Fast cloning and production of dehydrogenases enzymes using a Glycine auxotrophic *E.coli* M15 strain

Benito M. , Román R. , Caminal G. , García M. , Guillen M. , Sutton P.W. , Casablancas A. , Álvaro G. , González G.

Bioprocess Engineering and Applied Biocatalysis Research Group. Universitat Autònoma de Barcelona (UAB)

Mario.benito@uab.cat

INTRODUCTION

- **BIOCON-CO2** is developing an innovative and sustainable route for converting CO2 and ethanol into lactic acid for biodegradable polymers and feed additives. The multi-enzymatic cascade reaction system requires the activity of Alcohol dehydrogenase (ADH), Pyruvate decarboxylase (PDC) and Lactate dehydrogenase (LDH), creating an internal cofactor (NADH) regeneration loop.
- The techno-economic feasibility of such a process depends, among other factors, on the availability of robust biocatalysts (high activity and stability, low cost, high reuse ratio…). A modified *E.coli* glycine auxotrophic strain (M15 ΔglyA) [1] is selected to demonstrate its use as universal platform for enzyme production.
- We have used an in-house developed expression system combining this *E.coli* strain and SLIC-based vectors, allowing us to generate new plasmid vectors in a simple and effective way [2], aiming to produce Alcohol and Lactate dehydrogenase. High number of colonies were generated in all cases tested, confirming the robustness and reproducibility of the technique. Protocols of high cell density cultures -using antibiotic free media- developed in the past, have been validated with this work.

METHODS & RESULTS

One-Step Sequence- and Ligation-Independent Cloning (SLIC)

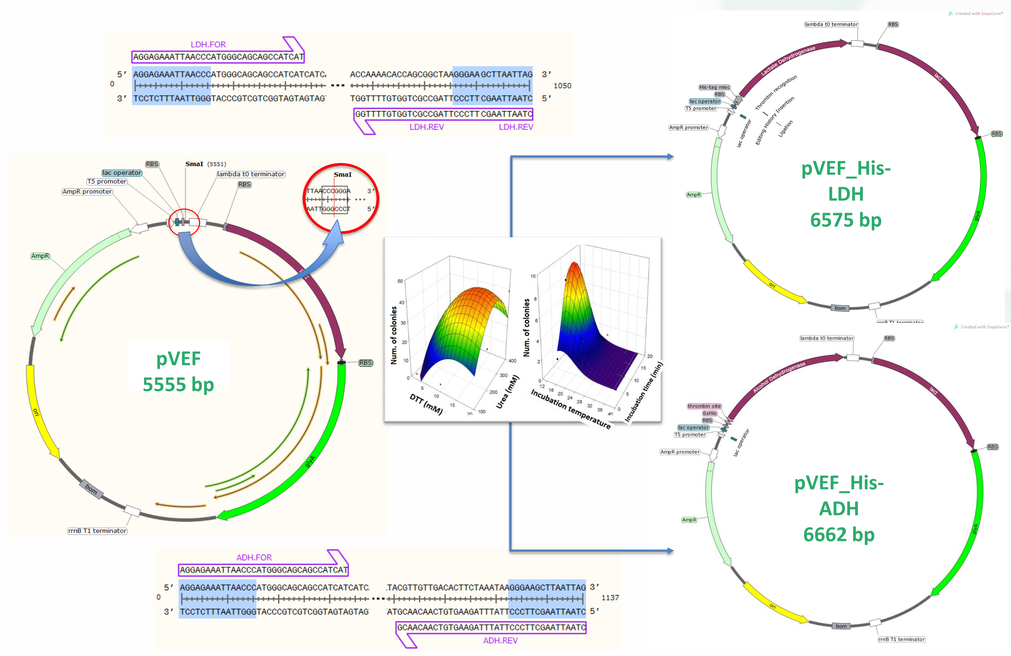

Figure 1. Schematic diagrams of one-step SLIC. Partial sequences of the insert are included, containing both forward and reverse primers used to amplify the insert, as well as homologous regions (inside blue box).

Figure 2. Analysis of PCR products from plasmid DNAs recovered from independent colonies derived from transformation with pVEF_His_ADH (left) and pVEF_His-LDH (right).

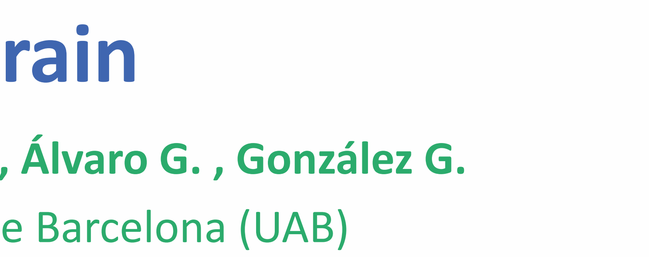

Figure 3. Plasmid digested with XhoI and analysed on agarose gel (left). Comparison respect theoretical restriction pattern (right).

- New plasmid were successfully obtained by SLIC technique (Fig. 1), under best conditions previously determined, obtaining from **20 to 40 colonies** per case.
- *E.coli* competent cells from **DH5α** and **Top10** strains were transformed with SLIC products, and harvested in petri dishes (LB medium).
- **100 % efficiency** was observed (Fig. 2), and one colony of each case (red circle) was picked up to analyse restriction pattern with **XhoI** enzyme (Fig. 3).
- Plasmid DNAs obtained were sequenced, assessing their integrity along the process.
- Afterwards, *E.coli* M15ΔglyA was transformed with the resulting plasmids.

Production of Alcohol and Lactate dehydrogenase with *E.coli* M15 ΔglyA

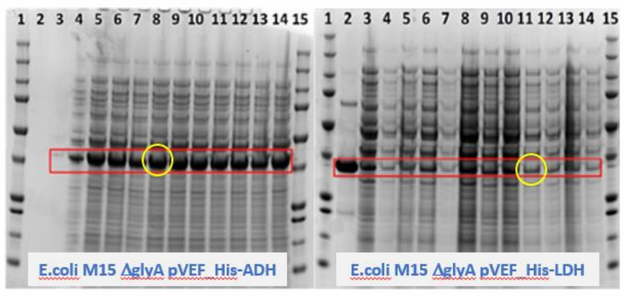

Figure 4. Screening performed with 24 colonies derived from transformation of E.coli M15 ΔglyA (glycine auxotrophic strain) in 1 mL LB medium, induced with IPTG (1mM). Red box shows protein bands corresponding to ADH (left) and LDH (right). Yellow circles correspond to colonies chosen for stock generation.

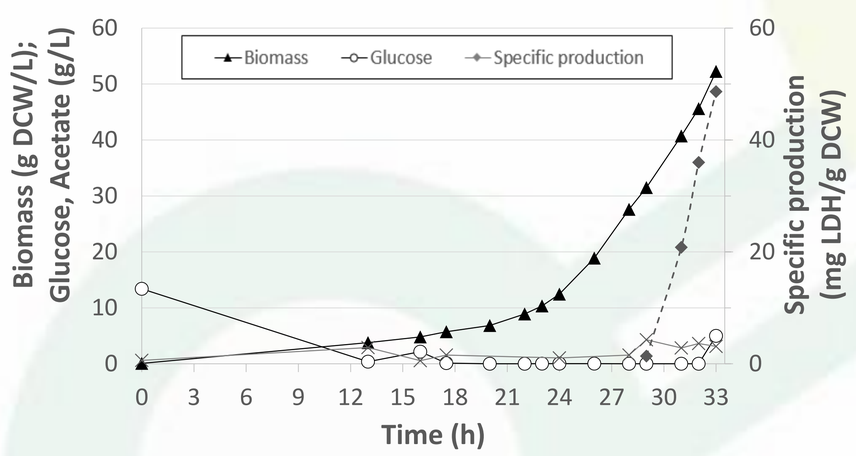

Figure 5. Production of Alcohol dehydrogenase (LDH) at bench-scale (2L), in high cell density culture, with minimum media. Induction (IPTG 0.4 mM) pointed with blue arrow.

- It was performed an screening with transformed *E.coli* M15ΔglyA in LB medium, induced with IPTG (Fig. 4).
- It was detected from 14 to 16% of relative production of ADH, and from 7 to 9% of LDH.
- Best cases were chosen for stock generation.
- When Fed-Batch strategy was applied for LDH production, about **2,5 g of LDH per liter** of culture were generated, corresponding to 48.7 mg of ADH per gram of DCW.

References: [1] Pasini M.; Robust microbial construction and efficient processes for recombinant enzymes production in Escherichia coli; Universitat Autònoma de Barcelona (December 2015).
[2] Jae-Yeon Jeong et al.; One-Step Sequence- and Ligation-Independent Cloning as a Rapid and Versatile Cloning Method for Functional Genomics Studies; Applied and EnvironmentalMicrobiology (August 2012); Vol. 78, Num. 15, pages 5440-5443.

Aknowledgements: The Department of Chemical, Biological and Environmental Engineering of Universitat Autònoma de Barcelona constitutes the Biochemical Engineering Unit of the Reference Network in Biotechnology and the research group 2017 SGR 1462, Generalitat de Catalunya. Authors also thank. Mario Benito acknowledges UAB for funding his Ph.D. grant.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement no. 761042 (BIOCON-CO2). This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.