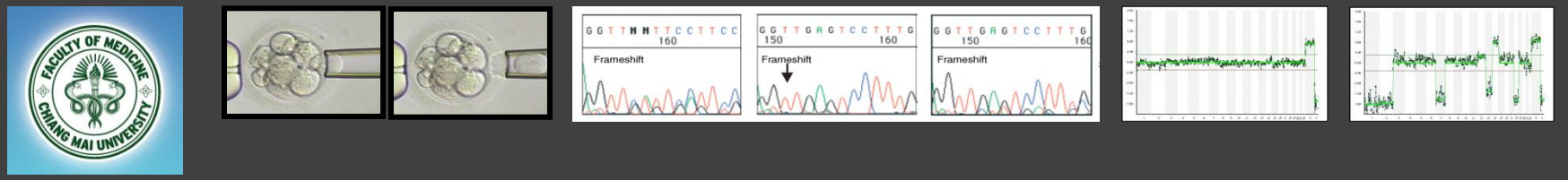
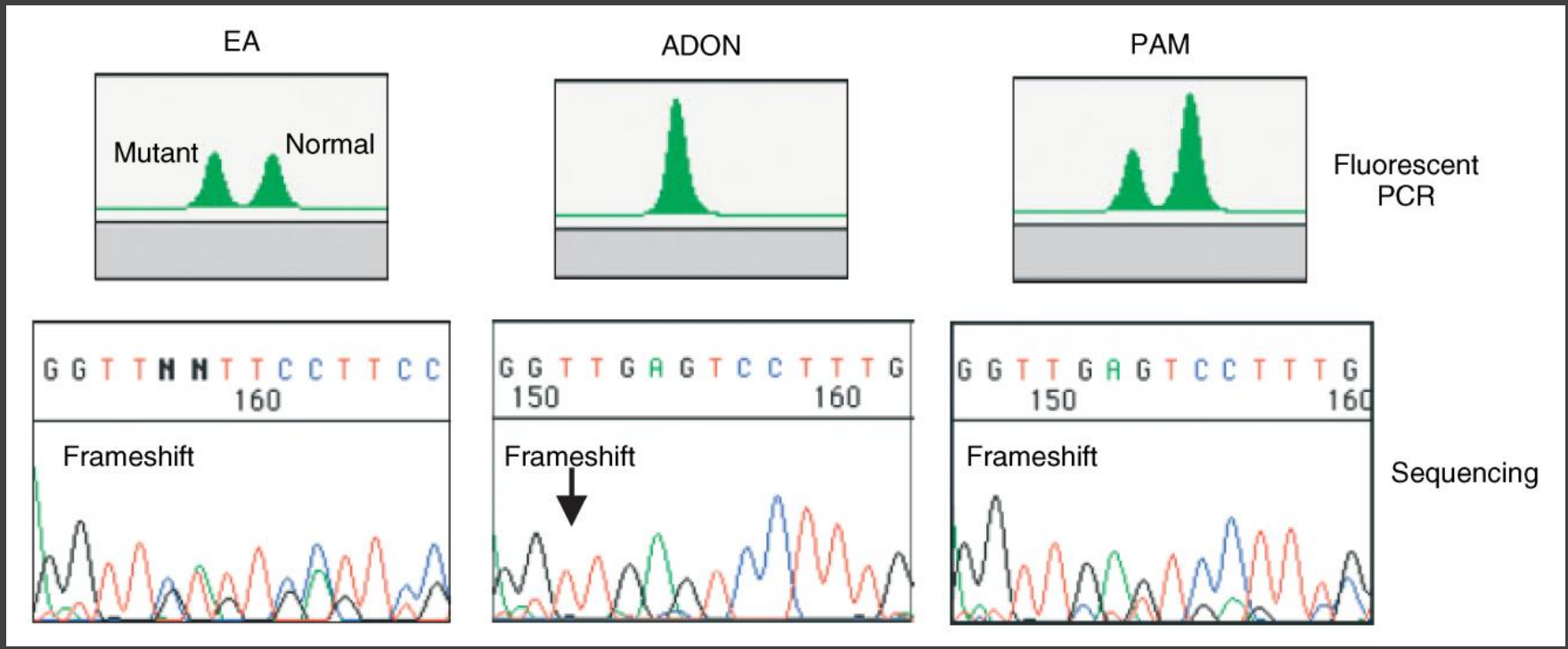
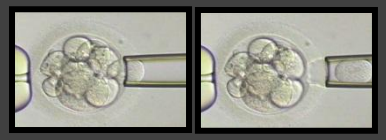
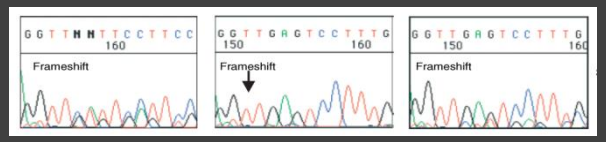
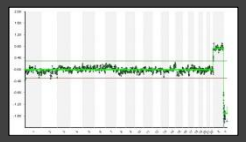


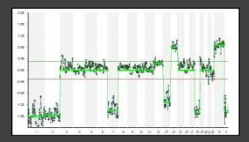
SINGLE CELL SEQUENCING
beta-Thalassemia codon 41-42



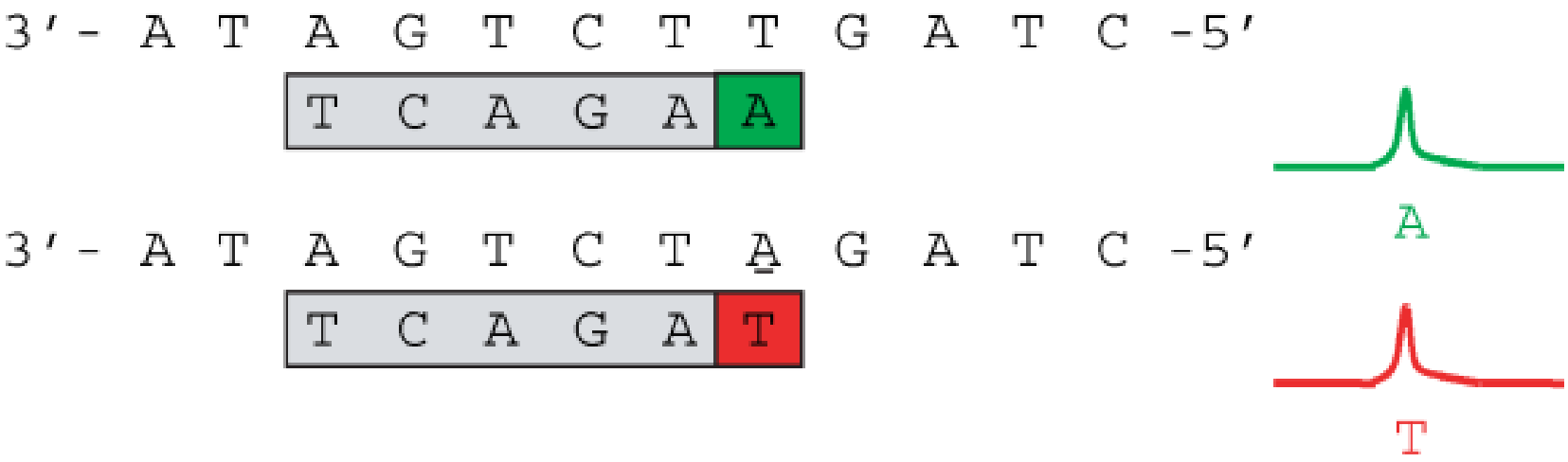


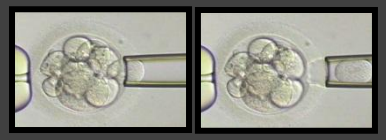


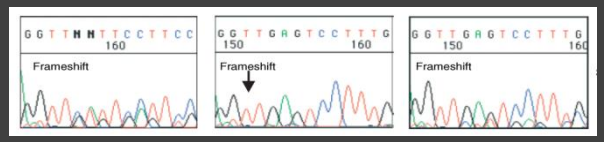


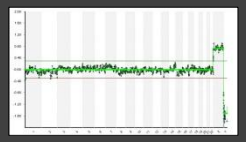


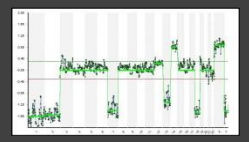
MINI-SEQUENCING



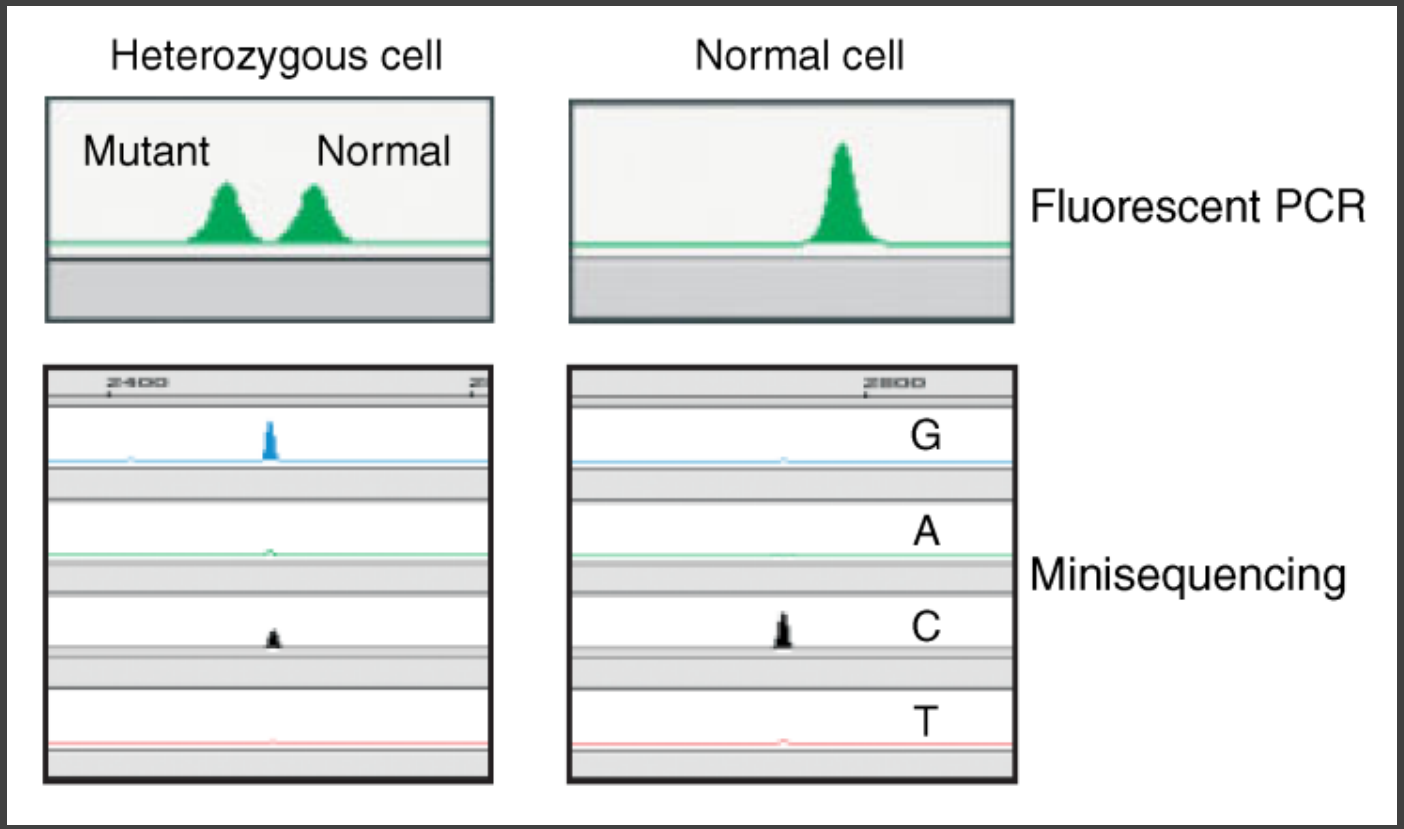


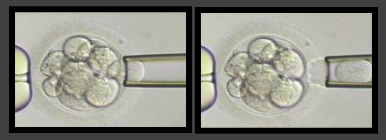
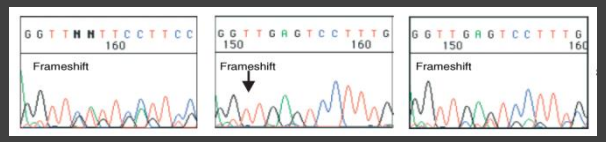


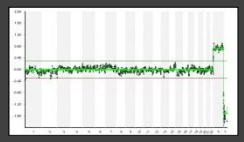
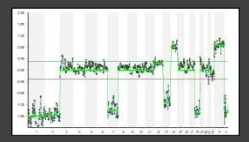




SINGLE CELL MINI-SEQUENCING
beta-Thalassemia codon 41-42



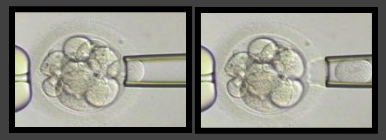
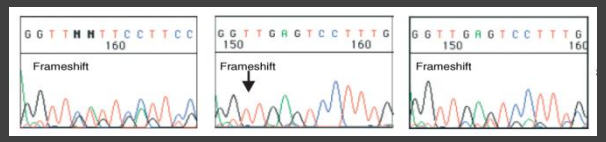



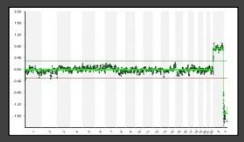
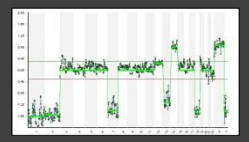



CONTAMINATION

- Dominant disorders
  - interpret normal as affected*

  - interpret affected as affected

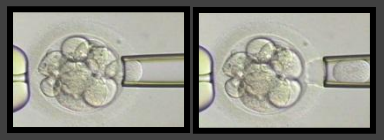



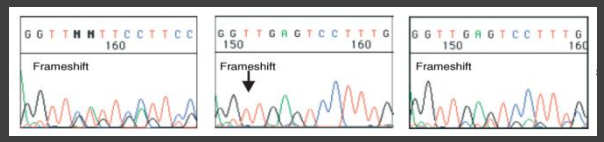



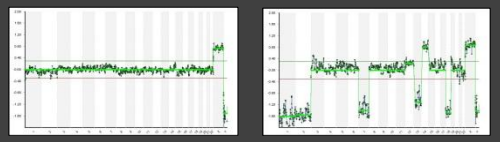
CONTAMINATION

- Recessive disorders
  - interpret normal as heterozygous (carrier)

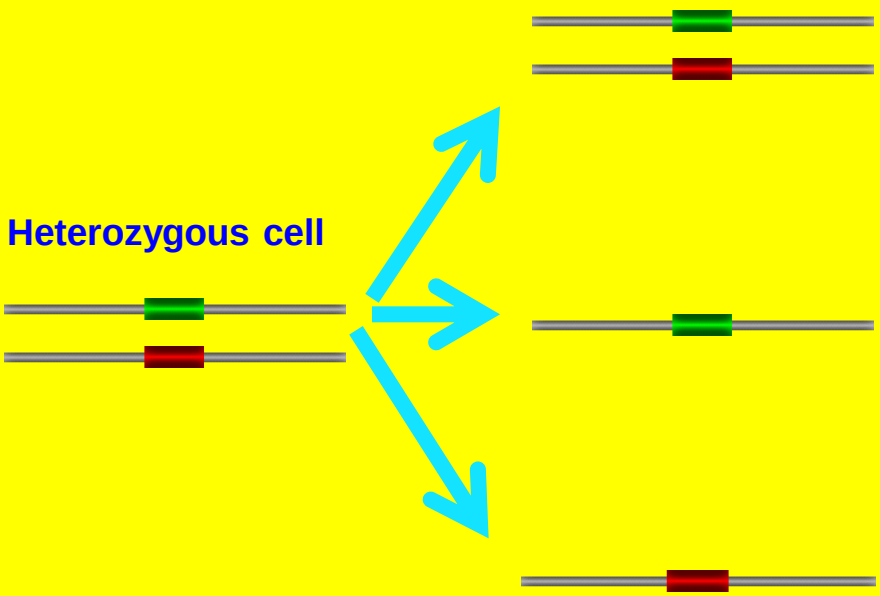
  - interpret affected as heterozygous (carrier)*







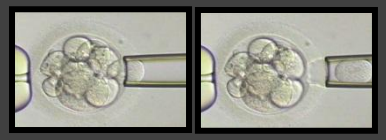
Allele Dropout

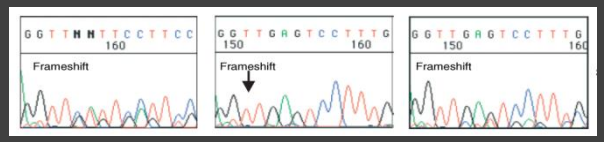


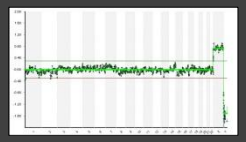
Amplification of both alleles
Heterozygous genotype predicted

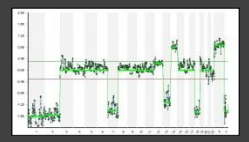
ADO of mutant allele
Amplification of normal allele only
Homozygous normal genotype predicted

ADO of normal allele
Amplification of mutant allele only
Homozygous affected genotype predicted

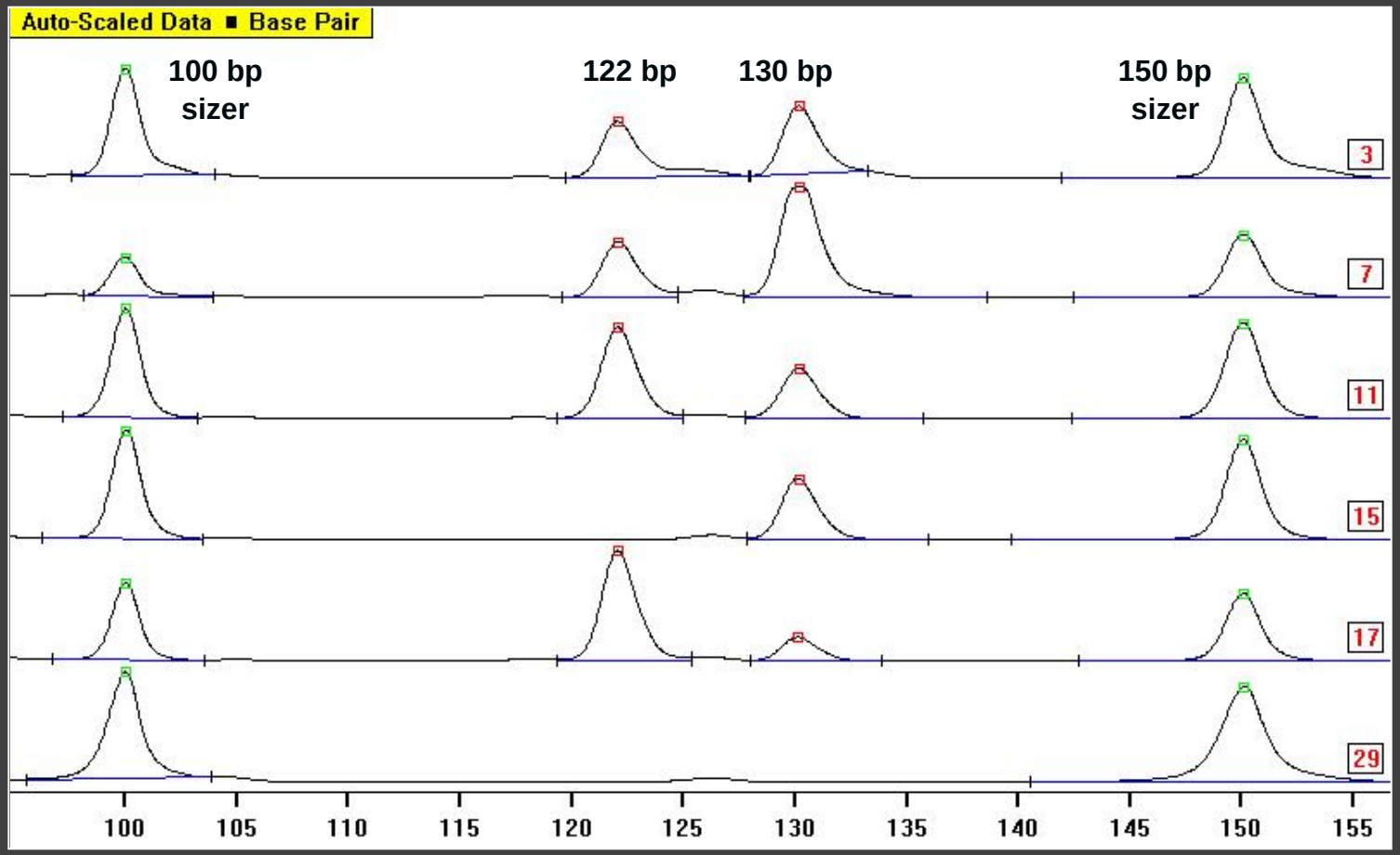


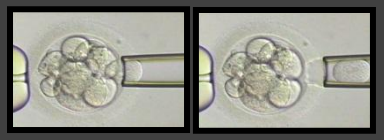
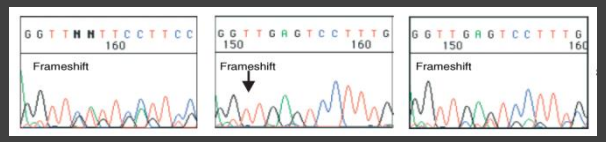


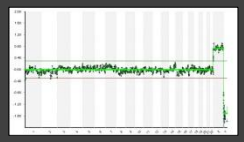
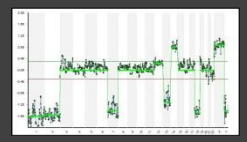




ALLELE DROP-OUT

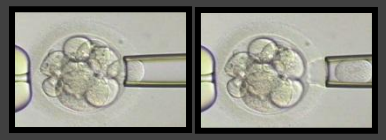
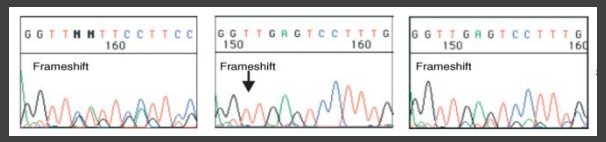


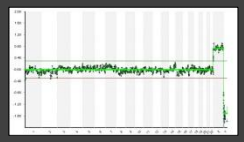
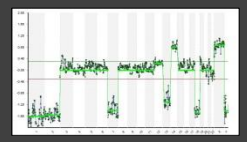



PGD for Single Gene Disorders

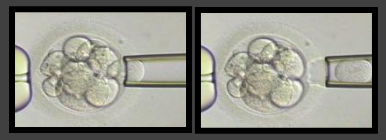
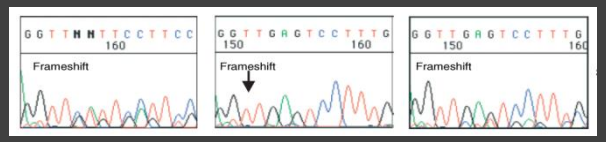
- Duchenne muscular dystrophy(Liu et al., 1995)
- Fragile X syndrome (Sermon et al., 1999)
- Tay Sachs disease (Gibbons et al., 1995)
- Marfan's syndrome (Harton et al., 1996)
- Myotonic dystrophy (Piyamongkol et al., 2001b)
- Charcot Marie Tooth type 1A (De Vos et al., 1998)
- Familial adenomatous polyposis coli (FAPC) (Ao et al., 1998)
- Huntington's chorea (Sermon et al., 1998)

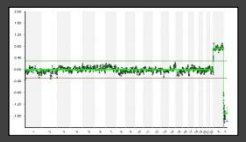
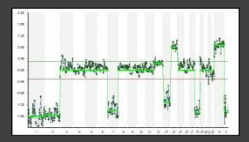



PGD for Single Gene Disorders

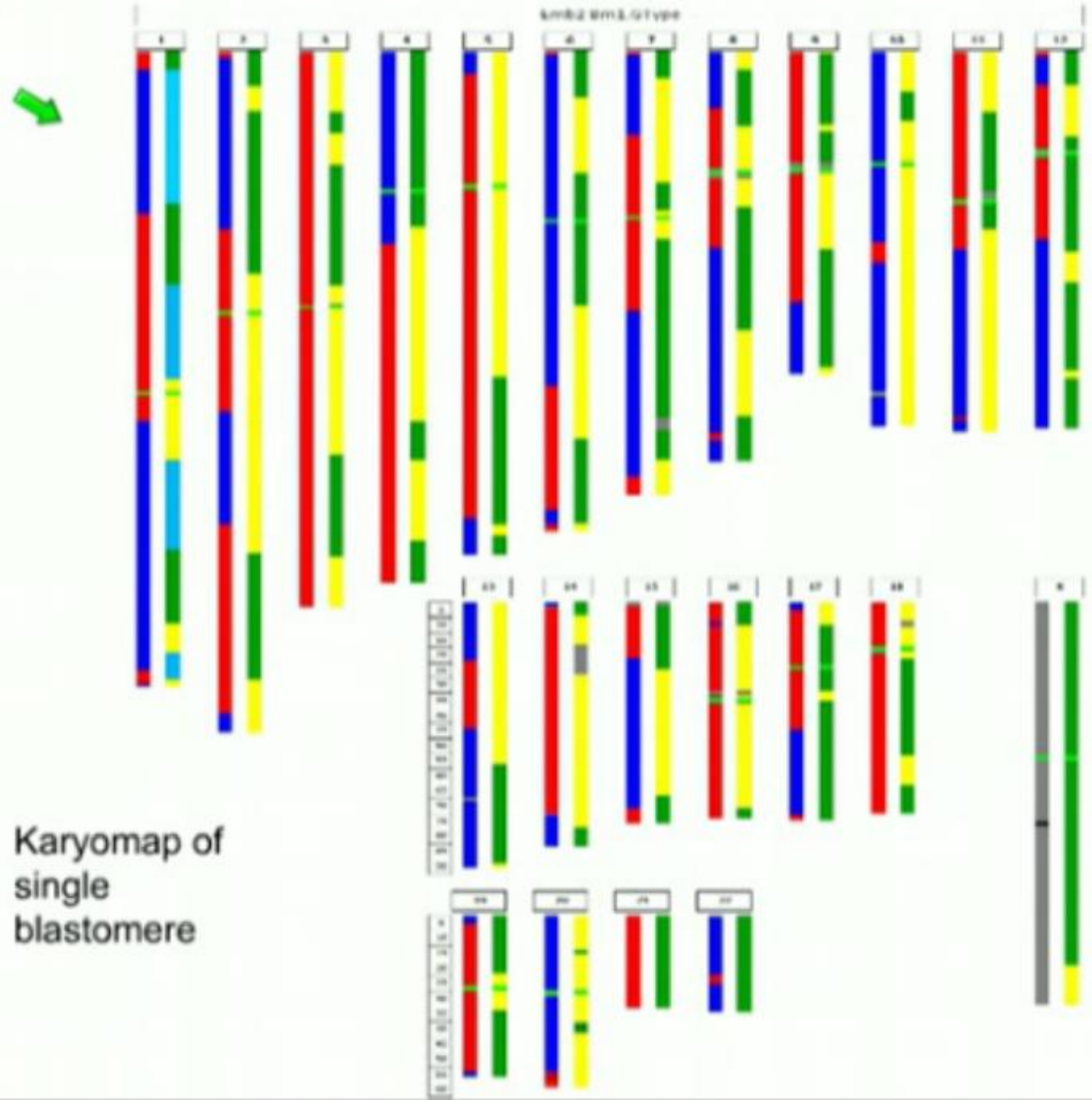
- Severe inherited skin diseases (McGrath and Handyside, 1998)
- Sickle cell anaemia (Rechitsky et al., 1998)
- Spinal muscular atrophy (Dreesen et al., 1998)
- β-thalassaemia (Kanavakis et al., 1999; Kuliev et al., 1999; Piyamongkol et al., 2006)
- Congenital adrenal hyperplasia (Van de Velde et al., 1999)
- Lesch Nyhan syndrome (Ray et al., 1999),
- Medium chain acyl CoA dehydrogenase (MCAD) deficiency (Ioulianos et al., 2000)

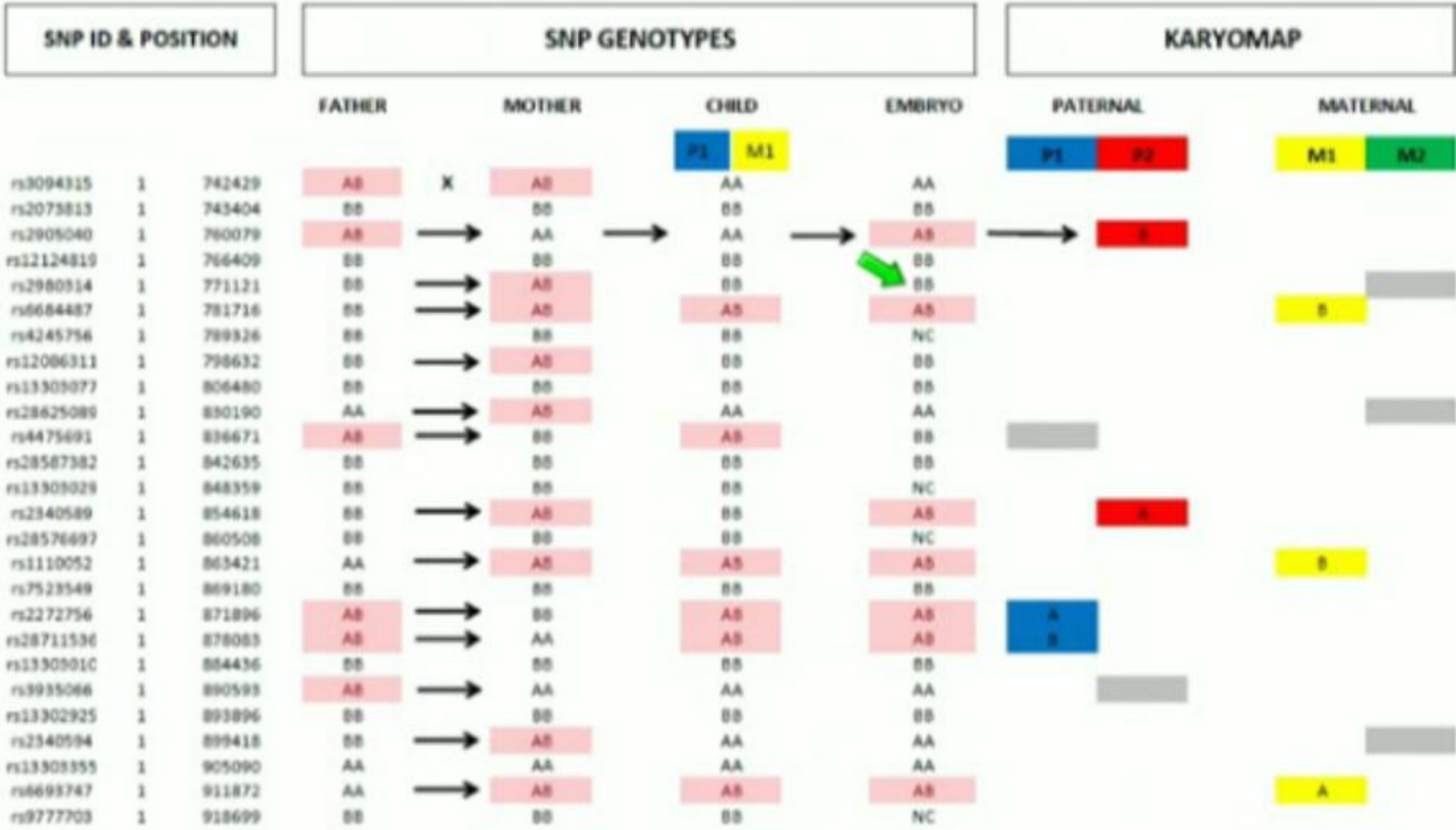



PGD of beta-Thalassaemia

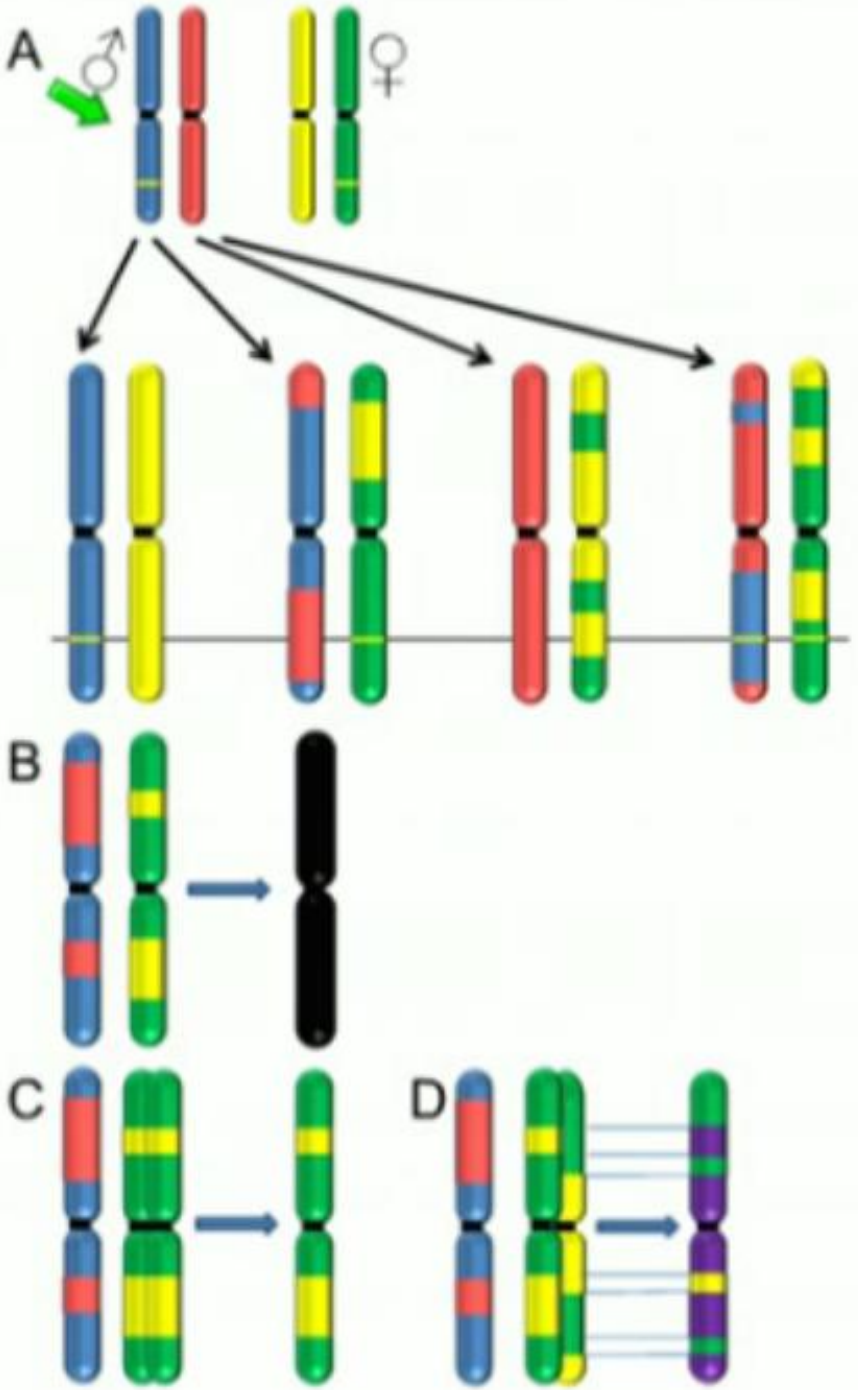
- Sexing - Handyside, 1989
- beta-Thalassaemia: RFLP - Kuliev, 1998
- beta-Thalassaemia: DGGE - Kanavakis, 1999
- beta-Thalassaemia: RFLP - De Rycke, 2001
- beta-Thalassaemia: RFLP & reverse dot-blot - Chamayou, 2002
- beta-Thalassaemia: sequencing - Hussey, 2002
- beta-Thalassaemia: WGA + reverse dot-blot - Jiao, 2003
- beta-Thalassaemia: F-PCR - Piyamongkol, 2006

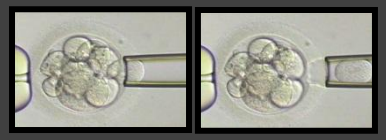
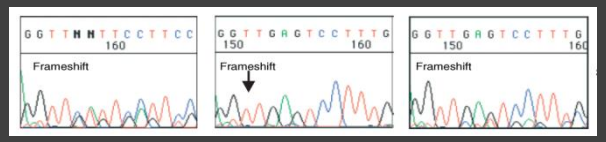


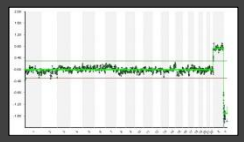
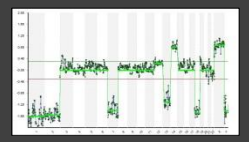
Karyomap of single blastomere


SNP ID & POSITION
SNP GENOTYPES
KARYOMAP
FATHER
MOTHER
CHILD
EMBRYO
PATERNAL
MATERNAL
P1
M1
P1
P2
M1
M2
rs3094315 1 742429 AB X AB AA AA
rs2073813 1 743404 BB BB BB BB
rs2905040 1 760079 AB AA AA AB P2
rs12124819 1 766409 BB BB BB BB
rs2980514 1 771121 BB AB BB BB
rs6684487 1 781716 BB AB AB AB M1
rs4245756 1 789326 BB BB BB NC
rs12086313 1 798632 BB AB BB BB
rs13303077 1 806480 BB BB BB BB
rs28625089 1 830190 AA AB AA AA
rs4475691 1 836671 AB BB AB BB P1
rs28587382 1 842635 BB BB BB BB
rs13303029 1 848359 BB BB BB NC
rs2340589 1 854618 BB AB BB AB P2
rs28576697 1 860508 BB BB BB NC
rs1110052 1 863421 AA AB AB AB M1
rs7523549 1 869180 BB BB BB BB
rs2272756 1 871896 AB BB AB AB P1
rs28711536 1 878083 AB AA AB AB P1
rs1330501C 1 884436 BB BB BB BB
rs9935066 1 890593 AB AA AA AA P2
rs13302925 1 893896 BB BB BB BB
rs2340594 1 899418 BB AB AA AA M2
rs13303355 1 905090 AA AA AA AA
rs6693747 1 911872 AA AB AB AB M1
rs9777703 1 918699 BB BB BB NC

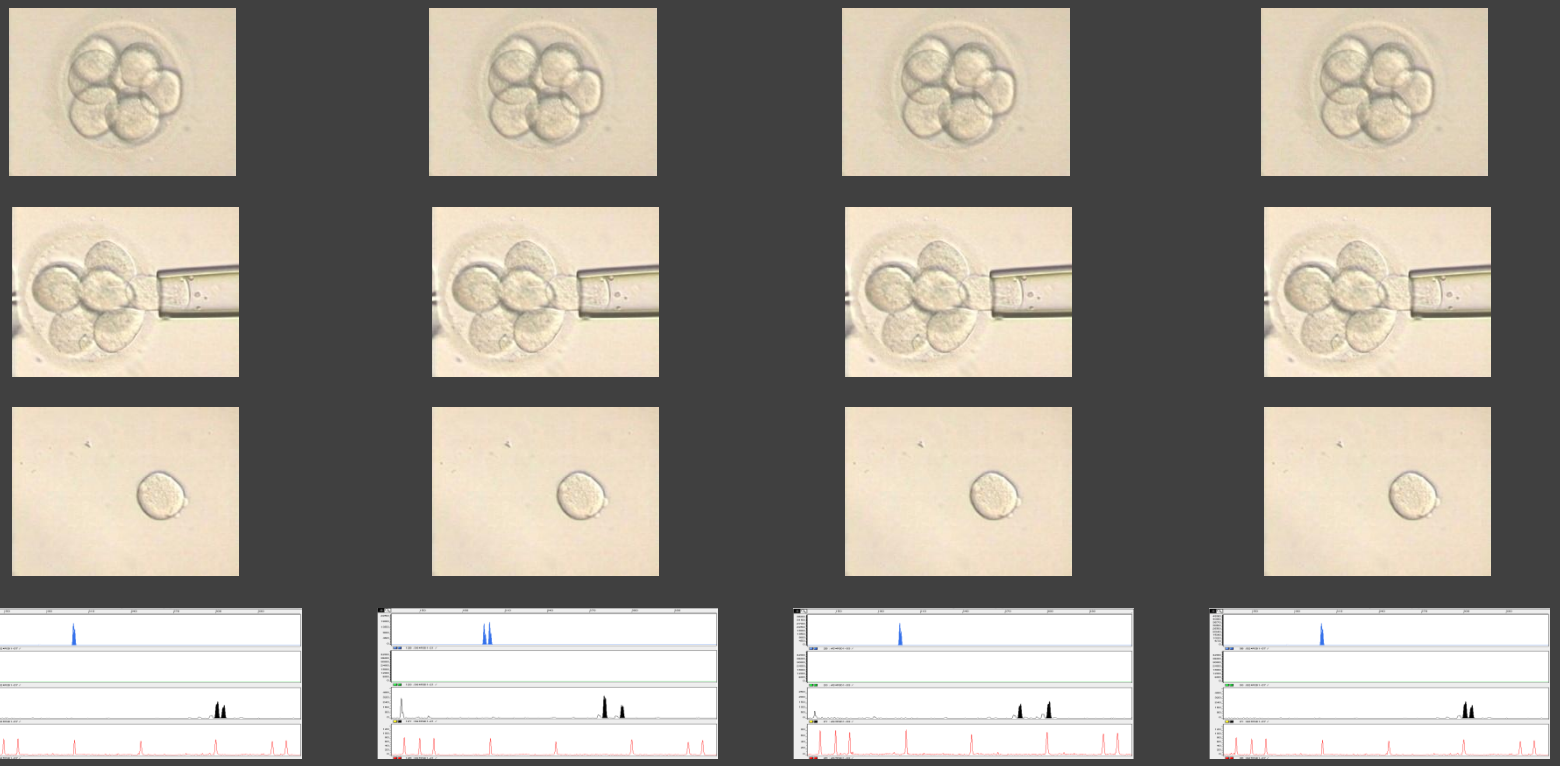
Karyomapping combines genome wide linkage based detection of single gene defects (A) with chromosomal aneuploidy including monosomy/deletions (B) and trisomies involving inheritance of two different meiotic chromosomes from one parent (D). Chromosome duplication is not detected (C).



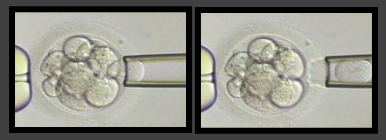



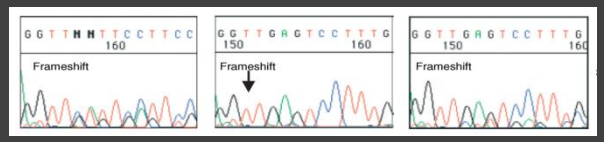



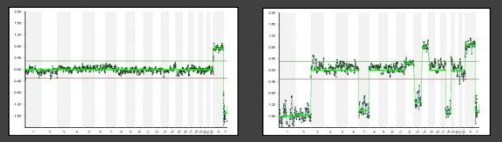
PGD of beta-thalassaemia codon41-42



normal	heterozygous	affected	normal
ET	ET		ET

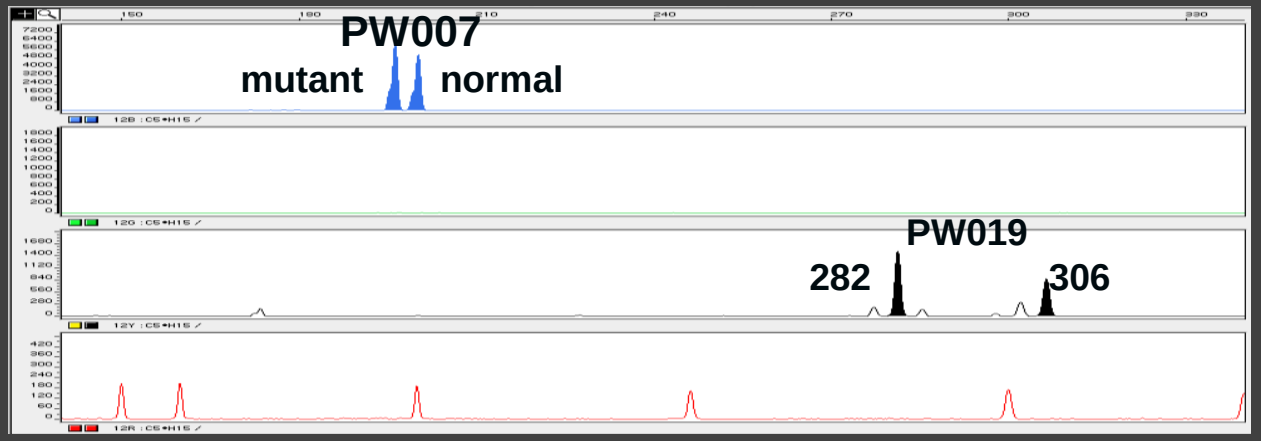




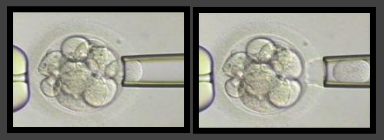


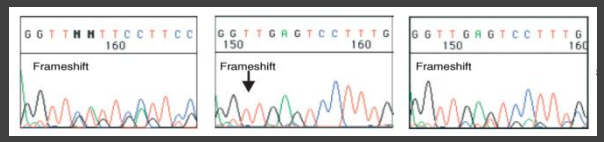
Work-up of PGD Protocol for beta-Thalassaemia codon 41-42

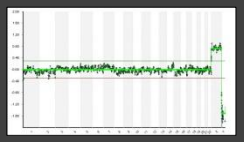
Father

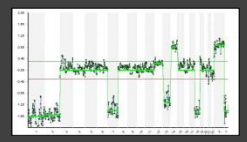


Mother







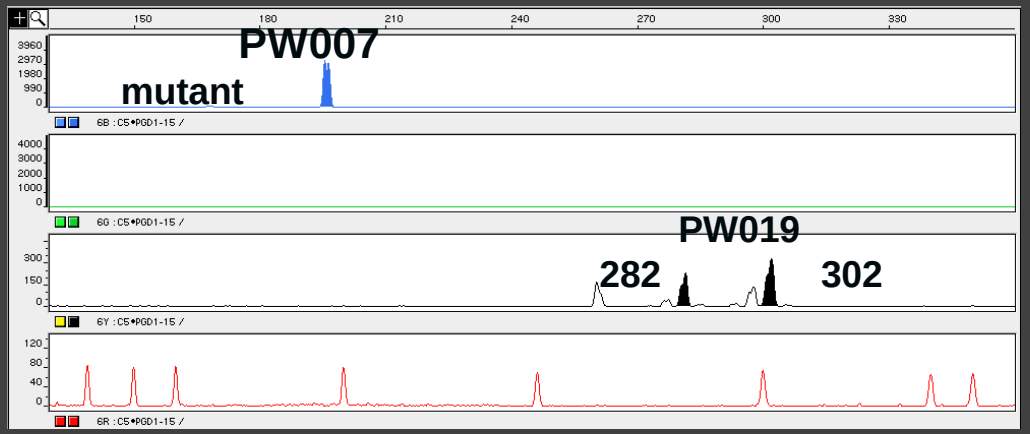


PGD of beta-Thalassaemia codon 41-42

Embryo 1

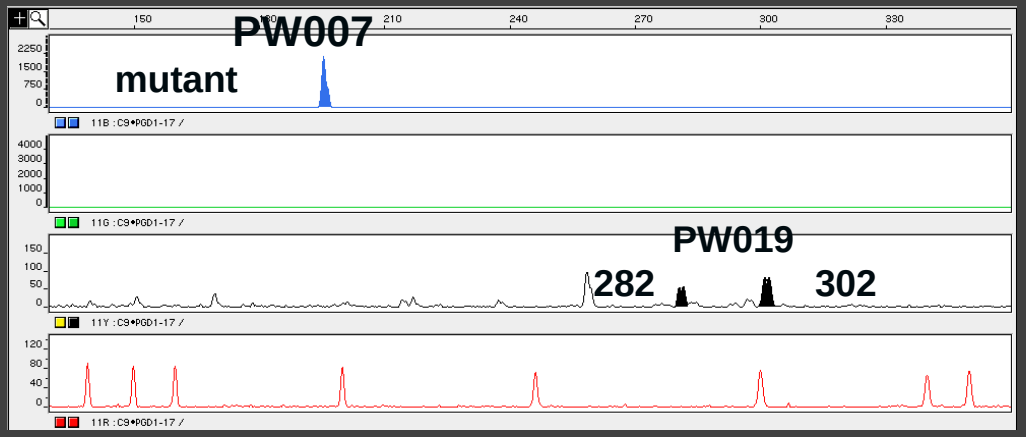
8-cell

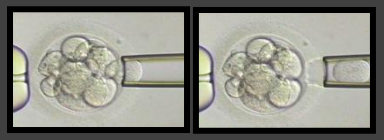
: Affected

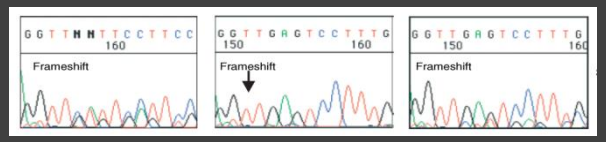


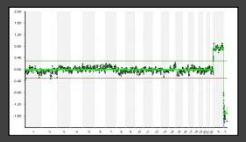
Confirmation -

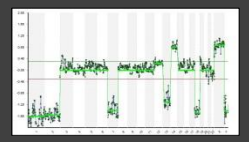
Affected





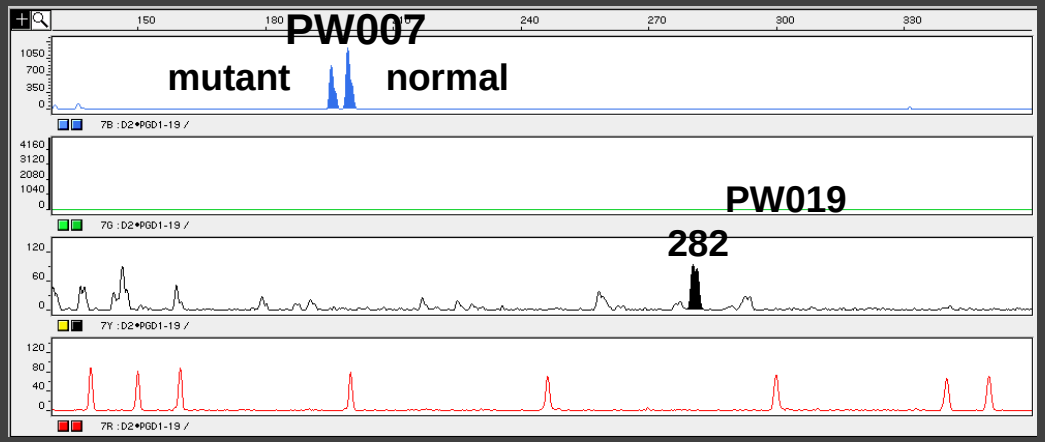




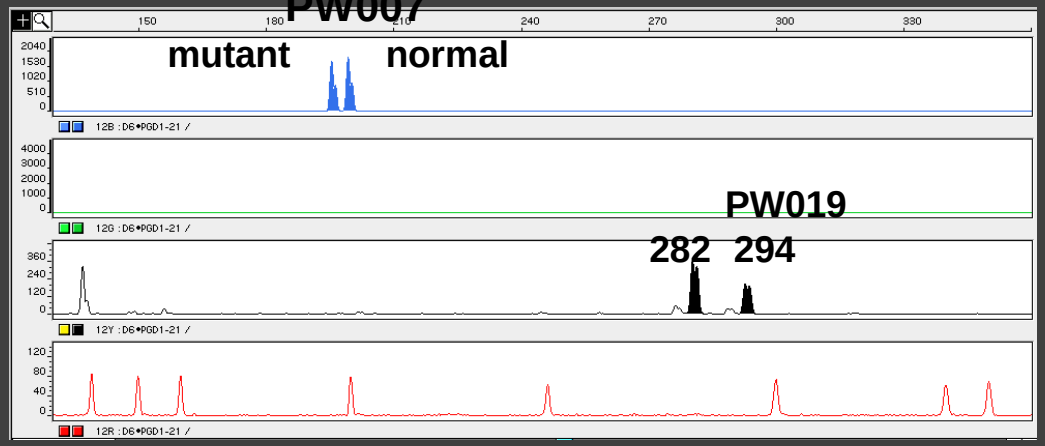


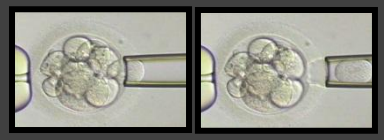
PGD of beta-Thalassaemia codon 41-42

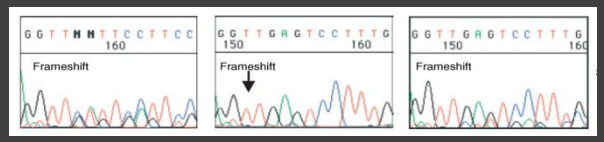
Embryo 8
8-cell
: Heterozygous

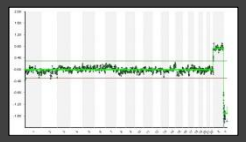


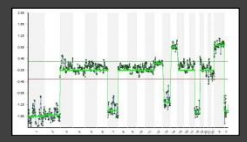
Confirmation -
Heterozygous





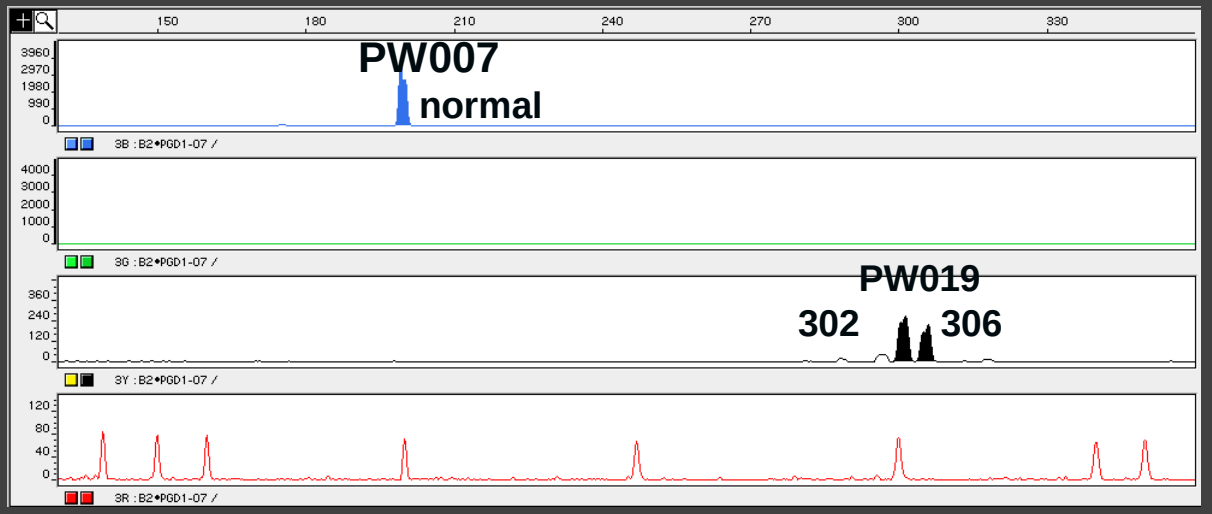




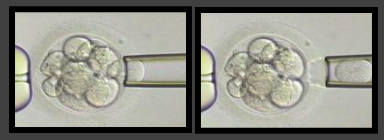


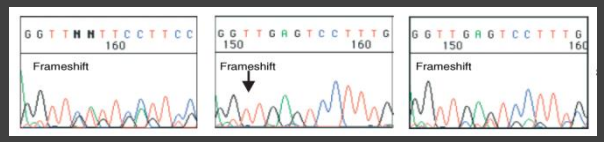
PGD of beta-Thalassaemia codon 41-42

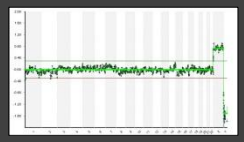
Embryo 4
8-cell
: Normal

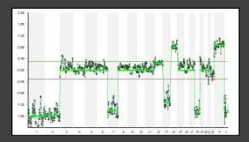


ET









Work-up of PGD Protocol for alpha-Thalassemia^{-SEA}