



2005 RNAi Report: A Revolutionary Technology

Biocompare Surveys and Reports

Published July 20, 2005

Table of Contents

I. Report Introduction.....	1
II. Market Overview.....	2
III. Survey Introduction.....	3
V. Conclusion/Recommendations.....	4
VI. Appendix I: Questionnaire.....	5
VIII. References.....	12

Report Introduction

The 2005 RNA interference (RNAi) Report contains a concise overview of the RNAi market, an introduction to the 2005 RNAi Survey, a comprehensive discussion of the survey results, as well as conclusions and recommendations that are based on both market analysis and survey data gathered from researchers in this area. The Market Overview focuses on the key applications for RNAi research in functional genomics: target validation, basic research, and therapeutics. The markets for each of these applications are growing rapidly and the Market Overview section provides their respective market forecasts for the next several years. Additionally, as RNAi drug development is currently in its infancy, these figures could likely skyrocket with successful clinical trials. The goals and methodology of the 2005 RNAi Survey are outlined in the Survey Introduction, with key findings from each survey question presented in the Discussion of Results. As this section comprises the bulk of the report, emphasis is placed on the specific applications identified in the Market Overview section. Additionally, important differences from the results of the 2004 RNAi: A Market Update Report are highlighted. Overall conclusions and recommendations regarding the current RNAi market are presented in the final section of the report. The detailed survey questionnaire, data, and tabulated results can be found in the appendices.

Market Overview

The prospect of using RNA interference (RNAi) for “functional genomics” studies has excited researchers worldwide. The ability to down-regulate protein levels in a gene specific manner makes RNAi one of the most exciting technologies around. The truth is, however, that the technique isn’t as simple as logic would have it. Methods for boosting transfection rates still need improvement. And for therapeutic purposes, RNAi faces the big pharmacokinetic challenge of delivery.

“The market for RNAi is, however, set to witness rapid initial growth because of its speedy adoption by researchers and for drug target validation by pharmaceutical and biotechnology companies,” said Dr. Raju Adhikari, a pharmaceutical and biotechnology analyst at Frost and Sullivan.

Very telling is \$48 million of revenue that the worldwide RNAi market generated in 2003, according to Frost & Sullivan. (1) That figure may rise to \$328 million by 2010, representing a CAGR of 31.5%. These figures factor in revenue from the sales of RNA oligonucleotides, vector-based siRNA, therapeutics and contract services.

Of the three application markets - target validation, basic research and therapeutics - target validation will be the most common use for RNAi for the foreseeable future, according to Frost & Sullivan. (2) In 2003, target validation efforts accounted for \$22.8 million of RNAi revenue in 2003. That figure could climb to \$146.4 million, representing nearly 50% of total RNAi revenues in 2010. Basic research and therapeutics, the other two applications, will generate \$97.6 million and \$56 million, respectively.

But other analysts forecast the RNAi market to be much larger. According to Select Biosciences, drug discovery efforts – which overlap with work in basic research and target validation – will generate \$400 million this year. By 2010, the RNAi market may rise to \$850 million and up to \$1.2 billion by 2015. (3) The future also promises healthy revenues for RNAi-based drugs, according to Select Biosciences. (3) In 2010, these drugs may bring in \$3.5 billion. By 2015, RNAi-based drugs will generate revenues of \$5.9 billion.

The wide variations on the estimates on revenues from RNAi-based drugs reflect the uncertainty of clinical trial results, which are just now getting off the ground. “The RNAi therapeutic market will grow to over \$10 billion in 2014 if the first few RNAi therapies in development are successful in human clinical trials,” said Zachary Zimmerman, senior research analyst at Life Science Insights.

Such optimism may well be rewarded. But gaining the prize means overcoming the many drug development challenges of pharmacokinetics, stability, delivery, assay development and toxicology. RNA is not that simple, after all.

Survey Introduction

The 2005 RNAi Survey is intended to help vendors of RNAi supplies and reagents gain a better understanding of how they fare in the RNAi marketplace. Data were gathered from questions regarding the techniques that customers are using for RNAi studies, what types of samples are used and how they are prepared, which systems, kits, and reagents they work with and plan to purchase, the important qualities of these products and their needed improvements, which companies they most frequently purchase from, and the importance of analysis software packages in their research. With this information, vendors will be better positioned to capitalize on the ever-changing, multi-million dollar RNAi market, primarily in terms of new product development for identified trends in RNAi research applications.

The 2005 RNAi Survey consisted of 38 questions. Of these, 2 were open-ended questions. Eighteen of the closed-ended questions included "other" as an answer choice, providing an opportunity for survey participants to compose a more appropriate answer to the particular question that was asked. Demographic information was obtained from answers to 6 of the survey questions and from the addresses submitted by survey participants. The survey was administered on-line from June 13th-17th, 2005 and the data tabulated and prepared for dissemination.

Conclusions and Recommendations

The data gathered from the 2005 RNAi Survey indicate that RNAi investigators are making use of a wide variety of kits and reagents, without consistent customer allegiance to a particular vendor. Clearly, the sweeping changes in the ranking of top vendors for these products over last year suggest the need for suppliers to pay attention to the latest scientific trends and to listen to the ever-changing needs of their customers. As Invitrogen was the clear favorite among RNAi companies in almost every category in which vendors were listed this year, the field is wide-open in terms of buyer loyalty. Indeed, suppliers who pay attention to both customer technical service and the education of their buyers will undoubtedly come out ahead.

The direction of RNAi research has shifted over the past year to more applied preclinical and therapeutic applications, a significant, but subtle, change. As seen from the doubling of RNAi researchers using RNAi for therapeutics, such a shift follows the three application markets described in the Market Overview. As the RNAi market is expected to double in the next 5 years, with RNAi-based drugs on the fast track, forecast figures will likely exceed expectations if the first RNAi therapeutics currently in development are successful in clinical trials.

Many challenges exist for the RNA community. While comprised of optimistic investors and innovative scientists, a win-win combination is certain if past trends are any indication. Advances in vector-based siRNA, miRNA, modified siRNA, and shRNA, viewed as trends in this exciting research area by survey participants, will likely raise expectations on investment returns for the foreseeable future.

Appendix I: Questionnaire

In what type of institution do you work?

- Private Research
- Government
- Biotech
- Pharmaceutical
- Clinical Diagnostic Testing
- Academic
- Other

Which title best applies?

- Professor/Instructor
- Process Engineer
- Business Development Director/Manager
- Research Director/VP of Research
- Department Head
- Technician/Research Assistant
- Account Manager
- Graduate Student
- Staff Scientist
- Principal Investigator
- President/CEO/Owner/VP
- Lab Director/Chief Scientist
- Postdoctoral Fellow
- Procurement Manager
- Consultant
- Other

Which best describes your purchasing authority?

- Authorize
- Recommend
- Evaluate
- No Purchase Role

Are you planning to start a new lab?

- Yes: Within 0 – 3 months
- Yes: Within 3 – 6 months
- Yes: Within 6 – 9 months
- Yes: Within 9 – 12 months
- Yes: In more than 12 months
- No

What is your principle area of research or work? (check all that apply)

- Bioinformatics
- Microbiology/Virology
- Immunology
- Genomics/Genetics
- Cell Biology
- Diagnostics/Pathology
- Drug Discovery
- Administration
- Biochemistry
- Marketing/Sales
- Pharmacology/Toxicology
- Molecular Biology
- Bioengineering
- Neuroscience
- Proteomics
- Purchasing
- None of the Above
- Other (please specify)

Please characterize your siRNA research.

- I am currently using siRNA in my research.
- I plan to use siRNA within 3 months.
- I plan to use siRNA within 6 months.
- I plan to use siRNA within 12 months.
- I do not work or plan to work with siRNA. (Exited from survey)

What is the goal of your RNAi research?

- Therapeutics
- Drug development
- Target identification (Screening)
- Target validation
- Functional genomics
- Basic research
- Kit development
- Other (please specify)

What species do you use in your siRNA experiments? (check all that apply)

- Rat
- Mouse
- *C. elegans*
- Human
- Zebrafish
- *Drosophila melanogaster*
- Plant
- Other (please specify)

Which of the following RNAi reagents, kits and services do you use in your lab? (check all that apply)

- Manufacturer-designed Single siRNA
- Custom-designed Single siRNA
- Manufacturer-designed siRNA Pools
- Custom-designed siRNA Pools
- siRNA Libraries
- Lipid-mediated Deliver Kits
- Antibody Specific to a siRNA Target
- Chemically Modified siRNAs
- Kits for preparing individual siRNAs by in vitro transcription
- Kits for preparing siRNA populations by Dicer
- Kits for preparing siRNA populations by RNAase III
- siRNA Expression Vectors – Plasmids
- siRNA Expression Vectors – Empty Viral Vectors
- siRNA Expression Template Kits – PCR-based
- Morpholinos for gene knockdown
- Electroporation buffers or kits

Where do you think improvements need to be made on siRNA kits and reagents? (check all that apply)

- Improve software for Identifying siRNA Oligos.
- Increase Transfection Efficiency
- Reduce Delivery Reagent Toxicity
- Increase siRNA Silencing Efficiency
- Reduce Off-Target Effects
- Increase siRNA Nuclease Resistance
- Increase Number or Type of RNAi Applications
- Other (please specify)

Where do you see the trends in the siRNA market? (check all that apply)

- microRNA
- Vector-based siRNA
- shRNA
- Modified siRNA
- Single-stranded siRNA
- Different siRNA Lengths

How do you measure gene silencing efficiency in your RNAi experiments? (check all that apply)

- Reporter gene assays (e.g. Luciferase)
- Quantitative RT-PCR/Real-Time quantitative PCR
- Northern blot
- Branched DNA (b-DNA)
- Western Blotting
- ELISA
- Microarray
- Other (please specify)

How many gene targets are you currently focusing on or planning to focus on within the next twelve months?

- Less than 10
- 11 – 100
- 101 – 500
- 500 +

Do you use RNAi starter kits?

- Yes
- No (skip next question)

Which supplier(s) of siRNA starter kits do you use? (check all that apply)

- Dharmacon
- Upstate
- Invitrogen
- Qiagen
- Other (please specify)

How do you typically generate your siRNA?

- In Vitro transcription
- Dicer/RNase III
- Expression in Cells from a siRNA/shRNA Expression Plasmid or Viral Vector
- Expression in Cells from a PCR-derived siRNA Expression Cassette
- Synthetic - from a Commercial Supplier
- Synthetic - Made In-house
- Other (please specify)

Which supplier(s) of synthetic siRNA do you use? (check all that apply)

- Ambion
- Bioneer
- B-Bridge
- Integrated DNA Technologies
- Dharmacon
- Qiagen
- Invitrogen
- OligoEngine
- Thermo Electron
- Proligo
- Eurogentec
- MWG
- Do Not Use Synthetic siRNA
- Other (please specify)

How do you prefer to buy your synthetic siRNA? (check all that apply)

- Annealed
- Single-stranded
- With modifications
- 96-well plate
- 24-well plate
- Packaged in Vials
- Libraries
- Do Not Use Synthetic siRNA
- Other (please specify)

Please rank the following siRNA features from 1 to 5 on a scale of importance to your research. (1 = Extremely important, 5 = Not at all important)

- Affordability
- Purity
- Quality documented by mass spectrometry
- Ready to use (i.e. no Desalting, Deprotecting, or Annealing)
- Timely Delivery

How many siRNA oligos do you plan to purchase over the next six months?

- 1 – 5
- 6 – 20
- 21 – 100
- 101 – 1000
- 1000 +
- None

Which of the following supplier(s) software do you use to design your siRNA? (check all that apply)

- Promega
- Mirus Bio
- Imgenex
- Qiagen
- MWG
- Invitrogen
- Dharmacon
- Genscript
- OligoEngine
- InvivoGen
- I Buy Manufacturer-designed siRNA
- Other (please specify)

Do you use proprietary software to design your siRNA?

- Yes
- No (skip next question)

Which supplier(s) proprietary software do you use? (open-ended)

Have you considered using advanced antisense oligos (e.g. Morpholinos) to avoid common siRNA problems (such as changing expression of non-targeted genes or triggering an interferon response)?

- I prefer to use siRNAs because they are widely used for gene knockdown
- I am unaware of the advantages of advanced antisense oligos for gene knockdown
- I am already using advanced antisense oligos for gene knockdown
- I am not using gene knockdown in my research

Which supplier(s) of siRNA construction kits do you use? (check all that apply)

- | | | |
|--------------|-------------|--------------------------------------|
| - Invitrogen | - NEB | - Do Not Use siRNA Construction Kits |
| - Imgenex | - Promega | - Other (please specify) |
| - Ambion | - Genlantis | |

Which supplier(s) of transfection kits or reagents do you use? (check all that apply)

- | | | |
|---------------------------|-------------------------|--------------------------------------|
| - Ambion | - Invitrogen | - New England Biolabs (NEB) |
| - BD Biosciences Clontech | - Mirus Bio | - Upstate |
| - B-Bridge International | - GenoSpectra | - Open Biosystems |
| - Bio-Rad | - OligoEngine | - Stratagene |
| - Imgenex | - Promega | - Ozbiosciences |
| - Novagen | - Dharmacon | - QBiogene |
| - Stratagene | - Roche Applied Science | - Do Not Use siRNA Transfection Kits |
| - Qiagen | - Thermo Electron | - Other (please specify) |
| - Genlantis | - Pepscan | |

Please rate the following siRNA transfection reagent features from 1 to 5 on a scale of importance to your research. (1 = Extremely important, 5 = Not at all important)

- Efficient siRNA Delivery in a Single Cell Line (high percentage of transfected cells)
- Efficient siRNA Delivery to a Variety of Different Cell Lines
- Efficient Silencing of Endogenous Gene (High Level of Knockdown)
- Reproducible Cellular Delivery
- Cell Viability
- Ease to Use Protocol
- Works in the Presence of Serum
- Price
- Effective at Multiple Cell Densities
- Effective at Multiple siRNA Concentrations

Into what cell type(s) are you transfecting siRNA? (check all that apply)

- Epithelial-like cells (HeLa, CaCo2)
- Fibroblast-like cells (HEK 293, Cos-7)
- Endothelial-like cells (HUVEC, BAEC)
- Hepatocyte-like cells (HEPA-1, HepG-2)
- Neuroblastoma (CLBPEC, SHEP)
- Leukemia cells/Lymphoblasts (Jurkat, K562)
- Melanoma
- Monocytes/macrophages
- Myotubes/myoblasts/muscle cells
- Keratinocytes
- Primary cells
- Other (please specify)

How do you typically transfect siRNA into cells?

- Traditional transfection procedure
- Reverse transfection procedure
- Electroporation
- Other (please specify)

Which supplier(s) of siRNA/shRNA expression vectors do you use? (check all that apply)

- Ambion
- BD Biosciences Clontech
- Imgenex
- Invitrogen
- Promega
- Stratagene
- OligoGene
- Mirus Bio
- InvivoGen
- GenScript
- Genlantis
- Upstate
- OligoEngine
- Open Biosystems
- Origene
- Epicentre
- Abgent
- Biovision
- I use a vector developed in my lab or by a colleague
- Other (Please specify)
- I do not use siRNA expression vectors
- Other (please specify)

Please rate the following siRNA company attributes from 1 to 5 on a scale of importance to you. (1 = Extremely important, 5 = Not at all important)

- Helpful Technical Support
- Offers Educational Materials
- Offers only siRNA Products
- Offers siRNA and Other Life Science Research Products
- Knowledgeable Sales Staff

If you were to purchase a siRNA library, how important would each of the following be in your decision?

- Delivery/Manufacture Time
- Price
- Customer-defined Number and Type of Gene Targets
- Manufacturer-defined Number and Type of Gene Targets
- Format Options (Packaged in vials or pre-dispensed in a plate)

Are you currently studying microRNA (miRNA)? If so, for how long?

- Yes: For less than 3 months
- Yes: Between 3 – 6 months
- Yes: Between 6 – 12 months
- Yes: For over 12 months
- No: But plan to in the next 12 months
- No: Do not plan to at all (skip next question)

What microRNA product(s) do you currently use or plan to use in the next twelve months? (check all that apply)

- Synthetic miRNA
- miRNA Inhibitors
- miRNA Detection Products
- miRNA Purification Products
- miRNA Microarrays
- Other (please specify)

If viral delivery of shRNA was gene target-specific, safe, easy and efficient would you prefer it over using a transfection reagent and synthetic siRNA?

- Yes
- No

If an efficient system to regulate the expression of shRNA in stably transfected cells were available, would you use it in your research?

- Yes
- No

If you could have an inducible siRNA that enables each of the following which TWO would be MOST useful to your research?

- Decoupling the transfection event and gene silencing
- Inducing cells with all compounds after transfection but without any gene silencing effect
- Activating siRNA as desired
- Having a real-time control for your siRNA
- Observing the desired phenotype only after at-will activation of siRNA
- Controlling the amount of gene knockdown
- Observing the effect of gene silencing only after at-will activation of siRNA

What RNAi product or service, currently not available commercially, would be help facilitate your RNA research? (open-ended)

References

1. "World RNAi Markets," Frost & Sullivan, July 2004.
2. "World RNAi Markets," Frost & Sullivan, July 2004.
3. "RNAi: Technologies, Markets and Companies," Select Biosciences, May 2005
4. Fire, A., S. Xu, M.K. Montgomery, S.A. Kostas, S.E. Driver, and C.C. Mello. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391: 806-811
5. Vionnet, O., P. Vain, S. Angell, and D.C. Baulcombe. 1998. Systemic spread of sequence-specific transgene RNA degradation in plants is initiated by localized introduction of ectopic promoterless DNA. *Cell* 95: 177-187
6. Waterhouse, P.M., M.W. Graham, and M.-B. Wang. 1998. Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. *Proc. Natl. Acad. Sci.* 95: 13959-13964