Data Analysis

KRAS Plus Kits

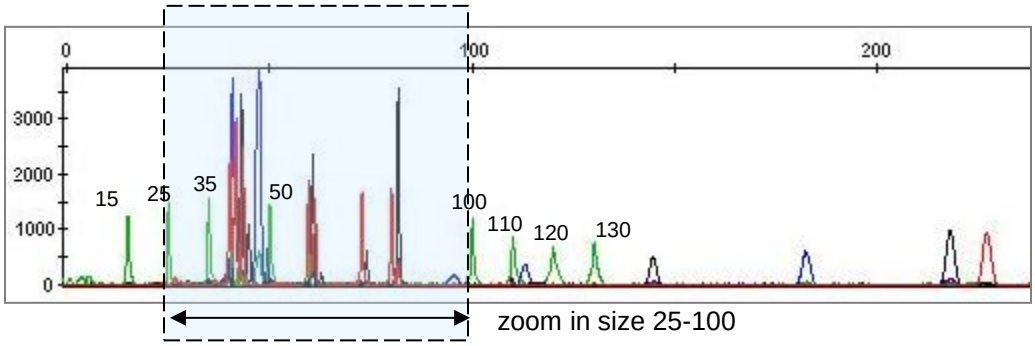
(KRAS codons 61, 117, 146)

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

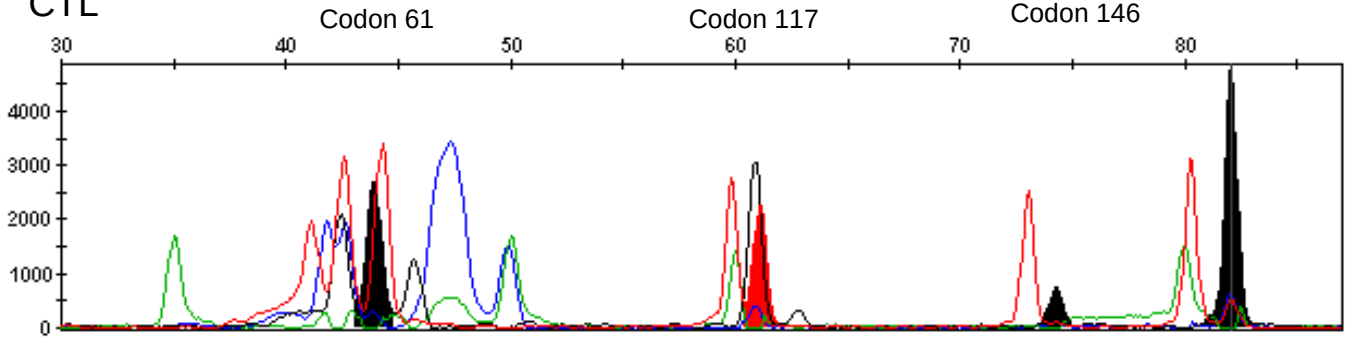
GeneMapper: www.trimgen.com/docs/PartIII-Data-Analysis-GeneMapper.pdf

KRAS Plus Data Analysis

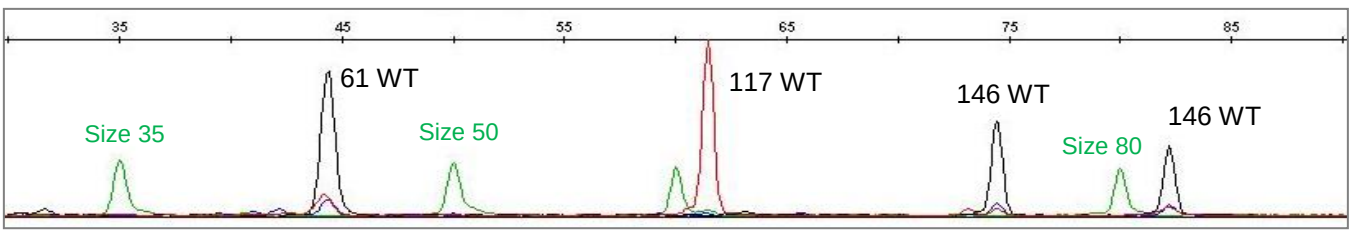
In sample plot window, zoom in on X-axis between size marker 25 and 100 (in between 2nd and 5th size makers). Peaks outside of this range will not be considered for data analysis. In the zoomed window, the CTL panel will show **three peak groups** for codon 61, 117 and 146 respectively. A wild type sample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



CTL



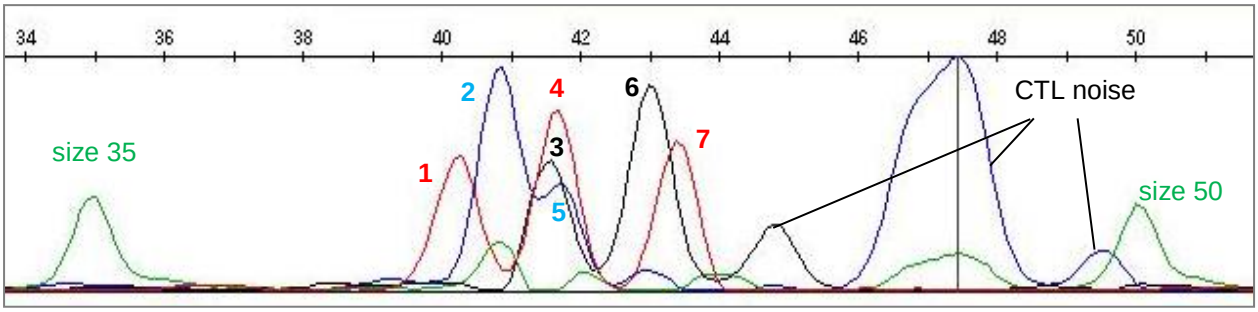
Sample (wild type)



Analysis of KRAS codon 61

Zoom in on X-axis (25-60)

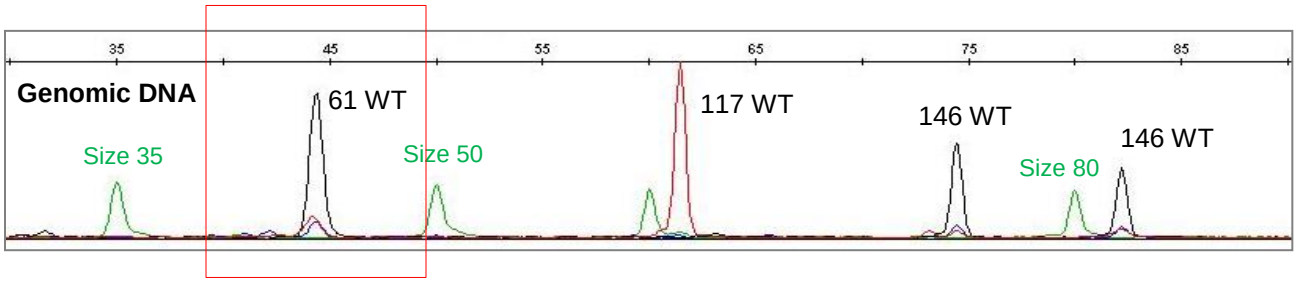
CTL of KRAS Codon 61 shows **7** peaks. The detailed mutation information is listed below:



From left to right:

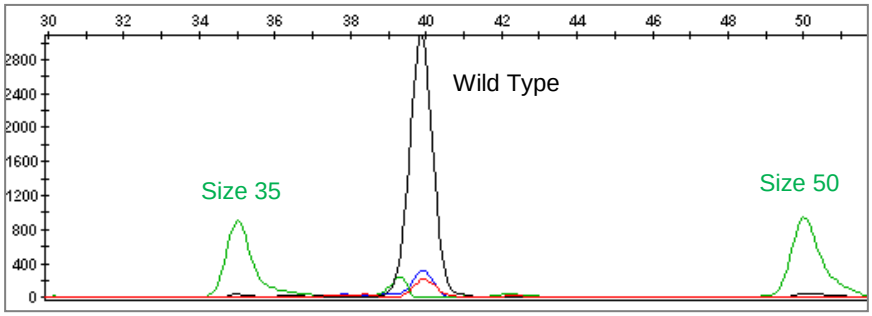
Peak #	Color	Genotype
1	Red	Q61H (CAA >CAT)
2	Blue	Q61R (CAA >CGA)
3	Black	Q61H (CAA >CAC)
4	Red	Q61L (CAA >CTA)
5	Blue	Q61E (CAA >GAA)
6	Black	Wild Type
7	Red	Q61K (CAA >AAA)

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.

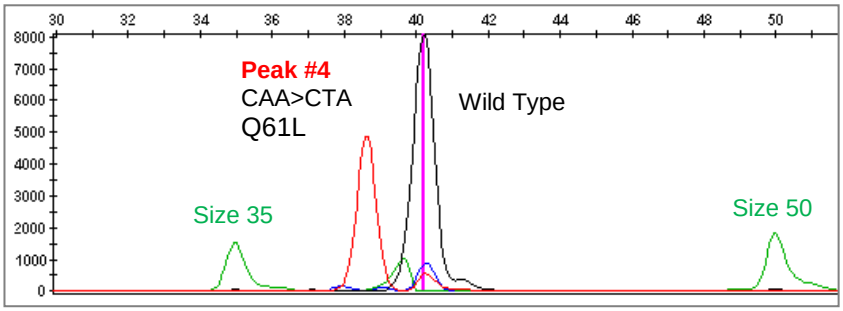


Example of mutations detected in FFPE samples

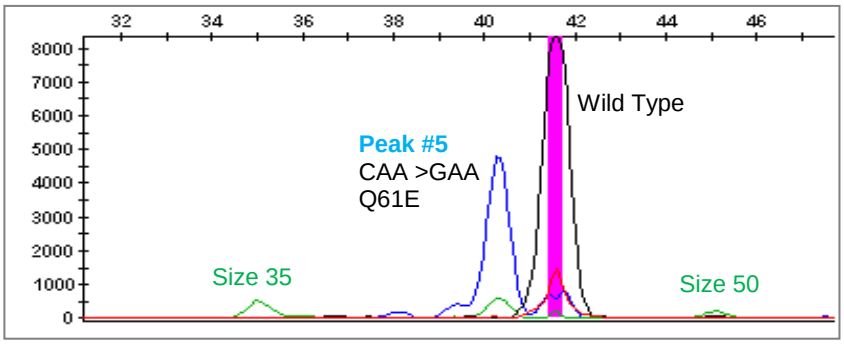
Sample 1 - **Wild Type**



Sample 2 - **Q61L (CAA >CTA) Mutation**



Sample 3 - **Q61E (CAA >GAA) Mutation**

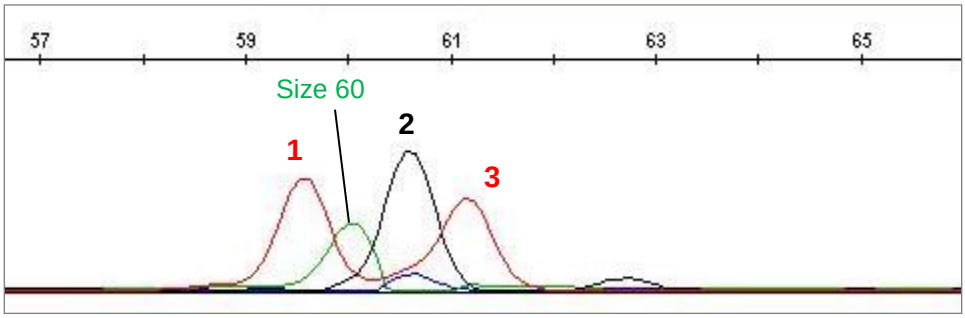


Note: The size of a particular wild type peak may be slightly different between test runs. For example, the size of the wild type peak of KRAS Codon 61 in sample 2 and sample 3 was different. This difference is generally caused by the performance of each capillary electrophoresis in difference runs.

Analysis of KRAS Codon 117

Zoom in on x-axis (50-70)

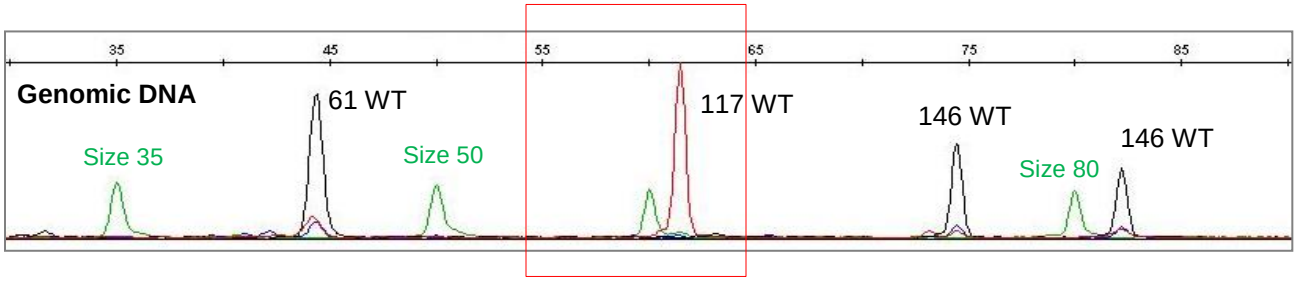
CTL of KRAS Codon 117 shows **3** peaks. Below is detailed mutation information.



From left to right:

Peak #	Peak Color	Genotype
1	**Red**	K117N (AAA >AAT)
2	**Black**	K117N (AAA >AAC)
3	**Red**	Wild Type

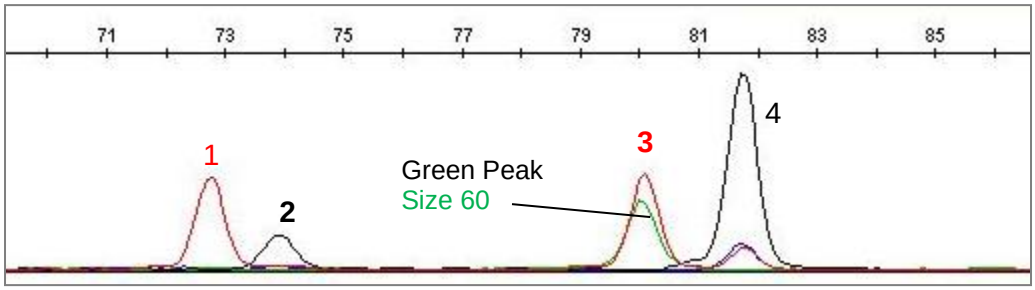
Sample DNA (wild type) will show **1 peak (red color)**, any additional peak that matches a mutation peak in the CTL panel (color and size) will be considered as a mutation.



Analysis of KRAS Codon 146

Zoom in on x-axis (65-90)

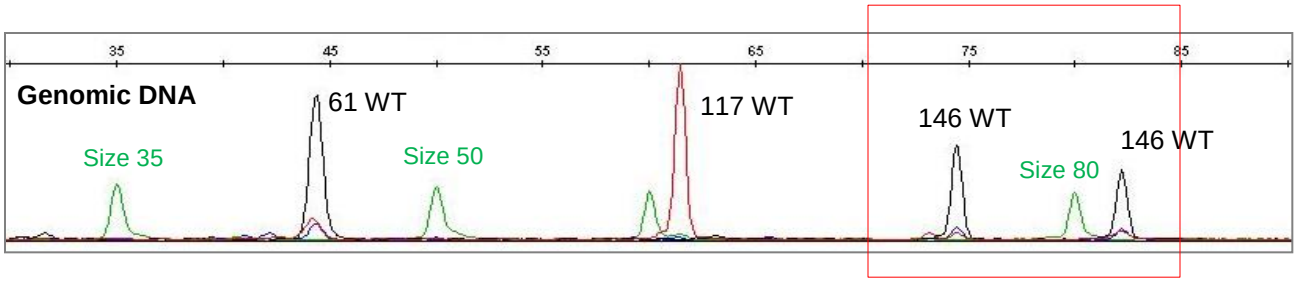
CTL of KRAS Codon 146 shows **4** peaks. Below is detailed mutation information.



From left to right:

Peak #	Peak color	Genotype
1	Red	A146V (GCA >GTA)
2	Black	Wild Type
3	Red	A146T (GCA >ACA)
4	Black	Wild Type

Sample DNA (wild type) will show **2 peaks (black color)**, any additional peak that matches a mutation peak in the CTL panel (color and size) will be considered as a mutation.



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.