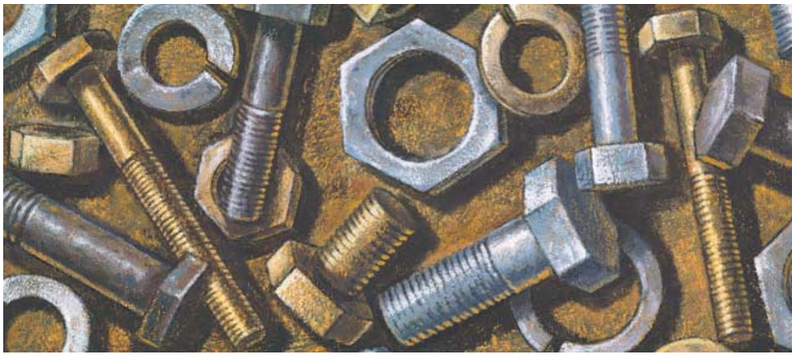
HIGHLIGHTS

TECHNOLOGY

Building the complete toolkit



Two groups have independently developed a general way of generating RNA interference (RNAi) libraries that will allow the high-throughput knock-down of expression of mammalian genes.

Seeing what happens when a gene does not work will remain, for the forseable future, our best way of determining the normal function of that gene. RNAi offers an easy, sequence-specific approach to knocking down gene expression but the time and money required to develop small interfering RNAs (siRNAs) that are complementary to each gene has always been a problem. Blau and colleagues and Hirose and colleagues solve this problem by developing stepwise protocols to create RNAi libraries from any given gene or pool of genes.

In both cases, the first steps are the digestion of the gene(s) of interest with an endonuclease and ligation of the resultant fragments to a hairpin-shaped oligonucleotide. The next crucial step is to engineer the recognition sequence for the *Mme1* restriction enzyme into the construct. This enzyme's recognition sequence is 20 nucleotides from where it cleaves DNA, so subsequent digestion with this enzyme generates fragments that are the right size to use as templates for functional siRNAs; that is, small enough to avoid the mammalian interferon response prompted by longer double-stranded DNAs (dsRNAs). The short single-stranded DNA (ssDNA) fragments are then extended into dsDNA. Another round of digestion to trim off the terminal hairpin sequences leaves clonable fragments that can then be transcribed from siRNA vectors.

Blau and colleagues were able to silence both transgenes (*GFP* again) and endogenous genes (*Oct3/4* and *MyoD*) with siRNAs from libraries that were generated using this new approach. They also went on to show the applicability of the approach to cDNA libraries. Importantly, they showed that they could generate an RNAi library from a cDNA library that broadly reflected the original expression profiles represented in the cDNA library. There seems no reason why this approach could not be routinely applied to genome-wide collections of cDNAs.

Shirane and colleagues also generated RNAi libraries from cDNAs of a marker gene (*GFP*) and an endogenous mammalian gene (type 1 *IP3R*), and used siRNA constructs from these libraries to knock down expression of these genes in human cells. They also developed a selection scheme that enabled high-throughput selection of efficient siRNA constructs from these RNAi libraries.

The demonstrated efficacy of this simple method for generating large, complex populations of siRNAs should prove to be a huge boost for research in mammalian functional genomics. We should expect to see a raft of papers in the near future that apply this technology for high-throughput knock-down of mammalian genes.

Nick Campbell

References and links

ORIGINAL RESEARCH PAPERS Sen, G. *et al.* Restriction enzyme-generated siRNA (REGS) vectors and libraries. *Nature Genet.* 4 Jan 2004 (doi:10.1038/ng1288) | Shirane, D. *et al.* Enzymatic production of RNAi libraries from cDNAs. *Nature Genet.* 4 Jan 2004 (doi:10.1038/ng1290)

FURTHER READING McManus, M. T. & Sharp, P. A. Gene silencing in mammals by small interfering RNAs. *Nature Rev. Genet.* **3**, 737–747 (2002)

WEB SITES

Helen Blau's laboratory:
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