BGene

Customizing Microorganisms

BELT21—an IP free strain for recombinant protein expression

BELT21 is a free-to-operate *E.coli* BL21 derivative, able to express proteins under the control of a T7 promoter.

The T7 expression system is widely used for the expression of recombinant proteins, which is an important issue in industrial biotechnology or scientific research. T7 expression system has been developped by Studier and Moffat in 1986 based on the expression of the T7 *gene 1*, coding for an RNA polymerase of bacteriophage T7, which is a very active polymerase specific to promoter T7. The transcription of this gene is controlled by *lac*UV5 promoter allowing repression by lacI and induction with isopropyo-b-D-1-thoigalactopyranoside (IPTG).

BL21(DE3) was originally constructed by infection of *E. coli* BL21 by a lambda phage (Studier and Moffatt, 1986) itself containing the T7 system cloned from the T7 phage (Davanloo, 1984).

Several reports mention that this construction could be unstable through spontaneous lysogenic induction (Rozanov, 1998, http://blog.gtptech.com/article/lysis-of-e-coli-expression-strain-by-spontaneous-excision-of-de3-prophage-318), encouraging BGene to develop an equivalent stable strain.

Construction of BELT21

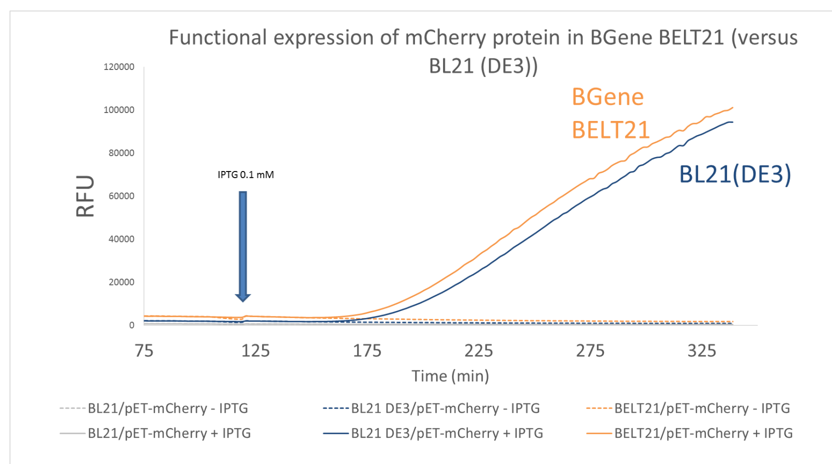
The starting material were the free-to-operate BL21 strain, and the T7 expression system, for which all patents are now expired.

BELT21 was constructed by genomic integration of a synthetic T7RNA polymerase gene into the chromosome of *Escherichia coli* BL21 strain. The T7RNA polymerase gene is regulated by the lacUV5 promoter, also synthesized and inserted.

Insertion of the *lacI* repressor gene downstream of the placUV5 was performed to obtain a perfect regulation of the expression of the T7RNA polymerase gene.

Control of the strain performance

The expression efficiency of a target gene was assessed by monitoring fluorescence intensity during expression of a fluorescent protein cloned into a pET plasmid, and shows that BELT21 is identical to BL21 (DE3) in terms of target gene expression.



Strain genotype

E. coli B, F-, *ompT*, *lon*, *hsdS* (r_{B-}, m_{B-}), *gal*, *dcm*, Δ(λ*B prophage), [*lacI* lacUV5-*T7]*

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