



Experimental Evolution of Vancomycin Resistance in *Clostridioides difficile*: Pathways and Mechanistic Insights

Twitter: @buddlejess

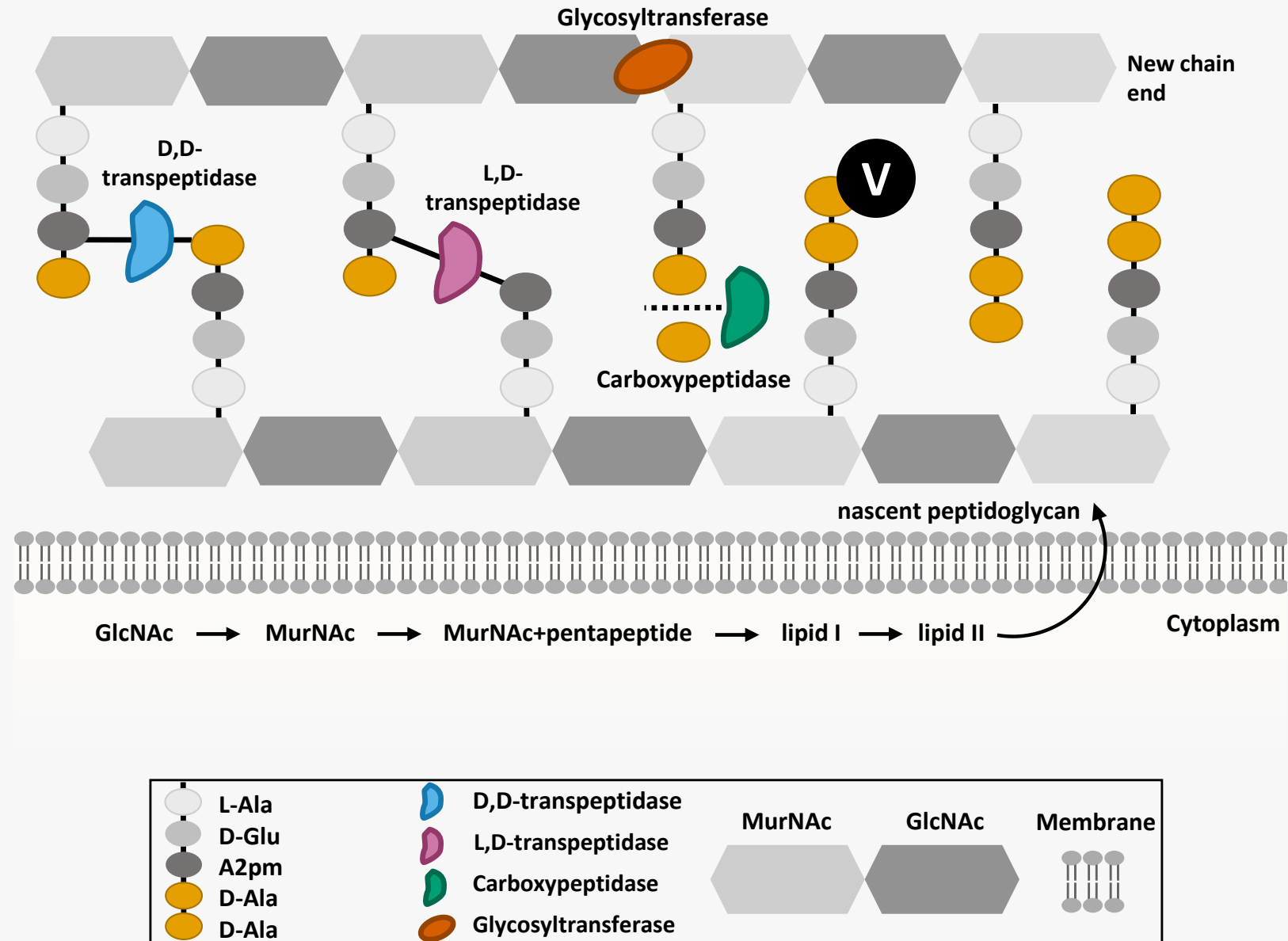
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Vancomycin

Recommended
front-line drug
(UK)



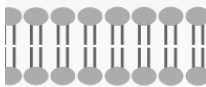
RESEARCH ARTICLE

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

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New chain end



Cytoplasm

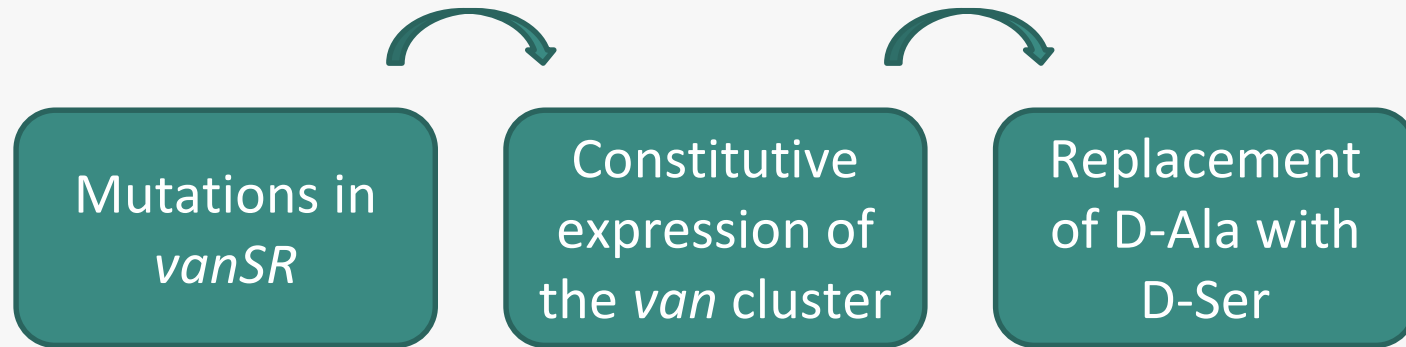
ne

 D-Ala

 Glycosyltransferase

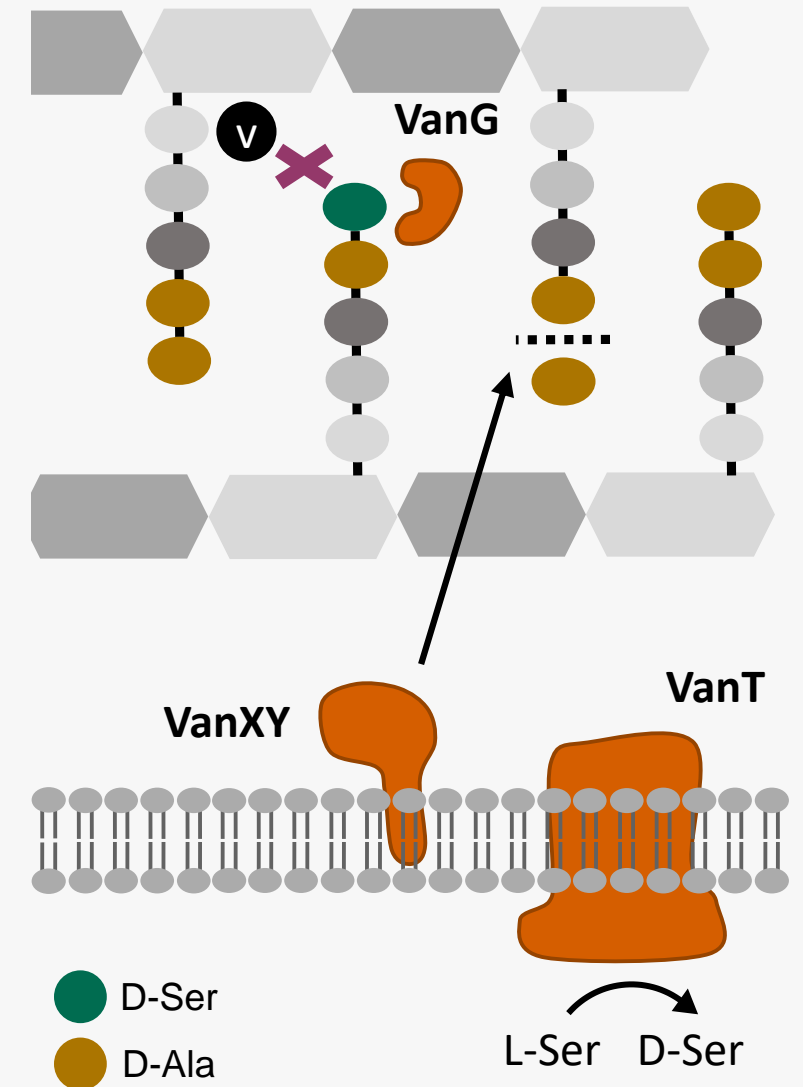
D-Ala to D-Ser Vancomycin Resistance

Previous experimental evolution showed vancomycin resistance in *C. difficile* is possible *in vitro*



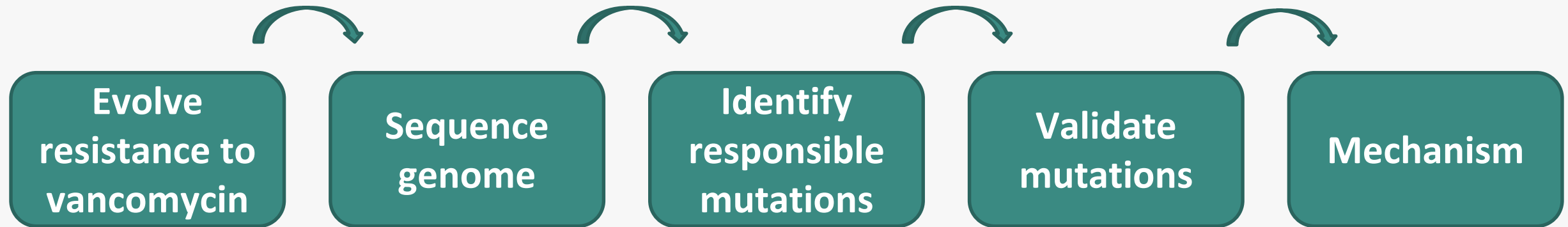
Unknowns:

Alternative pathways to resistance, routes to high-level resistance, fitness costs

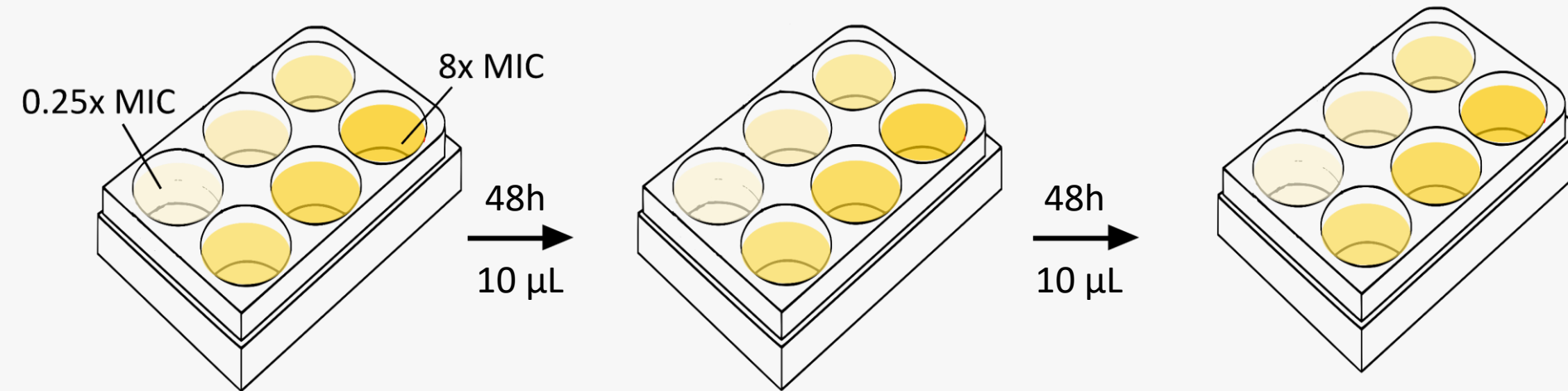


Aims

- i. Evolution – *rate, extent*
- ii. Phenotypic – *growth, fitness*
- iii. Genotypic – *mutations, routes, population dynamics*
- iv. Mechanistic – *novel mechanistic insights*



Evolution of R20291 (027) $\Delta PaLoc$

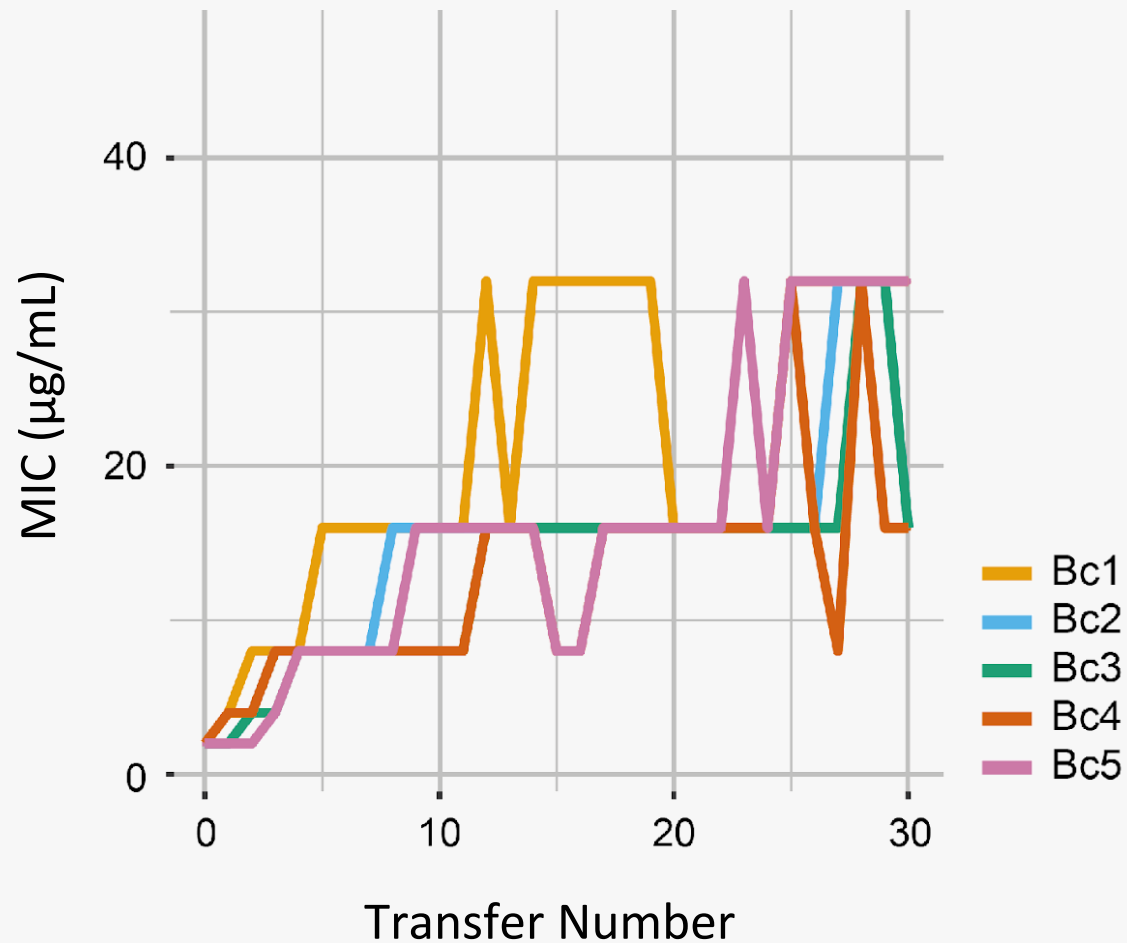


10 replicate lines
– genetically
barcoded
(Bc) 1-11

30 transfers

No-vancomycin
controls evolved
in parallel

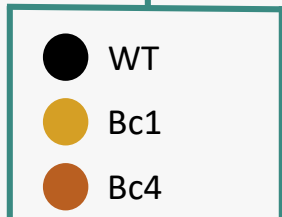
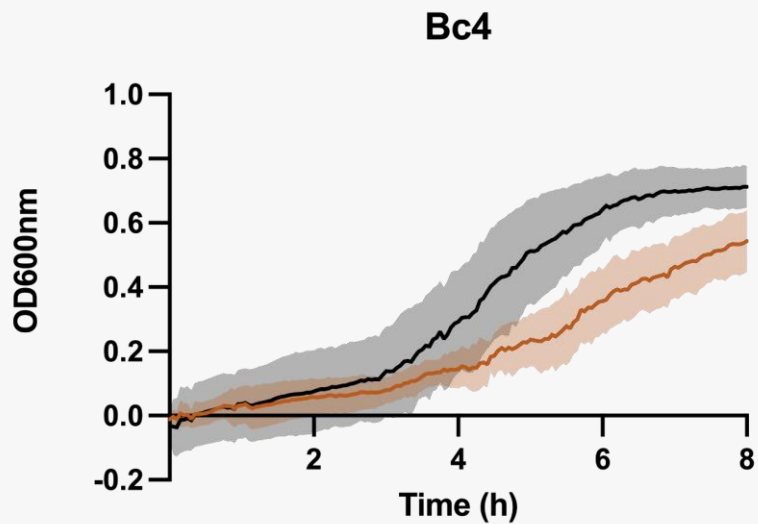
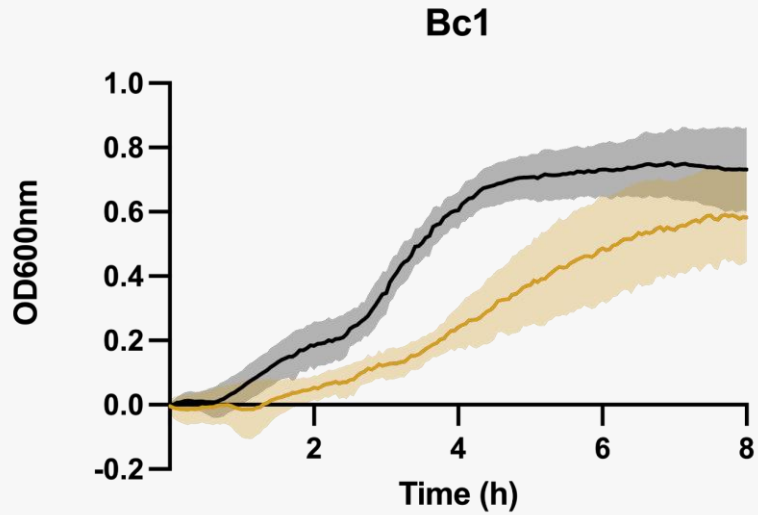
Vancomycin resistance evolves rapidly



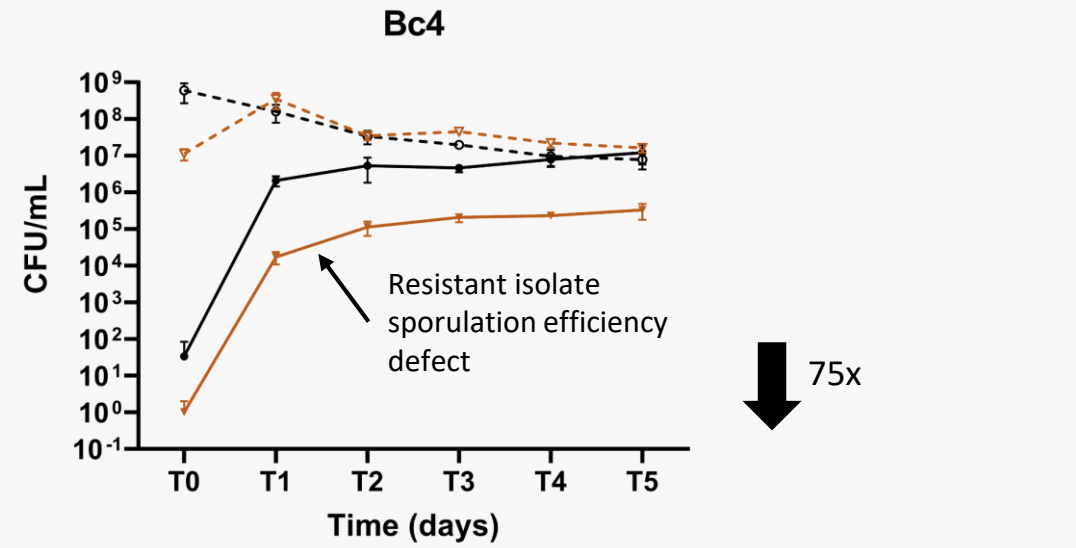
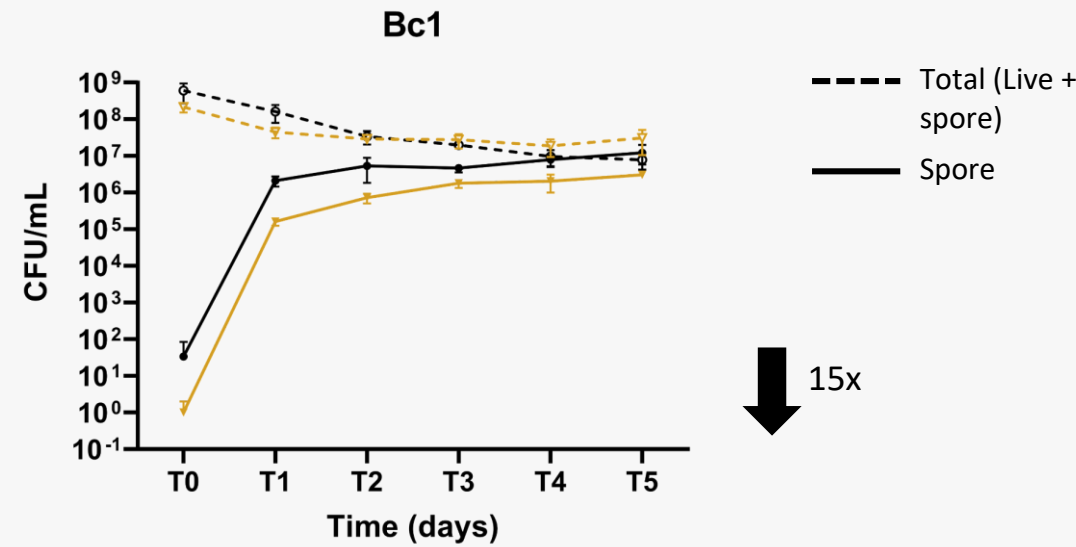
Replicate line	End point MIC (µg/mL)
Ancestral	1
Bc1	32
Bc2	16
Bc3	16
Bc4	16
Bc5	32

Resistance is accompanied by growth and sporulation defects

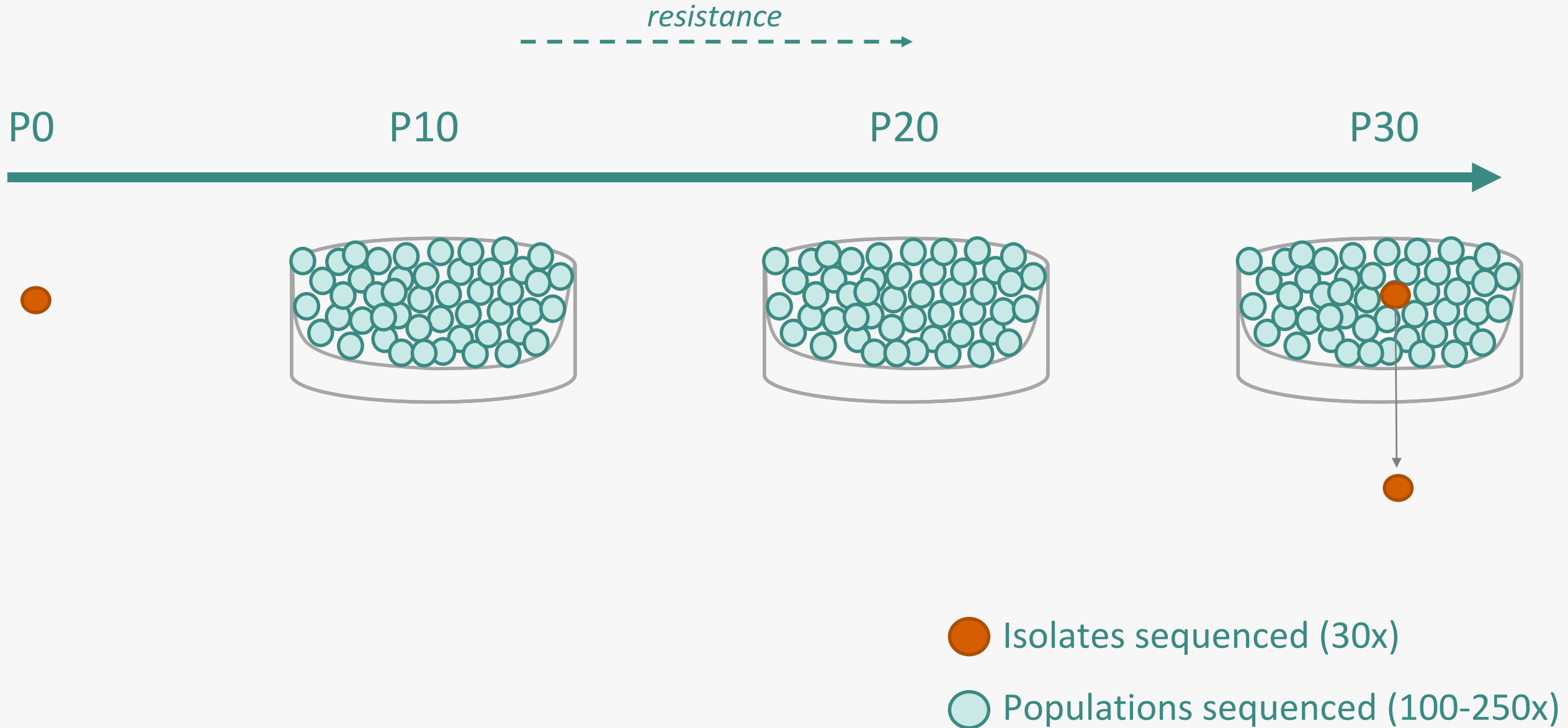
Growth



Sporulation



Genetic Characterisation of resistance: Sequencing



Genetic Characterisation of resistance: Sequencing

What do we want to gain from sequencing?

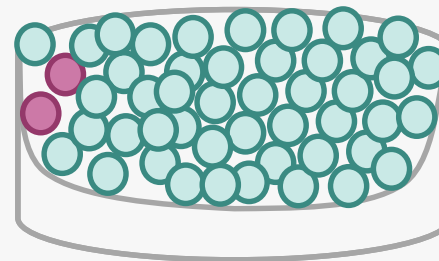
Isolates

- Mutations involved in an individual bacterium to promote resistance.

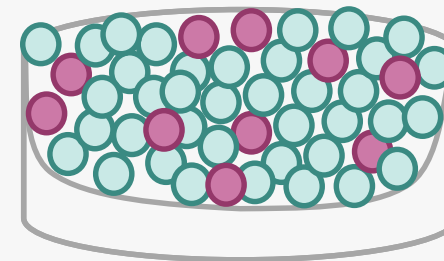


Populations

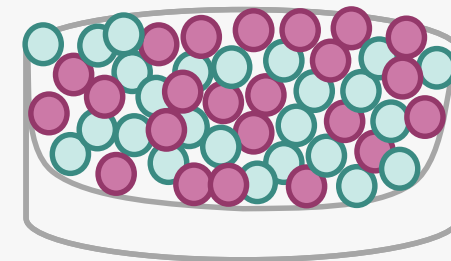
- Mutations involved in population resistance
- Frequency of these mutations in the population
- Frequency change over time (evolutionary dynamics)



5%

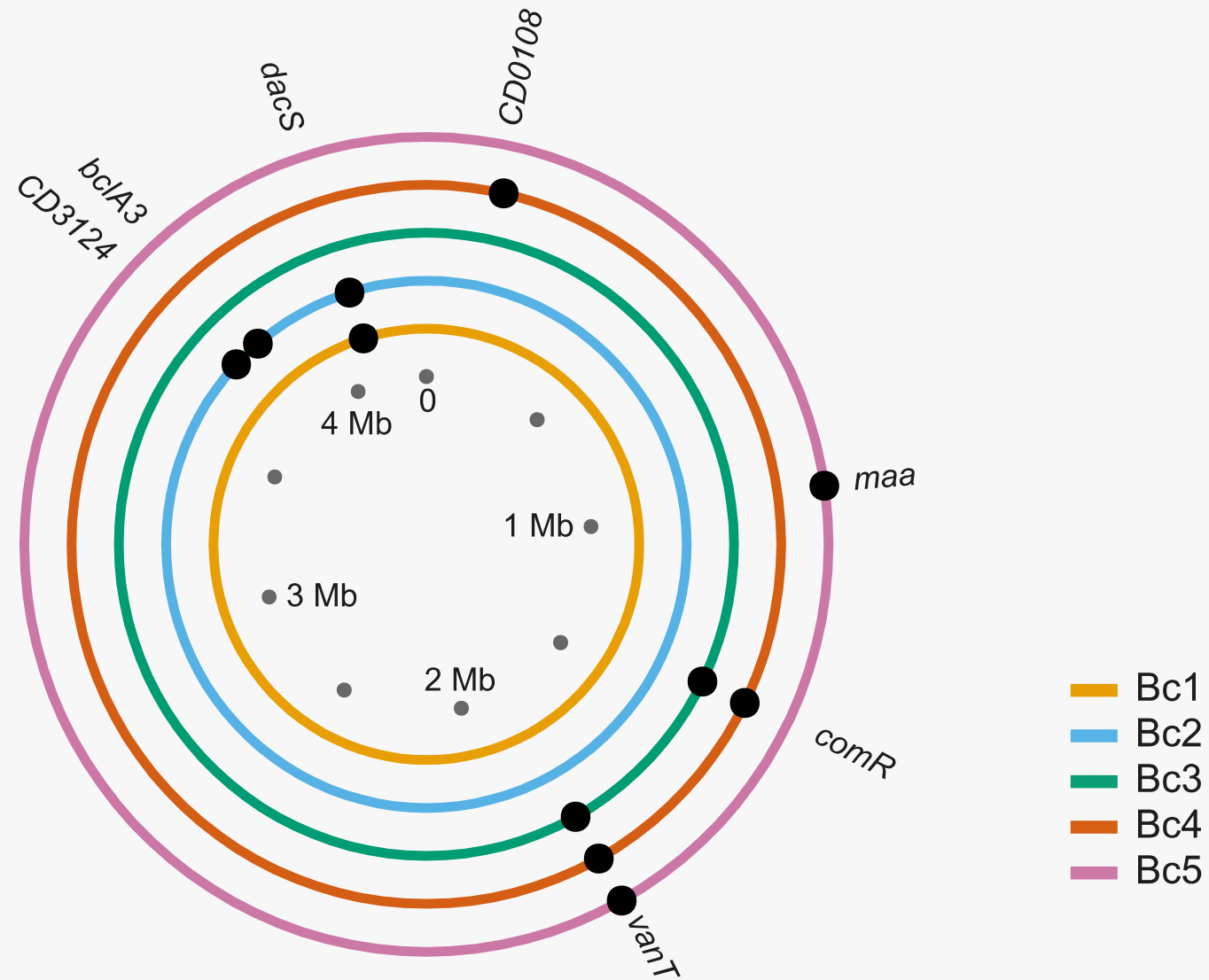


20%

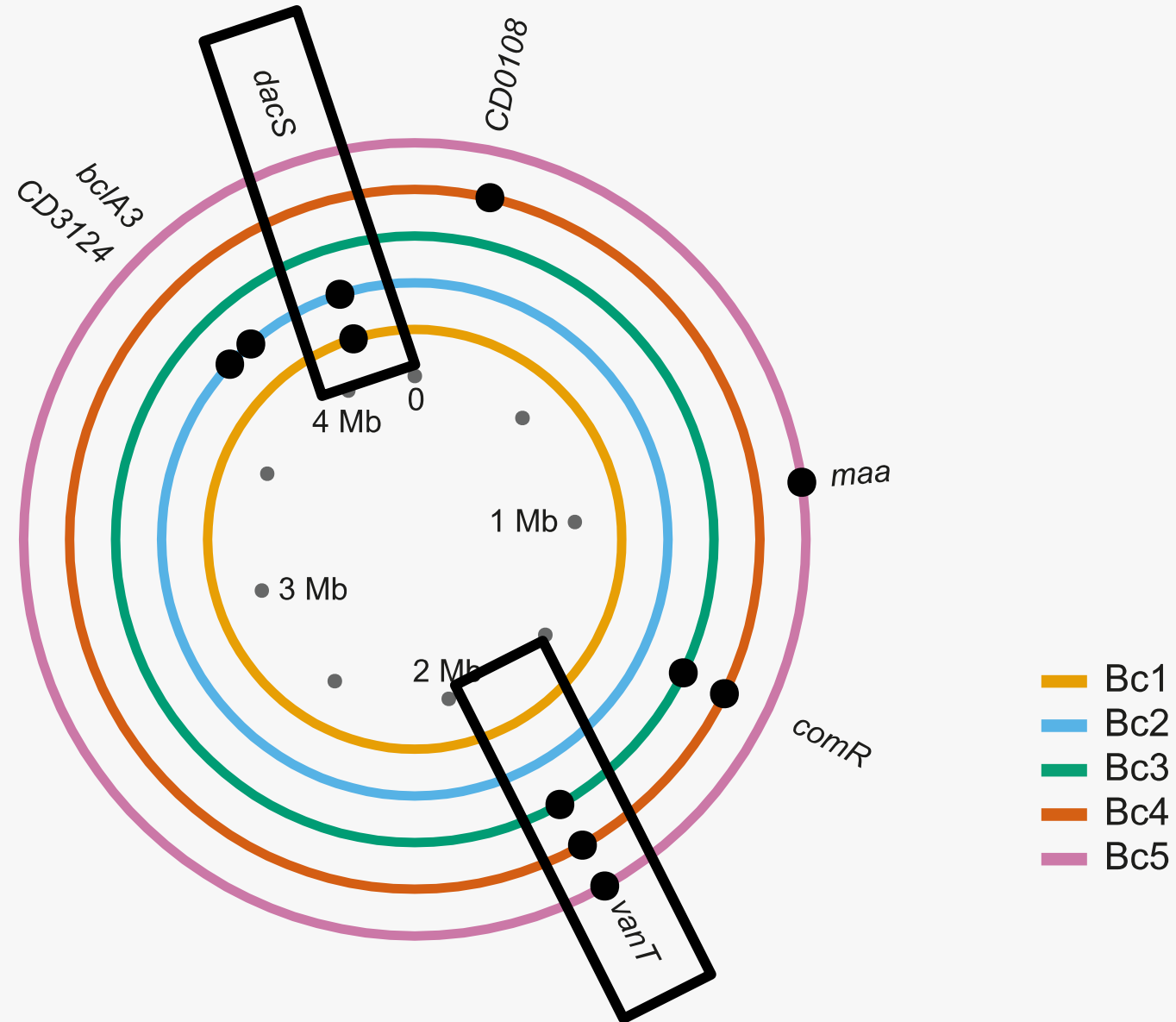


50%

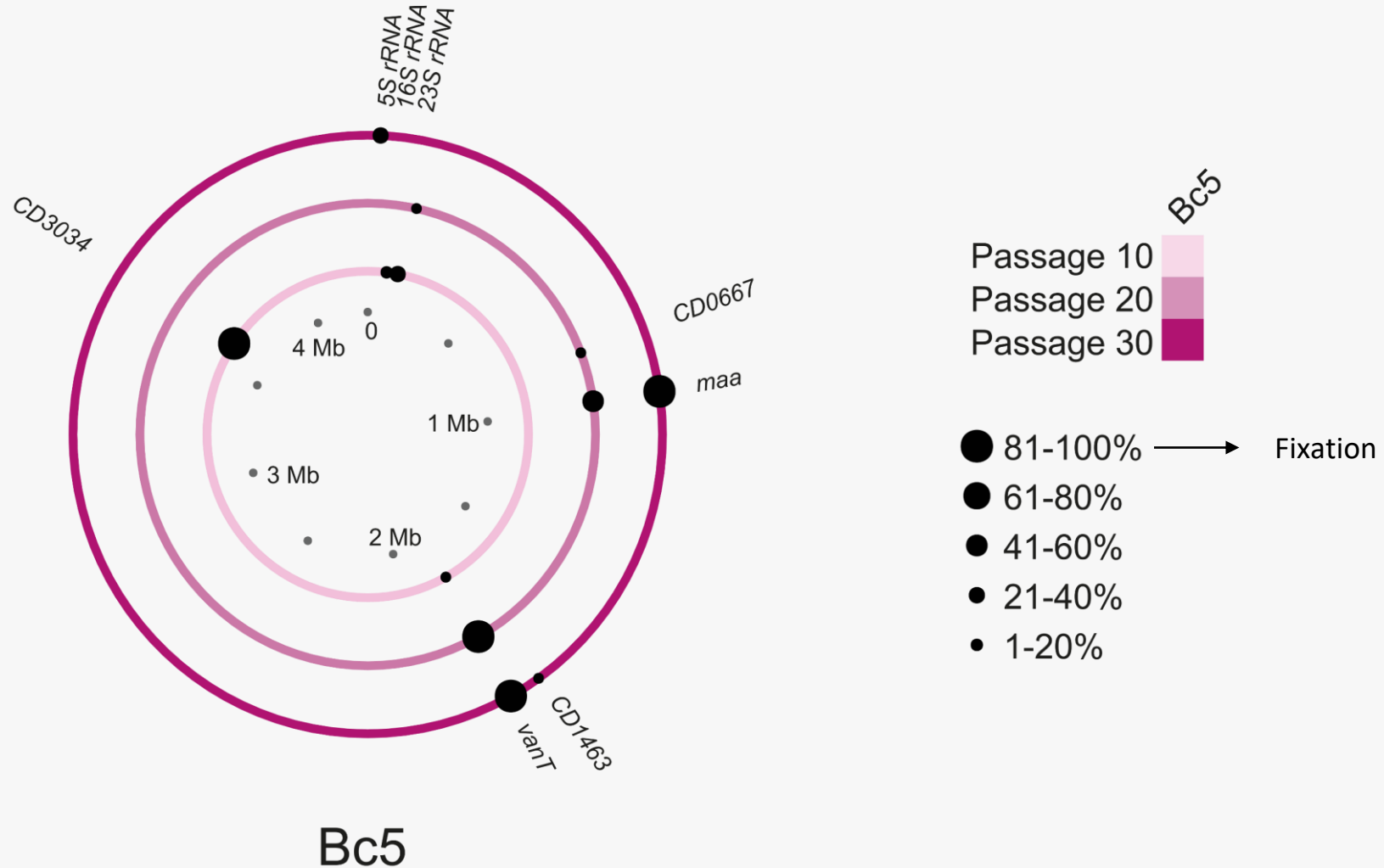
Resistance evolves in parallel in replicate lines



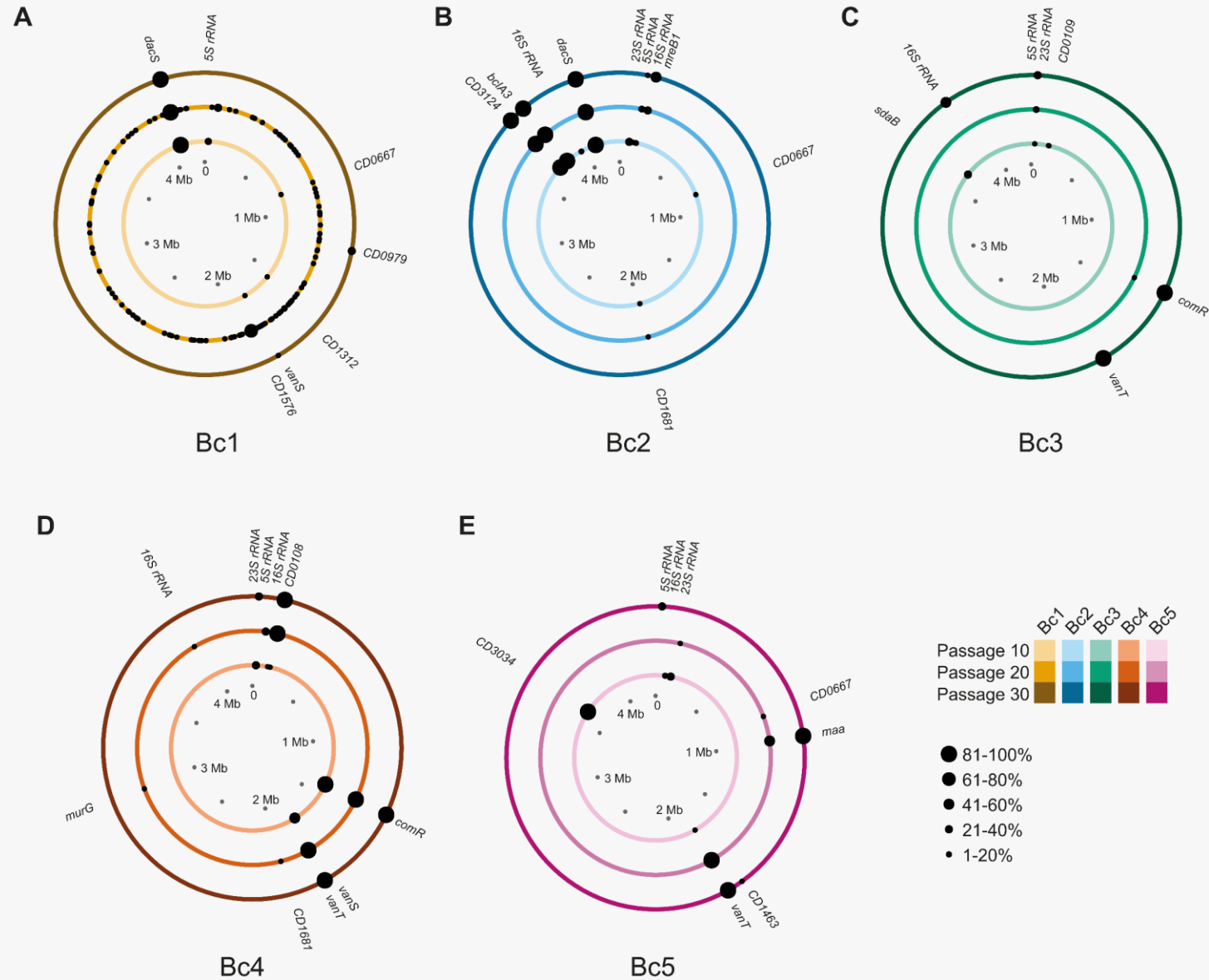
Resistance evolves in parallel in replicate lines



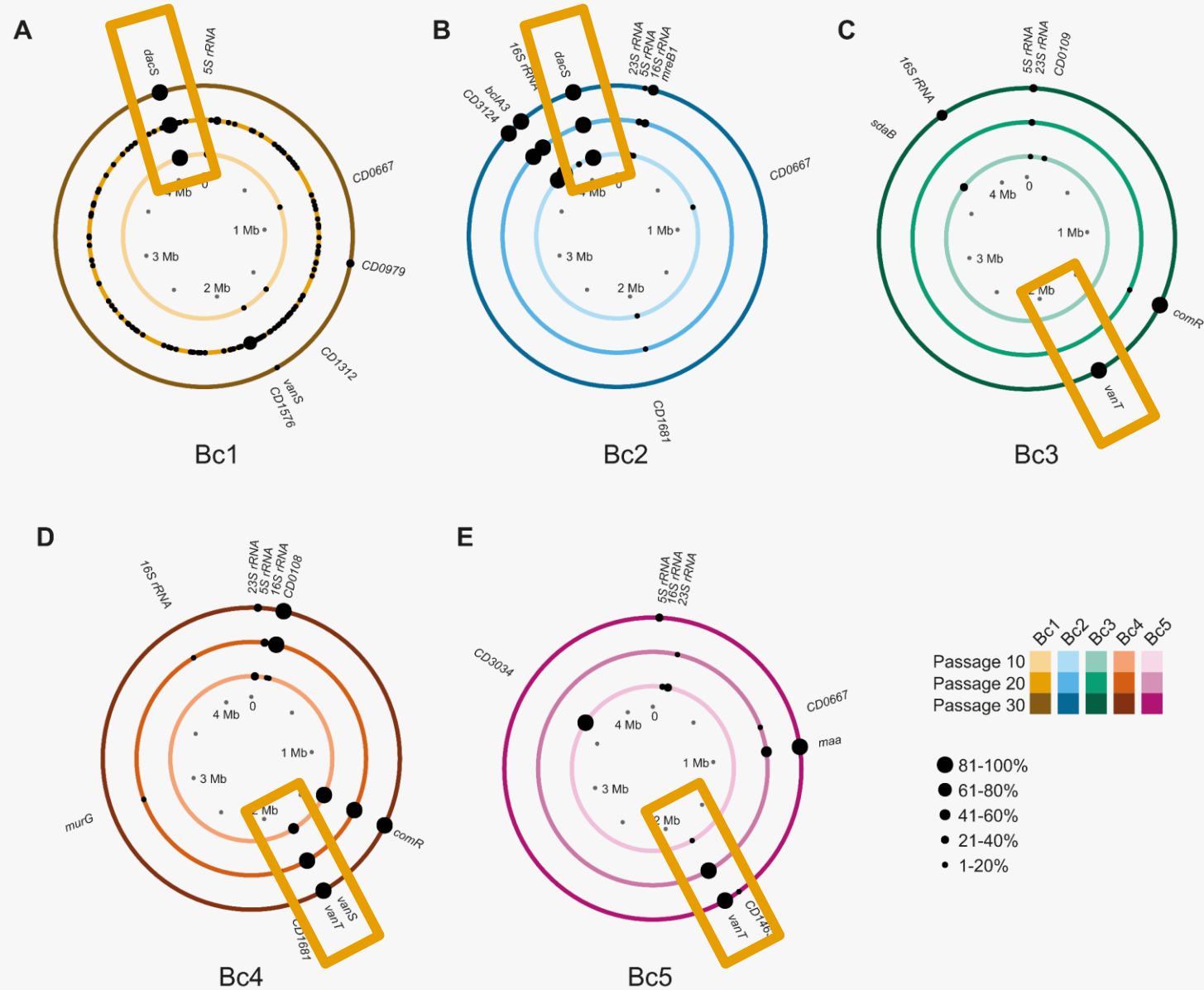
Population sequencing reveals evolutionary dynamics



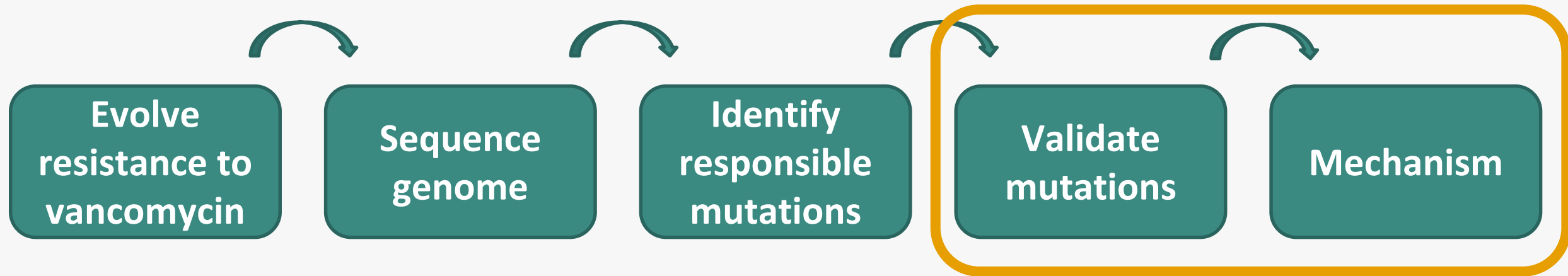
Population sequencing reveals evolutionary dynamics



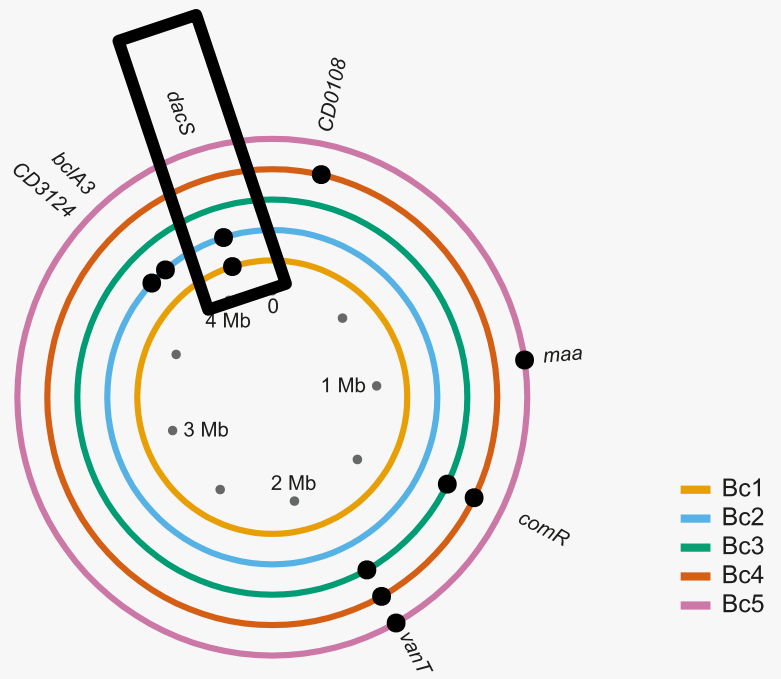
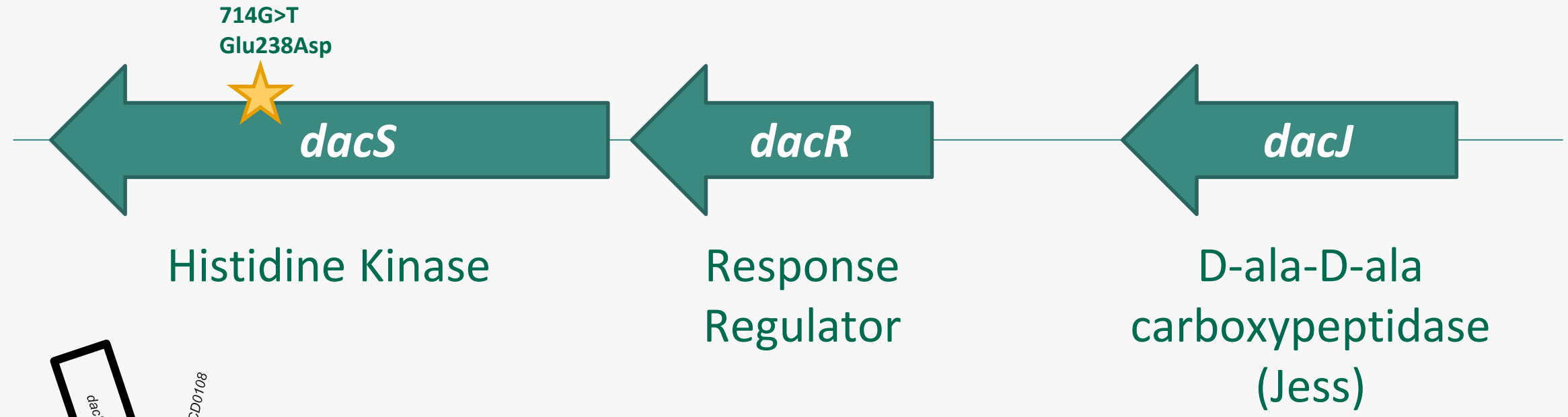
Population sequencing reveals evolutionary dynamics



Aims

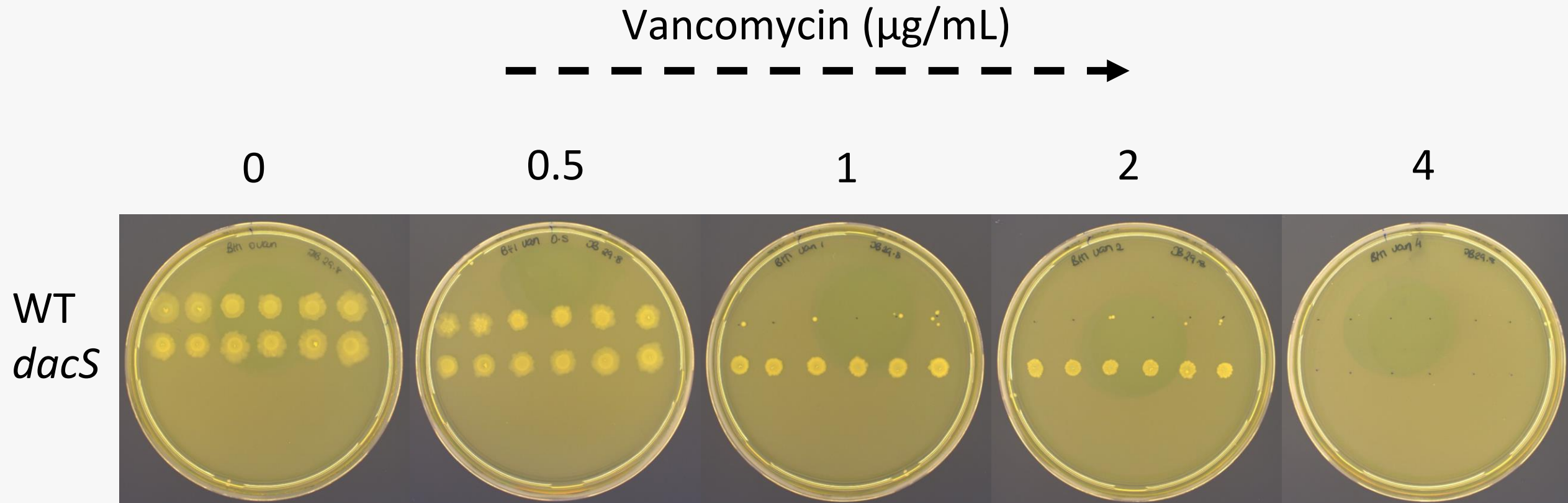


Validating the role of *dacS* in resistance

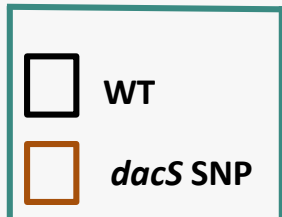
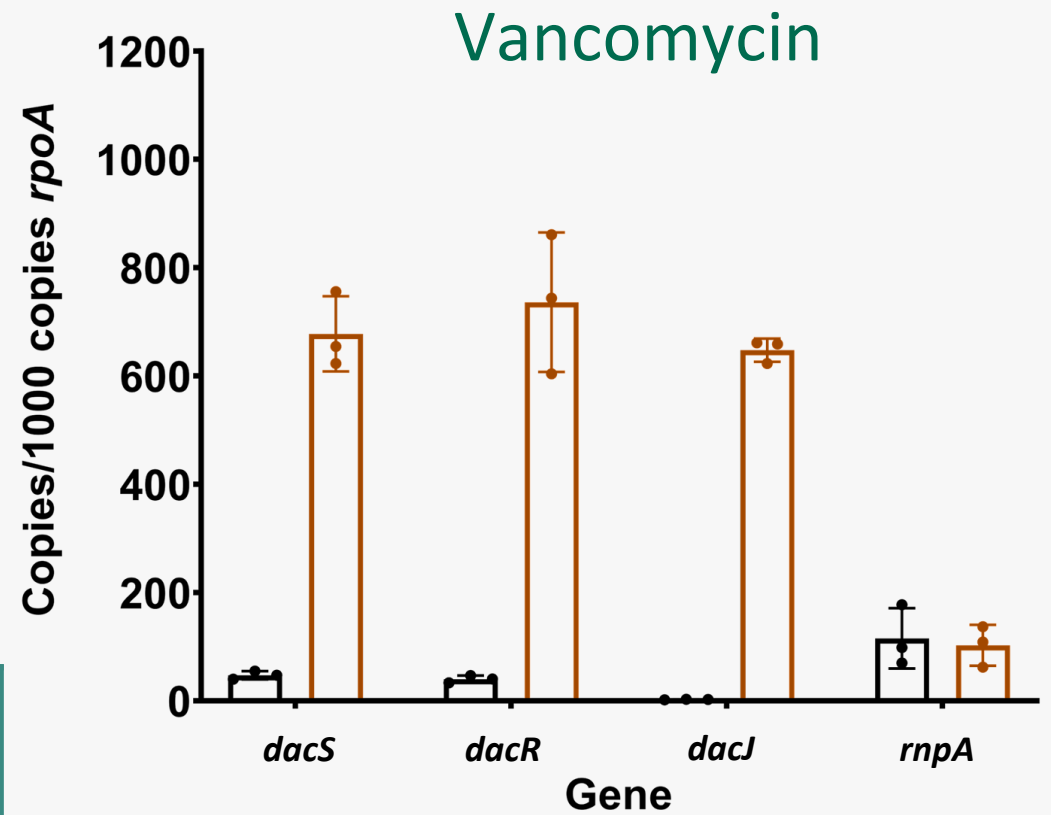
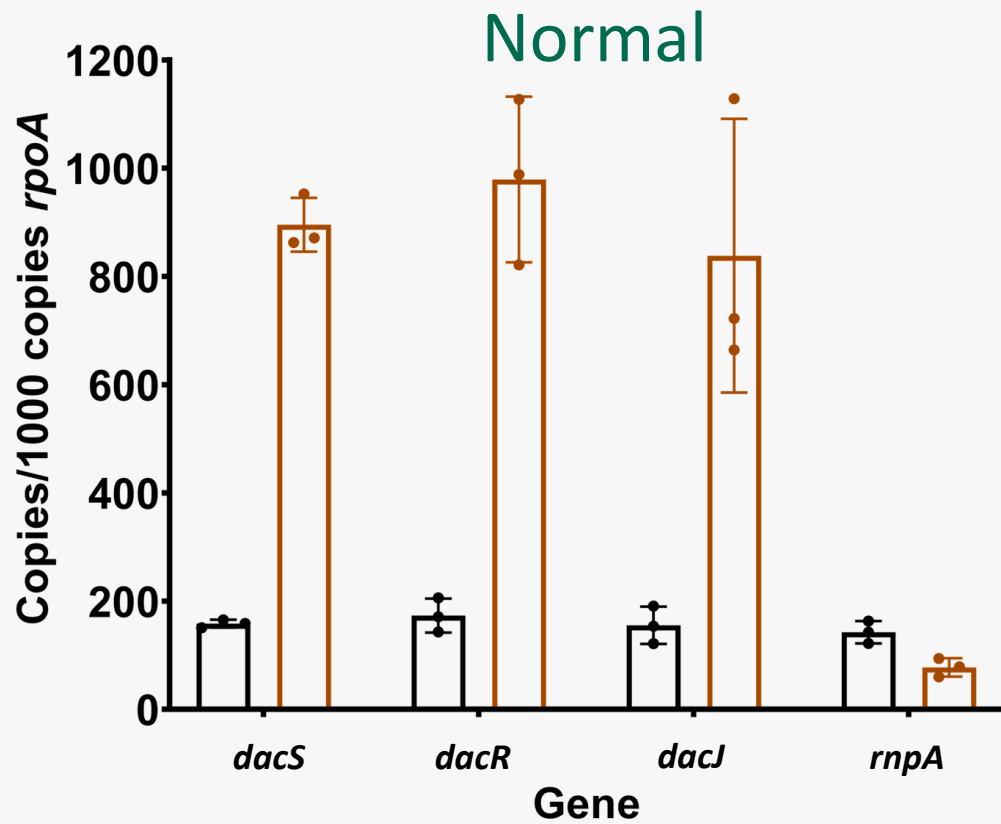


dacS SNP results in 4x increase in vancomycin resistance

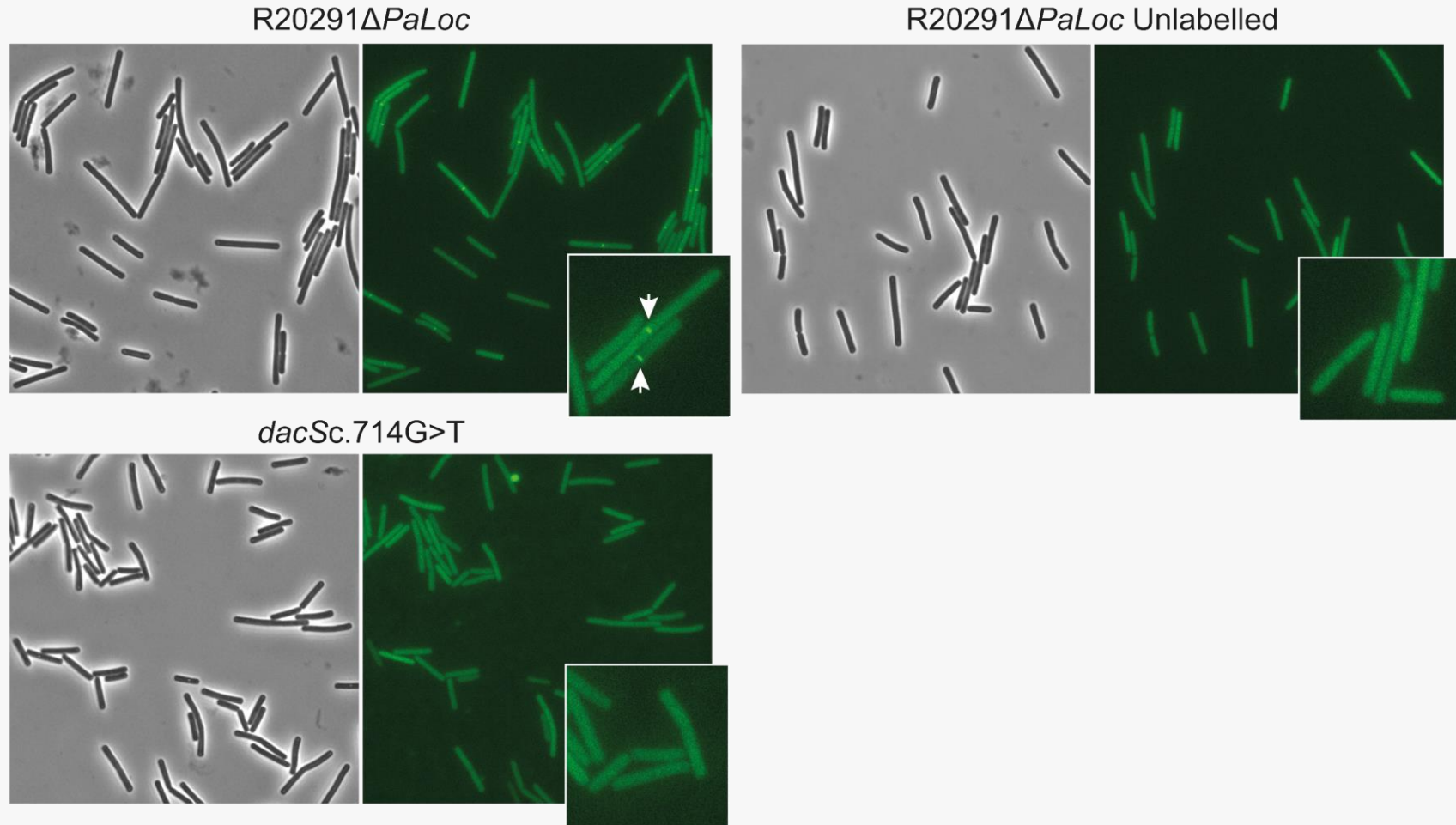
Recapitulated Bc1 *dacS* SNP in WT background to validate effects on resistance:



dacS SNP results in increased expression of *dacJRS*



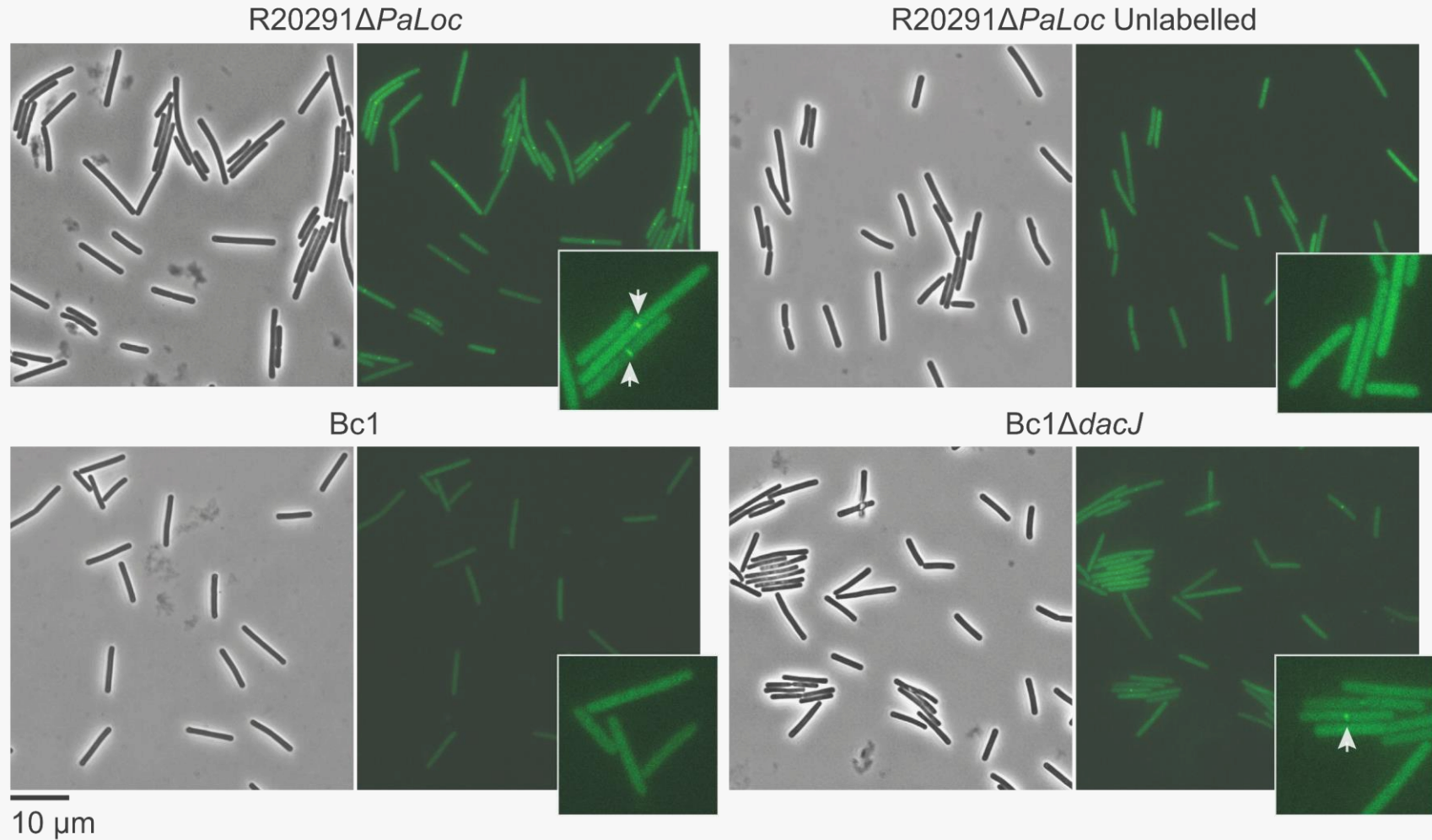
dacS mediated overexpression of *dacJ* depletes D-Ala



Overexpression of
dacJ results in
vancomycin binding
site depletion



Bc1 Δ *dacJ* partially restores vancomycin binding



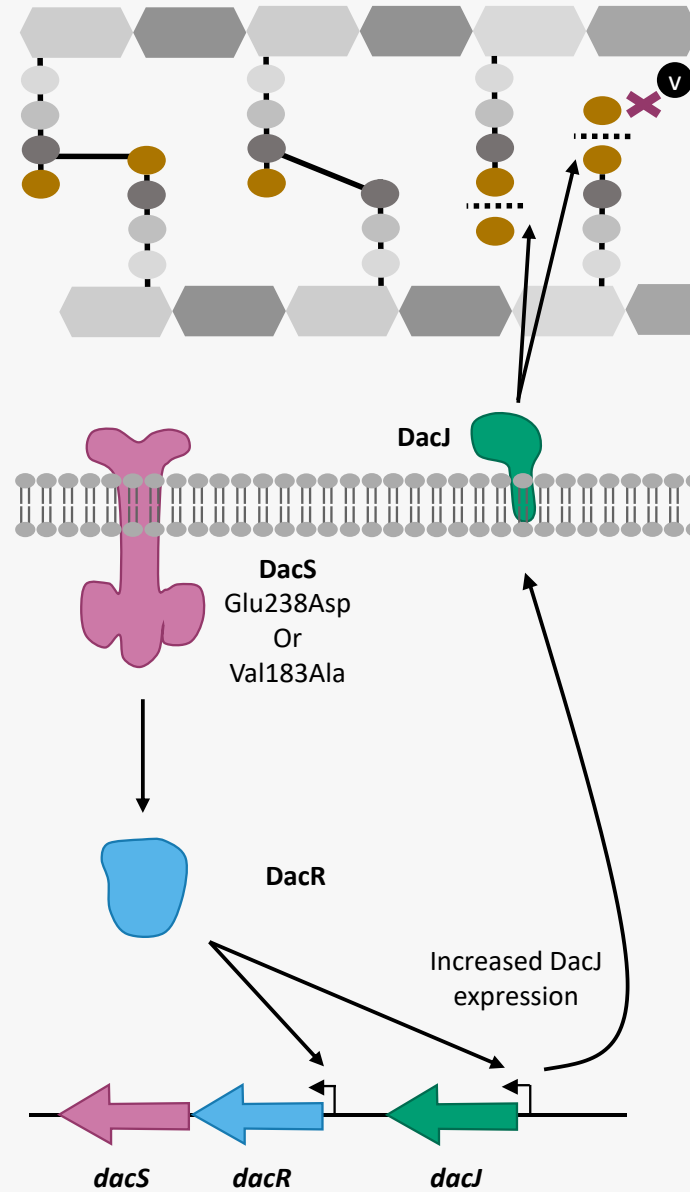
Bc1 Δ *dacJ*

8-fold reduction in
MIC

Partial restoration of
vancomycin binding



dacJRS Summary



dacJRS is not the sole mechanism of Bc1 resistance

Strain	End point MIC ($\mu\text{g}/\text{mL}$)
Ancestral WT	1
Bc1	16
Bc1 <i>dacS</i> SNP	4
Bc1 Δ <i>dacJ</i>	2

Bc1 Δ *dacJ* showed only *partial* restoration of vancomycin binding

No other vancomycin unique mutations in illumina data

dacJRS is not the sole mechanism of Bc1 resistance

Nanopore identified two additional InDels

Gene Name	Function	Mutation
<i>CDR20291_0979</i>		44 bp deletion
<i>vanS</i>	two-component sensor histidine kinase	30 bp insertion
<i>dacS</i>	two-component sensor histidine kinase	SNP

dacJRS is not the sole mechanism of Bc1 resistance

Isolate	Vancomycin ($\mu\text{g/mL}$)							
	0	0.5	1	2	4	8	16	MIC
R20291 Δ Paloc								1
Bc1								16
R20291 Δ PaLoc <i>dacSc.714G>T</i>								4
R20291 Δ PaLoc 1,197,357_1,197,400del								1
R20291 Δ PaLoc <i>vanSc.367_396dup</i>								2
R20291 Δ PaLoc <i>dacSc.714G>T</i> <i>vanSc.367_396dup</i>								16
R20291 Δ PaLoc <i>vanSc.367_396dup</i> 1,197,357_1,197,400del								2
R20291 Δ PaLoc <i>dacSc.714G>T</i> 1,197,357_1,197,400del								4
R20291 Δ PaLoc <i>dacSc.714G>T</i> <i>vanSc.367_396dup</i> 1,197,357_1,197,400del								16

dacJRS is not the sole mechanism of Bc1 resistance

dacS + *vanS*
mutations fully
recapitulate Bc1
resistance

Isolate	Vancomycin (µg/mL)							
	0	0.5	1	2	4	8	16	MIC
R20291Δ <i>PaLoc</i>								1
Bc1								16
R20291Δ <i>PaLoc</i> <i>dacSc.714G>T</i>								4
R20291Δ <i>PaLoc</i> 1,197,357_1,197,400del								1
R20291Δ <i>PaLoc</i> <i>vanSc.367_396dup</i>								2
R20291Δ <i>PaLoc</i> <i>dacSc.714G>T</i> <i>vanSc.367_396dup</i>								16
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R20291Δ <i>PaLoc</i> <i>dacSc.714G>T</i> 1,197,357_1,197,400del								4
R20291Δ <i>PaLoc</i> <i>dacSc.714G>T</i> <i>vanSc.367_396dup</i> 1,197,357_1,197,400del								16

dacS + *vanS*
work
synergistically

Implications of *dacS* + *vanS* synergy

Genetic determinants of resistance to antimicrobial therapeutics are rare in publicly available *Clostridioides difficile* genome sequences

[Baban Kolte](#) and [Ulrich Nübel](#)[✉]

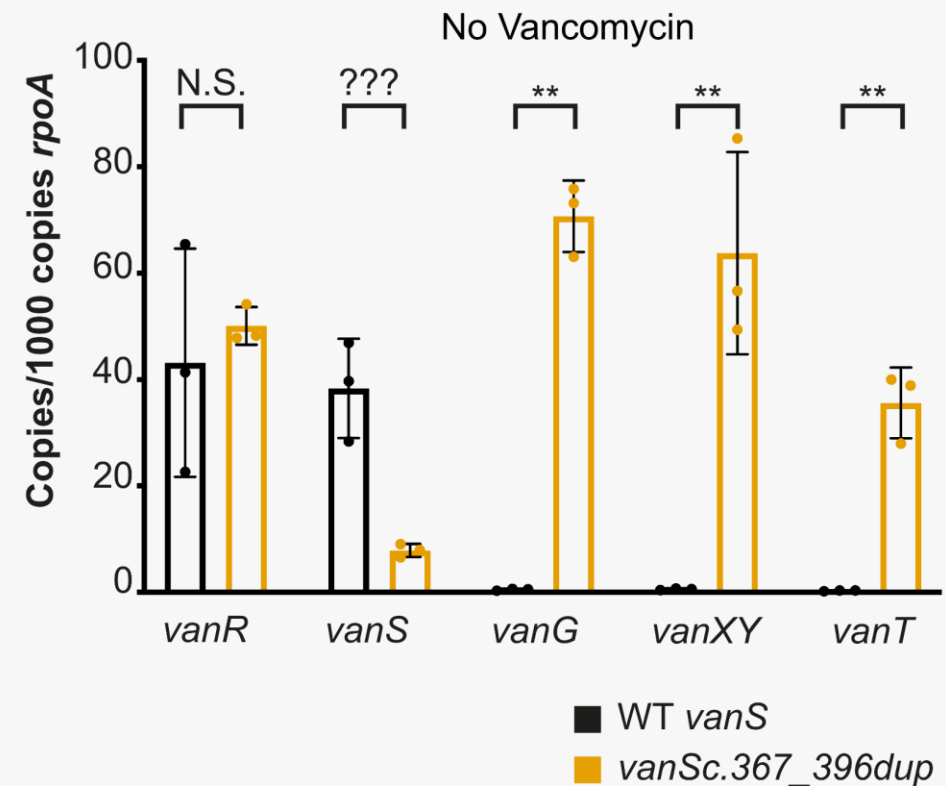
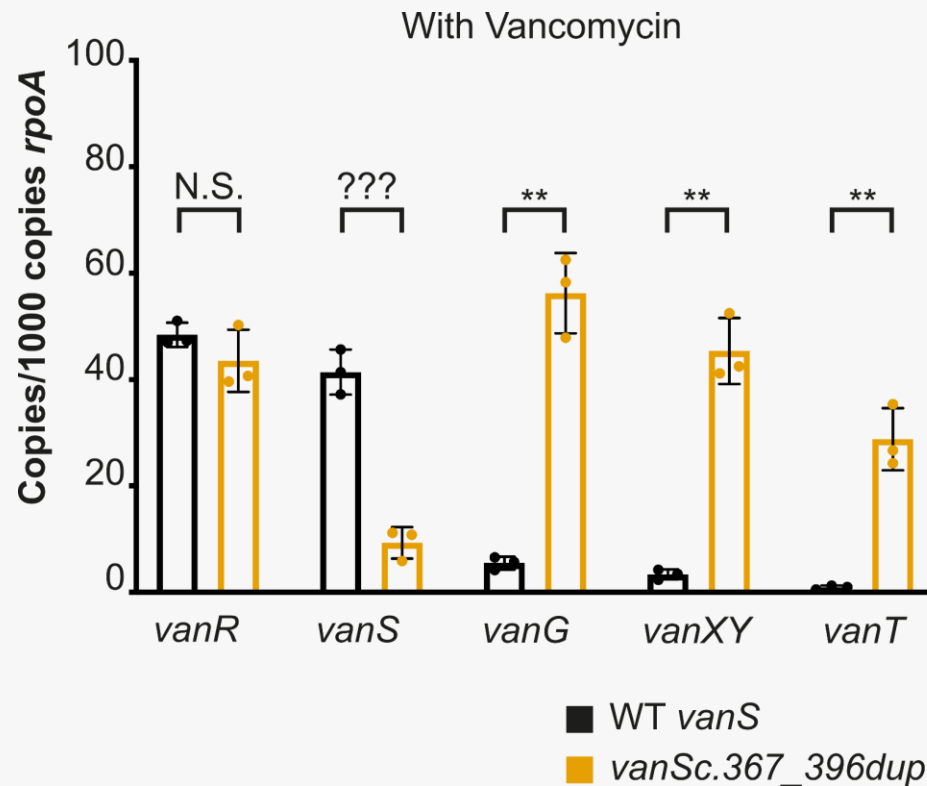
<i>vanS</i> -p.R314L	8	15	12	4×10^{-4}	4 (RT027), 178 (RT018), 2 (RT002)
<i>vanS</i> -p.R314H	8	this study	16	6×10^{-4}	4 (RT027), 22 (RT106), 86
<i>vanS</i> -p.S313F	8	15	13	5×10^{-4}	4 (RT027), 3 (RT001), 58 (RT012)
<i>vanS</i> -p.G319D	16/>8	15 /this study	45	2×10^{-3}	89, 3 (RT001), 4 (RT027)
<i>vanS</i> -p.T349I	8/2-8	15 /this study	3477	0.13	2 (RT002), 86 (RT005), 34 (RT014)

Only a single *dacS* SNP required to elevate *vanS* resistance dramatically

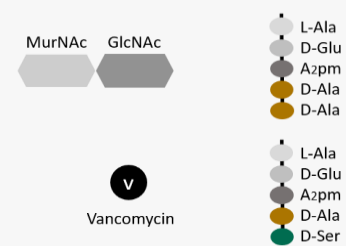
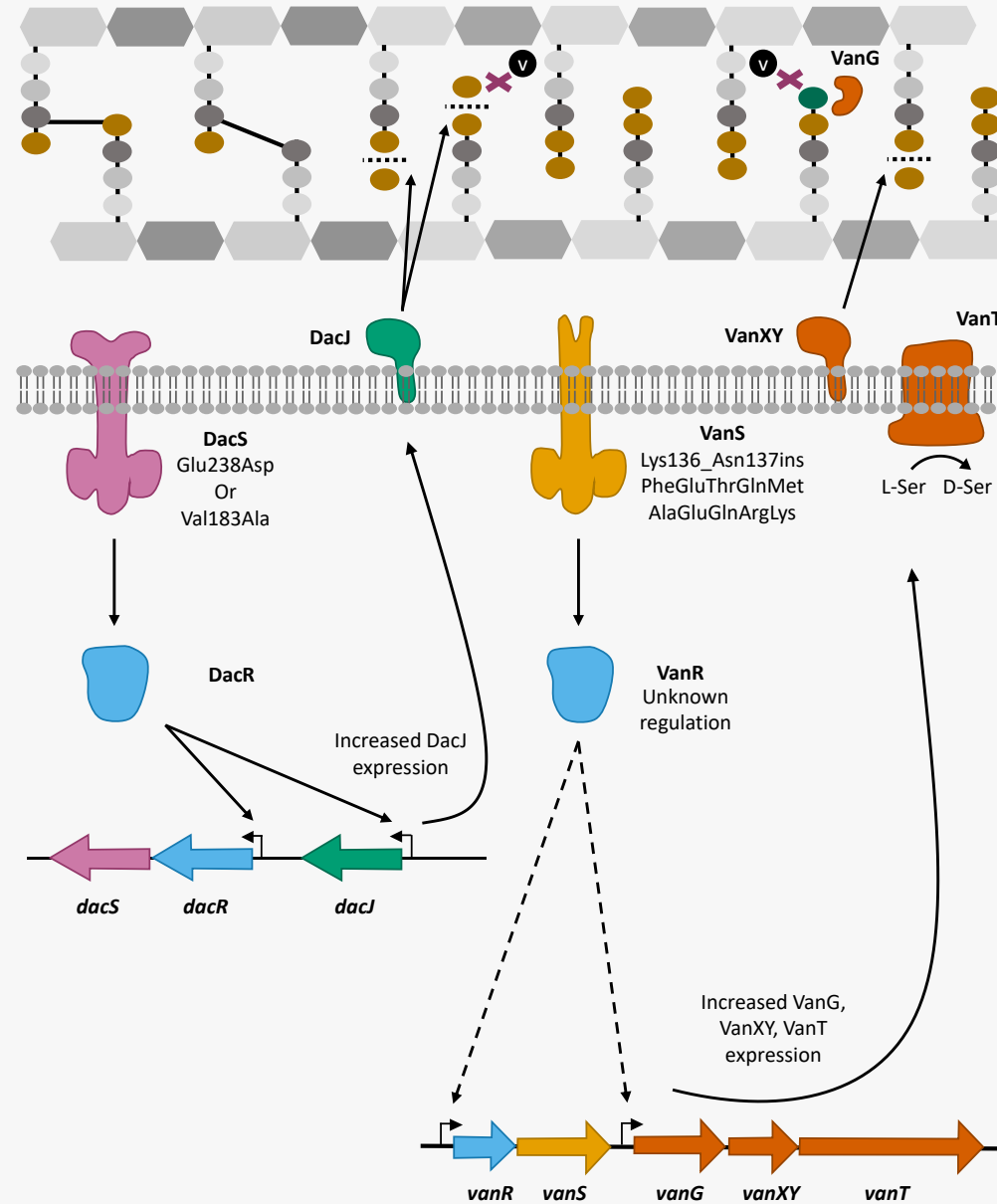
vanS insertions never reported – may not be captured

vanS mutation results in *van* constitutive expression

Like previously identified *vanS* SNPs, *vanSc.367_396dup* results in constitutive expression of *vanGXYT*









Summary



Read the paper here:

Multiple evolutionary pathways lead to vancomycin resistance in *Clostridioides difficile* 

 Jessica E. Buddle,  Rosanna C.T. Wright,  Claire E. Turner,
 Roy R. Chaudhuri,  Michael A. Brockhurst,  Robert P. Fagan

doi: <https://doi.org/10.1101/2023.09.15.557922>



Paper

#OpenToWork



CV website

PhD hand in: July
Scholarship end date: November

Please come chat to me! 😊

Acknowledgements

Robert P. Fagan^{1*}
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Anne S. Williams³
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Roy R. Chaudhuri¹
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Fagan Lab



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Paper

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