MANIPULATION OF DNA REPLICATION INITIATION IN *ESCHERICHIA COLI* BY CRISPR-CAS

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Introduction. One of the most important characteristics of DNA is the ability to replicate. DNA replication is a complex process, which includes a group of enzymatic reactions; this process is carried out in eukaryotic and prokaryotic cells[1]. DnaA and ori*C* are indispensable to initiate this process.
CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) is a region of inverted repeat sequences of approximately 33 pb associated to Cas proteins. Short sequences of foreign DNA are included in the CRISPRs array in the host genome. These sequences are transcribed to PrecrRNA and processed with Cas specific proteins to crRNA. When the foreign DNA (plasmid or viral) enters into the cell, it is recognized by Cas proteins carrying the corresponding CRISPR and leads to the destruction of this foreign genome[2].
The lambda phage specific integrative recombination is an event mediated by the integrase (Int) and the integration host factor (IHF). The integration of lambda DNA is effected by this site-specific recombinase through the recombination of site attP in the phage DNA and site attB in the *E. coli* chromosome[3].
It will be designed a control system to stop the replication initiation of the *E. coli* chromosome using the immune CRISPR-Cas system. This will be integrated on the chromosome by the integration system of lambda phage. This approach will benefit the production of DNA vaccines and increase the production of plasmids.

Methods. Based on the genome of *E.coli* K-12 the CRISPR synthetic gene was designed. Small random sequences of 33 pb from *dnaA* and ori*C* were included as spacers. Cas proteins were amplified from *E. coli* K-12 and joined to the synthetic CRISPR region.
The expression of CRISPR-Cas depends on pBAD promoter that only works in the presence of arabinose. The specific insertion of this system into the *E. coli* chromosome will be carried out by specific recombination with a synthetic attP.

Results. The CRISPR synthetic gene was designed with random fragments of *dnaA* and ori*C* (Fig. 1).

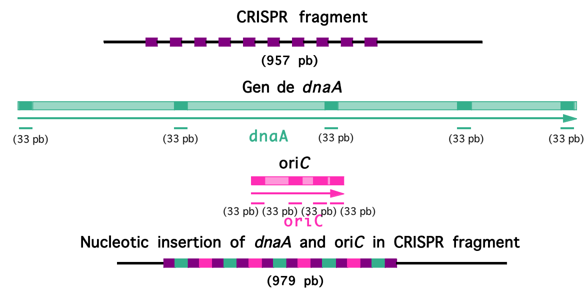

Fig.1 Design of CRISPR fragment that it has segments of 33 pb of *dnaA* and ori*C*.

Final construction of Control System, which has attP synthetic gene allowing the site-specific recombination, the CRISPR synthetic gene and Cas proteins (Fig. 2).

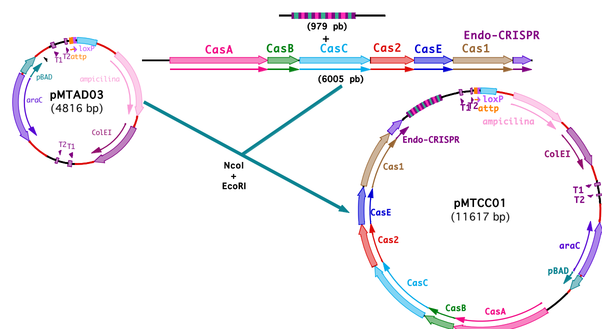

Fig.2 Final Control System designed in base of the bacterial immune system CRISPR-Cas.

Conclusions. A system to avoid the initiation of the chromosomal DNA replication in *E. coli* was designed and constructed.

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References.

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