



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

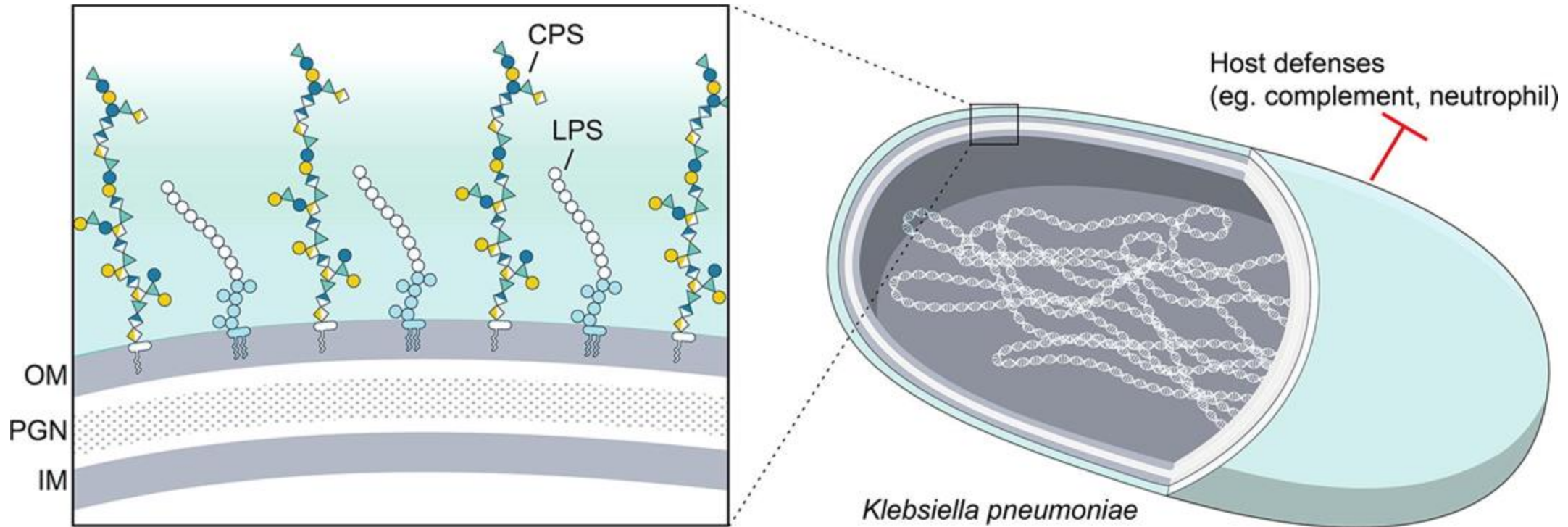
1. What are the capsule (K) and outer LPS (O) polysaccharides?
2. K and O loci
3. K and O typing with Kaptive
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K and O polysaccharides are surface molecules




- 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](#)

 **ELSEVIER**

Vaccine

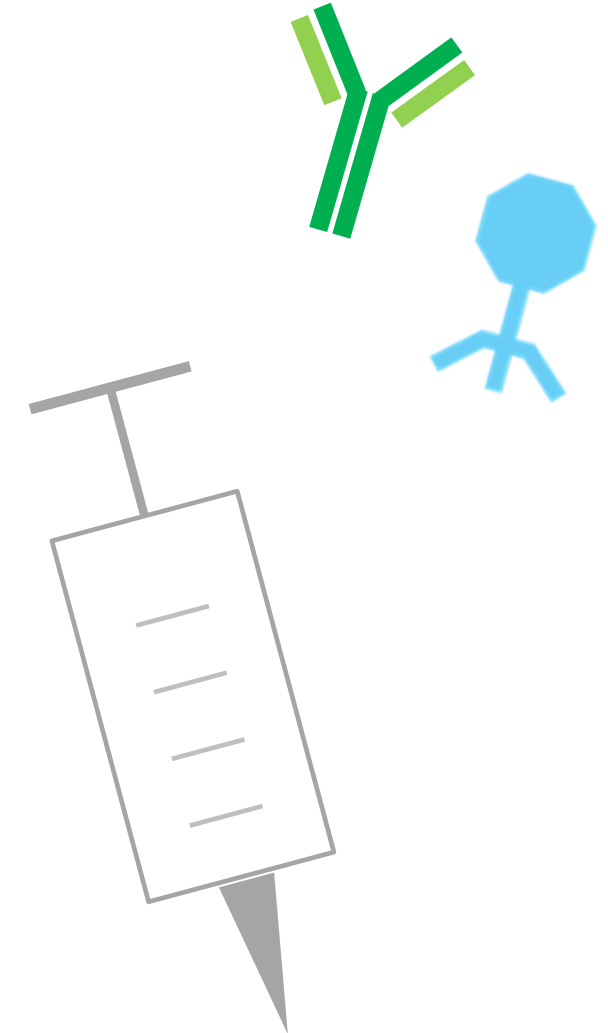
journal homepage: www.elsevier.com/locate/vaccine





Vaccine value profile for *Klebsiella pneumoniae*

Ziyaad Dangor^{a,*}, Nicole Benson^b, James A. Berkley^{c,d}, Julia Bielicki^{e,f}, Merijn W. Bijlma^{g,h}, Jonathan Broadⁱ, Ed T. Buurman^j, Alan Cross^k, Erin M. Duffy^j, Kathryn E. Holt^{l,m}, Pui-Ying Iroh Tamⁿ, Mark Jit^o, Konstantinos Karampatsas^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 \neq structure

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

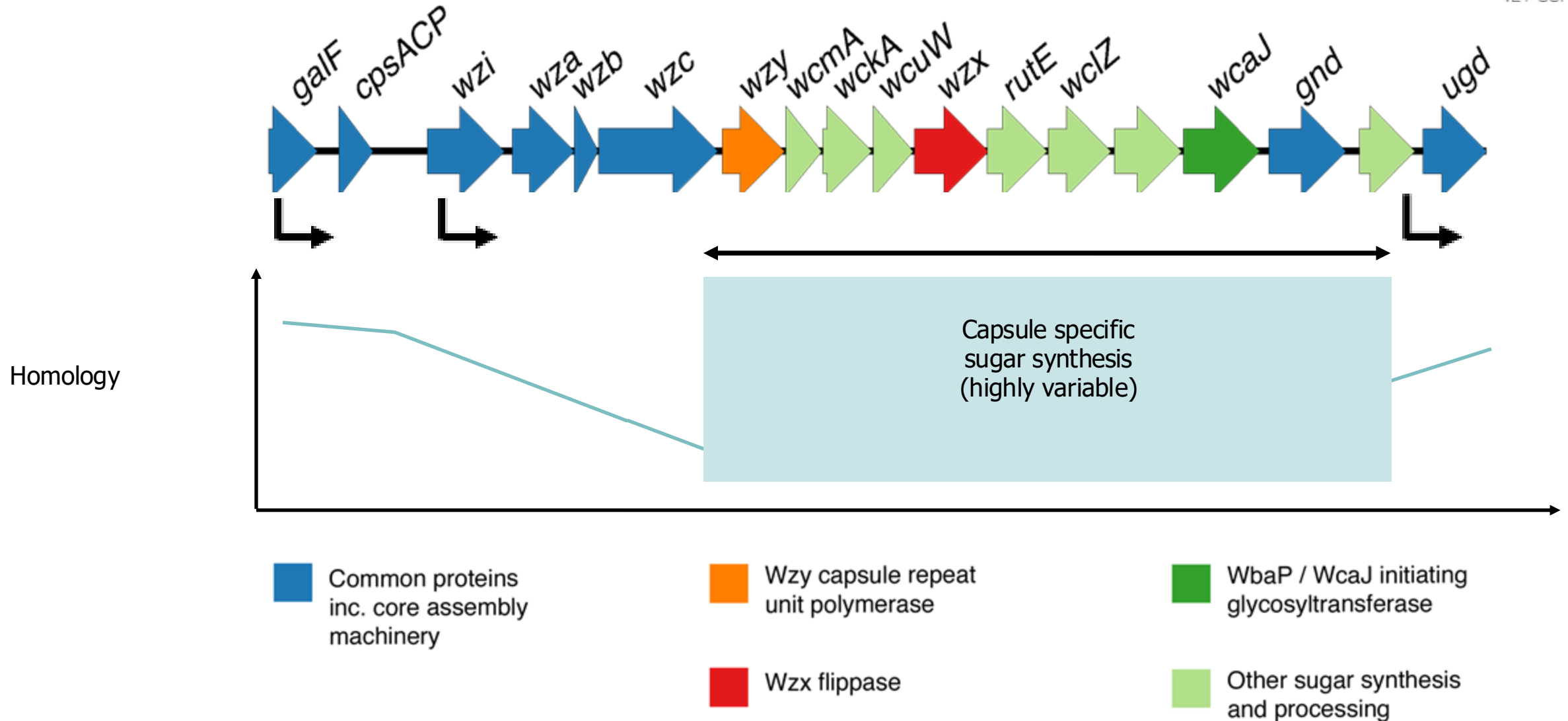
* +5 unpublished, Stanton *et al in prep*

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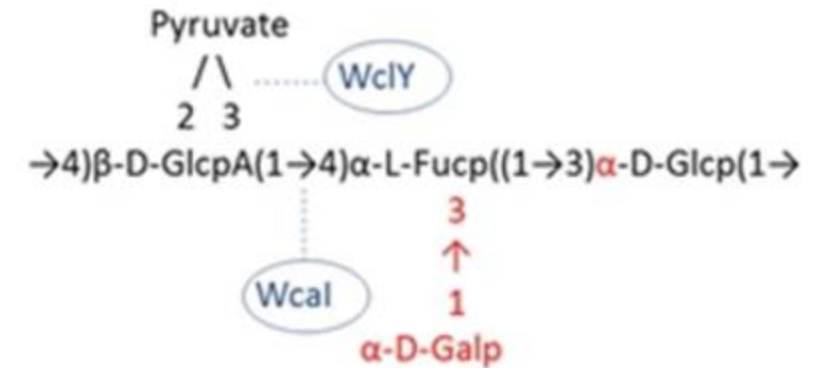
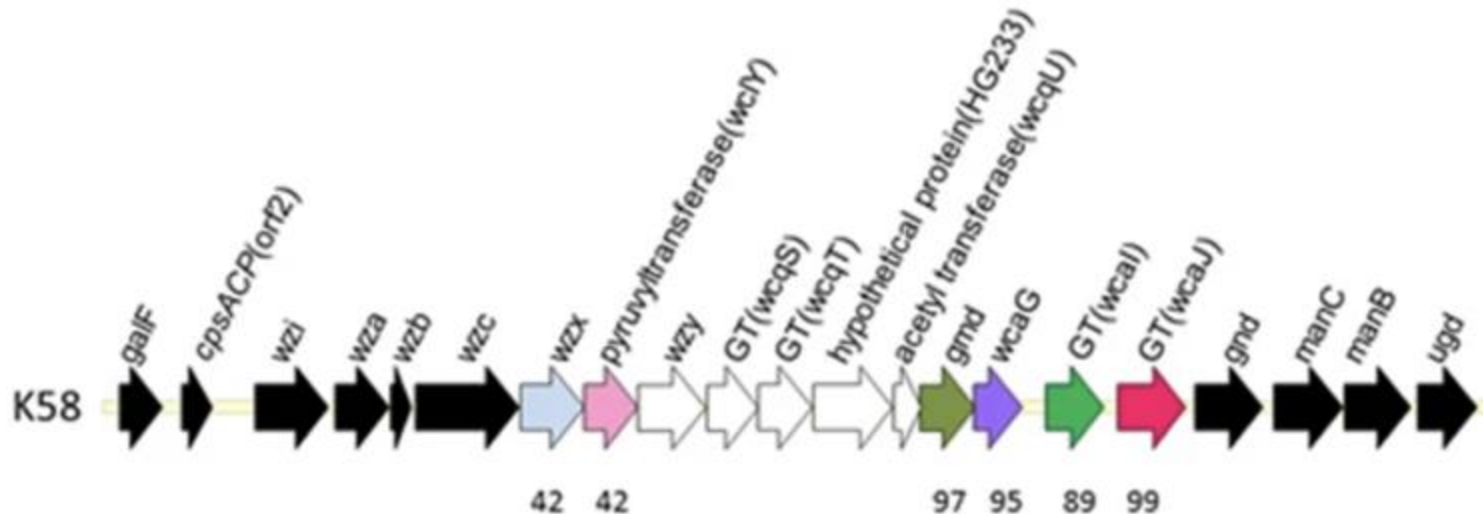
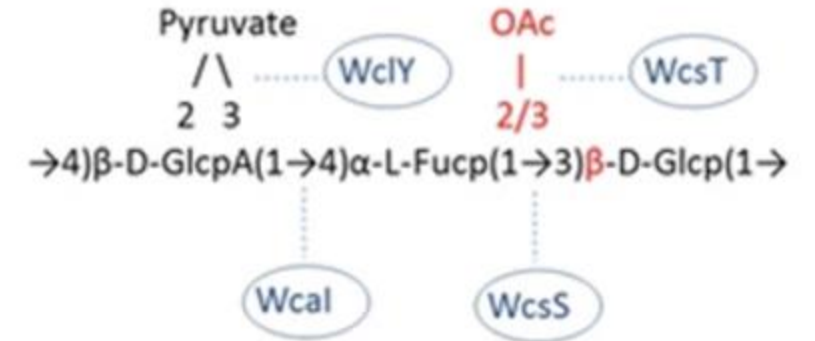
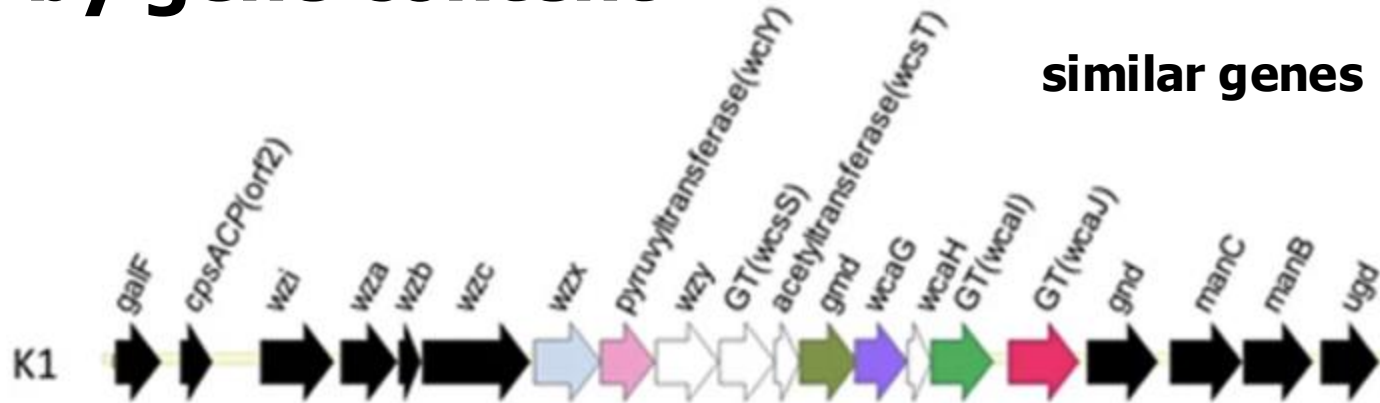
1. What are the capsule (K) and outer LPS (O) polysaccharides?
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The *Klebsiella* capsule (K) locus

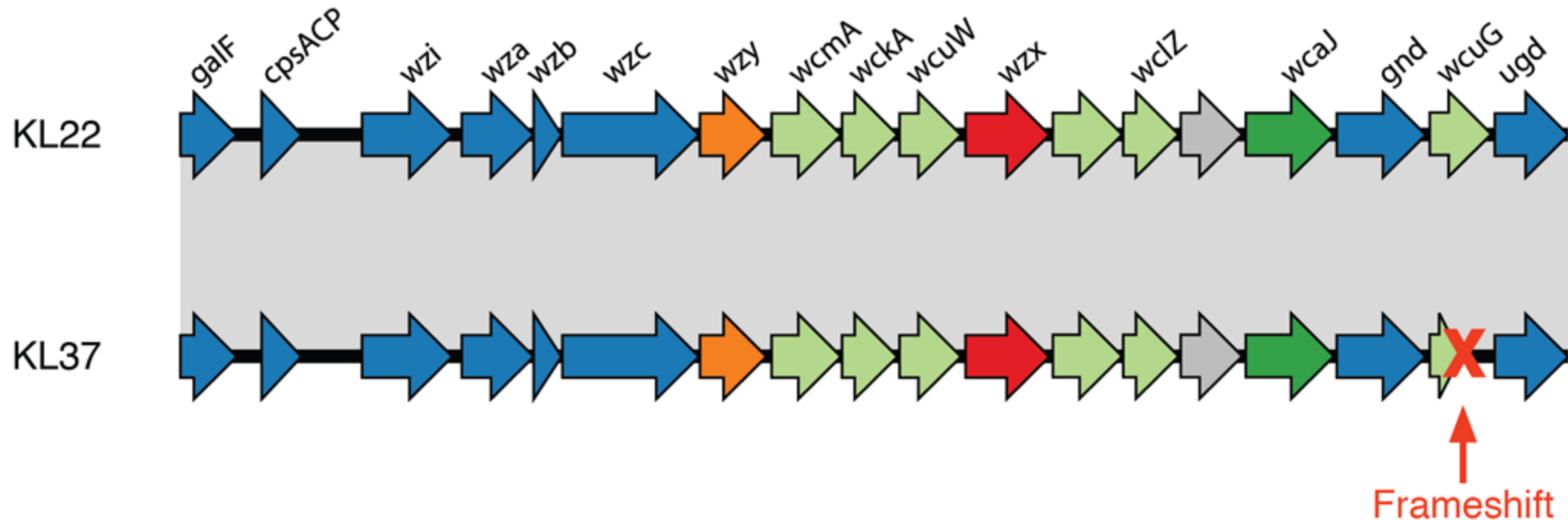


Serotype reference loci can (mostly) be distinguished by gene content

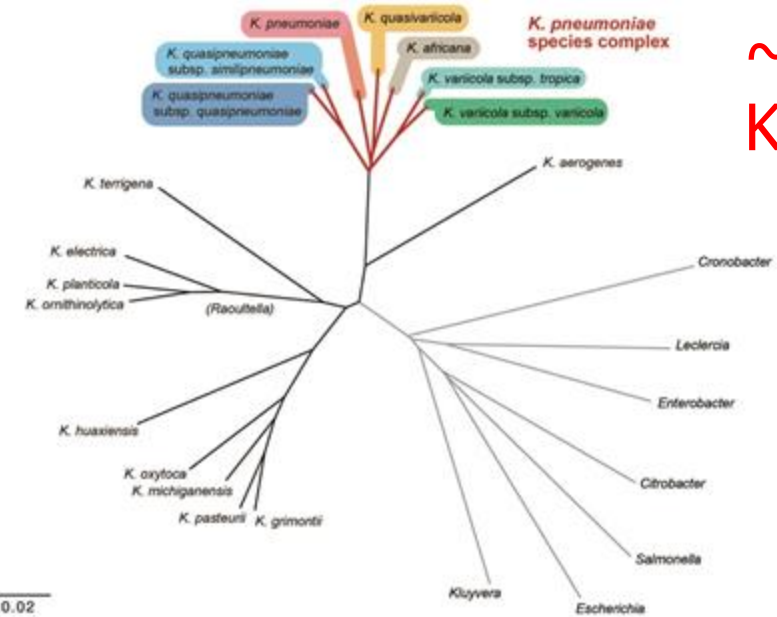
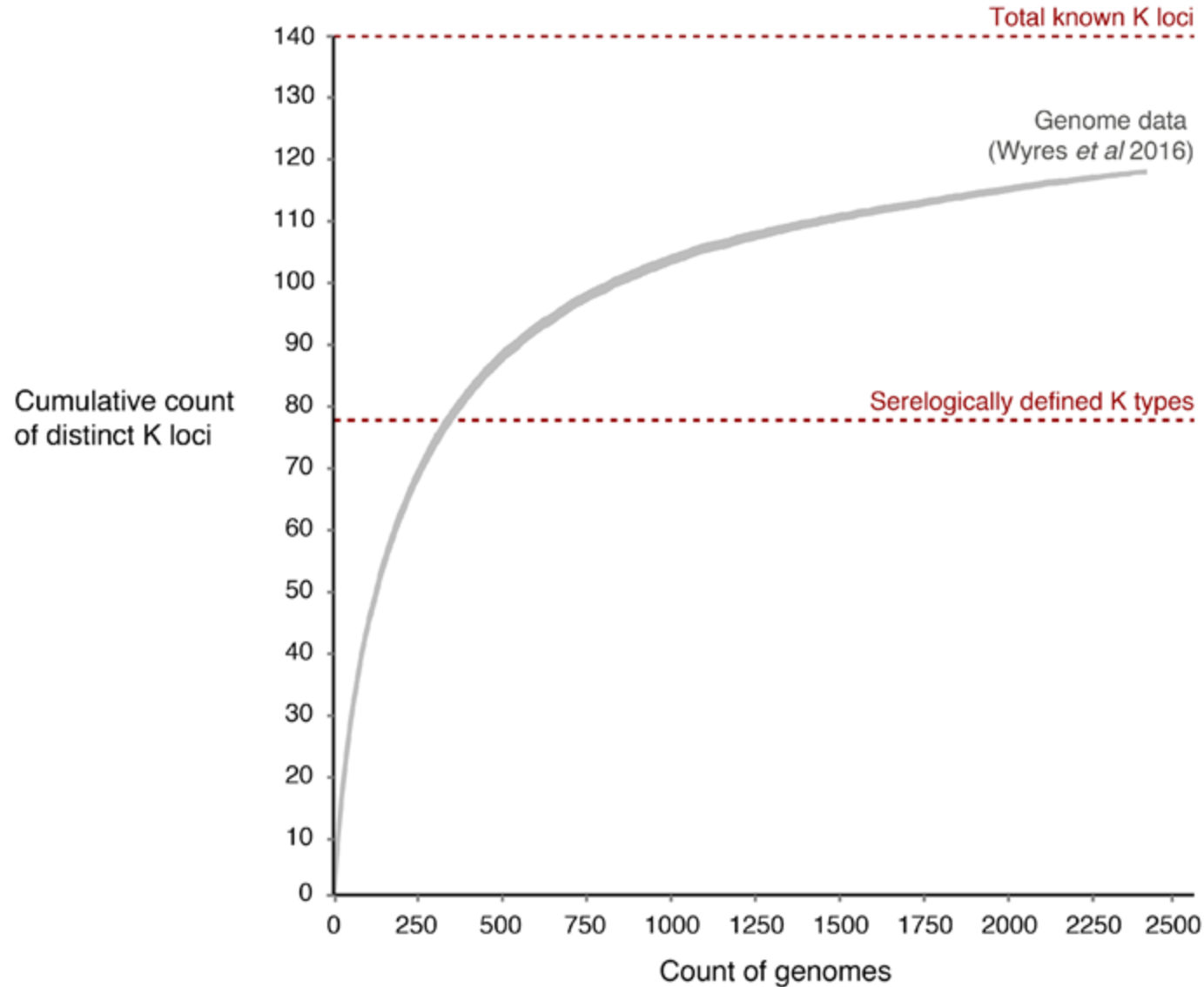
similar genes = similar capsule structure



Klebsiella K22 and K37 reference loci are the exception



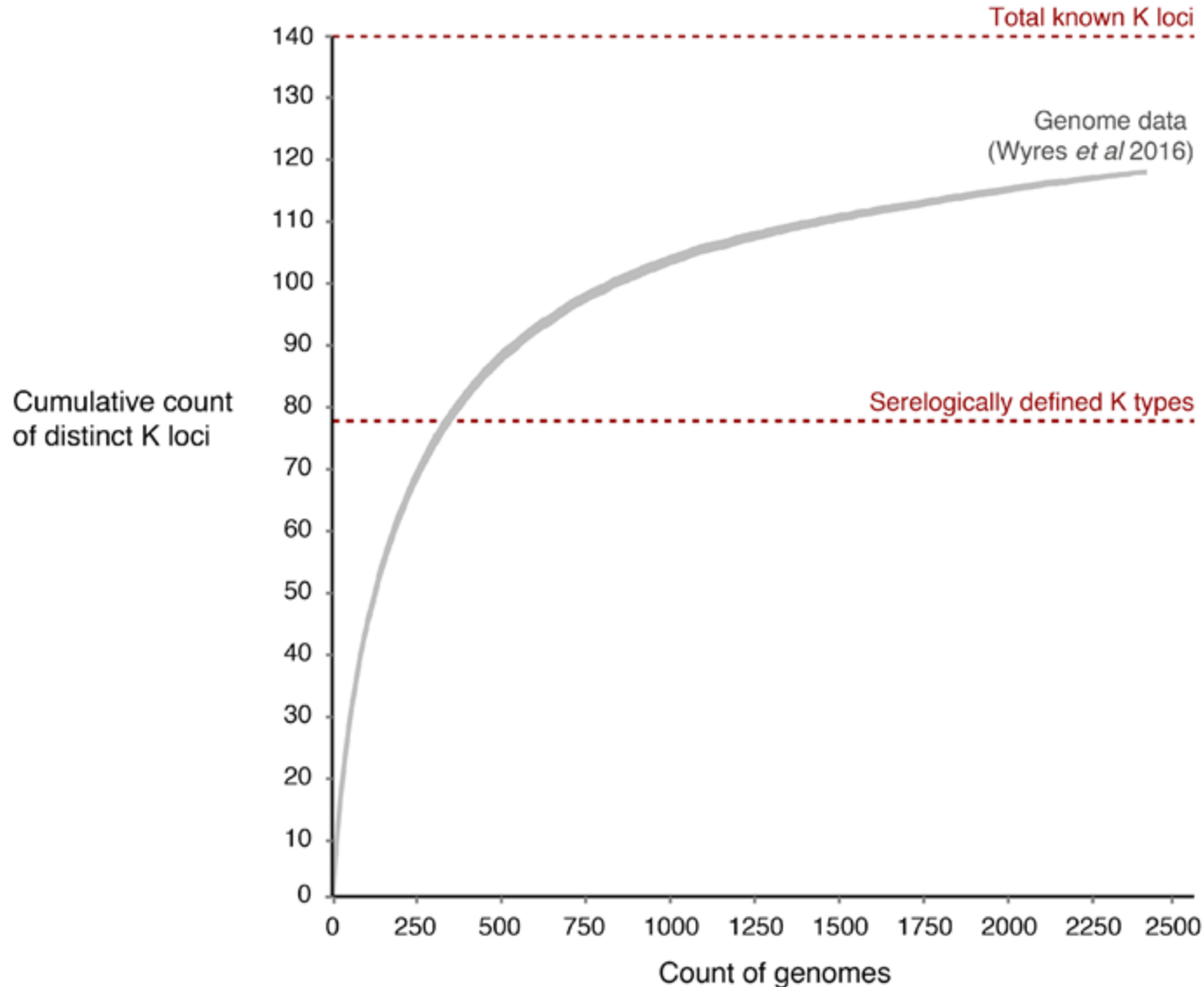
More than 60 additional K loci defined on the basis of gene content



~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

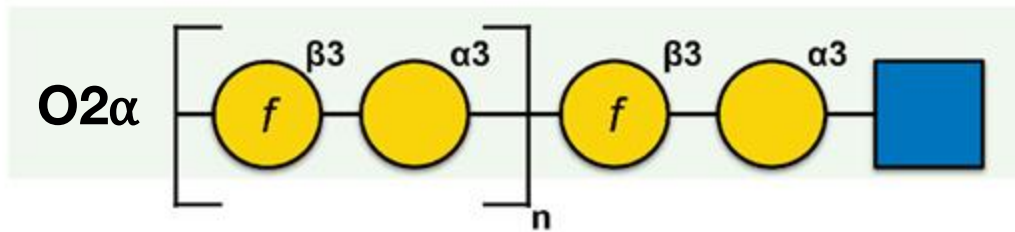


Genetics and Molecular Biology | Review

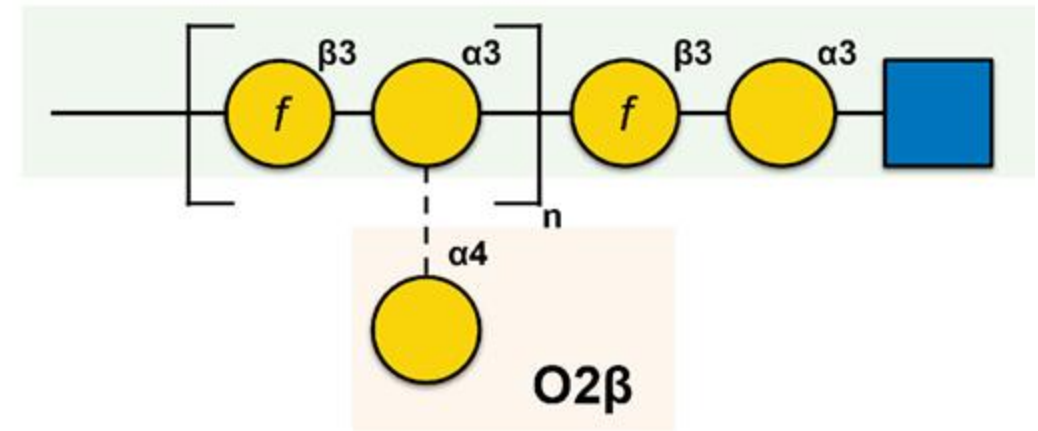
O-antigen polysaccharides in *Klebsiella pneumoniae*: structures and molecular basis for antigenic diversity

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



OL2_α



OL2_α + *gml*2_β

Nomenclature

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

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

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

Type = predicted **polysaccharide phenotype**

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

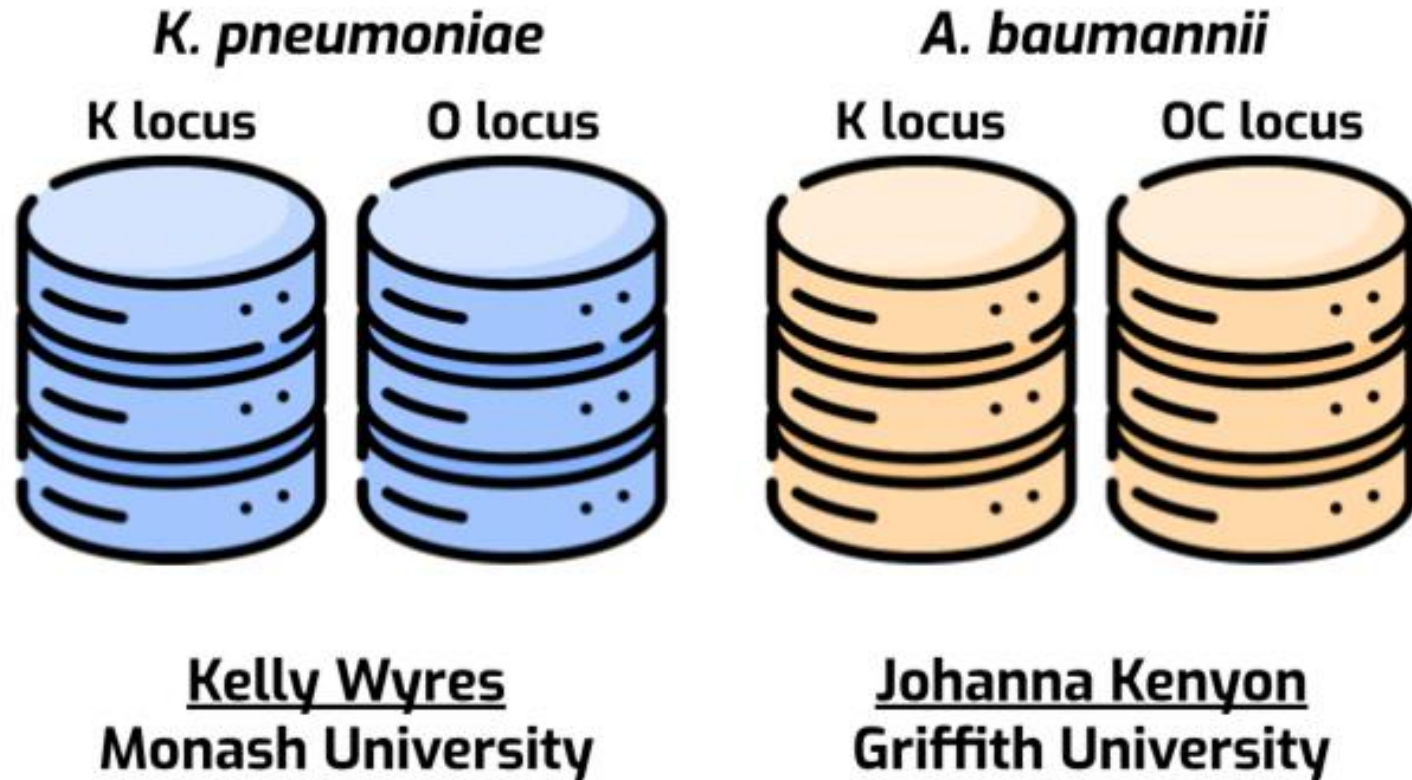
- locus + truncations within locus + additional genes

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Kaptive – databases and tools for bacterial surface polysaccharide locus typing



E. coli K (group 2 / 3)

Rebecca Gladstone
University of Oslo

In development:

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

Kaptive papers:

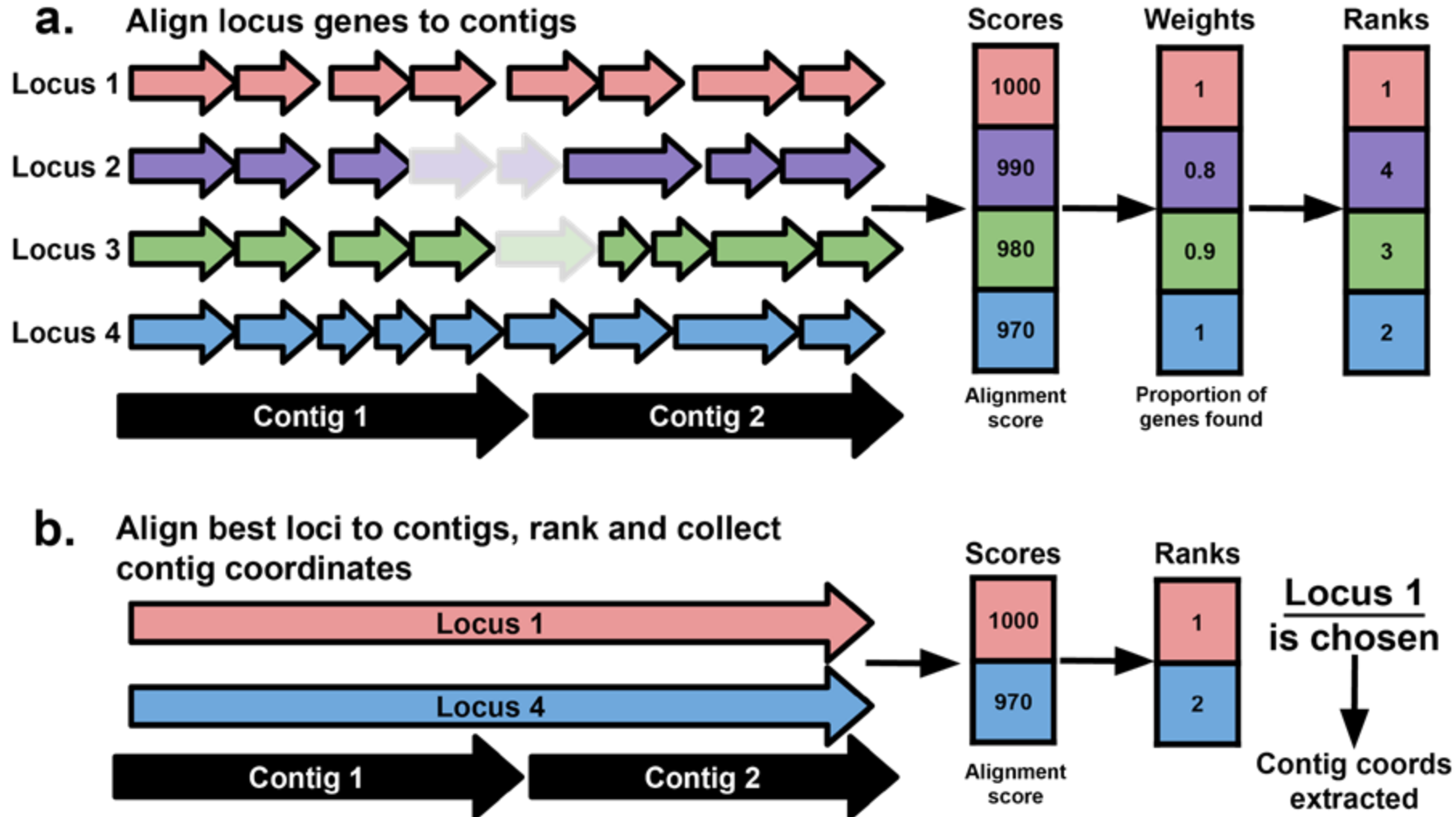
Wyres *et al Microbial Genom* 2016

Wick *et al JCM* 2018

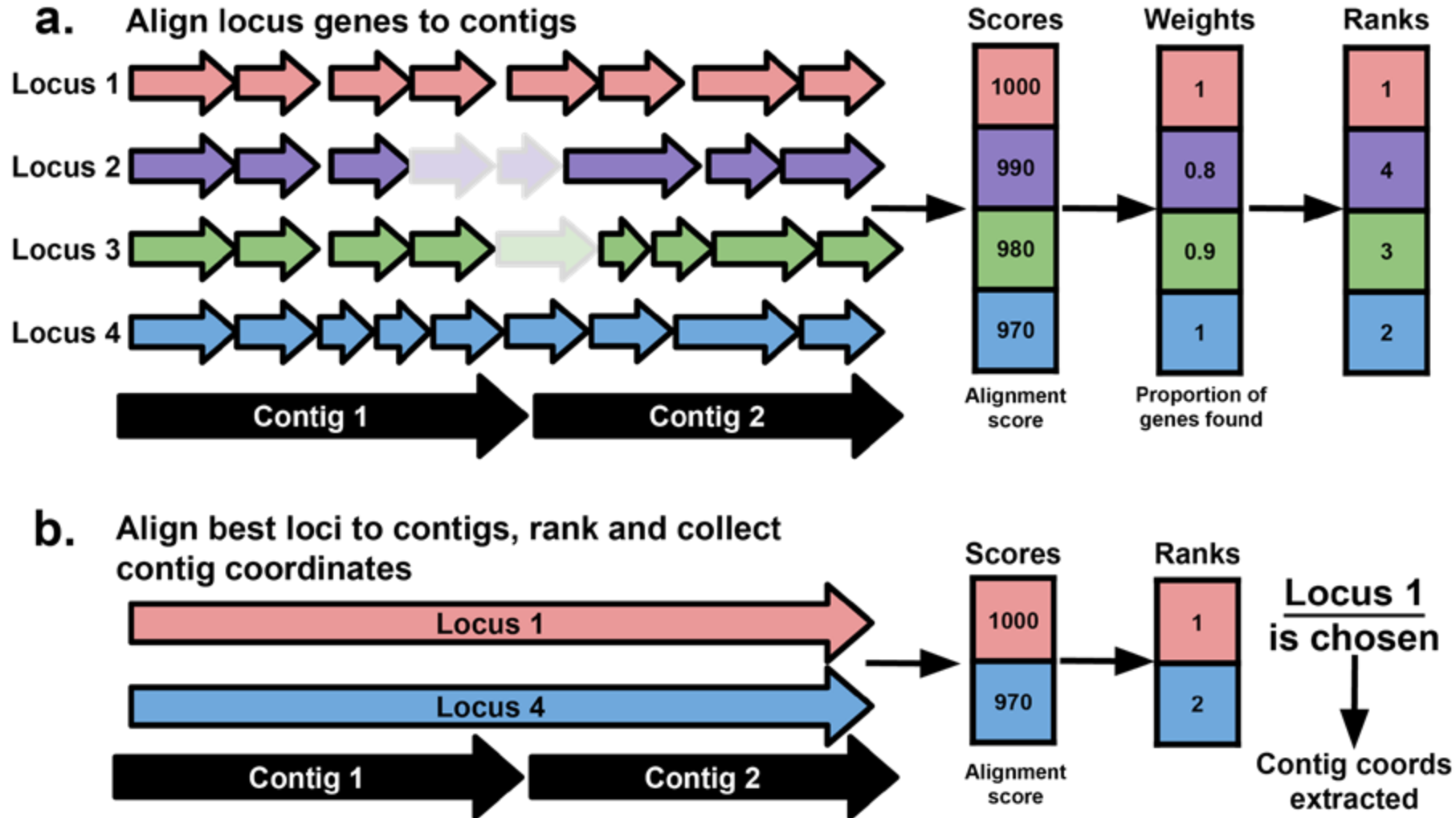
Lam *et al Microbial Genom* 2022

Stanton *et al Microbial Genom* 2025

How Kaptive (v3) works



How Kaptive (v3) works



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:

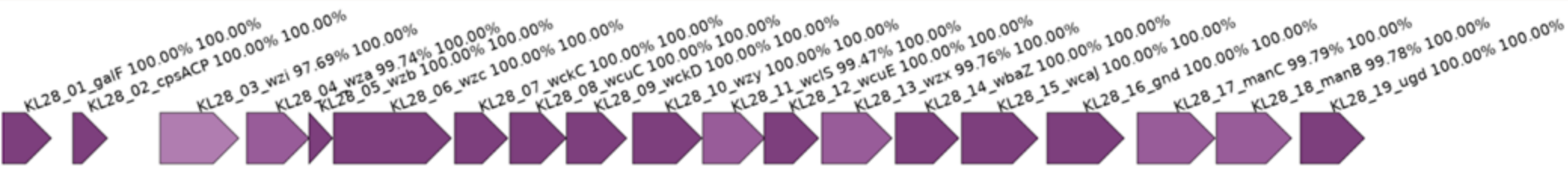
Assembly	Best match lo	Best match type	Match confidence	Problems	Identity	Coverage	Length discrepancy	Expected g
assembly_1	KL28	K28	Typeable		100.00%	100.00%	0 bp	19 / 19 (100
assembly_2	KL5	K5	Typeable		97.67%	100.04%	-8 bp	19 / 19 (100
CC1_001000415	KL1	unknown (K1.1)	Typeable		99.99%	99.99%	0 bp	99 / 99 (100

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
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KL28 reference ⓘ :



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

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

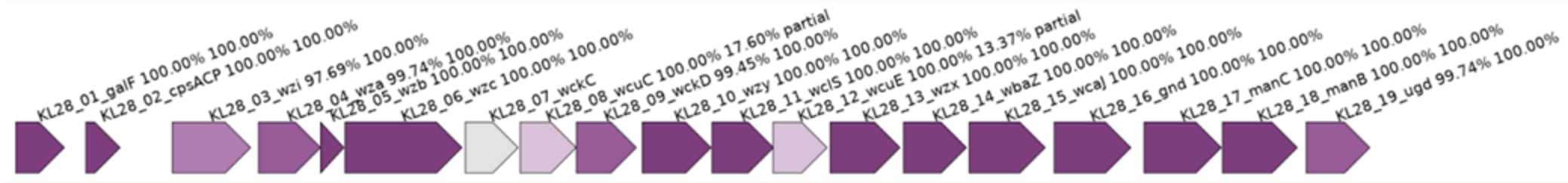
Contig name	Start position	End position
12	134125	158956

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
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KL28 reference ⓘ :



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

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

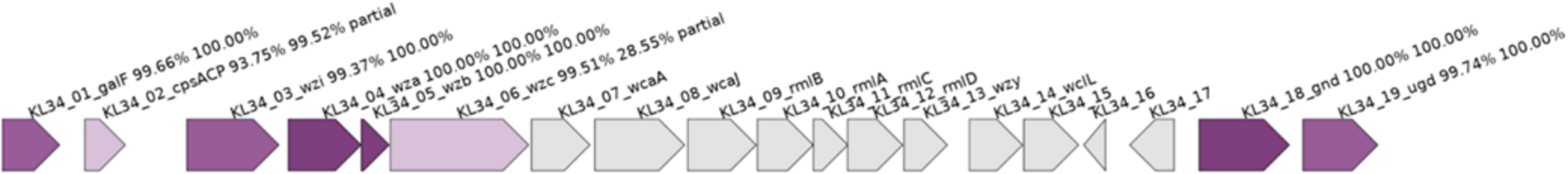
Contig name	Start position	End position
1	0	8256
66	0	3926
32	0	10690

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
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KL34 reference ⓘ :

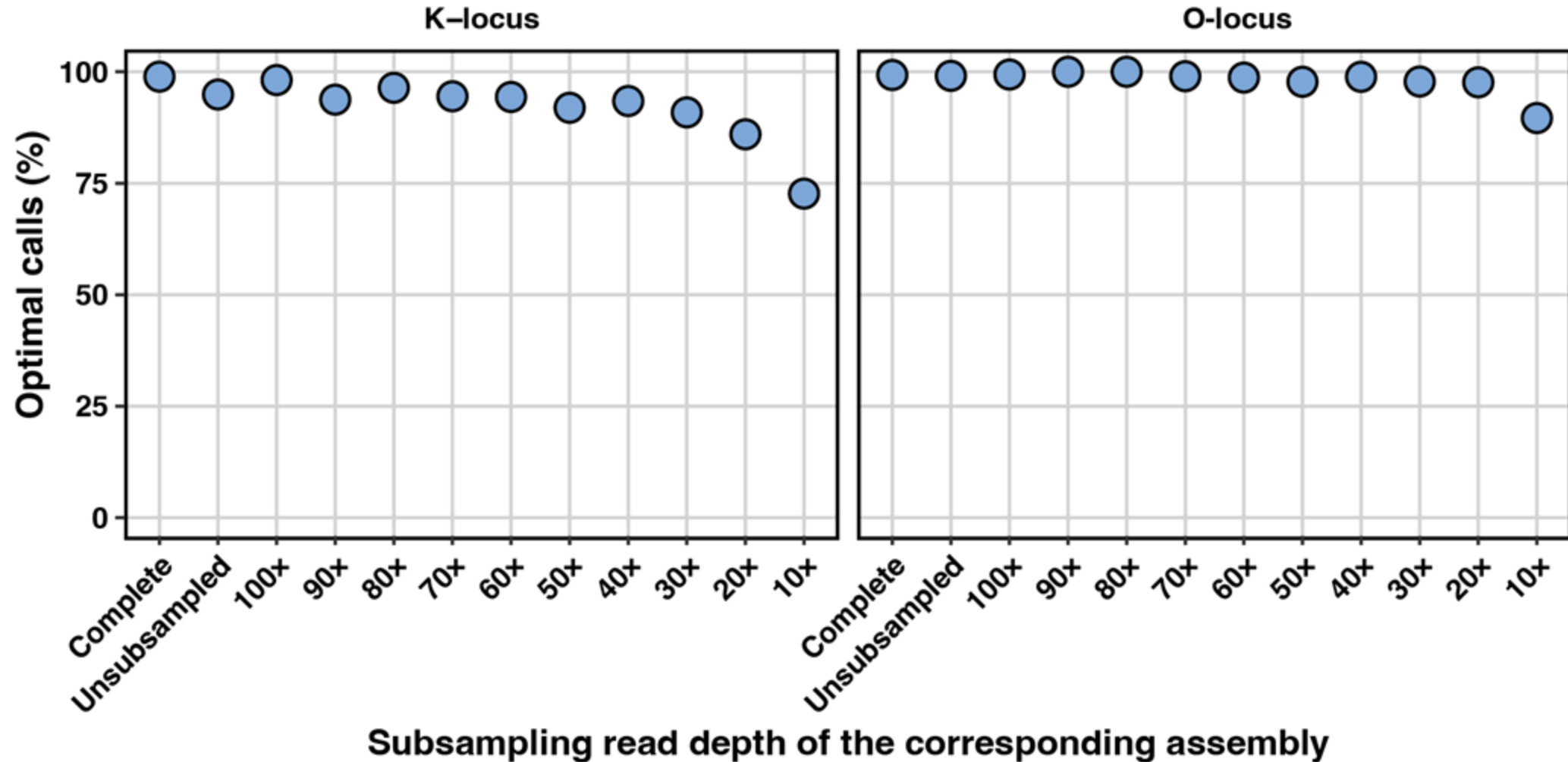


Other genes found in locus ⓘ : 0 ▼ Other genes found outside locus ⓘ : 4 ▼

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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- Lam *et al Nature Communications* 2021. PMID: 34234121
- 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

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