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Outside expertise

To the editor:

The article in the November issue by Emma Mills (*Nat. Biotechnol.* 20, 1171–1172; 2002) on the availability of contract technical workers and even senior executives “off the peg” leaves out some important aspects of contracting outside experts.

First, a typical year involves 1,800 formal work hours if one subtracts weekends, vacation, holidays, and sick leave. At \$100 per hour, this equals an annual salary of \$180,000 for a senior level industrial scientist. However, the typical compensation package also includes health insurance for the employee and family, employer contributions to a pension plan, and considerable stock options. This raises the hourly rate to nearly \$150, if not higher. This simple math shows that to contract a worker at that level of expertise and hourly rate makes economic sense. Add to this the ease of hiring and firing, the immediate availability of the expert, and the elimination of the management headaches of fitting senior level staff to an existing corporate structure, and you have a win-win situation even at a much higher hourly rate. Of course, the value that an outside expert may bring into moving a project ahead and solving critical problems is incalculable.

Second, an outside expert who does not get involved with personnel issues makes much more efficient use of his or her time and is approached more openly than a corporate manager. This greatly facilitates communication and often brings solutions to problems from within the organization.

Third, in contrast to the opinion expressed in the article, the simpler and more economical way to locate outside experts is by word-of-mouth recommendations based on reputation and credentials, not through an agency. Direct contact simplifies communication, builds trust and saves the considerable overhead that agencies charge.

Finally, today's means of communication make expert advice, problem solving, planning, and supervising experiments at a distance very feasible. I, for one, have brought several contracts to fruition with clients that I have yet to meet in person! Working with contract workers or even employees at a distance, saves the employer space and utility costs, and is already common practice in the computer industry. With the expanding role of information technology, it is becoming an important trend in the biotechnology industry, as well.

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RNAi targeting of DNA virus in plants

To the editor:

RNA interference (RNAi) is evolutionarily conserved in plants, animals, and fungi and is thought to protect cells against invasive nucleic acids, such as viruses and transposons¹. Recent reports have demonstrated that RNAi can be engineered to target viral RNA in plants^{2,3} and animals^{4–6}. Using blackgram (*Vigna mungo*) as a study system, we have discovered that the DNA of a replicating virus can also be a target of RNAi.

A key trigger of RNAi is double-stranded (ds) RNA. The RNase III-like enzyme, Dicer, processes dsRNA into 21–25 nt short-interfering (si) RNAs. These siRNAs guide an endonuclease complex to cleave a target RNA in a sequence-specific manner, resulting in post-transcriptional gene silencing (PTGS). In plants, dsRNA expressed from a transgene can induce not only PTGS, but also transcriptional gene silencing correlated with *de novo* methylation of cognate DNA in the genome^{7,8} by a hypothetical RNA-dependent DNA methylase (RdDM).

Many DNA viruses accumulate in the nucleus as minichromosomes. We hypothesized that this nuclear viral DNA, especially that of the promoter region, would be an alternative, or additional, target to achieve total virus resistance by RNAi. Here, we provide supportive evidence for this hypothesis by using dsRNA to target a geminivirus promoter to cure *V. mungo* plants of the viral disease.

To study the potency of RNAi against *Vigna mungo* yellow mosaic virus (VMYV), a bipartite DNA geminivirus, *V. mungo* plants were infected with the virus by cocultivation of germinating seeds with a

Figure 1. (A) Interfering DNA construct. Arrows indicate transcription start sites of the viral genes AC1 and AV2. (B) *Vigna mungo* plants, infected versus recovered. (C) Semi-quantitative PCR with viral DNA A-specific primers.

single *Agrobacterium tumefaciens* transformant harboring both A and B viral genomes⁹. Seven to 10 days post-inoculation, infected young seedlings were bombarded with 1 μ m gold particles covered with an interfering DNA construct. The latter was designed to express, under the control of the cauliflower mosaic virus 35S promoter and poly(A) signal, dsRNA cognate to the bidirectional promoter of VMYV DNA A (209 bp long, from position 2650 to 130, Accession No. AJ132575) with a synthetic plant intron¹⁰ inserted between the sense and antisense copy (Fig. 1A).

The bombardment resulted in complete recovery from infection (Fig. 1B). The curing effect lasted until natural senescence of the plant and correlated with the abolishment of viral DNA amplification. PCR with viral DNA A-specific primers detected much less viral DNA in the leaves bombarded with the construct than in those bombarded with a blank control, and only traces of viral DNA in the recovered upper leaves (Fig. 1C).

In three independent experiments, the intron-containing construct gave a higher incidence of complete recovery (68–77% of the seedlings) compared with a similar construct where the intron sequence was inverted (44–59%). Deletion of the 35S promoter from the former construct abolished the interference (90–100% infection, as with the blank control), thus ruling out a role for DNA-DNA interactions. Notably, the recovery of the whole plant from the viral disease indicates spreading of an interference signal, as in the case of PTGS¹¹, because only a few cells could have received the construct.

The promoter region of geminiviruses is not obligatorily transcribed, although fortuitous read-through transcription on the circular viral DNA cannot be excluded. We