Current Affairs: Utilizing Oxford Nanopore Sequencing Data to Detect Non-Canonical DNA Structures

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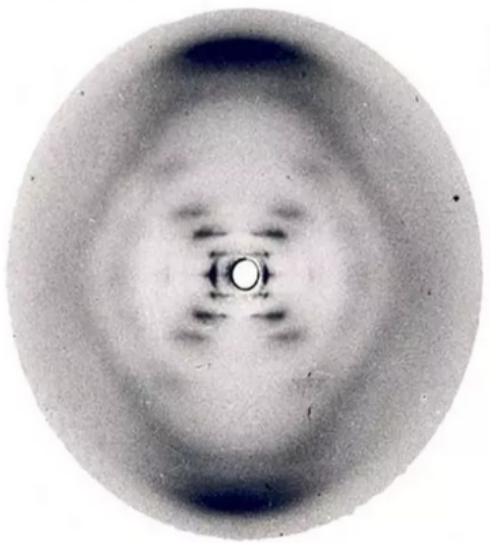
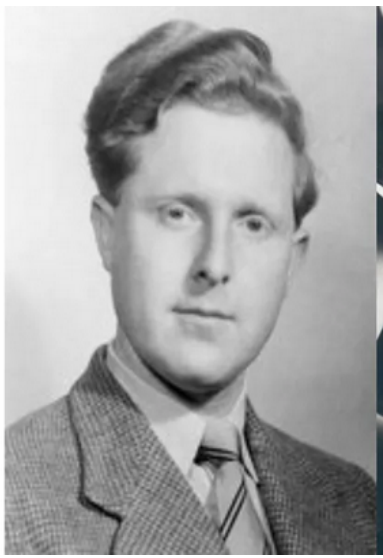
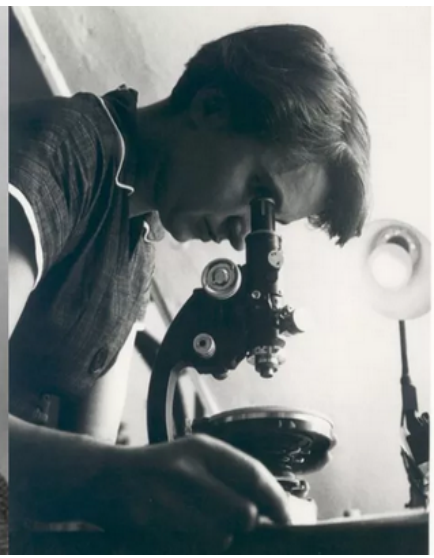
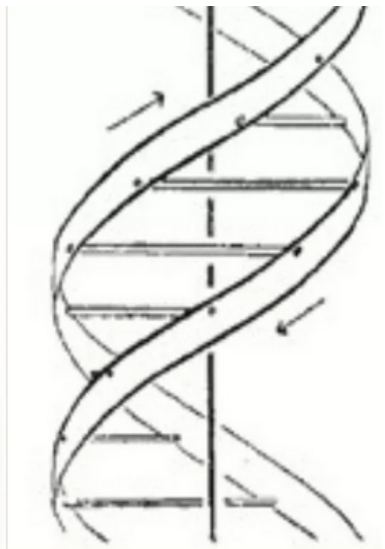
The Structure of DNA: A Brief History Lesson

- In 1953, Watson, Crick, Wilkins, Franklin, and Gosling were the first to describe the structure of DNA
- They discovered the right-handed double helix (canonical B-form DNA), the most common form found in cells









[1] Watson and Crick, 1953

DNA Can Adopt Alternative Structures

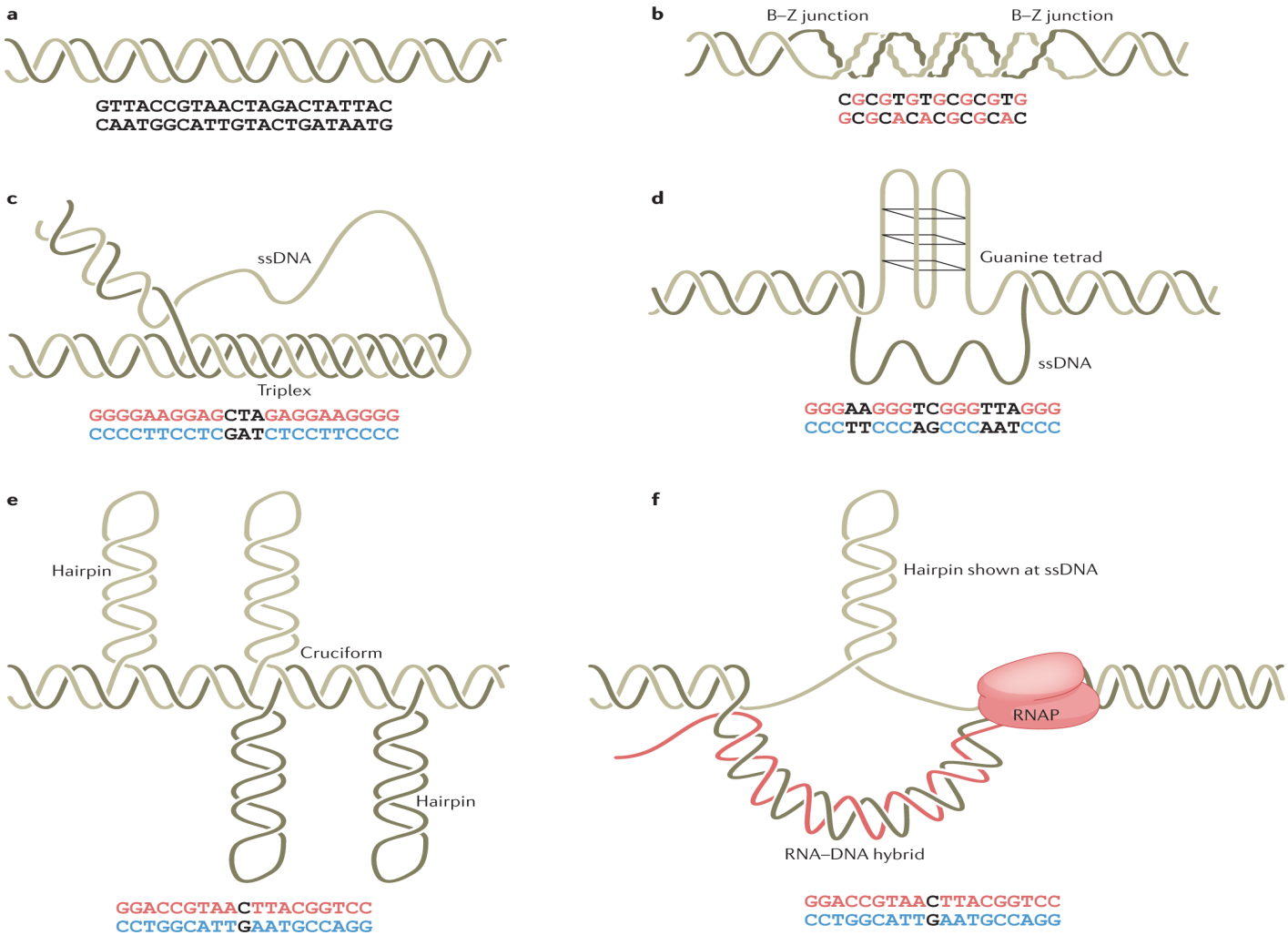
- Now, more than 15 types of DNA structure that differ from the canonical B-form have been reported (non-canonical or non-B form DNA)

- Through sequencing of the human genome, we now know over half the genome is composed of repetitive elements - these were initially thought to be 'junk DNA'

- A crucial feature of some repetitive sequences is their ability to fold into non-canonical DNA structures (non-B DNA)

[2] Wang and Vasquez, 2023

Types of Non-canonical DNA structures



[2] Figure from Wang and Vasquez, 2023

Non-Canonical DNA Structures are Involved in Biological Processes

Non-B DNA structures have been shown to co-localize with functional genomic loci (promoters, enhancers, etc) and genetic instability hotspots

This suggests a role for non-B DNA in vital cellular events such as;

- Regulation of transcription
- Regulation of DNA replication and recombination
- Regulating genome integrity

Diseases Associated with Non-Canonical DNA structures

Repeat Expansion Diseases: Expansions of non-B DNA structure-forming repeats have been implicated in many neurodegenerative and neuromuscular diseases.

Genetic Instability Diseases: Non-canonical DNA structures are associated with increased mutability (point mutations, deletions, insertions and chromosomal translocations)

- Enriched at chromosomal breakpoints in translocation-related cancers such as lymphomas and leukaemias.
- Can be recognized by DNA repair proteins, triggering error-generating repair processes
- G-quadruplexes are present within most human oncogenic promoters and at telomeres - a current theraputic target to downregulate transcription or block telomere elongation in cancer cells.

How are Non-B Structures Detected in the Genome?

Computational Approaches

- Sequence based computer algorithms
- Deep learning approaches
- Molecular dynamics simulations

Wet-lab Approaches

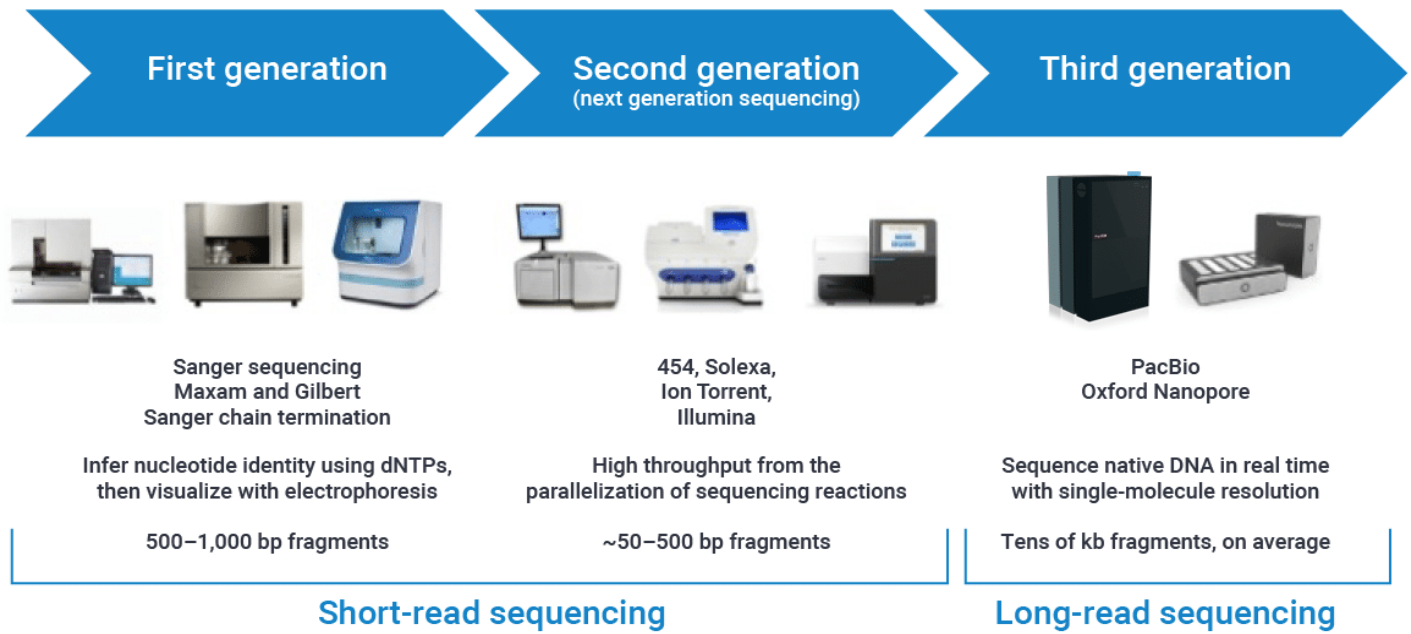
- circular dichroism spectra analysis
- Polymerase stop assays
- Immunoflourescence studies

These approaches are based primarily on DNA sequence motifs, which are necessary, but insufficient for formation and are not available for all non-B DNA structures

Third Generation Sequencing: A Promising New Approach

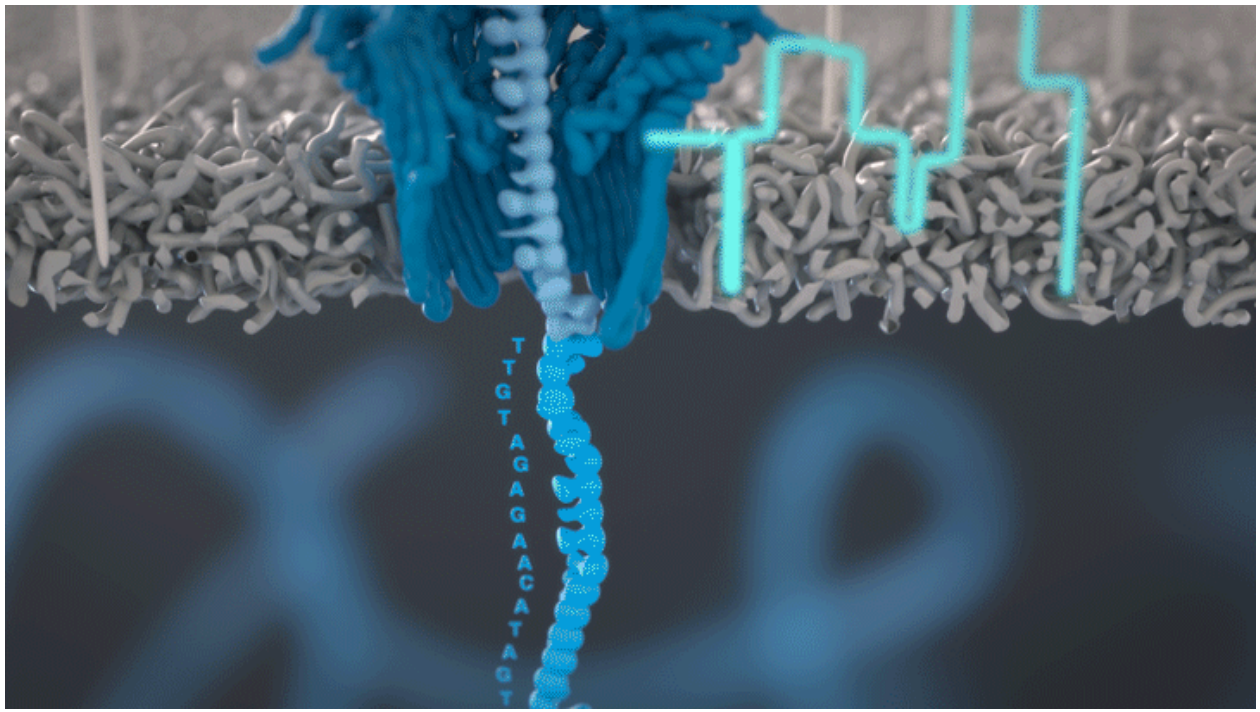
Single Molecule, Real Time Sequencing (SMRT): Pacbio's third generation sequencing machine

- Emits a fluorescent pulse when nucleotide is detected - the time interval between two pulses is called the interpulse duration (IPD)
- Guiblet et al (2018), showed that there is a significant divergence between IPDs in non-B DNA motif regions compared to B-DNA regions



Oxford Nanopore Sequencing Technology

ONT Sequencer



Inside the Nanopore

Predicting Non-B Structures From Nanopore Sequencing

A recently published paper utilized translocation times from ONT sequencing to predict non-B DNA structures (citation)

- Developed the first computational pipeline and a novel unsupervised deep statistical model for predicting non-B DNA structures

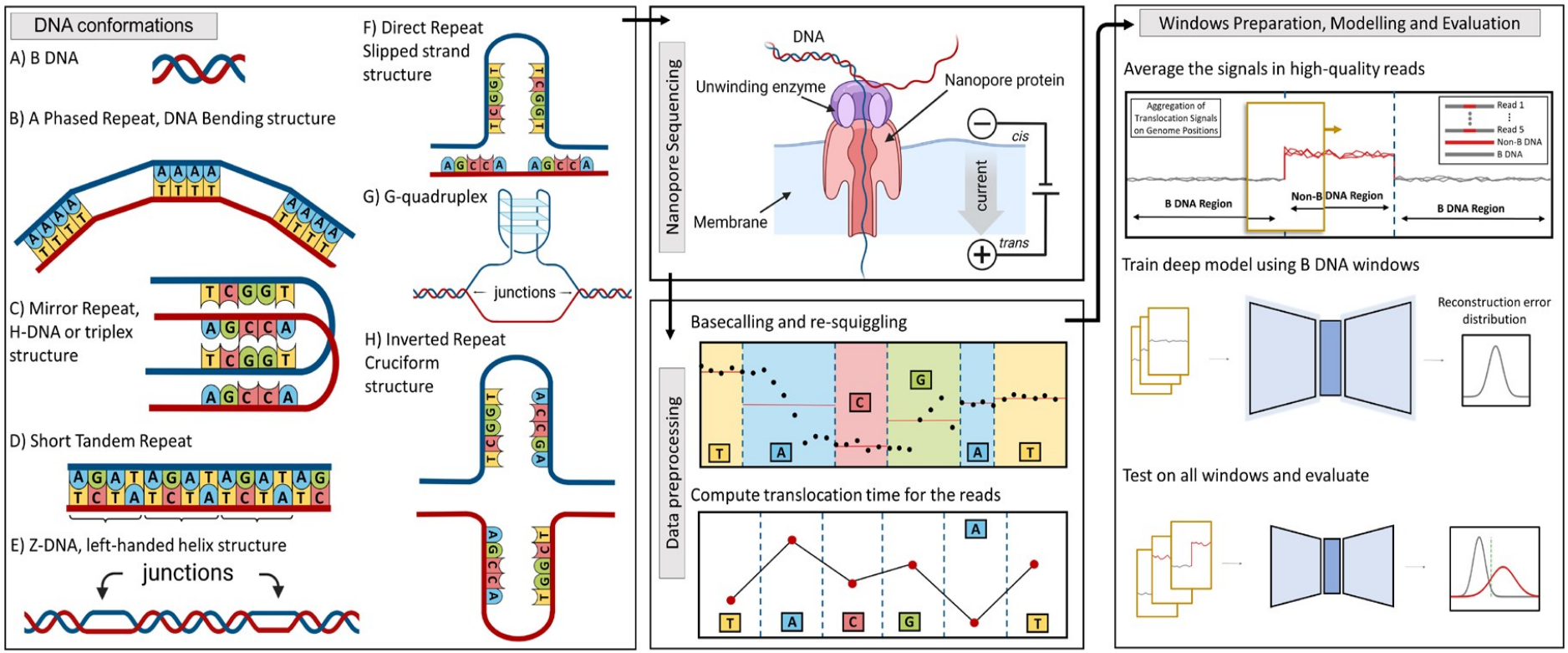
Benefits of unsupervised approach;

1. non-B database labels are noisy (just because motif is present does not mean structure is)
2. Even if high quality labeling for non-B DNA were available, substantially more B-DNA samples are available
3. Unknown non-B structures or non-B DNA without sequence motifs cannot be modelled by a supervised approach

[3] Hosseini et al., 2023

GoFAE-DND: Deep Statistical modelling of non-B DNA

Anomaly Detection Problem: Identifying patterns within data that deviate significantly from the norm or expected behaviour of the majority of the data



[3] Hosseini et al., 2023

Model Performance

At an FDR control level $\alpha = 0.2$, SVM and GoFAE-DND generated the most novelties, with GoFAE-DND yielding the most predictions for all non-B types besides G4

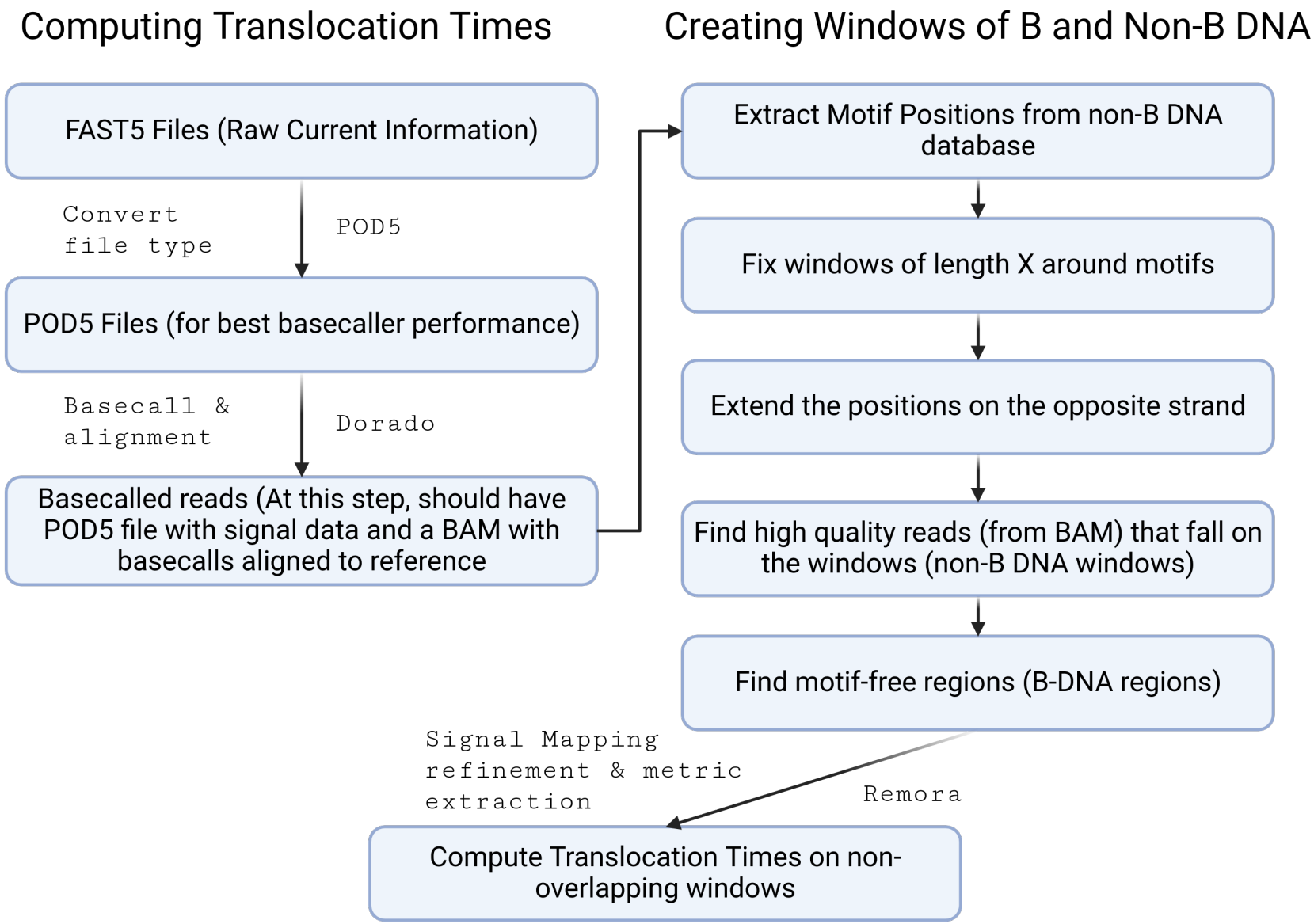
Datasets	Isolation Forest	Local Outlier Factor	One Class SVM	GoFAE-DND
A Phased Repeat	0 (0.00%)	0 (0.00%)	0 (0.00%)	5,137 (8.45%)
G-Quadruplex	3,003 (9.24%)	3 (0.00%)	12,364 (38.04%)	11,334 (34.87%)
Inverted Repeat	3 (0.00%)	0 (0.00%)	33,669 (4.26%)	41,950 (5.31%)
Mirror Repeat	0 (0.00%)	0 (0.00%)	0 (0.00%)	7 (0.01%)
Direct Repeat	0 (0.00%)	0 (0.00%)	0 (0.00%)	66 (0.16%)
Short Tandem Repeat	1 (0.00%)	143 (0.06%)	44,212 (18.65%)	112,631 (47.51%)
Z-DNA	0 (0.00%)	0 (0.00%)	0 (0.00%)	253 (1.86%)

The Objective

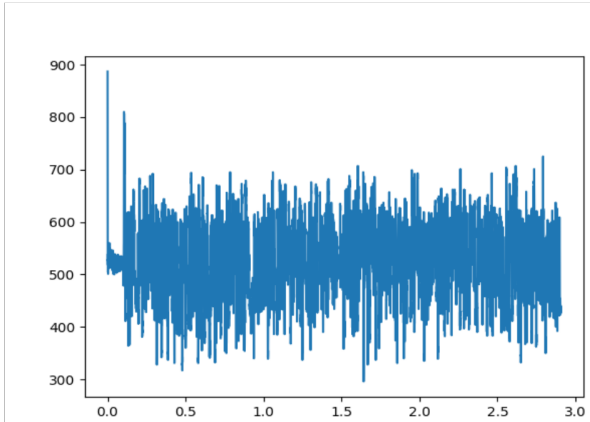
Given the dramatic increase in genome-scale data produced using ONT platforms, and the relevance of non-B structures in human cancers;

1. Utilize the model to analyze nanopore samples from the human pangenome reference consortium (HPRC)
2. Eventually improve the model, with an emphasis on the detection of G4 quadruplexes - Linking methylation, gene expression profiles which are available for HPRC samples

Preprocessing Workflow

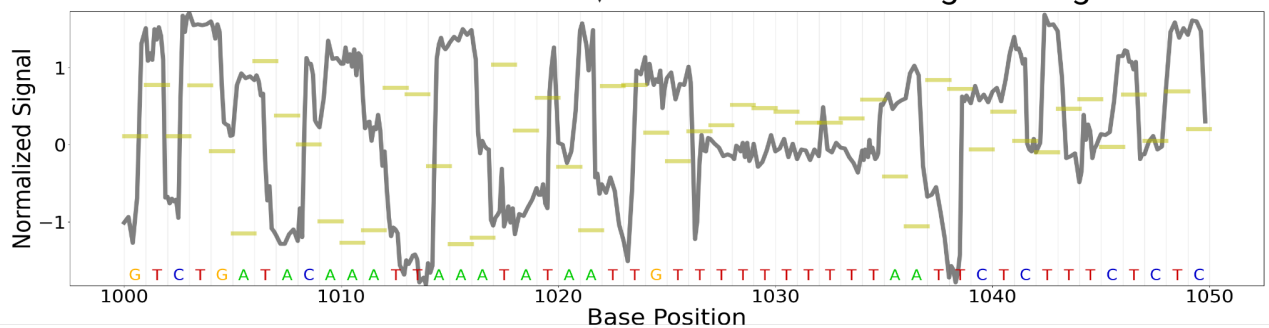


Preprocessing Visualization



Plotting raw signal data against time for a single read

After basecalling and alignment



After signal mapping refinement