Mass Spectrometry

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Mass spectrometry

- Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio of charged particles

- It can detect amino acid substitutions / post-translational modifications

MS History

- JJ Thomson built MS prototype to measure m/z of electron, awarded Nobel Prize in 1906
- MS concept first put into practice by Francis Aston, a physicist working in Cambridge England in 1919
- Designed to measure mass of elements
- Aston Awarded Nobel Prize in 1922

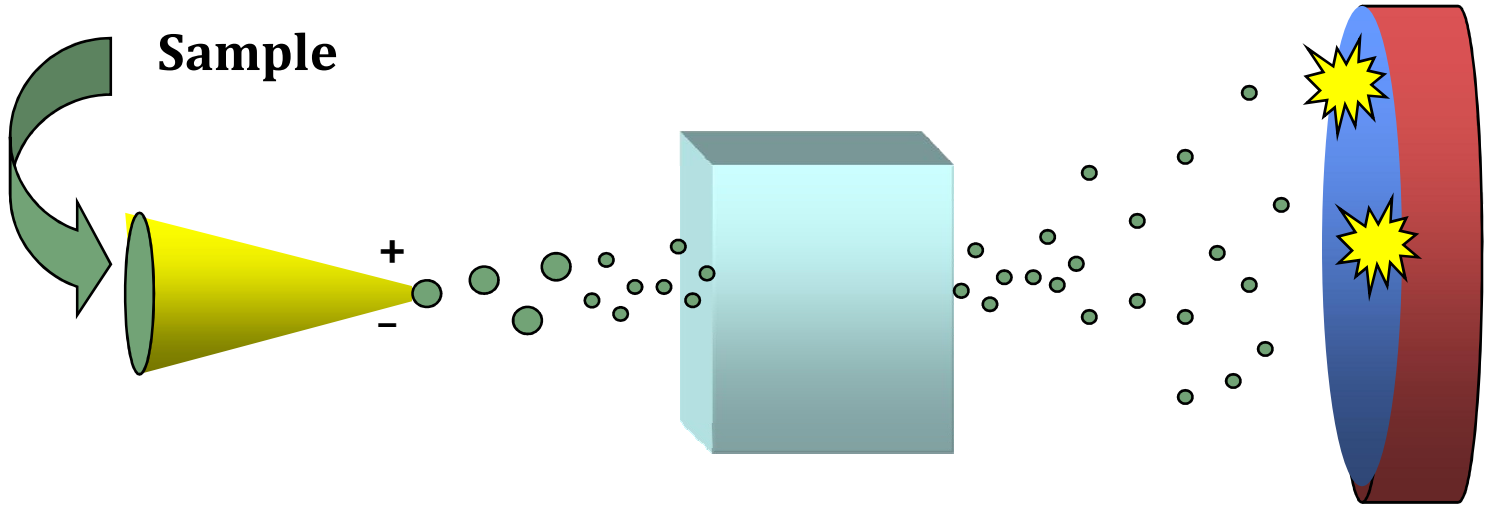
MS History

- 1948-52 - Time of Flight (TOF) mass analyzers introduced
- 1955 - Quadrupole ion filters introduced by W. Paul, also invents the ion trap in 1983 (wins 1989 Nobel Prize)
- 1968 - Tandem mass spectrometer appears
- Mass spectrometers are now one of the ***MOST POWERFUL ANALYTIC TOOLS IN CHEMISTRY***

MS Principles

- Find a way to “charge” an atom or molecule (ionization)
- Place charged atom or molecule in a magnetic field or subject it to an electric field and measure its speed or radius of curvature relative to its mass-to-charge ratio (mass analyzer)
- Detect ions using microchannel plate or photomultiplier tube

Mass Spec Principles



How does a mass spectrometer work?

Create ions → **Separate ions** → **Detect ions**

- **Ionization method**
  - **MALDI**
  - **Electrospray**

  (Proteins must be charged and dry)

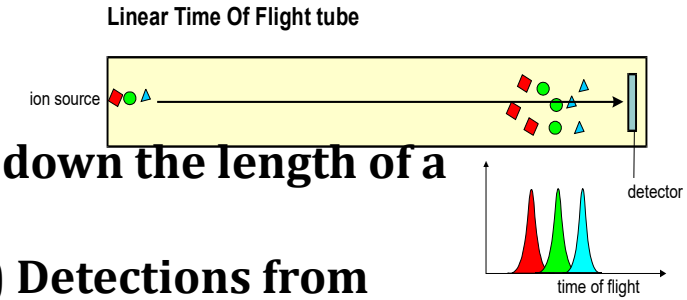
- **Mass analyzer**
  - **MALDI-TOF**
    - **MW**
  - **Triple Quadrapole**
    - **AA seq**
  - **MALDI-QqTOF**
    - **AA seq and MW**
  - **QqTOF**
    - **AA seq and protein modif.**

- **Mass spectrum**
- **Database analysis**

What sort of info is returned?

- Structural information can be generated
- Particularly using tandem mass spectrometers
- Fragment sample & analyse products
- Useful for peptide & oligonucleotide sequencing
- Plus identification of individual compounds in complex mixtures

Mass spectrometers



- **Time of flight (TOF) (MALDI)**
  - **Measures the time required for ions to fly down the length of a chamber.**
  - **Often combined with MALDI (MALDI-TOF) Detections from multiple laser bursts are averaged. Multiple laser**

- **Tandem MS- MS/MS**

  -separation and identification of compounds in complex mixtures

  - induce fragmentation and mass analyze the fragment ions.

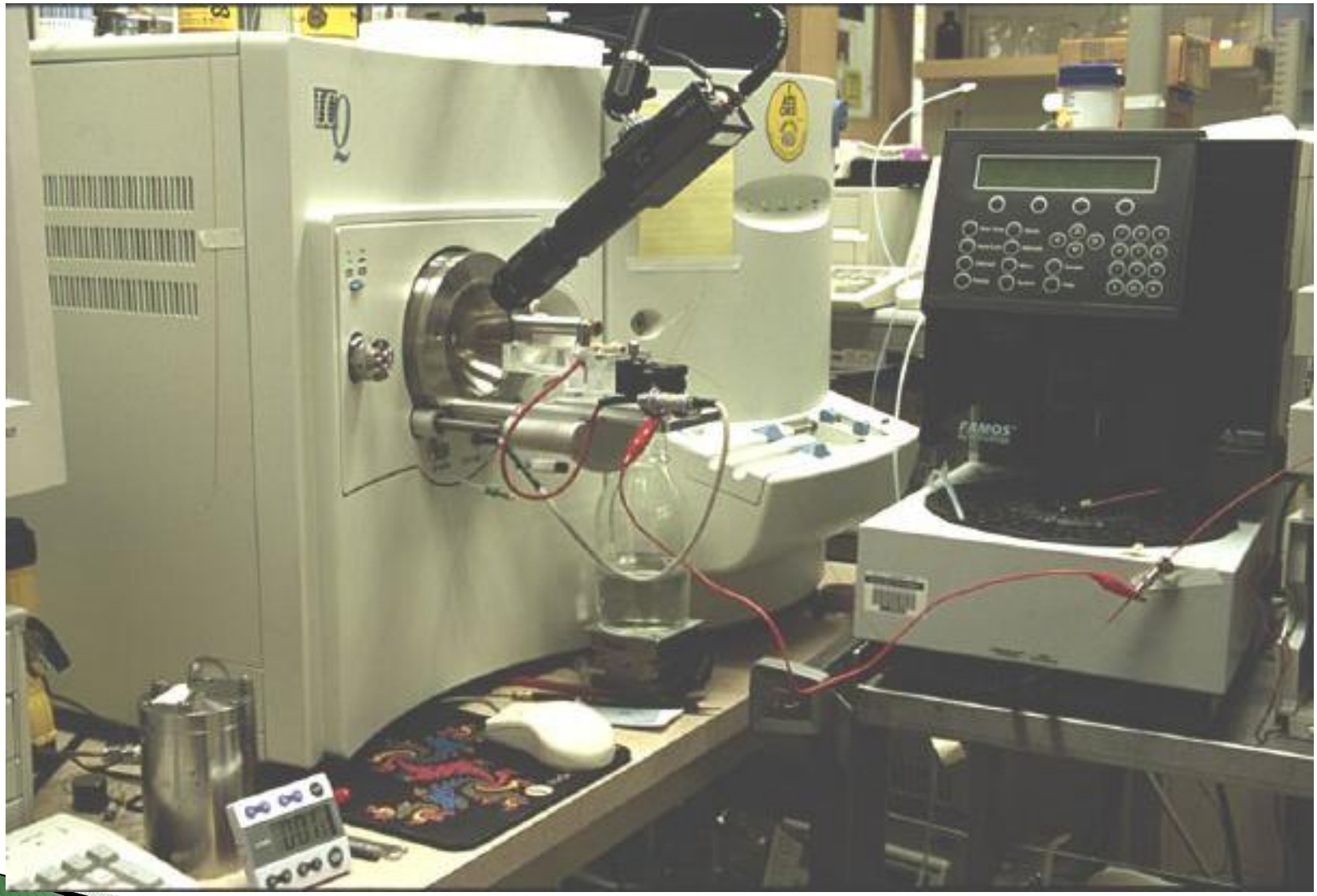
  - Uses two or more mass analyzers/filters separated by a collision cell filled with Argon or Xenon

- **Different MS-MS configurations**
  - **Quadrupole-quadrupole (low energy)**
  - **Magnetic sector-quadrupole (high)**
  - **Quadrupole-time-of-flight (low energy),**

    Time-of-flight-time-of-flight (low energy)

Typical Mass Spectrometer

LC/LC-MS/MS-Tandem LC, Tandem MS



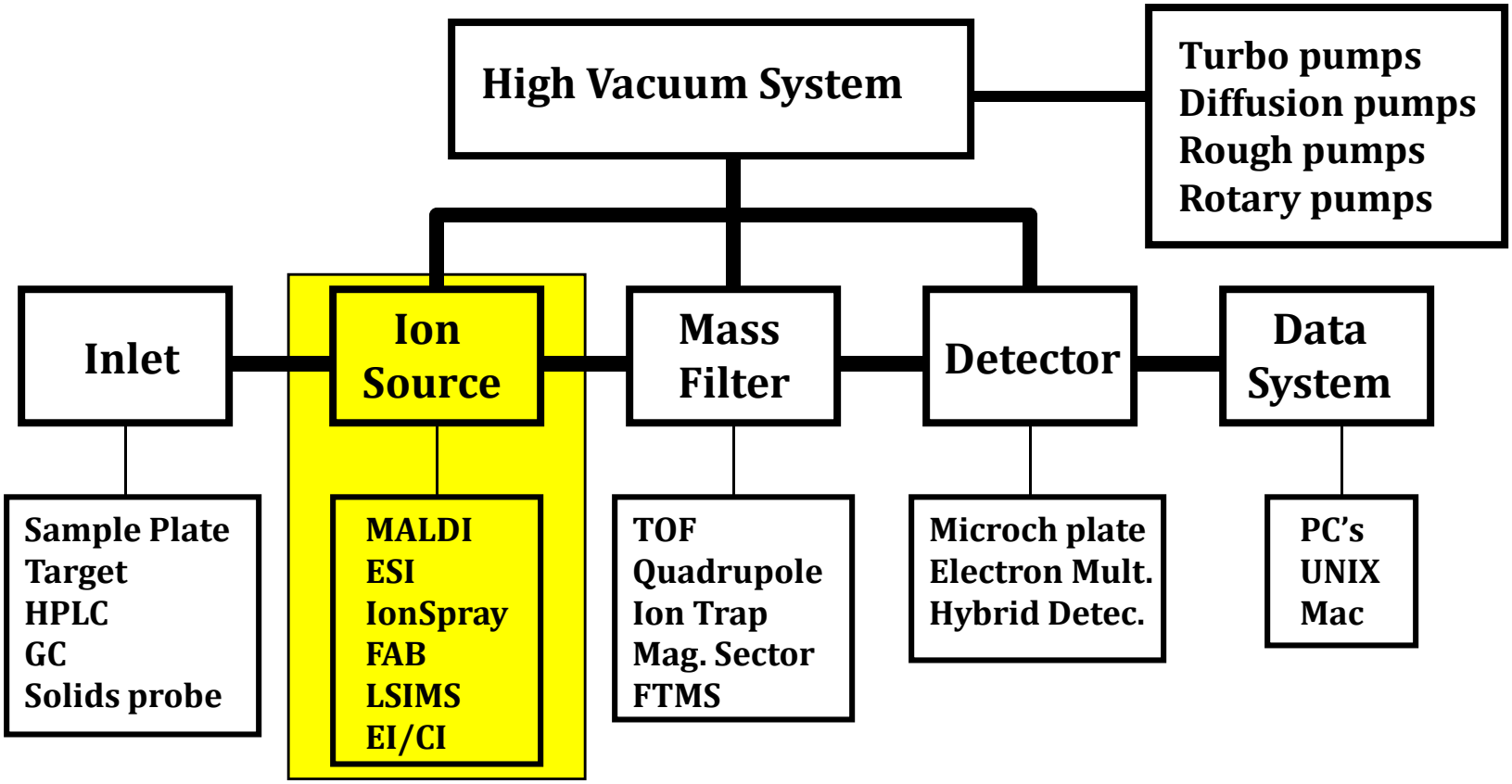
Typical Mass Spectrum

- Characterized by sharp, narrow peaks
- X-axis position indicates the m/z ratio of a given ion (for singly charged ions this corresponds to the mass of the ion)
- Height of peak indicates the relative abundance of a given ion (not reliable for quantitation)
- Peak intensity indicates the ion's ability to desorb or "fly" (some fly better than others)

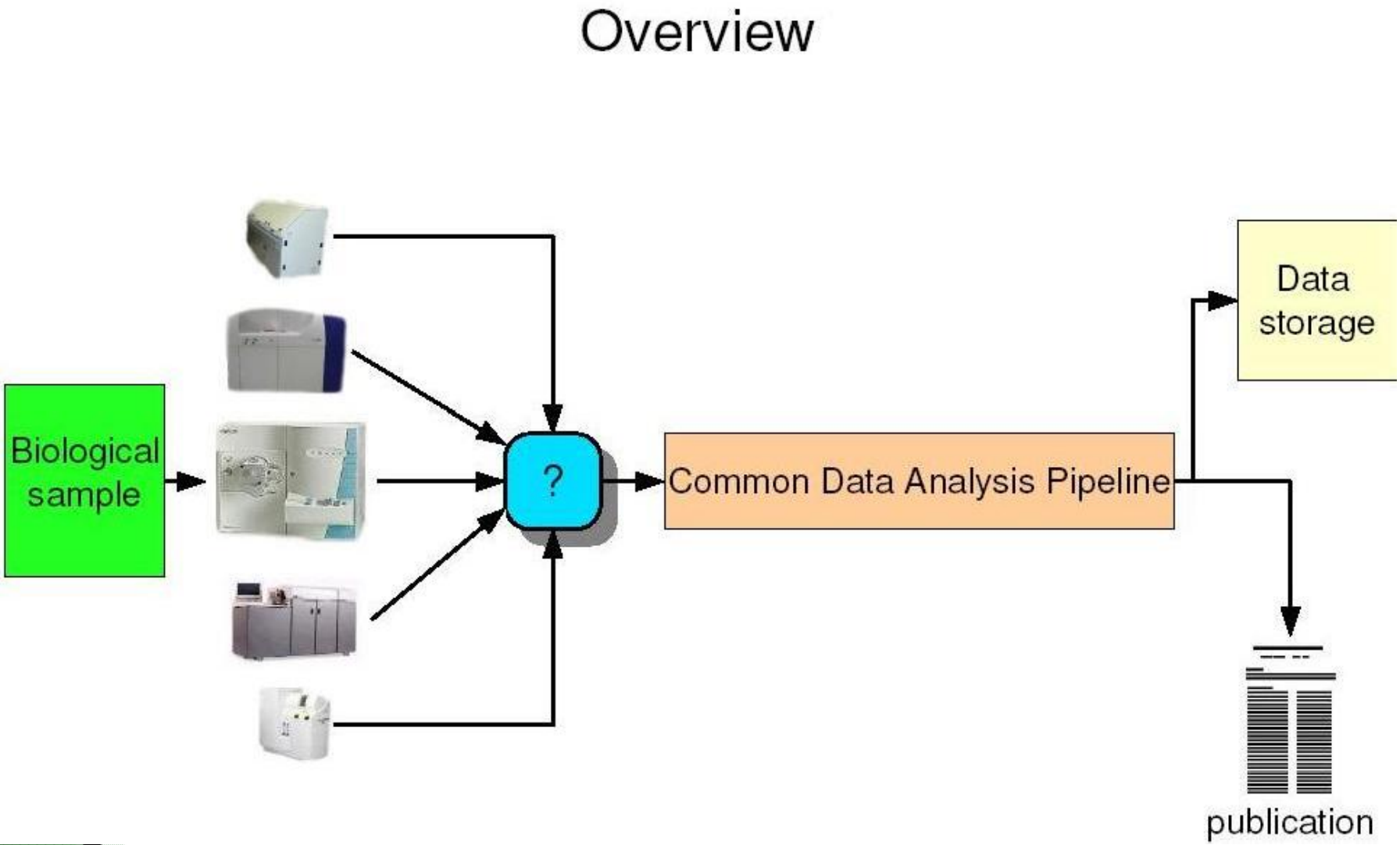
How does a Mass Spectrometer work?

- 3 fundamental parts: the ionisation source, the analyser, the detector
- Samples easier to manipulate if ionised
- Separation in analyser according to mass-to-charge ratios (m/z)
- Detection of separated ions and their relative abundance
- Signals sent to data system and formatted in a m/z spectrum

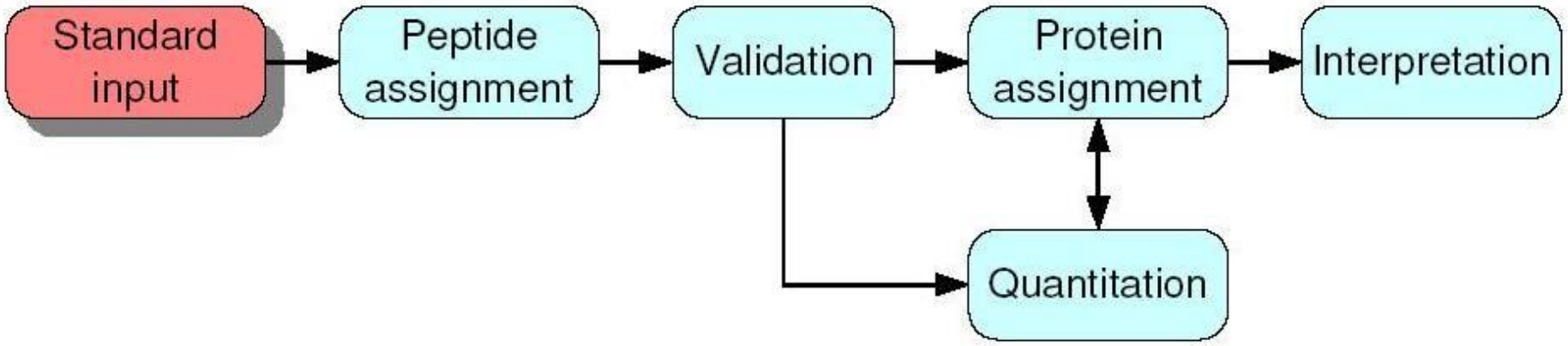
Mass Spectrometer Schematic



Data analysis



Common Data Analysis - Pipeline



Application

- Isotope ratio MS: isotope dating and tracking
- Trace gas analysis
- Atom probe
- Pharmacokinetics
- Protein characterization
- Glycan analysis
- Space exploration
- Respired gas monitor
- SNP Detection

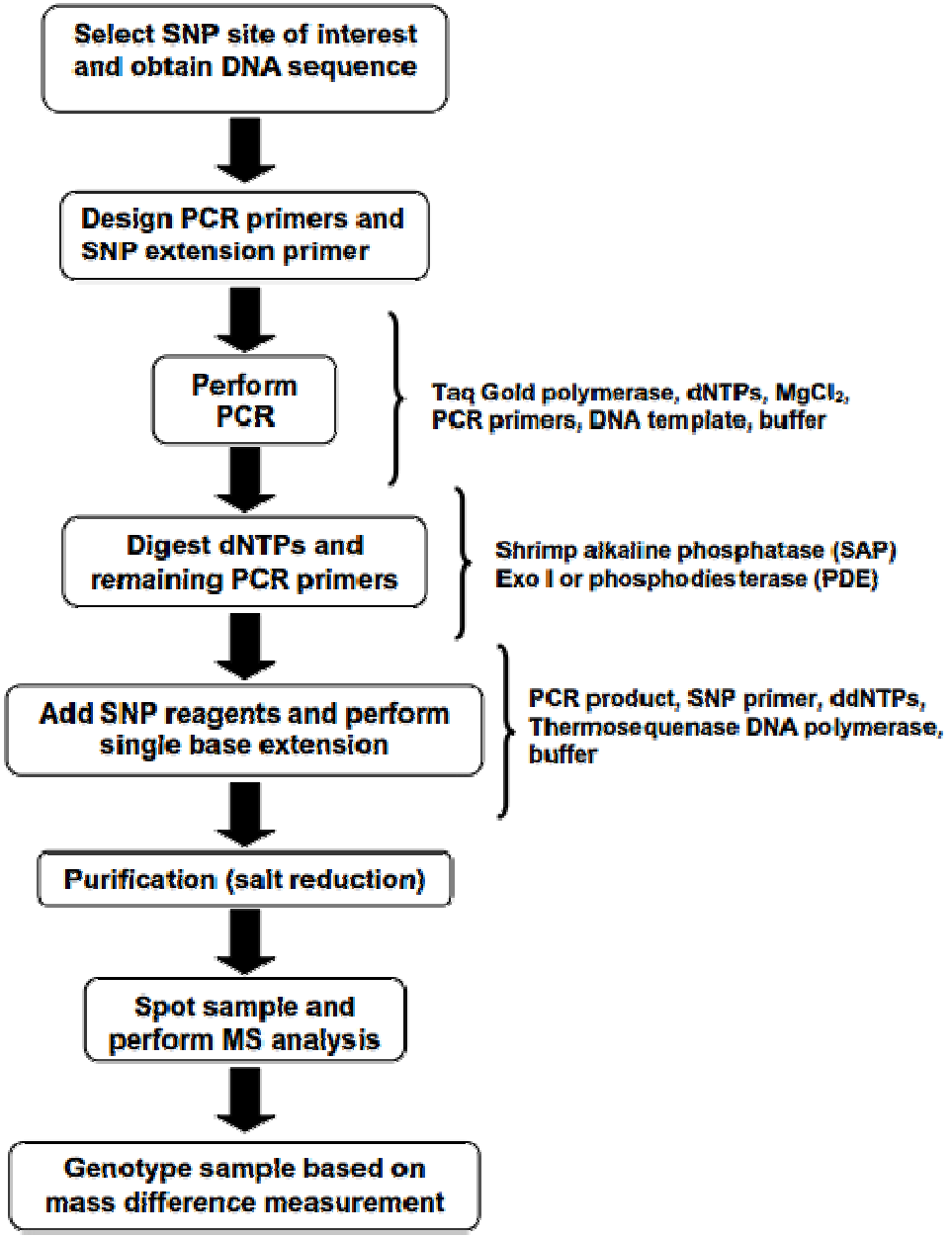
Primer Extension Assay

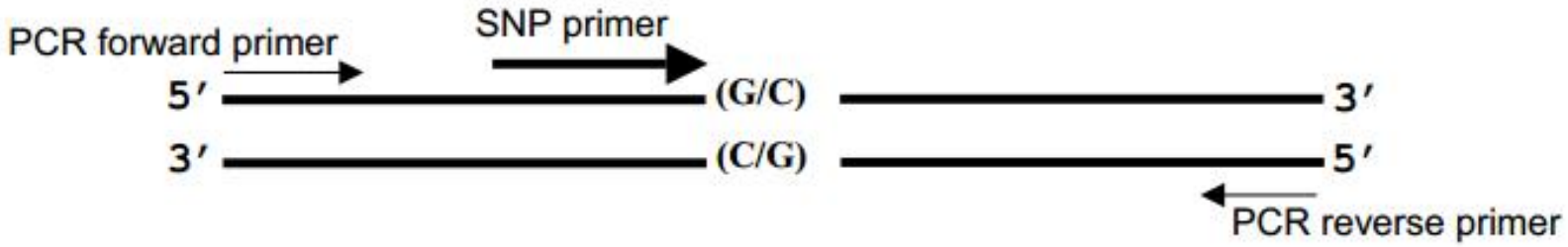
- The primer extension assay is often referred to as the single base extension, PinPoint or minisequencing assay . Variations upon this type of assay are popular for SNP detection, especially in conjunction with analysis by MALDI-TOF MS

Generic primer extension assay

The primary molecular biology techniques can be broken down into

(1) PCR amplification
(2) Phosphatase ddNTP digestion
(3) SNP primer extension.



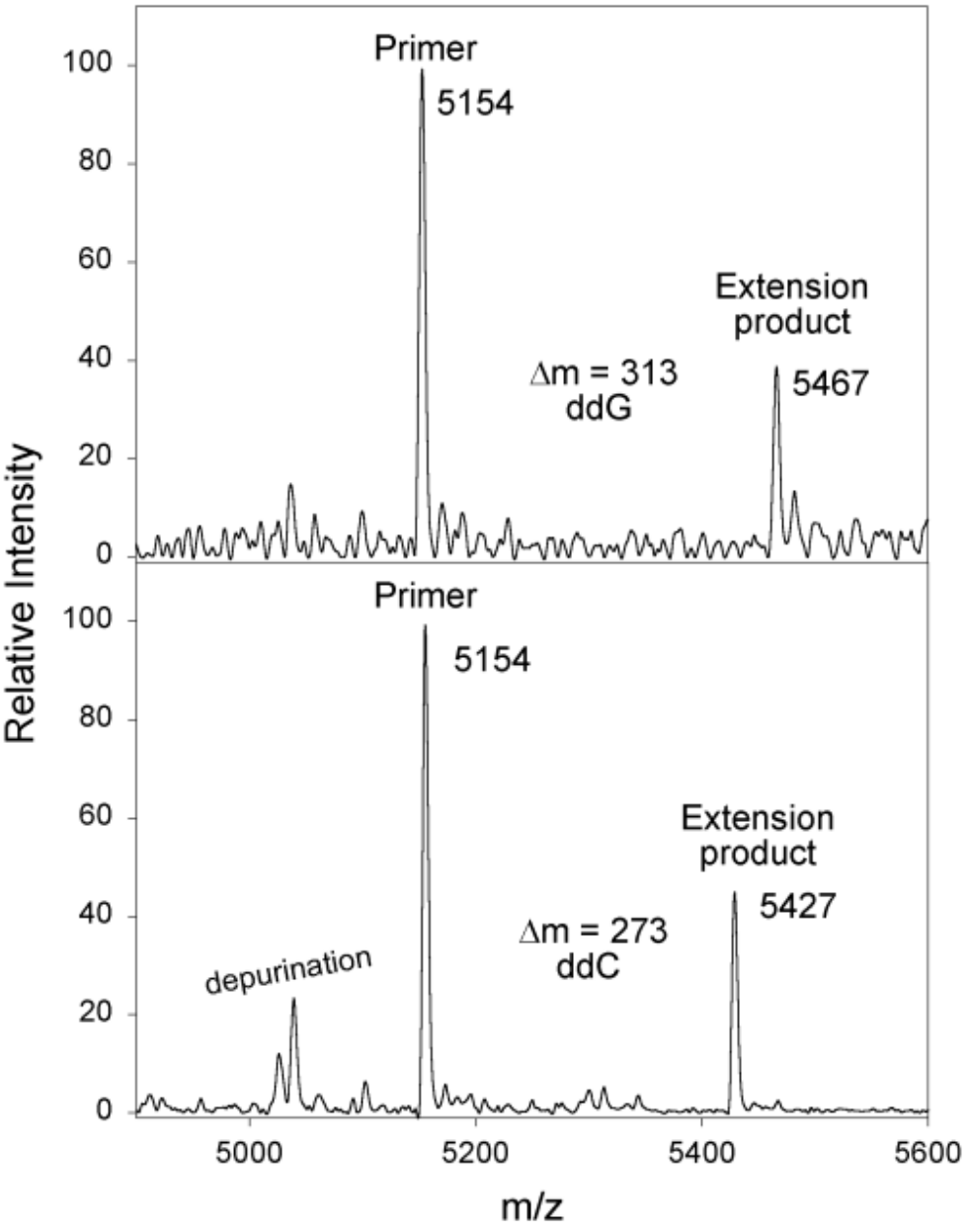


Possible extension products

5′-ACAGGTCTCTCATAATAddC-3′	5154 + 273
5′-ACAGGTCTCTCATAATAddG-3′	5154 + 313

Schematic of the M96 SNP marker and SNP primer. Shown is a section of the PCR amplicon for the region of DNA containing the M96 SNP (S). A primer for the primer extension assay is shown which is designed to hybridize to the "bottom" strand of the amplicon. The possible products of the primer extension assay are shown below with their corresponding masses. SNP primer was designed to have an annealing temperature of 58.6 ^{o}C

Single base extension mass spectra from two different individuals exhibiting different genotypes at the M96 marker. The SNP detection primer used in this assay has the sequence 5'-ACAGGTCTCTCATAATA-3' with an expected mass of 5154 Da The individual in the top panel has a G at the SNP site based on a mass difference of 313 Da between the primer and extension product. The sample in the lower panel exhibited a genotype of C at the same site because the primer and extension product differ by 273 Da. These results were collected on a Bruker BIFLEX III time-offlight mass spectrometer using experimental conditions described in the text. Mass spectra were smoothed and enhanced by the application of matrix convolution filters contained in the XMASS 5.0 analysis software package (Bruker Daltonics, Germany).



Mass spectra illustrating the results from variations on the primer extension assay. A hypothetical example of a C/T SNP marker from a heterozygous individual is used to demonstrate the effect on mass spectral data obtain from different assays. Modifications upon the basic primer extension assay enable improved resolution of heterozygotes, increased multiplexing potential, and greater sensitivity