

Technology Offer

A novel super-tight doxycyclin-regulatable episomal "one plasmid" vector

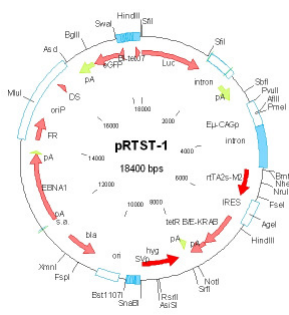
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TO 01-00453

The Challenge

The inducible expression of genes is very important for several scientific and industrial applications, such as the efficient production of proteins, and the evaluation of gene function and regulatory mechanisms. The so-called "tet-system" is based on bacterial tetracyclin-inducible promoters and trans-acting tetracycline-dependent transactivator or repressor proteins. It allows the control of gene expression in eukaryotic systems via application of small molecules, such as tetracycline or analogues (e.g. doxycycline). So far, the "leakiness" of the systems is problematic, often leading to basal expression in the absence of tetracycline.

The Technology

A novel doxycycline-inducible vector (pRTST-1) was constructed. It consists of all the elements needed for tetracycline-inducible gene expression on **one** EBV-derived episomally replicating plasmid. A bicistronic expression cassette drives simultaneous expression of an optimized reverse transactivator and a tet-repressor-KRAB fusion protein acting as a repressor in eucaryotic cells. The vector is characterized by (i) its very low background activity in the absence, (ii) its high inducibility in the presence of doxycycline, and (iii) its graded response to increasing doxycycline or tetracycline concentrations. Tight regulation is achieved by



binding of the repressor to the doxycycline-regulated bidirectional promoter in the absence of doxycycline, and combined relief of repression and binding of the reverse transactivator in the presence of doxycycline. In human B lymphoma lines stably transfected with pRTST-1, background of EGFP and luciferase expression was invariably very low, whereas inducibility varied among different clones and reached levels of 10.000 to 140.000-fold in individual single cells clones.

Doxycycline-inducible plasmid pRTST-1 Source: Georg W. Bornkamm, GSF

Commercial Opportunity

The one-plasmid system pRTST-1 can be used for tightly controlled expression of genes in cells transiently or stably transfected with this episomal vector. There are several advantages of this plasmid system:

- very low background
- gradual inducibility, dependent on doxycycline-concentration
- possibility to express in parallel a second protein (e.g. for protein interaction studies) or a surrogate marker
- use of pRTST-1 as integrating vector (e.g. mouse fibroblasts)

Patent situation

No patent was filed.

Relevant publication

Bornkamm et al. (2005), NAR 33, e137; Hölzel et al. (2007), NAR 35, e17

Contact:

Dr. Hubert Mueller
Technology Manager
Ascension GmbH

T: +49 (0)89 318814-32
F: +49 (0)89 318814-20
E: mueller@ascension.de



Berlin
Braunschweig
Hamburg
Hannover
Munich

Ascension GmbH
Herzogstrasse 64
D-80803 Munich
Germany

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