

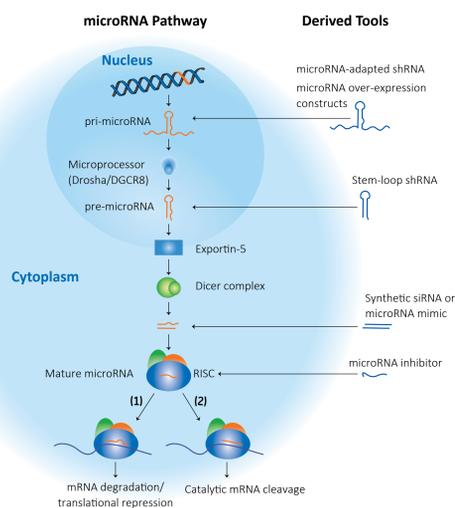
A new method for generating arrayed RNAi screening tools for any organism

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Abstract

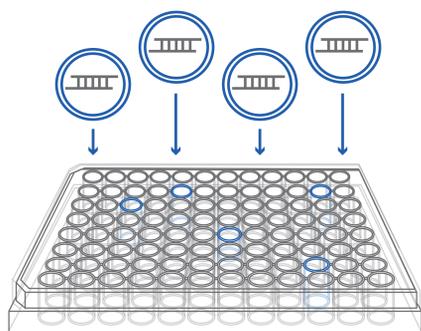
RNA interference (RNAi) using small interfering RNAs (siRNAs) is an important technology for down-regulation of gene expression and a powerful tool to study cellular processes and pathways. Previously, large collections of siRNAs were available only for traditional experimental model systems, such as human and mouse, and predominantly provided as chemically synthesized libraries. For studies in alternate species, siRNAs needed to be custom synthesized at a relatively high cost and very long manufacturing time. To improve the accessibility of these large screening libraries to researchers working in non-human or mouse model systems, we have recently developed a new cost-effective enzymatic manufacturing method to generate large collections of siRNAs, called Dharmacon™ Zoonome™ siRNAs (z-siRNAs). Like other well-established siRNA product lines, they are designed using the proprietary SMARTselection design algorithm for potent and specific gene expression knockdown, but are manufactured on-demand using a novel enzymatic method. Now available are customized arrayed collections of pools of four z-siRNAs to each gene target for screening hundreds or thousands of genes for any organism with an annotated genome. Here we show successful gene knockdown in *Cricetulus griseus* (Chinese hamster) and *Bos taurus* (bovine) cell lines using Zoonome siRNAs. With this new availability of large screening libraries, the power of unbiased functional screening in nearly any species can accelerate findings important to animal health, food safety, and infectious disease.

Harnessing the endogenous microRNA pathway



A schematic of the endogenous microRNA pathway, with points of entry for the three categories of RNAi tools. The endogenous microRNA pathway begins with genomic DNA transcription to produce pri-miRNA, which is then processed by the microprocessor complex containing the DROSHA protein and other proteins to a pre-miRNA hairpin structure and exported from the nucleus by Exportin-5. In the cytoplasm, the pre-miRNA is further processed by the Dicer complex to double-stranded anti-parallel RNA. One strand of this RNA duplex will load into the RISC complex and act to target a mRNA transcript for down-regulation by either (1) seed-mediated mRNA translational repression or (2) catalytic mRNA cleavage mediated by high sequence complementarity. The blue molecules to the right of the image illustrate different classes of RNAi research tools and where they enter the endogenous RNAi pathway.

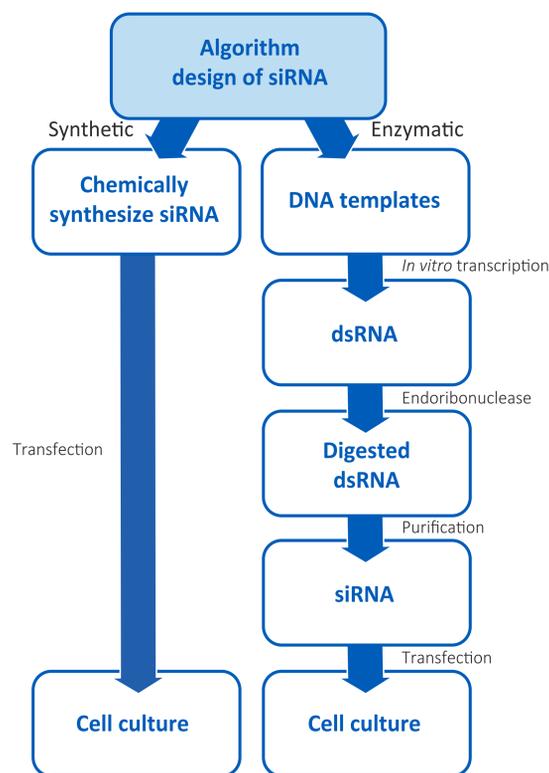
siRNA library screening for unbiased discovery



Screening with arrayed collections of siRNAs, targeting a particular gene family or a whole genome, provides a powerful, systematic approach to silencing many genes in one large experiment for unbiased functional analysis. The one-gene-per-well approach enables high content assays for the study of more complex phenotypes. siRNA libraries have become a standard tool for discovery efforts for the following interests:

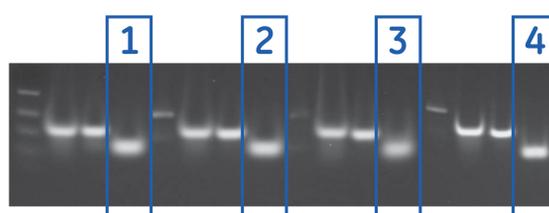
- Pathway analysis
- Drug resistance
- Disease progression
- Host/pathogen studies

An alternative to chemical synthesis for manufacture of siRNA



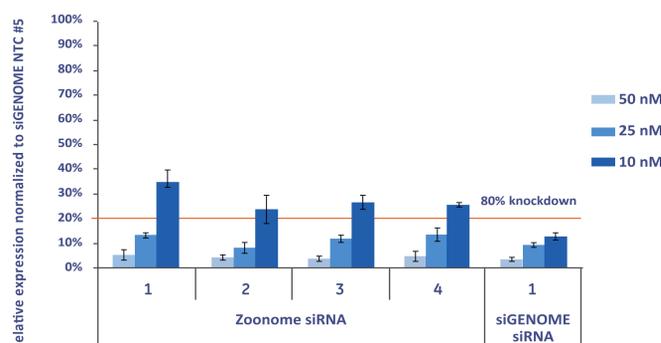
Although enzymatic methods require more processing steps, they can be carried out at smaller scales than chemical synthesis.

Zoonome siRNA generation produces reliable 21 mer duplexes



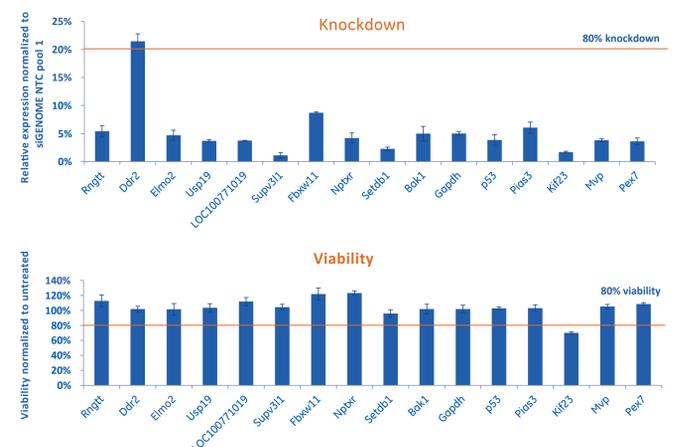
Zoonome siRNA pools sampled throughout the production workflow for four different genes were separated on a 4% agarose gel. Boxes outline the Zoonome siRNA 21 mer duplex.

Zoonome siRNA pools function similarly to synthetic siRNA in bovine cells



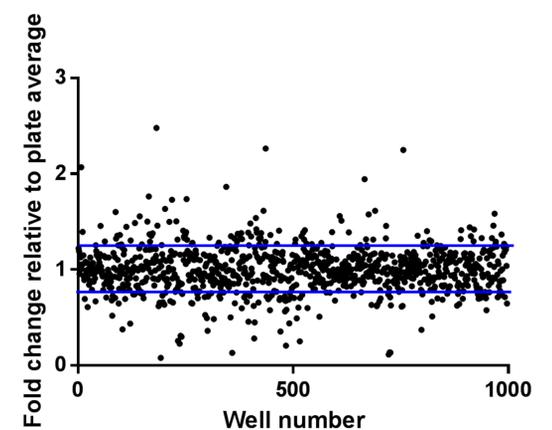
Side-by-side lipid-mediated transfection of four Zoonome siRNA pools and a synthetically generated siRNA pool targeting PPIB at three concentrations in MDBK cells. The cells were harvested 24 hours post-transfection for total RNA to determine mRNA expression by RT-qPCR for knockdown.

Functionality of Zoonome siRNA pools in CHO-K1 cells



Lipid-mediated transfection of Zoonome siRNA pools (25 nM) for sixteen different genes in CHO-K1 cells. The cells were assayed 48 hours post-transfection for viability by Resazurin colorimetric assay and harvested for total RNA to determine mRNA expression by RT-qPCR for knockdown.

Zoonome screening data in mosquito cells



Arrayed Zoonome siRNA screen performed in *Aedes aegypti* Aag2 cells. The siRNA were transfected at 50nM and a virus of interest was applied after transfection. Data was collected using a luciferase reporter encoded within the virus to show which genes influence replication of the virus. The blue lines indicate 1 standard deviation from the mean—dots above and below indicate genes that are considered significant hits. (Data provided by a collaborator).

Conclusions

- RNAi using siRNAs, especially in arrayed screening libraries, is a powerful tool to study gene regulation and function in cellular processes and pathways.
- The Zoonome siRNA library manufacturing method is a cost-effective way to enzymatically generate customized, on-demand, large collections of siRNAs, which offers a solution for large-scale RNAi studies in organisms other than human and mouse.
- Zoonome siRNA pools demonstrate potent knockdown with similar functionality compared to their synthetic siRNA counterparts.