Use of bioinformatics in Reproductive Sciences

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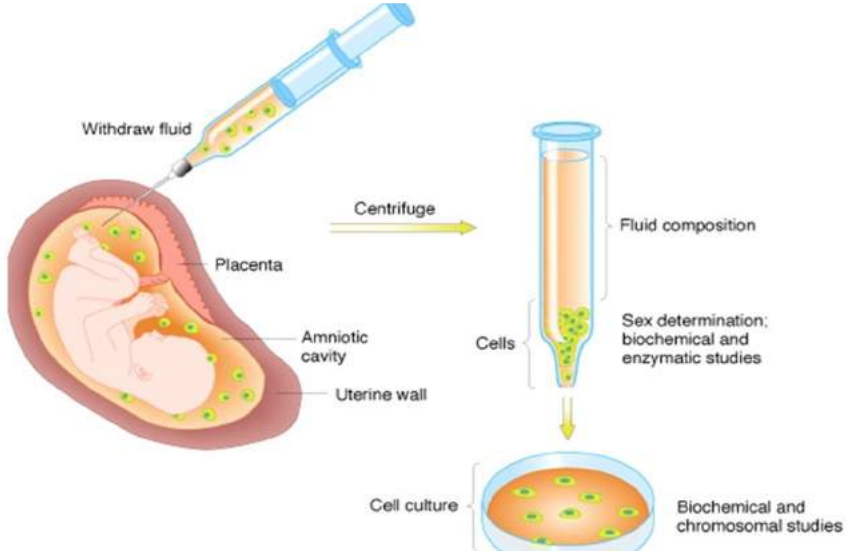
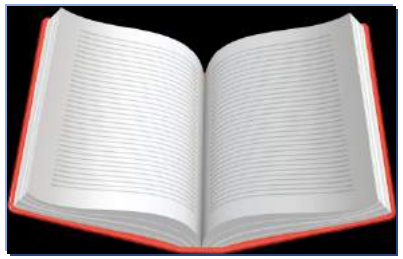
What is prenatal testing and why do it?

- Prenatal diagnosis of the common aneuploidies and monogenic disorders has been offered to pregnant women, initially to high-risk women
  - Advanced maternal age
  - Child with congenital anomalies or dysmorphology
  - Single gene disorder/ structural chromosamal abnormality
  - Consanguinity
- Invasive techniques:
  - CVS
  - Amniocentesis
  - Cordocentesis



Types of genetic disorders

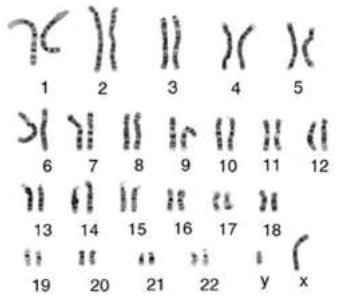


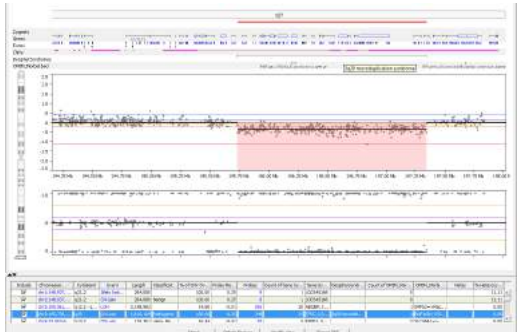
Our genome

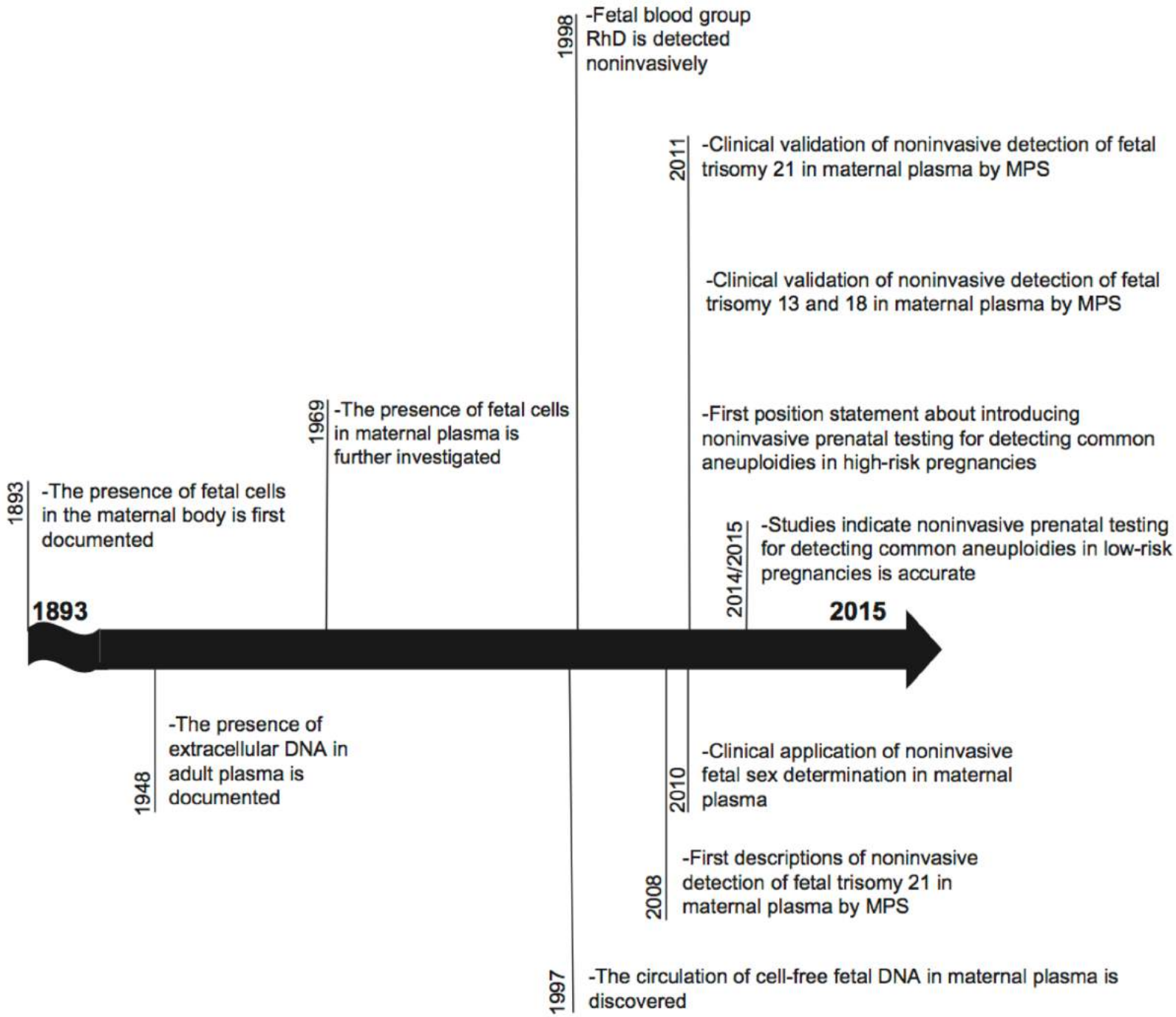
Chapter:
Chromosomal
Karyotype

Sentence:
Microdeletions/
microduplications
Chromosomal microarray

Letter:
Single gene mutations
Gene sequencing

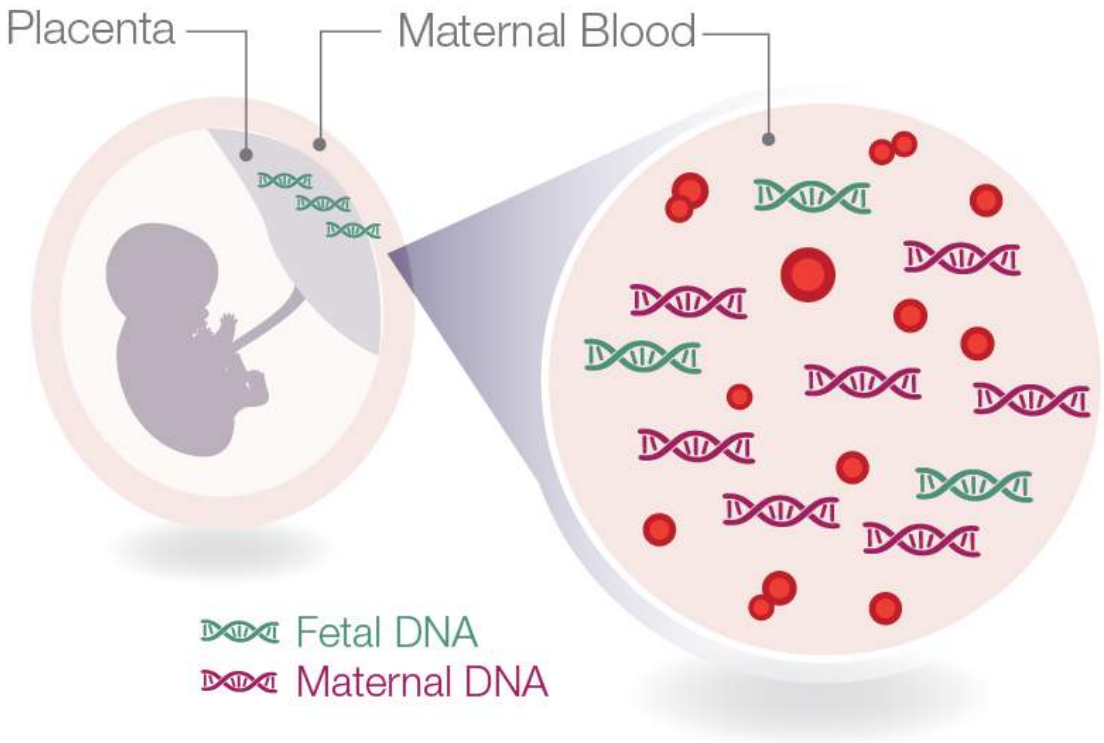




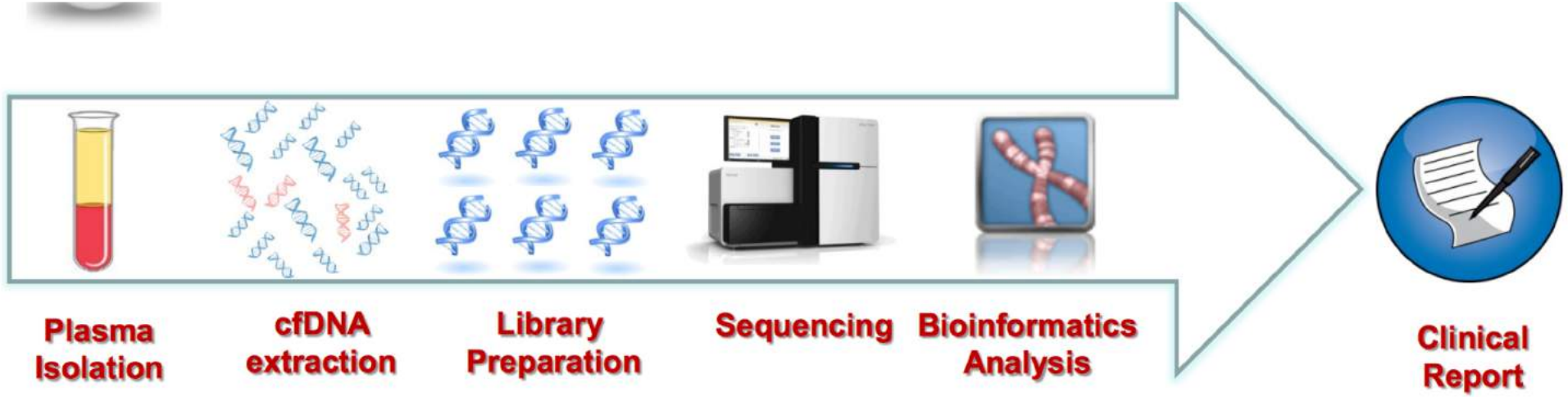

1893
-The presence of fetal cells in the maternal body is first documented
1893
1948
-The presence of extracellular DNA in adult plasma is documented
1969
-The presence of fetal cells in maternal plasma is further investigated
1997
-The circulation of cell-free fetal DNA in maternal plasma is discovered
1998
-Fetal blood group RhD is detected noninvasively
2008
-First descriptions of noninvasive detection of fetal trisomy 21 in maternal plasma by MPS
2010
-Clinical application of noninvasive fetal sex determination in maternal plasma
2011
-Clinical validation of noninvasive detection of fetal trisomy 21 in maternal plasma by MPS
-Clinical validation of noninvasive detection of fetal trisomy 13 and 18 in maternal plasma by MPS
-First position statement about introducing noninvasive prenatal testing for detecting common aneuploidies in high-risk pregnancies
2014/2015
-Studies indicate noninvasive prenatal testing for detecting common aneuploidies in low-risk pregnancies is accurate
2015

Cell free DNA (cfDNA)

- Released through apoptosis
- **Fetal**
  - Placental cells (trophoblasts) in the maternal circulation
  - Released into bloodstream as small DNA fragments (150–200 bp)
- Maternal blood contains both fetal, maternal cfDNA
- Fetal cfDNA reliably detected after 7+ weeks gestation



Non-Invasive prenatal testing



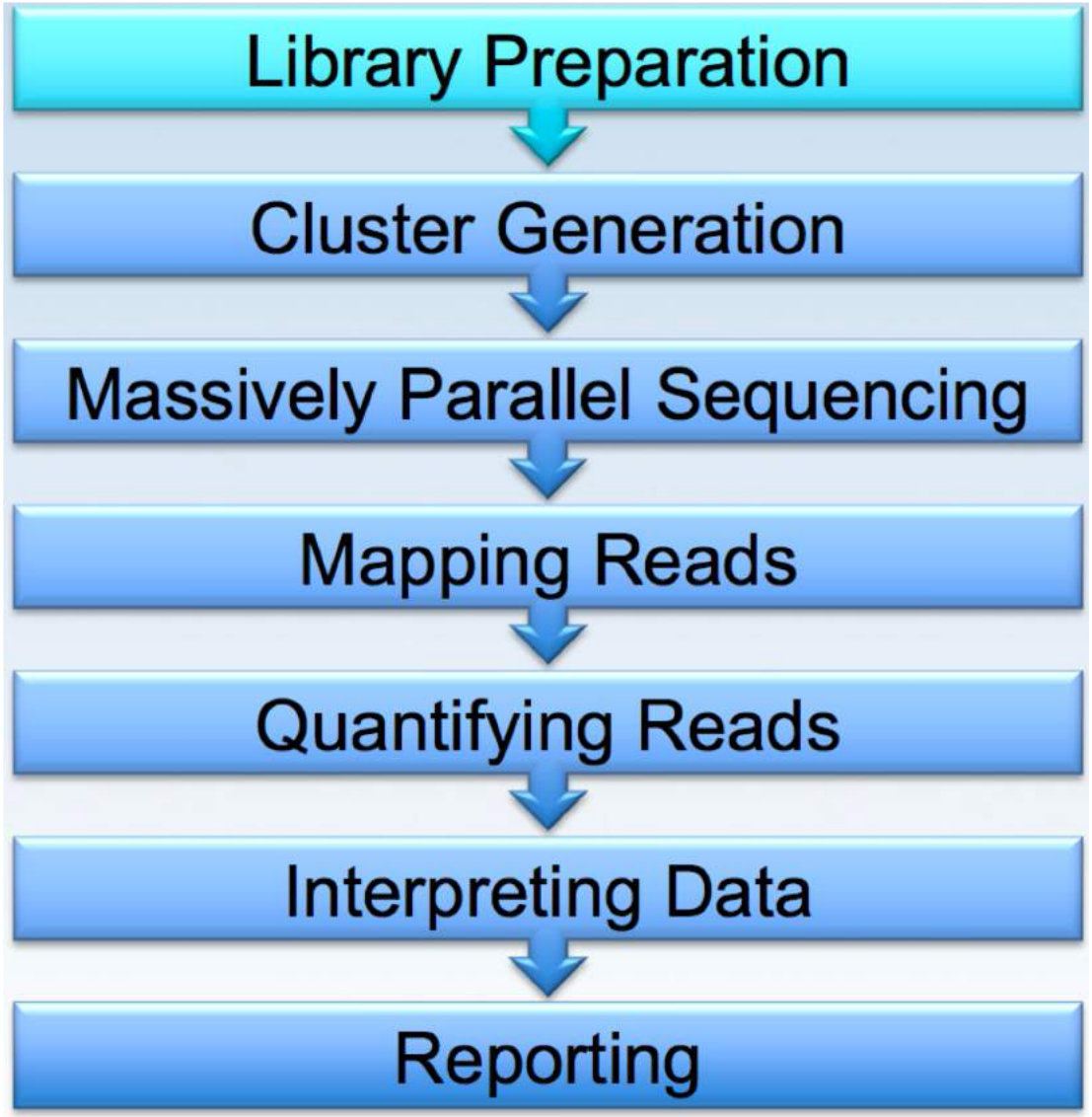
Assay Quality

– Lowers the limit of detection (LOD)
– Based on sequencing methodology and analysis method

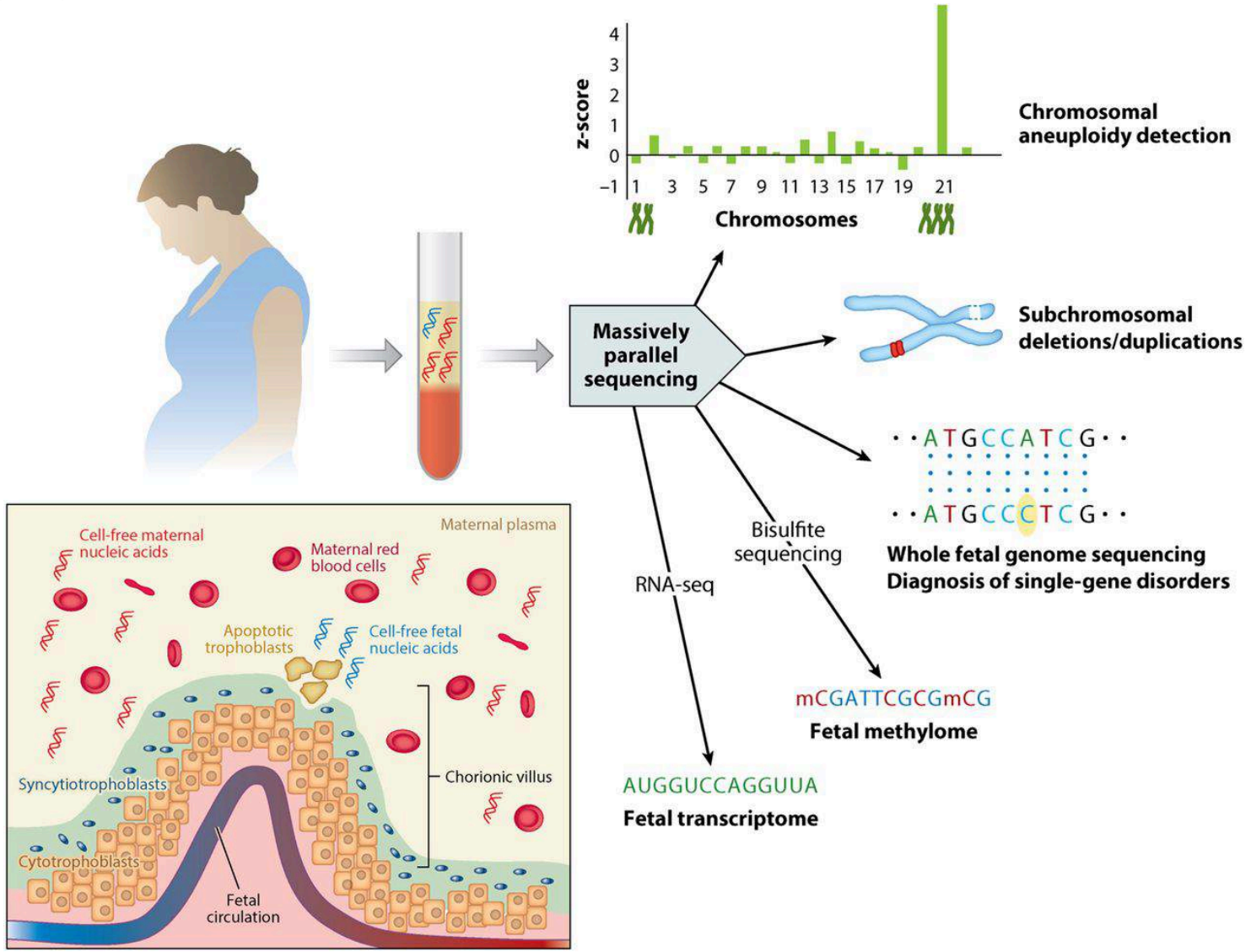
Fetal Fraction

– Lower fetal fraction demands a lower LOD

NIPT technology



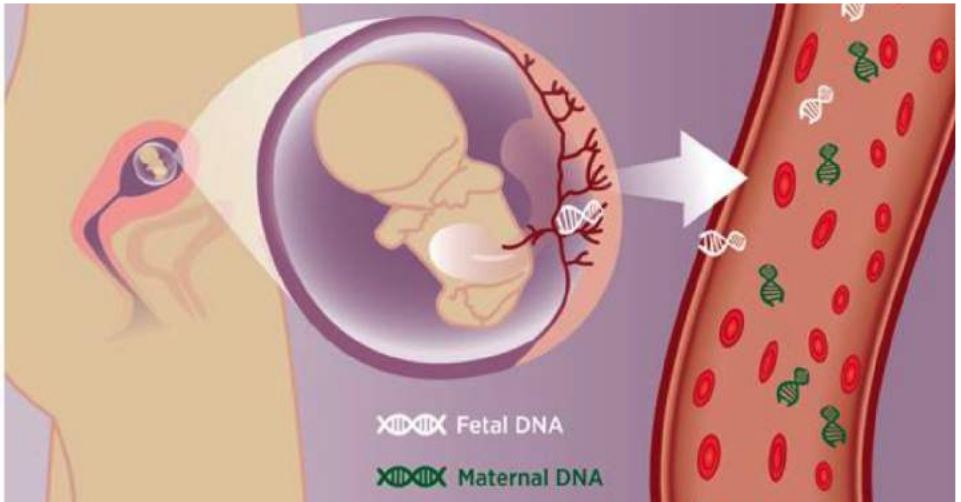
NIPT



Wong FCK, Lo YMD. 2016.
Annu. Rev. Med. 67:419–32

NIPT

- It is suggested that the half-life of fetal cfDNA in maternal plasma is roughly 1 h, which means a rapid clearance after birth
  - no misinterpretation due to the presence of fetal material of previous pregnancies
- Fetus-derived DNA has been identified by a variety of fetus- specific markers, such as chromosome Y-specific sequences, epigenetic markers, and SNPs
- Limitation- presence of a high background of maternal DNA
- 5–15% of cfDNA -from the fetus
  - only a small part of the fetal cfDNA is derived from a specific chromosome
  - chromosome 21- less than 1.5% of the total number of reads is derived from this chromosome
  - 0.15% of reads is derived from the fetal chromosome 21

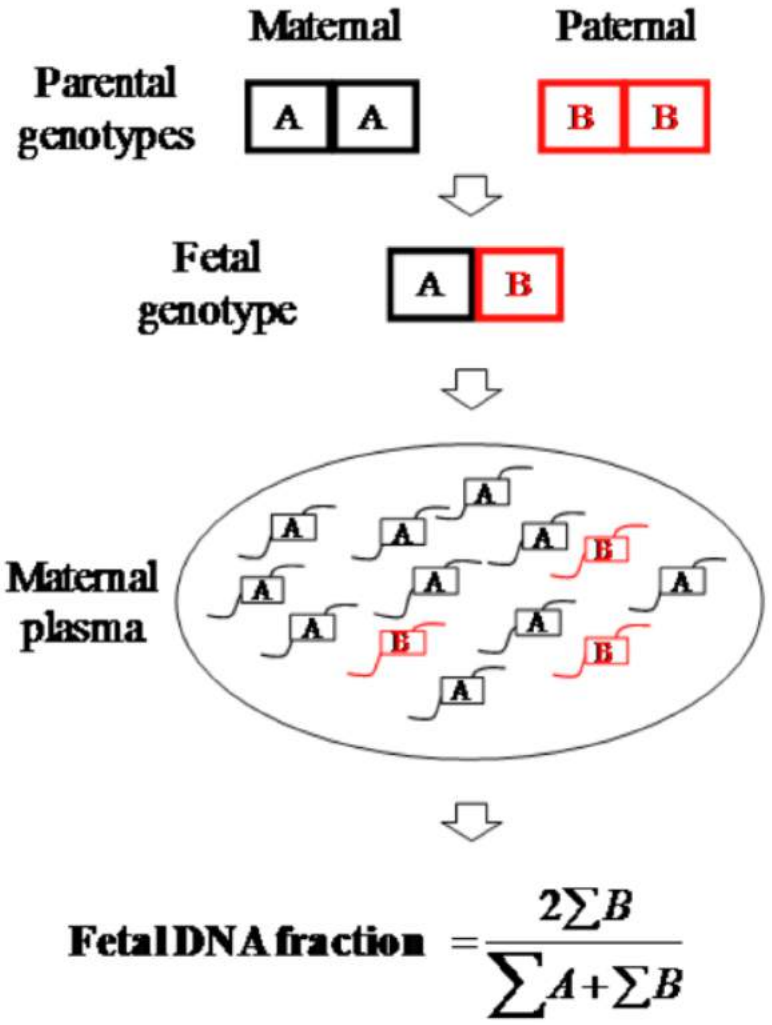


Targeted approach

- Focusing the test only on the chromosomes for the common aneuploidies (trisomy 13, 18, 21, X and Y)
- It will decrease sequencing costs, as smaller numbers of reads are needed

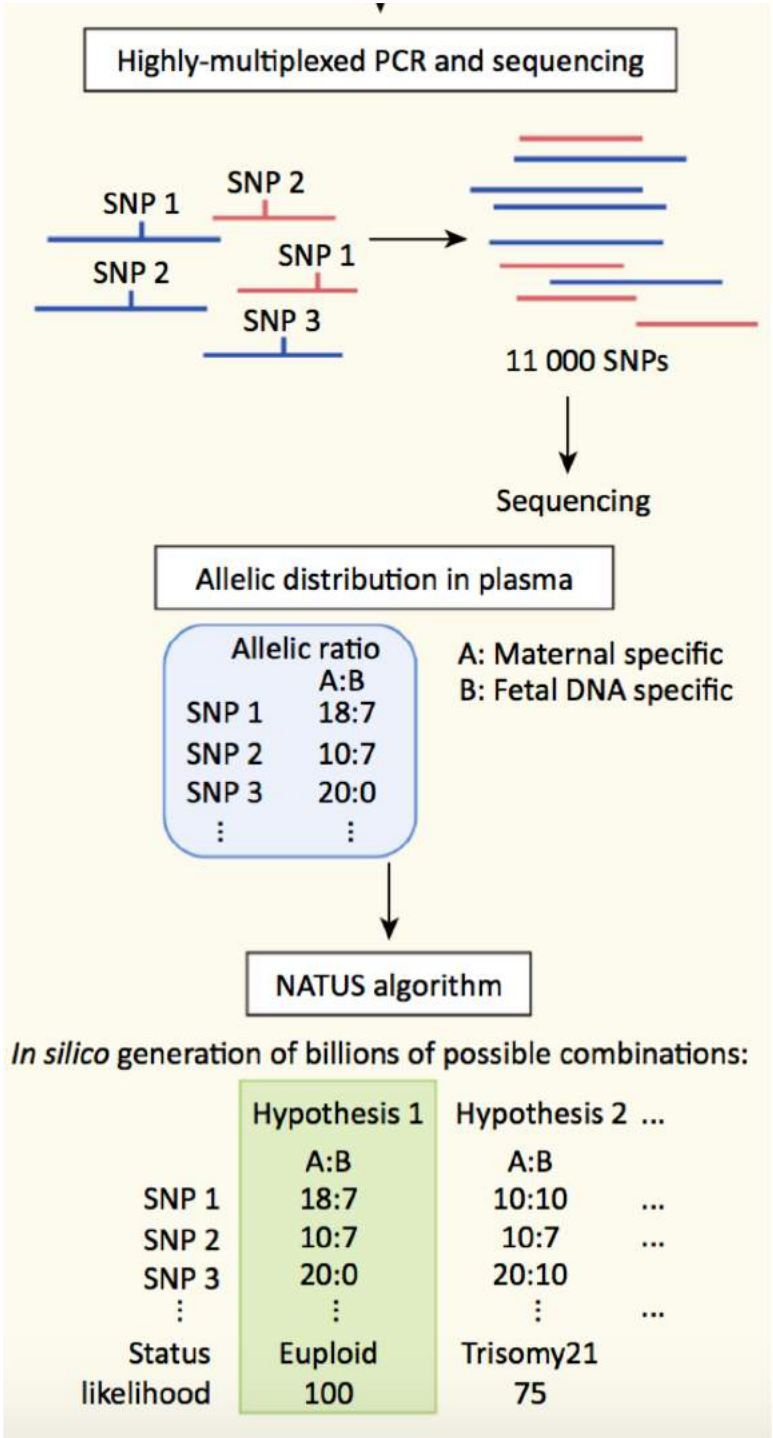
SNP-based targeted approach

- With parental genotypes
- Fetal genotype- heterozygous at SNP
  - Mother and father homozygous
- Fetal DNA fraction
- Feasibility- hindered
  - Parental genotypes are required

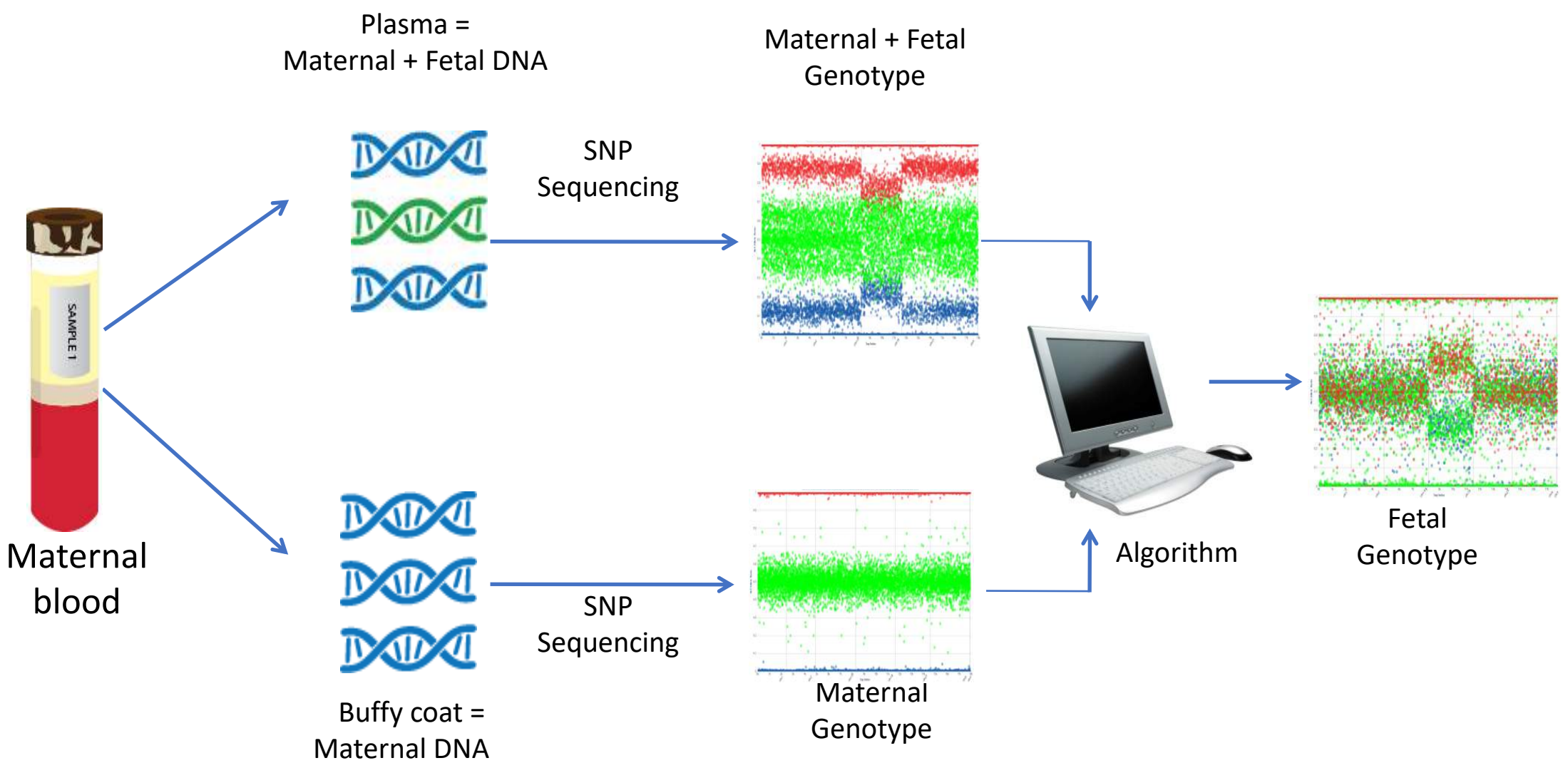


SNP-based targeted approach

- Use of hybridization- based capture of the genomic regions of interest, followed by MPS
- PCR-amplification of 19,488 SNPs on chromosomes 13, 18, 21, X, and Y followed by MPS – requires parental genotypes
- A risk is calculated based on these results, combined with maternal and gestational age prior risks



SNP-based targeted approach



http://www.panoramatest.com.hk/snp-testing-next-generation-of-NIPT

Cell-Free DNA Size-Based Approach

- To avoid the requirement of parental genotype information- fetal DNA fraction through the analysis of maternal plasma
- Counting of the DNA fragments in the plasma specimen
- MPS- millions of both fetal and maternal DNA fragments can be sequenced simultaneously and, each piece that maps to a discrete locus can be assigned to the chromosome from which it came.
- If fetal aneuploidy is present, there should be a relative excess or deficit for the chromosome in question.
  - difference in counts is small
  - counts need to be compared with the expected counts for euploid cases – normalization with disomic chromosomes within the same test run

Fetal Fraction Determination

CTTACCGTAATTCGGTCTAAAGTTCCAATAGGGGAG
Matches chromosome 12
Increment count

TACCGTATATTCGGTCTAGCAGTTCCAATAGGTGAC
Matches chromosomes 1 and 6
Discard

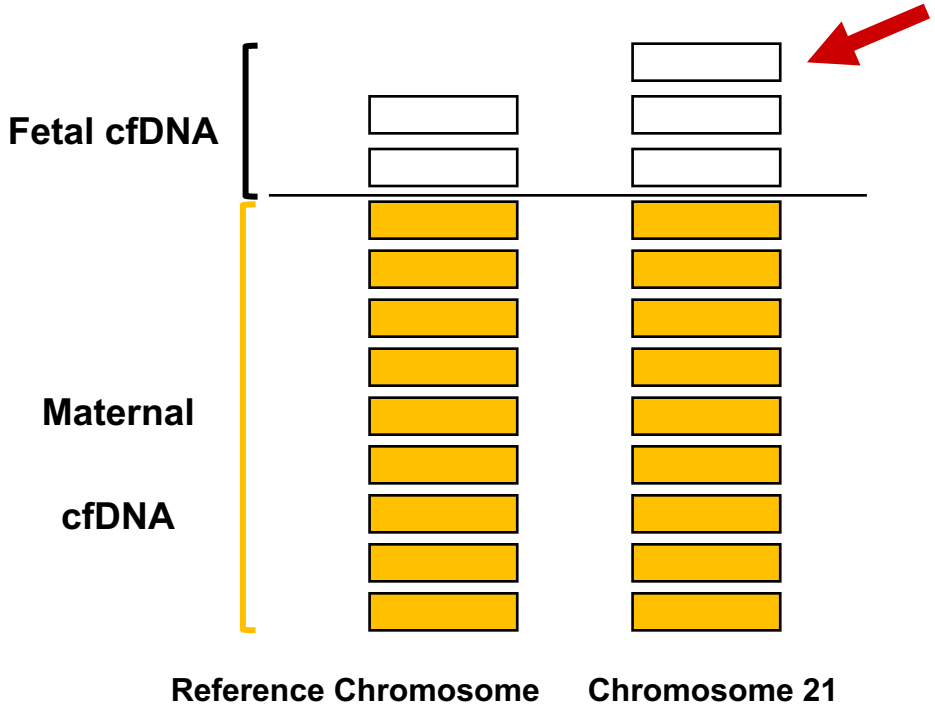
CCAGTATATTCGGTCTAGCAGTTCCAATAGGTGACT
Matches chromosome 3
Increment count

ACCGTAATTCGGTCTAAAGTTCCAATAGGGGAGCCT
Matches chromosome 12
Increment count

	Counts		Counts		Counts
1		9		17	
2		10		18	
3	1	11		19	
4		12	2	20	
5		13		21	
6		14		22	
7		15		X	
8		16		Y	

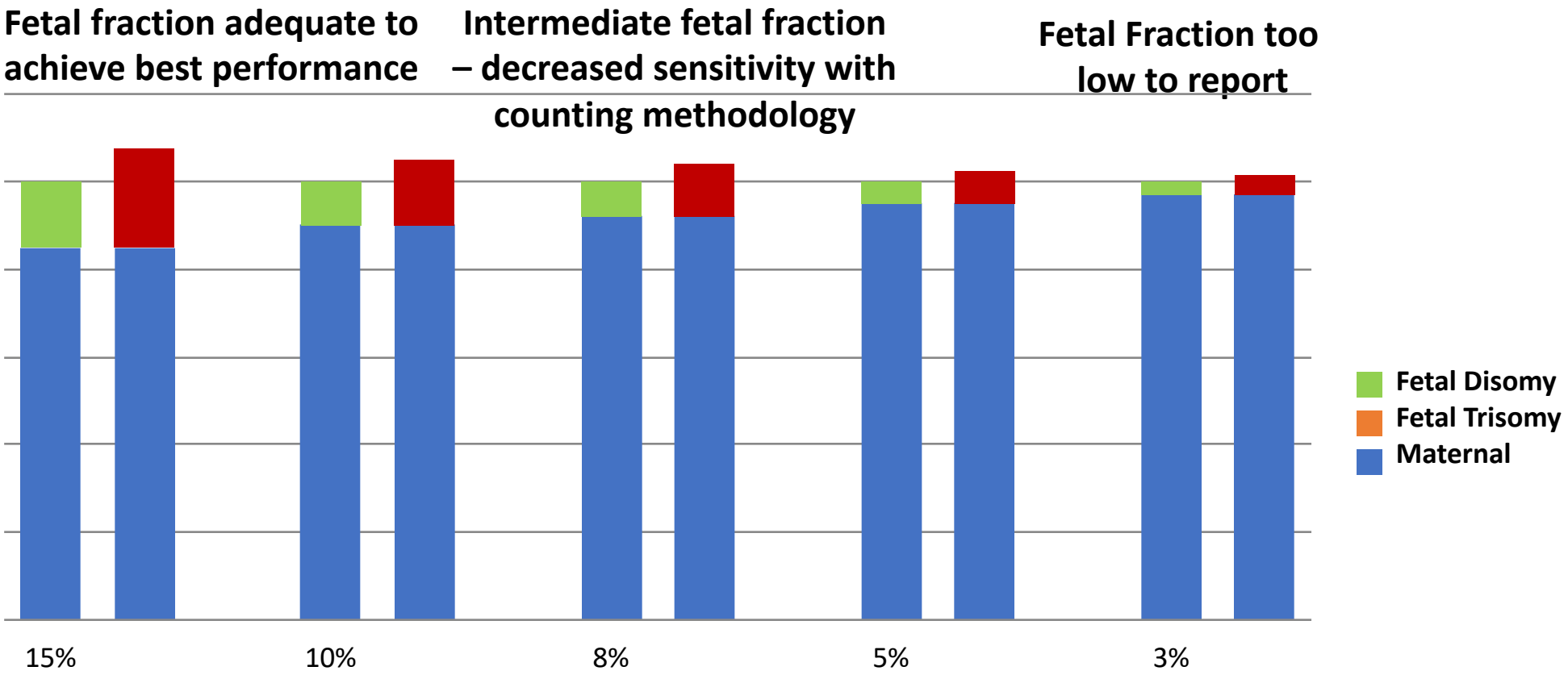
Fetal Fraction Determination

- Use of the difference in length between maternal and fetal cfDNA



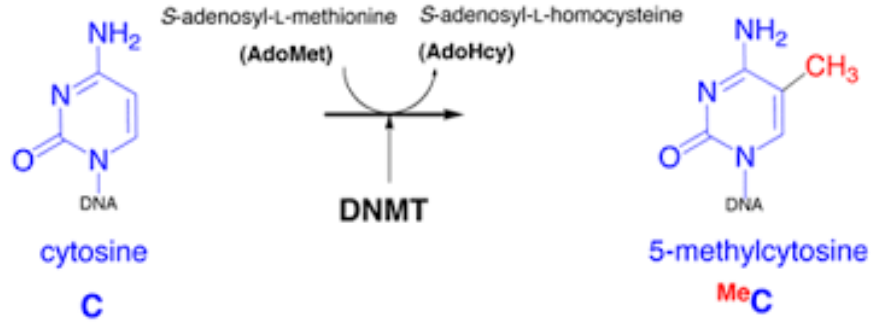
Fetal Fraction Determination

"An aneuploid sample with a lower fetal fraction has a higher probability of resulting in a false negative result."



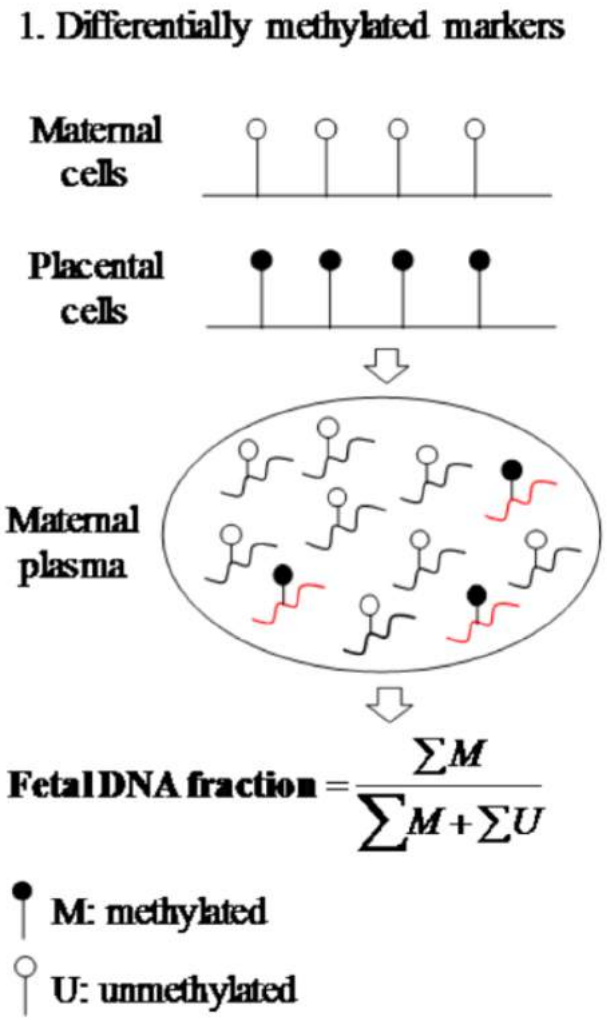
Methylation based targeted approach

- DNA methylation is a process by which a methyl group is added to 5’ carbon of cytosine nucleotides within a CpG
- Different organs have been suggested to show variable methylation profiles, which would allow us to identify the tissue of origin analyzing the regions with differential methylation states
- Placenta-specific methylation markers - methylation status changes with the progress of gestation
- Differences in methylation status between maternal and fetal DNA is used to determine the presence of trisomy 13, 18, 21 in the fetus



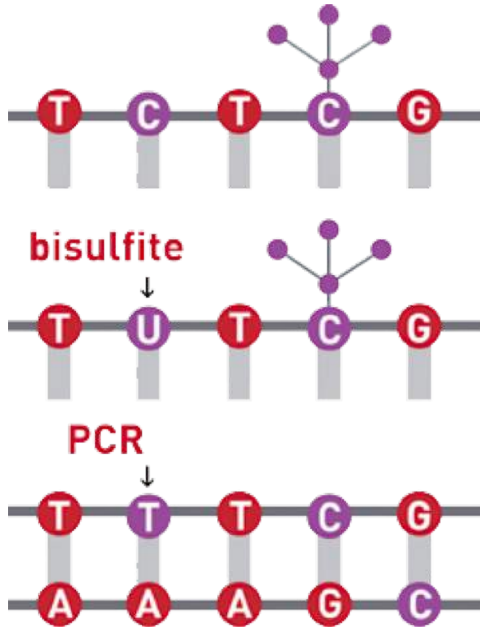
Methylation based targeted approach

- A methylation-sensitive restriction enzyme digest hypomethylated maternal-derived *RASSF1A* promoter sequences
- Methylated fetal-derived sequences remain unaffected
  - discrimination of the methylated fetal DNA molecules from the unmethylated maternal background for the calculation of fetal DNA fraction
- Based on differentially methylated regions - fetal DNA fraction in a plasma sample



Methylation based targeted approach

- Massively parallel bisulfite sequencing - estimate the fetal DNA fraction according to the ratio of fetal-derived DNA molecules within differentially methylated regions
- Sodium bisulfite treatment converts cytosine into uracil, while methylated cytosine is not affected.
- After MPS (where uracil is read as a thymine rather than a cytosine nucleotide), methylated cytosine nucleotides can be identified by comparing the modified DNA to the original reference sequence.



Methylation based targeted approach

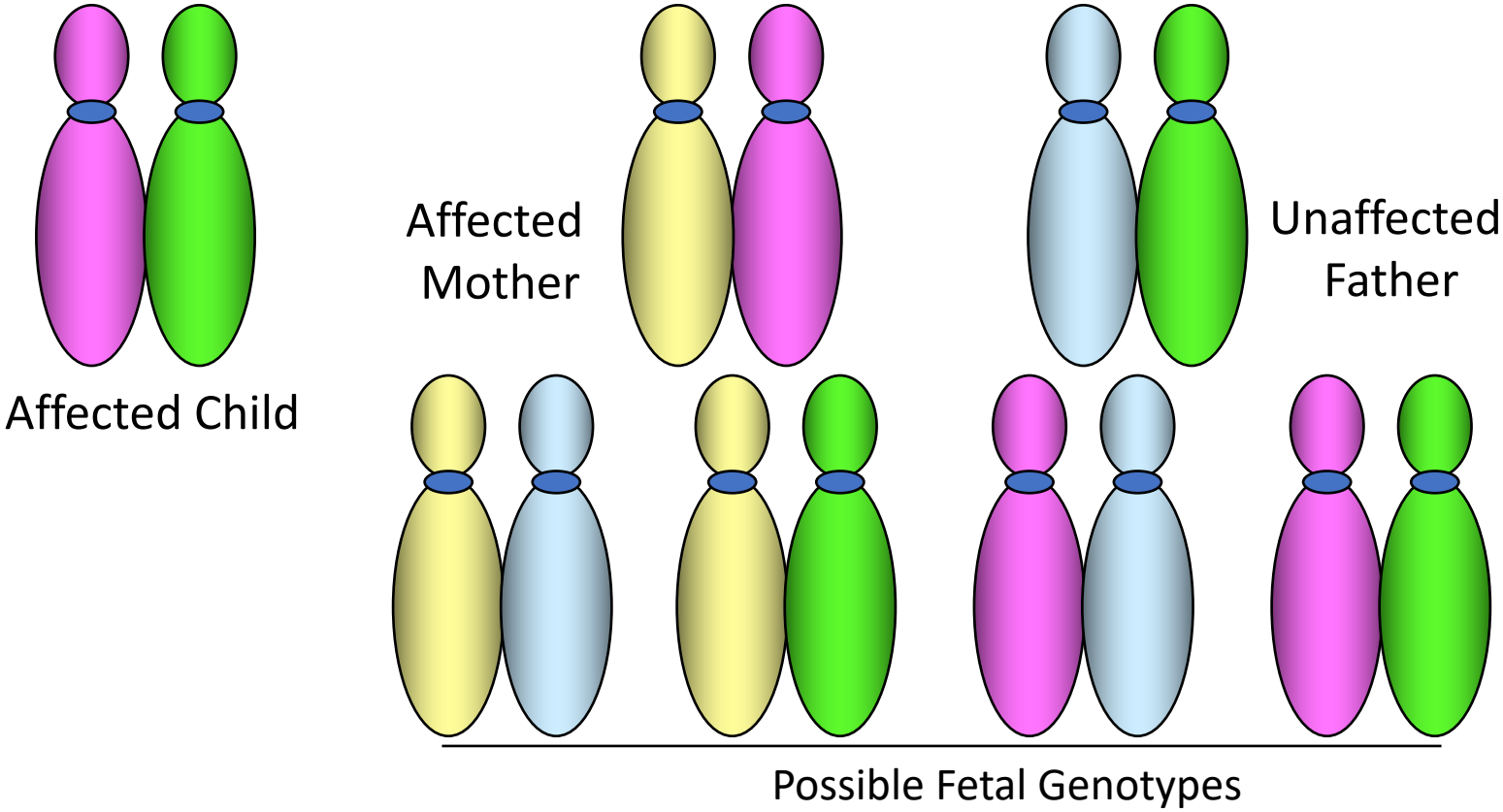
- Limitation: bisulfite conversion or digestion with methylation-sensitive restriction enzymes may affect the accuracy; genome-wide bisulfite sequencing is too expensive

NIPD for single gene disorders

- Single gene disorders- DIAGNOSIS
- Testing from paternally inherited and *de novo* mutation for many rare conditions
- Genetic maps from paternal and maternal DNA- fetal genome could be deduced

NIPD for recessive disorders

- Maternal inheritance, the analysis of the sequencing data is relatively more complicated.
  - fetal DNA is a minority in maternal plasma
  - analysis requires a quantitative approach based on SNP sites that are homozygous in the father and heterozygous in the mother
- DNA samples from the affected child and parents are analyzed to determine the haplotype structure of SNPs flanking the disease gene



Reasons of false positives and false negatives

- **False Negative:**
- Too little fetal DNA (usually reported as failed)
- Mosaicism
- **False positive:**

- Mosaicism
- Vanishing twin
- Maternal sex chromosome abnormality
- Neoplasia – apoptosis of cancer cells, aneuploidy common

Reasons for False-Negative Results

- Low Fetal Fraction
- False-negative rate increases with lower fetal fraction
- Threshold for the fetal fraction for accurate testing is around 4%
- The fetal fraction can be influenced by several factors.
- The four main factors are
  (1) maternal weight and/or BMI- lower fetal fraction
  (2) gestational age- before 7th week of gestation, higher risk
  (3) multiple gestations- lower fetal fraction
  (4) fetal aneuploidy- ? aneuploid fetus has lower fetal fraction

Reasons for False-Negative/ Positive Results

- True mosaicism – Presence of two or more karyotypically different cell lines in both the placenta and the fetus.
  - false positive or a false negative depending on the origin of the cfDNA

- Confined placental mosaicism – Presence of two or more karyotypically different cell lines that are confined to the placenta and not present in the fetus.
  - false positive
  - CPM is reported to be present in 1–2% of first trimester placentas
  - For chromosomes other than chromosome 21, 18, or 13, CPM is more common

- Fetal mosaicism – Presence of two or more karyotypically different cell lines that are present in the fetus but not present in the placenta.
  - false negative

Reasons for False-Negative Results

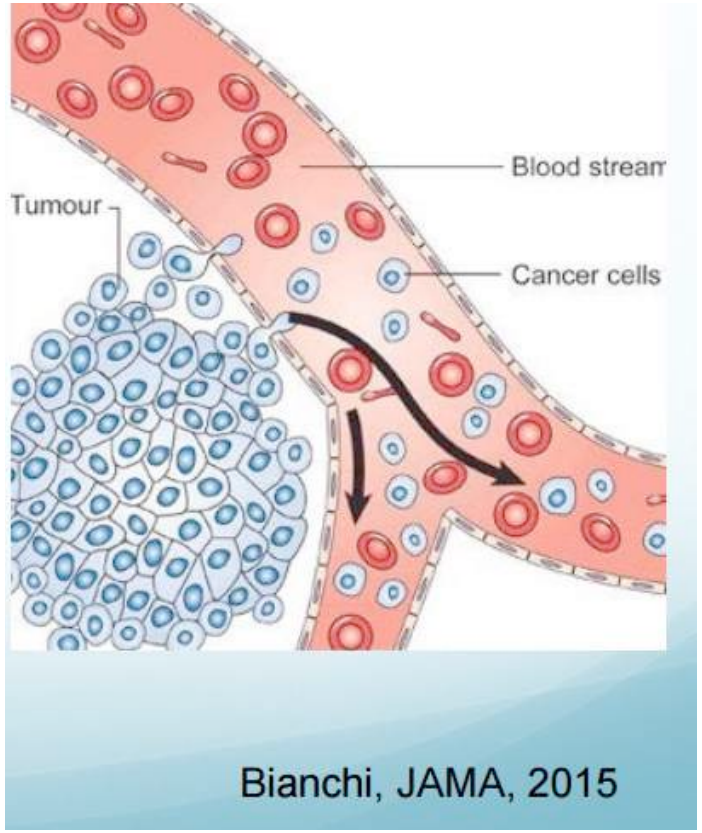
- Twin Pregnancies
- The fetal fraction per fetus in twin pregnancies is 30–50% lower than in singleton pregnancies
- More difficult to analyze because each fetus will release different amounts of cfDNA
- NIPT is not recommended for these pregnancies.

Reasons for False-Positive Results

- Maternal Findings: Chromosomal Abnormalities
- An aberrant NIPT result can also be the consequence of an abnormal karyotype or cell line in the pregnant woman.
- This finding can mistakenly be interpreted as an abnormal finding in the fetus.
- Triple X syndrome (47, XXX) is a relatively common chromosome abnormality (the prevalence is about 1 in 1000) which often goes undiagnosed and could lead to an abnormal NIPT result

Reasons for False-Positive Results

- Maternal Findings: Malignancy
- NIPT can incidentally detect an occult maternal malignancy.
- The incidence ranges from 0.07% to 0.1% for all malignant tumors, affecting approximately 1 in 1000 pregnancies
- The most common malignancies associated with pregnancy include cervical cancer, breast cancer, malignant melanoma, lymphomas, leukemias, ovarian cancer, and colorectal cancer



Reasons for False-Positive Results

- Vanishing Twin
- Sometimes during an ongoing multiple pregnancy the twin fetus dies in utero, resulting in an apparent singleton pregnancy.

Post-test Counseling:

- If a positive NIPT result:
  - Remember False Positives occur
  - Refer for genetic counseling
  - Always offer invasive testing for confirmation
  - If parents decline invasive testing, postnatal confirmation should be completed
- If a Negative NIPT result:
  - Remember False Negatives occur – especially in higher risk pregnancies
  - Always offer invasive testing if parents want to “know for sure”
- *“The tests should not be considered to be fully diagnostic and therefore are not a replacement for amniocentesis and CVS”*

SEQUENOM CMM
CENTER FOR MOLECULAR MEDICINE

Tel: **877-821-7266 (SCMM)**
Option 1: Customer Service, Reorder Supplies, Pre-Authorization
Option 2: Request for Sample Pick-up
Option 3: Billing Support
Option 4: Genetic Counselor Support

Printed: 05/16/2012 5:11 PM

Ordering Provider:	**Doe, Jane, MD**	Patient:	**Sample, Jane**
Provider Location:	**Grand Rapids**	DOB:	**09/13/1970**
Provider Phone:	**555-555-5555**	Patient ID:	**12345-01234**
Date Ordered:	**03/28/2012**	Specimen:	**1035600024**
Date Collected:	**03/29/2012**	Lab Director:	**Juan-Sebastian Saldivar, MD**
Date Received:	**03/30/2012**	Test Location:	**3595 John Hopkins Court**
Order ID:	**ORD12345-01234**		**San Diego, CA 92121-1121**

Final Report

MaterniT21™ PLUS Lab Report

Test Result

Negative

This specimen showed an expected representation of chromosome 21, 18 and 13 material.
Y chromosomal material was detected in this sample.

Interpretation

These results are consistent with an expected amount of fetal chromosome 21, 18 and 13 material. Y chromosomal material was detected, consistent with a male fetus. Clinical correlation is suggested.

About the Test

The MaterniT21 PLUS test analyzes circulating cell-free DNA extracted from a maternal blood sample.[2] The test is indicated for use in pregnant women with increased risk for chromosomal aneuploidy. Samples are also assessed for the presence of Y chromosomal material. However, sex chromosome aneuploidy is not designated by this result.

Additional Perspective

DNA test results do not provide a definitive genetic risk in all individuals. While results of this testing are highly accurate, infrequent errors may be due to unusual DNA sequences in the DNA analyzed or other causes. Based on the methodology and known performance characteristics, multiple gestations are not expected to influence test results.

Disclaimer

This LDT was developed and its performance characteristics determined by Sequenom Center for Molecular Medicine. It has not been cleared or approved by the U.S. Food and Drug

verifi prenatal test

Prenatal Aneuploidy Test Report

ANEUPLOIDY DETECTED
See Below

REPORT DATE AND TIME	
12/15/2012	10:45 AM

PROVIDER INFORMATION

Jane Doctor, MD
Local OBGYN Associates
123 Fake Street
Springfield, IL 12345

Phone: (415)123-1234
Fax: (415)123-1234

PATIENT INFORMATION

Name: Doe, Jane
DOB: 1/1/1975
Medical Record/Patient ID: 123456
Gestational Age at Draw (weeks): 15
Ordering Physician: Jane Doctor, MD
Client Sample ID: 12345678

PRENATAL ANEUPLOIDY TEST RESULTS

TEST	RESULT	INTERPRETATION
Chromosome 21	ANEUPLOIDY DETECTED	Result consistent with trisomy for chromosome 21
Chromosome 18	No aneuploidy detected	Result consistent with diploid chromosome 18
Chromosome 13	No aneuploidy detected	Result consistent with diploid chromosome 13
Sex Chromosomes	No aneuploidy detected	Result consistent with two sex chromosomes (XY)

Comments:
Genetic counseling is recommended. Clinical correlation with ultrasound findings and other screening tests is indicated. If definitive diagnosis is desired, chorionic villus sampling or amniocentesis is necessary.

SAMPLE

verifi prenatal test

Prenatal Aneuploidy Test Report

ANEUPLOIDY SUSPECTED
See Below

REPORT DATE AND TIME	
12/15/2012	10:45 AM

PROVIDER INFORMATION

Jane Doctor, MD
Local OBGYN Associates
123 Fake Street
Springfield, IL 12345

Phone: (415)123-1234
Fax: (415)123-1234

PATIENT INFORMATION

Name: Doe, Jane
DOB: 1/1/1975
Medical Record/Patient ID: 123456
Gestational Age at Draw (weeks): 15
Ordering Physician: Jane Doctor, MD
Client Sample ID: 12345678

PRENATAL ANEUPLOIDY TEST RESULTS

TEST	RESULT	INTERPRETATION
Chromosome 21	No aneuploidy detected	Result consistent with diploid chromosome 21
Chromosome 18	ANEUPLOIDY SUSPECTED (Borderline Value)	Result suggestive of trisomy for chromosome 18
Chromosome 13	No aneuploidy detected	Result consistent with diploid chromosome 13
Sex Chromosomes	No aneuploidy detected	Result consistent with two sex chromosomes (XY)

Comments:
Genetic counseling is recommended. Clinical correlation with ultrasound findings and other screening tests is indicated. If definitive diagnosis is desired, chorionic villus sampling or amniocentesis is necessary.

SAMPLE

Harmony™
PRENATAL TEST
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Ariosa DIAGNOSTICS
Ariosa Diagnostics, Inc.
5945 Optical Court
San Jose, CA 95138

Integrated GENETICS
LabCorp Specialty Testing Group

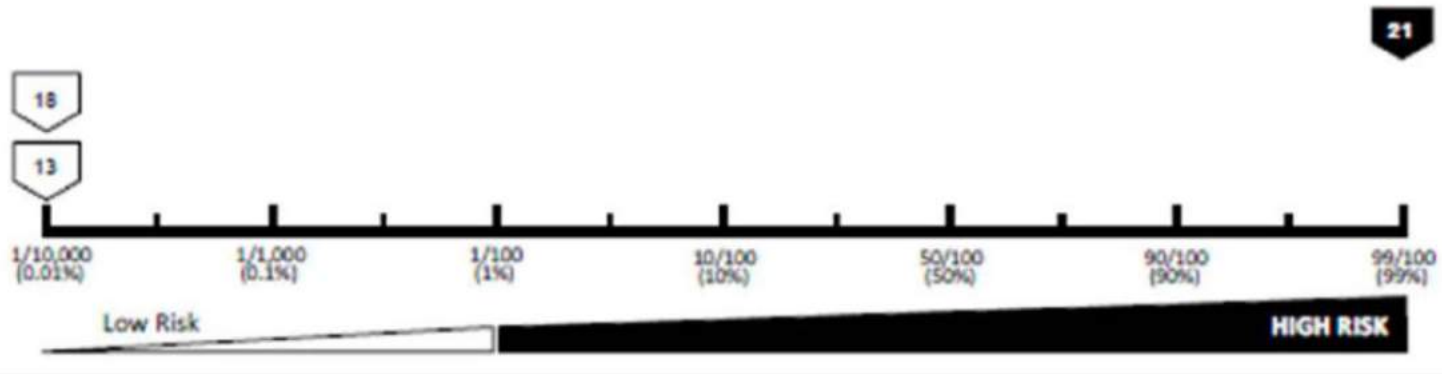
Questions
(800) 848 - 4436

Patient and Provider Information

PATIENT NAME:	Jane Doe	ACCOUNT #:	7654321
DATE OF BIRTH:	01/01/1970	CLINIC NAME:	The Clinic Offering Test
MRN:	1234567654321	REFERRING/ORDERING CLINICIAN:	Ordering Physician MD
SPECIMEN ID:	AD12345678-PAT	REFERRING/ORDERING CLINICIAN FAX #:	123-456-7890
GESTATIONAL AGE:	10 wks 5 days	OTHER CLINICIAN:	Genetic Counselor MA, CGC
COLLECTION DATE:	01/01/2012	OTHER CLINICIAN FAX #:	987-654-3210
RECEIVED DATE:	01/02/2012	REPORT DATE:	01/12/2012

Test Results

CHROMOSOME	RESULT	RISK SCORE	RECOMMENDATION
Trisomy 21 (T21)	**HIGH RISK**	**Greater than 99/100 (99%)**	**Genetic counselling and additional testing**
Trisomy 18 (T18)	Low Risk	Less than 1/10,000 (0.01%)	Review results with patient
Trisomy 13 (T13)	Low Risk	Less than 1/10,000 (0.01%)	Review results with patient



Disease
Chromosomal aneuploidy
Trisomy 21
Trisomy 18
Trisomy 13
Turner syndrome (45,X)
Klinefelter syndrome (47,XXY)
47,XYY syndrome
Triple X syndrome (47.XXX)
Triploidy (3N)
Subchromosomal aberration
22q11.2 deletion syndrome
DiGeorge syndrome
Prader–Willi syndrome
Angelman syndrome
1p36 deletion syndrome
Cri-du-chat syndrome
Wolf–Hirschhorn syndrome
22q11.2 duplication syndrome
De novo 22q11.2 deletion syndrome
De novo 22q11.2 microduplication
Translocation
Microdeletion
Microduplication
Monogenic diseases
β-Thalassemia
Congenital adrenal hyperplasia

What is Preimplantation Genetic Diagnosis?

- Preimplantation genetic diagnosis (PGD) is applied to couples at risk of transmitting an inherited disease or chromosomal imbalance to their offspring.
- Couples who have already been diagnosed with a
  - single gene disorder or
  - a chromosome imbalance

  can opt for PGD to select an embryo free from the mutation or an embryo with a balanced karyotype prior to implantation and pregnancy.

Preimplantation genetic screening

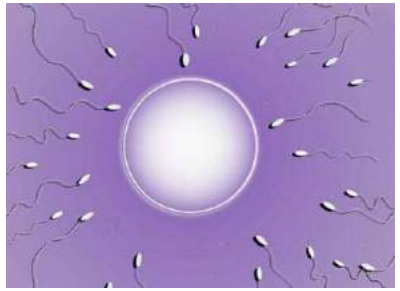
- To try and improve pregnancy rates
- Advanced maternal age
- Prior pregnancy /child chromosomally abnormal
- Multiple implantation failures (≥2 failed IVF)
- Recurrent miscarriage (≥2 miscarriages)
- Severe male factor (low sperm count)

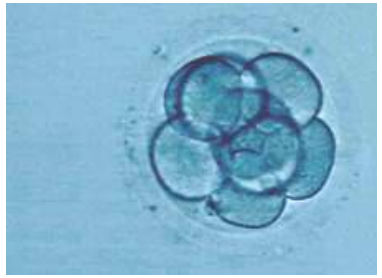
- Social sexing

How is PGT performed?

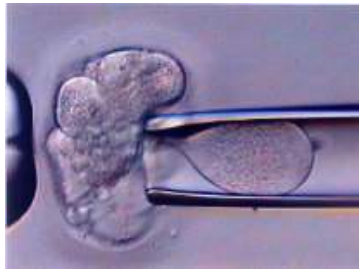
Ovarian Stimulation

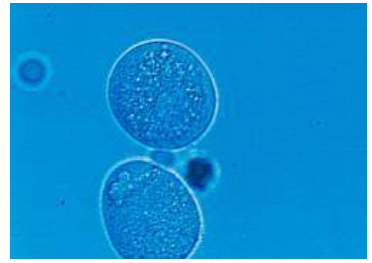
IVF

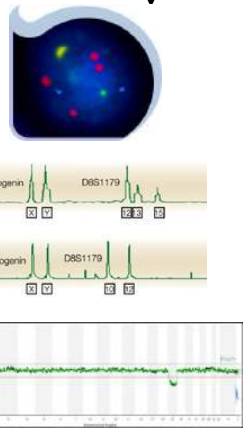




Blastomere Biopsy on Day 3 or 5/6







Genetic Analysis

Transfer of

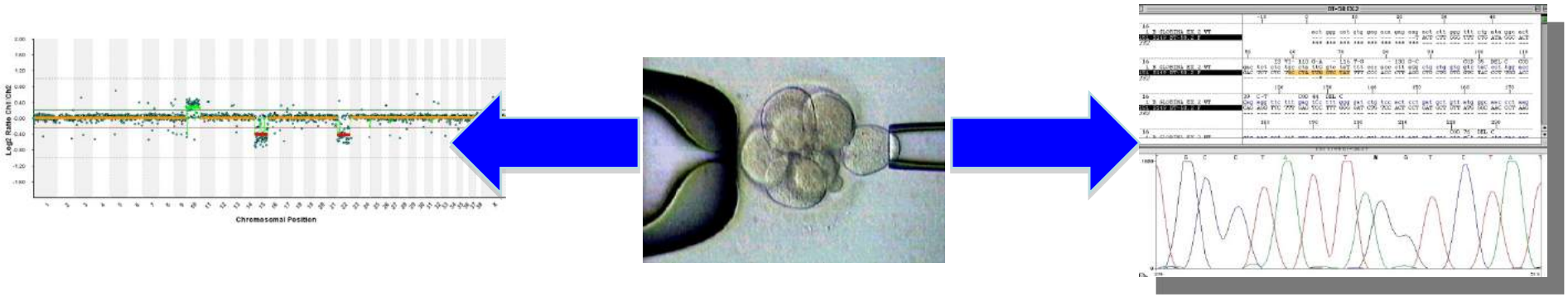
Unaffected Embryo

Outcome

Chromosomally Normal Baby

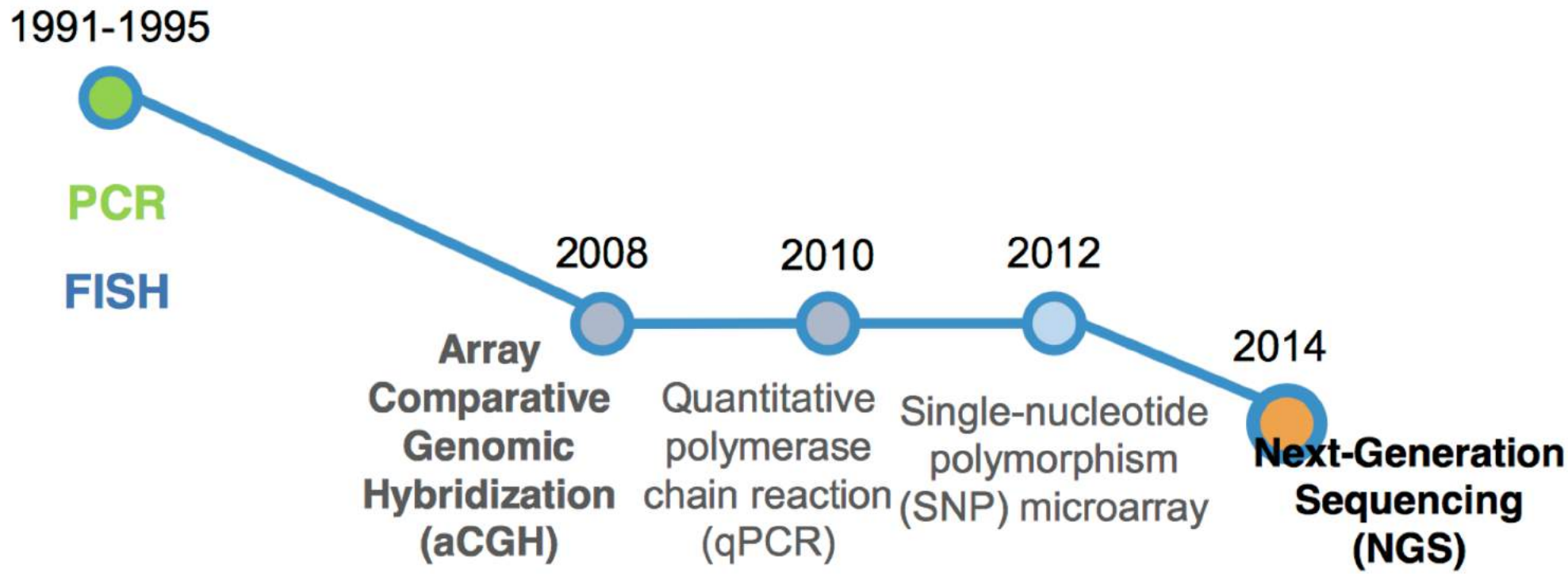
Preimplantation Genetic Testing

A procedure used in conjunction with *in vitro* fertilization (IVF) to screen for **specific genetic** or **chromosomal abnormalities** before transferring the fertilized eggs into the mother.



- PGS (Preimplantation Genetic Screening)
- (Numerical or structural chromosomal abnormalities)

- **PGD for Monogenic Diseases (PGD)**
- **PGD for late onset disorders**
- **PGD for inherited cancer predisposition**
- **with or w/o HLA typing**



Analysis of a identified single gene disease

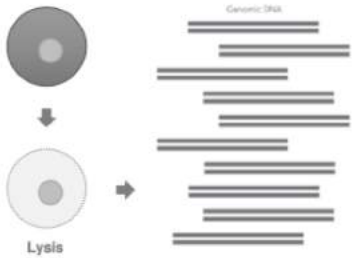
2-12 chromosomes

24 chromosomes

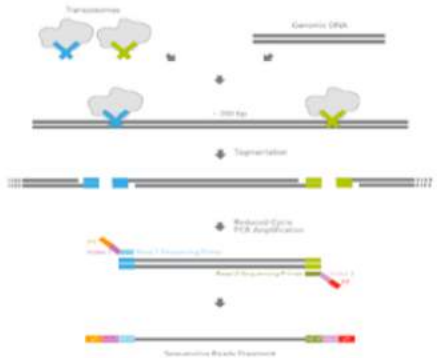
24 chromosomes
+
Mito DNA; mutations

igenomix

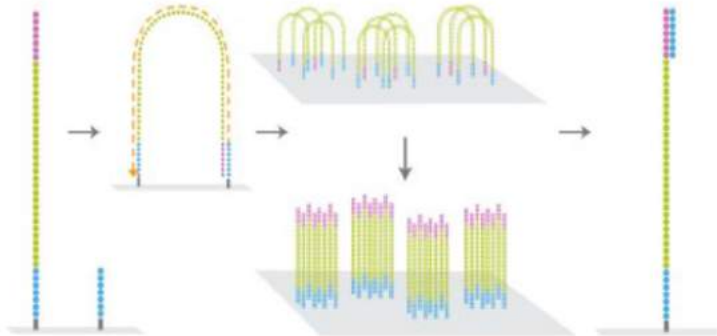
DNA amplification



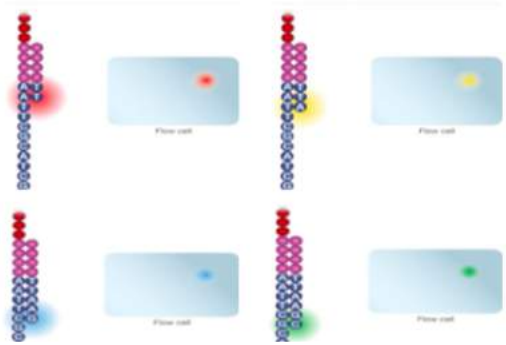
Tagmentation

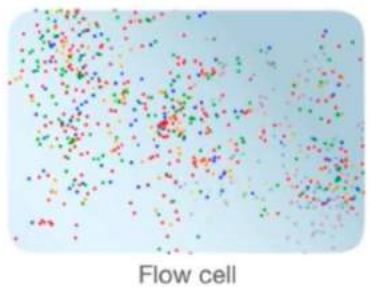


DNA library



Complementary nucleotide sequencing





Matching with Reference DNA

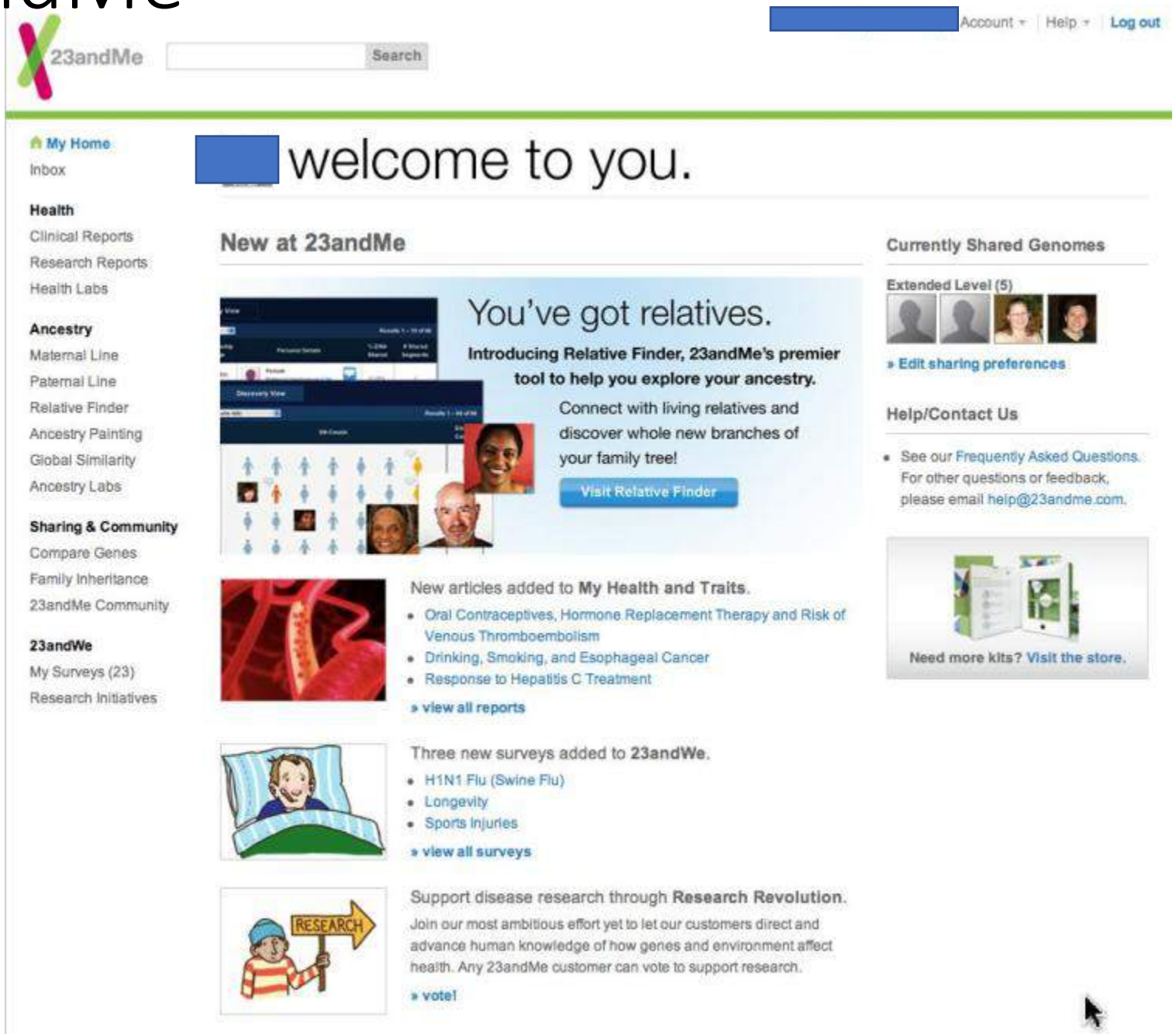
igenomix

23andMe Kit


23andMe


BIOHAZARD
SPECIMEN BAG
1) Insert Specimen(s) in Bag.
2) Peel off Blue Release Liner, then Seal Bag by Pressing Down on Glue Line
TEAR AT SCORE TO OPEN

23andMe


23andMe
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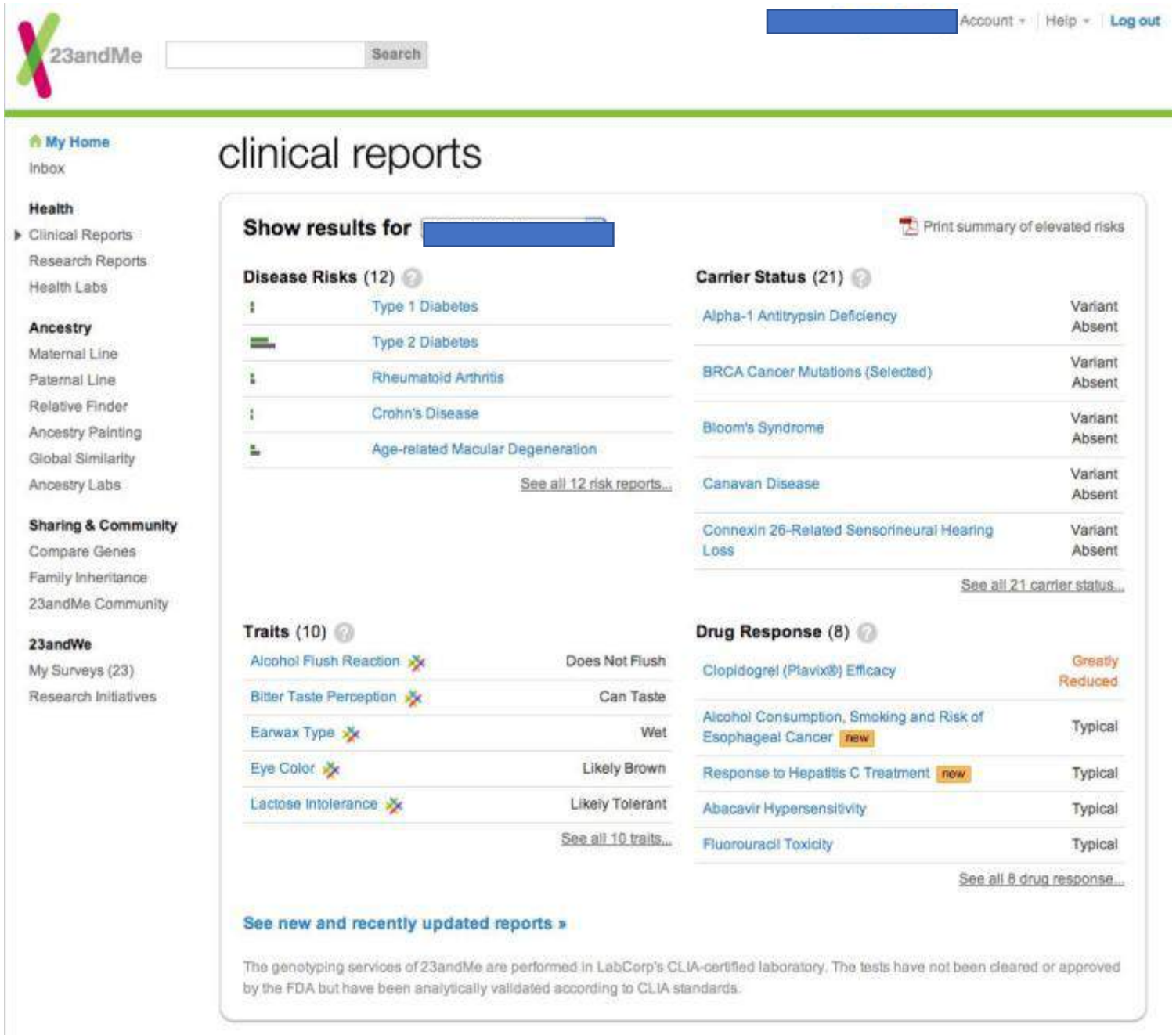
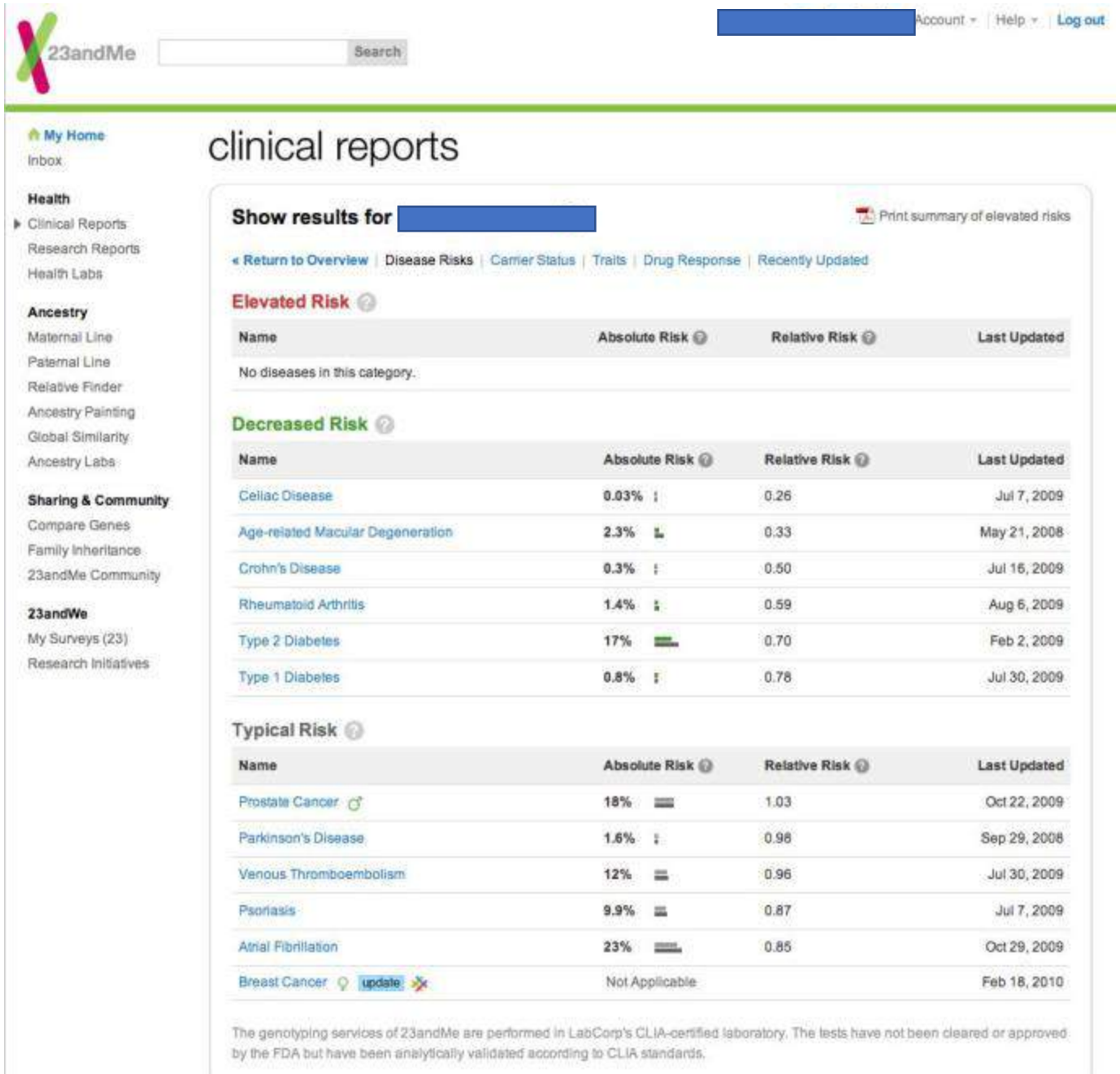
Health
Clinical Reports
Research Reports
Health Labs
Ancestry
Maternal Line
Paternal Line
Relative Finder
Ancestry Painting
Global Similarity
Ancestry Labs
Sharing & Community
Compare Genes
Family Inheritance
23andMe Community
23andWe
My Surveys (23)
Research Initiatives
[redacted] welcome to you.
New at 23andMe
You've got relatives.
Introducing Relative Finder, 23andMe's premier tool to help you explore your ancestry.
Connect with living relatives and discover whole new branches of your family tree!
Visit Relative Finder
New articles added to My Health and Traits.
Oral Contraceptives, Hormone Replacement Therapy and Risk of Venous Thromboembolism
Drinking, Smoking, and Esophageal Cancer
Response to Hepatitis C Treatment
» view all reports
Three new surveys added to 23andWe.
H1N1 Flu (Swine Flu)
Longevity
Sports Injuries
» view all surveys
RESEARCH
Support disease research through Research Revolution.
Join our most ambitious effort yet to let our customers direct and advance human knowledge of how genes and environment affect health. Any 23andMe customer can vote to support research.
» vote!
Currently Shared Genomes
Extended Level (5)
» Edit sharing preferences
Help/Contact Us
See our Frequently Asked Questions. For other questions or feedback, please email help@23andme.com.
Need more kits? Visit the store.

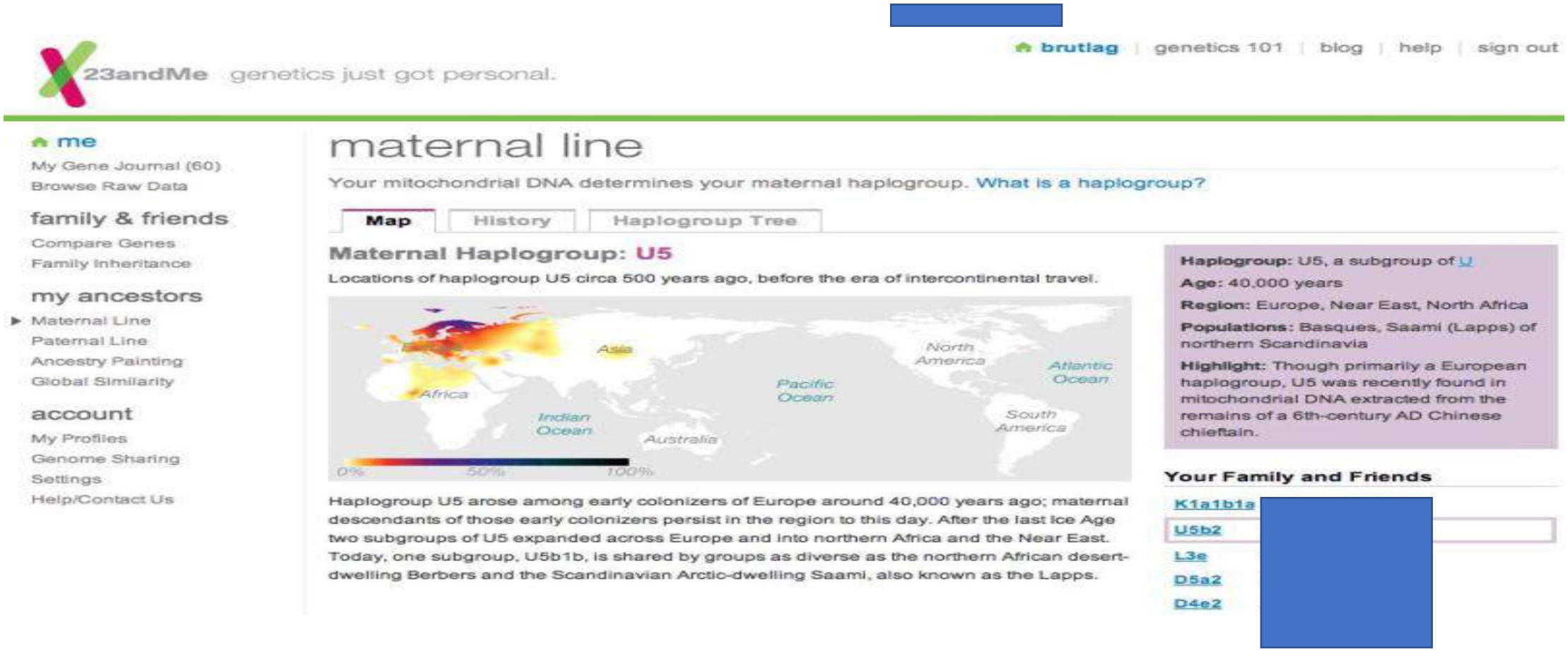
23andMe Clinical Reports



23andMe Clinical Reports



23andMe Maternal Inheritance



23andMe Paternal Inheritance

23andMe Ancestry