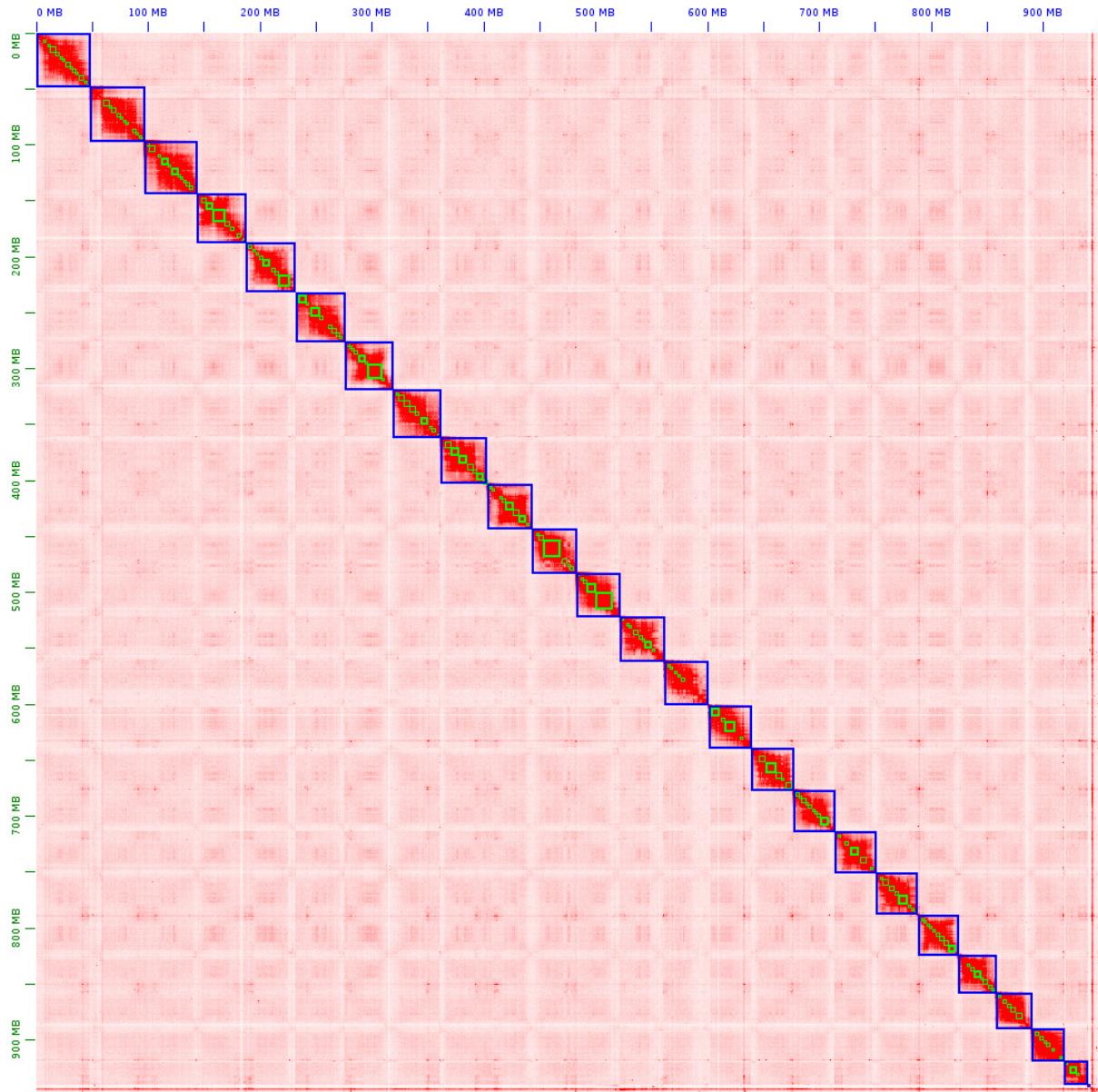


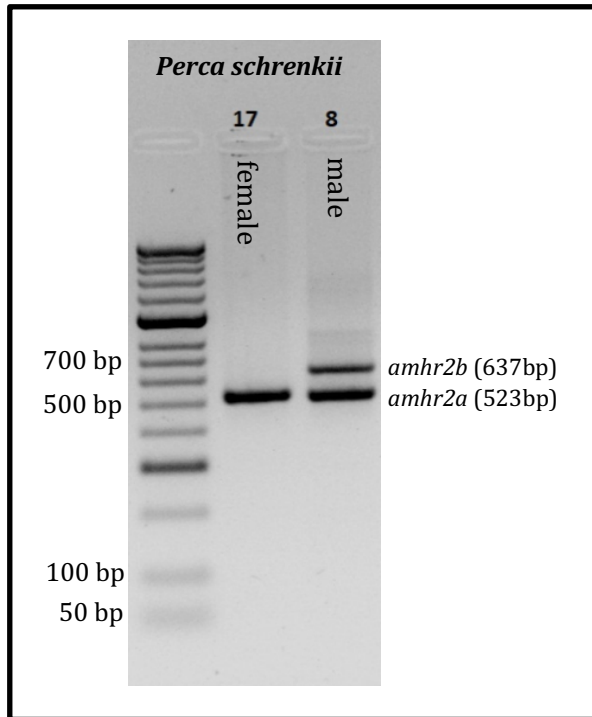
598 **Supplementary figures**



599

600 **Supplementary Figure 1: Hi-C map of *P. fluvialis* genome.**

601

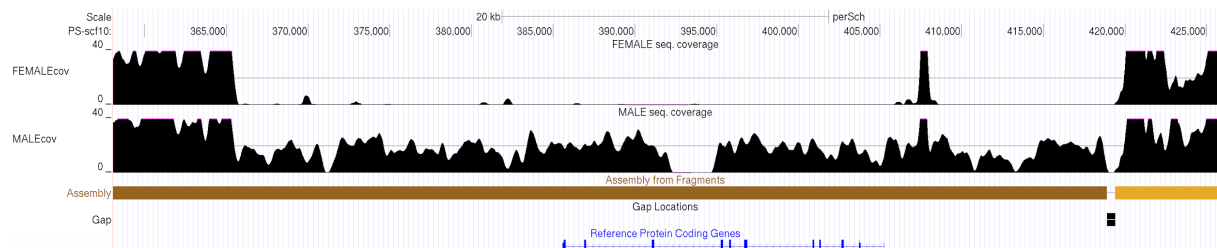


602

603 **Supplementary Figure 2:** A sex-specific 637 bp PCR product amplifies in *P. schrenkii* male (sample
604 id = 8), while it is absent in female (sample id = 17). The corresponding primer pair also works for sexing
605 of *P. flavescens*.

606

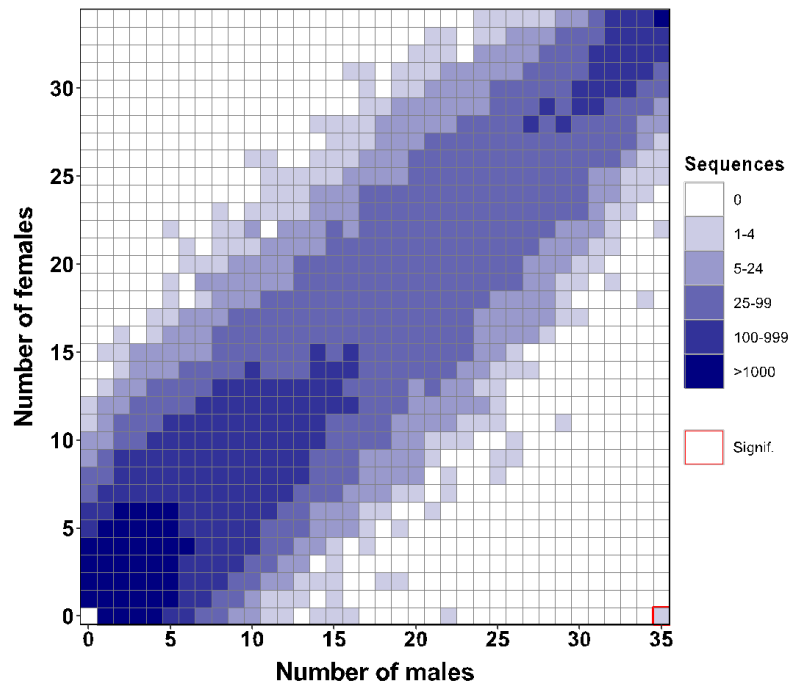
607



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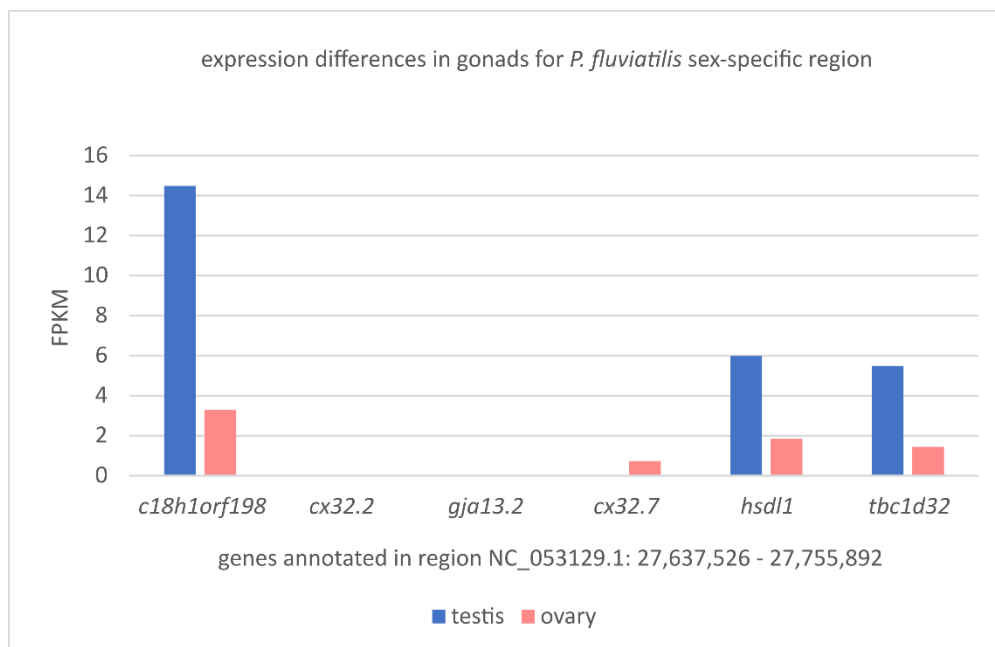
609 **Supplementary Figure 3:** Sex-specific sequencing coverage of *amhr2b* locus in *Perca schrenkii* in region
610 PS-scf10 / CM046795.1: 366,025-418,970. A female and a male genome were sequenced to
611 approximately 40x coverage using short-read sequencing. After filtering for unique mapping reads
612 (mapping quality 60), a clear coverage difference between females and males is visible. The ~53 kbp
613 region has virtually no coverage in females. In contrast males exhibit haploid coverage (about 20x),
614 which is in accordance with a X/Y SD system.

615



616

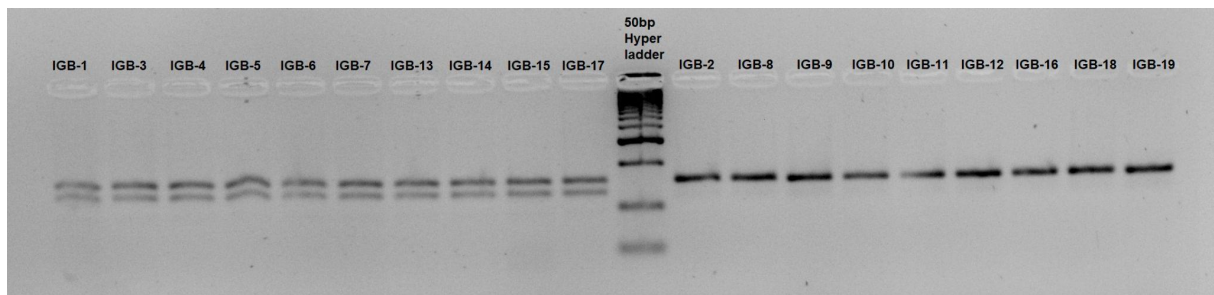
617 **Supplementary Figure 4: A single RAD sex-specific marker is significantly associated with male sex in**
 618 ***P. fluviatilis*.** Tile plot of the distribution of RADSex markers between *Perca fluviatilis* males (horizontal
 619 axis) and females (vertical axis) with a minimum read depth of 1 ($d = 1$). Color intensity (see color
 620 legend on the right) indicates the number of markers present for each of the corresponding number
 621 of males and females. A single significant marker at the lower right of the grid was present in all 35
 622 males and absent from all 34 females and is boxed with a red border (Chi-squared test, $p < .05$ after
 623 Bonferroni correction).



624

625 **Supplementary Figure 5: Expression of *hsd1* and neighboring genes in public RNAseq datasets (testis:**
 626 **SRR14461526, ovary: SRR14461527; age of both sampled individuals 9 month).** Here *hsd1* expression
 627 in testis is 3.25-fold higher than in ovary, for *tbc1d32* and *c18h1orf198* the ratio is 3.83 and 4.41,
 628 respectively.

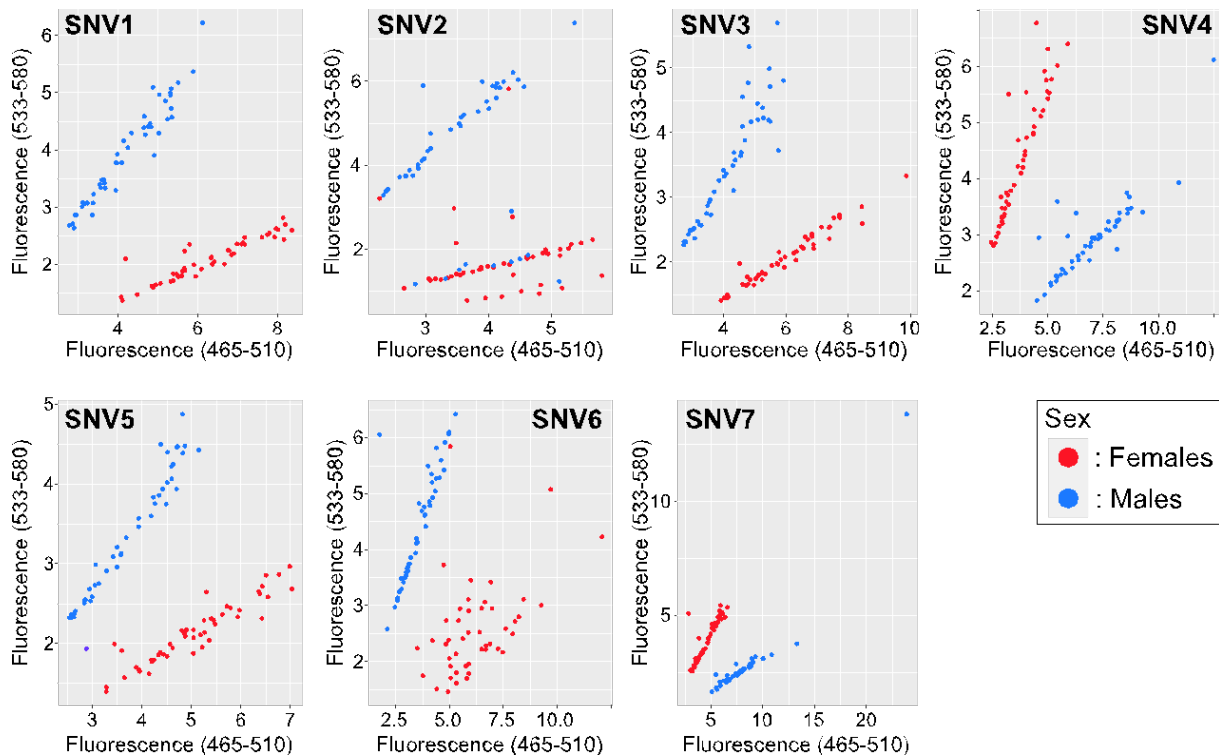
629



630

631 **Supplementary Figure 6: Sexing of *P. fluviatilis* using a 27 bp male specific deletion in Intron 3 of the *hsdI1* gene**
632 (10 males (left) and 9 females (right) from Lake Mueggelsee, Berlin. The simultaneous amplification in both males
633 and females of the X allele without the 27 bp deletion provides an internal control for this PCR. All XY male
634 samples (N = 10) produce two amplicons due to the small size difference of the X and Y amplified alleles, and all
635 XX females (N = 9) produced only the larger X amplicon. This *hsdI1* intronic indel variant is located near the variant
636 SNV5 (distance < 1.5 kbp).

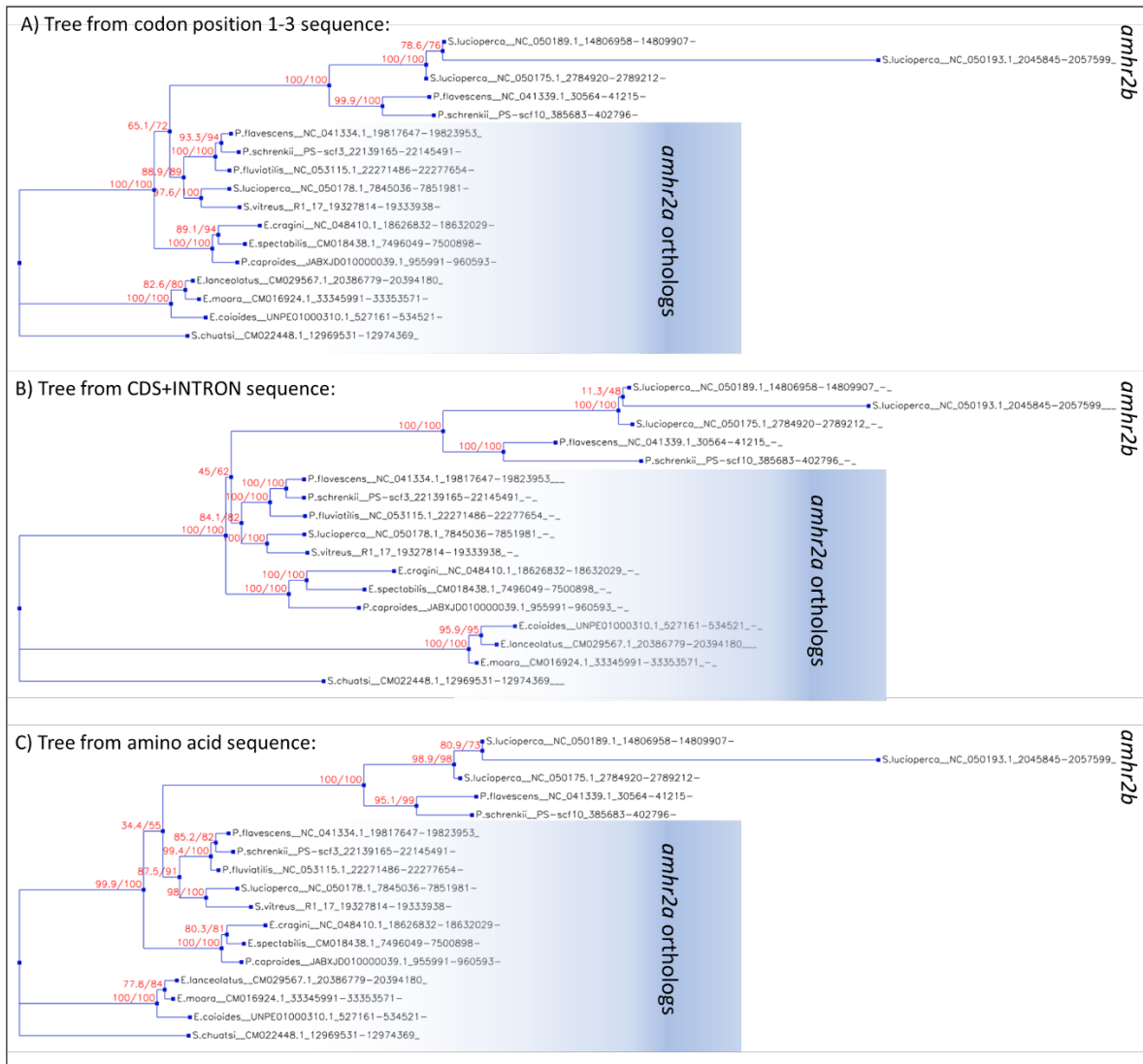
637



638

639 **Supplementary Figure 7: KASpar allele-specific PCR assays on seven single sex-specific nucleotide**
640 **variations (SNV ID#) in *P. fluviatilis*.** For each Single Nucleotide Variation (SNV), primer AL1 was
641 coupled to FAM fluorescent dye and primer AL2 was coupled to VIC fluorescent dye and the end-point
642 fluorescence of these two fluorescent dyes was respectively on the x- and y- axes. Male individuals are
643 represented by blue dots and females by red dots. Primers used for analysis can be found in Table 1.

644



645

646 **Supplementary Figure 8:** Additional gene trees for *amhr2*. A) Tree calculated from coding sequence.
 647 B) Tree calculated from coding plus intron sequence. C) Tree calculated from amino acid sequence.
 648 All trees share the same topology but differ in support values for some splits (SH-aLRT and UFBS
 649 tests).

676 **Supplementary Table 1:** Annotated repeats in *Perca sp.* and *Sander sp.* genomes (RepeatModeler *de*
677 *novo* analysis). Repeat elements mentioned in the manuscript have grey highlighting. The class “DNA”
678 is assigned to repeat elements that harbor signals of transposases, but miss further signals to classify
679 them with more detail.

	<i>P. fluviatilis</i>	<i>P. flavescens</i>	<i>P. schrenkii</i>	<i>S. lucioperca</i>	<i>S. vitreus</i>
length (no gaps)	951.053.269	877.041.836	893.440.234	901.028.039	785.350.625
Repeat class	bp masked	bp masked	bp masked	bp masked	bp masked
DNA	22.711.613	20.520.179	23.132.743	19.957.261	15.428.015
Academ	2.340.071	1.619.886	1.842.836	2.412.073	1.790.843
CMC-Chapaev-3	230.562	0	0	0	0
CMC-EnSpm	9.118.071	7.588.794	4.039.674	5.928.087	3.520.268
Crypton	841.031	547.307	748.707	772.498	402.911
Crypton-V	11.212	0	0	0	0
Dada	0	33.030	206.119	0	0
Ginger	0	15.288	0	0	0
IS3EU	1.282.033	531.773	496.067	288.665	509.614
Kolobok-Hydra	0	648.047	0	0	70.720
Kolobok-T2	1.252.506	1.976.511	2.668.034	1.586.132	1.222.046
MULE-MuDR	251.959	1.716.319	441.114	266.625	27.741
Maverick	2.187.699	1.402.756	266.487	1.342.717	0
Merlin	0	0	0	122.531	94.494
Novosib	0	0	0	0	82.199
P	467.516	442.149	411.688	765.209	555.234
PIF-Harbinger	12.989.790	12.205.497	12.181.221	12.830.055	8.037.073
PIF-ISL2EU	0	235.589	464.262	1.597.340	914.106
PiggyBac	3.844.887	2.011.288	2.742.549	3.776.961	2.299.194
Sola	1.885.532	229.560	163.435	77.871	0
TcMar	17.070	135.873	0	185.840	137.413
TcMar-Fot1	0	30.498	0	44.754	37.051
TcMar-ISRm11	4.570.634	3.171.866	2.722.899	2.158.133	1.830.923
TcMar-Stowaway	220.092	98.992	114.404	65.697	0
TcMar-Tc1	15.259.371	12.137.252	12.302.578	6.557.230	10.225.920
TcMar-Tc2	627.258	465.068	299.455	227.802	468.716
Zator	0	119.597	0	0	0
Zisupton	30.281	0	127.852	0	104.202

Zisupton-hAT-hybrid	0	0	0	0	339.346
hAT	9.251.059	7.244.672	9.113.922	8.460.295	6.356.798
hAT-Ac	26.161.464	27.815.352	29.153.525	30.578.355	27.361.032
hAT-Blackjack	1.252.051	459.611	338.327	514.383	437.930
hAT-Charlie	7.578.170	5.645.728	8.825.006	5.557.301	3.893.733
hAT-Tip100	3.892.297	2.083.169	5.060.148	2.183.415	2.136.454
hAT-Tol2	7.383.933	7.130.470	10.507.431	6.788.875	4.948.115
hAT-hAT5	1.008.628	1.141.196	1.066.203	873.439	560.575
hAT-hAT6	153.938	129.767	0	0	37.338
hAT-hobo	693.242	589.992	435.263	245.056	840.680
LINE	253.345	0	0	135.963	162.701
CR1	61.557	52.996	46.593	68.770	58.461
Dong-R4	0	161.938	0	0	0
I	902.480	1.009.950	1.721.862	451.473	448.921
I-Nimb	187.474	66.650	179.364	0	89.737
Jockey	1.281.097	0	0	249.376	0
L1	3.161.932	2.720.443	3.146.061	3.530.190	3.456.430
L1-Tx1	1.332.071	707.047	492.758	1.328.940	247.067
L2	24.295.832	18.778.236	18.931.729	15.671.956	10.981.470
Penelope	696.540	651.596	168.974	978.870	436.687
Proto2	432.031	406.555	335.607	288.595	210.869
R1	0	0	0	146.213	277.081
R2-Hero	258.797	37.021	152.400	101.778	204.860
R2-NeSL	139.492	0	191.055	106.574	62.871
RTE	1.012.896	0	0	0	0
RTE-BovB	3.915.100	11.058.368	4.053.280	5.607.772	8.522.404
RTE-RTE	0	0	0	169.573	0
RTE-RTEX	0	26.699	19.406	32.367	0
RTE-X	738.450	801.862	1.308.994	1.325.349	418.476
Rex-Babar	8.447.394	7.183.710	11.161.891	7.936.953	5.965.113
Tad1	0	0	0	138.056	320.275
LTR	569.316	146.042	61.769	0	0
Copia	180.685	275.175	422.246	471.447	0

DIRS	766.684	743.991	1.555.092	1.105.144	825.655
ERV1	750.875	1.123.315	980.225	1.469.763	126.774
ERVK	659.689	486.486	0	513.490	3.133.108
Gypsy	2.510.567	2.056.070	3.675.723	2.213.161	1.672.149
Ngaro	980.001	3.390.987	850.690	768.211	292.619
Pao	419.975	385.357	723.976	1.151.953	307.558
RC	0	0	0	0	0
Helitron	6.307.248	3.514.054	4.649.952	5.159.806	2.433.280
Retroposon	213.419	116.255	421.490	238.385	0
SINE	1.210.545	1.637.348	1.401.793	1.524.037	936.353
5S-Deu-L2	721.151	965.222	838.622	0	873.243
ID	0	0	19.012	29.728	116.970
MIR	1.185.268	1.020.008	1.163.355	2.103.257	382.739
tRNA	3.640.638	3.701.722	3.698.003	3.271.022	3.693.238
tRNA-Core	0	0	0	0	293.857
tRNA-Core-L2	0	0	0	89.805	106.872
tRNA-Deu-L2	0	0	0	42.616	0
tRNA-L2	461.844	0	103.308	223.271	301.117
tRNA-V	0	0	492.471	0	0
Unknown	149.784.304	121.893.498	134.020.433	144.621.637	113.225.894
total interspersed	354.992.667	305.241.677	326.860.083	319.430.101	255.255.533
Low_complexity	2.964.797	2.285.494	2.321.578	2.478.451	2.097.985
Satellite	700.110	1.425.777	1.023.206	656.699	1.718.620
Simple_repeat	30.391.090	32.172.324	29.527.186	34.124.020	27.469.062
rRNA	358.199	351.617	313.179	42.963	708.978
snRNA	0	0	0	9.265	0
Total	389.406.863	341.476.889	360.045.232	356.741.499	287.250.178

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