

Understanding vancomycin resistance in *C. difficile* : an evolutionary approach



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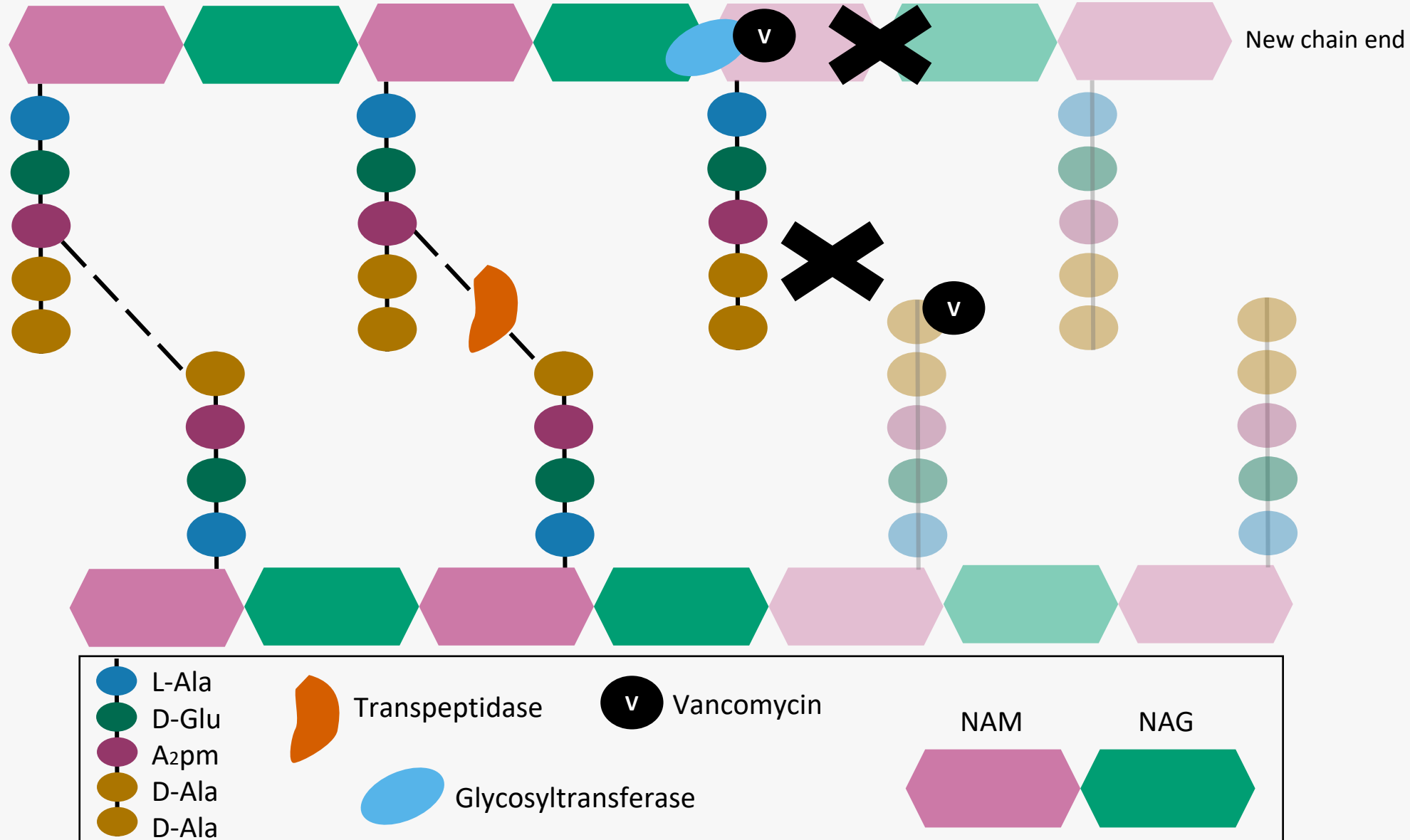
Jessica Buddle

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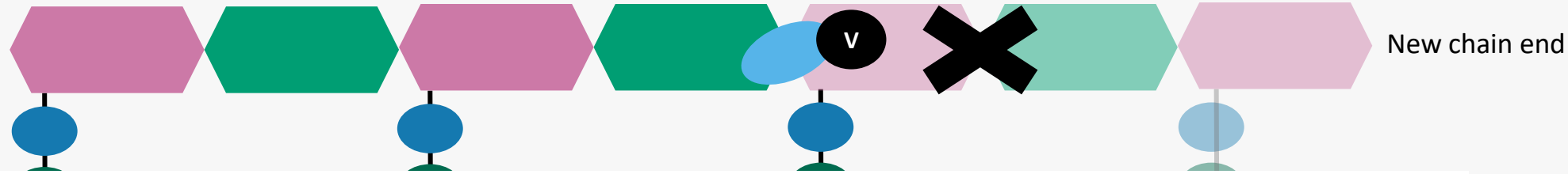
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Vancomycin

Recommended front-line drug (UK)



Vancomycin

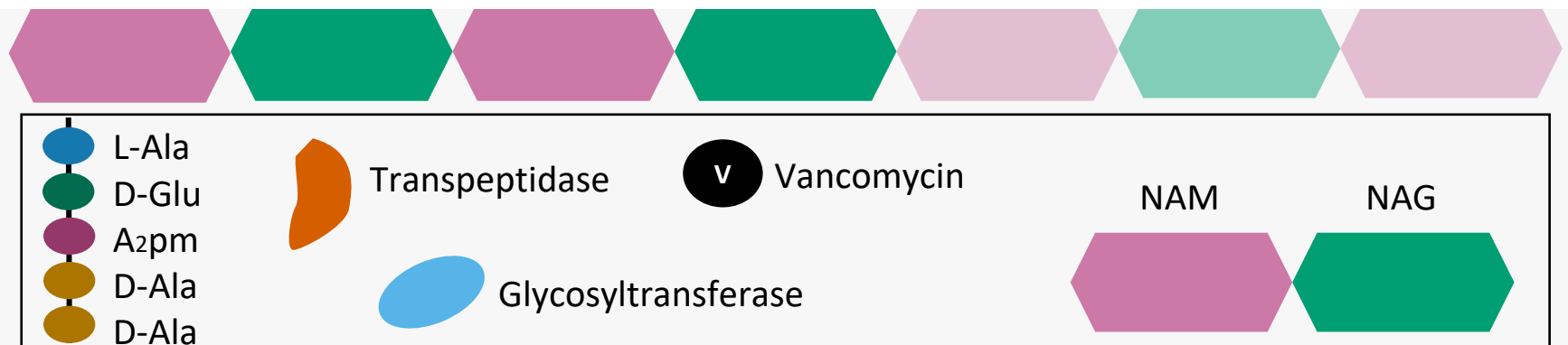


RESEARCH ARTICLE

Prevalence and antimicrobial resistance pattern of *Clostridium difficile* among hospitalized diarrheal patients: A systematic review and meta-analysis

Tebelay Dilnessa , Alem Getaneh, Workagegnehu Hailu, Feleke Moges, Baye Gelaw

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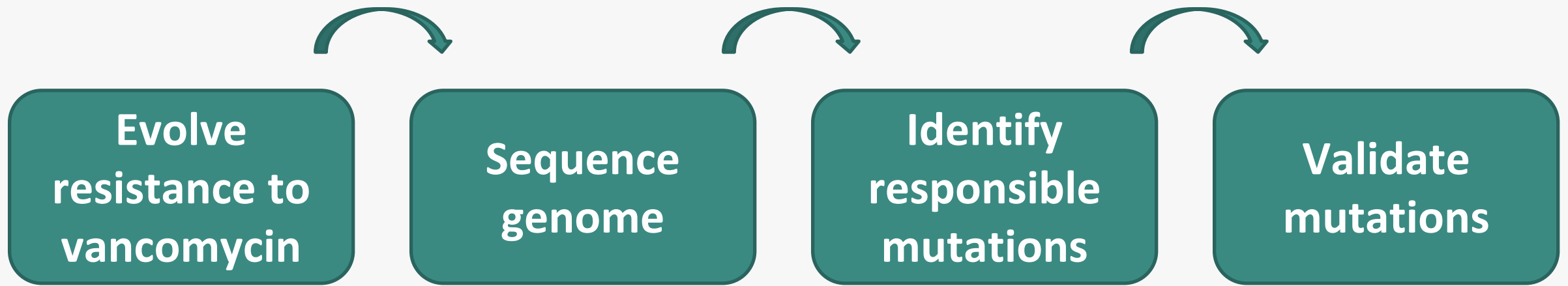


Rec
for
(UK

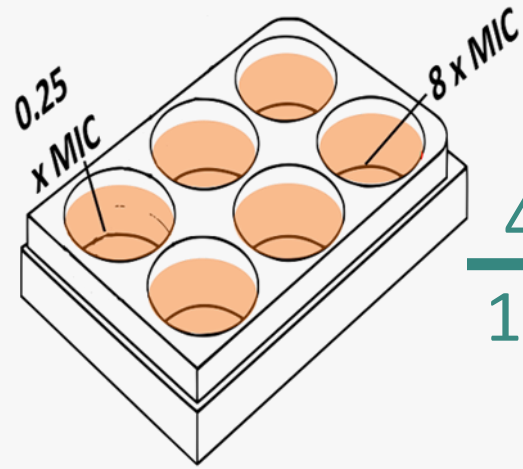
Aims

Characterise vancomycin resistance in *C. difficile*:

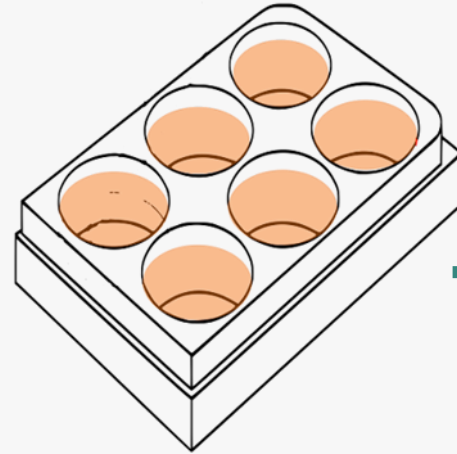
- Evolution – *rate, extent*
- Phenotype – *growth, fitness*
- Genetic – *mutations, routes, population dynamics*



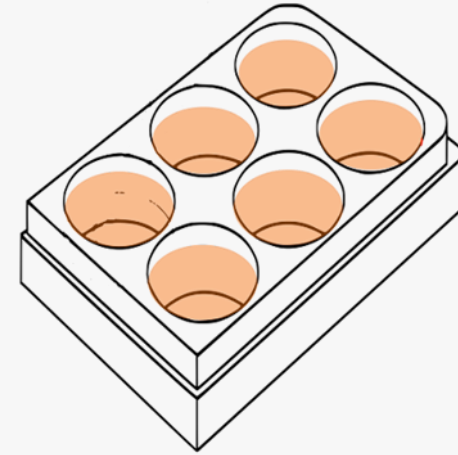
Evolution of R20291 (027) Δ PaLoc



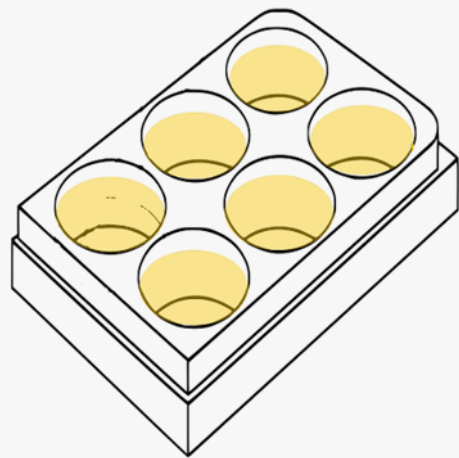
48h
10 μ L



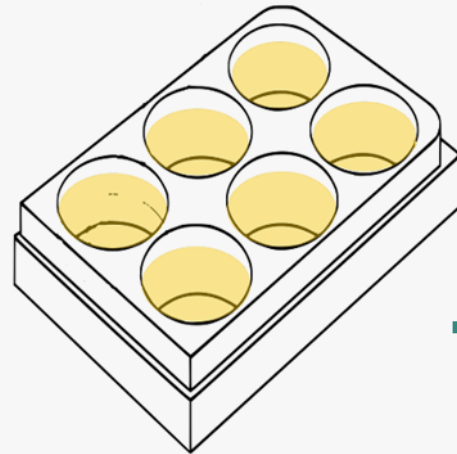
48h
10 μ L



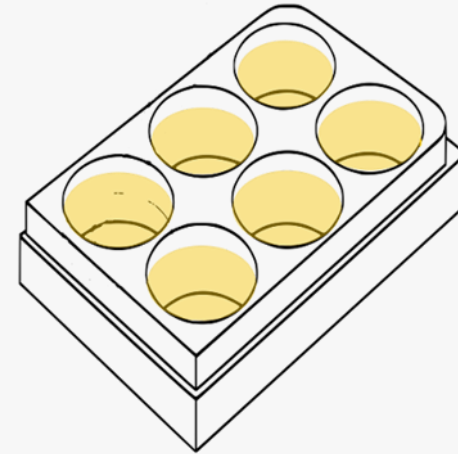
10 replicate lines
– genetically
barcoded
(Bc) 1-11



48h
10 μ L



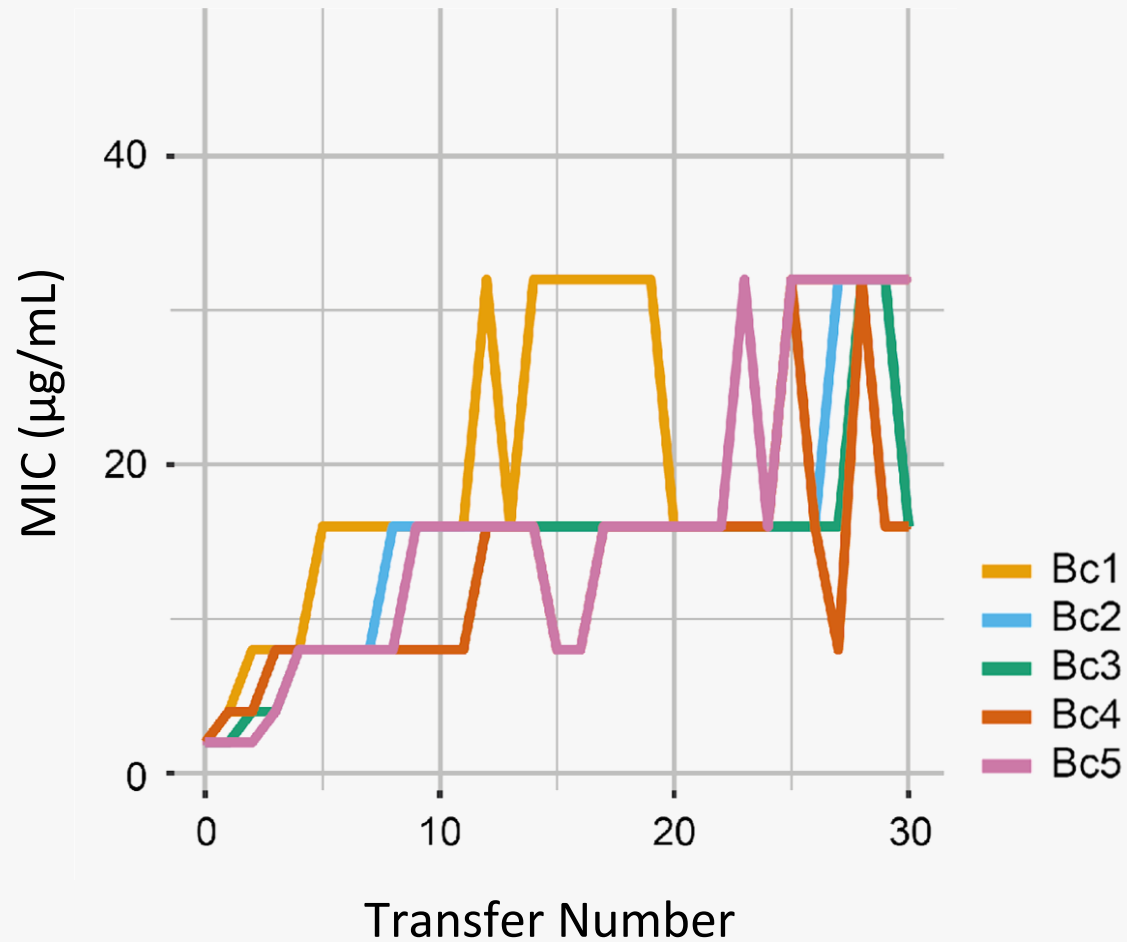
48h
10 μ L



30 transfers

No-vancomycin
controls evolved
in parallel

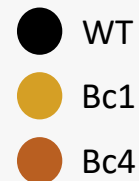
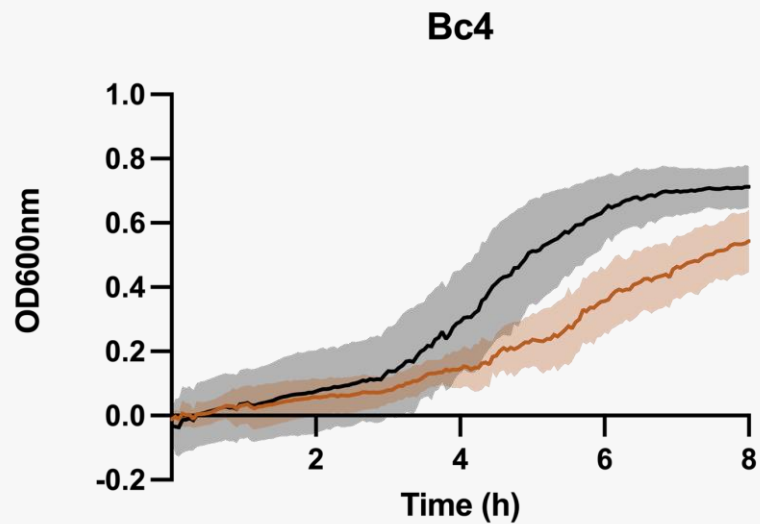
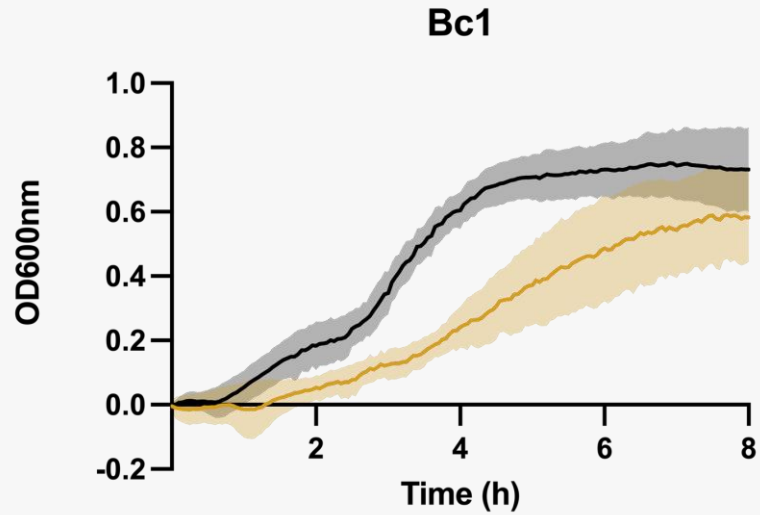
Vancomycin resistance evolves rapidly



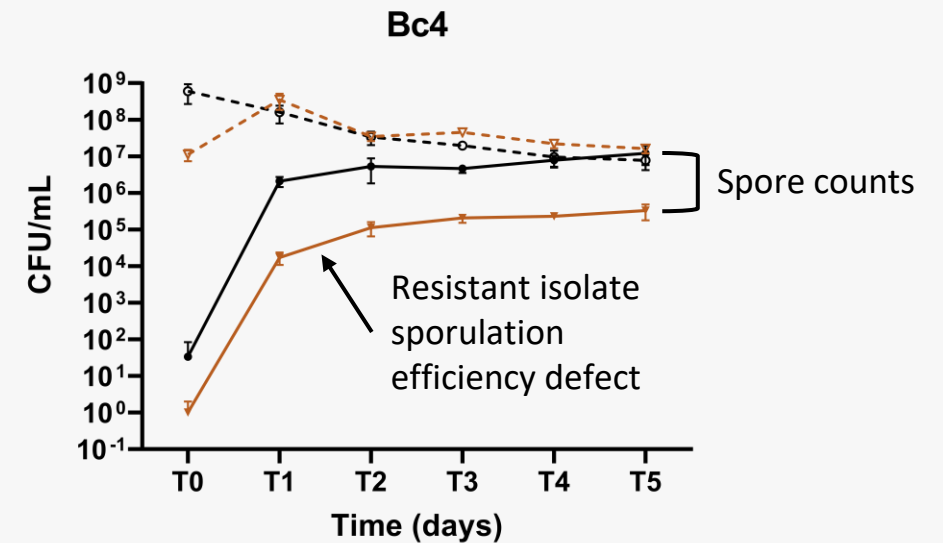
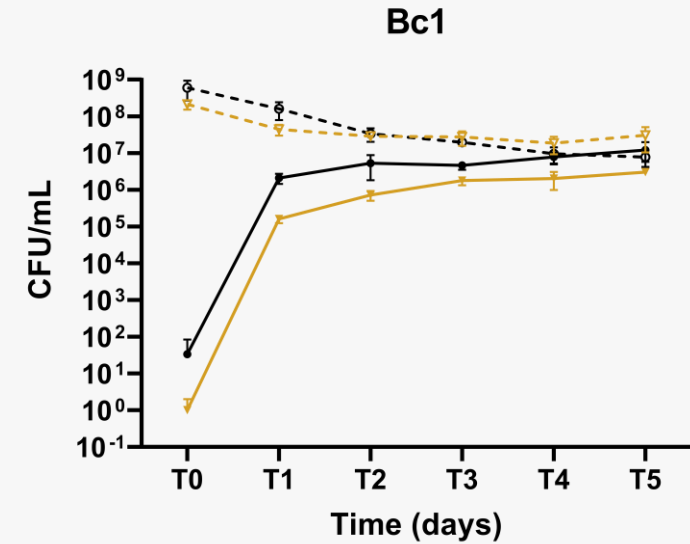
Replicate line	End point MIC (µg/mL)
Ancestral	1
Bc1	32
Bc2	16
Bc3	16
Bc4	16
Bc5	32

Resistance is accompanied by growth and sporulation defects

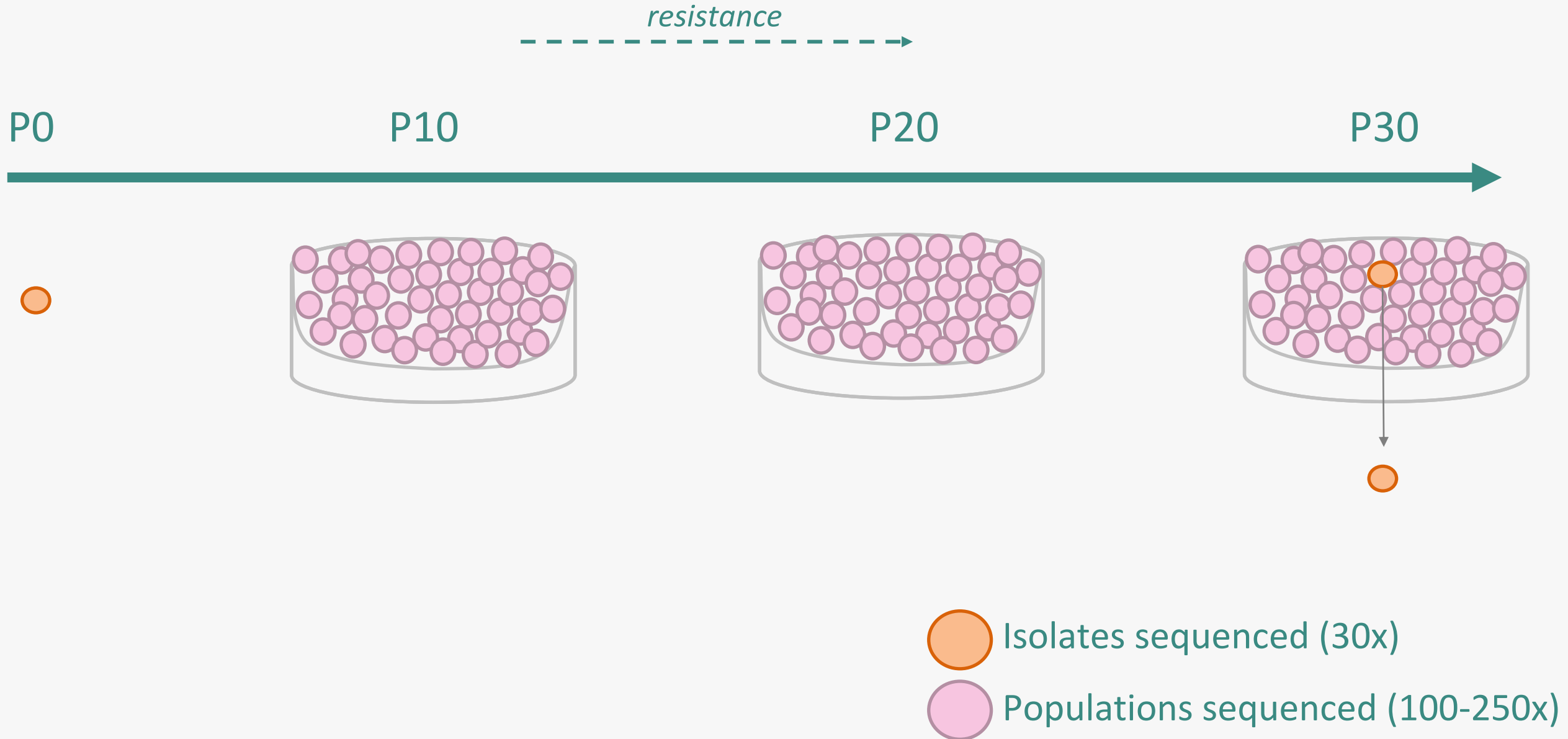
Growth



Sporulation



Genetic Characterisation of resistance: Sequencing



Genetic Characterisation of resistance: Sequencing

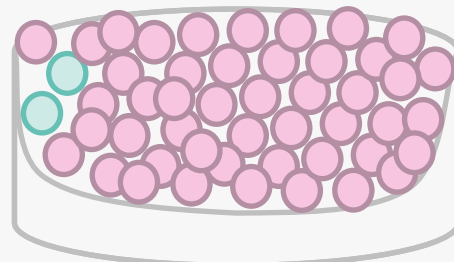
What do we want to gain from sequencing?

Isolates

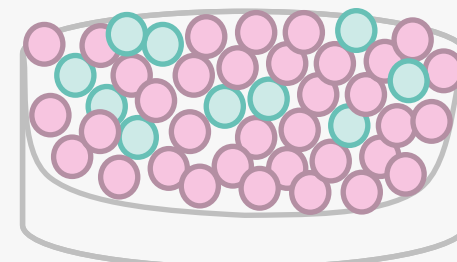
- Mutations involved in an individual bacterium to promote resistance.

Populations

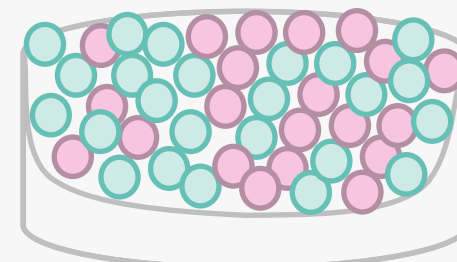
- Mutations involved in population resistance
- Frequency of these mutations in the population
- Frequency change over time (evolutionary dynamics)



5%

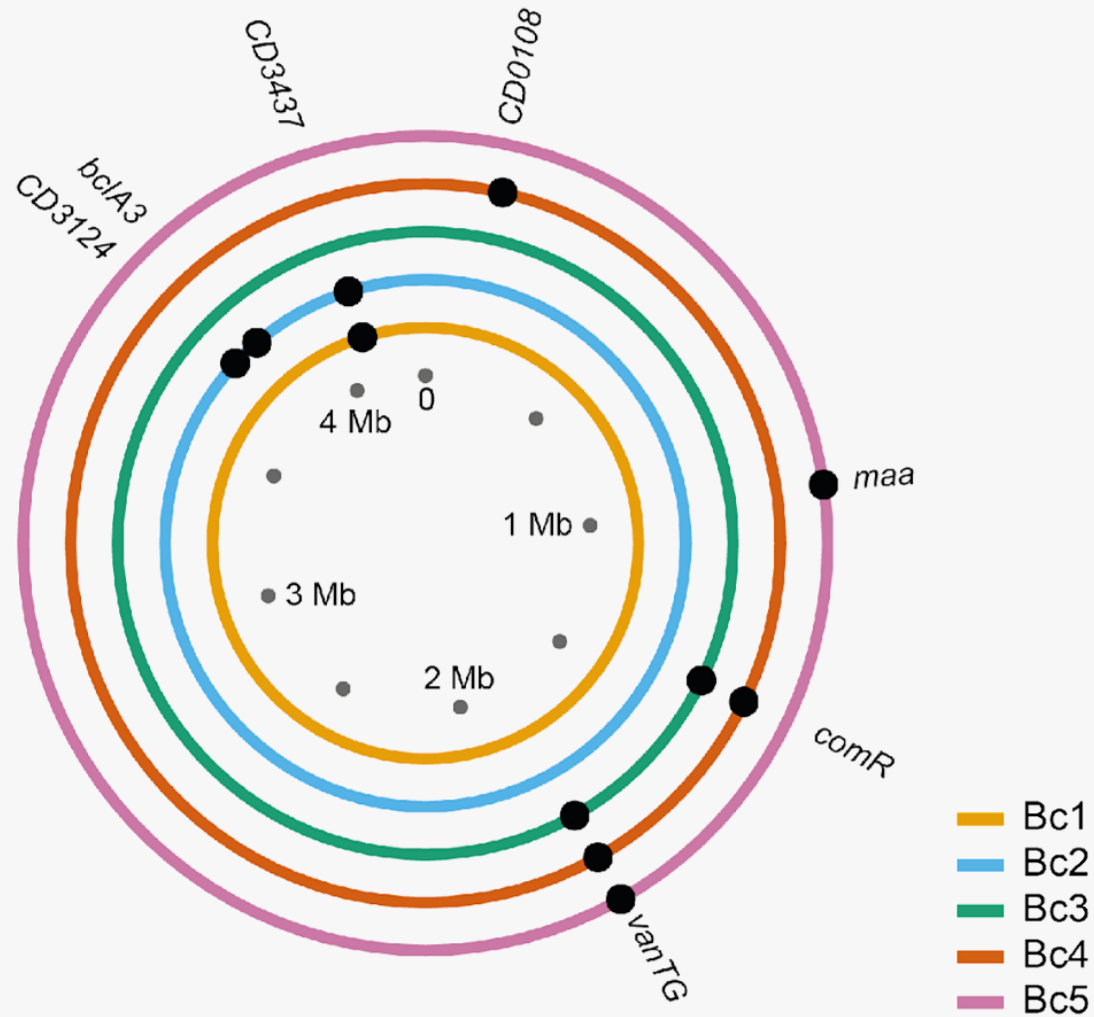


20%

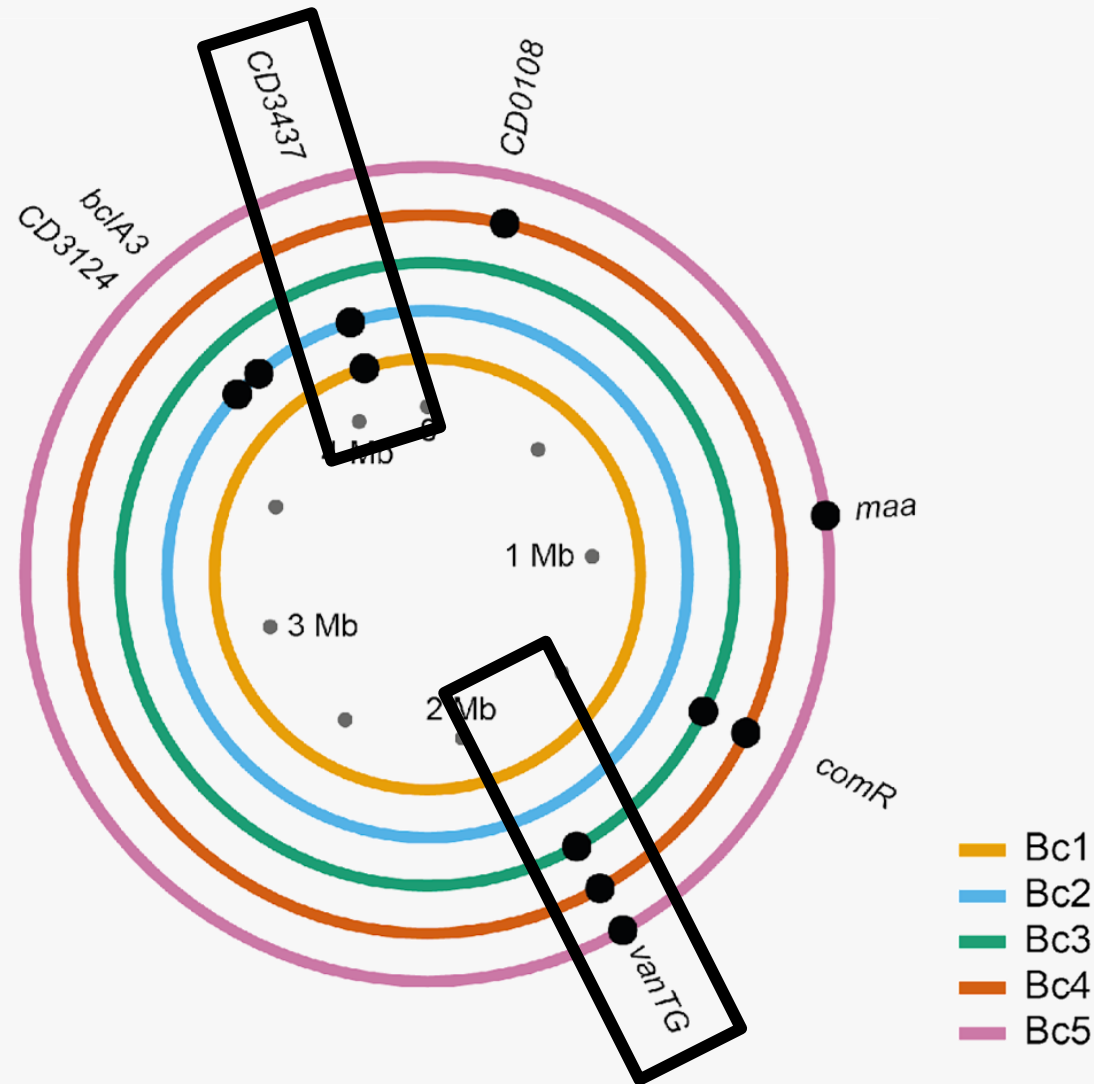


50%

Resistance evolves in parallel in replicate lines



Resistance evolves in parallel in replicate lines



Bc1: 3437

Bc3: *comR* + *vanTG*

Bc5: *maa* + *vanTG*

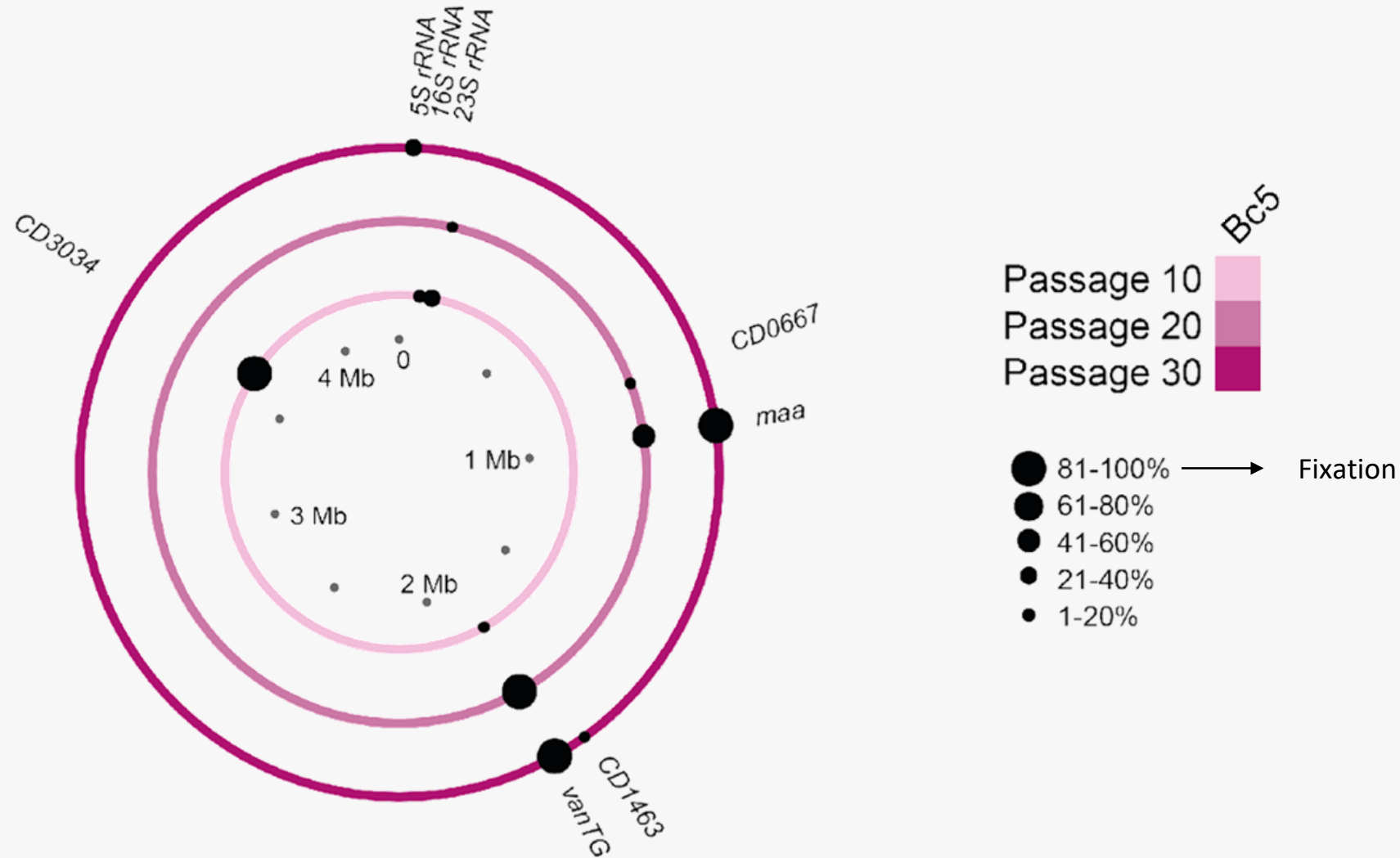


3437 alone

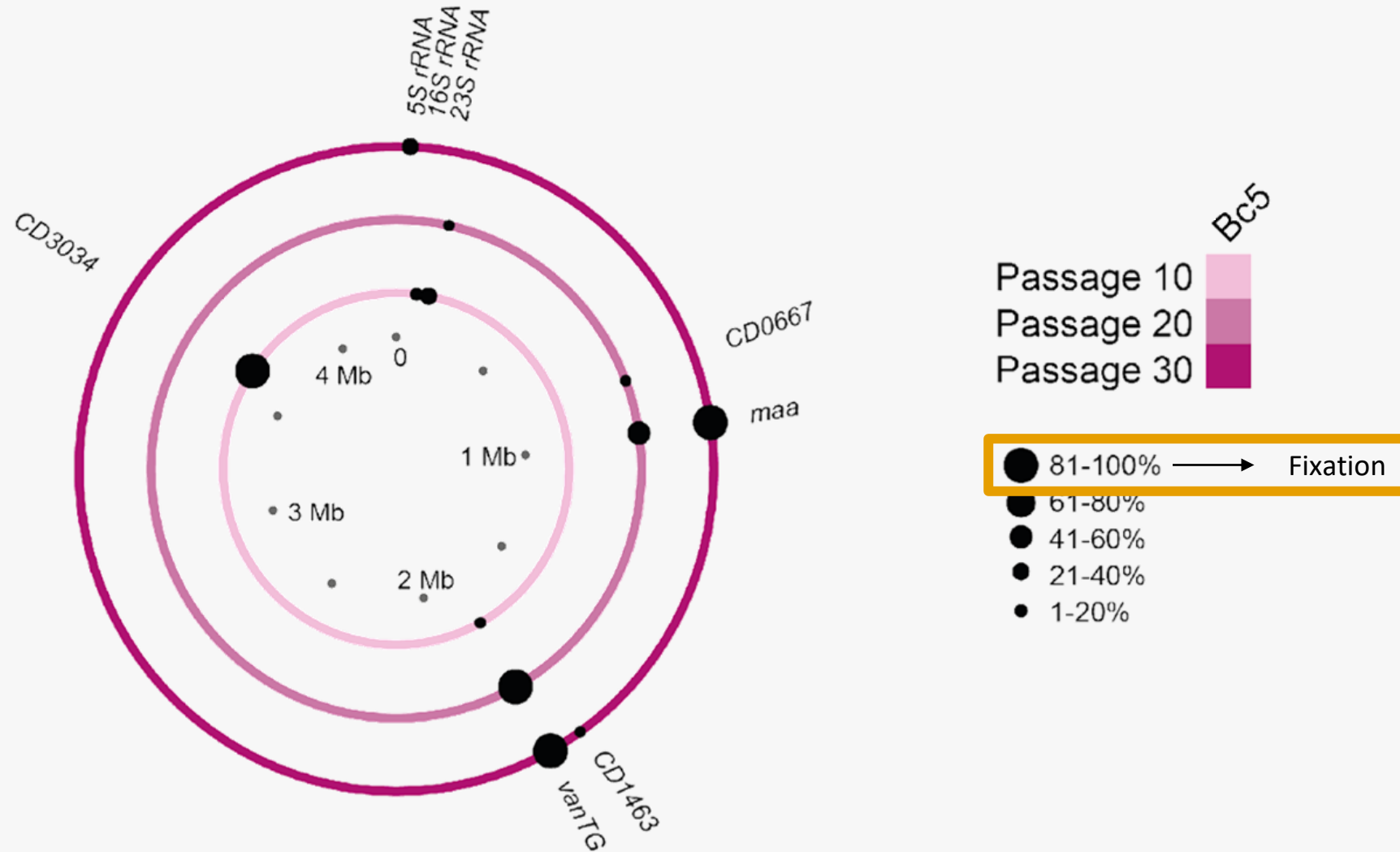
OR

vanTG with something else

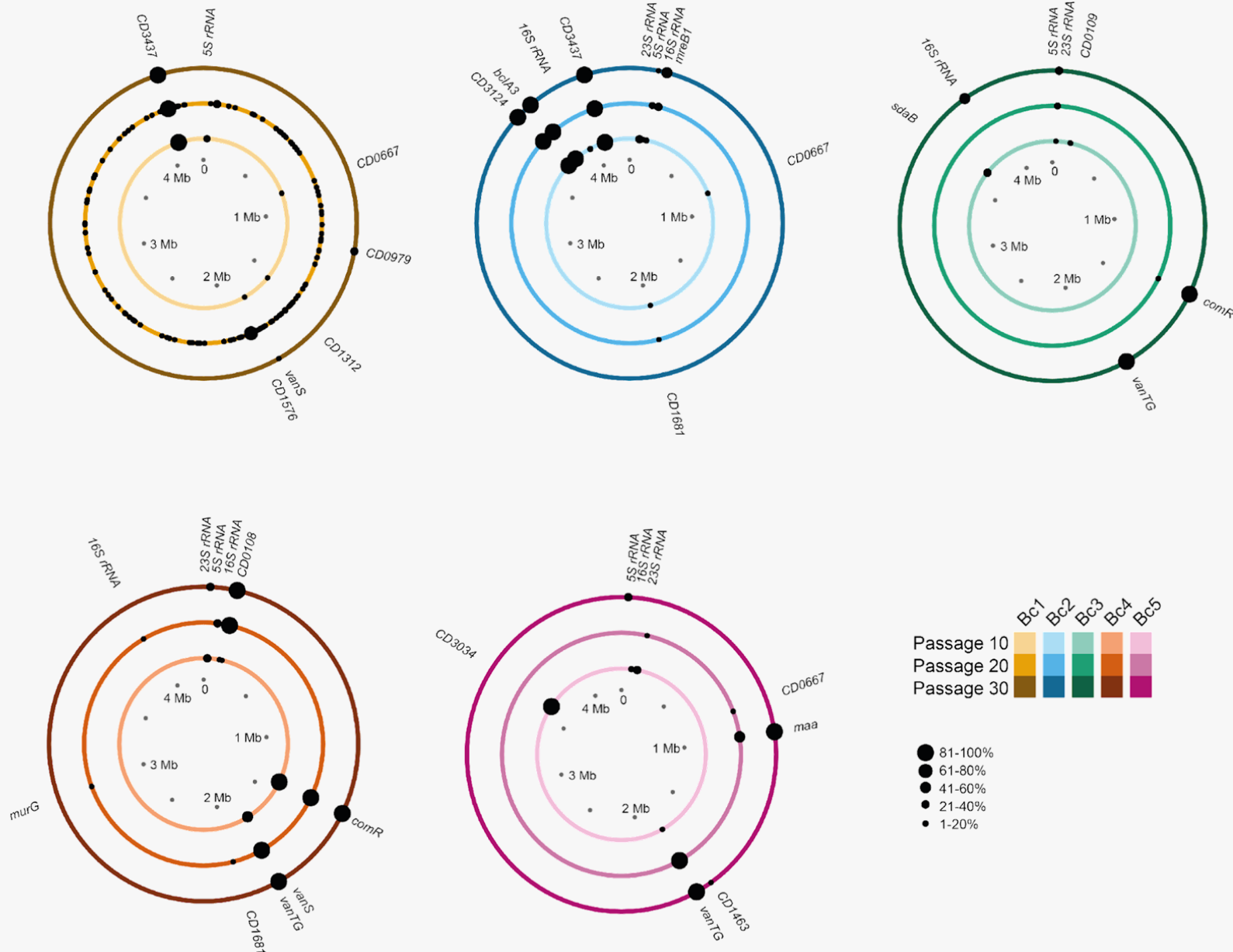
Population sequencing reveals evolutionary dynamics



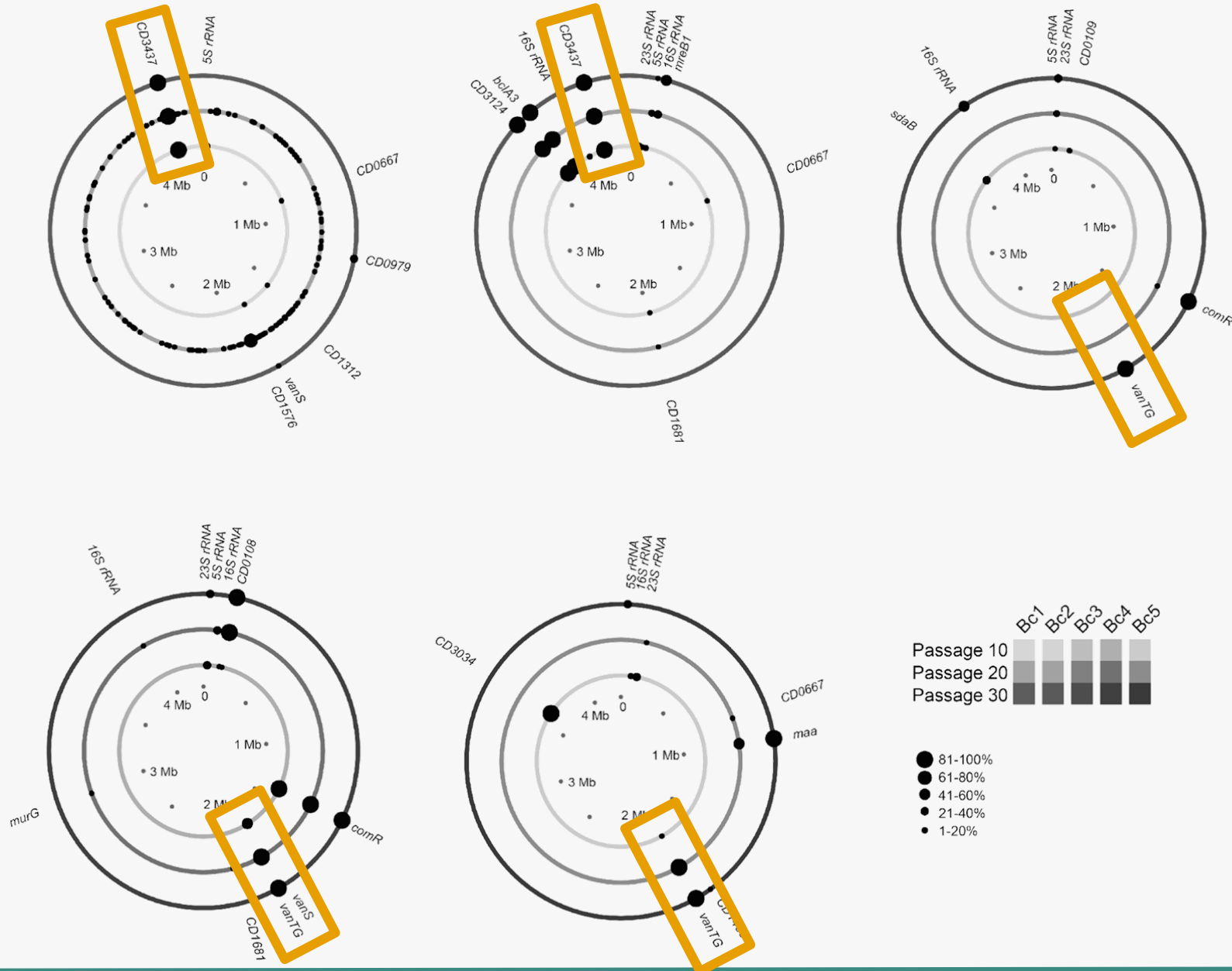
Population sequencing reveals evolutionary dynamics



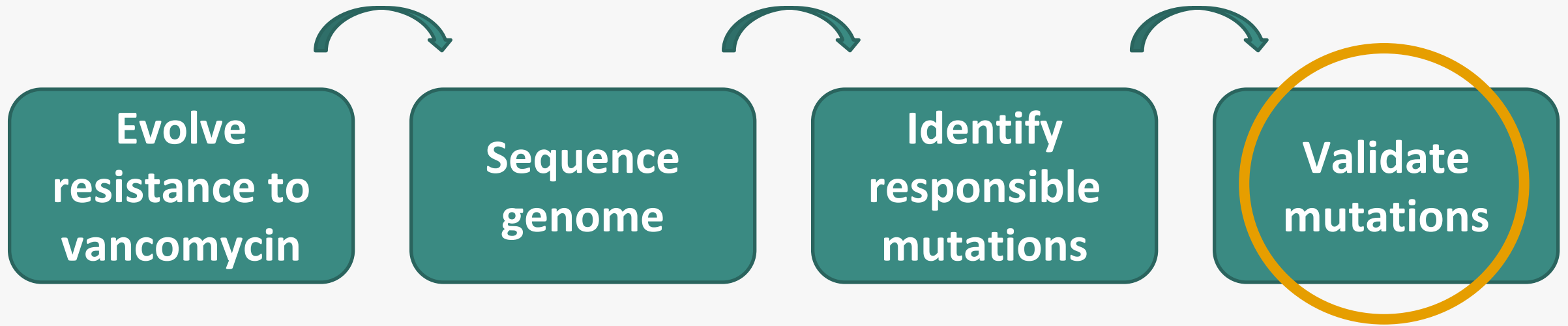
Population sequencing reveals evolutionary dynamics



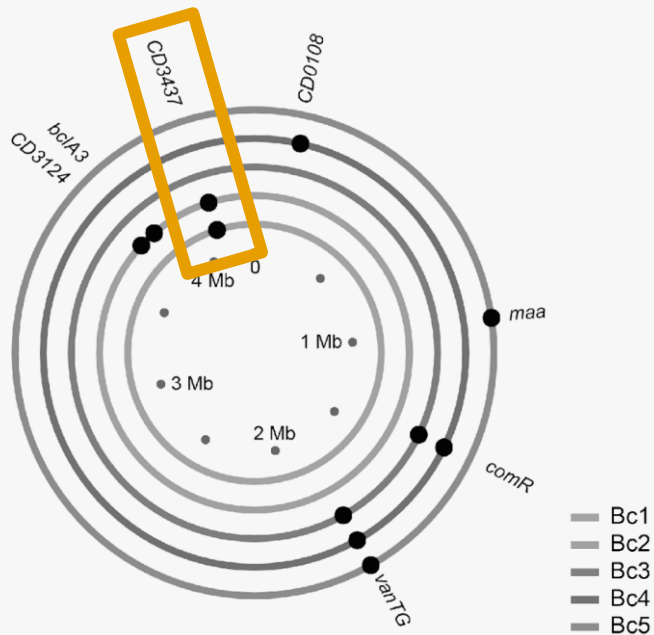
Population sequencing reveals evolutionary dynamics



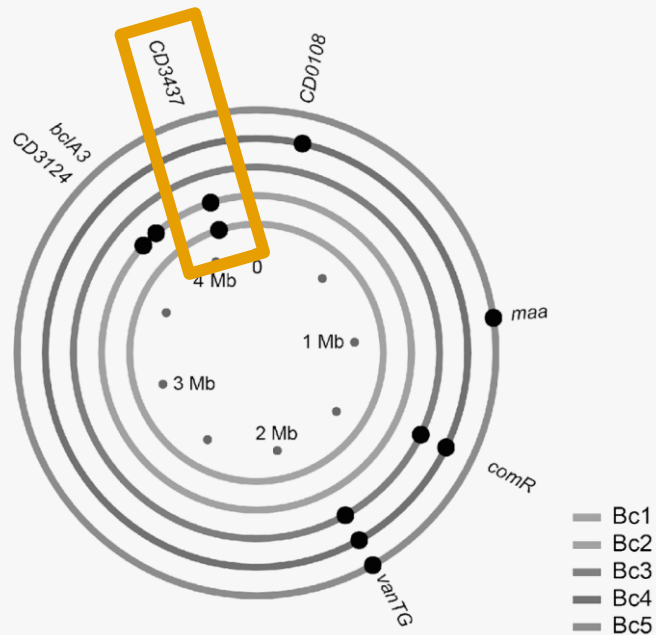
Aims



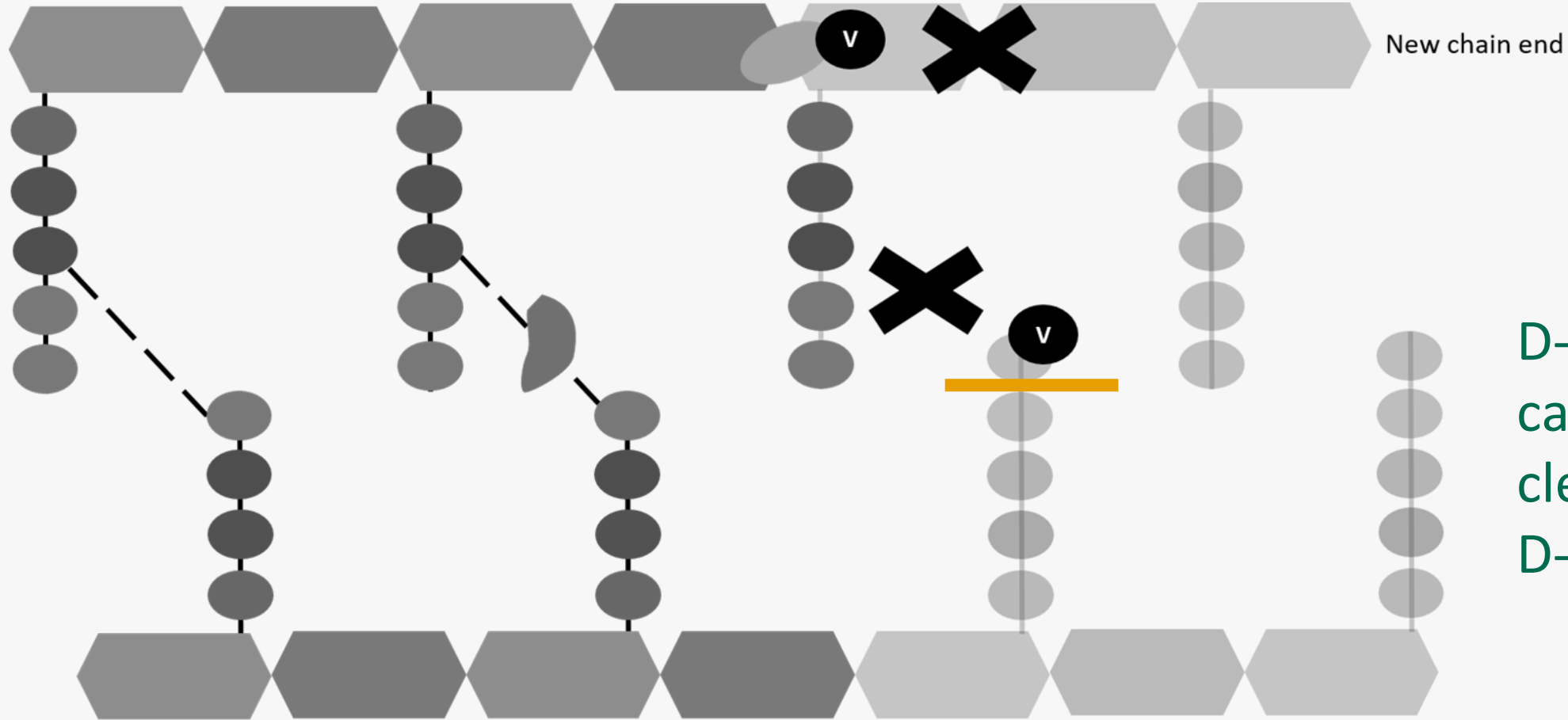
Validating the role of 3437 in resistance



Validating the role of 3437 in resistance



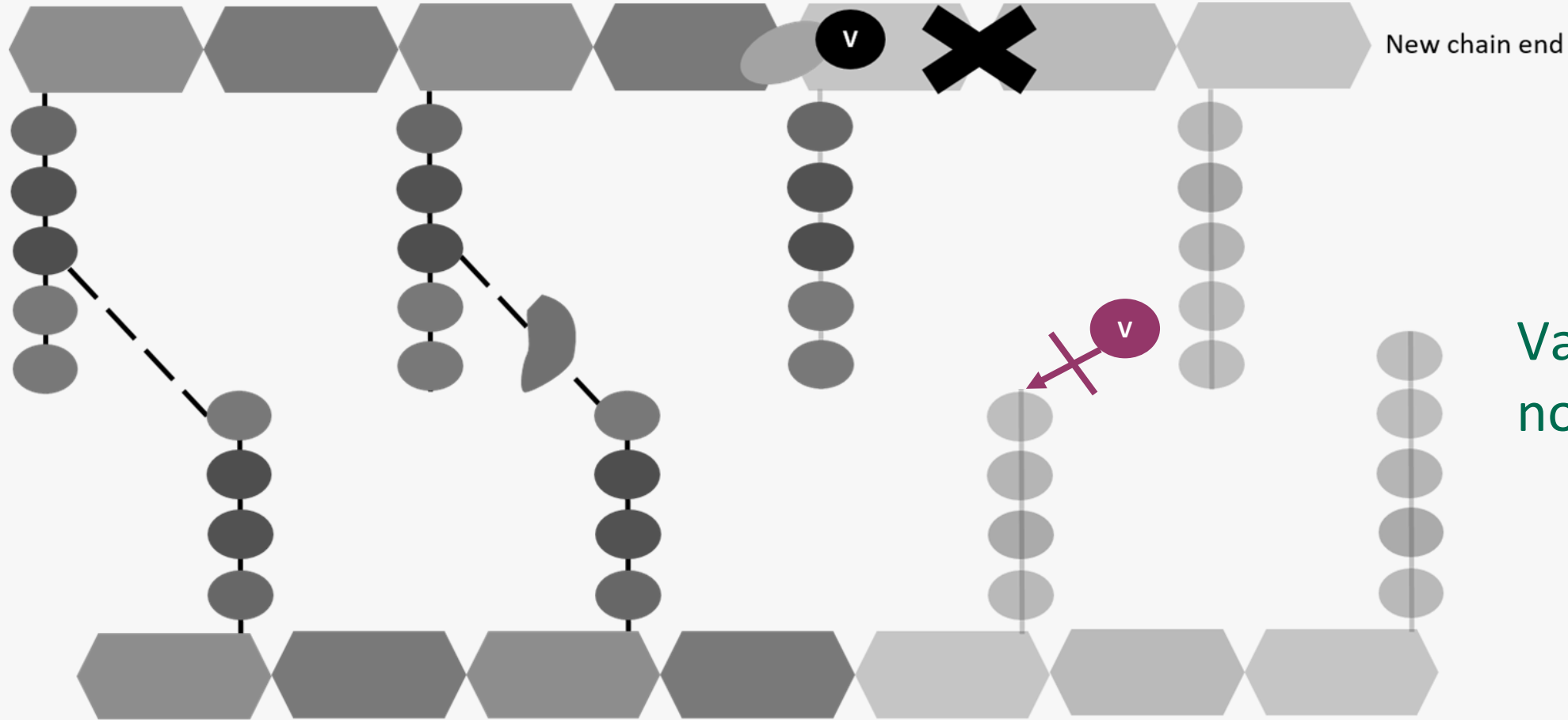
Validating the role of 3437 in resistance



D-ala-D-ala
carboxypeptidase
cleaves terminal
D-ala



Validating the role of 3437 in resistance

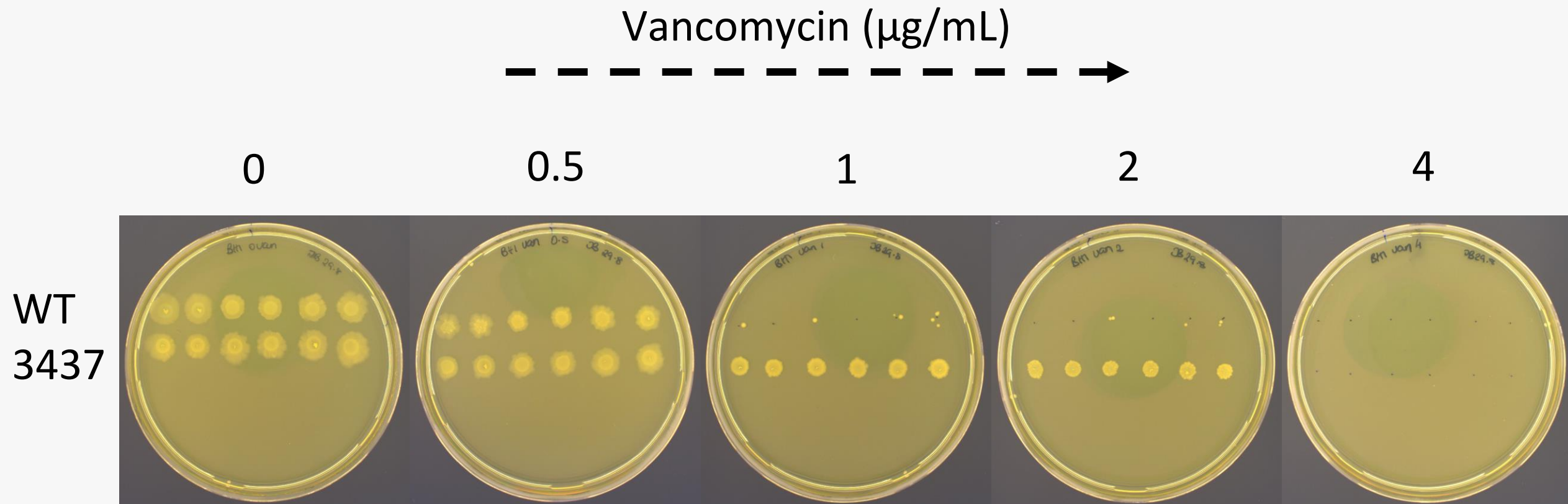


Vancomycin can no longer bind

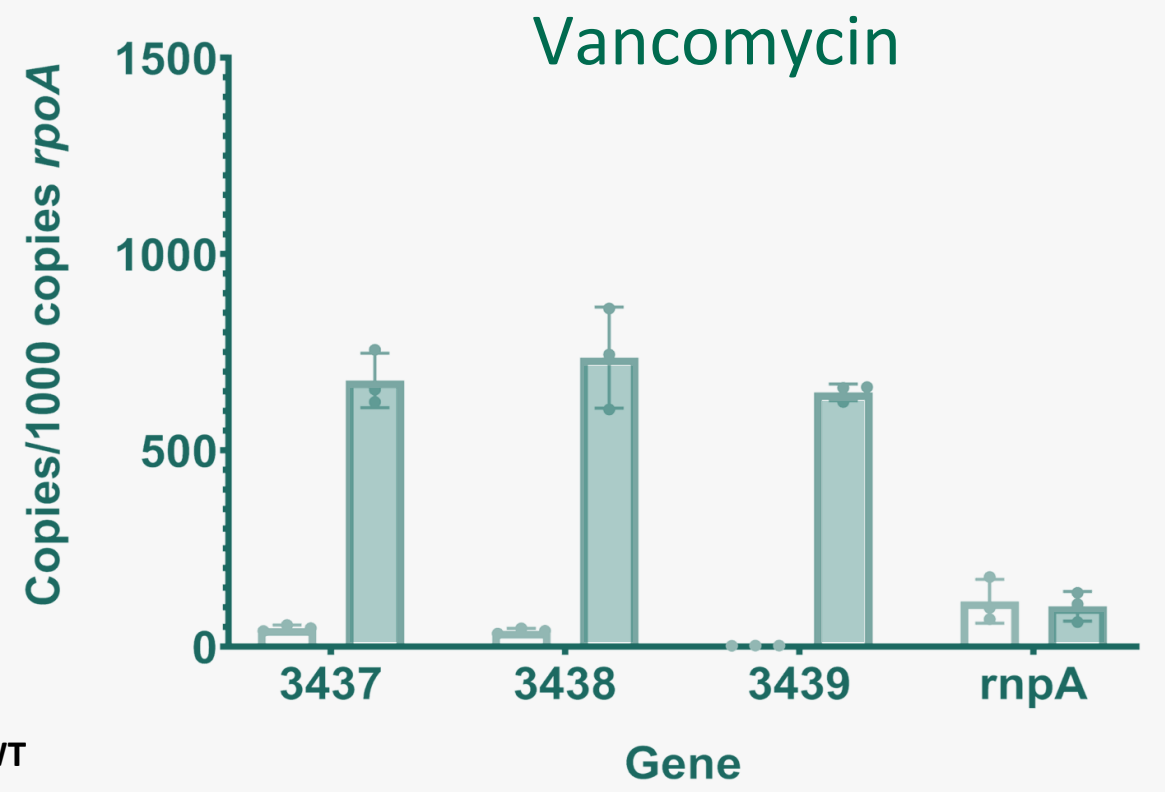
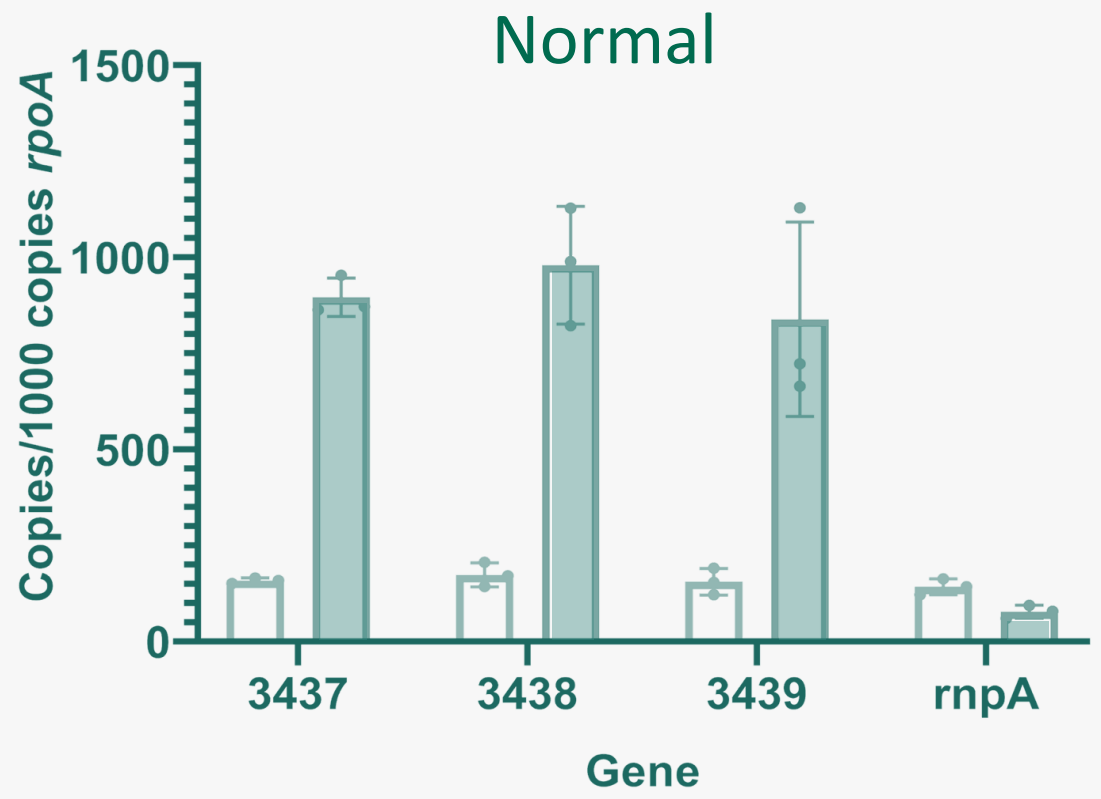
	L-Ala		Transpeptidase		Vancomycin		NAM		NAG
	D-Glu		Glycosyltransferase						
	A2pm								
	D-Ala								
	D-Ala								

3437 SNP results in 4x increase in vancomycin resistance

Recapitulated 3437 SNP in WT background to validate effects on resistance:



3437 SNP results in increased expression of 3437/8/9



□ WT

■ 3437 SNP

Summary

- Vancomycin resistance evolves rapidly in *C. difficile* in laboratory settings
- Resistance is accompanied by fitness costs
- 2 main routes to resistance – occurred in 10 replicate lines:
 - ***vanTG*** (fix late)
 - ***3437/8/9*** (fix early)
- *3437* SNP increases expression of *3439* D-ala-D-ala carboxypeptidase

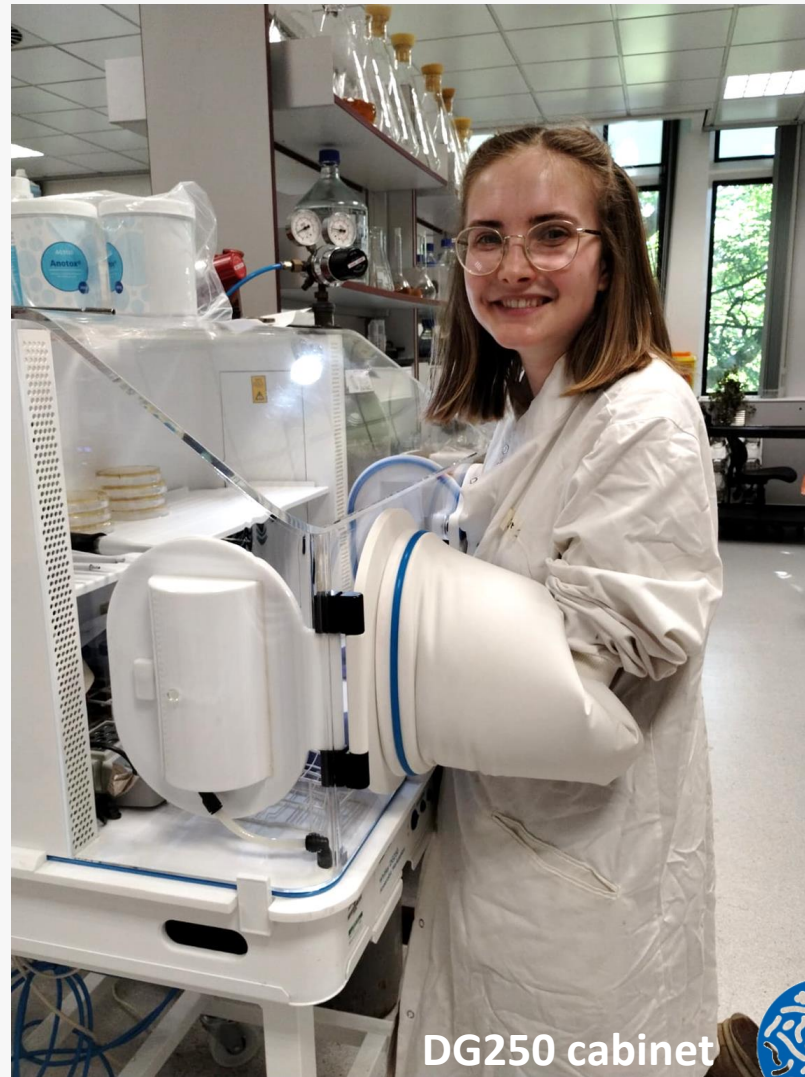
Acknowledgements

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ⁱ Sheffield ⁱⁱ Manchester

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