



Collectis announces the publication of a paper entitled “Computer design of obligate heterodimer meganucleases allows efficient cutting of custom DNA sequences” in *Nucleic Acids Research*

This publication describes the design of a new generation of engineered meganucleases

Biocitech, France, April 22nd 2008 - Collectis SA, the rational genome engineering company specializing in the production of meganuclease recombination systems and in meganuclease engineering, today announced the publication of a new paper in the high-profile *Nucleic Acids Research* journal (Fajardo-Sanchez et al., Computer design of obligate heterodimer meganucleases allows efficient cutting of custom DNA sequences, *Nucleic Acids Res.* 2008, Feb. 14th [Epub ahead of print]).

Researchers from the laboratory of Pr. Luis Serrano, formerly based at the European Molecular Biology Laboratory at Heidelberg (EMBL, Heidelberg, Germany) and now at Centro de Regulacion Genomica (CRG, Barcelona, Spain) succeeded in designing a novel generation of engineered meganucleases by computational means. Computational redesign of proteins relies on the use of software calculations of energy levels, in order to identify novel protein with altered binding or catalytic activities *in silico*. The laboratory of Pr. Luis Serrano, a world leader in the field, is conducting studies on meganucleases redesign, in collaboration with Collectis.

In this study, researcher from CRG modified two engineered meganucleases from Collectis' collection, in order to improve their specificity. Most engineered meganucleases so far are heterodimers derived from the dimeric I-CreI protein. Heterodimer formation is obtained by co-expression of two monomers in the targeted cell, and is actually associated with the formation of two homodimers recognizing different targets. This results in a loss of specificity, which can be solved only by the suppression of homodimer formation. To address this problem, CRG researchers have modified the protein-protein interface of the two proteins in order to abolish homodimerisation. This design could in principle be applied to every and any heterodimer produced by Collectis.

Pr. Luis Serrano, declared: « Protein design for research is already tough, but when doing it to obtain a product with possible industrial applications is a real challenge. Not only the designed proteins should behave as predicted in the test tube, they also should work « In vivo ». Thus special care should be taken not to affect any of the functional properties of the meganuclease targets, which makes the design more difficult. The combination of protein design with screening constitutes a very powerful tool to modify proteins and obtain new activities and specificities. Our collaboration with Collectis is a beautiful example of how this symbiosis could be achieved and shows how academy and industry can both benefit.»

In this work, the researchers focused on the redesign of the dimerization interface, i. e. on protein/protein interactions, but previous studies conducted by Pr. Luis Serrano' group, in collaboration with Collectis, had already shown the good predictive capabilities of FoldX for meganuclease/DNA interactions: the substrate of locally engineered I-CreI derivatives could be predicted relatively accurately (Arnould et al., 2006, *J. Mol. Biol.* 371:49-65). Ultimately, computational redesign could take a growing part in meganuclease overall redesign, replacing a large part of the current high throughput screening of meganucleases libraries with *in silico* screening.



Over the last decade, meganucleases have emerged as powerful tools for efficient and precise genome engineering. This technology is the world standard in gene targeting and is used to precisely substitute, delete, add or correct genetic sequences at a chosen location in any given genome. Meganuclease recombination systems (MRSs) address a wide range of applications spanning the fields of agricultural biotechnology, protein production and genomic research tools. However, meganucleases also provide new hope for novel therapeutic agents for curing monogenic inherited diseases and viral infections. The meganuclease technology as such and some of the principle uses of homologous recombination were discovered at Institut Pasteur in Paris, which then granted Collectis worldwide exclusive rights in 2000. Since then, Collectis has expanded the potential of this technology by developing meganucleases with tailored specificities, which are thus able to target selected genes in any given organism.

About Collectis

Collectis SA (www.collectis.com) is a world-leading company in genome engineering and genome surgery. The company is focused on developing and producing custom meganucleases for *in vivo* DNA surgery and also provides new tools for rational reverse genetics and targeted recombination. Collectis' products induce unique, site-directed, double-strand DNA breaks in a living cell and can be used in a wide range of biotechnological and therapeutic applications. To date, Collectis has entered into more than 48 deals on its genome engineering technologies with major players in the pharma, biotech and agrobiotech industries. Collectis is listed on the NYSE-Euronext Alternext market (ticker code: ALCLS). For more information on Collectis, visit our web site: www.collectis.com

About Collectis' technology

A meganuclease is a (protein) molecule that cuts DNA at a highly precise site on a chromosome. Once DNA is broken, it has to be repaired by the cell's natural endogenous maintenance systems. By providing a specifically engineered DNA molecule (called a repair matrix) which will be used as a template to repair the break, one can channel the repair pathway into an insertion, deletion or correction process. Thus, meganucleases can be used to trigger precise modification of specific genes in a variety of cells and organisms. By combining the meganuclease's capacity to cut DNA and DNA's ability to undergo repair, Collectis is creating new generations of products for a wide spectrum of applications - including human health, since many genetic diseases result from a single mutation in a specific gene. Meganucleases can specifically target this same gene. In parallel, a DNA repair matrix (prepared by Collectis and including a non-mutated copy of this gene) will be introduced into the cell. Upon cleavage by the meganuclease, the repair matrix will be used as a template to restore a correct gene. By erasing the mutation, gene correction addresses the very cause of the disease, rather than its consequences.

About Collectis' R&D policy

Collectis' policy is to foster research excellence in order to offer new solutions for genome engineering. To date, the major outcome of this effort has been the production of custom meganucleases that cleave genes of interest; this capability vastly expands the range of potential applications and is a prerequisite for therapeutic use. While the core activity (i.e. the protein engineering itself) is usually conducted solely by Collectis, upstream studies are often performed in collaboration with other major players in this field.

This effort has resulted in a growing body of know-how, of which part has been disclosed (after IP protection) in peer-reviewed journals in order to disseminate the company's achievements to a broad scientific audience. The publications listed below testify to Collectis' technical progress and its recognition by the scientific community.

Prieto J, et al. (2007) Generation and analysis of mesophilic variants of the thermostable archaeal I-Dmol homing endonuclease. *J Biol Chem.* 2007; Nov 12; ; 283(7):4364-74

Arnould S, et al. (2007) Engineered I-CreI derivatives cleaving sequences from the human XPC gene can induce highly efficient gene correction in mammalian cells. *J. Mol. Biol.* 371:49-65.

Prieto J et al. (2007) The C-terminal loop of the homing endonuclease I-CreI is essential for site recognition, DNA binding and cleavage. *Nucleic Acids Res.* 35:3262-71.

Paques F & Duchateau P (2007). Meganucleases and DNA double-strand break-induced recombination: perspectives for gene therapy. *Curr Gene Ther.* 7:49-66 (Review).

Smith J et al. (2006). A combinatorial approach to create artificial homing endonucleases cleaving chosen sequences. *Nucleic Acids Res* 34: e149.



Gouble A et al. (2006) Efficient in toto targeted recombination in mouse liver by meganuclease-induced double-strand break. *J Gene Med.* 8: 616-622.

Arnould S et al. (2006) Engineering of large numbers of highly specific homing endonucleases that induce recombination on novel DNA targets. *J. Mol. Biol.* 355:443-58

Chames P et al. (2005) In vivo selection of engineered homing endonucleases using double-strand break induced homologous recombination. *Nucleic Acids Res.* 33:e178.

Perez C et al. (2005) Factors affecting double-strand break-induced homologous recombination in mammalian cells. *Biotechniques.* 39:109-15.

Epinat JC et al. (2003) A novel engineered meganuclease induces homologous recombination in yeast and mammalian cells. *Nucleic Acids Res.* 31:2952-62.

Collectis' Forward-Looking Statements

This communication expressly or implicitly contains certain forward-looking statements concerning Collectis SA and its business. Such statements involve certain known and unknown risks, uncertainties and other factors, which could cause the actual results, financial condition, performance or achievements of Collectis SA to be materially different from any future results, performance or achievements expressed or implied by such forward-looking statements. Collectis SA is providing this communication as of this date and does not undertake to update any forward-looking statements contained herein as a result of new information, future events or otherwise.

For a discussion of risks and uncertainties which could cause actual results, financial condition, performance or achievements of Collectis SA to differ from those contained in the forward-looking statements please refer to the Risk Factors ("Facteurs de Risque") section of the prospectus approved by the French Autorité des Marchés Financiers ("AMF") on January 22nd, 2007 under visa number 07-023, available on the websites of the AMF (<http://www.amf-france.org>) and Collectis (<http://www.collectis.com>).

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