Docket #: S02-028

Recombinant Dicer efficiently converts large dsRNAs into siRNAs suitable for gene silencing

Applications

- · The loss-of-function phenotype of any gene in many mammalian cell culture models and vertebrate animal systems can be determined using d-siRNAs.
- · Large-scale, high-throughput functional screening can be accomplished using d-siRNAs.
- · d-siRNAs can be used in a high-throughput fashion to validate the genetic targets of drugs and small molecules.
- · d-siRNAs may be used for therapeutic applications.

Advantages

- ·Simple: siRNAs can be produced by a variety of methods: chemical synthesis, by in vitro transcription from a short DNA template, or by transfection of DNA expression construct that gives rise to a siRNA or short-hairpin-RNA (shRNA) in vivo. The advantage of using d-siRNAs instead of the currently available siRNAs is dependent on which method is used to generate the siRNA, but in general using d-siRNAs is advantageous because they are comprised of a pool of siRNAs. In certain instances, individual siRNAs evoke a cytotoxic response thus another potent sequence must be determined. The complexity of the d-siRNA pool avoids this issue.
- ·Cost effective: With our technology, for any single gene at least 300 different d-siRNAs can be produced from a ~600 base pair dsRNA at a cost equal to that required to produce an in vitro transcribed siRNA or in vivo encoded siRNA or shRNA. In addition, this cost is a few orders of magnitude less expensive than a

chemically synthesized siRNA. The current price of a single chemically synthesized siRNA is around $500. The major difference in cost lies in the fact that when single siRNAs are used three to eight siRNAs may be required to attain a high probability of silencing any particular gene.

- ·Time efficient: d-siRNAs can be generated in two days and the phenotype can be assayed in less than one week.
- ·Improved gene silencing: To determine the target region of a single siRNA an educated guess must be made, but d-siRNAs obviate the need to guess. Although the rules for what makes a potent siRNA are better characterized there is a substantial chance that any single 21-nucleotide region selected from the mRNA will be ineffective in initiating mRNA cleavage or inhibiting translation. d-siRNAs target a large region (at least 500 base pairs) thereby improving the chances of silencing a gene because if there is secondary structure or protein binding in the target region silencing will not be as efficient with a single siRNA.
- ·Improved scale-up: Using d-siRNAs for gene silencing, scales up well for studying gene function in a high-throughput, large-scale fashion (e.g. screening of cDNA libraries).Whereas, any method using a single siRNA does not scale up well because one or more siRNA would need to be designed and generated for each gene in the genome. d-siRNAs could be generated from a cDNA library either from individual cDNAs or from pools of cDNAs, and only one pool of d-siRNAs needs to be generated for efficient gene silencing making this approach less expensive and more efficient than using single siRNAs.
- ·More selective: Each siRNA within the complex pool of d-siRNAs is ~1/300th the concentration thereby decreasing the likelihood of off-target effects. Furthermore, d-siRNAs will cause destruction of a large portion of the targeted mRNA decreasing the chances of generating a truncated protein. Single siRNAs cause cleavage at one site and the resulting N-terminal truncated protein may act as a dominant negative or constitutively active protein rather than as a true protein-null.
- ·Unbiased: d-siRNAs can be generated from unknown sequences providing a means to study uncharacterized gene products or genomic elements. In a therapeutic setting, very little information about the genome of the pathogen (e.g. virus or parasite) must be known. Furthermore, in the case rapidly mutating virions the genomic material could be repeatedly isolated from the infected cells, and converted into d-siRNAs avoiding the complications of potency involved with mismatches between the siRNA and sense RNA.

Publications

- Jason W. Myers, Joshua T. Jones,Tobias Meyer, and James E. Ferrell, Jr. Recombinant Dicer efficiently converts large dsRNAs into siRNAs suitable for gene silencing. *Nat. Biotechnol.*, March 2003 Volume 21 Number 3 pp 324 - 328.
- Chuck C. Fink, Karl-Ulrich Bayer, Jason W. Myers, James E. Ferrell, Jr., Howard Schulman, and Tobias Meyer. Selective regulation of neurite extension and synapse formation by the beta but not the alpha isoform of CaMKII. *Neuron*, July 2003 Volume 39 pp 283-297.
- Josh T. Jones, Jason W. Myers, James E. Ferrell, Jr., Tobias Meyer. Accuracy of the mitotic clock probed with a live cell mitosis biosensor. *Nat. Biotechnol.*, March 2004-in press.
- U.S. Application: 20030224432

Patents

- Published Application: WO2003093430
- Published Application: 20030224432
- Published Application: 20090286287

Innovators

- Jason Myers
- James Ferrell

Licensing Contact

Cheryl Cathey

Senior Licensing and Strategic Alliance Manager

Email