## SMP: RNAi

# Project: RNAi Research, Development and Service Unit

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## Introduction

Since its foundation in 1999 Cenix BioScience GmbH has focused on the genomic application of RNAi. Thus, having successfully applied the technology in genome scale screens for *C. elegans* and Drosophila cells and in larger panels of genes in Human cancer cells *(1-3),* Cenix has developed a unique expertise of handling all steps involved from dsRNA design and production to fully automated HT screening and data analysis *(4).* Moreover, along this process, Cenix has learned about all possible bottlenecks and recognized the essential steps that require careful quality control and process tracking. As this SMP's main industrial partner, Cenix will implement and standardize all newly developed advanced RNAi applications to establish a unique RNAi service platform able to serve NGFN laboratories. Thus members of the NGFN will be able to access high quality RNAi screens through this facility without investment in large-scale facilities themselves. This will provide a clear path for technology transfer within this SMP, and provide a standardized interface for RNAi-based screening within the NGFN.

The primary goal of the present subproject is to establish a service platform to provide access to advanced RNAi research capabilities to NGFN laboratories. This will make use of Cenix's existing infrastructures and expertise in this area, and will be achieved in two steps, as follows:

**Offering of RNAi research services already established at CENIX**

- Within the SMP, Cenix will provide its services to partners of the subprojects for design and testing of constructs to be used in the mouse, in order to ensure streamlined and standardized workflow for the *in vivo* RNAi applications.
- Through an alliance with Ambion Inc. (Texas, USA) for siRNA synthesis, Cenix offers mammalian cell based screening services with highly potent, highly specific siRNA reagents, designed by Cenix, at a very competitive pricing.
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**Integration of esiRNA/SPOT-RNAi screening capabilities within Cenix offerings to NGFN**

A new method to generate small interfering RNAs (esiRNAs) by enzymatic processing of larger dsRNAs (please see subproject 1 „RNAi in vitro development and production"), will be combined with the implementation of solid phase transfection arrays for cell based assays to generate of a novel cost effective HT screening platform. This platform, developed in subproject 2 „Genome-scale RNAi on cell chips" will be brought to industry standards at Cenix. This includes:

- Production of esiRNA Arrays
- Establishment of genome scale solid phase transfection screens in Human cells
- Automated data acquisition and analysis
- Development of a Laboratory Information Management System (LIMS) for cell arrays
- Further development of the Cenix database to integrate array screening data and enable export of RNAi data to customer's databases.

Once established, Cenix will offer this screening service to all NGFN partners.

## Project Status

This project has been funded since Jan 2005. In this first half year of funding, experimental and IT work packages have been defined and started through intensive exchange with the other platform partners.

**SPOT-RNAi cell arrays:**

During the first 18 months of the project, Cenix will implement and further develop the SPOT-RNAi (Solid Phase Optimized Transfection-RNAi) screening capabilities established by the partner labs at the EMBL (see subproject 2 „Genome-scale RNAi on cell chips"). To make this platform applicable in a variety of assays and cell lines, protocols involving different types of transfection reagents and multiple cell types will be developed. Initial experiments addressing these needs are on the way.

The RNAi reagents used for the initial testing are chemically synthesized siRNAs. As soon as validation and standardisation of production for esiRNAs (see „RNAi in vitro development and production") have been completed, arrays using esiRNAs can be produced and tested (anticipated for beginning of 2006).

Automated microscopy, already established at Cenix, is currently being adapted for array technology. Image analysis will initially be performed using rule sets available in the current Cenix set up. More advanced analysis algorithms, developed by the Eils group, will be implemented once they have passed initial testing at the EMBL laboratories.

After completion of the setup phase, Cenix will be able to take on the first screening projects for NGFN partners, which is anticipated for the second half of 2006.

### Construction and implementation of a Laboratory Information Management (LIM) System and definition of annotation and exchange standards for RNAi screening data

Making best use of already available expertise, Cenix is building a LIM-System to guarantee a fully streamlined screening process. Development work includes:

- Quality control for arrays
- Tracking of individual arrays and their content
- Handling of transfection experiments
- Handling of data acquisition
- Browsing and filtering of large data sets

A major aim of the SMP-RNAi is to develop standards for RNAi experimentation. Cenix as the service partner has commited to provide not only standardized screening services, but also to contribute in defining data annotation and exchange standards for RNAi experimentation, making use of its 5 years experience in RNAi informatics.

Once implemented, these data standards will on one hand serve Cenix as an exchange format for the dissemination of screening data to its clients, and on the other hand facilitate the construction of an RNAi data repository to be built by the SMP's subproject „Bioinformatics". In initial discussions with the Eils group heading this project, a basic structure for this database has been outlined. Bioinformaticans and programmers throughout the platform will closely cooperate to make this task most efficient and to guarantee user-friendly data dissemination of the project data to the NGFN partners.

## Outlook

HT-RNAi screening is the best tool available today for realising the full potential of the Human Genome Project, offering an efficient method of rapidly identifying, among the tens of thousands of genes, those which harbour therapeutic potential by better understanding their functions in the body. Furthermore, for more detailed analyses of loss of function phenotypes in vivo, RNAi also offers the promise of a new, faster, more cost-effective, and more versatile alternative to current transgenic mouse knock out technologies.

The new technologies to be developed, will allow basic life science and clinical research labs in Germany to exploit the whole range RNAi technology can offer, from genome scale studies in human cells to identify and characterize novel disease relevant drug targets, to detailed in vivo studies of those targets with maximal pathophysiological relevance in living mice.

The present project offers to integrate cutting edge academic expertise with biotech-based capabilities, generating ideal synergies to advance the field in a maximally cost-efficient manner, securing Germany's place at the forefront of the most active aspect of genomics research today. The project also provides a clear model for technology transfer from the basic research arena to the commercial sphere in a way that will benefit both academia and industry research by creating and/or strengthening research service offerings available to all.

*Lit.: **1**. Sönnichsen B et al. Full genome RNAi profiling of early embryogenesis in C. elegans. Nature. 2005 434: 462-69. **2**. Kiger A et al. A functional genomic analysis of cell morphology using RNA interference. J Biol. 2003 2:2. **3**. Pelkmans L et al. Genome-wide analysis of human kinases in clathrin- and caveolae/raft-mediated endocytosis. Nature. 2005 436:78-86. **4**. Sachse C et al. High throughput RNA interference strategies for target discovery and validation by using synthetic short interfering RNAs: functional genomics investigations of biological pathways. Methods in Enzymology. 2005 392:242-77.*