Vibrio natriegens - A new chassis for Synthetic Biology

Waiting for cells to grow is an enormous time sink for synthetic biologists. Cloning cycles with the current standard, *Escherichia coli*, typically take up to three days. In our project Vibrigens - Accelerating Synbio, we established the tools to turn *Vibrio natriegens* into the next generation chassis for synthetic biology, ready to be used reliably. By taking advantage of its unbeaten doubling time of 7 minutes, we substantially reduced waiting time and made one-day-cloning a reality. We built and characterized a flexible golden-gate-based part collection, consisting of more than 100 parts, which enables the creation of complex pathways in a short amount of time. Our engineered *V. natriegens* strains VibriClone and VibriExpress are designed for cloning and protein expression applications, respectively. Moreover, we established the first synthetic metabolic pathway in this organism by producing the platform chemical 3-Hydroxypropionate and along the way developed an accelerated workflow for metabolic engineering.

iGEM MARBURG

presentation

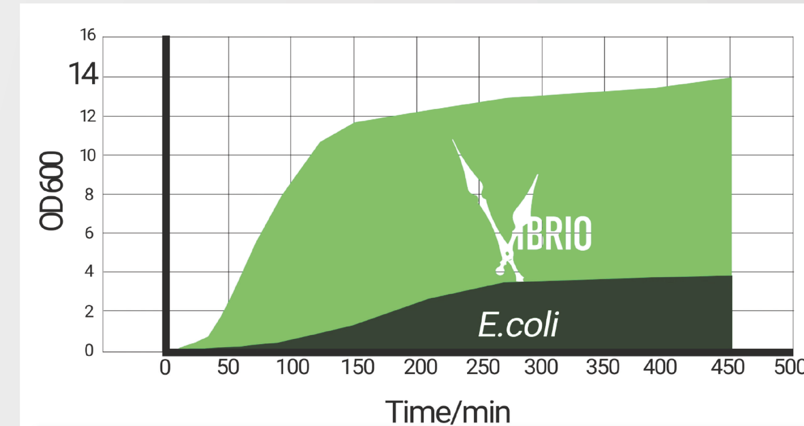
Friday 3:15 PM - Session G - Room 304

VIBRIGENS

VIBRIO NATRIEGENS

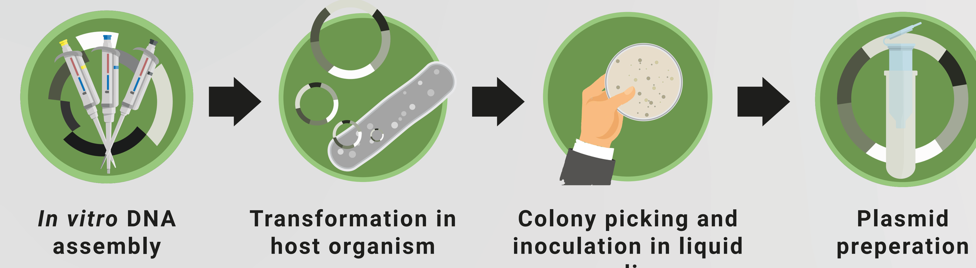
Doubling Time	< 10 min
Temperatuer	37°C
Media	LBv2 (2%NaCl)
Mutation Rate	~1,8E-09
Strain	ATCC14048
Safety	BSL1

Vibrio natriegens is a gram-negative, rod-shaped bacterium that was first isolated from salt marshes in 1958 (Payne 1958). *V. natriegens* is classified as a BSL1 organism and can be easily cultivated at 37 °C in standard LB medium supplemented with 1,5 % NaCl (Lee *et al.* 2016). The most remarkable properties of this bacterium are its doubling time of seven minutes under optimal conditions and its ability to grow to much higher cell densities compared to *E. coli*.



The figure shows the growth behavior of *V. natriegens* (wild type strain ATCC14048) in comparison to *E. coli* (NEB Turbo). Both organisms were grown at 37 °C in baffled flask with LB medium supplemented with 1.5 % NaCl for *V. natriegens* and *E. coli*, respectively. The experiment was set up in triplicates from exponentially growing precultures which were diluted to a starting OD_{600} of 0.05. Samples were taken for measurement in 10 minute intervals.

ONE DAY CLONING

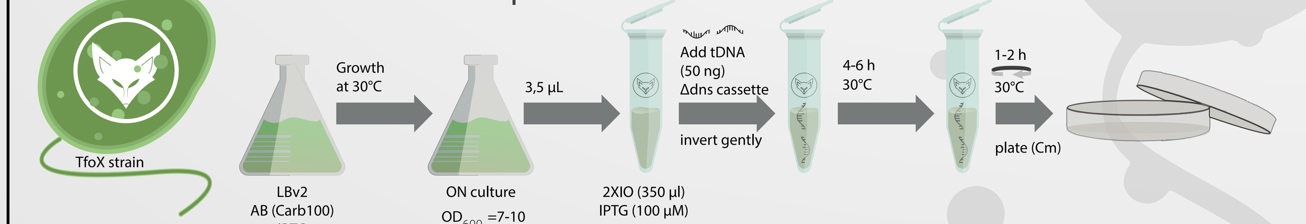


Highlights

- Gibson Cloning 5 fragments
- Golden Gate Cloning 8 parts
- Aqua Cloning 3 fragments

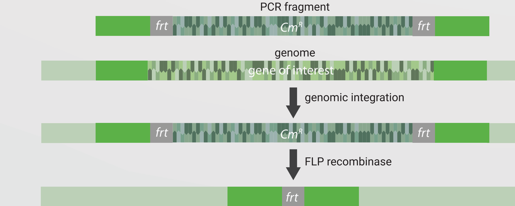
STRAIN ENGINEERING

Natural Competence



Natural transformation with linear and plasmid DNA induced by TfoX.

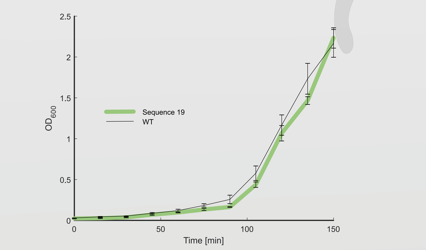
Site Specific Recombination



We designed a workflow for fast genomic modifications in *V. natriegens* using the Flp/*frt* system for excision of antibiotic markers and combining this technique with the natural competence of *V. natriegens* for the uptake of linear DNA from their environment.

Integration

We identified 15 possible sites of which two turned out to be siutable of integration.

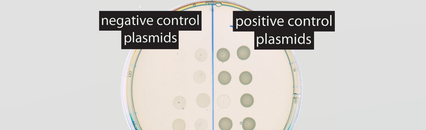


CRISPR/Cas9

We aimed to design a convenient system that allows easy adaption of CRISPR/Cas9 to target various sequences. We provided a first indication of a successful mutation via CRISPR/Cas9.

VibriInteract

Successful result for Vibrio2Hybrid with VibriInteract



Highlights

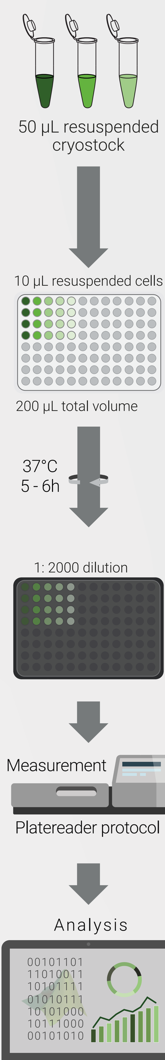
- We designed three custom tailored strains for specific applications
- Establishing the Flp/*frt* system in *Vibrio natriegens* for the first time
- Identification and characterization of 15 genomic integration sites

VibriClone · VibriExpress · VibriInteract

MEASUREMENT

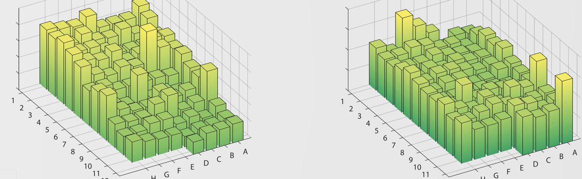
A new chassis requires rethinking existing procedures. We developed a new measurement workflow for platereader measurements with *V. natriegens*.

Workflow



Experiment

Setting up a 96 well plate with OD_{600} distribution for *Vibrio natriegens* following the common *E. coli* workflow (left, consecutive) vs diluting all wells uniformly 1:2000 (right, simultaneous). Shown is the 1:40 dilution.

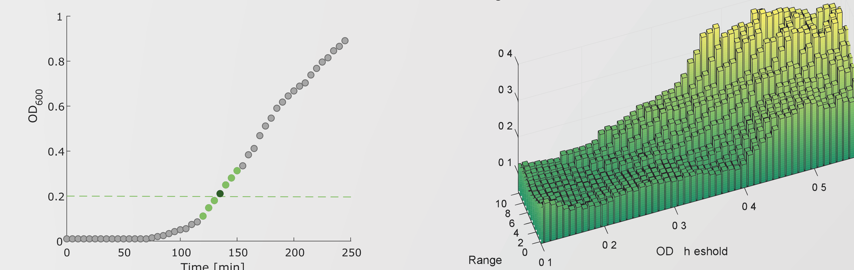


Reporter

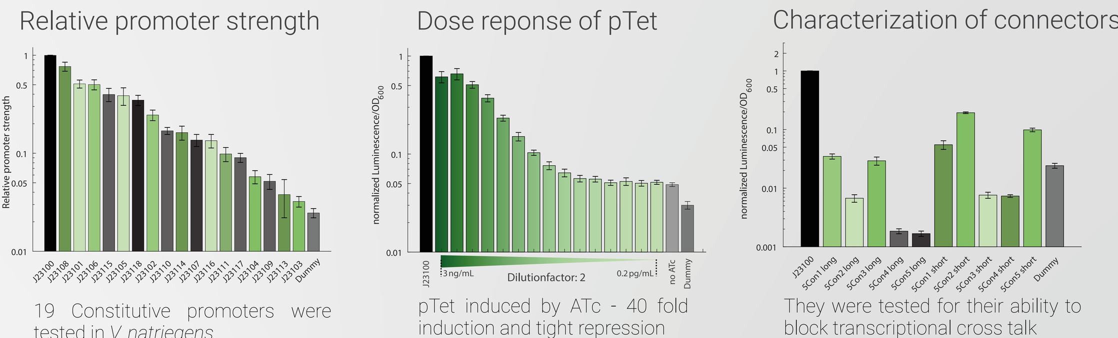
Outstanding sensitivity compared to fluorescence reporters with maximum dynamic range combining the lux operon with ColE1 Method applicable with GFP.

Analysis

The time point first reaching $OD_{600} = 0.2$ is determined for each well independently and three measurement events before and after are included for calculating the final data.



Results



19 Constitutive promoters were tested in *V. natriegens*

pTet induced by ATc - 40 fold induction and tight repression

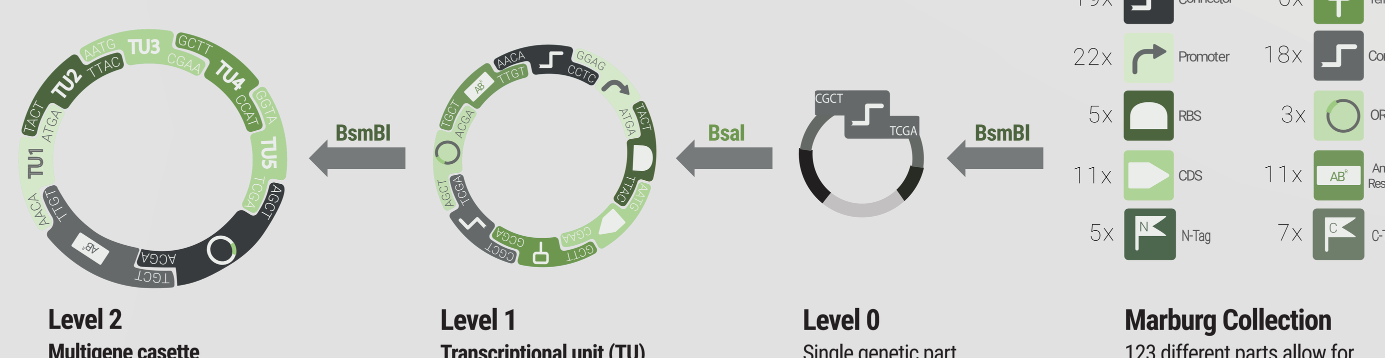
They were tested for their ability to block transcriptional cross talk

MARBURG COLLECTION

Highlights

- 123 different parts
- Most flexible bacterial toolbox
- Proven reliability
- Connectors for insulating single TUs
- Novel entry vectors with fluorescence dropout cassettes

CLICK 'N' CLONE
User-friendly software tool to generate pipetting protocols or picking lists for robots



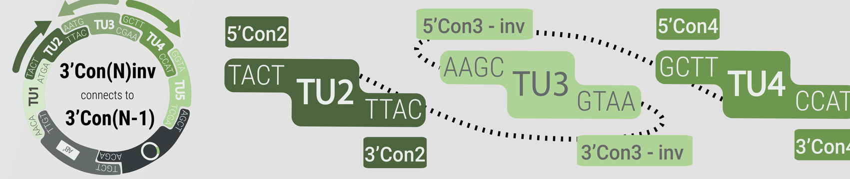
Level 2 — **Multigene casette** built out of level 1 plasmids

Level 1 — **Transcriptional unit (TU)** built out of level 0 plasmids

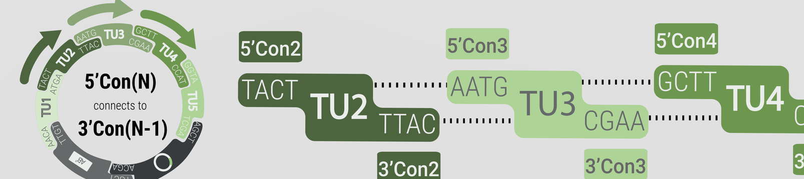
Level 0 — Single genetic part

Marburg Collection — 123 different parts allow for flexible cloning

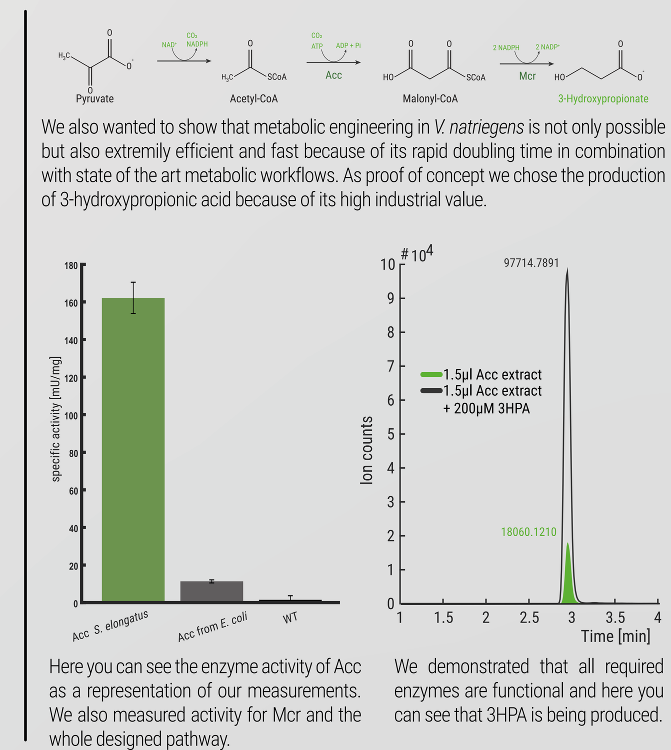
Inverse Set-up



Default Set-Up for Marburg Connectors



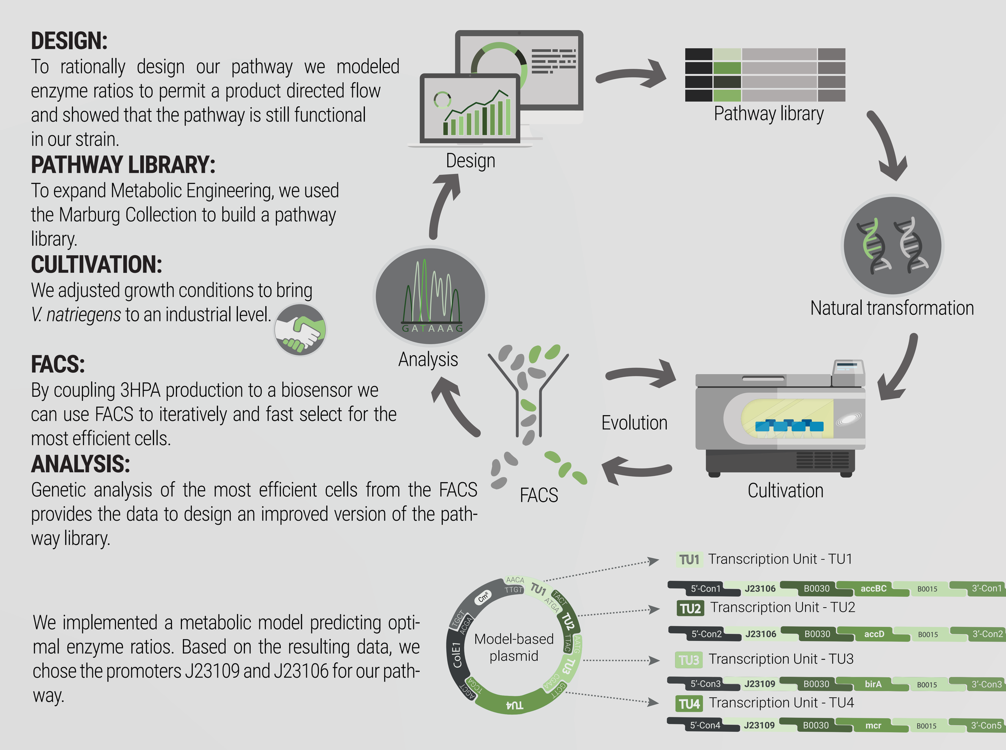
METABOLIC ENGINEERING



We also wanted to show that metabolic engineering in *V. natriegens* is not only possible but also extremily efficient and fast because of its rapid doubling time in combination with state of the art metabolic workflows. As proof of concept we chose the production of 3-hydroxypropionic acid because of its high industrial value.

Here you can see the enzyme activity of Acc as a representation of our measurements. We also measured activity for Mcr and the whole designed pathway.

We demonstrated that all required enzymes are functional and here you can see that 3HPA is being produced.



DESIGN:
To rationally design our pathway we modeled enzyme ratios to permit a product directed flow and showed that the pathway is still functional in our strain.

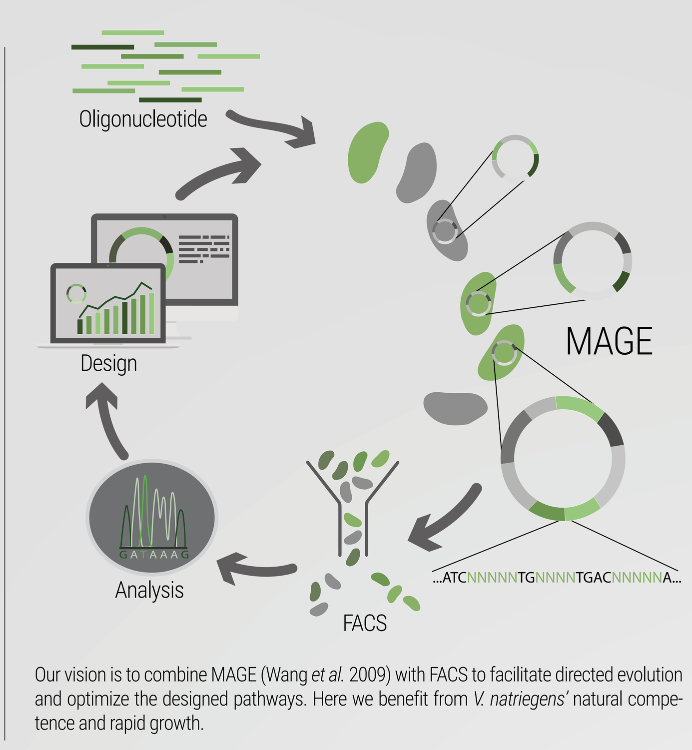
PATHWAY LIBRARY:
To expand Metabolic Engineering, we used the Marburg Collection to build a pathway library.

CULTIVATION:
We adjusted growth conditions to bring *V. natriegens* to an industrial level.

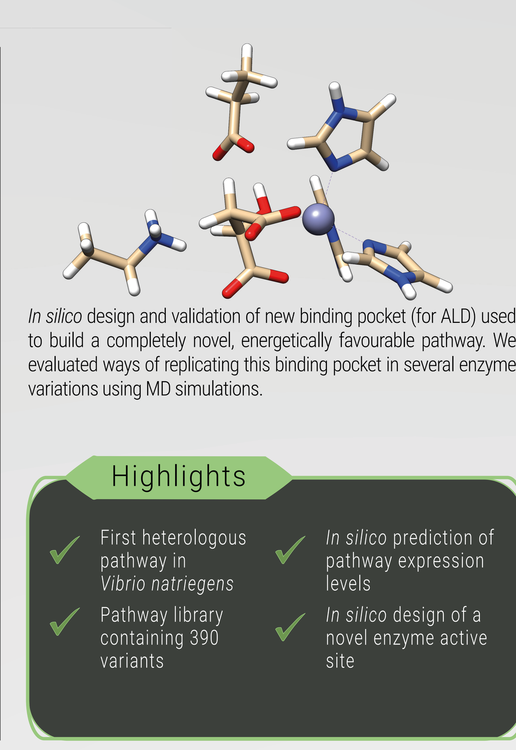
FACS:
By coupling 3HPA production to a biosensor we can use FACS to iteratively and fast select for the most efficient cells.

ANALYSIS:
Genetic analysis of the most efficient cells from the FACS provides the data to design an improved version of the pathway library.

We implemented a metabolic model predicting optimal enzyme ratios. Based on the resulting data, we chose the promoters J23109 and J23106 for our pathway.



Our vision is to combine MAGE (Wang *et al.* 2009) with FACS to facilitate directed evolution and optimize the designed pathways. Here we benefit from *V. natriegens*' natural competence and rapid growth.



In silico design and validation of new binding pocket (for ALD) used to build a completely novel, energetically favourable pathway. We evaluated ways of replicating this binding pocket in several enzyme variations using MD simulations.

Highlights

- First heterologous pathway in *Vibrio natriegens*
- Pathway library containing 390 variants
- *In silico* prediction of pathway expression levels
- *In silico* design of a novel enzyme active site

VIBRIGENS - INTERLAB

Could you imagine working with *V. natriegens* in the future?

No / Yes

We wished to test the reliability of our chassis in many different laboratories. In return we received valuable feedback for the improvement of our project.

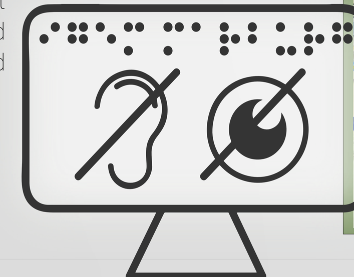
Highlights

- Integrating feedback from iGEMers
- Advertising and establishing *V. natriegens*
- More precise protocols & measurements
- Building a community

Integrated Human Practice Symbol

ACCESSIBLE SCIENCE

Orientating and reading without sight. Blindfold self-test and Braille.



As part of our project we (and other teams) designed a barrier-free website to take this step to remove barriers in communication!

Tools for better experience in the lab for the visually impaired: Chemophone (top) Optophone (bottom)

Feeling the growth curve of V. natriegens

Highlights

- 14 teams
- Poster sessions and expert presentations
- After iGEM workshop
- Collaboration with german association for SynBio