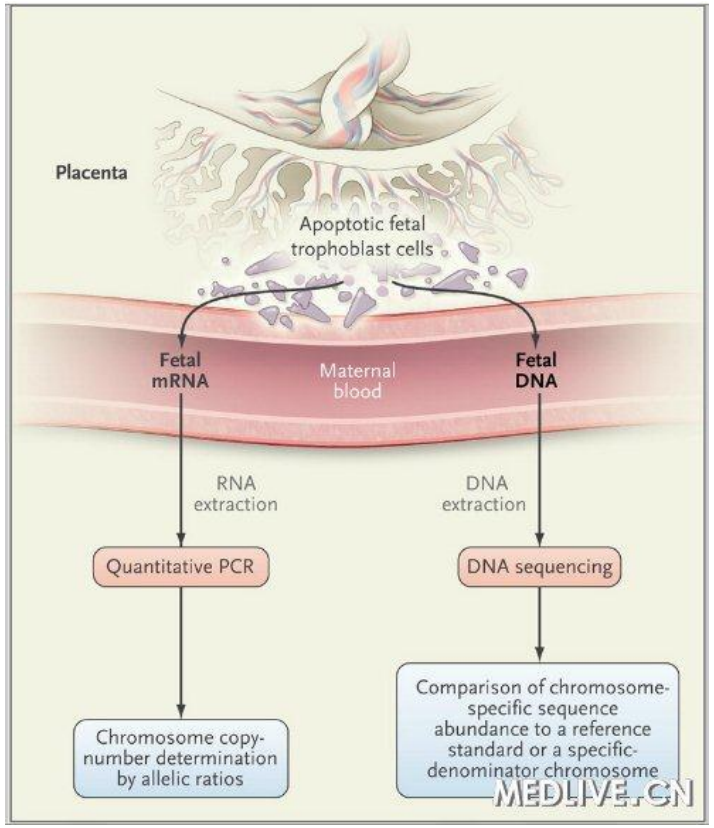
- Detection of cell-free DNA
- NIPT technique
- Indication and limitations
- Reporting policy

Non-invasive prenatal testing (NIPT)

Detection of cell-free fetal DNA (cfDNA) in maternal plasma in 1997 (1)

- → shedding of trophoblast cells
- → Micro-particles of fragmented DNA into maternal bloodstream
- → short half life (2 h clearance)

- Median prevalence of 3% to 10% of total cfDNA in 1st and 2nd trimester (2)
- Reliable detection from 11-12 weeks
- Increasing during pregnancy



(1) Lo YMD, Corbetta N, Chamberlain PF, Rai V, Sargent IL, et al. (1997) Presence of fetal DNA in maternal plasma and serum. Lancet 350: 485–487.

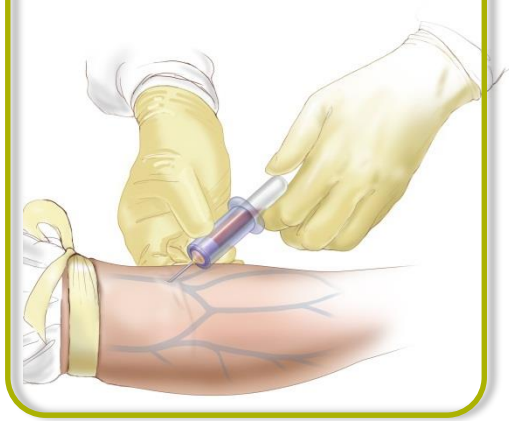
(2) Lun FMF, Chiu RWK, Chan KCA, Leung TY, Lau TK, Lo YMD. 2008 Microfluidics digital PCR reveals a higher than expected fraction of fetal DNA in maternal plasma. Clin. Chem. 54, 1664–1672.

Overview

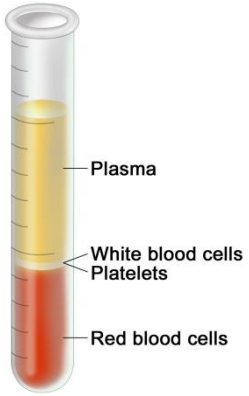
- Detection of cell-free DNA
- NIPT technique
- Indication and limitations
- Reporting policy

Overview NIPT technique

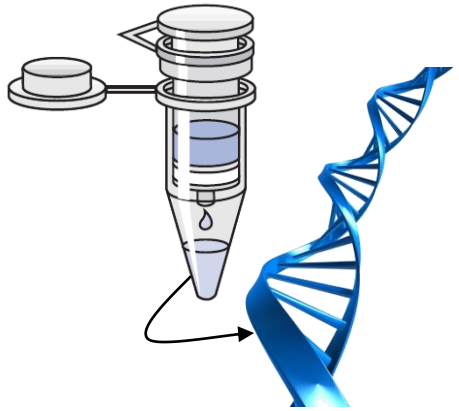
1. Phlebotomy



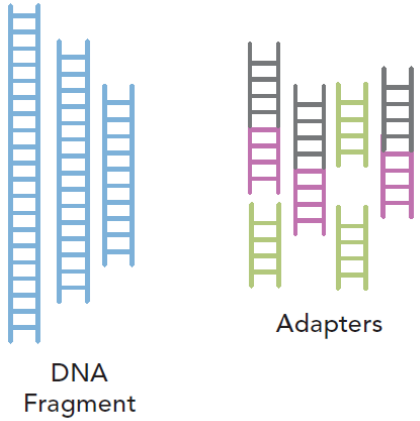
2. Plasma isolation



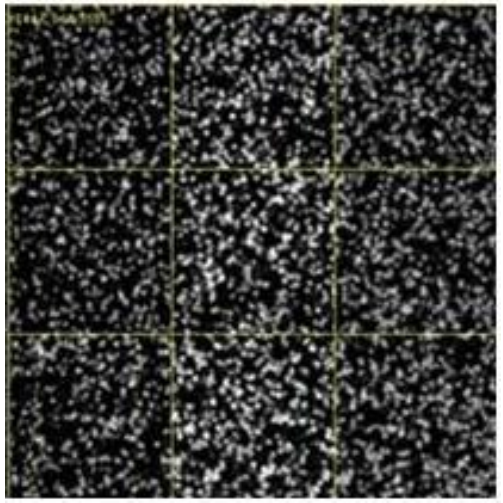
3. cfDNA extraction



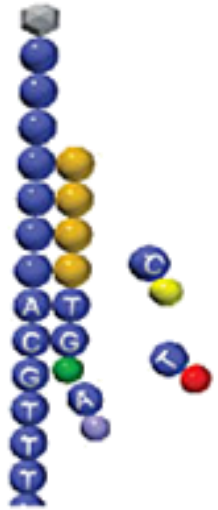
4. Library preparation



5. Cluster generation



6. Sequencing

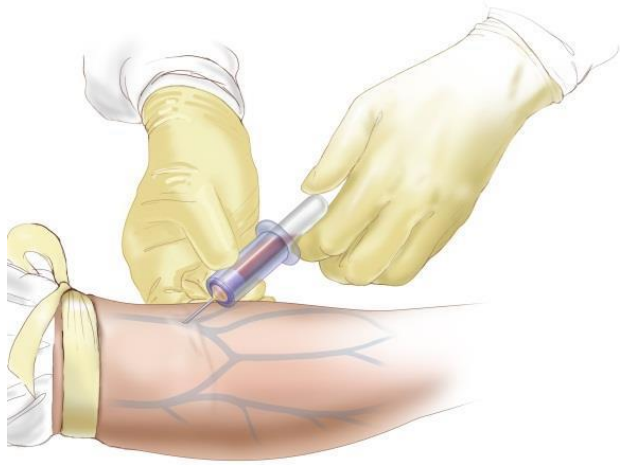


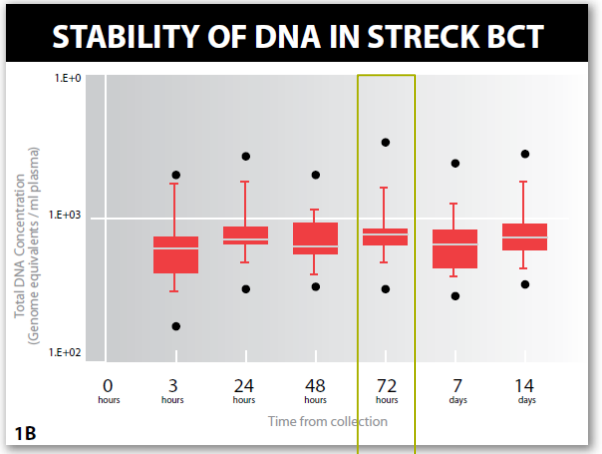
7. Data-analysis

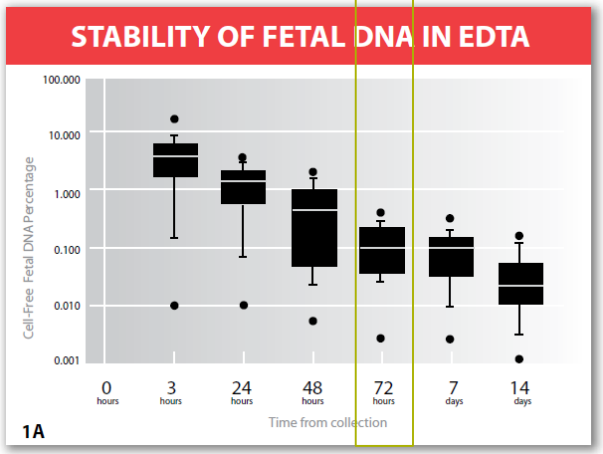
"Data don't make any sense,
we will have to resort to statistics."

8. Reporting

NIPT technique – Phlebotomy (1)







- Collection of maternal peripheral blood (from 12 weeks gestation) in 10 ml EDTA tubes (Streck tubes) with a proprietary stabilizing agent

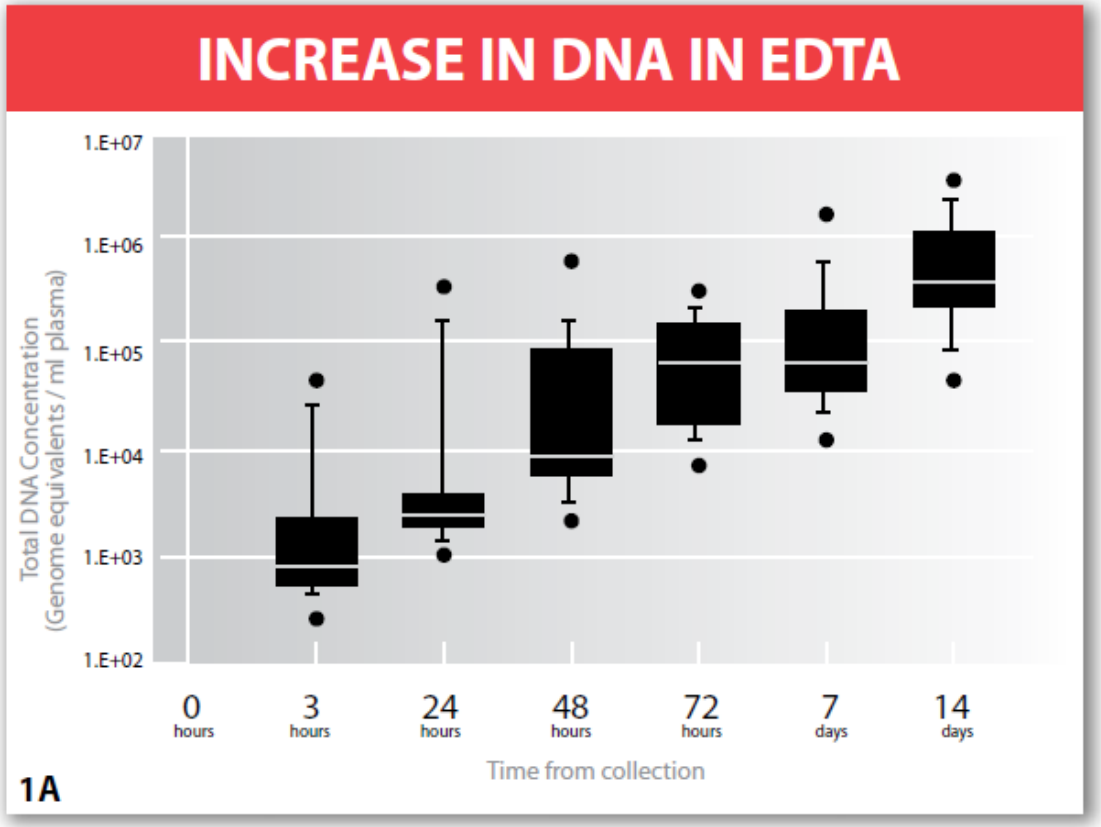
  → Inhibits gDNA release from nucleated (maternal) cells
  → Inhibits nuclease activity

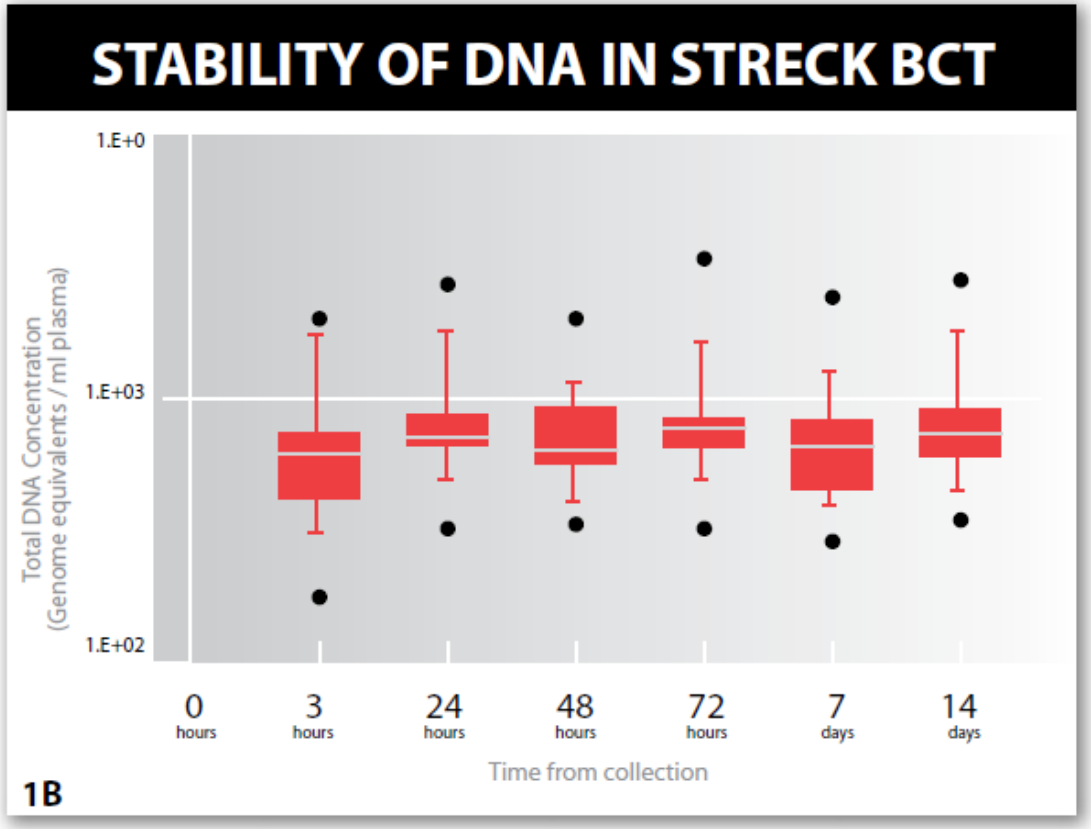
Universitair Ziekenhuis Brussel

Centrum voor Medische Genetica

NIPT technique – Phlebotomy (2)

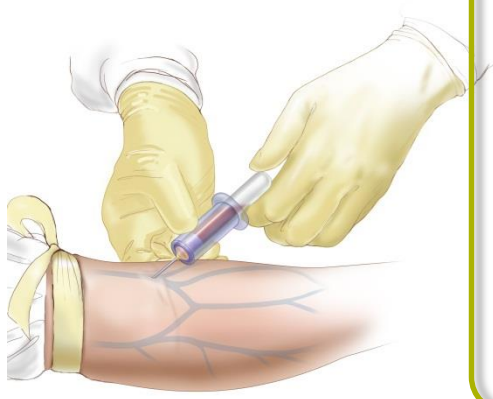
- gDNA release from nucleated (maternal) cell over time (0-14 days)



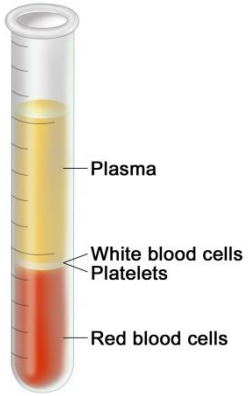


Overview NIPT technique

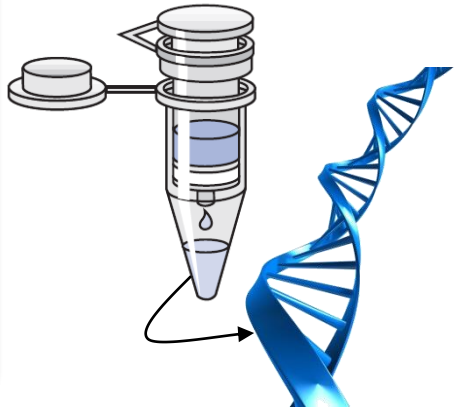
1. Phlebotomy



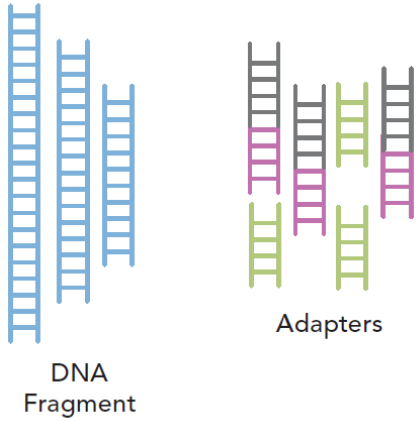
2. Plasma isolation



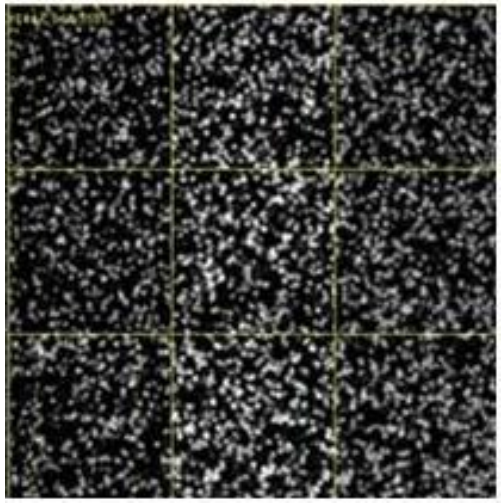
3. cfDNA extraction



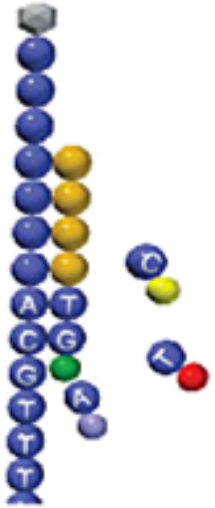
4. Library preparation



5. Cluster generation



6. Sequencing



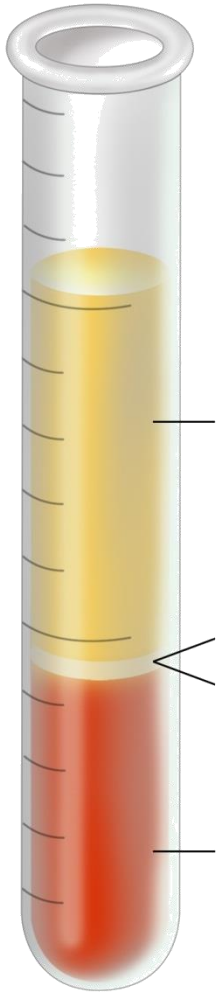
7. Data-analysis

"Data don't make any sense,
we will have to resort to statistics."

8. Reporting

NIPT technique – Plasma isolation (1)

2. Plasma isolation



- Centrifuge whole blood
- Collect plasma
- Recentrifuge plasma fraction

source of maternal & fetal cell-free DNA (cfDNA)

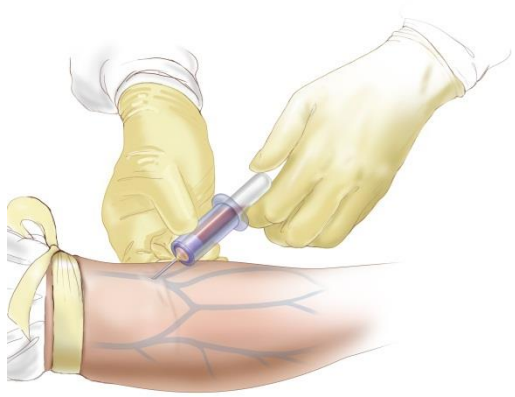
source of maternal genomic DNA (gDNA)

Universitair Ziekenhuis Brussel

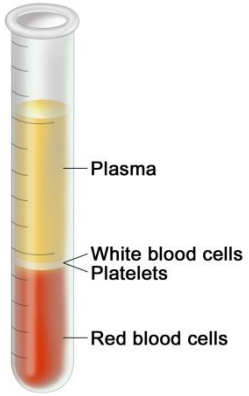
Centrum voor Medische Genetica

Overview NIPT technique

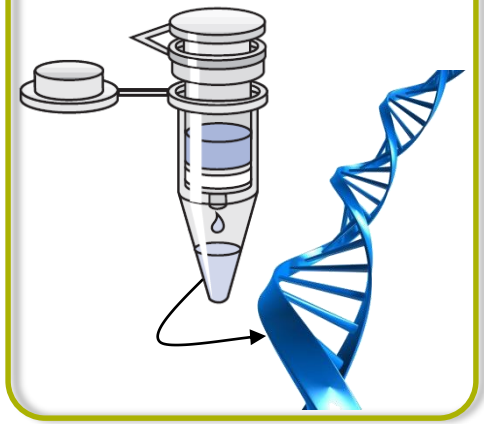
1. Phlebotomy



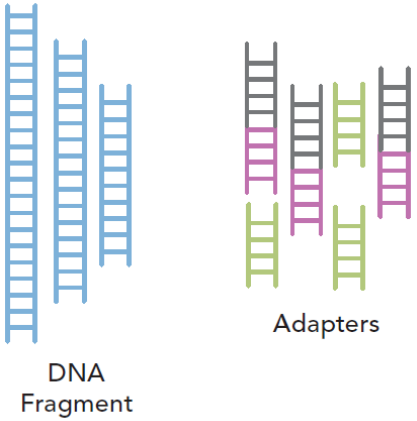
2. Plasma isolation



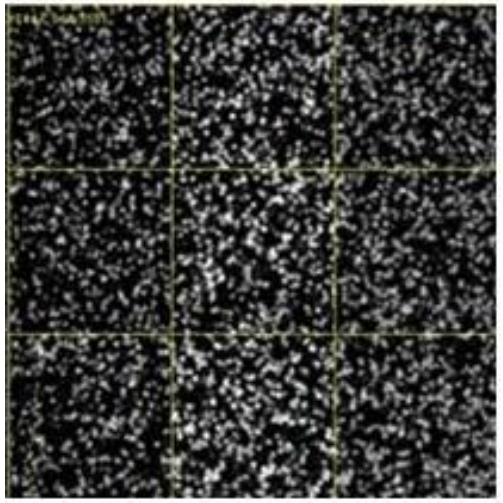
3. cfDNA extraction



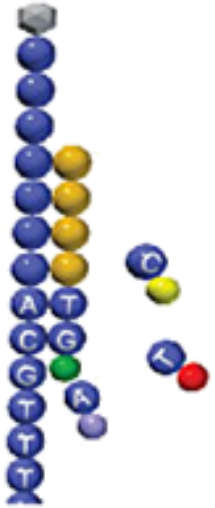
4. Library preparation



5. Cluster generation



6. Sequencing



7. Data-analysis

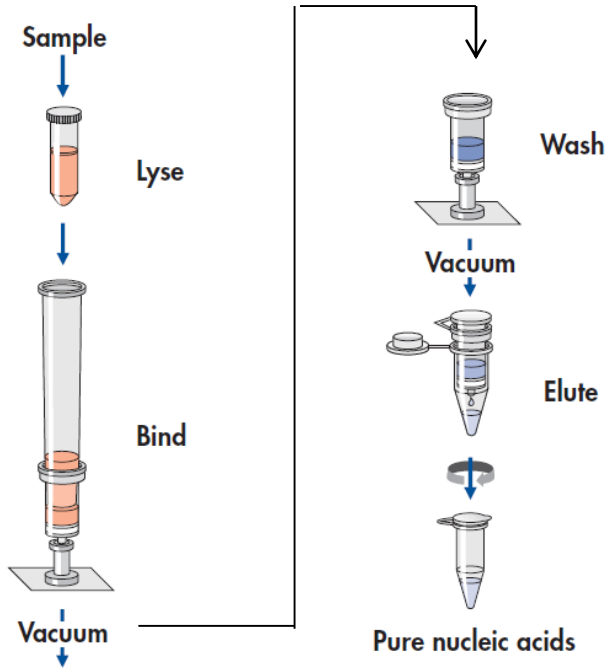
"Data don't make any sense,
we will have to resort to statistics."

8. Reporting

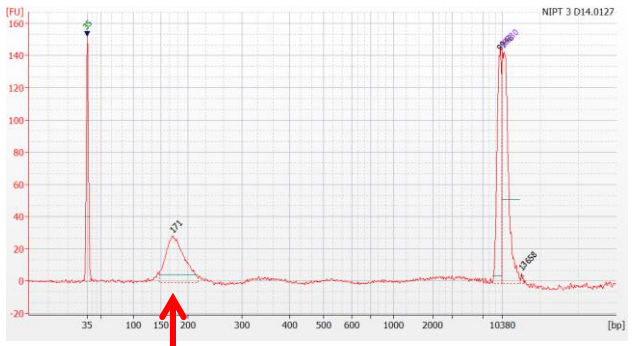
NIPT technique – cfDNA extraction (3)

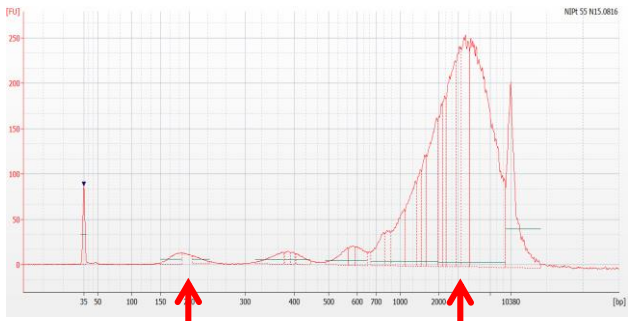
Extract cf DNA from plasma

- obtain pure DNA, depleted of proteins, macromolecules & salts



DNA quantification & qualification



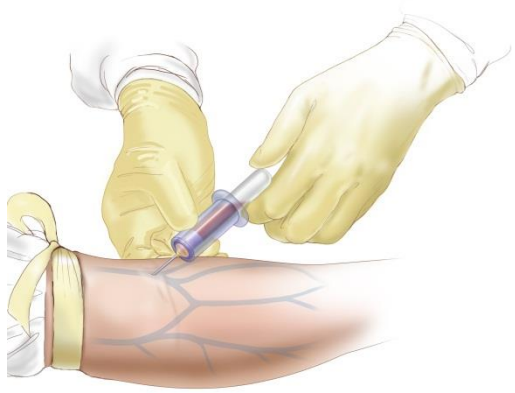


Universitair Ziekenhuis Brussel

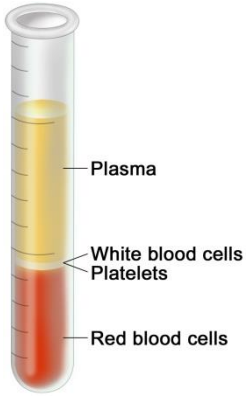
Centrum voor Medische Genetica

Overview NIPT technique

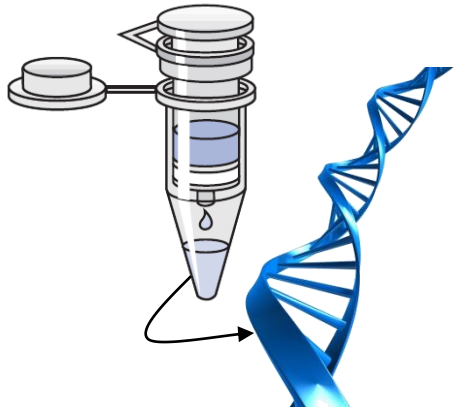
1. Phlebotomy



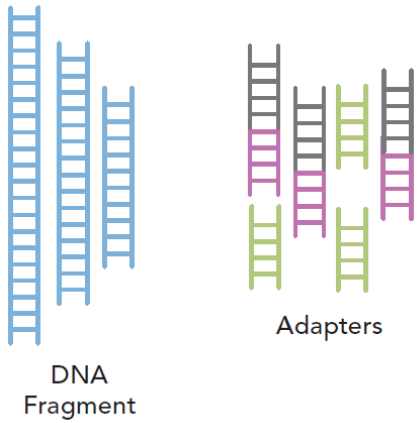
2. Plasma isolation



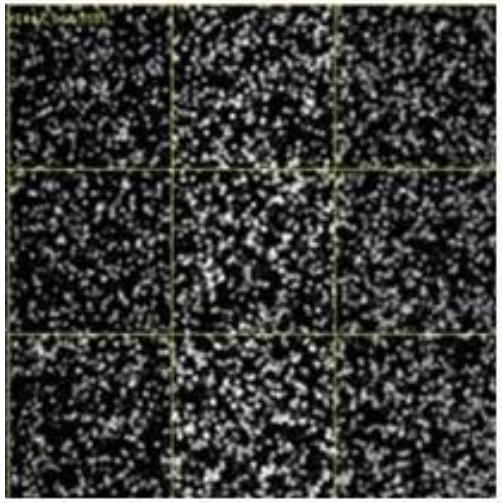
3. cfDNA extraction



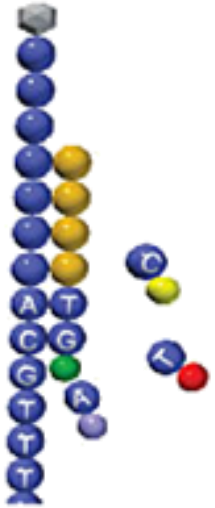
4. Library preparation



5. Cluster generation



6. Sequencing



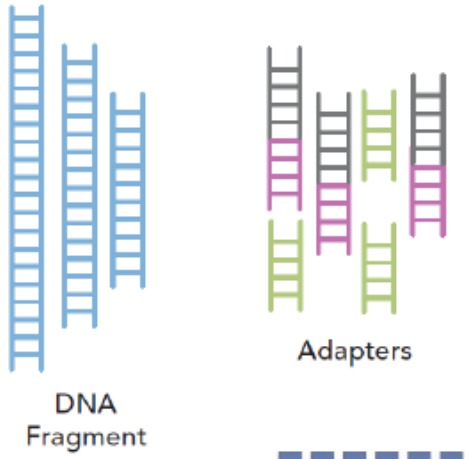
7. Data-analysis

"Data don't make any sense,
we will have to resort to statistics."

8. Reporting

NIPT technique – library prep (1)

4. Library preparation

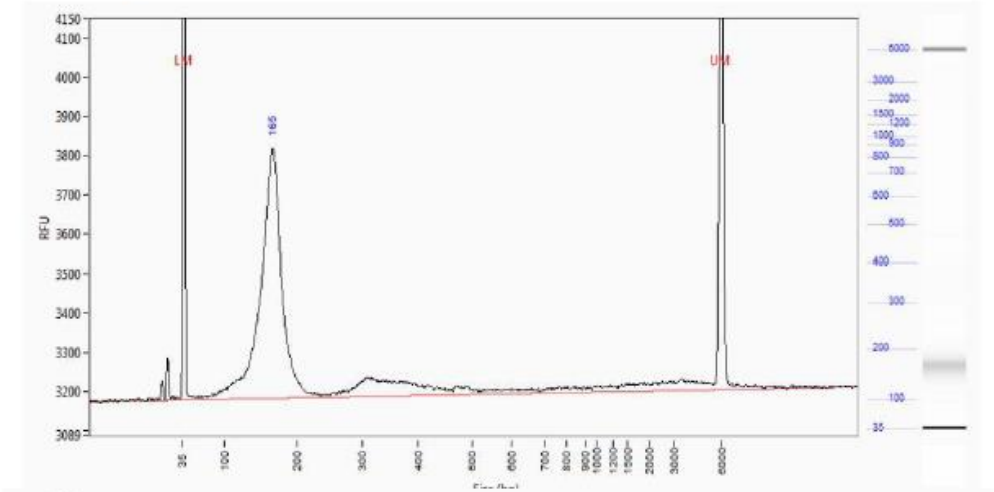


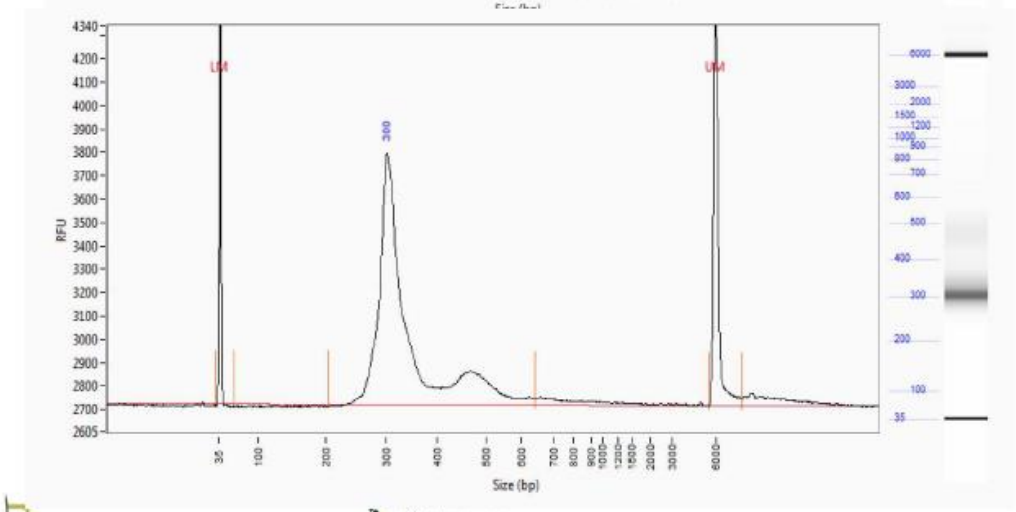
The cfDNA has to be modified for the sequencing instrument (eg. HiSeq) to be able to read the DNA sequences of each individual fragment. The following actions are required :

- **End repair**
- **Adenylation (3')**
- **Adapter ligation**
- **Library purification (x2)**
- **PCR amplification**
- **Library purification**
- **Library validation**

NIPT technique – library prep (2)

Library validation





Average size cfDNA fragments = 150-170 bp

Average size cfDNA fragments + adapters = ca. 300 bp

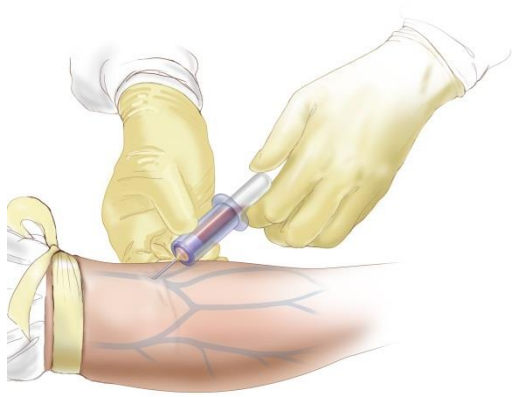
- *Validated libraries are pooled in equal ratios (equimolar).*
- *Because of the unique adapter (1 adapter per cfDNA sample), the instrument can discriminate*

Universitair Ziekenhuis Brussel

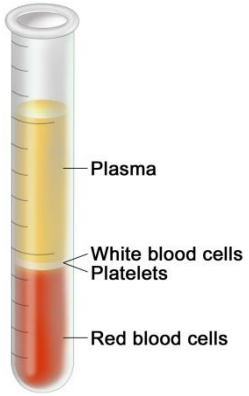
Centrum voor Medische Genetica

Overview NIPT technique

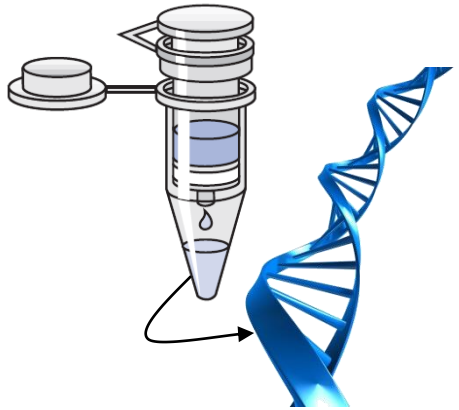
1. Phlebotomy



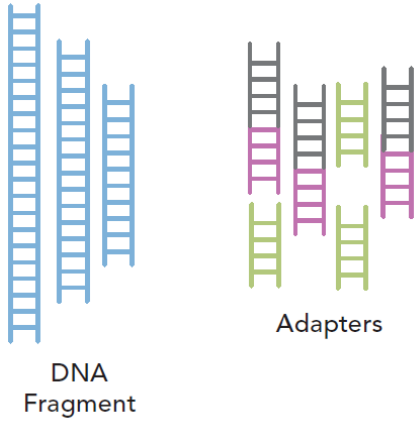
2. Plasma isolation



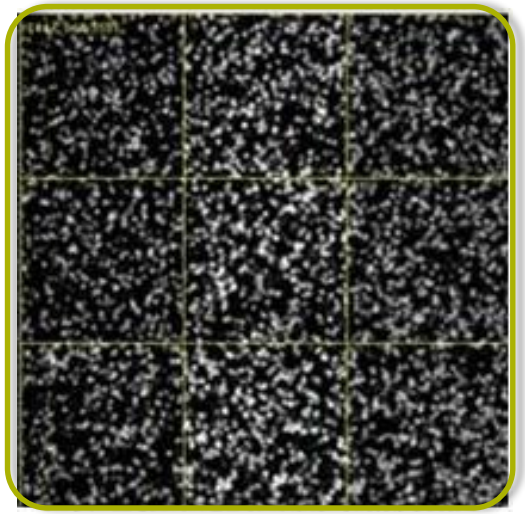
3. cfDNA extraction



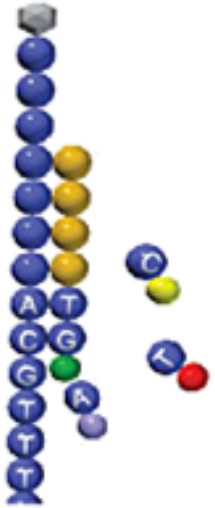
4. Library preparation



5. Cluster generation



6. Sequencing



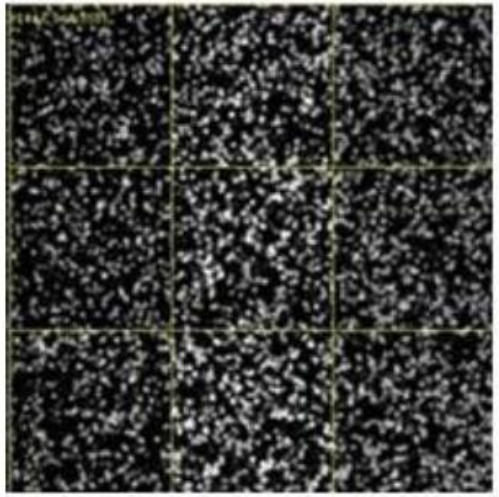
7. Data-analysis

"Data don't make any sense,
we will have to resort to statistics."

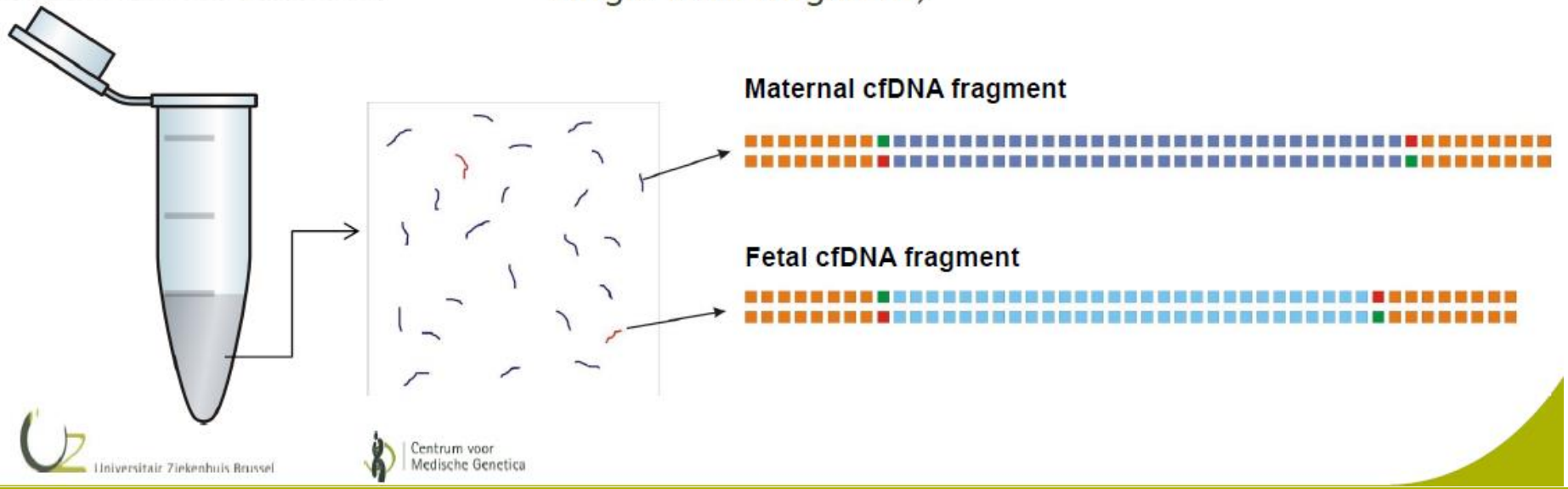
8. Reporting

NIPT technique –cluster gen. (1)

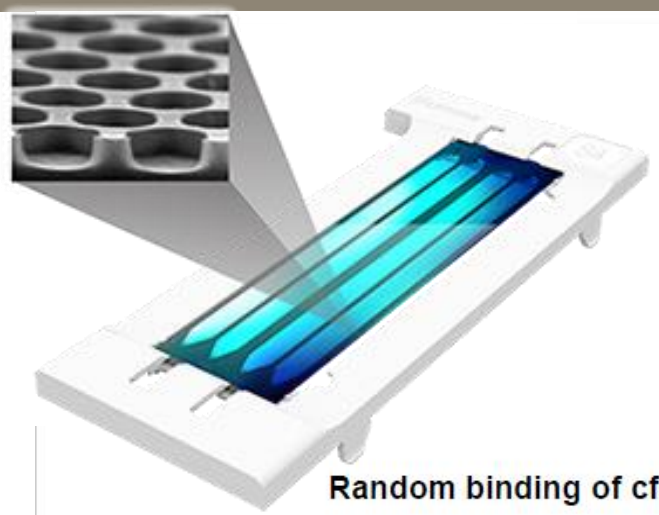
5. Cluster generation



- The pooled library sample has to be attached to the glass slide (flowcell), so every unique fragment binds to a random but distinct zone on this slide
- Per zone, there will be 1 unique fragment that will be amplified, so multiple copies of the same fragment exist on that zone (=cluster generation)
  - → Amplification needed to overcome current detection limitations (not possible to detect/genotype a single DNA fragment)

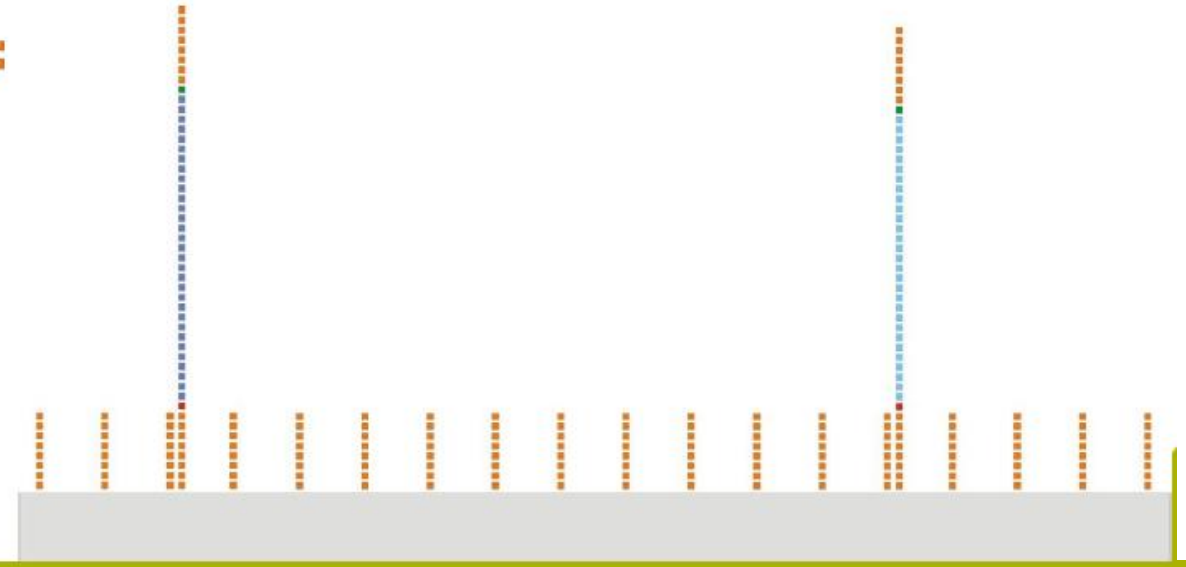


NIPT technique –cluster gen. (2)



Glass slide coated with DNA fragments that recognize the adapters

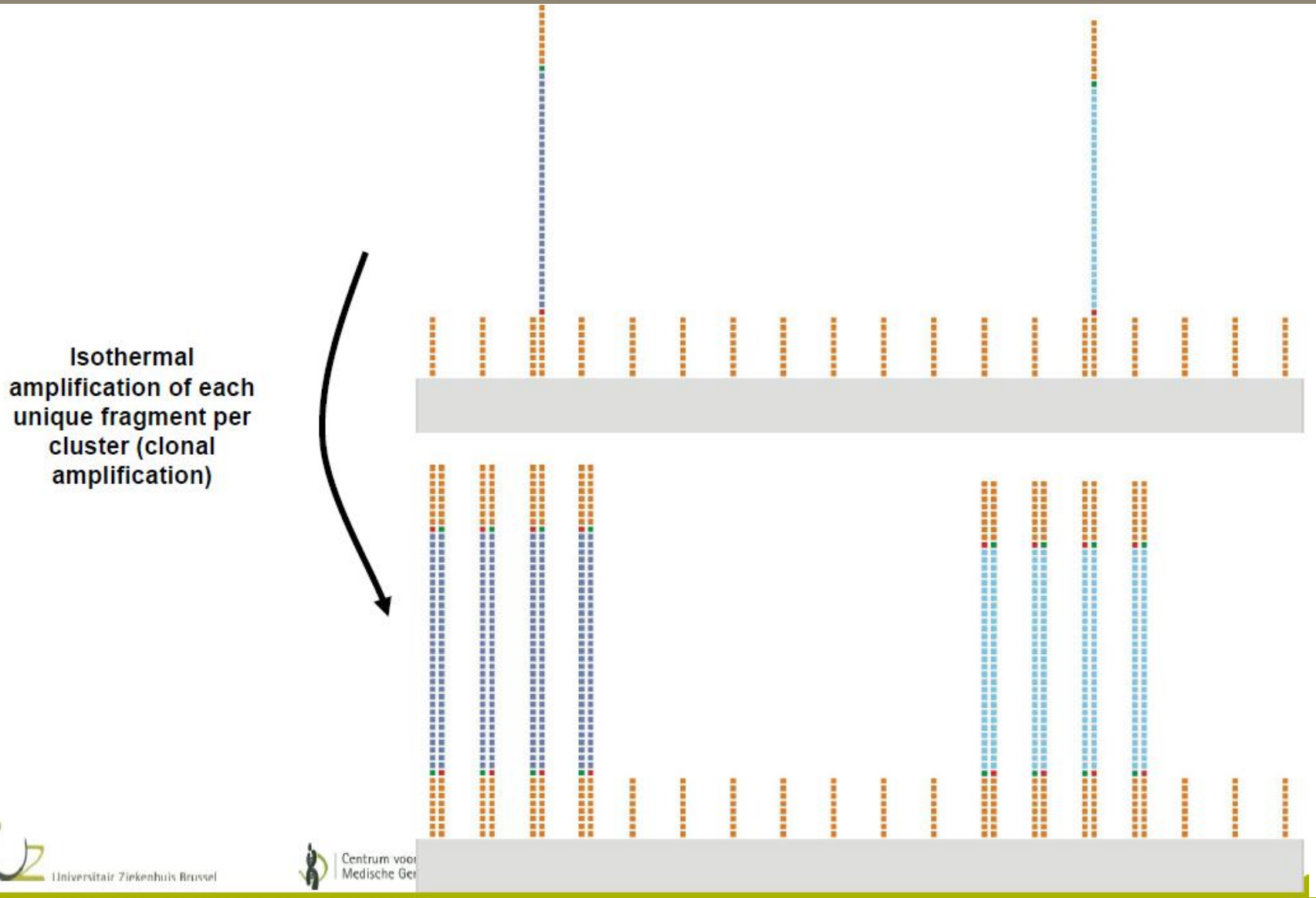
Random binding of cfDNA fragments to probes on flowcell



Universitair Ziekenhuis Brussel

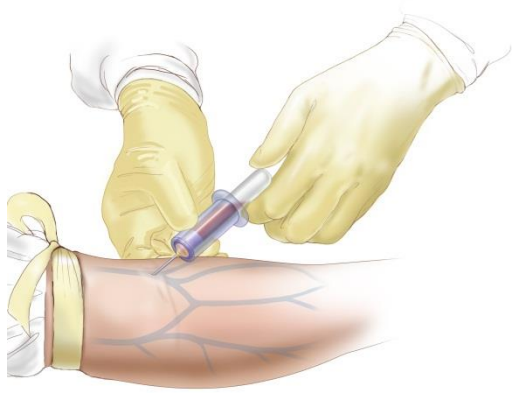
Centrum voor Medische Genetica

NIPT technique –cluster gen. (3)

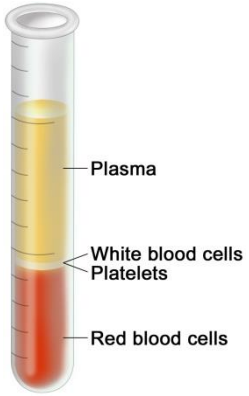


Overview NIPT technique

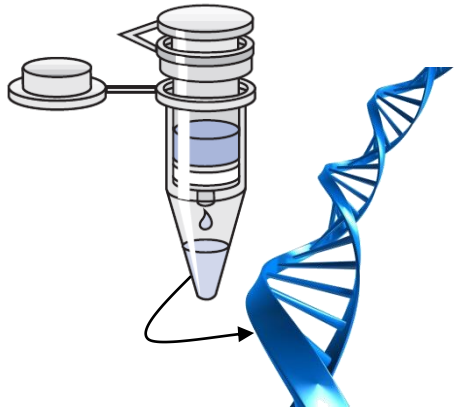
1. Phlebotomy



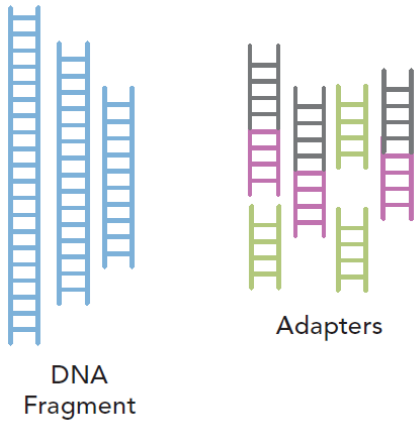
2. Plasma isolation



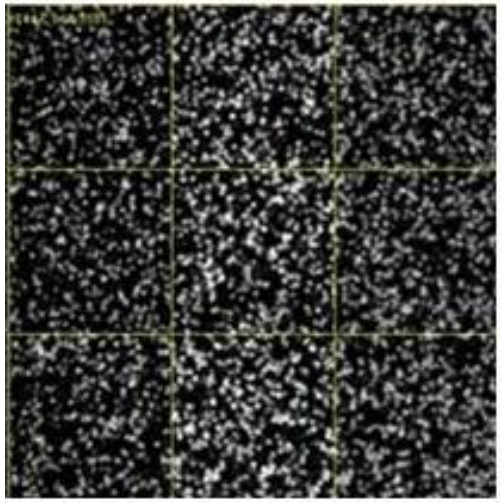
3. cfDNA extraction



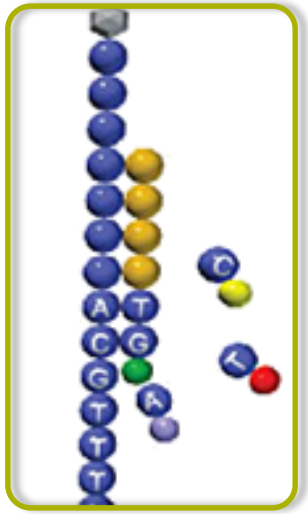
4. Library preparation



5. Cluster generation



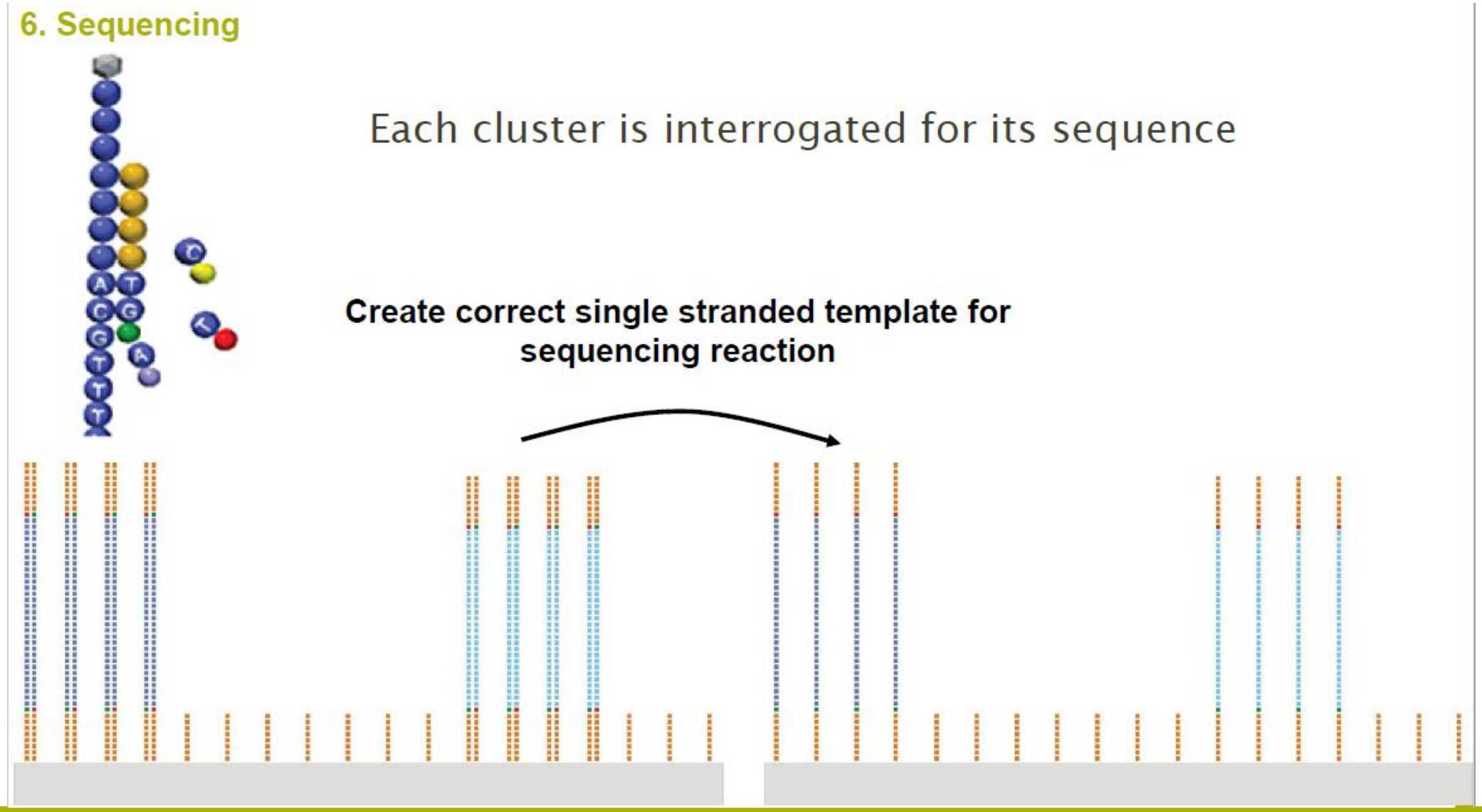
6. Sequencing



7. Data-analysis

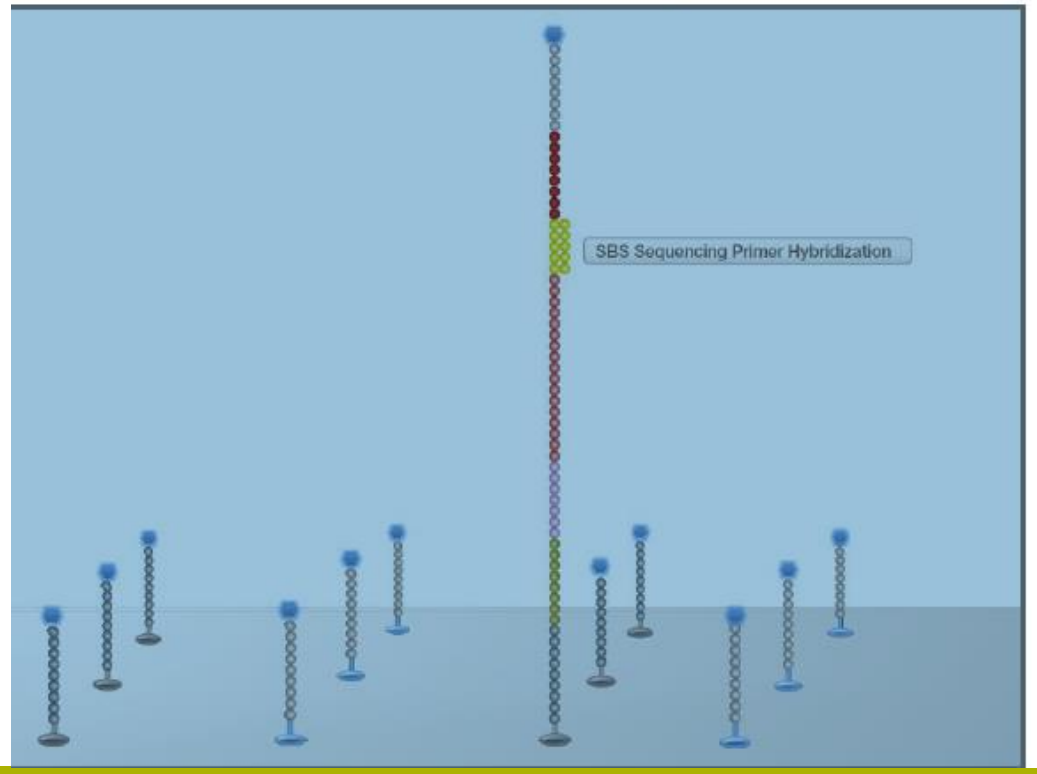
8. Reporting

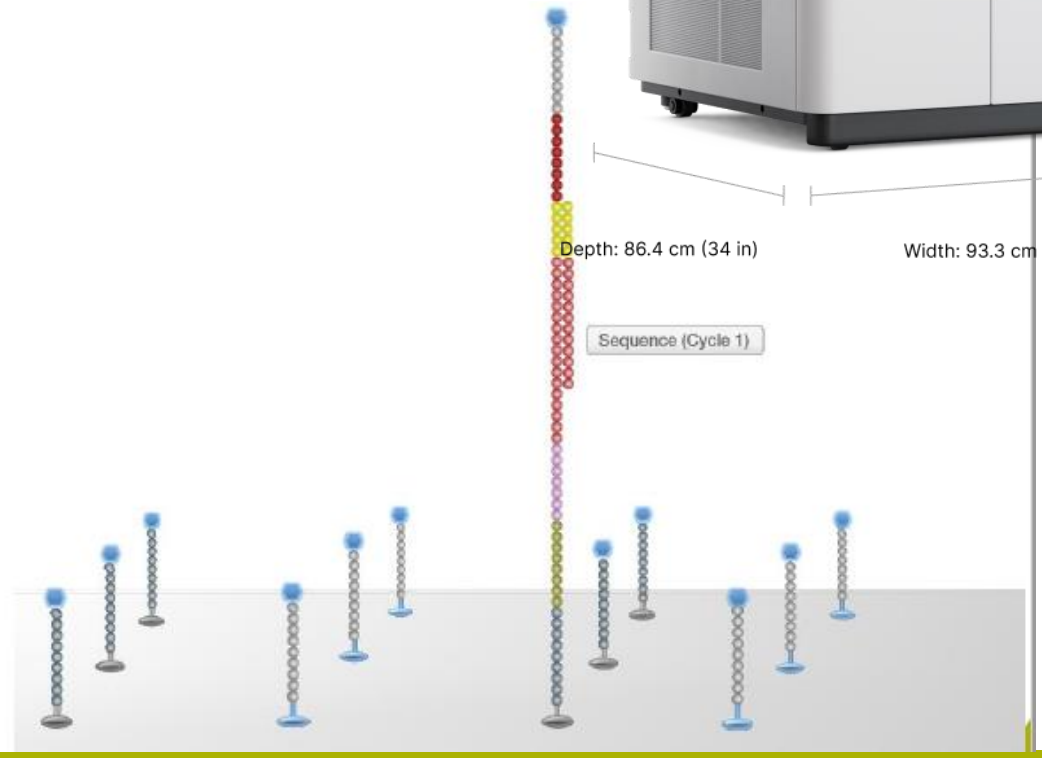
NIPT technique –Sequencing (1)



NIPT technique –Sequencing (2)

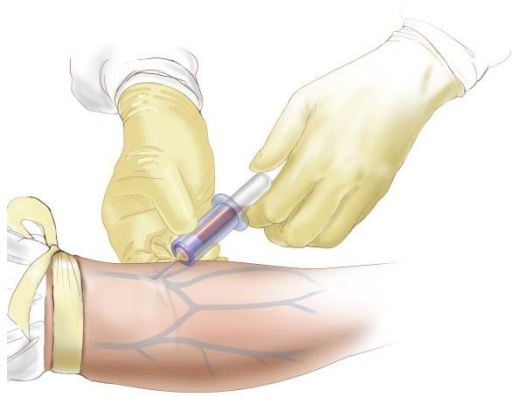
- Anneal sequencing primer
- Sequencing By Synthesis (SBS)
  - → Iteration of DNA polymerisation (1 base per cycle), laser scanning and decapping



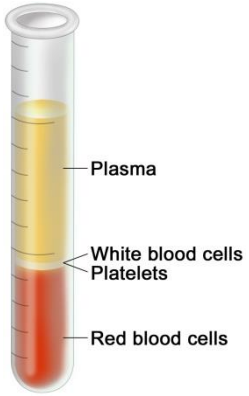


Overview NIPT technique

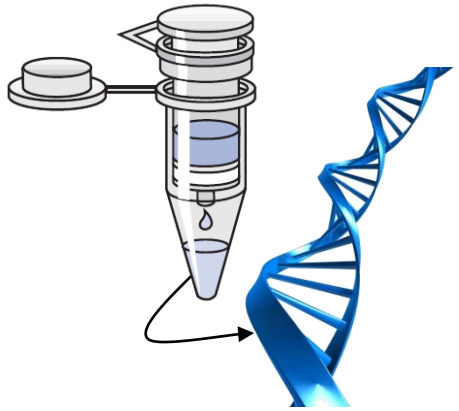
1. Phlebotomy



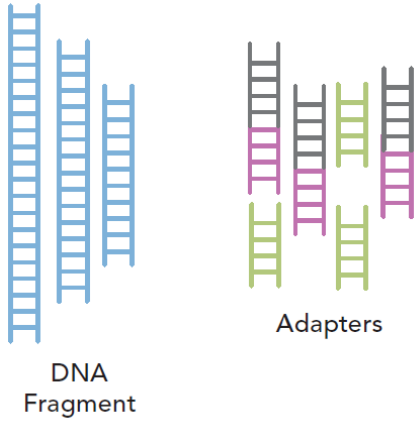
2. Plasma isolation



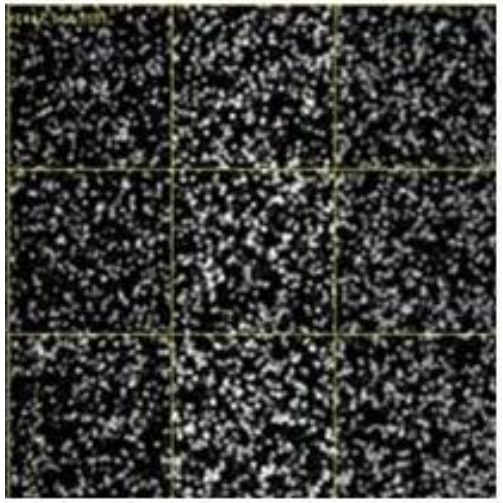
3. cfDNA extraction



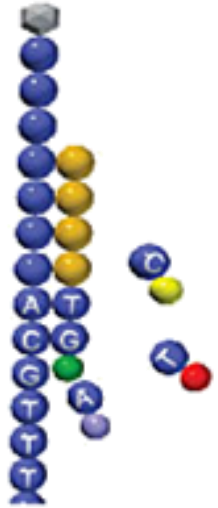
4. Library preparation



5. Cluster generation



6. Sequencing



7. Data-analysis

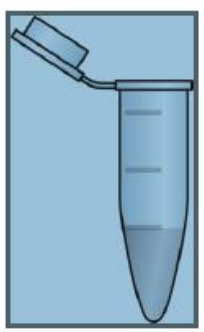
8. Reporting

NIPT technique – data analysis (1)

- Resolution of detection is determined by coverage (number of reads).
- Higher resolution has high impact on total cost.

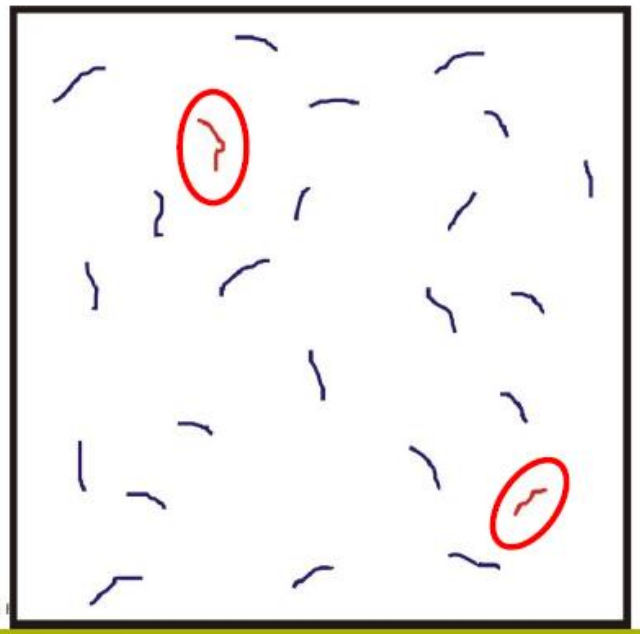
NIPT technique – data analysis (2)

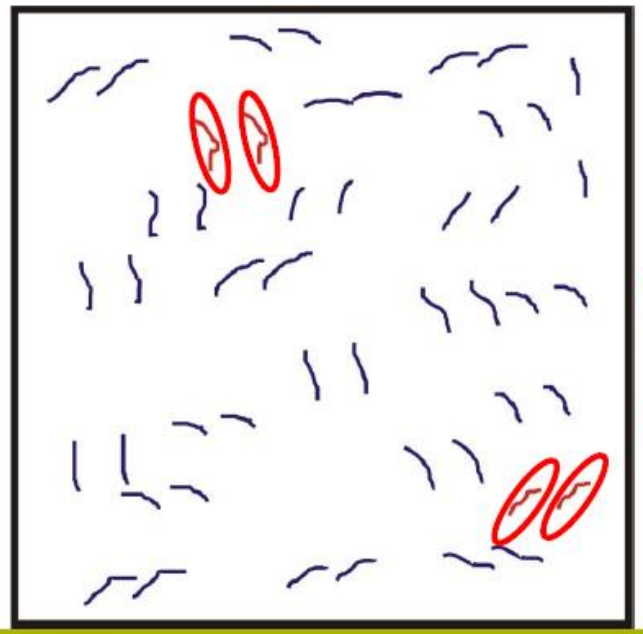
- Z-score calculation
  - → Step 1 : normalize for amount of data
    - Divide number of fragments for chr N by total number of fragments



Red : 2
Total : 24

% red = 8,3%





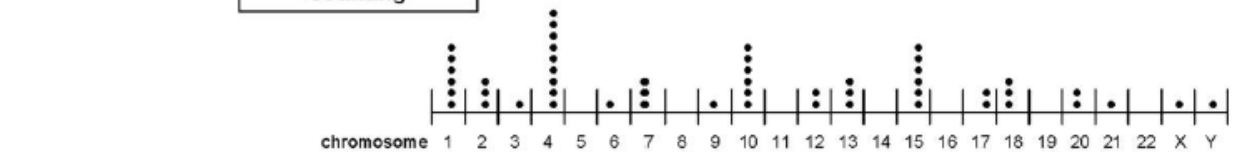
Red : 4
Total : 48

% red = 8,3%

NIPT technique – data analysis (3)

- Z-score calculation
  - → Step 2 : determine the number of standard deviations from mean (control SDs should be established for comparison purpose)

Sequence counting

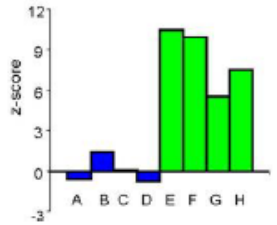


% representation of unique sequences mapped to a chromosome

$$\%\ \text{chrN} = \frac{\text{Unique count for chrN}}{\text{Total unique count}}$$

Disease status determination

$$\text{chrN z-score for test sample} = \frac{\%\ \text{chrN}_{\text{sample}} - \text{mean}\ \%\ \text{chrN}_{\text{reference}}}{\text{S.D.}\ \%\ \text{chrN}_{\text{reference}}}$$



Universitair Ziekenhuis Brussel

NIPT technique – data analysis parameters

- **Z-score**: $\%chrN_{sample} \sim \% chrN_{reference}$
- **ZZ-score**: meta-Z-score @ genome level
  - within sample normalization of the Z scores
  - $\%chrN_{sample} \sim \% chrALL_{sample}$
- **BM-score**: Z-score @ subchromosomal level
  - overlapping 5Mb subchromosomal bins
  - $\%chrN(5Mb)_{sample} \sim \% chrN(5Mb)_{reference}$

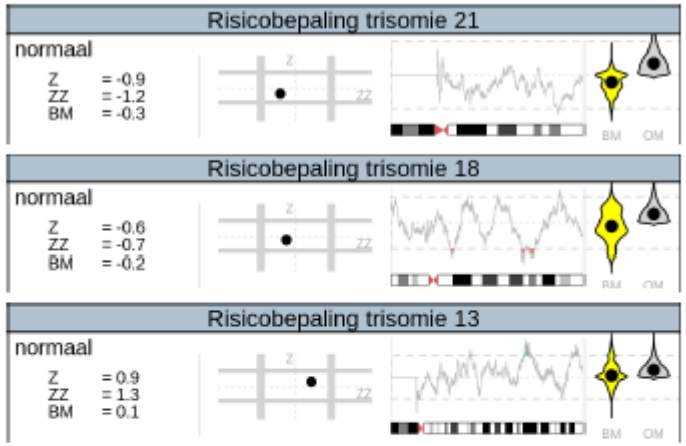
→ Gives extra power on the detection of
  - aneuploidy
  - CNV

NIPT technique: examples (1)

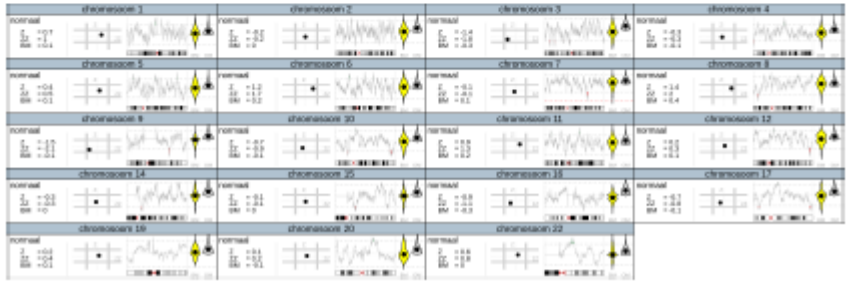
- Normal result

Resultaten niet-invasieve prenatale test (NIPT)[1,2]

Resultaat	Std dev	#Reads	#ReadsChrY	FF(ChrX)	FF(ChrY)	Geslacht
geslaagd	0.7	12665148	2854(60)	10.25%	7.41%	man



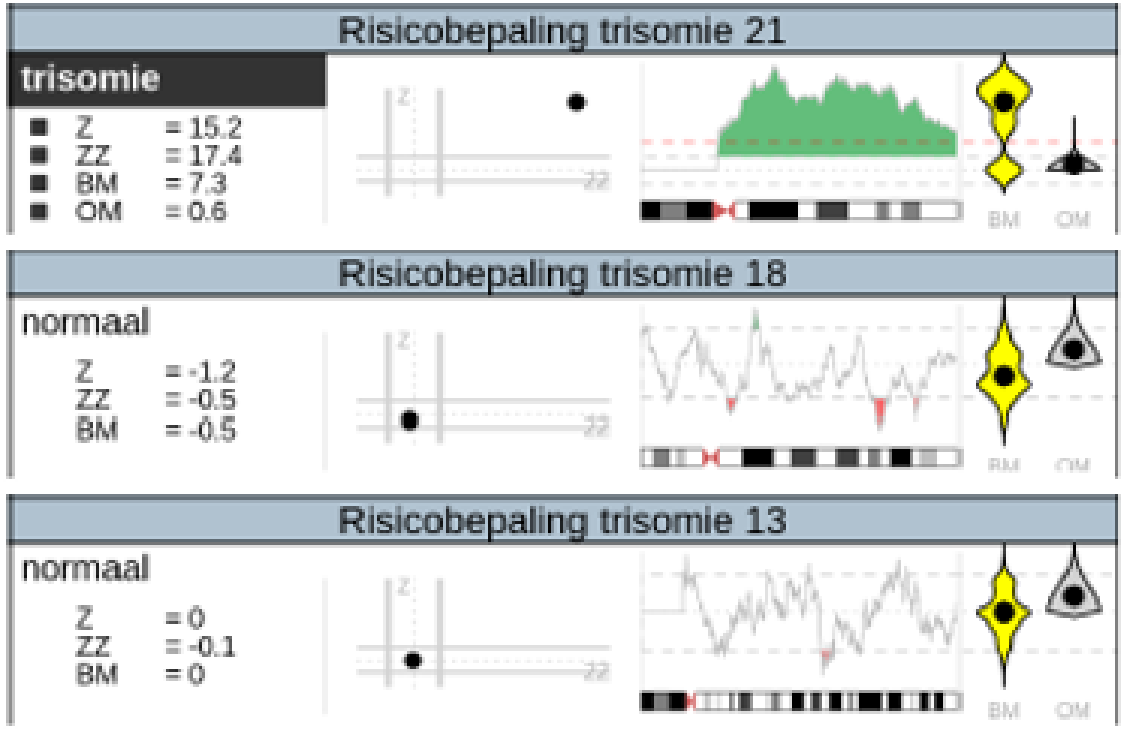
Andere autosomen



NIPT technique: examples (2)

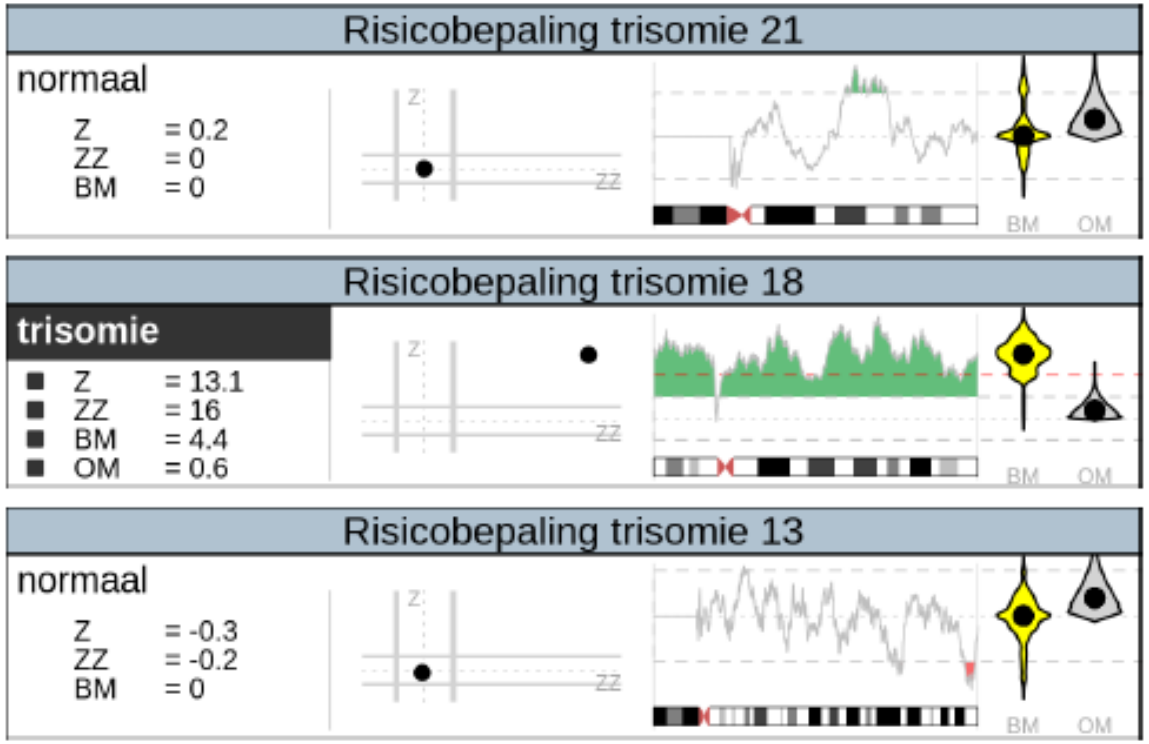
- Trisomy 21

Resultaat	Std dev	#Reads	#ReadsChrY	FF(ChrX)	FF(ChrY)	Geslacht
geslaagd	0.85	12046487	4264(104)	13.44%	12.14%	man



- Trisomy 18

Resultaat	Std dev	#Reads	#ReadsChrY	FF(ChrX)	FF(ChrY)	Geslacht
geslaagd	0.76	11368540	2776(62)	10.94%	7.57%	jongen



Universitair Ziekenhuis Brussel

Centrum voor Medische Genetica

Claimed accuracy (1)

- The sensitivity and specificity of the test vary per chromosome

  → Near 100% sensitivity and specificity

K. Van Den Bogaert et al.

Table 1. Performance of noninvasive prenatal screening (NIPS) as a first-tier screening test.

	Incidence	Sensitivity		Specificity		PPV		NPV	
	%	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Trisomy 21	0.32	98.91	97.24–99.58	99.98	99.97–99.99	92.39	89.34–94.61	100.00	99.99–100.00
Trisomy 18	0.07	97.47	91.23–99.30	99.99	99.98–99.99	84.62	75.82–90.61	100.00	100.00–100.00
Trisomy 13	0.06	100.00	90.36–100.00	99.97	99.96–99.98	43.90	33.67–54.68	100.00	100.00–100.00

CI confidence interval, *NPV* negative predictive value, *PPV* positive predictive value.

(https://doi.org/10.1038/s41436-021-01101-4)

Claimed accuracy (2)

- **Positive predictive value**, or **precision rate**, is the proportion of positive test results that are true positives (such as correct diagnoses).
- Reflects the probability that a positive test reflects the underlying condition being tested for. Its value strongly depends on the prevalence of the outcome of interest.

(https://doi.org/10.1038/s41436-021-01101-4)

Claimed accuracy (3)

K. Van Den Bogaert et al.

Table 1. Performance of noninvasive prenatal screening (NIPS) as a first-tier screening test.

	Incidence	Sensitivity		Specificity		PPV		NPV	
	%	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Trisomy 21	0.32	98.91	97.24–99.58	99.98	99.97–99.99	92.39	89.34–94.61	100.00	99.99–100.00
Trisomy 18	0.07	97.47	91.23–99.30	99.99	99.98–99.99	84.62	75.82–90.61	100.00	100.00–100.00
Trisomy 13	0.06	100.00	90.36–100.00	99.97	99.96–99.98	43.90	33.67–54.68	100.00	100.00–100.00

CI confidence interval, *NPV* negative predictive value, *PPV* positive predictive value.

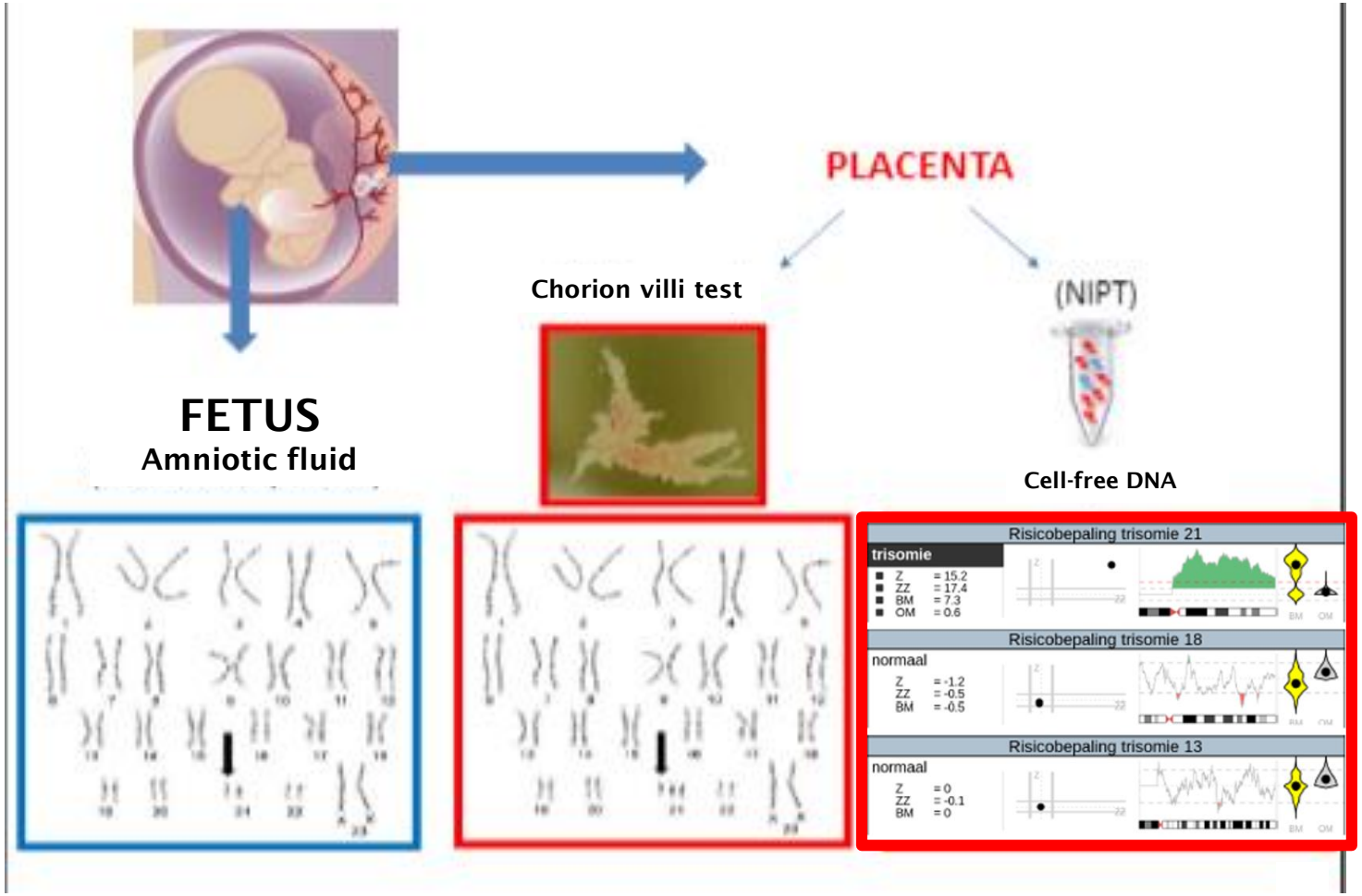
(https://doi.org/10.1038/s41436-021-01101-4)

Positive Predictive Value < 100%

- NIPT needs to be confirmed by a diagnostic prenatal invasive method, preferable amniotic fluid (AC).

Why?

Placental mosaicism



Overview

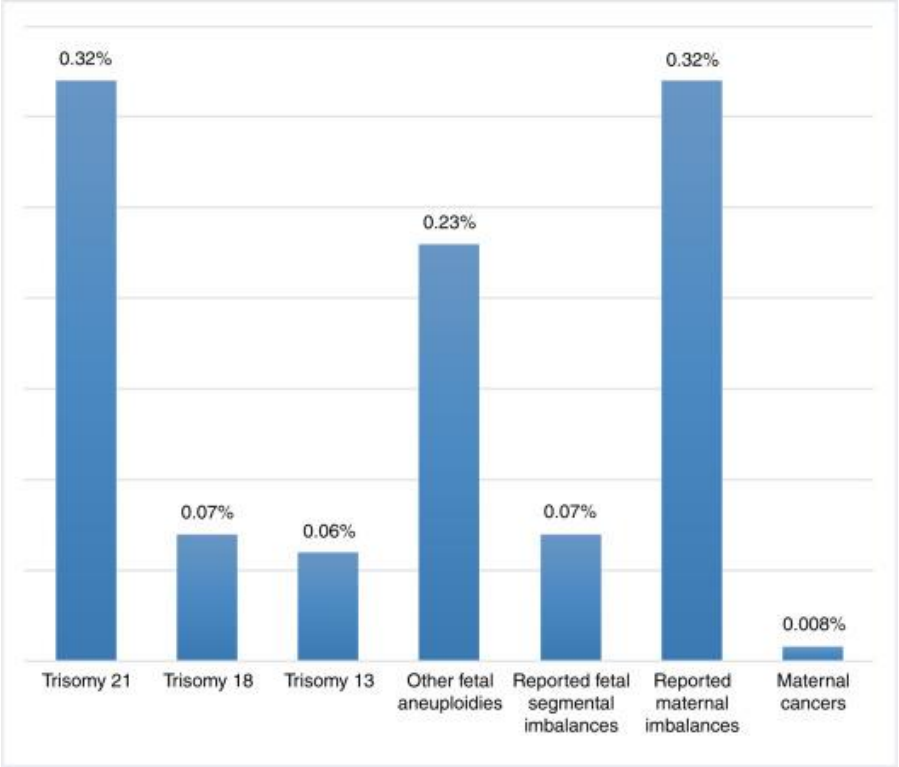
- Detection of cell-free DNA
- NIPT technique
- Indication and limitations
- Reporting policy

Indication and limitations

- First-tier screening test for T13, 18, 21
  - → early, accurately and safely
    - First trimester, no miscarriage risk
  - → NIPT is a better screening than first trimester combined screening using US and biochemical markers
    - Higher sensitivity and specificity
    - Result is independent from US findings
  - → Since 2017 reimbursed for all pregnant women (Belgium)

Indication and limitations

- RATs
- SCAs (Klinefelter syndrome, Turner syndrome)
- CNVs (maternal, fetal)



Percentage of pregnancies with a fetal or maternal imbalance from first-tier genome-wide NIPS.

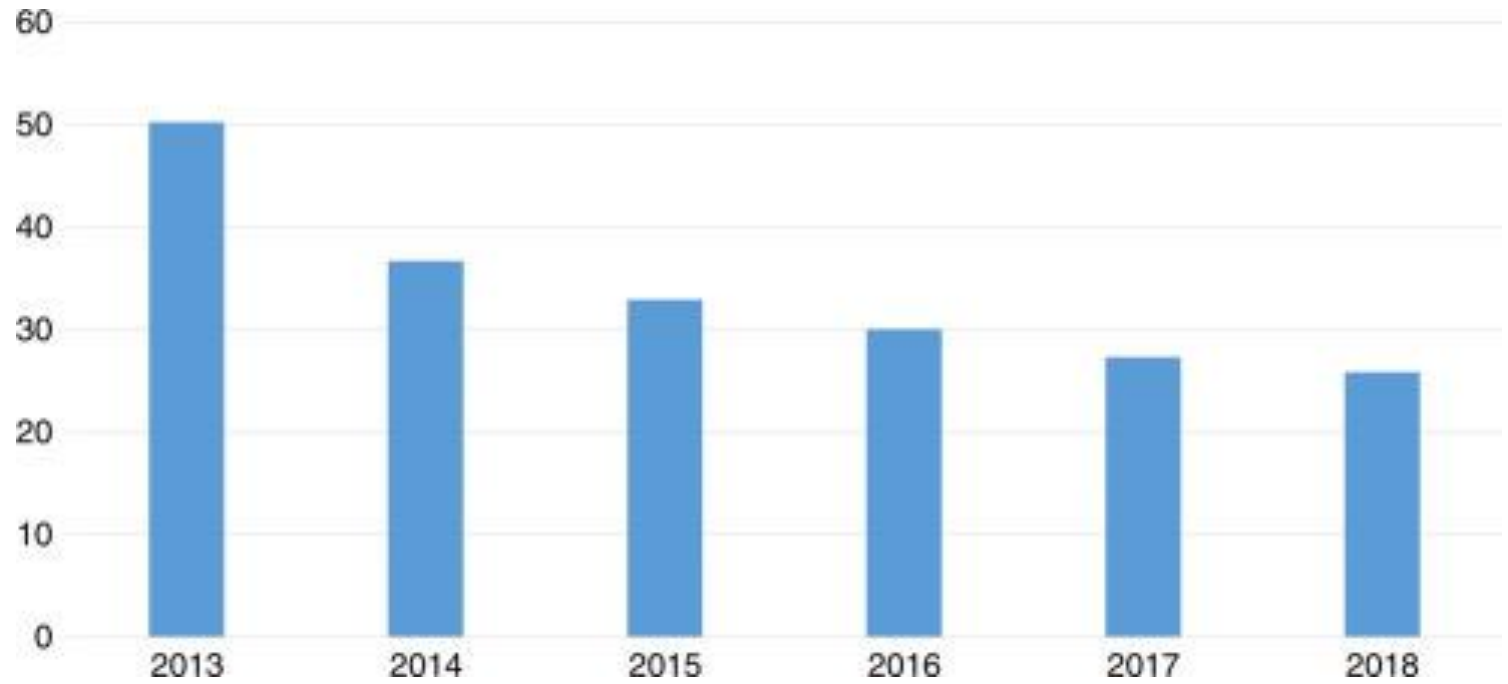
Van Den Bogaert et al., 2021

Universitair Ziekenhuis Brussel

Centrum voor Medische Genetica

Indication and limitations

- Reduction in the number of invasive test



Number of invasive procedures normalized per 1,000 live births performed from 2013 to 2018 in Belgium (RIZIV-INAMI registration).

Van Den Bogaert et al., 2021

NIPT limitations (1)

- Limitations
  - → US remains crucial and preferably performed before NIPT (twins, malformations, etc.)
  - → Maternal BMI (fetal fraction), heparin therapy
  - → Parent with chromosomal anomaly
  - → Placental mosaïcism
  - → Vanishing twin
  - → Transplantation, blood transfusion

NIPT limitations (2)

- NIPT will fail on:
  - → balanced translocations
  - → polyploidy (e.g. triploidy)
- NIPT might fail on:
  - → unbalanced translocations
  - → sub chromosomal aberrations
  - →(placental) mosaicism
  - → maternal chromosomal abnormalities, …
- NIPT might not detect:
  - → micro-deletions or –duplications
  - → mosaic chromosomal aberrations
- NIPT is not able to detect monogenic abnormalities

Universitair Ziekenhuis Brussel

Centrum voor Medische Genetica

Overview

- Detection of cell-free DNA
- NIPT technique
- Indication and limitations
- Reporting policy

Reporting guidelines BeSHG

- NIPT good clinical practice
  - → **first tier prenatal screening tool** for fetal trisomy 13, 18 and 21.
  - → **pre-test counselling** with information about the different screening options and their possibilities and limitations is required.
  - → NIPT does not replace the first trimester **fetal ultrasound** for measurement of the nuchal translucency (NT) and identification of fetal malformations
  - → Referral of a patient with a positive NIPT for **invasive prenatal diagnosis** by amniocentesis is necessary.
  - → **Fetal fraction** as a standard quality control parameter that is taken into account while interpreting all NIPT results.

https://www.college-genetics.be/

Universitair Ziekenhuis Brussel

Centrum voor Medische Genetica

Reporting guidelines BeSHG

- Incidental findings (fetal vs. maternal)

In reporting incidental findings, emphasis should be on the risk on causing potentially **serious harm for maternal or fetal health** when this finding is not reported. Therefore, incidental findings should only be reported if (i) they are considered **technically valid**, (ii) there is validated evidence on the **associated phenotype** and (iii) they are considered **clinically relevant and actionable.**

→ **Fetal: autosomal aneuploidies, subchromosomal aneuploidies**

→ **Maternal: pathogenic CNVs, CNVs causing late-onset genetic disorders, effect on future pregnancy, malignancy**

https://www.college-genetics.be/

Universitair Ziekenhuis Brussel

Centrum voor Medische Genetica