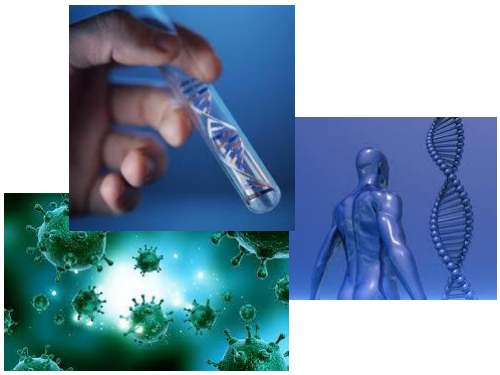
HUMAN CAPITAL
NATIONAL COHESION STRATEGY

UAM

EUROPEAN SOCIAL FUND

MOLECULAR DIAGNOSTICS



Molecular Diagnostics

Molecular diagnostics is >$3 billion market world wide and growing at >20% annually

The aim is to detect:

- Viruses
- Bacteria
- Fungi
- Parasites
- Proteins
- Nucleic acids

In water, plants, soil and organisms

Diagnostic test

Sensitivity the test must be able to **detect very small amounts of target** even in the presence of other molecules.

Specificity the test yields a **positive result for the target molecule only.**

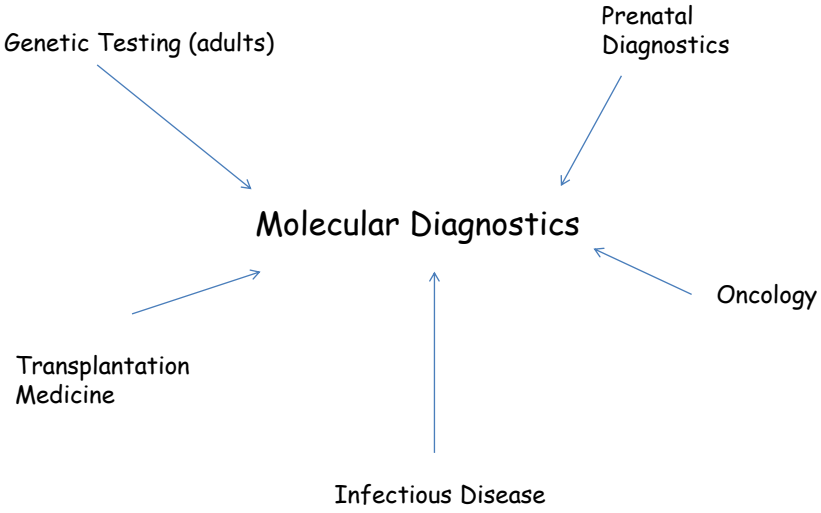
Simplicity the test must be able to **run efficiently and inexpensively** on a routine basis.

Diagnostic test – the definitions

Term	Definition	Complications in molecular tests	Calculation
Analytical sensitivity	Refers to the proportion of assays with the genotype that have a positive test result (false-negative rate of the assay)	Allele drop out; preferential amplification; mosaicism	True positives / (true positives + false negatives)
Analytical specificity	Refers to the proportion of assays without the genotype that have a negative test result (false-positive rate of the assay)		True negatives / (true negatives + false positives)
Clinical sensitivity	Refers to the proportion of people with a disease who have a positive test result (false-negative rate of diagnosis)	Variable penetrance; variable expressivity	True positives / (true positives + false negatives)
Clinical specificity	Refers to the proportion of people without a disease who have a negative test result (false-positive rate of diagnosis)		True negatives / (true negatives + false positives)
Positive predictive value (PPV)	Refers to the likelihood that a patient has the disease given that the test result is positive		True positives / (true positives + false positives)
Negative predictive value (NPV)	Refers to the likelihood that a patient does not have the disease given that the test result is negative		True negatives / (true negatives + false negatives)
Clinical utility	Refers to the value of the test for determining treatment, patient management and family planning	Depends on health-care system and environment	Subjectively determined on the basis of reports supporting use and economic benefits
Personal utility	Refers to the value of the test for personal and family choices	Depends on personal vantage	Subjectively determined from an individual's perspective

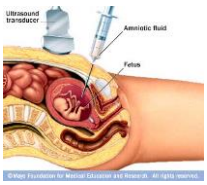
Katsanis et al.; Nature Genetics, 2013

Fields of interest



Molecular Diagnostics

Pre-natal testing



Is the baby healthy?

Disease predisposition

What diseases is this patient at risk for?

Disease detection

Does this patient have the disease ?

Molecular Diagnostics

Drug selection

What drugs should the doctor prescribe?

Recurrence monitoring

Does the patient have a recurrent disease?

Molecular Diagnostics

- Genetic Testing
- Prenatal diagnostics
- Oncology
- Transplantation Medicine
- Infectious Disease

- Progress is being made in all of these areas
- Each of these areas are commercially attractive

Genetic Testing: a bit confusing terminology….

Genetic testing = DNA-based tests

techniques used to test for genetic disorders which involves direct examination of the DNA

BUT ALSO

Other genetic tests include biochemical tests:

for gene products as enzymes and other proteins

for microscopic examination of stained or fluorescent chromosomes.

Genetic tests in the clinics

Test	Description	Example	Embryo or blastocyst (pre-implantation genetic diagnosis)	Fetus (prenatal testing)	Child	Adult
Newborn screening	Targeted tests for recessive genetic disorders	Phenylketonuria, cystic fibrosis, sickle-cell anaemia	Not applicable	Not applicable	Tests provided at birth vary by country and state or region	Not applicable
Diagnostic testing	Confirmatory test or differential diagnosis testing for a symptomatic individual	Skeletal dysplasias, thalassaemias, craniosynostoses	Specimen type and limited available amount for sampling may restrict platform selection (for example, WES or WGS versus SNP or STR typing)		Where treatment is desired, turnaround time may restrict platform selection	
			Turnaround time necessary may restrict platform selection			
Carrier testing	Targeted testing for asymptomatic individuals potentially carrying one or more recessive mutation	Cystic fibrosis, thalassaemias, Tay–Sachs disease	Applied typically for rare disease but applicable for other familial mutations		Carrier testing of minors is considered in the context of individual paediatric cases[164,165]	According to standard of care
Predictive testing	Tests for variants causing or associated with diseases or disorders with a hereditary component, usually with adult-onset symptoms	Most cancers, cardiovascular disease, diabetes	Some have discouraged genetic testing of asymptomatic minors for adult-onset conditions			According to standard of care
Pre-symptomatic testing	Tests for variants causing or associated with diseases or disorders known to be inherited in the family, often with adult-onset symptoms	Huntington's disease, haemo-chromatosis, Alzheimer's disease	Some have discouraged genetic testing of asymptomatic minors for adult-onset conditions[152,153]			According to standard of care
			Interpretation of VUSs will depend on presenting phenotypes in the family			
Pharmaco-genetics	Targeted tests for variants associated with pharmaceutical dosage choice or adverse reactions	DNA tests for abacavir, warfarin, carbamazepine	Application not currently conducted but theoretically feasible	Application not currently conducted, but conceivably applicable for screening treatment approaches *in utero*	Pharmacogenetic testing is considered in context of individual paediatric cases[166]	According to standard of care

SNP, single-nucleotide polymorphism; STR, short tandem repeat; WES, whole-exome sequencing; WGS, whole-genome sequencing; VUS, variant of unknown significance.

Katsanis et al.; Nature Genetics, 2013

Genetic testing - samples

Blood sample (most common for adult testing);

Mouth washes or buccal scrapes (non-invasive);

Chorionic villus biopsy samples (fetal DNA);

Hair, semen (forensics)

One or two cells removed from 8-cell embryo (in vitro fertilization)

Archived pathological specimens (tumor samples in paraffin blocks);

Paper cards with blood drops

Genetic testing - methodology

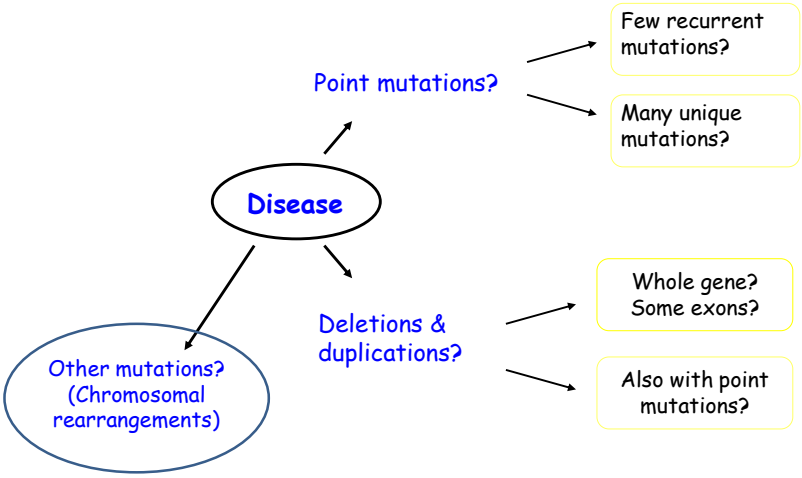
DNA Diagnostic Systems include:

- DNA Hybridization
- DNA Sequencing
- PCR
- Restriction endonuclease analysis
- RAPD (random amplified polymorphic DNA)
- DNA fingerprinting

OLD methods !!!!!!!

Genetic Testing

Types of Mutations Tested



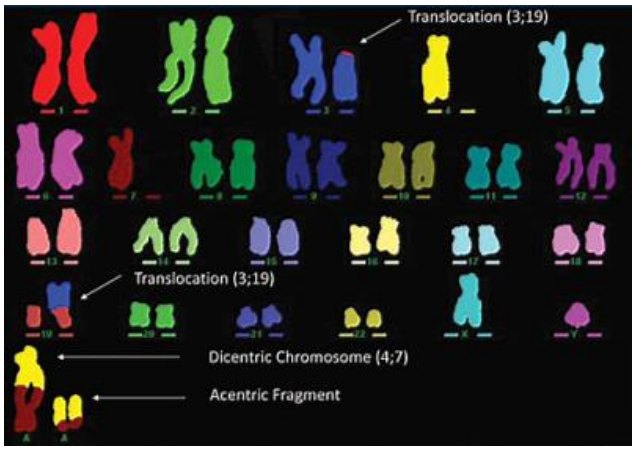
•High throughput testing for genetic disorders including single nucleotide polymorphisms (SNPs) markers, insertions, deletions

Hybridization methods - Spectral karyotyping (SKY)

Visualization of all human chromosomes at one time by labeling each pair of chromosomes in a different fluorescent color.

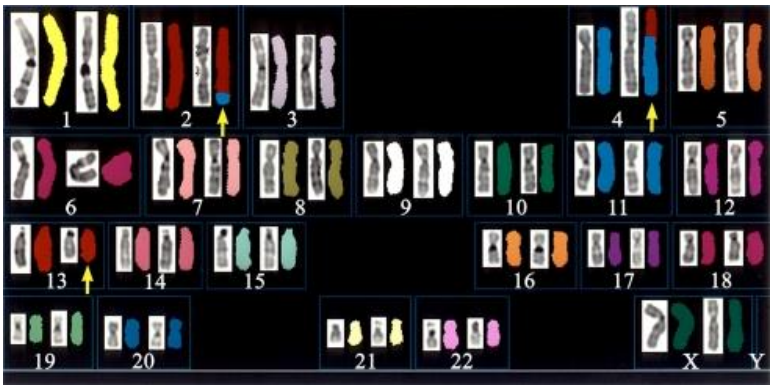
SKY involves the preparation of a large collection of short sequences of ssDNA called probes that hybridize to specific chromosomes

Spectral karyotyping (SKY)



The limit of resolution is in a range of 5-10 Mb

SKY - diagnosis of 13q syndrome



Imataka et al. 2012

13Q deletion syndrome is a chromosome disorder where one of the arms or the whole arm of the chromosome is missing at birth

Depending on which band of the arm is missing, many symptoms can occur: Global Developmental Delay, Small stature(weight and height), Low Muscle Tone, Seizures, Deafness, Blindness, Reflux, Cleft Palate

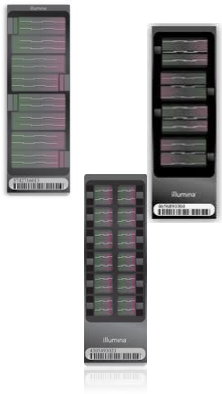
Whole Genome SNP Genotyping

Omni Whole-Genome Arrays

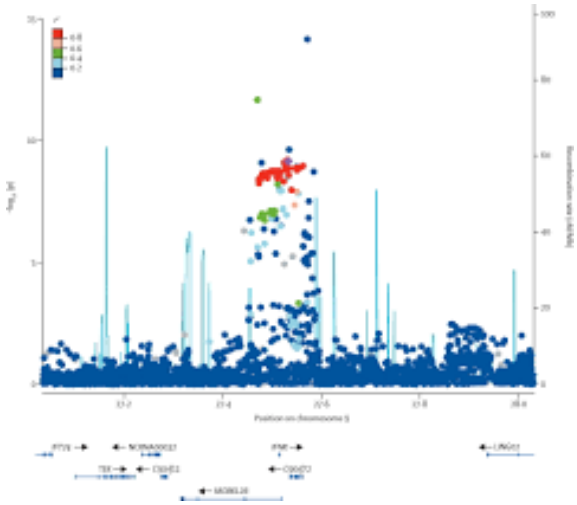
BeadChip	Array Format	Markers per Samp
HumanOmni5-Quad	4	~ 4.3 million
HumanOmni2.5S	8	~ 2.5 million
HumanOmni2.5-8	8	~ 2.5 million
HumanOmni1S	8	~ 1.25 million
HumanOmni1-Quad	4	~ 1.1 million
HumanOmniExpress	12	~ 700,000
HumanCytoSNP-12	12	~ 300,000

Omni Semi-Custom Whole-Genome Arrays

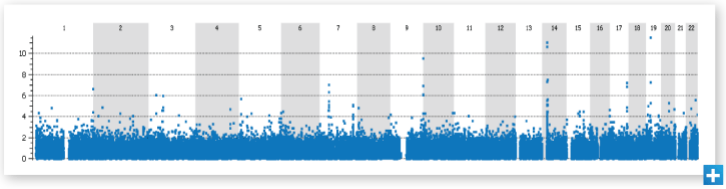
BeadChip	Array Format	Markers per Sample
HumanOmni5-Quad+	4	~ 4.3 million (fixed) up to 500K (custom)
HumanOmni2.5S+	8	~2.5 million (fixed) up to 500K (custom)
HumanOmniExpress+	12	~700,000 (fixed) up to 200K (custom)

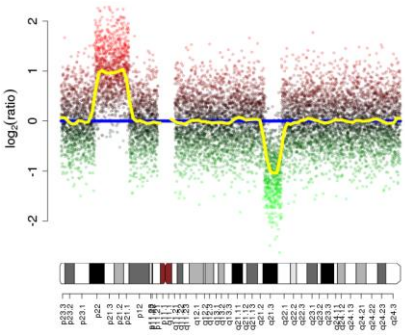


GWAS - association study



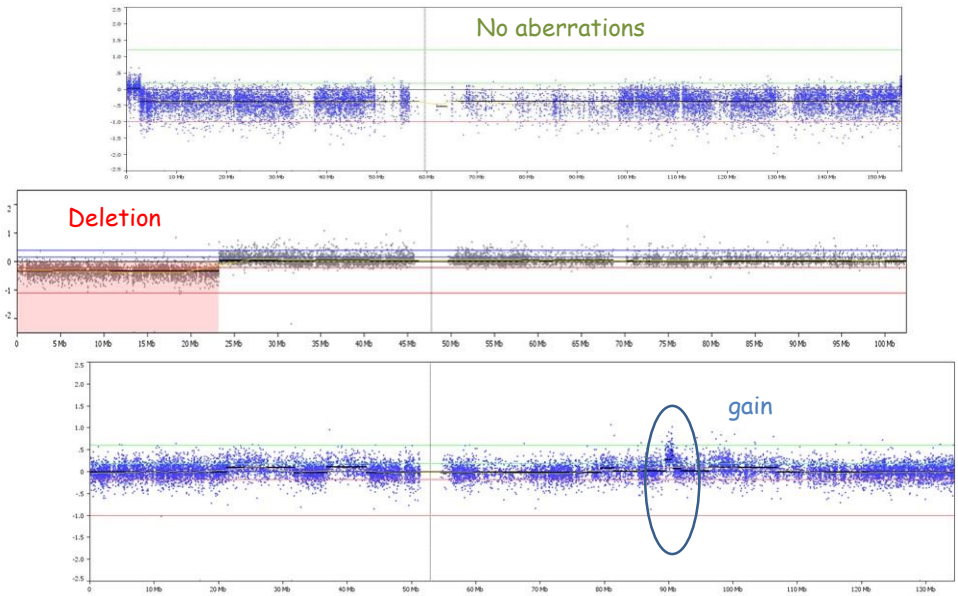
SNP chips and CNVs



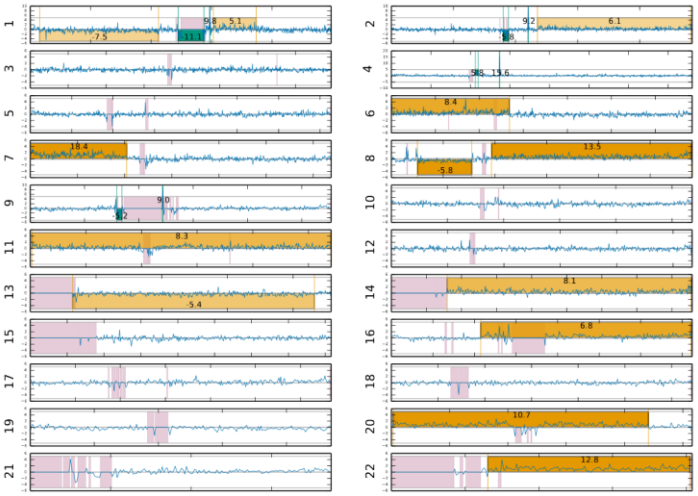


Chromosome 8

Analysis of chromosomal aberrations using GWAS

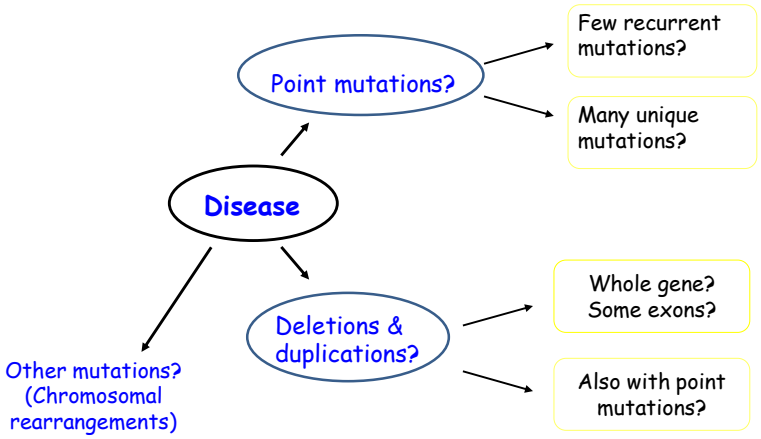


Analysis of chromosomal aberrations using GWAS

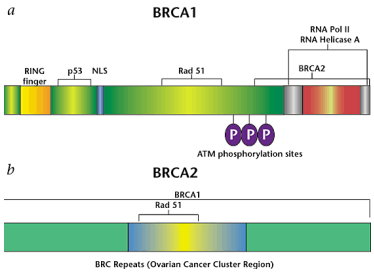


Genetic Testing

Types of Mutations Tested



Molecular diagnostics – single gene tests

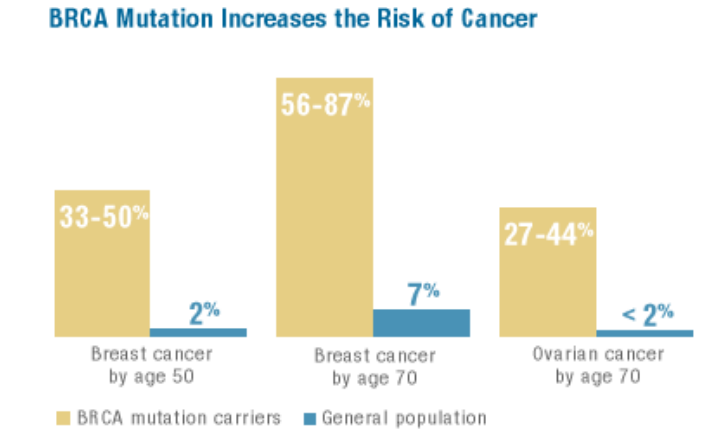


Women who have inherited mutations in these genes face a much higher risk of developing breast cancer and ovarian cancer compared with the general population.

The BRCA gene test isn't routinely performed on women at average risk of breast and ovarian cancers.

Inherited BRCA gene mutations are responsible for about 5 percent of breast cancers and about 10 to 15 percent of ovarian cancers.

BRCA1&2 mutations



Molecular diagnostics – single gene tests

After having a BRCA gene test performed, you learn whether you carry an inherited BRCA gene mutation and receive an **estimate of your personal risk** of breast cancer and ovarian cancer.

How do you perform the test?

Multiple gene testing

TruSight One
Targeting > 4,800 genes; enabling labs to expand and streamline their assay portfolio and sequencing portfolio

TruSight HLA
Accurate, unambiguous, phase-resolved HLA typing in a single assay

TruSight Myeloid
Uses expert-defined content to identify somatic mutations in myeloid malignancies

TruSight Cancer
Targeting genes previously linked to a predisposition towards cancer

TruSight Tumor 15
Focused panel assesses common somatic variants in solid tumors

TruSight Cardio
Focusing on identifying inherited cardiac conditions

TruSight Inherited Disease
Focusing on severe, recessive pediatric onset diseases

TruSight Autism
Assisting in the investigation of genomic features associated with autism

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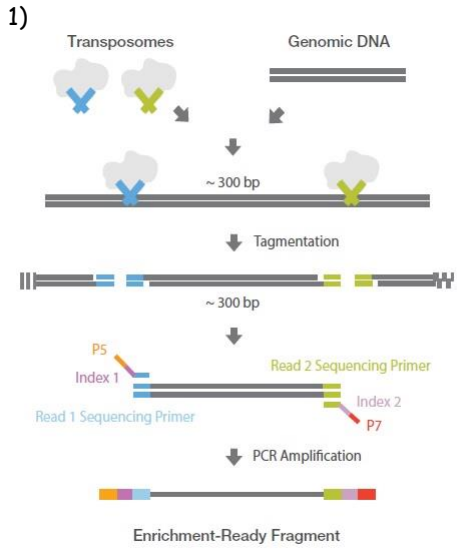
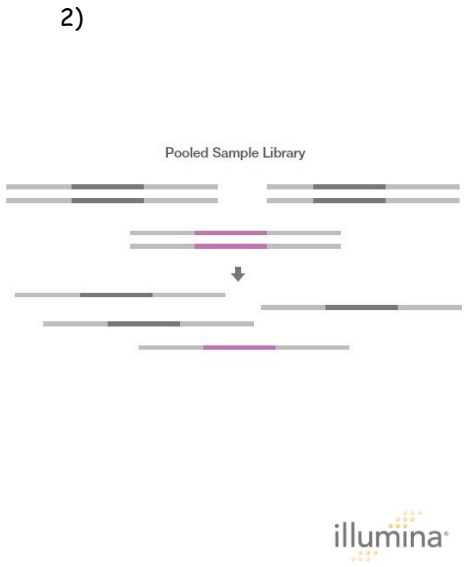
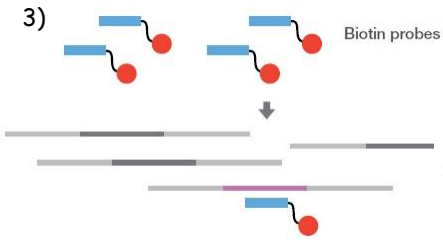
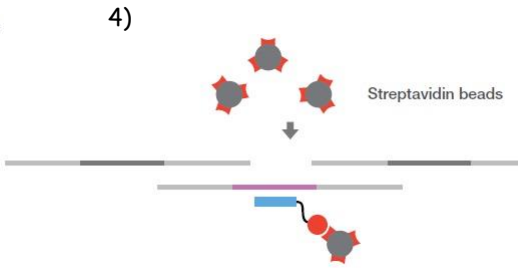
Multiple gene testing – sequence capture approach

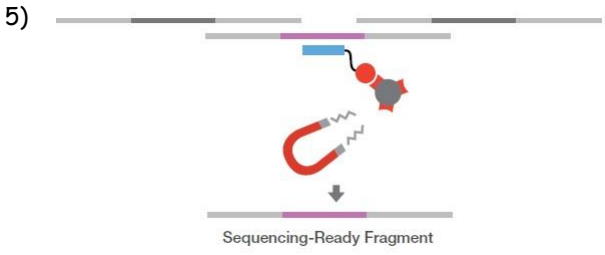




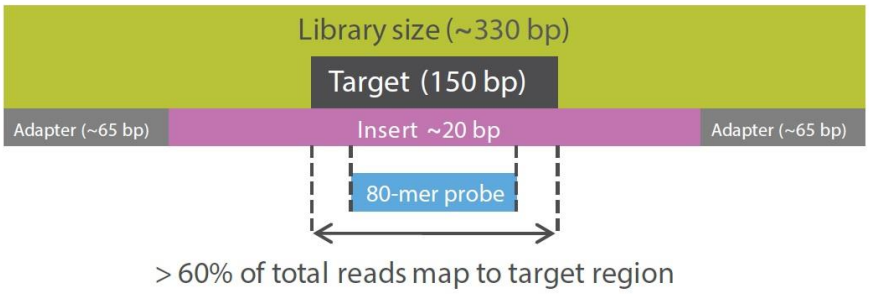
Multiple gene testing – sequence capture approach







Multiple gene testing – sequence capture approach



With an approximately 500 bp DNA library (insert size of 300 bp), the probe will enrich 250–650 bp centered around its midpoint.

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TruSight Cardio Kit – Inherited Cardiac Conditions

174 genes – 17 ICCs

Cardiac Condition	No. of Genes Covered
Aortic Valve Disease	3
Marfan Syndrome	3
Loeys-Dietz Syndrome	4
Short QT Syndrome	4
Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)	6
Familial Hypercholesterolemia	7
Restrictive Cardiomyopathy	9
Non-Compaction Cardiomyopathy	10
Noonan Syndrome	11
Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)	11
Brugada Syndrome	13
Structural Heart Disease	15
Long QT Syndrome	15
Familial Aortic Aneurysm	16
Familial Atrial Fibrillation	21
Hypertrophic Cardiomyopathy	47
Dilated Cardiomyopathy	59

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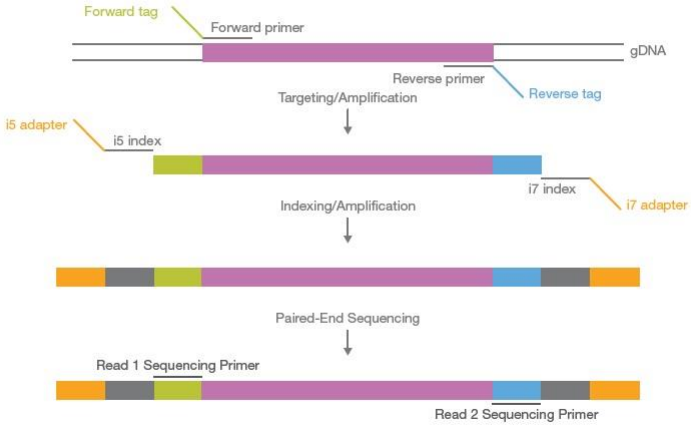
Multiple gene testing – amplicon sequencing



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TruSight Tumor 15

AKT1	*GNA11*	*NRAS*
BRAF	*GNAQ*	*PDGFRA*
EGFR	*KIT*	*PIK3CA*
ERBB2	*KRAS*	*RET*
FOXL2	*MET*	*TP53*

Parameter	Details
Panel Size	44 kb
Content	250 amplicons
Amplicon Size	Average ~150–175 bp
DNA Input Requirement	20 ng total (10 ng × 2 reactions)
Library Preparation Time	7 hours total time, 3.5 hours hands-on time
Sequence Run Time	27 hours on the MiSeq System
Sequence Run	2 × 151 bp
Sample Throughput	8 samples per run using MiSeq v3 chemistry
Variant Frequency	5%
Amplicon Coverage	93.5% of bases covered at ≥ 500×

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Multiple gene testing

	TruSight® Cancer	TruSight Tumor 15	TruSight Myeloid	TruSeq® Amplicon Cancer Panel
Key use	Germline mutation detection	Focused panel to assess relevant solid tumor somatic variants in a simple, sample-to-data workflow	Somatic mutation detection in myeloid malignancies	Somatic mutational hotspots in a broad spectrum of cancers
Catalog number	FC-121-0202/TG-141-1002	OP-101-1001/OP-101-1002	FC-130-1010	FC-130-1008/TG-130-1008 (and WG-321-1001 for FFPE QC)
Workflow	Enrichment	Amplicon	Amplicon	Amplicon
Genomic content	255 kb (~4000 probes, 94 genes)	44 kb (250 amplicons, 15 genes)	~141 kb (568 amplicons, 54 genes)	> 35 kb (212 amplicons, 48 genes)
DNA input	50 ng	20 ng	50 ng	150 ng (250 ng for FFPE)
FFPE compatible	Possible but not supported	Yes	Possible but not supported	Yes
Read length	2 × 151 bp	2 × 151 bp	2 × 151 bp	2 × 151 bp
Sequencing depth	20×	≥ 500× minimum coverage	~500× coverage	~1000× average coverage
Kit size	8, 16, 48, 96, or 288 samples	24 samples	96 samples	96 samples
Ideal instrument	MiSeq® or NextSeq® Series	MiSeq Series	MiSeq or NextSeq Series	MiSeq or NextSeq Series
Alignment and variant calling	MiSeq Reporter Enrichment workflow or BaseSpace® Enrichment Apps	MiSeq Reporter with Somatic Variant Caller	MiSeq Reporter with Somatic Variant Caller; BaseSpace TruSeq Amplicon - App	MiSeq Reporter with Somatic Variant Caller; BaseSpace TruSeq Amplicon - App
Filtering and annotation	VariantStudio	VariantStudio; Predefined Variant Report	VariantStudio	VariantStudio (with IA parts)

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Multiple gene testing - Illumina ADME panel

Genetic variability associated with drug response and variability

ABCB1	CYP2C19	DPYD	SLC22A1	TPMT
ABCC2	CYP2C8	GSTM1	SLC22A2	UGT1A1
ABCG2	CYP2C9	GSTP1	SLC22A6	UGT2B15
CYP1A1	CYP2D6	GSTT1	SLCO1B1	UGT2B17
CYP1A2	CYP2E1	NAT1	SLCO1B3	UGT2B7
CYP2A6	CYP3A4	NAT2	SLCO2B1	VKORC1
CYP2B6	CYP3A5	SLC15A2	SULT1A1	

GoldenGate Assay

- absorbtion
- distibution
- metabolism
- excretion

134 markers in 34 genes

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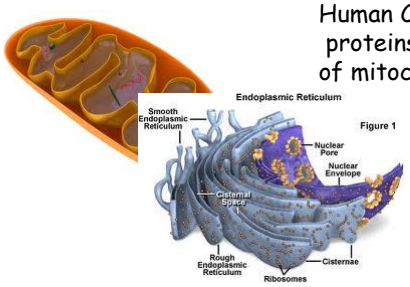
Drug transport & metabolism

The cytochrome P450 superfamily (officially abbreviated as CYP) is a large and diverse group of enzymes that catalyze the oxidation of organic substances.

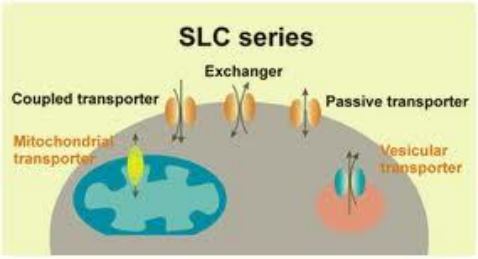


Human CYPs are primarily membrane-associated proteins located either in the inner membrane of mitochondria or in the endoplasmic reticulum of cells.

Multi-drug transporters belong to the superfamily of ATP Binding Cassette (ABC) proteins, present in organisms from bacteria to humans, located in the plasma membrane of the cells or in the membrane of different cellular organelles, and mediate the translocation of various molecules across these barriers (48 genes, ATP-dependent efflux pumps)

Drug transport & metabolism

The **solute carrier** (SLC) group of membrane transport proteins include over 300 members organized into 52 families.



Most members of the SLC group are located in the cell membrane

NAT1 & 2 arylamine N-acetyltransferases – catalyze the transfer of an acetyl group from acetyl-CoA to various arylamine and hydrazine substrates, take part in drug metabolism

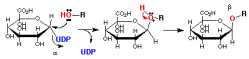
Thiopurine methyltransferase (TPMT) involved in metabolism of the thiopurine drugs such as azathioprine

Drug transport & metabolism

UDP glucuronosyltransferases (UGT) – glucuronidate : bilirubin,
steroids :testosterone and estrogen;
serotonin, drugs such as acetaminophen and morphine

Glucuronidation

- UDP-glucuronic acid reacts with alcohol (OH), carboxylic acids (CO_2H), amines and amides (NH_2),and thiols (SH)
- For example:



GSTM1 is a human glutathione S-transferase - detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress, by conjugation with glutathione

VKORC1 – reductase, originally identified as an enzyme activating vitamin K

Genodieta – test DNA

Współczesna dietetyka

Genodiet Slim

Identyfikuje różne warianty kodu DNA, które wpływają na indywidualną zdolność metabolizmu tłuszczu i węglowodanów. Jest więc największym sprzymierzeńcem w osiągnięciu i utrzymaniu odpowiedniej dla naszego organizmu masy ciała, ale również do zapobiegania chorobom metabolicznym takim jak: cukrzyca, miażdżyca itp.

6 profili dietetycznych:

- **A** Metabolizm lipidów jest wyższy niż węglowodanów. Obecność pewnych genów w tym genotypie oznacza, że osoba powinna skupić się na dobrych tłuszczach.
- **B** Wskazane jest kontrola spożycia węglowodanów
- **C** Utracie masy sprzyja zmniejszenie spożycia węglowodanów. Tłuszcze jednonienasycone mogą być bardzo korzystne.
- **D** Tłuszcze nasycone powodują przyrost masy ciała i wzrost poziomu cholesterolu.
- **E** Mniejsza wrażliwość na węglowodany, niż na lipidy. Dieta bogata w zbyt dużą ilość lipidów spowoduje wzrost masy ciała i obwodu w pasie.
- **F** Mniejsza wrażliwość na tłuszcze, ale bardzo duża na węglowodany.

Human Glucose Metabolism PCR Array

profiles the expression of 84 key genes involved in the regulation and enzymatic pathways of glucose and glycogen metabolism

Glucose Metabolism:

Glycolysis: ALDOA, ALDOB, ALDOC, BPGM, ENO1, ENO2, ENO3, GALM, GCK, GPI, HK2, HK3, PFKL, PGAM2, PGK1, PGK2, PGM1, PGM2, PGM3, PKLR, TPI1.

Gluconeogenesis: FBP1, FBP2, G6PC, G6PC3, PC, PCK1, PCK2.

Regulation: PDK1, PDK2, PDK3, PDK4, PDP2, PDPR.

TCA Cycle: ACLY, ACO1, ACO2, CS, DLAT, DLD, DLST, FH, IDH1, IDH2, IDH3A, IDH3B, IDH3G, MDH1, MDH1B, MDH2, OGDH, PC, PCK1, PCK2, PDHA1, PDHB, SDHA, SDHB, SDHC, SDHD, SUCLA2, SUCLG1, SUCLG2.

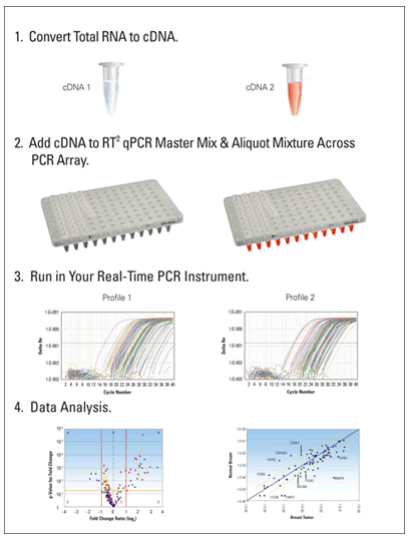
Pentose Phosphate Pathway: G6PD, H6PD, PGLS, PRPS1, PRPS1L1, PRPS2, RBKS, RPE, RPIA, TALDO1, TKT.

Glycogen Metabolism:

Synthesis: GBE1, GYS1, GYS2, UGP2.

Degradation: AGL, PGM1, PGM2, PGM3, PYGL, PYGM.

Regulation: GSK3A, GSK3B, PHKA1, PHKB, PHKG1, PHKG2.



Should we believe this?

SKINDNA

Home | Test Categories | Learning Centre | Media New | About Us | Contact Us

SkinDNA ™ is a revolutionary DNA laboratory test that examines 16 genetic markers (SNPs) in 5 categories associated with skin aging.

1. Firmness + Elasticity
2. Wrinkling (A.G.E.)
3. Sun Damage + Pigmentation
5. Free Radical Damage
4. Sensitivity + Inflammation

Your DNA results are used to scientifically create a personalised guide provide you with a unique regime tailored specifically to you. This allows you to advance beyond the 'one-size-fits-all' suggestions - using the right skincare ingredients targeted to your own genetic blueprint.

We know that not every skincare product is going to suit everyone no matter how much the cosmetic industry tells you. And this is why there are SO many brands out there and literally thousands of different products.

Let SkinDNA ™ take the guesswork out of skin care, instead using science to identify the most suitable skincare ingredients - based on your DNA.

Should we believe this?

SKINDNA

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1 in 3 people have a genetic variation that predisposes their skin to accelerated collagen breakdown.

The SkinDNA ™ Genetic Test can help identify if you are a carrier of key genetic variations associated with accelerated collagen loss - even before the signs have become visible.

Keeping the skin firm, plump and wrinkle-free, Collagen is the principle structural protein of the skin. Like many components of the body, collagen undergoes continuous turnover, being produced and recycled on an ongoing basis throughout your life. When you are younger, your body makes more collagen than it loses, but after about the age of 40, collagen loss can accelerate, leading to a decline in the health and appearance of your skin.

Our genetic predispositions play a big role in determining both the speed of collagen production and breakdown. Key variations in this genetic category can identify if the rise and fall of collagen is in balance, or if the breakdown of collagen predominates, which can result in the appearance of premature wrinkling, aging and sagging of the skin.

Genetic Markers SkinDNA ™ Test for in this category.

Gene Table

SkinDNA ™ Gene Descriptor	Genotype	Description
s700298/ **Collagen Breakdown**	1G1G	Involved in slowing the breakdown and degradation of Collagen fibers found in the extracellular matrix of human tissue. Chromosome Location: 11q21-q22
s706371/ **Collagen Protection**	TT	Assists in protecting existing collagen from unnecessary degradation and aids in normalising skin cell functions disrupted by oxidative stress including MMP-1 production Chromosome Location: 3q21.3

Multiple gene testing- targeted assays

Amplicon sequencing
Sequence capture

Small sequencers

MiSeq

~35GB

Ion Proton

Multiple gene testing- targeted assays

Amplicon sequencing
Sequence capture

MiniSeq

~16GB

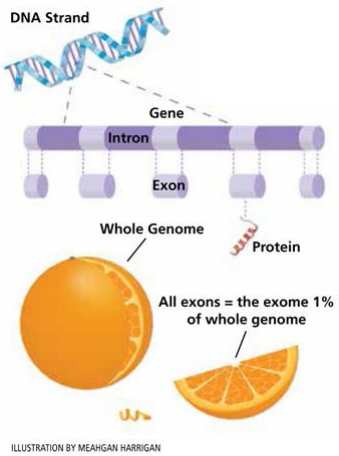
Whole genome exome sequencing (WES)

- challenging regions of the genome with current sequencing platforms:

  no high GC content fragments, large repeat regions, centromeres, telomeres

- focuses on just the protein coding sequences 1 – 2% of the genome

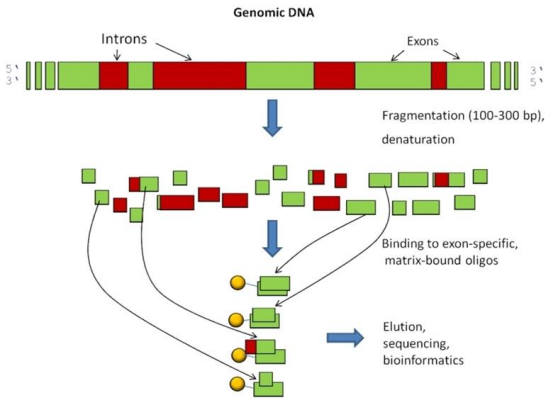
- WES samples are typically sequenced to a higher depth (100X vs 30X)



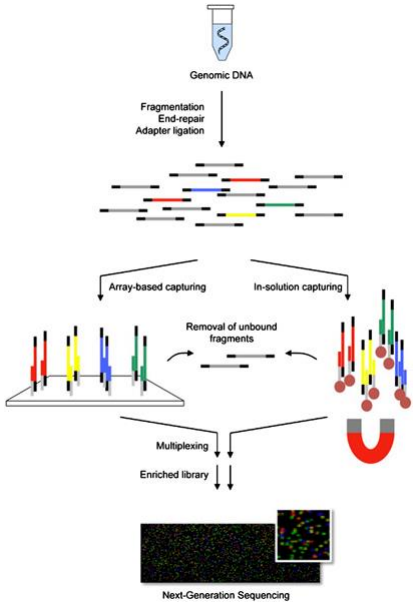
- The amount of sequence needed for a 100X exome sample is ~5-6Gb,

  substantially less than the ~90Gb needed for WGS

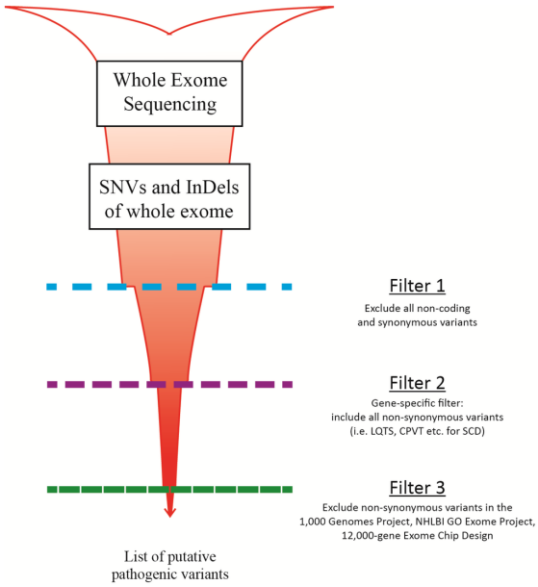
Whole exome sequencing –the principle



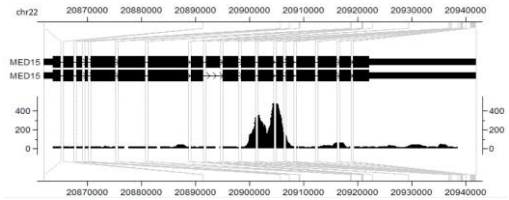
Whole exome sequencing –the principle



WES – the data



Disadvantages of WES



- The enrichment steps involved in WES lead to non-uniform coverage:

'hot spots' with too much coverage (a waste of sequencing power)

regions with too little coverage (leading to missed variant calls)

a region dense with SNPs can interfere with the capture process, as the enrichment baits may not hybridize as efficient

- Maximally 2x100bp reads

the longer reads available for whole genome sequencing allows for better determination of copy number variations, rearrangements and other structural variations

Who should consider WES??

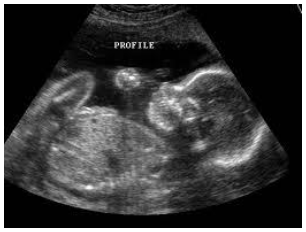
- Individuals who have had extensive genetic testing but a diagnosis has not yet been identified
- Individuals who very likely have a genetic condition but the diagnosis is unclear
- Individuals who have a genetic condition that could be caused by many different genes
- Individuals with an undiagnosed disorder who want to be as aggressive about determining if the cause is genetic

Genetic Testing - Pre-natal Diagnostics

130 million live births worldwide per year

8 million live births in US and Europe per year

6% of all babies are born with birth defects over 900 fetal genetic disorders



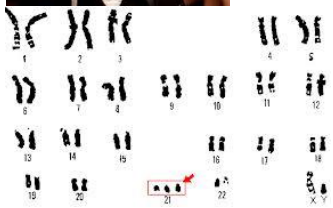
only non-invasive method: Ultrasound

No accurate, non-invasive prenatal diagnostic tests ("NIPD") available

Genetic Testing - Pre-natal Diagnostics

Down syndrome is the most common chromosomal abnormality

= trisomy 21



Although risk increases with age, 80% of Down births are in women <35 years old

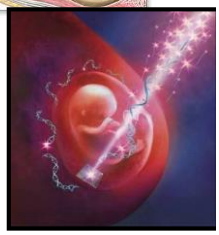
Sensitivity????

Even though limited to high risk mothers, still a $600 million market in US and $1.5 billion market worldwide

Other diseases : Cystic fibrosis?

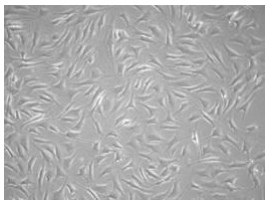
Genetic Testing - Pre-natal Diagnostics

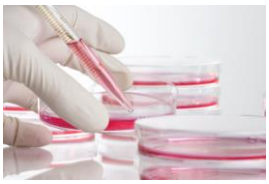
at the moment invasive methods available -> require a certain amount of fetal cells



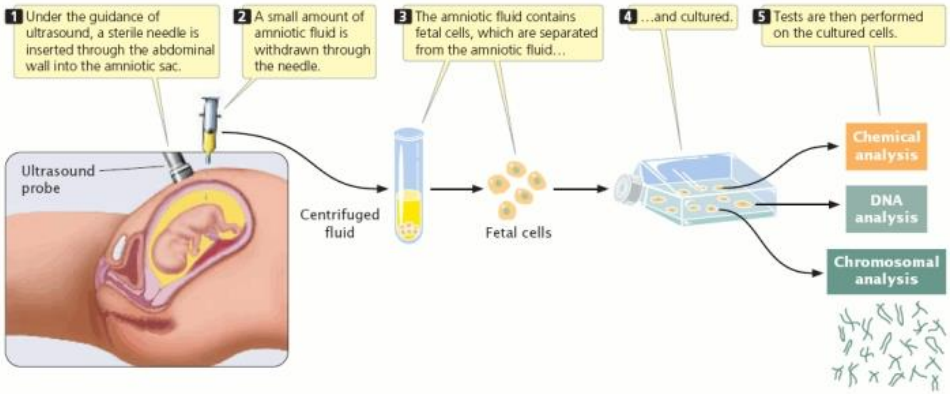
Amniotic stem cells are collected from amniotic fluid extracted during a genetic amniocentesis, a prenatal diagnosis procedure typically performed during the 2nd trimester of pregnancy.

cells are heterogeneous cell population of exfoliated fetal and amniotic cells





Genetic Testing - Pre-natal Diagnostics



a risk of miscarriage or infection that is estimated at about 1 in 400

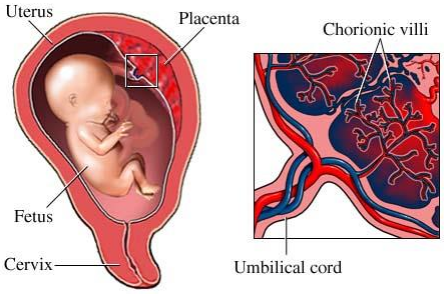
In vitro steps – chromosomal instability

w/o cell culture - small DNA amounts

Genetic Testing - Pre-natal Diagnostics

fetal blood cells gain the access to maternal circulation through the placental villi.

Ordinarily, only a very small number of fetal cells enter the maternal circulation in this fashion



it is difficult to get many fetal blood cells.

There may not be enough to reliably determine anomalies of the fetal karyotype or assay for other abnormalities

Whole Genome SNP Genotyping

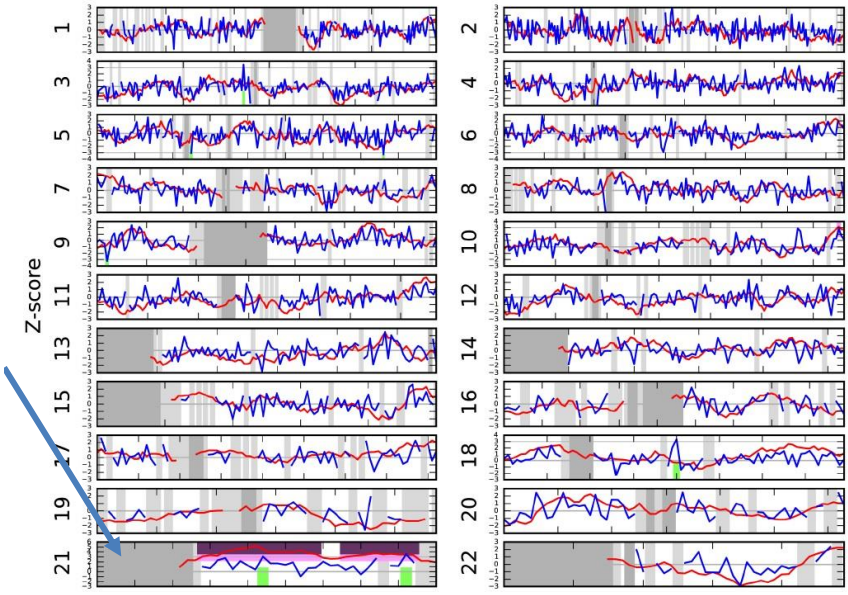
Omni Whole-Genome Arrays

BeadChip	Array Format	Markers per Samp
HumanOmni5-Quad	4	~ 4.3 million
HumanOmni2.5S	8	~ 2.5 million
HumanOmni2.5-8	8	~ 2.5 million
HumanOmni1S	8	~ 1.25 million
HumanOmni1-Quad	4	~ 1.1 million
HumanOmniExpress	12	~ 700,000
HumanCytoSNP-12	12	~ 300,000

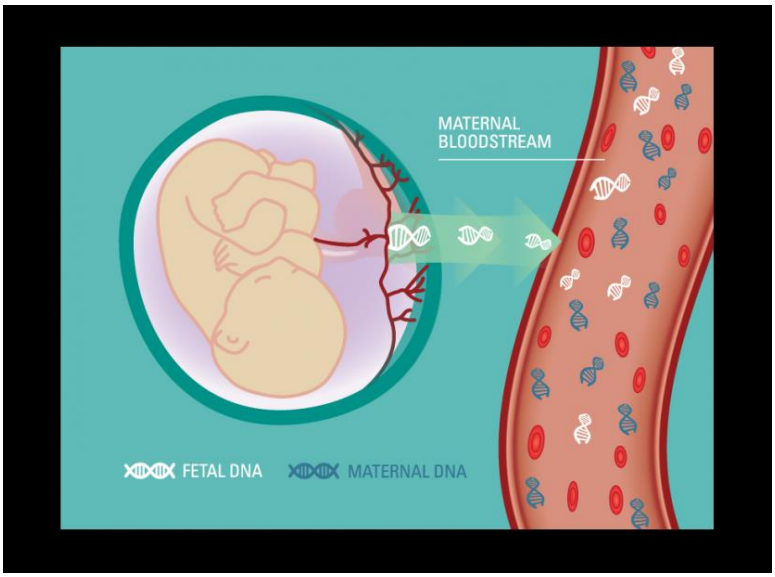
Omni Semi-Custom Whole-Genome Arrays

BeadChip	Array Format	Markers per Sample
HumanOmni5-Quad+	4	~ 4.3 million (fixed) up to 500K (custom)
HumanOmni2.5S+	8	~2.5 million (fixed) up to 500K (custom)
HumanOmniExpress+	12	~700,000 (fixed) up to 200K (custom)

Analysis of chromosomal aberrations using GWAS
Pre-natal Diagnostics



Genetic Testing - Pre-natal Diagnostics- NIPT



Free fetal DNA

Genetic Testing - Pre-natal Diagnostics

1-5% of isolated DNA

~ 5-10 mln aligned reads

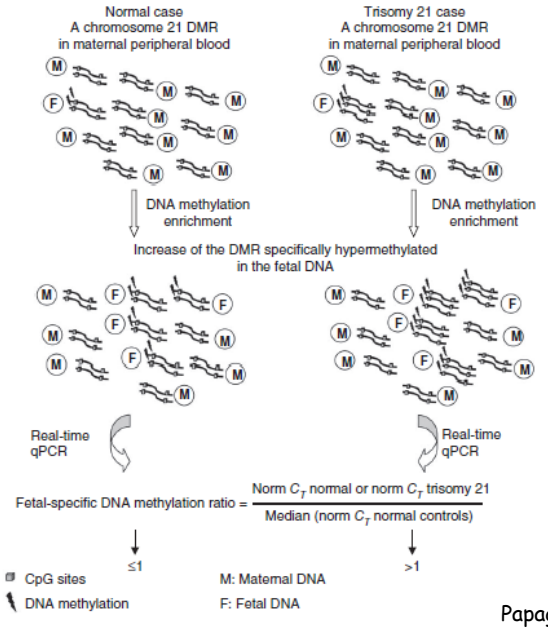
NIPT using cell-free DNA in maternal blood

- since 2014: **TRIDENT study** in the Netherlands
  - Trisomy 21
  - Trisomy 18
  - Trisomy 13

Patients with increased risk after first trimester combination test may choose between NIPT and invasive array testing.

ONLY LARGE CHROMOSOMAL REARRANGEMENTS

MeDiP -methylated DNA immunoprecipitation



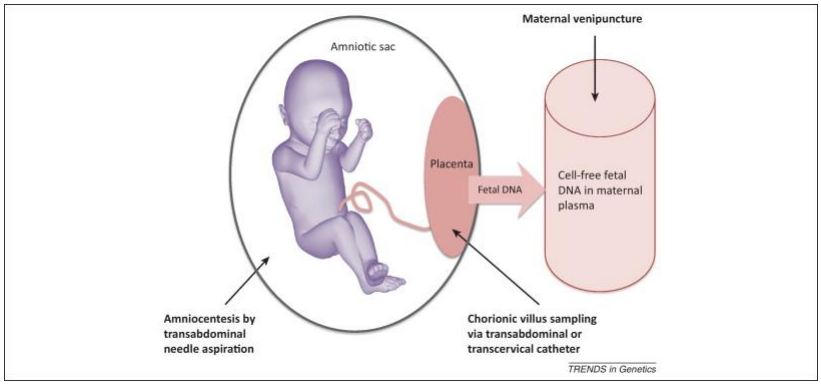
Assay based on free fetal DNA (ffDNA) in the maternal circulation during pregnancy (maternal plasma)

fetal-specific methylated regions located on chromosome 21

antibody specific for 5-methylcytidineto capture methylated sites and therefore enrich for fetal-specific methylated DNA

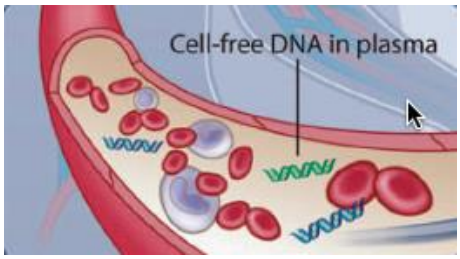
Papageorgiou et al., Nature Medicine, 2012

Genetic Testing - Pre-natal Diagnostics

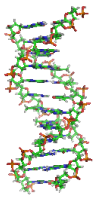


Hui et al., 2013

Diagnostics – novel approaches



Cell free DNA (cfDNA)

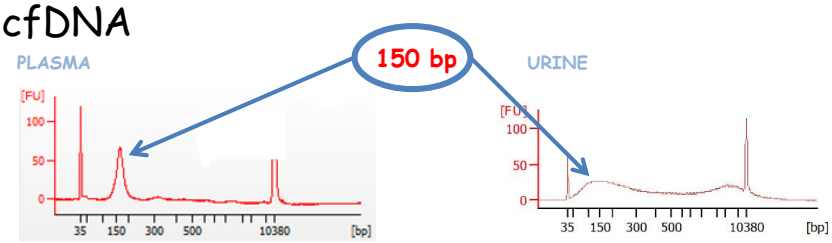


- Discovered in 1948 in blood by Mendel and Metais
- Recent studies revealed that concentrations of cfDNA is very low in healthy individuals
  - And is higher in cancer patients and patients with autoimmune diseases
- In healthy individuals cfDNA are longer than 200 bp.
- The length of „true" cell free DNAs is shorter than 150 bp in cancer

cfDNA concentration in plasma and urine of ccRCC patients

mean concentration of cfDNA (ng/µl)	
plasma	urine
7,09	0,885

Quality of cfDNA



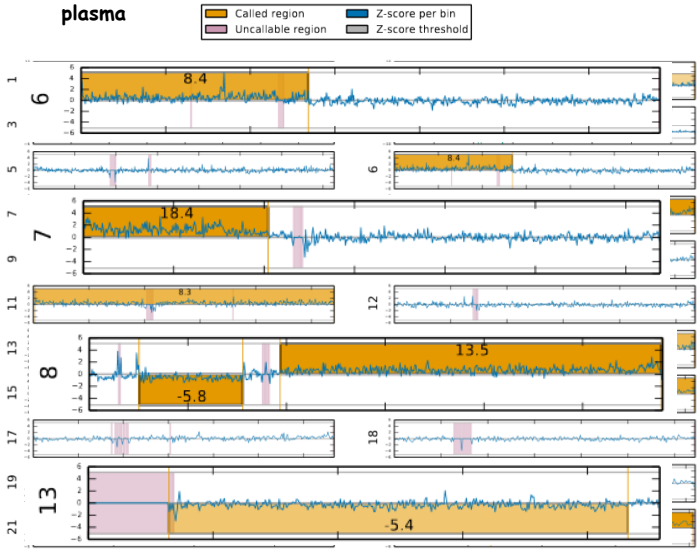
Shallow NGS Sequencing

Libraries sequenced using HiScanSQ

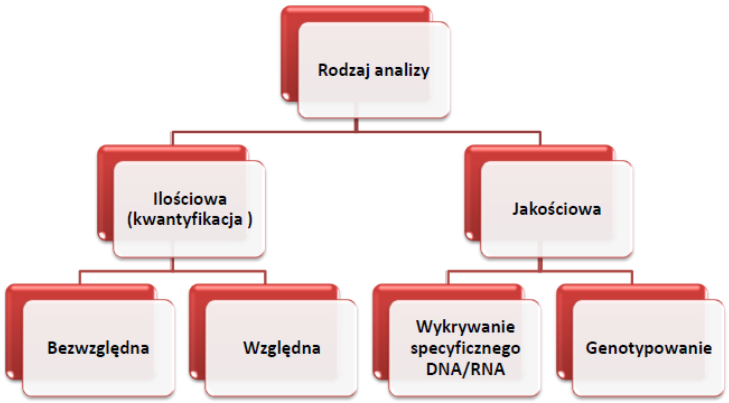
SR50

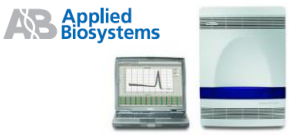
Mean Reads Identify PF	Mean Aligned Reads
8 895 148	8 541 721

Results- Wisecondor

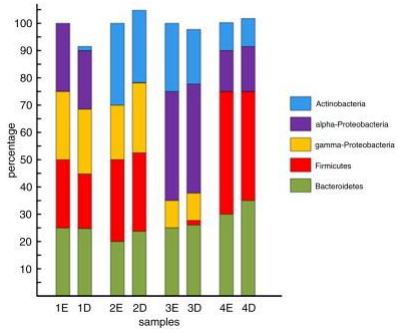


Real-Time PCR in molecular diagnostics





Panbacterial qPCR



Amplification and sequencing of the gene encoding 16S ribosomal RNA using universal primers

rapid and wide-spectrum detection of bacteria

all clinical samples can be tested

detection of bacteria responsible for :
Meningitis
Endocarditis
Arthritis
osteitis
deep abscesses and suppurations

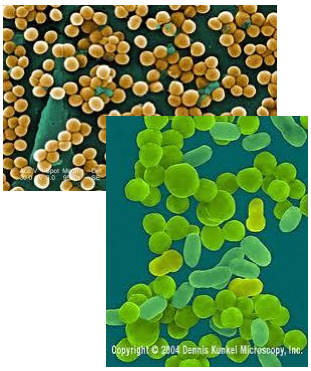
qPCR for Easy-to-Grow Bacteria

Staphylococcus Aureus,

a wide range of infections in humans including skin and soft tissue infections, arthritis, osteitis, deep abscesses, pneumonia, endocarditis, urinary tract infections, enteritis and bacteremia

methicillin-resistant *S. aureus* (MRSA) is a major nosocomial pathogen

Application: clinical samples and blood cultures



Haemophilus Influenzae, primarily involved in upper and lower respiratory tract infections, serotype b (Hib) predominate and are the more severe. Some qPCR tests also allow determination of the particular bacterial strains

Application: cerebrospinal fluid and sputum samples

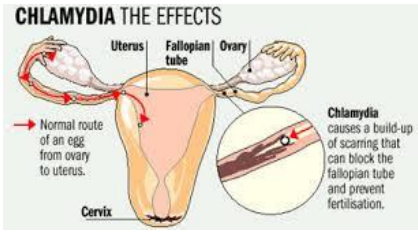
qPCR for Slow-Growing, Fastidious-Growing & Uncultivable Bacteria

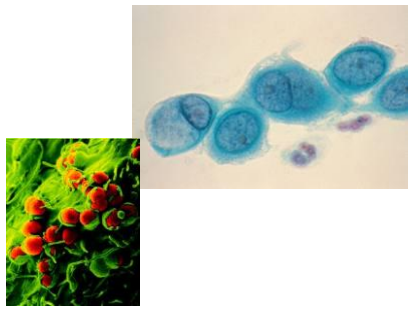
Chlamydia Trachomatis & Neisseria Gonorrhoeae

two main etiological agents of sexually transmitted infections (STIs).

Both organisms may cause obstetrical complications and infection in the neonate.

Application: urogenital samples





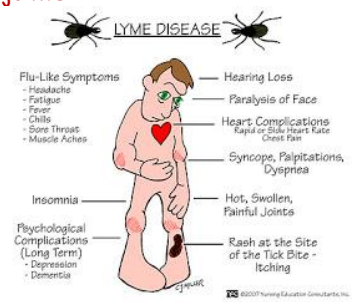
2.8 mln new cases per year in the US

qPCR for Slow-Growing, Fastidious-Growing & Uncultivable Bacteria

Borrelia burgdorferi, Borrelia afzelii* and *Borrelia garinii are responsible for most cases of Lyme disease.

These spirochetes are transmitted to humans via tick bites.
The disease should be diagnosed during the acute phase (a influenza-like illness) to prevent evolution to complications, including articular, neurological and cardiac involvement.

Application: synovial fluid or synovial membrane biopsies of affected joints



Multiplex qPCR for Diagnosis of Syndromes & Other Specific Clinical Situations

multiplex qPCR tests

Bacteremia

Bacteremia is an urgent situation that needs rapid detection and identification of the involved bacterial species in order to optimize antibiotic therapy and improve patient's prognosis

E. coli, *S. aureus* and *S. pneumoniae*.

rapid identification and evaluation of specific antibiotic resistances in bacteria grown in blood cultures

Bio-threat Agents.

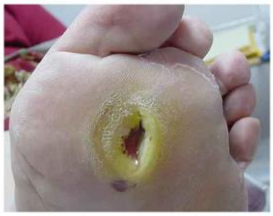
early characterization of the involved pathogen and implementation of adapted therapeutic and prophylactic measures

B. anthracis, *Y. pestis*, *F. tularensis*, *B. melitensis*, *Rickettsia* sp., *C. burnetii*

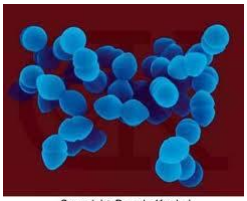
qPCR for Detection of Antibiotic Resistance Gene Determinants

In patients suffering from bacterial infections, quick determination of the antibiotic susceptibilities of the involved strain is of primary importance for rapid adaptation of the antibiotic therapy

The most widely-used commercial qPCR tests are those allowing detection of the *mecA* gene responsible for resistance to methicillin in *S. aureus*,



Also for vanA and *vanB* genes responsible for resistance to glycopeptides in *Enterococcus* species



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Quantification of Bacterial Load Using qPCR

these tests quantify DNA from both viable and nonviable bacteria,

results may be higher than those obtained by quantitative culture.

A high correlation between both methods was reported for *S. pneumoniae,* that causes many types of pneumococcal infections also other than pneumonia

In-house qPCR tests have been used for quantification of the bacterial load in clinical samples to differentiate infection from commensalism and to determine disease severity and patients' prognosis

HUMAN CAPITAL
NATIONAL COHESION STRATEGY

UAM

EUROPEAN SOCIAL FUND

Thank you for your attention