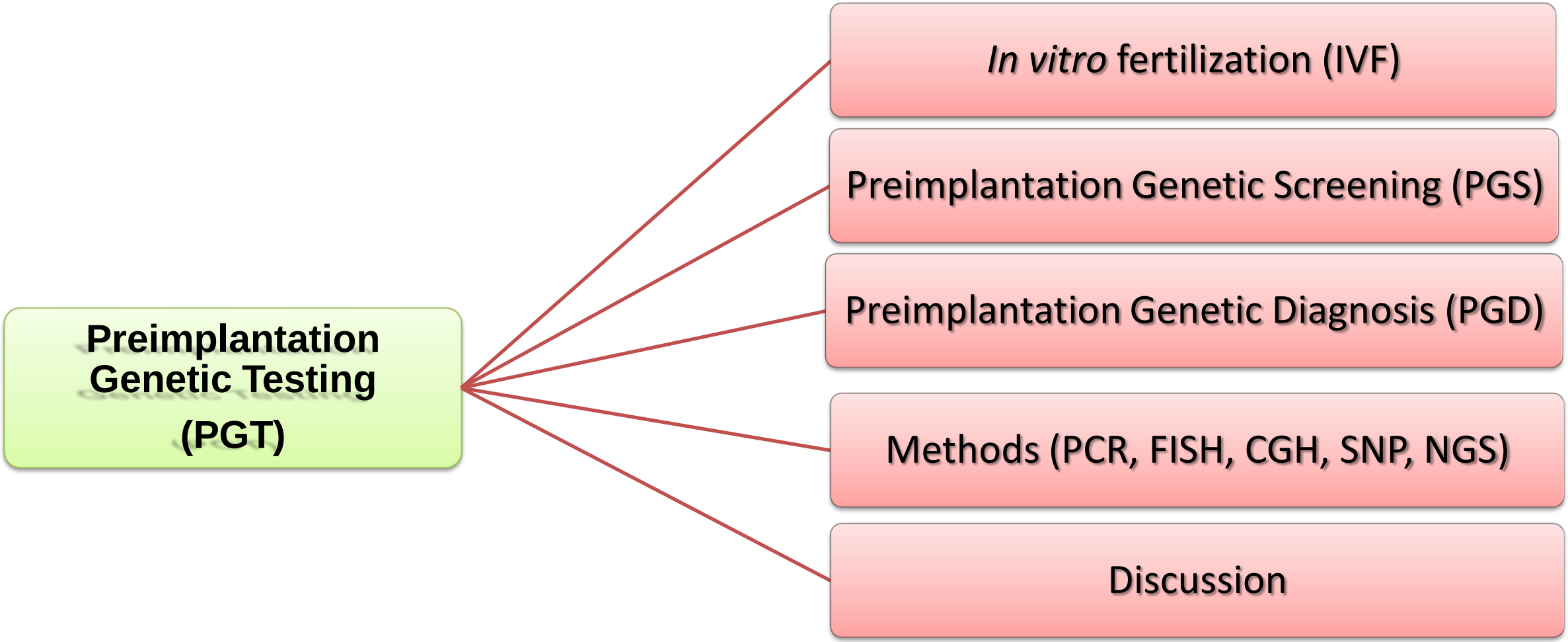
Preimplantation Genetic Screening (PGS) and Preimplantation Genetic Diagnosis (PGD)

Sofiva Genomics

Lab Director / General Manager
Double Hong, Ph.D.

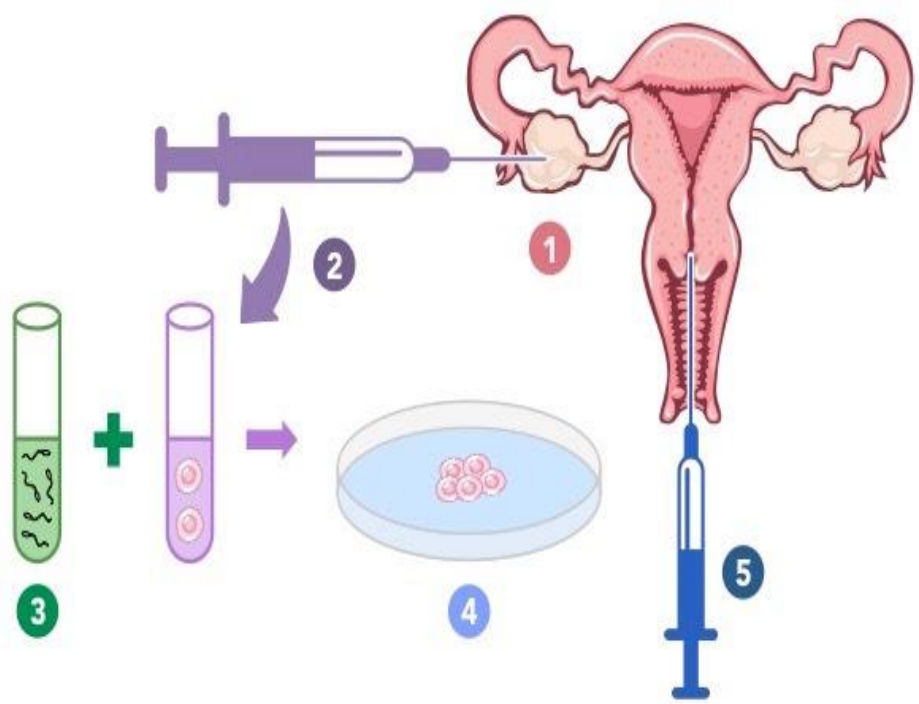
Outline


Preimplantation Genetic Testing (PGT)
In vitro fertilization (IVF)
Preimplantation Genetic Screening (PGS)
Preimplantation Genetic Diagnosis (PGD)
Methods (PCR, FISH, CGH, SNP, NGS)
Discussion

In vitro fertilization (IVF) Procedure

Procedure

1、Stimulation phase
2、Egg retrieval
3、Collect sperm
4、*In vitro* fertilization (IVF)
5、Embryo transfer
6、Implantation



http://ib.bioninja.com.au/standard-level/topic-6-human-physiology/66-hormones-homeostasis-and/in-vitro-fertilisation.html

in vitro fertilization (IVF) + genetic testing

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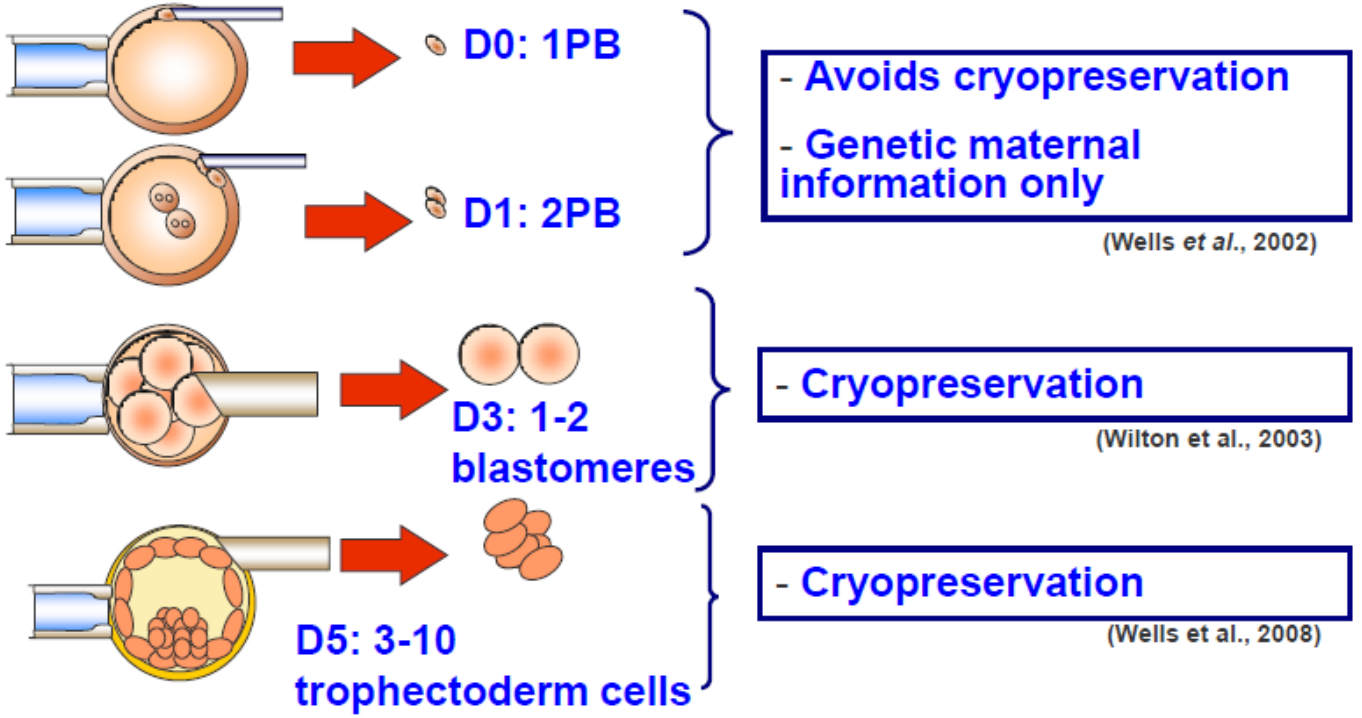
Procedure

1、Stimulation phase
2、Egg retrieval
3、Collect sperm
4、*In vitro* fertilization (IVF)
(Genetic Testing)
5、Embryo transfer
6、Implantation



Genetic testing before implantation : preimplantation genetic testing (PGT)

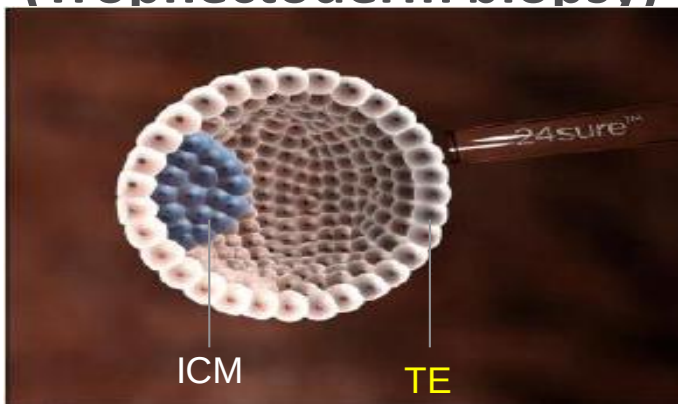
Biopsy Procedures

Polar body

Blastomere

1 cell

Blastocyst (Trophectoderm biopsy)



8-10 cells

	Polar body	Blastomere	Blastocyst
Advantages	• Non-invasive	• Detect both maternal and paternal errors • Well-established biopsy protocols	• Non-invasive • Detect both maternal and paternal errors • Mosacism might be detected
Limitations	• Large number of cells to test • Only maternal error can be detected	• Invasive • Mosacisim can lead to screening errors	• Biopsy skills • Blastomere culture protocols

Two Types of Preimplantation Genetic Testing

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✓ Preimplantation Genetic Screening (PGS)

- preimplantation genetic testing for **aneuploidy** and abnormal copy number of chromosomes (defined as **PGT-A**)

✓ Preimplantation genetic diagnosis (PGD)

- preimplantation genetic testing for **monogenic disorders** (defined as **PGT-M**)

PGS vs PGD

	Preimplantation Genetic Screening PGS	Preimplantation Genetic Diagnosis PGD
Item	Abnormal copy number of chromosomes	Single gene disorder
Technology	FISH Array-CGH NGS	Specific probe (primer) PCR Sanger sequencing STR marker
Indications	✓ Advanced maternal age ✓ History of recurrent early pregnancy loss ✓ Repeated IVF failure ✓ Infertility	✓ Known single gene disorders family history ✓ HLA typing

Preimplantation Genetic Screening, PGS

PGT-A

(also known as aneuploidy screening)

PGS detects aneuploidy among IVF embryos

Aneuploidy exists across all ages and increases with maternal age

Chromosomal aneuploidy is known to be a major cause of IVF failure

Indications for PGS

- ✓Women of advanced maternal age (>34 yo)
- ✓History of recurrent early pregnancy loss
- ✓Repeated IVF failure
- ✓Severe male infertility
- ✓Sex selection

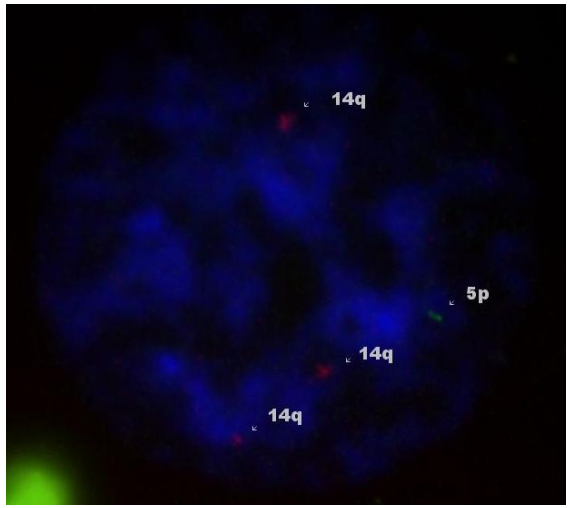
In the past……

chromosomal abnormalities

Embryo biopsy

Polar body
Single blastomere
Blastocyst

FISH



Genetic testing for specific region

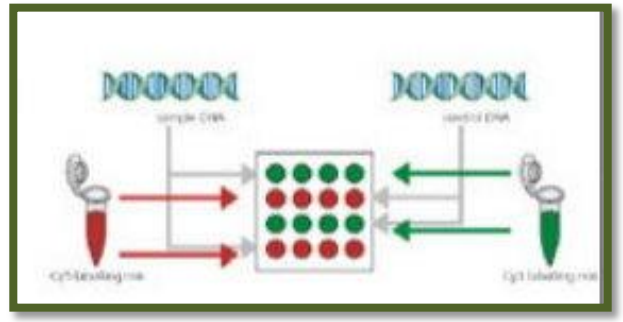
only a few chromosomes can be detected simultaneously by FISH

Array-based PGS
NGS-based PGS

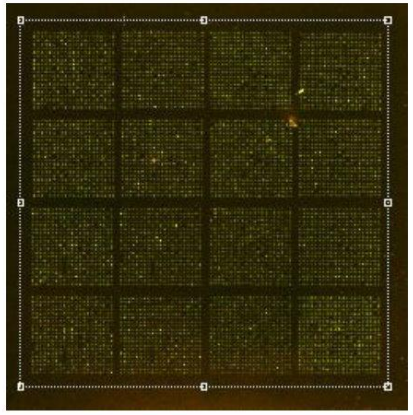
Embryo Biopsy

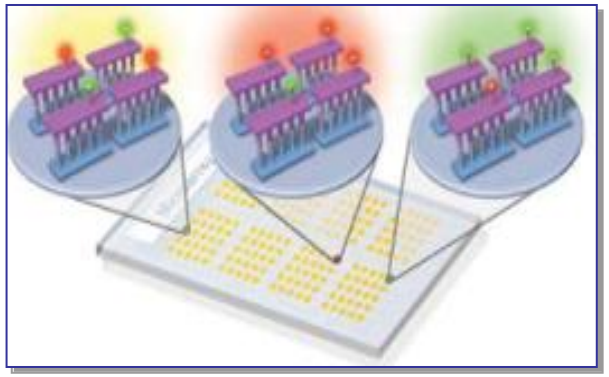
day 3 or day 5

Embryo(s)



Whole Genome Amplification (WGA)

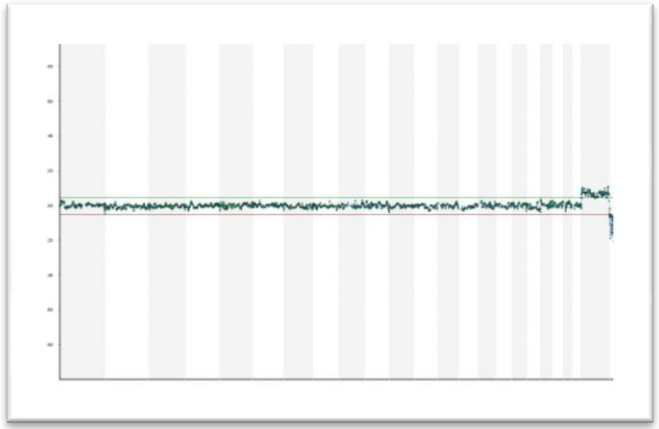


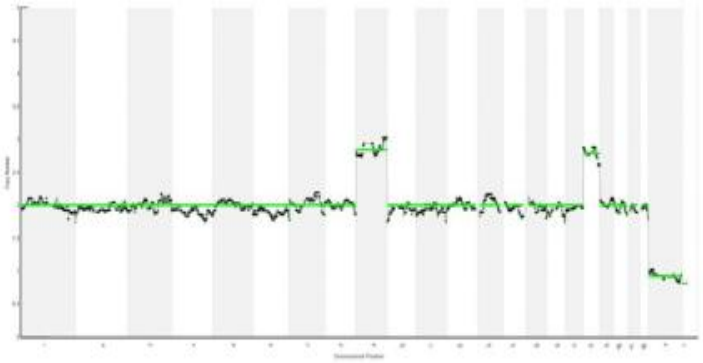


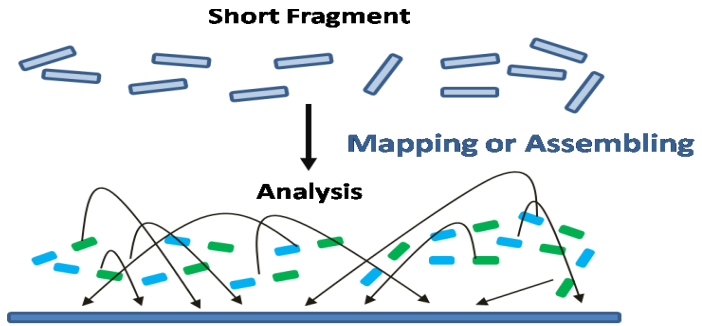
array-comparative genomic hybridization (array-CGH)

OR

Next Generation Sequencing (NGS)







permit visualization of all 23 chromosomes

Development of preimplantation Genetic Screening, PGS

FISH

PGS was originally performed using fluorescence *in situ* hybridization (FISH). Because FISH does not screen all 24 chromosomes, its efficacy and accuracy for detecting euploid embryos is limited.

Arrays

As new technologies were developed and applied to PGS, implantation rates improved. The array-based 24sure™ technology facilitated investigation of all 24 chromosomes in the early embryo, significantly improving PGS success.[7,8]

NGS

Next-generation sequencing (NGS), the latest technological breakthrough, is setting a new standard in PGS, with reliable PGS results[9], streamlined workflows, higher throughput capabilities, and customizable assays for easy portfolio expansion.

	PGS
FISH	Traditional genetic testing platform (chr 13,18,21,X,Y)
Array	Automated array technology Detect 23 pairs of chromosome
NGS	Latest technology Detect 23 pairs of chromosome High-throughput Easier experimental operation

NGS platform for PGS

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Ion PGM System - Thermo Fisher

Ion Proton Sequencer - Thermo Fisher

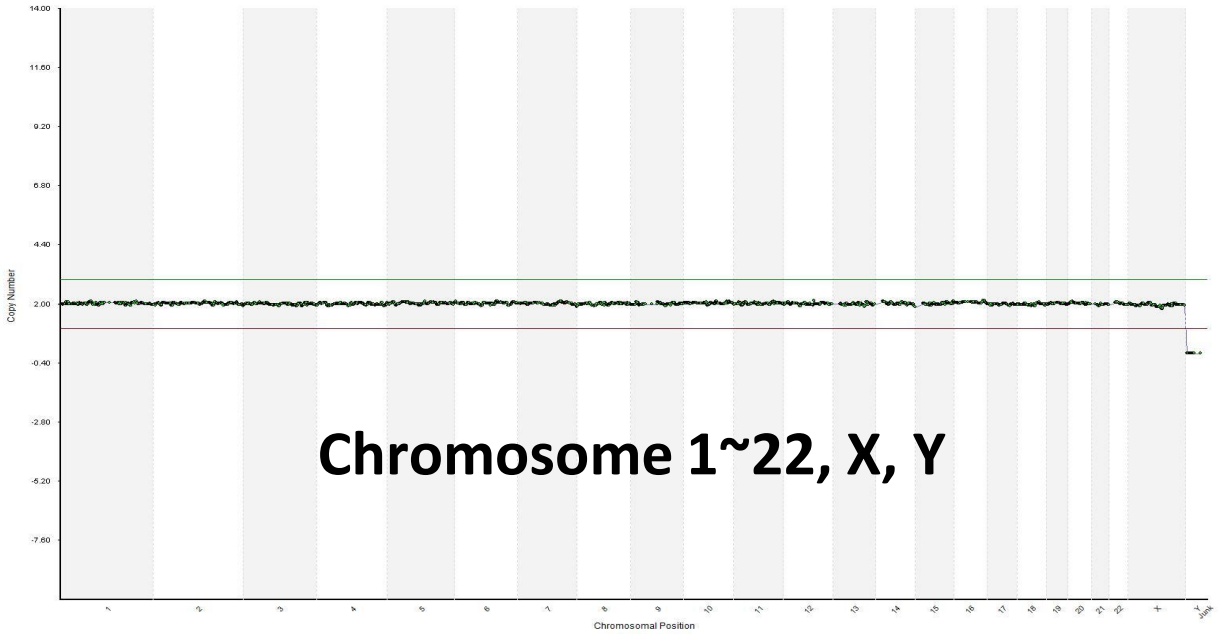
Miseq - Illumina

Analyze data

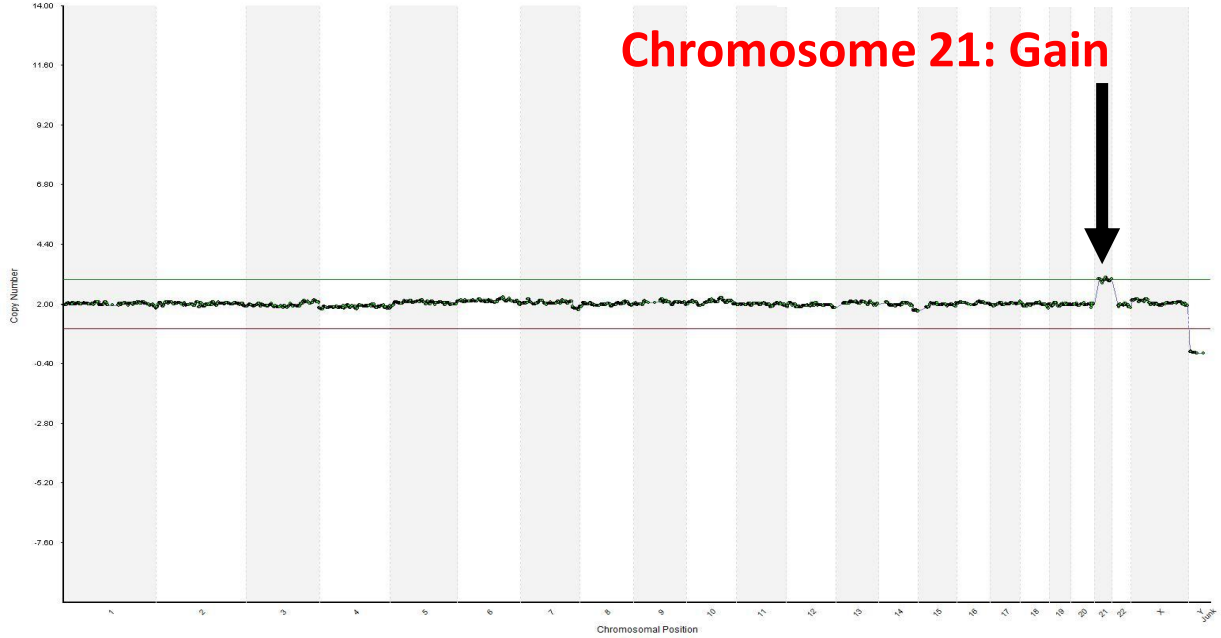
Copy number

Green line: 3 copies

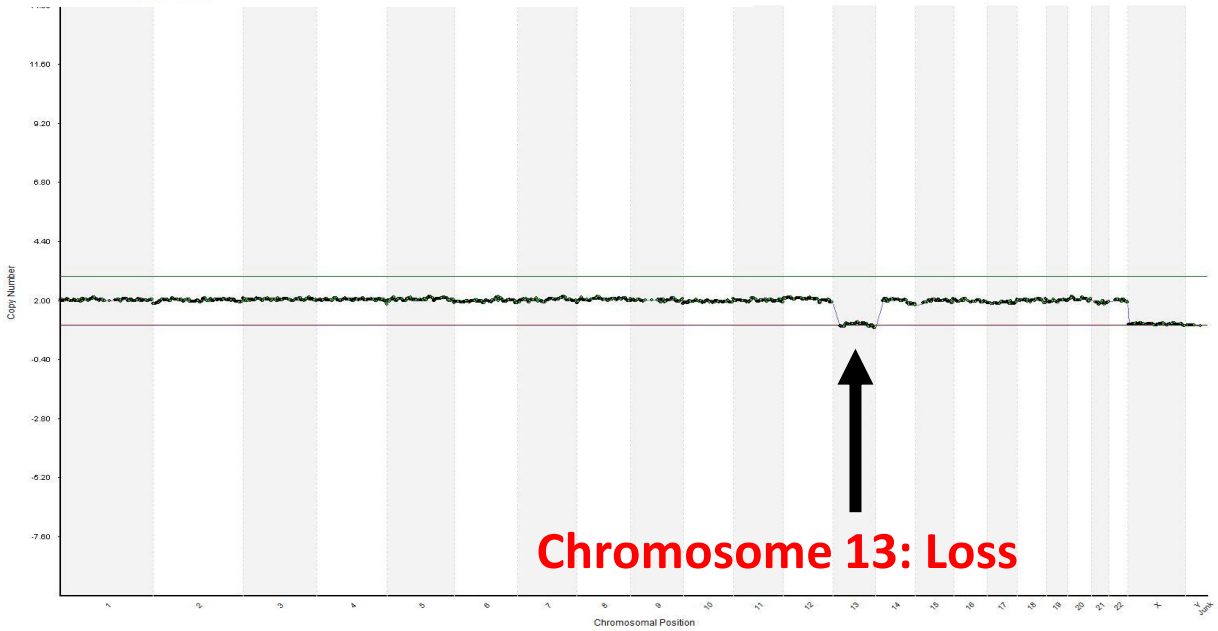
Red line: 1 copy



Chromosome 1~22, X, Y

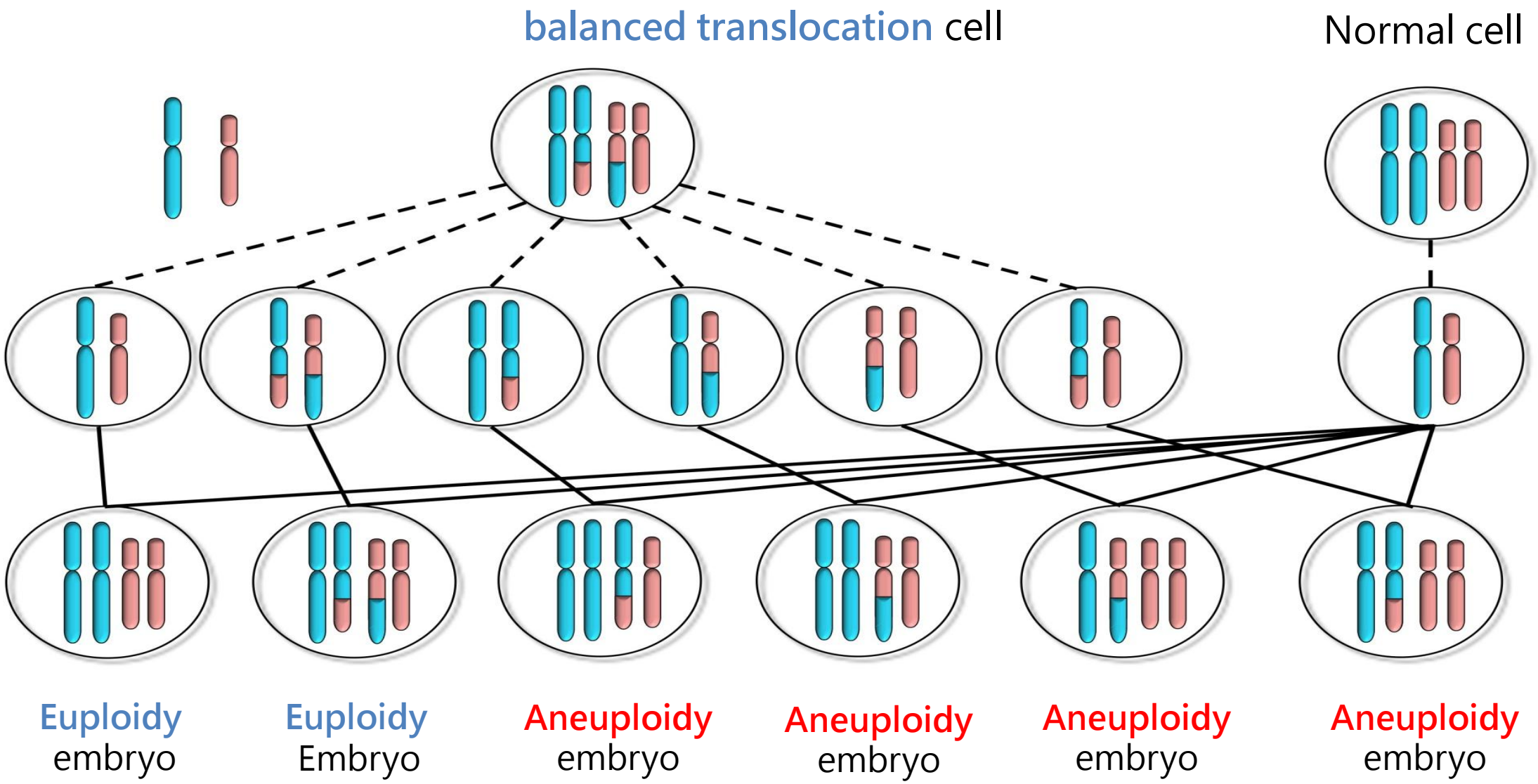


Chromosome 21: Gain

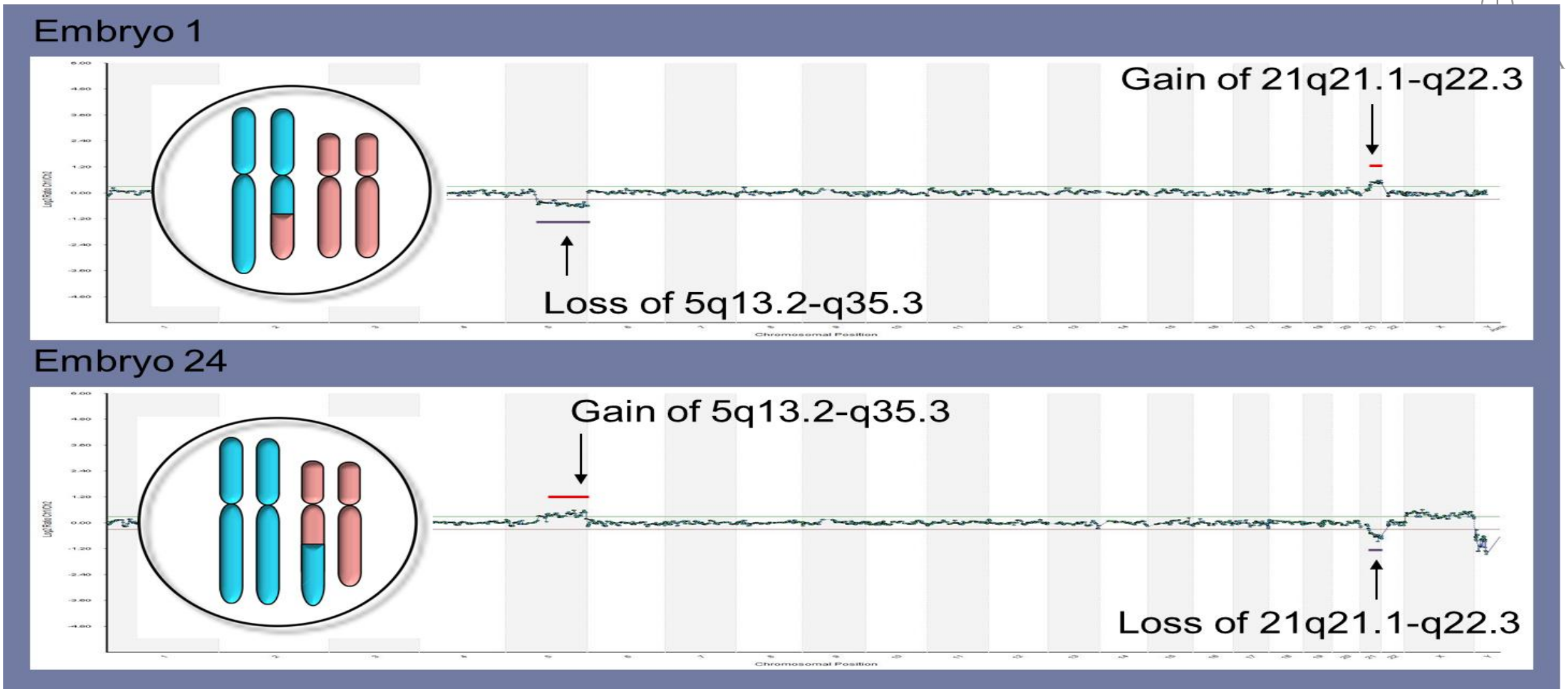


Chromosome 13: Loss

Example for reciprocal translocation for PGS

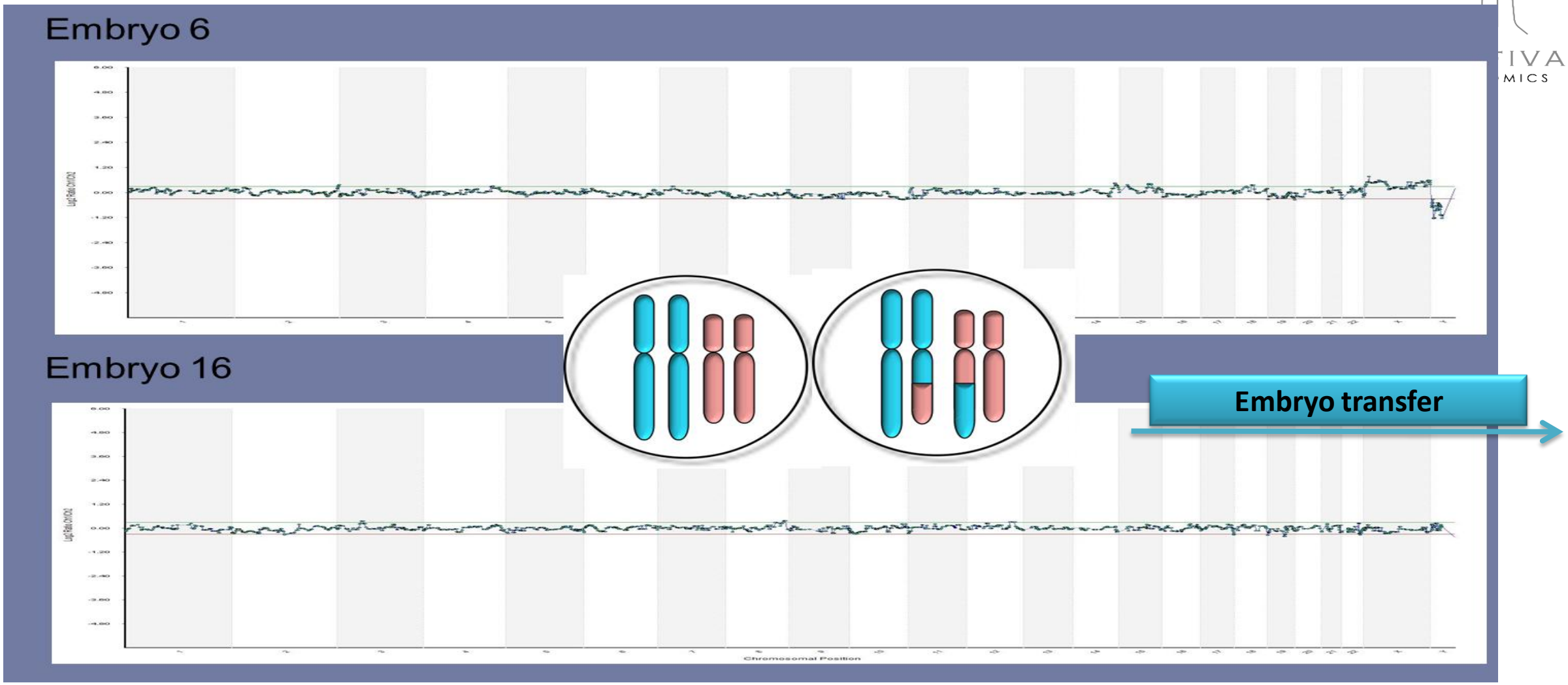


PGS result for case 46XY,t(5;21)(q11.2;q11.2)



Aneuploidy Embryo，**Not** transfer

PGS result for case 46XY,t(5;21)(q11.2;q11.2)



Euploidy Embryo，can transfer

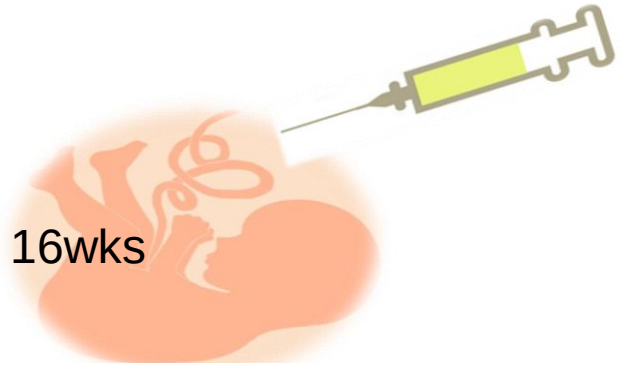
PGS case results

Total : 12 embryos
Abnormal: 10 embryos
Normal: 2 embryos

Embryo transfer

(No 6、16)

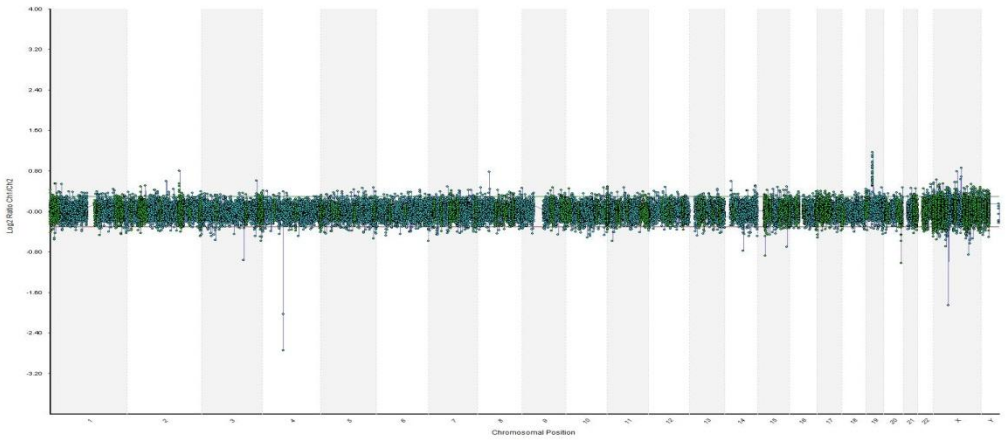
pregnancy

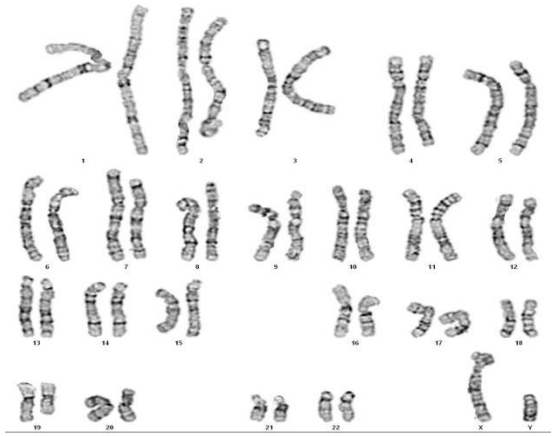


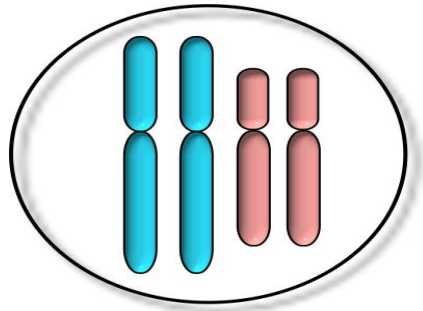
Confirmed by AF

Array CGH: arr(1-22)x2, (X)x1, (Y)x1

Chromosome: 46,XY

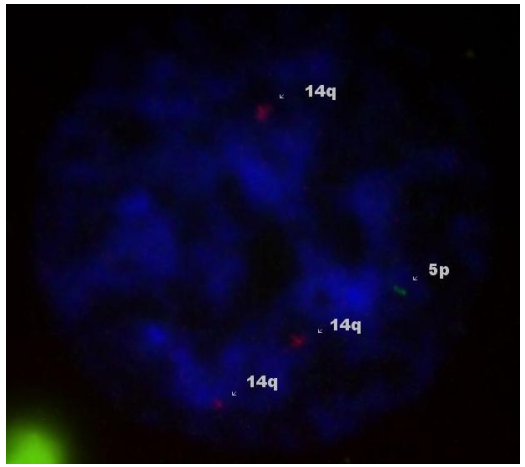


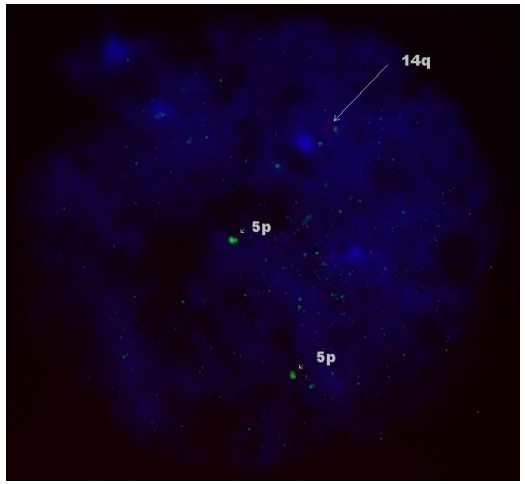




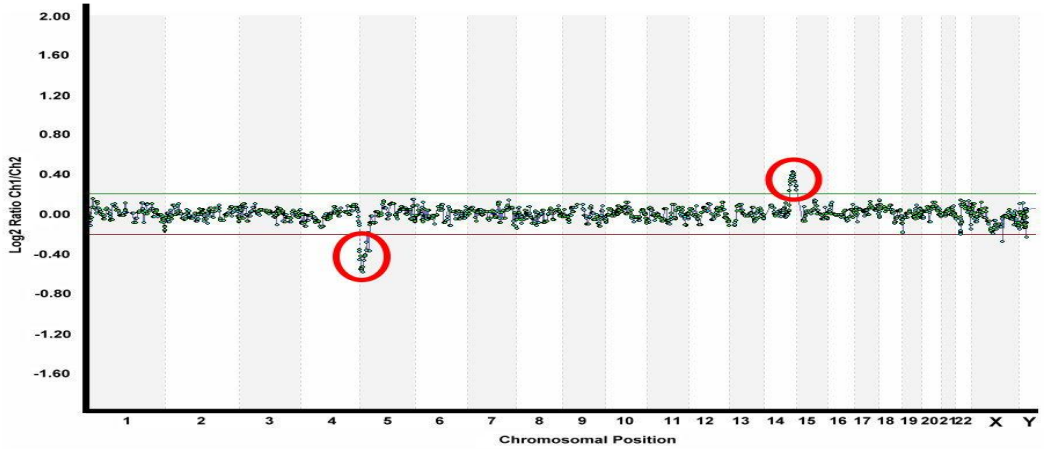
FISH vs aCGH

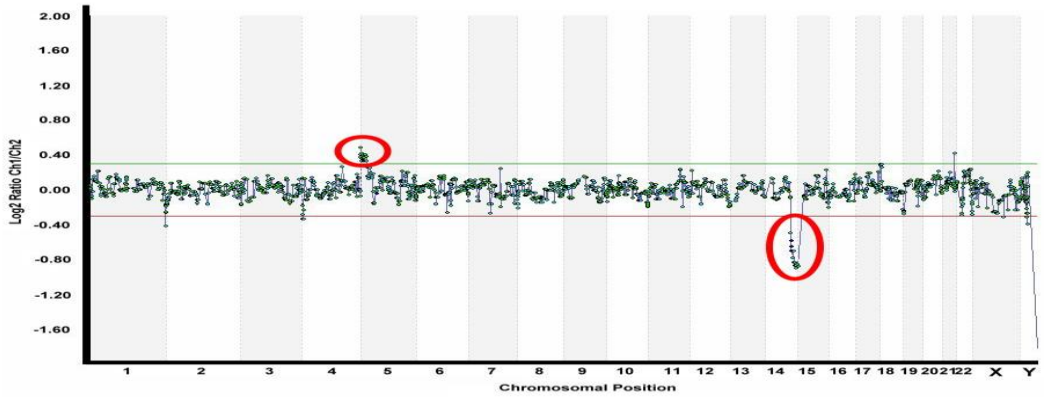
FISH





aCGH

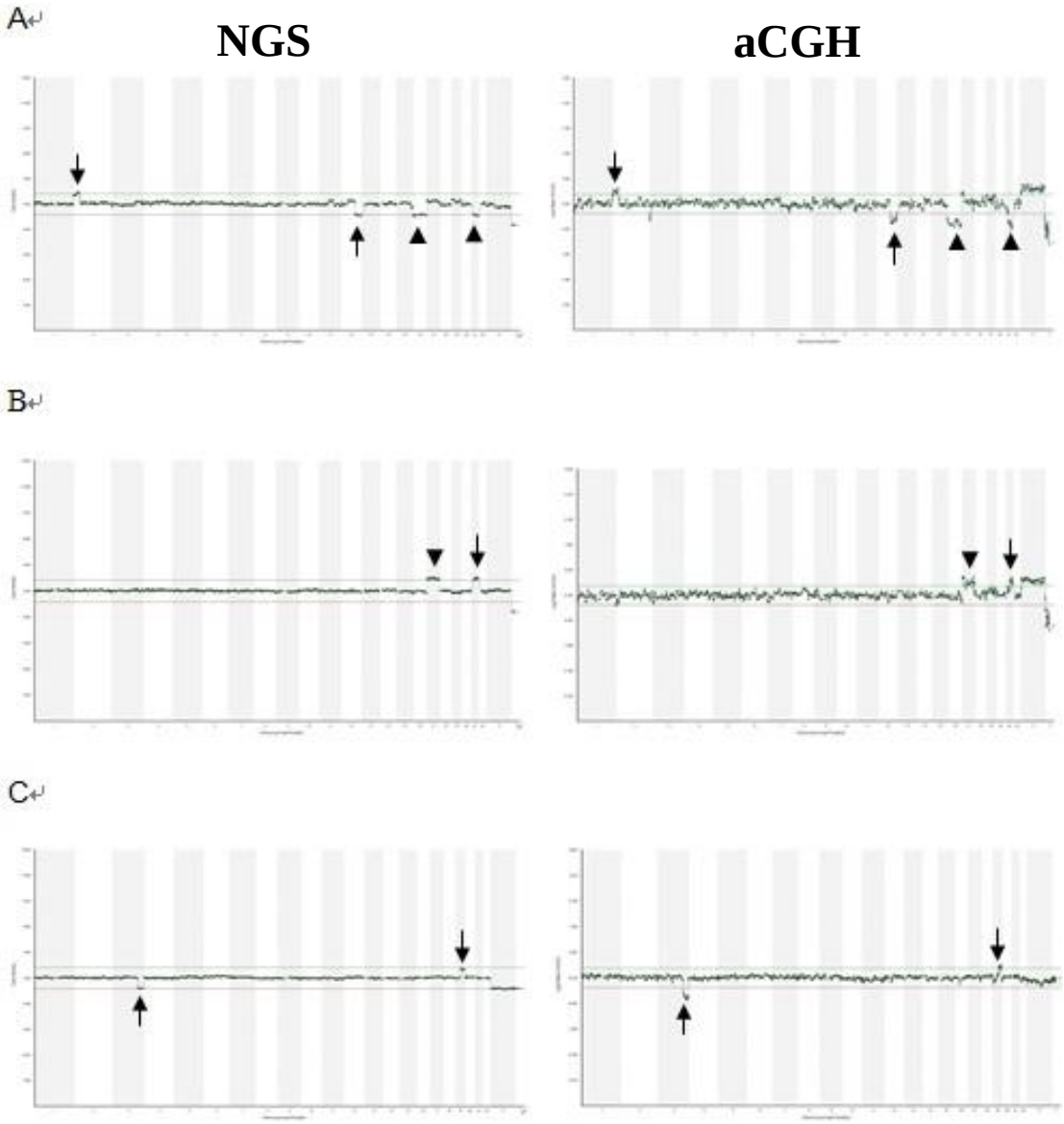




G: Cytocell 5p telomere probe
R: Cytocell 14q telomere probe

(5p,14q) balanced translocation

NGS vs aCGH



1. NGS vs aCGH : 100% sensitivity
2. Resolution: same
3. Handling time for technician: NGS is easier

Preimplantation Genetic Diagnosis, PGD
PGT-M

- One or both genetic parents carry a gene mutation
- Testing is performed to determine specific mutation

- Indication for PGD

✓ With known single gene disorders

Autosomal dominant

Autosomal recessive

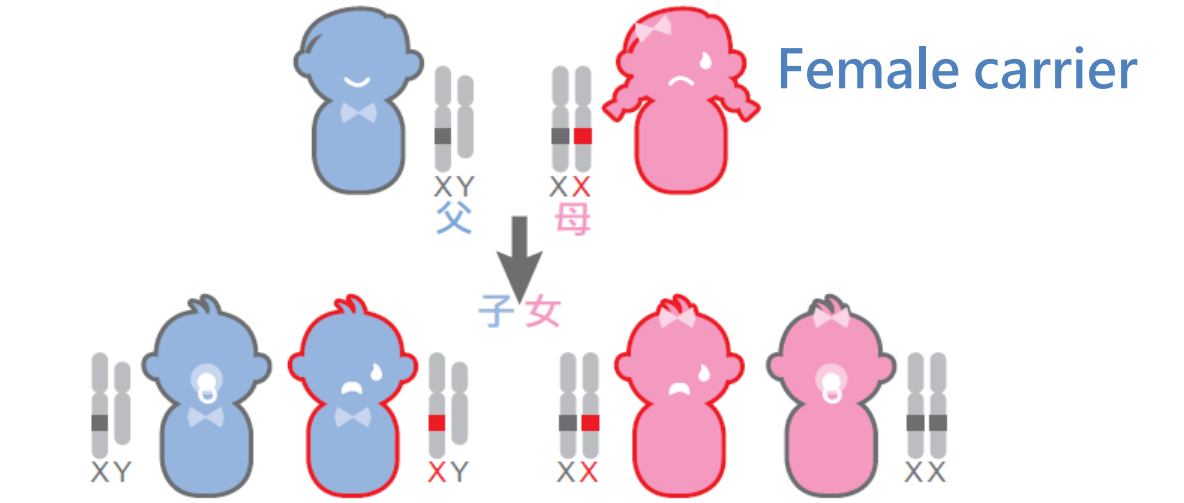
X-linked disorders

✓ Carriers of mutations

✓ Human Leukocyte antigen (HLA) matching

Clinical application of Preimplantation Genetic Diagnosis, PGD

- First took place in October 1989
- Haemophilia (X-liked disorder)
- Sex determination



Male WT Male affected Female carrier Female WT

In the past……

Single gene disorder

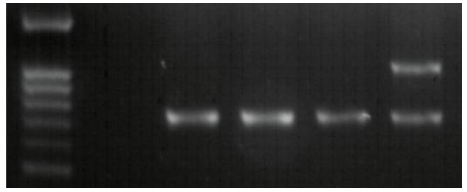
Embryo biopsy

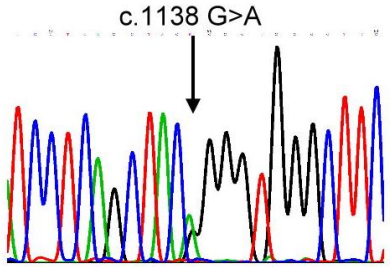
PCR

Gel electrophoresis

Sequencing

PGD for specific region



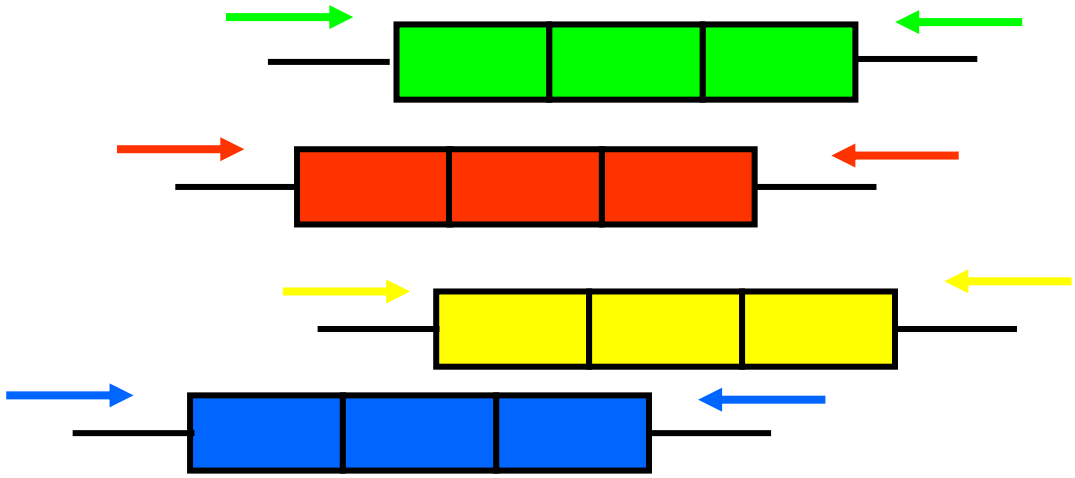


specific inherited disorders - single gene

selection of normal embryos for transfer

Modified PCR-based research

Optimized PGD-PCR protocols
Nested PCR
Multiplex PCR
Fluorescent PCR

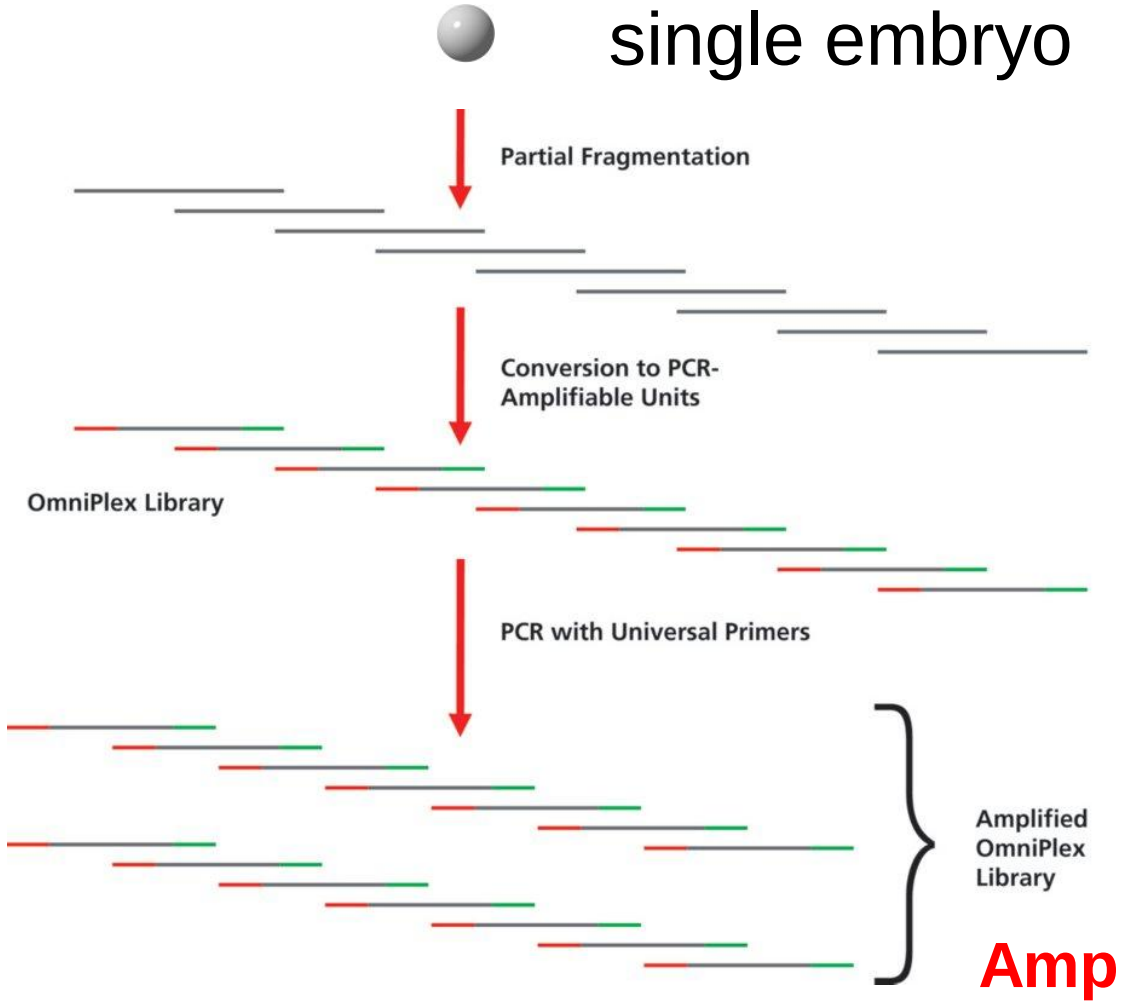


Multiple genes

Improve to target multiple regions

Still restrict to specific regions

Whole genome amplification (WGA)

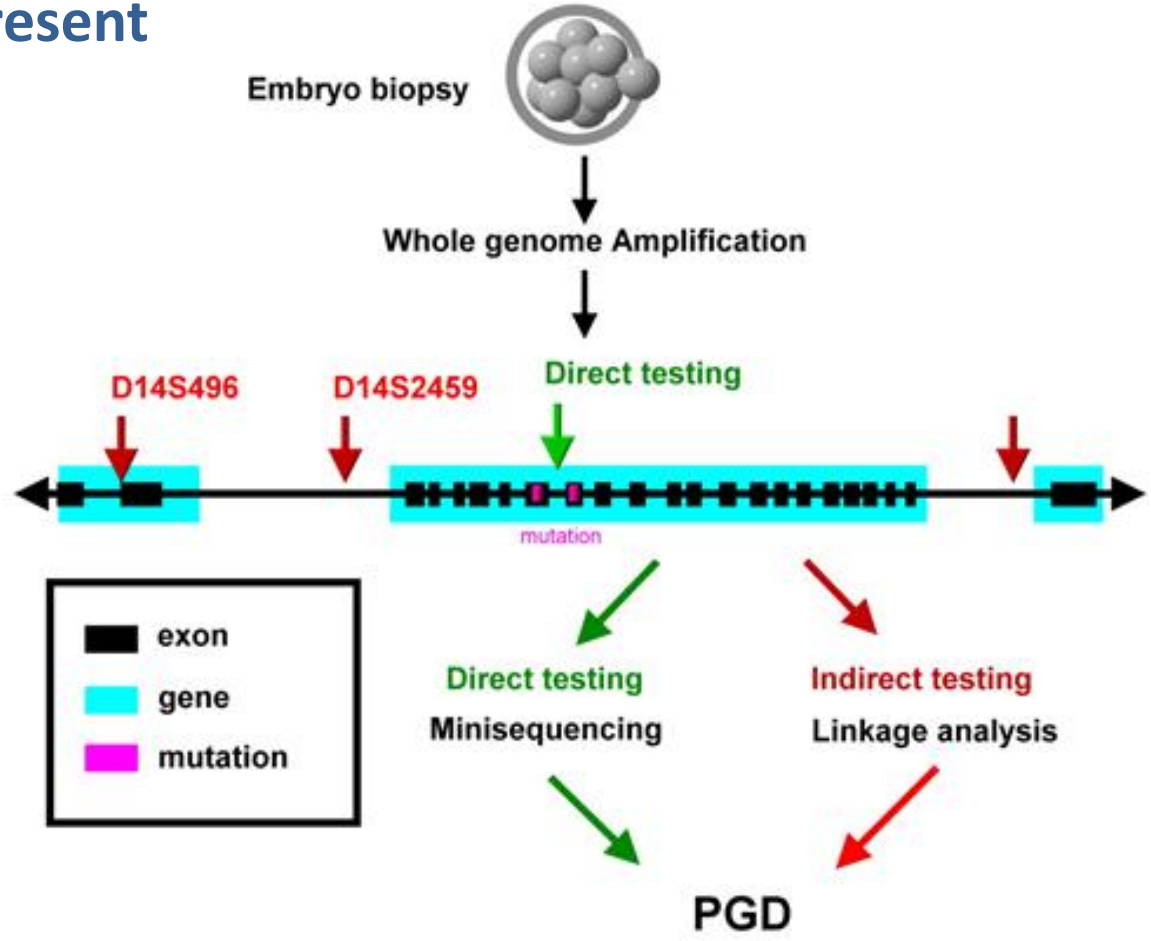


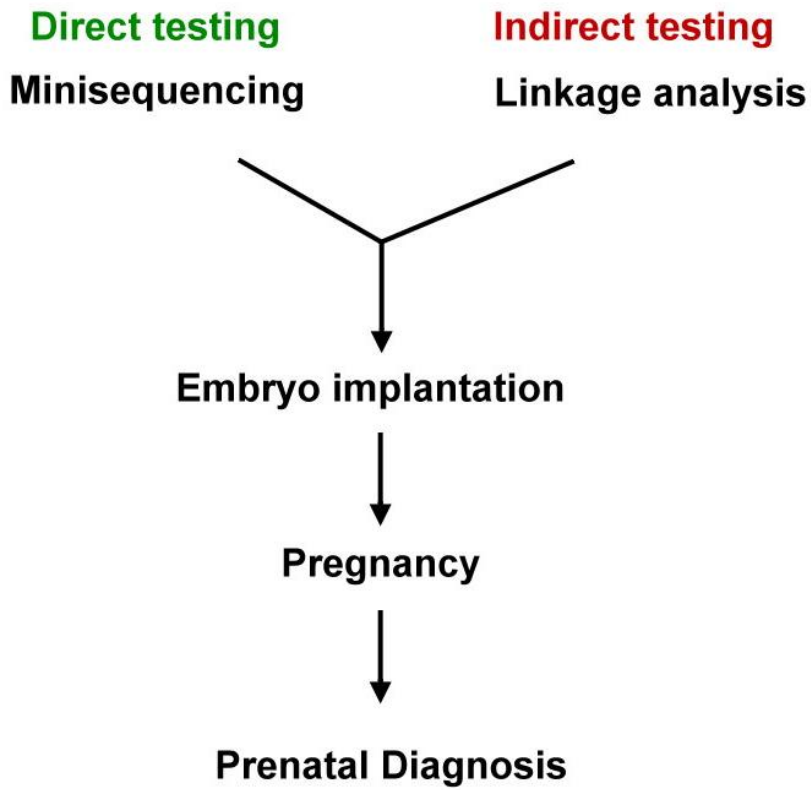
Amplify the entire genome from single cell

Further analysis for multiple loci

Direct and indirect diagnosis

In the present

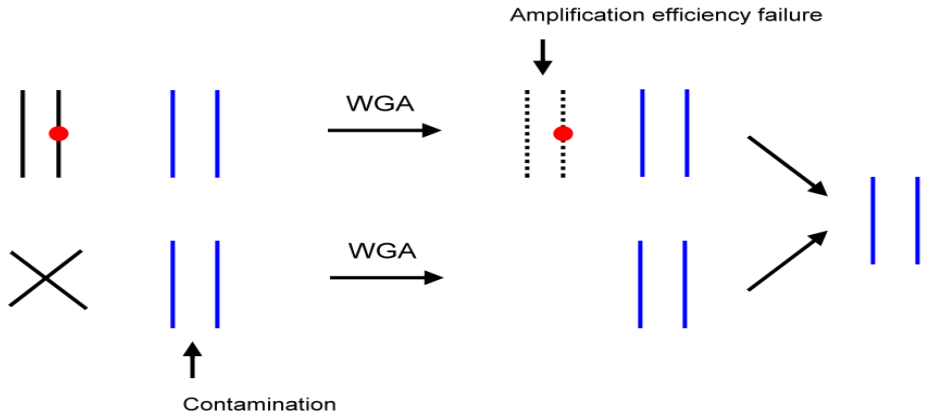




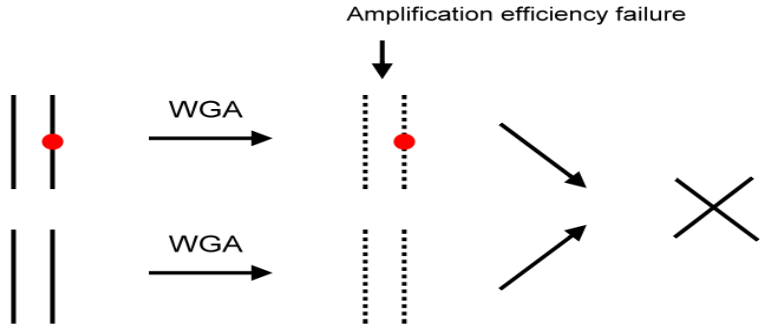
multi-loci

The advantages of STR marker

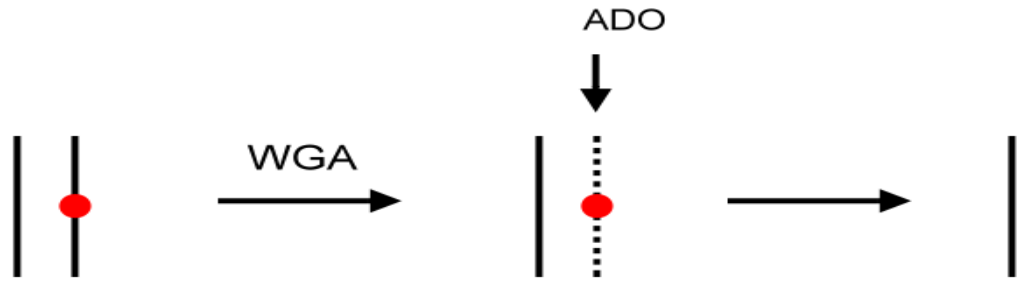
✓ to monitor contamination



✓ to monitor WGA experiment

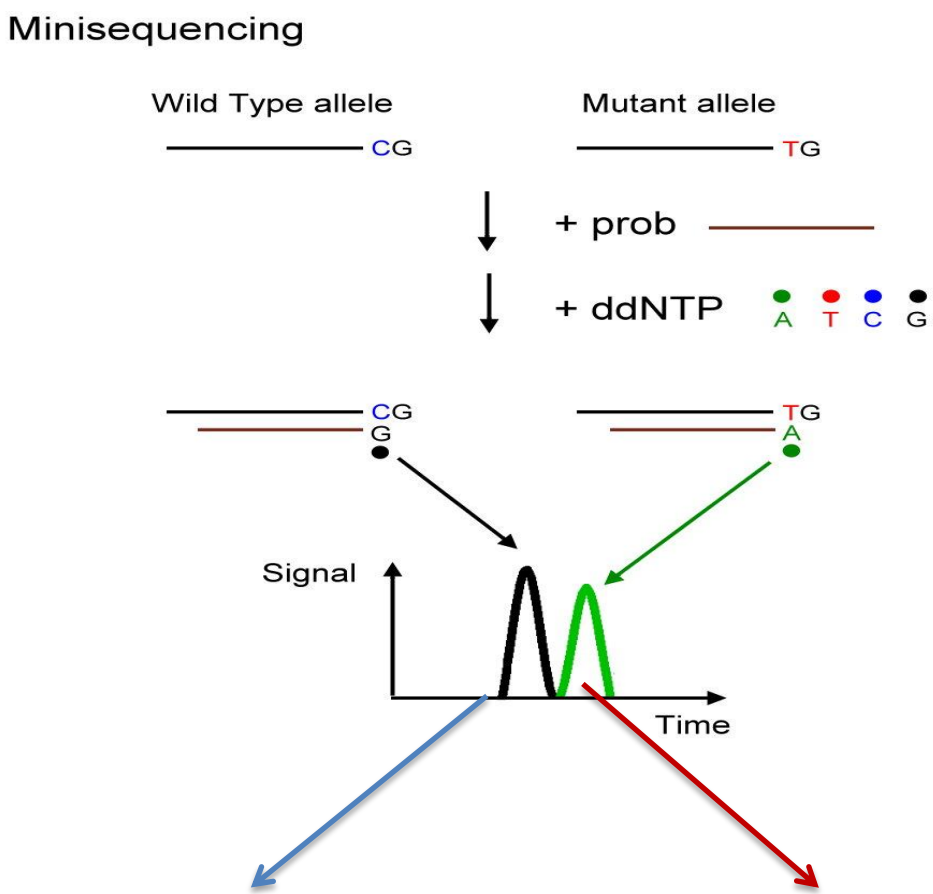


✓ to monitor Allele drop-out (ADO)



Example for PGD results

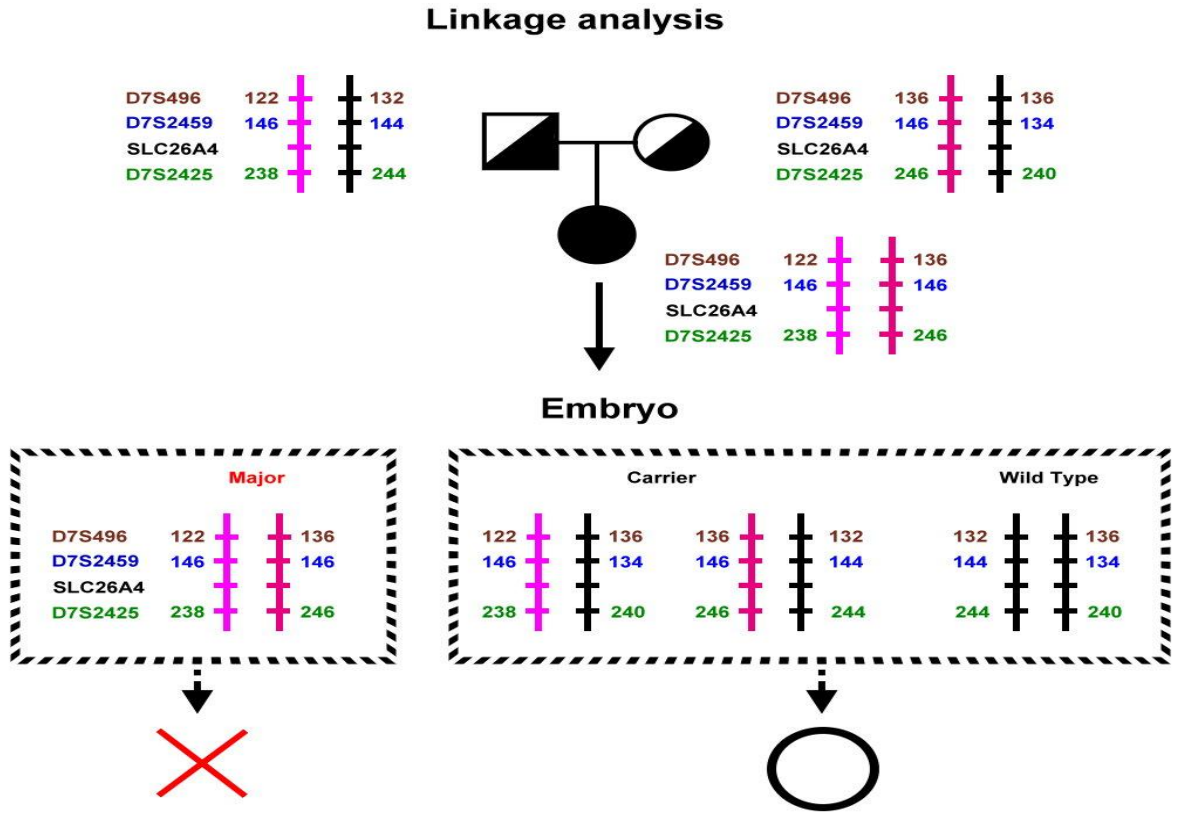
Direct genotyping



WT genotype

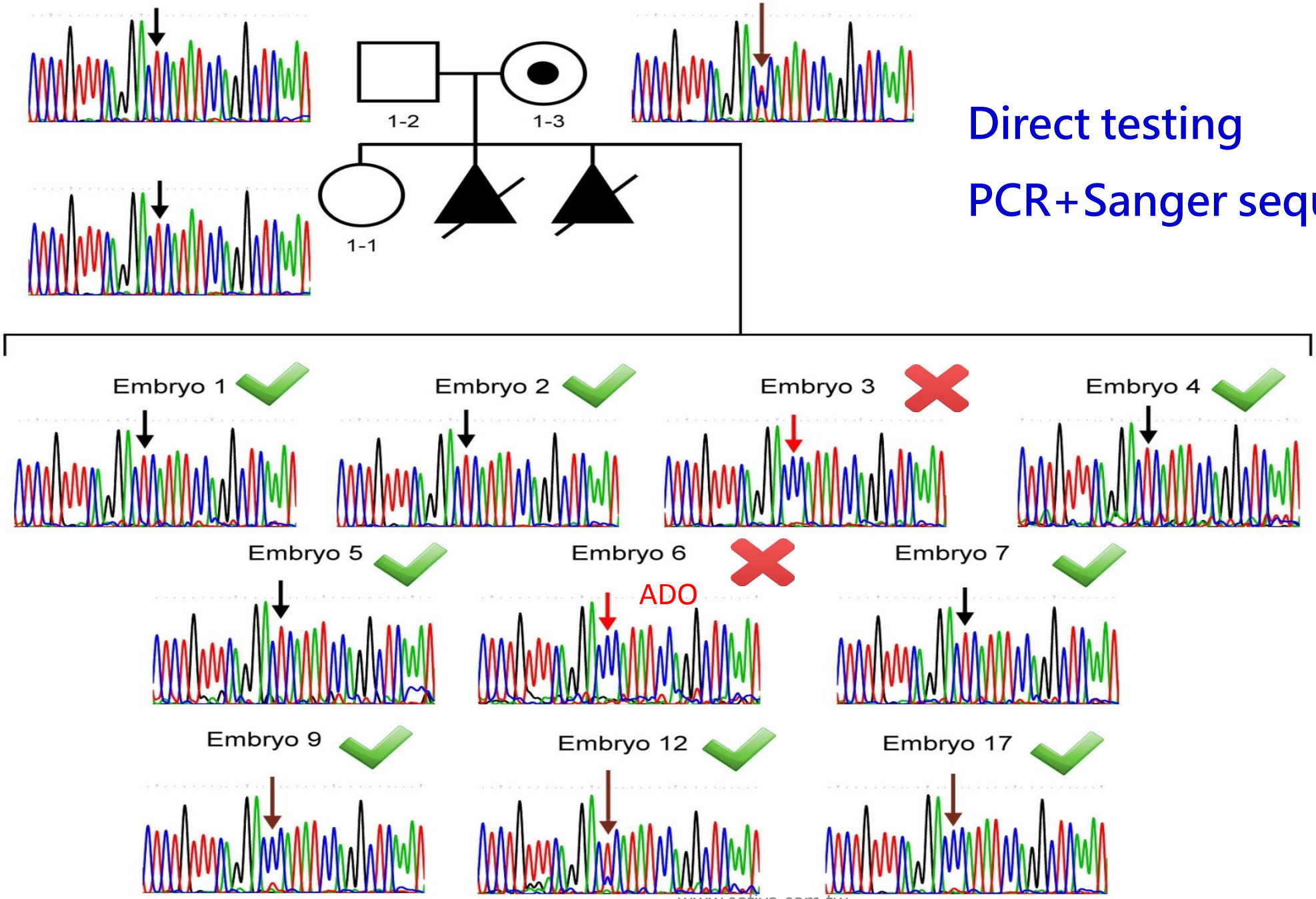
Abnormal genotype

Linkage analysis



Abnormal embryo
Not transfer

Wile type embryo
Can transfer



Direct testing

PCR+Sanger sequencing

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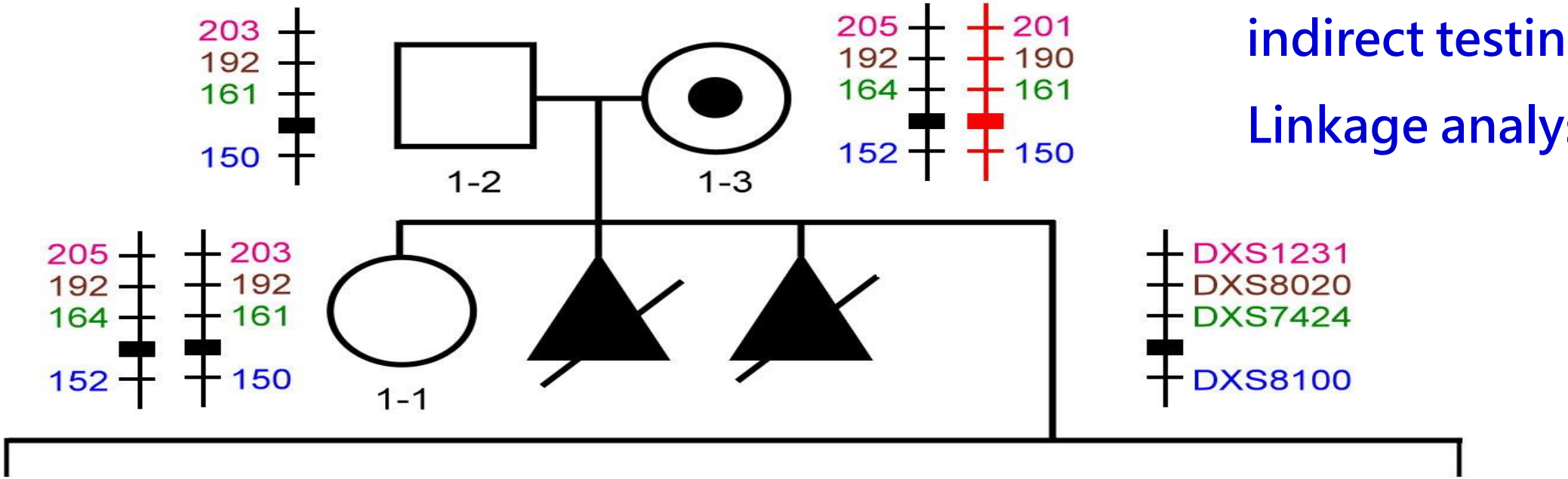
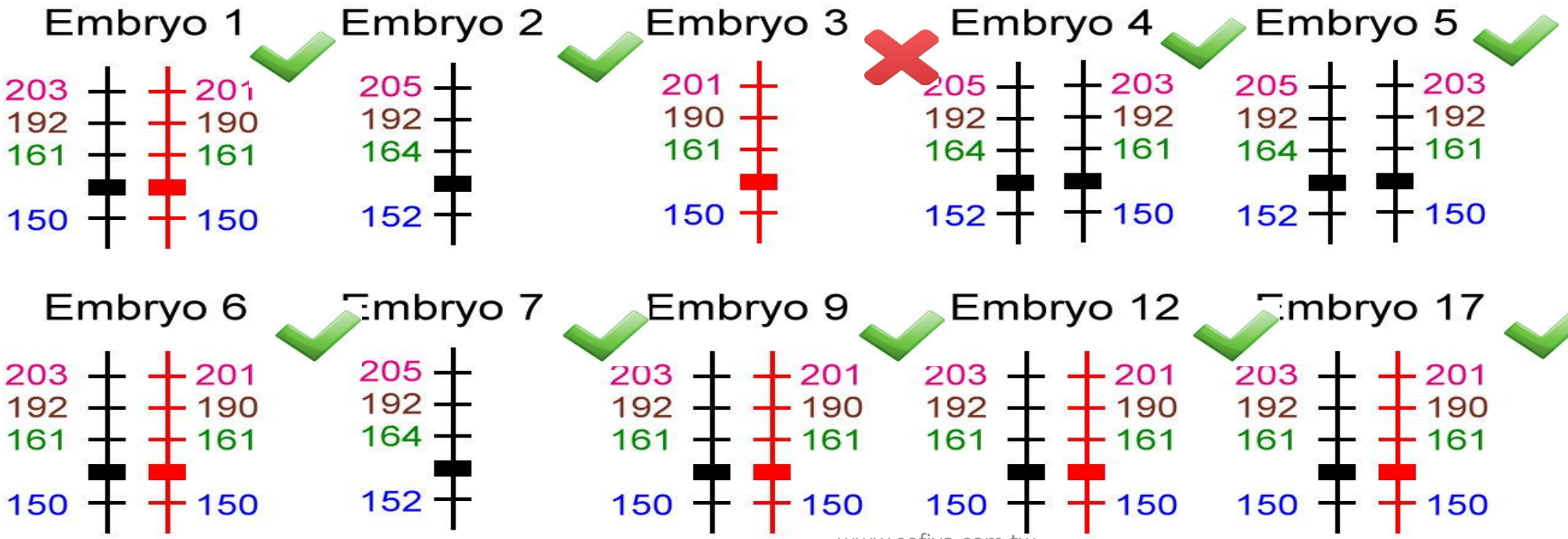
indirect testing

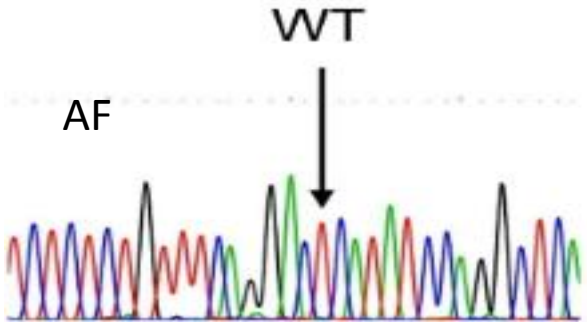
Linkage analysis

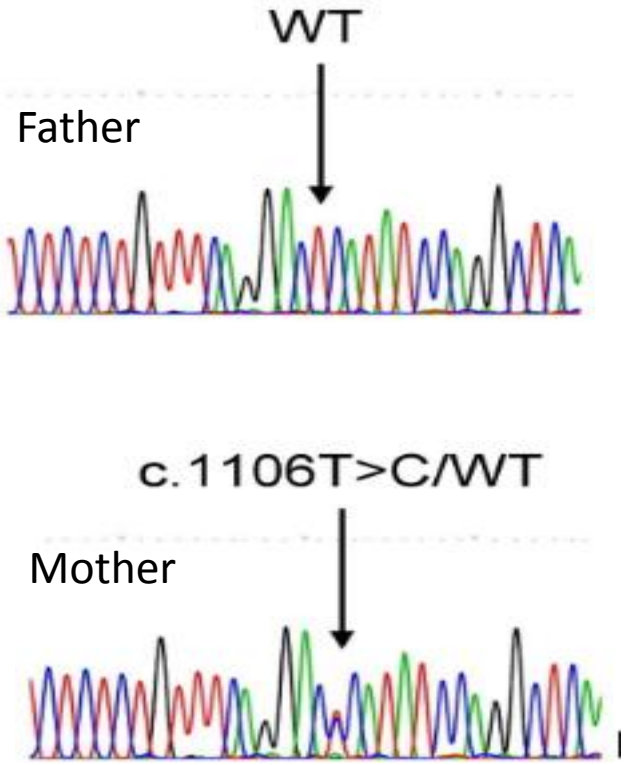




PGD case results
Total: 11 embryos

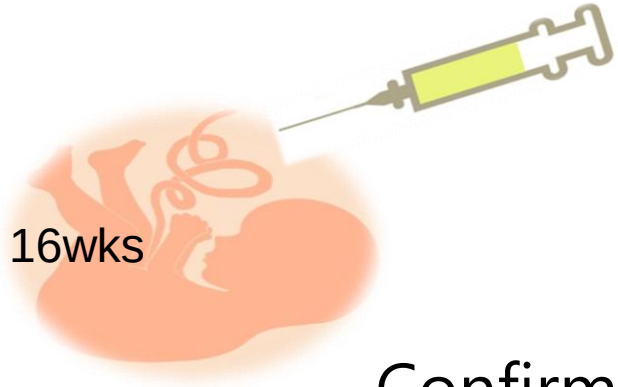
Major: 1 embryo
Wild Type : 4 embryos
Carrier: 5 embryos
No signal : 1 embryo





Embryo transfer

pregnancy



Confirmed by AF

Clinical case in Taiwan – Hearing Loss

Original Paper

Audiology & Neurotology

Audiol Neurotol 2010;15:311–317
DOI: 10.1159/000284349

Received: August 7, 2009
Accepted after revision: Decemb
Published online: February 17, 2

Preimplantation Genetic Diagnosis (Embryo Screening) for Enlarged Vestibular Aqueduct due to *SLC26A4* Mutation

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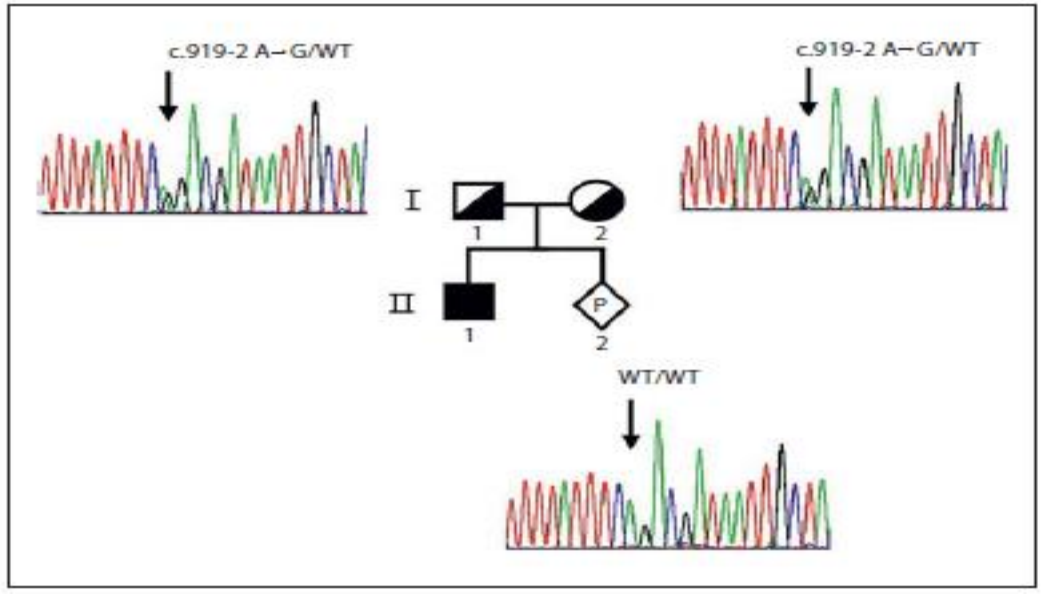

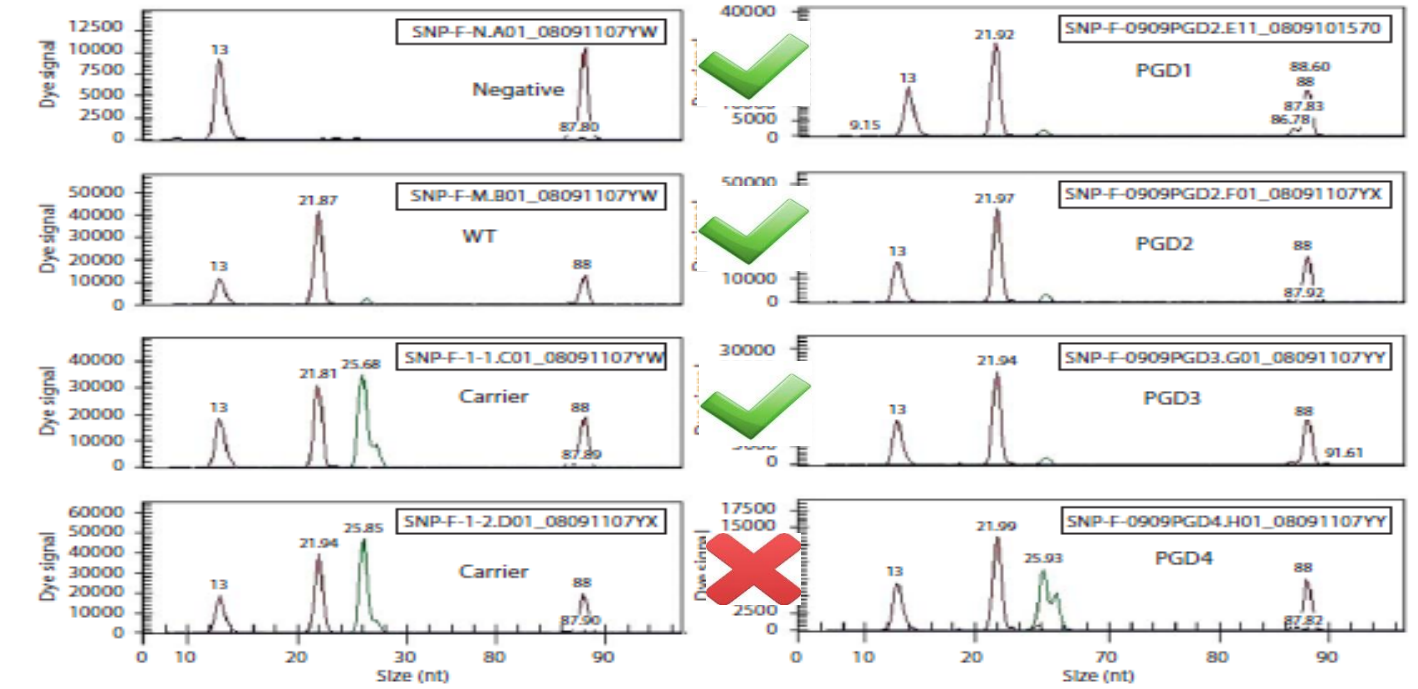

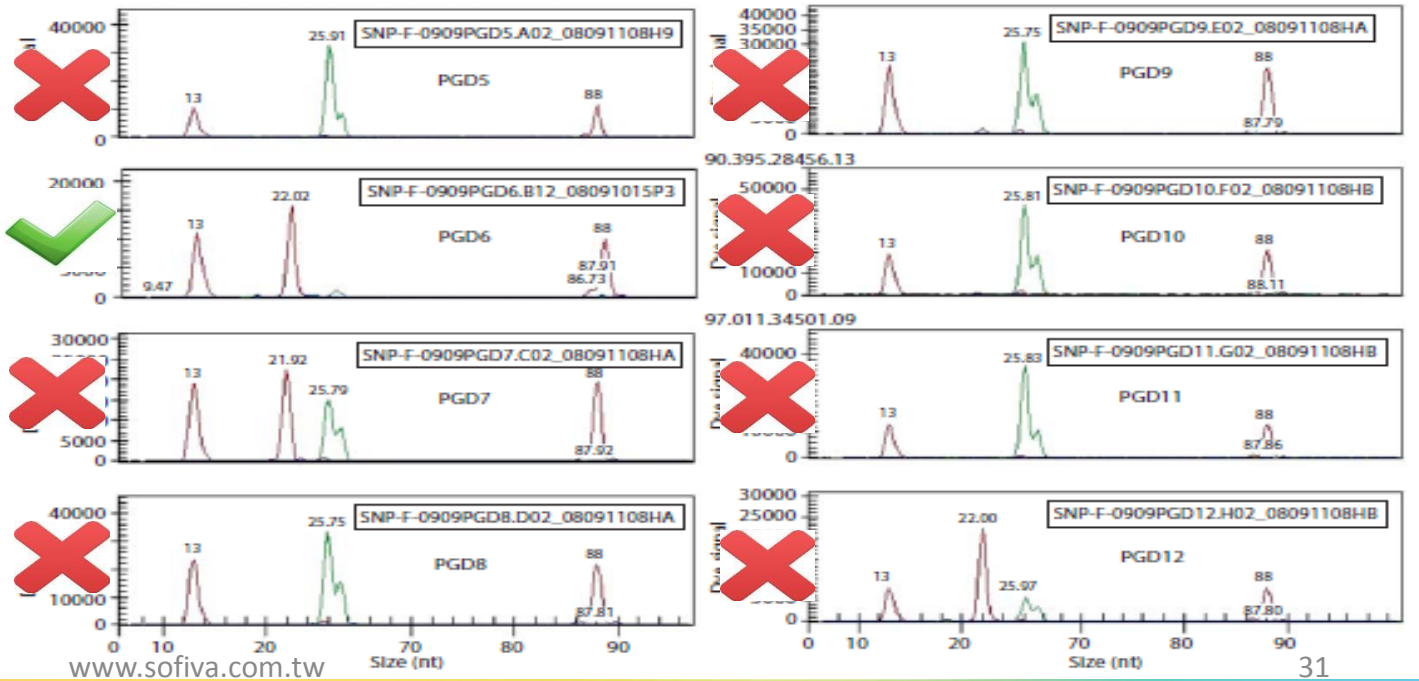

Clinical case - HLA typing & beta thalassemia

ine - Vol 17 No 5. 2008 699-705 Reproductive BioMedicine Online; www.rbmonline.com/Article/3440 on web 1 October 2008

Case report

PGD of β-thalassaemia and HLA haplotypes using OmniPlex whole genome amplification

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Dr Shee-Uan Chen

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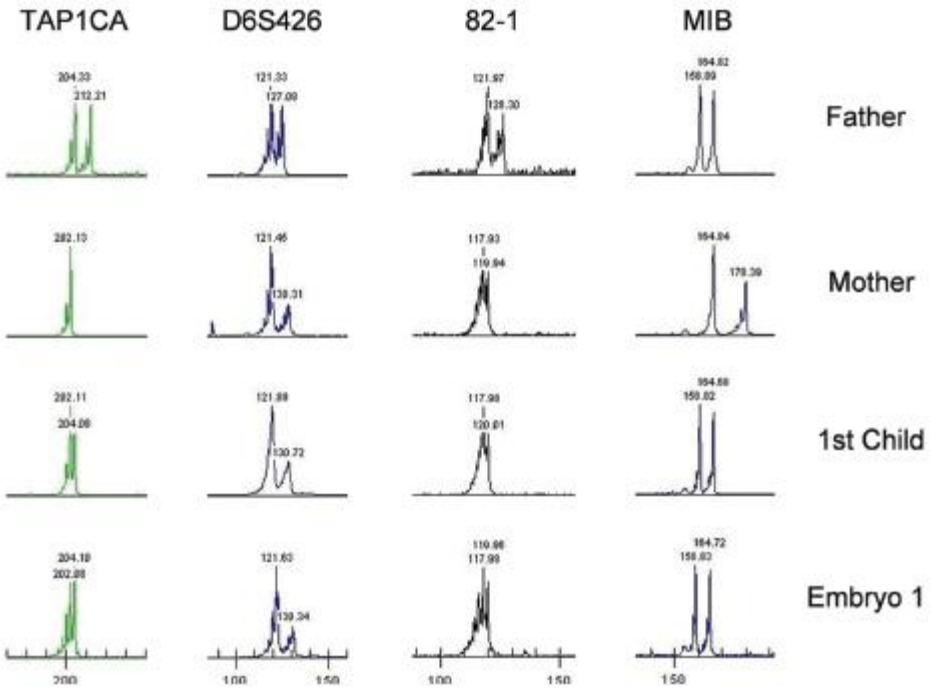
Abstract

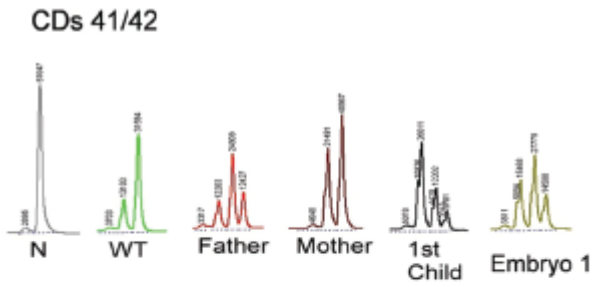
A strategy was developed using the OmniPlex technology of whole genome amplification for preimplantation genetic diagnosis (PGD) of single gene diseases and human leukocyte antigen (HLA) haplotypes. The amplified genomic DNA library was subsequently examined separately for mutation analysis with mini-sequence and for short tandem repeat (STR) markers within the HLA loci. To evaluate the reliability of the protocol prior to PGD, tests of 50 single lymphocytes revealed an amplification efficiency of 92–96% and allele drop-out (ADO) rate of 6–16%. The strategy was validated in one β-thalassaemia family having an affected boy. The couple underwent three cycles of ovarian stimulation and intracytoplasmic sperm injection for PGD. On 16 embryos tested, the amplification efficiency was 88–94% and ADO was 6–19%. Two cycles of embryo transfer were performed, and one pregnancy was achieved. The genotypes of the fetus were shown to be unaffected and HLA-identical, in agreement with PGD, by chorionic villus sampling. The cord blood stem cells from the newborn can be used to treat the affected sibling. This study demonstrates the first successful application of OmniPlex whole genome amplification in PGD of a single gene disorder for selecting unaffected and HLA-compatible embryos.

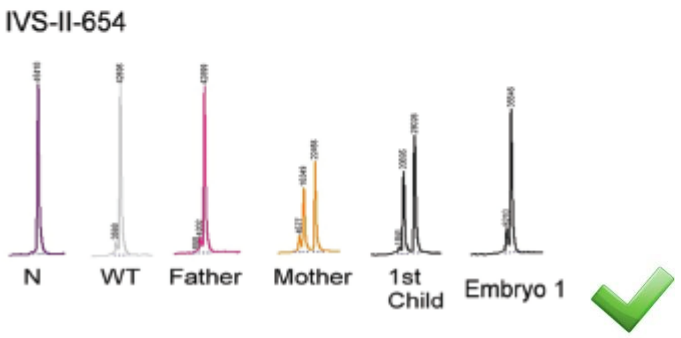
Keywords: *HLA, preimplantation genetic diagnosis, β-thalassaemia, whole genome amplification*

HLA typing



HBB genotyping



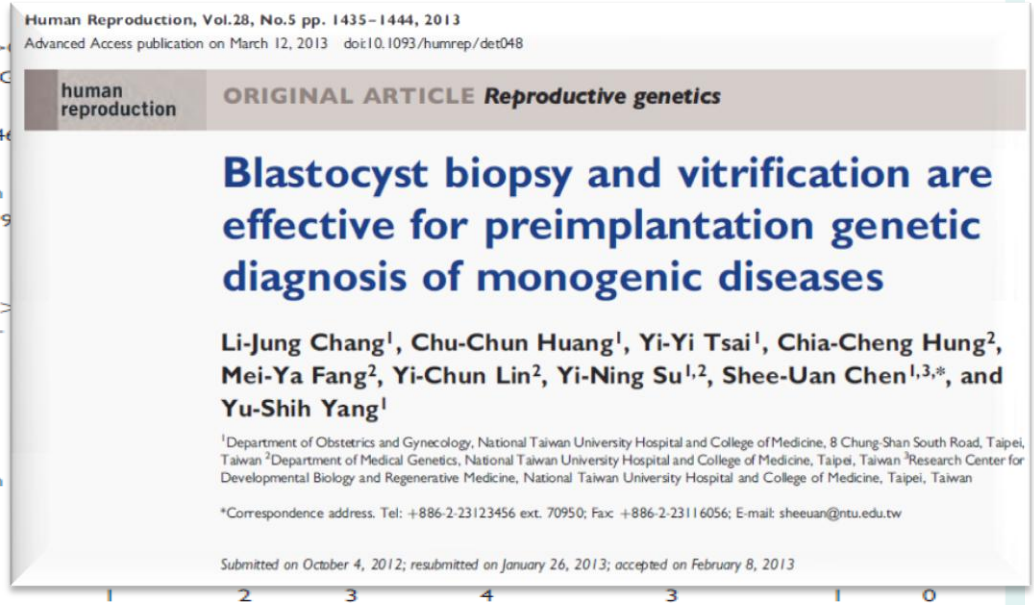


PGD for single gene disorder in Sofiva lab

Table I The hereditary modes, mutation sites of monogenic diseases, OPU treatment cycles, genotyping results of lymphocyte pretests and PGD of blastocysts of 33 couples.

Case	Diseases	Modes	Husband	Wife	OPU cycles	Lymphocyte test		PGD of blastocysts			
						AF	ADO	Unaffected	Affected	AF	ADO
1	α-Thalassemia	AR	SEA	SEA	1	1	2	6	2	1	0
2	Neurofibromatosis type 1	AD	Normal	NF1 c.6709 C>T	1	3	7	9	3	0	1
3	Spinal muscular atrophy	AR	SMN1:SMN2 = 1:3	SMN1:SMN2 = 1:3	1	1	2	10	5	0	2
4	Duchenne muscular dystrophy	XR	Normal	DMD deletion exon 48–52	2	2	3	11	3	0	1
5	β-Thalassemia	AR	654	654	1	3	3	4	2	1	0
6	Osteogenesis Imperfecta	AD	COL1A1 c.1064_1068 del CTGGT	Normal	2	1	1	10	6	0	0
7	α-Thalassemia	AR	SEA	SEA							
8	Congenital deafness	AR	SLC26A4 c.916_917 ins G	SLC26A4 c.919-2 A>							
9	Congenital deafness	AR	SLC26A4 c.919-2 A>G	SLC26A4 c.1579 A>C							
10	Spinocerebellar ataxia type 3	AD	AXTN3 (CAG)n: 14/69	Normal							
11	Duchenne muscular dystrophy	XR	Normal	DMD deletion exon 4							
12	α-thalassemia	AR	SEA	SEA							
13	Hemophilia A	XR	Normal	F8 intron 22 inversion							
14	Spinocerebellar ataxia type 6	AD	Normal	CACNA1A (CAG)n: 9							
15	α-Thalassemia	AR	SEA	SEA							
16	α-Thalassemia	AR	SEA	SEA							
17	Osteopetrosis	AR	TCIRG1 c.1213 G>A	TCIRG1 c.196 + 5 G>							
18	Bardet-Biedl syndrome	AR	BBS2 c.534 + 1 G>T	BBS2 c.534 + 1 G>T							
19	Spinocerebellar ataxia type 3	AD	ATXN3 (CAG)n: 14/62	Normal							
20	α-Thalassemia	AR	SEA	SEA							
21	Neurofibromatosis type 1	AD	Normal	NF1 c.889-1 G>T							
22	Marfan's syndrome	AD	FBN1 c.2 T>A	Normal							
23	Hemophilia A	XR	Normal	F8 intron 22 inversion							
24	Ornithine transcarbamylase deficiency	XR	Normal	OTC c.805G>A							
25	Retinoblastoma	AD	Normal	RB1 c.1960 G>T							
26	α-Thalassemia	AR	FIL	SEA							
27	α-Thalassemia	AR	SEA	SEA	1	2	3	4	3	1	0
28	Retinoblastoma	AD	Normal	RB1 c.862-2 A>G	1	2	2	1	1	0	1
29	Spinocerebellar ataxia type 3	AD	ATXN3 (CAG)n: 14/73	Normal	1	2	1	6	1	1	0
30	Alzheimer's disease	AD	Normal	PSEN1 c.438 G>A	1	3	0	3	2	0	0
31	Duchenne muscular dystrophy	XR	Normal	DMD duplication 19-44	1	3	3	7	1	1	1
32	α-Thalassemia	AR	SEA	SEA	1	1	3	0	1	1	0
33	Spinocerebellar ataxia type 3	AD	Normal	ATXN3 (CAG)n: 14/74	1	1	2	2	7	0	0

OPU, ovum pick-up; AD, autosomal dominant; AR, autosomal recessive; XR, X-linked recessive; AF, amplification failure; ADO, allele drop-out. Fifty samples of lymphocyte tests were performed for each case.



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human reproduction ORIGINAL ARTICLE *Reproductive genetics*

Blastocyst biopsy and vitrification are effective for preimplantation genetic diagnosis of monogenic diseases

Li-Jung Chang[1], Chu-Chun Huang[1], Yi-Yi Tsai[1], Chia-Cheng Hung[2], Mei-Ya Fang[2], Yi-Chun Lin[2], Yi-Ning Su[1,2], Shee-Uan Chen[1,3,*], and Yu-Shih Yang[1]

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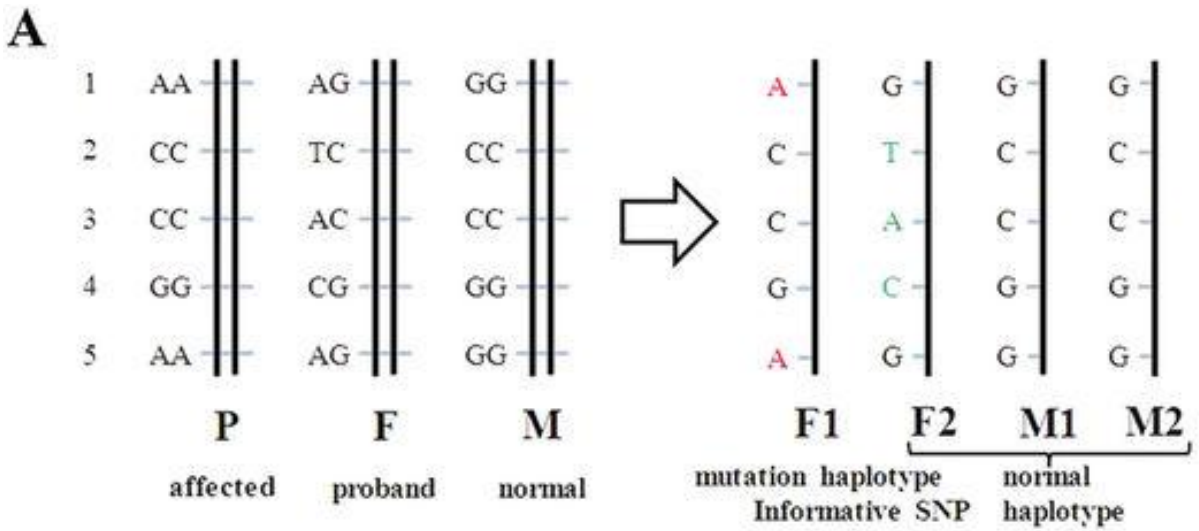
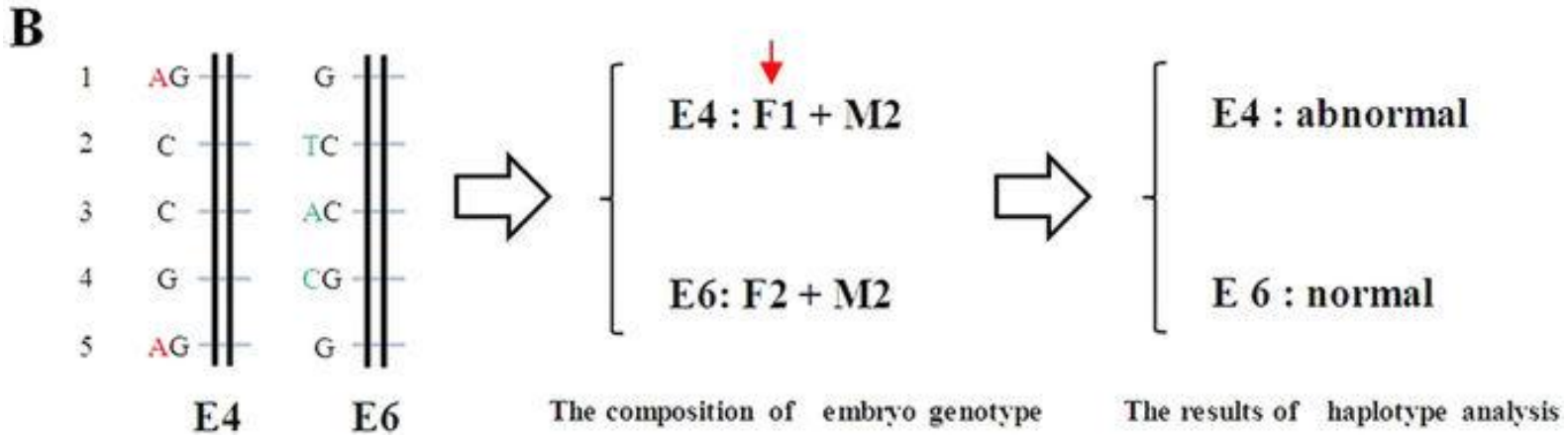
Genome-wide karyomapping for PGD





SNP-array based

haplotype analysis

Scientific Reports **volume6**, Article number: 25488 (2016)

Traditional PGD vs SNP-based PGD

	Traditional PGD	SNP-based PGD
Technology	Specific probe (primer) PCR Sanger sequencing STR marker	SNP array
Mutation site	Need to know	✓ Not need to know
Coverage	Specific gene / locus	✓ Any sites coverage by SNP probes
Disadvantage	Take time to design probes Separate designs when multiple loci	~ 90 % sensitivity Error rate 1% ~10% depends on different disease

Thanks for your attention