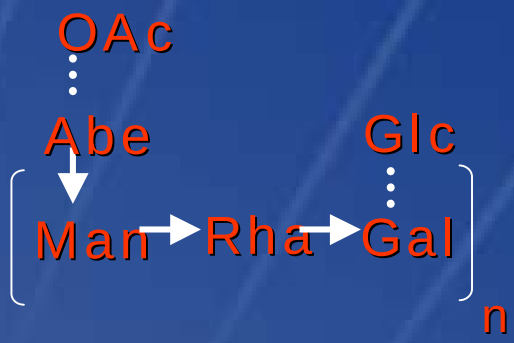
# Development and evaluation of PCR-based diagnostics for identification of *Salmonella* O antigens based on the *rfb* locus

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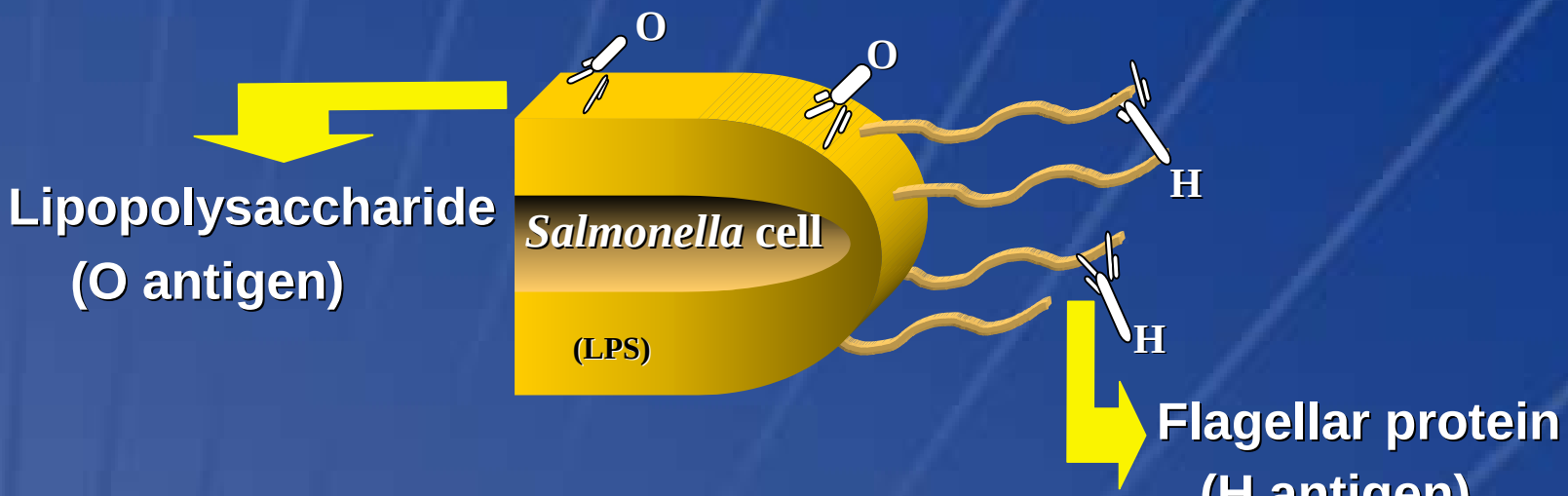
# What is serotyping ?

- Subtyping based on antigenic reactivity of:




- Designated by names or formulas

(subspecies O: Phase1 H : Phase 2 H)

*S.* Typhimurium: I 1,4,[5],12: i: 1,2

O- antigens H-antigens

2501 serotypes

# Why is it important ?

- Serotyping is very useful for the epidemiologic classification of strains
- It is the basis of the National *Salmonella* Surveillance System

## Technical issues

- Reagents required are expensive and time consuming to produce and quality control
  - 250 O and H grouping, typing and single factor reagents needed to identify all serotypes
  - 350 stock strains and antigens used to evaluate and quality control antisera

# Main Objective

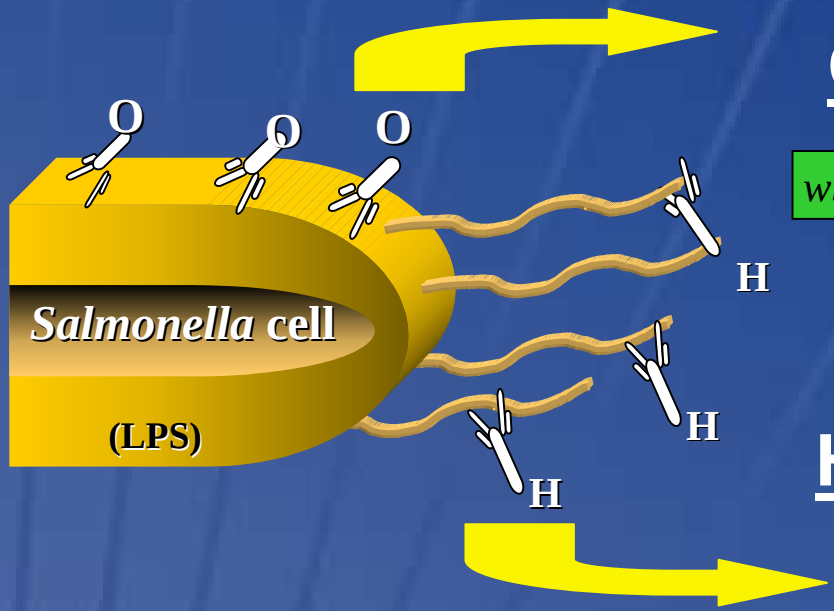
- Develop a DNA-based system that can replace traditional methods

"Molecular Serotyping"

- The new system should correlate with the current serotyping scheme so that results from the two systems can be compared

# Molecular serotyping goals

- Characterize and/or sequence genes required for biosynthesis of:



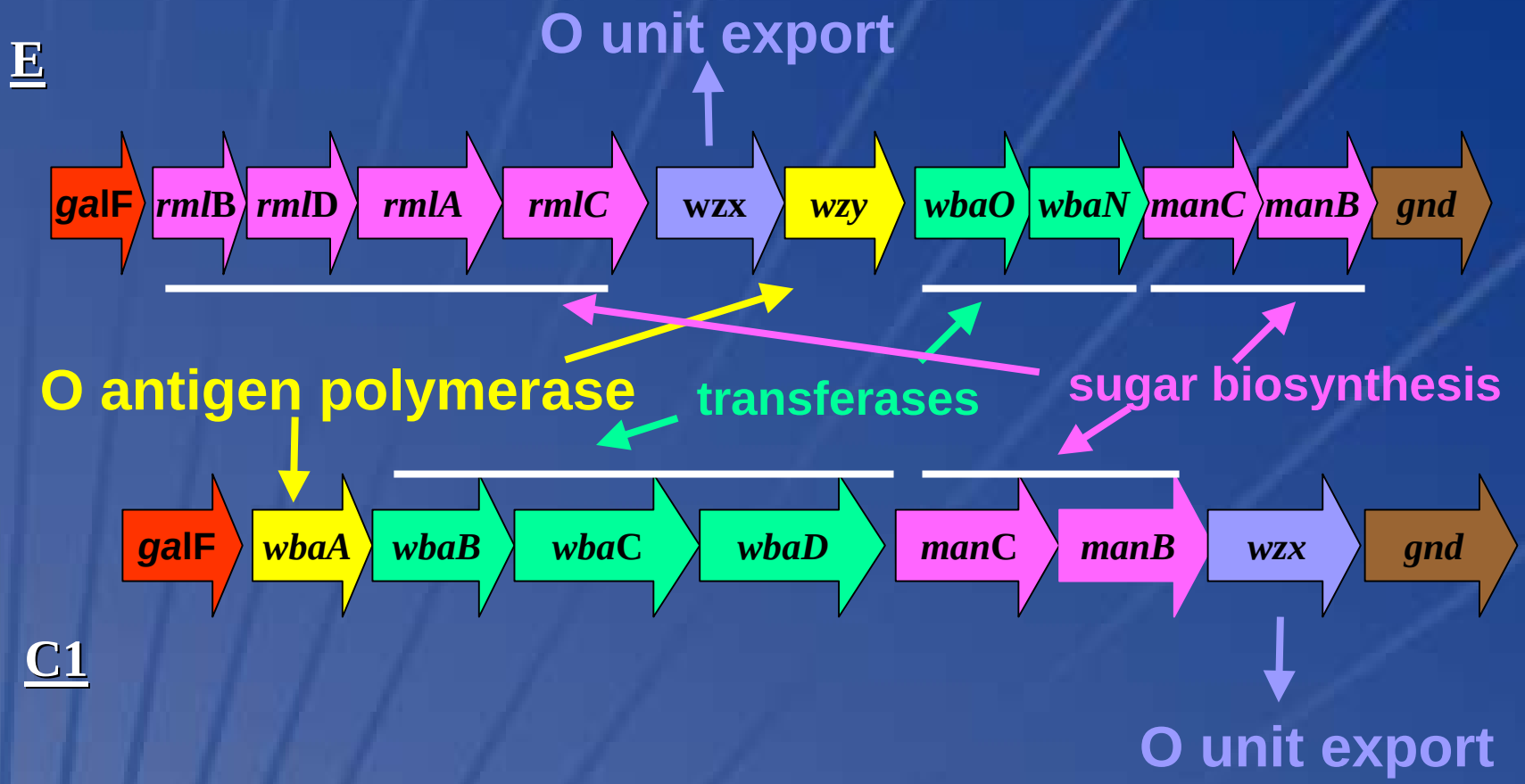

## O antigen (*rfb* gene cluster)


## H antigen (flagellin genes)


- Identify antigen-specific sequences
- Develop the target sequence into a molecular probe using appropriate technology

# *Salmonella* O antigen gene clusters

- O antigens are encoded primarily by the *rfb* region



# Background

- *rfb* gene cluster
  - has been partially/fully sequenced for 9 serogroups ($A/D_A$, $B$, $B_B$, $C$, $C_2$, $D_1$, $D_2$, $D_3$, $E_1$ and O:54)
  - no specific PCR has yet been described for serogroups $C_1$, E or O:54

Luke et al (1998) - Selective amplification of sugar biosynthesis genes (*abe*, *prt*) to identify serogroups

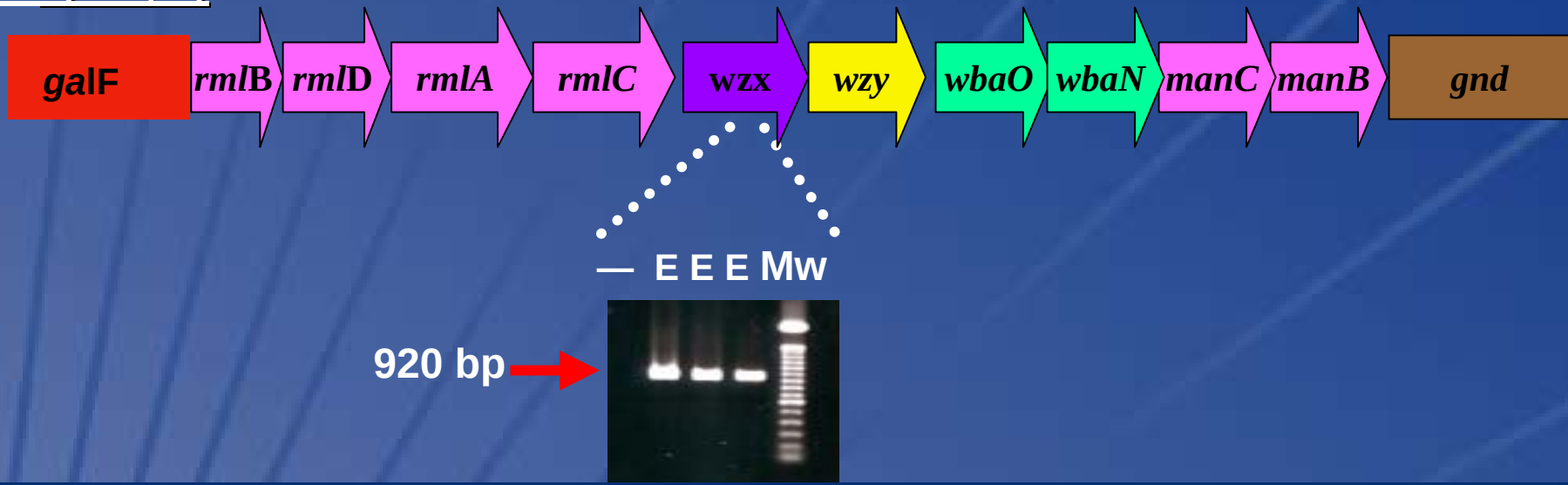
# AIMS

- Develop serogroup specific PCR's for those *rfb* gene clusters already sequenced
- Characterization of *rfb* gene clusters from additional serogroups

# Serogroup identification by PCR (1)

**O:6,7 (C1)**


**O:3,10 (E)**



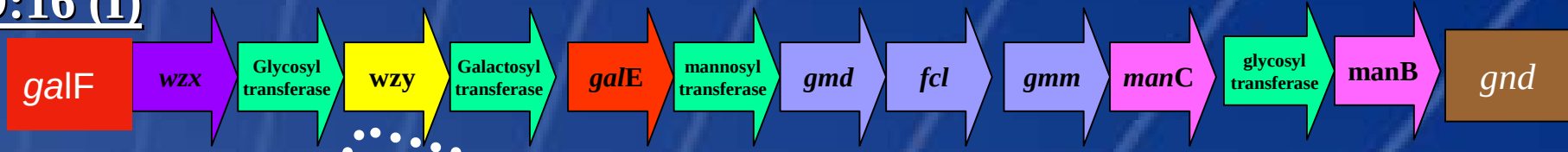
# DNA sequence analysis of *rfb* gene clusters

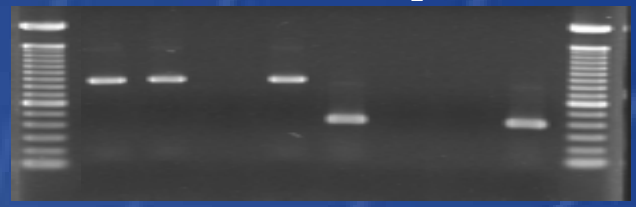
| Serogroup | Serotype | *rfb* size |
|---|---|---|
| O:6,14 (H) | *S.* Sundsvall | 8.436 kb |
| O:16 (I) | *S.* Gaminara | 12.891 kb |
| O:17 (J) | *S.* Jangwani | 9.195 kb |
| O:18 (K) | *S.* Cerro | 9.135 kb |
| O:35 (O) | *S.* Ealing | 13.642 kb |
| O:38 (P) | *S.* Roan | 6.013 kb |
| O:41 (S) | *S.* Ipswich | 9.578 kb |
| O:48 (Y) | *S.* Marina | 13.079 kb |

Characterization of *rfb* gene clusters from additional serogroups for which we currently have no sequence information

# Serogroup identification by PCR (2)

## O:16 (I)

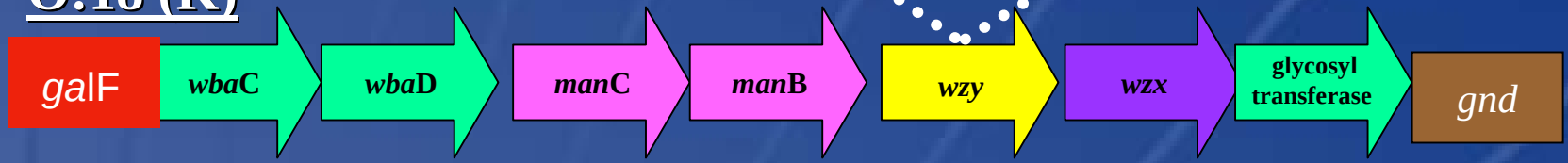


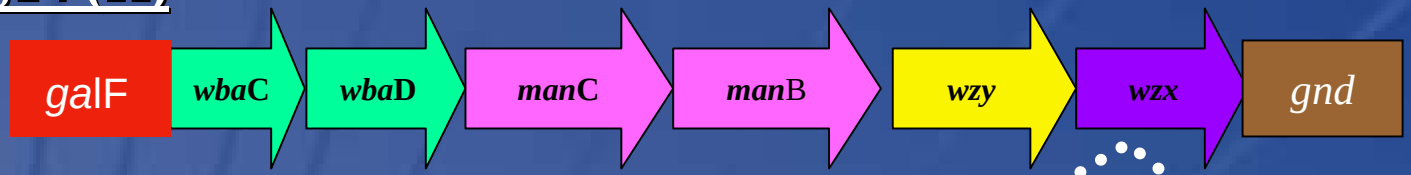

810 bp

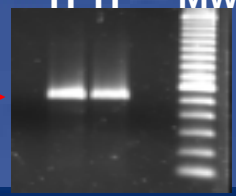
380 bp

## O:18 (K)




## O:6,14 (H)




501 bp

| Serogroup | Serotype | Target | Primers evaluated against 46 serogroups (398 strains) |
|---|---|---|---|
| $C_1$ (6,7,14) | *S.* Montevideo | *wbaA* | y |
| E (3,10,15) | *S.* Anatum | *wzx* | y |
| O:54 | *S.* Borreze | *wbbF* | y |
| H (0:6,7,24,25) | *S.* Sundsvall | *wzx* | y |
| I (O:16) | *S.* Gaminara | *wzy* | y |
| J (O:17) | *S.* Jangwani | *wzy* | y |
| K (O:18) | *S.* Cerro | *wzy* | y |
| O (O:35) | *S.* Ealing | *wzx* | y |
| P (O:38) | *S.* Roan | *wzy* | y |
| S (O:41) | *S.* Ipswich | *wzx* | y |
| Y (O:48) | *S.* Marina | *wzx* | y |
| A/D | *S.* Paratyphi A / *S.* Typhi | *prt* | y |
| B | *S.* Typimurium | *abe* | y |
| $C_2$ | *S.* Muenchen | *abe* | y |

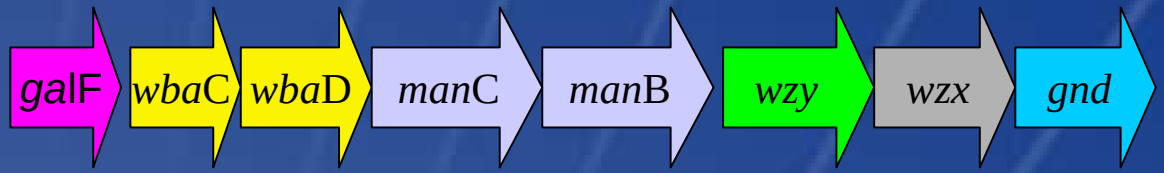
# Phase I objectives

- Molecular identification of top 100 serotypes
  - 98% of clinical isolates

| | | | |
|---|---|---|---|
| O:11 (F) | *S.* Abaetetuba | O:30 (N) | *S.* Urbana |
| O:13 (G) | *S.* Poona | O:40 (R) | *S.* Johannesburg |
| O:21 (L) | *S.* Minnesota | O:43 (U) | *S.* Houten |
| $O:28_{ab}$ (M) | *S.* Pomona | O:47 (V) | *S.* Bere |
| $O:28_{ac}$ (M) | *S.* Dakar | O:50 (Z) | *S.* Flint |

# Phase I objectives

## PCR identification of *Salmonella* O antigens

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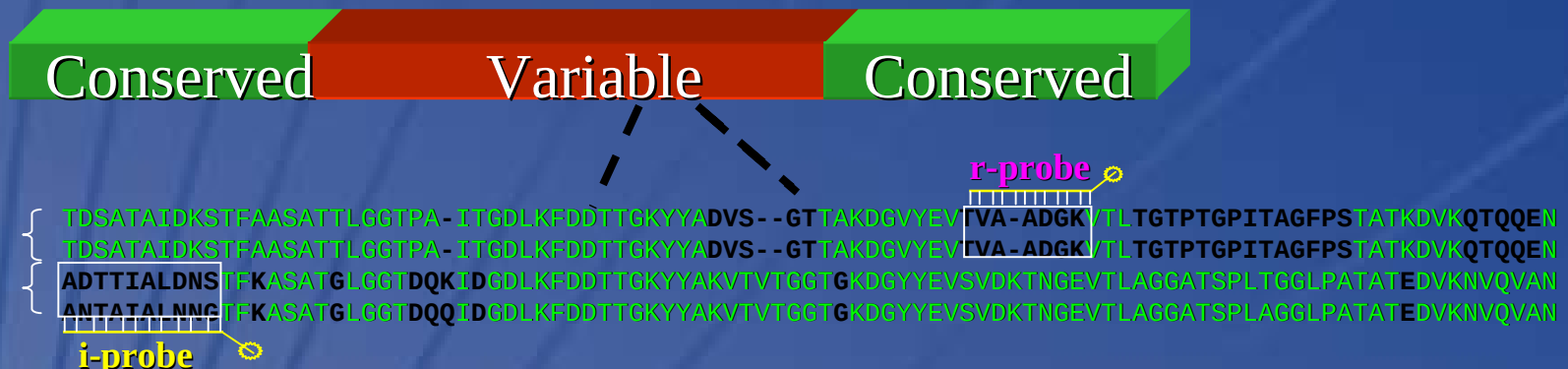
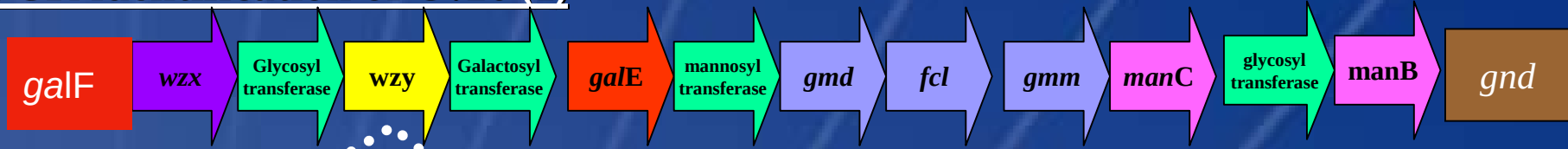
## EIA identification of *Salmonella* H antigens

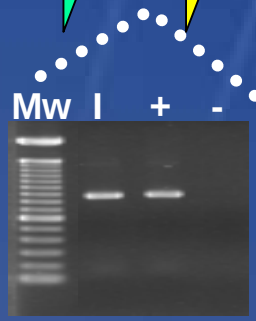


# Molecular serotyping

## S. Gaminara
## 16: d: 1,7

**PCR identification of O:16 (I)**

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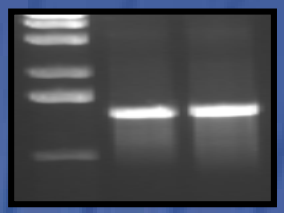
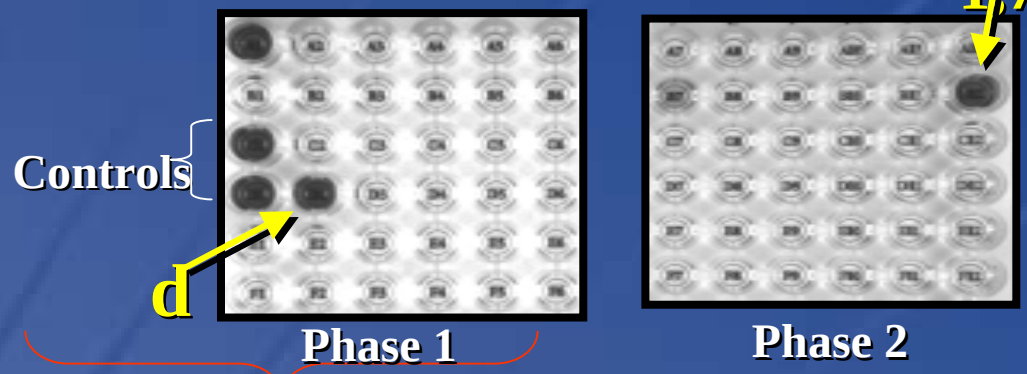
**EIA identification of H antigens d and 1,7**




**Result: 16: d: 1,7**

# Conclusions……so far

- Rapid identification of 15 *Salmonella* serogroups based on PCR amplification of specific gene targets within the *rfb* gene clusters
  - sensitive and specific
  - includes identification of the serotype in ‘rough’ isolates
  - attainable by non-specialized laboratories