**Data Analysis**

NRAS Plus Kits

(NRAS codons 61, 117, 146)

Open GeneMapper software and follow the online instruction to add data for analysis:

GeneMapper: www.trimgen.com/docs/PartIII-Data-Analysis-GeneMapper.pdf

NRAS Plus Data Analysis

In sample plot window, zoom in on X-axis between size marker 25 and 100 (in between 2nd and 5th size makers). Peaks outside of this range will not be considered for data analysis. In the zoomed window, the CTL panel will show **three peak groups** for codon 61, 117 and 146 respectively.   A wild type sample will show **three peaks**: one for codon 61, one for codon 117 and one for codon 146.
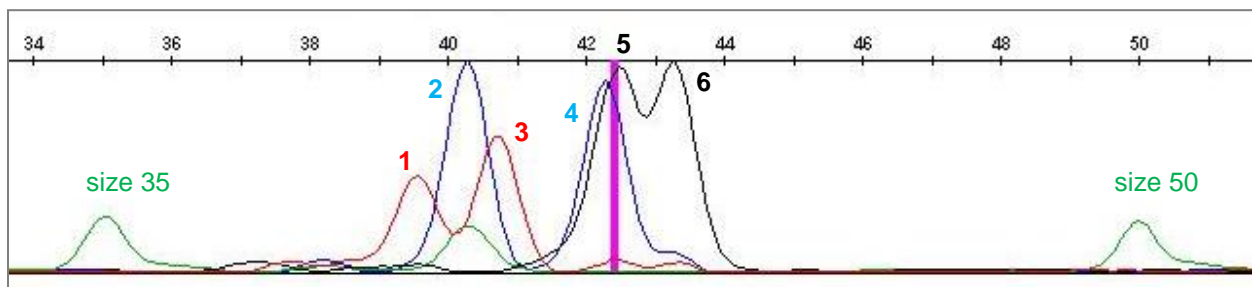

zoom in size 25-100

NRAS CTL   Codon 61   Codon 117   Codon 146
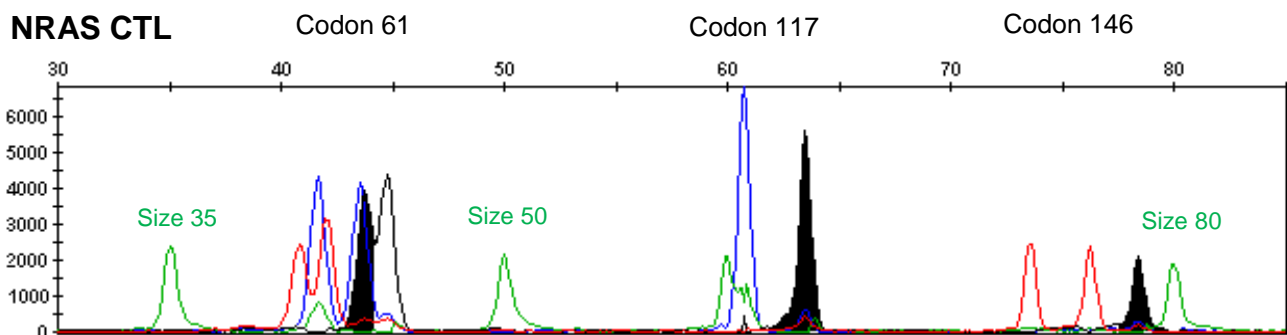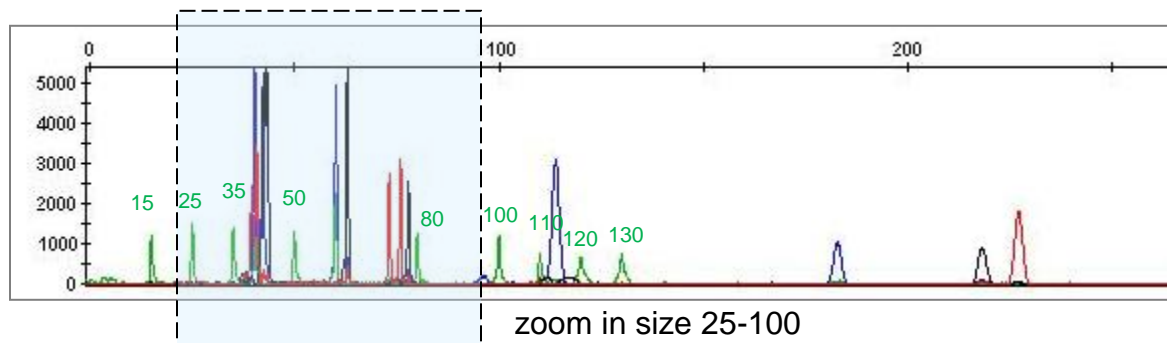
NRAS sample (wild type)
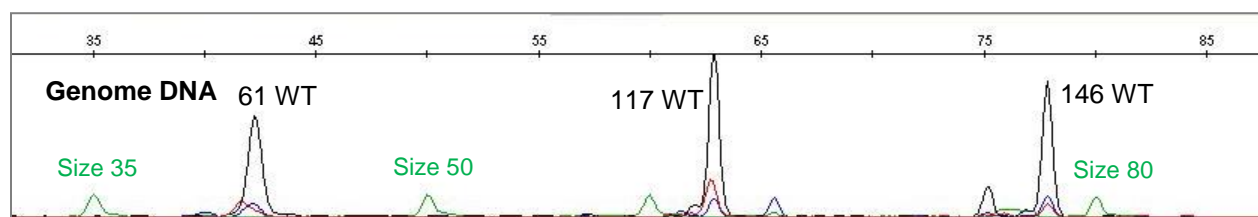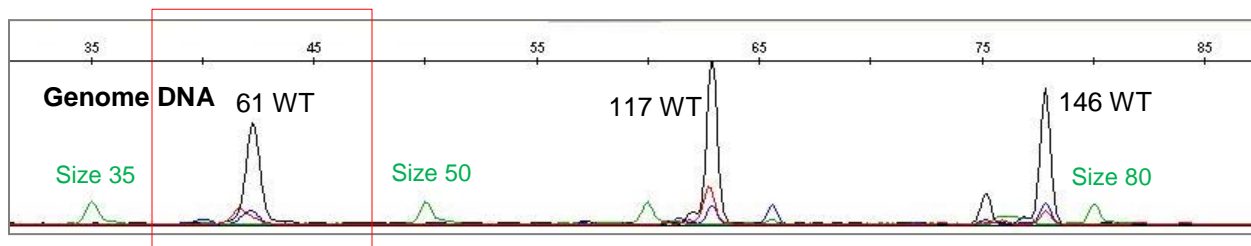
Analysis of NRAS Codon 61

Zoom in on x-axis (25-60)

CTL of NRAS Codon 61 shows **6** peaks. Below is detailed mutation information.
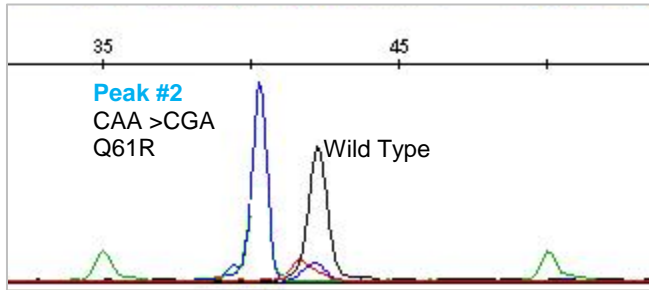

From left to right:

Peak #	Peak color	Genotype
1	**Red**	Q61H (CAA >CAT)
2	**Blue**	Q61R (CAA >CGA)
3	**Red**	Q61L (CAA >CTA)
4	**Blue**	Q61K (CAA >AAA)
5	Black	Wild Type
6	Black	Q61P (CAA >CCA)

Sample DNA (wild type) will show **1 peak (black color)**, any additional peak that matches a mutation peak in the CTL panel (color and size) will be considered as a mutation.

Example of mutations detected in FFPE samples

Sample 1 – **NRAS Q61R (CAA >CGA) Mutation**

Peak #2
CAA >CGA
Q61R
Wild Type

Sample 2 – **NRAS Q61H (CAA >CAT) Mutation**

Peak #1
CAA >CAT
Q61H
Wild Type

Analysis of NRAS Codon 117

Zoom in on x-axis (50-70)

CTL of NRAS Codon 117 shows <u>**3** peaks</u>. Below is detailed mutation information.

From left to right:		
Peak #	**Peak color**	**Genotype**
1	**Blue**	K117R (AAG >AGG)
2	**Black**	Wild Type

Sample DNA (wild type) will show <u>**1 peak (black color)**</u>, any additional peak that matches a mutation peak in the CTL panel (color and size) will be considered as a mutation.
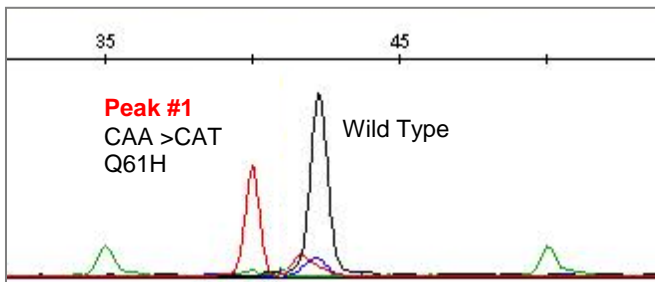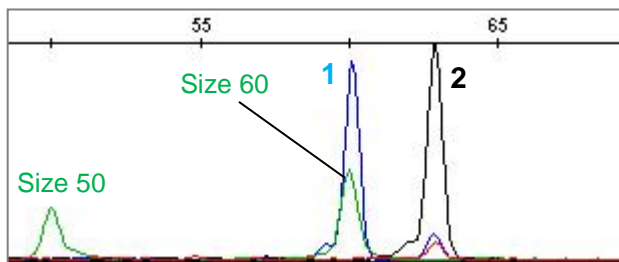

Non-specific pull-up peaks

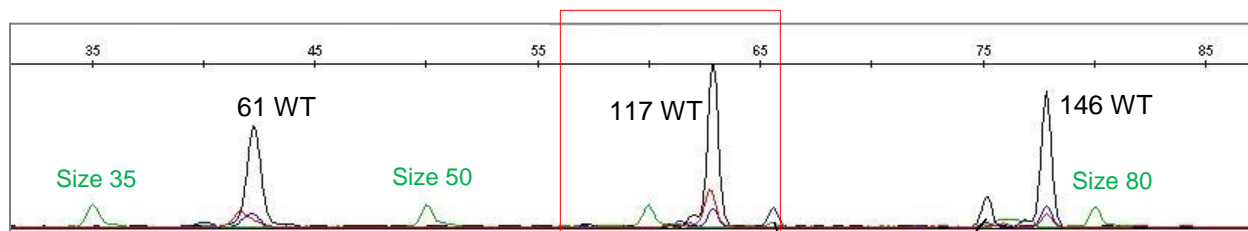# Analysis of NRAS Codon 146

Zoom in on x-axis (65-90)

CTL of NRAS Codon 146 shows <u>**3 peaks.**</u> Below is detailed mutation information.

From left to right:

Peak #	Peak color	Genotype
1	**Red**	A146V (GCC >GTC)
2	**Red**	A146T (GCC >ACC)
3	**Black**	Wild Type

Sample DNA (wild type) will show <u>**1 peak (black color)**</u>, any additional peak that matches a mutation peak in the CTL panel (color and size) will be considered as a mutation.
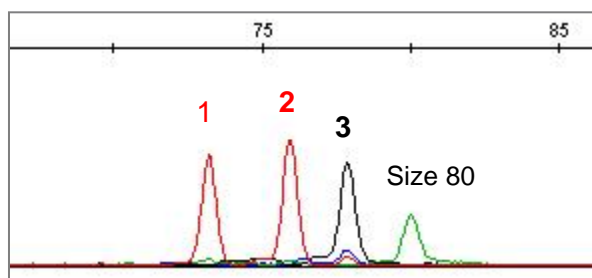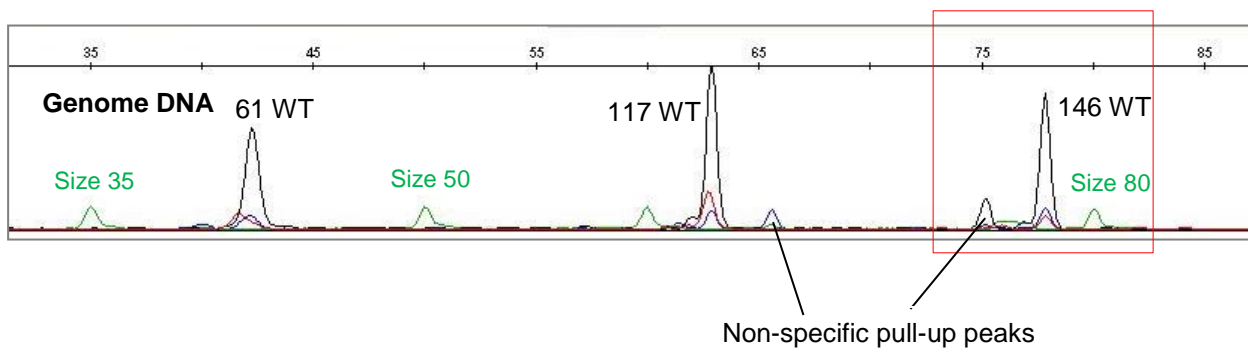

Non-specific pull-up peaks

Low Signal

The peak height represents signal intensity. The height of a wild type peak is usually above 1000 rfu (Y-axis). If the signal intensity is too low (below 200 rfu), the method cannot detect the low level of mutations.

The cause of low signal:
PCR amplification failure due to:
- poor DNA quality
- low DNA concentration
- existence of PCR inhibitors

The solution to resolve the issue:
Purify final ST products with TF Spin Filter tip (TrimGen Cat # TF-50). After purification, load 5-10 ul of the purified product to the sequencer. In most cases, this step increases the signal 3-5 times.

If this step does not increase signal, you need to re-run the PCR with more DNA.

Note: PCR may fail again if the sample contains PCR inhibitors. Cleaning the sample with the TF Spin Filter tip will help remove most PCR inhibitors.