# Supplementary information for:

## Donkey genome and insight into the imprinting of fast karyotype evolution

Jinlong Huang[1]†, Yiping Zhao[1]†, Dongyi Bai[1]†, Wunierfu Shiraigol[1]†, Bei Li[1]†, Lihua Yang[1]†, Jing Wu[1]†, Wuyundalai Bao[1]†, Xiujuan Ren[1], Burenqiqige Jin[1], Qinan Zhao[1], Anaer Li[1], Sarula Bao[1], Wuyingga Bao[1], Zhencun Xing[1], Aoruga An[1], Yahan Gao[1], Ruiyuan Wei[1], Yirugeletu Bao[1], Taoketao Bao[1], Haige Han[1], Haitang Bai[1], Yanqing Bao[1], Yuhong Zhang[1], Dorjsuren Daidiikhuu[1], Wenjing Zhao[2], Shuyun Liu[2], Jinmei Ding[2], Weixing Ye[3], Fangmei Ding[3], Zikui Sun[3], Yixiang Shi[3], Yan Zhang[4]*, He Meng[2]*, Manglai Dugarjaviin[1]*

[1]College of Animal Science, Inner Mongolia Agricultural University, 306 Zhaowuda Road, Hohhot 010018, P.R. China.

[2]School of Agriculture and Biology, Shanghai Jiaotong University; Shanghai Key Laboratory of Veterinary Biotechnology, 800 Dongchuan Road, Shanghai 200240, P. R. China.

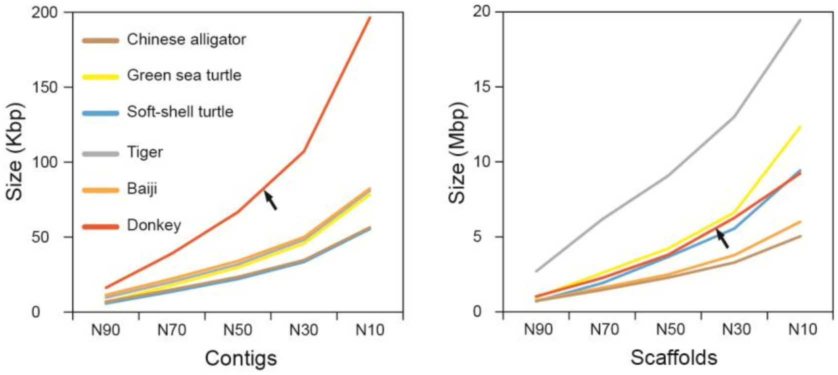
[3]Shanghai Personal Biotechnology Limited Company, 218 Yindu Road, Shanghai 200231, P.R. China.

[4]SRA Inc. 6003 Executive Blvd. Suite 400, Rockville, MD20852, USA.

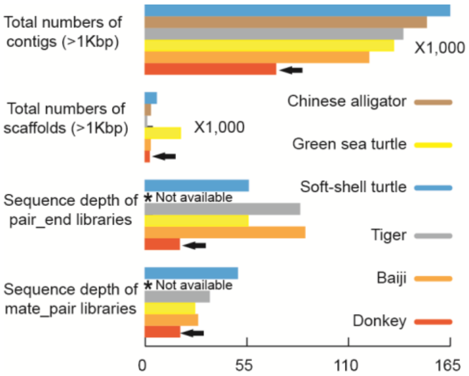
† These authors contributed equally to this work.

* For correspondence: Manglai Dugarjaviin (dmanglai@imau.edu.cn); He Meng (menghe@sjtu.edu.cn); Yan Zhang (yan_zhang@sra.com).

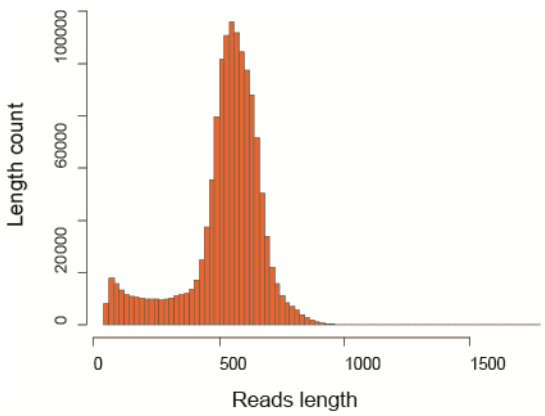
**Supplementary Figure 1.** Comparison of the size of contigs and scaffolds for the donkey genome assembly with other genome assemblies. The arrow indicates the donkey genome assembly.



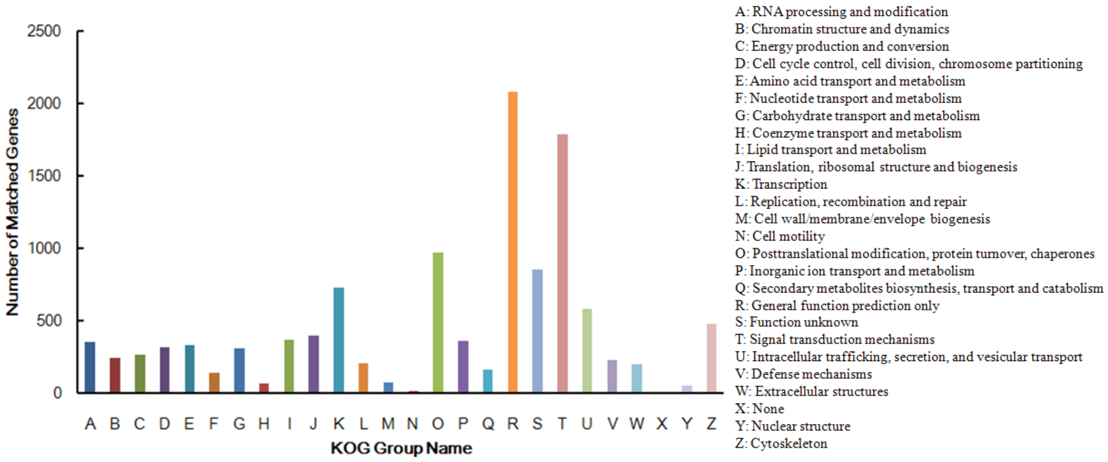
**Supplementary Figure 2.** Comparison of the sequence coverage and number of contigs and scaffolds for the donkey genome with other genomes. The arrow indicates the donkey genome.



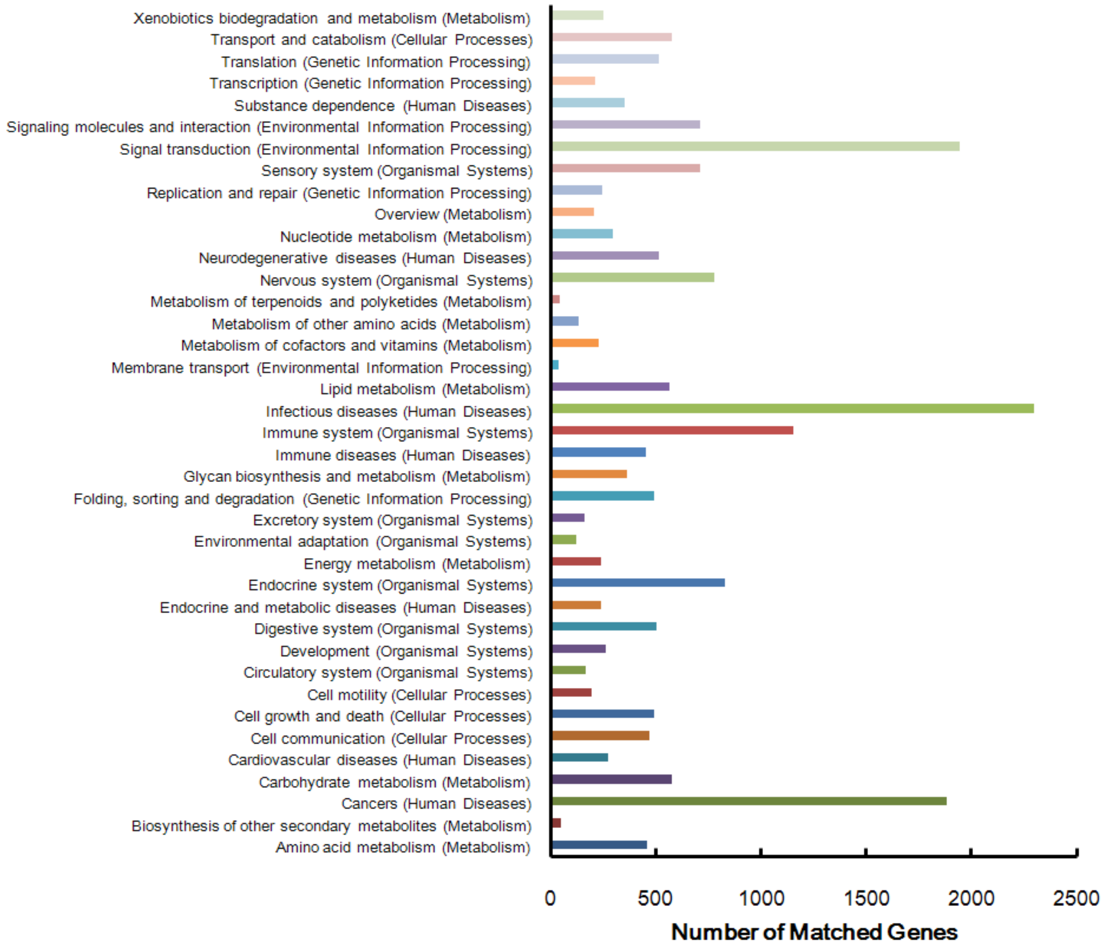
**Supplementary Figure 3.** Distribution of the transcriptome sequencing read lengths. RNA-seq was performed using the 454 FLX+ platform, and 1,390,416 reads were generated at an average length of 522 bp.



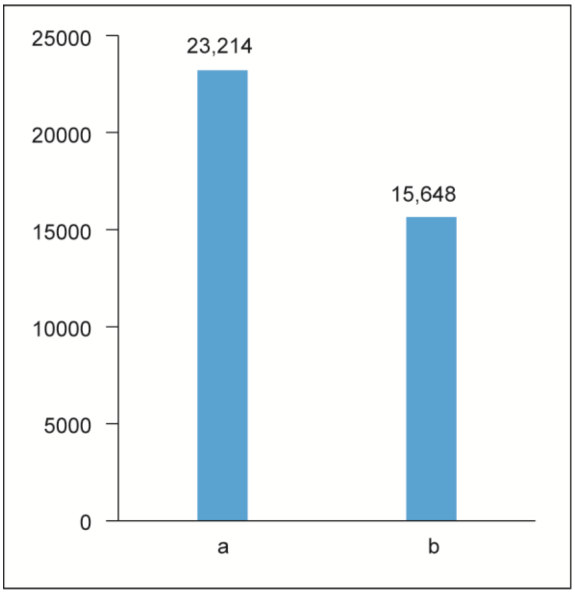
**Supplementary Figure 4.** KOG (EuKaryotic Orthologous Groups) assignments for donkey genes.



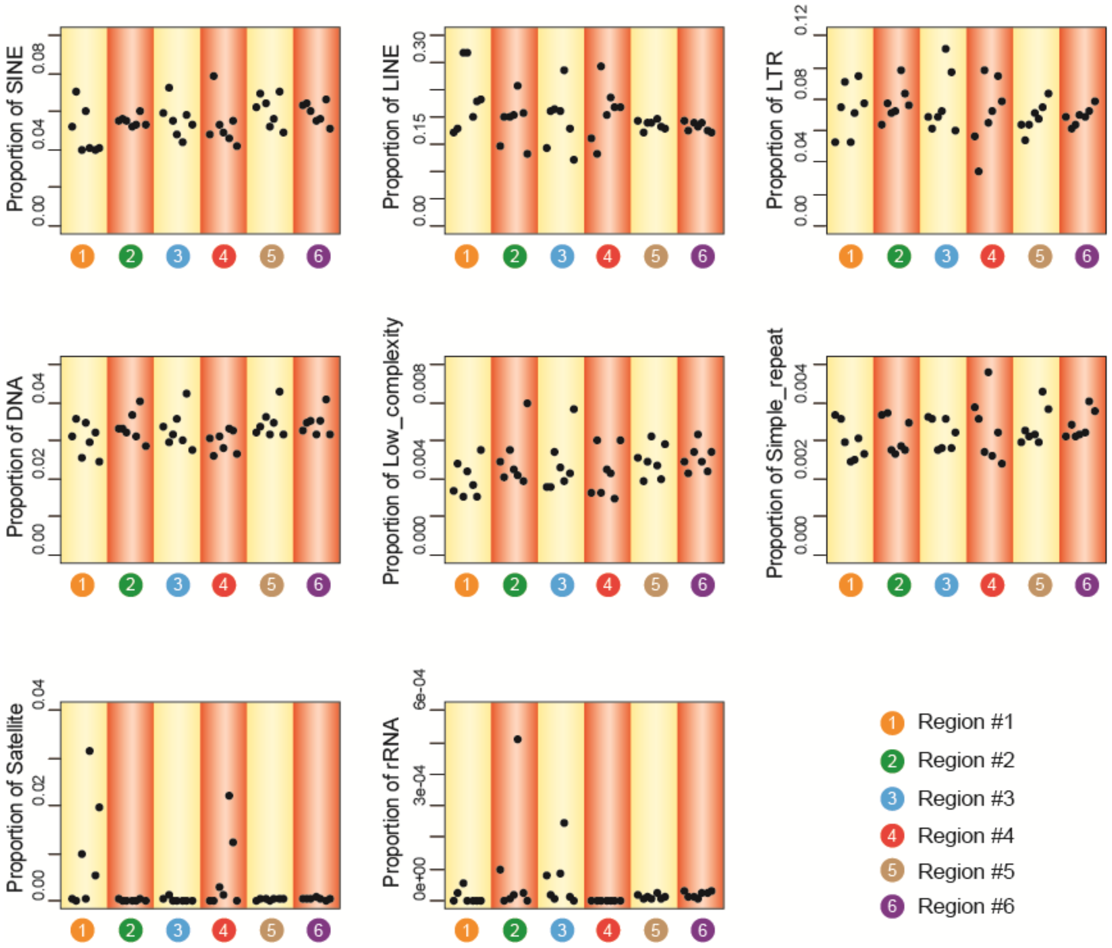
**Supplementary Figure 5.** KEGG (Kyoto Encyclopedia of Genes and Genomes) assignments for donkey genes.



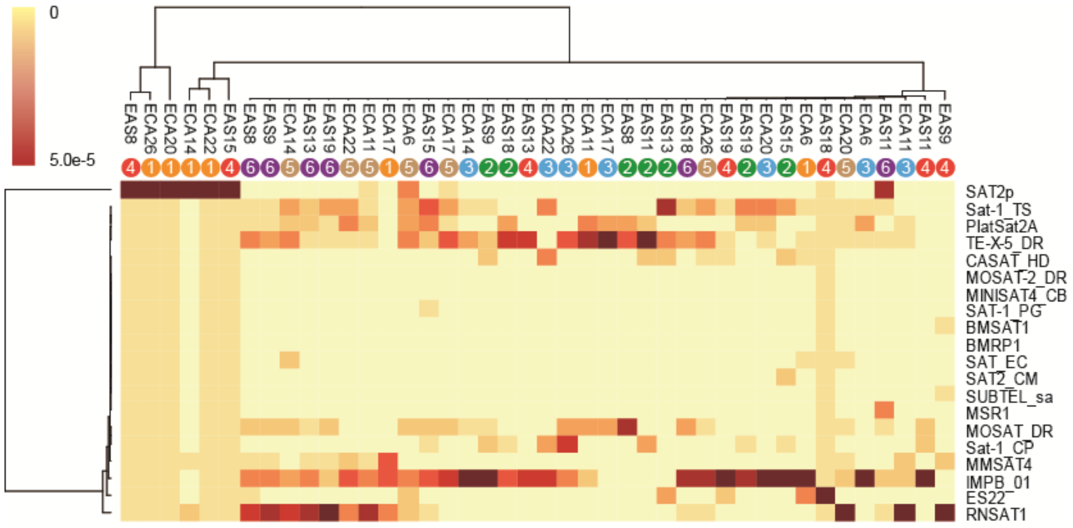
**Supplementary Figure 6.** RNA-seq Annotated genes in the donkey genome. (a), number of genes annotated according to the corresponding criteria; (b), number of genes confirmed by RNA-seq results.



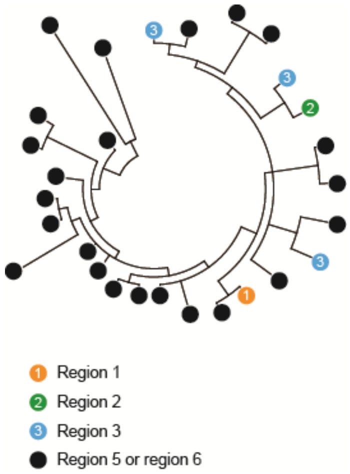
**Supplementary Figure 7.** Eight types of repetitive sequences in different regions (regions #1-6) of seven pairs of chromosomes.



**Supplementary Figure 8.** Hierarchical clustering results of 20 types of satellite sequences.



**Supplementary Figure 9.** Neighbor-joining tree for conservative 5SrRNA.



**Supplementary Table 1.** Genome sequencing summary for donkey. PE: Paired-end, standard genomic library sequenced using paired-end reads; SE: single-end; MP: Mate-paired.

| Library Type | Number of library | Insert size | platform | Reads length | Raw data (bp) |
|---|---:|---|---|---|---:|
| PE400 | 1 | 400 bp | MiSeq | Paired-End, 2×251 bp | 13,108,951,900 |
| PE450 | 3 | 450 bp | MiSeq | Paired-End, 2×251 bp | 31,738,312,460 |
| PE700 | 3 | 700 bp | MiSeq | Paired-End, 2×251 bp | 25,043,864,854 |
| PE1000 | 1 | 1000 bp | MiSeq | Paired-End, 2×251 bp | 3,971,185,456 |
| SE1500 | 1 | 1500~1900 bp | 454 FLX+ | Single-End,average 510bp | 389,938,235 |
| MP3K | 2 | 3000 bp | HiSeq2000 | Mate-Paired, 2×100 bp | 27,790,797,200 |
| MP5K | 2 | 5000 bp | HiSeq2000 | Mate-Paired, 2×100 bp | 24,393,026,800 |
| MP8K | 2 | 8000 bp | HiSeq2000 | Mate-Paired, 2×100 bp | 23,417,575,400 |
| MP12K | 1 | 12000 bp | HiSeq2000 | Mate-Paired, 2×100 bp | 10,020,707,400 |
| MP15K | 1 | 16000 bp | HiSeq2000 | Mate-Paired, 2×100 bp | 2,888,852,600 |

**Supplementary Table 2.** Qualified data for the donkey genome.

| Library | clean data (bp) | Read1 length | Read2 length |
|---|---:|---:|---:|
| PE400 | 10,321,705,614 | 242 | 219 |
| PE450 | 21,732,933,747 | 231 | 196 |
| PE700 | 16,246,446,690 | 230 | 184 |
| PE1000 | 1,544,607,013 | 186 | 133 |
| SE1500 | 389,938,235 | Single-End, average 510bp | |
| MP3K | 15,594,147,909 | 84 | 83 |
| MP5K | 14,258,628,782 | 86 | 85 |
| MP8K | 13,179,898,660 | 85 | 84 |
| MP12K | 5,877,899,778 | 85 | 84 |
| MP15K | 1,248,444,268 | 83 | 79 |

**Supplementary Table 3.** Genome sequencing summary for Asiatic wild ass.

| Library Type | Number of library | Insert size | platform | Reads length | Raw data (bp) |
|---|---|---|---|---|---|
| PE500 | 1 | 500 bp | HiSeq2000 | Paired-End, 2×100 bp | 32,703,961,600 |

**Supplementary Table 4.** Qualified data for the Asiatic wild ass genome.

| Library | clean data (bp) | Read1 length | Read2 length |
|---|---|---|---|
| PE500 | 28,556,773,659 | 97 | 96 |

**Supplementary Table 5.** Assembly results for the donkey genome.

| | Contigs | Scaffolds |
|---|---|---|
| Min Sequence length (bp) | 200 | 1,002 |
| Max Sequence length (bp) | 773,221 | 17,059,037 |
| N10 (bp) | 196,594 | 9,235,695 |
| N30 (bp) | 107,128 | 6,261,484 |
| N50 (bp) | 66,737 | 3,803,025 |
| N70 (bp) | 39,038 | 2,254,583 |
| N90 (bp) | 16,178 | 1,021,074 |
| N number (bp) | 0 | 33,114,414 |
| Total Sequence length (bp) | 2,324,805,719 | 2,357,920,133 |
| Total Sequence Number | 71,732 | 2,166 |

**Supplementary Table 6.** The completeness of 248 core eukaryotic genes in genome assemblies of donkey and other published genomes sequences using CEGMA.

| Species | Complete(%)[a] | Partial(%)[b] |
|---|---:|---:|
| Donkey | 57.26 | 92.34 |
| Baiji | 66.13 | 92.74 |
| Chinese alligator | 45.56 | 90.32 |
| Tiger | 52.42 | 90.32 |
| Green sea turtle | 37.90 | 86.69 |
| Soft-shell turtle | 34.68 | 83.87 |

[a] 'Complete' refers to predicted proteins in the set of 248 CEGs (Core Eukaryotic Genes) that could be aligned to the HMM of the KOG for that proteinfamily given alignment lengthsmore than 70% of the protein length.

[b] 'Partial' refers to incomplete proteins that exceed a pre-computed minimum alignment score. A protein deemed to be 'Complete' will also be included in the 'Partial' set.

**Supplementary Table 7.** Summary of RNA-seq analysis of the female donkey.

| Reads number | Average length | Raw data |
|---|---|---|
| 1,390,416 | 522bp | 725.8M |

**Supplementary Table 8.** Number of heterozygous (within an individual) SNPs and Indels in the donkey and Asiatic wild ass genomes.

| | donkey | Asiatic wild ass |
|---|---|---|
| Heterozygous SNP | 2,187,070 | 3,321,087 |
| Heterozygous INDEL | 247,822 | 213,735 |

**Supplementary Table 9.** Enrichment analysis of expansive gene families in the donkey genome. (MF: molecular function; CC: cell component; BP: biological process)

| Functional category | Term ID | Term description | Involved gene number | P_values |
|---|---|---|---|---|
| BP | GO:0050911 | detection of chemical stimulus involved in sensory perception of smell | 34 | 3.2E-11 |
| BP | GO:0007186 | G-protein coupled receptor signaling pathway | 38 | 1.54E-09 |
| BP | GO:0044699 | single-organism process | 4 | 0.000956 |
| BP | GO:0007608 | sensory perception of smell | 3 | 0.006588 |
| BP | GO:0035023 | regulation of Rho protein signal transduction | 4 | 0.01058 |
| BP | GO:0022617 | extracellular matrix disassembly | 3 | 0.01104 |
| BP | GO:0030509 | BMP signaling pathway | 3 | 0.01853 |
| BP | GO:0007411 | axon guidance | 6 | 0.01889 |
| BP | GO:0006805 | xenobiotic metabolic process | 3 | 0.02215 |
| BP | GO:0001501 | skeletal system development | 3 | 0.0428 |
| BP | GO:0008152 | metabolic process | 5 | 0.0466 |
| CC | GO:0005634 | nucleus | 18 | 0.000362 |
| CC | GO:0005604 | basement membrane | 5 | 0.00221 |
| CC | GO:0005796 | Golgi lumen | 4 | 0.002335 |
| CC | GO:0005886 | plasma membrane | 47 | 0.002908 |
| CC | GO:0070062 | extracellular vesicular exosome | 5 | 0.003173 |
| CC | GO:0016021 | integral to membrane | 63 | 0.01051 |
| CC | GO:0043202 | lysosomal lumen | 3 | 0.01686 |
| CC | GO:0030018 | Z disc | 4 | 0.01866 |
| CC | GO:0019898 | extrinsic to membrane | 3 | 0.02825 |
| MF | GO:0004984 | olfactory receptor activity | 36 | 3.52E-12 |
| MF | GO:0004930 | G-protein coupled receptor activity | 39 | 1.39E-11 |
| MF | GO:0005200 | structural constituent of cytoskeleton | 4 | 0.004218 |
| MF | GO:0005488 | binding | 4 | 0.005734 |
| MF | GO:0005201 | extracellular matrix structural constituent | 3 | 0.006588 |
| MF | GO:0016887 | ATPase activity | 4 | 0.01143 |
| MF | GO:0005089 | Rho guanyl-nucleotide exchange factor activity | 4 | 0.01636 |
| MF | GO:0005515 | protein binding | 11 | 0.02146 |
| MF | GO:0005509 | calcium ion binding | 15 | 0.02923 |
| MF | GO:0005516 | calmodulin binding | 4 | 0.04014 |
| MF | GO:0004725 | protein tyrosine phosphatase activity | 4 | 0.04205 |
| KEGG | map04740 | Olfactory transduction | 26 | 5.36E-08 |
| KEGG | map04974 | Protein digestion and absorption | 4 | 0.01327 |
| KEGG | map04512 | ECM-receptor interaction | 3 | 0.02613 |
| KEGG | map05320 | Autoimmune thyroid disease | 3 | 0.02825 |
| KEGG | map05152 | Tuberculosis | 6 | 0.0288 |
| KEGG | map05146 | Amoebiasis | 4 | 0.03301 |

**Supplementary Table 10.** Enrichment analysis of expansive gene families in the Thoroughbred horse genome. (MF: molecular function; BP: biological process)

| Functional category | Term ID | Term description | Involved gene number | P_values |
|---|---|---|---|---|
| BP | GO:0006952 | defense response | 3 | 0.011694 |
| BP | GO:0006950 | response to stress | 3 | 0.028023 |
| BP | GO:0006953 | acute-phase response | 2 | 0.03524 |
| BP | GO:0002526 | acute inflammatory response | 2 | 0.041051 |
| MF | GO:0004857 | enzyme inhibitor activity | 2 | 0.025568 |
| MF | GO:0030234 | enzyme regulator activity | 2 | 0.045455 |
| KEGG | ecb04740 | Olfactory transduction | 17 | 4.28E-13 |
| KEGG | ecb05322 | Systemic lupus erythematosus | 6 | 7.15E-05 |

**Supplementary Table 11.** Enrichment analysis of rapidly evolving genes in the donkey genome. (BP:biological process)

| Functional category | Term ID | Term description | Mean of ω (Donkey) | Mean of ω (Horse) | p_values | Gene number | Fold change in mean ω (Donkey) vs. ω (Horse) |
|---|---|---|---|---|---|---|---|
| BP | GO:0009060 | aerobic respiration | 0.468311429 | 0.212797 | 0.027965 | 7 | 2.200741149 |
| BP | GO:0030900 | forebrain development | 0.4581714 | 0.216707 | 0.00671 | 20 | 2.114239754 |
| BP | GO:0045619 | regulation of lymphocyte differentiation | 0.500157083 | 0.250011 | 0.02467 | 10 | 2.000538309 |
| BP | GO:0006099 | tricarboxylic acid cycle | 0.459216364 | 0.246425 | 0.016761 | 6 | 1.863510263 |
| BP | GO:0046356 | acetyl-CoA catabolic process | 0.459216364 | 0.246425 | 0.016761 | 6 | 1.863510263 |
| BP | GO:0006357 | regulation of transcription from RNA polymerase II promoter | 0.309431321 | 0.166096 | 0.016169 | 65 | 1.862962067 |
| BP | GO:0007050 | cell cycle arrest | 0.34912775 | 0.188365 | 0.020903 | 14 | 1.853461559 |
| BP | GO:0032774 | RNA biosynthetic process | 0.297064828 | 0.162725 | 0.043553 | 29 | 1.825568057 |
| BP | GO:0045944 | positive regulation of transcription from RNA polymerase II promoter | 0.254304259 | 0.144119 | 0.027416 | 36 | 1.764542694 |
| BP | GO:0006417 | regulation of translation | 0.393015333 | 0.2268 | 0.010715 | 18 | 1.732873538 |
| BP | GO:0051254 | positive regulation of RNA metabolic process | 0.261971181 | 0.154286 | 0.010306 | 47 | 1.69796081 |
| BP | GO:0045893 | positive regulation of transcription, DNA-dependent | 0.262990559 | 0.155364 | 0.013977 | 46 | 1.692738235 |
| BP | GO:0010608 | posttranscriptional regulation of gene expression | 0.359831739 | 0.224674 | 0.015445 | 24 | 1.601574328 |
| BP | GO:0051187 | cofactor catabolic process | 0.359052 | 0.225092 | 0.015928 | 7 | 1.595134434 |
| BP | GO:0000723 | telomere maintenance | 0.469709375 | 0.300447 | 0.002098 | 8 | 1.563369148 |
| BP | GO:0070727 | cellular macromolecule localization | 0.373786111 | 0.239228 | 0.048083 | 38 | 1.562466928 |
| BP | GO:0017148 | negative regulation of translation | 0.462737857 | 0.300145 | 0.006696 | 8 | 1.541714362 |
| BP | GO:0048534 | hemopoietic or lymphoid organ development | 0.363756941 | 0.239752 | 0.002764 | 31 | 1.517220228 |
| BP | GO:0030097 | hemopoiesis | 0.394863067 | 0.26126 | 0.001245 | 30 | 1.51137741 |
| BP | GO:0007548 | sex differentiation | 0.424734118 | 0.287558 | 0.002834 | 21 | 1.477039898 |
| BP | GO:0002520 | immune system development | 0.346672447 | 0.236993 | 0.003585 | 32 | 1.462798246 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| BP | GO:0006281 | DNA repair | 0.396609732 | 0.276214 | 0.005421 | 32 | 1.435877436 |
| BP | GO:0048608 | reproductive structure development | 0.430420952 | 0.30198 | 0.004686 | 18 | 1.425327087 |
| BP | GO:0045137 | development of primary sexual characteristics | 0.429110476 | 0.305384 | 0.005081 | 18 | 1.405149172 |
| BP | GO:0008406 | gonad development | 0.453081316 | 0.329392 | 0.008153 | 16 | 1.375508613 |
| BP | GO:0015031 | protein transport | 0.294934756 | 0.214471 | 0.038238 | 65 | 1.375176152 |
| BP | GO:0045184 | establishment of protein localization | 0.294226721 | 0.214088 | 0.044942 | 65 | 1.374324411 |
| BP | GO:0032268 | regulation of cellular protein metabolic process | 0.318470519 | 0.231948 | 0.041033 | 43 | 1.373023745 |
| BP | GO:0032504 | multicellular organism reproduction | 0.344703741 | 0.253658 | 0.019033 | 46 | 1.358928849 |
| BP | GO:0048609 | reproductive process in a multicellular organism | 0.344703741 | 0.253658 | 0.019033 | 46 | 1.358928849 |
| BP | GO:0006974 | response to DNA damage stimulus | 0.35513028 | 0.261345 | 0.029436 | 36 | 1.358856405 |
| BP | GO:0009968 | negative regulation of signal transduction | 0.251353462 | 0.188143 | 0.02522 | 24 | 1.335968851 |
| BP | GO:0065003 | macromolecular complex assembly | 0.298143231 | 0.225823 | 0.035578 | 58 | 1.320253487 |
| BP | GO:0008585 | female gonad development | 0.470596667 | 0.358097 | 0.010106 | 11 | 1.314160981 |
| BP | GO:0043933 | macromolecular complex subunit organization | 0.294688915 | 0.225482 | 0.039921 | 61 | 1.306926975 |
| BP | GO:0006259 | DNA metabolic process | 0.344672128 | 0.264876 | 0.023344 | 47 | 1.301256548 |
| BP | GO:0033554 | cellular response to stress | 0.331512551 | 0.257648 | 0.042435 | 50 | 1.286689072 |
| BP | GO:0046545 | development of primary female sexual characteristics | 0.4517768 | 0.352777 | 0.016781 | 11 | 1.280630699 |
| BP | GO:0046660 | female sex differentiation | 0.4517768 | 0.352777 | 0.016781 | 11 | 1.280630699 |
| BP | GO:0022613 | ribonucleoprotein complex biogenesis | 0.267161806 | 0.209294 | 0.004872 | 23 | 1.276492038 |
| BP | GO:0003006 | reproductive developmental process | 0.352671519 | 0.279023 | 0.030695 | 27 | 1.263949535 |
| BP | GO:0032200 | telomere organization | 0.451973529 | 0.360221 | 0.002607 | 8 | 1.254713207 |
| BP | GO:0006284 | base-excision repair | 0.346288824 | 0.277465 | 0.032216 | 6 | 1.24804374 |
| BP | GO:0006730 | one-carbon metabolic process | 0.389817222 | 0.317026 | 0.018309 | 15 | 1.229608198 |
| BP | GO:0006396 | RNA processing | 0.285309672 | 0.233234 | 0.017031 | 51 | 1.223277994 |
| BP | GO:0006446 | regulation of translational initiation | 0.470618 | 0.398308 | 0.0186 | 8 | 1.181542927 |
| BP | GO:0021537 | telencephalon development | 0.33231 | 0.281946 | 0.029594 | 10 | 1.17862995 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| BP | GO:0022402 | cell cycle process | 0.34776256 | 0.31212 | 0.002263 | 57 | 1.114196606 |
| BP | GO:0007049 | cell cycle | 0.334022819 | 0.301825 | 0.010578 | 70 | 1.106676614 |
| BP | GO:0051090 | regulation of transcription factor activity | 0.302910278 | 0.273746 | 0.044262 | 13 | 1.106536159 |
| BP | GO:0022602 | ovulation cycle process | 0.369524091 | 0.33527 | 0.008096 | 11 | 1.102170167 |
| BP | GO:0021987 | cerebral cortex development | 0.423459412 | 0.391195 | 0.005638 | 8 | 1.082477358 |
| BP | GO:0021543 | pallium development | 0.339452273 | 0.31452 | 0.033161 | 8 | 1.079269306 |
| BP | GO:0000087 | M phase of mitotic cell cycle | 0.373287273 | 0.347318 | 0.016474 | 25 | 1.074771021 |
| BP | GO:0000280 | nuclear division | 0.382009176 | 0.359573 | 0.02407 | 24 | 1.062397591 |
| BP | GO:0007067 | mitosis | 0.382009176 | 0.359573 | 0.02407 | 24 | 1.062397591 |
| BP | GO:0048285 | organelle fission | 0.3691325 | 0.353044 | 0.036003 | 24 | 1.045571211 |
| BP | GO:0000278 | mitotic cell cycle | 0.334097154 | 0.321872 | 0.026409 | 36 | 1.037982159 |
| BP | GO:0000279 | M phase | 0.38629 | 0.3752 | 0.014312 | 34 | 1.029558667 |
| MF | GO:0003702 | RNA polymerase II transcription factor activity | 0.425302785 | 0.129383 | 0.02382 | 26 | 3.287173155 |
| MF | GO:0003714 | transcription corepressor activity | 0.381889412 | 0.143207 | 0.034927 | 17 | 2.666694051 |
| MF | GO:0003712 | transcription cofactor activity | 0.327390175 | 0.170799 | 0.007749 | 38 | 1.916816119 |
| MF | GO:0008134 | transcription factor binding | 0.306913459 | 0.173941 | 0.019749 | 48 | 1.764473792 |
| MF | GO:0043565 | sequence-specific DNA binding | 0.317935251 | 0.186359 | 0.043653 | 53 | 1.706033969 |
| MF | GO:0005504 | fatty acid binding | 0.355241333 | 0.218435 | 0.012361 | 8 | 1.626304738 |
| MF | GO:0046983 | protein dimerization activity | 0.288388679 | 0.196181 | 0.04488 | 48 | 1.470012311 |
| MF | GO:0016563 | transcription activator activity | 0.265449921 | 0.183474 | 0.042536 | 38 | 1.446800697 |
| MF | GO:0008373 | sialyltransferase activity | 0.506252 | 0.396232 | 0.041709 | 5 | 1.27766561 |
| MF | GO:0004527 | exonuclease activity | 0.330923214 | 0.268063 | 0.024515 | 9 | 1.234500217 |
| MF | GO:0004518 | nuclease activity | 0.351341695 | 0.294962 | 0.037417 | 18 | 1.191142095 |
| MF | GO:0019904 | protein domain specific binding | 0.268009897 | 0.231765 | 0.037055 | 20 | 1.156388727 |

**Supplementary Table 12.** Enrichment analysis of rapidly evolving genes in the Thoroughbred horse genome. (MF: molecular function; BP: biological process)

| Functional category | Term ID | Term description | Mean of ω (Donkey) | Mean of ω (Horse) | p_values | Gene number | Fold change in mean ω (Horse) vs. ω (Donkey) |
|---|---|---|---|---|---|---|---|
| BP | GO:0042254 | ribosome biogenesis | 0.187661667 | 0.353428 | 0.035248 | 15 | 1.883327264 |
| BP | GO:0045494 | photoreceptor cell maintenance | 0.282578333 | 0.510645 | 0.041551 | 4 | 1.807091839 |
| BP | GO:0021532 | neural tube patterning | 0.128933 | 0.219653 | 0.041551 | 4 | 1.703621261 |
| BP | GO:0001947 | heart looping | 0.271165833 | 0.407191 | 0.046821 | 4 | 1.501630306 |
| BP | GO:0019932 | second-messenger-mediated signaling | 0.183038831 | 0.246639 | 0.001296 | 20 | 1.347467254 |
| BP | GO:0008654 | phospholipid biosynthetic process | 0.226798857 | 0.284659 | 0.036526 | 9 | 1.255115924 |
| BP | GO:0051345 | positive regulation of hydrolase activity | 0.236316842 | 0.296162 | 0.00288 | 16 | 1.253243118 |
| BP | GO:0051348 | negative regulation of transferase activity | 0.192748286 | 0.240657 | 0.026713 | 9 | 1.24855511 |
| BP | GO:0051640 | organelle localization | 0.253836875 | 0.316034 | 0.021295 | 9 | 1.245026949 |
| BP | GO:0060041 | retina development in camera-type eye | 0.30531375 | 0.357851 | 0.040076 | 5 | 1.172077085 |
| BP | GO:0006916 | anti-apoptosis | 0.364841875 | 0.421109 | 0.044915 | 14 | 1.154223491 |
| BP | GO:0006289 | nucleotide-excision repair | 0.30892619 | 0.356283 | 0.016082 | 7 | 1.153295979 |
| BP | GO:0043086 | negative regulation of catalytic activity | 0.214188333 | 0.247005 | 0.007771 | 20 | 1.153214072 |
| BP | GO:0007030 | Golgi organization | 0.287156364 | 0.330975 | 0.041551 | 4 | 1.152593456 |
| BP | GO:0009062 | fatty acid catabolic process | 0.256997333 | 0.289177 | 0.043807 | 5 | 1.125215307 |
| BP | GO:0010498 | proteasomal protein catabolic process | 0.288727838 | 0.317876 | 0.036526 | 9 | 1.100955449 |
| BP | GO:0043161 | proteasomal ubiquitin-dependent protein catabolic process | 0.288727838 | 0.317876 | 0.036526 | 9 | 1.100955449 |
| BP | GO:0016042 | lipid catabolic process | 0.252559385 | 0.276944 | 0.005337 | 15 | 1.096550027 |
| BP | GO:0051336 | regulation of hydrolase activity | 0.238613131 | 0.251643 | 0.027565 | 21 | 1.054608491 |
| BP | GO:0051321 | meiotic cell cycle | 0.413962143 | 0.431993 | 0.004068 | 11 | 1.043557008 |
| BP | GO:0048536 | spleen development | 0.25211 | 0.26125 | 0.031969 | 4 | 1.036254016 |
| BP | GO:0006913 | nucleocytoplasmic transport | 0.2808754 | 0.290537 | 0.031725 | 12 | 1.034399595 |
| BP | GO:0051169 | nuclear transport | 0.276276275 | 0.285749 | 0.034355 | 12 | 1.034286509 |
| BP | GO:0022403 | cell cycle phase | 0.362079272 | 0.364713 | 3.37E-05 | 34 | 1.007274028 |
| MF | GO:0008227 | amine receptor activity | 0.115142632 | 0.183432 | 0.016507 | 6 | 1.593085921 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| MF | GO:0005543 | phospholipid binding | 0.230389107 | 0.333719 | 0.030285 | 13 | 1.448503657 |

**Supplementary Table 13.** Repeat sequences in the donkey genome.

| | Number of elements | Length occupied | Percentage of sequence |
|---|---|---|---|
| SINEs: | 964330 | 175869828bp | 7.46% |
| Alu/B1 | 0 | 0bp | 0.00% |
| MIRs | 560212 | 86172367bp | 3.65% |
| | | | |
| LINEs: | 934805 | 539350954bp | 22.87% |
| LINE1 | 516000 | 413293333bp | 17.53% |
| LINE2 | 357376 | 109854592bp | 4.66% |
| L3/CR1 | 48393 | 11794134bp | 0.50% |
| RTE | 12552 | 4285769bp | 0.18% |
| | | | |
| LTR elements: | 380349 | 170193797bp | 7.22% |
| ERVL | 107069 | 53651613bp | 2.28% |
| ERVL-MaLRs | 175016 | 65729822bp | 2.79% |
| ERV_classI | 72276 | 42697897bp | 1.81% |
| ERV_classII | 1747 | 1173838bp | 0.05% |
| | | | |
| DNA elements: | 388813 | 81092370bp | 3.44% |
| hAT-Charlie | 224818 | 45590121bp | 1.93% |
| TcMar-Tigger | 75515 | 17447332bp | 0.74% |
| | | | |
| Satellites: | 422 | 1247289bp | 0.05% |
| | | | |
| Simple repeats: | 198517 | 10559725bp | 0.45% |
| | | | |
| Low complexity: | 265131 | 11227865bp | 0.48% |

**Supplementary Table 14.** Pericentromeric regions in the Thoroughbred horse and donkey genomes.

| Chromosomes of Thoroughbred horse | Region #1 (Thoroughbred horse) | Region #2 (donkey) | Region #3 (Thoroughbred horse) | Region #4 (donkey) |
|---|---|---|---|---|
| 6 | 20M-30M | scaffold1449:0-21669;<br>scaffold213:0-1335911;<br>scaffold328:0-2333015;<br>scaffold388:1072241-2001066 | 5.5M-9M | scaffold200:0-3629144;<br>scaffold355:0-2187355;<br>scaffold50:4049253-7518837; |
| 11 | 26M-30M | scaffold491:0-1546321;<br>scaffold66:4865946-6958850 | 45M-51M | scaffold486:0-210156;<br>scaffold9:6066945-9526757;<br>scaffold976:0-312072 |
| 14 | 0-4M | scaffold1807:0-1449;<br>scaffold219:0-1703000;<br>scaffold394:1319215-1971954;<br>scaffold45:0-7860578 | 43M-53M | scaffold1342:0-51966;<br>scaffold1462:0-19272;<br>scaffold1633:0-3551;<br>scaffold481:0-1589768;<br>scaffold848:0-508249;<br>scaffold864:0-481037;<br>scaffold918:0-402223 |
| 17 | 0-4M | scaffold110:1468912-5198093;<br>scaffold783:0-45959;<br>scaffold830:396350-542364 | 10M-14M | scaffold1587:0-5766;<br>scaffold206:0-811194 |
| 20 | 0-4M | scaffold1017:0-261445;<br>scaffold1033:0-240055;<br>scaffold1112:0-170579;<br>scaffold1188:0-119121;<br>scaffold1254:0-86521;<br>scaffold1264:0-81445;<br>scaffold1345:0-49439;<br>scaffold1534:0-9464;<br>scaffold1543:0-8915;<br>scaffold18:4145634-4801528;<br>scaffold244:0-1002888;<br>scaffold818:0-568079;<br>scaffold951:0-342753;<br>scaffold986:0-303444 | 30M-34M | scaffold450:0-1720145;<br>scaffold615:0-1105635;<br>scaffold750:0-719256;<br>scaffold838:0-533065 |
| 22 | 0-4M | scaffold606:0-1138699;<br>scaffold71:0-4364170;<br>scaffold71:6704483-6776457;<br>scaffold867:0-474124 | 13.5M-19.5M | scaffold1000:0-284273;<br>scaffold117:810785-1183002;<br>scaffold1395:0-34955;<br>scaffold20:8467469-9235695;<br>scaffold28:852521-858424;<br>scaffold413:0-1876501;<br>scaffold712:0-811768; |

|  |  |  |  |  |
|---|---|---|---|---|
|  |  |  |  | scaffold905:0-419880 |
| 26 | 0-4M | scaffold62:2583134-6478310 | 10M-14M | scaffold1133:0-152466;<br>scaffold1229:0-97236;<br>scaffold123:1539637-2102274;<br>scaffold344:1045554-2246623;<br>scaffold714:0-804170;<br>scaffold826:0-551156 |

**Supplementary Table 15.** Noncoding sequences in the donkey genome.

| ncRNA Type | Copy | Average length (bp) | Total length (bp) |
|---|---:|---:|---:|
| snoRNA | 512 | 110 | 56165 |
| CD-box | 284 | 86 | 24408 |
| HACA-box | 207 | 135 | 28044 |
| scaRNA | 21 | 177 | 3713 |
| miRNA | 1198 | 89 | 84673 |
| snRNA | 530 | 111 | 58745 |
| Cis-reg | 172 | 79 | 13578 |
| lncRNA | 189 | 147 | 27819 |
| Other | 256 | 239 | 61209 |
| Summary | 3590 | 109 | 390402 |

**Supplementary Table 16.** Enrichment analysis of target genes from expanded miRNA families in the donkey genome.

| Term description | Involved gene number | P_values |
|---|---:|---:|
| Neurotrophin signaling pathway | 29 | 9.11E-07 |
| Ubiquitin mediated proteolysis | 29 | 7.33E-06 |
| Chronic myeloid leukemia | 20 | 9.25E-06 |
| ErbB signaling pathway | 21 | 2.57E-05 |
| Endometrial cancer | 15 | 6.83E-05 |
| Pathways in cancer | 49 | 8.44E-05 |
| Insulin signaling pathway | 26 | 1.27E-04 |
| Glioma | 16 | 1.74E-04 |
| Prostate cancer | 19 | 3.67E-04 |
| Colorectal cancer | 18 | 5.27E-04 |
| Renal cell carcinoma | 16 | 5.91E-04 |
| Fc gamma R-mediated phagocytosis | 19 | 8.36E-04 |
| Acute myeloid leukemia | 14 | 8.68E-04 |
| Focal adhesion | 31 | 0.001361967 |
| Type II diabetes mellitus | 12 | 0.001474374 |
| MAPK signaling pathway | 38 | 0.001590571 |
| Aldosterone-regulated sodium reabsorption | 11 | 0.001722184 |
| Wnt signaling pathway | 24 | 0.003721774 |
| Non-small cell lung cancer | 12 | 0.00472718 |
| Progesterone-mediated oocyte maturation | 16 | 0.005083663 |
| Axon guidance | 21 | 0.005416773 |
| Pancreatic cancer | 14 | 0.006565047 |
| Melanogenesis | 17 | 0.008251476 |
| mTOR signaling pathway | 11 | 0.010503824 |
| Long-term potentiation | 13 | 0.010621187 |
| Adherens junction | 14 | 0.011557011 |
| Melanoma | 13 | 0.014832715 |
| Endocytosis | 25 | 0.020519368 |
| VEGF signaling pathway | 13 | 0.022284553 |
| Oocyte meiosis | 16 | 0.042579033 |
| Hedgehog signaling pathway | 10 | 0.043454872 |
| Small cell lung cancer | 13 | 0.048597727 |

**Supplementary Table 17.** Summary of small RNA-seq analysis of the female donkey.

| Annotation | Unique sequences |
|---|---:|
| rRNA | 17514 |
| tRNA | 4635 |
| snoRNA | 3943 |
| miRNA | 22100 |
| Intron | 1130 |

**Supplementary Table 18.** Novel miRNA identified in the donkey genome.

| miRNA | Sequences | Abundance |
|---|---|---|
| eas-m0001-5p | CTGTAAACACCCGACTGGAAG | 2113 |
| eas-m0002-5p | GCTTGGCACCTCGTAAGCACTC | 1916 |
| eas-m0003-5p | AGAGATCAGAGGCGCAGAGTG | 593 |
| eas-m0004-3p | ACCTTGCGCTACTCAGGTCTGCT | 579 |
| eas-m0005-3p | TCACTCCTCTCCTCCCGTCTTC | 384 |
| eas-m0006-5p | CTGCCCCAAGAAGCCTGGACTTG | 250 |
| eas-m0007-3p | ATTCAACGGGTCTTTATTGAG | 230 |
| eas-m0008-3p | CGGGGCCGCCCCCGCGGGCT | 198 |
| eas-m0009-3p | AGTCGGTGCCATCTGGGTAGAG | 161 |
| eas-m0010-3p | AGTCGGTGCCATCTGGGTAGAG | 161 |
| eas-m0011-3p | CCAGTTACCGCTTCCGCTACCG | 129 |
| eas-m0012-3p | GGGGCTGACCCAATGGCACAGT | 108 |
| eas-m0013-3p | ACGGGCTGCAGCGCCGACGCC | 93 |
| eas-m0014-5p | GGCTCTGTGAGGTCGGCTCAA | 92 |
| eas-m0015-3p | TGCCTGGGCCTCTGGAACCA | 72 |
| eas-m0016-5p | CTGTCAACCATCCAGCTGTTTG | 71 |
| eas-m0017-3p | TAAATGGTCCCCTAGTGCTCTG | 50 |
| eas-m0018-5p | GTTGATCAGAGAACATACATTT | 47 |
| eas-m0019-3p | ATTCGGCACCTTCTGGGTAGCG | 39 |
| eas-m0020-5p | TGTACATGGTAGGCTTTCATTC | 31 |
| eas-m0021-5p | TTTCCCCTAGTTCTCTTGCATA | 28 |
| eas-m0022-5p | TTCCTCTCTGCCCCATAGGGTGT | 26 |
| eas-m0023-3p | CTGCTGAGCTAGCACTTCCCGAG | 25 |
| eas-m0024-3p | GTTGCAGCTGCCTGGGAGTGATT | 22 |
| eas-m0025-3p | CTTGCCCTTCTTCCTCTGAC | 21 |
| eas-m0026-5p | ACTTTTTGGTCTGAGTGCGTCC | 17 |
| eas-m0027-3p | TTTTCGCCGTCTGAGTCCGCCG | 15 |
| eas-m0028-5p | ATTTCTGGACAAATGCTCCCTGC | 14 |
| eas-m0029-5p | CAAGGATTCAACCAACTTCGGA | 14 |
| eas-m0030-3p | TCAACAGACATTAATTGGGCGCC | 14 |
| eas-m0031-3p | GCCAGCTTGAGCCCACCTACATA | 13 |
| eas-m0032-3p | ACCCATATACCCCTCGGATACA | 13 |
| eas-m0033-3p | TTCCGTCCCCCACTCGCCTCCTG | 12 |
| eas-m0034-5p | CTAGTAGGTGTTCAGTAAGTGT | 12 |
| eas-m0035-5p | GTCTCTGGTTCTTGTCGTAC | 12 |
| eas-m0036-5p | TATGGAACTTCAGACAATAGGGC | 12 |
| eas-m0037-5p | CTGTTGGCCTTTGGACTGGAA | 11 |
| eas-m0038-5p | GGCAGGGCTGAGACCTGGACTG | 11 |
| eas-m0039-3p | CTGCACTTGAAGCTGAGCCTG | 11 |
| eas-m0040-3p | CTGCCCACCTGTCACCTGCCCTG | 10 |

**Supplementary Table 19.** Enrichment analysis of target genes of novel miRNAs identified in the donkey genome.

| eas-m0001-5p | | |
|---|---|---|
| **Term description** | **Involved gene number** | **P_values** |
| Cell cycle | 19 | 0.003777517 |
| Axon guidance | 19 | 0.003777517 |
| Progesterone-mediated oocyte maturation | 15 | 0.003954445 |
| Phosphatidylinositol signaling system | 13 | 0.007166977 |
| Oocyte meiosis | 16 | 0.010888807 |
| Inositol phosphate metabolism | 10 | 0.019918559 |
| Fc epsilon RI signaling pathway | 12 | 0.022480045 |
| Pathways in cancer | 33 | 0.023978817 |
| Dilated cardiomyopathy | 12 | 0.029576939 |
| Regulation of actin cytoskeleton | 23 | 0.030818307 |
| Toll-like receptor signaling pathway | 13 | 0.036258183 |
| Pancreatic cancer | 11 | 0.0364988 |
| Small cell lung cancer | 12 | 0.038152009 |
| Hypertrophic cardiomyopathy (HCM) | 11 | 0.047178673 |
| Fc gamma R-mediated phagocytosis | 12 | 0.048329925 |
| Arrhythmogenic right ventricular cardiomyopathy (ARVC) | 10 | 0.063468053 |
| Colorectal cancer | 11 | 0.064435642 |
| MAPK signaling pathway | 27 | 0.065649692 |
| Insulin signaling pathway | 16 | 0.075517024 |
| Endometrial cancer | 8 | 0.076494069 |
| Focal adhesion | 21 | 0.079113204 |
| Wnt signaling pathway | 15 | 0.082256427 |
| Tight junction | 15 | 0.0867579 |
| Non-small cell lung cancer | 8 | 0.091031401 |
| Ubiquitin mediated proteolysis | 15 | 0.091412913 |
| Neurotrophin signaling pathway | 15 | 0.096221864 |
| **eas-m0016-5p** | | |
| **Term description** | **Involved gene number** | **P_values** |
| Long-term potentiation | 13 | 0.001378065 |
| Renal cell carcinoma | 12 | 0.003009046 |
| ErbB signaling pathway | 14 | 0.003287375 |
| GnRH signaling pathway | 14 | 0.00679383 |
| Progesterone-mediated oocyte maturation | 13 | 0.009853494 |
| Insulin signaling pathway | 17 | 0.015168116 |
| Vascular smooth muscle contraction | 14 | 0.017674888 |
| Ubiquitin mediated proteolysis | 16 | 0.01980017 |
| Gap junction | 12 | 0.02228724 |
| Adipocytokine signaling pathway | 10 | 0.025114651 |

| | | |
|---|---|---|
| Starch and sucrose metabolism | 7 | 0.026855112 |
| MAPK signaling pathway | 26 | 0.03094091 |
| Long-term depression | 10 | 0.033050373 |
| Calcium signaling pathway | 18 | 0.033397315 |
| Oocyte meiosis | 13 | 0.044055391 |
| Prostate cancer | 11 | 0.047093949 |
| mTOR signaling pathway | 8 | 0.049203698 |
| Phosphatidylinositol signaling system | 10 | 0.049860494 |
| Glioma | 9 | 0.052277234 |
| Apoptosis | 11 | 0.054250943 |
| Cell cycle | 14 | 0.058345612 |
| Pathways in cancer | 28 | 0.060085423 |
| Regulation of autophagy | 5 | 0.069761567 |
| Acute myeloid leukemia | 8 | 0.070011686 |
| Dilated cardiomyopathy | 10 | 0.071523731 |
| **eas-m0026-5p** | | |
| **Term description** | **Involved gene number** | **P_values** |
| Arrhythmogenic right ventricular cardiomyopathy (ARVC) | 22 | 0.001035209 |
| Lysine degradation | 15 | 0.001293655 |
| Viral myocarditis | 19 | 0.001974023 |
| ECM-receptor interaction | 22 | 0.011386531 |
| Purine metabolism | 34 | 0.013871071 |
| Dilated cardiomyopathy | 21 | 0.014841824 |
| Hypertrophic cardiomyopathy (HCM) | 20 | 0.01668494 |
| Cell cycle | 29 | 0.020138058 |
| TGF-beta signaling pathway | 20 | 0.02554295 |
| Adherens junction | 18 | 0.037288349 |
| Tight junction | 28 | 0.050349253 |
| Oocyte meiosis | 24 | 0.055208192 |
| ErbB signaling pathway | 20 | 0.059477187 |
| Spliceosome | 27 | 0.067245732 |
| Focal adhesion | 39 | 0.067366587 |
| Calcium signaling pathway | 33 | 0.076447739 |
| Regulation of actin cytoskeleton | 39 | 0.076963619 |
| Alanine,aspartate and glutamate metabolism | 9 | 0.087357931 |
| Axon guidance | 26 | 0.088707488 |
| Phosphatidylinositol signaling system | 17 | 0.095772967 |
| **eas-m0028-5p** | | |
| **Term description** | **Involved gene number** | **P_values** |
| Pathways in cancer | 81 | 0.00203275 |
| Hypertrophic cardiomyopathy (HCM) | 25 | 0.006917152 |
| Dilated cardiomyopathy | 26 | 0.007219962 |
| Viral myocarditis | 19 | 0.025235539 |

| | | |
|---|---|---|
| Oocyte meiosis | 30 | 0.034415761 |
| Arrhythmogenic right ventricular cardiomyopathy (ARVC) | 21 | 0.036532845 |
| Aminoacyl-tRNA biosynthesis | 14 | 0.045881186 |
| Insulin signaling pathway | 36 | 0.047876863 |
| Pancreatic cancer | 21 | 0.056727446 |
| Cell adhesion molecules (CAMs) | 33 | 0.057029293 |
| Lysine degradation | 13 | 0.065338637 |
| Tryptophan metabolism | 14 | 0.066480641 |
| Mismatch repair | 9 | 0.067457113 |
| Butanoate metabolism | 11 | 0.091084451 |
| Jak-STAT signaling pathway | 33 | 0.092000511 |
| **eas-m0035-5p** | | |
| **Term description** | **Involved gene number** | **P_values** |
| Calcium signaling pathway | 48 | 0.001628761 |
| ErbB signaling pathway | 26 | 0.017741458 |
| ABC transporters | 15 | 0.018817179 |
| Axon guidance | 34 | 0.02797493 |
| Purine metabolism | 39 | 0.031169087 |
| ECM-receptor interaction | 24 | 0.037540082 |
| Focal adhesion | 49 | 0.037973594 |
| Long-term potentiation | 20 | 0.059922542 |
| Insulin signaling pathway | 35 | 0.063812474 |
| Oocyte meiosis | 28 | 0.078933815 |
| Endocytosis | 46 | 0.080488547 |
| Dorso-ventral axis formation | 9 | 0.080982595 |
| Fatty acid metabolism | 12 | 0.086577966 |
| Aminoacyl-tRNA biosynthesis | 13 | 0.086640066 |
| Regulation of actin cytoskeleton | 47 | 0.090198103 |
| Valine,leucine and isoleucine biosynthesis | 5 | 0.092771473 |