Professur für Systembiotechnologie

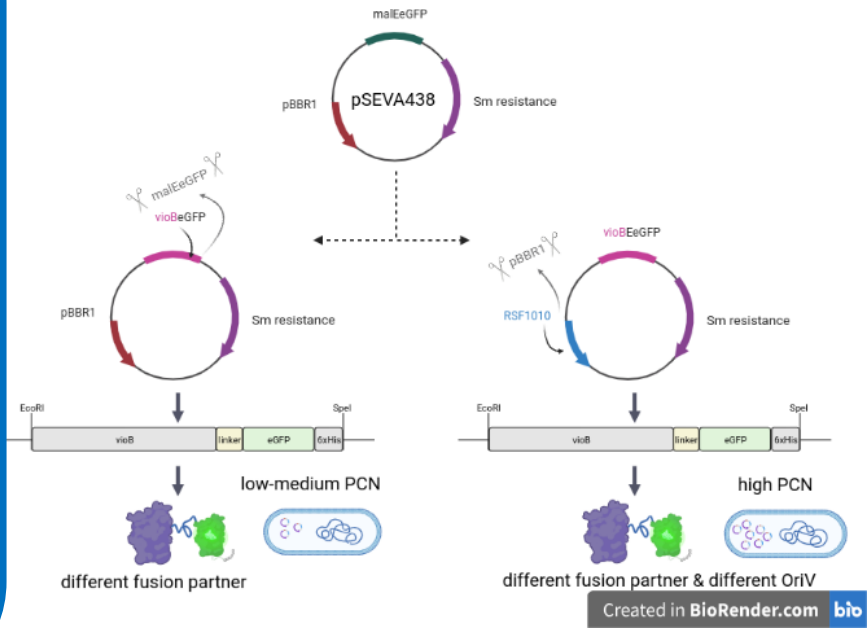
Metabolic burden in *P. putida*: Heterologous protein production of VioBeGP

Keywords: metabolic burden, protein fusion, *P. putida*, VioB

Project overview

We study metabolic burden in *P. putida* through inducible plasmid based expression. Production of the fusion protein MBPeGFP, reduces the growth rate significantly in *P. putida* and therefore indicates *metabolic burden.* MBP toxicity, however, could also be a possible explanation. The task at hand therefore is to remove MBP and fuse eGFP to VioB and study the effects of heterologous protein production during growth experiments. Moreover, the burden attributed to plasmid replication and maintenance is to be studied by increasing the plasmid copy number.

[For more detailed information, see CHEAP project on the SBT Website]



Tasks & Methods

- Genetic fusion of *vioB* and *eGFP* (Overlap-extension PCR); amplification of RSF1010 (PCR)
- Restriction enzyme based cloning (digestion, ligation and transformation in *E. coli*)
- Plasmid-Transfer into *P. putida* (Triparental conjugation)
- Growth experiments in shake flask scale (cultivation and spectrophotometric measurements)

Details

- Bachelorthesis or Masterthesis (English or German)
- Experience with the following is advantageous, but not necessary:
  - Working in sterile conditions
  - Preparation of LB-Agar plates
  - Plasmid prep & Gel purification
  - Agarose gel electrophoresis
  - PCR
- **Start: as soon as possible** (latest: July 2021)

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