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# Assessing Telomere Length Using Long Read Sequencing

*Dr Claire Hill, Dr Gareth McKay, Prof Bernadette McGuinness, Prof Amy Jayne McKnight  
Centre for Public Health, Queen's University Belfast, Northern Ireland*

*International Biomarker Network Meeting – 9<sup>th</sup> April 2025*



NORTHERN IRELAND COHORT  
FOR THE LONGITUDINAL  
STUDY OF AGEING

**SHAPING A  
BETTER  
WORLD  
SINCE 1845**

# TELOMERE LENGTH (TL)



- **Protective caps** at chromosome ends.
- Repeating noncoding nucleotide sequences of **(TTAGGG) $n$** .
- **Attrition** upon every cellular reproductive cycle.
- Evidence suggests that short telomeres trigger DNA damage responses that lead to **cellular senescence**.

# TELOMERE LENGTH (TL) AND DISEASE

## Telomere attrition



## Molecular mechanisms

DNA damage response



Oxidative stress



Inflammation



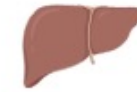
Senescence



## Age-related diseases



Diabetes



Liver disease



Hyperuricemia and gout



COPD



Cardiomyopathy

Atherosclerosis

Hypertension



Kidney disease



Neurodegenerative diseases



Osteoporosis

## Info from:

*Clin Exp Med* **25**, 72 (2025)

The relationship between telomere length and aging-related diseases

Xuanqi Huang<sup>1</sup> · Leyi Huang<sup>1</sup> · Jiaweng Lu<sup>1</sup> · Lijuan Cheng<sup>2,3</sup> · Du Wu<sup>4</sup> · Linmeng Li<sup>5</sup> · Shuting Zhang<sup>1</sup> · Xinyue Lai<sup>1</sup> · Lu Xu<sup>1,3</sup>



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# MEASURING TELOMERE LENGTH

**qPCR**

Measures ratio of telomere repeat copy number to single gene copy number.

- + High-throughput, easy to use and requires <50ng DNA.
- Can be highly variable.

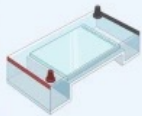


(A)

**TRF**

Uses restriction enzymes and Southern blotting.

- + Historically gold standard
- Low-throughput, labour intensive and unable to measure telomeres <2 kb in length, or single telomeres.




(A)

**STELA**

Uses ligation-PCR and Southern blotting.

- + Small amount of DNA needed.
- Low throughput and labour intensive. The technique is limited to a subset of chromosomes for which primers are available.




(A)

**U-STELA**

STELA but across all chromosomes.

- + Small amount of DNA needed. Able to measure shortest telomere.
- Can't distinguish chromosomes. Unable to measure telomeres >8kb. Low throughput and labour intensive.




(A)

**TESLA**

Uses restriction enzymes and Southern blotting.

- + Can measure all chromosomes. Measures shortest telomere. Able to measure telomeres up to 80 Kb. Small amount of DNA needed.
- Can't distinguish chromosomes. Low throughput and labour intensive.

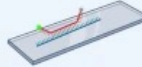


(A)

**TCA**

DNA fibres stretched on glass slide. Fluorescent probes used to visualise telomeres.

- + Highly accurate (shearing reduced by embedding DNA in agarose). Measure telomeres from ~1 kb up to ~80 kb.
- Costly and complex. Methods still being established.




(A)

**LRS**

Harnesses next-generation sequencing.

- + Measures at base pair resolution. Can measure individual chromosomes. Can measure ultra-long telomeres. Highly accurate.
- High amount of DNA needed. Costly and complex. Methods still being established.




(A)

**STAR**

Digital PCR

- + Low amount of DNA needed. High throughput. Can measure ultra-long telomeres. Measures shortest of telomeres. Can determine method of telomere elongation.
- Costly and complex.



(A)

(A) Absolute telomere length measurement possible

Editorial | [Open access](#) | Published: 22 October 2024

## Insights into the length and breadth of methodologies harnessed to study human telomeres

[Tiernan Coulter](#), [Claire Hill](#)  & [Amy Jayne McKnight](#) 

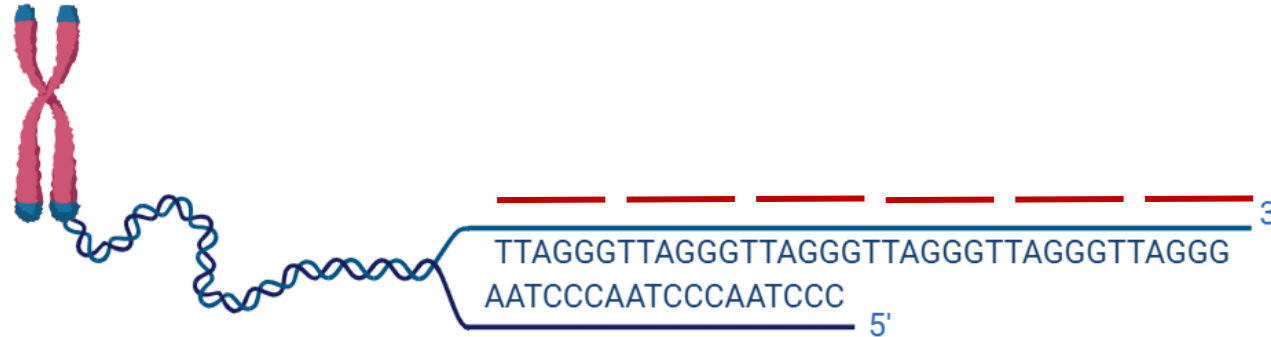
[Biomarker Research](#) 12, Article number: 127 (2024) | [Cite this article](#)

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# QUANTITATIVE POLYMERASE CHAIN REACTION



- Measured via **qPCR** (quantitative polymerase chain reaction).
- Relative ratio of telomere repeat copy number to a single copy gene *36B4*.
- DNA derived from whole blood.
- 3 technical triplicates.

•  **NICOLA** N= 2,971

# EPIGENETICALLY-PREDICTED TL

## DNAmTL

- Trained using **experimentally measured telomere length** (Southern Blot)
- Outperforms measured telomere length in predicting mortality and age-related conditions.

-  **NICOLA** N = 1,870

[www.aging-us.com](http://www.aging-us.com)

AGING 2019, Vol. 11, No. 16

### DNA methylation-based estimator of telomere length

Ake T. Lu<sup>1</sup>, Anne Seeboth<sup>2</sup>, Pei-Chien Tsai<sup>3,4,5</sup>, Dianjanyi Sun<sup>6,7</sup>, Austin Quach<sup>1</sup>, Alex P. Reiner<sup>8</sup>, Charles Kooperberg<sup>8</sup>, Luigi Ferrucci<sup>9</sup>, Lifang Hou<sup>10</sup>, Andrea A. Baccarelli<sup>11</sup>, Yun Li<sup>12</sup>, Sarah E. Harris<sup>13,14</sup>, Janie Corley<sup>13,14</sup>, Adele Taylor<sup>13,14</sup>, Ian J. Deary<sup>13,14</sup>, James D. Stewart<sup>15</sup>, Eric A. Whitset<sup>15,16</sup>, Themistocles L. Assimes<sup>17,18</sup>, Wei Chen<sup>7</sup>, Shengxu Li<sup>19</sup>, Massimo Mangino<sup>3</sup>, Jordana T. Bell<sup>3</sup>, James G. Wilson<sup>20</sup>, Abraham Aviv<sup>21</sup>, Riccardo E. Marioni<sup>2,13</sup>, Kenneth Raj<sup>22\*</sup>, Steve Horvath<sup>1,23\*</sup>

Article available at:



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The next step...

# LONG-READ SEQUENCING TO MEASURE TL

## Oxford Nanopore (ONT):

- Detects differences in **ionic current** as bases pass through a **pore**.
- Absolute **chromosome-specific** telomere measurement pipeline - "Telo-Seq"
- **Global TL:** ~300–500 telomeric reads
- **Chromosome specific TL:** ~10X coverage per arm, 92 telomere arms ∴ 1K telomeric reads
- Multiplexing coming soon.

## Telo-seq

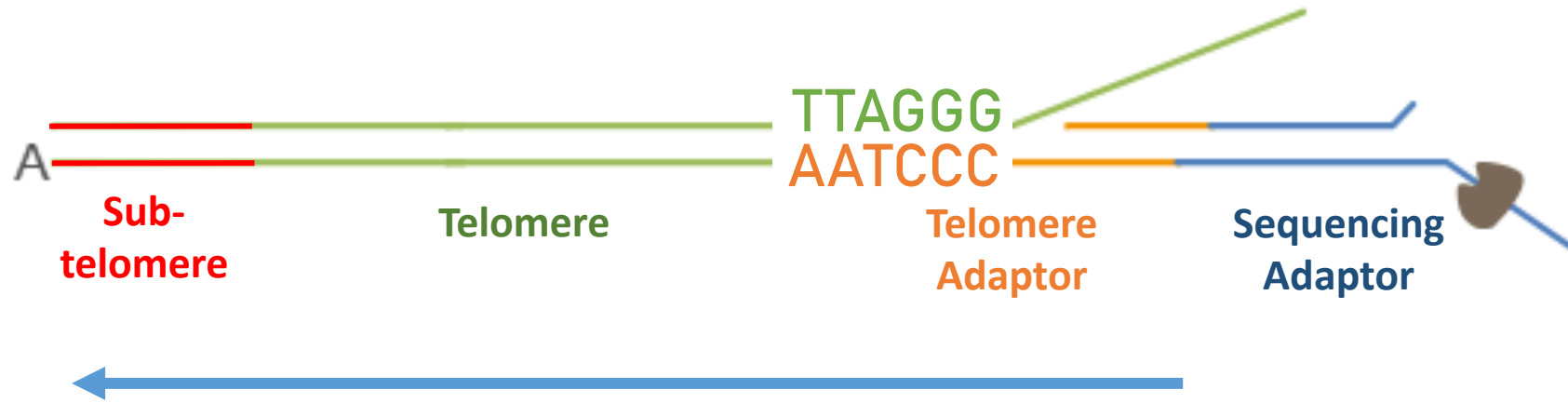


Library	Telomeric reads (K)		% reads on target	
	Mean	SD	Mean	SD
Telo-Seq 15 µg input	38.09	9.20	4.32 %	0.06 %
Telo-Seq 10 µg input	22.98	0.53	3.10 %	0.25 %
Telo-Seq 5 µg input	5.98	2.28	2.66 %	0.52 %
Telo-Seq 1 µg input	0.73	0.15	1.03 %	0.28 %
LSK114 1 µg input	0.11	0.00	0.42 %	0.58 %





# ONT TO MEASURE TL



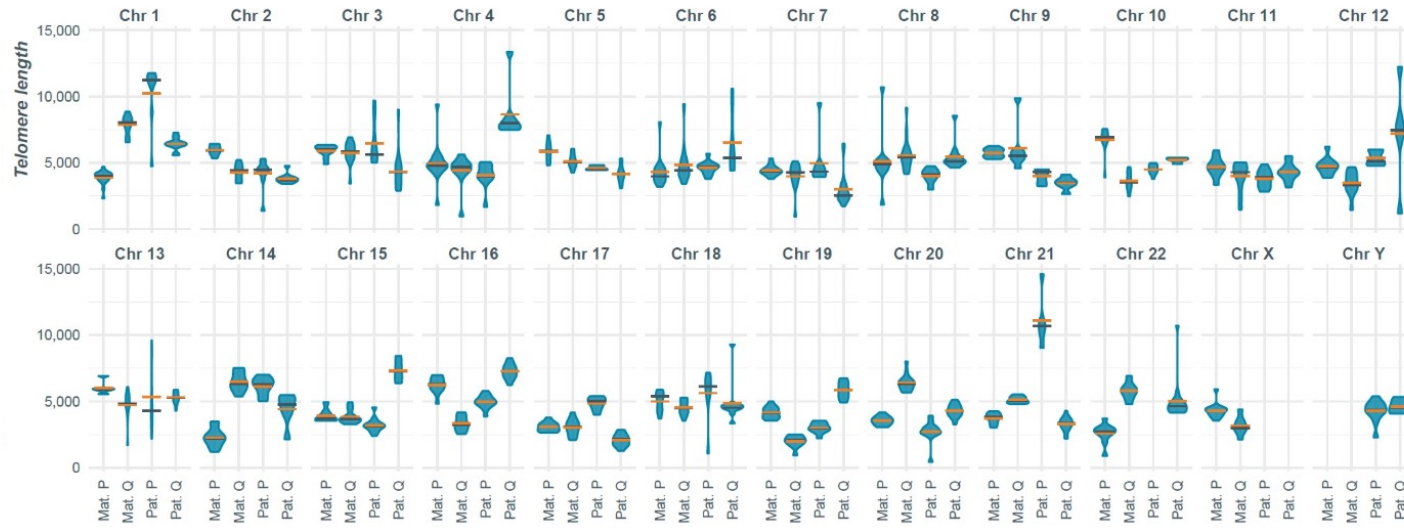
*Sequencing through the telomere into the sub-telomere, which allows alignment to individual chromosomes.*

Adapted from Oxford Nanopore

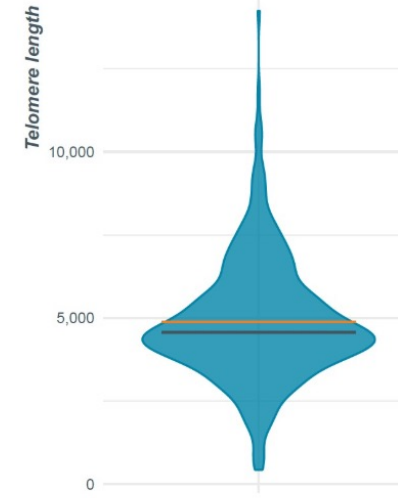
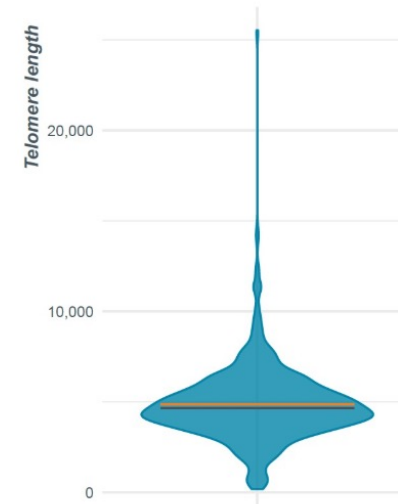
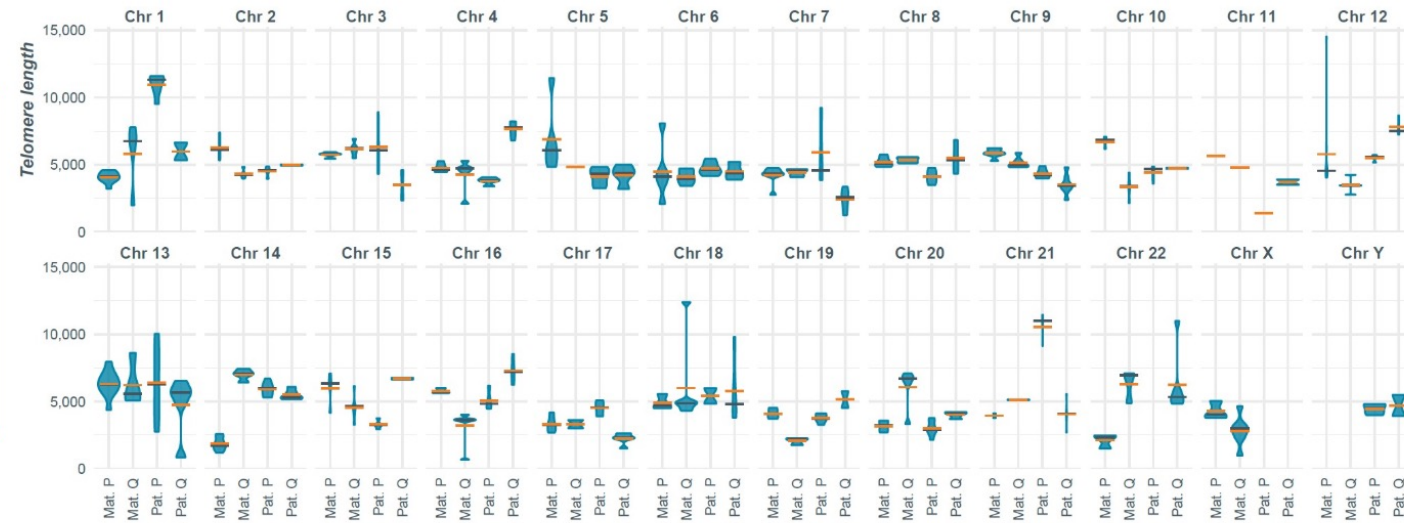
<https://nanoporetech.com/document/requirements/TELO-seq#analysis-pipeline>

# ONT CHROMOSOME LEVEL TL

1 K reads



400 reads



Telo-seq



Median Mean



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# ALTERNATIVE ONT METHOD

## **Telomouse—a mouse model with human-length telomeres generated by a single amino acid change in RTEL1**

[Riham Smoom](#), [Catherine Lee May](#), [Vivian Ortiz](#), [Mark Tigue](#), [Hannah M. Kolev](#), [Melissa Rowe](#), [Yitzhak Reizel](#), [Ashleigh Morgan](#), [Nachshon Egyes](#), [Dan Lichtental](#), [Emmanuel Skordalakes](#), [Klaus H. Kaestner](#) & [Yehuda Tzfat](#)

*Nature Communications* **14**, Article number: 6708 (2023) | [Cite this article](#)

***NIKRF Summer Studentship – Tiernan Coulter –***  
for us to test and utilise this protocol in human samples.

ONT now have their own protocol which we will utilise –  
good field support and validation.

This study developed their own method for chromosome-specific telomere measures with Oxford Nanopore.

**Article available at:**





# PACBIO TO MEASURE TL

## PacBio:

- Single-molecule real time (SMRT) sequencing, detecting the incorporation of **fluorescently-labelled DNA bases**.
- Telomere sequences (using ‘Telobait’ oligos) can be enriched prior to PacBio sequencing, with the option to multiplex.

Article | [Open access](#) | Published: 17 January 2023

### **High-throughput telomere length measurement at nucleotide resolution using the PacBio high fidelity sequencing platform**

[Cheng-Yong Tham](#), [LaiFong Poon](#), [TingDong Yan](#), [Javier Yu Peng Koh](#), [Muhammad Khairul Ramlee](#), [Vania Swee Imm Teoh](#), [Suihan Zhang](#), [Yi Cai](#), [Zebin Hong](#), [Gina S. Lee](#), [Jin Liu](#), [Hai Wei Song](#), [William Ying Khee Hwang](#), [Bin Tean Teh](#), [Patrick Tan](#), [Lifeng Xu](#), [Angela S. Koh](#), [Motomi Osato](#)  & [Shang Li](#) 

[Nature Communications](#) **14**, Article number: 281 (2023) | [Cite this article](#)

Article available at:



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# TELOGATOR2

- A flexible TL analysis tool useful for Oxford Nanopore and PacBio sequencing.
  - PacBio Revio HiFi
  - PacBio Sequel II
  - Nanopore R10
  - Nanopore Telo-seq
- Aligning reads to Telomere-to-Telomere reference genomes for accurate TL quantification, chromosome-specific TL and characterisation of **telomere variant repeats**.

## Characterization of telomere variant repeats using long reads enables allele-specific telomere length estimation

Research | [Open access](#) | Published: 17 May 2024

Volume 25, article number 194, (2024) [Cite this article](#)

[Zachary Stephens](#) & [Jean-Pierre Kocher](#)

Article available at:



Github link:



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# UNCOVERING NEW INSIGHTS 1

- Heterogeneity in **telomere variant sequences (TVSs)** which may have implications in disease.
  - These are deviations from the canonical telomeric repeats (TTAGGG)<sub>n</sub>.
  - Can alter the binding of shelterin, a protein complex protecting chromosome ends.
  - Distribution is unique to each individual and may be an inherited factor impacting telomere degradation, ageing and disease.

Article | [Open access](#) | Published: 17 January 2023

## High-throughput telomere length measurement at nucleotide resolution using the PacBio high fidelity sequencing platform

[Cheng-Yong Tham](#), [LaiFong Poon](#), [TingDong Yan](#), [Javier Yu Peng Koh](#), [Muhammad Khairul Ramlee](#), [Vania Swee Imm Teoh](#), [Suihan Zhang](#), [Yi Cai](#), [Zebin Hong](#), [Gina S. Lee](#), [Jin Liu](#), [Hai Wei Song](#), [William Ying Khee Hwang](#), [Bin Tean Teh](#), [Patrick Tan](#), [Lifeng Xu](#), [Angela S. Koh](#), [Motomi Osato](#)  & [Shang Li](#) 

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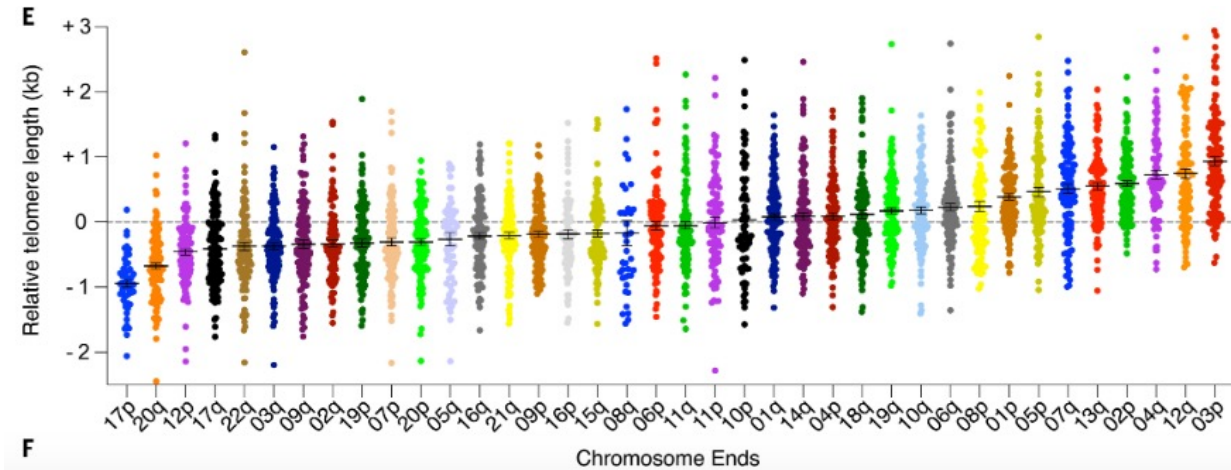
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# UNCOVERING NEW INSIGHTS 2

- Oxford Nanopore – developed their own protocol
- TL in 147 individuals revealed that certain **chromosome ends were consistently longer or shorter.**
- The same rank order was found in newborn cord blood
  - telomere length is determined at birth
  - chromosome-specific TL differences are maintained as they shorten with age.



## Human telomere length is chromosome end-specific and conserved across individuals

KAYARASH KARIMIAN , ALJONA GROOT , VIENNA HUSO , RAMIN KAHIDI , KAR-TONG TAN , SAMANTHA SHOLES , REBECCA KEENER .

JOHN F. MCDYER , JONATHAN K. ALDER , [...], AND CAROL W. GREIDER  [+2 authors](#) [Authors Info & Affiliations](#)

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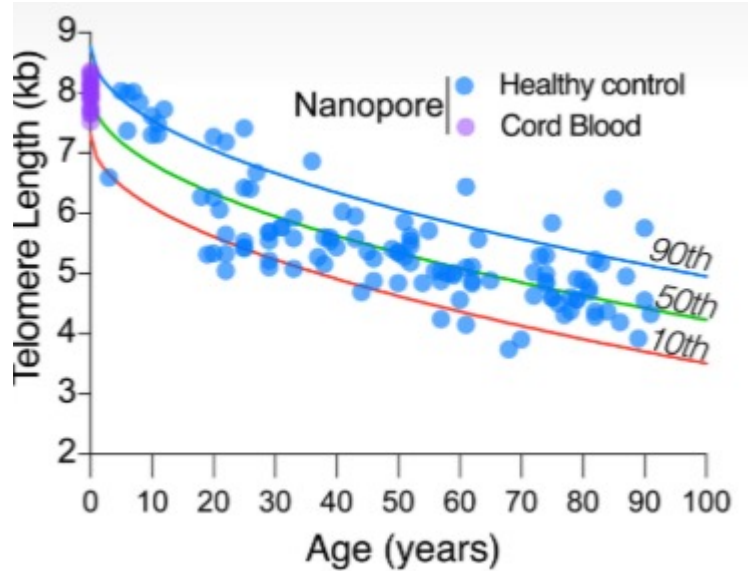
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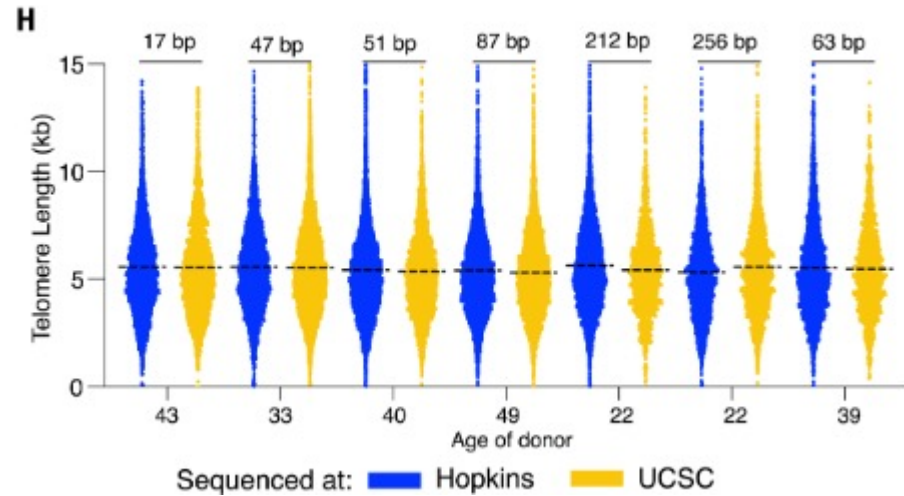
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# UNCOVERING NEW INSIGHTS 3

## Change in TL with advancing age



## Intra-lab variability



## Human telomere length is chromosome end-specific and conserved across individuals

KAYARASH KARIMIAN [ID](#), ALJONA GROOT [ID](#), VIENNA HUSO [ID](#), RAMIN KAHIDI [ID](#), KAR-TONG TAN [ID](#), SAMANTHA SHOLES [ID](#), REBECCA KEENER [ID](#),

JOHN F. MCDYER [ID](#), JONATHAN K. ALDER [ID](#), [...], AND CAROL W. GREIDER [ID](#) [+2 authors](#) [Authors Info & Affiliations](#)

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Article available at:



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Opportunities...

# FUTURE WORK

- Chromosome-specific TL in **population-based cohorts**
  - Larger sample size (largest to date N=147)
  - Longitudinal data - changes over time
  - Sex-differences
- **Biological insights** from chromosome-specific TL
  - Ageing
  - Association with disease
  - Association with comorbidities
  - Sex-inequalities
- We have Southern Blot + qPCR-based + methylation measurements for some samples for comparison with long-read sequencing methods.

Other forms of chromosome  
degradation...

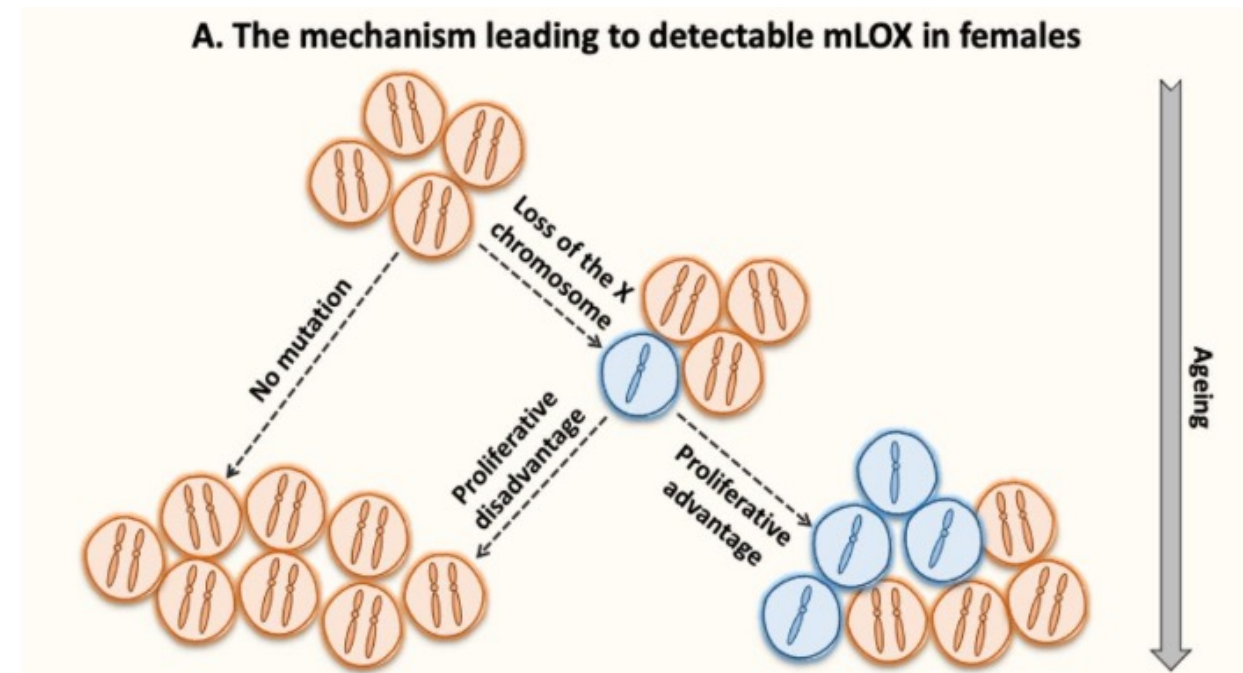
# MOCHA PIPELINES

Github link:



## Mosaic loss

- Specifically interested in **mosaic loss of sex chromosome (mLOX/mLOY)**
- A portion of cell have lost an X or Y chromosome from the pair.
- Most common chromosomal mutation acquired over the life course (Forsbert *et al.*, 2017).
- Input - Genotype array data
- Assess probe intensity across the genome to identify mosaic loss events



Liu *et al.*, 2024

# THANK YOU!

- NICOLA participants and researchers
- Prof Amy Jayne McKnight
- Dr Gareth McKay
- Prof Bernadette McGuinness
- Dr Laura Smyth
- Dr Claire Potter
- Jill Kilner
- Katie Quinn
- Tiernan Coulter
- Collaborators within HRS and TILDA
- QUB Genomics Core
  - Courtney Ward
  - Hossein Esfandiary
  - Sergio Salveti



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STUDY OF AGEING



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