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Differential display technology: a general guide

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Abstract. The 10 years since the invention of differential display technology (DD) has produced a massive amount of literature detailing problems and improvements to the technique, successful gene expression studies and studies done using genes found through the use of DD. In this review we summarise the results of 10 years of research that has focussed on improving DD and discuss how some of the problems associated with DD can be resolved or minimised. In addition to discussing DD, we address issues related to other differential gene expression analysis techniques and try to illustrate how these techniques can be used to complement one's use of DD. This review also serves as an introduction to the taxa-specific DD review articles that are found in this issue.

Key words. Differential display technology; gene expression; microarray; SAGE; subtractive Hybridisation.

Introduction to the differential display technique

Ten years ago, the differential display (DD) technique was developed for studying eukaryotic gene expression [1]. Although there are now many differential expression analysis techniques available, PubMed continues to log hundreds of papers per year in which DD was utilised in primary research. In this introductory review we discuss a large body of DD research (over 3800 publications), thus making the research accessible and leading to more sensible experimental designs that take into account potential problems.

The power of differential display lies in its simplicity. It is a combination of three frequently used molecular biology techniques that allow one to visualise and compare gene expression patterns between two or more samples (see fig. 1 in S. Stein, this issue) [1]. It requires reverse transcription of polyadenylated (poly-A) RNA, polymerase chain reaction (PCR) of the reverse-transcribed complementary DNA (cDNA) using special DD primers and polyacrylamide gel electrophoresis. First, high-quality total RNA is reverse transcribed using reverse transcriptase and one of three anchor primers designed to anneal to the 3′ poly-A tail of messenger RNA (mRNA). The resulting cDNA species are subsequently used as templates in a PCR, utilising the same anchor primer from the reverse transcription reaction in combination with an arbitrary primer. Because nonspecific binding of the primers is maximised and small oligonucleotides (10–13 bp) are used, an expression fingerprint with several cDNA species can be visualised after running the DD product on a polyacrylamide gel (see [2] for review). If fingerprints from two or more samples are visualised side by side, then the samples can easily be compared, and differences in gene expression can be seen (see reviews in this issue for examples). The genes responsible for the differences can subsequently be isolated and characterised. One can conceivably study gene expression comparisons using DD for any number of treatment conditions, phenotypes or genotypes. In addition to the original DD protocol, several modified versions exist. For a comprehensive review of the methods, see [3, 4].

After DD is performed, bands are selected, cut from the gel and confirmed as differentially expressed. Confirmed bands need to be sequenced so the gene responsible for the band can be characterized. Selection of bands is subjective; it depends on experience of the researcher with DD and preference for certain changes in gene expression. The reviews in this issue contain many examples of band selection. There are slightly different protocols for

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extracting and PCR reamplifying the cDNA bands from fluorescent DD (FDD) [5] and from isotope DD [2]. It is important to confirm the differential expression of the gene using Northern blot, quantitative real-time PCR, in situ hybridisation, or RNase protection assay, for false positives have been reported to make up a significant portion of the differentially expressed bands [6]. Typically, bands are cloned before sequencing is done; however, protocols exist that detail direct sequencing of bands from a gel [7, 8]. Problems with direct sequencing arise when multiple cDNA species migrate through the gel at the same rate or when two or more bands are in close proximity. If this occurs, then cloning is necessary. Although not universal, mRNAs have polyadenylation signals (AATAAA) upstream of where the poly-A tails are added. The presence of a polyadenylation signal in a sequence confirms that an mRNA transcript has been identified [2].

Guidelines for using DD

Differential display is only capable of determining the 3′ region of the gene, so full-length cDNA needs to be isolated by probing a cDNA library or by doing rapid amplification of cDNA ends (5′-RACE). Using cDNA libraries is more reliable than 5′-RACE, but it requires more labour. Once the 5′ region is known, the polypeptide produced from this gene can be characterised. There are as many ways to characterise a polypeptide as there are experimental designs. The reviews in this issue are intended to direct readers toward investigations similar to their own so ideas can be easily accessed.

Once the first few primer combinations have been tested, one should examine the results of the differential display. Although experimental designs may vary, a redesign of the experiment should be invoked when more than 5% of the transcripts are differentially expressed or if no differences exist [3, 9]. Matz also suggests using a few DD primer combinations as a method to evaluate the experimental design of any study using differential gene expression analysis [3].

Differential display experiments need to be designed according to a few guidelines, which significantly reduces false differences and increases the probability of finding useful genes [4, 10]:

- **Drastically different conditions should not be compared.** When comparing two different types of tissue (i.e., muscle vs. brain), many distinct genes are going to be expressed from each. These data will indicate little about the function of the tissues other than that they are drastically different from one another. For the same reasons, one should take care not to focus on extreme conditions of a cell type; it may result in many superfluous bands or cause one to miss subtle changes. For example, many gene expression differences caused by stress or drug treatment may have durations of less than 1 h after induction (see table 1 in [6]). If this is overlooked, then important target genes may be missed. An extreme treatment may also cause a massive cascade of events that are only distantly related to the initial treatment, thus increasing the workload of the researcher right off the bat.
- **Always repeat the DD reactions for each sample** to reduce the number of false positives due to reverse transcription and PCR artefacts. False positives have been noted to make up a large portion of the bands examined from DD experiments. As Ivanova and Ivanov point out in their review of DD in yeast (this issue), PCR artefacts may cause false-positive bands. Performing DD at least twice for each sample can control false positives.
- **Multiple samples should be used for each DD experiment.** As Simon points out in his review of DD in birds and amphibians (this issue), one should use multiple individuals for sampling to differentiate within-population variation from condition and treatment variation. We do not, however, recommend pooling multiple samples, for within-population differences may be masked by or may mask differences in gene expression.

Sensitivity of DD to low-abundance transcripts is a concern for many researchers. Bertioli et al. [11] investigated DD sensitivity in leaves infected with the tobacco mosaic virus and in rabbit globin mRNAs because the expression levels of transcripts were known. They found that DD is very biased toward higher-abundance transcripts. However, several problems arise from their procedure to test DD's ability to detect particular transcripts. First, they designed perfectly matched and mismatched arbitrary primers to a gene known to encompass 0.01% of the total mRNA in uninfected tobacco leaves and 1% of the total mRNA population of infected leaves. When they examined the leaves using DD with the designed primers, they did not find any differentially expressed bands. The problem arises when one takes into account the large number of mRNAs a small (10 bp) primer can detect. Other genes with a similar sequence can mask or outcompete the low-abundance gene, whereas a different primer may have been able to detect the target gene. This should be considered when calculating the number of primer combinations one needs to use. In another study, long, sequence-specific arbitrary primers were successfully used with DD to isolate known differentially expressed genes [12]. Bertioli et al. [11] used only one or two, 2-base anchor primers to reverse-transcribe the mRNA into cDNA. This can cause a major problem if the

Table 1. Comparison of Gene Expression Analysis Techniques.

Technique	Sample comparisons	Expression detection	RNA needed	Detectable differences
Differential display	any number	up and down	≤5 μg of total RNA for 240 reactions	1.1-fold or more
Subtractive hybridisation	two	one direction	1–5 μg of poly-A RNA per reaction	>10-fold
RDA	two	one direction	1–5 μg of poly-A RNA per reaction	>15-fold
SSH	two	one direction	1X–5 μg of poly-A RNA per reaction	>10-fold
SAGE	any number	up and down	≥5 μg of total RNA per sample	all differences
DNA Microarray	two (more with normalisation)	up and down	≥10 μg of poly-A RNA per slide	>2-fold

Technique	Threshold of transcript detection	Detection of differences in high copy number transcripts	Complexity
Differential display	low or medium	no	low
Subtractive hybridisation	medium	yes	high
RDA	medium	yes	high
SSH	medium	yes	high
SAGE	low to medium	yes	low
DNA microarray	NA	yes	depends on starting point

Technique	Cost	Redundancy	Detection of novel genes	False positive rate	Detection of differential splicing	Global genetic analysis
Differential display	low to medium	medium	yes	high	yes	no
Subtractive hybridisation	low	medium to high	yes	low	yes	no
RDA	low	medium to high	yes	low	yes	no
SSH	low	low	yes	low	yes	no
SAGE	low to medium	high	no	NA	no	yes
DNA Microarray	high	NA	generally, no	NA	no	yes

mRNA of interest is not in the population of cDNA being tested. The rationale for selecting the anchor primers was not presented in their paper. Finally, subsequent to the publication of this paper, several improvements to the DD method were proposed and implemented [2, 4, 10, 13].

In contrast to Bertioli et al.'s paper, Wan et al. [9, 14] found that DD could detect mRNA species that have a prevalence of as little as 1 in 200,000 transcripts and, on average, detects mRNAs with a prevalence of 1 in 20,000 transcripts. Some argue that because the system studied [response of HeLa cells to interferon (IFN)-γ] has a higher proportion of differentially expressed mRNA species, detection of any one species is more likely [3]. This argument does not take away the fact that DD can detect low-abundance mRNA species. Again, this must be incorporated into the calculation to determine how many primers one should use. In addition, several other investigations prove that many of the genes detected with DD are expressed in low abundance [14–17]. It should be noted, however, that in at least one comprehensive study (240 primer combinations or ~95% coverage of the genome) a gene expected to be differentially expressed was not detected [18]. This raises questions concerning how to calculate percent coverage of the genome with DD.

We are very interested in knowing the threshold needed to detect a difference in gene expression. Routinely, articles are written which provide evidence that twofold changes are easily detected [17–19], and some show that differences as little as 1.1 or 1.5 are detectable [17, 18]. These numbers should be used cautiously, for the detection threshold may be a factor of total amount of transcript present in the samples. It should also be noted that Northern blots were used to measure the relative amounts of the mRNA species between samples except in [19], where quantitative PCR was used. Northern blots are not good for quantitative comparisons. Matz and Lukyanov [3] point out that differences in abundant transcripts may not be detected as differences in DD, for an upper limit with detection may be reached. Methods to counter this problem need to be developed.

DD was designed as a method to understand eukaryotic gene expression. Investigators use DD to simultaneously examine up- and downregulated genes in the context of multiple samples. It is most useful when looking for low- to medium-abundance, novel genes. If the other techniques can be used to examine more than two samples, they need normalisation with housekeeping genes; with DD the controls are built in as a consequence of the protocol (comparison of the ~95% of the bands that are not differentially expressed between samples). However, a drawback of the protocol is that the analysis of large populations of mRNA (i.e. functional genomics) with DD

would require about 240 different primer combinations per sample to achieve 95% coverage of the genome [20]. If extensive gene coverage is desired, one should also consider using other methods for global analysis of gene expression, such as microarrays and SAGE. However, as discussed below, these techniques have major drawbacks when used with organisms for which little or no genomic information is available.

Differential gene expression techniques

Other gene expression analysis techniques exist which complement the DD technique or can be used in place of DD. In this section we will briefly discuss other popular gene expression analysis techniques. The focus will be on the following five areas: (i) description, (ii) complexity, (iii) cited problems, (iv) appropriateness and (v) complementation with DD. The information in the following sections is summarised in table 1.

Subtractive hybridisation

Subtractive hybridization [9, 21, 22] is used to study differences in DNA or cDNA abundance in both eukaryotes and prokaryotes. Two samples can be compared at once and are termed the tester and driver. The driver cDNA population is used to hybridise to the cDNAs in the tester population, and the driver/tester hybrids and driver cDNAs are removed. In theory, this should leave only tester species that do not hybridise to any of the driver species.

The protocol is technically difficult and labour intensive, but the screening is simple once the subtracted library has been made [9]. Only one-way comparisons can be analysed, so two subtraction experiments have to be done to get both up- and downregulated genes. Small differences may be masked, because 25 times more driver than tester is used in the hybridisation reactions [9]. It has also been documented that there is high redundancy of nonrelevant clones, but this can be controlled by normalisation [6].

According to the research of Wan and Erlander [9], subtractive hybridisation can detect low- and medium-abundance mRNA transcripts. Subtractive hybridisation is also good at detecting differences in high-abundance transcripts [3]. It has been recommended that both DD and subtractive hybridisation should be used in any single study so the most diverse differences can be found [9].

Modifications to the original subtractive hybridisation technique have greatly simplified the protocol and improved sensitivity ([23–26] among others). We will focus our discussion on suppression subtractive hybridisation (SSH) [23] and representational differences analysis (RDA) [24, 27, 28], for they have been used most often. Both RDA and SSH are PCR-based subtraction techniques that overcome the need to physically separate single-stranded (ss) and double-stranded (ds) cDNAs. RDA is less technically difficult than subtractive hybridisation, but still requires several rounds of subtraction [23]. The SSH method improves on RDA by suppressing nontarget DNA amplification in the PCR reactions and by introducing a normalisation step into the protocol. These improvements of SSH over RDA eliminate the steps needed to physically separate ss and ds cDNAs [23]. Although SSH and RDA greatly reduce the time required to perform subtractive hybridisation, it remains a technically demanding gene expression analysis method.

Serial analysis of gene expression SAGE

The serial analysis of gene expression (SAGE) technique is designed for studying functional genomics of eukaryotic organisms [29–31]. SAGE is accomplished by concatemerizing several short (10–14 bp) cDNA tags and cloning them into a vector for sequencing. Each clone produces around 35 tags that are entered into a database and analysed. The tags are used to identify corresponding genes; the frequency with which each gene occurs is used to determine the gene's expression level [6, 30].

If a lab has a 96-well sequencing apparatus, then one sequencing gel can be used to analyse thousands of genes in one run of the machine; therefore, SAGE analysis is relatively fast, straightforward and cost-effective [6, 31]. Because the tags are small genes they can only be identified if they have been deposited in quality gene-banking services (e.g. SAGEmap expression database) [6].

SAGE produces many redundant sequences as a consequence of its protocol, so several hundred to several thousand sequences need to be investigated to sample the cDNA population [31, 32]. Stollberg et al. [31] estimate 650,000 tags (~17,500 clones) will be needed to investigate a cDNA population of 56,000 transcripts. For this reason, SAGE is better suited for examinations of specific cell types or tissue with little cellular diversity [31]. Stollberg et al. [31] simulated a SAGE experiment in their investigation and found that SAGE caused a 'significant underestimation of the number of active genes in a preparation and in the fraction of genes expressed at low copy number'. Also, unique SAGE tags may be caused by a sequencing error, so not all unique SAGE tags are present in the cDNA population.

The SAGE technique is most useful in providing global transcription profiles of well-characterized organisms [6, 29–32] or specific cell types or tissue with low cellular diversity [31]. Because few well-characterized organisms exist with a good genetic database, differential display could be used to collect data, which can be used in subsequent SAGE experiments. It is also possible to use SAGE to quickly find a general global gene expression

pattern that can be used to determine difference between two conditions. This information can be used to design a more appropriate comparison.

DNA microarray technology

Microarrays are microscope-slide-size glass plates dotted with many thousand genes that can be analysed in one hybridisation experiment. The two most common types of microarrays are DNA fragment and oligonucleotide arrays [33, 34]. DNA species used to spot the DNA fragment arrays are usually cloned DNA fragments obtained through cDNA libraries [33, 34] or are collected from gene expression analysis techniques [24, 35–37]. Oligonucleotide arrays require accurate sequencing data for the entire genome of the organism of interest (for global analysis) to direct oligonucleotide synthesis [34, 38]. The two samples of mRNA that are being compared are labelled with two different fluorescent tags. The labelled mRNA species are simultaneously hybridised to the array. The arrays are analysed by an array reader, which is used to determine the relative intensity of the different fluorescent signals. The intensity of the signals indicates whether a gene is upregulated, downregulated or unchanged.

DNA microarrays are the most technically demanding and expensive gene expression analysis method. As an internal control, clones and oligonucleotides are spotted up to three times per chip [39]. Due to the cloning genome sequencing and microarray equipment costs, DNA microarrays are very expensive to manufacture. However, labs studying model organisms or closely related species can use arrays that are mass produced to reduce the initial cost of using microarray technology. Another limiting factor that should be considered when doing microarray is that a large amount of poly-A RNA is needed for each hybridisation. This poses a particular problem when investigating systems in which tissue or cells exist only in small quantities.

DNA microarray technology used to study global expression patterns is very complicated and has many problems that need to be resolved. The complexity of examining several thousand genes at once is evident, because computer programs that perform cluster analysis are required [35], and the analysis of the data is unwieldy, prone to human errors and liable to inconsistencies in probe hybridisation [35, 38, 40–43]. We do not believe microarray is a good method to look for novel genes, for it is limited by what has been put on the microarray slide – it is biased by that which is already known. Microarray also does not have the capacity to differentiate between alternate splicings of a gene. Microarray is particularly prone to human error, for contamination of clones used to spot the slides can occur, and the wrong products can be put on the slides [34, 38].

Recently, there has been a misconception that microarray technology may replace all other competitive methodologies such as DD, SAGE, and so on these methods should, however, be used to complement one another. DNA microarray technology, like SAGE, should be used to study global gene expression patterns owing to the fact that many genes can be analysed at once. Gibbs [41] calculates that the entire human genome will fit on less than 300 slides. The major advantage of microarray technology is that it can be used to investigate global transcription patterns quickly [6, 41]. Because a lot of information is needed prior to creating microarray slides, other gene expression analysis techniques should be used to design microarray slides (e.g. differential display [35], RDA [24] and SSH [36]).

Summary

Differential display continues to play an integral role in investigations of gene expression. This introduction provides an explanation and description of suggested guidelines one should follow when planning DD experiments. Ultimately, a well-designed experiment that takes the limits and the benefits of the techniques being used into account will reduce the time, money and frustration needed to finish the project. The authors hope that this introductory review and the following taxa-specific reviews will contribute to more sensible experimental designs that build on others' experiences with DD.

The authors of the taxa-specific reviews focus on the issues associated with DD experiments on yeast, plants, insects, birds and reptiles, and humans. It should be noted that although the reviews address considerations specific for each taxa, most of the information in each is generally applicable to all eukaryotic species.

1 Liang P. and Pardee A. B. (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science **257:** 967–971
2 Liang P. and Pardee A. B. (1997) Differential display – a general protocol. In: Methods in Molecular Biology: Differential Display Methods and Protocols, pp. 3–11, P. Liang and Pardee A. B. (eds), Humana Press, Totowa, NJ
3 Matz M. V. and Lukyanov S. A. (1998) Different strategies of differential display: areas of application. Nucleic Acids Res. **26:** 5537–5543
4 Cho Y., Prezioso V. R. and Liang P. (2002) Systematic analysis of intrinsic factors affecting differential display. Biotechniques **32:** 1–4
5 Ito T. and Sakaki Y. (1997) Fluorescent differential display. In: Methods in Molecular Biology: Differential Display Methods and Protocols, pp. 37–44, Liang P. and Pardee A. B. (eds), Humana Press, Totowa, NJ
6 Lievens S., Goormachtig S. and Holsters M. (2001) A critical evaluation of differential display as a tool to identify genes involved in legume nodulation: looking back and looking forward. Nucleic Acids Res. **29:** 3459–3468

7 Wang X. and Feuerstein G. Z. (1997) Direct sequencing of differential display PCR products. In: Methods in Molecular Biology: Differential Display Methods and Protocols, pp. 69–76, Liang P. and Pardee A. B. (eds), Humana Press, Totowa, NJ

8 Martin K. J., Kwan C. P. and Sager R. (1997) A direct-sequencing-based strategy for identifying and cloning cDNAs from differential display gels. In: Methods in Molecular Biology: Differential Display Methods and Protocols, pp. 77–85, Liang P. and Pardee A. B. (eds), Humana Press, Totowa, NJ

9 Wan J. S. and Erlander M. G. (1997) Cloning differentially expressed genes by using differential display and subtractive hybridization. In: Methods in Molecular Biology: Differential Display Methods and Protocols, pp. 45–68, Liang P. and Pardee A. B. (eds), Humana Press, Totowa, NJ

10 Liang P. (1998) Factors ensuring successful use of differential display. Methods Enzymol. **16:** 361–364

11 Bertioli D. J., Schlichter U. H. A., Adams M. J., Burrows P. R., Steinbiss H. H. and Antoniw J. F. (1995) An analysis of differential display shows a strong bias towards high copy number messenger RNAs. Nucleic Acids Res. **23:** 4520–4523

12 Jurecic R. (1996) Differential mRNA display using anchored oligo-dT and long sequence-specific primers as arbitrary primers. Trends Genet. **12:** 502–504

13 Cho Y., Meade J. D., Walden J. C., Chen X., Guo Z. and Liang P. (2001) Multicolor fluorescent differential display. Biotechniques **30:** 562–572

14 Wan J. S., Sharp S. J., Poirier G. M. C., Wagaman P. C., Chambers J., Pyati J. et al. (1996) Cloning differentially expressed mRNAs. Nat. Biotechnol. **14:** 1685–1691

15 Guimaraes M. J., McClanahan T. and Bazan J. F. (1997) Application of differential display in studying developmental processes. In: Methods in Molecular Biology: Differential Display Methods and Protocols, pp. 175–194, Liang P. and Pardee A. B. (eds), Humana Press, Totowa, NJ

16 Vogel A. M. and Gerster T. (1997) Expression of a zebrafish *Cathepsin L* gene in anterior mesendoderm and hatching gland. Dev. Genes. Evol. **206:** 477–479

17 Liao V. H. and Freedman J. H. (1998) Cadmium-regulated genes from the nematode *Caenorhabditis elegans*. J. Biol. Chem. **273:** 31962–31970

18 Kuno N., Muramatsu T., Hamazato F. and Furuya M. (2000) Identification by large-scale screening of phytochrome-regulated genes in etiolated seedlings of *Arabidopsis* using a fluorescent differential display technique. Plant Physiol. **122:** 15–24

19 Simon H. G., Kittappa R., Khan P. A., Tsilfidis C., Liversage R. A. and Oppenheimer S. (1997) A novel family of T-box genes in urodele amphibian linb development and regeneration: candidate genes involved in vertebrate forelimb/hindlimb patterning. Development. **124:** 1355–1366

20 Liang P. and Pardee A. B. (1994) Method of differential display. Methods Mol. Gen., pp. **5:** 3–16

21 Duguid J. R. and Dinauer M. C. (1990) Library subtraction of in vitro cDNA libraries to identify differentially expressed genes in scrapie infection. Nucleic Acids Res. **18:** 2789–2792

22 Hara E., Kato T., Nakada S., Sekiya S. and Oda K. (1991) Subtractive cDNA cloning using oligo(Dt)30-latex and PCR: isolation of cDNA clones specific to undifferentiated human embryonal carcinoma cells. Nucleic Acids Res. **19:** 7097–7104

23 Diatchenko L., Lau Y. F. C., Campbell A. P., Chenchik A., Moqadam F., Huang B. et al. (1996) Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries. Proc. Natl. Acad. Sci. USA **93:** 6025–6030

24 Welford S. M., Gregg J., Chen E., Garrison D., Sorensen P. H., Denny C. T. et al. (1998) Detection of differentially expressed genes in primary tumor tissues using representational differences analysis coupled to microarray hybridization. Nucleic Acids Res. **26:** 3059–3065

25 Jiang H. P., Kang D. C., Alexandre D. and Fisher P. B. (2000) RaSH, a rapid subtraction hybridization approach for identifying and cloning differentially expressed genes. Proc. Natl. Acad. Sci. USA **97:** 12684–12689

26 Yang M. H. and Sytkowski A. J. (1996) Cloning differentially expressed genes by linker capture subtraction. Anal. Biochem. **237:** 109–114

27 Lisitsyn N., Lisitsyn N. and Wigler M. (1993) Cloning the differences between two complex genomes. Science. **259:** 946–951

28 Hubank M. and Schatz D. G. (1994) Identifying differences in mRNA expression by representational difference analysis of cDNA. Nucleic Acids Res. **22:** 5640–5648

29 Velculescu V. E. (1999) Tantalizing transcriptomes: SAGE and its use in global gene expression analysis. Science **286:** 1491–1492

30 Velculescu V. E., Zhang L., Vogelstein B. and Kinzler K. W. (1995) Serial analysis of gene expression. Science **270:** 484–487

31 Stollberg J., Urschitz J., Urban Z. and Boyd C. D. (2000) A quantitative evaluation of SAGE. Genome Res. **10:** 1241–1248

32 Gilles P. (2001) Generating a genome-wide expression profile with SAGE technology. Expression. **23:** 12–13

33 Schena M., Shalon D., Davis R. W. and Brown P. O. (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science **270:** 467–470

34 Kehoe D. M., Villand P. and Somerville S. (1999) DNA microarrays for studies of higher plants and other photosynthetic organisms. Trends Plant Sci. **4:** 38–41

35 Martin K. J., Graner E., Li Y., Price L. M., Kritzman B. M., Fournier M. V. et al. (2001) High-sensitivity array analysis of gene expression for the early detection of disseminated breast tumor cells in peripheral blood. Proc. Natl. Acad. Sci. USA **98:** 2646–2651

36 Yang G. P., Ross D. T., Kuang W. W., Brown P. O. and Weigel R. J. (1999) Combining SSH and cDNA microarrays for rapid identification of differentially expressed genes. Nucleic Acids Res. **27:** 1517–1523

37 Trenkle T., Welsh J. and McClelland M. (1999) Differential display probes for cDNA arrays. Biotechniques **27:** 554–560

38 Knight J. (2001) When the chips are down. Nature **410:** 860–861

39 Kling J. (2002) Roll-your-own microarrays. The Scientist **16:** 51

40 Brenner S. (1999) Sillycon valley fever. Curr. Biol. **9:** R671–R671

41 Gibbs W. W. (2001) Shrinking to enormity: DNA microarrays are reshaping basic biology, but scientists fear they may soon drown in the data. Sci. Am. **284:** 33–34

42 Wooster R. (2000) Cancer classification with DNA microarrays: is less more? Trends Genet. **16:** 327–329

43 Mir K. U. (2000) The hypothesis is there is no hypothesis Trends Genet. **16:** 63–64