

Transcriptome characterization of human mammary cell lines expressing different levels of *ERBB2* by serial analysis of gene expression

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Abstract. Over-expression of *ERBB2*, a member of the family of transmembrane receptor tyrosine kinases, occurs in 15-30% of primary breast tumors and is associated with poor prognosis and chemoresistance to a variety of anticancer drugs. In this study, aiming to identify differentially-expressed genes involved in erbB2-mediated transformation of the breast, we generated SAGE libraries from two human mammary cell lines, derived from normal luminal cells, expressing different levels of erbB2. The parental cell line HB4a expresses basal levels and the C5.2 expresses high levels of erbB2. A total of 161,632 tags was generated by sequencing, 81,684 from HB4a cells (30,854 unique tags) and 79,948 from C5.2 cells (30,568 unique tags). The comparison between the HB4a and C5.2 libraries revealed 334 distinct transcripts more expressed in HB4a cells and 328 distinct transcripts more expressed in C5.2 cells. The expression pattern of some of these transcripts was further validated by RT-PCR. The C5.2 cell line, which over-express *ERBB2*, showed in comparison to HB4a cells a higher percentage of genes involved in transport, RNA processing, apoptosis and protein folding. A higher percentage of the genes more expressed in HB4a cells compared to C5.2 were found to be involved in signal transduction and cytoskeleton organization. The use of SAGE analysis allowed us to identify a significant number of genes implicated in different cellular pathways up- or down-regulated in the presence of *ERBB2* over-expression, including genes not previously implicated in breast cancer that could be considered as potential candidate markers for prognosis and therapy.

Introduction

The *ERBB2* gene, also known as HER2/NEU, encodes a 185-kDa transmembrane glycoprotein with intrinsic tyrosine kinase activity that belongs to the epidermal growth factor receptor (EGFR) family (1). This family of receptor tyrosine kinases (RTKs) comprises four members, *EGFR* (*ERBB1*), *ERBB2* (*HER2*), *ERBB3* and *ERBB4* that play important roles in several signal transduction pathways regulating cell proliferation, differentiation, cytoskeletal rearrangement and survival (1,2). The RTKs are cell surface allosteric enzymes activated by the ligand-binding that induces receptor monomers to form homo- or heterodimers. Receptor dimerization induces autophosphorylation on tyrosine residues creating binding sites for several adaptor proteins and enzymes, such as Grb2 and Shc, leading to the activation of intracellular signal transduction that includes Ras/Raf/mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI-3K) pathways (3,4). Although no specific ligand for *ERBB2* has been identified it is believed to play a key role in the *EGFR* family signaling since *ERBB2* is the preferred and more effective heterodimerization partner for all other family members (5,6).

Clinical and experimental evidence indicates that *ERBB2* over-expression plays important role in the malignant transformation, tumorigenesis and metastasis, however the mechanisms or *ERBB2* target genes involved are not fully known. In breast cancer, *ERBB2* over-expression usually due to gene amplification has been shown to occur in 15-30% of invasive ductal tumors, where it correlates with patient poor prognosis and resistance to hormone- and chemotherapy (7,8). Induced over-expression of *ERBB2* increases tumorigenic and metastatic potential of breast cancer cells and transgenic mice that over-express *ERBB2* display high incidence of mammary tumors and metastasis (9,10). Both *in vitro* and *in vivo* experimental results demonstrated that tyrosine kinase inhibitors, anti-sense oligonucleotides, monoclonal antibodies and siRNA are able to abolish the effects of *ERBB2* gene in mammary tumorigenesis (11,12). Therefore, *ERBB2* also proved to be an excellent target for therapeutic approaches. One example is the humanized antibody for erbB2 called Herceptin, approved for clinical application, has been successfully used for the treatment of breast cancer patients,

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both alone or in combination with other chemotherapeutic regimens (13,14).

Up-regulation of cyclin D1 and down-regulation of p27^{kip1} has been shown to be associated with cell proliferation and survival during *ERBB2* malignant transformation (15,16). Mammary tumors from transgenic mice that over-express *ERBB2* under the control of the endogenous promoter show up-regulation of several transcriptional factors associated with the differentiation status of the tumors (17). In fact, gene expression changes in response to *ERBB2* over-expression may have a significant impact on several biological processes, such as proliferation, differentiation, cell adhesion, apoptosis and angiogenesis.

The identification of *ERBB2* target genes could provide additional insights into the malignant transformation and contribute to improve diagnosis, prognosis and treatment of breast cancer. Differential cDNA hybridization and cDNA microarray techniques have been successfully used to identify differentially-expressed genes in breast cancer cells and tumors (18-22). In the present study, aiming to identify additional *ERBB2* target genes, we generated SAGE libraries from two human mammary cell lines, HB4 and C5.2, expressing different levels of *ERBB2* (23). Consistent with the role played by *ERBB2* signaling in behavior of mammary epithelial cells and tumors, using this approach we were able to identify potential *ERBB2* target genes implicated in a variety of cellular processes including cell proliferation, apoptosis and cytoskeleton organization.

Materials and methods

Cell culture. The mammary cell lines HB4a, C3.6 and C5.2 were kindly provided by Dr Michael O'Hare from Ludwig Institute for Cancer Research, London, UK. The parental HB4a is a normal mammary luminal epithelial cell line established as previously described (24). C3.6 and C5.2 cell lines were obtained by the transfection of HB4a cells with full-length normal human *ERBB2* cDNA derived from the breast cancer cell line BT474 (23). These cells were cultured in RPMI-1640 supplemented with 10% FCS, 2 mM glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin and 5 µg/ml hydrocortisone and insulin in a 5% CO₂ humidifier incubator at 37°C.

RNA extraction. For total RNA extraction the cells were washed twice with PBS, harvested and homogenized in a solution containing guanidine isothiocyanate (4 M guanidine isothiocyanate, 25 mM sodium citrate pH 7.0, 0.5% sarcosyl and 100 mM β-mercaptoethanol) and extracted as described (25). The quality of the RNA samples was determined by 1% agarose gel electrophoresis and ethidium bromide staining. All RNA samples were treated with DNase I for 1 h at 37°C to eliminate genomic DNA contamination.

Serial analysis of gene expression (SAGE). Total RNA (25 µg) isolated from HB4a and C5.2 cell lines were used to generate SAGE libraries. SAGE was carried out using the I-SAGE kit (Invitrogen) according to the manufacturer's protocol based on the original SAGE method (26). Concatemered ditags were cloned into pZERO-1 and the amplified inserts were sequenced using ET Dye Terminator Kit (Amersham Bio-

Table I. Summary of SAGE analysis in human mammary luminal cells HB4a and C5.2.

	HB4a	C5.2
Total no. of tags	81,684	79,948
Unique tags	30,854	30,568
Annotated tags	19,314	19,181
Tag-gene	7,698	7,616

sciences) and forward M13 primer and analyzed with a Mega BACE 1000 automated sequencer (Amersham Biosciences). Tag frequency tables were obtained from sequences by SAGE™ analysis software, with minimum tag count set to one, maximum ditag length set to 28, and other parameters set as default. The annotation was based on two specific tools, SAGEmap (<http://www.ncbi.nlm.nih.gov/SAGE>) and CGAP SAGE Genie (<http://cgap.ncbi.nlm.nih.gov/SAGE>).

Semi-quantitative RT-PCR. Total RNA (10 µg) was reverse-transcribed using the High Capacity cDNA Archive kit (Applied Biosystems). PCR amplification was performed in 25 µl volume using 50 ng of cDNA, 0.6 µM of each primer, 1.5 mM MgCl₂, 125 µM of each deoxynucleotide triphosphate, 50 mM KCl, 10 mM Tris-HCl pH 8.0, and 0.2 unit of *Taq* DNA polymerase (Invitrogen, Carlsbad, USA) and consisted of 28 and 32 cycles of denaturation for 30 sec at 94°C, annealing for 1 min at 55°C and extension for 1 min at 72°C, followed an extension of 5 min at 72°C. The expression of glyceraldehyde-3-phosphate dehydrogenase (*GAPD*) gene was used as an internal control for cDNA input. The PCR products were separated by electrophoresis on 2% agarose gels stained by ethidium bromide.

Results

Aiming to identify genes involved in *erbB2*-mediated transformation of the breast we compared the gene expression profiles of two human mammary luminal epithelial cell lines expressing different levels of *erbB2* using SAGE technique. Two independent SAGE libraries were constructed from HB4a (the parental cell line showing basal expression levels of *erbB2*) and C5.2 (showing high expression of *erbB2*) cell lines.

A total of 161,632 tags was generated by sequencing, 81,684 tags derived from HB4a and 79,948 derived from C5.2 cell lines (Table I). Excluding redundancy the sequence analysis identified 30,854 and 30,568 distinct tags from HB4a and C5.2 cells, respectively. In HB4a SAGE library, 19,314 from 30,854 unique tags correspond to UniGene clusters representing known genes, hypothetical proteins and expressed sequence tags (ESTs), the remaining 11,540 tags showed no matches in the CGAP SAGE Genie. In HB4a SAGE library, 19,181 from 30,568 unique tags correspond to UniGene clusters representing known genes, hypothetical proteins and expressed sequence tags (ESTs), the remaining 11,387 tags showed no matches in the CGAP SAGE Genie.

The comparison between the two SAGE libraries was performed using the tools found in our website (<http://gdm>).

Table II. List of the top 50 genes up-regulated in HB4a vs. C5.2 mammary cells.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome localization
AAAATATTTT	Hs.509765	Actinin, alpha 1 (ACTN1)	23.00	1.02	14q24.1-q24.2
AAAGCCAAGA	Hs.74047	Electron-transfer-flavoprotein, beta polypeptide (ETFB)	18.00	0.00	19q13.3
AAGTGAGATG	Hs.272822	RuvB-like 1 (<i>E. coli</i>) (RUVBL1)	15.00	0.00	3q21
AAGAGTTACG	Hs.55041	Mitochondrial ribosomal protein L2 (MRPL2)	15.00	1.02	6p21.3
AGAAATGTAT	Hs.282113	SNF1-like kinase (SNF1LK)	14.00	1.02	21q22.3
AGTGTGATAC	Hs.118820	Hypothetical protein BC007882 (LOC152217)	14.00	1.02	3q29
AGGAAAAGAT	Hs.523829	Polymerase (DNA-directed), delta 4 (POLD4)	12.00	1.02	11q13
CTTGACATAC	Hs.171695	Dual specificity phosphatase 1 (DUSP1)	12.00	1.02	5q34
AGGGCTACGG	BF571807	EST	12.00	1.02	
CCAGCCCAGC	Hs.29802	Slit homolog 2 (<i>Drosophila</i>) (SLIT2)	11.00	1.02	4p15.2
ACTGCGAGGA	Hs.110477	Dolichyl-phosphate mannosyltransferase polypeptide 3 (DPM3)	10.00	0.00	1q22
ATGTCTTTTC	Hs.462998	Insulin-like growth factor binding protein 4 (IGFBP4)	10.00	1.02	17q12-q21.1
GGCCTTTTTT	Hs.75307	H1 histone family, member X (H1FX)	10.00	1.02	3q21.3
CTACGTGCTC	Hs.521056	ATP synthase, mitochondrial F0 complex, sub f, isoform 2 (ATP5J2)	9.00	1.02	7q22.1
AATTTGCAAC	Hs.420272	H2A histone family, member Y (H2AFY)	9.00	1.02	5q31.3-q32
CTTGATTCCC	Hs.518374	Quiescin Q6 (QSCN6)	9.00	1.02	1q24
CACTTGAAAA	Hs.7753	Calumenin (CALU)	9.00	1.02	7q32
TTGGGAGCAG	Hs.445403	Isoleucine-tRNA synthetase (IARS)	9.00	1.02	9q21
ACGATTGATG	Hs.528320	Apolipoprotein A-I binding protein (APOA1BP)	9.00	1.02	1q22
AAAATAAAAA	Hs.517373	Protoporphyrinogen oxidase (PPOX)	9.00	1.02	1q22
CGTTCCTGCG	Hs.504609	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein (ID1)	9.00	1.02	20q11
ATGTGAAGAG	Hs.111779	Secreted protein, acidic, cysteine-rich (osteonectin) (SPARC)	141.00	17.34	5q31.3-q32
GATCCCAAAC	BF686592	EST	8.00	0.00	
TTCTTGCTTA	Hs.425777	Ubiquitin-conjugating enzyme E2L 6 (UBE2L6)	8.00	0.00	11q12
AAGAAAACCTG	Hs.352671	Crystallin, zeta (quinone reductase)-like 1 (CRYZL1)	8.00	0.00	21q21.3
CTTGGTTCTC	Hs.501578	Shadow of prion protein (Sprn)	8.00	0.00	10q26.3
AATACCTCGT	Hs.414579	Scotin (SCOTIN)	8.00	0.00	3p21.31
GTTGCTGCC	Hs.9234	Seven transmembrane domain protein (NIFIE14)	8.00	0.00	19q13.1
TTAAAGATTT	Hs.133892	Tropomyosin 1 (alpha) (TPM1)	8.00	0.00	15q22.1
GCCAAGATGC	Hs.515164	Growth arrest and DNA-damage-inducible, gamma interacting protein 1 (GADD45GIP1)	16.00	2.04	19p13.13
AACATTCTAA	Hs.530436	Syntaxin binding protein 3 (STXBP3)	8.00	1.02	1p13.3
TATCAATATT	Hs.213424	Secreted frizzled-related protein 1 (SFRP1)	8.00	1.02	8p12-p11.1
AAGGAGTCCC	Hs.333427	CD320 antigen (8D6A)	8.00	1.02	19p13.3-p13.2
CTCTAGAACC	Hs.292579	Phosphatidylserine synthase 1 (PTDSS1)	8.00	1.02	8q22
GCATCTGTTT	Hs.520189	ELOVL family member 5 (ELOVL5)	8.00	1.02	6p21.1-p12.1
AACCAGAGGT	T69505	EST	8.00	1.02	
TAACAAAGAA	Hs.405537	Protein kinase, interferon-inducible double stranded RNA dependent activator (PRKRA)	8.00	1.02	2q31.2
GAATAAAATA	Hs.269592	Putative NFκB activating protein (FLJ20241)	8.00	1.02	19p13.12
GACTCTTCAG	Hs.534293	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3 (SERPINA3)	8.00	1.02	14q32.1
TGATTTATTC	Hs.465870	Kelch-like ECH-associated protein 1 (KEAP1)	8.00	1.02	19p13.2
ATGGCAACAG	Hs.505654	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide) (ITGA5)	8.00	1.02	12q11-q13
TGGCTTAAAT	Hs.521171	Hypoxia-inducible protein 2 (HIG2)	8.00	1.02	7q32.2
AAAGTGCATC	Hs.193326	Fibroblast growth factor receptor-like 1 (FGFRL1)	8.00	1.02	4p16
GACCTCCTGC	Hs.502872	Mitogen-activated protein kinase kinase kinase 11 (MAP3K11)	8.00	1.02	11q13.1-q13.3
GATTTCAACC	Hs.371698	RNA export 1 homolog (<i>S. pombe</i>) (RAE1)	7.00	0.00	20q13.32
GTGGGGGGAG	Hs.446374	HLA class II region expressed gene KE2 (HKE2)	7.00	0.00	6p21.3
AATGACTGAA	Hs.93659	Protein disulfide isomerase-associated 4 (ERP70)	7.00	0.00	7q35
ATGCGGGAGA	Hs.534521	Beta-casein-like protein (BCLP)	7.00	0.00	1p35-p34
CACTCTATCC	Hs.258551	Aspartyl aminopeptidase (DNPEP)	7.00	0.00	2q35
TACAAACCTG	Hs.513626	Metallothionein 1F (functional) (MT1F)	7.00	0.00	16q13

Table III. List of the top 50 genes up-regulated in C5.2 vs. HB4 mammary cells.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome localization
CTGTGTTTAG	Hs.452583	Transcribed locus, strongly similar to NP_061322.2 matrin 3	0.00	230.52	5q31.2
CCAGGAACCT	Hs.446352	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (ERBB2)	0.00	85.68	17q11.2-q12
GCAGTTCCC	AI624297	EST	1.00	19.38	
TAAGTAAAGT	Hs.368260	Hypothetical protein MGC11242 (MGC11242)	0.00	16.32	17q21.32
CTATTTAGGG	Hs.7736	Mitochondrial ribosomal protein L27 (MRPL27)	0.00	16.32	17q21.3-q22
TCTTCTCCCT	Hs.506748	Hepatoma-derived growth factor (HDGF)	1.00	14.28	1q21-q23
CCCAGCTAAT	Hs.200395	Centromere protein H (CENPH)	2.00	25.50	5p15.2
TGATTGGTGG	Hs.74615	Platelet-derived growth factor receptor, alpha polypeptide (PDGFRA)	1.00	12.24	4q11-q13
GCCCCAATA	Hs.445351	Lectin, galactoside-binding, soluble, 1 (galectin 1) (LGALS1)	2.00	23.46	22q13.1
TACCATCGAT	BI226498	EST	1.00	11.22	
GGCAGAGACC	Hs.467133	Nucleoporin 62 kDa (NUP62)	1.00	11.22	19q13.33
GCTCTGCCTC	Hs.252549	Cathepsin Z (CTSZ)	0.00	10.20	20q13
CACGCAATGC	Hs.515053	Amino-terminal enhancer of split (AES)	1.00	10.20	19p13.3
GATTTTGTAG	Hs.494604	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member B (ANP32B)	2.00	18.36	9q22.32
TAGCTTCCTT	Hs.44276	Homeo box C10 (HOXC10)	1.00	9.18	12q13.3
TAAACATTGT	Hs.468140	DKFZP564F0522 protein (DKFZP564F0522)	1.00	9.18	2p22.3
GTAGACACCT	Hs.418133	Intracellular membrane-associated calcium-independent phospholipase A2 gamma (IPLA2 GAMMA)	2.00	17.34	7q31
ATGGCAGGAG	Hs.194121	RNA terminal phosphate cyclase-like 1 (RCL1)	0.00	8.16	9p24.1-p23
TAAATACAGT	Hs.503043	Carnitine palmitoyltransferase 1A (liver) (CPT1A)	0.00	8.16	11q13.1-q13.2
TGTATCACTG	BF932124	EST	0.00	8.16	
CATTGTAATA	BE871060	EST	0.00	8.16	
CCTAAGGCTA	Hs.108371	E2F transcription factor 4, p107/p130-binding (E2F4)	0.00	8.16	16q21-q22
TGGATCAACC	Hs.74034	Caveolin 1, caveolae protein, 22 kDa (CAV1)	0.00	8.16	7q31.1
CAGGAACCTG	BE468107	EST	0.00	8.16	
TCACAGCTGT	Hs.255935	B-cell translocation gene 1, anti-proliferative (BTG1)	2.00	16.32	12q22
CTACCAGGCC	Hs.334848	Hypothetical protein FLJ13291 (FLJ13291)	1.00	8.16	16q22.1
TCTCAATTCT	Hs.467637	Cell division cycle 42 (GTP binding protein, 25 kDa) (CDC42)	1.00	8.16	1p36.1
GCTTTCATTG	Hs.2704	Glutathione peroxidase 2 (GPX2)	1.00	8.16	14q24.1
TTTTGATAAA	Hs.388927	YY1 transcription factor (YY1)	1.00	8.16	14q
TCTGCTAAAAG	Hs.475685	High-mobility group box 1 (HMGB1)	1.00	8.16	13q12
AAGAGTTGGG	Hs.536652	Transcribed locus	1.00	8.16	
TAAGCAGATG	Hs.306425	Inhibitor of Bruton agammaglobulinemia tyrosine kinase (IBTK)	1.00	8.16	6q14.1
GCGAAACCCC	Hs.288945	Hypothetical protein FLJ13448 (FLJ13448)	1.00	8.16	2q33.1
CAGAATAATA	Hs.204475	HIV TAT specific factor 1 (HTATSF1)	1.00	8.16	Xq26.1-q27.2
GATTTGTAGC	BE279179	EST	2.00	15.30	
ACCGCCTGTG	Hs.79625	Chromosome 20 open reading frame 149 (C20orf149)	2.00	15.30	20q13.33
TCCTTTGCAA	Hs.81892	KIAA0101 (KIAA0101)	0.00	7.14	15q22.31
CTACATAATA	Hs.432385	DEAD (Asp-Glu-Ala-Asp) box polypeptide 7 (DDX7)	0.00	7.14	21
CTGCCTCCTT	Hs.472847	Chromosome 20 open reading frame 35 (C20orf35)	0.00	7.14	20q13.12
GCTACAGGTA	Hs.349306	Hypothetical protein FLJ31951 (FLJ31951)	0.00	7.14	5q33.3
TTCAAGAAAC	Hs.458974	Transcribed locus	0.00	7.14	8
CTCATAAGAA	Hs.549495	Transcribed locus, strongly similar to XP_529639.1	0.00	7.14	14
TTAGCTTGTT	BU074305	EST	0.00	7.14	
CAGGATCCAG	Hs.447477	Family with sequence similarity 10, member A5 (FAM10A5)	0.00	7.14	11p15.1
GCAGATCGGG	BF336989	EST	0.00	7.14	
GCGGAGAGAG	Hs.546104	Transcribed locus	3.00	21.42	X
AGTAGGTGGC	Hs.459311	Hypothetical protein DKFZp547K1113 (DKFZp547K1113)	2.00	14.28	15q26.1
CGAATAAAAT	Hs.139896	Macrophage erythroblast attacher (MAEA)	2.00	14.28	4p16.3
TACAATAAAC	Hs.507910	Progesterone receptor membrane component 2 (PGRMC2)	1.00	7.14	4q26
GTTGTGATGT	Hs.374477	Ewing sarcoma breakpoint region 1 (EWSR1)	1.00	7.14	22q12.2

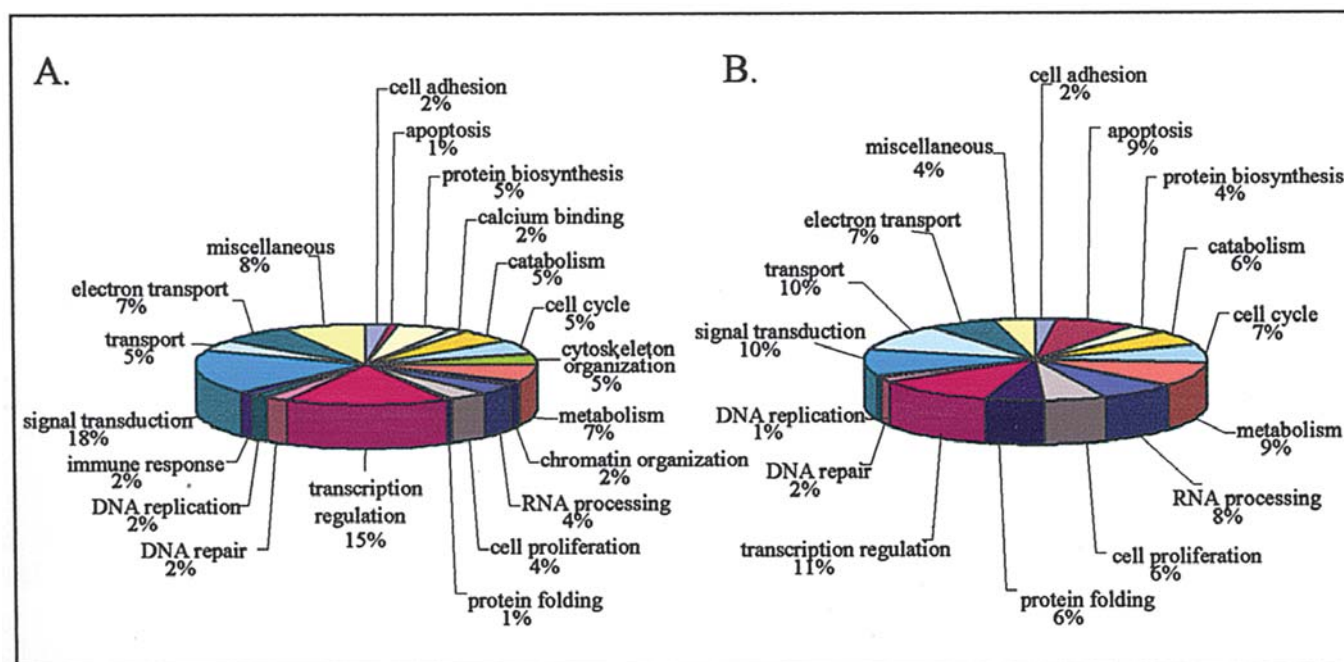


Figure 1. Distribution of the more expressed transcripts from HB4a (A) and C5.2 (B) cell lines classified by functional categories using Gene Ontology nomenclature.

fmrp.usp.br/cgi-bin/gc/uploadFace.pl). Using a cut-off at ≥ 4 -fold changes between the transcripts from HB4a and C5.2 libraries we detected 334 distinct genes more expressed in HB4a and 328 distinct genes more expressed in C5.2. The list of the 50 most expressed genes in the HB4a and C5.2 cells is shown in Tables II and III, respectively. A complete list of the differentially-expressed genes (cut-off ≥ 4 -fold changes) are shown as supplementary Tables II and III). Public available databases (e.g. Gene Ontology, Locus Link, UniGene cluster) were used to annotate by biological function the genes more expressed in each cell line. No functional roles could be assigned for 70 (21%) of the transcripts more expressed in HB4a cells and 95 (29%) of the transcripts more expressed in C5.2. The majority of the other transcripts could be classified using Gene Ontology nomenclature (Fig. 1). C5.2 cell line, which over-expressed the *erbB2* gene, showed in comparison to HB4a cells a higher percentage of genes involved in apoptosis (C5.2, 9% vs. HB4a, 1%), transport (C5.2, 10% vs. HB4a, 5%), RNA processing (C5.2, 8% vs. HB4a, 4%) and protein folding (C5.2, 6% vs. HB4a, 1%). A higher percentage of the genes more expressed in HB4a cells compared to C5.2 were found to be involved in signal transduction (HB4a, 18% vs. C5.2, 10%) and cytoskeleton organization (HB4a, 5% vs. C5.2, 0%).

Based on tag abundance and functional annotation, 9 of the differentially-expressed genes were selected for validation by RT-PCR. Those included 5 genes up-regulated (*MATR3*, *MGC11242*, *HDGF*, *CENPH* and *ANP32B*) and 4 down-regulated (*ETFB*, *RUVBL1*, *SPARC* and *SFRP1*) in C5.2 as compared to HB4a cells. The cell line C3.6, which shows moderate expression of the *erbB2* gene, was also included in the validation analysis. The expression pattern of the 9 genes selected from SAGE technique was confirmed by RT-PCR analysis (Fig. 2).

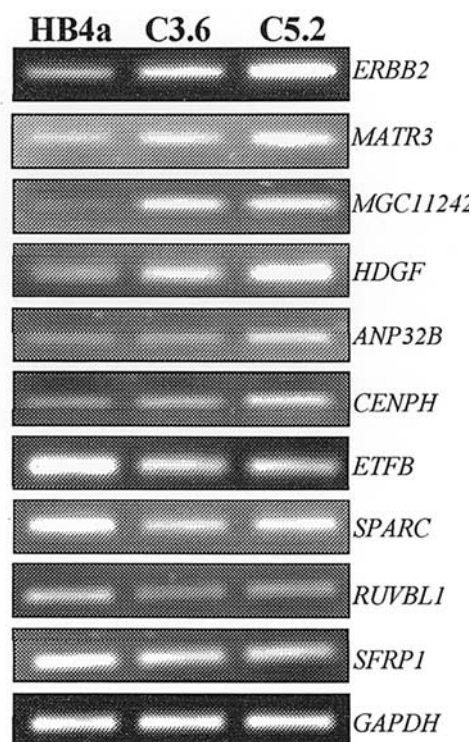


Figure 2. Validation by RT-PCR analysis of 9 differentially-expressed genes from HB4a and C5.2 SAGE libraries.

Discussion

ERBB2 over-expression activates important cell signaling pathways including ras/raf/MAPK and PI3K/AKT leading to the activation and repression of downstream genes especially involved in cell proliferation and survival implicated in

malignant transformation. Transgenic mice over-expressing *ERBB2* develop mammary tumors with high incidence and *ERBB2* over-expression has been demonstrated in 15-30% of human breast cancer in association with poor prognosis and resistance to hormone- and chemotherapy (1,27). The identification of *ERBB2* target genes may provide new insights into the tumorigenic process of the breast that could also have impact on diagnosis, prognosis and treatment of the disease.

In the present study, we used serial analysis of gene expression (SAGE) to characterize the transcriptome of two human mammary epithelial cell lines expressing different levels of *ERBB2* and to identify *ERBB2* regulated genes. The SAGE technique offers the opportunity of both high-throughput and quantitative analysis of gene expression profiles of a cell or tissue including known as well as unknown genes. The use of SAGE approach has enabled us to confirm previous data reported by others and to extend the number of new candidate genes up- or down-regulated by *ERBB2* in human mammary cells. Comparison of our SAGE results with the data reported by Mackay *et al* (20) in the same cell lines (HB4a and C5.2) but using a cDNA microarray containing 6,000 different transcripts showed many similarities. Not all, but several of the 61 transcripts identified by these authors as up-regulated, such as *LGALS1*, *CRIP1*, *S100P*, *MGC11242*, *SQLIE*, *HSPB1*, *DNAJB6* or down-regulated, such as *MYL9*, *SPARC*, *FNI*, *TAGLN*, *SPINT2*, *GTF3B*, *PMSD8* in the C5.2 cell line, which expresses high levels of *ERBB2*, showed the same pattern in our SAGE libraries, indicating that both platforms, cDNA microarrays and SAGE, are very sensitive methods to determine gene expression profiling. However, the use of SAGE technique allowed us to identify a large number of transcripts not previously identified as *ERBB2* regulated genes including those with unknown function. The relative abundance of the differentially-expressed transcripts in our SAGE libraries was validated by RT-PCR analysis. All nine genes analyzed by RT-PCR displayed the same expression pattern observed in the SAGE analysis, such as *MATR3*, *MGC11242*, *HDGF*, *ANP32B* and *CENPH* found to be up-regulated and *ETFB*, *SPARC*, *RUVBL1* and *SFRP1* found to be down-regulated in cells over-expressing *ERBB2*. However, the differences in the transcript levels were more evident in SAGE libraries, this lack of complete concordance could be due to the lower sensitivity of semi-quantitative RT-PCR to detect transcript abundance.

The malignant transformation involves changes in cell proliferation and survival, cell shape and cytoskeleton and alterations of cell adhesion. Several lines of evidence have implicated *ERBB2* in cell proliferation, differentiation and apoptosis. We further divided the most up-regulated and down-regulated genes into functional categories. Over-expression of *ERBB2* affected the expression of genes involved in different biological processes. Interestingly, the C5.2 cell line that over-express *ERBB2* showed a higher percentage of genes involved in cell cycle/proliferation, apoptosis and protein folding and lower percentage of genes involved in cytoskeleton organization than HB4a cell line.

A higher number of apoptosis-related genes were observed in the C5.2 library than in the HB4a library. Most of these genes (*PBP*, *PDCD4*, *ANP32B*, *BTG1*, *BNIP1*) play pro-apoptotic activity and show elevated expression probably in

response to the oncogenic stress experienced by the C5.2 cells. However, the ability of these genes to sensitize C5.2 cells to apoptosis is possible overcome by *ERBB2* over-expression that evokes activation of the PI3K and AKT and promotes cellular survival (28,29). Active PBK/AKT has been shown to protect against apoptosis through phosphorylation and inhibition of proapoptotic proteins such as Bad and Foxo (30-32). However, if this is the case, the proapoptotic genes identified here requires further experimental studies.

In our SAGE analysis, several genes encoding for chaperone and co-chaperone proteins such as Hsp90, Hsp70, Hsp40 and Hsp27 were detected as up-regulated in the C5.2 cell line, which over-express *ERBB2*. Heat shock proteins are an important family of endogenous protective proteins expressed in all tissues and induced under various stresses and different signals (33). Hsp70 and Hsp90 participate in the folding of numerous proteins including oncoproteins and also play critical roles in maintaining the estrogen receptor conformation for proper ligand binding (34,35). High expression of Hsp90, Hsp70 and Hsp27 in human tumors, including breast cancer, is correlated with poor prognosis and therapy resistance (36). Furthermore, those heat shock proteins have anti-apoptotic functions. The chaperone Hsp90 has been shown to stabilize anti-apoptotic proteins such as survivin and *RIP* and to inhibit pro-apoptotic kinase ASK1 (apoptosis signal regulator kinase 1) (37-39). Moreover, inhibition of Hsp90 by geldanamycin induces growth arrest and apoptosis in cells over-expressing *ERBB2* (40,41). Depletion of Hsp70 induces massive cell death in different tumorigenic cell lines revealing its anti-apoptotic function (42,43). There is also evidence showing that Hsp27 display anti-apoptotic activity. Hsp27 expression protects from apoptosis induced by TNF- α and chemically-induced cell death (44,45). Although the differential expression pattern of chaperone protein observed here could reflect the cellular adaptation to several types of stress, these differences may in part account for the increased cell survival and drug resistance associated with *ERBB2* over-expression.

Altered cytoskeleton organization is a hallmark of transformed cells, which acquire a more rounded shape and increased cell invasive and metastatic potential. In our SAGE analysis we identified down-regulation of genes associated with cytoskeleton organization such as *ACTN1* and *TPM1*. This finding is consistent with the disorganized and irregular shape displayed by the cell line C5.2 on Matrigel in comparison to the parental cell line HB4a (23). The role played by the *ACTN1* in cancer is unclear. The *ACTN1* encodes for a member of the α -actinin family that bind actin and play a key role in the assembly and maintenance of stress fibers in non-muscle cells (46,47). α -actinin 1 is believed to link actin cytoskeleton to adhesion plaques via integrins and other actin-binding proteins (48-50). The expression of α -actinin 1 has also been found to be down-regulated in SV40-transformed NIH3T3 cells (51), indicating that loss of α -actinin 1 may play a role in malignant transformation. A recent study showed that *erbB2* induces integrin inactivation in breast cancer cells through actin cytoskeletal rearrangement dependent on PI3K and PKB pathways (52).

Tropomyosin 1 (TPM1) one of the genes found to be down-regulated in the cell line with *ERBB2* over-expression

is a member of a family of actin binding proteins that play important function in microfilament stabilization (53,54). *TPM1* expression is down-regulated in several transformed cells, including breast cancer cells (54-56) and restoration of its expression is able to revert the malignant phenotype of oncogene-transformed cells (57). Primary breast tumors express low or undetectable levels of *TPM1* (58). In fact, *TPM1* has been shown to suppress the malignant phenotype of breast cancer cells. Restoration of *TPM1* expression in MCF-7 cells decreases cell proliferation, inhibits anchorage-independent growth and alters interaction of E-cadherin-catenin complex contributing to the stability of cell-cell adherens junctions (59). Down-regulation of both *ACTN1* and *TPM1* expression could be a result of the activation of ERBB2 signaling pathways that induces actin reorganization and motility, enhances the invasiveness and metastatic potential and chemoresistance of cancer cells (60).

Using serial analysis of gene expression (SAGE), we identified a large number of differentially-expressed genes in human mammary epithelial cells over-expressing *ERBB2* that are associated with different biological processes. Several of these genes are new or hypothetical proteins not previously implicated in breast cancer. Although, further experimental and clinical studies are required, some of these genes could be considered as potential candidate markers of the aggressive phenotype displayed by breast tumors over-expressing *ERBB2* and potential therapeutic targets of the disease.

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Table II supplementary. List of the genes down-regulated in C5.2 vs. HB4a mammary cells.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome region
AAAATATTTT	Hs.509765	Actinin, alpha 1 (ACTN1)	23.00	1.02	14q24.1-q24.2
AAAGCCAAGA	Hs.74047	Electron-transfer-flavoprotein, beta polypeptide (ETFB)	18.00	0.00	19q13.3
AAGTGAGATG	Hs.272822	RuvB-like 1 (<i>E. coli</i>) (RUVBL1)	15.00	0.00	3q21
AAGAGTTACG	Hs.55041	Mitochondrial ribosomal protein L2 (MRPL2)	15.00	1.02	6p21.3
AGAAATGTAT	Hs.282113	SNF1-like kinase (SNF1LK)	14.00	1.02	21q22.3
AGTGTGATAC	Hs.118820	Hypothetical protein BC007882 (LOC152217)	14.00	1.02	3q29
AGGAAAAGAT	Hs.523829	Polymerase (DNA-directed), delta 4 (POLD4)	12.00	1.02	11q13
CTTGACATAC	Hs.171695	Dual specificity phosphatase 1 (DUSP1)	12.00	1.02	5q34
AGGGCTACGG	BF571807	EST	12.00	1.02	
CCAGCCCAGC	Hs.29802	Slit homolog 2 (<i>Drosophila</i>) (SLIT2)	11.00	1.02	4p15.2
ACTGCGAGGA	Hs.110477	Dolichyl-phosphate mannosyltransferase polypeptide 3 (DPM3)	10.00	0.00	1q22
ATGTCTTTTC	Hs.462998	Insulin-like growth factor binding protein 4 (IGFBP4)	10.00	1.02	17q12-q21.1
GGCCTTTTTT	Hs.75307	H1 histone family, member X (H1FX)	10.00	1.02	3q21.3
CTACGTGCTC	Hs.521056	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit f, isoform 2 (ATP5J2)	9.00	1.02	7q22.1
AATTTGCAAC	Hs.420272	H2A histone family, member Y (H2AFY)	9.00	1.02	5q31.3-q32
CTTGATTCCC	Hs.518374	Quiescin Q6 (QSCN6)	9.00	1.02	1q24
CACTTGAAAA	Hs.7753	Calumenin (CALU)	9.00	1.02	7q32
TTGGGAGCAG	Hs.445403	Isoleucine-tRNA synthetase (IARS)	9.00	1.02	9q21

Table II supplementary. Continued.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome region
ACGATTGATG	Hs.528320	Apolipoprotein A-I binding protein (APOA1BP)	9.00	1.02	1q22
AAAATAAAAA	Hs.517373	Protoporphyrinogen oxidase (PPOX)	9.00	1.02	1q22
CGTTCCTGCG	Hs.504609	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein (ID1)	9.00	1.02	20q11
ATGTGAAGAG	Hs.111779	Secreted protein, acidic, cysteine-rich (osteonectin) (SPARC)	141.00	17.34	5q31.3-q32
GATCCCAAAC	BF686592	EST	8.00	0.00	
TTCTTGCTTA	Hs.425777	Ubiquitin-conjugating enzyme E2L 6 (UBE2L6)	8.00	0.00	11q12
AAGAAAACCTG	Hs.352671	Crystallin, zeta (quinone reductase)-like 1 (CRYZL1)	8.00	0.00	21q21.3
CTTGGTTCTC	Hs.501578	Shadow of prion protein (Sprn)	8.00	0.00	10q26.3
AATACCTCGT	Hs.414579	Scotin (SCOTIN)	8.00	0.00	3p21.31
GTTGCTGCC	Hs.9234	Seven transmembrane domain protein (NIFIE14)	8.00	0.00	19q13.1
TTAAAGATTT	Hs.133892	Tropomyosin 1 (alpha) (TPM1)	8.00	0.00	15q22.1
GCCAAGATGC	Hs.515164	Growth arrest and DNA-damage-inducible, gamma interacting protein 1 (GADD45GIP1)	16.00	2.04	19p13.13
AACATTCTAA	Hs.530436	Syntaxin binding protein 3 (STXBP3)	8.00	1.02	1p13.3
TATCAATATT	Hs.213424	Secreted frizzled-related protein 1 (SFRP1)	8.00	1.02	8p12-p11.1
AAGGAGTCCC	Hs.333427	CD320 antigen (8D6A)	8.00	1.02	19p13.3-p13.2
CTCTAGAACC	Hs.292579	Phosphatidylserine synthase 1 (PTDSS1)	8.00	1.02	8q22
GCATCTGTTT	Hs.520189	ELOVL family member 5, elongation of long chain fatty acids (ELOVL5)	8.00	1.02	6p21.1-p12.1
AACCAGAGGT	T69505	EST	8.00	1.02	
TAACAAAGAA	Hs.405537	Protein kinase, interferon-inducible double stranded RNA dependent activator (PRKRA)	8.00	1.02	2q31.2
GAATAAAATA	Hs.269592	Putative NFκB activating protein (FLJ20241)	8.00	1.02	19p13.12
GACTCTTCAG	Hs.534293	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3 (SERPINA3)	8.00	1.02	14q32.1
TGATTTATTC	Hs.465870	Kelch-like ECH-associated protein 1 (KEAP1)	8.00	1.02	19p13.2
ATGGCAACAG	Hs.505654	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide) (ITGA5)	8.00	1.02	12q11-q13
TGGCTTAAAT	Hs.521171	Hypoxia-inducible protein 2 (HIG2)	8.00	1.02	7q32.2
AAAGTGCATC	Hs.193326	Fibroblast growth factor receptor-like 1 (FGFRL1)	8.00	1.02	4p16
GACCTCCTGC	Hs.502872	Mitogen-activated protein kinase kinase kinase 11 (MAP3K11)	8.00	1.02	11q13.1-q13.3
GATTTCAACC	Hs.371698	RNA export 1 homolog (<i>S. pombe</i>) (RAE1)	7.00	0.00	20q13.32
GTGGGGGGAG	Hs.446374	HLA class II region expressed gene KE2 (HKE2)	7.00	0.00	6p21.3
AATGACTGAA	Hs.93659	Protein disulfide isomerase-associated 4 (ERP70)	7.00	0.00	7q35
ATGCGGGAGA	Hs.534521	Beta-casein-like protein (BCLP)	7.00	0.00	1p35-p34
CACTCTATCC	Hs.258551	Aspartyl aminopeptidase (DNPEP)	7.00	0.00	2q35
TACAAACCTG	Hs.513626	Metallothionein 1F (functional) (MT1F)	7.00	0.00	16q13
AGTATTCATA	Hs.466854	Pregnancy specific beta-1-glycoprotein 5 (PSG5)	7.00	0.00	19q13.2
GGGGCTGTGG	Hs.371718	General transcription factor IIIC, polypeptide 1, alpha 220 kDa (GTF3C1)	7.00	0.00	16p12
AGGACAGAAG	Hs.528670	Matrix metalloproteinase 25 (MMP25)	7.00	0.00	16p13.3
TCCTCTACCT	Hs.32018	SNAP-associated protein (SNAPAP)	7.00	0.00	1q21.3
CACCTTGTGA	Hs.515223	cDNA FLJ14241 fis, clone OVARC1000533	7.00	0.00	19
ATACTTTAAT	Hs.480653	Annexin A5 (ANXA5)	14.00	2.04	4q26-q28
TTTGGAGCAT	Hs.298198	Chemokine-like factor super family 3 (CKLFSF3)	7.00	1.02	16q22.1
AATGGCACTT	Hs.26023	Forkhead box J3 (FOXJ3)	7.00	1.02	1pter-q31.3
ACCTCTCTAA	Hs.94392	Zinc finger protein 580 (ZNF580)	7.00	1.02	19q13.42
TATTTATTGA	Hs.518250	Coatamer protein complex, subunit gamma (COPG)	7.00	1.02	3q21.3
GATTATGTAA	Hs.512597	CGI-41 protein (CGI-41)	7.00	1.02	1q23.1
GCTGGGGCCC	Hs.65256	Leucine-rich repeat LGI family, member 4 (LGI4)	7.00	1.02	19q13.12
CACTTTTGGG	Hs.334851	LIM and SH3 protein 1 (LASP1)	7.00	1.02	17q11-q21.3
CATTCAGTTG	Hs.502773	Membrane-type 1 matrix metalloproteinase cytoplasmic tail binding protein-1 (MTCBP-1)	7.00	1.02	2p25.2
CCCAGCTGGA	Hs.258798	Chromosome 10 open reading frame 86 (C10orf86)	7.00	1.02	10q26.13
CGGGGTTCTT	Hs.374262	Cofactor required for Sp1 transcriptional activation, subunit 8, 34 kDa (CRSP8)	7.00	1.02	9q34.1-q34.3
ACATAATAAA	Hs.373550	TGFB-induced factor (TALE family homeobox) (TGIF)	7.00	1.02	18p11.3
GAAAGATGCT	Hs.398989	Brain expressed X-linked 2 (BEX2)	7.00	1.02	Xq22

Table II supplementary. Continued.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome region
AGACCATATT	Hs.524899	Sin3-associated polypeptide, 18 kDa (SAP18)	7.00	1.02	13q12.11
AGTGTCTGTG	Hs.8867	Cysteine-rich, angiogenic inducer, 61 (CYR61)	7.00	1.02	1p31-p22
AGAAATAAAG	Hs.82927	Adenosine monophosphate deaminase 2 (isoform L) (AMPD2)	7.00	1.02	1p13.3
GAGCAAACGG	Hs.369785	Hypothetical protein MGC2749 (MGC2749)	7.00	1.02	19p13.11
TGGGAAGAGG	Hs.37982	Neural precursor cell expressed, developmentally down-regulated 9 (NEDD9)	7.00	1.02	6p25-p24
ATCTTGTTAC	Hs.203717	Fibronectin 1 (FN1)	34.00	5.10	2q34
ATTAAAGAAT	Hs.22011	C1q and tumor necrosis factor related protein 6 (C1QTNF6)	13.00	2.04	22q13.1
AACTACATAG	Hs.9527	Chromosome 2 open reading frame 28 (C2orf28)	19.00	3.06	2p23.3
AGTCTAGCTA	Hs.170835	Three prime repair exonuclease 2 (TREX2)	6.00	0.00	Xq28
AATGTTCTGT	Hs.439200	KIAA0090 (KIAA0090)	6.00	0.00	1p36.13
GAATCTGGAG	Hs.546348	Leukotriene B4 12-hydroxydehydrogenase (LTB4DH)	6.00	0.00	9q31.3
AGACCTCCTT	Hs.412468	Kelch domain containing 3 (KLHDC3)	6.00	0.00	6p21.1
TTGACCCTGG	Hs.420559	Phosphatidylserine decarboxylase (PISD)	6.00	0.00	22q12.2
GTGCGGAGGA	Hs.332053	Serum amyloid A1 (SAA1)	6.00	0.00	11p15.1
GAGAAATAAA	Hs.501140	KIAA1598 (KIAA1598)	6.00	0.00	10q25.3
GAGGACACAG	Hs.16426	Podocalyxin-like (PODXL)	6.00	0.00	7q32-q33
GAAAATAAAG	Hs.531561	Epithelial membrane protein 2 (EMP2)	6.00	0.00	16p13.2
AAGGGGCAAG	Hs.332012	Immunoglobulin superfamily, member 8 (IGSF8)	6.00	0.00	1q23.1
AAAATAAAGC	Hs.293818	Nei like 2 (<i>E. coli</i>) (NEIL2)	6.00	0.00	8p23.1
GAAATAAAGT	Hs.482034	Hypothetical protein FLJ21657 (FLJ21657)	6.00	0.00	5p12
ATTCTTACAG	Hs.70312	Cytochrome c oxidase subunit VIIa polypeptide 2 (liver) (COX7A2)	6.00	0.00	6q12
AGTCAGCTGG	Hs.26139	Epidermal growth factor receptor pathway substrate 8 (EPS8)	6.00	0.00	12q13
CTGATCCCCC	Hs.343571	CCR4-NOT transcription complex, subunit 3 (CNOT3)	6.00	0.00	19q13.4
TTGAATTCTT	Hs.288936	Mitochondrial ribosomal protein L9 (MRPL9)	6.00	0.00	1
AGGAAAGCTT	Hs.412601	Zinc finger protein 527 (ZNF527)	6.00	0.00	19q13.1
AAAAATAAAT	Hs.521124	Mitochondrial ribosomal protein S28 (MRPS28)	6.00	0.00	8q21.1-q21.2
ATTAATGAAT	Hs.18259	XPA binding protein 1 (XAB1)	6.00	0.00	2p23.3
AATACACATC	Hs.464734	Small nuclear ribonucleoprotein D1 polypeptide 16 kDa (SNRPD1)	6.00	0.00	18q11.2
TTCACAGTGC	Hs.437385	Adaptin-ear-binding coat-associated protein 2 (FLJ10420)	6.00	0.00	1p36.13
CGCATTAAG	Hs.107101	Hypothetical protein FLJ31031 (FLJ31031)	6.00	0.00	1p36.33
TAACATACAA	Hs.152536	Proteasome regulatory particle subunit p44S10 (P44S10)	6.00	0.00	3p14.1
ACCGCCGTGG	Hs.513803	Cytochrome b-245, alpha polypeptide (CYBA)	6.00	0.00	16q24
TACTCGGTTG	Hs.549298	Hypothetical protein FLJ34236 (FLJ34236)	6.00	0.00	12q13.3
GTTGGGTAGA	Hs.440263	TP53 regulating kinase (TP53RK)	6.00	0.00	20q13.2
TCTTCTAAAA	Hs.108112	Polymerase (DNA directed), epsilon 3 (p17 subunit) (POLE3)	6.00	0.00	9q33
TGTATTCAGC	Hs.21765	Fatty acid desaturase 3 (FADS3)	6.00	0.00	11q12-q13.1
ATGGTTAAAG	Hs.143728	Wiskott-Aldrich syndrome-like (WASL)	6.00	0.00	7q31.3
GCAATAATGG	Hs.499620	Gem (nuclear organelle) associated protein 4 (GEMIN4)	12.00	2.04	17p13
CCTTGACCAA	Hs.369052	Selenoprotein T (SELT)	6.00	1.02	3q25.1
TATACCAATC	Hs.379858	Dimethylarginine dimethylaminohydrolase 1 (DDAH1)	6.00	1.02	1p22
GACCAGCCCA	Hs.75799	Protease, serine, 8 (prostasin) (PRSS8)	6.00	1.02	16p11.2
AAGGGGAACT	Hs.180591	Nucleoporin 35 kDa (NUP35)	6.00	1.02	2q32.1
AAGTGATTCT	Hs.502829	Splicing factor 1 (SF1)	6.00	1.02	11q13
AGCGTGTGAT	Hs.515259	Single stranded DNA binding protein 4 (SSBP4)	6.00	1.02	19p13.1
ATCCCTCAGT	Hs.496487	Activating transcription factor 4 (tax-responsive enhancer element B67) (ATF4)	6.00	1.02	22q13.1
GCCACTACCC	Hs.242458	Spastic paraplegia 21 (autosomal recessive, Mast syndrome) (SPG21)	6.00	1.02	15q21-q22
TGCCTGTGGT	Hs.370129	Hypothetical gene supported by AK129923 (HMFNO672)	6.00	1.02	8p21.1
GCTATGCTCC	Hs.478469	Presenilin associated, rhomboid-like (PSARL)	6.00	1.02	3q27.1
CAAGGAGATC	Hs.7471	BBP-like protein 1 (BLP1)	6.00	1.02	8p11.23
CAGCCTGGGG	Hs.72026	Protease, serine, 21 (testisin) (PRSS21)	6.00	1.02	16p13.3
CAGCTGTCCC	Hs.432448	Keratin 16 (focal non-epidermolytic palmoplantar keratoderma) (KRT16)	6.00	1.02	17q12-q21
TTCTTCTCGT	Hs.474005	SMT3 suppressor of mif two 3 homolog 3 (yeast) (SUMO3)	6.00	1.02	21q22.3
CCCCCTCCGG	Hs.83753	Small nuclear ribonucleoprotein polypeptides B and B1 (SNRPB)	6.00	1.02	20p13

Table II supplementary. Continued.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome region
CCTGTCCAGT	Hs.4253	Hypothetical protein MGC2574 (MGC2574)	6.00	1.02	11q12.2
GTCTAGTCAA	Hs.129621	KIAA0179 (KIAA0179)	6.00	1.02	21q22.3
ACTGTTCTCT	Hs.437831	Chromosome 14 open reading frame 32 (C14orf32)	6.00	1.02	14q22.2-q22.3
CCGAAAAAGT	Hs.24763	RAN binding protein 1 (RANBP1)	6.00	1.02	22q11.21
GGTCCTGTTT	Hs.435255	UBX domain containing 1 (UBXD1)	6.00	1.02	19p13
AAATTGTTCC	Hs.531089	Proteasome (prosome, macropain) subunit, alpha type, 3 (PSMA3)	6.00	1.02	14q23
AATATTGTCC	Hs.283322	Hypothetical protein HSPC138 (HSPC138)	6.00	1.02	11q14.2
TGGAAGAAAC	Hs.93235	Chromosome 6 open reading frame 125 (C6orf125)	17.00	3.06	6p21.31
CCCCAATGC	Hs.115232	Splicing factor 3a, subunit 2, 66 kDa (SF3A2)	11.00	2.04	19p13.3-p13.2
TTGTGATGG	Hs.425801	Dual specificity phosphatase 23 (DUSP23)	11.00	2.04	1q23.2
AAAATAAAGA	Hs.73722	APEX nuclease (multifunctional DNA repair enzyme) 1 (APEX1)	75.00	14.28	14q11.2-q12
ATTTTGATAA	Hs.472330	Chromosome 20 open reading frame 3 (C20orf3)	16.00	3.06	20p11.22-p11.21
GAGTGGAGAG	Hs.410316	H-rev107-like protein 5 (HRLP5)	5.00	0.00	11q13.2
GAGTTCGACC	Hs.348553	Chromosome 19 open reading frame 33 (C19orf33)	5.00	0.00	19q13.2
CATAATAGTG	CD102897	EST	5.00	0.00	
TATTAATAA	Hs.131683	Cytoplasmic polyadenylation element binding protein 3 (CPEB3)	5.00	0.00	10q23.32
GTCTTTAGGA	Hs.2057	Uridine monophosphate synthetase (orotate phosphoribosyl transferase and orotidine-5'-decarboxylase) (UMPS)	5.00	0.00	3q13
CACAGGCAAA	Hs.355983	Basic leucine zipper and W2 domains 1 (BZW1)	5.00	0.00	2q33
CCCAGCAGTC	BE075489	EST	5.00	0.00	
CCCATCCGTC	AV649630	EST	5.00	0.00	
TAATTTAAAA	Hs.301613	JTV1 gene (JTV1)	5.00	0.00	7p22
GCCCAGCTCA	Hs.78466	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 8 (PSMD8)	5.00	0.00	19q13.2
GAGGAGATGG	Hs.546454	Hypothetical protein DKFZp434N035 (DKFZp434N035)	5.00	0.00	22q11.21
TTATATGAAA	Hs.488624	Postmeiotic segregation increased 2-like 2 (POM121)	5.00	0.00	7q11.23
CCGCTGATCC	Hs.492618	Exostoses (multiple) 1 (EXT1)	5.00	0.00	8q24.11-q24.13
GGCAGAGGGC	Hs.477498	Eukaryotic elongation factor, selenocysteine-tRNA-specific (SELB)	5.00	0.00	3q21.3
ATTTAGTTGG	Hs.268963	Ubiquitin associated protein 1 (UBAP1)	5.00	0.00	9p22-p21
AATATTCATA	Hs.210385	Hect [homologous to the E6-AP (UBE3A) carboxyl terminus] domain and RCC1 (CHC1)-like domain (RLD) 1 (HERC1)	5.00	0.00	15q22
TTTTAAAAAT	Hs.370408	Catechol-O-methyltransferase (COMT)	5.00	0.00	22q11.21-q11.23
AGAACAGAAA	Hs.467898	Adenylate cyclase 3 (ADCY3)	5.00	0.00	2p24-p22
TTCTCTCCCC	Hs.106909	Anaphase promoting complex subunit 13 (ANAPC13)	5.00	0.00	3q22.1
GATTTTCTAC	Hs.532399	KIAA0663 gene product (KIAA0663)	5.00	0.00	1q32.1
AAATCGATGA	Hs.496943	Hypothetical protein MGC29729 (MGC29729)	5.00	0.00	Xq28
AGAGCATATC	Hs.430425	Guanine nucleotide binding protein (G protein), beta polypeptide 1 (GNB1)	5.00	0.00	1p36.33
TTCACCTGTG	AW833547	EST	5.00	0.00	
TCTCTGCTCA	Hs.515134	CTL2 protein (CTL2)	5.00	0.00	19p13.1
TCTGTCTCA	Hs.272062	Protein tyrosine phosphatase, receptor type, F (PTPRF)	5.00	0.00	1p34
TTCCGTTTCT	Hs.288969	HSCARG protein (HSCARG)	5.00	0.00	16p13.3
AACCAATACA	Hs.146161	Endothelin converting enzyme 2 (ECE2)	5.00	0.00	3
CGCCGAACAC	BF127130	EST	5.00	0.00	
GTGCCCTTGA	BF308334	EST	5.00	0.00	
AAGATAAACT	Hs.426324	Tumor suppressor candidate 3 (TUSC3)	5.00	0.00	8p22
GGATGCAATG	BI192884	EST	5.00	0.00	
TAGGGCTCTC	Hs.524571	Cell division cycle associated 8 (CDCA8)	5.00	0.00	1p34.3
AAATAAAGAT	Hs.146766	cDNA FLJ41437 fis, clone BRHIP2007928	5.00	0.00	22
TATTGTATAT	Hs.461896	V-crk sarcoma virus CT10 oncogene homolog (avian) (CRK)	5.00	0.00	17p13.3
AGCCCTCCTG	BI089668	EST	5.00	0.00	
AACCTACAGA	Hs.532755	Likely ortholog of mouse gene trap locus 3 (GTL3)	5.00	0.00	16q21
TCCCACGTTT	Hs.534465	Presenilin enhancer 2 homolog (<i>C. elegans</i>) (PEN2)	5.00	0.00	19q13.12
CTTTTCAAGA	Hs.510402	Membrane cofactor protein (CD46, trophoblast-lymphocyte cross-reactive antigen) (MCP)	5.00	0.00	1q32
TTACTCTTTC	Hs.2533	Aldehyde dehydrogenase 9 family, member A1 (ALDH9A1)	5.00	0.00	1q23.1
CCTGGTCCAA	AA650073	EST	20.00	4.08	
ACCTTCCTAG	Hs.6454	Regulator of G-protein signalling 19 interacting protein 1 (RGS19IP1)	15.00	3.06	19p13.1

Table II supplementary. Continued.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome region
GAACGCCTAA	Hs.173381	Stromal cell-derived factor 2-like 1 (DPYSL2)	10.00	2.04	8p22-p21
AATCTTGTTT	Hs.417795	Dermokine (ZD52F10)	10.00	2.04	19q13.12
TATGCTTAGT	Hs.444818	CGG triplet repeat binding protein 1 (CGGBP1)	10.00	2.04	3p12-p11.1
ATCTCTTTCC	Hs.355570	Hypothetical protein FLJ30656 (FLJ30656)	10.00	2.04	17q21.31
TGCCTCTGTC	Hs.75514	Nucleoside phosphorylase (NP)	10.00	2.04	14q13.1
AAGAAGCAGG	Hs.523262	Chromosome 1 open reading frame 8 (C1orf8)	10.00	2.04	1p36-p31
ACTAACTGTG	Hs.16003	Retinoblastoma binding protein 4 (RBBP4)	10.00	2.04	1p35.1
GTGTAGTTGA	BI464015	EST	10.00	2.04	
AACTCTGGAC	Hs.134406	Thioredoxin-like 4B (TXNL4B)	5.00	1.02	16q22.2
GTGCCCGTGC	Hs.524219	Triosephosphate isomerase 1 (TPI1)	5.00	1.02	12p13
AAGGATGCGG	Hs.112444	TAF11 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 28 kDa (TAF11)	5.00	1.02	6p21.31
CTGCCCCAC	Hs.164410	Chromosome 16 open reading frame 7 (C16orf7)	5.00	1.02	16q24
ACCAGCTCCC	Hs.22546	Cytochrome b, ascorbate dependent 3 (CYBASC3)	5.00	1.02	11q12.2
CTTTTCTTCT	Hs.479814	Polymerase (RNA) II (DNA directed) polypeptide B, 140 kDa (POLR2B)	5.00	1.02	4q12
TCCAGGGCTC	Hs.22824	MYB binding protein (P160) 1a (MYBBP1A)	5.00	1.02	17p13.3
GAGGCATATG	Hs.553531	CGI-96 protein (CGI-96)	5.00	1.02	22q13.2-q13.31
GAGTGAAAGA	Hs.5811	Chromosome 21 open reading frame 59 (C21orf59)	5.00	1.02	21q22.1
ATCTTAAACA	Hs.473838	Down syndrome critical region gene 2 (DSCR2)	5.00	1.02	21q22.3
CACGAAGATG	Hs.150693	Activated leukocyte cell adhesion molecule (ALCAM)	5.00	1.02	3q13.1
GGAAGATGTT	Hs.288284	PQ loop repeat containing 1 (PQLC1)	5.00	1.02	18q23
TTCACAGATT	Hs.431101	Guanine nucleotide binding protein (G protein), gamma 12 (GNG12)	5.00	1.02	1p31.2
GGCCAAACAG	Hs.520149	Mitochondrial ribosomal protein S18A (MRPS18A)	5.00	1.02	6p21.3
GGCCCCAAAG	AI608743	EST	5.00	1.02	
CCATCCGAAA	AW799595	EST	5.00	1.02	
TACACAGAAC	Hs.24283	Zinc finger, DHHC-type containing 20 (FLJ25952)	5.00	1.02	13q12.11
CGCCTTTACT	Hs.47062	Polymerase (RNA) II (DNA directed) polypeptide I, 14.5 kDa (POLR2I)	5.00	1.02	19q12
CTCCATCGGC	Hs.65238	Ring finger protein 40 (RNF40)	5.00	1.02	16p11.2-p11.1
AATTAACCTC	Hs.436219	Aldehyde dehydrogenase 1 family, member B1 (ALDH1B1)	5.00	1.02	9p11.1
TTGAATCCCC	Hs.112341	Protease inhibitor 3, skin-derived (SKALP) (PI3)	5.00	1.02	20q12-q13
GAAATGCCTT	Hs.252682	Torsin family 1, member B (torsin B) (TOR1B)	5.00	1.02	9q34
AGATCCTACT	Hs.546253	Farnesyl-diphosphate farnesyltransferase 1 (FDFT1)	5.00	1.02	8p23.1-p22
TCAGAGAAGG	Hs.534350	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1 (SMARCB1)	5.00	1.02	22q11.23
GACTTGCCCC	Hs.473648	Phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase (GART)	5.00	1.02	21q22.1
TCTTCCCCAG	Hs.529132	Selenoprotein W, 1 (SEPW1)	5.00	1.02	19q13.3
ATCTTAGTCA	Hs.465763	Rho/rac guanine nucleotide exchange factor (GEF) 18 (ARHGEF18)	5.00	1.02	19p13.3
ATGGTTCTCA	Hs.440382	Ret finger protein (RFP)	5.00	1.02	6p22
GGAGTGTGCT	Hs.504687	Elongation factor Tu family protein (MYL9)	5.00	1.02	20q11.23
CCCTAGGTTG	Hs.3989	Plexin B2 (PLXNB2)	5.00	1.02	22q13.33
TTTTAATGTA	Hs.352341	Stress 70 protein chaperone, microsome-associated, 60kDa (STCH)	5.00	1.02	21q11.1
GTCCCTCTCA	Hs.459857	Calcium regulated heat stable protein 1, 24 kDa (CARHSP1)	5.00	1.02	16p13.2
CGCTGTGGGG	Hs.7486	Ethylmalonic encephalopathy 1 (ETHE1)	5.00	1.02	19q13.31
CGTTCATTC	Hs.216226	Synaptogyrin 1 (SYNGR1)	5.00	1.02	22q13.1
CTTCCGTAGC	Hs.436417	Decreased expression in renal and prostate (DERPC)	5.00	1.02	16q22.1
ACCTCCCACC	Hs.118666	FAD-synthetase (PP591)	5.00	1.02	1q22
ACTGACTATC	Hs.520037	Sialidase 1 (lysosomal sialidase) (NEU1)	5.00	1.02	6p21.3
AGGGGAATGG	Hs.441737	Junctophilin 2 (JPH2)	5.00	1.02	20q13.12
TCTAAAAAGG	Hs.16622	Zinc finger protein 185 (LIM domain) (ZNF185)	5.00	1.02	Xq28
ATTACACCAC	Hs.512607	Membrane interacting protein of RGS16 (MIR16)	5.00	1.02	16p12-p11.2
TGTTCTGCTA	AW367805	EST	5.00	1.02	
CCAAGGAATG	Hs.469941	Hypothetical protein LOC130074 (LOC130074)	5.00	1.02	2q21.2
AAATAGGTTT	Hs.419640	Parkinson disease (autosomal recessive, early onset) 7 (PARK7)	5.00	1.02	1p36.33-p36.12
GGTGTGGGTG	BC039061	EST	5.00	1.02	
AACATCAAAC	Hs.518609	Actin related protein 2/3 complex, subunit 5, 16 kDa (ARPC5)	5.00	1.02	1q25.3

Table II supplementary. Continued.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome region
TTTTACTCA	Hs.212395	CDKN1A interacting zinc finger protein 1 (CIZ1)	5.00	1.02	9q34.1
CTAGCCCTCA	BF678697	EST	5.00	1.02	
TAAAAAAGGT	Hs.122934	Hypothetical protein FLJ23861 (FLJ23861)	5.00	1.02	2q34
ACTATCTCTA	Hs.379754	Hypothetical protein LOC340061 (LOC340061)	5.00	1.02	5q31.2
GAAACAATGG	Hs.553542	Erythrocyte membrane protein band 4.1 like 4A (EPB41L4A)	5.00	1.02	5q22.2
TATGTATGTT	Hs.442592	Casein kinase 1, alpha 1 (CSNK1A1)	5.00	1.02	5q32
AGATGTGTGG	Hs.515848	Hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit (HADHB)	5.00	1.02	2p23
GACCACGAAT	Hs.148641	Cathepsin H (CTSH)	5.00	1.02	15q24-q25
GAGGATTTTA	Hs.79088	Reticulocalbin 2, EF-hand calcium binding domain (RCN2)	5.00	1.02	15q23
GATAGGTCGG	Hs.446075	Aconitase 1, soluble (ACO1)	5.00	1.02	9p22-q32
TGGCCCCAGG	Hs.110675	Apolipoprotein C-I (APOC1)	5.00	1.02	19q13.2
CAAGGTCATT	Hs.510833	Tight junction protein 1 (zona occludens 1) (TJP1)	5.00	1.02	15q13
CCATCTCTAG	BU955322	EST	5.00	1.02	
AGGAGCAAAG	Hs.515785	Biliverdin reductase B [flavin reductase (NADPH)] (BLVRB)	24	5.1	19q13.1-q13.2
GGAGGCAGGT	Hs.474083	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 2 (BAGALT2)	14	3.06	1p34-p33
GTTTAAATCG	Hs.333786	Proteasome (prosome, macropain) subunit, alpha type, 2 (PSMA2)	18	4.08	7p14.1
TTTTCTGAAA	Hs.435136	Thioredoxin (TXN)	18	4.08	9q31
CTTCCTTGCC	Hs.2785	Keratin 17 (KRT17)	9	2.04	17q12-q21
CCCCTCTGAG	Hs.12341	Adenosine deaminase, RNA-specific (ADAR)	9	2.04	1q21.1-q21.2
ATCCAGTCTG	Hs.517029	Chromosome 20 open reading frame 102 (C20orf102)	9	2.04	20q11.23
TGGAGAAAGA	Hs.445394	Vacuolar protein sorting 29 (yeast) (VPS29)	9	2.04	12q24
TAAAGTGCTC	Hs.462550	Phosphatidylinositol glycan, class S (PIGS)	9	2.04	17p13.2
CTTATTCCTT	Hs.418533	BUB3 budding uninhibited by benzimidazoles 3 homolog (yeast) (BUB3)	9	2.04	10q26
CTTCTATGTA	Hs.117825	Poly(ADP-ribose) polymerase family, member 4 (PARP4)	9	2.04	13q11
GAGGGTATAC	Hs.274184	Transcription factor binding to IGHM enhancer 3 (TFE3)	9	2.04	Xp11.22
CAGTCTCTCA	Hs.539100	Ribosomal protein S10 (RPS10)	61	14.3	6p21.31
GGCTCCCACT	Hs.509736	Heat shock 90 kDa protein 1, beta (HSPCB)	13	3.06	6p12
ATTCTGCCTC	Hs.369279	NACHT, leucine rich repeat and PYD containing 2 (NALP2)	13	3.06	19q13.42
ATGTACTAAA	Hs.518123	TRK-fused gene (TFG)	13	3.06	3q12.2
TTTTATATAA	Hs.473788	OTU domain, ubiquitin aldehyde binding 1 (OTUB1)	17	4.08	11q13.1
ATTAAACCAT	Hs.222494	Hypothetical LOC79954 (FLJ14075)	4	0	2p25.1
TACTATTAAT	Hs.6710	Mannose-P-dolichol utilization defect 1 (MPDU1)	4	0	17p13.1-p12
CAGGAGCCCC	Hs.444202	Solute carrier family 9 (sodium/hydrogen exchanger), isoform 8 (SLC9A8)	4	0	20q13.13
GGCACTTATG	Hs.513034	Unc-51-like kinase 3 (<i>C. elegans</i>) (ULK3)	4	0	15q24.1
CCGTCAAGGG	AA095497	EST	4	0	
GATAATTTTT	Hs.190722	HSPC142 protein (HSPC142)	4	0	19p13.11
ATTTTTGGTG	Hs.549585	Prolyl endopeptidase-like (KIAA0436)	4	0	2p22.1
GAATCAAGCT	Hs.17296	Potassium channel tetramerisation domain containing 14 (KCTD14)	4	0	11q14.1
GCTCTGGTGT	Hs.445097	Snf2-related CBP activator protein (SRCAP)	4	0	16p11.2
GCAAAAAAAT	Hs.522310	N-acetylneuraminic acid synthase (sialic acid synthase) (NANS)	4	0	9p24.1-p23
CCAACCTTGG	Hs.438409	XPMC2 prevents mitotic catastrophe 2 homolog (<i>Xenopus laevis</i>) (XPMC2H)	4	0	9q34.2
AGATCTACAA	Hs.514009	Chromosome 17 open reading frame 35 (C17orf35)	4	0	17p11.2
GTGAACACAG	Hs.274135	Hypothetical protein DKFZp434K1815 (DKFZp434K1815)	4	0	7q22.1
TCAATAAATA	Hs.4276	Basic helix-loop-helix domain containing, class B, 9 (BHLHB9)	4	0	Xq23
TGTGGCCAC	Hs.114198	Mitogen-activated protein kinase kinase 5 (MAP2K5)	4	0	15q23
TAAGGACGAG	Hs.554886	Hypothetical protein FLJ22457 (FLJ22457)	4	0	1p13.3
TGTTCTATGA	Hs.360033	DNA replication complex GINS protein PSF1 (KIAA0186)	4	0	20p11.21
TAAACAAAGA	Hs.553497	Phosphatidylinositol glycan, class H (PIGH)	4	0	14q11-q24
CCCCACATCC	Hs.84753	Hypothetical protein FLJ12442 (FLJ12442)	4	0	3p21.1
GAAATACACG	BG960366	EST	4	0	
TTCATTATAG	Hs.533514	Acetylserotonin O-methyltransferase-like (ASMTL)	4	0	Xp22.3
GATGGCTGCT	BE894663	EST	4	0	
AGAGCACTCT	Hs.355263	Transcribed locus	4	0	4

Table II supplementary. Continued.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome region
CAAAGGAATC	AA089931	EST	4	0	
TTTTTACAGT	Hs.477155	ATPase, H ⁺ transporting, lysosomal 70 kDa, V1 subunit A (ATP6V1A)	4	0	3q13.2-q13.31
TTCTGAAGAC	Hs.477352	Protein disulfide isomerase family A, member 5 (PDIR)	4	0	3q21.1
CCACTGCATT	Hs.513102	SCAN domain containing 2 (SCAND2)	4	0	15q25-q26
TCACAAAAGA	Hs.12646	Poly(ADP-ribose) polymerase family, member 12 (ZC3HDC1)	4	0	7q34
TGAGTTTTAC	Hs.58373	Hypothetical protein LOC283951 (LOC283951)	4	0	16p13.3
GAGAGGACAT	Hs.403933	F-box protein 32 (FBX032)	4	0	8q24.13
CCCCTCCCA	Hs.150540	Hypothetical protein BC002942 (BC002942)	4	0	22q13.33
TACATCATAA	Hs.122386	cDNA FLJ30952 fis, clone HCASM1000130	4	0	8
CTTTTCTTAT	AA610382	EST	4	0	
CAGAGTGACT	Hs.506652	PWP1 homolog (<i>S. cerevisiae</i>) (PWP1)	4	0	12q23.3
CAGCATCTGG	Hs.98669	ADP-ribosylhydrolase like 1 (ADPRHL1)	4	0	13q34
CAGCTTCCCT	Hs.528024	Microtubule associated monooxygenase, calponin and LIM domain containing 3 (MICAL3)	4	0	22q11.21
GTTAAGATTT	Hs.516954	Rho guanine nucleotide exchange factor (GEF) 11 (ARHGEF11)	4	0	1q21
AAAATGAAGA	Hs.418520	Chromosome 6 open reading frame 51 (C6orf51)	4	0	6q21
ACTCTTCTAA	Hs.498397	Saccharopine dehydrogenase (putative) (CGI-49)	4	0	1q44
GTAATTACTION	Hs.436502	Hypothetical protein LOC51249 (LOC51249)	4	0	1p34.1
AGAAATCACT	Hs.438289	L-3-hydroxyacyl-Coenzyme A dehydrogenase, short chain (HADHSC)	4	0	4q22-q26
TTCTAGACCA	Hs.372286	Cullin 3 (CUL3)	4	0	2q36.3
AATTAATTGT	Hs.466391	Chromosome 19 open reading frame 2 (C19orf2)	4	0	19q12
CCTGGGTCCC	Hs.287692	CD33 antigen-like 3 (CD33L3)	4	0	18q12.3
TTTTTTACAA	Hs.546316	Phosphatidylinositol-4-phosphate 5-kinase, type I, alpha (PIP5K1A)	4	0	1q22-q24
ATATCTTTGC	Hs.464985	Ras-like without CAAX 2 (RIT2)	4	0	18q12.3
TTGAAATAAA	Hs.418581	FLJ20160 protein (FLJ20160)	4	0	2q32.2
GTGAGGGCAC	Hs.107382	DEAH (Asp-Glu-Ala-His) box polypeptide 37 (DHX37)	4	0	12q24.31
ACAGGTGGAG	BI059321	EST	4	0	
GGAACCTCCA	Hs.75782	General transcription factor IIIC, polypeptide 2, beta 110 kDa (GTF3C2)	4	0	2p23.3
TCAGAGTCTT	BQ331290	EST	4	0	
GACTTACTAT	Hs.20848	WD repeat, SAM and U-box domain containing 1 (WDSAM1)	4	0	2q24.2
AAGAAAAGAA	Hs.299208	Coenzyme Q3 homolog, methyltransferase (yeast) (COQ3)	4	0	6q16.3
ACCCACTCTA	Hs.352614	PLSC domain containing protein (LOC254531)	4	0	15q14
GCCACCCCGT	Hs.461047	Glucose-6-phosphate dehydrogenase (G6PD)	4	0	Xq28
TTAATACAAT	Hs.126115	Phosphatidylinositol glycan, class B (PIGB)	4	0	15q21-q22
TCCTACAATC	Hs.556063	Opposite strand transcription unit to STAG3 (GATS)	4	0	7q22.1
AAAAATCCTA	Hs.116014	Chromosome 14 open reading frame 126 (C14orf126)	4	0	14q12
GAAACAAGAA	Hs.285887	UDP glycosyltransferase 2 family, polypeptide B4 (UGT2B4)	4	0	4q13
AGTAAGCTCC	Hs.172788	Armadillo repeat containing, X-linked 3 (ARMCX3)	4	0	Xq21.33-q22.2
AGTAATTCTT	T61002	EST	4	0	
GTAATAAAGG	BF246877	EST	4	0	
TGGCCAGCTC	Hs.477128	Steroid sensitive gene 1 (URB)	4	0	3q13.2
TTATTTTCCT	Hs.363492	DEAD (Asp-Glu-Ala-Asp) box polypeptide 18 (DDX18)	4	0	2q14.1
AGTCTCCCT	Hs.250429	Suppressor of Ty 6 homolog (<i>S. cerevisiae</i>) (SUPT6H)	4	0	17q11.2
CTGACTTAAA	Hs.104741	PDZ binding kinase (PBK)	4	0	8p21.2
GGCTAAGGAG	BI017905	EST	4	0	
TTCCCTGGCC	BU153762	EST	4	0	
TGGTATTTTCG	Hs.355398	Aldehyde dehydrogenase 16 family, member A1 (MGC10204)	4	0	19q13.33
GCACACAGAA	Hs.124186	Ring finger protein 2 (RNF2)	4	0	1q25.3
TGTCTGGATG	Hs.149443	Cytochrome b-561 domain containing 2 (CYB561D2)	4	0	3p21.3
CCAGTGTCTT	AW809533	EST	4	0	
TGAGTATAACC	Hs.115660	DNA cross-link repair 1B (PSO2 homolog, <i>S. cerevisiae</i>) (DCLRE1B)	4	0	1p13.2
CCCATCGTTC	CD239116	EST	4	0	
GCCATAAAAT	Hs.1908	Proteoglycan 1, secretory granule (PRG1)	4	0	10q22.1
AAGCATTAAA	Hs.550753	Protein kinase, cAMP-dependent, regulatory, type I, beta (PRKAR1B)	4	0	7pter-p22
AGGGATGGCC	Hs.515139	Transmembrane emp24 domain containing 1 (TMED1)	4	0	19p13.2

Table III supplementary. List of the genes up-regulated in C5.2 vs. HB4a mammary cells.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome region
CTGTGTTTAG	Hs.452583	Transcribed locus, strongly similar to NP_061322.2 matrin 3 (<i>Homo sapiens</i>)	0.00	230.52	5q31.2
CCAGGAACCT	Hs.446352	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian) (ERBB2)	0.00	85.68	17q11.2-q12
GCAGGTTCCC	AI624297	EST	1.00	19.38	
TAAGTAAAGT	Hs.368260	Hypothetical protein MGC11242 (MGC11242)	0.00	16.32	17q21.32
CTATTTAGGG	Hs.7736	Mitochondrial ribosomal protein L27 (MRPL27)	0.00	16.32	17q21.3-q22
TCTTCTCCCT	Hs.506748	Hepatoma-derived growth factor (high-mobility group protein 1-like) (HDGF)	1.00	14.28	1q21-q23
CCCAGCTAAT	Hs.200395	Centromere protein H (CENPH)	2.00	25.50	5p15.2
TGATTGGTGG	Hs.74615	Platelet-derived growth factor receptor, alpha polypeptide (PDGFRA)	1.00	12.24	4q11-q13
GCCCCAATA	Hs.445351	Lectin, galactoside-binding, soluble, 1 (galectin 1) (LGALS1)	2.00	23.46	22q13.1
TACCATCGAT	BI226498	EST	1.00	11.22	
GGCAGAGACC	Hs.467133	Nucleoporin 62 kDa (NUP62)	1.00	11.22	19q13.33
GCTCTGCCTC	Hs.252549	Cathepsin Z (CTSZ)	0.00	10.20	20q13
CACGCAATGC	Hs.515053	Amino-terminal enhancer of split (AES)	1.00	10.20	19p13.3
GATTTTGTAG	Hs.494604	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member B (ANP32B)	2.00	18.36	9q22.32
TAGCTTCCTT	Hs.44276	Homeo box C10 (HOXC10)	1.00	9.18	12q13.3
TAAACATTGT	Hs.468140	DKFZP564F0522 protein (DKFZP564F0522)	1.00	9.18	2p22.3
GTAGACACCT	Hs.418133	Intracellular membrane-associated calcium-independent phospholipase A2 gamma (IPLA2 GAMMA)	2.00	17.34	7q31
ATGGCAGGAG	Hs.194121	RNA terminal phosphate cyclase-like 1 (RCL1)	0.00	8.16	9p24.1-p23
TAAATACAGT	Hs.503043	Carnitine palmitoyltransferase 1A (liver) (CPT1A)	0.00	8.16	11q13.1-q13.2
TGTATCACTG	BF932124	EST	0.00	8.16	
CATTGTAATA	BE871060	EST	0.00	8.16	
CCTAAGGCTA	Hs.108371	E2F transcription factor 4, p107/p130-binding (E2F4)	0.00	8.16	16q21-q22
TGGATCAACC	Hs.74034	Caveolin 1, caveolae protein, 22 kDa (CAV1)	0.00	8.16	7q31.1
CAGGAACCTG	BE468107	EST	0.00	8.16	
TCACAGCTGT	Hs.255935	B-cell translocation gene 1, anti-proliferative (BTG1)	2.00	16.32	12q22
CTACCAGGCC	Hs.334848	Hypothetical protein FLJ13291 (FLJ13291)	1.00	8.16	16q22.1
TCTCAATTCT	Hs.467637	Cell division cycle 42 (GTP binding protein, 25 kDa) (CDC42)	1.00	8.16	1p36.1
GCTTTCATTG	Hs.2704	Glutathione peroxidase 2 (GPX2)	1.00	8.16	14q24.1
TTTTGATAAA	Hs.388927	YY1 transcription factor (YY1)	1.00	8.16	14q
TCTGCTAAAG	Hs.475685	High-mobility group box 1 (HMGB1)	1.00	8.16	13q12
AAGAGTTGGG	Hs.536652	Transcribed locus	1.00	8.16	
TAAGCAGATG	Hs.306425	Inhibitor of Bruton agammaglobulinemia tyrosine kinase (IBTK)	1.00	8.16	6q14.1
GCGAAACCCC	Hs.288945	Hypothetical protein FLJ13448 (FLJ13448)	1.00	8.16	2q33.1
CAGATAATA	Hs.204475	HIV TAT specific factor 1 (HTATSF1)	1.00	8.16	Xq26.1-q27.2
GATTTGTAGC	BE279179	EST	2.00	15.30	
ACCGCCTGTG	Hs.79625	Chromosome 20 open reading frame 149 (C20orf149)	2.00	15.30	20q13.33
TCCTTTGCAA	Hs.81892	KIAA0101 (KIAA0101)	0.00	7.14	15q22.31
CTACATAATA	Hs.432385	DEAD (Asp-Glu-Ala-Asp) box polypeptide 7 (DDX7)	0.00	7.14	21
CTGCCTCCTT	Hs.472847	Chromosome 20 open reading frame 35 (C20orf35)	0.00	7.14	20q13.12
GCTACAGGTA	Hs.349306	Hypothetical protein FLJ31951 (FLJ31951)	0.00	7.14	5q33.3
TTCAAGAAAC	Hs.458974	Transcribed locus	0.00	7.14	8
CTCATAAGAA	Hs.549495	Transcribed locus, strongly similar to XP_529639.1	0.00	7.14	14
TTAGCTTGTT	BU074305	EST	0.00	7.14	
CAGGATCCAG	Hs.447477	Family with sequence similarity 10, member A5 (FAM10A5)	0.00	7.14	11p15.1
GCGATCGGG	BF336989	EST	0.00	7.14	
GCGGAGAGAG	Hs.546104	Transcribed locus	3.00	21.42	X
AGTAGGTGGC	Hs.459311	Hypothetical protein DKFZp547K1113 (DKFZp547K1113)	2.00	14.28	15q26.1
CGAATAAAAT	Hs.139896	Macrophage erythroblast attacher (MAEA)	2.00	14.28	4p16.3
TACAATAAAC	Hs.507910	Progesterone receptor membrane component 2 (PGRMC2)	1.00	7.14	4q26
GTTGTGATGT	Hs.374477	Ewing sarcoma breakpoint region 1 (EWSR1)	1.00	7.14	22q12.2
GTTGCAAGTG	BQ709743	EST	1.00	7.14	
CTGTTGATGC	BG570533	EST	1.00	7.14	
TACAAAACCA	Hs.79110	Nucleolin (NCL)	1.00	7.14	2q12-qter

Table III supplementary. Continued.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome region
CGACCCCTCTC	Hs.400625	Glutamate-rich WD repeat containing 1 (GRWD1)	1.00	7.14	19q13.32
TAACCTCAGG	Hs.18166	KIAA0870 protein (KIAA0870)	1.00	7.14	8q24.3
TGAAGAGAAT	Hs.364260	Forkhead box R2 (FOXR2)	1.00	7.14	Xp11.22
GCCGCCATCA	Hs.212102	Protein disulfide isomerase-associated 6 (TXNDC7)	1.00	7.14	2p25.1
AAAGGGGGCA	Hs.516539	Heterogeneous nuclear ribonucleoprotein A3 (HNRPA3)	1.00	7.14	2q31.2
GCTGGGAGGG	Hs.325650	EH-domain containing 2 (EHD2)	1.00	7.14	19q13.3
GGAATGAGGG	Hs.189810	Sulfotransferase family 4A, member 1 (SULT4A1)	1.00	7.14	22q13.2-q13.31
CTCTTCAGGA	Hs.30954	Phosphomevalonate kinase (PMVK)	1.00	7.14	1p13-q23
GGATGGGTGT	Hs.513330	Chromosome 16 open reading frame 51 (C16orf51)	1.00	7.14	16p13.13-p12.3
TCAAGAAATT	Hs.152978	Proteasome (prosome, macropain) activator subunit 3 (PA28 gamma; Ki) (PSME3)	2.00	13.26	17q21
TACTGCTCGG	Hs.553141	Transcribed locus	2.00	13.26	7
GAAAAAATGT	Hs.194329	Transcription elongation factor A (SII)-like 4 (TCEAL4)	2.00	13.26	Xq22.2
CCGATTTTAA	Hs.26570	Hypothetical protein FLJ20422 (FLJ20422)	0.00	6.12	19p13.11
TGGGAAGGGA	Hs.15961	Chromosome 9 open reading frame 23 (C9orf23)	0.00	6.12	9p13.3
CTGTTGTTGG	AI275403	EST	0.00	6.12	
CATAGGTTAA	BF348753	EST	0.00	6.12	
GTGGCAGCGC	Hs.348326	Stathmin-like 3 (STMN3)	0.00	6.12	20q13.3
GTGAAACTCC	Hs.522394	Heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa) (HSPA5)	0.00	6.12	9q33-q34.1
GAGGACGAAG	Hs.50425	Inactive progesterone receptor, 23 kDa (TEBP)	0.00	6.12	12q13.3
GCAGTTCCT	Hs.297729	Transcribed locus	0.00	6.12	15
ACCACGCCGT	Hs.436329	Scribbled homolog (<i>Drosophila</i>) (SCRIB)	0.00	6.12	8q24.3
TCCACTACCA	Hs.405692	CGI-116 protein (CGI-116)	0.00	6.12	12q23.3
TAGTTAAGCC	Hs.458598	UTP14, U3 small nucleolar ribonucleoprotein, homolog A (yeast) (UTP14A)	0.00	6.12	Xq25
TCCATCCCTT	Hs.7527	Small fragment nuclease (DKFZP566E144)	0.00	6.12	11q23.1-q23.2
CTGGCCGACT	Hs.513883	Proline-, glutamic acid-, leucine-rich protein 1 (PELP1)	0.00	6.12	17p13.2
GAGTCAGCAT	Hs.166463	Heterogeneous nuclear ribonucleoprotein U (scaffold attachment factor A) (HNRPU)	0.00	6.12	1q44
CTAAAGACTT	Hs.176247	Dipeptidylpeptidase 10 (DPP10)	0.00	6.12	2q14.1
CCTGAGCTGG	Hs.97910	Hypothetical protein LOC253264 (LOC253264)	0.00	6.12	10q22.2
GCAGAAGAGG	Hs.546465	Leukocyte receptor cluster (LRC) member 9 (LENG9)	0.00	6.12	19q13.4
CCCGGGGCCT	Hs.90691	Nucleophosmin/nucleoplasm, 3 (NPM3)	0.00	6.12	10q24.31
GCCTGCTGGG	Hs.433951	Glutathione peroxidase 4 (phospholipid hydroperoxidase) (GPX4)	2.00	12.24	19p13.3
CAGTTTGTAC	Hs.530331	Pyruvate dehydrogenase (lipoamide) alpha 1 (PDHA1)	2.00	12.24	Xp22.2-p22.1
TTTGTAGATG	Hs.184233	Heat shock 70 kDa protein 9B (mortalin-2) (HSPA9B)	2.00	12.24	5q31.1
ACAAGGTGCT	BU853523	EST	2.00	12.24	
TAAAATAAGA	Hs.69293	Hexosaminidase B (beta polypeptide) (HEXB)	2.00	12.24	5q13
GCGCAGACTT	Hs.14745	Chromosome 10 open reading frame 9 (C10orf9)	1.00	6.12	10p11.21
GGAGAAGATG	Hs.492516	Prefoldin 2 (PFDN2)	1.00	6.12	1q23.3
TTAAGAGGGA	Hs.531550	Transducer of ERBB2, 1 (TOB1)	1.00	6.12	17q21
AGACATAAAT	CA429050	EST	1.00	6.12	
CACGGACACG	Hs.500756	Glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1) (GOT1)	1.00	6.12	10q24.1-q25.1
CTGAGCTGTA	Hs.8737	WD repeat domain 6 (WDR6)	1.00	6.12	3p21.31
CCATAAATGT	Hs.74052	CDNA FLJ40823 fis, clone TRACH2011093	1.00	6.12	
TGAAGCAGTA	Hs.232543	Programmed cell death 4 (neoplastic transformation inhibitor) (PDCD4)	1.00	6.12	10q24
GAAGGGATCA	Hs.501345	C-terminal binding protein 2 (CTBP2)	1.00	6.12	10q26.13
TAGCAGAGGC	Hs.513266	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10, 22 kDa (NDUFB10)	1.00	6.12	16p13.3
AAGACAGGGC	BE080148	EST	1.00	6.12	
GAGCCTGTAA	Hs.406377	Hypothetical protein DKFZp762N1910 (DKFZp762N1910)	1.00	6.12	11q12.3
TCCGTGGTTG	Hs.201641	Brain abundant, membrane attached signal protein 1 (BASP1)	1.00	6.12	5p15.1-p14
GGCTGGGGGG	AV699465	EST	1.00	6.12	
TTCCCCTTCC	Hs.368376	Signal recognition particle receptor ('docking protein') (SRPR)	1.00	6.12	11q24.3

Table III supplementary. Continued.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome region
GCGCAGAGGT	Hs.356799	cDNA clone IMAGE:6726542, partial cds	12.00	68.34	
TGGCTGGGAA	Hs.534373	Vesicle-associated membrane protein 8 (endobrevin) (VAMP8)	2.00	11.22	2p12-p11.2
CTTAATCCTG	Hs.221847	Solute carrier family 38, member 2 (SLC38A2)	2.00	11.22	12q
GTGTGTGTGT	Hs.2549	Adrenergic, beta-3-, receptor (ADRB3)	0.00	5.10	8p12-p11.2
CCAGGAACCC	BQ898423	EST	0.00	5.10	
TTGTAAGAAC	Hs.513280	Transcribed locus	0.00	5.10	16
CCTCTAGTCC	Hs.488778	cDNA FLJ26938 fis, clone RCT07169	0.00	5.10	7
TGATTTACC	UV723019	EST	0.00	5.10	
AGGTTTCCTC	Hs.12970	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 3 (PSMD3)	0.00	5.10	17q21.1
TCTTCTCCTG	Hs.128553	Wingless-type MMTV integration site family member 2 (WNT2)	0.00	5.10	7q31
GAGCTGTGGT	Hs.282931	Solute carrier family 17 (sodium phosphate), member 4 (SLC17A4)	0.00	5.10	6p22-p21.3
TGCAGAGTAA	Hs.512065	Similar to cytochrome c oxidase (LOC285849)	0.00	5.10	
TGGGGTTCCA	BE962013	EST	0.00	5.10	
TCCACTGGCC	Hs.441783	Chromosome 14 open reading frame 78 (C14orf78)	0.00	5.10	14q32.33
GTCACCCCA	Hs.10082	Potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4 (KCNN4)	0.00	5.10	19q13.2
CACTGTGTGT	Hs.532987	Chromosome 18 open reading frame 22 (C18orf22)	0.00	5.10	18q23
GTGAAACTGC	Hs.405942	Hypothetical protein LOC339229 (LOC339229)	0.00	5.10	17q25.3
GAGGATTTGG	Hs.370102	Chromosome 10 open reading frame 70 (C10orf70)	0.00	5.10	10q21.1
TCTTTCCCA	Hs.268557	Pleckstrin homology-like domain, family A, member 3 (PHLDA3)	0.00	5.10	1q31
GGGCCCTTGG	Hs.525610	Chromosome 14 open reading frame 172 (C14orf172)	0.00	5.10	14q32.32
TATCTGTCTA	Hs.436687	SET translocation (myeloid leukemia-associated) (SET)	10.00	51.00	9q34
CAAGATAAAT	Hs.5258	Melanoma antigen family D, 1 (MAGED1)	2.00	10.20	Xp11.23
TACCATCAAG	Hs.515955	Similar to cysteine and histidine-rich domain (CHORD)-containing, zinc-binding protein 1	2.00	10.20	2p21
GACAGCTGAG	Hs.175473	Adenylate kinase 1 (AK1)	2.00	10.20	9q34.1
TGTGAACAAC	Hs.534331	Nudix (nucleoside diphosphate linked moiety X)-type motif 1 (NUDT1)	2.00	10.20	7p22
GCAAAAAAAAA	Hs.284491	Pyridoxal (pyridoxine, vitamin B6) kinase (PDXK)	2.00	10.20	21q22.3
TTAAGGGATG	Hs.521008	Tumor necrosis factor, alpha-induced protein 9 (TNFAIP9)	2.00	10.20	7q21.12
GAAGCTTTGC	Hs.523560	Heat shock 90 kDa protein 1, alpha (HSPCA)	1.00	5.10	14q32.33
GGCTGGTCCC	Hs.380801	Pecanex-like 3 (<i>Drosophila</i>) (PCNXL3)	1.00	5.10	11q13.1
CATAACCTTC	Hs.405144	Splicing factor, arginine/serine-rich 3 (SFRS3)	1.00	5.10	6p21
GTGGAATAAA	Hs.512776	Latent transforming growth factor beta binding protein 2 (LTBP2)	1.00	5.10	14q24
CCCCCGGCTA	Hs.144722	cDNA FLJ43899 fis, clone TEST14009928	1.00	5.10	9
GGTGAGACCT	Hs.433863	Prostatic binding protein (PBP)	1.00	5.10	12q24.23
GATTCATAAA	Hs.372688	Rho-related BTB domain containing 2 (RHOBTB2)	1.00	5.10	8p21.3
CTGGTAATAA	AI524746	EST	1.00	5.10	
GTTCGGGCCG	Hs.502914	Dipeptidylpeptidase 3 (DPP3)	1.00	5.10	11q12-q13.1
AGGAACTGTA	Hs.495473	Notch homolog 1, translocation-associated (<i>Drosophila</i>) (NOTCH1)	1.00	5.10	9q34.3
GTGTCCGGGG	Hs.459795	Leucine zipper domain protein (FLJ22386)	1.00	5.10	16p13.3
CACATCTCTG	Hs.355606	Hypothetical protein MGC23909 (MGC23909)	1.00	5.10	5q14.2
TATATATATC	Hs.414407	Kinetochore associated 2 (KNTC2)	1.00	5.10	18p11.32
TACCGCCCGT	Hs.238513	Chromosome 7 open reading frame 21 (C7orf21)	1.00	5.10	7q36.1
GGAAACTGAT	Hs.83916	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13 kDa (NDUFA5)	1.00	5.10	7q32
AGTGCTCACT	Hs.540696	Solute carrier family 6 (neurotransmitter transporter, creatine), member 8 (SLC6A8)	1.00	5.10	Xq28
AATAAATTCC	Hs.439671	Neuroblastoma, suppression of tumorigenicity 1 (NBL1)	1.00	5.10	1p36.13-p36.11
CTTCGAAACT	Hs.464572	NADH dehydrogenase (ubiquinone) flavoprotein 2, 24 kDa (NDUFV2)	1.00	5.10	18p11.31-p11.2
TAAACCTGTC	Hs.435610	WW domain containing adaptor with coiled-coil (WAC)	1.00	5.10	10
CCGATCACCG	Hs.429180	Eukaryotic translation initiation factor 2, subunit 2 beta, 38 kDa (EIF2S2)	1.00	5.10	20pter-q12
TTCCCAAGGC	Hs.437846	Smoothed homolog (<i>Drosophila</i>) (SMO)	1.00	5.10	7q32.3
CAGCTTGCAA	Hs.105465	Small nuclear ribonucleoprotein polypeptide F (SNRPF)	1.00	5.10	12q23.1
GGTGTGTCCG	Hs.386939	Ubiquitin specific protease 7 (herpes virus-associated) (USP7)	1.00	5.10	16p13.3
GAGTGGCTAT	Hs.299055	GDP dissociation inhibitor 2 (GDI2)	1.00	5.10	10p15

Table III supplementary. Continued.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome region
TGCCCAGCAA	Hs.235116	G protein-coupled receptor kinase 6 (GRK6)	1.00	5.10	5q35
CCCTTGACCC	Hs.79769	Protocadherin 1 (cadherin-like 1) (PCDH1)	1.00	5.10	5q32-q33
TTGTTCTTTG	Hs.483408	Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform (PPP2CA)	1.00	5.10	5q31.1
CTTGAGTCAC	Hs.214043	Chromosome 6 open reading frame 79 (C6orf79)	1.00	5.10	6p24.3-p23
TGGGCAAACC	BE967503	EST	1.00	5.10	
GGAGCACTGT	Hs.528952	Tripartite motif-containing 25 (TRIM25)	1.00	5.10	17q23.1
AGTGGAGGTG	Hs.499891	Heterogeneous nuclear ribonucleoprotein H3 (2H9) (HNRPH3)	1.00	5.10	10q22
CAGTATCCCA	Hs.474978	Transducer of ERBB2, 2 (TOB2)	1.00	5.10	22q13.2-q13.31
TATTTATTCA	Hs.490795	Family with sequence similarity 62 (C2 domain containing) member B (CHR2SYT)	1.00	5.10	7q36.3
GATGAGTGGA	Hs.744	Ferredoxin 1 (FDX1)	1.00	5.10	11q22
GGAGCGTGGG	BE538756	EST	1.00	5.10	
GCATTTAAAA	Hs.552946	<i>Homo sapiens</i> , clone IMAGE:4045462, mRNA	1.00	5.10	15
GTGCTGGTGC	Hs.9613	Angiopoietin-like 4 (ANGPTL4)	1.00	5.10	19p13.3
AGCCTGACTG	Hs.289108	Family with sequence similarity 3, member A (FAM3A)	1.00	5.10	Xq28
GAGCGCAGCG	Hs.493202	Cleavage and polyadenylation specific factor 1, 160 kDa (CPSF1)	1.00	5.10	8q24.23
TAGGACAAC	Hs.511034	Transcribed locus, weakly similar to XP_574882.1 PREDICTED: similar to H3 histone, family 3B (<i>Rattus norvegicus</i>)	1.00	5.10	
GTAGTACTG	AA581135	EST	1.00	5.10	
GAGGGACTCC	Hs.75238	Chromatin assembly factor 1, subunit B (p60) (CHAF1B)	1.00	5.10	21q22.13
CTAGCCAGCA	Hs.501420	Nicalin homolog (zebrafish) (NCLN)	1.00	5.10	19p13.3
TTGTTCTGCT	Hs.458358	TSPY-like 1 (TSPYL1)	1.00	5.10	6q22-q23
CTGCATAGAT	Hs.494529	Fanconi anemia, complementation group C (FANCC)	1.00	5.10	9q22.3
GTGTGAGTGT	Hs.490347	Makorin, ring finger protein, 1 (MKRN1)	1.00	5.10	7q34
TCAGCCGCTA	Hs.434213	Chromosome 9 open reading frame 89 (C9orf89)	1.00	5.10	9q22.31
GGAATATGCA	Hs.224764	Chromosome 20 open reading frame 155 (C20orf155)	1.00	5.10	20p13-p12.3
ATTCAGCACC	Hs.11125	Signal peptidase complex subunit 1 homolog (<i>S. cerevisiae</i>) (SPCS1)	1.00	5.10	3p21.1
AAGTACGAGG	Hs.445081	NS5ATP13TP2 protein (NS5ATP13TP2)	1.00	5.10	11q23.3
GCAGACCCAC	Hs.15277	Chromosome 16 open reading frame 33 (C16orf33)	3	14.3	16p13.3
TAAACTGTTT	Hs.291219	cDNA FLJ37429 fis, clone BRAWH2001666	15	69.4	19
CCAACGTGTA	AI272324	EST	2	9.18	
CCACACAAGC	Hs.484363	Ring finger protein 130 (RNF130)	2	9.18	5q35.3
GCCAAGAATC	Hs.503047	Mitochondrial ribosomal protein L21 (MRPL21)	2	9.18	11q13.3
GAGTCGTAAT	Hs.370292	BRCA2 and CDKN1A interacting protein (BCCIP)	2	9.18	10q26.1
TGGATCCTCG	Hs.5268	Zinc finger, DHHC-type containing 4 (ZDHHC4)	3	13.3	7p22.1
CCCAAGCTAG	Hs.520973	Heat shock 27 kDa protein 1 (HSPB1)	7	29.6	7q11.23
GCTCTGTTCA	Hs.18192	Serine/arginine repetitive matrix 1 (SRRM1)	0	4.08	1p36.11
AAGGTGGAGT	Hs.9573	ATP-binding cassette, sub-family F (GCN20), member 1 (ABCF1)	0	4.08	6p21.33
TGTCATCACA	Hs.116479	Lysyl oxidase-like 2 (LOXL2)	0	4.08	8p21.3-p21.2
TAAGCCATAT	Hs.43818	Hypothetical protein LOC221710 (LOC221710)	0	4.08	6p24.1
GTAAATGAGC	Hs.247324	Mitochondrial ribosomal protein S14 (MRPS14)	0	4.08	1q23-1q25
TAAAAAGAGA	Hs.11108	Cdk5 and Abl enzyme substrate 1 (CABLES1)	0	4.08	18q11.2
GGCTATACAG	Hs.531111	YLP motif containing 1 (YLP1)	0	4.08	14q24.3
TTTGGGCCTA	Hs.70327	Cysteine-rich protein 1 (intestinal) (CRIP1)	0	4.08	14q32.33
CAGCCTGTCTG	Hs.26890	Cat eye syndrome chromosome region, candidate 5 (CECR5)	0	4.08	22
GTGATCTTCT	BE926316	EST	0	4.08	
TTGCCGTTT	Hs.471917	Proteasome (prosome, macropain) inhibitor subunit 1 (PI31) (PSMF1)	0	4.08	20p13
GAGGAAAGCT	Hs.145726	BCL2/adenovirus E1B 19 kDa interacting protein 1 (BNIP1)	0	4.08	5q33-q34
TGGAGACTGG	Hs.550499	Dynamin 1-like (DNM1L)	0	4.08	12p11.21
TATGTAATAT	Hs.435122	Phosphatidic acid phosphatase type 2A (PPAP2A)	0	4.08	5q11
TTAGCACTTC	Hs.488591	Williams-Beuren syndrome chromosome region 17 (WBSCR17)	0	4.08	7q11.23
TTGGTCTCC	BX393351	EST	0	4.08	
TAAATCTACA	Hs.288981	Nucleotide binding protein-like (NUBPL)	0	4.08	14q12
GGATTGGCTG	Hs.549560	cDNA FLJ30538 fis, clone BRAWH2001238	0	4.08	19
CTGACCGGTG	Hs.505806	Pre-B-cell leukemia transcription factor interacting protein 1 (PBXIP1)	0	4.08	1q22

Table III supplementary. Continued.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome region
TTGTGATGTA	Hs.187199	Metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA) (MALAT1)	0	4.08	11q13.1
TGCTGGAGAA	Hs.120573	Hypothetical protein DKFZp434K1172 (DKFZp434K1172)	0	4.08	13q14.3
AGCCGTGTAT	Hs.493771	Chromosome 9 open reading frame 25 (C9orf25)	0	4.08	9p13.3
TATAAAGGGT	Hs.25956	Sclerostin domain containing 1 (SOSTDC1)	0	4.08	7p21.1
TGACCTCCAG	Hs.494705	KIAA0859 (KIAA0859)	0	4.08	1q24-q25.3
GTCCAACAAC	CB857419	EST	0	4.08	
TGACTGTGCT	Hs.524116	Neurogranin (protein kinase C substrate, RC3) (NRGN)	0	4.08	11q24
TATGAACTGA	Hs.177766	Poly(ADP-ribose) polymerase family, member 1 (PARP1)	0	4.08	1q41-q42
GTTGTGGAGG	Hs.73853	Bone morphogenetic protein 2 (BMP2)	0	4.08	20p12
ACAAGCATAT	Hs.10056	Cytosolic sialic acid 9-O-acetyltransferase homolog (CSE-C)	0	4.08	11q24
ACACTCAATA	CA417269	EST	0	4.08	
AGAATCACTT	Hs.192127	Hypothetical protein MGC32020 (MGC32020)	0	4.08	19q13.11
TTGGCTTTTC	Hs.550788	AB14 precursor RNA	0	4.08	1
TAAATATAT	Hs.22109	Bromo adjacent homology domain containing 1 (BAHD1)	0	4.08	15q15.1
GTGGATTCAT	Hs.293736	Activity-dependent neuroprotector (ADNP)	0	4.08	20q13.13
GTGGCGTGTG	Hs.551542	Prenylcysteine oxidase 1 (PCYOX1)	0	4.08	2p13.3
TGCCTTAGTA	Hs.499000	DnaJ (Hsp40) homolog, subfamily C, member 1 (DNAJC1)	0	4.08	10p12.31
TGTCACACAC	Hs.20879	Zinc finger protein 691 (LOC51058)	0	4.08	1p34.2
CCGGGGAGCA	Hs.27059	GDP-mannose pyrophosphorylase A (GMPPA)	0	4.08	2q35
AATCGCTAAT	Hs.59486	Hydroxysteroid dehydrogenase like 2 (HSDL2)	0	4.08	9q32
CGTTTAATGT	BU933772	EST	0	4.08	
TGTGTGTGTG	Hs.330741	Zinc finger protein 670 (MGC12466)	0	4.08	1q44
GGCGGGGGCG	BQ227822	EST	0	4.08	
ACTTATTATG	Hs.156316	Decorin (DCN)	0	4.08	12q13.2
GCACCTAGTG	Hs.485041	Tripartite motif-containing 26 (TRIM26)	0	4.08	6p21.3
GGGAACGCAG	Hs.438454	F-box protein 25 (FBX025)	0	4.08	8p23.3
CATACTTTTA	Hs.24643	Hypothetical protein FLJ31413 (FLJ31413)	0	4.08	7q36.1
AGCAAGCCCC	Hs.222510	DAZ associated protein 1 (DAZAP1)	0	4.08	19p13.3
TGTAGGTATT	Hs.497741	Centromere protein F, 350/400 kDa (mitosin) (CENPF)	0	4.08	1q32-q41
CACCATCAAA	Hs.268488	Leucine rich repeat containing 47 (KIAA1185)	0	4.08	1p36.32
GTGGTGGCAG	Hs.546325	G protein-coupled receptor, family C, group 5, member A (GPCR5A)	0	4.08	12p13-p12.3
AAACTCGGGT	AW992502	EST	0	4.08	
TATAAGGTGG	Hs.223141	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21 (DDX21)	0	4.08	10q21
AGCTTGCGCT	Hs.193832	G patch domain containing 4 (GPATC4)	0	4.08	1q22
TGGAGAGCAA	AI301461	EST	0	4.08	
GCTTTACTTG	BX490890	EST	0	4.08	
AATGCTGGCA	Hs.490745	DnaJ (Hsp40) homolog, subfamily B, member 6 (DNAJB6)	0	4.08	7q36.3
AGGATGTGGC	Hs.546308	TEA domain family member 1 (SV40 transcriptional enhancer factor) (TEAD1)	0	4.08	11p15.4
CAAATAAACT	Hs.462956	PPAR binding protein (PPARBP)	0	4.08	17q12-q21.1
TTGCACTTAA	Hs.175414	Uncharacterized hypothalamus protein HT008 (HT008)	0	4.08	17q23.3
ACAGCCTGCA	Hs.153752	Cell division cycle 25B (CDC25B)	0	4.08	20p13
CCCCGTATGG	Hs.111801	Arsenate resistance protein ARS2 (ARS2)	0	4.08	7q21
TGTTTTTATG	Hs.3192	6-pyruvoyl-tetrahydropterin synthase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) (PCBD)	4	16.3	10q22
CTTCCTGTAC	Hs.488861	Uroplakin 3B (UPK3B)	4	16.3	7q11.2
CGGCTCAAGT	Hs.515500	SUMO-1 activating enzyme subunit 1 (SAE1)	2	8.16	19q13.32
CTTAAGGATT	Hs.530412	PAI-1 mRNA binding protein (PAI-RBP1)	2	8.16	1p31-p22
CAGCTGGGGC	Hs.172550	Polypyrimidine tract binding protein 1 (PTBP1)	2	8.16	19p13.3
GACTCTCTCA	Hs.472667	Catenin, beta like 1 (CTNBL1)	2	8.16	20q11.23-q12
ACTCGAATAT	Hs.546296	Secreted and transmembrane 1 (SECTM1)	2	8.16	17q25
GCAGGAGAAG	Hs.528574	Topoisomerase (DNA) I, mitochondrial (TOP1MT)	2	8.16	8q24.3
TTACTTCCCC	Hs.502745	Fatty acid desaturase 2 (FADS2)	2	8.16	11q12-q13.1
GTGAAACCGT	Hs.533772	Meteorin, glial cell differentiation regulator (METRN)	2	8.16	16p13.3
TGATGTCTGG	Hs.517155	Transmembrane, prostate androgen induced RNA (TMEPAI)	2	8.16	20q13.31-q13.33

Table III supplementary. Continued.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome region
GGAAGTCCTG	Hs.369397	Transforming growth factor, beta-induced, 68 kDa (TGFB1)	2	8.16	5q31
ATTCTTCGGA	Hs.122363	WIPI49-like protein 2 (WIPI-2)	1	4.08	7p22.1
ATTGTATGAC	Hs.377001	mRNA; cDNA DKFZp762L214 (from clone DKFZp762L214)	1	4.08	16
CAAATAAACCC	Hs.495728	Pirin (iron-binding nuclear protein) (PIR)	1	4.08	Xp22.2
TTTATTTCTA	Hs.349656	Scavenger receptor class B, member 2 (SCARB2)	1	4.08	4q21.1
GCCCTTCCTG	Hs.520955	Williams-Beuren syndrome chromosome region 16 (WBSCR16)	1	4.08	7q11.23
AATGAGTTTG	Hs.492555	Enhancer of yellow 2 homolog (<i>Drosophila</i>) [e(y)2]	1	4.08	8q23.1
CACTACACGG	Hs.227729	FK506 binding protein 2, 13kDa (FKBP2)	1	4.08	11q13.1-q13.3
GTTCAGAACT	Hs.524969	Ubiquitin-fold modifier 1 (Ufm1)	1	4.08	13q13.3
GTTGAAACCC	Hs.149425	Solute carrier family 28 (sodium-coupled nucleoside transporter), member 3 (SLC8A3)	1	4.08	9q22.2
CAGATTTGCA	Hs.510328	DEAD (Asp-Glu-Ala-Asp) box polypeptide 24 (DDX24)	1	4.08	14q32
GGCACGTGGT	BU928325	EST	1	4.08	
CCACTCTGGC	Hs.516119	Glucosidase I (GCS1)	1	4.08	2p13-p12
TGTACCTAAC	Hs.533770	Solute carrier family 38, member 1 (SLC38A1)	1	4.08	12q13.11
AGTCTATGA	Hs.531330	COBW domain containing 2 (CBWD1)	1	4.08	9p24.3
GAGGGACCCA	Hs.447531	Mesoderm posterior 1 (MESP1)	1	4.08	15q26.1
TATAAAATGG	Hs.459538	Aldehyde dehydrogenase 1 family, member A3 (ALDH1A3)	1	4.08	15q26.3
GGGGACTGGT	Hs.282982	Solute carrier family 25 (mitochondrial carrier), member 18 (SLC25A18)	1	4.08	22q11.2
GGGGCAGTGA	Hs.38512	KIAA1285 protein (KIAA1285)	1	4.08	7q36.1
GGGGCGGGGT	Hs.474970	Zinc finger CCCH-type containing 7B (RoXaN)	1	4.08	22q13.2
GGTCGGAAAA	BU536065	EST	1	4.08	
GATTGATGTC	Hs.491912	COP9 constitutive photomorphogenic homolog subunit 5 (Arabidopsis) (COPS5)	1	4.08	8q13.2
GCAACAAATT	Hs.479602	Amyloid beta (A4) precursor protein-binding, family B, member 2 (Fe65-like) (APBB2)	1	4.08	4p14
ATGGCAGAAG	Hs.552746	Hypothetical protein LOC284889 (LOC284889)	1	4.08	22q11.23
TTGGGTTCCA	H53496	EST	1	4.08	
TCTGCAAAAA	Hs.405880	Mitochondrial ribosomal protein S21 (MRPS21)	1	4.08	1
GCCTACCCGA	Hs.23582	Tumor-associated calcium signal transducer 2 (TACSTD2)	1	4.08	1p32-p31
GTGGCGGGTG	Hs.288520	Hypothetical gene FLJ00060 (FLJ00060)	1	4.08	19q13.42
ACCCACAGTG	Hs.67896	Opioid growth factor receptor (OGFR)	1	4.08	20q13.3
TGCTGCCTCA	Hs.30792	Hook homolog 2 (<i>Drosophila</i>) (HOOK2)	1	4.08	19p13.13
TAAGATTCA	Hs.373763	Heterogeneous nuclear ribonucleoprotein R (HNRPR)	1	4.08	1p36.12
TACATTTCAA	Hs.368542	Protein convertase subtilisin/kexin type 5 (PCSK5)	1	4.08	9q21.3
TATGTTTTAG	Hs.61812	Protein tyrosine phosphatase, non-receptor type 12 (PTPN12)	1	4.08	7q11.23
ATCCGCTGC	Hs.514557	cDNA FLJ41626 fis, clone DFNES1000107	1	4.08	17
GATTTAAATC	Hs.501857	RAB6 interacting protein 1 (RAB6IP1)	1	4.08	11p15.4
CGAGACTGGT	Hs.75438	Quinoid dihydropteridine reductase (QDPR)	1	4.08	4p15.31
CGTGAGCCAC	Hs.462303	Tripartite motif-containing 16 (TRIM16)	1	4.08	17p11.2
GCAGCTAATT	Hs.202011	GK001 protein (GKD01)	1	4.08	17q23.3
AATAAAGCAT	BM468964	EST	1	4.08	
GCTTAATGTG	Hs.334885	GTP binding protein 3 (mitochondrial) (GTPBP3)	1	4.08	19p13.11
ACTAACACCT	CF456631	EST	1	4.08	
CATCCAAGGC	Hs.462392	Hypothetical protein FLJ20308 (FLJ20308)	1	4.08	17p11.2
TGGGGAAAAG	Hs.445652	Transformer-2 alpha (TRA2A)	1	4.08	7p15.3
AGCACCTCAG	BF378578	EST	1	4.08	
GAATAATCTT	Hs.480815	Short coiled-coil protein (SCOC)	1	4.08	4q31.1
AGCCACCGTG	Hs.162233	Chromodomain helicase DNA binding protein 4 (CHD4)	1	4.08	12p13
GATACTTGAC	Hs.473082	Zinc finger protein 64 homolog (mouse) (ZFP64)	1	4.08	20q13.2
ATCATTCCCT	Hs.531788	Dpy-30-like protein (LOC84661)	1	4.08	2p22.3
TTGCCTGTAT	Hs.546305	Transcription elongation factor B (SIII), polypeptide 1 (15 kDa, elongin C) (TCEB1)	1	4.08	8q21.11
TCCAGAATAC	Hs.463607	Family with sequence similarity 33, member A (FAM33A)	1	4.08	17q23.2
TTGGTTTTGT	Hs.131434	Coiled-coil-helix-coiled-coil-helix domain containing 3 (CHCHD3)	1	4.08	7q32.3-q33
CTGACGGGTA	Hs.63795	Hypothetical protein DKFZp434L0117 (DKFZP434L0117)	1	4.08	1p36.11

Table III supplementary. Continued.

TAG	UniGene	Description (locus name)	HB4a	C5.2	Chromosome region
CAAGCCATCC	BF938487	EST	1	4.08	
CACAACCTGG	BE170306	EST	1	4.08	
GTGGCGGGCA	Hs.369632	Hypothetical protein FLJ38482 (FLJ384820)	1	4.08	4q32.3
GTGTTCTGG	Hs.162877	Protein kinase C and casein kinase substrate in neurons 2 (PACSIN2)	1	4.08	22q13.2-13.33
CACTGCCTGT	Hs.325309	Chromosome 9 open reading frame 54 (C9orf54)	1	4.08	9q34.11
ACACCTCTAA	Hs.149185	CNDP dipeptidase 2 (metallopeptidase M20 family) (CNDP2)	1	4.08	18q22.3
ACAGTGTTAA	Hs.385913	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member E (ANP32E)	1	4.08	1q21.2
ACATTCCAAG	Hs.297324	Tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory) (TIMP3)	1	4.08	22q12.1-q13.2
CTTGACACAC	H47931	EST	1	4.08	
TGGAACCTGTA	Hs.303727	Chromosome 10 open reading frame 4 (C10orf4)	1	4.08	10q23.33
TAAATATAAA	Hs.416998	Mitochondrial ribosomal protein L18 (MRPL18)	1	4.08	6q25.3
AGAAAGGGAG	Hs.436181	Homeo box B7 (HOXB7)	1	4.08	17q21.3
GGCAGAGGTT	H82959	EST	1	4.08	
TGTCTTTATA	Hs.551978	cDNA FLJ25632 fis, clone STM03991	1	4.08	18
ATATGTATAT	Hs.502328	CD44 antigen (homing function and Indian blood group system) (CD44)	1	4.08	11p13
TCACAACAAA	AW875846	EST	1	4.08	