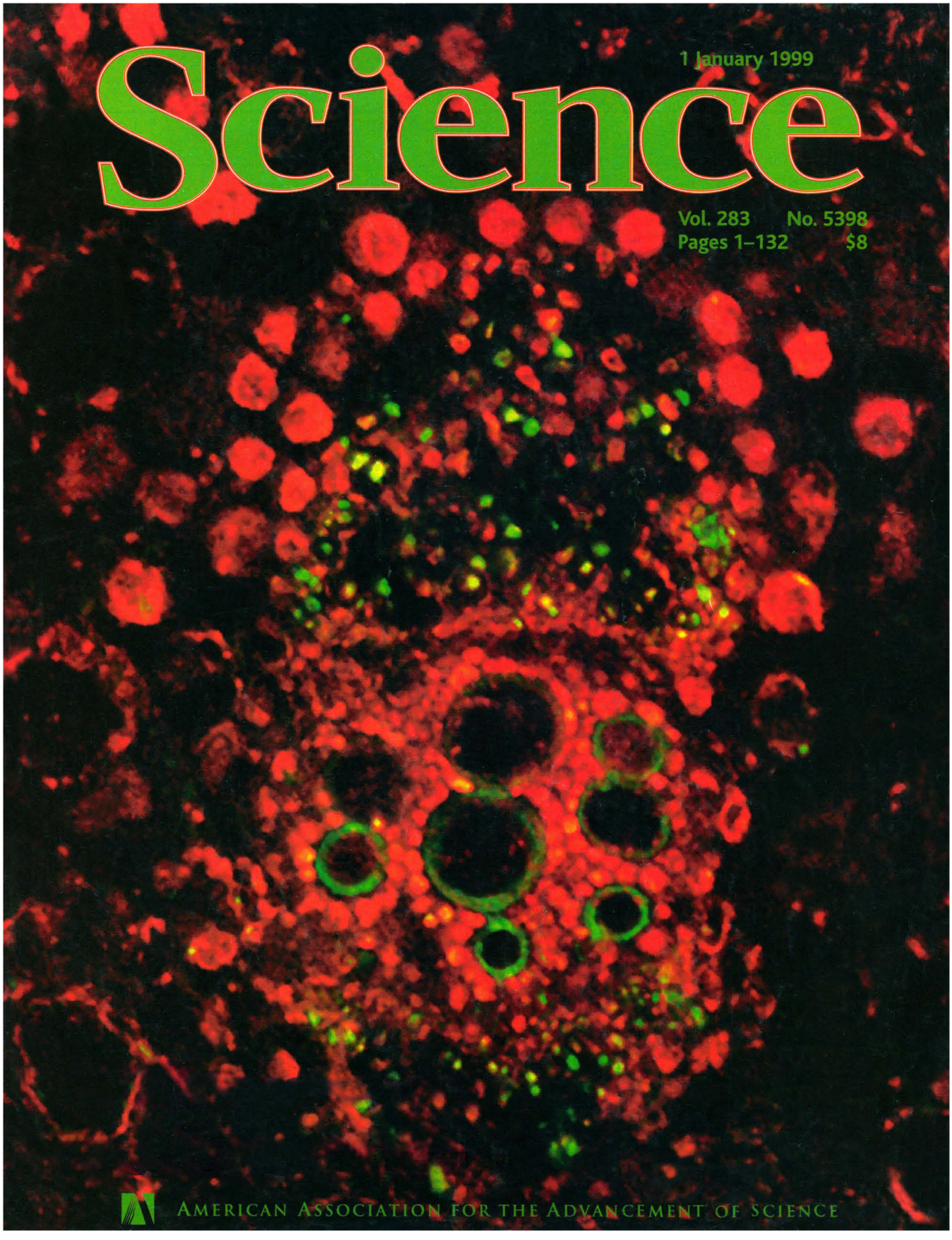

1 January 1999

# Science

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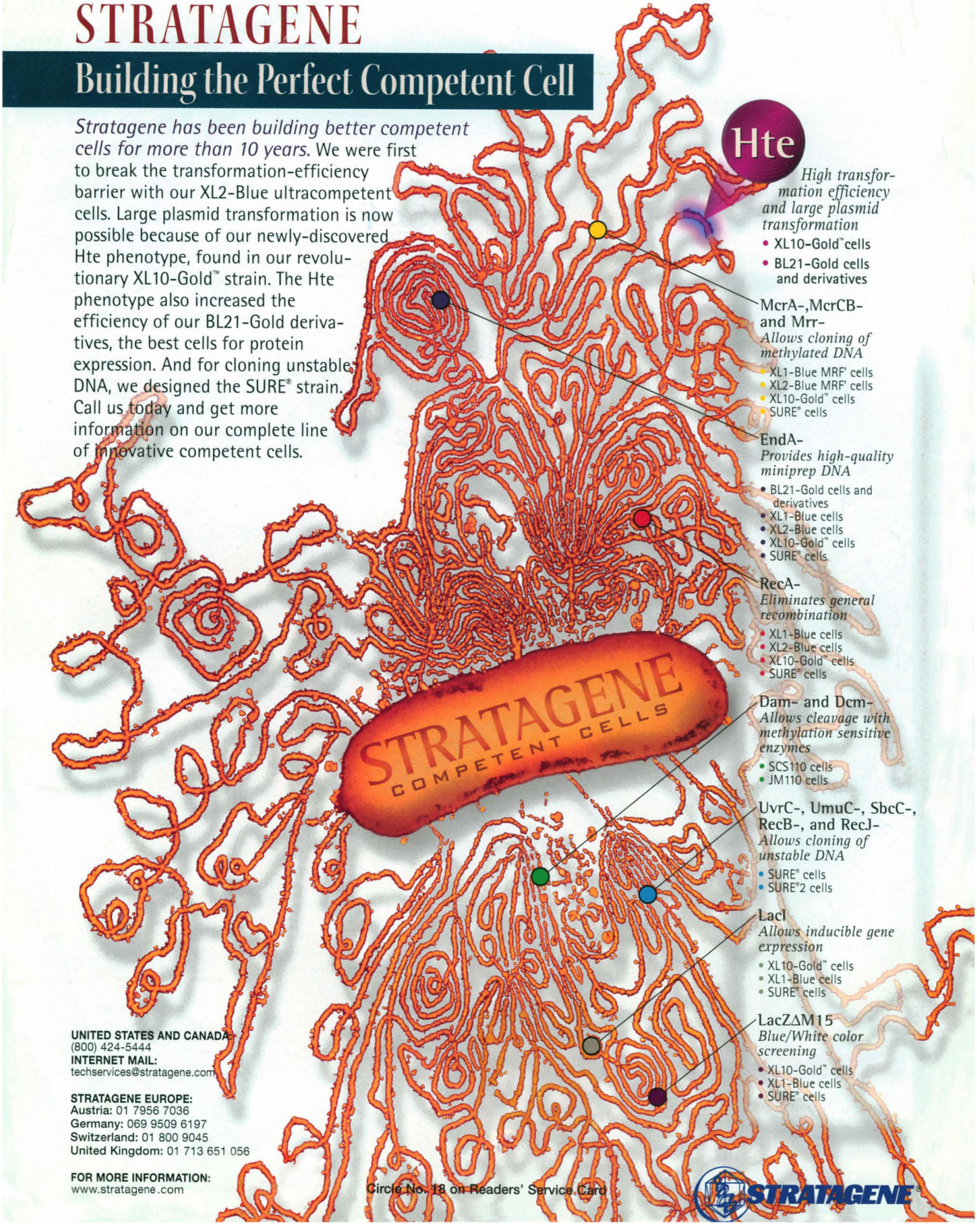


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THE MJ RESEARCH NOTEBOOK

*Volume VIII...No. 4* *A Bulletin of Technological Advance in Molecular Biology* *Winter 1999*

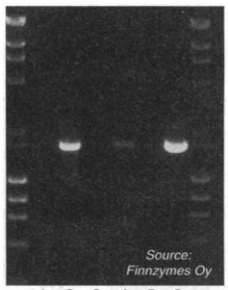
# AMPLIFY GC-RICH TEMPLATES WITH EASE

## ENZYME BLASTS THROUGH SECONDARY STRUCTURES

Some templates are difficult to amplify because they are GC-rich or contain long complementary areas that easily form loops. These secondary structures can prevent primer annealing and/or DNA synthesis, and thus they inhibit PCR and other amplification reactions.

DyNAzyme EXT is a particularly capable enzyme mix for driving amplification reactions in these tough circumstances. But sometimes, even EXT isn't enough. Fortunately, the enzyme is also tolerant of extra additives.

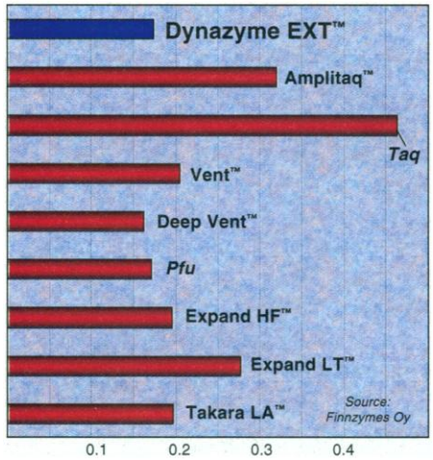
Often special "helping" solutions are employed to change the melting characteristics of DNA and thus minimize the effects of secondary structure. These additives may be DMSO, formamide, glycerol, betaine, etc. EXT was tested alongside of two market-leading enzymes with and without their proprietary helping solutions. The result? Just look at the gel—DyNAzyme EXT with 5% DMSO (an ordinary lab reagent) was significantly more effective in amplifying a 69% GC-rich template than either of the competing enzymes with their proprietary solutions.




PCR on TGF-β1 1.9kb region (69% GC-rich)

#1 "Q" *taq*, #2 "Q" *taq* + sol.
#3 "C" *taq*, #4 "C" *taq* + sol.
#5 EXT, #6 EXT + 5% DMSO

# *DyNAzyme EXT™ Polymerase Exhibits Extraordinary Fidelity*




OD(450), lower signal means higher accuracy

## Fidelity Assayed in Actual PCR Reactions

The data above represent relative fidelity among a number of polymerases. It was collected using the immobilized mismatch binding protein method (IMBP)†. This technique, which detects errors through the binding of a repair protein, does not yield absolute numbers for error rates, but it is extremely effective in showing relative performance. It assays accuracy during actual PCR by measuring the number of accumulated errors in the final product, and it is able to detect almost all common mistakes. For more info, see www.genecheck.com.

†Wagner, R. & Dean. A. 1998. *The use of immobilized mismatch binding protein for the optimization of PCR fidelity* in "PCR Methods Manual," Innis M. Gelfand, D. and Sninsky, J. eds. in press

## BROAD TOLERANCE FOR VARYING REACTION CONDITIONS

### Get the Best of Both Worlds–Accuracy & Robust Reactions

WATERTOWN, Mass. — Since the spring of 1998, MJ RESEARCH has distributed the PCR-licensed DNA polymerases made by Finnzymes Oy of Finland. These enzymes have their origin in the thermophile *T. brockianus*, and they exhibit many superior properties to *Taq* polymerase in driving PCR reactions (especially in thermal stability and yield).

However, one question that was frequently asked is, "What's the fidelity compared to *Taq*?" Unfortunately, determining error rates is very laborious and condition dependent, and making comparisons among enzymes is not easily done. But now solid data exists—and the results are so good, the Finns insisted on repeating the experiments over and over again.

The assay used was IMBP *(see accompanying article)*, and DyNAzyme EXT* was particularly outstanding in its fidelity characteristics. EXT is a cocktail with a small amount of proofreading enzyme, and it is great for long PCR, high-fidelity TA cloning, and difficult templates. Yet in this fidelity assay, it performed as well or better than every other polymerase tested—including proofreading enzymes considered to be the "gold standards" of accuracy! But unlike those enzymes, EXT has the finesse to amplify templates as long as 40kb, the versatility to amplify templates that are high in GC content, as well as the robustness to withstand widely varying reaction conditions.

All DyNAzyme enzymes come licensed by Hoffmann-LaRoche to perform PCR reactions in research.** DyNAzyme is available as native enzyme, recombinant enzyme, or as the EXT cocktail. All are available separately or in kits, and they come with a variety of buffers.

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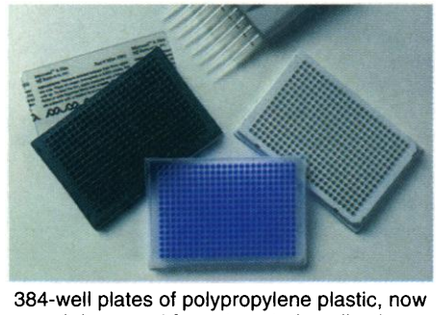
# *384-well Format Comes Into Its Own*

## Plates Now Flatter & Bar Coded

MJ Research has been involved in the development of the 384-well format since 1993. In fact, the V-well design generally used in thermal cycling was developed by MJ engineers. It is a true 4x96-well configuration, which allows use of the same dispensing/harvesting equipment as with 96 wells (the frame is just shifted).

The newly improved Microseal 384 plate features a rigid, robot-friendly design with an industry-standard footprint and locator holes for secure handling. The flatter upper surface provides more reliable automated liquid dispensing, and the plates are much less likely to stick in the block after cycling. Serialized bar code labels (code39) are a new, low-cost option.



384-well plates of polypropylene plastic, now much improved for automated applications

*EXT licensed under US Pat. 5,436,149 owned by TaKaRa Shuzo Co. Ltd.

** Purchase of DyNAzyme polymerase is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) process for research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased, i.e. an authorized thermal cycler.

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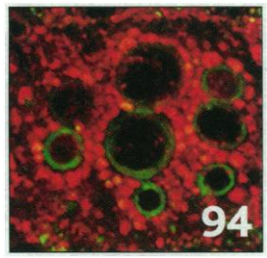
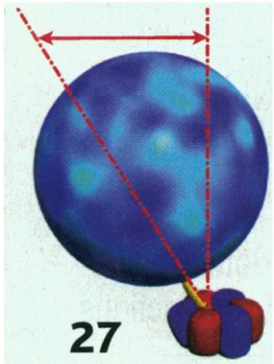



**Cover** Localization of *CmPP16* messenger RNA (mRNA) in the vascular tissue of pumpkin plants (image width ~500 μm). In this cross section of a petiole showing a vascular bundle, the green fluorescence identifies *CmPP16* mRNA within companion cells and sieve elements of the phloem. CmPP16 has properties similar to plant viral movement proteins and appears to mediate mRNA entry into the phloem transport system. [Image: R. Ruiz-Medrano, B. Xoconostle-Cázares, W. J. Lucas]



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Little engines that can

## NEWS

### NEWS OF THE WEEK



### NEWS FOCUS



## RESEARCH

### REPORTS



### DEPARTMENTS



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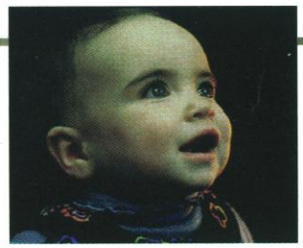


**40**
Learning by the rules

### EDITORIAL



### LETTERS



### POLICY FORUM

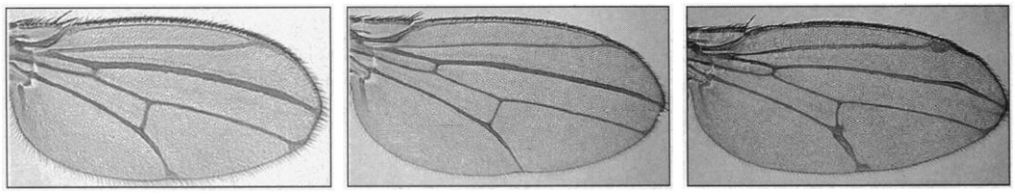


### BOOKS *ET AL.*



### PERSPECTIVES





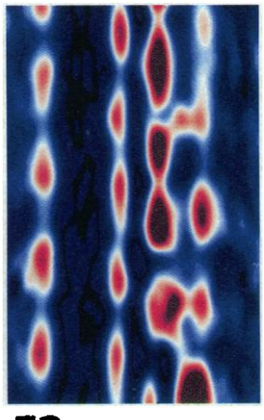
**91**
Soluble Notch ligand



### TECHNICAL COMMENTS







**52**
Resolving discrete electron states in nanotubes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microarray system to look at temporal changes in gene expression when quiescent human fibroblasts are exposed to serum. They find clustering of genes that suggests clues to function and a possible recapitulation of many of the steps of wound repair.

### DRUG PRODUCTION IN VIVO

Gene therapy will be most useful if the expression of the therapeutic proteins can be controlled and regulated. Ye *et al.* (p. 88) used an adeno-associated virus system in which erythropoietin expression is under the control of two chimeric proteins that are reconstituted in vivo into an inducible transcription factor complex. Stable, expression was observed in immune-competent mice for 6 months and in rhesus monkeys for 3 months.

### NOTCH ON THE MOVE

Throughout development, the Notch signaling pathway functions in directing cell fate, patterning, and morphogenesis. Previous work indicated that the Notch cell surface receptor is activated by direct cell contact with a ligand-expressing cell, with Notch forming a complex with transmembrane ligands such as Delta and Serrate. Qi *et al.* (p. 91) used biochemical and genetic analyses to show that the metalloprotease Kuzbanian cleaves the Notch ligand Delta. This work is contrary to previous models showing that Notch ligands act exclusively as membrane-bound molecules. Instead, the ligand can be found as an active diffusable ligand, possibly permitting Notch signaling that is not restricted to adjacent cells.

### PASSING RNA BETWEEN PLANT CELLS

Certain viruses spread through their infected host plants with the aid of virally encoded proteins that shepherd nucleic acids from one cell to the next through the plasmodesmata, channels connecting neighboring cells. Xoconostle-Cázares *et al.* (p. 94; see the cover and the news story by Strauss) show that the pumpkin plant encodes its own protein with similarities to these viral movement proteins. Thus, the marked mobility of viral components between cells may be a reflection of physiological processes already present, but perhaps regulated with more discrimination, in the normal plant. These processes may allow RNA molecules to be carried far from the cell that synthesized them.

## TECHNICAL COMMENT SUMMARIES

### Gene Targeting in Human Cells Without Isogenic DNA

The full text of this comment can be seen at www.sciencemag.org/cgi/content/full/283/5398/9a

"Because gene targeting in human somatic cells is rapidly gaining acceptance," J. M. Sedivy *et al.* "have compiled gene targeting data available to date" to assess whether the procedure's success depends on the use of isogenic DNA, which would be a significant technical impediment. They found "numerous examples of high-efficiency gene targeting using nonisogenic DNA," and "thus envision the rapid emergence of a library of tested and optimized gene targeting vectors that will be available for widespread gene analysis in the large number of human experimental cell systems."

### Endocranial Capacity of Early Hominids

The full text of these comments can be seen at www.sciencemag.org/cgi/content/full/283/5398/9b

G. C. Conroy *et al.* (Reports, 12 June, p. 1730) used transaxial computed tomography (CT) scans to measure the endocranial capacity of an early hominid skull (Stw 505) from Sterkfontein, South Africa, tenatively assigned *Australopithecus africanus.* The capacity was estimated to be about 515 cubic centimeters, "markedly less than anecdotal reports of endocranial capacity exceeding 600 cubic centimeters."

C. A. Lockwood and W. H. Kimbel comment that not all "sources of postmortem distortion have been taken into account" and that the reported measure underestimates the actual capacity "perhaps by as much as 10 to 15%." J. Hawks and M. H. Wolpoff estimate endocranial volume "by stepwise multiple regression" with the use of "seven linear measurements" to arrive at a capacity of 598 cubic centimeters.

In response, Conroy *et al.* discuss how they accounted for "the obvious displacement of the left parietal-temporal bones" in their study. They state that several of the seven linear measurements used by Hawks and Wolpoff may well be "values that have been overestimated in the paloeanthropological literature and are themselves in need of reassessment" (see related Letters to the Editor, p. 34).

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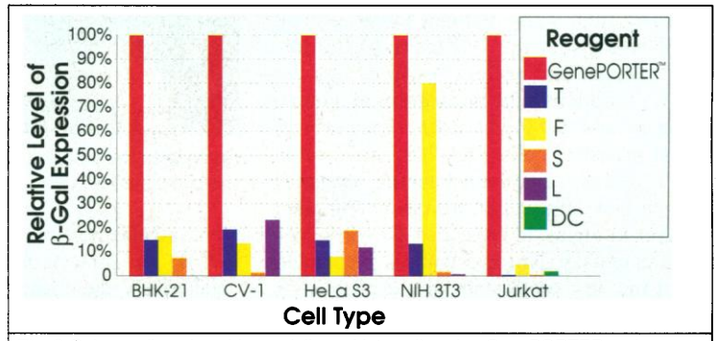


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