# Bicolor angelfish (*Centropyge bicolor)* provides the first chromosome-level genome of the Pomacanthidae family

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## Abstract

The Bicolor Angelfish, *Centropyge bicolor,* is a tropical coral reef fish. It is named for its striking two-color body. However, a lack of high-quality genomic data means little is known about the genome of this species. Here, we present a chromosome-level *C. bicolor* genome constructed using Hi-C data. The assembled genome is 650 Mbp in size, with a scaffold N50 value of 4.4 Mbp, and a contig N50 value of 114 Kbp. Protein-coding genes numbering 21,774 were annotated. Our analysis will help others to choose the most appropriate *de novo* genome sequencing strategy based on resources and target applications. To the best of our knowledge, this is the first chromosome-level genome for the Pomacanthidae family, which might contribute to further studies exploring coral reef fish evolution, diversity and conservation.

## Data Description

### Background

*Centropyge bicolor* (NCBI:txid109723; FishbaseID: 5454; urn:lsid:marinespecies.org:taxname:211780) (Figure 1), also known as the Bicolor, Two-Colored, or Pacific Rock Beauty Angelfish, is a showy coral reef fish commonly

distributed in the Indo–Pacific ocean (from East Africa to the Samoan and Phoenix Islands, north to southern Japan, south to New Caledonia; throughout Micronesia). As a member of the Pomacanthidae family, it is similar to those of the Chaetodontidae (Butterflyfishes) but is distinguished by the presence of strong preopercle spines. *C. bicolor* has clear boundaries between its body colors, so might be a good model in which to study body color development in coral fish[1].

### Context

Although the availability of genetic, and especially genomic resources, remains limited for the Pomacanthidae family, we assembled the first *C. bicolor* reference genome. This will provide valuable information for genetic studies of this coral reef fish, and will contribute to studies in body color diversity. With the whole genome sequence of *C. bicolor*, it might be possible to explore the genetic mechanisms of body color development in coral reef fish by comparative genomic methods.

## Methods and Results

A protocols collection for BGISEQ-500, stLFR and Hi-C library construction is available in protocols.io (Figure 2) [2].


**Figure 2.** Protocols for BGISEQ-500, stLRF and Hi-C library preparation and construction, and genome assembly, for the Bicolor Angelfish, *Centropyge bicolor*[2].

## Sample collection and genome sequencing

A *C. bicolor* individual was collected from the market in Xiamen, Fujian Province, China. DNA was extracted from fresh muscle tissue according to a standard protocol. Single-tube long fragment read (stLFR)[2] and Hi-C libraries were constructed following the manufacturers' instructions[2,3] to sequence and assemble the genome. We obtained 130.47 Gbp (gigabase pairs; ~197×) raw stLFR data and 134.57 Gbp (~203.20×) raw Hi-C data (Table 1) using the BGISEQ-500 platform in 100-bp (basepair) paired-end mode.

Low-quality reads (sequences with more than 40% of bases with a quality score lower than 8), polymerase chain reaction (PCR) duplications, adaptor sequences and reads with a high (greater than 10%) proportion of ambiguous bases (Ns) occurring in stLFR data were filtered using SOAPnuke (v1.6.5; RRID:SCR_015025)[4].We obtained

62.6 Gbp (~91.67×) clean data (Table 1) to assemble the draft genome. Meanwhile, HiC-Pro (v. 2.8.0)[5] was used for the quality control of raw Hi-C data, and 42.51 Gbp (~ 64.19×) valid data were used to assemble the genome to the chromosome-level (Table 1).

**Table 1**. Statistics of DNA sequencing data.

| Libraries | Read length | Raw data | | Valid data | |
|---|---|---|---|---|---|
| | | Total bases (Gbp) | Sequencing depth (×) | Total bases (Gbp) | Sequencing depth (×) |
| stLFR | 100:100 | 130.47 | 197.00 | 60.71 | 91.67 |
| Hi-C | 100:100 | 134.57 | 203.20 | 42.51 | 64.19 |

Sequencing depth = Total bases / Genome size, where the genome size is the result of *k*-mer estimation, as shown in Table 2.

## Genome assembly

Using GenomeScope software with stLFR clean data, *k*-mer distribution was used to understand the genome complexity before genome assembly[6]. The genome size of *C. bicolor* was estimated as 662.27 Mbp (megabase pairs), with 37.6% repeat sequences

and 1.16% heterozygous sites (Table 2, Figure 3).

**Table 2**. Statistical information of 17-mer analysis.

| ***k*-mer** | ***k*-mer number** | ***k*-mer Depth** | **Heterozygosity (%)** | **Genome size (Mbp)** |
|---|---|---|---|---|
| 17 | 50,994,645,240 | 77 | 1.16 | 662.27 |

The genome size, G, was defined as G = K_num/K_depth, where K_num is the total number of *k*-mers, and K_depth is the most frequently occurring *k*-mer.

We reformatted the clean stLFR data into 10× Genomics format using an in-house script(https://github.com/BGI-Qingdao/stlfr2supernova_pipeline) and assembled the draft genome using Supernova (v.2.0.1 , RRID:SCR_016756)[7] with default parameters. The draft genome was 681 Mbp, with a contig N50 of 115.5 Kbp (kilobase pairs) and scaffold N50 of 4.4 Mbp (Table 3), which is similar to the estimated genome size.

**Table 3.** Statistics of the draft assembly with stLFR data.

| **Statistics** | **Contig** | **Scaffold** |
|---|---|---|

| | | |
|---|---|---|
| Total number (#) | 40,442 | 29,065 |
| Total length (bp) | 655,705,062 | 681,285,455 |
| Gap (N) (bp) | 0 | 25,580,393 |
| Average length (bp) | 16,213.47 | 23,440.06 |
| N50 length (bp) | 115,524 | 4,424,004 |
| N90 length (bp) | 6,029 | 7,618 |
| Maximum length (bp) | 1,148,507 | 21,943,074 |
| Minimum length (bp) | 48 | 940 |
| GC content (%) | 41.74 | 41.74 |

To obtain the chromosome-level genome, we used Juicer (v3, RRID:SCR_017226)[8] to build a contact matrix and 3dDNA(v. 170123) [9]to sort and anchor scaffolds with the parameters: "–m haploid –s 4 –c 24". There are 24 distinct contact blocks, which correspond to 24 chromosomes, representing 96% of the whole genome (Figure 4A, Figure 5, Table 4). On evaluating the completeness of the genome and gene set using Benchmarking Universal Single-Copy Orthologs (BUSCO,(v.3.0.2 , RRID:SCR_015008)) [10]and a vertebrata database, our assembly maintained a score of 96.2% (Table 5). We also identified putative homologous chromosomal regions

between *C. bicolor* and *Oryzias latipes* by MCscanx[11](Figure 6).

**Table 4.** Statistics of the chromosome-level genome.

| Statistics | Contig | Scaffold |
|---|---|---|
| Total number (#) | 40,778 | 28,555 |
| Total length (bp) | 655,705,062 | 680,873,932 |
| Gap (N) (bp) | 0 | 25,168,870 |
| Average length (bp) | 16,079.87 | 23,844.30 |
| N50 length (bp) | 113,563 | 21,943,074 |
| N90 length (bp) | 5,988 | 7,542 |
| Maximum length (bp) | 1,148,507 | 28,105,280 |
| Minimum length (bp) | 43 | 43 |
| GC content (%) | 41.74 | 41.74 |

**Table 5.** Statistics of the BUSCO assessment.

| Types of BUSCOs | Gene set | Assembly |
|---|---|---|

| | Number | Percentage (%) | Number | Percentage (%) |
|---|---|---|---|---|
| Complete BUSCOs | 2,408 | 93.1 | 2,486 | 96.2 |
| Complete single-copy BUSCOs | 2,348 | 90.8 | 2,438 | 94.3 |
| Fragmented BUSCOs | 81 | 3.1 | 64 | 2.5 |
| Missing BUSCOs | 97 | 3.8 | 36 | 1.3 |
| Total BUSCO groups searched | 2,586 | 100 | 2,586 | 100 |

In addition, we cut off partial stLFR reads (25 M) for assembly by MitoZ with default parameters[12], and obtained a 16,961-bp circular mitochondrial genome of *C. bicolor*. Thirteen protein-coding genes, 24 tRNA genes and three rRNA genes were annotated by GeSeq[13] (Figure 4B).

## Genomic annotation

For the annotation of repeats, we carried out homolog annotation and *ab initio* prediction independently. RepeatMasker (v.4.0.6 , RRID:SCR_012954)[14], RepeatProteinMask (a module from RepeatMasker) and trf (Tandem Repeats Finder,

v.4.07b)[15] were used to identify known repetitive sequences by comparing the whole genome with RepBase[16]. LTR_FINDER (v.1.06, RRID:SCR_015247) [17][15] and RepeatModeler (v.1.0.8, RRID:SCR_015027) [18] were used in *de novo* prediction. We also classified transposable elements (TEs) from the integration of all repeats. In total, we identified 124 Mbp (18.32% of the entire genome) of repetitive sequences (Figure 4A, Table 6), including 110 Mbp of TEs (Figure 4A, Table 7).

**Table 6.** Statistics of repetitive sequences.

| Type | Repeat size (bp) | Percentage of genome (%) |
|---|---|---|
| TRF | 14,165,095 | 2.08 |
| RepeatMasker | 43,423,877 | 6.38 |
| RepeatProteinMask | 12,503,750 | 1.84 |
| *De novo* | 110,871,693 | 16.28 |
| Total | 124,708,977 | 18.32 |

**Table 7.** Statistics of transposable elements.

| | Repbase TEs, n | Protein TEs, n | *De novo* TEs, n | Combined TEs, n |
|---|---|---|---|---|

| | (%) | (%) | (%) | (%) |
|---|---|---|---|---|
| **DNA** | 27,163,851 (3.990) | 1,068,990 (0.157) | 61,731,447 (9.067) | 70,925,963 (10.417) |
| **LINE** | 10,228,332 (1.502) | 6,956,340 (1.022) | 20,006,579 (2.938) | 26,714,285 (3.924) |
| **SINE** | 856,125 (0.126) | 0 (0.000) | 497,024 (0.073) | 1,187,676 (0.174) |
| **LTR** | 10,971,817 (1.611) | 4,485,808 (0.659) | 16,270,071 (2.390) | 23,101,529 (3.393) |
| **Other** | 10,041 (0.001) | 0 | 0 | 10,041 (0.001) |
| **Unknown** | 0 | 0 | 14,054,230 (2.064) | 14,054,230 (2.064) |
| **Total** | 43,423,877 (6.378) | 12,503,750 (1.836) | 99,265,690 (14.579) | 109,868,166 (16.136) |

Homolog-based and *ab initio* prediction were used to identify the protein-coding genes. Augustus (v.3.3, RRID:SCR_008417)[19] was used in *ab initio* prediction basing on a repeat-masked genome[20]. Protein sequences of *Astatotilapia calliptera*, *Danio rerio*, *Larimichthys crocea*, and *Oreochromis niloticus* were downloaded from the National Center for Biotechnology Information (NCBI) GenBank database and aligned

to the *C. bicolor* genome for homolog gene annotation with Genewise (v2.4.1, RRID:SCR_015054)[21]. Finally, we used GLEAN[22] to integrate all the above evidence and obtained a total of 21,774 genes, which contained 11 exons on average and had an average coding sequence (CDS) length of 1,575 bp (Table 8).

**Table 8.** Statistics of the predicted genes in the bicolor angelfish genome.

| | Gene set | Gene number | Average transcript length (bp) | Average CDS length (bp) | Average intron length (bp) | Average exon length (bp) | Average exons per gene |
|---|---|---|---|---|---|---|---|
| **Homolog** | *Astatotilapia calliptera* | 51,174 | 21,762.29 | 2,259.23 | 1,691.33 | 180.29 | 12.53 |
| | *Danio rerio* | 22,005 | 27,982.75 | 1,570.36 | 3,438.82 | 180.90 | 8.68 |
| | *Larimichthys crocea* | 47,419 | 19,884.78 | 2,139.39 | 1,575.94 | 174.50 | 12.26 |
| | *Oreochromis niloticus* | 47,067 | 17,771.04 | 1,906.97 | 1,608.29 | 175.53 | 10.86 |
| ***De novo*** | Augustus | 34,470 | 9,675.42 | 1,335.20 | 1,344.81 | 185.40 | 7.20 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **GLEAN** | 21,774 | 14,024.40 | 1,906.28 | 1,206.07 | 172.55 | 11.05 |

The GLEAN gene set is the integrated result of *de novo* gene predictions and homolog gene predictions.

To predict gene functions, 21,774 genes were aligned against several public databases, including TrEMBL[23], SwissProt[23], KEGGViewer[24] and InterProScan[25]. As a result, 99.67% of all genes were predicted functionally (Table 9, Figure 7).

**Table 9.** Statistics of the functional annotation.

| Database | Number | Percentage (%) |
|---|---|---|
| Total | 21,774 | 100.00 |
| SwissProt | 20,784 | 95.45 |
| KEGG | 19,168 | 88.03 |
| TrEMBL | 21,688 | 99.61 |
| Interpro | 20,153 | 92.56 |
| Overall | 21,702 | 99.67 |

## Phylogenetic analysis

We downloaded the gene data of seven representative teleost fishes from NCBI to

study the phylogenetic relationships between *C. bicolor*. These seven fishes were: *Danio rerio*, *Gasterosteus aculeatus*, *Gadus morhua*, *Larimichthys crocea*, *Oryzias latipes*, *Oreochromis niloticus* and *Tetraodon nigroviridis*. For each dataset, the longest transcripts were selected and aligned to each other by BLASTP (v2.9.0, RRID:SCR_001010)[26] (E-value ≤ 1e-5). TreeFam (v.2.0.9, RRID:SCR _013401) [27] was used to cluster gene families, with default parameters. Among all 20,706 clustered gene families, there were 4,450 common single-copy families and 57 families specific to *C. bicolor* (Table 10). With single-copy sequences, we used PhyML (v.3.3,RRID:SCR_014629)[28] to construct the phylogenetic tree of *C. bicolor* and the seven other fishes mentioned above, setting *D. rerio* as an outgroup.

**Table 10. Statistics of gene family clustering.**

| Species | Total genes | Unclustered genes | Families | Unique families | Average number of genes per family |
|---|---|---|---|---|---|
| *Centropyge bicolor* | 21,774 | 694 | 16,219 | 57 | 1.3 |
| *Danio rerio* | 30,067 | 2,188 | 18,575 | 726 | 1.5 |
| *Gasterosteus aculeatus* | 20,756 | 784 | 15,921 | 16 | 1.25 |
| *Gadus morhua* | 19,987 | 535 | 15,630 | 9 | 1.24 |
| *Larimichthys crocea* | 24,403 | 610 | 17,273 | 55 | 1.38 |

| | | | | | |
|---|---|---|---|---|---|
| *Oryzias latipes* | 19,535 | 1,048 | 14,805 | 87 | 1.25 |
| *Oreochromis niloticus* | 21,431 | 180 | 15,780 | 14 | 1.35 |
| *Tetraodon nigroviridis* | 19,544 | 901 | 14,803 | 57 | 1.26 |

Based on the phylogenetic tree and single-copy sequences, the divergence time between different species was estimated by MCMCTREE with parameters of "--model 0 --rootage 500 -clock 3". The results showed that *C. bicolor* was formed ~34.95 million years ago, when differentiated from the common ancestor with *L.crocea* (Figure 8).

## Analysis of bicolor formation in teleosts

Current studies suggest that different pigment cells produce different pigments. Some types of pigment cells already have been identified in teleost[29]. *C. bicolor* has an attractive body color with clear color boundaries, but the molecular mechanism underlying this remains unknown. Compared with other teleost, there are 1,081 expanded gene families and 57 specific gene families in *C. bicolor* (Figure 9). Functional enrichment analysis showed that notable expansion occurred in those gene families related to visual development and enzyme metabolism (Figure 9).

## Re-use Potential

Coral reef fishes, with distinctive color patterns and color morphs, are important for understanding the adaptive evolution of fishes. In this study, we firstly assembled a high-quality, chromosome-level genome of *C. bicolor*, with a length of 681 Mbp, and annotated 21,774 genes. This is the first genome of a fish from the Pomacanthidae family. These genomic data will be useful for genome-scale comparisons and further studies on the mechanisms underlying colorful body development and adaptation.

### Data Availability

The data sets supporting the results of this article are available in the GigaScience Database, doi: 10.5524/100802. Raw reads from genome sequencing and assembly are deposited at the China National Gene Bank under reference number CNP0001160, which contains sample information (CNS0315939), Hi-C raw data (CNX0286336) and stLFR raw data (CNX0286337). The project also has been deposited at NCBI under accession ID PRJNA702283.

## Declarations

### List of Abbreviations

bp: base pair; BUSCO: Benchmarking Universal Single-Copy Orthologs; Gbp:

gigabase pair; Kbp: kilobase pair; KEGG: Kyoto Enyclopedia of Genes and Genomes; Mbp: megabase pair; NCBI: National Center for Biotechnology Information; stLFR: single-tube long fragment reads; TE: transposable element.

## Ethical Approval

All resources used in this study were approved by the Institutional Review Board of BGI (IRB approval No. FT17007). This experiment has passed the ethics audit of the Beijing Genomics Institute (BGI) Gene Bioethics and Biosecurity Review Committee.

## Consent for Publication

Not applicable.

## Competing Interests

The authors declare that they have no competing interests.

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## Authors' Contributions

H.Z. and G.F. designed this project. M.Z. prepared the samples. S.L., S.P., W.X., C.W. and C.M. conducted the experiments. C.L., X.Y., L.S., R.Z. and Q.L. did the analyses. C.L., X.Y., L.S., R.Z. wrote and revised the manuscript. All authors read and approved the final version of the manuscript.

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## Figure Legends

**Figure 1.** Photograph of *Centropyge bicolor*.

**Figure 2.** Protocols for BGISEQ-500, stLRF and Hi-C library preparation and construction, and genome assembly, for the Bicolor Angelfish, *Centropyge bicolor*[2].

**Figure 3.** The 17-mer depth distribution of *Centropyge bicolor*.

The estimated genome size is 662.27 Mbp and the heterozygosity is 1.16%.

**Figure 4.** Annotation of the *Centropyge bicolor* genome. (**A**) Basic genomic elements of the *Centropyge bicolor* genome. LTR, long terminal repeat; LINE, long interspersed nuclear elements; SINE, short interspersed elements. (**B**) Physical map of mitochondrial assembly.

**Figure 5.** Heat map of interactive intensity between chromosome sequences.

**Figure 6.** Homologous chromosomal regions between *Centropyge bicolor* and *Oryzias latipes*.

**Figure 7.** Venn diagram of orthologous gene families.

Four teleost species (*Centropyge. bicolor, Larimichthys crocea, Oreochromis niloticus, and Danio rerio*) were used to generate the Venn diagram based on gene family cluster analysis.

**Figure 8.** Comparative analysis of the *Centropyge bicolor* genome.

(**A**) The protein-coding genes of the eight species were clustered into 17,849 gene families. Among these gene families, 4,450 were single-copy gene families. (**B**) Phylogenetic analysis of *Centropyge bicolor* (Cbi.), *Danio rerio* (Dre.), *Gasterosteus aculeatus* (Gac.), *Gadus morhua* (Gmo.), *Larimichthys crocea* (Lcr.), *Oryzias latipes* (Ola.), *Oreochromis niloticus* (Oni.), and *Tetraodon nigroviridis* (Tni.) using single-copy gene families. The species differentiation time between *Centropyge bicolor* and *Larimichthys crocea* was ~34.95 million years.

**Figure 9.** Statistics of gene function enrichment (Gene Ontology) for expanded genes of *Centropyge. bicolor*.

Nodes are colored by *q*-value (adjusted *p*-value). Node size is shown according to its enriched gene number.


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# Protocols for "Bicolor Angelfish (*Centropyge bicolor*) genome provided first chromosome-level reference of Pomacanthidae family and clues for bi-color body formation"

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1 *Works for me*

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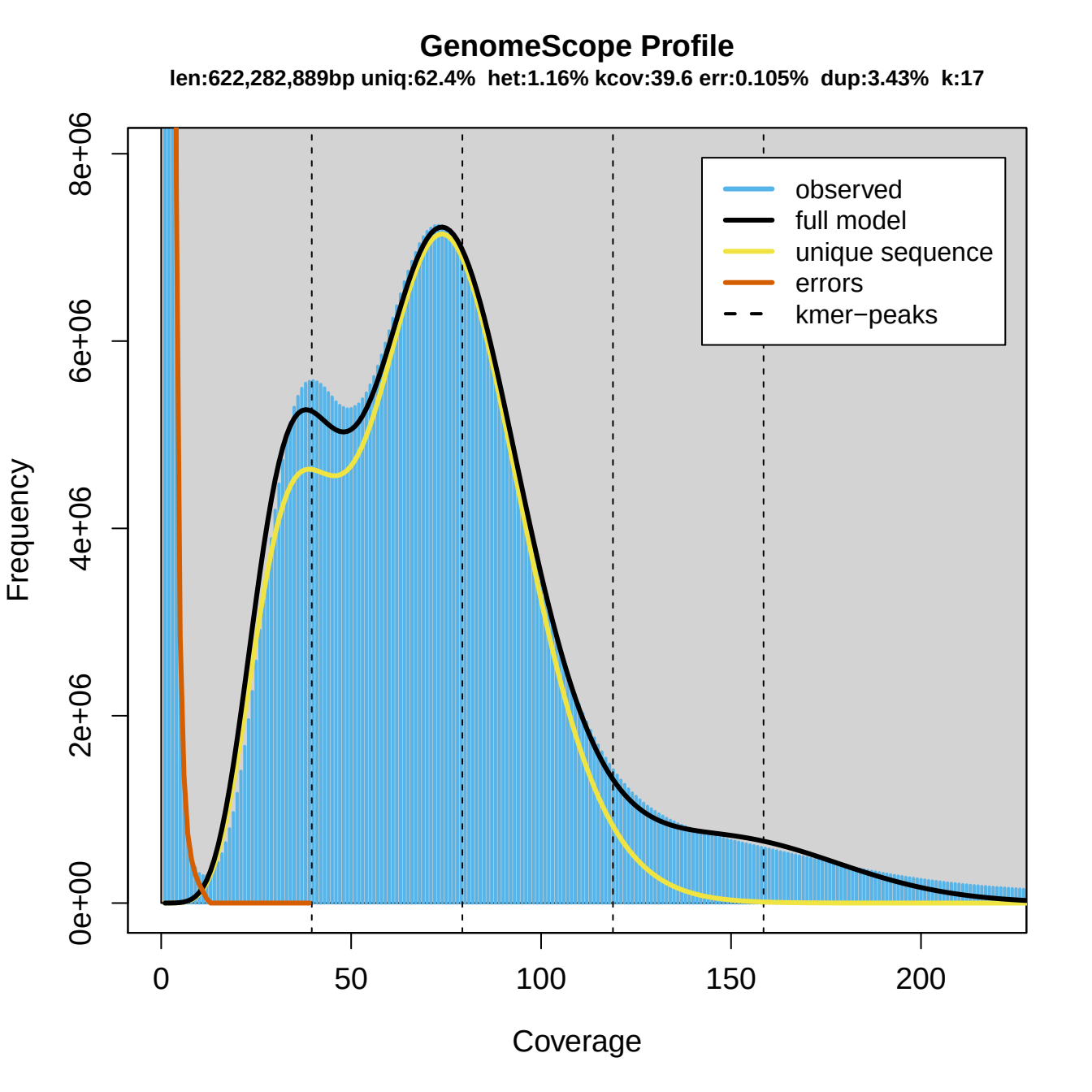
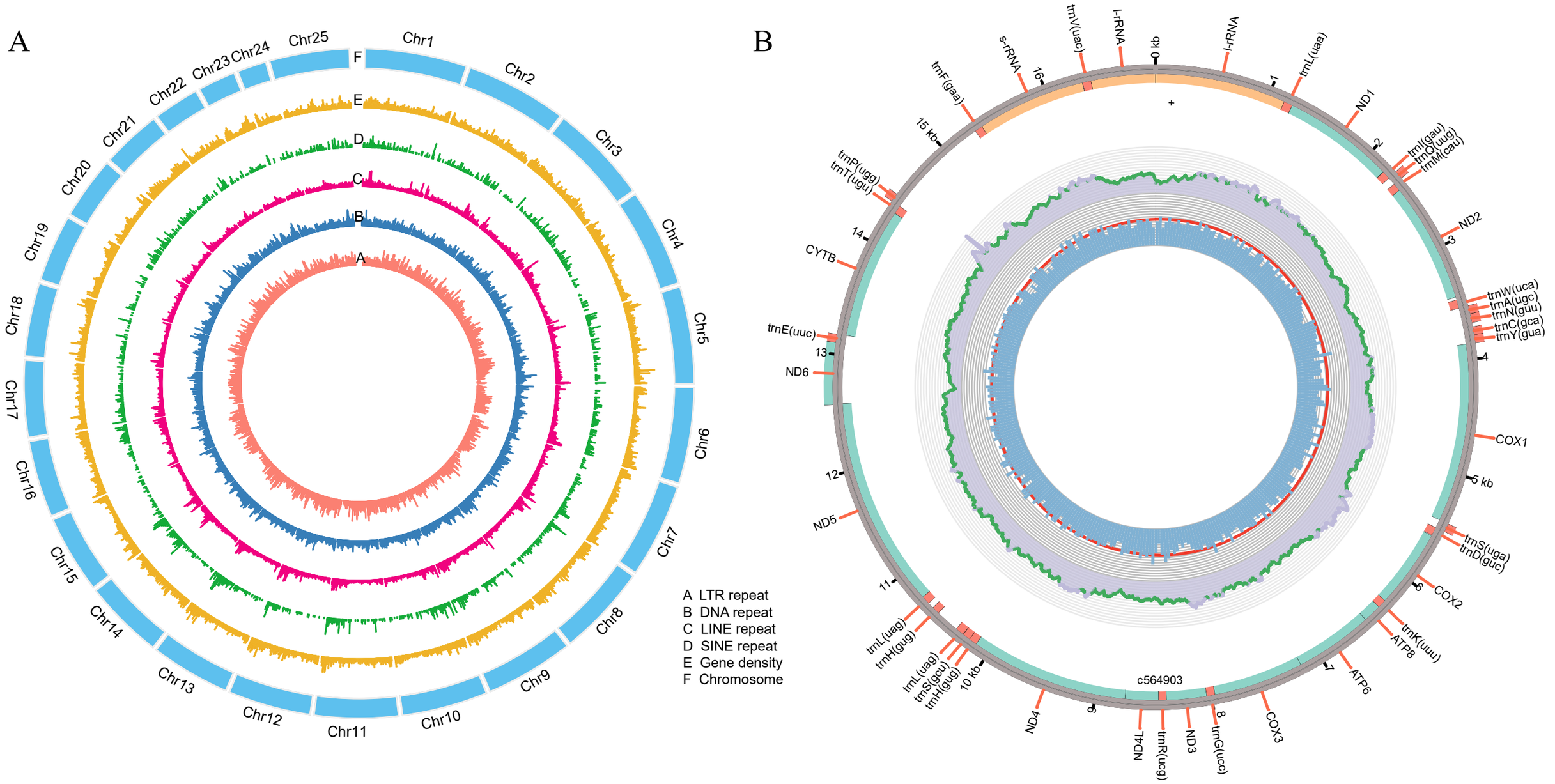
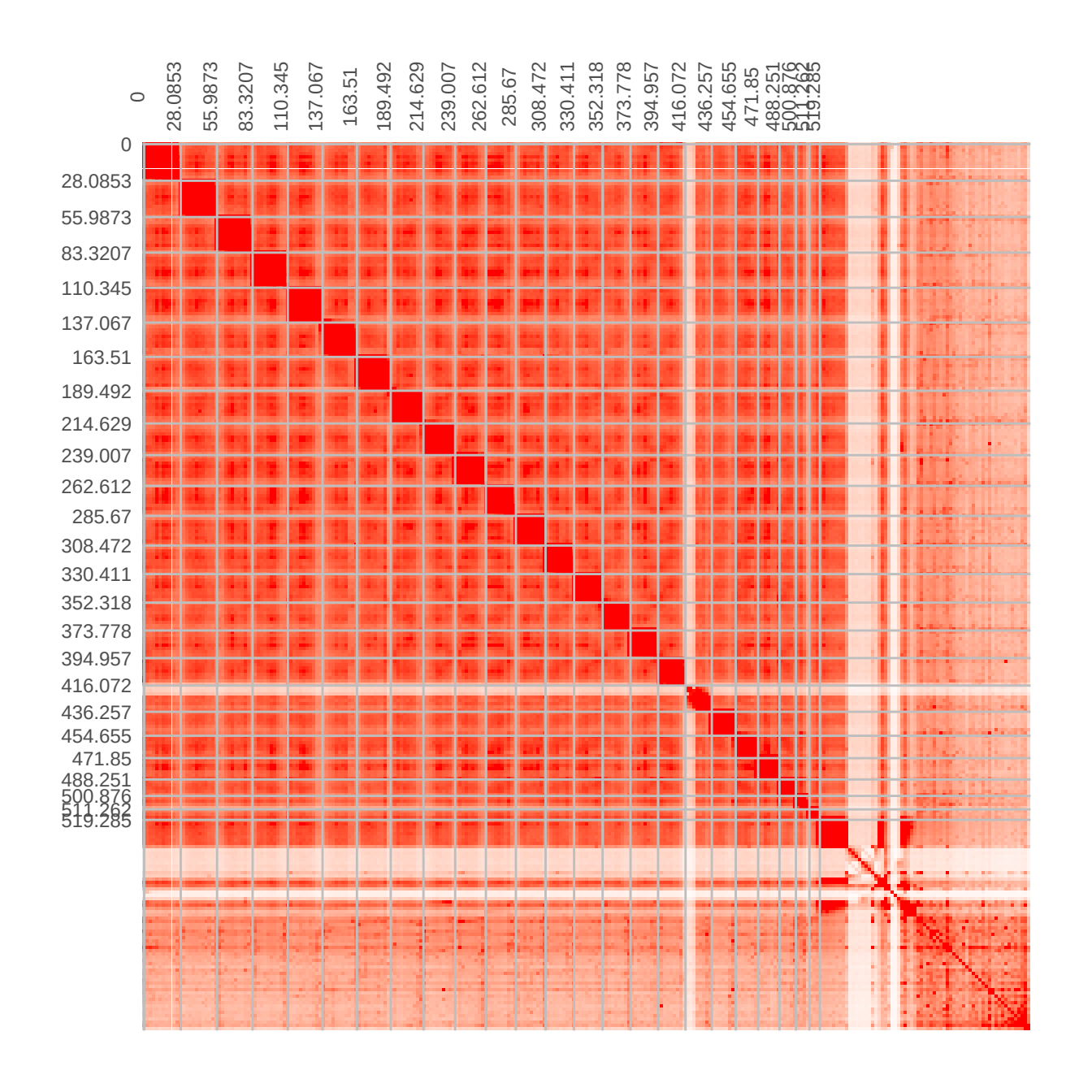
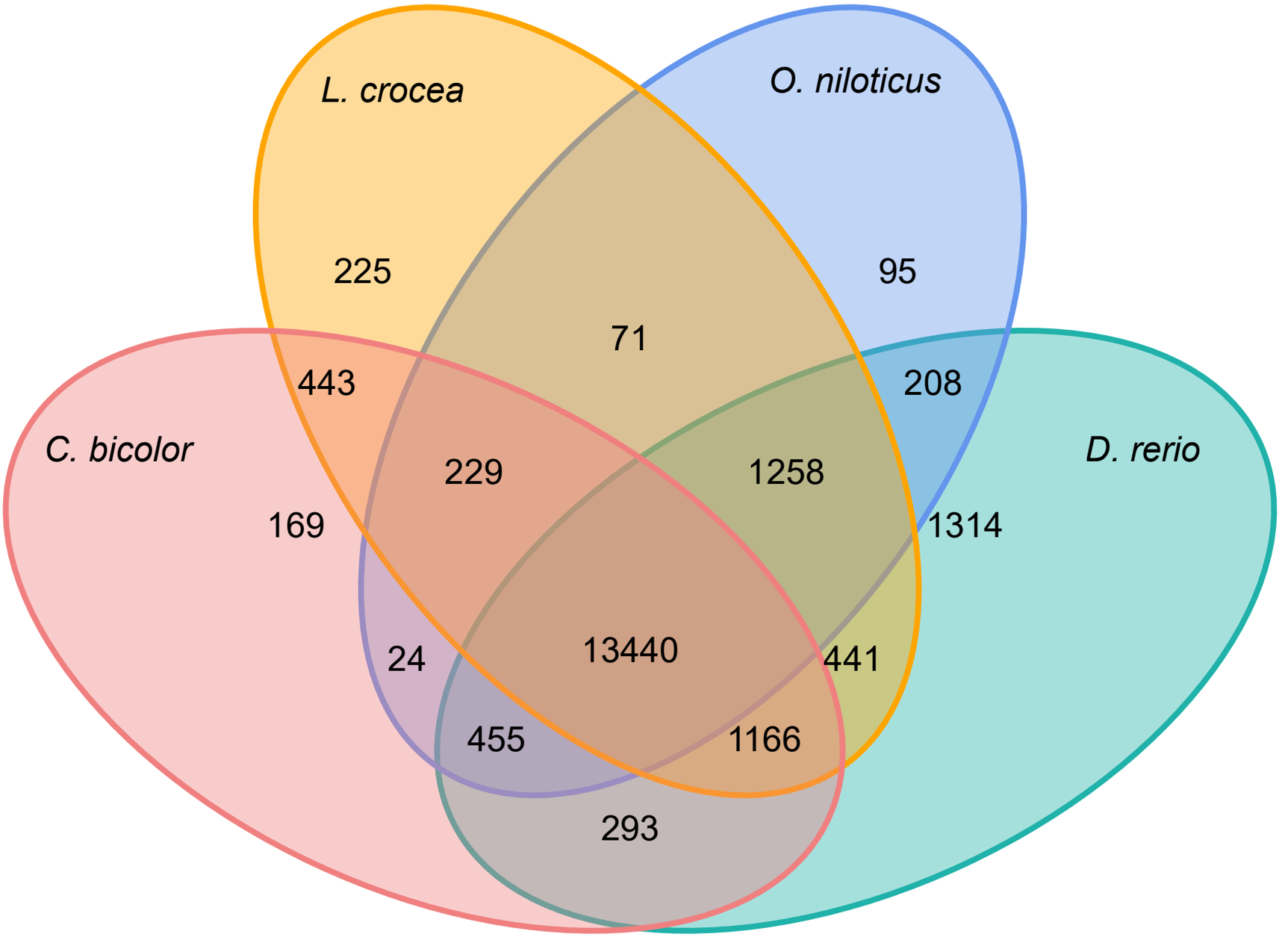
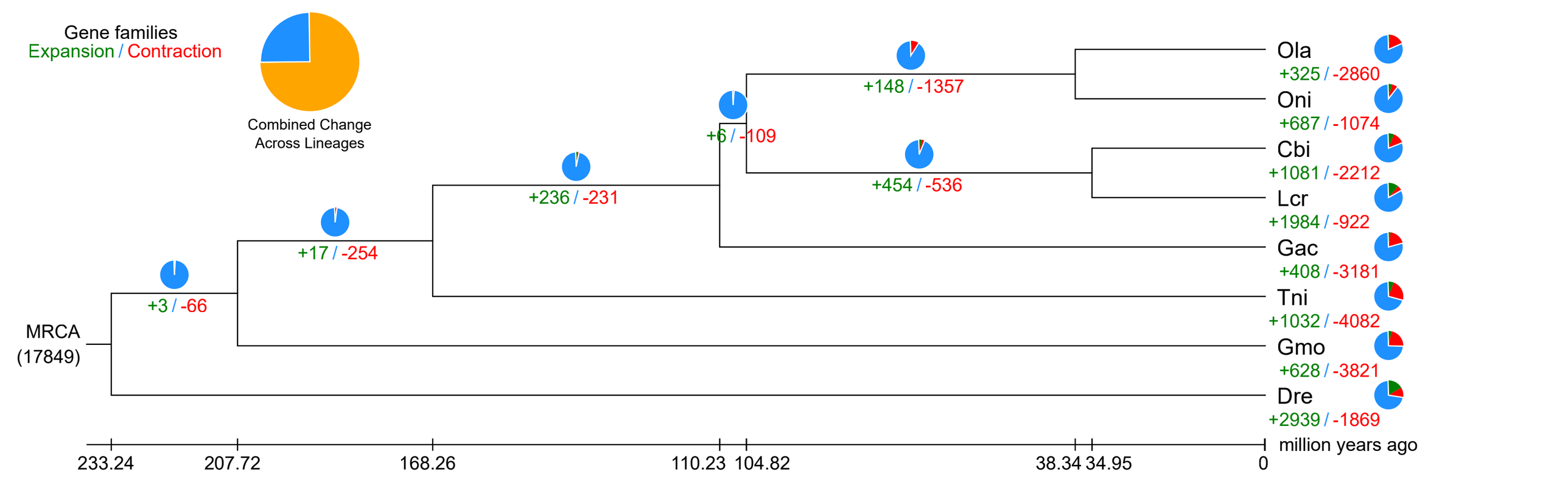

GenomeScope Profile
len:622,282,889bp uniq:62.4% het:1.16% kcov:39.6 err:0.105% dup:3.43% k:17
observed
full model
unique sequence
errors
kmer−peaks
Frequency
0e+00
2e+06
4e+06
6e+06
8e+06
0
50
100
150
200
Coverage

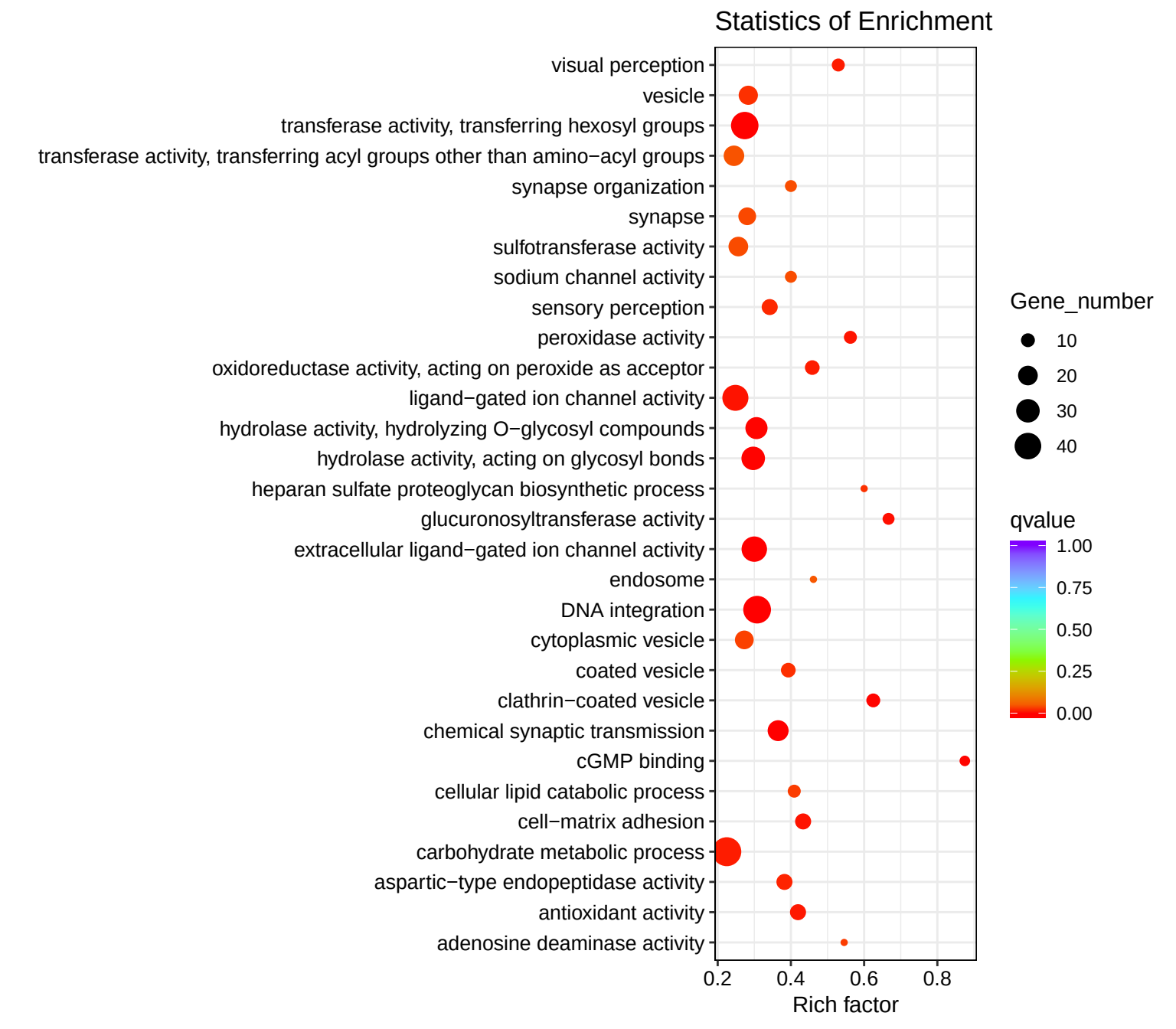

A
Chr1
Chr2
Chr3
Chr4
Chr5
Chr6
Chr7
Chr8
Chr9
Chr10
Chr11
Chr12
Chr13
Chr14
Chr15
Chr16
Chr17
Chr18
Chr19
Chr20
Chr21
Chr22
Chr23
Chr24
Chr25
F
E
D
C
B
A
A LTR repeat
B DNA repeat
C LINE repeat
D SINE repeat
E Gene density
F Chromosome
B
0 kb
+
l-rRNA
trnL(uaa)
ND1
trnI(gau)
trnQ(uug)
trnM(cau)
ND2
trnW(uca)
trnA(ugc)
trnN(guu)
trnC(gca)
trnY(gua)
COX1
5 kb
trnS(uga)
trnD(guc)
COX2
trnK(uuu)
ATP8
ATP6
COX3
trnG(ucc)
ND3
trnR(ucg)
ND4L
ND4
10 kb
trnL(uag)
trnS(gcu)
trnH(gug)
trnL(uag)
trnH(gug)
ND5
ND6
trnE(uuc)
CYTB
trnP(ugg)
trnT(ugu)
15 kb
trnF(gaa)
s-rRNA
trnV(uac)
l-rRNA
c564903


0
28.0853
55.9873
83.3207
110.345
137.067
163.51
189.492
214.629
239.007
262.612
285.67
308.472
330.411
352.318
373.778
394.957
416.072
436.257
454.655
471.85
488.251
500.876
511.262
519.285




Number of gene families


Gene families
Expansion / Contraction
Combined Change
Across Lineages
MRCA
(17849)
+3 / -66
+17 / -254
+236 / -231
+6 / -109
+148 / -1357
+454 / -536
Ola
+325 / -2860
Oni
+687 / -1074
Cbi
+1081 / -2212
Lcr
+1984 / -922
Gac
+408 / -3181
Tni
+1032 / -4082
Gmo
+628 / -3821
Dre
+2939 / -1869
233.24
207.72
168.26
110.23
104.82
38.34
34.95
0
million years ago


Statistics of Enrichment
visual perception
vesicle
transferase activity, transferring hexosyl groups
transferase activity, transferring acyl groups other than amino−acyl groups
synapse organization
synapse
sulfotransferase activity
sodium channel activity
sensory perception
peroxidase activity
oxidoreductase activity, acting on peroxide as acceptor
ligand−gated ion channel activity
hydrolase activity, hydrolyzing O−glycosyl compounds
hydrolase activity, acting on glycosyl bonds
heparan sulfate proteoglycan biosynthetic process
glucuronosyltransferase activity
extracellular ligand−gated ion channel activity
endosome
DNA integration
cytoplasmic vesicle
coated vesicle
clathrin−coated vesicle
chemical synaptic transmission
cGMP binding
cellular lipid catabolic process
cell−matrix adhesion
carbohydrate metabolic process
aspartic−type endopeptidase activity
antioxidant activity
adenosine deaminase activity
0.2
0.4
0.6
0.8
Rich factor
Gene_number
10
20
30
40
qvalue
1.00
0.75
0.50
0.25
0.00