nature genetics

ARTICLES

Personalized copy number and segmental duplication maps using next-generation sequencing

Can Alkan[1,2], Jeffrey M Kidd[1], Tomas Marques-Bonet[1,3], Gozde Aksay[1], Francesca Antonacci[1], Fereydoun Hormozdiari[4], Jacob O Kitzman[1], Carl Baker[1], Maika Malig[1], Onur Mutlu[5], S Cenk Sahinalp[4], Richard A Gibbs[6] & Evan E Eichler[1,2]

Despite their importance in gene innovation and phenotypic variation, duplicated regions have remained largely intractable owing to difficulties in accurately resolving their structure, copy number and sequence content. We present an algorithm (mrFAST) to comprehensively map next-generation sequence reads, which allows for the prediction of absolute copy-number variation of duplicated segments and genes. We examine three human genomes and experimentally validate genome-wide copy number differences. We estimate that, on average, 73–87 genes vary in copy number between any two individuals and find that these genic differences overwhelmingly correspond to segmental duplications (odds ratio = 135; $P < 2.2 \times 10^{-16}$). Our method can distinguish between different copies of highly identical genes, providing a more accurate assessment of gene content and insight into functional constraint without the limitations of array-based technology.

The human genome is enriched for gene-rich segmental duplications that vary extensively in copy number[1–4]. Variation in the content and copy number of these duplicated genes has been associated with recurrent genomic rearrangements as well as with a variety of diseases, including color blindness, psoriasis, HIV susceptibility, Crohn's disease and lupus glomerulonephritis[5–10]. Despite recent technological advances in copy number detection, a global assessment of genetic variation of these regions has remained elusive. Commercial SNP microarrays frequently bias against probe selection within these regions[11–13]. Array comparative genomic hybridization (array CGH) approaches have limited power to discern copy number differences, especially as the underlying number of duplicated genes increases and the difference in copy number with respect to a reference genome becomes vanishingly small[3,14,15]. Even sequence-based strategies such as paired-end mapping[16,17] frequently cannot unambiguously assign end sequences in duplicated regions, making it impossible to distinguish allelic and paralogous variation. Consequently, duplicated regions have been largely refractory to standard human genetic analyses.

One promising approach for assessing copy number variation has involved measuring the depth of coverage of whole-genome shotgun (WGS) sequencing reads aligned to the human reference genome[1]. Recent applications of this approach to next-generation sequencing (NGS) technology[18–22] have provided high-resolution mapping of copy number alterations. However, most of these approaches assay only the 'unique' regions of the genome[21,23,24]. For example, MAQ reports only unique alignments and arbitrarily selects one position in the case of tied map positions, not reporting any sequence variation[23]. Although it is possible to run MAQ with an option to return all possible map locations of the sequence reads, it reports only the anchoring position and does not return any information on sequence variation. Here, we develop a read-mapping algorithm to rapidly assay copy number variation and experimentally verify its ability to accurately predict copy number in some of the most complex and duplicated regions of three human genomes.

RESULTS

Algorithm development

We developed mrFAST (micro-read fast alignment search tool) to (i) effectively map large amounts of short sequence read data to the human genome reference assembly, (ii) calculate accurate read depth and (iii) return all possible single-nucleotide differences within both unique and duplicated portions of the genome (**Supplementary Figs. 1** and **2a**). We have shown previously that the ability to place reads to all possible locations in the reference genome is critical to accurately predicting the absolute copy number of duplicated sequences[1].

mrFAST is designed for short sequence reads (>25 bp). It uses a seed-and-extend method similar to BLAST[25] and implements a hash table to create indices ($n = 300$ indices of 10 Mb each) of the reference genome that can efficiently use the main memory of the system (**Supplementary Fig. 1**). For each read, the first, middle and last k-mers are interrogated in the hash table to place initial seeds, where k is the ungapped seed length (we set $k = 12$ by default). A rapid version of the edit distance[26] computation[27] is then performed

[1]Department of Genome Sciences, University of Washington School of Medicine, Seattle, Washington, USA. [2]Howard Hughes Medical Institute, Seattle, Washington, USA. [3]Institut de Biologia Evolutiva (UPF-CSIC), Barcelona, Catalonia, Spain. [4]School of Computing Science, Simon Fraser University, Burnaby, British Columbia, Canada. [5]Department of Electrical and Computer Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania, USA. [6]Baylor College of Medicine, Houston, Texas, USA. Correspondence should be addressed to E.E.E. (eee@gs.washington.edu).

Received 16 April; accepted 23 July; published online 30 August 2009; doi:10.1038/ng.437

Table 1 Summary statistics for three human genome libraries

Genome	Platform	Number of reads	Number of mapped reads[a]	Autosomes		Chromosome X	
				Reads per 5 kb	s.d.	Reads per 5 kb	s.d.
JDW	454	509,667,772	159,293,568	694.93	170.64	400.58	179.30
NA18507	Illumina	1,776,928,308	556,713,986	2,393.52	542.80	1,427.50	615.84
YH	Illumina	1,315,249,404	375,234,167	1,645.51	358.44	971.58	475.31

[a]Reads mapped against the human reference genome (build35) using mrFAST. Average read length is 36 bp in the JDW and NA18507 genomes and 35 bp in the YH genome.
We inspected three different WGS libraries from three individuals. Approximately 74 million 454-based reads from the JDW genome were rendered into 36-bp reads (**Supplementary Note**). In total, we processed 4.9 billion reads (206 Gb), and approximately 1.4 billion reads (~28.5%) were mapped to repeat-masked human genome build35 (see duplication maps of three individual genomes and **Supplementary Note**).

to extend the seed to discover all possible map locations, allowing ≤2 indels. We optionally exclude most of the 'non-extendable' seeds, bypassing the high cost of the edit distance computation. For this analysis, we selected an edit distance threshold of two mismatches or indels to account for allelic variants and sequencing error. Moreover, querying three distinct *k*-mers guarantees discovery of all possible locations of reads within an edit distance of 2 if the length is ≥35 bp and $k = 12$. As a benchmark, mapping of one human genome (21-fold) against the repeat masked reference genome was achieved in 13.5 h using a 100-CPU cluster.

Personal duplication maps

We tested the utility of mrFAST to accurately construct duplication maps by obtaining WGS sequence data from three human males from the NCBI short-read archive and European Read Archive (see URLs section). These included a genome sequence generated with 454 Life Sciences GS-FLX sequence data (for an individual of European descent, JDW)[20] and two genome sequences generated with Illumina WGS data (for a Yoruba African individual, NA18507, and a Han Chinese individual, YH)[18,22] (**Table 1**). All loci were first masked for high-copy common repeat elements (retroposons and short high-copy repeats) using RepeatMasker[28], Tandem Repeats Finder[29] and WindowMasker[30]. We initially assessed the dynamic range response of shotgun sequence data mapped by mrFAST by determining the read depth for a set of 32 duplicated and unique loci where copy number status had been previously confirmed using experimental methods[1]. Using these benchmark loci, we determined the average read depth and variance for 5-kb (unmasked) regions for autosomal and X chromosomal loci (**Table 1**). For each of the three libraries, we found that read depth strongly correlated with the known copy number ($R^2 = 0.83$–0.90; **Fig. 1a**). Owing to the known sequencing biases of high-throughput sequencing technologies in GC-rich and GC-poor regions[31], we also applied a statistical correction to normalize the read depth based on the GC content of each window (Online Methods and **Supplementary Note**).

We next assessed the ability of mrFAST read depth to accurately predict the boundaries of known duplicated sequences. We selected a set of 961 autosomal duplication intervals (745 intervals ≥20 kb) that were predicted both by the analysis of the human genome assembly[32] and by an independent assessment of Celera capillary WGS sequences[1,33] where the 20-kb threshold was applied. We reasoned that duplications detected by both methods probably represented a set of true positive duplications whose boundaries would remain largely invariant in additional human genomes. We mapped each of the three WGS sequence libraries (JDW, NA18507 and YH) to the human reference genome (build35) using mrFAST and identified all intervals where at least six out of seven consecutive windows showed an excess depth of coverage (number of reads greater than or equal to the mean plus 3 s.d.). A threshold of 3 s.d. corresponds to a diploid copy number of approximately 3.5, which means that a fraction of sequences with a hemizygous duplication may be missed by this approach. We compared the predicted sizes of intervals in each genome with the duplications predicted from the assembly[34] and determined that the boundaries of known duplications could be accurately predicted ($R^2 = 0.92$; **Fig. 1b**). Because sequence coverage directly affects the power to detect duplications by read depth, we computed the fraction of high-confidence duplication intervals that could be detected at various WGS sequence coverages (**Fig. 1c**). At

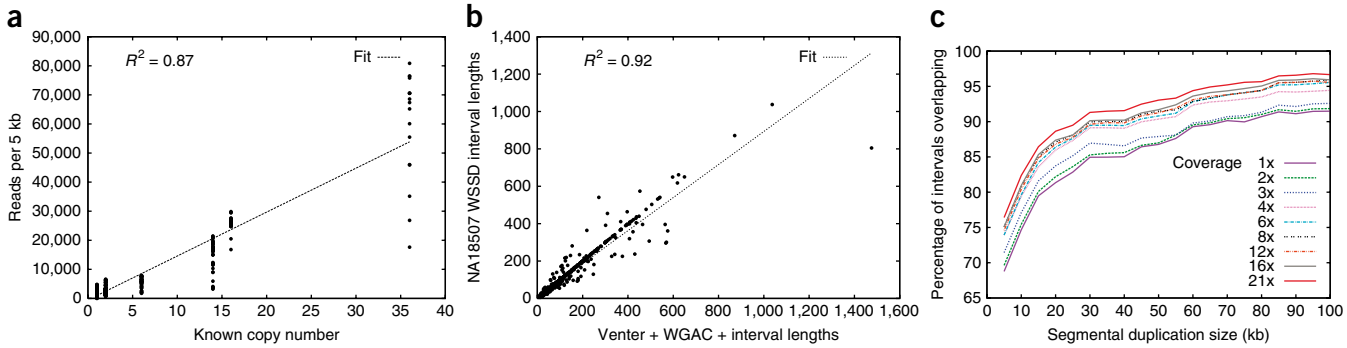

Figure 1 Correlation of predicted and known segmental duplications in NA18507. (**a**) mrFAST sequence read depth per 5-kb window along the human genome correlates well ($R^2 = 0.87$) with the known copy number of duplicated sequences. (**b**) Predicted duplication interval length versus the assembly-based length of known duplications (whole-genome assembly comparison; ≥94% sequence identity)[34] shows that boundaries of duplications can be accurately predicted. A few intervals show discrepancy in boundary prediction; this is largely due to deletion polymorphism in the NA18507 genome within duplications (confirmed by array CGH). WSSD, whole-genome shotgun sequence detection[1]; Venter, J. Craig Venter, whose genome has previously been sequenced with Sanger sequencing. (**c**) A cumulative plot of the fraction of duplication intervals detected as a function of read depth (sequence coverage). The segmental duplication size is given in cumulative intervals (≥10 kb, ≥20 kb, etc.) and represents the set of intervals identified both within the reference assembly (build35) and the Celera whole-genome shotgun sequence reads. As expected, the sensitivity of our method increases with greater genome coverage; the most marked difference in detection is observed between three- and fourfold coverage. WGAC, whole-genome assembly comparison.

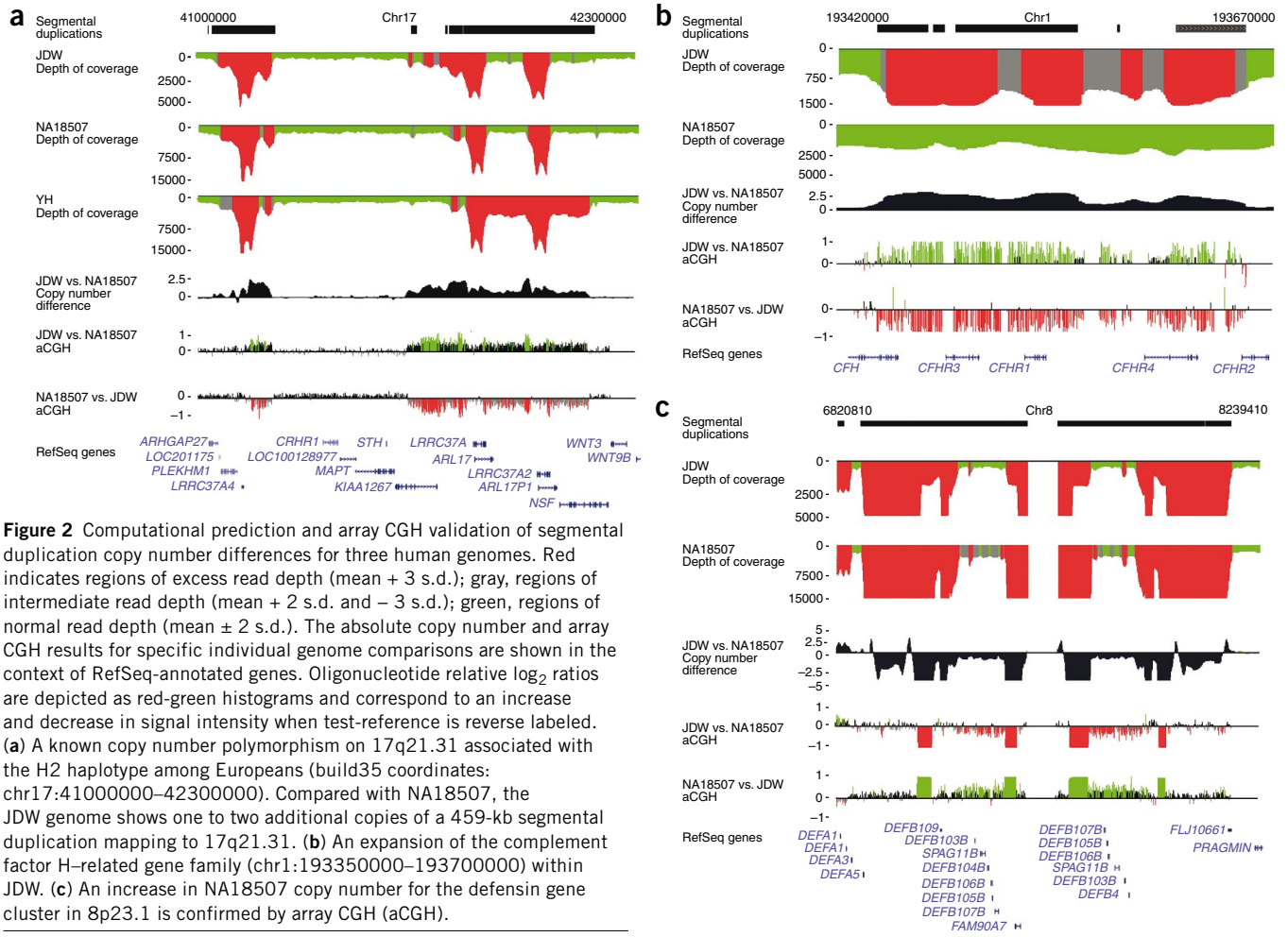

Figure 2 Computational prediction and array CGH validation of segmental duplication copy number differences for three human genomes. Red indicates regions of excess read depth (mean + 3 s.d.); gray, regions of intermediate read depth (mean + 2 s.d. and – 3 s.d.); green, regions of normal read depth (mean ± 2 s.d.). The absolute copy number and array CGH results for specific individual genome comparisons are shown in the context of RefSeq-annotated genes. Oligonucleotide relative $\log_2$ ratios are depicted as red-green histograms and correspond to an increase and decrease in signal intensity when test-reference is reverse labeled. (**a**) A known copy number polymorphism on 17q21.31 associated with the H2 haplotype among Europeans (build35 coordinates: chr17:41000000–42300000). Compared with NA18507, the JDW genome shows one to two additional copies of a 459-kb segmental duplication mapping to 17q21.31. (**b**) An expansion of the complement factor H–related gene family (chr1:193350000–193700000) within JDW. (**c**) An increase in NA18507 copy number for the defensin gene cluster in 8p23.1 is confirmed by array CGH (aCGH).

20-fold sequence coverage, we found that >90% of segmental duplications larger than 20 kb were accurately predicted. Notably, the most substantial increase in yield occurred between three- and fourfold sequence coverage, suggesting that the majority of copy number variable sequences >20 kb will be accurately predicted from the 1000 Genomes Project (see URLs section below) where WGS sequence data with at least fourfold sequence coverage are available. We also performed benchmark analyses to compare the segmental duplication detection power of mrFAST with different edit distance parameters, as well as against some of the other available read mapping tools (**Supplementary Note**).

As an independent and more sensitive test within unique regions of the genome, we compared copy number variant (CNV) genotype calls for NA18507, with calls recently assessed by another group using the Affymetrix 6.0 platform[35]. We found that 250/282 (88.7%) of CNVs >10 kb and 120/128 (93.8%) of CNVs >20 kb were consistent between the two platforms (**Supplementary Note**). In two of the most extreme cases of discrepancy, we found that the Affymetrix 6.0 genotypes probably misassigned absolute copy numbers, possibly owing to an incorrect assignment of the population average genotype based on fluorescence intensities. These results highlight the potential of mrFAST read depth to provide precise estimates of copy number across all genomic regions.

We constructed duplication maps for each of the three genomes and estimated the absolute copy number of each duplication interval larger than 20 kb in length. We considered a given segment to be duplicated within an individual if the median estimated copy number for that individual was >2.5 (diploid copy number; see **Supplementary Note**). We compared the extent of overlap among duplicated sequences (**Fig. 2** and Online Methods) and reclassified duplicated sequences as shared or specific to an individual based on the predicted copy numbers in the analysis of these three genomes (**Supplementary Note**). We defined a total of 725 non-overlapping duplication intervals across the three individuals that total 84.76 Mb.

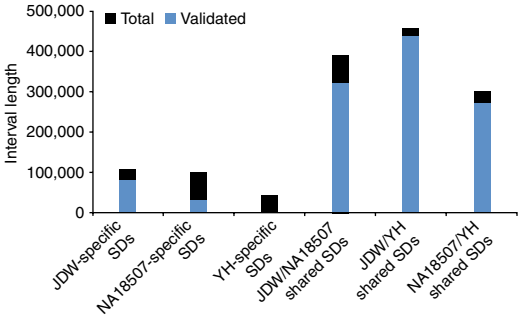

Figure 3 Validation of individual specific segmental duplications. The number of duplicated base pairs predicted and validated in NA18507, JDW and YH (autosomes only) are shown. The heights of the bars represent the sum of computationally predicted interval lengths; blue bars correspond to the experimentally validated portion. Only duplicated intervals >20 kb were considered for validation. SD, segmental duplication.

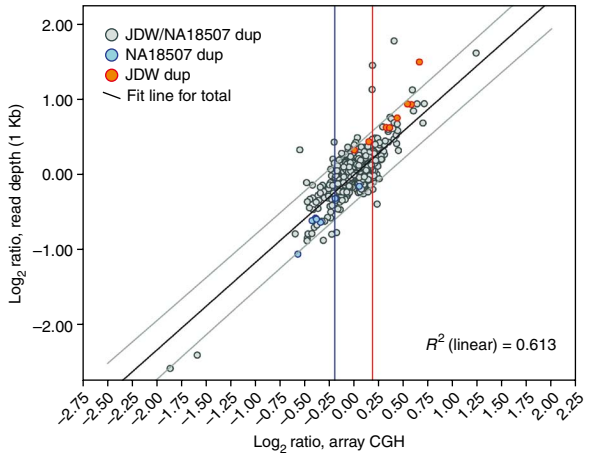

Figure 4 Correlation between computational and experimental copy number for NA18507 and JDW. We computed the copy number for each shared (gray) and individual-specific duplication interval (blue or orange) based on the depth of coverage of aligned WGS data against the human reference assembly (build35). Based on these computational estimates of copy number, we calculated a predicted $\log_2$ copy number ratio for each autosomal duplication interval >20 kb (and with <80% of total common repeat content). These values were plotted against the experimental $\log_2$ ratios determined by oligonucleotide array CGH. The vertical lines indicate the threshold used for the validated calls (**Supplementary Note**).

Only 25 duplication intervals were not predicted in all three individuals, suggesting that the vast majority (97% of the intervals and 98% by base pair) of large segmental duplications are shared (**Fig. 3** and **Supplementary Fig. 3**).

Experimental validation

We designed two targeted oligonucleotide microarrays to validate predicted differences in copy number by array CGH. Using DNA from each of the sequenced genomes, we performed three pairwise array CGH experiments. We validated 68% (17/25) of duplication intervals not shared in all three individuals, which implied that only 1.1 Mb of duplicated regions would be unique to one of them (**Fig. 3** and **Supplementary Note**). Notably, ~80% of these validated 'individual-specific' duplications mapped within 2 kb of shared human duplications, suggesting that sequences adjacent to ancestral duplication blocks have the highest probability of segmental duplication. We also performed a reciprocal analysis of intervals (>20 kb) predicted to be deleted in one or more of the individuals and confirmed 28 deletions (or 1.4 Mb of deletion) (**Supplementary Note**).

Regardless of the NGS platform, the pattern of read depth was reproducible for 48% of the shared duplications (44,711/94,070; **Supplementary Fig. 4**). However, among the remaining 52% of duplications, read depth did not correlate between individuals. This suggests that shared duplications show the greatest extremes of copy number variation between individuals (**Supplementary Fig. 5**). Using absolute estimates of copy number, we calculated an *in silico* $\log_2$ ratio for each of the three genome-wide comparisons and compared it with the experimental values determined by array CGH (**Fig. 4** and **Supplementary Fig. 6**). Overall, we found a positive correlation with copy number predictions (R^2 = ~0.52–0.63, depending on the pairwise comparison). We note that the ability of array CGH to discriminate absolute differences diminishes as the duplication copy number increases[14].

We selected 11 duplicated loci that showed copy number differences between the YH and NA18507 genomes and performed FISH analysis on interphase nuclei (**Fig. 5** and **Supplementary Note**) from immortalized cell lines from YH and NA18507. The FISH results were highly consistent with the absolute copy number predicted by mrFAST. For

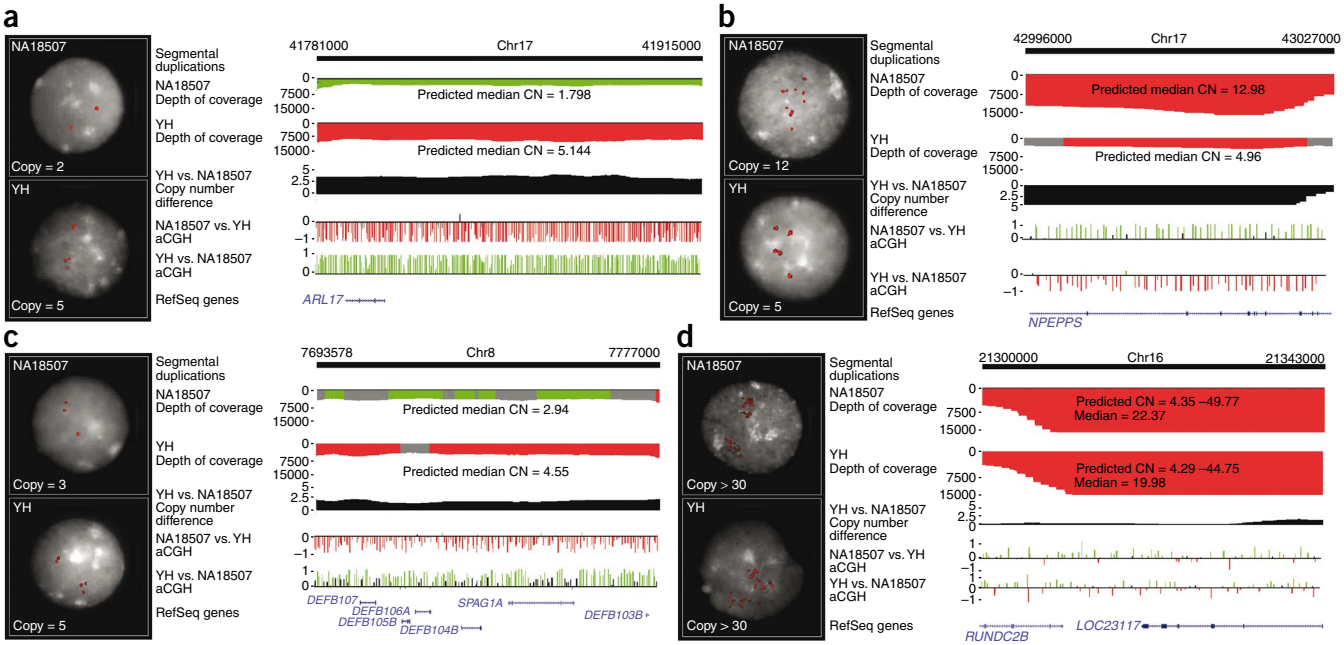

Figure 5 FISH validation. (**a**) Sequence read depth predicts five copies of this segment of 17q21.31 in the YH genome and two 'unique' copies in NA18507. Array CGH shows a higher copy number in the YH genome, and FISH on interphase nuclei confirms the absolute copy number difference between the two genomes. (**b**) Similarly, interphase FISH confirms five copies of *NPEPPS* in YH versus 12 copies in NA18507. (**c**) YH is predicted and validated to have two more copies of the defensin gene family cluster of 8p23.1. (**d**) Owing to the known mosaic architecture[38] for this high-copy locus (>30 copies), both array CGH and FISH cannot accurately estimate copy number difference between the NA18507 and YH genomes, despite the fact that sequence depth predicts approximately two more copies in NA18507. aCGH, array CGH; CN, copy number.

Table 2 The 30 genes with the most variable copy numbers in the three human genomes

Gene	Transcript ID	Gene size (bp)	Duplicated bp[a]	JDW copy number	NA18507 copy number	YH copy number	Copy number range
DUX4	NM_033178	8,205	8,205	248	97	196	151
DUB3	NM_201402	1,593	1,593	139	186	122	64
FAM90A7	NM_001136572	18,865	18,865	7	44	36	38
PRR20	NM_198441	3,022	3,022	28	22	11	17
HRNR	NM_001009931	12,112	7,721	19	8	15	12
TBC1D3	NM_032258	10,897	10,897	26	29	17	11
TP53TG3	NM_016212	3,200	3,200	16	7	6	10
WASH1	NM_182905	15,229	15,229	26	16	20	10
ZNF717	NM_001128223	48,227	24,791	36	27	32	9
OR4F17	NM_001005240	918	918	18	13	9	9
C2orf78	NM_001080474	32,959	26,245	9	7	14	7
PCDHB8	NM_019120	2,590	2,508	12	6	8	6
TCEB3C	NM_145653	1,877	1,877	23	18	17	6
PCDHB7	NM_018940	3,714	2,333	10	4	7	6
OR4F16	NM_001005277	937	937	18	17	12	6
FOXD4L5	NM_001126334	3,109	3,108	40	43	38	6
FOXD4L4	NM_199244	3,107	3,106	40	43	38	6
MST1	NM_020998	4,816	4,776	11	6	11	5
MGC50273	NM_214461	6,701	6,701	33	28	32	5
AMY1A	NM_004038	8,871	8,871	6	11	10	5
AMY2A	NM_000699	8,395	8,395	6	10	11	5
POTEB	NM_207355	31,415	31,415	17	21	22	5
NPEPPS	NM_006310	92,199	62,993	4	8	3	5
NBPF1	NM_017940	49,571	49,533	43	48	46	5
OR2A1	NM_001005287	931	931	6	5	9	4
FAM86B2	NM_001137610	10,727	10,727	20	17	21	4
GOLGA6	NM_001038640	12,694	12,694	17	13	17	4
LOC283767	NM_001001413	9,757	9,757	26	22	26	4
FLG	NM_002016	23,029	10,606	13	9	13	4
BAGE	NM_001187	41,142	40,371	18	17	14	4

[a]'Duplicated bp' corresponds to the number of base pairs in the gene that intersect with segmental duplications. This value is equal to the gene size for genes that are completely in segmental duplications or smaller if the gene partially overlaps with known duplications.

Based on the estimated and validated copy numbers of genes in the RefSeq database, we calculated the maximum copy number difference between each pair of the three genomes analyzed. The 30 validated genes with the highest copy number range are shown.

cases where the copy number was >15, FISH was unable to provide a precise estimate of copy number difference because of the technical limitations of this procedure (**Fig. 5d** and **Supplementary Note**). With one exception, interphase FISH analysis showed that differences in copy number involved local changes in copy number, suggesting that duplicative transpositions to new locations were exceedingly rare.

Copy number polymorphic genes

This analysis validated 68 gene families as completely or partially copy number–variable among these three individual genomes (**Supplementary Table 1**). This included a complete duplication of the complement factor H–related complex (consisting of four genes, *CFHR1* through *CFHR4*) within the JDW genome (**Fig. 2b**). We also confirmed one additional copy of the 8p23.1 defensin gene family (*DEFB103B*) in the YH genome compared with NA18057 and one additional copy in NA18507 compared with JDW. We predict about twice as many copies of the amylase (*AMY1A*) gene family in NA18507 ($n = 9$) and YH ($n = 10$) as in JDW ($n = 5$). As expected[7], the African genome (NA18507) showed the greatest number of *CCL3L1* copies ($n = 7$) compared with either JDW ($n = 3$) or YH ($n = 5$). We also validated increases in gene segments of functional relevance. For example, we found ten fewer copies of the kringle IV domain of the lipoprotein(a) gene (*LPA*) in NA18507 (22 copies versus 35 in JDW and 26 in YH)—a polymorphism known to be associated with risk of coronary heart disease[36].

Although many of these differences are consistent with previous studies, the analysis also confirmed differences in rapidly evolving human and great ape gene families that have been previously difficult to ascertain. For example, our results suggested a higher copy number for the *TBC1D3* gene family in NA18507 (29 copies) than in the other two genomes (26 copies in JDW and 17 in YH). Similarly, we predicted absolute differences in the copy number of *NPIP* (a member of the morpheus gene family) between three human genomes. Unlike FISH or array CGH, sequencing data provide very high specificity to assess the presence or absence of individual paralogous genes. We examined three gene families (morpheus, opsin and *CFHR*) in more detail by identifying single-nucleotide variants that distinguish the different paralogs. Despite the high degree of sequence identity among the duplicated genes, we found approximately 300 distinct paralogous sequence variants per duplicated gene (one variant per 91 bp) (**Supplementary Table 2**). We determined which specific duplicate genes were present in each individual, providing, to our knowledge, the first accurate assessment of specific genes, as opposed to copy number differences in the aggregate (**Supplementary Fig. 7**). Because we tracked all single-nucleotide differences using mrFAST, we were able to assess the relative proportion of disruptive stop codons

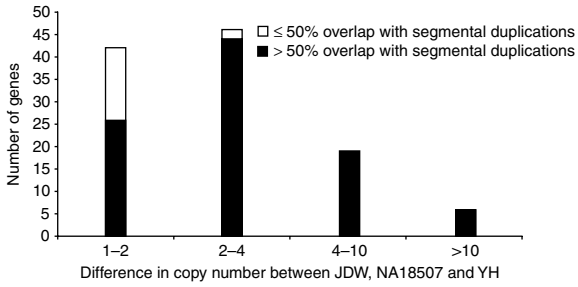

Figure 6 Copy number differences between unique and duplicated regions. The 113 genes that vary in copy number were partitioned based on copy number difference and on their intersection with annotated segmental duplications (WGAC[34]). Duplicated genes show a greater extent of copy number variation than genes mapping to unique regions of the genome.

providing a first-pass approximation of the functional constraint on each polymorphic gene family (**Supplementary Table 3**). These data suggest that the systematic identification of unique paralogous sequence variants for all duplicated gene families combined with NGS data will be a powerful approach to genotype these complex regions of the genome. However, longer sequence reads will be necessary to accurately assess phase.

Our experimental analysis found that 97% (66/68) of the validated genic copy number differences among the three genomes corresponded to regions annotated as segmental duplications (providing strong evidence that functional copy number polymorphisms will be similarly biased in their genomic distribution). Because we considered only the largest (>20 kb) regions in our initial analysis, we repeated the copy number estimate on a gene-by-gene basis, removing the length threshold. We analyzed 17,610 nonredundant RefSeq transcripts[37] (**Supplementary Note**) and calculated the absolute copy number for each sample based on the median depth of coverage for each of the corresponding gene segments in the genome (**Supplementary Note**). Based on this computational analysis, we predict that 3.8% of genes (662/17,601) show a difference of at least one copy (**Supplementary Tables 4**,**5**), with an average of 394 predicted gene copy number differences between two individuals (see **Table 2** for the 30 validated genes with the largest copy number differences). To validate these predicted gene differences, many of which are <20 kb, we interrogated the three samples using a customized oligonucleotide microarray targeted toward these gene regions. We conservatively validated 113 genes (**Supplementary Table 6**) as variable in copy number among these three individuals (73–87 genes between two human genomes). Although there are almost certainly real copy number differences that were not validated by array CGH (**Supplementary Note**), 84% (95/113) of the validated changes mapped to segmental duplications. Thus, genes that are duplicated (having a 50% overlap with annotated duplications of at least 90% identity) were significantly more likely to show copy number difference (odds ratio = 135; $P < 2.2 \times 10^{-16}$ using Fisher's exact test). Moreover, these variably duplicated genes showed a greater copy number range than the nonduplicated CNV genes (median copy number difference of 2.8 for variably duplicated genes versus 1.2 for nonduplicated CNV genes). Notably, 97% (69/71) of the genes with a copy number difference ≥2 mapped to previously reported segmental duplications[1,32,34] (**Fig. 6**).

DISCUSSION

NGS platforms are fundamentally altering genetic and genomic research. Compared to other methods, these platforms offer the ability to obtain an unprecedented amount of sequence information in a low-cost, high-throughput fashion. The main drawback of existing technologies is the comparably short sequence read lengths they produce. As a result, some regions of the human genome—particularly duplication- or repeat-rich regions—have already begun to be excluded as part of standard NGS analyses. We specifically designed our new mapping algorithm, mrFAST, to address this limitation. By considering all possible map locations for a read in an efficient manner, we have been able to apply the high potential of NGS to some of the most structurally complex and dynamic regions of the human genome. By including these regions, we provide one of the first comprehensive estimates of absolute copy number differences among three human genomes.

We draw three major conclusions from our computational and experimental analyses. First, NGS read depth can be used to accurately predict absolute copy number, such that even multicopy differences (5 versus 12; **Fig. 5**) can be reliably predicted between different individuals. Second, the duplication status of the largest segmental duplications (>20 kb in length) is largely invariant, with only 3% of the duplications being specific to an individual. Third, the most extreme copy number variation corresponds to genes embedded within segmental duplications, and most of these differences involve tandem changes in copy as opposed to duplications to new locations. We have validated 113 complete genes as copy number variable among these three individuals. Several of the validated loci are of known biomedical relevance related to color blindness (for example, opsin variation (**Supplementary Fig. 2d**), psoriasis (**Supplementary Note**) and age-related macular degeneration (**Fig. 2b**). In addition, several human genes with the most variable copy number (**Table 2** and **Supplementary Fig. 2b**,**f**) correspond to rapidly evolving gene families that emerged within the common ancestor of human and African great apes (for example, *TBC1D*, *LRRC37*, *GOLGA* and *NBPF*). Members of these gene families correspond to the core duplicons that have been implicated in the expansion of intrachromosomal segmental duplications during hominid evolution[38]. Although the function of these genes is largely unknown, the ability to use NGS to accurately predict their copy number provides the ability to make genotype and phenotype correlations in these complex areas of the genome.

Copy number differences, including variable duplications of entire genes, are now recognized as making substantial contributions to variation in human phenotypes. The ability to accurately and systematically determine the absolute copy number for any genomic segment is a notable first step toward a true and complete picture of individual genomes and phenotypes. In light of the sensitivity and specificity of read depth approaches, we anticipate that this strategy will eventually replace array CGH–based methods. The next challenge will be further definition of the sequence content and structural organization of these dynamic and important regions of the human genome.

URLs. NCBI short-read archive: http://www.ncbi.nlm.nih.gov/Traces/sra/sra.cgi; European Read Archive: ftp://ftp.era.ebi.ac.uk/; 1000 Genomes Project: http://www.1000genomes.org; mrFAST: http://mrfast.sourceforge.net.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

We thank D. Bentley for early access to the Illumina WGS dataset for NA18507; J. Wang for the YH DNA and the cell line; M. Egholm and B. Simen for the JDW

DNA and J.D. Watson for permission to analyze his genome. We also thank M. Shumway, P. Flicek and R. Leinonen for technical assistance in transferring large sequence datasets; E. Tüzün for help in parallelizing mrFAST for computation clusters through message passing interface; S. Girirajan for assistance with experiments and T. Brown for her help in manuscript preparation. J.M.K. is supported by a US National Science Foundation Graduate Research Fellowship. T.M.-B. is supported by a Marie Curie fellowship (FP7). This work was supported, in part, by U.S. National Institutes of Health grant HG004120 to E.E.E. E.E.E. is an investigator of the Howard Hughes Medical Institute.

AUTHOR CONTRIBUTIONS

C.A., J.M.K., T.M.-B. and E.E.E. designed the study, performed analytical work and wrote the manuscript. C.A., F.H. and O.M. designed and implemented the mrFAST algorithm. C.A., J.M.K., G.A. and J.O.K. performed computational analysis. T.M.-B., F.A., C.B. and M.M. performed validation experiments. R.A.G. advised on handling of JDW data analysis. S.C.S. and E.E.E. obtained funding for the study.

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1. Bailey, J.A. *et al.* Recent segmental duplications in the human genome. *Science* **297**, 1003–1007 (2002).
2. Iafrate, A.J. *et al.* Detection of large-scale variation in the human genome. *Nat. Genet.* **36**, 949–951 (2004).
3. Redon, R. *et al.* Global variation in copy number in the human genome. *Nature* **444**, 444–454 (2006).
4. Kidd, J.M. *et al.* Mapping and sequencing of structural variation from eight human genomes. *Nature* **453**, 56–64 (2008).
5. Fanciulli, M. *et al.* FCGR3B copy number variation is associated with susceptibility to systemic, but not organ-specific, autoimmunity. *Nat. Genet.* **39**, 721–723 (2007).
6. Aitman, T.J. *et al.* Copy number polymorphism in Fcgr3 predisposes to glomerulonephritis in rats and humans. *Nature* **439**, 851–855 (2006).
7. Gonzalez, E. *et al.* The influence of CCL3L1 gene-containing segmental duplications on HIV-1/AIDS susceptibility. *Science* **307**, 1434–1440 (2005).
8. Fellermann, K. *et al.* A chromosome 8 gene-cluster polymorphism with low human beta-defensin 2 gene copy number predisposes to Crohn disease of the colon. *Am. J. Hum. Genet.* **79**, 439–448 (2006).
9. Yang, Y. *et al.* Gene copy-number variation and associated polymorphisms of complement component C4 in human systemic lupus erythematosus (SLE): low copy number is a risk factor for and high copy number is a protective factor against SLE susceptibility in European Americans. *Am. J. Hum. Genet.* **80**, 1037–1054 (2007).
10. Hollox, E.J. *et al.* Psoriasis is associated with increased beta-defensin genomic copy number. *Nat. Genet.* **40**, 23–25 (2008).
11. Estivill, X. *et al.* Chromosomal regions containing high-density and ambiguously mapped putative single nucleotide polymorphisms (SNPs) correlate with segmental duplications in the human genome. *Hum. Mol. Genet.* **11**, 1987–1995 (2002).
12. Locke, D.P. *et al.* Linkage disequilibrium and heritability of copy-number polymorphisms within duplicated regions of the human genome. *Am. J. Hum. Genet.* **79**, 275–290 (2006).
13. Cooper, G.M., Zerr, T., Kidd, J.M., Eichler, E.E. & Nickerson, D.A. Systematic assessment of copy number variant detection via genome-wide SNP genotyping. *Nat. Genet.* **40**, 1199–1203 (2008).
14. Locke, D.P. *et al.* Large-scale variation among human and great ape genomes determined by array comparative genomic hybridization. *Genome Res.* **13**, 347–357 (2003).
15. Sharp, A.J. *et al.* Segmental duplications and copy-number variation in the human genome. *Am. J. Hum. Genet.* **77**, 78–88 (2005).
16. Tuzun, E. *et al.* Fine-scale structural variation of the human genome. *Nat. Genet.* **37**, 727–732 (2005).
17. Korbel, J.O. *et al.* Paired-end mapping reveals extensive structural variation in the human genome. *Science* **318**, 420–426 (2007).
18. Wang, J. *et al.* The diploid genome sequence of an Asian individual. *Nature* **456**, 60–65 (2008).
19. Campbell, P.J. *et al.* Identification of somatically acquired rearrangements in cancer using genome-wide massively parallel paired-end sequencing. *Nat. Genet.* **40**, 722–729 (2008).
20. Wheeler, D.A. *et al.* The complete genome of an individual by massively parallel DNA sequencing. *Nature* **452**, 872–876 (2008).
21. Chiang, D.Y. *et al.* High-resolution mapping of copy-number alterations with massively parallel sequencing. *Nat. Methods* **6**, 99–103 (2009).
22. Bentley, D.R. *et al.* Accurate whole human genome sequencing using reversible terminator chemistry. *Nature* **456**, 53–59 (2008).
23. Li, H., Ruan, J. & Durbin, R. Mapping short DNA sequencing reads and calling variants using mapping quality scores. *Genome Res.* **18**, 1851–1858 (2008).
24. Hillier, L.W. *et al.* Whole-genome sequencing and variant discovery in *C. elegans*. *Nat. Methods* **5**, 183–188 (2008).
25. Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **215**, 403–410 (1990).
26. Levenshtein, V.I. Binary codes capable of correcting deletions, insertions and reversals. *Sov. Phys. Dokl.* **10**, 707–710 (1966).
27. Ukkonen, E. On approximate string matching. in *Fundamentals of Computation Theory, Proceedings of the 1983 International FCT Conference* 487–495 (Springer-Verlag, London, 1983).
28. Smit, A.F.A., Hubley, R. & Green, P. RepeatMasker Open-3.0. <http://www.repeatmasker.org> (1996–2004).
29. Benson, G. Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Res.* **27**, 573–580 (1999).
30. Morgulis, A., Gertz, E.M., Schaffer, A.A. & Agarwala, R. WindowMasker: window-based masker for sequenced genomes. *Bioinformatics* **22**, 134–141 (2006).
31. Smith, D.R. *et al.* Rapid whole-genome mutational profiling using next-generation sequencing technologies. *Genome Res.* **18**, 1638–1642 (2008).
32. She, X. *et al.* Shotgun sequence assembly and recent segmental duplications within the human genome. *Nature* **431**, 927–930 (2004).
33. Istrail, S. *et al.* Whole-genome shotgun assembly and comparison of human genome assemblies. *Proc. Natl. Acad. Sci. USA* **101**, 1916–1921 (2004).
34. Bailey, J.A., Yavor, A.M., Massa, H.F., Trask, B.J. & Eichler, E.E. Segmental duplications: organization and impact within the current human genome project assembly. *Genome Res.* **11**, 1005–1017 (2001).
35. McCarroll, S.A. *et al.* Integrated detection and population-genetic analysis of SNPs and copy number variation. *Nat. Genet.* **40**, 1166–1174 (2008).
36. Lackner, C., Cohen, J.C. & Hobbs, H.H. Molecular definition of the extreme size polymorphism in apolipoprotein(a). *Hum. Mol. Genet.* **2**, 933–940 (1993).
37. Pruitt, K.D., Tatusova, T. & Maglott, D.R. NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res.* **35**, D61–D65 (2007).
38. Jiang, Z. *et al.* Ancestral reconstruction of segmental duplications reveals punctuated cores of human genome evolution. *Nat. Genet.* **39**, 1361–1368 (2007).

ONLINE METHODS

Computational analyses. Details regarding the mrFAST algorithm are described at length in the **Supplementary Note**. mrFAST is freely available to not-for-profit institutions (see URLs section). Segmental duplication maps were constructed from approximately sixfold 454 sequence coverage of the JDW genome, 21-fold Illumina sequence coverage of NA18507 and 16-fold Illumina sequence coverage of YH. 454-based WGS sequence reads of JDW (average length, 266 bp) were broken into 36-bp sequences to make the read-length properties comparable among the three sequence libraries (**Supplementary Note**). Sequence reads were mapped using mrFAST against human genome reference build35 (**Supplementary Note**) to define duplication intervals and calculate absolute copy numbers. Read depth was normalized with respect to GC content via a LOESS-based smoothing technique (**Supplementary Note**). For cross-sample comparisons, the duplication status of each individual over each interval was reassessed based on the estimated absolute copy number (**Supplementary Note**).

Array CGH validation. We performed array CGH to confirm duplications specific to individual genomes and to confirm copy number differences for shared duplications. A total of six experiments were performed in duplicate with dye-reversals performed between test and reference: NA18507 versus JDW, NA18507 versus YH and JDW versus YH. The $\log_2$ relative hybridization intensity was calculated for each probe. In this analysis, we restricted our analysis to regions >20 kb that contained at least 20 probes. We used a heuristic approach to calculate $\log_2$ thresholds of significance for each comparison, dynamically adjusting the thresholds for each hybridization to result in a false discovery rate of <1% in the control regions[39].

FISH analysis. Metaphase spreads were obtained from lymphoblast cell lines from NA18507 (Coriell Cell Repository) and YH (Han Chinese)[18]. FISH experiments were performed using fosmid clones[4] (**Supplementary Note**) directly labeled by nick-translation with Cy3-dUTP (Perkin-Elmer) as described previously[40] with minor modifications (**Supplementary Note**). Digital images were captured using a Leica DMRXA2 epifluorescence microscope equipped with a cooled CCD camera (Princeton Instruments). 4,6-diamidino-2-phenylindole (DAPI) and Cy3 fluorescence signals, detected with specific filters, were recorded separately as grayscale images. Pseudocoloring and merging of images was performed using Adobe Photoshop software. A minimum of 50 interphase cells were scored for each probe.

39. Marques-Bonet, T. *et al.* A burst of segmental duplications in the genome of the African great ape ancestor. *Nature* **457**, 877–881 (2009).
40. Lichter, P. *et al.* High-resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. *Science* **247**, 64–69 (1990).

doi:10.1038/ng.437