**Supplementary Table 1 - List of assays used for qPCR analysis of candidate reference genes. Information on RPLP0 reference gene analysis are reported within the text.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene symbol** | **Assay ID** | **Probe modification** | **Functional class** |
| *18S* | Hs99999901\_s1 | FAM-NFQ | Ribosomal RNA |
| *ACTB* | Hs99999903\_m1 | FAM-NFQ | Actin cytoskeleton |
| *B2M* | Hs00187842\_m1 | FAM-NFQ | Major histocompatibility complex |
| *GAPDH* | Hs99999905\_m1 | FAM-NFQ | Glycolytic pathway |
| *HPRT1* | Hs02800695\_m1 | FAM-NFQ | Purine salvage pathway |
| *PGK1* | Hs99999906\_m1 | FAM-NFQ | Glycolytic pathway |
| *RPL13A* | Hs01926559\_g1 | FAM-NFQ | Ribosomal protein |
| *TBP* | Hs00427620\_m1 | FAM-NFQ | TATA-box binding protein |
| *UBC* | Hs00824723\_m1 | FAM-NFQ | Ubiquitin pathway |

**Supplementary Table 2 – List of primers used for qPCR analysis of circular RNA for validation of hybridization array results. The primers were used for qPCR detection of circular RNAs with a SYBR green chemistry.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **CircRNA ID** | **best\_transcript** | **GeneSymbol** | **circRNA boundary (circRNA length)** | **Forward primer sequence** | **Reverse primer sequence** | **Amplicon length (Tm°C)** | **Design strategy** |
| hsa\_circRNA\_104574 | NM\_171982 | *TRIM35* | Exon 3 end-exon 3 start (231 bp) | 5'-AGATGAAGGAGGACGACGTTTC-3' | 5'-GCTCCTCCACTCTCAAGAACTC-3' | 108 bp (81°C) | Divergent primers |
| hsa\_circRNA\_100395 | NM\_014458 | *KLHL20* | Exon 11 end- Exon 7 start (671 bp) | 5'-AGTGGATTGGTGGTTGGTGC-3' | 5'-GACCCCAACTCCGCATCTC-3' | 119 bp  (79°C) | Backspliced junction-specific primer |
| hsa\_circRNA\_101015 | NM\_002336 | *LRP6* | Exon 2 end-exon 2 start (394 bp) | 5'-TTAGATCCTTCAAGTGGCGGC-3' | 5'-CATCCTCCAAGCCTCCAACTAC-3' | 119 bp (79°C) | Backspliced junction-specific primer |
| hsa\_circRNA\_405396 | NM\_020843 | *SCAPER* | Exon 15 end - Exon 9 start (1094 bp) | 5'-AAGAAGCTAAGAACGGAAAGATGC-3' | 5'-CTTGTGGCTTCTGTTGCCAAT-3' | 117 bp (78°C) | Backspliced junction-specific primer |
| hsa\_circRNA\_023461 | NM\_015242 | *ARAP1* | Exon 22 end - Exon 5 beginning (2542 bp) | 5'- GATGAACGTGCACAACCTGG-3' | 5'- TCCGCCAACTAAACACCACC-3' | 128 bp  (82°C) | Backspliced junction-specific primer |

**Supplementary Table 3 – List of assays used for qPCR analysis of microRNA (miRNA) for validation of RNAseq results. All assays are TaqMan Advanced miRNA Assays from Thermo Fisher. hsa-miR-361-5p and hsa-miR-186-5p were used as reference miRNA for normalization of gene expression.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Mature miRNA ID** | **Assay ID** | **Probe modification** | **Stem-loop Accession no.** | **Mature miRNA sequence** |
| hsa-miR-361-5p | 478056\_mir | FAM-NFQ | MI0000760 | UUAUCAGAAUCUCCAGGGGUAC |
| hsa-miR-186-5p | 477940\_mir | FAM-NFQ | MI0000483 | CAAAGAAUUCUCCUUUUGGGCU |
| hsa-miR-205-5p | 477967\_mir | FAM-NFQ | MI0000285 | UCCUUCAUUCCACCGGAGUCUG |
| hsa-miR-429 | 477849\_mir | FAM-NFQ | MI0001641 | UAAUACUGUCUGGUAAAACCGU |
| hsa-miR-1246 | 477881\_mir | FAM-NFQ | MI0006381 | AAUGGAUUUUUGGAGCAGG |
| hsa-miR-197-3p | 477959\_mir | FAM-NFQ | MI0000239 | UUCACCACCUUCUCCACCCAGC |
| hsa-miR-1298-5p | 479452\_mir | FAM-NFQ | MI0003938 | UUCAUUCGGCUGUCCAGAUGUA |
| hsa-miR-200b-3p | 477963\_mir | FAM-NFQ | MI0000342 | UAAUACUGCCUGGUAAUGAUGA |
| hsa-miR-200c-3p | 478351\_mir | FAM-NFQ | MI0000650 | UAAUACUGCCGGGUAAUGAUGGA |
| hsa-miR-494-5p | 478944\_mir | FAM-NFQ | MI0003134 | AGGUUGUCCGUGUUGUCUUCUCU |
| hsa-miR-9-3p | 478211\_mir | FAM-NFQ | MI0000466 | AUAAAGCUAGAUAACCGAAAGU |
| hsa-miR-16-1-3p | 478727\_mir | FAM-NFQ | MI0000070 | CCAGUAUUAACUGUGCUGCUGA |

**Supplementary Table 4 - List of differentially regulated miRNAs - 3D (trypsinised) vs. 2D (monolayer) day 0 samples. Highlighted in bold the miRNAs with both p-value and False Discovery Rate (FDR) <0.05.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Up-regulated miRNA (3D vs 2D)** | | | |
| **Mature\_ID** | **Fold\_Change** | **p\_value** | **FDR** |
| hsa-miR-1298-5p | 58.27581241 | 0.00668543 | 0.291682365 |
| hsa-miR-205-5p | 42.66541728 | 0.004049485 | 0.291682365 |
| hsa-miR-219a-2-3p | 40.73265421 | 0.005463637 | 0.291682365 |
| hsa-miR-429 | 30.62081755 | 0.024134274 | 0.569418016 |
| hsa-miR-338-3p | 28.83744381 | 0.016189341 | 0.434333888 |
| hsa-miR-383-5p | 19.9516949 | 0.011369157 | 0.357654728 |
| hsa-miR-9-3p | 18.4033642 | 0.005484 | 0.291682365 |
| hsa-miR-9-3p | 17.5850591 | 0.006306965 | 0.291682365 |
| hsa-miR-9-3p | 17.5850591 | 0.006306965 | 0.291682365 |
| hsa-miR-9-5p | 14.83355127 | 0.007613798 | 0.291682365 |
| hsa-miR-9-5p | 14.82092766 | 0.00763275 | 0.291682365 |
| hsa-miR-9-5p | 14.81956171 | 0.007636787 | 0.291682365 |
| hsa-miR-203a-3p | 12.357523 | 0.008499354 | 0.291682365 |
| hsa-miR-375-3p | 9.67492676 | 0.037370193 | 0.688158435 |
| hsa-miR-200c-3p | 7.536591415 | 0.003531205 | 0.291682365 |
| hsa-miR-338-5p | 7.266168837 | 0.029842017 | 0.638094369 |
| hsa-miR-129-1-3p | 6.70921998 | 0.044299822 | 0.760144671 |
| hsa-miR-200b-3p | 6.677579559 | 0.014342869 | 0.401069117 |
| **hsa-miR-4531** | **6.109283641** | **2.09852E-05** | **0.005281283** |
| hsa-miR-6510-3p | 4.234478455 | 0.024095466 | 0.569418016 |
| **hsa-miR-16-1-3p** | **2.921183298** | **2.15621E-06** | **0.001627937** |
| **hsa-miR-410-5p** | **2.770662161** | **1.21343E-05** | **0.004580704** |
| hsa-miR-4791 | 2.559073029 | 0.010254044 | 0.336600127 |
| hsa-miR-1248 | 2.522015746 | 0.014303087 | 0.401069117 |
| hsa-miR-3182 | 2.37792286 | 0.014249121 | 0.401069117 |
| hsa-miR-1261 | 2.283591538 | 0.001169687 | 0.110389224 |
| hsa-miR-330-5p | 2.26815046 | 0.038508866 | 0.692242701 |
| hsa-miR-136-5p | 2.178427266 | 0.04720133 | 0.784951606 |
| hsa-miR-1277-5p | 2.100062919 | 0.033457041 | 0.646329707 |
| hsa-miR-1291 | 1.918733885 | 0.032116008 | 0.638094369 |
| **hsa-miR-21-3p** | **1.869152254** | **0.000161122** | **0.017378154** |
| **hsa-miR-30e-5p** | **1.71438815** | **0.000127227** | **0.016009392** |
| hsa-miR-494-5p | 1.701154627 | 0.006109194 | 0.291682365 |
| hsa-miR-222-5p | 1.661019142 | 0.008414381 | 0.291682365 |
| hsa-miR-29b-3p | 1.514458794 | 0.048422479 | 0.784951606 |
| hsa-miR-29b-3p | 1.513624256 | 0.048864537 | 0.784951606 |
| **Down-regulated miRNA (3D vs 2D)** | | | |
| **Mature\_ID** | **Fold\_Change** | **p\_value** | **q\_value** |
| hsa-miR-10401-3p | 0.64035475 | 0.030055983 | 0.638094369 |
| hsa-miR-197-3p | 0.625359984 | 0.025436283 | 0.581951322 |
| hsa-miR-495-3p | 0.612486811 | 0.031076498 | 0.638094369 |
| hsa-miR-12135 | 0.603862054 | 0.041382257 | 0.726595447 |
| hsa-miR-4521 | 0.599248409 | 0.008147302 | 0.291682365 |
| **hsa-miR-1246** | **0.442577077** | **4.3031E-05** | **0.006497684** |
| **hsa-miR-1290** | **0.275411902** | **3.15713E-05** | **0.005959083** |

**Supplementary Table 5 - List of differentially regulated piwi-RNAs - 3D (trypsinised) vs. 2D (monolayer) day 0 samples. \*LTR derived; \*\*LINE derived; \*\*\*SINE derived. Highlighted in bold the piRNAs with both p-value and False Discovery Rate (FDR) <0.05.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Up-regulated piRNA (3D vs 2D)** | | | | | | | |
| **ID** | **NCBI/EMBL name** | **GenBank accession no.** | **fold\_change** | **p\_value** | **FDR** | **length** | **Identified in** |
| piR-hsa-6842 | piR-44721 | DQ576609 | 54.46021 | 0.023873889 | 0.241231022 | 32 | [28, 29] |
| piR-hsa-6840 | piR-44719 | DQ576607 | 51.8911 | 0.02531562 | 0.241231022 | 30 | [28, 29] |
| piR-hsa-6841 | piR-44720 | DQ576608 | 51.8911 | 0.02531562 | 0.241231022 | 31 | [28, 29] |
| piR-hsa-5632 | piR-43452 | DQ575340 | 30.58512 | 0.012993133 | 0.195117448 | 31 | Downregulated in bladder cancer [28, 29] |
| piR-hsa-21707 | piR-58555 | DQ591443 | 25.0602 | 0.012910376 | 0.195117448 | 29 | [28] |
| piR-hsa-27489\* | piR-35280 | DQ597214 | 6.955108 | 0.029882221 | 0.241231022 | 27 | [28] |
| piR-hsa-13940 | piR-50786 | DQ583674 | 6.87107 | 0.034835312 | 0.271348744 | 30 | [28] |
| **piR-hsa-4047** | **piR-41880** | **DQ573768** | **5.82895** | **0.000613554** | **0.038916841** | **30** | **[28]** |
| piR-hsa-27137 | piR-35466 | DQ597400 | 5.212595 | 0.044629542 | 0.308571636 | 26 | [28] |
| piR-hsa-12790 | piR-32679 | DQ582567 | 4.9447 | 0.027121866 | 0.241231022 | 29 | [28] |
| piR-hsa-24684 | piR-60577 | DQ594465 | 4.861851 | 0.040741804 | 0.296546898 | 32 | [28] |
| piR-hsa-28527 | piR-36378 | DQ598312 | 4.819944 | 0.046643268 | 0.309098672 | 29 | Upregulated in breast cancer [28, 30] |
| piR-hsa-15572 | piR-52373 | DQ585261 | 3.944318 | 0.011656035 | 0.195117448 | 27 | Upregulated in bladder cancer [28, 29, 31] |
| piR-hsa-27725 | piR-35545 | DQ597479 | 3.888503 | 0.015696818 | 0.195117448 | 28 | [28] |
| piR-hsa-23289 | piR-33123 | DQ593011 | 3.444631 | 0.024982582 | 0.241231022 | 28 | [28, 29] |
| piR-hsa-24000 | piR-33864 | DQ593752 | 3.363849 | 0.011543085 | 0.195117448 | 28 | [28] |
| piR-hsa-23662 | piR-33526 | DQ593414 | 3.339749 | 0.021381609 | 0.241231022 | 27 | [28] |
| **piR-hsa-23327** | **piR-33161** | **DQ593049** | **3.261308** | **0.000349952** | **0.038916841** | **29** | **[28]** |
| **piR-hsa-27621** | **piR-35412** | **DQ597346** | **3.167974** | **0.000570278** | **0.038916841** | **31** | **[28]** |
| **piR-hsa-1243** | **piR-31104** | **DQ570992** | **3.161594** | **0.000581123** | **0.038916841** | **32** | **[28, 29]** |
| piR-hsa-1344 | piR-31686 | DQ571574 | 3.09659 | 0.002563848 | 0.081310621 | 32 | [28] |
| piR-hsa-23230 | piR-33064 | DQ592952 | 3.09659 | 0.002563848 | 0.081310621 | 30 | [28] |
| piR-hsa-27622 | piR-35413 | DQ597347 | 2.937921 | 0.001643788 | 0.071794932 | 32 | [28] |
| piR-hsa-23619 | piR-33468 | DQ593356 | 2.841356 | 0.037383508 | 0.28132674 | 30 | [28] |
| piR-hsa-30374\*\*\* | piR-38240 | DQ600174 | 2.748511 | 0.026551115 | 0.241231022 | 30 | Upregulated in bladder cancer [28, 31] |
| piR-hsa-27140 | piR-35469 | DQ597403 | 2.622904 | 0.011217909 | 0.195117448 | 32 | [28] |
| piR-hsa-5770 | piR-43604 | DQ575492 | 2.586464 | 0.008732706 | 0.195117448 | 29 | [28] |
| piR-hsa-27139 | piR-35468 | DQ597402 | 2.560312 | 0.013084156 | 0.195117448 | 31 | [28] |
| piR-hsa-25786\*\* | piR-61651 | DQ595539 | 2.420887 | 0.021265331 | 0.241231022 | 27 | [28] |
| piR-hsa-27620 | piR-35411 | DQ597345 | 2.368114 | 0.001481681 | 0.071794932 | 30 | [28] |
| piR-hsa-1242 | piR-31103 | DQ570991 | 2.366578 | 0.001512092 | 0.071794932 | 31 | [28, 29] |
| piR-hsa-27623 | piR-35414 | DQ597348 | 2.362805 | 0.004871483 | 0.127231672 | 32 | [28] |
| **piR-hsa-23231** | **piR-33065** | **DQ592953** | **2.263859** | **0.000269495** | **0.038916841** | **30** | **[28, 29]** |
| piR-hsa-20613\*\* | piR-57516 | DQ590404 | 2.153437 | 0.01416368 | 0.195117448 | 31 | [28] |
| piR-hsa-27493 | piR-35284 | DQ597218 | 2.125892 | 0.04556139 | 0.308571636 | 30 | Upregulated in breast cancer [28-30] |
| piR-hsa-28223 | piR-36074 | DQ598008 | 2.093154 | 0.014521243 | 0.195117448 | 30 | [28] |
| piR-hsa-27619 | piR-35410 | DQ597344 | 2.073673 | 0.002169267 | 0.08026289 | 29 | [28] |
| piR-hsa-28131 | piR-35982 | DQ597916 | 2.025496 | 0.015802483 | 0.195117448 | 31 | [28, 29] |
| piR-hsa-1207 | piR-31068 | DQ570956 | 2.025112 | 0.015820334 | 0.195117448 | 31 | [28, 29] |
| piR-hsa-24672 | piR-60565 | DQ594453 | 1.890001 | 0.004565686 | 0.126697797 | 31 | [28, 29] |
| piR-hsa-25046 | piR-60852 | DQ594740 | 1.880096 | 0.009125832 | 0.195117448 | 27 | [28] |
| piR-hsa-5934 | piR-43768 | DQ575656 | 1.83712 | 0.036458886 | 0.279099062 | 27 | [28] |
| piR-hsa-26686 | piR-34536 | DQ596470 | 1.624619 | 0.018317784 | 0.21981341 | 31 | [28] |
| piR-hsa-26685 | piR-34535 | DQ596469 | 1.584407 | 0.029068428 | 0.241231022 | 30 | [28] |
| piR-hsa-26684 | piR-34534 | DQ596468 | 1.582195 | 0.028802831 | 0.241231022 | 29 | Upregulated in bladder cancer  [28, 31] |
| piR-hsa-26681 | piR-34531 | DQ596465 | 1.582018 | 0.028812413 | 0.241231022 | 29 | [28, 29] |
| piR-hsa-26682 | piR-34532 | DQ596466 | 1.579044 | 0.029423923 | 0.241231022 | 30 | Upregulated in bladder cancer [28, 29, 31] |
| piR-hsa-26683 | piR-34533 | DQ596467 | 1.574046 | 0.028499216 | 0.241231022 | 30 | [28, 29] |
| **Down-regulated piRNA (3D vs 2D)** | | | | | | | |
| **ID** | **NCBI/EMBL name** | **GenBank accession no.** | **fold\_change** | **Pv** | **Fdr** | **length** | **Identified in** |
| piR-hsa-32298 | N/A | N/A | 0.178681919 | 0.045372819 | 0.308571636 | 27 | [32] |
| piR-hsa-15615 | piR-52416 | DQ585304 | 0.22241129 | 0.03404473 | 0.269926075 | 31 | [28, 29] |
| piR-hsa-27007 | piR-34871 | DQ596805 | 0.28413596 | 0.001778703 | 0.071794932 | 27 | [28] |
| piR-hsa-1100 | piR-30961 | DQ570849 | 0.313365538 | 0.021828631 | 0.241231022 | 31 | [28] |
| piR-hsa-12485 | piR-32374 | DQ582262 | 0.314147928 | 0.025189493 | 0.241231022 | 31 | [28] |
| piR-hsa-23588 | piR-33437 | DQ593325 | 0.318941821 | 0.010914859 | 0.195117448 | 28 | [28] |
| piR-hsa-27400 | piR-35176 | DQ597110 | 0.330217483 | 0.026684792 | 0.241231022 | 32 | [28] |
| **piR-hsa-7116** | **piR-44892** | **DQ576780** | **0.349968739** | **0.000504515** | **0.038916841** | **27** | **[28]** |
| piR-hsa-23087 | piR-59892 | DQ592780 | 0.355530146 | 0.045868757 | 0.308571636 | 30 | [28] |
| piR-hsa-26491 | piR-34341 | DQ596275 | 0.398562123 | 0.013706341 | 0.195117448 | 30 | [28] |
| **piR-hsa-23326** | **piR-33160** | **DQ593048** | **0.425134463** | **0.000438758** | **0.038916841** | **30** | **[28]** |
| piR-hsa-13150 | piR-32939 | DQ582827 | 0.581430243 | 0.043727257 | 0.308571636 | 26 | [28, 29] |
| piR-hsa-23248 | piR-33082 | DQ592970 | 0.633234036 | 0.022768789 | 0.241231022 | 32 | [28] |
| piR-hsa-32182 | N/A | N/A | 0.633526115 | 0.009513007 | 0.195117448 | 26 | [32] |

**Supplementary Table 6 - List of differentially regulated circular RNAs - 3D (trypsinised) vs. 2D (monolayer) day 0 samples.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Up-regulated circRNA (3D vs 2D)** | | | | | | | | | | | | |
| **CircRNA ID** | **Alias** | **circRNA\_type** | **best\_transcript** | **GeneSymbol** | **FC (abs)** | **P-value** | **FDR** | **MRE1** | **MRE2** | **MRE3** | **MRE4** | **MRE5** |
| hsa\_circRNA\_101066 | hsa\_circ\_0026337 | exonic | NM\_014191 | SCN8A | 2.2015902 | 0.02174316 | 0.999288827 | hsa-miR-22-5p | hsa-miR-181d-5p | hsa-miR-181b-5p | hsa-miR-181a-5p | hsa-miR-181c-5p |
| hsa\_circRNA\_000543 | hsa\_circ\_0000326 | intronic | TCONS\_l2\_00004572 | XLOC\_l2\_002352 | 1.7486891 | 0.001579531 | 0.999288827 | hsa-miR-338-3p | hsa-miR-9-3p | hsa-miR-16-2-3p | hsa-miR-320b | hsa-miR-320a |
| hsa\_circRNA\_104574 | hsa\_circ\_0083756 | exonic | NM\_171982 | TRIM35 | 1.7134615 | 0.01364327 | 0.999288827 | hsa-miR-21-5p | hsa-miR-205-5p | hsa-miR-873-5p | hsa-miR-558 | hsa-miR-495-5p |
| hsa\_circRNA\_100395 | hsa\_circ\_0015278 | exonic | NM\_014458 | KLHL20 | 1.6923705 | 0.027100892 | 0.999288827 | hsa-miR-141-3p | hsa-miR-588 | hsa-miR-660-3p | hsa-miR-136-5p | hsa-miR-200a-3p |
| hsa\_circRNA\_089761 | hsa\_circ\_0089761 | exonic | uc022bqs.1 | JA760602 | 1.6558348 | 0.034020011 | 0.999288827 | hsa-miR-3529-3p | hsa-miR-6891-3p | hsa-miR-554 | hsa-miR-5196-3p | hsa-miR-384 |
| hsa\_circRNA\_101015 | hsa\_circ\_0000378 | exonic | NM\_002336 | LRP6 | 1.6372244 | 0.015499695 | 0.999288827 | hsa-miR-640 | hsa-miR-135b-5p | hsa-miR-499a-3p | hsa-miR-543 | hsa-miR-135a-5p |
| hsa\_circRNA\_089763 | hsa\_circ\_0089763 | exonic | uc022bqt.1 | JA760600 | 1.6356325 | 0.026333323 | 0.999288827 | hsa-miR-6803-3p | hsa-miR-6856-3p | hsa-miR-7111-3p | hsa-miR-454-5p | hsa-miR-670-3p |
| hsa\_circRNA\_405396 |  | exonic | NM\_020843 | SCAPER | 1.6063116 | 0.007743907 | 0.999288827 | hsa-miR-4778-3p | hsa-miR-22-5p | hsa-miR-1248 | hsa-miR-4659a-3p | hsa-miR-4659b-3p |
| hsa\_circRNA\_101205 | hsa\_circ\_0006078 | exonic | NM\_023928 | AACS | 1.5827427 | 0.010299423 | 0.999288827 | hsa-miR-488-3p | hsa-miR-653-3p | hsa-miR-552-5p | hsa-miR-767-5p | hsa-miR-197-3p |
| hsa\_circRNA\_023461 | hsa\_circ\_0023461 | exonic | NM\_015242 | ARAP1 | 1.5056374 | 0.003669466 | 0.999288827 | hsa-miR-6720-5p | hsa-miR-4512 | hsa-miR-6836-5p | hsa-miR-766-3p | hsa-miR-3918 |

**Supplementary Table 7 - microRNA-binding sites in differentially expressed circular RNA identified in 3D (trypsinised) vs. 2D (monolayer) day 0 samples.**

|  |  |  |
| --- | --- | --- |
| **CircRNA ID** | **Binding site for** | **miRNA regulation in 3D vs 2D** |
| circRNA\_104574 | miR-205-5p | Up |
| circRNA\_101205 | miR-197-3p | Down |
| circRNA\_100395 | miR-136-5p | Up |
| circRNA\_000543 | miR-338-3p miR-9-3p | Up |
| circRNA\_405396 | miR-1248 | Up |

**Supplementary Table 8 - Summary of results of geNorm, NormFinder, and comparative ΔCt analyses for the stability of candidate reference genes in 3D (trypsinised) vs. 2D (monolayer) day 0 samples. Genes were ranked from the most to the less stable, based on the value calculated by the algorithms.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **geNorm** | | | **NormFinder** | | | **Comparative ΔCt** | | |
| **Ranking order** | **Gene name** | **M value** | **Ranking order** | **Gene name** | **Stability value** | **Ranking order** | **Gene name** | **Mean std dev** |
| **#1** | ***HPRT1*** | **0.173** | **#1** | ***PGK1*** | **0.038** | **#1** | ***HPRT1*** | **0.173** |
| **#1** | ***PGK1*** | **0.173** | **#2** | ***HPRT1*** | **0.043** | **#1** | ***PGK1*** | **0.173** |
| **#1** | ***TBP*** | **0.173** | **#3** | ***TBP*** | **0.050** | **#1** | ***TBP*** | **0.173** |
| #2 | *UBC* | 0.197 | #4 | *B2M* | 0.054 | #2 | *UBC* | 0.197 |
| #3 | *B2M* | 0.212 | #5 | *UBC* | 0.057 | #3 | *B2M* | 0.212 |
| #4 | *ACTB* | 0.216 | #6 | *18S* | 0.082 | #4 | *ACTB* | 0.216 |
| #5 | *RPLP0* | 0.234 | #7 | *ACTB* | 0.093 | #5 | *RPLP0* | 0.234 |
| #6 | *RPL13A* | 0.241 | #8 | *RPLP0* | 0.095 | #6 | *RPL13A* | 0.241 |
| #7 | *18S* | 0.244 | #9 | *RPL13A* | 0.110 | #7 | *18S* | 0.244 |
| #8 | *GAPDH* | 0.248 | #10 | *GAPDH* | 0.127 | #8 | *GAPDH* | 0.248 |

**Supplementary Table 9 - Summary of results of geNorm and NormFinder analyses for the stability of selected miRNA endogenous controls in 3D (trypsinised) vs. 2D (monolayer) day 0 samples.**

|  |  |  |  |
| --- | --- | --- | --- |
| **geNorm** | | **NormFinder** | |
| **Endogenous control** | **M value** | **Endogenous control** | **Stability value** |
| hsa-miR-361-5p | 0.110 | hsa-miR-361-5p | 0.045 |
| hsa-miR-186-5p | 0.110 | hsa-miR-186-5p | 0.045 |
| Geometric mean | 0.073 | Geometric mean | 0.010 |