An Irish Experience of Whole Genome Sequencing and a Food Borne Outbreak

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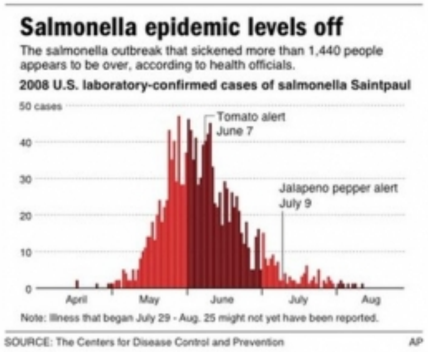
Salmonella serovars and food-borne outbreaks

- *Salmonella* is the second most common bacterial cause of foodborne gastroenteritis worldwide.
- In the European Union (EU)/European Economic Area (EEA), salmonellosis continues to be the second most commonly reported bacterial gastrointestinal infection after *Campylobacter.*
- *Salmonella* remains the predominant cause of foodborne outbreaks.
- In 2010, a total of 102,323 confirmed salmonellosis cases were reported by the 29 EU/EEA countries resulting in an estimated overall burden in the EU alone of up to €3 billion per year.



Salmonella epidemic levels off

The salmonella outbreak that sickened more than 1,440 people appears to be over, according to health officials.

2008 U.S. laboratory-confirmed cases of salmonella Saintpaul

Note: Illness that began July 29 - Aug. 25 might not yet have been reported.

SOURCE: The Centers for Disease Control and Prevention AP

Salmonella Agona and food-borne outbreaks

- In 2010 *Salmonella enterica* serovar Agona became the 10th most frequently reported nontyphoidal *Salmonella* serovar in humans in the EU, increasing 15% on 2009 (ECDC).

- It has caused a number of human disease outbreaks involving a range of foodstuffs including:
  - ready-to-eat savory snacks (UK, US and Israel, 1996)
  - Malt-O-Meal Rice/Wheat Cereals (US, 1998 and 2008)
  - fennel-aniseed-caraway infusion (Germany, 2002-2003)
  - infant milk formula (France, 2005)
  - Fresh Imported Papayas (US, 2011)
  - Curry Leaves at a Street Spice Festival (Newcastle, UK, 2013)

Salmonella Agona and food-borne outbreaks

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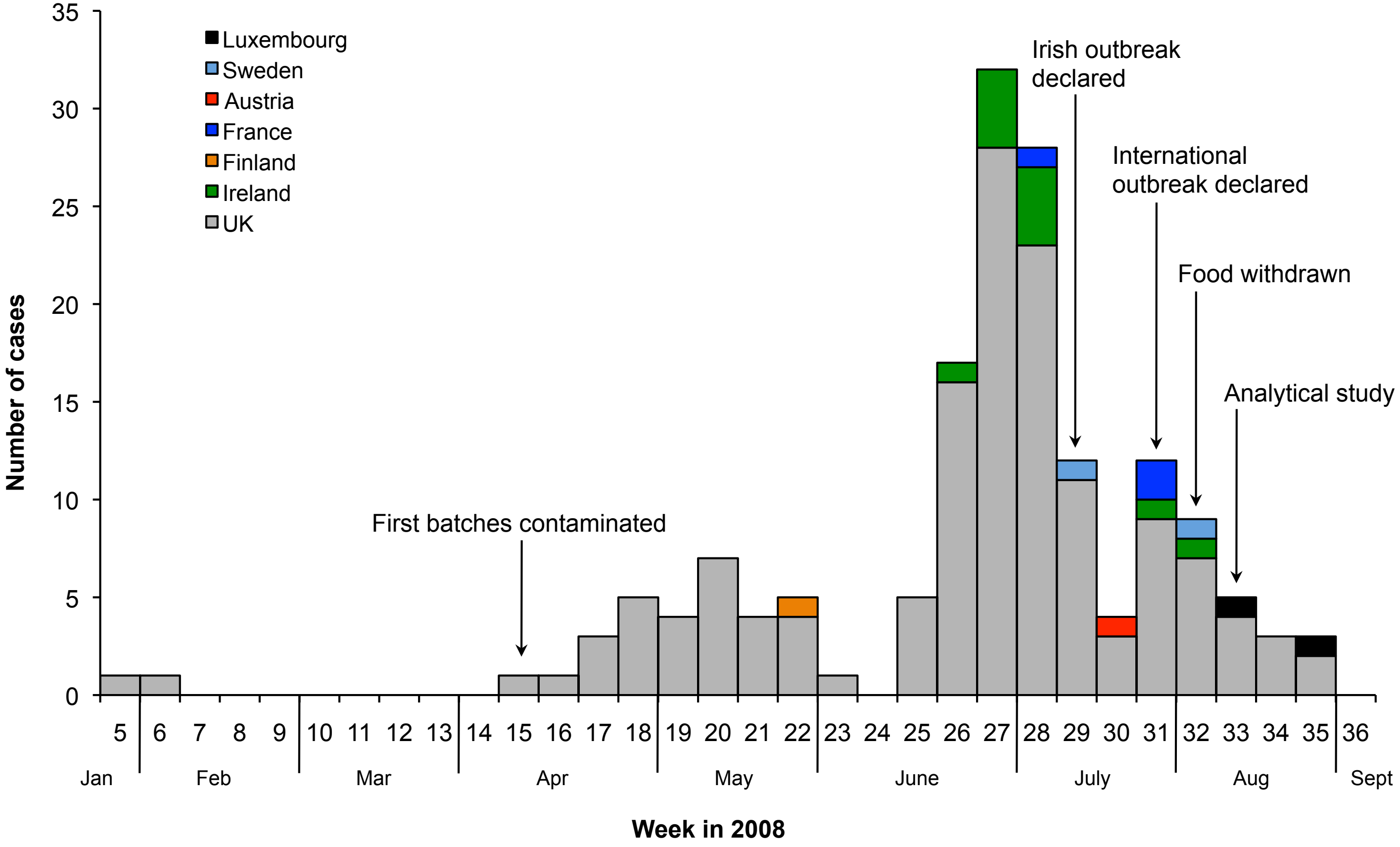
- In 2008 there was a pan-European outbreak caused by *S.* Agona with a new phage type 39, and Pulsed-Field Gel Electrophoresis (PFGE) pattern, SAGOXB.0066.

- 161 cases between the 1st week in April and the 1st week August 2008 and 2 deaths.

Events timeline of pan-European *Salmonella* Agona outbreak 2008.



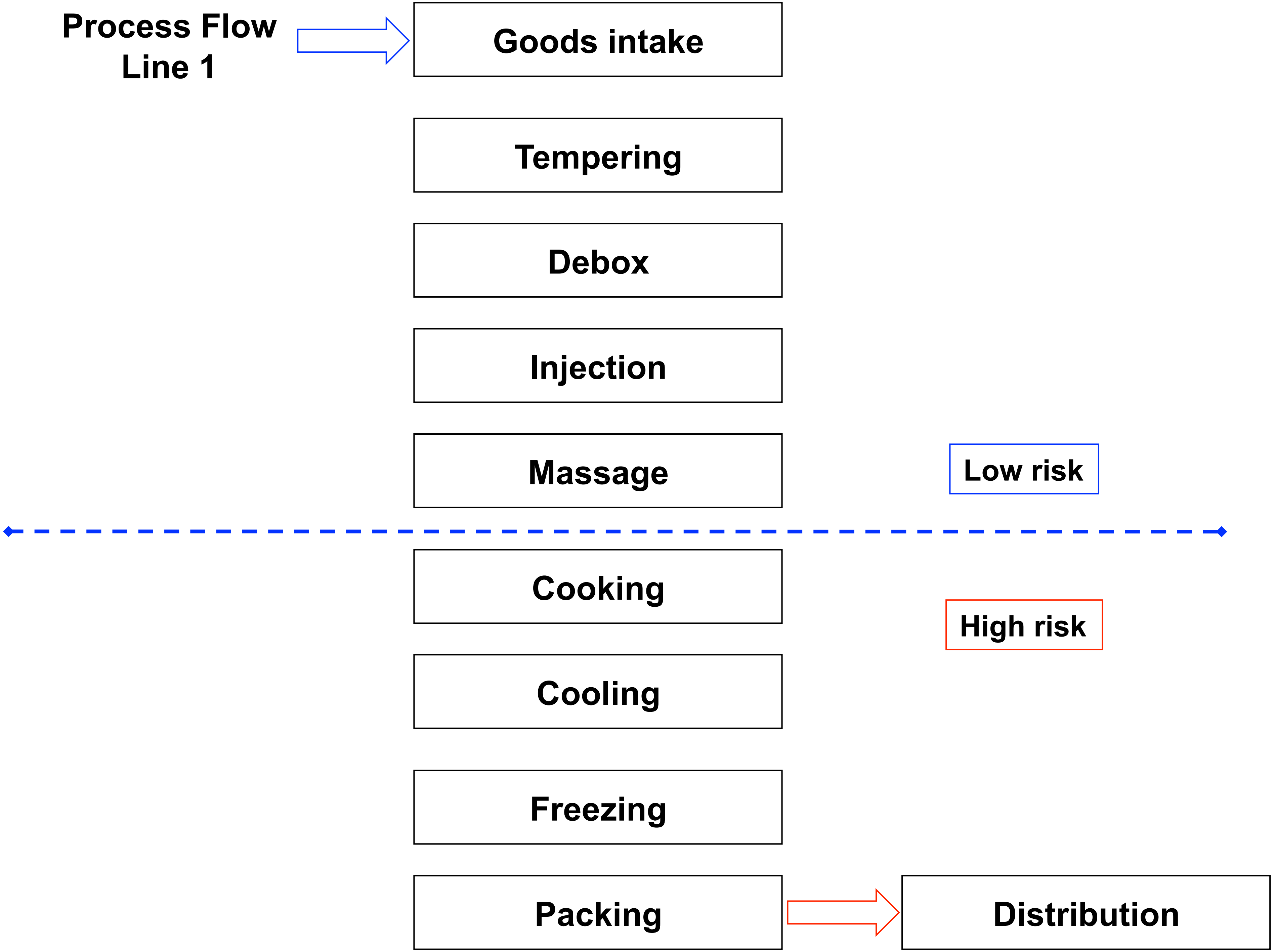
***Salmonella* Agona in Ireland - 3 to 5 cases per year**

July 15: **6 *S.* Agona** isolates reported by the Irish National *Salmonella* Reference Laboratory between June 30 and July 12.

July 17: **47 cases** in the United Kingdom (UK) since February. New phage type 39 and PFGE pattern *SAGOXB.0066*

July 18: **International outbreak declared**.

Process Flow
Line 1



Goods intake

Tempering

Debox

Injection

Massage

Low risk

Cooking

High risk

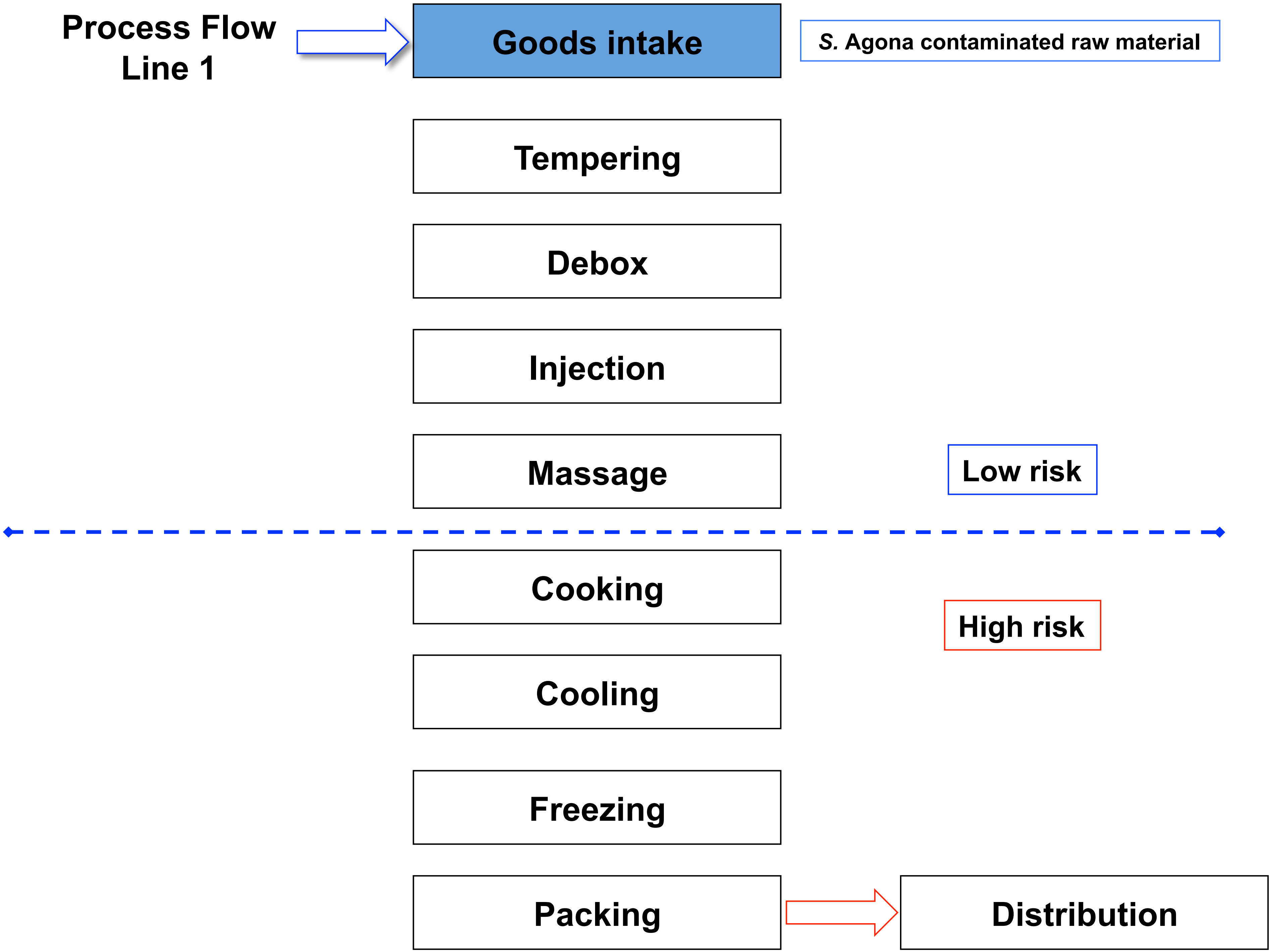
Cooling

Freezing

Packing

Distribution

Process Flow
Line 1



Goods intake

S. Agona contaminated raw material

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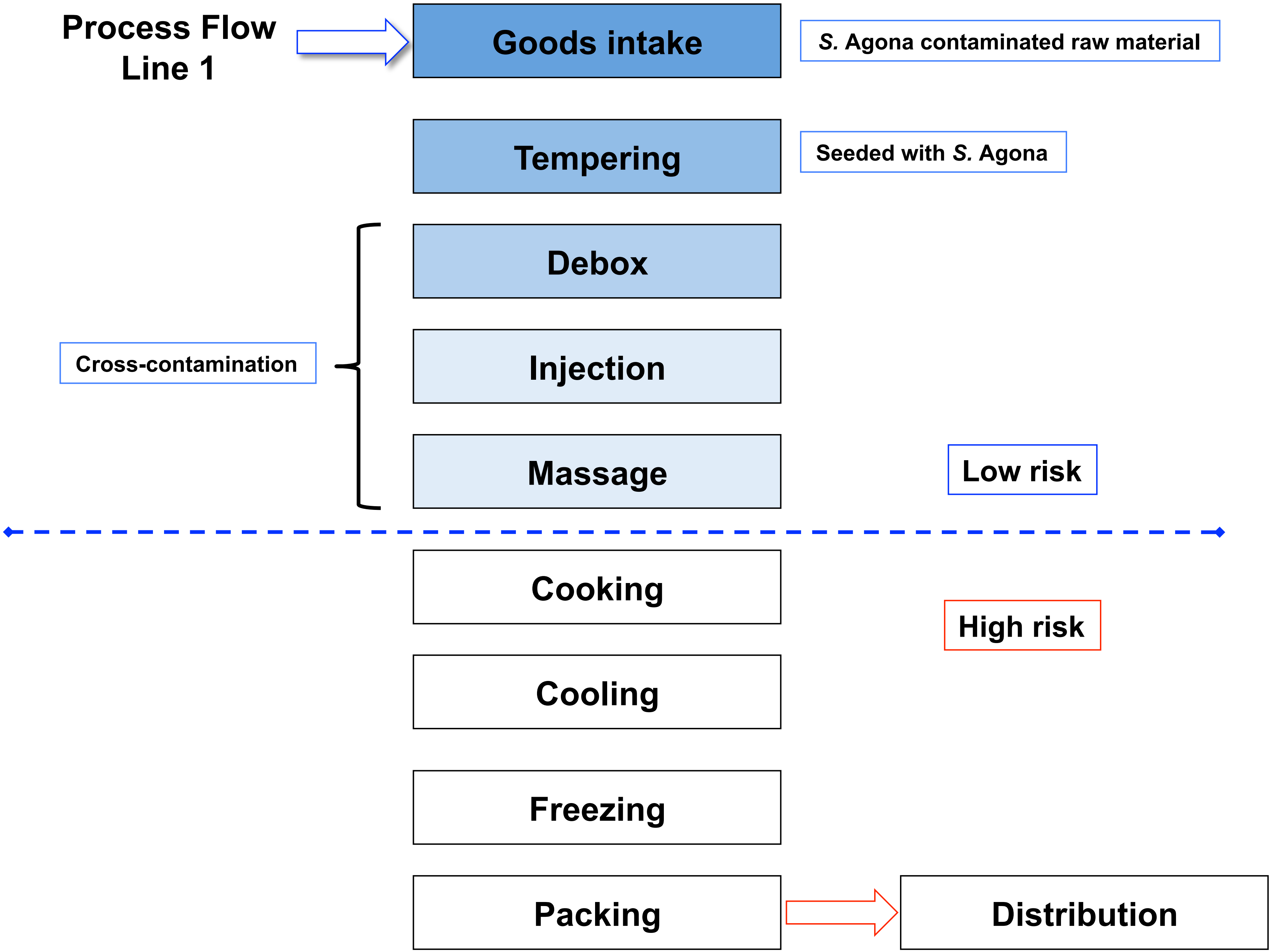

Goods intake

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Tempering

Seeded with *S.* Agona

Cross-contamination

Debox

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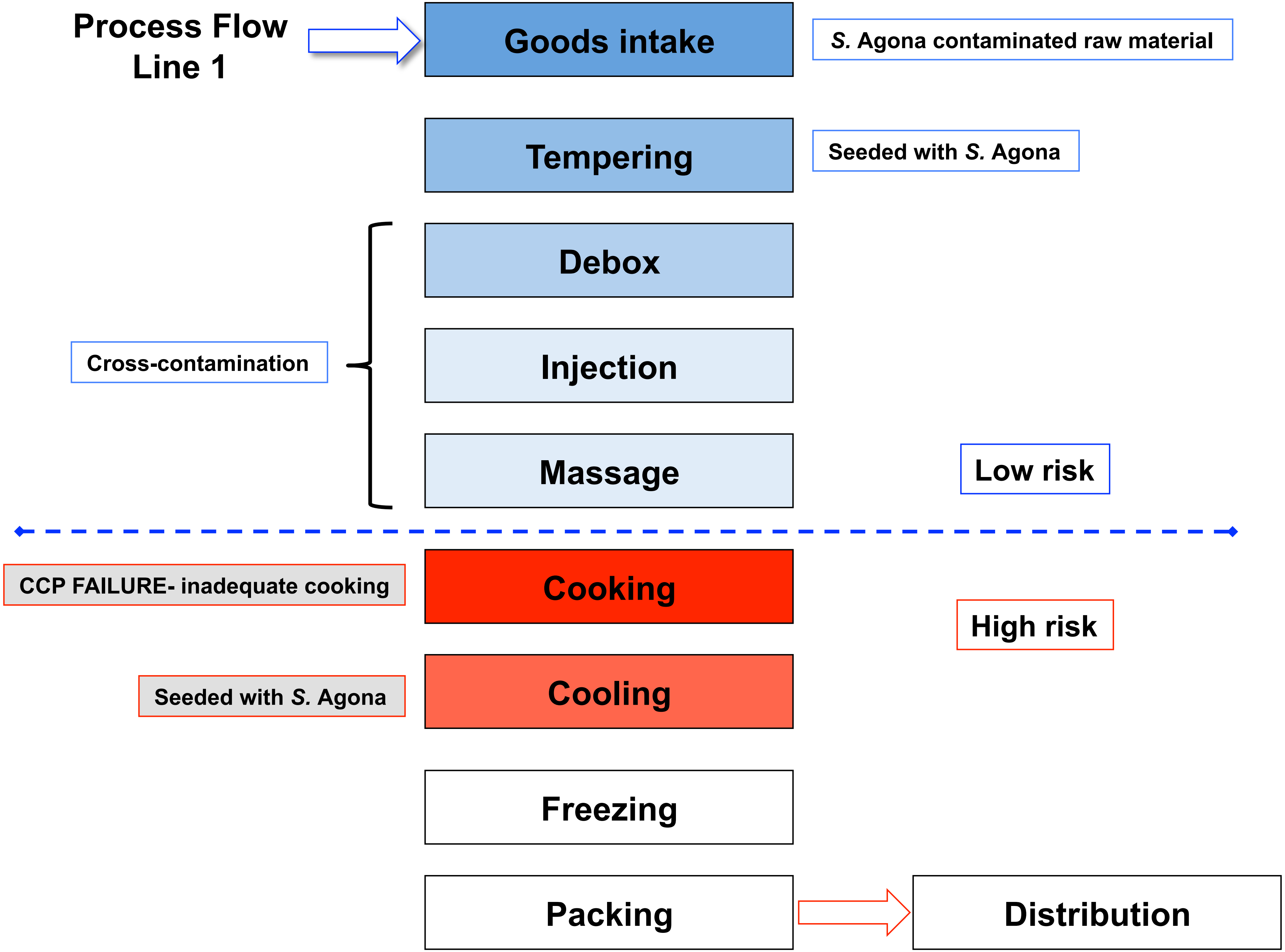

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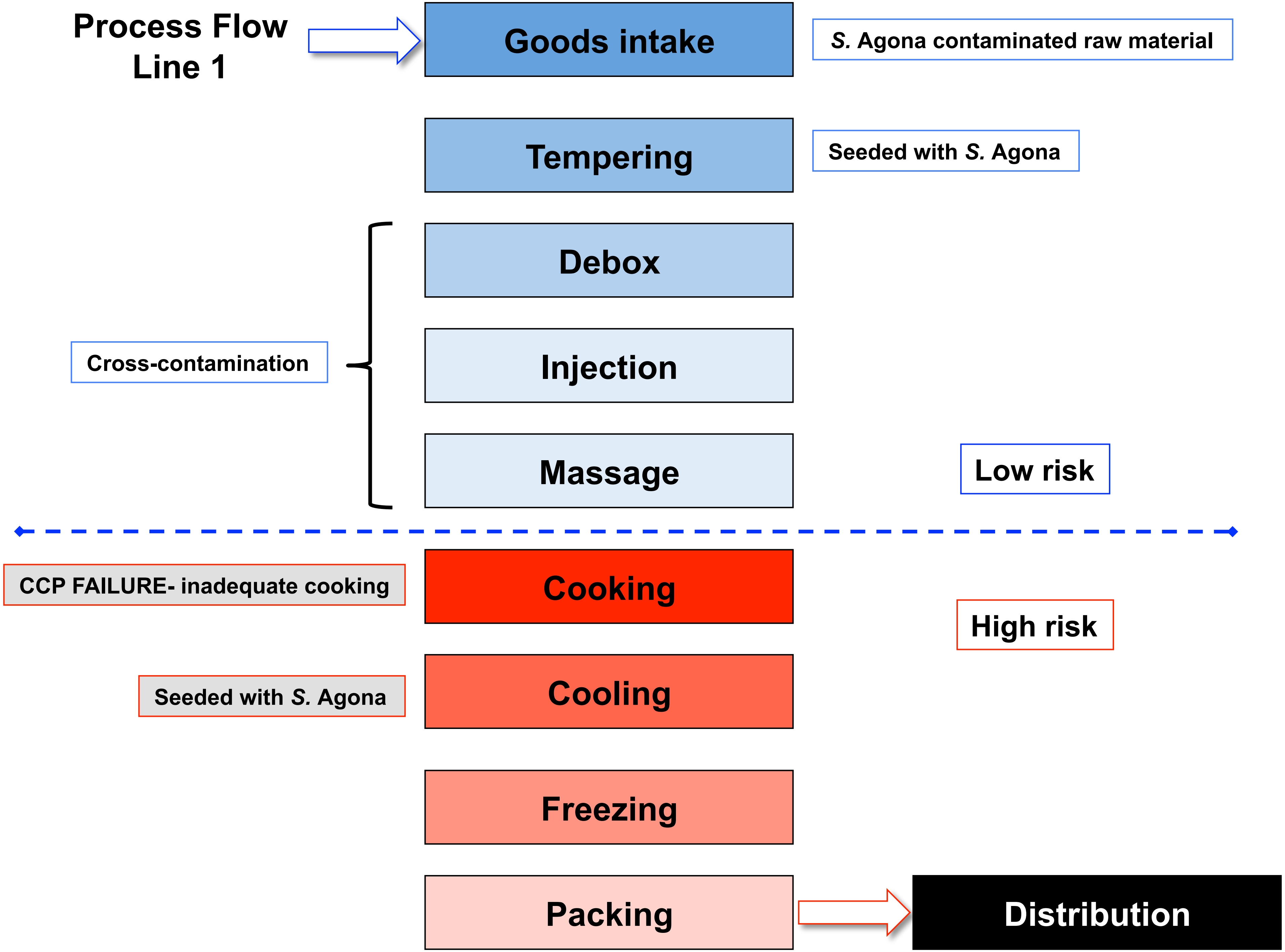

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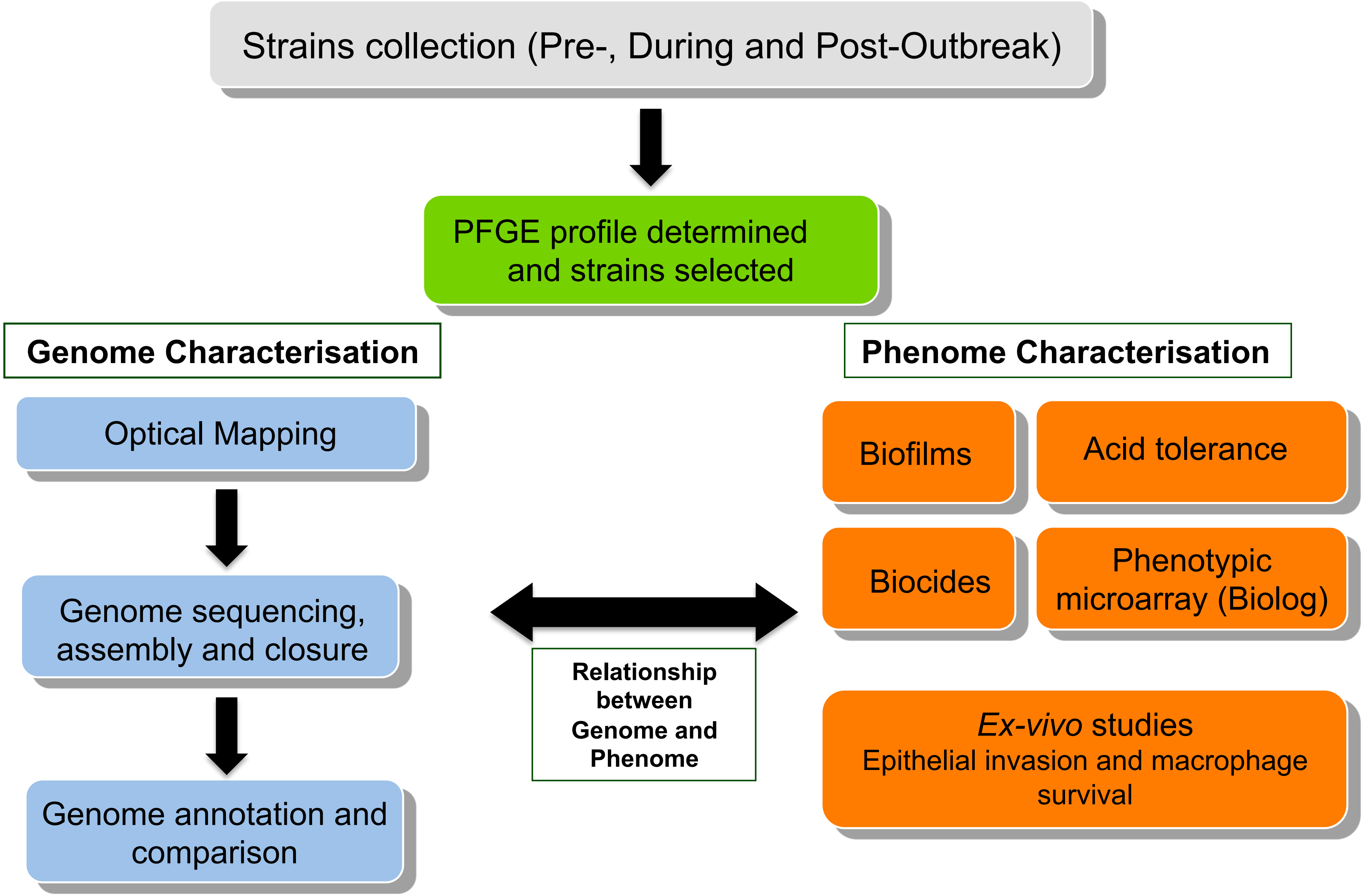
Experimental Outline



Strains collection (Pre-, During and Post-Outbreak)

PFGE profile determined and strains selected

Genome Characterisation

Optical Mapping

Genome sequencing, assembly and closure

Genome annotation and comparison

Relationship between Genome and Phenome

Phenome Characterisation

Biofilms

Acid tolerance

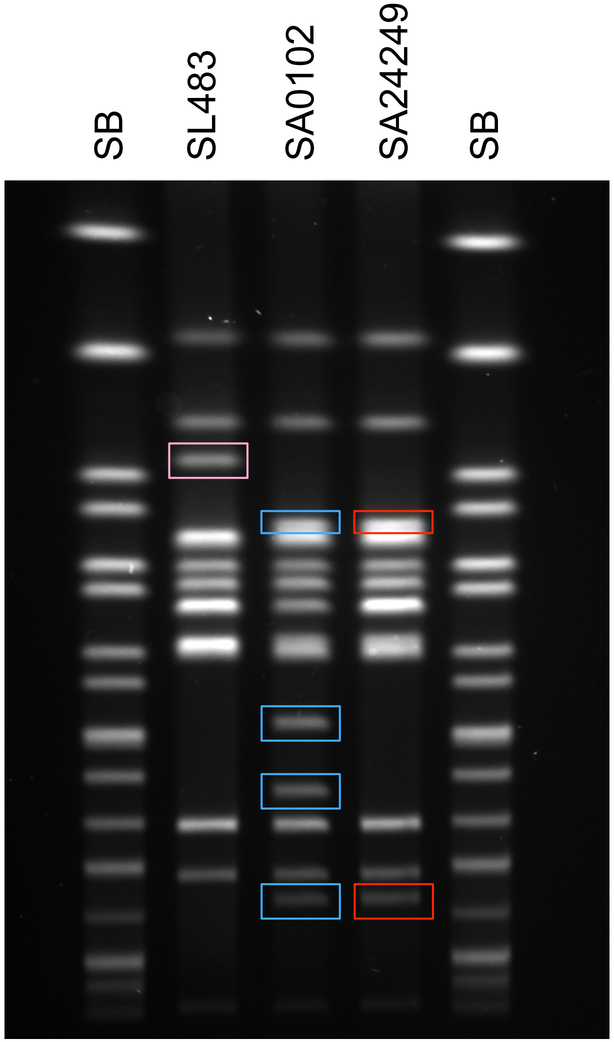
Biocides

Phenotypic microarray (Biolog)

Ex-vivo studies
Epithelial invasion and macrophage survival

PFGE-typing of the *S.* Agona outbreak strain showed a new PFGE profile designated SAGOXB.0066

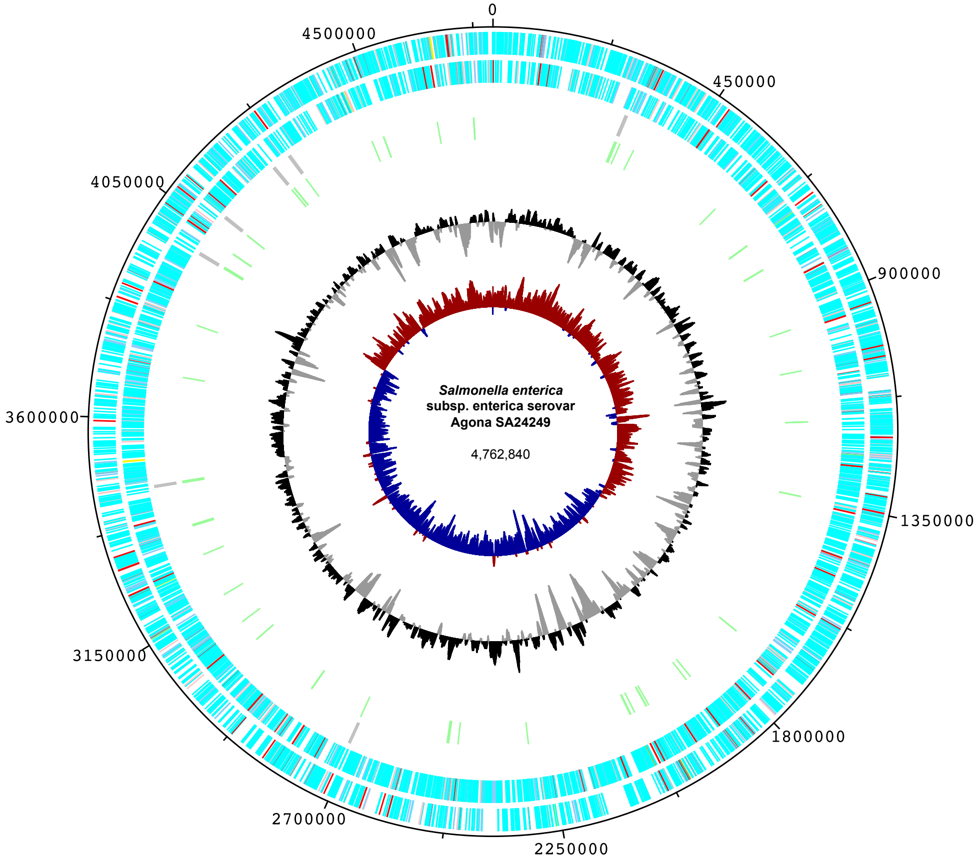
- 5 band difference between SL483 and ‘factory’ isolate.
- 3 band difference between SL483 and ‘outbreak’ strain.
- 2 band difference between the ‘factory’ and ‘outbreak’ strains.



SB – *Salmonella* Braenderup ladder
SL483 – sequenced strain
SA0102 – ‘factory’ isolate
SA24249 – ‘outbreak’ isolate

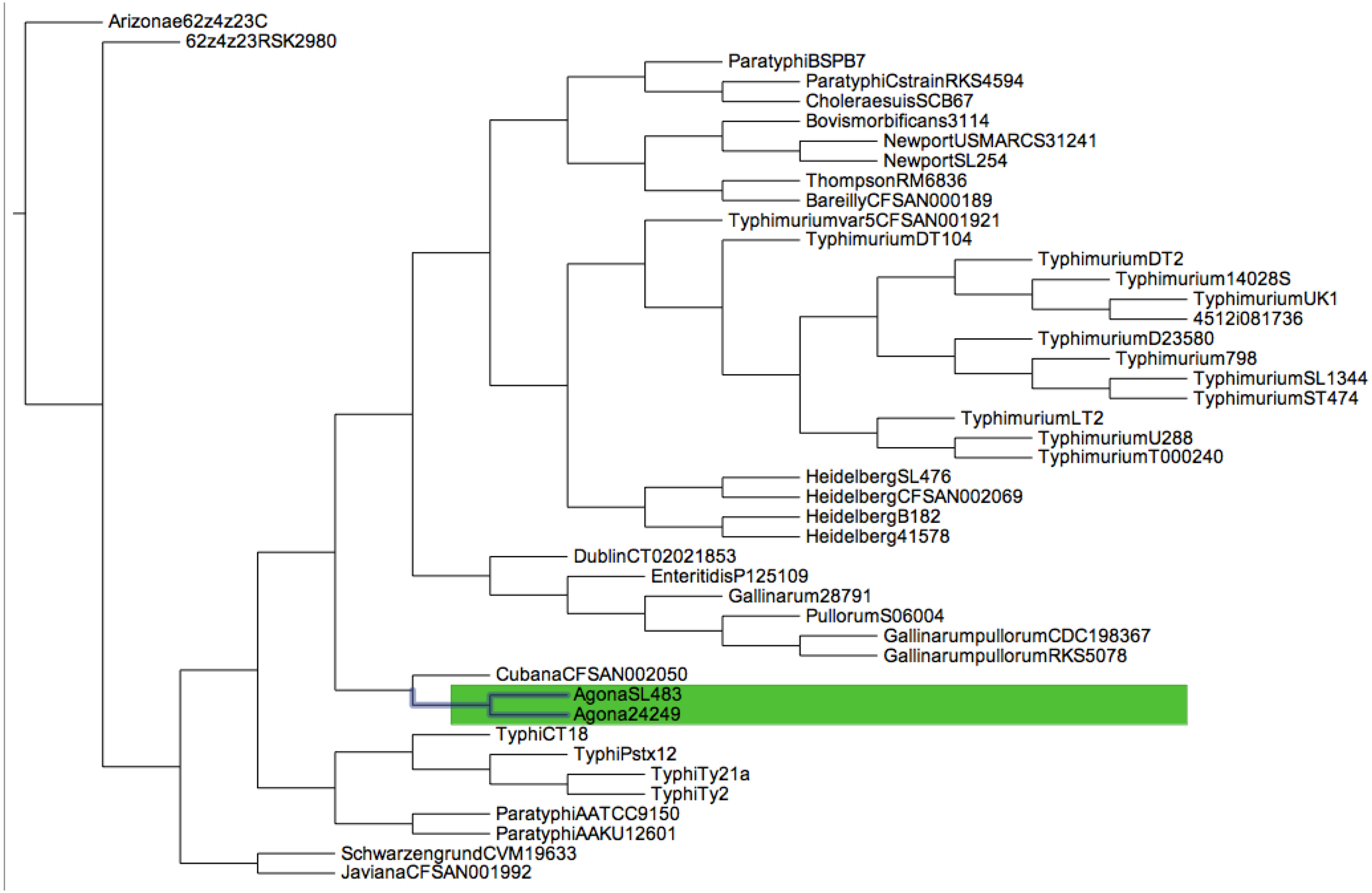
Complete Genome of *S.* Agona SA24249 PFGE-type SAGOXB.0066

- The *S.* Agona SA24249 genome is composed of a 4,762,840-bp chromosome with a G-C content of 52.1%.
- The strain did not contain any plasmids.
- The chromosome contains 4,712 open reading frames (ORFs), including 38 pseudogenes.
- 7 rDNA loci (rRNA) and 84 tRNA gene loci.
- Comparison of SA24249 to the only other complete S. Agona genome (SL483) revealed high colinearity.



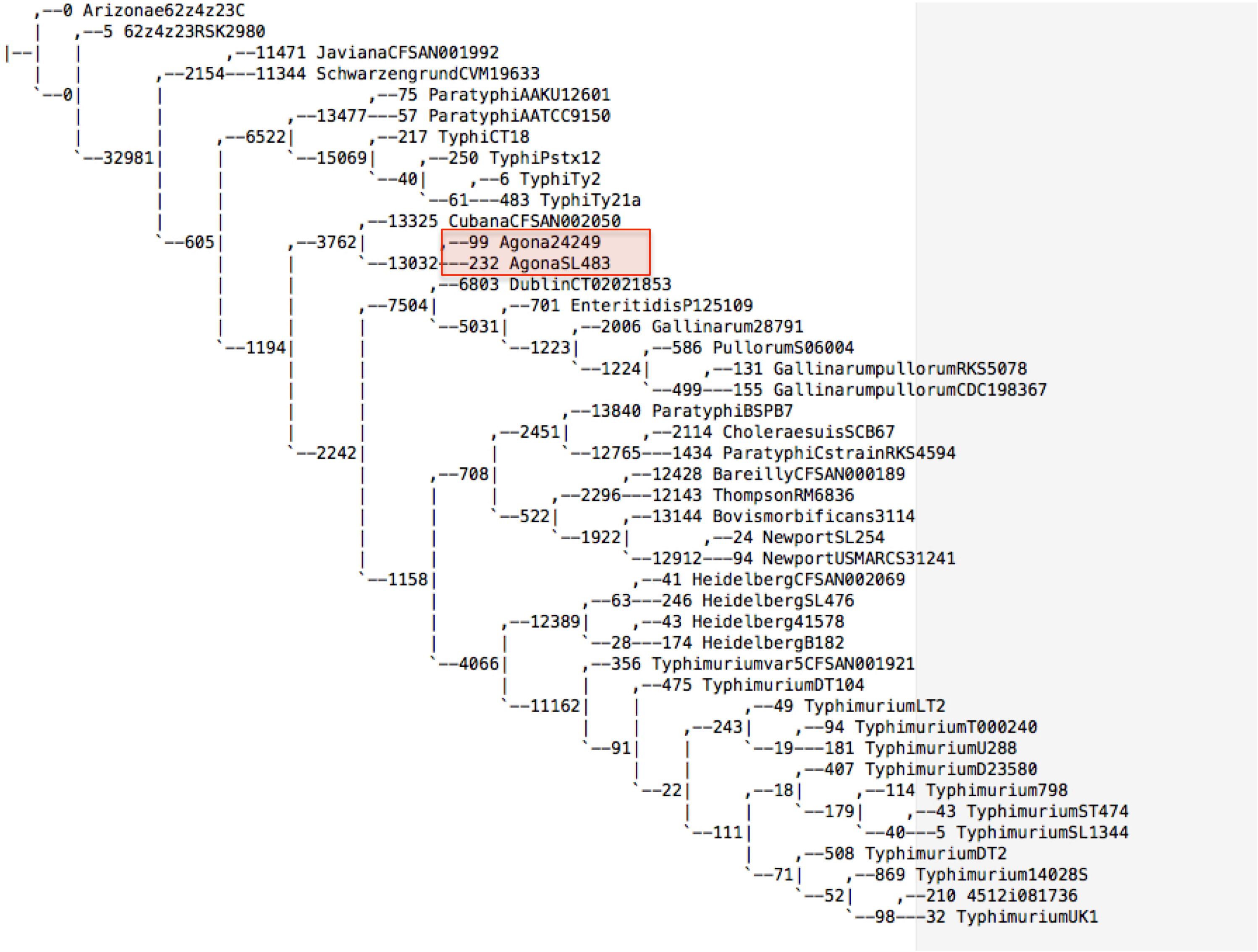
Circular representation of the *S. enterica* serovar Agona SA24249 genome. From the outside inward, the outer circle 1 indicates the size in base pairs (Mb). Circles 2 and 3 show the positions of CDS transcribed in clockwise and anticlockwise directions, respectively. The grey bars on circle 3 and green bars on circle 4 indicate ribosomal DNA loci and tRNAs, respectively. Circle 5 shows a plot of G+C content. Circle 6 shows a plot of GC skew ([G - C]/[G + C].

Cluster Analysis of *S.* Agona outbreak strain



- **Phylogenetic relationship** of *S.* Agona SA24249 with *S.* Agona strain SL483 and the 38 complete *Salmonella* genomes.

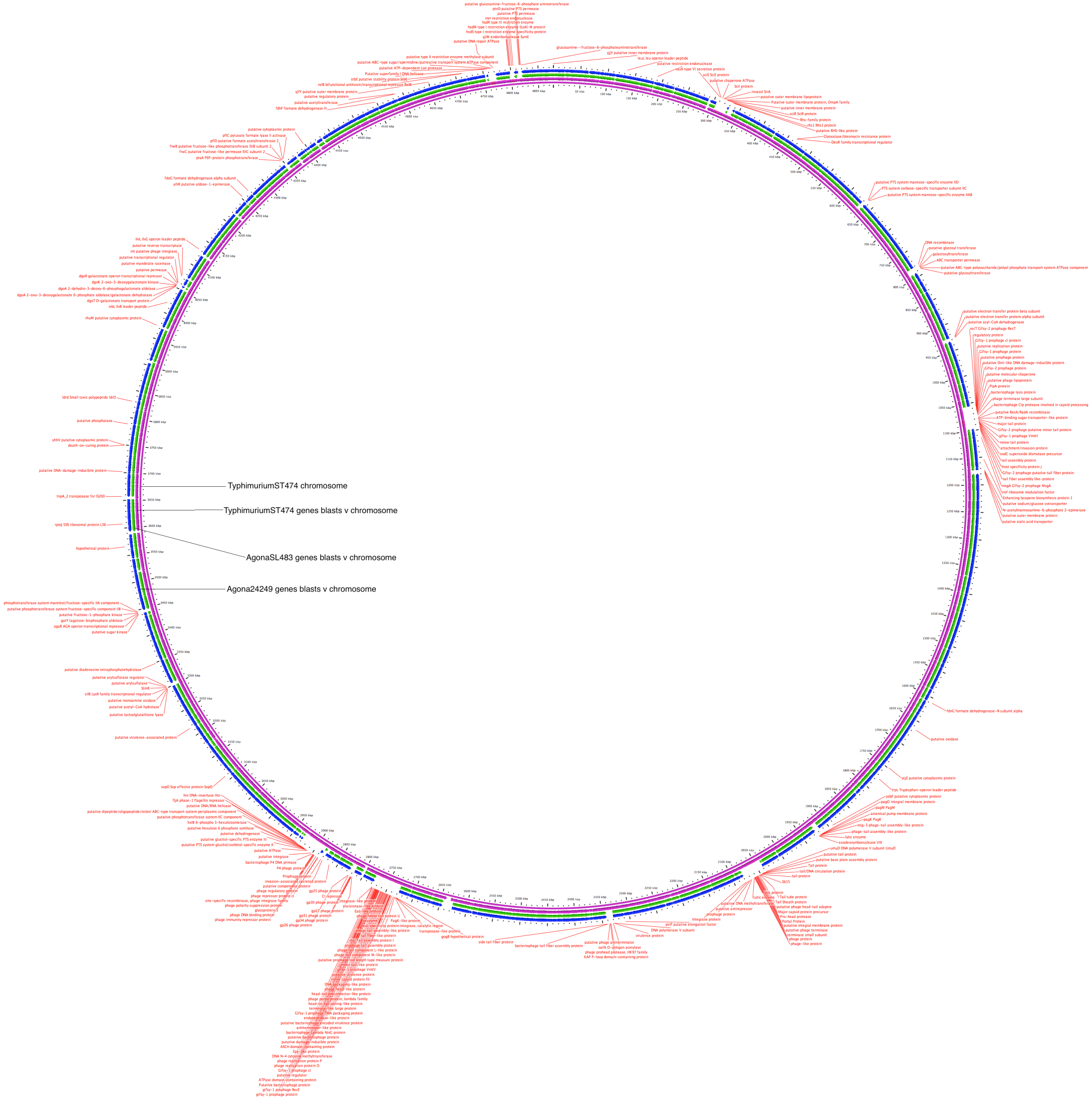
SNP Tree Analysis of *S.* Agona outbreak strain



```
   ,--0 Arizonae62z4z23C
   |   ,--5 62z4z23RSK2980
|--|   |              ,--11471 JavianaCFSAN001992
   |   |       ,--2154---11344 SchwarzengrundCVM19633
   `--0|       |                    ,--75 ParatyphiAAKU12601
       |       |            ,--13477---57 ParatyphiAATCC9150
       |       |     ,--6522|       ,--217 TyphiCT18
       `--32981|     |      `--15069|    ,--250 TyphiPstx12
               |     |              `--40|    ,--6 TyphiTy2
               |     |                   `--61---483 TyphiTy21a
               |     |             ,--13325 CubanaCFSAN002050
               `--605|      ,--3762|       ,--99 Agona24249
                     |      |      `--13032---232 AgonaSL483
                     |      |             ,--6803 DublinCT02021853
                     |      |      ,--7504|      ,--701 EnteritidisP125109
                     |      |      |      `--5031|      ,--2006 Gallinarum28791
                     `--1194|      |             `--1223|      ,--586 PullorumS06004
                            |      |                    `--1224|     ,--131 GallinarumpullorumRKS5078
                            |      |                           `--499---155 GallinarumpullorumCDC198367
                            |      |                   ,--13840 ParatyphiBSPB7
                            |      |            ,--2451|       ,--2114 CholeraesuisSCB67
                            `--2242|            |      `--12765---1434 ParatyphiCstrainRKS4594
                                   |      ,--708|            ,--12428 BareillyCFSAN000189
                                   |      |     |     ,--2296---12143 ThompsonRM6836
                                   |      |     `--522|      ,--13144 Bovismorbificans3114
                                   |      |           `--1922|       ,--24 NewportSL254
                                   |      |                  `--12912---94 NewportUSMARCS31241
                                   `--1158|                   ,--41 HeidelbergCFSAN002069
                                          |              ,--63---246 HeidelbergSL476
                                          |      ,--12389|    ,--43 Heidelberg41578
                                          |      |       `--28---174 HeidelbergB182
                                          `--4066|       ,--356 Typhimuriumvar5CFSAN001921
                                                 |       |    ,--475 TyphimuriumDT104
                                                 `--11162|    |          ,--49 TyphimuriumLT2
                                                         |    |    ,--243|    ,--94 TyphimuriumT000240
                                                         `--91|    |     `--19---181 TyphimuriumU288
                                                              |    |          ,--407 TyphimuriumD23580
                                                              `--22|     ,--18|     ,--114 Typhimurium798
                                                                   |     |    `--179|    ,--43 TyphimuriumST474
                                                                   `--111|          `--40---5 TyphimuriumSL1344
                                                                         |    ,--508 TyphimuriumDT2
                                                                         `--71|    ,--869 Typhimurium14028S
                                                                              `--52|    ,--210 4512i081736
                                                                                   `--98---32 TyphimuriumUK1
```

- **SNP based relationship** of *S.* Agona SA24249 with *S.* Agona strain SL483 and the 38 complete *Salmonella* genomes.

Blast analysis of *S.* Agona outbreak strain



Blast analysis of *S.* Agona SA24249 and SL483 against *S.* Typhimurium strain 4/74.

Inside PINK ring is 4/74 genome
Outside PINK ring is 4/74 translated
GREEN ring is SL483 translated
BLUE ring is SA24249 translated

Gives you the genes absent in *S.* Agona SA24249 compared to *S.* Typhimurium strain 4/74

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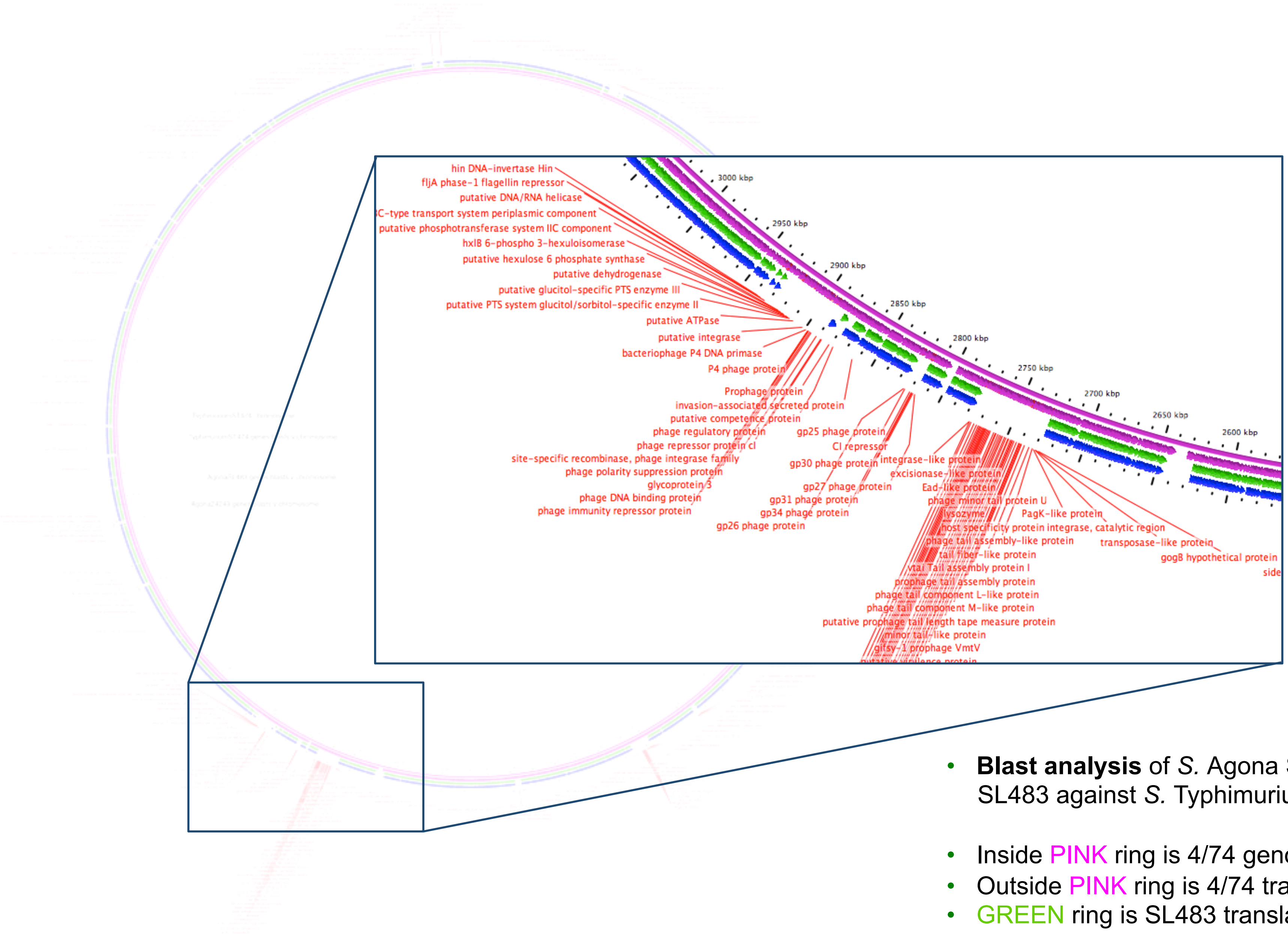

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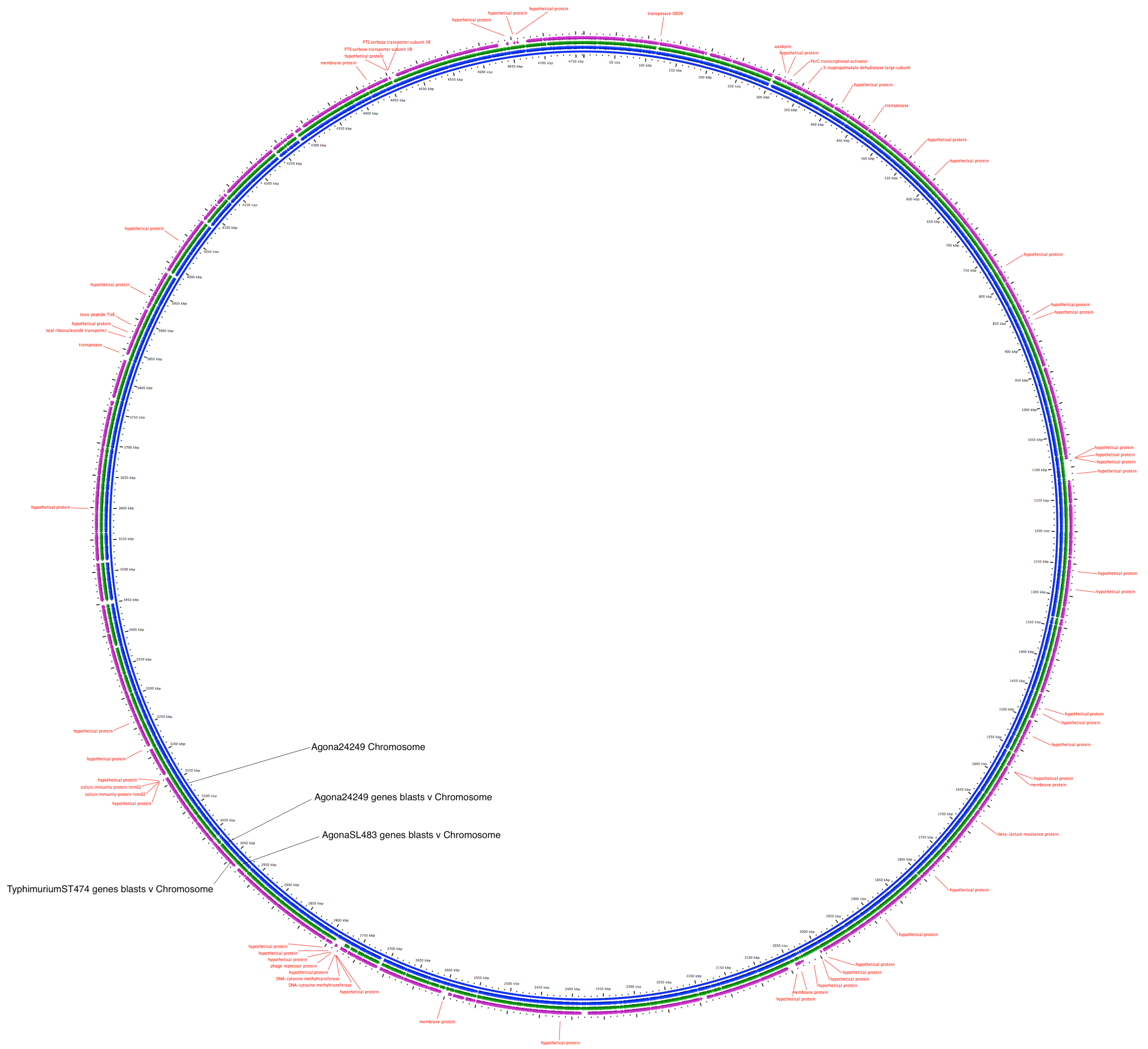

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- **Gives you the genes absent in *S.* Typhimurium strain 4/74 compared to *S.* Agona SA24249.**

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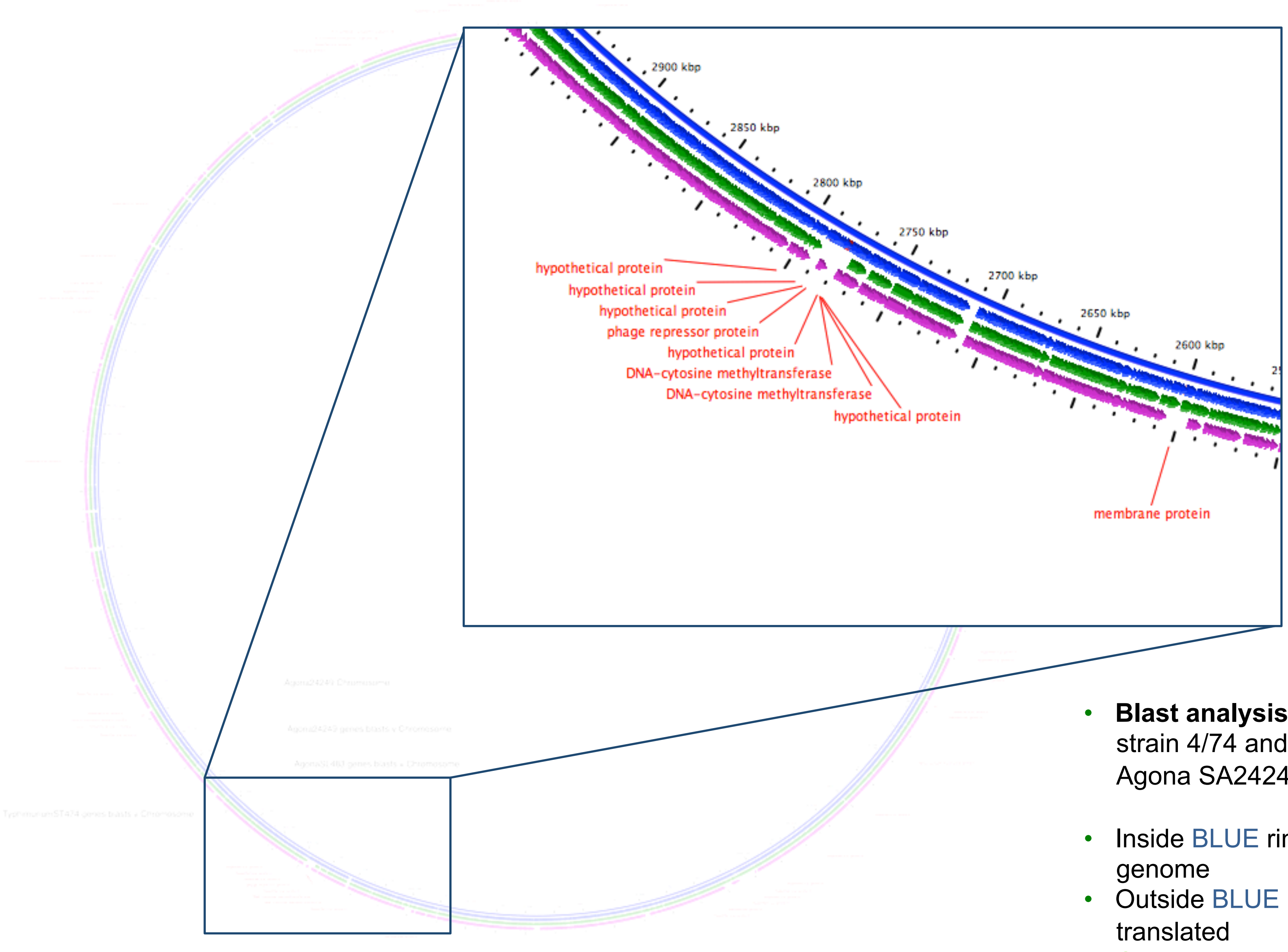

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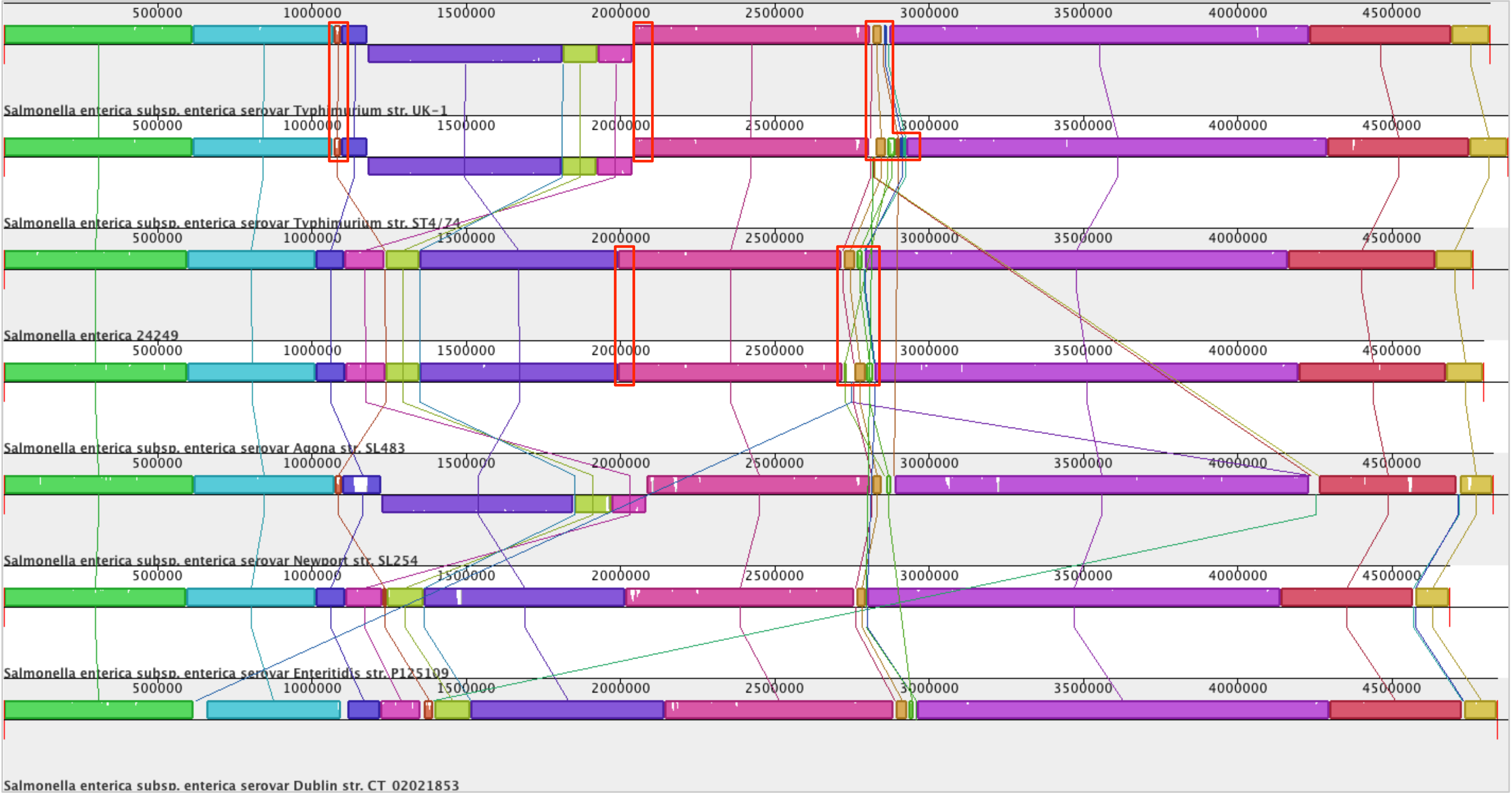
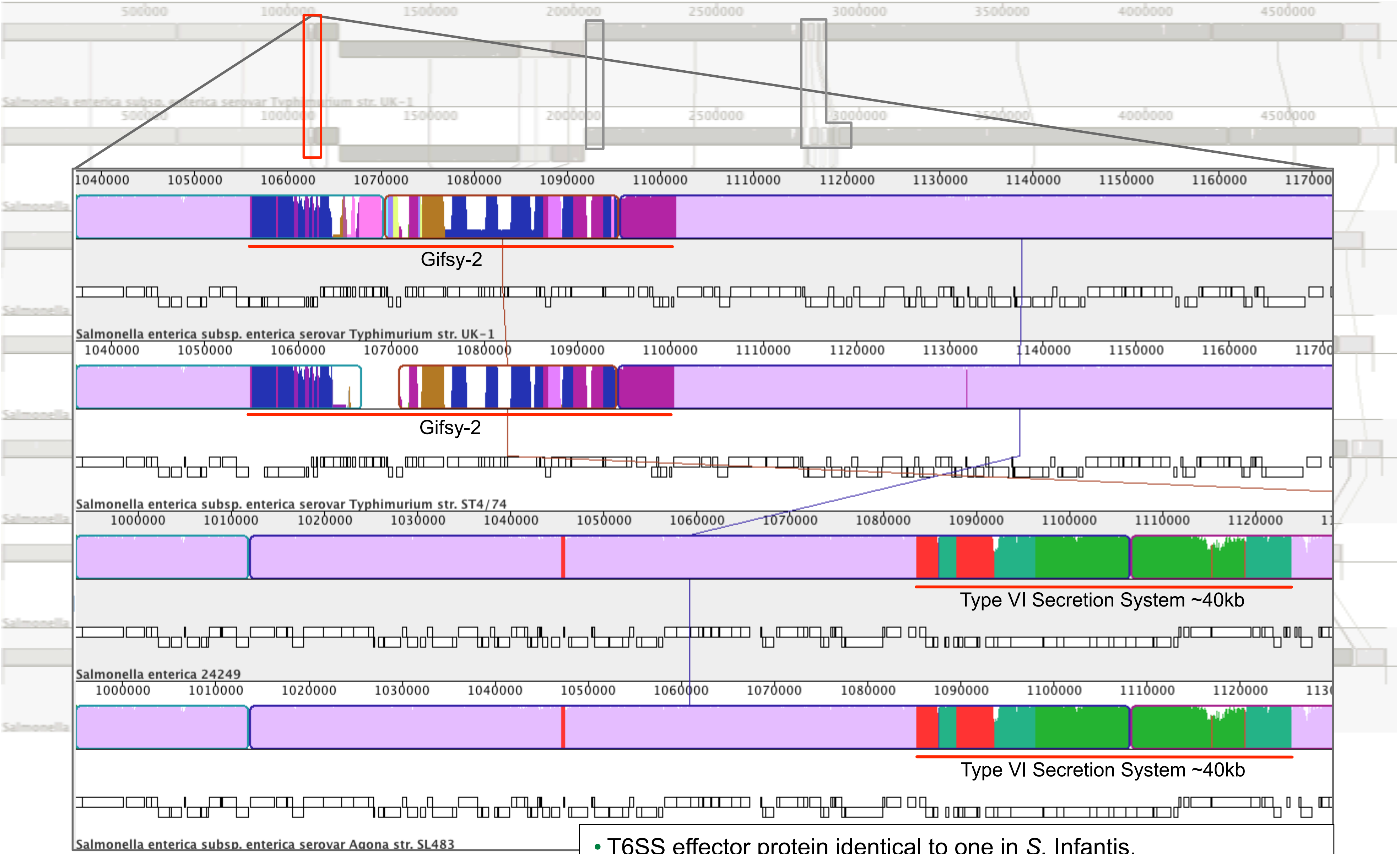
Multiple Genome Aligment of *S.* Agona SA24249 with MAUVE



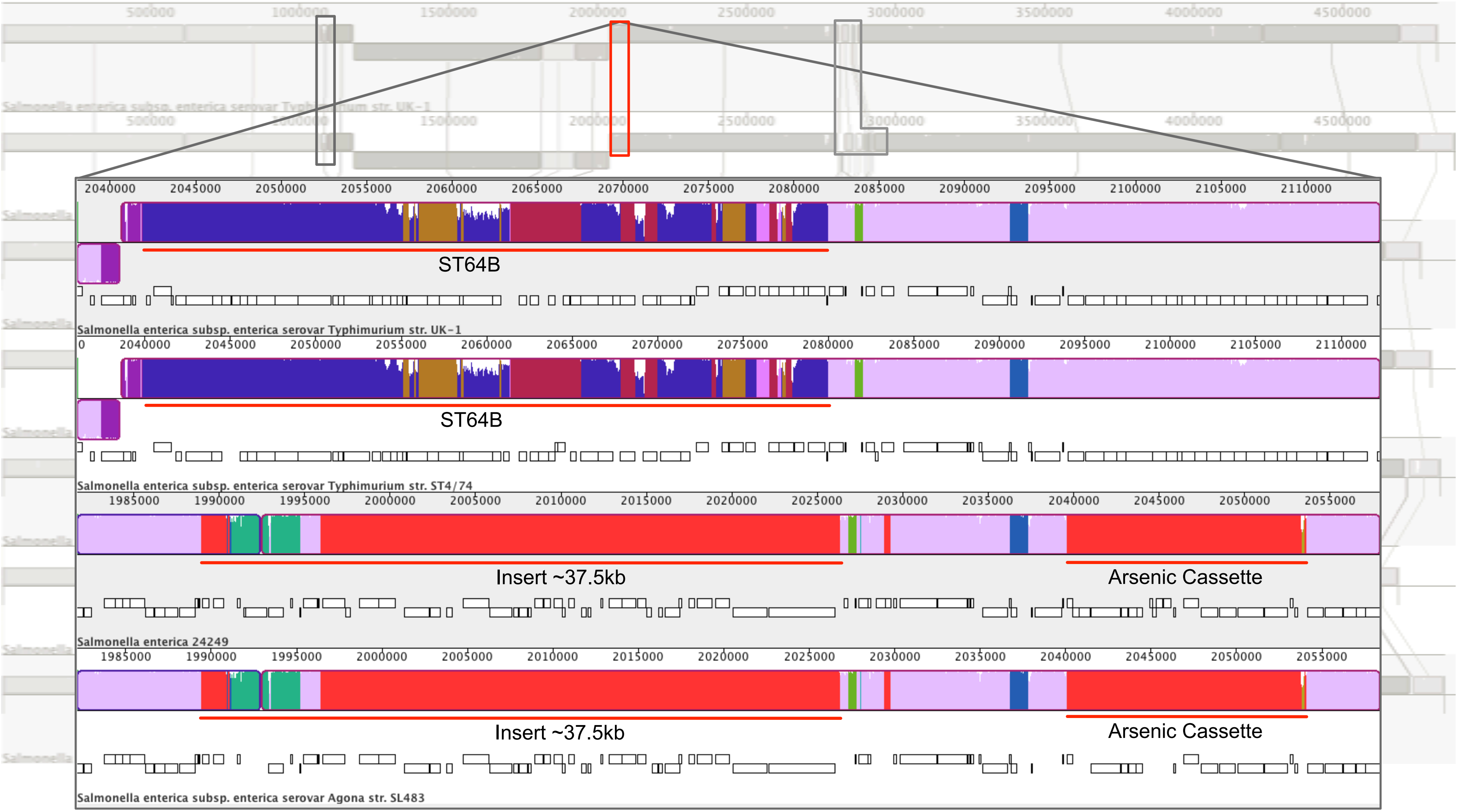
- Three main areas of difference between *S.* Typhimurium ST4/74 and *S.* Agona SA24249.
- Mainly phage regions.

Multiple Genome Aligment of *S.* Agona SA24249 with MAUVE



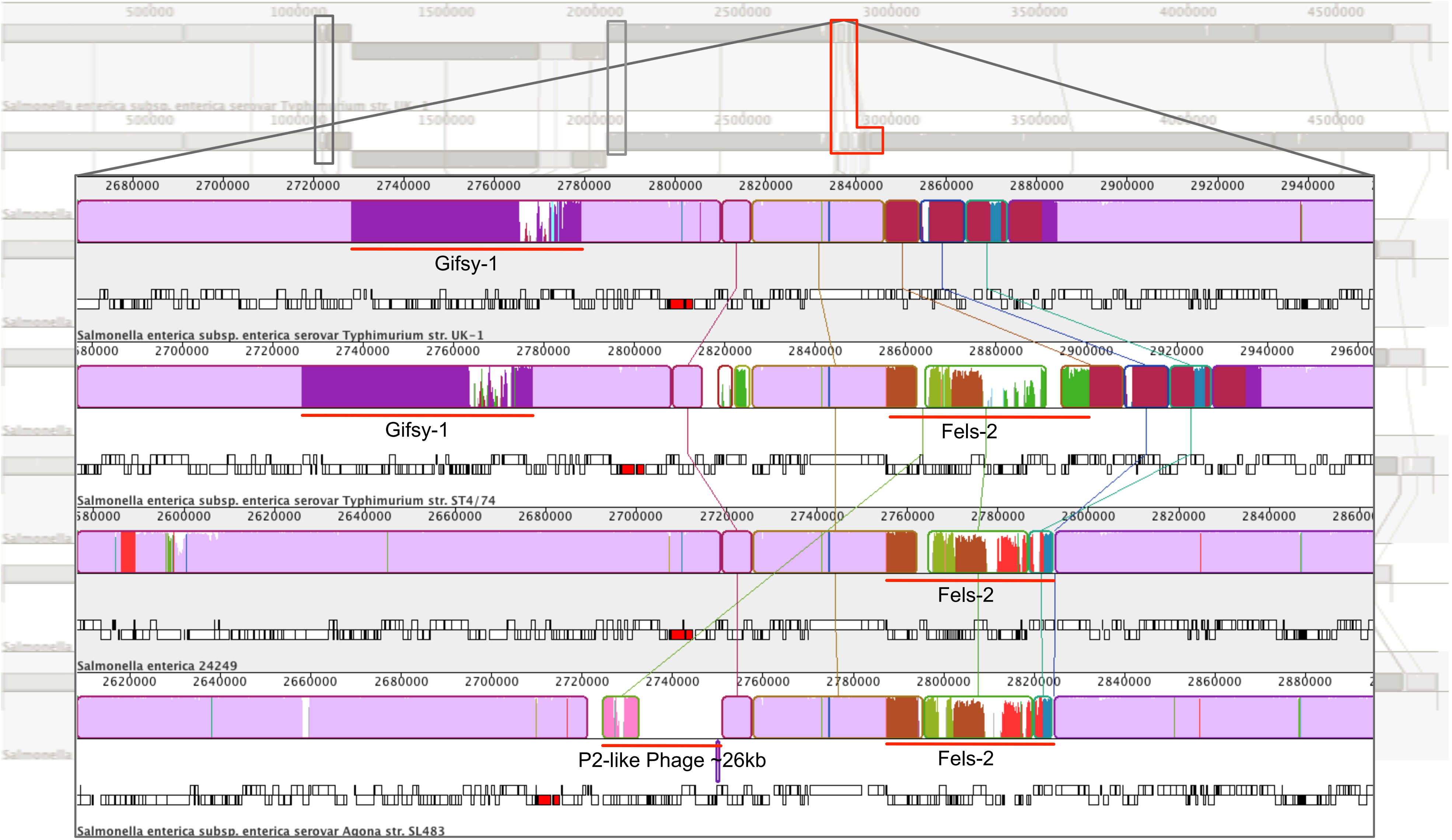
- T6SS effector protein identical to one in *S.* Infantis.
- T6SS protein IcmF similar to *S.* Dublin/Gallinarum

Multiple Genome Aligment of *S.* Agona SA24249 with MAUVE



- *Salmonella* phage ST64B encodes a member of the SseK/NleB effector family (Brown *et al*. (2011) PLoS ONE).
- Arsenic resistance cassette ubiquitous in *S.* Agona sequenced to date as well as other atypical serovars e.g. Newport, Montevideo, St. Paul, etc.

Multiple Genome Aligment of *S.* Agona SA24249 with MAUVE



- Gifsy-1 prophage not present in the *S*. Agona, novel Fels-2-like prophage and a P2-like *S.* Agona prophage in SL483 not present in SA24249.

S. Agona SA24249 SPI-2 Pseudogene

- In *S.* Agona SA24249 gene *sscB*, is a pseudogene.
- SscB acts as the chaperone for SseF, an effector for the *Salmonella* pathogenicity island 2 (SPI-2).
- *sscB* gene is required for efficient *Salmonella* replication in macrophages (Dai S & Zhou D. J Bacteriol. 2004).

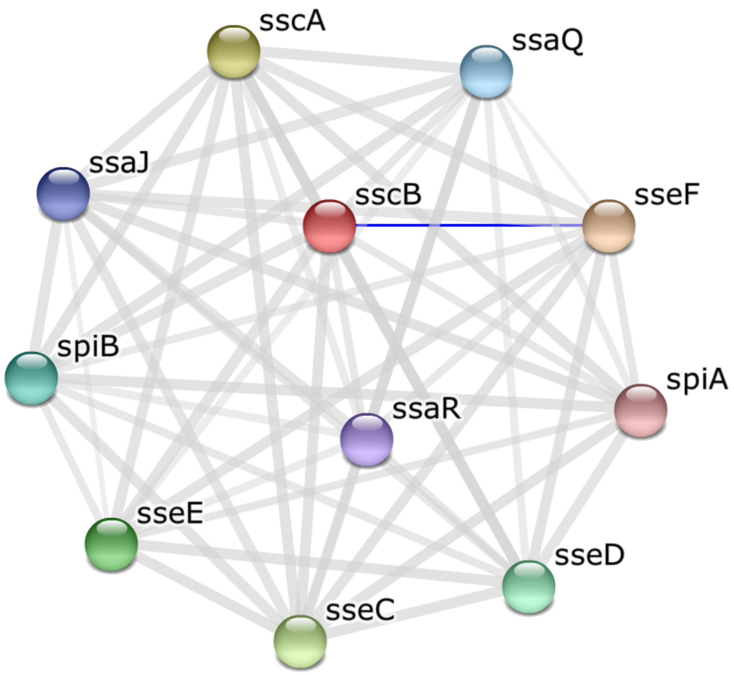
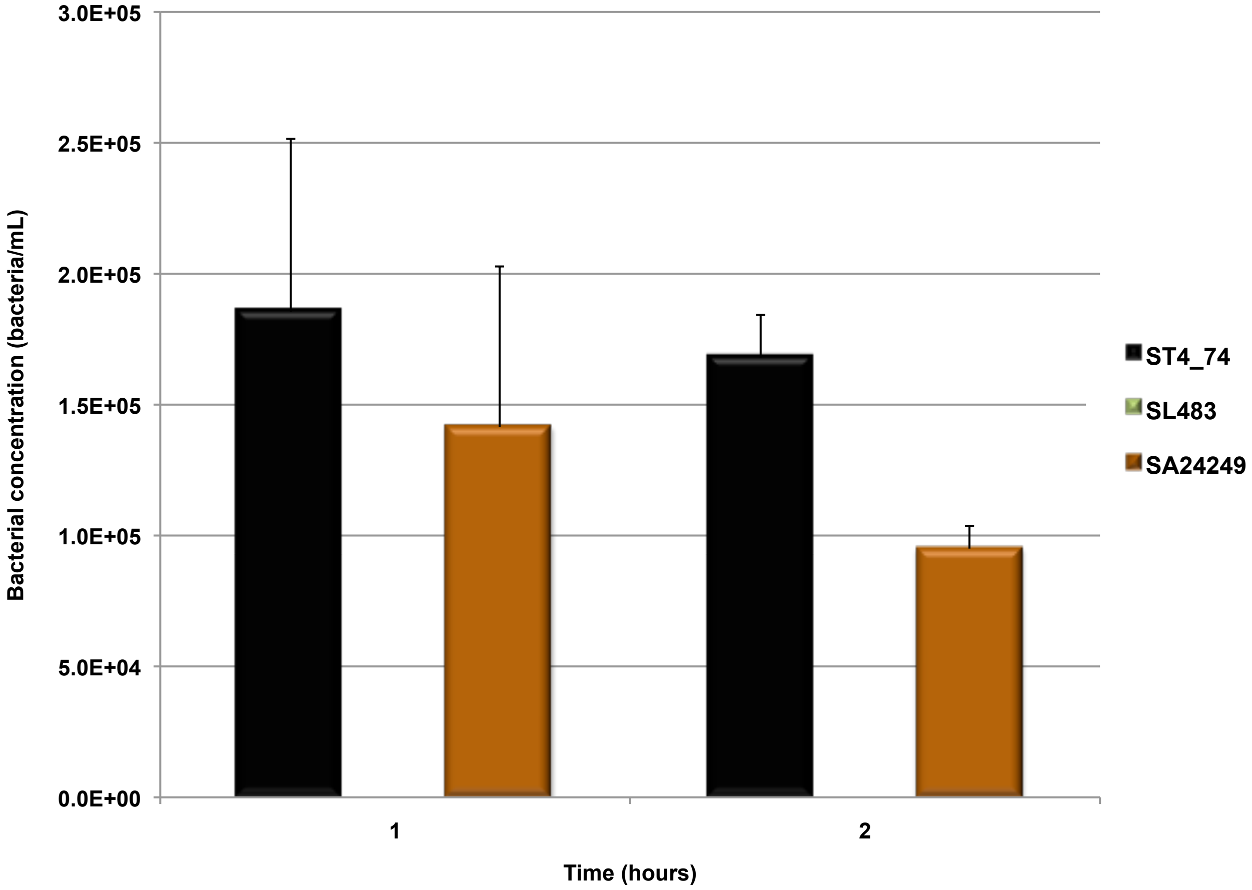


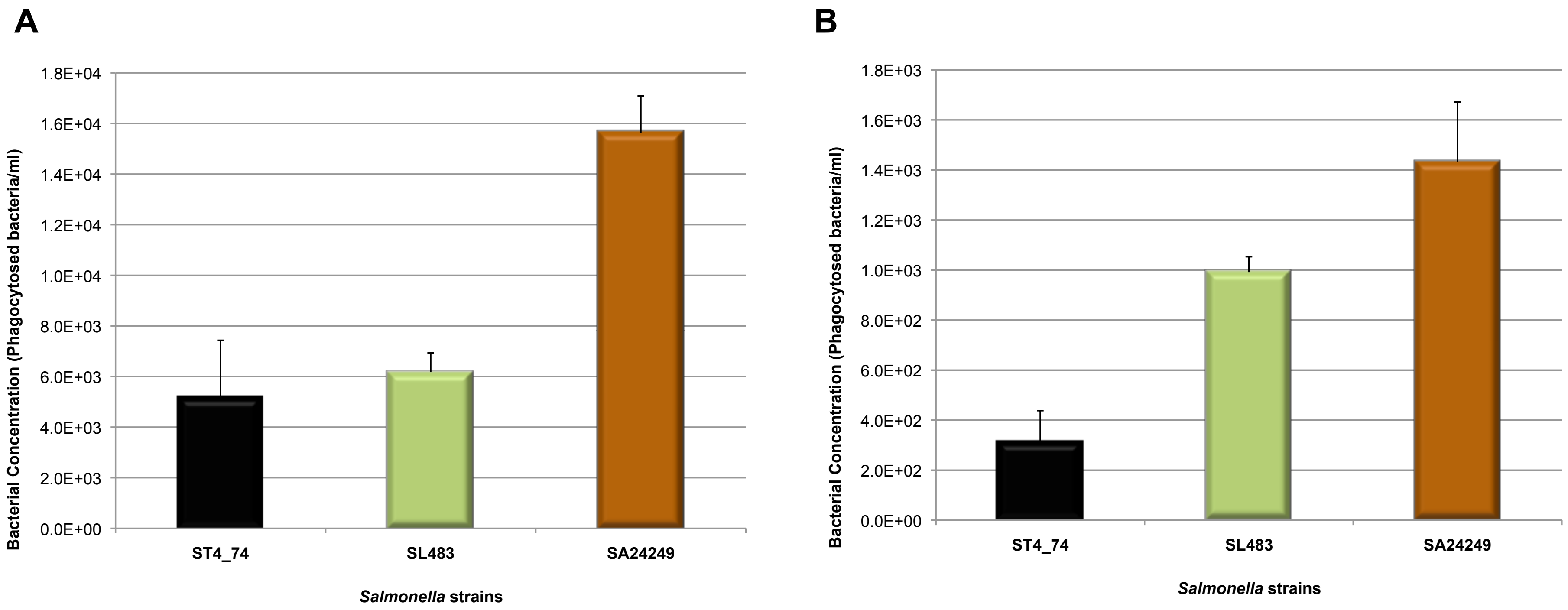
Image from STRING 9.05

Survival in Acidic Conditions



- *S.* Agona strain SL483 showed no survival after 1h of exposure to pH 2.5.
- *S.* Agona strain SA24249 showed good tolerance to pH 2.5 when compared with *S.* Typhimurium ST4_74.
- SL483 is an *rpoS* mutant with an IS10 insertion, while the *rpoS* region in SA24249 is identical to S. Typhimurium except for a silent mutation at amino acid 158.

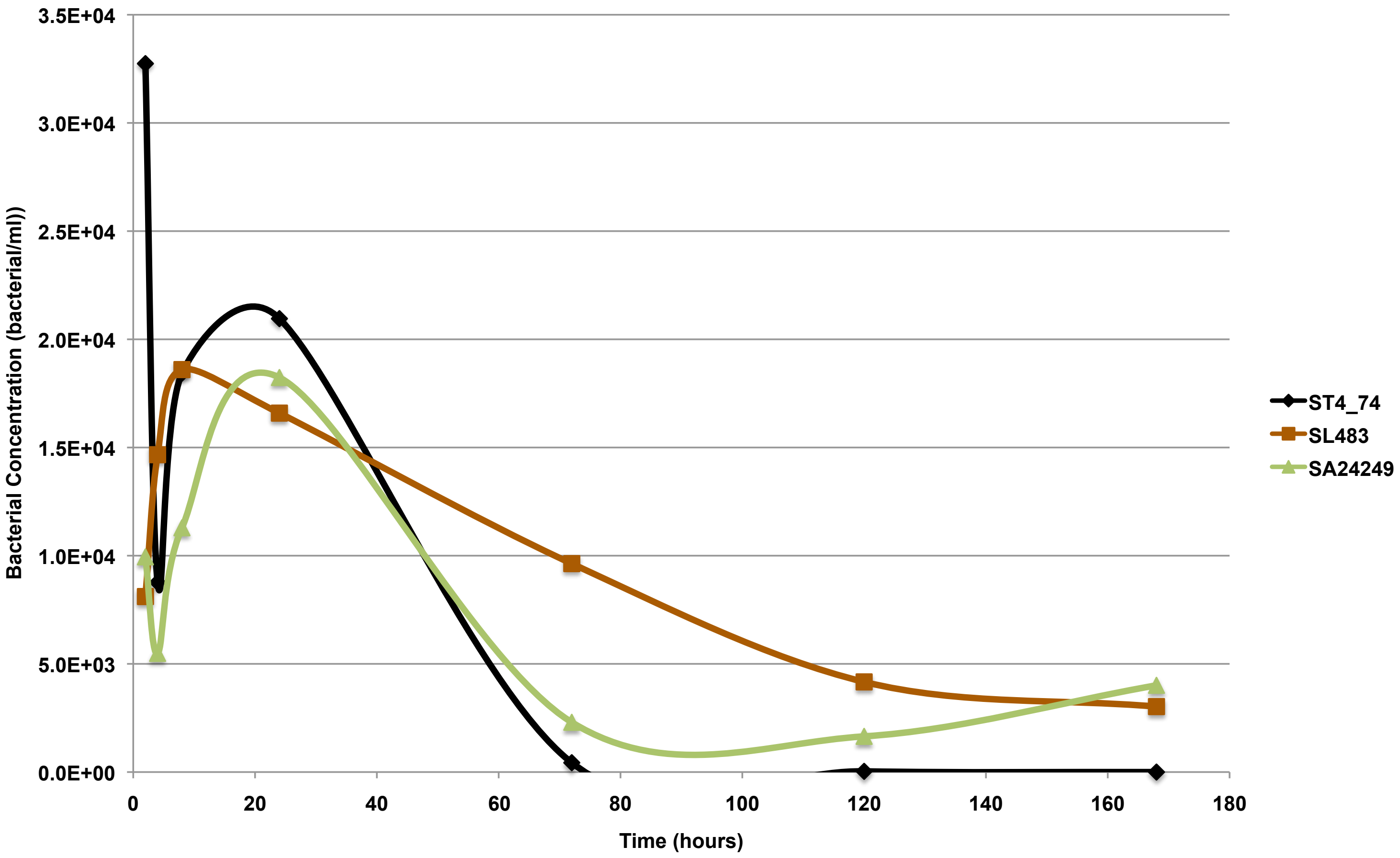
Ex-vivo Studies - Adherence and Invasion of Human epithelial cells



- *S.* Agona strain SA24249 showed an increased ability to adhere (A) and invade (B) human epithelial Caco-2 cells when compared with *S.* Typhimurium ST4_74 and *S*. Agona SL483 after 2h.

Adherence and Invasion ability of different strains of *Salmonella* Agona. These assays were performed in Caco-2 cells. *Salmonella* Typhimurium ST4_74 was used as a reference. All the assays were conducted in triplicate.

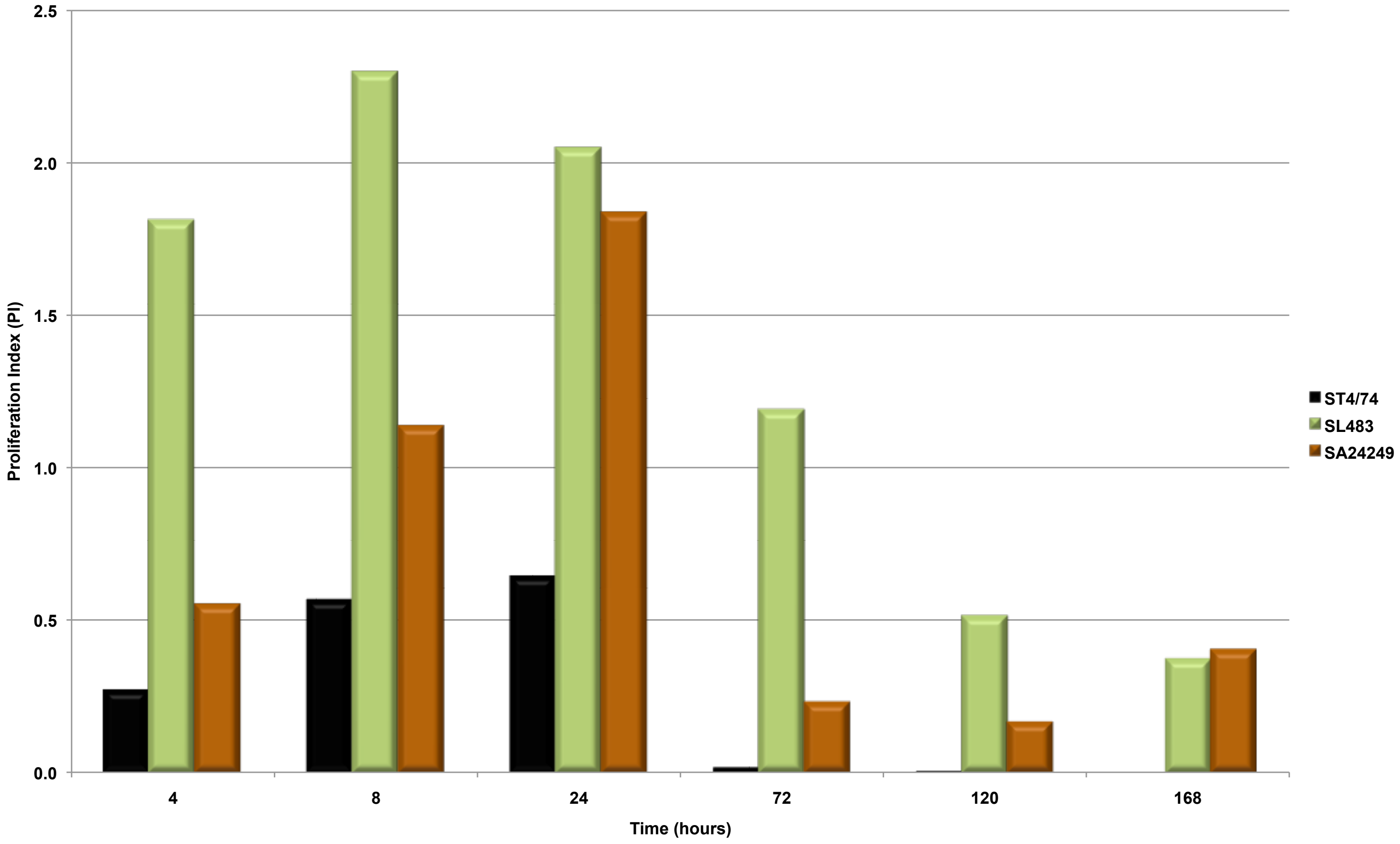
Ex-vivo Studies - Survival in human macrophage



- In the macrophage survival assays all 3 *Salmonella* strains showed similar survival after 24h.
- By extending the assay to 7 days *S.* Agona SL483 and SA24249 exhibited an ability to survive and persist inside the macrophages, while *S.* Typhimurium ST47_4 was cleared between 72h and 120h PI.

***Salmonella* infection of human macrophages.** The human macrophage cell line THP-1 was used for the infection assays. All the assays were conducted in triplicate.

Ex-vivo Studies - Survival in human macrophage



- Both *S.* Agona strains were able to proliferate significantly better than *S.* Typhimurium ST4_74 in the human macrophage THP-1.
- *S.* Typhimurium ST4_74 was cleared between 72h and 120h PI.
- After 10 days PI, both *S.* Agona strains SL483 and SA24249 could be recovered.

***Salmonella* infection of human macrophages.** The human macrophage cell line THP-1 was used for the infection assays. All the assays were conducted in triplicate.

Conclusions and Future Perspectives

- Genomics is going to produce tens to hundreds of thousands of genomes in the coming years.
- Critical in allowing source tracking for surveillance and outbreak investigation. However, it is not the whole story.
- Understanding the links between the genetic make up of pathogens and their phenotype is critical to assessing the potential risks.
- Multi-omics approaches using transcriptomics, proteomics and metabolomics are being used to shed new light on
- Help to provide insights into their evolution, biology, and ecological fitness.
- These studies will also aid in elucidating the mechanisms employed by pathogens as they adapt to the variety of conditions encountered throughout their life cycle, from the food-processing environment to *in vivo* during infection.
- Genomics will aid in the development of novel preventative and control strategies, which in turn will ultimately lead to a safer food supply.

Acknowledgements

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