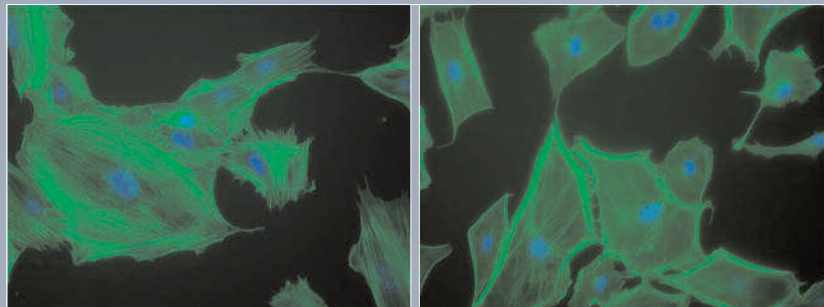
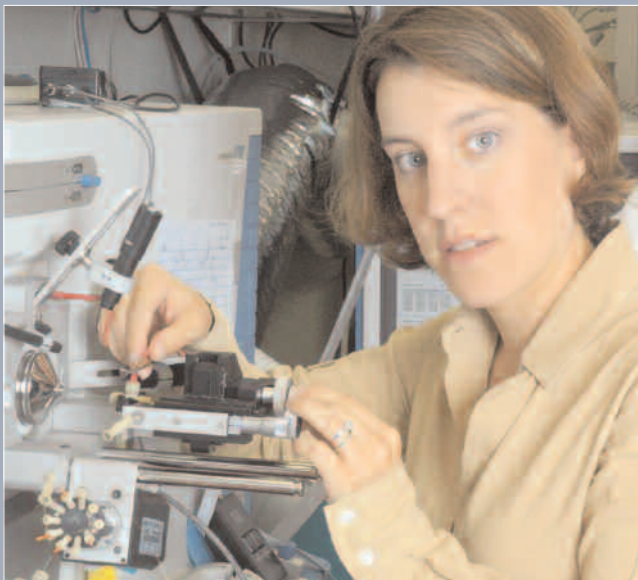


## Translational Research Institute



Inhibition of stress fiber formation in Swiss 3T3 cells by rho kinase  
Inhibitor for potential treatment of spinal cord Injury. Work performed by  
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*Head, Drug Discovery*

## Chairman's Overview

The Translational Research Institute, headed by Pat Griffin, professor of biochemistry, merges drug discovery efforts at Scripps Florida with advanced technology platforms to rapidly identify and validate biological pathways that can be targeted for therapeutic intervention. The technology platforms are grouped into genomics, cell-based screening, and proteomics cores.

The goal of the drug discovery operation is to discover and develop small-molecule therapeutic agents for unmet medical needs in neurodegeneration, Parkinson's disease, acute respiratory distress, spinal cord injury, cardiovascular disease, cancer, and metabolic disorders such as insulin resistance and type 2 diabetes. Therapeutic areas and targets, which include G protein-coupled receptors, proteases, channels, and kinases, are selected on the basis of unmet needs and the ability to attract funding. The drug discovery operation is fully integrated with the following groups: Lead Identification, headed by Peter Hodder, director and associate professor; Medicinal Chemistry, headed by William Roush, executive director and professor of biochemistry; Discovery Biology, headed by Phil LoGrasso, senior director and associate professor of biochemistry; CNS Disorders, headed by Claes Wahlestedt, director and professor of biochemistry; and Drug

Metabolism and Pharmacokinetics, headed by Mike Cameron, associate director.

The Lead Identification department enables high-throughput screening-related drug discovery research. Using state-of-the-art automation and instrumentation, the department is responsible for developing and executing biochemical or cell-based high-throughput screening assays in a miniaturized 1536-well microtiter plate format. In addition to its support of internal Scripps Research objectives, the group participates in the National Institutes of Health's Molecular Library Screening Center Network, where qualified assays are screened against the network's high-throughput screening compound library. Several internal and external investigators have accessed the department's expertise via collaborative or core-charge mechanisms.

The Genomics platform is headed by Mathew Pletcher, assistant professor of biochemistry. The Genomics core oversees genotyping and gene expression profiling technology platforms. The services provided by this core allow Scripps Research investigators to query the genome at both the genetic and transcriptional levels for the genes that underlie common diseases. In collaboration with Scripps Florida colleagues, the core has been involved in projects seeking to identify the genes responsible for pathologies such as addiction and alcoholism, lupus, autism, obsessive-compulsive disorder, obesity, and prion pathogenesis.

The Cell-Based Screening platform is headed by Mike Conkright, assistant professor of Cancer Biology. The Cell-Based Screening platform leverages high-throughput technologies toward a systematic description of the function of genes encoded by the human genome and a more comprehensive understanding of the genetic basis for human disease. The Cell-Based Screening group provides investigators with access to genome-wide collections of cDNAs and siRNAs that can be used to interrogate cellular models of signal transduction pathways and phenotypes.

The Proteomics platform is headed by Jennifer Caldwell-Busby, associate director. The core focuses on the application of liquid chromatography and state-of-the-art mass spectrometry technology to the identification, quantitation, and characterization of proteins and their modifications. The laboratory is involved in scientific collaborations that use novel technologies to identify biologically important proteins and modifications. Large-scale differential analysis is being used to map the pathways related to insulin sensitization and adipogenesis.

Another project uses targeted peptide scanning to determine the rate of degradation of key proteins involved in cellular regulation. Still other projects are using chromatographic enrichment techniques to identify

sites of phosphorylation and other post-translational modifications. The Proteomics Lab works with collaborating scientists to create experiments that will provide meaningful mass spectrometric results.

## INVESTIGATORS' REPORTS

### Proteomics Laboratory

J.A. Caldwell-Busby, V. Cavett, J.D. Tipton

**T**he Proteomics Laboratory provides proteomics services and expertise to scientific collaborators at all Scripps Research facilities, universities within the state of Florida, and other educational institutions. We use cutting-edge mass spectrometry technology to identify proteins, map modifications that occur after translation, and do relative quantitation experiments with a variety of samples.

In its lifetime, a protein can have several locations and functions within a cell. Location, function, and 3-dimensional structures of proteins are all influenced by static and dynamic chemical modifications that occur after translation. These modifications vary from small methyl and acetyl groups, which are a part of the histone codes, to large lipid and glycosylation modifications, which act as cellular markers and signaling molecules. With mass spectrometry, we can detect both the small and the large changes in mass that occur in proteins because of these modifications, and we can identify the specific amino acids modified.

Relative changes in protein levels between multiple samples provide biologically relevant information about cellular pathways and proteins of interest. Large-scale studies of this type require rigorous sample preparation methods and highly tuned algorithms for comparing different mass spectrometric analyses. We are currently validating methods for both sample fractionation and data analysis for these types of large-scale differential protein experiments.

Mass spectrometers at the facility include an ion-trap spectrometer, which is used mostly to identify proteins and peptides, and a triple quadrupole mass spectrometer, which is used for relative quantitation experiments. A new addition is a mass spectrometer that can be used to perform accurate mass and high-resolution experiments. Each mass spectrometer is interfaced to nanoflow electrospray ionization sources and capillary high-performance liquid chromatography columns.

Data analysis is performed primarily via an automated workflow on a cluster maintained by the bioinformatics group. Automation of the front-end processing allows for a more thorough review of the resultant data and more time for development of innovative software in collaboration with information technology groups at Scripps Research and beyond.

#### PUBLICATIONS

**Kirkland, P.A., Busby, J., Stevens, S., Jr., Maupin-Furlow, J.A.** Trizol-based method for sample preparation and isoelectric focusing of halophilic proteins. *Anal. Biochem.* 351:254, 2006.

### Drug Discovery: The Lead Identification Department

P. Hodder, P. Baillargeon, P. Chase, C. Chung, F. Madoux, D. Minond, K. Ryan, L. Scampavia, T. Spicer

**T**he Lead Identification Department is responsible for developing and executing biological and biochemical high-throughput screening assays and for supporting downstream medicinal chemistry efforts. The anchors of the department are 2 fully automated robotic platforms (Fig. 1). One supports screening of 384- and 1536-well microtiter plates in a variety of assay formats. The other is used to manage the library of compounds used for drug discovery at Scripps Research.

This past year, we completed implementation of the ultra-high-throughput screening (uHTS) operation and successfully completed several uHTS-related collaborations. Table 1 provides a list of the assay formats we can use in screening.

#### INFRASTRUCTURE ENHANCEMENTS

The library of compounds for drug discovery contains more than 600,000 unique small molecules. Most of the compounds have been acquired from commercial suppliers. This past year, we augmented our collection with compounds derived from internal research efforts. Our newest additions include collections of kinase-specific and click chemistry compounds; the click chemistry compounds were generously donated by V. Fokin and K.B. Sharpless, Department of Chemistry. Through our involvement in the National Institutes of Health

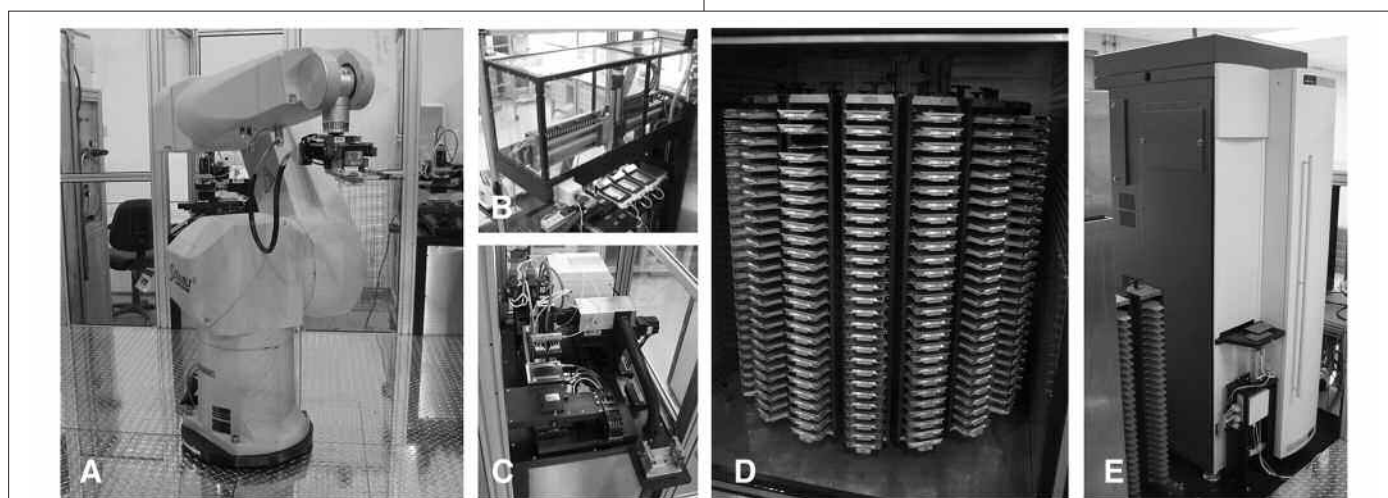
**Table 1.** Examples of uHTS-compatible assay formats for some popular drug discovery targets and indications.

Desired target or indication	Measured species	Assay formats
G protein-coupled receptors	Reporter gene	$\beta$ -Lactamase Luciferase Melanophore
	Second messenger	Homogeneous fluorescence resonance energy transfer (cAMP) Fluorescence imaging plate reader (intracellular calcium)
Nuclear hormone receptor	Reporter gene	$\beta$ -Lactamase Luciferase
Protease	Labeled substrate	Fluorescence dequenching Homogeneous fluorescence resonance energy transfer Fluorescence polarization
Kinase	Labeled substrate	Homogeneous fluorescence resonance energy transfer Fluorescence polarization
Antifungal or antibiotic	Cell viability	Luminescence (ATP activity)
Apoptosis	Cell viability	Luminescence (ATP activity)
Protein-protein interactions	Labeled protein	Homogeneous fluorescence resonance energy transfer Enzyme-linked immunosorbent assay
Protein folding	Labeled protein	Fluorescence dequenching

Roadmap Initiative, we have also acquired approximately 100,000 compounds to support HTS collaborations associated with the Molecular Library Screening Center Network (MLSCN). This collection contains scaffolds

that might be useful chemical probes for relevant biological or biochemical targets.

This past year we acquired specialized equipment and software to further automate the selection and



**Fig. 1.** The Scripps Research uHTS platform. A, The entire robot occupies a footprint of 220 square feet. B, A 1536-pin tool is used to transfer test compounds rapidly from compound plates to assay plates. C, Two liquid handlers capable of dispensing up to 32 different reagents and washing 1536-well plates are integrated onto the platform. D, Three incubators, each capable of holding approximately 700,000 samples in 1536-well format are used to store assay and compound plates. E, An imaging plate reader measures absorbance, luminescence, and fluorescence (with time resolution) on 1536-well plates. Not shown is a compound management robot capable of storing approximately 700,000 samples in a 384-well format for selecting the best or most desirable compounds; it occupies a footprint of 198 square feet.

reformatting of medicinal chemistry compounds. We also subdivided our collection of compounds into smaller collections on the basis of known scaffold templates and target classes. This subdivision has enabled us to screen subsets of the Scripps collection of compounds. Additionally, we acquired a specialized liquid chromatography–mass spectrometry platform that allows us to rapidly determine the structure and purity of collections of small-molecule compounds, important for quality control and structure-activity efforts.

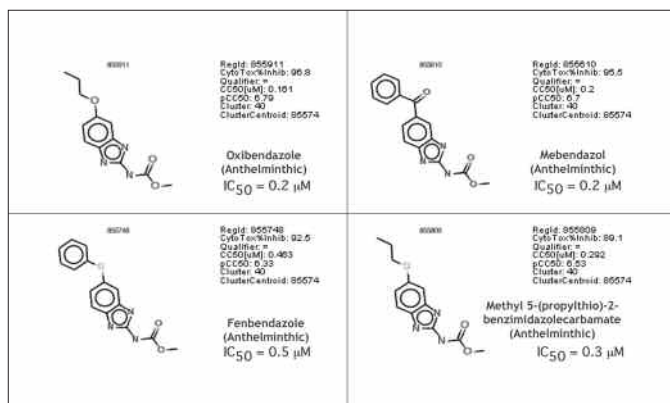
Through collaborations with the Bioinformatics Department, we have installed a database to manage the data produced by uHTS activities. We also developed or helped develop several software tools that interface with our uHTS and compound management platforms. Most important among these tools are those that enable us to automatically create lists of compounds of interest, track sample locations, and generate uHTS summary reports.

#### RESEARCH COLLABORATIONS

In collaboration with various internal and external partners, we have successfully developed and executed several biological and biochemical uHTS assays. Our assays chiefly come from scientists at Scripps Research involved in drug discovery, the MLSCN, and the Florida Access to Technologies program.

The first assay completed on the uHTS platform was under the auspices of the MLSCN. In this campaign, a collection of compounds from the National Institutes of Health was tested for the effects of the compounds on the proliferation and viability of a Jurkat cell line. Because the collection contains several probes with known cytotoxic activity, it was expected that the assay would unambiguously indicate the cytotoxic compounds.

The uHTS campaign was conducted in 3 phases. In the first phase, the primary screen, we determined if any compounds in the collection were active. In the second phase, we selected active compounds, sorted them into a new compound plate, and then tested them in triplicate to confirm activity. In the third phase, we reformatted compounds with confirmed activity so that potency could be determined. In the potency assay, 63 compounds had  $IC_{50}$  values less than  $10 \mu M$ . As expected, clustering these compounds by structural similarity revealed several chemotherapeutic agents and other compounds with structural similarity to known cytotoxic agents (Fig. 2). These findings and all other MLSCN-generated assay data are available for public viewing at the PubChem Web site: <http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=364>.



**Fig. 2.** Results of a uHTS campaign to determine the effects of the MLSCN collection of compounds on the proliferation and viability of Jurkat cells. Representative structure-activity relationships derived from in silico analysis of active compounds are shown. A cluster of 4 antiparasitic agents, 2-carbamoyl-benzimidazoles, is shown, along with percentages of inhibition and  $IC_{50}$  values. Also found in the screen were known apoptosis inducers (vincristine and vinblastine); chemotherapeutic agents (paclitaxel and docetaxel, doxorubicin and daunorubicin); and adenosine, pyrimidoindole, phenylthiazidine, and benzimidazole derivatives.

We have also been actively screening the Scripps collection of compounds against drug discovery targets internal to Scripps Research. For example, in one uHTS campaign, we used homogeneous time-resolved fluorescence resonance energy transfer to discover inhibitors of a serine-threonine kinase. The primary screen was conducted in a miniaturized assay volume of  $10 \mu L$ /well. More than 400,000 discrete compounds were tested for activity, with a throughput of approximately 8000 tests/hour (Fig. 3).

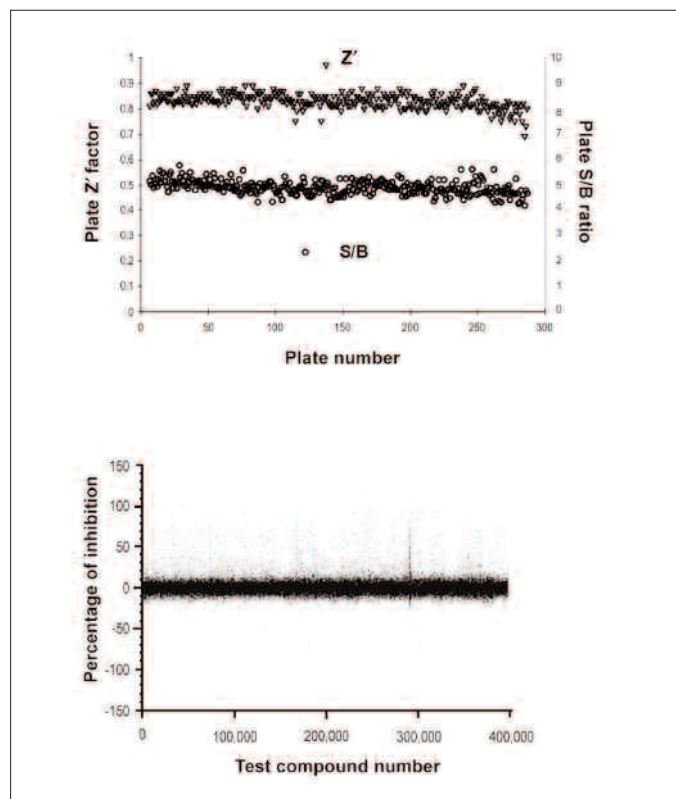
From the primary uHTS campaign, 4220 compounds were identified as compounds of interest and were retested to confirm activity and selectivity to the kinase of interest. After inspection of the resulting data, 1177 of the compounds were selected for titration assays in order to determine  $IC_{50}$  values. From this set of compounds, several novel scaffolds were identified that will complement future medicinal chemistry efforts.

As part of our commitment to the State of Florida, we participate in the Access to Technologies program, an initiative to help Florida academicians develop uHTS-compatible assays. Currently, we are collaborating with B. Dunn, University of Florida in Gainesville, and H. Weissbach, Florida Atlantic University in Boca Raton, to develop assays for discovering novel antimalarials and activators of antioxidant enzymes.

#### PUBLICATIONS

Hodder, P.S. Building a uHTS laboratory. *Drug Discov. World* 31, 2005/2006, Winter Issue.

Hodder, P.S. Integration of miniaturisation technologies into the high throughput screening laboratory. *Eur. Pharm. Rev.* 2:47, 2005.



**Fig. 3.** A, Summary data from a uHTS campaign done with the Scripps Research robotic screening platform. Each data point represents the results from one 1536-well plate.  $Z'$  measures the assay window, and S/B measures the signal-to-background ratio from each plate. The well-resolved traces are indicative of high-quality data. B, A graph of the individual percentage of inhibition values for each of the 400,000 compounds tested in the primary uHTS campaign for a serine-threonine kinase.

## Development of Protein Kinase Inhibitors

C. Liang, M. Koenig, T. Vojkovsky, Y. He

Our goal is to discover protein kinase inhibitors that can be used as therapeutic agents for the treatment of human diseases such as cancer and arthritis. Protein kinases are a class of enzymes that catalyze the transfer of the  $\gamma$ -phosphate from ATP to protein substrates. These enzymes play critical roles in signal transduction for a number of cellular functions. In particular, they regulate most of the hallmarks of cancer: cell proliferation, cell survival, cell motility/metastasis, cell cycle/division, and angiogenesis. Protein kinases are also implicated in inflammatory diseases such as

arthritis and asthma. For these reasons, protein kinases are being investigated as valuable therapeutic targets by virtually every pharmaceutical company, and according to estimates, about 25% of all current pharmaceutical research is devoted to these targets.

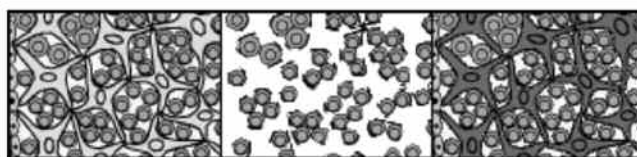
We successfully completed 2 drug discovery projects in the past year. The compounds from one project have been exclusively licensed to a large pharmaceutical company for further development; those from the other are being considered by another company. We also made significant progress in our collaboration with the NeoRx Corporation, Seattle, Washington, to discover novel multitargeted protein kinase inhibitors for the treatment of cancer. We have filed 2 invention disclosures covering 2 novel classes of compounds as inhibitors of protein kinases of great interest to NeoRx. Further optimizations of these compounds are under way.

## Identification of Biomarkers in Tissues of Different Cell Types

N.F. Tsinoremas, M. Gosink

Biomarkers are increasingly important in the development of personalized medicine. However, identification of suitable biomarkers between individuals and disease states is complicated by the incredible heterogeneity within tissues affected by disease. These underlying tissues can play a crucial role in the development, regulation, and progression of disease in adjacent tissues. The identification of particular biomarkers within a single cell type in a mixture of cells currently involves extensive and laborious "wet biology" experimentation to purify a critical cell type so that potential markers can be isolated. Once this cell type has been isolated, a number of approaches, including expression profiling, are used to identify genes specific to the cell type.

We have developed an algorithmic approach to isolate the expression pattern of a single set of tissues from a mixture of tissues (Fig. 1, left). With this approach,



**Fig. 1.** Isolating the expression profile of a single tissue from a mixture of tissues.

some tissue types from a mixed sample can be isolated (Fig. 1, middle). However, we are interested in the other tissues that cannot be purified from the mixture (Fig. 1, right, red blood cells). In our approach, computational methods are used to identify the part of the expression profile of a mixed sample that is due to the tissue type that can be purified. Once this proportion is known, a simple calculation can be performed to determine the expression profile of the genes in the remaining tissues. This approach can also be used to deconvolute or improve the resolution of expression patterns in wild-type vs mutant genes or normal vs diseased tissues when the gene or disease affects only a subset of tissues.

#### IMPROVEMENT OVER STRAIGHT SUBTRACTION

When the interesting subset of tissues in a mixture makes up a significant part of the total mixture, genes with dramatic changes in expression can be identified by performing a simple fold-change calculation between mixed and purified tissues. However, this approach breaks down when the interesting tissue makes up only a small percentage of the mixture. For example, in a mixture of 2 tissues in which the tissue of interest accounts for 5% of the total, a gene with an expression that is 10 times higher in the tissue of interest than in the overall mixture would have only a 1.45-fold change by the standard calculation.

In addition to increased sensitivity, the method can be expanded to allow algorithmic removal of multiple tissues. A combined fold-change calculation between the mixed sample and the purified tissue samples is not possible, and sequential subtraction of each tissue from the mixed sample would result in oversubtraction for most gene signals. In order to use this method, the amount each tissue is contributing to the expression profile of the mixed sample is first calculated on the basis of genes that are strongly expressed in one tissue in the sample but not in the other tissues. Once the individual contribution fractions are calculated, the expression profile of the remaining tissue of interest is calculated as before.

#### APPLICATIONS OF THE IN SILICO SUBTRACTION METHOD

In collaboration with H. Petrie, Department of Biochemistry, we are using this approach to identify genes expressed in the stroma of the thymus. Thymic stroma cells play a critical role in the maturation of T cells after the T cells leave the bone marrow. The stroma cells display numerous signaling molecules and secrete many factors involved in this process. Although a few

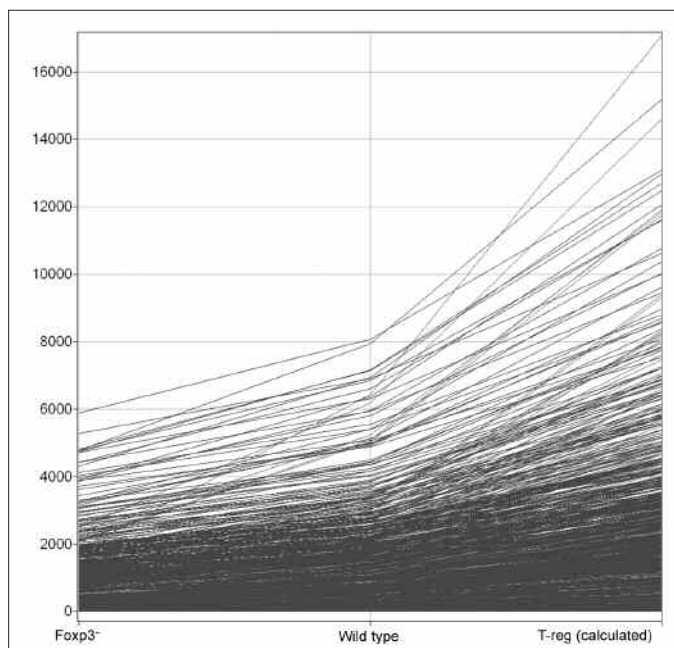
of the major genes in the signaling process have been identified, most have not. The thymus contains a mixture of tissues, including the stromal cells and a significant number of lymphocytes/thymocytes. Currently, the stroma cells cannot be purified from the thymic tissue; however, thymocytes can be isolated from the thymus, and from these cells an expression profile can be determined. This simplification method (deconvolution) is being used to electronically subtract the expression profile of isolated thymocytes from the expression profile of the cells that make up the whole thymus. The calculated stromal cell expression profile has revealed not only genes known to be involved in thymocyte maturation but also genes known to be involved in the immune response (by mutational analysis) but by unknown mechanisms.

We are also applying this *in silico* subtraction method to understand the development of regulatory T cells that suppress the immune response to self-antigens. Although these cells play a critical role in preventing autoimmune disease, they constitute only a small fraction of all T cells, and they are difficult to isolate. However, the transcription factor Foxp3 is a master regulator of the development of regulatory T cells. Expression profiles of T cells from mice lacking the gene for Foxp3 have been reported, and a limited number of genes with significant differences in expression between Foxp3<sup>-</sup> and wild-type T cells have been identified. We reanalyzed the expression data from this experiment and identified many more genes that are upregulated in regulatory T cells (Table 1 and Fig. 2).

**Table 1.** Number of genes identified as having 2-fold or greater expression in regulatory T cells.

Method	No. of genes
Standard fold-change calculation	1355
Deconvolution method	7657

An analysis of these gene sets indicates that although both sets have a significant number of genes annotated by gene ontologies as involved in immune responses, only the set identified by using the deconvolution method had a significant number of genes involved in cell-mediated immune responses. It is thought that in order to exert their activity, regulatory T cells must interact directly with other T cells. In addition, the genes for IL-6 signal transducer and transforming growth factor- $\beta$  receptor 2 were induced more than 2-fold in regulatory T cells when the new method was



**Fig. 2.** Signals for genes that changed by 2-fold or more in regulatory T (T-reg) cells.

used but not when the standard fold-change calculations were used. Recent evidence indicates that IL-6 and transforming growth factor- $\beta$  play important roles in the differentiation of regulatory T cells.