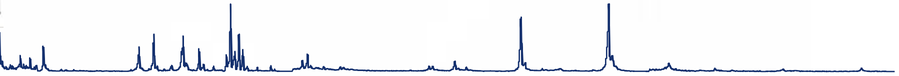
VITEK MS™

MALDI-TOF for bacterial identification



BIOMÉRIEUX
UNIVERSITY

Identifying microorganisms: new solutions for the future

The most common pratice in clinical routine:

?

1900

1970

2000

Identificazione rapida batteri aerobi, anaerobi, micobatteri e miceti

MALDI-TOF

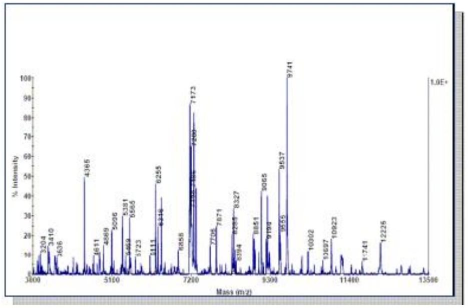
(Matrix Assisted Laser Desorption Ionization – Time of Flight)

VITEK MS™

TECNOLOGIA MALDI-TOF

(matrix assisted laser desorption/ionisation - time of flight)

Lanciato a Maggio 2011 in Europa, il VITEK® MS consiste in uno strumento per la **spettrometria di massa** e una soluzione informatica progettati per l'identificazione rapida di microrganismi basata sulla tecnologia MALDI-TOF.
L'identificazione microbica viene raggiunta tramite l'ottenimento di spettri, analizzati con l'ausilio del database presente nel VITEK®MS.



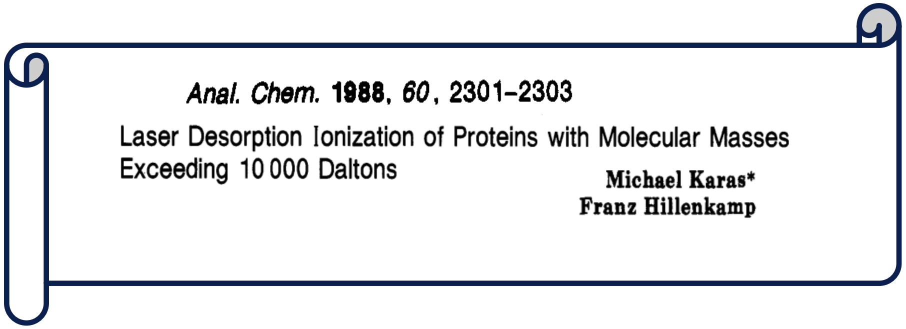
I picchi di questi spettri vengono confrontati con il profilo tipico di una specie, **genere o famiglia di microrganismo**, per dare un'identificazione microbica. 1946 specie identificabili

The Inventors of MALDI

Matrix Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry

Developed in 1980‘s by :

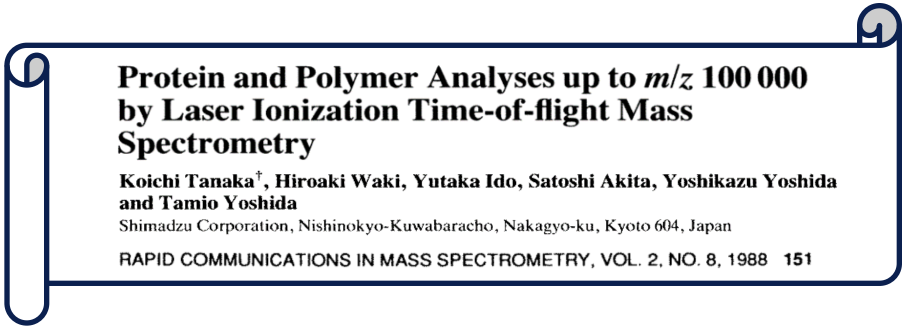
Karas & Hillenkamp in Germany and Tanaka et al in Japan.



Anal. Chem. **1988**, *60*, 2301–2303

Laser Desorption Ionization of Proteins with Molecular Masses Exceeding 10 000 Daltons

Michael Karas*
Franz Hillenkamp



Protein and Polymer Analyses up to *m/z* 100 000 by Laser Ionization Time-of-flight Mass Spectrometry

Koichi Tanaka†, Hiroaki Waki, Yutaka Ido, Satoshi Akita, Yoshikazu Yoshida and Tamio Yoshida
Shimadzu Corporation, Nishinokyo-Kuwabaracho, Nakagyo-ku, Kyoto 604, Japan

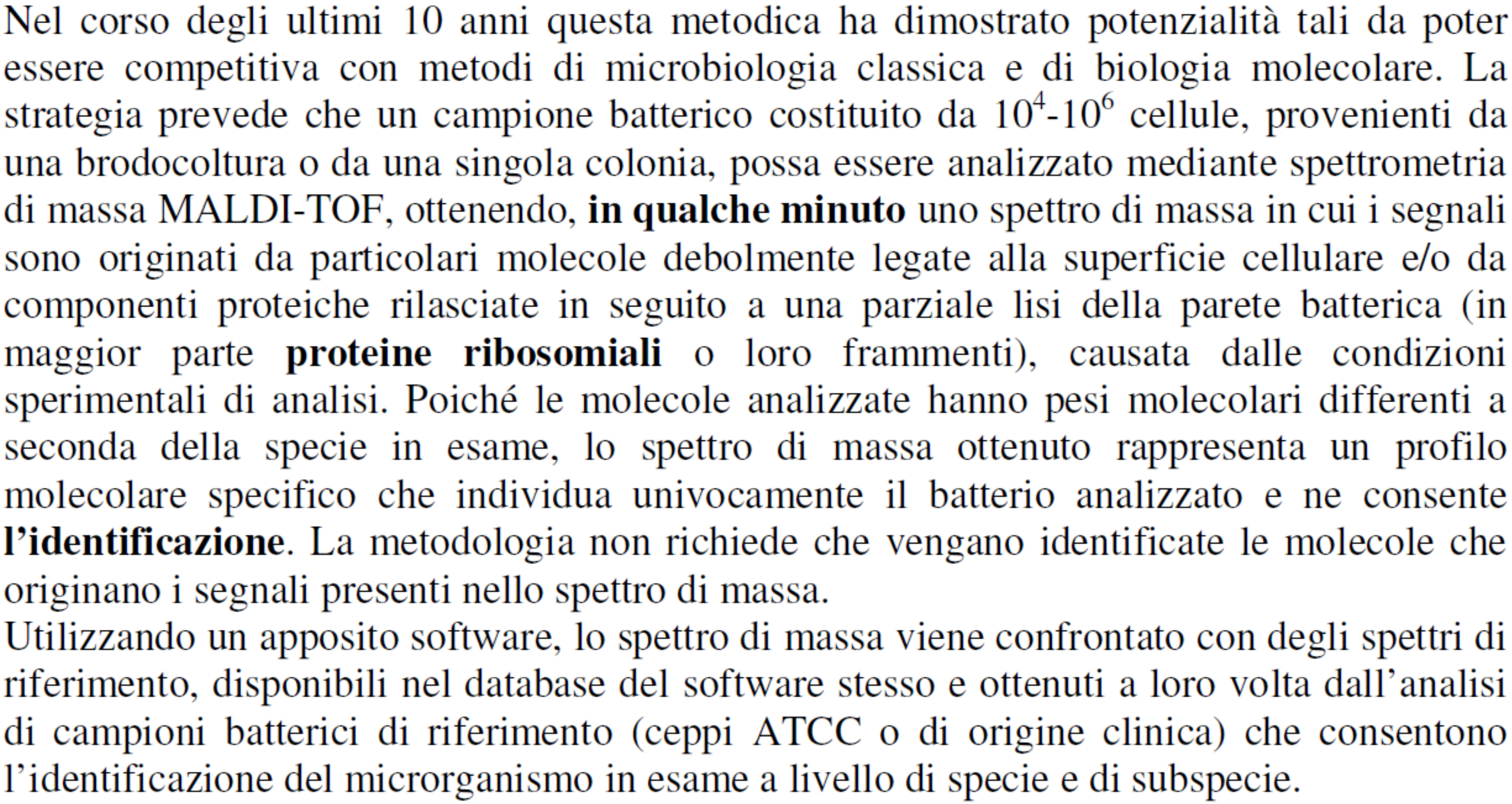
RAPID COMMUNICATIONS IN MASS SPECTROMETRY, VOL. 2, NO. 8, 1988 **151**

First commercial apparatus in 1991

Nobel Prize for Chemistry to K. Tanaka in 2002

MALDI-TOF

(Matrix Assisted Laser Desorption Ionization – Time of Flight)



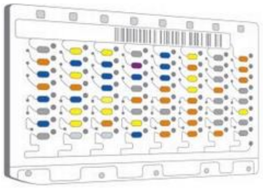
Nel corso degli ultimi 10 anni questa metodica ha dimostrato potenzialità tali da poter essere competitiva con metodi di microbiologia classica e di biologia molecolare. La strategia prevede che un campione batterico costituito da 10^4-10^6 cellule, provenienti da una brodocoltura o da una singola colonia, possa essere analizzato mediante spettrometria di massa MALDI-TOF, ottenendo, **in qualche minuto** uno spettro di massa in cui i segnali sono originati da particolari molecole debolmente legate alla superficie cellulare e/o da componenti proteiche rilasciate in seguito a una parziale lisi della parete batterica (in maggior parte **proteine ribosomiali** o loro frammenti), causata dalle condizioni sperimentali di analisi. Poiché le molecole analizzate hanno pesi molecolari differenti a seconda della specie in esame, lo spettro di massa ottenuto rappresenta un profilo molecolare specifico che individua univocamente il batterio analizzato e ne consente **l'identificazione**. La metodologia non richiede che vengano identificate le molecole che originano i segnali presenti nello spettro di massa.

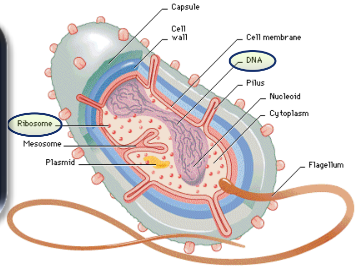
Utilizzando un apposito software, lo spettro di massa viene confrontato con degli spettri di riferimento, disponibili nel database del software stesso e ottenuti a loro volta dall'analisi di campioni batterici di riferimento (ceppi ATCC o di origine clinica) che consentono l'identificazione del microrganismo in esame a livello di specie e di subspecie.

Current Identification Methods

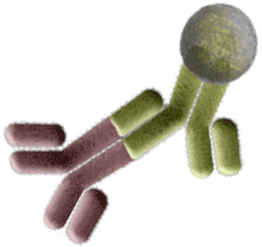
sensory

biochemical

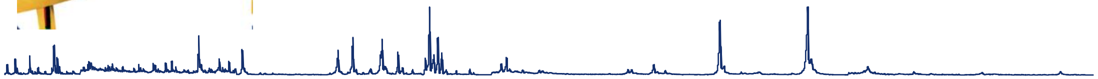




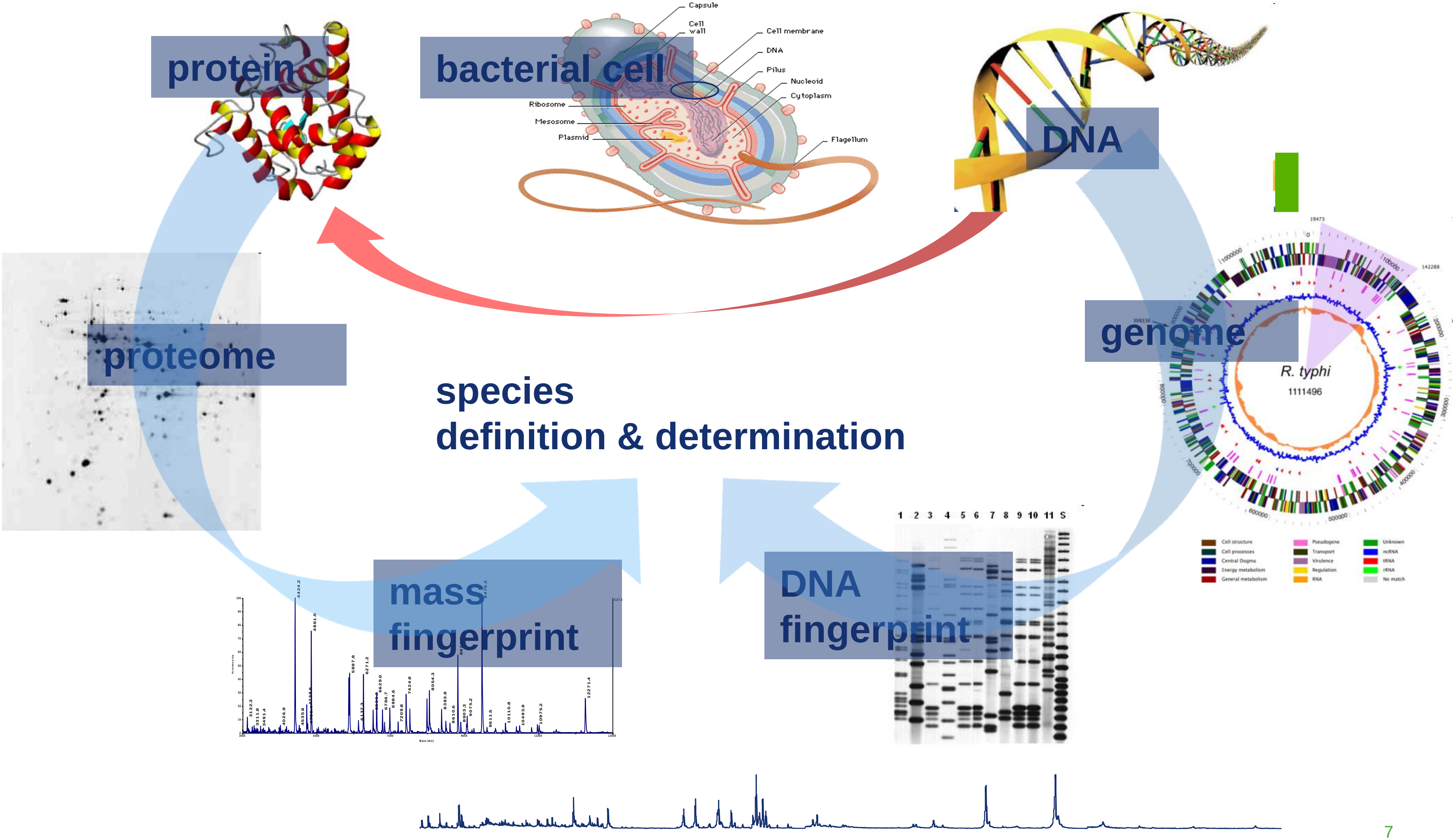
immunological



molecular



Modern microbial taxonomy: proteomics & genomics



protein

bacterial cell

DNA

genome

proteome

species
definition & determination

mass fingerprint

DNA fingerprint

The MALDI Concept

The concept:

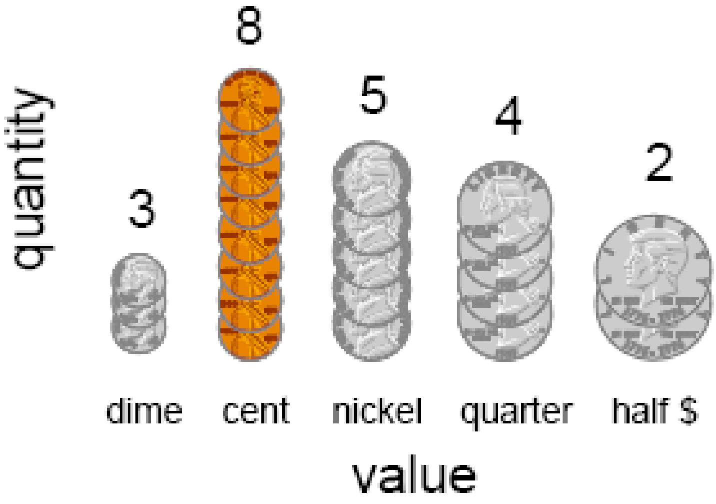
- mixing of an analyte and a matrix

The result:

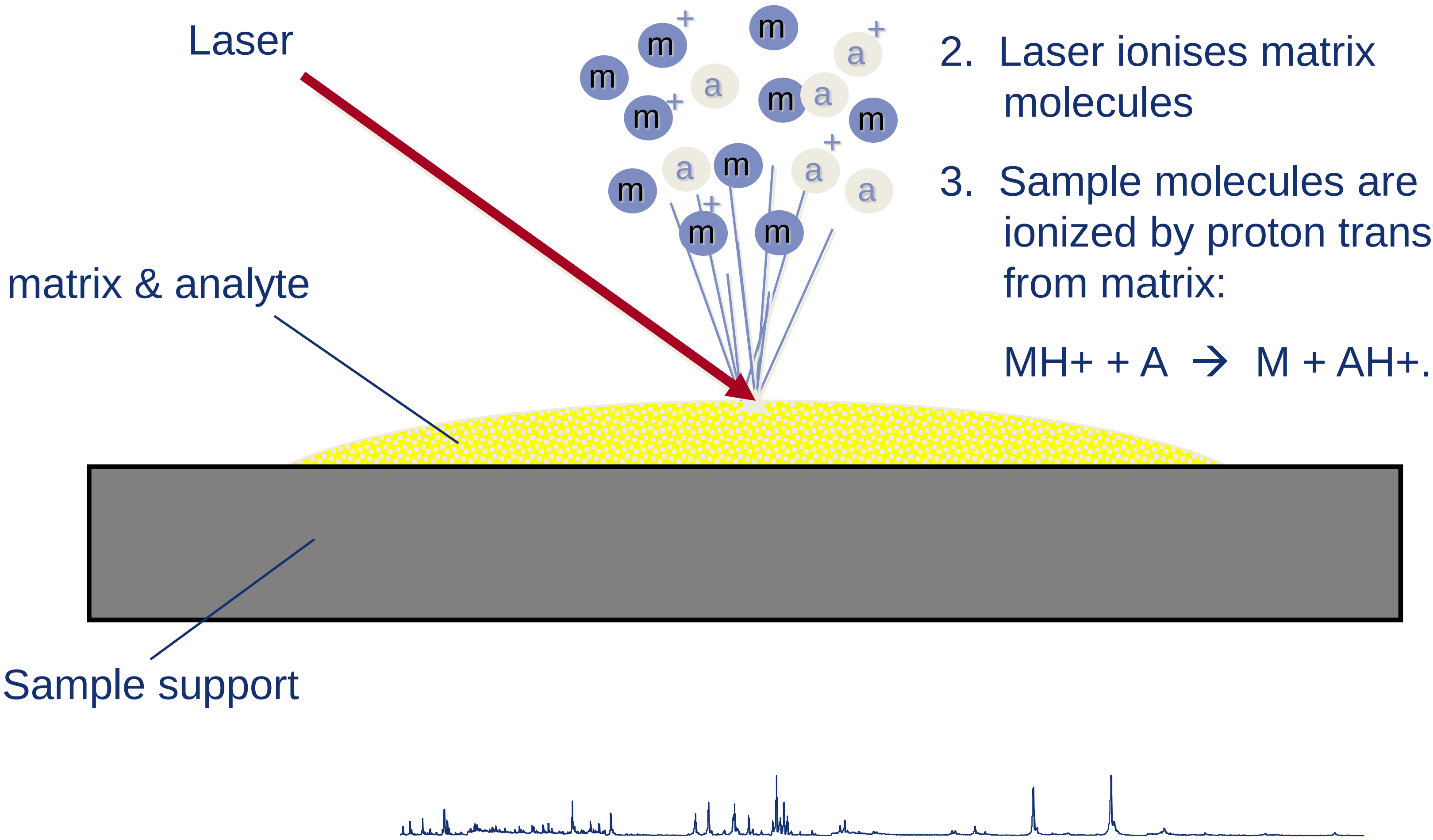
- Generation of gas phase ions

Concept : Mass analysis

- Sorting and Counting
  - *Pocket change (mixture of coins)*
  - *Penny, dime, nickel, quarter, half $*
  - *Sorting change by value or size*
  - *Concept of visual interpretation*
  - *Mixture of molecules*
  - *Molecules of different weight, size*
  - *Separation by mass*
  - *spectrum*



MALDI Mechanism



Laser

matrix & analyte

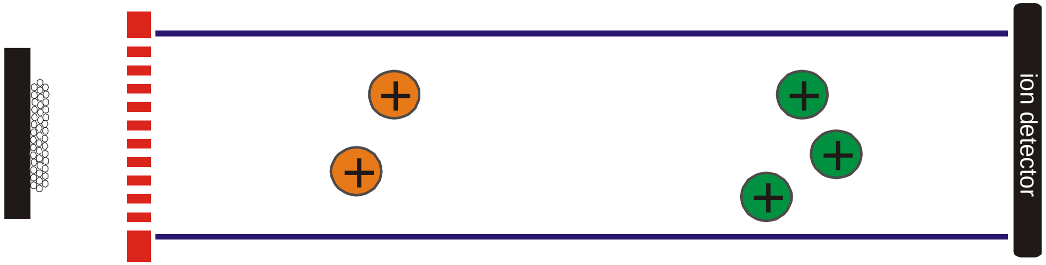
Sample support

1. Sample (A) is mixed with excess matrix (M) and dried on a MALDI target
2. Laser ionises matrix molecules
3. Sample molecules are ionized by proton transfer from matrix:

   MH+ + A → M + AH+.

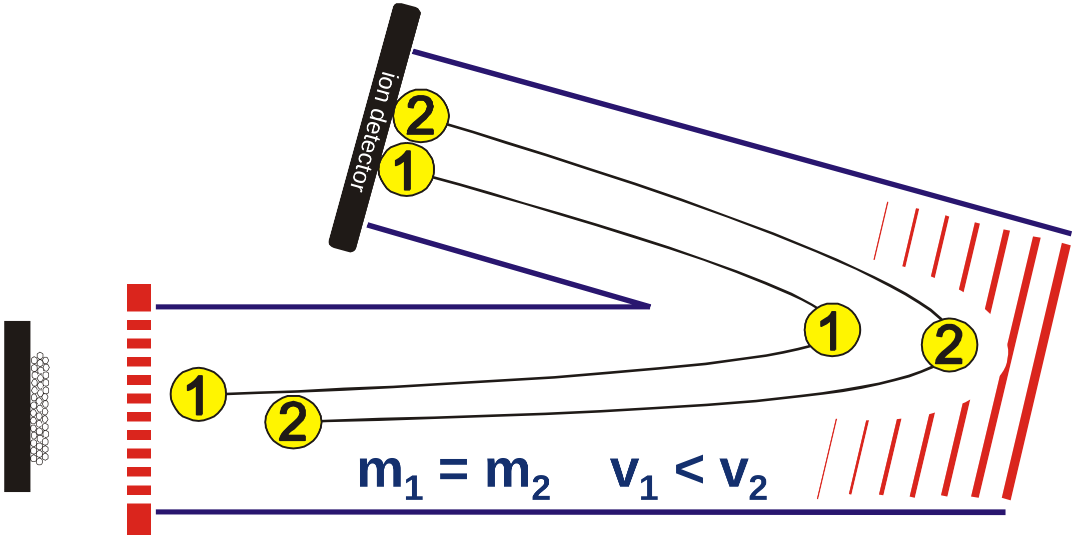
MALDI-TOF MS:
Linear and reflector modes

Linear time-of-flight mass spectrometer



- mass range up to 350 kDa
- high sensitivity
- low resolution

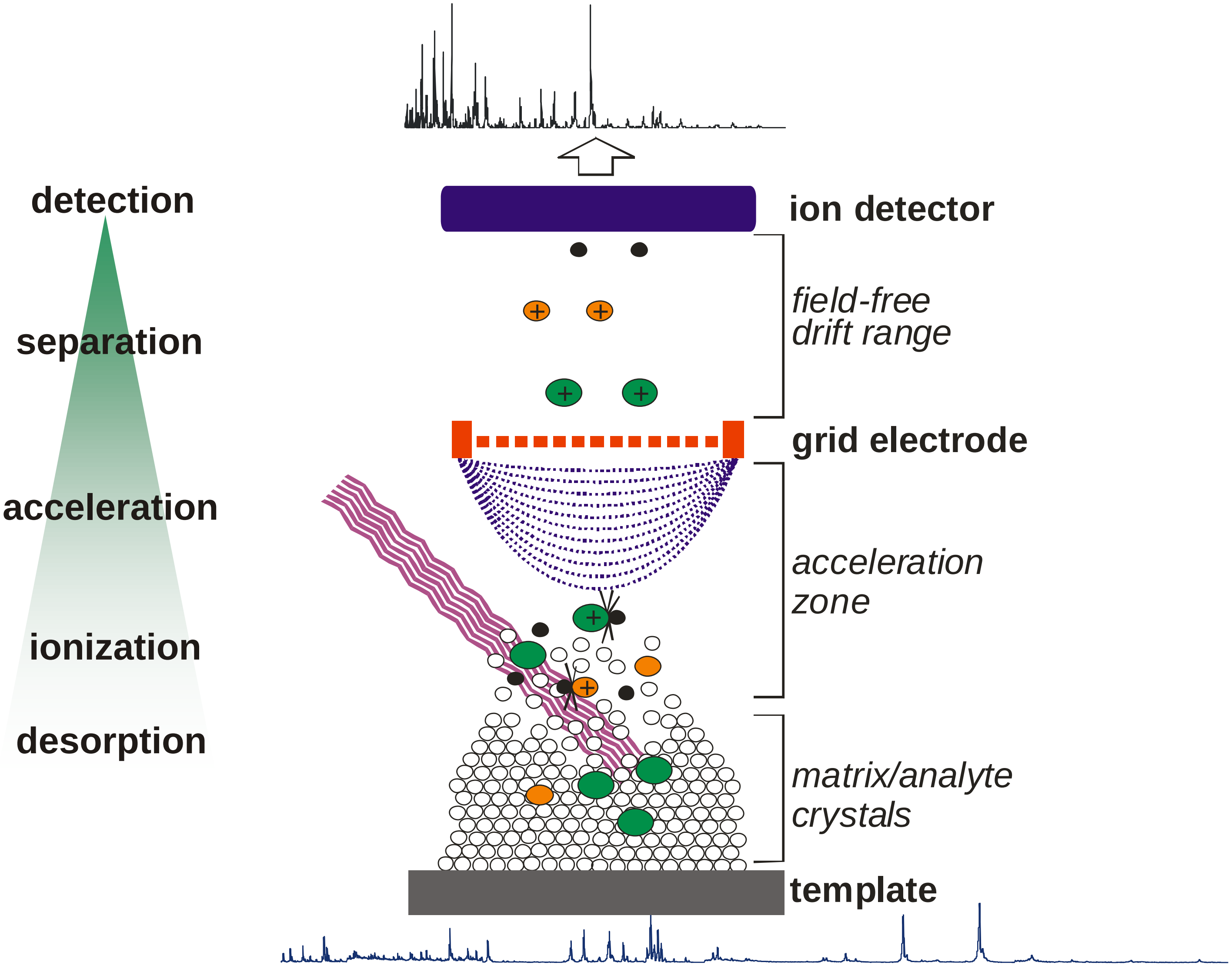
Reflector time-of-flight mass spectrometer



$m_1 = m_2 \quad v_1 < v_2$

- mass range up to 5000 Da
- low sensitivity
- high resolution

MALDI-TOF MS: basic principles
At a glance



detection

separation

acceleration

ionization

desorption

ion detector

field-free drift range

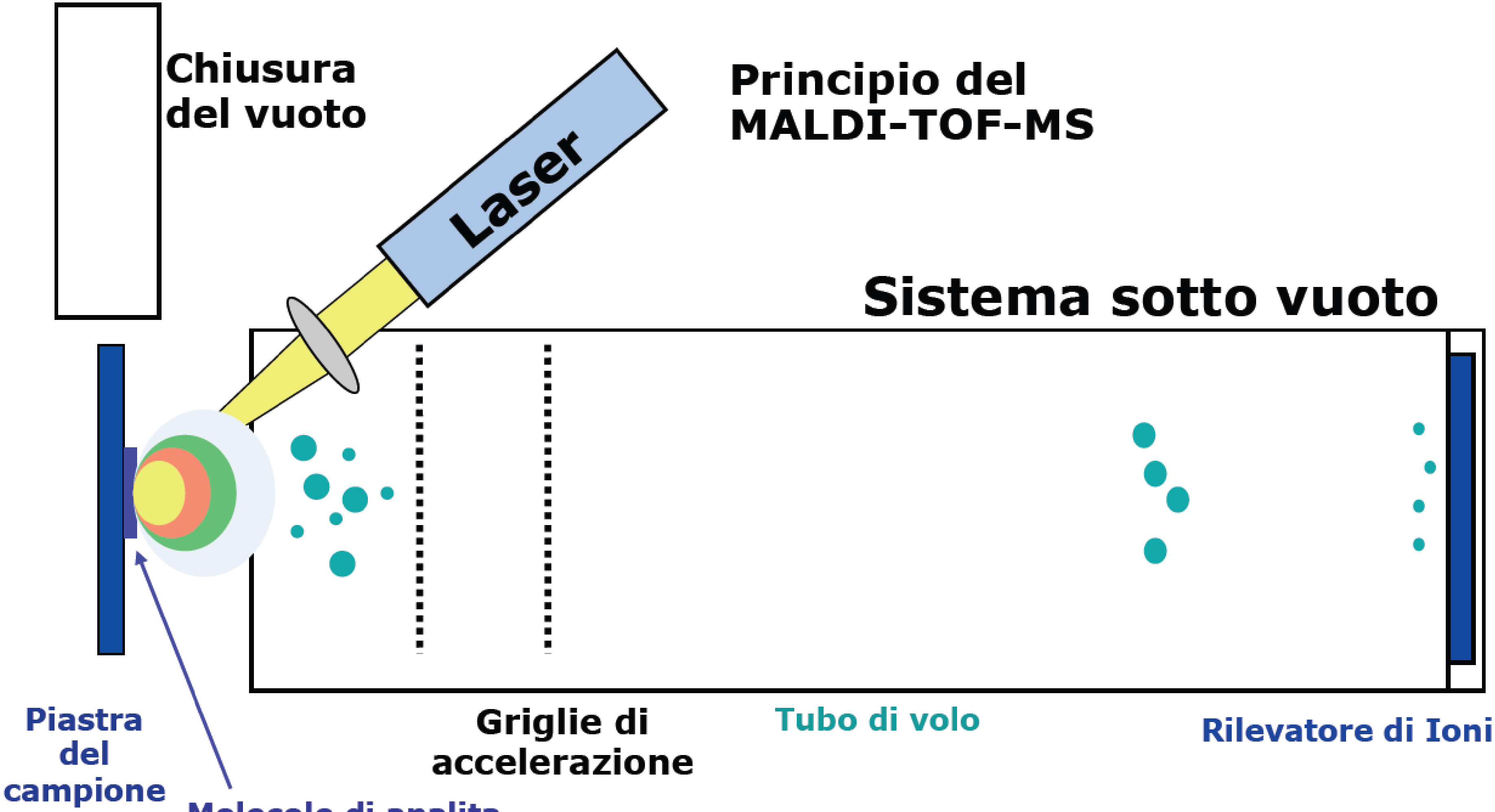
grid electrode

acceleration zone

matrix/analyte crystals

template

Principio del MALDI-TOF-MS



Chiusura del vuoto

Laser

Sistema sotto vuoto

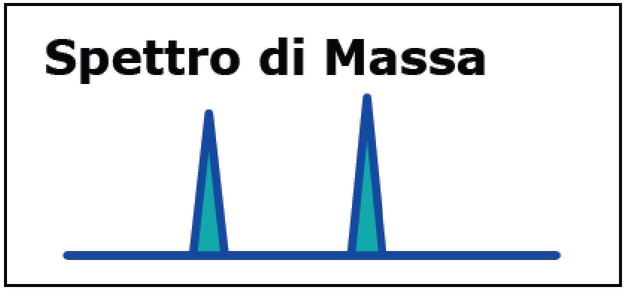
Piastra del campione

Griglie di accelerazione

Tubo di volo

Rilevatore di Ioni

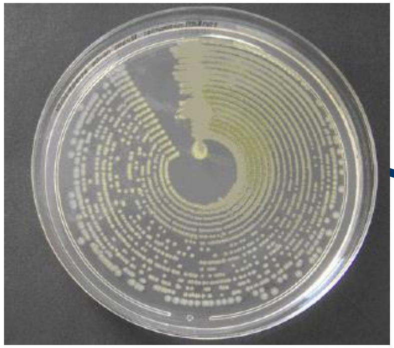
Molecole di analita immerse nella matrice



Spettro di Massa

Lo spettro di massa riflette essenzialmente le molecole di analita

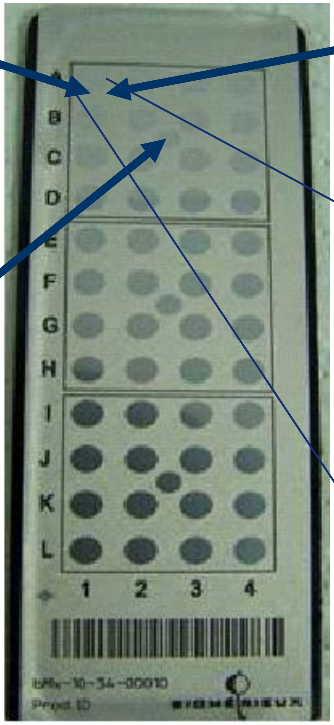
The Workflow :
Step 1 – Sample preparation



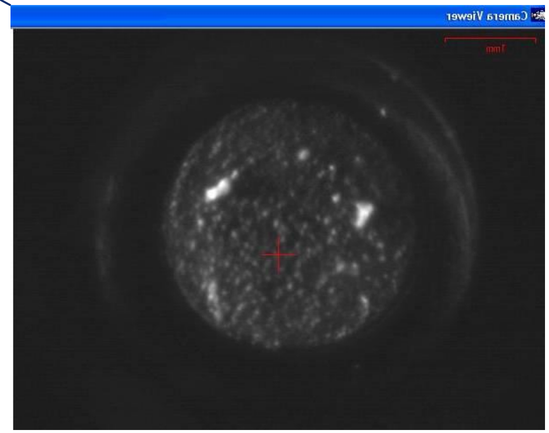
selection and transfer of cell from a single colony

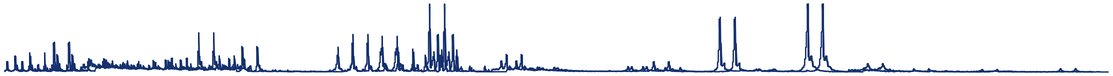
selection and transfer of cell from a single colony of calibrant strain

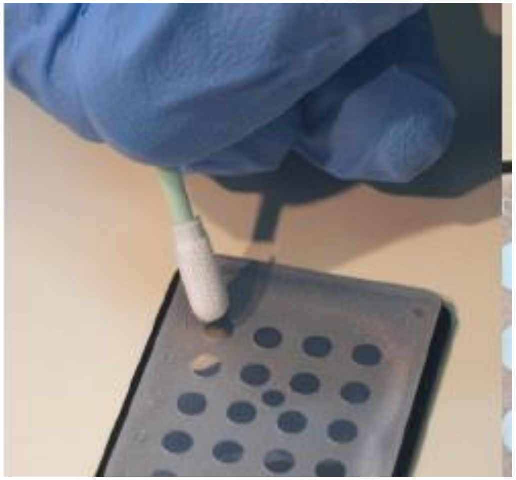
VITEK MS-DS target slide with 48 positions

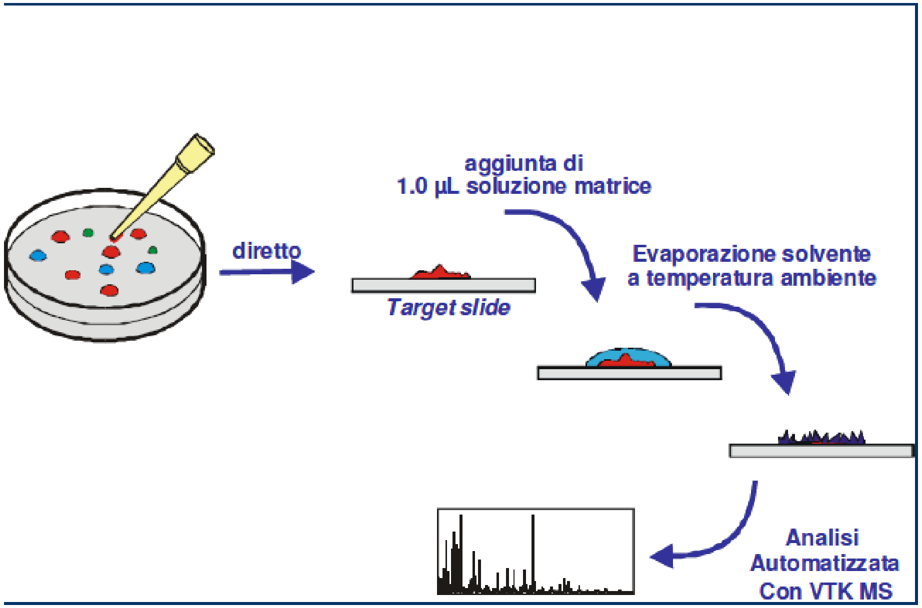


addition of 1µL Matrix solution









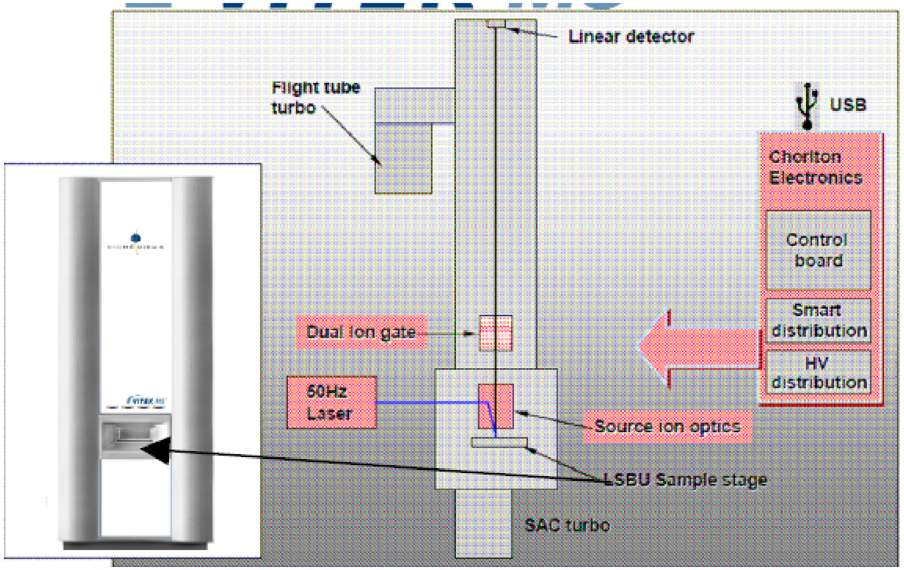
diretto

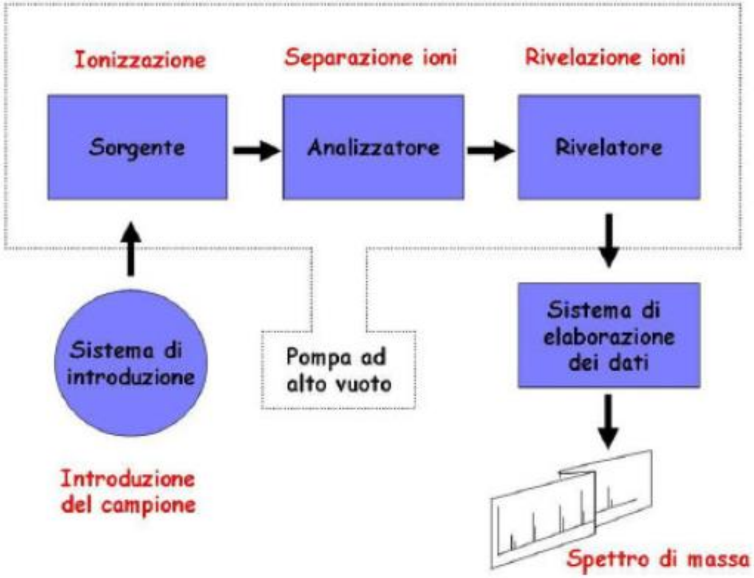
Target slide

aggiunta di
1.0 µL soluzione matrice

Evaporazione solvente
a temperatura ambiente

Analisi
Automatizzata
Con VTK MS





Ionizzazione

Separazione ioni

Rivelazione ioni

Sorgente → Analizzatore → Rivelatore

Sistema di introduzione

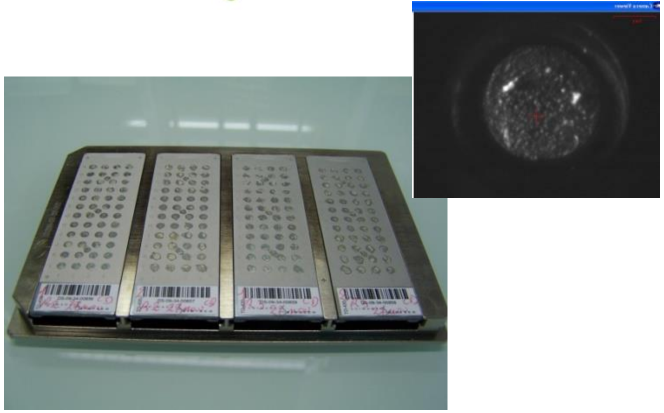
Introduzione del campione

Pompa ad alto vuoto

Sistema di elaborazione dei dati

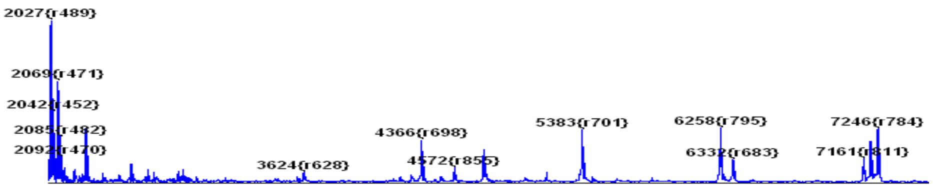
Spettro di massa

The Workflow : Step 2 – Measurement

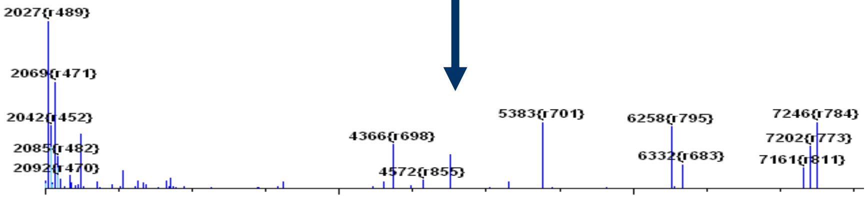


automated spectrum acquisition ~1 min depending on the sample quality

ionisation of intact proteins and molecular weight measurement

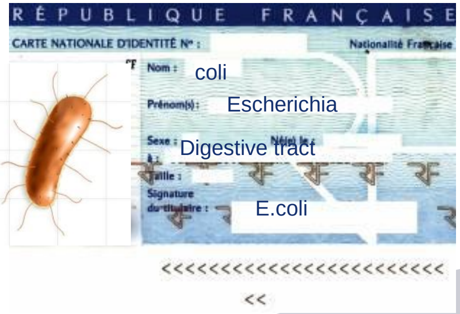


Spectrum processing and Peak list creation



Peak list transfer

The Workflow :
Step 3 – Analysis - Identification



RÉPUBLIQUE FRANÇAISE

CARTE NATIONALE D'IDENTITÉ N° :

Nationalité Française

Nom : coli

Prénom(s) : Escherichia

Sexe : Né(e) le : Digestive tract

à :

Taille :

Signature du titulaire : E.coli

<<<<<<<<<<<<<<<<<<<<<<<<<

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- Comparison with the database
- Computation with an algorithm
- Identification of the microorganism