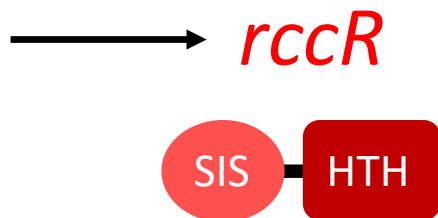


One ligand, two regulators and three binding sites: how KDPG controls primary carbon metabolism in *Pseudomonas*

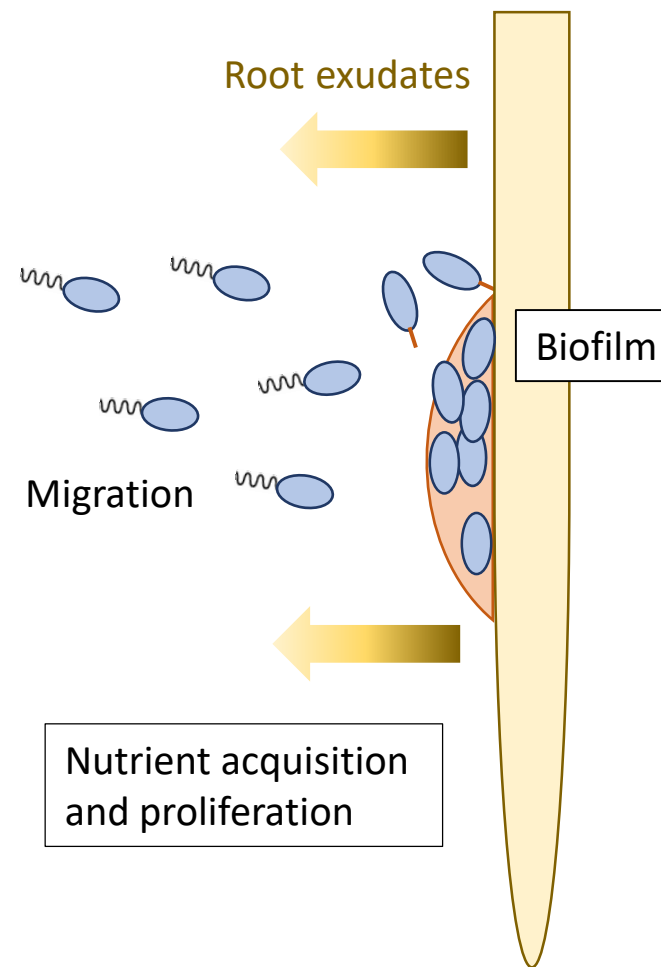


The complex process of rhizosphere colonisation

Functional Genome *in vivo* expression
technology (IVET) screening



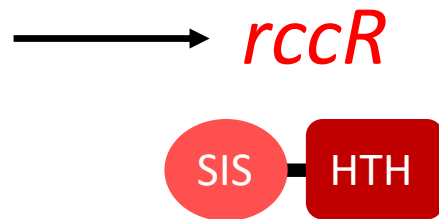
HexR - (43% identity, > 70% similarity)



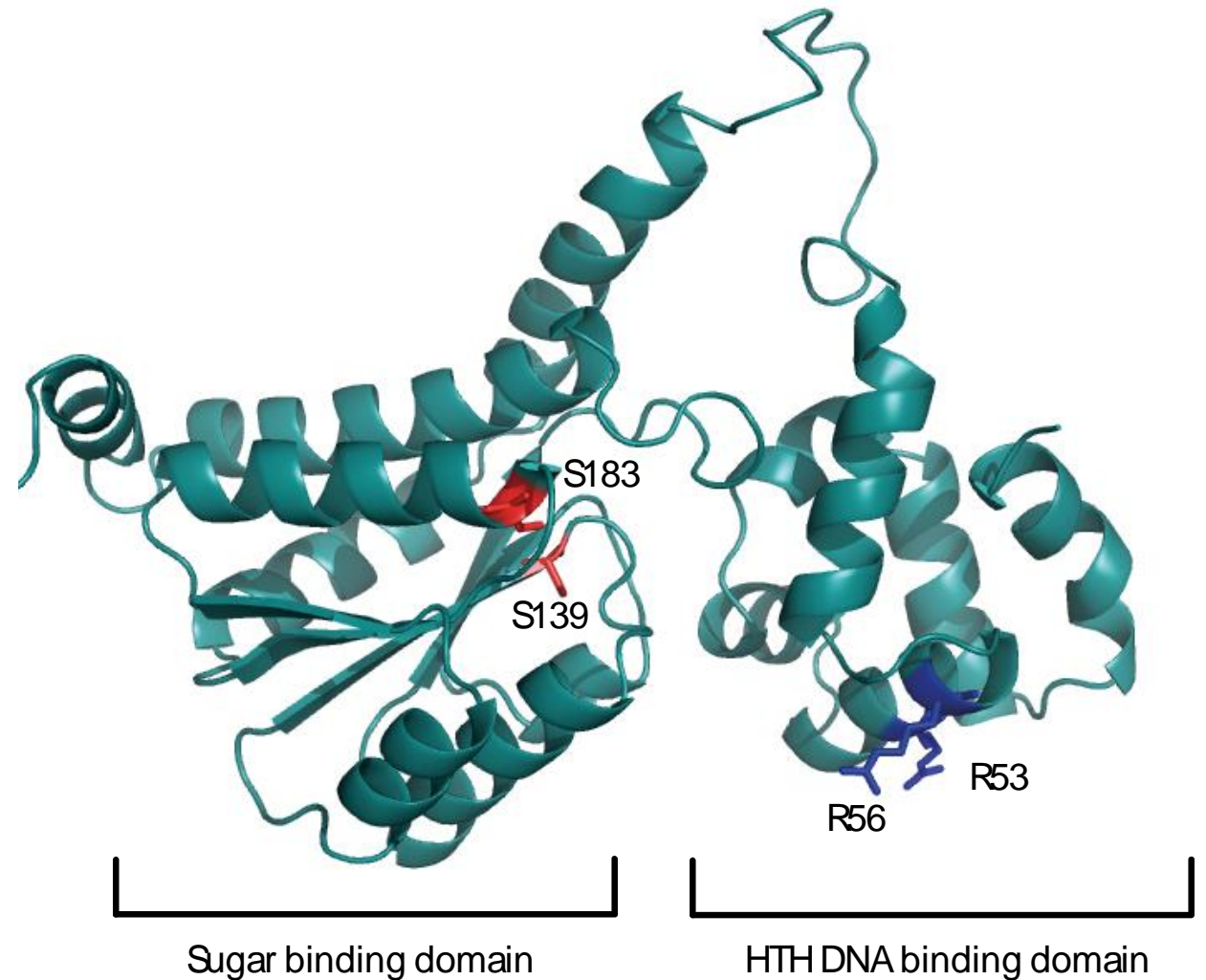
 *Pseudomonas fluorescens*

RccR: an RpiR-family transcription factor

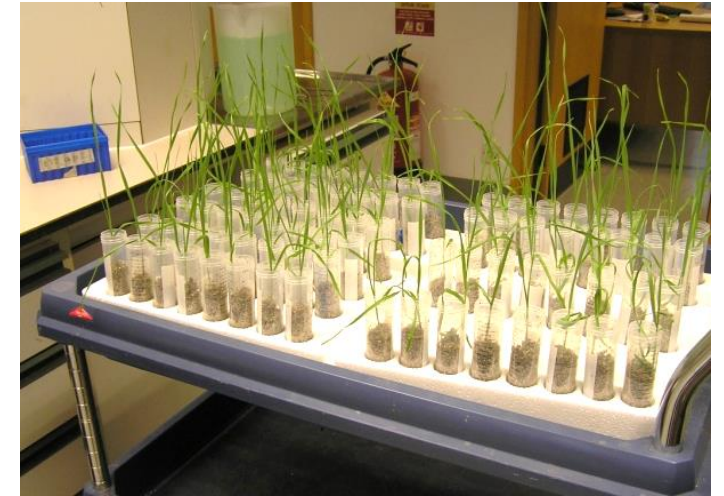
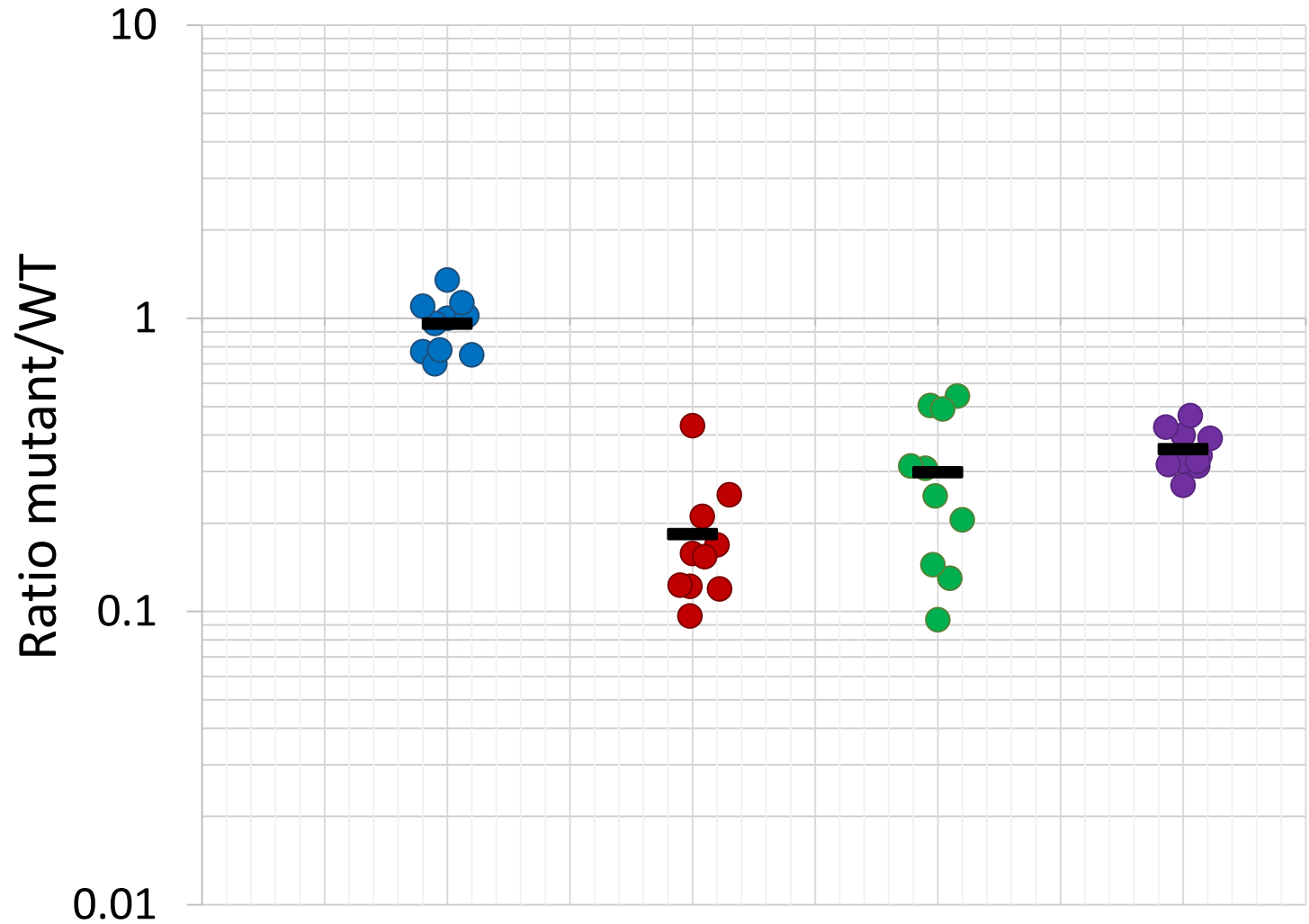
Functional Genome *in vivo* expression
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HexR - (43% identity, > 70% similarity)

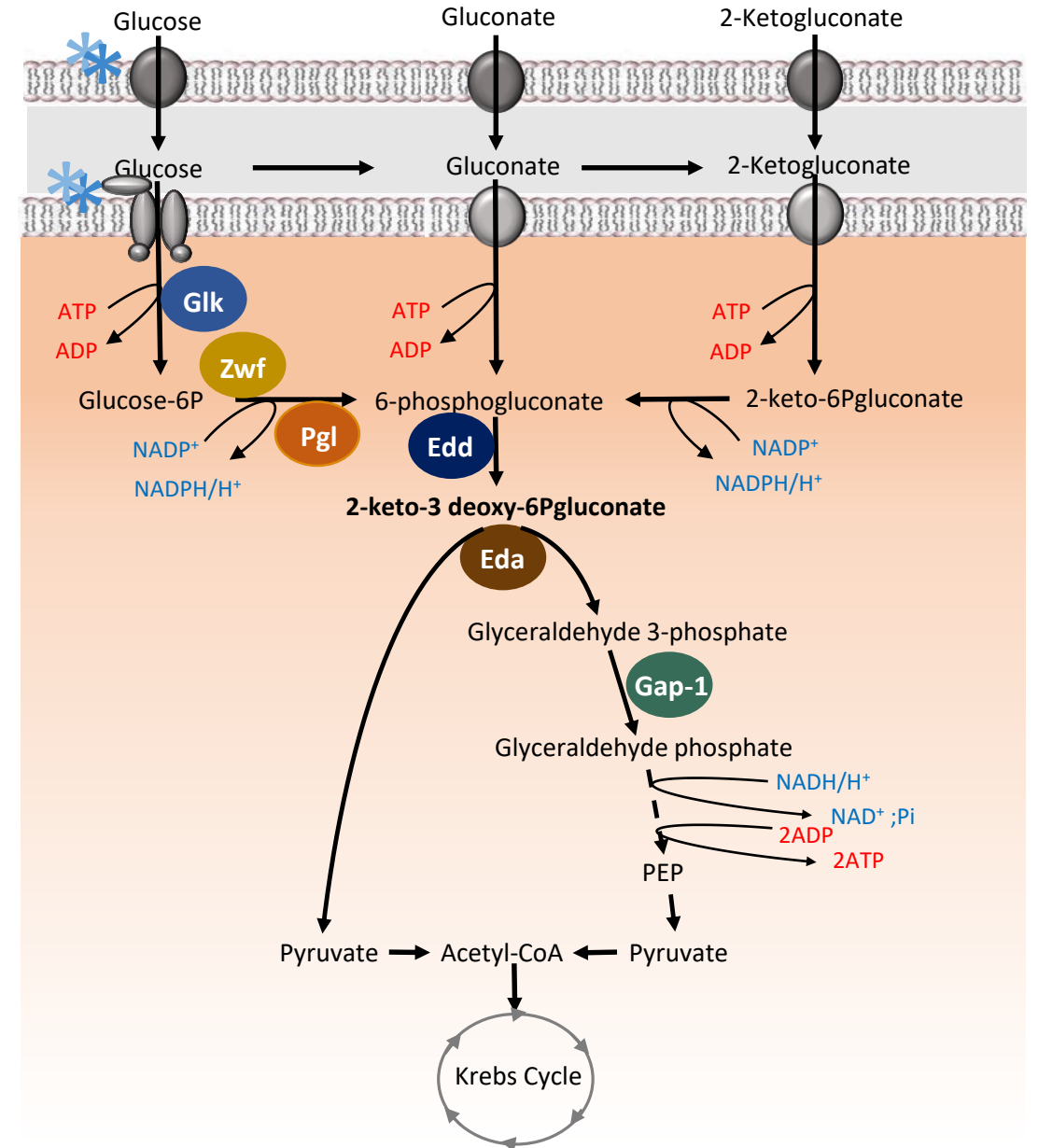
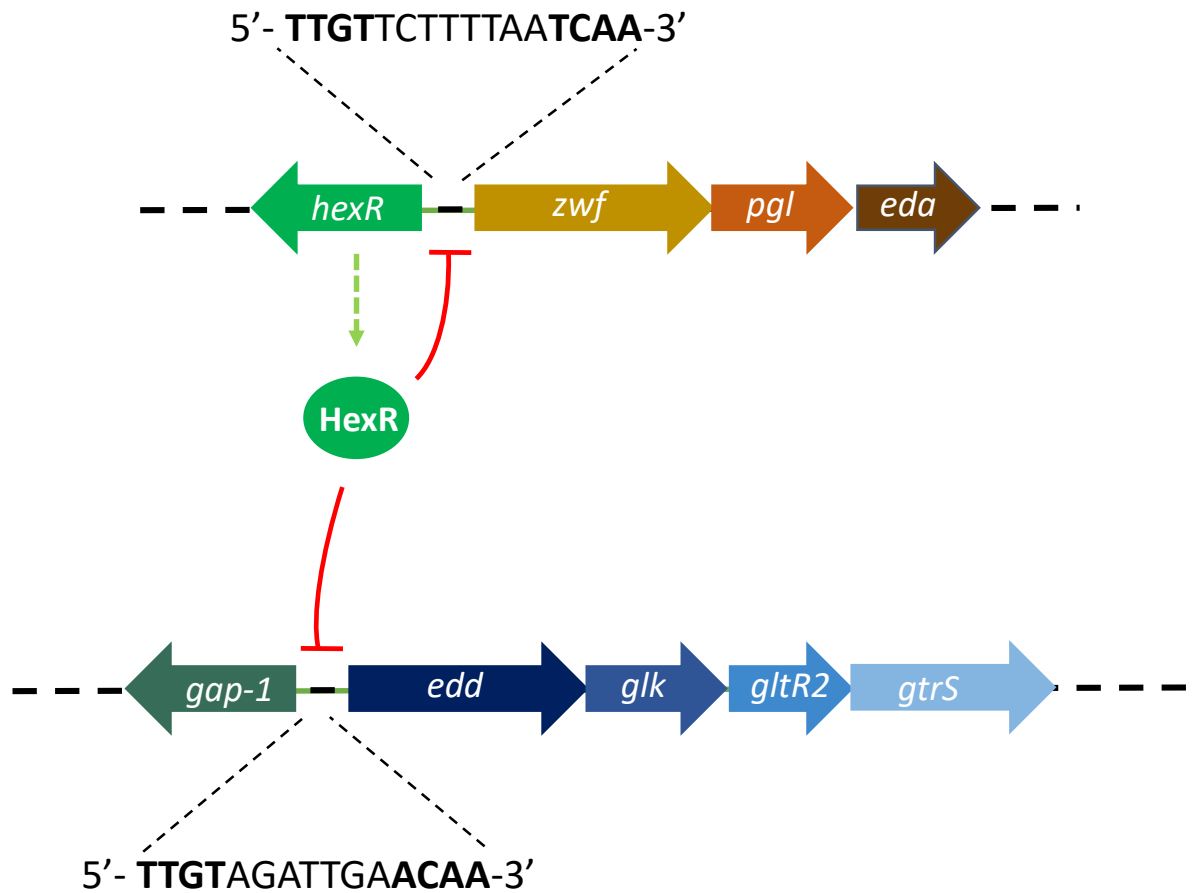


RccR and HexR are important for wheat rhizosphere colonization



- **SBW25**
- **$\Delta rccR$**
- **$\Delta hexR$**
- **$\Delta rccR\Delta hexR$**

HexR controls the Entner-Doudoroff pathway in *P. putida*

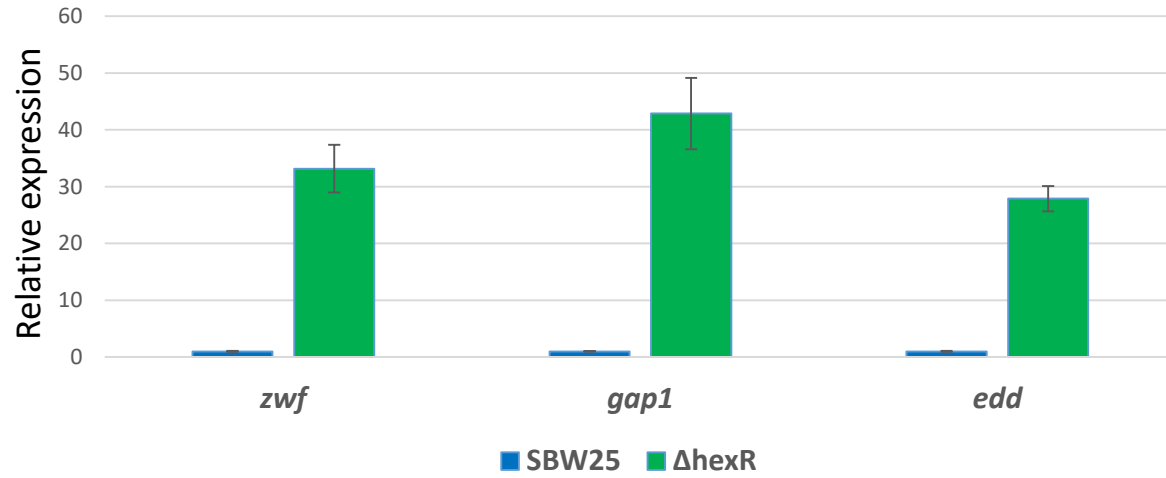


Daddaoua et al. *JBC* (2009) 284(32): 21360–21368

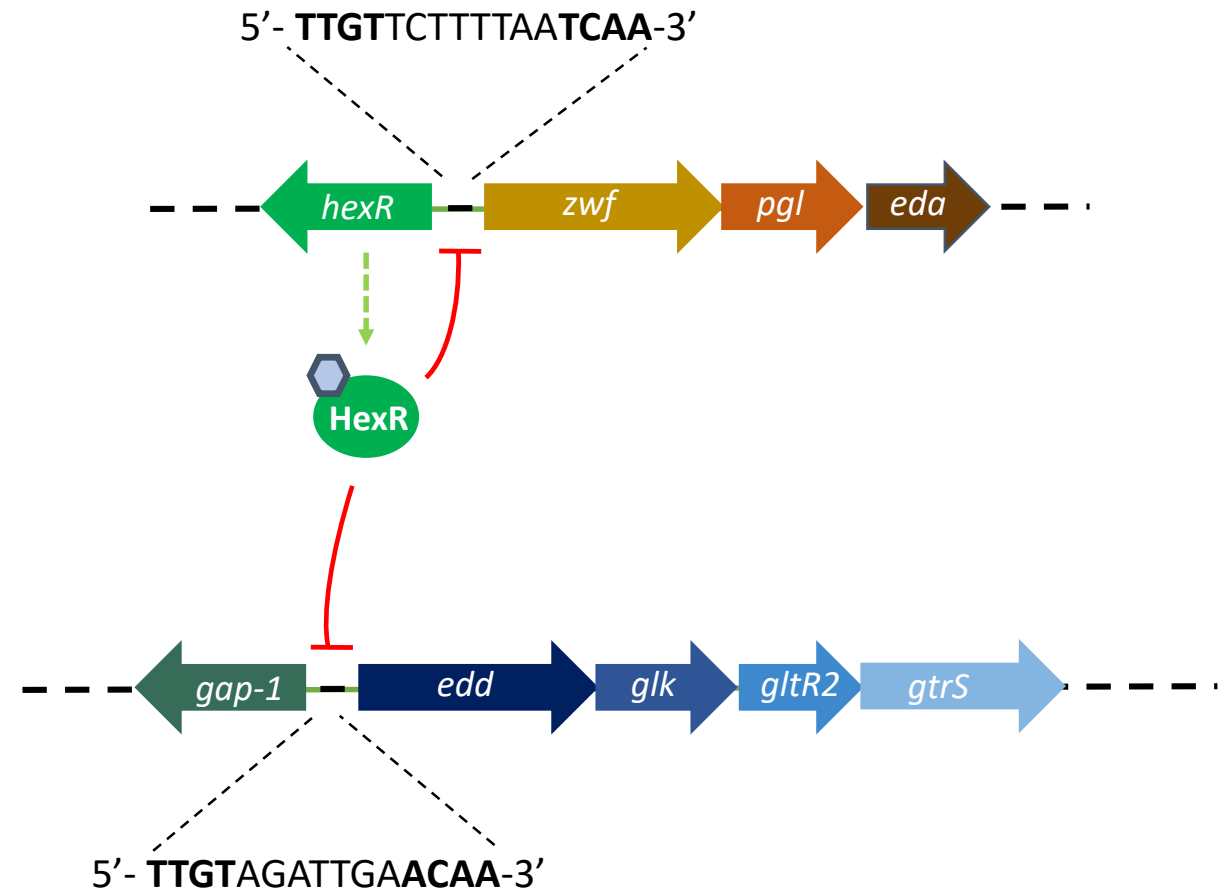
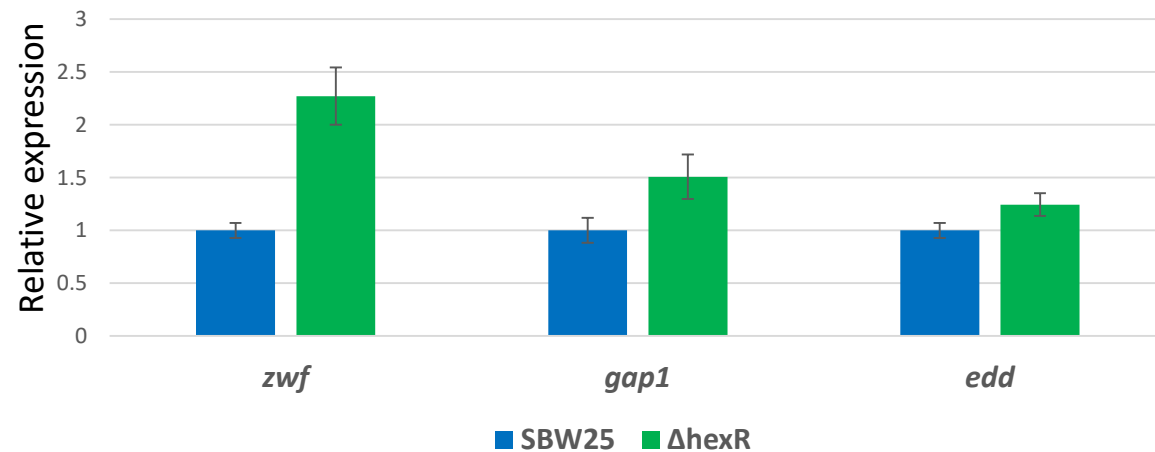
Campilongo et al. *PLoS Genetics* (2017) 13 (6), e1006839

HexR controls the Entner-Doudoroff pathway in *P. fluorescens*

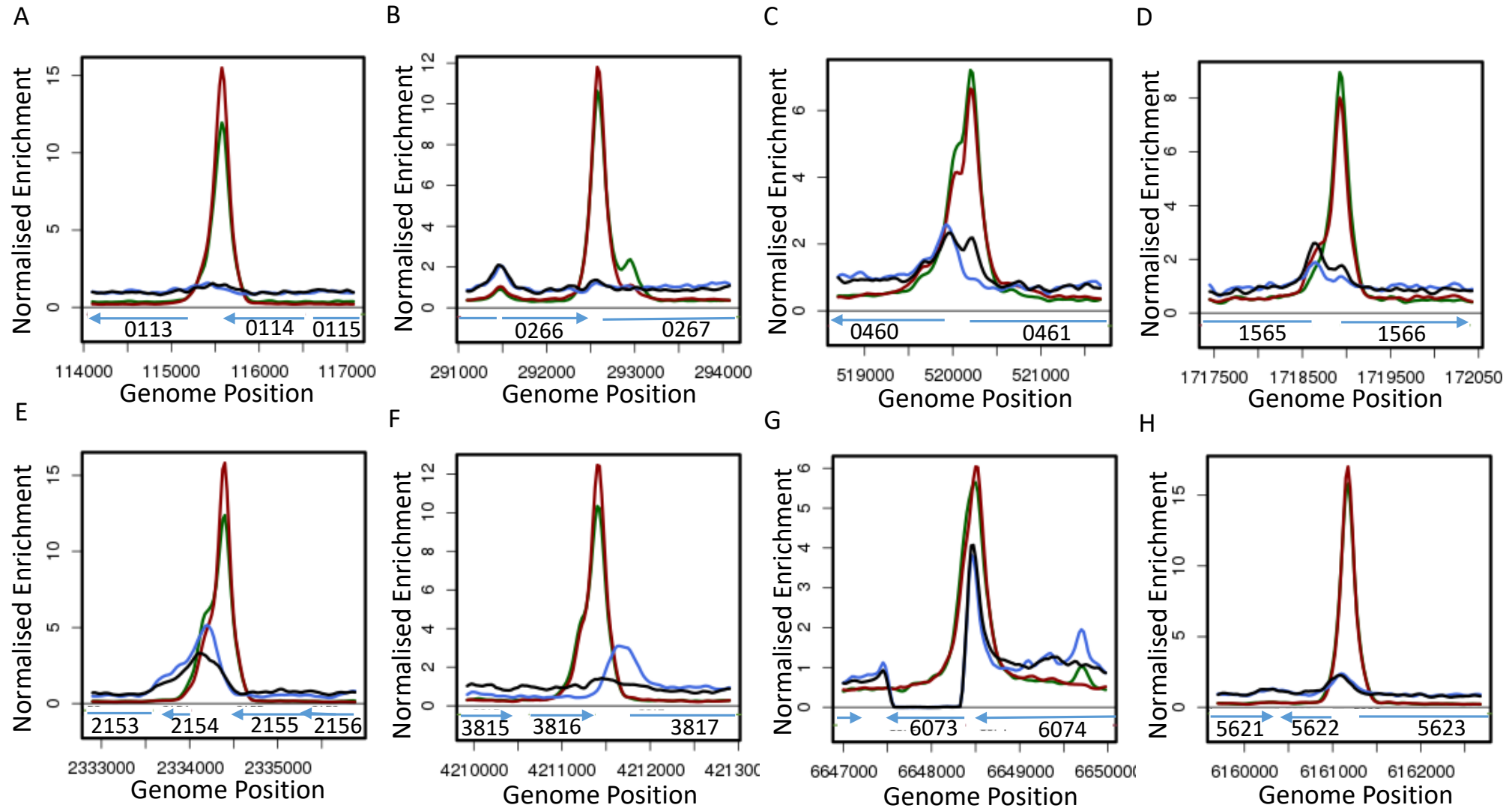
Acetate



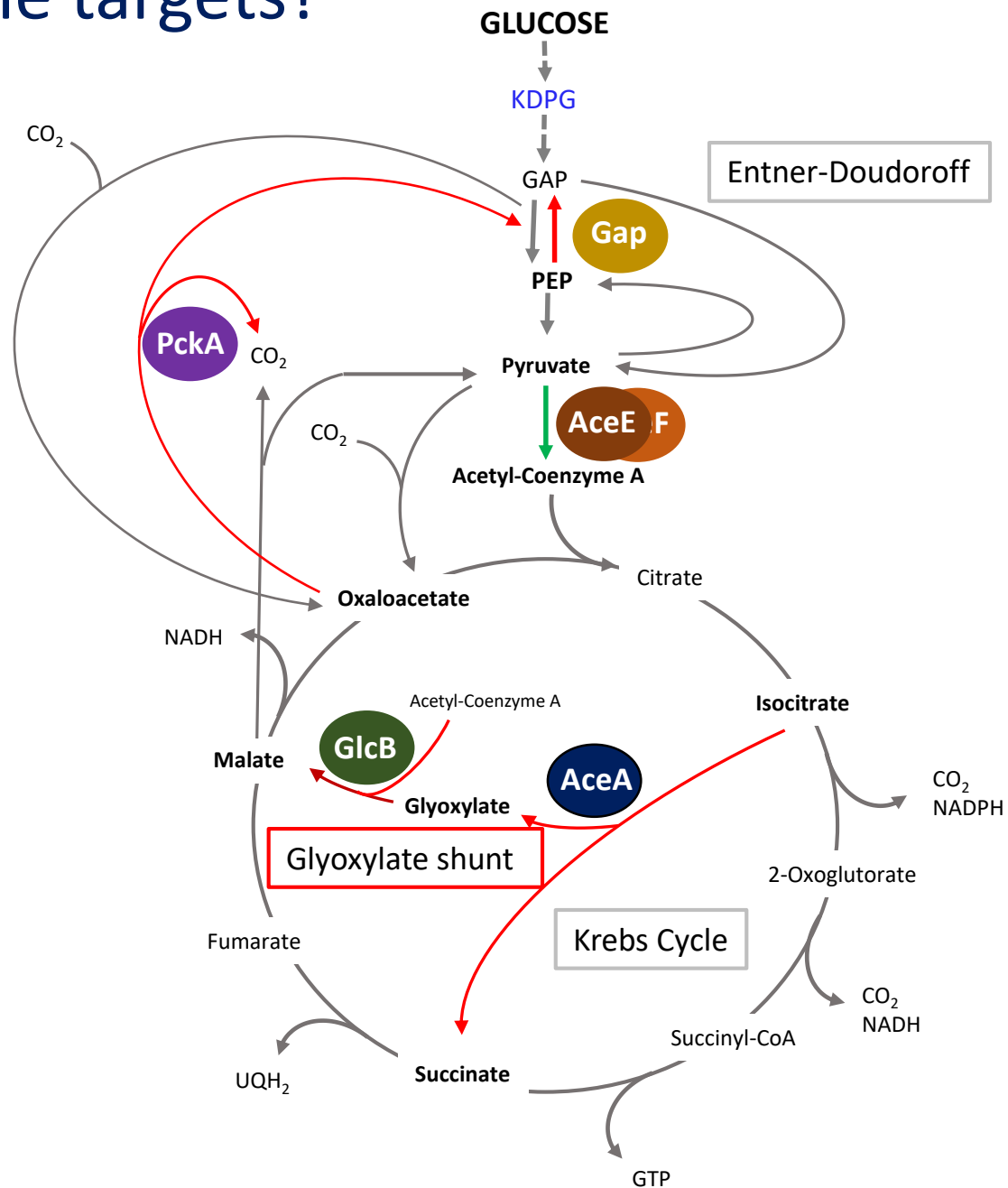
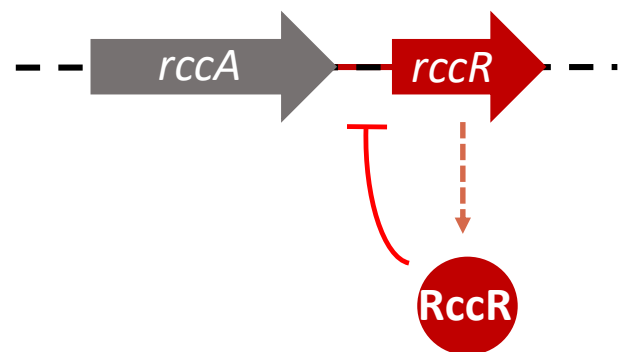
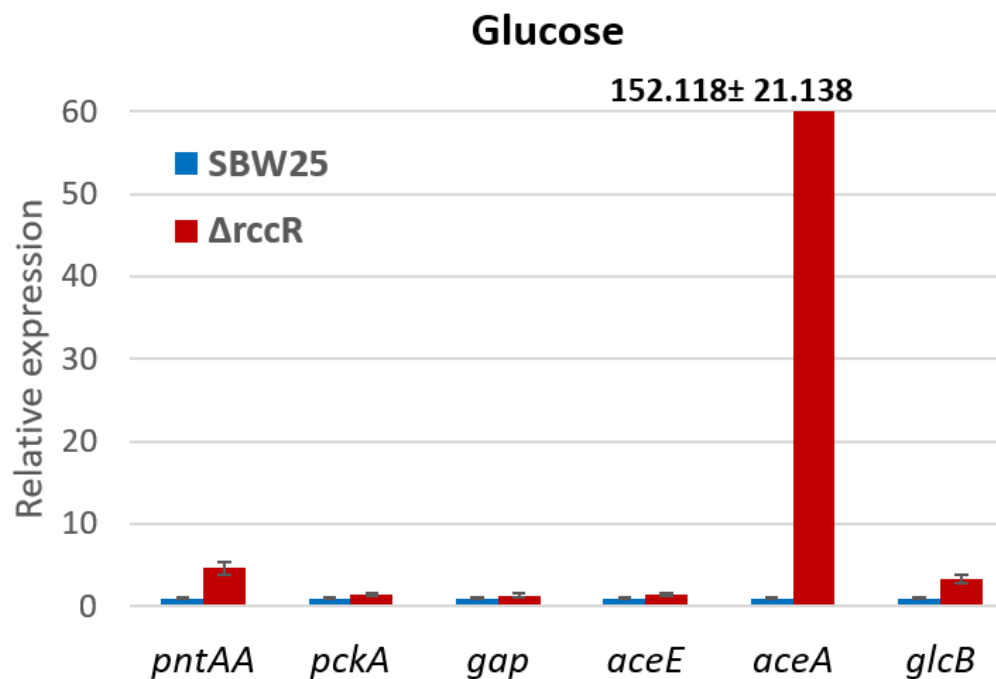
Glucose



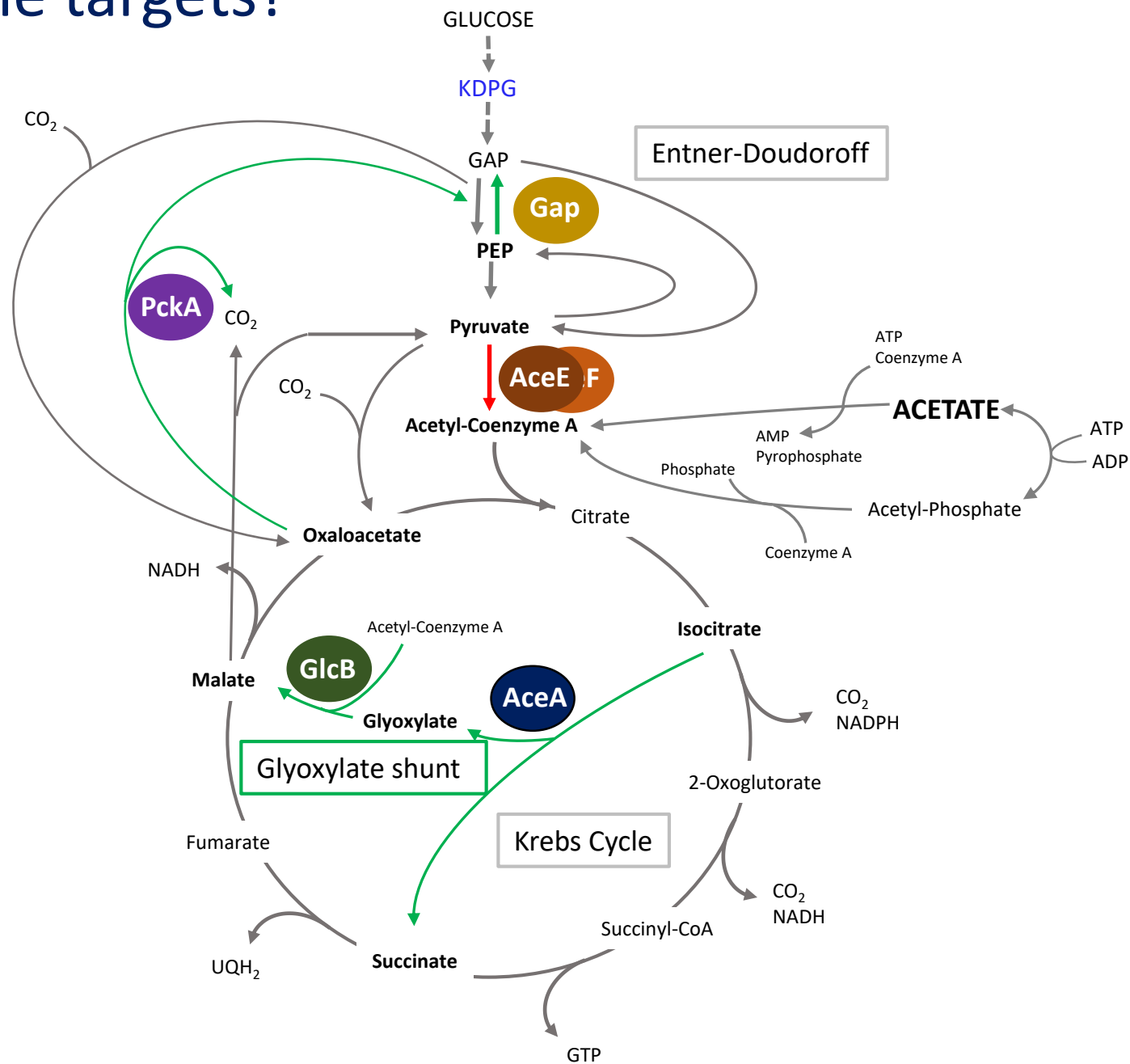
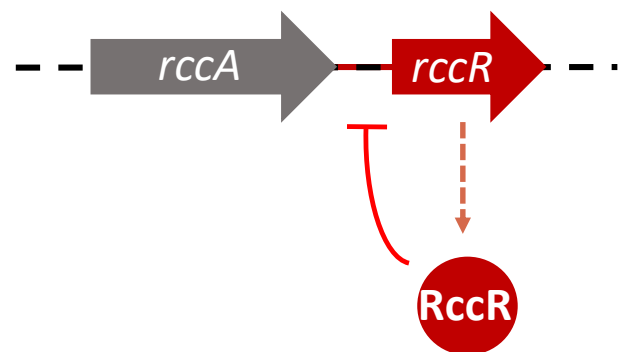
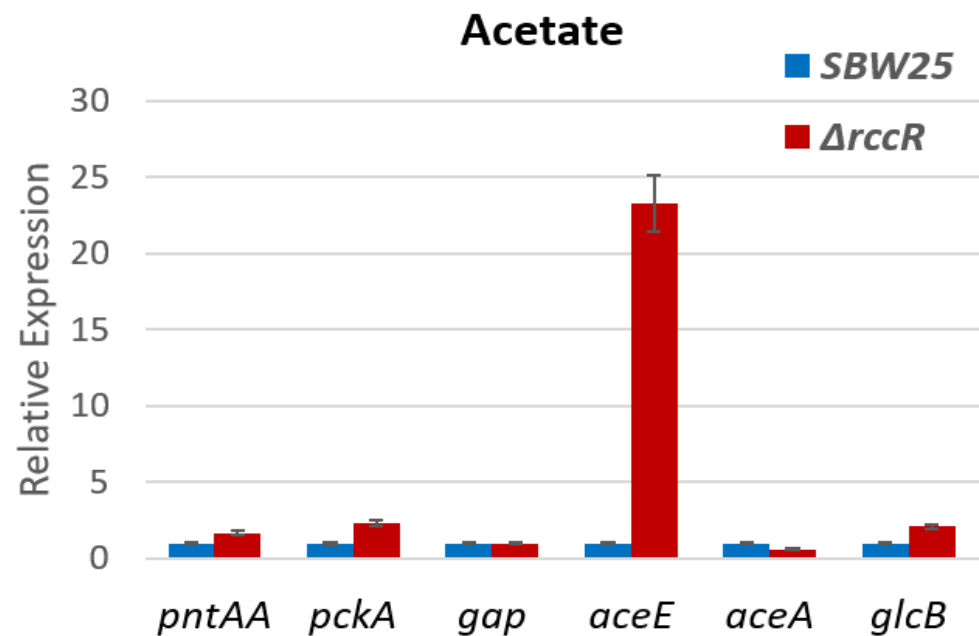
RccR binds to primary carbon metabolism loci



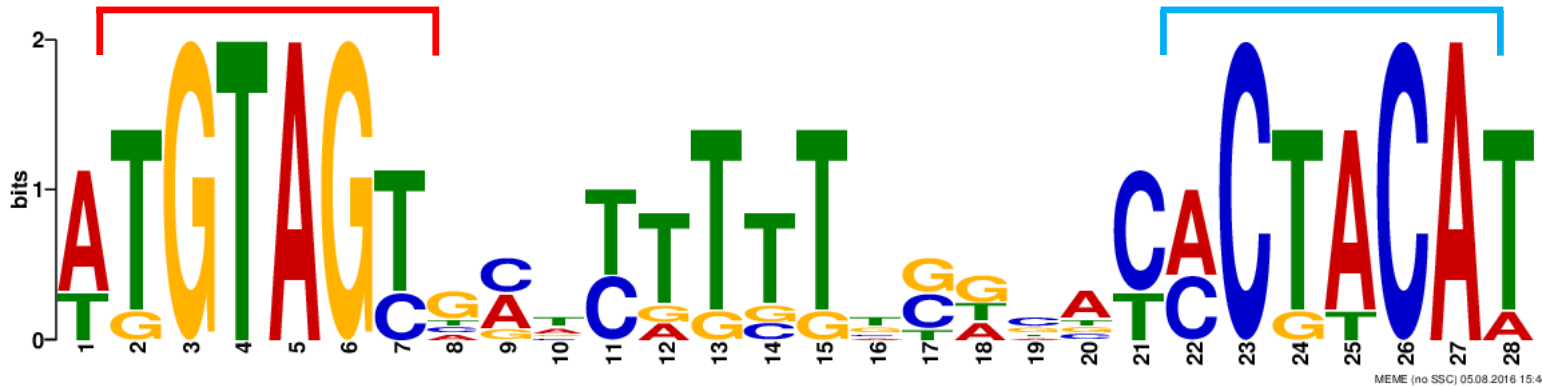
How does RccR regulate its gene targets?



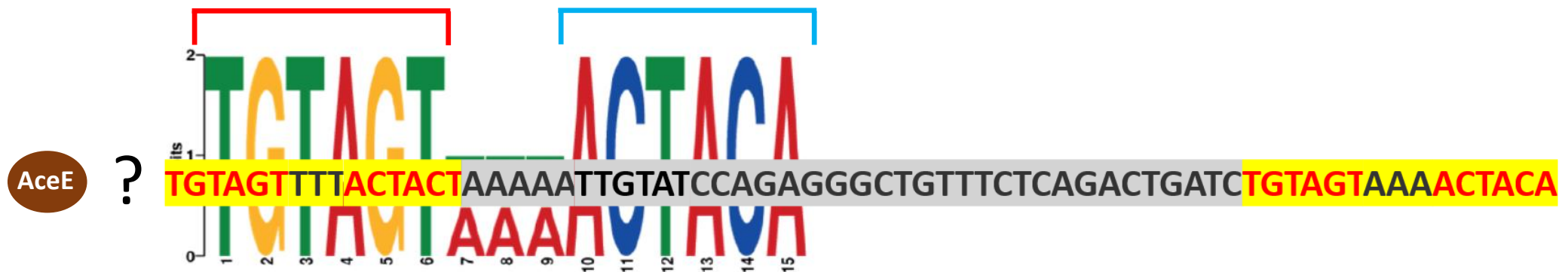
How does RccR regulate its gene targets?



Does RccR recognise a specific DNA consensus sequence?

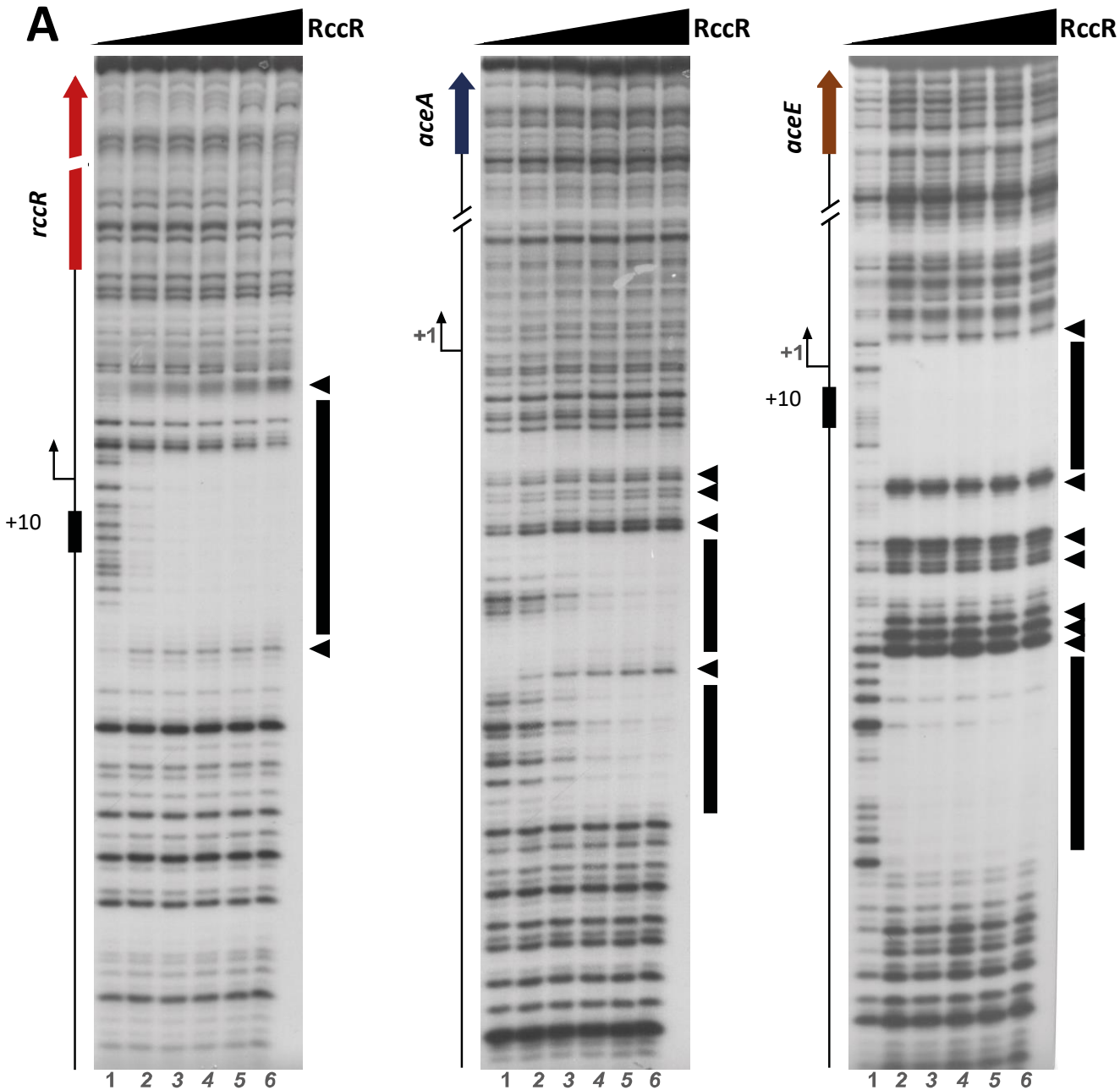


Gene	Strand	<i>p</i> -value	Sequence
AceA	-	7.49e-13	GTCAAATGTT TTGTAGTGC TTTTTTTTCAAGCACTACAT CTTTAGTCTA
2154 PFLU2154	+	9.68e-13	GTCAACGGTT ATGTAGTGATT ATTTTTT GCTCACTACAT AAATTGCTGT
GlcB	-	7.93e-12	AGATTTTCATT ATGTAGTGCAC GGTTGGGCATACTACAT GATGACTTGC
Gap	+	1.55e-11	GGGCGGCATA ATGTAGCCC TTTTTTTCAGCCCCTACAT GTGGAAGGTT
PckA	-	9.92e-11	CTTCACAGCC AGGTAGTGGTCTTC GGCGGACCCCTACAT GCACGTGGGA
PntAA	-	1.22e-10	ACAGGGTGAC TTGTAGTTAAT TTTTTCGTCACCC GT CAT AATCCCTTGA
RccR	+	6.86e-10	AGGGCCAAGG ATGTAGCAAGCTT <u>GTAGTTATACTACAA</u> GAATTGCCGT



Gene	Strand	<i>p</i> -value	Sequence
AceE	+	7.24e-9	TTCGGCGTCC TGTAGTTTTACTACA AATCGTCGGC
RccR	-	7.24e-9	CGGCAATTCT TGTAGTATAACTACA AGCTTGCTAC

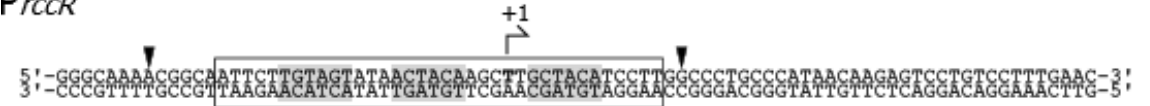
Does RccR bind to these two DNA binding sites?



DNase I Foot printing – Dr Davide Roncarati

B

P_{rccR}



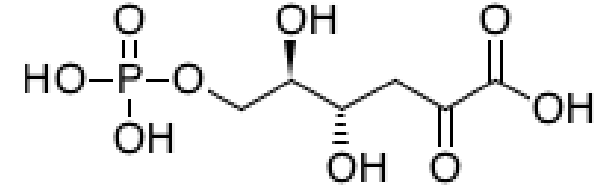
P_{aceA}



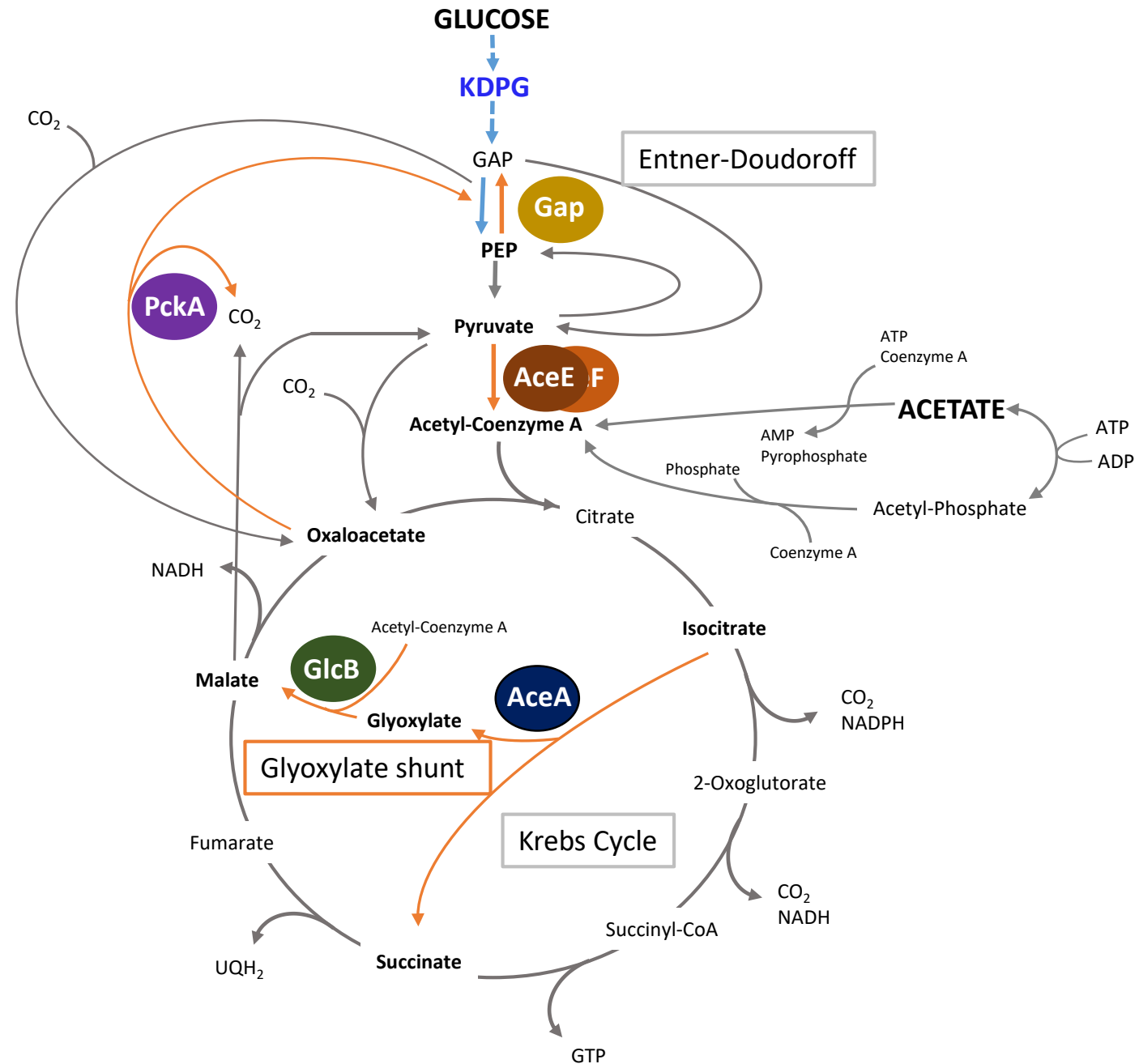
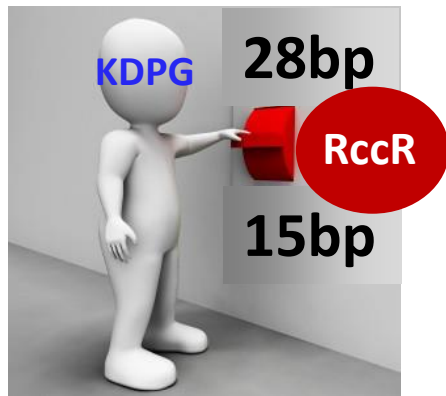
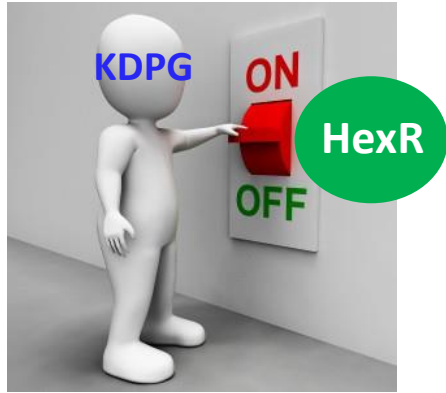
P_{aceE}



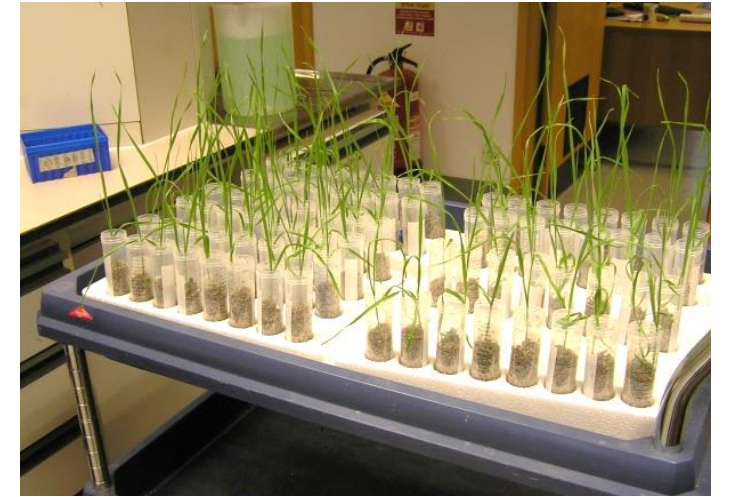
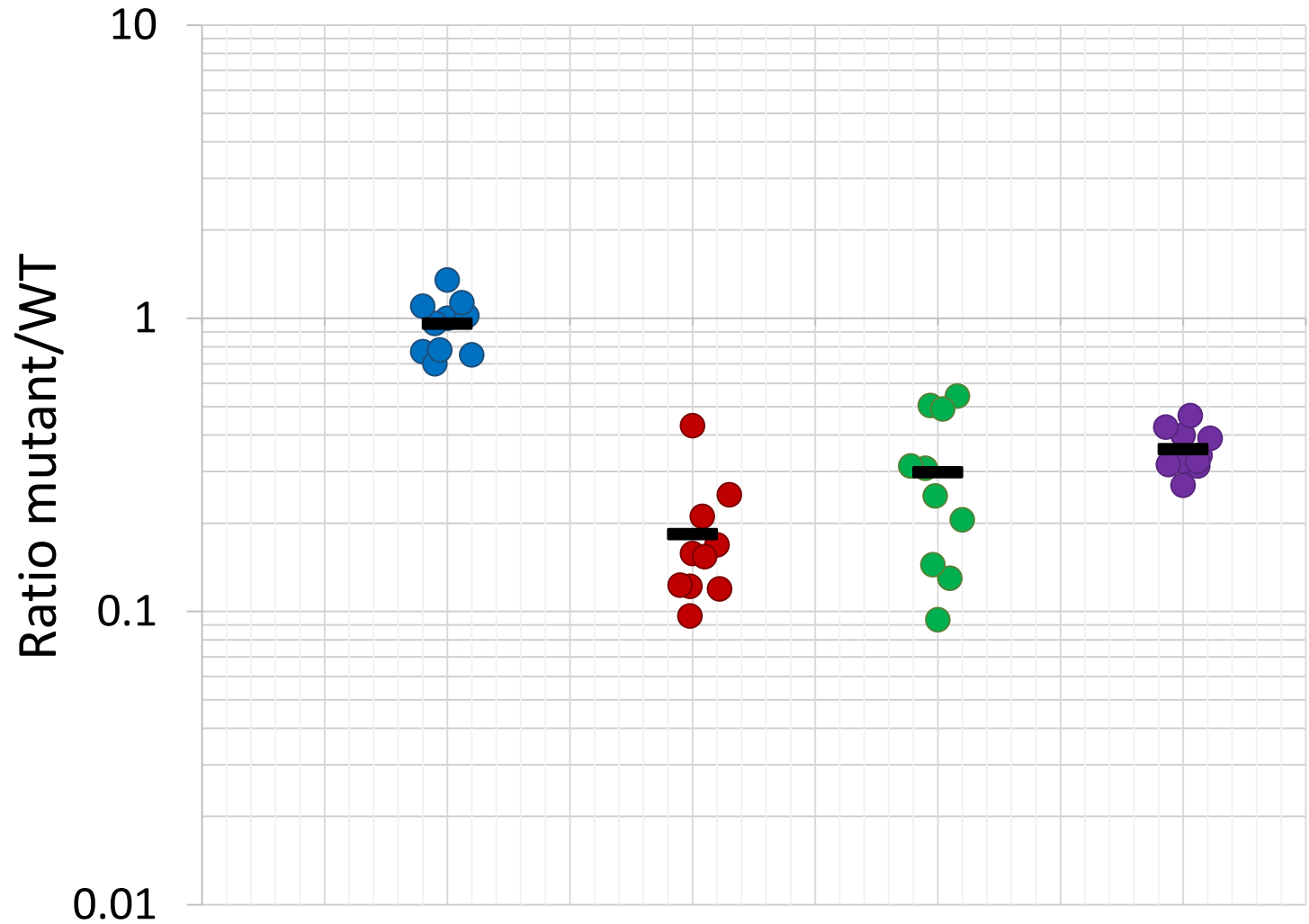
What is the key effector of RccR?



KDPG controls central carbon metabolism in *Pseudomonas*

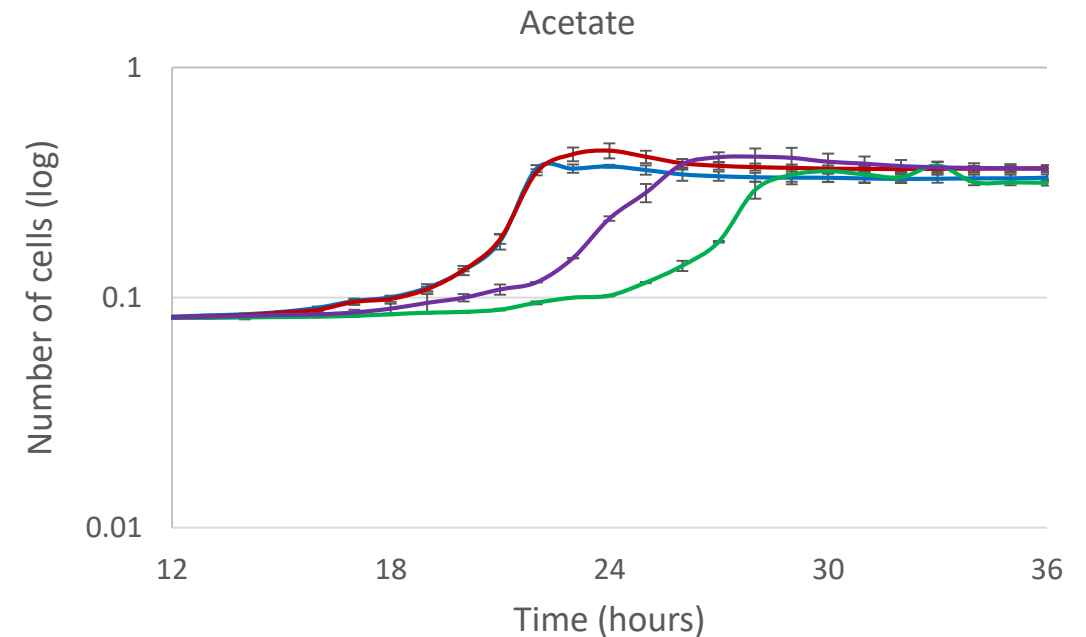
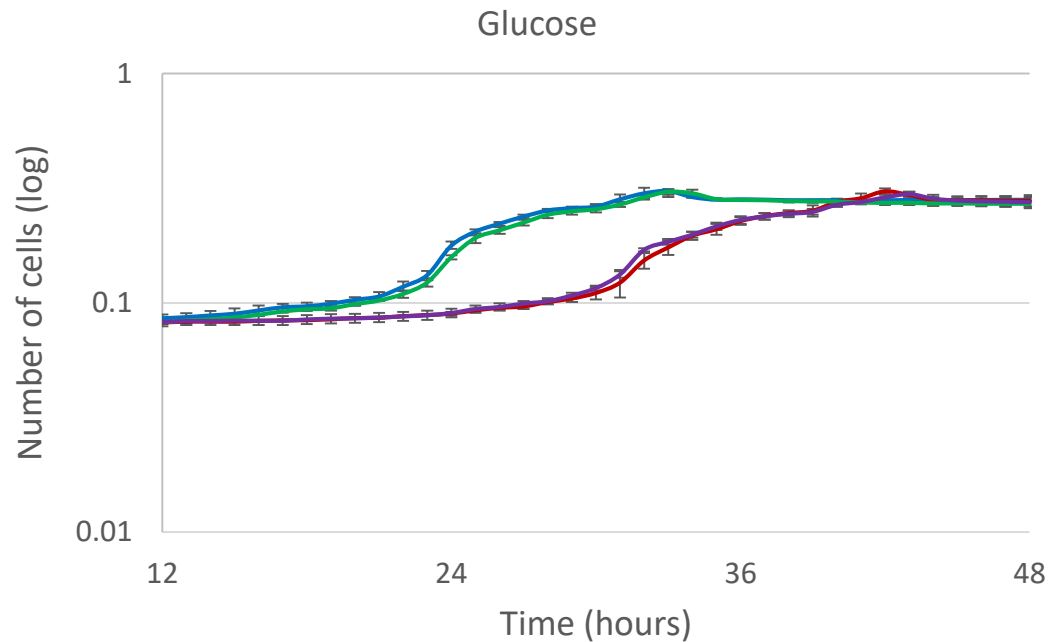


RccR and HexR are important for wheat rhizosphere colonization



- SBW25
- $\Delta rccR$
- $\Delta hexR$
- $\Delta rccR\Delta hexR$

P. fluorescens rccR and *hexR* mutants show specific growth defects in defined media



- SBW25
- $\Delta rccR$
- $\Delta hexR$
- $\Delta rccR\Delta hexR$

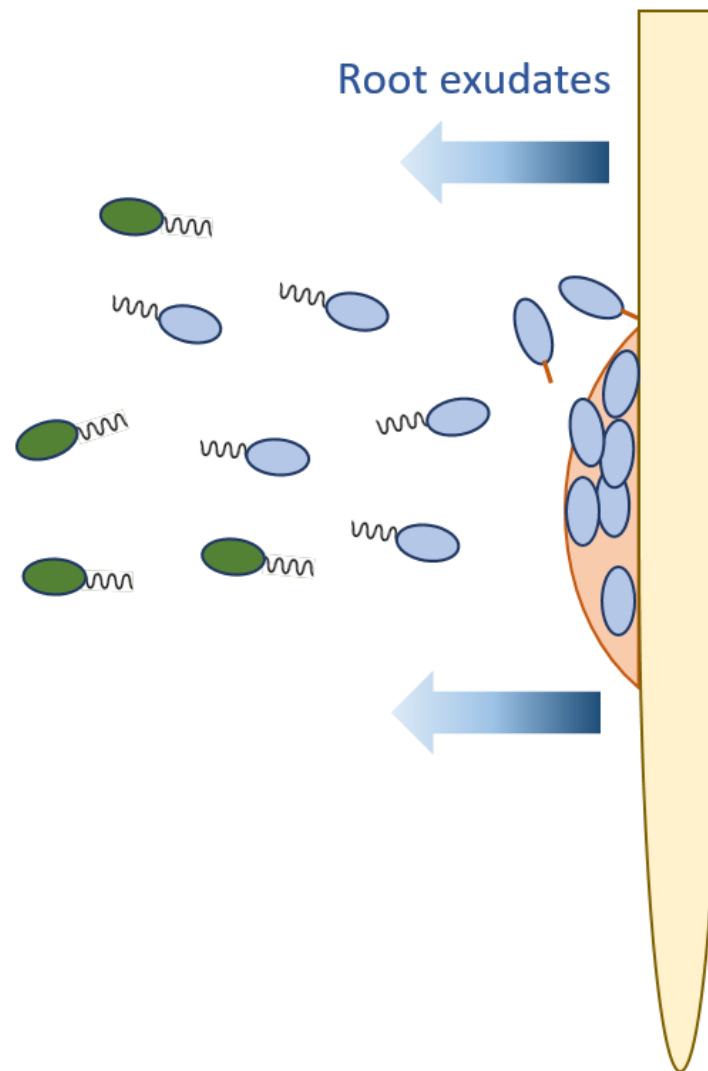
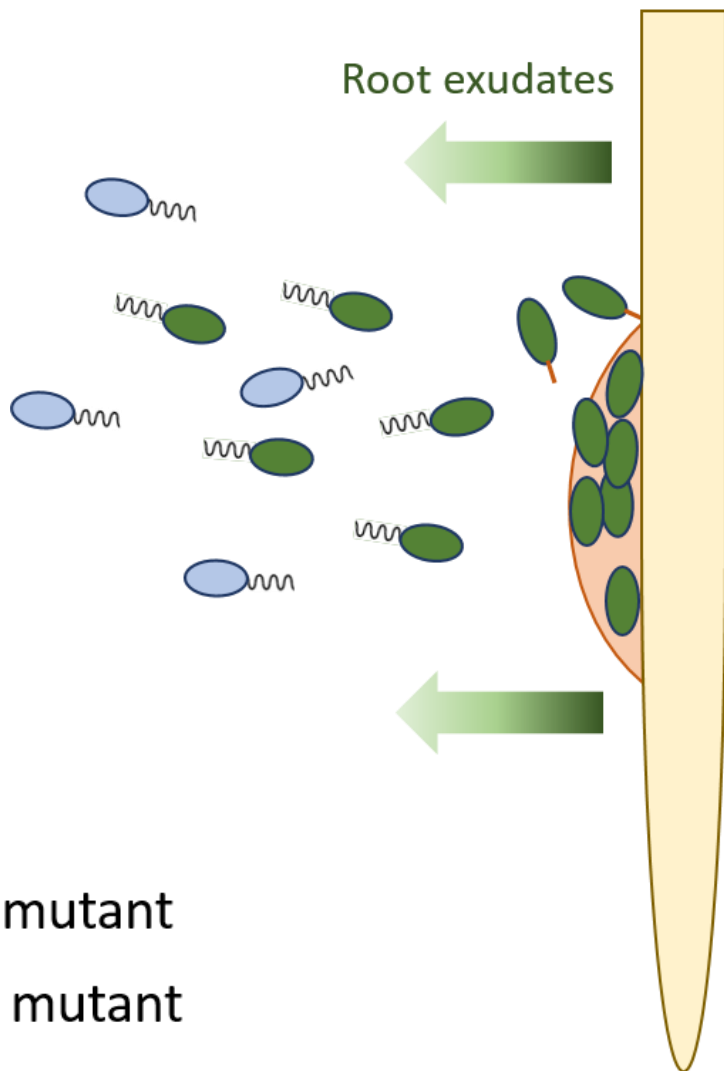
Pseudomonas mutants: plant carbon secretion sensors

C6 Sugars

C2 Sugars

Root exudates

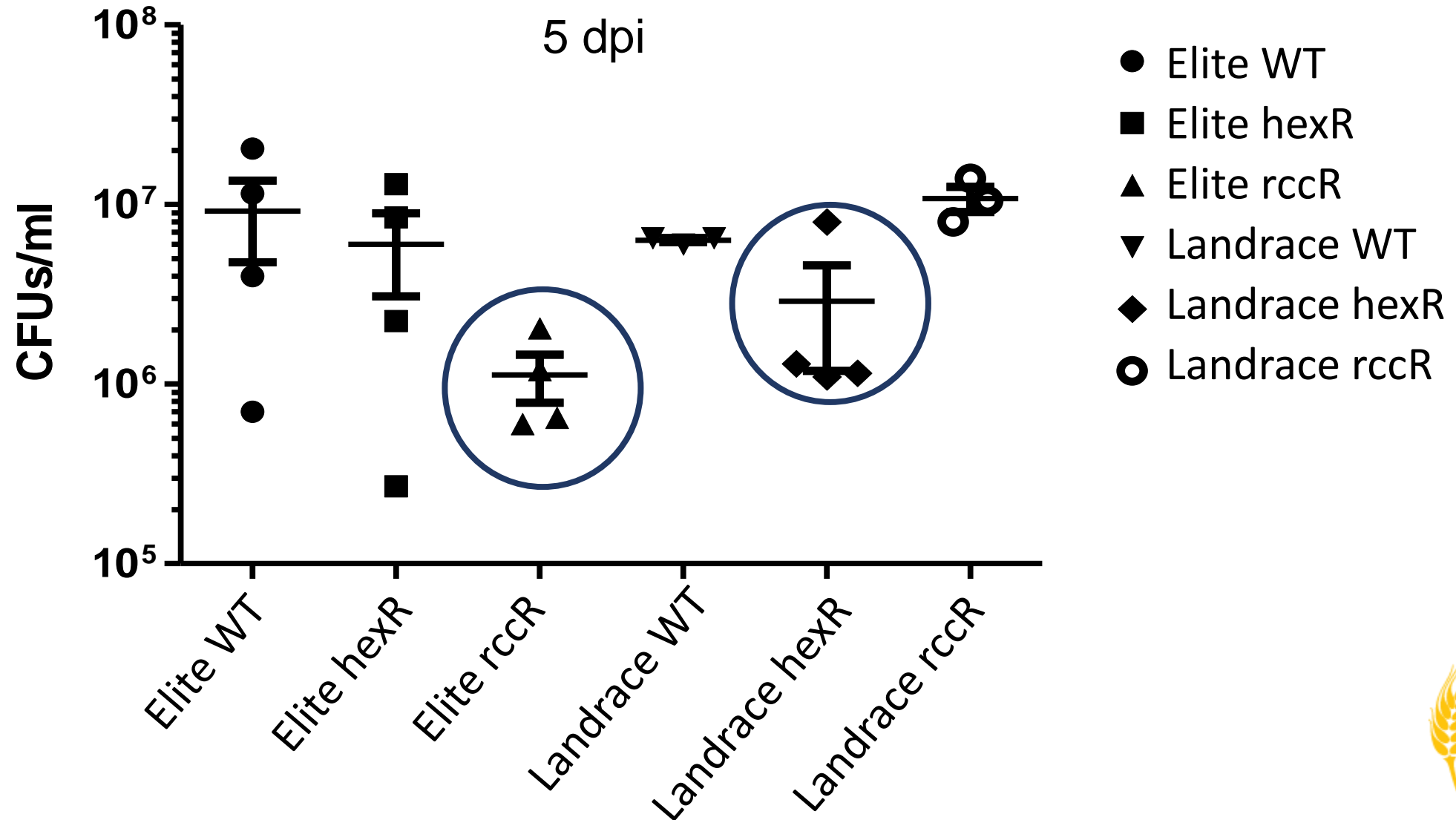
Root exudates



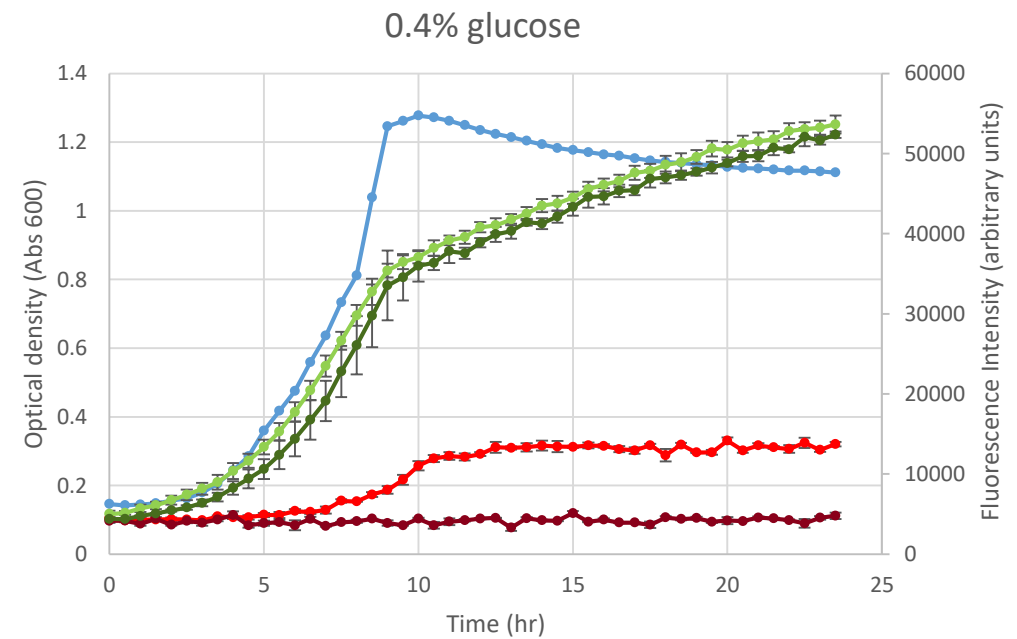
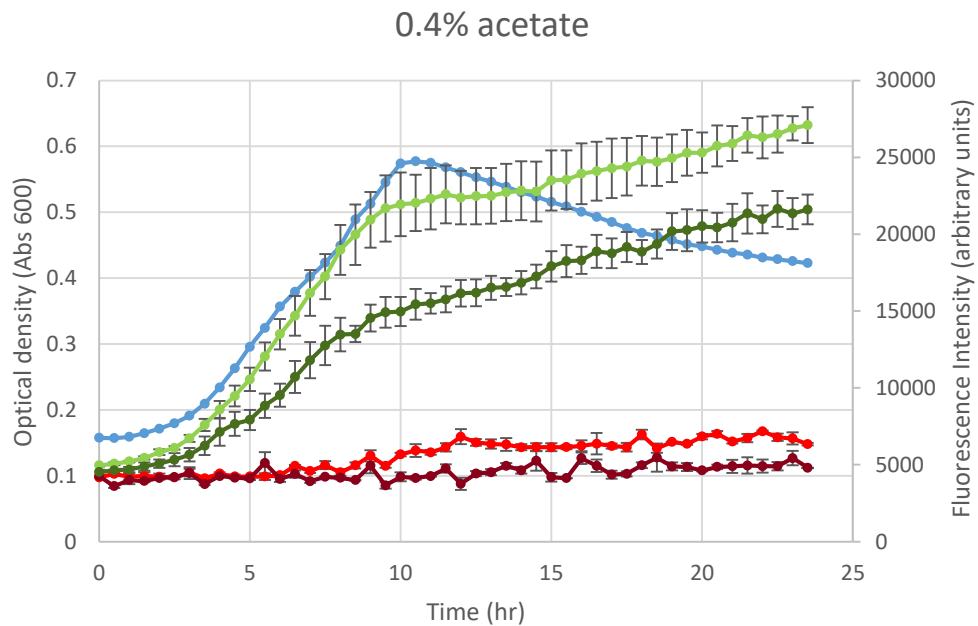
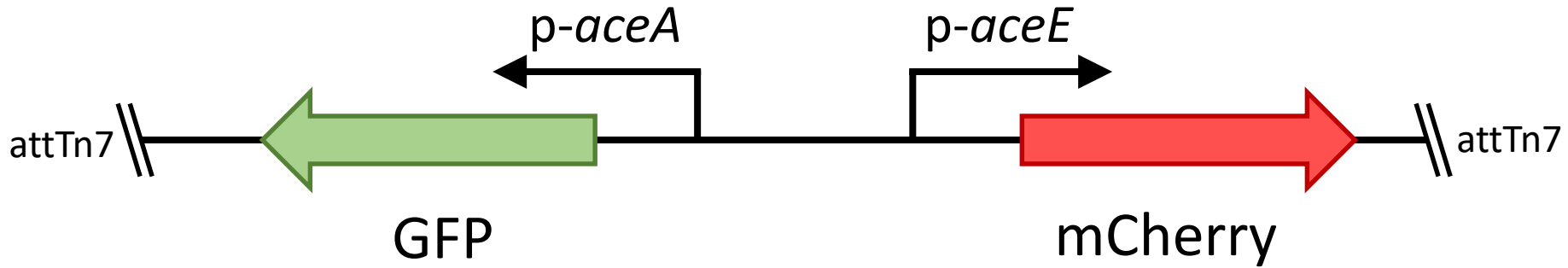
 *rccR* mutant

 *hexR* mutant

Different barley genotypes are colonised differently by *Pseudomonas hex/rcc* mutants



An *rccR*-based biosensor for plant carbon secretion



Thank you for listening!



John Innes Centre

Unlocking Nature's Diversity

Rosaria Campilongo

Clare Stevenson

Govind Chandra

Richard Little

Lucia Grenga

Eleftheria Trampari

Rowena Fung

Alba Pacheco Moreno

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The Malone Lab

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