

Synthetic microbes on the loose: a series of biocontainment strategies for cell factories

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Type of thesis: Experimental

Required competences: Basic molecular biology cloning techniques (bacterial cell growth culturing, transformation, PCR, gel electrophoresis, restriction-ligation, assembly...), basic oligonucleotide design.

Acquired competences: SEVA-Brick cloning and assembly, fluorescence analysis, knock out, knock in, recombineering, CRISPR/Cas, fitness assays, escape frequency analysis.

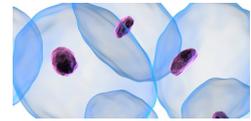
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Description

Synthetic Biology has brought gene modification to the next level. Many microbes, especially those ones of industrial interest are being redesigned to substantially enhance their commercial value and properties. In this context, Safety-by-design aims at considering biosafety issues in the early stages of the engineering of these biological entities^{1,2}.

Here, we address this issue by developing bespoke and advanced biological safeguards and by deploying robust risk assessment methods in the versatile cell factory *P. putida*³. These biocontainment strategies are useful as a tool for application and regulation of GMOs.

We will be working in the design and construction of different safeguards to limit horizontal gene transfer and towards strain isolation, such as synthetic auxotrophy, essential gene riboregulation or engineered addiction^{4,5}.



References

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2. van de Poel, I. & Robaey, Z. Safe-by-Design: from Safety to Responsibility. *NanoEthics* **11**, 297–306 (2017).
3. Martínez-García, E., Nikel, P. I., Aparicio, T. & de Lorenzo, V. Pseudomonas 2.0: genetic upgrading of *P. putida* KT2440 as an enhanced host for heterologous gene expression. *Microbial Cell Factories* **13**, (2014).
4. Gallagher, R. R., Patel, J. R., Interiano, A. L., Rovner, A. J. & Isaacs, F. J. Multilayered genetic safeguards limit growth of microorganisms to defined environments. *Nucleic Acids Research* **43**, 1945–1954 (2015).
5. Lopez, G. & Anderson, J. C. Synthetic Auxotrophs with Ligand-Dependent Essential Genes for a BL21(DE3) Biosafety Strain. *ACS Synthetic Biology* **4**, 1279–1286 (2015).