

# Genetic Diversity of Shiga toxin producing Escherichia coli O26:H11

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Platform IdentityPath

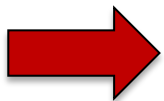
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# Background STEC O26:H11/H-

- *Escherichia coli* O26:H11 is the most common serotype in human infections after O157:H7
- Main reservoir is cattle :
  - Meat and dairy products are frequently contaminated with O26 strains
  - Adulterant in non-intact beef and components  
(FSIS Notice 47-13; [www.fsis.usda.gov](http://www.fsis.usda.gov))
- Not all *E. coli* O26 are the same :
  - Non virulent, ETEC, EPEC, STEC / EHEC (+ EHEC-like)



## Phylogenetic analysis :

Extensive investigation into relatedness of STEC O26:H11 isolated in humans in France



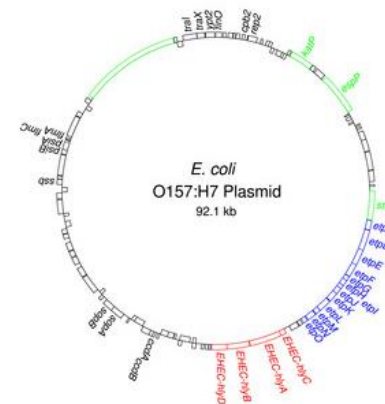
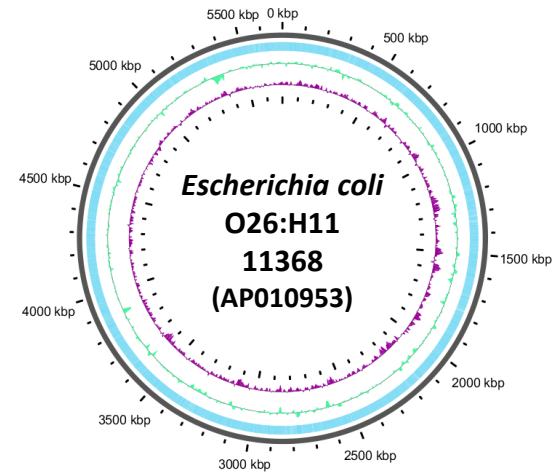
# Characterization of EHEC O26:H11/H-

EHEC O26:H11 can be distinguished from other related strains using a set of specific genetic markers

- *wzx*<sub>O26</sub>, *fliC*<sub>H11</sub>
- *Stx1a* or *stx1a* + *stx2a*
- *eae-beta*
- *arcA* allele 14
- *espK* (OI-50)
- CRISPR<sub>O26</sub>
- Plasmid genes combination  
*ehxA*+/*KatP*+/*espP*+/*etpD*-

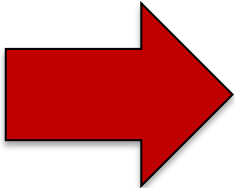
## - MLST ST21

Zhang WL et al. JCM 2000.  
Leomil L et al. FEMS Microbiol. Lett. 2005.  
Miko A et al. FEMS Microbiol. Lett. 2010.  
Bugarel M et al. AEM 2011.  
Delannoy S et al. JCM 2012.  
Bielaszewska M et al. CID 2013  
Delannoy S et al. JCM 2015



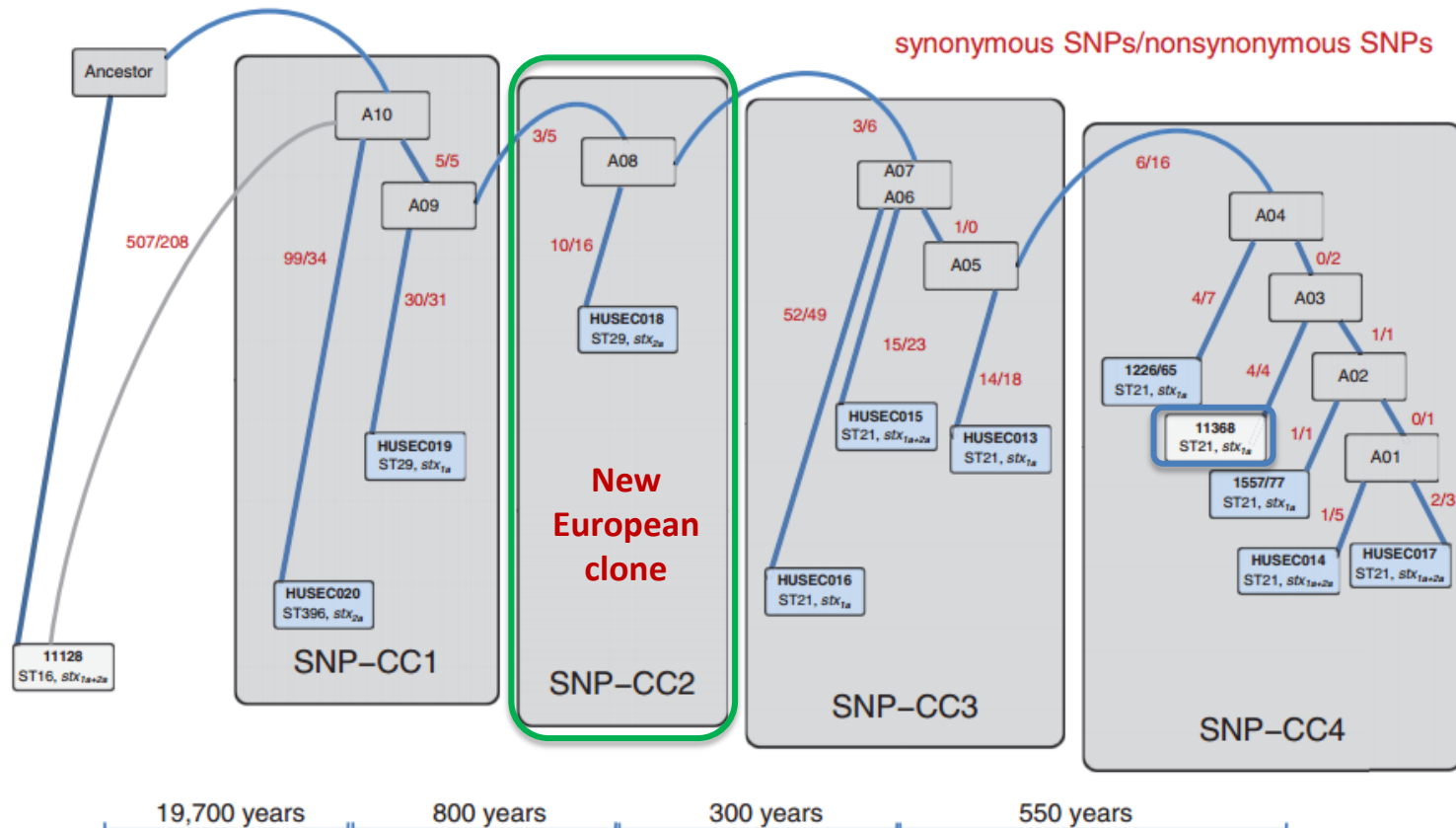
# Emergence of a new *stx2a* O26:H11 clone in Europe

“New European” clone : --> different genetic characteristics

- 
- *wzx*<sub>O26</sub>, *fliC*<sub>H11</sub>
  - ***Stx2a* only**
  - *eae-beta*
  - Plasmid genes combination  
*ehxA*+/*KatP*-/*esP*-/*etpD*+
  - **MLST ST29**
  - **More virulent**
  - **Other genetic markers?**

# Evolutionary phylogenetic relationship of EHEC O26:H11 based on SNPs

- Identification of **48 phylogenetically informative SNPs** that cluster EHEC O26:H11 in **4 Clonal Complexes (CC)**
- The **“New European”** clone belongs to the **SNP-CC2**



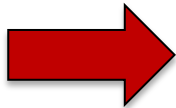
# Is the new stx2a O26:H11 clone (The New European clone) circulating in France ?



Hôpital universitaire  
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ASSISTANCE  
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DE PARIS



We collected French clinical isolates of EHEC O26:H11  
which are positive for **stx2 only**.

# The new, highly pathogenic, European EHEC O26:H11 clone (stx2a+, ST29) is circulating in France

- **EHEC O26:H11 positive for stx2 alone (n=23)** were isolated from pediatric patients with HUS between 2010 and 2013 in France.
- 7 / 23 strains belong to **ST21**
- 16 / 23 strains belong to **ST29** --> some strains are characteristic of the 'New European clone'

MLST and CRISPR typing of the O26:H11 French clinical isolates

Strain	ST	CRISPR1	CRISPR1 Allele	CRISPR2a	CRISPR2a allele	CRISPR Type (CT)
30993	21	A 7A 26A 21A 20A 10E 4F	113	C 7C 8C 9C 10C 11C 12D 13D 14A 15E	4	4
32876	21	A 7A 26A 21A 20A 10E 4F	113	C 7C 8C 9C 10C 11C 12D 13D 14A 15E	4	4
31493	21	A 7A 11A 8A 9A 26A 21A 20A 10E 4F	11	C 7C 8C 9C 10C 11C 12D 13D 14A 15E	4	1
33116	21	A 7A 11A 8A 9A 26A 21A 20A 10E 4F	11	C 7C 8C 9C 10C 11C 12D 13D 14A 15E	4	1
34195	21	A 7A 11A 8A 9A 26A 21A 20A 10E 4Y	11	C 7C 8C 9C 10C 11C 12D 13D 14A 15E	4	1
36079	21	A 7A 11A 8A 9A 10E 4F	114	C 7C 8C 9C 10C 11C 12D 13D 14A 15E	4	6
36084	21	A 7A 11A 8A 9A 10E 4F	114	C 7C 8C 9C 10C 14A 15E	119	7
33344	29	A 7A 11A 8A 9A 21A 20A 10E 4F	69	C 7C 8C 9C 10C 11C 12D 13D 14A 15E	4	5
31131	29	A 7A 11A 8A 9A 26A 21A 20A 10E 4F	11	C 7C 8C 9C 10C 11C 12D 13D 14A 15E	4	1
34130	29	A 7A 11A 8A 9A 26A 21A 20A 10E 4F	11	C 7C 8C 9C 10C 11C 12D 13D 14A 15E	4	1
36708	29	A 7A 11A 8A 9A 26A 21A 20A 10E 4F	11	C 7C 8C 9C 10C 11C 12D 13D 14A 15E	4	1
33618*	29	A 7A 21A 20A 10E 4F	29	W 210C 8C 11C 211C 212C 12D 13D 14A 15E	71	3
34586*	29	A 7A 21A 20A 10E 4F	29	W 210C 8C 11C 211C 212C 12D 13D 14A 15E	71	3
34629*	29	A 7A 21A 20A 10E 4F	29	W 210C 8C 11C 211C 212C 12D 13D 14A 15E	71	3
36293*	29	A 7A 21A 20A 10E 4F	29	W 210C 8C 11C 211C 212C 12D 13D 14A 15E	71	3
36348*	29	A 7A 21A 20A 10E 4F	29	W 210C 8C 11C 211C 212C 12D 13D 14A 15E	71	3
32802*	29	A 7A 21A 20A 10E 4F	29	W 210C 211C 212C 12D 13D 14A 15E	67	2
36493*	29	A 7A 21A 20A 10E 4F	29	W 210C 211C 212C 12D 13D 14A 15E	67	2
31132	29	A 7A 21A 20A 10E 4F	29	W 210C 211C 212C 12D 13D 14A 15E	67	2
34620	29	A 7A 21A 20A 10E 4F	29	W 210C 211C 212C 12D 13D 14A 15E	67	2
34760	29	A 7A 21A 20A 10E 4F	29	W 210C 211C 212C 12D 13D 14A 15E	67	2
34827	29	A 7A 21A 20A 10E 4F	29	W 210C 211C 212C 12D 13D 14A 15E	67	2
34870	29	A 7A 21A 20A 10E 4F	29	W 210C 211C 212C 12D 13D 14A 15E	67	2



# 12 strains = “ New French clone” significantly different from the previously described “European clone”

**Plasmid and virulence gene profiles of the O26:H11 French clinical isolates**

Designation	MLST	Strain	stx2 subtype	ehxA	katP	espP	etpD	arcA-allele2	Z2098	espK	espV	SP_O26_C	SP_O26_D	SP_O26_E	
EHEC O26:H11	ST21	30993	stx2a	+	+	+	-	+	+	+	+	+	+	-	
	ST21	32876	stx2a	+	+	+	-	+	+	+	+	+	+	-	
	ST21	31493	stx2a	+	+	+	-	+	+	+	+	+	+	-	
	ST21	33116	stx2a	+	+	+	-	+	+	+	+	+	+	-	
	ST21	34195	stx2a	+	+	+	-	+	+	+	+	+	+	-	
	ST21	36079	●	stx2a	+	+	+	-	+	+	+	+	+	+	-
	ST21	36084	●	stx2a	+	+	+	-	+	+	+	+	-	+	-
New European clone (Bielazewska et al. 2013)	ST29	33344	stx2a	+	-	-	+	+	+	+	+	+	+	-	
	ST29	31131	stx2a	+	-	-	+	+	+	+	+	+	+	-	
	ST29	34130	stx2a	+	-	-	+	+	+	+	+	+	+	-	
	ST29	36708	●	stx2a	+	-	-	+	+	+	+	+	+	-	
	ST29	33618	stx2d	-	-	-	-	-	-	-	-	-	+	+	
	ST29	34586	stx2d	-	-	-	-	-	-	-	-	-	+	+	
	ST29	34629	stx2d	-	-	-	-	-	-	-	-	-	+	+	
	ST29	36293	●	stx2d	-	-	-	-	-	-	-	-	+	+	
	ST29	36348	●	stx2d	-	-	-	-	-	-	-	-	+	+	
	ST29	32802	stx2d	-	-	-	-	-	-	-	-	-	-	+	
New French clone (Delannoy et al. 2015)	ST29	36493	●	stx2d	-	-	-	-	-	-	-	-	-	+	
	ST29	31132	stx2a	-	-	-	-	-	-	-	-	-	-	+	
	ST29	34620	stx2a	-	-	-	-	-	-	-	-	-	-	+	
	ST29	34760	stx2a	-	-	-	-	-	-	-	-	-	-	+	
	ST29	34827	●	stx2a	-	-	-	-	-	-	-	-	-	+	
	ST29	34870	●	stx2a	-	-	-	-	-	-	-	-	-	+	

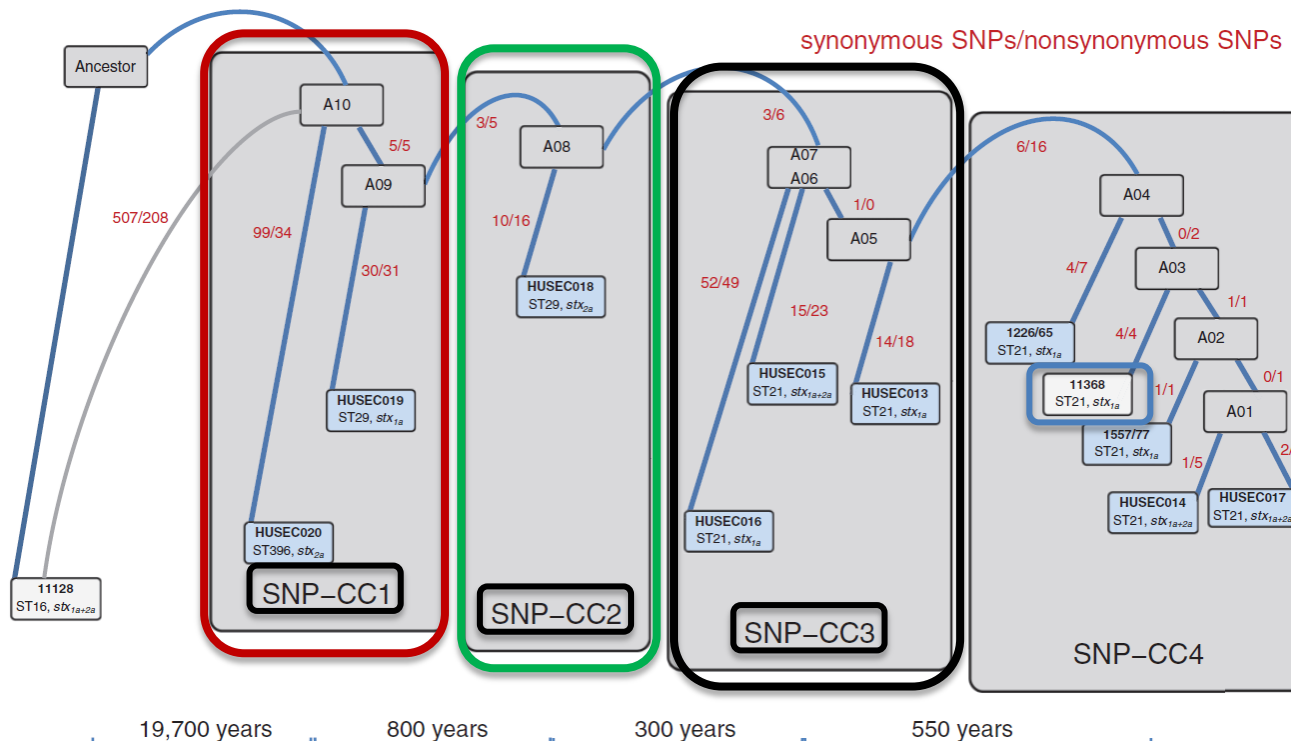


# The New French clone (CC1, ST29, SP\_O26-E<sup>POS</sup>)

## The European clone (CC2, ST29, SP\_O26-E<sup>NEG</sup>)

➤ Identification of 48 phylogenetically informative SNPs that cluster EHEC O26:H11 in 4 clonal complexes (CC):

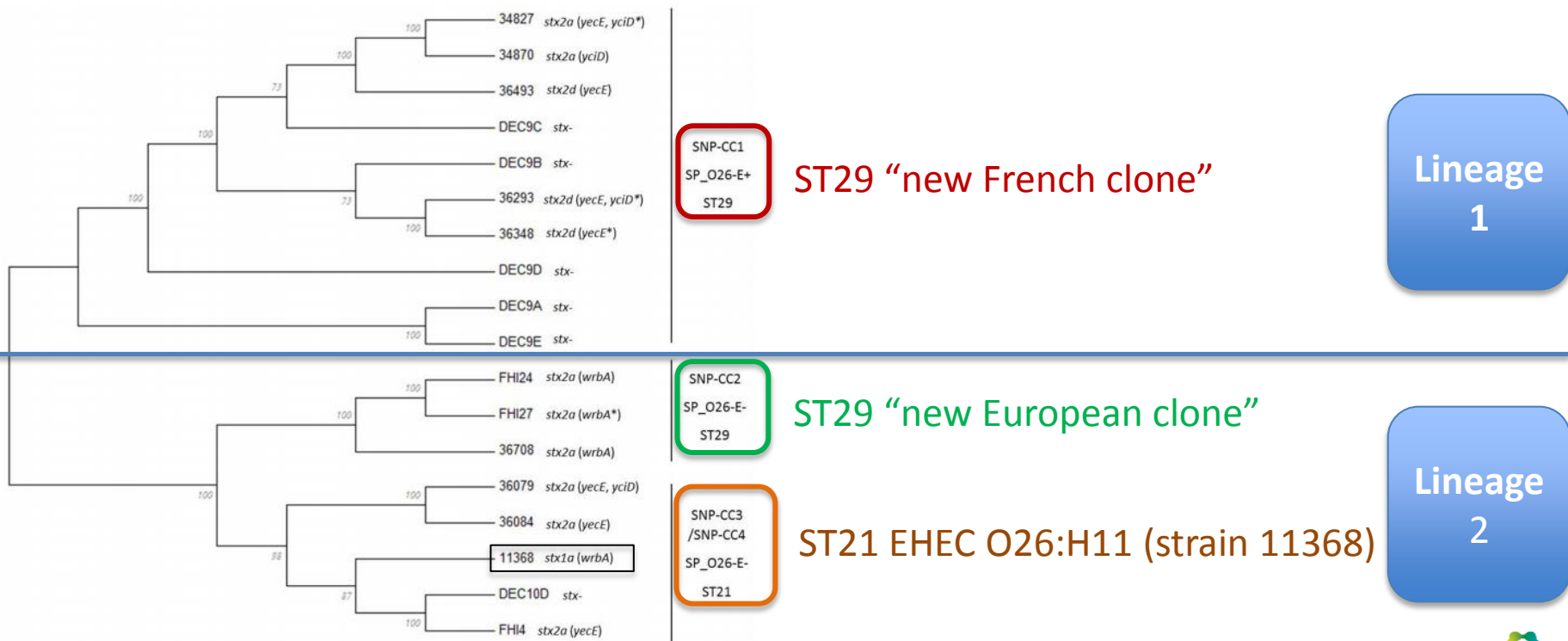
- The French strains belong to **CC1, CC2, CC3**
- The **new European** clone belongs to **SNP-CC2**
- The **new French** clone belongs to **SNP-CC1**



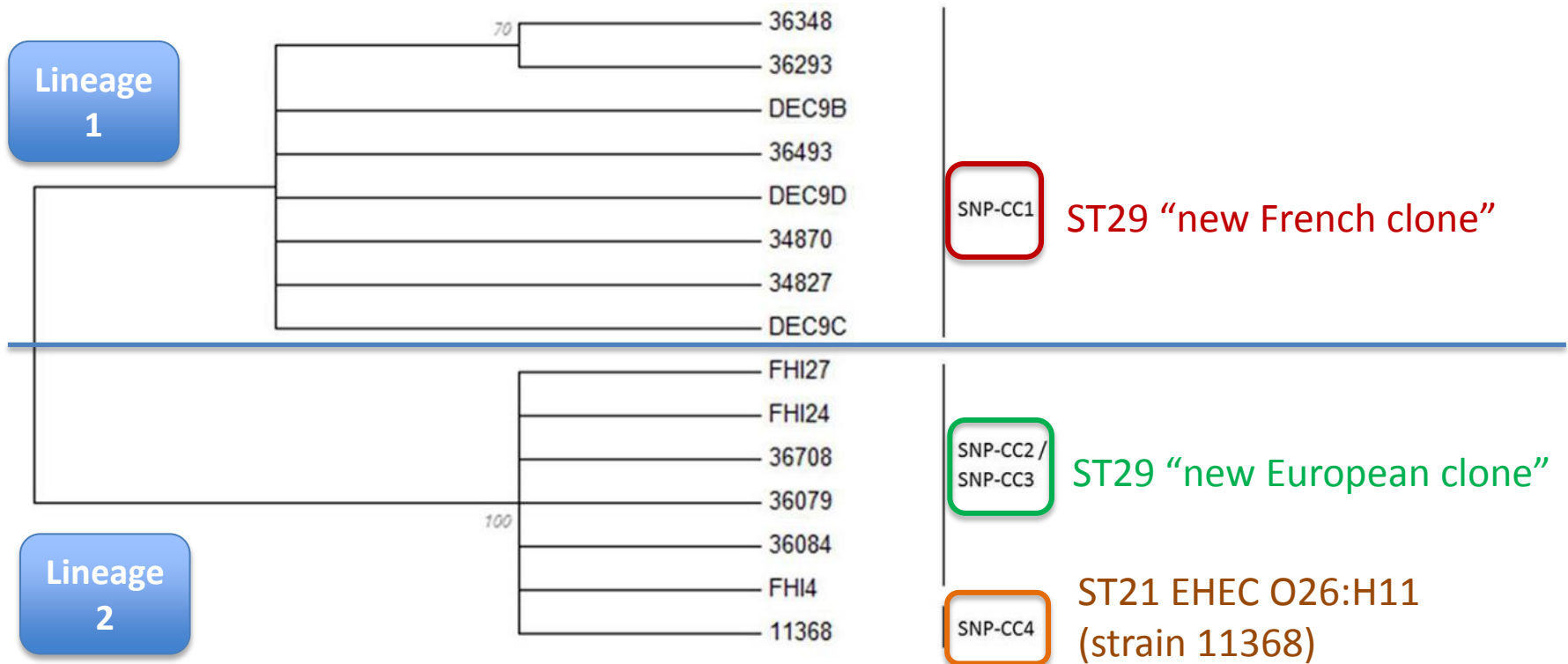
# Whole-genome SNPs (wgSNP) analysis using CSI Phylogeny 1.2 on the CGE server.

A phylogenic analysis distributed the strains in two lineages :

- Both the ST21 strains (SNP-CC3 and SNP-CC4) and the ST29 “new European clone” (SNP-CC2) were found to belong to the same lineage.
- The ST29 “new French clone” (SNP-CC1) was found to belong to a distinct lineage .



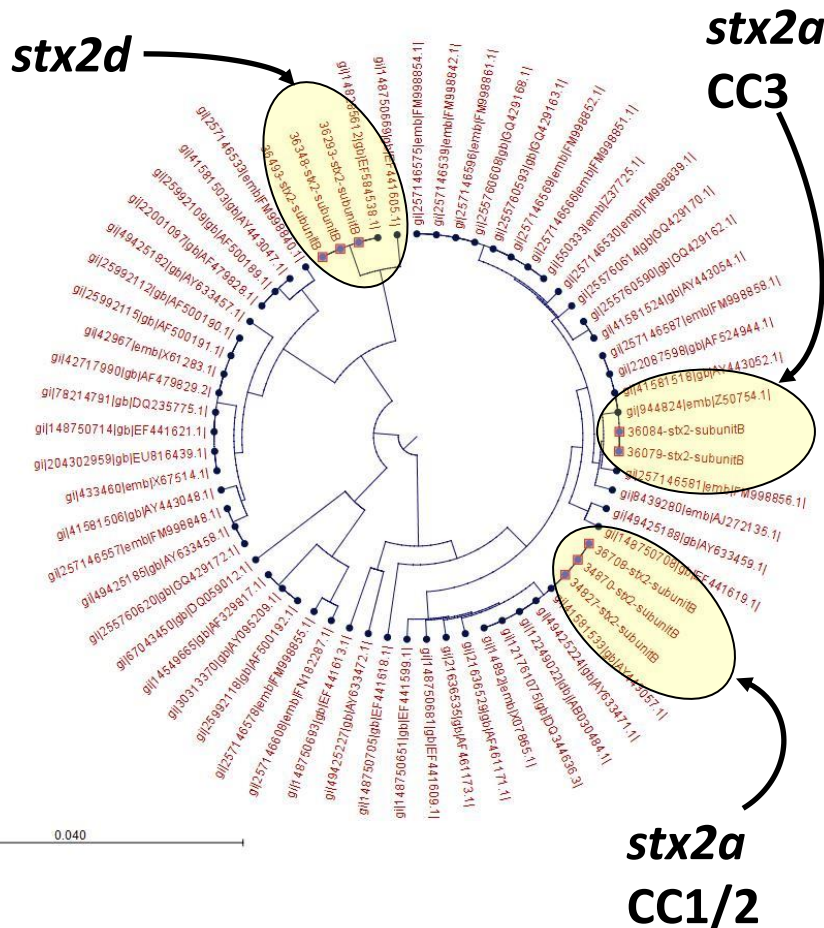
# Evolutionary relationships of the O26:H11 strains based on the concatenated ORFs of the eight *cas* genes.



- The *cas* gene tree is congruent with the wgSNP analysis.
- It is not however congruent with the MLST phylogeny (as ST29 is split between the two branches).
- Interestingly, a simple qPCR assay targeting the CRISPR array specific to SNP-CC1 (SP\_O26-E) can distinguish between the two main lineages.

# The *stx2a* gene sequence of ST21 (CC3) and ST29 (CC1/2) are different suggesting separate acquisition events

## Sequence analysis of the *stx2* subunits

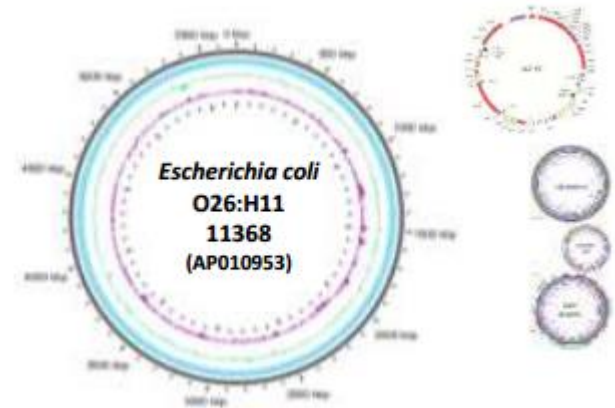


- *stx2d* sequences identical to strain 06-5231 (O55:H7).
- *stx2a* sequences from CC3 - ST21 identical to O157:H- strain 258/98.
- *stx2a* sequences from CC1/2 - ST29 identical to the 2011 O104:H4 epidemic strain.

# The 'Mobilome' accounts for 20% of the genome of *E. coli*

## Reference Genome O26:H11 – 11368 (accession AP010953) :

- Total size of the Genome (chromosome + plasmids) = **5 855 531 bp** including :
  - 21 prophages (810 311 bp)
  - 9 Integrated elements (292 371 bp)
  - 4 plasmids (158 291 bp)



### Genetic Mobile Elements ( "mobilome" )

= **1 260 973 bp**

= **21.5% of the Genome**

'Short reads' sequencing technologies may be problematic to access the complete 'mobilome' :

Repeated elements



difficulties to perform the assembly

# Human strains of EHEC O26:H11, stx2 Positive, circulating in France

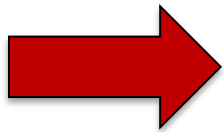
## Whole Genome Sequencing of 8 clinical isolates representative of the O26:H11 stx2+ circulating in France :

- Libraries prepared with the Nextera XT kit
- Illumina Sequencing with the MiSeq (paired-end 2x150bp or 2x300 bp)
- Assembly with the CLC Genomic workbench

TABLE 1 NCBI accession numbers and assembly metrics of the O26:H11 *E. coli* draft genomes

Isolate	ST	stx subtype	No. of contigs	Genome size (bp)	$N_{50}$ (bp)	Median read depth ( $\times$ )	No. of coding sequences (per PGAAP)
36084	ST21	stx <sub>2a</sub>	205	5,235,007	96,218	45	5,209
36708	ST29	stx <sub>2a</sub>	192	5,526,827	114,644	84	5,579
34827	ST29	stx <sub>2a</sub>	223	5,604,044	73,150	38	5,674
34870	ST29	stx <sub>2a</sub>	196	5,498,010	65,024	38	5,524
36348	ST29	stx <sub>2d</sub>	208	5,570,467	94,008	71	5,573
36293	ST29	stx <sub>2d</sub>	207	5,458,923	75,621	45	5,460
36493	ST29	stx <sub>2d</sub>	204	5,422,929	78,448	47	5,389

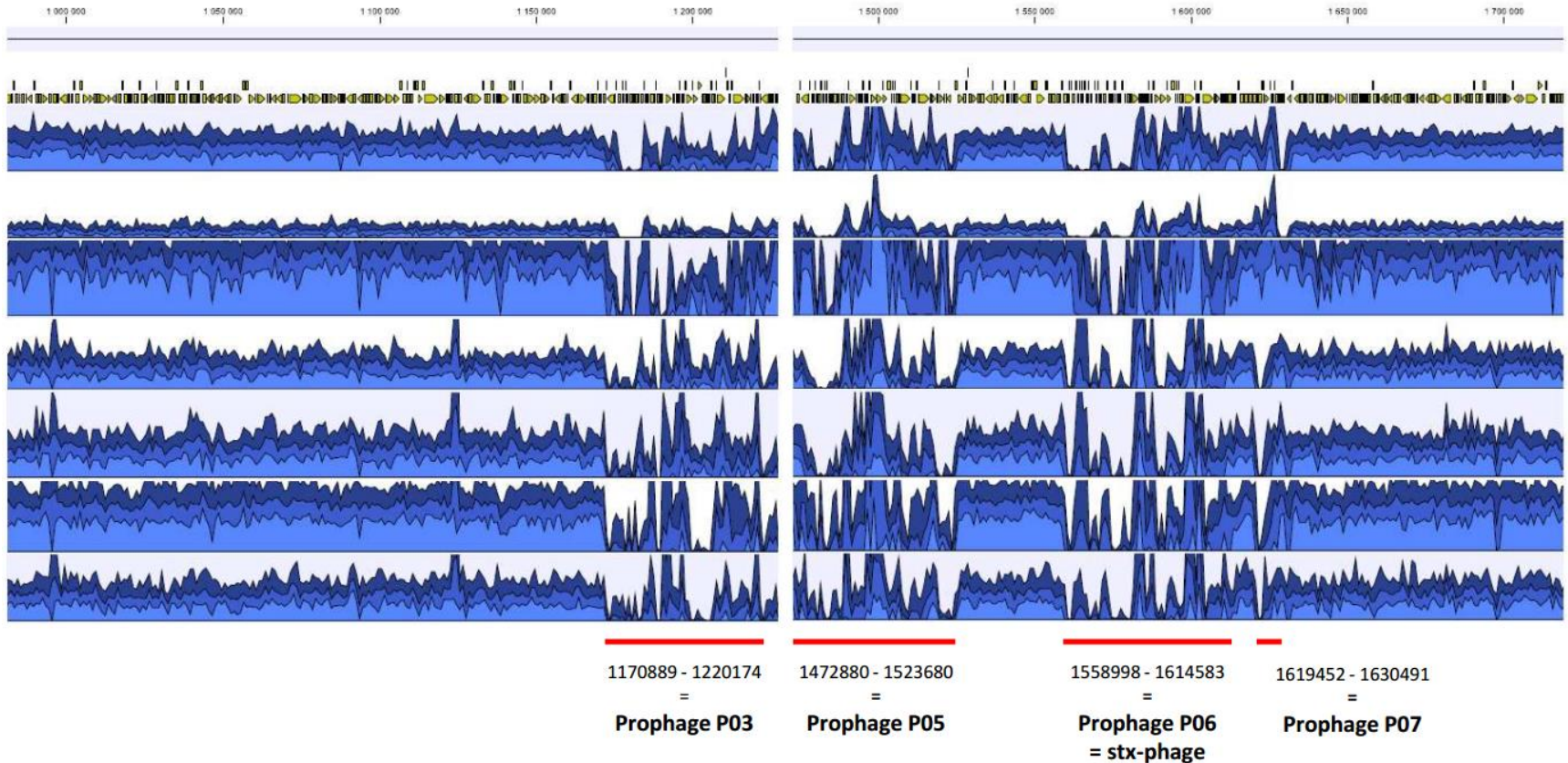
Delannoy et al. Genome Announc. 2015, 3(4): 00852-15



**Data fragmentation :**  
**About 200 contigs for a genome of 5.5 Mbp**

# Data Fragmentation = Mobilome

## Short reads mapping on O26:H11 reference genome 11368



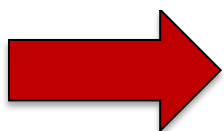
- Mapping the short reads of the French isolates to the reference strain 11368 genome sequence indicated a high degree of conservation across the chromosomal backbones of all isolates. In fact, **92–98% of the reference genome was conserved**
- **Most of the divergence appears to be concentrated in mobile genetic elements (MGEs) such as prophages, integrated elements, and plasmids**

# Long Reads sequencing with the PacBio

Whole Genome Sequencing of 8 clinical isolates representative of the O26:H11 stx2a+ circulating in France :

- PacBio RSII Sequencing (Get PlaGe Platform – INRA Toulouse)
- Reads length = 10 kbp
- Assembly with the CLC Genomic workbench + genome finishing module (long reads *de novo* assembly, mapping Illumina short reads, consensus extraction)

Isolate	Genome size (bp)	Nb de contigs	$N_{50}$ (bp)
34827	5,933,672	34	785311
34870	5,798,318	27	649134
36079	5,735,195	23	468179
36084	5,849,490	39	482424
36293	5,790,328	33	727839
36493	5,758,521	29	446336
36708	5,894,762	39	634585
36348	5,978,287	41	424063



**About 30 contigs for a genome of 5.8 Mbp**



# Identification of the duplication of the phage *stx*

Example: Strain 34827 (O26:H11, *stx2a*, ST29, CC1)

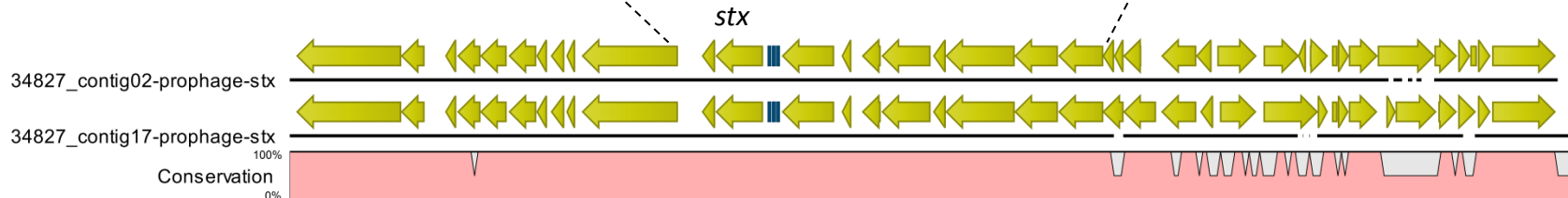
## Illumina Assembly :

One portion of the phage *stx* (10 kb) is on an isolated contig



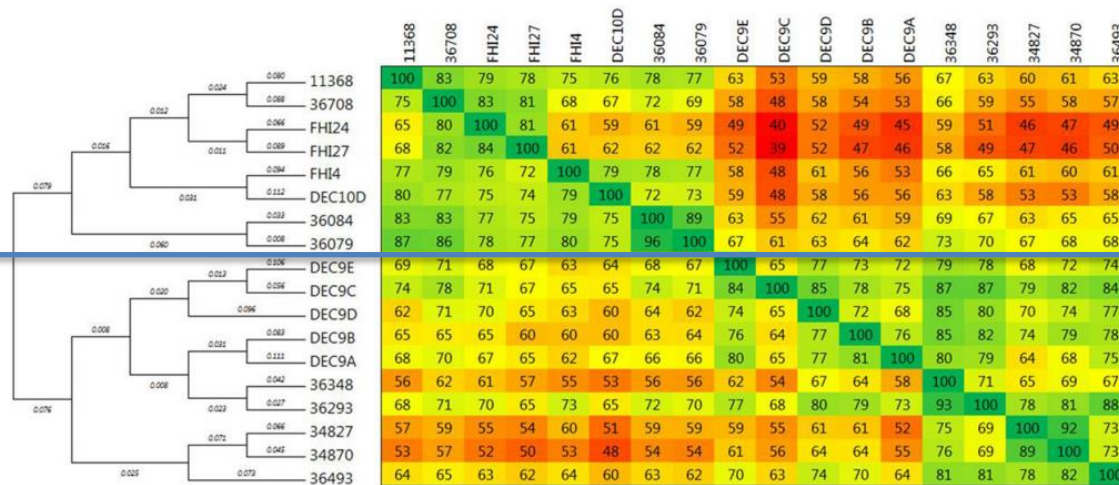
## PacBio Assembly :

Identification of 2 phages *stx* with their chromosomal environment



# The PacBio sequencing allowed a detailed analysis of the Mobile Genetic Elements (MGEs) of the strains.

- Many MGEs were identified in each strain, including a **large number of prophages** and up to **four large plasmids**, representing overall **8.7–19.8%** of the total genome size.
- Analysis of the prophage pool of the strains shows a great diversity with a **complex history of recombination**.
- Each clonal complex (SNP-CC) is characterized by a unique set of plasmids and phages, including the *stx*-prophages, suggesting **evolution through separate acquisition events**.

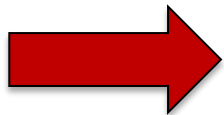


Phylogenetic relationships of the phageome of O26:H11/H- strains.

The phage regions of the isolates as determined with PHASTER were compared using Gegenees 2.1 and Mega 6.06.

# Conclusions

- The new, highly pathogenic, **European EHEC O26:H11 clone (stx2+, ST29, CC2)** is circulating in France.
- 12 French EHEC O26:H11 strains differ significantly from the European clone and belong to a different lineage and CC = **The 'New French' clone (stx2+, ST29, CC1)**
- There is a congruence between the **CRISPR sequence (SP\_O26-E)** and the phylogenetic background (**CC1**).
- The **PCR test SP\_O26-E** could be a useful addition for rapid investigation of the **new French O26:H11 clone**.
- First description of the association of **stx2d and eae** in the same O26:H11 clinical strains
- Overall, the MGEs appear **to play a major role** in O26:H11 intra-serotype clonal diversification.



**Continuing evolution of EHEC O26:H11**



# The Mobilome; A Major Contributor to *Escherichia coli* stx2-Positive O26:H11 Strains Intra-Serotype Diversity

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**Thanks!**



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