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Interactions of intergenic microRNAs with mRNAs of genes involved in carcinogenesis

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Abstract:
miRNAs regulate gene expression by binding with mRNAs of many genes. Studying their effects on genes involved in oncogenesis is important in cancer diagnostics and therapeutics. The RNAHybrid 2.1 program was used to predict the strong miRNA binding sites ($p < 0.0005$) in target mRNAs. The program Finder 2.2 was created to verify 784 intergenic miRNAs (ig-miRNA) origin. Among 54 considered oncogenes and tumor suppressor genes, 47 genes are the best targets for ig-miRNAs. Accordingly, these genes are strongly regulated by 111 ig-miRNAs. Some miRNAs bind several mRNAs, and some mRNAs have several binding sites for miRNAs. Of the 54 mRNAs, 21.8%, 43.0%, and 35.2% of the miRNA binding sites are present in the 5'UTRs, CDSes, and 3'UTRs, respectively. The average density of the binding sites for miRNAs in the 5'UTR was 4.4 times and 4.1 times greater than in the CDS and the 3'UTR, respectively. Three types of interactions between miRNAs and mRNAs were identified, which differ according to the region of the miRNA bound to the mRNA: 1) binding occurs predominantly via the 3'-region of the miRNA; 2) binding occurs predominantly through the central region of the miRNA; and 3) binding occurs predominantly via the 5'-region of the miRNA. Several miRNAs effectively regulate only one gene, and this information could be useful in molecular medicine to modulate translation of the target mRNA. We recommend described new sites for validation by experimental investigation.

Keywords: microRNA, mRNA, oncogene, human, oncogenesis

Background:
Increasing interest in the biological role of miRNA has resulted in a number of publications dedicated to these unique regulators of gene expression **[1].** Correlations have been shown between changes in miRNA concentration and the development of certain cancers, such as lung **[2]**, esophageal **[3]**, stomach **[4]**, colon **[5]**, liver **[6]**, prostate **[7]**, and breast **[8, 9]**. Cancer diagnostic methods have been developed based on changes in miRNA expression levels during the stages of oncogenesis **[10-14]**. Searching for binding sites for miRNAs in mRNAs is an important challenge. The conventional point of view is that miRNAs bind primarily to the 3'UTR of mRNA, while other regions of mRNA (specifically the 5'UTR and CDS) do not have a significant role in the regulation of translation by miRNAs **[15]**. However, there are publications demonstrating miRNA and mRNA interactions in the 5'UTR and CDS **[16-17].** Let-7 inhibits mRNA translation of the *lin-41* gene in *Zebrafish* embryos by binding to a site in the 3'UTR. If this binding site for let-7 appears in both the protein-coding sequence and the 5'UTR, it is likely to also be functional in the 5'UTR **[18].** In *Drosophila*, it has been shown that binding sites in the 5'UTR and CDS are similarly functional. MiR-2 represses translation initiation in the 5'UTR and stimulates mRNA deadenylation **[19].** Another article presented data using a reported gene assay that confirmed the presence of binding sites for miR-181a in the coding sequence of *ZNF* mRNA in several cell lines **[20].** Information about the number of miRNA binding sites and the features of miRNA binding could improve the current understanding of interactions between miRNAs and mRNAs. The number of genes regulated by an individual miRNA and the number of miRNAs affecting the expression of an individual mRNA remain unknown **[21].** In this research, the

binding sites of more 700 intergenic miRNAs within the 5'UTRs, CDSes, and 3'UTRs of mRNAs for 54 human oncogenes were investigated. These genes participate in the development of esophageal, stomach, colon, liver, breast, prostate, and ovarian cancers. The aims of the present work are as follows: a) to identify features of interactions between miRNAs and different regions of mRNAs; b) to establish differences in the ability of miRNAs to bind to different mRNAs; and c) to determine features of nucleotide interactions during the formation of complexes between miRNA and mRNA

Methodology:

Nucleotide sequences of mRNAs of 54 human genes (*Homo sapiens* Genome build 37.2.) were obtained from Genbank. This set comprises well-known oncogenes and tumor suppressor genes provided by literature review. Nucleotide sequences of ig-miRNAs were obtained from the miRBase database. The program Finder 2.2 was created and used to find genomic origin of ig-miRNAs. All miRNA:mRNA pairs were found by RNAHybrid 2.1 program that provides position of potential binding sites, the free energy value (ΔG) of binding and interaction schemes. As a comparative quantitative criterion of binding was inserted the $\Delta G/\Delta G_m$ value (%), where ΔG_m equals the binding energy for a miRNA with a completely complementary nucleotide sequence. Searching for miRNA binding sites was performed along the total mRNA sequence. miRNA binding sites were selected for each target sequence based on the ΔG value and its standard deviation. The binding ability of ig-miRNAs, in-miRNAs, and ex-miRNAs to the 5'UTRs, CDSes, and 3'UTRs of the 54 mRNAs was estimated with a significance of $p < 0.0005$ (Student's criterion). The density of the binding sites in the 5'UTR, CDS, and 3'UTR per 1000 nucleotides was computed as the number of sites divided by the nucleotide length of the region and multiplied by 10^3 (s/l).

Results:

Interactions between intergenic miRNAs and the mRNAs of genes involved in oncogenesis

The interactions between 784 intergenic miRNAs and the mRNAs of 54 protein-coding human genes were explored. It was revealed that 47 mRNAs are targets for these miRNAs, while the mRNAs of seven genes (*ABCB1*, *MSH2*, *MSH3*, *MYC*, *PROM1*, *SNAI1*, and *TNFSF10*) have no binding sites for ig-miRNAs based on established interaction criteria (see Materials and Methods). Only 111 miRNAs out of the 784 ig-miRNAs bind to these 47 mRNAs with a high interaction energy, at a total of 165 binding sites. **Table 1 (see supplementary material)** represents the characteristics of ig-miRNA binding sites that have an $\Delta G/\Delta G_m$ value is greater than or equal to 75% ($p<0.0005$). Each mRNA binds one or several ig-miRNAs. Some of these mRNAs are bound by six or more ig-miRNAs. For example, the mRNAs of the *AXIN1*, *CCND1*, *CDH1*, *FLCN*, *MMP2*, *SMAD4*, and *SRC* genes have from 6 to 13 sites for ig-miRNAs. This number is greater than the average number of ig-miRNA binding sites for the 47 genes, which is 3.3. **Table 1 (see supplementary material)** shows that each of the mRNAs has one binding site for a miRNA. Several miRNAs associate with several mRNAs. For example, miR-4472 binds to the mRNAs of seven genes, and miR-1279 has five target mRNAs. There is no relation between the length of the mRNA and the number of ig-miRNA binding sites because the correlation coefficient equals -0.061. The number of binding sites is not proportional to mRNA length, and consequently the established binding sites are not random. Shuffling the mRNA nucleotide sequences reduced the number of binding sites. Of the binding sites for ig-miRNAs, 21.8%, 43.0%, and 35.2% are located in the 5'UTRs, CDSes, and 3'UTRs of the 47 mRNAs, respectively. The density of the binding sites for miRNAs varies among mRNAs. Data from Additional **Table 1 (see supplementary material)** shows that the density of sites varies from 0.14 s/l (for the mRNA of *BRCA1*) to 3.09 s/l (for the mRNA of *SRC*), with an average density of 0.89 s/l for all 47 mRNAs. The mRNA of *SRC* has the highest binding site density, showing an important role for intergenic miRNAs in the regulation of the expression of this gene.

The mRNAs of the studied genes have different numbers of binding sites for ig-miRNAs in their 5'UTRs, CDSes, and 3'UTRs. The average binding site densities for ig-miRNAs in the 5'UTR, CDS, and 3'UTR of the 47 mRNAs are 2.60 s/l, 0.65 s/l, and 0.68 s/l. The average number of binding sites for miRNAs in the 5'UTR is 4.4 times more than in the CDS and 4.1 times more than in the 3'UTR. These data show that miRNAs can bind not only in the 3'UTR but also in the 5'UTR and CDS of target mRNAs. mRNAs of the *GNAS*, and *PTEN* genes interact with five ig-miRNAs at sites all located in their 5'UTRs. The mRNAs of *CCND1*, *MTHFR*, *SMAD4*, and *SRC* associate with ig-miRNAs preferably in their 3'UTRs **Table 4 (see supplementary material)**. These data indicate the specificity of the interactions between mRNAs and miRNAs. The nucleotide sequences for binding sites between some miRNAs and mRNAs are shown in **Table 2 (see supplementary material).** These interaction schemes represent different examples of the miRNA contribution to the interaction energy in various regions of the mRNA. There are three types of interaction that differ according to the contribution of the ig-miRNA binding regions to interaction energy: 1) where binding occurs predominantly through the 5'-region of the miRNA (miR-4455:*ABCG2* mRNA and miR-1279:*PTPN12* mRNA); 2) where binding occurs predominantly through the 3'-region of the miRNA (miR-1972:*FLCN* mRNA and miR-4455:*CD44* mRNA); and 3) where binding occurs predominantly through the central region of the miRNA (miR-1285:*TP53* mRNA and miR-320f:*APC-1* mRNA). First type of interaction has some nucleotides, which are not complementary in 3'-part of miRNA site. Second type of binding has not complementary base pairs in 5'-part of miRNA. Third type of interaction may have not complementary base pairs in both ends of miRNA. Therefore, all parts of the ig-miRNA can contribute to the interaction energy between the miRNA and the mRNA. Data from Table 2 show that ig-miRNAs can bind in the 5'UTR, CDS, and 3'UTR with a high interaction energy.

Discussion:

The number of miRNAs identified in humans is constantly increasing, and currently more than 1500 miRNAs are known. Most studies use near 300 well-described miRNAs; our investigation provided for 784 miRNAs the best binding sites with 54 target genes. The identification of target genes for miRNAs depends on the quality of prediction data *in silico*. Several miRNAs are able to interact with multiple mRNAs **[22]**. This data suggests that the majority of human genes are regulated by miRNAs. It is known that many miRNAs appear

to have a tissue-specific pattern of gene expression, and the concentration of a miRNA might be lower than would be necessary to repress the expression of its target mRNA **[23]**. In this case, miRNAs that are completely complementary to a given mRNA do not affect its expression. Intronic miRNAs comprise 45% of the total number of miRNAs and their expressions directly depend on the transcription of host gene. miRNA expression may increase a hundred-fold under environmental condition and mutation, and might alter the gene expression of target mRNAs **[11]**. In the 54 mRNAs, 21.8%, 43.0%, and 35.2% of the miRNAs bind within the 5'UTRs, CDSes, and 3'UTRs, respectively, of their target genes **Table 1 (see supplementary material)**. These results suggest that 3'UTR, the 5'UTR and the CDS regions of mRNA may all be involved in translation regulation by miRNAs. While considering miRNA sites only in 3'UTR, we lose 1.8 times more sites in 5'UTR and CDS. While miRNA binding sites are perfectly complementary to mRNAs in plants, animal miRNA binding sites are rarely fully complementary. Weak sites have 6 complementary base pairs **[24]**. Based on our data, the human genome contains many strong miRNA binding sites in many genes. The miR-4693-5p: *APC-1* mRNA complex has a $\Delta G/\Delta G_m$ of 89.8%; the miR-378b: *MLH3* mRNA has a $\Delta G/\Delta G_m$ of 90.6%; the miR-1260: *MTHFR* mRNA complex has a $\Delta G/\Delta G_m$ of 90.4%; and the miR-212: *MMP2* mRNA complex has a $\Delta G/\Delta G_m$ of 81.7% (further information can be found in **Table 1(see supplementary material)**. Each of these sites has about 13–14 or more complementary base pairs. **Table 2 (see supplementary material)** shows interaction schemes for miRNA binding sites with 15–22 complementary base pairs. There are several miRNA sites with near perfect complementarity in human genome. For example, the miR-1285:TP53 mRNA pair has 19 complementary base pairs and a $\Delta G/\Delta G_m$ of 98.7%, and the miR-1972: *FLCN* mRNA pair has 22 complementary base pairs and a $\Delta G/\Delta G_m$ of 89.9%. Most verified sites have fewer complementary base pairs and hybridization free energy than the sites described in this paper.

To confirm validity of existence of binding sites, experimentally well-established miRNAs sites were searched from the literature data. The analysis of such data has shown that researchers used sites with low hybridization energy and of $\Delta G/\Delta G_m$ value. Majority of these miRNA and target gene pairs were reported as being important in cancer development. That may influence to choice of miRNA:target mRNA pair. In our work we used more strict threshold level for selection and chose significant sites with $p<0.0005$. For example, luciferase experiments **[21]** for *AXIN2* gene showed sites of miR-34a using wild and mutant site for this miRNA. Hybridization energy of described site is -24.3 kcal/mol and $\Delta G/\Delta G_m$ value equals 52.2% **Table 3 (see supplementary material)**. We found 5 interaction sites for *AXIN2* with $\Delta G/\Delta G_m$ value varying from 69.6 to 86.7% and suppose that they might work more effectively. Nagel Remco *et al.* showed regulation expression of *APC* gene by miR-135a in MCF-7 cells **[25].** But selected pair of miRNA and mRNA has a low interaction energy (-16.9 kcal/mol) with a low ratio of $\Delta G/\Delta G_m$ (43.1%) **Table 3 (see supplementary material).** We recommend using miR-302f and miR-4693-5p that have sites with larger $\Delta G/\Delta G_m$ values 89.8 and 99.6% respectively. Olive V. *et al.* validated interaction sites of *PTEN* and mir-17-92 cluster **[26].** Mir-17-92 transcribes polycistronic primary transcript that encodes six mature miRNAs: miR-17, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92a. Luciferase reporter assay provided two miR-19b binding sites in 3'UTR of *PTEN* mRNA the specifically regulate gene expression of *PTEN* gene, but other members of cluster do not inhibit expression of this gene. Mutations in miR-19b binding sites decrease inhibiting effect. **Table 4 (see supplementary material)** represents one out of two binding sites of miR-19b. Both sites score poor hybridization energy (-16.9 and -16.4 kcal/mol) and $\Delta G/\Delta G_m$ value (39.5 and 38.3%). According to our data, there are stronger sites in this gene for the following miRNAs: miR-3187-5p, miR-3195, miR-3676, miR-3677-5p and miR-4472. Also were experimentally verified association sites for miR-141 and *PTEN* gene **[27].** In 3'UTR of *PTEN* mRNA has been validated location of miR-141 site. Described site has low hybridization energy (-16.2 kcal/mol) and $\Delta G/\Delta G_m$ value (39.2 %).

Conclusions:

Present research data suggest that the miRNAs described here may regulate gene expression more effectively than miRNAs studied in other experimental investigations. *ADAM29, BAX, MSH2, PIK3CA* genes have only one strong potential site for considered miRNAs. We recommend that described new sites are validated by experimental investigation. Several miRNAs affect only one gene, and this information could be useful in molecular medicine to modulate translation of the target mRNA.

Conflict of interest:

The authors declare that they have no conflict of interest.

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Supplementary material:

Table 1: Features of 47 mRNAs binding with intergenic miRNAs

5'UTR

ABCG2: miR-4455, 88.3, miR-4472, 81.8. ***ADAM29:*** miR-4284, 84.0. ***ALCAM:*** miR-21*, 85.0, miR-4472, 80.8. ***AXIN1:*** miR-1268, 83.2. ***AXIN2:*** miR-125b-1, 75.3. ***BAD:*** miR-1538, 74.0. ***CD44:*** miR-1268, 81.7, miR-4455, 84.9. ***CDH1:*** miR-1587, 84.6, miR-4481, 85.6, miR-4507, 91.0, miR-4710, 83.9. ***ENG:*** miR-1587, 77.6, miR-4327, 78.6. ***FLCN:*** miR-125b-1, 76.9. ***GNAS:*** miR-1268, 84.6, miR-1587, 79.2, miR-4466, 81.7, miR-4507, 77.5, miR-4787-5p, 75.2. ***MMP2:*** miR-212, 81.7, miR-1205, 77.7, miR-3130-5p, 76.1, miR-4665-3p, 75.9, ***PIK3CA:*** miR-4787-5p, 79.0. ***KLF12:*** miR-466, 80.0. ***PMS1:*** miR-4746-3p, 81.4. ***PTEN:*** miR-3187-5p, 74.9, miR-3195, 85.9, miR-3676, 77.8, miR-3677-5p, 75.0, miR-4472, 82.3. ***SMAD4:*** miR-3195, 83.0. ***TGFBR2:*** miR-4472, 81.6.

CDS

ABCC2: miR-1246, 82.4, miR-4455, 83.3. ***APC-1:*** miR-302f, 99.6. ***APC-2:*** miR-302f, 99.6 and ***APC-3:*** miR-302f, 99.6. ***AXIN1:*** miR-324-5p, 74.4, miR-365*, 81.2, miR-1204, 78.9, miR-1587, 79.4, miR-4307, 85.5. ***AXIN2:*** miR-4488, 86.7. ***BAD:*** miR-3180, 79.5, miR-4665-5p, 79.3. ***BRAF:*** miR-1260b, 84.2, miR-4458, 82.8. ***BRCA1:*** miR-3613-5p, 80.5. ***BRCA2:*** miR-4801, 79.2. ***BUB1:*** miR-4727-3p, 76.8. ***CDH1:*** miR-4472, 84.7, miR-4488, 85.3. ***DLC1:*** miR-4307, 78.6, miR-4472, 81.6, miR-4736, 83.1. ***DLC1:*** miR-4307, 78.6, miR-4472, 81.6, miR-4736, 83.1. ***ENG:*** miR-4456, 82.2. ***EP300:*** miR-4481, 83.4, miR-4483, 85.6, miR-4717-3p, 76.8. ***FLCN:*** miR-150*, 81.9, miR-515-3p, 78.4, miR-4508, 85.2, miR-4727-5p, 84.0. ***FZD7:*** miR-194*, 75.3, miR-4516, 82.0. ***KIT:*** miR-544, 77.3, miR-4776-3p, 74.0. ***KLF12:*** miR-4328, 82.6. ***MET:*** miR-4307, 81.3. ***MLH1:*** miR-320a, 76.5, miR-320c, 82.6, miR-2113, 81.0, miR-4795-3p, 75.1. ***MLH3***: miR-384, 77, miR-1205, 77, miR-4795-3p, 75. ***MMP2:*** miR-154, 80.1, miR-4665-5p, 74.7. ***MMP9:*** miR-3186-5p, 78.2, miR-4443, 82.1, miR-4530, 80.3. ***MSH6:*** miR-141*, 76.8, miR-1279, 89.4, miR-4527, 76.7, miR-4787-5p, 77.6. ***MTHFR:*** miR-720, 86.4. ***MUTYH-a:*** miR-331-5p, 77.6, miR-4278, 80. ***PMS1:*** miR-9, 75.6, miR-4464, 80.5. ***PMS2:*** miR-1279, 83.0. ***PTPN12:*** miR-548m, 76.3, miR-1279, 86.5. ***SRC:*** miR-4278, 82.5, miR-4327, 78.2, miR-4521, 75. ***TGFBR2:*** miR-377*, 78.2 and ***VDR:*** miR-4298, 75.3, miR-4650-5p, 78.9. ***ZEB1:*** miR-1279, 89.4, miR-3613-5p, 78.9, miR-4307, 79.9, miR-4732-3p, 76.3.

3'UTR

ALCAM: miR-1279, 88.3. ***APC-1:*** miR-4693-5p, 89.8. ***APC-2:*** miR-4693-5p, 89.8. ***APC-3:*** miR-4693-5p, 89.8. ***AXIN2:*** miR-339-5p, 75.5, miR-760, 78.1. ***BAX:*** miR-4275, 86.8. ***BRCA2:*** miR-4650-5p, 84.2. ***CCND1:*** miR-223, 76.0, miR-507, 78.1, miR-1260b, 78.7, miR-3180-5p, 74.5, miR-4481, 84.4, miR-4487, 84.1. ***CDH1:*** miR-1285, 78.8. ***CTNNB1:*** miR-4708-5p, 78.0. ***ENG:*** miR-4472, 84.2. ***FLCN:*** miR-1285, 78.1, miR-1972, 89.9. ***KIT:*** miR-3147, 73.3. ***KLF12:*** miR-221*, 79.9, miR-4704-3p, 76.5. ***KRAS:*** miR-499a-3p, 77.9, miR-548ak, 77.7. ***MLH3:*** miR-221, 75.4, miR-378b, 90.6, miR-378d, 77. ***MMP2:*** miR-3202, 76.9. ***MTHFR:*** miR-513b, 75.4, miR-513c, 77.0, miR-1260, 90.4, miR-4269, 81.0, miR-4456, 83.6, miR-4665-5p, 74. ***PTPN12:*** miR-4711-3p, 80. ***SMAD4:*** miR-513a-5p, 83.9, miR-1268, 82.7, miR-1285, 77.0, miR-1972, 80.4, miR-4645-5p, 79.7. ***SRC:*** miR-129-5p, 76, miR-302f, 88.1, miR-320a, 81.0, miR-320b, 85.5, miR-320c, 85.5, miR-320d, 80.5, miR-466, 74.9, miR-568, 85.1, miR-4436a, 77.5, miR-4466, 80.0. ***TGFBR2:*** miR-30b*, 75.6 and ***TP53:*** miR-1285, 98.7, miR-2392, 78.7, miR-4430, 80. ***VDR:*** miR-1275, 84.3, miR-1587, 83.6, miR-4507, 81.6.

Table 2: Schemes of interaction miRNAs with mRNAs

Schemes of interaction of ig-miRNAs with some mRNAs

```
mRNA FLCN  5' G                    C   3'      mRNA TP53 5' U                          C 3'
               UGAGCCACUGUGCCUGGCC                            GGGUCUCGCUUUGUUGCCCAGG
               ACUCGGUGACACGGACCGG                            UCCAGAGUGAAACAACGGGUCU
miRNA-1972 3'                     ACU 5'      miR-1285  3'                              5'
3'UTR,3374   ΔG = -202  ΔG/ΔGm = 89.9         3'UTR,2298   ΔG = -192  ΔG/ΔGm = 98.7

mRNA PTPN12  5'  C                A 3'        mRNA APC-1  5' G                   G 3'
                  AAAGGAGCAAUAUGA                            GGACAUGGGGGCAGUUA
                  UUUCUUCGUUAUACU                            UUUGUACCUUCGUUAAU
miR-1279     3' UC                  5'        miR-302f    3'                        5'
CDS,927   ΔG = -102  ΔG/ΔGm = 86.5           CDS,2287  ΔG = -115  ΔG/ΔGm = 99.6

mRNA CD44 5' C         G      C 3'            mRNA ABCG2  5'  C                G 3'
              GGAGGCACA GCACCC                             GAGCGCACGCAUCCU
              UUUUUGUGU UGUGGG                             UUUGUGUGUGUGGGA
miR-4455  3'           G      A 5'            miR-4455    3' UU                  5'
5'UTR,46   ΔG = -112  ΔG/ΔGm = 84.9           5'UTR,416   ΔG = -117  ΔG/ΔGm = 88.3
```

Interaction the first position of site - nt, interaction energy (ΔG) - kJ/mol; ΔG/ΔGm value - %.

Table 3: Schematic representation of several experimentally validated complex of miRNAs and target mRNAs

```
AXIN2     5'         U       G        U 3'          PTEN      5'             U     G    G  G 3'
                   UUGAGA ACUGCCA                                          UGGAU UGCA CA
                   GAUUCU UGACGGU                                          ACCUA ACGU GU
miR-34a   3' UGUUGGUC       G          5'          miR-19b   3' AGUCAAAACGU     A          5'

3'UTR, 3086    ΔG = -24.3 ΔG/ΔGm = 52.2            3-utr,  4827 ΔG = -16.9  ΔG/ΔGm = 39.4

APC-1      5'              U          U 3'         PTEN      5'              U          A 3'
                         GGAAGCCAUA                                          GCAGUGUUG
                         UUUUCGGUAU                                          UGUCACAAU
miR-135a   3' AGUGUAUCCUUAU             5'         miR-141   3' GGUAGAAAUGGUC            5'
3'UTR, 8852    ΔG = -16.9  ΔG/ΔGm = 43.11          3'UTR,3686 ΔG = -16.2  ΔG/ΔGm = 39.22
```

Interaction energy (ΔG) - kcal/mol; ΔG/ΔGm value - %

Table 4: Interaction features of ig-miRNAs with 47 genes

gene	gene length	5'U length	CDS length	3'U length	Sites with p<0.0005	sites in 5'UTR	sites in CDS	sites in 3'UTR	s/length	s/5'U	s/CDS	s/3'U
ABCC2	5051	139	4638	274	2	0	2	0	0.40	0.00	0.43	0.00
ABCG2	4431	493	1968	1970	2	2	0	0	0.45	4.06	0.00	0.00
ADAM29	3325	670	2463	192	1	1	0	0	0.30	1.49	0.00	000
ALCAM	4741	540	1752	2449	3	2	0	1	0.63	3.70	0.00	0.41
APC1	10840	193	8533	2114	2	0	1	1	0.18	0.00	0.12	0.47
APC2	10732	85	8533	2114	2	0	1	1	0.19	0.00	0.12	0.47
APC3	11027	380	8533	2114	2	0	1	1	0.18	0.00	0.12	0.47
AXIN1	3675	389	2589	697	6	1	5	0	1.63	2.57	1.93	0.00
AXIN2	4234	289	2531	1414	4	1	1	2	0.94	3.46	0.40	1.41
BAD	970	82	507	381	3	1	2	0	3.09	12.20	3.94	000
BAX	810	69	580	161	1	0	0	1	1.23	0.00	0.00	6.21
BRAF	2944	61	2302	581	2	0	2	0	0.68	0.00	0.87	0.00
BRCA1	7224	232	5592	1400	1	0	1	0	0.14	0.00	0.18	0.00
BRCA2	11386	227	10258	901	2	0	1	1	0.18	0.00	0,10	1.11
BUB1	3502	112	3259	131	1	0	1	0	0.29	0.00	0.31	0.00
CCND1	4289	209	889	3191	6	0	0	6	1.40	0.00	0.00	1.88
CD44	4589	434	1087	3068	2	2	0	0	0.44	4.61	0.00	0.00
CDH1	4815	125	2649	2041	7	4	2	1	1.45	32.00	0.76	0.49
CTNNB1	3720	268	2346	1106	1	0	0	1	0.27	0.00	0.00	0.90
DLC1	7479	444	4587	2448	3	0	3	0	0.40	0.00	0.65	0.00
ENG	3060	413	1977	670	4	2	1	1	1.31	4.84	0.51	1.49
EP300	8761	395	7246	1120	3	0	3	0	0.34	0.00	0.41	0.00
FLCN	3687	504	1740	1443	7	1	4	2	1.90	1.98	2.30	1.39
FZD7	3851	61	1725	2065	2	0	2	0	0.52	0.00	1.16	0.00
GNAS	1907	356	1185	366	5	5	0	0	2.62	14.04	0.00	0.00
KIT	5174	87	2932	2155	3	0	2	1	0.58	0.00	0.68	0.46
KLF12	10909	222	1209	9478	4	1	1	2	0.37	4.50	0.83	0.21
KRAS	5297	181	568	4548	2	0	0	2	0.38	0.00	0.00	0.44
MET	6676	187	4227	2262	1	0	1	0	0.15	0.00	0.24	0.00
MLH1	2662	198	2272	192	4	0	4	0	1.50	0.00	1.76	0.00
MLH3	7896	216	4363	3317	6	0	3	3	0.76	0.00	0.69	0.90
MMP2	3549	311	1983	1255	7	4	2	1	1.97	12.86	1.01	0.80
MMP9	2387	19	2124	244	3	0	3	0	1.26	0.00	1.41	0.00
MSH6	4328	152	4084	92	4	0	4	0	0.92	0.00	0.98	0.00
MTHFR	7150	229	1972	4949	7	0	1	6	0.98	0.00	0.51	1.21
MUTYH-alpha	1945	216	1650	79	2	0	2	0	1.03	0.00	1.21	0.00
PIK3CA	3712	157	3207	348	1	1	0	0	0.27	6.37	0.00	0.00
PMS1	3538	529	2799	210	3	1	2	0	0.85	1.89	0.71	0.00
PMS2	2836	87	2589	160	1	0	1	0	0.35	0.00	0.39	0.00
PTEN	5572	1031	1212	3329	5	5	0	0	0.90	4.85	0.00	0.00
PTPN12	3225	91	2343	791	3	0	2	1	0.93	0.00	0.85	1.26
SMAD4	8769	538	1660	6571	6	1	0	5	0.68	1.86	0.00	0.76
SRC	4097	449	1612	2036	13	0	3	10	3.17	0.00	1.86	4.91
TGFBR2	4704	382	1779	2543	3	1	1	1	0.64	2.62	0.56	0.39
TP53	2586	197	1182	1207	3	0	0	3	1.16	0.00	0.00	2.49
VDR	5060	401	1434	3225	5	0	2	3	0.99	0.00	1.39	0.93
ZEB1	6268	391	3327	2551	5	0	4	1	0.80	0.00	1.20	0.39
Average	5093	302	2978	1864	3	0.77	1.51	1.23	0.89	2.55	0.65	0.68