

# Comprehensive Newborn Screening for Severe Combined Immunodeficiency in Manitoba, Canada

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# Our Unique Populations

- **Aboriginal**
- **First Nations**
- **Inuit**
- **Métis**
- **Mennonite**
- **Hutterite**
- **Amish**



# Severe Combined Immunodeficiency

- Is the most profound form of the Primary Immunodeficiency Diseases (PID)(~ 200 congenital disorders)
- Characterized by profound impairment in T-cell development and function and lack of an adaptive immune system
- Fatal within the 1<sup>st</sup> year without BMT
- Early treatment is associated with better outcome
- “Classic SCID”
  - Clinical features – chronic diarrhea, pneumonia, failure to thrive, persistent thrush
  - And one of:
    - Absolute lymphocyte count  $< 3 \times 10^9/L$
    - Family history
  - Exclusion – HIV infection or cystic fibrosis

# SCID Immunophenotype TBNK Classification

- T<sup>-</sup> B<sup>+</sup> NK<sup>+</sup>  
Common gamma chain; JAK<sub>3</sub> defect
- T<sup>-</sup> B<sup>-</sup> NK<sup>+</sup>  
RAG<sub>1</sub>, RAG<sub>2</sub> – Omenn's syndrome
- T<sup>-</sup> B<sup>+</sup> NK<sup>-</sup>  
CD3δ defect  
Di George Syndrome (del22q11.2)
- T<sup>-</sup> B<sup>-</sup> NK<sup>-</sup>  
ADA
- T<sup>+</sup> B<sup>+</sup> NK<sup>+</sup>
  - Mennonites
  - First Nations



# Comparative Summary

	Classic	Mennonite	Northern Cree
Age at presentation	< 1 year	6-18 months	2-6 months
Clinical picture	Infection	Respiratory	Overwhelming sepsis
Lymphocyte count	< 2	5-14	4-9
Immunoglobulins	Decreased or absent	Normal or increased	Absent
T+ B+ NK+	Absent T cells	Present	Present
CD8+ cells	Absent	Decreased	Normal
Mitogen response	Absent	Absent	Low to normal
Mutation	Multiple	ZAP70	IKBKB

# TRECS

- Extra-chromosomal T-cell receptor excision circles
- TRECs are formed during maturation of T lymphocytes in the thymus (TCR gene rearrangement; normal cutting and splicing events with end-joining of genomic DNA segments)
- Newborn babies normally have high numbers of naïve T-cells and TRECs
- Typically, SCID babies have few or no naïve T-cells and TRECs
- Detects not only SCID, but other forms of T cell lymphopenia
  - Absent in X-SCID
  - Decreased / variable in other SCID variants
- Decreased in premature infants
- Accepted test for newborn screening



# Retrospective Pilot Study

- Overall incidence of SCID/PID in Manitoba is ~1:15,000, 3X more frequent than the national average and is overrepresented in 2 groups : Mennonites (*ZAP70*) and First Nations (*IKBKB*) babies that have T cells
- 
- Challenge: TREC method would likely not flag these babies unless their TRECs were significantly decreased
- Retrospective pilot study\* to establish normative TREC data and determine whether archived newborn specimens from 18 SCID/PID babies born between 1992 and 2010 could be identified from 982 normal, age matched control specimens by the TREC assay
- \* [Jilkina et. al. Molecular Genetics and Metabolism Reports 1 \(2014\) 324-333](#)

# Retrospective Pilot Study

- 18 SCID/PID babies all requiring BMT
- (5) T-cell NEG. SCID - 3 ADA, 1 CD3, 1 Clinical SCID
- (8) T-cell POS. SCID - 5 ZAP70, 3 IKBKB
- (1) T-cell NEG. PID - CHH
- (4) T-cell POS. PID - 2 CVID, 1 WAS, 1 XLP
  
- Screen Positive (9)
- TREC/ul (0) - 3 ADA, 1 CHH, 1 CD3
- TREC/ul (< 252) - 1 Clinical SCID (no molecular diagnosis)
- - 2 ZAP70 zeta chain-associated protein kinase
- - 1 CVID
- False Positive - 5 preterm babies, 1 twin, 4 unknown
  
- Screen Negative
- TREC/ul (> 252) - 3 ZAP70, 1 WAS, 3 IKBKB, 1 CVID, 1 XLP

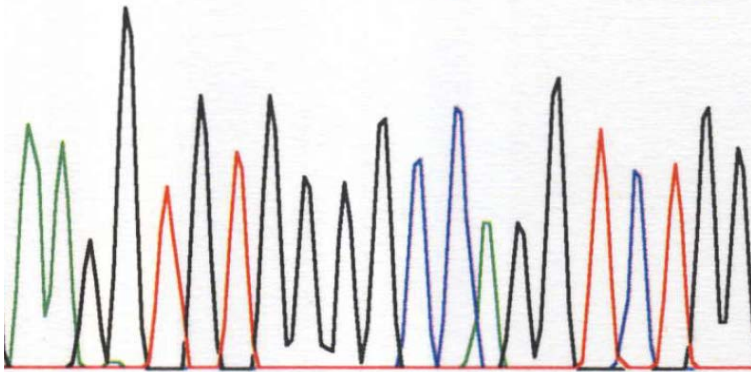


# IKBKB Sequencing

## Exon 13

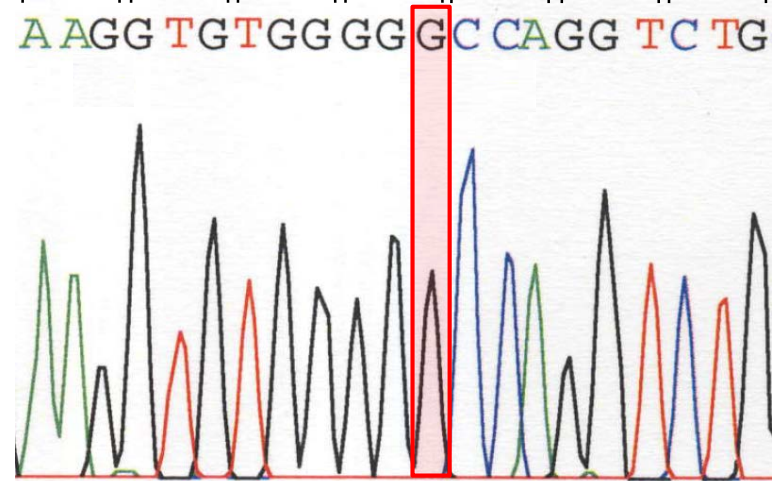
### Control

428 429 430 431 432 433 434  
Lys Val Trp Gly Gln Val Trp  
AAGG TGTGGGGCCAGG TCTGG



### F1P1

428 429 430 431 432 433 434  
Lys Val Trp Gly **Pro Gly Leu**  
AAGG TGTGGGGG **GC** CAGG TCTG



c.1292\_1293insG

p.Gln432ProfsX62

# ZAP 70 MUTATION



Normal 3' intronic sequence

cggcttgagcag

normal splice acceptor sequence

Mennonite mutation

cagcttgagcag

new splice acceptor sequence

The newly created splice acceptor sequence results in the insertion of 9 nts to the mRNA and three additional amino acids to the protein product (inactivating the kinase).

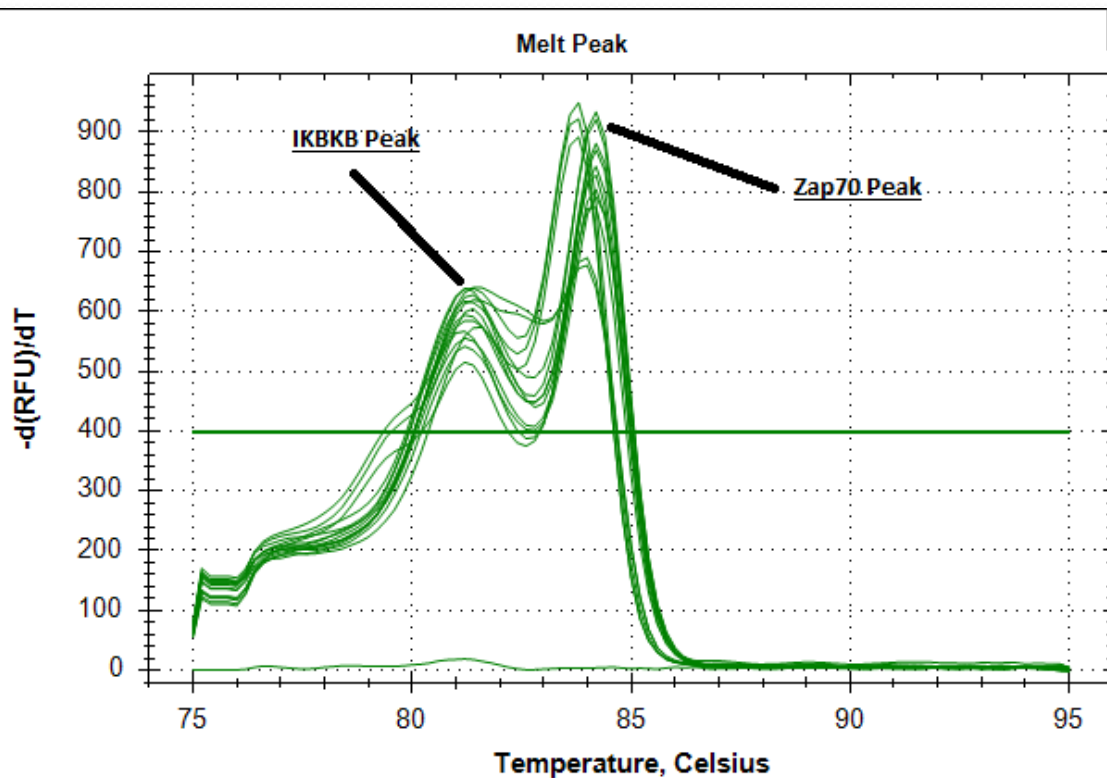


# High Resolution Melt Analysis

- Designed primers to produce short amplicons containing the respective mutation sites utilizing the same extract of DNA prepared for the TREC assay
- Amplify a 60bp fragment flanking the mutated IKBKB sequence
- Amplify 96bp fragment flanking the mutated Zap70 sequence
- Goal: use melt curves to exploit the difference in melt temperature ( $T_m$ ) of the amplicons created by the presence of the mutation in order to genotype patient samples
- Amplified in the presence of SYBR green dye, which binds double stranded DNA

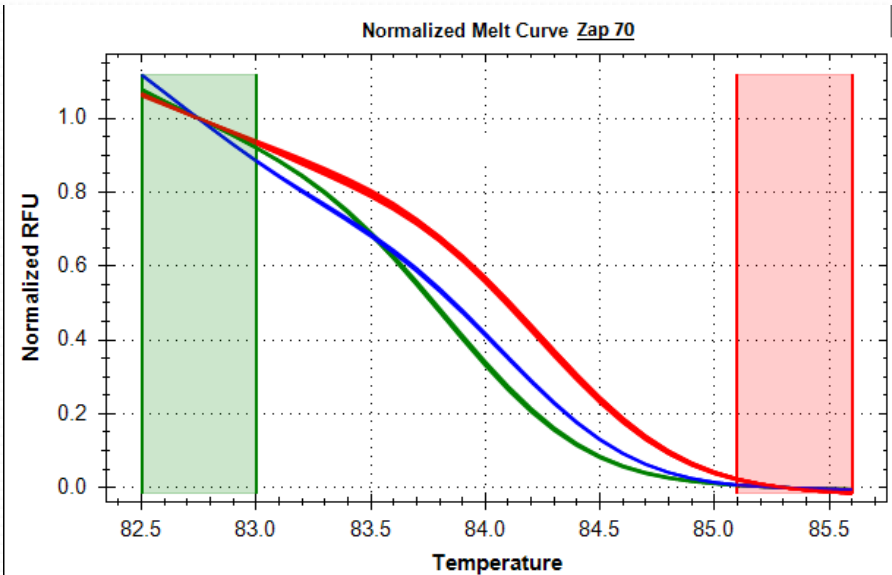
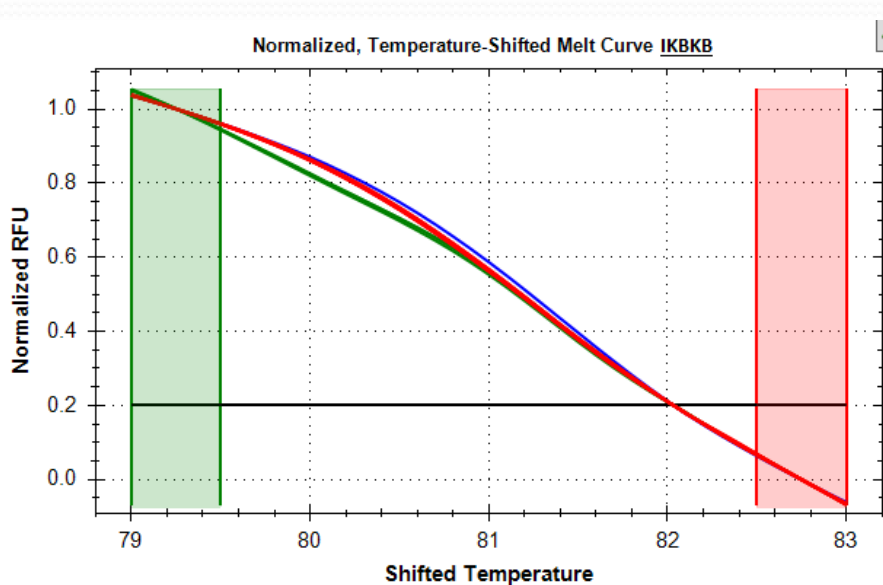
# Raw Melt Curve

- Software produces a raw melt curve adjusted by the negative first derivative to visualize the dissociation temperatures (50% dissociation) of the amplicons.



# Precision analysis

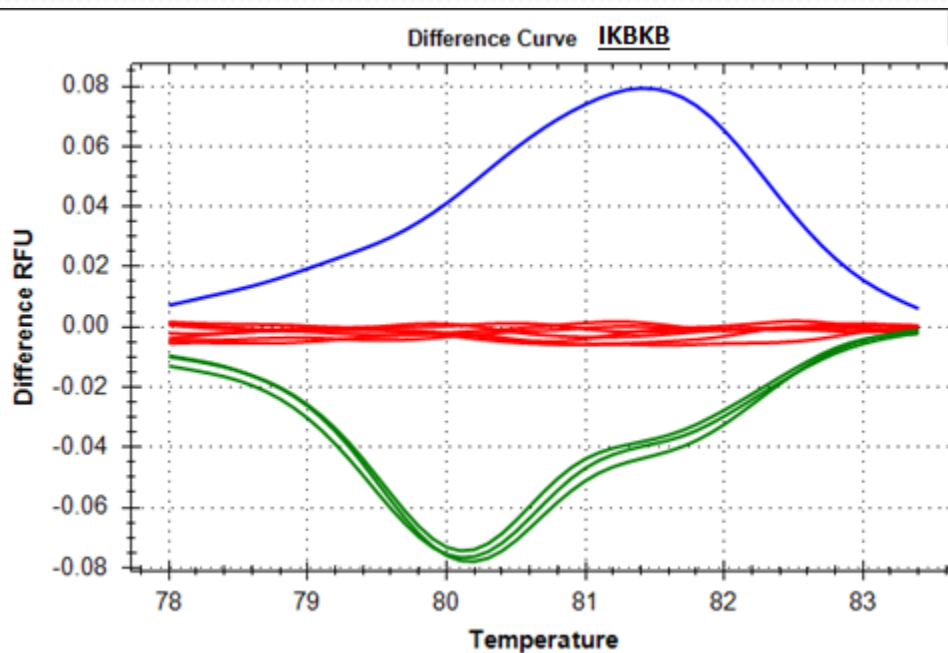
- HRM software manipulates raw fluorescent data by normalization to correct for background signal
- Areas of stable pre- and post-melt fluorescence intensity are set to relative values of 1.0 and 0
- Produces Normalized Melt Curve for clear differentiation of both sets of amplicons





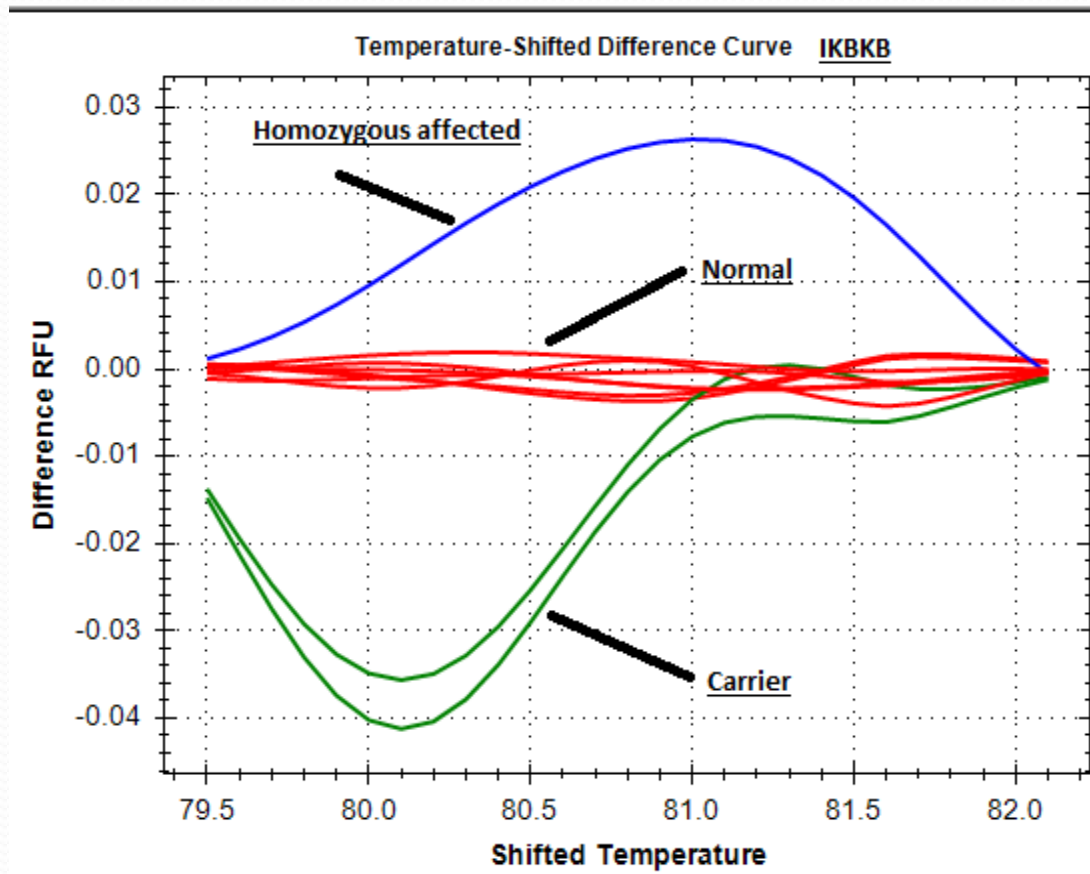
# Difference Curve

- Software generates an easy to read difference curve to visually magnify the melt profile differences between different clusters of the same genotype
  - Includes percent confidence of cluster assignment

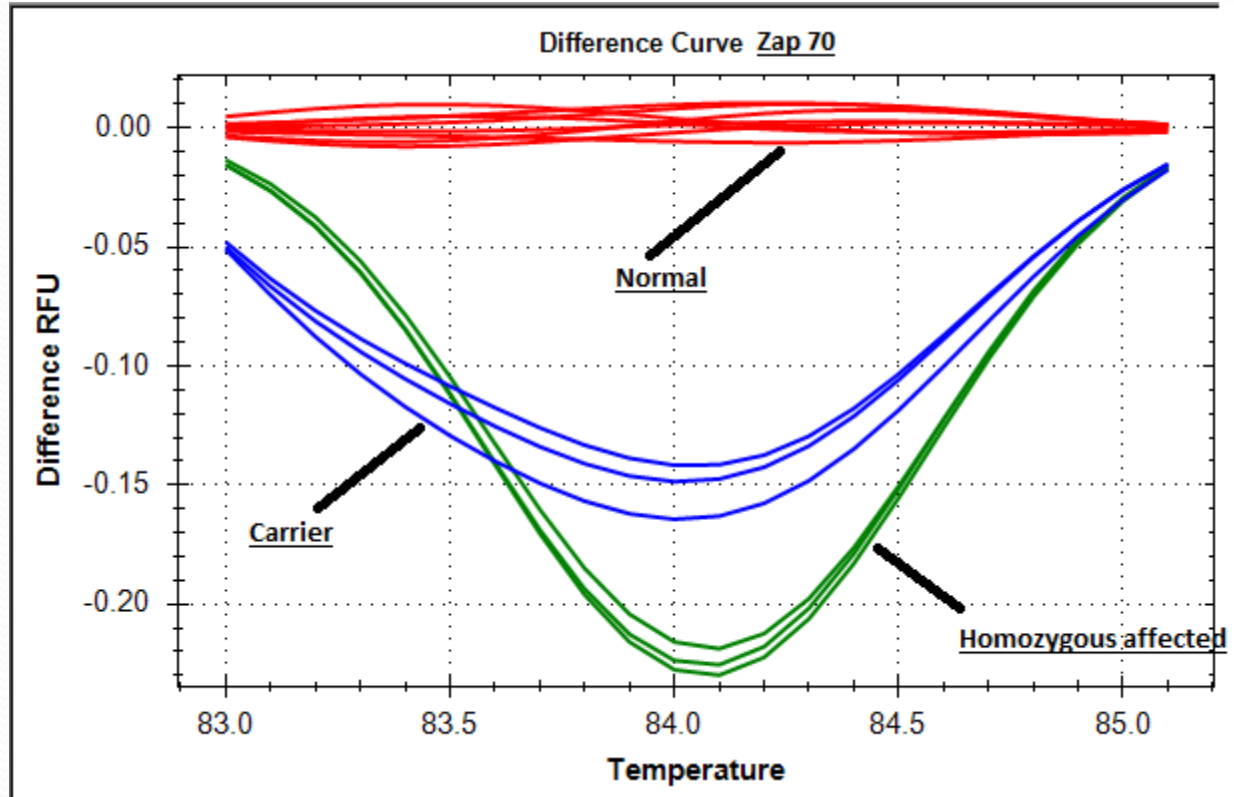


Well	Content	Sample	Cluster	Percent Confidence
A02	Unkn		Cluster 1	99.7
A04	Unkn		Cluster 1	99.8
B01	Unkn		Cluster 1	99.9
B02	Unkn		Cluster 1	99.9
D02	Unkn		Cluster 1	99.9
D04	Unkn		Cluster 1	99.9
F02	Unkn		Cluster 1	99.9
A01	Unkn		Cluster 2	99.5
D01	Unkn		Cluster 2	99.0
F01	Unkn		Cluster 2	99.6
D03	Unkn		Cluster 3	99.7
G01	Unkn		<Excluded>	N/A

# IKBKB



# Zap 70



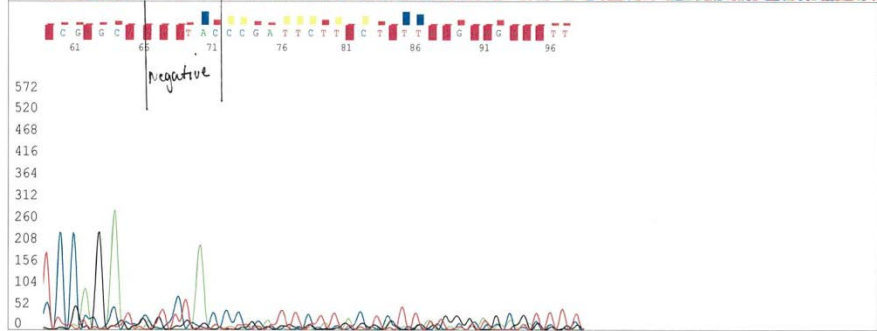
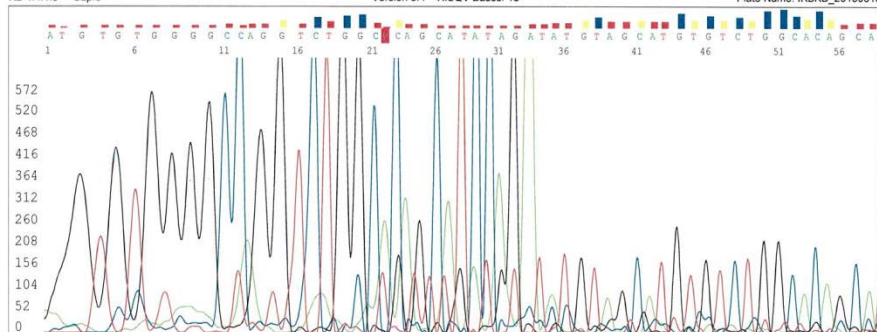
# IKBKB Product Sequencing



S/N G:304 A:191 T:128 C:184  
KB bcp  
KB 1.4.1.8 Cap:6

CPL#2F03062015-06-10-13-21-30  
CPL#2  
KB\_3500\_POP6\_BDTV3.mob  
Pts 1233 to 2600 Pk1 Loc:1233  
Version 5.4 HISQV Bases: 16

Inst Model/Name 3500/3500 Instrument  
Jun 10, 2015 04:55PM, CDT  
Jun 10, 2015 05:32PM, CDT  
Spacing:13.7 Pts/Panel/800  
Plate Name: IKBKB\_20150610



Printed on: Thu Jun 11, 2015 09:30AM, CDT

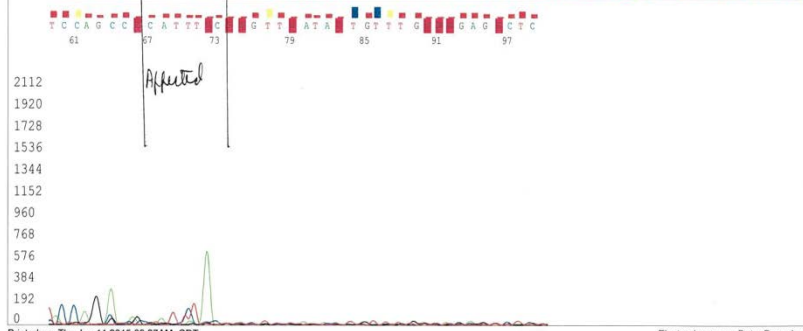
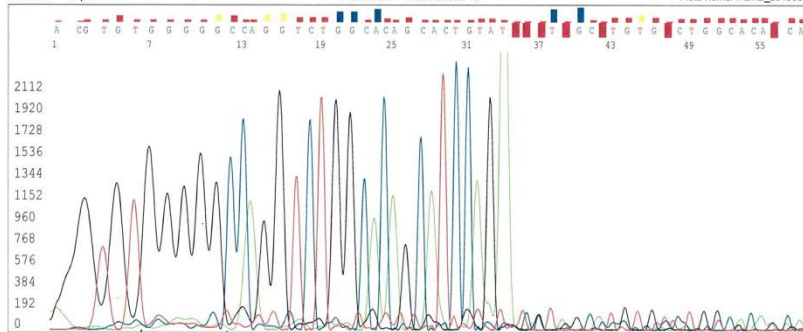
Electropherogram Data Page 1 of 1



S/N G:393 A:187 T:84 C:159  
KB bcp  
KB 1.4.1.8 Cap:8

CPL#4H03082015-06-10-13-21-30  
CPL#4  
KB\_3500\_POP6\_BDTV3.mob  
Pts 1223 to 2600 Pk1 Loc:1223  
Version 5.4 HISQV Bases: 10

Inst Model/Name 3500/3500 Instrument  
Jun 10, 2015 04:55PM, CDT  
Jun 10, 2015 05:32PM, CDT  
Spacing:13.74 Pts/Panel/800  
Plate Name: IKBKB\_20150610



Printed on: Thu Jun 11, 2015 09:27AM, CDT

Electropherogram Data Page 1 of 1

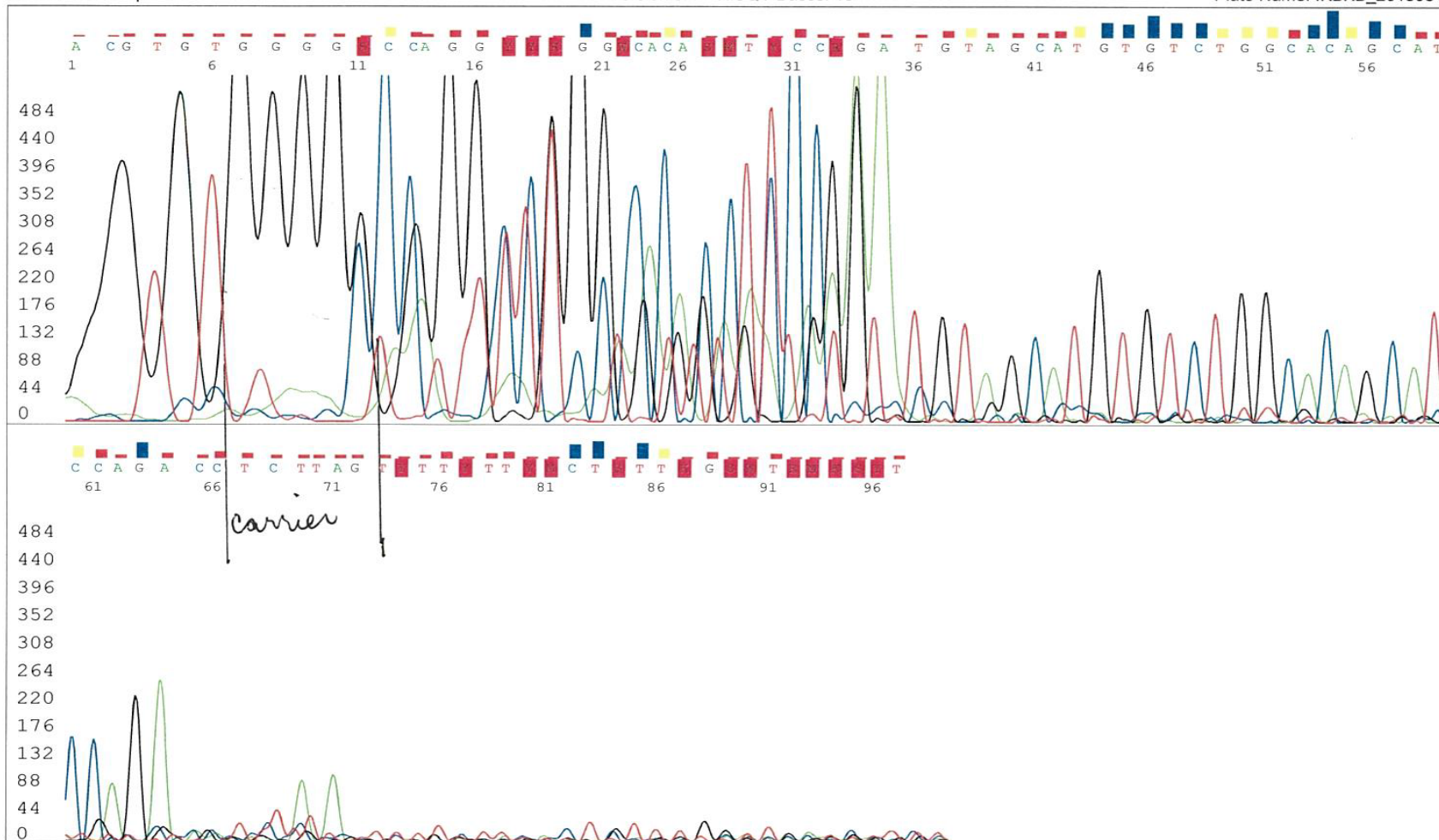
# IKBKB Heterozygote



S/N G:571 A:298 T:188 C:293  
KB.bcp  
KB 1.4.1.8 Cap:7

CPL#3G03072015-06-10-13-21-30  
CPL#3  
KB\_3500\_POP6\_BDTv3.mob  
Pts 1225 to 2600 Pk1 Loc:1225  
Version 5.4 HiSQV Bases: 16

Inst Model/Name 3500/3500 Instrument  
Jun 10,2015 04:55PM, CDT  
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Spacing:13.87 Pts/Panel800  
Plate Name: IKBKB\_20150610





# Conclusions

- Universal application of the multiplex high resolution DNA melt analysis for mutation genotyping in combination with TRECs quantification on all newborns is planned for implementation and should provide comprehensive detection of SCID in the newborn population of Manitoba.
- Screen positive criteria, follow up investigation and downstream protocols are under development.