

Comparison of oligonucleotide-microarray and serial analysis of gene expression (SAGE) in transcript profiling analysis of megakaryocytes derived from CD34⁺ cells

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Abbreviations: SAGE, serial analysis of gene expression

Abstract

For the comprehensive analysis of transcript expression, the array-based hybridization analysis and the serial analysis of gene expression (SAGE) are commonly used platforms. The SAGE is based on a high-throughput sequencing of ditags derived from the transcript. DNA microarrays are a powerful tool for monitoring thousands of transcripts simultaneously, whereas the Genechip (Affimatrix microarray) technology is based on the hybridization of a single probe or other manufacturer's microarrays (cDNA- or oligonucleotide-microarray) procedures include the competitive hybridization of two probes. In this study, the quantitative accuracy of expression using oligonucleotide-microarray was determined by comparing data set from the SAGE. In previous study the microSAGE was performed for the megakaryocytes and non-megakaryocytes derived from human cord blood CD34⁺ cells by *ex vivo* expansion using thrombopoietin, and a total of 38,909 tags representing 8,976 unique genes were obtained. On the identical RNA, expression profiling was also carried out using oligonucleotide-microarray (MAGIC II 10K chip, Macrogen). The most frequently expressed genes in human megakaryocytes were identified as platelet factor 1 followed by annexin A1, ribosomal protein S23. The majority of the 50 most highly expressed genes in the CD34⁺-derived megakaryocytes were those involved in protein synthesis, e.g., ribosomal proteins. The expression level through the single channel of oligonucleotide-microarray and SAGE have a fairly good cor-

relation in terms of absolute analyses and that the correlation is higher for the genes with higher expression levels.

Keywords: oligonucleotide microarray; transcript expression analysis

Introduction

For the analysis of the global gene expression, several methods such as DNA microarray and serial analysis of gene expression (SAGE) were developed. DNA microarrays are technology to profile the expression of thousands of transcripts simultaneously (Brown and Botstein, 1999; Lockhart and Winzeler, 2000; Noordewier and Warren, 2001), and two different types of microarray technologies are available; Genechips (Affymetrix platform) and spotted microarrays. The Genechips produced by photolithography and hybridize the single cRNA probe have certain advantages over spotted microarray, such as that cross-hybridization could be avoided, quality control by sequence validation of PCR clones is not required, and noncompetitive nature of hybridization make small amount of RNA sample detectable (Kane *et al.*, 2000; Lockhart and Winzeler, 2000). As this platform is not accessible for every laboratory, spotted microarrays of longer oligonucleotides (50-70 mer) are becoming attractive and alternative platform (Hughes *et al.*, 2001).

The spotted microarrays are manufactured by spotting the cDNAs or synthetic oligonucleotides to the glass plate. Oligonucleotide-microarrays were recently preferred over cDNA microarray due to the easiness of target gene design and the reliability of hybridization (Southern *et al.*, 1999). Oligonucleotide-libraries covering large parts of the transcriptome of several organisms are now available from many companies. Quantitation of transcripts using oligonucleotide-microarrays is based on the competitive hybridization of each labeled cRNA probes to the oligonucleotides anchored on the glass. In short, the total RNA from two different samples was reverse-transcribed into cDNAs, which were labeled with fluorescent dyes Cy3 (blue) and Cy5 (red). The cDNA (or cRNA) labeled with Cy3 and Cy5 are mixed together and hybridized against oligonucleotides on the same array. The two populations compete for the same targets or probe spots on

the array. The spot intensity at the two wavelengths is determined. A ratio or log ratio between the two fluorescent intensities is calculated. The most advantage of the microarray platform in global analysis of gene expression is the speed of data acquisition, but the gene with low level of expression would not be discriminated from that of no expression.

SAGE is a high throughput sequencing based technique used to estimate the relative expression levels of thousands of transcripts by sequencing concatamers of short sequence tags derived from transcript sample. SAGE developed by Velculescu is based on the principle that a nucleotide sequence of 9-10 bases (a gene tag) at a specific location within a transcript represents a specific gene (Velculescu *et al.*, 1995). These tags, which contain sufficient information to identify a gene uniquely, are generated, concatenated and cloned. By sequencing the clones, the identification and the abundance of a transcript are established. The number of tags for a given sequence by the total tags gives the absolute abundance of the tag. It allows for the establishment of both a representative and comprehensive different gene expression profile in various cell types and organs under physiological and pathological states. The data set by SAGE platform has been used construct the transcript expression databases (<http://www.sagenet.org>).

It has been a great concern whether the expression analysis using microarray has quantitative accuracy, and whether it has reliable correlation comparing SAGE data set. Although microarray technology has previously shown to be correlated well with other analytical methods including SAGE, but the microarray technology used was always the Affymetrix Genechip based on the single probe hybridization (Ishii *et al.*, 2000; Evans *et al.*, 2002). As the other microarray procedures adopted the competitive nature the hybridization of two probes, they have been applied to evaluate only the relative changes of expression. This necessitates the evaluation of the competitive hybridization-based microarray using two probes by comparing other platforms of global transcript analysis. The accuracy of spotted microarray in comparison with SAGE has not been done. Previously, we have performed microSAGE in megakaryocytes and non-megakaryocytes derived from human CB CD34⁺ cells by *ex vivo* expansion using thrombopoietin (Kim *et al.*, 2002). Total 38,909 tags representing 8,976 unique genes were obtained. In this study, the quantitative accuracy of oligonucleotide-microarray (MAGIC II chip; Macro-gen) was determined by comparing transcript profile to that of SAGE method on the identical mRNA specimens. From this study it is observed that the gene expression levels from oligonucleotide-microarray fairly correlated well with the SAGE for the moderate- to high expressed transcripts.

Materials and Methods

Preparation of cells and mRNA

The procedures used to prepare samples are described previously (Kim *et al.*, 2002). Briefly, cord blood (CB) was obtained from full-term deliveries with informed consent. Cord blood mononuclear cells were isolated by centrifugation on a FicollHypaque (density, 1.077; APB, Upsalla, Sweden) density centrifugation. The CD34⁺ cell fraction was positively isolated using an anti-CD34 monoclonal antibody (QBEND 10; Miltenyi Biotech, Bergisch Gladbach, Germany) The CD34⁺ cells were cultured in serum-free essential media supplemented with bovine serum albumin, insulin, and transferrin (StemCell Technologies, Vancouver, BC, Canada), and were stimulated with recombinant human TPO (50 ng/ml; Kirin Brewery, Maebashi, Japan) alone. After 10 days, megakaryocytes fraction was separated from non-megakaryocytes fraction using an anti-CD61 (GPIIb/IIIa) monoclonal antibody (Dako, Copenhagen, Denmark). Purity of each separated fraction was verified by flow cytometry with a different antibody reacting with megakaryocytes (FITC-conjugated anti-human CD41; BD). Total RNA from these cells was isolated using TRIZOL (Gibco BRL, NY) according to the manufacturer's instructions. The same RNA was used in both protocols.

SAGE protocol

The microSAGE protocol has been described previously (Datson *et al.*, 1999; Kim *et al.*, 2002). Biotinylated oligo dT primer annealed 10 µg of total RNA was converted to cDNA with a cDNA synthesis kit (Gibco BRL) in streptavidine coated PCR tube (Roche; Mannheim, Germany; <http://biochem.boehringer-mannheim.com>). The cDNA was cleaved with *Nla* III, and was ligated to the oligonucleotide containing recognition sites for *BsmF* I. After ligation, the bound cDNA was released from the matrix by digestion with *BsmF* I. SAGE tag overhangs were filled with Klenow enzyme, and tags from two pools were combined and ligated to each other. The ligation product was amplified by PCR, concatemerized and cloned into the *Sph*I site of pZero-1 (Invitrogen, Calsbad, CA). Clones were sequenced with the BigDye terminator kit and analyzed using ABI 3,700 automated sequencer (Perkin-Elmer, Branchberg). Sequence files were analyzed by means of SAGE analysis software version 4.12. Sequence files were analyzed, and statistical analysis of the data was performed by the use of SAGE software, version 4.12 (courtesy of Victor Velculescu and Ken Kinzler, Johns Hopkins University School of Medicine) (Velculescu, Zhang *et al.*, 1995). The identity of the mRNAs corresponding to the SAGE tags was determined through inspection and comparison

with the SAGEmap (www.ncbi.nlm.nih.gov/SAGE/SAGE-tag.cgi) and UniGene (www.ncbi.nlm.nih.gov/UniGene) databases. SAGE tags that had no reliable matches and multiple matches in UniGene clusters were excluded from the list. Mitochondrial genes and ESTs were also omitted. If more than one gene tag corresponded to the same UniGene cluster, then the higher ranked tag was selected.

Microarray protocol

Experimental procedures for microarray were performed according to the MacroGen MAGIC II-10K technical Manual. The identical total RNA as in the SAGE protocol was used to generate cDNA. Total RNA (5 μ g) was converted into double stranded cDNA using the cDNA synthesis system (Roche) using T7-(dT)₂₄ primer. The each cDNA was purified using RNeasy kit (Qiagen, Valencia, <http://www.qiagen.com>). Non-megakaryocytes selected by CD61 antibody were used as reference in the experiment. Each Cy3-(for non-megakaryocytes), or Cy5-(for megakaryocytes) labeled cRNA was synthesized using the Megascript T7 kit (Ambion, Austin), using Cy3-CTP and Cy5-CTP (APB, Uppsala Sweden). The cRNA was cleaned using RNeasy (Qiagen). Labeled 15 μ g of each cRNA was mixed and fragmented by heating to 94°C for 15 min. Fragmented cRNA was hybridized with MAGIC II-10 K microarray (MacroGen, Seoul, Korea) for 16 h at 42°C. Arrays were then washed and scanned with an Array scanner (APB). Acquired images were processed and analyzed statistically for interpretation of analyzed spot intensity results using Imagen v4.1 software (Roche). Non-biological factors that may

contribute to variability of data were minimized using global normalization/scaling with data from all probe sets, and normalization between the microarrays was also carried out. Each chip contains a total of 10,368 elements of which 10,108 are unique genes/clusters. The length of oligonucleotides was 50-mer.

Results

Total of 38,909 SAGE tags representing 8,976 unique genes were obtained from megakaryocytes. After normalization of fluorescence intensities from the microarray, 8,889 transcripts were used for the exact comparison to that of SAGE using UniGene accession number. Resulting total 1,168 transcripts were matched to each system. For the CD34⁺-derived megakaryocytes, the top 50 transcripts in terms of cy5 fluorescence intensity were listed in Table 1. The most frequently expressed genes in human megakaryocytes were identified as platelet factor 1 followed by annexin A1, ribosomal protein S23. The majority of the 50 most highly expressed genes in the CD34⁺-derived megakaryocytes were those involved in protein synthesis, e.g., ribosomal proteins.

The correlation between the probe intensity observed in oligonucleotide-microarray and the actual mRNA abundance in terms of absolute analyses was shown in Figure 1. Comparing the logarithmic scale of the fluorescent intensities and tag frequencies for the 1,168 Unigenes, the Spearman's coefficient is 0.425 ($P = 0.0001$, Figure 1A). Oligonucleotide-microarray intensity scores are one or two orders of mag

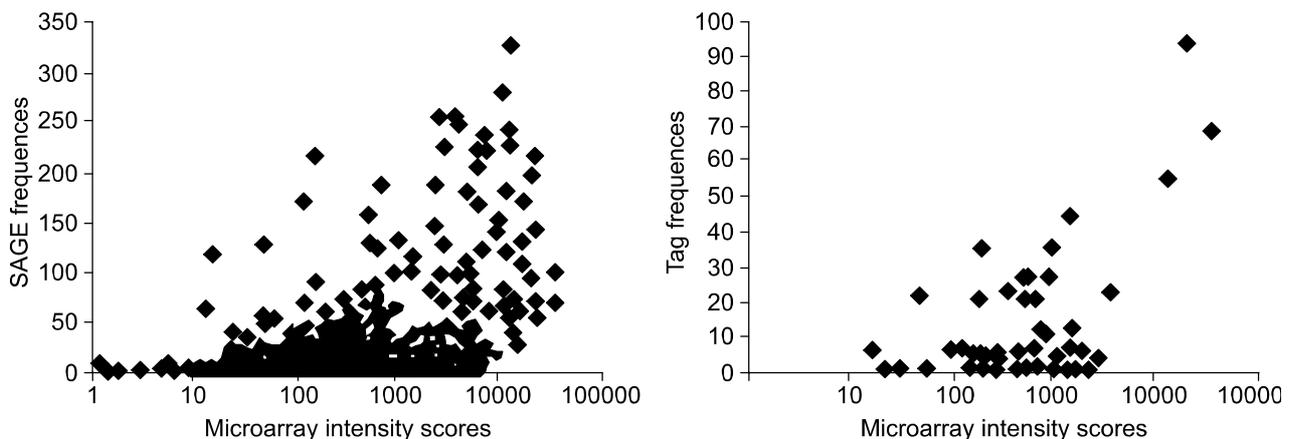


Figure 1. Scatter graph of intensity values in oligonucleotide-microarray and the number of tags in SAGE. Intensity scores and the number of tags are plotted in a logarithmic scale on the abscissa and the ordinate, respectively. Genes with no tag and tags that match multiple genes were excluded. GeneChip scores are one or two orders of magnitude higher than SAGE frequencies. (A) For the CD34⁺-derived megakaryocytes, the coefficient was as high as 0.425 ($n = 1,168$, $P < 0.0001$). (B) For the transcript without any significant change (expression fold-changes < 2) in microarray, the coefficient was as high as 0.657 ($n = 73$, $P < 0.0001$).

Table 1. Transcript profile in CD61⁺ megakaryocytes derived from CD34⁺ cells.

Intensity	UniGene	Description	Tags
38,457	Hs.81564	plateletfactor 4	69
35,717	Hs.75510	annexinA11	117
22,942	Hs.3463	ribosomalprotein S23	218
22,083	Hs.73797	G protein alpha 15 (Gq class)	72
21,200	Hs.2164	pro-plateletbasic protein	94
17,021	Hs.184014	ribosomalprotein L31	172
16,453	Hs.76847	alphaglucosidase II alpha subunit	130
16,160	Hs.418072	ferritin,heavy polypeptide 1	62
14,599	Hs.54673	Tumornecrosis factor superfamily, member 13	65
14,023	Hs.169238	fucosyltransferase3	30
13,543	Hs.380843	ribosomalprotein S6	227
13,391	Hs.76686	glutathioneperoxidase 1	55
13072	Hs.389335	ribosomalprotein L13a	329
12,817	Hs.406511	ribosomalprotein, large, P0	242
12,552	Hs.168383	intercellularadhesion molecule 1 (CD54),	118
11,381	Hs.182740	ribosomalprotein S11	33
10,977	Hs.301636	peroxisomalbiogenesis factor 6	83
10,926	Hs.434029	ribosomalprotein S12	283
9,778	Hs.432645	red cell anion exchanger 3' non-coding region	153
9,230	Hs.301547	ribosomalprotein S7	142
7,696	Hs.334807	ribosomalprotein L30	235
7,647	Hs.234518	ribosomalprotein L23	222
7,442	Hs.430150	ferritin,light polypeptide	21
7,277	Hs.409045	ribosomalprotein L6	124
6,536	Hs.406682	ribosomalprotein L26	124
6,458	Hs.426460	ribosomalprotein L9	222
6,190	Hs.65588	DAZassociated protein 1	168
5,541	Hs.235422	ribosomalprotein L14	27
5,415	Hs.412900	ribosomalprotein L10	47
5,410	Hs.169476	glyceraldehyde-3-phosphatedehydrogenase	6
5,338	Hs.419463	ribosomalprotein L23a	23
5,288	Hs.5174	ribosomalprotein S17	41
5,215	Hs.180450	ribosomalprotein S24	82
5,188	Hs.334842	tubulin,alpha, ubiquitous	39
5,183	Hs.397609	ribosomalprotein S16	74
5,032	Hs.256184	eukaryotictranslation elongation factor 1 gamma	100
5,027	Hs.5308	ubiquitinA-52 residue ribosomal protein fusion product 1	23
4,769	Hs.326249	ribosomalprotein L22	110
4,531	Hs.426035	RAN,member RAS oncogene family	35
4,520	Hs.14376	actin,gamma 1	62
4,324	Hs.169793	ribosomalprotein L32	74
4,108	Hs.283781	musclespecific gene	21

Table 1. Continued.

Intensity	UniGene	Description	Tags
4,079	Hs.431927	ribosomalprotein L21	249
3,793	Hs.279652	mitochondrialribosomal protein L4	23
3,767	Hs.48516	beta-2-microglobulin	41
3,731	Hs.10306	naturalkiller cell group 7 sequence	97
3,715	Hs.356371	ribosomalprotein L28	256
3,691	Hs.233936	myosinregulatory light chain	23
3,526	Hs.115808	leukocyte-associatedlg-like receptor 1	23
3,410	Hs.76067	heatshock 27kDa protein 1	6

RU, resonance unit; RF, rheumatoid factor; ESR, erythrocyte sedimentation rate; ND, not done 1:100 diluted joint fluids were injected onto the CM5 sensor chips followed by the running buffer. Specific binding signals were obtained by subtraction of nonspecific signals from binding on BSA-immobilized flow cells. Laboratory test results were shown.

nitude higher than SAGE frequencies due to amplification of RNA. Genes ranked high by the SAGE method generally showed high-intensity scores in microarray analysis. This graph suggests that the two analytical procedures, oligonucleotide-microarray and SAGE, have a fairly good correlation in terms of absolute analyses and that the correlation is higher for genes with higher expression levels.

Conventionally using the spotted-microarray, the further analysis has been carried out for the transcript with the expression fold-changes greater than 2. Transcripts without any significant change and the fold-changes less than 2 were excluded. Figure 1b shows the scatter graph of the log intensity values in oligonucleotide-microarray and the SAGE tag numbers. The coefficient became as high as 0.657 ($n = 73$, $P < 0.001$). The scatter graph of fold changes in both analytical procedures indicates that the comparative analyses have a better correlation among those genes with high SAGE tags, but have a relatively poor correlation among genes with lower SAGE tags. The relatively poor correlation in the range of low tag numbers may be attributable to the limited number of tags caused by extensive exclusion of genes from the list.

Discussion

The spotted-microarray was used for the study of transcriptional activity only in terms of fold changes. So this makes the spotted-microarray uneasy to compare the each fluorescent intensities of microarray with the expression level of other platforms, such as SAGE. Theoretical basis of using the intensity from the single channel of cy5 (or cy3 as well) in comparing SAGE frequencies is as follows. The amount

of fluorescence-labelled cRNA used for hybridization on microarray is about 20 μg . If the frequency of the RNA for the moderately expressed species is 1/3,000, its amount would be around 10 fmole (Rininge *et al.*, 2000). As 50 fmol of oligonucleotides were overlaid for each spot on the microarray, the amount would be more than that of the moderate to low expressed transcripts. So, it is supposed that the hybridization mechanism would not be so competitive in the experimental condition using two probes, excluding a couple of the highly-expressed transcripts.

As a whole, spotted oligonucleotide-microarray technology is reasonably reliable for the analysis of expression profiling of genes with higher expression levels and greater changes in expression by SAGE and the MAGIC II chip. The coefficient was 0.42, which is somewhat lower than that of the other report using GeneChip (Ishii *et al.*, 2000). The GeneChip technology, which is based on the single probe hybridization, was described to be moderately quantitative in terms of comparative analyses (Evans *et al.*, 2002). When compared for the transcript with expression-fold changes greater than 2, the coefficient was 0.67. If the transcripts with low tag numbers were excluded, the coefficient would be higher. The detection efficiency of microarray for the transcripts with the low tag numbers (for example 1 or 2 tags) was highly variable, as 0 to 100% (Evans *et al.*, 2002). So the reduction of correlation was mainly ascribe to the irrelevance between the low SAGE tags and microarray fluorescence intensities. The detailed causes might come from the limitations and drawbacks of each technology.

Microarray is a high throughput method for profiling gene expression but many variables influence the outcome of the experiment. It may under perform due to probe design issues, such as; distance of the tar-

get sequence from the poly A tail; secondary structures with the target sequence; variations in pin geometry; leading to different amounts of DNA hybridizing within different non-specific hybridization; variations in exposure of different portions of the slide to the hybridization solution; efficiencies in dye incorporation; and cross-reactivity of the probe with other transcripts, each of which may influence detection (Schuchhardt, *et al.*, 2000). Since there are a large number of factors that contribute to experimental error and biological variation, replicate experiments are needed to normalize the data (Lee *et al.*, 2000).

On the other hand, SAGE may under-perform because specific transcripts may be missed due to the absence of a recognition site for the anchoring enzyme or CG-content bias (Margulies *et al.*, 2001). In addition, incorrect tag counts can arise from incomplete digestion or alternative polyadenylation, giving rise to multiple tags derived from a single transcript. Moreover, some of the transcript can be matched multiply. To increase tag length, *Rsa* I and *Bsm* FI were often used in SAGE library construction. That would generate 14 bp tags (Ryo *et al.*, 2000), together with GTAC (*Rsa* I site sequence), total 18 bp stretch should be conveniently used for matching Unigene library. Also, sequencing errors, especially in the population of tags encountered only once, can contribute to the differences between SAGE and MAGIC II chip data. In this study, the number of gene tags analyzed was not sufficient to cover the entire set of mRNAs in a single cell type. Therefore, statistical significance is not robust for those genes expressed at a low level. Namely, even when 300,000 tags were analyzed, there was a 92% chance of detecting a tag for transcripts when the expression on average was at least three copies per cell (Zhang *et al.*, 1997).

The fold-changes of expression megakaryocytes/non-megakaryocytes were compared. The correlation could not be observed between two platforms, but the pattern in terms of increasing or decreasing was kept (data not shown). The overall expression fold-changes by MAGIC II chip had tendency of high value than that by SAGE. The causes of such pattern might be from the high incorporation efficiencies of Cy5-UTP, and the preference of the Cy5-labelled probes in the hybridization procedure. The higher quantum yield of the Cy5 fluorophore might also be contributed (Gruber *et al.*, 2000; t Hoen *et al.*, 2003).

This is the first report showing their quantitative analyses in spotted microarray using competitive hybridization. This study suggested that the expression intensities from a single channel of oligonucleotide-microarray technology reliable to detect medium-to-high abundant transcripts in absolute expression analyses, and would be appropriate as a platform to build a gene expression database, but the determi-

ning the fold-changes of expression needs other technologies of transcript analysis.

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References

- Brown PO, Botstein D. Exploring the new world of the genome with DNA microarrays. *Nat Genet* 1999;21:33-7
- Datson NA, van der Perk-de Jong J, van den Berg MP, de Kloet ER, Vreugdenhil E. MicroSAGE: a modified procedure for serial analysis of gene expression in limited amounts of tissue. *Nucleic Acids Res* 1999;27:1300-7
- Evans SJ, Datson NA, Kabbaj M, Thompson RC, Vreugdenhil E, De Kloet ER, Watson SJ, Akil H. Evaluation of Affymetrix Gene Chip sensitivity in rat hippocampal tissue using SAGE analysis. *Serial Analysis of Gene Expression. Eur J Neurosci* 2002;16:409-13
- Gruber HJ, Hahn CD, Kada G, Riener CK, Harms GS, Ahrer W, Dax TG, Knaus HG. Anomalous fluorescence enhancement of Cy3 and cy3.5 versus anomalous fluorescence loss of Cy5 and Cy7 upon covalent linking to IgG and non-covalent binding to avidin. *Bioconjug Chem* 2000;11:696-704
- Hughes TR, Mao M, Jones AR, Burchard J, Marton MJ, Shannon KW, Lefkowitz SM, Ziman M, Schelter JM, Meyer MR, Kobayashi S, Davis C, Dai H, He YD, Stephanians SB, Cavet G, Walker WL, West A, Coffey E, Shoemaker DD, Stoughton R, Blanchard AP, Friend SH, Linsley PS. Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. *Nat Biotechnol* 2001;19:342-7
- Ishii M, Hashimoto S, Tsutsumi S, Wada Y, Matsushima K, Kodama T, Aburatani H. Direct comparison of GeneChip and SAGE on the quantitative accuracy in transcript profiling analysis. *Genomics* 2000;68:136-43
- Kane MD, Jatkoa TA, Stumpf CR, Lu J, Thomas JD, Madore SJ. Assessment of the sensitivity and specificity of oligonucleotide (50mer) microarrays. *Nucleic Acids Res* 2000; 28:4552-7
- Kim JA, Jung YJ, Seoh JY, Woo SY, Seo JS, Kim HL. Gene expression profile of megakaryocytes from human cord blood CD34⁺ cells *ex vivo* expanded by thrombopoietin. *Stem Cells* 2002;20:402-16
- Lee ML, Kuo FC, Whitmore GA, Sklar J. Importance of replication in microarray gene expression studies: statistical methods and evidence from repetitive cDNA hybridizations. *Proc Natl Acad Sci USA* 2000;97:9834-9
- Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, Mittmann M, Wang C, Kobayashi M, Horton H, Brown EL. Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat Biotechnol* 1996;14:1675-80
- Lockhart DJ, Winzeler EA. Genomics, gene expression and

DNA arrays. *Nature* 2000;405:827-36

Margulies EH, Kardia SL, Innis JW. Identification and prevention of a GC content bias in SAGE libraries. *Nucleic Acids Res* 2001;29:E60

Noordewier MO, Warren PV. Gene expression microarrays and the integration of biological knowledge. *Trends Biotechnol* 2001;19:412-5

Rininger JA, DiPippo VA, Gould-Rothberg BE. Differential gene expression technologies for identifying surrogate markers of drug efficacy and toxicity. *Drug Discov Today* 2000;5:560-8

Ryo A, Kondoh N, Wakatsuki T, Hada A, Yamamoto N, Yamamoto M. A modified serial analysis of gene expression that generates longer sequence tags by nonpalindromic cohesive linker ligation. *Anal Biochem* 2000;277:160-2

Schuchhardt J, Beule D, Malik A, Wolski E, Eickhoff H, Lehrach H, Herzog H. Normalization strategies for cDNA microarrays. *Nucleic Acids Res* 2000;28:E47

Southern E, Mir K, Shchepinov M. Molecular interactions on microarrays. *Nat Genet* 1999;21(suppl 1):5-9

Hoehn PA, de Kort F, van Ommen GJ, den Dunnen JT. Fluorescent labelling of cRNA for microarray applications. *Nucleic Acids Res* 2003;31:e20

Velculescu VE, Zhang L, Vogelstein B, Kinzler KW. Serial analysis of gene expression. *Science* 1995;270:484-7

Zhang L, Zhou W, Velculescu VE, Kern SE, Hruban RH, Hamilton SR, Vogelstein B, Kinzler KW. Gene expression profiles in normal and cancer cells. *Science* 1997;276:1268-72