Klebsiella pneumoniae genomic epidemiology and antimicrobial resistance

In silico serotyping of *Klebsiella*

<Creation date of the training material>

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
4. How to use Kaptive
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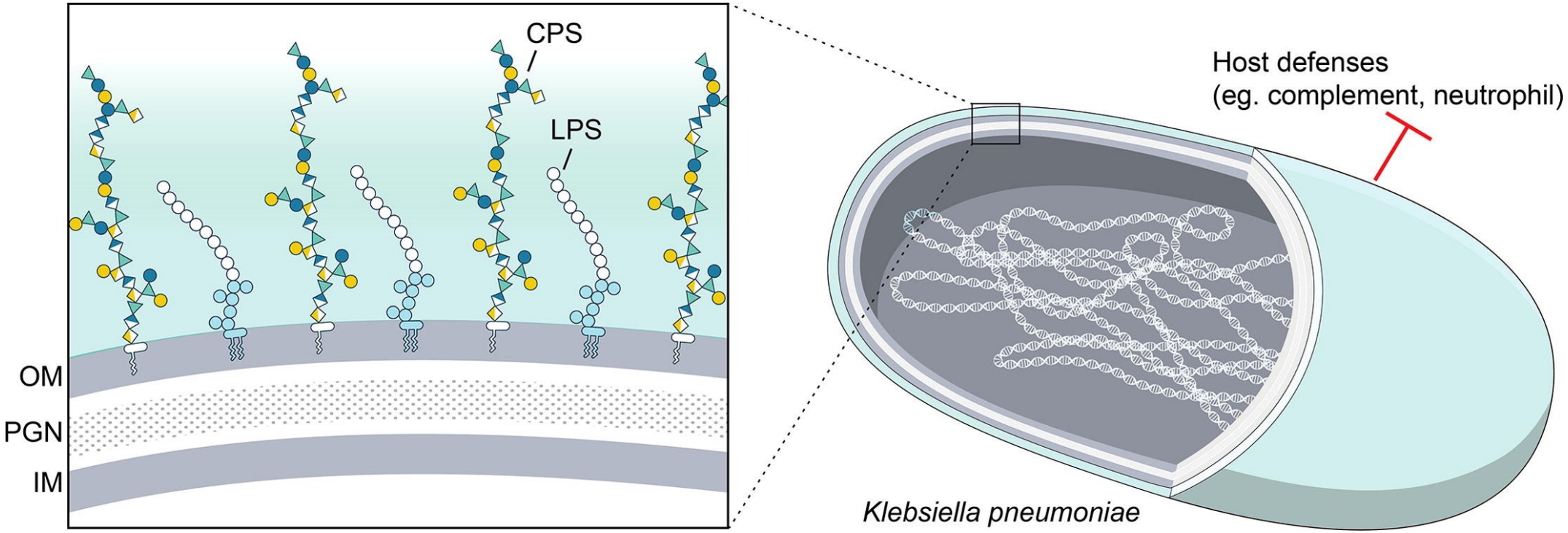
K and O polysaccharides are surface molecules

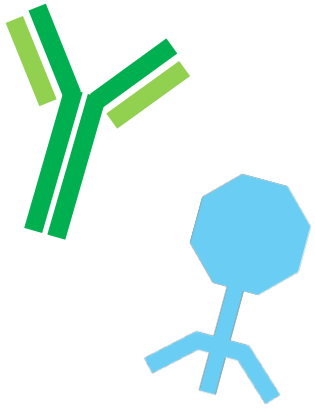


- Protects bacteria from desiccation
- Required for virulence
- Immunogenic

Image from: Opoku-Temeng *et al Comput Struct Biotechnol J* 2019

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

Vaccine value profile for *Klebsiella pneumoniae*

Ziyaad Dangor [a,*], Nicole Benson [b], James A. Berkley [c,d], Julia Bielicki [e,f], Merijn W. Bijsma [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 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 ≠ structure

		Klebsiella	*E. coli*
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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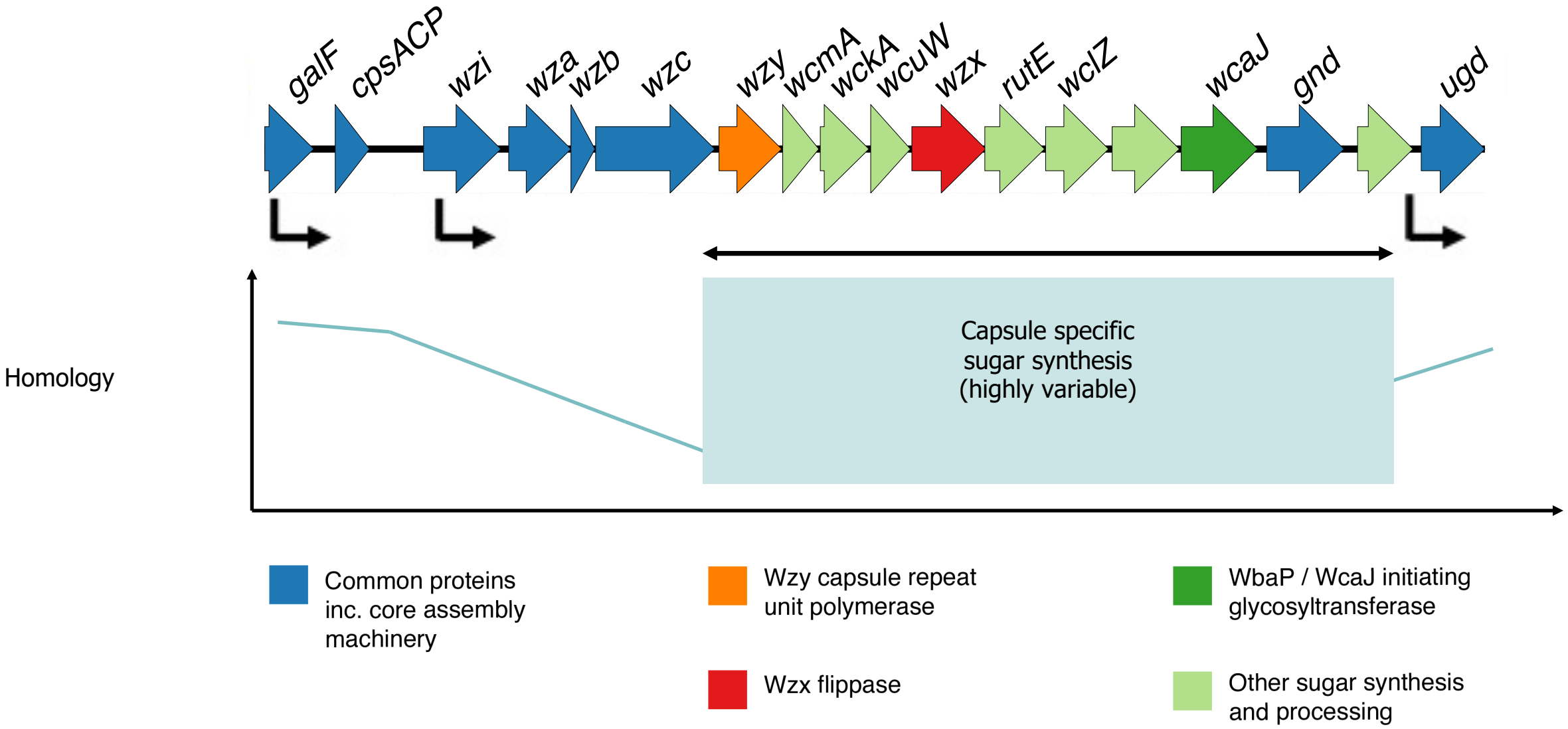
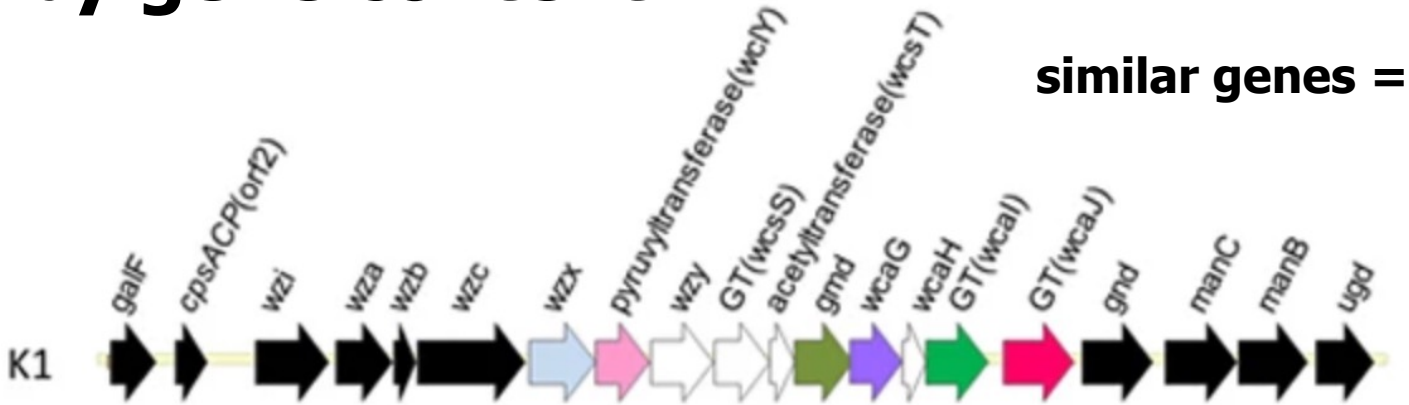
The *Klebsiella* capsule (K) locus

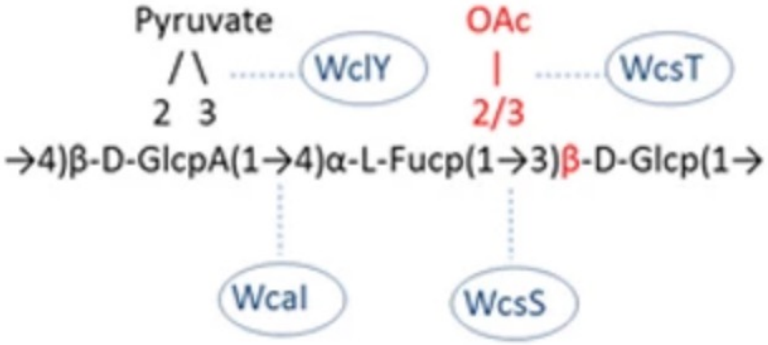


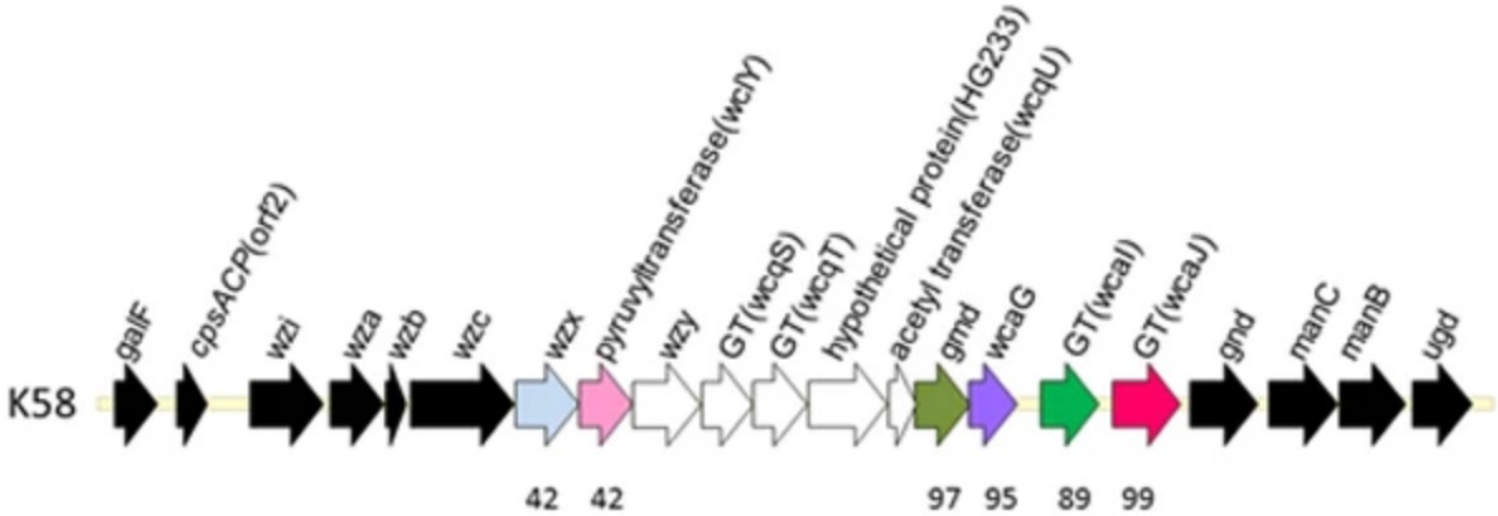
Wyres *et al. Microbial Genomics* 2016

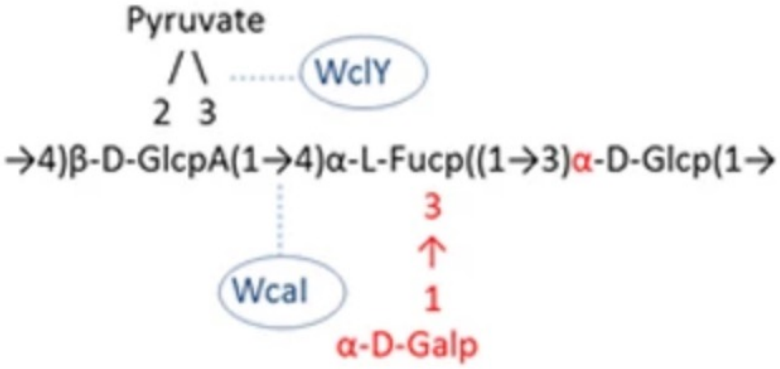
Serotype reference loci can (mostly) be distinguished by gene content

similar genes = similar capsule structure



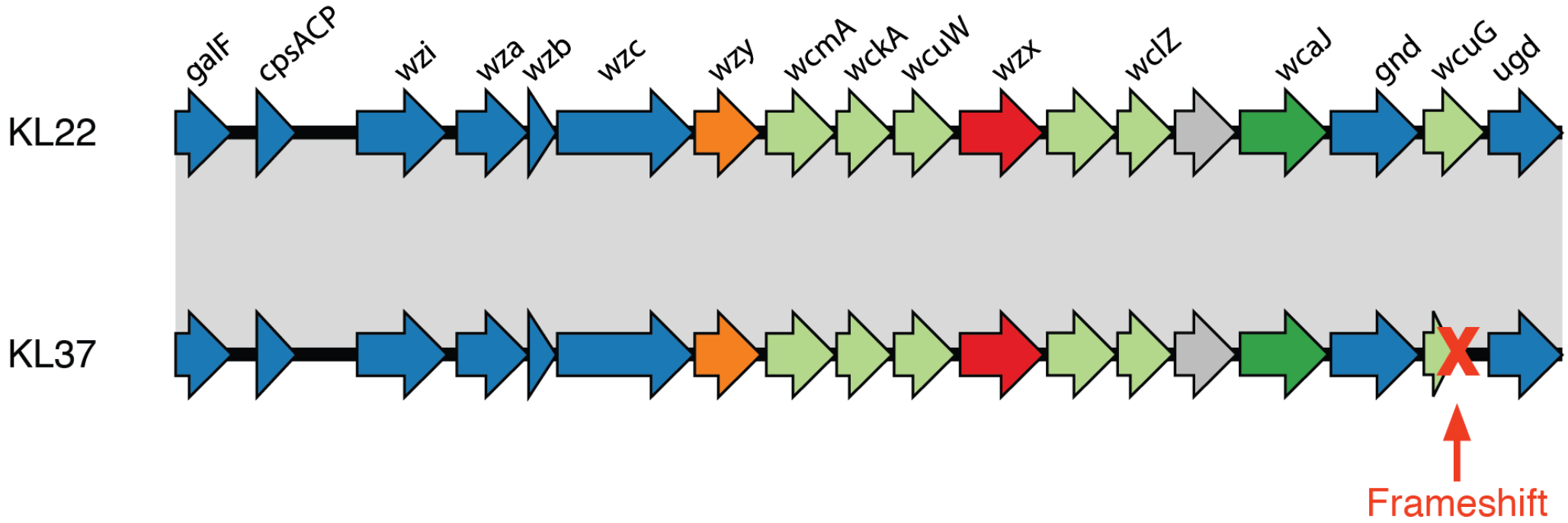






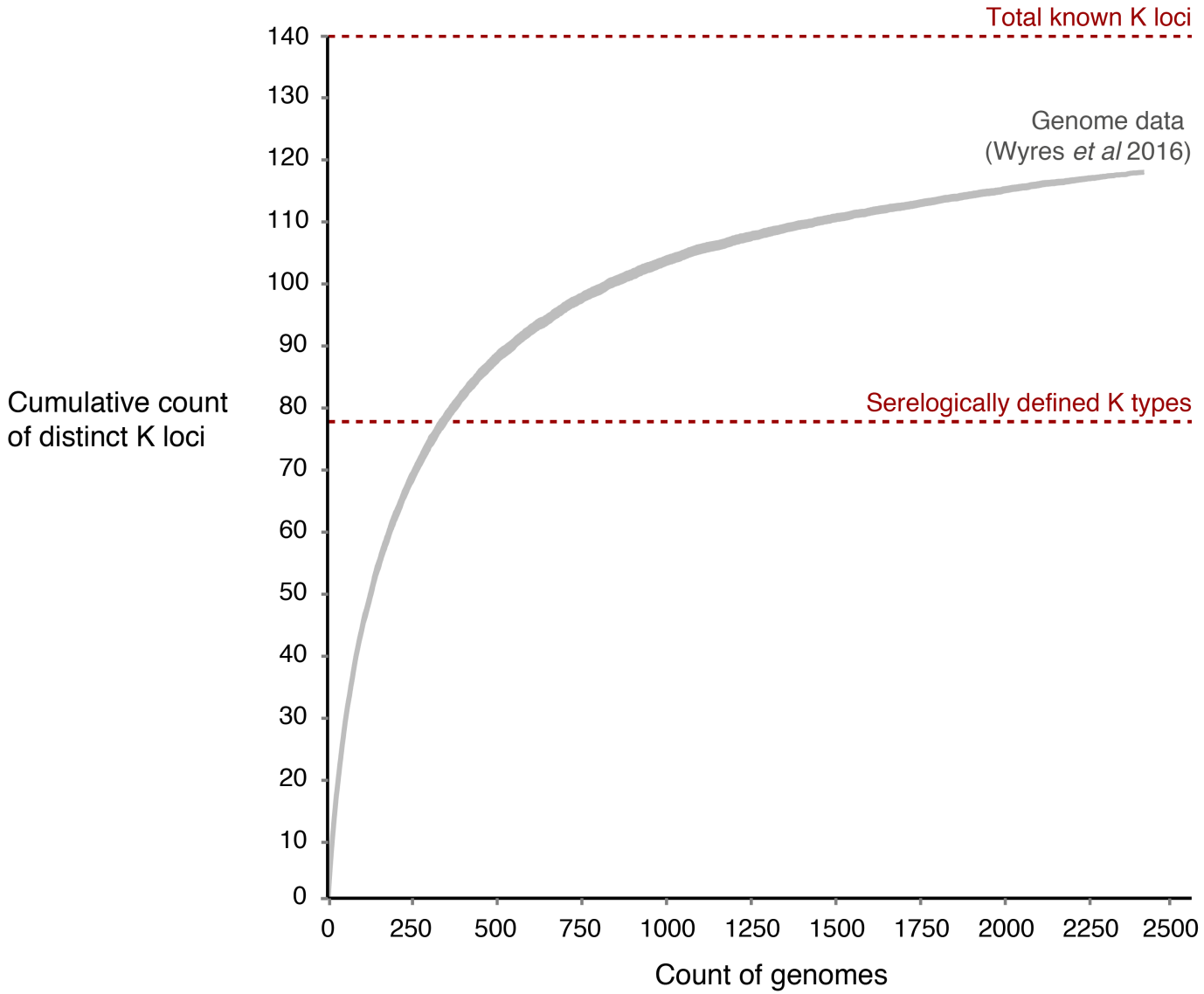
Pan *et al Sci Rep* 2015

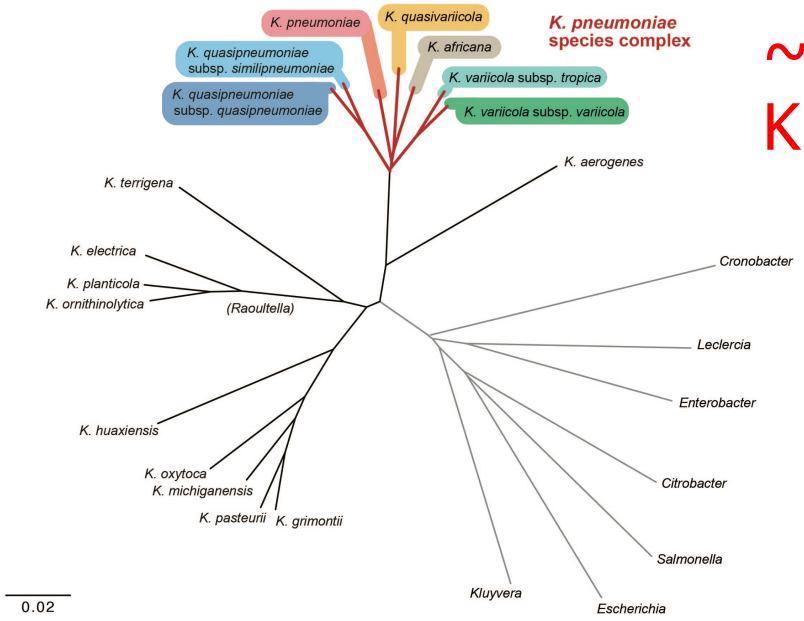
Klebsiella K22 and K37 reference loci are the exception



Pan *et al Sci Rep* 2015 – genetic analysis of K-loci for the 79 capsular serotypes

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

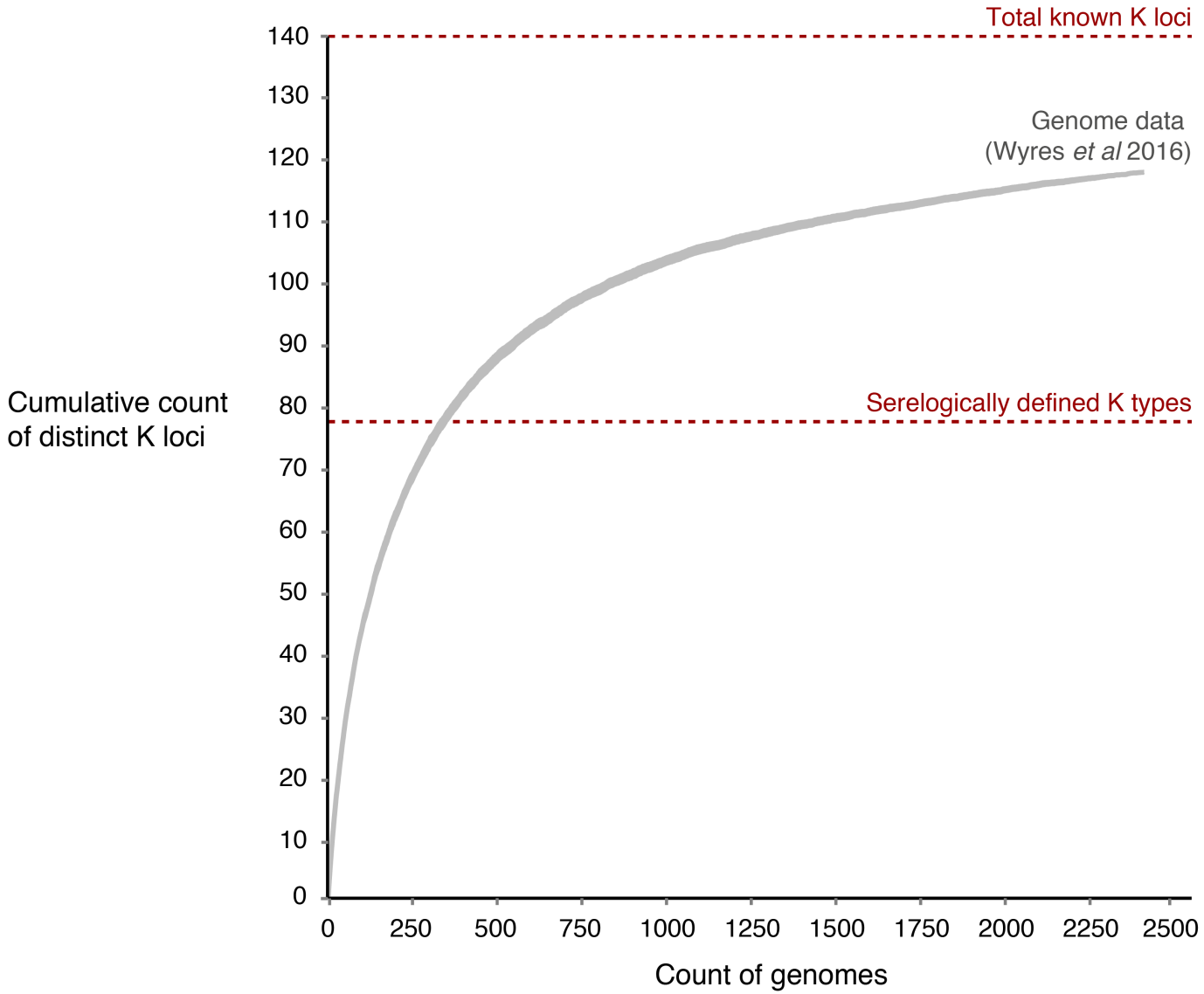




~2500 KpSC

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

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology and Molecular Biology Reviews

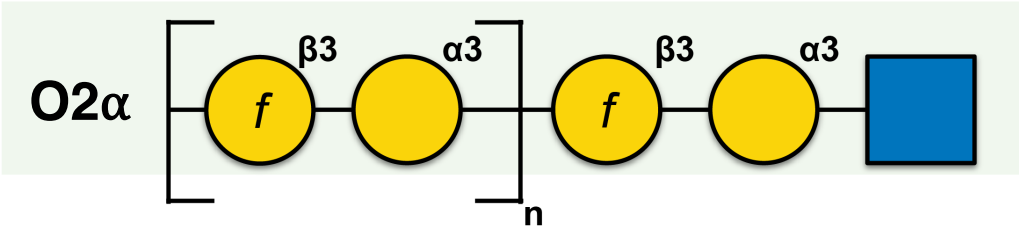
Check for updates

Genetics and Molecular Biology | Review

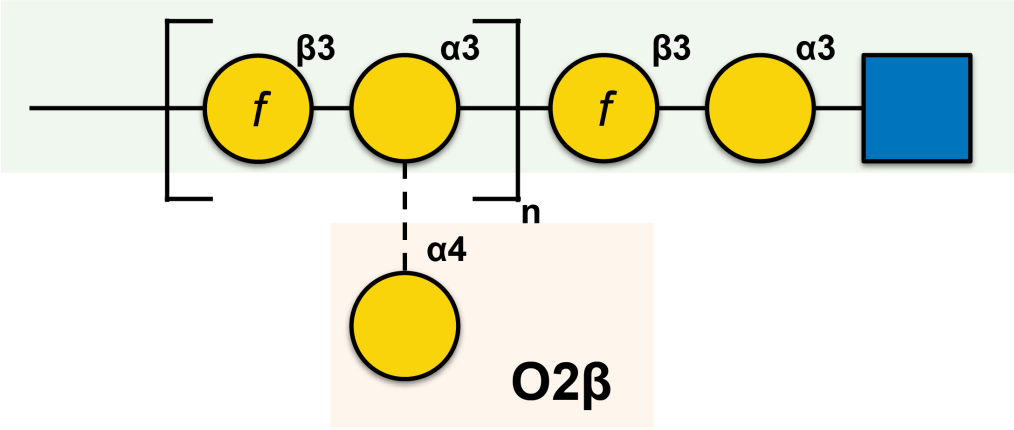
O-antigen polysaccharides in *Klebsiella pneumoniae*: structures and molecular basis for antigenic diversity

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 Kowalczyk[1]

Klebsiella O structure variation requires O loci & additional genes



OL2α



OL2α + *gml2β*

Whitfield *et al MMBR* 2025

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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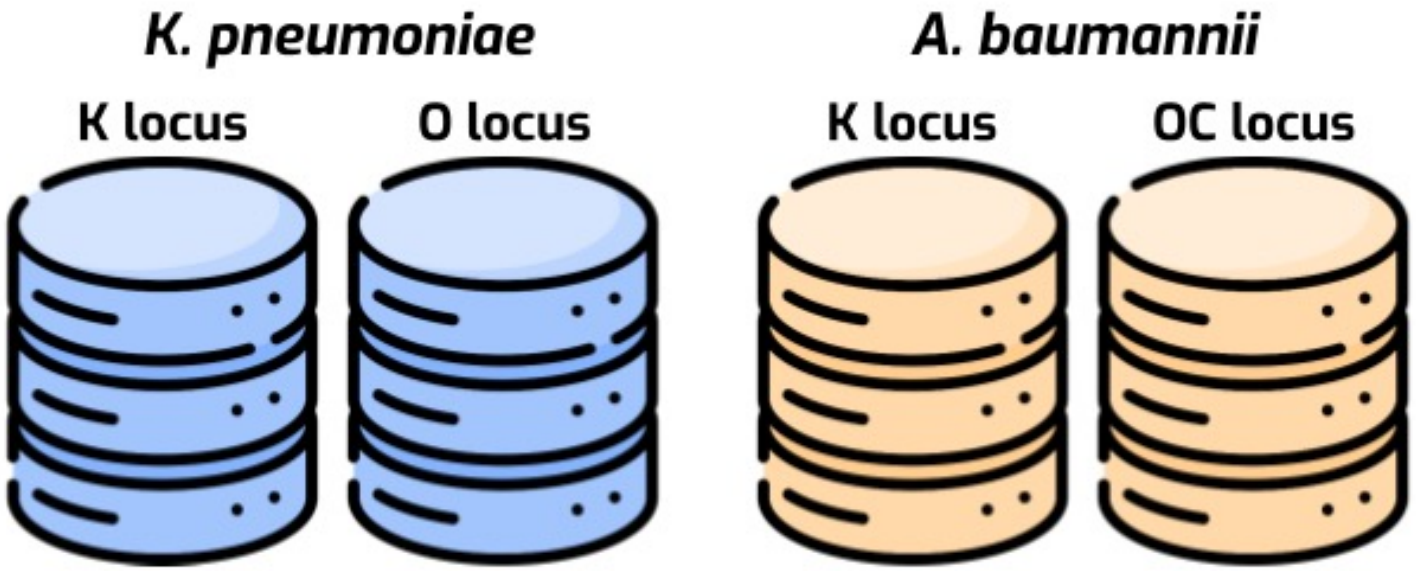
Kaptive – databases and tools for bacterial surface polysaccharide locus typing



Kelly Wyres
Monash University

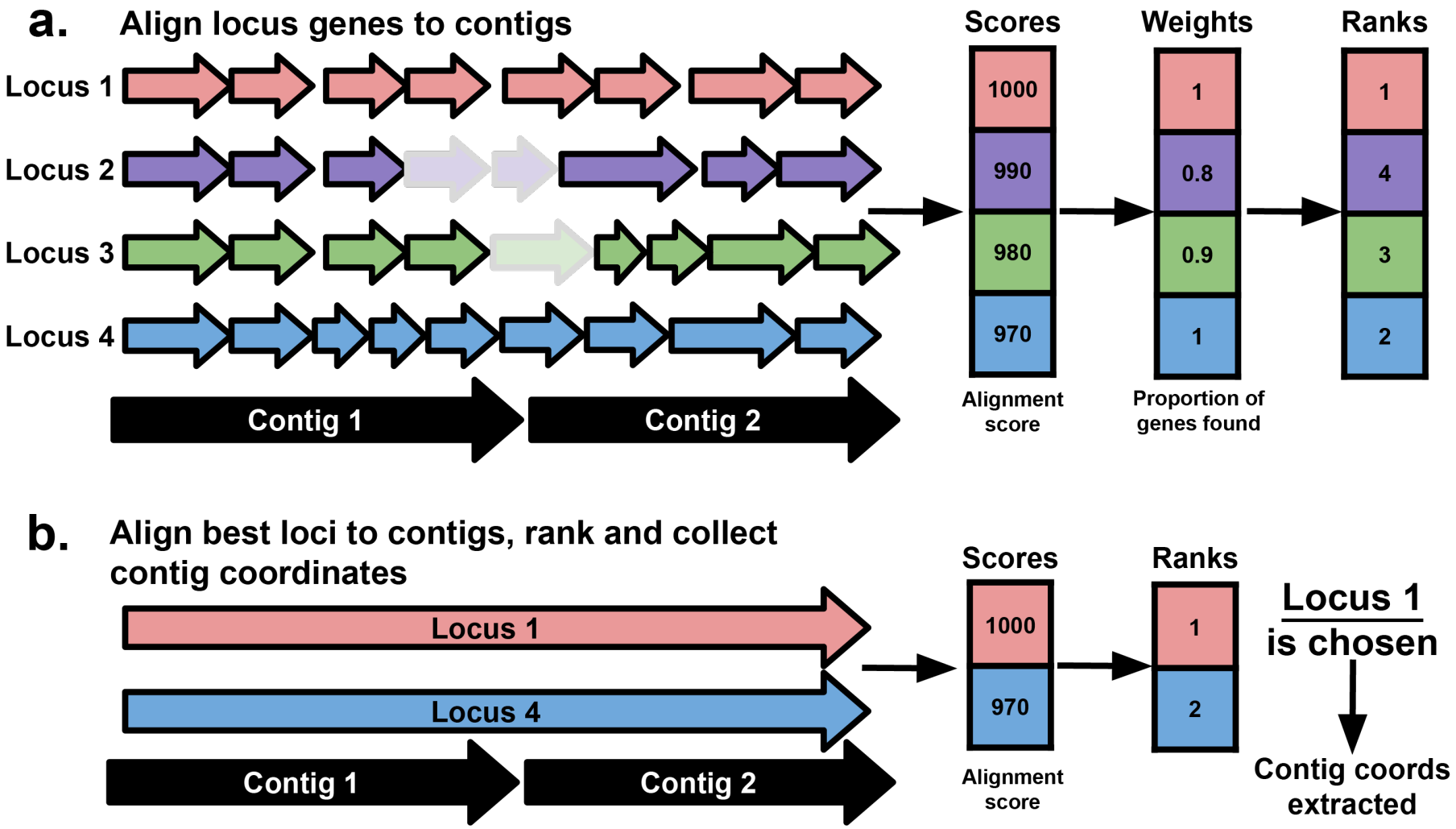
Johanna Kenyon
Griffith University

***E. coli* K (group 2 / 3)**
Rebecca Gladstone
University of Oslo

In development:
***E. coli* K (group 1 / 4)**
***Klebsiella oxytoca* K and O**

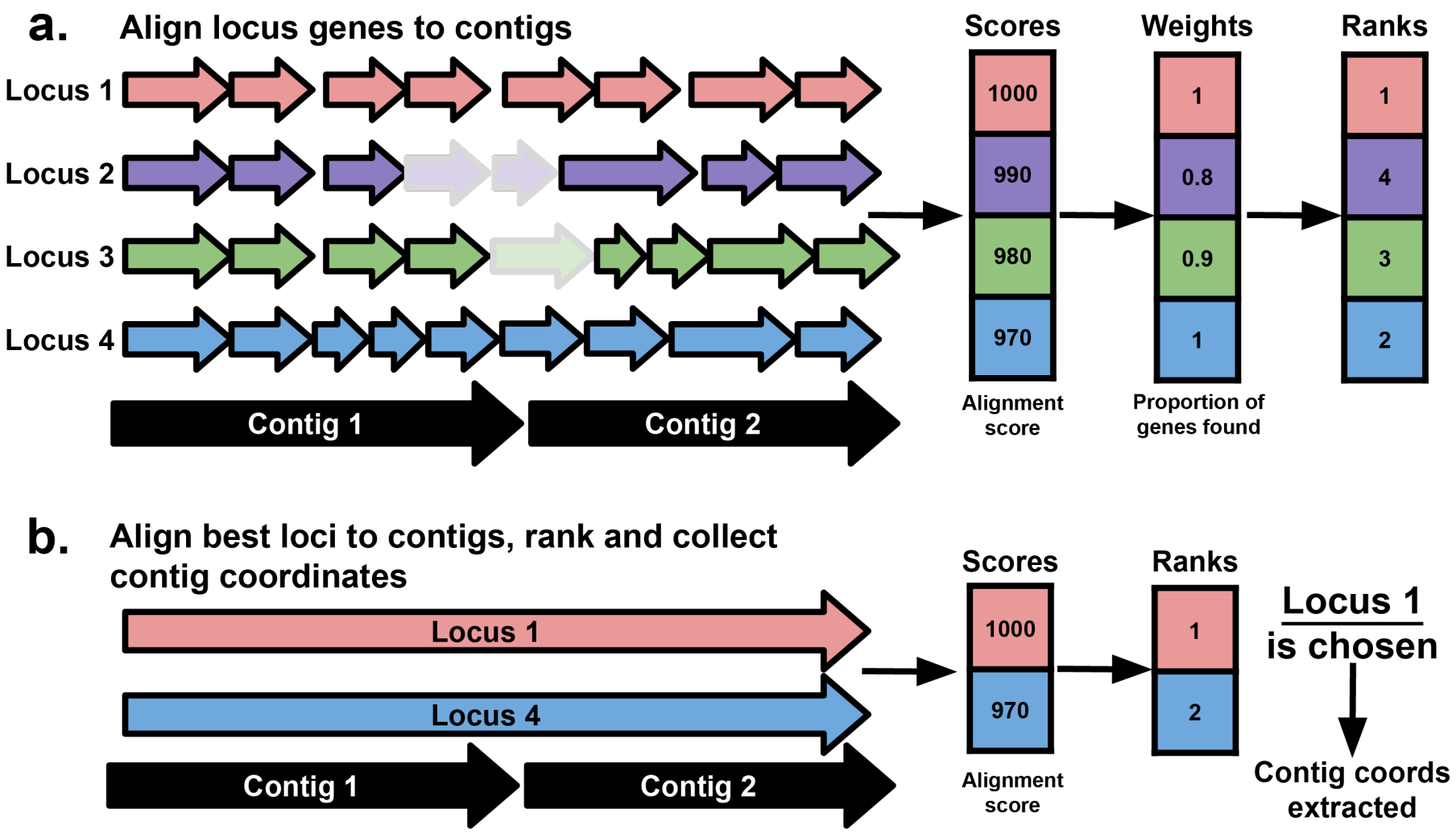
Kaptive papers:
Wyres *et al Microbial Genom* 2016
Wick *et al JCM* 2018
Lam *et al Microbial Genom* 2022
Stanton *et al Microbial Genom* 2025

How Kaptive (v3) works



Stanton *et al Microbial Genom* 2025

How Kaptive (v3) works



Calculate Cov and ID per gene

↓

Report missing and extra genes in locus

↓

Apply phenotype logic

Stanton *et al Microbial Genom* 2025

Kaptive confidence scoring

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

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

Stanton *et al Microbial Genom* 2025

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Multiple ways to access Kaptive

	Klebsiella		*E. coli*	
	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

KLEBORATE

Pathogenwatch

Partial *E. coli* K locus db: Gladstone *et al bioRxiv* 2025

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Interpreting Kaptive output

Full documentation:
https://kaptive.readthedocs.io/en/latest/

Tutorial:
https://tinyurl.com/2s3rs3ny

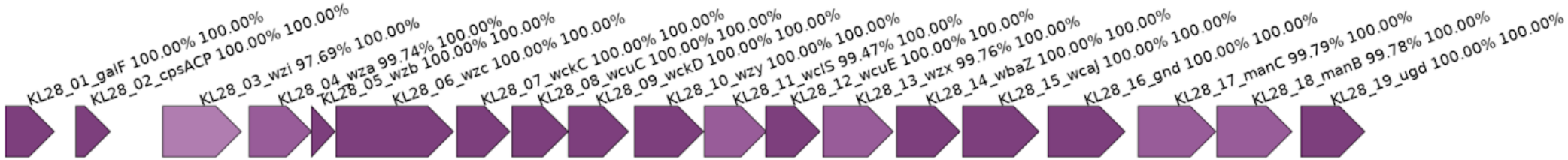
Output table:

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

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 ▼

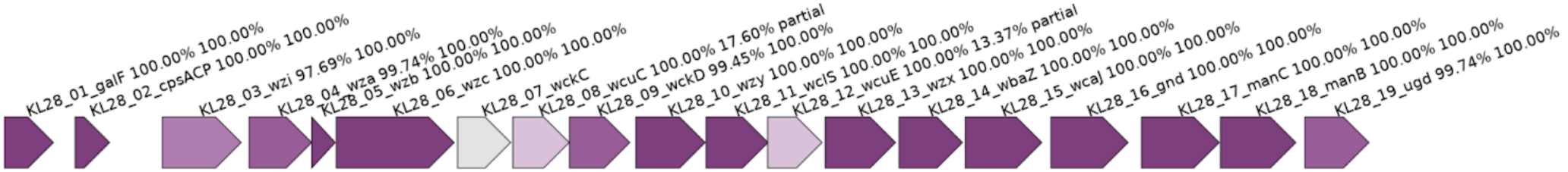
Assembly pieces ⓘ : Download as FASTA

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 ℹ :** Typeable **Cov ℹ :** 91.36% **ID ℹ :** 99.80% **Genes:** 19 / 20

KL28 reference ℹ :



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

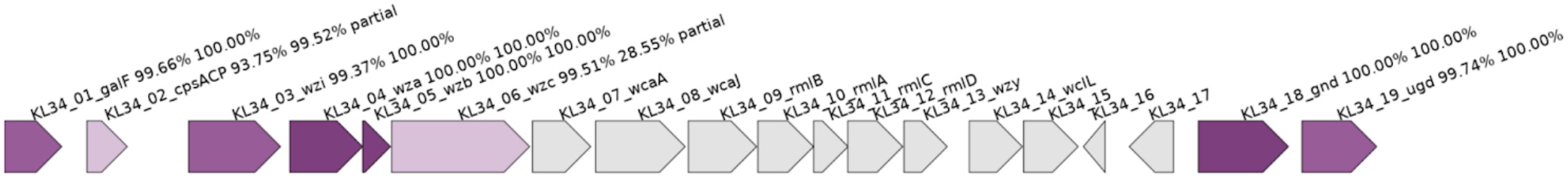
Assembly pieces ℹ : Download as FASTA

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 ℹ :** Untypeable **Cov ℹ :** 42.20% **ID ℹ :** 99.00% **Genes:** 8 / 19

KL34 reference ℹ :

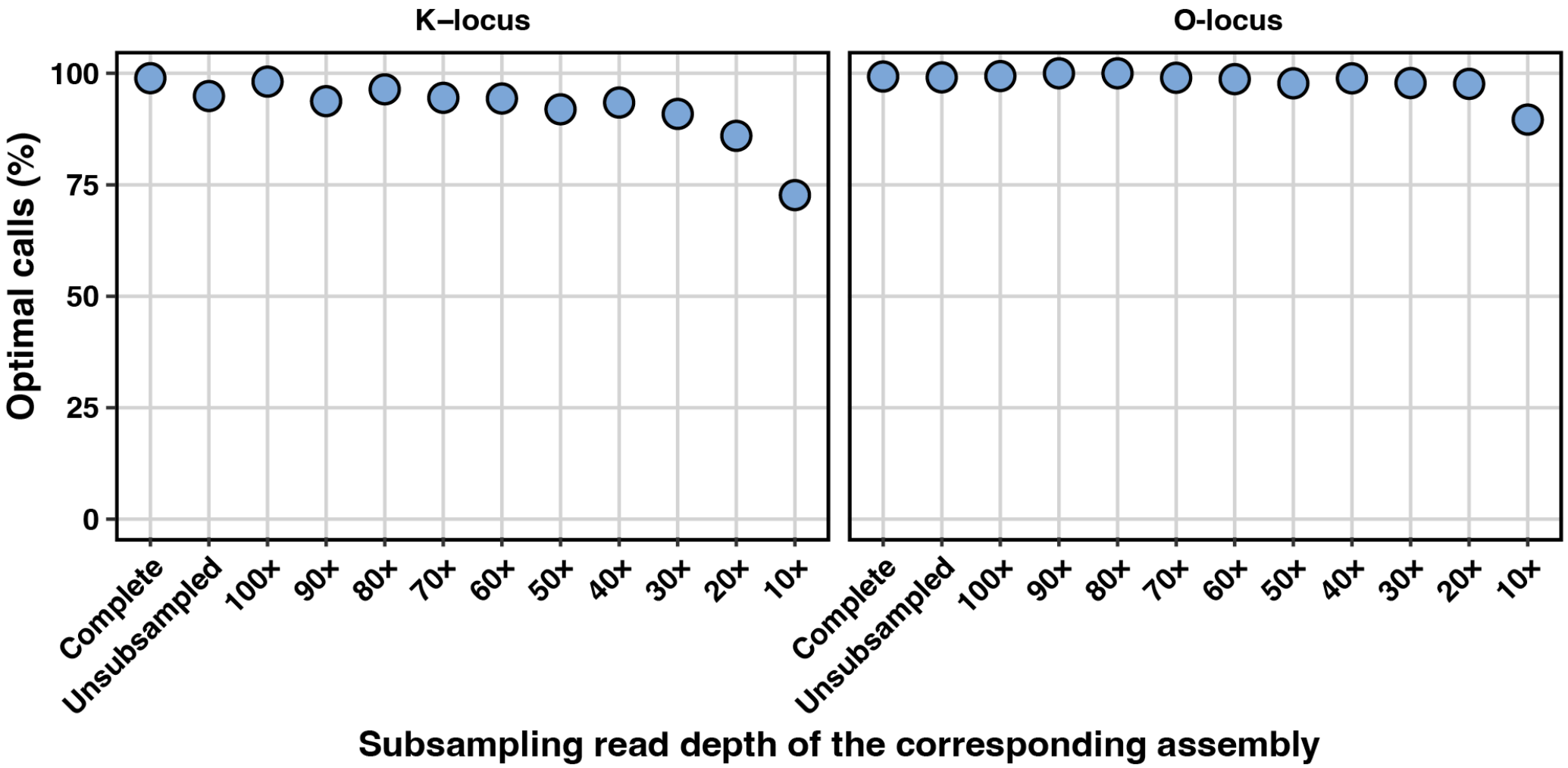


Other genes found in locus ℹ : 0 ▼ **Other genes found outside locus ℹ :** 4 ▼

Assembly pieces ℹ : 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



Adapted from: Stanton *et al Microbial Genom* 2025

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	**476**	555	64	86	**596 (84.5)**

Stanton *et al* Technical Report *in prep*

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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Zangor *et al Vaccine* 2024. PMID: 38503661
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Wyres *et al Microb Genom* 2016. PMID: 28348840
Lam *et al Microb Genom* 2022. PMID: 35311639
Whitfield *et al MMBR* 2025. PMID: 40116577
Wyres *et al PLoS Genetics* 2019. PMID: 30986243
Gorrie *et al Nature Communications* 2022. PMID: 35641522
David *et al Nature Microbiology* 2019. PMID: 31358985
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

Acknowledgements

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