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An improved pig reference genome sequence to enable pig genetics and genomics research

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Abstract:	The domestic pig (Sus scrofa) is important both as a food source and as a biomedical model given its similarity in size, anatomy, physiology, metabolism, pathology and pharmacology to humans. The draft reference genome (Sscrofa10.2) of a purebred Duroc female pig established using older clone-based sequencing methods was incomplete and unresolved redundancies, short range order and orientation errors and associated misassembled genes limited its utility. We present two annotated highly contiguous chromosome-level genome assemblies created with more recent long read technologies and a whole genome shotgun strategy, one for the same Duroc female (Sscrofa11.1) and one for an outbred, composite breed male (USMARCv1.0). Both assemblies are of substantially higher (>90-fold) continuity and accuracy than Sscrofa10.2. These highly contiguous assemblies plus annotation of a further 11 short read assemblies provide an unprecedented view of the genetic make-up of this important agricultural and biomedical model species. We propose that the improved Duroc assembly (Sscrofa11.1) become the reference genome for genomic research in pigs.

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Experimental design and statistics Full details of the experimental design and statistical methods used should be given in the Methods section, as detailed in our Minimum Standards Reporting Checklist. Information essential to interpreting the data presented should be made available in the figure legends. Have you included all the information requested in your manuscript?	Yes
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An improved pig reference genome sequence to enable pig genetics and genomics research

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Abstract

The domestic pig (*Sus scrofa*) is important both as a food source and as a biomedical model given its similarity in size, anatomy, physiology, metabolism, pathology and pharmacology to humans. The draft reference genome (Sscrofa10.2) of a purebred Duroc female pig established using older clone-based sequencing methods was incomplete, and unresolved redundancies, short range order and orientation errors and associated misassembled genes limited its utility. We present two annotated highly contiguous chromosome-level genome assemblies created with more recent long read technologies and a whole genome shotgun strategy, one for the same Duroc female (Sscrofa11.1) and one for an outbred, composite breed male (USMARCv1.0). Both assemblies are of substantially higher (>90-fold) continuity and accuracy than Sscrofa10.2. These highly contiguous assemblies plus annotation of a further 11 short read assemblies provide an unprecedented view of the genetic make-up of this important agricultural and biomedical model species. We propose that the improved Duroc assembly (Sscrofa11.1) become the reference genome for genomic research in pigs.

Keywords

Pig genomes, reference assembly, pig, genome annotation

Background

High quality, richly annotated reference genome sequences are key resources and provide important frameworks for the discovery and analysis of genetic variation and for linking genotypes to function. In farmed animal species such as the domestic pig (*Sus scrofa*, NCBI:txid9823) genome sequences have been integral to the discovery of molecular genetic variants and the development of single nucleotide polymorphism (SNP) chips [1] and enabled efforts to dissect the genetic control of complex traits, such as growth, feed conversion, body composition, reproduction, behaviour and responses to infectious diseases [2].

Genome sequences are not only an essential resource for enabling research but also for applications in the life sciences. Genomic selection, in which associations between thousands of SNPs and trait variation as established in a phenotyped training population are used to choose amongst selection candidates for which there are SNP data but no phenotypes, has delivered genomics-enabled genetic improvement in farmed animals [3] and plants. From its initial successful application in dairy cattle breeding, genomic selection is now being used in many sectors within animal and plant breeding, including by leading pig breeding companies [4, 5].

The domestic pig (*Sus scrofa*) has importance not only as a source of animal protein but also as a biomedical model. The choice of the optimal animal model species for pharmacological or toxicology studies can be informed by knowledge of the genome and gene content of the candidate species including pigs [6]. A high quality, richly annotated genome sequence is also essential when using gene editing technologies to engineer improved animal models for research or as sources of cells and tissue for xenotransplantation and potentially for improved productivity [7, 8].

The highly continuous pig genome sequences reported here are built upon a quarter of a century of effort by the global pig genetics and genomics research community including the development of

recombination and radiation hybrid maps [9, 10], cytogenetic and Bacterial Artificial Chromosome (BAC) physical maps [11, 12] and a draft reference genome sequence [13].

The previously published draft pig reference genome sequence (Sscrofa10.2), developed under the auspices of the Swine Genome Sequencing Consortium (SGSC), has a number of significant deficiencies [14-17]. The BAC-by-BAC hierarchical shotgun sequence approach [18] using Sanger sequencing technology can yield a high quality genome sequence as demonstrated by the public Human Genome Project. However, with a fraction of the financial resources of the Human Genome Project, the resulting draft pig genome sequence comprised an assembly, in which long-range order and orientation is good, but the order and orientation of sequence contigs within many BAC clones was poorly supported and the sequence redundancy between overlapping sequenced BAC clones was often not resolved. Moreover, about 10% of the pig genome, including some important genes, were not represented (e.g. *CD163*), or incompletely represented (e.g. *IGF2*) in the assembly [19]. Whilst the BAC clones represent an invaluable resource for targeted sequence improvement and gap closure as demonstrated for chromosome X (SSCX) [20], a clone-by-clone approach to sequence improvement is expensive notwithstanding the reduced cost of sequencing with next-generation technologies.

The dramatically reduced cost of whole genome shotgun sequencing using Illumina short read technology has facilitated the sequencing of several hundred pig genomes [17, 21, 22]. Whilst a few of these additional pig genomes have been assembled to contig level, most of these genome sequences have simply been aligned to the reference and used as a resource for variant discovery.

The increased capability and reduced cost of third generation long read sequencing technology as delivered by Pacific Biosciences and Oxford Nanopore platforms, have created the opportunity to generate the data from which to build highly contiguous genome sequences as illustrated recently for cattle [23, 24]. Here we describe the use of Pacific Biosciences (PacBio) long read technology to

149 establish highly continuous pig genome sequences that provide substantially improved resources for
150 pig genetics and genomics research and applications.
151

Results

Two individual pigs were sequenced independently: a) TJ Tabasco (Duroc 2-14) i.e. the sow that was the primary source of DNA for the published draft genome sequence (Sscrofa10.2) [13] and b) MARC1423004 which was a crossbred barrow (i.e. castrated male pig) from a composite population (approximately ½ Landrace, ¼ Duroc and ¼ Yorkshire) at the USDA Meat Animal Research Center. The former allowed us to build upon the earlier draft genome sequence, exploit the associated CHORI-242 BAC library resource [25] and evaluate the improvements achieved by comparison with Sscrofa10.2. The latter allowed us to assess the relative efficacy of a simpler whole genome shotgun sequencing and Chicago Hi-Rise scaffolding strategy [26]. This second assembly also provided data for the Y chromosome, and supported comparison of haplotypes between individuals. In addition, full-length transcript sequences were collected for multiple tissues from the MARC1423004 animal, and used in annotating both genomes.

Sscrofa11.1 assembly

Approximately sixty-five fold coverage (176 Gbp) of the genome of TJ Tabasco (Duroc 2-14) was generated using Pacific Biosciences (PacBio) single-molecule real-time (SMRT) sequencing technology. A total of 213 SMRT cells produced 12,328,735 subreads of average length 14,270 bp and with a read N50 of 19,786 bp (Table S1). Reads were corrected and assembled using Falcon (v.0.4.0) [27], achieving a minimum corrected read cutoff of 13 kbp that provided 19-fold genome coverage for input resulting in an initial assembly comprising 3,206 contigs with a contig N50 of 14.5 Mbp.

The contigs were mapped to the previous draft assembly (Sscrofa10.2) using Nucmer [28]. The long range order of the Sscrofa10.2 assembly was based on fingerprint contig (FPC) [12] and radiation hybrid physical maps with assignments to chromosomes based on fluorescent *in situ* hybridisation data. This alignment of Sscrofa10.2 and the contigs from the initial Falcon assembly of the PacBio data provided draft scaffolds that were tested for consistency with paired BAC and fosmid end sequences

and the radiation hybrid map [9]. The draft scaffolds also provided a framework for gap closure using PBJelly [29], or finished quality Sanger sequence data generated from CHORI-242 BAC clones from earlier work [13, 20].

Remaining gaps between contigs within scaffolds, and between scaffolds predicted to be adjacent on the basis of other available data, were targeted for gap filling with a combination of unplaced contigs and previously sequenced BACs, or by identification and sequencing of BAC clones predicted from their end sequences to span the gaps. The combination of methods filled 2,501 gaps and reduced the number of contigs in the assembly from 3,206 to 705. The assembly, Sscrofa11 (GCA_000003025.5), had a final contig N50 of 48.2 Mbp, only 103 gaps in the sequences assigned to chromosomes, and only 583 remaining unplaced contigs (Table 1). Two acrocentric chromosomes (SSC16, SSC18) were each represented by single, unbroken contigs. The SSC18 assembly also includes centromeric and telomeric repeats (Tables S2, S3; Figs. S1, S2), albeit the former probably represent a collapsed version of the true centromere. The reference genome assembly was completed by adding Y chromosome sequences from other sources (GCA_900119615.2) [20] because TJ Tabasco (Duroc 2-14) was female. The resulting reference genome sequence was termed Sscrofa11.1 and deposited in the public sequence databases (GCA_000003025.6) (Table 1).

The medium to long range order and orientation of Sscrofa11.1 assembly was assessed by comparison to an existing radiation hybrid (RH) map [9]. The comparison strongly supported the overall accuracy of the assembly (Fig. 1a), despite the fact that the RH map was prepared from a cell line of a different individual. There is one major disagreement between the RH map and the assembly on chromosome 3, which will need further investigating. The only other substantial disagreement on chromosome 9, is explained by a gap in the RH map [9]. The assignment and orientation of the Sscrofa11.1 scaffolds to chromosomes was confirmed with fluorescent *in situ* hybridisation (FISH) of BAC clones (Table S4, Fig. S3). The Sscrofa11.1 and USMARCv1.0 assemblies were searched using BLAST [30] with sequences

204 derived from the BAC clones which had been used as probes for the FISH analyses. For most BAC
205 clones these sequences were BAC end sequences [12], but in some cases these sequences were
206 incomplete or complete BAC clone sequences [13, 20]. The links between the genome sequence and
207 the BAC clones used in cytogenetic analyses by fluorescent *in situ* hybridization are summarised in
208 Table S4. The fluorescent *in situ* hybridization results indicate areas where future assemblies might be
209 improved. For example, the Sscrofa11.1 unplaced scaffolds contig 1206 and contig1914 may contain
210 sequences that could be added to end of the long arms of SSC1 and SSC7 respectively.
211
212 The quality of the Sscrofa11 assembly, which corresponds to Sscrofa11.1 after the exclusion of SSCY,
213 was assessed as described previously for the existing Sanger sequence based draft assembly
214 (Sscrofa10.2) [14]. Alignments of Illumina sequence reads from the same female pig were used to
215 identify regions of low quality (LQ; regions with high GC normalised coverage, prevalence of
216 improperly paired reads and prevalence of reads with improper insert sizes) or low coverage (LC;
217 regions with low GC normalised coverage) (Table 2).. The analysis confirms that Sscrofa11 represents
218 a significant improvement over the Sscrofa10.2 draft assembly. For example, the Low Quality Low
219 Coverage (LQLC) proportion of the genome sequence has dropped from 33.07% to 16.3% when
220 repetitive sequence is not masked, and falls to 1.6% when repeats are masked prior to read alignment.
221 The remaining LQLC segments of Sscrofa11 have an average GC content of 61.6%. Thus, these regions
222 may represent sequence where short read coverage is low due to the known systematic bias of the
223 short read platform against extreme GC content sequences, rather than deficiencies of the assembly.
224
225 The Sscrofa11.1 assembly was also assessed visually using gEVAL [31]. The improvement in short range
226 order and orientation as revealed by alignments with isogenic BAC and fosmid end sequences is
227 illustrated for a particularly poor region of Sscrofa10.2 on chromosome 12 (Fig. S4). The problems in
228 this area of Sscrofa10.2 arose from failures to order and orient the sequence contigs and resolve the
229 redundancies between these sequence contigs within BAC clone CH242-147O24 (ENA: FP102566.2).

The improved contiguity in Sscrofa11.1 not only resolves these local order and orientation errors, but also facilitates the annotation of a complete gene model for the *ABR* locus. Further examples of comparisons of Sscrofa10.2 and Sscrofa11.1 reveal improvements in contiguity, local order and orientation and gene models (Fig. S5 to S7).

USMARCv1.0 assembly

Approximately sixty-five fold coverage of the genome of the MARC1423004 barrow was generated on a PacBio RSII instrument. The sequence was collected during the transition from P5/C3 to P6/C4 chemistry, with approximately equal numbers of subreads from each chemistry. A total of 199 cells of P5/C3 chemistry produced 95.3 Gbp of sequence with mean subread length of 5.1 kbp and subread N50 of 8.2 kbp. A total of 127 cells of P6/C4 chemistry produced 91.6 Gbp of sequence with mean subread length 6.5 kbp and subread N50 of 10.3 kbp, resulting in an overall average subread length, including data from both chemistries, of 6.4 kbp. The reads were assembled using Celera Assembler 8.3rc2 [32] and Falcon [27]). The resulting assemblies were compared and the Celera Assembler result was selected based on better agreement with a Dovetail Chicago® library [26] (i.e. there was a lower proportion of conflicting links between read pairs from the Chicago® library), and was used to create a scaffolded assembly with the HiRise™ scaffolder consisting of 14,818 contigs with a contig N50 of 6.372 Mbp (GenBank accession GCA_002844635.1; Table 1). The USMARCv1.0 scaffolds were therefore completely independent of the existing Sscrofa10.2 or new Sscrofa11.1 assemblies, and they can act as supporting evidence where they agree with those assemblies. However, chromosome assignment of the scaffolds was performed by alignment to Sscrofa10.2, and does not constitute independent confirmation of this ordering. The assignment of these scaffolds to individual chromosomes was confirmed post-hoc by FISH analysis as described for Sscrofa11.1 above. The FISH analysis revealed that several of these chromosome assemblies (SSC1, 5, 6-11, 13-16) are inverted with respect to the cytogenetic convention for pig chromosome (Table S4; Figs. S3, S8 to S10). After

255 correcting the orientation of these inverted scaffolds, there is good agreement between the
256 USMARCv1.0 assembly and the RH map [9] (Fig. 1b, Table S5).
257
258 Sscrofa11.1 and USMARCv1.0 are co-linear
259 The alignment of the two PacBio assemblies reveals a high degree of agreement and co-linearity, after
260 correcting the inversions of several USMARCv1.0 chromosome assemblies (Fig. S11). The agreement
261 between the Sscrofa11.1 and USMARCv1.0 assemblies is also evident in comparisons of specific loci
262 (Figs. S5 to S7) although with some differences (e.g. Fig. S6). The whole genome alignment of
263 Sscrofa11.1 and USMARCv1.0 (Fig. S11) masks some inconsistencies that are evident when the
264 alignments are viewed on a single chromosome-by-chromosome basis (Figs. S8 to S10). It remains to
265 be determined whether the small differences between the assemblies represent errors in the
266 assemblies, or true structural variation between the two individuals (see discussion of the *ERLIN1*
267 locus below).
268
269 Pairwise comparisons amongst the Sscrofa10.2, Sscrofa11.1 and USMARCv1.0 assemblies using the
270 Assemblytics tools [33] revealed a peak of insertions and deletion with sizes of about 300 bp (Figs.
271 S12a to S12c). We assume that these correspond to short interspersed nuclear elements (SINEs). Both
272 Sscrofa11.1 and USMARCv1.0 assemblies have more differences against Sscrofa10.2 (33,347 and
273 44,023 respectively) than against each other (28,733). This is despite the fact that Sscroffa11.1 and
274 Sscrofa10.2 represent the same pig genome. While some differences between Sscrofa10.2 and
275 Sscrofa11.1 may be due to differences in which haplotype has been captured in the assembly, the
276 reduction in low quality and low coverage regions and the dramatic decrease in differences versus
277 USMARCv1.0 leads us to conclude that the majority are improvements in the Sscrofa11.1 assembly.
278 The differences between the Sscrofa11.1 and USMARCv1.0 will represent a mix of true structural
279 differences and assembly errors that will require further research to resolve. The Sscrofa11.1 and
280 USMARCv1.0 assemblies were also compared to 11 Illumina short read assemblies [17] (Table S6).

Repetitive sequences, centromeres and telomeres

The repetitive sequence content of the Sscrofa11.1 and USMARCv1.0 was identified and characterised. These analyses allowed the identification of centromeres and telomeres for several chromosomes. The previous reference genome (Sscrofa10.2) that was established from Sanger sequence data and a minipig genome (minipig_v1.0, GCA_000325925.2) that was established from Illumina short read sequence data were also included for comparison. The numbers of the different repeat classes and the average mapped lengths of the repetitive elements identified in these four pig genome assemblies are summarised in Figures S13 and S14, respectively.

Putative telomeres were identified at the proximal ends of Sscrofa11.1 chromosome assemblies of SSC2, SSC3, SSC6, SSC8, SSC9, SSC14, SSC15, SSC18 and SSCX (Fig S1; Table S2). Putative centromeres were identified in the expected locations in the Sscrofa11.1 chromosome assemblies for SSC1-7, SSC9, SSC13 and SSC18 (Fig S2, Table S3). For the chromosome assemblies of each of SSC8, SSC11 and SSC15 two regions harbouring centromeric repeats were identified. Pig chromosomes SSC1-12 plus SSCX and SSCY are all metacentric, whilst chromosomes SSC13-18 are acrocentric. The putative centromeric repeats on SSC17 do not map to the expected end of the chromosome assembly.

Completeness of the assemblies

The Sscrofa11.1 and USMARCv1.0 assemblies were assessed for completeness using two tools, BUSCO (Benchmarking Universal Single-Copy Orthologs) [34] and Cogent [35]. BUSCO uses a database of expected gene content based on near-universal single-copy orthologs from species with genomic data, while Cogent uses transcriptome data from the organism being sequenced, and therefore provides an organism-specific view of genome completeness. BUSCO analysis suggests both new assemblies are highly complete, with 93.8% and 93.1% of BUSCOs complete for Sscrofa11.1 and USMARCv1.0

306 respectively, a marked improvement on the 80.9% complete in Sscrofa10.2 and comparable to the
307 human and mouse reference genome assemblies (Table S7).
308
309 Cogent is a tool that identifies gene families and reconstructs the coding genome using full-length,
310 high-quality (HQ) transcriptome data without a reference genome and can be used to check
311 assemblies for the presence of these known coding sequences [35]. PacBio transcriptome (Iso-Seq)
312 data consisting of high-quality isoform sequences from 7 tissues (diaphragm, hypothalamus, liver,
313 skeletal muscle (*longissimus dorsi*), small intestine, spleen and thymus) [36] from the pig whose DNA
314 was used as the source for the USMARCv1.0 assembly were pooled together for Cogent analysis.
315 Cogent partitioned 276,196 HQ isoform sequences into 30,628 gene families, of which 61% had at
316 least 2 distinct transcript isoforms. Cogent then performed reconstruction on the 18,708 partitions.
317 For each partition, Cogent attempts to reconstruct coding 'contigs' that represent the ordered
318 concatenation of transcribed exons as supported by the isoform sequences. The reconstructed contigs
319 were then mapped back to Sscrofa11.1 and contigs that could not be mapped or map to more than
320 one position are individually examined. There were five genes that were present in the Iso-Seq data,
321 but missing in the Sscrofa11.1 assembly. In each of these five cases, a Cogent partition (which consists
322 of 2 or more transcript isoforms of the same gene, often from multiple tissues) exists in which the
323 predicted transcript does not align back to Sscrofa11.1. NCBI-BLASTN of the isoforms from the
324 partitions revealed them to have near perfect hits with existing annotations for *CHAMP1*, *ERLIN1*,
325 *IL1RN*, *MB*, and *PSD4* for other species.
326
327 *ERLIN1* is missing from its predicted location on SSC14 between *CHUK* and *CPN1* gene in Sscrofa11.1.
328 There is good support for the Sscrofa11.1 assembly in the region from the BAC end sequence
329 alignments suggesting this area may represent a true haplotype. Indeed, a copy number variant (CNV)
330 nsv1302227 has been mapped to this location on SSC14 [37] and the *ERLIN1* gene sequences present
331 in BAC clone CH242-513L2 (ENA: CT868715.3) were incorporated into the earlier Sscrofa10.2

assembly. However, an alternative haplotype containing *ERLIN1* was not found in any of the assembled contigs from Falcon and this will require further investigation. The *ERLIN1* locus is present on SSC14 in the USMARCv1.0 assembly (30,107,816-30,143,074; note the USMARCv1.0 assembly of SSC14 is inverted relative to Sscrofa11.1). Of eleven short read pig genome assemblies [17] that have been annotated with the Ensembl pipeline (Ensembl release 98, September 2019) [38, 39] *ERLIN1* sequences are present in the expected genomic context in all eleven genome assemblies. As the *ERLIN1* gene is located at the end of a contig in eight of these short read assemblies, it suggests that this region of the pig genome presents difficulties for sequencing and assembly and the absence of *ERLIN1* in the Sscrofa11.1 is more likely to be an assembly error.

The other 4 genes are annotated in neither Sscrofa10.2 nor Sscrofa11.1. Two of these genes, *IL1RN* and *PSD4*, are present in the original Falcon contigs, however they were trimmed off during the contig QC stage because of apparent abnormal Illumina, BAC and fosmid mapping in the region which was likely caused by the repetitive nature of their expected location on chromosome 3 where a gap is present. The *IL1RN* and *PSD4* genes are present in the USMARCv1.0, albeit their location is anomalous, and are also present in the 11 short read assemblies [17]. *CHAMP1* (ENSSSCG00070014091) is present in the USMARCv1.0 assembly in the sub-telomeric region of the q-arm, after correcting the inversion of the USMARCv1.0 scaffold and is also present in all 11 short read assemblies [17]. After correcting the orientation of the USMARCv1.0 chromosome 11 scaffold there is a small inversion of the distal 1.07 Mbp relative to the Sscrofa11.1 assembly; this region harbours the *CHAMP1* gene. The orientation of the Sscrofa11.1 chromosome 11 assembly in this region is consistent with the predictions of the human-pig comparative map [40]. The myoglobin gene (*MB*) is present in the expected location in the USMARCv1.0 assembly flanked by *RASD2* and *RBFOX2*. Partial *MB* sequences are present distal to *RBFOX2* on chromosome 5 in the Sscrofa11.1 assembly. As there is no gap here in the Sscrofa11.1 assembly it is likely that the incomplete *MB* is a result of a misassembly in this region. This interpretation is supported by a break in the pairs of BAC and fosmid end sequences that

map to this region of the Sscrofa11.1 assembly. Some of the expected gene content missing from this region of the Sscrofa11.1 chromosome 5 assembly, including *RASD2*, *HMOX1* and *LARGE1* is present on an unplaced scaffold (AEMK02000361.1). Cogent analysis also identified 2 cases of potential fragmentation in the Sscrofa11.1 genome assembly that resulted in the isoforms being mapped to two separate loci, though these will require further investigation. In summary, the BUSCO and Cogent analyses indicate that the Sscrofa11.1 assembly captures a very high proportion of the expressed elements of the genome.

Improved annotation

Annotation of Sscrofa11.1 was carried out with the Ensembl annotation pipeline and released via the Ensembl Genome Browser (Ensembl release 90, August 2017) [38, 41]. Statistics for the annotation as updated in June 2019 (Ensembl release 98, September 2019) are listed in Table 3. This annotation is more complete than that of Sscrofa10.2 and includes fewer fragmented genes and pseudogenes.

The annotation pipeline utilised extensive short read RNA-Seq data from 27 tissues and long read PacBio Iso-Seq data from 9 adult tissues. This provided an unprecedented window into the pig transcriptome and allowed for not only an improvement to the main gene set, but also the generation of tissue-specific gene tracks from each tissue sample. The use of Iso-Seq data also improved the annotation of UTRs, as they represent transcripts sequenced across their full length from the polyA tract.

In addition to improved gene models, annotation of the Sscrofa11.1 assembly provides a more complete view of the porcine transcriptome than annotation of the previous assembly (Sscrofa10.2; Ensembl releases 67-89, May 2012 – May 2017) [42] with increases in the numbers of transcripts annotated (Table 3). However, the number of annotated transcripts remains lower than in the human

and mouse genomes. The annotation of the human and mouse genomes and in particular the gene content and encoded transcripts has been more thorough as a result of extensive manual annotation.

Efforts were made to annotate important classes of genes, in particular immunoglobulins and olfactory receptors. For these genes, sequences were downloaded from specialist databases and the literature in order to capture as much detail as possible (see supplementary information for more details).

These improvements in terms of the resulting annotation were evident in the results of the comparative genomics analyses run on the gene set. The previous annotation had 12,919 one-to-one orthologs with human, while the new annotation of the Sscrofa11.1 assembly has 15,544. Similarly, in terms of conservation of synteny, the previous annotation had 11,661 genes with high confidence gene order conservation scores, while the new annotation has 15,958. There was also a large reduction in terms of genes that were either abnormally short or split when compared to their orthologs in the new annotation.

The Sscrofa11.1 assembly has also been annotated using the NCBI pipeline [43]. We have compared these two annotations. The Ensembl and NCBI annotations of Sscrofa11.1 are broadly similar (Table S8). There are 17,676 protein coding genes and 1,700 non-coding genes in common. However, 540 of the genes annotated as protein-coding by Ensembl are annotated as non-coding or pseudogenes by NCBI and 227 genes annotated as non-coding by NCBI are annotated as protein-coding (215) or as pseudogenes (12) by Ensembl. The NCBI RefSeq annotation can be visualised in the Ensembl Genome Browser by loading the RefSeq GFF3 track and the annotations compared at the individual locus level. Similarly, the Ensembl annotated genes can be visualised in the NCBI Genome Browser. Despite considerable investment there are also differences in the Ensembl and NCBI annotation of the human reference genome sequence with 20,444 and 19,755 protein-coding genes on the primary assembly,

409 respectively. The MANE (Matched Annotation from NCBI and EMBL-EBI) project was launched to
410 resolve these differences and identify a matched representative transcript for each human protein-
411 coding gene [44]. To date a MANE transcript has been identified for 12,985 genes.
412
413 We have also annotated the USMARCv1.0 assembly using the Ensembl pipeline [38] and this
414 annotation was released via the Ensembl Genome Browser (Ensembl release 97, July 2019) [39](see
415 Table 3 for summary statistics). More recently, we have annotated a further eleven short read pig
416 genome assemblies [17] (Ensembl release 98, September 2019) [39]; see Tables S6 and S11 for
417 summary statistics for the assemblies and annotation, respectively.
418
419 SNP chip probes mapped to assemblies
420 The probes from four commercial SNP chips were mapped to the Sscrofa10.2, Sscrofa11.1 and
421 USMARCv1.0 assemblies. We identified 1,709, 56, and 224 markers on the PorcineSNP60, GGP LD and
422 80K commercial chips that were previously unmapped and now have coordinates on the Sscrofa11.1
423 reference (Table S9). These newly mapped markers can now be imputed into a cross-platform,
424 common set of SNP markers for use in genomic selection. Additionally, we have identified areas of the
425 genome that are poorly tracked by the current set of commercial SNP markers. The previous
426 Sscrofa10.2 reference had an average marker spacing of 3.57 kbp (Stdev: 26.5 kbp) with markers from
427 four commercial genotyping arrays. We found this to be an underestimate of the actual distance
428 between markers, as the Sscrofa11.1 reference coordinates consisted of an average of 3.91 kbp
429 (Stdev: 14.9 kbp) between the same set of markers. We also found a region of 2.56 Mbp that is
430 currently devoid of suitable markers on the new reference.
431
432 A Spearman's rank order (rho) value was calculated for each assembly (alternative hypothesis: rho is
433 equal to zero; $p < 2.2 \times 10^{-16}$): Sscrofa10.2: 0.88464; Sscrofa11.1: 0.88890; USMARCv1.0: 0.81260. This
434 rank order comparison was estimated by ordering all of the SNP probes from all chips by their listed

435 manifest coordinates against their relative order in each assembly (with chromosomes ordered by
436 karyotype). Any unmapped markers in an assembly were penalized by giving the marker a "-1" rank in
437 the assembly ranking order.
438
439 In order to examine general linear order of placed markers on each assembly, the marker rank order
440 (y axis; used above in the Spearman's rank order test) was plotted against the rank order of the probe
441 rank order on the manifest file (x axis) (Fig. S15). The analyses revealed some interesting artefacts that
442 suggest that the SNP manifest coordinates for the porcine 60K SNP chip are still derived from an
443 obsolete (Sscrofa9) reference in contrast to all other manifests (Sscrofa10.2). Also, it confirms that
444 several of the USMARCv1.0 chromosome scaffolds are inverted with respect to the canonical
445 orientation of pig chromosomes. The large band of points at the top of the plot corresponds to marker
446 mappings on the unplaced contigs of each assembly. These unplaced contigs often correspond to
447 assemblies of alternative haplotypes in heterozygous regions of the reference animal [24]. Marker
448 placement on these segments suggests that these variants are tracking different haplotypes in the
449 population, which is the desired intent of genetic markers used in Genomic Selection.
450

Discussion

We have assembled a superior, extremely continuous reference assembly (Sscrofa11.1) by leveraging the excellent contig lengths provided by long reads, and a wealth of available data including Illumina paired-end, BAC end sequence, finished BAC sequence, fosmid end sequences, and the earlier curated draft assembly (Sscrofa10.2). The pig genome assemblies USMARCv1.0 and Sscrofa11.1 reported here are 92-fold to 694-fold respectively, more continuous than the published draft reference genome sequence (Sscrofa10.2) [13]. The new pig reference genome assembly (Sscrofa11.1) with its contig N50 of 48,231,277 bp and 506 gaps compares favourably with the current human reference genome sequence (GRCh38.p12) that has a contig N50 of 57,879,411 bp and 875 gaps (Table 1). Indeed, considering only the chromosome assemblies built on PacBio long read data (i.e. Sscrofa11 - the autosomes SSC1-SSC18 plus SSCX), there are fewer gaps in the pig assembly than in human reference autosomes and HSAX assemblies. Most of the gaps in the Sscrofa11.1 reference assembly are attributed to the fragmented assembly of SSCY. The capturing of centromeres and telomeres for several chromosomes (Tables S2, S3; Figs. S1, S2) provides further evidence that the Sscrofa11.1 assembly is more complete. The increased contiguity of Sscrofa11.1 is evident in the graphical comparison to Sscrofa10.2 illustrated in Figure 2.

The improvements in the reference genome sequence (Sscrofa11.1) relative to the draft assembly (Sscrofa10.2) [13] are not restricted to greater continuity and fewer gaps. The major flaws in the BAC clone-based draft assembly were i) failures to resolve the sequence redundancy amongst sequence contigs within BAC clones and between adjacent overlapping BAC clones and ii) failures to accurately order and orient the sequence contigs within BAC clones. Although the Sanger sequencing technology used has a much lower raw error rate than the PacBio technology, the sequence coverage was only 4-6 fold across the genome. The improvements in continuity and quality (Table 2; Figs. S5 to S7) have yielded a better template for annotation resulting in better gene models. The Sscrofa11.1 and USMARCv1.0 assemblies are classed as 4|4|1 and 3|5|1 [10^X: N50 contig (kbp); 10^Y: N50 scaffold

(kbp); Z = 1|0: assembled to chromosome level] respectively compared to Sscrofa10.2 as 1|2|1 and the human GRCh38p5 assembly as 4|4|1 [45].

The improvement in the complete BUSCO (Benchmarking Universal Single-Copy Orthologs) genes indicates that both Sscrofa11.1 and USMARCv1.0 represent superior templates for annotation of gene models than the draft Sscrofa10.2 assembly and are comparable to the finished human and mouse reference genome sequences (Table S7). Further, a companion bioinformatics analysis of available Iso-seq and companion Illumina RNA-seq data across the nine tissues surveyed has identified a large number (>54,000) of novel transcripts [36]. A majority of these transcripts are predicted to be spliced and validated by RNA-seq data. Beiki and colleagues identified 10,465 genes expressing Iso-seq transcripts that are present on the Sscrofa11.1 assembly, but which are unannotated in current NCBI or Ensembl annotations [36].

Whilst the alignment of the Sscrofa11.1 and USMARCv1.0 assemblies revealed that several of the USMARCv1.0 chromosome assemblies are inverted relative to Sscrofa11.1 and the cytogenetic map. Such inversions are due to the agnostic nature of genome assembly and post-assembly polishing programs. Unless these are corrected post-hoc by manual curation, they result in artefactual inversions of the entire chromosome. However, such inversions do not generally impact downstream analysis that does not involve the relative order/orientation of whole chromosomes.

Whether the differences between Sscrofa11.1 and USMARCv1.0 in order and orientation within chromosomes represent assembly errors or real chromosomal differences will require further research. The sequence present at the telomeric end of the long arm of the USMARCv1.0 chromosome 7 assembly (after correcting the orientation of the USMARCv1.0 SSC7) is missing from the Sscrofa11.1 SSC7 assembly, and currently located on a 3.8 Mbp unplaced scaffold (AEMK02000452.1). This unplaced scaffold harbours several genes including *DIO3*, *CKB* and *NUDT14* whose orthologues map

503 to human chromosome 14 as would be predicted from the pig-human comparative map [40]. This
504 omission will be corrected in an updated assembly in future.
505
506 We demonstrate moderate improvements in the placement and ordering of commercial SNP
507 genotyping markers on the Sscrofa11.1 reference genome which will impact future genomic selection
508 programs. The reference-derived order of SNP markers plays a significant role in imputation accuracy,
509 as demonstrated by a whole-genome survey of misassembled regions in cattle that found a correlation
510 between imputation errors and misassemblies [46]. The gaps in SNP chip marker coverage that we
511 identified will inform future marker selection surveys, which are likely to prioritize regions of the
512 genome that are not currently being tracked by marker variants in close proximity to potential causal
513 variant sites. In addition to the gaps in coverage provided by the commercial SNP chips there are
514 regions of the genome assemblies that are devoid of annotated sequence variation as hitherto
515 sequence variants have been discovered against incomplete genome assemblies. Thus, there is a need
516 to re-analyse good quality re-sequence data against the new assemblies in order to provide a better
517 picture of sequence variation in the pig genome.
518
519 The cost of high coverage whole-genome sequencing (WGS) precludes it from routine use in breeding
520 programs. However, it has been suggested that low coverage WGS followed by imputation of
521 haplotypes may be a cost-effective replacement for SNP arrays in genomic selection [47]. Imputation
522 from low coverage sequence data to whole genome information has been shown to be highly accurate
523 [48, 49]. At the 2018 World Congress on Genetics Applied to Livestock Production Aniek Bouwman
524 reported that in a comparison of Sscrofa10.2 with Sscrofa11.1 (for SSC7 only) for imputation from
525 600K SNP genotypes to whole genome sequence overall imputation accuracy on SSC7 improved
526 considerably from 0.81 (1,019,754 variants) to 0.90 (1,129,045 variants) (Aniek Bouwman, pers.
527 comm). Thus, the improved assembly may not only serve as a better template for discovering genetic
528 variation but also have advantages for genomic selection, including improved imputation accuracy.

Advances in the performance of long read sequencing and scaffolding technologies, improvements in methods for assembling the sequence reads and reductions in costs are enabling the acquisition of ever more complete genome sequences for multiple species and multiple individuals within a species. For example, in terms of adding species, the Vertebrate Genomes Project [50] aims to generate error-free, near gapless, chromosomal level, haplotyped phase assemblies of all of the approximately 66,000 vertebrate species and is currently in its first phase that will see such assemblies created for an exemplar species from all 260 vertebrate orders. At the level of individuals within a species, smarter assembly algorithms and sequencing strategies are enabling the production of high quality truly haploid genome sequences for outbred individuals [24]. The establishment of assembled genome sequences for key individuals in the nucleus populations of the leading pig breeding companies is achievable and potentially affordable. However, 10-30x genome coverage short read data generated on the Illumina platform and aligned to a single reference genome is likely to remain the primary approach to sequencing multiple individuals within farmed animal species such as cattle and pigs [21, 51].

There are significant challenges in making multiple assembled genome resources useful and accessible. The current paradigm of presenting a reference genome as a linear representation of a haploid genome of a single individual is an inadequate reference for a species. As an interim solution the Ensembl team are annotating multiple assemblies for some species such as mouse and dog [52, 53, 54]. We have implemented this solution for pig genomes, including eleven Illumina short-read assemblies [17] in addition to the reference Sscrofa11.1 and USMARCv1.0 assemblies reported here (Ensembl release 98, September 2019) [39, 41]. Although these additional pig genomes are highly fragmented (Table S6) with contig N50 values from 32 – 102 kbp, the genome annotation (Table S11) provides a resource to explore pig gene space across thirteen genomes, including six Asian pig

554 genomes. The latter are important given the deep phylogenetic split of about 1 million years between
555 European and Asian pigs [13].
556
557 The current human genome reference already contains several hundred alternative haplotypes and it
558 is expected that the single linear reference genome of a species will be replaced with a new model –
559 the graph genome [55-57]. These paradigm shifts in the representation of genomes present challenges
560 for current sequence alignment tools and the 'best-in-genome' annotations generated thus far. The
561 generation of high quality annotation remains a labour-intensive and time-consuming enterprise.
562 Comparisons with the human and mouse reference genome sequences which have benefited from
563 extensive manual annotation indicate that there is further complexity in the porcine genome as yet
564 unannotated (Table 3). It is very likely that there are many more transcripts, pseudogenes and non-
565 coding genes (especially long non-coding genes), to be discovered and annotated on the pig genome
566 sequence [36]. The more highly continuous pig genome sequences reported here provide an improved
567 framework against which to discover functional sequences, both coding and regulatory, and sequence
568 variation. After correction for some contig/scaffold inversions in the USMARCv1.0 assembly, the
569 overall agreement between the assemblies is high and illustrates that the majority of genomic
570 variation is at smaller scales of structural variation. However, both assemblies still represent a
571 composite of the two parental genomes present in the animals, with unknown effects of haplotype
572 switching on the local accuracy across the assembly.
573
574 Future developments in high quality genome sequences for the domestic pig are likely to include: (i)
575 gap closure of Sscrofa11.1 to yield an assembly with one contig per (autosomal) chromosome arm
576 exploiting the isogenic BAC and fosmid clone resource as illustrated here for chromosome 16 and 18;
577 and (ii) haplotype resolved assemblies of a Meishan and White Composite F1 crossbred pig (i.e. the
578 offspring of a Meishan sire and a White Composite dam that is approximately ½ Landrace, ¼ Duroc
579 and ¼ Yorkshire) currently being sequenced. Beyond this haplotype resolved assemblies for key

genotypes in the leading pig breeding company nucleus populations and of miniature pig lines used in biomedical research can be anticipated in the next 5 years. Unfortunately, some of these genomes may not be released into the public domain. The first wave of results from the Functional Annotation of ANimal Genomes (FAANG) initiative [58, 59], are emerging and will add to the richness of pig genome annotation.

In conclusion, the new pig reference genome (Sscrofa11.1) described here represents a significantly enhanced resource for genetics and genomics research and applications for a species of importance to agriculture and biomedical research.

Methods

Additional detailed methods and information on the assemblies and annotation are included in the Supplementary Materials.

Preparation of genomic DNA

DNA was extracted from Duroc 2-14 cultured fibroblast cells passage 16-18 using the Qiagen Blood & Cell Culture DNA Maxi Kit. DNA was isolated from lung tissue from barrow MARC1423004 using a salt extraction method.

Genome sequencing and assembly

Genomic DNAs from the samples described above were used to prepare libraries for sequencing on Pacific Biosciences RS II sequencer (PacBio RS II Sequencing System, RRID:SCR_017988) [60]. For Duroc 2-14 DNA P6/C4 chemistry was used, whilst for MARC1423004 DNA a mix of P6/C4 and earlier P5/C3 chemistry was used.

Reads from the Duroc 2-14 DNA were assembled into contigs using the Falcon v0.4.0 assembly pipeline (Falcon, RRID:SCR_016089) following the standard protocol [27]. Quiver v. 2.3.0 [61] was used to correct the primary and alternative contigs. Only the primary pseudo-haplotype contigs were used in the assembly. The reads from the MARC1423004 DNA were assembled into contigs using Celera Assembler v8.3rc2 (Celera assembler, RRID:SCR_010750) [32]. The contigs were scaffolded as described in the results section above.

Fluorescence *in situ* hybridisation

Metaphase preparations were fixed to slides and dehydrated through an ethanol series (2 mins each in 2×SSC, 70%, 85% and 100% ethanol at RT). Probes were diluted in a formamide buffer (Cytocell) with Porcine Hybloc (Insight Biotech) and applied to the metaphase preparations on a 37°C hotplate

before sealing with rubber cement. Probe and target DNA were simultaneously denatured for 2 mins on a 75°C hotplate prior to hybridisation in a humidified chamber at 37°C for 16 h. Slides were washed post hybridisation in 0.4x SSC at 72°C for 2 mins followed by 2x SSC/0.05% Tween 20 at RT for 30 secs, and then counterstained using VECTASHIELD anti-fade medium with DAPI (Vector Labs). Images were captured using an Olympus BX61 epifluorescence microscope with cooled CCD camera and SmartCapture (Digital Scientific UK) system.

Analysis of repetitive sequences, including telomeres and centromeres

Repeats were identified using RepeatMasker (v.4.0.7, RRID:SCR_012954) [62] with a combined repeat database including Dfam (v.20170127) [63] and RepBase (v.20170127) [64]. RepeatMasker was run with "sensitive" (-s) setting using sus scrofa as the query species (-- species "sus scrofa"). Repeats which showed greater than 40% sequence divergence or were shorter than 70% of the expected sequence length were filtered out from subsequent analyses. The presence of potentially novel repeats was assessed by RepeatMasker using the novel repeat library generated by RepeatModeler (v.1.0.11, RRID:SCR_015027) [62].

Telomeres were identified by running Tandem Repeat Finder (TRF) [65] with default parameters apart from Mismatch (5) and Minscore (40). The identified repeat sequences were then searched for the occurrence of five identical, consecutive units of the TTAGGG vertebrate motif or its reverse complement and total occurrences of this motif were counted within the tandem repeat. Regions which contained at least 200 identical hexamer units, were >2kbp of length and had a hexamer density of >0.5 were retained as potential telomeres.

Centromeres were predicted using the following strategy. First, the RepeatMasker output, both default and novel, was searched for centromeric repeat occurrences. Second, the assemblies were searched for known, experimentally verified, centromere specific repeats [66, 67] in the Sscrofa11.1

642 genome. Then the three sets of repeat annotations were merged together with BEDTools (BEDTools,
643 RRID:SCR_006646) [68] (median and mean length: 786 bp and 5775 bp, respectively) and putative
644 centromeric regions closer than 500 bp were collapsed into longer super-regions. Regions which were
645 >5kbp were retained as potential centromeric sites.
646
647 Long read RNA sequencing (Iso-Seq)
648 The following tissues were harvested from MARC1423004 at age 48 days: brain (BioSamples:
649 SAMN05952594), diaphragm (SAMN05952614), hypothalamus (SAMN05952595), liver
650 (SAMN05952612), small intestine (SAMN05952615), skeletal muscle – *longissimus dorsi*
651 (SAMN05952593), spleen (SAMN05952596), pituitary (SAMN05952626) and thymus
652 (SAMN05952613). Total RNA from each of these tissues was extracted using Trizol reagent
653 (ThermoFisher Scientific) and the provided protocol. Briefly, approximately 100 mg of tissue was
654 ground in a mortar and pestle cooled with liquid nitrogen, and the powder was transferred to a tube
655 with 1 ml of Trizol reagent added and mixed by vortexing. After 5 minutes at room temperature, 0.2 ml
656 of chloroform was added and the mixture was shaken for 15 seconds and left to stand another
657 3 minutes at room temperature. The tube was centrifuged at 12,000 x g for 15 minutes at 4°C. The
658 RNA was precipitated from the aqueous phase with 0.5 ml of isopropanol. The RNA was further
659 purified with extended DNase I digestion to remove potential DNA contamination. The RNA quality
660 was assessed with a Fragment Analyzer (Advanced Analytical Technologies Inc., IA). Only RNA samples
661 of RQN above 7.0 were used for library construction. PacBio Iso-Seq libraries were constructed per
662 the PacBio Iso-Seq protocol. Briefly, starting with 3 µg of total RNA, cDNA was synthesized by using
663 SMARTer PCR cDNA Synthesis Kit (Clontech, CA) according to the Iso-Seq protocol (Pacific Biosciences,
664 CA). Then the cDNA was amplified using KAPA HiFi DNA Polymerase (KAPA Biotechnologies) for 10 or
665 12 cycles followed by purification and size selection into 4 fractions: 0.8-2 kbp, 2-3 kbp, 3-5 kbp and
666 >5 kbp. The fragment size distribution was validated on a Fragment Analyzer (Advanced Analytical
667 Technologies Inc, IA) and quantified on a DS-11 FX fluorometer (DeNovix, DE). After a second round

of large scale PCR amplification and end repair, SMRT bell adapters were separately ligated to the cDNA fragments. Each size fraction was sequenced on 4 or 5 SMRT Cells v3 using P6-C4 chemistry and 6 hour movies on a PacBio RS II sequencer (Pacific Bioscience, CA). Short read RNA-Seq libraries were also prepared for all nine tissue using TruSeq stranded mRNA LT kits and supplied protocol (Illumina, CA), and sequenced on a Illumina NextSeq500 platform using v2 sequencing chemistry to generate 2 x 75 bp paired-end reads.

The Read of Insert (ROI) were determined by using *consensustools.sh* in the SMRT-Analysis pipeline v2.0, with reads which were shorter than 300 bp and whose predicted accuracy was lower than 75% removed. Full-length, non-concatemer (FLNC) reads were identified by running the classify.py command. The cDNA primer sequences as well as the poly(A) tails were trimmed prior to further analysis. Paired-end Illumina RNA-Seq reads from each tissue sample were trimmed to remove the adaptor sequences and low-quality bases using Trimmomatic (v0.32, RRID:SCR_011848) [69] with explicit option settings: *ILLUMINACLIP:adapters.fa: 2:30:10:1:true LEADING:3 TRAILING:3 SLIDINGWINDOW: 4:20 LEADING:3 TRAILING:3 MINLEN:25*, and overlapping paired-end reads were merged using the PEAR software (v0.9.6; PEAR, RRID:SCR_003776) [70]. Subsequently, the merged and unmerged RNA-Seq reads from the same tissue samples were *in silico* normalized in a mode for single-end reads by using a Trinity (v2.1.1, RRID:SCR_013048) [71] utility, *insilico_read_normalization.pl*, with the following settings*: --max_cov 50 --max_pct_stdev 100 --single*. Errors in the full-length, non-concatemer reads were corrected with the preprocessed RNA-Seq reads from the same tissue samples by using proovread (v2.12; Proovread, RRID:SCR_017331) [72]. Untrimmed sequences with at least some regions of high accuracy in the *.trimmed.fq* files were extracted based on sequence IDs in *.untrimmed.fa* files to balance off the contiguity and accuracy of the final reads.

<u>Short read RNA sequencing (RNA-Seq)</u>

694 In addition to the Illumina short read RNA-seq data generated from MARC1423004 and used to correct
695 the Iso-Seq data (see above), Illumina short read RNA-seq data (PRJEB19386) were also generated
696 from a range of tissues from four juvenile Duroc pigs (two male, two female) and used for annotation
697 as described below. Extensive metadata with links to the protocols for sample collection and
698 processing are linked to the BioSample entries under the Study Accession PRJEB19386. The tissues
699 sampled are listed in Table S10. Sequencing libraries were prepared using a ribodepletion TruSeq
700 stranded RNA protocol and 150 bp paired end sequences generated on the Illumina HiSeq 2500
701 platform (Illumina HiSeq 2500 System, RRID:SCR_016383) in rapid mode.
702
703 Annotation
704 The assembled genomes were annotated using the Ensembl pipelines (Ensembl, RRID:SCR_002344)
705 [38] as detailed in the Supplementary materials. The Iso-Seq and RNA-Seq data described above were
706 used to build gene models.
707
708 Mapping SNP chip probes
709 The probes from four commercial SNP chips were mapped to the Sscrofa10.2, Sscrofa11.1 and
710 USMARCv1.0 assemblies using BWA MEM [73] and a wrapper script [74]. Probe sequence was derived
711 from the marker manifest files that are available on the provider websites: Illumina PorcineSNP60 [1,
712 75]; Affymetrix Axiom™ Porcine Genotyping Array [76]; Gene Seek Genomic Profiler Porcine – HD
713 beadChip [77]; Gene Seek Genomic Profiler Porcine v2– LD Chip [77]. In order to retain marker
714 manifest coordinate information, each probe marker name was annotated with the chromosome and
715 position of the marker's variant site from the manifest file. All mapping coordinates were tabulated
716 into a single file, and were sorted by the chromosome and position of the manifest marker site. In
717 order to derive and compare relative marker rank order, a custom Perl script [78] was used to sort and
718 number markers based on their mapping locations in each assembly.
719

Availability of supporting data

The genome assemblies are deposited at NCBI under accession numbers GCA_000003025 (Sscrofa11.1) and GCA_002844635.1 (USMARCv1.0). The associated BioSample accession numbers are SAMN02953785 and SAMN07325927, respectively. Iso-seq and RNA-Seq data used for analysis and annotation are available under accession numbers PRJNA351265 and PRJEB19386, respectively. Supporting data and materials are available in the *GigaScience* GigaDB database [100].

List of abbreviations

BAC: bacterial artificial chromosome; BLAST: Basic Local Alignment Search Tool; BLASTN: BLAST search of nucleotide database(s); bp: base pairs; BUSCO: Benchmarking Universal Single-Copy Orthologs; BWA: Burrows-Wheeler Aligner; CCD: charged couple device; cDNA: complementary DNA; CNV: copy number variation; DAPI: 4′,6-diamidino-2-phenylindole; DNA: Deoxyribonucleic Acid; DNase: deoxyribonuclease; ENA: European Nucleotide Archive; FAANG: Functional Annotation of Animal Genomes; FISH: fluorescent *in situ* hybridisation; FLNC: full-length, non-concatemer; FPC: fingerprint contig; g: relative centrifugal force; Gbp: gigabase pairs; GFF3: ge; eral feature format, version 3; GC: guanine-cytosine; h: hours; HQ: high quality; ID: identity; Iso-Seq: long read RNA sequencing using PacBio technology; kbp: kilobase pairs; LC: low coverage; LQ: low quality; LQLC: low quality, low coverage; MANE: Matched Annotation from NCBI and EMBL-EBI; Mbp: megabase pairs; mg: milligrams; µg: micrograms; ml: millilitres; mins: minutes; mRNA: messenger RNA; NCBI: National Center for Biotechnology Information; PacBio: Pacific Biosciences; polyA: poly adenine; PCR: polymerase chain reaction; QC: quality control; RefSeq: NCBI Reference Sequence Database; RH: radiation hybrid; RNA: Ribonucleic Acid; RNA-Seq: high-throughput short-read RNA sequencing; ROI: read of interest; RQN: RNA quality number; RT: room temperature; secs: seconds; SGSC: Swine Genome Sequencing Consortium; SINE: Short interspersed nuclear element; SMRT: single-molecule real-time; SNP: single nucleotide polymorphism; SSC: saline sodium citrate; SSCn: Sus scrofa chromosome n; Stdev: standard deviation; TRF: Tandem Repeat Finder.

746 **Consent for publication**

747 Not applicable

748 **Competing interests**

749 R.H. K.K. and E.T. are employed by Pacific Biosciences; all other authors declare that they have no
750 competing interests.

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764

765 **Authors' contributions**

766 A.L.A. and T.P.L.S. conceived, coordinated and managed the project; A.L.A., P.F., D.A.H., T.P.L.S. M.W.
767 supervised staff and students performing the analyses; D.J.N., L.R., L.B.S., T.P.L.S. provided biological
768 resources; R.H., K.S.K. and T.P.L.S. generated PacBio sequence data; H.A.F., T.P.L.S. and R.T. generated
769 Illumina WGS and RNA-Seq data; N.A.A., C.A.S., B.M.S. provided SSCY assemblies; D.J.N, and T.P.L.S.
770 generated Iso-Seq data; G.H., R.H., S.K., A.M.P., A.S.S, A.W. generated sequence assemblies; A.W.
771 polished and quality checked Sscrofa11.1; W.C., G.H., K.H., S.K., B.D.R., A.S.S., S.G.S., E.T. performed

quality checks on the sequence assemblies; R.E.O'C. and D.K.G. performed cytogenetics analyses; L.E. analysed repeat sequences; H.B., H.L., N.M., C.K.T. analysed Iso-Seq data; D.M.B. and G.A.R. analysed sequence variants; B.A., K.B., C.G.G., T.H., O.I., F.J.M. annotated the assembled genome sequences; A.W. and A.L.A drafted the manuscript; all authors read and approved the final manuscript.

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Supplementary materials

Supplementary materials for this article include:

Supplementary Methods and Information

Table S1. Pacific Biosciences read statistics.

Table S2. Predicted telomeres.

Table S3. Predicted centromeres.

Table S4. Assigning scaffolds to chromosomes.

Table S5 Alignment of Radiation Hybrid maps and genome assemblies.

Table S6. Assemblytics comparisons, assembly statistics.

Table S7. BUSCO results.

Table S8. Annotation statistics. (Ensembl-NCBI comparison)

Table S9. Commercial SNP chip probes.

Table S10. Tissue samples.

Table S11. Ensembl annotation statistics for 13 pig genome assemblies

Figure S1. Predicted telomeres.

Figure S2. Predicted centromeres.

Figure S3. Fluorescent *in situ* hybridisation assignments.

Fig. S4. Improvement in local order and orientation and reduction in redundancy.

Fig. S5. Assembly comparisons in gEVAL (SSC15).

Fig. S6. Assembly comparisons in gEVAL (SSC5).

Fig. S7. Assembly comparisons in gEVAL (SSC18).

Fig. S8. Order and orientation of SSC18 assemblies.

Fig. S9. Order and orientation of SSC7 assemblies.

Fig. S10. Order and orientation of SSC8 assemblies.

Fig. S11. Assembly alignments.

Figure S12. Assemblytics results.

Figure S13. Counts of repetitive elements in four pig assemblies.

Figure S14. Average mapped length of repetitive elements in four pig genomes.

Figure S15. Assembly SNP rank concordance versus reported chromosomal location.

819 **References**

820 1. Ramos AM, Crooijmans RPMA, Affara NA, Amaral AJ, Archibald AL, Beever JE, et al. Design of a
821 high density SNP genotyping assay in the pig using SNPs identified and characterized by next
822 generation sequencing technology. PLoS One 2009;**4**:e6524.
823 2. Hu ZL, Park CA, ReecyJM, Developmental progress and current status of the Animal QTLdb. Nucleic
824 Acids Res 2016;**44**:D827–33.
825 3. Meuwissen T, Hayes, B, Goddard M, Accelerating Improvement of Livestock with Genomic
826 Selection. Annu Rev Anim Biosci 2013;**1**:221–37.
827 4. Christensen OF, Madsen P, Nielsen B, Ostersen T, Su G, Single-step methods for genomic
828 evaluation in pigs. Animal 2012;**6**:1565–71.
829 5. Cleveland M, Hickey JM, Practical implementation of cost-effective genomic selection in
830 commercial pig breeding using imputation. J Anim Sci 2013;**91**:3583–92.
831 6. Vamathevan JJ, Hall MD, Hasan S, Woollard PM, Xu M, Yang Y, et al. Minipig and beagle animal
832 model genomes aid species selection in pharmaceutical discovery and development. Toxicol Appl
833 Pharmacol 2013;**270**:149–57.
834 7. Klymiuk N, Seeliger F, Bohlooly M, Blutke A, Rudmann DG, Wolf E, Tailored Pig Models for
835 Preclinical Efficacy and Safety Testing of Targeted Therapies. Toxicol Pathol 2016;**44**:346–57.
836 8. Wells KD, Prather RS, Genome-editing technologies to improve research, reproduction, and
837 production in pigs. Mol Reprod Dev 2017;**84**:1012–7.
838 9. Servin B, Faraut T, Iannuccelli N, Zelenika D, Milan D, High-resolution autosomal radiation hybrid
839 maps of the pig genome and their contribution to the genome sequence assembly. BMC Genomics
840 2012;**13**:585.
841 10. Tortereau F, Servin B, Frantz L, Megens HJ, Milan D, Rohrer G, et al. A high density recombination
842 map of the pig reveals a correlation between sex-specific recombination and GC content. BMC
843 Genomics 2012;**13**:586.

11. Yerle M, Lahbib-Mansais Y, Mellink C, Goureau A, Pinton P, Echard G, et al. The PiGMaP consortium cytogenetic map of the domestic pig (Sus scrofa domestica). Mamm Genome 1995;**6**:176–86.
12. Humphray SJ, Scott CE, Clark R, Marron B, Bender C, Camm N, et al. A high utility integrated map of the pig genome. Genome Biol 2017;**8**:R139.
13. Groenen MAM, Archibald AL, Uenishi H, Tuggle CK, Takeuchi Y, Rothschild MF, et al. Analyses of pig genomes provide insight into porcine demography and evolution. Nature 2012;**491**:393–8.
14. Warr A, Robert C, Hume D, Archibald AL, Deeb N, Watson M. Identification of Low-Confidence Regions in the Pig Reference Genome (Sscrofa 10.2). Front Genet 2015;**6**:338.
15. O'Connor RE, Fonseka G, Frodsham R, Archibald AL, Lawrie M, Walling GA et al. Isolation of subtelomeric sequences of porcine chromosomes for translocation screening reveals errors in the pig genome assembly. Anim Genet 2017;**48**:395–403.
16. Dawson HD, Chen C, Gaynor B, Shao J, Urban Jr. JF. The porcine translational research database: a manually curated, genomics and proteomics-based research resource. BMC Genomics 2017;**18**:643.
17. Li M, Chen L, Tian S, Lin Y, Tang Q, Zhou X, et al. Comprehensive variation discovery and recovery of missing sequence in the pig genome using multiple de novo assemblies. Genome Res 2017;**27**:865–74.
18. Schook LB, Beever JE, Rogers J, Humphray S, Archibald A, Chardon P, et al. Swine Genome Sequencing Consortium (SGSC): A strategic roadmap for sequencing the pig genome. Comp Funct Genomics 2005;**6**:251–5.
19. Robert C, Fuentes-Utrilla P, Troup K, Loecherbach J, Turner F, Talbot R, et al. Design and development of exome capture sequencing for the domestic pig (Sus scrofa). BMC Genomics 2014;**15**:550.
20. Skinner BM, Sargent CA, Churcher C, Hunt T, Herrero J, Loveland JE, et al. The pig X and Y Chromosomes: structure, sequence, and evolution. Genome Res 2016;**26**:130–9.

21. Frantz LAF, Schraiber JG, Madsen O, Megens HJ, Cagan A, Bosse M, et al. Evidence of long-term gene flow and selection during domestication from analyses of Eurasian wild and domestic pig genomes. Nat Genet 2015;**47**:1141-8.
22. Groenen MAM. A decade of pig genome sequencing: a window on pig domestication and evolution. Genet Sel Evol 2016;**48**:23.
23. van Dijk EL, Jaszczyszyn Y, Naquin D, Thermes C. The Third Revolution in Sequencing Technology. Trends Genet 2018;**34**:666-81.
24. Koren S, Rhie A, Walenz BP, Dilthey AT, Bickhart DM, Kingan SB, et al. De novo assembly of haplotype-resolved genomes with trio binning. Nat. Biotechnol 2018;**36**:1174-82.
25. CHORI-242: Porcine (Sus scrofa) BAC Library https://bacpacresources.org/library.php?id=124 Accessed 17 Apr 2020.
26. Putnam NH, O'Connell BL, Stites JC, Rice BJ, Blanchette M, Calef R, et al. Chromosome-scale shotgun assembly using an in vitro method for long-range linkage. Genome Res 2016;**26**:342–50.
27. Chin CS, Peluso P, Sedlazeck FJ, Nattestad M, Concepcion GT, Clum A, et al. Phased diploid genome assembly with single-molecule real-time sequencing. Nat Methods 2016;**13**:1050–4.
28. Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, et al. Versatile and open software for comparing large genomes. Genome Biol 2004;**5**:R12.
29. English AC, Richards S, Han Y, Wang M, Lee V, Qu J, et al. Mind the Gap: Upgrading Genomes with Pacific Biosciences RS Long-Read Sequencing Technology. PLoS One 2012;**7**:e47768.
30. Altschul SF, Gish W, Miller W, et al. Basic Local Alignment Search Tool. J Mol Biol 1990;**215**:403–10.
31. Chow W, Brugger K, Caccamo M, Sealy I, Torrance J, Howe K. gEVAL-a web-based browser for evaluating genome assemblies. Bioinformatics 2016;**32**:2508–10.
32. Berlin K, Koren S, Chin CS, Drake JP, Landolin JM, Phillippy AM. Assembling large genomes with single-molecule sequencing and locality-sensitive hashing. Nat Biotechnol 2015;**33**:623–30.

33. Nattestad M, Schatz MC. Assemblytics: A web analytics tool for the detection of variants from an assembly. Bioinformatics 2016;**32**:3021-3.
34. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 2015;**31**:3210–2.
35. Tseng E. Cogent: COding GENome reconstruction Tool. 2017 https://github.com/Magdoll/Cogent
36. Beiki H, Liu H, Manchanda N, Nonneman D, Smith TPL, Reecy JM et al. Improved annotation of the domestic pig genome through integration of Iso-Seq and RNA-seq data. BMC Genomics 2019;**20**:344.
37. Long Y, Su Y, Ai H, Zhang Z, Yang B, Ruan G, et al. A genome-wide association study of copy number variations with umbilical hernia in swine. *Anim Genet* 2016;**47**:298–305.
38. Cunningham F, Achuthan P, Akanni W, Allen J, Amode MR, Armean IM, et al. Ensembl 2019. Nucleic Acids Res 2019;**47(D1)**:D745-51.
39. Ensembl pig strains genome annotation Release 98, September 2019 http://www.ensembl.org/Sus_scrofa/Info/Strains
40. Meyers SN, Rogatcheva MB, Larkin DM, Yerle M, Milan D, Hawken RJ, et al. Piggy-BACing the human genome: II. A high-resolution, physically anchored, comparative map of the porcine autosomes. Genomics 2005;**86**:739-52.
41. Ensembl pig genome annotation Release 98, September 2019 http://www.ensembl.org/Sus_scrofa/Info/Index Accessed 15 October 2019.
42. Ensembl archive http://may2017.archive.ensembl.org/Sus_scrofa/Info/Index Accessed 15 April 2020.
43. NCBI annotation report 106 https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Sus_scrofa/106/ Accessed 15 April 2020.

44. MANE (Matched Annotation from NCBI and EMBL-EBI) https://www.ensembl.org/info/genome/genebuild/mane.html Accessed 15 April 2020.
45. gEVAL: Genome Evaluation Browser. https://geval.sanger.ac.uk/ Accessed 15 October 2019.
46. Utsunomiya ATH, Santos DJ, Boison SA, Utsunomiya YT, Milanesi M, Bickhart DM, et al. Revealing misassembled segments in the bovine reference genome by high resolution linkage disequilibrium scan. BMC Genomics 2016;**17**:705.
47. Hickey JM. Sequencing millions of animals for genomic selection 2.0. J Anim Breed Genet 2013;**130**:331-2.
48. Le SQ, Durbin R. SNP detection and genotyping from low-coverage sequencing data on multiple diploid samples. Genome Res 2011;**21**:952-60.
49. Li Y, Sidore C, Kang HM, Boehnke M, Abecasis GR. Low-coverage sequencing: Implications for design of complex trait association studies. Genome Res 2011;**21**:940-51.
50. Vertebrate Genomes Project https://vertebrategenomesproject.org/
51. Daetwyler HD, Capitan A, Pausch H, Stotthard P, van Binsbergen R, Brøndum RF, et al. Whole-genome sequencing of 234 bulls facilitates mapping of monogenic and complex traits in cattle. Nat Genet 2014;**46**:858-65.
52. Lilue J, Doran AG, Fiddes IT, Abrudan M, Armstrong J, Bennett R, et al. Sixteen diverse laboratory mouse reference genomes define strain-specific haplotypes and novel functional loci. Nat Genet 2018;**50**:1574-83.
53. Ensembl mouse strain annotation http://www.ensembl.org/Mus_musculus/Info/Strains Accessed 15 April 2020.
54. Ensembl dog breed annotation http://www.ensembl.org/Canis_familiaris/Info/Strains Accessed 15 April 2020.
55. Baier U, Beller T, Ohlebusch E. Graphical pan-genome analysis with compressed suffix trees and the Burrows-Wheeler transform. Bioinformatics 2015;**32**:497–504.

945 56. Chaisson MJP, Wilson RK, Eichler EE. Genetic variation and the de novo assembly of human
946 genomes. Nat Rev Genet 2015;**16**:627–40.
947 57. Garrison E, Sirén J, Novak AM, Hickey G, Eizenga JM, Dawson ET, et al. Variation graph toolkit
948 improves read mapping by representing genetic variation in the reference. Nat Biotechnol
949 2018;**36**:875-9.
950 58. Andersson L, Archibald AL, Bottema CD, Brauning R, Burgess SC, Burt DW, et al. Coordinated
951 international action to accelerate genome-to-phenome with FAANG, the Functional Annotation
952 of Animal Genomes project. Genome Biol 2015;**16**:57.
953 59. Foissac S, Djebali S, Munyard K, Vialaneix N, Rau A, Muret K, et al. Multispecies annotation of
954 transcriptome and chromatin structure in domesticated animals. BMC Biol 2019;**17**:108
955 60. Pendleton M, Sebra R, Pang AA, Ummat A, Franzen O, Rausch T, et al. Assembly and diploid
956 architecture of an individual human genome via single-molecule technologies. Nat Methods
957 2015;**12**:780–6.
958 61. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, et al. Nonhybrid, finished
959 microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 2013;**10**:563-
960 9.
961 62. RepeatMasker http://www.repeatmasker.org Accessed 17 April 2020
962 63. Hubley R, Finn RD, Clements J, Eddy SR, Jones TA, Bao W, et al. The Dfam database of repetitive
963 DNA families. Nucleic Acids Res 2016;**44**:D81–9.
964 64. Bao W, Kojima KK, Kohany O. Repbase Update, a database of repetitive elements in eukaryotic
965 genomes. Mob DNA **6**(1). doi: 10.1186/s13100-015-0041-9. (2015).
966 65. Benson G. Tandem repeats finder: A program to analyze DNA sequences. Nucleic Acids Res
967 1999;**27**:573–80.
968 66. Miller JR, Hindkjær J, Thomsen PD. A chromosomal basis for the differential organization of a
969 porcine centromere-specific repeat. Cytogenet Cell Genet 1993;**62**:37–41.

67. Riquet J, Mulsant P, Yerle M, Cristobal-Gaudy MS, Le Tissier P, Milan D, et al. Sequence analysis and genetic mapping of porcine chromosome 11 centromeric S0048 marker. Cytogenet Cell Genet 1996;**74**:127-32.
68. Quinlan AR, Hall IM. BEDTools: A flexible suite of utilities for comparing genomic features, Bioinformatics 2010;**26**:841–2.
69. Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 2014;**30**:2114–20.
70. Zhang J, Kobert K, Flouri T, Stamatakis A. PEAR: A fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 2014;**30**:614–20.
71. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 2011;**29**:644–52.
72. Hackl T, Hedrich R, Schultz J, Förster F. Proovread: Large-scale high-accuracy PacBio correction through iterative short read consensus. Bioinformatics 2014;**30**:3004–11.
73. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009;**25**:1754-60.
74. Bickhart D. 2019. Align and order SNP probes. https://github.com/njdbickhart/perl_toolchain/blob/master/assembly_scripts/alignAndOrderSnpProbes.pl
75. Illumina PorcineSNP60 Beadchip https://emea.illumina.com/products/by-type/microarray-kits/porcine-snp60.html
76. Axiom™ Porcine Genotyping Array https://www.thermofisher.com/order/catalog/product/550588#/550588
77. Gene Seek Genomic Profiler Porcine https://genomics.neogen.com/uk/ggp-porcine
78. Bickhart D. 2019. Pig genome SNP sort rank order. https://github.com/njdbickhart/perl_toolchain/blob/master/assembly_scripts/pigGenomeSNPSortRankOrder.pl

79. Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, et al. Integrative genomics viewer. Nat Biotechnol 2011;**29**:24–6.
80. Koren S, Walenz BP, Berlin K, JMiller JR, Bergman NH, Phillippy AM. Canu: Scalable and accurate long-read assembly via adaptive κ-mer weighting and repeat separation. Genome Res 2017;**27**:722–36.
81. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *ArXiv:1303.3997v1 [q-bio.GN]* (2013).
82. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, et al. Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS ONE 2014;**9**:e112963.
83. Anderson SI, Lopez-Corrales NL, Gorick B, Archibald AL. A large-fragment porcine genomic library resource in a BAC vector. Mamm Genome 2000;**11**:811–4.
84. Morgulis A, Gertz EM, Schäffer AA, Agarwala R. A Fast and Symmetric DUST Implementation to Mask Low-Complexity DNA Sequences. J Comp Biol 2006;**13**:1028-40.
85. Down TA, Hubbard TJP. Computational detection and location of transcription start sites in mammalian genomic DNA. Genome Res 2002;**12**:458–61.
86. Lowe TM, Eddy SR. TRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 1996;**25**:955–64.
87. Burge C, Karlin S. Prediction of complete gene structures in human genomic DNA. J Mol Biol 1997;**268**:78–94.
88. O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 2016;**44**:D733-45.
89. Slater GSC, Birney E. Automated generation of heuristics for biological sequence comparison. BMC Bioinformatics 2005;**6**:31.

90. Frankish A, Diekhans M, Ferreira AM, Johnson R, Jungreis I, Loveland J, et al. GENCODE reference annotation for the human and mouse genomes. Nucleic Acids Res 2019;**47**:D766-73.
91. Li H. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics 2018;**34**:3094-3100.
92. She R, Chu JS, Uyar B, Wang J, Wang K, Chen N. genBlastG: Using BLAST searches to build homologous gene models. Bioinformatics 2011;**27**:2141–3.
93. Lefranc MP, Giudicelli V, Duroux P, Jabado-Michaloud J, Folch G, Aouinti S, et al. IMGT®, the international ImMunoGeneTics information system® 25 years on. Nucleic Acids Res 2015;**43**:D413–22.
94. Lewis SE, Searle SMJ, Harris N, Gibson M, Iyer V, Richter J, et al. Apollo: a sequence annotation editor. Genome Biol 2002;**3**:research0082.1.
95. Griffiths-Jones S, Bateman A, Marshall M, Khanna A, Eddy SR. Rfam: An RNA family database', Nucleic Acids Res 2003;**31**:439–41.
96. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res 2006;**34**:D140–4.
97. Lorenz R, Bernhart SH, Höner Zu Siederdissen C, Tafer H, Flamm C, Stadler PF, et al. ViennaRNA Package 2.0. Algorithms Mol Biol 2011;**6**:26.
98. Nawrocki EP, Eddy SR. Infernal 1.1: 100-fold faster RNA homology searches. Bioinformatics 2013;**29**:2933-5.
99. Papatheodorou I, Fonesca NA, Keays M, Tang YA, Barrera E, Bazant W, et al. Expression Atlas: Gene and protein expression across multiple studies and organisms. Nucleic Acids Res 2018;**46**:D246-51.
100. Warr A; Affara N; Aken B; Beiki H; Bickhart DM; Billis K; Chow W; Eory L; Finlayson HA; Flicek P; Girón CG; Griffin DK; Hall R; Hannum G; Hourlier T; Howe K; Hume DA; Izuogu O; Kim K; Koren S; Liu H; Manchanda N; Martin FJ; Nonneman DJ; O'Connor RE; Phillippy AM; Rohrer GA; Rosen BD; Rund LA; Sargent CA; Schook LB; Schroeder SG; Schwartz AS; Skinner BM; Talbot R; Tseng E; Tuggle CK; Watson M; Smith TPL; Archibald AL (2020): Supporting data for "An improved pig

reference genome sequence to enable pig genetics and genomics research" *GigaScience* Database. http://dx.doi.org/10.5524/100732

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Figure 1. Assemblies and radiation hybrid map alignments. Plots illustrating co-linearity between radiation hybrid map and a) Sscrofa11.1 and b) USMARCv1.0 assemblies (autosomes only).

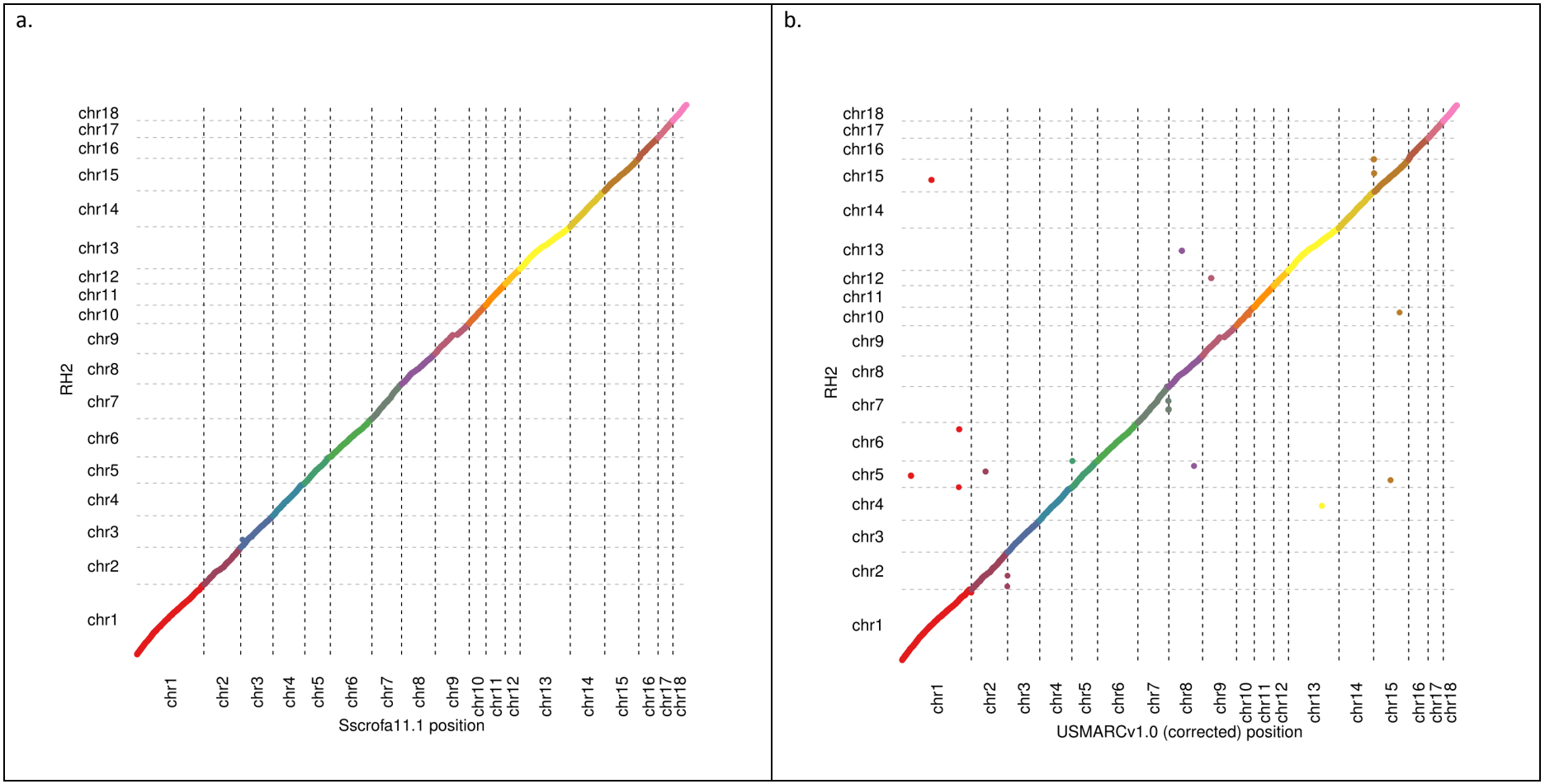
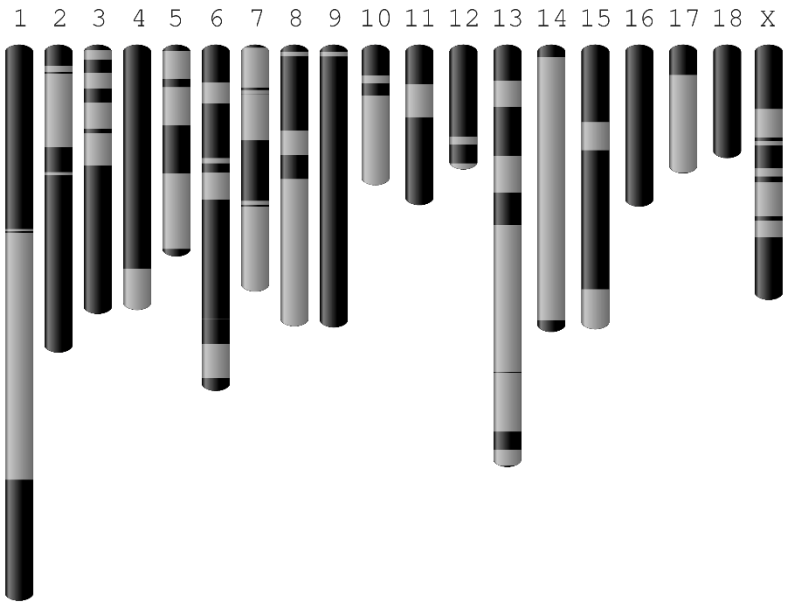


Figure 2. Visualisation of improvements in assembly contiguity. Graphical visualisation of contigs for Sscrofa11 (top) and Sscrofa10.2 (bottom) as alternating dark and light grey bars.



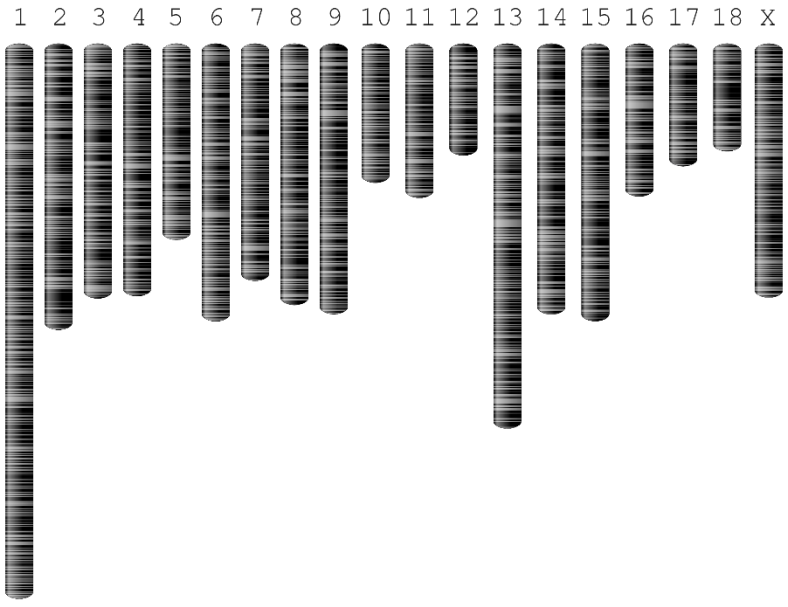


Table 1. Assembly statistics. Summary statistics for assembled pig genome sequences and comparison with current human reference genome. (source: NCBI, https://www.ncbi.nlm.nih.gov/assembly/; * includes mitochondrial genome.

Assembly	Sscrofa10.2	Sscrofa11	Sscrofa11.1	USMARCv1.0	GRCh38.p12
Total sequence length	2,808,525,991	2,456,768,445	2,501,912,388	2,755,438,182	3,099,706,404
Total ungapped length	2,519,152,092	2,454,899,091	2,472,047,747	2,623,130,238	2,948,583,725
Number of scaffolds	9,906	626	706	14,157	472
Gaps between scaffolds	5,323	24	93	0	349
Number of unplaced scaffolds	4,562	583	583	14,136	126
Scaffold N50	576,008	88,231,837	88,231,837	131,458,098	67,794,873
Scaffold L50	1,303	9	9	9	16
Number of unspanned gaps	5,323	24	93	0	349
Number of spanned gaps	233,116	79	413	661	526
Number of contigs	243,021	705	1,118	14,818	998
Contig N50	69,503	48,231,277	48,231,277	6,372,407	57,879,411
Contig L50	8,632	15	15	104	18
Number of chromosomes*	*21	19	*21	*21	24

Table 2. Summary of quality statistics for SSC1-18, SSCX. Quality measures and terms as defined [14].

	Mean (Sscrofa11)	Std (Sscrofa11)	Bases (Sscrofa11)	% genome (Sscrofa11)	% genome (Sscrofa10.2)
High Coverage	50	7	119,341,205	4.9	2.6
Low Coverage (LC)	50	7	185,385,536	7.5	26.6
% Properly paired	86	6.8	95,508,007	3.9	4.95
% High inserts	0.3	1.6	40,835,320	1.72	1.52
% Low inserts	8.2	4.3	114,793,298	4.7	3.99
Low quality (LQ)	-	-	284,838,040	11.6	13.85
Total LQLC	-	-	399,927,747	16.3	33.07
LQLC windows that do not intersect RepeatMasker regions			39,918,551	1.6	

Table 3. Annotation statistics. Ensembl annotation of pig (Sscrofa10.2, Sscrofa11.1, USMARCv1.0), human (GRCh38.p12) and mouse (GRCm38.p6) assemblies.

	Sscrofa10.2	Sscrofa11.1	USMARCv1.0	GRCh38.p13	GRCm38.p6
	Ensembl (Release 89)	Ensembl (Release 98)	Ensembl (Release 97)	Ensembl (Release 98)	Ensembl (Release 98)
Coding genes	21,630 (Incl. 10 read through)	21,301	21,535	20,444 incl 667 read through	22,508 incl 270 read through
Non-coding genes	3,124	8,971	6,113	23,949	16,078
small non-coding genes	2,804	2,156	2,427	4,871	5,531
long non-coding genes	135 (incl 1 read through)	6,798	3,307	16,857 incl 304 read through	9,985 incl 75 read through
misc. non-coding genes	185	17	379	2,221	562
Pseudogenes	568	1,626	674	15,214 incl 8 read through	13,597 incl 4 read through
Gene transcripts	30,585	63,041	58,692	227,530	142,446
Genscan gene predictions	52,372	46,573	152,168	51,756	57,381
Short variants	60,389,665	64,310,125		665,834,144	83,761,978
Structural variants	224,038	224,038		6,013,113	791,878

Figure

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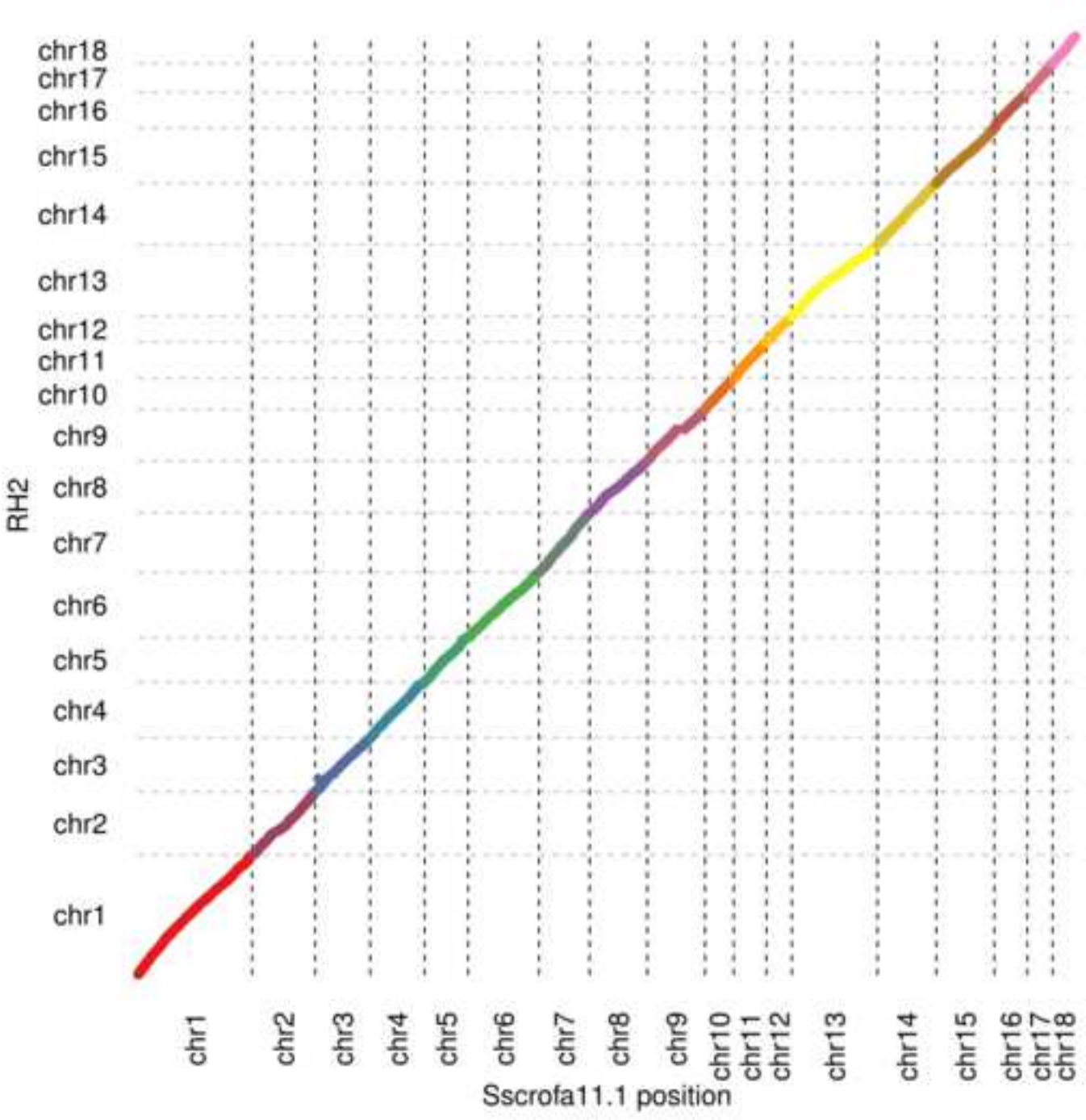

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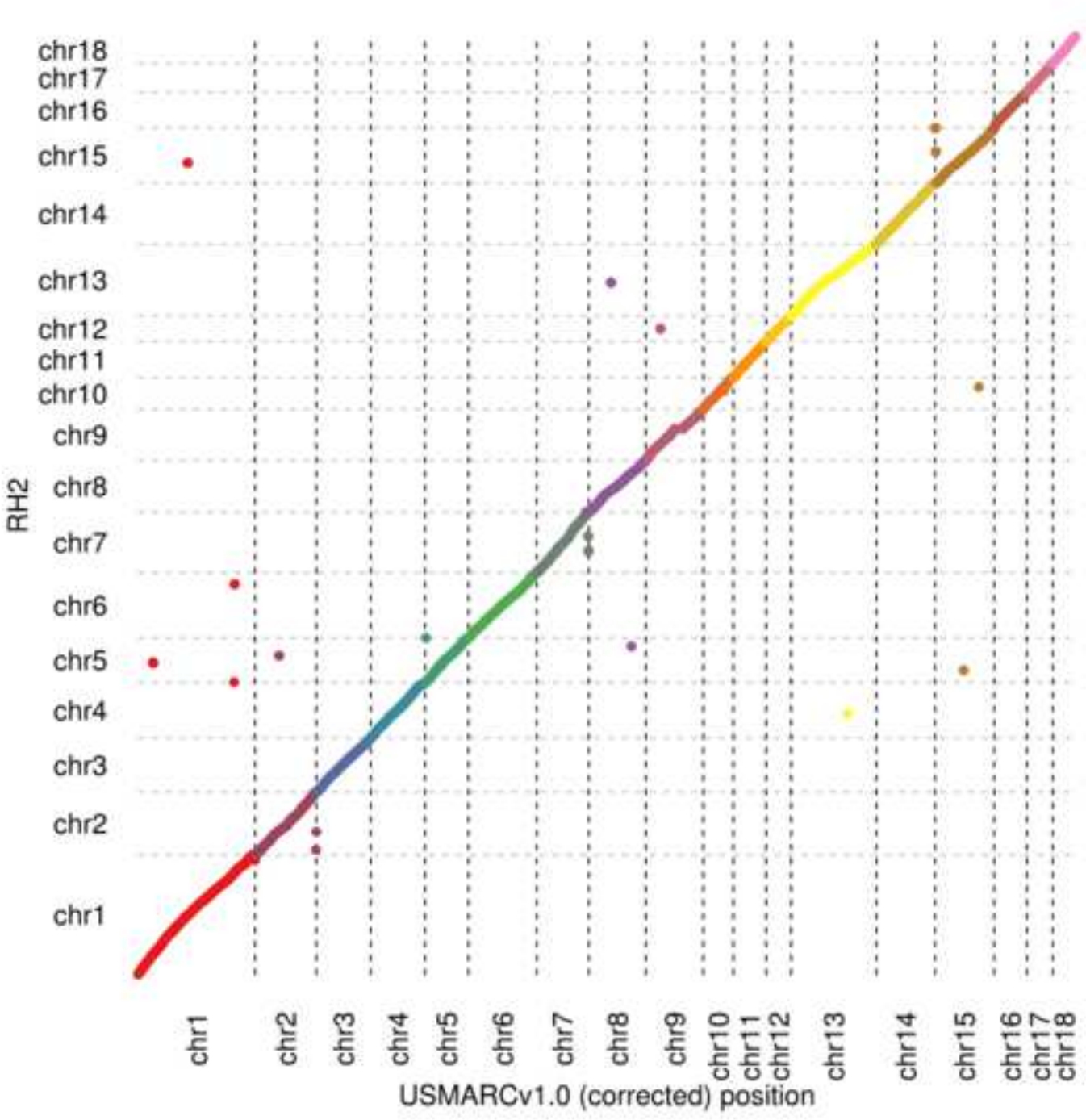

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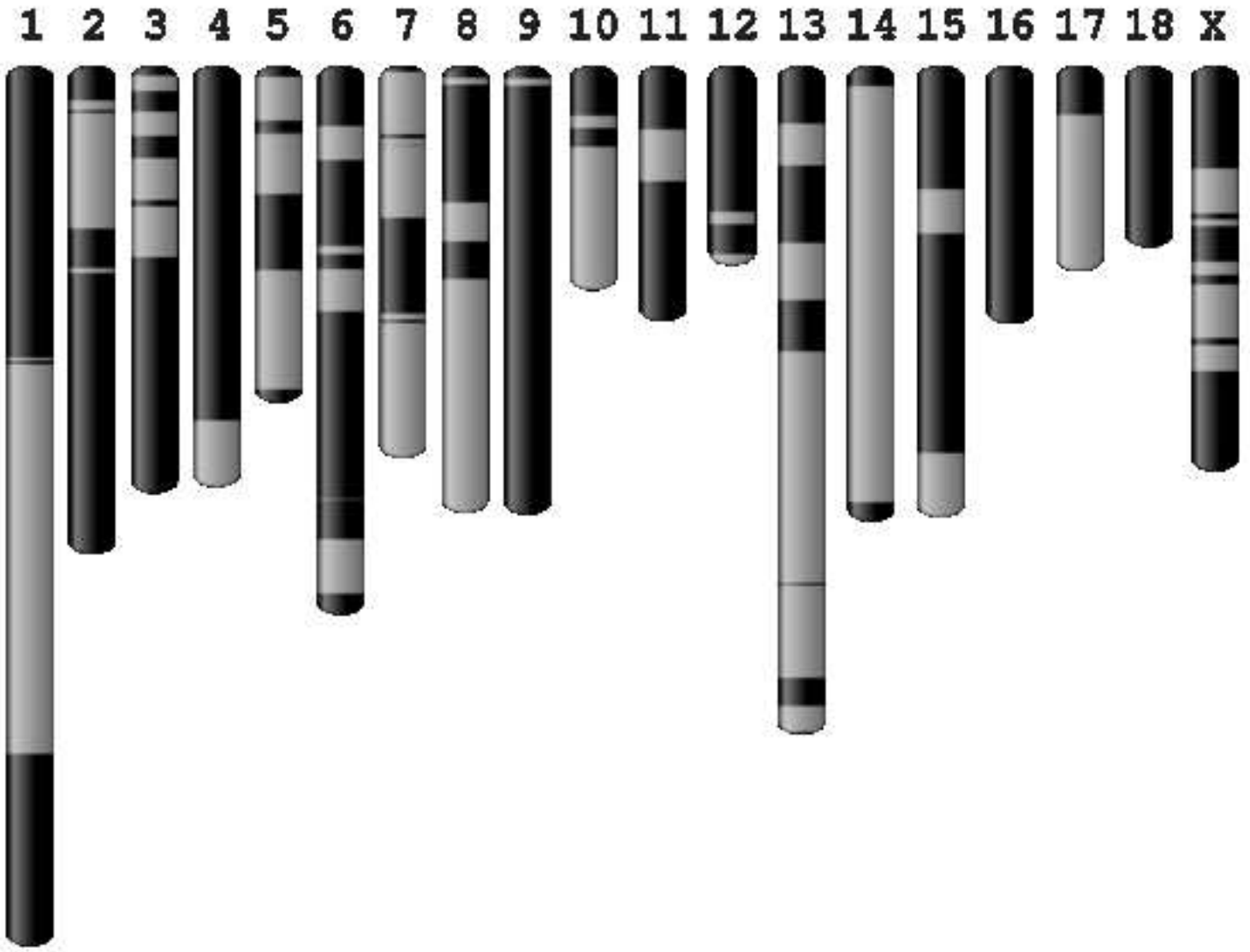

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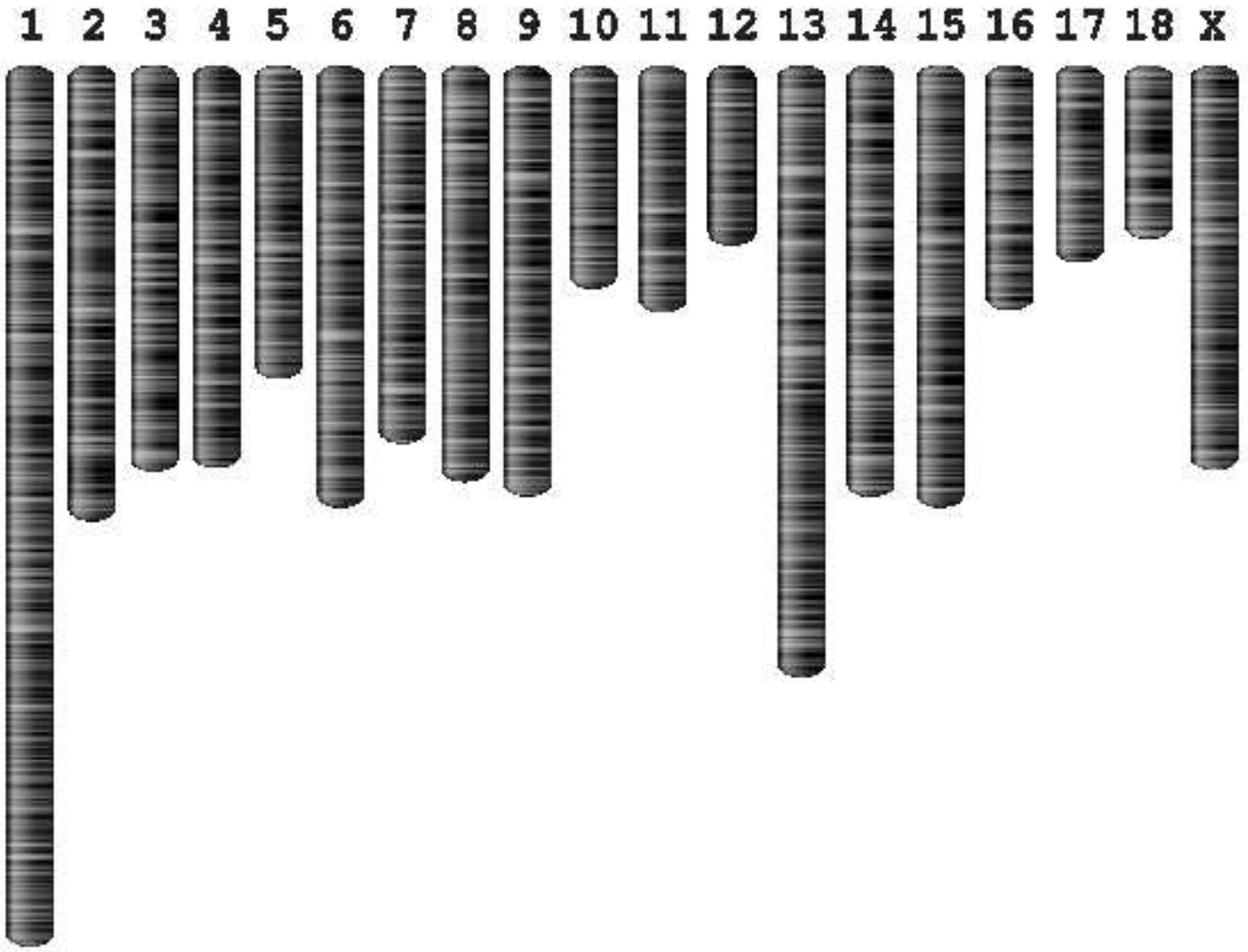

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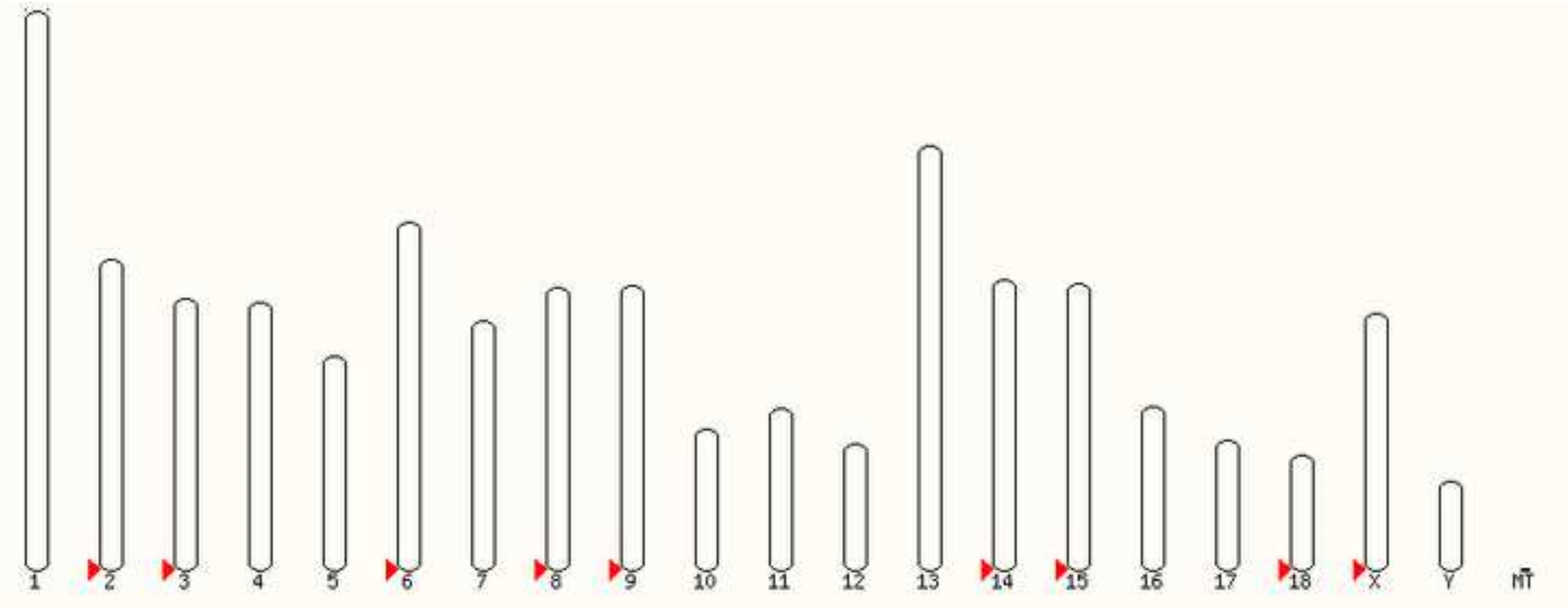

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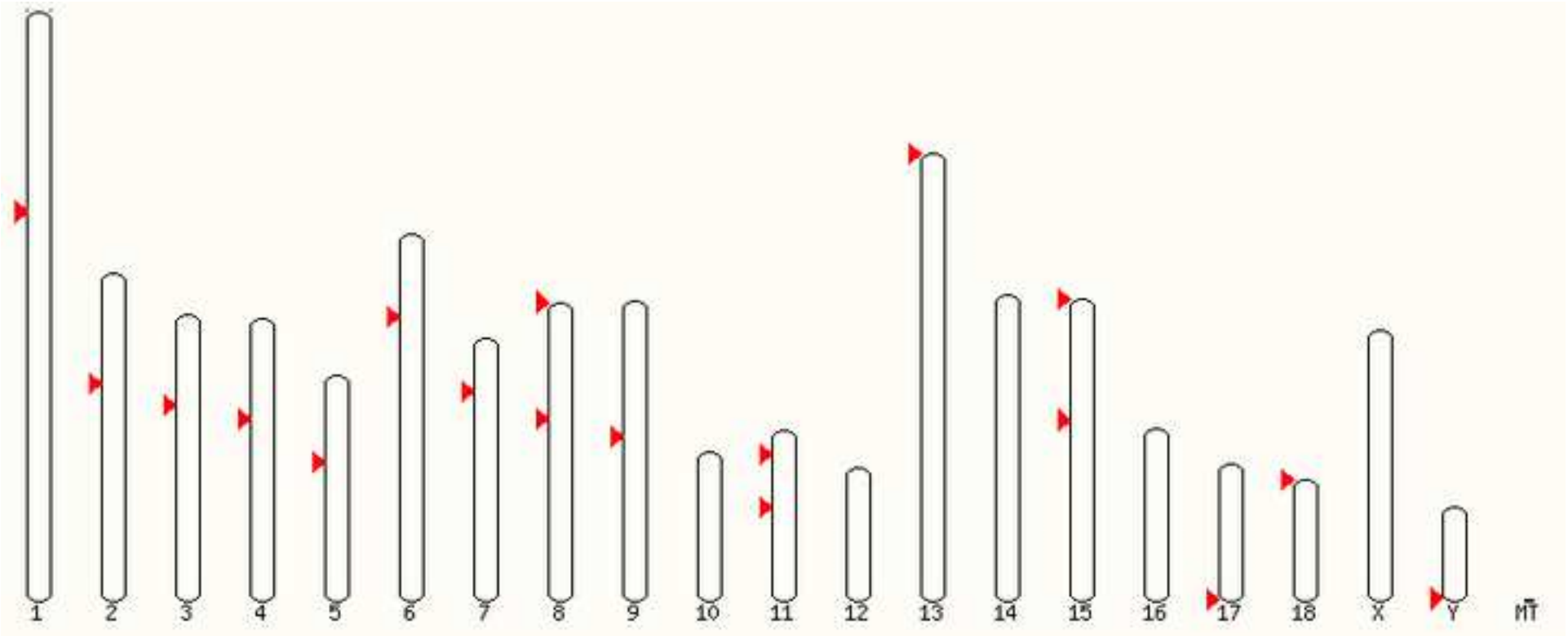

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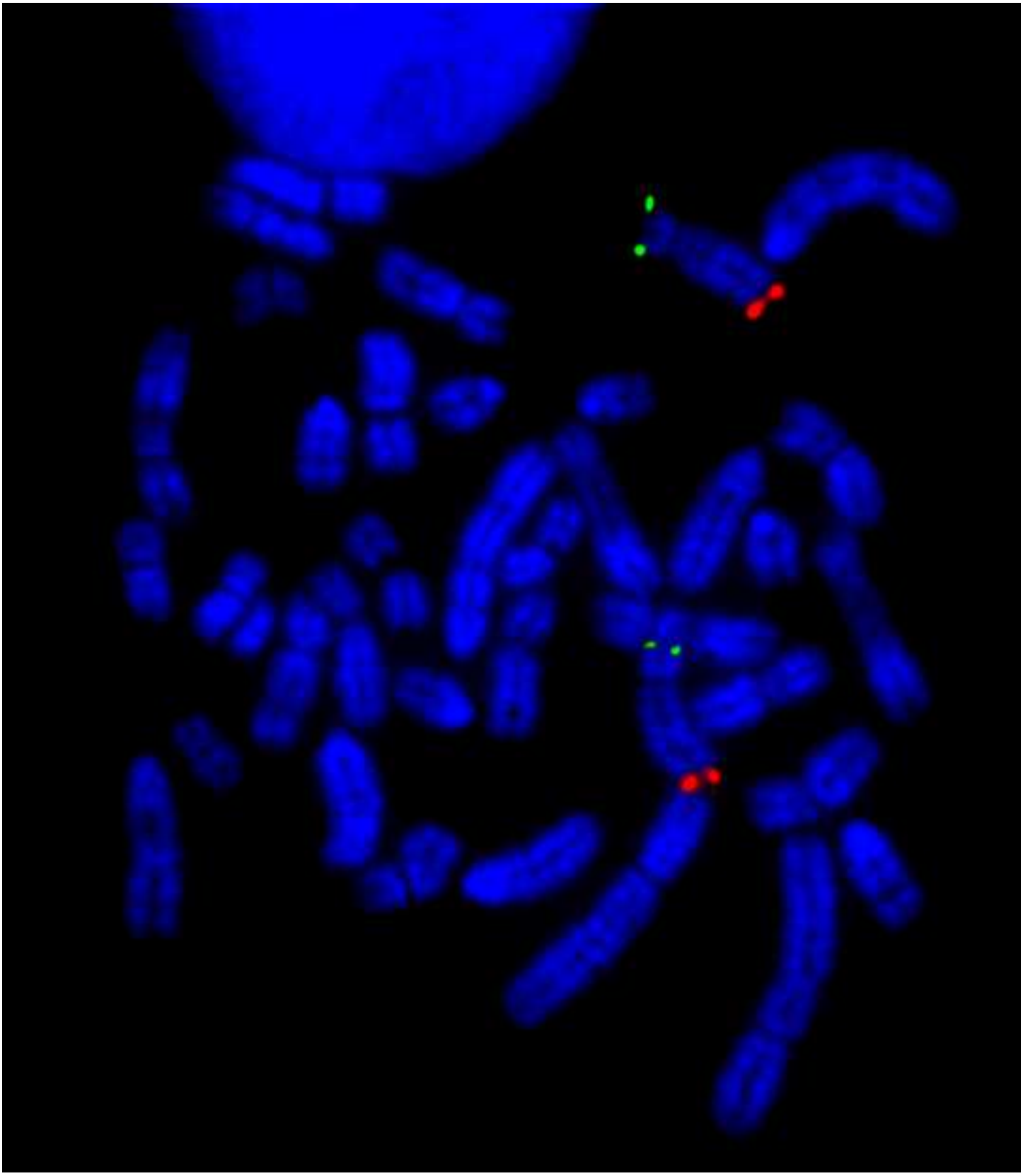

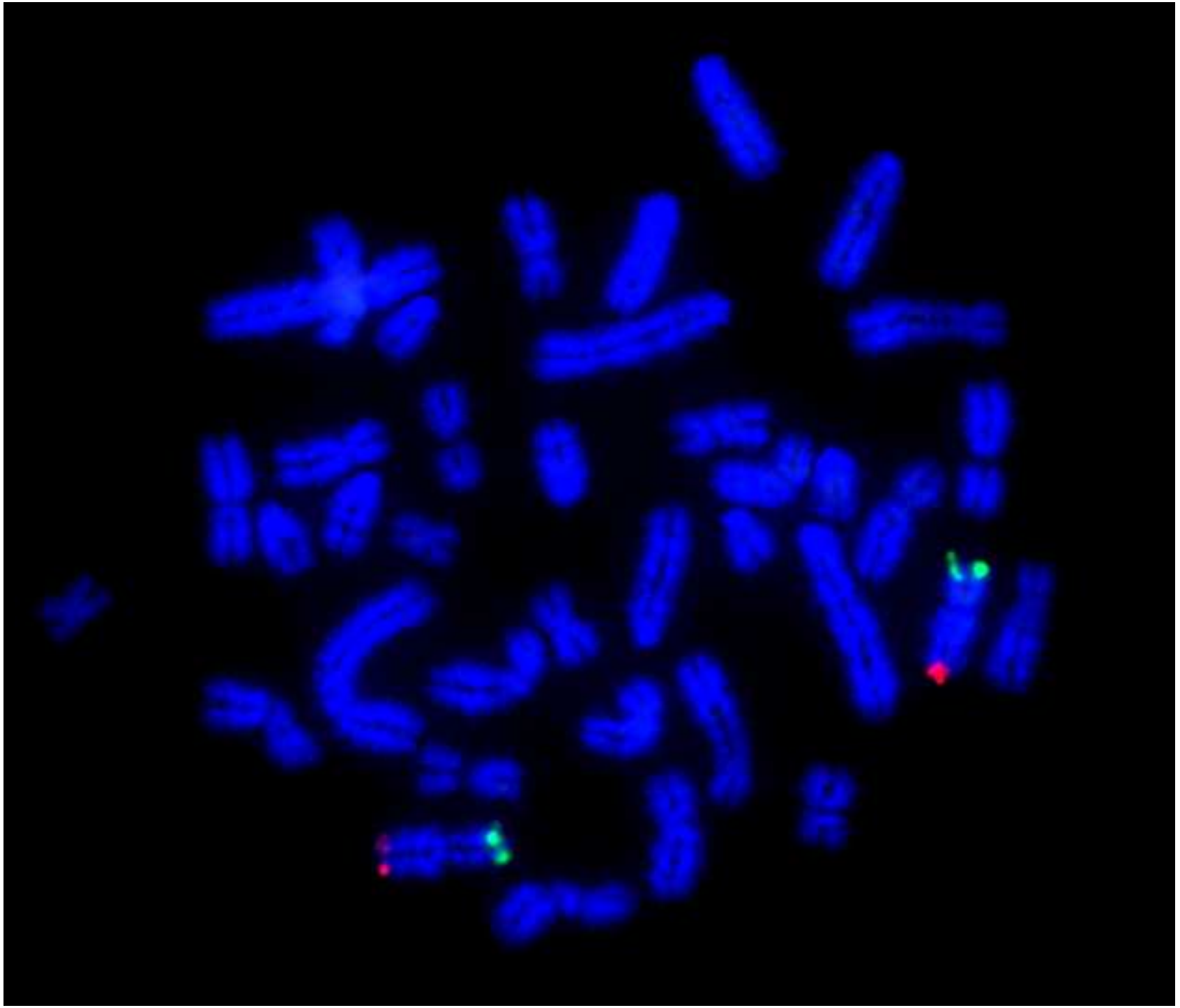

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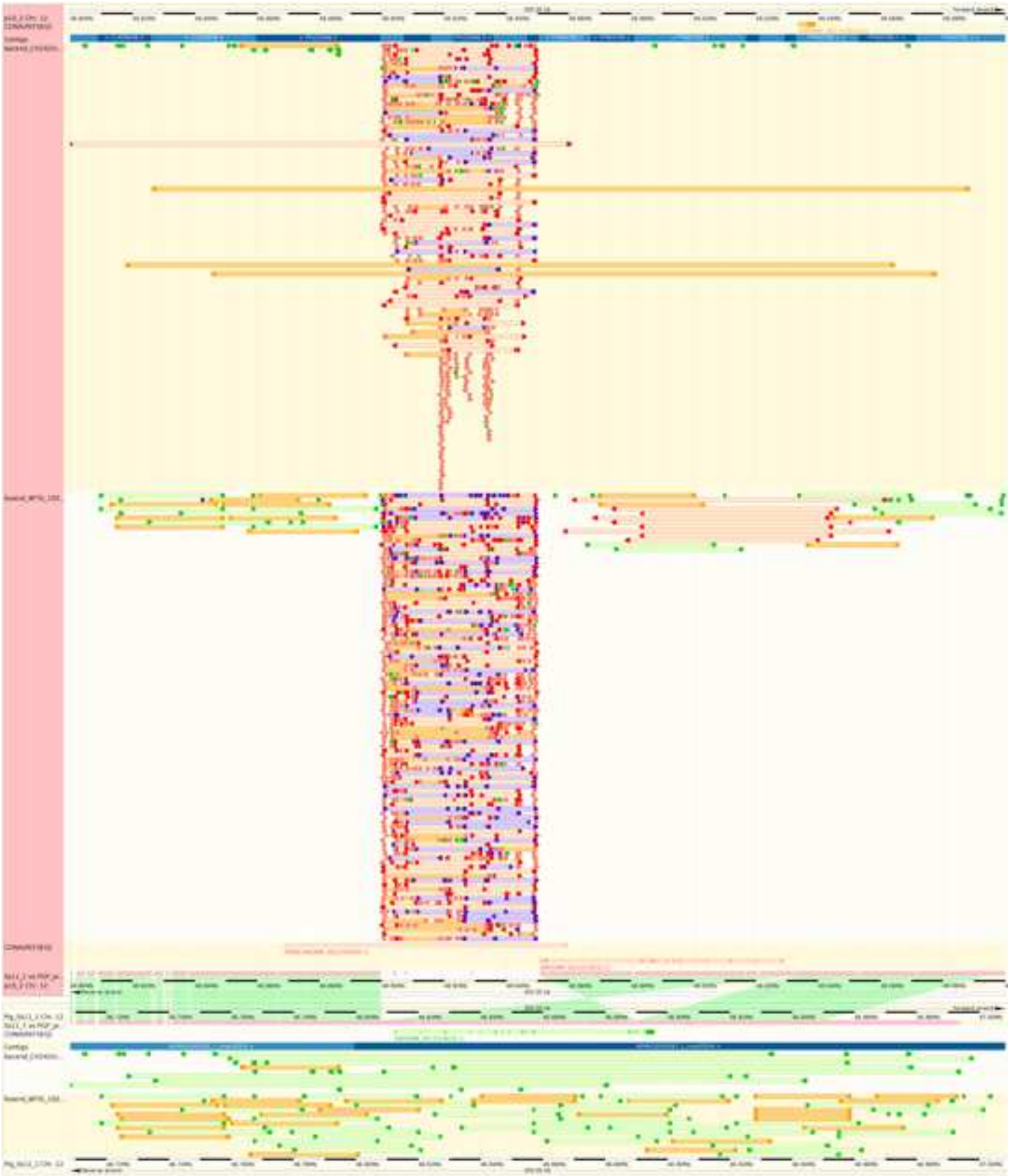

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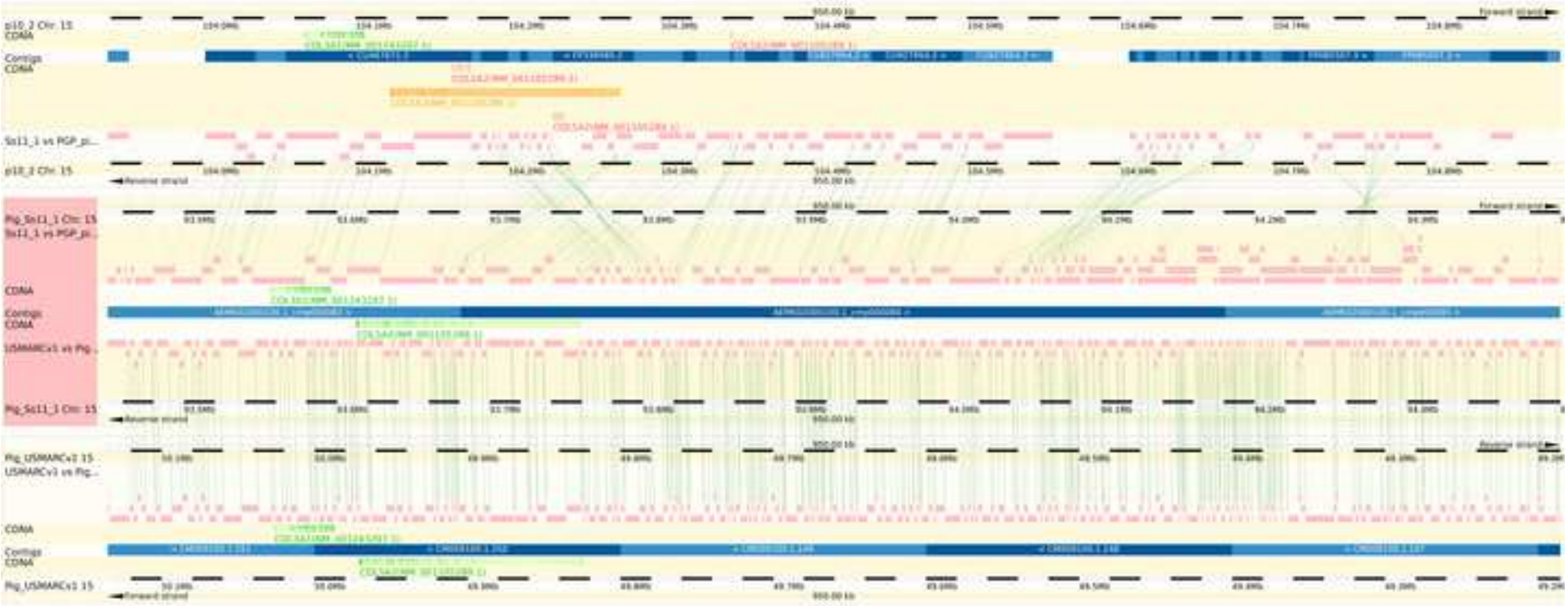

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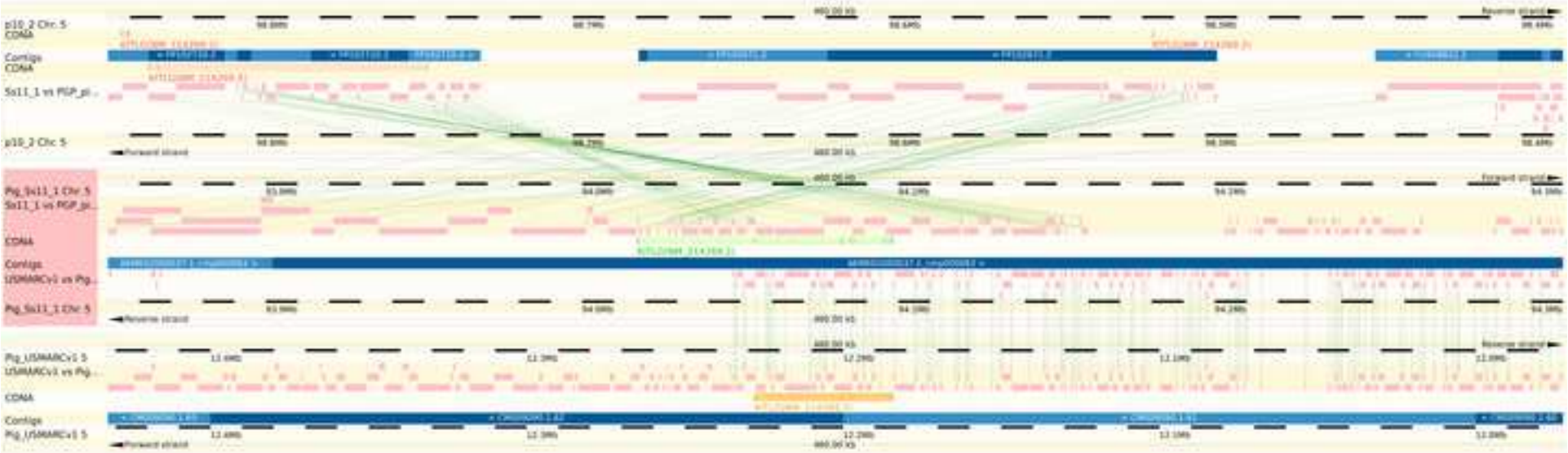

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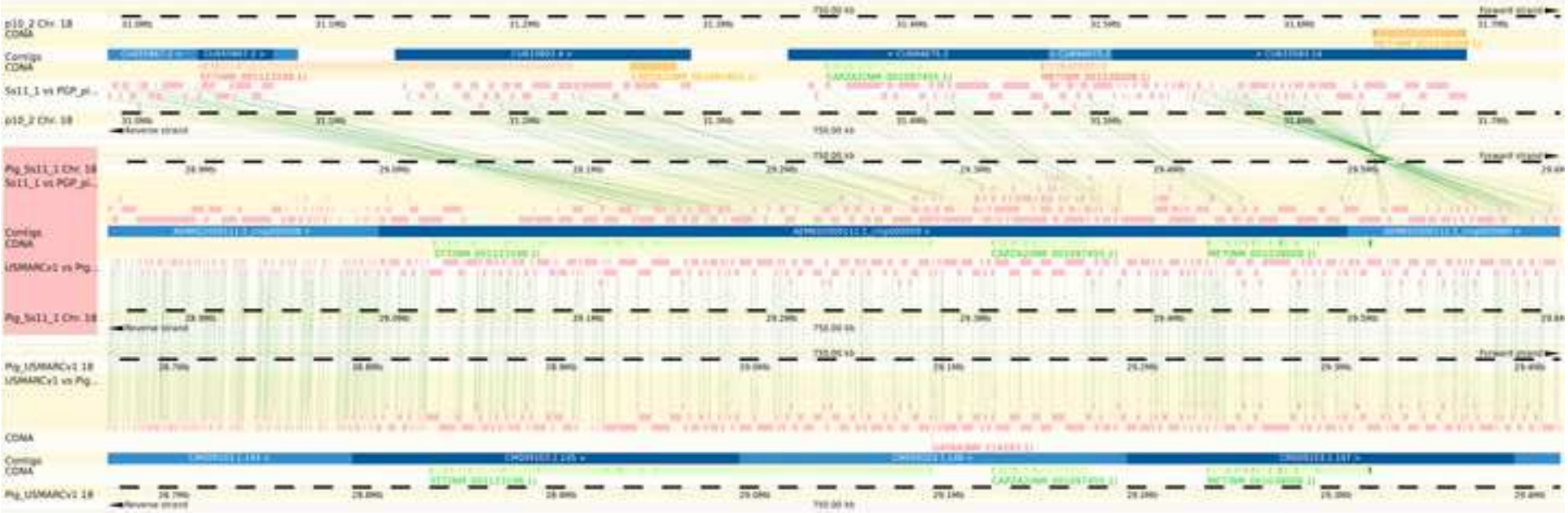

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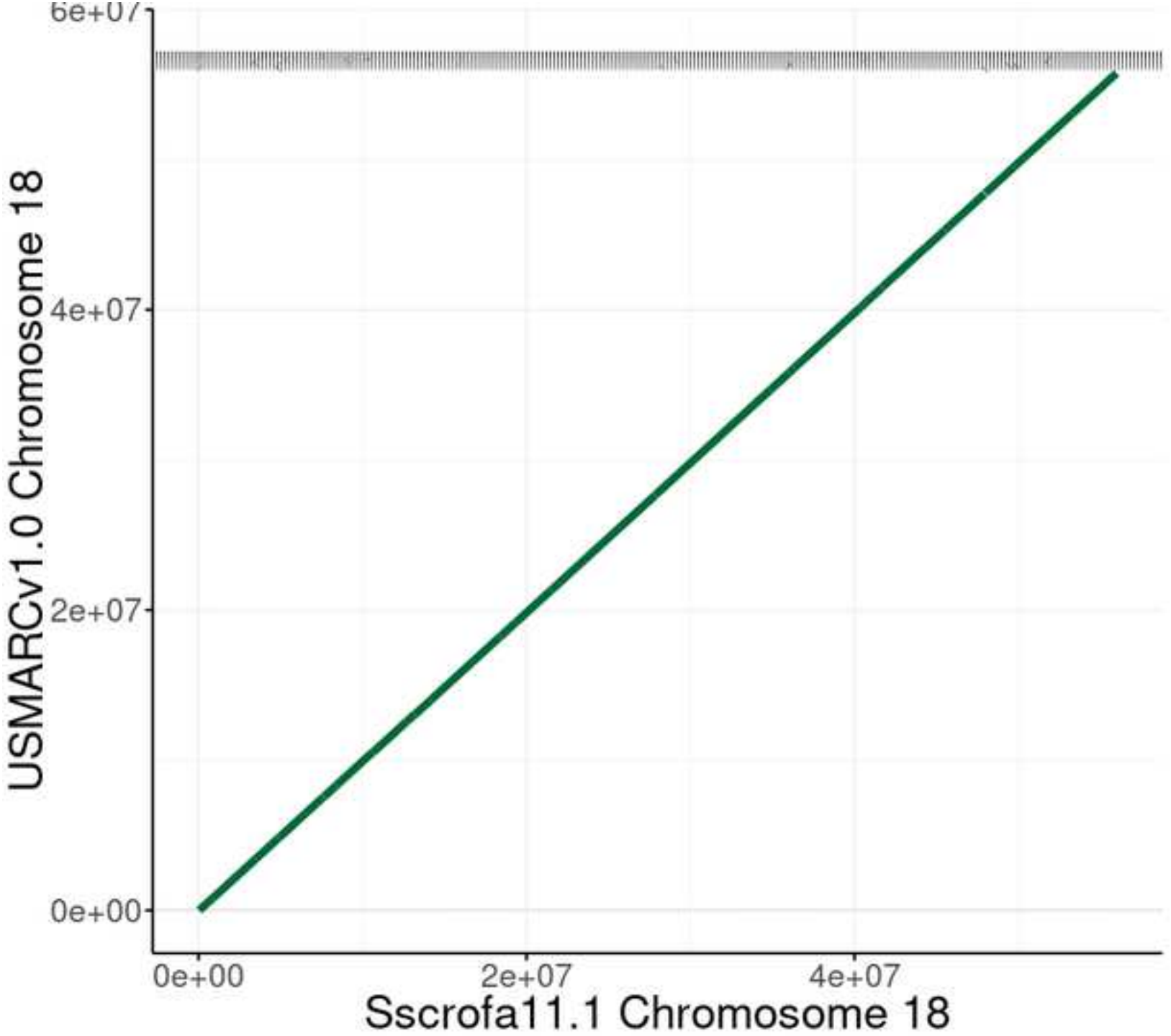

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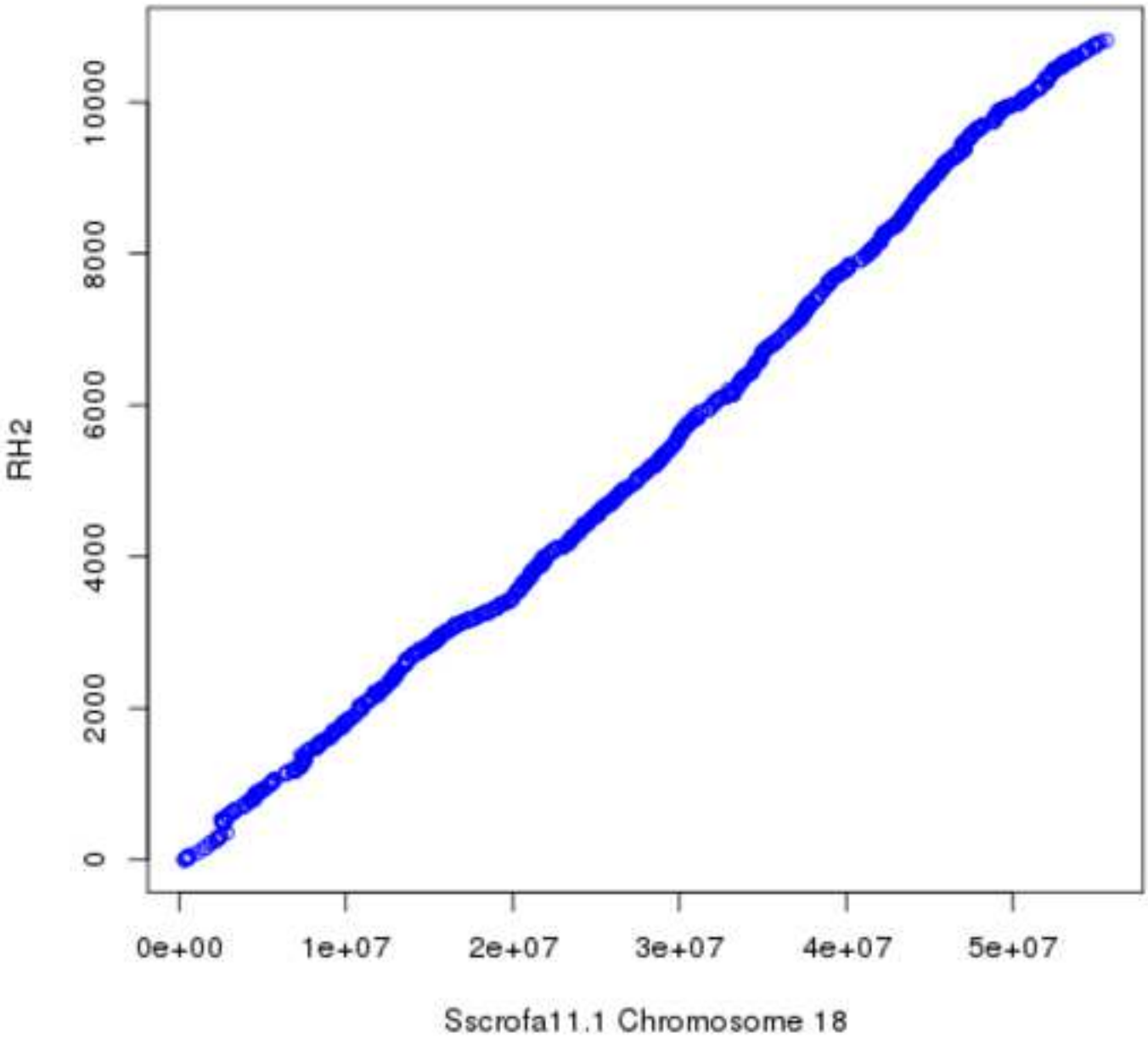

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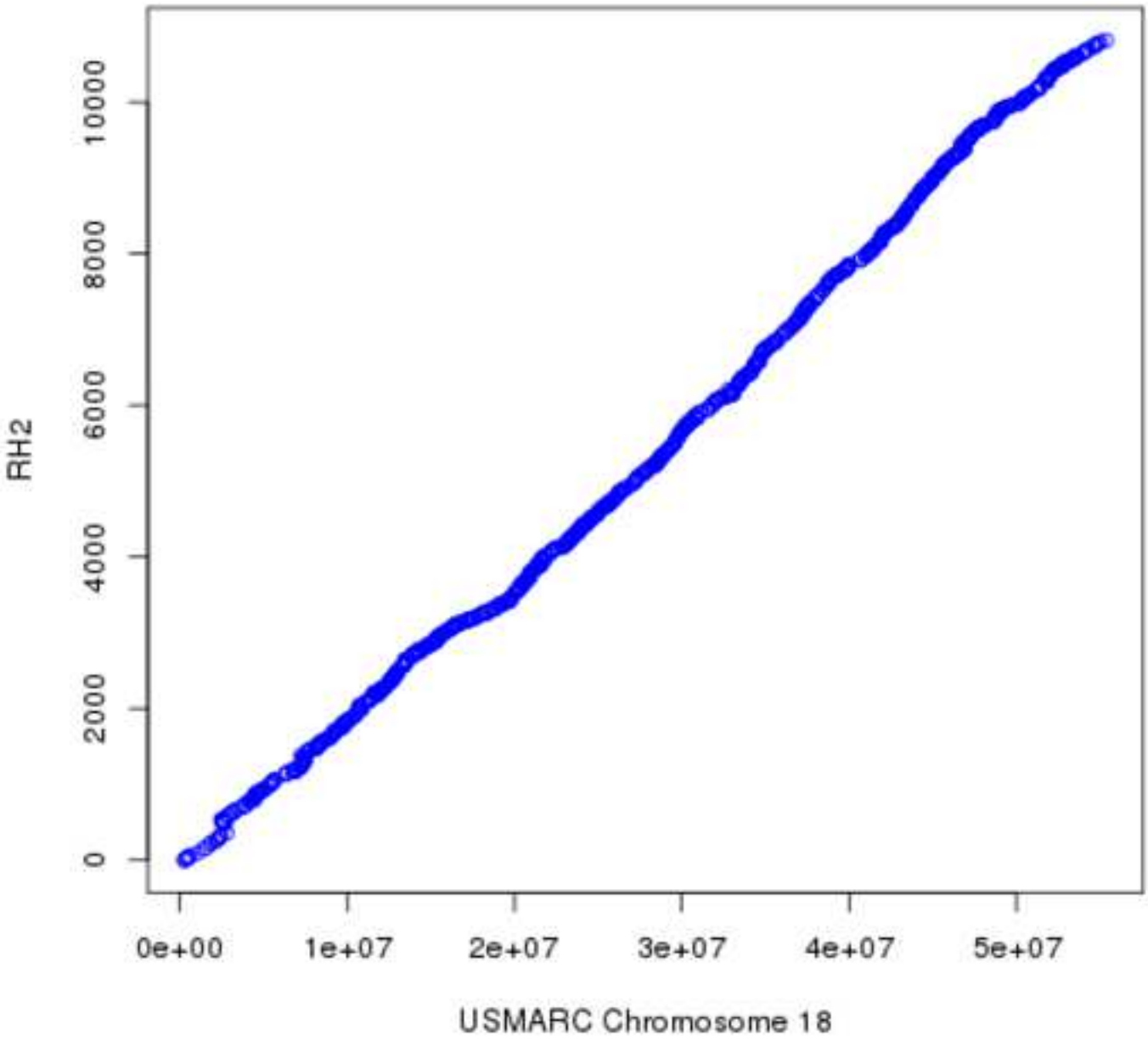

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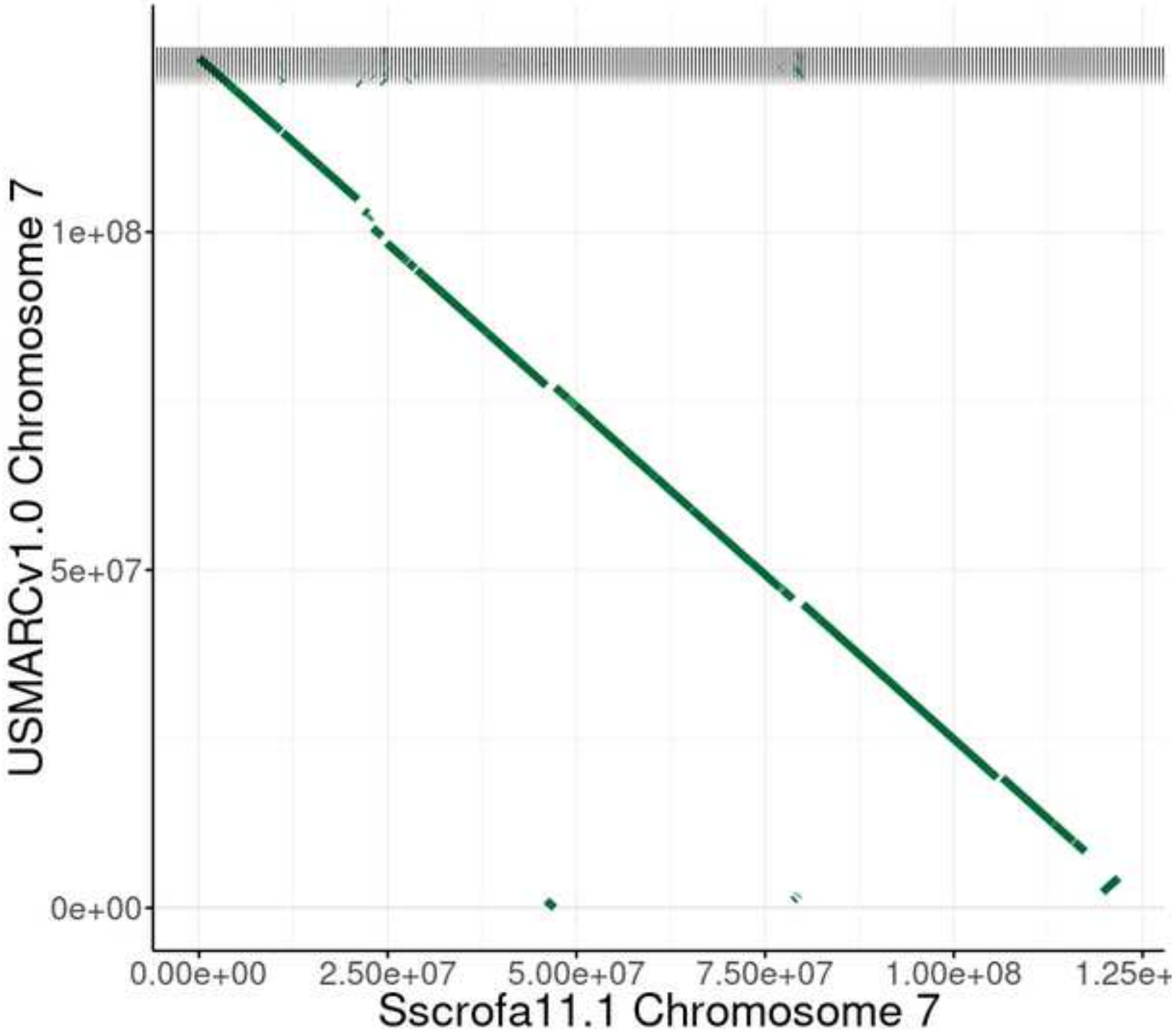

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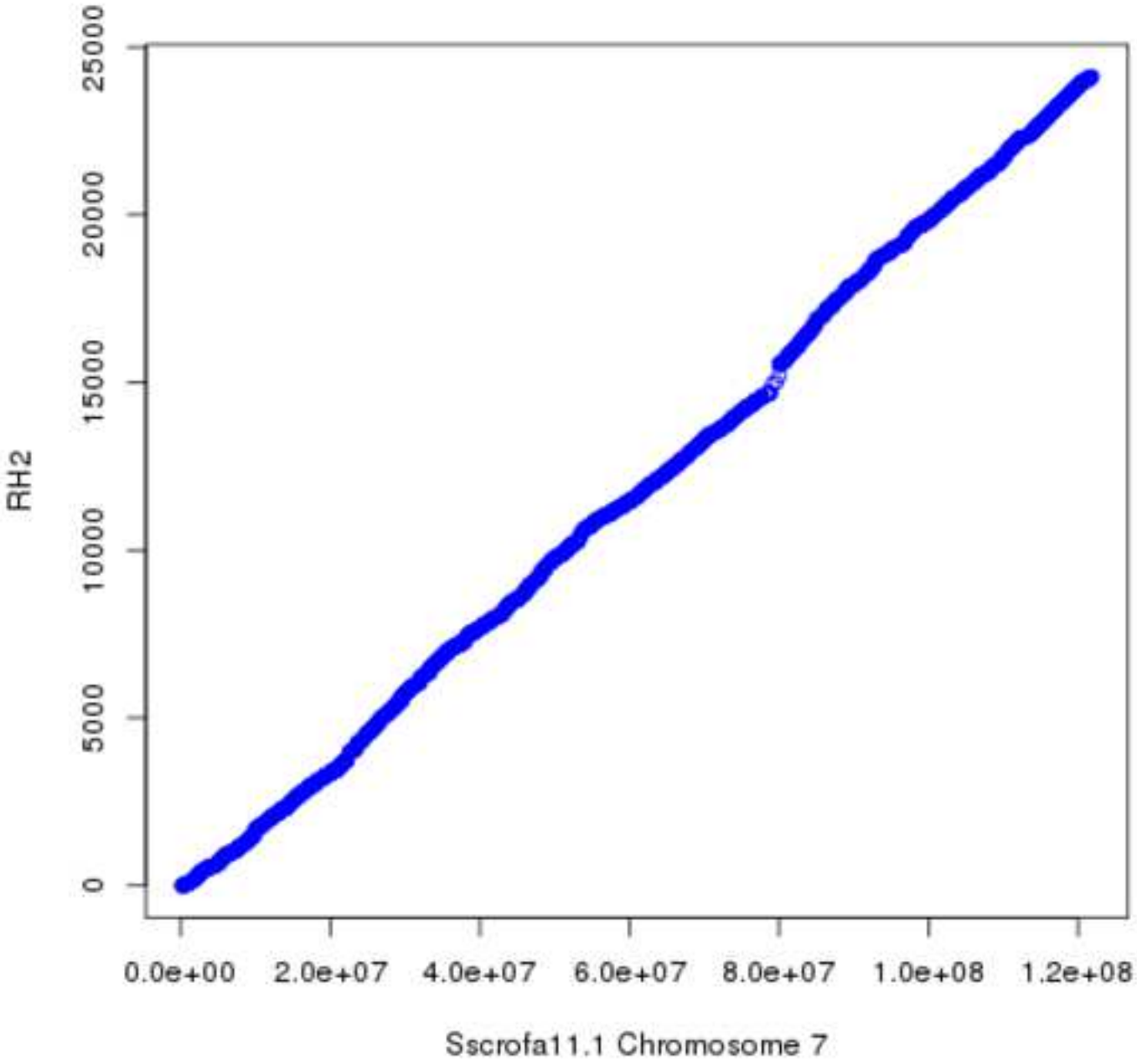

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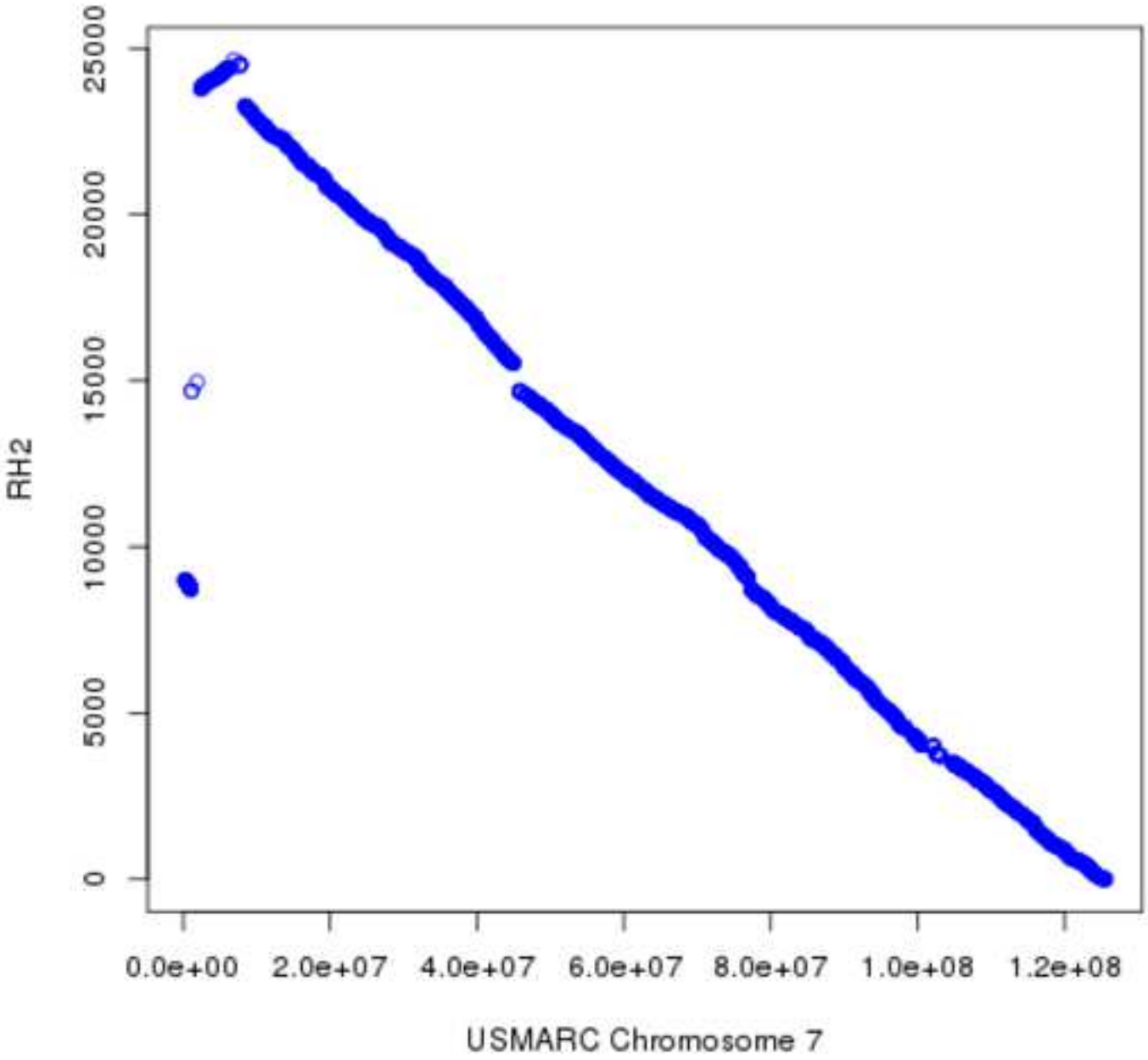

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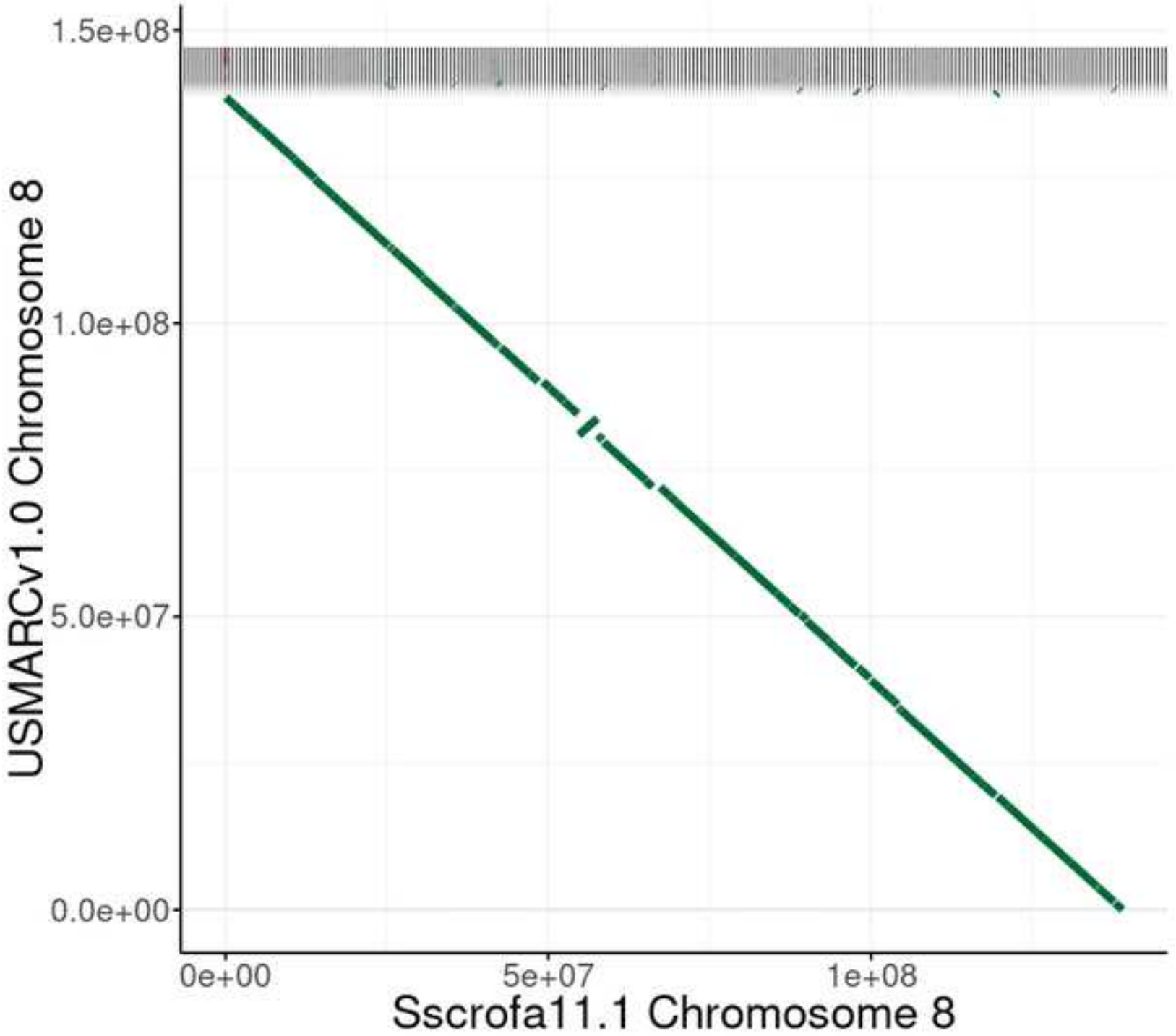

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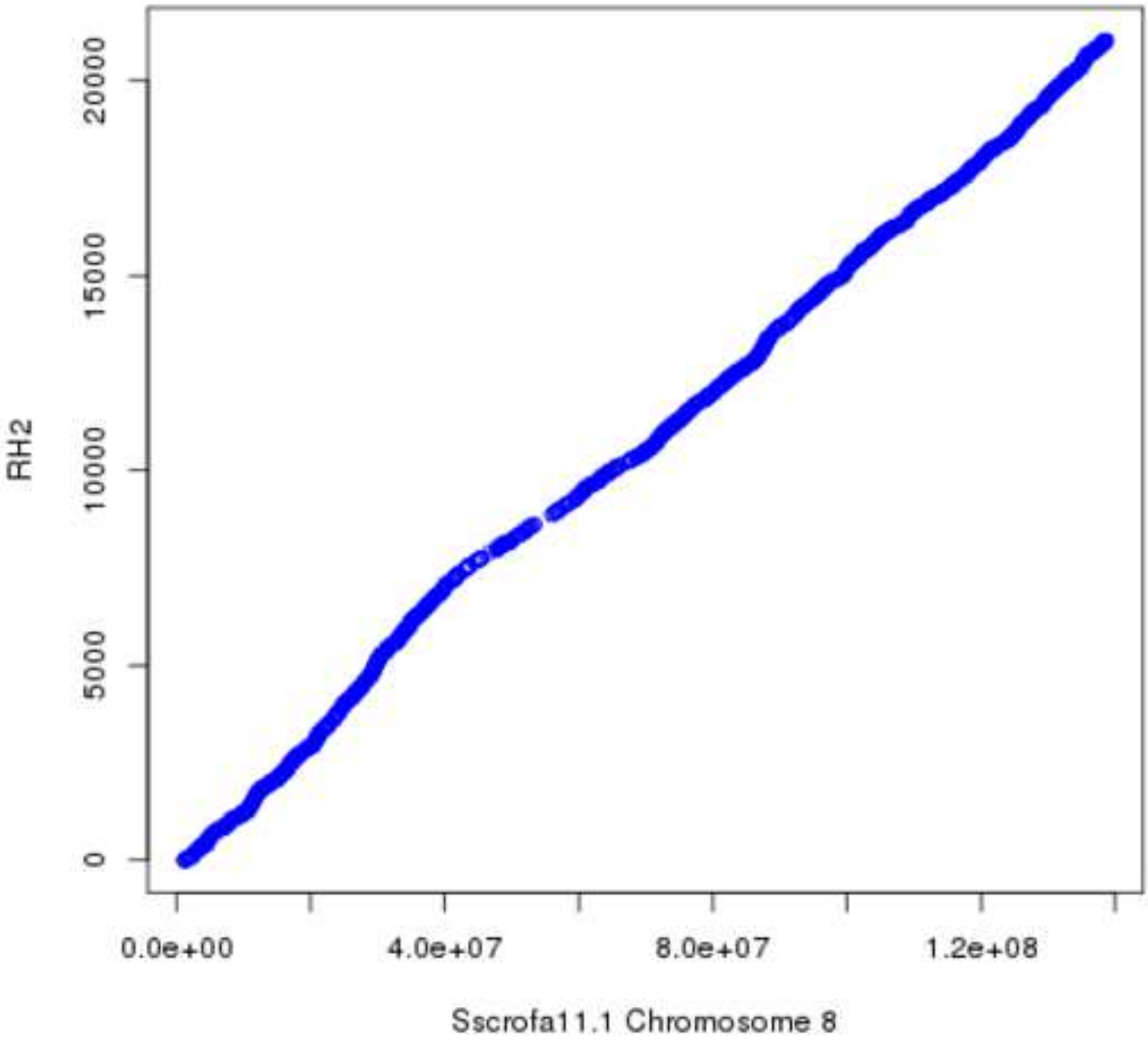

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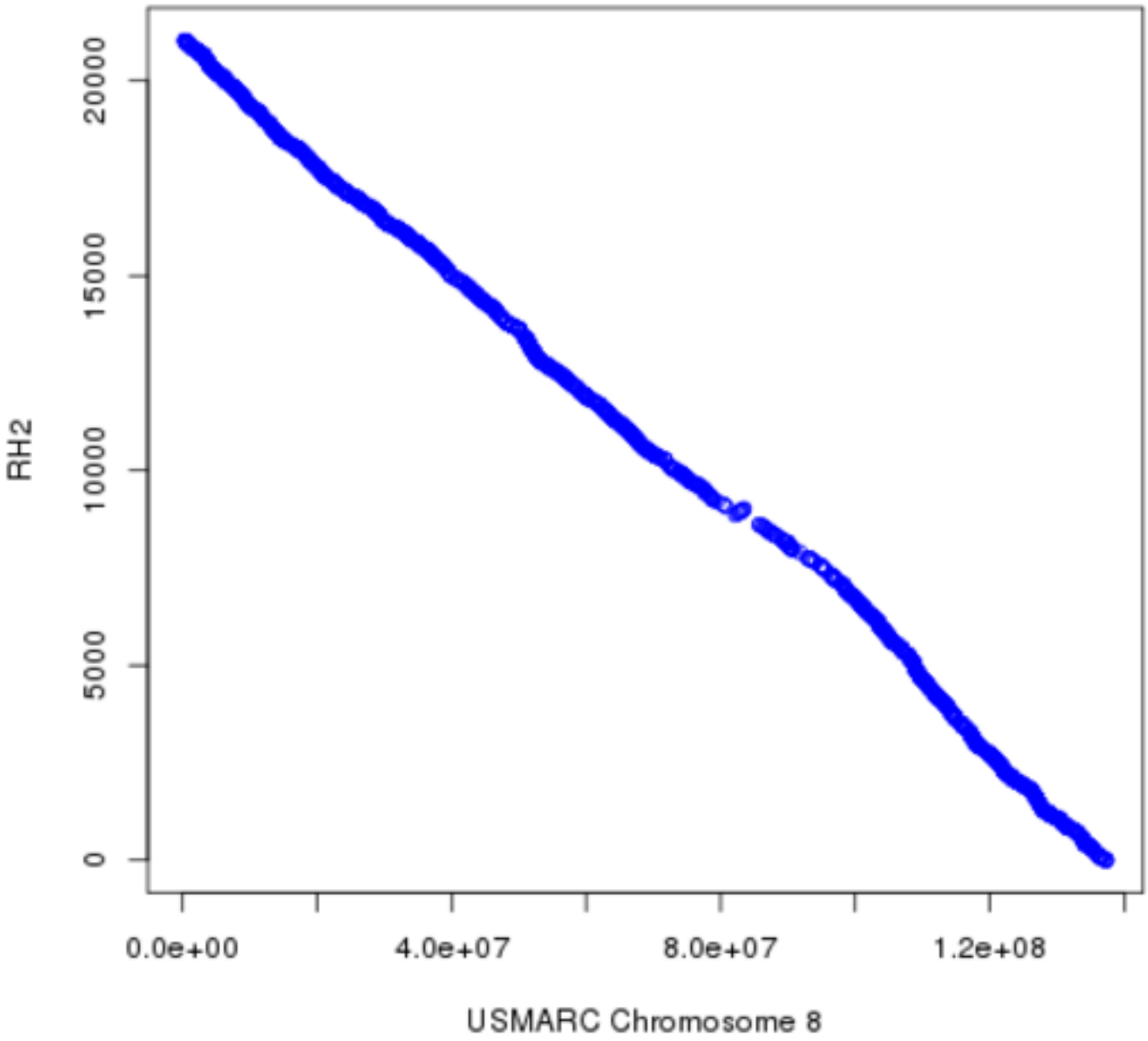

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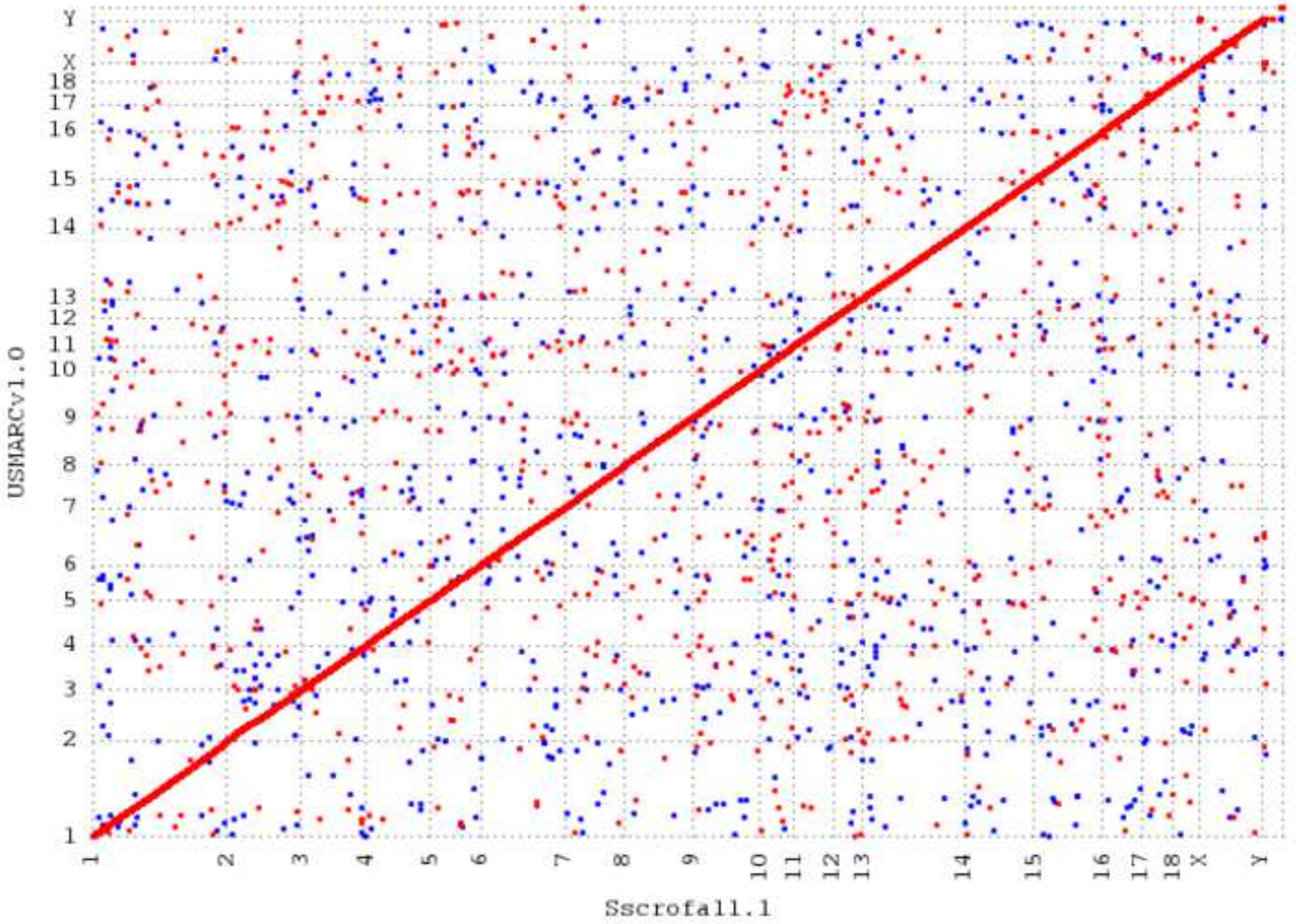

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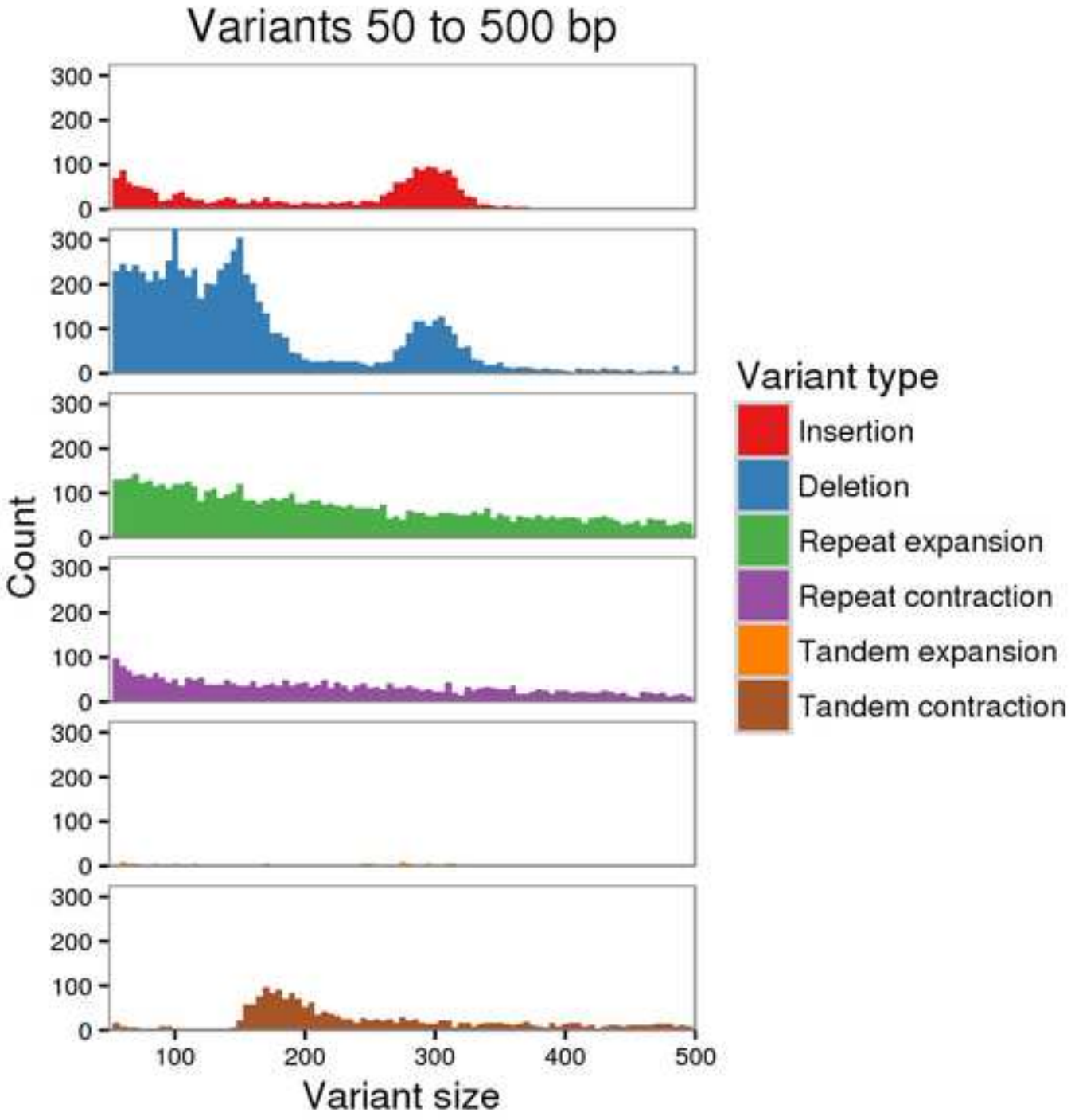

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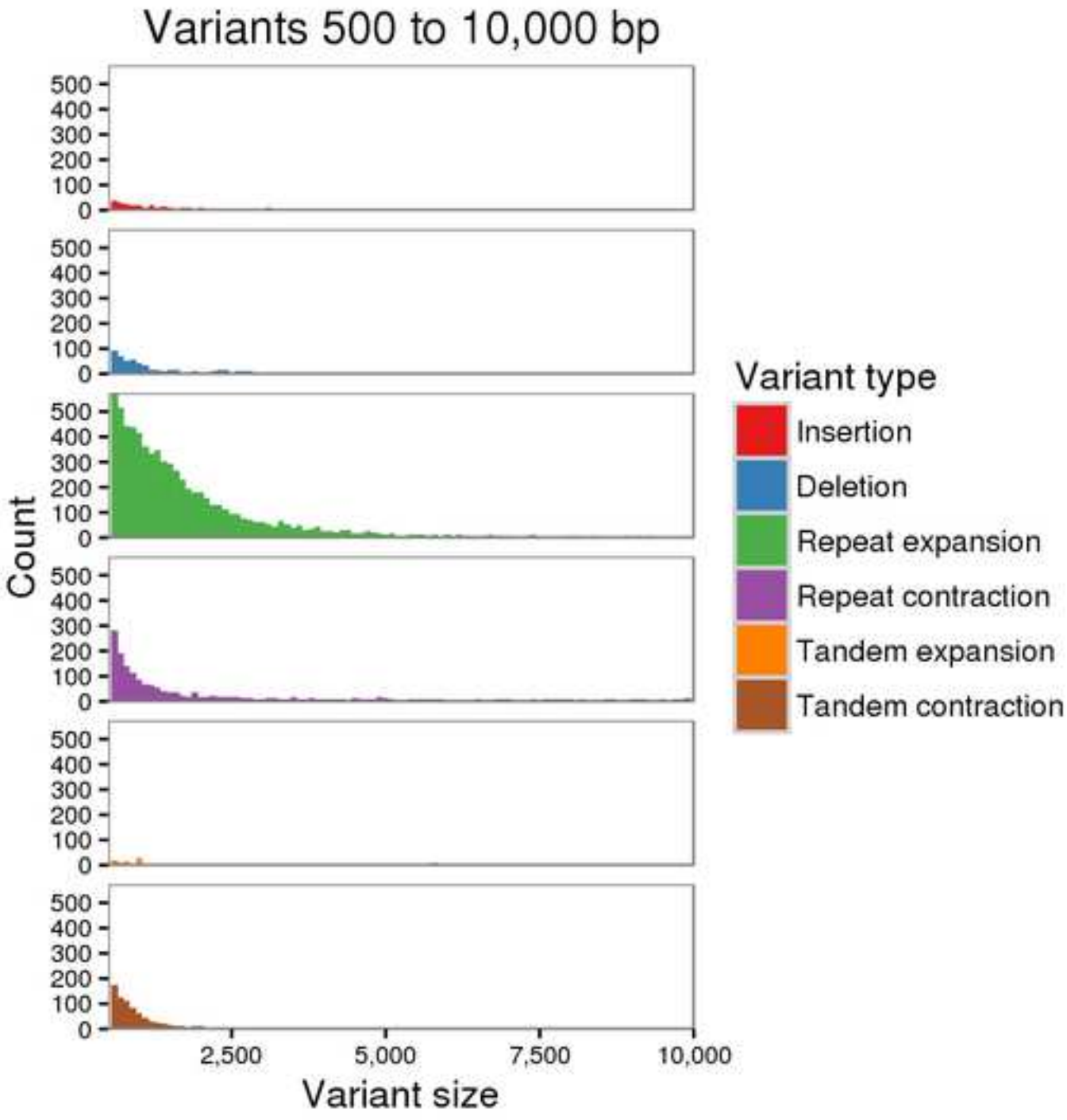

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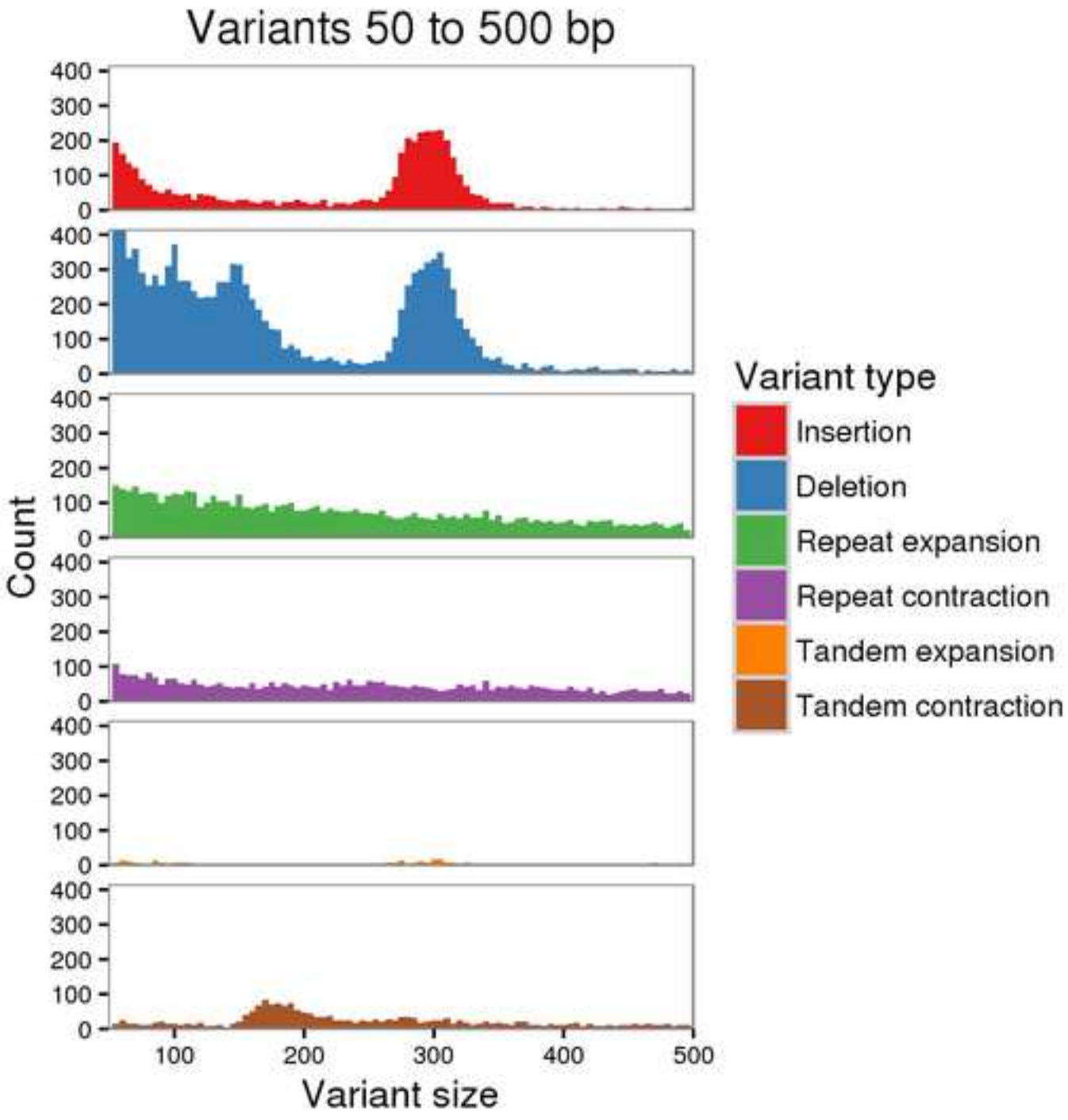

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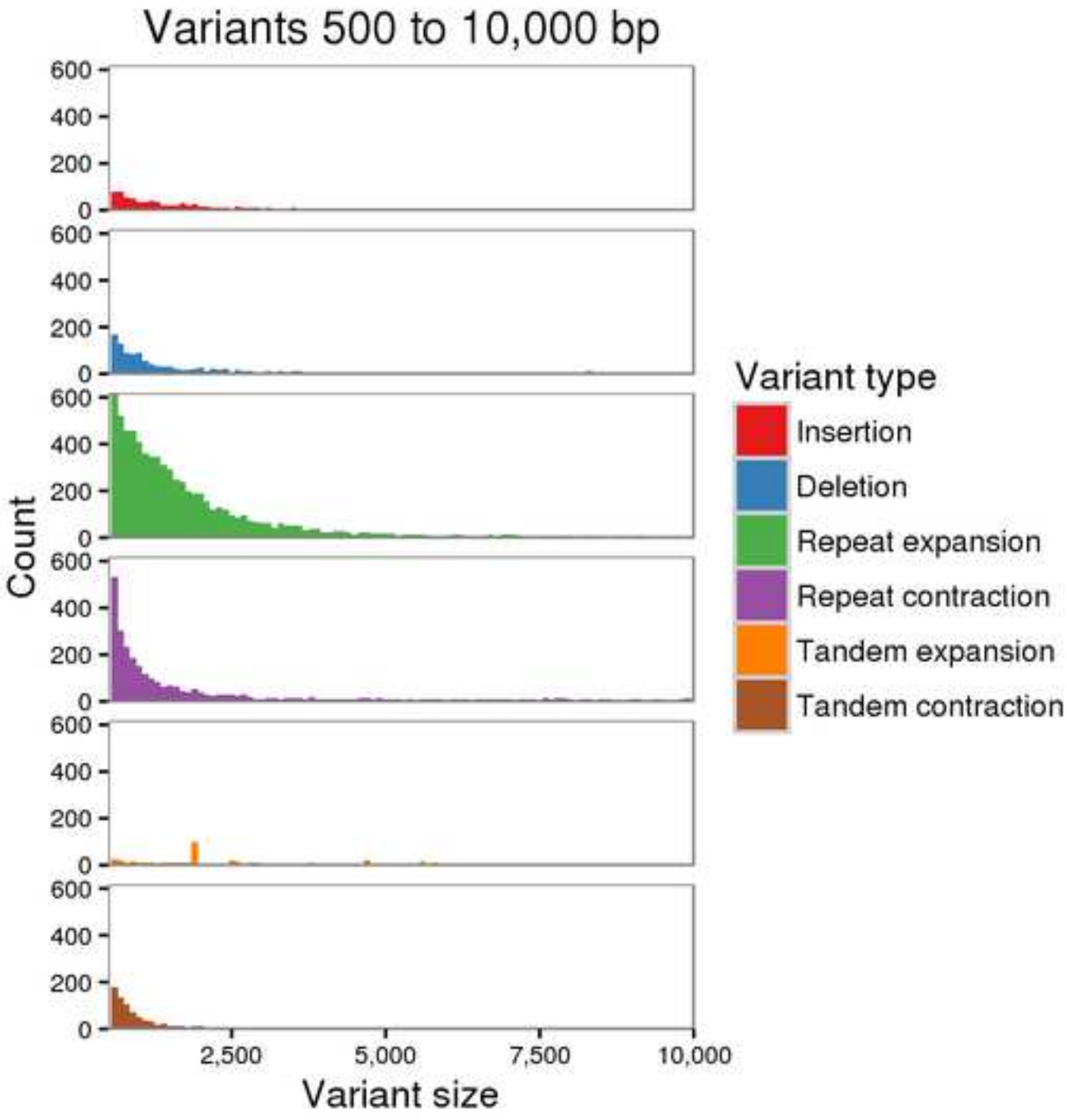

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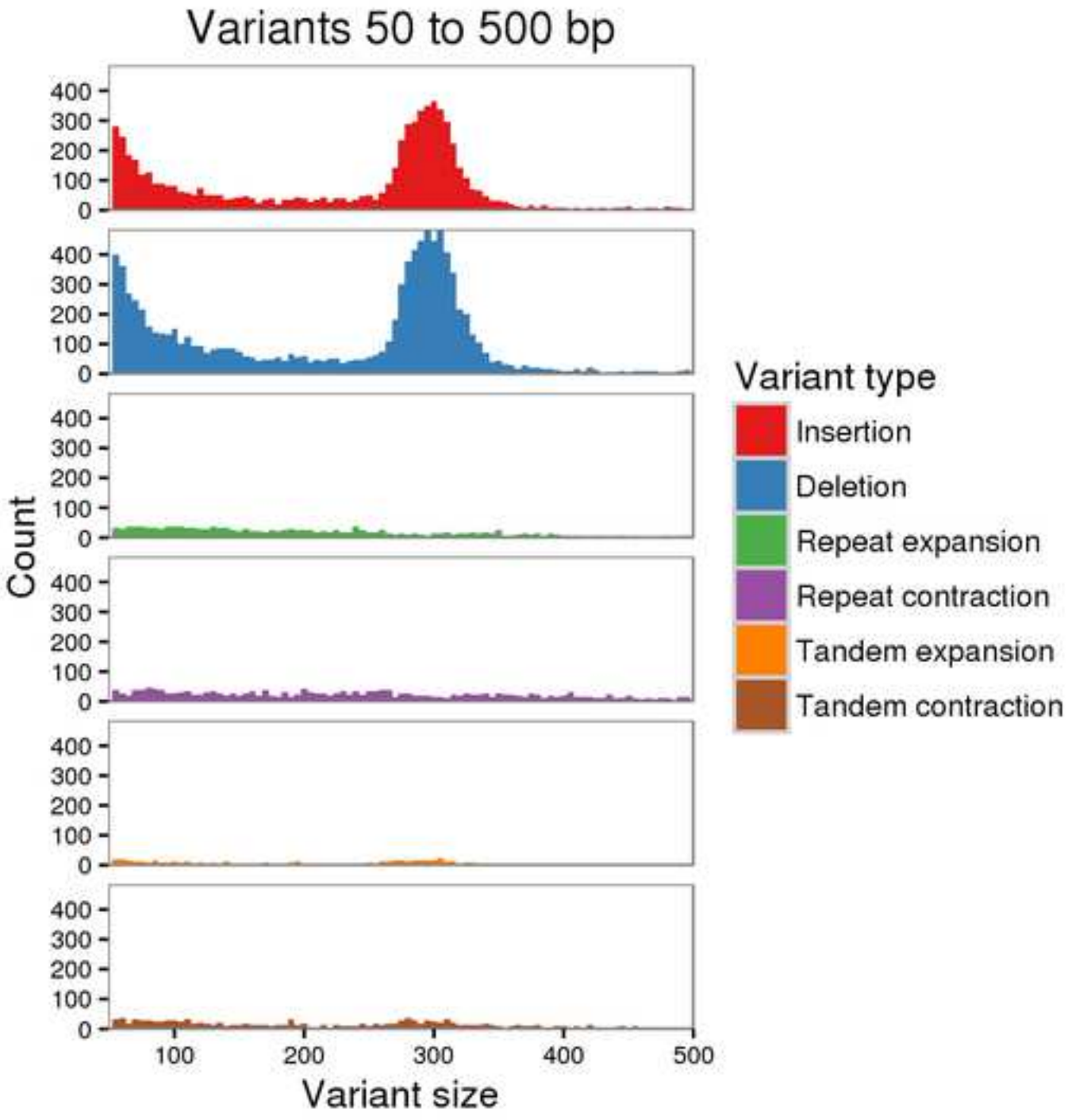

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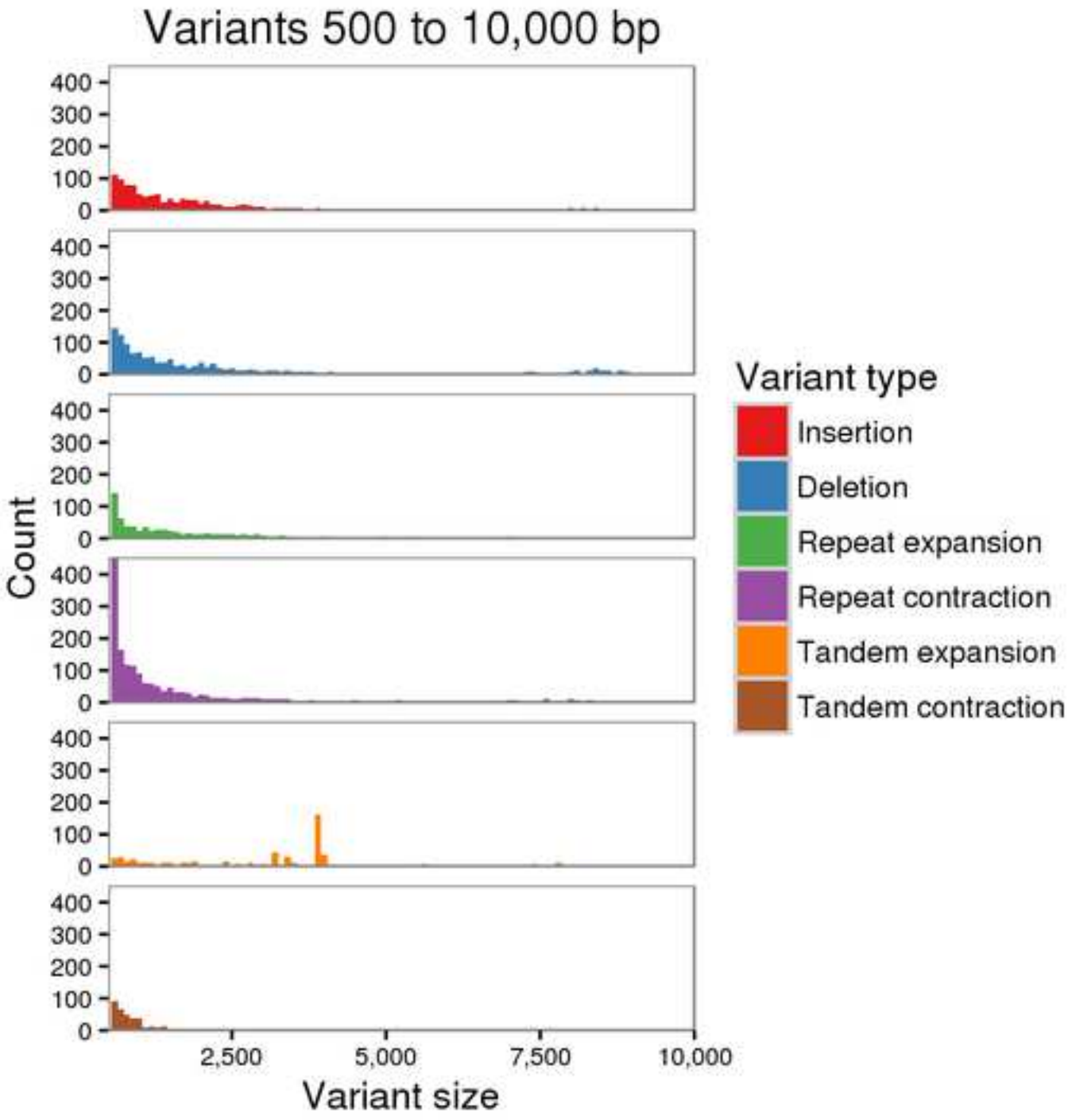

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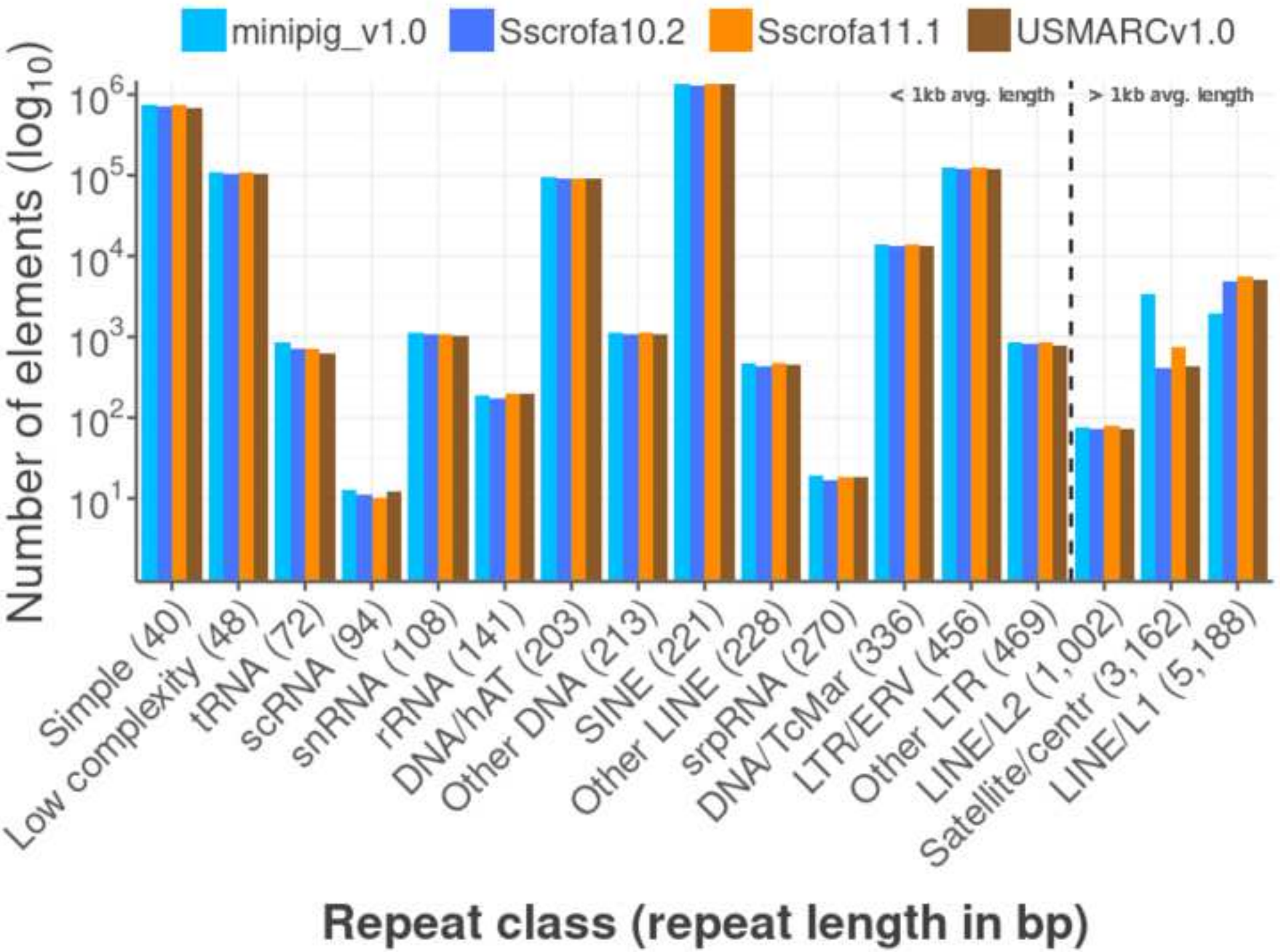

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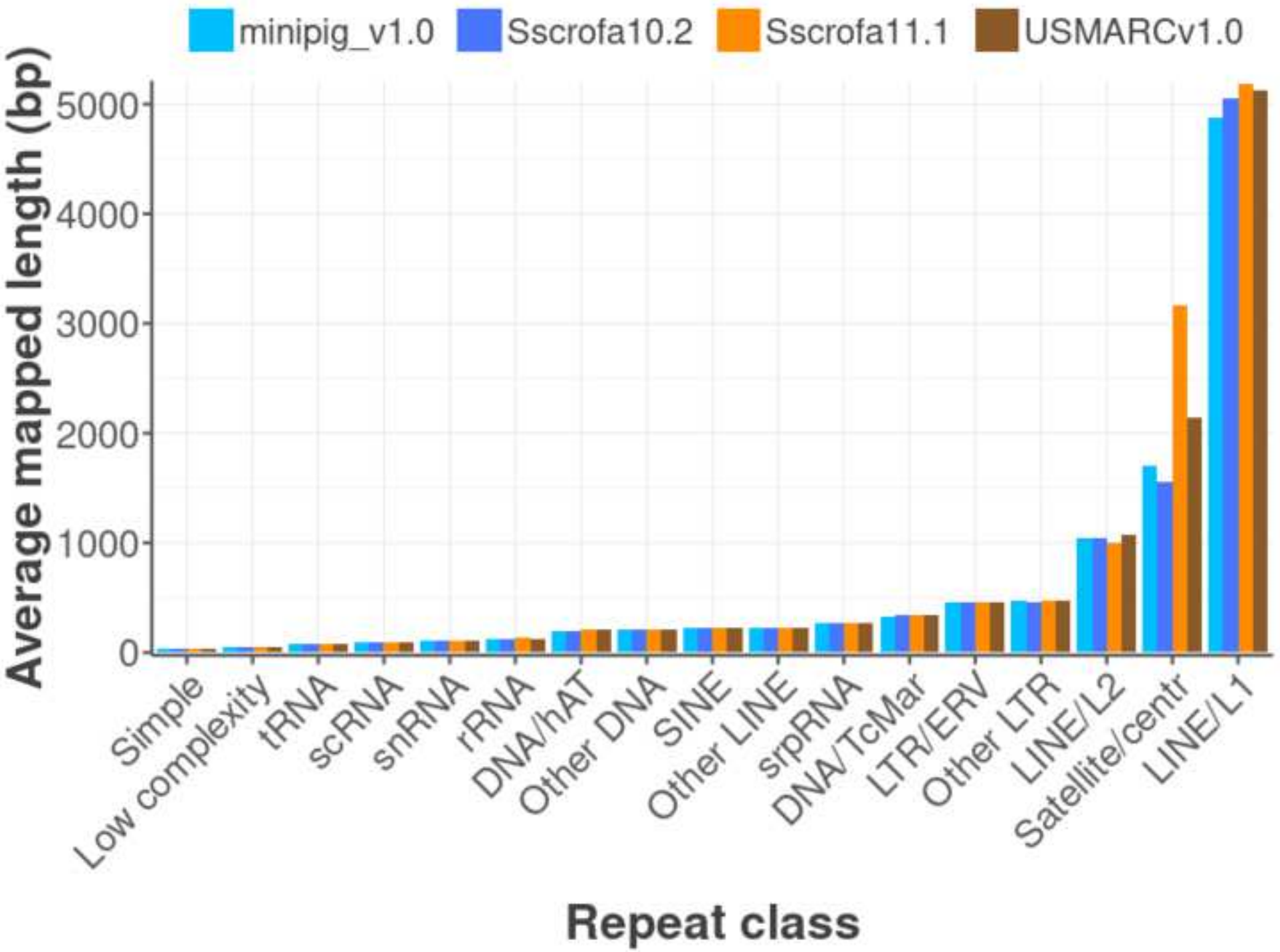

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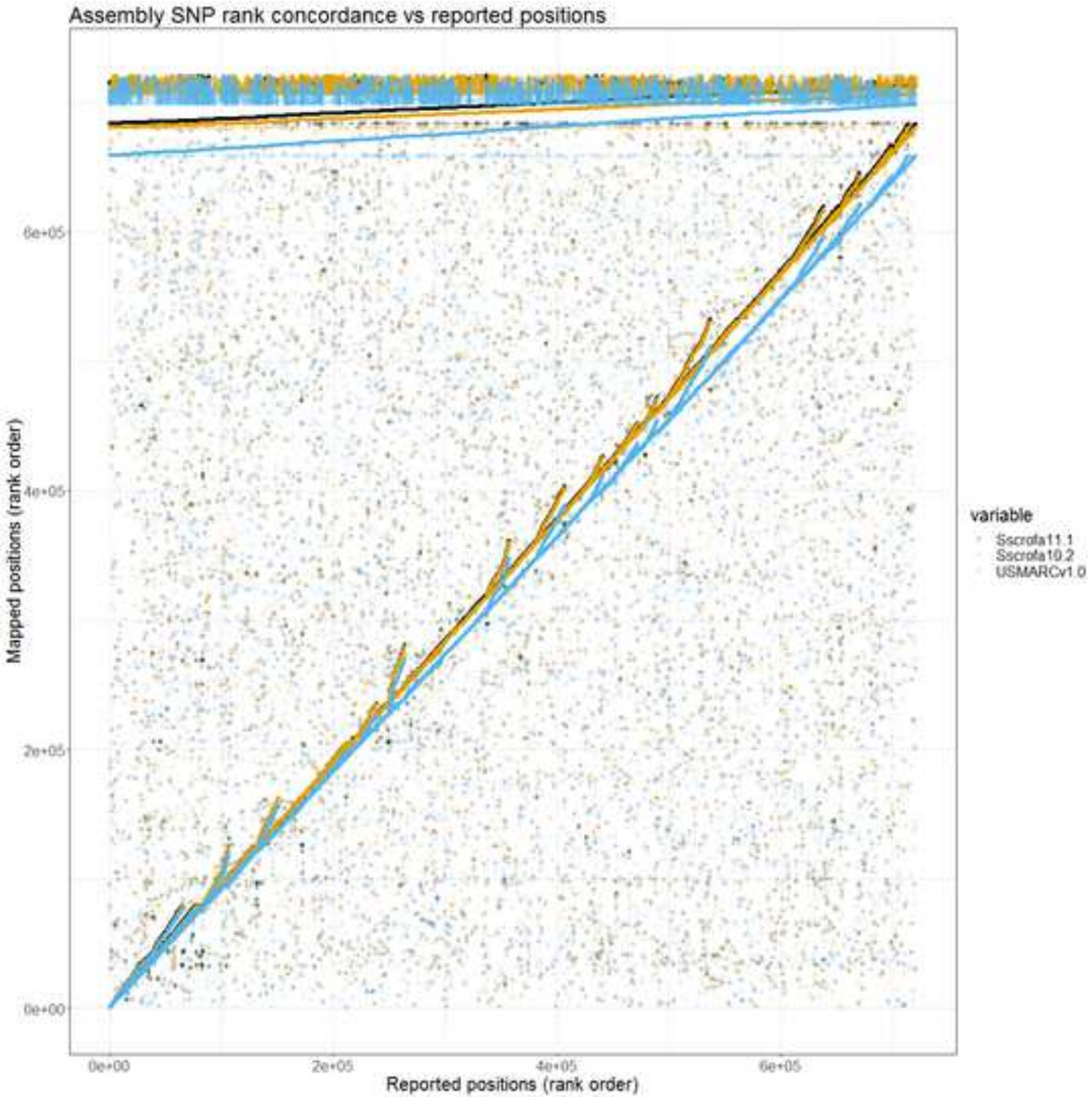

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Dear Editors

I am pleased to submit a final revised version of the manuscript entitled “An improved pig reference genome sequence to enable pig genetics and genomics research”.

The questions and issues raised by the reviewers have been addressed as described previously.

The results from the Assemblytics comparisons of the 13 pig genome assemblies available in GigaDB (http://dx.doi.org/10.5524/100732).

The figures are integrated into the manuscript and supplementary materials. I have also uploaded copies of each figure image. These images are also available in the associated GigaDB dataset (http://dx.doi.org/10.5524/100732).

Yours sincerely

Alan L. Archibald