Chromosomal expression as an antibiotic-free alternative to plasmid expression

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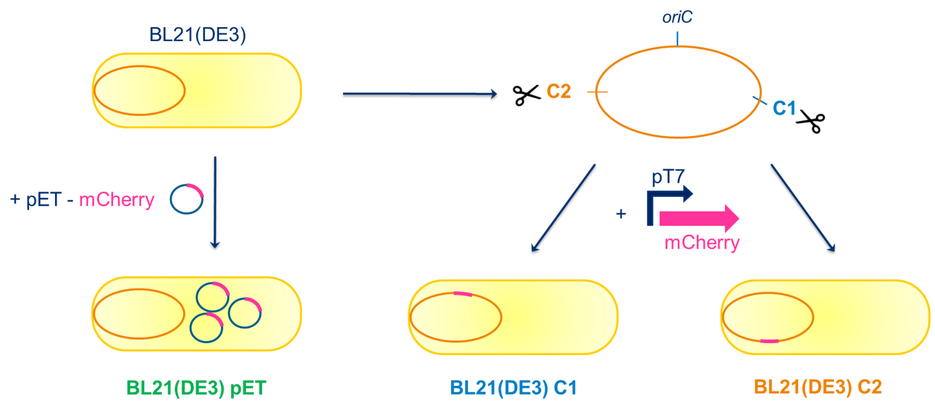
INTRODUCTION

BGene is an innovative company specialized in **customized genomic engineering solutions** for laboratories using micro-organisms for research and bioproduction.

BGene provides services around the **design**, **construction**, and **optimization** of tailor-made bacterial strains. BGene is able to modify and improve the microbial genome efficiently, leaving **no scar nor antibiotic resistance gene**.

The objectives of this study were to compare the expression of a target gene, cloned either in an expression plasmid or in a single copy on two different targets in the chromosome.

The inserted DNA contained the gene of interest and an inducible promoter (pT7). Regarding the litterature (Sabido et al, 2013 and Schulga et al, 2016 among others), plasmid replication and target protein synthesis can cause a metabolic burden leading to plasmid loss, or great variations in the plasmid copy number when no selective pressure is applied. These above-mentioned issues could be overcome by integration of the target gene in the chromosome, even in a single copy (Striedner et al 2009).



Principle of the sudy : one gene expressed from a plasmid (pET) or from the chromosome (2 different targets)

METHODS

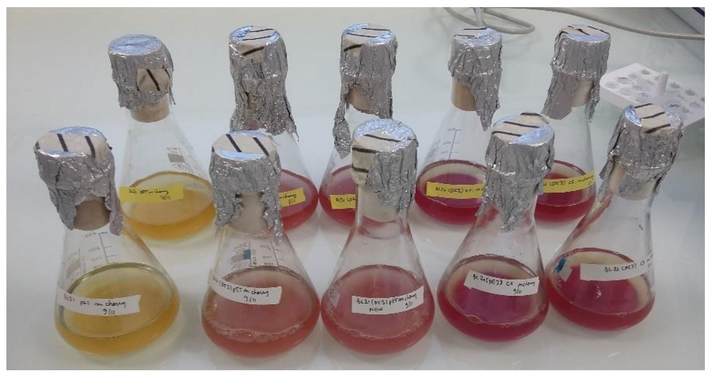
Fluorescence spectroscopy

One single clones for the 3 different strains were grown overnight in LB medium and diluted in 150µL LB without any antibiotic at an OD of 0.1. in a 96 wells plate. 6 replicates for each strains were allowed to grow at 37°C until OD reaches 0.6, 3 of the replicates were then induced by adding 0.1 mM IPTG.

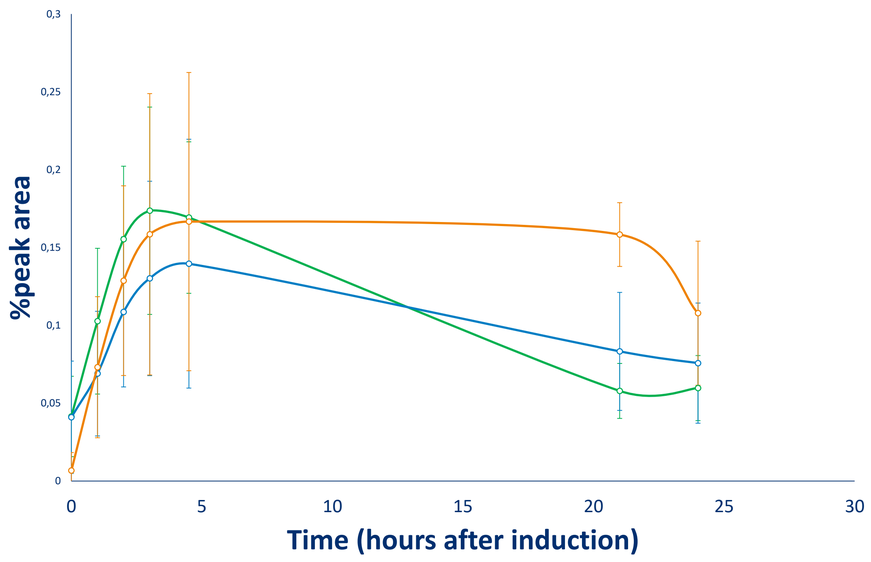
Flask culture and SDS-PAGE analysis

One single clones for the 3 different strains were grown overnight in LB medium and diluted in 50 mL LB without any antibiotic at an OD of 0.1. Each cultures were allowed to grow at 37°C until OD reaches 0.6 and induced by adding 0.1 mM IPTG. At different time points after induction (1, 2, 3, 4.5, 21 and 24 hrs), 500 µL were aliquoted and lysed with Laemmli sample Buffer and loaded on a SDS-PAGE gel (12% acrylamide).

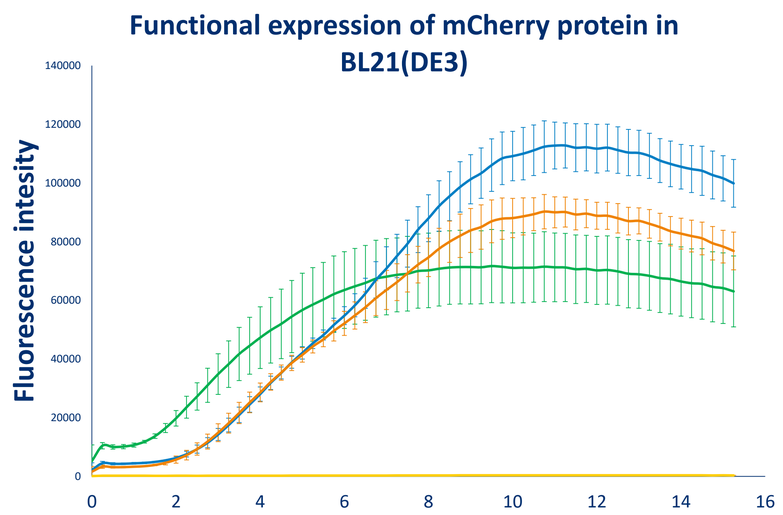
The 28.8 kDa band intensity was calculated with ImageJ software as the percentage of peak area out of total area of the lane.



From left to right: erlen flasks cultures after 24hrs : BL21 – pETmCherry, BL21(DE3) – pETmCherry1, BL21(DE3) – pETmCherry2, BL21(DE3) –C1::T7mCherry, BL21(DE3) – C2::T7mCherry

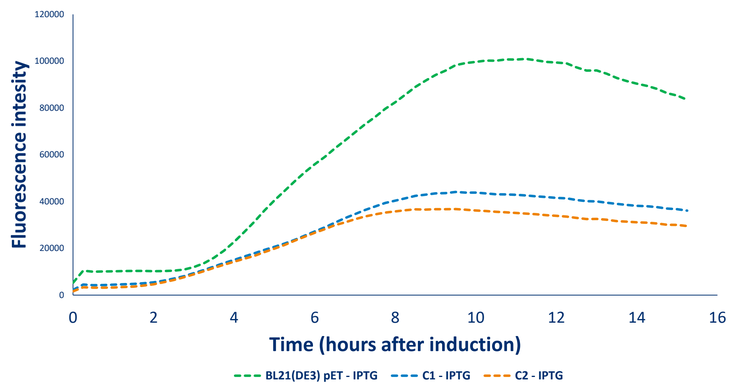


RESULTS

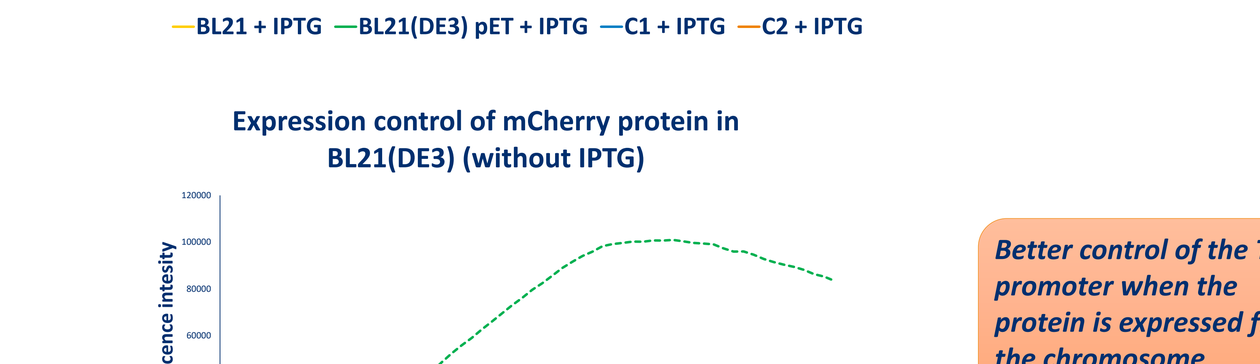


***Plasmid**: Fluorescence arises more rapidly but reaches a plateau also more rapidly (6h)*

Chromosome**: fluorescence continues rising after 6 hours, **more reproducible results



Better control of the T7 promoter when the protein is expressed from the chromosome



12% acrylamide SDS-PAGE analysis at different time points after induction with IPTG

Plasmid production starts earlier but decreases after 4.5 hours. Plasmid loss ?

After 20hrs, expression from the chromosome is higher than from plasmid, especially for the target C2

CONCLUSIONS: ADVANTAGES OF CHROMOSOMAL EXPRESSION VERSUS PLASMID EXPRESSION

- Better expression of the recombinant protein (quantity and quality), even when the gene is in a single copy.
- No expression decrease due to plasmid loss.
- No antibiotic needed
- Better control of T7 promoter
- Better reproducibility