The Use of New Molecular Genetic

Technologies in Reporductive Medicine

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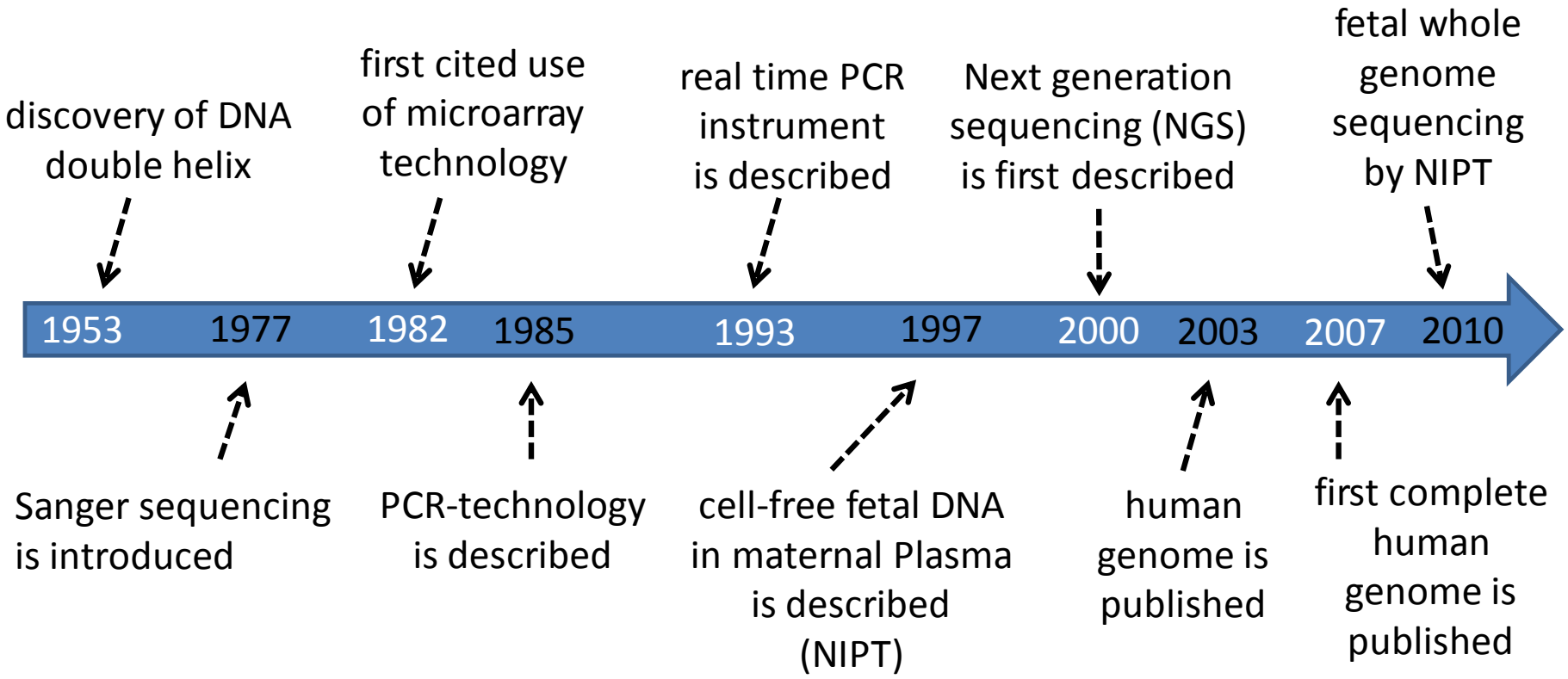
Landmarks of Molecular Genetic Technologies



How can new molecular genetic technologies help an infertile couple to get a healthy baby?

Chromosome anomalies are a main reason for pregnancy losses

approximately 0.6% of all newborn have a chromosome anomaly

10%–15% of clinically recognized pregnancies end with a first trimester loss

using assisted reproductive technologies (ART) miscarriage rates of 36-69% were reported and in up to 83% of miscarriages aneuploidy was detected

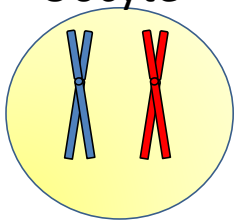
Nayak et all, 2011

Chromosome aneuploidies contribute to the vast majority of pregnancy losses in both natural as well as ART conceptions

Solution of this problem: **Identification of embryos without a chromosome anomalies**

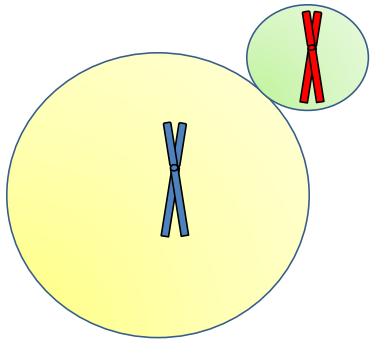
Origin of non disjunction in human

Oocyte



oocyte
46 chromosomes
each consists of
2 chromatids

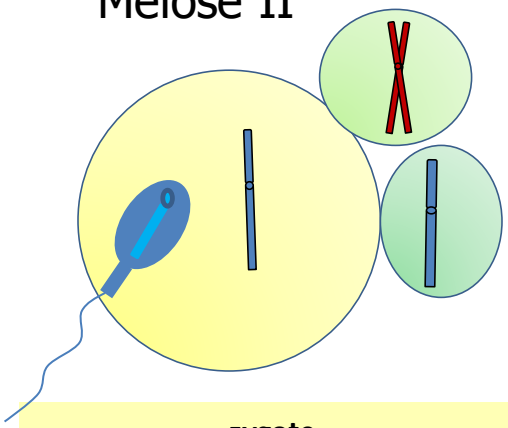
Meiose I



oocyte
23 chromosomes
each consists of
2 chromatids

first polar body
23 chromosomes
each consists of
2 chromatids

Meiose II

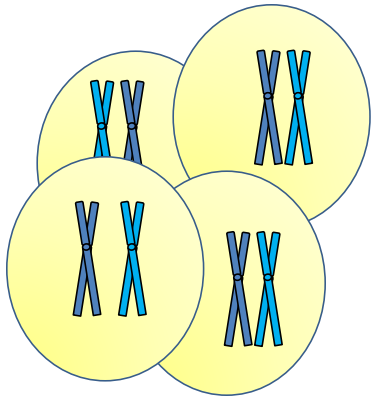


zygote
23 chromosomes each consists of
1 chromatid
23 chromosomes of the sperm

1. polar body
23 chromosomes
each consists of
2 chromatids

2. polar body
23 chromosomes
each consists of
1 chromatid

Mitose

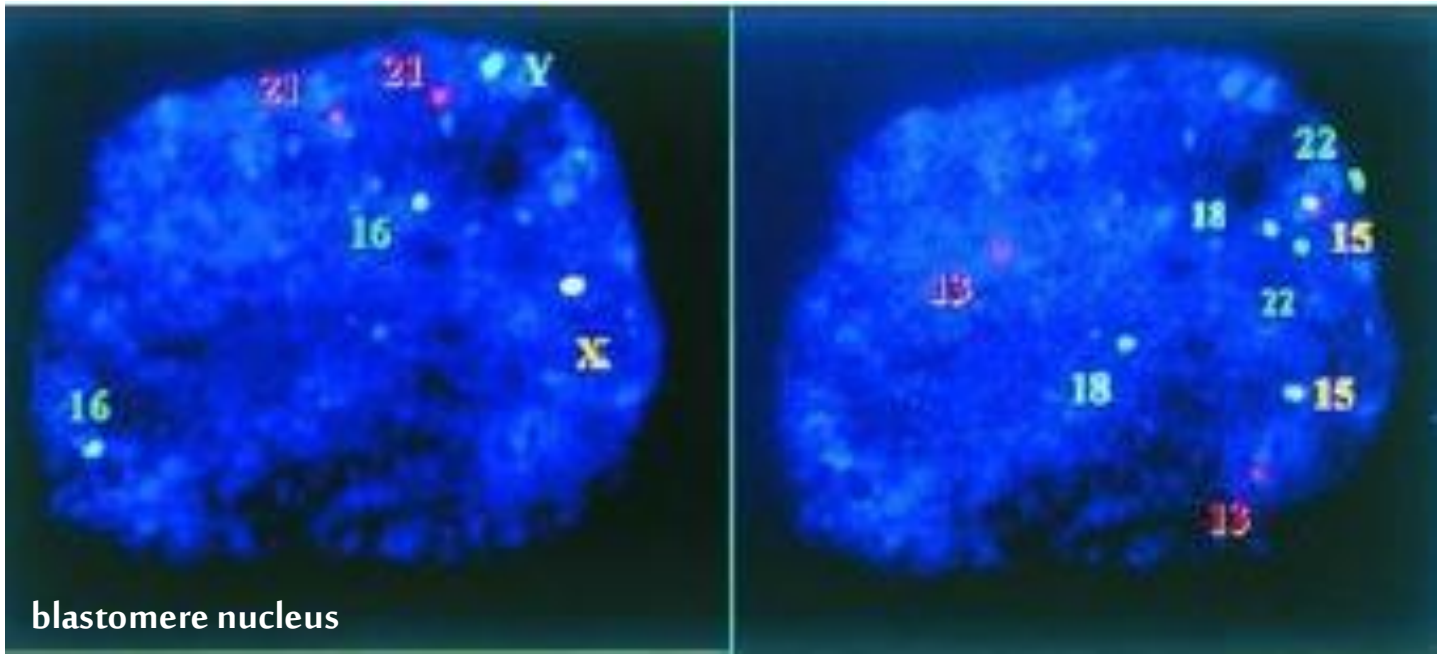


embryo
46 chromosomes each
consists of 1 chromatid
(before mitosis each
chromosome consists of 2
chromatids)

division errors for chromosome 21	maternal origin ~ 90 %	MI error: 69% MII error: 22%	mitotic origin ~ 5%
	paternal origin 5-8%	MI error: 2% MII error: 3%	

Nicolaidis and Petersen 1998 Hum Rep

Fluorescence in situ Hybridisation (FISH)



The image on the left shows the result after the first round of FISH using probes for chromosomes X (yellow), Y (aqua), 16 (green), and 21 (orange); and the one on the right shows the result after the second round of FISH using probes for chromosomes 13 (orange), 15 (yellow), 18 (aqua), and 22 (green). The blue is DAPI used as counterstaining.

http://quizlet.com/14732443/molecular-diagnosis-onward-flash-cards/

using FISH technology only few chromosomes can be tested for aneuploidy

DATA from the Literature

Hum Reprod. 2008 (12):2813-7.

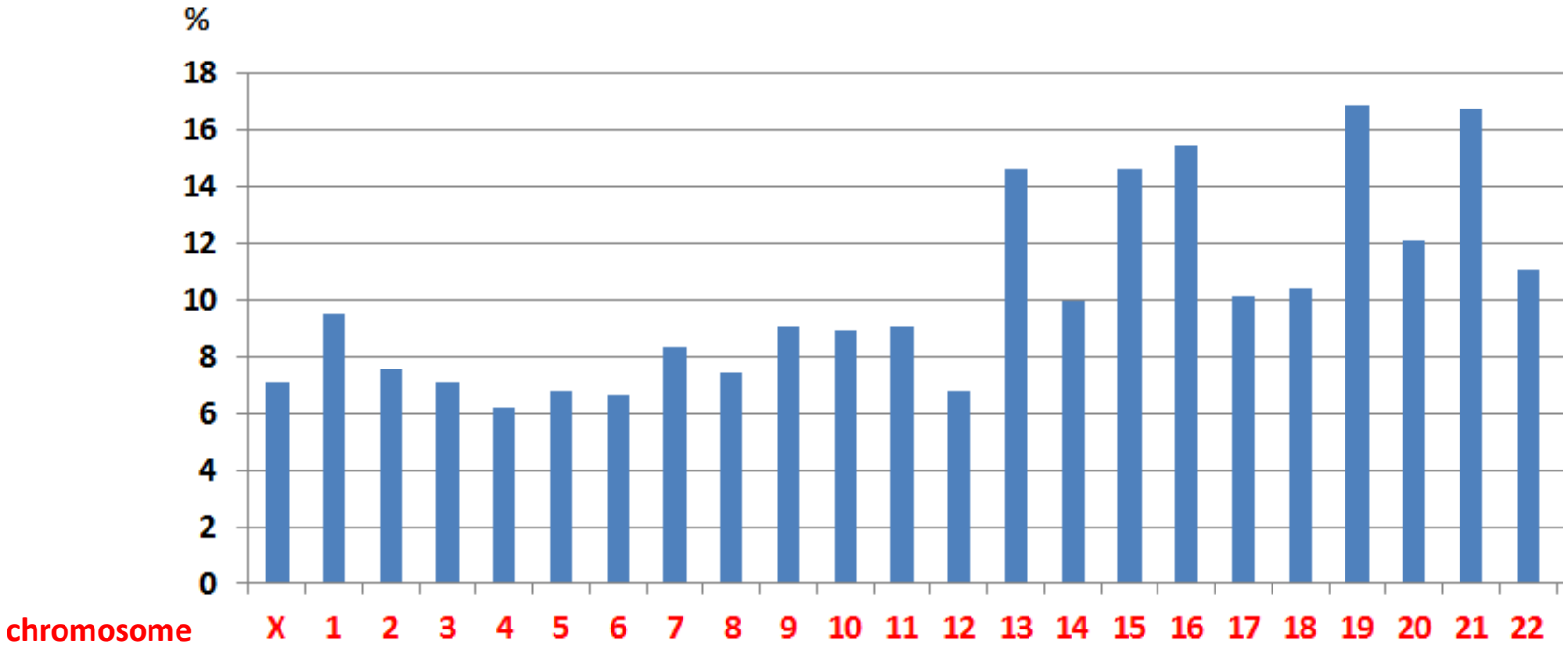
No beneficial effect of preimplantation genetic screening in women of advanced maternal age with a high risk for embryonic aneuploidy.

Twisk M, Mastenbroek S, Hoek A, Heineman MJ, van der Veen F, Bossuyt PM, Reprint S, Korevaar JC.

In the PGS group, a single blastomere was aspirated from each embryo and analysed for aneuploidies using probes for chromosomes 1, 13, 16, 17, 18, 21, X and Y.

Frequency of chromosome aneuploidies

polar bodies of 886 oocytes were analysed by array-CGH; 600 (68%) oocytes were aneuploid, meiotic seperation errors of 6-18% were observed for all chromosomes



the need for new technologies allowing to analyse all chromosomes

- array-CGH technology
- real time or quantitative PCR
- NGS

Single Nucleotide Polymorphism (SNP)

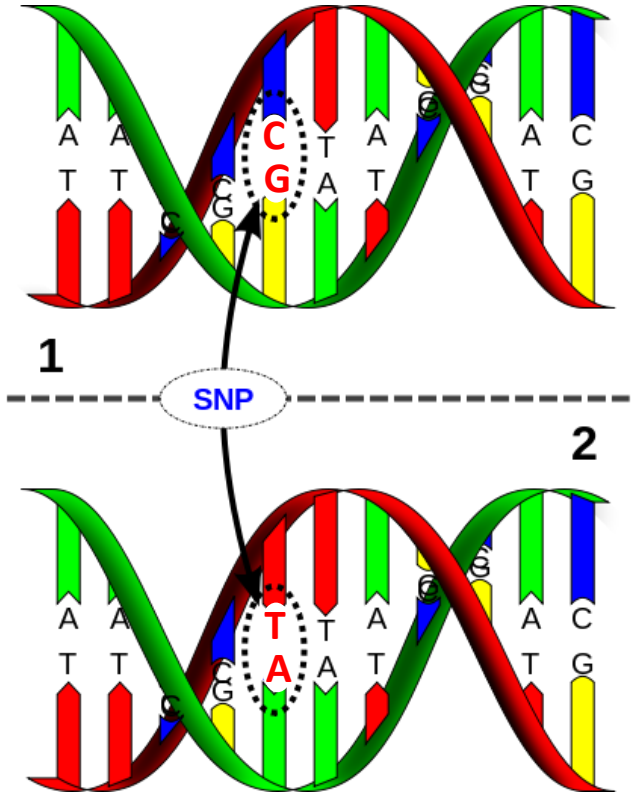
a single nucleotide alteration in genomic DNA found in > 1% of the population (<1% it is called variation)

SNPs are found every 100-300 bps of the genome

accounts for ~ 90% of genomic variation

2/3 SNPs are C – T

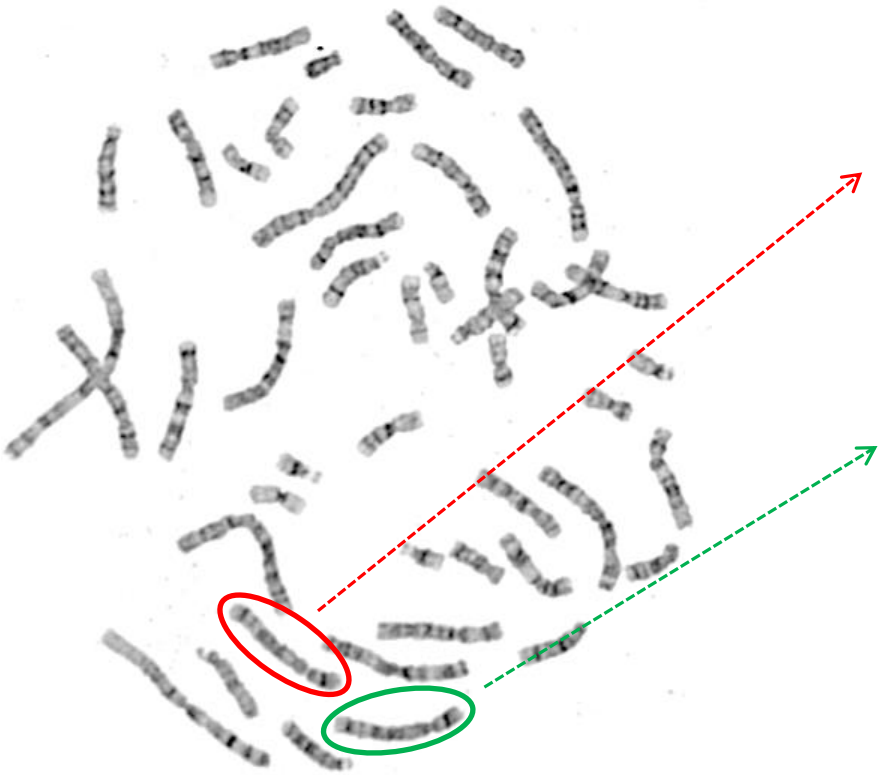
SNPs are evolutionarily stable



http://de.wikipedia.org/wiki/Single_Nucleotide_Polymorphism

Oligonucleotide and SNP

human metaphase



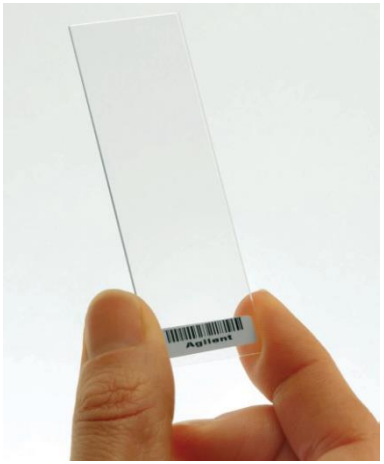
exon 4 of NSD1 gene
chromosome 5 (only forward strand is shown)

TTTCCAACCGGAGGCC**C**TATCGGCAGTACTACGT
GGAGGCTTTTGGAGATCCTTCTGAGAGAGCCTGGG
TGGCTGGAAAAGCAATCGTCATGTTTGAAGGCAGA
CATCAATTCGAAGAGCTACCTGTCCTTAGGAGAAG
AGGGAAACAGAAAGAAAAAGGATATAGGCATAAG

TTTCCAACCGGAGGCC**T**TATCGGCAGTACTACGT
GGAGGCTTTTGGAGATCCTTCTGAGAGAGCCTGGG
TGGCTGGAAAAGCAATCGTCATGTTTGAAGGCAGA
CATCAATTCGAAGAGCTACCTGTCCTTAGGAGAAG
AGGGAAACAGAAAGAAAAAGGATATAGGCATAAG

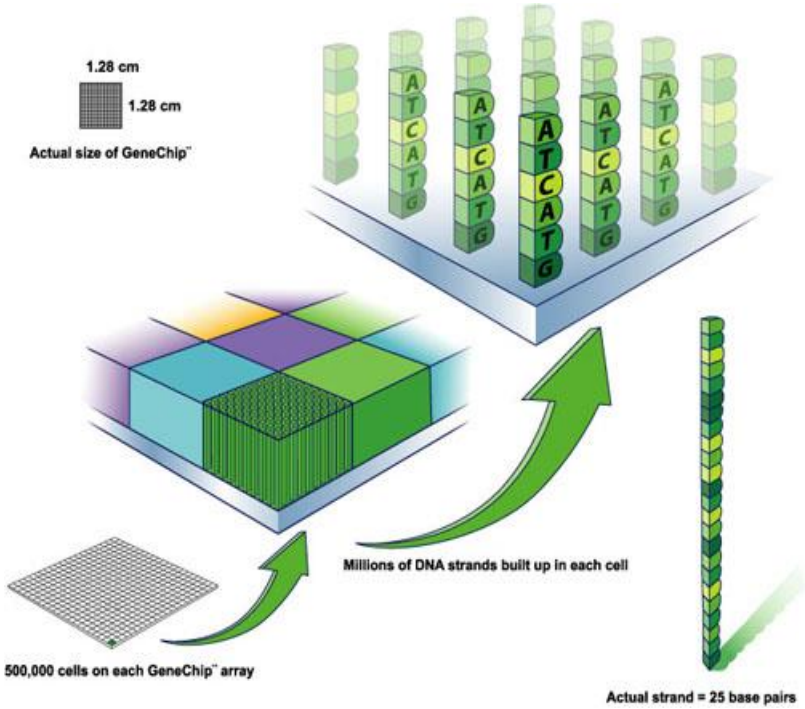
rs148455858 SNP
Alleles **C**/**T** | Ancestral: **C**
Location Chromosome **5: 177204136**
(forward strand)

Microarray - Oligonucleotide/SNP-Array



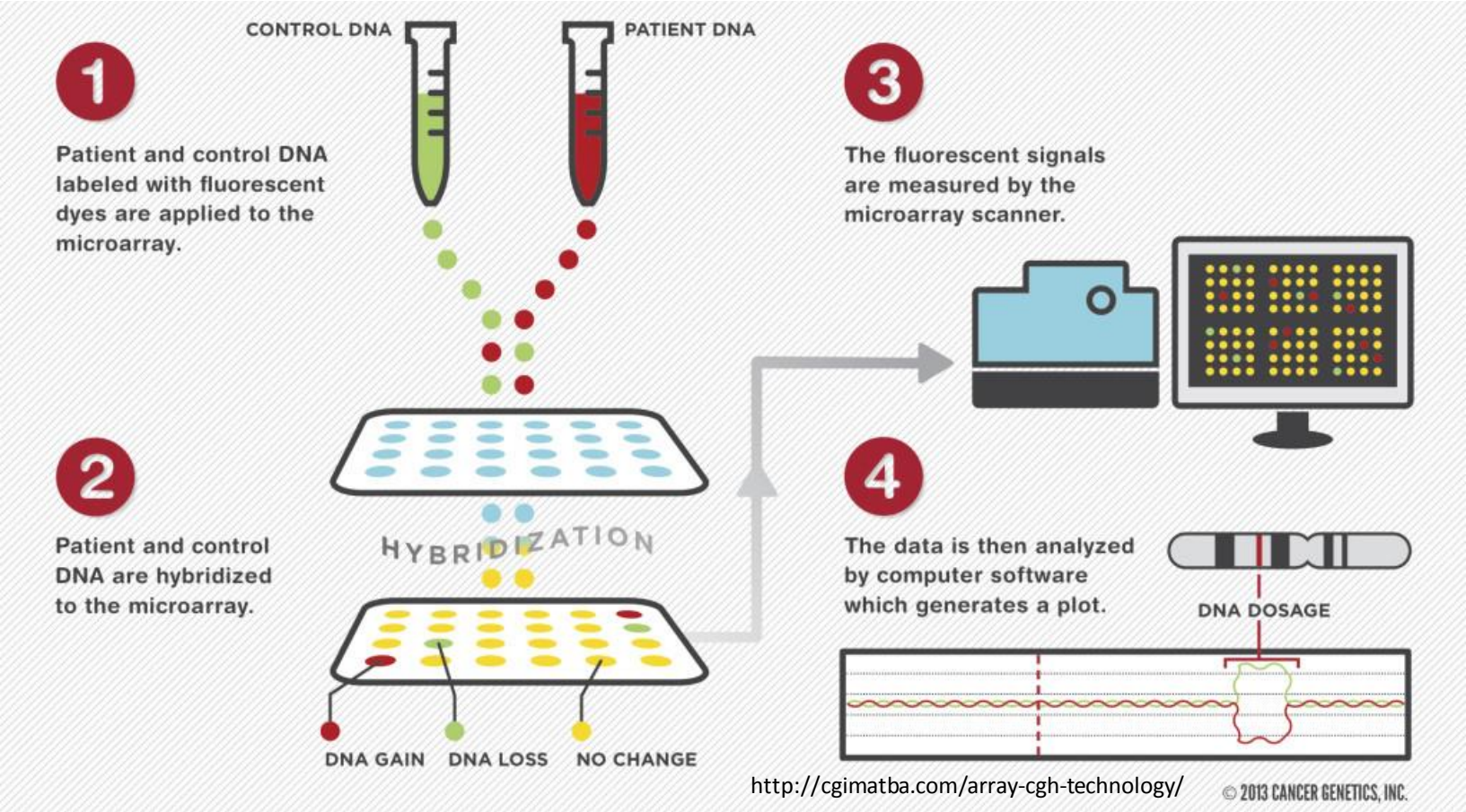
Microarray is a dual color array containing 60-mer oligonucleotide

4x 180K chip
169,991 unique probes
110,712 (CGH) + 59,647 (SNP



CytoScan 750K Array
750 000 markers
550 000 unique non-polymorphic probes
200 000 SNPs

The process of array-CGH



Agilent 500 – 1000 ng DNA
handling time ~ 1 day
hybridization ~ 24 hours
scanning and analysis 2-4 hours

Affimetrix 250 – 500 ng DNA
handling time ~ 1- 2 days
hybridization ~ 24 hours
scanning and analysis 2-4 hours

Microarray - Oligonucleotide/SNP-Array

The oligonucleotide /SNP array can detect with high sensitivity gains and losses of DNA (by detecting large stretches of SNPs homozygosity).

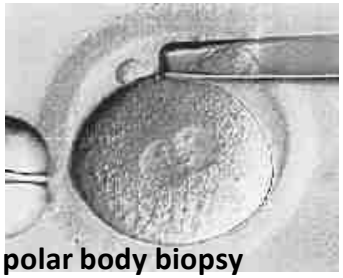
Regions of SNP homozygosity indicate:

1. chromosomal deletion
2. a possible consanguinity of the parents
3. uniparental isodisomy (complete or partial)

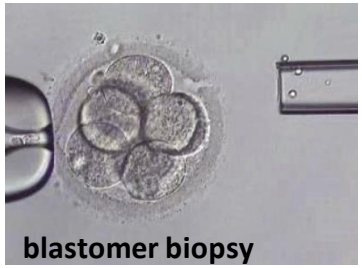
The SNP array test does not detect all changes of the chromosomes. In particular, it is not able to find structural rearrangements of the chromosomes and it cannot detect small changes in the DNA (point mutations, deletion or duplications of few nucleotides)

array-CGH analysis in PID

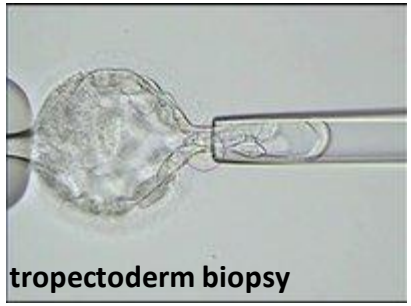
1 ml blood (several million white blood cells)
➔ 100 µg DNA (no amplification step necessary)



polar body biopsy



blastomer biopsy



tropectoderm biopsy

Whole Genome Amplifikation (time: 3h)

DNA:
few picogram

DNA:
5-10 microgram

array-CGH analysis in PID

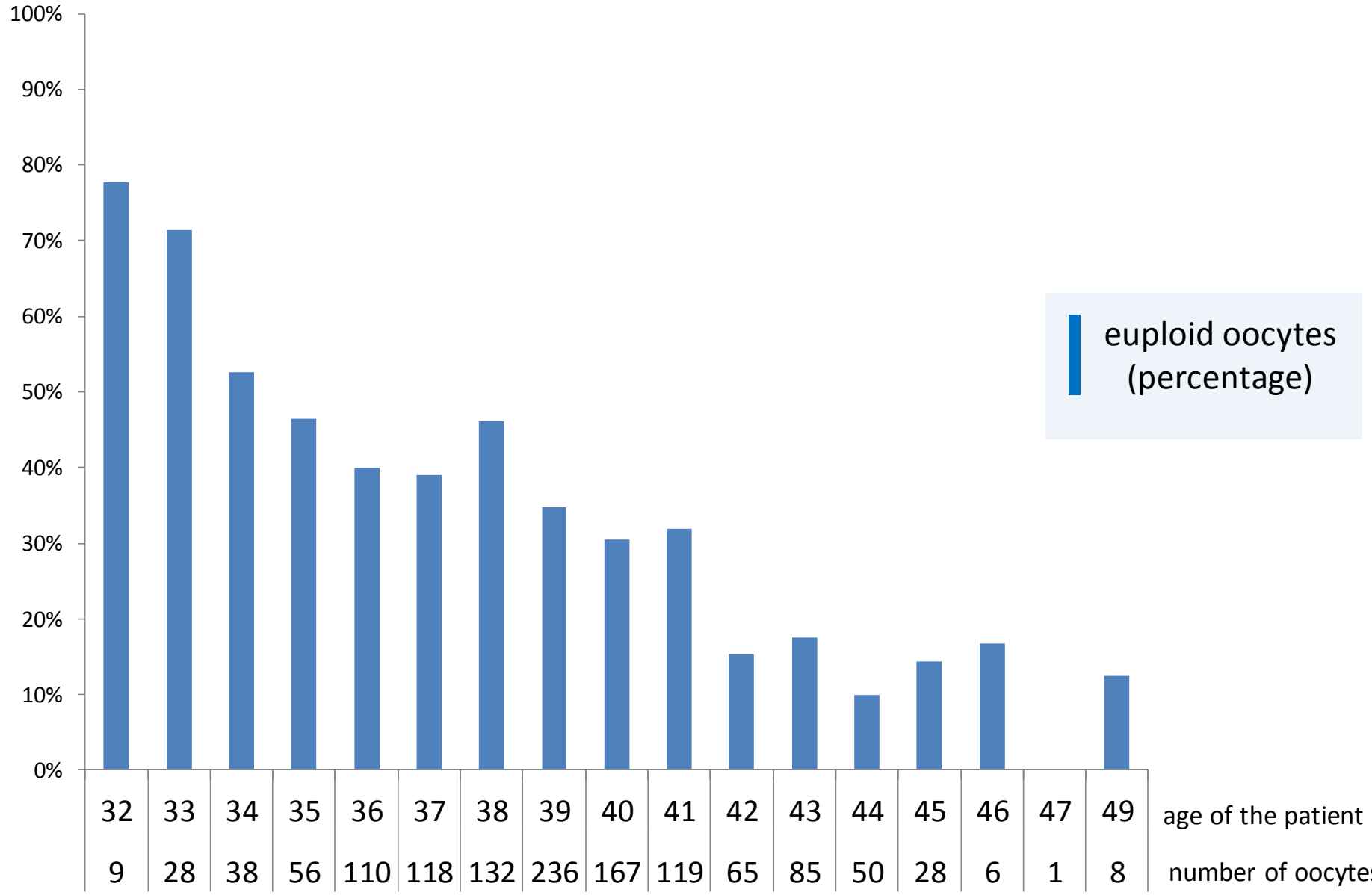
complete time after biopsy 10-23h

DNA labelling (time: 3h)
200-400 ng DNA

hybridisation (3.5-16.5h)

analysis (30min)

array-CGH analysis using DNA from polar bodies



Comprehensive Chromosome Screening is highly predictive of clinical success

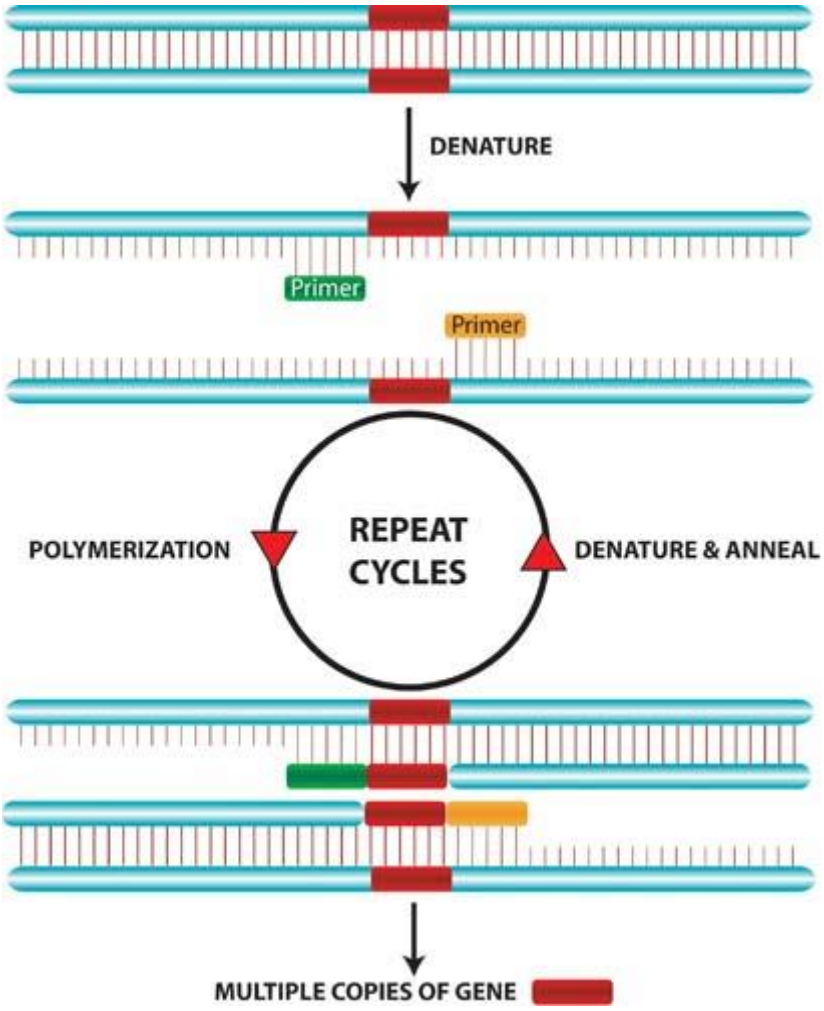
blinded nonselective study

48.2% of euploid blastocytes resulting in ongoing implantation per transfer
93.5% of aneuploid predicted blastocystes failed to implant

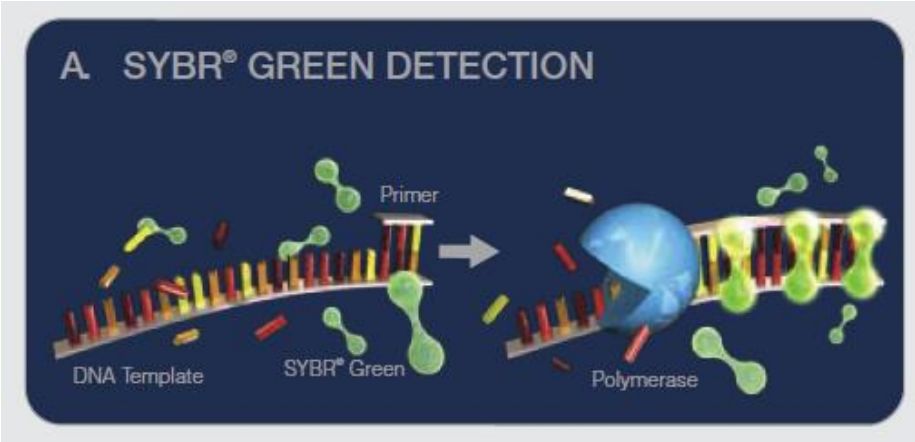
29.2% of euploid blastomere resulting in ongoing implantation per transfer
98.1% of aneuploid predicted blastomere failed to implant

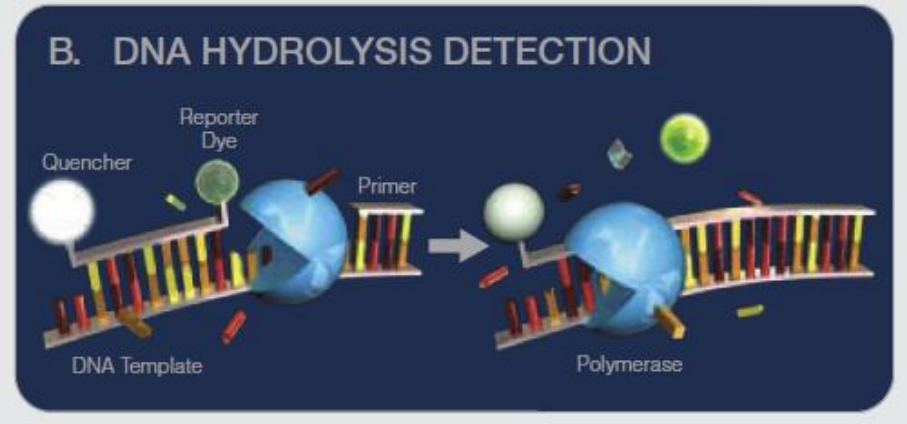
Scott et al., 2012, Fertil Steril. 97(4):870-5

PCR – quantitative PCR



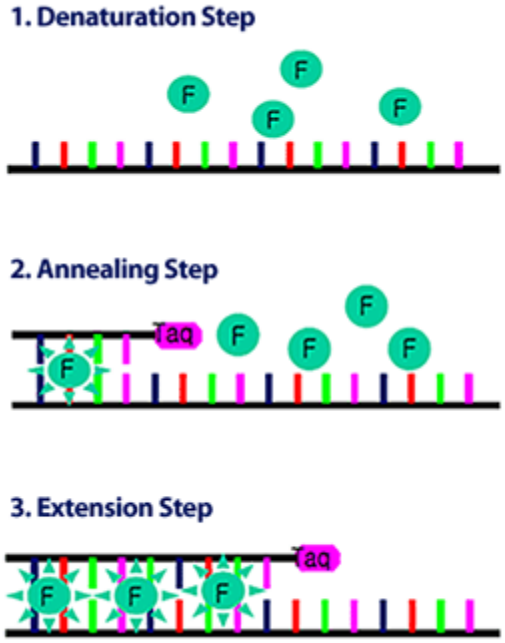
http://info.gbiosciences.com/blog/bid/155569/Using-Polymerase-Chain-Reaction-to-Identify-Different-Strains-of-an-Organism

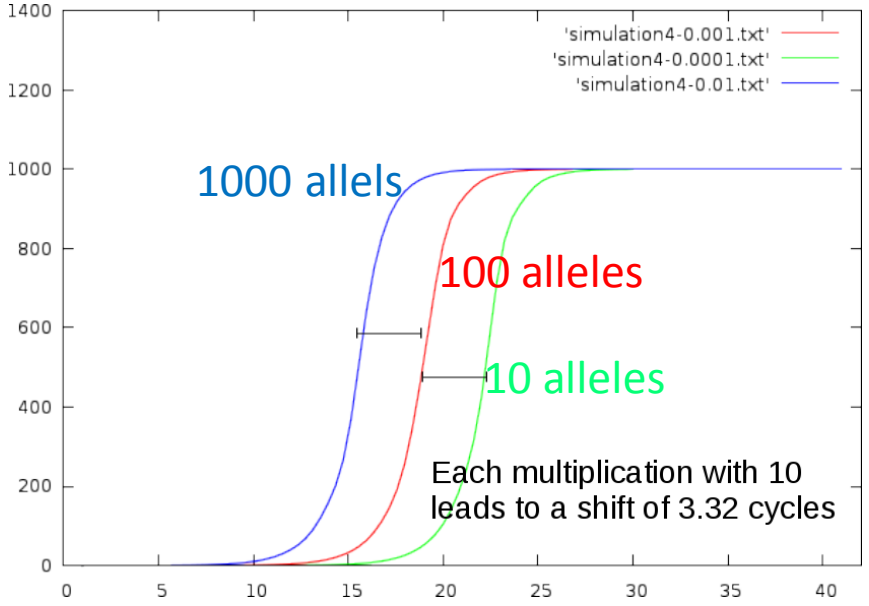




http://www.sabiosciences.com/pathwaymagazine/pathways8/real-time-pcr-for-systems-biology.php

PCR – quantitative PCR





if you start your PCR with more DNA, the fluoreszence signal is detectable after less cycles

NGS machines

Chip PI
output up to 10 Gb
200bp
reads 60-80 millions
time 4hr

High Output Flow Cell
output 100 Gb / 2x 150bp
reads 400 millions / time 29hr

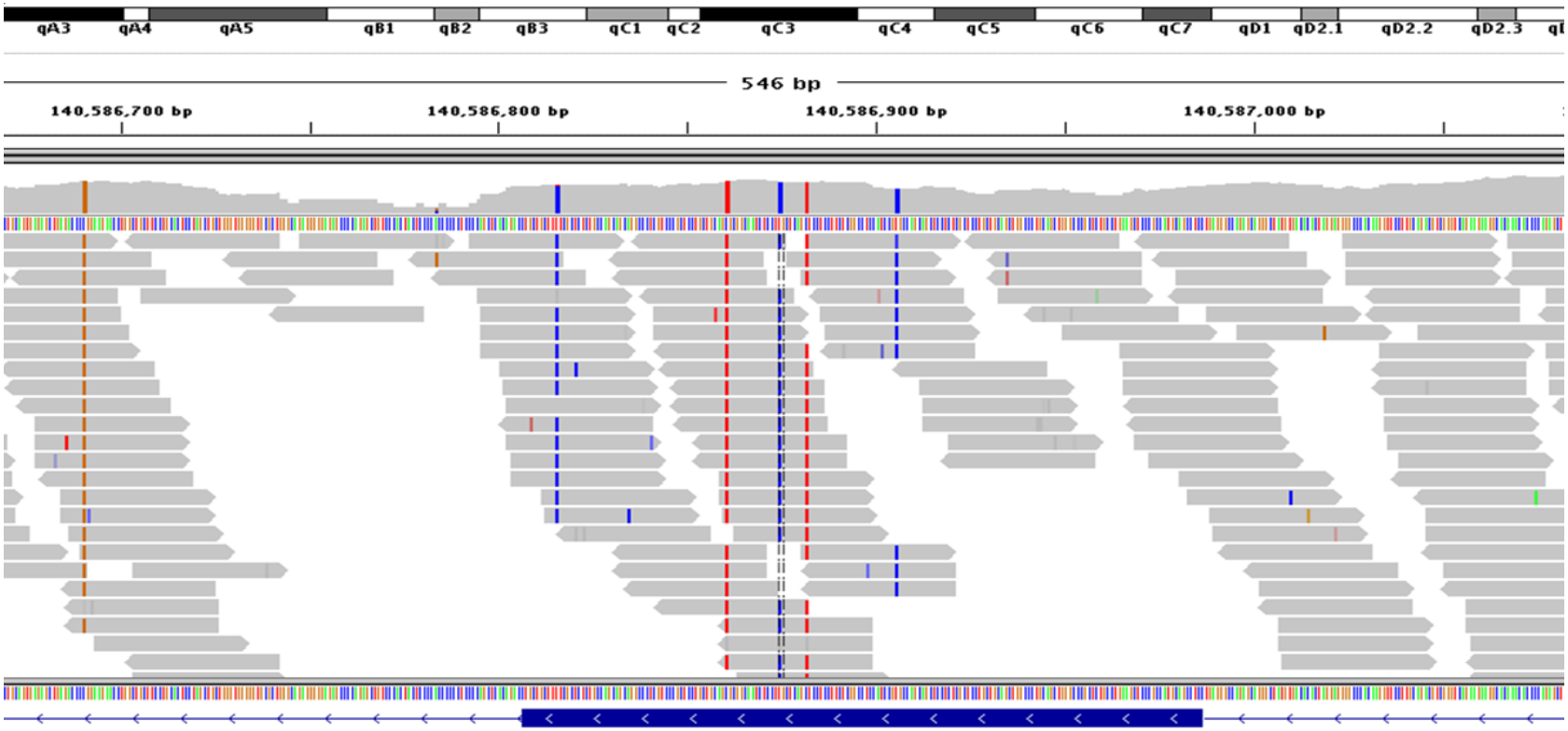
Chip 316
output 300-600 Mb / 200bp
reads 2-3 millions / time 3hr

Kit v3
output 3.5 Gb
2x 75bp
reads 20 millions
time 21hr

Coverage / Amount of Data

		10X coverage	100x coverage
whole genome	**Genome:**		
 DNA extraction and fragmentation	~ 3-6 GB	30 GB	300 GB
exome	**Exome:**		
 exons are amplified by PCR or enriched using cDNA (~25 000 genes)	~ 30-50 MB	500 MB	5GB
targeted sequencing	**200 Gene**		
 targeted sequences are amplified by PCR (thousands of amplicons)	~ 400 kb	4 MB	40 MB

Coverage

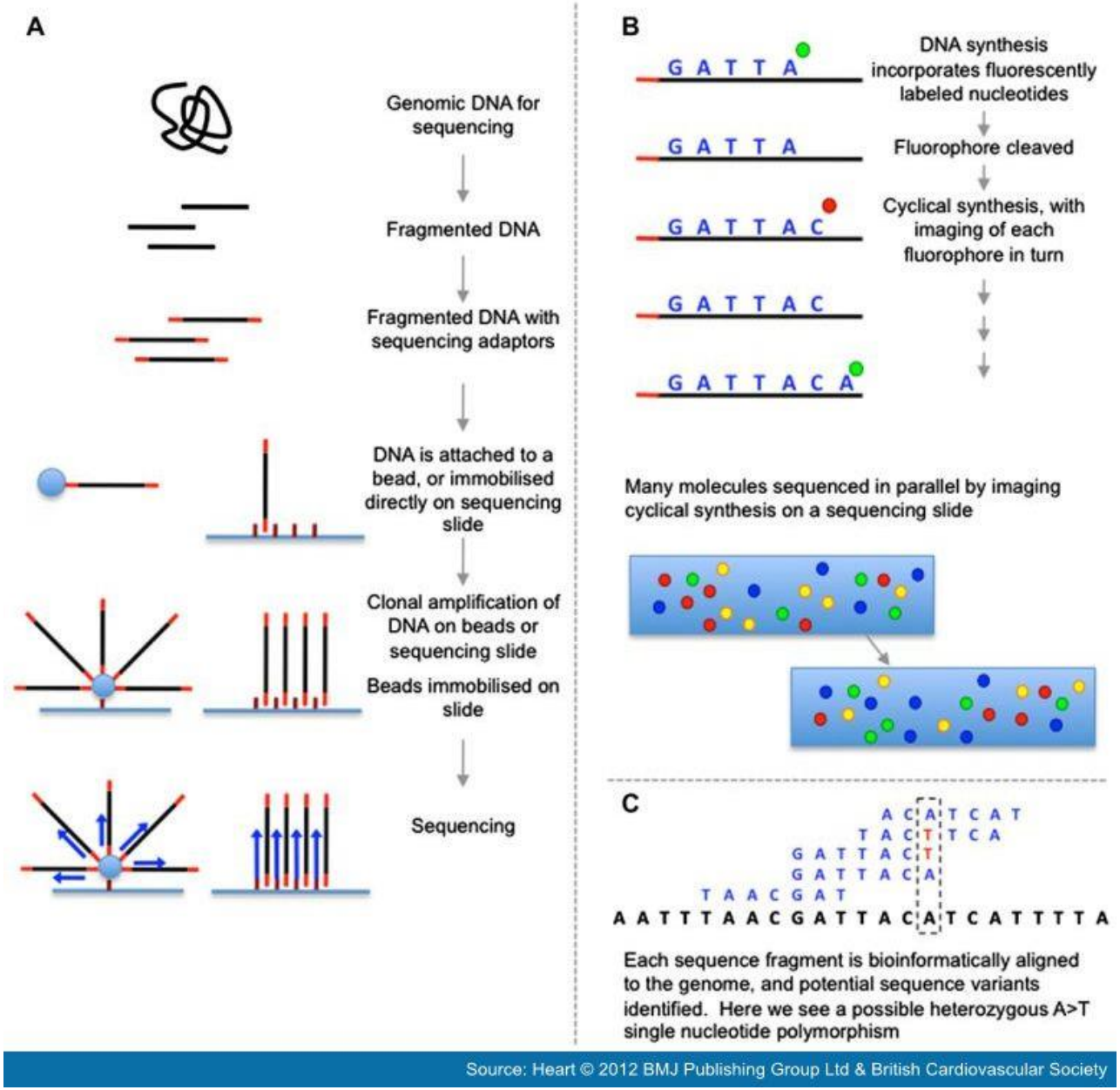

qA3
qA4
qA5
qB1
qB2
qB3
qC1
qC2
qC3
qC4
qC5
qC6
qC7
qD1
qD2.1
qD2.2
qD2.3
546 bp
140,586,700 bp
140,586,800 bp
140,586,900 bp
140,587,000 bp

NGS-next generation sequencing

Using „Barcodes“ (2 - 96x probes) it is posssible to analyse DNA probes of several patients in one run ➔ lower costs

CGGATTACCATGTG 1

CGGATGACCATGTG 2



Source: Heart © 2012 BMJ Publishing Group Ltd & British Cardiovascular Society

Fields of application of NGS in reproductive medicine

- panel sequencing: increased nuchal translucency (NT), cardiac anomalies and a normal karyotype indication for Noonan syndrome (PTPN11, KRAS, RAF1, BRAF and MAP2K1)

- targeted sequencing: screening of infertile couples for mutations frequently found in the population (cystic fibrosis, sickle cell disorder, hemophilia, hot spot mutations)

- aneuploidy screening: screening of polar bodies, blastomers or trophoblasts for aneuploidy

Wells et al., 2014, J Med Genet. Aug;51(8):553-62.
Clinical utilisation of a rapid low-pass whole genome sequencing technique for the diagnosis of aneuploidy in human embryos prior to implantation.

- NIPT fetal cell free DNA found in the plasma of the pregnant mother can be isolated and analysed

Outlook

NIPT /NGS	non invasive methods will strongly increase if costs for the test decrease, more women will use NIPT aneuploidy screening it is already possible to analyse the whole genome of a fetus using cell free DNA; therefore, also monogenetic disorders will be analysed by NIPT in future
infertile couples	trophectoderm cells will be routinely screened for chromosome anomalies using array CGH or NGS technology; more genetic markers should be identified that are involved in the process of implantation and they may be tested in order to increase the pregnancy rate (mtDNA)

➔ **genetic analyses will play a very relevant role in reproductive medicine**