RESEARCH ARTICLE

HUMAN GENOMICS

The complete sequence of a human genome

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Since its initial release in 2000, the human reference genome has covered only the euchromatic fraction of the genome, leaving important heterochromatic regions unfinished. Addressing the remaining 8% of the genome, the Telomere-to-Telomere (T2T) Consortium presents a complete 3.055 billion–base pair sequence of a human genome, T2T-CHM13, that includes gapless assemblies for all chromosomes except Y, corrects errors in the prior references, and introduces nearly 200 million base pairs of sequence containing 1956 gene predictions, 99 of which are predicted to be protein coding. The completed regions include all centromeric satellite arrays, recent segmental duplications, and the short arms of all five acrocentric chromosomes, unlocking these complex regions of the genome to variational and functional studies.

The current human reference genome was released by the Genome Reference Consortium (GRC) in 2013 and most recently patched in 2019 (GRCh38.p13) (*1*). This reference traces its origin to the publicly funded Human Genome Project (*2*) and has been continually improved over the past two decades. Unlike the competing Celera effort (*3*) and most modern sequencing projects based on "shotgun" sequence assembly (*4*), the GRC assembly was constructed from sequenced bacterial artificial chromosomes (BACs) that were ordered and oriented along the human genome by means of radiation hybrid, genetic linkage, and fingerprint maps. However, limitations of BAC cloning led to an underrepresentation of repetitive sequences, and the opportunistic assembly of BACs derived from multiple individuals resulted in a mosaic of haplotypes. As a result, several GRC assembly gaps are unsolvable because of incompatible structural polymorphisms on their flanks, and many other repetitive and polymorphic regions were left unfinished or incorrectly assembled (*5*).

The GRCh38 reference assembly contains 151 mega–base pairs (Mbp) of unknown sequence distributed throughout the genome, including pericentromeric and subtelomeric regions, recent segmental duplications, ampliconic gene arrays, and ribosomal DNA (rDNA) arrays, all of which are necessary for fundamental cellular processes (Fig. 1A). Some of the largest reference gaps include human satellite (HSat) repeat arrays and the short arms of all five acrocentric chromosomes, which are represented in GRCh38 as multimegabase stretches of unknown bases (Fig. 1, B and C). In addition to these apparent gaps, other regions of GRCh38 are artificial or are otherwise incorrect. For example, the centromeric alpha satellite arrays are represented as computationally generated models of alpha satellite monomers to serve as decoys for resequencing analyses (*6*), and sequence assigned to the short arm of chromosome 21 appears falsely duplicated and poorly assembled (*7*). When compared with other human genomes, GRCh38 also shows a genome-wide deletion bias that is indicative of incomplete assembly (*8*). Despite finishing efforts from both the Human Genome Project (*9*) and GRC (*1*) that improved the quality of the reference, there was limited

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