NanoBaseLib: A Multi-Task Benchmark Dataset for Nanopore Sequencing *Supplementary Material*

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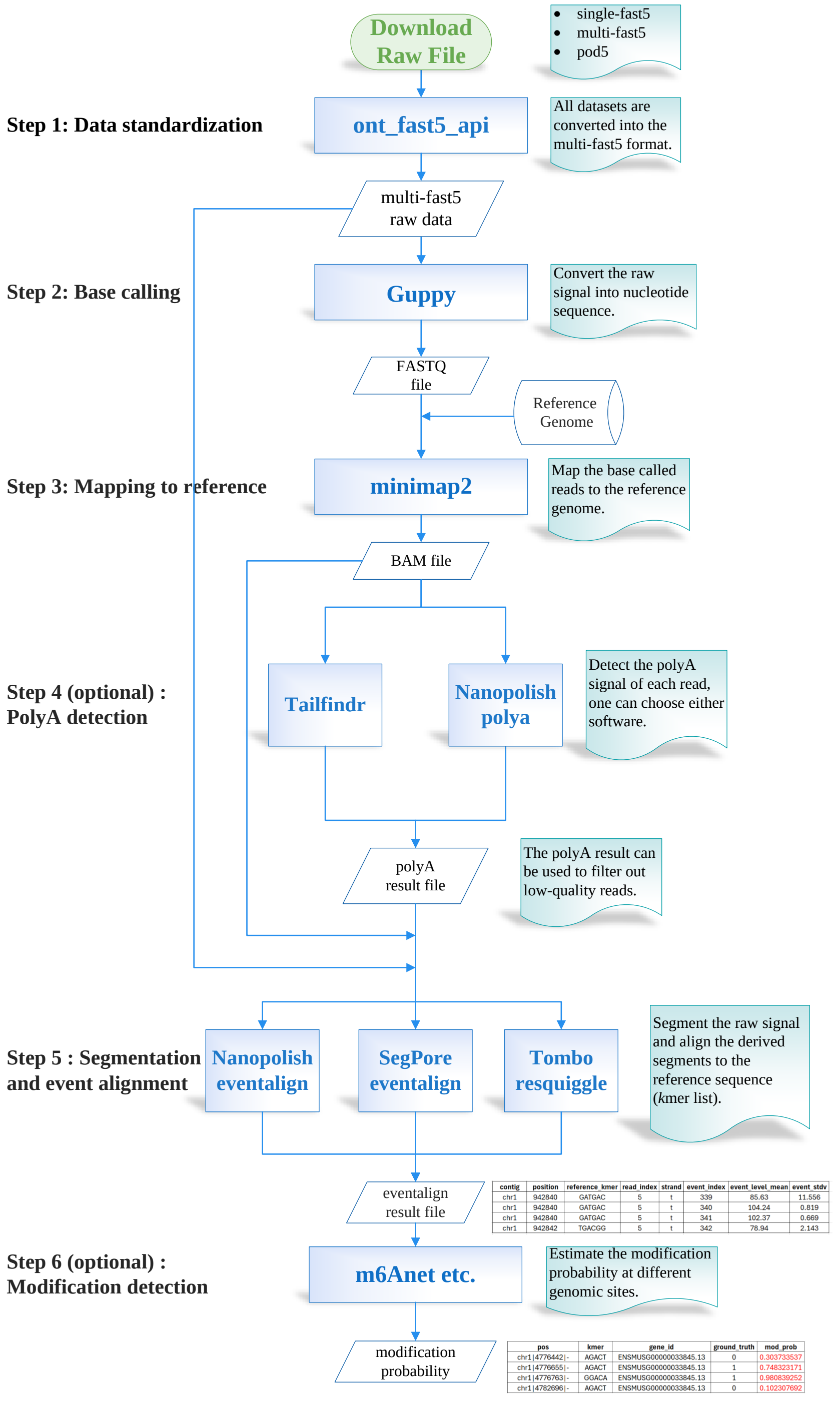
1 Dataset Checklist

1. Submission introducing new datasets must include the following in the supplementary materials:
   (a) Dataset documentation and intended uses. Recommended documentation frameworks include datasheets for datasets, dataset nutrition labels, data statements for NLP, and accountability frameworks. A: Available on the dataset website (https://nanobaselib.github.io/dataset.html).
   (b) URL to website/platform where the dataset/benchmark can be viewed and downloaded by the reviewers. A: The raw data download instruction: https://nanobaselib.github.io/raw.html. The benchmarks and processed datasets are available at https://doi.org/10.5281/zenodo.10889896.
   (c) URL to Croissant metadata record documenting the dataset/benchmark available for viewing and downloading by the reviewers. You can create your Croissant metadata using e.g. the Python library available here: https://github.com/mlcommons/croissant A: NA.
   (d) Author statement that they bear all responsibility in case of violation of rights, etc., and confirmation of the data license. A: Yes.
   (e) Hosting, licensing, and maintenance plan. The choice of hosting platform is yours, as long as you ensure access to the data (possibly through a curated interface) and will provide the necessary maintenance. A: The dataset is hosted on Zenodo. The website is hosted on GitHub, where it will be maintained and regularly updated.
2. To ensure accessibility, the supplementary materials for datasets must include the following:
   (a) Links to access the dataset and its metadata. This can be hidden upon submission if the dataset is not yet publicly available but must be added in the camera-ready version. In select cases, e.g when the data can only be released at a later date, this can be added afterward. Simulation environments should link to (open source) code repositories. A: https://nanobaselib.github.io or https://doi.org/10.5281/zenodo.10889896.
   (b) The dataset itself should ideally use an open and widely used data format. Provide a detailed explanation on how the dataset can be read. For simulation environments, use existing frameworks or explain how they can be used. A: Available on the dataset website.
   (c) Long-term preservation: It must be clear that the dataset will be available for a long time, either by uploading to a data repository or by explaining how the authors themselves will ensure this. A: Zenodo is a long-time storage open repository.
   (d) Explicit license: Authors must choose a license, ideally a CC license for datasets, or an open source license for code (e.g. RL environments). A: The processed dataset is licensed under CC BY license.
   (e) Add structured metadata to a dataset's meta-data page using Web standards (like schema.org and DCAT): This allows it to be discovered and organized by anyone. If you use an existing data repository, this is often done automatically. A: The dataset structure is available on the dataset website (https://nanobaselib.github.io/dataset.html).
   (f) Highly recommended: a persistent dereferenceable identifier (e.g. a DOI minted by a data repository or a prefix on identifiers.org) for datasets, or a code repository (e.g. GitHub, GitLab,...) for code. If this is not possible or useful, please explain why. A: DOI: 10.5281/zenodo.10889896.
3. For benchmarks, the supplementary materials must ensure that all results are easily reproducible. Where possible, use a reproducibility framework such as the ML reproducibility checklist, or otherwise guarantee that all results can be easily reproduced, i.e. all necessary datasets, code, and evaluation procedures must be accessible and documented. A: The code for the benchmarks is available at GitHub (https://github.com/nanobaselib/NanoBaseLib).
4. For papers introducing best practices in creating or curating datasets and benchmarks, the above supplementary materials are not required. A: NA.

2 Appendix Figures

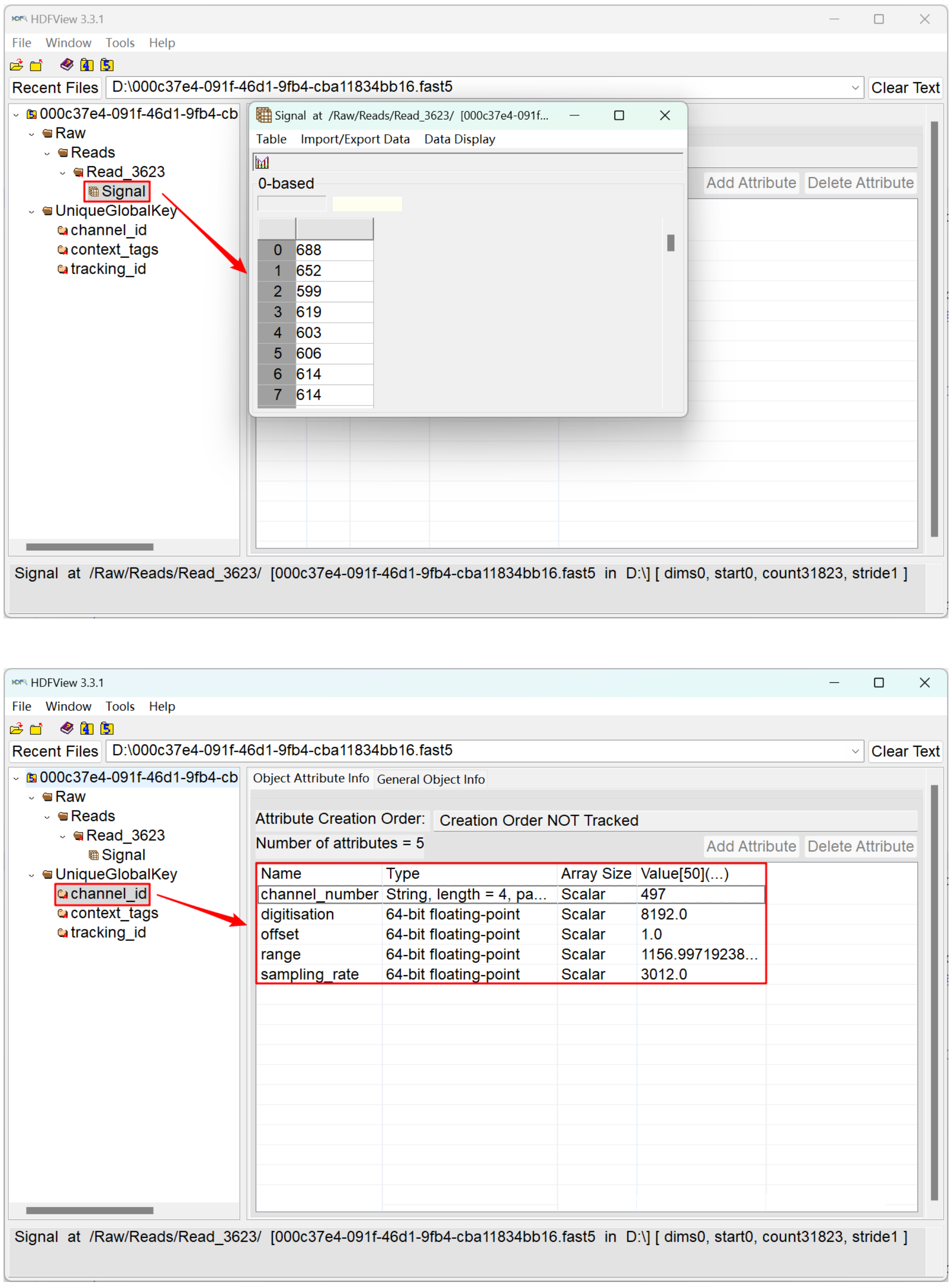


Download Raw File
single-fast5
multi-fast5
pod5
Step 1: Data standardization
ont_fast5_api
All datasets are converted into the multi-fast5 format.
multi-fast5 raw data
Step 2: Base calling
Guppy
Convert the raw signal into nucleotide sequence.
FASTQ file
Reference Genome
Step 3: Mapping to reference
minimap2
Map the base called reads to the reference genome.
BAM file
Step 4 (optional) : PolyA detection
Tailfindr
Nanopolish polya
Detect the polyA signal of each read, one can choose either software.
polyA result file
The polyA result can be used to filter out low-quality reads.
Step 5 : Segmentation and event alignment
Nanopolish eventalign
SegPore eventalign
Tombo resquiggle
Segment the raw signal and align the derived segments to the reference sequence (kmer list).
eventalign result file
Step 6 (optional) : Modification detection
m6Anet etc.
Estimate the modification probability at different genomic sites.
modification probability

contig	position	reference_kmer	read_index	strand	event_index	event_level_mean	event_stdv
chr1	942840	GATGAC	5	t	339	85.63	11.556
chr1	942840	GATGAC	5	t	340	104.24	0.819
chr1	942840	GATGAC	5	t	341	102.37	0.669
chr1	942842	TGACGG	5	t	342	78.94	2.143

pos	kmer	gene_id	ground_truth	mod_prob
chr1|4776442|-	AGACT	ENSMUSG00000033845.13	0	0.303733537
chr1|4776655|-	AGACT	ENSMUSG00000033845.13	1	0.748323171
chr1|4776763|-	GGACA	ENSMUSG00000033845.13	1	0.980839252
chr1|4782696|-	AGACT	ENSMUSG00000033845.13	0	0.102307692

Figure 1: NanoBaseLib dataset processing workflow.


HDFView 3.3.1
File Window Tools Help
Recent Files
Clear Text
Table Import/Export Data Data Display
Add Attribute Delete Attribute
Object Attribute Info General Object Info

D:\000c37e4-091f-46d1-9fb4-cba11834bb16.fast5

```
000c37e4-091f-46d1-9fb4-cb
  Raw
    Reads
      Read_3623
        Signal
  UniqueGlobalKey
    channel_id
    context_tags
    tracking_id
```

Signal at /Raw/Reads/Read_3623/ [000c37e4-091f...

0-based

0	688
1	652
2	599
3	619
4	603
5	606
6	614
7	614

Signal at /Raw/Reads/Read_3623/ [000c37e4-091f-46d1-9fb4-cba11834bb16.fast5 in D:\] [dims0, start0, count31823, stride1]

Attribute Creation Order: Creation Order NOT Tracked

Number of attributes = 5

Name	Type	Array Size	Value[50](...)
channel_number	String, length = 4, pa...	Scalar	497
digitisation	64-bit floating-point	Scalar	8192.0
offset	64-bit floating-point	Scalar	1.0
range	64-bit floating-point	Scalar	1156.99719238...
sampling_rate	64-bit floating-point	Scalar	3012.0

Signal at /Raw/Reads/Read_3623/ [000c37e4-091f-46d1-9fb4-cba11834bb16.fast5 in D:\] [dims0, start0, count31823, stride1]

Figure 2: Illustration of the single fast5 file. The top panel displays the raw signal, while the bottom panel presents some meta information.

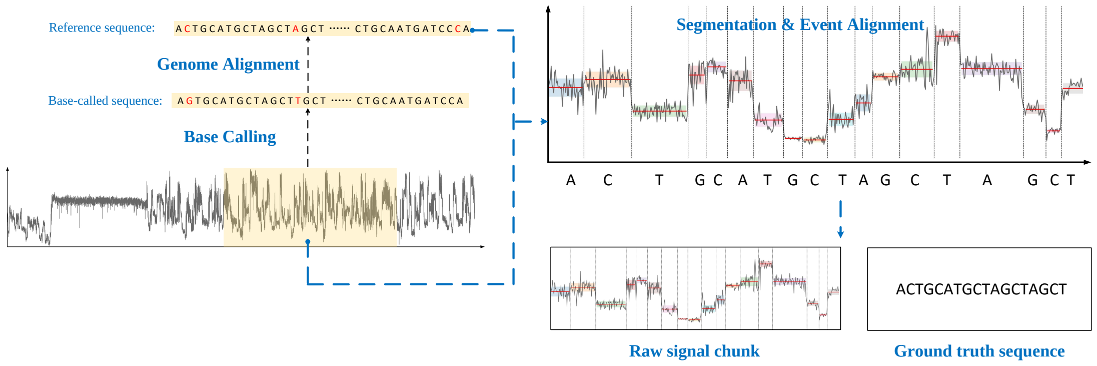

Reference sequence:
ACTGCATGCTAGCTAGCT ······ CTGCAATGATCCCA
Genome Alignment
Base-called sequence:
AGTGCATGCTAGCTTGCT ······ CTGCAATGATCCA
Base Calling
Segmentation & Event Alignment
A C T G C A T G C T A G C T A G C T
ACTGCATGCTAGCTAGCT
Raw signal chunk
Ground truth sequence

Figure 3: Illustration of Base Calling (BC) task ground truth acquisition. We perform base calling (using Guppy v6.0.1) firstly, then align the base-called sequence with the reference genome and run Nanopolish "eventalign". Finally, we extract the matched raw signal segments and reference sequence fragments as the ground truth.

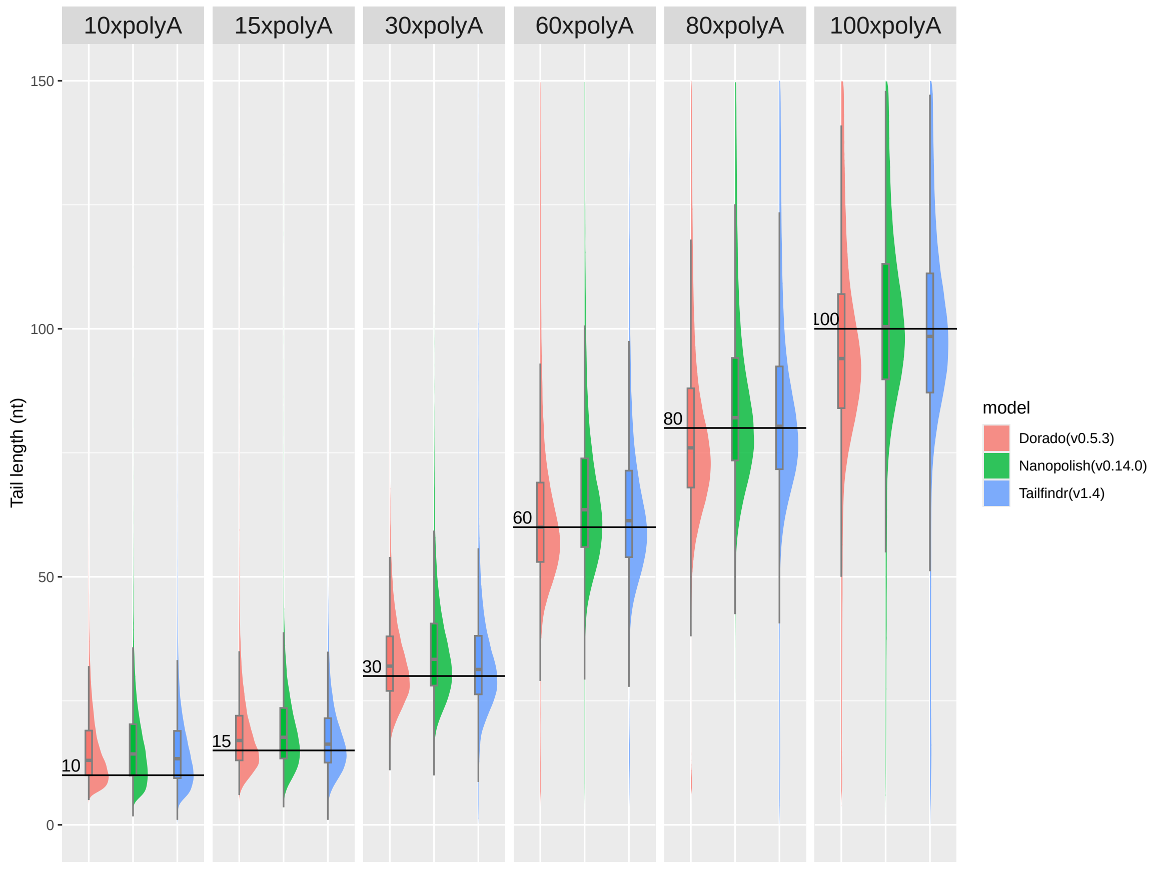
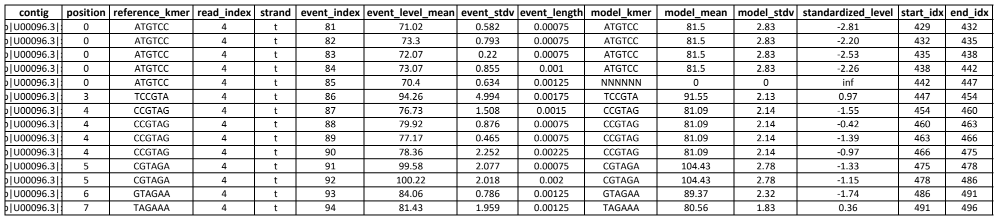


Figure 4: PolyA tail length distribution on test dataset ont_polya_standard. The black line and the numbers (10, 15, 30, 60, 80, 100) represent the ground truth. The results are based on Nanopolish (v0.14), Tailfindr (v1.4), and Dorado (v0.5.3).



contig	position	reference_kmer	read_index	strand	event_index	event_level_mean	event_stdv	event_length	model_kmer	model_mean	model_stdv	standardized_level	start_idx	end_idx
b|U00096.3|	0	ATGTCC	4	t	81	71.02	0.582	0.00075	ATGTCC	81.5	2.83	-2.81	429	432
b|U00096.3|	0	ATGTCC	4	t	82	73.3	0.793	0.00075	ATGTCC	81.5	2.83	-2.20	432	435
b|U00096.3|	0	ATGTCC	4	t	83	72.07	0.22	0.00075	ATGTCC	81.5	2.83	-2.53	435	438
b|U00096.3|	0	ATGTCC	4	t	84	73.07	0.855	0.001	ATGTCC	81.5	2.83	-2.26	438	442
b|U00096.3|	0	ATGTCC	4	t	85	70.4	0.634	0.00125	NNNNNN	0	0	inf	442	447
b|U00096.3|	3	TCCGTA	4	t	86	94.26	4.994	0.00175	TCCGTA	91.55	2.13	0.97	447	454
b|U00096.3|	4	CCGTAG	4	t	87	76.73	1.508	0.0015	CCGTAG	81.09	2.14	-1.55	454	460
b|U00096.3|	4	CCGTAG	4	t	88	79.92	0.876	0.00075	CCGTAG	81.09	2.14	-0.42	460	463
b|U00096.3|	4	CCGTAG	4	t	89	77.17	0.465	0.00075	CCGTAG	81.09	2.14	-1.39	463	466
b|U00096.3|	4	CCGTAG	4	t	90	78.36	2.252	0.00225	CCGTAG	81.09	2.14	-0.97	466	475
b|U00096.3|	5	CGTAGA	4	t	91	99.58	2.077	0.00075	CGTAGA	104.43	2.78	-1.33	475	478
b|U00096.3|	5	CGTAGA	4	t	92	100.22	2.018	0.002	CGTAGA	104.43	2.78	-1.15	478	486
b|U00096.3|	6	GTAGAA	4	t	93	84.06	0.786	0.00125	GTAGAA	89.37	2.32	-1.74	486	491
b|U00096.3|	7	TAGAAA	4	t	94	81.43	1.959	0.00125	TAGAAA	80.56	1.83	0.36	491	496

Figure 5: Illustration of Nanopolish “eventalign” output

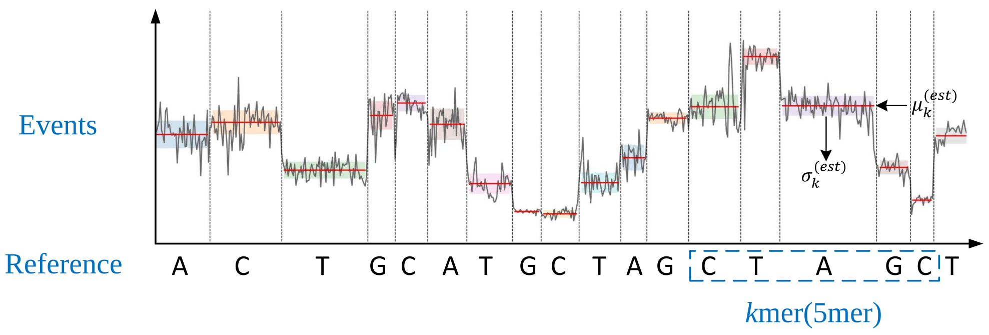


Figure 6: Illustration of segmentation and event alignment.

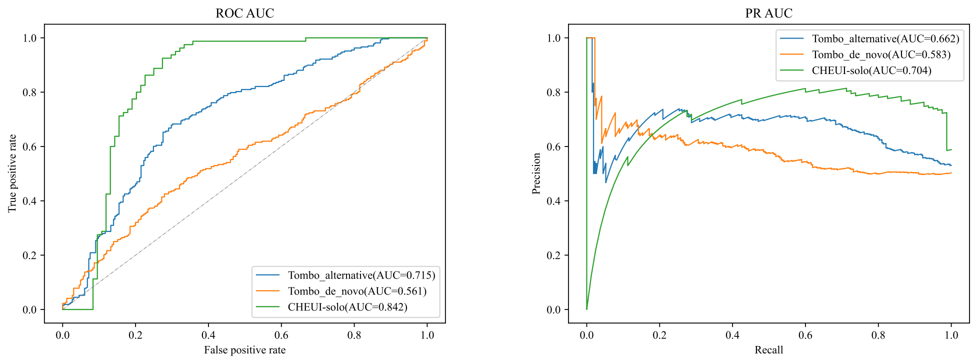


Figure 7: m5C modification detection benchmark on test dataset ecoli_eligos (IVT_m5C and IVT_normalC).

3 Appendix Tables

Table 1: NanoBaseLib dataset comprehensive information.

Dataset	Accession	Sample	Kit	Flowcell
ont_polya_standard	PRJEB28423	10xpolyA	rna001	flo-min106
		15xpolyA	rna001	flo-min106
		30xpolyA	rna001	flo-min106
		60xpolyA	rna001	flo-min106
		80xpolyA	rna001	flo-min106
		100xpolyA	rna001	flo-min106
eGFP_polyA_DNA	PRJEB31806	dna_rep1_sqklsk108_flipflop	lsk108	flo-min106
		dna_rep2_sqklsk109_flipflop	lsk109	flo-min106
eGFP_polyA_RNA	PRJEB31806	rna_rep1_sqkrna001_plus_rt	rna001	flo-min106
		rna_rep2_sqkrna001_plus_rt	rna001	flo-min106
		rna_rep3_sqkrna002_minus_rt	rna002	flo-min106
lambda_phage	PRJNA926802	VER5940	lsk109	flo-flg001
NA12878	PRJEB23027	FAB42828	lsk108	flo-min106
		FAF04090	lsk108	flo-min106
		FAF09968	lsk108	flo-min106
curlcake	PRJNA511582	m6A-mod-rep1	rna001	flo-min106
		m6A-mod-rep2	rna001	flo-min106
		non-mod-rep1	rna001	flo-min106
		non-mod-rep2	rna001	flo-min106
scBY4741_m5C	PRJNA563591	m5C_modified	rna001	flo-min106
scBY4741_hm5C	PRJNA548268	hm5C_modified	rna001	flo-min106
scBY4741_pU	PRJNA549001	pU_modified	rna001	flo-min106
hct116	PRJEB44348	HCT-WT-rep1	rna002	flo-min106
		HCT-WT-rep2	rna002	flo-min106
		HCT-WT-rep3	rna002	flo-min106
hek293t_wt	PRJEB40872	HEK293T-WT-rep1	rna001	flo-min106
		HEK293T-WT-rep2	rna002	flo-min106
		HEK293T-WT-rep3	rna002	flo-min106
hek293t_ko	PRJEB40872	HEK293T-WT-rep1	rna001	flo-min106
		HEK293T-WT-rep2	rna002	flo-min106
		HEK293T-WT-rep3	rna002	flo-min106
mESCs_eligos	PRJNA497103	mESCs_Mettl3_WT	rna002	flo-min106
		mESCs_Mettl3_KO	rna002	flo-min106
ecoli_eligos	PRJNA497103	IVT_Inosine	rna002	flo-min106
		IVT_m5C	rna002	flo-min106
		IVT_m6A	rna002	flo-min106
		IVT_normalA	rna002	flo-min106
		IVT_normalC	rna002	flo-min106
dinopore_ivt	SRP363295	gBlock_pureI	rna001	flo-min106
		gBlock_G	rna001	flo-min106
dinopore_xenopus	SRP363295	rep3_stage1_20200812	rna002	flo-min106
		rep3_stage1_20201005	rna002	flo-min106
		rep3_stage9_20200812	rna002	flo-min106
		rep3_stage9_20201008	rna002	flo-min106

Table 2: NanoBaseLib dataset statistics. Avg. L_{signal} represents the average raw current signal length. Avg. L_{base} represents the average base sequence length, which are from Guppy 6.0.1.

Dataset	**Type**	**Sample**	**#Reads**	**Avg.** L_{signal}	**Avg.** L_{base}
ont_polya_standard	RNA	10xpolyA	92,428	59001.85	1207.22
		15xpolyA	91,084	56518.49	1216.28
		30xpolyA	63,886	54111.54	1192.65
		60xpolyA	108,314	57397.07	1172.57
		80xpolyA	409,634	47166.28	859.32
		100xpolyA	279,895	61938.01	1173.39
eGFP_polyA_DNA	cDNA	dna_rep1_sqklsk108_flipflop	484,000	8956.69	763.46
		dna_rep2_sqklsk109_flipflop	280,428	21619.23	1667.14
eGFP_polyA_RNA	RNA	rna_rep1_sqkrna001_plus_rt	922,826	57068.67	1126.53
		rna_rep2_sqkrna001_plus_rt	1,452,042	50103.37	928.41
		rna_rep3_sqkrna002_minus_rt	592,571	30888.61	465.02
lambda_phage	DNA	VER5940	113,514	116272.62	9561.99
NA12878	DNA	FAB42828	33,633	131148.91	6810.35
		FAF04090	62,833	509826.89	17801.22
		FAF09968	21,947	334920.97	53615.01
curlcake	RNA	m6A-mod-rep1	134,374	69745.77	850.16
		m6A-mod-rep2	638,860	58341.88	835.01
		non-mod-rep1	66,736	57930.60	866.98
		non-mod-rep2	846,595	61719.51	1066.53
scBY4741_m5C	RNA	m5C_modified	415,453	40792.42	539.89
scBY4741_hm5C	RNA	hm5C_modified	111,015	81528.20	1022.88
scBY4741_pU	RNA	pU_modified	42,386	46652.89	475.18
hct116	RNA	HCT-WT-rep1	987,488	66363.12	1217.43
		HCT-WT-rep2	1,015,893	57524.51	1023.03
		HCT-WT-rep3	1,673,394	65628.29	1153.23
hek293t_wt	RNA	HEK293T-WT-rep1	1,040,661	60169.77	939.80
		HEK293T-WT-rep2	1,396,000	54077.71	1077.61
		HEK293T-WT-rep3	513,561	56785.55	1005.06
hek293t_ko	RNA	HEK293T-WT-rep1	1,490,210	58140.70	952.63
		HEK293T-WT-rep2	1,815,589	52569.78	993.85
		HEK293T-WT-rep3	1,677,075	50185.96	970.32
mESCs_eligos	RNA	mESCs_Mettl3_WT	3,163,286	33202.35	526.23
		mESCs_Mettl3_KO	1,527,561	28350.74	437.70
ecoli_eligos	RNA	IVT_Inosine	811,953	32978.04	845.43
		IVT_m5C	573,674	45397.06	719.52
		IVT_m6A	1,482,437	41642.13	708.29
		IVT_normalA	383,209	33499.75	620.83
		IVT_normalC	452,806	44566.76	731.75
dinopore_ivt	RNA	gBlock_pureI	165,628	29869.74	450.32
		gBlock_G	150,405	32047.08	641.17
dinopore_xenopus	RNA	rep3_stage1_20200812	1,451,289	46688.45	917.23
		rep3_stage1_20201005	1,812,200	27213.72	532.63
		rep3_stage9_20200812	1,560,032	44621.79	894.37
		rep3_stage9_20201008	1,251,130	31185.45	448.15

Table 3: NanoBaseLib task overview.

Task	Input	Output	Category	Typical Model
Base calling (BC)	Raw current signal sequence	Nucleotide sequence	Supervised Learning, Generative Model	CNN + LSTM + CTC-CRF [1] UNet + GRU + CE [2] CNN + Transformer + CTC [3] ResNet CNN + CTC [4]
PolyA detection (PD)	Raw current signal sequence	PolyA tail length and borders	Unsupervised or Supervised Learning, Predictive Model	Hidden Markov model[5, 6]
Segmentation and event alignment (SA)	Raw signal and reference sequence	Event alignment results	Unsupervised Learning, Predictive Model	Hidden Markov model [7, 8]
Modification detection (MD)	Event alignment results	Modification probability for each site & read	Supervised Learning, Predictive Model, Multiple Instance Learning	SVM [9] CNN [10]

Table 4: Data preprocessing software overview and limitations.

Software	Version	Link	Limitation
bedtools	2.30.3	`https://bedtools.readthedocs.io/en/latest/`	NA
Bonito	0.7.3	`https://github.com/nanoporetech/bonito`	NA
causalcall	NA	`https://github.com/scutbioinformatic/causalcall`	Only for DNA
CHEUI	NA	`https://github.com/comprna/CHEUI`	m6A and m5C
Dorado	0.5.3	`https://community.nanoporetech.com/downloads`	NA
Epinano	1.2.0	`https://github.com/novoalab/EpiNano`	NA
Guppy	6.0.1	`https://community.nanoporetech.com/downloads`	NA
h5py	1.8.18	`https://www.h5py.org/`	NA
minimap2	2.24	`https://github.com/lh3/minimap2`	NA
MINES	NA	`https://github.com/YeoLab/MINES`	Only for m6A
m6Anet	1.0	`https://github.com/GoekeLab/m6anet`	Only for m6A
Nanopolish	0.14.0	`https://github.com/jts/nanopolish`	NA
Nanom6A	2.0	`https://github.com/gaoyubang/nanom6A`	Only for m6A
ont-fast5-api	4.0.2	`https://pod5-file-format.readthedocs.io`	NA
Rodan	NA	`https://github.com/biodlab/RODAN`	Only for RNA
SegPore	1.0	`https://github.com/guangzhaocs/SegPore`	Only for RNA
Tailfindr	1.4	`https://github.com/adnaniazi/tailfindr`	NA
Tombo	1.5.1	`https://nanoporetech.github.io/tombo`	NA

Table 5: ONT basecaller configuration parameters

Basecaller	Version	Sample	Configure
Guppy	2.3.1	DNA	`dna_r9.4.1_450bps.cfg`
Guppy	2.3.1	RNA	`rna_r9.4.1_70bps.cfg`
Guppy	4.5.4	DNA	`dna_r9.4.1_450bps_hac.cfg`
Guppy	4.5.4	RNA	`rna_r9.4.1_70bps_hac.cfg`
Guppy	6.0.1	DNA	`dna_r9.4.1_450bps_hac.cfg`
Guppy	6.0.1	RNA	`rna_r9.4.1_70bps_hac.cfg`
Bonito	0.7.3	DNA	`dna_r9.4.1_e8_hac@v3.3`
Dorado	0.5.3	DNA	`dna_r9.4.1_e8_hac@v3.3`
Dorado	0.7.0	DNA	`dna_r9.4.1_e8_hac@v3.3`
Dorado	0.5.3	RNA	`rna002_70bps_hac@v3`
Dorado	0.7.0	RNA	`rna002_70bps_hac@v3`

4 Appendix Methods

4.1 Pros and Cons of Nanopore Sequencing

Pros. Compared with Illumina sequencing, Nanopore sequencing has three advantages. First, it sequences long reads (10-100 kb), where a "read" refers to a measured DNA/RNA sequence composed of the nucleotide bases adenine (A), cytosine (C), guanine (G), thymine (T) or uracil (U). The read length of Illumina sequencing is less than 300 bp, which is $\sim$1% of a long read. Long reads greatly facilitate genome assembly by reducing ambiguities in the genome backbone, especially in repetitive genomic regions. Second, the sequencing library preparation is generally simpler than second-generation sequencing, which makes it ideal for pathogen surveillance in wild environments. Third, the current signals directly carry chemical information on DNA and RNA molecules, e.g., DNA/RNA modifications, which makes it perfect for epi-genetic applications, such as epi-genetic disease studies [11], RNA structures [12], and mRNA vaccine development [13].

Cons. The most significant disadvantage of Nanopore sequencing compared with Illumina sequencing is its low accuracy in base calling. The average base calling accuracy of Nanopore RNA sequencing is around 90% [14], while the accuracy is 99.9% for Illumina cDNA sequencing [15]. This makes Nanopore sequencing not ideal for many classic biological applications, e.g., detection of single nucleotide variants (SNVs). The costs of Nanopore sequencing are higher than Illumina sequencing, while the throughput is lower. Also, current methods could not provide a satisfactory alignment of the raw current signal and the reference sequences, which limits its performance in DNA/RNA modification estimation. In short, there is plenty of room for improvement in various computational tasks of Nanopore sequencing data analysis. We believe these disadvantages could be overcome by developing new machine learning models.

4.2 Raw signal processing and normalization

Appendix Figure 2 illustrates the structure of a single-fast5 file from Nanopore sequencing. In the above panel, the "Signal" data represents the raw current passing through the pore (type: int16). Oxford Nanopore Technology employs different pores (proteins) in various products. A single flow cell in the sequencing device can contain between 512 and 2675 pores, each referred to as a channel. As shown in the below panel, the fast5 file also includes attributes associated with the channel through which the read passes. These parameters include `channel_number` (the channel number from which the read was acquired), `digitisation` (the digitisation is the number of quantisation levels in the Analog to Digital Converter (ADC)), `offset` (the ADC offset error), `range` (the full scale measurement range in pico amperes), and `sampling_rate` (sampling frequency of the ADC).

The raw signal can be converted into pico Ampere (pA) values using attributes available in the `channel_id` group by the equation:

$$signal\_in\_pico\_ampere = \frac{(raw\_signal\_value + offset) \times range}{digitisation}. \tag{1}$$

To improve the accuracy of analyses for various downstream tasks, some tools aim to further standardize or normalize the signal (pA).

Tombo. Tombo uses median shift and MAD (median absolute deviation) scale parameters to normalize the signal [16]:

$$norm\_signal = \frac{signal\_in\_pico\_ampere - median}{MAD}. \tag{2}$$

Nanopolish. For each read, Nanopolish estimates a scale parameter to standardize the signal. You can add the `--scale-events` option in the nanopolish `eventalign` command to enable this feature [5].

SegPore. SegPore first detects the polyA tail and calculates its mean (μ_{polyA}) and standard deviation (σ_{polyA}). These values are then used to standardize the signal [7]:

$$stand\_signal = \frac{signal\_in\_pico\_ampere - \mu_{polyA}}{\sigma_{polyA}} \times \sigma_{stand\_polyA} + \mu_{stand\_polyA} \tag{3}$$

where the μ_{stand_polyA} and σ_{stand_polyA} represent the mean and standard deviation of the kmer "AAAAA" from ONT's standard kmer table [17].

4.3 Segmentation and event alignment

Appendix Figure 5 is an example of the Nanopolish `eventalign` output, which illustrates the segmentation and event alignment results. Each line represents an "event", which contains mapped chromosome/transcript t (`contig`), the location on the chromosome/transcript p (`position`), the corresponding $k$mer s (`reference_kmer`), the estimated mean of this "event" $\mu_s^{(est)}$ (`event_level_mean`), the estimated std of this "event" $\sigma_s^{(est)}$ (`event_stdv`), `model_mean`, `model_stdv`, and other information. Columns `model_mean` and `model_stdv` are from a standard $k$mer parameter table, which is provided by ONT [17]. This $k$mer table contains the standard mean $\mu_s^{(ref)}$ and std $\sigma_s^{(ref)}$ for each $k$mer s. For example, the standard mean is 81.5 and std is 2.83 for $k$mer `ATGTCC` (Row 1$\sim$ 4 in Appendix Figure 5).

Given the eventalign results, we use two metrics to evaluate the performance of the segmentation and event alignment task: (1) the average std $\hat{\sigma}$, and (2) the average log-likelihood $\hat{L}$. Assuming there are N reads in total, and the nth read has K_n events. We denote the kth event of the nth read by $e_{n,k} = \big(t_{n,k}, p_{n,k}, s_{n,k}, \mu_{s_{n,k}}^{(est)}, \sigma_{s_{n,k}}^{(est)}, \mu_{s_{n,k}}^{(ref)}, \sigma_{s_{n,k}}^{(ref)}, \cdots\big)$, where $t_{n,k}$ is the mapped chromosome/transcript, $p_{n,k}$ is the mapped genomic location, $s_{n,k}$ is the corresponding $k$mer. The average std $\hat{\sigma}$ is defined as

$$\hat{\sigma} = \frac{1}{N}\sum_{n=1}^{N}\Big\{\frac{1}{K_n}\sum_{k=1}^{K_n}\sigma_{s_{n,k}}^{(est)}\Big\} \tag{4}$$

and the average log-likelihood $\hat{L}$ is defined as

$$\hat{L} = \frac{1}{N}\sum_{n=1}^{N}\Big\{\frac{1}{K_n}\sum_{k=1}^{K_n}\log\mathcal{N}\Big(\mu_{s_{n,k}}^{(est)}|\mu_{s_{n,k}}^{(ref)}, \sigma_{s_{n,k}}^{(ref)}\Big)\Big\} \tag{5}$$

As shown in Appendix Figure 6, the red line represents the event mean $\mu_k^{(est)}$ and the shaded area represents the std $\sigma_k^{(est)}$ for event k. A poorly segmented raw signal corresponding to an event will exhibit a large standard deviation. Therefore, a smaller $\hat{\sigma}$ indicates lower variations within the raw signal segment, signifying better performance.

If an event is aligned to the correct reference $k$mer, the mean $\mu_{n,k}^{(est)}$ will be close to the reference $\mu_{s_{n,k}}^{(ref)}$ and the log-likelihood $\hat{L}$ will be large. So higher $\hat{L}$ means more similar results to ONT's estimates and better performances.

4.4 Potential negative societal impact

All raw datasets were collected from public resources, and we only preprocessed them using a standard pipeline. However, the datasets could still be exploited for unforeseen purposes, such as commercialization. In which, the anonymized genetic data might be used by companies to develop targeted products, potentially compromising privacy or leading to social inequities, like expensive drugs limited to specific populations. Additionally, the data might be repurposed for unintended research, possibly contradicting the original data providers' intent or causing societal harm.

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