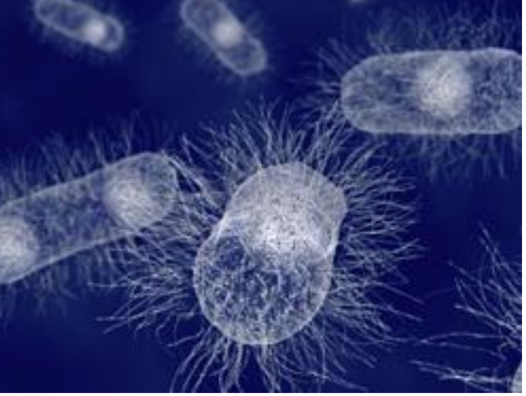

Whole genome sequencing (WGS) applications to VTEC surveillance and outbreak investigations

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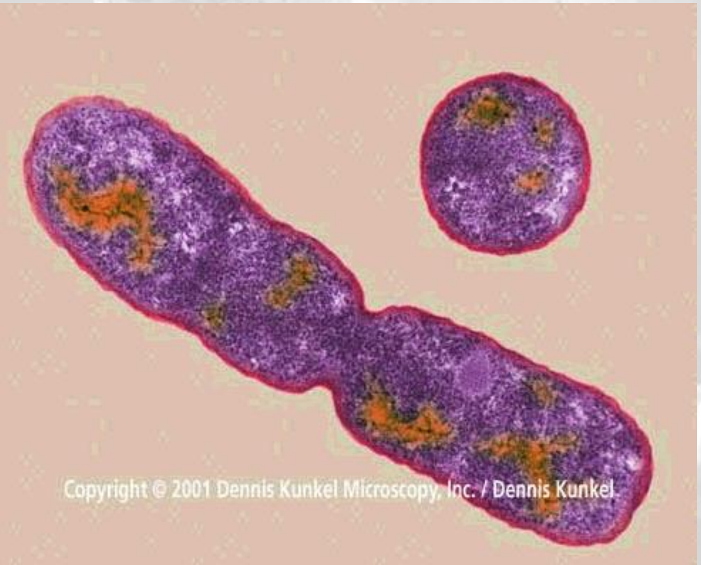


Real-Time Surveillance of VTEC using WGS

VTEC
→

→ **Conventional typing**

↓

→ **WGS typing**

Real-Time Surveillance of VTEC using WGS

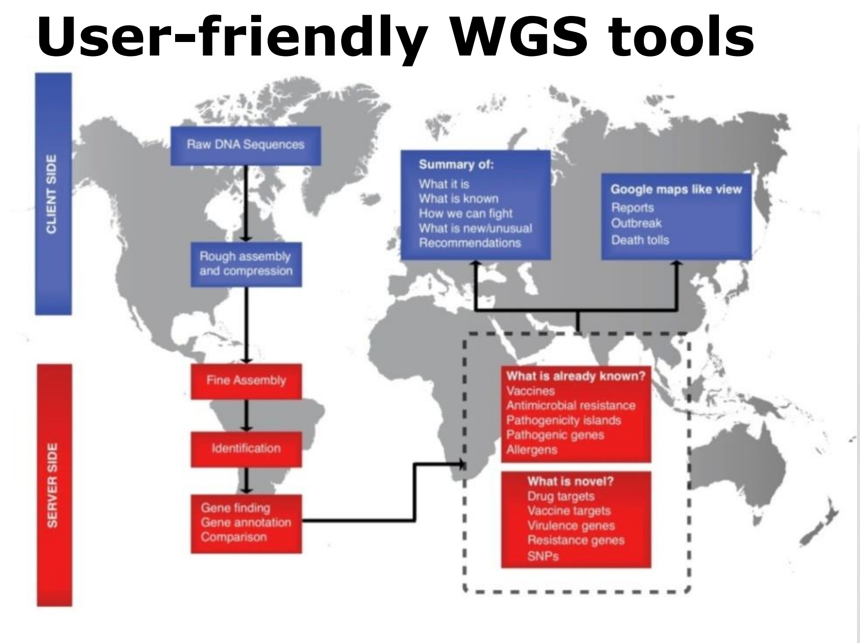
Conventional:

- Serotyping
- PCR, Hybridisation
- VCA
- Haemolysis
- PFGE...

Whole genome sequencing:

- Determination of the complete DNA sequence of the organism at once

Real-Time Surveillance of VTEC using WGS



CGE (Center for Genomic Epidemiology)

http://www.genomicepidemiology.org/

Main Service – Pipeline
CGE (In development)
Sequence Typing
MLST (Works)
pMLST (Works)
Resistance - Virulence - Plasmids – Pathogen
ResFinder (Works)
PlasmidFinder (Works)
VirulenceFinder (Works)
PathogenFinder (Works)
Phylogenetic Tree
snpTree (Works)
NDtree (Works)
TreeViewer (Works)
Species Finding
KmerFinder (Works)
SpeciesFinder (Works)
TaxonomyFinder (This program is in development)
Read2Type (This service is not implemented on the new server)
Tapir (This service is not implemented on the new server)
Genome Assembly
Assembler (Works)
Other
PanFunPro (in development)
MGmapper (in development)
GeneticDiseaseProject (stalled indefinitely)
NetFCM (in development)
Restriction-ModificationFinder (in development)

Real-Time Surveillance of VTEC using WGS

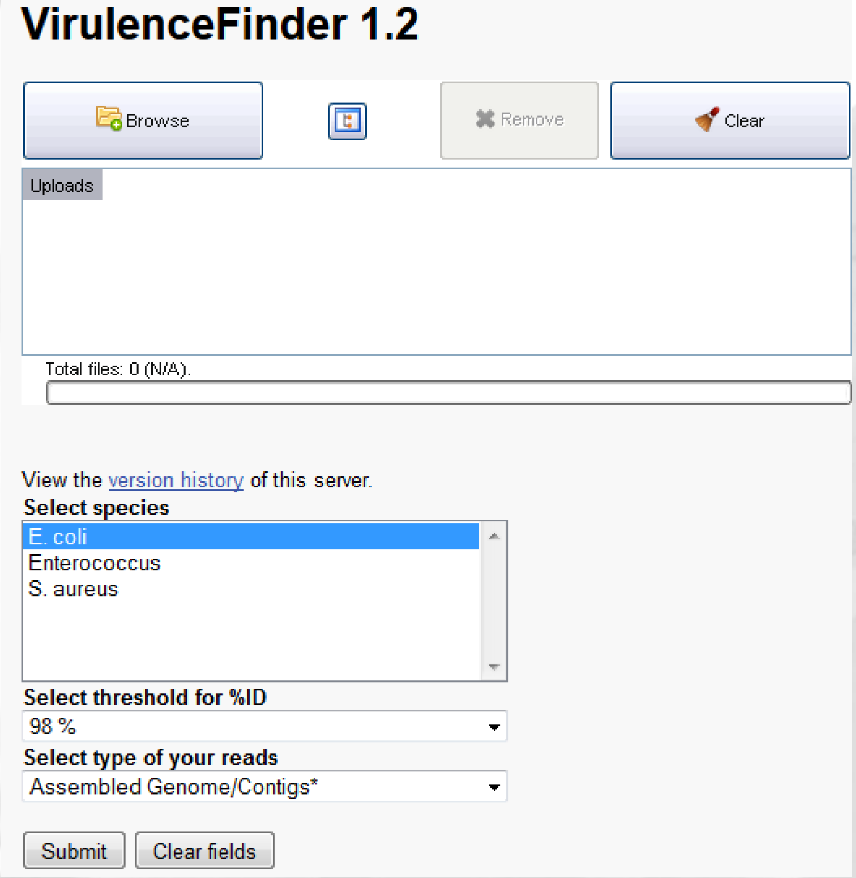
Gene	Description	Variants in database
astA	Heat-stable enterotoxin 1	11
bfpA	Major subunit of bundle-forming pili	5
cba	Colicin B	15
ccl	Cloacin	4
cdtB	Cytolethal distending toxin B	14
celb	Endonuclease colicin E2	10
cfa_c	Colonisation factor antigen I	4
cif	Type III secreted effector	4
cma	Colicin M	19
cnf1	Cytotoxic necrotizing factor	7
cofA	Longus type IV pilus subunit	1
eae	Intimin	45
eatA	Serine protease autotransporters of Enterobacteriaceae (SPATE)	3
efa1	EHEC factor for adherence	11
ehxA	Enterohaemolysin	12
epeA	Serine protease autotransporters of Enterobacteriaceae (SPATE)	1
espA	Type III secretions system	23
espB	Secreted protein B	14
espC	Serine protease autotransporters of Enterobacteriaceae (SPATE)	3
espF	Type III secretion system	13
espI	Serine protease autotransporters of Enterobacteriaceae (SPATE)	2
espJ	Prophage-encoded type III secretion system effector	2
espP	Putative exoprotein precursor	4
etpD	Type II secretion protein	3
f17-A	Subunit A of F17 fimbrial protein	7
f17-G	Adhesin subunit of F17 fimbriae	9
fanA	Involved in biogenesis of K99/F5 fimbriae	1
fasA	Fimbriae 987P/F6 subunit	1
fedA	Fimbrial protein F107 subunit A	3
fedF	Fimbrial adhesin AC precursor	6
fim41a	Mature Fim41a/F41 protein	2
gad	Glutamate decarboxylase	70
hlyE	Avian *E.coli* haemolysin	1
iha	Adherence protein	19
ipaD	Invasion protein *Shigella flexneri*	9
ipaH9.8	Invasion plasmid antigen	8
ireA	Siderophore receptor	4
iroN	Enterobactin siderophore receptor protein	13
iss	Increased serum survival	14
K88ab	K88/F4 protein subunit	10
katP	Plasmid-encoded catalase peroxidase	1
lngA	Longus type IV pilus	2
lpfA	Long polar fimbriae	11

76 different genes

stx1:14A,18B variants

stx2:114A,43B variants

eae: 45 variants



VirulenceFinder 1.2

Total files: 0 (N/A).

View the version history of this server.

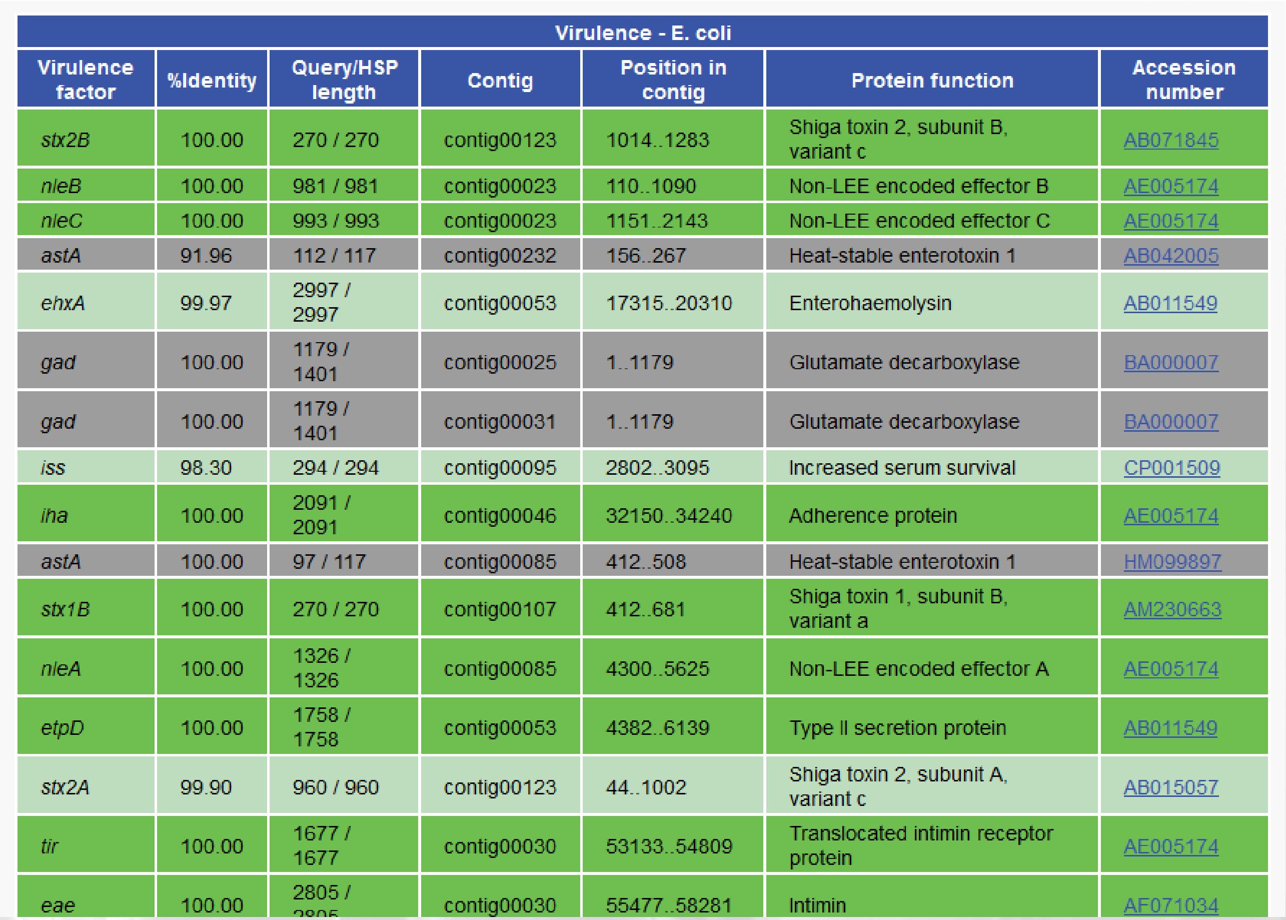
Select species

- E. coli
- Enterococcus
- S. aureus

Select threshold for %ID: 98 %

Select type of your reads: Assembled Genome/Contigs*

Real-Time Surveillance of VTEC using WGS



Virulence - E. coli						
Virulence factor	**%Identity**	**Query/HSP length**	**Contig**	**Position in contig**	**Protein function**	**Accession number**
stx2B	100.00	270 / 270	contig00123	1014..1283	Shiga toxin 2, subunit B, variant c	AB071845
nleB	100.00	981 / 981	contig00023	110..1090	Non-LEE encoded effector B	AE005174
nleC	100.00	993 / 993	contig00023	1151..2143	Non-LEE encoded effector C	AE005174
astA	91.96	112 / 117	contig00232	156..267	Heat-stable enterotoxin 1	AB042005
ehxA	99.97	2997 / 2997	contig00053	17315..20310	Enterohaemolysin	AB011549
gad	100.00	1179 / 1401	contig00025	1..1179	Glutamate decarboxylase	BA000007
gad	100.00	1179 / 1401	contig00031	1..1179	Glutamate decarboxylase	BA000007
iss	98.30	294 / 294	contig00095	2802..3095	Increased serum survival	CP001509
iha	100.00	2091 / 2091	contig00046	32150..34240	Adherence protein	AE005174
astA	100.00	97 / 117	contig00085	412..508	Heat-stable enterotoxin 1	HM099897
stx1B	100.00	270 / 270	contig00107	412..681	Shiga toxin 1, subunit B, variant a	AM230663
nleA	100.00	1326 / 1326	contig00085	4300..5625	Non-LEE encoded effector A	AE005174
etpD	100.00	1758 / 1758	contig00053	4382..6139	Type II secretion protein	AB011549
stx2A	99.90	960 / 960	contig00123	44..1002	Shiga toxin 2, subunit A, variant c	AB015057
tir	100.00	1677 / 1677	contig00030	53133..54809	Translocated intimin receptor protein	AE005174
eae	100.00	2805 / 2805	contig00030	55477..58281	Intimin	AF071034

DTU

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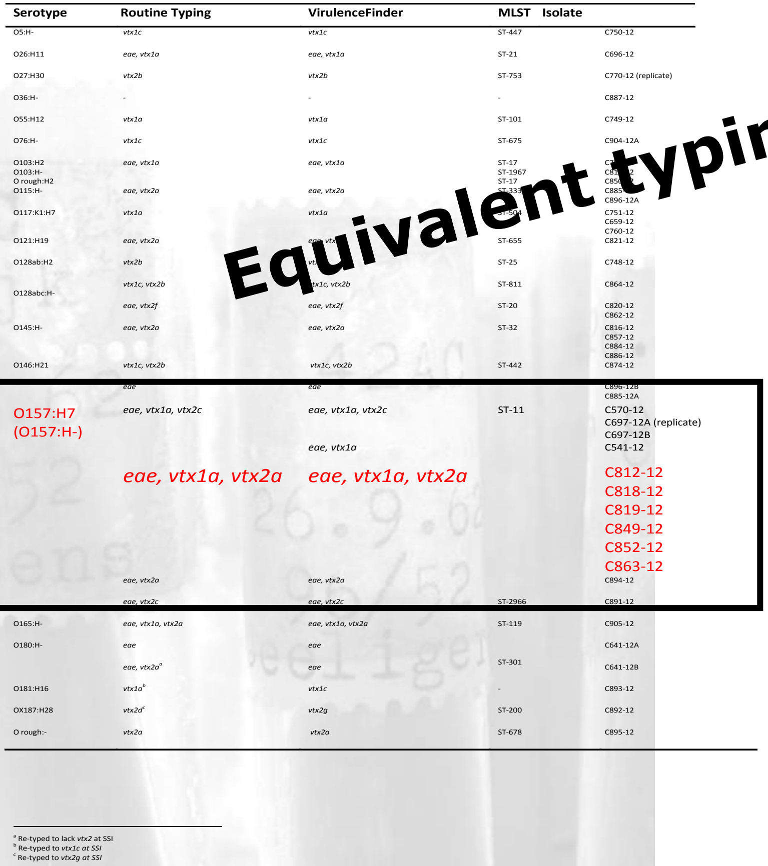
Real-Time Surveillance of VTEC using WGS

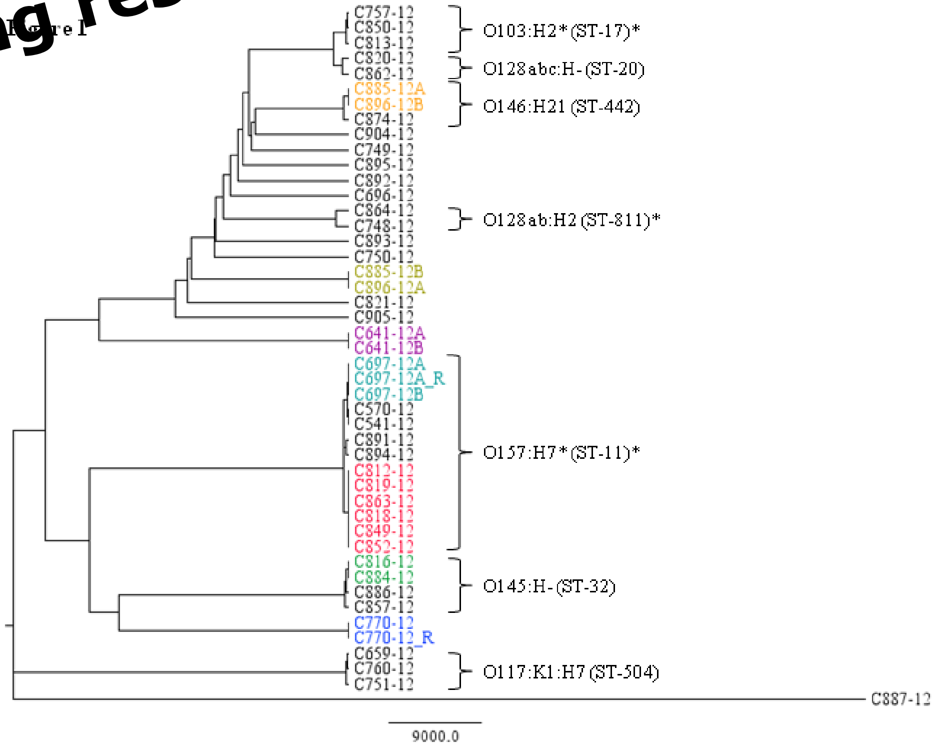


Serotype	Routine Typing	VirulenceFinder	MLST	Isolate
O5:H-	*vtx1c*	*vtx1c*	ST-447	C750-12
O26:H11	*eae, vtx1a*	*eae, vtx1a*	ST-21	C696-12
O27:H30	*vtx2b*	*vtx2b*	ST-753	C770-12 (replicate)
O36:H-	-	-	-	C887-12
O55:H12	*vtx1a*	*vtx1a*	ST-101	C749-12
O76:H-	*vtx1c*	*vtx1c*	ST-675	C904-12A
O103:H2 O103:H- O rough:H2	*eae, vtx1a*	*eae, vtx1a*	ST-17 ST-1967 ST-17	[illegible] [illegible] [illegible]
O115:H-	*eae, vtx2a*	*eae, vtx2a*	ST-333	[illegible] C896-12A
O117:K1:H7	*vtx1a*	*vtx1a*	ST-504	C751-12 C659-12 C760-12
O121:H19	*eae, vtx2a*	[illegible]	ST-655	C821-12
O128ab:H2	*vtx2b*	[illegible]	ST-25	C748-12
O128abc:H-	*vtx1c, vtx2b*	*vtx1c, vtx2b*	ST-811	C864-12
	eae, vtx2f	*eae, vtx2f*	ST-20	C820-12 C862-12
O145:H-	*eae, vtx2a*	*eae, vtx2a*	ST-32	C816-12 C857-12 C884-12 C886-12
O146:H21	*vtx1c, vtx2b*	*vtx1c, vtx2b*	ST-442	C874-12
	eae	*eae*		C896-12B C885-12A
O157:H7 (O157:H-)	*eae, vtx1a, vtx2c*	*eae, vtx1a, vtx2c*	ST-11	C570-12 C697-12A (replicate) C697-12B
		eae, vtx1a		C541-12
	eae, vtx1a, vtx2a	*eae, vtx1a, vtx2a*		C812-12 C818-12 C819-12 C849-12 C852-12 C863-12
	eae, vtx2a	*eae, vtx2a*		C894-12
	eae, vtx2c	*eae, vtx2c*	ST-2966	C891-12
O165:H-	*eae, vtx1a, vtx2a*	*eae, vtx1a, vtx2a*	ST-119	C905-12
O180:H-	*eae*	*eae*	ST-301	C641-12A
	eae, vtx2a[a]	*eae*		C641-12B
O181:H16	*vtx1a*[b]	*vtx1c*	-	C893-12
OX187:H28	*vtx2d*[c]	*vtx2g*	ST-200	C892-12
O rough:-	*vtx2a*	*vtx2a*	ST-678	C895-12

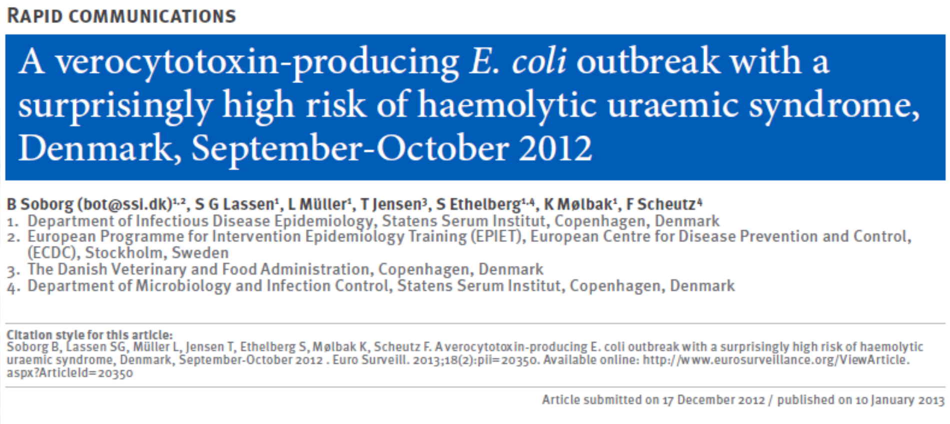
---

[a] Re-typed to lack *vtx2* at SSI
[b] Re-typed to *vtx1c at SSI*
[c] Re-typed to *vtx2g at SSI*

Equivalent typing results



Real-Time Surveillance of VTEC using WGS



RAPID COMMUNICATIONS

A verocytotoxin-producing *E. coli* outbreak with a surprisingly high risk of haemolytic uraemic syndrome, Denmark, September-October 2012

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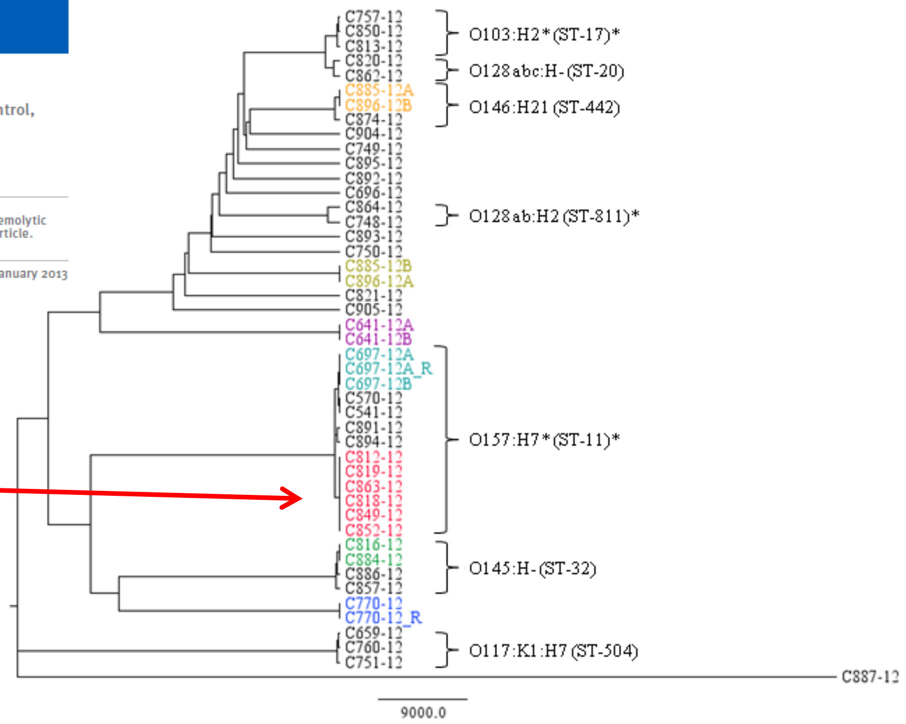

Outbreak detection

Real-Time Surveillance of VTEC using WGS

JCM
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Real-Time Whole-Genome Sequencing for Routine Typing, Surveillance, and Outbreak Detection of Verotoxigenic *Escherichia coli*

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Fast and accurate identification and typing of pathogens are essential for effective surveillance and outbreak detection. The current routine procedure is based on a variety of techniques, making the procedure laborious, time-consuming, and expensive. With whole-genome sequencing (WGS) becoming cheaper, it has huge potential in both diagnostics and routine surveillance. The aim of this study was to perform a real-time evaluation of WGS for routine typing and surveillance of verocytotoxin-producing *Escherichia coli* (VTEC). In Denmark, the Statens Serum Institut (SSI) routinely receives all suspected VTEC isolates. During a 7-week period in the fall of 2012, all incoming isolates were concurrently subjected to WGS using IonTorrent PGM. Real-time bioinformatics analysis was performed using web-tools (www.genomicepidemiology.org) for species determination, multilocus sequence type (MLST) typing, and determination of phylogenetic relationship, and a specific VirulenceFinder for detection of *E. coli* virulence genes was developed as part of this study. In total, 46 suspected VTEC isolates were characterized in parallel during the study. VirulenceFinder proved successful in detecting virulence genes included in routine typing, explicitly verocytotoxin 1 (*vtx1*), verocytotoxin 2 (*vtx2*), and intimin (*eae*), and also detected additional virulence genes. VirulenceFinder is also a robust method for assigning verocytotoxin (*vtx*) subtypes. A real-time clustering of isolates in agreement with the epidemiology was established from WGS, enabling discrimination between sporadic and outbreak isolates. Overall, WGS typing produced results faster and at a lower cost than the current routine. Therefore, WGS typing is a superior alternative to conventional typing strategies. This approach may also be applied to typing and surveillance of other pathogens.

Bacterial pathogens still pose a major threat to public health, and in order to limit their spread and prevent infectious disease outbreaks, accurate and rapid diagnostics and classification of isolates are of great importance. In current routine practice, isolation and identification are mostly performed at clinical microbiological laboratories, and verification and further characterization are performed for a few selected pathogens at national, or regional, reference laboratories, using a variety of species-specific methods. Typing and surveillance of bacterial pathogens rely mainly on well-established, standardized phenotypic and molecular typing methods, such as serotyping and pulsed-field gel electrophoresis (PFGE) (1, 2). However, to obtain sufficient discrimination between isolates, it is typically necessary to combine typing results from several different typing techniques, both phenotypic and genotypic. As a result, it is laborious, time-consuming, and expensive to perform proper typing for surveillance and outbreak detection.

As the cost of whole-genome sequencing (WGS) has decreased and benchtop sequencing machines enable fast turnaround, it has become increasingly attractive for use in routine diagnostics and typing, and the approach has already been found useful in retrospective outbreak investigations (3, 4).

Although WGS provides detailed information that will, in theory, enable diagnostics and typing solely on the basis of the features in the bacterial genome, it is a challenge to define and extract the appropriate information from the large amount of sequence data that is generated. Thus, to facilitate the use of WGS data for routine diagnostics, typing, and surveillance, it is important that the sequence data can be automatically and quickly converted to clinically relevant information that can be easily interpreted by physicians and public health professionals with limited bioinformatics skills. To achieve this, the Center for Genomic Epidemiology (CGE) provides public, user-friendly web-tools for rapid handling of WGS data and extraction of relevant information, useful for diagnostics, surveillance, and outbreak investigations for the global medical society (www.genomicepidemiology.org).

In this study, as a proof of concept, we demonstrate the usefulness of WGS for routine typing, surveillance, and outbreak detection of verocytotoxin-producing *Escherichia coli* (VTEC) infections in Denmark. VTEC, also known as Shiga toxin-producing *E. coli* (STEC), is a gastrointestinal pathogen, which is typically spread by ingestion of contaminated food or water or person-to-person contact. Rapid and reliable diagnostics and detection of outbreak clusters are of utmost importance for control. VTEC infections cause bloody diarrhea and in some cases hemolytic-uremic syndrome (HUS), which is characterized by kidney failure, thrombocytopenia, and microangiopathic hemolytic anemia, and can be fatal to young children. VTEC pathogenicity is facili-

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The authors have paid a fee to allow immediate free access to this article.

Real-Time Surveillance of VTEC using WGS

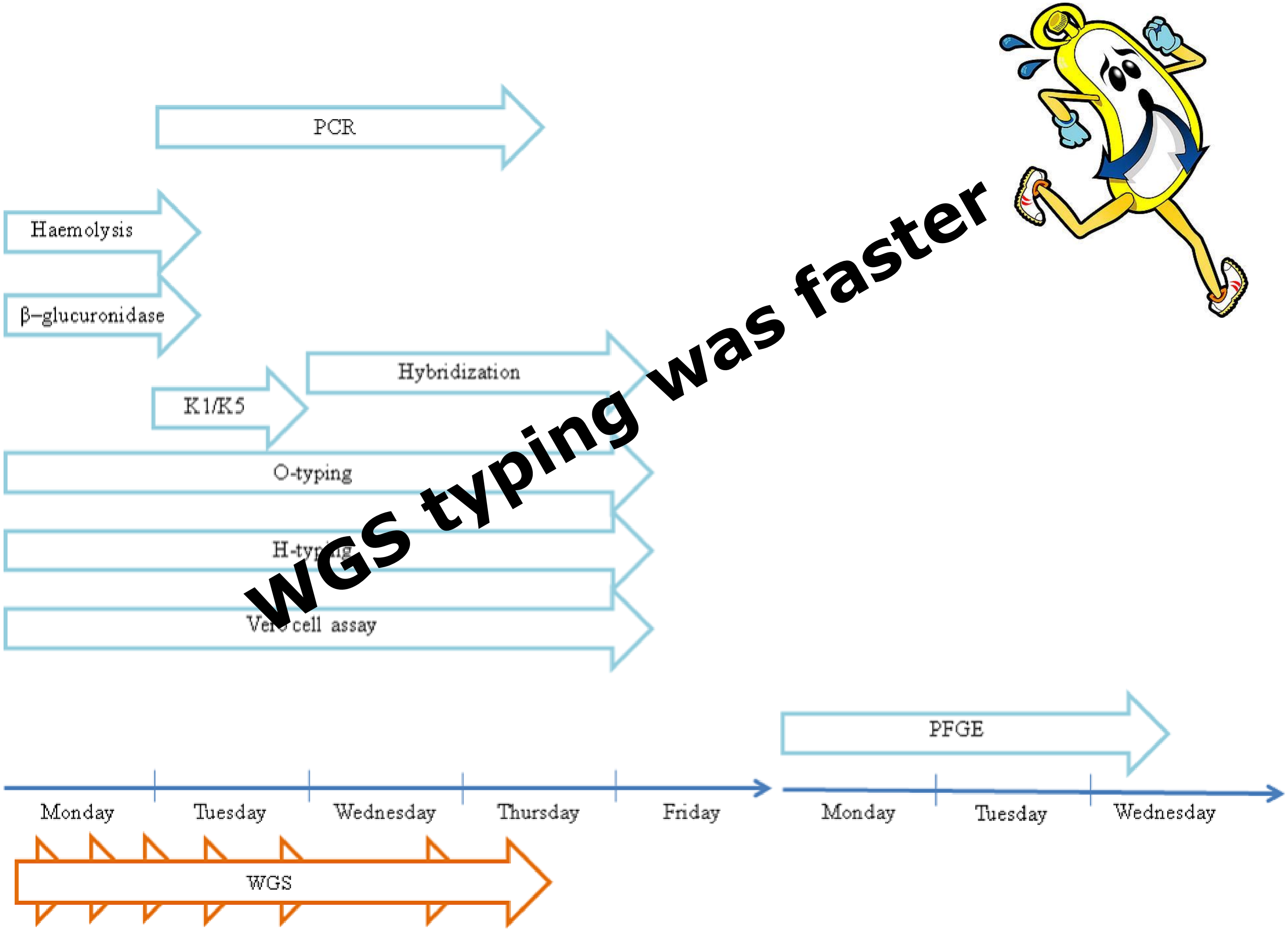


Figure 3

WGS typing was faster

Real-Time Surveillance of VTEC using WGS

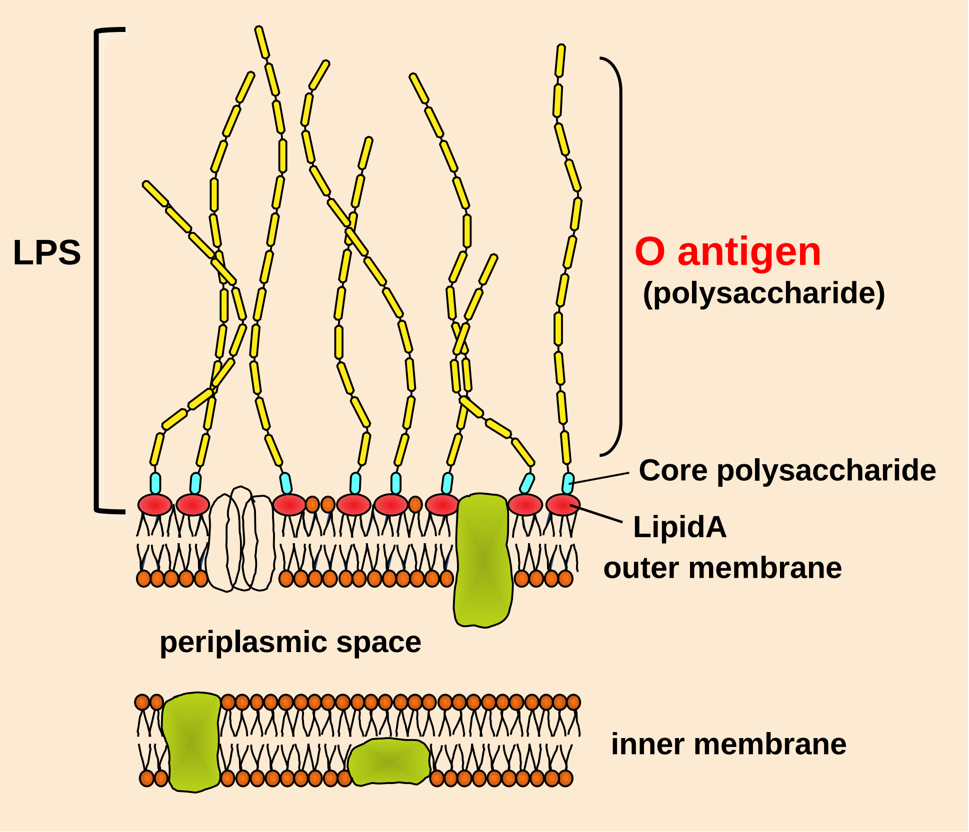
Reviewer comment:

”It appears that VTEC serotype was determined solely by conventional agglutination methods. Could the investigators add "molecular serotyping" to the WGS output by using the flagellar and O-antigen associated genes?”

SerotypeFinder: O

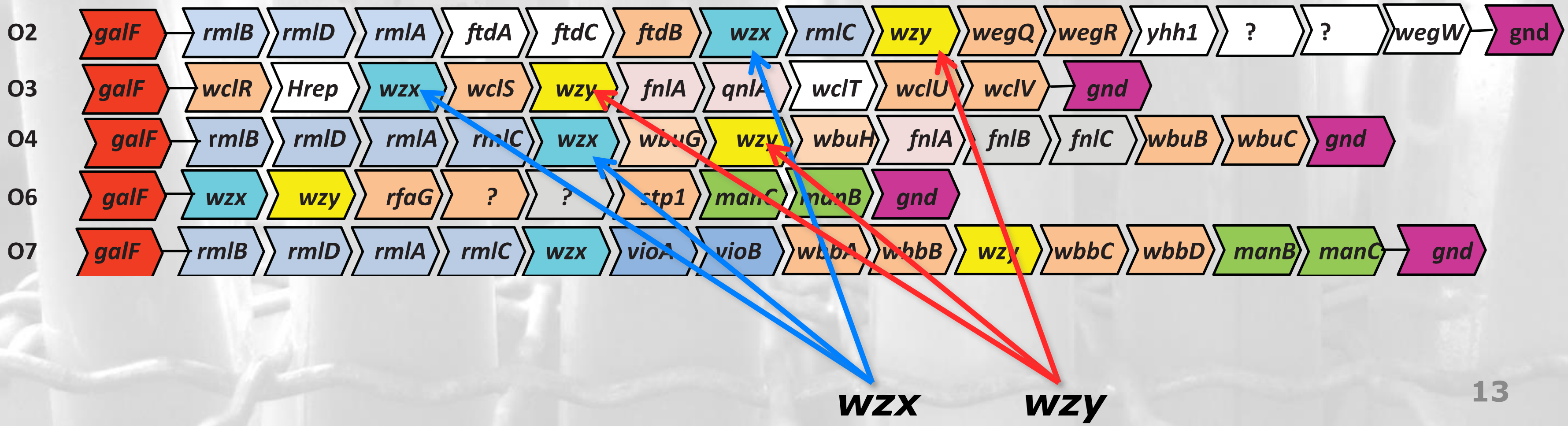
- ***wzx* and *wzy***
  (O-antigen flippase and polymerase)
- ***wzm* and *wzt***
  (ABC transporter)

Anna Maria Malberg Tetzschner
Master student

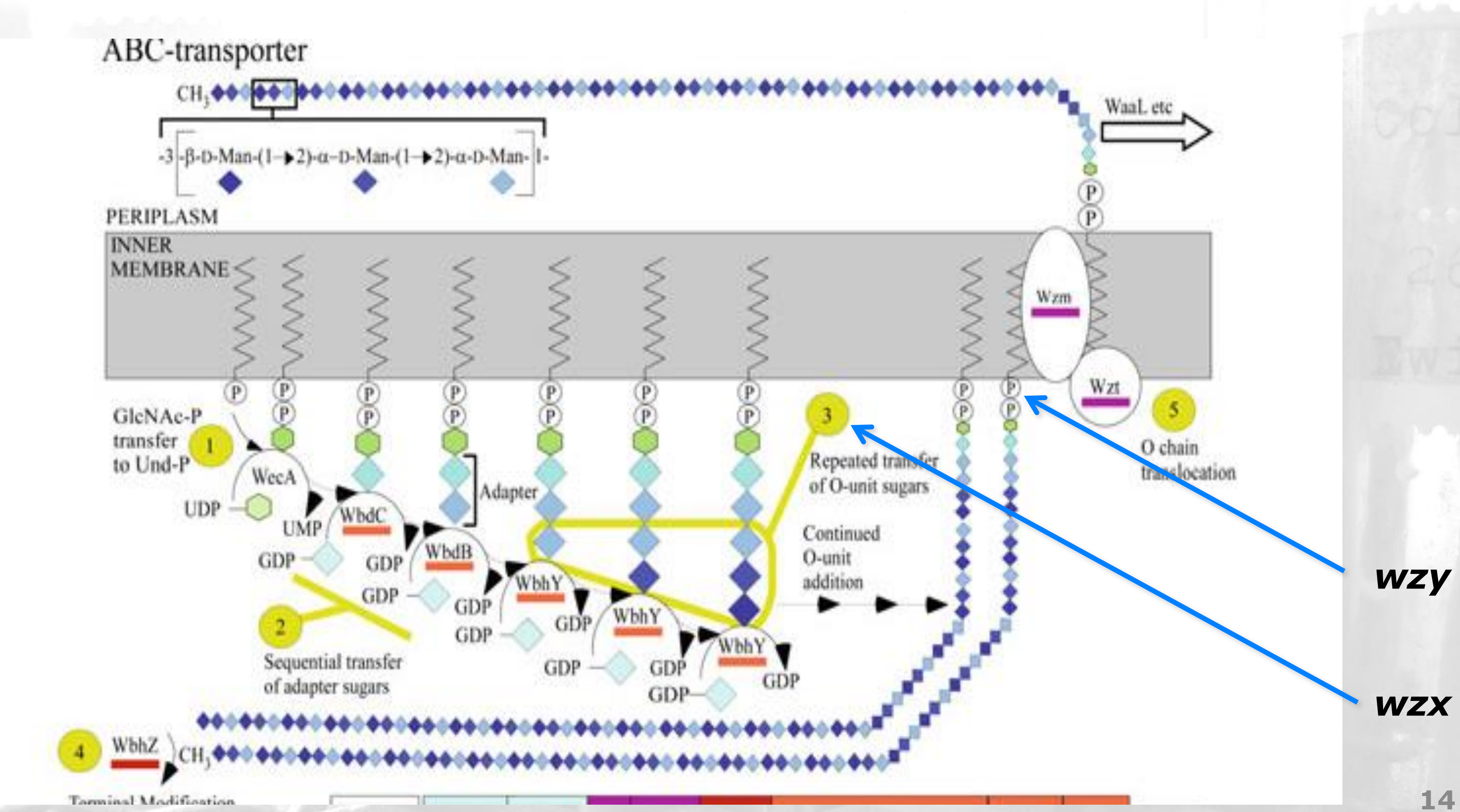


O-antigen gene cluster

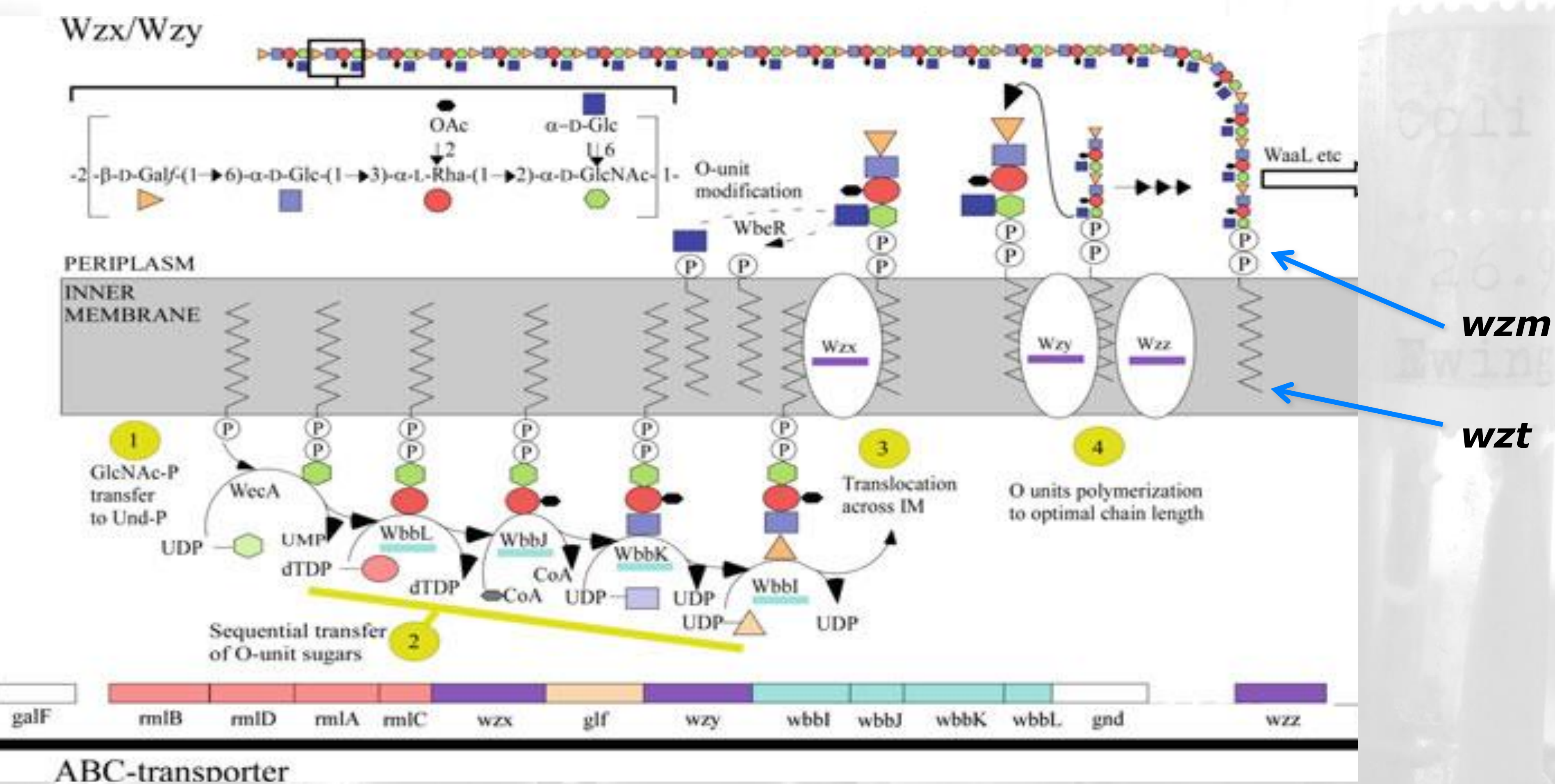
- Cluster on chromosome
  - – between *galF* & *gnd*, (*his* & *gnd* for O8 & O9)
- Three gene classes:
  - – Nucleotide sugar genes
  - – Sugar transferase genes
  - – O-unit processing genes: *wzx, wzy, wzm* & *wzt*



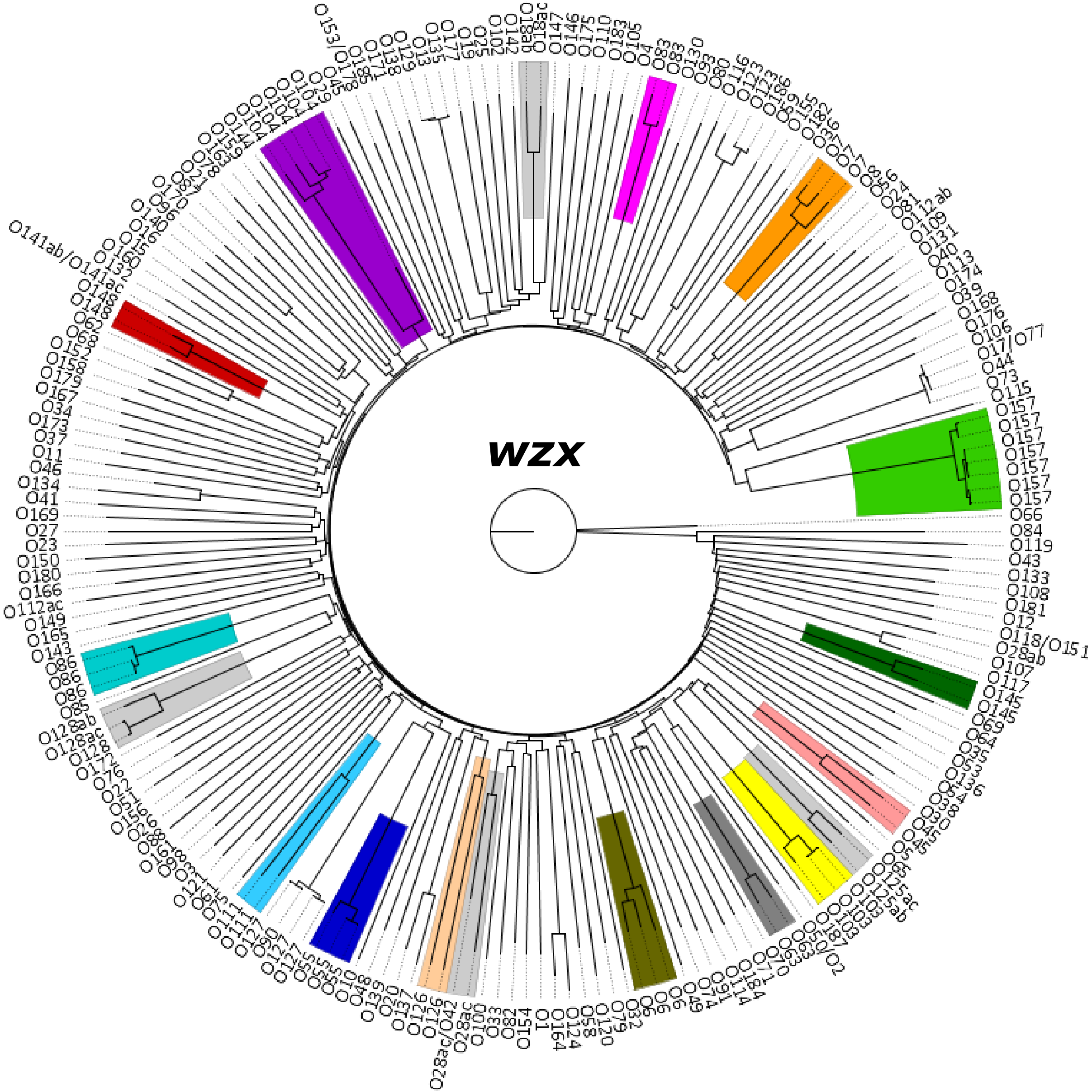
wzy-dependent



wzy-independent

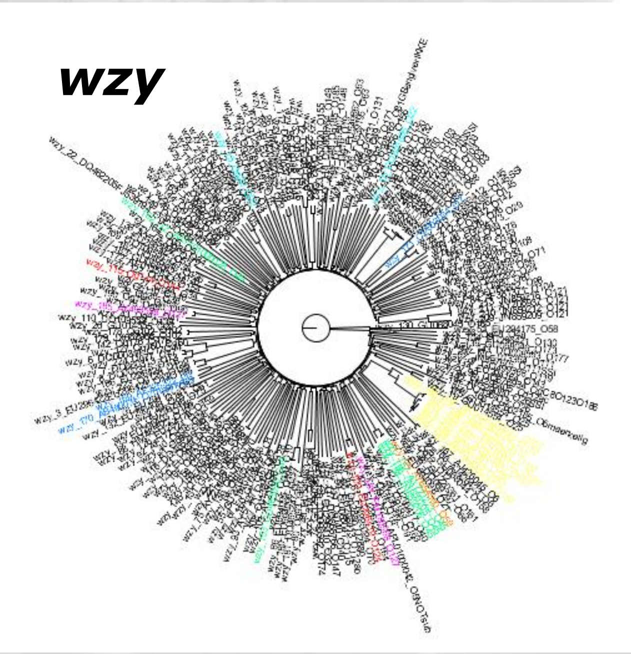


SerotypeFinder: O-Typing

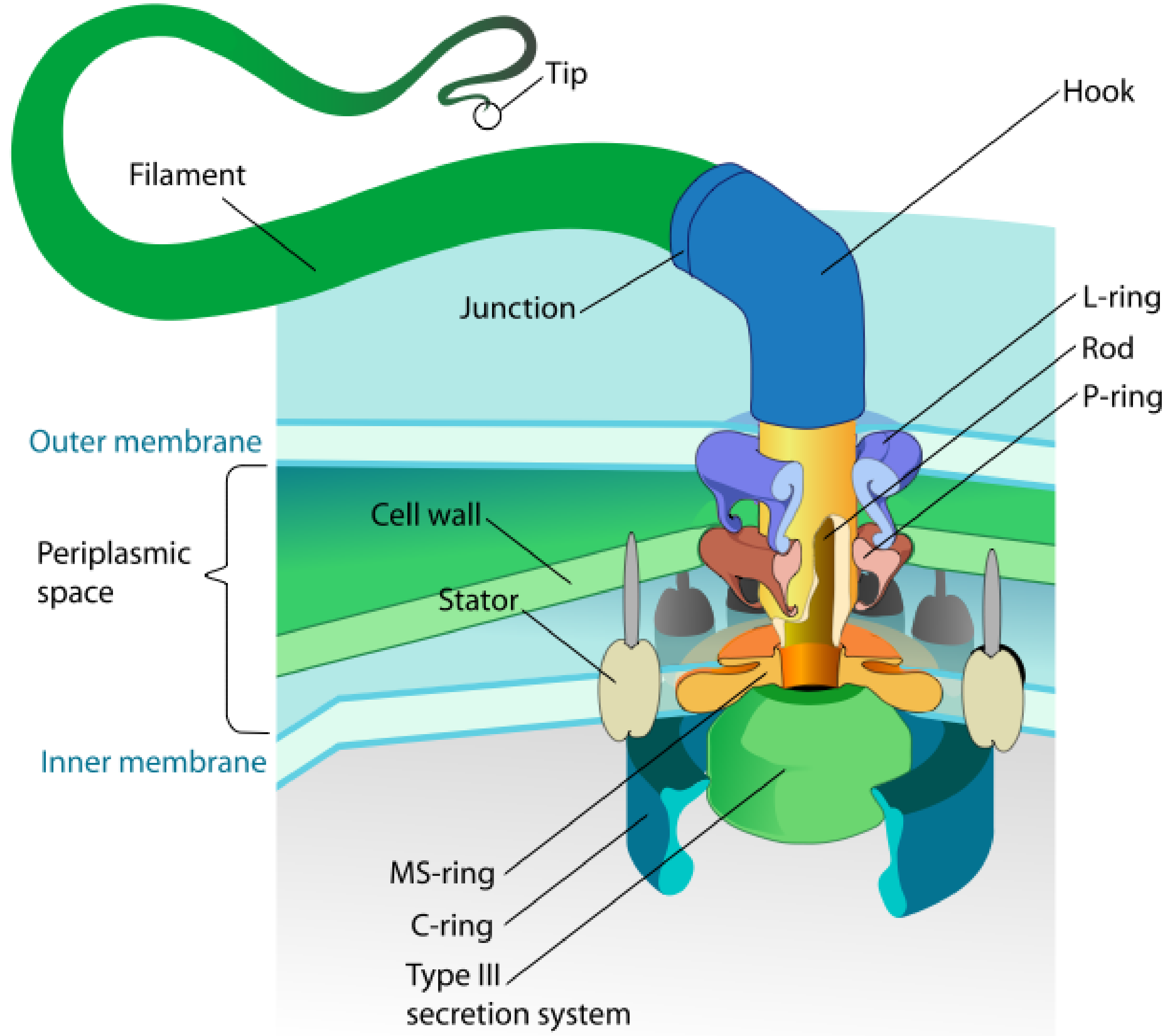


Two types are not in database:
O57
O14

Probably because of a deletion of these genes



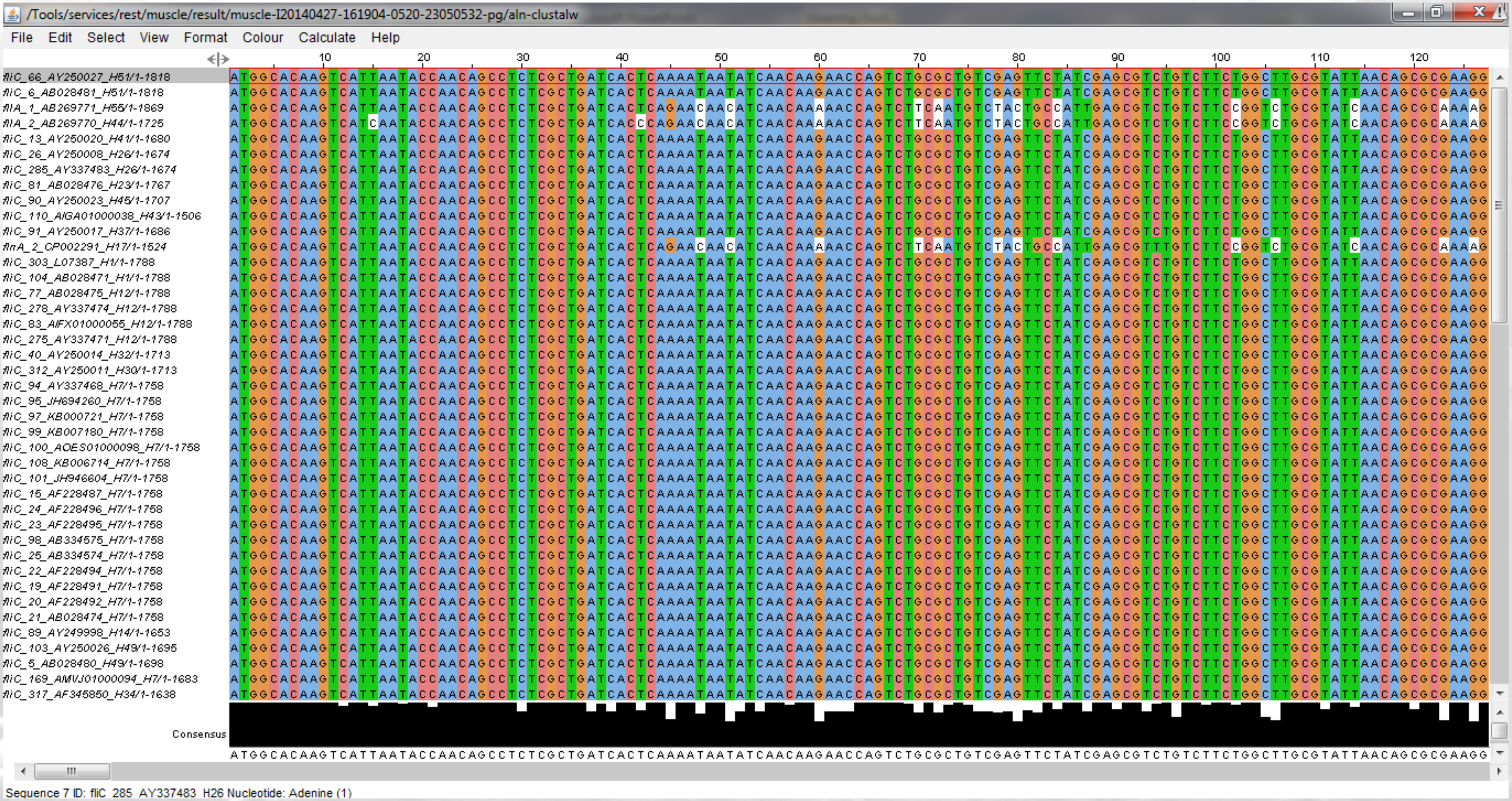
***SerotypeFinder:* -H**



- ***fliC*** (flagellin)
- ***flkA*, *flnA*, *flmA*, *fllA***

SerotypeFinder

- **Collection of genes with serotype information from NCBI**



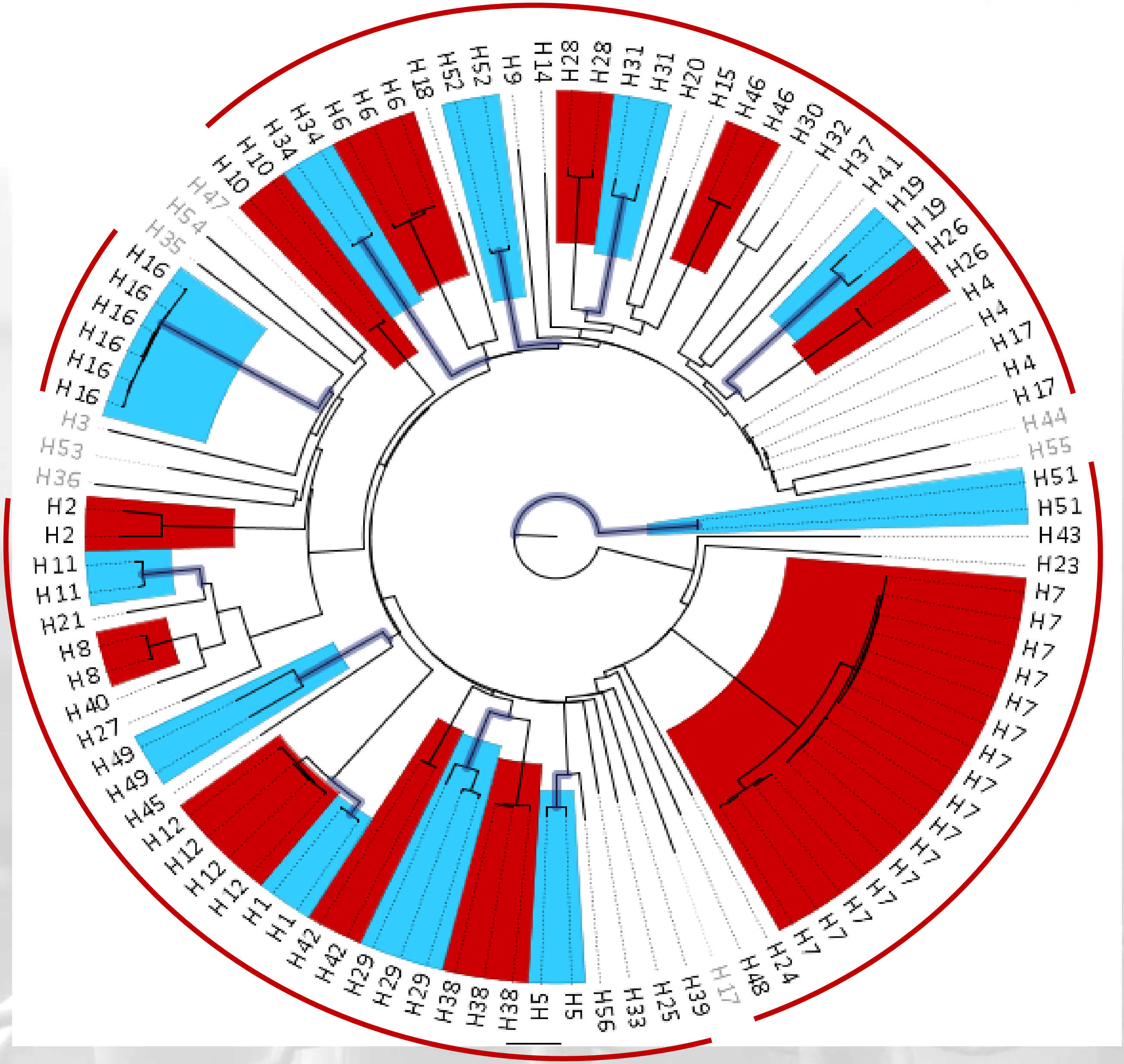
/Tools/services/rest/muscle/result/muscle-I20140427-161904-0520-23050532-pg/aln-clustalw

```
fliC_66_AY250027_H51/1-1818         ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_6_AB028481_H51/1-1818          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliA_1_AB269771_H55/1-1869          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAGAACAACATCAACAAAAACCAGTCTTCAATGTCTACTGCCATTGAGCGTCTGTCTTCCGGTCTGCGTATCAACAGCGCAAAAG
fliA_2_AB269770_H44/1-1725          ATGGCACAAGTCATCAATACCAACAGCCTCTCGCTGATCACCCAGAACAACATCAACAAAAACCAGTCTTCAATGTCTACTGCCATTGAGCGTCTGTCTTCCGGTCTGCGTATCAACAGCGCAAAAG
fliC_13_AY250020_H41/1-1680         ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_26_AY250008_H26/1-1674         ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_285_AY337483_H26/1-1674        ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_81_AB028476_H23/1-1767         ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_90_AY250023_H45/1-1707         ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_110_AIGA01000038_H43/1-1506    ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_91_AY250017_H37/1-1686         ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
flnA_2_CP002291_H17/1-1524          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAGAACAACATCAACAAAAACCAGTCTTCAATGTCTACTGCCATTGAGCGTTTGTCTTCCGGTCTGCGTATCAACAGCGCAAAAG
fliC_303_L07387_H1/1-1788           ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_104_AB028471_H1/1-1788         ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_77_AB028475_H12/1-1788         ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_278_AY337474_H12/1-1788        ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_83_AIFX01000055_H12/1-1788     ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_275_AY337471_H12/1-1788        ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_40_AY250014_H32/1-1713         ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_312_AY250011_H30/1-1713        ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_94_AY337468_H7/1-1758          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_95_JH694260_H7/1-1758          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_97_KB000721_H7/1-1758          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_99_KB007180_H7/1-1758          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_100_AOES01000098_H7/1-1758     ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_108_KB006714_H7/1-1758         ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_101_JH946604_H7/1-1758         ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_15_AF228487_H7/1-1758          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_24_AF228496_H7/1-1758          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_23_AF228495_H7/1-1758          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_98_AB334575_H7/1-1758          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_25_AB334574_H7/1-1758          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_22_AF228494_H7/1-1758          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_19_AF228491_H7/1-1758          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_20_AF228492_H7/1-1758          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_21_AB028474_H7/1-1758          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_89_AY249998_H14/1-1653         ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_103_AY250026_H49/1-1695        ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_5_AB028480_H49/1-1698          ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_169_AMVJ01000094_H7/1-1683     ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
fliC_317_AF345850_H34/1-1638        ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG

                          Consensus
                                    ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGG
```

Sequence 7 ID: fliC 285 AY337483 H26 Nucleotide: Adenine (1)

SerotypeFinder: -H



All 53 H-types represented

***fliC* genes**

SerotypeFinder: Evaluation -H

- 132 isolates –serotyped and sequenced- tested with tool
- Correct H-type assigned (100%)

Isolate	**H type**	%ID	Length
042_O44**H18**	**H18**	100.00	1665/1665
11128_O111**H-**	**H8**	100.00	1479/1479
11368_O26**H11**	**H11**	99.93	1467/1459
12009_O103**H2**	**H2**	100.00	1494/1494
2362-75_O55**H6**	**H6**	100.00	1647/1647
536_O6K15**H31**	**H31**	100.00	1668/1668
53638_O144	**H30**	100.00	1713/1713
55989_O104**H4**	**H4**	100.00	1050/1050
86-24_O157**H7**	**H7**	100.00	1758/1758
B7A_O148**H28**	**H28**	100.00	1740/1740
BL21_roughK12	**H48**	100.00	1497/1497
C1214-90_O26**H11**	**H11**	99.93	1467/1459
C1244_91_O55**H-**	**H7**	100.00	1758/1758
C12-92_O55**H7**	**H7**	100.00	1758/1758
C154-11_O55**H7**	**H7**	100.00	1758/1758
C155_11_O26**H11**	**H11**	99.93	1467/1459
C166_11_O26**H-**	**H11**	99.93	1467/1459
C170_11_O128ab**H2**	**H2**	100.00	1494/1494
C213_10_O127**H40**	**H40**	98.51	1479/1479
C2139_99_O111**H7**	**H7**	100.00	1758/1758
C238_91_O103**H25**	**H25**	100.00	1332/1332
C260_92_O127K-**H40**	**H40**	98.51	1479/1479
C262_10_O114**H49**	**H49**	98.70	1695/1695
C283_09_O177**H-**	**H25**	97.04	1332/1316
C295_10_O55**H34**	**H34**	98.96	1638/1638
C341_10_O128ab**H-**	**H2**	100.00	1494/955

SerotypeFinder: Preliminary validation of O:H

Number of genomes

	for validation	**with detected genes**	**with consistent WGS -and conventional results**
O-typing	535	507[a] (~95%)	502[b] (~99%)
H-typing	437	436 (~100%)	434 (~100%)

a) In 43 genomes, genes were either found by reference mapping, or only one gene was used for prediction.

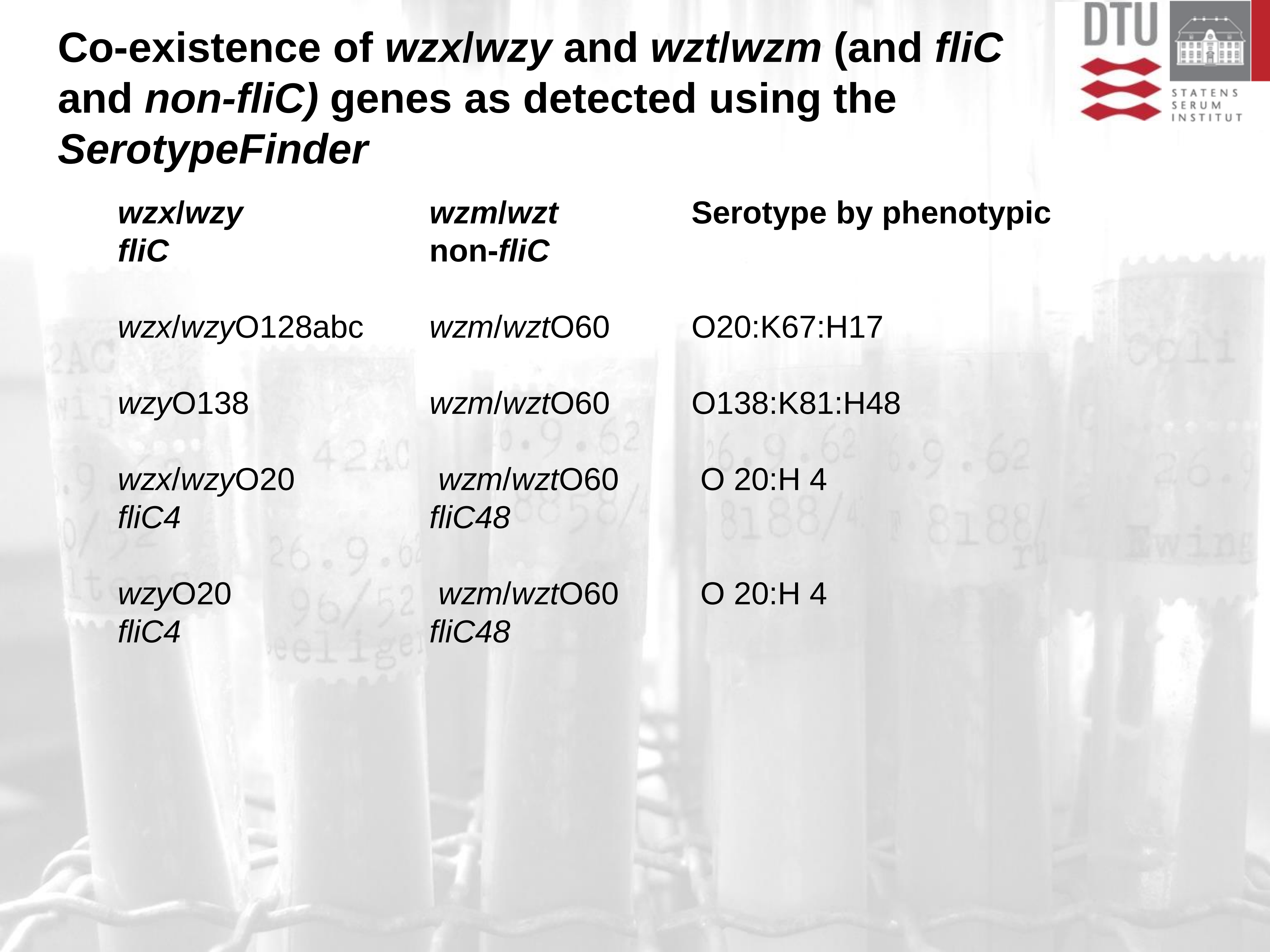
b) *b)* 11 predictions were ambiguous between the two O-processing genes (O118/O151(7), O164/O124, O134/O46, O90/O127, O162/O101).

Co-existence of *fliC* and *non-fliC* genes as detected using the *SerotypeFinder*

fliC	**non-*fliC***	**Serotype by phenotypic tests**
fliC2	*flkA35*	O128abc:H35
		O?:K?:H35
fliC2	*flkA47*	OX185:H47
		O128abc:H47
fliC11	*flkA47*	**O156**:H47
fliC21	*flkA36*	O86:H36
fliC21	*flkA47**	O86:**H47**
fliC21	*flmA54**	O161:**H54**
fliC25	*fllA55*	O21:H55
fliC27	*flkA36*	O26:H36
		O51:H36
fliC27	*flmA54*	O117:H54
fliC38	*fllA55**	O75:**H55**
		O75:H55
fliC40	*flkA53*	O148:H53

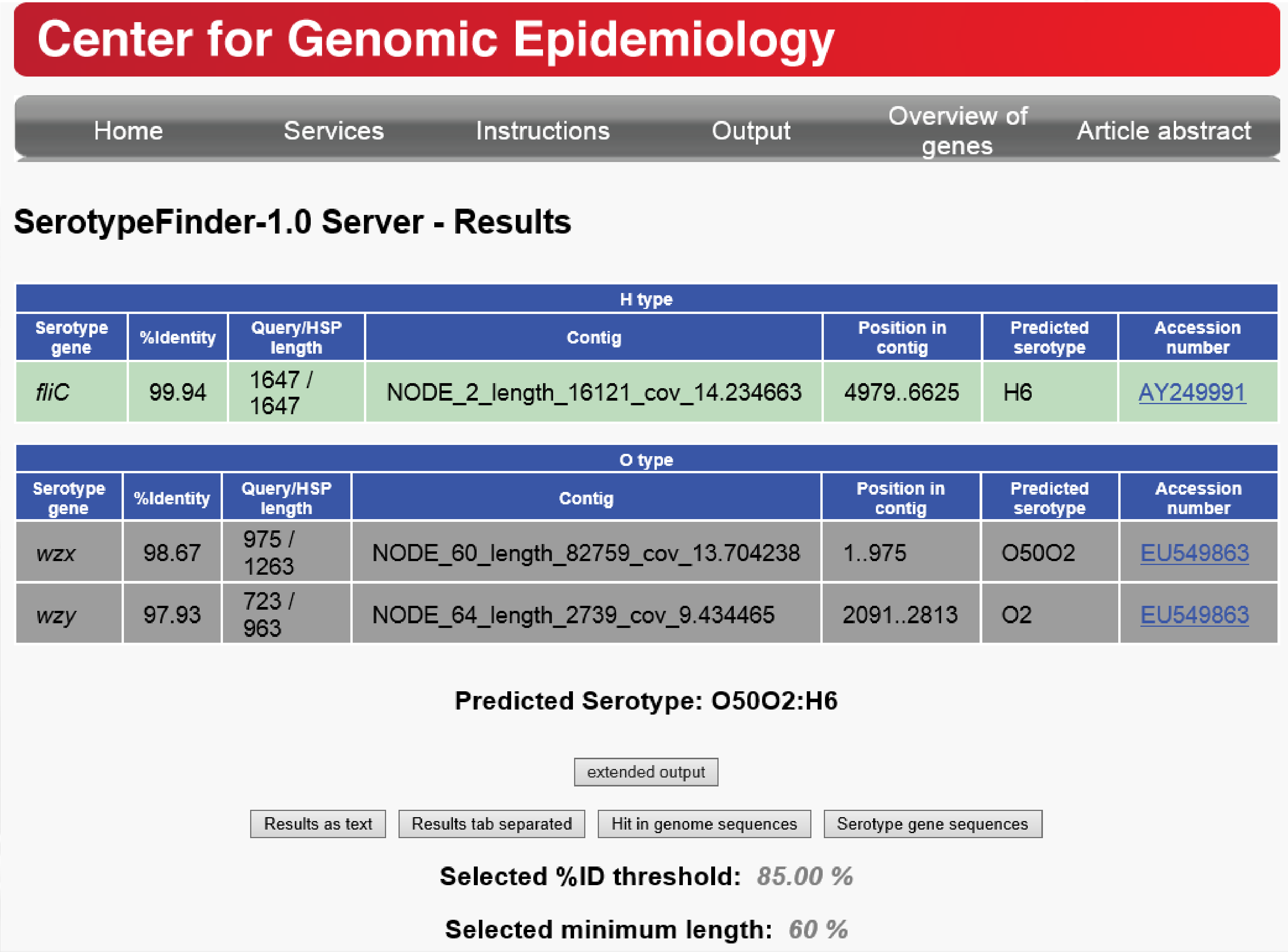
*** Previously described combinations**

Co-existence of *wzx/wzy* and *wzt/wzm* (and *fliC* and *non-fliC)* genes as detected using the *SerotypeFinder*



wzx/wzy ***fliC***	***wzm/wzt*** **non-*fliC***	**Serotype by phenotypic**
*wzx/wzy*O128abc	*wzm/wzt*O60	O20:K67:H17
*wzy*O138	*wzm/wzt*O60	O138:K81:H48
*wzx/wzy*O20 *fliC4*	*wzm/wzt*O60 *fliC48*	O 20:H 4
*wzy*O20 *fliC4*	*wzm/wzt*O60 *fliC48*	O 20:H 4

O:H serotyping



SerotypeFinder-1.0 Server - Results

H type

Serotype gene	%Identity	Query/HSP length	Contig	Position in contig	Predicted serotype	Accession number
fliC	99.94	1647 / 1647	NODE_2_length_16121_cov_14.234663	4979..6625	H6	AY249991

O type

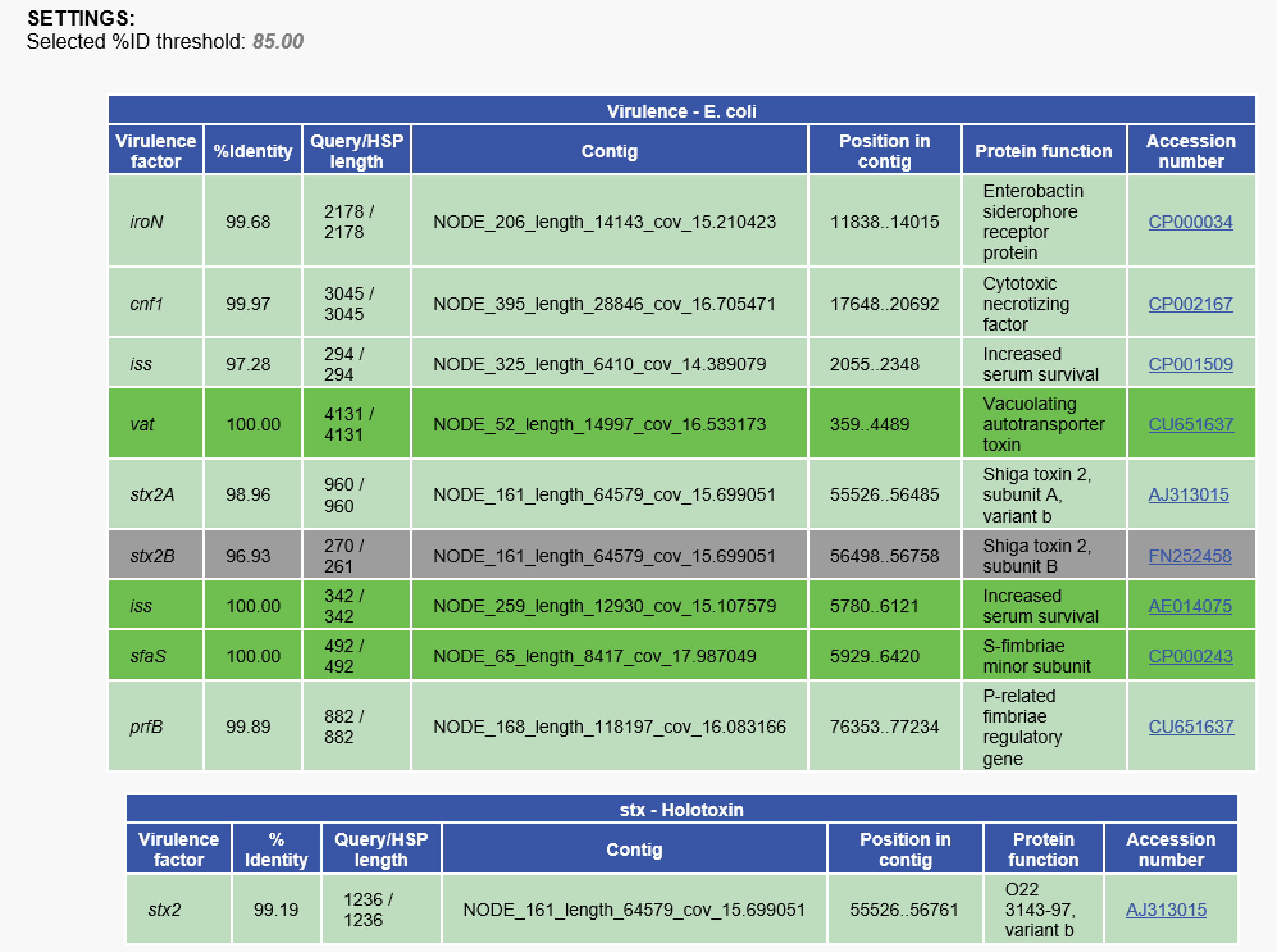
Serotype gene	%Identity	Query/HSP length	Contig	Position in contig	Predicted serotype	Accession number
wzx	98.67	975 / 1263	NODE_60_length_82759_cov_13.704238	1..975	O50O2	EU549863
wzy	97.93	723 / 963	NODE_64_length_2739_cov_9.434465	2091..2813	O2	EU549863

Predicted Serotype: O50O2:H6

Selected %ID threshold: *85.00 %*

Selected minimum length: *60 %*

Virulence genes and vtx *subtyping*



SETTINGS:
Selected %ID threshold: *85.00*

Virulence - E. coli						
Virulence factor	**%Identity**	**Query/HSP length**	**Contig**	**Position in contig**	**Protein function**	**Accession number**
iroN	99.68	2178 / 2178	NODE_206_length_14143_cov_15.210423	11838..14015	Enterobactin siderophore receptor protein	CP000034
cnf1	99.97	3045 / 3045	NODE_395_length_28846_cov_16.705471	17648..20692	Cytotoxic necrotizing factor	CP002167
iss	97.28	294 / 294	NODE_325_length_6410_cov_14.389079	2055..2348	Increased serum survival	CP001509
vat	100.00	4131 / 4131	NODE_52_length_14997_cov_16.533173	359..4489	Vacuolating autotransporter toxin	CU651637
stx2A	98.96	960 / 960	NODE_161_length_64579_cov_15.699051	55526..56485	Shiga toxin 2, subunit A, variant b	AJ313015
stx2B	96.93	270 / 261	NODE_161_length_64579_cov_15.699051	56498..56758	Shiga toxin 2, subunit B	FN252458
iss	100.00	342 / 342	NODE_259_length_12930_cov_15.107579	5780..6121	Increased serum survival	AE014075
sfaS	100.00	492 / 492	NODE_65_length_8417_cov_17.987049	5929..6420	S-fimbriae minor subunit	CP000243
prfB	99.89	882 / 882	NODE_168_length_118197_cov_16.083166	76353..77234	P-related fimbriae regulatory gene	CU651637

stx - Holotoxin						
Virulence factor	**% Identity**	**Query/HSP length**	**Contig**	**Position in contig**	**Protein function**	**Accession number**
stx2	99.19	1236 / 1236	NODE_161_length_64579_cov_15.699051	55526..56761	O22 3143-97, variant b	AJ313015

Virulence genes and vtx *subtyping*

```
stx2: WARNING, Identity: 99.19%, Query/HSP: 1236/1236, Contig name: NODE_9_length_64883_cov_15.699967, Position: 55830..57065

Virulence gene seq: atgaagtgtatattgttaaaatgggtactgtgcctgttactgggcttttcttcggtatcc
Hit in genome:      atgaagtgtatattgttaaaatggatactgtgcctgttgctgggcttttcttcggtatcc

Virulence gene seq: tattcccgggaatttacgatagacttttcgactcaacaaagttatgtctcttcgttaaat
Hit in genome:      tattcccgggaatttacgatagacttttcgactcaacaaagttatgtctcttcgttaaat

Virulence gene seq: agtatacggacagaaatatcgactcctcttgagcatatatctcaggggaccacatcggtg
Hit in genome:      agtatacggacagaaatatcgactcctcttgagcatatatctcaggggaccacatcggtg

Virulence gene seq: tctgttattaaccacaccccaccgggcagttattttgctgtggatatacgagggcttgat
Hit in genome:      tctgttattaaccacaccccaccgggcagttattttgctgtggatatacgagggcttgat

Virulence gene seq: gtctatcaggcgcgttttgaccatcttcgtctgattattgagcaaaataatttatatgtg
Hit in genome:      gtctatcaggcgcgttttgaccatcttcgtctgattgttgagcaaaataatttatatgtg

Virulence gene seq: gccggatttgttaatacggcaacaaatactttctacagattttcagattttgcacatata
Hit in genome:      gccggattcgttaatacggcaacaaatactttctacagattttctgattttgcacatata

Virulence gene seq: tcagtgcccggtgtgacaactgtttccatgacaacggacagcagttataccactctgcaa
Hit in genome:      tcagtgcccggtgtgacaactgtttccatgacaacggacagcagttataccactctgcaa

Virulence gene seq: cgtgtcgcagcgctggaacgttccggaatgcaaatcagtcgtcactcactggtttcatca
Hit in genome:      cgtgtcgcagcgctggaacgttccggaatgcaaatcagtcgtcactcactggtttcatca

Virulence gene seq: tatctggcgttaatggagtttagtggaaatgccatgaccagagatgcatccagagcagtt
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Virulence gene seq: ctgcgttttgtcactgtcacagcagaagccttacggttcaggcaaatacagagagaattt
Hit in genome:      ttgcgttttgttactgtcacagcagaagccttacggttcaggcaaatacagagagaattt

Virulence gene seq: cgtctggcactgtctgaaactgctcctgtttatacgatgacaccggaagaaatggacctc
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Virulence gene seq: acactgaactgggggagaatcagcaatgtgcttccggagtttcggggagagggtggtgtc
Hit in genome:      acactgaactgggggcgaatcagcaatgtgcttccggagtttcggggagagggtggtgtc

Virulence gene seq: agagtggggcgaatatcctttaataatatatcagcgatactgggcacagtggcggttata
Hit in genome:      agagtggggcgaatatcctttaataatatatcagcgatactgggcacagtggcggttata

Virulence gene seq: ctgaattgccatcatcagggggcgcgttccgttcgcgccgtgaatgaagagatacaacca
Hit in genome:      ctgaattgccatcatcagggggcacgttccgttcgcgccgtgaatgaagagatacaacca

Virulence gene seq: gaatgtcagataactggcgacaggccagttataaggataaacaatactttatgggaaagt
Hit in genome:      gaatgtcagataactggcgacaggccagttataaggataaacaatactttatgggaaagt
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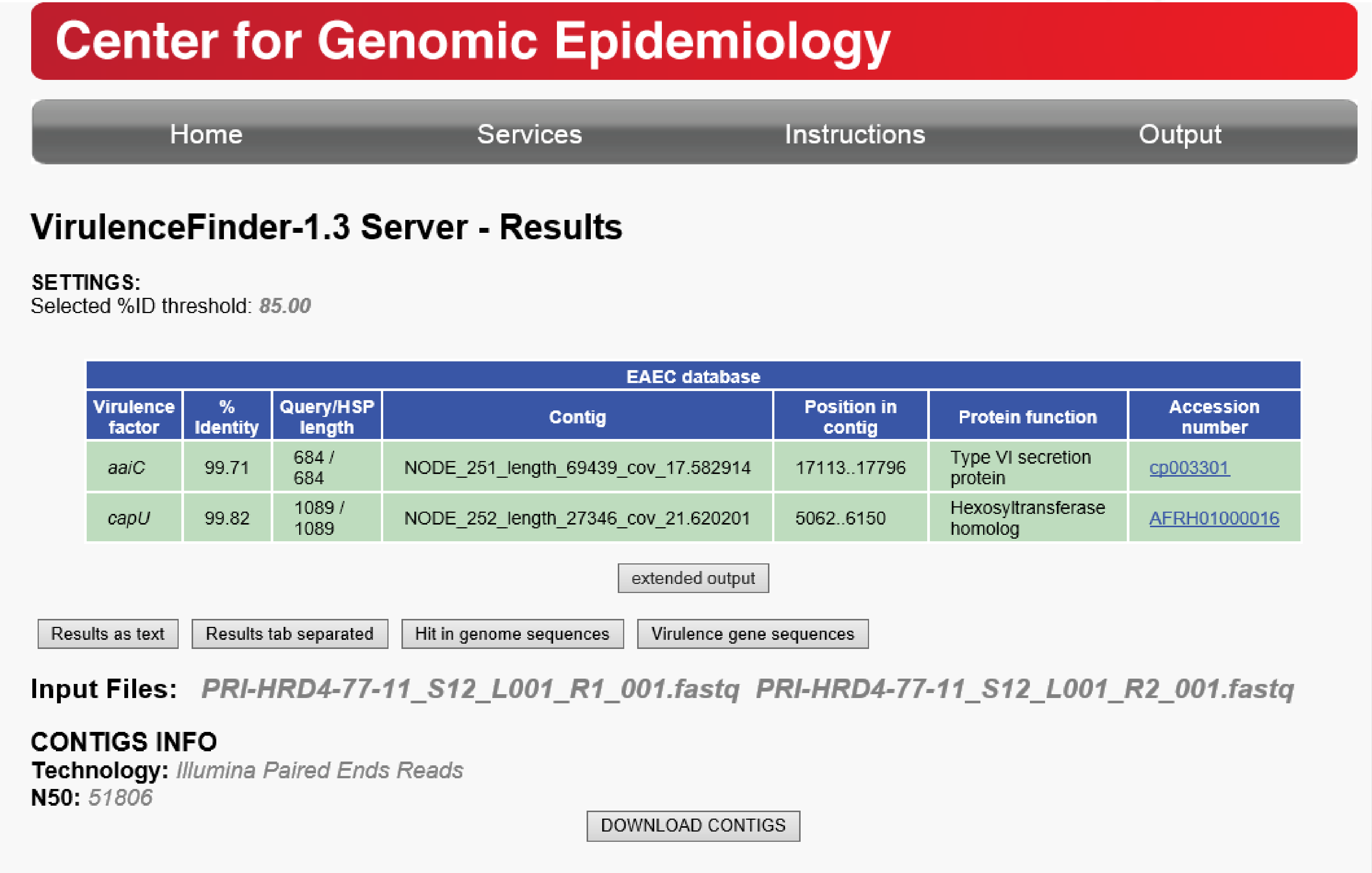
EAggECFinder



VirulenceFinder-1.3 Server - Results

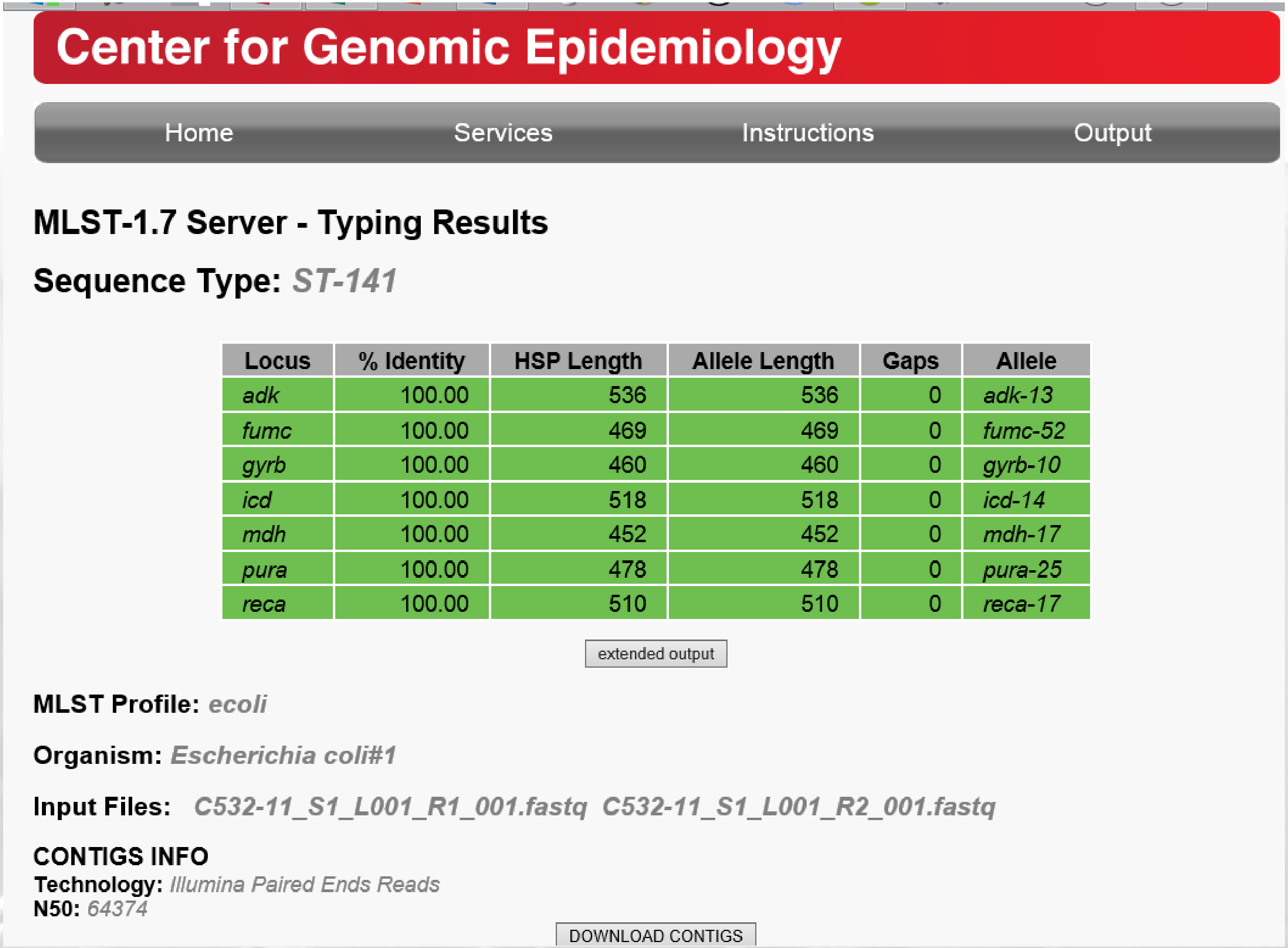
SETTINGS:
Selected %ID threshold: *85.00*

EAEC database						
Virulence factor	**% Identity**	**Query/HSP length**	**Contig**	**Position in contig**	**Protein function**	**Accession number**
aaiC	99.71	684 / 684	NODE_251_length_69439_cov_17.582914	17113..17796	Type VI secretion protein	cp003301
capU	99.82	1089 / 1089	NODE_252_length_27346_cov_21.620201	5062..6150	Hexosyltransferase homolog	AFRH01000016

Input Files: *PRI-HRD4-77-11_S12_L001_R1_001.fastq PRI-HRD4-77-11_S12_L001_R2_001.fastq*

CONTIGS INFO
Technology: *Illumina Paired Ends Reads*
N50: *51806*

MLST typing



MLST-1.7 Server - Typing Results

Sequence Type: *ST-141*

Locus	% Identity	HSP Length	Allele Length	Gaps	Allele
adk	100.00	536	536	0	*adk-13*
fumc	100.00	469	469	0	*fumc-52*
gyrb	100.00	460	460	0	*gyrb-10*
icd	100.00	518	518	0	*icd-14*
mdh	100.00	452	452	0	*mdh-17*
pura	100.00	478	478	0	*pura-25*
reca	100.00	510	510	0	*reca-17*

MLST Profile: *ecoli*

Organism: *Escherichia coli#1*

Input Files: *C532-11_S1_L001_R1_001.fastq* *C532-11_S1_L001_R2_001.fastq*

CONTIGS INFO
Technology: *Illumina Paired Ends Reads*
N50: *64374*

Conclusion

- In combination with other available WGS-typing tools, *E. coli* O:H serotyping can be performed solely from WGS-data
- Faster and cheaper than current routine typing procedures
- WGS-typing is already a superior alternative to conventional typing strategies.

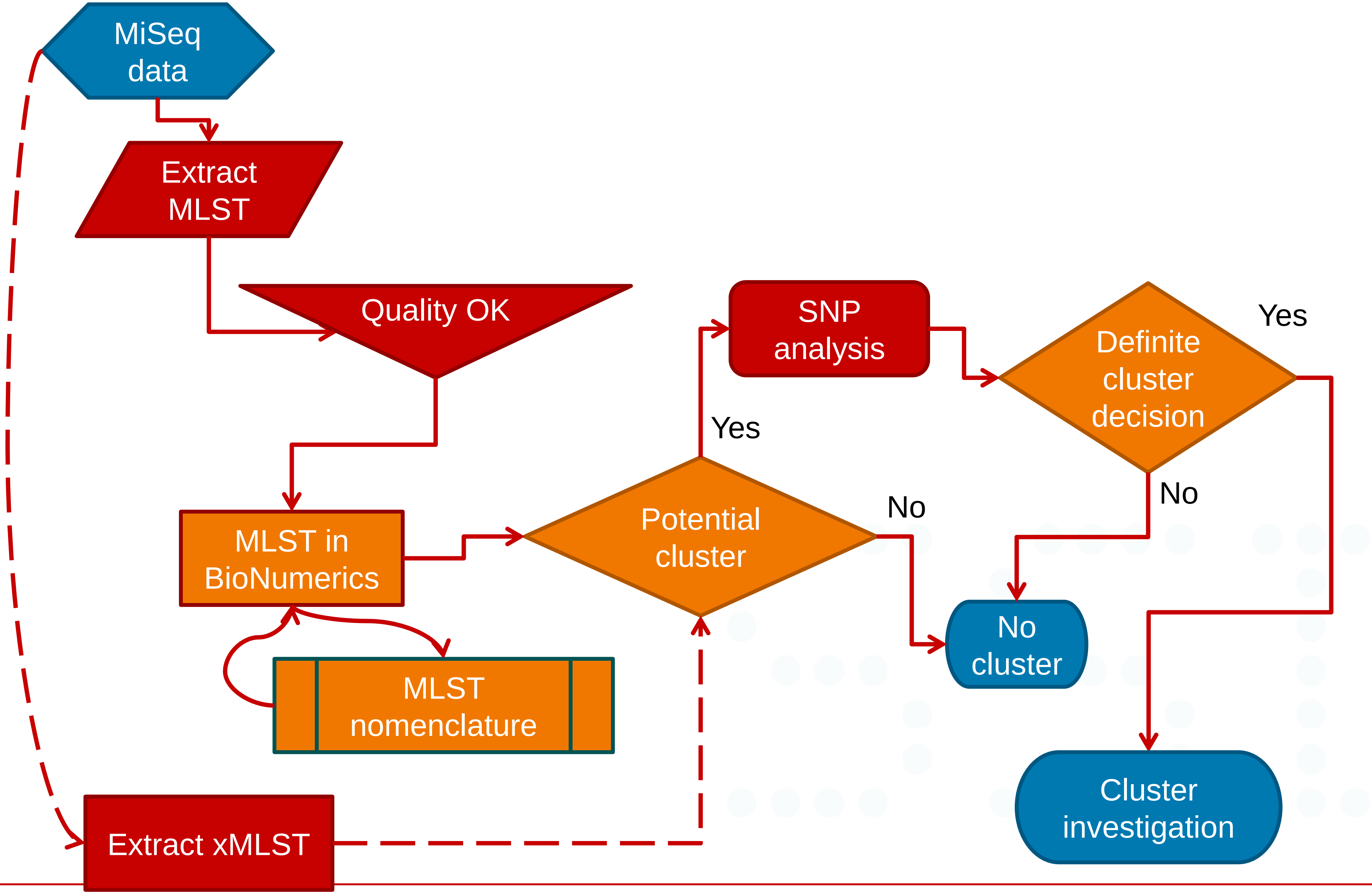
SerotypeFinder: Further Evaluation

- 3 isolates per type –sequenced and serotyped for evaluation
- Real-time ROUTINE surveillance of VTEC within this year

Surveillance before 2014

- **Real-time typing of human isolates:**
  - ***Salmonella***
    - **Serotyping, antimicrobial resistance and MLVA (WGS, PFGE and phage typing ad hoc)**
  - **VTEC**
    - **Serotyping, virulence profiling, PFGE and WGS (SNP, O-/H-serotyping, virulence finder)**
  - ***Listeria***
    - **Molecular serotyping, WGS (PFGE)**
  - ***Campylobacter***
    - **None (PFGE, flaA and MLST ad hoc)**
  - ***Yersinia***
    - **None (PFGE ad hoc)**
  - ***Shigella***
    - **None (PFGE ad hoc)**

LISTERIA – ANALYSIS WORK FLOW



MiSeq data

Extract MLST

Quality OK

MLST in BioNumerics

MLST nomenclature

Extract xMLST

Potential cluster

Yes

No

SNP analysis

Definite cluster decision

Yes

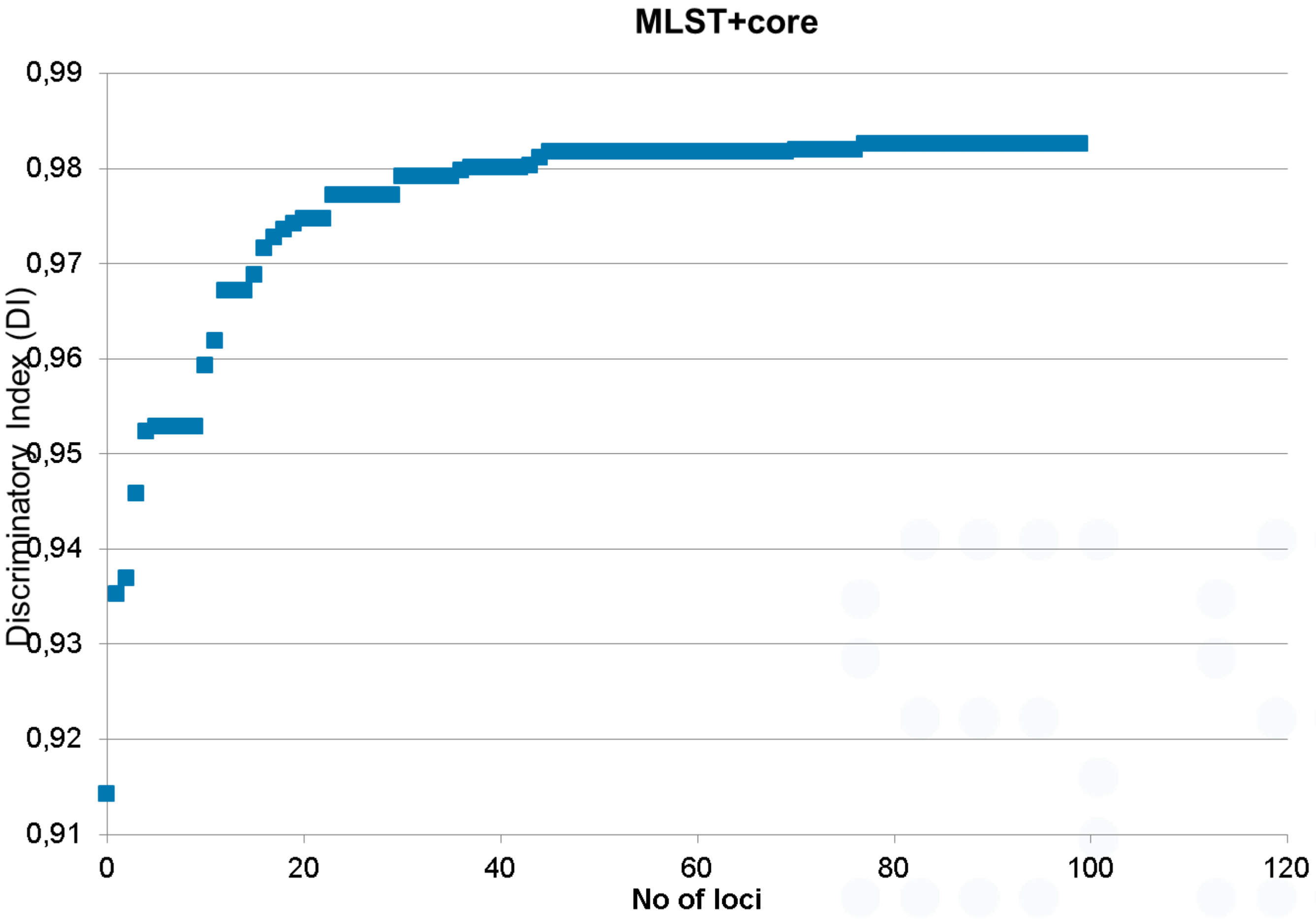
No

No cluster

Cluster investigation

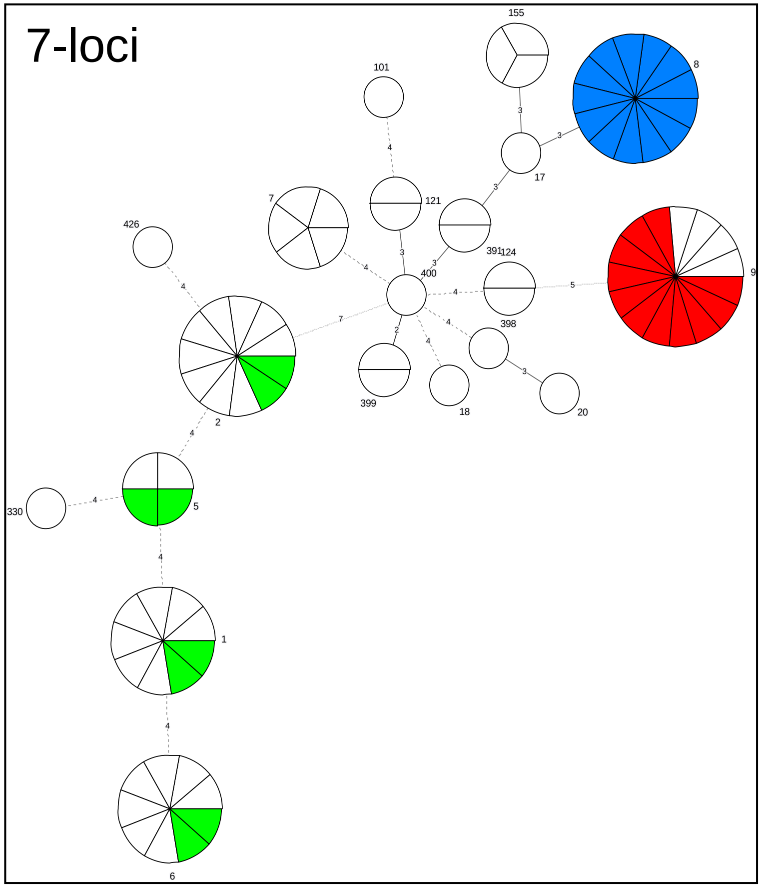
xMLST -ADDING FROM CORE GENOME

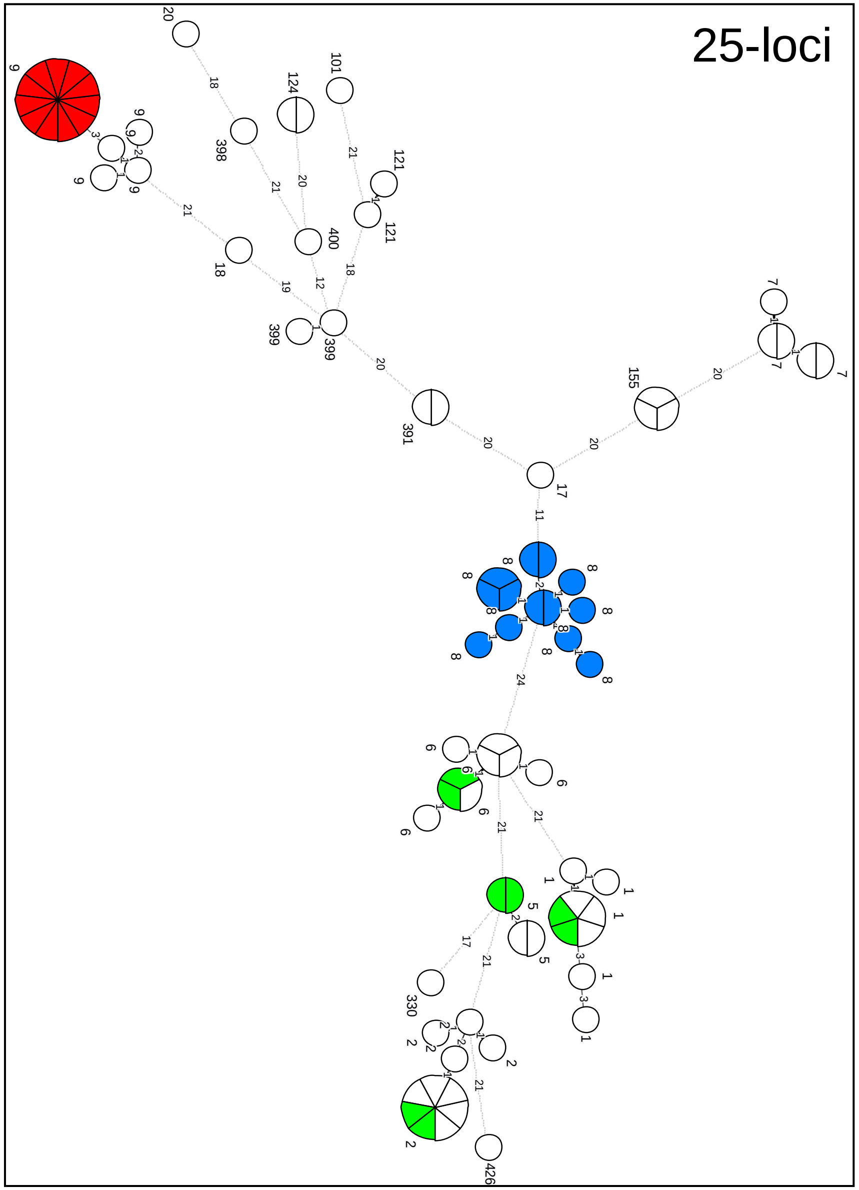
Thesis: Emilie Bak



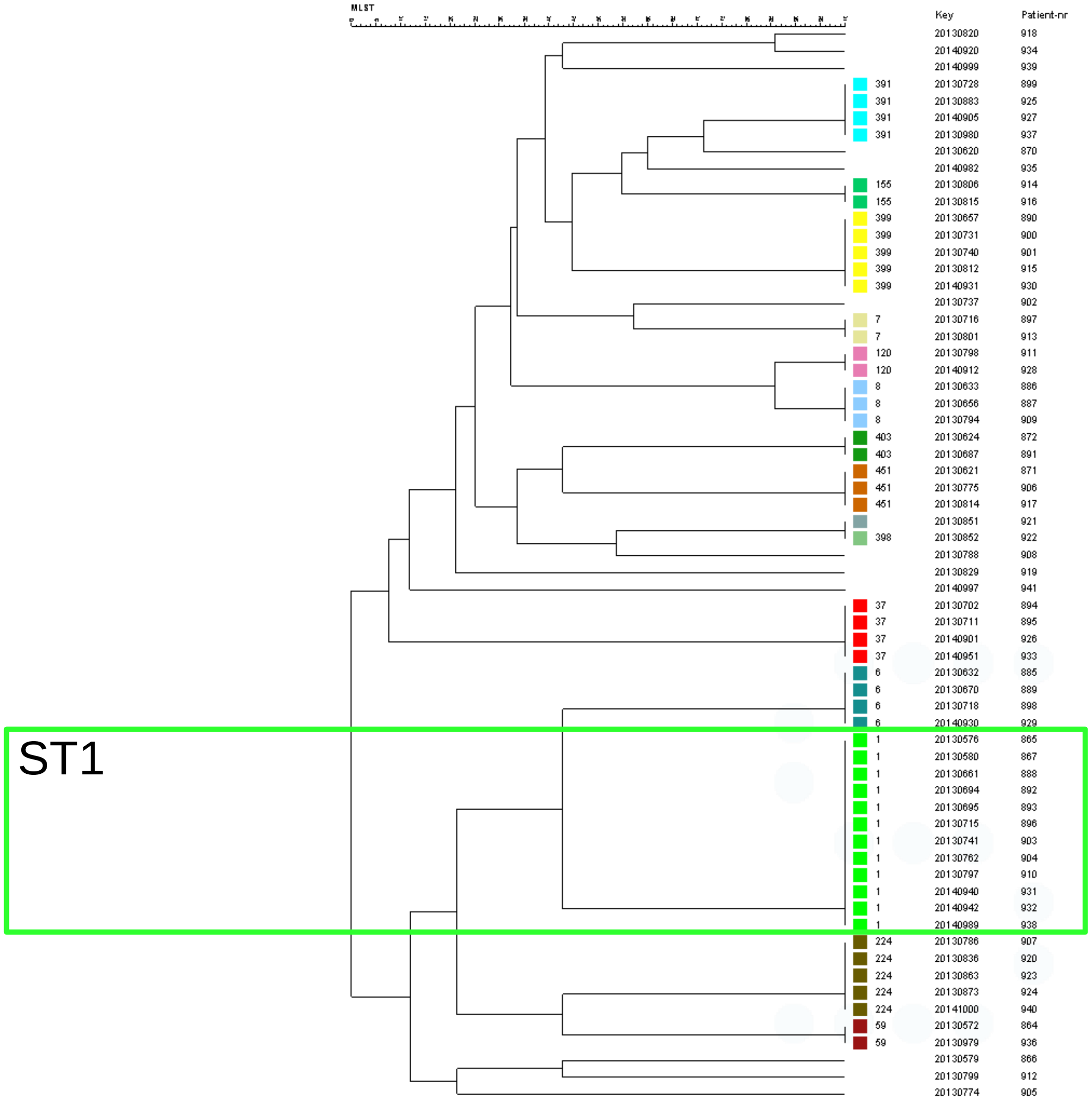
Increase in discriminatory index when adding one gene at the time

WGS: 7-LOCI AND 25-LOCI MLST





MLST BASED TREE *LISTERIA* 2013-2014



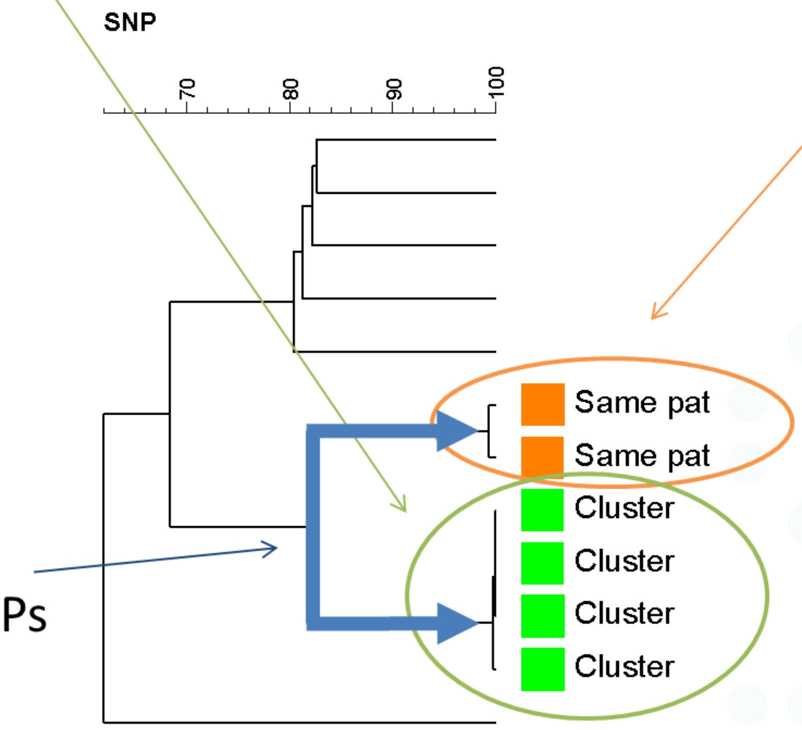
	Key	Patient-nr
	20130820	918
	20140920	934
	20140999	939
391	20130728	899
391	20130883	925
391	20140905	927
391	20130980	937
	20130620	870
	20140982	935
155	20130806	914
155	20130815	916
399	20130657	890
399	20130731	900
399	20130740	901
399	20130812	915
399	20140931	930
	20130737	902
7	20130716	897
7	20130801	913
120	20130798	911
120	20140912	928
8	20130633	886
8	20130656	887
8	20130794	909
403	20130624	872
403	20130687	891
451	20130621	871
451	20130775	906
451	20130814	917
	20130851	921
398	20130852	922
	20130788	908
	20130829	919
	20140997	941
37	20130702	894
37	20130711	895
37	20140901	926
37	20140951	933
6	20130632	885
6	20130670	889
6	20130718	898
6	20140930	929
1	20130576	865
1	20130580	867
1	20130661	888
1	20130694	892
1	20130695	893
1	20130715	896
1	20130741	903
1	20130762	904
1	20130797	910
1	20140940	931
1	20140942	932
1	20140989	938
224	20130786	907
224	20130836	920
224	20130863	923
224	20130873	924
224	20141000	940
59	20130572	864
59	20130979	936
	20130579	866
	20130799	912
	20130774	905

ST1 GROUP, SNP ANALYSIS

4 patients
19 weeks
1 SNPs

1 patient
14 weeks
3 SNPs

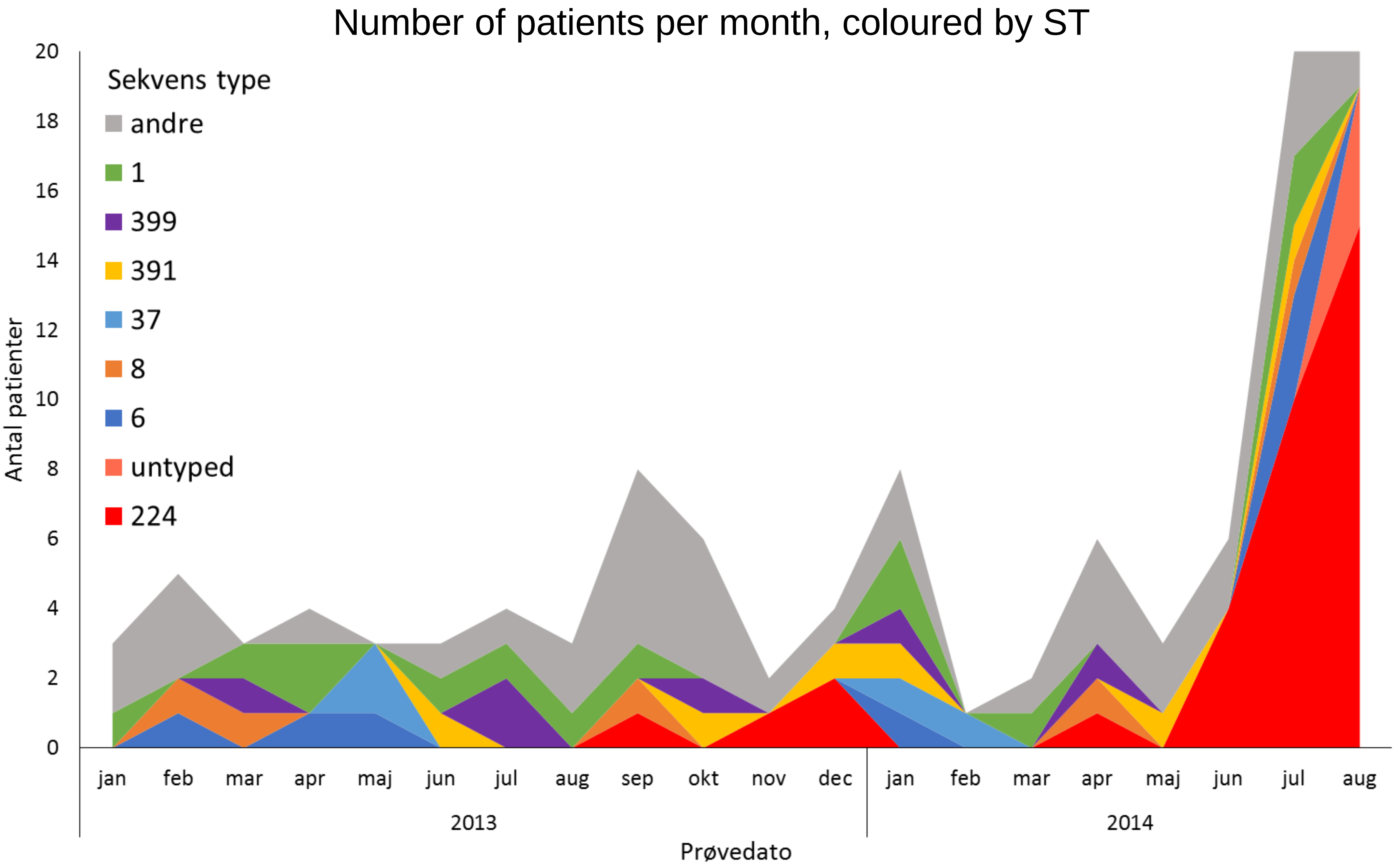
(12 entries)



85 SNPs

Key	Patient-nr
20140942	932
20130661	888
20130741	903
20140989	938
20130580	867
20130576	865
20130695	893
20130694	892
20130762	904
20130797	910
20130715	896
20140940	931

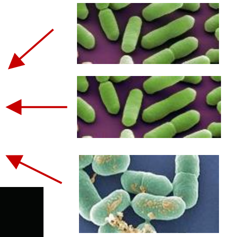
PATIENTS WITH LISTERIA 2013-14 – ST-TYPES



Genome sequencing ties patients to ”*rullepølse*”

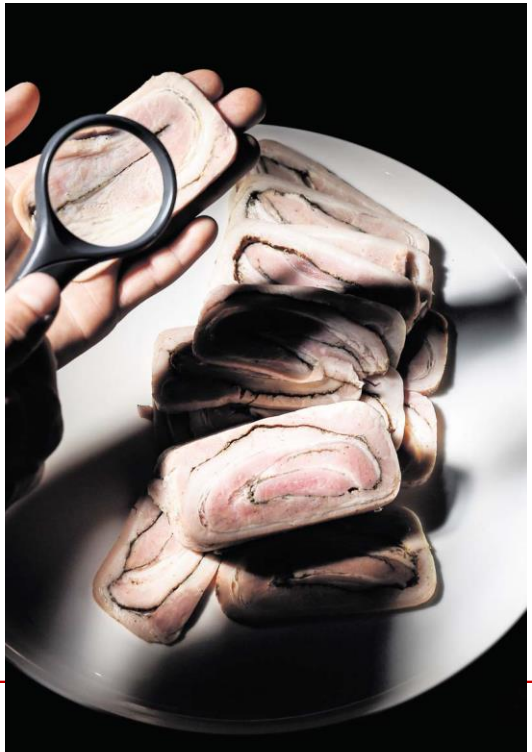
- WGS shows that *Listeria* from ”*rullepølse*” & patients are identical

Rullepølse: T C G C C A C G A A C C T G C G G C G C G G G C T T T G G C T G T C A G T A C C T G A A G C A C T T T A G
Patient 1: T C G C C A C G A A C C T G C G G C G C G G G C T T T G G C T G T C A G T A C C T G A A G C A C T T T A G
Patient 2: C C G C C A T G A A C C G G C T G A A C G G G C T T T G G C C G T C A G T A C C T G A A G C A C T T T T G

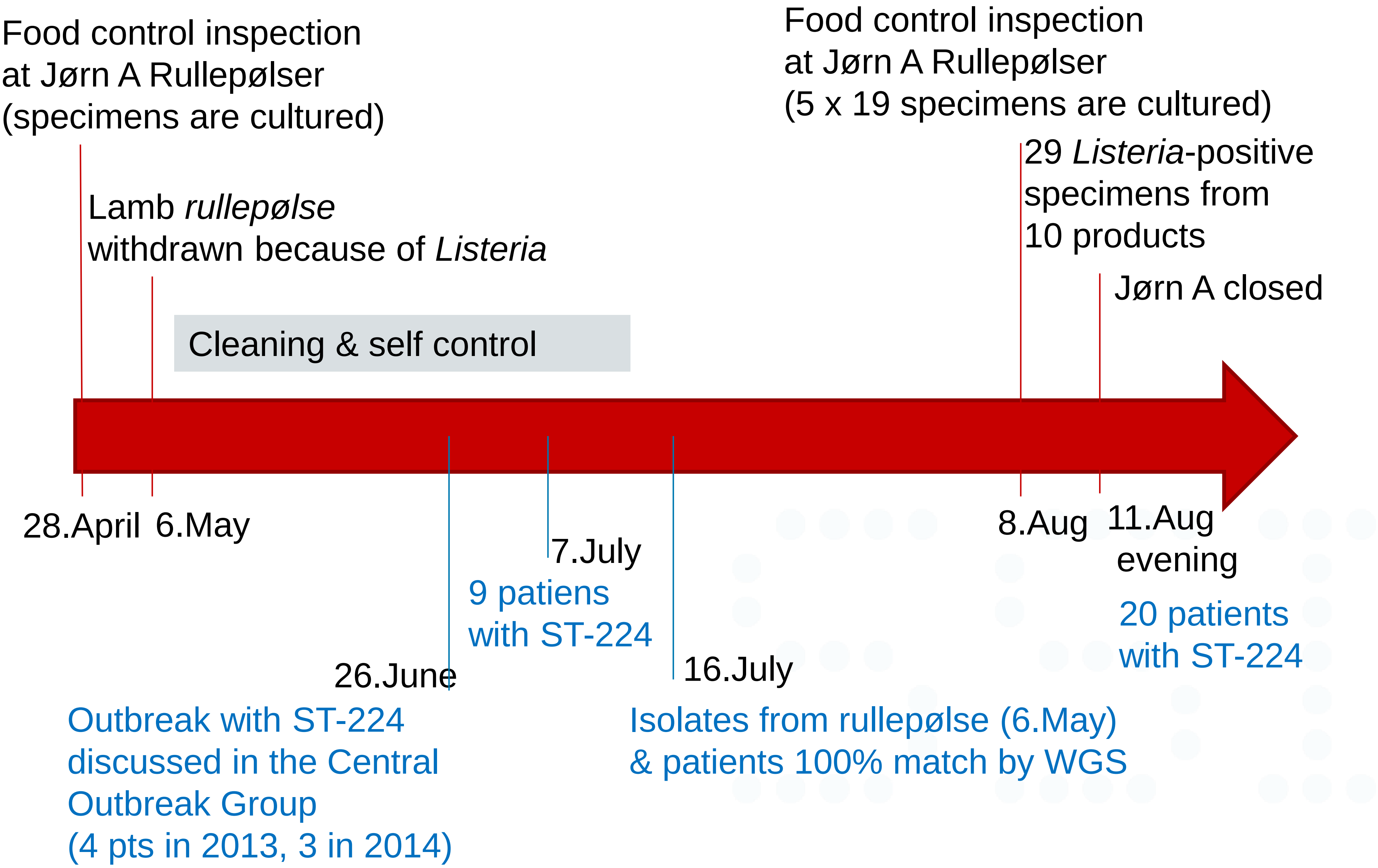


Nach Schleswig-Holstein verkauft

Dänische Killer-Wurst auch in Deutschland



TIMELINE



Food control inspection
at Jørn A Rullepølser
(specimens are cultured)

28.April

Lamb *rullepølse*
withdrawn because of *Listeria*

6.May

Cleaning & self control

26.June

Outbreak with ST-224
discussed in the Central
Outbreak Group
(4 pts in 2013, 3 in 2014)

7.July

9 patiens
with ST-224

16.July

Isolates from rullepølse (6.May)
& patients 100% match by WGS

Food control inspection
at Jørn A Rullepølser
(5 x 19 specimens are cultured)

8.Aug

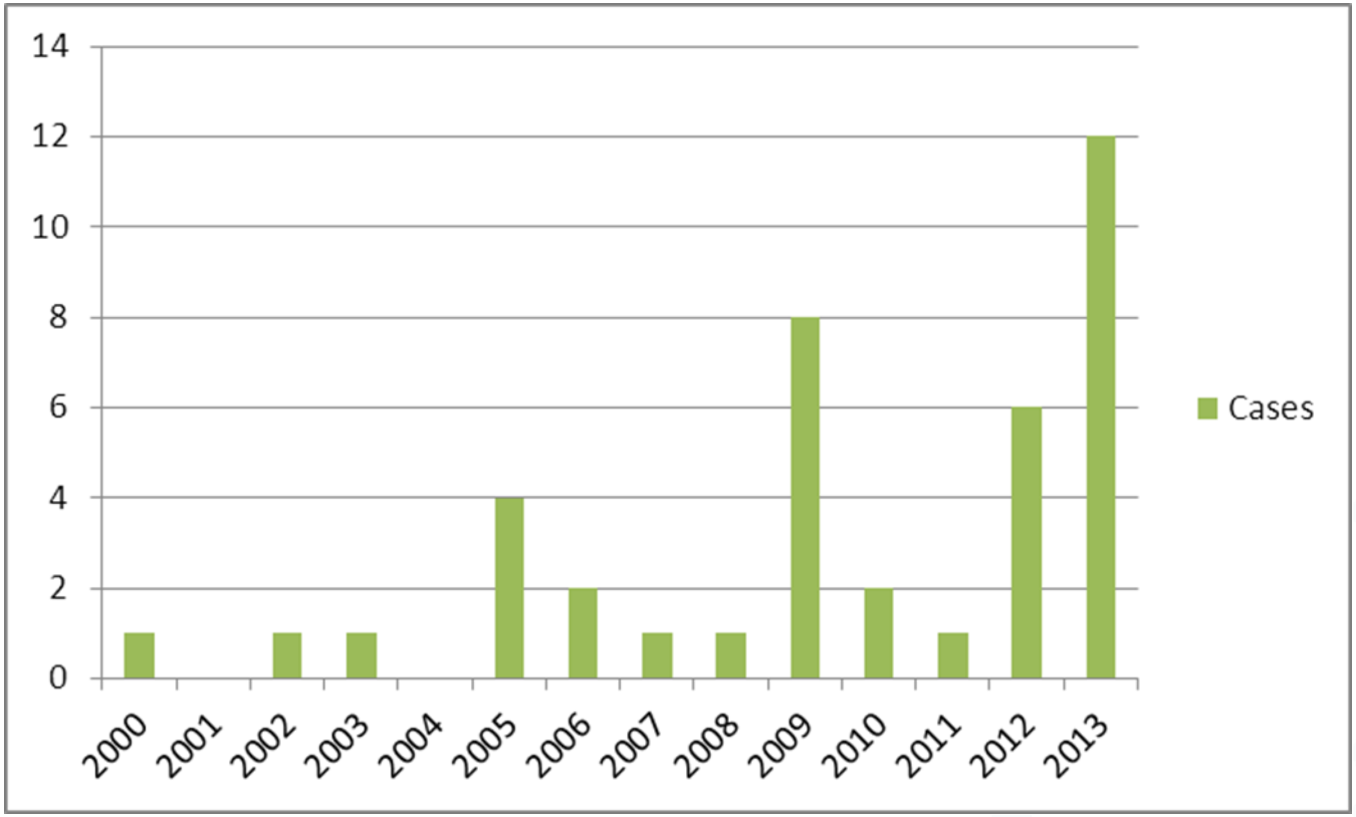
29 *Listeria*-positive
specimens from
10 products

11.Aug
evening

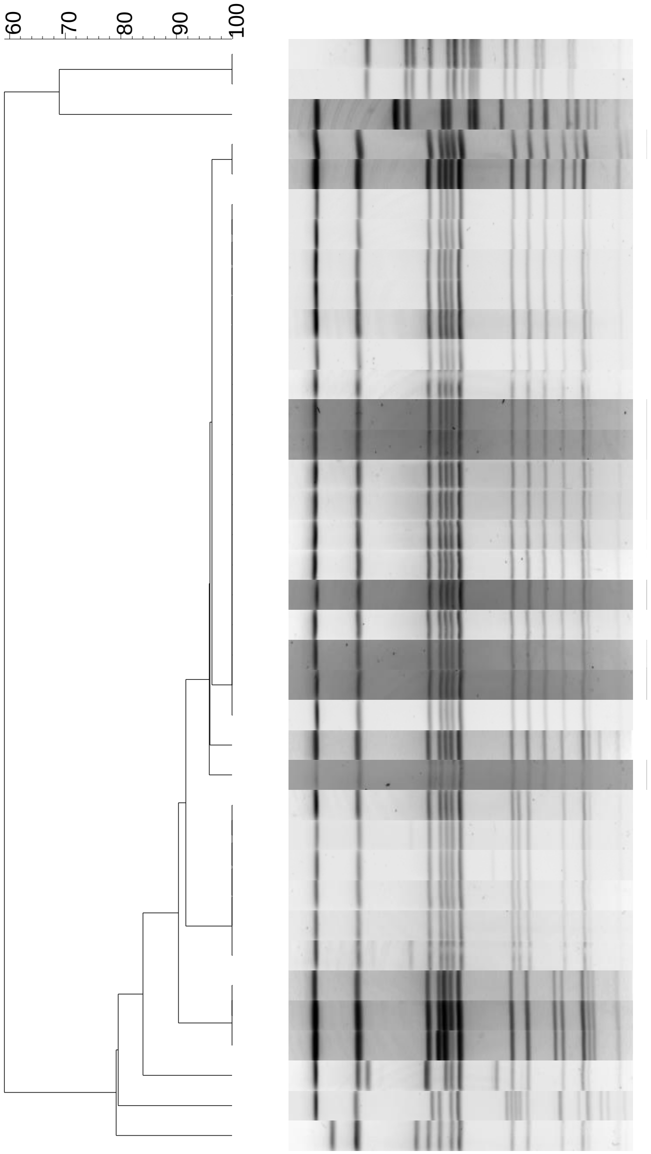
Jørn A closed

20 patients
with ST-224

How can we use WGS in outbreaks?



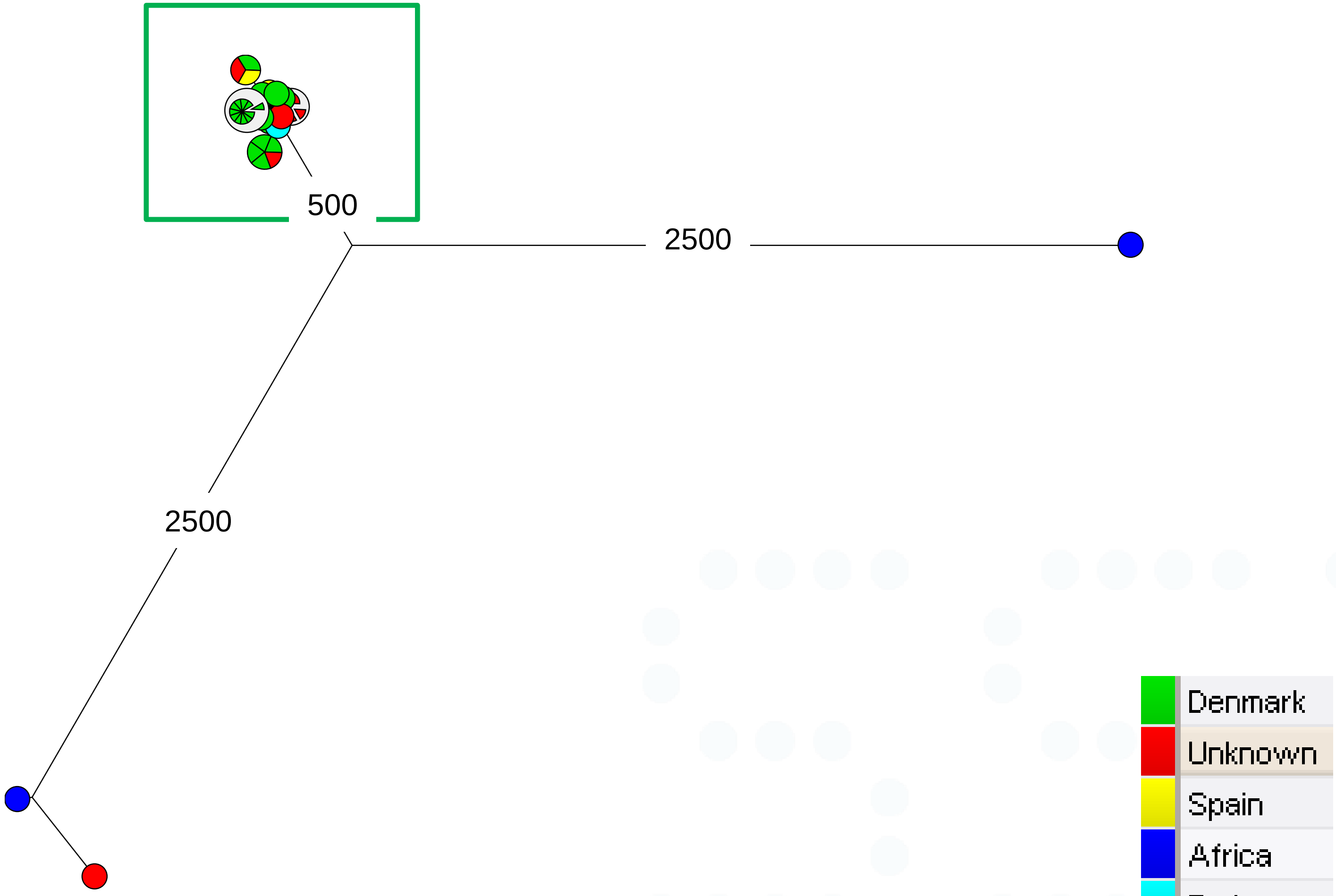
PFGE



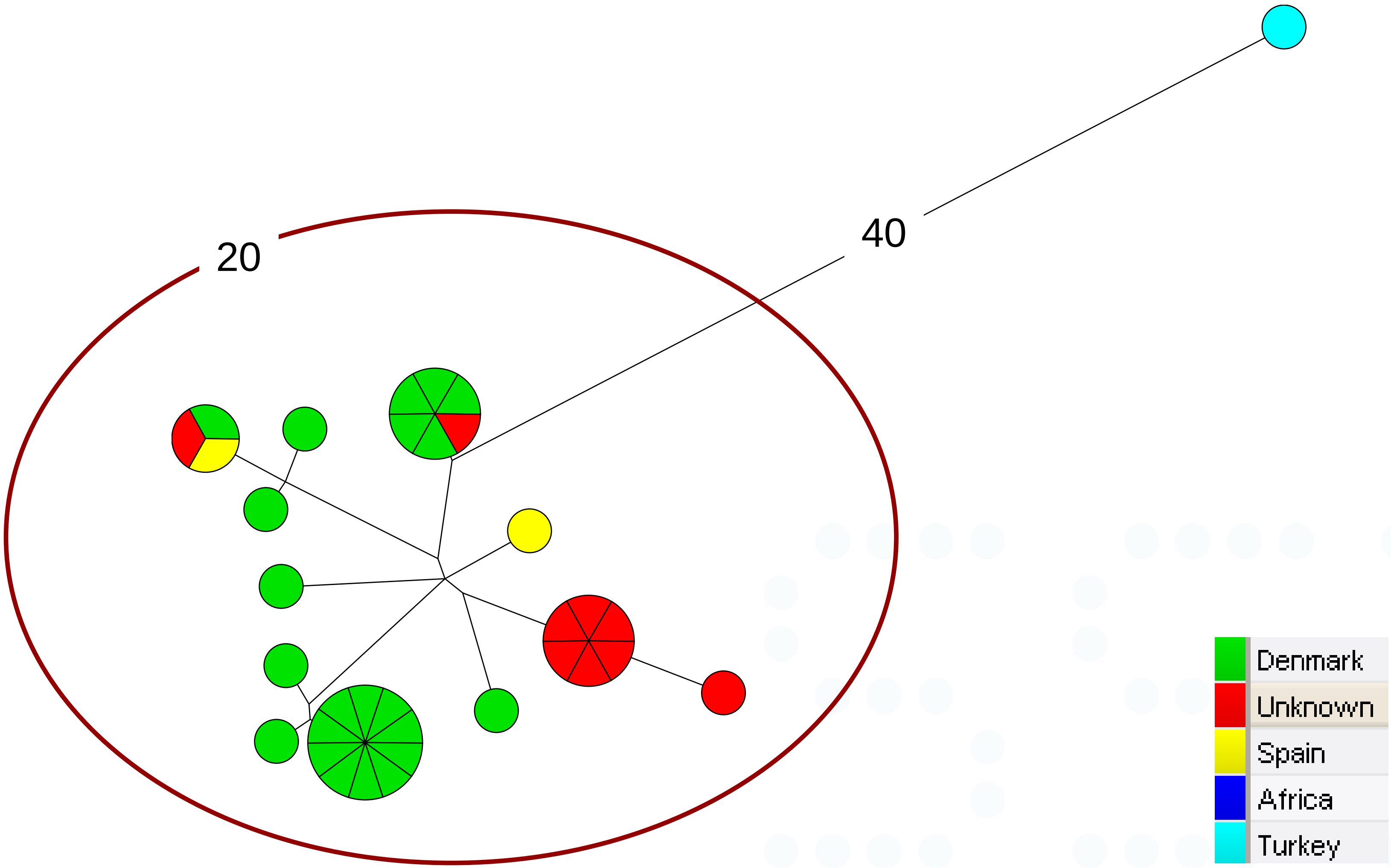
Year	PFGEType	PatientTravel
2006	MIKX01.0013.DK	
2009	MIKX01.0013.DK	Africa
2013	MIKX01.0004.DK	Africa
2012	MIKX01.0001.DK	No Travel
2012	MIKX01.0001.DK	No Travel
2009	MIKX01.0002.DK	
2009	MIKX01.0002.DK	No Travel
2009	MIKX01.0002.DK	No Travel
2009	MIKX01.0002.DK	No Travel
2009	MIKX01.0002.DK	No Travel
2009	MIKX01.0002.DK	No Travel
2011	MIKX01.0002.DK	
2013	MIKX01.0002.DK	No Travel
2013	MIKX01.0002.DK	No Travel
2013	MIKX01.0002.DK	No Travel
2013	MIKX01.0002.DK	No Travel
2013	MIKX01.0002.DK	No Travel
2013	MIKX01.0002.DK	No Travel
2013	MIKX01.0002.DK	No Travel
2013	MIKX01.0002.DK	No Travel
2013	MIKX01.0002.DK	No Travel
2013	MIKX01.0002.DK	No Travel
2010	MIKX01.0002.DK	Spain
2012	MIKX01.0006.DK	No Travel
2013	MIKX01.0007.DK	No Travel
2005	MIKX01.0008.DK	
2005	MIKX01.0008.DK	
2005	MIKX01.0008.DK	
2005	MIKX01.0008.DK	
2006	MIKX01.0008.DK	
2007	MIKX01.0008.DK	
2012	MIKX01.0003.DK	
2012	MIKX01.0003.DK	No Travel
2012	MIKX01.0003.DK	Spain
2008	MIKX01.0009.DK	No Travel
2009	MIKX01.0010.DK	Turkey
2010	MIKX01.0011.DK	No Travel

MAXIMUM LIKELIHOOD TREE

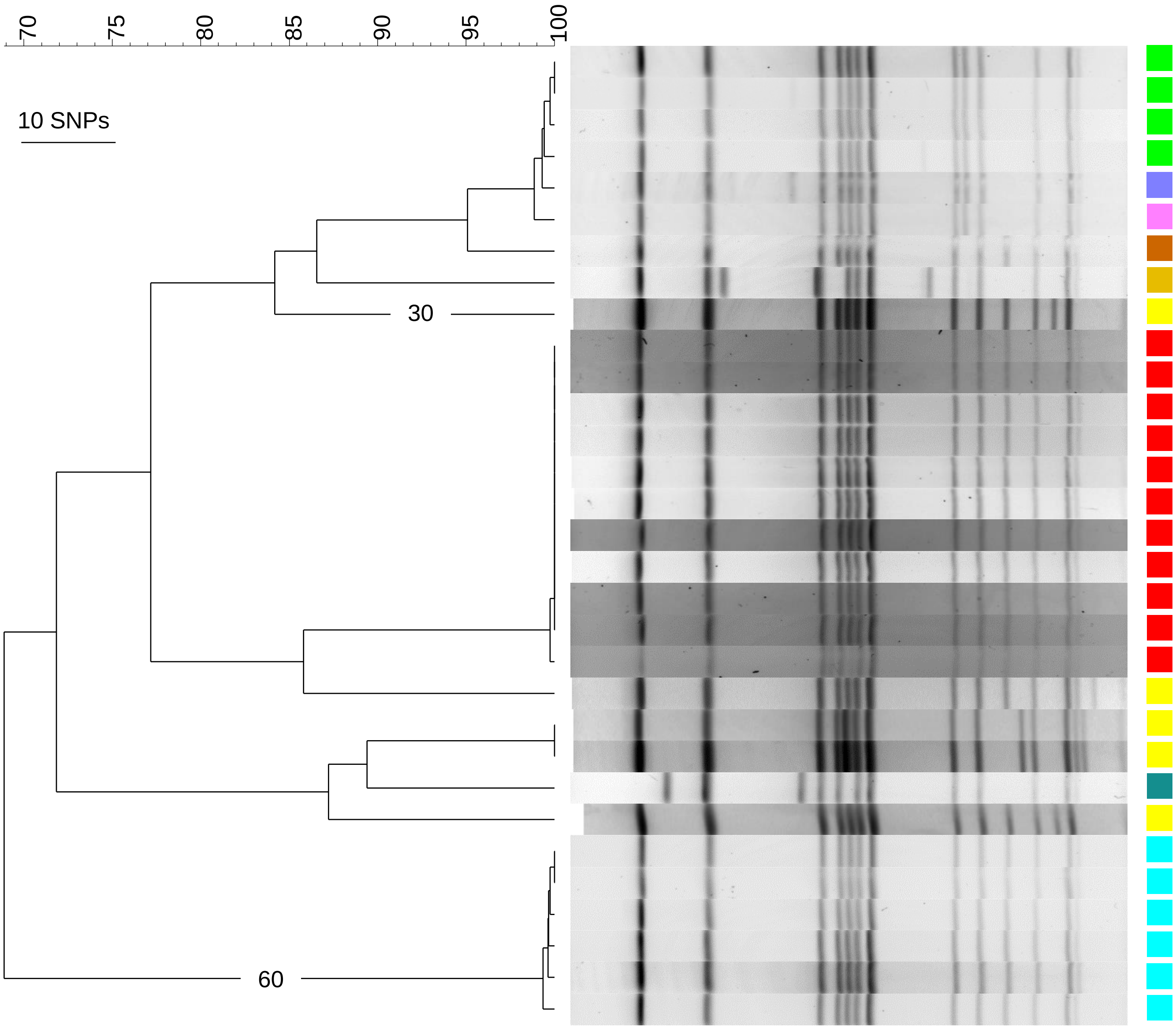
Mikawasima isolates 2005-2013



CLOSELY RELATED ISOLATES

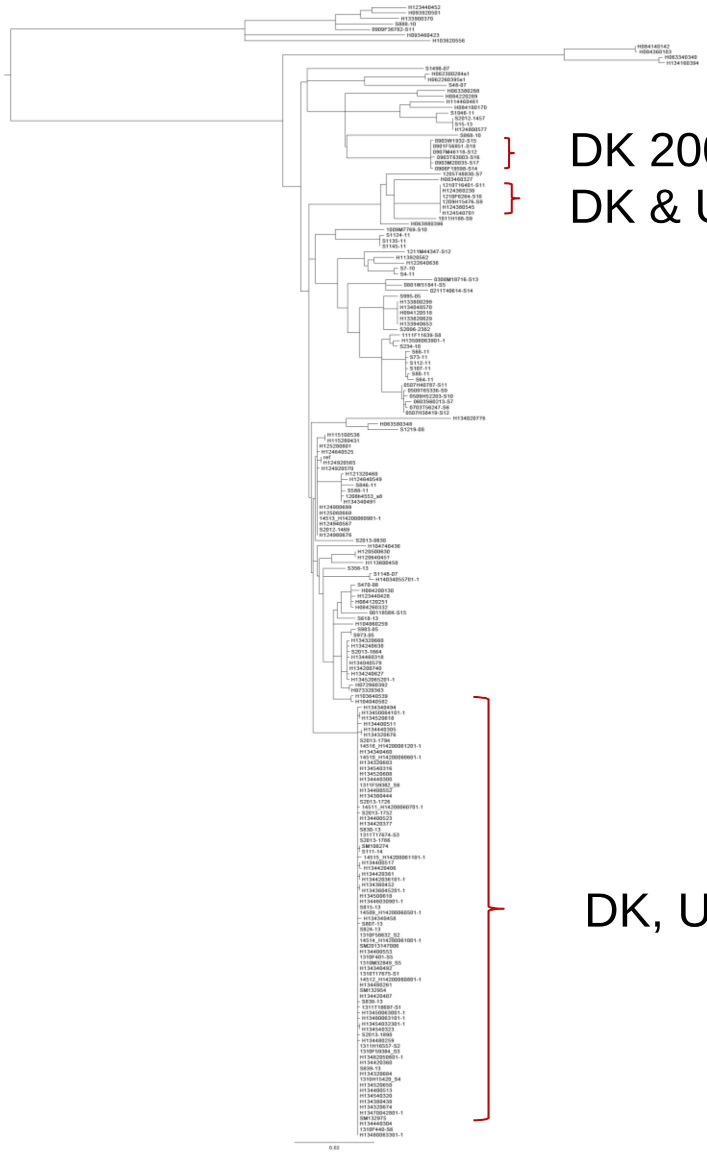


SNP TREE



Year	PFGEType
2005	MIKX01.0008.DK
2005	MIKX01.0008.DK
2005	MIKX01.0008.DK
2005	MIKX01.0008.DK
2007	MIKX01.0008.DK
2006	MIKX01.0008.DK
2011	MIKX01.0002.DK
2008	MIKX01.0009.DK
2012	MIKX01.0001.DK
2013	MIKX01.0002.DK
2013	MIKX01.0002.DK
2013	MIKX01.0002.DK
2013	MIKX01.0002.DK
2013	MIKX01.0002.DK
2013	MIKX01.0002.DK
2013	MIKX01.0002.DK
2013	MIKX01.0002.DK
2013	MIKX01.0002.DK
2013	MIKX01.0002.DK
2013	MIKX01.0007.DK
2012	MIKX01.0006.DK
2012	MIKX01.0003.DK
2012	MIKX01.0003.DK
2010	MIKX01.0011.DK
2012	MIKX01.0001.DK
2009	MIKX01.0002.DK
2009	MIKX01.0002.DK
2009	MIKX01.0002.DK
2009	MIKX01.0002.DK
2009	MIKX01.0002.DK
2009	MIKX01.0002.DK

INTERNATIONAL DIMENSIONS



DK 2009

DK & UK 2012

DK, UK & SE 2013

SURVEILLANCE FROM 2014

- Real-time typing of human isolates:
  - *Salmonella*
    - Serotyping, antimicrobial resistance and MLVA (PFGE and **WGS ad hoc**)
  - VTEC
    - **WGS from 2015**
      - Serotyping, virulence profiling and PFGE **ad hoc**
      - **Development of WGS based O-/H-typing and virulence finder**
  - *Listeria*
    - **WGS**

QUESTIONS