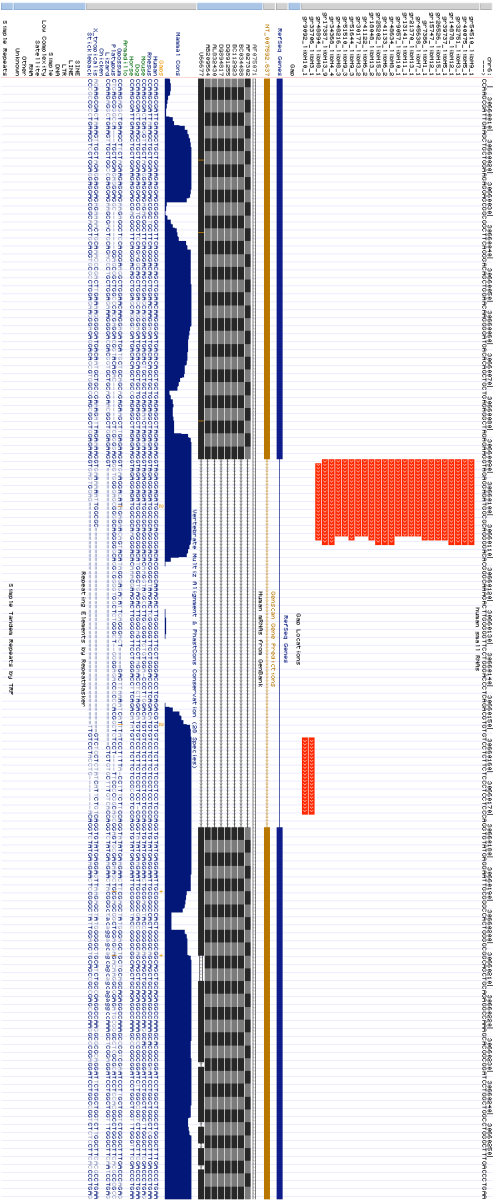


hsa-mir-877: human, cow, dog, elephant, mouse, rat, rhesus, tenrec

```

>Human chr6 30660087-30660172
GTAGAGGATGCGCAGGGGACACCGGCAAAAGACTTGGGGTTCCCTGGGACCCTCAGACGTTGTTCCCTCCCTCCAG
-35.80 ...(((((((.....((((((((((((((((((((((((((((((((((((((((((((((((((((((((
Rhesus chr4 30166021-30166106
GTAGAGGATGCGCAGGGGACACCGGCTAAGACTCCGGGGTTCCCTGGGACCCTCAGACATGTTGTTCCCTCCCTCCAG
-36.41 ...(((((((.....((((((((((((((((((((((((((((((((((((((((((((((((((((((((
Rat chr20 2958183-2958267
GTAGAGGATGCGCAGGGGACACCAAGTTAGCCCTTCCGGGTTGTGGCCCTTGACATGTTGTTCCCTCCCTCCAG
-35.00 ...(((((((.....((((((((((((((((((((((((((((((((((((((((((((((((((((((((
Mouse chr17 59608553-59608637
GTAGAGGATGCGCAGGGGACACAAGTTAGCCCTTCCGGGTTGTGGACCCTTGACATGTTGTTCCCTCCCTCCAG
-35.60 ...(((((((.....((((((((((((((((((((((((((((((((((((((((((((((((((((((((
Dog chr12 3408300-3408384
GTAGAGGATGCGCAGGGGACATGGGCTGAGACTTGGGAGTTCCTAGAACCTCTAGACATGTTGTTCCCTCCCTCCAG
-32.50 ...(((((((.....((((((((((((((((((((((((((((((((((((((((((((((((((((((((
Cow chr23 26513259-26513343
GTAGAGGATGCGCAGGGGACACCGGCTAAGACTGGGGCTCCCGGACCCCCCAACATGTTGTTCCCTCCCTCCAG
-38.30 ...(((((((.....((((((((((((((((((((((((((((((((((((((((((((((((((((((((
Elephant scaffold_82320 103-188
GTAGAGGATGCGCAGGGGACACAGGCTATGACATGGGGCTCCCTGGGGCCCTTGAAACATGTTGTTCCCTCCCTCCAG
-35.70 ...(((((((.....((((((((((((((((((((((((((((((((((((((((((((((((((((((((
Tenrec scaffold_315780 12230-12314
GTAGAGGATGCGCAGGGGACACCGGGGAGACTGGGCTGGGCTGCTCCCAACATGTTGTTCCCTCCCTCCAG
-36.20 ...(((((((.....((((((((((((((((((((((((((((((((((((((((((((((((((((((((

```



NM_025160.1: human, chimp, cow, dog, hedgehog, opossum, rhesus, shrew

```

>Human      chr1      222685906-222686005
GTAAGGTAGGGCATATAGAGCTGTGTGACGTGCTAAATAATTAACAGATTTGGAGTGTGACATTTTGTTCCTTTATACCAATTTTTCACG
-17.10      (((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((
chimp      chr1      20504905-205050004
GTAAGGTAGGGCATATAGAGCTGTGTGACGTGCTAAATAATTAACAGATTTGGAGTGTGACATTTTGTTCCTTTATACCAATTTTTCACG
-17.10      (((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((
rhesus     chr1      82202846-82202945
GTAAGGTAGGGCATATAGAGCTGTGTGACGTGCTAAATAATTAACAGATTTGGAGTGTGACATTTTGTTCCTTTATACCAATTTTTCACG
-16.20      (((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((
shrew      scaffold_236018 46395-46494
GTAAGGTAGGGCATATAGAGCTGTGTGACGTGCTAAATAATTAACAGATTTGGAGTGTGACATTTTGTTCCTTTATACCAATTTTTCACG
-14.80      (((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((
hedgehog  scaffold_31531 7725-7827
GTAAGGTAGGGCATATAGAGCTGTGTGACGTGCTAAATAATTAACAGATTTGGAGTGTGACATTTTGTTCCTTTATACCAATTTTTCACG
-25.10      (((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((
dog        chr7      41061071-41061170
GTAAGGTAGGGCATATAGAGCTGTGTGACGTGCTAAATAATTAACAGATTTGGAGTGTGACATTTTGTTCCTTTATACCAATTTTTCACG
-27.10      (((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((
cow        chr16     24511052-24511151
GTAAGGTAGGGCATATAGAGCTGTGTGACGTGCTAAATAATTAACAGATTTGGAGTGTGACATTTTGTTCCTTTATACCAATTTTTCACG
-15.90      (((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((
>Opossum   chr2      135883898-135884001
GTAAGGTAGGGCATATAGAGCTGTGTGACGTGCTAAATAATTAACAGATTTGGAGTGTGACATTTTGTTCCTTTATACCAATTTTTCACG
-16.72      (((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((

```

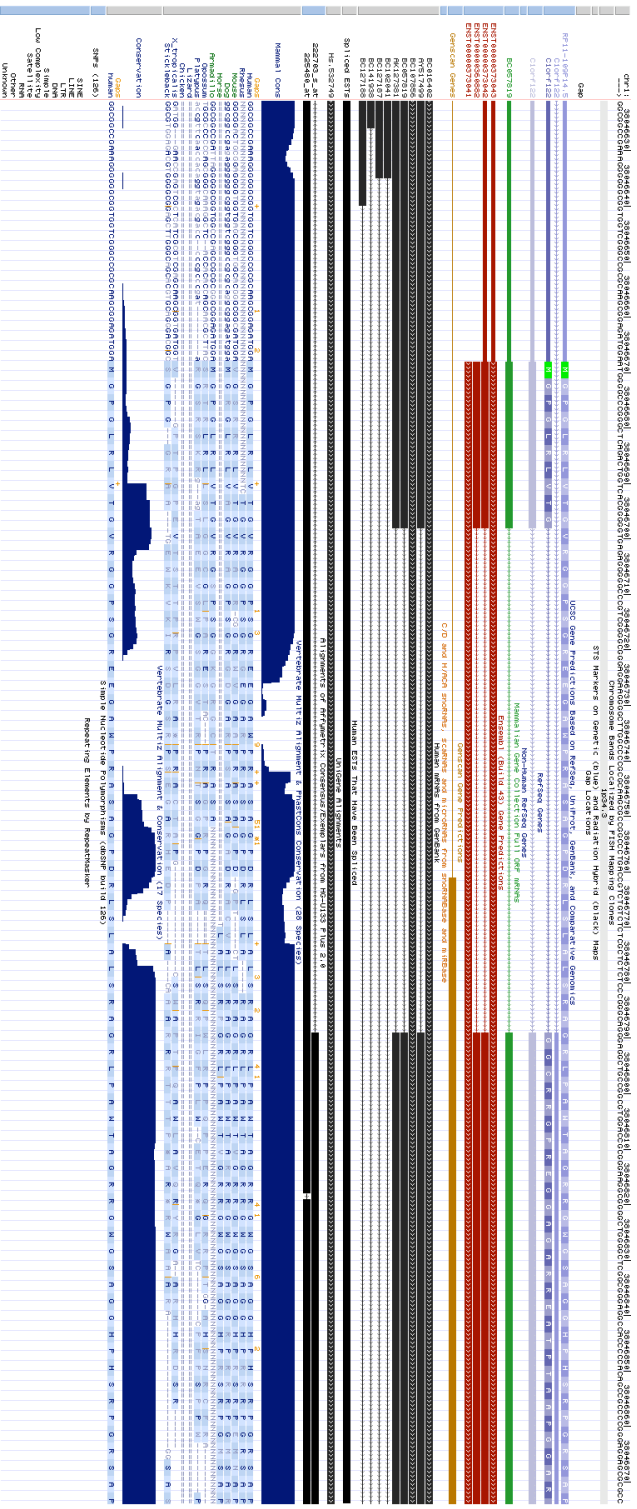
Genome browser interface showing a genomic track for NM_025160.1. The track includes various annotations such as RefSeq, Ensembl, and RepeatMasker. A large blue block indicates a region of high conservation or a specific feature. The bottom section shows a detailed alignment of the sequence with various species, including human, chimp, cow, dog, hedgehog, opossum, rhesus, and shrew. The alignment is presented as a grid of characters (A, C, G, T) with gaps represented by dashes. The track also displays coordinates and other genomic data.


```

NM_198446.0: human, chimp, cow, hedgehog, mouse, rat
>Human chr1 38046698-38046788
GTGAGAGGGGGCCGTCGGGGCGGAGAGAGGGGCTTTGGCCCCCGCAAGCCGCTGACCCGTCCTCCCGGGCAG
-45.90 .((( (((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((
>Chimp chr1 38385021-38385111
GTGAGAGGGGGCCGTCGGGGCGGAGAGGGGCTTTGGCCCCCGCAAGCCGCTGACCCGTCCTCCCGGGCAG
-45.50 .((( (((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((
>Rat chr1 28912879-28912965
GTGAGAGGGGGCCGTCGGGGCGGAGAGGGGCTTTGGCCCCCGCAAGCCGCTGACCCGTCCTCCCGGGCAG
-47.70 .((( (((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((
>Mouse chr1 30676876-30676963
GTGAGAGGGGGCCGTCGGGGCGGAGAGGGGCTTTGGCCCCCGCAAGCCGCTGACCCGTCCTCCCGGGCAG
-43.50 .((( (((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((
>Hedgehog scaffold_273501 14459-14549
gtgagagggggccgctcggggcgaggaggggctcgaactccggcgagggccgctgaccgctctcccccggcgag
-43.80 .((( (((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((
>Cow chr3 11512244-11512333
GTGAGAGGGGGCCGTCGGGGCGGAGAGGGGCTTTGACCCGCGCAAGCCGCTGACCCGTCCTCCCGGGCAG
-44.21 .((( (((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((((

```

Intron has coding potential, but has evidence of multiple spliced ESTs



NM_002912_7 Chimp Rhesus Rabbit Dog Horse

```
>Chimp      chr6      113298181-113298267
GTAAGTGGTAAATTAATTAATGATTCACAGACTAAATTTAAATTCCTTTGTTATCTGTTAATAAGGATACTTTAATTAAGCAG
-9.71      .....(((.....(((.....(((.....(((.....(((.....))).....))).....))).....))).....)))..... [ ]
>Rhesus    chr4      15103584-15103670
GTAAGTGGTAAATTAATTAATGATTCACAGACTAAATTTAAATTCCTTTGTTATCTGTTAATAAGGATACTTTAATTAAGCAG
-9.71      .....(((.....(((.....(((.....(((.....(((.....))).....))).....))).....))).....)))..... [ ]
>Rabbit    scaffold 2(73828-73912)
GTAAGTGGTAAATTAATTAATGATTCACAGAAAAAATTTAATTCGATTTGTTAATAAGGATACTTTAATTAAGCAG
-10.00     (((.....(((.....(((.....(((.....(((.....))).....))).....))).....))).....)))..... [ ]
>Dog       chr12     70913400-70913484
GTAAGTGGTAAATTAATTAATGATTCACAGACTAAATTTAATTCCTTTGTTATCTGTTAATAAGGATACTTTAATTAAGCAG
-13.50     .....(((.....(((.....(((.....(((.....(((.....))).....))).....))).....))).....)))..... [ ]
>Horse     chr10     55495795-55495881
GTAAGTGGTAAATTAATTAATGATTCACAGACTAAATTTAATTCCTTTGTTATCTGTTAATAAGGATACTTTAATTAAGCAG
-10.40     .....(((.....(((.....(((.....(((.....(((.....))).....))).....))).....))).....)))..... [ ]
```

Gene models and conservation tracks for the region 113298181-113298267 on chromosome 6 of the Chimp genome.

Gene Models:

- USC Gene Predictions:** Based on RefSeq, UniProt, GenBank, and Comparative Genomics. Includes *USC1* (NM_001001001.1) and *USC2* (NM_001001002.1).
- RefSeq Genes:** *USC1* (NM_001001001.1) and *USC2* (NM_001001002.1).
- Non-RefSeq Genes:** *USC1* (NM_001001001.1) and *USC2* (NM_001001002.1).
- Ensembl (Build 43) Gene Predictions:** *USC1* (ENSGM00000000000.1) and *USC2* (ENSGM00000000000.1).
- Genes and Regions from Ensembl and RefSeq:** *USC1* (NM_001001001.1) and *USC2* (NM_001001002.1).
- UniProt Alignments:** *USC1* (P00000) and *USC2* (P00000).
- NCBI Conserved Domains:** *USC1* (PF00000) and *USC2* (PF00000).
- Vertebrate Multiple Alignment:** Conservation scores for Chimp, Rhesus, Rabbit, and Dog.

Conservation: Conservation scores for Chimp, Rhesus, Rabbit, and Dog. The scores are shown as a bar chart and a line plot.

Repeat Elements: Repeating elements by RepeatMasker. The elements are shown as black bars on the sequence.

Sequence: The DNA sequence is shown in the bottom track, with the reference sequence (Chimp) in uppercase and the other species in lowercase.

Figure S3. Small RNA Libraries Used For Mirtron Discovery.

library	mapped reads	Description	reference
mouse_lib1	23703	brain	1
human_lib1	5476	brain	1
human_lib2	3202	skin	1
human_lib3	2177	heart	1
human_lib4	3265	lung	1
human_lib7	11939	umbilical cord blood-derived endothelial progenitor cells	1
human_lib8	3490	foreskin-derived microvascular endothelial cells	1
chimp_libPT0	153316	mix of brain regions	2
human_libH0	118275	fetal brain	2
human_libH1	24167	amygdale	3
human_libH2	18613	cerebellum	3
human_libH3	44954	cingulate gyrus	3
human_libH4	48351	superior frontal gyrus	3
human_libH5	22352	superior occipital gyrus	3
human_libH6	33855	medial temporalis gyrus	3
human_libH7	44521	Hippocampus	3
human_libH8	35007	hypothalamus	3
human_libH9	44337	locus coeruleus	3
human_libH10	21306	superior parietal gyrus	3
human_libH11	38095	substantia nigra	3
human_libH12	35052	spinal-cord	3
human_libH13	43885	thalamus (pulvinar)	3
human_libH14	21648	caudate nucleus	3
human_libH15	39199	putamen	3
macaque_lib1	25856	superior frontal gyrus	3
macaque_lib2	37528	medial temporalis gyrus	3
macaque_lib3	37922	superior occipital gyrus	3
macaque_lib4	44343	superior parietal gyrus	3
macaque_lib5	28875	hippocampus	3
macaque_lib6	39652	cerebellum	3
macaque_lib7	30530	cingulate gyrus	3
macaque_lib8	38037	substantia nigra	3
macaque_lib9	34601	caudate nucleus	3
macaque_lib10	27400	putamen	3
macaque_lib11	23220	locus coeruleus	3
macaque_lib12	41084	amygdale	3
macaque_lib13	26796	thalamus (pulvinar)	3
macaque_lib14	24731	spinal-cord	3
macaque_lib15	33140	hypothalamus	3
	1,333,900		

1. Berezikov et al. Genome Research 16, 1289-1298.
2. Berezikov et al. Nat Genet 38, 1375-1377.
3. Berezikov et al. Manuscript in preparation.

Figure S4. List of Officially Recognized Mammalian Mirtrons and the Extent of Their Cloning Evidence.

```

classI: miR/miR*cloned from multiple mammals and conserved across mammals

# mir-877
# arms: 2
# species: 4
# libraries: 32
# reads: 148
hsa ...(((((((.(.(.(.((((((((((.(.....((((((((((.....)))))))))).))))))))))....
hsa AGAAGGTAGAGGAGATGGCGCAGGGGACACGGGCAAGACTTGGGGTCTCTGGGACCTCAGACGTGTCTCTCTCCCTCCCTCCAGGTGTA
hsa >>>>+++++
hsa ....GTAGAGGAGATGGCGCAGGGAC..... 1 human_libH0
hsa ....GTAGAGGAGATGGCGCAGG..... 1 human_libH1
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH1
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH10
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH10
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH12
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH12
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_lib4
hsa ....GTAGAGGAGATGGCGCAGGGAC..... 8 human_libH13
hsa ....GTAGAGGAGATGGCGCAGGG..... 2 human_libH13
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH13
hsa ....GTAGAGGAGATGGCGCAGG..... 1 human_libH13
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH15
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH15
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH7
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH15
hsa ....GTAGAGGAGATGGCGCAGG..... 2 human_libH3
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH3
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH3
hsa ....GTAGAGGAGATGGCGCAGGG..... 2 human_libH4
hsa ....GTAGAGGAGATGGCGCAGGG..... 2 human_libH4
hsa ....GTAGAGGAGATGGCGCAGGGAC..... 1 human_libH4
hsa ....GTAGAGGAGATGGCGCAGGGAC..... 1 human_libH4
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH8
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH5
hsa ....GTAGAGGAGATGGCGCAGGG..... 2 human_libH6
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH6
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH7
hsa ....GTAGAGGAGATGGCGCAGG..... 2 human_libH8
hsa ....GTAGAGGAGATGGCGCAGGG..... 2 human_libH8
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH8
hsa ....GTAGAGGAGATGGCGCAGGG..... 3 human_libH0
hsa ....GTAGAGGAGATGGCGCAGG..... 3 human_libH9
hsa ....GTAGAGGAGATGGCGCAGGG..... 2 human_libH9
hsa ....GTAGAGGAGATGGCGCAGGG..... 1 human_libH9
ptr ...(((((((.(.(.(.((((((((((.(.....((((((((((.....)))))))))).))))))))))....
ptr AGAAGGTAGAGGAGATGGCGCAGGGACACGGGCAAGACTTGGGGTCTCTGGGACCTCAGACGTGTCTCTCTCCCTCCCTCCAGGTGTA
ptr >>>>+++++
ptr ....GTAGAGGAGATGGCGCAGG..... 1 chimp_libPT0
ptr ....GTAGAGGAGATGGCGCAGGG..... 1 chimp_libPT0
mm1 ...(((((((.(.(.(.((((((((((.(.....((((((((((.....)))))))))).))))))))))....
mm1 AGAAGGTAGAGGAGATGGCGCAGGGACACGGGCAAGACTTGGGGTCTCTGGGACCTCAGACGTGTCTCTCTCCCTCCCTCCAGGTGTA
mm1 >>>>+++++
mm1 ....GTAGAGGAGATGGCGCAGGG..... 1 macaque_lib10
mm1 ....GTAGAGGAGATGGCGCAGGG..... 1 macaque_lib10
mm1 ....GTAGAGGAGATGGCGCAGGG..... 3 macaque_lib11
mm1 ....GTAGAGGAGATGGCGCAGGG..... 2 macaque_lib1
mm1 ....GTAGAGGAGATGGCGCAGGG..... 1 macaque_lib11
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 1 macaque_lib11
mm1 ....GTAGAGGAGATGGCGCAGG..... 1 macaque_lib11
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 4 macaque_lib12
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 1 macaque_lib12
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 1 macaque_lib12
mm1 ....GTAGAGGAGATGGCGCAGGG..... 1 macaque_lib12
mm1 ....GTAGAGGAGATGGCGCAGG..... 1 macaque_lib1
mm1 ....GTAGAGGAGATGGCGCAGGG..... 1 macaque_lib13
mm1 ....GTAGAGGAGATGGCGCAGG..... 1 macaque_lib13
mm1 ....GTAGAGGAGATGGCGCAG..... 1 macaque_lib13
mm1 ....GTAGAGGAGATGGCGCAGGG..... 1 macaque_lib14
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 1 macaque_lib14
mm1 ....GTAGAGGAGATGGCGCAGGG..... 1 macaque_lib15
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 1 macaque_lib15
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 6 macaque_lib3
mm1 ....GTAGAGGAGATGGCGCAGGG..... 2 macaque_lib3
mm1 ....GTAGAGGAGATGGCGCAGG..... 2 macaque_lib3
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 1 macaque_lib3
mm1 ....GTAGAGGAGATGGCGCAGGG..... 9 macaque_lib4
mm1 ....GTAGAGGAGATGGCGCAGG..... 2 macaque_lib4
mm1 ....GTAGAGGAGATGGCGCAGGG..... 2 macaque_lib4
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 1 macaque_lib4
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 1 macaque_lib4
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 1 macaque_lib4
mm1 ....GTAGAGGAGATGGCGCAGGG..... 5 macaque_lib5
mm1 ....GTAGAGGAGATGGCGCAGGG..... 1 macaque_lib5
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 1 macaque_lib5
mm1 ....GTAGAGGAGATGGCGCAGGG..... 1 macaque_lib5
mm1 ....GTAGAGGAGATGGCGCAGG..... 1 macaque_lib5
mm1 ....GTAGAGGAGATGGCGCAGGG..... 3 macaque_lib6
mm1 ....GTAGAGGAGATGGCGCAGGG..... 3 macaque_lib6
mm1 ....AGGAGATGGCGCAGGGAC..... 1 macaque_lib6
mm1 ....GTAGAGGAGATGGCGCAGG..... 1 macaque_lib6
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 4 macaque_lib10
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 3 macaque_lib8
mm1 ....GTAGAGGAGATGGCGCAGGGAC..... 2 macaque_lib8
mm1 ....GTAGAGGAGATGGCGCAGGG..... 2 macaque_lib8
mmu ...(((((((.(.(.(.((((((((((.(.....((((((((((.....)))))))))).))))))))))....
mmu AAGAGGTAGAGGAGATGGCGCAGGGACACAGGTAGGCTTGGGGTCTGTGG-ACCTTGGACATGTCTCTCTCCCTCCCTCCAGGTGTA
mmu >>>>+++++
mmu ....GTAGAGGAGATGGCGCAGGG..... 3 mouse_lib1
hsa chromosome:6:30660083:30660178:1 ENST00000376575 ENSG00000204580 DDR1 Epithelial discoidin domain-containing receptor 1 precursor (EC 2.7.10.1) (Epithelial discoidin domain receptor 1) (Tyrosine kinase DDR) (Discoidin receptor tyrosine kinase) (Tyrosine-protein kinase CAK) (Cell adhesion kinase) (TRK E) (Protein-tyrosine ki [Source:UniProt/SWISSPROT;Acc:Q08345]
ptr chromosome:6:31202460:31202555:1 ENSPTR00000033105 ENSPTRG00000017930 ABCF1 ATP-binding cassette sub-family F member 1 (ATP-binding cassette 50) (TNF-alpha-stimulated ABC protein) [Source:UniProt/SWISSPROT;Acc:Q9N871]
mm1 chromosome:4:30166017:30166112:1 ENSMMU00000002661 ENSMUG00000001886 Q5TM60 MACMU ATP-binding cassette, sub-family F (GCN20), member 1. [Source:UniProt/SPTREMBL;Acc:Q5TM60]
mmu chromosome:17:35568778:35568872:-1 ENSMUST000000087205 ENSMUG000000038762 Abcf1 ATP-binding cassette, sub-family F (GCN20), member 1 [Source:MarkerSymbol;Acc:MGI:1351658]

```


Figure S7. Correlation between Number of Mirtron Small RNA Reads and Number of Human ESTs.

mirtron	host gene	host transcript	location	total reads	ESTs in the region	ESTs in the entire gene
hsa-mir-877	ENSG00000204580	ENST00000376575	chromosome:6:30660083:30660178:1	59	55	13804
hsa-mir-1224	ENSG00000145198	ENST00000273794	chromosome:3:185441882:185441976:1	58	9	45
hsa-mir-1225	ENSG00000008710	ENST00000382481	chromosome:16:2080192:2080291:1	3	45	742
hsa-mir-1226	ENSG00000132153	ENST00000348968	chromosome:3:47866044:47866128:1	143	92	568
hsa-mir-1227	ENSG00000104886	ENST00000326631	chromosome:19:2185056:2185153:1	5	111	263
hsa-mir-1228	ENSG00000123384	ENST00000338962	chromosome:12:55874549:55874631:1	6	7	951
hsa-mir-1229	ENSG00000161013	ENST00000376937	chromosome:5:179157879:179157957:1	16	298	1287
hsa-mir-1231	ENSG00000134369	ENST00000367302	chromosome:1:200044357:200044458:1	3	15	618
hsa-mir-1233	ENSG00000175676	ENST00000328919	chromosome:15:20996204:20996295:1	3	55	128
hsa-mir-1234	ENSG00000071894	ENST00000349769	chromosome:8:145596279:145596372:1	3	35	482
hsa-mir-1236	ENSG00000204356	ENST00000375429	chromosome:6:32032590:32032701:1	2	165	316
hsa-mir-1237	ENSG00000162302	ENST00000334205	chromosome:11:63892645:63892756:1	2	23	206
hsa-mir-1238	ENSG00000130734	ENST00000309469	chromosome:19:10523793:10523885:1	2	38	222
human_block172399	ENSG00000107331	ENST00000371605	chromosome:9:139030140:139030239:1	3	24	493
human_block107544	ENSG00000088247	ENST00000201886	chromosome:19:6366308:6366395:1	2	71	602
human_block96092	ENSG00000141258	ENST00000268989	chromosome:17:2222294:2222391:1	2	11	389
human_block168305	ENSG00000179950	ENST00000349157	chromosome:8:144971936:144972024:1	1	202	861
human_block108536	ENSG00000105655	ENST00000338128	chromosome:19:18408053:18408144:1	1	83	595
human_block2497	ENSG00000121753	ENST00000373658	chromosome:1:31974574:31974765:1	1	18	175
human_block145951	ENSG00000078269	ENST00000367122	chromosome:6:158405766:158405850:1	1	6	300
human_block100511	ENSG00000108840	ENST00000336057	chromosome:17:39511555:39511693:1	1	109	366
human_block168304	ENSG00000179950	ENST00000349157	chromosome:8:144970973:144971072:1	1	142	861
human_block127094	ENSG00000198355	ENST00000360612	chromosome:22:48742513:48742596:1	1	70	346
human_block94727	ENSG00000102904	ENST00000388833	chromosome:16:66417476:66417560:1	1	7	56
human_block88730	ENSG00000184916	ENST00000347004	chromosome:14:104688155:104688251:1	1	5	95
human_block713	ENSG00000171608	ENST00000377346	chromosome:1:9703302:9703391:1	1	11	229
human_block168233	ENSG00000104518	ENST00000262580	chromosome:8:144715659:144715765:1	1	75	429
human_block110912	ENSG00000130726	ENST00000341753	chromosome:19:63753459:63753560:1	1	359	1532
human_block29395	ENSG00000173442	ENST00000309295	chromosome:11:65148409:65148522:1	1	25	1146
human_block110710	ENSG00000125503	ENST00000376393	chromosome:19:60295720:60295806:1	1	33	284
human_block185010	ENSG00000198753	ENST00000361971	chromosome:X:152693451:152693533:1	1	16	117
human_block109083	ENSG00000089351	ENST00000318014	chromosome:19:40192935:40193032:1	1	64	379
human_block91836	ENSG00000008710	ENST00000382481	chromosome:16:2083736:2083817:1	1	18	742
human_block152130	ENSG00000197150	ENST00000377904	chromosome:7:150378969:150379063:1	1	5	492
human_block91683	ENSG00000185615	ENST00000219406	chromosome:16:275012:275089:1	1	17	59

Fig. S8. Minimum Free Energies (MFEs) of 5 nt Terminal Duplexes of Mirtronic miRNAs.

```
-----
>hsa-mir-877
GUAGAGGAGAUGGGCGAGGGGACACGGGCAAAGACUUGGGGUUCCUGGACCUCACAGCUGUGUCCUCUUCUCCUCCUCCAG
...(((((((...((...(((.....(((.....)))))))).).)))))).... (-39.50)
>left
GUAGA&UCCAG
...((&)).... ( 1.20)
>right
GGGAC&GUCCU
((((&))) ( -5.40)
```

Dominant arm: 5'
Cloning: consistent

```
-----
>hsa-mir-1224
GUGAGGACUCGGGAGGUGGAGGGUGGCCGCCGGGGCGGCGUUCUUCAGCUCGCUUCUCCCCCACCUCUCUCUCCUCAG
.(((((((...(((.....(((.....)))))))).).)))))).... (-45.00)
>left
GUGAG&CUCAG
.((((&))). ( -3.00)
>right
GAGGG&CCCCC
(.((&)).) ( -2.20)
```

Dominant arm: 3'
Difference between arms is small.
Cloning: 3' arm is more abundant in chimp,
in human and macaque - 5' arm is slightly more abundant.

```
-----
>hsa-mir-1225
GUGGGUACGGCCAGUGGGGGGAGAGGGACAGCCUGGGCUCUGCCAGGGUGCAGCCGGACUGACUGAGCCCCUGGCCGCCCCAG
.(((((((...(((.....(((.....)))))))).).)))))).... (-48.20)
>left
GUGGG&CCCAG
.((((&))). ( -5.10)
>right1
GGGG&ACUGAGCC
((((&...)) ( 0.60)
>right2
GG&UGAGC
((&...)) ( 8.30)
```

Dominant arm: 3'
Cloning: more 5' reads but difference in clone numbers is small

```
-----
>hsa-mir-1226
GUGAGGGCAUGCAGGCCUGGAUGGGGACGUGGGAUGGUCCAAAAGGGUGGCCUCACCAGCCUUGUUCUCCUAG
...(((((((...(((.....(((.....)))))))).).)))))).... (-36.90)
>left
GUGAG&CUAG
...((&)).. ( 1.40)
>right1
UGGG&CCUCA
((((&))) ( -4.40)
>right2
GGAUGG&UCACC
(.((&))) ( -0.70)
```

Dominant arm: 5'
Cloning: inconsistent, mostly 3' reads


```
>hsa-mir-1227
GUGGGGCCAGGCGGUGGUGGGGCACUGCUGGGGUGGGGCACAGCAGCCAUGCAGAGCGGGCAUUUGACCCCGUGCCACCCUUUUCCCCAG
.(((((((((...((((((((((((((...((((((...))))))...))))))...))))))...))))). (-36.70)
>left
GUGGG&CCCAG
.((((&))))). (-5.10)
>right1
GGUGG&CCCGUGCC
(((((&)...))) (-1.30)
>right2
GU&CGUGC
((&...)) ( 1.90)
```

Dominant arm: 3'
Cloning: consistent

```
>hsa-mir-1228
GUGGGCGGGGGCAGGUGUGUGGUGGGUGGUGGCCUGCGGUGAGCAGGGCCUCACACCUGCCUGCCCCCAG
..(((((((...((((((((((((((...((((((...))))))...))))))...))))))...))))). (-46.00)
>left
GUGGG&CCCCCAG
..((((&)))....) (-3.30)
>right1
GUGUG&CAC
((..&)) (-0.80)
>right2
UGUGU&UCACA
((((&.))) (-2.50)
```

Dominant arm: 3'
Cloning: consistent

```
>mml-mir-1230
GUGGGUGGGGGCAUCUCGAGGAGGUGGGGGGUGUGGCGCCAGCGGAUGACUCCGAGCGGCUCUUUCCCCAG
.(((((((...((((((((((((((...((((((...))))))...))))))...))))))...))))). (-38.40)
>left
GUGGG&CCCAG
.((((&))))). (-5.10)
>right
CGGAG&CUCCG
(((((&)))) (-6.10)
```

Dominant arm: 5'
Cloning: consistent

Figure S9. MiRbase 10.0 Drosophila, human and mouse mature miRNAs annotated with a 5' G nucleotide.

dme-miR-1017	GAAGCTCTACCCAAACTCATCC						
dme-miR-263a	GTTAATGGCACTGGAAGAATTCAC						
dme-miR-306*	GGGGTCACTCTGTGCCTGTGC						
dme-miR-309	GCACCTGGGTAAGTTTCTCCTA						
hsa-miR-138-2*	GCTATTCTGACACACAGGAGTT	hsa-miR-138-1*	GCTACTTCAACAACCAGGGGCC				
hsa-miR-139-3p	GCAGACGGCCCTCTTGAAGT						
hsa-miR-143*	GGTGCAGTCTGCATCTCTGGT						
hsa-miR-144*	GGATATCATATACATGTAAG						
hsa-miR-145	GTCCAGTTTTCCAGGAATCCCT	hsa-miR-145*	GGATTCCTGGAAACTACTGTTCT	mmu-miR-145	GTCCAGTTTTCCAGGAATCCCT		
hsa-miR-147b	GTGTGCGGAAATGCTCTGCCTA	hsa-miR-147	GTGTGTGGAAATGCTCTGCG	mmu-miR-147	GTGTGCGGAAATGCTCTGCCTA		
hsa-miR-183*	GTGAATTACCGAAGGCCATAA	mmu-miR-183*	GTGAATTACCGAAGGCCATAA				
hsa-miR-186*	GCCTCAAGGTGAATTTTTGGG	mmu-miR-186*	GCCTCAAGGTGAATTTTTGGG				
hsa-miR-187*	GGCTACAACACAGGACCCGGGC						
hsa-miR-191*	GCTGCGCTTGGATTTCTGCC	mmu-miR-191*	GCTGCACTTGGATTTCTGCC				
hsa-miR-198	GGTCCAGAGGGGAGATAGTTTC						
hsa-miR-203	GTGAATGTTTAGACCACTAG	mmu-miR-203	GTGAATGTTTAGACCACTAG				
hsa-miR-23a*	GGGGTCTCTGGGATGGGATTT						
hsa-miR-296-3p	GAGGGTTGGGTGAGGCCTCTCC	mmu-miR-296-3p	GAGGGTTGGGTGAGGCCTCTCC				
hsa-miR-296-1*	GCTGGTTTCATATGGTGTTAGA	mmu-miR-296*	GCTGGTTTCATATGGTGTTAGA				
hsa-miR-330-3p	GCAAAAGCACAGGCGCTGCAGAGA	mmu-miR-330*	GCAAAAGCACAGGCGCTGCAGAGA				
hsa-miR-331-3p	GCCCTGGGCGCTATCTAGAA	mmu-miR-331-3p	GCCCTGGGCGCTATCTAGAA				
hsa-miR-337-5p	GAAGCGCTTCATACAGGAGTT	mmu-miR-337-5p	GAAGCGCTTCATACAGGAGTT				
hsa-miR-33b	GTGCATTGCTGTTGCATTGC	hsa-miR-33a	GTGCATTGCTGTTGCATTGCA	mmu-miR-33	GTGCATTGCTGTTGCATTGCA		
hsa-miR-345	GCTGACTCTAGTCCAGGGCTC	mmu-miR-345-5p	GCTGACTCTAGTCCAGGGCTC				
hsa-miR-370	GCCTGCTGGGTGGAACCTGGT	mmu-miR-370	GCCTGCTGGGTGGAACCTGGT				
hsa-miR-373	GAAGTGCCTCGATTTGGGGGT						
hsa-miR-376a*	GTAGATTCTCTCTATGAGTA	mmu-miR-376b*	GTGGATATCTCTATGTTTA	mmu-miR-376e*	GTGGATATCTCTCTATGTTTA	mmu-miR-376a*	GGTAGATTCTCTCTATGAGT
hsa-miR-382	GAAGTGTTCGTGGTGGATTCG	mmu-miR-382	GAAGTGTTCGTGGTGGATTCG				
hsa-miR-409-3p	GAAGTGTTCGTGGTGGATTCG	mmu-miR-409-3p	GAAGTGTTCGTGGTGGATTCG				
hsa-miR-455-3p	GCAGTCCATGGCGATATACAC	mmu-miR-455	GCAGTCCACGGGCATATACAC				
hsa-miR-485-3p	GTACATACAGGCTCTCTCTCT						
hsa-miR-489	GTGACATCACATACAGGCGAGC						
hsa-miR-505*	GGGAGCCAGGAAGTATGATGT						
hsa-miR-511	GTGCTTTTGTCTGCACTCA						
hsa-miR-515-3p	GAGTGCCTCTTTGGAGCGTT						
hsa-miR-518f	GAAGCGCTTCTCTTAGAGG	hsa-miR-518a-3p	GAAGCGCTTCTCTTAGAGG				
hsa-miR-523	GAACGCGCTTCCCTATAGAGGT						
hsa-miR-524-3p	GAAGCGCTTCCCTTAGAGG						
hsa-miR-525-3p	GAAGCGCTTCCCTTAGAGG						
hsa-miR-528b*	GAAGTGTTCCTTTAGAGGC						
hsa-miR-539	GGAGAAATATCCTGTGGTGT	mmu-miR-539	GGAGAAATATCCTGTGGTGT				
hsa-miR-551a	GCAGCCACTCTGGTTTCCA	hsa-miR-551b	GCAGCCACTCTGGTTTCCAG	hsa-miR-551b*	GAATCAAGCGTGGGTGAGACC	mmu-miR-551b	GCGACCATACTGGTTTCCAG
hsa-miR-554	GCTAGTCTGACTCCAGCATG						
hsa-miR-556-5p	GATGAGCTATGTAATATGAG						
hsa-miR-557	GTTTGACAGGTTGGGCTTGTCT						
hsa-miR-560	GCCTGCGCGCGCGCGCGCC						
hsa-miR-565	GGCTGCGCTCGGATGCTGTTT						
hsa-miR-566	GGCGCCTGTGATCCAC						
hsa-miR-572	GTCCGCTCGCGGTGGCCCA						
hsa-miR-575	GAGCCAGTTGGACAGGAGC						
hsa-miR-590-5p	GAGCTTATCATAAAGTGACG	mmu-miR-590-5p	GAGCTTATCATAAAGTGACG				
hsa-miR-595	GAAGTGTCCGCTGGTGTGCT						
hsa-miR-599	GTGTGTGCTTATCAAC						
hsa-miR-602	GACACGGGCGACAGCTCGGCC						
hsa-miR-607	GTCAAAATCCAGATCTATAAC						
hsa-miR-611	GCAGGACCCCTCGGCTCTGAC						
hsa-miR-612	GCTGGGACGGCTCTGAGCTCCTT						
hsa-miR-614	GAACCGCTGTTCTTCCAGGTTGG						
hsa-miR-615-5p	GGGGGTCCCCGGTCTCGGATC	mmu-miR-615-5p	GGGGGTCCCCGGTCTCGGATC				
hsa-miR-619	GACCTGGACATGTTTGCCCGAGT						
hsa-miR-621	GGCTAGCAACAGCGCTTACCT						
hsa-miR-625*	GACTATGAACTTTCCCGCTCA						
hsa-miR-627	GTGAGTCTTAAGAAAGAGGGA						
hsa-miR-629*	GTTCTCCAGATTAACCCAGC						
hsa-miR-632	GTGTCTGCTCTCTGGGA						
hsa-miR-642	GTCCCTCTCAAATGTTCTTG						
hsa-miR-647	GTGGCTGCACTCACTTCTTC						
hsa-miR-653	GTGTTGAAACAATCTCACTG	mmu-miR-653	GTGTTGAAACAATCTCACTG				
hsa-miR-657	GGCAGGTTCTCAACCTCTTAGG						
hsa-miR-658	GGCGGAGGGAAGTAGTCCGTTGGT						
hsa-miR-674	GCACCTGAGATGGGAGTGGTGA	mmu-miR-674	GCACCTGAGATGGGAGTGGTGA				
hsa-miR-768-5p	GTGTGGAGTGAAGTATGATGAT						
hsa-miR-801	GATTGCTCTGCTGCGGAATCGAC	mmu-miR-801	GATTGCTGCTGCGGAATCGAC				
hsa-miR-873	GCAGGAACCTGTGAGTCTCCT	mmu-miR-873	GCAGGAACCTGTGAGTCTCCT				
hsa-miR-877	GTAGAGGATGGCCAGAGG	mmu-miR-877	GTAGAGGATGGCCAGAGG				
hsa-miR-887	GTGAACGGGCGCCATCCCGAGG						
hsa-miR-888*	GACTGACACCTCTTTGGTGAA						
hsa-miR-920	GGGAGCTGTGGAAGCAGTA						
hsa-miR-922	GCAGCAGAGAAATAGACTACGTC						
hsa-miR-923	GTCAAGCGAGGAAAAGAACT						
hsa-miR-929-2*	GGTGGGATTTGTTGCATTAC						
mmu-miR-133a*	GCTGGTAAATGGAACCAAT						
mmu-miR-146b*	GCCCTAGGACTAGTCTGGT						
mmu-miR-207	GCTTCTCTGCTCTCTCCCTC						
mmu-miR-24-2*	GTGCCACTGAGCTGAAACAGT	mmu-miR-24-1*	GTGCCACTGAGCTGATATCAGT				
mmu-miR-291b-5p	GATCAAAGTGGAGGCCCTCTCC						
mmu-miR-298	GGCAGAGGAGGGCTGTTCTTCCC						
mmu-miR-302c*	GCTTTAAGATGGGTACCTGCG						
mmu-miR-374*	GSTTGATATGATGTTCCGAG						
mmu-miR-434-5p	GCTGAGCTATGGTTGAACCA						
mmu-miR-465a-3p	GATCAGGCGCTTCTAAGTAGA						
mmu-miR-466b-5p	GATGTGTGTACATGTACATG	mmu-miR-466e-5p	GATGTGTGTGTACATGTACATA	mmu-miR-466e-5p	GATGTGTGTGTACATGTACATA		
mmu-miR-467b	GTAAGTGCCTGCATATATG						
mmu-miR-503*	GAGTATTGTTCCACTGCCTGG						
mmu-miR-666-3p	GGCTGACGCTGATCGCTGCT						
mmu-miR-678	GTCTGCTGCAAGGACTGAGG						
mmu-miR-679	GCAGCTGTGAGTACTCTTCTG						
mmu-miR-680	GGCATCTGCTGACATGGGG						
mmu-miR-693-3p	GCAGCTTTCAGATGGCTGTA						
mmu-miR-696	GCTGTGCTTGTCTGGG						
mmu-miR-705	GGTGGGAGTGGGGGGGCA						
mmu-miR-709	GGAGGCAGAGGCAGAGGA						
mmu-miR-711	GGGACCGGGGAGAGTAAAG						
mmu-miR-742	GAAAGCCACATGCTGGGTAAA						
mmu-miR-743a	GAAAGACACCAAGCTGAGTAGA	mmu-miR-743b-3p	GAAAGACATCATGCTGAATAGA				
mmu-miR-759	GCAGAGTGAACAATTTGAC						
mmu-miR-761	GCAGCAGGTTGAACTGACACA						
mmu-miR-762	GGGGTGGGGCCGGACAGAGC						
mmu-miR-764-5p	GGTGCCTACATGCTCTCT						
mmu-miR-805	GAATGATCAGGACATAGGG						
mmu-miR-878-3p	GCATGACACCACACTGGGTAGA						
mmu-miR-879*	GCTTATGGCTTCAAGCTTTCGG						

