Efficient Gene Silencing with High Selectivity using Mission™ shRNA Lentiviral Vectors

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Overview

The RNAi Consortium (TRC) is a collaborative effort among several academic laboratories (including the Broad Institute, the Whitehead Institute for Biomedical Research, the Dana Farber Cancer Institute, Massachusetts General Hospital, Massachusetts Institute of Technology, Harvard Medical School, Columbia University, and Washington University School of Medicine), as well as major biotechnology and pharmaceutical institutes such as Sigma-Aldrich, Eli Lilly, Novartis, Bristol-Myers Squibb and others. The goal of the consortium is to validate methods and tools that will enable the scientific community to use RNAi in both mouse and human genes. To that end, reagents have been generated consisting of short hairpin sequences cloned into a lentiviral vector that can be used for a wide range of in vitro and in vivo studies. When completed, the Broad RNAi lentiviral library will consist of 150,000 custom-designed shRNAs, targeting 15,000 human (MISSION TRC - Hs 1.0) and 15,000 mouse (MISSION TRC - Mm 1.0) genes. Applications for this library will range from investigations into a single gene or pathway to genome-wide functional screens.

Background

While siRNAs have become the most common form of RNA inhibition, they possess several qualities that may be less than optimal for a given experiment. The use of shRNAs requires a cell line that can be readily transfected. This trait is often missing from a number of cell types critical to today’s biomedical research, including primary cell cultures, stem cell lines, and even a wide array of human tumor cell lines. Another consideration is the transient nature of siRNA transfection. As cells are successfully transfected with siRNA continue to divide, the concentration of siRNA is rapidly diluted, resulting in the gradual reaccumulation of target RNA. The long term silencing of a majority of cell lines can be achieved, however, through use of shRNAs in a lentiviral-transduction fashion.

We have examined the variable knockdown resulting after infection of the human lung tumor cell line A549 by four clones that are designed to target JAK1. Regulation of shRNA in A549 epithelial cells has been linked to regulation of JAK1 (Yokota et al., 2004) making manipulation of this gene particularly interesting in this cell line background. In addition to JAK1, we sought to examine the specificity of inhibition by shRNA using the gene AKT as an example. While all three isoforms of AKT (AKT1, AKT2, and AKT3) are expressed in A549 cells, inhibition of each individual isoform is believed to result in a different phenotype (Sithanandam et al., 2005). We transduced A549 cells with shRNAs to two of the three AKT isoforms, and examined down-regulation of the targeted isoform, as well as the two related, but non-targeted isoforms.

Conclusion

RNA interference technology has been one of the key biological breakthroughs of the last decade and has revolutionized basic biology and gene function studies, but it also holds promise to revolutionise drug discovery and therapeutics. The Mission™ TRC shRNA libraries overcome the limitations of synthetic siRNAs by providing a system for long term silencing and phenotypic observation, and the ability to generate lentiviral particles for infection and integration of primary cells, dividing cells, and nondividing cells. The libraries provide excellent specificity to target genes and high efficiency of transfection.

References
