

LECTURE SERIES

Klebsiella pneumoniae Genomic Epidemiology and Antimicrobial Resistance

In silico serotyping of *Klebsiella*

Tom Stanton, Monash University

Intended Learning Objectives

Specific objectives of this session:

1. Understand the biology and genetics of the capsule (K) and LPS (O) antigens and rationale of serotyping.
2. To understand how Kaptive, a tool for *in silico* serotyping, works.
3. How to install and use Kaptive via the command-line and web-based interfaces.
4. Understand how to interpret and use Kaptive results.

Outline

This session consists of the following elements

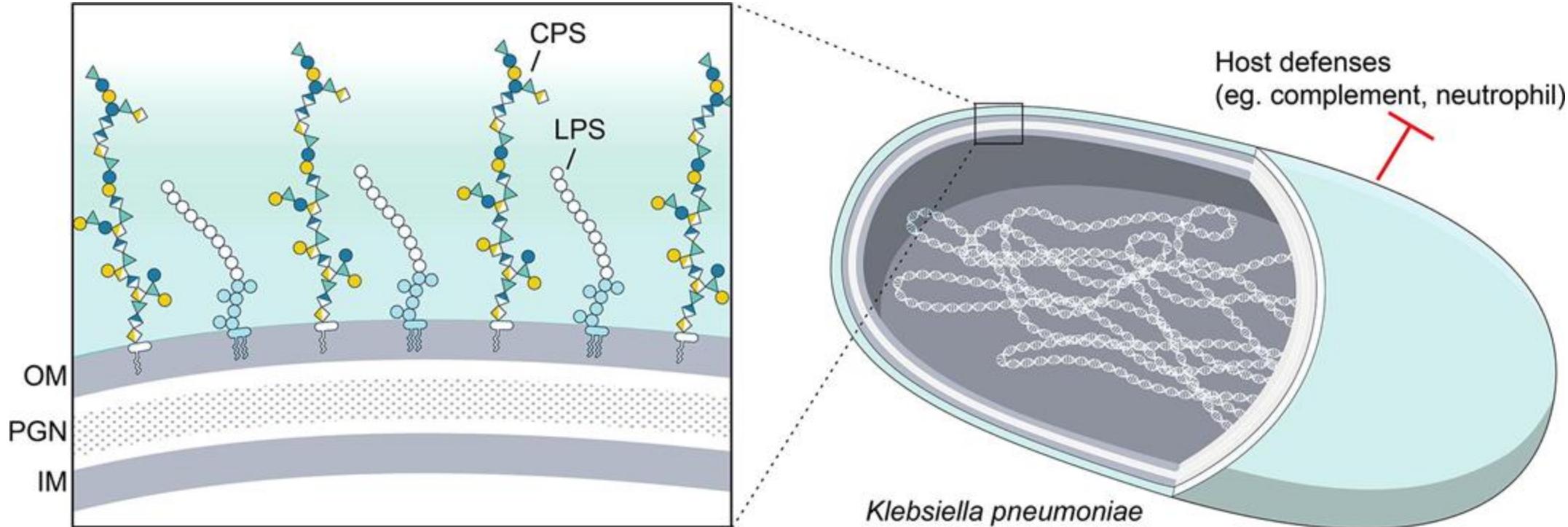
1. What are the capsule (K) and outer LPS (O) polysaccharides?
2. K and O loci
3. K and O typing with Kaptive
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K and O polysaccharides are surface molecules



- Protects bacteria from desiccation
- Required for virulence
- Immunogenic

K and O polysaccharides are targets for novel control strategies



- Vaccines
- Mono-clonal antibodies
- Phage receptors

Vaccine 42 (2024) S125–S141

Contents lists available at ScienceDirect

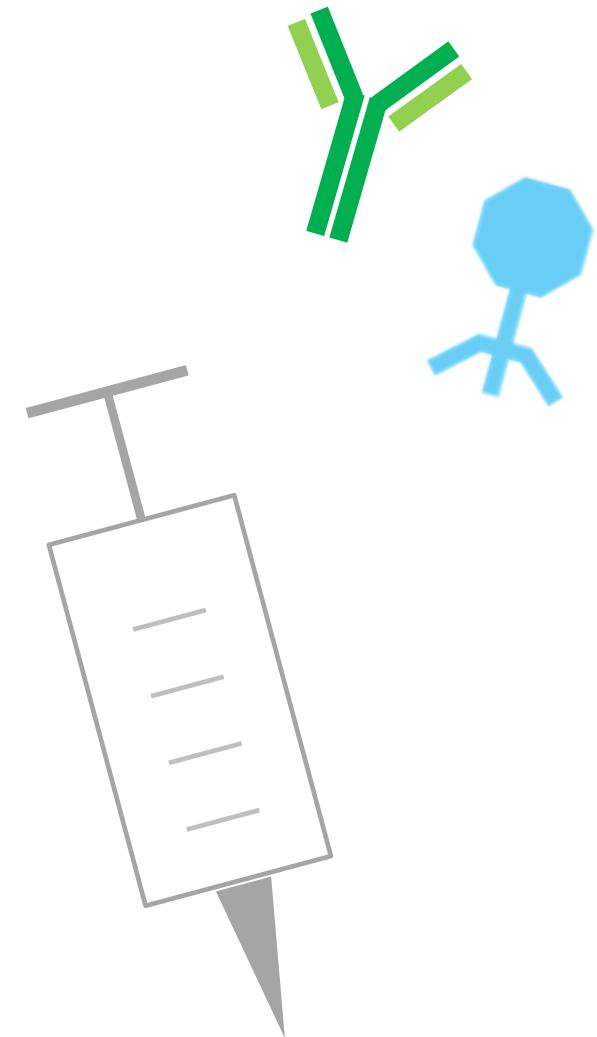
Vaccine

journal homepage: www.elsevier.com/locate/vaccine

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Vaccine value profile for *Klebsiella pneumoniae*

Ziyaad Dangor ^{a,*}, Nicole Benson ^b, James A. Berkley ^{c,d}, Julia Bielicki ^{e,f}, Merijn W. Bijlsma ^{g,h}, Jonathan Broad ⁱ, Ed T. Buurman ^j, Alan Cross ^k, Erin M. Duffy ^j, Kathryn E. Holt ^{l,m}, Pui-Ying Iroh Tam ⁿ, Mark Jit ^o, Konstantinos Karampatas ^e, Michael Katwere ^p, Gaurav Kwatra ^{q,r,a}, Ramanan Laxminarayan ^s, Kirsty Le Doare ^{e,t,u}, Robert Mboizi ^o, Francesca Micoli ^v, Catrin E. Moore ^e, Eve Nakabembe ^w, Nichola R. Naylor ^{t,x}, Seamus O'Brien ^y, Courtney Olwagen ^a, Denasha Reddy ^a, Charlene Rodrigues ^{l,z,aa}, David A. Rosen ^{ab}, Manish Sadarangani ^{ac,ad}, Padmini Srikantiah ^b, Sharon M. Tennant ^k, Mateusz Hasso-Agopsowicz ^{ae}, Shabir A. Madhi ^a



***Klebsiella* and *E. coli* produce highly diverse K and O polysaccharides**

Serological typing:

- Technically challenging
- Labour intensive
- Many strains untypeable (*Klebsiella*)

Serotype ≠ structure

		<i>Klebsiella</i>	<i>E. coli</i>
K	serotypes	77*	80
	structures	89	80
O	serotypes	9	184
	structures	22	197

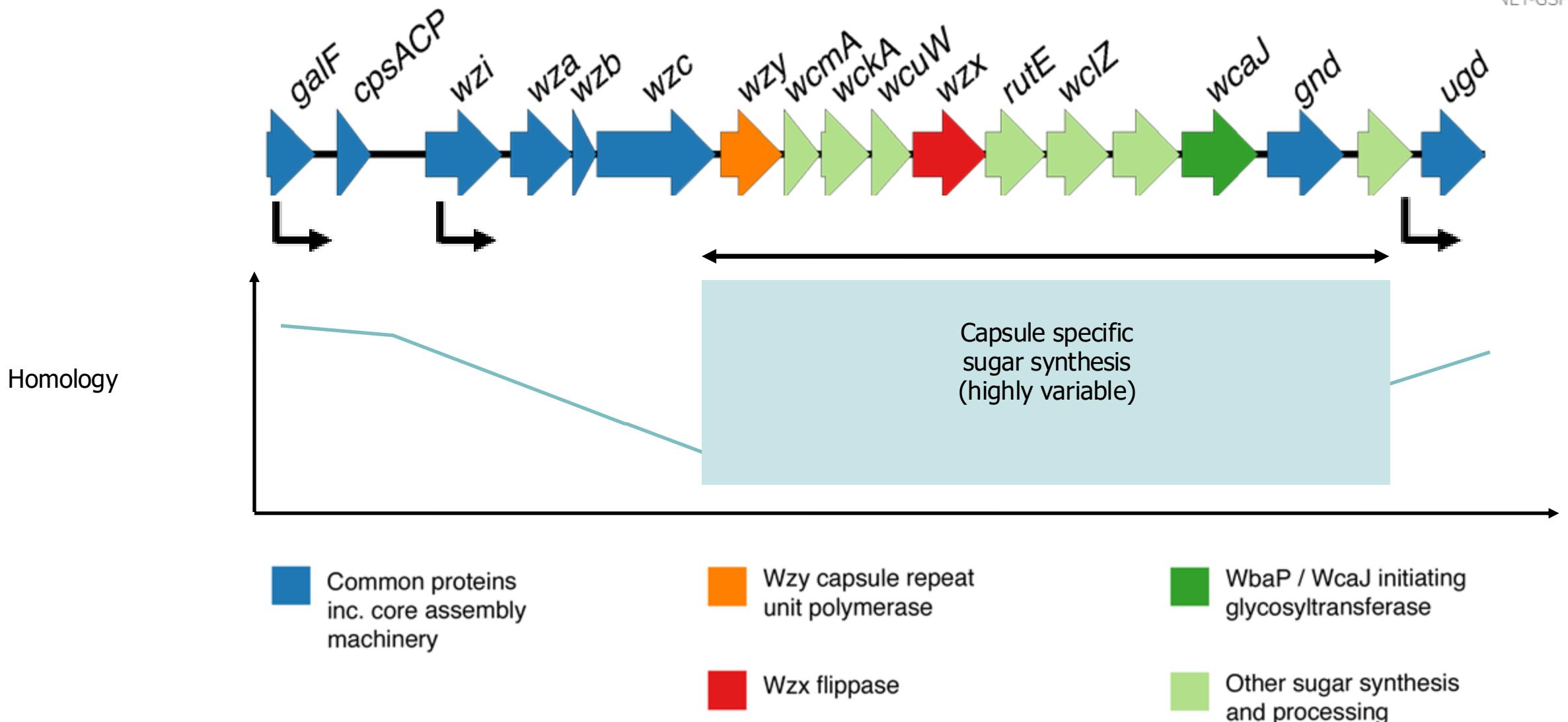
* +5 unpublished, Stanton *et al in prep*

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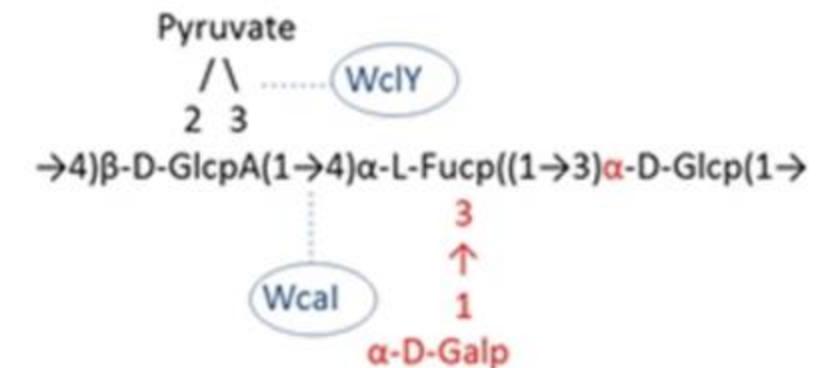
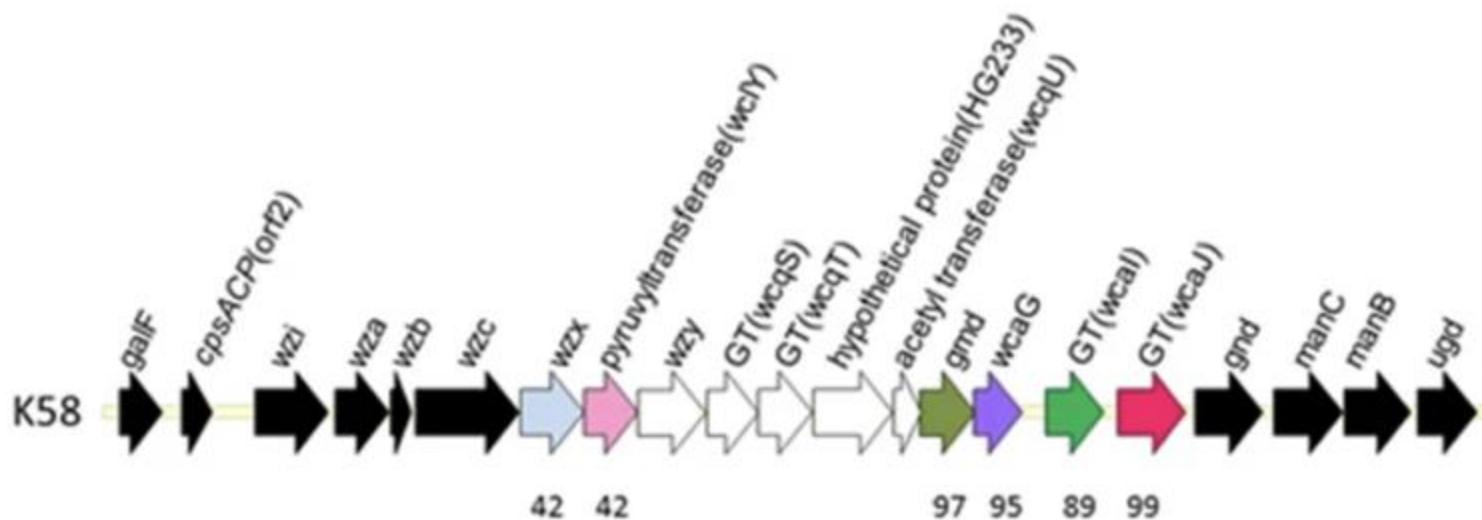
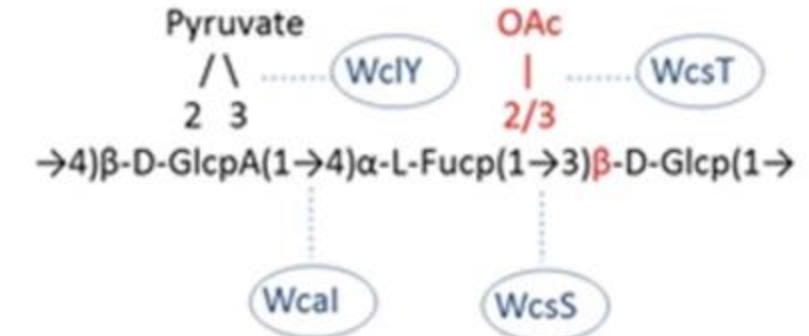
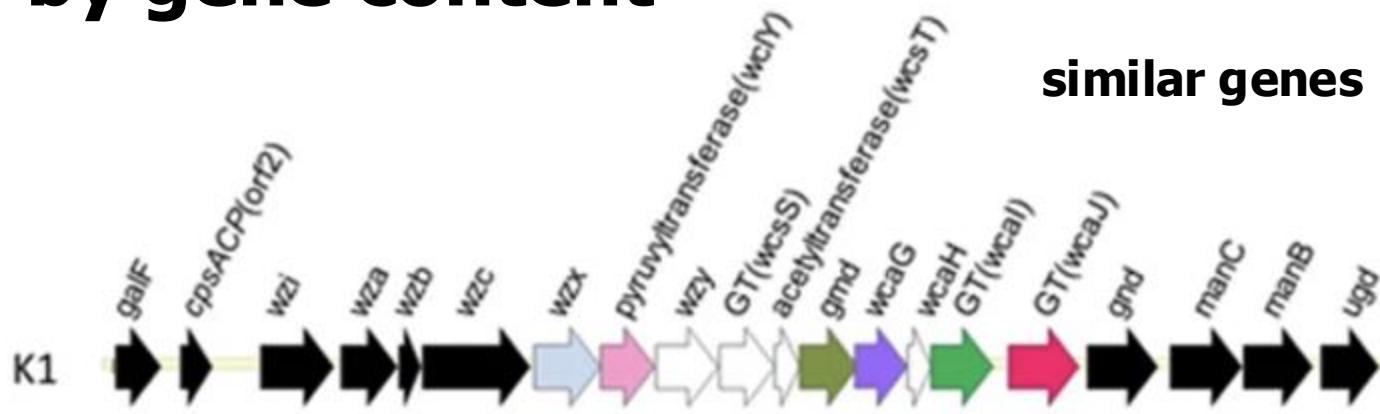
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The *Klebsiella* capsule (K) locus

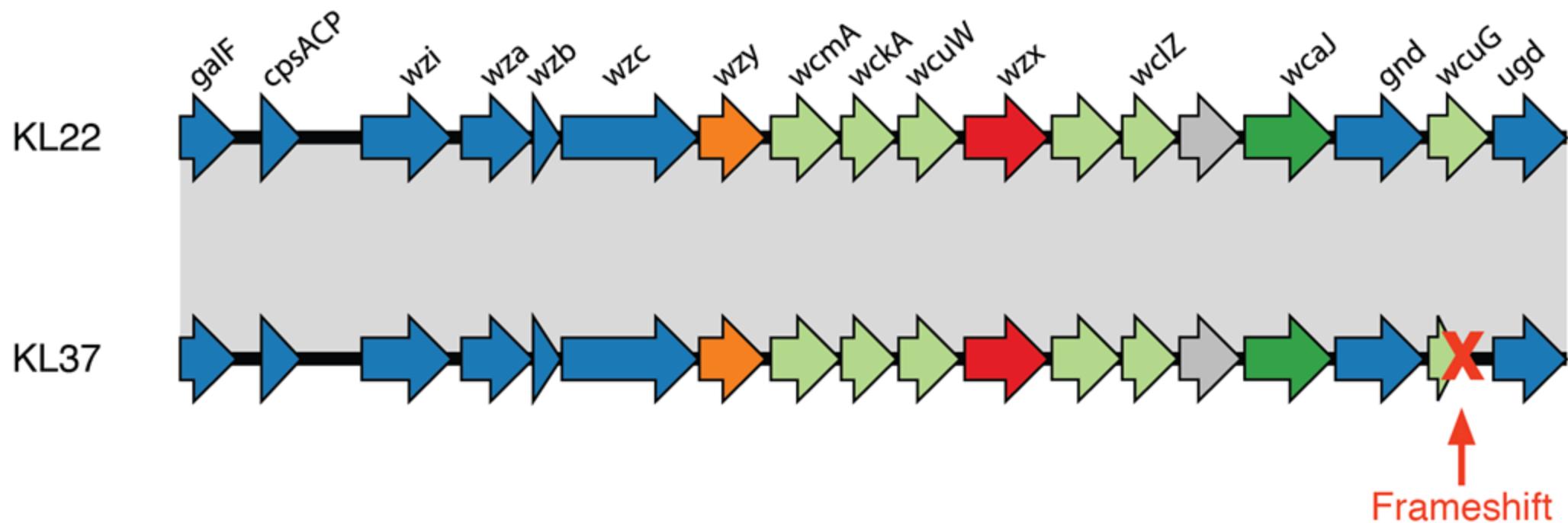


Serotype reference loci can (mostly) be distinguished by gene content

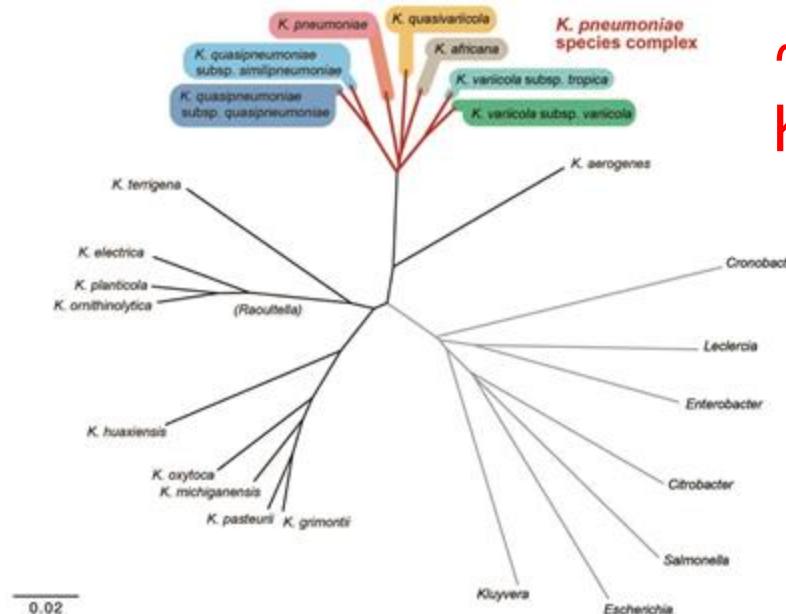
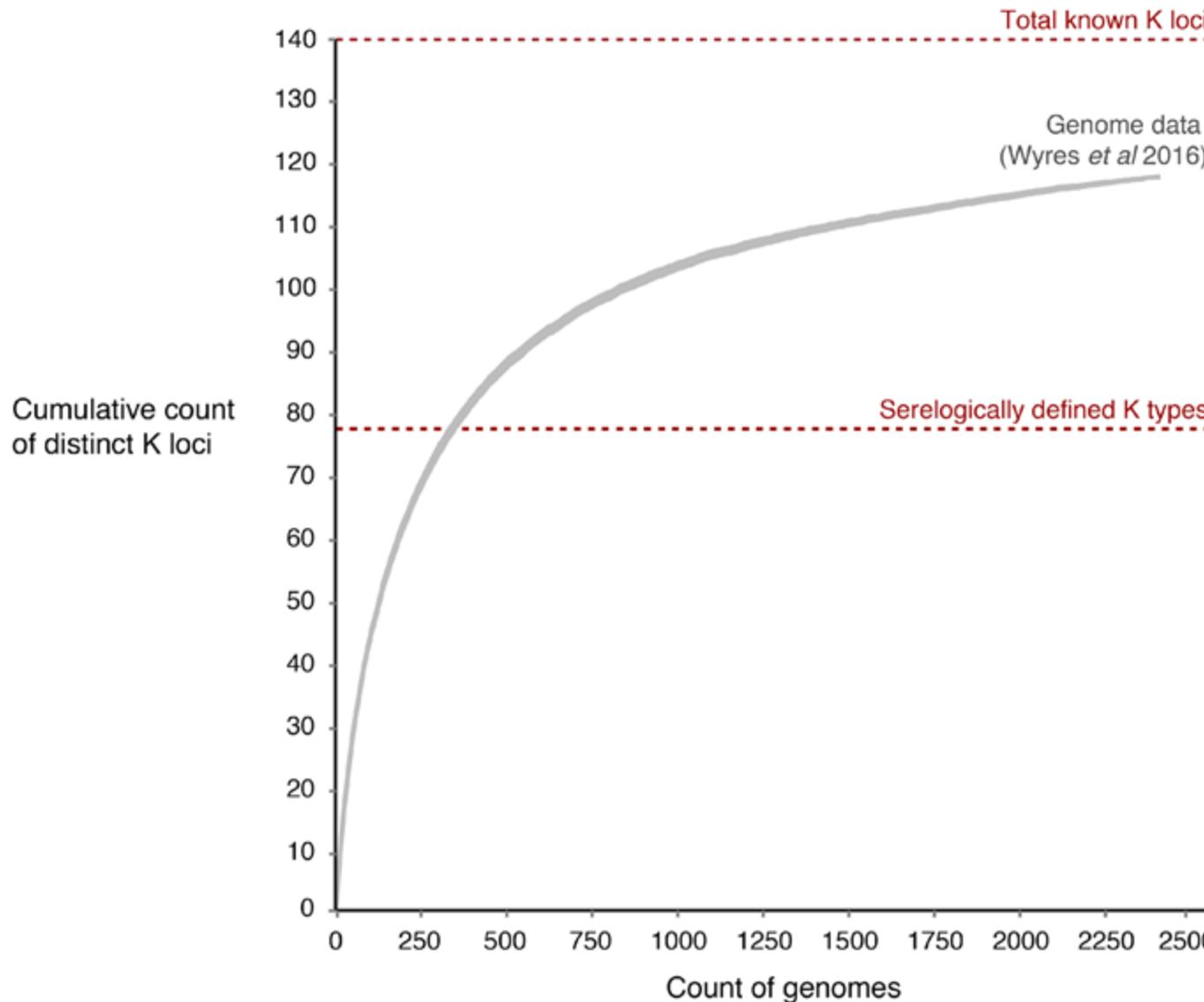
similar genes = similar capsule structure



***Klebsiella* K22 and K37 reference loci are the exception**



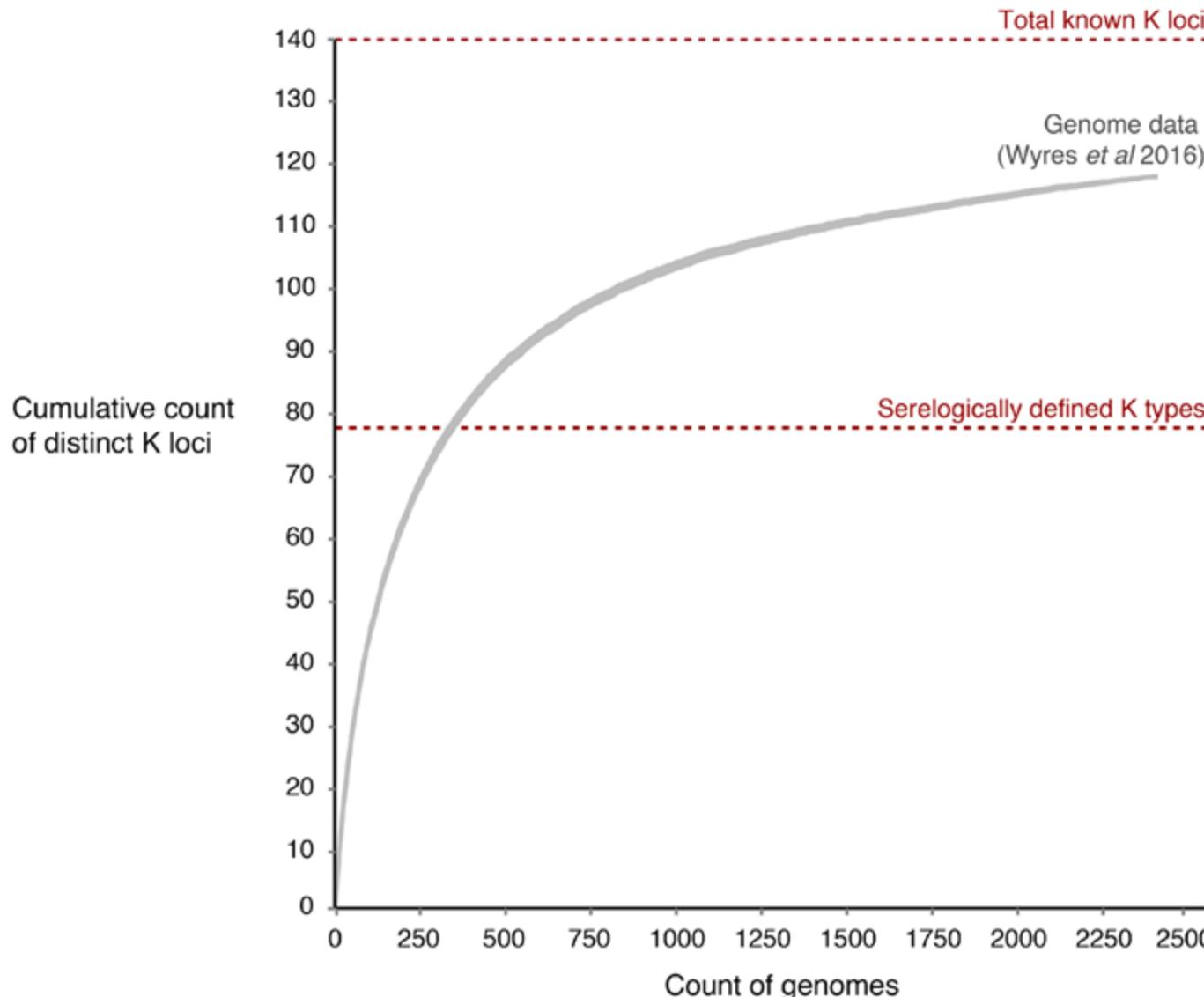
More than 60 additional K loci defined on the basis of gene content



~2500
KpSC

Papers defining additional loci:
Chen et al *mBio* 2014
Wyres et al *Genome Biol Evol* 2015
Follador et al *Microb Genom* 2016
Wyres et al *Microb Genom* 2016

More than ~~60~~ 80 additional K loci defined on the basis of gene content



>160 K loci to-date
from ~13,000 KpSC

Papers defining additional loci:
Chen et al *mBio* 2014
Wyres et al *Genome Biol Evol* 2015
Follador et al *Microb Genom* 2016
Wyres et al *Microb Genom* 2016
Wyres et al *Genome Med* 2020
Gorrie et al *Nature Commun* 2021
Lam et al *Microb Genom* 2022

Growing support that 'novel' K loci encode distinct polysaccharides



High resolution structures available for KL106 and KL107:

- Bellich *et al* *International Journal of Biological Macromolecules* 2019, 2020
- Kubler-Kielb *et al* *Carbohydr Res* 2013

Lower resolution structures available for 9 'novel' KpSC loci:

- Nonne *et al* *Communications Biology* 2025

***Klebsiella* O structure variation requires O loci & additional genes**



Microbiology and Molecular
Biology Reviews

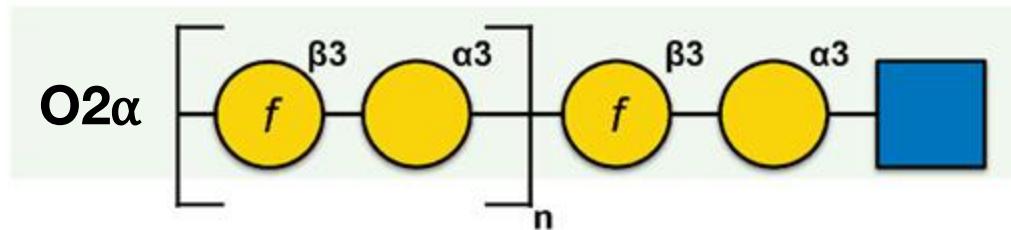


Genetics and Molecular Biology | Review

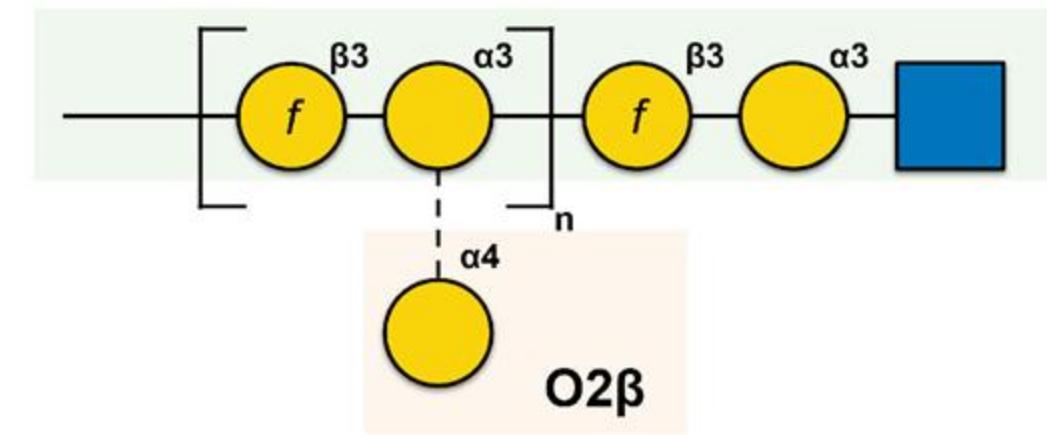
O-antigen polysaccharides in *Klebsiella pneumoniae*: structures and molecular basis for antigenic diversity

Chris Whitfield,¹ Steven D. Kelly,¹ Tom D. Stanton,^{2,3} Kelly L. Wyres,^{2,3} Bradley R. Clarke,¹ Taylor J. B. Forrester,¹ Agnieszka Kowalczyk¹

Klebsiella O structure variation requires O loci & additional genes



OL2α



OL2α + *gmI2β*

Nomenclature

Locus = **set of genes** encoding the synthesis / export machinery for the polysaccharide backbone

e.g. KL1, KL2, KL3, OL1, OL2, OL3

- in KpSC KL $x<100$ corresponds to original serotype reference locus,
KL $x>100$ corresponds to 'novel' loci

Type = predicted **polysaccharide phenotype**

e.g. K1, K2, K3, O1, O2, O3

- locus + truncations within locus + additional genes

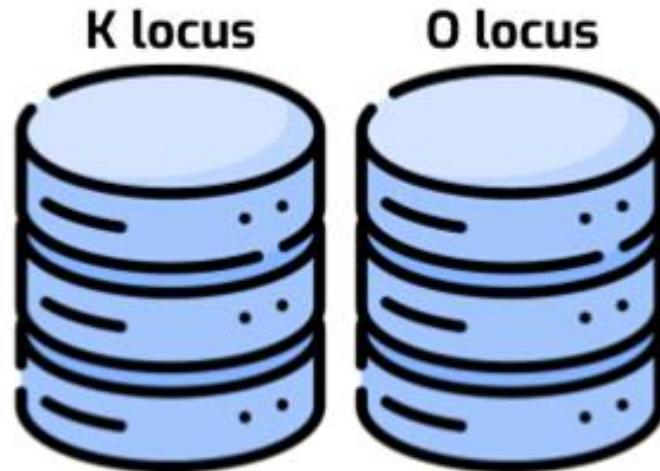
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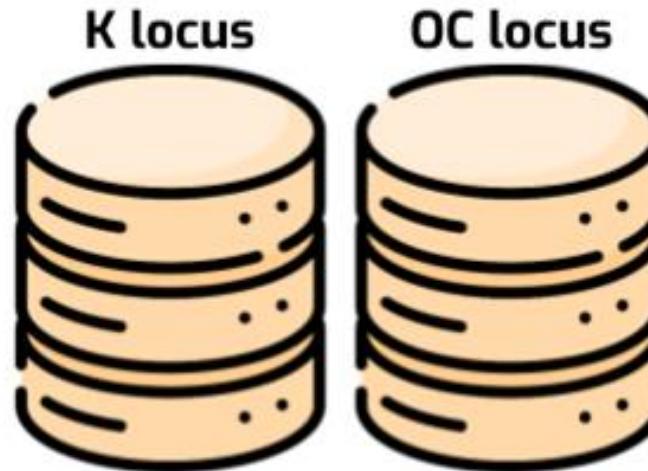
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Kaptive – databases and tools for bacterial surface polysaccharide locus typing

K. pneumoniae



A. baumannii



E. coli K (group 2 / 3)

Rebecca Gladstone
University of Oslo

In development:

E. coli K (group 1 / 4)
Klebsiella oxytoca K and O

Kelly Wyres
Monash University

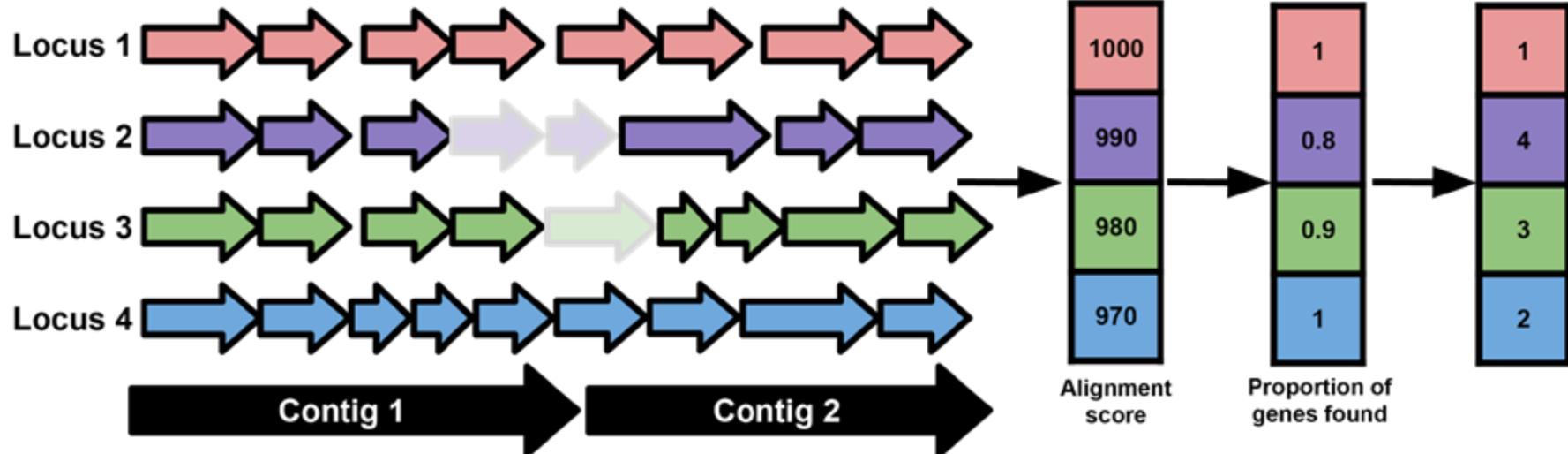
Johanna Kenyon
Griffith University

Kaptive papers:
Wyres *et al* *Microbial Genom* 2016
Wick *et al* *JCM* 2018
Lam *et al* *Microbial Genom* 2022
Stanton *et al* *Microbial Genom* 2025

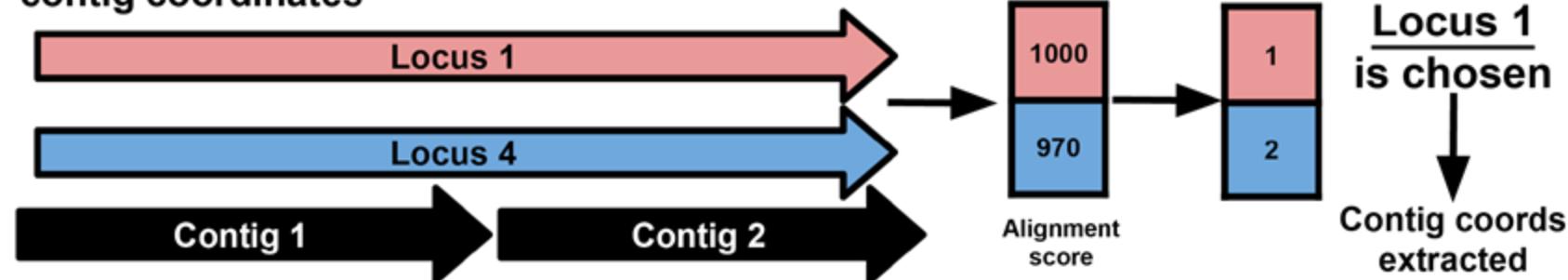
How Kaptive (v3) works



a. Align locus genes to contigs



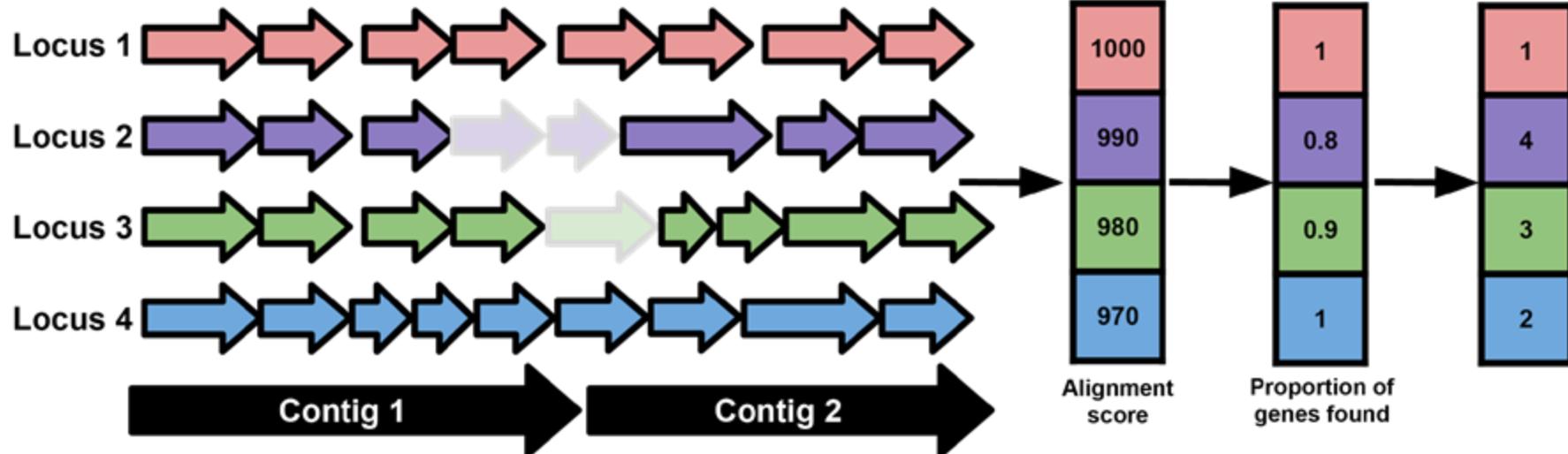
b. Align best loci to contigs, rank and collect contig coordinates



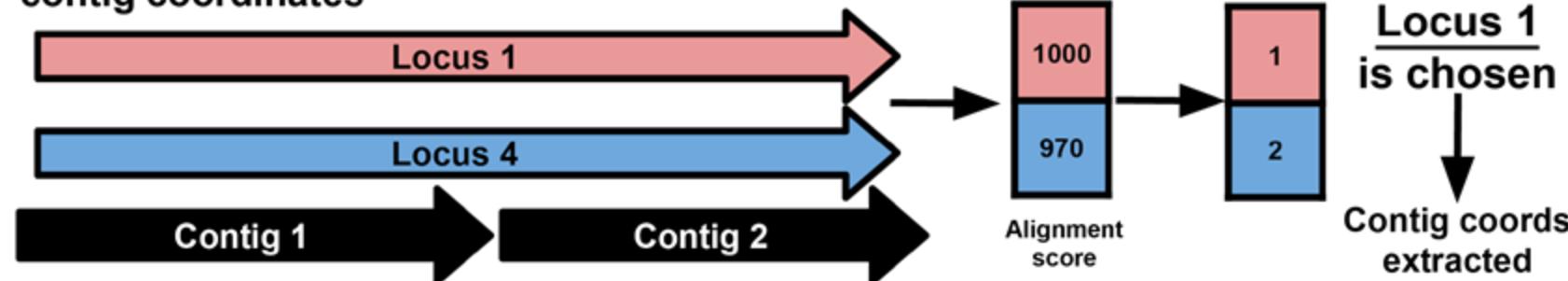
How Kaptive (v3) works



a. Align locus genes to contigs



b. Align best loci to contigs, rank and collect contig coordinates



- Calculate Cov and ID per gene
- ↓ Report missing and extra genes in locus
- ↓ Apply phenotype logic

Kaptive confidence scoring



Confidence	Fragmented	# Genes below identity threshold	Expected genes found (%)	# Extra genes
<i>Typeable</i>	No	0	100	0
	<i>Or if locus is fragmented</i>			
	Yes	0	≥50	≤1
<i>Untypeable</i>	Does not meet the above criteria			

K or O loci marked 'Untypeable' should be excluded from analyses

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Multiple ways to access Kaptive



	<i>Klebsiella</i>		<i>E. coli</i>	
	K	O	K	O
Command line interface: github/klebgenomics/kaptive	✓	✓	Partial db	X
Via command line Kleborate	✓	✓	X	ECTyper
Kaptive Web: kaptive-web.erc.monash.edu/	✓	✓	X	X
Pathogen.watch: pathogen.watch.org	✓	✓	X	X

Complete *E. coli* K locus db in progress



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Interpreting Kaptive output



Full documentation:

<https://kaptive.readthedocs.io/en/latest/>

Tutorial:

<https://tinyurl.com/2s3rs3ny>

Output table:

Assembly	Best match ID	Best match type	Match confidence	Problems	Identity	Coverage	Length discrepancy	Expected g...
assembly_1	KL28	K28	Typeable		100.00%	100.00%	0 bp	19 / 19 (100%)
assembly_2	KL5	K5	Typeable		97.67%	100.04%	-8 bp	19 / 19 (100%)
contaminant					00.00%	00.00%	0 bp	00 / 00 (0%)



Interpreting Kaptive output – Example of a great match

C_ERR4920513 Best locus: KL28 Predicted type: K28 Match confidence ⓘ : Typeable Cov ⓘ : 100.0% ID ⓘ : 99.80% Genes: 19 / 19

KL28 reference ⓘ :

Other genes found in locus ⓘ : 0 ▾ Other genes found outside locus ⓘ : 6 ▾

Assembly pieces ⓘ : [Download as FASTA](#)

Contig name	Start position	End position
12	134125	158956



Interpreting Kaptive output – Example of a good match

H_ERR4920541 Best locus: KL28 Predicted type: K28 Match confidence ⓘ : Typeable Cov ⓘ : 91.36% ID ⓘ : 99.80% Genes: 19 / 20

KL28 reference ⓘ :

KL28_01_galF 100.00% 100.00%
KL28_02_cpsACP 100.00% 100.00%
KL28_03_wzl 97.69% 100.00%
KL28_04_wza 99.74% 100.00%
KL28_05_wzb 100.00% 100.00%
KL28_06_wzc 100.00% 100.00%
KL28_07_wckC
KL28_08_wcuC 100.00% 17.60% partial
KL28_09_wckD 99.45% 100.00%
KL28_10_wzy 100.00% 100.00%
KL28_11_wclS 100.00% 13.37% partial
KL28_12_wcuE 100.00% 100.00%
KL28_13_wzx 100.00% 100.00%
KL28_14_wbaZ 100.00% 100.00%
KL28_15_wcaJ 100.00% 100.00%
KL28_16_gnd 100.00% 100.00%
KL28_17_manC 100.00% 100.00%
KL28_18_manB 100.00% 100.00%
KL28_19_ugd 99.74% 100.00%

Other genes found in locus ⓘ : 0 ▾ Other genes found outside locus ⓘ : 4 ▾

Assembly pieces ⓘ : [Download as FASTA](#)

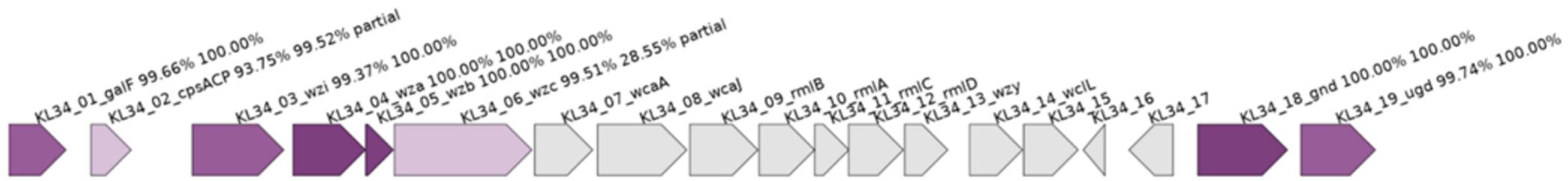
Contig name	Start position	End position
1	0	8256
66	0	3926
32	0	10690



Interpreting Kaptive output – Example of an untypeable result

ERR4920573 Best locus: KL34 Predicted type: K34 Match confidence ⓘ : Untypeable Cov ⓘ : 42.20% ID ⓘ : 99.00% Genes: 8 / 19

KL34 reference ⓘ :



Other genes found in locus ⓘ : 0 ▾ Other genes found outside locus ⓘ : 4 ▾

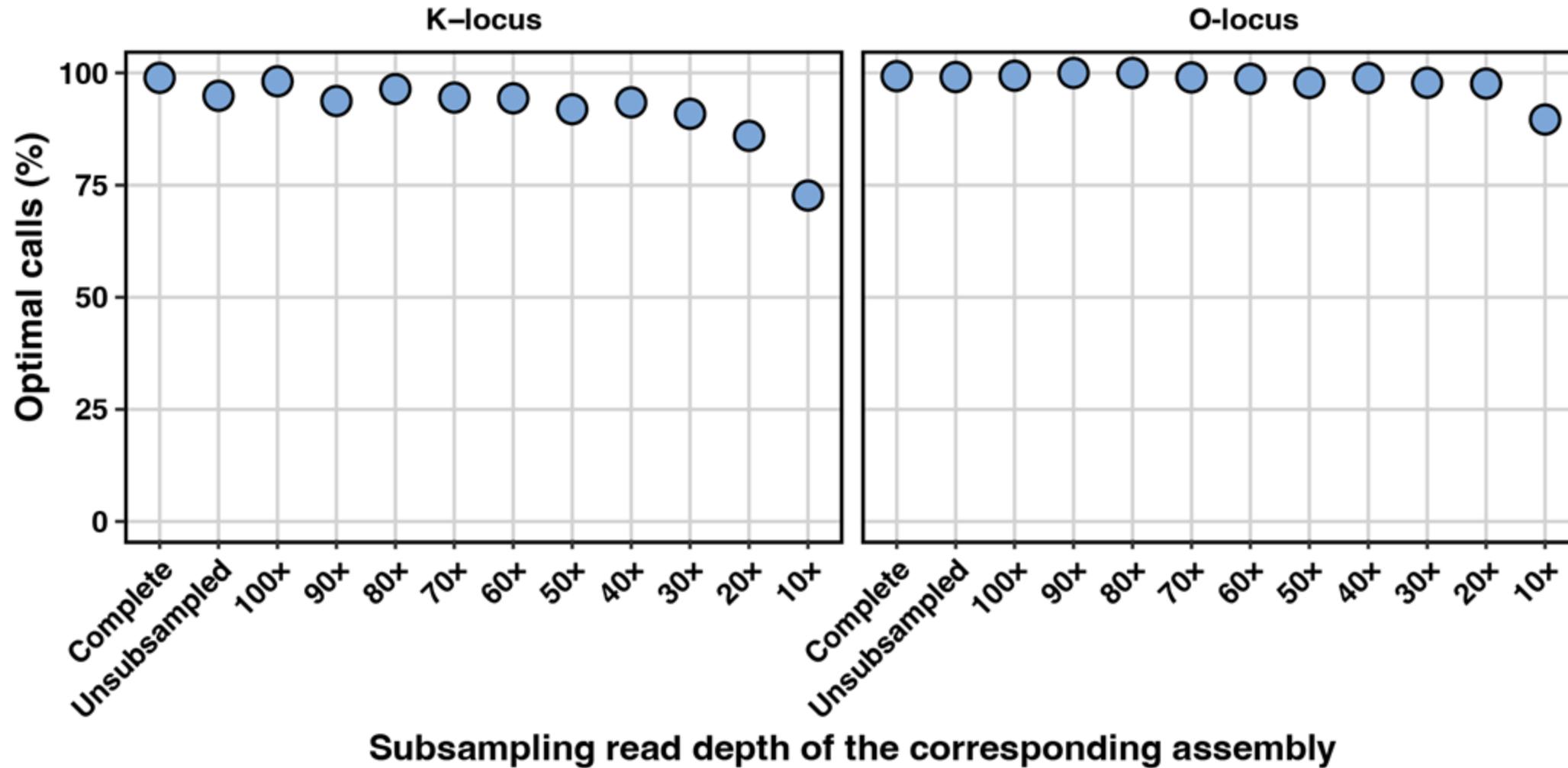
Assembly pieces ⓘ :

[Download as FASTA](#)

Contig name	Start position	End position
321	0	1913
233	0	4622
290	0	2904



Kaptive can type K- and O-loci with a very high accuracy





Kaptive can predict *K. pneumoniae* K types with high accuracy

Table 2: Serological typing outcomes for *K. pneumoniae* with matched K locus types

	N	Serological outcome			Total expected results (%)
		+ve match	+ve mismatch	-ve	
K locus associated with known serotype	634	555	34	45	555 (87.5)
K locus NOT associated with known serotype	71	n/a	30	41	41 (57.8)
Total	705	555	64	86	596 (84.5)

In summary

List of learning points in this session:

- The K and O antigens of *Kpn* are attractive vaccine targets.
- Distinct antigens are encoded by distinct sets of genes, so we can perform *in silico* serotyping from genome data with Kaptive.
- Kaptive is easy to install and interpret when the results are typeable!
- Sero-epidemiology can be performed at large scale quickly with Kaptive.

Further reading

Specific further reading for this session

Stanton TD, Hetland MAK, Löhr IH, Holt KE, Wyres KL. Fast and accurate *in silico* antigen typing with Kaptive 3. *Microb Genom.* 2025 Jun;11(6):001428. doi: 10.1099/mgen.0.001428. PMID: 40553506; PMCID: PMC12188004.

References

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- Wyres *et al* *Microb Genom* 2016. PMID: 28348840
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- Heinz *et al* *Genome Medicine* 2024. PMID: 38711148
- Stanton *et al* *Microbial Genomics* 2025. PMID: 40553506
- Stanton & Keegan *et al* *medRxiv* 2025. DOI: 10.1101/2025.06.28.25330253



KlebNET-GSP

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