# High resolution acquisition on TIMS-QTOF for multiplexed proteomics

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# Overview

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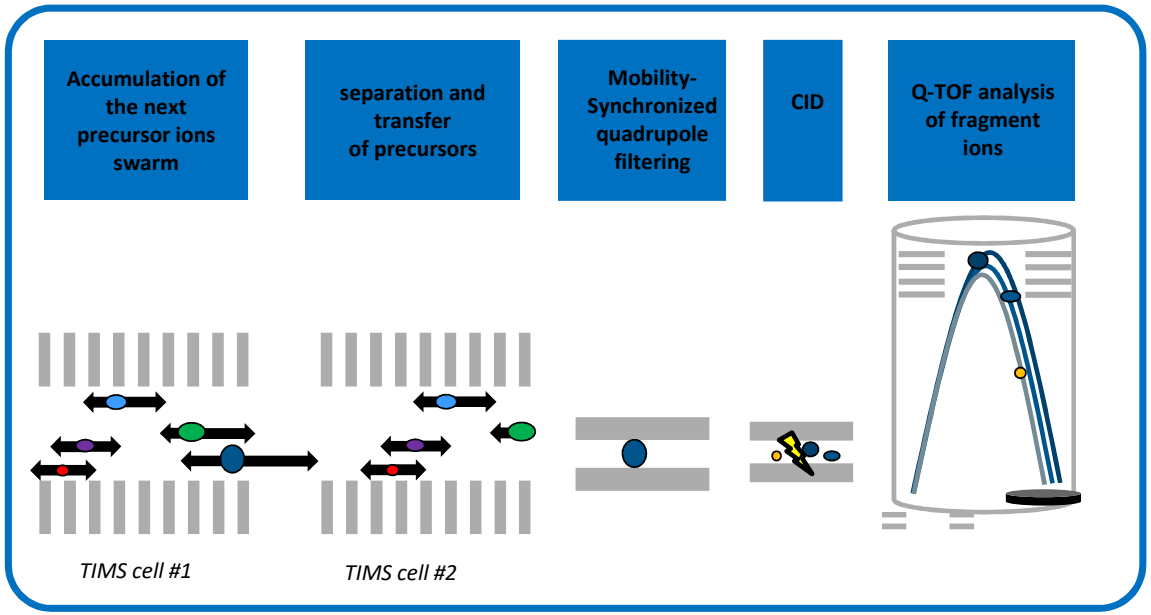
## Abstract

prm-PASEF is a new targeted acquisition method that fully exploits the multiplexing capability and the high resolution of the TIMS-TOF mass spectrometer. Multiple peptides can be sequentially measured from a single ion mobility scan without compromising the sensitivity. We evaluated parameters including sensitivity, reproducibility, accuracy and dynamic range using AQUA peptides spiked in a Hela cell lysate. Finally, we applied the method to quantify the mutations and isoforms of the Ras oncoproteins family in cancer cell lines.

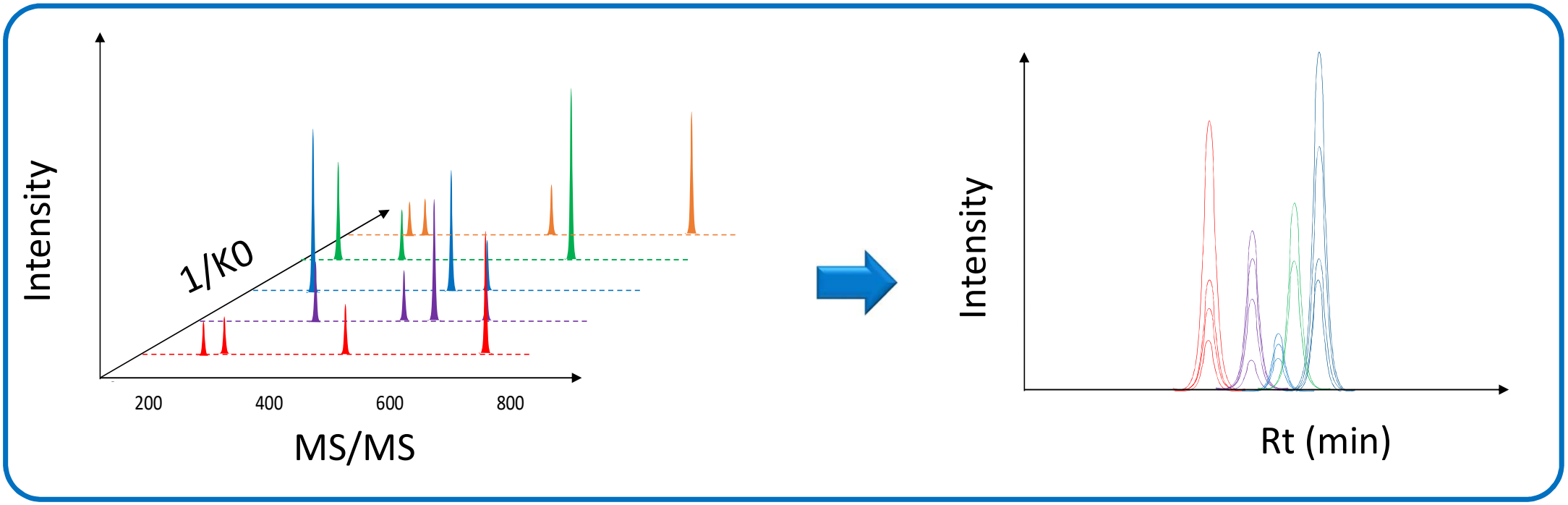
## prm-PASEF acquisition




*In the TIMS cells the ions are accumulated and separated according to their shape and size. While the first TIMS cell accumulates the next ion swarm, the second cell elutes the precursor ions into the QTOF mass spectrometer. The targeted peptides are filtered by the quadrupole, fragmented in the collision cell and the resulting fragment ions analyzed at high resolution (60,000).*

# Overview

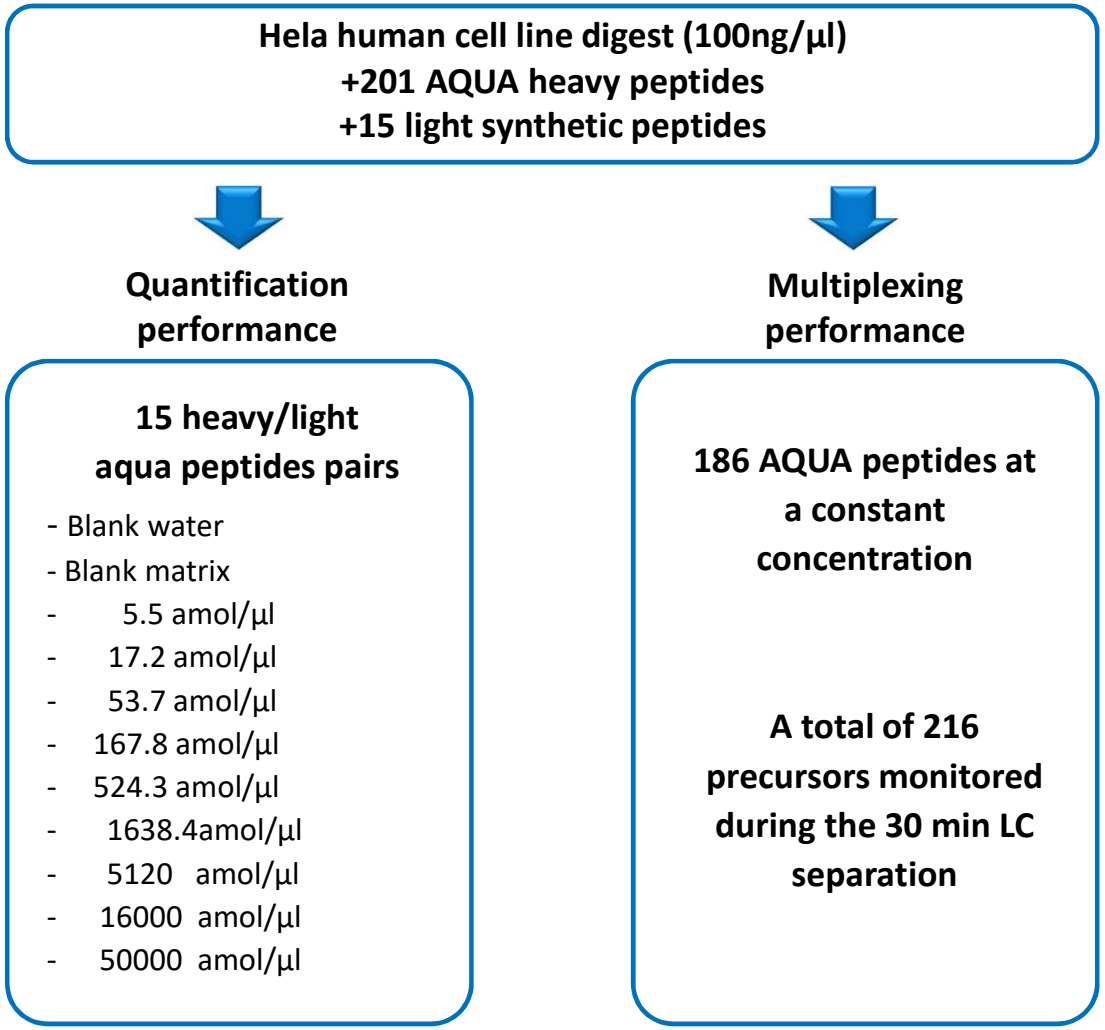
## prm-PASEF data processing




*Extracted ion chromatograms (XICs) are generated from the signal of fragment-ions.*
*The quantification is based on the area under the peak of the XICs.*

# Material and Methods

Samples were separated by nano-HPLC (nanoElute, Bruker Daltonics) on 250 mm pulled emitter columns (IonOpticks, Australia) with a 30 min gradient. Peptides were analyzed on a timsTOF Pro instrument (Bruker Daltonics) operated in prm-PASEF acquisition mode (prototype). Data were processed with Skyline-daily.



# Results

## prm-PASEF a new highly parallelized acquisition method

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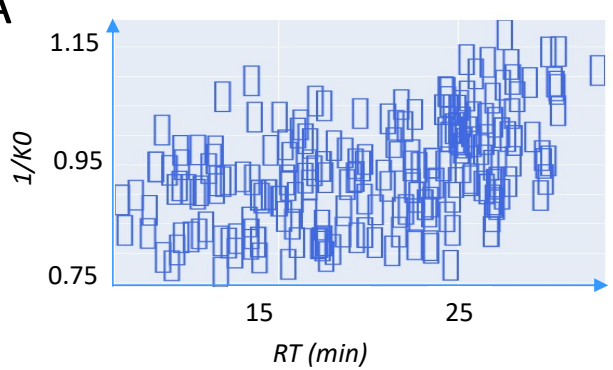
A

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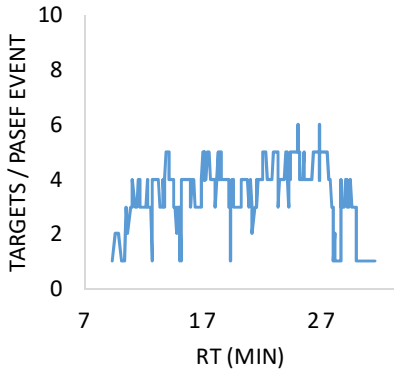


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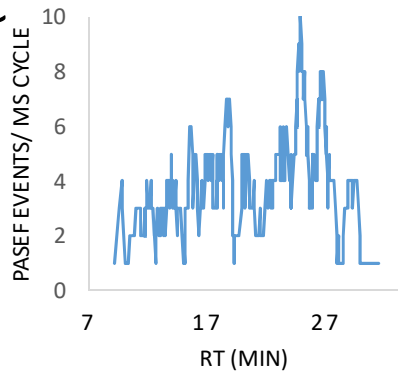
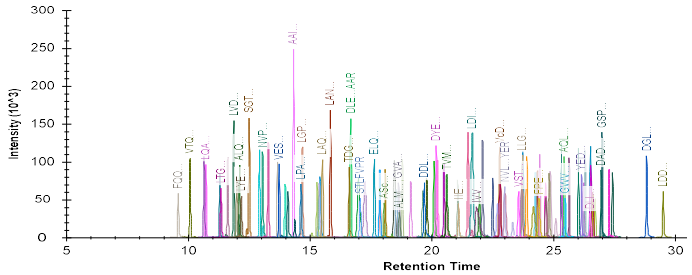



*A) Representation of PASEF-PRM isolation boxes in the ion mobility (1/K0) and chromatography retention time (min) dimensions.*
*B) Visualization of the number of targets per PASEF event across the chromatography separation.*
*C) Visualization of the PASEF event per MS cycle across the chromatography separation.(1 PASEF event = 100ms)*

D

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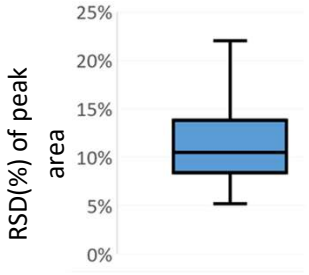


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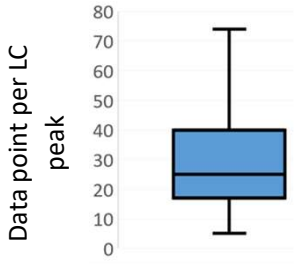


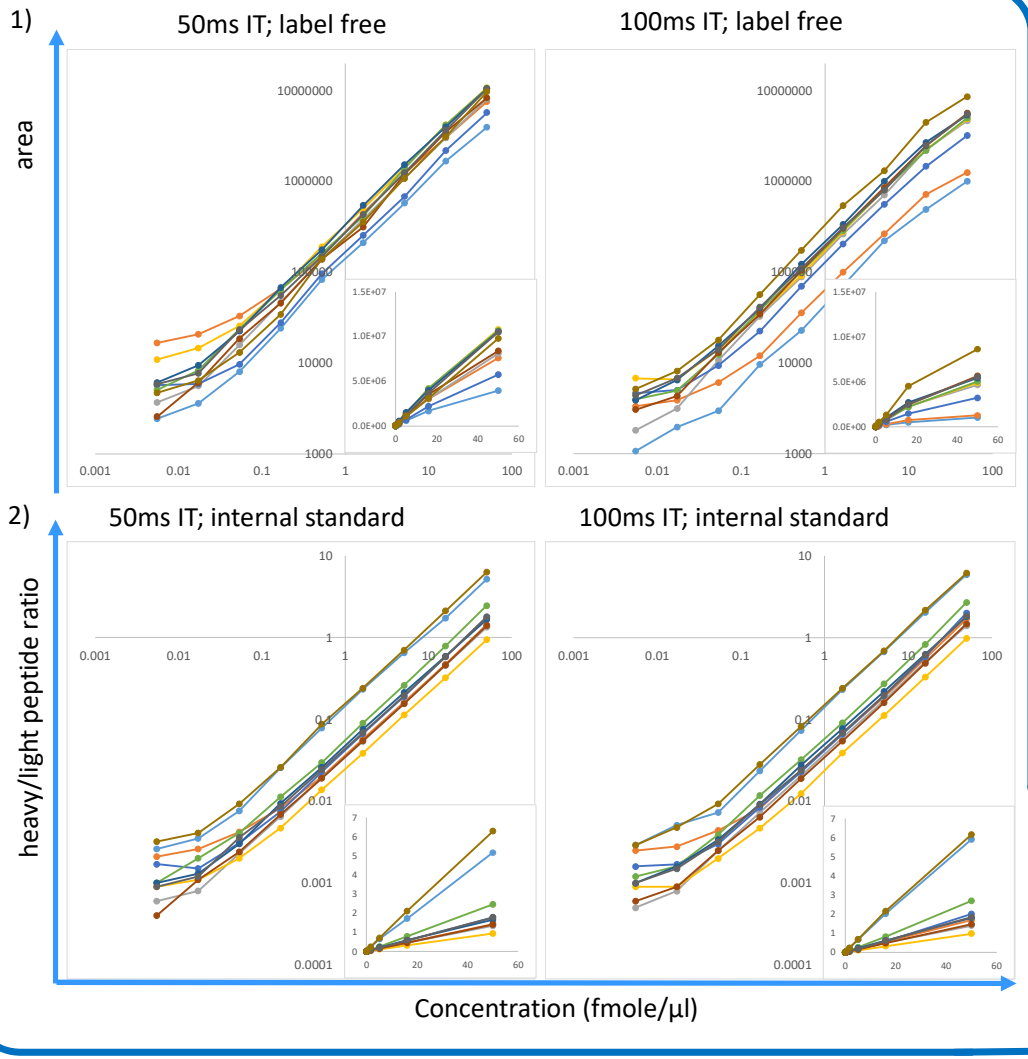

*D) Representative prm-PASEF traces of the targeted 216 precursors in all LC-(prm-PASEF) runs*
*E) RSD(%) of the peak areas of all peptides monitored in 30 prm-PASEF runs (label free data)*
*F) Number of data points across the chromatographic peaks*

Several peptides ions can be targeted from a single (100ms) ion mobility separation (PASEF event)

It enables high-multiplexed targeted acquisition without compromising the quantification performance and number of data points per LC peaks

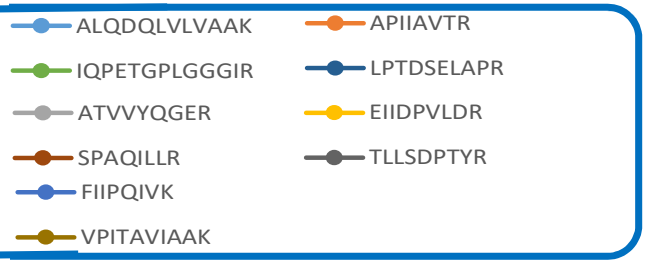
# Results

## prm-PASEF quantification performance




| TIMS accumulation time | 50ms | 100ms |
|---|---|---|
| ALQDQLVLVAAK | 53.7 | 167.8 |
| APIIAVTR | 53.7 | 167.8 |
| ATVVYQGER | 17.2 | 17.2 |
| EIIDPVLDR | 53.7 | 53.7 |
| FIIPQIVK | 53.7 | 53.7 |
| IQPETGPLGGGIR | 17.2 | 17.2 |
| LPTDSELAPR | 17.2 | 17.2 |
| SPAQILLR | 53.7 | 17.2 |
| TLLSDPTYR | 53.7 | 17.2 |
| VPITAVIAAK | 53.7 | 53.7 |

***The limits of quantification (LOQ)*** *in amol of ten AQUA peptides were defined as lowest concentration point within 80%<accuracy<120% associated to a signal higher that the mean(blanks)+3×SD(blanks)*




prm-PASEF demonstrated limits of quantification down to 17.2 amole/ul

Synthetic peptide standards (SIL) play a critical role to extend the linearity range and the accuracy of the method

# Results

## application to screening Ras mutants in cancer cell lines

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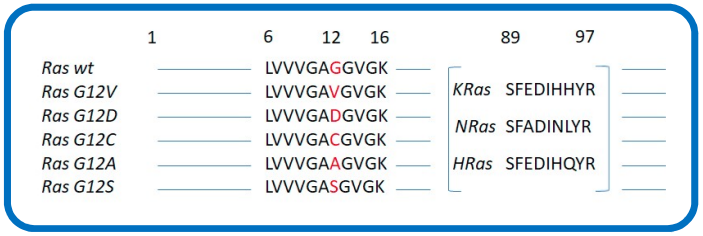
-Epidermoid carcinoma (A431)
-Lung carcinoma (H1975, A549)
-NSCL carcinoma (H3255)
-Lung adenocarcinoma (HCC827)
-Colorectal carcinoma (HT29, SW480, SW620)
Patient derived colorectal cancer (T20, T18)

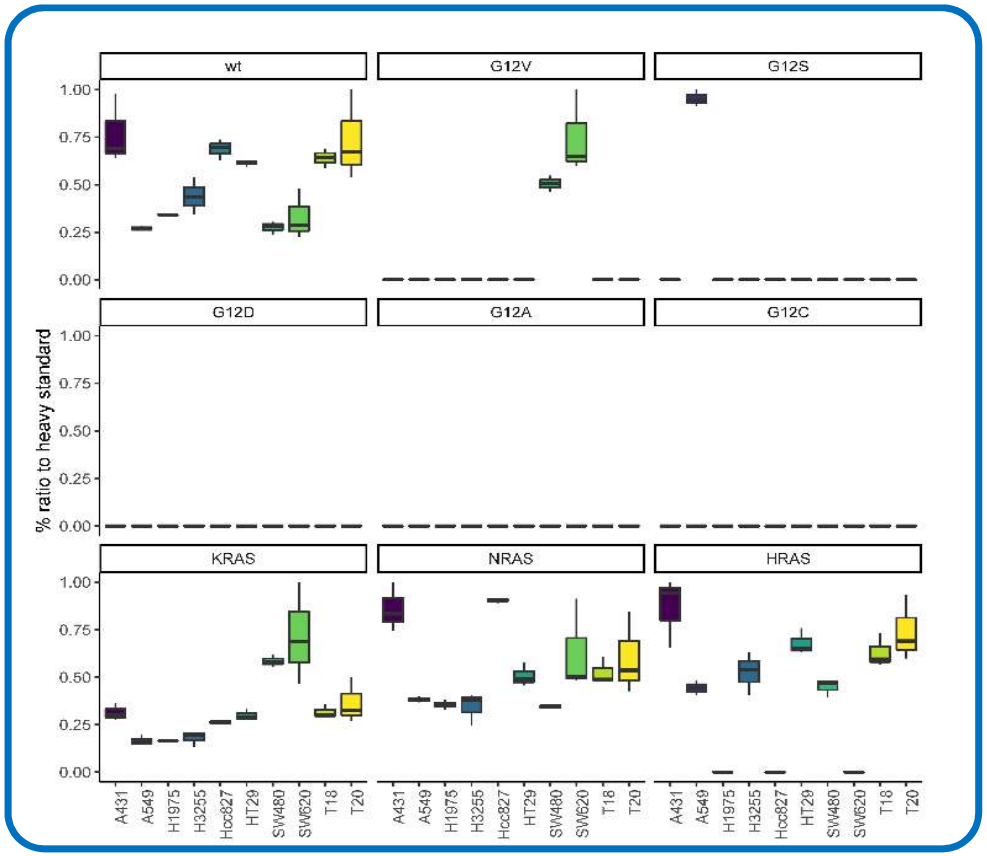
Trypsin proteolysis

Addition isotope labeled peptides

Fast-LC-pasef PRM analysis
25ng injected 10min gradient




*Signature peptides used for the relative quantification of the Ras mutation and isoforms.*




*Ras mutants and isoform profiling from the cell lines samples*

The three Ras isoforms, including disease-relevant mutations, were quantified from 25 ng of protein material with a 10min gradient.

The Ras mutations G12V and G12S were detected in the expected cancer cell lines.

# Conclusions

- prm-PASEF takes advantage of the trapped ion mobility technology for the targeted proteomics analysis.
- The sensitivity and selectivity of the acquisition method is improved by the ion mobility filtering and time focusing effect that happens in the TIMS cells.
- The parallelization of targets acquisition during a prm-PASEF cycle allows to maximize the number of peptides quantified in a single analysis while keeping an excellent chromatographic peak profile and quantification performance.
- Disease relevant mutations can be screened in biological samples at high throughput (10min gradient) showing a potential for routine testing of patient samples.

# Acknowledgments

We thanks Dr. Garry Kruppa, Bruker Daltonics for making this collaboration possible and fruitful discussions.