

Characterization of an *stx2k*-positive strain isolated from a pediatric patient in Italy: identification of a mosaic bacteriophage

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Strains isolated in China from samples from:

- Patients with diarrhea
- Animals
- Raw meat

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Escherichia coli strains producing a novel Shiga toxin 2 subtype circulate in China



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RESEARCH ARTICLE



High Prevalence and Persistence of *Escherichia coli* Strains Producing Shiga Toxin Subtype 2k in Goat Herds

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First *stx2k* strain in Europe

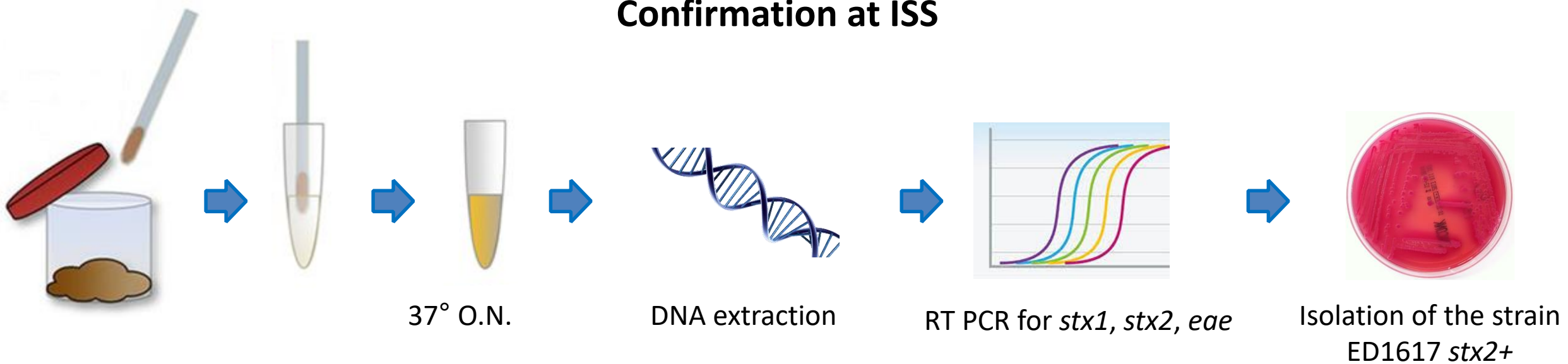
August 2022: pediatric patient with diarrhoea resident in Lazio region



Screening for gastrointestinal pathogens with Allplex™ Gastrointestinal Panel Assay (multiplex Real Time PCR)

➔ ***stx+***

Confirmation at ISS



Sequencing and bioinformatic analysis



Short reads Ion Torrent S5
Long reads Nanopore



WGS analysis

(<https://w3.iss.it/site/aries/>)



E. coli serotyper

E. coli virulotyper

E. coli Shiga toxin typer

Assembly (SPAdes)

MLST (T. Seeman)

Gene annotation (Prokka)

cgMLST (chewBBACA

Innuendo scheme)

Mauve (Multiple Genome Alignment)

Assembly statistics and basic characterization

Parameter	Ion Torrent contigs	Combined contigs (Ion Torrent + Nanopore)
Number of contigs	54	11
Total length	4807079	4881374
Largest contig	498470	1181512
N50	202043	770931

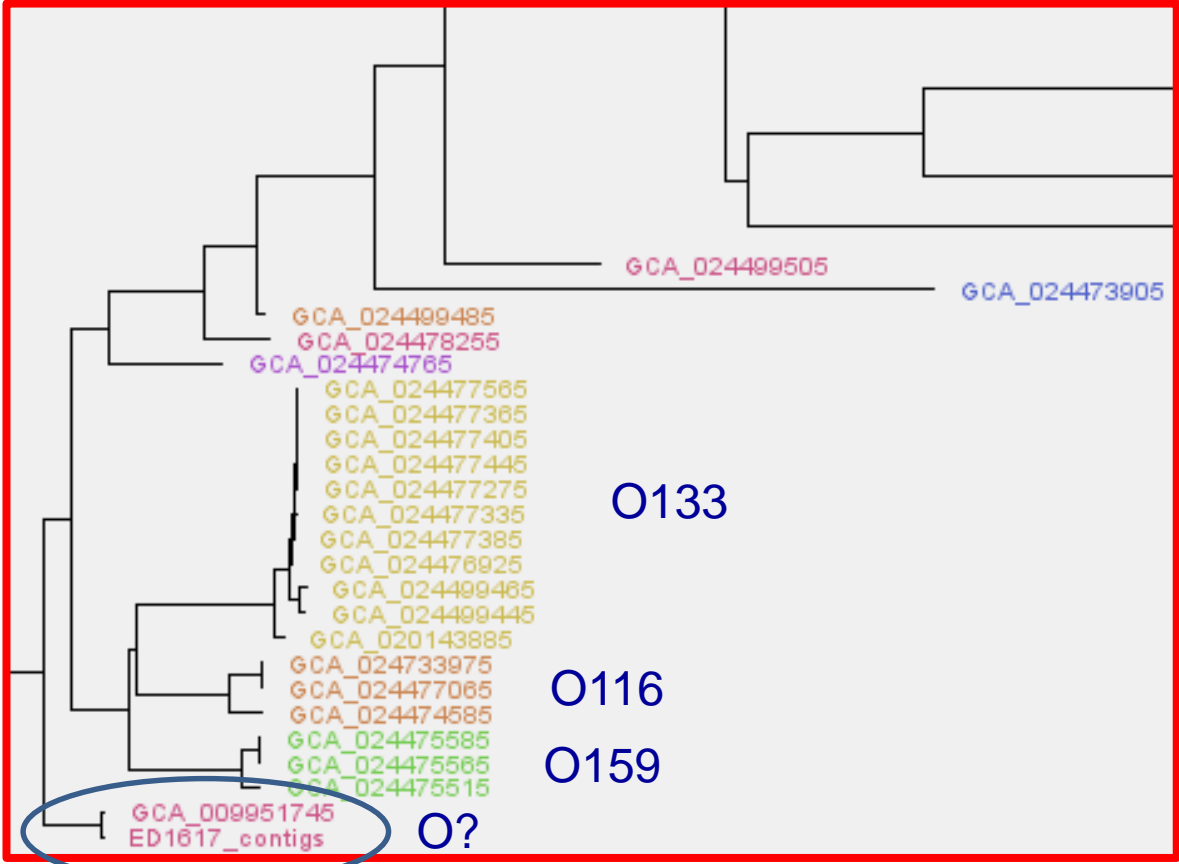
Virulotyper ***stx2k*** (100%), *gad*, *lpfa*, *ompt*, *terc*

MLST ST155

Serotyping **ONT:H21 (89% sequence identity with *wzxO155*)**

Comparison with genomes from 187 *stx2k*-positive strains from the literature

Serogroup	Colour
O100	Red
O116	Brown
O133	Olive
O134	Light Green
O159	Green
O16	Light Green
O174	Teal
O184	Light Blue
O22	Blue
O48	Dark Blue
O8	Purple
O86	Dark Purple
O93	Magenta
O?	Pink

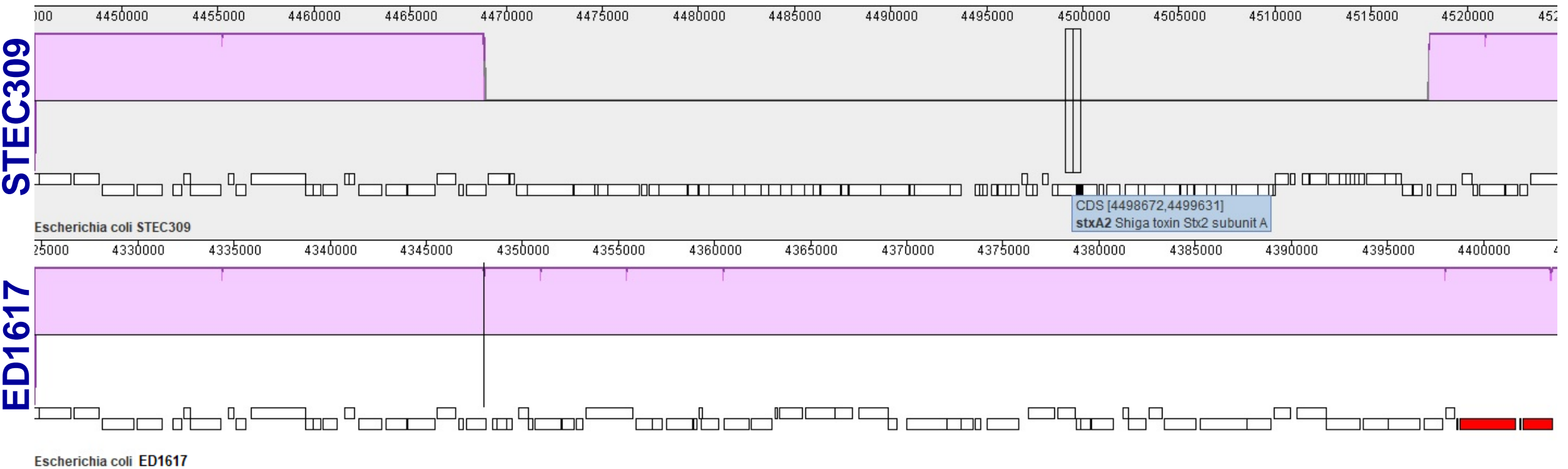


7 allelic differences with STEC309 strain, isolated from a patient in China in 2013

Alignment of sequences of *stx2k*-phages from STEC309 and ED1617

Insertion site of *stx2k*-phage in STEC309: *dusA*

dusA locus is free in the genome of ED1617



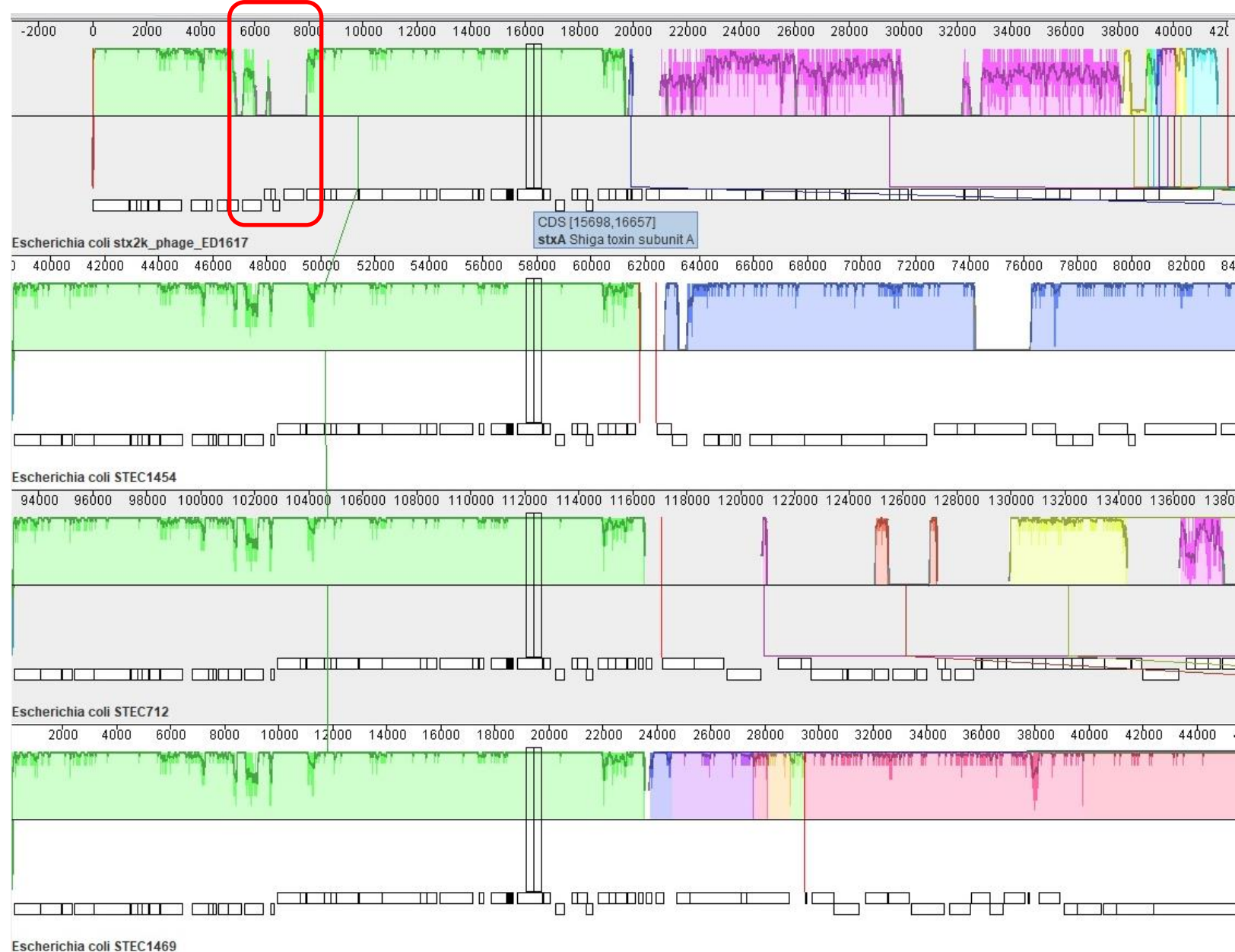
Alignment of sequences of *stx2k*-phages from Group 3

Insertion site in ED1617: *yecE*
as in *stx2k*-phages of Group 3

Same genetic arrangement
of the region upstream *stx*
genes

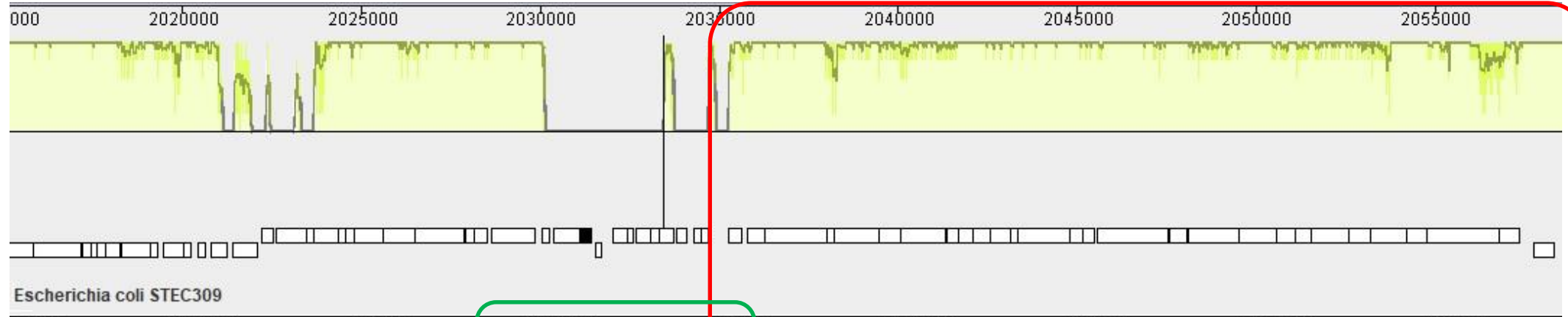
Insertion of about 2000 bp
encoding four hypothetical
proteins

ED1617

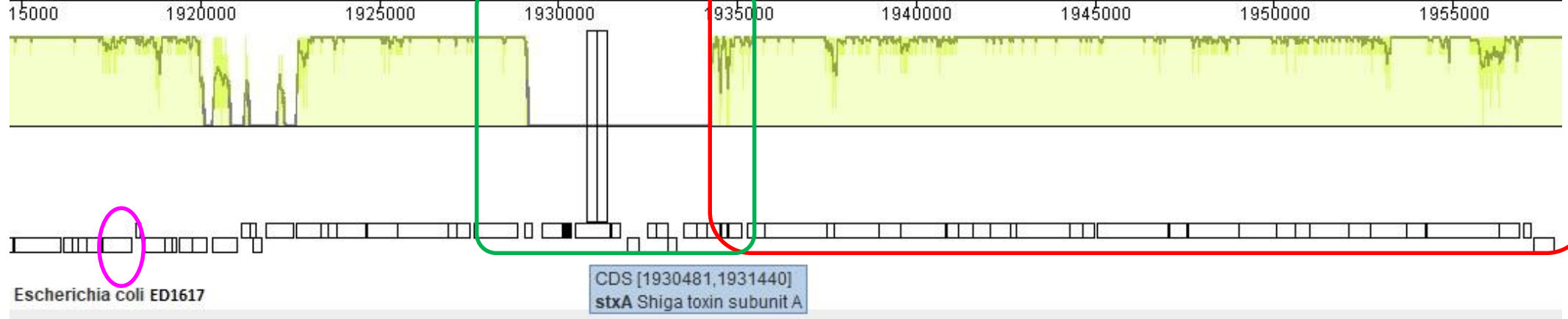


Escherichia coli STEC1469

STEC309



ED1617



PCR confirmation of the **region surrounding *stx* genes**

Region downstream *stx*: $\geq 97\%$ of nucleotidic identity with region encoding structural proteins of another phage integrated in the chromosome of STEC309 strain

Integrase-coding gene: not functional

Conclusions

- First description of an *stx2k*-positive strain in Europe
- The isolation from a strain affected by diarrhoea highlights the importance of surveillance of cases of milder disease compared to HUS
- Persistent strains harbouring *stx2k*
- Possibility of recombination events among phages, putatively favoured by defective state of integrase-coding genes

Possible emergence of novel phages able to spread *stx2k* genes



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Centro Nazionale per la Prevenzione e il Controllo delle Malattie. 2019.
«Azioni mirate a ridurre l'impatto di salute delle infezioni da *E. coli* produttore di Shigatossina (STEC) nella popolazione pediatrica e a supporto dei programmi di controllo One-Health del rischio epidemico da STEC»

Thank you for your attention!

