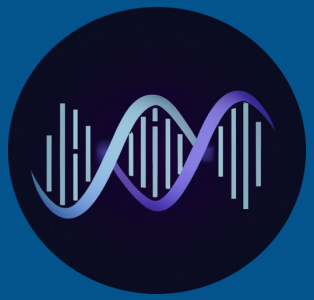
ISMB/ECCB 2023



RawHash

Enabling Fast and Accurate Real-Time Analysis of Raw Nanopore Signals for Large Genomes

Can Firtina

Nika Mansouri Ghiasi Joel Lindegger Gagandeep Singh

Meryem Banu Cavlak Haiyu Mao Onur Mutlu

Paper

Code

SAFARI

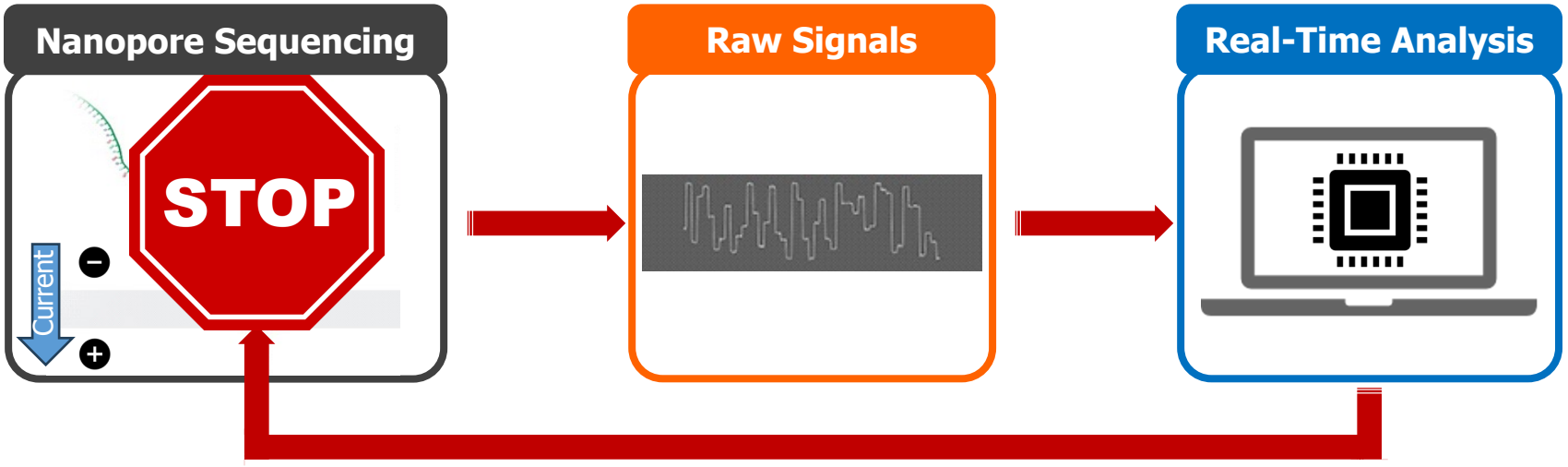
ETH zürich

Nanopore Sequencing

Nanopore Sequencing: a widely used sequencing technology

- Can sequence large fragments of nucleic acid molecules (up to >2Mbp)
- Offers high throughput
- Cost-effective
- Enables **real-time genome analysis**

Real-Time Analysis with Nanopore Sequencing



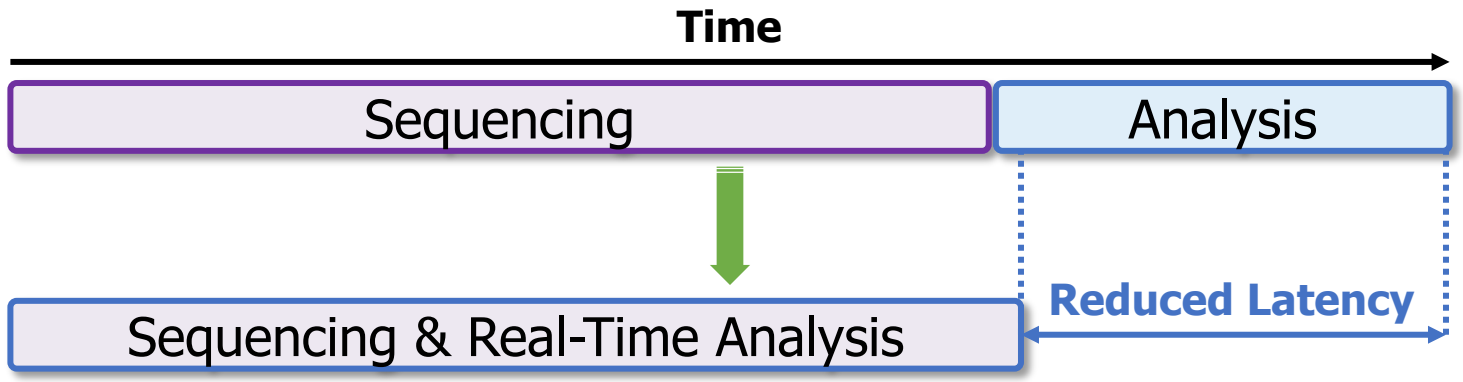
Raw Signals: Ionic current measurements generated at a certain **throughput**

Real-Time Analysis: Analyzing all raw signals by **matching the throughput**

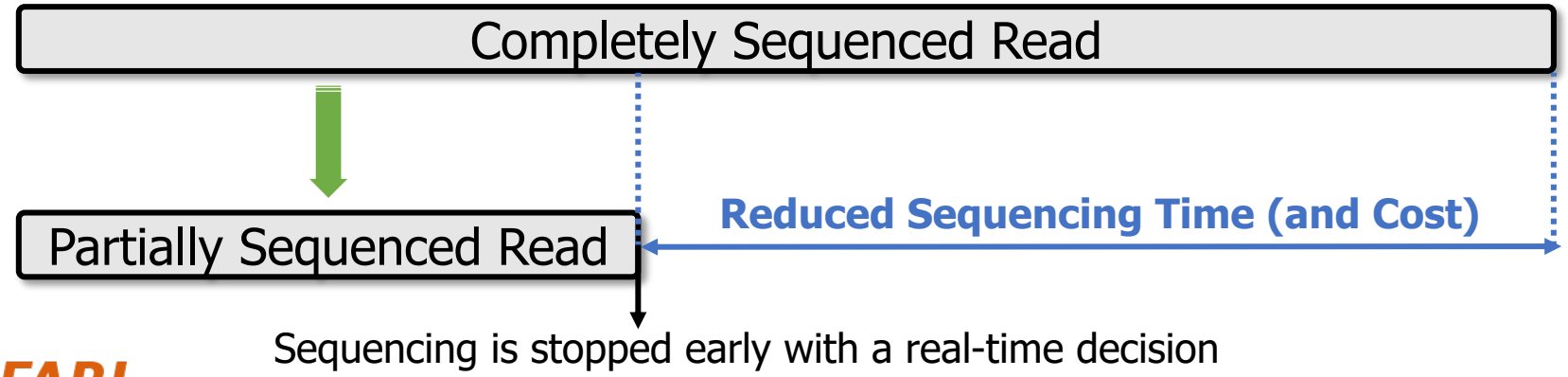
Real-Time Decisions: Stopping sequencing **early** based on real-time analysis

Benefits of Real-Time Genome Analysis

✓ **Reducing latency** by overlapping the sequencing and analysis steps



✓ **Reducing sequencing time and cost** by stopping sequencing early



Challenges in Real-Time Genome Analysis

Rapid analysis to match the nanopore sequencer throughput

Timely decisions to stop sequencing as early as possible

Accurate analysis from noisy raw signal data

Power-efficient computation for scalability and portability

Executive Summary

Problem: Real-time analysis of nanopore raw signals is **inaccurate** and **inefficient for large genomes**

Goal: Enable **fast** and **accurate** real-time analysis of raw signals for **large genomes**

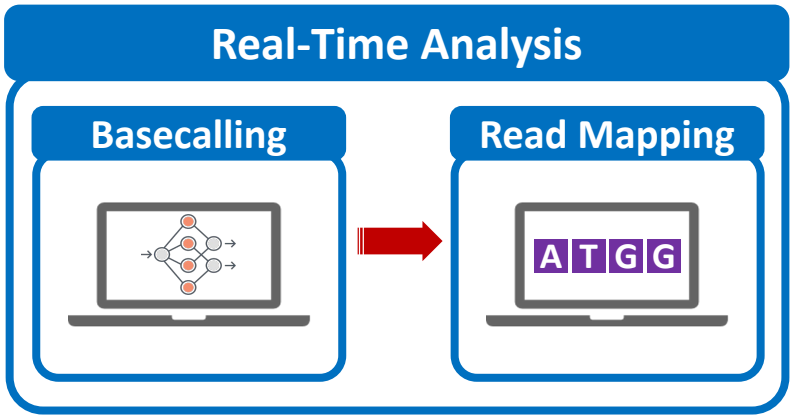
Key Contributions:
1) The **first hash-based mechanism** that can quickly and accurately analyze raw nanopore signals for **large genomes**

2) The novel **Sequence Until** technique can accurately and **dynamically stop the entire sequencing of all reads at once** if further sequencing is not necessary

Key Results: Across 3 use cases and 5 genomes of varying sizes, RawHash provides
- **25.8× and 3.4× better average throughput** compared to two state-of-the-art works
- **1.14× – 2.13× more accurate mapping results** for **large genomes**
- Sequence Until **reduces the sequencing time and cost by 15×**

Existing Solutions

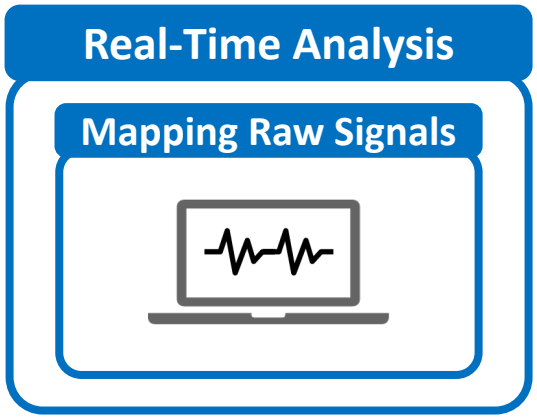
1. Deep neural networks (**DNNs**) for translating **signals** to **bases**



Less noisy analysis from basecalled sequences

Costly and power-hungry computational requirements

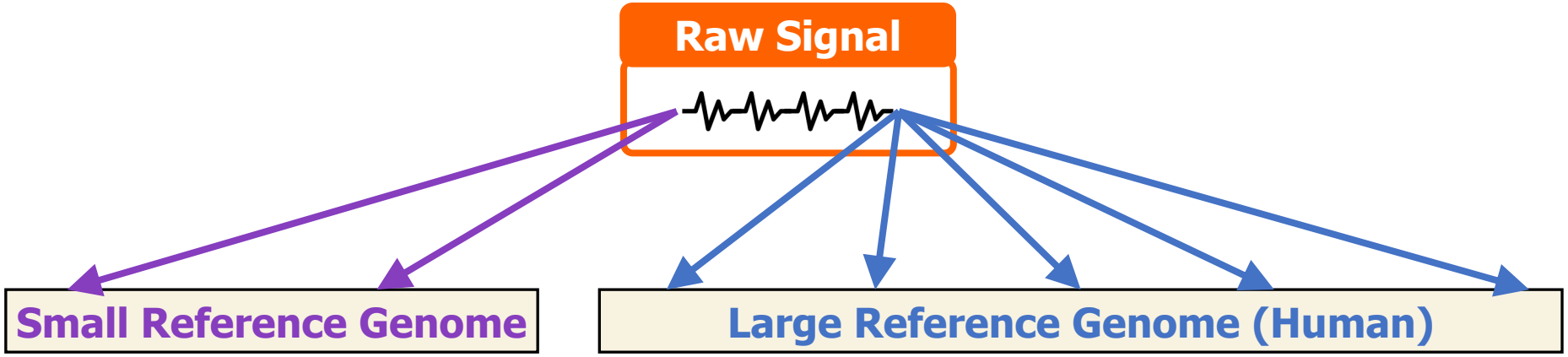
2. Mapping **signals** to reference genomes **without** basecalling



Raw signals contain richer information than bases

Efficient analysis with better scalability and portability

The Problem – Mapping Raw Signals



Fewer candidate regions in **small genomes**

Substantially **larger number of regions** to check **per read** as the genome size increases

Accurate mapping

Problem: Probabilistic mechanisms on **many regions → inaccurate mapping**

High throughput

Problem: Distance calculation on **many regions → reduced throughput**

The Problem – Mapping Raw Signals

Existing solutions are
inaccurate or inefficient
for large genomes

Accurate mapping

on **many regions → inaccurate mapping**

High throughput

Problem: Distance calculation
on **many regions → reduced throughput**

Outline

Background

RawHash

Evaluation

Conclusion

Goal

Enable **fast and accurate real-time analysis** of raw nanopore signals **for large genomes**

RawHash

The **first hash-based search mechanism**
to quickly and accurately map raw nanopore signals
to reference genomes

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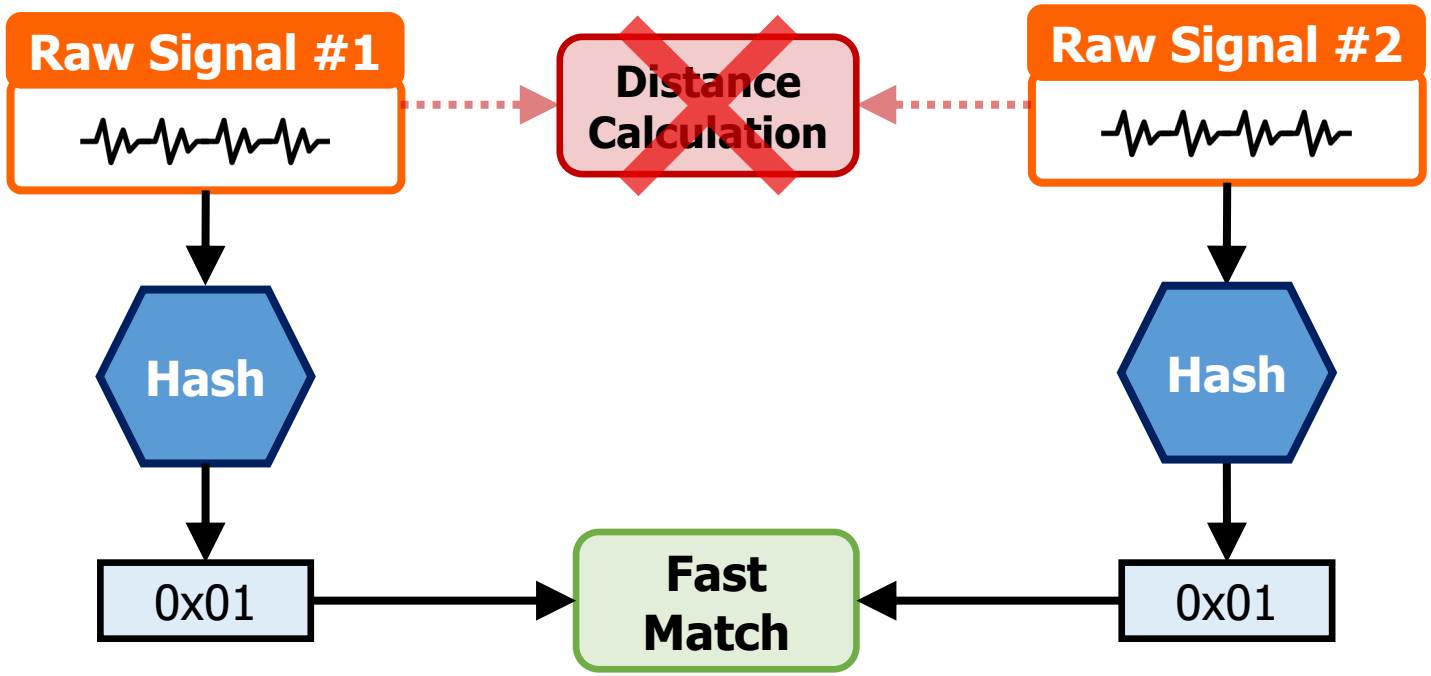
RawHash – Key Idea

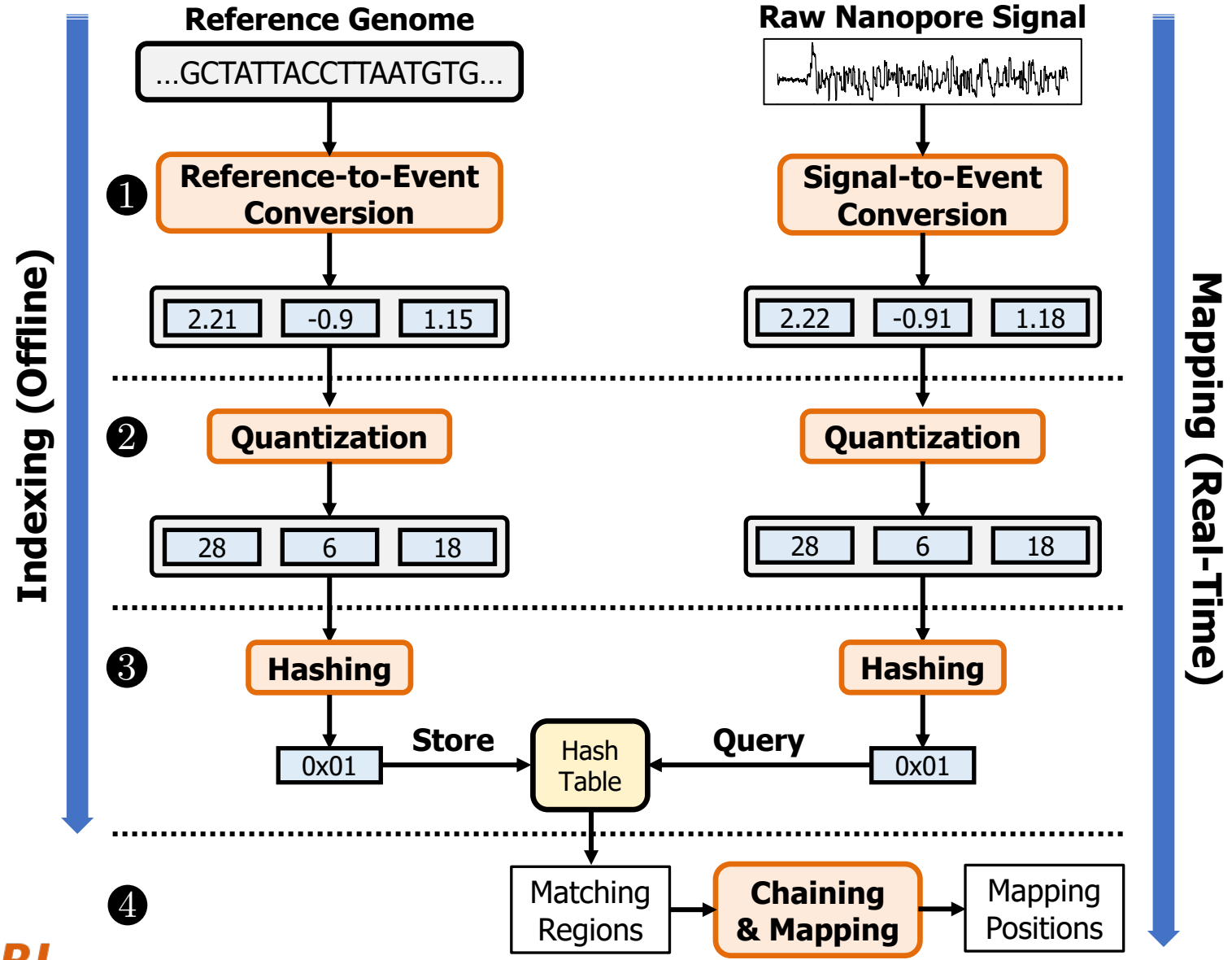
Key Observation: **Identical** nucleotides generate **similar** raw signals



Challenge #1: Generating the **same** hash value for **similar enough** signals

Challenge #2: **Accurately** finding similar regions **as few as possible**

RawHash Overview



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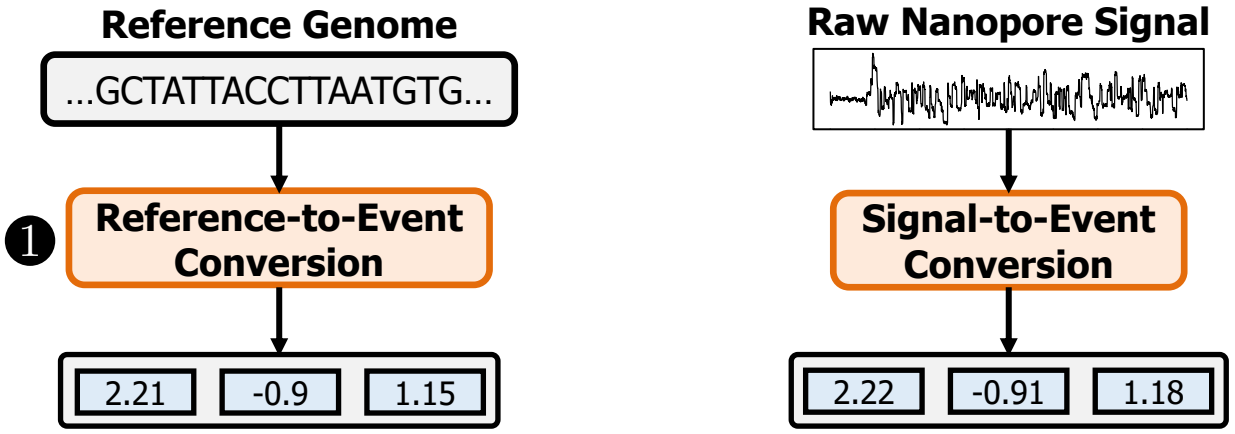
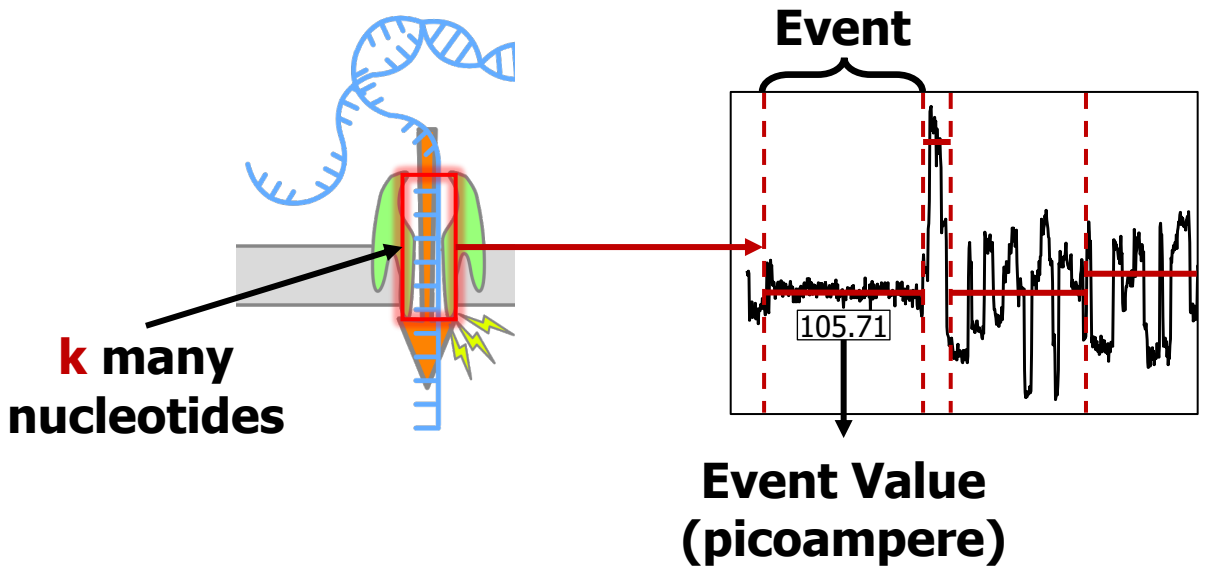


Events in Raw Nanopore Signals

- **Event:** A **segment** of the raw signal
  - Corresponds to a **particular k**-mer

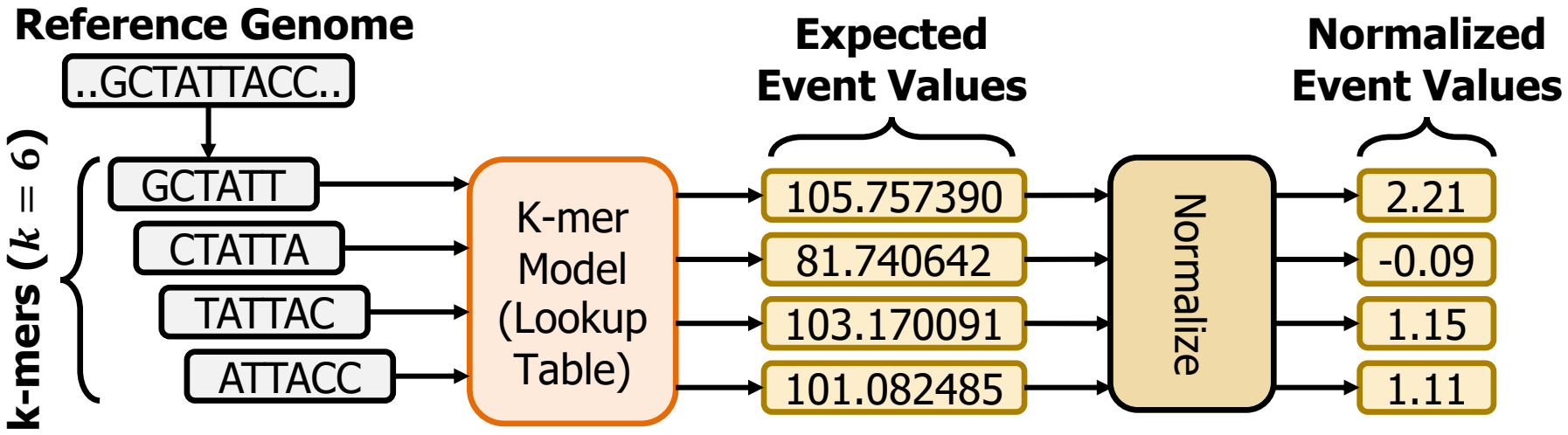
- **Event detection** finds these segments to identify **k**-mers
  - Start and end positions are marked by abrupt signal changes
  - Statistical methods identify these abrupt changes
  - **Event value:** average of signals **within an event**



Reference-to-Event Conversion

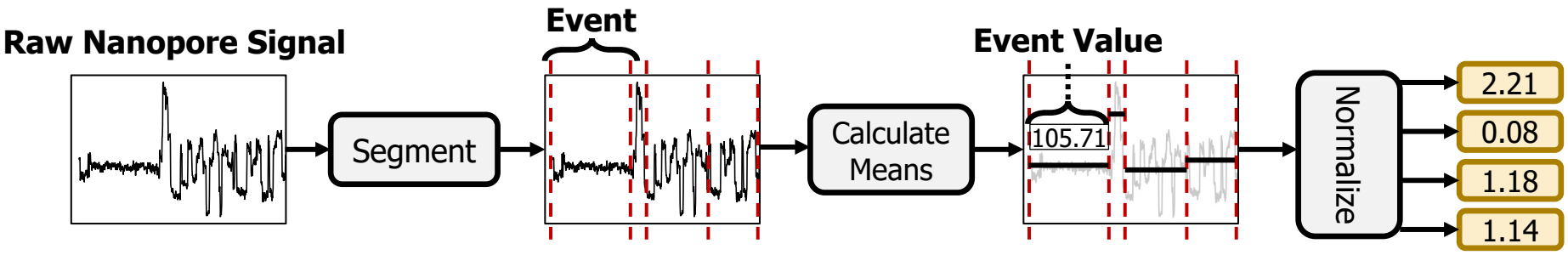
- **K-mer model:** Provides **expected** event values **for each k-mer**
  - Preconstructed based on nanopore sequencer characteristics

- Use the **k-mer model** to convert **all k-mers** of a reference genome to their **expected** event values



Signal-to-Event Conversion

- **Event detection:** Identifies signal regions corresponding to specific k-mers
  - Uses statistical test (**segmentation**) to spot abrupt signal changes



- Consecutive events ➔ consecutive k-mers

Signal-to-Event Conversion

- **Event detection:** Identifies signal regions corresponding to specific k-mers
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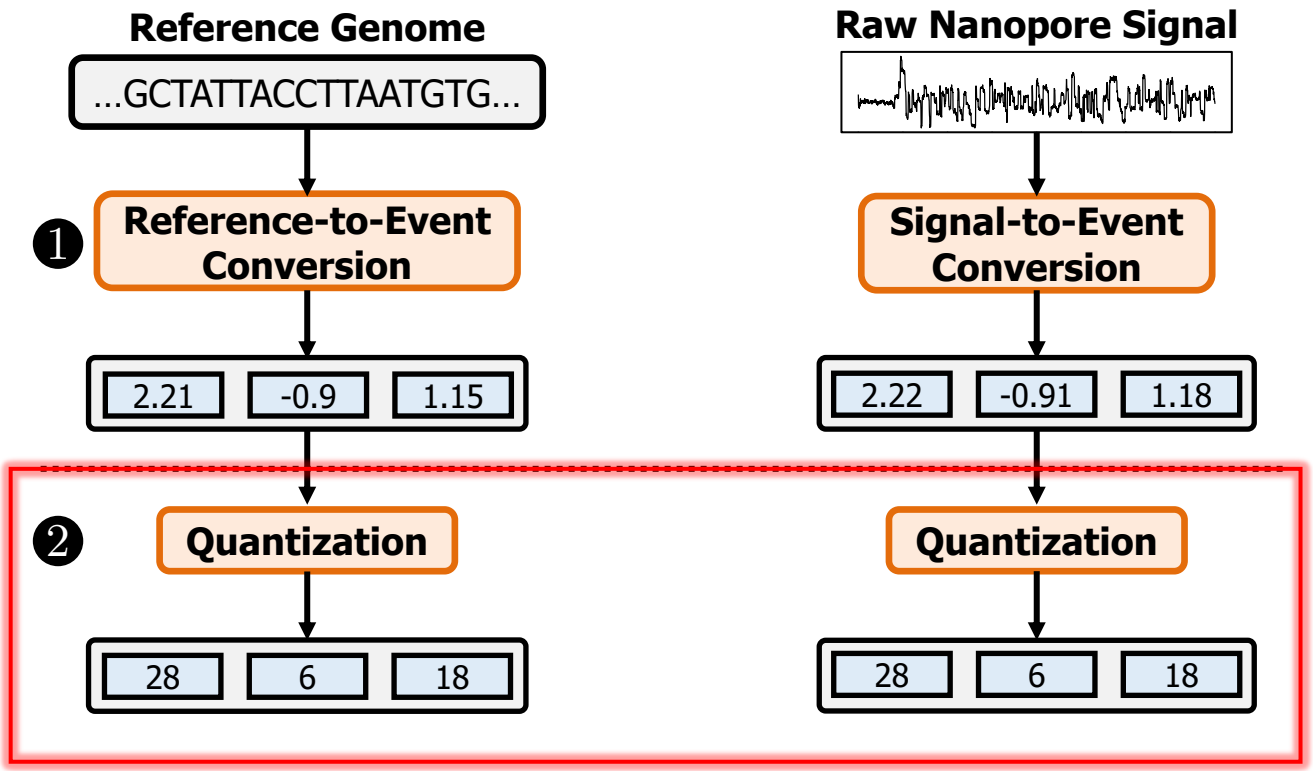
Can we match events (k-mers) between reference genome and raw signals?

- Consecutive events ➔ consecutive k-mers

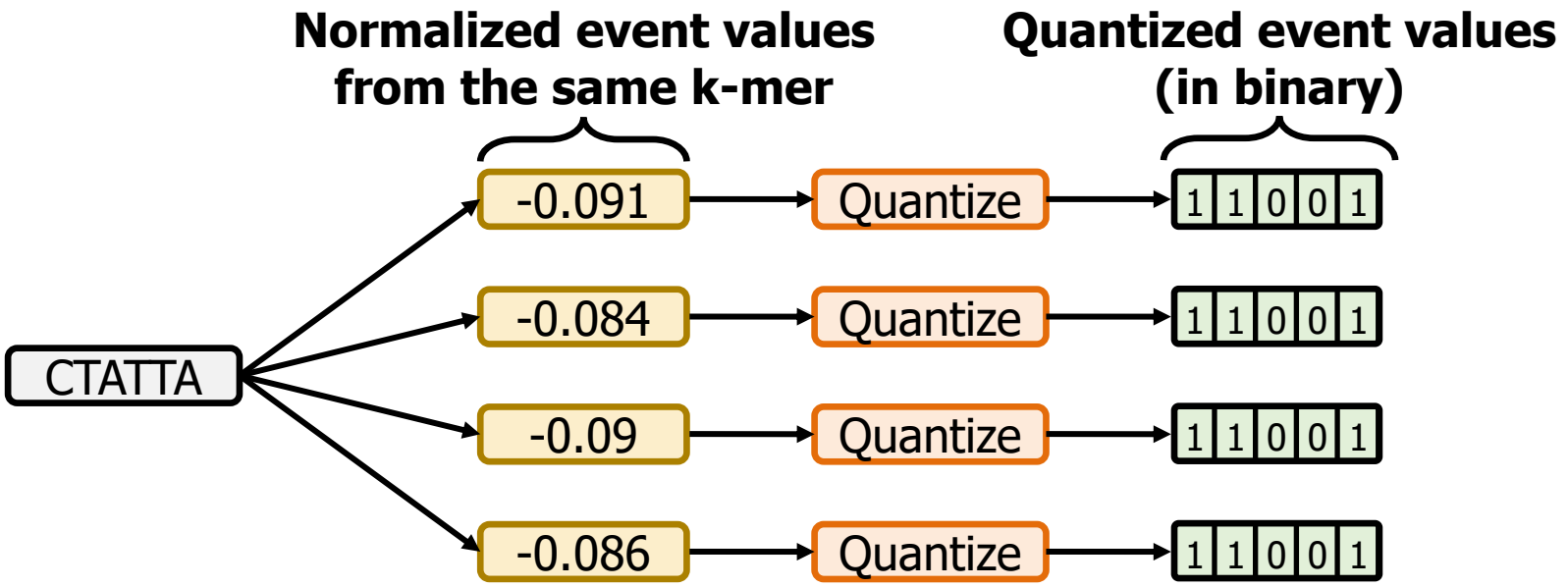
RawHash Overview



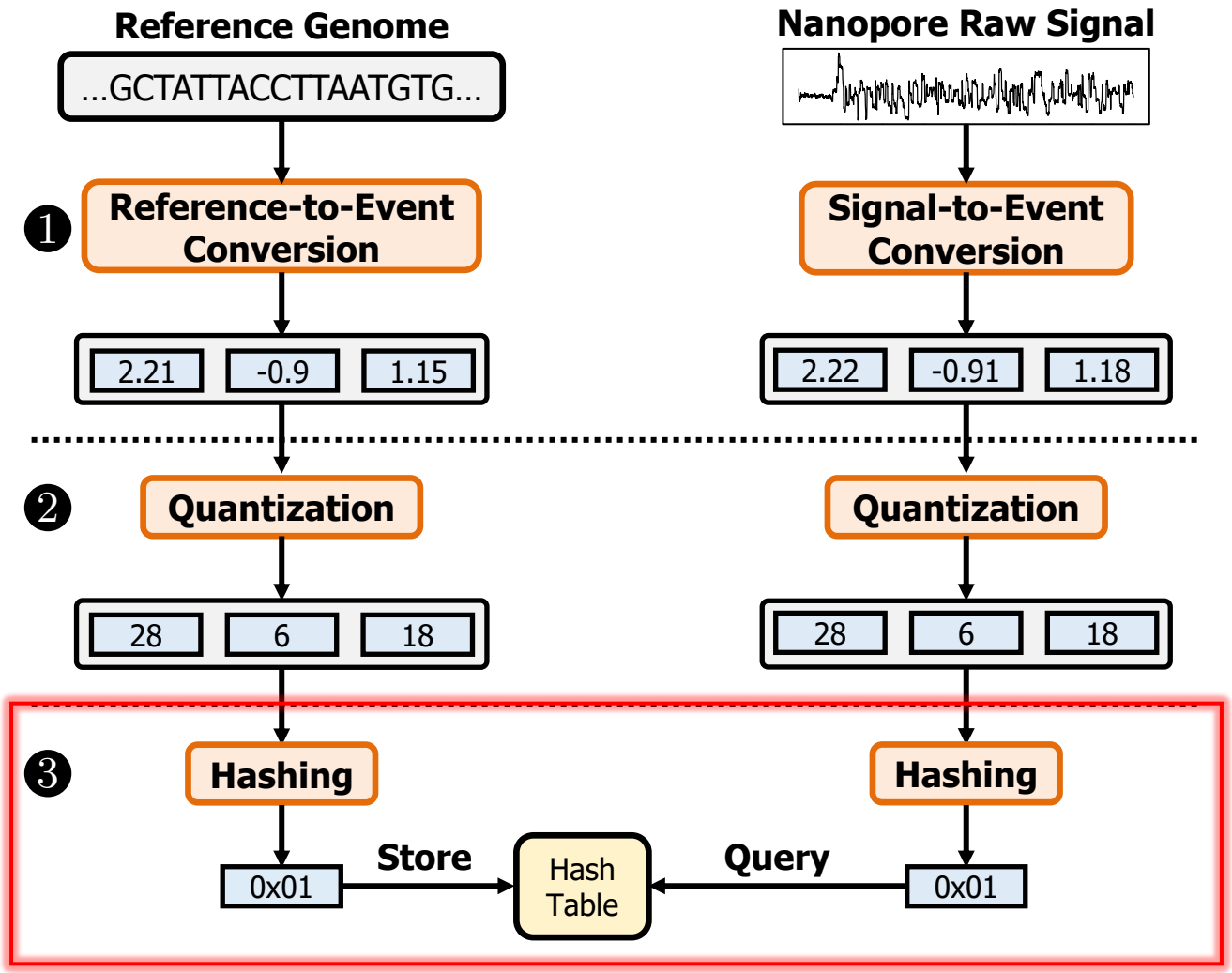
Quantizing the Event Values

- **Observation:** Slight differences in raw signals from identical k-mers
  - **Challenge:** Direct event value matching is not feasible and accurate

- **Key Idea:** Quantize the event values
  - Enables assigning **identical quantized values** to **similar event values**



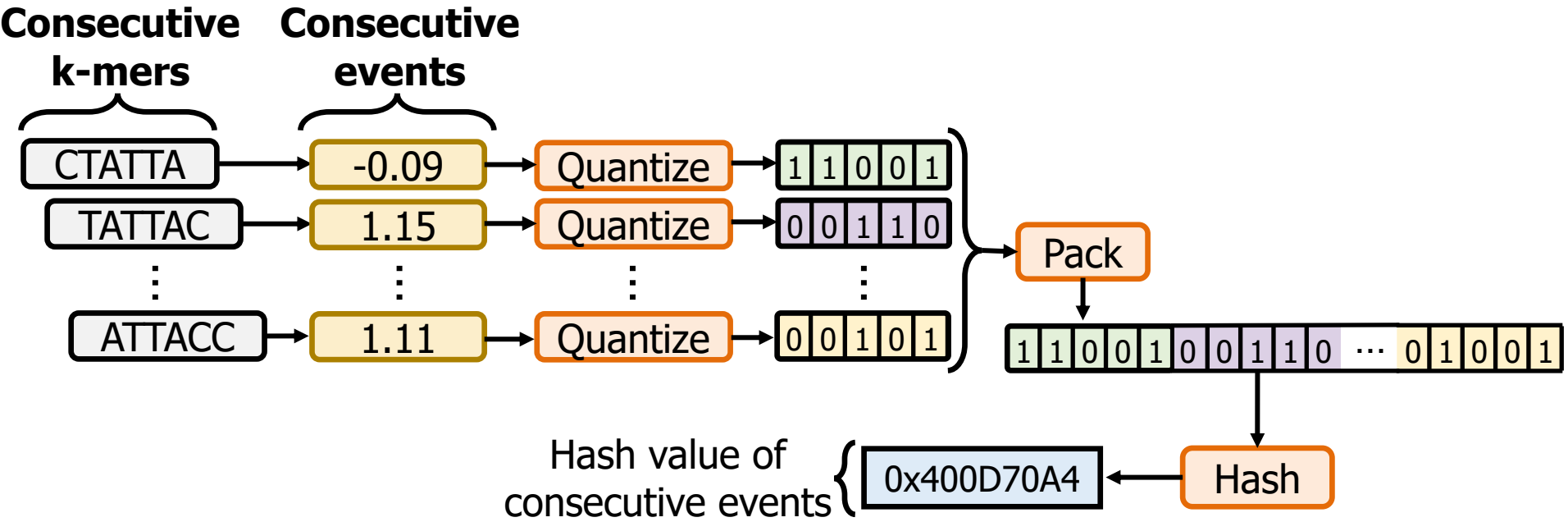
RawHash Overview



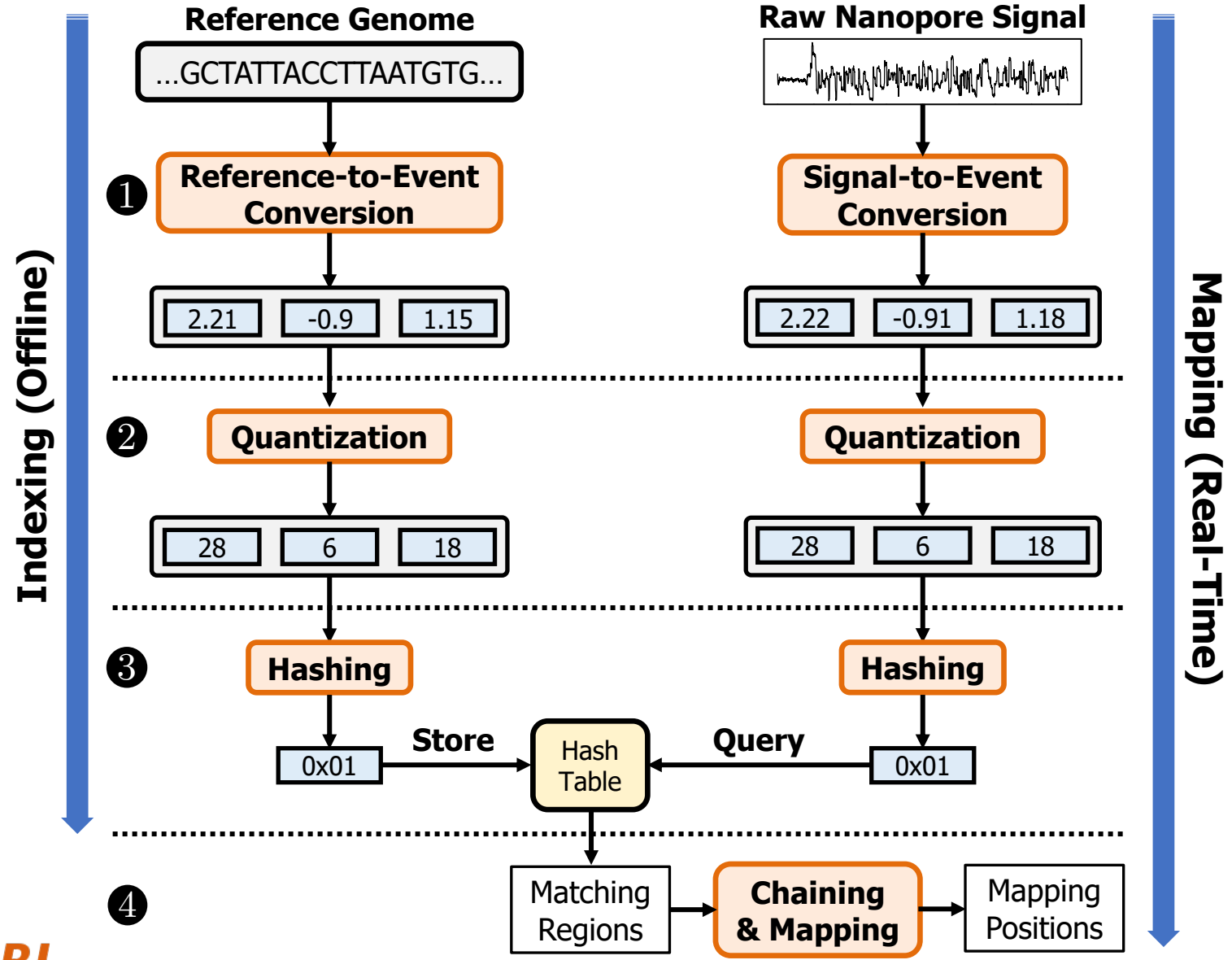
Hashing for Fast Similarity Search

- Each event usually represents a very small k-mer (6 to 9 characters)
  - **Challenge:** Short k-mers are likely to appear in many locations

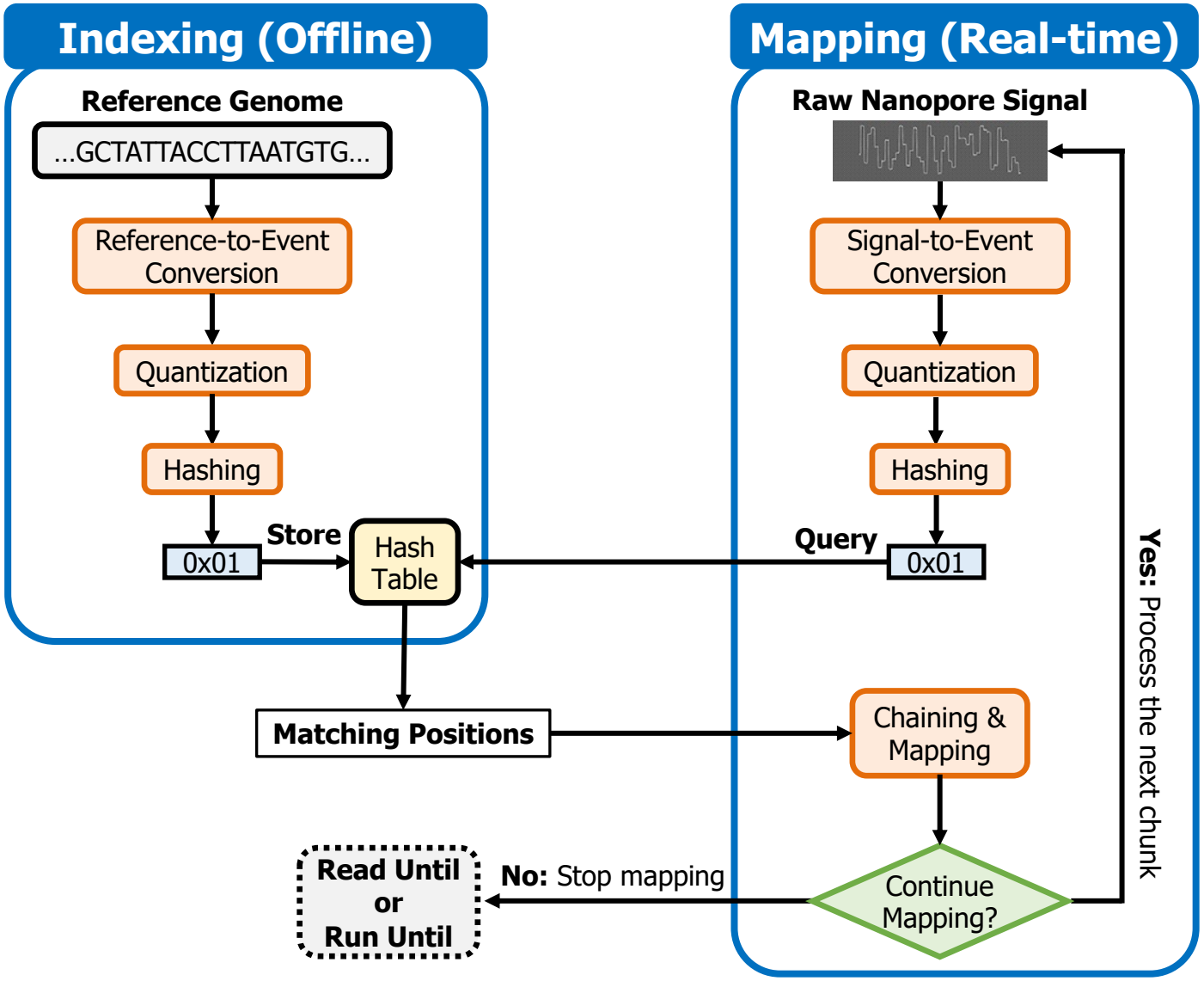
- **Key Idea:** Create longer k-mers from many **consecutive events**
- **Key Benefit:** Directly match hash values to quickly identify similarities



RawHash Overview



Real-Time Mapping using Hash-based Indexing



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Evaluation Methodology

- Compared to **UNCALLED** [Kovaka+, Nat. Biotech. 2021] and **Sigmap** [Zhang+, ISMB/ECCB 2021]
  - **CPU baseline:** AMD EPYC 7742 @2.26GHz
  - **32 threads** for each tool

- **Use cases** for real-time genome analysis:
  1. Read mapping
  2. Relative abundance estimation
     - **Benefits of Sequence Until**
  3. Contamination analysis

Evaluation Methodology

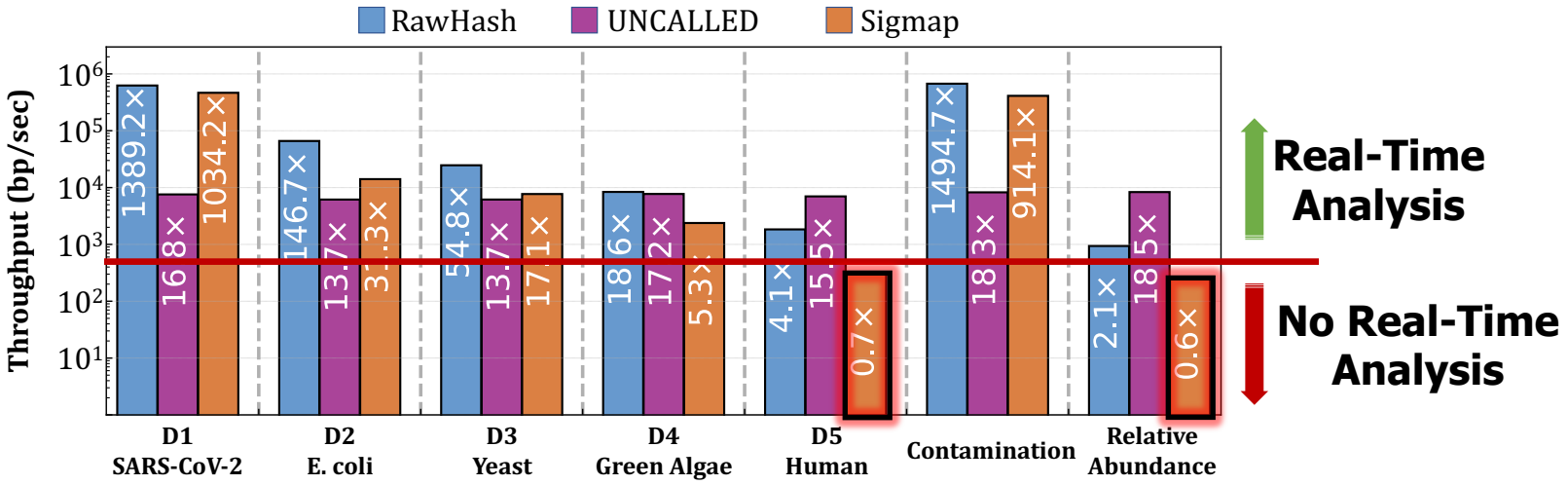
- Evaluation metrics:
  - **Throughput** (bases processed per second)
  - Potential reduction in **sequencing time and cost**
  - **Accuracy**
    - **Baseline:** Mapping basecalled reads using minimap2
    - Precision, recall, and F1 scores
    - Relative abundance estimation distance to ground truth

- **Datasets:**

	Organism	Reads (#)	Bases (#)	Genome Size
Read Mapping				
D1	*SARS-CoV-2*	1,382,016	594M	29,903
D2	*E. coli*	353,317	2,365M	5M
D3	*Yeast*	49,989	380M	12M
D4	*Green Algae*	29,933	609M	111M
D5	*Human HG001*	269,507	1,584M	3,117M
Relative Abundance Estimation				
	D1-D5	2,084,762	5,531M	3,246M
Contamination Analysis				
	D1 and D5	1,651,523	2,178M	29,903

Throughput

- **Real-time analysis requires** faster throughput than sequencer
  - Throughput of a nanopore sequencer: **~450 bp/sec (data generation speed)**

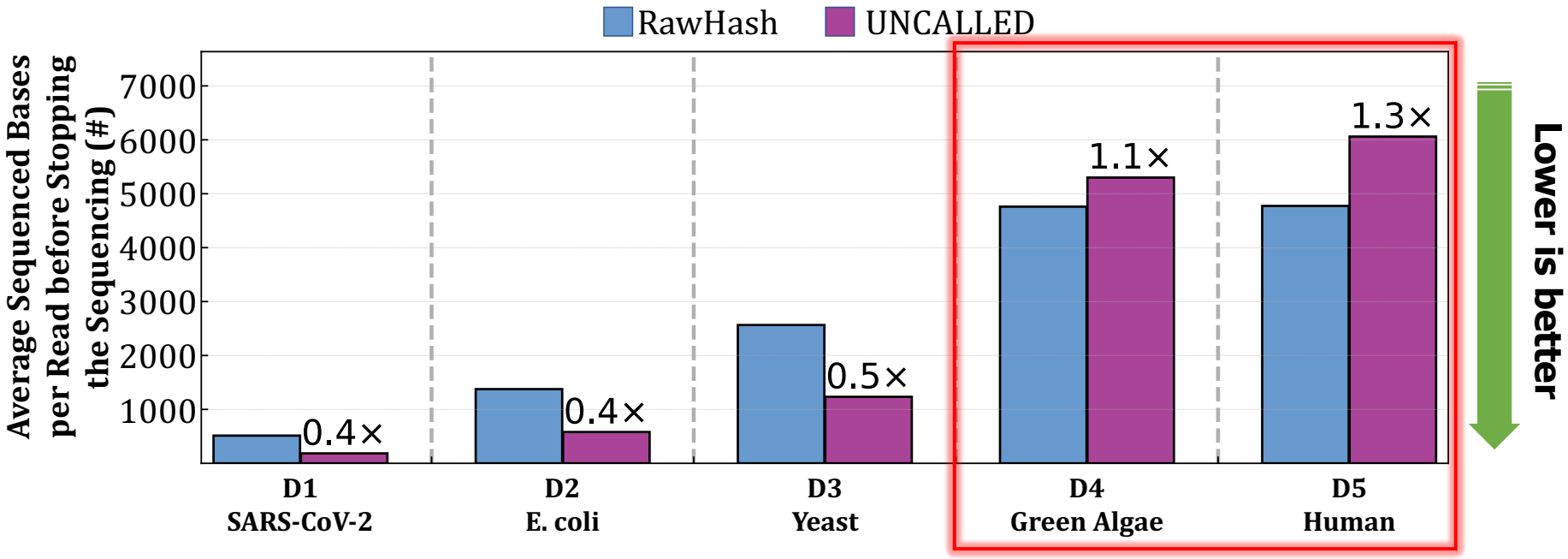


25.8× and 3.4× better average throughput compared to **UNCALLED and Sigmap**, respectively

Sigmap **cannot** perform real-time analysis **for large genomes**

Sequencing Time

- Fewer bases to sequence ➔
  - Reduction in sequencing time and cost



RawHash **reduces sequencing time and cost**

for large genomes up to **1.3**× compared to UNCALLED

Mapping Accuracy

- Read mapping accuracy of each tool and each use case

Dataset		UNCALLED	Sigmap	RawHash
Read Mapping				
D1	Precision	0.9547	**0.9929**	0.9868
SARS-CoV-2	Recall	**0.9910**	0.5540	0.8735
	F_1	**0.9725**	0.7112	0.9267
D2	Precision	0.9816	**0.9842**	0.9573
E. coli	Recall	**0.9647**	0.9504	0.9009
	F_1	**0.9731**	0.9670	0.9282
D3	Precision	0.9459	0.9856	**0.9862**
Yeast	Recall	**0.9366**	0.9123	0.8412
	F_1	0.9412	**0.9475**	0.9079
D4	Precision	0.8836	**0.9741**	0.9691
Green Algae	Recall	0.7778	**0.8987**	0.7015
	F_1	0.8273	**0.9349**	0.8139
D5	Precision	0.4867	0.4287	**0.8959**
Human HG001	Recall	0.2379	0.2641	**0.4054**
	F_1	0.3196	0.3268	**0.5582**

Dataset		UNCALLED	Sigmap	RawHash
Relative Abundance Estimation				
D1-D5	Precision	0.7683	0.7928	**0.9484**
	Recall	0.1273	0.2739	**0.3076**
	F_1	0.2184	0.4072	**0.4645**
Contamination Analysis				
D1, D5	Precision	**0.9378**	0.7856	0.8733
	Recall	**0.9910**	0.5540	0.8735
	F_1	**0.9637**	0.6498	0.8734

For Large Genomes: RawHash provides the **best accuracy** in all metrics, resulting in **1.14×** - **2.13×** improvement in F_1 score

Relative Abundance Estimation Accuracy

- Estimating the ratio of genomes in a sample in real-time
  - **Distance:** Euclidean distance compared to the ground truth distance
  - The dataset includes a large reference genome

	Estimated Relative Abundance Ratios					
Tool	***SARS-CoV-2***	***E. coli***	***Yeast***	***Green Algae***	***Human***	**Distance**
Ground Truth	0.0929	0.4365	0.0698	0.1179	0.2828	N/A
UNCALLED	0.0026	0.5884	0.0615	0.1313	0.2161	0.1895
Sigmap	0.0419	0.4191	0.1038	0.0962	0.3390	0.0877
RawHash	0.1249	0.4701	0.0957	0.0629	0.2464	**0.0847**

RawHash provides the **best relative abundance estimation** closest to the ground truth estimation

Real Implementation of Sequence Until

- Running RawHash by using
  - **RawHash (100%):** The entire sample **without Sequence Until**
  - **RawHash (7%):** RawHash **with Sequence Until** where Sequence Until dynamically stops the entire sequencing after sequencing **7% of the sample**

	Estimated Relative Abundance Ratios in 50,000 Random Reads					
Tool	***SARS-CoV-2***	***E. coli***	***Yeast***	***Green Algae***	***Human***	**Distance**
RawHash (100%)	0.0270	0.3636	0.3062	0.1951	0.1081	N/A
RawHash + Sequence Until (7%)	0.0283	0.3539	0.3100	0.1946	0.1133	0.0118

Sequence Until enables sequencing **only 7% (~1/15)** of the entire sample **with high accuracy**

Simulating Sequence Until

- Real relative abundance results using the entire set of reads

	Estimated Relative Abundance Ratios					
Tool	***SARS-CoV-2***	***E. coli***	***Yeast***	***Green Algae***	***Human***	**Distance**
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- Simulating the benefits of Sequence Until by
  - Using **a random portion** (25%, 10%, 1%, …) of the sample

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Ground Truth	0.0929	0.4365	0.0698	0.1179	0.2828	N/A
UNCALLED (25%)	0.0026	0.5890	0.0613	0.1332	0.2139	0.1910
RawHash (25%)	0.0271	0.4853	0.0920	0.0786	0.3170	**0.0995**
UNCALLED (10%)	0.0026	0.5906	0.0611	0.1316	0.2141	0.1920
RawHash (10%)	0.0273	0.4869	0.0963	0.0772	0.3124	**0.1004**
UNCALLED (1%)	0.0026	0.5750	0.0616	0.1506	0.2103	0.1836
RawHash (1%)	0.0259	0.4783	0.0987	0.0882	0.3088	**0.0928**
UNCALLED (0.1%)	0.0040	0.4565	0.0380	0.1910	0.3105	0.1242
RawHash (0.1%)	0.0212	0.5045	0.1120	0.0810	0.2814	**0.1136**
UNCALLED (0.01%)	0.0000	0.5551	0.0000	0.0000	0.4449	0.2602
RawHash (0.01%)	0.0906	0.6122	0.0000	0.0000	0.2972	**0.2232**

Simulating Sequence Until

- Real relative abundance results using the entire set of reads

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UNCALLED and RawHash benefit from **Sequence Until** significantly **by up to 100×** reductions in sequencing time and costs

Tool	***SARS-CoV-2***	***E. coli***	***Yeast***	***Green Algae***	***Human***	**Distance**
Ground Truth	0.0929	0.4365	0.0698	0.1179	0.2828	N/A
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RawHash (0.01%)	0.0906	0.6122	0.0000	0.0000	0.2972	**0.2232**

More in the Paper

- **More Results**
  - **Mapping time** per read
  - Overall **computational resources** required by each tool
    - Peak memory usage, CPU time and real time in the indexing and mapping steps
  - **Performance breakdown** of the steps in RawHash

- **Details of all mechanisms and configurations**
  - Details of the **quantization** and **hashing** mechanism
  - Details of the **parameter configurations**
  - Trade-offs between the **DNN-based approaches** and raw signal mapping approaches

RawHash

- Can Firtina, Nika Mansouri Ghiasi, Joel Lindegger, Gagandeep Singh, Meryem Banu Cavlak, Haiyu Mao, and Onur Mutlu,
  "RawHash: Enabling Fast and Accurate Real-Time Analysis of Raw Nanopore Signals for Large Genomes"
  *Proceedings of the 31st Annual Conference on Intelligent Systems for Molecular Biology (**ISMB**) and the 22nd European Conference on Computational Biology (**ECCB**)*, Jul 2023
  [arXiv preprint]
  [Source Code]

Bioinformatics, 2023, **39**, i297–i307
https://doi.org/10.1093/bioinformatics/btad272
ISMB/ECCB 2023

OXFORD

RawHash: enabling fast and accurate real-time analysis of raw nanopore signals for large genomes

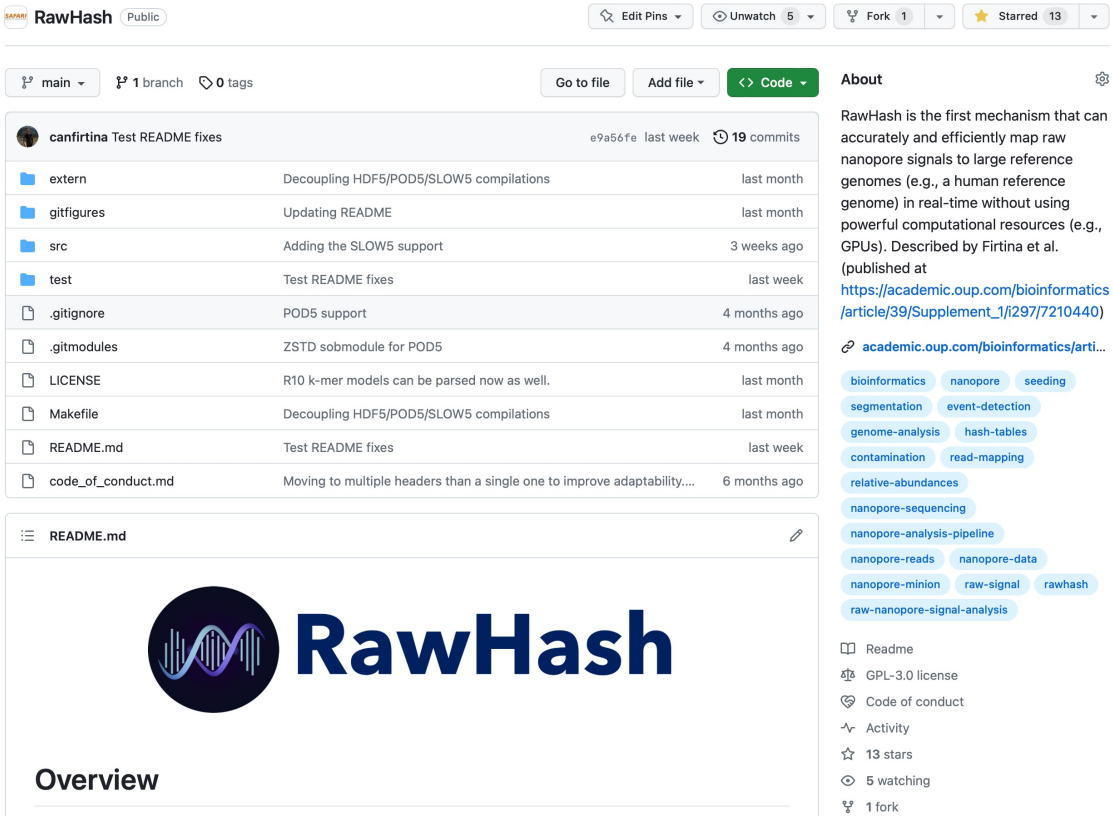
Can Firtina [1,*], Nika Mansouri Ghiasi [1], Joel Lindegger [1], Gagandeep Singh [1], Meryem Banu Cavlak [1], Haiyu Mao [1], Onur Mutlu [1,*]

[1]Department of Information Technology and Electrical Engineering, ETH Zurich, 8092 Zurich, Switzerland

*Corresponding author. Department of Information Technology and Electrical Engineering, ETH Zurich, Gloriastrasse 35, 8092 Zurich, Switzerland.
E-mail: firtinac@ethz.ch (C.F.), omutlu@ethz.ch (O.M.)

RawHash Source Code

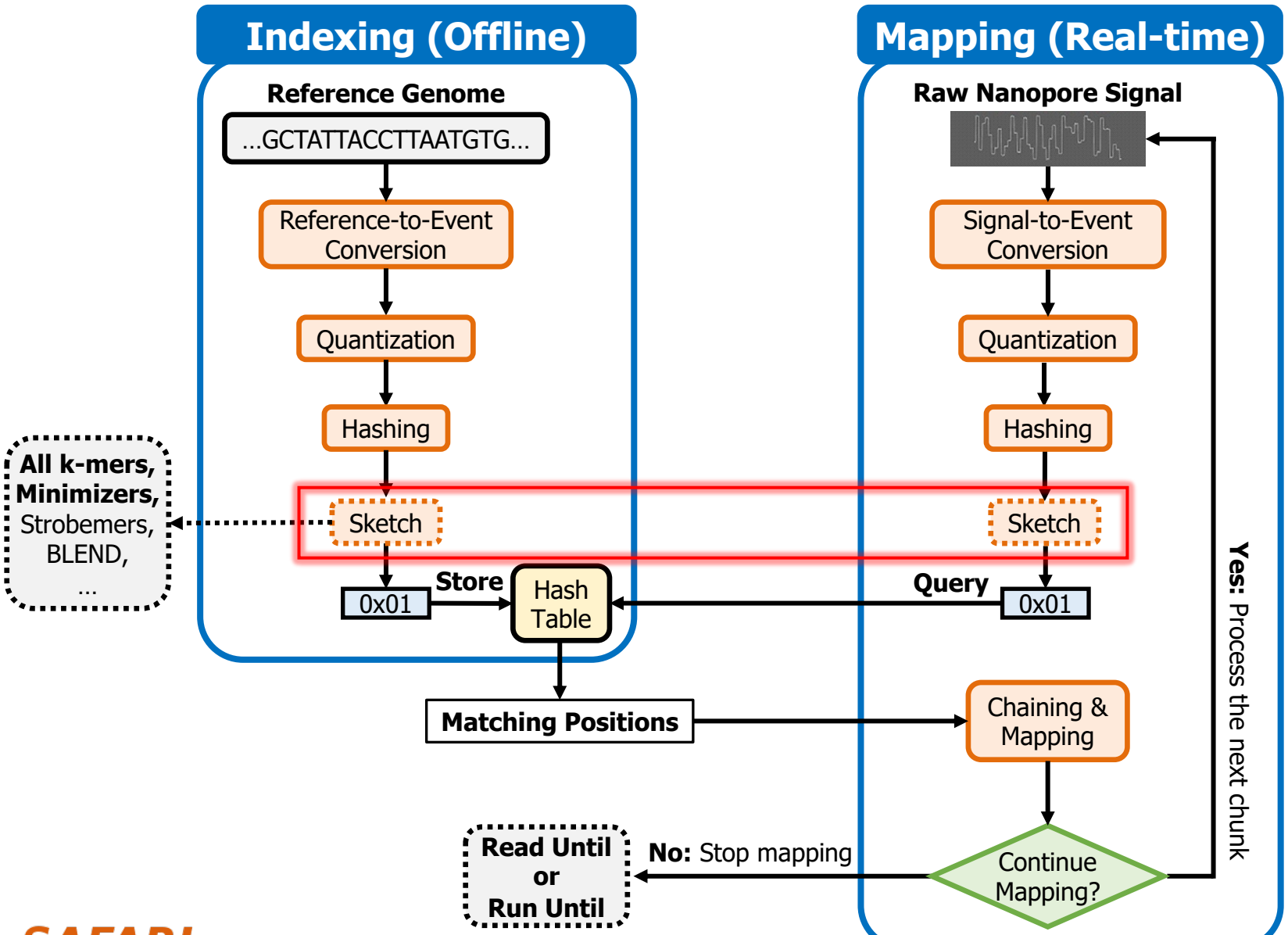
- Supports **all major raw signal file formats and flow cell versions**
  - FAST5, POD5, S/BLOW5 file formats
- Easy-to-use scripts
  - To download all the datasets
  - To reproduce all of our results
- You can write your outlier function for Sequence Until
  - Easily integrate Sequence Until
- Upcoming Feature:
  - Integrating the MinKNOW API



https://github.com/CMU-SAFARI/RawHash

SAFARI

Sketching with Hash-based Indexing



Outline

Background

RawHash

Evaluation

Conclusion

Conclusion

Key Contributions:
1) The **first hash-based mechanism** that can quickly and accurately analyze raw nanopore signals for **large genomes**

2) The novel **Sequence Until** technique can accurately and **dynamically stop the entire sequencing of all reads at once** if further sequencing is not necessary

Key Results: Across 3 use cases and 5 genomes of varying sizes, RawHash provides
- **25.8× and 3.4× better average throughput** compared to two state-of-the-art works
- **1.14× – 2.13× more accurate mapping results** for **large genomes**
- Sequence Until **reduces the sequencing time and cost by 15×**

Many opportunities for analyzing raw nanopore signals in real-time:
- Many hash-based **sketching techniques** can now be used for raw signals
- **Indexing is very cheap:** Many future use cases with the on-the-fly index construction
- We should rethink the algorithms to perform downstream analysis fully using raw signals

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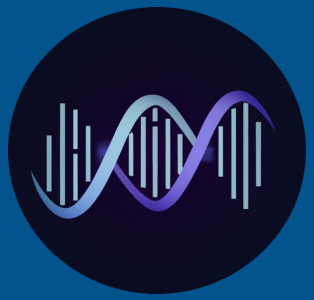


RawHash

Enabling Fast and Accurate Real-Time Analysis of Raw Nanopore Signals for Large Genomes

Can Firtina

Nika Mansouri Ghiasi Joel Lindegger Gagandeep Singh

Meryem Banu Cavlak Haiyu Mao Onur Mutlu

Paper

Code

SAFARI

ETH zürich

Fast and Accurate Real-Time Genome Analysis

- Can Firtina, Melina Soysal, Joel Lindegger, and Onur Mutlu,
  "RawHash2: Accurate and Fast Mapping of Raw Nanopore Signals using a Hash-based Seeding Mechanism"
  Preprint on ***arxiv***, September 2023.
  [arXiv version]
  [RawHash2 Source Code]

RawHash2: Accurate and Fast Mapping of Raw Nanopore Signals using a Hash-based Seeding Mechanism

Can Firtina Melina Soysal Joel Lindegger Onur Mutlu
ETH Zürich

Optimizations in RawHash2 (1)

- **More sensitive** chaining implementation with penalty scores
  - Benefits: Enables filtering dissimilar regions quickly
  - Downside: Additional computations with costly log operations

- Weighted mapping decisions
  - Benefit #1: 'Learned' mapping decisions based on the weights chosen from empirical analysis
  - Benefit #2: Faster and more accurate decisions

- Frequency filters
  - Filters the seeds that frequently appear before chaining
  - Benefits: Reduced workload on chaining without significantly affecting accuracy
  - Downside: Less sensitive mapping due to removed seeds

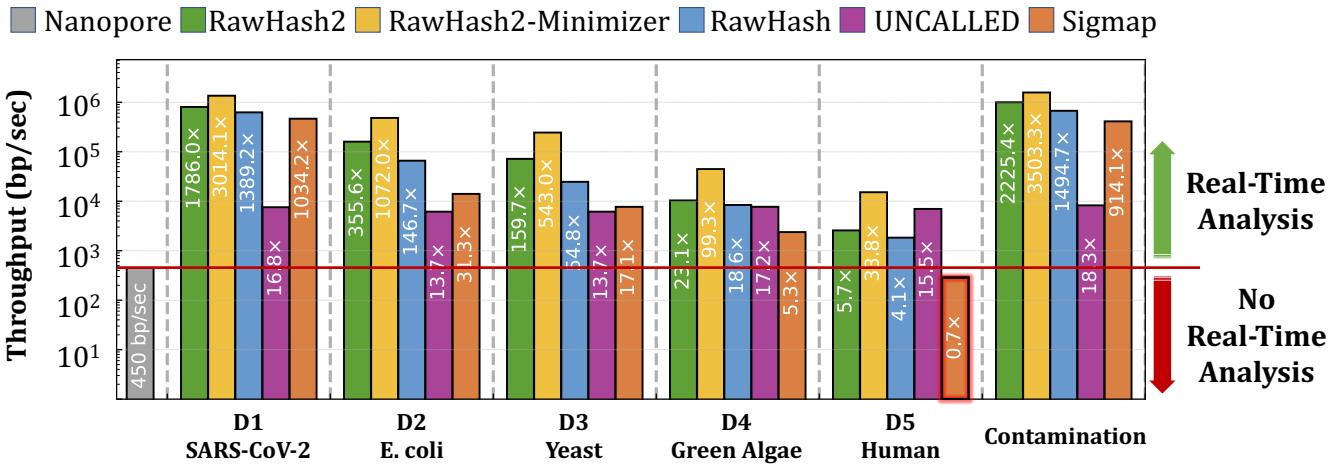
Optimizations in RawHash2 (2)

- New sketching techniques such as **minimizers** and **BLEND**
  - Enables integration of widely studied sketching techniques
  - Benefits: Can take advantage of these techniques (e.g., reduced storage requirements)

- Support for the recent improvements in the technology
  - Support for **new data formats**: POD5 and S/BLOW5
  - Support for **newer nanopore chemistry** versions: R10.4

Results – Throughput

- **Real-time analysis requires** faster throughput than sequencer
  - Throughput of a nanopore sequencer: **~450 bp/sec (data generation speed)**



2.3× better average throughput RawHash

Results – Accuracy

Dataset		UNCALLED	Sigmap	RawHash	RawHash2	RawHash2-Minimizer
			Read Mapping			
D1	Precision	0.9547	**0.9929**	0.9868	0.9857	0.9602
SARS-CoV-2	Recall	**0.9910**	0.5540	0.8735	0.8842	0.7080
	F_1	**0.9725**	0.7112	0.9267	0.9322	0.8150
D2	Precision	0.9816	0.9842	0.9573	**0.9864**	0.9761
E. coli	Recall	**0.9647**	0.9504	0.9009	0.8934	0.7805
	F_1	**0.9731**	0.9670	0.9282	0.9376	0.8674
D3	Precision	0.9459	0.9856	**0.9862**	0.9567	0.9547
Yeast	Recall	**0.9366**	0.9123	0.8412	0.8942	0.7792
	F_1	0.9412	**0.9475**	0.9079	0.9244	0.8581
D4	Precision	0.8836	**0.9741**	0.9691	0.9264	0.9198
Green Algae	Recall	0.7778	**0.8987**	0.7015	0.8659	0.6711
	F_1	0.8273	**0.9349**	0.8139	0.8951	0.7760
D5	Precision	0.4867	0.4287	**0.8959**	0.8830	0.8111
Human HG001	Recall	0.2379	0.2641	0.4054	**0.4317**	0.1862
	F_1	0.3196	0.3268	0.5582	**0.5799**	0.3028
			Contamination			
D1 and D5	Precision	0.9378	0.7856	0.8733	**0.9393**	0.9330

RawHash2 is more accurate than RawHash **in all cases**

Results – Average Sequencing Length

Tool	*SARS-CoV-2*	*E. coli*	*Yeast*	*Green Algae*	*Human*	*Contamination*
Average sequenced base length per read						
UNCALLED	**184.51**	**580.52**	**1,233.20**	5,300.15	6,060.23	1,582.63
RawHash	513.95	1,376.14	2,565.09	4,760.59	4,773.58	742.56
RawHash2	488.46	1,234.39	1,715.31	**2,077.39**	**3,441.43**	**681.94**
RawHash2-Minimizer	566.42	1,763.76	2,339.41	2,891.55	4,090.68	787.82
Average sequenced number of chunks per read						
Sigmap	**1.01**	**2.11**	**4.14**	5.76	10.40	2.06
RawHash	1.24	3.20	5.83	10.72	10.70	2.41
RawHash2	1.18	2.93	4.02	**4.84**	**7.78**	**1.68**
RawHash2-Minimizer	1.39	4.16	5.45	6.66	9.17	1.89

RawHash2 uses fewer bases to sequence than RawHash in all cases

RawHash2 uses the smallest number of bases to sequence for larger genomes

Fast and Accurate Real-Time Genome Analysis

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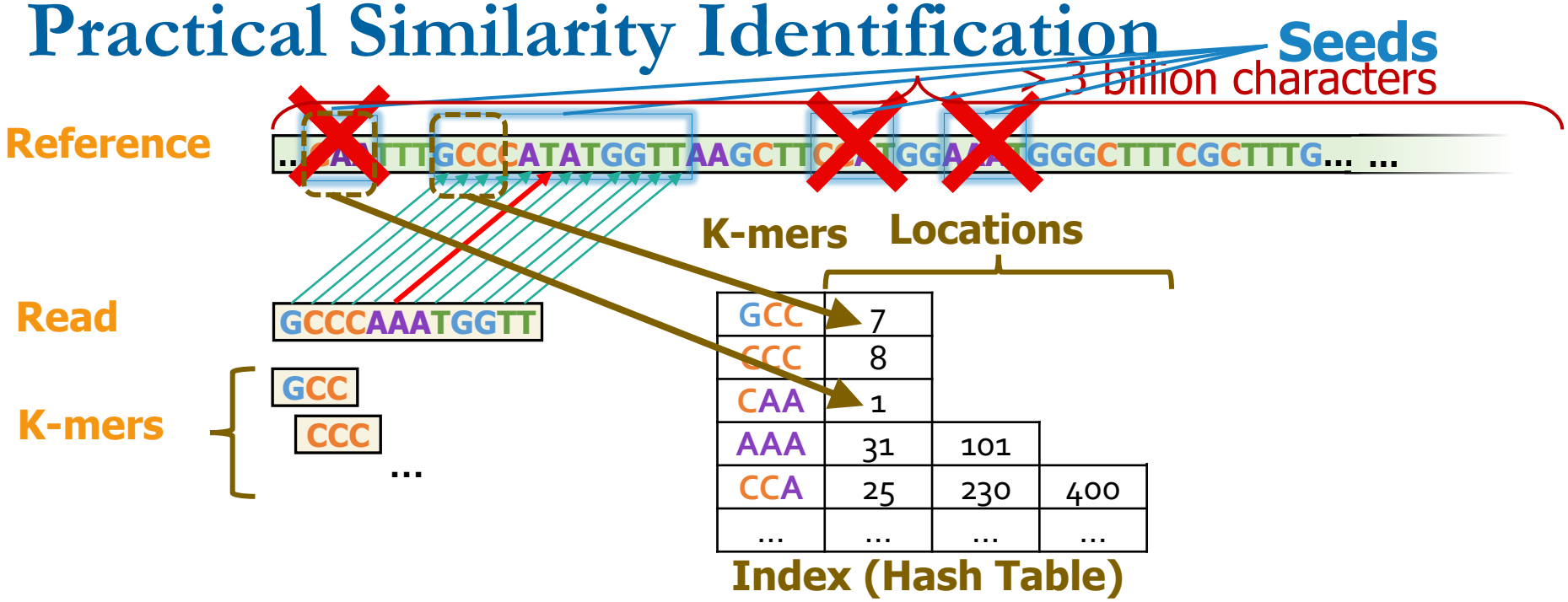
Backup Slides

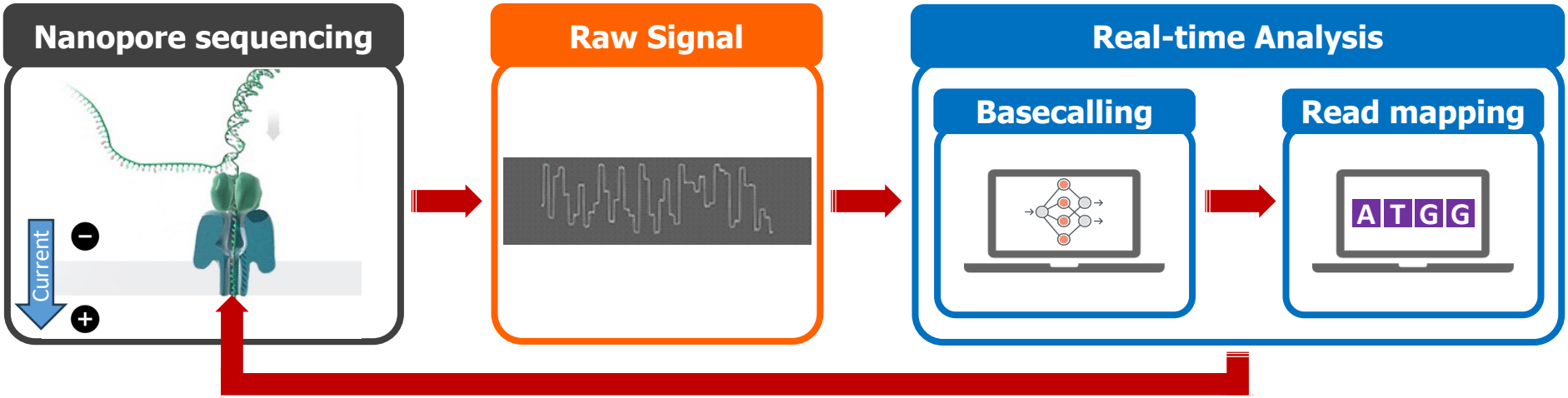
Practical Similarity Identification



Seeding	Determine potential matching regions (seeds) in the reference genome
Seed Filtering (e.g., Chaining)	Prune some seeds in the reference genome
Alignment	Determine the exact differences between the read and the reference genome

Existing Solutions – Real-time Basecalling

Deep neural networks (**DNNs**) for translating **signals** to **bases**

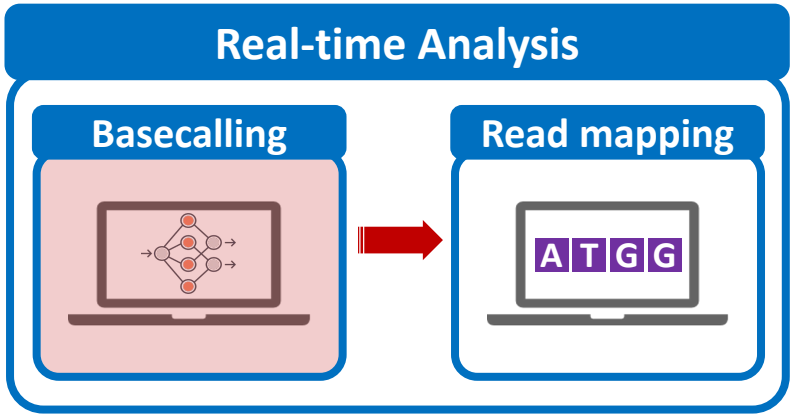


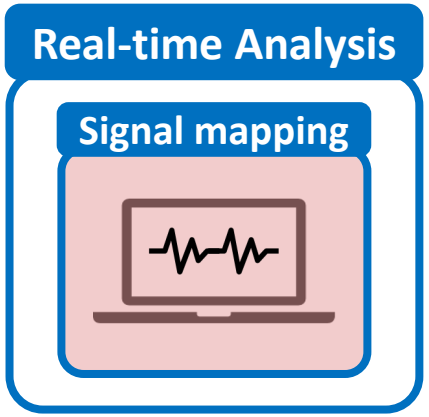
DNNs provide **less noisy analysis** from basecalled sequences

Costly and power-hungry computational requirements

The Problem

The existing solutions are **ineffective for large genomes**





Costly and energy-hungry computations to basecall each read:
Portable sequencing becomes challenging with resource-constrained devices

Larger number of reference regions **cannot be handled accurately or quickly**, rendering existing solutions **ineffective for large genomes**

Applications of Read Until

Depletion: Reads mapping to a particular reference genome is ejected

- Removing contaminated reads from a sample
- Relative abundance estimation
- Controlling low/high-abundance genomes in a sample
- Controlling the sequencing of depth of a genome

Enrichment: Reads **not** mapping to a particular reference genome is ejected

- Purifying the sample to ensure it contains only the selected genomes
- Removing the host genome (e.g., human) in contamination analysis

Applications of Run Until and Sequence Until

Run Until: Stopping the sequencing without informative decision from analysis

- Stopping when reads reach to a particular depth of coverage
- Stopping when the abundance of all genomes reach a particular threshold

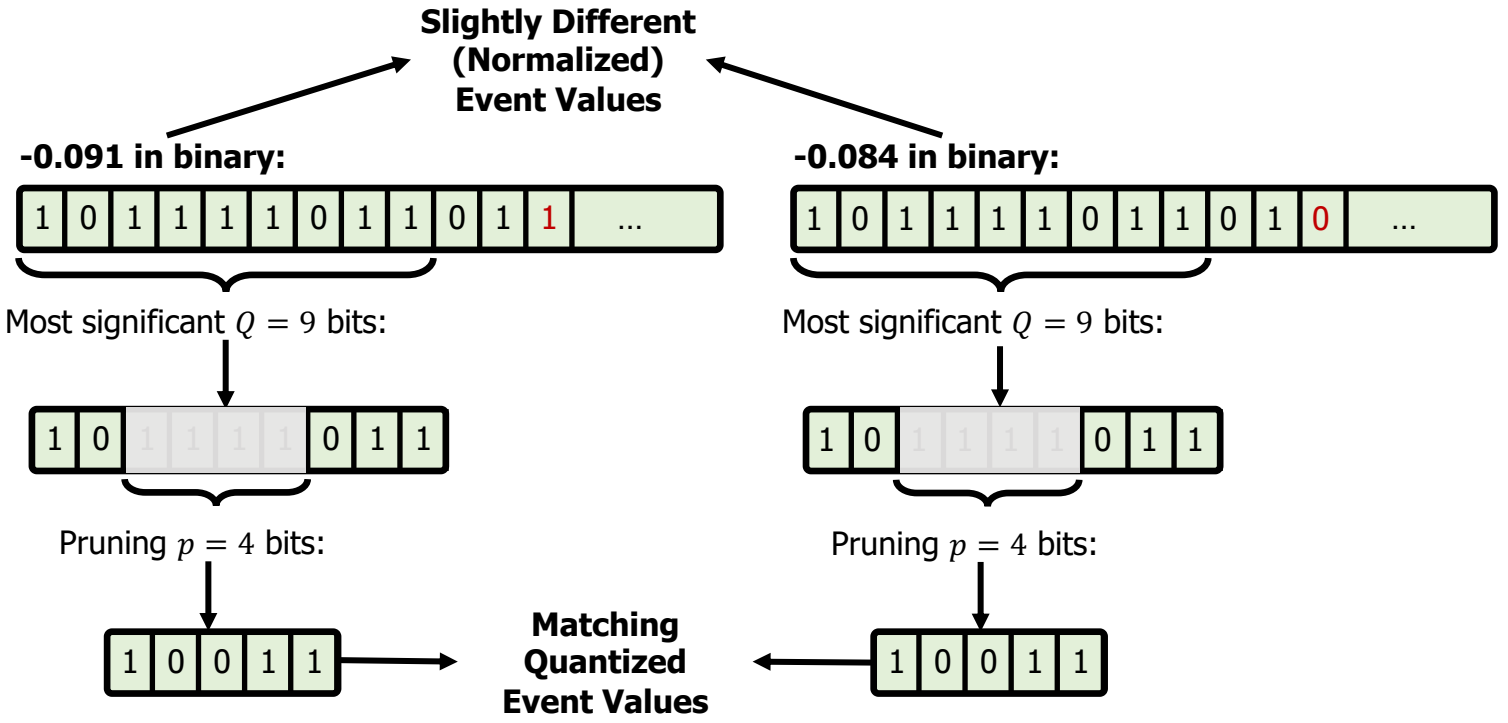
Sequence Until: Stopping the sequencing based on information decision

- Stopping when relative abundance estimations do not change substantially (for high-abundance genomes)
- Stopping when finding that the sample is contaminated with a particular set of genomes
- …

Details: Quantizing the Event Values

- **Observation:** Identical k-mers generate similar raw signals
  - **Challenge:** Their corresponding event values can be slightly different

- **Key Idea:** Quantize the event values
  - To enable assigning the **same quantized value** to the **similar event values**



Average Sequenced Bases and Chunks

Tool	*SARS-CoV-2*	*E. coli*	*Yeast*	*Green Algae*	*Human*
Average sequenced base length per read					
UNCALLED	**184.51**	**580.52**	**1,233.20**	5,300.15	6,060.23
RawHash	513.95	1,376.14	2,565.09	**4,760.59**	**4,773.58**
Average sequenced number of chunks per read					
Sigmap	**1.01**	**2.11**	**4.14**	**5.76**	**10.40**
RawHash	1.24	3.20	5.83	10.72	10.70

RawHash **reduces sequencing time and cost for large genomes**

up to **1.3**× compared to UNCALLED

Although Sigmap processes less number of chunks than RawHash, it fails to provide real-time analysis capabilities for large genomes

Breakdown Analysis of the RawHash Steps

	Fraction of entire runtime (%)				
Tool	***SARS-CoV-2***	***E. coli***	***Yeast***	***Green Algae***	***Human***
File I/O	0.00	0.00	0.00	0.00	0.00
Signal-to-Event	21.75	1.86	1.01	0.53	0.02
Sketching	0.74	0.06	0.04	0.03	0.00
Seeding	3.86	4.14	3.52	6.70	5.39
Chaining	73.50	93.92	95.42	92.43	94.46
Seeding + Chaining	77.36	98.06	98.94	99.14	99.86

The entire runtime is **bottlenecked by the chaining step**

Required Computation Resources in Indexing

Tool	*Contamination*	*SARS-CoV-2*	*E. coli*	*Yeast*	*Green Algae*	*Human*	*Relative Abundance*
			CPU Time (sec)				
UNCALLED	8.72	9.00	11.08	18.62	285.88	4,148.10	4,382.38
Sigmap	0.02	0.04	8.66	24.57	449.29	36,765.24	40,926.76
RawHash	0.18	0.13	2.62	4.48	34.18	1,184.42	788.88
			Real time (sec)				
UNCALLED	1.01	1.04	2.67	7.79	280.27	4,190.00	4,471.82
Sigmap	0.13	0.25	9.31	25.86	458.46	37,136.61	41,340.16
RawHash	0.14	0.10	1.70	2.06	15.82	278.69	154.68
			Peak memory (GB)				
UNCALLED	0.07	0.07	0.13	0.31	11.96	48.44	47.81
Sigmap	0.01	0.01	0.40	1.04	8.63	227.77	238.32
RawHash	0.01	0.01	0.35	0.76	5.33	83.09	152.80

The indexing step of RawHash is **orders of magnitude faster** than the indexing steps of UNCALLED and Sigmap, especially **for large genomes**

RawHash requires **larger memory space** than UNCALLED

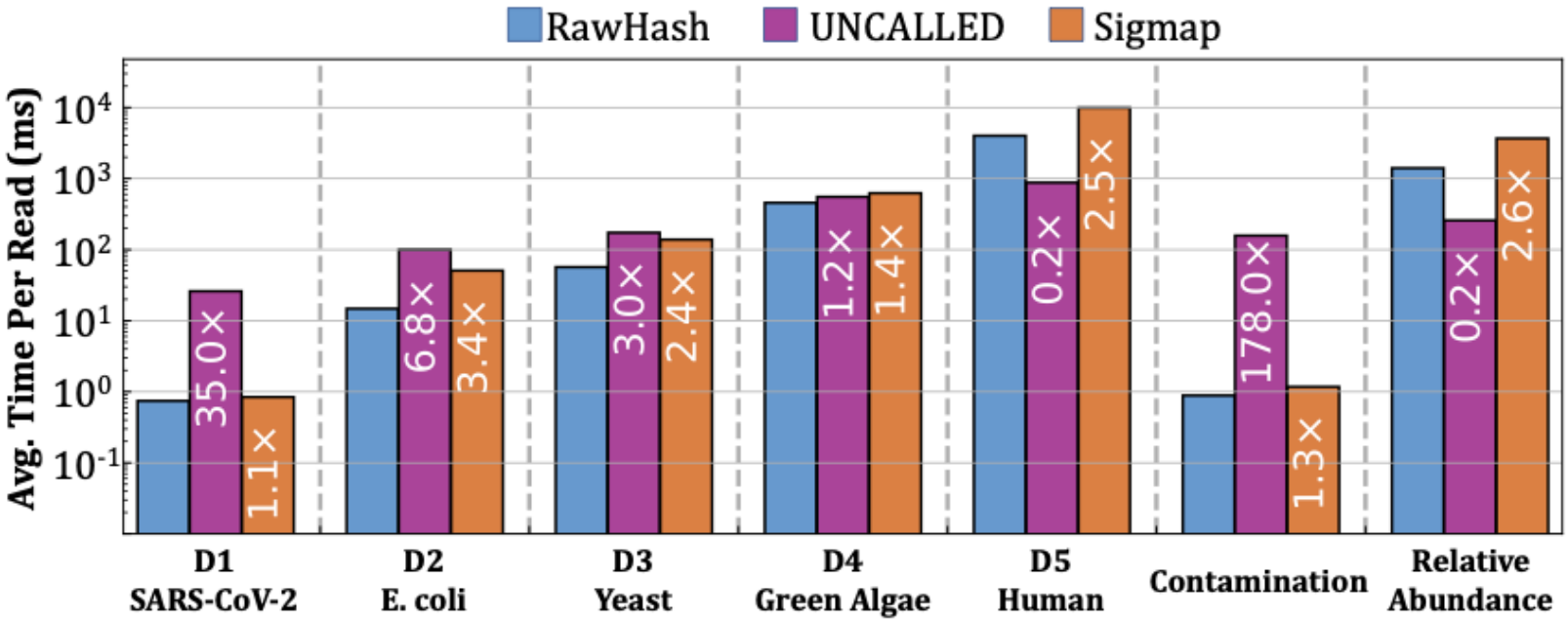
Required Computation Resources in Mapping

Tool	*Contamination*	*SARS-CoV-2*	*E. coli*	*Yeast*	*Green Algae*	*Human*	*Relative Abundance*
				CPU Time (sec)			
UNCALLED	265,902.26	36,667.26	35,821.14	8,933.52	16,769.09	262,597.83	586,561.54
Sigmap	4,573.18	1,997.84	23,894.70	11,168.96	31,544.55	4,837,058.90	11,027,652.91
RawHash	3,721.62	1,832.56	8,212.17	4,906.70	25,215.23	2,022,521.48	4,738,961.77
				Real time (sec)			
UNCALLED	20,628.57	2,794.76	1,544.68	285.42	2,138.91	8,794.30	19,409.71
Sigmap	6,725.26	3,222.32	2,067.02	1,167.08	2,398.83	158,904.69	361,443.88
RawHash	3,917.49	1,949.53	957.13	215.68	1,804.96	65,411.43	152,280.26
				Peak memory (GB)			
UNCALLED	0.65	0.19	0.52	0.37	0.81	9.46	9.10
Sigmap	111.69	28.26	111.11	14.65	29.18	311.89	489.89
RawHash	4.13	4.20	4.16	4.37	11.75	52.21	55.31

The mapping step of RawHash is **significantly faster than Sigmap** for all genomes, and **faster than UNCALLED for small genomes**

RawHash requires **larger memory space** than UNCALLED

Average Mapping Time per Read



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Parameter Configurations

Tool	*Contamination*	*SARS-CoV-2*	*E. coli*	*Yeast*	*Green Algae*	*Human*	*Relative Abundance*
RawHash	-x viral -t 32	-x viral -t 32	-x sensitive -t 32	-x sensitive -t 32	-x fast -t 32	-x fast -t 32	-x fast -t 32
UNCALLED	map -t 32						
Sigmap	-m -t 32						
Minimap2	-x map-ont -t 32						

Preset (-x)	**Corresponding parameters**	Usage
viral	-e 5 -q 9 -l 3	Viral genomes
sensitive	-e 6 -q 9 -l 3	Small genomes (i.e., $< 50M$ bases)
fast	-e 7 -q 9 -l 3	Large genomes (i.e., $> 50M$ bases)

Versions

Tool	Version	Link to the Source Code
RawHash	0.9	https://github.com/CMU-SAFARI/RawHash/tree/8042b1728e352a28fcc79c2efd80c8b631fe7bac
UNCALLED	2.2	https://github.com/skovaka/UNCALLED/tree/74a5d4e5b5d02fb31d6e88926e8a0896dc3475cb
Sigmap	0.1	https://github.com/haowenz/sigmap/tree/c9a40483264c9514587a36555b5af48d3f054f6f
Minimap2	2.24	https://github.com/lh3/minimap2/releases/tag/v2.24

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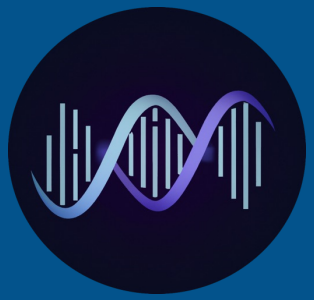

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