MINISTRY OF DEFENSE
MILITARY MEDICAL UNIVERSITY

# STUDY ON PROCEDURE DEVELOPMENT FOR PRE-IMPLANTATION GENETIC DIAGNOSIS IN IVF-ICSI

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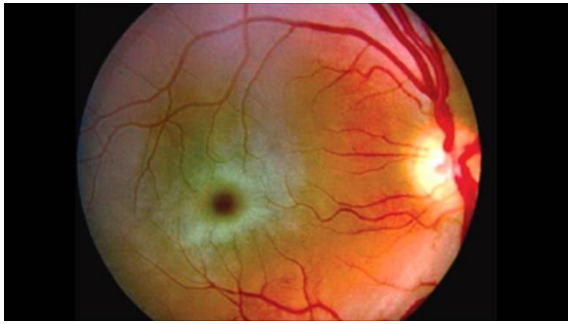
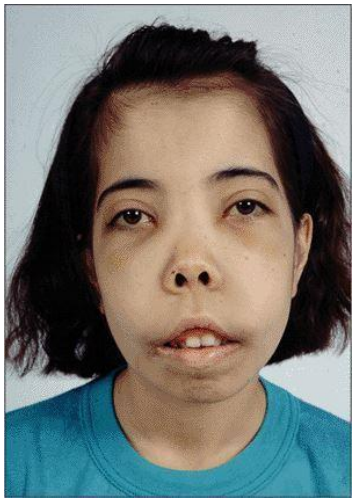
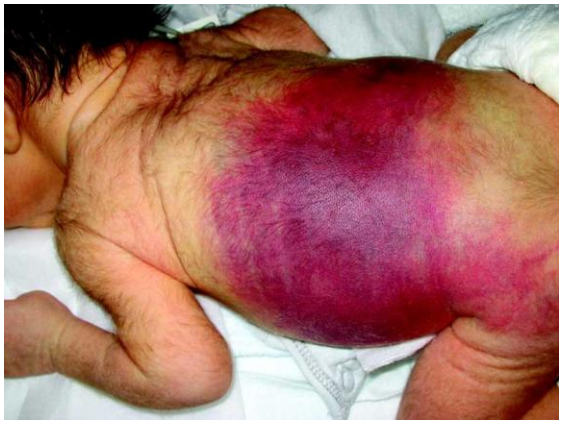
# INSTRODUCTION
## INHERITED DISEASES

MORE THAN 4.000 INHERITED DISEASES







# INSTRODUCTION
# PREVENTIVE STEPS


Carrier, chromosome aberrations
Rh incompatibility

•Family history: CF, FragileX, DMD, hemophilia, mental redartation, CAH,
•Race
……..

Avoid hazard agents
Preventive medicamens
Prenatal screening and diagnosis

# OBJECTIVES

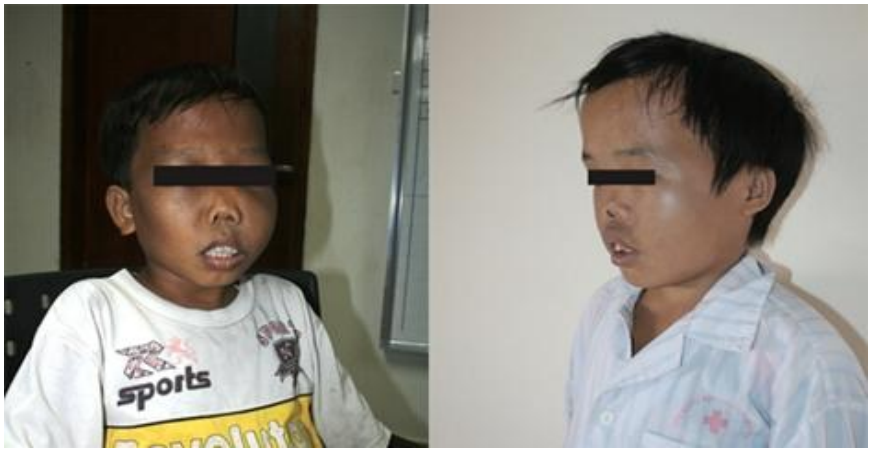
1. **Developed Pre-Implantation Genetic Diagnosis procedures.**
2. **Application of the PGD procedures on some monogenics disorders in Vietnam.**

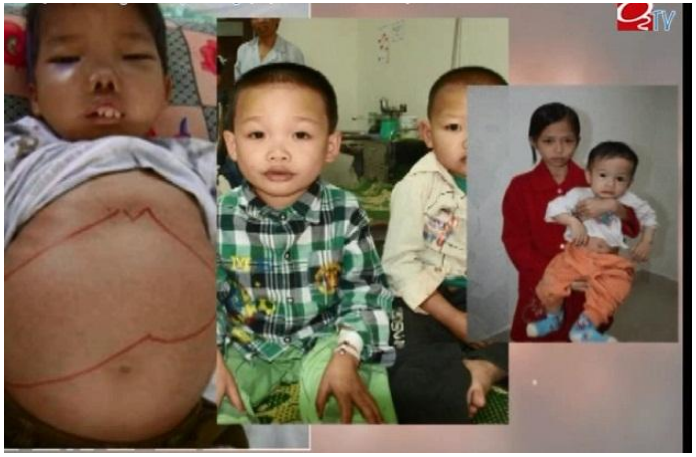
# OVERVIEW
# PGD VS PGS

| **PGD (pre - implantation genetic diagnosis):** | **PGS (pre-implantation genetic screening):** |
| --- | --- |
| **Detection known mutation in embryos** | **Usually for chromosome aberrations (maybe)** |

# THALASSEMIA

Thalassemia is the most common monogenic disorder in over the wordl. In VN, about 5 millions carriers or patients. About 2.000 thalassemia babies annually.



Dependent on alpha or beta globin deficiency , there are 2 main kinds: alpha thalassemia and beta-thalassemia.

# α-THALASSEMIA

| Thalassemia types | *α-thalassemia* | Manifest |
|---|---|---|
| α thalassemia silent | 1 of 4 copy gene del. | No clinical manifest. |
| α thalassemia minor | 2 of 4 copy gene del. | Clinical and hematological manifest |
| HbH | 3 of 4 copy gene del. | Moderate thalassemia. |
| Hb Bart's | All 4 copy gene del. | Serious leading to died. |

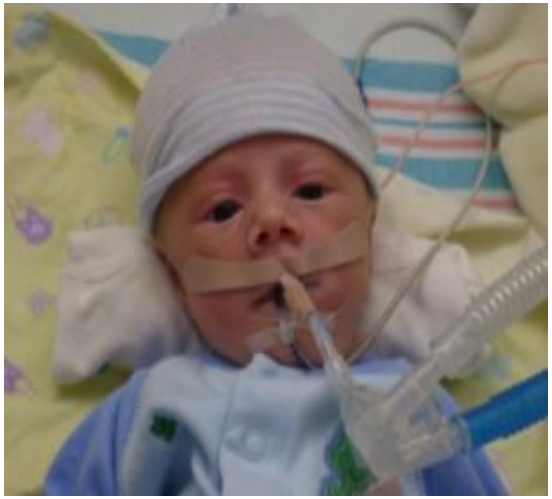
# CLASSIFICATION OF β THALASSEMIA(genetic)

| CLASSIFICATION | GENOTYPE | CLINICAL SEVERITY |
|---|---|---|
| β thal minor/trait | β/β+, β/β0 | Silent |
| β thal intermedia | β+ /β+, β+/β0 | Moderate |
| β thal major | β0/ β0 | Severe |

# SPINAL MUSCULAR ATROPHY (SMA)

- Incidence: 1 / 6.000  to 1/10.000 babies
- An autosomal recessive, second to CF.
- SMN1 (SMNt) mutation carrier incidence in west EU from 2% to 3% of population.

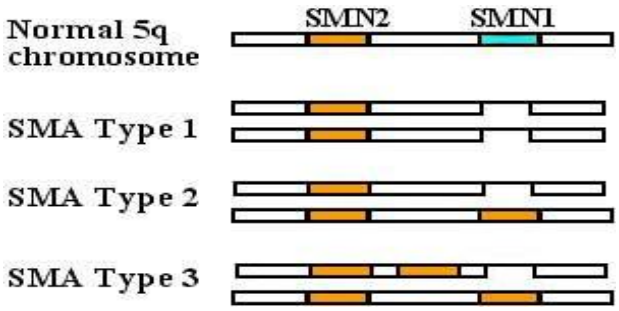
# CLINICAL SMA



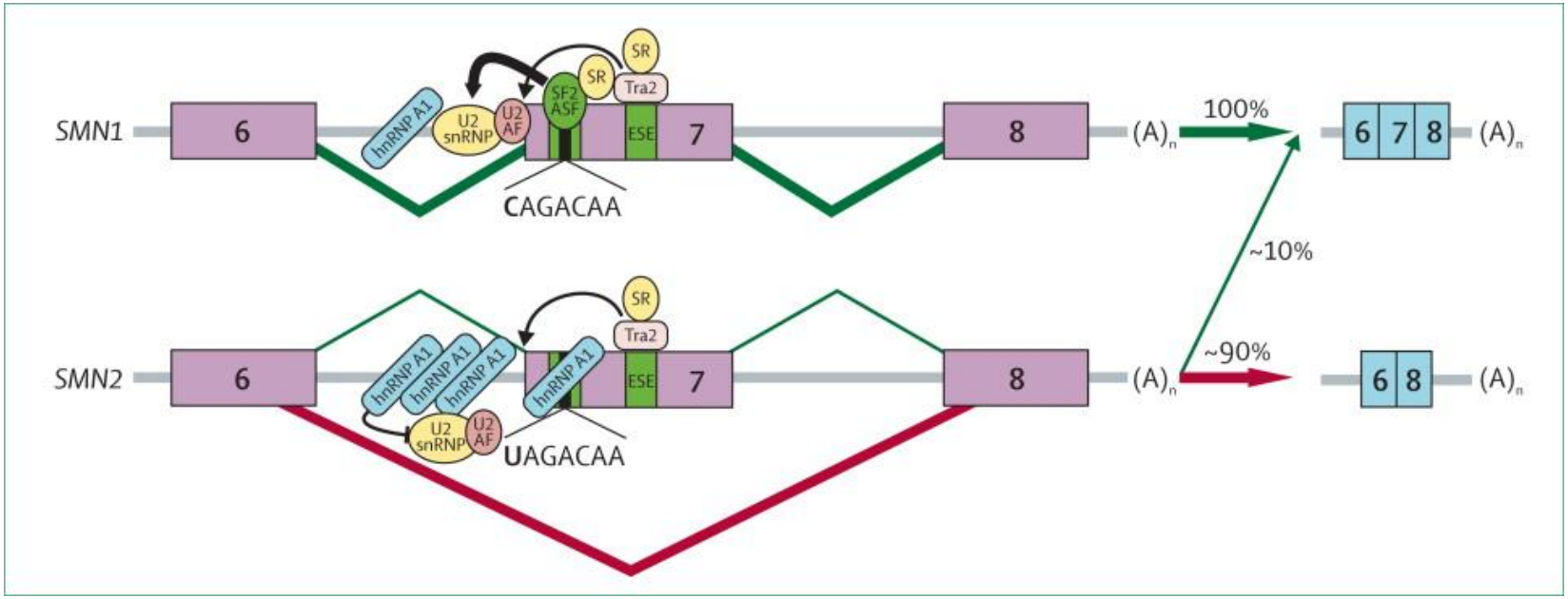
Clinical feature in type I
(2 gene copy deletion)

Clinical feature in type II
(1 gene copy deletion)

Clinical feature in type III (1 gene copy deletion+ repeated sequence).

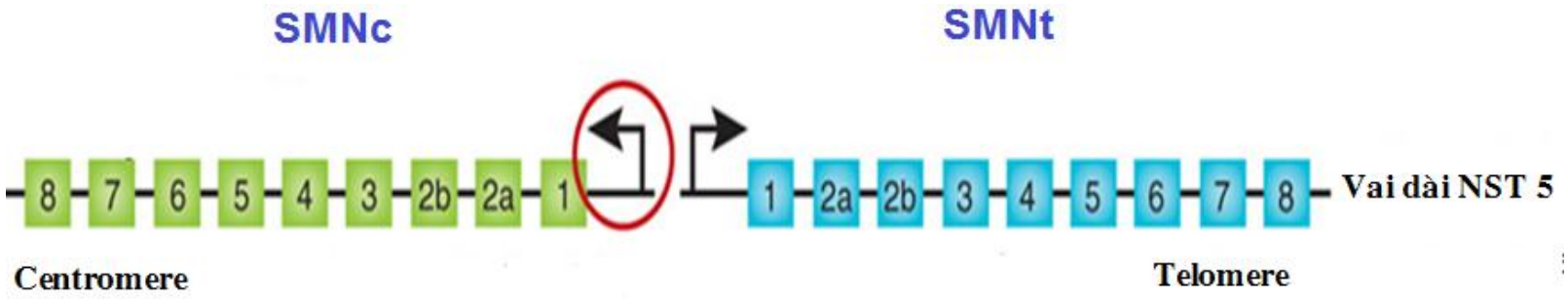


# SMA GENETICS

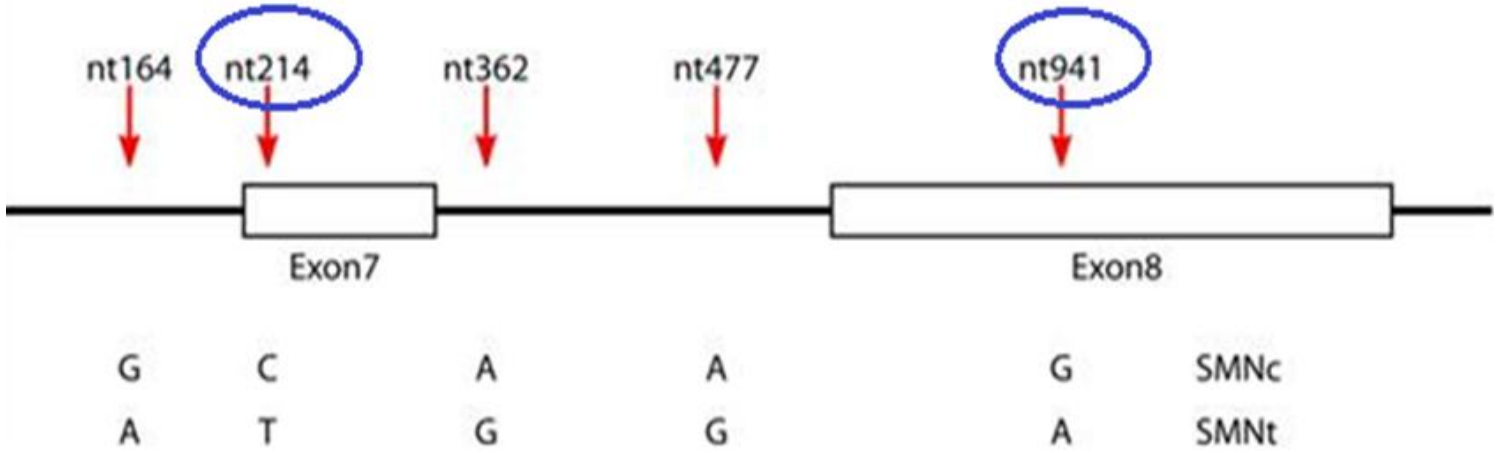


# SMA GENETICS

**In 1995, Judith Melki discribed SMN gene:** SMN gene have 9 exons coding for SMA protein with 294 amino acids. There are 2 similar gene copies : SMNt (SMN1) and SMNc (SMN2).




***SMN gene structrure.***




**The difference between SMNt and SMNc**

# PGD SITUATION FOR THALASSEMIA

- **Zexu Jiao et al**. (China), 2003: Nested PCR (single cell) + hybridization. Of 28 embryos, 24 embryos had been diagnosed, gene amplification successful rate was 86,8%, 3 embryos had been transferred, 1 case with pregnancy.
- **Wen Wang et al**. Singapore) 2009: first successful in PGD for SMA in Singapore. Nested PCR (single cell)+ minisequencing.
- **Yan-Wen Xu, 2009 (**China**):** Nested PCR + gap PCR for SEA. Results: of 472 embryos, amplification successful rate was 82,6. ADO rate was 16,4%. 25 cases with clinical pregnancy (successessful rate was 24,0%).

# PGD SITUATION FOR SMA

- **Dreesen et al**. (The Nertherland), 1998: nested PCR (single cell)+ RE. *DraI* to differenciate exon 7/SMNt from SMNc. Of 25 embryos, sucescessful rate up to 100% .
- **Daniels et al**. (US, UK and Canada cor-operation), 2001; **Ce'line Moutou et al.,** (France), 2003 : nested PCR (single cell ) + RE. *HinfI.* Of 34 embryos, successessful rate 91%.
- **Fiorentino F. et al.**, 2003: nested PCR (single cell)+ minisequencing. Kết quả 14 phôi, successessful rate was 92,90%.

## SUBJECTS AND METHODS

### 1. FOR DEVELOPMENT OF PGD PROCEDURES

- 43 blood samples from 16 families with thalassemia, 30 surplus embryos (normal) using TripAssay and minisequencing methods.
- 17 SMA families and surplus 30 embryos using PCR-RFLP and Minisequencing methods.

# SUBJECTS AND METHODS

## 2. APPLICATION PGD ON COUPLES

- **Subjects:**

- 62 couple having thalassemia babies (30 from NHOP and 32 from VMMU),13 subjected to PGD.

-17 couple having SMA babies (from NHOP ), 3 subjected to PGD.

- Samples: 5ml peripheral blood /EDTA, embryo biosy samples.

***Methods:**

- Whole genome amplification (WGA): Omniplex (Sigma) kits.

- Thal screening: TripAssay kit, Vienna Lab, Austria, Minisequencing (SnaPshot, AB, USA).

- SMA screening: RFLP-PCR ( DraI and DeI, Thermo), Minisequencing.

- Embryo vitrification (Kuwayama method, Japan, 2005).

# RESULTS AND DISCUSSION

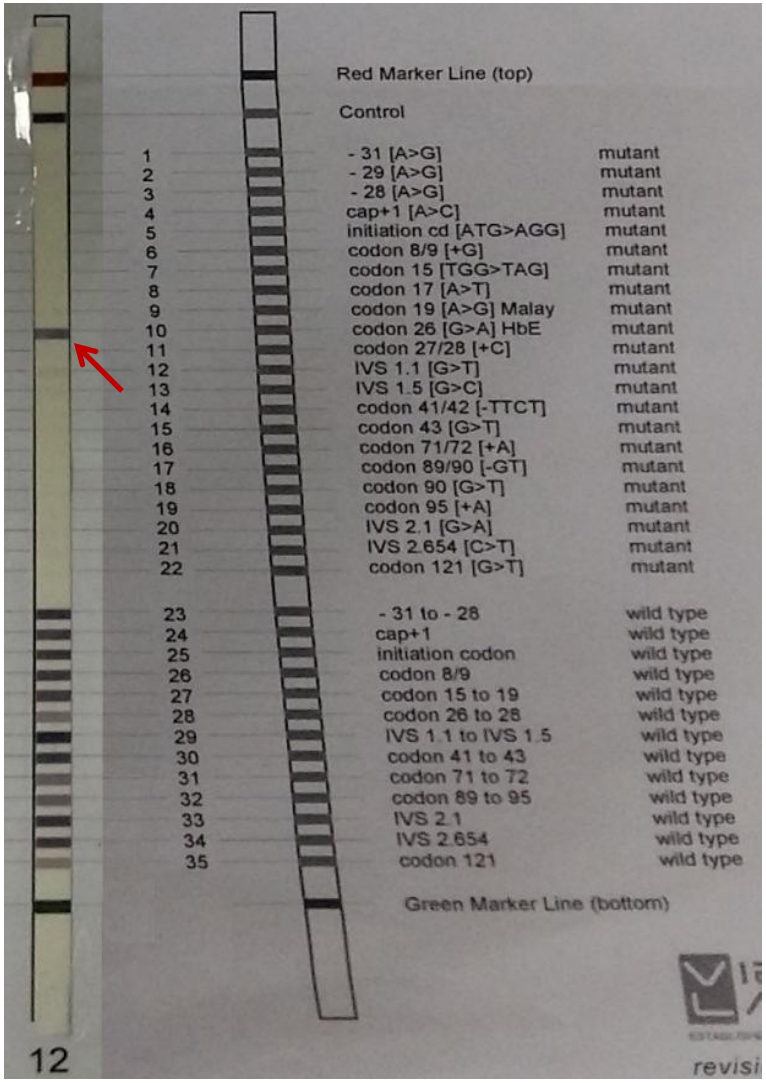
## 1. Development of PGD procedures

### *1.1. PDG for Thalassemia on surplus embryos*

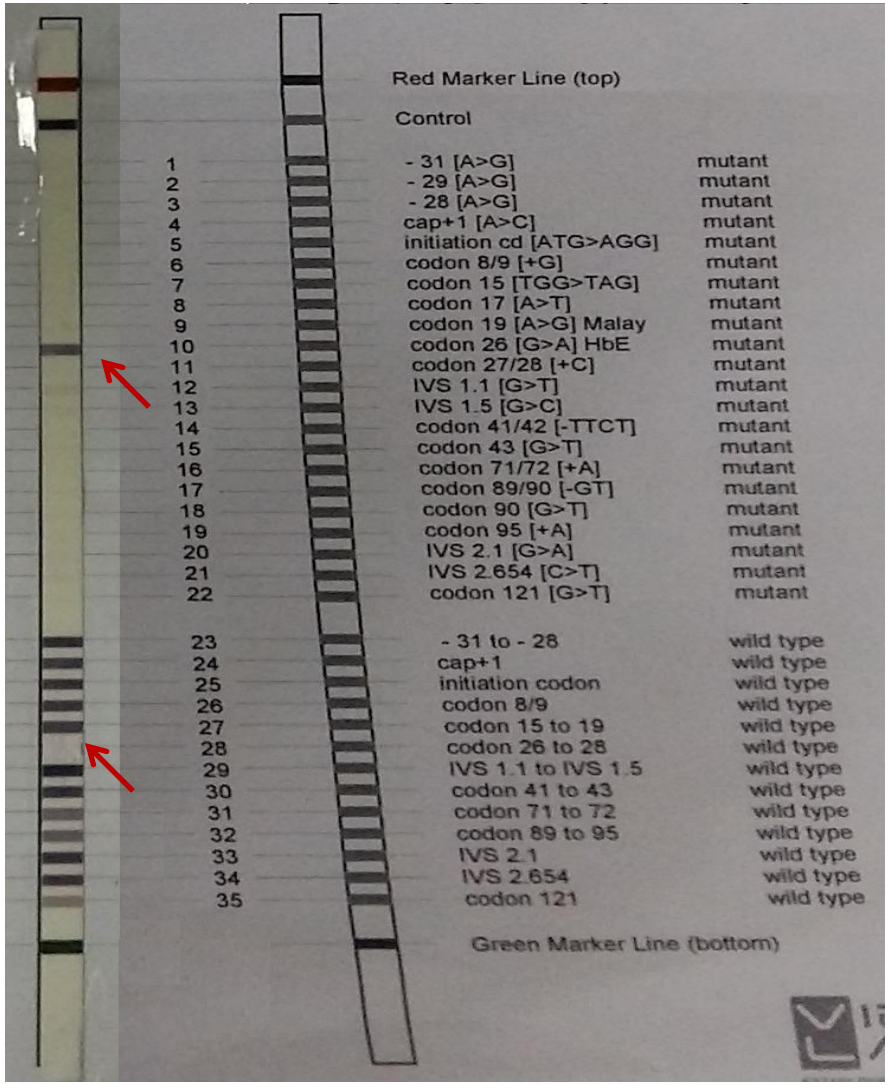
- Screening using TripAssay Kit
- Beta thalassemia screening using Minisequencing.
- Alpha Thalassemia screening using Gap-PCR.

# Screening on thalassemia families

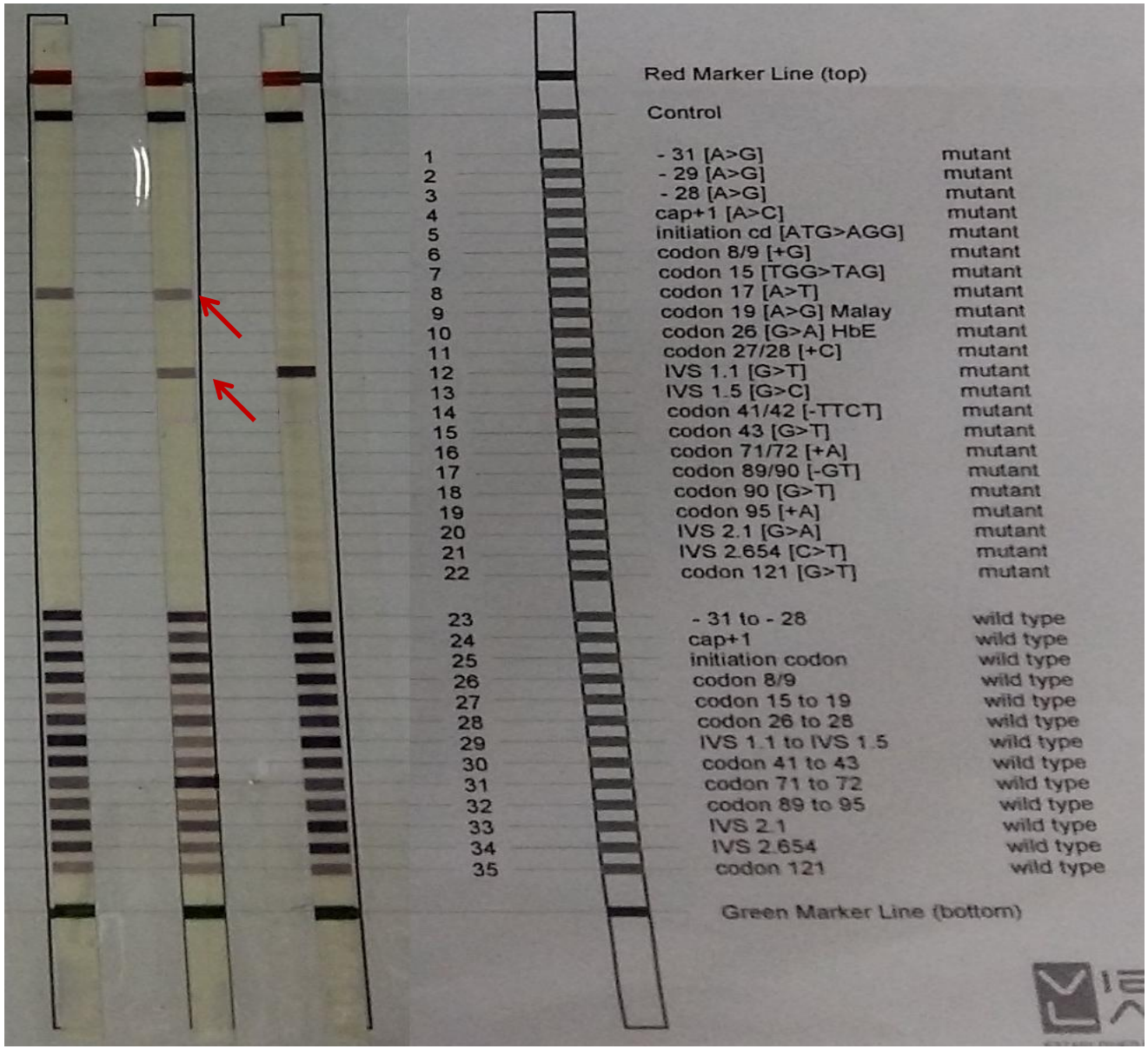
| Number | Code | Type mutation on single cell | Number | Code | Type mutation on single cell |
|---|---|---|---|---|---|
| 1 | THB07 | Cd26 | 23 | THM35 | Cd26 |
| 2 | THM07 | Cd26 | 24 | THC35 | Cd26/ Cd71/72 |
| 3 | THC07 | Cd26/Cd26 | 25 | THB48 | Cd41/42 |
| 4 | THB09 | IVS1-1 | 26 | THM48 | IVS1-1 |
| 5 | THM09 | Cd17 | 27 | THC48 | Cd41/42/IVS1-1 |
| 6 | THC09 | Cd17/ IVS1-1 | 28 | THB49 | Cd17 |
| 7 | THB16 | Cd17 | 29 | THM49 | Cd17 |
| 8 | THM16 | Cd26 | 30 | THC49 | Cd17/Cd17 |
| 9 | THC16 | Cd17/Cd26 | 31 | THB38 | 3.7 |
| 10 | THB20 | IVS1-1 | 32 | THM38 | SEA |
| 11 | THM20 | Cd17 | 33 | THC38 | 3.7/SEA |
| 12 | THC20 | IVS1-1/ Cd17 | 34 | THB56 | SEA |
| 13 | THB24 | Cd17 | 35 | THM56 | SEA |
| 14 | THM24 | Cd17 | 36 | THB59 | SEA |
| 15 | THC24 | Cd17/Cd17 | 37 | THM59 | SEA |
| 16 | THC26 | Cd17 | 38 | THB60 | SEA |
| 17 | THB26 | IVS2-654 | 39 | THM60 | SEA |
| 18 | THM26 | Cd17/ IVS2-654 | 40 | THB61 | SEA |
| 19 | THB29 | Cd41/42 | 41 | THM61 | SEA |
| 20 | THM29 | Cd26 | 42 | THB62 | SEA |
| 21 | THC29 | Cd26/ Cd41/42 | 43 | THM62 | SEA |
| 22 | THB35 | Cd71/72 | | | |



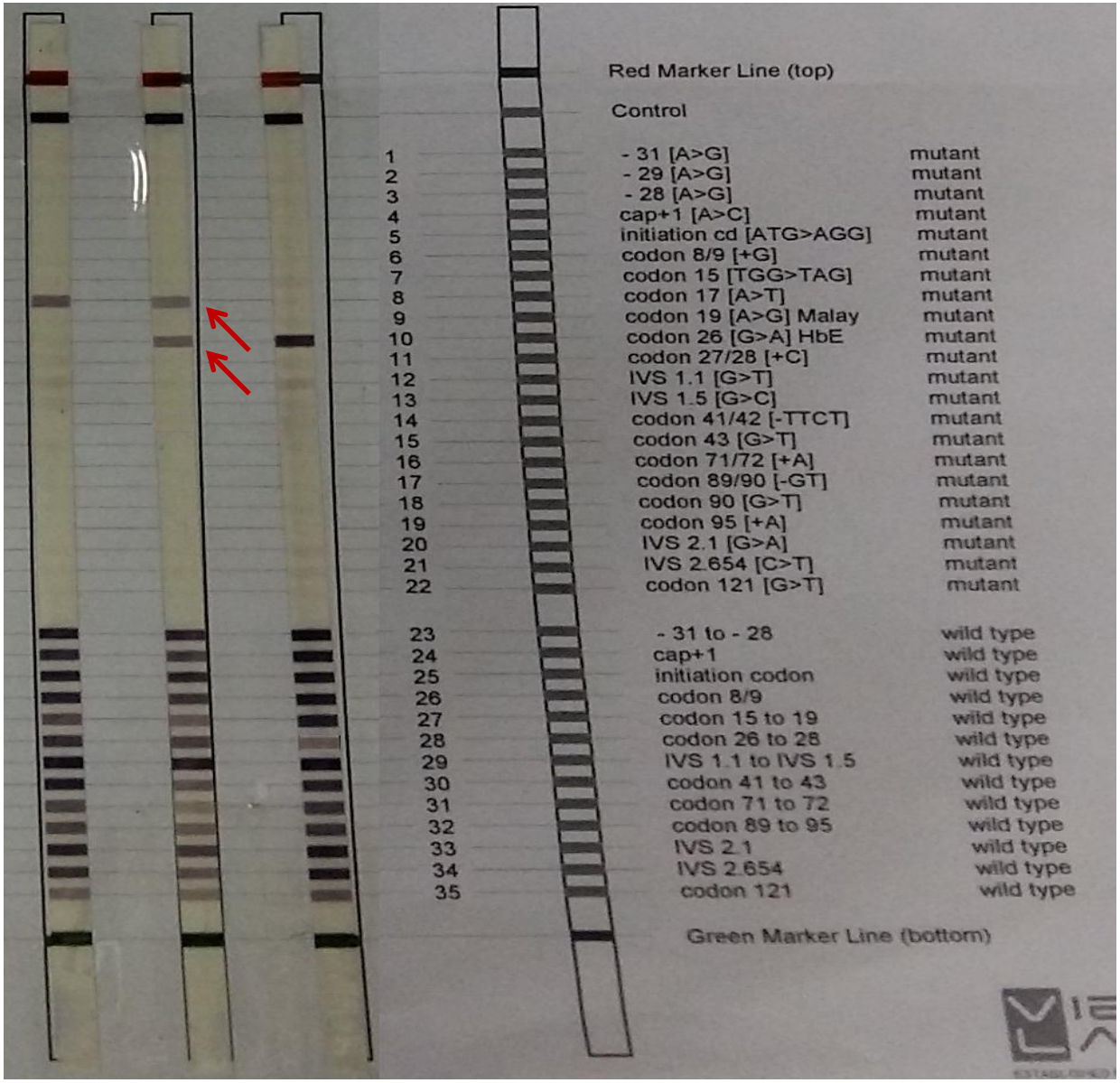

**Cd26 herterozygous**



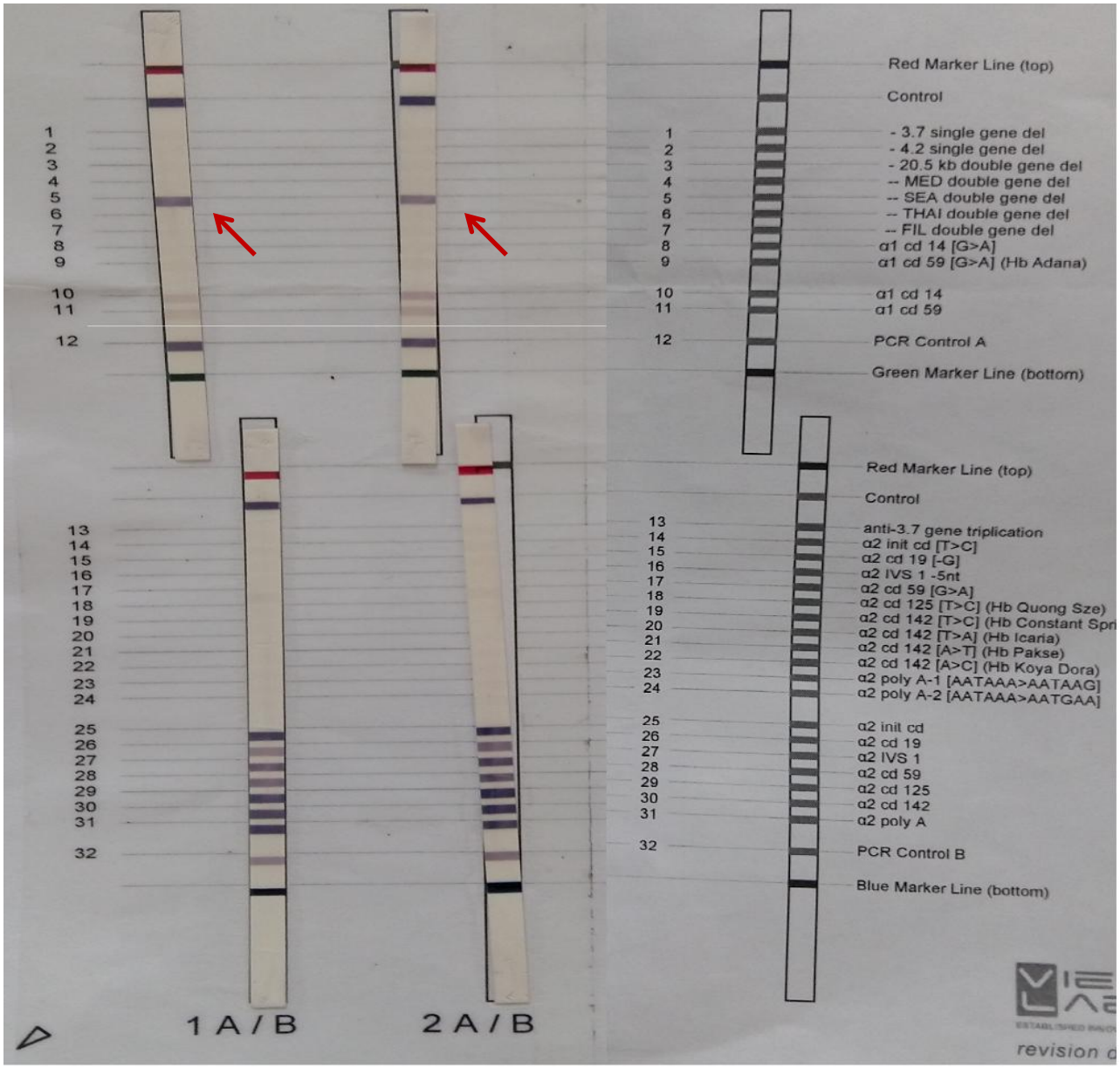

**Cd26 homozygous**




**Cd17 and IVS1.1**
**From left to right side: THM20, THC20, THB20.**

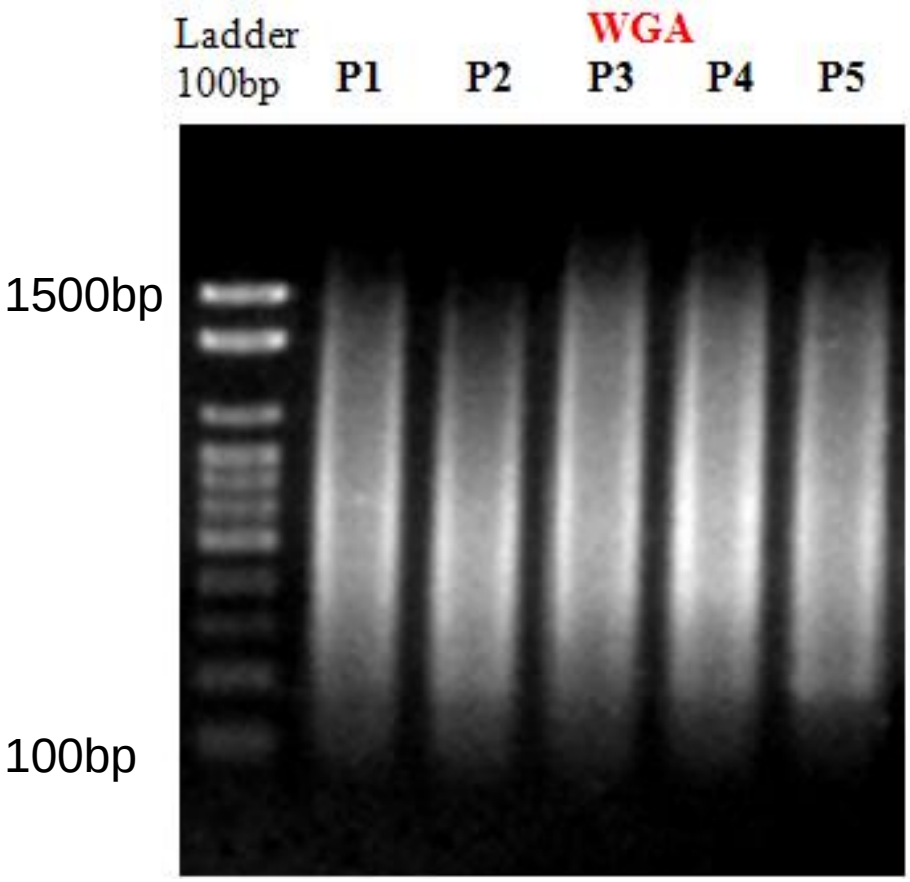



**Cd17 and Cd26 From left to right side :THB16, THC16, THM16**

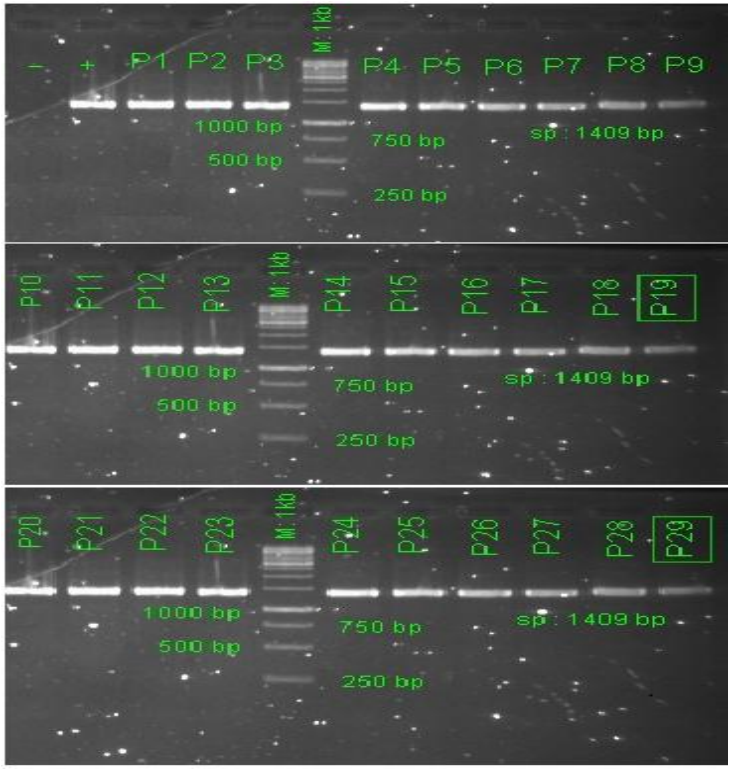



**Alpha thalassemia from left to right: THB60, THM60 (SEA)**

# Beta Thalassemia screening using Minisequencing



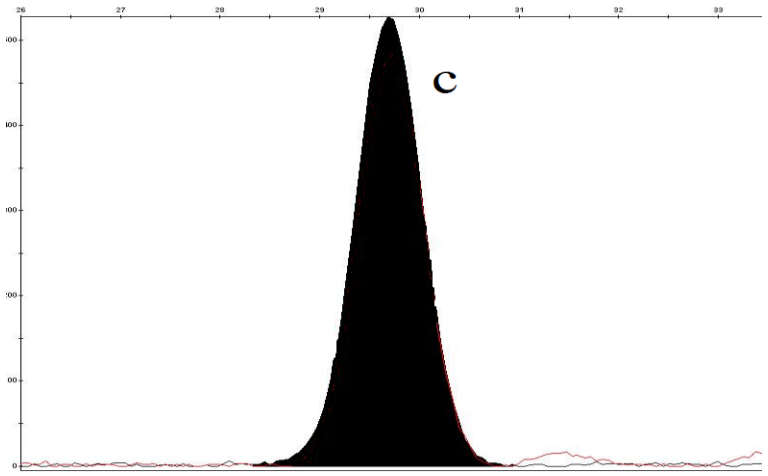

Electrophoresis image on 2% gel agarose of WGA products (WGA4, GenomePlex, Sigma) from single cell: P1: E1; P2: E 2; P3: E 3; P4: E 4; P5: E 5



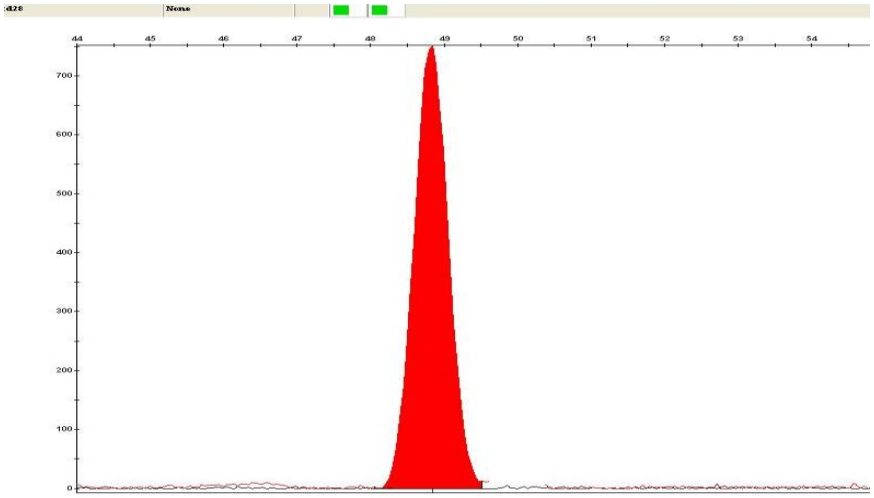

Electrophoresis image on 2% gel agarose of β-globin amplification product

## 3.1.3. Development of PDG procedure for thalassemia

*3.1.3.2. Development of PDG procedure for thalassemia using Minisequencing*



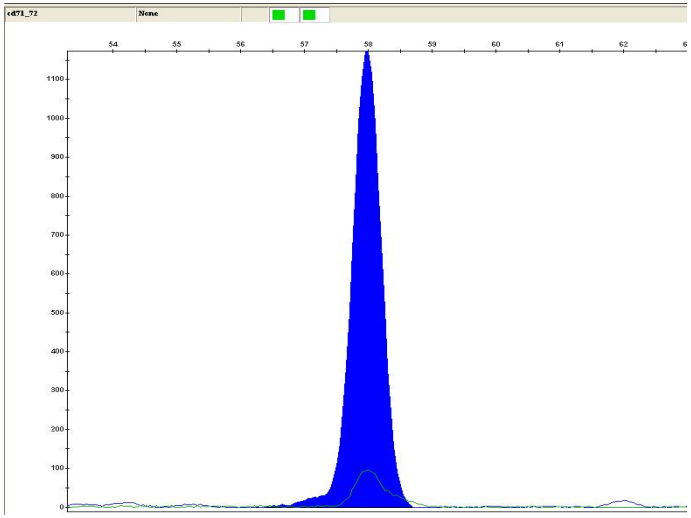

Minisequencing on IVS 2-645 sample P1-P30. only one black peak denoted to nucleotide **C** (norral)



Minisequencing on Cd28 sample P1-P30. only one red peak denoted to Nucleotide **T** in red (normal)

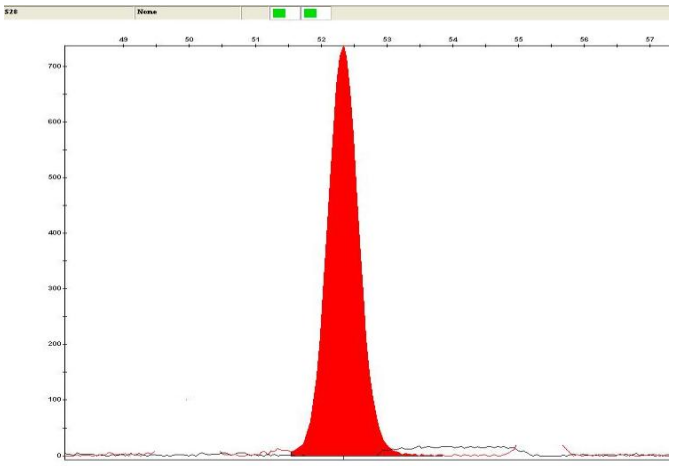
## 3.1.3. Development of PDG procedure for thalassemia

*3.1.3.2. Development of PDG procedure for thalassemia using Minisequencing*



Minisequencing on Cd71/72 P1-P30 sample P1-P30. only one blue peak

denoted to nucleotide **G** (normal)



Minisequencing on Cd71/72 P1-P30 sample SNP28 P1-P30. only one red peak denoted to nucleotide **T**

(normal)

# *Beta* thalassemia mutation dectetion on surplus using *Minisequencing*

| Number | Code | Type mutation on single cell | Number | Code | Type mutation on single cell |
|---|---|---|---|---|---|
| **1** | P1 | Normal | **16** | P16 | Normal |
| **2** | P2 | Normal | **17** | P17 | Normal |
| **3** | P3 | Normal | **18** | P18 | Normal |
| **4** | P4 | Normal | **19** | P19 | Normal |
| **5** | P5 | Normal | **20** | P20 | Normal |
| **6** | P6 | Normal | **21** | P21 | Normal |
| **7** | P7 | Normal | **22** | P22 | Normal |
| **8** | P8 | Normal | **23** | P23 | Normal |
| **9** | P9 | Normal | **24** | P24 | Normal |
| **10** | P10 | Normal | **25** | P25 | Normal |
| **11** | P11 | Normal | **26** | P26 | Normal |
| **12** | P12 | Normal | **27** | P27 | Normal |
| **13** | P13 | Normal | **28** | P28 | Normal |
| **14** | P14 | Normal | **29** | P29 | Normal |
| **15** | P15 | Normal | **30** | P30 | Normal |

# *1.2.* Development of PDG procedure for thalassemia

**PDG work up for thalassemia**

**Step 1: ADN isolation from whole peripheral blood.**

**Step 2: exon 7- SMN amplification.**

**Step 3: *PCR –RFLP* and Minisequencing for mutation detection.**

***SMA PGD on surplus embryos***

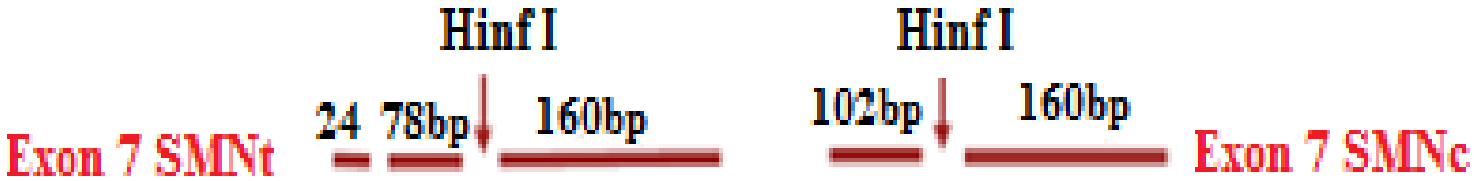
**Step 1: WGA**

**Step 2: Nhân exon 7- SMN**

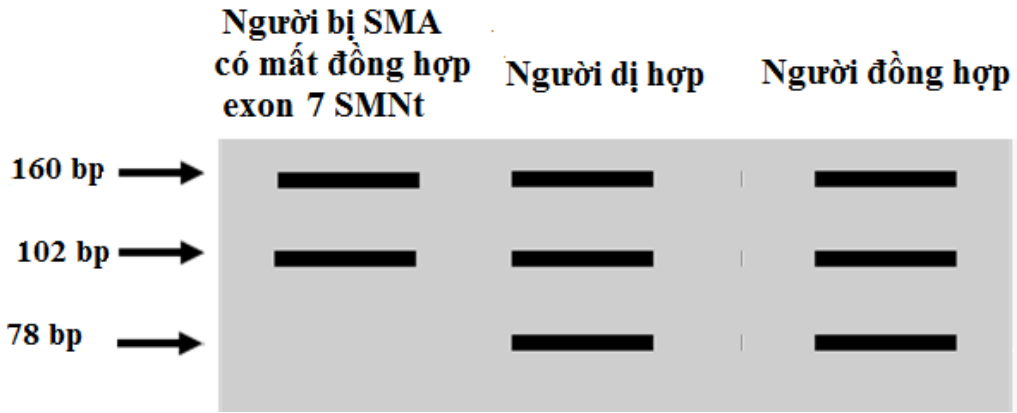
**Step 3: PCR-RFLP and Minisequencing for mutation detection**

***RFLP-PCR on embryos***

- ***RE treatment***
- To differenciate exon 7- SMNt from exon 7- SMNc, using ***Hinf I,*** Thermo scientific.
- Treatment PCR product with ***Hinf I*** for 2-3hs. ***Hinf I*** cut both exon 7 SMNt and SMNc

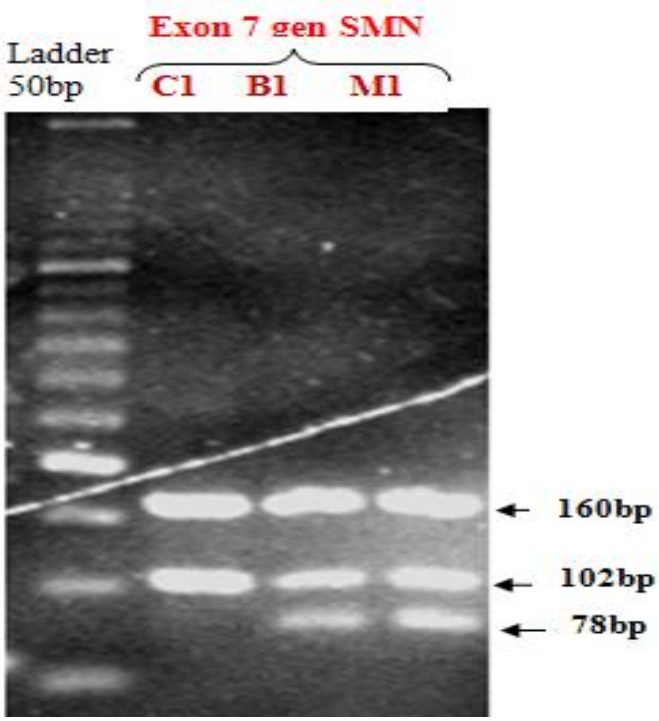



*Site of cut on exon 7 SMNt and SMNc of enzyme Hinf I.*

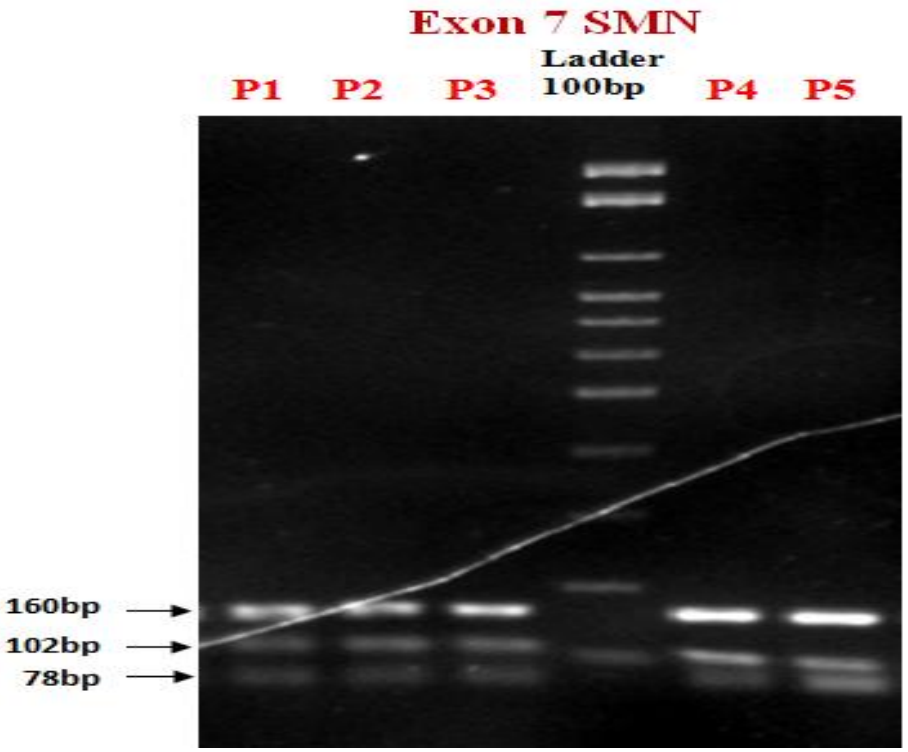


## 3.1.3. Developed PGD procedure for SMA

### *3.1.3.1. Screening SMN mutation using RFLP-PCR on embryos*




Electrophoresis image on on gel 1% agarose gel of SMN gene exon 7 amplification PCR product in Family C1.
B1: father1; M1: mother1; C1: patient1




Electrophoresis image on gel 3% agarose gel of WGA PCR product from normal one.
P1: E1; P2E2; P3: E3; P4: E4; P5: E5 5

# PGD procedure for SMA using Minisequencing

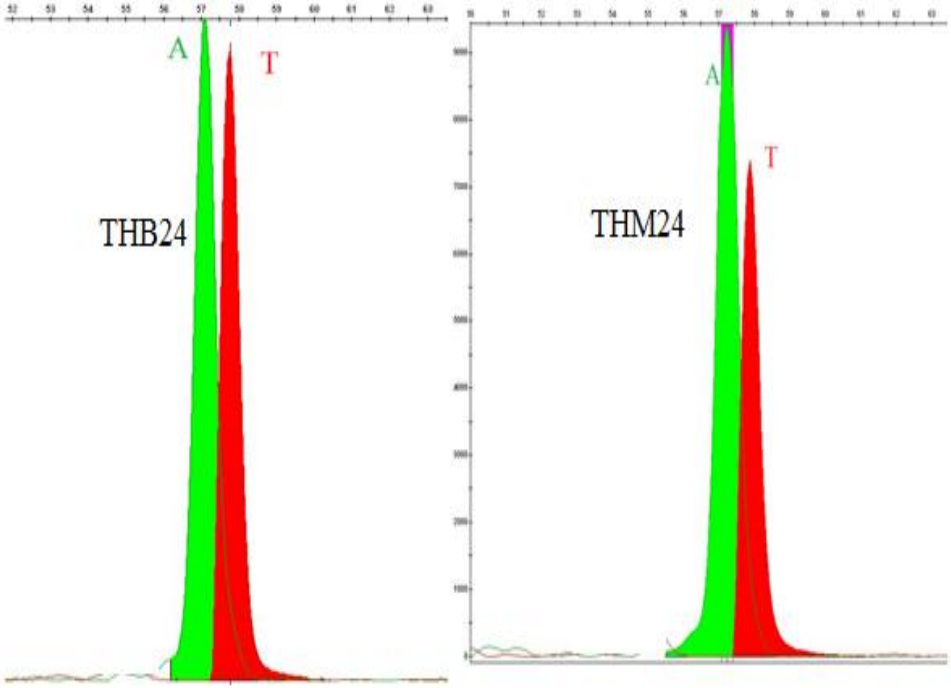
## Families enroled in SMN screening *(-) exon 7 homozygous deletion; (+) with exon 7.*

| No. | Code | exon 7 gene SMNt amp | | | No. | Code | exon 7 gene SMNt amp | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Father | Mother | Baby | | | Father | Mother | Baby |
| 1 | SMA1 | + | + | - | 10 | SMA11 | + | + | - |
| 2 | SMA2 | + | + | - | 11 | SMA12 | + | + | - |
| 3 | SMA3 | + | + | - | 12 | SMA13 | + | + | - |
| 4 | SMA4 | + | + | - | 13 | SMA14 | + | + | - |
| 5 | SMA5 | + | + | - | 14 | SMA15 | + | + | - |
| 6 | SMA6 | + | + | - | 15 | SMA16 | + | + | - |
| 7 | SMA7 | + | + | - | 16 | SMA17 | + | + | - |
| 8 | SMA8 | + | + | - | 17 | SMA18 | + | + | - |
| 9 | SMA10 | + | + | - | | | | | |

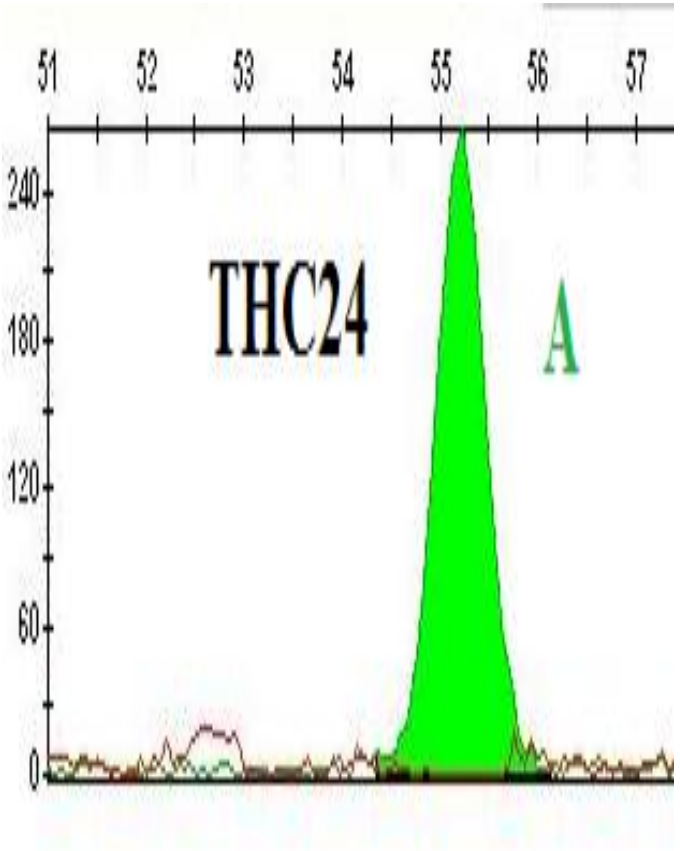
# 2. Application of PGD procedure for thalassemia on embryos

Rerults of Beta thalassemia mutation detection on embryos.

| Number | Code | Mutation type | | Code | Mutation type | | Code | Mutation type | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | THC24 | Cd17 | Cd17 | THB24 | Cd17 | + | THM24 | Cd17 | + |
| | P1_24 | + | + | P2_24 | Cd17 | Cd17 | P3_24 | Cd17 | Cd17 |
| | P4_24 | Cd17 | + | P5_24 | Cd17 | + | P6_24 | Cd17 | + |
| | P1_24_L2 | Cd17 | + | P2_24_L2 | Cd17 | Cd17 | | | |
| | | | | | | | | | |
| 2 | THC48 | Cd41/42 | IVS1.1 | THB48 | Cd41/42 | + | THM48 | IVS1.1 | + |
| | P1_48 | IVS1.1 | + | P2_48 | IVS1.1 | + | | | |
| | P1.L2_48 | IVS1.1 | + | P2.L2_48 | + | + | | | |
| | | | | | | | | | |
| 3 | THC51 | Cd41/42 | IVS1.1 | THB51 | Cd41/42 | + | THM51 | IVS1.1 | + |
| | P1_51 | + | + | | | | | | |
| | | | | | | | | | |
| 4 | THC57 | Cd17 | Cd26 | THB57 | Cd17 | + | THM57 | Cd26 | + |
| | P1_57 | Cd26 | + | P2_57 | Cd17 | + | | | |
| 5 | THC58 | Cd26 | Cd17 | THB58 | Cd26 | + | THM58 | Cd17 | + |
| | P1_58 | + | + | | | | | | |

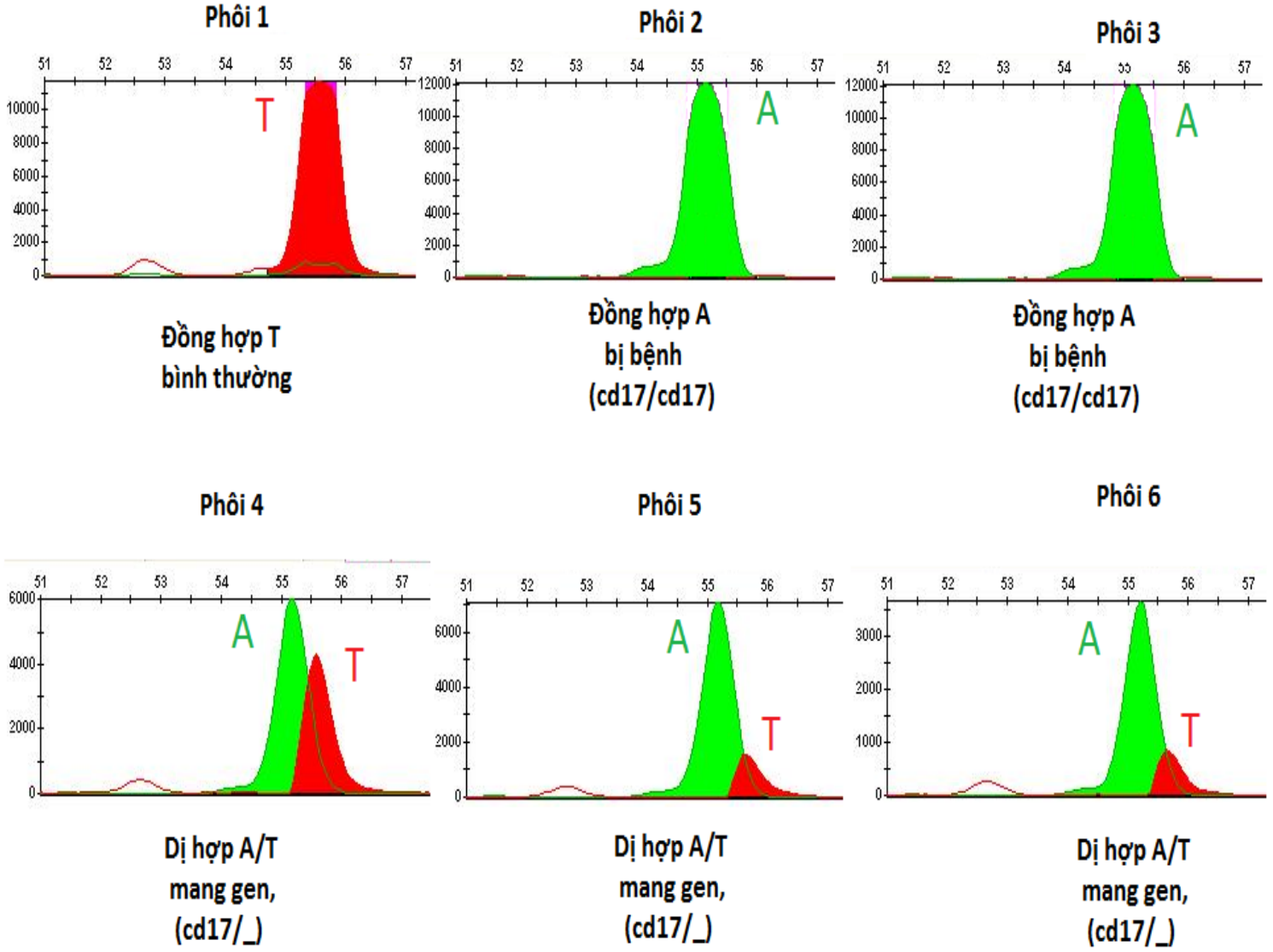



**Minisequencing in THB24, THM24.**

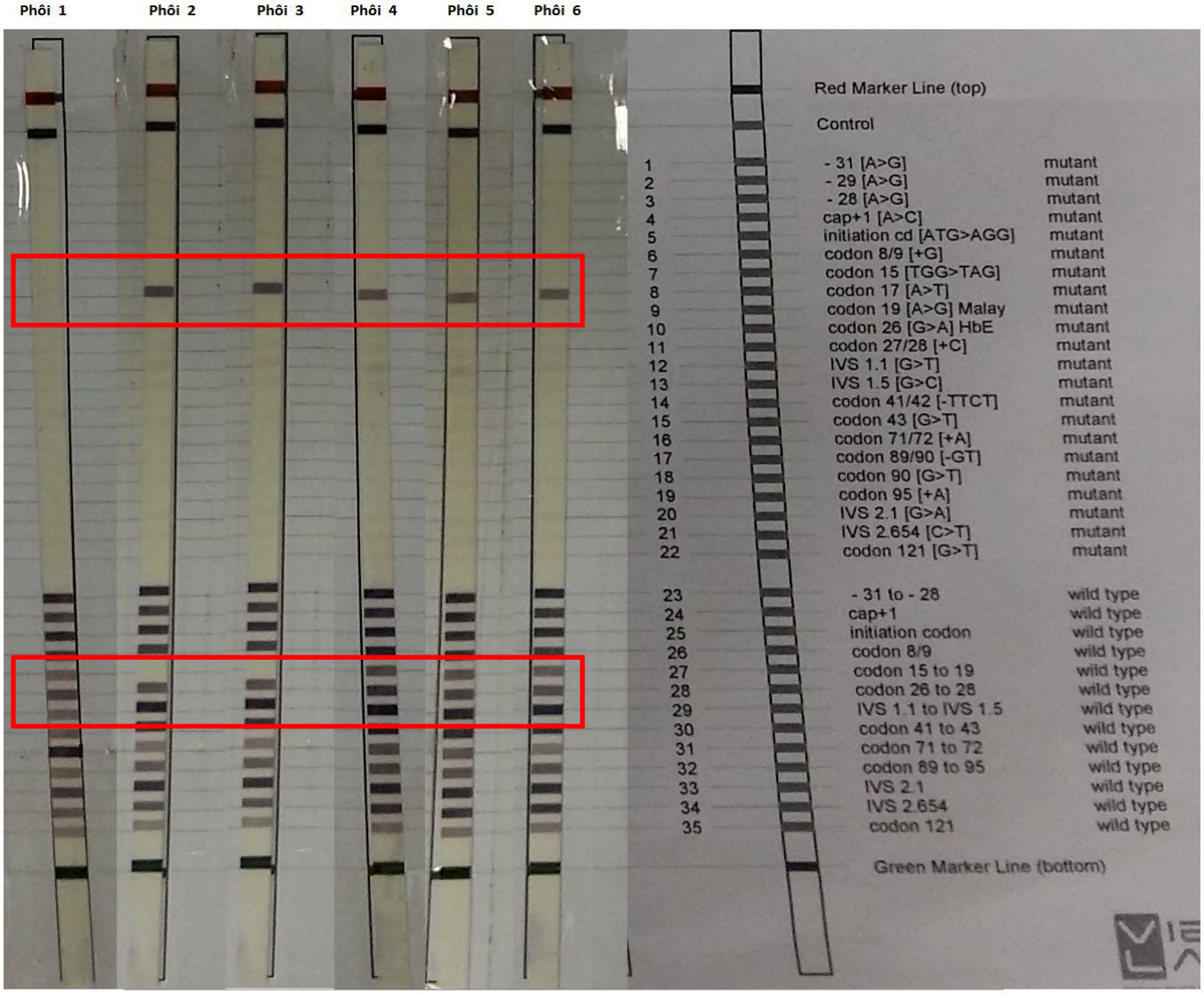



**Minisequencing in THC24.**

Cd17/Cd17



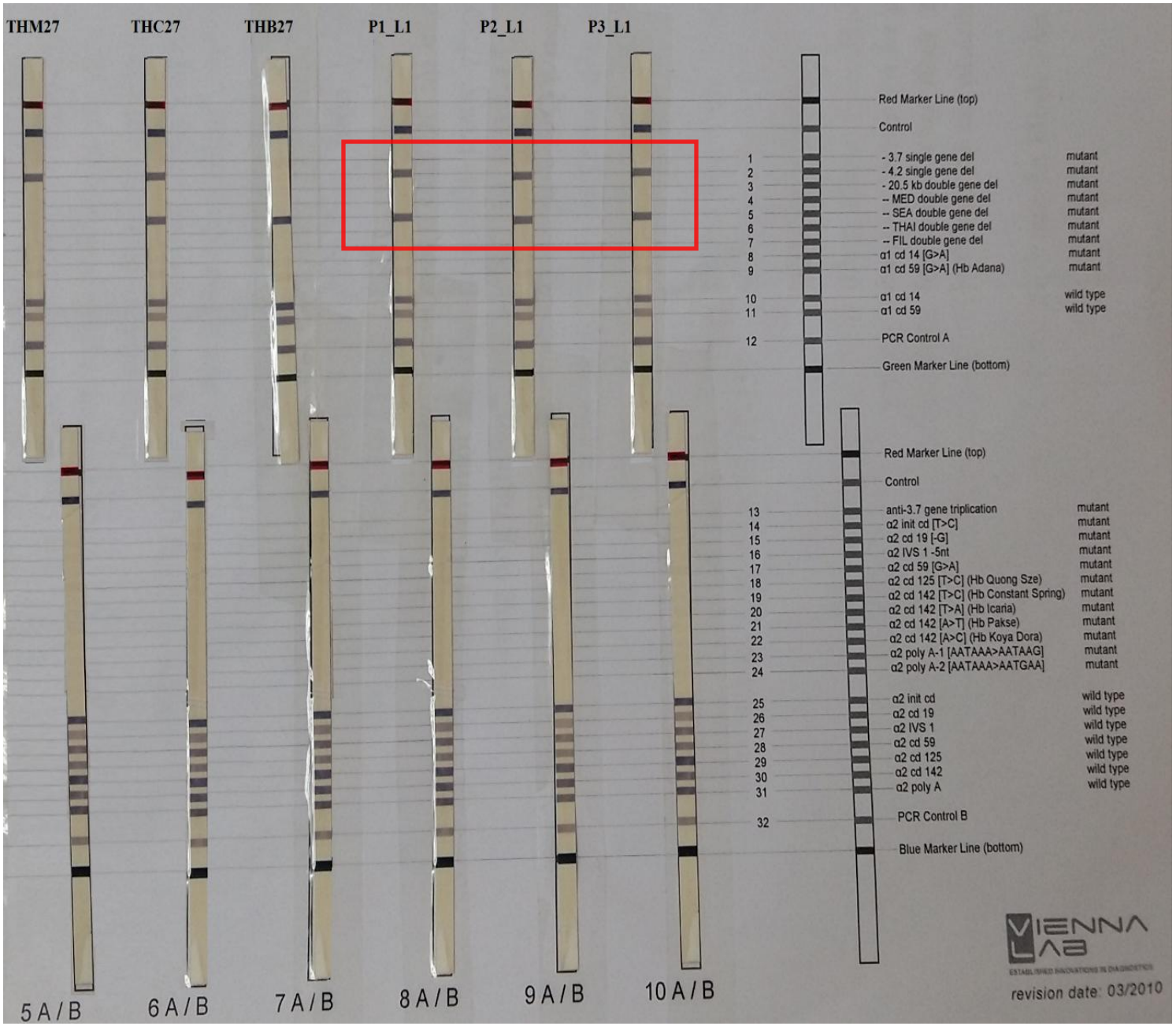

**Minisequencing on 6 embryos from family 24.**




**TripAssay on 6 embryos in Family 24 (Cd17)**
**E1 normal, E2,3 affected, E4,5,6 heterozygous**

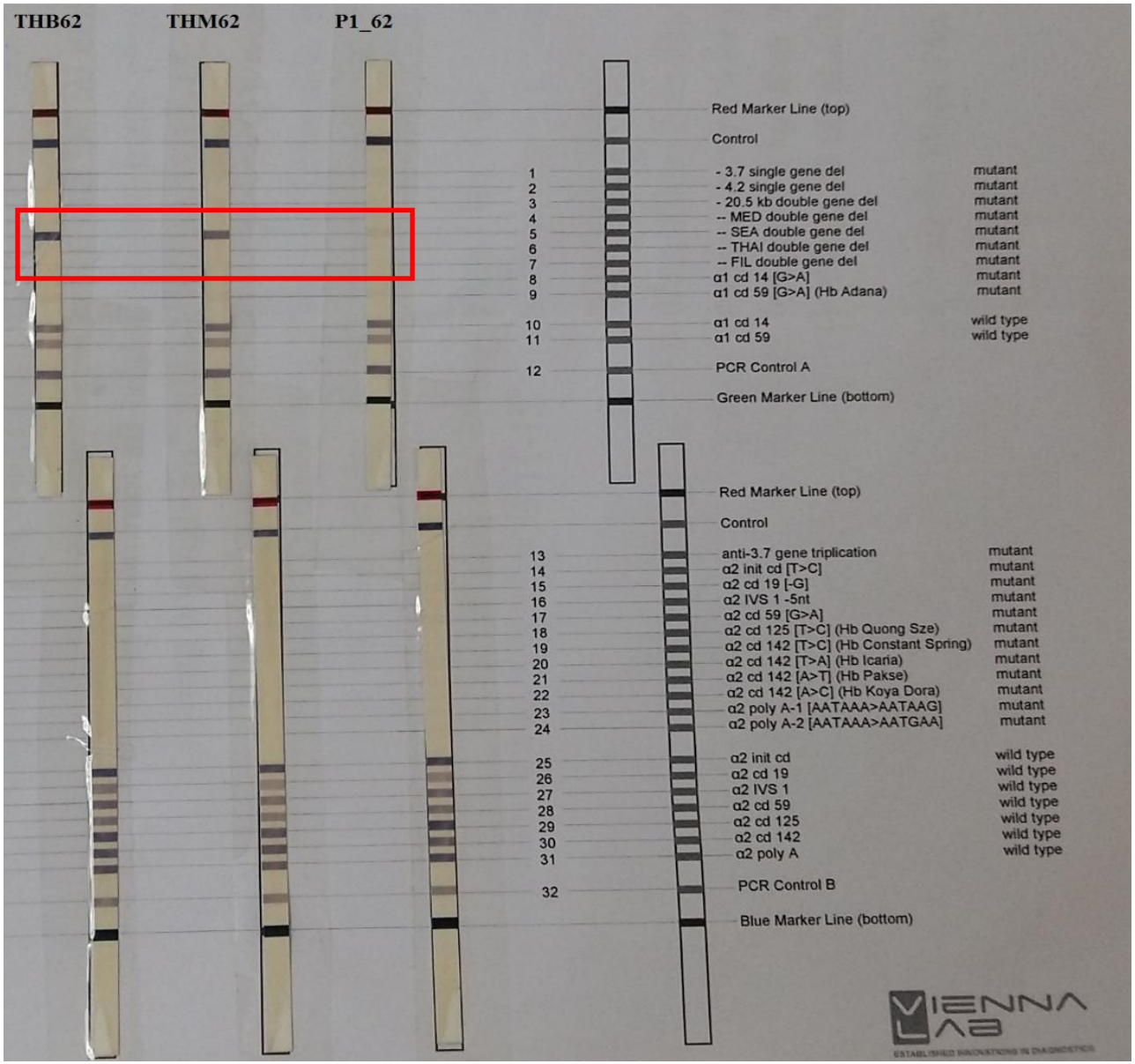
# Alpha thalassemia mutation Detection on embryos

| Number | Code | Mutation type | | Code | Mutation type | | Code | Mutation type | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | THC23 | SEA | HBC | THB23 | SEA | + | THM23 | + | HBC |
| | P1_23 | + | + | P2_23 | + | SEA | P3_23 | SEA | SEA |
| | P4_23 | SEA | + | P5_23 | + | + | | | |
| | | | | | | | | | |
| 2 | THC27 | SEA | 4.2 | THB27 | SEA | + | THM27 | 4.2 | + |
| | P1_27 | SEA | 4.2 | P2_27 | SEA | 4.2 | P3_27 | SEA | 4.2 |
| | P1_L2_24 | - | - | P2_L2_27 | - | - | | | |
| | | | | | | | | | |
| 3 | THC38 | 3.7 | SEA | THB38 | 3.7 | + | THM38 | SEA | + |
| | F 38 no emb biosied | | | | | | | | |
| 4 | THC | - | - | THB56 | SEA | + | THM56 | SEA | + |
| | P1_56 | + | + | P2_56 | SEA | SEA | P3_56 | SEA | + |
| | P4_56 | SEA | SEA | | | | | | |
| | | | | | | | | | |
| 5 | THC | - | - | THB59 | SEA | Cd26 | THM59 | SEA | + |
| | P1_59 | + | + | P2_59 | SEA | + | P3_59 | SEA | + |
| | | | | | | | | | |
| 6 | THC | - | - | THB60 | SEA | + | THM60 | SEA | + |
| | P1_60 | + | + | P2_60 | SEA | SEA | P3_60 | SEA | + |
| | | | | | | | | | |
| 7 | THC | - | - | THB62 | SEA | + | THM62 | SEA | + |
| | P1_62 | + | + | P2_62 | SEA | + | | | |




3 embryos affected

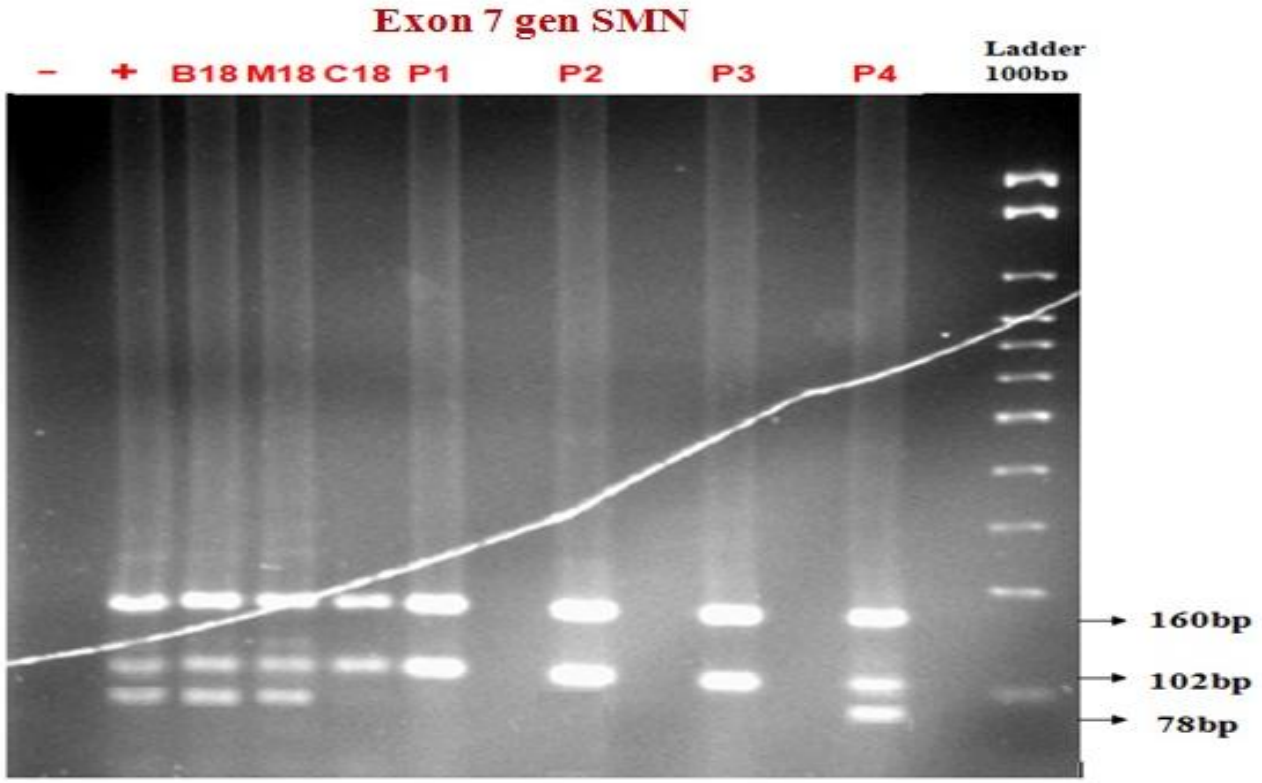
**TripAssay in family 27 (4.2 and SEA)**




**TripAssay in family 62 (SEA)**

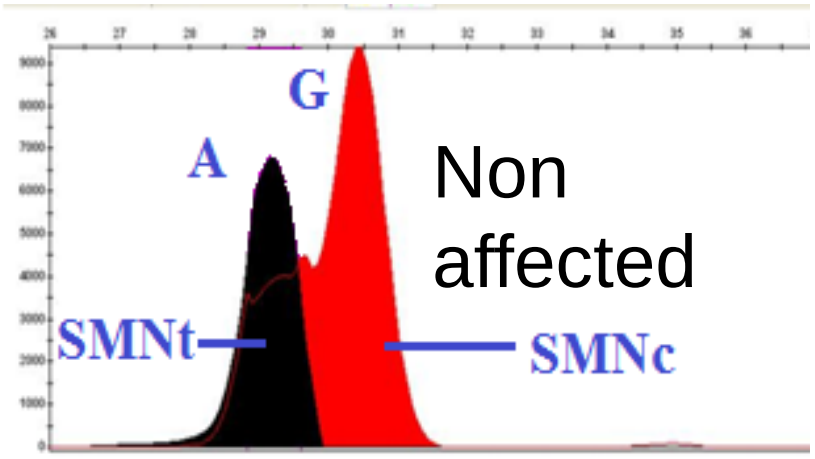
# *2.2. Application of PGD procedure for SMA*

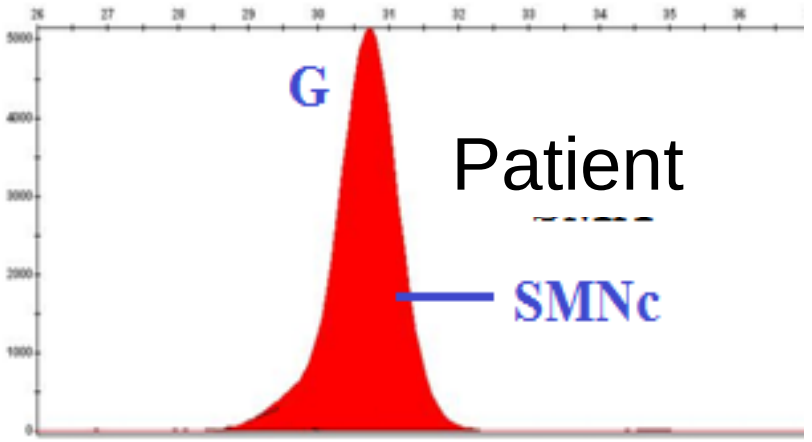
**For 3 families with SMA.**




Electrophoresis image on 3% agarose gel of exon 7 gen SMN PCR product from SMA18 Family.

Besides PCR-RFLP, Minisequencing was carried out for SMA mut detection based on the difference in nucleotid 214 on exon 7/ SMNt gene (T), but in exon 7/gen SMNc gene (C).

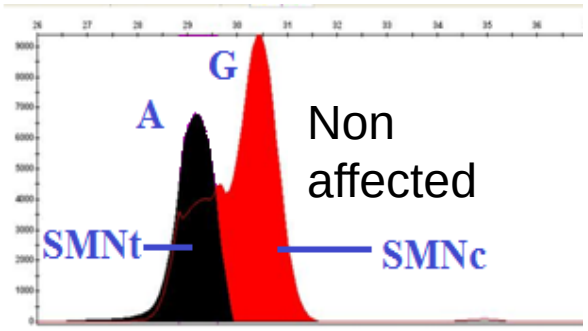


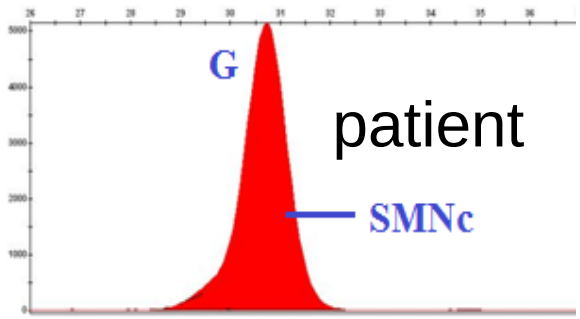



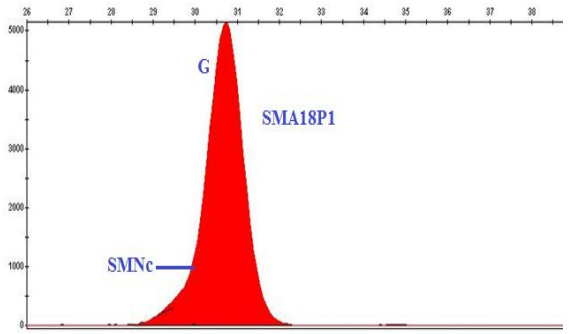

A: Who have both exon 7/gen SMNt gene and exon 7/gen SMNc gene - normal;

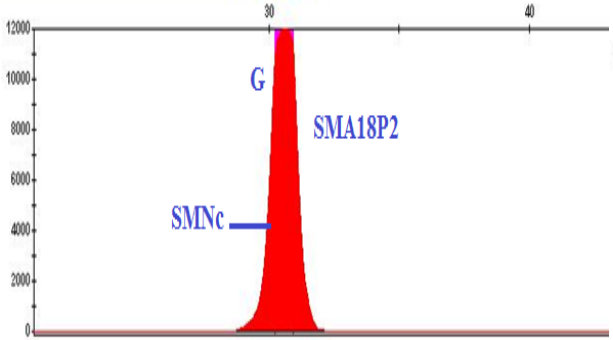
B: Who have only exon 7/SMNc gene - SMA.

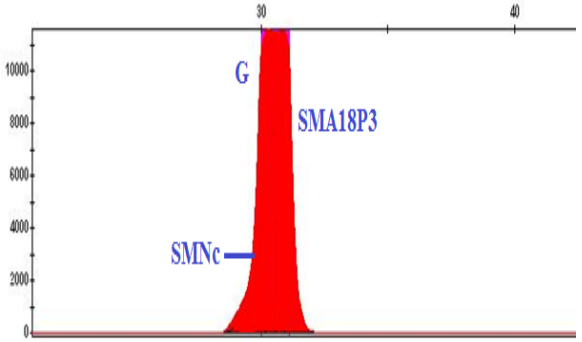
# Minisequencing results:

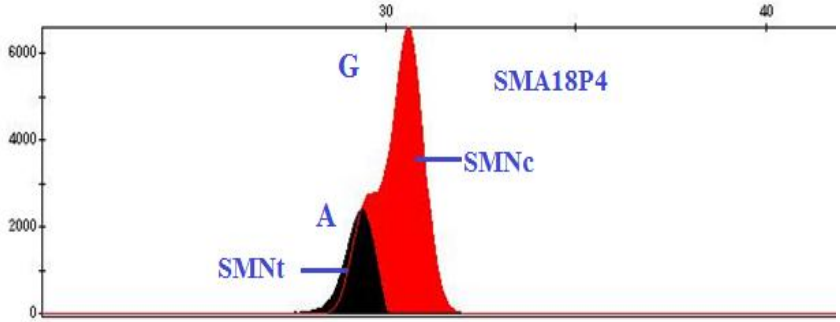



Minisequencing result totally in accordance with PCR-RFLP result.

# PGD rerults on three SMA families

| Code | No. of ovul | No. of EMB | Biopsied EMB | Normal EMB | EMB Trans | Results |
|---|---|---|---|---|---|---|
| SMA1 | 10 | 8 | 5 | 4 | 1 | Baby taking home |
| SMA2 | 5 | 3 | 0 | | | |
| SMA18 | 9 | 7 | 5 | 1 | 1 | Baby taking home |

- Dreesen et al. (The Netherland), 1998: 2 embryos x 2 times: 1 baby.
- Daniels et al. , US,UK, Canada , 2001, 3/5 successful, 6 babies.
- Ce'line Moutou et al. ., Pháp, 2003. 4 cases, no baby.
- FiorentinoF. et al. , 2003: 3 cases, no pregnancy.
- Girardet A. et al. . , 2008: 1 case, 1 baby.

# CONCLUSION

1. A Pre-Implantation Genetic Diagnosis has been developed on in vitro fertilization embryos.
   - PGD procedures for muscular dystrophy.
   - PGD procedures for Thalassemia.

# CONCLUSION

2. Applied PGD for some of the most common genetic diseases in Vietnam on IVF-ICSI embryos.

- Applying PGD for 17 families participating in the study, including 3 families who had eggs, 8 embryos, 2 embryos transferred giving 2 healthy babies.
- Applied PGD for the Thalassemia on 80 families, 25 of whom participated in IVF and 60 embryos. Transferring embryos in six cases gave healthy baby and many were pregnant, some preparing to transfer embryos.

# PROPOSALS

- Application PGD for thalassemia and SMA.
- Combination of PGD with PGS to improve IVF-ICSI successful rate.

# THANK YOU VERY MUCH