

The NEB Transcript

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SNP Maps and the Promise of Pharmacogenomics

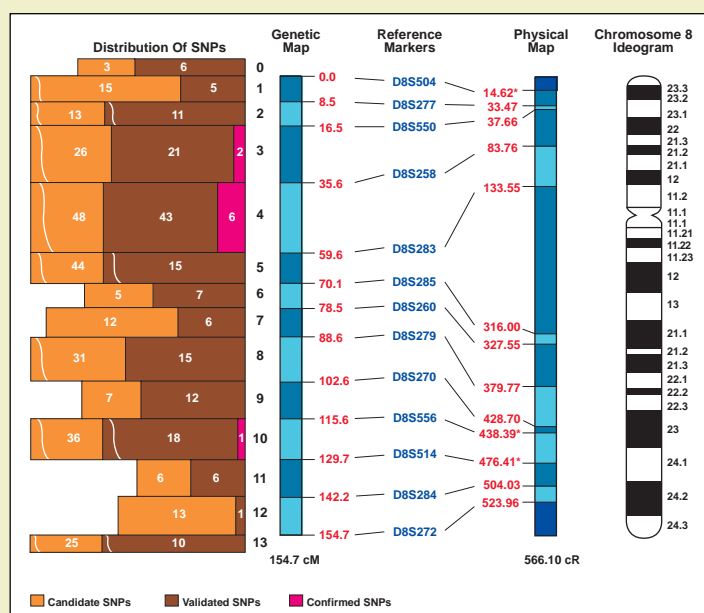
Nadia S. Halim and David Altshuler, Ph.D., Whitehead Institute for Biomedical Research

The draft sequence of the human genome, recently published in *Nature* (1) by the Human Genome Project public consortium and in *Science* (2) by a private company, represents a major milestone in science. The nearly-completed map of the human genome—the genetic blueprint for a human being—is more than 95 percent complete and covers 96 percent of the genome. Embedded within our genomes are the sequences of the approximately 30,000 genes that underlie human biology and medicine. One of the central goals of the post-genome sequencing era will be to relate individual genes to specific diseases and to find the variations in our genes that influence an individual's risk of becoming ill. As most common diseases and many drug responses have been shown to be influenced by inherited differences in our genes, studying genetic variance can improve our understanding and treatment of disease. One promising approach is to focus on common genetic variations that exist in the human population.

If a region of the human genome is sequenced from two randomly chosen individuals, 99.9 percent of the examined DNA will be identical. Of the 0.1 percent that differ, more than 80 percent will be single nucleotide polymorphisms (SNPs) (3): a single base is swapped for an alternate (rather than many nucleotides being altered), and both versions are observed in the general population at frequencies greater than 1 percent. As SNPs constitute the bulk of human genetic variation, they can be used to track inheritance of genes in traditional family-based linkage studies. SNPs can also be used to test, by way of epidemiological

association, susceptibilities to common diseases such as cancer, diabetes and heart disease.

Based on the promise of SNP research, ten pharmaceutical companies put aside their differences and invested millions to create The SNP Consortium, an international collaboration of academic centers, pharmaceutical companies and a private foundation in 1999. The consortium set an initial goal of discovering 300,000 SNPs that would



Distribution of SNPs on Chromosome 8.
 Source: The National Cancer Institute's Cancer Genome Anatomy Project
<http://lpg.nci.nih.gov/html-snp/imagemaps.html>

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New Restriction Enzymes

BceA I (cleavage site revised)

A C G G C (N)₁₂ ▼
T G C C G (N)₁₄ ▲

#R0623S	25 units	\$55
#R0623L	125 units	\$220

BfrB I (*Nsi I* neoschizomer)

A T G C A T
T A C G T A

#R0625S	250 units	\$55
#R0625L	1,250 units	\$220

BspCN I (Note: cleavage site varies)

C T C A G (N)₁₀ ▼ and C T C A G (N)₉ ▼
G A G T C (N)₈ ▲ G A G T C (N)₇ ▲

#R0624S	100 units	\$55
#R0624L	500 units	\$220

Hpy99 I (r , new recognition sequence)

C G W C G ▼
▲ G C W G C

#R0615S	100 units	\$55
#R0615L	500 units	\$220

Hpy188 I (r , new recognition sequence)

T C N G A
A G N C T

#R0617S	1,000 units	\$55
#R0617L	5,000 units	\$220

Hpy188 III (r , new recognition sequence)

T C N N G A
A G N N C T

#R0622S	500 units	\$55
#R0622L	2,500 units	\$220

HpyCH4 IV (r , *Mae II* isoschizomer)

A C G T
T G C A

#R0619S	500 units	\$55
#R0619L	2,500 units	\$220

HpyCH4 V (r , *CviR I* isoschizomer)

T G C A
A C G T

#R0620S	100 units	\$55
#R0620L	500 units	\$220

(r = recombinant enzyme)

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be available to the public without charge by April of 2001. The ultimate hope is that such a SNP map will increase the efficiency of drug development and the efficacy of therapeutic compounds.

Far outstripping its original goal, The SNP Consortium in collaboration with the International Human Genome Sequencing Consortium has created a catalogue of more than 1.4 million SNPs—approximately one SNP every 2,000 to 3,000 bases, pinpointing the exact location of each in the human genome (4). This publicly available SNP map, and others created by private companies, promise to advance our knowledge of the links between genes and disease. The SNP effort will serve as the bedrock of pharmacogenomics, the emerging field of personalized medicine in which drug treatments and preventative strategies are specifically tailored to suit an individual's genetic profile. Comprehensive SNP maps will also facilitate attempts to trace human history through gene analysis. By drawing on genetic, anthropological and archaeological data, looking specifically at inherited groups of genes and SNPs, researchers can learn about the movements of human populations over time.

The SNPs most likely to have a direct impact on the protein product of a gene are those that change the amino acid sequence and variants in gene regulatory regions, which control protein expression levels. Of the 1.4 million SNPs currently on the public map, about 60,000 (4 percent) are located in protein coding regions called exons. Relatively few of these transform amino acids since many are "silent" changes. The number of regulatory SNPs is completely unknown. It is possible, however, to use SNPs to track associations to disease without necessarily finding each functionally important SNP up-front, due to a phenomenon called linkage disequilibrium (LD).

Linkage disequilibrium refers to the observation that SNPs in a certain region often track together in the population, such that nearby SNPs can serve as proxies for each other in a disease study. This means that a subset of SNPs spaced throughout the genome might allow a comprehensive test of common genetic variation across the entire genome. Although the specific number (density) of SNPs needed for such an approach is not known, it is believed that the 1.4 million SNPs in the public domain will offer a sufficient number for LD studies at most regions of the genome.

In cases where a single base change in the genome sequence is necessary and sufficient to cause disease, it has become possible to identify the causal change and enhance our understanding of disease. The first such example was sickle cell anemia, which is caused by the substitution of the nucleotide thymine for adenine at a single position in the gene that produces the hemoglobin molecule that causes the disease. Later, using ever more powerful approaches, literally hundreds of human diseases have been explained by genetic approaches. However, nearly all of these diseases have been rare. For the common diseases responsible for most morbidity and mortality, the genetic contributions are no less certain but have proven more difficult to discover.

This is because in most cases the influence of gene variants is subtle. Geneticists believe that a number of mutations scattered across the genome, coupled with environmental factors, influence the risk of contracting many common diseases. Even if the causal mutations are themselves common in the population, their effects will be difficult to discover. As the relevant SNPs constitute just a handful out of the millions in the human genome, fast and efficient tools will be needed for comprehensive studies. Furthermore, as the effects of any given SNP may be modest, it will be necessary to study large patient samples to observe associations in a reproducible fashion.

To narrow down the search from a genome-wide catalogue of SNPs, scientists often prioritize genes based on hypotheses about gene function. This so-called “candidate gene” approach takes advantage of prior knowledge about disease pathogenesis, family-based linkage data or gene expression patterns to identify genes relevant to disease. Researchers then look for SNPs in those particular genes and compare their frequency in affected and control individuals. For instance, genetic association studies have been used to establish important links between polymorphic variation in the coagulation factor gene F5 and deep-vein thrombosis (5) and in the chemokine receptor gene CCR5 and susceptibility to HIV infection (6). These “susceptibility genes” may directly influence an individual’s likelihood of developing the disease.

Whole genome studies, in which a researcher surveys the entire genome of affected and control individuals to look for SNPs, offers an alternative to the candidate gene approach. This strategy becomes absolutely necessary when no clues are available about the molecular basis for a disease. It can also provide new hypotheses about the disease for researchers to pursue.

Both candidate gene and whole genome approaches may yield some SNPs that fall within genes and actually cause disease, but most will play no obvious role in disease onset or drug response. LD can be used to track down a susceptibility gene, without any prior information about the gene. In principle, when strong LD is present between a SNP marker and a nearby disease-causing SNP, both may show a similar association with the disease. This approach can be very effective in isolated and recently expanded populations, but LD is not predictable and other populations may not exhibit close associations.

LD has been successfully used in linkage studies of families with multiple affected

individuals to uncover genes for monogenic diseases—those caused by a single gene and passed through Mendelian genetics (7,8). Although LD has not been successfully applied in whole gene studies to evaluate unrelated individuals for either disease or drug response to date, the current high-density SNP maps will facilitate large association studies.

The gargantuan job of associating SNPs to a specific disease or drug response is going to require detecting many SNPs at once in an efficient, cost-effective manner. Toward this end, several genotyping platforms have been developed (9), although none has yet emerged as the standard for future work. Certain methods

(continued on page 4)

Introducing NEB's Quick Ligation Kit

ligate cohesive or blunt ends in just 5 minutes at room temperature

NEB introduces a new kit to help save time when performing DNA ligations. The Quick Ligation™ Kit includes Quick T4 DNA Ligase and a PEG-containing reaction buffer, both of which have been optimized to enable ligation in 5 minutes or less (see graph). Detailed protocols for a variety of standard ligation reactions are included with the kit.

Advantages:

- **Fast**—5 minutes for cohesive or blunt ends
- **Convenient**—ligation performed at room temperature
- **Easy**—includes enzyme, buffer and protocols
- **Flexible**—suitable for all common ligation reactions

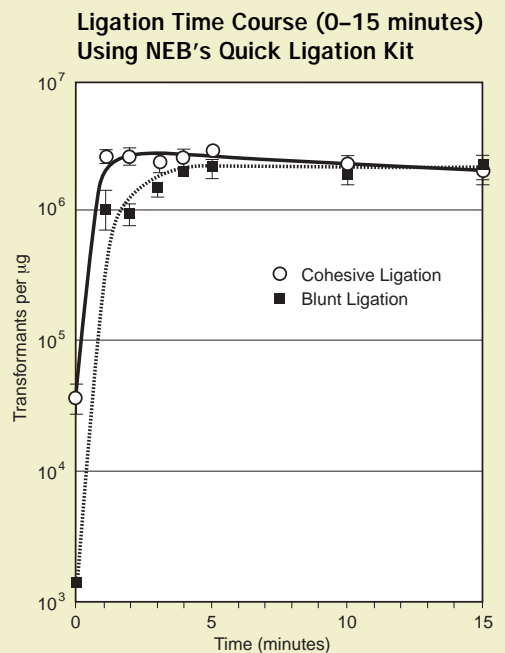
Ordering Information:

Quick Ligation Kit

#M2200S	30 rxns	\$90
#M2200L	150 rxns	\$360

The System Includes:
Quick T4 DNA Ligase,
QuickLigation Buffer (licensed under U.S. patent 4,582,802) and protocols

For more information, visit the NEB website at: www.neb.com



Ligation Time Course: LITMUS 28 vector (NEB #N3628S) was cut with either EcoR V (blunt) or Hind III (cohesive), treated with calf intestinal alkaline phosphatase and gel purified. Blunt inserts from a Hae III digest of ϕ X174 DNA and cohesive inserts from a Hind III digest of λ DNA were ligated into the respective vectors at a 3:1 insert:vector ratio using the Quick Ligation Kit. Ligation products were transformed into chemically competent *E. coli* DH-5 α cells and grown overnight on LB-amp plates at 37°C.

Increased Units/\$

At NEB, enzyme production is linked to basic research in the cloning and overexpression of restriction/modification enzyme systems. Presently, over 120 recombinant restriction endonucleases are available from NEB, as well as numerous recombinant modifying enzymes for DNA and protein.

Alw I (r , 2.5X more units/\$)

#R0513S	250 units	\$55
#R0513L	1,250 units	\$220

Bcl I (r , 1.5X more units/\$)

#R0160S	3,000 units	\$50
#R0160L	15,000 units	\$200

BsrF I (r , 3.3X more units/\$)

#R0562S	500 units	\$55
#R0562L	2,500 units	\$220

I-Ceu I (r , 2.5X more units/\$)

#R0699S	250 units	\$60
#R0699L	1,250 units	\$240

Mly I (r , 10X more units/\$)

#R0610S	1,000 units	\$55
#R0610L	5,000 units	\$220

Mse I (r , 2.5X more units/\$)

#R0525S	500 units	\$55
#R0525L	2,500 units	\$220

Ple I (r , 10X more units/\$)

#R0515S	250 units	\$55
#R0515L	1,250 units	\$220

Pml I (2X more units/\$)

#R0532S	2,000 units	\$55
#R0532L	10,000 units	\$220

Sss I Methylase (r , 2X more units/\$)

#M0226S	100 units	\$55
#M0226L	500 units	\$220

Furin (2X more units/\$)

#P8077S	50 units	\$100
#P8077L	250 units	\$400

(r = recombinant enzyme)

(continued from page 3)

are based on modifications of the traditional DNA sequencing approach, where a single base (that of the SNP) is sequenced in each patient. This approach, termed single base extension (SBE) (10), can use a range of detection methods, such as radioactivity, fluorescence resonance energy transfer (FRET) or fluorescence polarization (FP). More recently, SBE-tagged arrays on glass slides have been used to genotype large numbers of SNPs in parallel (11). DNA chip-based microarray (12) and mass spectrometry genotyping (13) technologies are the latest development in the genotyping arena. Which of these methods, or others, will become most useful is not yet clear. A method that involves the pooling of patient DNA samples has been suggested to reduce the overall number of genotypes needed. Pooling, however, presents technical challenges and prohibits subgroup analysis (14).

At a current average price of one dollar per genotype, SNP detection in large-scale genotyping studies is still prohibitively expensive. Even at one cent per genotype, the cost per person in a typical association study testing 100,000 SNPs will be about \$1,000, possibly adding \$1 million to the cost of a clinical trial (15). Significant advances will be necessary to make extensive genotyping a standard part of clinical trials.

Though the current SNP maps will be invaluable tools for finding statistically significant associations between a SNP and drug response or disease, there are still many significant technical and analytical challenges that must be overcome before the promise of SNPs can be fulfilled. Scientists don't fully understand the genetic architecture of common traits underlying disease and drug response. Association studies are complicated by many unknowns that contribute to a disease, such as the number of genes, the number of variants in each gene, and the frequency of a variant within a population. The location of a variant SNP, whether it is in the coding region, regula-

tory region or the non-coding region of the genome, and how this contributes to disease is still not clear. The interaction of individual SNPs and degree to which they travel together in LD is also under intense investigation.

Other issues that must be addressed to unlock the full potential of SNPs involve the groups of individuals being studied. The sample sizes in association studies need to be large enough to achieve adequate statistical power. The large number of SNPs and the low probability that any given one causes disease will require larger groups of people to be studied to determine a statistically valid disease association. This raises the problem of accurately phenotyping individuals because in different patients the same disease may manifest itself in different ways. It will also be necessary to develop better genotyping technologies and better analytical and statistical tools to fully interpret the data (16).

New ethical issues will have to be tackled as SNP technology improves and becomes more widely used. Current genetic tests typically look for single-disease genes. But SNPs may give rise to genetic tests that produce a long list of diseases to which a person is susceptible. Not only will a person have to cope with this information, but access to the information will have to be protected so it is not misused by employers or insurers. Researchers are just beginning to grapple with the question of how to keep sensitive phenotypical and genotypical information confidential.

With the first round of human genome sequencing complete, attention is now focused on identifying functions for each of the 30,000 or more human genes and determining which play a role in disease. The recognition that most variation among individuals is attributable to a finite number of common SNPs has led researchers to the task of characterizing and cataloging this shared universe of genetic differences.

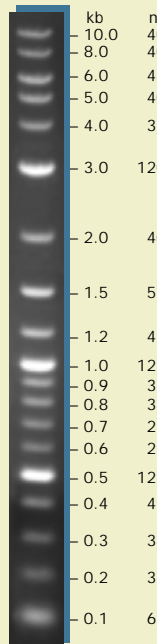
Figuring out how to relate this encyclopedia of human genetic variation to disease will be one of the great challenges of 21st century biomedicine. Although this task appears quite demanding, it offers tremendous opportunities in our search to understand and ultimately target a broad array of diseases.

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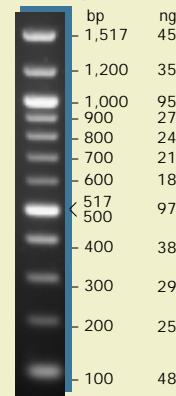
DNA Ladders: 100 bp, 1 kb and 2-Log

2-Log DNA Ladder



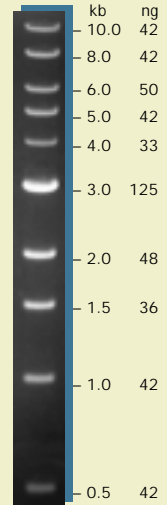
1.0 µg of 2-Log DNA Ladder on 1.0% TBE Agarose Gel

100 bp DNA Ladder



0.5 µg of 100 bp DNA Ladder on 1.3% TAE Agarose Gel

1 kb DNA Ladder



0.5 µg of 1 kb DNA Ladder on 1.0% TAE Agarose Gel

DNA Ladders from NEB Feature:

- Uniform Band Intensities
- Easy-to-Identify Reference Bands
- No Extra High Molecular Weight Bands
- Defined Mass Profile for Sample Quantification

Free Samples:

A limited quantity of 2-Log DNA Ladder samples are available free-of-charge. Simply return the attached reply card with an indication in the comments area that you would like to receive a 2-Log DNA Ladder sample. International customers without reply cards, please contact your local NEB distributor.

Ordering Information:

2-Log DNA Ladder

#N3200S	100 µg	\$55
#N3200L	500 µg	\$220

1 kb DNA Ladder

#N3232S	100 µg	\$55
#N3232L	500 µg	\$220

100 bp DNA Ladder

#N3231S	50 µg	\$55
#N3231L	250 µg	\$220

For more information, visit the NEB website at: www.neb.com

Properties of Exonucleases and Endonucleases

Richard Whitaker, Ph.D. and Elisabeth Raleigh, Ph.D., New England Biolabs, Inc.

Advances in molecular biology research require that new methods of manipulating DNA are devised while earlier ones are improved. New England Biolabs is committed to providing a wide variety of essential

reagents that serve to advance the frontier of DNA-based manipulations.

The table below is intended to be used as a guideline. Not all reported activities and properties for each exonuclease or endonuclease are

listed. The amount of enzyme, substrate and time of incubation can have a dramatic effect upon the desired outcome of the experiment.

For further information, please refer to our Catalog, website <www.neb.com> or the product technical datacard which accompanies each enzyme.

Enzyme	Polarity	DNA Substrate		Activity without 5' Phosphate	Initiate at ds DNA with ¹ :				Partial Digestion	
		ss	ds		5' ext	3' ext	blunt	nick	Generates Extension ²	Products Produced ³
Lambda Exo	5'→3'	+/-	+	+/- ⁴	+/-	+	+	-	3'	ss DNA, dNMP
T7 Exo	5'→3'	-	+	+	+/-	+	+	+	3'	ss DNA, dNMP, dinucleotide
Exo III	3'→5'	-	+	+	+	+/-	+	+	5'	ss DNA, dNMP
RecJ _f	5'→3'	+	-	+	+/- ⁵	-	+/- ⁶	-	NA	dNMP
Exo I	3'→5'	+	-	+	-	+/- ⁵	+/- ⁶	NR	NA	dNMP, dinucleotide
Exo T	3'→5'	+	-	+	-	+ ⁷	+/- ⁶	NR	NA	dNMP
BAL-31 Nucl	3'→5'; endo ⁸	+	+	+	+	+	+	+	NA	ss DNA, dNMP
Mung Bean Nucl	endo ⁹	+	-	+	+	+	-	-	NA	ss DNA, ds DNA
T7 Endo I	endo ¹⁰	-	+	NA	NA	NA	NA	+/-	NA	ds DNA

Table Legend:

+ activity
- no significant activity
+/- activity greatly reduced relative to preferred substrate
NR not reported
NA not applicable
ss single-stranded
ds double-stranded
ext extension
dNMP deoxyribonucleoside monophosphate

Footnotes:

¹ The ability to act on short extensions and blunt ends operationally distinguishes these enzymes; such ends are conveniently generated by restriction digestion.

² Partial digestion of ds DNA by Lambda Exonuclease, T7 Exonuclease and Exonuclease III will produce ds DNA products with ss extensions. If digestion goes to completion, ss DNA will be produced.

³ Complete hydrolysis of the preferred substrate will generate the listed products. It has been reported that the first product hydrolyzed from dsDNA by T7 Exonuclease is a dinucleotide. Subsequent hydrolytic cleavage by T7 Exonuclease releases dNMP. Exonuclease I releases dNMP from ss DNA, except at the last hydrolytic step where a dinucleotide is produced.

⁴ Lambda Exonuclease initiates degradation very poorly without a 5' phosphate. Thus, if linear ds DNA has a 5' phosphate at one end and lacks a 5' phosphate on the other end, then Lambda Exonuclease will preferentially degrade the DNA from the phosphorylated end.

⁵ RecJ_f is not suitable for making 5' extensions blunt and Exo I is not suitable for making 3' extensions blunt. Both RecJ_f and Exo I appear to require long extensions to initiate.

⁶ Depending upon the DNA sequence and amount of exonuclease, RecJ_f, Exonuclease I and Exonuclease T may remove a few nucleotides from blunt termini.

⁷ Exo T can be used to make 3' extensions blunt, but it was 2–4 fold less efficient than DNA Polymerase I, Klenow Fragment plus dNTP on a test substrate.

⁸ BAL-31 Nuclease has been reported as having ss endonuclease activity as well as 3'→5' ds exonuclease activity. Thus, any linear DNA is a substrate for BAL-31 Nuclease.

⁹ Mung Bean Nuclease is an endonuclease specific for ss DNA.

¹⁰ T7 Endonuclease I recognizes and cleaves non-perfectly matched DNA, cruciform DNA structures, Holliday structures or junctions. It will act more slowly on nicked ds DNA.

Ordering Information:

Lambda Exonuclease	r	RecJ _f	new, r	BAL-31 Nuclease	
#M0262S	1,000 units \$55	#M0264S	250 units \$55	#M0213S	50 units \$50
#M0262L	5,000 units \$220	#M0264L	1,250 units \$220	#M0213L	250 units \$200
T7 Exonuclease	r	Exonuclease I (<i>E. coli</i>)	r	Mung Bean Nuclease	
#M0263S	1,000 units \$50	#M0293S	3,000 units \$55	#M0250S	1,500 units \$50
#M0263L	5,000 units \$200	#M0293L	15,000 units \$220	#M0250L	7,500 units \$200
Exonuclease III (<i>E. coli</i>)	r	Exonuclease T	new, r	T7 Endonuclease I	
#M0206S	5,000 units \$50	#M0265S	250 units \$55	#M0292S	250 units \$55
#M0206L	25,000 units \$200	#M0265L	1,250 units \$220	#M0292L	1,250 units \$220

r = recombinant enzyme

Why choose a recombinant enzyme from NEB?

At New England Biolabs, enzyme production is linked to basic research in the cloning and overexpression of restriction/modification enzyme systems. Our focus on providing the largest selection of recombinant enzymes has resulted in lower \$/unit cost and improved purity and consistency of product. We are the only company which continues an aggressive research program in the cloning and overexpression of these essential reagents. Presently, over 120 recombinant restriction endonucleases are available from NEB, as well as numerous recombinant modifying enzymes for both DNA and protein.

Purity

Once an enzyme system is cloned, the choice of expression vector and strain background allows tight control over the production environment. For restriction endonucleases, this eliminates enzymes known to contaminate native preparations. Although our recombinant enzymes and native enzymes are manufactured to meet the same rigorous quality control standards, it is recombinant enzymes that produce a more pure product with less processing time.

Consistency

Typically, the yields obtained for recombinant and overexpressed enzymes are significantly larger than those produced by native strains. Larger lots means greater product consistency and less lot-to-lot variation. Further, this large lot capability is ideal for customers that have a need for bulk quantities of enzymes. It simplifies their quality assurance procedures by reducing the time and effort normally spent qualifying enzymes supplied from different lots.

Expertise

NEB focuses on the analysis of restriction/modification systems at the molecular level. Our goal is to understand the regulation of these specialized systems and how they interact with DNA. This expertise is available to our customers as part of the NEB commitment to customer service.

Magnetic Beads: Protein A, Protein G, Streptavidin

New England Biolabs now supplies Protein A, Protein G and Streptavidin-coated magnetic beads and a Magnetic Separation Rack for the selective purification of proteins and nucleic acids. Magnetic bead technology offers several advantages over traditional affinity column chromatography or centrifugation, including the ability to work in small volumes, minimal sample loss and gentle treatment of samples.

Recombinant Protein A and ProteinG magnetic beads exhibit strong specific binding for the Fc region of IgG antibodies while the antigen binding sites are left free. The bead matrices consist of purified recombinant Protein A or Protein G covalently coupled to a 1 μm nonporous supermagnetic bead. The proteins are coupled via a linkage that is stable over a wide pH range. This permits the immunomagnetic purification of IgG from ascites, serum or cell culture supernatants. The immunocomplex can then be directly eluted into SDS-PAGE gel loading buffer. In addition, specific antibodies can be chemically cross-linked to the Protein A or ProteinG coated surface to create a

reusable immunoprecipitation bead, avoiding co-elution of antibody with target antigen.

Streptavidin magnetic beads are available for the capture of biotin-labeled antigens, antibodies and nucleic acids. Captured substrates can be used as ligands in subsequent experiments including mRNA isolation using biotinylated Oligo d(T)₁₈ (NEB #S1325S).

Advantages of Magnetic Beads:

- **Convenient**—no centrifugation required; matrix can be regenerated without loss of binding capacity
- **Efficient**—minimal sample loss during pipetting because magnetic beads concentrate at the side of the tube instead of the bottom
- **Flexible**—Protein A and Protein G magnetic beads allow for the small-scale purification or immunoprecipitation of IgG species

Ordering Information:

Magnetic Separation Rack

#S1506S holds 6-tubes \$175

Special Offer: for a limited time, rack includes free samples of Streptavidin and Protein A Magnetic Beads.

Protein A Magnetic Beads

#S1425S 1 ml \$125

Capacity: > 400 μg human IgG/ml

Protein G Magnetic Beads

#S1430S 1 ml \$150

Capacity: > 400 μg human IgG/ml

Streptavidin Magnetic Beads

#S1420S 20 mg \$200

Capacity: > 500 pmol single-stranded 20 bp biotinylated oligonucleotide/mg



NEB's Magnetic Separation Rack holds six 1.5 ml Eppendorf tubes and, for a limited time, is supplied with free samples of Streptavidin and Protein A Magnetic Beads.

For more information, visit the NEB website at: www.neb.com

Neglected Flowers: The Decline of Honey Bees in North America

Margaret Goddard and Douglas Taron, Ph.D., Peggy Notebaert Nature Museum

As a follow up to our 00/01 Catalog's theme of Nature's Services, Margaret Goddard and Douglas Taron of the Peggy Notebaert Nature Museum, address current threats to honey bees (Apis mellifera).

America has traditionally been depicted as the land of plenty. Images of “amber waves of grain” and mountains rising “above the fruited plane” are celebrated in our anthems. Rich natural resources and the hard work of people over the generations account for much of this bounty. But credit is also due to heroes who are rarely the subject of ballads—pollinators that make agriculture possible by enabling flowering plants to reproduce. An agent, or vector, which varies for different plant species, deposits grains of pollen on the stigma of a flower. Wind, insects, birds and bats pollinate a wide array of species in their respective habitats.

Insects, by and large, don't get a lot of respect in our society, yet honey bees and other insect pollinators make a big contribution to the American economy and to the environment as a whole. Honey bees (*Apis mellifera*) are especially useful for pollinating crop monocultures, in part because they forage in groups due to their dependence upon the hive. Honey bee populations have dipped sharply in the last half century owing to a variety of threats—a disturbing trend that has attracted little public notice, despite the importance of this species to our agricultural system.

Imported to America from Europe in the 1600s, honey bees are generalist pollinators: they visit the flowers of many species during a season, but stick to the most rewarding species during a given day. In this way, the honey bee reaches flowers at their prime during the bloom cycle and

communicates its nectar findings to other foragers in the hive. Thus, a large area of a single flowering species will be combed for pollen and nectar by a large group of insects and thoroughly pollinated in the process. Although other bee species are also effective pollinators, they forage in smaller numbers than honey bees. Furthermore, they are poorly suited to the domestication and relocation required to facilitate the pollination of large crop acreages.

**“An estimated
1.6 million honey bee colonies
have died since the
appearance of these
pathogens”**

Overall, honey bees pollinate about 15 percent of the world's crops. In the United States, honey bees pollinate more than 90 cultivated crops—mainly fruits, vegetables, and nuts—serving as the principal pollinators both for commercial farms and

backyard gardens. Apple and almond growers, for example, rely heavily on the labors of the honey bee. The almond crop in California's Central Valley alone, which is pollinated by commercial honey bees, is worth about \$800 million a year. According to one analysis based on increases in crop yield and quality, managed honey bees provide anywhere from about \$2 to \$15 billion in agricultural services each year in the United States.

Apis mellifera is unquestionably valuable, regardless of which figure is more accurate, yet it is also a species in decline. The U.S. population of managed honey bees has decreased four-fold since the end of World War II, from about 6 million colonies to 1.5 million today. Meanwhile, wild honey bees have fared even worse: about 90 percent of the population has been wiped out in that time, with feral colonies barely hanging on. Pollination biologist Gary Nabhan calls the loss of honey bee colonies, which fell sharply in the 1990s, “one of the most severe declines U.S. agriculture has ever experienced in such a short period.” The abruptness of the drop in honey bee populations in the United



Immature stage (white) and adult female (brown) varroa mites on a drone bee that was removed from its cell just prior to emerging on its own. ©Dr. Scott Camazine

States has raised questions about potential impacts on natural ecosystems as well as on agriculture.

For decades, the chief menace to honey bees has come from a handful of fronts: industrial pollution, the related problems of habitat destruction and ecosystem fragmentation, the excessive use of chemical pesticides and harsh winter weather. A new peril has emerged since the 1980s, with the discovery of two pathogens that had never been seen before in the United States: Varroa and tracheal mites. An estimated 1.6 million honey bee colonies have died in this country since the appearance of these pathogens. Although the bee losses may not be solely attributable to the mites, this new threat warrants special consideration.

Varroa (*Varroa jacobsoni*) are external mites that lay their eggs alongside bee larvae inside the brood cell. The Varroa larva attaches to the bee larva, and the adult mite attaches to the exoskeleton of the adult bee. Varroa was most likely introduced to the United States through uninspected shipments of bees. Early hypotheses of a South American origin have been revised in light of molecular marker studies, and the mites are now thought to have originated in eastern Russia (1). Tracheal mites (*Acarapis woodi*) live and reproduce inside the breathing tubes of the honey bee, blocking oxygen flow. Both mites feed on the blood of the bee, eventually leading to the demise of their host, although the precise cause of death is still unknown. Researchers describe the effects of infestation as "parasitic mite syndrome."

One possibility is that mite infestation could weaken the colony, with a virus or other pathogen then taking advantage of the impaired immune systems. A researcher at Pennsylvania State University reports losses in managed bee colonies of 80%, 53%, and 40% percent during the winter of 1995–

1996 in Maine, Pennsylvania, and Delaware, respectively (2). Further examination revealed that those beekeepers who did not treat their colonies for mites or disease lost 71.6% of their colonies compared to a 25% loss when colonies were treated with a combination of antibiotics, miticide and Fumidil-B for nosema (2). Resistance to the current miticide Apistan has begun to appear after only five years of use (2). Additional mite management strategies under development include the selective breeding of resistance to both mites and viruses (3).

If there was ever any doubt, the mite episode has made it abundantly clear that measures to protect honey bees and other key pollinators are urgently needed. Surprisingly, these issues are not a high priority for most conservationists. The fact that many of the most important pollinators are insects—and therefore receive less attention than more celebrated creatures like right whales, bald eagles, and condors—has not helped the cause. The 2000 World Conservation Union (IUCN) Red List, a database of species threatened with extinction worldwide, lists only one bee species and 2,135 species of birds, leaving no doubt that invertebrates are significantly underrepresented on the agendas of many wildlife protection groups.

On a more hopeful note, greater public awareness concerning the plight of honey bees may help spur conservation efforts directed towards other, less familiar pollinators. All told, more than 100,000 species of insects, worms, birds, mammals, marsupials and reptiles—including bees, butterflies, moths, wasps, beetles, birds, bats, opossums and geckos—provide essential pollination services. These animals help perpetuate not only our croplands and gardens but also our forests, pastures and meadows.

Of course, it is not realistic to wage a campaign to "save" 100,000 different animal species, but it would still be prudent to identify the world's most important pollinators and then devise strategies to protect them. With an effective course of action, we might continue to reap the fruits of our agricultural wealth, while doing our part to maintain a planet with healthy ecosystems and varied life forms.

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Doug Taron is Curator of Biology for the Peggy Notebaert Nature Museum, part of the Chicago Academy of Sciences. Although trained as a biochemist, Doug now spends his time managing the biological components of the Museum's exhibits. In particular, he runs Butterfly Haven, a 2700 square foot greenhouse where visitors can walk among butterflies from around the world.

Margaret Goddard is a multimedia artist, writer, beekeeper and exhibit technology consultant who began working with honeybees in 1995. Her exhibits place an enclosed colony of live bees in close proximity to the observer to allow for an intimate view that provides the full sensory experience of the hive interior for safe reflection and observation opportunities not possible in the wild. Two versions of these exhibits have been presented at the School of the Art Institute of Chicago (1995) and at the Portland Institute for Contemporary Art (1997).

Ligation Detection Reaction (LDR): Enabling Multiplexed Detection of Known Polymorphisms

Jodene Fitness, Ph.D., Wellcome Trust Centre for Human Genetics, Oxford University

Ligation Detection Reaction (LDR) provides an elegant technique for the multiplexed typing of SNPs, micro-deletions and insertions. Developed by Francis Barany (1), LDR utilizes the ability of DNA ligase to preferentially seal adjacent oligonucleotides hybridized to target DNA in which there is perfect complementation at the nick junction.

To type a bi-allelic SNP, three probes are designed, one common and two allelic (Figure 1). The common probe anneals to the PCR amplified template immediately downstream of the nucleotide in question. One allelic probe has at its 3' end the nucleotide corresponding to the wildtype allele. The other has at its 3' end the nucleotide corresponding to the variant allele. These two allelic probes compete to anneal to the template adjacent to the common probe. This generates a double stranded region containing a nick (missing phosphodiester bond) at the nucleotide position to be tested. Only the allelic probe

with perfect complementation to the template will be ligated to the common probe by the DNA ligase. Utilization of thermostable Taq DNA Ligase enables repeated thermal cycles, resulting in a linear increase in ligation product (Figure 1).

The allelic probes can be designed to have unique lengths, so that the wildtype and variant ligation products can be separated on the basis of size. Alternatively, the allelic probes can be labelled with different fluorescent dyes, enabling the ligation products to be discriminated by their color.

This approach can equally be applied to the typing of micro-deletions and insertions, as well as SNPs. For example, LDR can be used to achieve the sometimes difficult task of distinguishing a string of 7 Ts from a string of 8 Ts (2).

Multiplexed LDR

One of the most useful aspects of LDR is that sets of probes for a number of polymorphic sites can be multiplexed together, enabling several polymorphisms to be typed simultaneously. Furthermore, these polymorphisms need not be present in the same PCR amplified template *i.e.*, several polymorphic sites located on different templates can be investigated simultaneously.

Multiplexing a number of reactions in the same tube requires that none of the LDR probes will anneal to each other, that the LDR probes all share similar annealing temperatures and that each ligation product can be differentiated either by length or the label used.

Applications of Multiplexed LDR

Multiplexed LDR is very amenable to diagnosis and carrier screening of inherited disorders in which many different mutations may result in the same disease. Cystic Fibrosis (CF) is one such disease. Although there are over 400 mutant CF alleles known,

30 mutations account for over 96% of CF chromosomes worldwide. Four multiplexed, single color LDR assays, each containing seven or eight mutations, can be used to detect the presence or absence of all 30 mutations (3). Alternatively, a single three color assay can be used to screen for all 30 mutations simultaneously (3).

Congenital Adrenal Hyperplasia (CAH), an inherited inability to synthesize cortisol, is another disorder in which a number of different mutations in one gene may result in more or less the same disease. Over 90% of CAH cases are due to Steroid 21-Hydroxylase Deficiency (21-OHD) and nine mutations account for the majority of 21-OHD alleles (4). Severe CAH occurs in approximately 1/15000 live births (5) and without treatment can result in neonatal death due to hyponatremic dehydration and shock. Thus, in many countries, newborns are screened for CAH using a simple, inexpensive hormonal ELISA, which measures the cortisol precursor 17-hydroxyprogesterone (17-OHP) in spots of dried blood stored on Guthrie cards (filter paper). A high 17-OHP level is normally an indicator of CAH, but as cortisol production is up-regulated in response to stress, elevated 17-OHP levels may also occur in neonates that are sick, premature or otherwise stressed. This results in a number of false positives (between 0.02 and 0.2% depending on the screening program) that cannot be accurately retested until the baby is older or healthier (6). A delayed diagnosis is undesirable because the onset of a potentially lethal salt-wasting crisis can occur very rapidly. In 1995, Day *et al.* described a multiplexed LDR assay that enabled detection of the presence or absence of the nine common 21-OHD mutations simultaneously (2). This LDR assay can be used on DNA obtained from Guthrie card blood samples and therefore it could be performed immediately on newborns with high 17-OHP levels, enabling earlier initiation of treatment, and thus providing

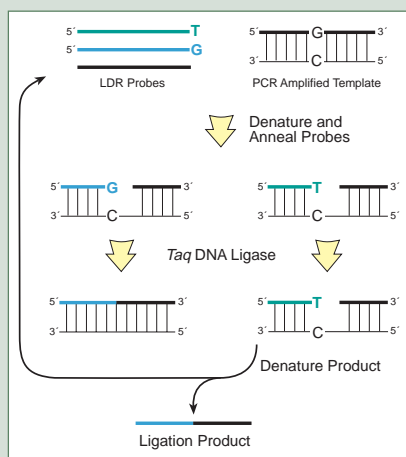


Figure 1: LDR Typing of a G → T SNP.

The allelic probe corresponding to the wildtype allele is shown in blue and has a G at its 3' end. The common probe is shown in bold black. In this example, the template has been amplified from an individual homozygous for the wildtype G allele. Thus only the wildtype allelic probe is ligated to the common probe, generating a single type of ligation product. The thermal cycling is repeated a few times in order to generate sufficient ligation product for detection.

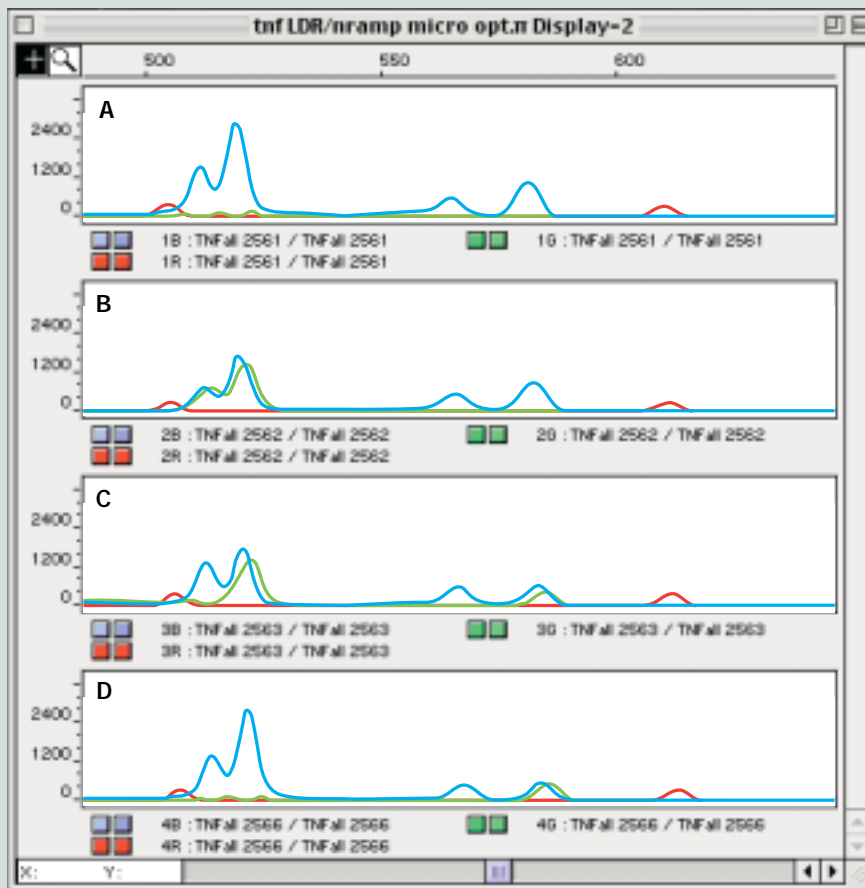


Figure 2: Chromatograms of TNF α Promoter Multiplexed LDR Products. LDR Probes were designed for four polymorphic sites in the promoter region of TNF α (-238, -308, -857, -863). Poly (A)⁺ tailing of the allelic probes ensured the ligation product corresponding to each of the four polymorphic sites had a unique length. Each wildtype allelic probe was labeled with 6-Fam (blue). Each variant allelic probe was labeled with Tet (green). The fluorescently labeled ligation products obtained after multiplexed LDR were electrophoresed and detected using an ABI Prism 373 DNA Sequencer. Internal size standards (GS-350) were included so that the size of the ligation products could be accurately determined. The panels show fluorescence chromatograms; the fluorescence count is on the Y axis and the length in base pairs is on the X axis. The blue peaks represent the wildtype ligation products. The green peaks represent the variant ligation products. The red peaks are the size standards. Panel A shows the chromatogram of an individual homozygous wildtype for each of the four sites. Panels B, C and D show the chromatograms of individuals heterozygous at one or more of the four sites.

a useful adjunct to the hormonal newborn screen for CAH (7).

Our lab is largely concerned with the genetics of host susceptibility to a number of infectious diseases including Tuberculosis, Leprosy, HIV, Hepatitis B and C, Pneumococcus, Staphylococcus, and Malaria, in several different populations from around the world. Since its introduction to the lab two years ago, we have routinely used multiplexed LDR to type sets of polymorphisms in genes that are candidates for disease susceptibility, including, for example, the gene for the cytokine TNF α (Figure 2). Part of the

appeal of the LDR technique was that no particularly specialized equipment was necessary—just thermal cyclers and preferably a fluorescent DNA sequencer for analysis. Usually from three to five polymorphisms are included in one assay. Even if the probes for several polymorphisms cannot be multiplexed together, due, for example, to incompatible annealing temperatures, the products can still be pooled together, enabling multiplexed analysis, and thus saving costs on gel runs and size standards. In our lab, the LDR approach is certainly much preferred to dot blotting.

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Taq DNA Ligase for LDR

Taq DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini of two adjacent oligonucleotides which are hybridized to a complementary target DNA. The ligation will only occur if the oligonucleotides are perfectly paired to the complementary target DNA and have no gaps between them; therefore, a single-base substitution can be detected.

Utilization of thermostable Taq DNA Ligase for Ligation Detection Reaction (LDR) enables repeated thermal cycle reactions which linearly increase product. In addition, performing ligation reactions at higher temperatures increases annealing stringency.

Ordering Information:

Taq DNA Ligase		r
#M0208S	2,000 units	\$65
#M0208L	10,000 units	\$260

For more information, visit the NEB website at: www.neb.com

r = recombinant enzyme

The Sensitivity of Restriction Enzymes to Methylated DNA

Richard J. Roberts, Ph.D., New England Biolabs, Inc.

For most bacteria and archaea, DNA methylation is ubiquitous. It has proven both fascinating and intellectually rewarding to the few molecular biologists, like myself, who study it. In eukaryotes, 5-methylcytosine often occurs in CG or CNG sequences, where it can impact a variety of biological processes (1). For most working molecular biologists though, all of this DNA methylation can be an irritation, because it sometimes prevents restriction enzymes from cleaving as expected. However, the annoyance can be short-lived because several resources exist that can both minimize its impact and help to recognize it when it occurs. In REBASE (rebase.neb.com/rebase) (2), there is a wealth of information about the known impact of methylation on cleavage by restriction enzymes. Furthermore, many of the common cloning systems employ bacterial strains that are devoid of the DNA methylases that might cause problems. Nevertheless, methylation effects that come from the DNA being cloned or from the use of poorly characterized cloning hosts can still bring surprises.

In 1975, my laboratory serendipitously discovered an enzyme, *Msp* I, from a contaminant that appeared during fermentation. At first it seemed that *Msp* I was merely an isoschizomer of *Hpa* II. It recognized the sequence C/CGG and, just like *Hpa* II, cut between the two C residues. However, in 1978, while studying the cleavage of human DNA containing the β -globin gene, Waalwijk and Flavell discovered that *Hpa* II and *Msp* I, while superficially appearing to be identical, responded differently to DNA methylation (3). When the central CG residue in the CCGG recognition sequence was fully methylated with 5-methylcytosine, as occurs in many CG sequences in mammalian DNA, *Hpa* II failed to cleave it,

whereas *Msp* I cleaved as expected, irrespective of the state of methylation. This was the first pair of enzymes ever discovered that showed this differential sensitivity to methylation.

Soon others were found, such as *Mbo* I, which will cleave GATm5C, but is unable to cleave Gm6ATC (4). In contrast, *Sau*3A I can cleave Gm6ATC, but cannot cleave GATm5C (5). As more enzymes were discovered and tested for their abilities to cleave methylated DNA, many examples were found of restriction enzymes that were blocked by methylation at sites other than those normally methylated by the natural protective DNA methyltransferase, the so-called cognate methyltransferase. This was put to positive use when several clever schemes were devised for generating “new specificities” for restriction enzymes by using the fact that selected sequences could be protected by non-cognate methylation (6).

At first it seemed that the effects of non-cognate methylation were all or nothing, either they blocked or were cleaved completely. However, it soon became apparent that in many cases these non-cognate methylation events serve not to block cleavage completely, but rather just to impair cleavage, either slowing it down so that complete digestion could only be achieved with a large excess of restriction enzyme, or in some cases, slowing digestion to a point where it seemed that only partial cleavage could ever be achieved. Clearly, all of these effects were really caused by the unusual methylation affecting the rate of cleavage. However, because rates have rarely been quantitated, various qualitative parameters have been used to describe the effects. When considering the effects of these heterologous methylation events it should be noted that often methylation

may only be present on one strand of the restriction enzyme recognition site. Thus, when DNA is grown in normal *E. coli* strains, it is methylated by the Dam methylase, which forms Gm6ATC (7). If one of these Dam methylated sites overlaps with another restriction enzyme’s recognition site, say *Mbo* II (GAAGA), then cleavage is blocked completely in Dam methylated strains. In this case, the sequence GAAGm6ATC that contains the overlap has just one methyl group in the strand shown, but this is still sufficient to block cleavage completely.

Beginning in 1981, McClelland began compiling lists showing how restriction enzyme cleavage was affected by methylation (8). Similar data was incorporated into the restriction enzyme compilations put together by Kessler (9) and these efforts continued until 1994 (10). I have recently gone back and rigorously checked all of this earlier information against the original literature or have communicated with the original contributing authors of unpublished data. Errors have been removed and all of the reliable methylation sensitivity information has been incorporated into REBASE, which is now the only up-to-date source. For the most current and accurate data, readers are referred to the REBASE web site where clicking on the “Methylation Sensitivity” icon will lead to various compilations of the data, all displayed in full double-stranded format. Data about enzymes from individual companies that supply restriction enzymes can be found from the “Supplier” pages including data on the sensitivity to Dam, Dcm, CpG and the strain specific methylation found in *E. coli* K and B strains.

Two kinds of methylation that are commonly encountered, and which might cause unexpected results, are the products of the Dam (Gm6ATC) and Dcm (Cm5CWGG) methylases of *E. coli* (7). Most manufacturers of restriction enzymes are aware of these

potential methylations and test their enzymes for sensitivity. Often they just report the results as sensitive or insensitive. Rarely do they report impairment of cleavage or differentiate between the effects of full and hemi-methylation. For this reason it is always wise to check REBASE, where these effects are clearly differentiated and displayed in double-stranded form.

Several other kinds of methylation may also be encountered. Occasionally, *E. coli* strains are employed for cloning that still carry the methylases from either the *EcoK* I (11) or *EcoB* I (12) restriction systems. These both lead to adenine methylation as shown below:

```

m6
AACNNNNNNNGTGC
TTGNNNNNNNCACG
m6

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m6
TGANNNNNNNNTGCT
ACTNNNNNNNNACGA
m6

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These methylations may also lead to confusion, but because they occur infrequently they are rarely mentioned in catalogs. These effects are also included in REBASE, whenever they have been tested experimentally. An additional common source of problems comes when analyzing DNA from mammalian sources, where most occurrences of the dinucleotide CG actually occur as m5CG. This modification frequently affects restriction enzyme cleavage, as was observed for *Hpa* II above. Here though things get very complicated because not only does one need to consider double-stranded methylation when the CG lies completely within the recognition sequence, but often it may also overlap the sequence when the C forms the terminal residue. Then, it is only when the next base is a G, that it might be important, and the methylation will only be in one strand. In this case the usual printed representation that appears in catalogs can be quite confusing. For instance, when considering *Ban* I (recognition sequence: GGYRCC) five different cases need to be considered.

(continued on page 15)

Try NEBcutter

a new program for restriction enzyme site mapping

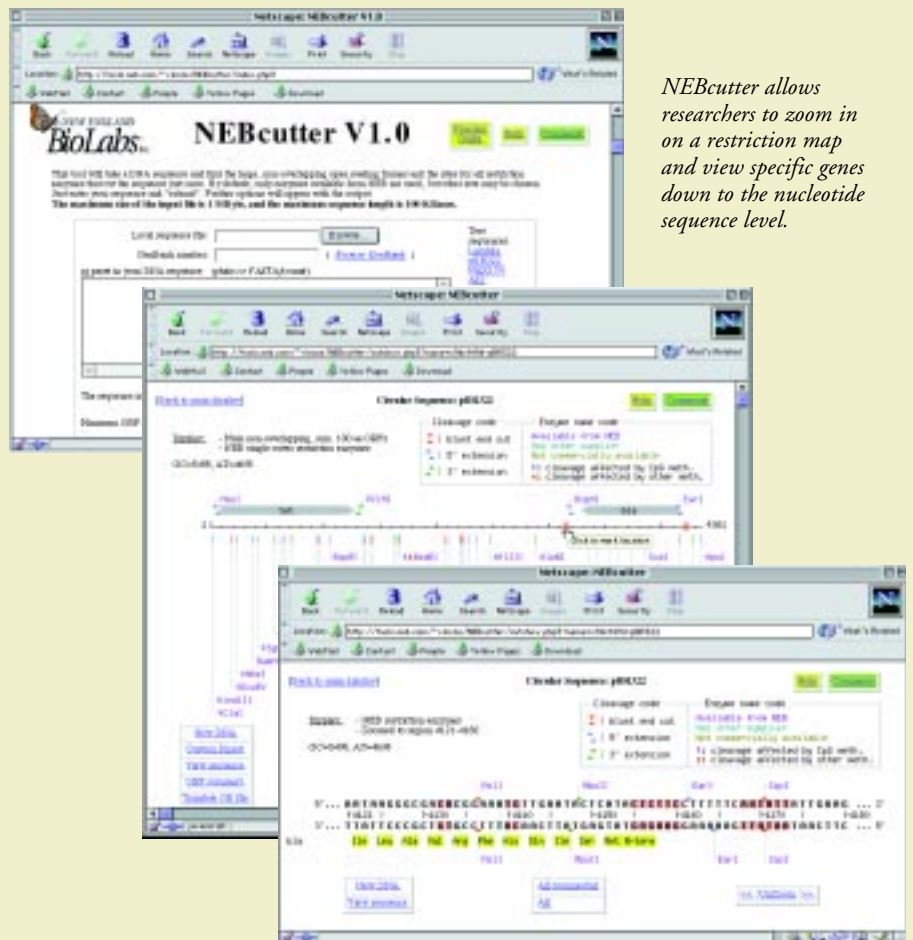
NEBcutter, a new computer program available on the NEB website, will find restriction enzyme recognition sites in DNA sequences. The program accepts sequences retrieved from a local file or from NCBI as a Genbank file via its accession number. It also accepts an input sequence pasted into a designated field. NEBcutter can calculate a restriction map using NEB enzymes or all commercially available restriction enzymes included in REBASE, the restriction enzyme database.

When presented with a sequence, NEBcutter will find the largest open reading frames within the sequence and indicate those restriction enzymes

that flank the open reading frame and could be used to excise the gene. It also locates the positions of all restriction enzymes that cut only once within the sequence chosen. Other options include custom digestion with enzymes of your choice and various displays of those digests, including computer-generated gel displays.

A unique feature of this program is that it is fully aware of the information on the methylation sensitivity of restriction enzymes and alerts a user to overlapping methylation by *dam*, *dcm*, etc.

We welcome comments so that future versions can incorporate additional useful features.



NEBcutter allows researchers to zoom in on a restriction map and view specific genes down to the nucleotide sequence level.

Phospho-specific Antibodies in Immunohistochemistry

Brad Smith, Ph.D., Cell Signaling Technology, Inc.

The variety of diagnostic tools available to the research scientist, clinician and pathologist seeking to characterize tumors and develop cancer therapies has increased greatly in the last few years. Immunohistochemical analysis of patient tissues has long been a technique used by pathologists to aid in classification. As drug therapies become increasingly targeted to specific cellular proteins and cellular events, the characterization of signaling pathways in tumors will become critical in determining the most efficacious treatment. Combining the specificity of immunohistochemistry with the power of activation-state specific antibodies will provide powerful new

tools for pathology, drug development and basic cancer research.

Gene expression analysis by cDNA microarrays and other molecular techniques has redefined the concept of tumor profiling. Using a broad range of activation-state specific antibodies and immunohistochemical analysis will provide function-based tumor profiling. Such an analysis will reveal to the scientist which pathways are activated in a tumor and which may make good drug targets. One example of such an application is the analysis of the ErbB2/HER2 MAP kinase pathway in breast cancer. Using Cell Signaling Technology (CST) phosphorylation-specific antibodies, the researcher can easily determine the activation status of this critical pathway

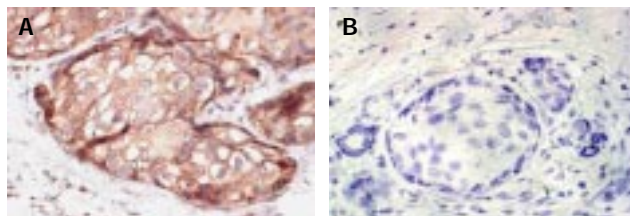
and predict the effectiveness of treatments such as herceptin. Another important example is the analysis of the Akt survival pathway and the predicted effectiveness of drugs that inhibit this pathway.

Determining the efficacy of a drug treatment in the preclinical setting is of great importance to the biotechnology industry. CST cleavage-specific antibodies have great utility in allowing the researcher to easily detect apoptosis in a morphological context. Similarly, CST phosphorylation-specific antibodies may be used to determine pathway inhibition. These applications take advantage of the high sensitivity and cellular and tissue specificity allowed by immunohistochemistry in comparison to other standard techniques such as Western blot analysis.

The range of antibodies offered by CST provides many opportunities for studying novel sites for drug intervention. Beyond the obvious pathways implicated in tumorigenesis, mechanisms that promote metastases or deregulate apoptosis may be analyzed using immunohistochemistry. For example, chromatin regulation via histone acetylation, HDAC function and the regulation of such proteins as Rb may provide critical targets for future drug development.

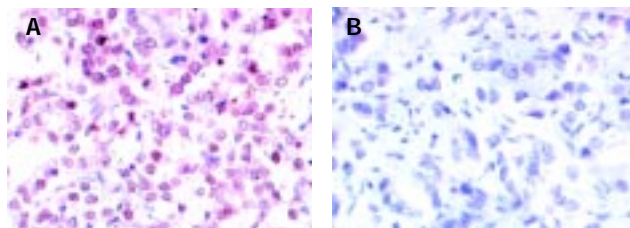
In summary, while standard immunohistochemical analysis of tissue sections has been a well accepted

Phospho-HER2/ErB2 (Tyr1248) Ab



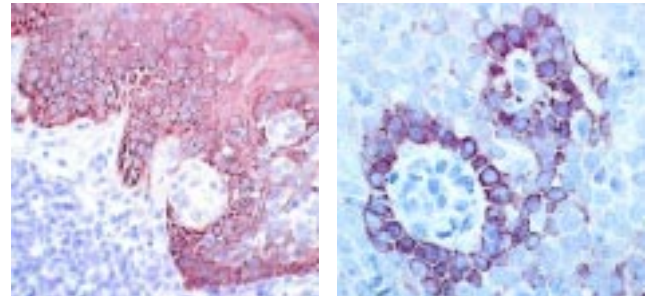
Immunohistochemical staining of paraffin-embedded human breast tumor with Phospho-HER2/ErB2 (Tyr1248) Antibody showing cytoplasmic and membrane staining (A). Antigen-specific phospho-peptide preabsorption blocks immunohistochemical staining of phosphorylated HER2/ErB2 in human breast tumor (B).

Phospho-Akt (Ser473) Ab (IHC Specific)



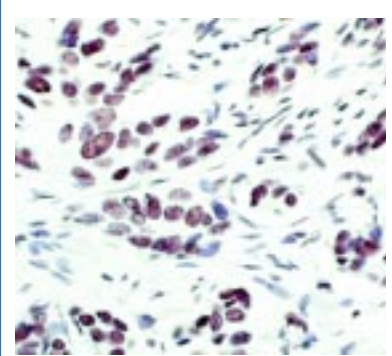
Immunohistochemical staining of phosphorylated Akt in formalin-fixed, paraffin-embedded human prostate tumor using Phospho-Akt (Ser473) Antibody (IHC Specific) against phosphorylated Akt (S473) showing nuclear and cytoplasmic staining. In image A, the antibody is pre-absorbed with an irrelevant peptide and in image B, the antibody is pre-absorbed with the phospho-Akt S473 peptide demonstrating the specificity of the immunostaining.

Cleaved Caspase-3 (Asp175) Ab



Immunohistochemical staining of paraffin-embedded human tonsil with Cleaved Caspase-3 (Asp175) Antibody showing perinuclear staining of select epithelial cells (low and high magnification).

Acetyl-Histone H3 (Lys9) Ab



Immunohistochemical staining of formalin fixed, paraffin-embedded human breast carcinoma with Acetyl-Histone H3 (Lys9) Antibody showing nuclear localization of acetylated histone.

technique in pathology and research, new antibodies developed by CST offer powerful new immunohistochemical tools for exploring the underlying mechanisms of cancer. These tools will be useful to the pathologist characterizing tumors, the clinician prescribing therapies and the research scientist developing new drugs.

Cell Signaling Technology (CST) is a new company from New England Biolabs. CST and NEB maintain many close scientific collaborations.

For more information about CST antibodies, visit <www.cellsignal.com>.

(continued from page 13)

A final cautionary note applies to investigators working with bacterial DNAs. It is usual that such DNAs are methylated, because most will contain their own restriction systems and their genomes will be modified by the protective methylases. As a result, their DNAs may be refractory to cleavage by certain restriction enzymes. It is always worth checking REBASE to see if there are known restriction/modification systems in your organism and also, whether such systems might cause problems if they overlap with your chosen restriction enzymes. Anyone who has dealt with DNA from *Neisseria gonorrhoeae* will know that because of the extensive methylation in that organism, it is very difficult to find restriction enzymes that cleave it (13). A look at the "Genomes" section of REBASE will give some idea of the general problems likely to be encountered. Of the 34 bacteria and archaea whose genomes had been completely sequenced by December 2000, 193 potential methylase genes are found, of which we suspect more than half will be active. Methylation is common and can lead to unexpected problems, but some help is already at hand and more is coming. We have launched a new computer program, NEBcutter (see page 13), that will take an input DNA sequence and predict the restriction enzyme recognition sites. NEBcutter knows everything that REBASE knows about DNA methylation and will take it into account when helping you to choose restriction enzymes for your experiments.

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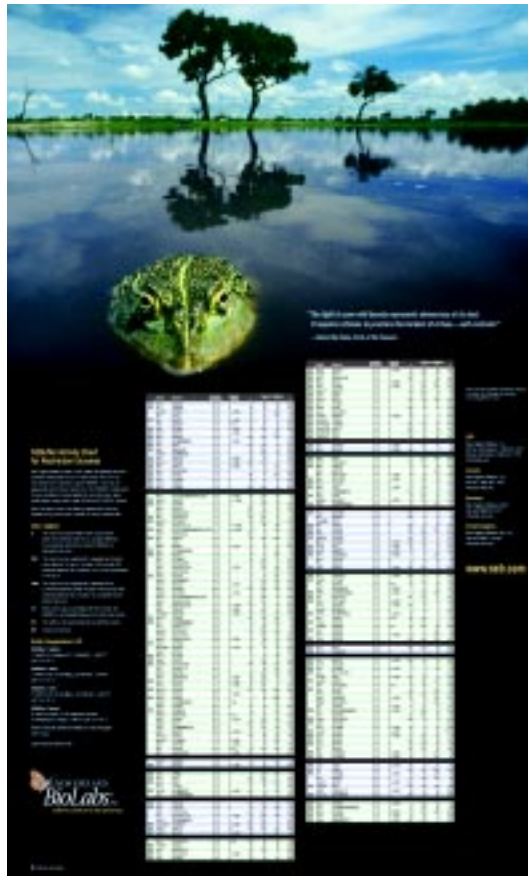
The NEB Transcript is designed and edited by the employees of New England Biolabs, Inc. It is intended to offer technical information and ideas that are useful and thought-provoking. We encourage you to share your comments with us.

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