Supplementary Material for

Increased alignment sensitivity improves the usage of genome alignments for comparative gene annotation

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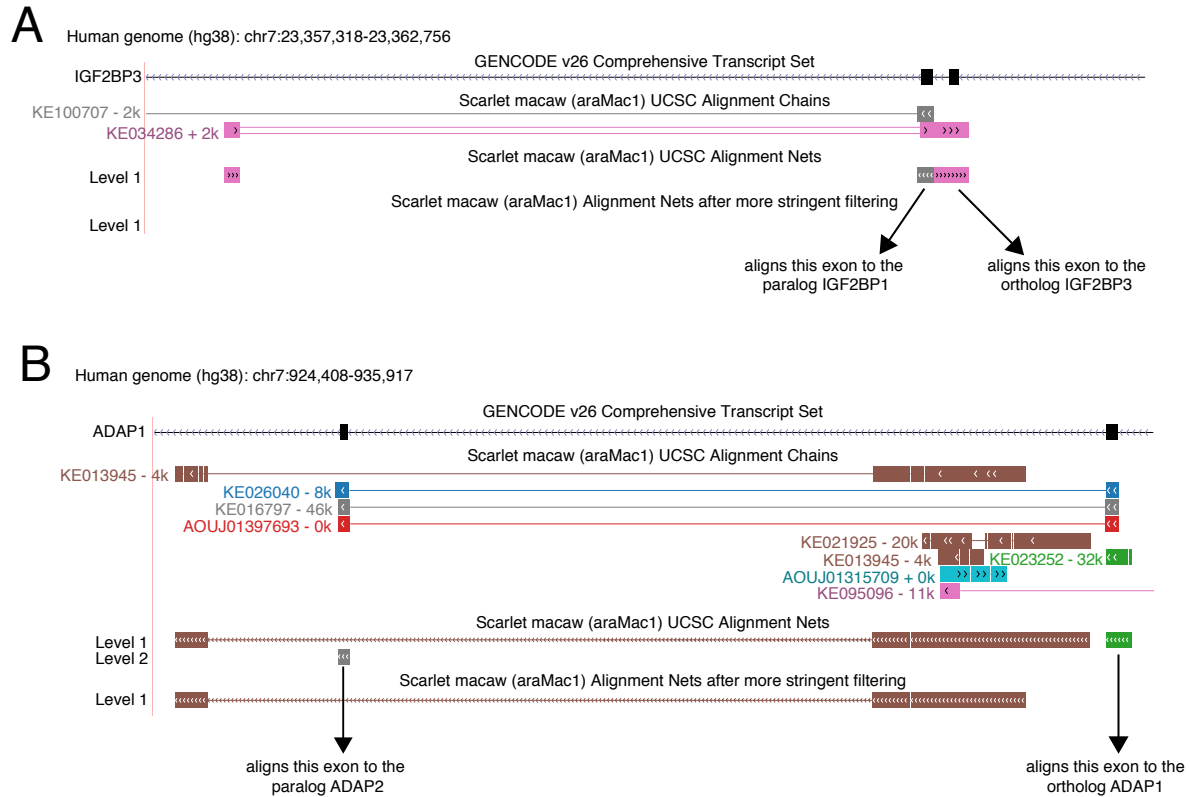Tel: +49 351 210 2781
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Supplementary Figure 1: Scarlet macaw alignments that are discarded by our more stringent filters contain paralogous exon alignments that would lead to incorrect gene annotations.

The examples in (A) and (B) illustrate why our more stringent filtering approach results in fewer annotated exons and genes for fragmented genome assemblies (Figure 5, main text) that result in short co-linear alignment nets. The panels show UCSC genome browser screenshots of the human genome and the co-linear alignment chains and nets to the scarlet macaw. Each box in an alignment chain or net represents aligning sequence.

(A) Our more stringent net filter thresholds remove alignments to two exons of *IGF2BP3*, contributing to the lower number of annotated exons in the scarlet macaw. However, the grey alignment likely aligns an exon of the paralog *IGF2BP1*, as revealed by aligning the respective scarlet macaw sequence to the well annotated chicken genome. Thus, removing this net increases the specificity in the gene annotation by avoiding a mis-annotation. The pink alignment of the second exon likely aligns the orthologous gene, illustrating that these fragmented assemblies result in short chains and nets that make it hard to distinguish orthologous and paralogous alignments.

(B) Another example illustrating short alignment chains and nets in the scarlet macaw. The green net that spans only a single exon is likely an alignment to the ortholog of *ADAP1*. However, the grey alignment aligns the upstream exon to the paralog *ADAP2*.

Assembly	Species	Scientific name	Net type	Alignment computed	% of human (hg38) genome in aligning blocks containing this species	Notes
hg38	Human	Homo sapiens	Reference species	-	-	
panTro4	Chimp	Pan troglodytes	syntenic	no (download from UCSC)	87.053% (2793773646 bp)	
panPan1	Bonobo	Pan paniscus	syntenic	no (download from UCSC)	86.857% (2787476067 bp)	not in UCSC 100-way but in the UCSC primate alignment
gorGor3	Gorilla	Gorilla gorilla gorilla	syntenic	no (download from UCSC)	81.109% (2603032702 bp)	
ponAbe2	Orangutan	Pongo pygmaeus abelii	syntenic	no (download from UCSC)	81.790% (2624886526 bp)	
nomLeu3	Gibbon	Nomascus leucogenys	syntenic	no (download from UCSC)	80.404% (2580390048 bp)	
rheMac3	Rhesus	Macaca mulatta	syntenic	no (download from UCSC)	75.857% (2434452580 bp)	
macFas5	Crab-eating macaque	Macaca fascicularis	syntenic	no (download from UCSC)	80.148% (2572180197 bp)	
papAnu2	Baboon	Papio anubis	syntenic	no (download from UCSC)	78.855% (2530680298 bp)	
chlSab2	Green monkey	Chlorocebus sabaeus	syntenic	no (download from UCSC)	79.547% (2552877571 bp)	
nasLar1	Proboscis monkey	Nasalis larvatus	syntenic	no (download from UCSC)	59.926% (1923212200 bp)	not in UCSC 100-way but in the UCSC primate alignment
rhiRox1	Golden snub-nosed monkey	Rhinopithecus roxellana	syntenic	no (download from UCSC)	79.578% (2553881926 bp)	not in UCSC 100-way but in the UCSC primate alignment
calJac3	Marmoset	Callithrix jacchus	syntenic	no (download from UCSC)	67.510% (2166594286 bp)	
saiBol1	Squirrel monkey	Saimiri boliviensis	syntenic	no (download from UCSC)	66.644% (2138792419 bp)	not in UCSC 100-way but in the UCSC primate alignment
tarSyr2	Tarsier	Tarsius syrichta	syntenic	no (download from UCSC)	53.523% (1717714322 bp)	
otoGar3	Bushbaby	Otolemur garnettii	syntenic	no (download from UCSC)	51.911% (1665976796 bp)	
micMur2	Mouse lemur	Microcebus murinus	syntenic	yes	54.845% (1760139027 bp)	assembly updated (micMur1 is in UCSC primate alignment)
tupChi1	Chinese tree shrew	Tupaia chinensis	syntenic	no (download from UCSC)	44.085% (1414807345 bp)	
speTri2	Squirrel	Spermophilus tridecemlineatus	syntenic	yes	48.520% (1557148621 bp)	
jacJac1	Lesser Egyptian jerboa	Jaculus jaculus	syntenic	yes	34.884% (1119528205 bp)	
micOch1	Prairie vole	Microtus ochrogaster	syntenic	yes	32.532% (1044048968 bp)	
perManBai1	Prairie deer mouse	Peromyscus maniculatus bairdii	syntenic	yes	36.198% (1161713393 bp)	species added
criGri1	Chinese hamster	Cricetulus griseus	syntenic	yes	34.265% (1099649614 bp)	
mesAur1	Golden hamster	Mesocricetus auratus	syntenic	yes	31.228% (1002208949 bp)	
mm10	Mouse	Mus musculus	syntenic	yes	32.896% (1055730834 bp)	
rn6	Rat	Rattus norvegicus	syntenic	yes	32.261% (1035338911 bp)	
nanGal1	Blind mole rat	Nannospalax galili	syntenic	yes	39.773% (1276420898 bp)	species added
hetGla2	Naked mole-rat	Heterocephalus glaber	syntenic	yes	46.886% (1504708927 bp)	
cavPor3	Guinea pig	Cavia porcellus	syntenic	yes	43.503% (1396146209 bp)	
chiLan1	Chinchilla	Chinchilla lanigera	syntenic	yes	46.489% (1491948983 bp)	
octDeg1	Brush-tailed rat	Octodon degus	syntenic	yes	41.127% (1319878019 bp)	
oryCun2	Rabbit	Oryctolagus cuniculus	syntenic	yes	43.445% (1394260530 bp)	
ochPri3	Pika	Ochotona princeps	syntenic	yes	34.458% (1105866449 bp)	
susScr3	Pig	Sus scrofa	syntenic	yes	43.977% (1411351436 bp)	
vicPac2	Alpaca	Vicugna pacos	syntenic	yes	47.915% (1537730338 bp)	
camFer1	Bactrian camel	Camelus ferus	syntenic	yes	48.351% (1551735184 bp)	
turTru2	Dolphin	Tursiops truncatus	min score 100k	yes	48.411% (1553639543 bp)	
orcOrc1	Killer whale	Orcinus orca	syntenic	yes	49.937% (1602606513 bp)	
phyCat1	Sperm whale	Physeter catodon	min score 100k	yes	50.800% (1630320179 bp)	species added
balAcu1	Minke whale	Balaenoptera acutorostrata	syntenic	yes	50.799% (1630272577 bp)	species added
panHod1	Tibetan antelope	Pantholops hodgsonii	syntenic	yes	45.582% (1462842254 bp)	
bosTau8	Cow	Bos taurus	syntenic	yes	45.834% (1470932026 bp)	
bisBis1	Bison	Bison bison bison	syntenic	yes	44.359% (1423605716 bp)	species added
oviAri3	Sheep	Ovis aries	syntenic	yes	45.517% (1460762110 bp)	
capHir1	Domestic goat	Capra hircus	syntenic	yes	45.000% (1444182511 bp)	
equCab2	Horse	Equus caballus	syntenic	yes	54.276% (1741862725 bp)	
cerSim1	Rhinoceros	Ceratotherium simum	syntenic	yes	55.225% (1772316407 bp)	
felCat8	Cat	Felis catus	syntenic	yes	51.061% (1638680977 bp)	
canFam3	Dog	Canis lupus familiaris	syntenic	yes	50.326% (1615105515 bp)	
musFur1	Ferret	Mustela putorius furo	syntenic	yes	49.359% (1584081766 bp)	
ailMel1	Panda	Ailuropoda melanoleuca	syntenic	yes	51.345% (1647822381 bp)	
ursMar1	Polar bear	Ursus maritimus	syntenic	yes	51.593% (1655756012 bp)	species added
odoRosDiv1	Pacific walrus	Odobenus rosmarus divergens	syntenic	yes	51.621% (1656658543 bp)	
lepWed1	Weddell seal	Leptonychotes weddellii	min score 100k	yes	50.883% (1632970808 bp)	
manPen1	Chinese pangolin	Manis pentadactyla	min score 100k	yes	44.856% (1439551201 bp)	species added
pteAle1	Black flying-fox	Pteropus alecto	syntenic	yes	47.629% (1528548624 bp)	
pteVam1	Megabat	Pteropus vampyrus	min score 100k	yes	42.944% (1378203172 bp)	
eptFus1	Big brown bat	Eptesicus fuscus	syntenic	yes	38.604% (1238910861 bp)	
myoDav1	Davids myotis bat	Myotis davidii	syntenic	yes	37.842% (1214472498 bp)	
myoLuc2	Microbat	Myotis lucifugus	syntenic	yes	37.902% (1217018404 bp)	
eriEur2	Hedgehog	Erinaceus europaeus	syntenic	yes	25.506% (818548418 bp)	
sorAra2	Shrew	Sorex araneus	syntenic	yes	27.284% (875607470 bp)	
conCri1	Star-nosed mole	Condylura cristata	syntenic	yes	36.794% (1180817074 bp)	
loxAfr3	Elephant	Loxodonta africana	syntenic	yes	44.849% (1439316697 bp)	
eleEdw1	Cape elephant shrew	Elephantulus edwardii	syntenic	yes	27.037% (867708181 bp)	
triMan1	Manatee	Trichechus manatus latirostris	syntenic	yes	44.578% (1430630850 bp)	
chrAsi1	Cape golden mole	Chrysochloris asiatica	syntenic	yes	33.343% (1070066279 bp)	
echTel2	Tenrec	Echinops telfairi	syntenic	yes	29.651% (951570829 bp)	
oryAfe1	Aardvark	Orycteropus afer afer	syntenic	yes	40.029% (1284635867 bp)	
dasNov3	Armadillo	Dasypus novemcinctus	syntenic	yes	45.058% (1446055144 bp)	
monDom5	Opossum	Monodelphis domestica	min score 10k	yes	16.439% (527575746 bp)	
sarHar1	Tasmanian devil	Sarcophilus harrisii	min score 10k	yes	13.527% (434129438 bp)	
macEug2	Wallaby	Macropus eugenii	min score 10k	yes	9.786% (314070130 bp)	
ornAna2	Platypus	Ornithorhynchus anatinus	min score 10k	yes	7.610% (244238785 bp)	assembly updated

Supplementary Table 1 (part 1): All mammals included in the 144-vertebrate alignment.

Assembly	Species	Scientific name	Net type	Alignment computed	% of human (hg38) genome in aligning blocks containing this species	Notes
zonAlb1	White-throated sparrow	Zonotrichia albicollis	min score 10k	yes	4.886% (156805456 bp)	
geoFor1	Medium ground finch	Geospiza fortis	min score 10k	yes	4.862% (156033541 bp)	
serCan1	Canary	Serinus canaria	min score 10k	yes	5.188% (166499082 bp)	species added
taeGut2	Zebra finch	Taeniopygia guttata	min score 10k	yes	6.621% (212475807 bp)	
ficAlb2	Collared flycatcher	Ficedula albicollis	min score 10k	yes	5.350% (171700154 bp)	
pseHum1	Tibetan ground jay	Pseudopodoces humilis	min score 10k	yes	5.494% (176307062 bp)	
corCor1	Hooded crow	Corvus cornix cornix	min score 10k	yes	4.878% (156535011 bp)	species added
corBra1	American crow	Corvus brachyrhynchos	min score 10k	yes	4.936% (158418176 bp)	species added
manVit1	Golden-collared manakin	Manacus vitellinus	min score 10k	yes	4.835% (155178743 bp)	species added
melUnd1	Budgerigar	Melopsittacus undulatus	min score 10k	yes	4.955% (159020493 bp)	
amaVit1	Parrot	Amazona vittata	min score 10k	yes	3.852% (123631020 bp)	
araMac1	Scarlet macaw	Ara macao	min score 10k	yes	3.409% (109406414 bp)	
falPer1	Peregrine falcon	Falco peregrinus	min score 10k	yes	5.165% (165758579 bp)	
falChe1	Saker falcon	Falco cherrug	min score 10k	yes	5.121% (164335455 bp)	
picPub1	Downy woodpecker	Picoides pubescens	min score 10k	yes	4.380% (140552870 bp)	species added
halLeu1	Bald eagle	Haliaeetus leucocephalus	min score 10k	yes	5.548% (178055539 bp)	species added
aquChr2	Golden eagle	Aquila chrysaetos canadensis	min score 10k	yes	5.646% (181199086 bp)	species added
egrGar1	Little egret	Egretta garzetta	min score 10k	yes	4.892% (157004660 bp)	species added
nipNip1	Crested ibis	Nipponia nippon	min score 10k	yes	5.320% (170743201 bp)	species added
pygAde1	Adelie penguin	Pygoscelis adeliae	min score 10k	yes	4.881% (156644132 bp)	species added
aptFor1	Emperor penguin	Aptenodytes forsteri	min score 10k	yes	5.251% (168523562 bp)	species added
chaVoc2	Killdeer	Charadrius vociferus	min score 10k	yes	4.954% (158985295 bp)	species added
opiHoa1	Hoatzin	Opisthocomus hoazin	min score 10k	yes	4.589% (147272093 bp)	species added
calAnn1	Anna's hummingbird	Calypte anna	min score 10k	yes	4.794% (153857566 bp)	species added
cucCan1	Common cuckoo	Cuculus canorus	min score 10k	yes	4.934% (158337416 bp)	species added
colLiv1	Rock pigeon	Columba livia	min score 10k	yes	4.956% (159075555 bp)	
galGal4	Chicken	Gallus gallus	min score 10k	yes	5.308% (170335631 bp)	
melGal1	Turkey	Meleagris gallopavo	min score 10k	yes	5.675% (182131850 bp)	
anaPla1	Mallard duck	Anas platyrhynchos	min score 10k	yes	4.551% (146045350 bp)	
tinGut2	White throated tinamou	Tinamus guttatus	min score 10k	yes	4.413% (141615668 bp)	species added
strCam1	Ostrich	Struthio camelus australis	min score 10k	yes	4.929% (158185647 bp)	species added
allMis1	American alligator	Alligator mississippiensis	min score 10k	yes	6.788% (217838578 bp)	
gavGan0	Gharial	Gavialis gangeticus	min score 10k	yes	6.814% (218675397 bp)	species added
cheMyd1	Green seaturtle	Chelonia mydas	min score 10k	yes	6.978% (223955076 bp)	
chrPic2	Painted turtle	Chrysemys picta bellii	min score 10k	yes	7.363% (236301642 bp)	
pelSin1	Chinese softshell turtle	Pelodiscus sinensis	min score 10k	yes	5.867% (188280315 bp)	
apaSpi1	Spiny softshell turtle	Apalone spinifera	min score 10k	yes	4.275% (137184865 bp)	
anoCar2	Lizard	Anolis carolinensis	min score 10k	yes	4.015% (128844558 bp)	
pytBiv1	Burmese python	Python bivittatus	min score 10k	yes	4.079% (130897768 bp)	species added
HLtupMer3	Tupinambis	Tupinambis merianae	min score 10k	yes	4.365% (140100325 bp)	species added
xenTro7	X. tropicalis	Xenopus tropicalis	min score 10k	yes	3.586% (115088552 bp)	
nanPar1	Tibetan frog	Nanorana parkeri	min score 10k	yes	2.542% (81588078 bp)	species added
latCha1	Coelacanth	Latimeria chalumnae	min score 10k	yes	2.746% (88116320 bp)	
oreNil2	Nile tilapia	Oreochromis niloticus	min score 10k	yes	1.686% (54123951 bp)	
neoBri1	Princess of Burundi	Neolamprologus brichardi	min score 10k	yes	1.581% (50742798 bp)	
hapBur1	Burton's mouthbreeder	Haplochromis burtoni	min score 10k	yes	1.598% (51290353 bp)	
mayZeb1	Zebra mbuna	Maylandia zebra	min score 10k	yes	1.625% (52156056 bp)	
punNye1	Pundamilia nyererei	Pundamilia nyererei	min score 10k	yes	1.605% (51494905 bp)	
stePar1	Bicolor damselfish	Stegastes partitus	min score 10k	yes	1.586% (50913521 bp)	species added
oryLat2	Medaka	Oryzias latipes	min score 10k	yes	1.727% (55412849 bp)	
xipMac1	Southern platyfish	Xiphophorus maculatus	min score 10k	yes	1.692% (54285951 bp)	
poeFor1	Amazon molly	Poecilia formosa	min score 10k	yes	1.696% (54436979 bp)	species added
poeRet1	Guppy	Poecilia reticulata	min score 10k	yes	1.670% (53610154 bp)	species added
cypVar1	Sheepshead minnow	Cyprinodon variegatus	min score 10k	yes	1.750% (56170064 bp)	species added
HLnotFur1	Turquoise killifish	Nothobranchius furzeri	min score 10k	yes	1.788% (57377141 bp)	species added
gasAcu1	Stickleback	Gasterosteus aculeatus	min score 10k	yes	1.750% (56160799 bp)	
notCor1	Black rockcod	Notothenia coriiceps	min score 10k	yes	1.481% (47531241 bp)	species added
dicLab1	European sea bass	Dicentrarchus labrax	min score 10k	yes	1.681% (53952519 bp)	species added
tetNig2	Tetraodon	Tetraodon nigroviridis	min score 10k	yes	1.526% (48980179 bp)	
fr3	Fugu	Takifugu rubripes	min score 10k	yes	1.545% (49590257 bp)	
takFla1	Yellowbelly pufferfish	Takifugu flavidus	min score 10k	yes	1.332% (42753135 bp)	
cynSem1	Tongue sole	Cynoglossus semilaevis	min score 10k	yes	1.564% (50179432 bp)	species added
gadMor1	Atlantic cod	Gadus morhua	min score 10k	yes	1.297% (41631253 bp)	
esoLuc1	Northern pike	Esox lucius	min score 10k	yes	1.793% (57537814 bp)	species added
danRer10	Zebrafish	Danio rerio	min score 10k	yes	3.312% (106303948 bp)	
astMex1	Astyanax mexicanus	Mexican tetra	min score 10k	yes	1.882% (60394303 bp)	
lepOcu1	Spotted gar	Lepisosteus oculatus	min score 10k	yes	2.505% (80391398 bp)	
calMil1	Elephant shark	Callorhinchus milii	min score 10k	yes	2.619% (84038668 bp)	species added
petMar2	Lamprey	Petromyzon marinus	min score 10k	yes	1.032% (33115344 bp)	
letCam1	Arctic lamprey	Lethenteron camtschaticum	min score 10k	yes	1.198% (38460097 bp)	species added

Supplementary Table 1 (part 2): All non-mammalian genomes included in our 144-vertebrate alignment.

The table lists all species, alignment net filtering thresholds, for which species we computed alignments, percentage and absolute number of bases in the human genome in aligning blocks that include this species, and which species we added compared to the UCSC hg38 100-way alignment.

runtime in CPU hours

Assembly	Species name	lastz	chaining	highly sensitive alignments
micMur2	Mouse_lemur	134.3	8	-
speTri2	Squirrel	998.7	14.9	-
jacJac1	Lesser_Egyptian_jerboa	1038.1	25.4	-
micOch1	Prairie_vole	1134.4	70.8	-
perManBai1	Prairie_deer_mouse	1183.5	65.3	-
criGri1	Chinese_hamster	1269.7	123.3	-
mesAur1	Golden_hamster	1049.1	51.1	-
mm10	Mouse	1001.2	8.9	-
rn6	Rat	1180.5	127.5	-
nanGal1	Blind_mole_rat	896.8	12.6	-
hetGla2	Naked_mole_rat	1068.8	15.9	-
cavPor3	Guinea_pig	1412.2	29.8	-
chiLan1	Chinchilla	1033	15.1	-
octDeg1	Brush_tailed_rat	1256.1	19.4	-
oryCun2	Rabbit	927	15.2	-
ochPri3	Pika	860.4	17.7	-
susScr3	Pig	952.8	13.9	-
vicPac2	Alpaca	849.7	13.8	-
camFer1	Bactrian_camel	870.5	22.7	-
turTru2	Dolphin	843.9	7.8	-
orcOrc1	Killer_whale	1011.7	99.2	-
phyCat1	Sperm_whale	908.1	20.1	-
balAcu1	Minke_whale	926.1	30.8	-
panHod1	Tibetan_antelope	1075.4	32	-
bosTau8	Cow	1028.7	41	-
bisBis1	Bison	1003	30.6	-
oviAri3	Sheep	1042.4	45.6	-
capHir1	Domestic_goat	1049	49.6	-
equCab2	Horse	1052.7	29.1	-
cerSim1	White_rhinoceros	1025.5	10.2	-
felCat8	Cat	980.4	55.5	-
canFam3	Dog	966.6	17.6	-
musFur1	Ferret	947.1	26.7	-
ailMel1	Panda	854	3.6	-
ursMar1	Polar_bear	935.8	39.3	-
odoRosDiv1	Pacific_walrus	1000.1	43.4	-
lepWed1	Weddell_seal	887.5	28.3	-
manPen1	Chinese_pangolin	919.8	20.1	-
pteAle1	Black_flying_fox	972.5	47.3	-
pteVam1	Megabat	808.3	9.3	-
eptFus1	Big_brown_bat	894.9	26.9	-
myoDav1	Davids_myotis_bat	948.7	46.7	-
myoLuc2	Microbat	834.6	9.2	-
eriEur2	Hedgehog	908.3	36.5	-
sorAra2	Shrew	1138.2	101	-
conCri1	Star_nosed_mole	947.9	15.2	-
loxAfr3	Elephant	1141.8	18.8	-
eleEdw1	Cape_elephant_shrew	834.8	10.1	-
triMan1	Manatee	1098.3	17.2	-
chrAsi1	Cape_golden_mole	1655.5	30.1	-
echTel2	Tenrec	1152.4	36.3	-
oryAfe1	Aardvark	1564.1	39	-
dasNov3	Armadillo	1130.8	19.6	-
monDom5	Opossum	1800.8	150.3	362.1
sarHar1	Tasmanian_devil	913.5	10.8	59.3
macEug2	Wallaby	870.8	20.8	51.7
ornAna2	Platypus	583.4	16.4	47.9
zonAlb1	White_throated_sparrow	428.7	2	19.8
geoFor1	Medium_ground_finch	444.4	2.3	22
serCan1	Canary	478.4	3.2	26.2
taeGut2	Zebra_finch	801.8	10.7	62.3
ficAlb2	Collared_flycatcher	476.7	10.1	52.9
pseHum1	Tibetan_ground_jay	467	2.4	25.3
corCor1	Hooded_crow	420.4	1.6	22.2

runtime in CPU hours

Assembly	Species name	lastz	chaining	highly sensitive alignments
corBra1	American_crow	477.8	1.8	20.5
manVit1	Golden_collared_manakin	494.2	2	21.4
melUnd1	Budgerigar	450.7	2.3	41.4
amaVit1	Parrot	465.6	2.1	7
araMac1	Scarlet_macaw	436.8	1.7	5.6
falPer1	Peregrine_falcon	446.4	2	21
falChe1	Saker_falcon	545.2	2	20.2
picPub1	Downy_woodpecker	427.7	1.8	16.4
halLeu1	Bald_eagle	470.7	2.5	48.4
aquChr2	Golden_eagle	472.9	2.1	25.4
egrGar1	Little_egret	445.2	2.3	19.3
nipNip1	Crested_ibis	467	1.8	21.8
pygAde1	Adelie_penguin	571.8	1.8	18.7
aptFor1	Emperor_penguin	453.4	2	22
chaVoc2	Killdeer	475.3	2.6	20.4
opiHoa1	Hoatzin	439.9	2.3	20.5
calAnn1	Annas_hummingbird	450.2	2.2	45.6
cucCan1	Common_cuckoo	532.5	2.6	21.5
colLiv1	Rock_pigeon	459.5	2.1	20.3
galGal4	Chicken	414.4	5.1	74.8
melGal1	Turkey	620.8	2.5	28.9
anaPla1	Mallard_duck	424.5	1.8	15.9
tinGut2	White_throated_tinamou	431.7	2	13.1
strCam1	Ostrich	474.7	2.4	18.7
allMis1	American_alligator	838.5	5.9	49.9
gavGan0	Gharial	1550.7	337.2	435.2
cheMyd1	Green_seaturtle	789.4	12.1	45.6
chrPic2	Painted_turtle	1151.3	8.4	64.3
pelSin1	Chinese_softshell_turtle	660.5	5.1	39.6
apaSpi1	Spiny_softshell_turtle	637.6	3.9	24.6
anoCar2	Lizard	545.2	19	72
pytBiv1	Burmese_python	638.4	13.3	31.8
HLtupMer3	Tupinambis	856.3	7.8	78.1
xenTro7	X_tropicalis	704.2	80.4	148.9
nanPar1	Tibetan_frog	600.3	6.4	33
latCha1	Coelacanth	586	4.8	17.8
oreNil2	Nile_tilapia	275.3	2.3	11.2
neoBri1	Princess_of_Burundi	227.7	1.6	8.2
hapBur1	Burtons_mouthbreeder	270.6	1.8	26.4
mayZeb1	Zebra_mbuna	256	1.7	8.6
punNye1	Pundamilia_nyererei	232.1	1.6	8.4
stePar1	Bicolor_damselfish	255.9	1.3	7
oryLat2	Medaka	203.4	3	12.2
xipMac1	Southern_platyfish	209	1.8	8.6
poeFor1	Amazon_molly	271.3	2.6	23.4
poeRet1	Guppy	234.4	4.9	15.4
cypVar1	Sheepshead_minnow	279.9	1.9	10.9
HLnotFur1	Turquoise_killifish	373.6	11	21.3
gasAcu1	Stickleback	229.9	2.3	10.5
notCor1	Black_rockcod	204.8	1.3	5.7
dicLab1	European_sea_bass	245.4	4.1	50.8
tetNig2	Tetraodon	97.9	1.3	7.8
fr3	Fugu	132	1.1	19.3
takFla1	Yellowbelly_pufferfish	113.9	1.1	4.8
cynSem1	Tongue_sole	164.3	1.3	8.4
gadMor1	Atlantic_cod	182.2	1	6.3
esoLuc1	Northern_pike	257.3	2.9	12.5
danRer10	Zebrafish	952.5	42.2	95.4
astMex1	Mexican_tetra_cavefish	285.6	3.8	35.6
lepOcu1	Spotted_gar	318.5	3.9	31.7
calMil1	Elephant_shark	492	63.8	104.7
petMar2	Lamprey	136.8	2.4	3.6
letCam1	Arctic_lamprey	206.8	1.9	5.8
SUM		89308	2724	2946
TOTAL		94977		

Supplementary Table 2: Runtimes in CPU hours for all pairwise alignments.
The runtimes of chainCleaner (typically not more than 10 minutes) and the netting step sum to less than 50 CPU hours in total and are not listed here.