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Poster Presentation



# ColE1 derived RNA I as a key molecule in a novel antibiotic free plasmid addiction system

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## Background

Today there are various plasmid selection systems available, whereas the most common approach comprises the use of antibiotic resistance genes and the addition of the corresponding drug into the medium. Bacterial vectors carrying a ColE1-type origin, e.g. pBR322, the pET-series [1] or the pUC-vectors are the most commonly used plasmids for recombinant protein production. For the application of plasmid DNA in gene therapy or DNA vaccination antibiotic resistance genes on the plasmid backbone are highly unwanted. To avoid these resistance genes and other additional sequences that can be used for plasmid maintenance, we intend to exploit the plasmid replication machinery in a novel plasmid addiction system. The mechanism is based on RNA/RNA antisense interaction involving the naturally occurring RNA I derived from the plasmid's origin of replication (see Figure 1). The plasmid replicational regulatory network consisting of RNA I/RNA II and their impact on Plasmid Copy Number (PCN) was linked to the transcription of an regulatory target gene, present on the bacterial chromosome.

Consequently, the strategy presented in this work can be applied for conventional protein expression vectors as well as for therapeutical plasmids.

## Results

In our experiments we found that RNA I was capable of silencing engineered target genes by RNA antisense reaction completely. On the mRNA transcript of the reporter gene *gfp* (green fluorescent protein) RNA II-like sequences were added near the ribosomal binding site. Thus, suppression of a chromosomally inserted *gfp* gene could be achieved by mere presence of the ColE1-type plasmid pBR322 in shaker flask experiments and fed-batch fermentation processes.

Conditionally lethal bacterial hosts were created by inserting an inducible promoter on the genome in front of an essential gene, e.g. *murA* [2] by homologous recombination [3]. By integration of an IPTG inducible expression cassette on the bacterial genome containing the tet-repressor gene (*tetR*), we were able to inhibit growth upon addition of IPTG, when the essential gene was set under control of the corresponding promoter, pLtetO [4].

For the design of engineered hosts depending on a ColE1-type plasmid in the cell the *tetR* transcript was combined with RNA I complementary sequences. Thus, the plasmid's replication machinery provides an essential advantage to the host cells.

## Conclusion

Basically, the discrete reactions of the plasmid addiction system were proven to be functional. We demonstrated that the regulatory mechanism of ColE1 plasmid replication is a useful tool for gene silencing of a designed target gene. Moreover, the essential gene *murA* was shown to be an efficient target for the selection system.

The strategy of regulating gene expression by plasmid replication implicates a novel strategy for plasmid selection in recombinant protein production processes and for gene therapeutic applications.

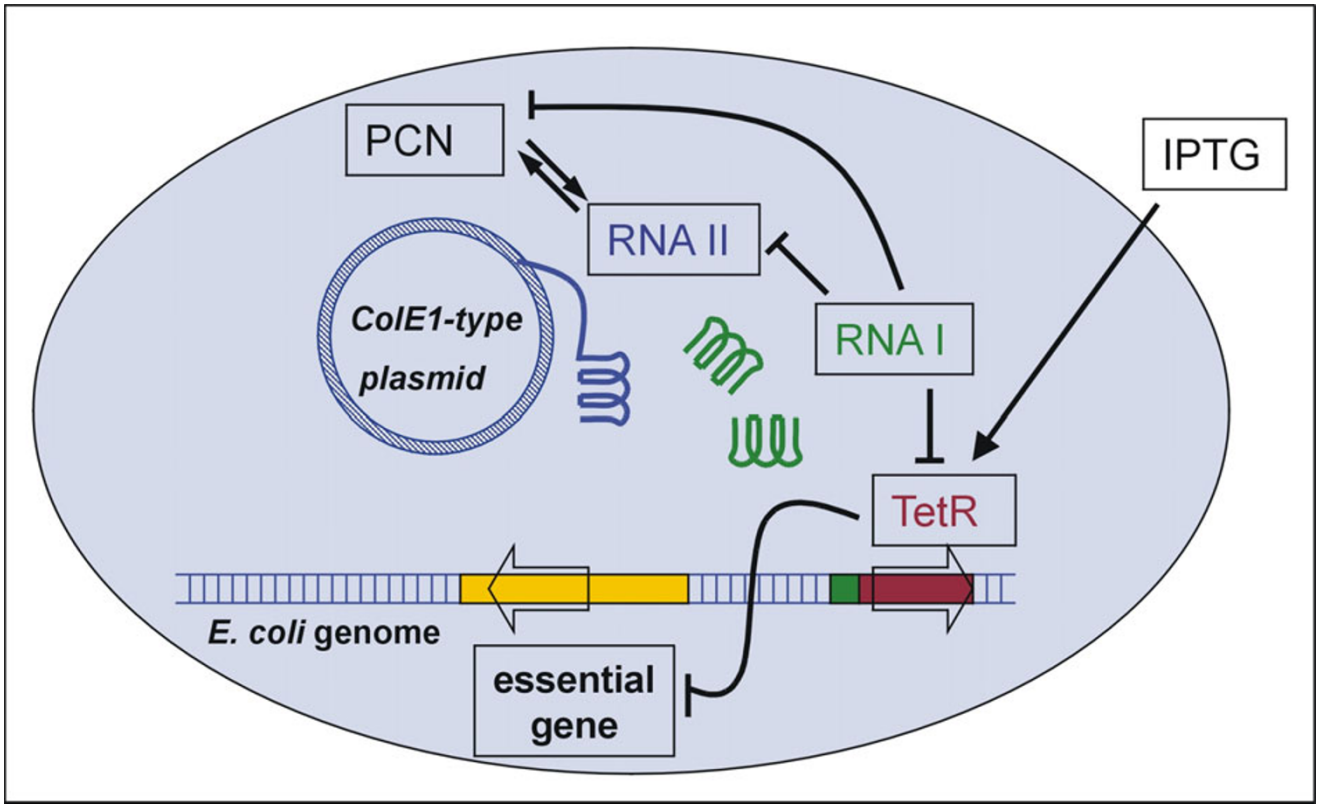


**Figure 1**
Concept of RNA I mediated plasmid addiction system: The ColE1-type plasmid indicated in Figure 1 can be a common protein expression vector or a therapeutical plasmid.

## Acknowledgements
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