

# IZS

TERAMO

/

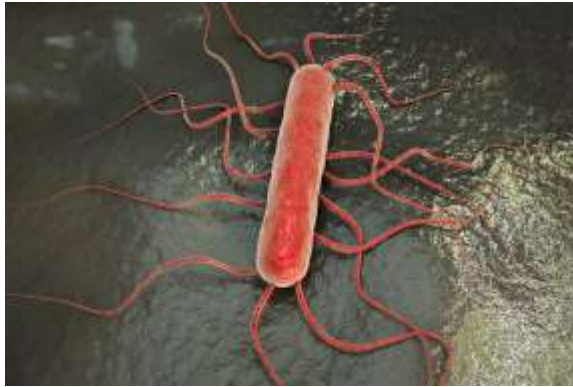
LABORATORIO  
NAZIONALE  
DI RIFERIMENTO PER  
*LISTERIA*  
*MONOCYTOGENES*

## Proteomic profiling of a virulent *Listeria* *monocytogenes* strain grown under several stress conditions

Doc. Federica D'Onofrio ([fdonofrio@unite.it](mailto:fdonofrio@unite.it))



## *Listeria monocytogenes* PhD aims



- ✓ Identify discriminant biomarkers to implement in surveillance and diagnostic fields to prevent listeriosis outbreaks
- ✓ Assessment of stress tolerance and virulence potential of different *Listeria monocytogenes* strains by means of biomolecular techniques

Strain isolated from pork meat product  
(*Coppa di testa*)

(Marche outbreak 2015 - 2016)



## Listeria monocytogenes ST7

### Growth conditions:

\*C1 (control): 37°C, pH 7.0, NaCl 0.5%;

C2: 37°C, pH 5.5, NaCl 7%;

C3: 12°C, pH 7.0, NaCl 0.5%;

\*C4: 12°C, pH 5.5, NaCl 7%

### Culture media:

Brain Heart Infusion (BHI) broth (NaCl 0.5%, pH 7.0)

modified BHI broth (NaCl 7%, pH 5.5)

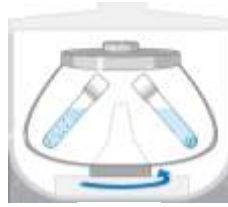
Technical and biological triplicates

Bacteria cells collection during exponential growth  
phase ( $OD_{600} = 0.6$ )

\* : condition used for RNAseq experiment

## *Listeria* *monocytogenes* 1/2a

### Proteins extraction and purification protocol



Wash by sterile ice-cold phosphate-buffered saline (PBS)  
0.1 M pH 7.0 (5,600 x g for 15 min at 4°C)



Proteins extraction:  
CellLytic B Cell Lysis Reagent (Sigma Aldrich) e  
CellLytic IB Inclusion Body Solubilization Reagent  
(Sigma Aldrich)

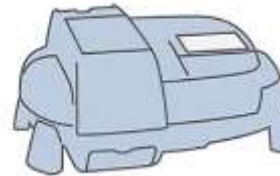


Protein precipitation by “TCA  
protocol”



Solubilization solution: SDS 5% (w/v) containing 0.1N  
NaOH

Read at 562 nm



Spectrophotometer

Quantification of protein concentration: Pierce™  
BCA Protein Assay Kit (Thermo Scientific).

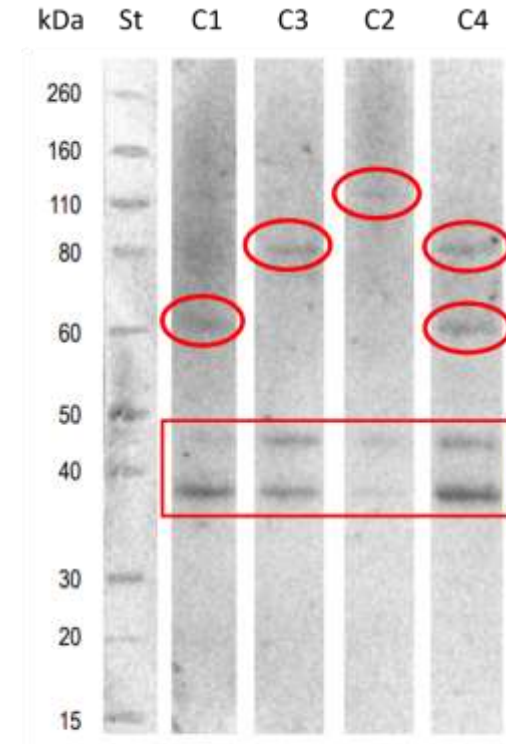
## IMMUNOBLOTTING

Positive serum against *L. monocytogenes* collected from naturally infected sheep



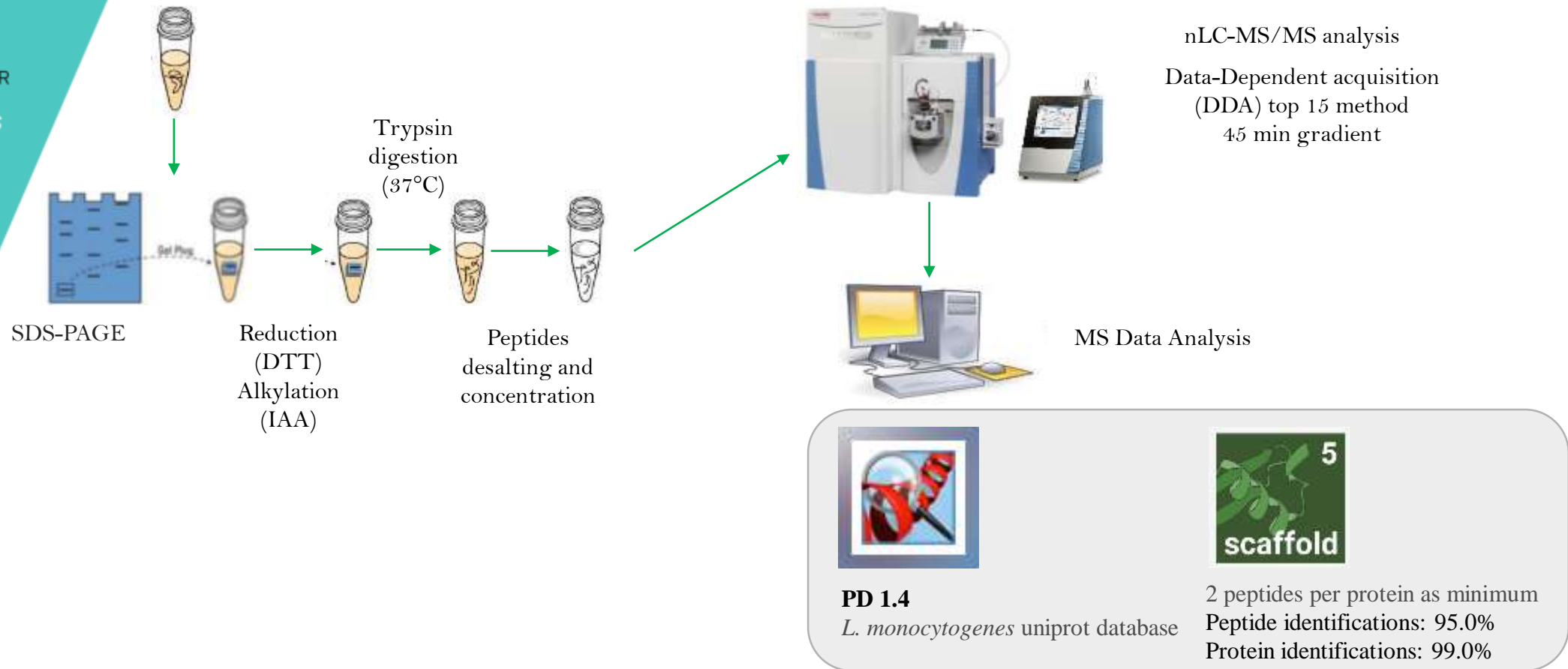
**Ag-Ab immunocomplexes identified:**

- 38 and 45 kDa for all conditions
- 60 kDa in C1 e C4
- 80 kDa in C3 e C4
- 110 kDa in C2

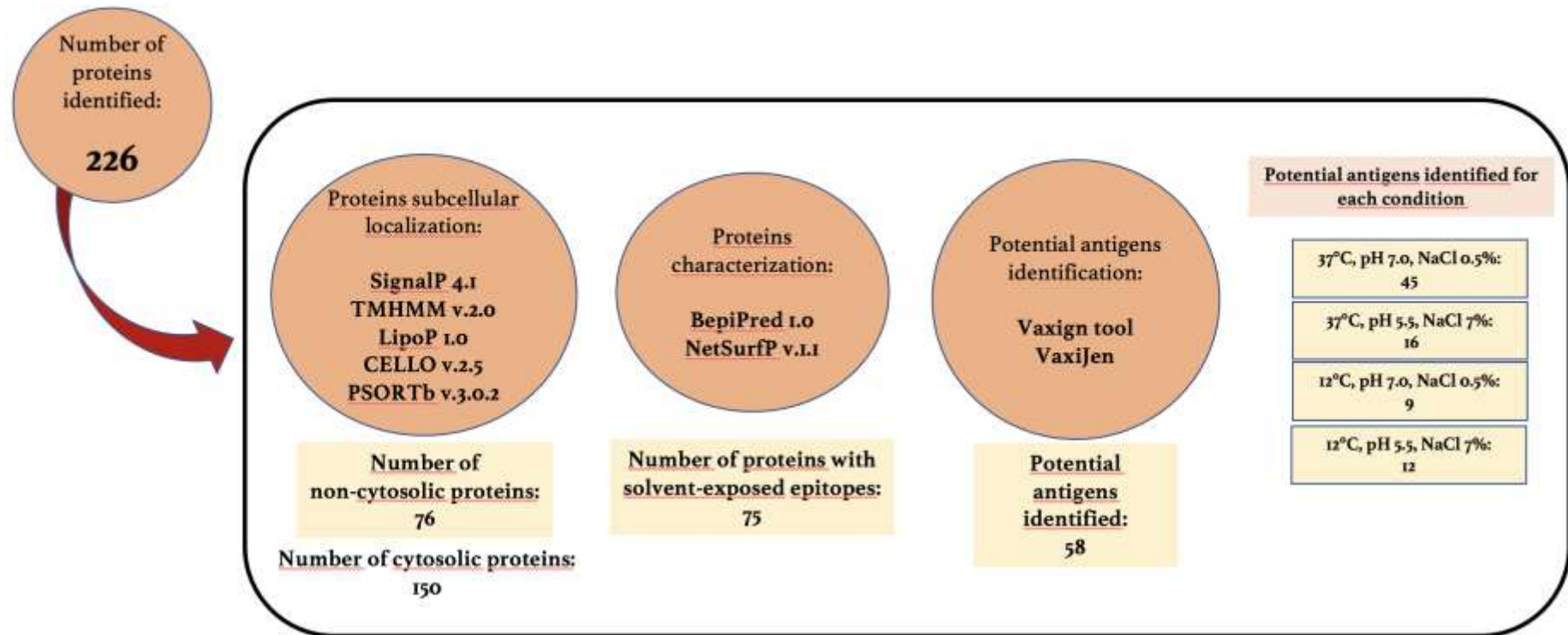


St = standard; C1 = (37°C, pH 7,0, NaCl 0.5%);  
C3 = (12°C, pH 7,0, NaCl 0.5%); C2 = (37°C, pH  
5,5, NaCl 7%); C4 = (12°C, pH 5,5, NaCl 7%)

## Protein digestion and Mass spectrometry analysis



## PROTEINS IDENTIFICATION BY BIOINFORMATIC ANALYSIS



**TABLE 1** List of *L. monocytogenes* immunogenic proteins identified

Gene	Protein	Function	Function	Localization	MW (kDa)	Condition
<i>Imo2679</i>	Q8Y3Z9	Histidine kinase	Adaptation to high-osmolarity conditions by regulating the expression of a high-affinity potassium uptake system encoded by the <i>kdpABC</i> genes	Membrane	100	C2
<i>Imo0699</i>	Q8Y942	Flagellar motor switch protein - FliM	Part of the switch complex that is involved in switching the direction of the flagella rotation	Membrane	38	C3
<i>Imo0723</i>	Q8Y919	<i>Imo0723</i>	Methyl-accepting chemotaxis protein	Membrane	66	C3
<i>Imo1053</i>	Q8Y864	PdhB protein	Highly like pyruvate dehydrogenase (E1 beta-subunit)	Membrane	35	C4
<i>trxB</i>	O32823	Thioredoxin reductase	Protection against oxidative stress by regulating the expression of thioredoxins	Membrane	34	C4
<i>Imo0132</i>	Q8YAJ3	<i>Imo0132</i>	DNA repair and maintenance	Membrane	55	C4

C2 (37°C, pH 5.5, 7% NaCl); C3 (12°C, pH 7.0, 0.5% NaCl); and C4 (12°C, pH 5.5, 7% NaCl). MW, molecular weight.

Received: 28 February 2022 | Revised: 6 June 2022 | Accepted: 25 July 2022

DOI: 10.1002/pmic.202200062

RESEARCH ARTICLE

Proteomics  
Proteomics and Systems Biology

## Immunoproteome profiling of *Listeria monocytogenes* under mild acid and salt stress conditions

Federica D'Onofrio<sup>1</sup> | Pierina Visciano<sup>1</sup> | Ivanka Krasteva<sup>2</sup> | Marina Torresi<sup>2</sup> |  
Manuela Tittarelli<sup>2</sup> | Francesco Pomilio<sup>2</sup> | Luigi Iannetti<sup>2</sup> | Tiziana Di Febo<sup>2</sup> |  
Antonello Paparella<sup>1</sup> | Maria Schirone<sup>1</sup> | Mirella Luciani<sup>2</sup>



**Next step:  
combine proteomic data with RNAseq data**

## *Listeria monocytogenes 1/2a*

### Comparison of C1 - opt vs C4 - stress

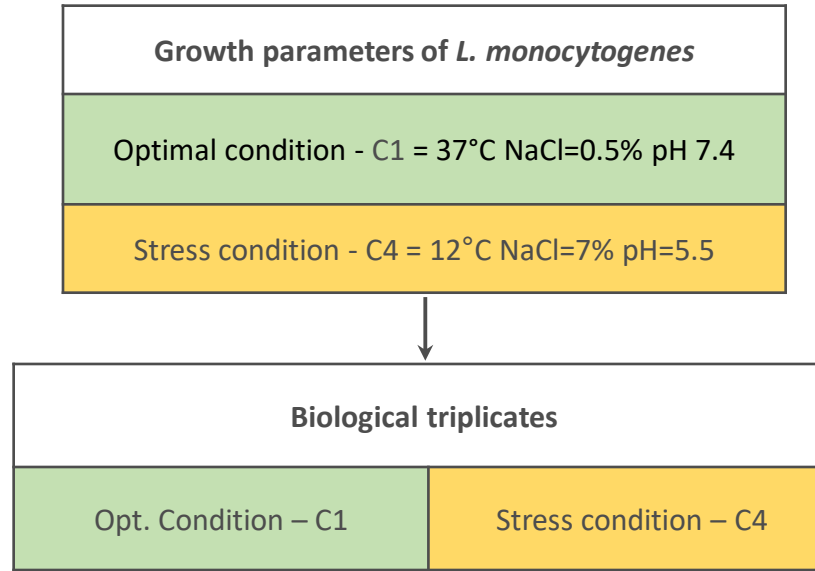
121 proteins included  
exclusively in C1

214 common proteins in  
C1 and C4

32 proteins included  
exclusively in C4

Platform: Illumina  
Nextseq 500

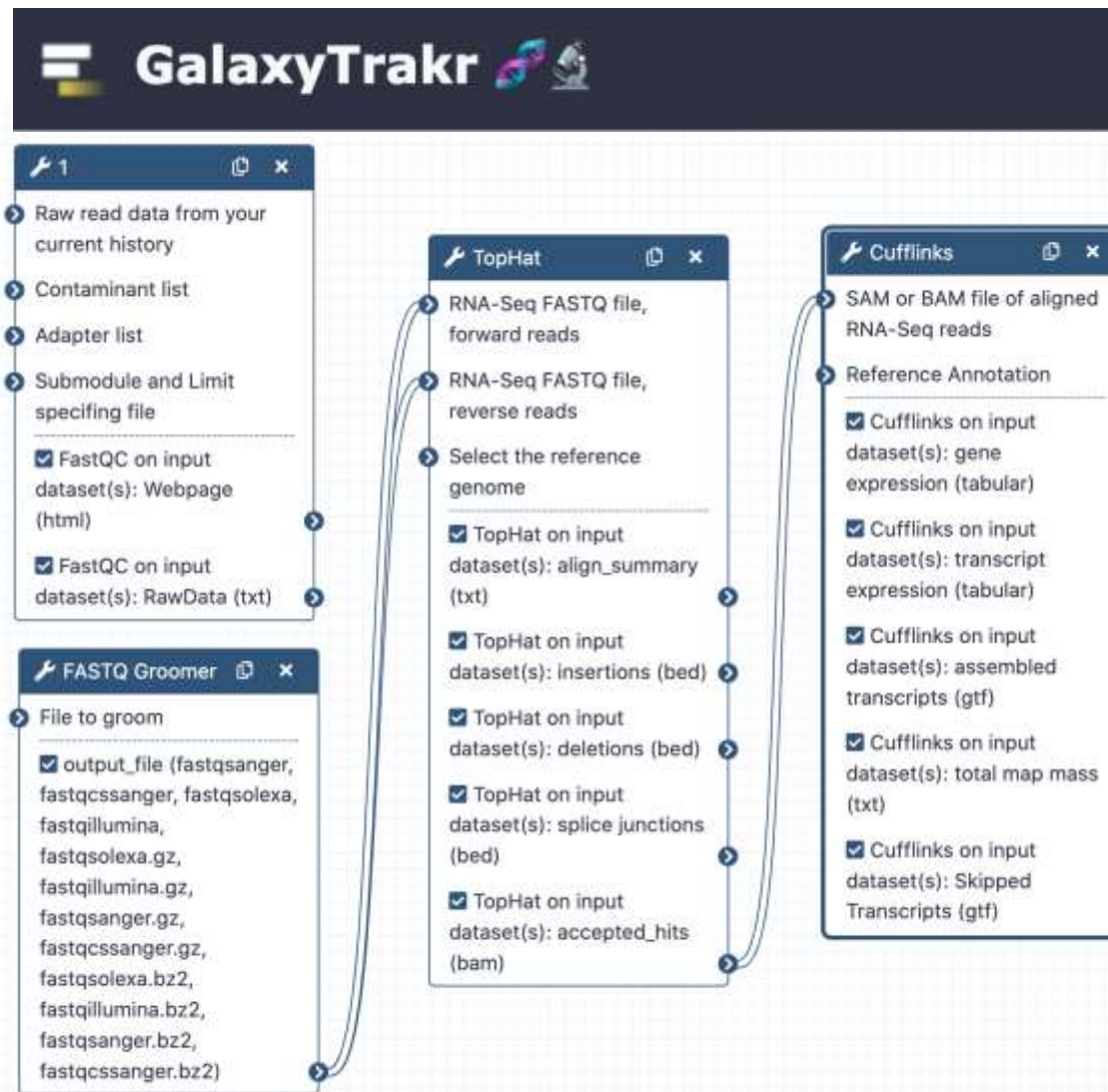
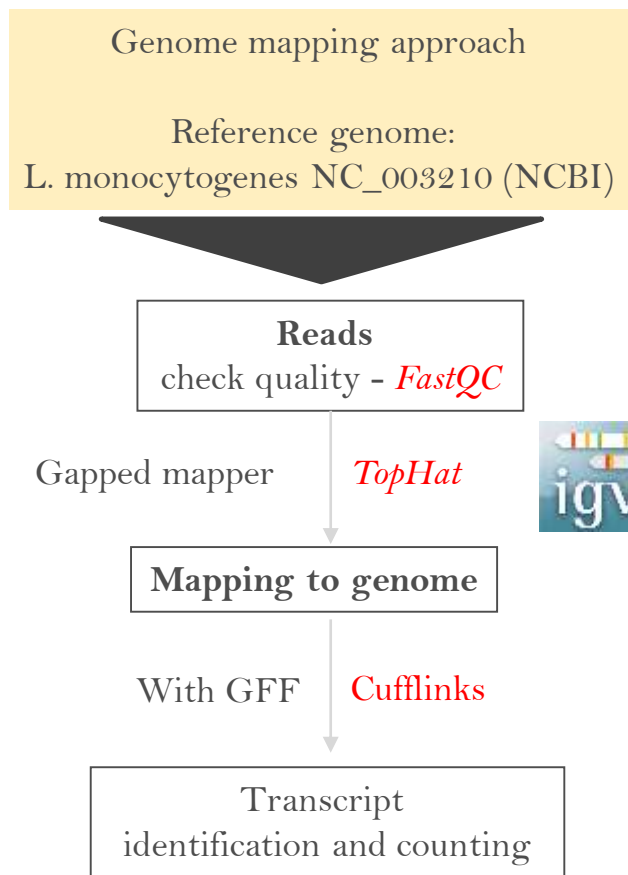
## RNAseq analysis



AIMS:

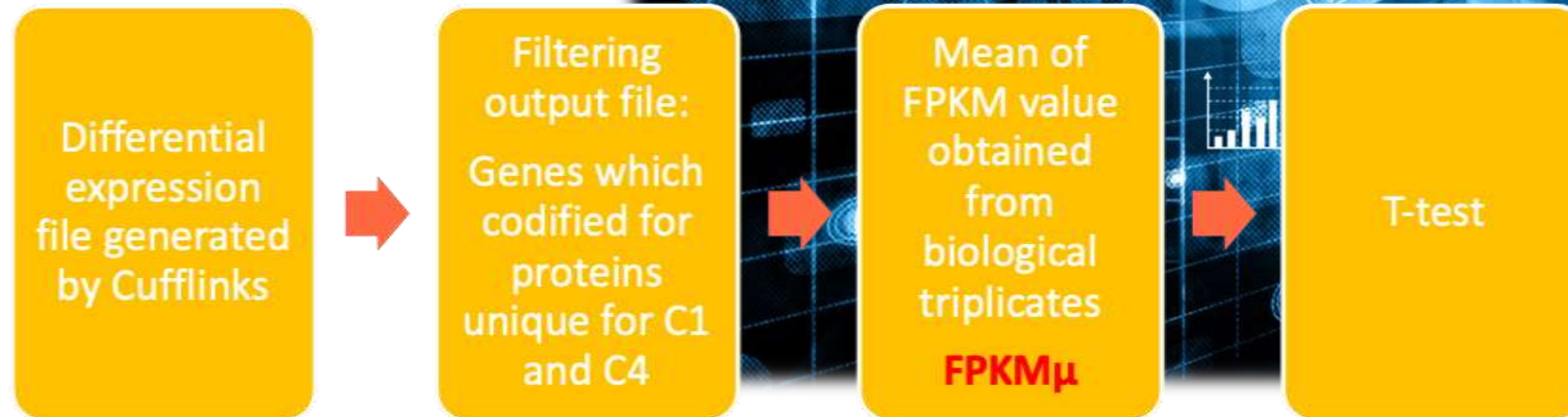
Elaborate a pipeline to connect proteomic and transcriptomic data  
Integration of RNA-seq with proteomics will confirm proteomic data?

## RNAseq analysis



Trapnell et al.; 2016

## Data analysis



**FPKM** (Fragments Per Kilobase of transcript per Million mapped reads)

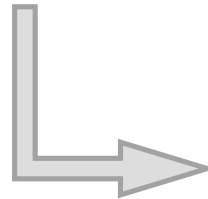
*FPKM values provide a measure of the expression level of each transcript.*

## PROTEOMIC AND TRANSCRIPTOMIC DATA COMPARISON

	A	B	C	D	E	F	G	H	I	J	K
1	tracking_id	gene_id	gene_short_name	locus	length	coverage	FPKM	FPKM_conf_lo	FPKM_conf_hi	FPKM_status	
2	gene	lmo2115	gene-lmo2115	lmo2115	NC_003210.1:2194398-2196339	1941	164,407	70,3992	66,1298	74,6686	OK
3	gene	lmo0279	gene-lmo0279	lmo0279	NC_003210.1:302007-304140	2133	604,035	381,005	363,843	398,168	OK
4	gene	lmo1645	gene-lmo1645	lmo1645	NC_003210.1:1691971-1695043	3072	44,2413	21,2996	19,6972	22,902	OK
5	gene	lmo1076	gene-lmo1076	lmo1076	NC_003210.1:1106040-1107759	1719	1077,73	462,925	454,28	471,57	OK
6	gene	lmo2631	gene-lmo2631	rplD	NC_003210.1:2704432-2705056	624	5342,74	2417,66	2311,23	2524,09	OK
7	gene	lmo0288	gene-lmo0288	lmo0288	NC_003210.1:311829-313662	1833	339,608	148,131	140,43	155,831	OK
8	gene	lmo1079	gene-lmo1079	lmo1079	NC_003210.1:1110768-1113627	2859	1477,65	640,011	632,391	647,632	OK
9	gene	lmo1085	gene-lmo1085	lmo1085	NC_003210.1:1118931-1121022	2091	850,62	357,051	350,247	363,854	OK
10	gene	lmo1404	gene-lmo1404	mutL	NC_003210.1:1432838-1434644	1806	1384,36	659,755	649,711	669,799	OK
11	gene	lmo1571	gene-lmo1								
12	gene	lmo1438	gene-lmo1								

Significativity test:  
T-test

				C1 Rep1	C1 Rep2	C1 Rep3	C4 Rep1	C4 Rep3		FPKM C1 mean value	FPKM C4 mean value	t-test		
13	gene	lmo0590	gene-lmo0	lmo2115	64,5415	70,3992	69,6334	304,377	167,547	68,19	235,96	0,046200	Legend	
14	gene	lmo1455	gene-lmo1	lmo0279	378,429	381,005	354,407	155,182	95,5475	371,28	125,36	0,002174	Confirmed	
15	gene	lmo2760	gene-lmo2	lmo1645	19,2975	21,2996	19,6663	41,84	21,2631	20,09	31,55	0,233740	Unconfirmed	
16	gene	lmo2757	gene-lmo2	lmo1076	431,207	462,925	413,076	265,56	198,484	435,74	232,02	0,007355		
17	gene	lmo1915	gene-lmo1	lmo2631	2235,95	2417,66	2148,26	717,999	730,299	2.267,29	724,15	0,000637		
18	gene	lmo1606	gene-lmo1	lmo0288	126,547	148,131	133,172	205,483	113,976	135,95	159,73	0,546503		
				lmo1079	608,166	640,011	687,24	321,957	174,655	645,14	248,31	0,007865		
				lmo1085	380,75	357,051	347,476	360,658	176,312	361,76	268,49	0,274197		
				lmo1404	617,406	659,755	606,705	329,46	240,993	627,96	285,23	0,003114		
				lmo1571	4486,35	3687,94	3837,1	1159,76	2959,62	4.003,80	2.059,69	0,078911		
				lmo1438	701,865	676,36	664,285	528,394	603,407	680,84	565,90	0,035238		
				lmo0590	23,6566	25,0927	33,3316	300,022	162,776	27,36	231,40	0,028422		
				lmo1455	745,881	765,35	785,078	489,993	600,094	765,44	545,04	0,014896		
				lmo2760	14,7563	15,916	15,1961	66,0063	39,6701	15,29	52,84	0,031533		
				lmo2757	359,304	349,01	351,986	392,211	441,993	353,43	417,10	0,043843		
				lmo1915	227,454	227,717	228,087	257,653	159,334	227,75	208,49	0,635539		
				lmo1606	535,776	532,019	501,853	229,209	139,342	523,22	184,28	0,002590		
				lmo1814	594,921	593,819	521,611	262,216	190,834	570,12	226,53	0,003586		
				lmo0354	857,846	983,069	919,283	316,763	164,868	920,07	240,82	0,002666		
				lmo1286	414,219	429,869	416,16	230,269	154,993	420,08	192,63	0,004216		
				lmo2558	529,166	541,569	485,971	576,57	675,161	518,90	625,87	0,087319		
				lmo1299	984,088	1415,98	1425,24	387,573	196,187	1.275,10	291,88	0,016335		
				lmo0988	416,277	441,606	449,504	424,671	402,838	435,80	413,75	0,244957		
				lmo2655	13178,2	13164,3	12348,6	1874,01	2572,85	12.897,03	2.223,43	0,000153		
				lmo2335	3049,88	3031,1	2898,85	459,847	359,473	2.993,28	409,66	0,000047		
				lmo2689	40,0109	43,5794	54,4311	308,322	213,713	46,01	261,02	0,009175		
				lmo0641	1174,64	1088,25	1081,02	510,556	570,726	1.114,64	540,64	0,001028		



## Gene set upregulated at 12°C, 5.5 pH, 7.0% NaCl

Locus	Gene	Functional annotation or prediction	Comments	Transcripts quantification FPKM C1	Transcripts quantification FPKM C4	P-value
lmo0823	lmo0823	oxidoreductase	Bacteriocins resistance; Cold tolerance (Melian et al., 2021)	268,822	1244,840	0,00327
lmo1525	lmo1525	recombination protein RecS	Cold tolerance (Mudcharee Julotok, 2008). DNA repair and recombination (Kidane et al., 2012).	262,997	670,483	0,000487
lmo0919	lmo0919	antibiotic ABC transporter ATP-binding protein	Antibiotic resistance. (Brodiazhenko et al., 2022)	37,903	213,880	0,0004593
lmo1910	lmo1910	oxidoreductase	Stress response; Dessication response. (Kragh et al., 2020)	45,692	207,284	0,0080064
lmo2338	pepC	aminopeptidase C	Cold tolerance (Reid Aaron Ivy, 2010)	166,004	297,383	0,0025717
lmo0956	lmo0956	putative N-acetyl-glucosamine-6 phosphate deacetylase	Amino-sugar metabolism; TAs biosynthesis; influence positively cell wall murein content; influence positively the sensitivity to cell wall hydrolase, mutanolysin and peptide antibiotic (colistin). (Popowska et al., 2012)	389,760	500,824	0,012392
lmo2005	lmo2005	oxidoreductase	Acidic stress related gene. (Bowman et al., 2010)	57,120	1114,505	0,0120352
lmo2695	lmo2695	dihydroxyacetone kinase subunit DhaK	PrfaA and sigB regulated; virulence/stress associated; involved in the utilization of glycerol as carbon source. (Abram et al., 2008)	110,415	1782,460	0,0007862
lmo0613	lmo0613	oxireductase	Increase resistance to oxidative and antibiotic stress (Pleitner et al., 2014)	52,789	656,194	0,0030489
lmo2478	trxB	thioredoxin reductase	Oxidative stress response (D'Onofrio et al., 2022)	739,527	4632,305	0,022374
lmo2157	sepA	hypotetical protein	Prfa regulated; Deletion decreases virulence (Chatterjee et al., 2006) Produced in presence of acid shock (Neuhaus et al., 2013)	313,169	656,823	0,0090629
lmo1053	PdhB	pyruvate dehydrogenase subunit E1 beta	DNA repair and maintenance (D'Onofrio et al., 2022)	945,332	2907,955	0,0146895

Gene set significantly upregulated (p-value < 0.05) in C4 compared to optimal condition C1  
FPKM: Fragments Per Kilobase Million; TA: Teichoic acids

## CONCLUSIONS

In response to low temperature, acidic shock and high osmolarity, *L. monocytogenes* adapts to hostile environment activating genes which codify for proteins involved in virulence and stress response pathways.

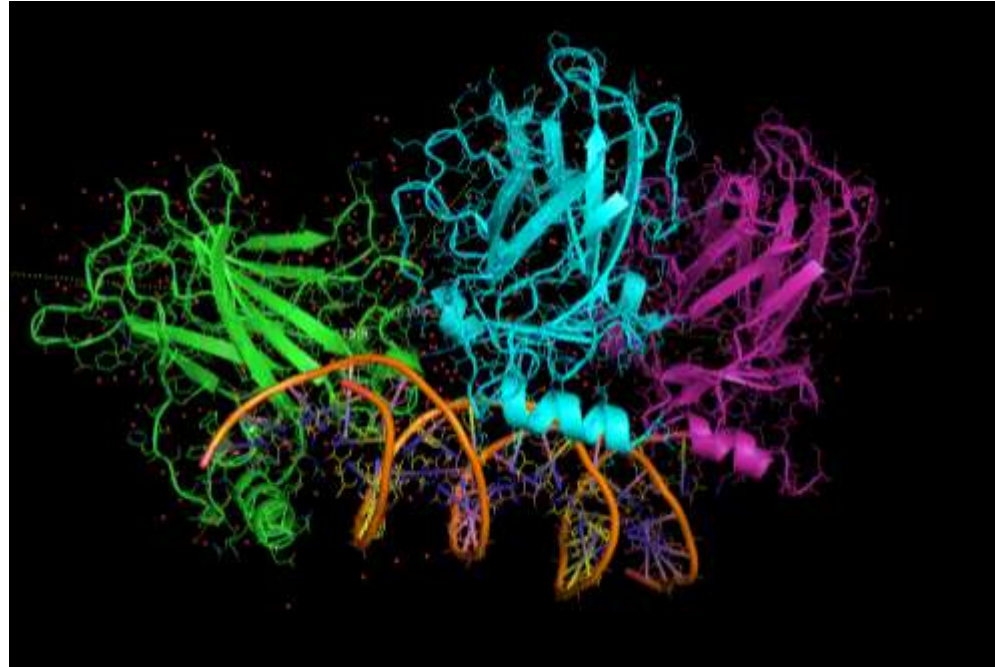
Transcriptomic and Proteomic results can be correlated to give a wider information spectra about *L. monocytogenes* biomolecular factors.

# IZS

TERAMO

/

LABORATORIO  
NAZIONALE  
DI RIFERIMENTO PER  
*LISTERIA*  
*MONOCYTOGENES*



*THANKS FOR  
THE ATTENTION*

Special thanks to:

Facoltà di Bioscienze e Tecnologie Agro-Alimentari e Ambientali, Università degli Studi di Teramo  
Reparto di Immunologia e Sierologia - Istituto Zooprofilattico Sperimentale  
dell'Abruzzo e del Molise "G. Caporale"

Reparto di Igiene e Tecnologie degli Alimenti - Istituto Zooprofilattico Sperimentale  
dell'Abruzzo e del Molise "G. Caporale"

Reparto di Biologia molecolare e tecnologie omiche - Istituto Zooprofilattico Sperimentale  
dell'Abruzzo e del Molise "G. Caporale"

UCD School of Biosystems and Food Engineering Agriculture and Food Science Centre

