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A draft annotation and overview of the human genome

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A draft annotation and overview of the human genome

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Abstract

The recent draft assembly of the human genome provides a unified basis for describing genomic structure and function. The draft is sufficiently accurate to provide useful annotation, enabling direct observations of previously-inferred biological phenomena. We report a functionally annotated human gene index placed directly on the genome. The index is based on the integration of public transcript, protein, and mapping information, supplemented with computational prediction. Such a global approach has been described only for chromosomes 21 and 22, which together account for 2.2% of the genome. We estimate that the genome contains 65,000-75,000 transcriptional units, with exonic sequences comprising 4%.

Background

The sequence of the human nuclear genome has been completed in draft form by an international public consortium consisting of 16 sequencing centers and associated computational facilities (http://www.nhgri.gov/HGP). A private commercial version of the genome has also been sequenced and assembled using a whole genome shotgun approach [1]. Many lower organisms have been sequenced to date (http://www.tigr.org/tdb/mdb/mdbcomplete.html), but the 3.2 billion base pair human genome is ~25 times as large as the largest currently finished genomes, *Drosophila* at 120 Mb [2] and *Arabidopsis* at 115 Mb [3].

The current public human sequence is primarily based on ~23,000 accessioned bacterial artificial chromosome (BAC) clones covering 97% of the euchromatic portion of the genome (http://genome.wustl.edu/gsc/human/Mapping). The sequence of these clones is approximately 93% complete to at least 4x coverage (http://www.ncbi.nlm.nih.gov/genome/seq). Thirty percent of the genome is in finished form, including the entire sequence of chromosomes 21 and 22 (http://www.ncbi.nlm.nih.gov/genome/seq/HsHome.shtml). These clones represent the most complete sequence information available, with overlapping clones positioned on a framework map using restriction fingerprinting. However, reduction to a single consensus sequence permits placement of genes and other chromosomal structures in their proper positional context. Recently, the consortium has distributed a working draft assembly of the entire genome that removes redundancies, orients sequence fragments and clearly indicates gaps arising from sequencing and assembly. The total assembled length is 3.08 billion bp – about 4% smaller than estimates of genome size based on flow cytometry [4], presumably due to the exclusion of constitutive heterochromatic regions and centromeres. Major gaps (50 kb-200 kb) comprise 16% of the assembly, while minor gaps (100 or fewer bp) and low quality calls comprise 0.5%.

Results

Combine and conquer

Functional annotation of the genome is primarily hampered by the lack of a unified transcript index. Current transcript information still largely consists of anonymous and highly redundant ESTs. The situation is further complicated by extensive splicing variation and elusive expression. To address these problems, the Ensembl consortium relies initially on computational prediction, followed by confirmation with EST/protein alignments (http://www.ensembl.org/Docs/wiki/html/EnsemblDocs/ScienceDocumentation.html). However, pure computational approaches can give differing results [5], and may miss 20% or more of transcript-supported exons [6]. Other gene identification approaches rely on selecting and grouping ESTs into putative gene indices [7, 8], or consensus sequences [9, 10]. These approaches

emphasize internal consistency and result in limited EST populations that only partially overlap. The genome sequence serves as a powerful arbiter of the quality of EST evidence, and will enable consolidation of additional exons into transcriptional units. Thus, we adopt a more inclusive approach.

Our approach is to combine the major public cDNA, EST and protein databases, resolve redundancies, and place the resulting exonic sequences uniquely on the genome using the program Blast. We refer to these genomic segments (technically *high-scoring segment pairs*, http://www.ncbi.nlm.nih.gov/BLAST/blast_help.html) as "exons," although the alignment evidence awaits future biological confirmation. Splicing evidence was carefully maintained within genomic clones, and across clones using the fingerprint map. For a given transcript, only the best match to genomic sequence (using splicing evidence, length and high sequence identity) was preserved, resulting in a unique location for each exonic unit within each database. We have successfully applied this approach to integrate UniGene consensus sequences into the human genome draft (Zhou et al., in press).

To compile a truly unique exonic index, redundancies must also be resolved across transcript databases. We grouped the databases into ranked categories and ordered them within categories. Transcripts with known boundary information (using the UTR-DB database) [11] or full-length cDNAs in the HTDB database [12] were given precedence over other records. Consensus transcripts were given precedence over individual ESTs because they provide greatly improved specificity, splicing evidence and transcript integrity. We assembled UniGene-based human (Zhou et al., in press), mouse, and rat consensus transcripts. Collectively, the databases represent almost all public information on known genes, transcripts and relevant homologous sequences. When aligned segments overlapped, only the segments from the highest-ranked categories were used. After resolution of overlapping exons, a new exonic index of contiguous spliced components was formed. Each member of this new index inherited the rank of its highest-ranked exon, in order to facilitate subsequent identification of transcriptional units. Our approach also ensures that known genes are represented only once in the final gene map.

Table 1 describes the identification of exonic sequence via the public databases. Not all human transcript records could be placed on the genome, reflecting sequence gaps and the draft quality of the genomic clones. The percentage placement of known genes (80%-89%) suggests that unsequenced regions will contribute substantial numbers of additional genes. The varying placement percentages among transcript databases reflect varying sequence quality and differing transcript lengths. Unique exons are those that have no overlap with those already placed by a higher-ranked database. Rodent transcripts provided a modest number of additional exons. Finally,

additional placements were possible using protein homology. The percent placement was relatively low because all proteins from different species were considered, with specificity assured by using appropriately stringent criteria.

When all of the databases are considered, 613,183 unique exons were placed, including 299,014 in complete open reading frames (ORFs) and 55,860 in partial ORFs. The total putative exonic lengths add to 106 Mb, or about 4% of the sequenced genome. At least 30-40% of the known genes or transcript indices contain one or more internal transcripts, suggesting alternative splicing, internal genes or occasional artifacts (misassembly or genomic contamination). The prevalence of alternative splicing remains unknown, but may occur frequently [13]. "Sandwiched" transcripts were merged with their flanking indices, unless both the internal and the flanking sequences were distinct known genes (<150 apparent internal genes). In addition, we observed a small number of apparently overlapping genes (~530 on opposite strands) [14].

We assessed three *ab initio* gene prediction methods by comparing their predicted exons to the ones identified by transcripts and proteins. Genscan, Grail and Fgene were used across the genomic clones to identify potential exons. Approximately 70% of the 299,014 exons in ORFs with either transcript or protein support were identified by at least one of the programs, but a very large number (847,283) of unconfirmed exons were also identified. A summary of the gene prediction analyses appears on our web site (http://pandora.med.ohio-state.edu/Annotation). The large apparent false positive rate implies that pure computational gene prediction is not yet a practical alternative to experimental evidence.

Transcriptional units

Our consolidated exonic index is of inherent biological interest, but it is desirable to further identify transcriptional boundaries to create a putative gene index. We employed an approach designed to minimize fragmentation of exons and provide conservative gene counts (see Methods). The following criteria were used to identify gene boundaries: (1) known 5′ or 3′ UTR sequences in UTR-DB; (2) full-length cDNAs in HTDB; (3) exons in partial ORFs as possible boundaries of coding regions; (4) exons without continuous ORFs as additional UTR sequences; (5) CpG islands; and (6) gene boundaries predicted by Genscan. Multiple in-frame exons in a continuous ORF were always considered part of a single gene, an approach that tends to consolidate exons rather than create spurious additional genes. Additional consolidation resulted from extension of boundaries for multiple exons not residing in ORFs until occurrence of genomic landmarks described above. The success of this approach depends largely on the extension and consolidation of overlapping

transcripts, and the integrity of ORFs and other genomic landmarks provided by the draft sequences.

Table 1 lists the number of genes added by each database to the cumulative sum. The total number of known genes in UTR-DB, HTDB and HINT is 16,673. This compares with 11,191 entries with at least partial functional annotation in UniGene (May '00 build) and 11,863 entries in the HUGO Human Gene Nomenclature database (http://www.gene.ucl.ac.uk/nomenclature). Approximately 48% of the transcriptional units were based on consensus transcripts and 28% based on individual ESTs. A total of 9,372 transcriptional units were based on singleton transcripts without splicing evidence, which can result from genomic contamination or other artifacts. A total of 1,437 units were supported only by rodent transcripts. An additional 3,154 units were identified based on protein homology. Our approach yields an overall estimate of 75,982 transcriptional units, with 66,610 supported by multiple transcripts or individual transcripts with splicing evidence. We observed that 45% of the gene units were associated with CpG islands (defined as 10 kb upstream or within the gene). For the 6,500 known genes with known 5′ boundaries, the value was 40%. The average genomic size of each transcriptional unit, including only transcript or protein-based exons, is ~ 12 kb. In total transcriptional units occupy about 900 Mb, corresponding to approximately 35% of the sequenced genome.

Gene map

The placement of transcriptional units is not without error, as most genomic clones are unfinished and the restriction fingerprint map can be subject to misassembly. To resolve placement errors, we used a relational database to integrate information from several independent maps, including Genemap '99, assembled genomic contigs, and fingerprint, radiation hybrid and cytogenetic maps (See Methods). Placement required a minimum of three concordant criteria. Together, a total of 75,982 transcriptional units were placed on the genome, providing an initial glimpse of a complete gene map. The map and associated functional annotation (see below) are available at http://pandora.med.ohio-state.edu/Annotation.

Functional annotation

SWISS-PROT, TrEMBL, PIR and Pfam were used to annotate our unified gene index, because functional keywords in these databases are standardized [15] (Table 2). We used the classification schema developed by the International Gene Ontology Consortium to assign each keyword to an appropriate ontological description (http://www.geneontology.org; and see http://pandora.med.ohio-

state.edu/Annotation for keyword assignments). Clear functional roles and biological processes were given priority over other keyword designations. Similarly, protein-based annotation was performed for HINT consensus transcripts. The transcriptional units resulted in a greater number of annotations (~23,000) than HINT transcripts (~11,000) because of the increased length of the included genomic sequence.

The annotation also allows us to assess the protein composition of human *vs*. other species. A BlastX result of $E < 10^{-20}$ was required in cross-species DNA-protein alignments to be considered homologous. A total of 20,892 human transcriptional units (30% of all units) are homologous to at least one other species; 5,792 (10%) were conserved across mammals (mouse or rat), *Drosophila*, and *C. elegans*. A total of 1,759 (3%) were conserved across all of these species and yeast. These values are very consistent with a recent comparative genomic survey [16].

Global tissue expression profiles

During the assembly of UniGene (Zhou et al., in press), we retained the library source for each EST, via links provided by UniGene to the IMAGE consortium (http://image.llnl.gov). Most of the 2,500 libraries comprising UniGene ESTs were derived from single tissues or embryonic stages, and we further standardized the library source annotation into 102 categories. Keywords and derived categories available at http://pandora.med.ohio-state.edu/Annotation. The most highly represented categories were various types of tumors (15.0% of all ESTs), fetal tissue (10.7%), embryo (6.2%), infant (5.1%), and testis (4.3%). We reasoned that some genes might exhibit highly tissue-specific expression, such that most of the ESTs comprising a transcript would be derived from the tissue. The identified genes are potential candidates for diseases of the involved tissues. Similar approaches have been used to identify candidate genes for pathologies of the prostate [17] and retina [18]. We explore here the global nature of tissue/source specificity. The result was 7,459 HINT transcripts highly significant tissue-specificity (11%). Many of these are known genes, and an examination of the most-specific transcripts revealed clear relationships to the associated tissue. For example, a search for retina-specific genes revealed that the 10 most significantly associated with retina include five known genes, all related to retina function. Four are implicated in retina pathology: *GNAT1* and *ARR* (night blindness), *RHO* (retinitis pigmentosa), and *GUCA1A* (cone dystrophy). Similar results were observed in numerous other tissues, although not as obviously related to pathology. The results appear especially striking for tissues with substantial EST representation, including brain, lung, liver, kidney, and testis, suggesting that putative tissue involvement can be inferred for many anonymous ESTs. Where possible, the tissue expression profile has been incorporated into the annotation of our gene index. Approximately half (50.5%) of

the tissue-specific clusters were from embryonic tissue libraries (while such tissue contributed 6.2% of all UniGene ESTs). This striking result is consistent with the highly regulated and specific nature of embryonic development [19]. The embryo category is followed by brain (9.7% brain-specific vs. 3.8% of ESTs) in number of tissue-specific clusters, kidney (5.5% vs. 3.5%), and testis (6.1% vs. 4.3%). We also examined the locations of the tissue-specific transcripts on the genome, and found no evidence of regional clustering (see description of regional functional clustering in Methods).

A global view of the human genome

In keeping with the longstanding clinical importance of cytogenetics, it is important to align Giemsa-staining G (dark) cytobands vs. R (pale) bands (ISCN 1995) to the assembly [20]. Cytoband boundaries on genomic sequence have been depicted with apparent precision [6, 21] but in fact are largely unknown. With only a few-fold genomic coverage, the gap sizes in unfinished sequence are difficult to estimate precisely. Thus, it is preferable to align the cytoband positions to the fixed assembly rather than the reverse. Such an "assembly-corrected" alignment was performed using genes/ESTs that have been mapped cytogenetically and also placed on the assembly. This alignment is approximate, as the resolution of conventional staining techniques and FISH is limited to 1-3Mb [22].

Density of genomic features

The resulting corrected ideograms and six major genomic features are plotted across the genome in Figure 1. Unique exons (as determined above), CpG islands, genomic GC content, *Alu* and LINE1 elements, and minisatellites are plotted as densities (proportion of bases belonging to feature) in 1 Mb intervals. The assembly-corrected ideogram clearly differs from the standard ideogram – e.g., in our representation 1p is longer than 1q. This may reflect more complete sequencing on 1p, or perhaps differing DNA packing densities on the two chromosome arms. Many of the chromosomes show a suggestive relationship between cytobands and exon density, consistent with the expectation that R bands are relatively gene rich. A more striking result is the expected positive correlation among exons, CpG islands, GC content, and minisatellites, which track each other closely on most chromosomes. Exon density is relatively high on chromosomes known to be gene rich (e.g., 17 and 19) [23], and low on chromosomes 4, 13, X, and Y.

A total of 48,000 CpG islands were found on the assembly using standard criteria [24] (see Figure 1 legend), with a median length of 336 bp. As sequencing gaps are filled, this number may increase. Considering the varying definitions of CpG islands (especially the minimum length of

CpG-rich region), this number is in close agreement with the estimate of 45,000 obtained by Antequera and Bird [25] using methylation-sensitive restriction enzymes. The CpG island density is also in agreement with a report of FISH karyotypes using CpG island probes [26] with contrasting fluorescent signal in late replicating regions. Extended regions of high CpG island density, such as the terminus of 1p and 1q21-q22, are apparent in the FISH assay. Short spikes of CpG islands (e.g., in 3p26 and 3p25 of Figure 1) do not obviously appear in the assay, perhaps because they are below the resolution of FISH or are part of transcriptionally active regions.

In contrast to exon and CpG island density, GC content shows limited variation – in the range 35%-55% for most 1 Mb intervals. The overall GC content is 41.1%. This compares with estimates in the range of 40%-41% based on density gradient centrifugation [27] and flow cytometry [28].

Consistent with previous reports [29] *Alu* repeats show an apparent positive correlation with exon, CpG and GC densities, while LINE1 densities do not show such correlation. Approximately 1.1 million *Alu* repeats were identified, as expected [30]. However, a total of 758,000 LINE1 repeats were identified – 40% higher than estimates based on a sampling of sequenced regions [30]. Minisatellites of the hypervariable family (20 bp-50 bp repeat size) are dispersed throughout the genome, but as expected [31] show sharp spikes in subtelomeric regions of most chromosomes.

Comparison of cytogenetic bands

We next examined the overall correspondence between cytobands and exonic density and other genomic features. Table 3 gives the average densities of features in the R bands vs. G bands based on the assembly-corrected alignment. Genomic intervals residing in R bands were significantly richer in exons, CpG islands, GC content, *Alu* repeats and minisatellites than those in G bands. The reverse is true for LINE1 elements. These observations accord with predictions based on a variety of indirect methods [32], or a selected set of genes [33], but only now may be investigated directly using the sequence of the entire genome. The increased exonic density in R bands was fairly modest (~30%), and may reflect attenuation due to alignment error. In addition, the analysis did not account for variation in staining intensity in G-bands [20]. However, the results across the chromosomes were fairly consistent, and the R/G exonic density ratio exceeded 2.0 on two chromosomes (13 and 21) and was below 1.0 on only one chromosome (Y). The increased density of CpG islands in R bands was more striking (59%), while GC content was only a few percent higher (42.2% vs. 39.8% in G bands), again consistent with previous observations [34]. The results for the cytobands are also reflected in pairwise correlations of the genomic features across 1 Mb intervals. These correlations do not depend on the cytoband alignment, and most features were

positively correlated. LINE1 elements again differed from other features, showing a negative correlation with exons, CpG islands, GC content and *Alu* repeats.

Gene density

We analyzed for each chromosome the exonic sequence as given in Table 1. Figure 2A shows the density of exonic sequence per chromosome. Chromosomes 19 and 17 are the richest (i.e., densest) in exonic sequence [23], by factors of 2.04 and 1.62, respectively, compared to the average for the genome. Chromosomes 4, 13, 21, X and Y are exon-poor. A similar pattern emerges in the density of transcriptional units across the chromosomes, as shown in figure 2B (Zhou et al., in press). Reports based on integrated radiation hybrid maps of ESTs [35, 36] indicated that chromosomes 1 and 22 were more gene-rich, but otherwise broadly agree with our results.

An intriguing clinical observation follows from these data and the tissue-specific observations. It had been noted [32] that the aneuploidies that are compatible with survival until birth (trisomies 13, 18, and 21, as well as X and Y aneuploidy) appeared to occur in relatively gene-poor chromosomes. Our data confirm these observations. However, the most obvious models for the deleterious effects of aneuploidy should instead depend on the total number of genes. In examining our HINT transcripts we have found that in fact the total number of embryo-specific transcripts is lowest on these 5 chromosomes (Figure 3). We suggest that trisomy of other chromosomes may exceed a limit of survivable dosage compensation during development.

Comparisons to genetic and RH maps

A total of 3,628 Genethon markers from the Marshfield map were localized via e-PCR [37] on the assembly, along with 28,350 Genebridge 4 markers/ESTs and 4,688 Stanford G3 markers appearing in Genemap ’99. Figure 4 shows the positions of markers on the Chromosome 1 assembly. The curves are nearly monotonically increasing, showing that the assembly is broadly correct, although localized orientation errors and outliers remain (plots for all chromosomes appear at http://pandora.med.ohio-state.edu/Annotation). These plots are immediately useful as they enable the placement of new markers on genetic maps without the need for mapping experiments. Some of the variation likely reflects estimation error in the published maps, and the curves are not completely monotone for finished chromosomes 21 and 22. However, other regions likely reflect errors in assembly, as the genetic and RH maps agree with each other but disagree with the assembly (e.g., the 130-148 Mb region is reversed on chromosome 5; a 15 Mb region of Xqter belongs at Xpter; numerous other isolated reversals and extensive reversals on chromosome 16). The genetic map shows a higher recombination rate per unit physical distance (i.e., higher slope) at

the telomeres, and a low male recombination rate (and thus sex-averaged rate) near the centromere (~130 Mb). Similar patterns hold for the entire genome. These observations agree with previous studies which had been limited to comparisons of genetic and RH maps [38], male/female meiotic ratios [39], or relatively few markers on well-sequenced chromosomes [39]. The plots offer an interesting perspective on positional cloning efforts. For example, examination of the plots reveals that the hemochromatosis gene *HFE*, at 28 Mb on 6p, lies at the edge of a recombination "cold spot" from 28-40 Mb. This fact complicated efforts to map the gene via linkage disequilibrium [40]. In contrast, the *NIDDM1* gene at 2qter (a region with higher recombination rate) was initially mapped to a 7 cM region, which fortunately was discovered to be only 1.7 Mb of sequence [41].

The radiation hybrid plots tend to be more linear, which is consistent with the model that radiation induces chromosomal breakpoints essentially uniformly [42]. However, jumps in the GB4 map occur at the centromere on most chromosomes. This may result from incomplete centromeric sequencing and assembly, so that a large centromeric gap might not appear as such. Alternatively, the jumps may reflect statistical difficulties in estimating breakpoint rates across the centromere. We note that no jump occurs in the G3 map, apparently because the higher radiation intensity produces insufficient marker pairs in the rescued hybrids that span the centromere. Thus the jump cannot be accurately estimated and was simply suppressed in the published map (http://www-shgc.stanford.edu/Mapping). A large unrecognized sequence gap would then appear as a flat region on G3 plot, which does not occur. An alternative possibility is that the jumps reflect increased radiation sensitivity at the centromere. This is worthy of additional investigation.

Clusters and compartments

The availability of the full assembly enables a comparison of the entire genome to itself for evidence of homology arising from duplications or insertions. We emphasize that the genome is still in draft form, and a complete description of these features will be a large and ongoing scientific and computational task. We used BlastN [43] to identify intra-chromosomal homology and to provide an initial look at the genomic landscape. Local duplication is a feature common to all chromosomes, as evidenced by the near-diagonal runs in dot-matrix plots in which the line of complete identity has been removed (Figure 5, full page plots for each chromosome at http://pandora.med.ohio-state.edu/Annotation). These runs vary across the chromosomes, and tend to be of high sequence identity, indicative of recent origin. More distant duplications also occur, and include large repetitive regions of high identity on chromosomes 10 and 17. The Y chromosome shows strong internal sequence similarity, some of which arises from strikingly long duplications (from several on the order of 100 kb to a duplication of almost 1 Mb near the q-

terminus of the euchromatic region). Near-duplicate sequences appear through the genome, producing a "plaid" appearance on many chromosomes. These sequences tend to have lower sequence similarity (blue in Figure 5), consistent with an ancient origin and accumulated mutations. As an example of functional duplication, we note that more than 60% of the entire zinc-finger (ZNF) families are mapped to chromosome 19, restricted to six tandemly duplicated gene clusters spanning the chromosome. More than one type of ZNF is found within each cluster, presumably resulting from sequence divergence. A majority of these ZNFs are densely populated within the 22-27 Mb region (see Figure 5). The remaining ZNFs are mapped to 15q21 (bZIP), 7q11 (KRAB), 11q13 (C_3HC_4), 11q23 (C_3HC_4), 6p21 (C_2H_2), 10p11 (KRAB), 10q11 (C_2H_2), 16p11 (C_2H_2), 9q22 (C_2H_2), and 3p21 (C_2H_2). Regions of high and striking similarity and the list of matching sequences with protein homology are provided at http://pandora.med.ohio-state.edu/Annotation.

Discussion

Comparison of gene counts

Our count of 66,000-75,000 transcriptional units on the genome is consistent with gene count estimates [25, 44] that had held sway until recent widely varying estimates [10, 45, 46]. Ewing and Green [10] examined 680 assumed genes on chromosome 22 and found matches to 2% of a selected set of assembled EST contigs. The sampling approach assumes that the 680 genes represent 2% of all genes, resulting in an overall count of 34,000. An examination of evolutionarily conserved regions in known genes on chromosome 22 in humans vs. the fish *T. nigorviridis* [45] results in an estimate of ~30,000 genes, assuming a uniform rate of conserved regions per true gene. These approaches resulted in similar estimates when applied to larger sets of mRNAs or known genes, and are similar to the current 33,000 genes reported by Ensembl as having Genscan computational support and EST confirmation. All of these estimates are carefully constructed and remarkably concordant, and we propose possible explanations for the difference from our results. The differences do not result entirely from the reliance on transcriptional evidence, as has been proposed [47].

Our estimate of 854 genes on chromosome 22 is 25% greater than that of Ewing and Green noted [10], but represents only 1.4% (rather than 2%) of our gene total. It was noted [10] that high expression on chromosome 22 could result in low gene count estimates by biasing the reference sample. In addition, known genes may be more highly expressed than unknown genes, which presumably aided their initial identification and characterization. Our evaluation of EST evidence supports the existence of both forms of bias. We have found that 5% of Ewing and Green's original set of EST contigs (selected with less stringent criteria than those used to estimate gene counts) map

to chromosome 22. An examination of UniGene transcripts (May '00) reveals that the known genes contain a median of 41 entries, while anonymous transcripts contain a median of just two entries. This is not entirely explained by the greater length of the known gene-like transcripts (having been correctly assembled as a single unit). In dividing the number of ESTs in the consensus by its length, we obtain a median of 0.017 entries/bp for known genes and 0.005 entries/bp for anonymous transcripts. On chromosome 22, the median number of ESTs per anonymous transcripts is three, which is significantly higher than that among other transcripts on the genome (geometric mean 3.76 vs. 3.11 for other chromosomes, $p<0.0001$, Wilcoxon rank-sum test). The estimate based on conserved regions [45] is calibrated using known genes. This approach also introduces bias, as such genes appear more likely to belong to the evolutionary core proteome. Known genes comprise 22% of all of our transcriptional units, but comprise 71% of our units which are conserved with rodents, *Drosophila* and *C. elegans*. A recent high gene estimate based on transcript evidence [46], again using chromosome 22, appears to result from less stringent alignment criteria, resulting in many putative genes.

As genomic annotation proceeds, the number of protein-encoding genes will become clearer. Our approach seems to rule out artifactual or genomic contamination as the predominant explanation for transcriptional units with unknown function or protein homology. Ensembl has recently listed a count of 170,160 'confirmed' exons, while we report 299,014 in complete ORFs and many more in untranslated regions, suggesting that our approach identifies considerable additional transcription. We point out that only 58% of known genes exhibit protein homology (Table 1), and e.g. a large proportion of transcriptional units have not been functionally classified in *Drosophila* [2]. We thus propose that most of the unclassified transcriptional units are in fact coding – the lack of protein homology may reflect difficulty in studying these proteins, or rapid gene evolution, and some portion is likely to function at the RNA level [48].

Clustering of ontological groups

We examined the locations of all transcriptional units that had been classified according to Gene Ontology (Table 2) for evidence of regional clustering. We applied a test that corrected for regional gene density, and found substantial evidence for regional clustering among the transcripts belonging to the same category (location plots for the top 60 ontological categories at http://pandora.med.ohio-state.edu/Annotation). Such clustering is pervasive – much of it likely to have arisen from duplication in which functional units have been preserved.

We also examined the runs of six or more gene units in which the ontological classifications occur in the same order (or the reverse) in multiple locations on the genome. A dot-matrix plot

across the genome appears at http://pandora.med.ohio-state.edu/Annotation. The plot shows clear evidence of local duplication, while the distant matches (even across chromosomes) are under investigation in the context of the complete sequence. We have noticed interesting associations among membrane proteins, ion channels, electron transporters, ATP binding cassettes, and genes involving metabolism on chromosomes 2, 5, and 7, suggesting that proximity may be important for regulating functionally coupled genes. This phenomenon is well established in lower organisms [49]. Similar physical-functional coupling has also been recently reported in yeast [50].

As an additional demonstration of the duplication phenomenon, we considered the occurrence of Pfam motifs within ORF, with only the best Pfam match retained per ORF (~1,930 of the 2,011 Pfam categories were represented). Matching successive runs of four or more (that occur at least three times on the genome) appear on http://pandora.med.ohio-state.edu/Annotation. Many of the runs occur on the near-diagonal. Most involve four identical Pfam categories in succession, or a double run of two categories, again pointing to local duplication.

Concluding remarks

The human genome is a capacious resource that will support years of intensive investigation. The quality of the draft sequence has now reached the point that genetic maps can truly be integrated into the genome. Analysis at the sequence level shows pervasive local and distant duplication, much of which preserves function. We have found evidence for a large number of transcriptional units (65,000-75,000) and performed initial annotation and classification. The effective study of transcription and protein function requires the compilation of all available evidence of transcription and protein homology. We have created such a resource to aid in this effort.

Materials and Methods

Exon identification The June 26, 2000 version of the repeat-masked draft sequences was downloaded from http://www.ensembl.org and blasted against cDNA and protein sequences by using the Blast program compiled from the NCBI toolkit (6.1) on a 32-node SGI Linux/Intel Cluster, with four 550MHz Pentium III Xeons processors and 2GB of RAM on each node. The following databases were used: Human UTR-DB (EBI) ftp://ftp.ebi.ac.uk/pub/database/UTR (v. 13); HTDB (Baylor University) http://www.hgsc.bcm.tms.edu/HTDB (v. 1); GenBank CDS (NCBI) ftp://ncbi.nlm.nih.gov/blast/db/nt.Z (only PRI mRNA sequences were used, v. 119); HINT (Ohio State University) http://pandora.med.ohio-state.edu/HINT; EG (University of Washington)

http://www.phrap.org/est_assembly; THC (TIGR) http://www.tigr.org/tdb/hgi (v. 4.5); dbEST (NCBI) ftp://ncbi.nlm.nih/blast/db/est_human.Z (v. 119); MINT (Ohio State University) http://pandora.med.ohio-state.edu/HINT; RINT (Ohio State University) http://pandora.med.ohio-state.edu/HINT; EMBL Rodent (EMBL) ftp://ftp.ebi.ac.uk/pub/databases/embl/release/rod.dat.gz (v. 63); SWISS-PROT (EMBL) http://www.ebi.ac.uk/SWISS-PROT (v. 39); TrEMBL (EMBL) http://www.ebi.ac.uk/SWISS-PROT (v. 14); PIR (MIPS-JIPID) http://pir.georgetown.edu (v. 65); and Pfam (Sanger Centre) http://www.sanger.ac.uk/Software/Pfam (v. 5.4). The Mouse and Rat Indices of Non-redundant Transcripts (MINT and RINT) were derived from Mouse and Rat UniGene (http://ncbi.nlm.nih.gov/unigene) using the same approach we have applied to human UniGene (Zhou et al., in press). Briefly, chimeric sequences were removed, UniGene transcripts were assembled into sequence contigs, and links to progenitor records retained.

The genome-wide hit expectation value was set at $E<10^{-25}$ (BlastN) or $E<10^{-15}$ (BlastX) to filter out non-specific high-scoring segment pairs (HSPs). Default parameters of Blast were used. The Blast report was parsed into field-specific tables using the program MSPcrunch (ftp://ftp.cgr.ki.se/pub/prog, Version 2.3). The resulting table was processed using a set of Perl scripts by first retaining only the HSPs that were spliced from the same transcripts on the same genomic contig. The same process was then applied to the HSPs on the genomic sequences, that spliced HSPs from the same transcripts were retained followed by the singleton HSPs that were both longer and higher in sequence identity over their overlapping counterparts, resulting in a unique placement for each cDNA segment on the genomic sequence.

Prediction of transcriptional units A set of Perl scripts was used to implement the algorithm described above. Genomic clones were ordered and oriented using the fingerprint map and draft assembly. Within unfinished clones, sequence contigs were further ordered and oriented according to Ensembl's assembly (ftp://ftp.sanger.ac.uk/pub/enembl/data/mysql/contig.txt.table.gz). This mapping produced the positional context necessary for consolidating fragmented exon units. Where necessary, small sequencing gaps (100 bp or fewer) were ignored and genomic clones were considered contiguous except where a large gap was indicated in the draft assembly ($\geq$50 kb). ORFs were determined using the program getorf (http://www.emboss.org).

Gene mapping A relational database was used to integrate multiple largely independent maps for the genomic clones, where transcripts had been placed. This integration thus results in a transcript map based on the order and position of genomic clones. Individual sequencing contigs within each unfinished clone were oriented using the Ensembl contig map

(ftp://ftp.sanger.ac.uk/pub/ensembl/data/mysql/contig.txt.table.gz). The fingerprint (http://genome.wustl.edu/gsc/human/Mapping, version June 15, 2000), GoldenPath assembly (Versions June 15 and September 5, 2000), and radiation hybrid maps (ftp://ncbi.nlm.nih.gov/repository/genemap/Mar1999) were used to place genomic clones into their chromosomal context. Since a substantial number of the clones in the working draft had not been physically typed with RH or genetic markers, the program e-PCR [37] and primers collected in the RHdb (http://corba.ebi.ac.uk/RHdb) and Genethon (http://www.genethon.org) were used under stringent criteria (mismatch=0, margin=50, and word size=7). Genetic mapping information was obtained from the Marshfield map (http://research.marshfieldclinic.org/genetics). In addition, Genemap'99 for cDNA was integrated into the genomic clones harboring HINT consensus transcripts. For the HINT consensus with more than one mapped EST, an averaged RH position was used. Cytogenetic bands were inherited from the original UniGene database. Furthermore, we incorporated a weighted composite quality score for the following four maps: Genemap'99 (the number of consistently mapped ESTs and their associated genomic clones), e-PCR (the number of consistently mapped STSs in a genomic clone), FPC (the supporting evidence in the original database), Blast (evidence of splicing). Based on such an integrated database schema, mapping information from sequence, clone, contigs, radiation hybrid, and cytogenetic positions for a given transcript could be obtained through a SQL join statement.

Tissue-specific transcripts

We noted the total number of ESTs contributed by each tissue to compute an expected proportion. For each HINT consensus transcript, we identified the tissue/source contributing the most ESTs to the consensus. The expected binomial distribution for the fixed number of ESTs in the consensus was used to compute a p-value, which was then Bonferroni-corrected for the 81 tissues X 67,000 HINT consensus transcripts.

Cytoband alignment G bands are known to be relatively AT rich, but the precise relationship between sequence and cytoband position is too poorly understood to be used for alignment. Genes/ESTs with cytoband position appearing in UniGene were placed on the full genome assembly. Cytoband cutpoints were used to create a scatterplot with the center of the cytoband forming the x-coordinate, and assembly position as the y-coordinate. Outliers were identified as points lying more than 2.5 standard errors outside of prediction intervals from a third degree polynomial regression fit. A Loess regression fit was used on the remaining points to estimate cytoband boundaries, with p and q arms fit separately. Centromeres and heterochromatic regions

were assumed not sequenced, based on a review of current clone frameworks. Primary sources for assignments of genes to heterochromatic regions were examined and in most cases deemed inconclusive. An exception is chromosome 19, which has a considerable number of genes assigned to 19q12 and finished sequence in the region. Scatterplots and regression fits for the entire genome are at http://pandora.med.ohio-state.edu/Annotation.

Genomic feature correlations. All 1 Mb intervals were combined to produce Table 3, but statistical tests were performed by computing ratios and correlations within each chromosome separately, in order to account for correlation of features within each chromosome. These statistics were then compared across the chromosomes to an appropriate null value using single sample t-tests. Some of the features were skewed, and pairwise comparisons were performed using Spearman rank correlations. A Bonferroni multiple-comparison procedure was applied to the 15 unique correlations.

Regional functional clustering

Apparently significant clustering can arise from the fact that genes exhibit regional clustering. To correct for this, we considered the physical order of all mapped transcripts and calculated the distances (in ranked location) between transcripts belonging to the same ontological category. Under the null hypothesis, the transcripts in a category should be distributed uniformly among all mapped transcripts with ontological classification, and the successive distances are approximately truncated exponential. Based on this, we compared the observed tenth percentile of successive distances to that under null hypothesis to compute a p-value. All tests were highly significant, with $p<0.0001$ for 59 of the 60 largest categories, and quantile-quantile plots with observed vs. expected distributions showed striking evidence of clustering. These tests were confirmed with permutation tests with empirical generations under the null hypothesis. As a conservative correction for the possibility that separate transcriptional units that might belong to the same gene, we considered successive distances for every other transcript. These tests were also significant, with $p<0.01$ for the 60 categories.

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Figure Legends

Figure 1: Overview map of features on the entire human genome, based on the working draft assembly (June 15, 2000 release) and finished sequences for chromosomes 21 and 22. Ideograms are oriented with the p-arm at the top, and are assembly-corrected to form an approximate cytogenetic alignment with the features of the draft assembly depicted to the right of each ideogram. Sequencing gaps at the centromeres and contiguous heterochromatic regions are represented by horizontal lines. Chromosome 19 is an exception, for which evidence suggests that both heterochromatic regions are at least partially sequenced. Genomic features are presented as densities (i.e., proportion of bp occupied by each feature) in non-overlapping 1 Mb intervals. The densities are corrected for sequencing gaps indicated in the draft assembly as 50 kb-200 kb segments of Ns, but (with the exception of GC content) are not corrected for sporadic Ns of lower quality base calls, because these would not interfere with assignment of the feature to the assembly. Exon density (red) is based on high scoring pairs from Table 2, not necessarily in ORFs. CpG island density (blue) based on standard definitions [24] of a run of at least 200 bases with GC content > 50% and observed over expected CpG > 0.6, and implemented using the program cpg (www.sanger.ac.uk/Software). GC content (green) is the number of G or C bases divided by the number of non-N bases in the 1 Mb interval. LINE1 (blue) and *Alu* (black) repeat elements were determined using RepeatMasker (www.phrap.org) and minisatellites of repeat size 20-50 bp by the etandem program of the EMBOSS suite (www.emboss.org). Density ranges were selected to illuminate features across the genome while preserving a common scale to facilitate comparison. A number of values exceed the range for the feature and are truncated, with a small dot of the corresponding color (•) placed under the ordinate. The data points for the figure are available at http://pandora.med.ohio-state.edu/Annotation.

Figure 2. Coding sequence density for human chromosomes. **(A)** Proportion of assembled sequence that is exonic provides direct confirmation of previously hypothesized patterns of gene density. **(B)** Transcriptional units per Mb. Additional plots and data are at http://pandora.med.ohio-state.edu/Annotation.

Figure 3. Total number of embryo-specific genes (based on HINT clusters) for each chromosome. Chromosomes 13, 18, 21 and Y are clearly lower than other chromosomes.

Figure 4. The correspondence between the genetic map and physical location (upper panel) and radiation hybrid maps vs. physical location (lower panel). The Genebridge 4 (GB4, black) radiation hybrid map shows a jump at the centromere, reflecting a sequencing gap and possible increased radiation sensitivity in the region. The jump for the Stanford G3 map (blue) is not easily estimated and is suppressed in the published map. Chromosome 1 is shown here for illustration, while the corresponding figures and data points for the entire genome are available at http://pandora.med.ohio-state.edu/Annotation.

Figure 5. Repeat-masked chromosome sequences were divided into 1 Mb segments and analyzed against the entire chromosomal sequence. Matches of at least 70% identity (both forward and reverse) and $E<10^{-25}$ are plotted. The diagonal line of complete identity has been removed to clarify features near the diagonal. Plots for each chromosome are available at http://pandora.med.ohio-state.edu/Annotation.

Tables

Table 1. Identification of exons on the genome after vector screening using transcript, rodent, and protein databases. The definition of a record varies according to the database, while 'exons' refer to high-scoring segment pairs in BlastN comparisons ($E<10^{-15}$ and sequence identity > 90%) to the genome. Unique Exons and all subsequent columns refer to placements that were possible after considering the preceding databases. Placement of rodent transcripts required evidence of splicing and sequence identity >80%. Protein homology required BlastX $E<10^{-15}$. Pfam hits required score > 20 using hmmpfam (http://hmmer.wustl.edu). CpG islands were identified using cpgreport (http://www.emboss.org) using standard criteria [24].

Category	Database	Total Records	Percent Placed	Unique Exons	Exon Length (bp)	Putative Genes (Non-Splicing Singletons)	Protein Homology (Pfam Hit)	CpG Islands
Known genes	UTR-DB	40,258	80%	19,195	6,925,762	10,007 (426)	5,701 (3,813)	3,866
	HTDB	15,305	89%	48,477	11,893,081	4,816 (148)	2,938 (1,943)	1,960
Consensus	HINT	87,000	77%	103,817	23,381,024	20,357 (959)	9,121 (6,453)	7,557
Transcripts	EG	62,064	80%	13,085	4,562,954	4,800 (154)	2,177 (1,679)	2,462
	THC	84,837	81%	38,806	12,406,081	8,604 (322)	2,907 (2,026)	3,983
Transcripts	GenBank CDS	110,222	81%	41,917	5,303,064	2,634 (227)	1,858 (1,607)	1,178
	DbEST Human	2,154,995	73%	273,881	32,288,385	20,073 (7,136)	5,377 (3,745)	11,807
Rodent	MINT	92,531	30%	8,284	866,046	777	123 (56)	486
Transcripts	RINT	37,367	46%	5,600	592,788	458	65 (32)	255
	EMBL Rodent	43,488	28%	5,819	724,630	202	68 (72)	135
Protein	SWISS-PROT	86,593	38%	27,526	9,858,797	1,648	1,648 (1,244)	158
Homology	TrEMBL	351,834	13%	22,670	4,385,497	1,185	1,185 (654)	92
	PIR	182,106	16%	4,106	1,355,644	321	321 (132)	20
Total				**613,183**	**114,543,753**	**75,982 (9,372)**	**33,489 (23,008)**	**33,959**

Table 2. Ontological classification of 22,339 human gene products. Each transcriptional unit and HINT transcript (in parentheses) was assigned to a unique biological function or process.

Biological function	Number of transcripts	Biological process	Number of transcripts
Transcription factor	958 (306)	Carbohydrate metabolism	281 (84)
Translation factor	62 (27)	Nucleotide and nucleic acid metabolism	173 (51)
RNA binding	142 (41)	DNA replication	240 (126)
Ribosomal protein	232 (130)	Transcription	1,059 (651)
Cell cycle regulator	42 (16)	RNA processing	204 (59)
Structural protein	145 (48)	Amino Acid and derivative metabolism	87 (29)
Cytoskeleton structural protein	329 (181)	Protein biosynthesis	264 (162)
Extracellular matrix	361 (87)	Protein modification	235 (88)
Actin binding	66 (25)	Protein targeting	26 (5)
Motor protein	245 (77)	Protein degradation	136 (45)
Chaperone	87 (27)	Proteolysis and peptidolysis	96 (36)
Enzyme	2,664 (1,404)	Lipid metabolism	424 (187)
Protein kinase	895 (484)	Monocarbon compound metabolism	9 (3)
Protein kinase inhibitor	19 (12)	Coenzyme and prosthetic group metabolism	92 (29)
Protein phsophatase	43 (7)	Steroid compound metabolism	40 (10)
Protein phsophatase inhibitor	17 (3)	Prostaglandin metabolism	12 (3)
Protease	441 (255)	Transport	549 (288)
Protease inhibitor	92 (37)	Electron transport	491 (273)
Enzyme activator	18 (3)	Ion transport	302 (90)
Enzyme inhibitor	14 (4)	Small molecular transport	19 (9)
Alkyl transfer	17 (3)	Neurotransmitter transport	9 (3)
Amide transfer	15 (3)	Ion homeostasis	201 (57)
Carbonyl transfer	191 (38)	Organelle organization and biogenesis	408 (254)
Hydroxyl transfer	13 (6)	Nuclear organization and biogenesis	1,380 (647)
Phosphoryl transfer	823 (281)	Cytoplasm organization and biogenesis	42 (20)
Oxireduction	148 (76)	Meiosis	15 (2)
Transmembrane protein	184 (48)	Mitosis	25 (6)
Receptor	921 (478)	Cell cycle	271 (100)
G protein-linked receptor	164 (106)	DNA packaging	15 (6)
Defense/immunity protein	353 (164)	DNA repair	132 (41)
Ligand binding or carrier	691 (331)	DNA recombination	31 (3)
Ion channel	245 (141)	Methylation	185 (53)
Oncogene	128 (42)	Signal transduction	1,231 (383)
Tumor suppressor	8 (6)	Growth regulation	15 (4)
Growth factor	95 (40)	Differentiation	24 (6)
Hormone	42 (14)	Apoptosis	160 (49)
Cell communication	247 (84)	Angiogenesis	11 (4)
Cell adhesion	433 (252)	Defense/immunity	112 (49)
		Detoxification	33 (15)
		Stress response	90 (41)
		Developmental process	278 (99)
		Neurogenesis and regeneration	147 (43)
		Physiological process	159 (43)
		Sensory perception	292 (65)
Functionally classified	**12,334 (5,204)**	**Process classified**	**10,005 (4,225)**

Table 3. **(Top)** Densities of features in major cytogenetic bands by Giemsa staining. Pale-staining (R) and dark-staining (G) bands are compared, with alignment of cytogenetic bands to sequence as described in text. All of the features except LINE1 elements are denser in the R bands. The true differences are likely to be larger, as errors in cytoband alignment will tend to understate the differences in the band types. The differences in the bands are highly significant at $p<0.001$ for all features except for minisatellites ($p=0.006$). **(Bottom)** Rank correlations of features, in 1 Mb intervals ($p=0.03$, corrected for multiple comparisons).

Density of features per Mb in Giemsa-staining cytogenetic bands

	R	**G**	**R/G ratio**
Exons	0.0415	0.0319	1.30
CpG islands	0.0119	0.0075	1.59
GC content	42.23%	39.76%	1.06
LINE1 repeats	0.1435	0.1602	0.90
***Alu* repeats**	0.1204	0.0937	1.28
Minisatellites	0.0090	0.0078	1.15

Correlation of features in 1 Mbase intervals

	Exon	**CpG**	**GC**	**LINE1**	***Alu***	**Minisatellite**
Exon	1.00	0.65	0.64	-0.26	0.73	0.19
CpG		1.00	0.73	-0.42	0.58	0.16
GC			1.00	-0.54	0.61	0.13
LINE1				1.00	-0.20	0.28
Alu					1.00	0.23
Minisatellite						1.00

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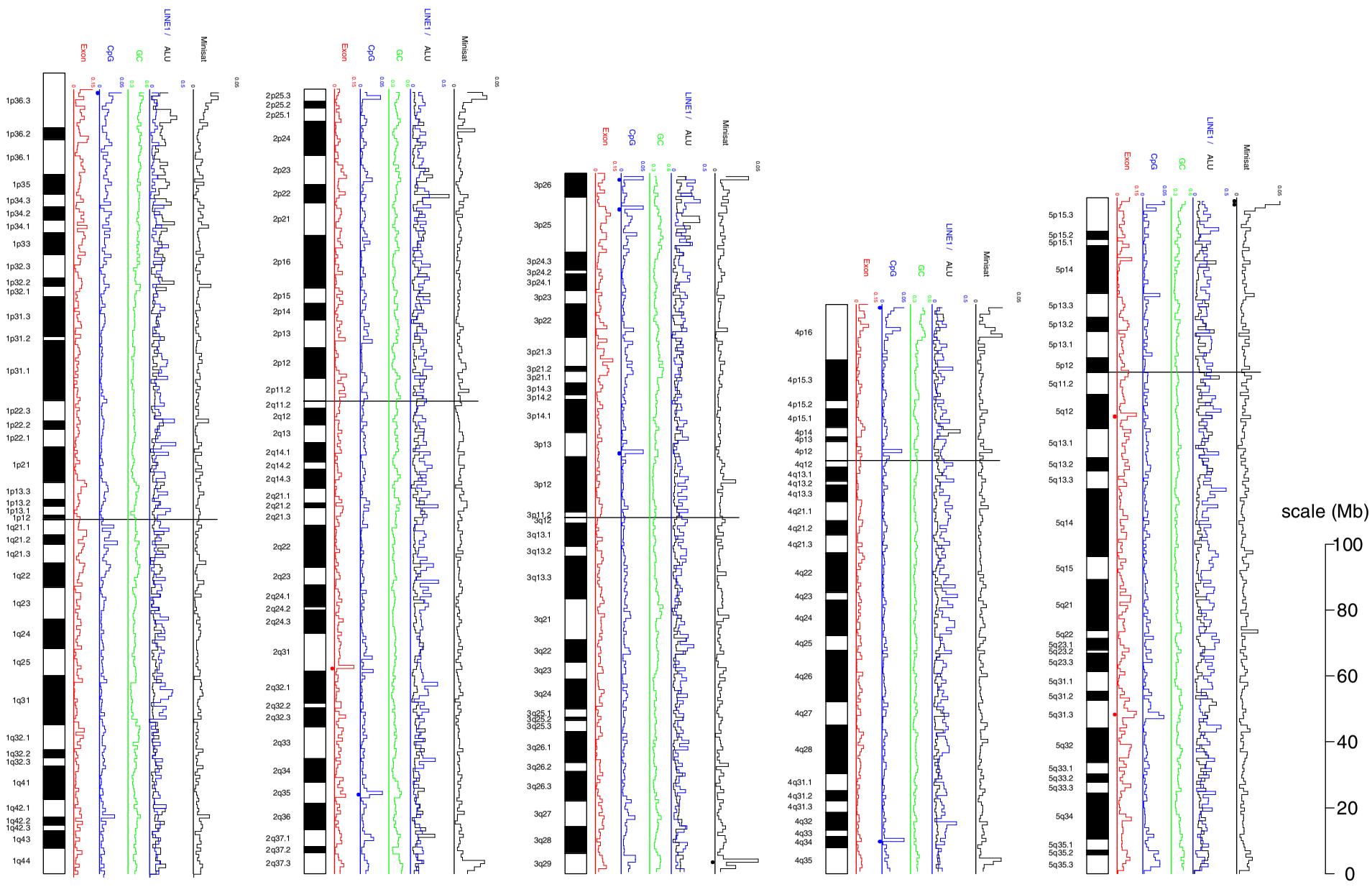
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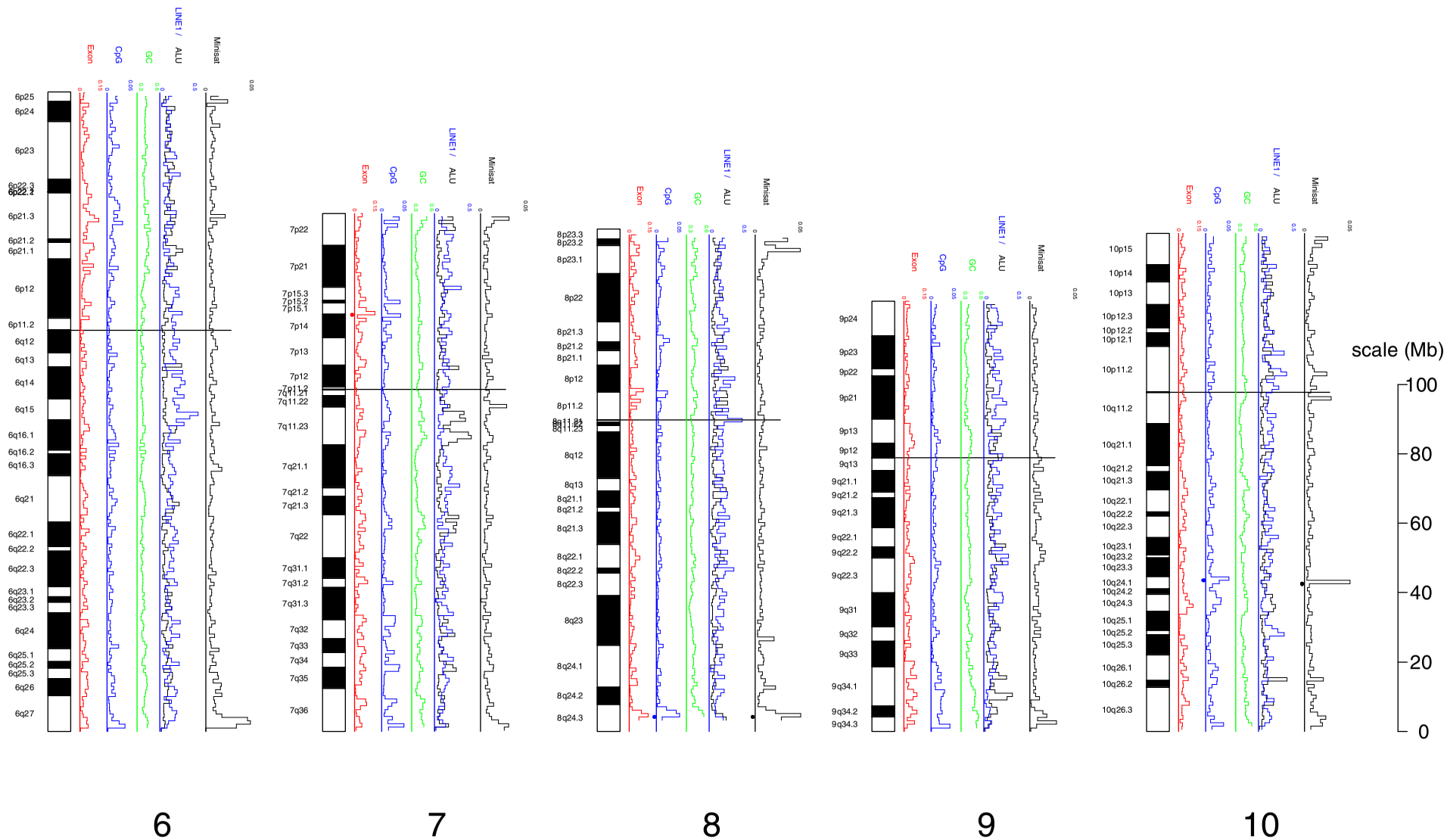
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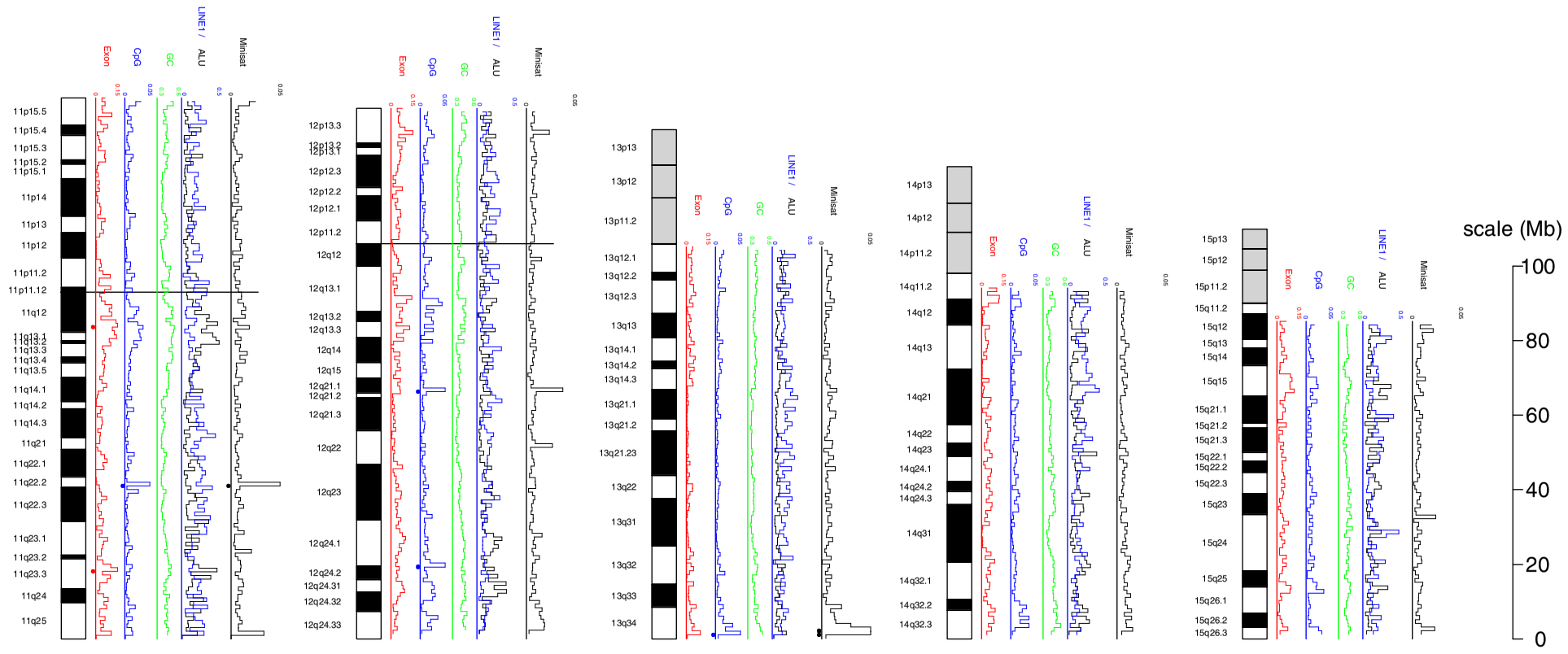
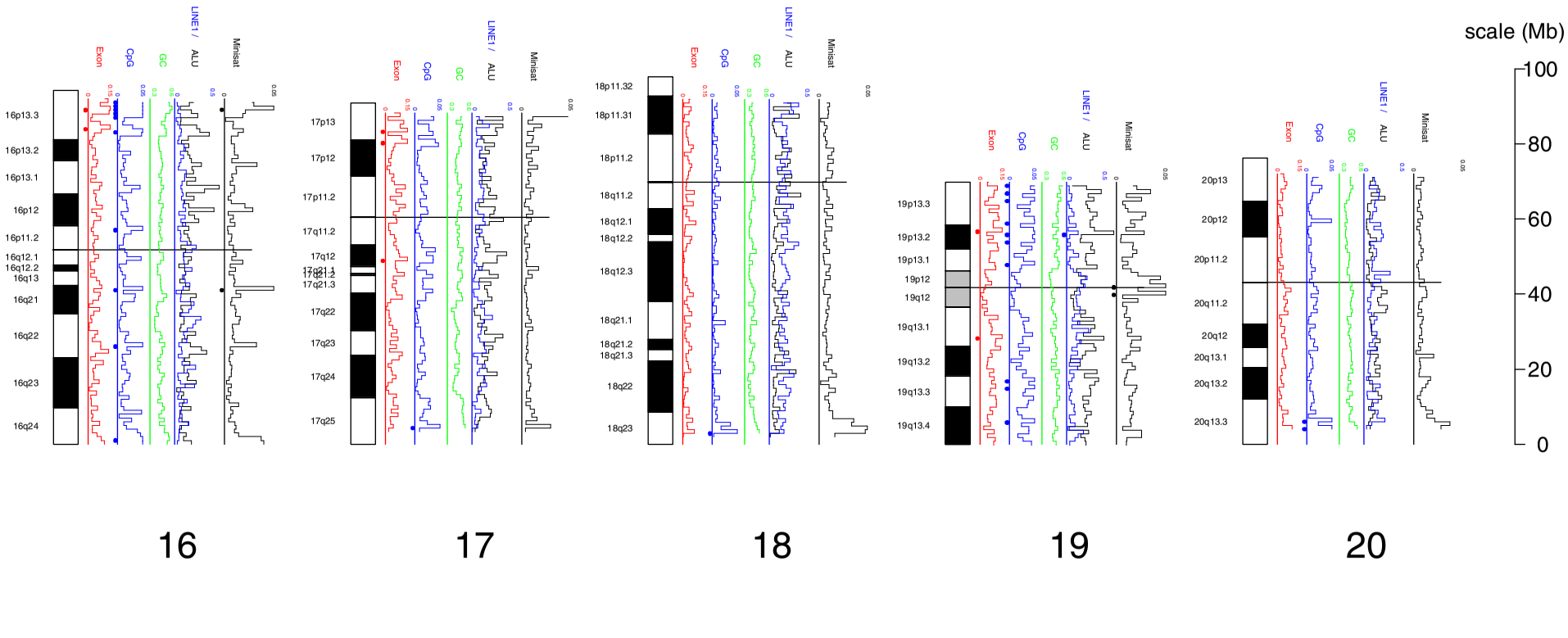
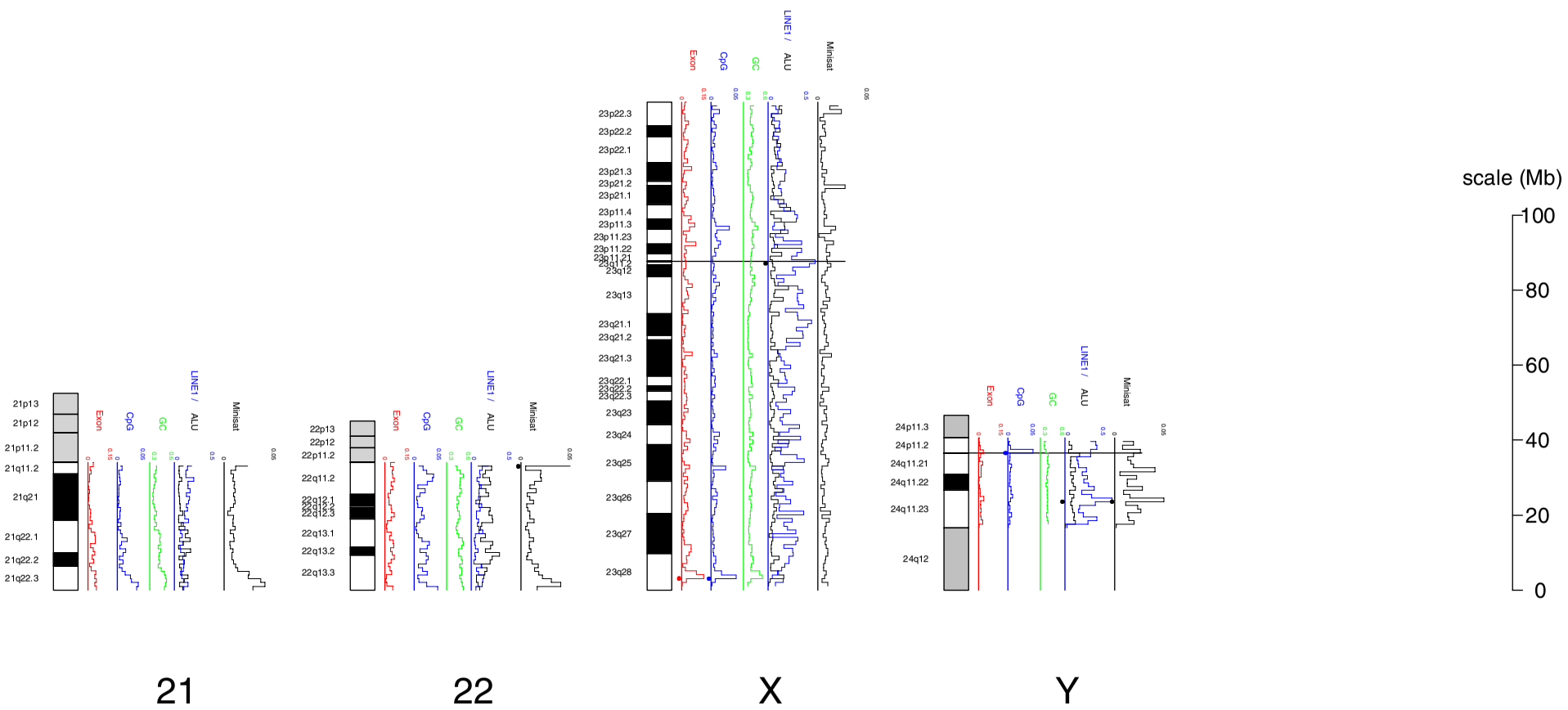
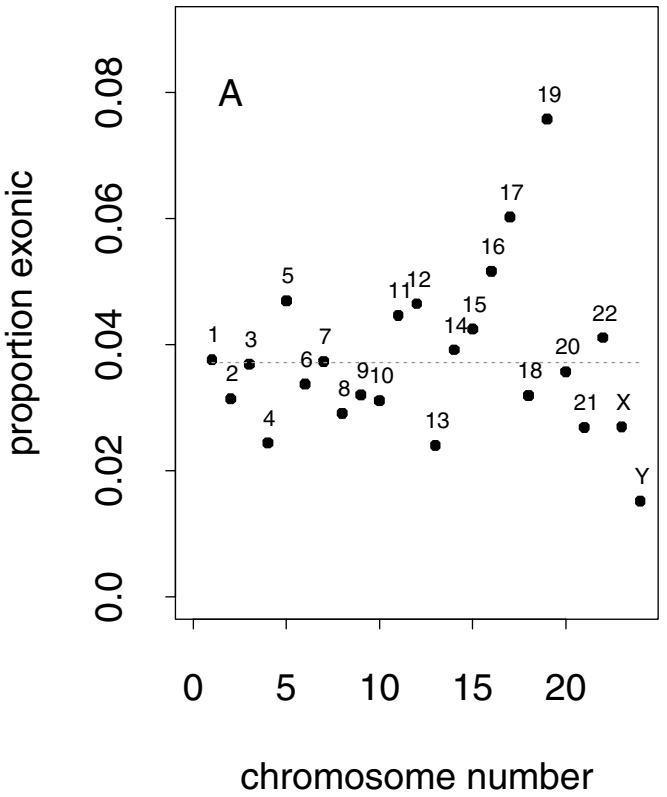
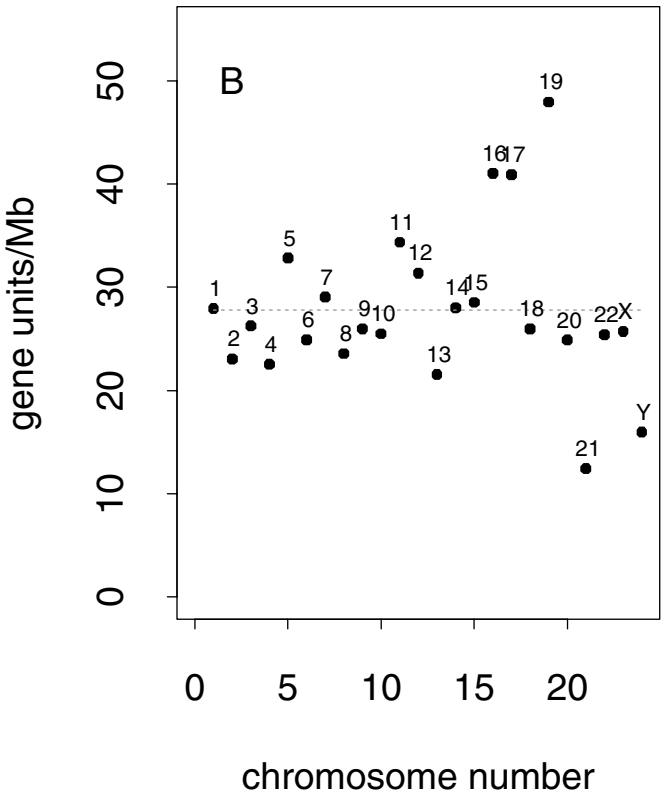
LINE1 / ALU
Minisat
scale (Mb)
100
80
60
40
20
0
6
7
8
9
10


Exon
CpG
GC
LINE1 / ALU
Minisat
scale (Mb)
100
80
60
40
20
0
11p15.5
11p15.4
11p15.3
11p15.2
11p15.1
11p14
11p13
11p12
11p11.2
11p11.12
11q12
11q13.1
11q13.2
11q13.3
11q13.4
11q13.5
11q14.1
11q14.2
11q14.3
11q21
11q22.1
11q22.2
11q22.3
11q23.1
11q23.2
11q23.3
11q24
11q25
12p13.3
12p13.2
12p13.1
12p12.3
12p12.2
12p12.1
12p11.2
12q12
12q13.1
12q13.2
12q13.3
12q14
12q15
12q21.1
12q21.2
12q21.3
12q22
12q23
12q24.1
12q24.2
12q24.31
12q24.32
12q24.33
13p13
13p12
13p11.2
13q12.1
13q12.2
13q12.3
13q13
13q14.1
13q14.2
13q14.3
13q21.1
13q21.2
13q21.23
13q22
13q31
13q32
13q33
13q34
14p13
14p12
14p11.2
14q11.2
14q12
14q13
14q21
14q22
14q23
14q24.1
14q24.2
14q24.3
14q31
14q32.1
14q32.2
14q32.3
15p13
15p12
15p11.2
15q11.2
15q12
15q13
15q14
15q15
15q21.1
15q21.2
15q21.3
15q22.1
15q22.2
15q22.3
15q23
15q24
15q25
15q26.1
15q26.2
15q26.3
11
12
13
14
15

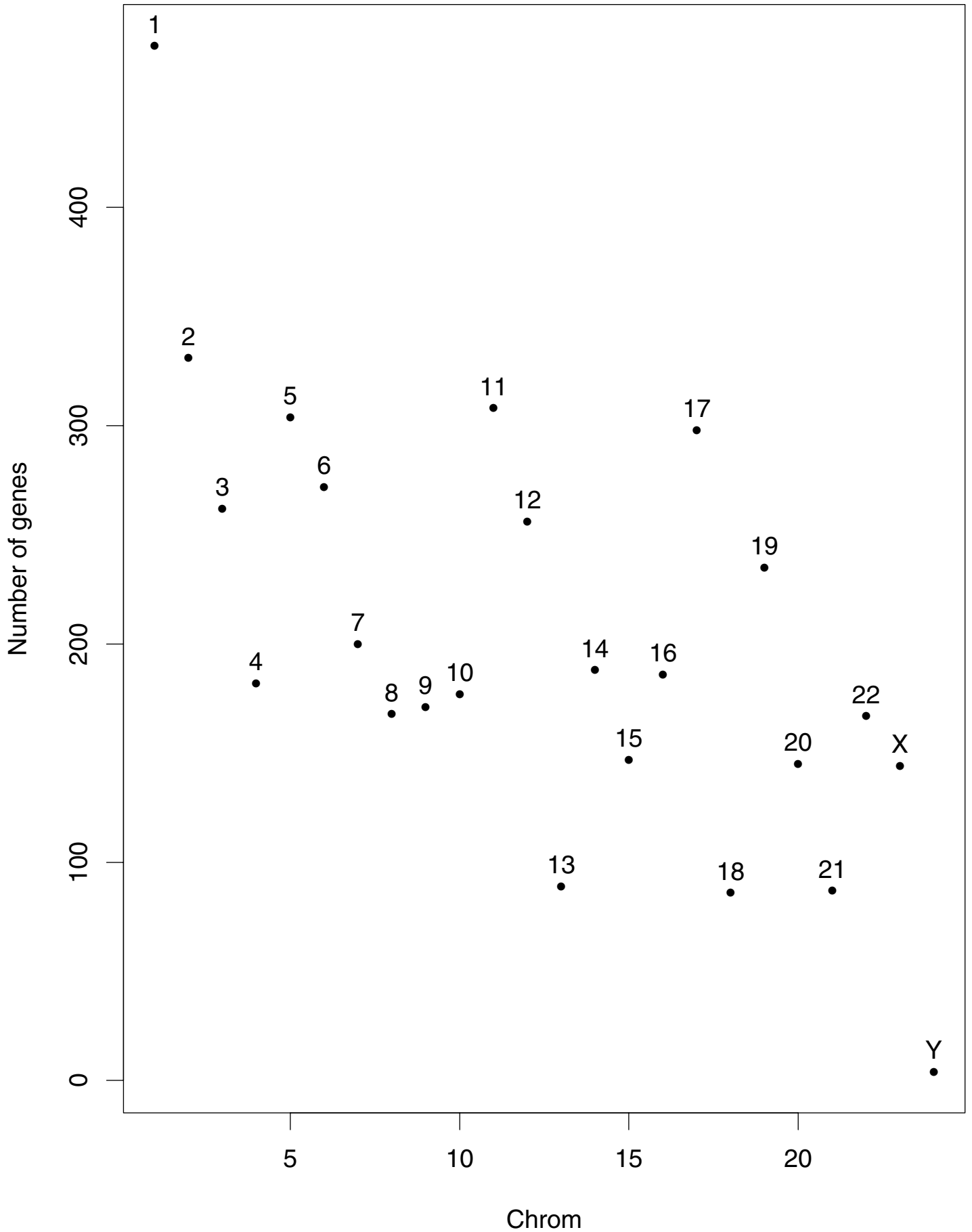

scale (Mb)
100
80
60
40
20
0
Exon
CpG
GC
LINE1 / ALU
Minisat
16p13.3
16p13.2
16p13.1
16p12
16p11.2
16q12.1
16q12.2
16q13
16q21
16q22
16q23
16q24
17p13
17p12
17p11.2
17q11.2
17q12
17q21.1
17q21.2
17q21.3
17q22
17q23
17q24
17q25
18p11.32
18p11.31
18p11.2
18q11.2
18q12.1
18q12.2
18q12.3
18q21.1
18q21.2
18q21.3
18q22
18q23
19p13.3
19p13.2
19p13.1
19p12
19q12
19q13.1
19q13.2
19q13.3
19q13.4
20p13
20p12
20p11.2
20q11.2
20q12
20q13.1
20q13.2
20q13.3
16
17
18
19
20

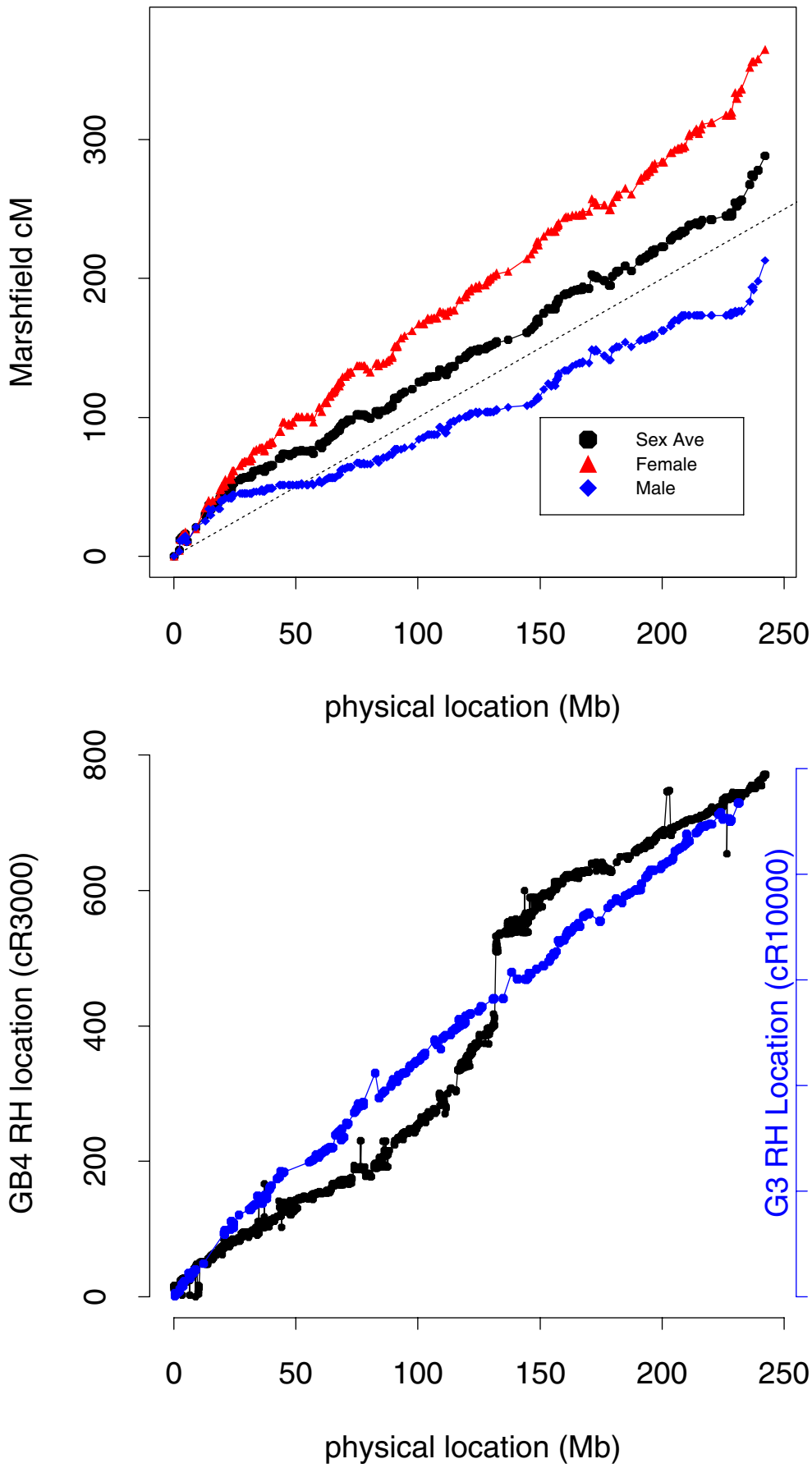

Exon
CpG
GC
LINE1 / ALU
Minisat
21p13
21p12
21p11.2
21q11.2
21q21
21q22.1
21q22.2
21q22.3
22p13
22p12
22p11.2
22q11.2
22q12.1
22q12.2
22q12.3
22q13.1
22q13.2
22q13.3
23p22.3
23p22.2
23p22.1
23p21.3
23p21.2
23p21.1
23p11.4
23p11.3
23p11.23
23p11.22
23p11.21
23q12
23q13
23q21.1
23q21.2
23q21.3
23q22.1
23q22.2
23q22.3
23q23
23q24
23q25
23q26
23q27
23q28
24p11.3
24p11.2
24q11.21
24q11.22
24q11.23
24q12
scale (Mb)
100
80
60
40
20
0
21
22
X
Y

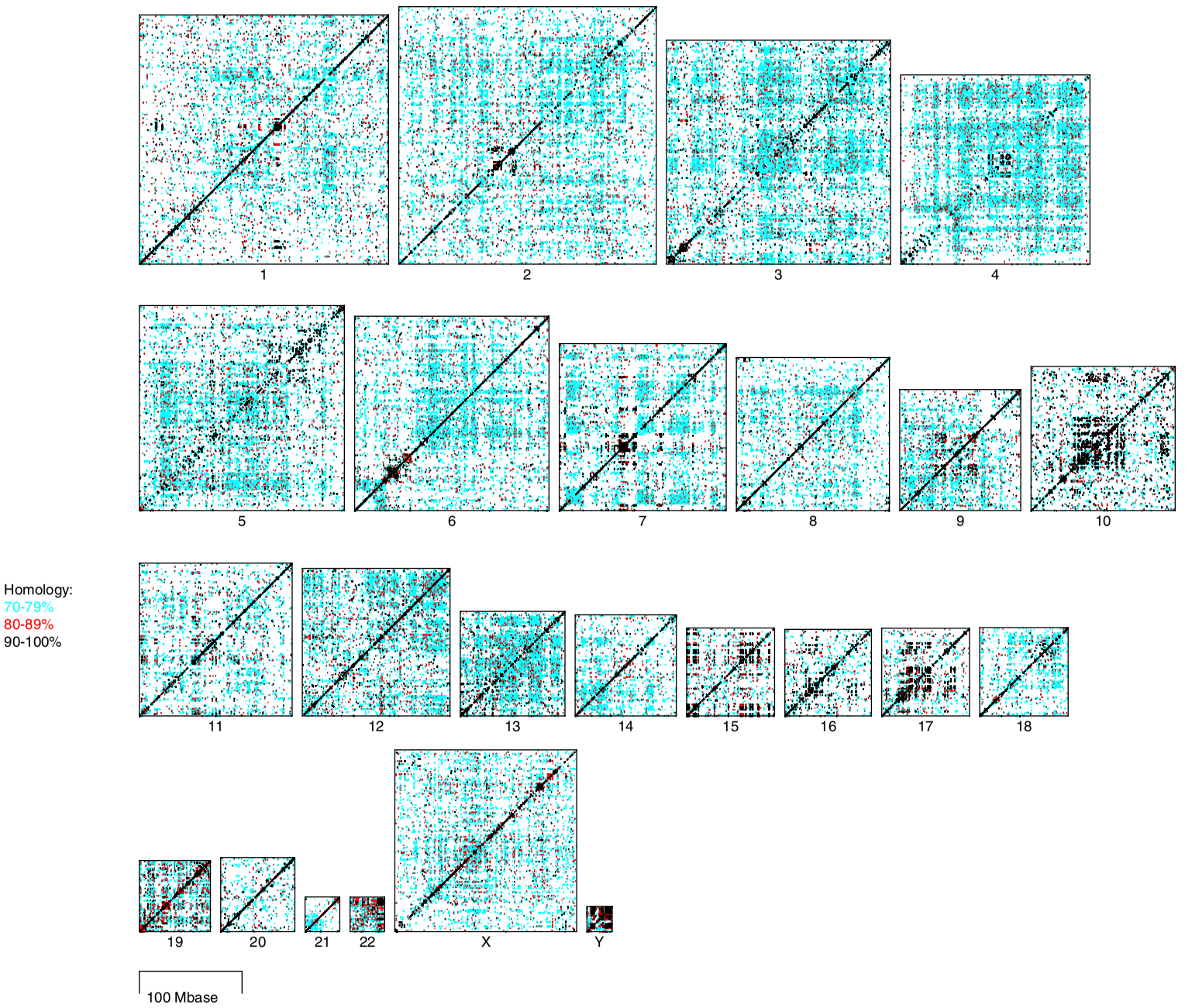

A
proportion exonic
0.0
0.02
0.04
0.06
0.08
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
0
5
10
15
20
chromosome number


B
gene units/Mb
0
10
20
30
40
50
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
0
5
10
15
20
chromosome number

Total number of embryo-specific genes




Marshfield cM
0
100
200
300
Sex Ave
Female
Male
0
50
100
150
200
250
physical location (Mb)
GB4 RH location (cR3000)
0
200
400
600
800
G3 RH Location (cR10000)
0
2000
4000
6000
8000
0
50
100
150
200
250
physical location (Mb)



Homology:
70-79%
80-89%
90-100%