



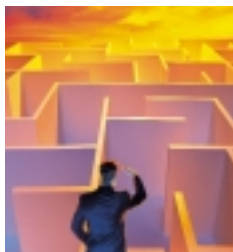
Convenient, Quantitative Gene Expression Analysis is Now in Sight



The I-SAGE™ Kit takes advantage of SAGE™ technology so you can:

- Find low-abundance transcripts
- Detect up- or down-regulated genes
- Measure a compound's effect on expression
- Identify novel genes

Quantitative Gene Expression and Novel Gene Discovery



Identifying all of the genes expressed in a tissue or cell line is critical in understanding pathogenesis and gene regulation. DNA arrays provide some clues but are limited by their finite collection of genes and the variability of hybridization. SAGE™ (Serial Analysis of Gene Expression) is the only technology that allows you to detect and quantify all of the genes expressed in a cell—even low-abundance transcripts—whether known or novel. The I-SAGE™ Kit provides pre-qualified, performance-guaranteed reagents so you can take advantage of SAGE™ in your own lab and get the gene discovery results you need.

Powerful SAGE™ technology in a convenient kit

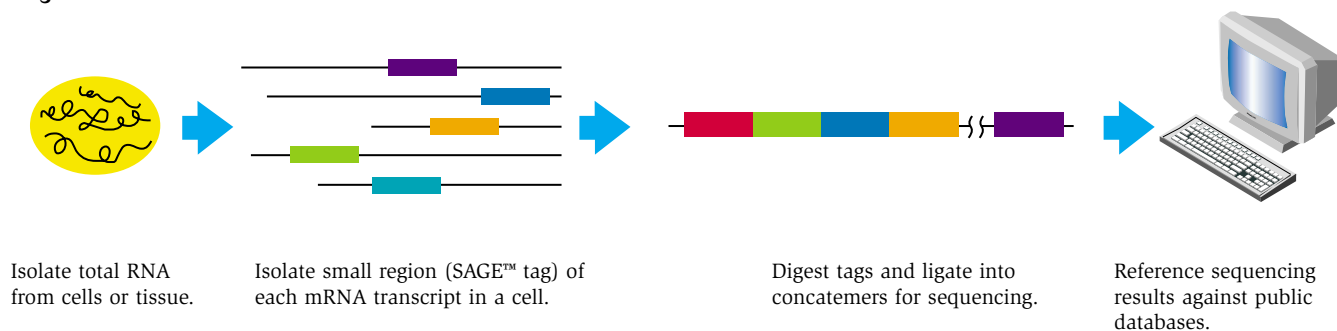
SAGE™ is the most powerful, unbiased method for quantitative, genome-wide gene expression analysis. SAGE™ technology can be used to:

- Compare expression between two cell lines (1)
- Measure expression of low-abundance transcripts (2)
- Discover new genes (3)
- Describe a transcriptome—the expression profile of all transcripts from a genome (4)

The I-SAGE™ Kit is the only kit that provides ready-to-use reagents for convenient construction of SAGE™ libraries.

The I-SAGE™ Kit is based on the original protocol described by Velculescu *et al* (5). The I-SAGE™ protocol allows construction of SAGE™ libraries by isolating "tags", defined 14 bp regions of each transcript, rather than the entire sequence. These small tags are sufficient to uniquely identify a transcript. The tags are ligated into concatemers, cloned, and sequenced in high-throughput to generate raw data for analysis. SAGE™ analysis software is used to list and count each tag from the raw data (Figure 1). The tags can then be matched to a reference database for identification and gene expression analysis.

Figure 1 – Overview of the SAGE™ method



Profiles include every gene

SAGE™ allows you to analyze and count every transcript that is expressed in a cell so you can produce a comprehensive and quantitative profile of gene expression. Unlike cDNA and oligo microarrays, SAGE™ is not limited to a collection of genes printed on a pre-made chip, slide, or filter. SAGE™ is not affected by the variability of probe labeling or background from hybridization. Compared to sequencing EST tags,

SAGE™ is amenable to high-throughput sequencing. And unlike differential display and subtractive hybridization procedures, gene amplification using the SAGE™ method is unbiased (Table 1). With the power of SAGE™ you can detect every transcript, even those expressed at low-copy number, which often results in the identification of rare and novel genes.

Table 1 – Comparison of genome-wide gene expression analysis methods

	Comprehensive	Quantitative	Detects low-abundance genes	Detects novel genes
SAGE™	+	+	+	+
Microarrays	–	±	±	–
EST analysis	±	±	±	±
Differential display	±	–	–	±

- + = Ideal for this type of analysis
- ± = Limited for this type of analysis
- = Not ideal for this type of analysis

SAGE™ extensively cited

SAGE™ can detect low-abundance transcripts, novel genes, and find proprietary targets, revealing a genome-wide expression profile for in-depth analysis. Visit www.invitrogen.com/SAGE

to view a list of references that depict the various fields—such as human, cancer, plant, and yeast studies—where SAGE™ has been used to successfully analyze gene expression patterns.

The proven advantage of SAGE™

SAGE™ is a highly efficient, accurate, and sensitive method for performing genome-wide expression analysis. The PCR step avoids bias because all templates are 100 bp, giving you quantitative, accurate results. SAGE™ quantifies mRNA from both known and unknown genes so there's no need to have prior knowledge of all transcripts. To demonstrate, SAGE™ was used to identify approximately 300,000 transcripts

present in both normal and cancer tissues. Out of these transcripts, 86% were expressed at less than five copies per cell (1). About 49% of the low-abundance transcripts discovered by SAGE™ did not match any entry in public databases. These represent undiscovered genes that had not been previously described or characterized.

Performance-guaranteed I-SAGE™ Kit

The I-SAGE™ Kit includes reagents for every step of the SAGE™ process organized into nine modules. Included are modules for cDNA synthesis, restriction digests, ditag formation and amplification, and cloning. Each component in the I-SAGE™ Kit is tested to ensure stringent performance specifications are met. These quali-

ty-tested reagents save you time because you don't have to rely on old reagents in your freezer or contact numerous suppliers to assemble your own kit. All components are tested to work in concert so you can construct high-quality SAGE™ libraries.

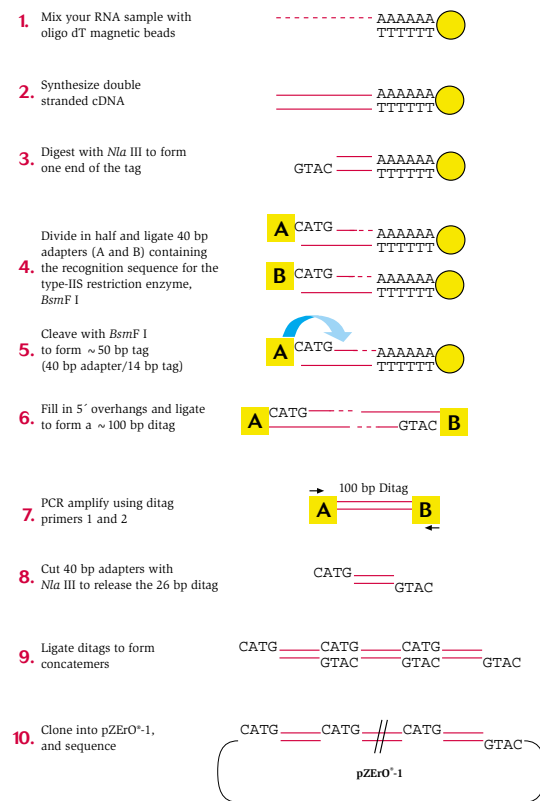
Figure 3 – The I-SAGE™ Kit



Strategic I-SAGE™ protocol

The construction of SAGE™ libraries with the I-SAGE™ Kit is broken down into 10 steps (Figure 2). The I-SAGE™ protocol begins with cDNA synthesis and restriction digestion to create individual 14 bp tags for concatemerization, cloning, and sequencing. Double-stranded cDNA is synthesized from 5 µg of total RNA on magnetic beads and digested with the restriction enzyme *Nla* III that cuts on average every 250 bases (Figure 2, Steps 1-3). This creates one end of the tag. An adapter that contains a site for the type II restriction enzyme *Bsm*F I is ligated to the tag (Figure 2, Step 4). Digestion with *Bsm*F I results in cleavage 14 bp away from its recognition site, releasing the tag from the bead and creating the other end of the tag (Figure 2, Step 5). The overhang from the *Bsm*F I digest is filled in and the tags are ligated together to form a 100 bp ditag containing the adapter and tag sequences. (Figure 2, Step 6). The ditags are PCR amplified to generate sufficient template for cloning (Figure 2, Step 7). PCR is unbiased because the starting material is a homogenous pool of short, uniform 100 bp templates. The 100 bp ditags are digested with *Nla* III a second time to release the adapter sequence from the ditag, leaving a CATG overhang for concatemerization (Figure 2, Steps 8 and 9). Approximately 10-20 ditags are ligated into the cloning vector pZErO®-1*, creating clones that contain 20-40 individual tags for high-throughput sequencing. Using the I-SAGE™ Kit protocol you can construct a SAGE™ library with > 100,000 tags for genome-wide expression analysis.

Figure 2 – The I-SAGE™ protocol



*The pZErO®-1 vector utilizes positive selection to eliminate high background when cloning. It also contains the small Zeocin™ resistance gene to reduce the overall vector size for increased ligation and transformation efficiencies. Please see p. 16 of the 2001 Invitrogen Catalog or www.invitrogen.com for more information.

Quantitative gene analysis software

When you purchase an I-SAGE™ Kit, you'll receive access to SAGE™ software for analyzing your sequencing data. SAGE™ software tabulates the occurrence of each tag sequence, or transcript, using the *Nla* III CATG as a punctuation reference and generates a report of each tag and its abundance level. You can identify the sequence of each SAGE™ tag by comparing it to the SAGEmap reference DNA database (www.ncbi.nlm.nih.gov/SAGE) (6). Tags with no entry in

the database can be used as probes to screen cDNA libraries for novel gene discovery. You can also compare tag counts from multiple projects and public gene expression databases (SAGEmap). Analysis of 20,000-100,000 tags, or 1,000-2,500 concatemer clones, will generate a relevant profile of gene expression within a cell, providing you with quantitative measurement of low-abundance transcripts for gene discovery.

Complete kit ensures results

The I-SAGE™ Kit provides all of the components necessary for constructing high-quality SAGE™ libraries. All reagents are pre-qualified and tested to ensure your success. You'll save time because you won't have to locate, order, and test each component for activity or compatibility. In addition, the purchase of an I-SAGE™ Kit includes free access to SAGE™ analysis

software for analysis and comparison of expression data through the Invitrogen web site. The I-SAGE™ Kit takes care of all of the preparation so you can save time, build high-quality SAGE™ libraries, and get the accurate genome-wide expression results you need for gene discovery.

Inquire about the convenient I-SAGE™ Kit

SAGE™ is a powerful gene expression monitoring tool that can enrich your gene discovery efforts. Using the I-SAGE™ Kit from Invitrogen, you'll discover novel and low-abundance transcripts with precise measure-

ment. Contact Invitrogen today and ask one of our highly trained Technical Service Representatives about the I-SAGE™ Kit.

Product	Quantity	Cat. no.	Price
I-SAGE™ Kit			
<i>without magnetic stand</i>	5 libraries	T5000-01	\$3100
<i>with magnetic stand</i>	5 libraries	T5001-01	\$3380
Invitrogen Magnetic Stand	1	R670-01	\$294
I-SAGE™ Ditag PCR Module	1000 PCRs	T5000-02	\$510

References:

- Zhang, L. *et al.* (1997) *Science* **276**: 1268-1272.
- Madden, S.L. *et al.* (1997) *Oncogene* **15**: 1079-1085.
- Polyak, K. *et al.* (1997) *Nature* **389**: 300-305.
- Velculescu, V. *et al.* (1997) *Cell* **88**: 243-251.
- Velculescu, V. *et al.* (1995) *Science* **270**: 484-487.
- Lash, A.E. *et al.* (2000) *Genome Research* **10**: 1051-1060.

Easy licensing

Invitrogen offers use of the SAGE™ technology through the I-SAGE™ Kit for non-commercial use in academic organizations. If you belong to a commercial organization and would like access to the I-SAGE™ Kit, please contact Genzyme Molecular Oncology at 508-271-2627 or SAGE@genzyme.com.

SAGE™ is a trademark of Genzyme Corporation. Genzyme is a registered trademark and service mark of Genzyme Corporation. The I-SAGE™ Kit is based on technology owned and licensed by Genzyme Corporation under U.S. Patent No. 5,695,937 and patents pending. For research purposes only. Inquiries regarding licenses should be made to Genzyme.



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