Tranquility

Setting new standards of healthcare

Vuk Devrnja
International Medical Officer | Genoma

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Non-Invasive Prenatal Testing (NIPT)

Noninvasive prenatal testing (NIPT) is a screening method for detecting certain specific chromosomal abnormalities in a developing baby.

Noninvasive prenatal testing is a sophisticated blood test that examines fetal DNA in the maternal bloodstream to determine whether a baby is at risk of Down syndrome, extra sequences of chromosome 13 (trisomy 13), extra sequences of chromosome 18 (trisomy 18) or a sex chromosome abnormality, such as Turner syndrome.

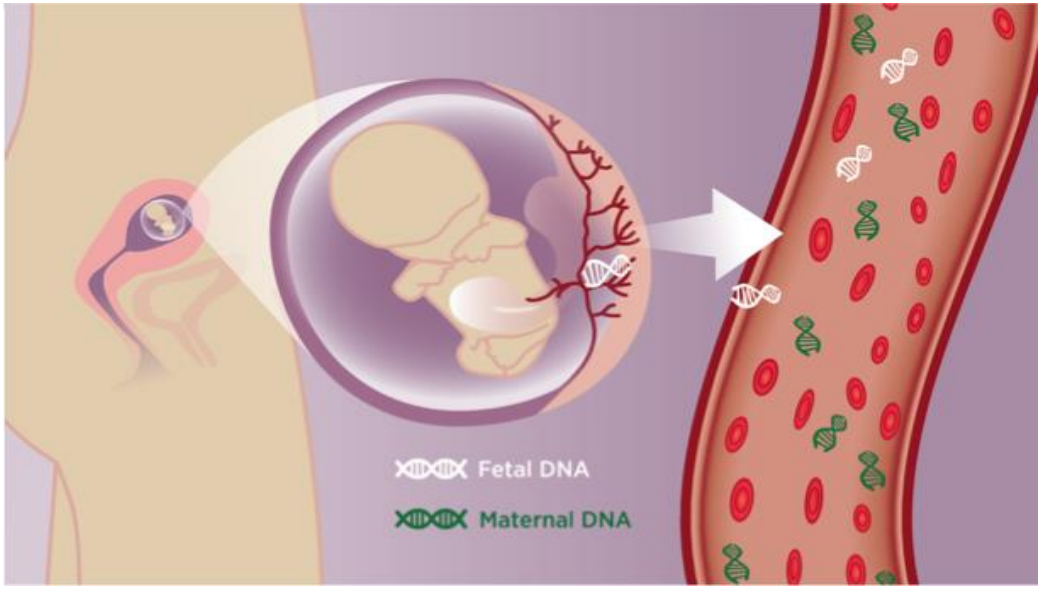
Non-Invasive Prenatal Testing (NIPT)

Principle of NIPT:

< 1 % of total DNA in maternal circulation is fetal

5-30 % of cell-free DNA in maternal blood stream is fetal

NIPT utilises these in order to determine potential chromosomal abnormalities without exposing the mother or child to risk of miscarriage of invasive diagnostic techniques.



Prenatal testing methods not based on DNA

Combined first-trimester testing (Triple Test):

Blood test – weeks 9-13

Ultrasound – weeks 11-12 of gestation

Triple test markers

Marker: HCG (blood test)
Full name: beta-human chorionic gonadotropin
Origin: Thophoblast
T21: (↑MoM: 1,8)

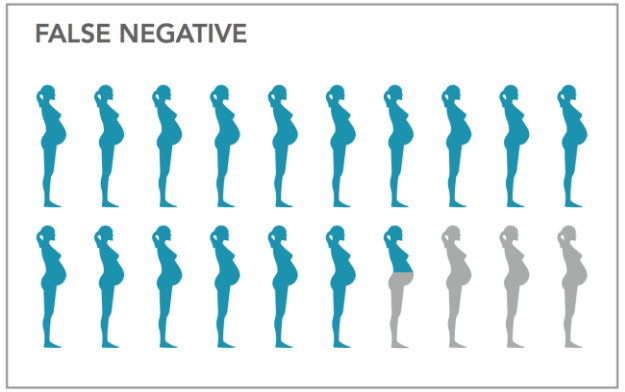
Marker: PAPP-A (blood test)
Full name: Pregnancy-associatedplasma protein A
Origin: Thophoblast and decidualized endometrial stroma cells
T21: (↓MoM: 0,4)

Marker: NT (ultrasound)
Full name: Nuchal translucenty thickness
T21: (↑MoM: 2-2,5)

Factors influencing Triple Test:

Gestational age, maternal weight, multiple pregnancy, IDDM, gravidity/parity, previous pregnancy

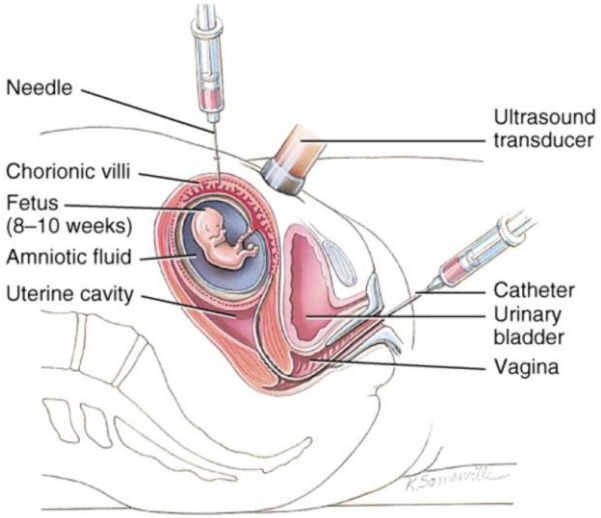
19 out of 20 triple test positive results are false and lead to unnecessary amniocentesis



3-4 out of 20 Down syndrome cases are not detected via triple test

Invasive testing based on DNA

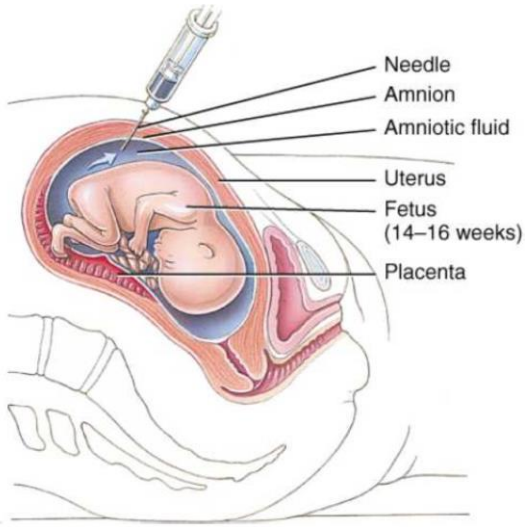
Chorion Villus Sampling



Performed after 10-13 weeks of gestation

Risks:
Vaginal bleeding
Amniotic fluid leakage
Infections
Chorio-amnionitis
Fetal loss: 0.5%

Amniocentesis



Performed after 14 weeks of gestation

Risks:
Transient vaginal spotting: 1-2%
Amniotic fluid leakage: 1-2%
Chorio-amnionitis: < 0.1%
Needle injuries to the fetus (rare when ultrasonographic guidance is used)
Fetal loss: 0.5-1%

Analysis method

Cytogenetics:

- **Karyotype**
- **Fluorescence in Sito Hybridation (FISH)**

Molecular:

- **Quantitative Fluorescence - Polymerase chain reaction (QF-PCR)**
- **Multiplex Ligation-Dependent Probe Amplification (MLPA)**
- **Comparative Genomic Hybridization (aCGH) and Single Nucleotide Polymorphism (SNP array)**

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Aneuploidies screening objectives

- ✓ Increased sensitivity
- ✓ Reduced fetal invasion
- ✓ Fewer fetal losses
- ✓ Reduce the anxiety of the couple
- ✓ Early
- ✓ Lower cost

Tranquility® - CE-IVD certified

- Tranquility is a non-invasive test of fetal DNA with CE-IVD certification for trisomies 21, 18 and 13 (also allows fetal sex determination).

- The entire process connected to trisomies 21, 18 and 13 (collection, preparation, sequencing, bioinformatic analysis and report) are in accordance with:

In Vitro Diagnostics Directive (98/79/EC)

Tranquility – Technology and expertise

Tranquility is based on whole genome sequencing, using Next Generation Sequencing (NGS) platforms.

Test is performed in the Genoma Swiss Biotechnology laboratories in Geneva.

This is the largest clinical genetics laboratory in Europe.

Tranquility – availability

- Tranquility can be performed
  any day of the week,
  365 days a year
  10th gestational week in singleton pregnancies
  12th gestational week in multigestational pregnancies

- Results are available **5 working days** after sample arrival to the laboratory

What is detected?

Chromosomal Aneuploidies	Gender
Trisomy 21	Male
Trisomy 18	Female
Trisomy 13	
Microdeletions	**Sex Chromosome Disorders**
Angelman Syndrome	Triple X Syndrome (XXX)
Cri Du Chat Syndrome	Jacobs Syndrome (XYY)
DiGeorge Syndrome	Klinefelter Syndrome (XXY)
Prader-Willi Syndrome	Turner Syndrome (45 X)
2q33.1 deletion Syndrome	

Microdeletion syndromes

Microdeletion syndrome	location	Size deleted	Incidence	Clinical features
DiGeorge syndrome	22q11.2 deletion	>3Mb (~85% of cases)	1/4,000	• Variable intellectual disability, • Heart defects (74% of individuals), • Palatal abnormalities • Immune deficiency • Normal lifespan.
Cri-du-chat Syndrome (5p-)	5p15.2	3.2 Mb – 30 Mb	1 in 20,000-50,000	• Significant intellectual disability, • Cat-like cry, dysmorphic features • 10% mortality in first year
Prader-Willi Syndrome	15q11.2-q13	5-6Mb	1 in 10,000-25,000	• Severe hypotonia • Feeding difficulties in early infancy, followed by excessive eating leading to morbid obesity. • Developmental delay • Hypogonadism, short stature, characteristic facial features
Angelman Syndrome	15q11.2-q13	5-6Mb	1 in 12,000	• Intellectual disability & developmental delay • Speech impairment, • Ataxia, seizures, dysmorphic features • Normal lifespan

Microdeletion detection challenges

Resolution of the analysis is determined by the number of reads:

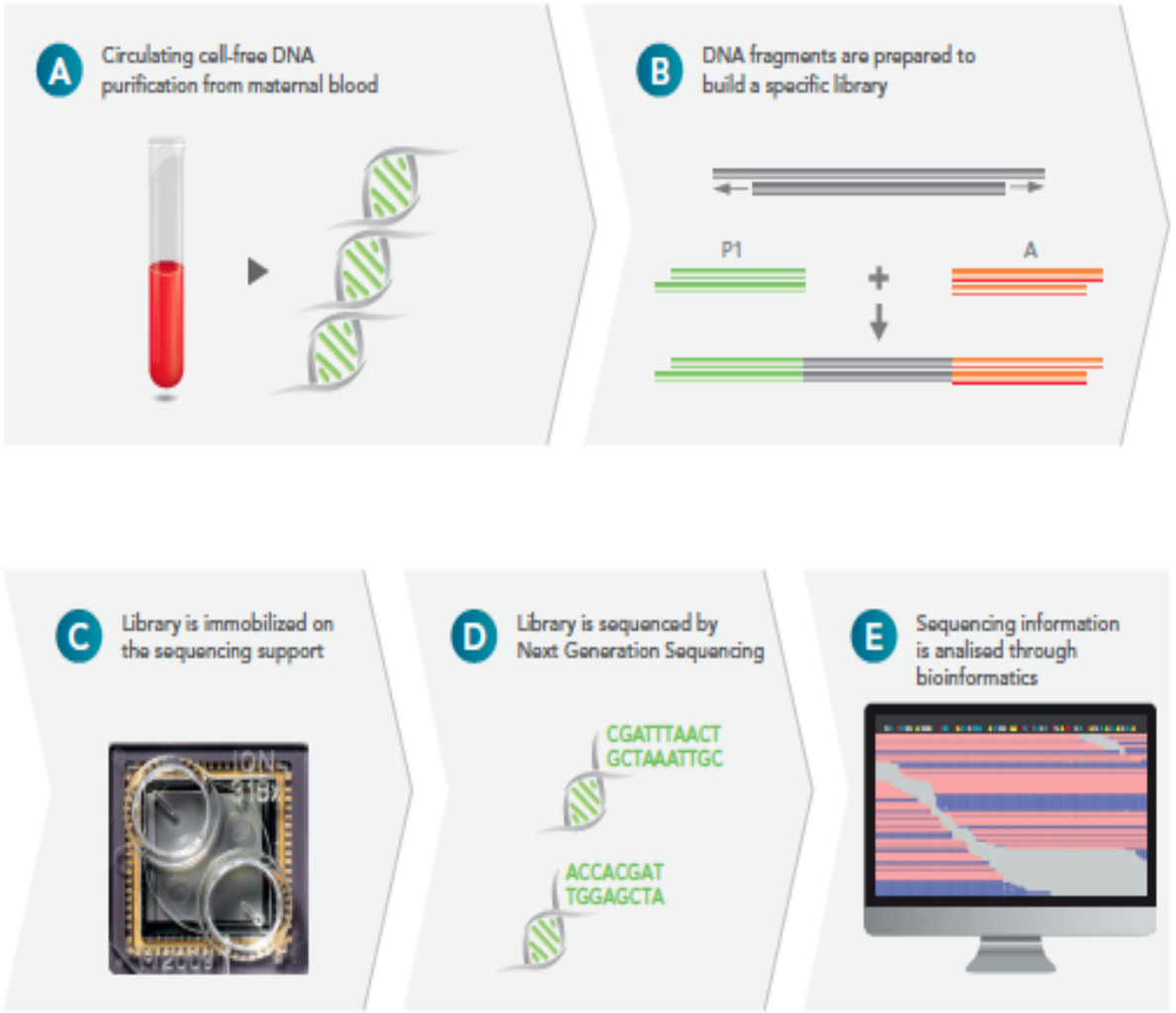
Uniquely mapped reads : longer reads have more chances to be unique

Resolution of 3 Mb would require 150M reads
Resolution of 2 Mb would require 192M reads
Resolution of 1 Mb would require 380M reads

-> too expensive for a screening test

Range of currently implemented tests: *from 2M to 25M*

Tranquility - process



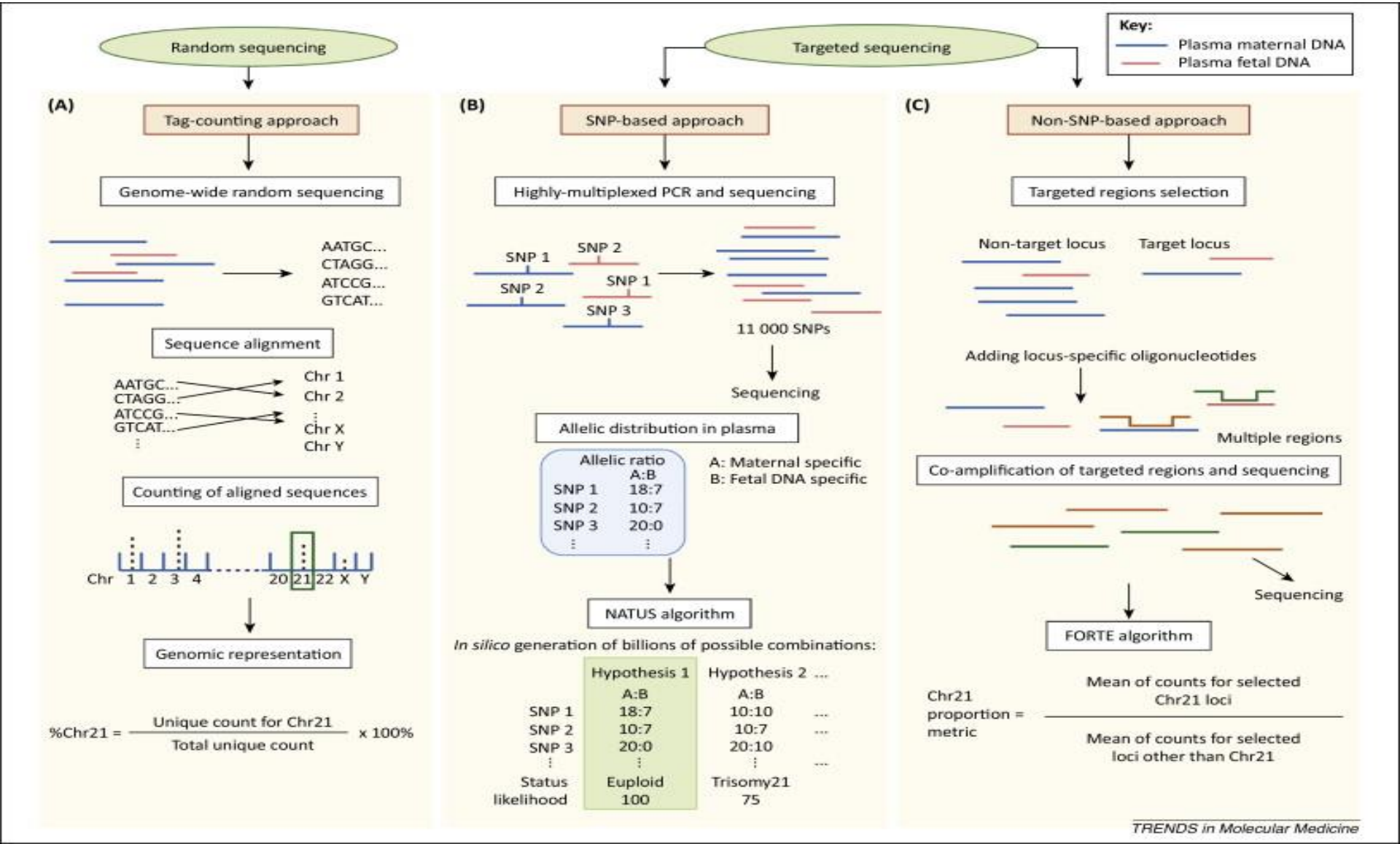
Testing process

Tranquility utilises Next Generation Sequencing (NGS): Innovative high tech method for genome sequencing, which can be used to detect chromosomal abnormalities.

In principle, bases of small fragment of DNA are sequentially identified from signals emitted as each fragment is re-synthesised from a DNA template strand.

NGS extends across millions of reactions in a massively parallel fashion, rather than being limited to a single or a few DNA fragments. This advance enables sequencing of large stretches of DNA base pairs spanning entire genomes, with instruments capable of producing hundreds of gigabases of data in a single sequencing run.

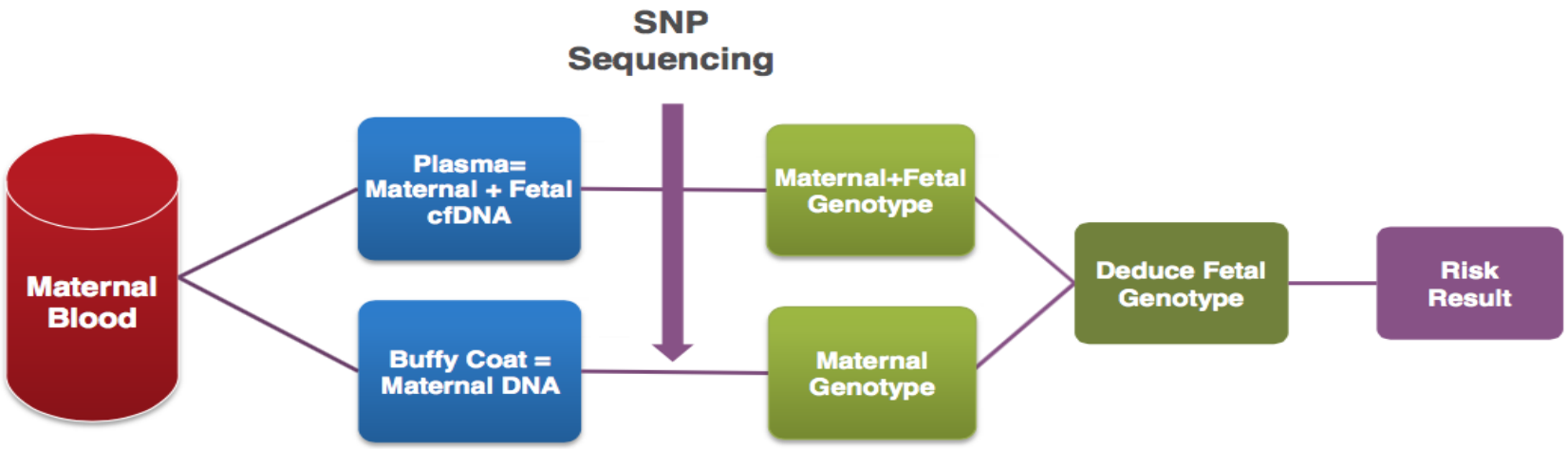
Technology comparisson



Technology comparisson

Targeted MPS (SNP-Based Method)

Complex, failure-prone method



- **Drawbacks**
  - High Assay Failure Rates
  - Slower, more complex workflow (slower TAT)
  - Difficult to analyze egg-donation, multiple gestation samples

Technology comparison

Genome-wide MPS (Tranquility)

- Low Assay failure rates
- Faster analysis times
- Ability to add new content to test menu

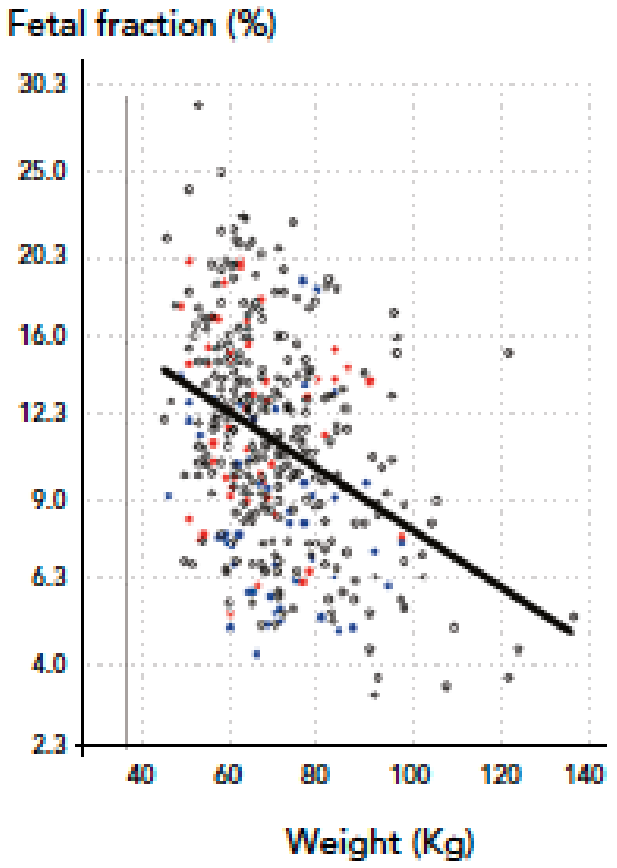
FetalDNA fraction- factors

Maternal weight

The amount of maternal cell-free DNA increases with an increase in body weight. Increased maternal weight may prevent a satisfiable isolation of cell-free-fetal DNA

Maternal health

Certain health conditions, such as infections, cancer or other conditions that may require blood transfusion, also may prevent satisfiable isolation of cell-free-fetal DNA.



Tranquility technical workflow

Bioinformatic analysis and report generation – InKaryo biotechnology

- Data imported automatically from Ion Proton and process on a CE-IVD certified software
- Calculation of risk of affected or unaffected pregnancy
- Process takes 5-7 minutes per sample
- A validity check based on sequencing data quality, fetal fraction estimate, number of sequencing reads is performed to ensure the validity of results

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Circulating Cell-free DNA Sequencing Report

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Advanced bioinformatics

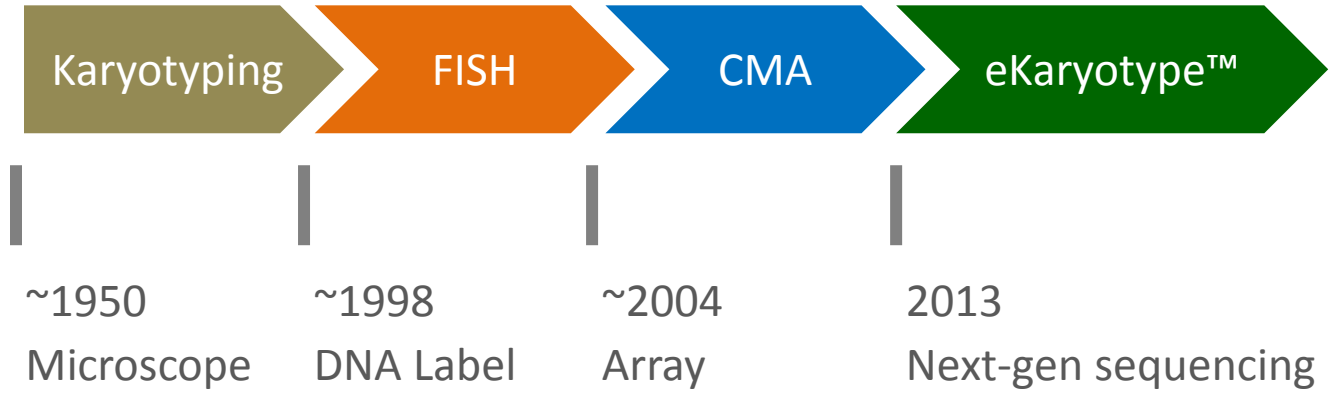
An advanced algorithm is utilised for analysing sequenced samples, evaluating the chromosome numbers, and other characteristics.

The most advanced bioinformatics platform used for analysis is called InKaryo and is the property of Genoma Swiss Biotechnology.

Calculating the fetal fraction and incorporating it into the bioinformatics algorithm allows Tranquility the highest levels of analytical performance as well as reliability and reproducibility.

Evolution of Cytogenetic Testing

- Highlights in e-karyotyping development



- From analog to digital

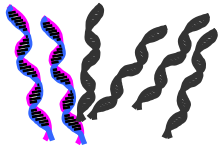
- NGS performance exceeds that of Chromosomal MicroArrays (CMA)

- Cost inflection point is occurring now

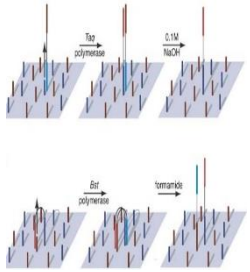
InKaryo eKaryotype workflow

Karyotype Specific Massive Parallel Shotgun Sequencing (KS-MPSS)

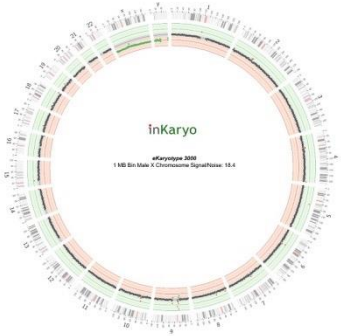
Sample Requisition



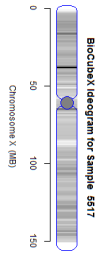
Library Preparation



Shotgun Sequencing



Bioinformatics



eKaryotyping

Proprietary Bioinformatics

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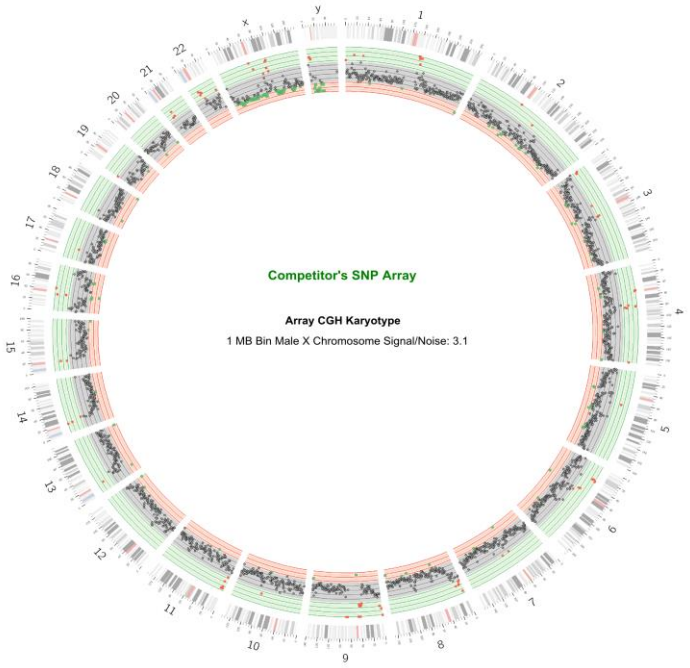
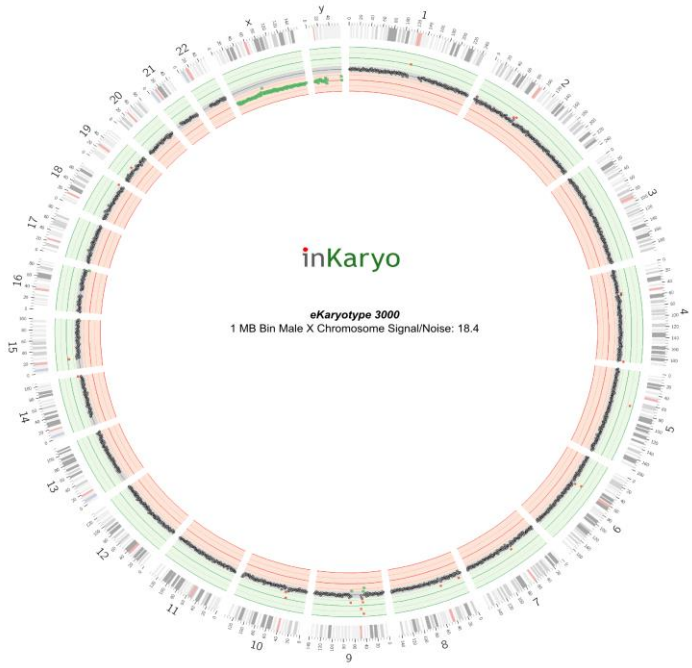
eKaryotype





CGH Array Karyotype:
The width of the central grey band represents the noise for quantifyi
2 copies of chromosome

InKaryo eKaryotype:
The central grey band almost becomes a line, indicating significant noise reduction in quantifying the 2 copies of chromosome bins.

Results

Tranquility™

Circulating Cell-free DNA Sequencing Report

Tranquility is a non-invasive prenatal testing that screens the genome of the fetus to detect trisomies 21, 18, 13, sex chromosome numerical aberrations (XO, XXX, XXY, XYY), microdeletions and that determines the sex of the fetus. It specifically analyses the circulating cell-free DNA (ccfDNA) that is present in maternal blood and which is made of DNA originating from maternal and fetal tissues in variable proportion. The fetal fraction which corresponds to the portion of fetal DNA, is experimentally measured in every sample to ensure that there is enough fetal DNA to perform a reliable analysis.

Patient information

Name: KEKSA Marina
Date of Birth: 14 March 1994
Email: kaksa.marina@yopmail.com
Phone: +33658415896

Address: 24 Rue de la grave d'Allan
69912 Tintamare
Country: Italy
Patient ID: AC1468841

Doctor information

Name: DEREIG Paul
Email: paul.dereig@yopmail.com
Phone: +33456865847

Address: 17 Rue des avalanches 74123 Nice
Country:

Data from patient

Type of pregnancy: Singleton pregnancy
Last menstrual period: 05 July 2015
Expected delivery date: 14 May 2016
Gestational age* (WW+D): 12+3
Method used to measure gestational age: Menstrual

Patient weight* (Kg): 68
Patient height (Cm): 164
In-vitro fertilization: No
Reason for performing Tranquility:

Analysis performed

- Chromosomes 21, 18 and 13 analysis
- Sex Chromosome analysis
- Microdeletions detection
- Sex of the fetus identification

Geneticist signature

Geneticist: Patrick Perrin, MD, FAMH, GLN 7601000867252

Genoma Laboratory Genoma S.A., Chemin des Aulx, 12, 1228 Plan-les-Ouates, Geneva, Switzerland, +41 58 1000 100
Laboratory Registered in Switzerland under the number 7601002104828
Directors: Thomas Pilo Pilo, Ph.D., Xiberg A, Ph.D

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Results

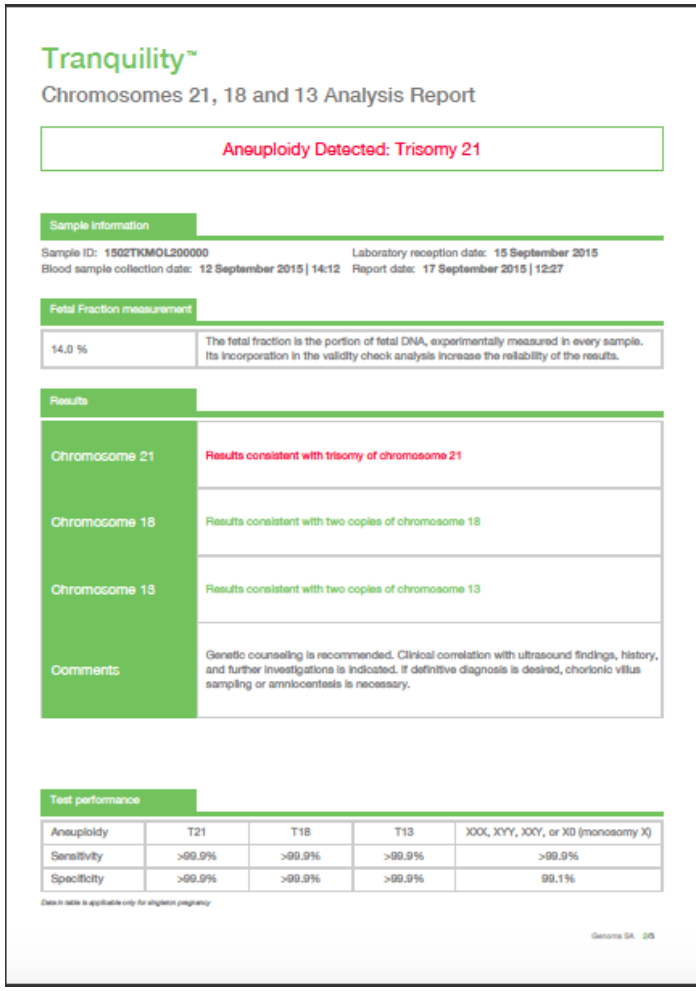
Results of Tranquility present the absence of risk that the fetus carries a chromosomal aneuploidy or microdeletion. If requested sex determination will be performed as well.

In twin pregnancies Tranquility will determine the risk of one of the feti having a trisomy 21, 18 or 13. If requested. Presence of Y chromosome will be determined.

Chromosome 21, 18, 13 (CE-IVD):

- “Aneuploidy not detected”

- “Aneuploidy detected”



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Chromosomes 21, 18 and 13 Analysis Report

Aneuploidy Detected: Trisomy 21

Sample information

Sample ID: 1502TKMOL200000
Blood sample collection date: 12 September 2015 | 14:12
Laboratory reception date: 15 September 2015
Report date: 17 September 2015 | 12:27

Fetal Fraction measurement

14.0 %	The fetal fraction is the portion of fetal DNA, experimentally measured in every sample. Its incorporation in the validity check analysis increase the reliability of the results.

Results

Chromosome 21	Results consistent with trisomy of chromosome 21
Chromosome 18	Results consistent with two copies of chromosome 18
Chromosome 13	Results consistent with two copies of chromosome 13
Comments	Genetic counselling is recommended. Clinical correlation with ultrasound findings, history, and further investigations is indicated. If definitive diagnosis is desired, chorionic villus sampling or amniocentesis is necessary.

Test performance

Aneuploidy	T21	T18	T13	XXX, XYY, XXY, or X0 (monosomy X)
Sensitivity	>99.9%	>99.9%	>99.9%	>99.9%
Specificity	>99.9%	>99.9%	>99.9%	99.1%

Data in table is applicable only for singleton pregnancy

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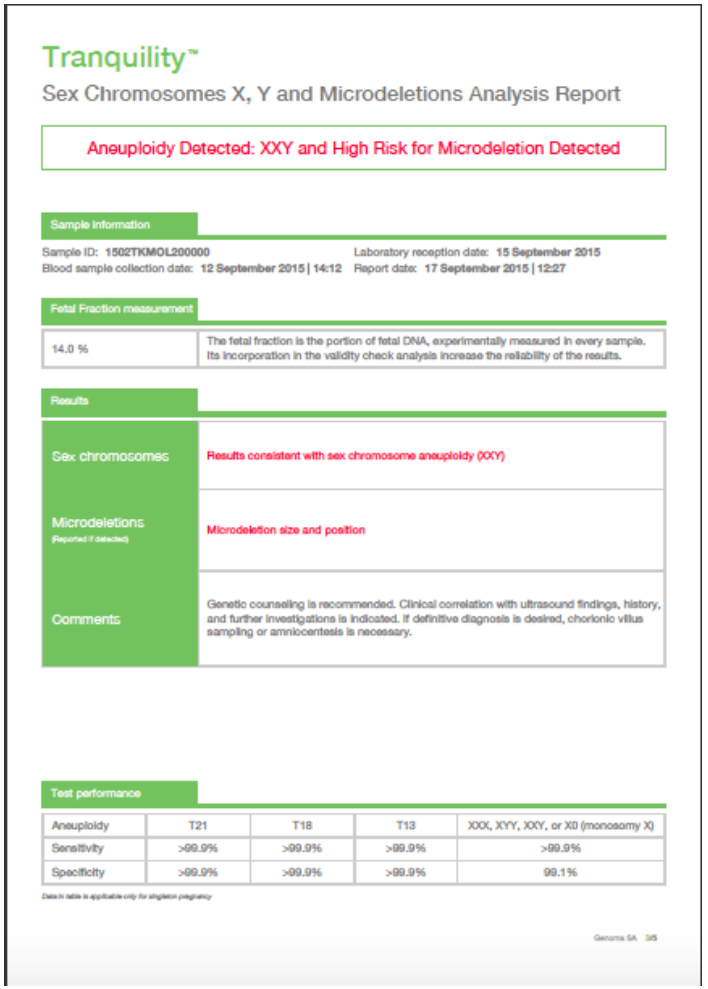
Results

Sex chromosome aneuploidies:

- "Results consistent with two sex chromosomes" – based on country
- "Result consistent with sex chromosome aneuploidy"
- Aneuploidy detected will be listed as: XXY, XXX, X, XYY.

Microdeletions:

"Detected" - one or more microdeletions has been detected. The report will contain the genomic position and size of microdeletion on a chromosome. If the microdeletion has previously been associated with the specific syndrome, such designation will be provided.



Tranquility™

Sex Chromosomes X, Y and Microdeletions Analysis Report

Aneuploidy Detected: XXY and High Risk for Microdeletion Detected

Sample information

Sample ID: 1502TKMOL200000 — Laboratory reception date: 15 September 2015
Blood sample collection date: 12 September 2015 | 14:12 — Report date: 17 September 2015 | 12:27

Fetal Fraction measurement

14.0 %	The fetal fraction is the portion of fetal DNA, experimentally measured in every sample. Its incorporation in the validity check analysis increase the reliability of the results.

Results

Sex chromosomes	Results consistent with sex chromosome aneuploidy (XXY)
Microdeletions (Reported if detected)	Microdeletion size and position
Comments	Genetic counseling is recommended. Clinical correlation with ultrasound findings, history, and further investigations is indicated. If definitive diagnosis is desired, chorionic villus sampling or amniocentesis is necessary.

Test performance

Aneuploidy	T21	T18	T13	XXX, XYY, XXY, or X0 (monosomy X)
Sensitivity	>99.9%	>99.9%	>99.9%	>99.9%
Specificity	>99.9%	>99.9%	>99.9%	99.1%

Data in table is applicable only for singleton pregnancy

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