

# Haplotype Diversity and Sequence Heterogeneity of Human Telomeres

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## Abstract

Telomeres are regions of repetitive nucleotide sequences capping the ends of eukaryotic chromosomes that protect against deterioration, and whose lengths can be correlated with age and adverse health risk factors. Given their length and repetitive nature, telomeric regions are not easily reconstructed from short-read sequencing, making telomere sequence resolution a very costly and generally intractable problem. Recently, long-read sequencing, with read lengths measuring in hundreds of Kbp, has made it possible to routinely read into telomeric regions and inspect their sequence structure. Here, we describe a framework for extracting telomeric reads from whole genome single-molecule sequencing experiments, prior-less *de novo* identification of telomere repeat motifs, and describing their sequence variation. We find that long telomeric stretches can be accurately captured with long-read sequencing, observe extensive sequence heterogeneity of human telomeres, discover and localize non-canonical motifs (both previously reported as well as novel), confirm the presence of the non-canonical motifs in short read sequencing experiments, and report the first motif composition maps of human telomeric haplotypes across populations on a multi-Kbp scale.

## Keywords

Telomere, telomeric haplotypes, long-read sequencing, telomere sequence heterogeneity

## Introduction

Telomeres are the functional ends of human chromosomes that naturally shorten with cell division and therefore with age [1]. Telomere length can also be influenced by a variety of lifestyle factors and environmental exposures (e.g., stress, exercise, air pollution, radiation) [2]. While human telomeres are known to consist largely of a conserved six-nucleotide repeat (TTAGGG) [3], several studies have identified variations of this motif in proximal telomeric regions [4–7]. However, such studies were performed with oligonucleotide hybridization, PCR, immunoprecipitation, and short-read sequencing, requiring prior assumptions about specific target motifs, custom sample preparation, and targeted sequencing, and therefore preventing *de novo* identification of motif variants and their localization. Thus, long-range maps of telomeric sequence variation in the human genome are still lacking. Such maps can provide insight into telomere biology and enable novel approaches to analyze the effects of health status, aging, and environment on telomere sequence and length.

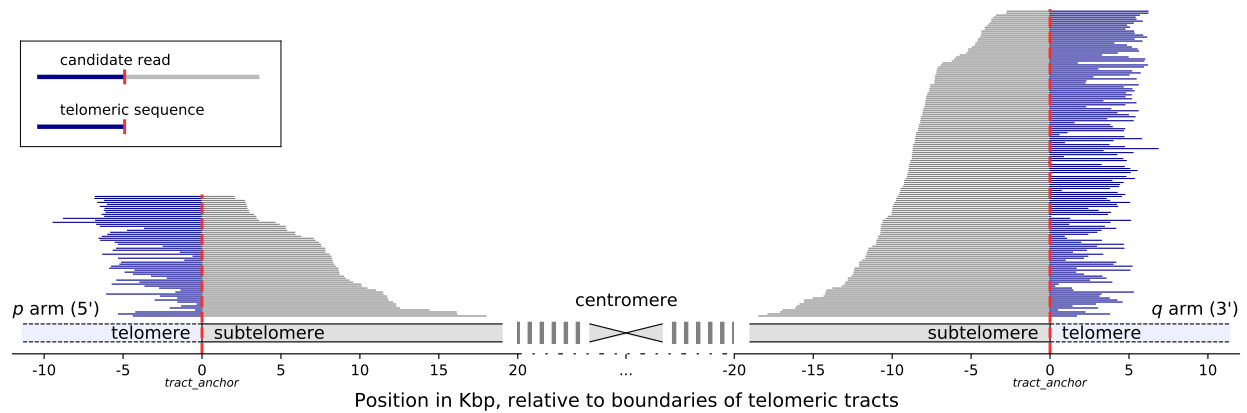
To improve our understanding of telomere sequence structure and variation, we developed *edgeCase*, a framework for alignment and *de novo* telomeric motif discovery which uses human whole genome long-read sequencing experiments, making it easily scalable. We have validated these methods using Genome in a Bottle [8] single-molecule real-time (SMRT) sequencing datasets generated with Pacific Biosciences circular consensus sequencing (PacBio CCS) [9, 10], and short-read Illumina [11] and 10X Genomics (Chromium) [12] datasets. These results provide evidence for multiple novel, non-canonical telomeric repeats, resolution of multiple chromosome-specific haplotypes with SMRT sequencing, and a new method for long-range characterization of the structure of telomeric sequences.

## Results

### **A telomere-annotated reference genome recovers telomeric reads from human long-read whole genome sequencing datasets**

We constructed an extended reference genome, *hg38ext*, that combines chromosome sequences of the *hg38* reference genome [13, 14] and human subtelomeric assemblies [15], resulting in a reference set annotated with boundaries of subtelomeric and telomeric tracts. The layout of this reference set is available in **Supplemental File S1**, and the set itself can be reproduced with a script available as **Supplemental File S2**. We then aligned PacBio CCS reads of seven Genome in a Bottle (GIAB) human subjects (HG001 through HG007) to *hg38ext*, and in total, observed reads mapping to the ends of chromosomes and extending into

telomeric regions on 10 *p* arms and 19 *q* arms, with 53–295 such reads on the *p* arms and 384–1119 on the *q* arms (**Supplemental Table S1**). Portions of reads contained in the telomeric regions were extracted for further analysis (**Figure 1**).



**Figure 1:** Mapping of candidate telomeric reads, illustrated with reads from the HG002 dataset aligning to chromosome 5. The chromosome is displayed schematically, centered around the centromere. Vertical red dashed lines denote the position of the boundary of the annotated telomeric tract. Coordinates are given in Kbp, relative to the positions of the telomeric tract boundaries. Statistics for all chromosomes of all seven datasets are provided in **Supplemental Table S1**.

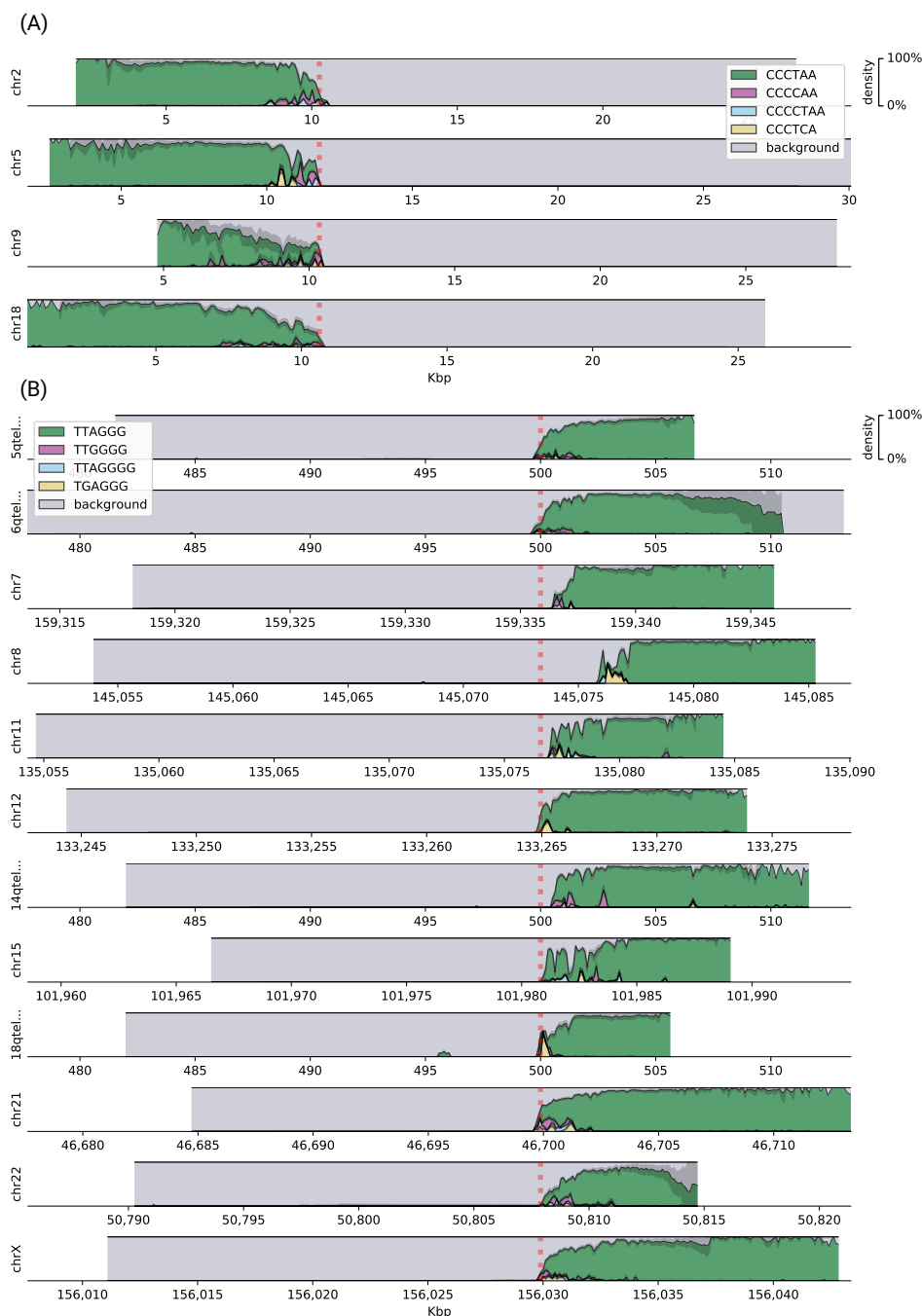
## Telomeric reads contain variations of the canonical motif

We performed *de novo* repeat discovery in the telomeric sequences for motifs of lengths 4 through 16, and identified motifs in repeat contexts that are statistically enriched in the seven datasets. The majority of motifs were either the canonical TTAGGG / CCCTAA, its variations (e.g., TTGGGG / CCCCAA), or a duplet of variants, such as TTAGGGTTAGGG (Table 1). CG-rich motifs were also observed on the *p* arms. The top enriched motif (TTAGGG / CCCTAA) explained 62.2%–82.5% of the telomeric repeat content on the *q* arms and 11.6%–36.3% on the *p* arms, and three more motifs (TTGGGG, TTAGGG, TGAGGG) each explained at least 1% of the repeat content in all seven datasets. These top motifs, as well as 11 less enriched ones, were confirmed in independently generated human short read and linked-read genomic datasets (**Supplemental Table S2**).

Arm	Motif	Percentage of sequence explainable by motif, %							Score		HG003	HG004	HG005	HG006	HG007	Combined adjusted p value
		HG001	HG002	HG003	HG004	HG005	HG006	HG007	HG001	HG002						
q	TTAGGG	74.5	82.5	80.1	81.7	75.7	77.5	62.2	0.6295	0.7126	0.6255	0.6497	0.6113	0.5988	0.4550	9.51e-113
	TTGGGG	2.5	3.4	2.8	2.8	2.4	3.1	6.6	0.0158	0.0229	0.0175	0.0179	0.0155	0.0197	0.0434	4.04e-58
	TTAGGGG	4.6	4.8	7.2	6.0	5.1	7.6	9.0	0.0152	0.0166	0.0200	0.0163	0.0161	0.0232	0.0279	4.22e-110
	TGAGGG	1.9	2.5	1.7	2.0	3.6	2.9	4.1	0.0128	0.0162	0.0102	0.0129	0.0230	0.0184	0.0265	1.15e-47
	TTGCGG	1.2	0.5	0.7	0.4	1.4	1.1	2.5	0.0080	0.0034	0.0043	0.0025	0.0095	0.0077	0.0168	7.68e-46
	TTAGGGTTAGGG	3.0	3.3	6.3	5.4	3.7	6.0	6.5	0.0043	0.0050	0.0090	0.0073	0.0053	0.0083	0.0092	2.76e-102
	TCAGGG	0.9	0.7	1.1	1.0	1.1	0.8	1.4	0.0065	0.0044	0.0078	0.0069	0.0082	0.0058	0.0087	1.22e-24
	TTAGG	1.8	1.6	3.4	4.2	2.0	3.2	1.9	0.0048	0.0041	0.0092	0.0110	0.0052	0.0084	0.0049	4.60e-94
	TAGGG	2.3	1.9	3.1	3.0	2.8	3.2	2.4	0.0050	0.0039	0.0067	0.0063	0.0058	0.0067	0.0048	5.75e-91
	TTAGGTTAGGG	2.7	2.6	5.2	6.5	2.8	4.9	2.5	0.0037	0.0034	0.0069	0.0088	0.0037	0.0065	0.0033	1.97e-89
	TAGGGC	0.5	0.4	0.6	0.6	0.8	0.2	1.3	0.0039	0.0032	0.0047	0.0047	0.0060	0.0014	0.0099	5.64e-42
	TTTAGGG	1.5	1.5	1.4	1.4	1.4	2.2	2.5	0.0048	0.0039	0.0029	0.0028	0.0034	0.0055	0.0058	2.32e-79
	TAGGGG	0.7	0.9	0.6	0.9	0.7	0.6	1.2	0.0035	0.0051	0.0028	0.0044	0.0034	0.0025	0.0060	2.68e-42
	TAGGGTTAGGG	3.1	2.6	3.9	4.0	3.5	3.8	2.9	0.0036	0.0031	0.0041	0.0041	0.0041	0.0040	0.0035	1.45e-84
	TTAAGGG	0.8	1.2	1.1	0.8	1.0	1.2	1.3	0.0022	0.0030	0.0032	0.0021	0.0029	0.0034	0.0032	4.87e-70
	TTGGG	1.4	0.9	1.9	1.7	1.8	1.9	1.4	0.0022	0.0013	0.0032	0.0026	0.0028	0.0028	0.0022	3.17e-70
	TTAGGGTTTAGGG	1.2	1.4	1.4	1.5	1.3	2.0	2.3	0.0011	0.0017	0.0013	0.0014	0.0016	0.0021	0.0033	5.17e-68
	TTGGGTTAGGG	1.7	1.0	2.1	1.9	1.9	2.0	1.1	0.0012	0.0007	0.0013	0.0014	0.0015	0.0014	0.0008	1.75e-53
	TTAGGGTTAAGGG	0.5	1.0	0.9	0.5	0.7	0.7	1.0	0.0005	0.0020	0.0009	0.0004	0.0006	0.0009	0.0007	1.03e-50
p	CCCTAA	21.5	36.3	19.9	17.1	32.0	16.9	11.6	0.1687	0.3113	0.1491	0.1258	0.2639	0.1255	0.0831	9.51e-113
	CCCCAA	1.5	1.6	1.4	1.1	1.8	1.1	1.4	0.0100	0.0104	0.0087	0.0073	0.0120	0.0073	0.0093	1.05e-73
	CCCCTAA	2.3	2.4	1.9	2.0	2.2	1.9	1.9	0.0075	0.0075	0.0054	0.0059	0.0067	0.0056	0.0061	9.17e-109
	CCCTCA	0.2	0.6	0.5	0.5	0.4	0.6	0.5	0.0009	0.0044	0.0033	0.0029	0.0025	0.0037	0.0035	1.05e-50
	CCCTAACCTAA	1.8	2.0	1.6	1.6	2.0	1.6	1.3	0.0029	0.0031	0.0023	0.0023	0.0029	0.0023	0.0022	1.46e-97
	GGCGCA	2.1	1.8	1.4	1.1	1.6	1.4	1.1	0.0028	0.0023	0.0019	0.0014	0.0022	0.0020	0.0016	2.35e-27
	CCGCG	1.1	0.8	0.7	0.5	0.8	0.9	0.9	0.0028	0.0020	0.0018	0.0013	0.0021	0.0022	0.0021	4.35e-100
	CCCTA	0.9	1.1	1.0	0.9	1.2	0.8	0.5	0.0020	0.0021	0.0022	0.0019	0.0026	0.0015	0.0010	2.38e-98
	CCTAA	0.8	1.0	0.9	0.9	0.6	0.6	0.4	0.0020	0.0026	0.0023	0.0023	0.0016	0.0016	0.0010	5.75e-100
	CCCTAACCTAA	1.1	1.6	1.3	1.2	0.9	0.9	0.5	0.0015	0.0021	0.0017	0.0016	0.0012	0.0012	0.0007	1.47e-80
	CCCTACCTAA	1.1	1.3	1.2	0.9	1.6	0.9	0.5	0.0012	0.0020	0.0012	0.0011	0.0021	0.0010	0.0007	6.67e-77

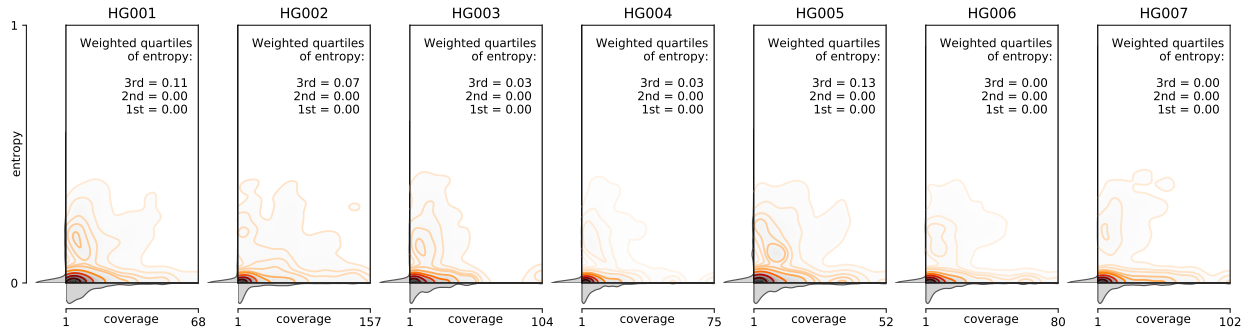
**Table 1:** Significantly enriched repeating motifs in telomeric regions of GIAB datasets HG001 through HG007. Only the motifs that explain at least 1% of the telomeric sequence on either arm of at least one dataset, with respect to reverse-complemented equivalence, are included.

We visualized the locations of the top four enriched motifs and their reverse complements on the chromosomal ends of the HG002 dataset (**Figure 2**), as it provided the deepest coverage among the assessed datasets (**Supplemental Table S1**). Only the chromosomal arms covered by at least 25 reads were plotted. Plots for the other six datasets are available as **Supplemental Figs. S1–S6**.



**Figure 2:** Densities of the top four enriched motifs at ends of chromosomal (A) p arms and (B) q arms of the HG002 dataset. *Background* represents the remaining sequence content (non-repeating sequence and not significantly enriched motifs). Only the arms covered by at least 25 reads are displayed. Reads are shown aligned to the contigs in the *hg38ext* reference set, and genomic coordinates are given in Kbp. Vertical red dashed lines denote the position of the boundary of the annotated telomeric tract.

Long reads on each arm agreed on the locations of different motifs within any given 10 bp window (the coverage-weighted median of normalized Shannon entropy was 0.00 for all data, and the coverage-weighted 3rd quartile was 0.00–0.13, [Figure 3](#)), indicating that locations of the variations are colinear among reads.



**Figure 3:** Distribution of motif entropies in 10 bp windows of of candidate PacBio CCS reads aligning to the same chromosomal arms in GIAB datasets HG001 through HG007, with respect to per-window coverage, and the coverage-weighted quartiles of the entropy values.

## Long-read sequencing resolves human telomeric haplotypes

Sequences of telomeric reads clustered by relative pairwise Levenshtein distances [16] with varying levels of heterogeneity depending on the dataset and the chromosomal arm to which they belonged. We examined the  $q$  arms of the HG002 dataset to investigate this heterogeneity, as they provided the deepest coverage (??), and found that, on 12 out of the 15 arms, reads clustered into two prominent groups per arm when maximizing the Bayesian information criterion [17] (see [Materials and Methods](#)). Pairwise distances between the reads within these clusters were significantly lower than those for out-of-cluster pairings, implying that distinct telomeric haplotypes are present. To quantify the differences between putative haplotypes, we calculated silhouette scores [18] for these clusterings (??), and generated motif density plots for the four chromosome arms with the highest such scores to visualize the differences in haplotypes (??).

## Discussion

Repeat-rich, low-complexity regions of the human genome such as telomeres have been historically recalcitrant to full mapping and annotation [19], mainly due to the alignment challenge they pose and to the read lengths required to span such areas [20]. The advent of long-read, single-molecule methods (third generation sequencing) has provided new opportunities to map the sequence composition of a previously "dark" area of the human genome, enabling research into the sequence composition and length dynamics [luxton2020]

of telomeres. Our results reaffirm that the canonical repeat (TTAGGG) is certainly the most dominant type of motif in telomeres, but also reveal a surprising diversity of repeat variations, which are confirmed by both short and long-read sequencing technologies. This diversity of repeats includes previously reported variants, as well as novel motifs that are characterized not only by nucleotide substitutions, but also insertions, deletions, and even motif pairing. Apart from these variations, CG-rich motifs were identified in telomeric regions of *p* arms, consistent with previously reported findings [21]. Moreover, while short read sequencing is able to identify such variants, it alone cannot reveal the relative locations of these motifs within telomeres, as repetitive short reads can neither be aligned outside of the reference genome nor provide enough overlap variability to be assembled *de novo*. Long SMRT reads, on the other hand, can be anchored to known subtelomeric sequences of the human genome and extend into the previously unmapped telomeric area. These results also highlight the need of better subtelomeric and telomeric annotations in the human genome. Four of the 40 subtelomeric assemblies [15] were homologous to regions in the reference genome far within the respective chromosomes (up to 586 Kbp into the reference sequence), and the canonical motif was present on the *q* arm of chr8 only after 2–3Kbp past the annotated boundary in all datasets, suggesting that the existing assemblies do not provide a completely accurate telomeric annotation, and that methods described herein could help to resolve these areas of reference genomes.

We observed PacBio CCS reads reaching up to 16 Kbp beyond the known regions of the genome, and resolving the underlying sequence with reasonable fidelity, measured both by the entropy of motif assignment and by pairwise Levenshtein distances between the reads belonging to the same chromosomal arms. While short reads also provided support for non-canonical motifs, the overlap between the short and the long reads was substantial, but not complete, which can be explained by the necessary bias towards the canonical motif during the selection of short reads. Therefore, telomeric regions with higher content of non-canonical repeats are less likely to be identified through the use of short reads, and instead, long reads appear to be more suitable for this purpose as well. The identified variations in long range contexts enable clustering of SMRT reads into distinct haplotypes at ends of chromosomes, and thus provide a new means of diplotype mapping and reveal the existence and motif composition of such diplotypes on a multi-Kbp scale.

## Materials and Methods

### The extended reference genome

We constructed the extended reference genome by performing an all-to-all alignment of all contigs in the *hg38* reference genome [13, 14] and the subtelomeric assemblies [15] with *minimap2* [22] using three settings

for assembly-to-reference mapping (*asm5*, *asm10*, *asm20*). Forty subtelomeric contigs mapped to ends of *hg38* chromosomes with a mapping quality of 60, one (XpYptel) mapped with the quality of 0 and was discarded; one (14qtel) mapped to the ALT version of chr14 (chr14\_KI270846v1\_alt) with the quality of 52, which, in turn, mapped to the main chr14 chromosome with the quality of 60. These data and the exact match and mismatch coordinates were used to create a combined reference (*hg38ext*) in which subtelomeric contigs informed the locations of the boundaries of the telomeric tracts (*tract\_anchor*). Such contigs that mapped fully within *hg38* chromosomes resulted in *tract\_anchor* annotations directly on those *hg38* chromosomes; partially mapping contigs were considered as forking from the *hg38* sequence and were similarly annotated by themselves.

## Detection of telomeric sequences in long-read datasets

Three subjects were selected for the analysis. The first individual (NA12878/HG001) came from the pilot genome of the HapMap project [23], while the other two, including the son from the Ashkenazi Jewish Trio (NA24385/HG002) and the son from the Chinese Trio (NA24631/HG005), are members of the Personal Genome Project, whose genomes are consented for commercial redistribution and reidentification [24]. These subjects are referred to hereafter as HG001, HG002, and HG005, respectively.

For subjects HG001 and HG005, Genome in a Bottle [8] PacBio\_SequellI\_CCS\_11kb datasets were used (one dataset per each subject). For subject HG002, a combination of two sequencing experiments was analyzed (PacBio\_CCS\_10kb and PacBio\_CCS\_15kb). The mean coverage was  $\sim 29x$ ,  $\sim 58x$ , and  $\sim 32x$  for subjects HG001, HG002, and HG005, respectively. Reads were mapped to *hg38ext* with *minimap2*, and reads that mapped to either end of either chromosome and overlapped the boundary of its telomeric tract were selected for further analysis. These reads had a portion of their sequence mapped to the reference contig and a portion extending beyond the reference (soft- or hard-clipped in the alignment file). Sequences past the *tract\_anchor* marker were extracted from the reads that had this marker within their mapped portion (from the 5' end to the marker on *p* arms and from the marker to the 3' end on *q* arms, accounting for forward and reverse mappings). To identify regions of the telomeres that are fully supported by both short and long reads, we extracted candidate telomeric reads from GIAB Illumina datasets (NIST\_NA12878\_HG001\_HiSeq\_300x, NIST\_HiSeq\_HG002\_Homogeneity-10953946, HG005\_NA24631\_son\_HiSeq\_300x; all three  $\sim 300x$  coverage) with *Telomerecat* [25], and selected those that mapped perfectly with *minimap2* (at least a 50bp-long exact match without insertions or deletions, allowing all secondary mappings) to the telomeric regions of the PacBio CCS candidates from the same subject's dataset.



## Detection of telomeric sequences in short- and linked-read datasets

To evaluate sequence motifs in datasets generated by technologies other than SMRT, we generated four whole-genome Illumina datasets (mean coverage  $\sim 104\times$ ) and three linked-read 10X datasets (mean coverage  $\sim 28\times$ ) for one individual at different timepoints aboard the International Space Station (ISS), and one additional linked-read 10X dataset (coverage  $\sim 47\times$ ) for another individual aboard the ISS. Blood samples were collected from astronaut subjects as described in [twins\_study]. For each sample, 1.2ng of sorted immune cell input was aliquoted for TruSeq PCR-free WGS (short read) and standard Chromium 10X whole genome (linked-read) preparation respectively, and sequenced across one S4 flow cell on an Illumina NovaSeq 6000. From these datasets, candidate telomeric short reads were selected using Telomerecat [25].

## Identification of repeat content

Overrepresentation of motifs of lengths  $k \in [4, 16]$  was tested within the candidate telomeric regions of PacBio CCS reads, as well as in the candidate reads from independently generated Illumina and 10X Chromium datasets. To target motifs in repeat contexts, doubled sequences (for example,  $k$ -mer ACGTACGT for motif ACGT) were counted with *jellyfish* [26], and counts of  $k$ -mers synonymous with respect to circular shifts (for example, ACGTACGT and CGTACGTA) were summed together. For each such  $k$ -mer, Fisher’s exact test was performed to determine whether its count is significant on the background of counts of other  $k$ -mers of the same length. Briefly, we considered  $k$ -mers with counts higher than 1.5 interquartile range above the third quartile of the distribution as potentially classifiable, and a  $2 \times 2$  contingency matrix  $C$  for the test was constructed as follows: row 0 contained counts of potentially classifiable  $k$ -mers, row 1 contained counts of remaining (non-classifiable)  $k$ -mers, columns 0 and 1 contained counts of single and remaining (background)  $k$ -mers, respectively, i.e.:  $C_{0,0}$  = count of target  $k$ -mer,  $C_{0,1}$  = sum of counts of other potentially classifiable  $k$ -mers,  $C_{1,0}$  = median count of  $k$ -mer,  $C_{1,1}$  = sum of counts of other non-classifiable  $k$ -mers. The resultant  $p$ -values for each motif among the samples were combined using the Mudholkar-George method [27] within each technology (PacBio CCS, Illumina, 10X Genomics), and the Bonferroni multiple testing correction was applied. Motifs in the long-read datasets for which  $k$ -mers yielded  $p$ -values below the cutoff of 0.05 were reported. Additionally, motifs that were significantly enriched in the datasets produced by all three technologies (PacBio, Illumina, 10X), with respect to reverse-complemented equivalence, were reported.

## Evaluation of sequence concordance in telomeric long reads

As telomeric reads contain long low-complexity regions and present an alignment challenge, we evaluated concordance of their sequences without realignment of their portions that extended past the reference sequence. To that end, for all reads mapping to the same chromosomal arm, we calculated densities of each identified motif in a rolling window starting from the innermost mapped position of each entire read. To evaluate whether the reads on the same arm agree on the positions of different motifs, for each read, we calculated motif densities in 10 bp windows with 10 bp smoothing to buffer insertions and deletions. For each window in each read, the motif with the highest density was selected to represent that window. Then, normalized Shannon entropy among all reads was calculated in each window as  $S = \frac{-\sum_i (p_i \ln p_i)}{\ln N}$ , where  $p_i$  is the frequency of each motif in the window and  $N$  is the number of motifs [28]. The value of normalized entropy was a metric bounded by  $[0, 1]$ , with 0 describing perfect agreement and 1 describing maximum randomness. For visualization, we performed 1000 rounds of bootstrap of the calculated density values in the 10 bp rolling windows, and selected the lower and the upper bounds of the 95% confidence interval of bootstrap. Of note, several chromosome arms had the *tract\_anchor* position further away from the end of the contig than others ( $\sim 79$ – $586$  Kbp into the chromosome sequence), and the reads mapping to these arms did not contain these motifs, suggesting that either their subtelomeric annotations were incorrect or large insertions or duplications were present in the reference genome; in light of this, reads mapping to the *p* arm of chr1, the *q* arm of chr4, and both arms of chr20 were removed from the study, and the analysis was repeated.

## Extraction of telomeric haplotypes from long-read datasets

Within groups of reads mapping to each chromosome arm, all relative pairwise Levenshtein distances were calculated. In short, to calculate the absolute distance between each pair of reads, the sequences in the overlapping positions of the reads were extracted; the distance then equaled the minimum number of single-character insertions, deletions, and substitutions required to make these sequences identical. The relative distance was computed as the absolute distance divided by the length of the overlap. Relative distances were then clustered using Ward’s method via the Euclidean metric.

## Data access

The NASA Life Sciences Data Archive (LSDA) is the repository for all human and animal research data, including the whole genome Illumina and 10X Chromium sequencing datasets from subjects aboard the ISS

that were used in this study. These datasets are protected by the terms of the Weill Cornell Medicine Internal Review Board (IRB) and can be made available to be shared upon request. LSDA has a public facing portal where data requests can be initiated ([lsda.jsc.nasa.gov/Request/dataRequestFAQ](https://lsda.jsc.nasa.gov/Request/dataRequestFAQ)); the LSDA team provides the appropriate processes, tools, and secure infrastructure for archival of experimental data and dissemination while complying with applicable rules, regulations, policies, and procedures governing the management and archival of sensitive data and information. The LSDA team enables data and information dissemination to the public or to authorized personnel either by providing public access to information or via an approved request process for information and data from the LSDA in accordance with NASA Human Research Program and JSC Institutional Review Board direction.

The software for identification of telomeric reads, *de novo* discovery of repeat motifs, haplotype inference and motif density visualization was implemented in Python and is freely available at [github.com/lankycyril/edgecase](https://github.com/lankycyril/edgecase).

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## Author contributions

S.M.B. and C.E.M. conceived the study. K.G., J.F., and C.E.M. developed the framework and analyzed the data. D.Bu., J.J.L., M.J.M., L.T., and K.A.G. participated in collection and processing of the ISS samples. D.Be., D.Bu., J.J.L., J.R., and C.M. analyzed the data. All authors edited the manuscript.

## Competing interests

The authors declare no relevant conflict of interest, although C.E.M. is a Co-Founder of Onegevity.

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