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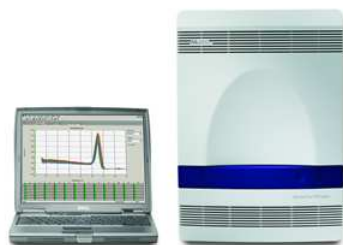
# Implementing whole genome sequencing within a Reference Laboratory – early experiences for *Salmonella* typing



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## Current Typing Methods for *Salmonella*

### Subspeciation



**Real time TaqMan® PCR assays**  
- target three different genes



**OmniLog® ID System (Biolog)**  
- phenotypic microarray



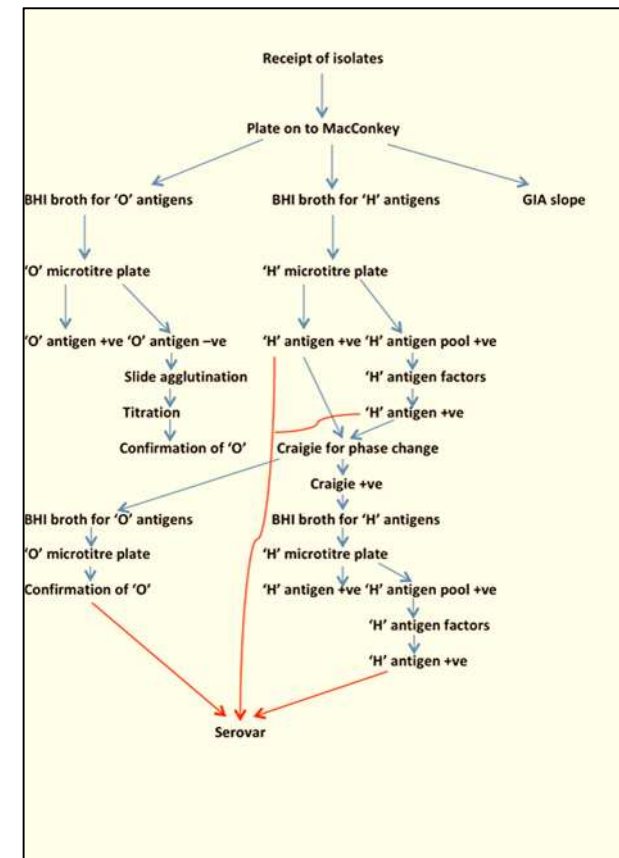
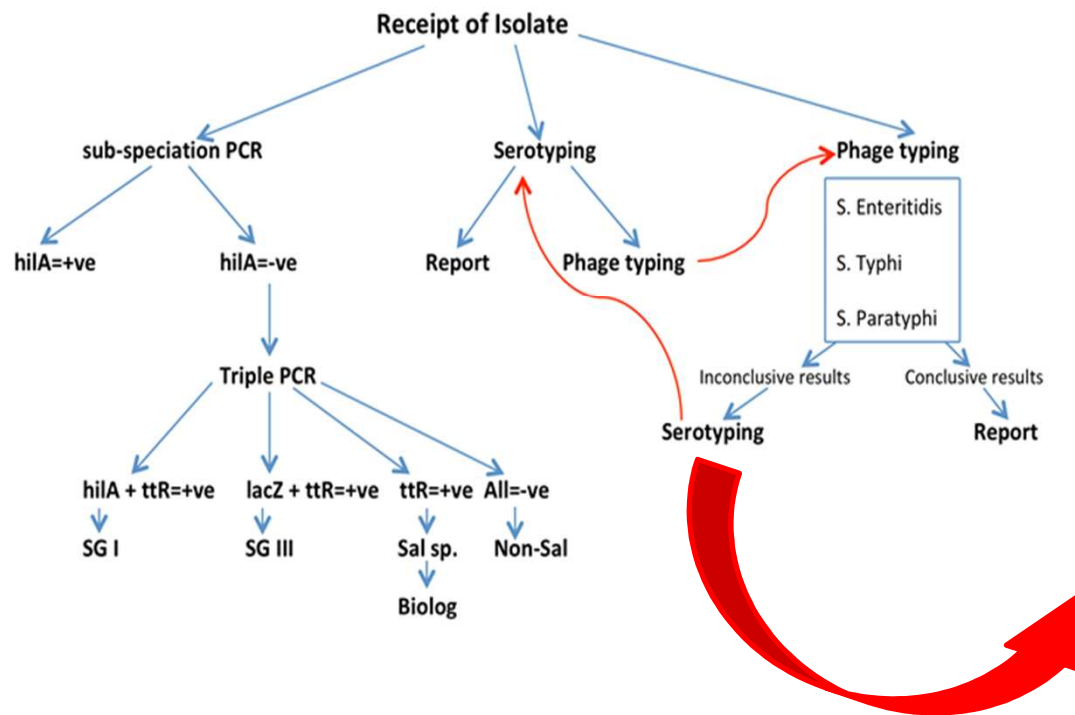
### Serotyping

**Agglutination with specific antisera  
against LPS & flagella (O & H antigens)**

- Slide agglutination
- Microtitre plates
- Dreyer's tubes



## Schematic representation of current workflow



- **46 'O' antigens and 114 'H' antigens**

## Serotyping is complex!!



## Implementation of WGS

- Priority organisms selected in 2013 - *Salmonella*
- PHE's WGS service opened 1<sup>st</sup> April 2014
- Practical implementation for routine services
  - reliable sample handling processes through the robotics
  - reproducible high-quality data
  - consistent linking of meta-data through the whole sample workflow
- ISO15189 Accreditation

# DNA Extraction Workflow in SRS



Samples arrive



Lysis &  
enzymatic  
steps



Run the  
QiaSymphony DNA extraction



Quantify  
DNA  
extracts

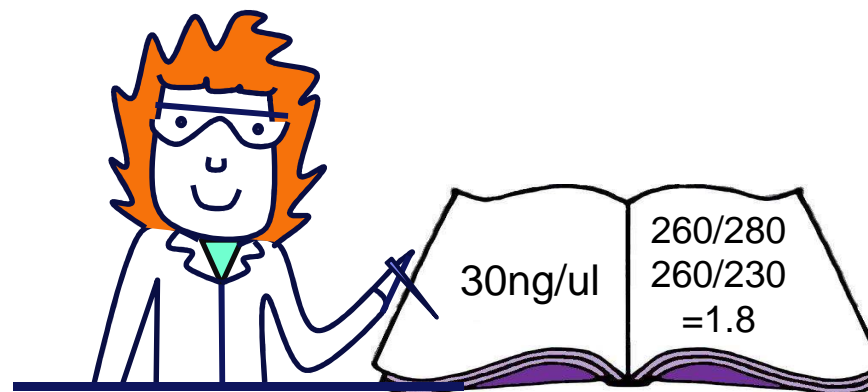
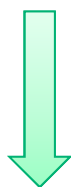


Transfer DNA to  
Sequencing  
Service



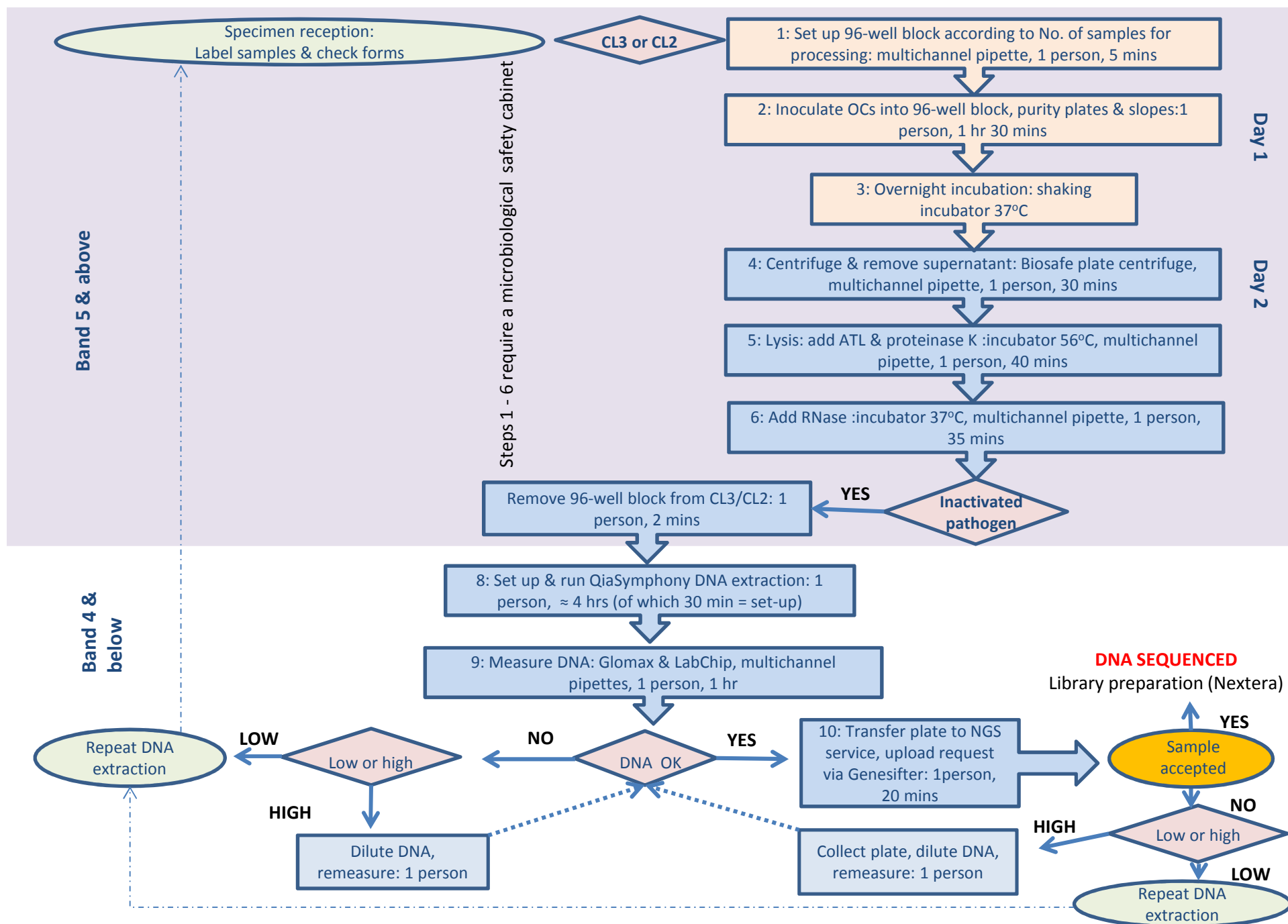
# WGS project – laboratory workflow

- Inoculate broth culture (overnight growth)
- Genomic Extraction - QiaSymphony
- Quantify & quality check DNA
  - LabChip/Glomax



**Trouble shooting!**

- Library preparation (Nextera)
- Sequence on HiSeq2500 (Rapid run)
- Automated Bioinformatics Analysis





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# PHE WGS Sequencing Service - the hardware

- Two HiSeq 2500 high-throughput sequencers
- Four MiSeq machines

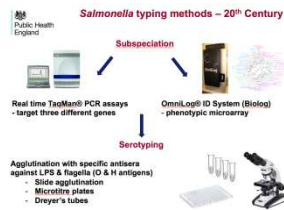


**NEW** HiSeq 2500

Capacity ~ 3,000 genomes per week



# Salmonella sample received

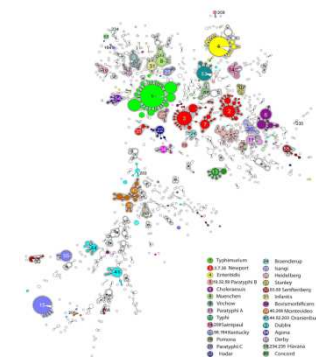


Reported to customer

# PHE Bioinformatics pipeline

- Quality trim
- KmerID to check purity
- Short read sequence typing to determine MLST

Sequencing of  
over 6000  
representative  
*Salmonella* –  
results compared  
with serotyping



– MLST/eBG-serotype



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# Infrastructure

Data storage warehouse  
Generators & coolers





# Nucleic Acid to Automated Report

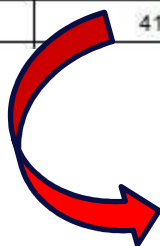
## Results for SRS : salmonella-typing

Submitter: SRS

Workflow: salmonella-typing

Date-Time of report: 13/09/2014-22:19.49

Sample	MLST ST	MLST profile	QC mean cons depth	QC max % non cons base	QC % coverage	QC min cons depth	predicted serotype
H14354083501-1	NOVEL_allele	191,22,*27,22,18,85,169	28.65	7.1	100	12	no ST-serotype
H14354083601-1	1541	197,187,10,234,8,65,22	37.86	5.3	100	15	Corvallis 1
H14354083701-1	592	189,70,68,132,175,9,172	26.79	9.4	100	12	Worthington 4
H14354083801-1	413	15,70,93,78,113,6,68	31.94	6.3	100	15	Mbandaka 15



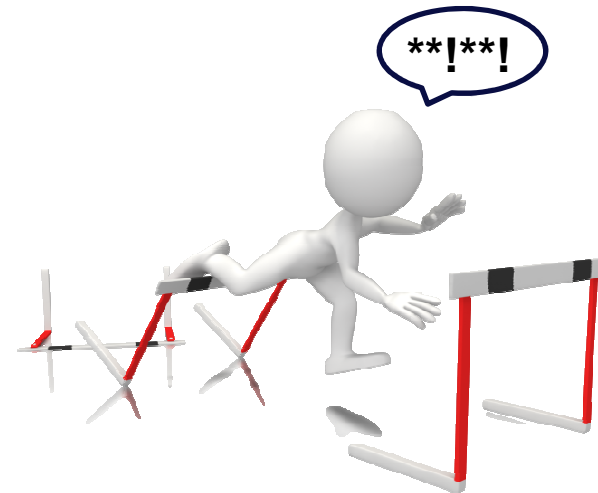
- ST 413 = *Salmonella* Mbandaka



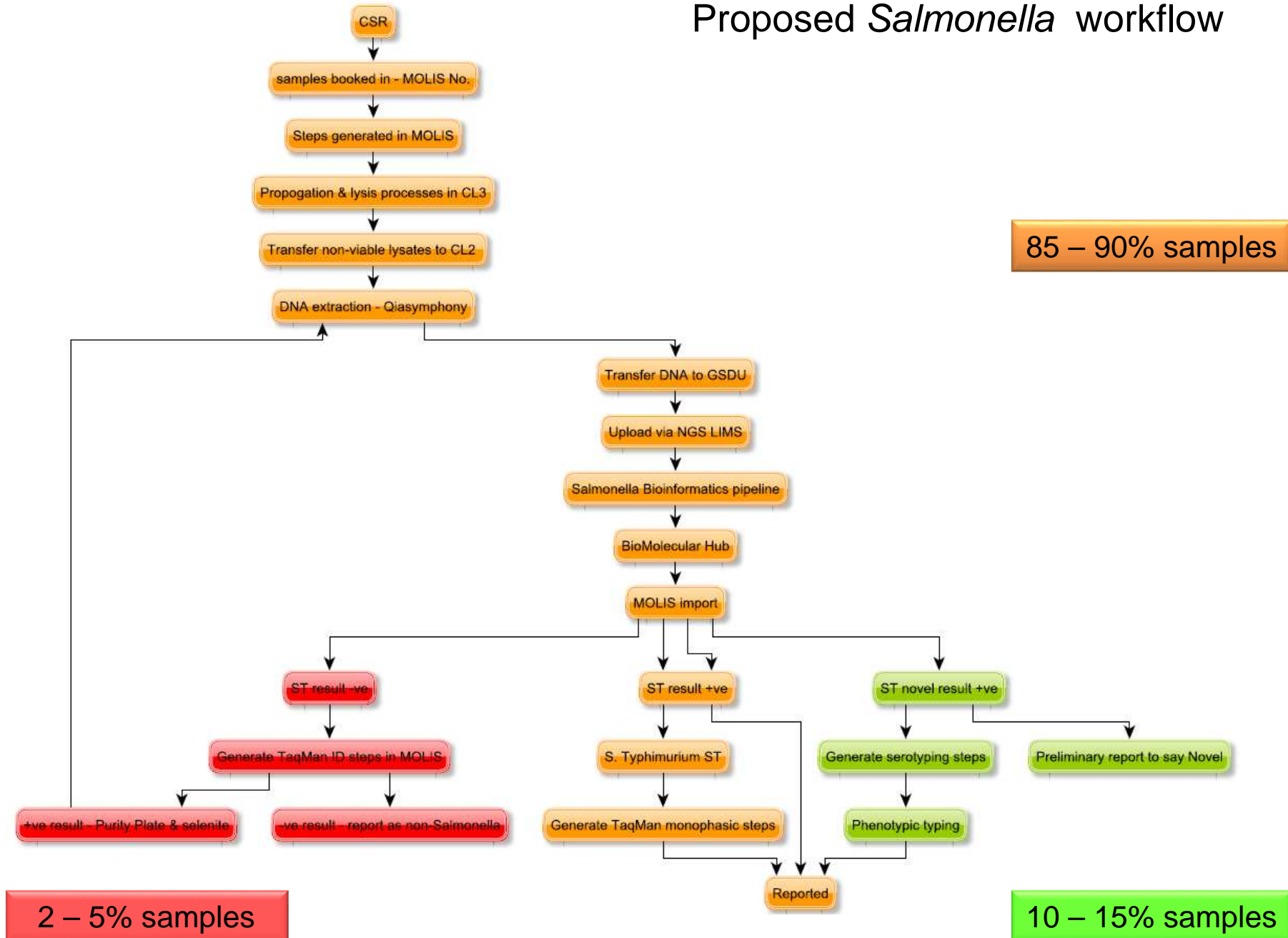
## Where do we go from here?

*Salmonella* are running in parallel for one year

- Opportunities:
  - Accuracy
  - Resolution
  - Communication
- Challenges:
  - Throughput
  - Quality/reproducibility
  - Communication
- still hurdles to overcome



## Proposed *Salmonella* workflow





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Watch this space!

Thank you for listening