HIGH YIELD PLASMID DNA PRODUCTION UNDER OXYGEN LIMITATION USING MICROAEROBICALLY INDUCED REPLICATION

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Key Words: Microaerobic Processes, Escherichia coli, Plasmid DNA, *rnaII*.

With the aim of increasing plasmid DNA (pDNA) production under oxygen limitation, a self-inducible replication system was created. An extra copy of the gene coding for *rnaII*, which is a positive control molecule for pMB1-derived replicons, was placed under control of the *lac* or *trp* promoters and cloned in plasmid pUC18. The modified plasmid pUC18-P_{trc} *rnaII* resulted in a strong overexpression of *rnaII* which in turn triggered the plasmid copy number in more than the double of that of pUC18. Based on this, a microaerobically-inducible plasmid was created by inserting an extra copy of *rnaII* under control of the microaerobic promoter from the *Vitreoscilla* hemoglobin (P_{vgb}). Such plasmid was tested in fed-batch cultures of the strain W3110 *recA*$^-$ in which dissolved oxygen was depleted for nearly 6 h. Upon oxygen depletion, *rnaII* was efficiently induced and pDNA titer increased steadily for pUC18- P_{vgb} *rnaII*, reaching nearly 400 mg/L. In contrast, only 200 mg/L of the unmodified pUC18 were obtained.

In order to improve cellular performance under oxygen limitations, engineered strains expressing the *Vitreoscilla* hemoglobin encoded in the chromosome, were created. The *vgb* gene was inserted in BL21 and W3110 strains and the performance of both strains were compared in biphasic aerobic-oxygen limited cultures. Interesting differences were observed in the kinetic behavior, metabolic fluxes distribution and gene expression levels when the vgb gene was expressed in BL21 or W3110 *recA*$^-$ *vgb*$^+$, therefore, this strain was used for production of the inducible plasmid. The amount of pUC18 produced by W3110 *recA*$^-$ *vgb*$^+$ under oxygen limitation doubled that of W3110 *recA*$^-$. However, when pUC18-P_{vgb} *rnaII* was used, the engineered strain produced only 20 mg/L. Moreover, the size of the obtained plasmid was strongly shortened. Plasmid sequencing revealed that an important fraction of the origin of replication was lost. These results demonstrate the feasibility of microaerobically-induced pDNA production, and that the performance of genetic constructions depend on the strain used. Furthermore, unexpected changes in plasmid fidelity can arise when using genetically modified strains.