

# **Update on the preparedness towards the use of NGS for characterization and typing of pathogenic *E. coli***

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**13th Annual Workshop of the National Reference  
Laboratories for *E. coli* in the EU**

Rome, October 19<sup>th</sup> 2018



Istituto Superiore di Sanità, Dep. of Food Safety, Nutrition and Veterinary Public Health  
European Union and National Reference Laboratory for *E. coli*, Rome, Italy



# PT-WGS1 – A voluntary laboratory exercise

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**General purpose to get info on the data produced by the network and on their usability for typing and surveillance/monitoring of STEC infections**

- to evaluate the **quality** of the sequences produced
- to evaluate the **inter laboratory/platform variability** in terms of SNPs in the genomes produced

## Strategy

WGS of the **strains** part of PT-PFGE6 by using the preferred NGS technology.

It was requested to submit the sequencing data as **fastq files with no additional bioinformatics performed**.

A dedicated webserver was used for the sequences files upload.

# Roadmap

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Shipment of strains

13/11/2017



DNA extraction



Sequencing



Submission of fastq data

Deadline:  
28/02/2018

Analysis of the data by EURL on ARIES webserver



Istituto Superiore di Sanità, Dep. of Food Safety, Nutrition and Veterinary Public Health  
European Union and National Reference Laboratory for *E. coli*, Rome, Italy



# Data submission process

The participants were asked to submit the sequencing data through the IRIDA database installed on ISS webserver and available at: <https://irida.iss.it/irida-aries/login>

## Login

Username

Password

[Reset Password](#) [Activate Account](#)

## Welcome to the IRIDA Platform

### Beta Release

Canada's Integrated Rapid Infectious Disease Analysis (IRIDA) platform is designed to equip public health workers with easy-to-use tools needed to analyse genomic data, without the complexity and technical barriers of traditional tools. IRIDA is free and open-source software. More information is available at [irida.ca](http://irida.ca).

Account details were provided to each participant together with detailed instructions for the submission of the data

The screenshot shows the IRIDA Platform interface. At the top, there is a navigation bar with links for Projects, Analysis, Users, Help, and various icons. Below the navigation bar, there is a sub-navigation bar with 'Details' and 'Files' tabs, where 'Details' is selected. The main content area displays a sample submission titled 'Sample - Strain1\_Labcode - Files'. It shows a file named 'Strain1\_Labcode.fastq' with a size of 362.6 MB and a date created of 07 Nov 2017. There are also delete and download icons for the file. To the left of the main content, there is a sidebar for the sample 'Strain1\_Labcode' showing details like ID (31), Organism (Escherichia coli), and creation/modification dates. At the bottom of the main content area, there is a button labeled 'Upload Sequence Files'.

The participants were requested to submit **only the fastq files** produced, with no additional result of bioinformatics analyses

# Methods

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Analysis of the data by EURL on ARIES webserver through EURL VTEC pipeline

- QC
- Trimming
- Serotyping
- MLST (7 genes)
- Identification of target genes (STEC virulence genes)

Assembly (w/o optimization) used for:

- Reference-free SNP-based phylogenetics (all SNPs and core SNPs)
- wgMLST – chewBBACA («Innuendo» EFSA-funded project)
- cgMLST – chewBBACA – fixed scheme
- cgMLST – chewBBACA – dynamic scheme

# Participants and material

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6 STEC strains (STEC O26)

21 Laboratories (18 EU NRLs; 3 IT OIs)

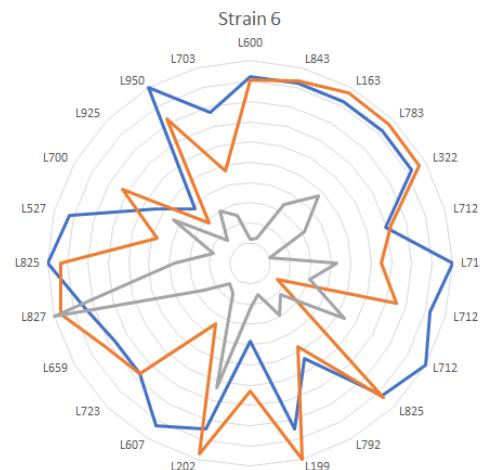
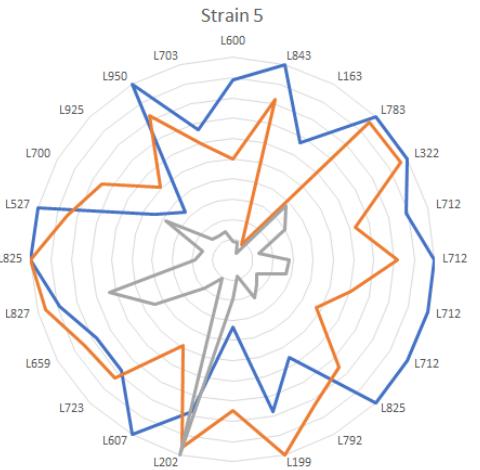
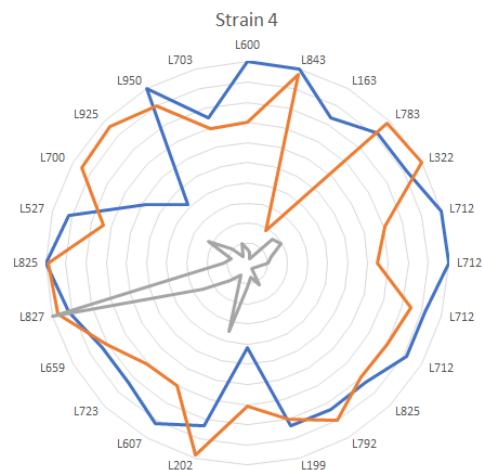
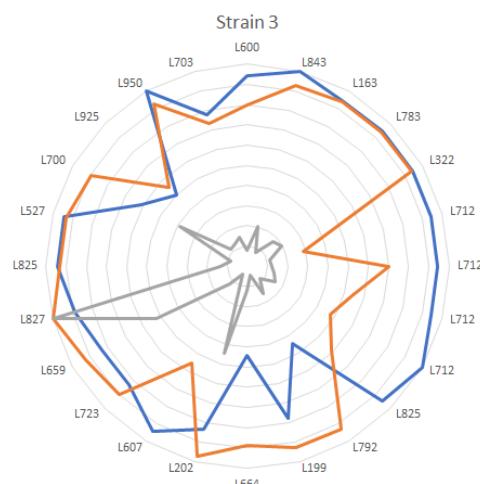
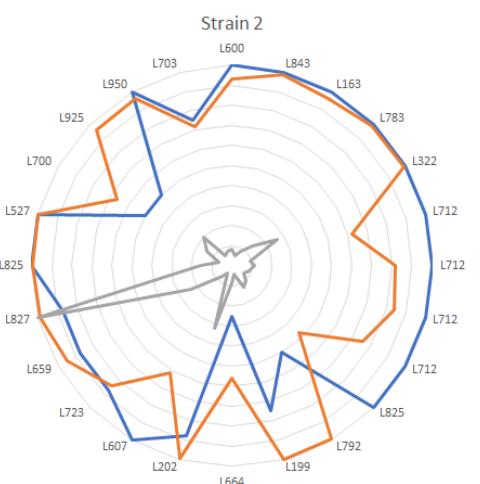
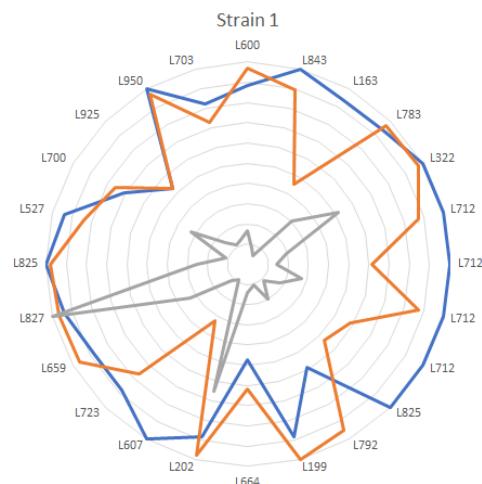
Number	Strain	Serotype	MLST	Genotype
1	ED 56	O26:H11	21	<i>stx1 eae ehxA</i>
2	ED 258	O26:H11	21	<i>stx1 eae</i>
3	ED 477	O26:H11	21	<i>stx2 eae ehxA</i>
4	ED 600	O26:H11	21	<i>stx1 eae</i>
5	ED 1014	O26:H11	21	<i>stx2 eae ehxA</i>
6	ED 1104	O26:H11	21	<i>stx2 eae ehxA</i>

# Overview of the data panel

- Assembly Coverage
- N50
- Depth

17 paired-end

4 single end



# Sequencing depth

Green: depth  $\geq 30x$

Yellow:  $20 \leq \text{depth} < 30$

Orange:  $10 \leq \text{depth} < 20$

Red: depth  $< 10$

$6.6x < \text{Depth} < 309.5x$

Mean 43.6x

Median 30.7x

Seven laboratories  
achieved a depth of  
30x or higher for all  
the six strains tested

Lab code	Strain1	Strain2	Strain3	Strain4	Strain5	Strain6
L163	8,5	25,5	18,9	7,6	6,6	37,5
L199	17,7	13,0	10,6	21,1	14,9	17,7
L202	109,3	100,3	106,3	105,6	181,3	70,9
L322	87,0	80,8	46,8	59,0	53,9	35,0
L527	19,3	20,7	20,6	25,2	28,1	20,9
L600	28,1	25,7	18,8	22,2	16,3	13,5
L607	14,4	13,1	11,2	20,0	18,7	19,3
L659	55,6	72,8	123,8	76,7	80,6	29,2
L664	23,6	26,9	27,9	34,8	34,7	24,9
L700	54,1	44,2	92,3	68,3	69,2	48,8
L703	20,9	22,7	35,1	31,2	25,6	27,7
L712_Qa	33,1	29,7	26,5	38,1	24,0	11,5
L712_Qb	23,6	35,6	28,3	31,9	50,5	48,0
L712_R	46,4	26,9	31,8	18,0	49,1	34,3
L712_Rb	30,7	20,6	37,0	13,4	25,1	60,2
L723	19,4	21,7	29,9	35,5	35,3	15,5
L783	51,2	43,9	41,7	53,0	68,2	53,0
L792	33,5	37,6	37,6	37,1	39,4	33,0
L825	43,0	48,9	31,9	35,3	33,5	41,9
L825b	18,6	31,3	21,3	10,3	30,6	23,9
L827	167,6	309,5	237,2	305,0	114,1	111,6
L843	13,6	17,0	48,8	17,4	16,1	14,5
L925	25,9	62,3	28,3	31,5	26,0	17,7
L950	18,9	18,9	29,9	12,1	24,9	33,2

# Multi Locus Sequence typing

Green: exact match of all the alleles, exact ST prediction

Orange: uncertain prediction of the correct ST

Red: Sequence Type Not Found (STNF), due to an unknown combination of the alleles of the seven loci

\* next to the ST indicates that there were mismatches against at least one of the alleles.

? indicates that there was uncertainty in at least one of the alleles.

15/144 ST Not Found

11/144 ST21 uncertain

Only 3 labs submitted more than one genome with STNF

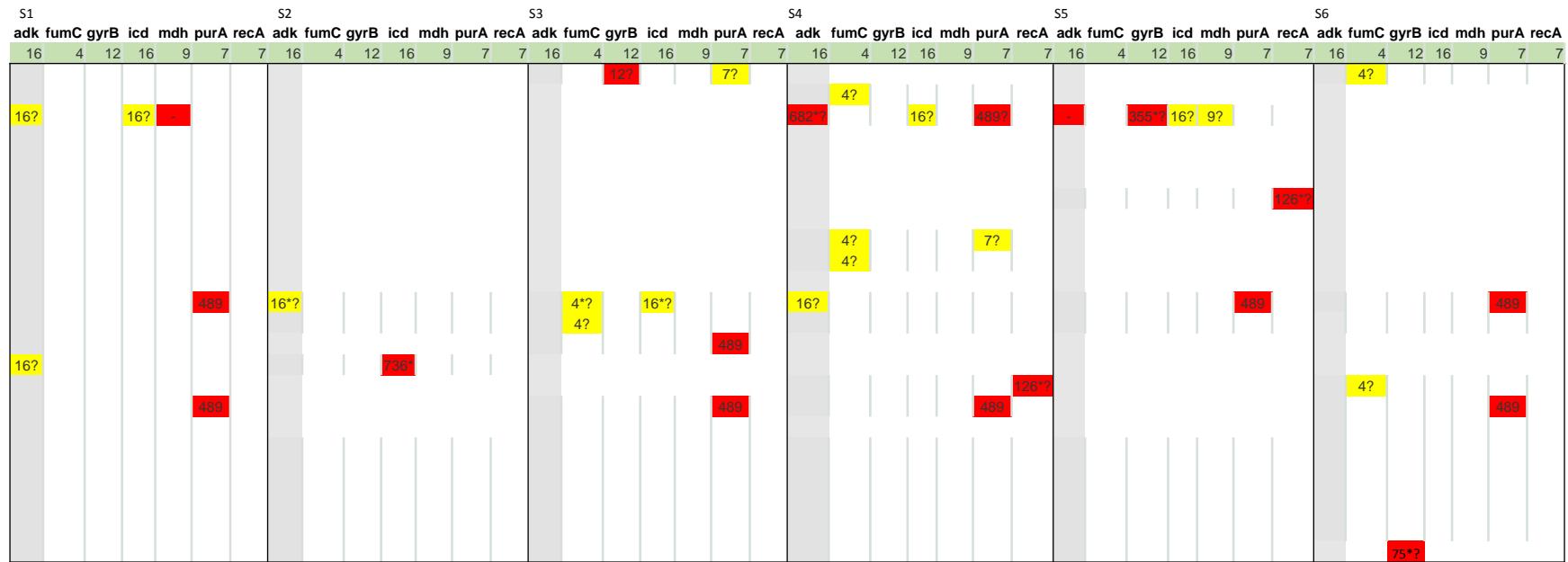
Lab code	Strain1	Strain2	Strain3	Strain4	Strain5	Strain6
True value	ST21	ST21	ST21	ST21	ST21	ST21
L163	STNF?	ST21	ST21	STNF*?	STNF*?	ST21
L199	STNF	ST21*?	ST21*?	ST21?	STNF	STNF
L202	ST21	ST21	STNF	ST21	ST21	ST21
L322	ST21	ST21	ST21	ST21	ST21	ST21
L527	ST21	ST21	ST21	ST21	ST21	ST21
L600	ST21	ST21	ST21?	ST21	ST21	ST21?
L607	ST21?	STNF*	ST21	ST21	ST21	ST21
L659	STNF	ST21	STNF	STNF	ST21	STNF
L664	ST21	ST21	ST21?	ST21	ST21	ST21
L700	ST21	ST21	ST21	ST21	ST21	ST21
L703	ST21	ST21	ST21	ST21	ST21	STNF*?
L712_Qa	ST21	ST21	ST21	ST21	ST21	ST21
L712_Qb	ST21	ST21	ST21	ST21	STNF*?	ST21
L712_R	ST21	ST21	ST21	ST21	ST21	ST21
L712_Rb	ST21	ST21	ST21	ST21?	ST21	ST21
L723	ST21	ST21	ST21	STNF*?	ST21	ST21?
L783	ST21	ST21	ST21	ST21	ST21	ST21
L792	ST21	ST21	ST21	ST21	ST21	ST21
L825	ST21	ST21	ST21	ST21	ST21	ST21
L825b	ST21	ST21	ST21	ST21?	ST21	ST21
L827	ST21	ST21	ST21	ST21	ST21	ST21
L843	ST21	ST21	ST21	ST21?	ST21	ST21
L925	ST21	ST21	ST21	ST21	ST21	ST21
L950	ST21	ST21	ST21	ST21	ST21	ST21

# Multi Locus Sequence typing: details

Errors in calling the alleles of some of the 7 housekeeping genes of MLST

Genes per each of the 6 strains

Labs



Allele purA 489: Systematic error?

# *In silico* serotyping

Green: exact serotype prediction

Red: Errors detected in serotype prediction

Lab code	Strain1	Strain2	Strain3	Strain4	Strain5	Strain6
True value	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L163	O26:H11	O26:H11	O26:H11	O26:H11	O?:H11	O26:H11
L199	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L202	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L322	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L527	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L600	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L607	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L659	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L664	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L700	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L703	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L712_Qa	O?:H11	O26:H11	O26:H11	O?:H11	O26:H11	O?:H11
L712_Qb	O26:H11	O?:H11	O?:H11	O26:H11	O26:H11	O26:H11
L712_R	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L712_Rb	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L723	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L783	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L792	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L825	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L825b	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L827	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L843	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L925	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11
L950	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11	O26:H11

4 runs by L712

2/4 runs problems in O-group



# *In silico* virulotyping – strains 1 and 2

## Green: exact prediction

Red: Error detected in the prediction

\*: absence of identification of *ehxA* gene in one of the two sequences provided for the same strain

Two different morphologies on agar plates

# *In silico* virulotyping – strains 3 and 4

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Lab code	Strain 3				Strain 4			
	<i>stx1</i>	<i>stx2</i>	<i>eae</i>	<i>ehxA</i>	<i>stx1</i>	<i>stx2</i>	<i>eae</i>	<i>ehxA</i>
True value	-	+	+	+	+	-	+	-
L163								
L199								
L202								
L322								
L527								
L600								
L607								
L659								
L664								
L700								
L703								
L712_Qa								
L712_Qb								
L712_R								
L712_Rb								
L723								
L783								
L792								
L825								
L825b								
L827								
L843								
L925								
L950								

3 <-> 4



★ 4 <-> 6

★ 3 <-> 4

# *In silico* virulotyping – strains 5 and 6

Lab code	Strain 5				Strain 6			
	<i>stx1</i>	<i>stx2</i>	<i>eae</i>	<i>ehxA</i>	<i>stx1</i>	<i>stx2</i>	<i>eae</i>	<i>ehxA</i>
True value	-	+	+	+	-	+	+	+
L163								
L199								
L202								
L322								
L527								
L600								
L607								
L659								
L664								
L700								
L703								
L712_Qa								
L712_Qb								
L712_R								
L712_Rb								
L723								
L783								
L792								
L825								
L825b								
L827								
L843								
L925								
L950								

★ 4 <-> 6

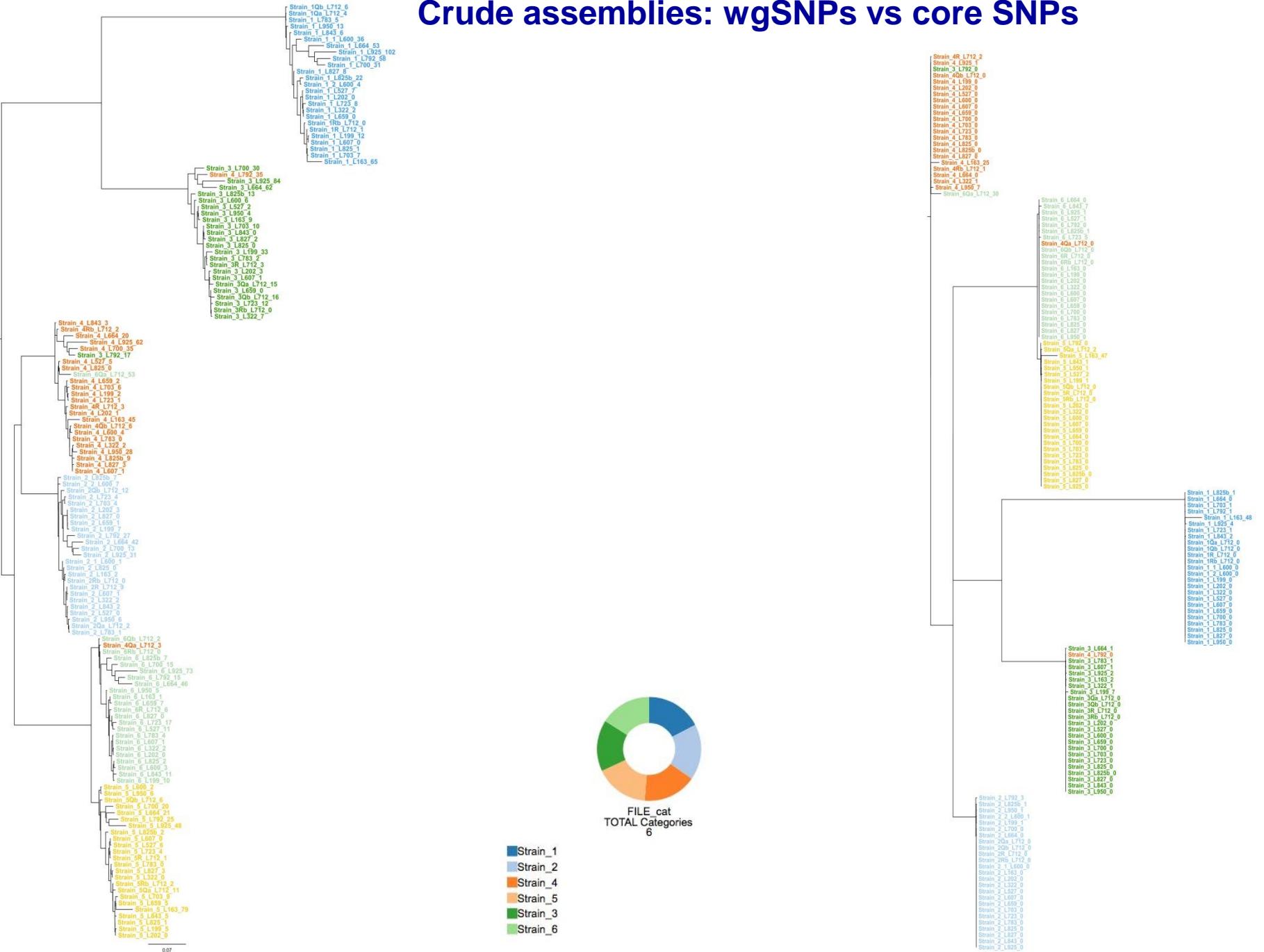
Lack of identification of *stx2* in *stx2*-positive samples was observed in 4 sequences (strain 5 for L163 and L925 and strain 6 for L703 and L792)

# Phylogenetic analysis

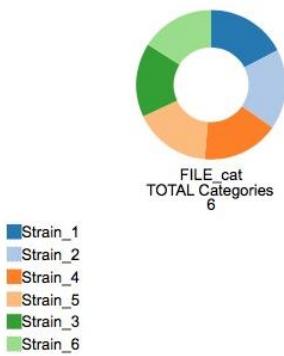
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- SNPs comparison through a reference-free, kmer-based approach (**whole genome SNPs and core SNPs**)
- whole genome MLST (**wgMLST**). INNUENDO Scheme 7601 genes
- core genome MLST (**cgMLST**), INNUENDO Scheme 2360 core genes
- dynamic core genome MLST (**dynamic cgMLST**), with a dynamic scheme of core genes shared by all the sequences analyzed.

## Crude assemblies: wgSNPs vs core SNPs



## Optimized assemblies: wgSNPs vs core SNPs

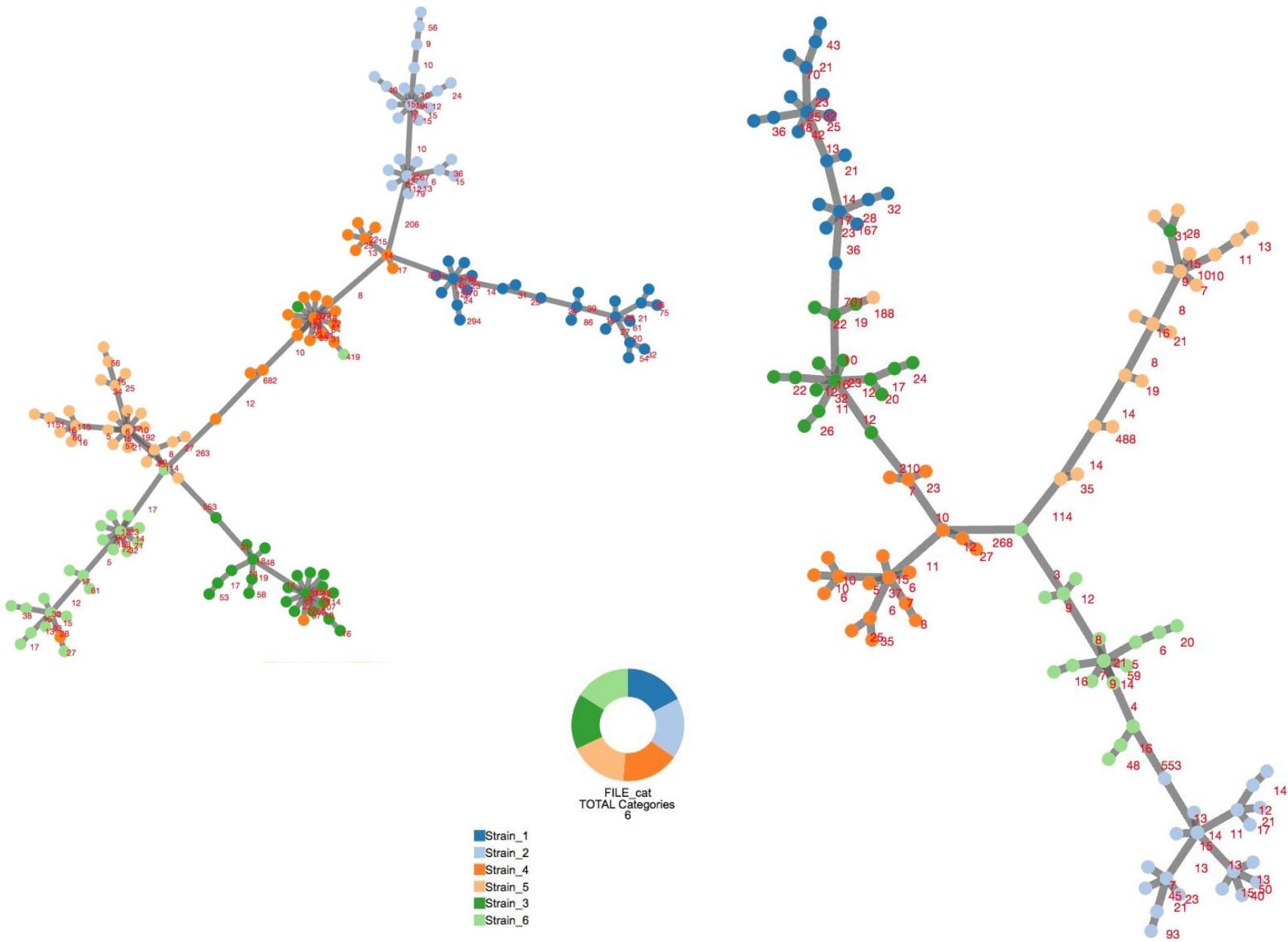


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Strain1\_L599.trim.spades3111.proc\_filt\_polished\_4  
Strain1\_L827.trim.spades3111.proc\_filt\_polished\_8  
Strain1\_L825.trim.spades3111.proc\_filt\_polished\_2  
Strain1\_L607.trim.spades3111.proc\_filt\_polished\_38  
Strain1\_L604.trim.spades3111.proc\_filt\_polished\_50  
Strain1\_L163.trim.spades3111.proc\_filt\_polished\_32  
StrainQa\_L712.trim.spades3111.proc\_filt\_polished\_12  
Strain1\_L527.trim.spades3111.proc\_filt\_polished\_0  
Strain1\_L607.trim.spades3111.proc\_filt\_polished\_1  
Strain2\_L609.trim.spades3111.proc\_filt\_polished\_7  
Strain1\_L720.trim.spades3111.proc\_filt\_polished\_159  
Strain3\_L722.trim.spades3111.proc\_filt\_polished\_2  
Strain3\_L232.trim.spades3111.proc\_filt\_polished\_5  
Strain3\_L255.trim.spades3111.proc\_filt\_polished\_8  
Strain3Qa\_L712.trim.spades3111.proc\_filt\_polished\_22  
Strain3\_L720.trim.spades3111.proc\_filt\_polished\_92  
Strain3\_L607.trim.spades3111.proc\_filt\_polished\_4  
Strain3Qb\_L712.trim.spades3111.proc\_filt\_polished\_4  
Strain3\_L607.trim.spades3111.proc\_filt\_polished\_26  
Strain3\_L600.trim.spades3111.proc\_filt\_polished\_7  
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Strains3\_L199.trim.spades3111.proc\_filt\_polished\_114  
Strain3\_L720.trim.spades3111.proc\_filt\_polished\_1  
Strain3\_L625.trim.spades3111.proc\_filt\_polished\_1  
Strain3\_L160.trim.spades3111.proc\_filt\_polished\_10  
Strain3\_L723.trim.spades3111.proc\_filt\_polished\_18  
Strain3\_L783.trim.spades3111.proc\_filt\_polished\_1  
Strain3\_L950.trim.spades3111.proc\_filt\_polished\_5  
Strain3\_L827.trim.spades3111.proc\_filt\_polished\_21  
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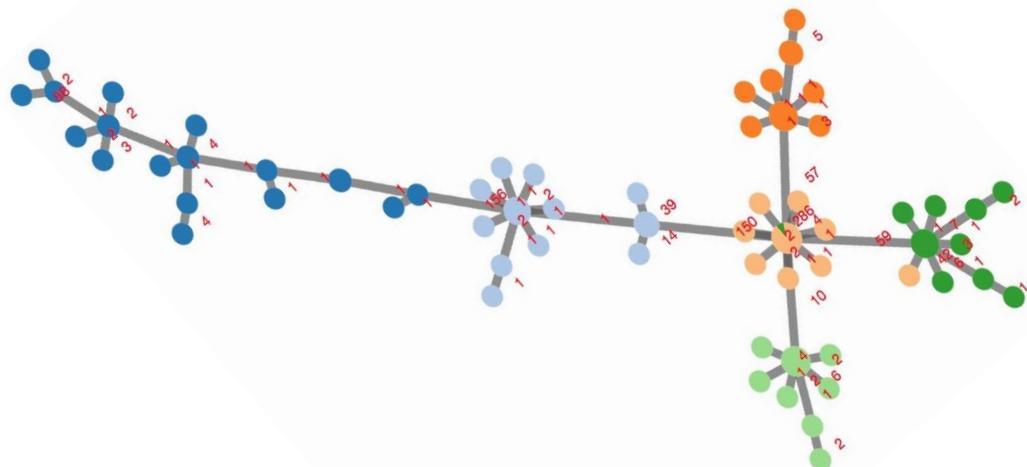
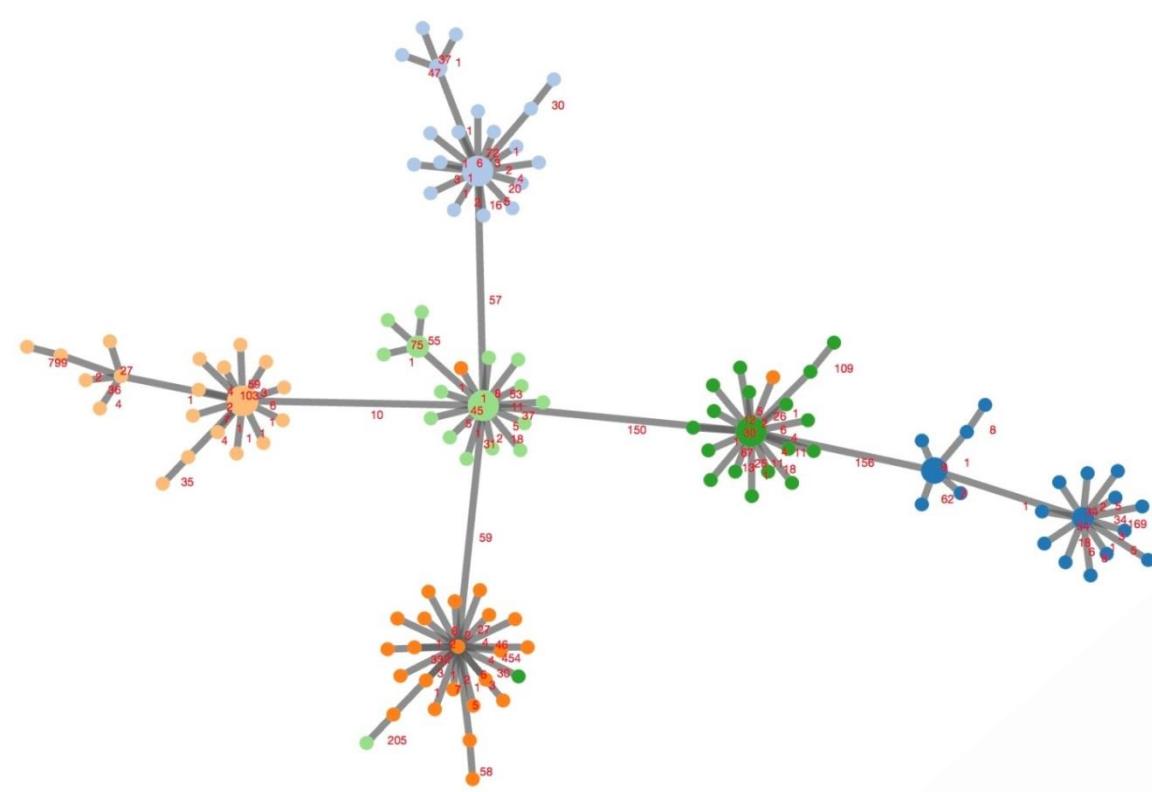
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int\_L783.trim.spades3111.proc\_filt\_polished\_8  
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int\_L607.trim.spades3111.proc\_filt\_polished\_3  
int\_L604.trim.spades3111.proc\_filt\_polished\_5  
rain4\_L712.trim.spades3111.proc\_filt\_polished\_3  
rain4Qb\_L712.trim.spades3111.proc\_filt\_polished\_9  
rain4Rb\_L712.trim.spades3111.proc\_filt\_polished\_5  
strain4\_L950.trim.spades3111.proc\_filt\_polished\_12  
strain4\_L828.trim.spades3111.proc\_filt\_polished\_76  
strain4\_L720.trim.spades3111.proc\_filt\_polished\_1  
strain4\_L199.trim.spades3111.proc\_filt\_polished\_6  
strain4\_L202.trim.spades3111.proc\_filt\_polished\_16  
strain4\_L609.trim.spades3111.proc\_filt\_polished\_9  
strain4\_L599.trim.spades3111.proc\_filt\_polished\_10  
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in2\_L604.trim.spades3111.proc\_filt\_polished\_13  
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rain2\_L202.trim.spades3111.proc\_filt\_polished\_3  
rain2Bb.trim.spades3111.proc\_filt\_polished\_2  
rain2Rb\_L712.trim.spades3111.proc\_filt\_polished\_1  
strain2\_L723.trim.spades3111.proc\_filt\_polished\_9  
strain2\_L199.trim.spades3111.proc\_filt\_polished\_11  
strain2\_L827.trim.spades3111.proc\_filt\_polished\_4  
strain2\_L720.trim.spades3111.proc\_filt\_polished\_6  
rain2L\_Qb\_L712.trim.spades3111.proc\_filt\_polished\_1  
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rain2\_L607.trim.spades3111.proc\_filt\_polished\_2  
rain2Qb\_L712.trim.spades3111.proc\_filt\_polished\_8  
rain2R\_L712.trim.spades3111.proc\_filt\_polished\_0  
strain2\_L827.trim.spades3111.proc\_filt\_polished\_10  
strain2\_L720.trim.spades3111.proc\_filt\_polished\_6  
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strain2\_L723.trim.spades3111.proc\_filt\_polished\_2  
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strain2\_L322.trim.spades3111.proc\_filt\_polished\_4  
strain6\_L607.trim.spades3111.proc\_filt\_polished\_2  
strain6\_L825.trim.spades3111.proc\_filt\_polished\_5  
strain1\_L163.trim.spades3111.proc\_filt\_polished\_3  
strain6\_L703.trim.spades3111.proc\_filt\_polished\_3  
strain4Qa\_L712.trim.spades3111.proc\_filt\_polished\_7  
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strain4\_L723.trim.spades3111.proc\_filt\_polished\_27  
strain6\_L659.trim.spades3111.proc\_filt\_polished\_21  
strain4\_L783.trim.spades3111.proc\_filt\_polished\_1  
strain5\_L527.trim.spades3111.proc\_filt\_polished\_3  
strain5\_L602.trim.spades3111.proc\_filt\_polished\_3  
strain5\_L727.trim.spades3111.proc\_filt\_polished\_12  
strain5\_L723.trim.spades3111.proc\_filt\_polished\_10  
strain5\_L689.trim.spades3111.proc\_filt\_polished\_2  
strain5Rb\_L712.trim.spades3111.proc\_filt\_polished\_2  
strain5\_L783.trim.spades3111.proc\_filt\_polished\_1  
strain5\_L527.trim.spades3111.proc\_filt\_polished\_3  
strain5\_L602.trim.spades3111.proc\_filt\_polished\_3  
strain5\_L727.trim.spades3111.proc\_filt\_polished\_3  
strain5\_L723.trim.spades3111.proc\_filt\_polished\_21  
strain5\_L322.trim.spades3111.proc\_filt\_polished\_4  
strain5\_L607.trim.spades3111.proc\_filt\_polished\_3  
strain5\_L720.trim.spades3111.proc\_filt\_polished\_3  
strain5\_L625.trim.spades3111.proc\_filt\_polished\_3  
strain5\_L823.trim.spades3111.proc\_filt\_polished\_3

StrainQa\_L12 trim spades3111 proc filt polluted\_2  
StrainQa\_L12 trim spades3111 proc filt polluted\_1  
Strain3\_L12 trim spades3111 proc filt polluted\_1  
Strain3\_L25B trim spades3111 proc filt polluted\_1  
Strain3\_L19 trim spades3111 proc filt polluted\_18  
Strain3\_L25 trim spades3111 proc filt polluted\_0  
Strain3Qa\_L12 trim spades3111 proc filt polluted\_0  
Strain3Qa\_L12 trim spades3111 proc filt polluted\_0  
Strain3RB\_L12 trim spades3111 proc filt polluted\_0  
Strain3RB\_L12 trim spades3111 proc filt polluted\_0  
Strain3\_L27 trim spades3111 proc filt polluted\_0  
Strain3\_L60 trim spades3111 proc filt polluted\_0  
Strain3\_L63 trim spades3111 proc filt polluted\_0  
Strain3\_L65 trim spades3111 proc filt polluted\_0  
Strain3\_L67 trim spades3111 proc filt polluted\_0  
Strain3\_L73 trim spades3111 proc filt polluted\_0  
Strain3\_L75 trim spades3111 proc filt polluted\_0  
Strain3\_L77 trim spades3111 proc filt polluted\_0  
Strain3\_L80 trim spades3111 proc filt polluted\_0

# wgMLST: crude assemblies vs optimized assemblies



# Fixed cgMLST: Crude assemblies vs Optimized assemblies



FILE\_cat  
TOTAL Categories  
6

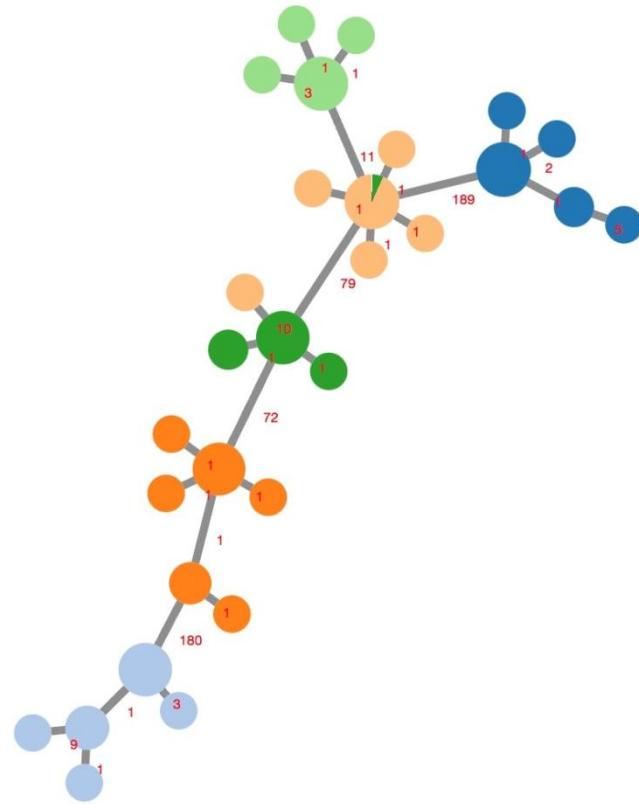
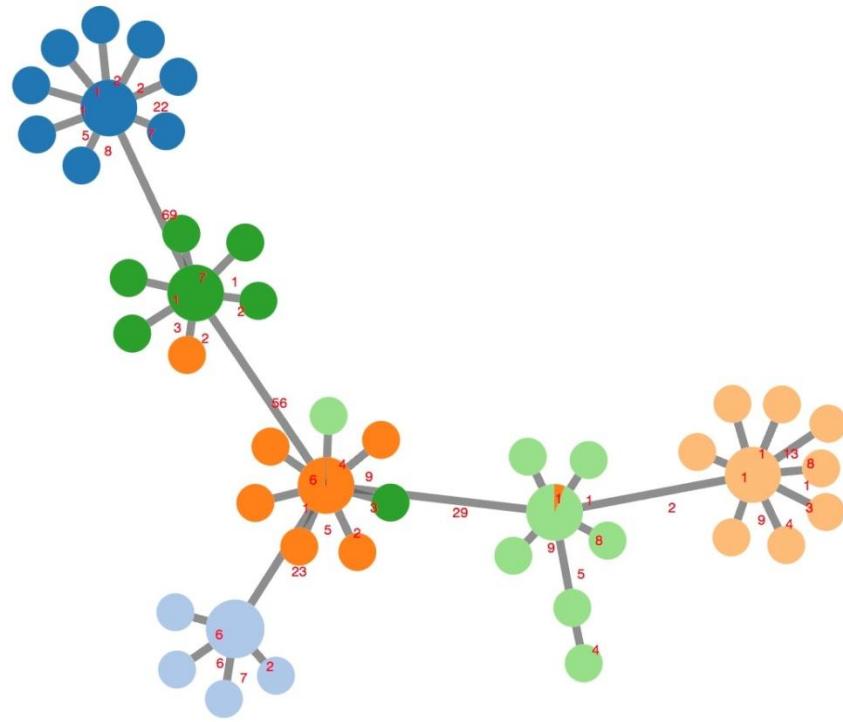
- Strain\_1
  - Strain\_2
  - Strain\_4
  - Strain\_5
  - Strain\_3
  - Strain\_6

# Dynamic cgMLST:

## Crude assemblies

vs

## Optimized assemblies



## 1<sup>st</sup> PT on WGS of STEC Take home messages

- Many of the NRLs in the *E. coli* Network do WGS
- A sequencing depth above 18 is desired to correctly type STEC (Sero/virulotyping; 7 genes MLST)
- Much more depth needed for phylogenetic analysis (>30)? Higher accuracy in the assembly (e.g. pilon) is of help in cleaning the cluster analysis
- Sub-cultivation may introduce variation in the genomic profiles and should be minimized
- Standardization should cover the entire process from the DNA preparation to sequencing
- *ad hoc* training will be deployed to cover the weaknesses identified in this PT exercise as well as in the next ones

# Courses on Bioinformatics for NGS data mining

22 attendees in 2015 (7 NRLs, EFSA)

22 attendees in 2016 (6 NRLs, EFSA, ECDC)



**EU Reference Laboratory for *E. coli***  
Department of Veterinary Public Health and Food Safety  
Unit of Foodborne Zoonoses  
Istituto Superiore di Sanità



Program for a 3-days training at the EURL-VTEC, Istituto Superiore di Sanità, Rome,  
on the use of bioinformatics tools for Next Generation Sequencing data mining  
for typing pathogenic *E. coli*

**Training in 2017**  
**11 attendees (9 NRLs)**

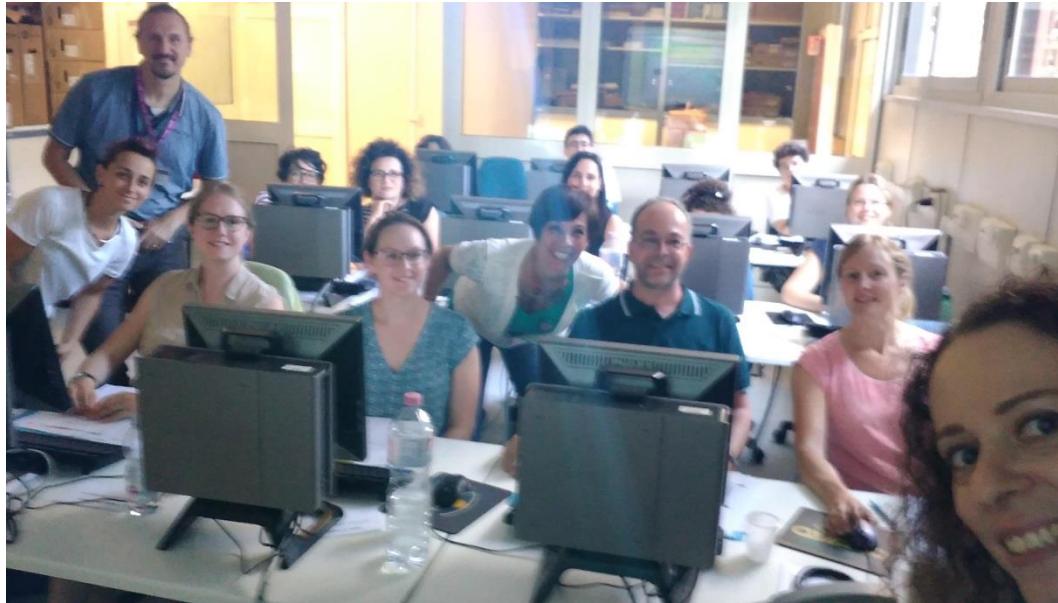


Istituto Superiore di Sanità, Dep. of Food Safety, Nutrition and Veterinary Public Health  
European Union and National Reference Laboratory for *E. coli*, Rome, Italy



# Courses on Bioinformatics for NGS data mining

16 attendees in 2018 (6 NRLs, 6 OLs, 4 ISS)



2019: Joint course on molecular typing (PFGE or NGS data analysis?)  
with EURL Listeria and EURL Salmonella