Increasing electroporation efficiency in the lithoautotrophic bacterium *Cupriavidus necator* H16

A roadmap for non-model bacteria domestication

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Non-model bacteria are a treasure trove of promising reactions and metabolisms, but their cumbersome engineering limits their use in an industrial context. For example, *Cupriavidus necator* H16 can grow autotrophically using CO_2, H_2 and O_2, but most engineering attempts rely on conjugation, especially when using large plasmids. Bacteria can use an array of defense systems to defend themselves from exogenous DNA elements, such as phages, mobile genetic elements or, in our case, recombinant DNA. To improve transformation efficiency, we need to identify and overcome these defense systems.

Is your organism easily transformed by electroporation?

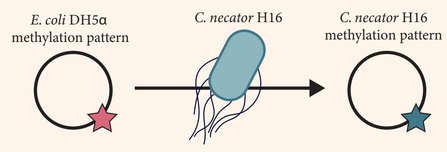
No

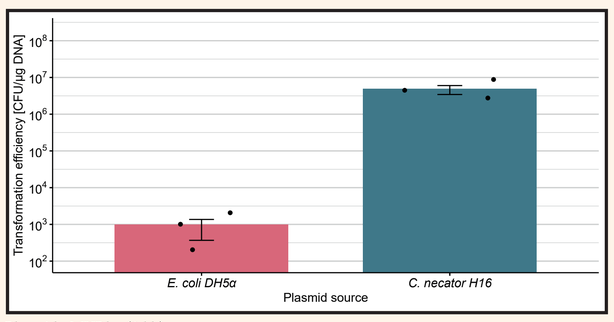
The culprits are often the native
Restriction-Modification systems (RM systems)

Bacteria mark their DNA as 'self' by methylating it in specific patterns. Then, they are able to degrade non-native DNA (incorrectly methylated)

How do I test for it?

Transform *C. necator* with the same plasmid extracted from *E. coli* DH5α (incorrect methylation pattern) and from *C. necator* (correct methylation pattern). Is there a difference?





Plasmid pMVRha (8 kb)

No difference!

Maybe the low efficiency is caused by other defense systems able to interfere with plasmid transformation
(e.g. Wadjet, pAgo, DdmDE...)

Yes

Lucky you!
Move ahead with all your high-throughput techniques, why are you even reading this poster?

Methylation status makes a difference!

Collect your genome information & methylome information

Several web services and databases are updated with recently discovered systems (REBASE, DefenseFinder, PADLOC, MicrobeMod)

Bioinformatic prediction of defense systems

Genes involved	Defense system	Predicted action
hsdR, *hsdS*, *hsdM*	Type I RM system	Degrades DNA which is unmethylated in the (putative) pattern: GAYCNNNNTTAG
mcrBP, *mcrCP*	Type IV RM system	Recognizes and degrades DNA methylated in the wrong pattern. Does not degrade unmethylated DNA
jetA, *jetB*, *jetC*, *jetD*	Wadjet system (type III)	Lowers plasmid stability and/or decrease transformation efficiency. Probably acts through sequence-independent restriction of small circular DNA molecules
+ 11 other putative defense systems		

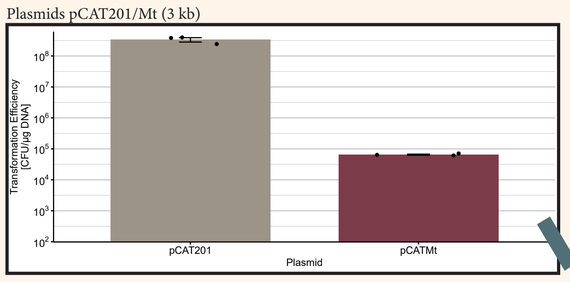
Manual curation and identification of the most promising targets

Experimental confirmation

Confirmation of the type I RM system presence and specificity

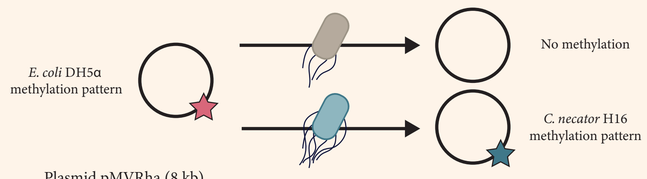
Insertion of a putative restriction site in a small plasmid and comparison of transformation efficiency

Plasmids pCAT201/Mt (3 kb)

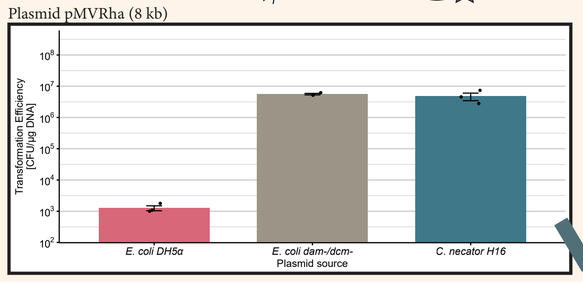


Confirmation of the type IV restriction enzymes presence and activity

De-methylation of plasmid by using a shuttle strain (*E. coli dam⁻/dcm⁻*)



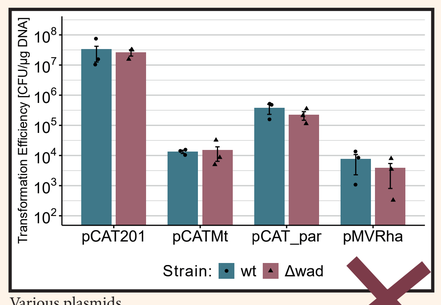
Plasmid pMVRha (8 kb)



Confirmation of the Wadjet system presence and activity

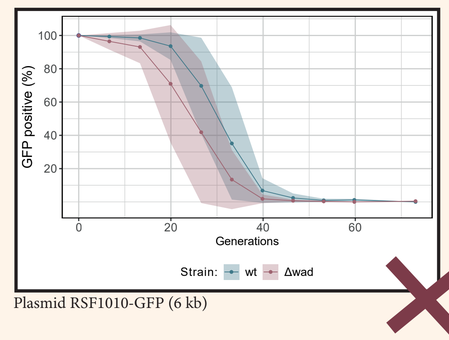
Deletion of the Wadjet operon (***C. necator* Δwad**) and comparison with ***C. necator wt***

Does it influence electroporation?



Various plasmids

Does it influence plasmid stability?



Plasmid RSF1010-GFP (6 kb)

Three strategies designed for high electroporation efficiency

The predicted Wadjet system is either a wrong prediction or inactive in the tested conditions

1. Restriction avoidance

Plasmid design

Remove type I restriction patterns by rational mutagenesis

+

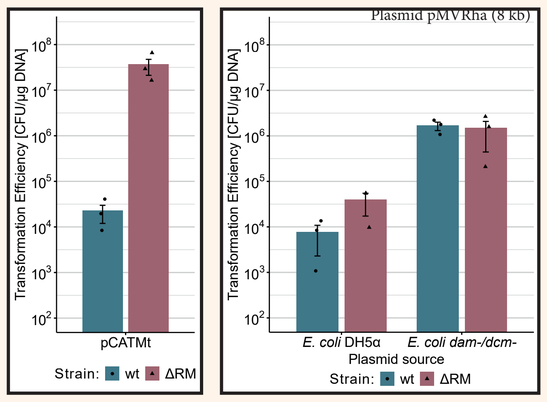
De-methylation

Remove incorrect methylation pattern using shuttle strain *E. coli dam⁻/dcm⁻*

Resulted in successful electroporation of both large plasmids (15 kb) and suicide plasmids

2. Strain engineering

Deletion of both enzymes from **C. necator H16**, obtaining a easier-to-use strain (***C. necator* ΔRM**)



pCATMt is not restricted anymore, but there is still another system able to target mis-methylated DNA (pMVRha extracted from *E. coli dam⁻/dcm⁻* is still uptaken more efficiently)

3. Strain engineering + restriction avoidance

Combines flexibility and efficiency. Currently the best way to transform *C. necator* H16 by electroporation

Strain *C. necator* ΔRM

It is not necessary to remove restriction sites from the plasmid anymore

+

De-methylation

Most plasmids can be transformed without de-methylation. For large plasmids or if many colonies are necessary, *E. coli dam⁻/dcm⁻* can be used to further increase electroporation efficiency

Read more about using C. necator to transform CO_2 into useful products

ITN-EJD ConCO₂rde

ConCO₂rde

Manuscript available!
It also includes a species-independent technique to increase cloning-electroporation efficiency

This is me, feel free to reach out if you are interested!

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