ICM-MS phenotyping in applied animal sciences: Two examples

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ICM-MS phenotyping in applied animal sciences:

Two examples

Journée Utilisateurs Life Science - Maison de l'Amérique Latine - Paris

BRUKER

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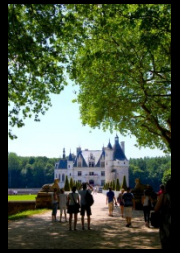
-Basic and applied animal sciences
-Reproduction and Behaviour
-Infectiology and Public Health
-Avian Research Unit




Intact Cells MALDI-TOF MS

- Identification of cell-specific peptide/protein MS fingerprints
- “Intact cell” means that whole cells are subjected directly to MS analysis without any preparatory steps
- Widely used in microbiology for biotyping
- Starting to be used in pathology and diagnostics

1

Phenotyping chicken male fertility

Why phenotyping male fertility?

Sperm quality tests evaluate individual sperm cells functions:

motility, integrity, acrosome reaction, etc.

Often poorly correlated with *real* fertility

A new paradigm of sperm quality testing is needed: global evaluation of sperm functions

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Background: Pilot study by Labas et al., 2014:

-Intact cell MALDI-TOF mass spectrometry (ICM-MS) = fast, reliable and relatively inexpensive tool to phenotype male fertility

olecular basis of fertility (top-down HRMS)

OBJECTIVES:

1- Acquisition of sperm cells ICM-MS profiles in a standardized and automated way.

2- Evaluation of the diagnostic performance of ICM-MS profiles comparison to predict fertility.

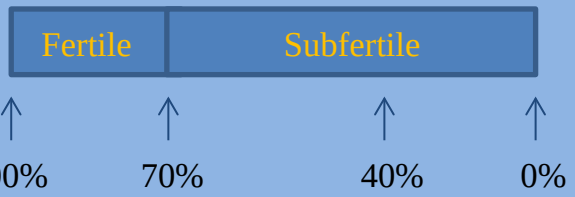
3- Identification of the endogenous peptides/proteins represented in the sperm cells ICM-MS spectra, particularly those linked with fertility.

Animal model and reference fertility rates

Our model is…..the chicken!!!

72 roosters of 2 divergent pure genetic lines: meat line (n=36) and a egg laying line (n=36).

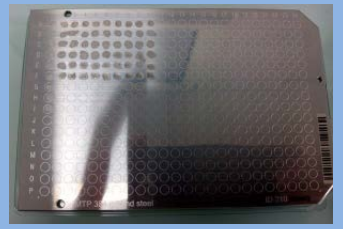
Fertility rates (% fertile/incubated eggs) : artificial insemination 10 females/male.



Fertility rates
cut-off values

Intact Cells MALDI-TOF MS

10^6 of fresh, washed (Tris-Sucrose) sperm cells + Matrix (20mg/mL Sinapinic acid, 2% TFA, 50% ACN)



12 spots / sample, 3 consecutive runs ---- 36 technical replicates

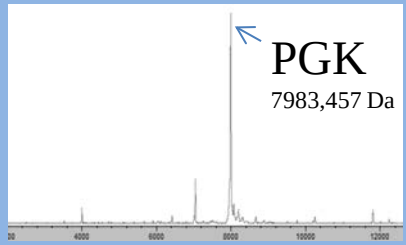
3 ejaculates per male (high day-to-day variability)

Spectra automatic acquisition in a Bruker UltrafleXtreme MALDI-TOF instrument (mass range 1-20 kDa).

flexAnalysis

External calibration : Peptide/protein mix calibrant

Internal calibration: Lock-mass using peak 7983,457 Da



Pre-processing: ClinProTools 3.0 (Bruker)

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Fertility-predictive models construction using ClinProTools 3.0

Model construction

N=8

Quick Classifier
Supervised Neural Network
Genetic Analyser

Validation

N=36

Classification as Fertile or Subfertile by >50% spectra from each male

Evaluation

Recognition capability: Ability of each model to correctly identify its component spectra

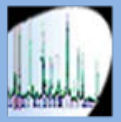
Cross-validation: Ability to handle variability among spectra

Diagnostic Accuracy

Sensitivity / Specificity

Positive and negative predictive values

Positive and negative likelihood ratios



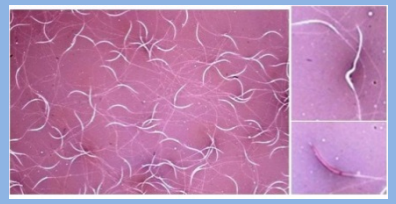
In-vitro sperm quality tests

- Viability
- Subjective mass motility evaluation
- Objective motility evaluation measured with computer-assisted sperm analysis (CASA) system with an HTM-IVOS

3 ejaculates per male

Diagnostic accuracy

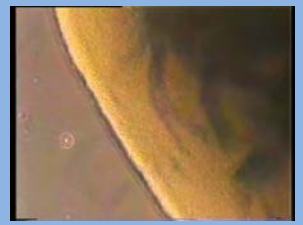
Sensitivity / Specificity
Positive and negative predictive values
Positive and negative likelihood ratios



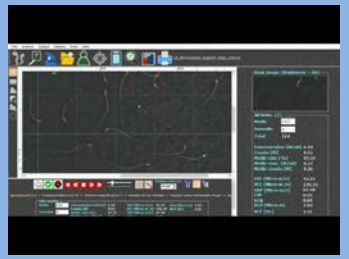
Viable

Not viable

Mass motility = 1/5



Mass motility = 5/5



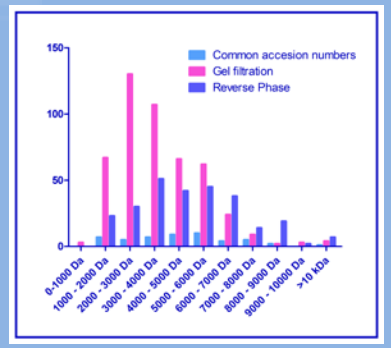
CASA System

Top-down HRMS protein identification

Sperm cells
protein extracts
Tris-Urea 6M
Sonication

Fractionation

Reverse Phase Chromatography
XBridge BEH C18, Waters



Gel Filtration Chromatography
Superdex 75 10/300 GL, GE Healthcare

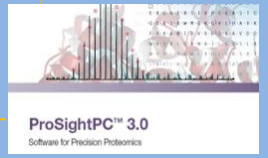
µLC-MS/MS:

Monolithic PS-DVB
PepSwift, Dionex

LTQ Orbitrap Velos,
Thermo Scientific

Target resolution 100000
Range 400-2000 m/z
Isolation: top 10 most intense ions with charge states ≥ 2
Fragmentation/ HCD 38% Collision energy
Ion selection thr: 500 counts; isol: m/z 3

Identification and structural characterization:
Search 1: Monoisotopic mass, 10ppm tolerance
Search 2: Average mass, 3 kDa tolerance
Search 3: Average mass, 25k Da tolerance
p-score ≤1 × 10−5



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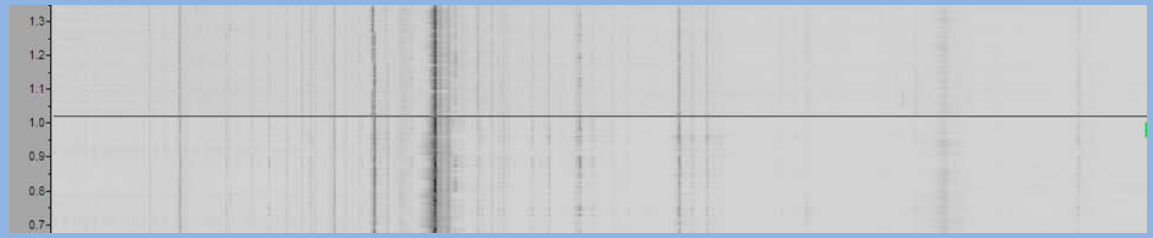
ICM-MS- based models vs. Traditional tests

85,5 % of shared m/z masses but differences in TIC values : no common model



Meat line (GA)

Recognition Capability	Cross Validation
99.4 %	**93.48 %**

Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
ICM-MS	84.6	55.0	55.0	84.6	1.9	0.3
Mass motility	69.2	55.0	50.0	73.3	1.5	0.6
Viability	76.9	35.0	43.5	70.0	1.2	0.7
% Motiles	61.5	40.0	40.0	61.5	1.0	1.0

Laying line (SNN)

Recognition Capability	Cross Validation
92.28 %	**80.3 %**

Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
ICM-MS	100.0	75.0	53.3	100.0	4.0	0.0
Mass motility	71.4	78.6	45.5	91.7	3.3	0.4
Viability	71.4	57.1	29.4	88.9	1.7	0.5
% Motiles	71.4	78.6	45.5	91.7	3.3	0.4

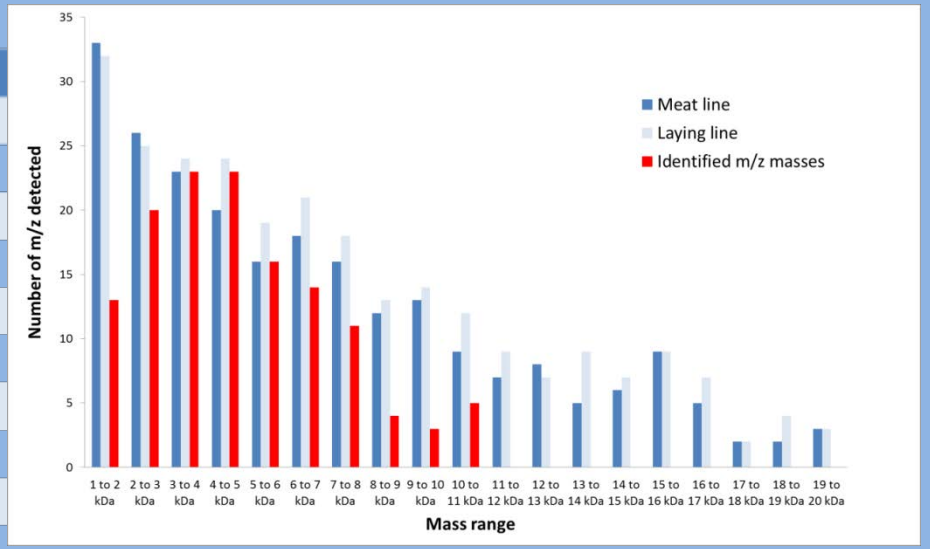
ICM-MS: Global analysis of sperm cells functions

GeneAnalytics
GENE SET ANALYSIS

GO - Biological Process
Respiratory Electron Transport Chain
Cellular Metabolic Process
Small Molecule Metabolic Process
Protein Polymerization
Microtubule Cytoskeleton Organization
Microtubule-based Process

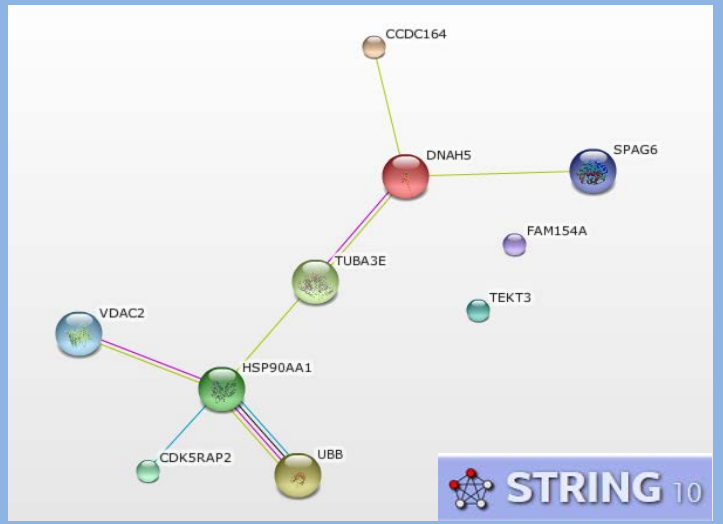
GO - Molecular Function
Structural Constituent of Cytoskeleton
ATPase Activity
Proton-transporting ATP Synthase Activity, Rotational Mechanism
Transmembrane Transporter Activity
Creatine Kinase Activity
MHC Class II Protein Complex Binding
Protein Binding Involved in Protein Folding
ATP Binding
Proton-transporting ATPase Activity, Rotational Mechanism

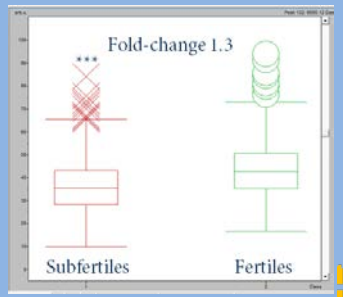
119/256 m/z masses identified

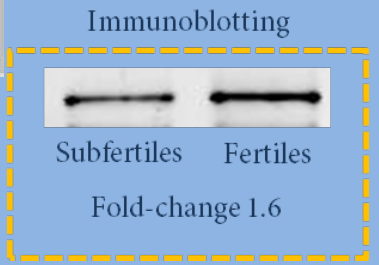


Masses employed to generate models

Mass (kDa)	*Gene Name*	*Ratio SubF/F*	*Protein name*	*Role associated with sperm cells functionality*
7035,24	LRD	1,8	Left-right dynein protein	Flagellar motility
6539,45	TUBA3E	1,7	Tubulin, Alpha 3e	Cell integrity and Flagellar motility
5226,48	CDK5RAP2	1,6	CDK5 regulatory subunit associated protein 2	Centrosome organization during fertilization
6695,03	HSP90AA	1,3	Heat shock protein 90 alpha	Chaperone, stabilisation of key proteins
2574,18	TEKT3	1,2	Tektin 3	Flagellar motility
8569,95	UBB	1,2	Ubiquitin	Protein degradation
4538,03	SPAG6	1,1	Sperm associated antigen 6	Cell integrity and Flagellar motility
4010,91	FAM154A	1,05	Family with sequence similarity 154, member A	Flagellar motility
6615,77	VDAC2	0,9	Voltage-dependent anion channel	Motility/capacitation/fertilization
4777,2	DRC1	0,9	Dynein Regulatory Complex Subunit 1 Homolog	Flagellar motility
2400,65	FER1L4	0,8	Fer-1-like protein 4 / Otoferlin	Membrane traffic/Sperm activation
2187,08	LV1L2	0,8	Ig lambda chain V-1 region-like	Motility







Conclusions

Fertility-predictive models can be generated from sperm cells ICM-MS spectra with high diagnostic performance

Allows for global testing of different sperm cell functions

Interpretation of molecular basis of fertility

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2

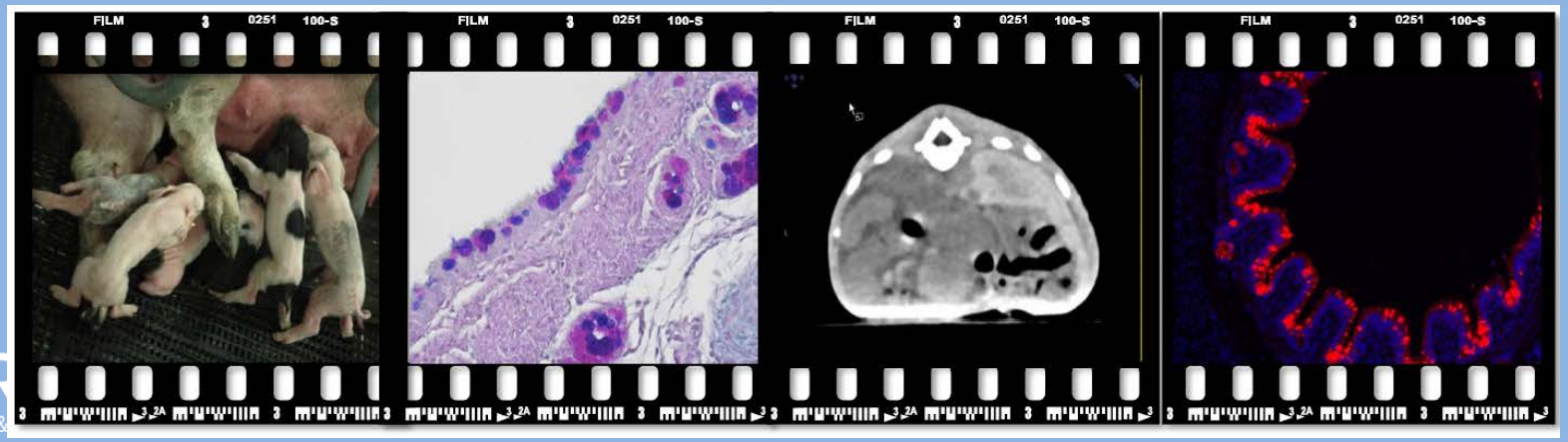
Phenotyping of Swine $CFTR^{+/+}$ vs. $CFTR^{-/-}$ neutrophils

Example 2: Swine Cystic Fibrosis-mutant neutrophils phenotyping (*Ignacio Caballero-Posadas*)

Caused by the presence of mutations in both copies of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) protein.

Affects mostly the lungs but also the pancreas, liver, kidneys, and intestine.

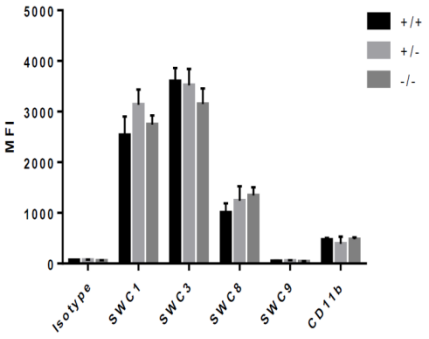
Pig transgenic model CFTR$^{-/-}$



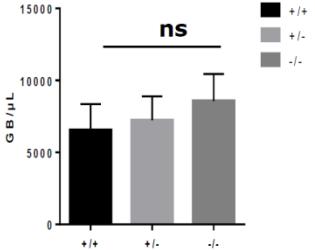
Altered neutrophil function?

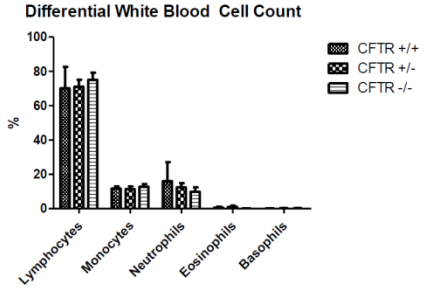
- Presence of CFTR → Phagolysosome (Painter et al. 2006, 2008, 2010)
- Defective killing (chlorination phagolysosome)
- Altered chemotaxis
- Increased oxidative burst
- Increased protease release

No differences in surface markers

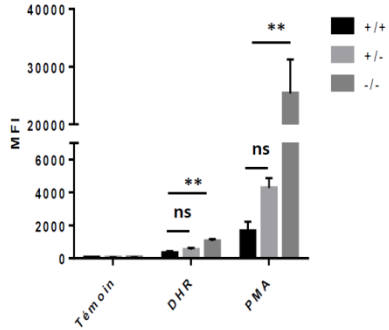


No difference in total and differential WBC count





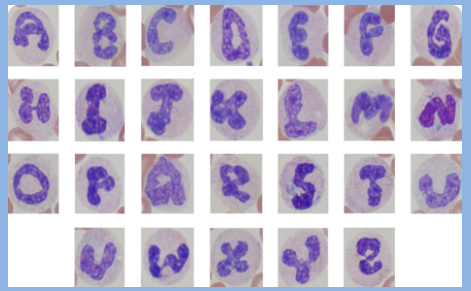
Increase in ROS secretion



$5x10^5$ cells/spot

7 $CFTR^{+/+}$ and 7 $CFTR^{-/-}$

8 technical replicates

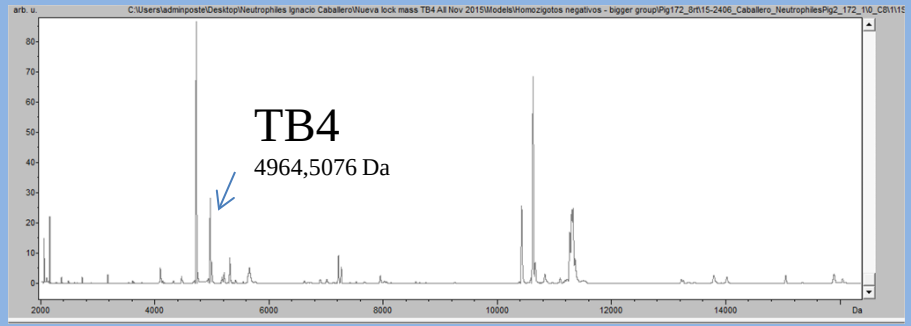


Spectra manual acquisition in a Bruker UltrafleXtreme MALDI-TOF instrument (mass range 1-20 kDa).

External calibration : Peptide/protein mix calibrant

Internal calibration: Lock-mass using peak 4964,5076 Da (TB4)

Pre-processing: ClinProTools 3.0 (Bruker)



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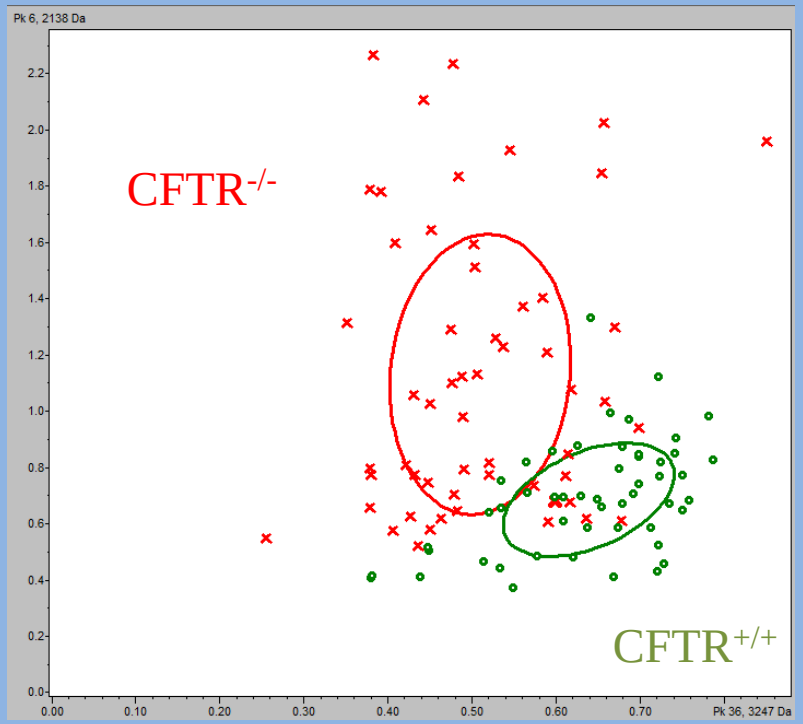
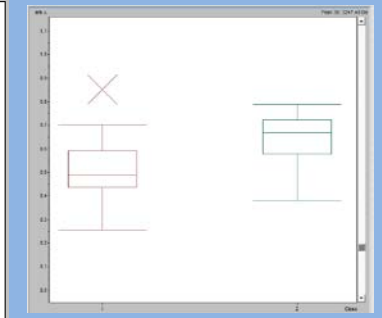
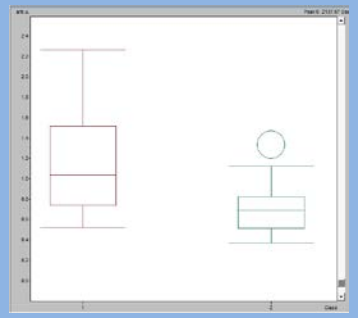
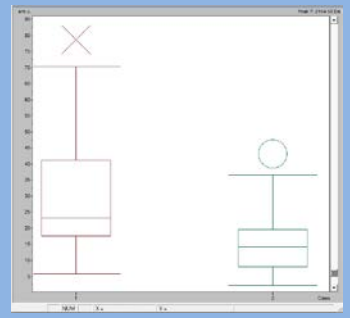
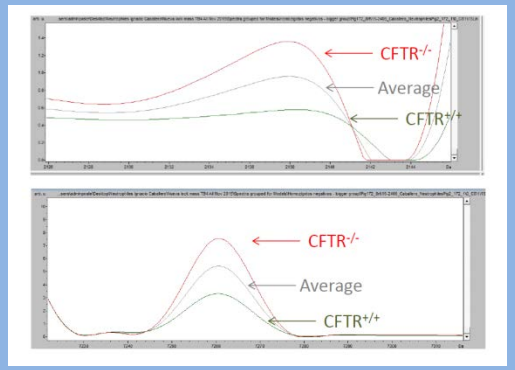








Index	Mass	DAve	PTTA
36	3247.48	0.14	< 0.000001
6	2137.67	0.5	0.0000036
7	2154.58	17.17	0.00000367
114	7260.62	4.2	0.00000367
28	2980.76	0.13	0.00000367



Genetic Analyser

Recognition Capability	Cross Validation
100 %	92.73 %

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Acknowledgements_

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Merci / Thank you!!!

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