Microarray pig-zoonosis V4:
Response analysis on mono-specific samples

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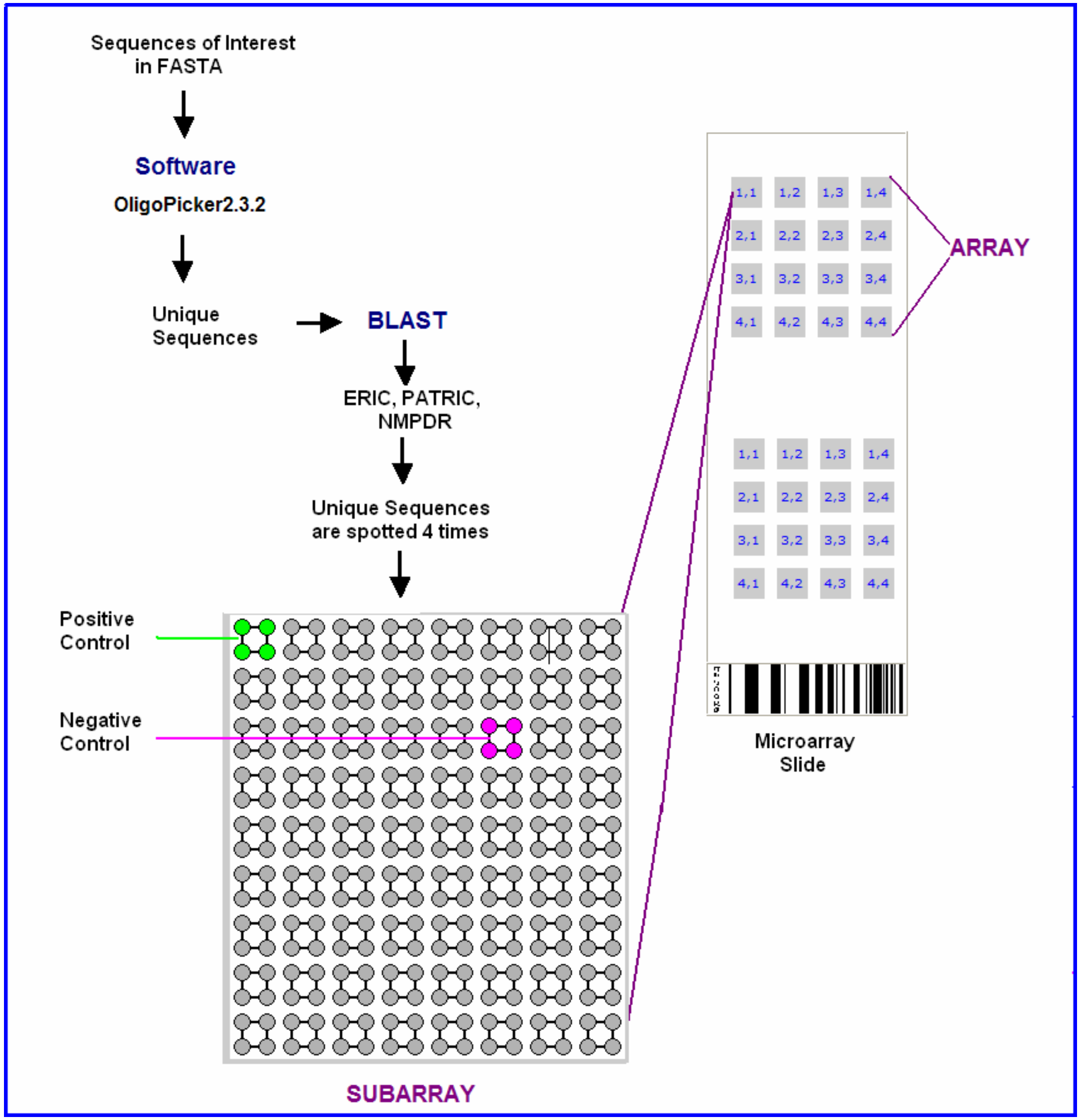
Development of a diagnostic microarray system for the detection of bacterial and viral zoonoses agents

Microarray Design

Hybridization patterns with mono-specific samples

Hybridization patterns with environmental samples

Microarray Design



(from Tonelli et al. 2010)

Collection of all sequences of interest in FASTA format

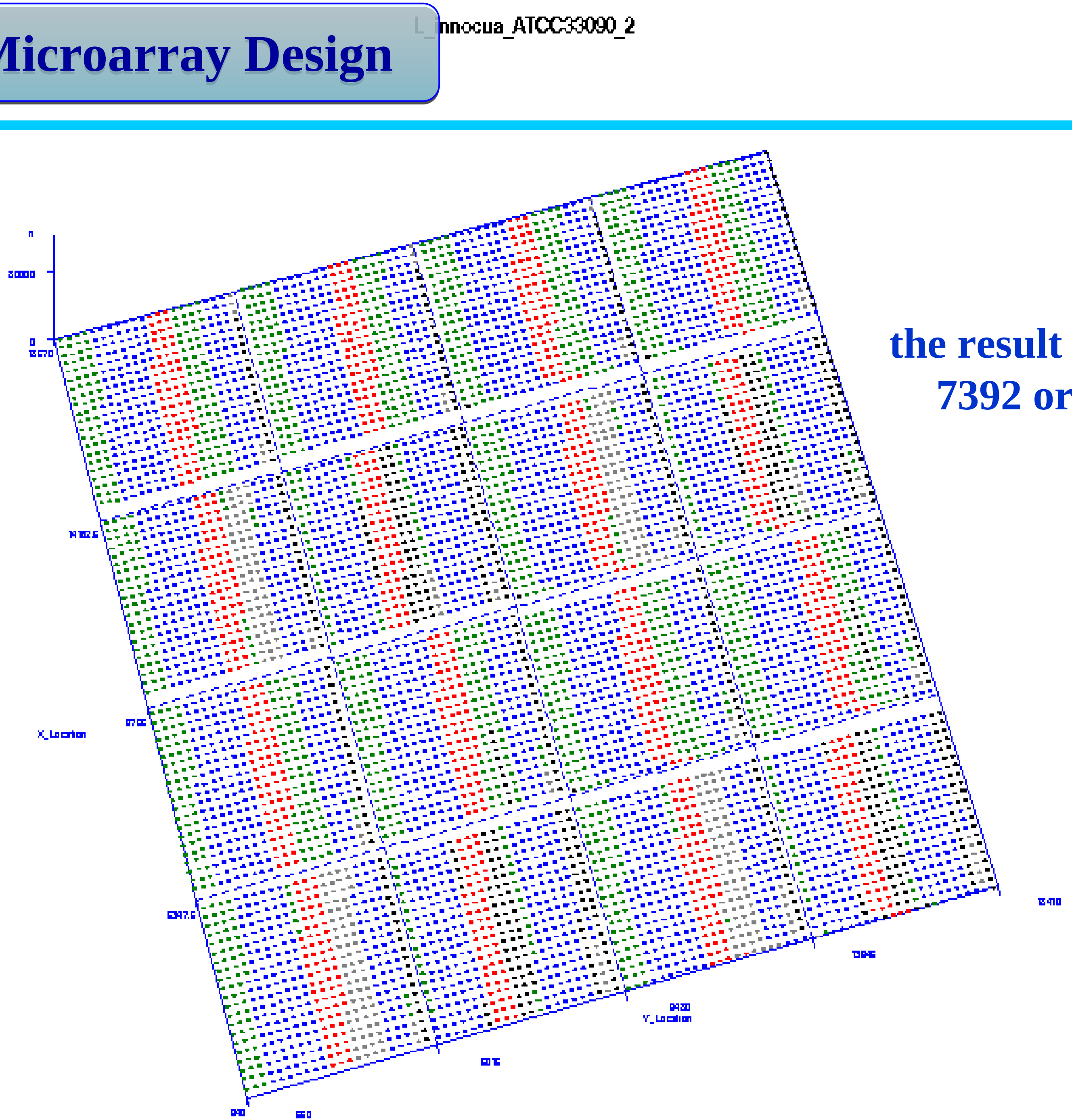
Choice of the oligonucleotides:
- length (influence the number of mismatch for sequence)
- contained in CG
- homology between target and non target-genes
- number of genes by species
- selectivity power of genes

Positioning of the oligonucleotides in the array (Spotting)

Microarray Design

Genus	n° Spot	n° Genes	n° Species
Arcobacter	6	2	1
Campylobacter	1419	473	19
Clostridium	339	113	30
Enterococcus	225	75	26
Erysipelothrix	30	10	1
Escherichia	18	6	1
Hafnia	3	1	1
Helicobacter	48	16	10
Listeria	348	116	6
Salmonella	1860	620	5
Staphylococcus	1116	372	37
Streptococcus	660	220	42
Vibrio	15	5	1
Yersinia	948	316	14
Controls	357	119	4
Total	7392	2464	198

Microarray Design



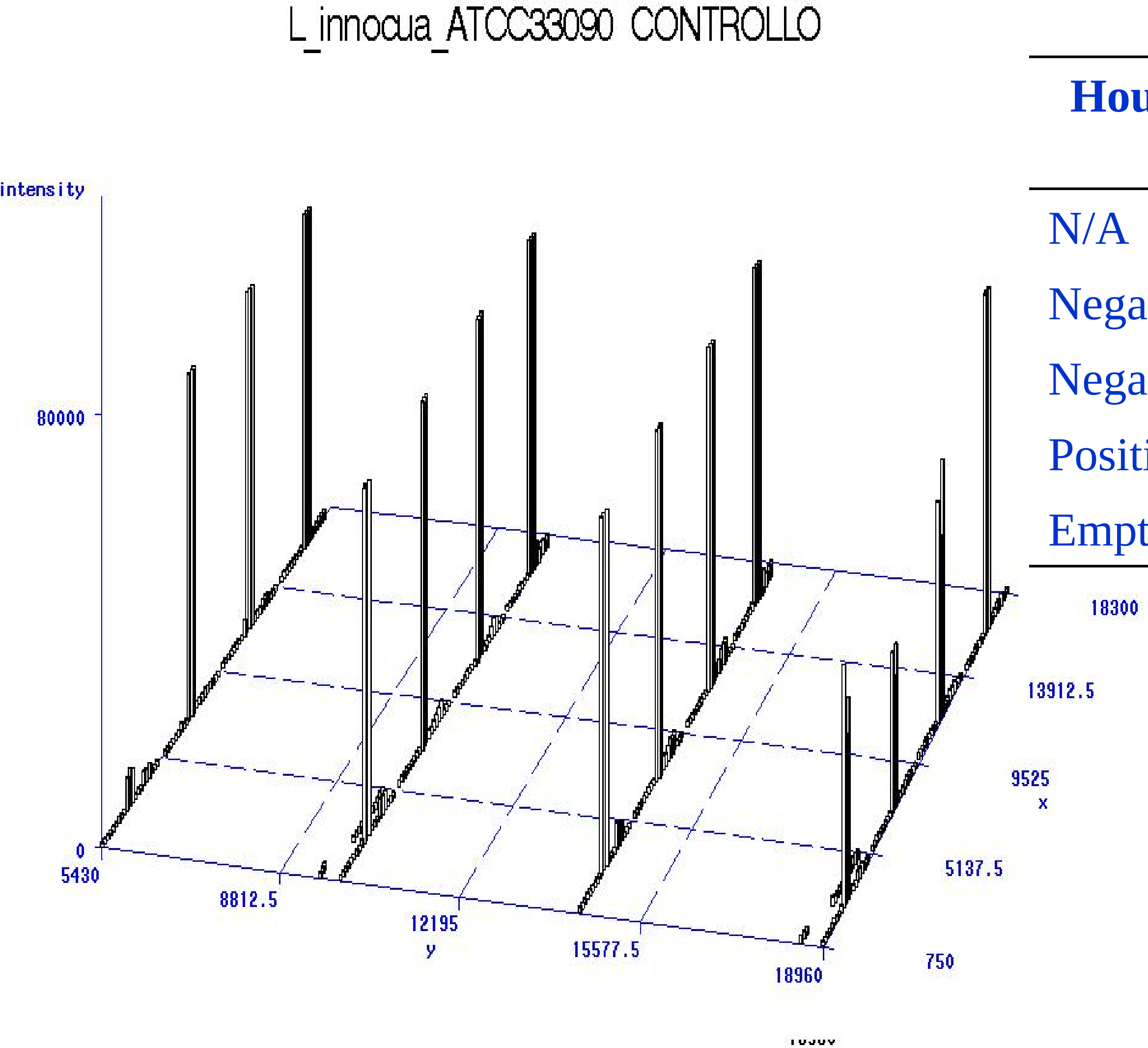
the result is a slide with 7392 orderly spots

Hybridization patterns with mono-specific samples

As first validation the array has been hybridized to mono-specific samples

Zoonotic Agents
Staphylococcus species
Vibrio species
Enterococcus species
Campylobacter species
Escherichia species
Listeria species
Listeria cheese project

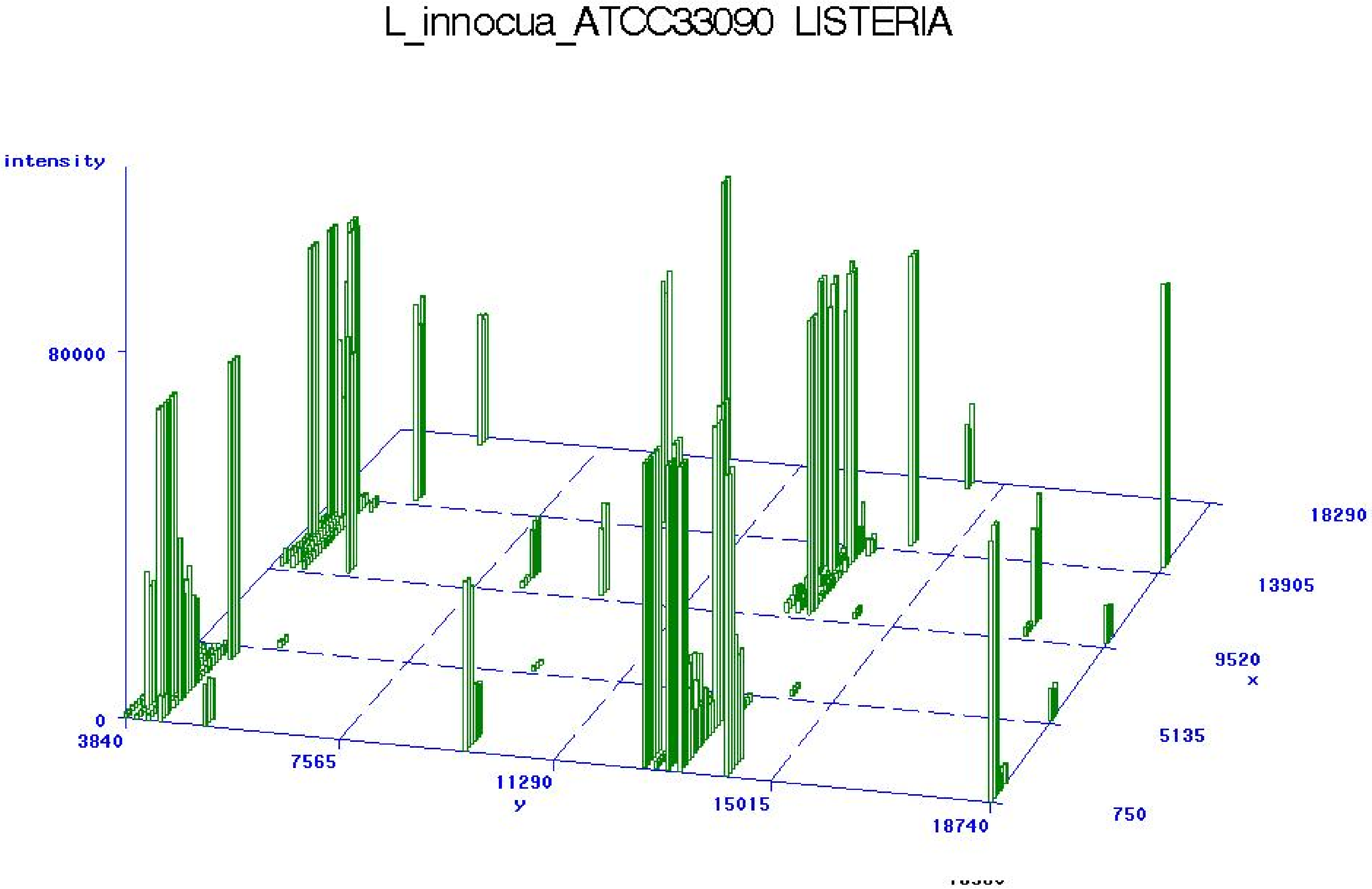
Microarray Design



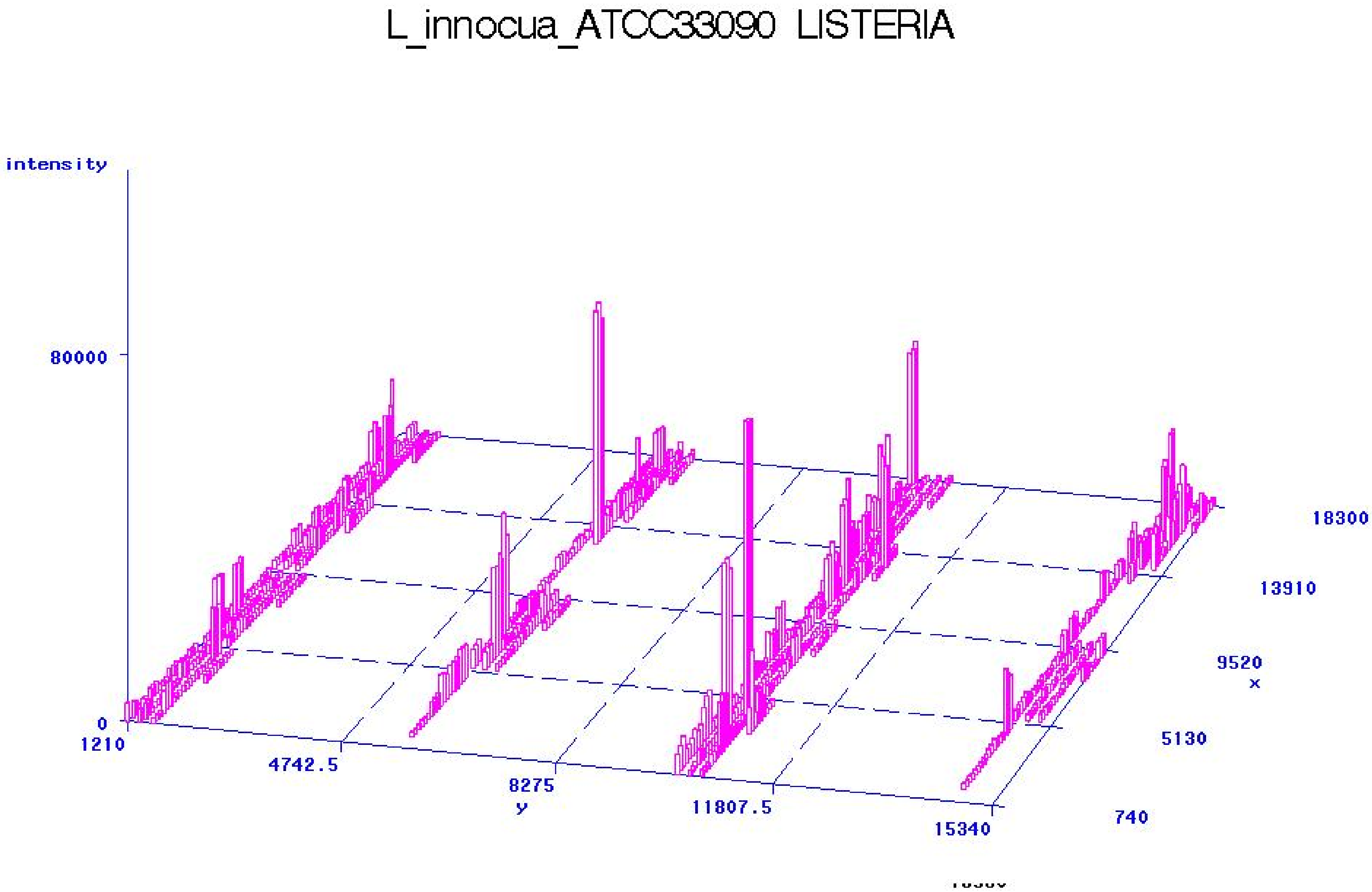
the array contains 357 spots of controls

Housekeeping type		Number
N/A		12
Negative control	Arabid	51
Negative control	gfp50	51
Positive control	EUB33835	51
Empty		192

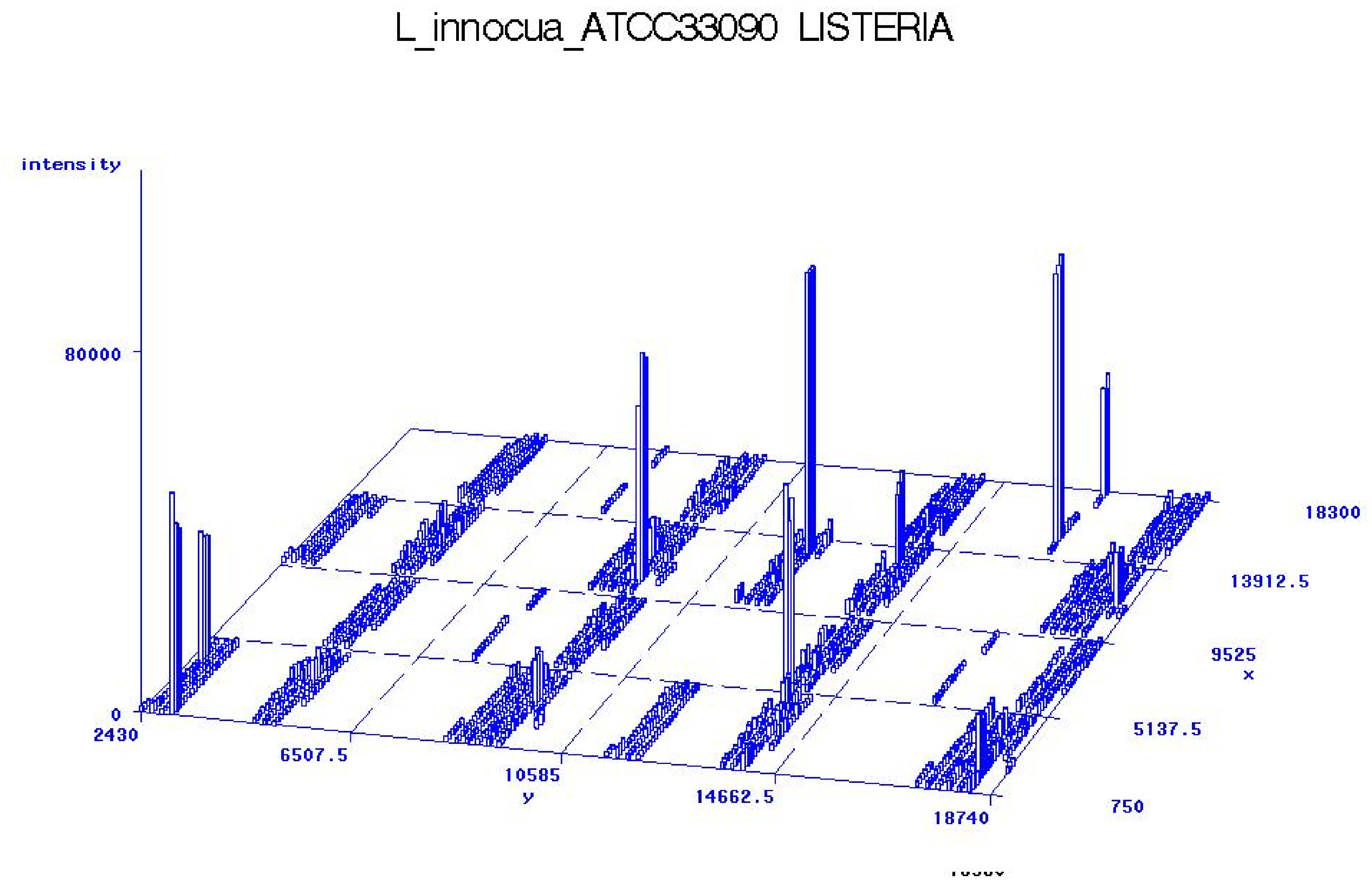
response of the genes of Listeria sp. when the array is hybridized with Listeria innocua



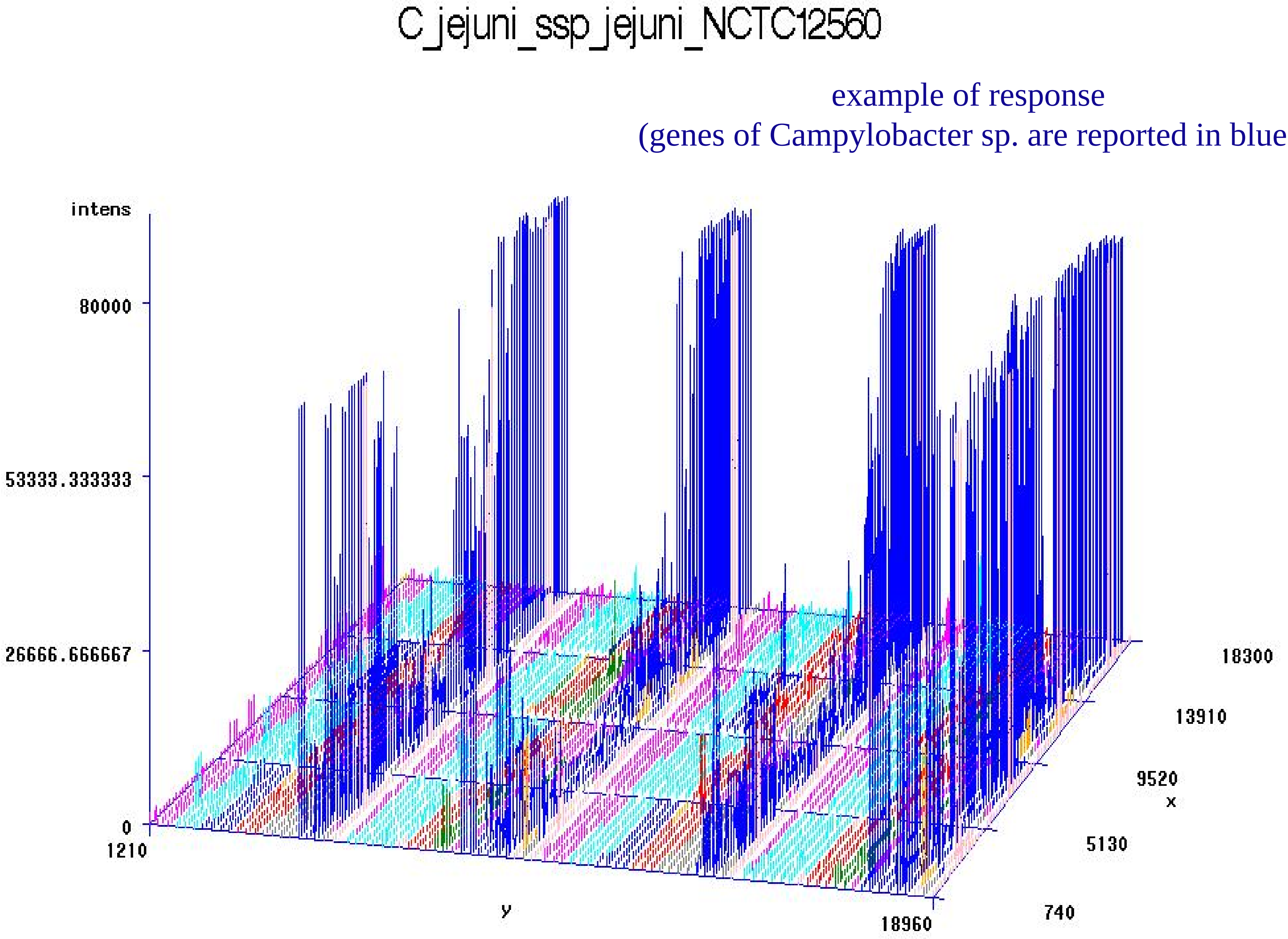
response of the genes of Campylobacter sp. when the array is hybridized with Listeria innocua



response of the genes of Staphylococcus sp. when the array is hybridized with Listeria innocua

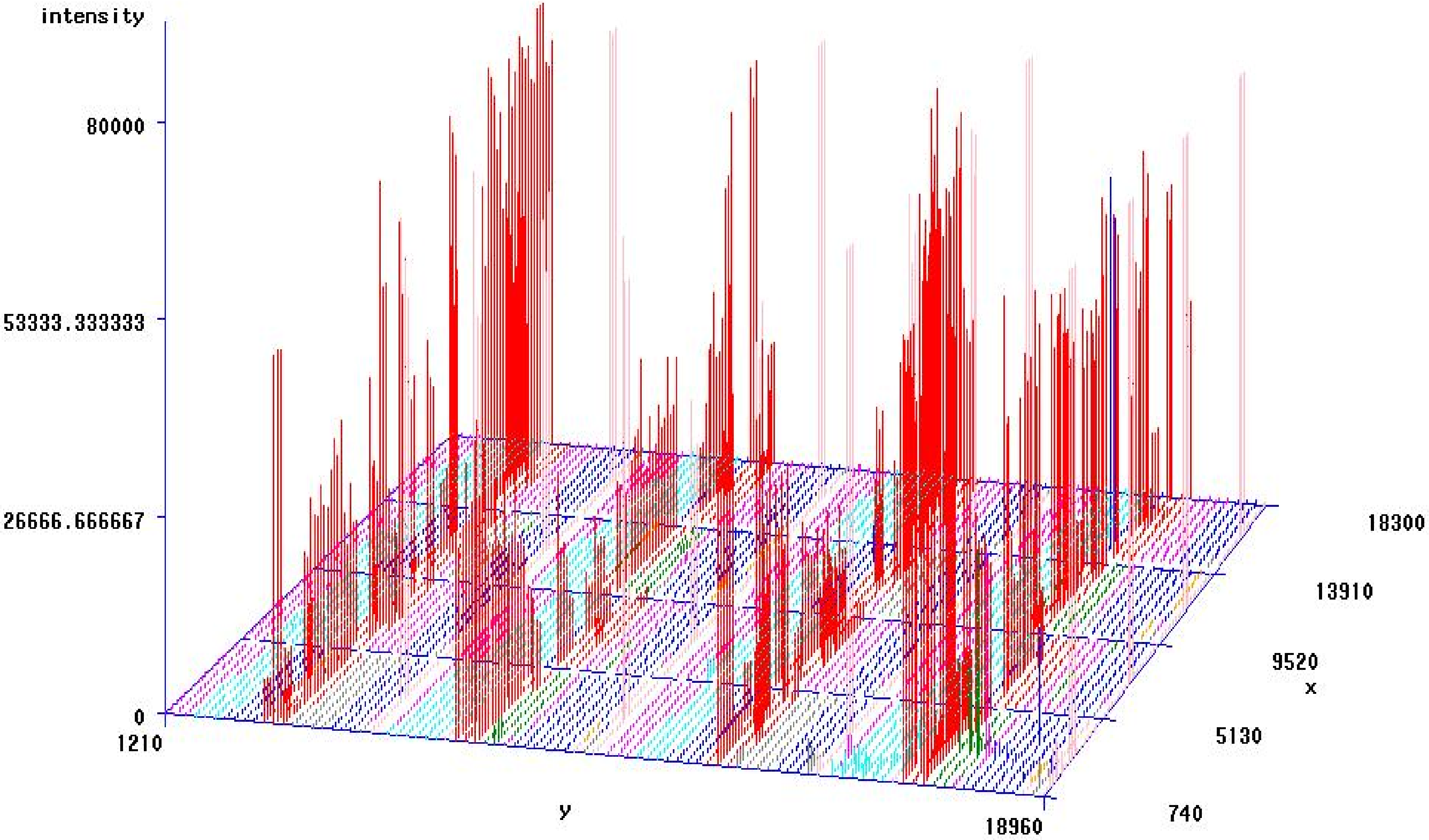


example of response
(genes of Campylobacter sp. are reported in blue)



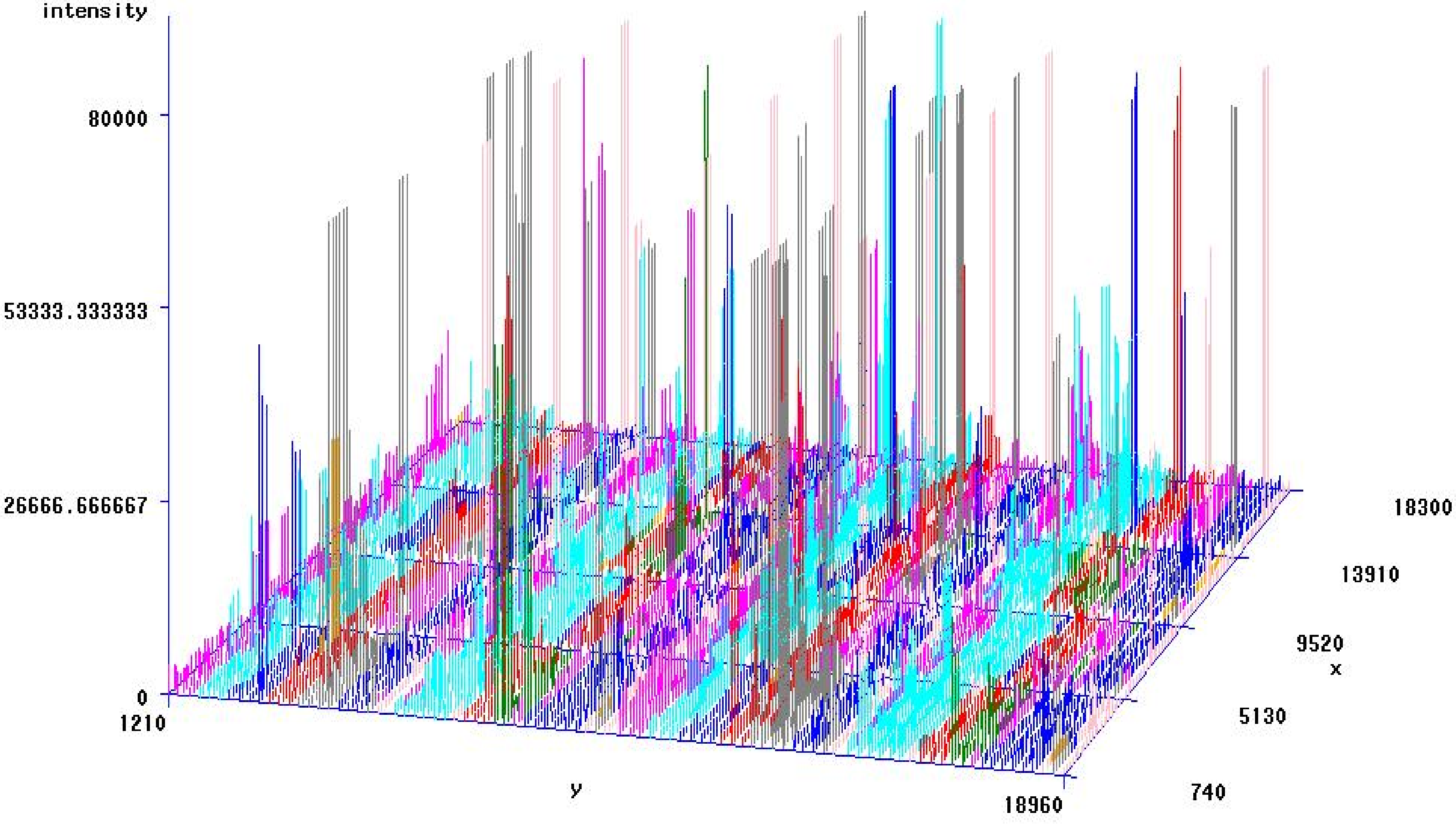
Staphylococcus_aureus_ATCC33862

example of response
(genes of Staphylococcus sp. are reported in red)



L_innocua_ATCC33090

example of response
(genes of Listeria sp. are reported in grey)



Hybridization patterns with mono-specific samples

MODEL

$$Y = \mu + F + e$$

μ = general mean

F = effect of classification factor

e = residual error

Intensity= μ + Genus + residual error

Hybridization patterns with mono-specific samples

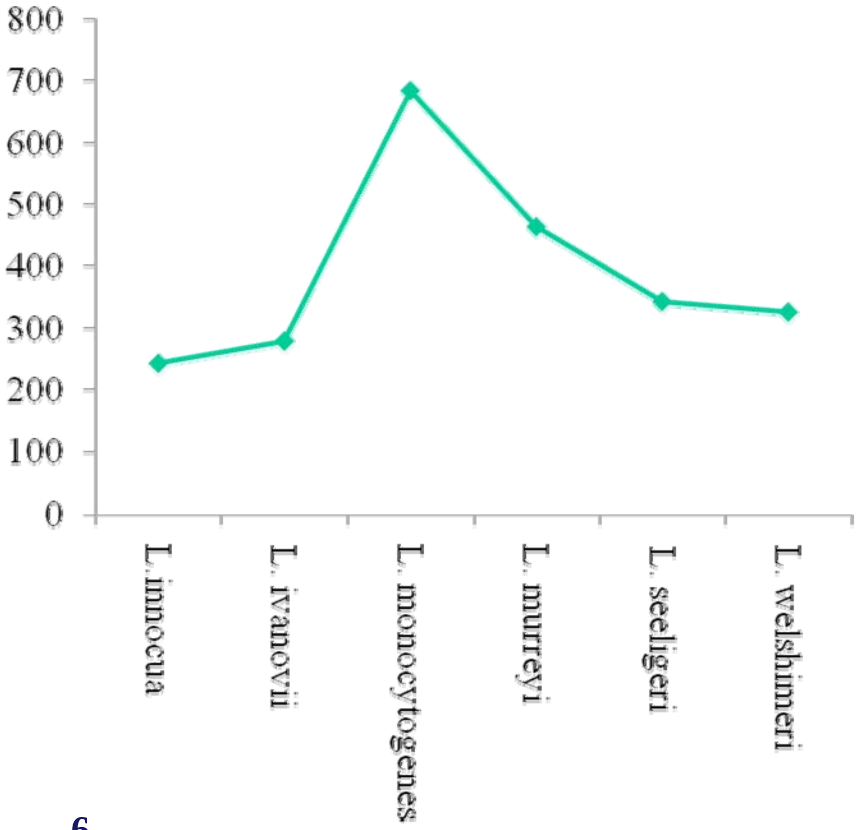
La procedura GLM

```
                    Variabile dipendente: accrescimento

                          Somma dei          Media        Valore
Origine            DF      quadrati       quadratica        F       Pr > F
Modello             2   30700.00000    15350.00000       7.59     0.0003
Errore             15   30350.00000     2023.33333
Totale corretto    17   61050.00000

        R-quadro    Var coeff    Radice MSE    Intensity Media
        0.502867    6.938017      44.98148        448.3333


                                                 Media        Valore
Origine            DF     Tipo I SS         quadratica        F       Pr > F
Intensity           5   30700.00000    15350.00000        7.59     0.0003

                                                  Media        Valore
Origine            DF    Tipo III SS        quadratica        F       Pr > F
Intensity           5   30700.00000    15350.00000        7.59     0.0003
```

Hybridization patterns with mono-specific samples

MODEL

LEVELS	ch1_Intensity LSMEAN	Numero LSMEAN
Listeria innocua	242.642414	1
Listeria ivanovii	278.123474	2
Listeria monocytogenes	683.617900	3
Listeria murrayi	463.034826	4
Listeria seeligeri	342.738803	5
Listeria welshimeri	325.289662	6



i/j	1	2	3	4	5	6
1		0.7364	<.0001	0.2752	0.6200	0.4612
2	0.7364		<.0001	0.3716	0.7548	0.6966
3	<.0001	<.0001		0.2530	0.0777	0.0002
4	0.2752	0.3716	0.2530		0.6549	0.5127
5	0.6200	0.7548	0.0777	0.6549		0.9339
6	0.4612	0.6966	0.0002	0.5127	0.9339	

Hybridization patterns with mono-specific samples

MODEL

Correction of the data

Plain	Correction	Non significant array	Non significant organism
1	row data	3	2
2	log2 (intensity)	5	4
3	intensity – background	5	3
4	Log2(intensity – background)	5	6
5	median of 3 spot	20	6
6	mean of 3 spot	5	3
7	intensity – back. and median	14	6
8	intensity – back. and mean	13	6
9	mean/dev.st of 3 spot	42	14
10	median/dev.st of 3 spot	42	14

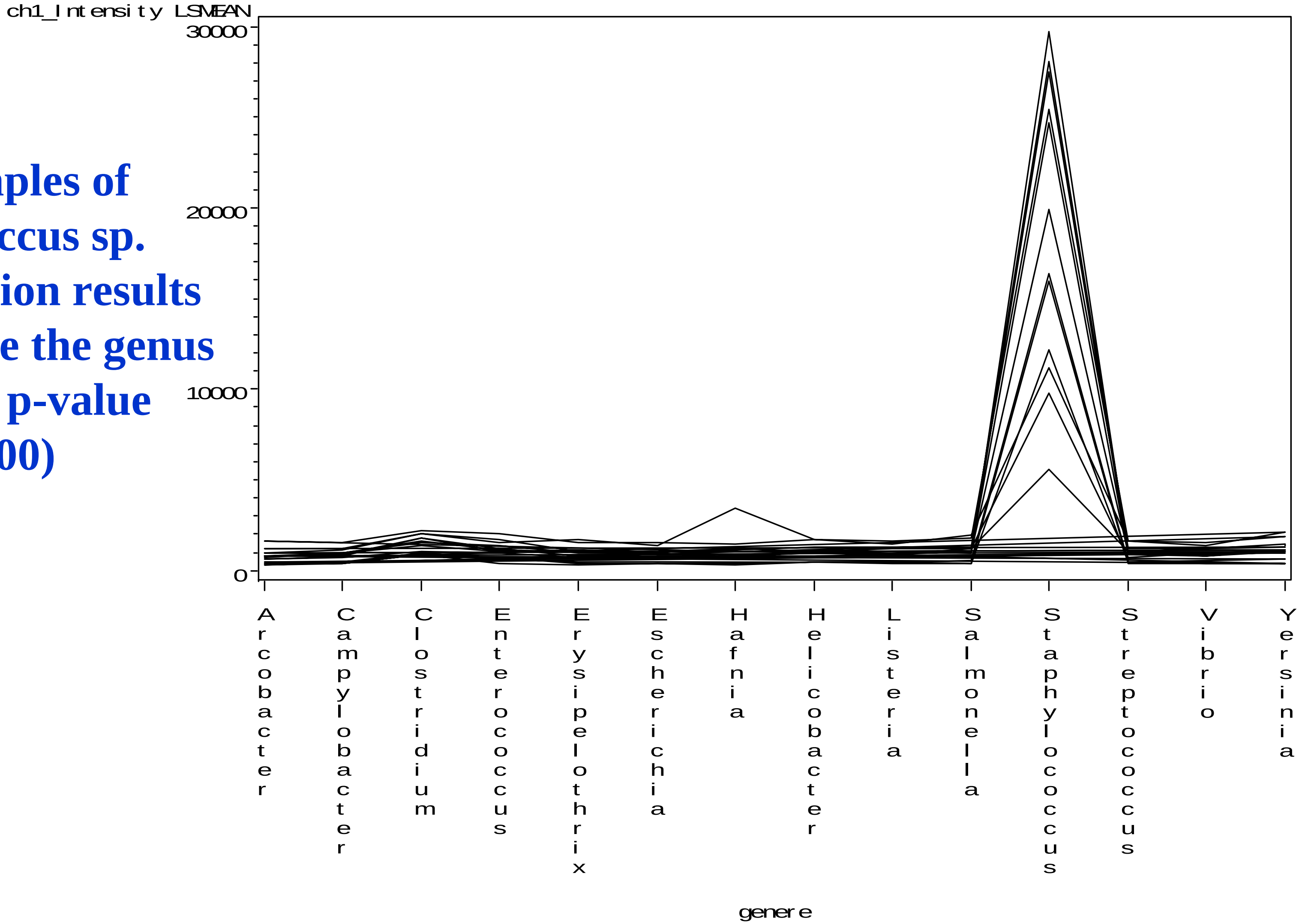
consequently, following analyses have been conducted with row data

Hybridization patterns with mono-specific samples

RESULTS

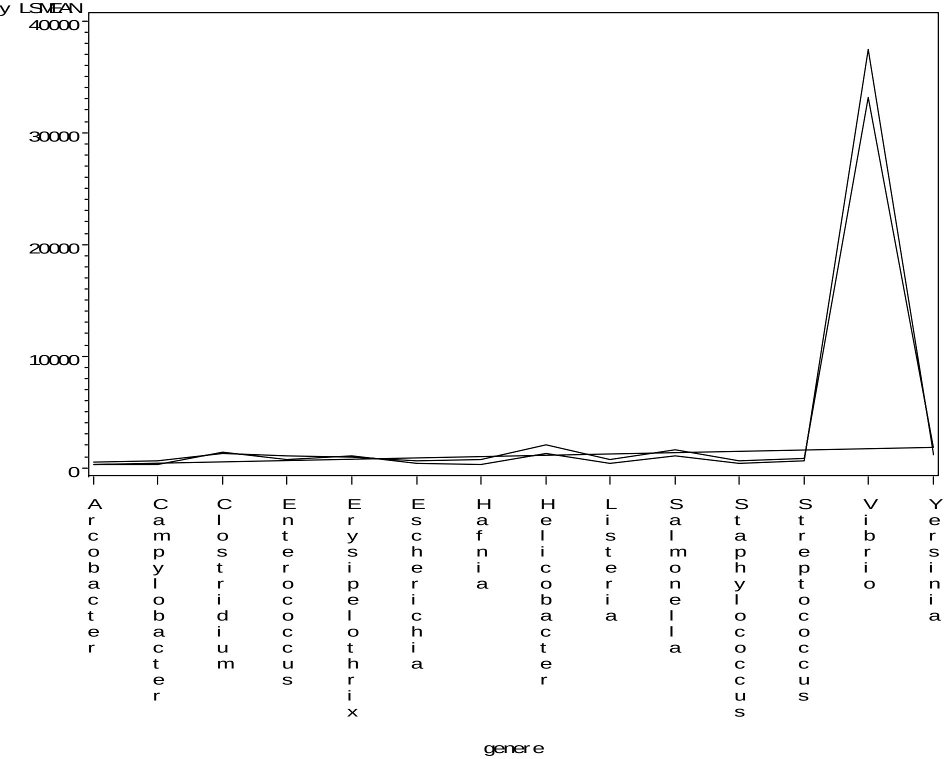
Staphylococcus

for all samples of Staphylococcus sp. the hybridization results clearly separate the genus with highli p-value ($p<0.000$)

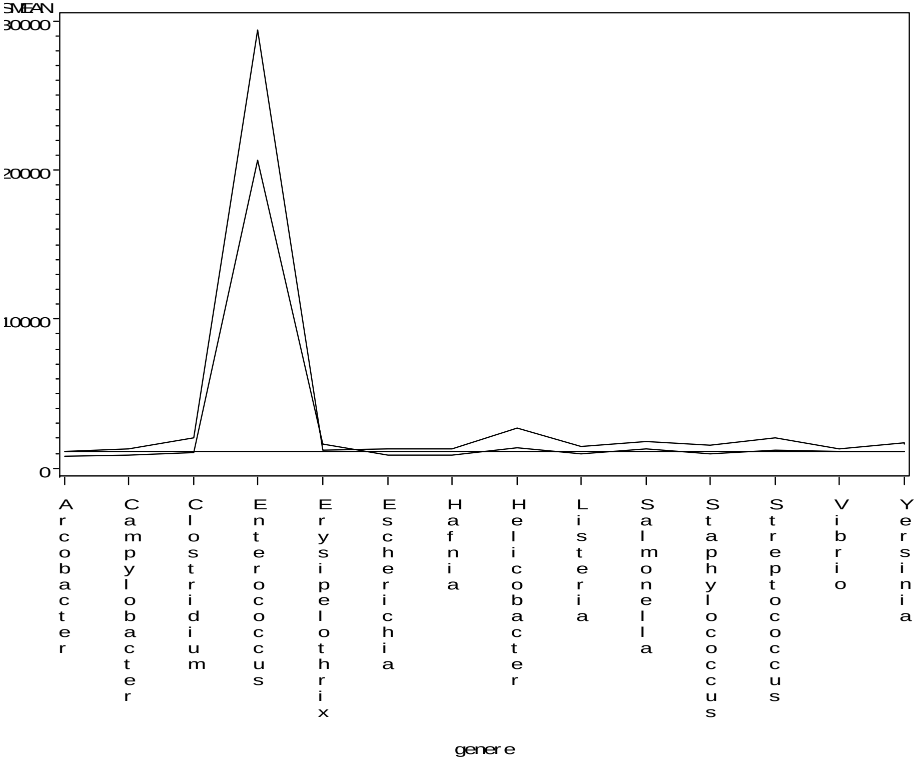


Hybridization patterns with mono-specific samples

Vibrio



Enterococcus

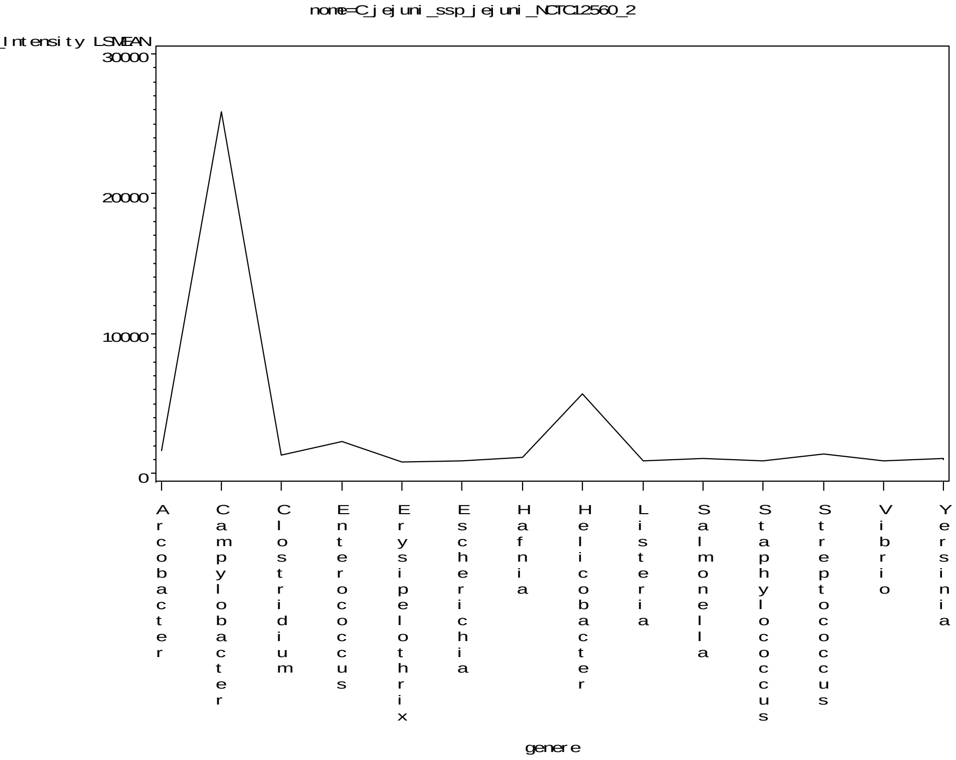


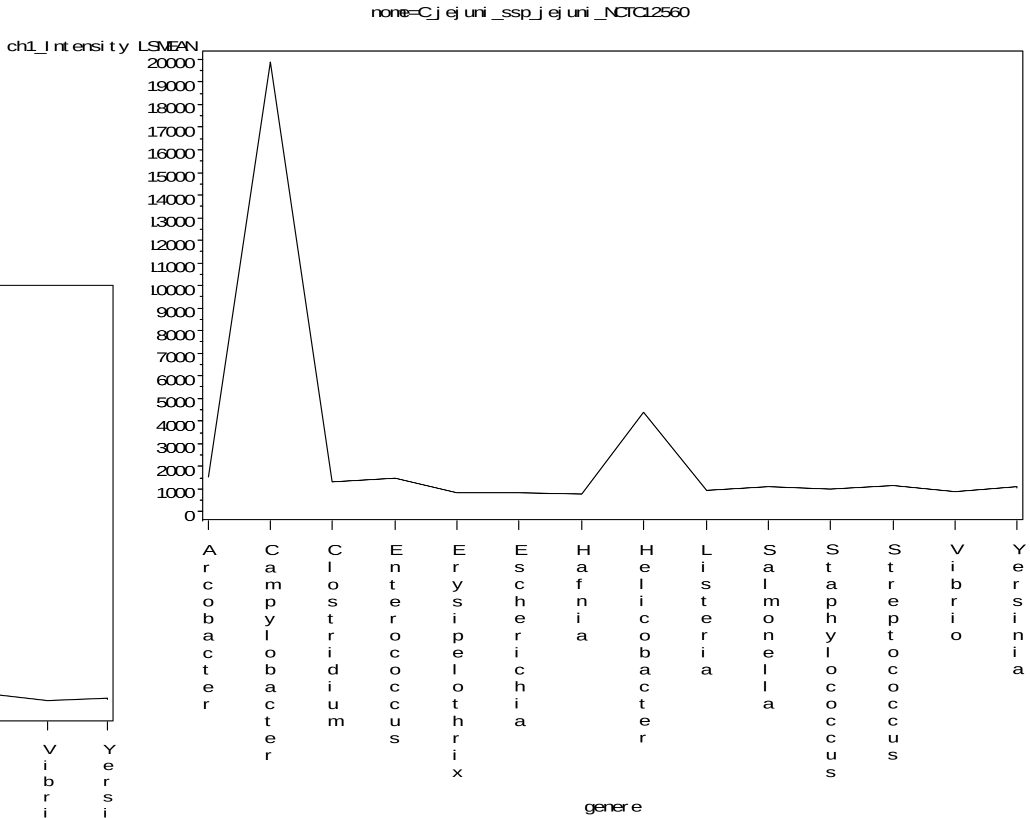
The separation is also clean for Vibrio sp. and Enterococcus sp.

Hybridization patterns with mono-specific samples

RESULTS

Camphylobacter



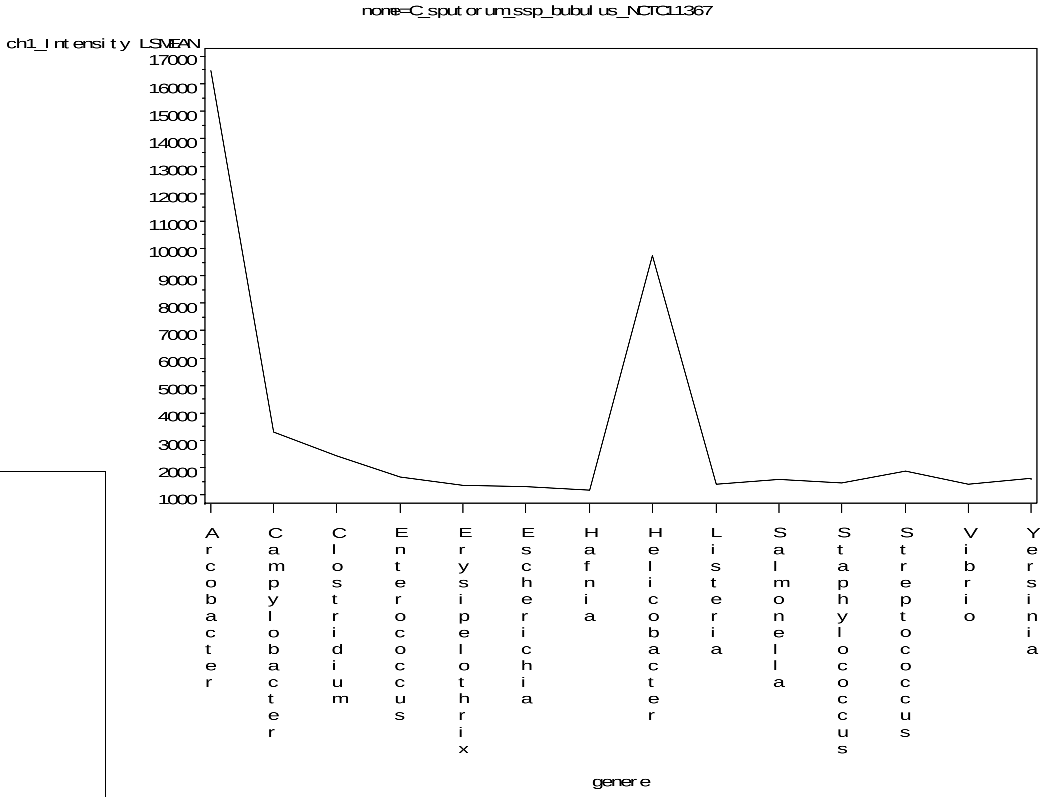


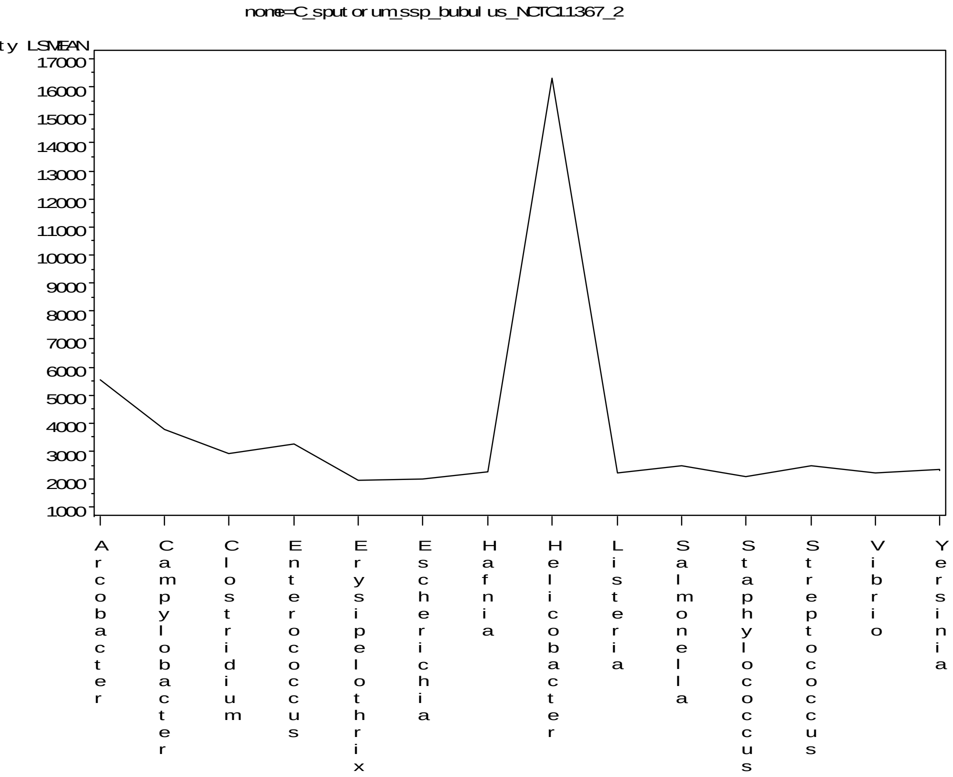
In the case of Campylobacter sp. the hybridization results show good resolution for C. jejuni

Hybridization patterns with mono-specific samples

RESULTS

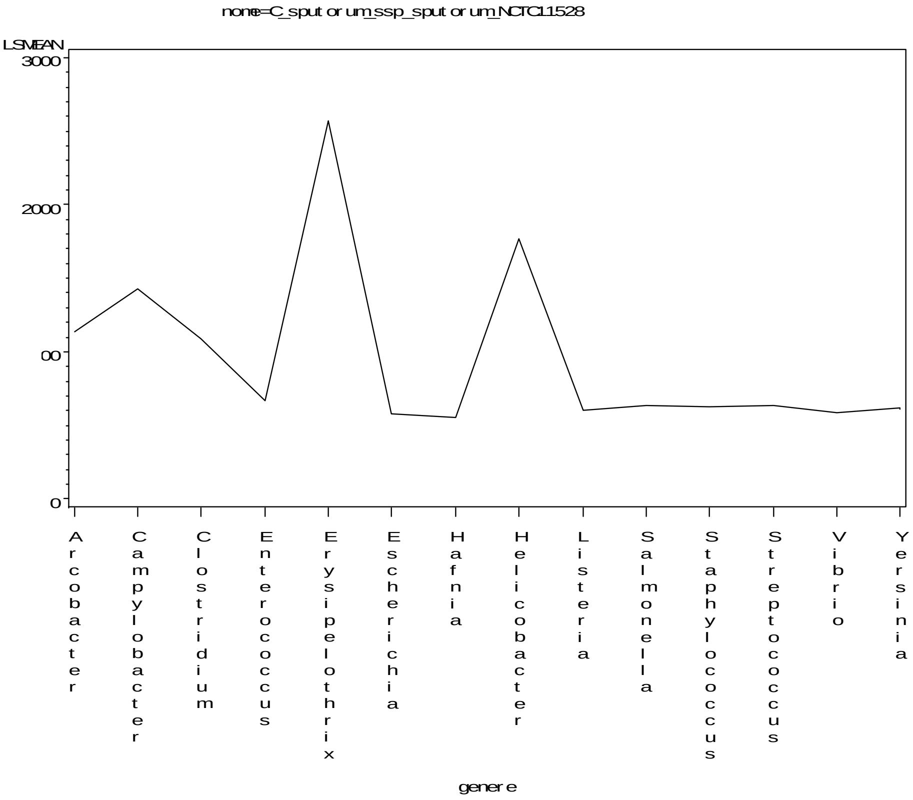
but contrasting patterns for
C. sputorum and C.upsaliensis

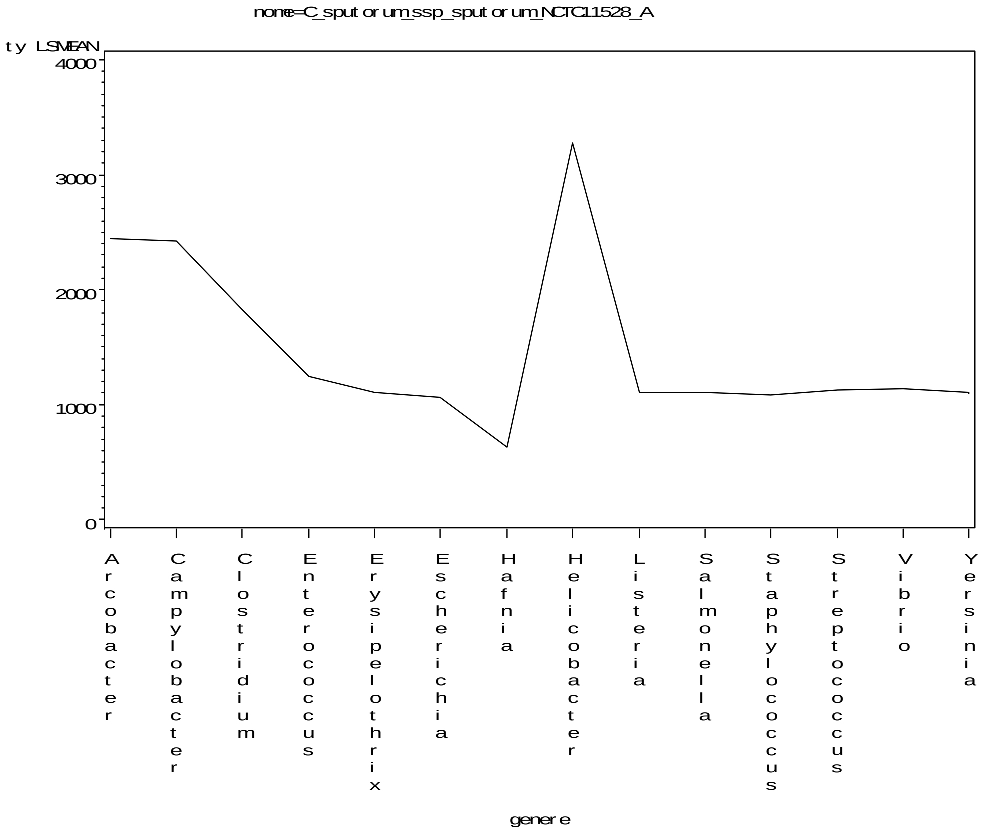




Hybridization patterns with mono-specific samples

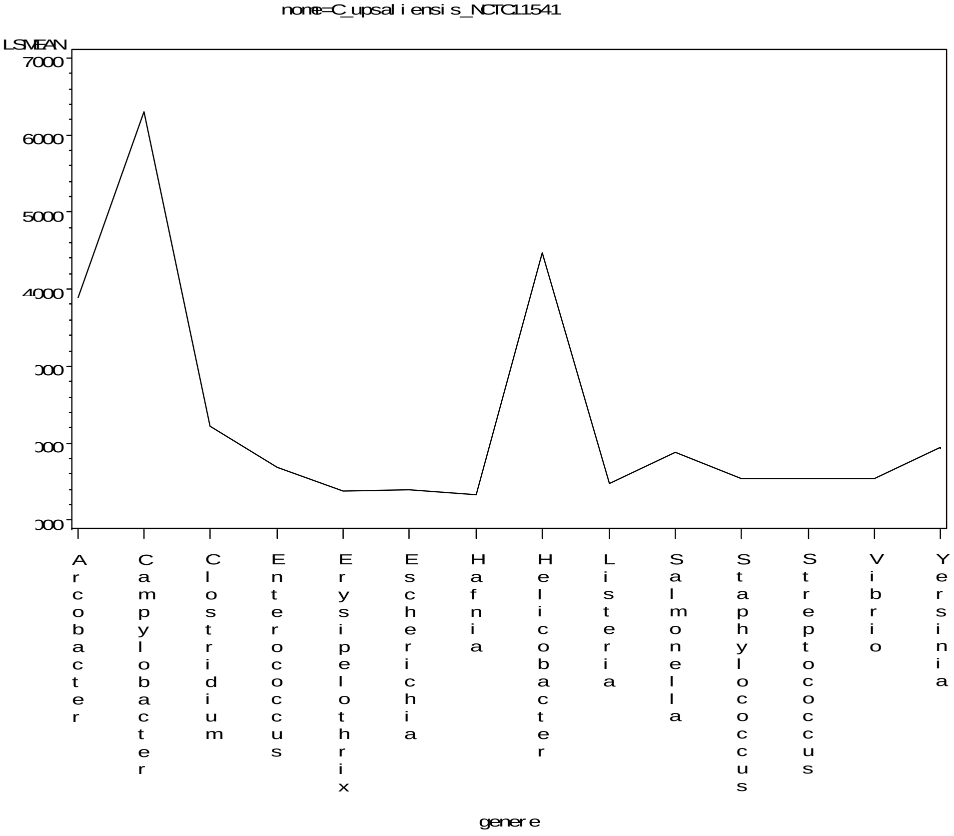
RESULTS

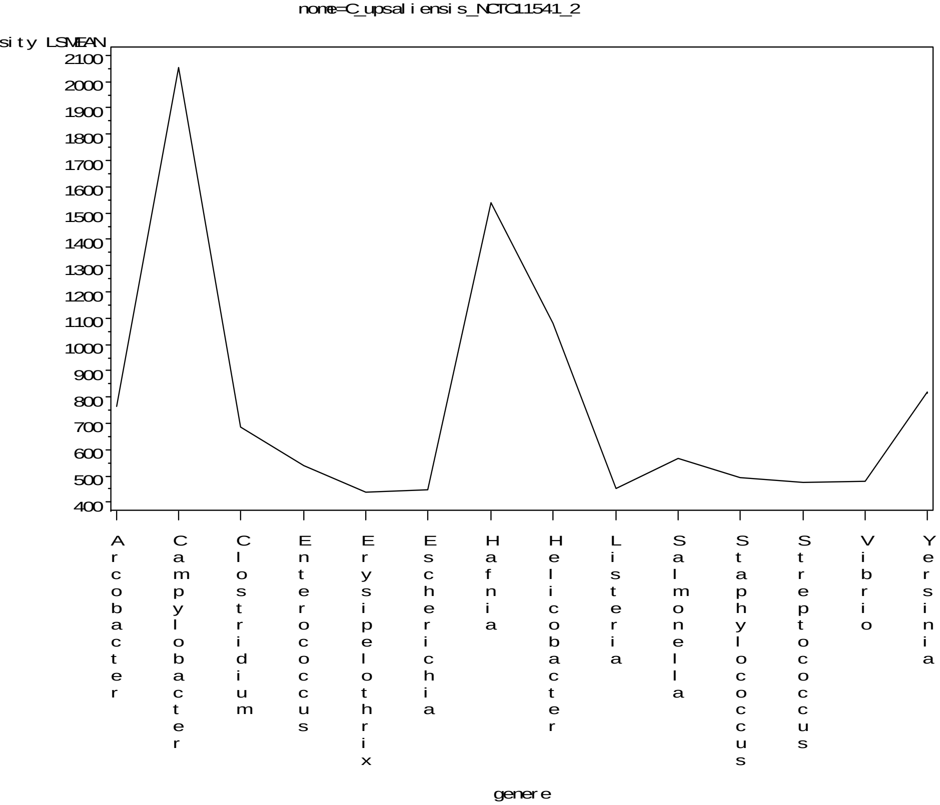




Hybridization patterns with mono-specific samples

RESULTS





Hybridization patterns with mono-specific samples

RESULTS

Helicobacter
Campylobacter
Arcobacter
Hafnia
Erysipelothrix

are the genus that mostly react

when the arrays are hybridized

with Campylobacter sp. samples

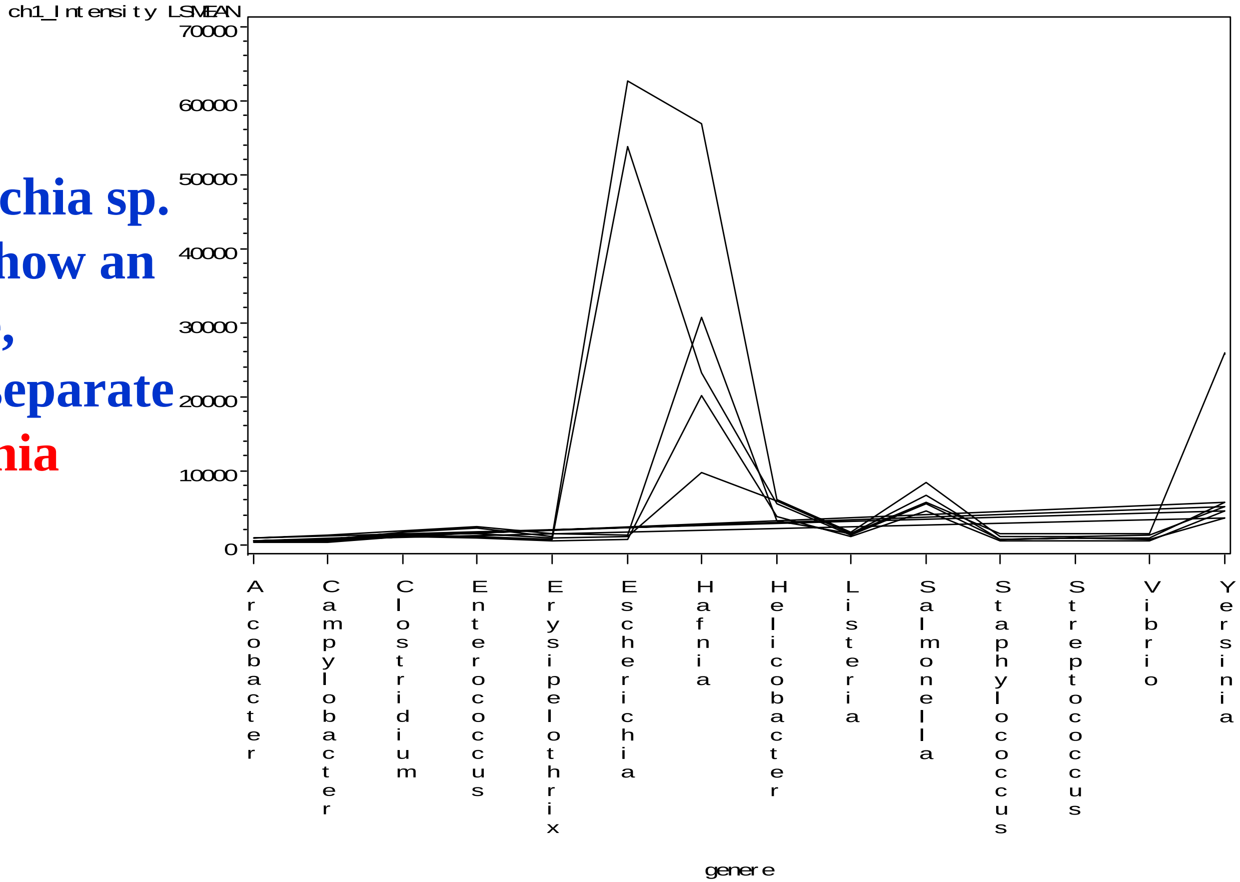
Organismo ibridizzato	Genere	p-value
C_sputorum_ssp_sputorum_NCTC11528_A	Erysipelothrix	0,055754
C_sputorum_ssp_bubulus_NCTC11367	Escherichia	0,093715
C_sputorum_ssp_sputorum_NCTC11528_A	Helicobacter	0,123145
C_sputorum_ssp_sputorum_NCTC11528_A	Escherichia	0,123212
C_sputorum_ssp_bubulus_NCTC11367_2	Escherichia	0,123295
C_sputorum_ssp_bubulus_NCTC11367	Vibrio	0,137295
C_sputorum_ssp_bubulus_NCTC11367_2	Enterococcus	0,146986
C_upsaliensis_NCTC11541	Hafnia	0,169748
C_sputorum_ssp_sputorum_NCTC11528_A	Vibrio	0,184806
C_sputorum_ssp_sputorum_NCTC11528	Escherichia	0,189894
C_sputorum_ssp_bubulus_NCTC11367_2	Vibrio	0,217498
C_upsaliensis_NCTC11541_2	Campylobacter	0,228154
C_sputorum_ssp_sputorum_NCTC11528	Vibrio	0,235477
C_upsaliensis_NCTC11541	Campylobacter	0,345405
C_sputorum_ssp_bubulus_NCTC11367_2	Campylobacter	0,368935
C_sputorum_ssp_sputorum_NCTC11528	Helicobacter	0,402555
C_sputorum_ssp_sputorum_NCTC11528_A	Hafnia	0,405986
C_sputorum_ssp_bubulus_NCTC11367	Hafnia	0,457731
C_sputorum_ssp_sputorum_NCTC11528	Hafnia	0,579974
C_sputorum_ssp_bubulus_NCTC11367_2	Hafnia	0,585699
C_upsaliensis_NCTC11541_2	Hafnia	0,733746
C_sputorum_ssp_sputorum_NCTC11528	Campylobacter	0,794605
C_sputorum_ssp_sputorum_NCTC11528_A	Campylobacter	0,993182

Hybridization patterns with mono-specific samples

RESULTS

Escherichia

**Also In the case of Escherichia sp.
the hybridization results show an
ambiguous response,
it is not possible to clearly separate
Escherichia from Hafnia**

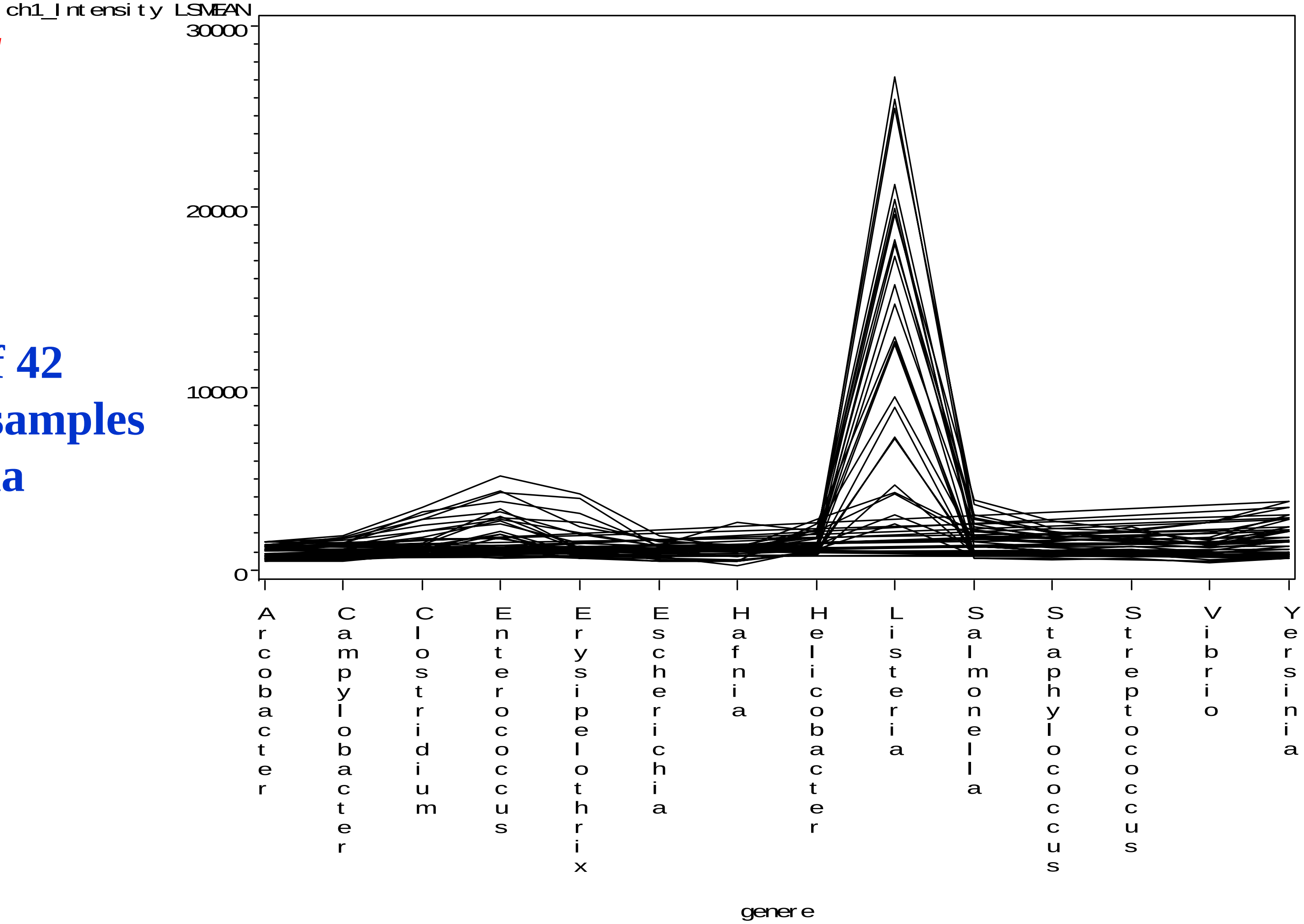


Hybridization patterns with mono-specific samples

RESULTS

Listeria

response of 42 mono-specific samples of Listeria

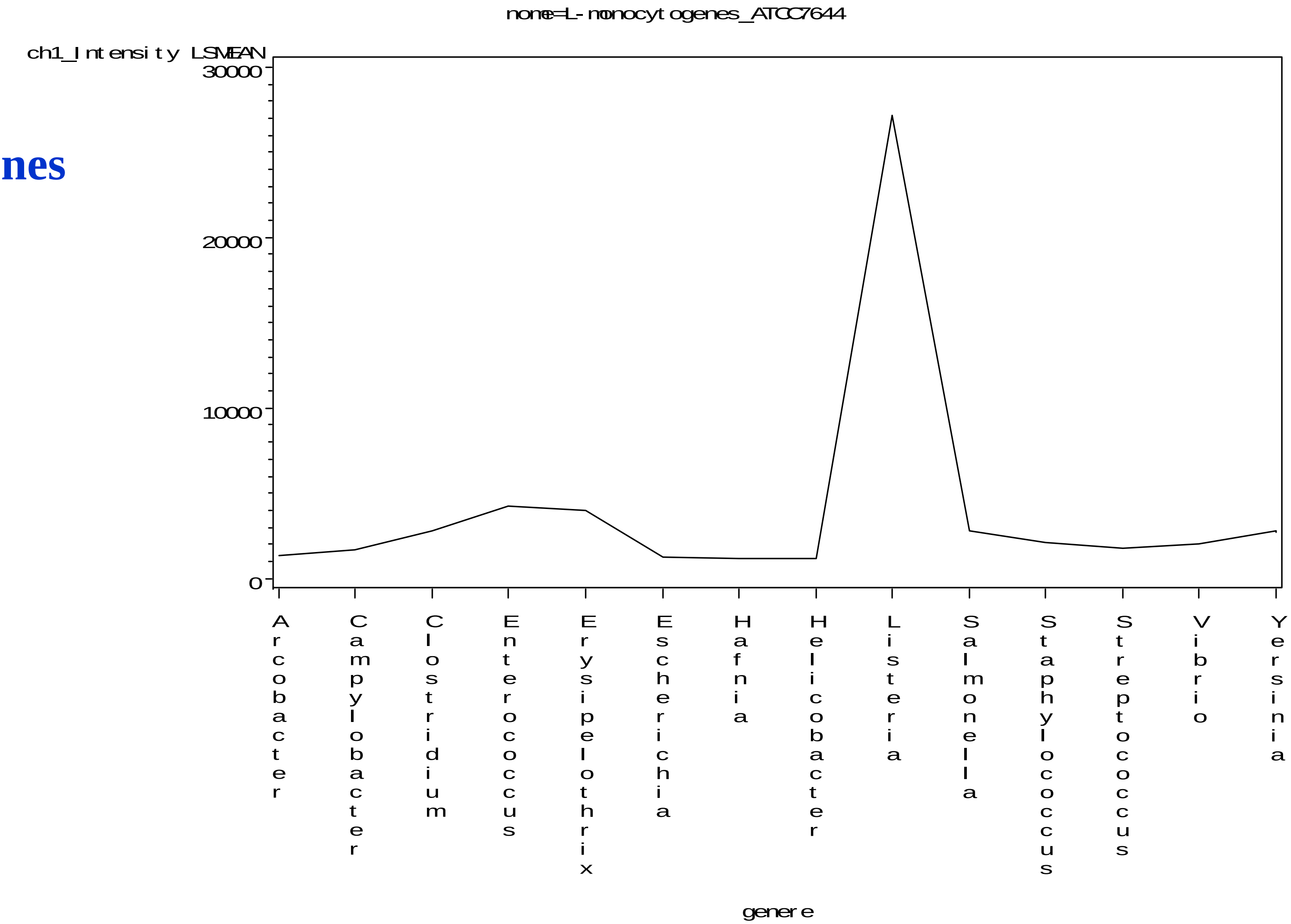


In almost all the cases it is possible to distinguish the hybridized genus with a highly p-value ($p<0.000$)

Hybridization patterns with mono-specific samples

RESULTS

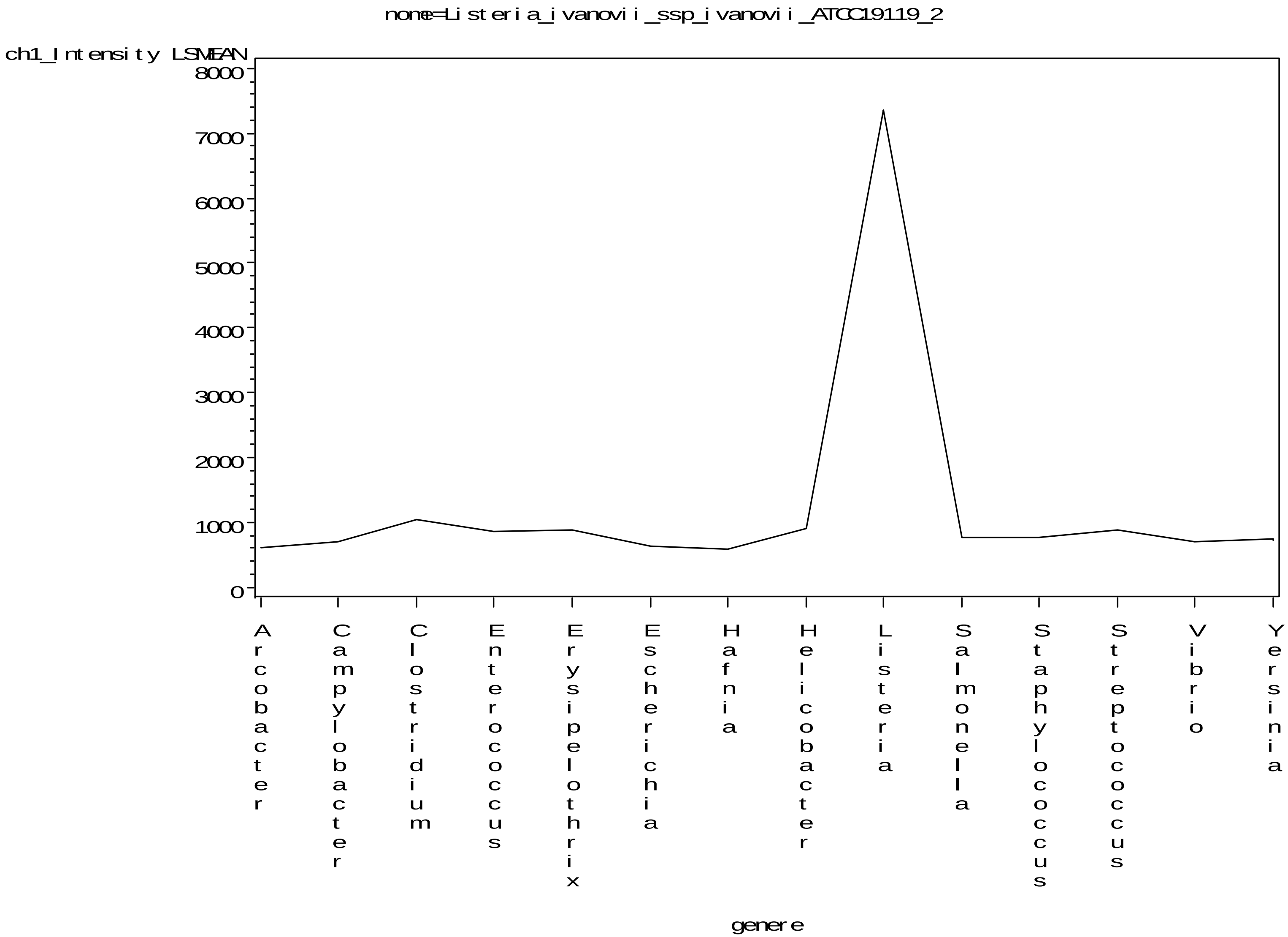
Listeria monocytogenes



Hybridization patterns with mono-specific samples

RESULTS

Listeria ivanovii



Hybridization patterns with mono-specific samples

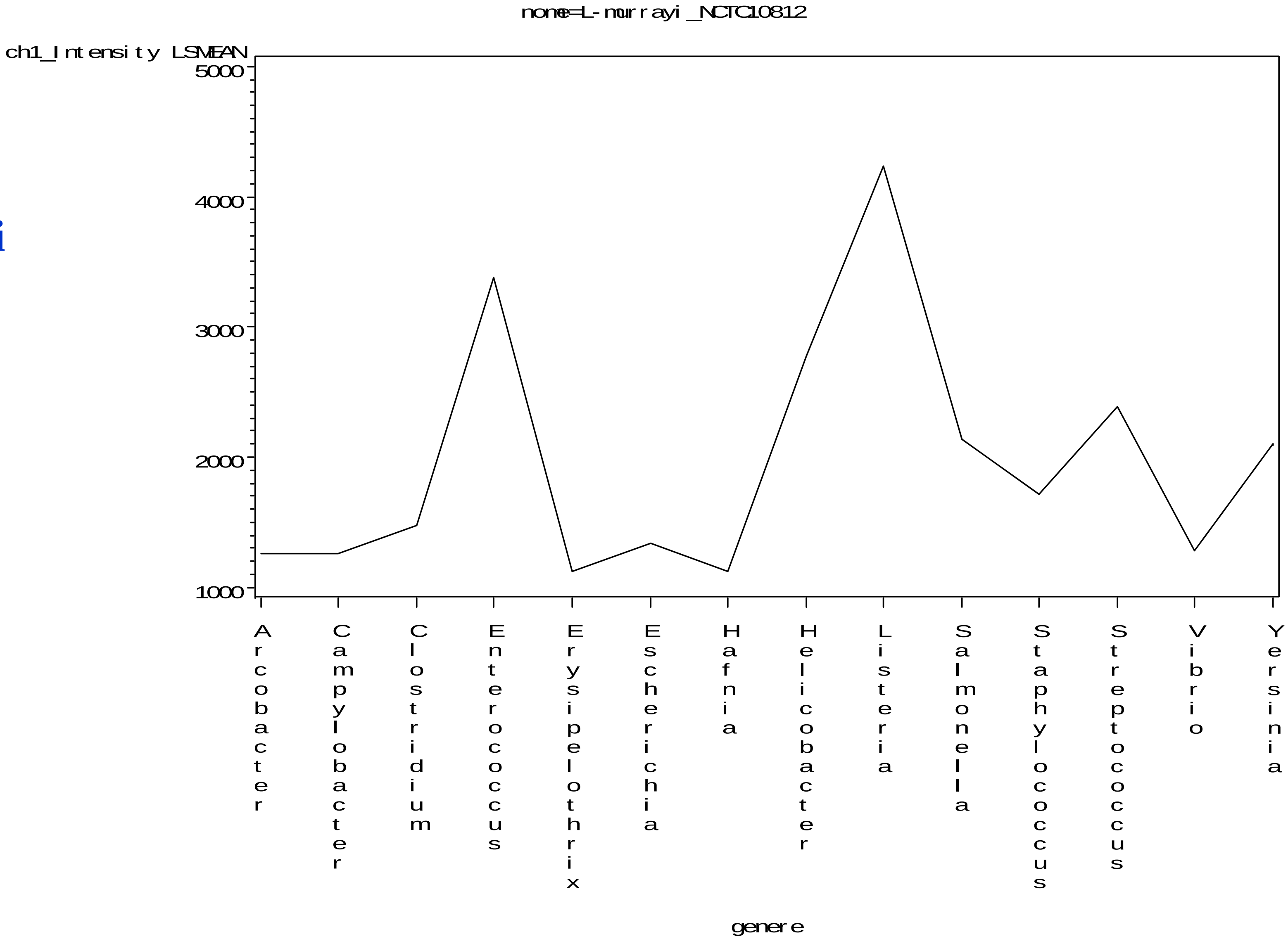
Hybridization results obtained with L. murrayi are less clear: Enterococcus and Hafnia don't statistically result different from Listeria

Organismo ibridizzato	Genere	p-value
L-murrayi_NCTC10812_2	Enterococcus	0,077
L-murrayi_NCTC10812	Enterococcus	0,110
L-murrayi_NCTC10812_2	Hafnia	0,121
L_grayi_ATCC19120_2	Hafnia	0,199
L-murrayi_NCTC10812	Hafnia	0,234
L_grayi_ATCC19120	Hafnia	0,400

Hybridization patterns with mono-specific samples

RESULTS

Listeria murrayi



Hybridization patterns with mono-specific samples

with the aim to verify the discriminating power of the array with regard to level - species the same model has been used for Listeria sp.

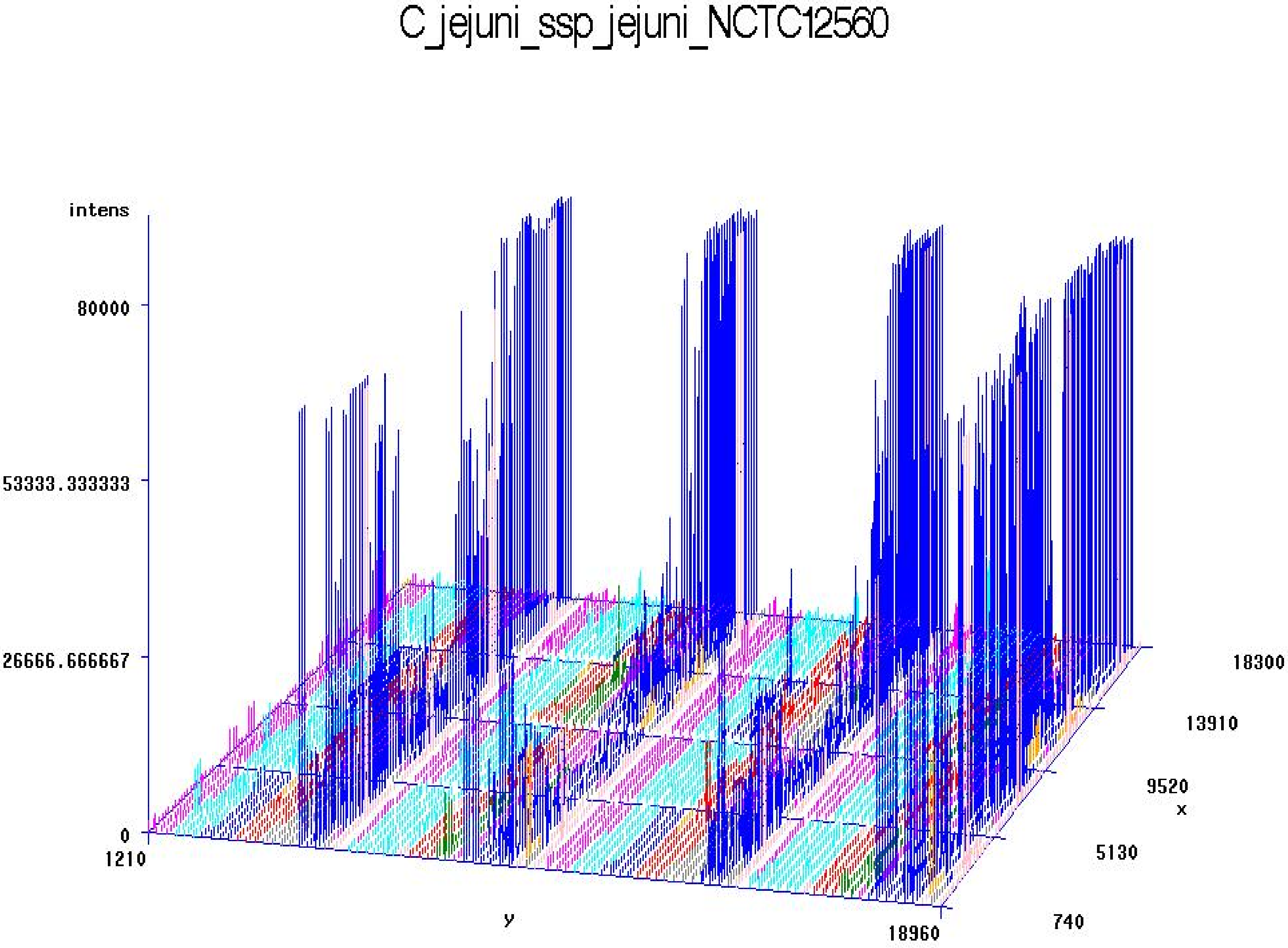
In this case:

Intensity = general mean + species + residual error

In this case, for every species, the average of the intensity of the genes of Listeria sp. has been calculated on all the arrays except those of the species in object

Such value has been taken out from the intensity of the species analysed with the purpose of "to polishing up" the signal from the response of the genes of a species if that one is lacking

Hybridization patterns with mono-specific samples



Hybridization patterns with mono-specific samples

RESULTS

Listeria monocytogenes

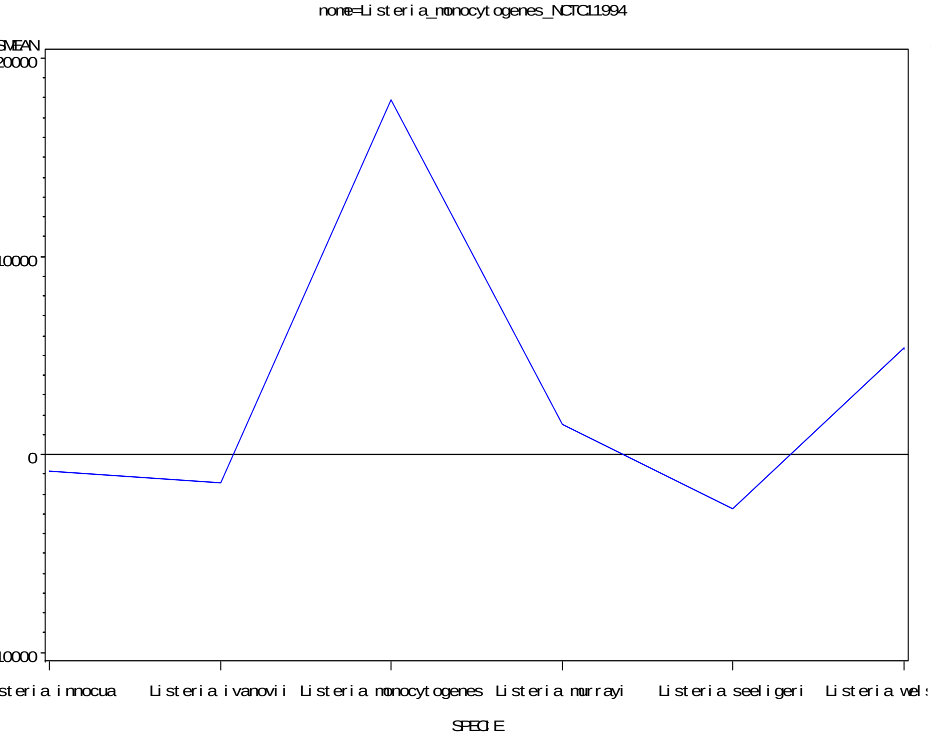
Array	Duplicato
Listeria_monocytogenes_ATCC10888	Listeria_monocytogenes_ATCC10888_2
Listeria_monocytogenes_NCTC11994	Listeria_monocytogenes_NCTC11994_2
Listeria_monocytogenes_Type1_ATCC984	Listeria_monocytogenes_Type1_ATCC984_2
Listeria_monocytogenes_USDA_FS15_H2426	Listeria_monocytogenes_USDA_FS15_H2426_2
Listeria_monocytogenes_serotype4b_ATCC13932	Listeria_monocytogenes_serotype4b_ATCC13932_2
Listeria_monocytogenes_serovar1_2a_NCTC10887	Listeria_monocytogenes_serovar1_2a_NCTC10887_2
Listeria_monocytogenes_serovar1a_NCTC4886	Listeria_monocytogenes_serovar1a_NCTC4886_2
Listeria_monocytogenes_serovar4c_NCTC4883	Listeria_monocytogenes_serovar4c_NCTC4883_2
Listeria_monocytogenes_serovar7_NCTC10890	Listeria_monocytogenes_serovar7_NCTC10890_2

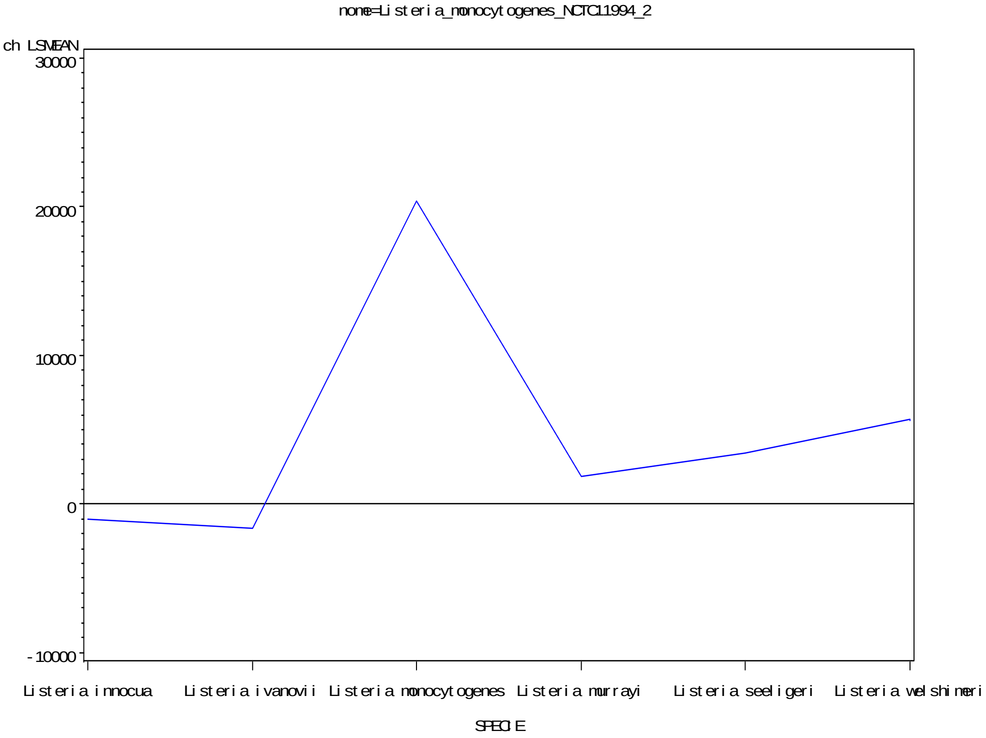
for nine samples of L. monocytogenes the species is perfectly individualized with good repeatability between arrays and replicas

Hybridization patterns with mono-specific samples

RESULTS

Listeria monocytogenes

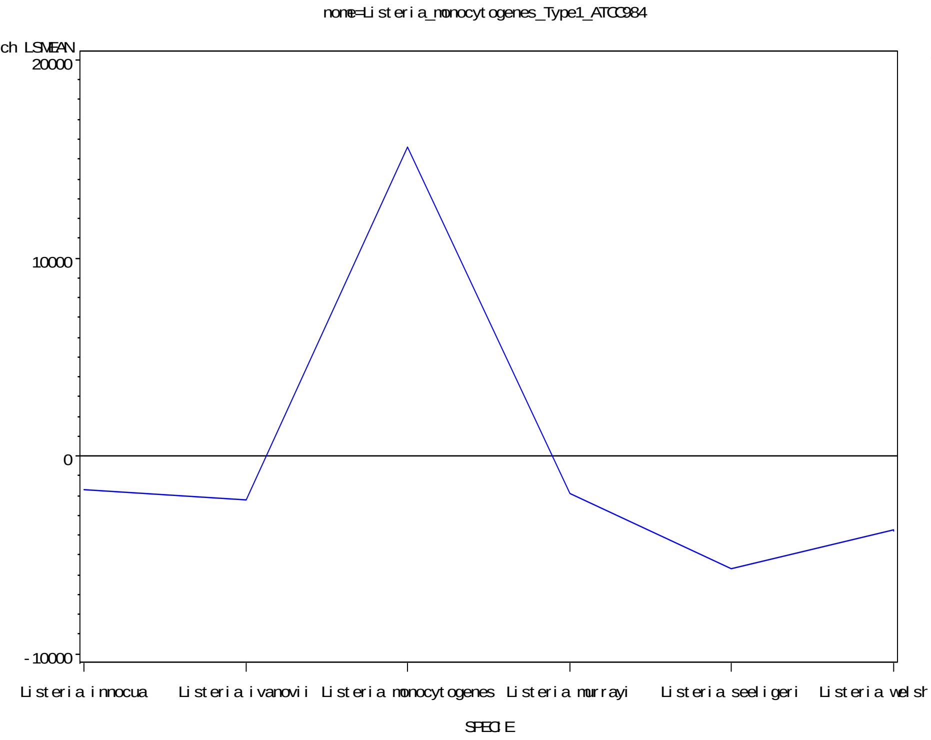


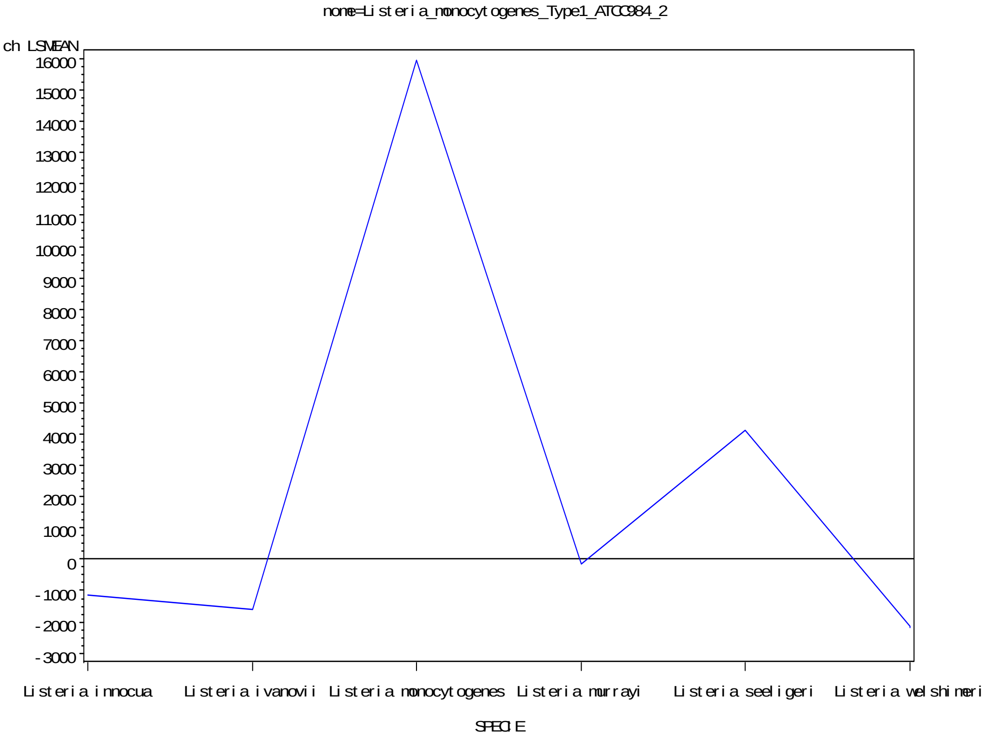


Hybridization patterns with mono-specific samples

RESULTS

Listeria monocytogenes

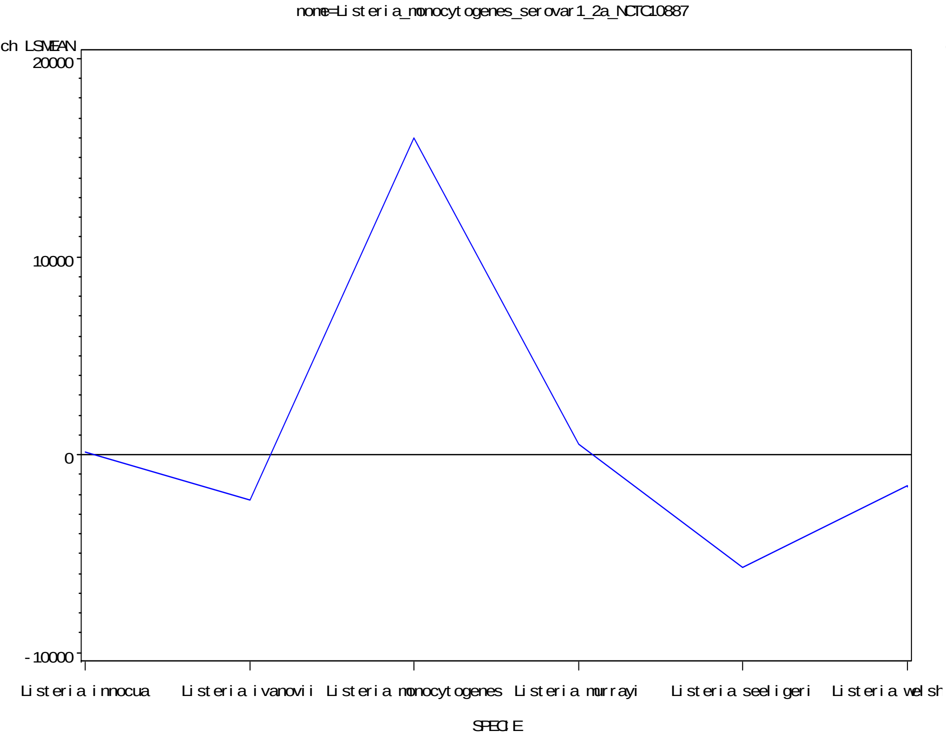


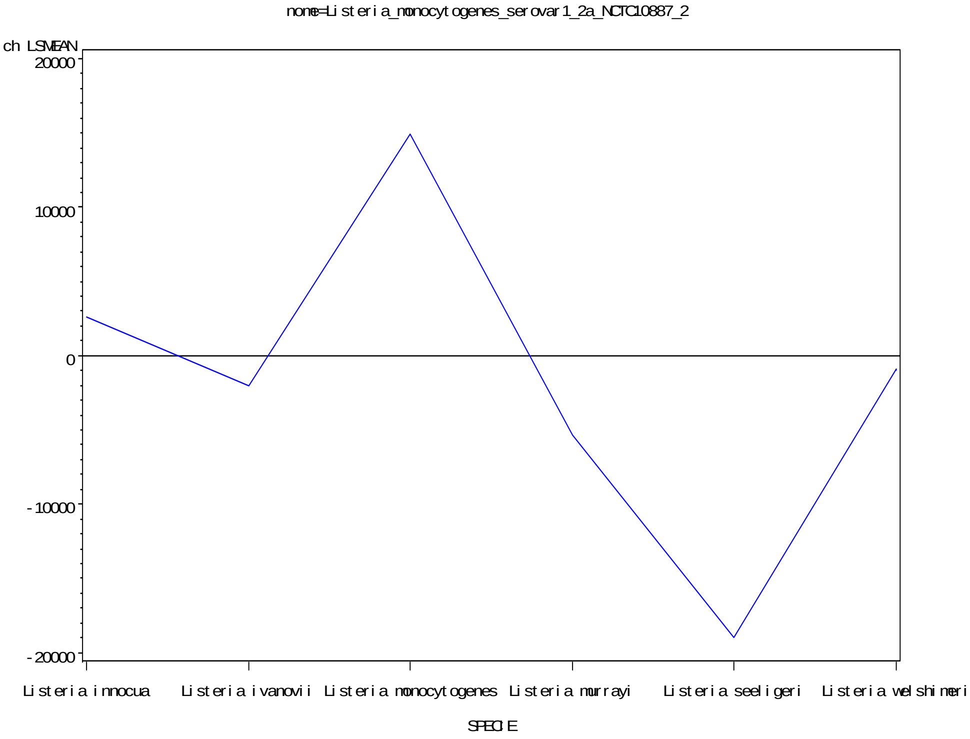


Hybridization patterns with mono-specific samples

RESULTS

Listeria monocytogenes

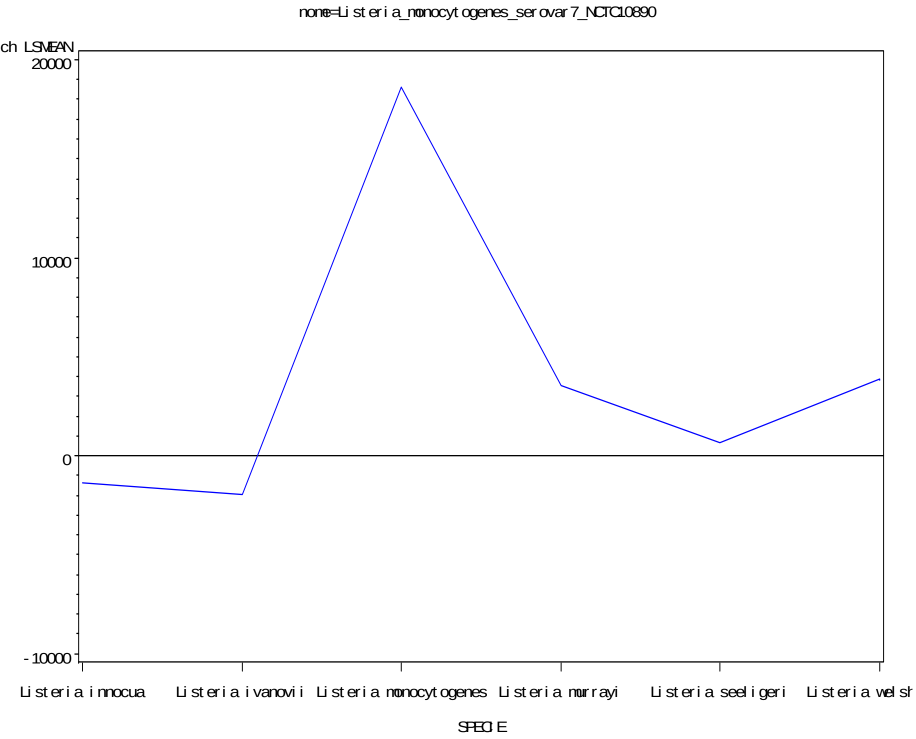


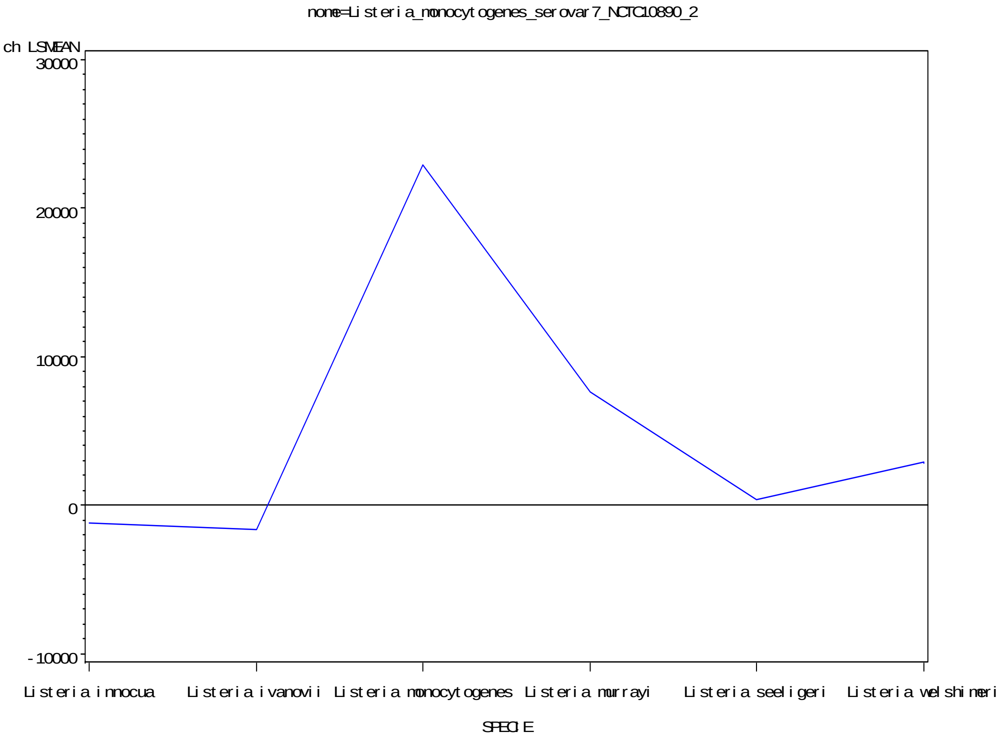


Hybridization patterns with mono-specific samples

RESULTS

Listeria monocytogenes





Hybridization patterns with mono-specific samples

RESULTS

Listeria monocytogenes

for the following samples a low repeatability was found between the array and his duplicate,

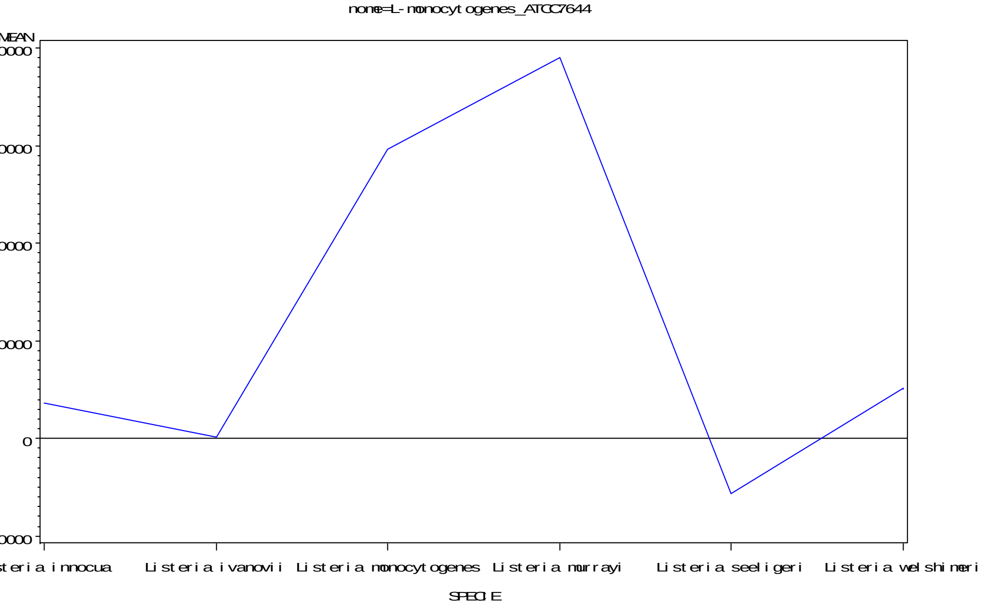
Array	**Duplicato**
L-monocytogenes_ATCC7644	L-monocytogenes_ATCC7644_2
L-monocytogenes_serovar3_NCTC5105	L-monocytogenes_serovar3_NCTC5105_2
L-monocytogenes_sierotype4_ATCC19115	L-monocytogenes_sierotype4_ATCC19115_2
L-monocytogenes_typeII_ATCC9525	L-monocytogenes_typeII_ATCC9525_2

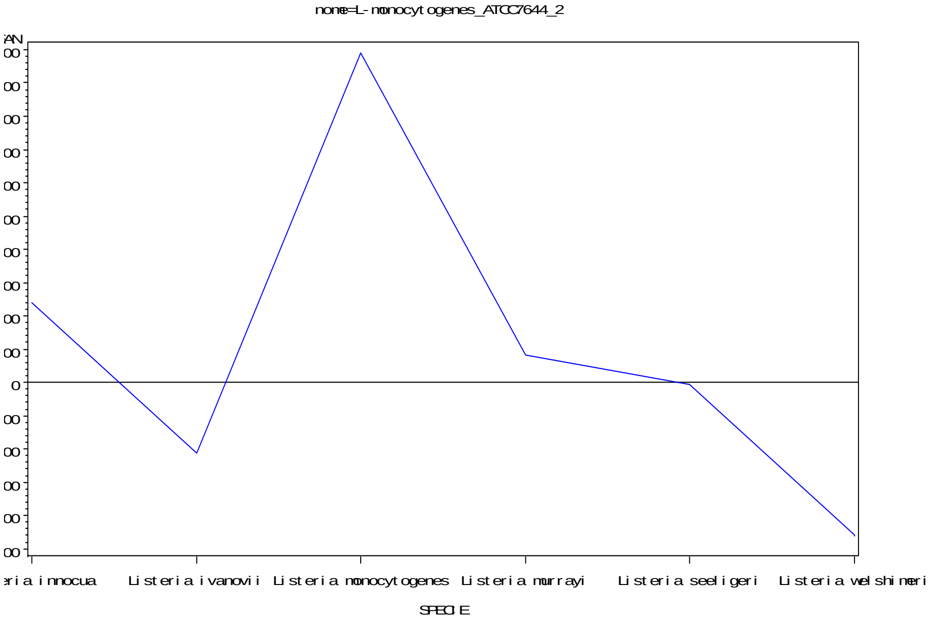
with a precise response in a case (L. monocytogenes is individualized in univocal way) and an ambiguous one in the other

Hybridization patterns with mono-specific samples

RESULTS

Listeria monocytogenes

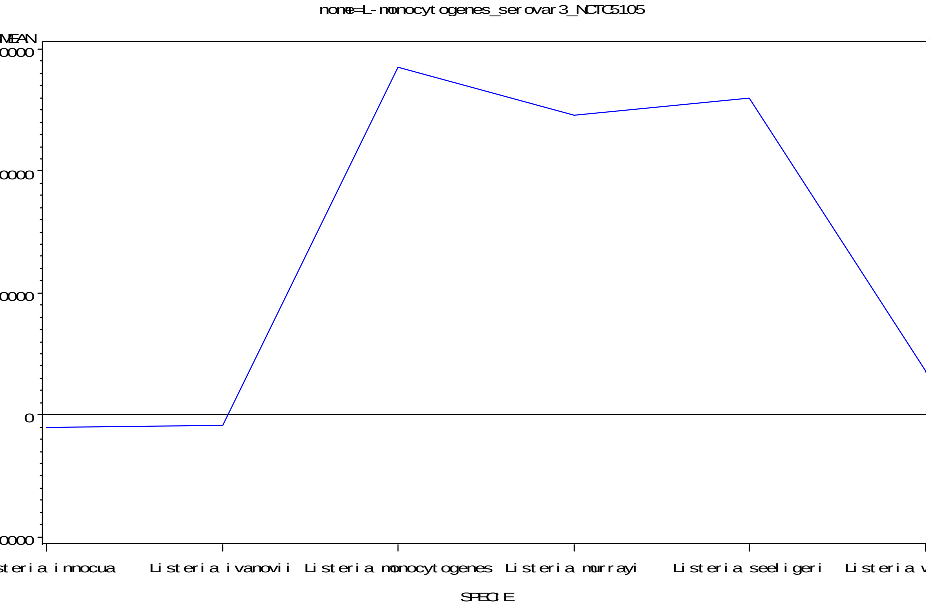


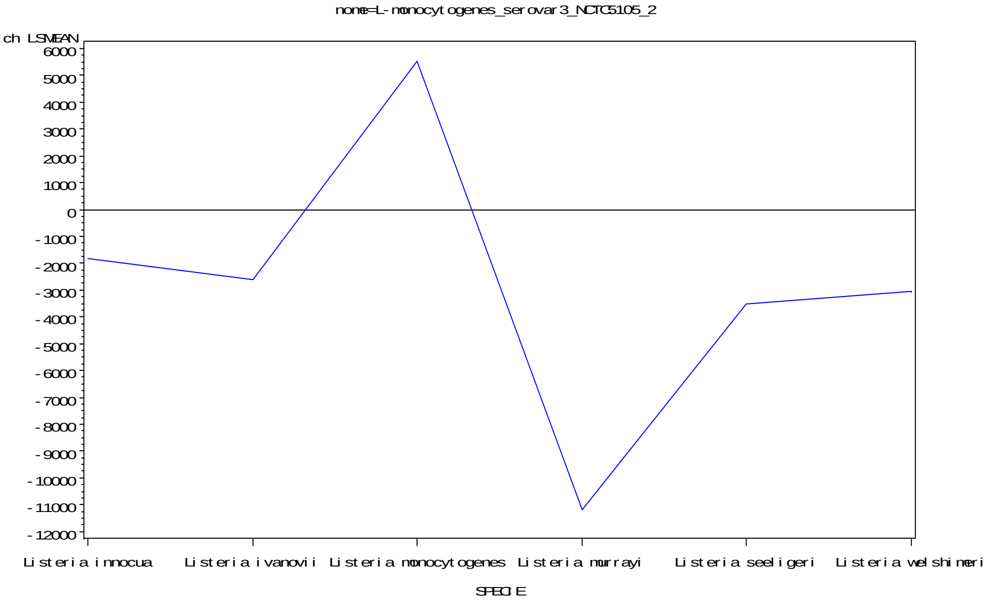


Hybridization patterns with mono-specific samples

RESULTS

Listeria monocytogenes

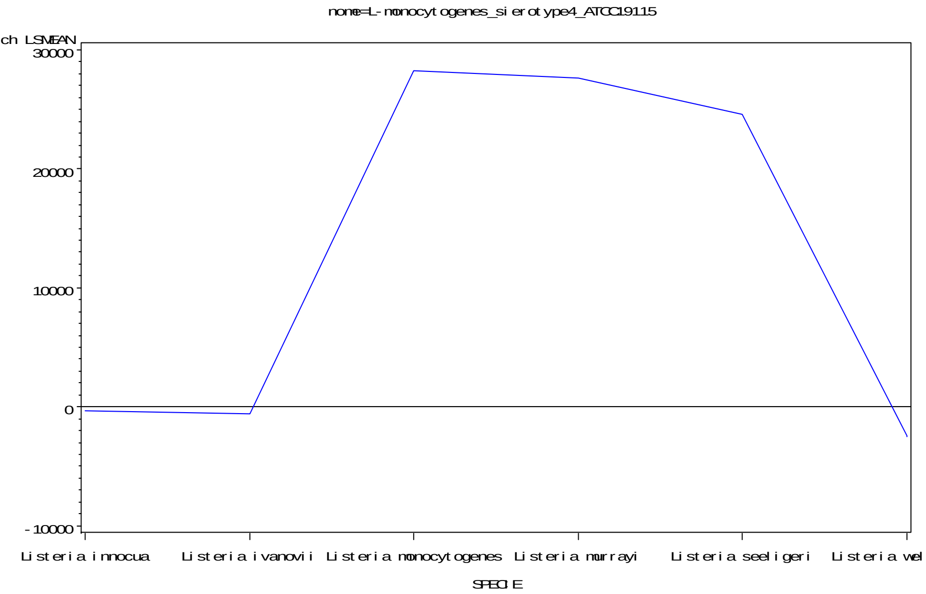


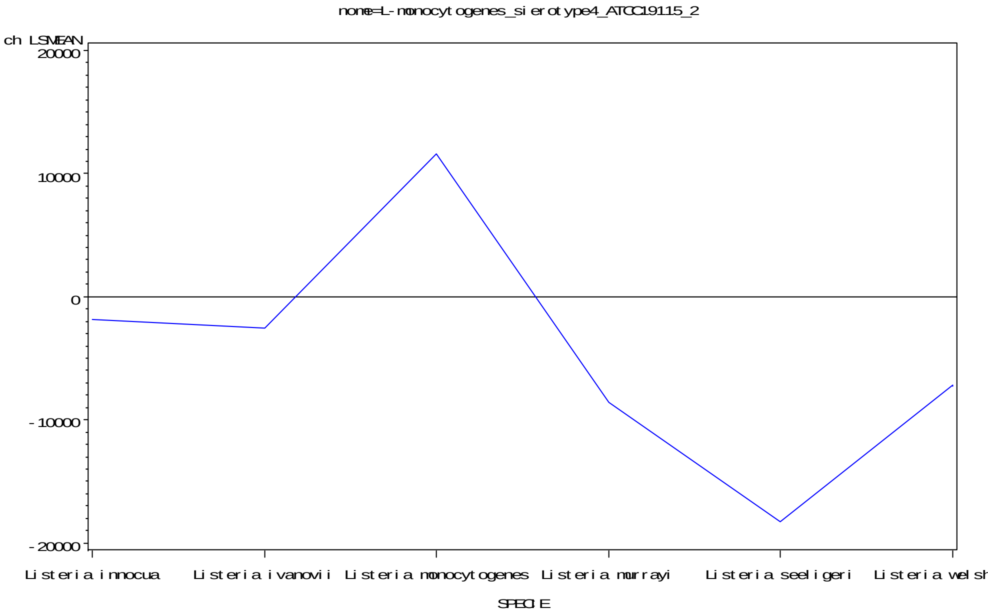


Hybridization patterns with mono-specific samples

RESULTS

Listeria monocytogenes

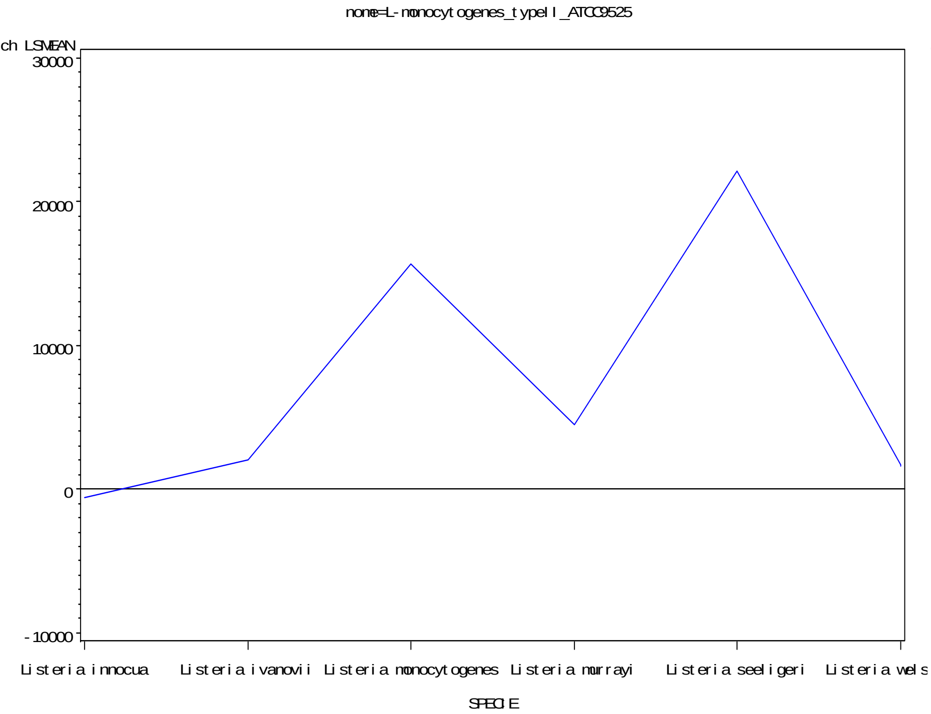


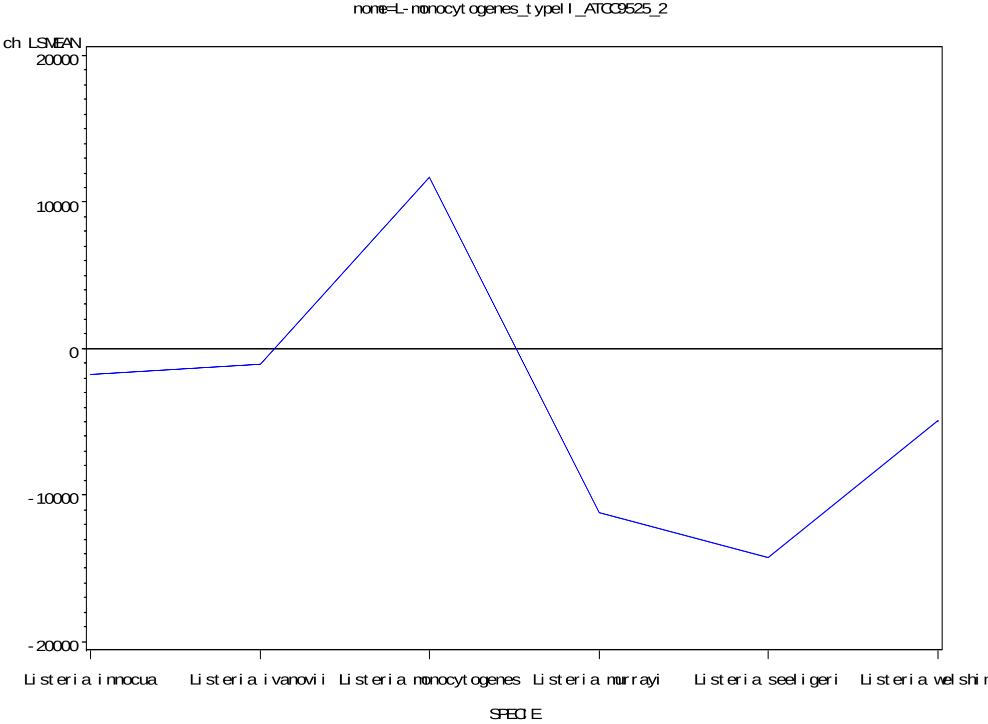


Hybridization patterns with mono-specific samples

RESULTS

Listeria monocytogenes



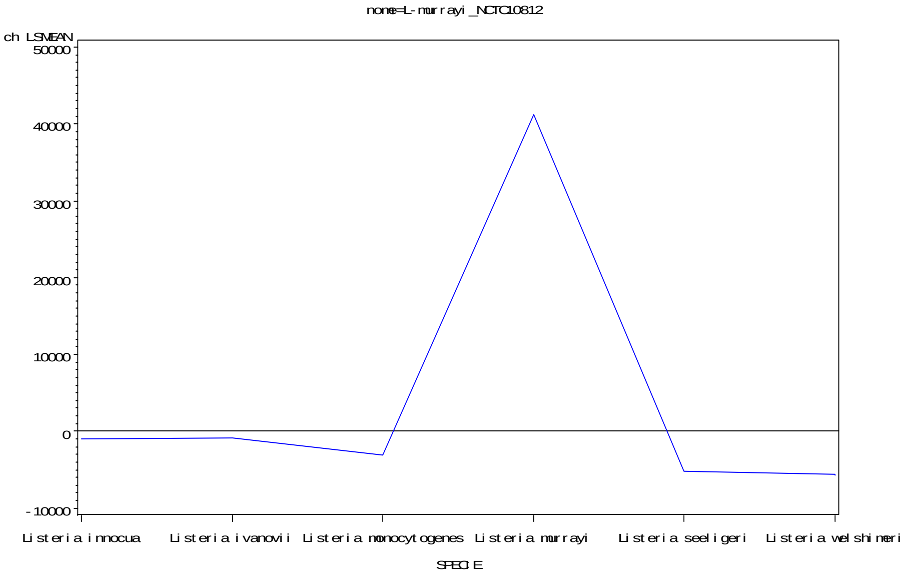


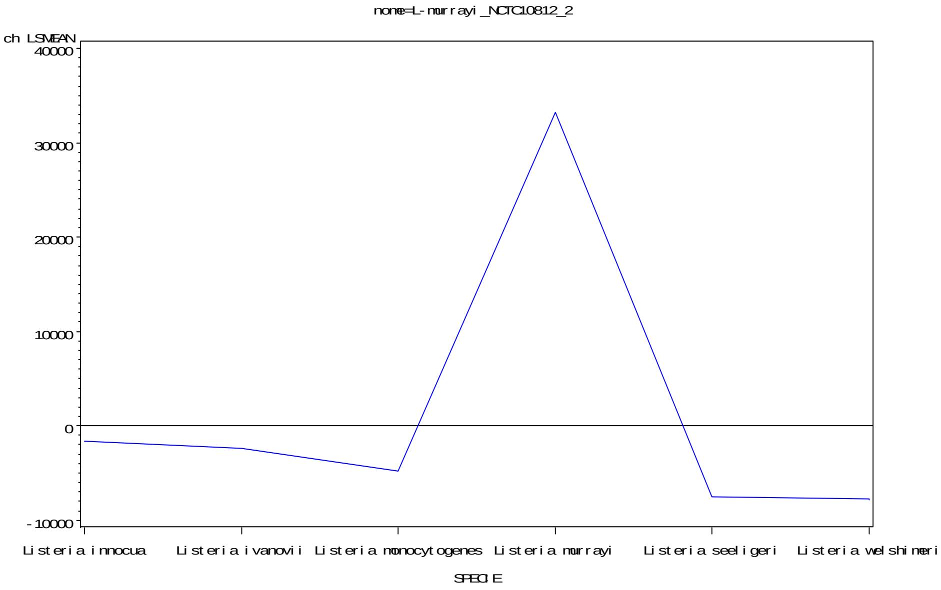
Hybridization patterns with mono-specific samples

RESULTS

Listeria murrayi

Array	Duplicato
L-murrayi_NCTC10812	L-murrayi_NCTC10812_2
L_grayi_ATCC19120	L_grayi_ATCC19120_2
L_grayi_ssp_grayi_NCTC10815	L_grayi_ssp_grayi_NCTC10815_2



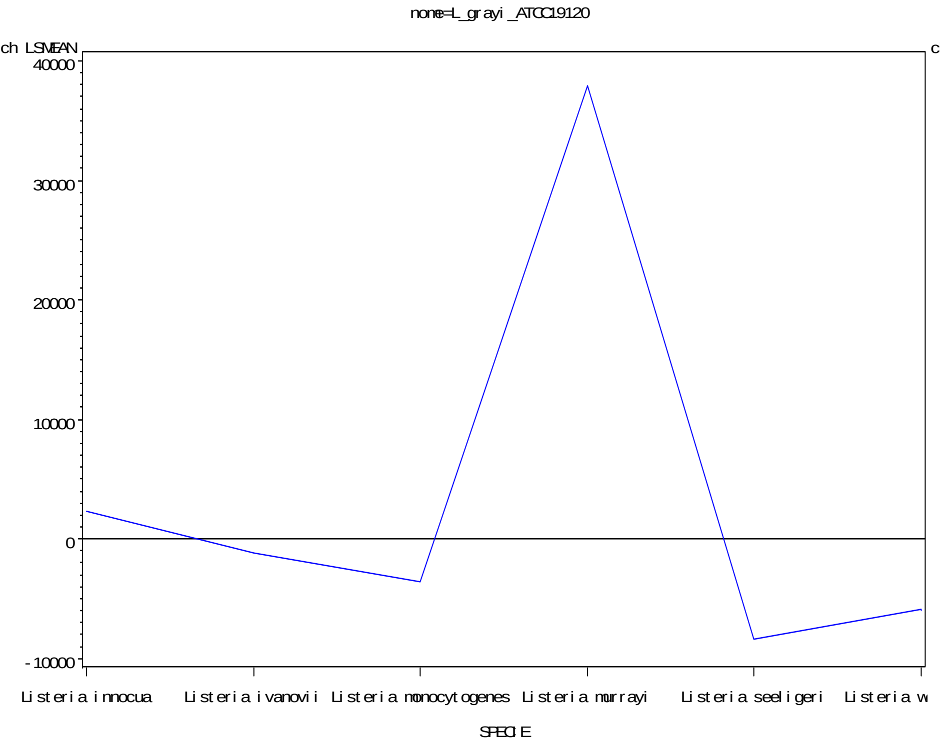


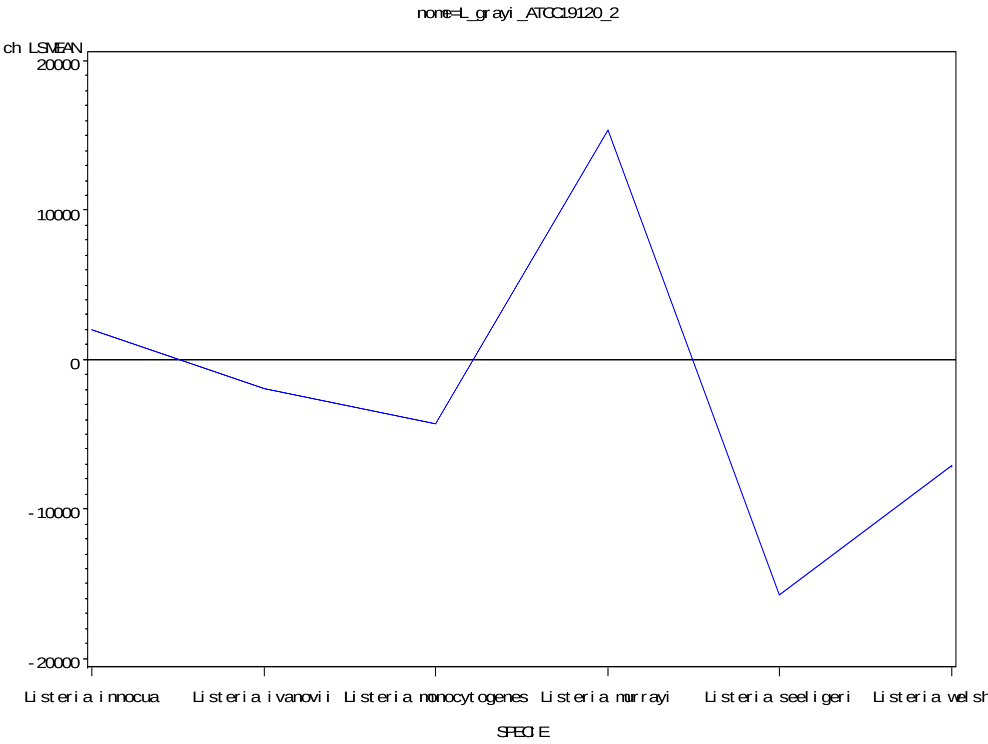
in the case of L. murrayi the species is perfectly individualized

Hybridization patterns with mono-specific samples

RESULTS

Listeria murrayi

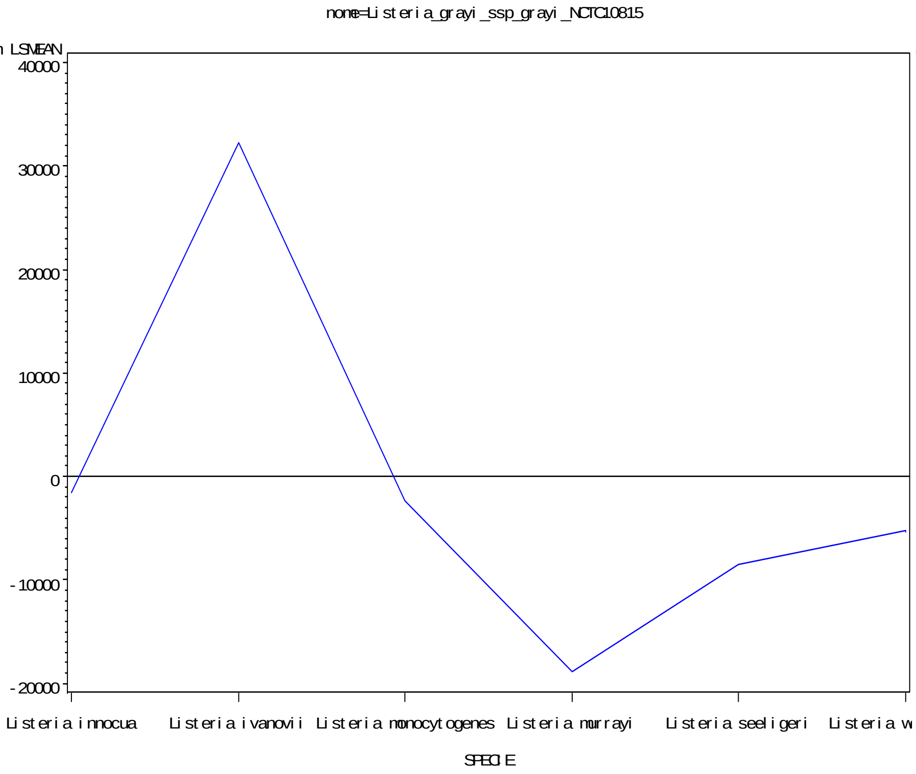


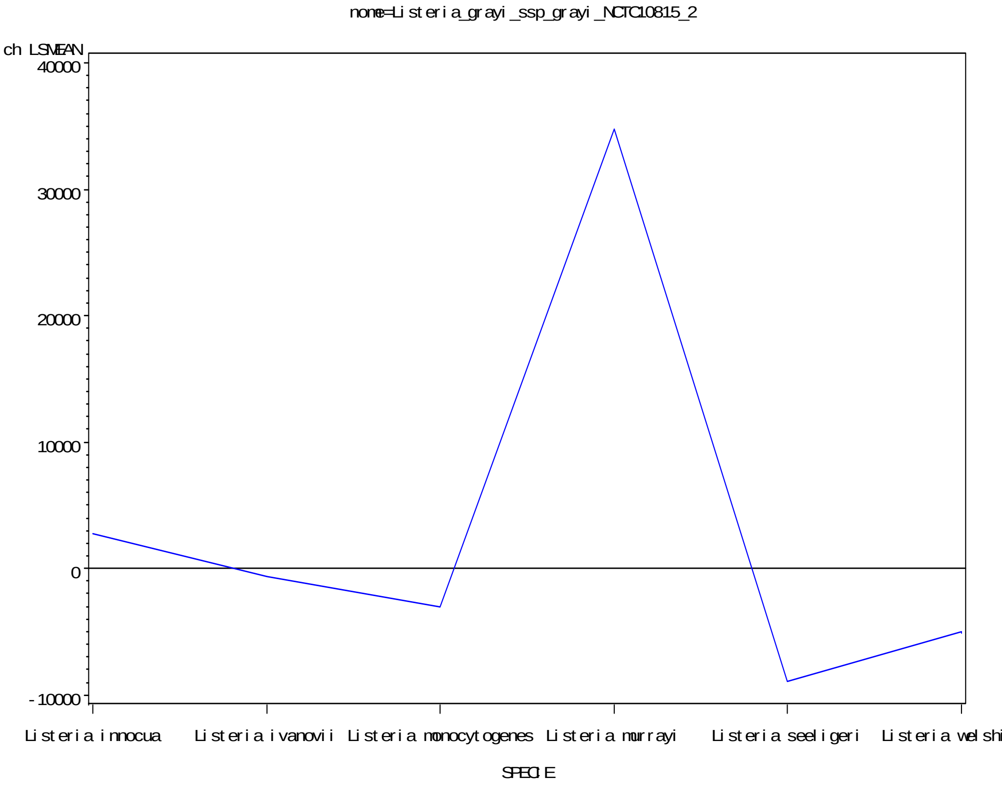


Hybridization patterns with mono-specific samples

RESULTS

Listeria murrayi





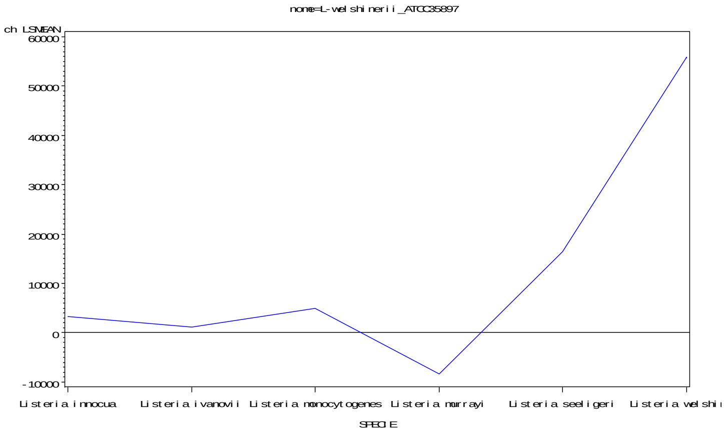
L. ivanovii ?

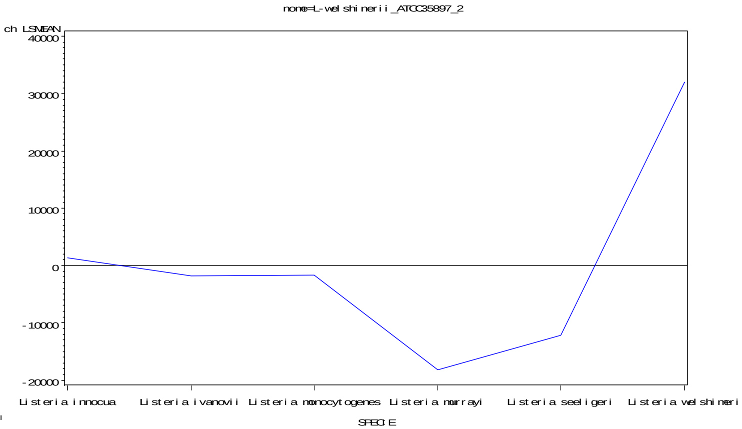
Hybridization patterns with mono-specific samples

RESULTS

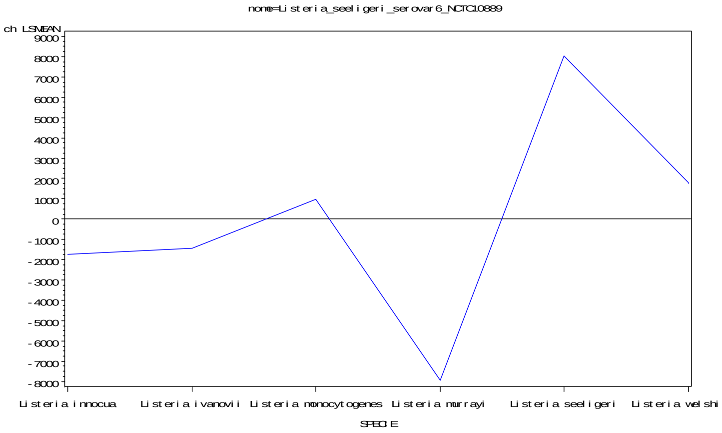
Array	**Dupliato**
L-welshinerii_ATCC35897	L-welshinerii_ATCC35897_2
L_seeligeri_serovar6_NCTC10889	L_seeligeri_serovar6_NCTC10889_2

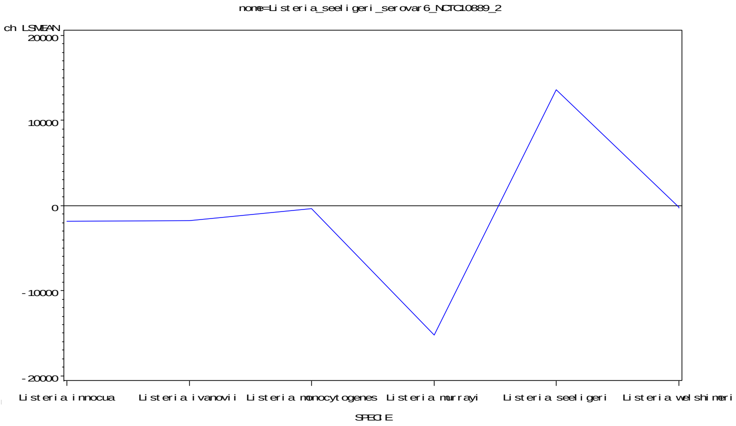
Listeria Welshinerii





Listeria Seeligeri



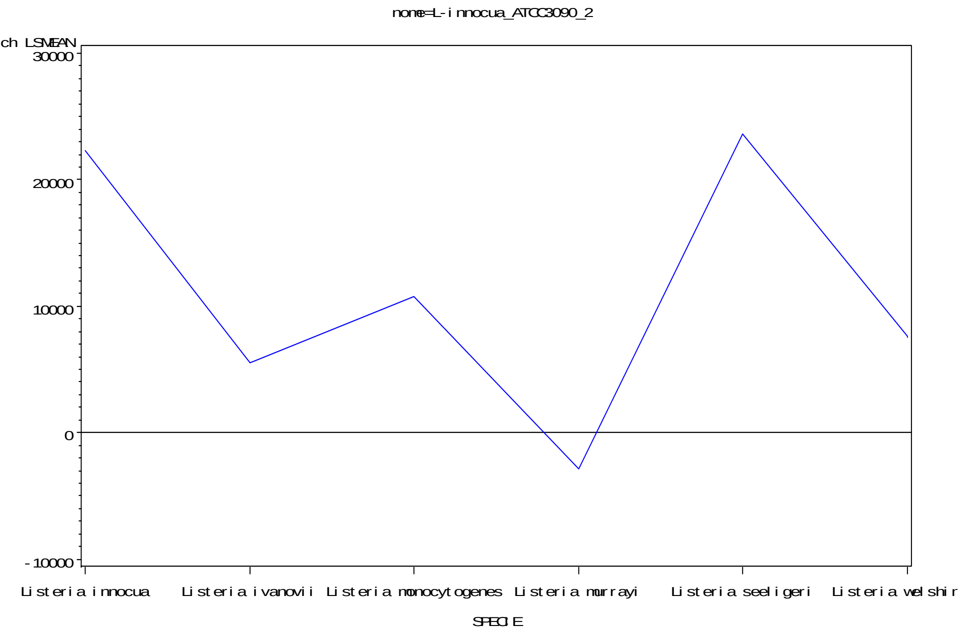


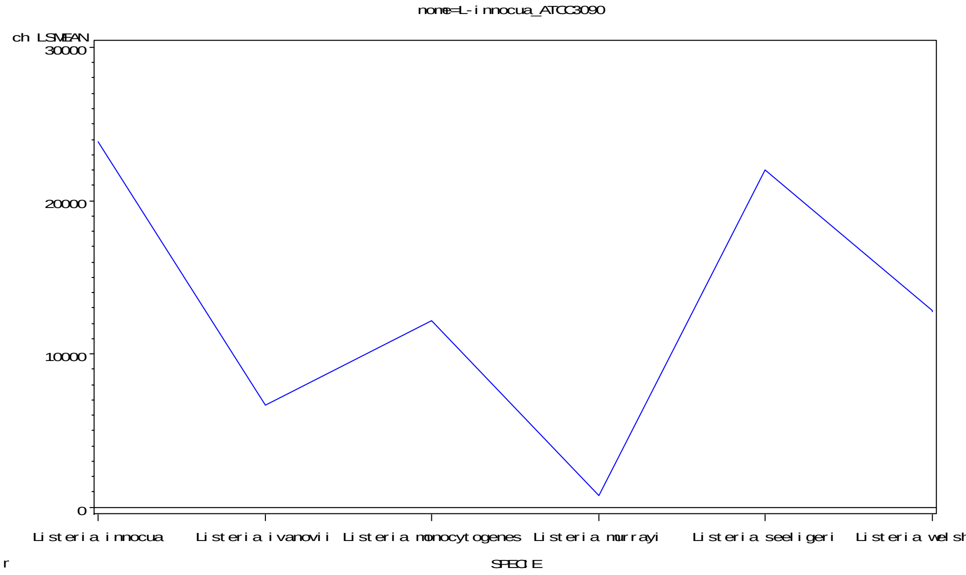
Same results was found for *L. ivanovii*

Hybridization patterns with mono-specific samples

RESULTS

Listeria innocua





L. innocua ***L. seeligerii***

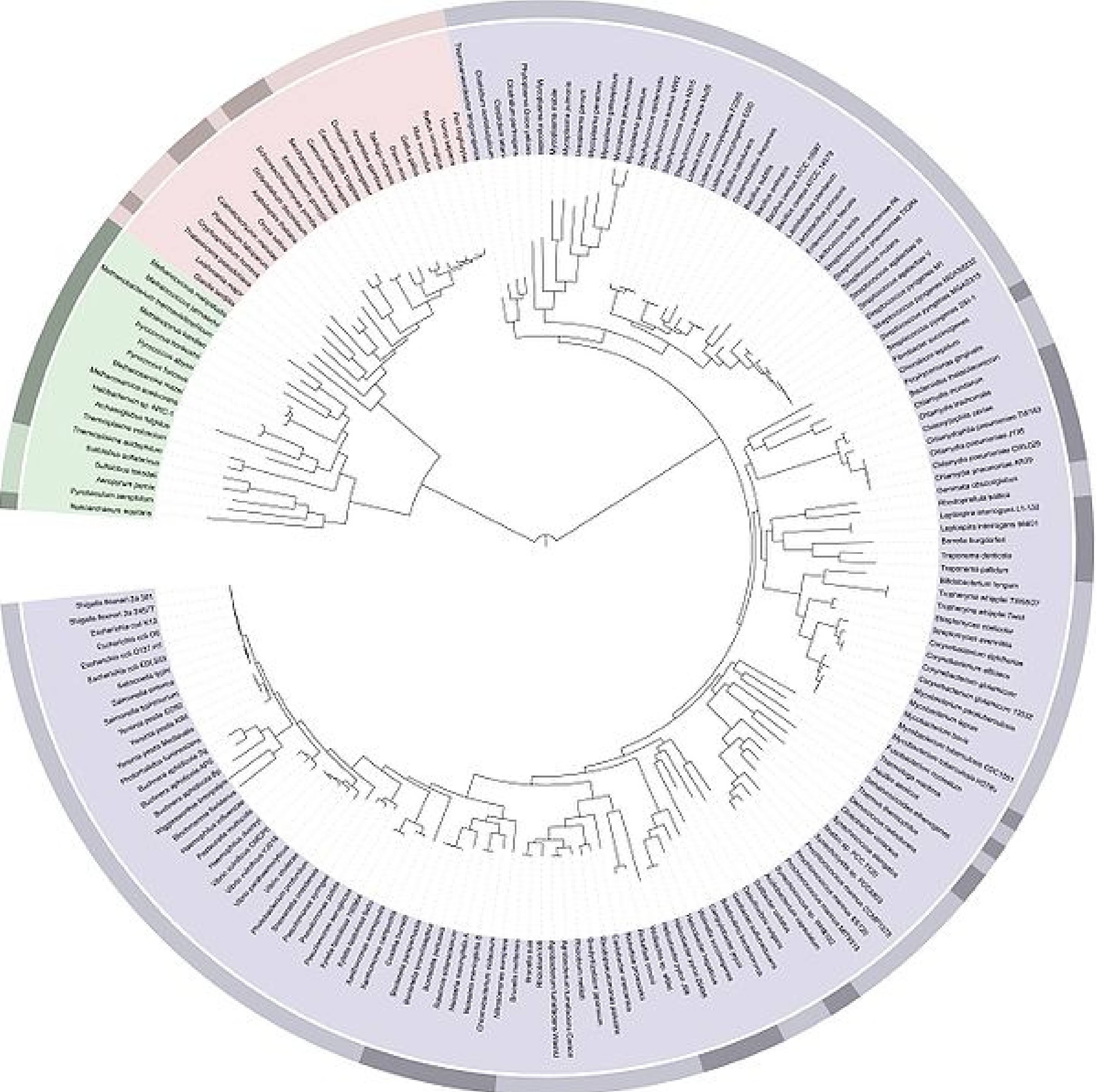
Hybridization patterns with mono-specific samples

Hybridization results of the microarray pig-zoonosis V4s with mono-specific samples show

- high discriminating power to level of genus
- high discriminating power to level of species (limitedly to the Listeria sp.)

We need further examinations to verify if it is possible to discriminate also the organisms with regard to subspecies and sierotype level

further investigations are necessary to establish the response with samples containing more species and/or genus contemporarily
(as meat or environmental samples)


Chlamydia muridarum
Chlamydia trachomatis
Chlamydophila caviae
Chlamydophila pneumoniae TW183
Chlamydia pneumoniae J138
Chlamydia pneumoniae CWL029
Chlamydia pneumoniae AR39
Gemmata obscuriglobus
Rhodopirellula baltica
Leptospira interrogans L1-130
Leptospira interrogans 56601
Borrelia burgdorferi
Treponema denticola
Treponema pallidum
Bifidobacterium longum
Tropheryma whipplei TW08/27
Tropheryma whipplei Twist
Streptomyces coelicolor
Streptomyces avermitilis
Corynebacterium diphtheriae
Corynebacterium efficiens
Corynebacterium glutamicum
Mycobacterium leprae
Mycobacterium bovis
Shigella flexneri 2a 301
Shigella flexneri 2a 2457T
Escherichia coli K12
Escherichia coli O6
Escherichia coli O157 H7
Escherichia coli EDL933
Salmonella typhi
Salmonella enterica
Salmonella typhimurium
Yersinia pestis KIM
Yersinia pestis CO92
Yersinia pestis Medievalis
Photorhabdus luminescens
Buchnera aphidicola
Nanoarchaeum equitans
Pyrobaculum aerophilum
Aeropyrum pernix
Sulfolobus tokodaii
Sulfolobus solfataricus
Thermoplasma acidophilum
Thermoplasma volcanium
Archaeoglobus fulgidus
Halobacterium sp. NRC-1