

Title (en)

METHOD FOR IDENTIFYING AND INHIBITING FUNCTIONAL NUCLEIC ACID MOLECULES IN CELLS

Title (de)

VERFAHREN ZUR IDENTIFIZIERUNG UND INHIBIERUNG FUNKTIONALER NUKLEINSÄUREMOLEKÜLE IN ZELLEN

Title (fr)

PROCEDE D'IDENTIFICATION ET D'INHIBITION DE MOLECULES FONCTIONNELLES D'ACIDE NUCLEIQUE DANS DES CELLULES

Publication

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Application

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Abstract (en)

[origin: WO9927135A2] Two methodologies are provided: the first provides a means for rapidly and efficiently identifying essential and functional genes; and the second provides a means for obtaining biologically active nucleic molecules (ribozymes, EGSs, and antisense) which can be used to inactivate functional genes. In the first method, a library of EGSs is prepared based on all possible known compositions. In a preferred embodiment, the EGSs are twelve or thirteen-mers for targeting bacterial RNase to cleave a substrate. This library is added to the cells containing the genes to be screened, for example, E. coli. Those cells in which the EGS causes a loss of viability, or other phenotype, are identified. The EGS(s) responsible for the loss of viability are analyzed, and the resulting sequence information used to identify the gene within the known genomic sequences. In the second method, nucleotide molecules with optimal biological activity, for example, directing cleavage of a gene of interest by RNase P, are rapidly identified through the use of a vector including two reporter genes, the first in phase with the gene of interest, and the second as a control to verify that the vector is present in a cell or to aid in selection of cells containing the vector. Those cells where the gene of interest is cleaved by the functional oligonucleotide molecule can then be identified by reference to reporter gene 1. The responsible functional oligonucleotide molecules is then isolated and characterized. These methods provide powerful tools for identifying essential genes whose sequence is known only as part of a genome with unknown function, as well as means for identifying functional oligonucleotide molecules, useful as diagnostic reagents and therapeutics.

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