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DESIGN OF MICROAEROBICALLY INDUCIBLE REPLICONS FOR HIGH-YIELD PLASMID DNA PRODUCTION IN MICROAEROBIC HIGH CELL-DENSITY CULTURES OF ESCHERICHIA COLI

Karim E. Jaén, Daniela Velázquez, Juan Carlos Sigala, Alvaro R. Lara
Universidad Autónoma Metropolitana-Cuajimalpa. Departamento de Procesos y Tecnología
alara@correo.cua.uam.mx

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Introduction. Due to the technical limitations during the operations of large-scale bioreactors, O_2 limitation can easily arise. This is a typical challenge for the scale-up of bioprocesses, since O_2 limitation cause undesirable physiological changes in *E. coli*. However, O_2 limitation can be used as an inducer for the production of recombinant products. We have previously characterized a set of promoters inducible by oxygen limitation [1]. Here, we applied synthetic biology strategies to develop microaerobically-inducible pUC-derived replicons. We used the *Vitreoscilla* hemoglobin promoter (P_{vgb}) to induce the expression of the positive control element of the pUC replicon, the rnaII molecule. Such system was tested in high cell-density cultures with O_2-limited regimes.

Methodology. The modified replicon was constructed by adding a second copy of *rnaII* in the backbone pUC18 (Fig. 1). To test the biological design, the P_{trc} was tested first to induce rnaII with IPTG in shake flasks. The microaerobically-inducible version was obtained by adding a second copy of *rnaII* under control of P_{vgb}. This construction was tested in high-cell density cultures in shake flasks at low O_2 transfer rates using a commercial medium with enzyme controlled glucose release. Fed-batch cultures were run to characterize the O_2 transfer rate during high cell-density cultures and the expression of *rnaII* and plasmid copy number upon O_2 depletion by RT-qPCR. Using extracellular rates, the metabolic fluxes under the different stages of the cultures were estimated to gain further knowledge on the physiology of the host.

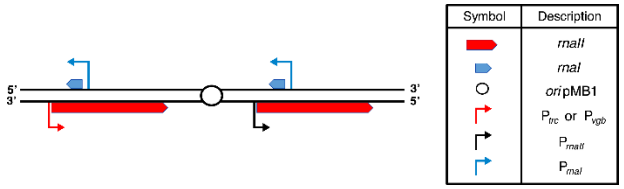

Fig. 1. Diagram of the inducible replicon.

Results. The induction of *rnaII* with IPTG conducted to increases of up to 5-fold plasmid yields in strains like BL21, DH5α and W3110. This proof of concept proved the validity of proposed molecular design. The microaerobically-inducible plasmid was then tested in high cell-density cultures of strain W3110. Upon O_2 depletion, *rnaII* was efficiently induced and pDNA titer increased steadily for the inducible plasmid, reaching a titer of *ca.* 400 mg/L. In contrast, only 200 mg/L of pUC18 were obtained (Fig. 2).

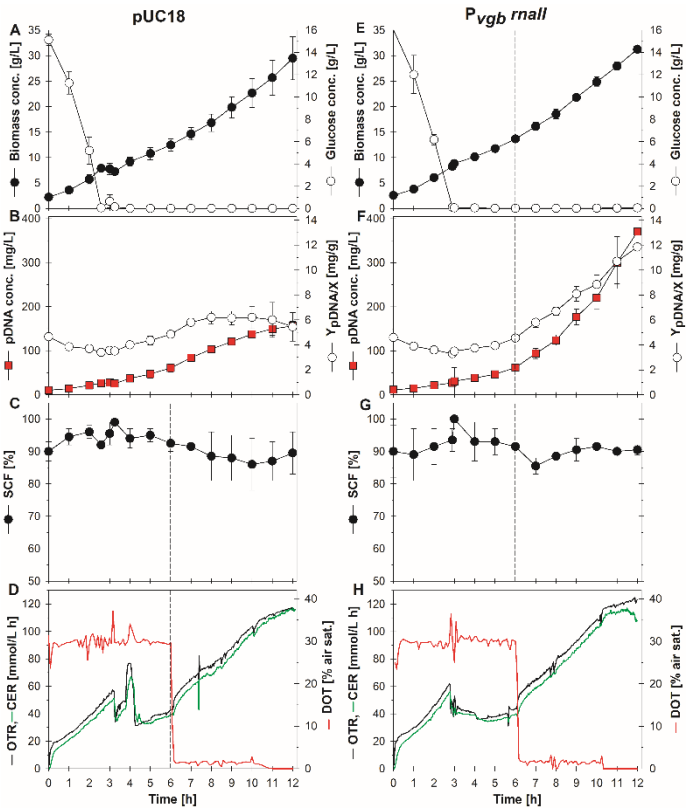

Figure 2. Production of pUC18 (left panel) and P_{vgb}*rnaII* (right panel) in fed-batch cultures of *E. coli* W3110recA- with a step-change to oxygen-limited regime. The time profiles of biomass and glucose concentrations (A, E). pDNA concentration and yield on biomass (B, F). Supercoiled pDNA fraction (C, G). Oxygen transfer rate, carbon dioxide evolution rate and dissolved oxygen tension (D, H).

Conclusions. The proposed system show the potential of applying synthetic biology strategies to overcome typical problems of bioreactors operation. We have demonstrated that bioreactor limitations can be used to induce the production of valuable biomolecules. Moreover, this strategy could be easily up-scaled.

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Bibliography.

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