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INSIDE THIS ISSUE

Genetic Footprinting Making Its Mark

As molecular biologists know, the popular methods to identify gene functionality are labor-intensive. They normally require isolating, storing and characterizing each created mutant individually, an arduous process when you are characterizing numerous mutants. Due to an original procedure invented by Dr. Patrick Brown, an associate professor and HHMI investigator in Stanford's biochemistry department, and Dr. Victoria Smith, a former post-doctoral fellow at Stanford now working at Genentech, this process may have just gotten easier.

Genetic footprinting is a fast, high-throughput method for analyzing gene functionality in microorganisms and for high-resolution genetic dissection of any cloned gene. The critical difference between this and other methods is that genetic footprinting allows mutations to be made, and their consequences for gene function determined, many thousands at a time rather than one at a time.

"Instead of making each mutant and analyzing it independently, you make the mutants and analyze them in parallel," said Rachel Crowley, the graduate student currently working with Brown on genetic footprinting applications. "It has the potential to give you a lot more information."

"We're not crippled by our assumptions," added Smith. "With this method one doesn't have to pick out the certain gene you think might code for something." Instead, all of the possibilities are examined.

Two applications

The genetic footprinting strategy can be applied in two distinct ways to attack two different experimental problems. The first procedure was developed by Smith and Brown to determine the functions of all of the thousands of genes in the genome of a microorganism. The entire genome is subjected to the genetic footprinting process.

Using this method, Smith and Brown examined the biological roles of virtually every gene on one specific chromosome of Brewer's yeast (*Sci-*

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OTL's Homepage Gets a Facelift and Adds Some Features

With all the places to visit on the Internet these days, just going online and doing a search can be daunting. However, OTL would like to persuade readers to visit its revamped web site at **www.stanford.edu/group/OTL**, which will help all those associated with or interested in OTL to understand and explore our office further.

At the homepage, users can select from the choices listed in Fig. 1. OTL's homepage naturally contains the basics, including descriptions of the office and staff, frequently asked questions, and directions to the office.

Trusty old *Brainstorm* is also now located on the web site. Back issues from the Winter of 1993 to Summer of 1997 will soon be accessible.

One of the most recent web site options is the "Featured Technology" category. "Featured Technology" highlights hot new technologies looking for a home. OTL will normally feature a new

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- **About the Office**
- **Revenue Chart**
- **FAQ**
- **OTL Staff**
- **Job Opportunities**
- **Brainstorm - The OTL Newsletter**
- **Featured Technology**
- **Corporate User Info**
- **University User Info**
- **Entrepreneurial Information**
- **Technology Transfer**
- **Sondius-XG®**
- **Submit Disclosures Here!**
- **Search the Available Technologies Database**

Fig. 1 - The OTL homepage menu. Just type in our address (www.stanford.edu/group/OTL) into your internet browser and bookmark it for future use! Note that our available technologies section now has a search engine.

Stanford University
Technology Transfer Through Licensing
Office of Technology Licensing

STANFORD TECHNOLOGY BRAINSTORM

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Stanford Technology BRAINSTORM is published quarterly in order to provide information about OTL and general information of interest to the licensing community, both within and outside Stanford.

OTL's services are available to any Stanford faculty, students, or staff who invent technologies which may benefit the public or be of commercial value.

To learn about a specific technology or to disclose one of your own, contact us at the above address.

Genetic Footprinting...

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ence, Smith et al., Vol. 274, pp. 2069-2074, Dec. 20, 1996). Continuing work in Dr. Brown's laboratory is extending this analysis to every gene in the entire genome of this organism.

The second application that is currently being used and perfected by Brown, Crowley and Ila Singh relates to understanding in detail how each tiny part of a gene affects the function of that gene or the gene product (*Proc. Natl. Acad. Sci. USA,* Singh et al., Vol. 94. pp. 1304-1309, Feb. 1997).

The second procedure has two separate uses. One is to determine how the disturbance of each part of the cloned gene or gene fragment affects any aspect of the gene's function. This is done by making and analyzing a comprehensive set of different, small local changes in the gene's sequence.

The other use begins with inserting gene fragments with known functions at every possible position into another gene. The molecular biologist then sees if any of the products both retain the function of the original gene while acquiring the properties brought to the gene by the inserted fragment.

The procedure

Both applications have similar procedural steps. The first involves insertional mutagenesis: introduction of a specific DNA into random sites in the targeted gene of interest, or into the entire genome of the microorganism being studied. The result is a library of mutant DNA molecules in the first application noted above and a population of cells harboring different insertion mutations in their genomes in the second case.

The mutagenized DNA molecules or mutant cells from above are then subjected to one or more selective conditions, a process called functional selection. Functional selection determines whether any of the mutations affect the function of the gene or fitness of the cell.

An example of functional selection for genetic footprinting analysis of the entire genome of a microorganism is the growth of cells in a medium lacking a nutrient required for cell survival. Cells that contain intact versions of the genes which synthesize the missing nutrient will survive and grow. In contrast, cells containing disrupted versions of these genes will fail to grow. The next step is to identify the genes which had a role in cell fitness during the selection.

Polymerase Chain Reaction (PCR) is used to identify where the mutations were made. Specific PCR products corresponding to the positions of the mutations in the targeted gene after selection (selected population) and the positions of mutations in each gene before selection (unselected population) are compared by gel electrophoresis.

Gel electrophoresis shows a number of "bands," each band representing a position where the gene sequence was disrupted by a mutation. The position and intensity of the bands indicates the location of each mutation and its abundance in the population, respectively.

The "footprint" shows itself as any missing bands on the gel. The presence of a band for the unselected population but not for the selected population infers that this mutation diminished the gene's ability to make the necessary gene product(s) for survival.

Idea conception

The idea for this method was planted in Brown's mind when he was working on retroviral integration. In 1991, Peter Pryciak, a graduate student in Harold Varmus' laboratory, was studying where retroviruses integrate into the host genome.

Pryciak found that not all sites in the genome were used equally as targets for retroviral integration in living cells. Brown noted that the distribution of integration events that were ultimately recovered might also reflect another factor. Some of these integrations might disrupt features of the target DNA site that were essential for its replication, resulting in a lower recovery of those DNA molecule, according to Brown.

"It occurred to me that this would be useful for a completely different application," said Brown. In other words, if it was applied to functional analysis of genes. Brown and his wife, geneticist Sue Klapholz, MD, PhD, began testing Brown's hypothesis at night in the laboratory.

A year after starting these experiments, Dr. Victoria Smith joined Brown's lab to aid him in his search. The first successful test of the method was performed by Smith in January, 1993. In a pilot experiment analyzing about a dozen genes in Baker's yeast, Smith saw the genetic footprints she was looking for.

"We needed it not just to work, but to work robustly," said Smith. From their first set of data, Brown and Smith knew they had struck gold. Comparing Smith's results with established results for this set of well-characterized genes showed flawless precision.

Future directions

Besides being highly sensitive and accurate, the genetic footprinting procedure is also simple to implement in most well-equipped molecular ge-

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A Sampling of Licenses Granted by OTL in the Last Quarter

Docket(s)	Title(s)	Uses	Licensee(s)	License Type
S76-047	"Monolithic Semiconductor Switching Device"	IGBT's	Matsushita	Non-exclusive
S83-075	"LSRE-1 Hybridoma"	Monoclonal Antibody	BioSource	Non-exclusive
S89-139	"Insect Steroid Receptors"	Protein Regulation	Pioneer Hi-Bred Intl. Bayer	Field Exclusive Non-exclusive
S93-138, S95-124	"Radio Surg.- Treatment Plan" "Image Registration"	Treatment Planning	Accuray	Non-exclusive
S94-140	"Antigen-Specific T Cells"	Diagnostics	Beckman Coulter	Field Exclusive
S95-022	"Nucleotide Delivery Device"	Gene Delivery	Mann, Dzau	Option
S95-024	"Detect DNA Heteroduplices"	Mutation Detection	Transgenomic	Non-exclusive
S97-072	GENSCAN™	Gene Identification	Darwin Molecular Corp., Pangea	Non-exclusive
S97-079	"293T Cell Line"	Very High Transfection Efficiency	Rigel Eli Lilly	Field Exclusive Non-exclusive
S97-150	"Edu. Prog. for Gifted Youth"	Education	MiraeNet Co.	Geo. Exclusive
S97-152	"Mouse Ab contra C Terminal"	Antibodies	Strategene	Non-exclusive

OTL Staff gets hooked...up to the Stanford Phone System

Therefore, all OTLers have new phone numbers! Please note these for future reference. Our area code is now 650.

Blanca Revuelta	723-0651	Kellyanne Ebisui	725-9116
Brenda Martino	725-9119	Kirsten Leute	725-9407
Celeste Beirne-Ryan	725-9120	Linda Chao	725-9408
Floyd Grolle	723-0690	Luis Mejia	723-0651
Glennia Campbell	725-9114	Maria Gladfelter	725-9410
Hans Wiesendanger	723-0651	Mary Watanabe	725-9411
Jessica Smith	723-1586	Mona Wan	725-9035
Jon Sandelin	725-9404	Sally Hines	725-9036
Katharine Ku	725-9037	Sandra Bradford	725-9037
Kay Ankerbrand	725-9388	Susana Ching	725-9038

Nifty Fifty Festivities for OTL

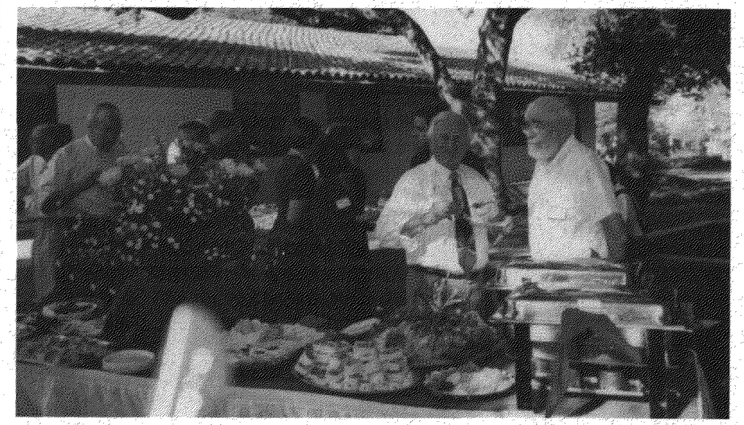
OTL's Big 5-0 festivities, held September 3, 1997, in Alumnae Grove at Stanford, was a rousing time complete with music, a video, terrific food and new and old friends. The Big 5-0 was in celebration of OTL achieving a year-end income of over $50 million (see page 4 for the figures).

Gary Leuenberger, owner of Enchanter Productions, pleased the crowd with his musical talent. His instruments included keyboards, a breath controller and the Yamaha VL 70M Physical Modeling box which incorporates Sondius-XG™ technology (see *Brainstorm*, Summer 1997). With these instruments, Gary provided music which included synthesized drum, flute, guitar and saxophone sounds. Overheard was the comment "I read about Sondius-XG™ in the OTL newsletter, but I had no idea how amazing it is!"

Also for entertainment, OTL showed a new video about the office entitled Connections. The video was produced by the Stanford Center for Professional Development (SCPD) and is being used to help introduce inventors, industry personnel and other interested parties to OTL.

However, the main entertainment was conversation. Inventors, lawyers, patent agents, associates and OTLers all converged to enjoy the beautiful weather and one anothers' company for a momentous occasion in OTL history.

Thank you for joining us in our celebration!



"The Spread" at the OTL Big 5-0 Party. Besides the aroma of good food, the atmosphere was full of music, chatter, laughter...and an occasional yellow jacket.

OTL's Homepage Gets a Facelift...

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technology in this section every month.

"Corporate User Info" describes many of the possible interactions between Stanford and industry. You can even see what OTL's boilerplate license agreements look like.

For the University members of the audience, the next category on the menu will be of interest. This section includes information on the OTL Graduate Fellowship Fund, the OTL Research Incentive Fund, the patent process, Stanford policies associated with technology transfer, a marketing abstract example and much more.

The marketing abstract example is of particular note. OTL encourages inventors to be more involved in the marketing of their inventions. Besides discussing possible industry interests with the Associate handling the invention, OTL would like to elicit the aid of the inventors to write the abstract for the marketing letters. Inventors know their technology best and therefore can write more succinct and comprehensive description.

"Entrepreneurial Information" is a page of links to sites to help Stanford's many budding entrepreneurs. OTL would like to help companies developing from the Stanford community and hope this site will be of assistance.

"Technology Transfer" is a page of links to other Universities, research centers, patent information sites, and technology transfer information sites.

Now linked to our site is the Sondius-XG™ homepage. This link will transport the user to Sondius-XG™ land, the future in sound synthesis.

Invention disclosures can now be submitted online, but to ensure the security of the information being transmitted, the submitter must have a Stanford SUNet ID. This electronic submission has been added to ease the disclosure process for our inventors and to facilitate the transfer of information to the OTL database.

Last on the list is "Search the Available Technologies Database." Though this database has been up and running for a while, a new search engine has recently been added that will facilitate finding particular technologies that are available for licensing from Stanford.

OTL currently has descriptions of many of its available technologies located in the online database. However, please contact OTL if the search does not prove fruitful. Some technologies have not yet been released to the web. The database is updated daily.

Please contact Mary Watanabe at mary@otlmail.stanford.edu with any comments or insights on our reformatted and updated homepage.

OTL Fiscal Year 1996-97
(Preliminary Figures)

Total Income: $51.8 Million (M)
Cohen-Boyer DNA Patents:
- **Total Income: $38.5 M**
- **New Licenses: 31**

All Other Technologies:
- **Total Income: $13.3 M**
- **New Licenses: 122**

Companies in which Stanford took equity: 8

Distribution:
- **OTL Budget: $1.8 M**
- **Other Institutions: $17.9 M**
- **SU Departments: $8.5 M**
- **SU Schools: $8.4 M**
- **Inventors: $7.6 M**
- **Research Incentive Fund: $3.7 M**

Genetic Footprinting...

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netics laboratories, relying on PCR, DNA sequencing gels, and transposons or a transposase enzymes. "All of the tools are there," said Smith.

A patent on the process issued in March of this year as U.S. Patent Number 5,612,180.

So why haven't the biotechnology companies been breaking down OTL's doors to license the technology? As Smith, Brown and Crowley all commented, it is often difficult to change mindsets and long practiced procedures. "People are still skeptical," said Crowley, "and they're not used to thinking about the advantages of looking at hundreds or thousands of different mutants in parallel."

Brown has a positive outlook for the future of the genetic footprinting procedure, due in part to its key features: it's fast, easily implemented and more thorough than other methods.

"I believe that once the key reagent - the transposase or integrase enzyme - becomes widely available," said Brown, "genetic footprinting will become the method of choice for rapidly dissecting the functional organization of cloned genes."

For more information on genetic footprinting, please contact Jessica Smith at (650) 723-1586 or jessica@otlmail.stanford.edu.

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