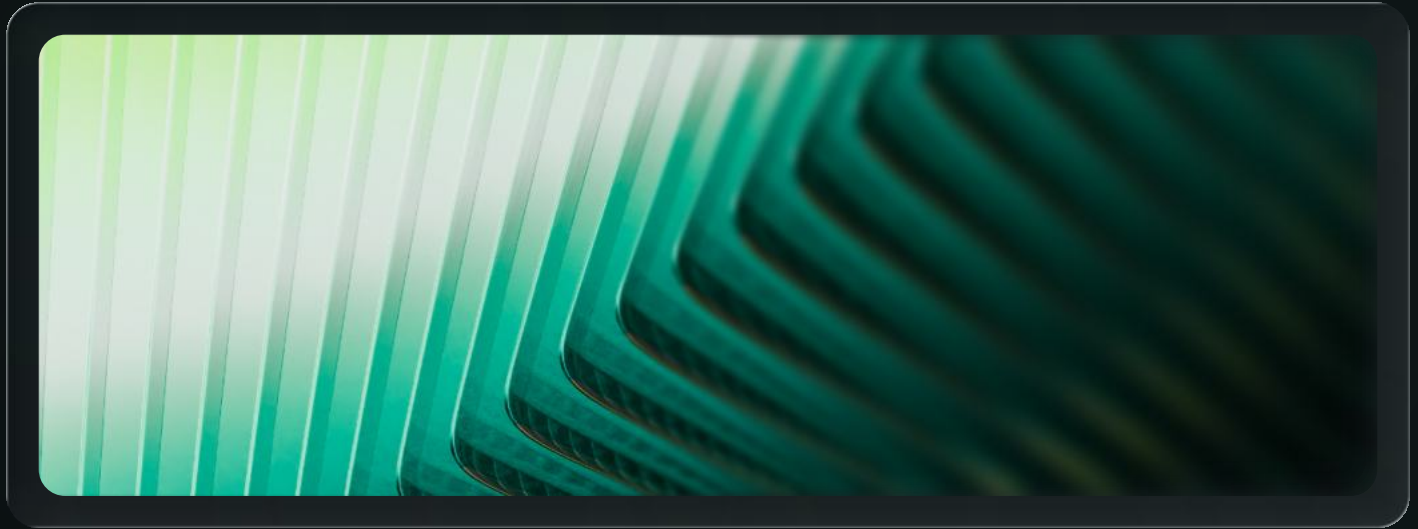




Arm-Resolved Telomere Profiling From Native DNA

Unlocking Banked Cohorts for Discovery and Scaling

Teloseq consolidates global and per-arm TL plus subtelomeric DMR/SV context from extracted genomic DNA in a single long-read workflow – unlocking banked cohorts for discovery and scaling to prospective blood-derived DNA for translational and clinical programs, without live-cell handling, fragile shipping windows, or multi-assay patchwork.

Snapshot

Study Goals

Establish feasibility and QC gates for Renew's Teloseq service; verify per-arm TL estimates and subtelomeric readouts; benchmark alignment and throughput

Approach

12 samples on ONT 's Teloseq protocol. Groups: GIAB HG002, GIAB HG005, healthy PBMC donor, CLL PBMC donor.

Outcomes

- Per-arm resolution achieved: $\geq 90\%$ of chromosome arms met ≥ 10 reads/arm, enabling reliable arm-level TL estimates per sample.
- Replicate consistency: GIAB lines (HG002/HG005) showed tight replicate concordance when aligned to matched references.
- Biology surfaced beyond globals: Healthy vs CLL showed a modest global TL shift but pronounced arm-specific differences, clarifying why global averages can mask risk-relevant short arms.
- Context, not just length: Reads extended into subtelomeres, supporting candidate DMR calls and visualization of indels/SVs for mechanistic interpretation.



Revolutionizing Epigenomic Insights

Beyond Live Cells: The Case for Change

Legacy telomere methods force trade-offs:

- Flow-FISH can be powerful, but it often depends on fresh/viable leukocytes, tight shipping windows, and site-level expertise. Failures and reschedules aren't rare, and the output is typically a global length measure (or a few cell-type bins), not arm-resolved biology.
- TRF/Southern and qPCR have their place, yet they also tend to produce averages with batch-and protocol-sensitive variability, and offer no subtelomeric view.

When the question is “Which chromosome arms are actually short?” or “What’s happening in the subtelomere near those repeats?”, these tools don’t see enough.

Long-read Teloseq flips this: by ligating adapters to the native 3' overhang and reading through telomeres into subtelomeres, a single ONT run yields per-arm telomere distributions and the surrounding sequence context, from high-molecular-weight DNA. No live-cell logistics. No multi-assay patchwork.

Why this Matters

- For Researchers: Arm-specific shortening and subtelomeric features reveal locus-level mechanisms (instability, regulation near repeats), sharpen biomarker discovery, and enable precise cohort stratification that global averages can obscure.
- For Clinicians: Arm-level deficits that hide inside a “normal” global TL can change risk assessment and monitoring plans, guiding follow-up testing and study enrollment decisions in a way not detectable with global assays.



Workflow



72 hour

Objective

Characterize utility of ONT's telomere sequencing protocol.

Protocol & Run

Teloseq libraries were prepared using ONT's standard barcoded ligation workflow, pooled, and sequenced on an Oxford Nanopore long-read platform.

Samples (N=12)

- GIAB HG002 (×3), GIAB HG005 (×3), Healthy PBMC donor (×3), CLL PBMC donor (×3)
- Input: 5 µg HMW DNA per sample

Analysis

- Per-arm telomere length estimation; global vs per-arm distributions
- Alignments to matched references
- Subtelomeric differentially methylated regions (DMRs) and structural variants (SVs) visualized in genome browser

QC / Acceptance

- Coverage target: ≥10 reads per arm in ≥90% of arms across samples
- Replicates: low variance within GIAB cell-line replicates



Replicate Precision Under Matches Reference

Across reference cell lines (highlighted by **HG002** and **HG005**) Teloseq shows **tight replicate concordance** under matched-reference alignment: **arm-level telomere length estimates are stable run-to-run**, with per-arm distributions overlapping within expected technical variance. Coverage gates are consistently met (**≥10 reads/arm in ≥90% of arms**), and replicate scatter aligns near identity ($r \geq 0.95$), establishing precision suitable for decision-grade reporting.

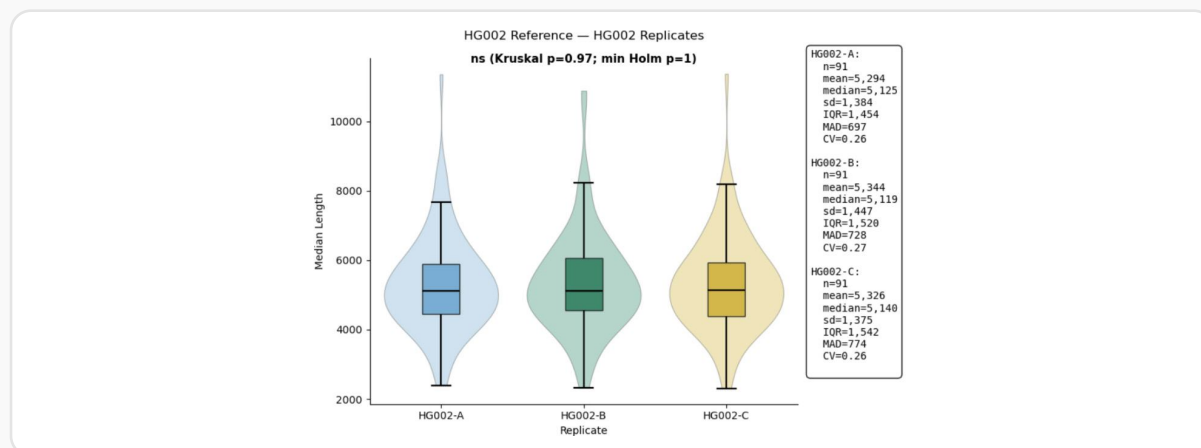


Figure 1A - Replicate Distributions (sample-level): Per-molecule telomere-length distributions for each replicate (N=3 per line), aggregated across all chromosome arms.

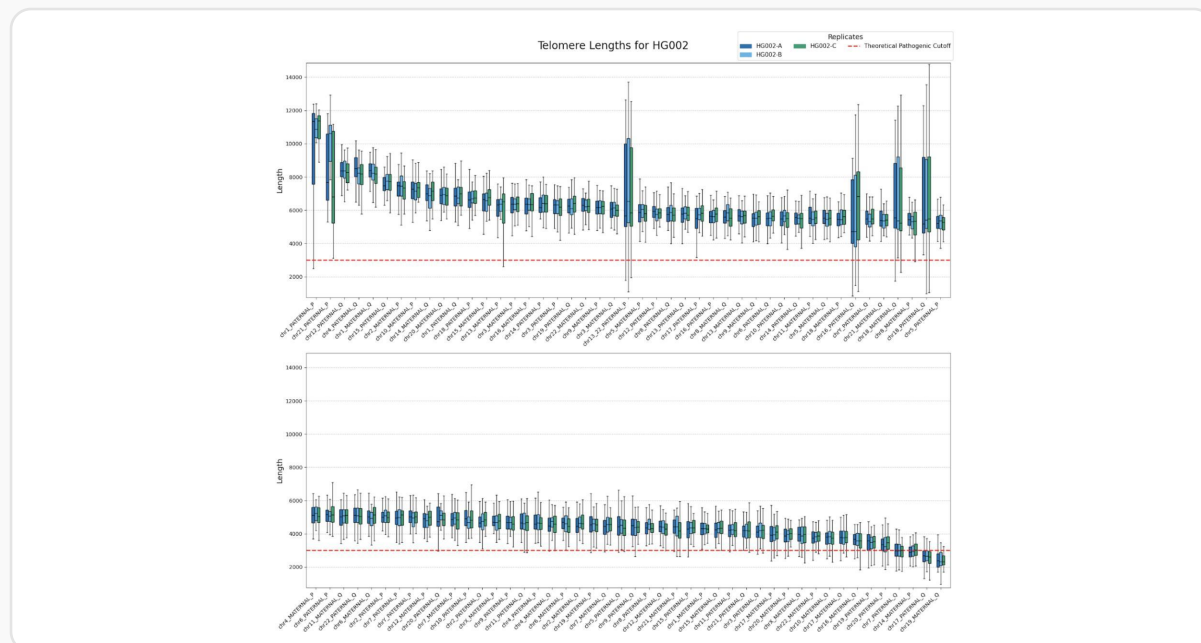


Figure 1A - Replicate Distributions (sample-level): Per-molecule telomere-length distributions for each replicate (N=3 per line), aggregated across all chromosome arms.



Healthy vs CLL: Decision-Relevant Arm Differences

Between the healthy and CLL PBMC donors, the global telomere-length shift is modest, but arm-level analysis exposes larger, decision-relevant differences. When we move from a single pooled metric to per-arm distributions, several chromosome arms show material shifts ($\Delta\text{median} \approx 2.5\text{--}5.5\text{ kb}$) that persist across replicates and remain significant after multiple-testing correction. This pattern explains how a “near-normal” global TL can mask risk-relevant short arms that matter for stratification and follow-up.

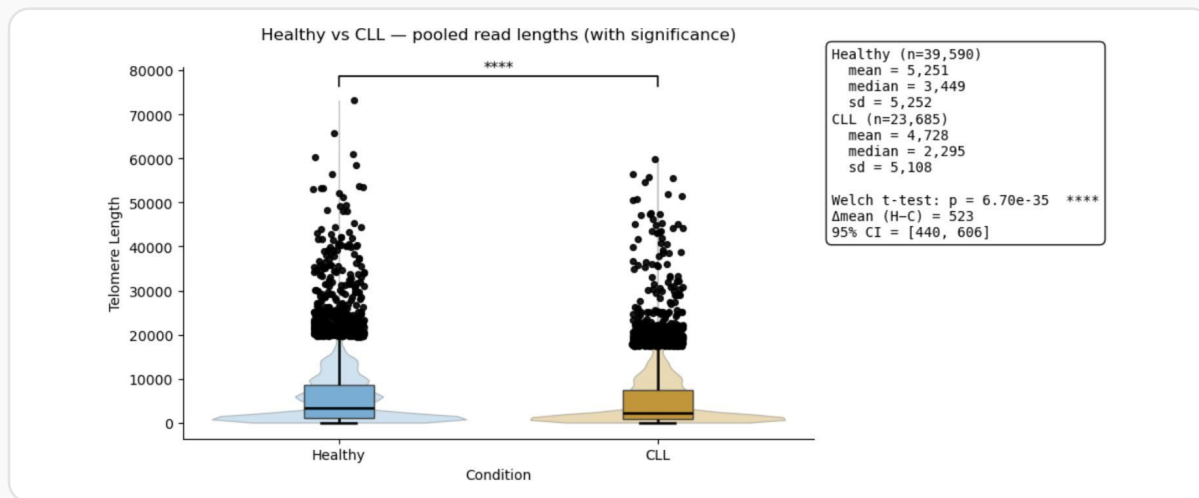


Figure 2A — Global comparison (healthy vs CLL) — Per-Molecule TL Violin/Box With Medians and IQRs; Welch’s t-test or Wilcoxon (two-sided) with effect size ($\Delta\text{median} = 1\text{ kb}$, $p \leq 0.05$, $r = 0.98$).

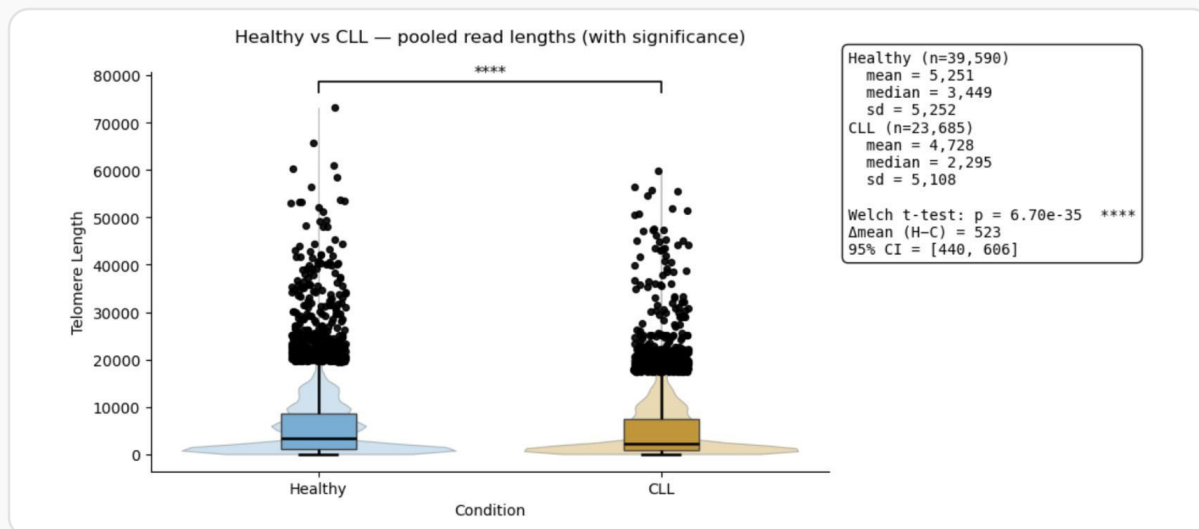


Figure 2B — Arm Level Differences — Violin Plot Of Per-Arm Median TL (Healthy – CLL) with 95% CIs; arms meeting coverage ≥ 10 reads in both donors are tested via Mann–Whitney with Benjamini–Hochberg FDR.

Beyond Length: Telomere-Adjacent Biology

Because Teloseq reads traverse the telomere–subtelomere boundary on single molecules, we can pair arm-level telomere length with adjacent subtelomeric context, calling candidate DMRs at CpG resolution and visualizing indels/SVs that differ by donor. Across replicates, these subtelomeric signals are consistent, co-localize with arms showing TL shifts, and provide mechanistic clues (regulatory state, structural instability) that global TL alone can't capture.



Figure 3A — Boundary Read-Through (IGV): Representative read stacks crossing from subtelomere into TTAGGG repeats with per-CpG methylation tracks.

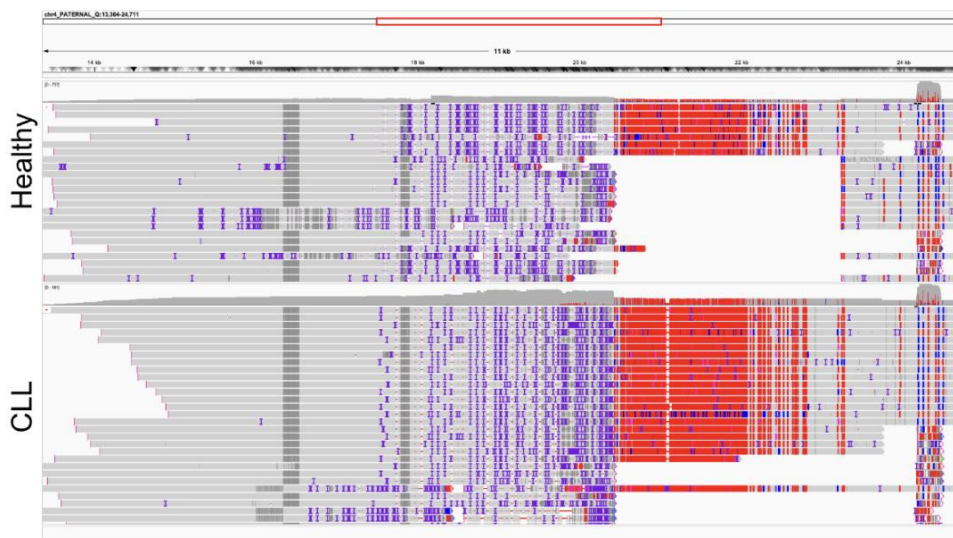


Figure 3B — Subtelomeric DMRs: Windowed methylation difference across the terminal 2 kb; significant clusters highlighted as candidate DMRs ($|\Delta\text{methylation}| \geq 0.5$, $\text{FDR} < 0.05$), annotated by chromosome arm.

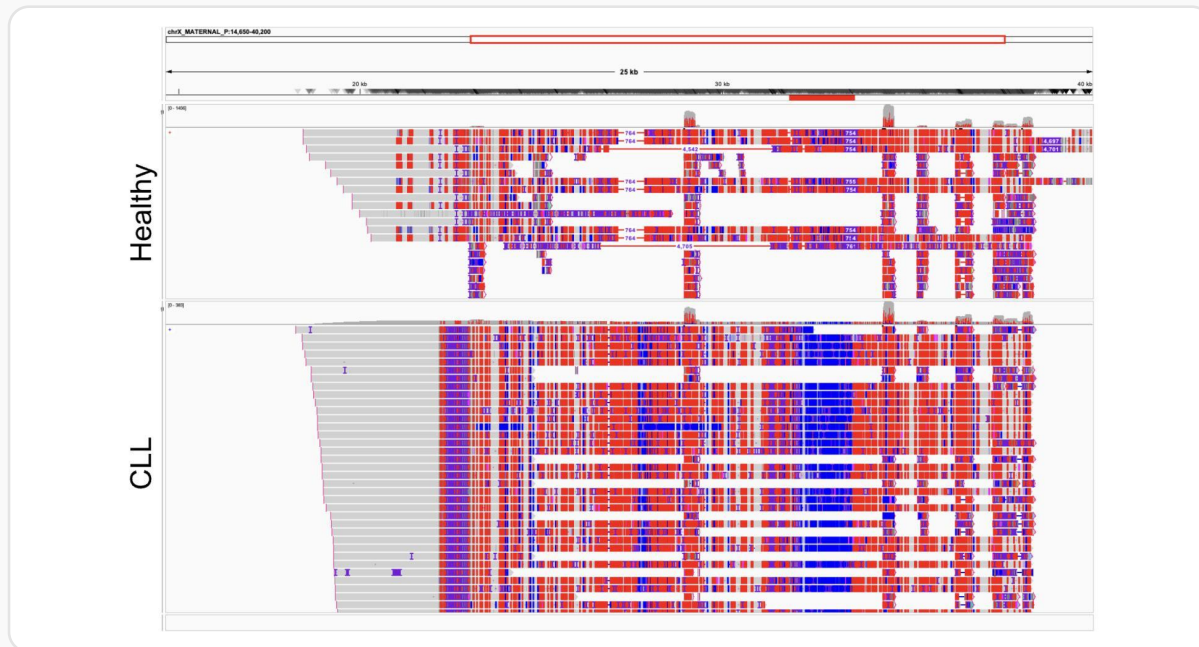


Figure 3C - Structural Variation Near Repeats: Split-read and depth support for a representative 750 bp within 5 kb of the telomere; breakpoints, and read support (n = 5).

Conclusion

Teloseq delivered decision-grade precision and resolution. Replicate concordance under matched-reference alignment was tight, and our coverage gates were consistently met (≥ 10 reads per arm in $\geq 90\%$ of arms), enabling reliable arm-level estimates. In donors, global TL difference (healthy vs CLL) concealed material, arm-specific shifts that persisted across replicates and multiple-testing correction, exactly the signal researchers and clinicians need for finer stratification and hypothesis development. Because the same long reads traverse into subtelomeres, length measurements arrive with adjacent methylation (DMR) and SV context, improving interpretability beyond a single pooled metric.

Unlock the full potential of your research with Renew

Visit renewbt.com

Contact us at info@renewbt.com