Genome-wide detection of segmental duplications and potential assembly errors in the human genome sequence

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Abstract

**Background:** Previous studies have suggested that recent segmental duplications, which are often involved in chromosome rearrangements underlying genomic disease, account for some 5% of the human genome. We have developed rapid computational heuristics based on BLAST analysis to detect segmental duplications, as well as regions containing potential sequence misassignments in the human genome assemblies.

**Results:** Our analysis of the June 2002 public human genome assembly revealed that 107.4 of 3,043.1 megabases (Mb) (3.53%) of sequence contained segmental duplications, each with size equal or more than 5 kb and 90% identity. We have also detected that 38.9 Mb (1.28%) of sequence within this assembly is likely to be involved in sequence misassignment errors. Furthermore, we have identified a significant subset (199,965 of 2,327,473 or 8.6%) of single-nucleotide polymorphisms (SNPs) in the public databases that are not true SNPs but are potential paralogous sequence variants.

**Conclusion:** Using two distinct computational approaches, we have identified most of the sequences in the human genome that have undergone recent segmental duplications. Near-identical segmental duplications present a major challenge to the completion of the human genome sequence. Potential sequence misassignments detected in this study would require additional efforts to resolve.

**Background**
Segments of DNA with near-identical sequence (segmental duplications or duplicons) in the human genome can be hot spots or predisposition sites for the occurrence of non-allelic homologous recombination or unequal crossing-over leading to genomic mutations such as deletion [1], duplication [1], inversion [2] or translocation [3,4]. These structural alterations, in turn, can cause dosage imbalance of genetic material or lead to the generation of new gene products resulting in diseases defined as genomic disorders [5].

Previous studies to identify segmental duplications in the human genome have analyzed older versions of the genome assembly, which contained higher amounts of unfinished
sequence and incorrectly mapped regions, and have used different computational approaches all performed by the same group [6-8]. With the human genome sequence now nearing completion, we have examined its content for segmental duplications using two distinct computational methods. In the first, we utilized the rapid BLAST2 [9] algorithms that allow direct chromosomal-wide sequence comparisons to be made. All BLAST results reported in table formats can be subsequently grouped, parsed and analyzed for the detection of duplicated sequences. In addition, we have shown previously that there is a strong correlation between ambiguously mapped SNPs (ambSNPs), as well as the density of SNPs, and segmental duplications [10]. AmbSNPs are SNPs that were annotated to map to two locations on a particular chromosome in the NCBI dbSNP. A subset of these ambSNPs are not true SNPs but are likely to be computer-generated nucleotide mismatches from paralogous copies of duplicated sequences and should be more appropriately labeled as paralogous sequence variants (PSVs) [10]. Another subset is likely to be false ambSNPs of genomic sequences that have been misassigned in genome assemblies. Here, we report our analysis of all potential PSVs in the human genome and their correlation with segmental duplications as detected by our BLAST analysis. Furthermore, we provide a critical assessment on the three latest human genome assemblies from our analysis of sequence misassignments as identified from this study.

Results and discussion

Human genome segmental duplication content

On the basis of the June 2002 (NCBI Build 30) human genome assembly, a total of 107.4 Mb (3.53%) of the human genome content (3,043.1 Mb) were found to be involved in recent segmental duplications by our BLAST analysis criteria (Table 1). This content is composed of more than 1,530 distinct intrachromosomal segmental duplications (80.3 Mb or 2.64% of the total genome, Figure 1) and 1,637 distinct interchromosomal duplications (43.8 Mb or 1.44% of the total genome). In addition, 29% of all duplications are located in unfinished regions of the current genome assembly. Our results are shown using the Generic Genome Browser [11,12]. We have also found that 38% of the duplications (52.3 Mb) can be considered as tandem duplications - defined here as two related duplicons separated by less than 200 kb.

In this study, we only analyzed large (size ≥ 5 kb) and recent (sequence identity ≥ 90%) duplications because we can achieve higher confidence and to prioritize those regions for their potential involvement in diseases. Previously, Bailey and colleagues [8] reported a total of 5.2% of the human genome involved in recent segmental duplications. The 1.6% discrepancy between our findings could be due to the difference in our detection criteria (size cutoff of 5 kb used in this study versus 1 kb used in Bailey et al. [8]). Moreover, we have identified 38.9 Mb of sequences (1.28% of the June 2002 genome assembly) likely to be artifactual duplications resulting from sequence misassignment errors present in the assembly. By comparing our results with those published previously [8], we found that 482/2579 clones that we identified to be involved in duplication were novel.

The molecular mechanism by which segmental duplications are created is still unclear at the moment. A recent report has suggested that Alu repeat clusters had a role as mediators of recurrent chromosomal rearrangements [13]. We have examined whether elevated amounts of repetitive elements could be found in duplkon junctions. We inspected all duplication borders from our results and calculated the occurrence of different repeat types within the 500 bp window outside each duplication junction. The whole-genome average frequencies were determined by sampling random 500 bp windows across the genome (excluding gap regions). Overall, we found that there are significant enrichment (or relative fold increase) for the presence of small ribonucleoprotein RNA (srpRNA), satellite, long terminal (LTR) and SINE/Alu repeats (see Additional data file). In addition, our data also showed that for some chromosomes the amount of duplicated sequence is higher in the pericentromeric and subtelomeric regions of chromosomes (Figure 1), supporting the hypothesis that these repeat-dense regions have made an important contribution to the evolution of the human genome [14].

Regions containing recently occurring segmental duplications can harbor rapidly evolving hominid-specific genes, as well as novel gene families that are unique to primates [15,16]. Using the National Center for Biotechnology Information RefSeq annotation, we identified 1,152 human genes that were mapped to duplicated regions. Of these, 475 genes were fully contained within duplicated regions and were best candidates for recent whole-gene duplication. We have carried out functional analysis of these 475 genes using the Gene Ontology Consortium database [17] and found that there is a significant increase in gene duplications for genes involved in immune defense (antibodies, blood-group antigens) and reproduction (pregnancy, sex differentiation) (see Additional data file).

Sequence misassignment errors in the human genome sequence assembly

We were aware that in silico detection methods, such as the ones used in this study, would not allow us to distinguish completely true duplications from artifactual duplications arising from misassigned sequences, especially in cases where sequence identity between two detected duplications exceeded 99.5% over a substantial length (> 5 kb) in regions composed of draft sequences. Although a small proportion of such results (duplications with > 99.5% identity) might represent unfinished regions of the genome that contain true duplications that have arisen very recently in the evolution of the human genome (such as the large and nearly perfect
palindromic repeats located in the AZFc region on chromosome Yq11.223 involved in male infertility [18]), we suspect that most of the duplications (> 99.5% identity and contain draft sequences) are in fact sequence misassignment errors in the genome assembly. An explanation for such errors would be when two identical sequences belonging to the same genomic location were misassigned to distinct regions in the genome assembly.

We have used the NCBI e-PCR [19] to evaluate our results (potential sequence misassignment errors) from the June 2002 human genome assembly. Using some of the largest interchromosomal misassignment errors detected in our study, we found that none of the STS markers located within these misassigned sequences maps to their incorrectly assigned chromosomes. For example, AC121339 is incorrectly mapped to 3q13.13 in the June 2002 genome assembly, as supported by a consensus number of chromosome X sequence-tagged site (STS) markers (Figure 2, Table 2).

From this genome assembly, we identified that a total of 38.9 Mb of sequences, representing 1.28% of the total sequence content, are involved in such potential errors (a full list of potentially misassigned sequences can be obtained from [12]) that would require additional effort and further sequencing to achieve resolutions. We also analyzed an

Table 1

| Chromosome | Size (bp) | Intra- % | Inter- % | Total % | Total duplications (bp) | % chromosome (previous) |
|------------|----------|---------|---------|---------|-------------------------|------------------------|
| 1          | 246,874,334 | 5,278,549 | 2.1 (4.4) | 2,854,898 | 1.2 (2.3) | 7,056,274 | 2.9 (5.7) | 4,369,406 | 1.8 |
| 2          | 240,681,600 | 4,917,160 | 2.0 (2.4) | 3,298,723 | 1.4 (1.6) | 6,892,585 | 2.9 (3.2) | 2,311,522 | 1.0 |
| 3          | 194,908,136 | 2,128,493 | 1.1 (2.3) | 1,654,201 | 0.8 (2.0) | 3,146,570 | 1.6 (3.2) | 3,979,610 | 2.0 |
| 4          | 192,019,378 | 2,599,650 | 1.4 (2.3) | 2,164,382 | 1.1 (2.2) | 4,061,432 | 2.1 (3.4) | 2,482,740 | 1.3 |
| 5          | 180,966,400 | 3,519,480 | 1.9 (2.0) | 1,464,945 | 0.8 (2.0) | 4,530,406 | 2.5 (2.8) | 2,297,998 | 1.3 |
| 6          | 170,309,517 | 2,358,252 | 1.4 (2.3) | 743,875 | 0.4 (1.3) | 2,877,392 | 1.7 (3.4) | 569,918 | 0.3 |
| 7          | 157,432,793 | 6,436,434 | 5.5 (6.3) | 2,614,326 | 1.7 (2.9) | 10,139,669 | 6.4 (7.8) | 205,130 | 0.1 |
| 8          | 143,874,322 | 2,318,984 | 1.6 (2.2) | 1,125,241 | 0.8 (2.0) | 2,877,392 | 1.7 (3.4) | 3,956,756 | 2.8 |
| 9          | 132,438,756 | 7,248,232 | 5.5 (7.1) | 4,801,871 | 3.6 (4.7) | 8,341,767 | 6.3 (8.2) | 1,589,734 | 1.2 |
| 10         | 134,416,750 | 3,519,480 | 2.6 (2.0) | 1,464,945 | 0.8 (2.0) | 4,530,406 | 2.5 (2.8) | 2,297,998 | 1.3 |
| 11         | 137,442,545 | 3,622,080 | 2.6 (3.3) | 1,670,412 | 1.2 (1.8) | 4,363,619 | 3.2 (4.4) | 2,028,875 | 1.5 |
| 12         | 131,300,572 | 1,894,547 | 1.4 (2.2) | 971,490 | 0.7 (1.2) | 2,816,187 | 2.1 (3.3) | 3,383,730 | 2.6 |
| 13         | 113,446,104 | 918,255 | 0.8 (1.9) | 1,202,102 | 1.1 (2.3) | 1,855,806 | 1.6 (3.4) | 146,198 | 0.1 |
| 14         | 104,324,908 | 531,219 | 0.5 (0.7) | 820,880 | 0.8 (1.6) | 1,335,177 | 1.3 (2.1) | 13,814 | 0.0 |
| 15         | 99,217,355 | 4,917,218 | 6.0 (6.6) | 2,228,116 | 2.7 (3.9) | 5,634,176 | 7.4 (9.8) | 2,113,843 | 2.6 |
| 16         | 81,671,585 | 4,917,218 | 6.0 (6.6) | 2,228,116 | 2.7 (3.9) | 5,634,176 | 7.4 (9.8) | 2,113,843 | 2.6 |
| 17         | 80,052,782 | 4,775,137 | 6.0 (7.1) | 646,968 | 0.8 (2.5) | 5,274,195 | 6.6 (8.5) | 2,145,614 | 2.7 |
| 18         | 77,516,809 | 525,636 | 0.7 (1.2) | 700,654 | 0.9 (1.9) | 1,226,290 | 1.6 (3.1) | 1,443,755 | 1.9 |
| 19         | 60,013,307 | 2,700,984 | 4.5 (6.8) | 704,757 | 1.2 (2.6) | 3,156,687 | 5.3 (8.1) | 335,190 | 0.6 |
| 20         | 62,842,997 | 592,441 | 0.9 (1.1) | 873,152 | 1.4 (1.8) | 1,052,248 | 1.7 (2.1) | 147,940 | 0.2 |
| 21         | 44,626,493 | 481,879 | 1.1 (1.4) | 1,303,776 | 2.9 (5.1) | 1,504,333 | 3.4 (5.2) | 0 | 0.0 |
| 22         | 47,748,585 | 1,741,766 | 3.6 (6.7) | 1,374,363 | 2.9 (7.4) | 2,770,386 | 5.8 (10.9) | 0 | 0.0 |
| X          | 14,924,981 | 2,625,206 | 1.8 (3.6) | 2,927,714 | 2.0 (2.3) | 5,518,712 | 3.7 (5.5) | 2,185,046 | 1.5 |
| Y          | 58,368,225 | 5,959,836 | 10.2 (28.4) | 3,524,276 | 6.0 (25.0) | 8,461,355 | 14.5 (40.7) | 56,204 | 0.1 |
| Un‡        | 1,391,854 | 179,709 | 12.9 (20.4) | 378,110 | 27.2 (32.6) | 407,013 | 29.2 (36.5) | 116,923 | 8.4 |
| Total      | 3,043,135,925 | 80,343,681 | 2.6 (3.8) | 43,769,191 | 1.4 (2.6) | 107,381,220 | 3.5 (5.2) | 38,870,017 | 1.3 |

*Previous data on segmental duplications distributed by chromosomes as reported in [8]. †Errors represent data that were detected as potential sequence misassignments. ‡Un, unmapped chromosome sequence.
Figure 1 (see legend on the following page)
additional two previous versions of the human genome assemblies, December 2001 and April 2002, and our results showed that there has been a dramatic reduction in potential errors in the latest human genome assembly compared to the two previous genome assemblies (Table 3). Furthermore, we examined the distribution of the amount of duplications in five different categories on the basis of their level of sequence identity to each other (Table 3). We observed a large reduction in duplications that fall within the 98-100% category, supporting the fact that the genome assemblies continue to improve and correct errors made. In addition, our data showed that there have been major improvements for chromosomes 5, 6, 7, 13, 14 and 19 over the last three genome assemblies. And for chromosomes that had reached finished status, such as chromosomes 20, 21 and 22, the number of errors was negligible.

Paralogous sequence variants in the human genome
We have previously shown a strong correlation between ambSNPs with segmental duplications [10]. AmbSNPs are SNPs that were annotated to map to two locations on a particular chromosome in the NCBI dbSNP. Here we show on a genome scale that ambSNPs most specifically correlate with intrachromosomal segmental duplications, suggesting they are paralogous sequence variants (PSVs) (Figure 1). These PSVs were perhaps mistakenly introduced into dbSNP by automated in silico-generated analysis, arising from nucleotide mismatches in paralogous copies of duplicated sequences. Overall, a surprisingly high proportion, 8.6% (199,965 of 2,327,473), of the refSNPs were annotated as ambSNPs from dbSNP (Build 108). A significant number of the ambSNPs (139,974 of 199,965 or 70.0%) are located within duplicated regions as identified by BLAST and should be regarded as PSVs (see Additional data file).

The non-identification by BLAST analysis of regions that contain ambSNPs could be due to one of three possibilities. First, the duplicated copy(s) could have been removed from the sequence assembly or the two have been conflated, that is, mistakenly thought to be the same sequence owing to their high sequence similarity. A second possibility is that the duplication is smaller than 5 kb and was excluded in our BLAST analysis. A third possibility is that a collection of ambSNPs could have been generated from misassigned sequences (identical sequences but misassigned to two different locations in the genome) in older assembly builds due to sequencing errors or true SNPs (with high polymorphism rate) in the sequence. We also observed that the density of ambSNPs generally correlates with the size of the putative duplication, although this might be affected by the level of sequence identity between duplications. For example, two duplicated sequences sharing 98% sequence identity compared to 95% over the same length might contain fewer PSVs as the number of base-pair mismatches


Examples of sequence misassignment errors

| Clone* | Location | Size of region involved (bp) | e-PCR results |
|--------|----------|----------------------------|---------------|
| AC121339† | 3q13.13 | 193,190 chrX |
| AC016003 | 17q21.31 | 181,582 chr9 |
| AC119723 | 3q22.11 | 159,924 chr6 |
| AC093007 | 3q12.11 | 169,882 chr6 |
| AC110578 | 8p23.21 | 160,554 chr15 |
| AC108862 | 11p15.3 | 156,150 chr18 |
| AC113009 | 8q23.11 | 155,171 chr11 |
| AC104765 | 8q12.11 | 150,029 chr18 |
| AC105412 | 2p13.11 | 144,924 chr5 |
| AC092744 | 12p12.31 | 144,009 chr4 |
| AC099061 | 12p1.31 | 140,516 chr15 |
| AC108735 | 3p24.31 | 136,005 chr16 |
| AC122869 | 3q23 | 120,057 chr12 |
| AC017027 | 1q32.1 | 116,265 chr5 |
| AC013530 | 3q26.11 | 99,768 chr8 |
| AC115093 | 11p15.4 | 98,715 chr1 |
| AC112921 | Xp22.2 | 96,272 chr3 |
| AC108094 | 16q21 | 94,953 chr17 |
| AC079186 | 8q12.1 | 78,771 chr7 |
| AC024573 | Unmapped | 56,016 chr2 |
| AC115093 | 11p15.4 | 53,858 chr1 |

*A full list can be obtained from [12]. †See Figure 2 for e-PCR results supporting sequence misassignment.

would be fewer in the former. In addition, we observed that regions identified by our BLAST method but do not contain ambSNPs often correspond to artifactual duplications generated from assembly errors.

**Duplicons related to genomic disorders**

The size, orientation, and contents of segmental duplications are highly variable and most of them show great organizational complexity. This is perhaps due to successive transposition and rearrangement events leading to the creation of segmental duplications [14]. In many cases, a contiguous duplcon is organized into multiple modules with different orientations and sizes. For example, one of the largest segmental duplicons detected in this study was 359 kb in size at the Williams-Beuren locus on 7q11.23 [20,21]. In this case, the two duplicons are separated by 1.6 Mb of intervening sequence with the telomeric duplcon comprising several separate smaller modules as compared to the primary duplcon. The results presented in our study (provided in tables available at [12]) would also allow rapid identification of new duplicons that are potentially responsible for chromosome rearrangements and genomic disorders. For example, the location of the duplicons on chromosomes 9q34/22q11 that have been suggested to mediate recombination leading to the Philadelphia chromosome [4] was identified in our analysis, as were other medically relevant chromosomal regions (Table 4) [22,23].

The characterization of most large segmental duplications is complicated by the fact that many of them (29% of all duplications) are only represented as draft sequences from the current genome assembly. Despite the fact that both BLAST and PSVs analyses allowed us to identify most segmental duplications involved in known genomic disorder mutations (Table 4), estimations of the size of rearranged regions were different from those previously reported [23]. In fact, with the exception of several small duplications and the segmental duplications on chromosome 22 [24], other regions containing duplications involved in genomic disorders were often erroneously assembled and misplaced. Furthermore, we have searched the Celera human genome C3 (publicly released version [25]) and C4 (subscription-based version) sequence assemblies for large duplications found on chromosome 7. We observed that most of them were not represented in large scaffolds, but instead were located in their sequence gaps, or only partially found at ends of scaffolds leading into gaps (see Table 4) [26]. This suggests that the whole-genome assembly approach [25] alone might not be able to finish such duplicated regions in mammalian genomes.

**Conclusions**

We have used two different computational approaches to identify the locations of all recent segmental duplications in the current human genome draft sequence. The fidelity of the results reflects the quality of the assembly examined and the parameters used. In addition, our approach has detected numerous potential sequence misassignment errors in the current genome annotation, allowing rapid error detection in future sequence assemblies. The segmental duplication map of the human genome should serve as a guide for investigation of the role of duplications in genomic disorders, as well as their contributions to normal human genomic variability [2,3,27]. It is clear that genomic regions containing segmental duplications present a major challenge to the completion of the human genome sequence by April 2003. Focused efforts including targeted sequencing of allele-specific clones, high-resolution fluorescence in situ hybridization, and expert curation would be required to validate the actual (or proposed) organization of these complex regions as well as to complete the human genome reference sequence.

**Materials and methods**

**Genome sequence and chromosome-wide BLAST**

We obtained the December 2001, April 2002, and June 2002 (NCBI Build 28, 29 and 30 respectively) human genome assemblies through the University of California, Santa Cruz...
Human Genome Browser [28]. All chromosome sequences were lower-case masked for highly repetitive elements by RepeatMasker (A.F.A. Smit and P. Green, unpublished). For each assembly build, each of the 25 masked chromosome sequences (including one unmapped chromosome sequence ‘ChrUn’) was compared against itself by chromosome-wide BLAST2 [9] to detect intrachromosomal segmental duplications (25 comparisons made), as well as pairwise comparisons to each of the other 24 chromosomes to detect interchromosomal segmental duplications (600 comparisons made). All BLAST results were subsequently parsed to eliminate low-quality and fragmented alignments under the

| Chr  | Length | Duplications | Errors | Length | Duplications | Errors | Length | Duplications | Errors |
|------|--------|--------------|--------|--------|--------------|--------|--------|--------------|--------|
| Chr1  | 2,564  | 99           | 115    | 2,459  | 68           | 60     | 2,469  | 71           | 44     |
| Chr2  | 2,413  | 70           | 45     | 2,468  | 79           | 57     | 2,407  | 69           | 23     |
| Chr3  | 2,048  | 49           | 90     | 2,047  | 29           | 73     | 1,949  | 31           | 40     |
| Chr4  | 1,914  | 39           | 44     | 1,970  | 51           | 49     | 1,920  | 41           | 25     |
| Chr5  | 1,848  | 55           | 90     | 1,896  | 55           | 112    | 1,810  | 45           | 23     |
| Chr6  | 1,783  | 58           | 56     | 1,828  | 43           | 153    | 1,703  | 29           | 6      |
| Chr7  | 1,638  | 130          | 48     | 1,605  | 119          | 27     | 1,574  | 101          | 2      |
| Chr8  | 1,457  | 35           | 66     | 1,484  | 33           | 43     | 1,439  | 26           | 40     |
| Chr9  | 1,330  | 83           | 38     | 1,291  | 75           | 27     | 1,324  | 83           | 16     |
| Chr10 | 1,421  | 74           | 51     | 1,385  | 72           | 39     | 1,344  | 63           | 13     |
| Chr11 | 1,414  | 51           | 84     | 1,341  | 43           | 36     | 1,374  | 44           | 20     |
| Chr12 | 1,396  | 30           | 83     | 1,342  | 24           | 32     | 1,313  | 28           | 34     |
| Chr13 | 1,151  | 29           | 21     | 1,136  | 22           | 15     | 1,134  | 19           | 1      |
| Chr14 | 1,065  | 27           | 8      | 1,054  | 23           | 10     | 1,043  | 13           | 0      |
| Chr15 | 991    | 62           | 30     | 1,000  | 54           | 20     | 992    | 56           | 17     |
| Chr16 | 938    | 65           | 44     | 932    | 67           | 32     | 817    | 60           | 21     |
| Chr17 | 839    | 66           | 46     | 811    | 46           | 29     | 801    | 53           | 21     |
| Chr18 | 818    | 16           | 59     | 809    | 12           | 32     | 775    | 12           | 14     |
| Chr19 | 769    | 45           | 28     | 730    | 34           | 12     | 600    | 32           | 3      |
| Chr20 | 630    | 10           | 5      | 628    | 12           | 4      | 628    | 11           | 1      |
| Chr21 | 446    | 18           | 3      | 446    | 16           | 2      | 446    | 15           | 0      |
| Chr22 | 478    | 28           | 0      | 477    | 29           | 1      | 477    | 28           | 0      |
| ChrX  | 1,517  | 54           | 40     | 1,518  | 61           | 23     | 1,492  | 55           | 22     |
| ChrY  | 584    | 86           | 2      | 584    | 95           | 2      | 584    | 85           | 1      |
| ChrUn | 74     | 10           | 1      | 125    | 11           | 43     | 14     | 4            | 1      |
| Total | 31,526 | 1,290        | 1,097  | 31,366 | 1,175        | 932    | 30,431 | 1,074        | 389    |

| % range | Duplication | Error | Duplication | Error | Duplication | Error |
|---------|-------------|-------|-------------|-------|-------------|-------|
| 90-92%  | 135         | 0     | 137         | 0     | 117         | 0     |
| 92-94%  | 334         | 0     | 334         | 0     | 311         | 0     |
| 94-96%  | 391         | 0     | 382         | 0     | 367         | 0     |
| 96-98%  | 451         | 0     | 444         | 0     | 418         | 0     |
| 98-100% | 884         | 1,097 | 724         | 932   | 665         | 389   |

All numbers shown in the table are x 100 kb. *Sequence similarity between duplication by five levels of percent identity.
Table 4
Segmental duplications involved in known genomic disorders and chromosome rearrangements identified by BLAST and ambSNP analyses

| Disorders                          | Band            | First copy       | Second copy(s)   | Identity |
|-----------------------------------|-----------------|------------------|------------------|----------|
|                                   |                 | Start*           | Size*            | ambSNPs* |         |
| Gaucher disease                   | 1q22            | 14810965         | 10,649           | 7        | 152776301 | -10,479 | 10 | 95.19 | S |
| Spinal muscular atrophy           | 5p14/5q13       | 21621854         | 79,183           | 1,032    | 69175603  | -79,149 | 1,190 | 98.22 | M |
| Williams-Beuren syndrome          | 7q11.23         | 70970126         | 359,416          | 380      | 72927299  | 111,773 | 56   | 99.60 | P |
| t(4;8)(p16;p23) Wolf-Hirschhorn syndrome | 4p16/8p23       | 8769778          | 99,609           | 3†       | 7156209   | -51,677 | 18   | 95.65 | P |
| inv dup(8p)                       | 8p23.1          | 7084847          | 138,560          | 123      | 7756853   | 126,769 | 229  | 99.16 | M |
| Prader-Willi syndrome and Angelman syndrome | 15q11/15q13     | 19709020         | 75,325           | 102      | 19961243  | 34,902  | 55   | 98.70 | P |
| Polycystic kidney disease 1       | 16p13           | 2164789          | 38,034           | 136      | 16249164  | 24,076  | 243  | 98.32 | P |
| Charcot-Marie-Tooth IA/Hereditary neuropathy with pressure palsy | 17p12/17p12     | 14440158         | 23,599           | 272      | 15837032  | 23,585  | 286  | 98.42 | P |
| Smith-Magen syndrome/dup(17)(p11.2-p11.1) | 17p12           | 18524425         | 152,700          | 547      | 20492073  | -147,255| 539  | 99.06 | M |
| Neurofibromatosis type 1          | 17q11.2         | 28686414         | 63,356           | 163      | 28952984  | -32,619 | 129  | 98.65 | P |
| DiGeorge syndrome and velocardiofacial syndrome | 22q11.21       | 15662253         | 155,811          | 471      | 18221385  | 155,996 | 322  | 99.42 | P |
| Chronic myeloid leukemia t(9;22)(p34;q11) | 9q34/22q11      | 12326365         | 36,956           | NA       | 20552124  | 26,424  | NA   | 91.81 | S |
| Emery-Dreifuss muscular dystrophy | Xq28            | 14762787         | 11,030           | 2        | 14767652  | 11,034  | 2    | 99.61 | S |
| Schwachman-Diamond syndrome       | 7q11.21         | 65091051         | 325,140          | 665      | 70647188  | 302,881 | 652  | 97.43 | P |
| Red green color blindness         | Xq28            | 148439480        | 21,144           | 61       | 148476598 | 21,834  | 58   | 99.82 | S |
| BRCA1 duplication                 | 17q21           | 40983970         | 43,221           | 66       | 62252214  | 431,52  | 66   | 99.85 | P |
| Male infertility AZFc microdeletion region 2 | Yq11.22         | 23322362         | 190,336          | 39†      | 23680552  | -185,149| 393† | 99.88 | P |
|                                   |                 | 23908727         | 94,194           | 282†     | 24794944  | -93,690 | 284† | 99.92 | P |
|                                   |                 | 24794944         | 93,690           | 247†     | 27460935  | -94,218 | 248† | 99.93 | P |

This table represents a partial list of all known genomic disorders and chromosome rearrangements. *Only the start coordinates (based on June 2002 assembly) for duplicons are shown. Results from BLAST analysis with chromosome coordinates and size of duplicon. For several genomic mutations (Williams-Beuren syndrome, Prader-Willi syndrome and Angelman syndromes, and DiGeorge syndrome) the duplicons shown are incomplete, most of which are composed of several duplication modules. The '-' sign indicates that the second duplicon is in the inverse orientation. †The number of ambSNPs (ambiguously mapped single-nucleotide polymorphisms) found within the genomic segment. NA, not applicable. The ambSNP analysis defines regions containing high densities of contiguous ambSNPs. For some of the segmental duplications involved in genomic disorders, the contiguous lengths of ambSNPs are much larger than those detected by BLAST. The specific sizes of the segmental duplications have to be resolved by detailed characterization of the different modules. ‡Celera representation: S, both copies found in large (> 500 kb) sequence scaffolds; P, partially hit, single copy found, or less than perfect alignments; M, missing from large sequence scaffolds, hitting numerous fragments. ‡SNPs with multiple locations were used for evaluating the density of ambSNPs.

following criteria: BLAST results having ≥ 90% sequence identity, ≥ 80 bp in length, and with expected value ≤ 10⁻⁹⁰. BLAST results parsing and duplication detection Each BLAST report was sorted by chromosomal coordinates. All identical hits (same coordinate alignments),
including suboptimal BLAST alignments recognized by multiple, overlapping alignments, as well as mirror hits (reverse coordinate alignments) from the BLAST results of the intrachromosomal set were removed. Contiguous alignments separated by a distance of less than 3 kb, then 5 kb, and subsequently 9 kb were joined (stepwise) into modules in order to traverse masked repetitive sequences and to overcome breaks in the BLAST alignments caused by insertions/deletions and sequence gaps. Such contiguous sequence alignment modules represent sequence similarity between the subject and query chromosome sequence in question (at their respective positional coordinates). This pairwise sequence comparison procedure serves as a rapid and robust way to detect duplication relationships. However, because of the use of masked sequences, our method would only yield a poor (on average 0.1-0.5 kb) resolution for the determination of the precise duplication alignment boundaries. Results were classified as either duplications or ‘questionable’ results based on sequencing status of the region and the percent sequence similarity between the detected alignments. Questionable duplications are results that fall within regions containing draft sequences with > 99.5 % detected sequence identity with another region. We consider these questionable duplications to be involved in potential sequence misassignment errors in the human genome assembly and would require further effort to achieve resolution.

Fine mapping of segmental duplications

Detailed information regarding segmental duplications as well as potential sequence misassignment errors identified by our analysis were presented using the Generic Genome Browser [11,12]. We have also summarized our results in table formats [12] that include information on size of duplications, chromosomal band locations, level of identity between duplicated copies, sequenced clones (accession numbers) and their sequencing status, as well as genes mapped to these regions. In addition, we have plotted the size of each intrachromosomal duplication (y-axis) against its chromosome position (x-axis) along each chromosome to indicate the intrachromosomal segmental duplication content of each chromosome (Figure 1) using the publicly available visualization tool GenomePixelizer [29,30]. Results generated from the detection of segmental duplications were subsequently converted into coordinate files as input for display using GenomePixelizer.

Paralogous sequence variants (PSV) density map

SNP mapping data from dbSNP were obtained through the NCBI ftp site [31]. Each chromosome SNP table, containing annotation regarding ambSNPs that have appeared twice in a particular chromosome, were extracted and sorted along with their corresponding chromosomal positions. The number of ambSNPs was tabulated along a 10-kb window to produce density plots of ambSNPs along the length of each chromosome (Figure 1).

Additional data file

An additional data file with tables describing the relative frequency increase of repeat types at duplcion junctions and the number of ambiguously mapped SNPs within segmental duplications (number of PSVs), respectively, as well as a figure showing functional profiling of genes involved in recent whole-gene duplication vs human genome average is available with the online version of this article.

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