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Conversion of CO_2 to the platform chemical 3-hydroxypropanoic acid (3-HP) using metabolically engineered strains of *Acetobacterium woodii*

Matthias H. Beck[1], Jonathan Baker[2], Anja Wiechmann[3], Nigel P. Minton[2], Volker Müller[3], Frank R. Bengelsdorf[1], and Peter Dürre[1]

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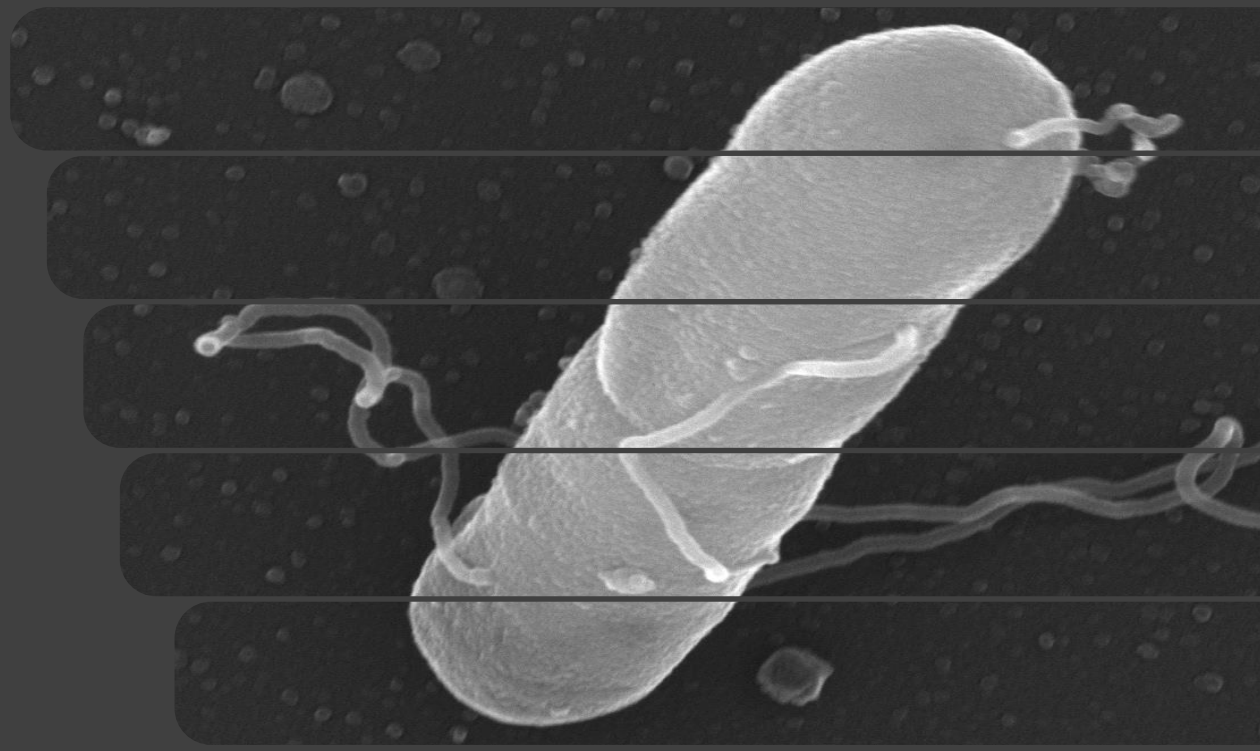
[2]: University of Nottingham, Clostridia Research Group, BBSRC/EPSRC Synthetic Biology Research Centre, School of Life Sciences, NG7 2RD, Nottingham, United Kingdom
[3]: Department of Molecular Microbiology and Bioenergetics, Institute of Molecular Biosciences, Johann Wolfgang Goethe University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany



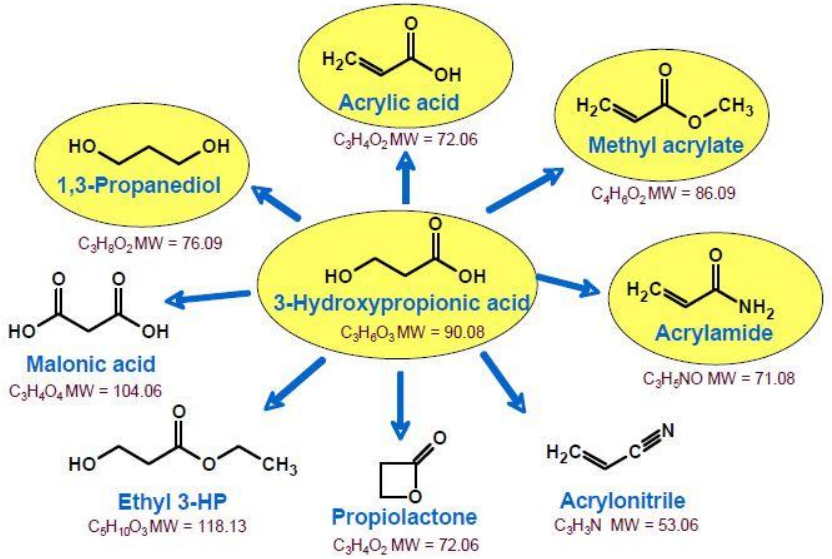
January 21, 2019

Why 3-HP?

- Listed in top 12 “value added chemicals from biomass”
  (US Department of Energy; DOE)
- Poly (3-hydroxypropionic acid)

➔No commercial production from petrochemical feedstocks

➔Bioproduction with sugar or glycerol as feedstocks

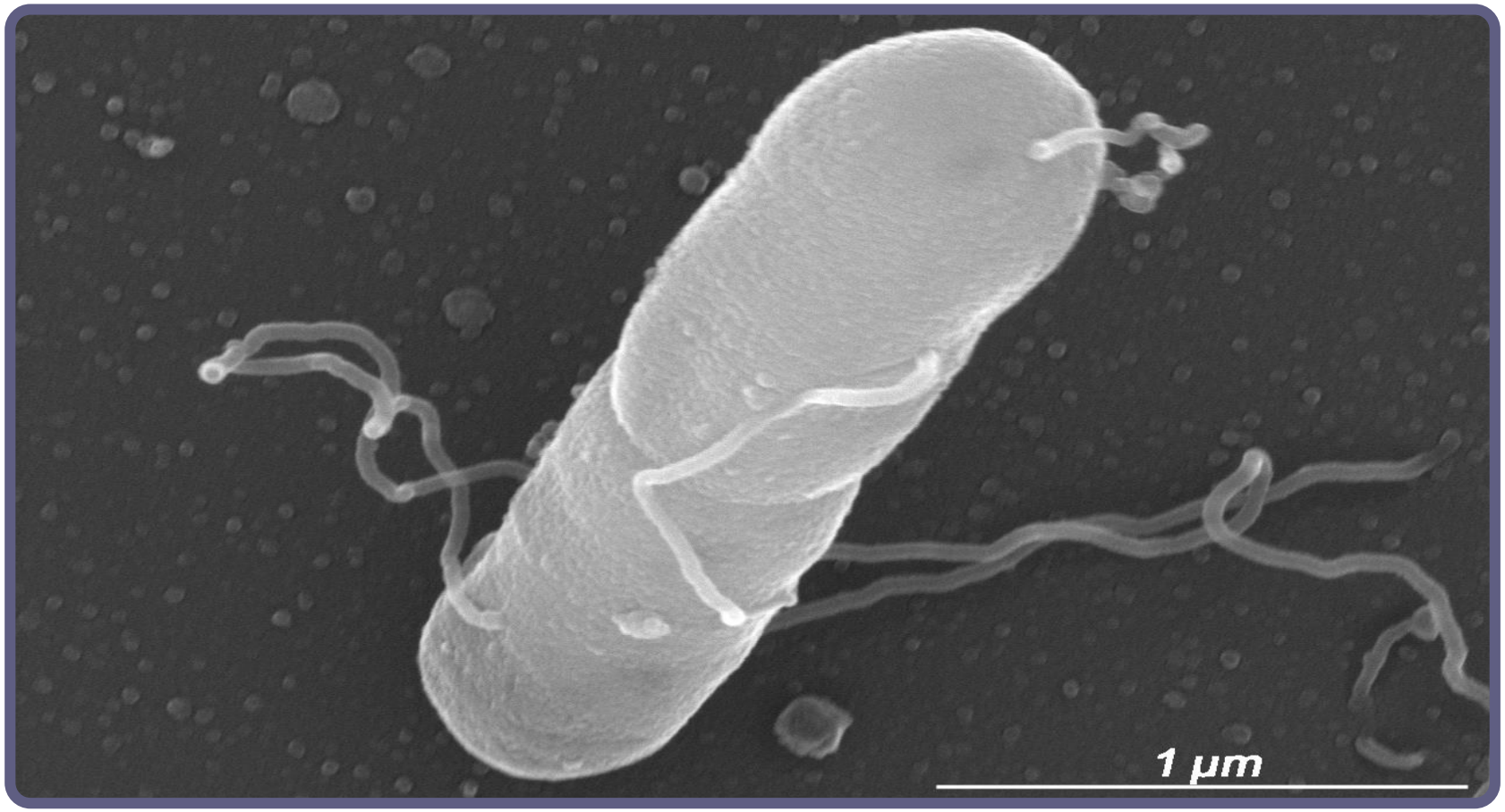


Werpy, 2004

www.pexels.com

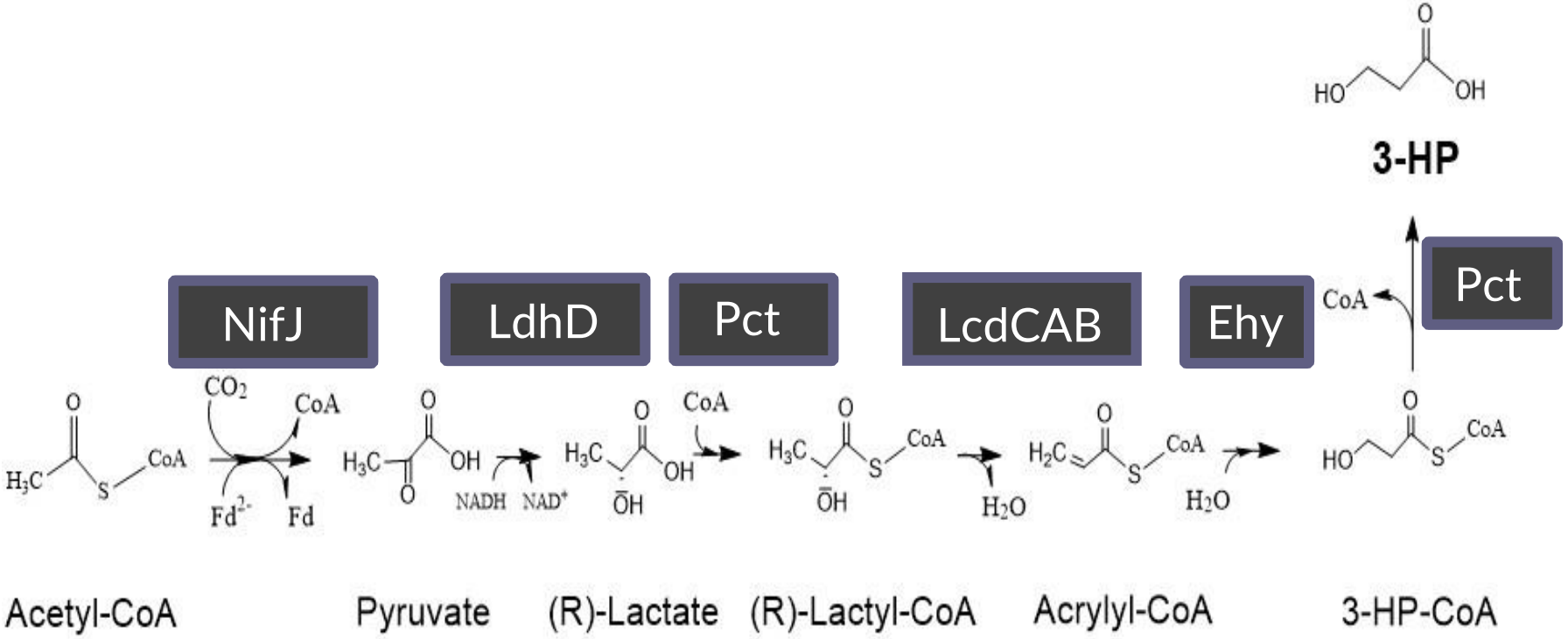
How to produce 3-HP from CO_2?

➔ recombinant *Acetobacterium woodii* strains



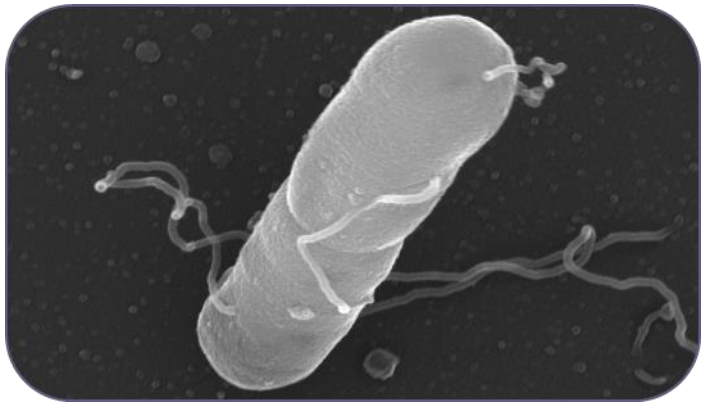
$2\ CO_2 + 4\ H_2 + HS\text{-}CoA$ ➔ Acetyl-CoA + $3\ H_2O$ **(via Wood-Ljungdahl pathway)**

Strategy: Synthetic biosynthesis pathway



NifJ	→ pyruvate:ferredoxin oxidoreductase:	*Acetobacterium woodii*
LdhD	→ lactate dehydrogenase:	*Leuconostoc mesenteroides*
Pct	→ propionyl-CoA transferase,	*Clostridium neopropionicum*
LcdCAB	→ lactyl-CoA dehydratase:	
Ehy	→ enoyl-CoA hydratase:	*Chloroflexus aurantiacus*

First approach: Operon synthesis



Constitutive expression does not work here!

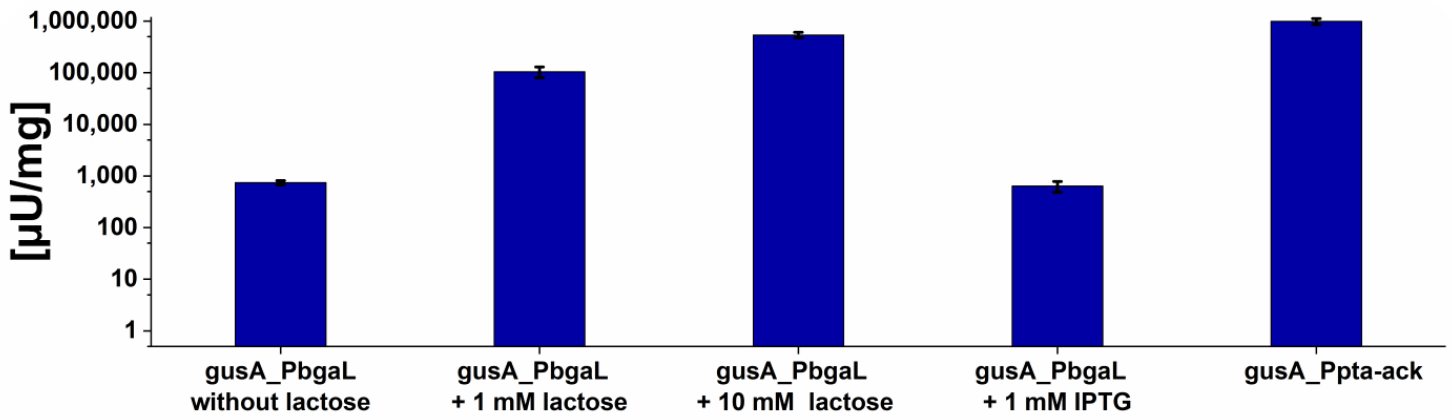
- Operon could not be synthesized as planned

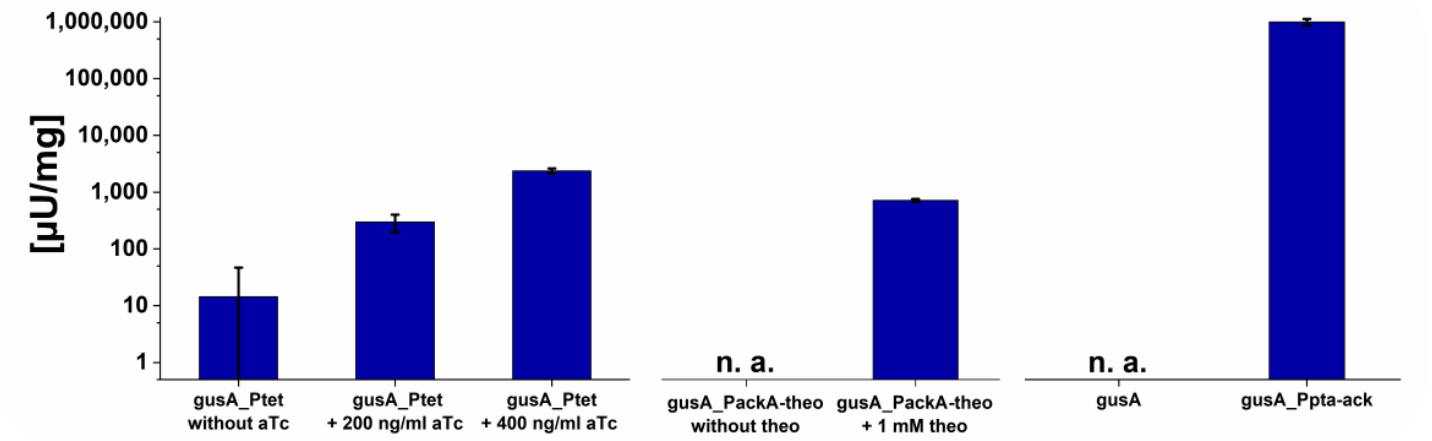
➔Some genes (e.g. *ldhD*) only intact without promoter

➔Requirement of inducible promoter systems

➔β-Glucuronidase (GusA) reporter assays

Establishment of an inducible promoter system





$P_{pta\text{-}ack}$: Constitutive [1]

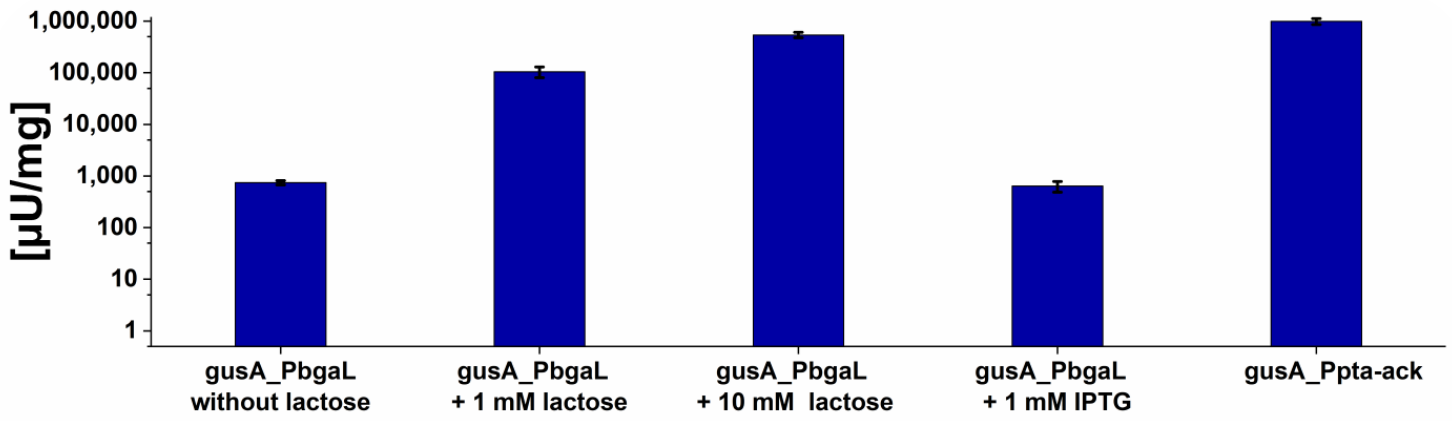
P_{bgaL}: Lactose inducible [2]

P_{tet}: Tetracycline inducible [3]

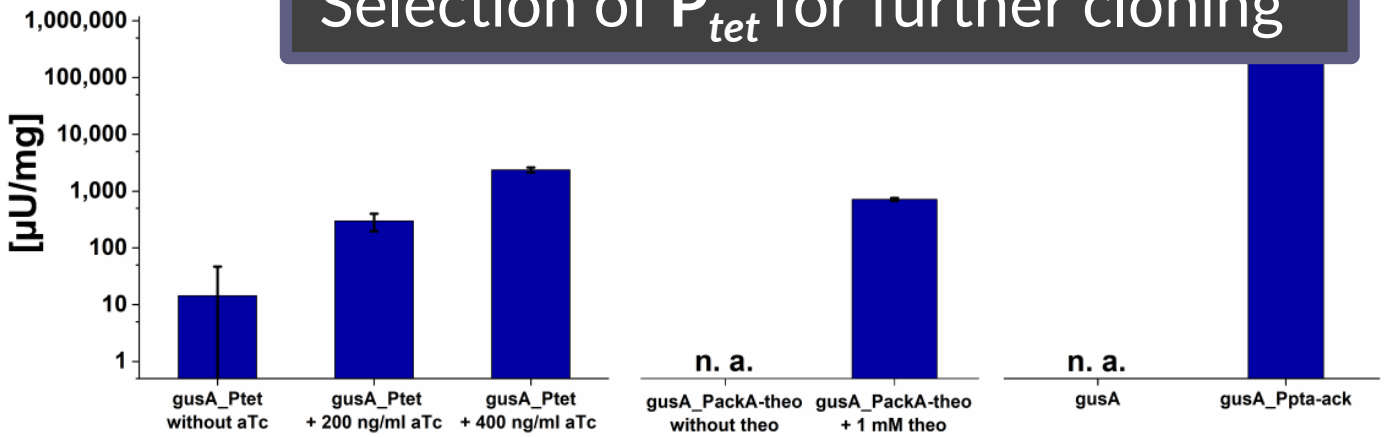
$P_{ackA\text{-}theo}$: Synthetic P_{ackA}-theophylline-dependent riboswitch construct [1; 4]

[1] Hoffmeister, Gerdom, Bengelsdorf, Linder, Flüchter, Öztürk, Blümke, May, Fischer, Bahl, Dürre. 2016. Metab Eng. **36**:37-47.
[2] Hartman, Liu, Melville. 2011. Appl Environ Microbiol. **77**:471-8.
[3] Ransom, Ellermeier, Weiss. 2015. Appl Environ Microbiol. **81**:1652-60.
[4] Seibold and Rückert. Unpublished.

Establishment of an inducible promoter system



Selection of P_{tet} for further cloning



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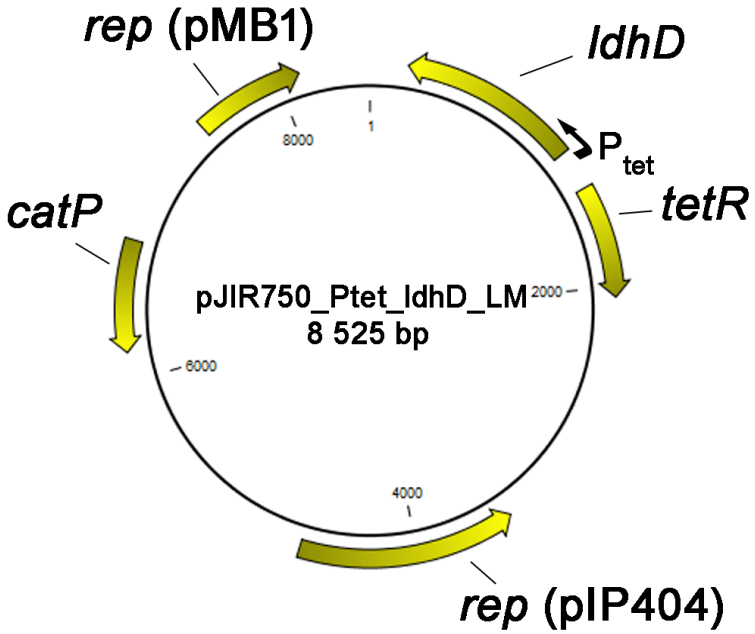
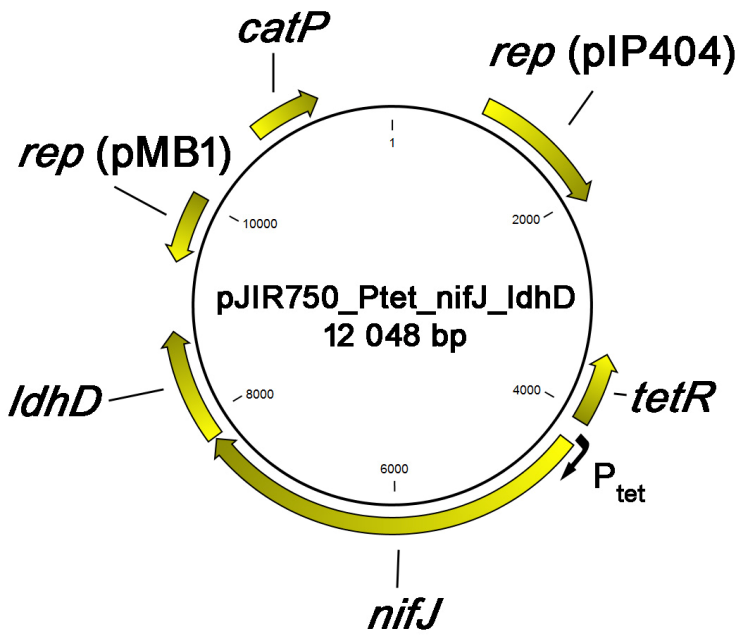
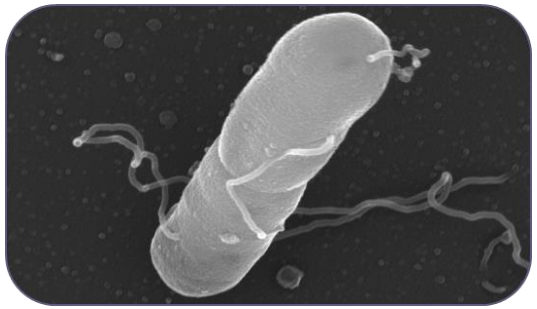
[4] Seibold and Rückert. Unpublished.

First plasmids constructed



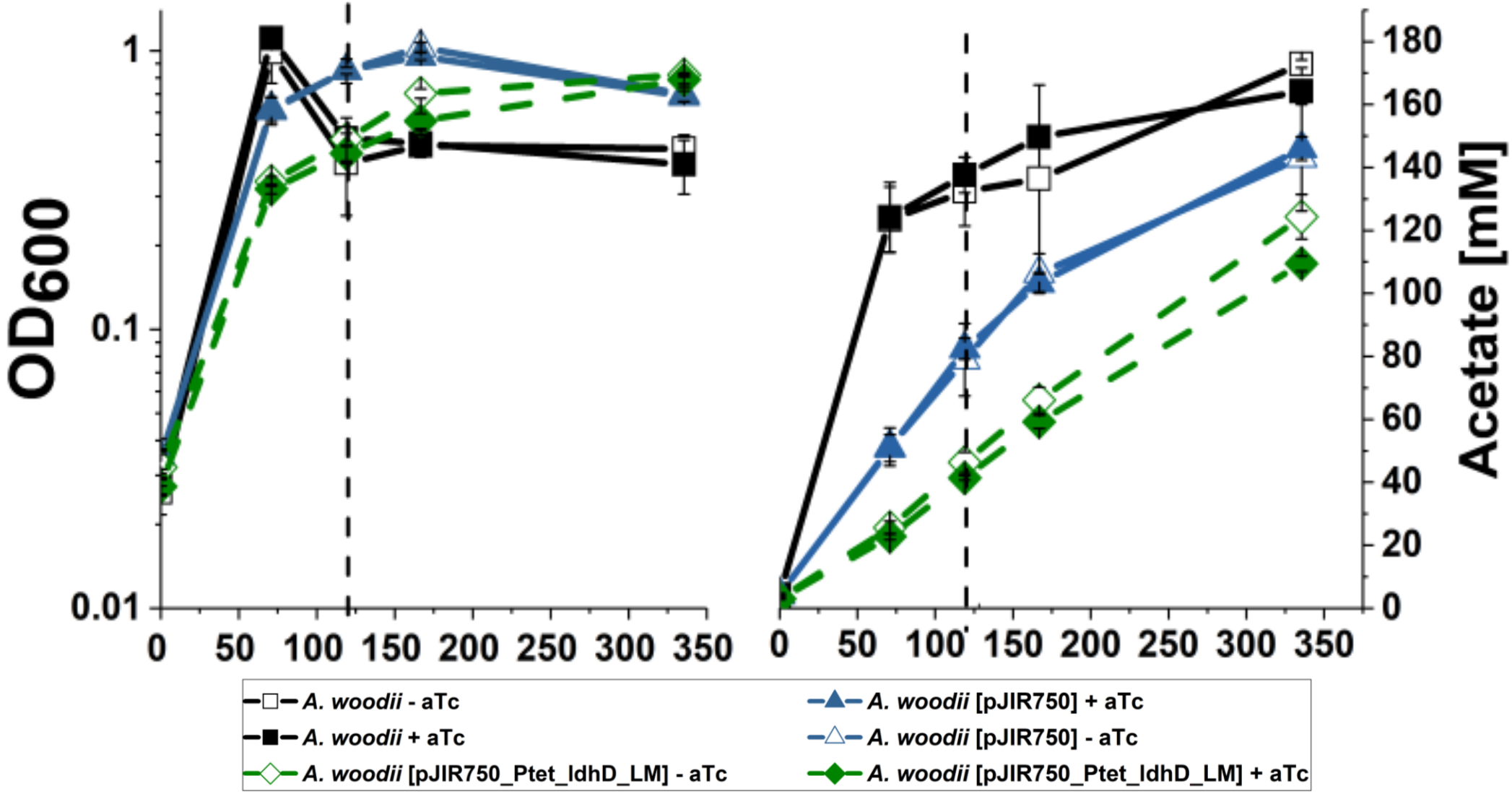


nifJ:
Acetobacterium woodii
ldhD:
Leuconostoc mesenteroides

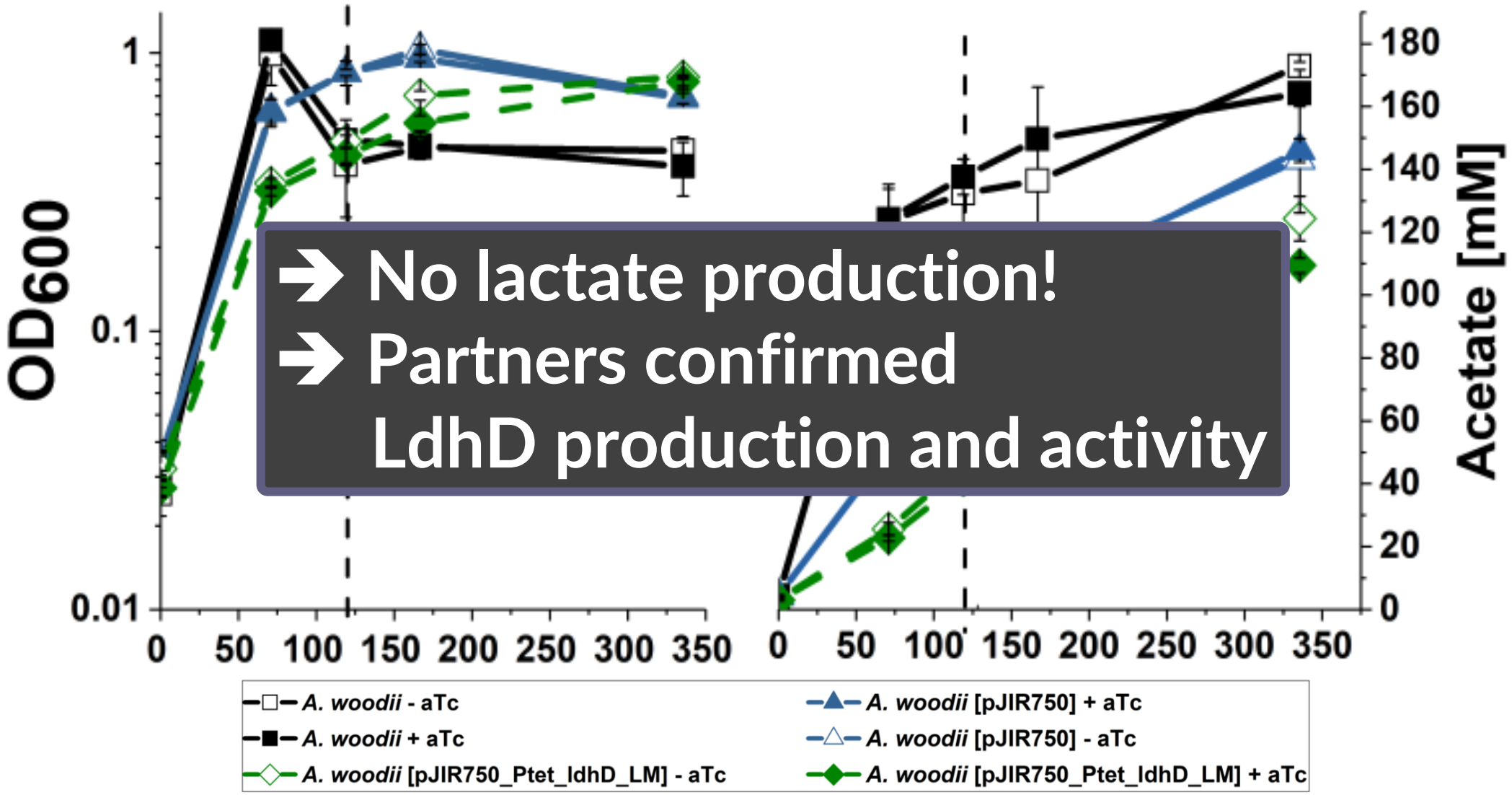


Let‘s start with the *ldhD* plasmid!

A. woodii [pJIR750_Ptet_ldhD_LM] on H_2 + CO_2



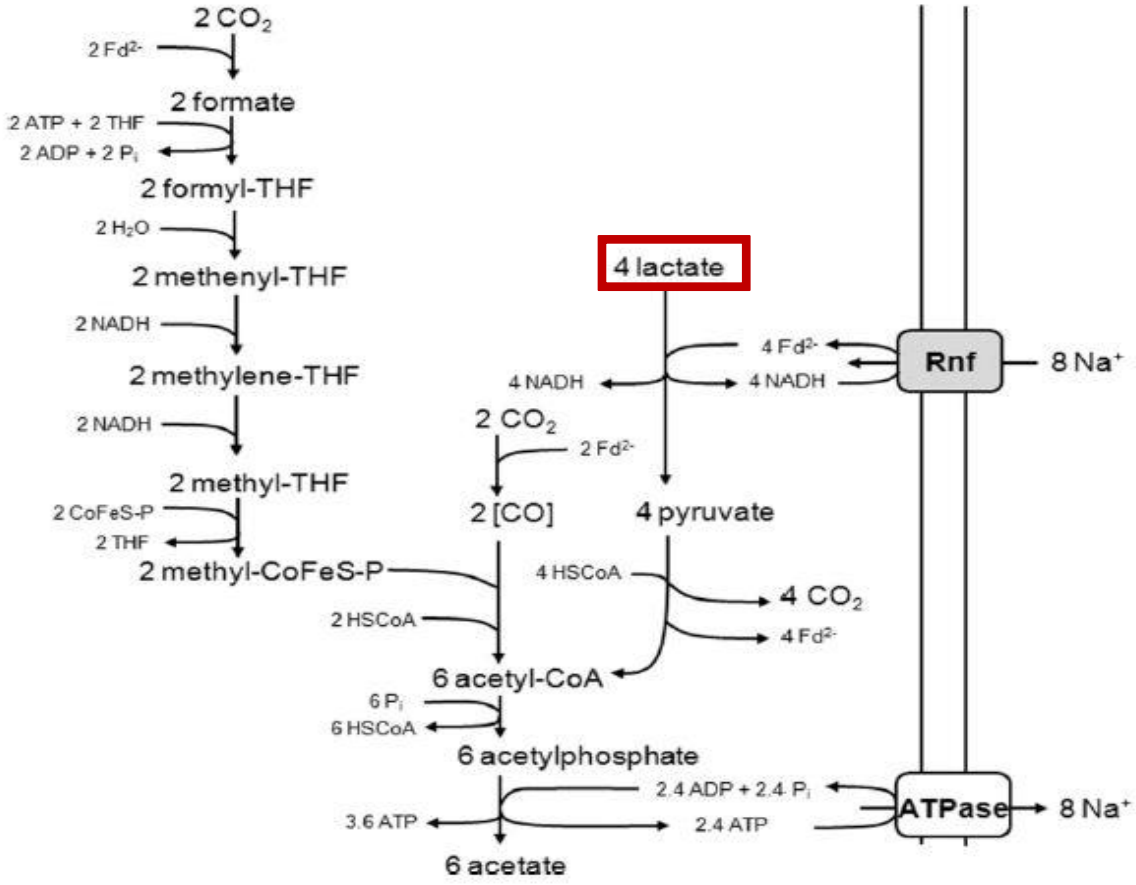
A. woodii [pJIR750_Ptet_ldhD_LM] on H_2 + CO_2



➔ No lactate production!

➔ Partners confirmed LdhD production and activity

Native lactate metabolism of *A. woodii*

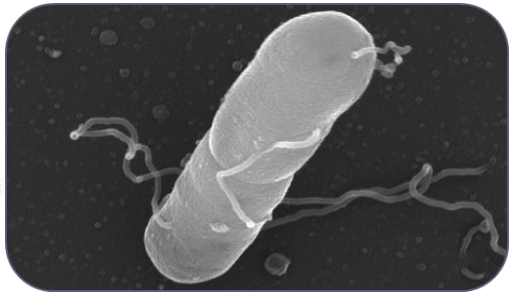


Weghoff *et al.*, 2014

environmental microbiology
Environmental Microbiology (2014)
doi:10.1111/1462-2920.12493

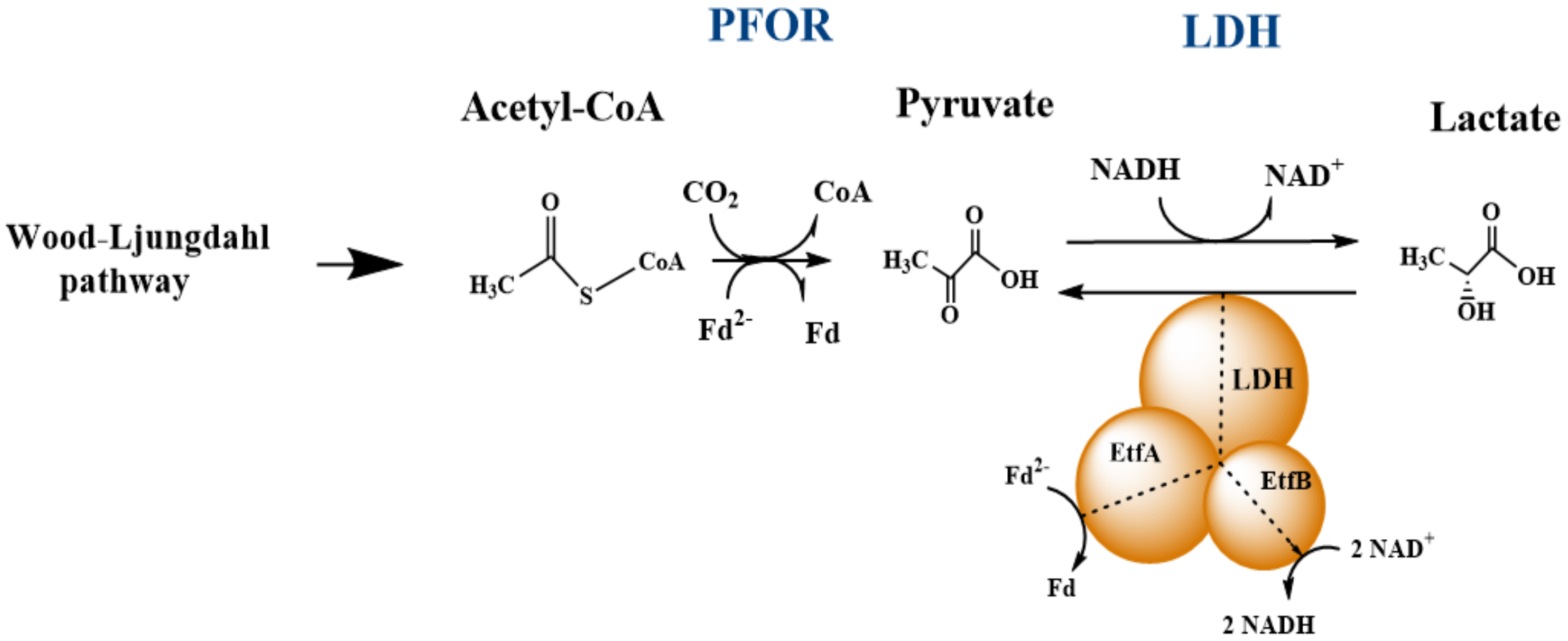
A novel mode of lactate metabolism in strictly anaerobic bacteria

Marie Charlotte Weghoff, Johannes Bertsch and Volker Müller. 2015.
Environ Microbiol. **17**:670-677.

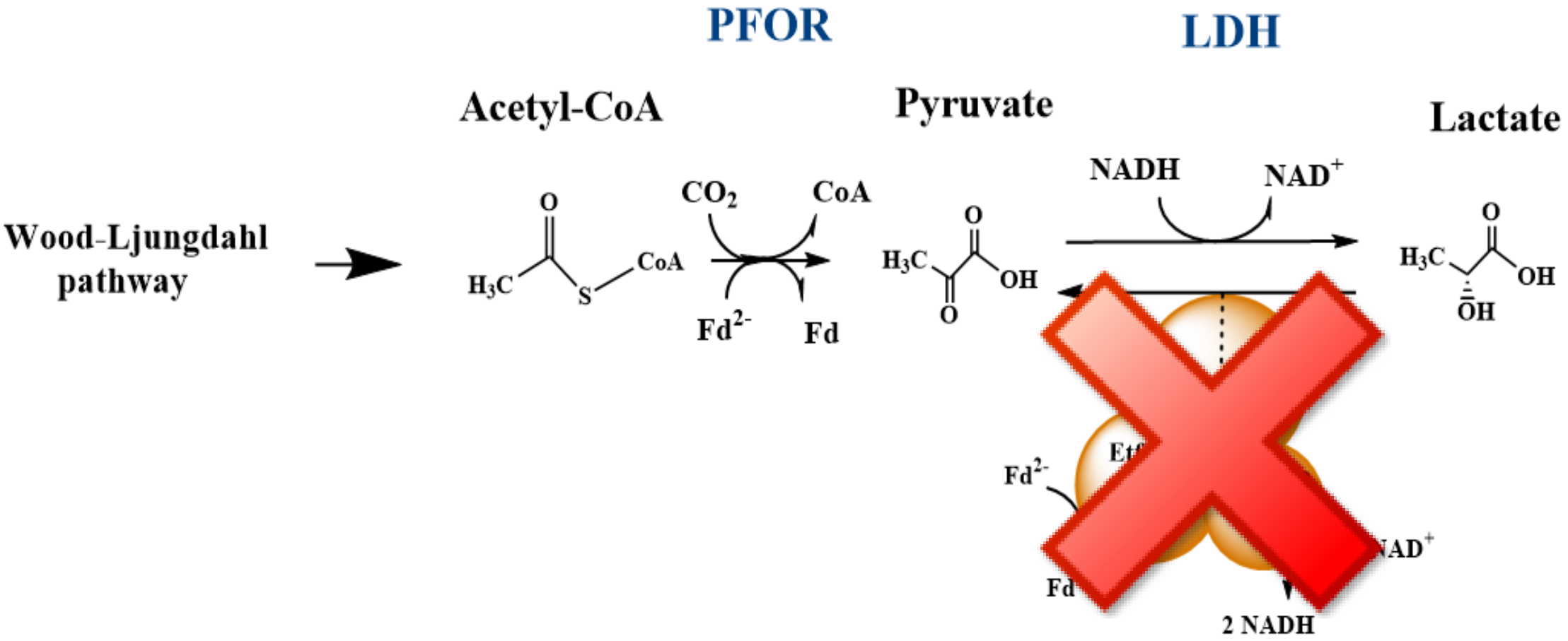


I like lactate as a substrate!

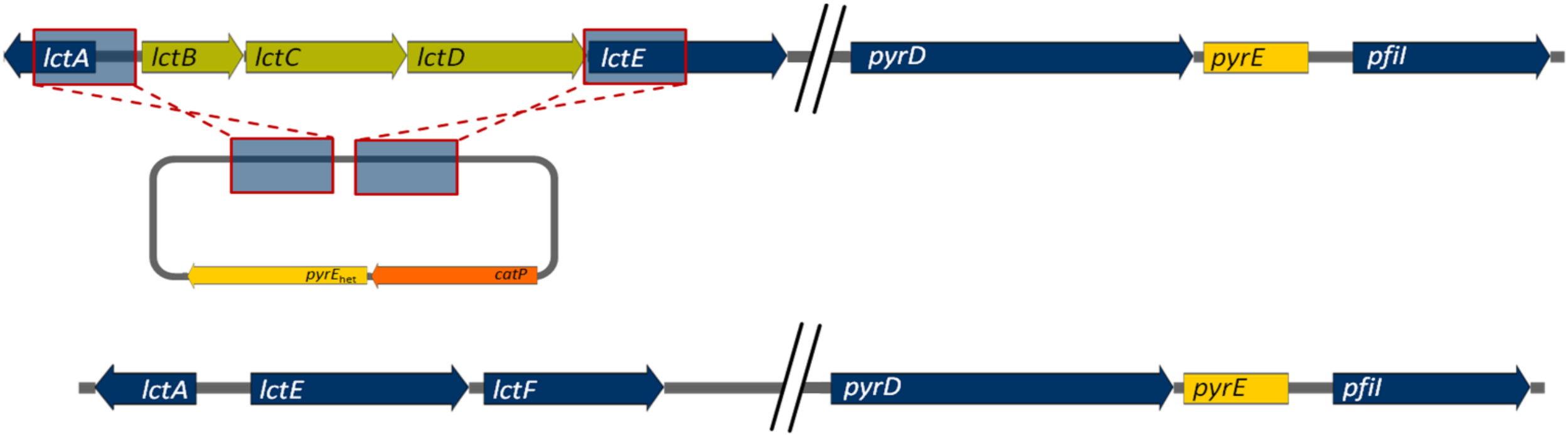
Idea: Knock-out of LDH/Etf complex genes (*lctBCD*)



Idea: Knock-out of LDH/Etf complex genes (*lctBCD*)

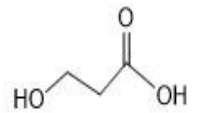


Method: Allele-Coupled Exchange *pyrE* linked K/O

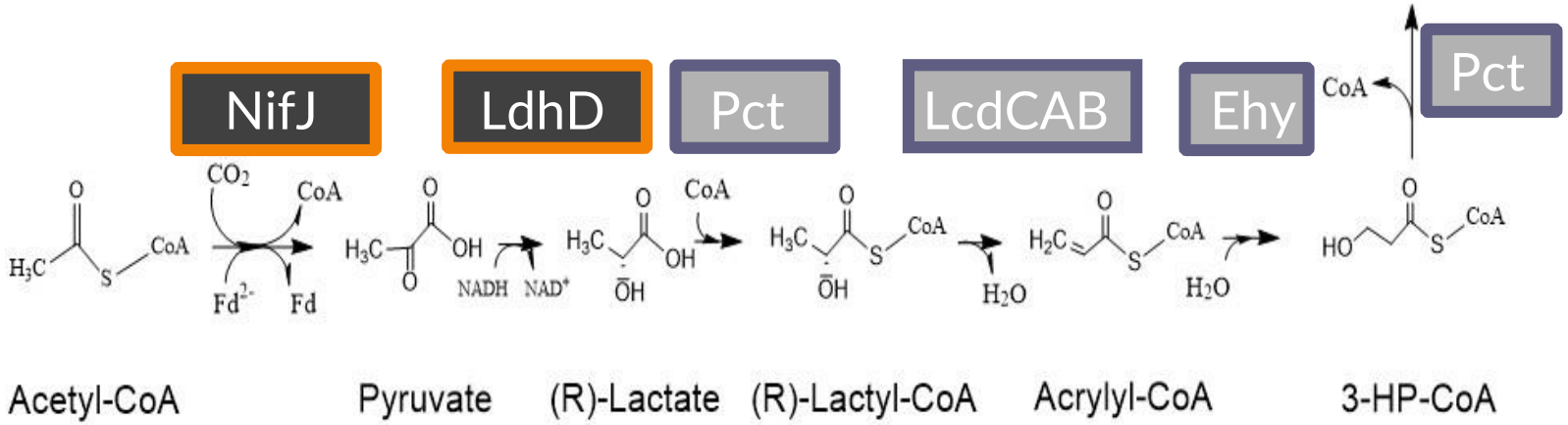


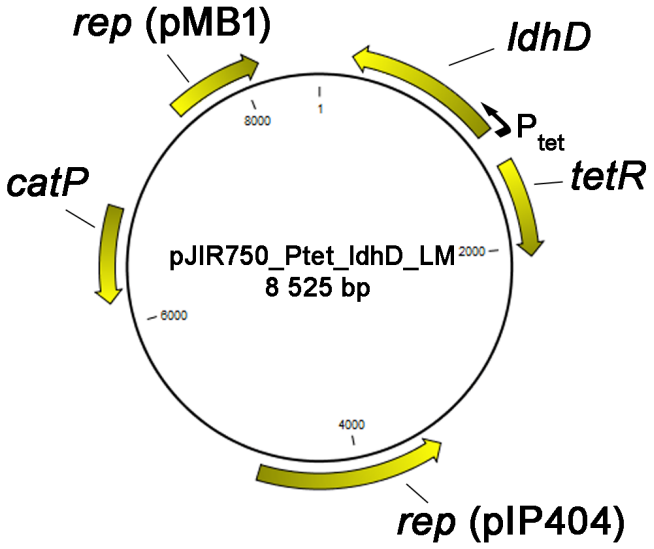
SynBio
SBRC Nottingham
a BBSRC/EPSRC Synthetic Biology Research Centre

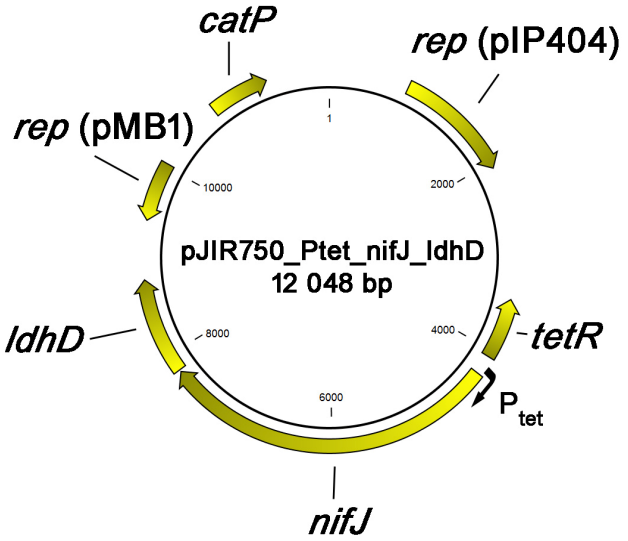
1st part: Lactate production



3-HP







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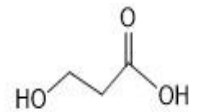
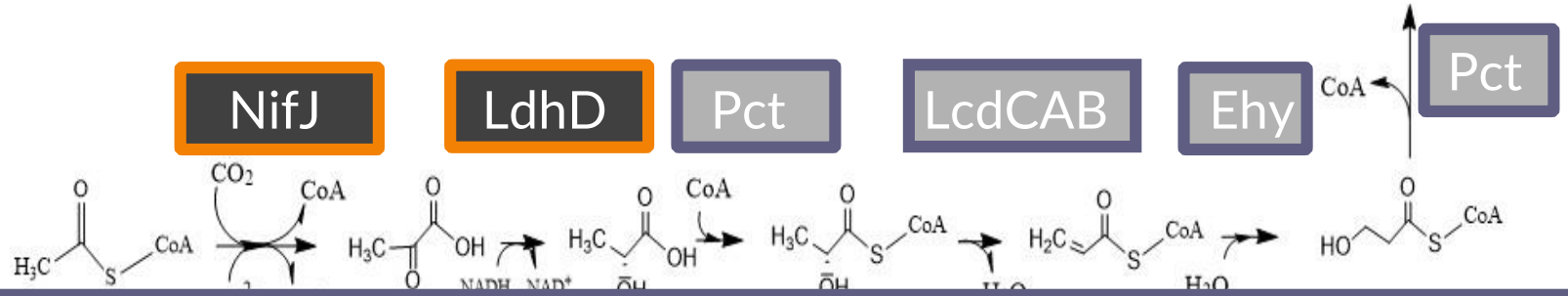
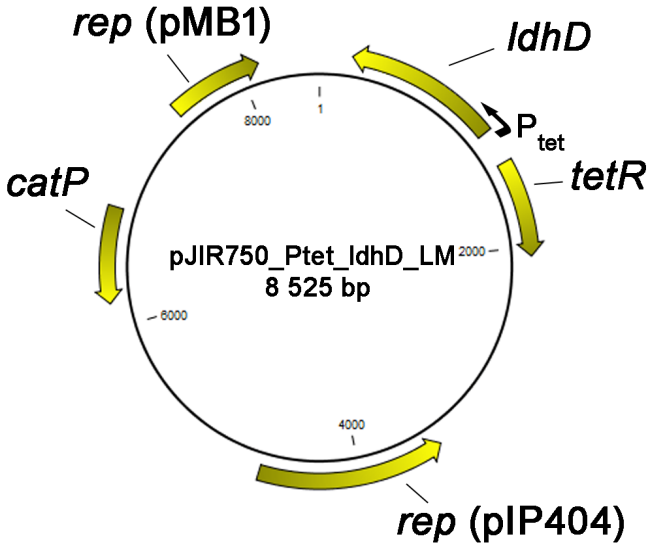
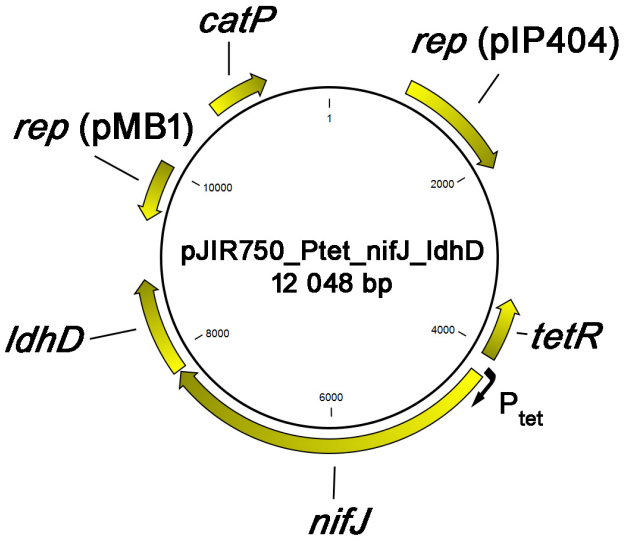


3-HP

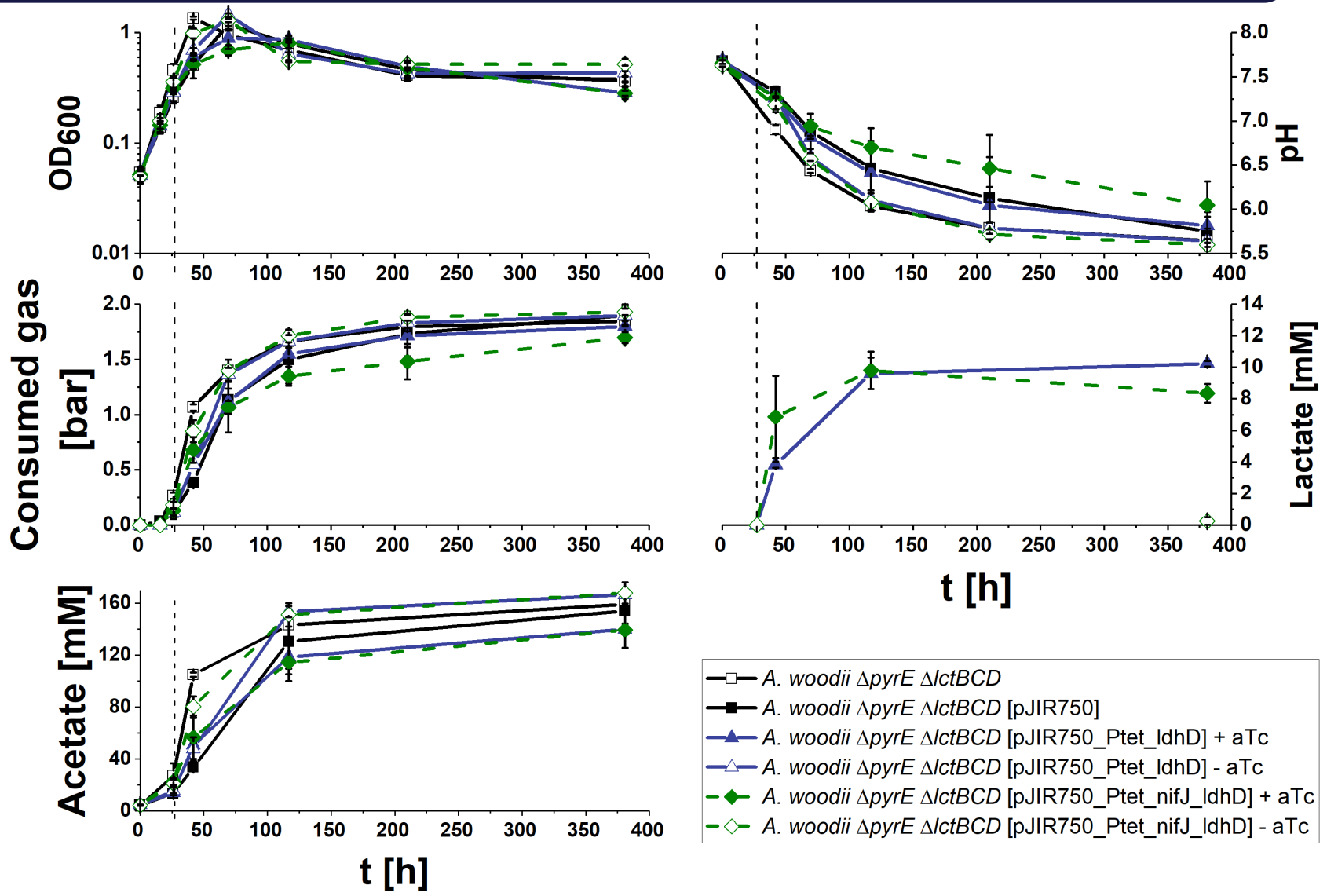


Using recombinant *A. woodii ΔpyrE ΔlctBCD*

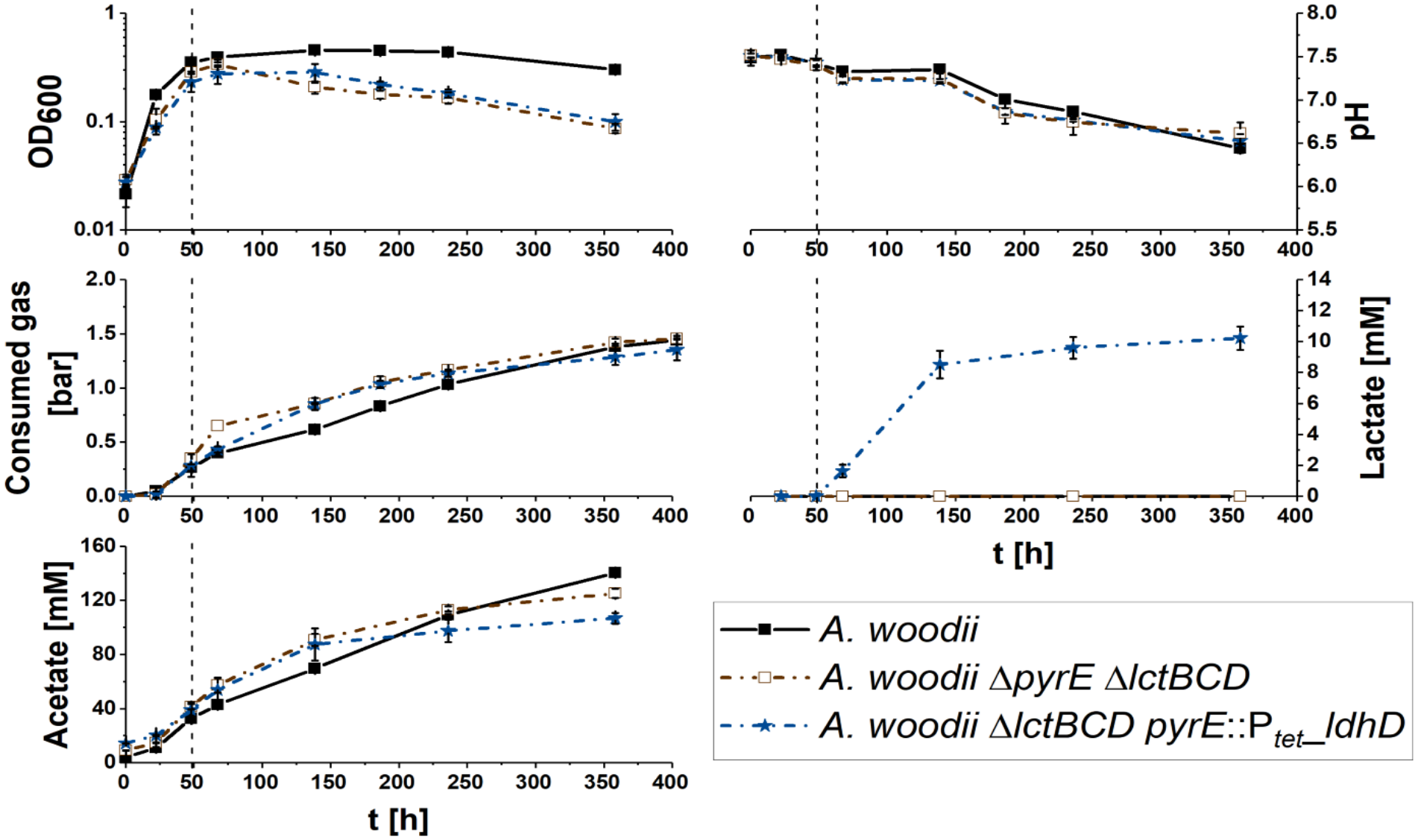




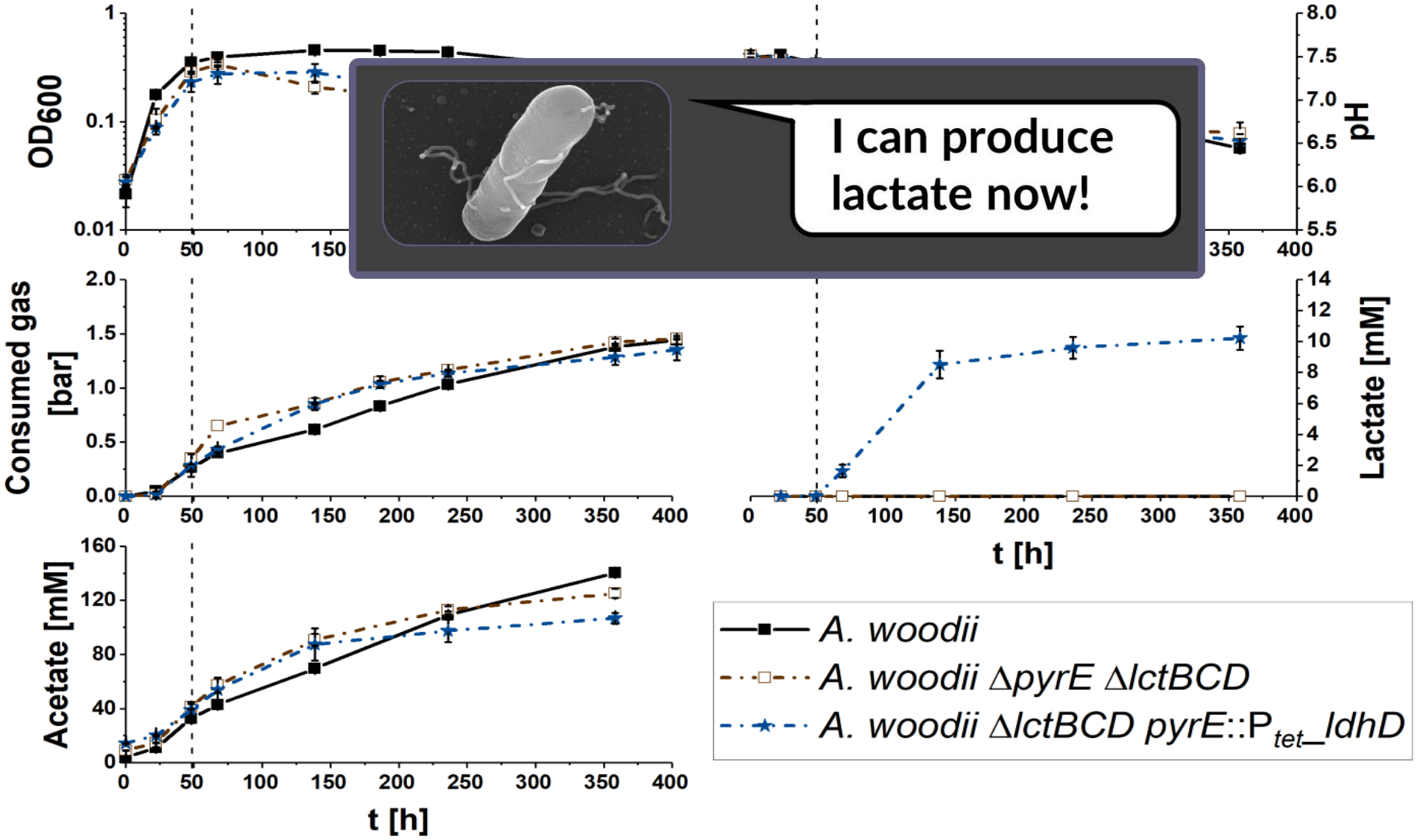
A. woodii ΔpyrE ΔlctBCD on H_2 + CO_2



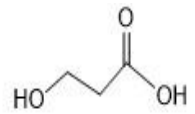
A. woodii ΔlctBCD pyrE::P$_{tet}$_ldhD on H_2 + CO_2



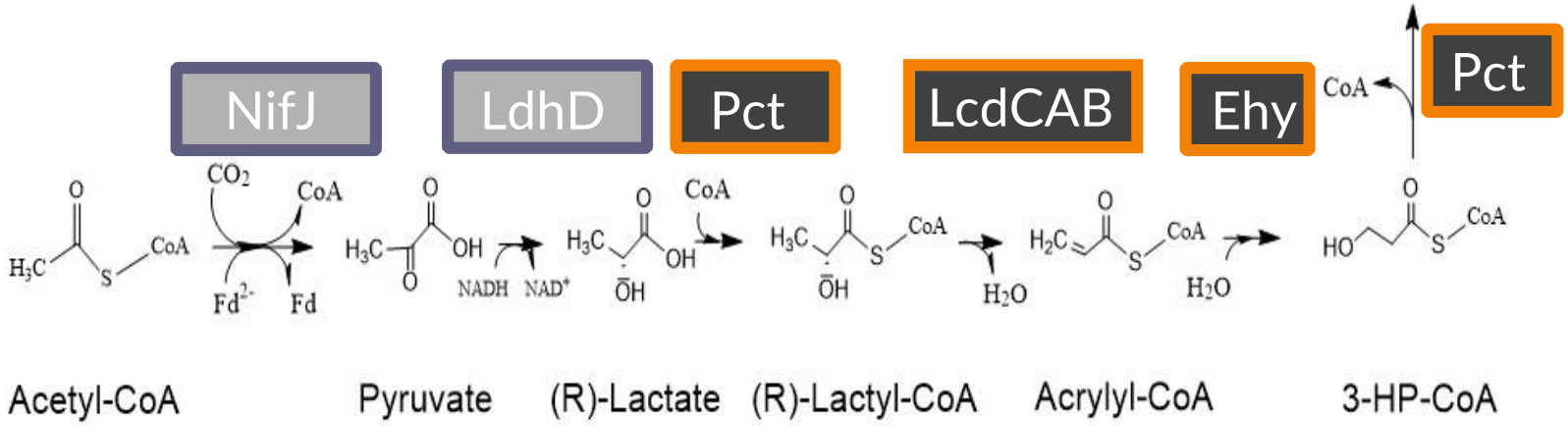
A. woodii ΔlctBCD pyrE::P$_{tet}$_ldhD on H_2 + CO_2

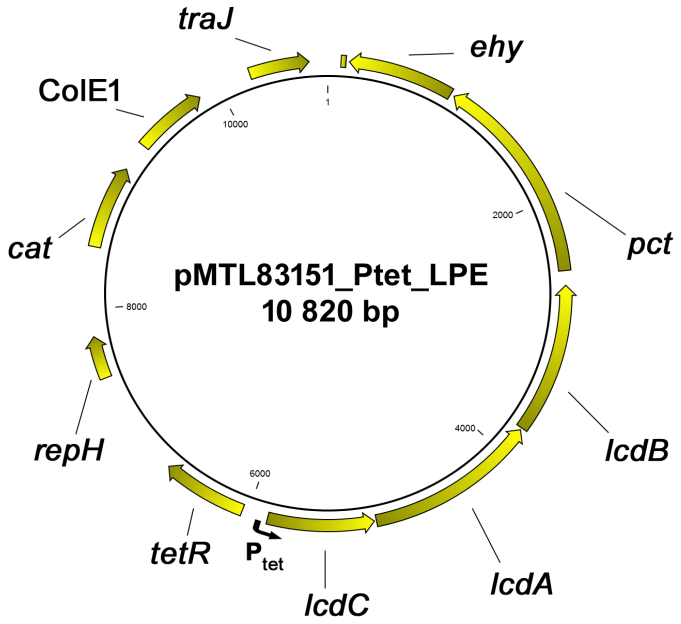


2nd part: Lactate ➔ 3-HP

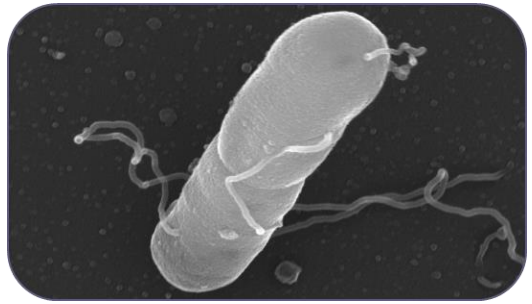


3-HP



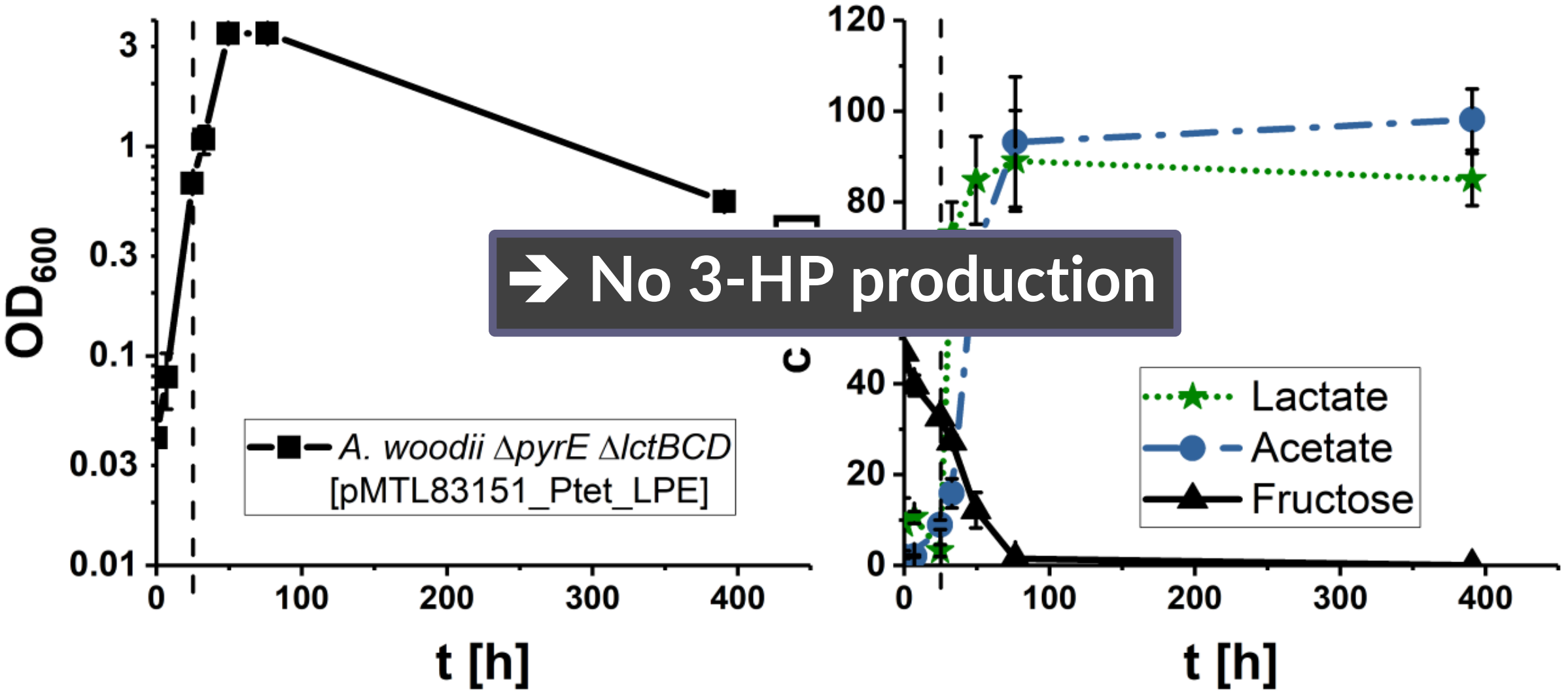


Pct, LcdCAB: *Clostridium neopropionicum*
Ehy: *Chloroflexus aurantiacus*



Let‘s try to convert lactate into 3-HP!

A. woodii ΔpyrE ΔlctBCD [pMTL83151_Ptet_LPE] on fructose + lactate added

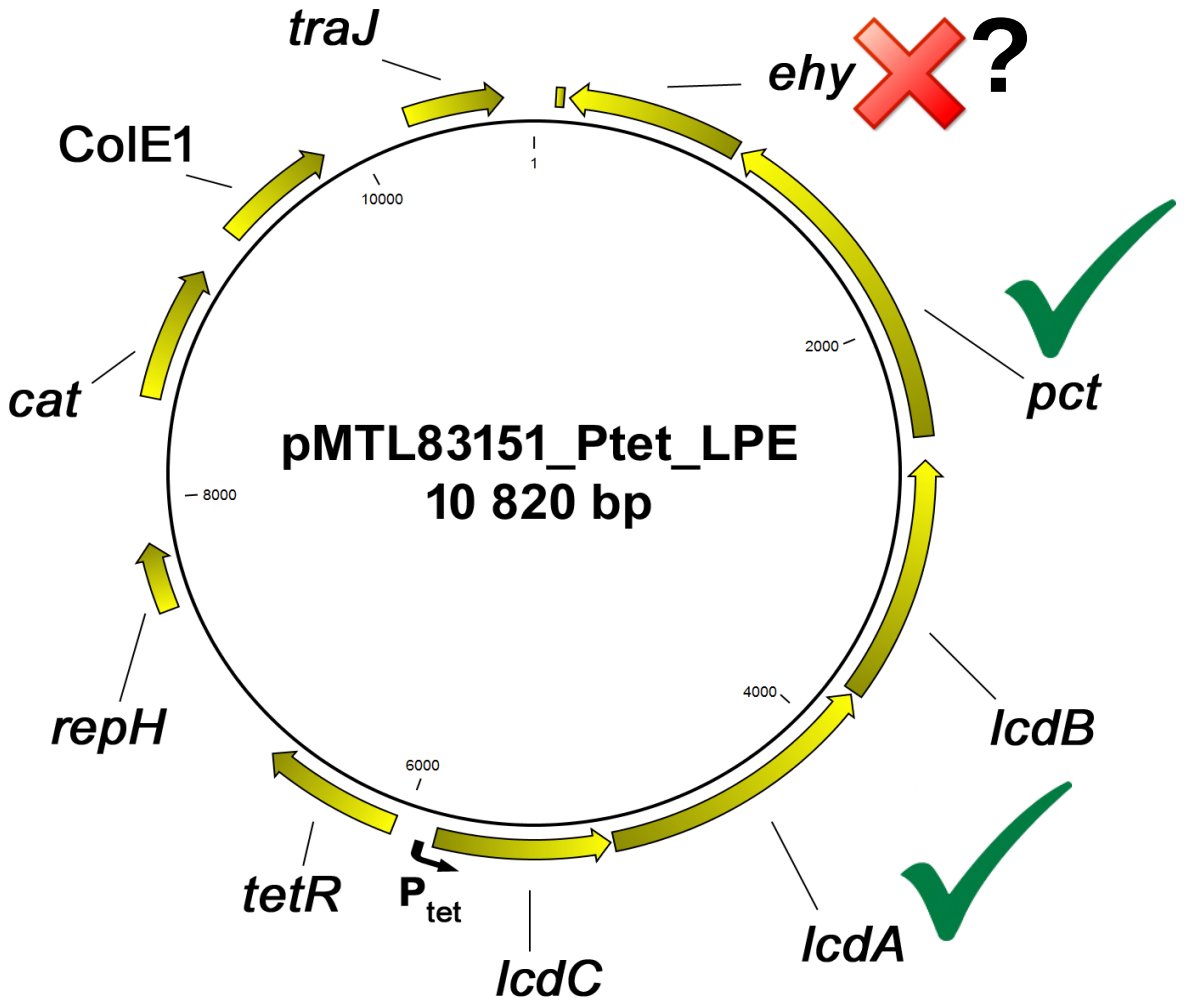


➔ No 3-HP production

A. woodii ΔpyrE ΔlctBCD [pMTL83151_Ptet_LPE]

- Partners confirmed production of Pct and parts of LcdCAB

➔**but not of Ehy**



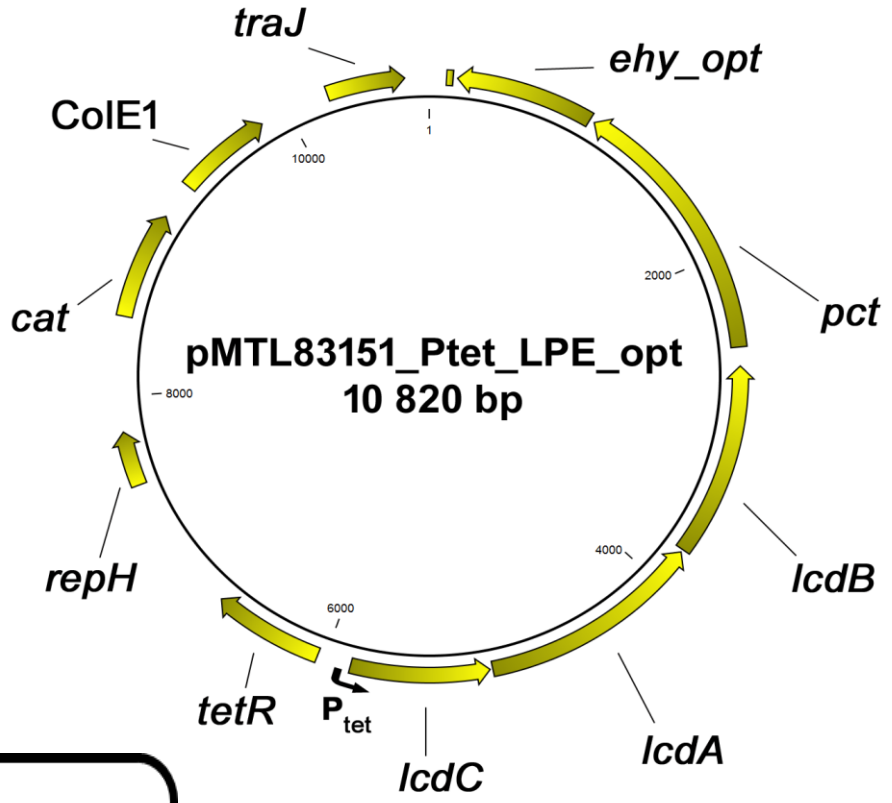
Idea: Codon optimization of *ehy*

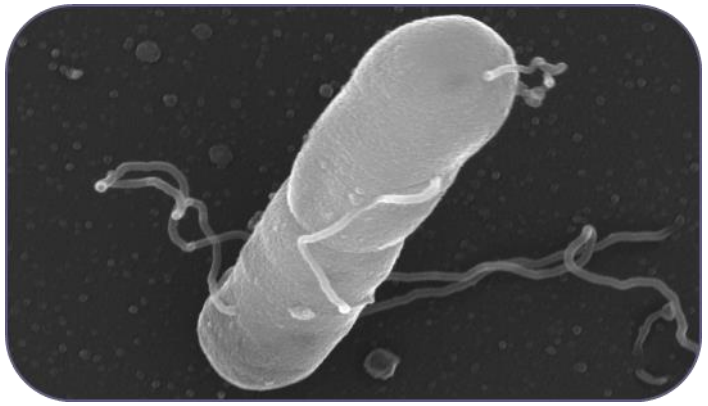
- *ehy* from *C. aurantiacus* (58.2 % G + C)

➔ *A. woodii* (39.3 % G + C)

➔Exchange of *ehy*

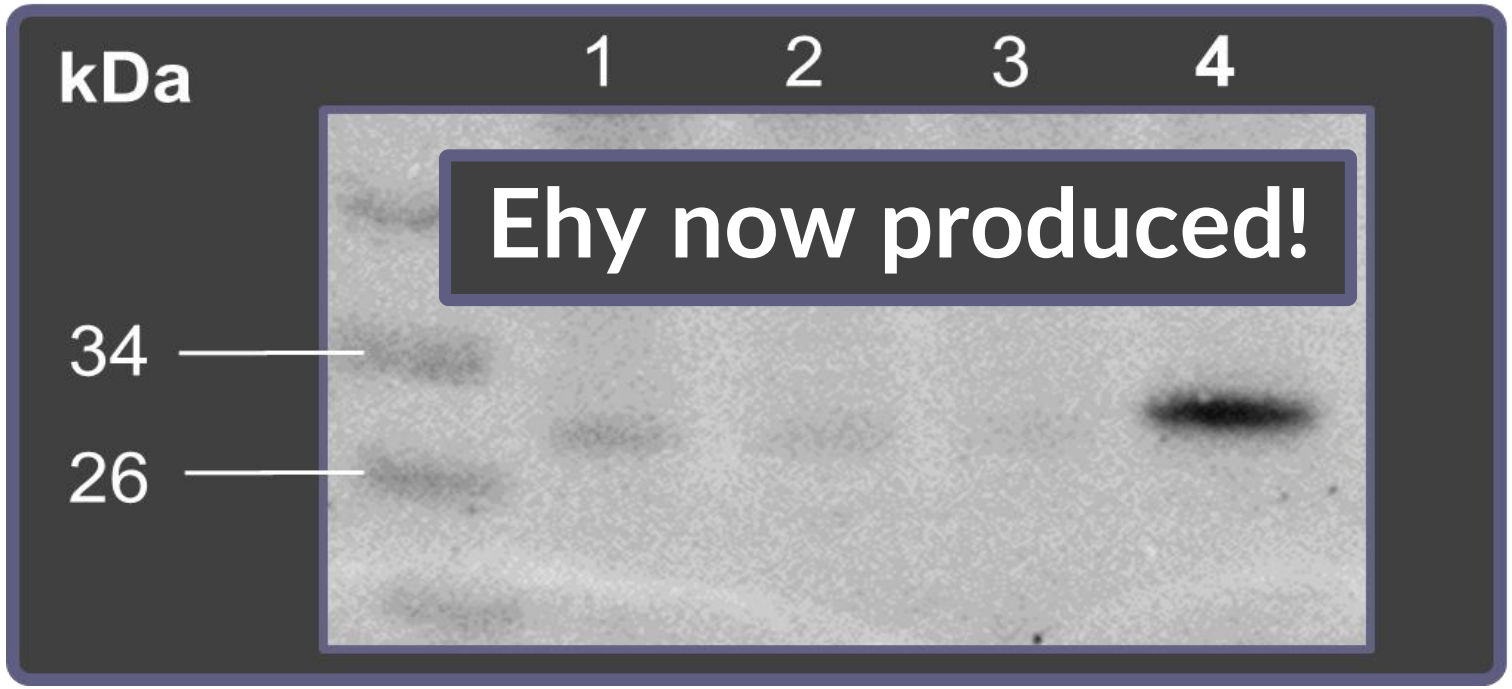
➔Western blot, AB against Ehy (27 kDa)





High G + C content can be a problem!

Idea: Codon optimization of *ehy*

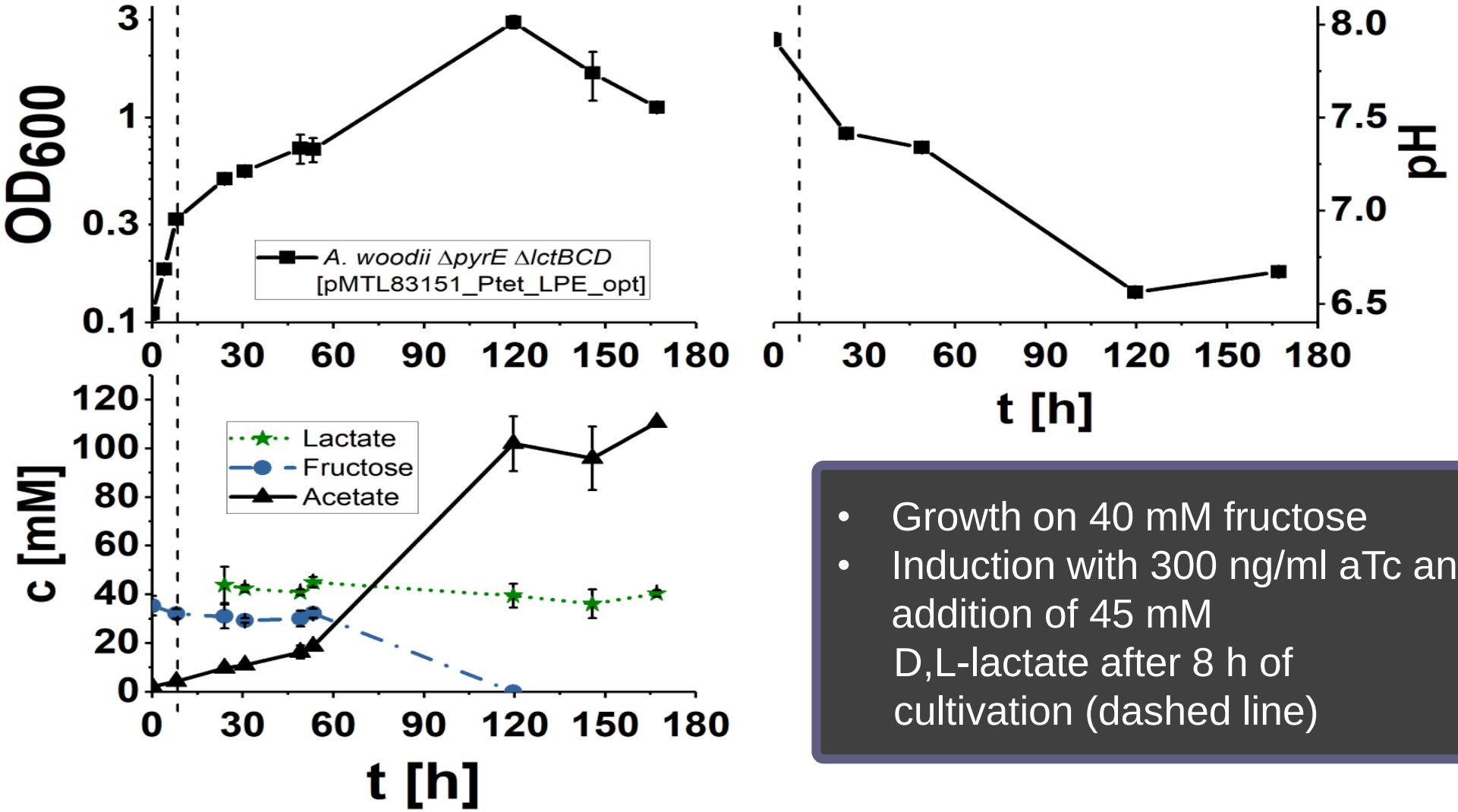


On fructose *A. woodii ΔpyrE ΔlctBCD*:

Parental strain (1), pMTL83151_MCS (2),

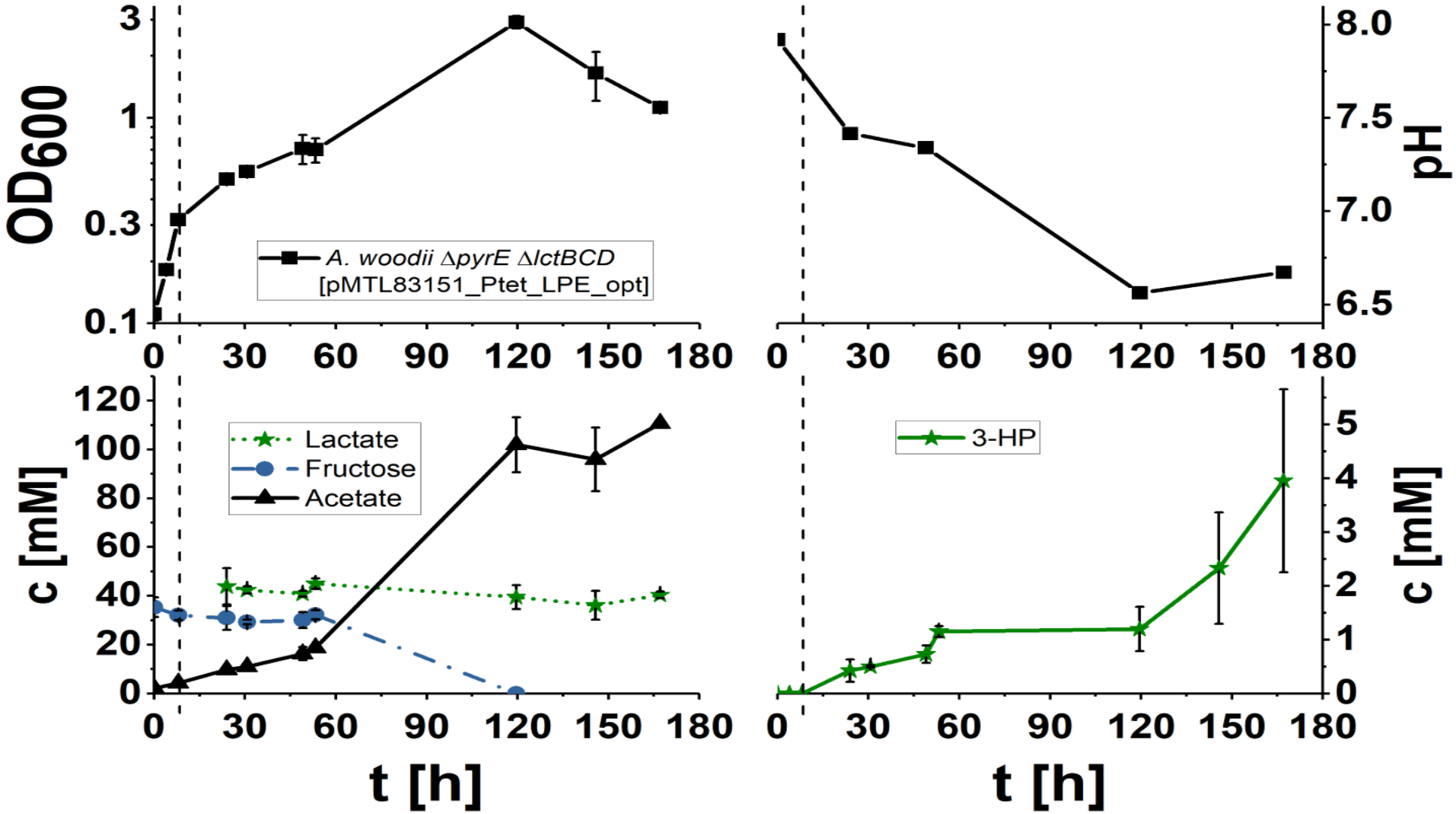
pMTL83151_Ptet_LPE + aTc (3), pMTL83151_Ptet_LPE_opt + aTc **(4)**

A. woodii ΔpyrE ΔlctBCD [pMTL83151_Ptet_LPE_opt]

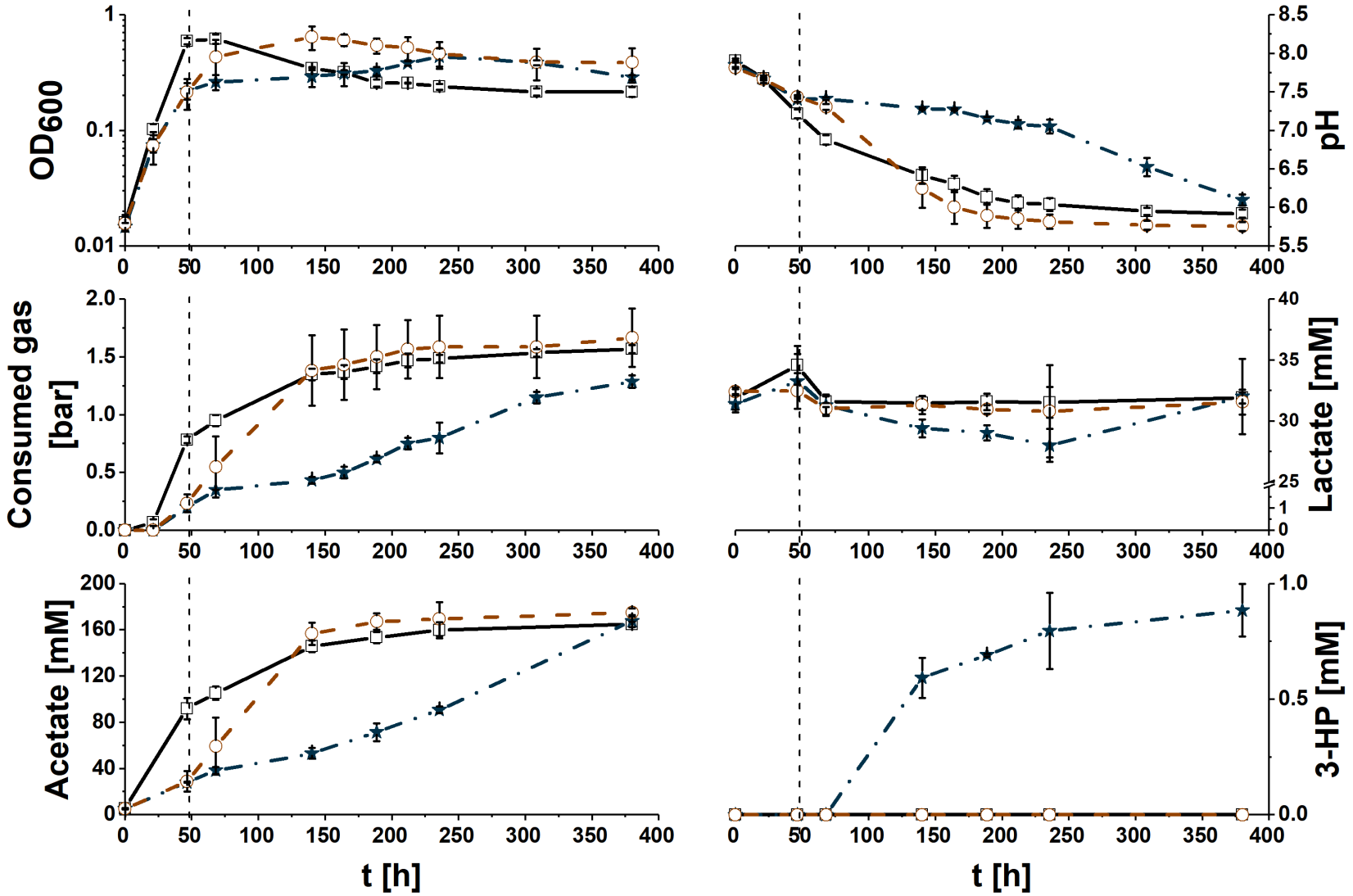


- Growth on 40 mM fructose
- Induction with 300 ng/ml aTc and addition of 45 mM D,L-lactate after 8 h of cultivation (dashed line)

A. woodii ΔpyrE ΔlctBCD [pMTL83151_Ptet_LPE_opt]

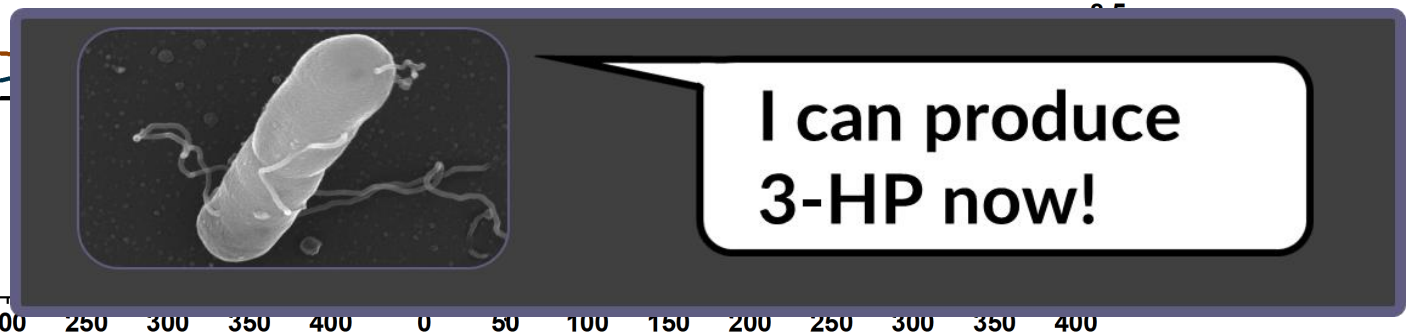


A. woodii ΔpyrE ΔlctBCD [pMTL83151_Ptet_LPE_opt] on H_2 + CO_2 + lactate added



A. woodii ΔpyrE ΔlctBCD
A. woodii ΔpyrE ΔlctBCD [pMTL83151_MCS]
A. woodii ΔpyrE ΔlctBCD [pMTL83151_Ptet_LPE_opt]

A. woodii ΔpyrE ΔlctBCD [pMTL83151_Ptet_LPE_opt] on H_2 + CO_2 + lactate added



A. woodii ΔpyrE ΔlctBCD
A. woodii ΔpyrE ΔlctBCD [pMTL83151_MCS]
A. woodii ΔpyrE ΔlctBCD [pMTL83151_Ptet_LPE_opt]

Summary

- Inducible promoters established
- Lactate metabolism circumvented
- Lactate production from CO_2 + H_2
- Codon optimized *ehy* and production of Ehy
- Conversion of lactate into 3-HP

Acknowledgement

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Alexander Tremel

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Jason Bromley

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