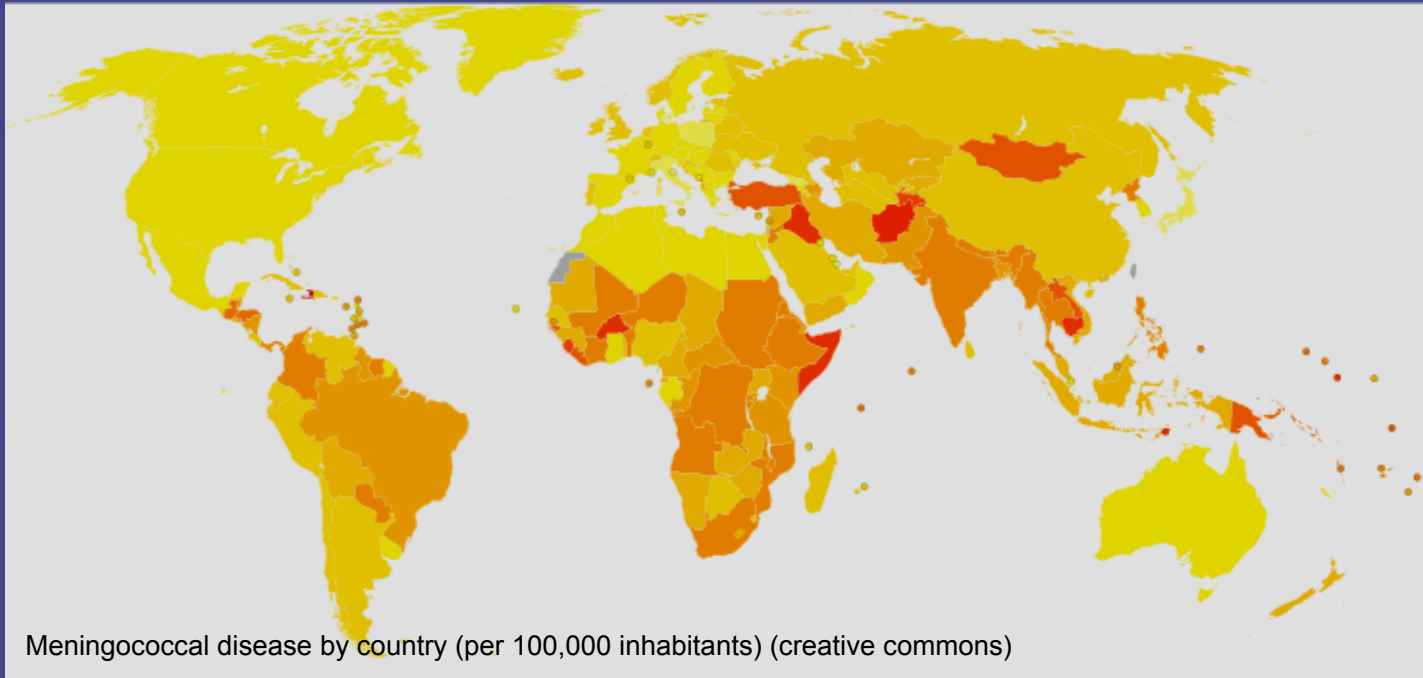


Household contact *Neisseria meningitidis* disease-carriage pairs: a tool to dissect virulence

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Specialist Science Solutions

Manaaki Tangata Taiao Hoki
protecting people and their environment through science

What do we do?

We are the Meningococcal Reference Laboratory for New Zealand.

We have an extensive isolate collection.

We use clinical, low passage meningococcal isolates to try to understand why some isolates cause disease and some do not.

Neisseria meningitidis: Pathogen or Commensal?

Carriage

- Prevalent in nasopharynx of general population (10-25%)
- Higher rates of carriage in crowded living
- Carriage may result in immunity
- Carriage strains highly diverse

Disease

- Small group of hypervirulent strains
- Causes endemic and epidemic disease

**Bacterial,
environmental
and host
factors all
contribute to
disease
development**

Neisseria meningitidis: Understanding Carriage vs. Disease



What bacterial factors contribute to disease development?

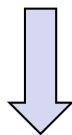
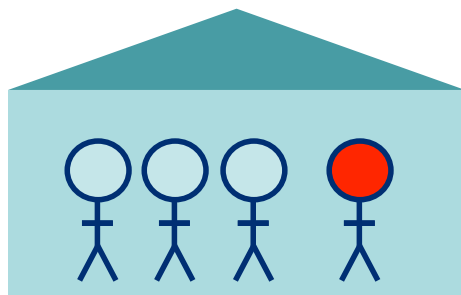


Problem: Comparing large number of meningococcal genomes has not yielded strictly disease-associated virulence factors.



Solution: Study closely related isolates.

Isolates from household contacts



Meningococcal isolate
from patients and rest
of the household

- 954 household contacts of 160 patients
- Isolates from 52 households were typed
- 28 households had healthy individuals carrying meningococcal isolates indistinguishable from the invasive meningococcus isolated from the patient.

Household Group	Disease Isolate	Strain type	ST	Clonal complex	Anatomical Site
1	96/294	B:4:P1.4	42	41/44	Blood
	CM149	B:4:P1.4	42	41/44	Throat
2	97/032	W:nt:P1.6	22	22	Blood
	CM101	W:nt:P1.6	22	22	Throat
	CM102	W:nt:P1.6	22	22	Throat
3	97/052	C:2b:P1.5,2	66	8	Blood
	CM111	C:2b:P1.5,2	66	8	Throat
	CM112	C:2b:P1.5,2	66	8	Throat
4	97/112	W:nt:P1.6	22	22	CSF
	CM209	W:nt:P1.6	22	22	Throat
5	97/165	C:2a:P1.2	4025	11	Blood
	CM131	C:2a:P1.2	4025	11	Throat
6	97/192	B:4:P1.4	154	41/44	CSF
	CM238	B:4:P1.4	154	41/44	Throat
	CM239	B:4:P1.4	154	41/44	Throat
	CM240	B:4:P1.4	154	41/44	Throat

The 97/192 Household

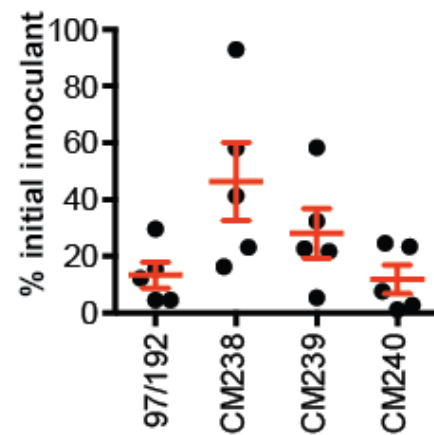
A MLST analysis of the 97/192 household

Isolates	Strain type	ST	Clonal Complex	Anatomical Site
97/192	B:4:P1.4	154	41/44	CSF
CM238	B:4:P1.4	154	41/44	Throat
CM239	B:4:P1.4	154	41/44	Throat
CM240	B:4:P1.4	154	41/44	Throat

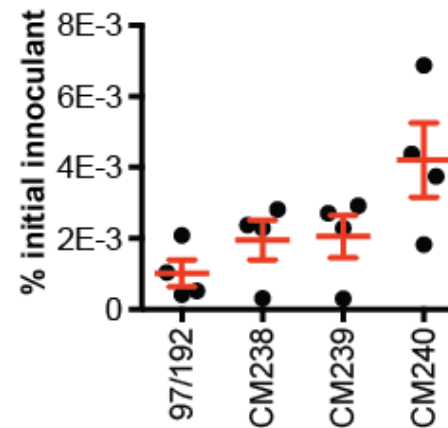
"97/" = disease associated isolate from patient

"CM" = carriage associated from healthy household members

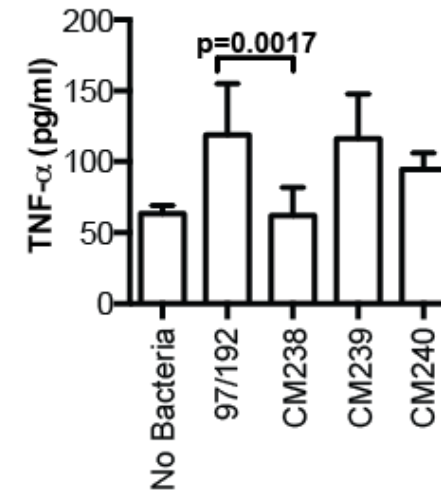
B Adhesion to 16HBE

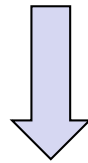
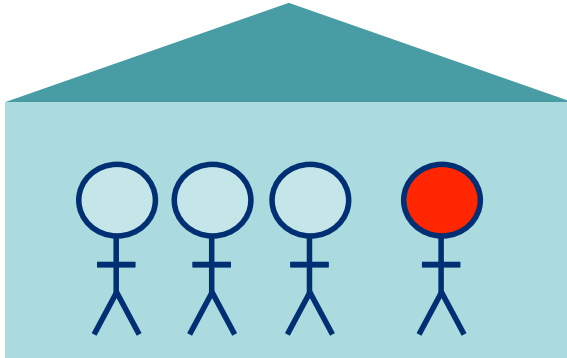


C Invasion into 16HBE

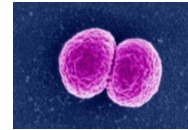


D TNF-α secretion





Meningococcal isolates
from patients and rest of the
household with the same MLST



The plan

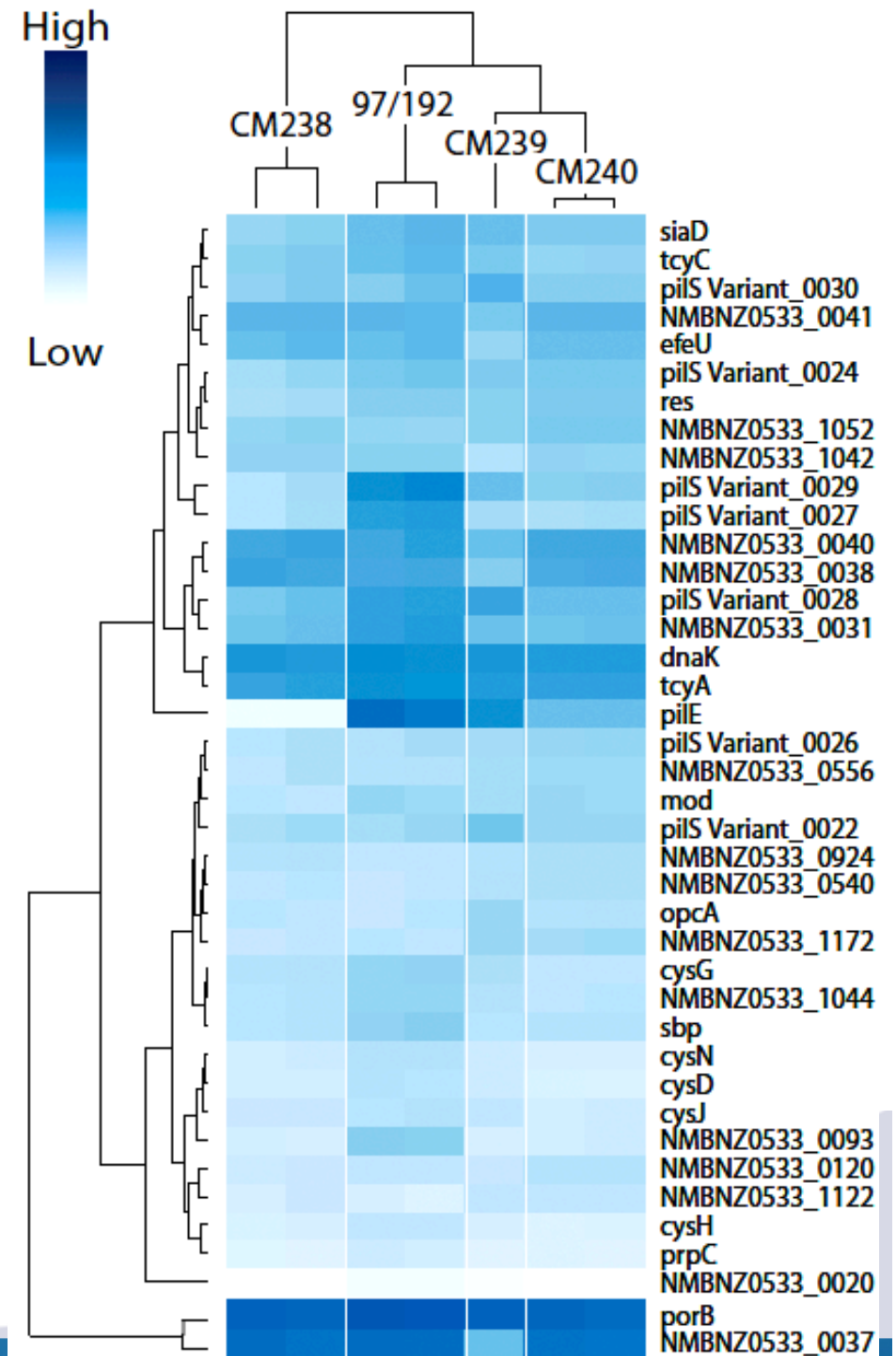
Transcriptome (RNA-seq)
analysis of bacteria in the
presence of epithelial cells

Whole genome shotgun
sequencing

Mutant analysis of candidate
genes

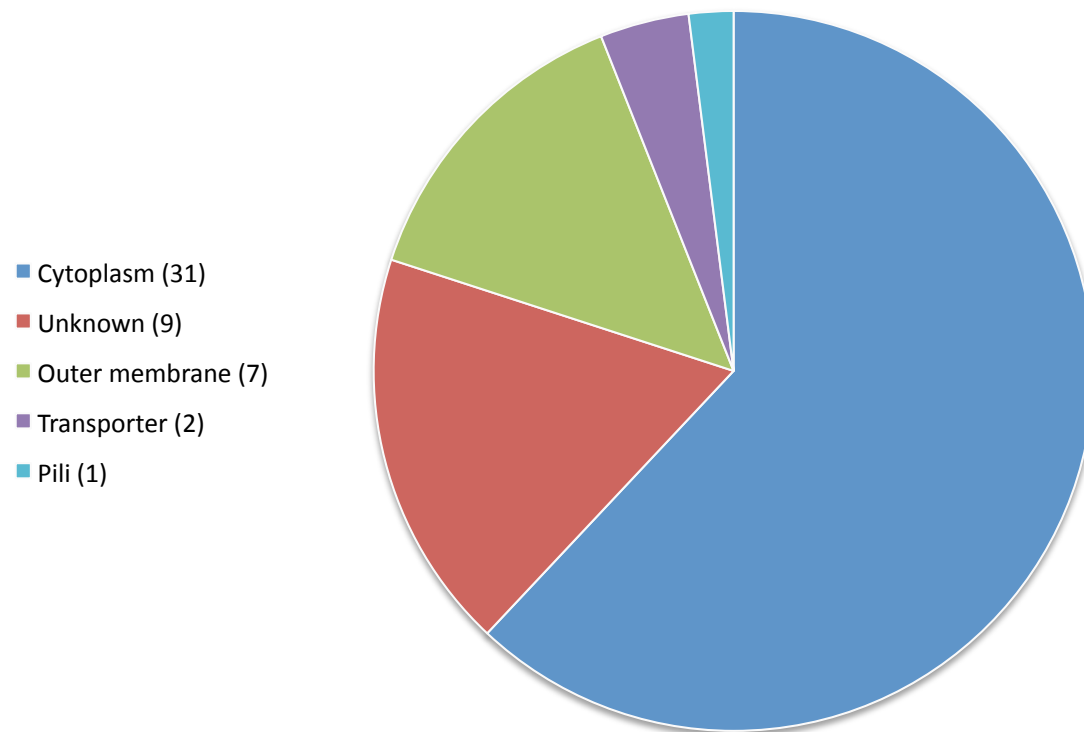
Differentially expressed transcripts between 97/192 and its carriage-associated isolates ($[\log_2] \geq 1$; adj $p < 0.05$)

- Not hundreds of transcripts
- Lower expression in carriage-associated isolates

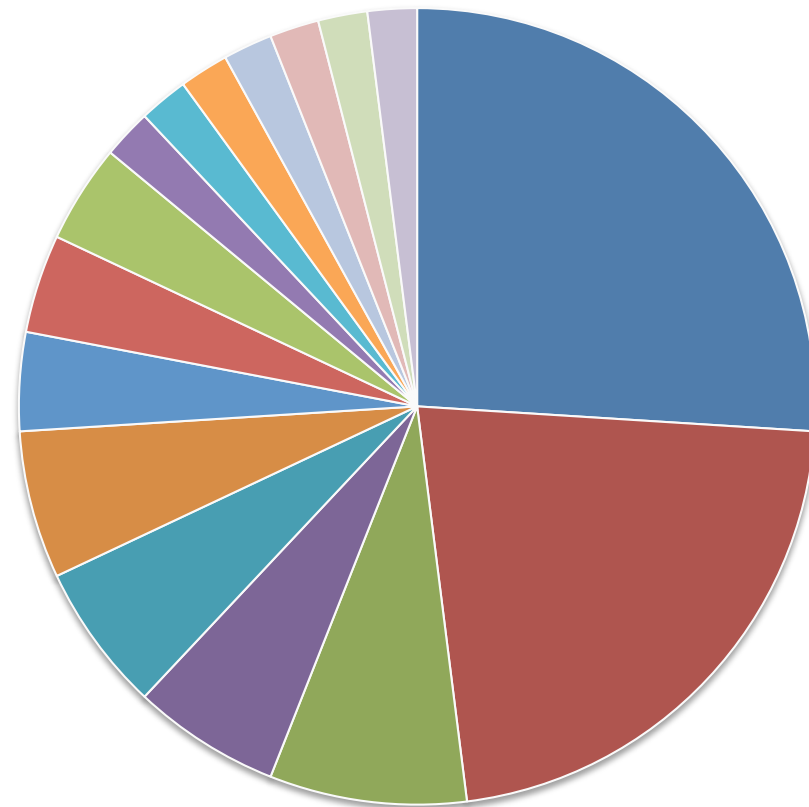
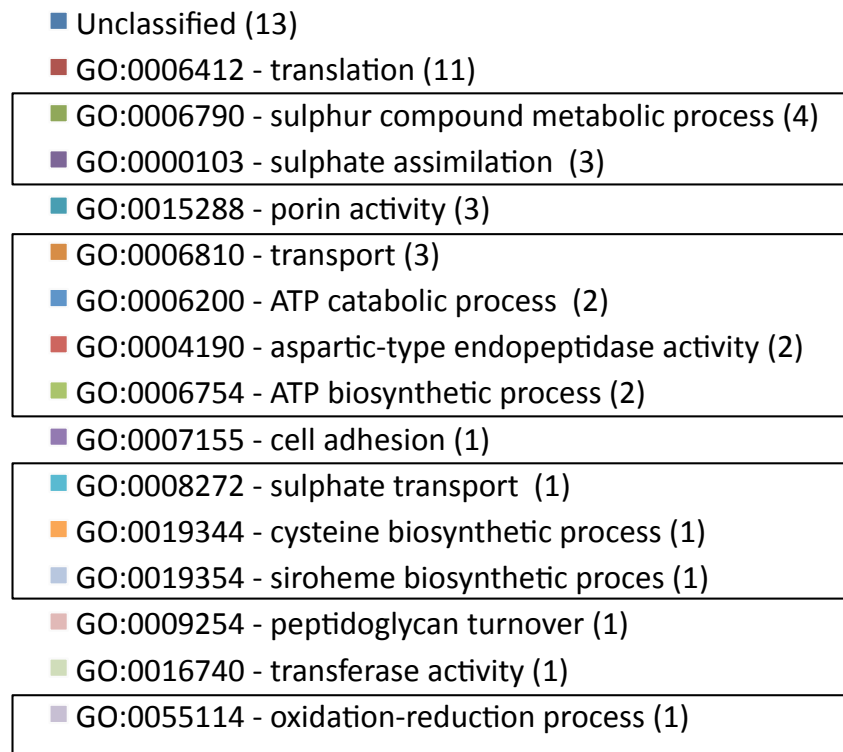


Bowtie 2, unique, DESeq2

Differentially expressed transcripts between 97/192 and its carriage-associated isolates ($[\log_2] \geq 1$; adj $p < 0.05$)



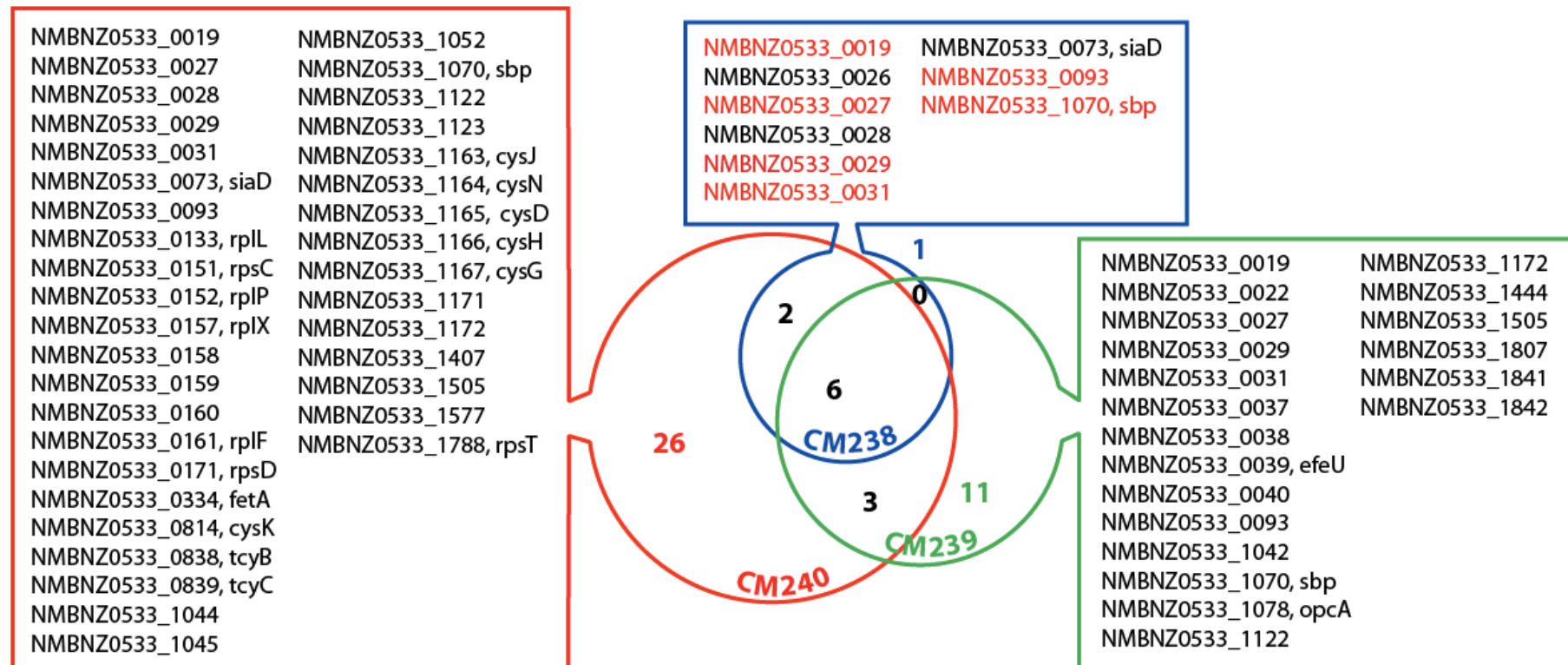
Differentially expressed transcripts between 97/192 and its carriage-associated isolates ($[\log_2] \geq 1$; adj $p < 0.05$)



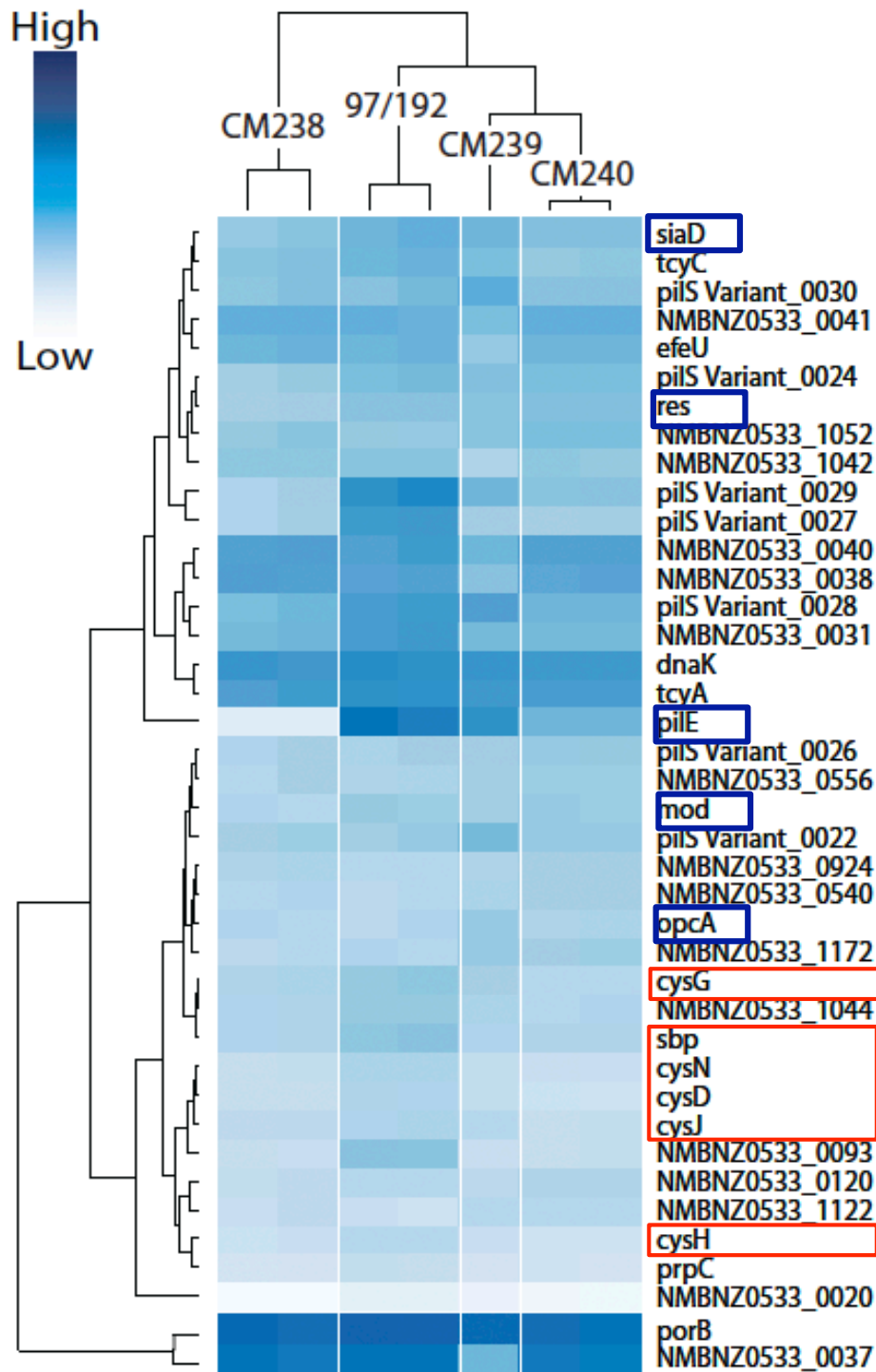
Gene Ontology assignment by <http://biocyc.org/>

Differentially expressed transcripts between 97/192 and its carriage-associated isolates ($[\log_2] \geq 1$; adj $p < 0.05$)

- Six transcripts were in common between all isolates (highlighted in red)



Bowtie 2, unique, DESeq2

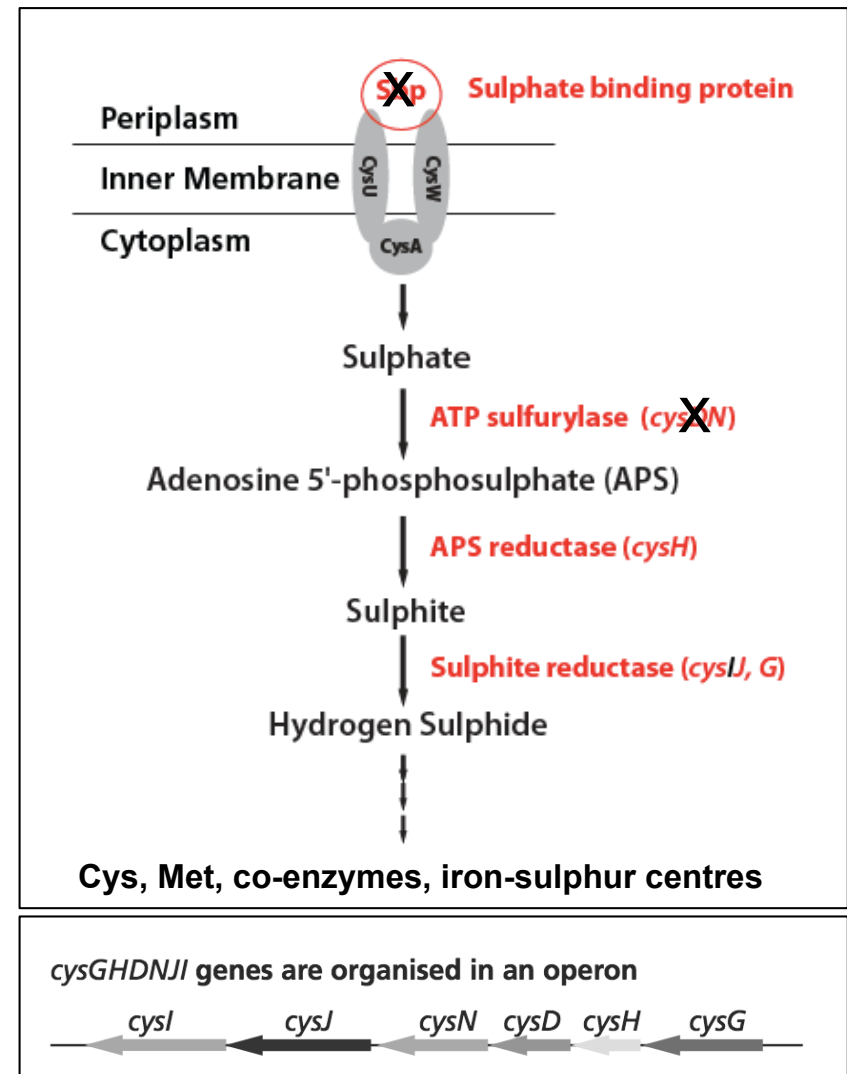


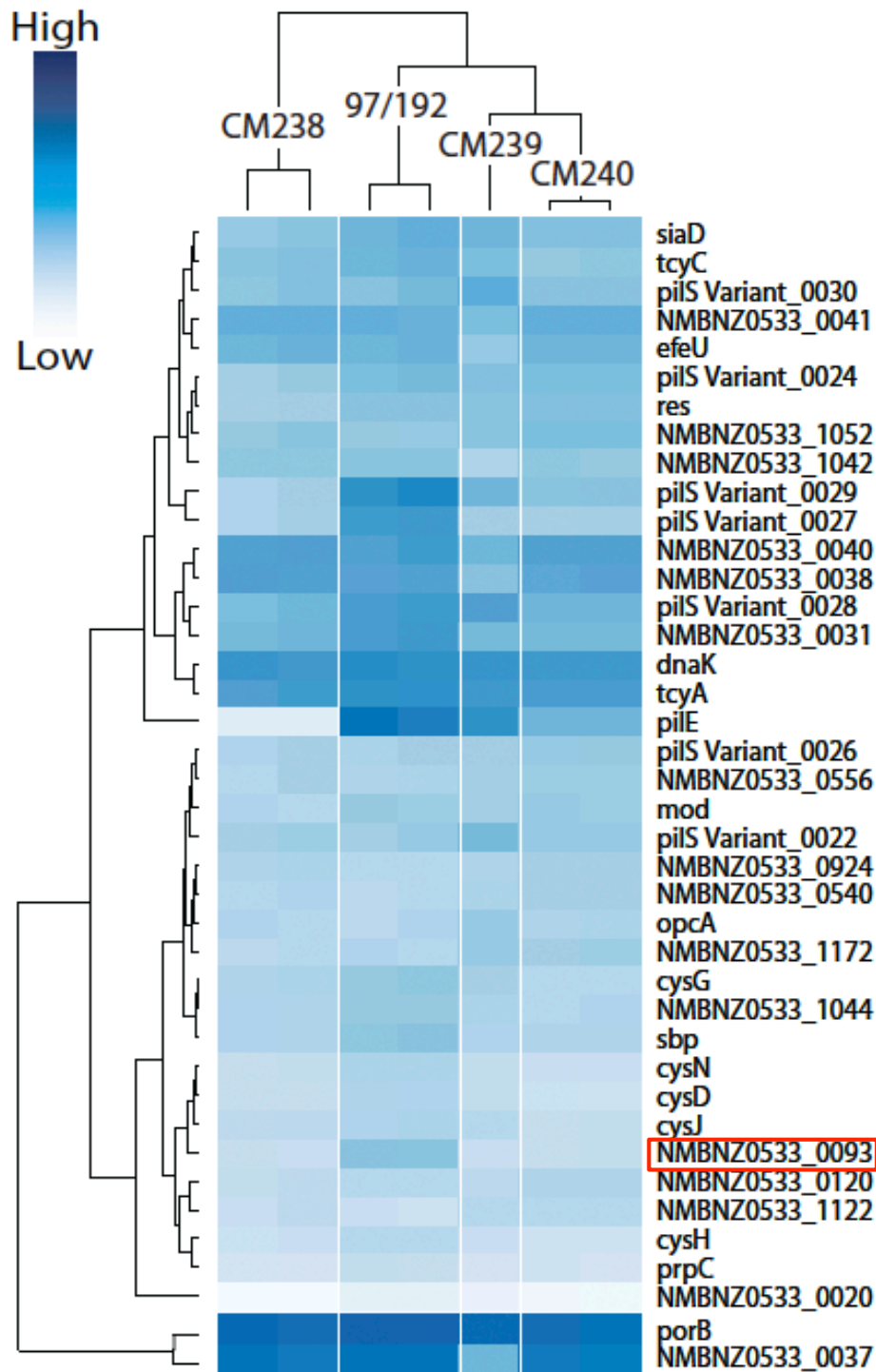
1 Sulphate assimilation pathway enzymes

Bowtie 2, unique, DESeq2

1 Sulphate assimilation pathway enzymes

- Lower expression in carriage-associated isolates: Sbp and 5 out of 6 enzymes
- Organised in an operon
- How it is regulated is not known: no genomic or expression differences in the putative transcription regulator *cysB*
- Maybe important for survival in low nutrient environments, such as the CSF, and under hypoxic conditions.





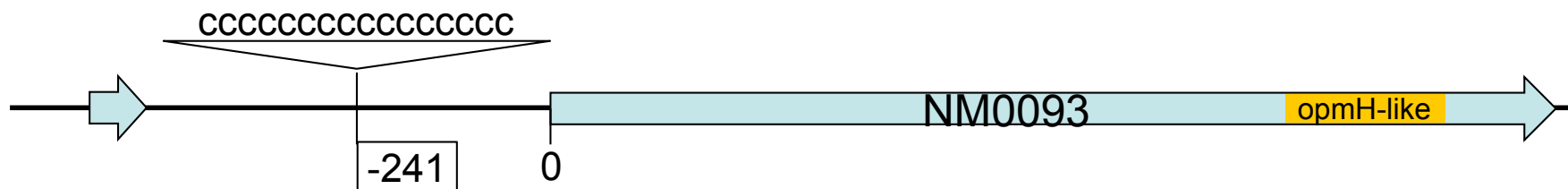
2 NM0093: conserved
hypothetical protein

Bowtie 2, unique, DESeq2



2 NM0093: Conserved hypothetical protein.

- Similar protein found in other bacteria
- Seemed to be serogroup B specific
- Maybe under phase-variation expression control



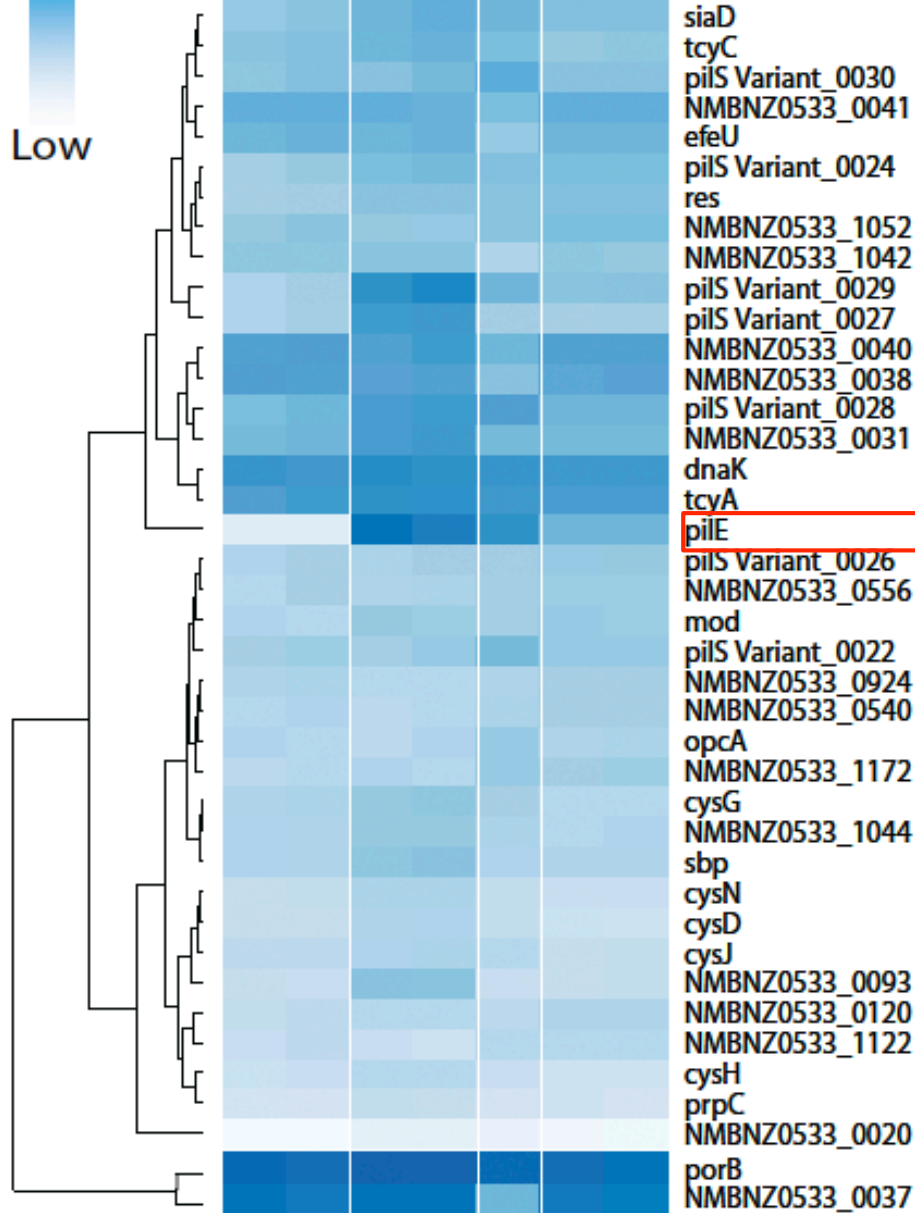
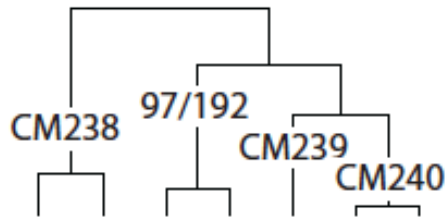
No. Isolates	Site	No. Cs
97/192	CSF	9
CM238	Throat	10
CM239	Throat	10
CM240	Throat	10

No. Isolates	Site	Type	Presence	No. Cs
2	Throat	True carriage	-	
1	Throat	True carriage	+	>16 (?)
3	CSF	NZ Epidemic	+	9 or 15
4	Blood	NZ Epidemic	+	14 or 16
5	Throat	NZ Epidemic	+	10, 14 or 16

High



Low

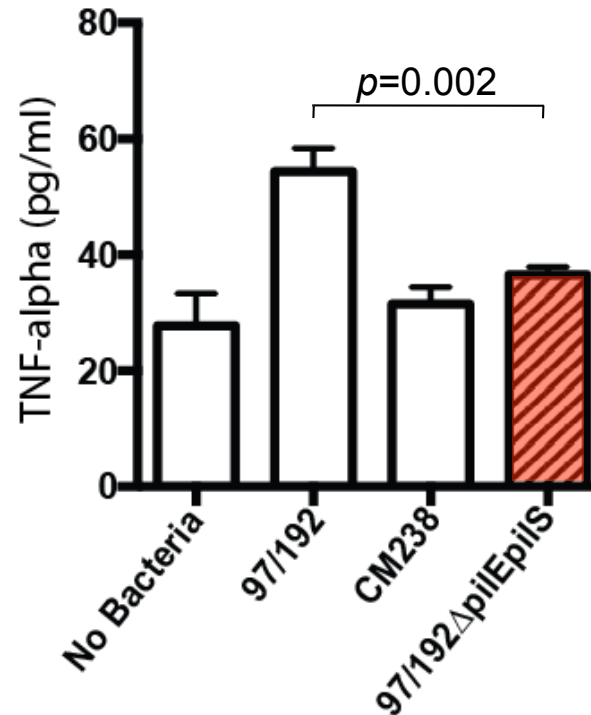
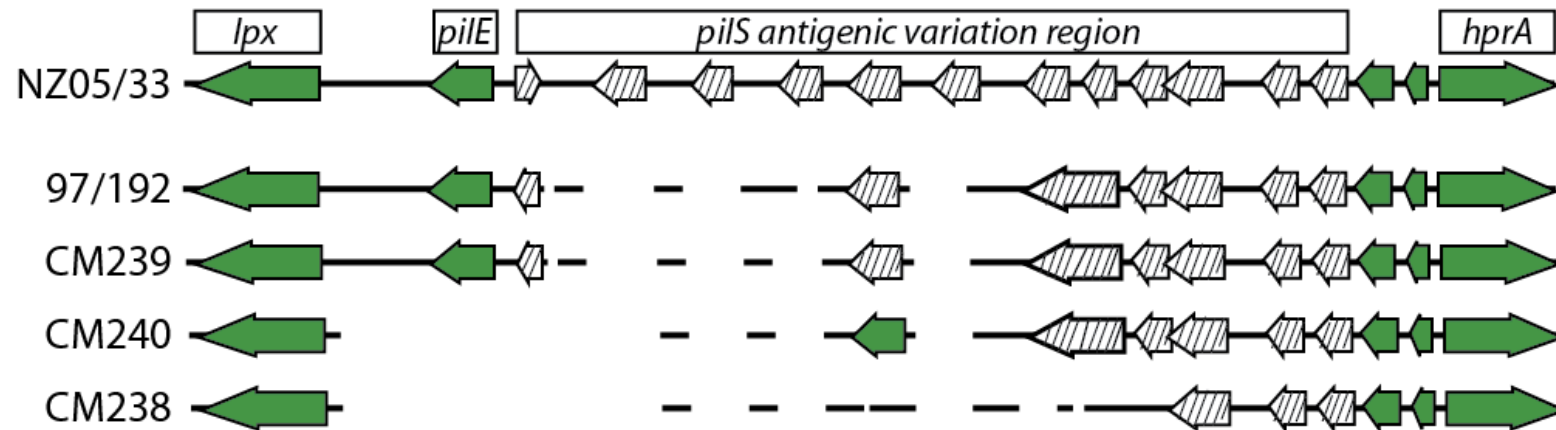


3 *pilE*: pili structural protein

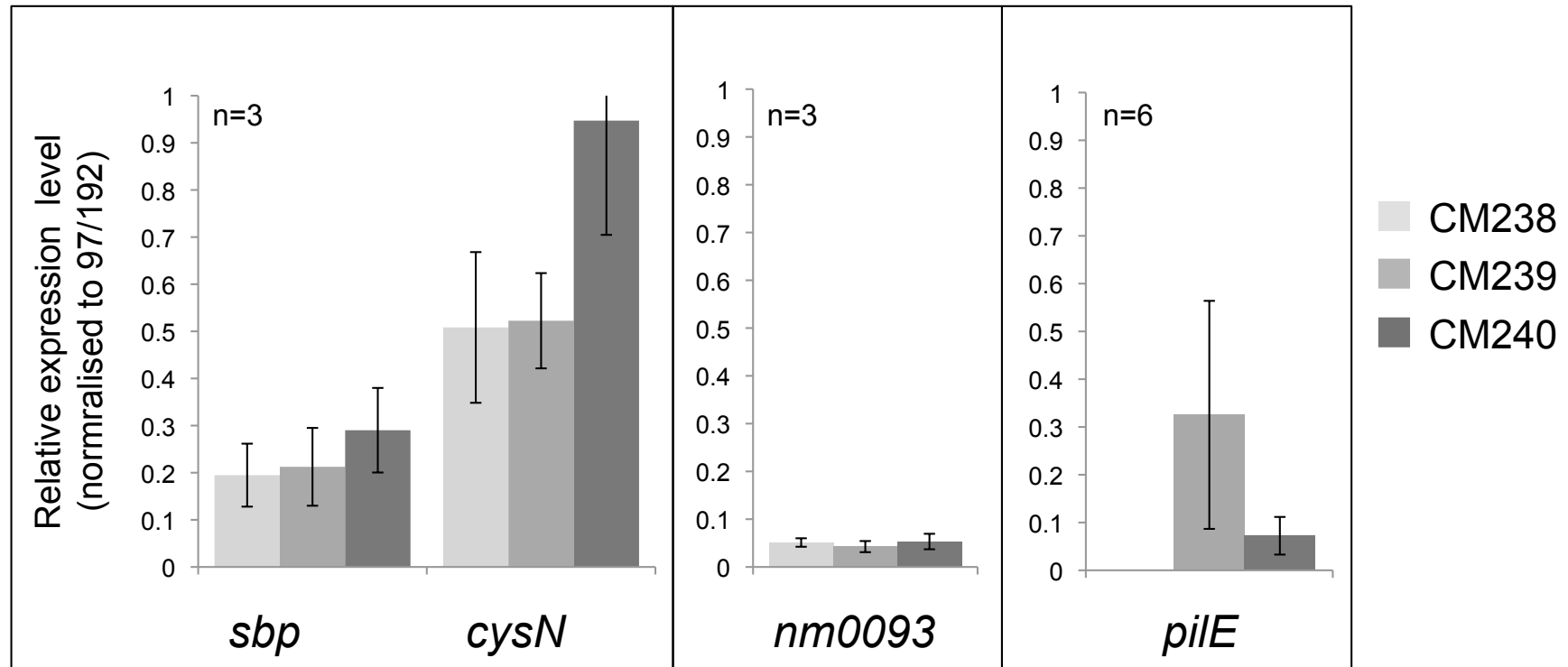
Bowtie 2, unique, DESeq2

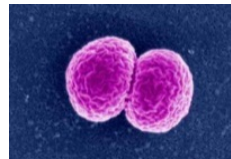
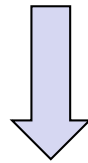
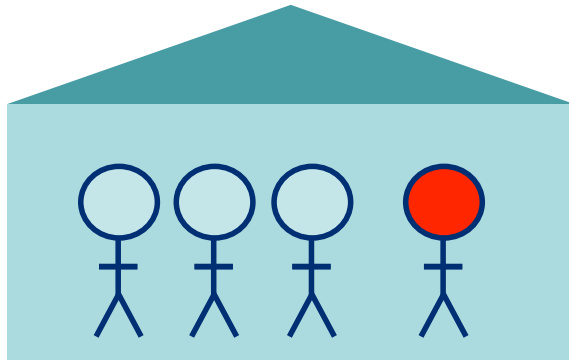


3 Genomic sequencing identified changes in the *pilE* antigenic variation region and mutagenesis suggests *pilE* is important for TNF-alpha stimulation

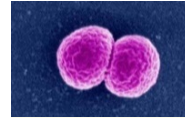


Real-time quantitative PCR validation: carriage-associated isolates normalised to 97/192





Meningococcal isolates
from patients and rest of the
household with the same MLST



The Plan

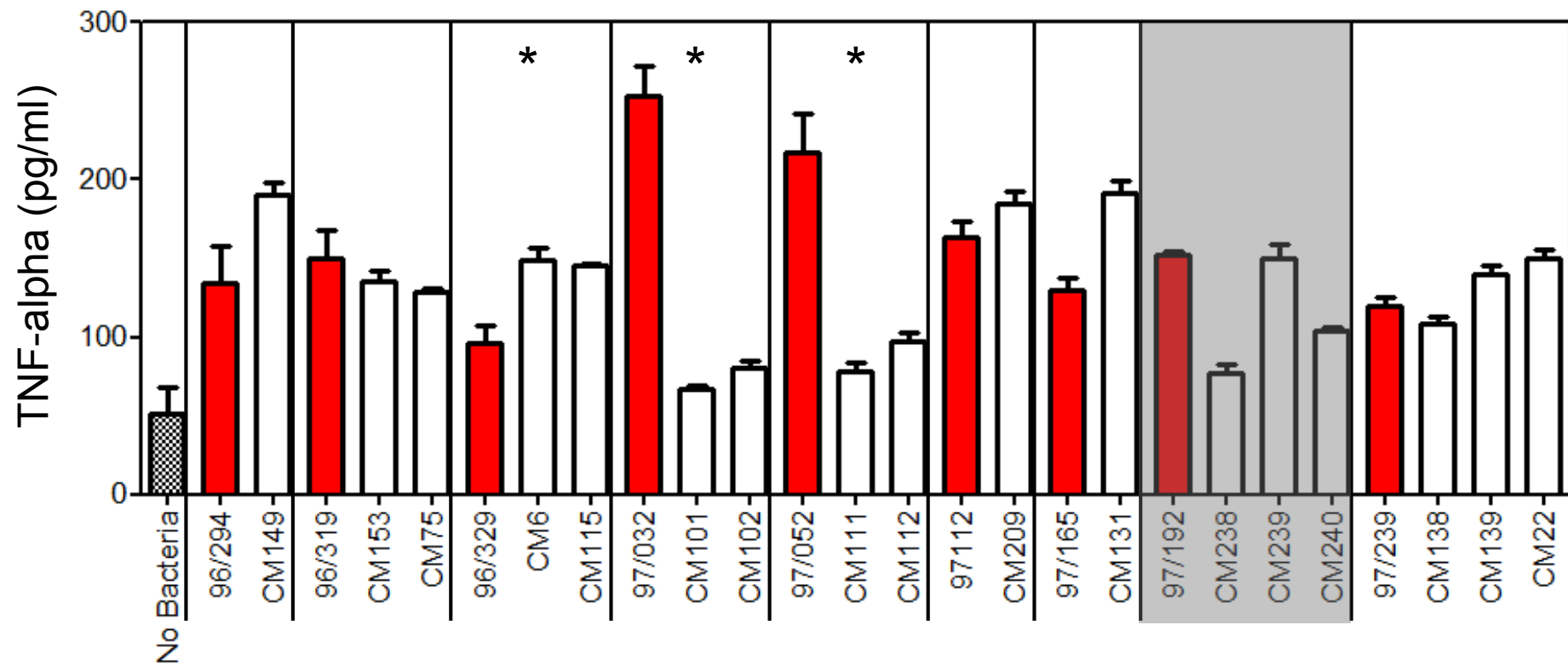
Expand phenotypic analysis
to more household groups

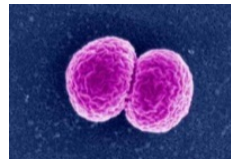
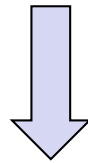
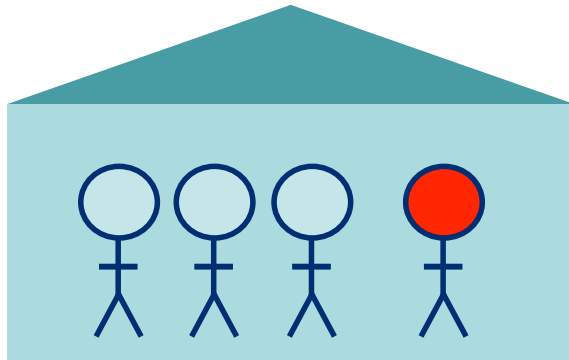
Whole genome shotgun
sequencing

Transcriptome analysis

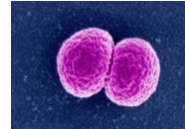
Look for consistent patterns

A quick look at TNF-alpha induction





Meningococcal isolates
from patients and rest of the
household with the same MLST

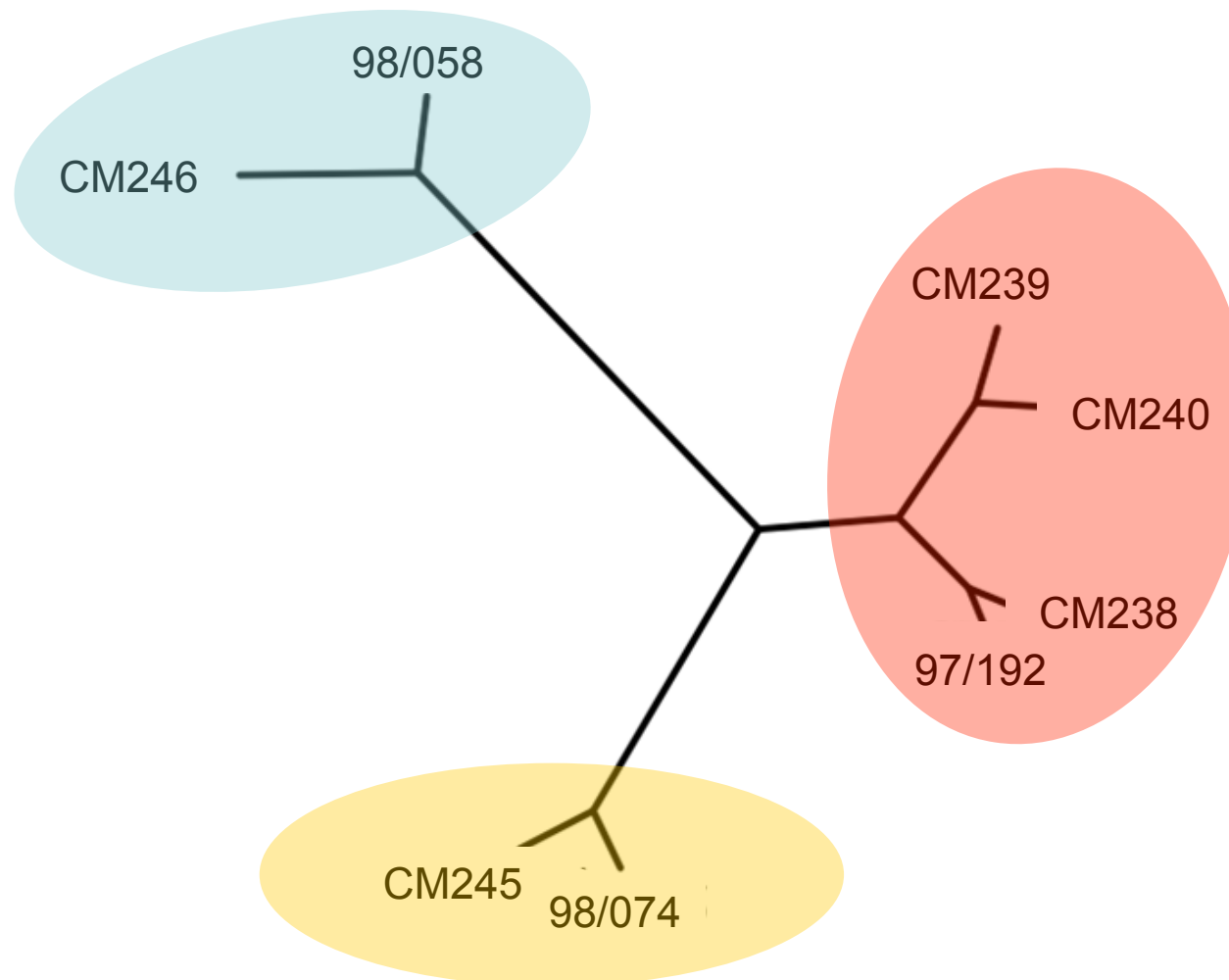


So Far

RNA-seq and WGS for 6
households, a total of 16
isolates of a variety of
serogroups commonly found
in NZ.

Are isolates from the same households more closely related to each other?

- SNP tree of isolates from 3 households
- Same strain type, clonal complex, MLST and FetA



Are there any themes?

- Lower expression in carriage-associated isolates
- *pilE*, 5 out of 6 household showed deletions or reduced expression in carriage-associated isolates
- Putting all the NZ epidemic strain isolates RNA-seq together: 2 blood, 1 CSF, 5 carriage-associated isolates (red highlights lower expression in carriage or blood isolates)

Invasive vs carriage

pilE

Putative lipoprotein
(NMBNZ0533_0037)

Chaperonin: *dnaJ*, *hslO*

tRNA-Ser

Iron acquisition: Lactoferrin binding protein (*lbpB*), FrpC Operon Protein (NMBNZ0533_1883)

CSF vs Blood

Putative lipoprotein (NMBNZ0533_0037)

Iron acquisition: ferrous iron permease (*efeU*), iron ABC transporter substrate-binding protein (NMBNZ0533_0040), ferric enterobactin receptor (*fetA*), lactoferrin binding protein (*lbpB*)

Hypothetical Protein (NMBNZ0533_0093, NMBNZ0533_0549, NMBNZ0533_0948, NMBNZ0533_1880, NMBNZ0533_2054)

tRNAs (tRNA-Ser, tRNA-Glu)

acyl-CoA dehydrogenase domain protein (NMBNZ0533_1044)

Sulphate binding protein (*sbp*)

DNA-binding protein (NMBNZ0533_1178)

L-lactate dehydrogenase (*lldA*)

Using the unique household contact isolate collection we found:

- Most gene expression differences seen are due to lower expression in carriage–associated isolates
- *pilE* expression is reduced in many carriage–associated isolates
- Expression differences in some usual suspects and pathways that haven't been studied before

A lot more to do:

- A closer look at the genome: nonsynonymous substitutions, SNP and nucleotide modifications
- Use real-time PCR to survey more isolates
- Looking to study more households: hope to compare across households and define factors that may contribute to virulence and survival in specific anatomical sites



Acknowledgments:

Core Funding from ESR

David Eccles, Victoria University of Wellington

Jane Clapham, ESR

Paul Gardner's Group, University of Canterbury

Joanna MacKichan, Victoria University of Wellington

Invasive Pathogens Lab, ESR

Miles Benton, ESR

Donia Macartney-Coxson, ESR

Potential PhD projects:

Bioinformatic analysis of household isolates

Dissect sulphate assimilation pathway in *Neisseria*

