

SUPPLEMENTARY MATERIAL

METHODS

Clinical samples

For phase I, a total of 333 urines from bladder washings (BW) from patients submitted to transurethral resection (TURB) for a primary or recurrent bladder tumor were consecutively collected prior to surgery. Of them, 29 samples were excluded from the study because of a screening failure, 19 because they showed an unclear pathological stage or grade and 41 were also excluded because they did not fulfill the RNA quality criteria (33 samples that had a housekeeping average CT > 27.5) or less than 100 ng of RNA were obtained (8 samples). In addition, 152 voided urine samples from healthy people and from patients with nonmalignant urological diseases (benign prostatic hyperplasia, urinary tract infections and urolithiasis) were collected as controls. Of them, 7 samples were excluded from the study due to the presence of other malignant urological diseases (screening failure) and 24 were also excluded as they did not fulfill the RNA quality criteria (15 samples with a housekeeping average CT > 27.5) or less than 100 ng of RNA were obtained (9 samples). However, we do not rule out the possibility that those samples with less than 100 ng of RNA could be properly quantified using cDNA pre-amplification protocols. Finally, 244 tumoral BW and 121 control urines were analyzed in the first phase of the study.

For phase II, 318 consecutively voided urine samples were collected: 163 from patients subjected to TURB for an incident or recurrent bladder tumor and 155 controls (healthy people and patients with nonmalignant urological diseases). Of the 163 tumoral urine samples, 50 were excluded from the study because a screening failure, 12 because they showed an unclear pathological stage or grade and 4 were also excluded as they yielded less than 100 ng of RNA. The policy that allowed this surprisingly high number of patients submitted to TURB without subsequent tumor confirmation by pathology (screening failures) has been corrected in our hospital after this study. Of 155 control urines, 22 samples were excluded from the study due to a screening failure and 19 were also excluded because they yielded less than 100 ng of RNA. Finally, 97 and 114 voided urine samples from bladder urothelial cell carcinoma (UCC) and control patients, respectively, were analyzed in this second phase of the study.

Selection of genes included in TaqMan Arrays (TA)

To set up the 384-TA, 345 target genes were selected from previous data obtained using Affymetrix microarrays (1). Some of the initially selected probe-sets from Affymetrix microarrays were finally not included in the definitive list of genes because

no available TA primers were found. Of the 345 target genes selected, 172 were differentially expressed between normal and tumoral bladder urothelium. Moreover, since we have demonstrated previously that gene expression profiles of high grade (HG) nonmuscle invasive bladder cancer (NMIBC) are more similar to muscle invasive bladder cancer (MIBC) than to low grade (LG) NMIBC (1), we also selected 173 genes that can differentiate LG NMIBC (named LG tumors) from HG NMIBC and MIBC (named HG tumors), in order to assess tumor aggressiveness. Genes were selected to be in 384-TA according to their ranking position in our previous Affymetrix lists (tumor vs control or LG vs HG lists); genes flagged as muscle in tumor-control comparisons were excluded (1). Additionally, 29 targets reported in the literature to be differentially expressed in UCC (2-12) and that were not inside the most differentially expressed genes in our lists, were also selected to set up 384-TA. As endogenous controls, seven genes were tested (*GUSB*, *HSPCB*, *PPIA*, *TEGT*, *HPRT1*, *ATP5B* and *18S rRNA*) (13,14).

After the analysis of tumor and control urines in 384-TA, data was normalized (Δ CT calculation) and statistically differentially expressed genes ($p < 0.05$) between tumor and control samples (diagnostic genes) and between LG and HG tumors (genes that predict tumor aggressiveness) were identified and then ranked according to their median log₂ ratio. Then, the 82 most differentially expressed genes ($p < 0.05$) from 384-TA, favoring up-regulated over down-regulated genes (1), altogether with 11 outliers (genes highly differentially expressed in a reduced number of patients or with special expression patterns) and the 3 most stable endogenous controls (*GUSB*, *PPIA*, *18S rRNA*) were selected to be analyzed by 96-TA. The same approach was used to further reduce the number of studied genes. The 39 most differentially expressed genes, together with 6 outlier genes and the 3 endogenous controls were used to build-up the 48-TA (Table 2).

During the course of the first phase, additional genes were reported in the literature to be differentially expressed in UCC samples and additional primers for TA became available. Thus, to set up 48-TA-urine used in phase II of the study, 6 additional genes (*BIRC5*, *CDH1*, *DLG7*, *MDK*, *MKI67*, *VEGFA*) were added and 6 genes that did show low differential expression levels (*ANK2*, *CYP24A1*, *GJB2*, *MMP1*, *NR2F1*, *PDZRN3*) were deleted from the new set of TA.

Selection of endogenous controls

To compare expression profiles between samples, normalization based on reference genes is needed to correct for the differences arising from variability in RNA quality and

total quantity in each assay. In order to identify the most stably expressed control genes in urine samples, we evaluated in 384-TA 6 candidate reference genes well-known in the literature to be suited to normalizing qRT-PCR data from bladder cancer tissue (*GUSB*, *HSPCB*, *PPIA*, *TEGT*, *HPRT1*, *ATP5B*) (13,14) and the endogenous control *18S rRNA* that is routinely present in TA. Then, we used the Ge-Norm software (15) to determine the minimum number of genes required to calculate a reliable normalization factor. We concluded that the best-performing endogenous control genes in our set of samples were *GUSB*, *PPIA* and *18S rRNA*, since they showed the lowest variability between them (data not shown). For pre-amplified cDNA samples only 2 reference genes were used, since *18S rRNA* TaqMan assay cannot be included in the pre-amplification pool as it is so highly expressed. A reference endogenous control CT for each tested sample was defined as the geometric average measured CT of the 3 or 2 reference genes, respectively. The maximum reference endogenous control CT that every sample must pass before being analyzed in our gene profile classifier was 27.5. In the set of samples from phase I, 48 of them (6%) did not pass this cutoff and therefore we considered that they did not fulfill the minimum RNA quality criteria and were excluded from the study. This requirement is important to guarantee the reliability of the result. On the other hand, the multiplex cDNA pre-amplification technique used in RNA from urine samples (phase II), allowed us to decrease CT values making the utilization of a cutoff unnecessary.

RESULTS

Biological processes for the genes of the 12+2 gene set panel

Inspection of the 12 genes included in the diagnostic signature reveals that 6 of them (*KRT20*, *TERT*, *IGF2*, *CTSE*, *MAGEA3* and *POSTN*) have been previously related with bladder cancer, although to our knowledge only *TERT* has been used in some bladder cancer detection test. Three genes have never been related to bladder cancer, although they have been related to other cancer types (*ANXA10*, *CRH* and *KLF9*). Finally, there are 3 genes which have been never related to cancer (*SLC1A6*, *AHNAK2* and *PPP1R14D*). Gene ontology analysis of the signature showed that the biological processes in which the 12 genes of the diagnostic panel are involved, can be summarized in the following functional categories: growth, signal transduction, synaptic transmission, regulation of transcription and skeletal system development. The two genes selected for prognosis are involved in cell-cell adhesion and DNA replication. This is in agreement with other gene signatures obtained in many other cancer entities

(16).

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FIGURE LEGENDS

Table 1S. Differentially expressed genes analyzed in the 3 steps of the first phase of this study. Primers/probe sets from Applied Biosystems used are listed. The assays highlighted in grey correspond to 48-TA and in yellow to 96-TA. The endogenous controls are highlighted in orange.

Table 2S. Comparison of the paired bladder washings and urines as samples to diagnose urothelial carcinoma. CT geometric means of the three endogenous controls selected to normalize gene expression values (*GUSB*, *PPIA* and *18S rRNA*) in paired BW and urine samples are shown. The CT geometric mean value indirectly shows RNA quality in each sample; lower values higher RNA quality. The R values obtained using expression values of **assessable** genes (CT<35) in each TA is also shown. (CT, Cycle threshold; BW, Bladder washing).

Table 1S

Gene symbol	Assay number	Gene Bank
ABCA8	Hs00200350_m1	NM_007168
ABL1	Hs00245445_m1	2 RefSeqs
ADAM19	Hs00224960_m1	2 RefSeqs
ADAMTS1	Hs00199608_m1	NM_006988
AEBP1	Hs00371239_m1	NM_001129
AIM2	Hs00175457_m1	NM_004833
AKR1B10	Hs00252524_m1	NM_020299
AL137566	Hs00612017_s1	AL137566
ANK2	Hs00153998_m1	
ANLN	Hs00218803_m1	NM_018685
ANXA1	Hs00167549_m1	NM_000700
ANXA10	Hs00200464_m1	NM_007193
AOC3	Hs00186647_m1	NM_003734
APOBEC3B	Hs00358981_m1	NM_004900
AREG	Hs00155832_m1	NM_001657
ARGBP2	Hs00221428_m1	NM_021069
ASAM	Hs00293345_m1	NM_024769
ASPM	Hs00411505_m1	NM_018136
ATF3	Hs00231069_m1	
ATP5B	Hs00266077_m1	NM_001686
ATP8B2	Hs00393111_m1	NM_020452
C2orf32	Hs00384403_m1	NM_015463
OLFML1	Hs00416948_m1	NM_198474
BAG2	Hs00188716_m1	NM_004282
MGC16635	Hs00536653_s1	NM_138433
LOC387882	Hs00329098_m1	NM_207376
BMP5	Hs00234930_m1	NM_021073
KLF9	Hs00230918_m1	NM_001206
BUB1B	Hs00176169_m1	NM_001211
C10orf3	Hs00216688_m1	NM_0181
C10orf87	Hs00332436_m1	NM_144587
C14orf78	Hs00746838_s1	
C2orf23	Hs00224761_m1	NM_022912
C6orf109	Hs00209864_m1	NM_015388
C7	Hs00175109_m1	NM_000587
CA2	Hs00163869_m1	NM_000067
CALD1	Hs00189021_m1	
CAPNS2	Hs00260517_s1	NM_032330
CAV1	Hs00184697_m1	NM_001753
CBFA2T1	Hs00231702_m1	4 RefSeqs
CCL11	Hs00237013_m1	NM_002986
CCL18	Hs00268113_m1	NM_002988
CCL2	Hs00234140_m1	NM_002982
CCNA2	Hs00153138_m1	NM_001237
CCNB1	Hs00259126_m1	NM_031966
CCNB2	Hs00270424_m1	NM_004701
CCND1	Hs00277039_m1	NM_053056
CCND2	Hs00277041_m1	NM_001759
CCNE1	Hs00233356_m1	2 RefSeqs
CDC2	Hs00364293_m1	NM_001786
CDC20	Hs00415851_q1	NM_001255

Gene symbol	Assay number	Gene Bank
FBN2	Hs00417208_m1	NM_001999
FBXO32	Hs00369714_m1	
FEN1	Hs00748727_s1	NM_004111
FGF3	Hs00173742_m1	NM_005247
FGFR1	Hs00241111_m1	9 RefSeqs
FGFR3	Hs00179829_m1	2 RefSeqs
FGL2	Hs00173847_m1	NM_006682
FLJ10159	Hs00215979_m1	NM_018013
FLJ10719	Hs00289551_m1	NM_018193
FLJ11029	Hs00383634_m1	NM_018304
FLJ13710	Hs00388227_m1	NM_024817
TSC	Hs00215487_m1	NM_017899
FLJ20701	Hs00374054_q1	
FLJ21986	Hs00227735_m1	NM_024913
FLJ23191	Hs00375503_m1	NM_024574
SPOCD1	Hs00375905_m1	NM_144569
FLJ31052	Hs00708284_s1	NM_152378
FLJ32569	Hs00611179_m1	NM_152491
FLJ38736	Hs00419054_m1	NM_182758
FN1	Hs00365058_m1	7 RefSeqs
FNBP1	Hs00390705_m1	NM_015033
FOS	Hs00170630_m1	NM_005252
FOSB	Hs00171851_m1	NM_006732
FOXF1	Hs00230962_m1	NM_001451
FOXM1	Hs00153543_m1	2 RefSeqs
FYN	Hs00176628_m1	3 RefSeqs
FZD7	Hs00275833_s1	NM_003507
GATA6	Hs00232018_m1	NM_005257
GEM	Hs00170633_m1	NM_005261
GGH	Hs00608257_m1	NM_003878
GHR	Hs00174872_m1	NM_000163
GJB2	Hs00269615_s1	NM_004004
GJB6	Hs00272726_s1	NM_006783
GKN1	Hs00219734_m1	NM_019617
GMNN	Hs00210707_m1	NM_015895
GPC6	Hs00170677_m1	NM_005708
GPM6B	Hs00383529_m1	4 RefSeqs
GPR124	Hs00262150_m1	NM_032777
GREM1	Hs00171951_m1	NM_013372
GSN	Hs00609276_m1	2 RefSeqs
GULP1	Hs00169604_m1	NM_016315
GUSB	Hs99999908_m1	NM_000181
H19	Hs00399294_q1	
HEPH	Hs00207710_m1	
CFH	Hs00164830_m1	NM_000186
HIST1H1C	Hs00271185_s1	NM_005319
HIST1H2BD	Hs00371070_m1	NM_138720
HIST1H2BG	Hs00374317_s1	NM_003518
HIST2H2AA	Hs00358508_s1	NM_003516
HIST2H2BE	Hs00269023_s1	NM_003528
HMGB2	Hs00357789_q1	NM_002129

Gene symbol	Assay number	Gene Bank
MYADM	Hs00414763_m1	NM_138373
MYBPC1	Hs00159451_m1	
MYC	Hs00153408_m1	NM_002467
NEK2	Hs00601227_mH	NM_002497
NEXN	Hs00332124_m1	NM_144573
NFIA	Hs00325656_m1	NM_005595
NFIB	Hs00232149_m1	NM_005596
NFIL3	Hs00356605_q1	NM_005384
FLJ22595	Hs00540047_s1	NM_025047
NMES1	Hs00260902_m1	
NNMT	Hs00196287_m1	NM_006169
NOPE	Hs00326335_m1	NM_020962
NQO1	Hs00168547_m1	NM_000903
NR2F1	Hs00818842_m1	NM_005654
NR4A1	Hs00172437_m1	NM_173157
NR4A2	Hs00428691_m1	4 RefSeqs
NR4A3	Hs00175077_m1	
NTNG2	Hs00287286_m1	NM_032536
NUSAP1	Hs00153533_m1	NM_016359
OAS1	Hs00242943_m1	2 RefSeqs
OLFML3	Hs00220180_m1	NM_020190
OSR2	Hs00369588_m1	NM_053001
PDGFC	Hs00211916_m1	NM_016205
PDGFRA	Hs00183486_m1	NM_006206
PDLIM3	Hs00205533_m1	NM_014476
PDZRN3	Hs00392900_m1	
PEG10	Hs00248288_s1	NM_015068
PFKFB3	Hs00190079_m1	NM_004566
Pfs2	Hs00211479_m1	NM_016095
PGM5	Hs00222671_m1	NM_021965
PLA2G2A	Hs00179898_m1	NM_000300
PLAGL1	Hs00243030_m1	NM_002656
PLCB4	Hs00168656_m1	
PLEKHC1	Hs00235033_m1	NM_006832
PLN	Hs00160179_m1	NM_002667
PLSCR4	Hs00220482_m1	NM_020353
PMP22	Hs00165556_m1	
POLQ	Hs00198196_m1	2 RefSeqs
POSTN	Hs00170815_m1	NM_006475
POU1F1	Hs00230821_m1	NM_000306
PPIA o CYC	Hs99999904_m1	3 RefSeqs
PPP1R12B	Hs00364078_m1	
PPP1R14D	Hs00214613_m1	NM_017726
PRC1	Hs00187740_m1	
PRKAR2B	Hs00176966_m1	NM_002736
PRL	Hs00168730_m1	NM_000948
PSAT1	Hs00795278_mH	2 RefSeqs
PTCH	Hs00181117_m1	NM_000264
PTEN	Hs00829813_s1	NM_000314
PTGS2	Hs00153133_m1	NM_000963
PTN	Hs00383235_m1	NM_002825

Gene symbol	Assay number	Gene Bank
CDC6	Hs00154374_m1	NM_001254
CDCA1	Hs00230097_m1	2 RefSeqs
CDCA3	Hs00229905_m1	NM_031299
CDH1	Hs00170423_m1	NM_004360
CDH11	Hs00156438_m1	2 RefSeqs
CDH19	Hs00253534_m1	NM_021153
CDKN2A	Hs00233365_m1	3 RefSeqs
CDKN2B	Hs00365249_m1	NM_078487
CDKN3	Hs00193192_m1	NM_005192
CEACAM6	Hs00366002_m1	NM_002483
CEACAM7	Hs00185152_m1	NM_006890
CENPA	Hs00156455_m1	NM_001809
CENPF	Hs00193201_m1	NM_016343
CFL2	Hs00368395_q1	
ChGn	Hs00218054_m1	NM_018371
CHI3L1	Hs00609691_m1	NM_001276
CKS2	Hs00854958_q1	NM_001827
CLCA2	Hs00197957_m1	NM_006536
CLCN3	Hs00156527_m1	NM_001829
CLIC3	Hs00362166_q1	NM_004669
CLIC4	Hs00749895_s1	NM_013943
COL1A1	Hs00385388_m1	
COL15A1	Hs00266332_m1	NM_001855
COL1A2	Hs00164099_m1	NM_000089
COL3A1	Hs00164103_m1	NM_000090
COL5A2	Hs00169768_m1	NM_000393
COL6A1	Hs00242448_m1	NM_001848
COL6A2	Hs00242484_m1	NM_001849
COLEC12	Hs00560477_m1	2 RefSeqs
CPA3	Hs00157019_m1	NM_001870
CFH	Hs00164830_m1	
CRH	Hs00174941_m1	NM_000756
CRTAC1	Hs00216208_m1	NM_018058
CTGF	Hs00170014_m1	NM_001901
CTSE	Hs00157213_m1	
CTTN	Hs00193322_m1	2 RefSeqs
VSIG2	Hs00204823_m1	NM_014312
CUGBP2	Hs00272516_m1	NM_006561
CXCL12	Hs00171022_m1	
CXCR4	Hs00237052_m1	NM_003467
CYBRD1	Hs00227411_m1	NM_024843
CYP24A1	Hs00167999_m1	NM_000782
CYR61	Hs00155479_m1	NM_001554
EBF	Hs00395513_m1	
D4S234E	Hs00205189_m1	NM_014392
DBC1	Hs00180893_m1	NM_014618
DCN	Hs00266491_m1	
DF	Hs00157263_m1	NM_001928
DKFZp434B0	Hs00230322_m1	NM_031476
DKFZp564O0	Hs00209875_m1	NM_015393
DKFZp586H2	Hs00405837_m1	NM_015430
DKK1	Hs00183740_m1	NM_012242
DLG7	Hs00207323_m1	NM_014750

Gene symbol	Assay number	Gene Bank
HMOX1	Hs00157965_m1	NM_002133
HN1	Hs00602957_m1	3 RefSeqs
HOP	Hs00261238_m1	3 RefSeqs
HOXB6	Hs00255831_s1	3 RefSeqs
HOXD4	Hs00429605_m1	NM_014621
HPRT	Hs99999909_m1	NM_000194
HRAS	Hs00610483_m1	NM_005343
HSPC150	Hs00204359_m1	NM_014176
HSPCB	Hs00607336_qH	NM_007355
HTR1F	Hs00265296_s1	NM_000866
IER3	Hs00174674_m1	NM_003897
IGF1	Hs00153126_m1	NM_000618
IGF2	Hs00171254_m1	NM_000612
IGFBP3	Hs00181211_m1	NM_000598
IGFBP5	Hs00181213_m1	NM_000599
IGHM	Hs00378512_m1	
IL6	Hs00174131_m1	NM_000600
INA	Hs00190771_m1	NM_032727
INHBA	Hs00170103_m1	NM_002192
IQGAP3	Hs00603642_m1	NM_178229
ITGA8	Hs00233321_m1	NM_003638
ITM2A	Hs00191609_m1	NM_004867
JAM2	Hs00221894_m1	NM_021219
JAM3	Hs00230289_m1	NM_032801
JUNB	Hs00357891_s1	NM_002229
KCNG1	Hs00158410_m1	NM_002237
KDELR3	Hs00423556_m1	2 RefSeqs
KIAA0101	Hs00207134_m1	NM_014736
KIAA0186	Hs00221421_m1	NM_021067
KIAA0992	Hs00363101_m1	NM_016081
KIF11	Hs00189698_m1	NM_004523
KIF14	Hs00208408_m1	NM_014875
KIF20A	Hs00194882_m1	NM_005733
KIF2C	Hs00199232_m1	NM_006845
KIF4A	Hs00602211_q1	NM_012310
KISS1	Hs00158486_m1	NM_002256
KLHL7	Hs00375239_m1	NM_018846
KPNA2	Hs00818252_q1	NM_002266
KRT14	Hs00265033_m1	NM_000526
KRT20	Hs00300643_m1	NM_019010
KRT7	Hs00818825_m1	NM_005556
LAF4	Hs00171448_m1	NM_002285
LEPR	Hs00174497_m1	NM_002303
LGALS1	Hs00169327_m1	NM_002305
LIFR	Hs00158730_m1	NM_002310
LOC83468	Hs00229917_m1	NM_031302
LOX	Hs00184700_m1	NM_002317
LPPR4	Hs00322721_m1	NM_014839
LUM	Hs00158940_m1	NM_002345
LY6D	Hs00170353_m1	NM_003695
MAD2L1	Hs00829154_q1	NM_002358
MAGEA3	Hs00366532_m1	NM_005362
MAGEA9	Hs00245619_s1	NM_005365

Gene symbol	Assay number	Gene Bank
PTPRC	Hs00236304_m1	3 RefSeqs
PTRF	Hs00396859_m1	NM_012232
RAB23	Hs00212407_m1	
RACGAP1	Hs00374747_m1	NM_013277
RAI2	Hs00253960_s1	NM_021785
RAMP	Hs00212788_m1	NM_016448
RASL12	Hs00275429_m1	NM_016563
RB1	Hs00153108_m1	NM_000321
RBM24	Hs00290607_m1	NM_153020
RECK	Hs00221638_m1	NM_021111
RFC3	Hs00161357_m1	2 RefSeqs
RGS1	Hs00175260_m1	NM_002922
RNASE4	Hs00377763_m1	
RODH	Hs00366258_m1	NM_003725
RPESP	Hs00541931_m1	NM_153225
RRM2	Hs00357247_q1	NM_001034
S100A10	Hs00741221_m1	NM_002966
SBLF	Hs00538997_m1	
SCN7A	Hs00161546_m1	NM_002976
SELL	Hs00174151_m1	NM_000655
SELM	Hs00369741_m1	NM_080430
SERPIN3	Hs00199468_m1	NM_006919
SETBP1	Hs00210209_m1	NM_015559
SFRP1	Hs00610060_m1	NM_003012
SLC1A6	Hs00192604_m1	NM_005071
SLIT2	Hs00191193_m1	NM_004787
SMAD6	Hs00178579_m1	NM_005585
SMOC2	Hs00405777_m1	NM_022138
SNX10	Hs00203362_m1	NM_013322
SOCS3	Hs00269575_s1	NM_003955
SOX4	Hs00268388_s1	NM_003107
SOX9	Hs00165814_m1	NM_000346
SPARCL1	Hs00190740_m1	NM_004684
SPON1	Hs00323883_m1	
SPP1	Hs00167093_m1	NM_000582
SPRR3	Hs00271304_m1	NM_005416
SRPX	Hs00196867_m1	NM_006307
STC1	Hs00174970_m1	NM_003155
STK6	Hs00269212_m1	NM_003600
STN2	Hs00263833_m1	NM_033104
SULF1	Hs00290918_m1	NM_015170
SULT1E1	Hs00193690_m1	NM_005420
TCF21	Hs00162646_m1	
TCF8	Hs00611018_m1	NM_030751
TCN1	Hs00169055_m1	NM_001062
TEAD2	Hs00366217_m1	NM_003598
TEGT	Hs00162661_m1	NM_003217
TERT	Hs00162669_m1	2 RefSeqs
TGFB11	Hs00210887_m1	NM_015927
TIMP2	Hs00234278_m1	NM_003255
TK1	Hs00177406_m1	NM_003258
TMPO	Hs00162842_m1	NM_003276
TNA	Hs00162844_m1	NM_003278

Gene symbol	Assay number	Gene Bank
<i>DOC1</i>	Hs00706279_s1	
<i>DPT</i>	Hs00170030_m1	NM_001937
<i>DPYSL3</i>	Hs00181665_m1	NM_001387
<i>DSCR1L1</i>	Hs00195165_m1	NM_005822
<i>DTR</i>	Hs00181813_m1	NM_001945
<i>DUSP1</i>	Hs00610256_q1	NM_004417
<i>E2F3</i>	Hs00605457_m1	NM_001949
<i>EBF</i>	Hs00395513_m1	NM_024007
<i>ECRG4</i>	Hs00260897_m1	NM_032411
<i>ECT2</i>	Hs00216455_m1	NM_018098
<i>EDNRA</i>	Hs00609865_m1	NM_001957
<i>EGR1</i>	Hs00152928_m1	NM_001964
<i>ENTPD3</i>	Hs00154325_m1	NM_001248
<i>EPHA3</i>	Hs00178327_m1	NM_005233
<i>EPHA7</i>	Hs00177891_m1	NM_004440
<i>ERBB2</i>	Hs00170433_m1	2 RefSeqs
<i>F3</i>	Hs00175225_m1	NM_001993
<i>FABP4</i>	Hs00609791_m1	NM_001442
<i>FABP6</i>	Hs00155029_m1	NM_001445
<i>FAP</i>	Hs00189476_m1	NM_004460
<i>FBLN1</i>	Hs00242545_m1	4 RefSeqs
<i>FBLN5</i>	Hs00197064_m1	NM_006329
<i>FBN1</i>	Hs00171191_m1	NM_000138

Gene symbol	Assay number	Gene Bank
<i>MAMDC2</i>	Hs00299196_m1	NM_153267
<i>MAN1C1</i>	Hs00220595_m1	NM_020379
<i>MAP1B</i>	Hs00195487_m1	2 RefSeqs
<i>MCM10</i>	Hs00218560_m1	2 RefSeqs
<i>MCM2</i>	Hs00170472_m1	NM_004526
<i>MDM2</i>	Hs00242813_m1	3 RefSeqs
<i>MELK</i>	Hs00207681_m1	NM_014791
<i>MFAP2</i>	Hs00250064_m1	2 RefSeqs
<i>MFAP4</i>	Hs00412974_m1	NM_002404
<i>TUBB6</i>	Hs00603164_m1	NM_032525
<i>MGC45780</i>	Hs00382351_m1	NM_173833
<i>MGC57827</i>	Hs00822131_m1	NM_207418
<i>MGP</i>	Hs00179899_m1	NM_000900
<i>MKI67</i>	Hs00606991_m1	NM_002417
<i>MMD</i>	Hs00202450_m1	NM_012329
<i>MMP1</i>	Hs00233958_m1	NM_002421
<i>MMP12</i>	Hs00159178_m1	NM_002426
<i>MRS2L</i>	Hs00252895_m1	NM_020662
<i>MRV11</i>	Hs00180652_m1	
<i>MSRB3</i>	Hs00827017_m1	NM_198080
<i>MST1R</i>	Hs00234013_m1	NM_002447
<i>MTHFD2</i>	Hs00759197_s1	NM_006636
<i>MUC7</i>	Hs00379529_m1	NM_152291

Gene symbol	Assay number	Gene Bank
<i>TNC</i>	Hs00233648_m1	NM_002160
<i>TNRC9</i>	Hs00300355_m1	
<i>TOP2A</i>	Hs00172214_m1	NM_001067
<i>TOPK</i>	Hs00218544_m1	NM_018492
<i>TP53</i>	Hs00153349_m1	NM_000546
<i>TPX2</i>	Hs00201616_m1	NM_012112
<i>TRIP13</i>	Hs00188500_m1	NM_004237
<i>TSPAN-2</i>	Hs00194836_m1	NM_005725
<i>TTK</i>	Hs00177412_m1	NM_003318
<i>TU3A</i>	Hs00200376_m1	NM_007177
<i>TYMS</i>	Hs00426591_m1	NM_001071
<i>UBD</i>	Hs00197374_m1	NM_006398
<i>UBE2C</i>	Hs00738962_m1	NM_181799
<i>UHRF1</i>	Hs00273589_m1	NM_013282
<i>URB</i>	Hs00736722_m1	
<i>WFDC1</i>	Hs00221849_m1	NM_021197
<i>WISP1</i>	Hs00365573_m1	2 RefSeqs
<i>ZAK</i>	Hs00370447_m1	2 RefSeqs
<i>ZBTB16</i>	Hs00232313_m1	NM_006006
<i>ZFP36</i>	Hs00185658_m1	NM_003407
<i>ZNF217</i>	Hs00232417_m1	NM_006526
<i>ZWINT</i>	Hs00199952_m1	
<i>ZYX</i>	Hs00170299_m1	NM_003461
<i>18S rRNA</i>	Hs99999901_s1	

Table 2S

	Sample name	CT geometric mean (PPIA, GUSB and 18S)		Number of valuable genes			Correlation coefficient (R)
		BW	Urine	BW	Urine	Both	
TA-384	1	19.45	23.31	340	321	308	0.83
	2	20.74	23.57	357	319	317	0.94
	3	21.11	21.88	317	304	292	0.91
TA-96	4	21.02	25.77	93	71	71	0.86
	5	22.34	22.27	89	85	85	0.92
	6	22.2	21.55	93	90	89	0.97
	7	21.22	27.5	96	80	80	0.83
	8	21.43	22.51	94	92	92	0.94
	9	21.36	24.39	88	83	83	0.9
	10	22.36	22.84	94	91	90	0.93
	11	23.37	26.78	72	61	54	0.8
	12	23.1	26.81	89	61	60	0.79
	13	24.17	21.71	89	85	82	0.81
	14	23.9	25.99	85	60	58	0.81
	15	21.48	24.7	94	78	78	0.87
TA-48	16	22.97	26.27	39	32	30	0.9
	17	20.00	26.95	46	26	26	0.89
	18	25.68	26.59	30	21	17	0.92
	19	19.84	19.9	48	47	47	0.92
	20	24.21	24.59	39	36	33	0.88
	21	21.63	23.24	44	44	42	0.88
	22	23.7	26.66	41	25	24	0.89
	23	21.15	26.82	45	36	36	0.89
	24	25.27	24.75	37	41	36	0.86
	25	24.01	25.83	40	27	25	0.91

	26	24.6	26.00	33	34	29	0.91
	27	23.4	24.1	41	30	30	0.95
	28	22.13	22.76	41	39	39	0.89
	29	24.41	24.96	27	34	23	0.92
	30	23.21	20.78	43	46	43	0.95