NanoMapper: An Efficient Read Mapping Algorithm Using Gapped Minimizer and FM-indexing for Oxford Nanopore Long Noisy Reads

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2. Why Mapper Is Needed?
3. What are the challenges?
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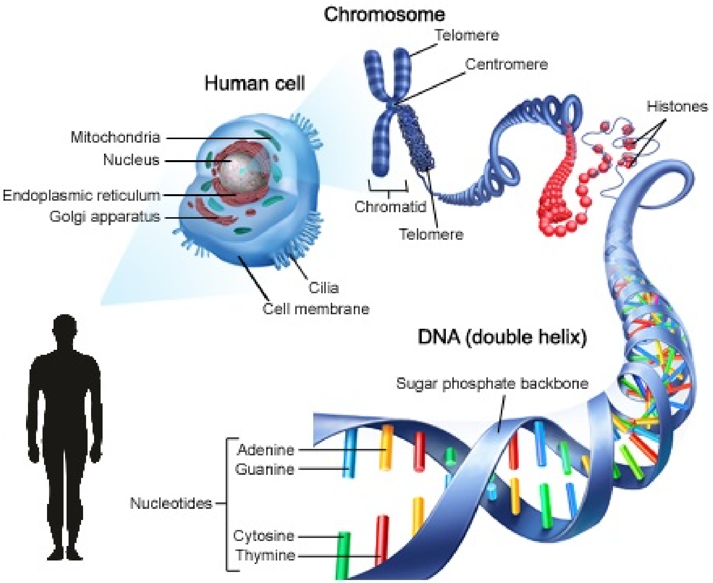
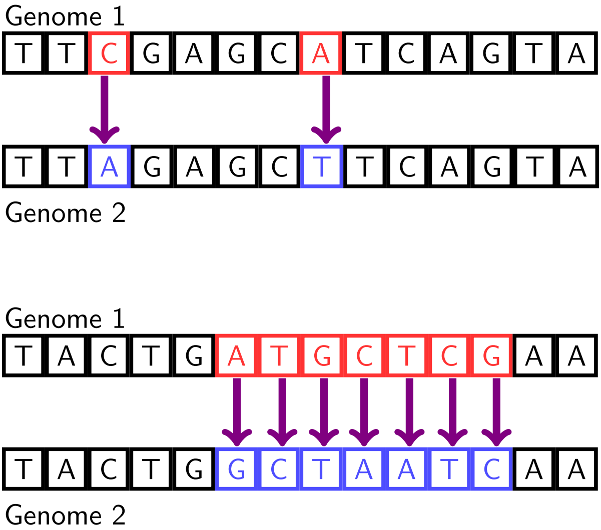
Human Cell To DNA Sequence



Mutation



Genome 1

T T C G A G C A T C A G T A

T T A G A G C T T C A G T A

Genome 2

Genome 1

T A C T G A T G C T C G A A

T A C T G G C T A A T C A A

Genome 2

Figure: Random mutations and Segment mutation.

Mapper

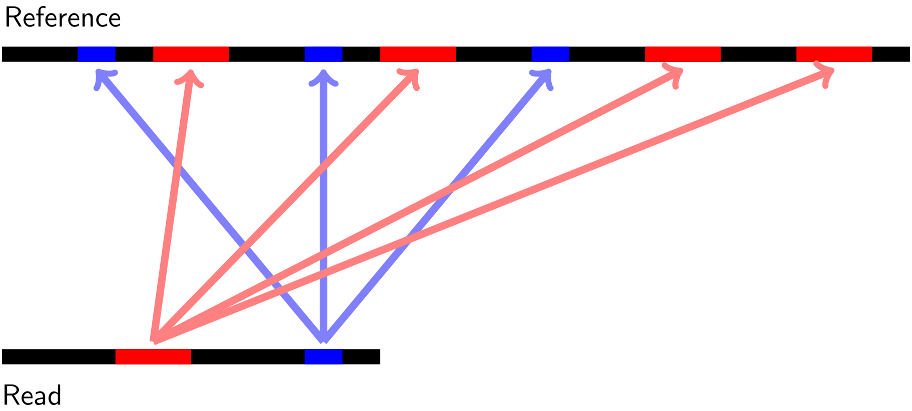


Figure: Mapping is just indicating the clusters of a large segment of the read in reference.

Aligner

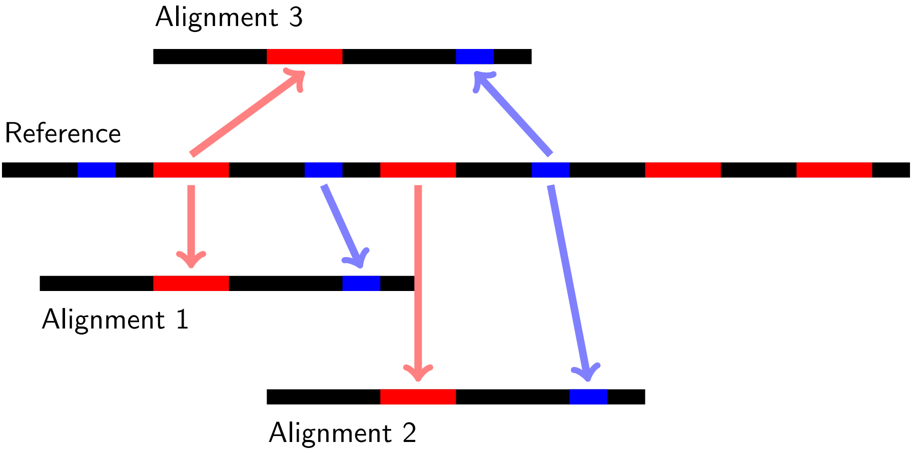


Figure: Three possible alignments based on the read mapping.

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Alignment Needs Mapping

- Variant Calling
  - Identification of causative genes , candidate genes, passenger and driver genes in many complex diseases, disorders and cancers.

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- DNA Binding
  - Finding DNA-Binding sites on specific reference genome sequence.

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  - Identification of causative genes , candidate genes, passenger and driver genes in many complex diseases, disorders and cancers.
- DNA Binding
  - Finding DNA-Binding sites on specific reference genome sequence.
- Gene Expression
  - Classification of human tumors, profiling breast cancer, Ontological analysis.

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Challenges Developing Mapper

- Illumina/Solexa Technologies Produces Short Reads – 30 - 300 BP

Challenge

Very Repetitive for Long Reference Like 3 billion BP

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- PacBio's Oxford Nanopore Technology Produces Long Reads – 10K - 60K

Challenge

The More The Length, The More The Noise

Challenges Developing Mapper

- Illumina/Solexa Technologies Produces Short Reads – 30 - 300 BP

Challenge

Very Repetitive for Long Reference Like 3 billion BP

- PacBio's Oxford Nanopore Technology Produces Long Reads – 10K - 60K

Challenge

The More The Length, The More The Noise

- Needs Huge Memory To Index Reference Sequence

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Minimap

- Used Minimizer and Min-sketch
- Tweaked for Miniasm
- Multi-threaded
- Not Tested Enough Yet

Output Format

The output format is PFA which is different than other tools.

BWA

- Two versions:
  - BWA / BWA-MEM for Illumina/Solexa reads
  - BWA-SW for Oxford Nanopore Reads of MinION instrument
- Used BWT
- Used Prefix-Trie Traversing Top-Down Fashion
- Applied Smith–Waterman-like Dynamic Programming
- Multi-threaded

Bowtie

- Two versions:
  - Bowtie
  - Bowtie 2
- Used BWT FM-index
- Quality-aware Backtracking Algorithms Enabling Mismatches
- Double Indexing – To Avoid the Excessive Use of Backtracking

MUMmer

- Three versions: 1, 2, 3
- Used Suffix-Tree

NanoBLASTer

- Used "seed-and-extend" Technique
- Dynamic Programming Based Extension Mechanism
- Faster than Leading Alignment Tools
- Less False Positive Rate

Limitation

Could not Handle Long Insertion or Deletion

Long Insertion

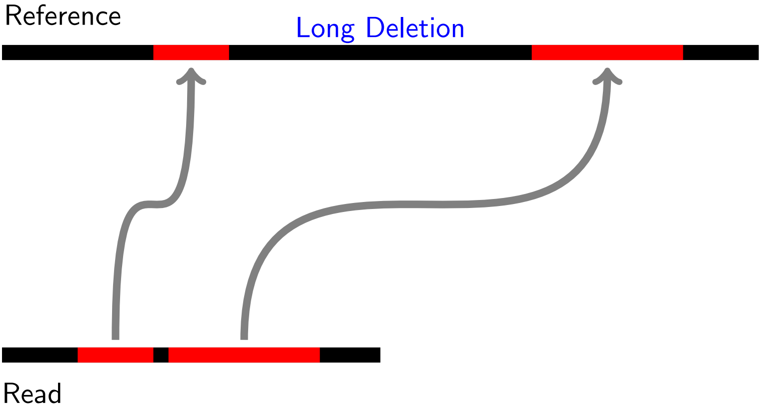

Figure: Mapping Two Long K-mers with Long Deletion in Read.

Long Deletion

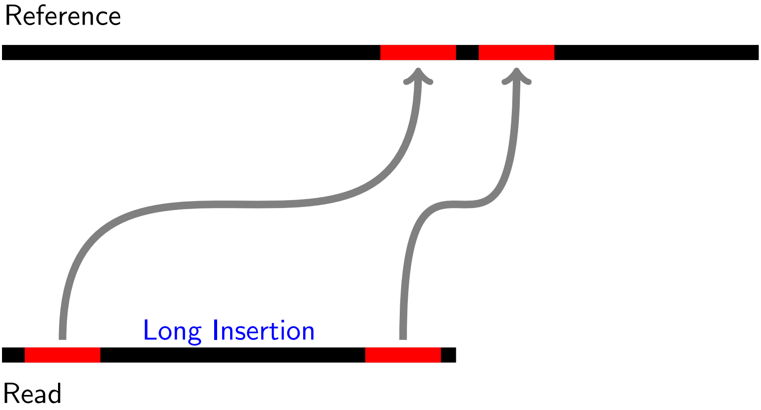

Figure: Mapping Two Long K-mer with Long Insertion in Read.

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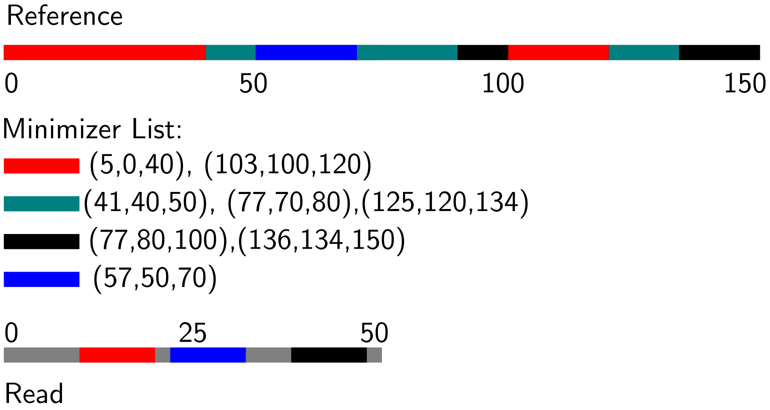
Minimizer Approach

There are two versions of this approach:

- Naive Window Technique
- Efficient Window Traversing Technique

Minimizer Approach



Minimizer List:

🟥 (5,0,40), (103,100,120)

🟩 (41,40,50), (77,70,80),(125,120,134)

⬛ (77,80,100),(136,134,150)

🟦 (57,50,70)

Figure: Mapping Minimizers.

Minimizer Approach : Result

Table: Minimizer Hits For Increasing Error Rate.

Error (%)	Found In Reference (%)	Found In Range (%)	Found Both In and Out of Range (%)
0	99.96	69.2	30.7
5	71.8	62.4	27.2
10	53.8	52.8	25.5
15	43.5	47.4	21.7
20	34.8	39.8	17.9
45	0.2	40	40

Gapped Minimizer

Problem

The More The Error, The More The Mismatches

Gapped Minimizer

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The More The Error, The More The Mismatches

Solution

Insert Gap in Both Reads and Reference To Neutralize Mismatches At Every Certain Number of Bases.

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Remark

In NanoMapper, Gaps are Added Every Third Base Assuming 33% Error.

Example: `ATCTGGTAATCATAGCGTAC`

With Gap: `AT_TG_TA_TC_TA_CG_AC`

BWT FM-index Approach

This approach also has two versions:

- Naive Approach
- Enhanced Approach

BWT FM-index Approach : Method

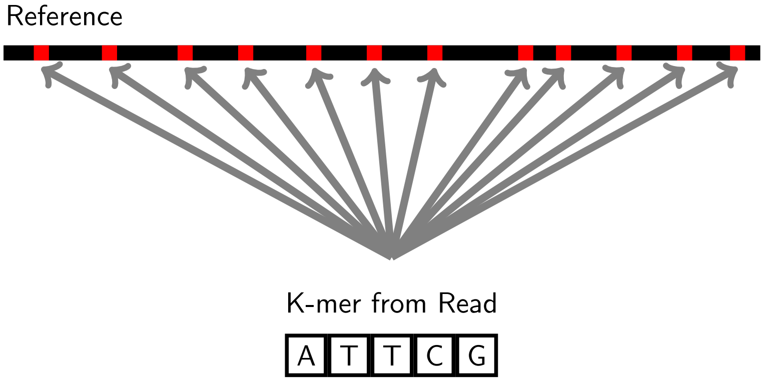
1. Index the Reference Genome
2. Take a Read
3. Take the Next K-mer. If There is not Any, Go to Step 9

9. Exit

BWT FM-index Approach : Method

1. Index the Reference Genome
2. Take a Read
3. Take the Next K-mer. If There is not Any, Go to Step 9
4. If it is not in the Reference, Go to Step 3
5. Let $i = 1$
6. If $(K + i)$-mer does not Exist, Go to Step 8
7. Do $i = i + 1$ and Go to Step 6.
8. Write the Locations of $(K + i - 1)$-mer To the Output and Go to Step 3
9. Exit

BWT FM-index Approach : Method



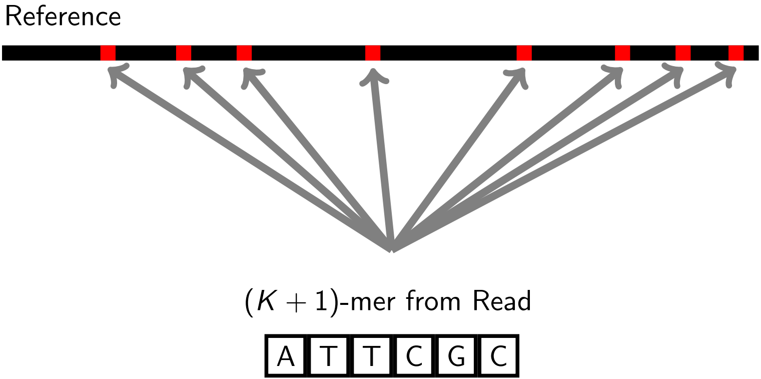
Reference

K-mer from Read

A T T C G

Figure: A K-mer with Value $K = K_{min}$ is Picked Up from Read and Indicated Where The K-mer is Found in the Reference.

BWT FM-index Approach : Method



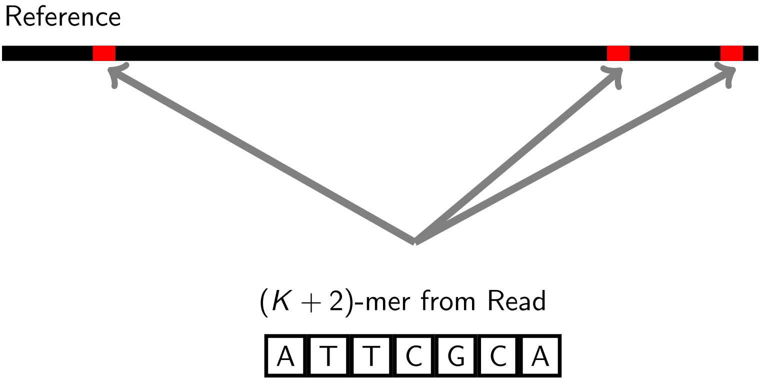
Reference

$(K+1)$-mer from Read

A T T C G C

Figure: Extending One Base in K-mer, The Locations of $(K+1)$-mer in the Reference is Reduced.

BWT FM-index Approach : Method



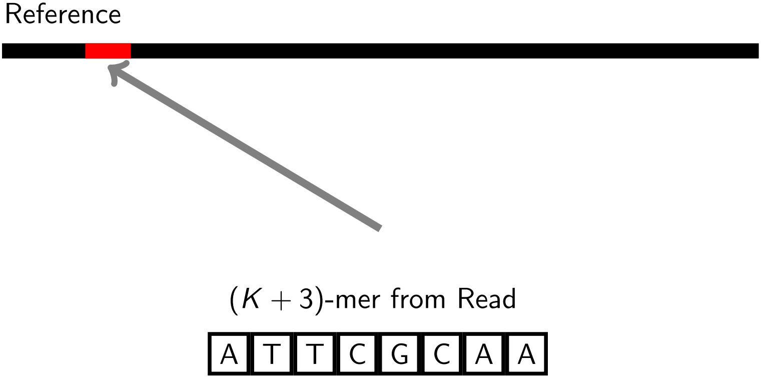
Reference

$(K + 2)$-mer from Read

A	T	T	C	G	C	A

Figure: Extending One More Base , The Locations of $(K + 2)$-mer in the Reference is Reduced And Now It is Only 3.

BWT FM-index Approach : Method



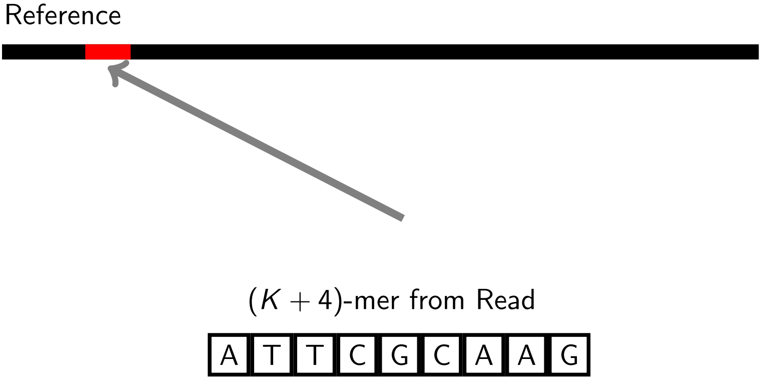
Reference

$(K+3)$-mer from Read

A	T	T	C	G	C	A	A

Figure: Continuing the Extension, $(K+3)$-mer is Created. The Count in Reference is Only One.

BWT FM-index Approach : Method



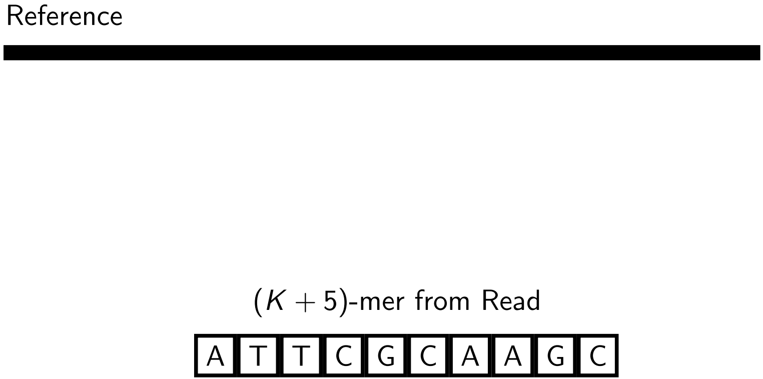
Reference

$(K+4)$-mer from Read

A T T C G C A A G

Figure: $(K+4)$-mer is Made By Appending One Base From Read. It has No Consequence in The Count in Reference.

BWT FM-index Approach : Method



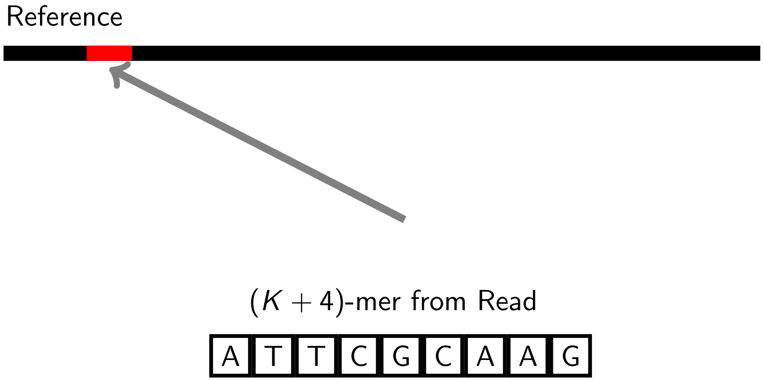
Reference

$(K+5)$-mer from Read

A	T	T	C	G	C	A	A	G	C

Figure: One Base Extension in $(K+4)$-mer, There is No Existence of $(K+5)$-mer in Reference. So, the Locations Got From $(K+4)$-mer Would be Considered as Final.

BWT FM-index Approach : Method



Reference

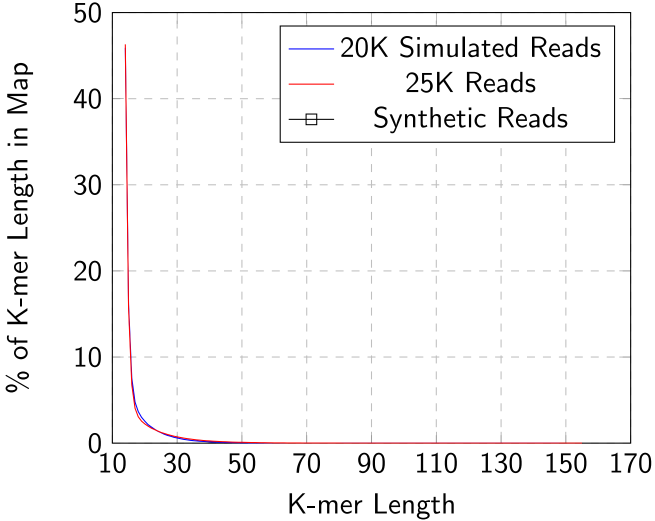
$(K + 4)$-mer from Read

A	T	T	C	G	C	A	A	G

Figure: $(K + 4)$-mer is Made By Appending One Base From Read. It has No Consequence in The Count in Reference.

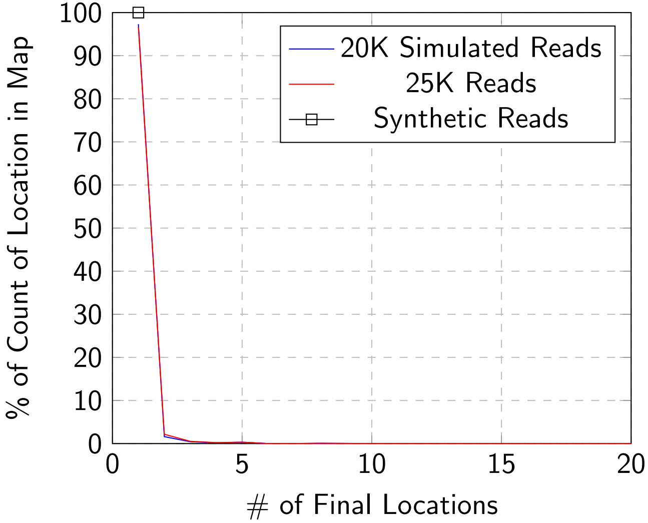
BWT FM-index Approach : Result

Which Length of K-mer Dominates the Mapping by What Percentage



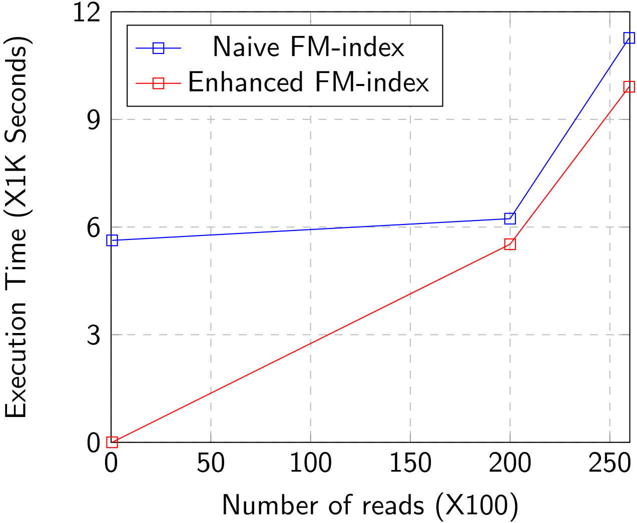
BWT FM-index Approach : Result

# of Locations Where the Final K-mers are Found VS Their Percentage



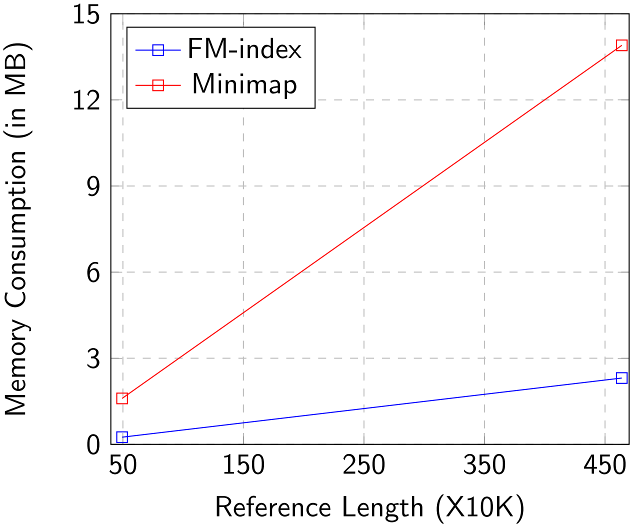
BWT FM-index Approach : Naive vs Enhanced

Exec. Time Comparison Between Naive vs Enhanced FM-index Approach



BWT FM-index Approach vs Minimap

Memory Requirement for Indexing: FM-index Approach vs Minimap



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Hotchpotch Recipe

- Minimizer
- Window Technique

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- Window Technique
- Gap Insertion
- Enhanced BWT FM-index

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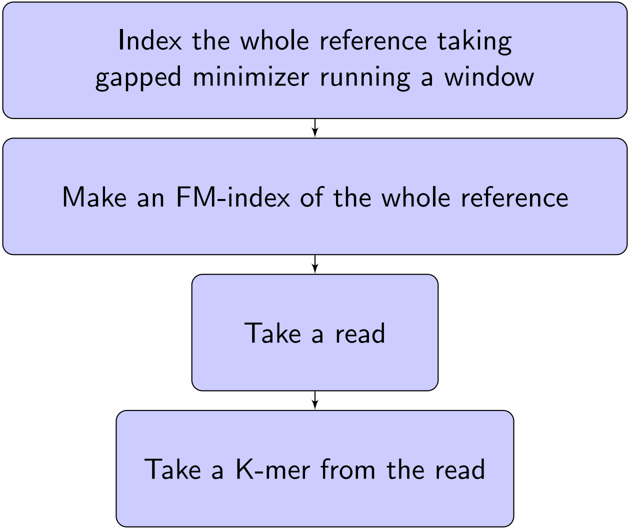
NanoMapper

A Hot Hotchpotch having All Above Characteristics

NanoMapper



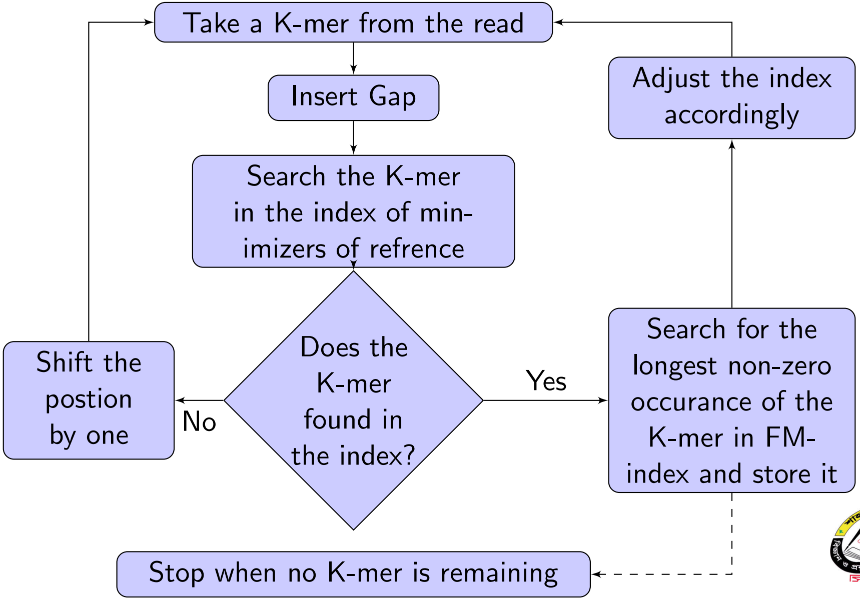
Index the whole reference taking gapped minimizer running a window

Make an FM-index of the whole reference

Take a read

Take a K-mer from the read

NanoMapper



Take a K-mer from the read

Insert Gap

Search the K-mer in the index of minimizers of refrence

Does the K-mer found in the index?

No

Shift the postion by one

Yes

Search for the longest non-zero occurance of the K-mer in FM-index and store it

Adjust the index accordingly

Stop when no K-mer is remaining

NanoMapper : Result

Table: Summary of Processing Reference genome

No	Reference Name	Reference Length	# of Minimizer	Indexing Time (Mini.)	Indexing Time (FM)	Memory Usage (MB)
1	E.Coli	4639211	682246	0.46	*1.76*	1.67
2	Synthetic	493290	12225	0.04	*0.16*	0.18

NanoMapper : Result

Table: Summary of Processing Read Sequences

No	Name of Data Set	Total Length of Reads	Time to Map (Naive)	Time to Map (Enhanced)
1	20K Simulated Reads	118335765	*19538.9 (5h 26m)*	*17051 (4h 44m)*
2	25K Reads	216906558	*9408.94 (2h 37m)*	*9869.78 (2h 45m)*
3	Synthetic Reads	500000	*3867.97 (1h 5m)*	*0.90*

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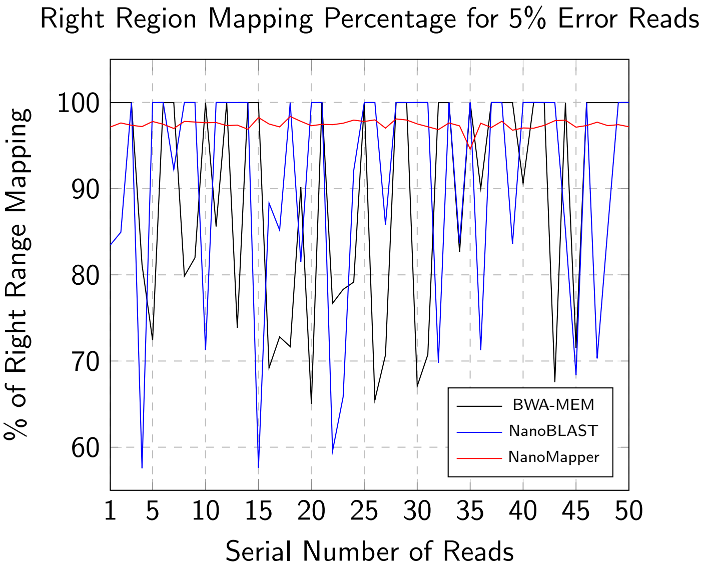
NanoMapper : Accuracy

Table: Mapping Comparison While 5% Error Added in Read Data

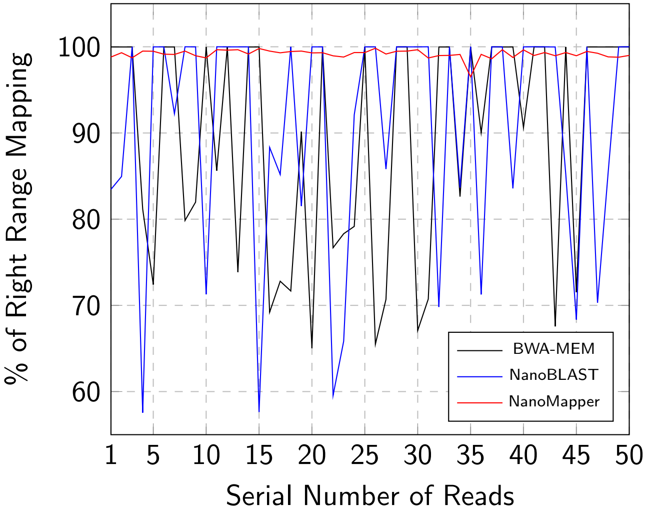
Name of the Tool	Right Position (%)	Wrong Position (%)
BWA-MEM	88.4	11.6
NanoBLAST	86.55	13.45
NanoMapper	97.42	2.58

NanoMapper : Accuracy



NanoMapper : Accuracy

Eliminating K-mers Having Length $\leq$ 15



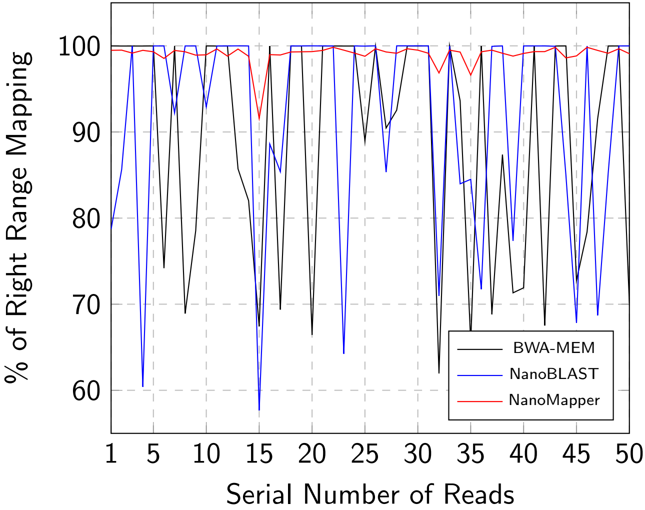
NanoMapper : Accuracy

Table: Mapping Comparison While 10% Error Added in Read Data Eliminating K-mer Having Length ≤ 15.

Name of the Tool	Right Position (%)	Wrong Position (%)
BWA-MEM	88.82	11.18
NanoBLAST	88.72	11.28
NanoMapper	99.02	0.98

NanoMapper : Accuracy

10% Error Reads Eliminating K-mers Having Length ≤ 15



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Future Work

- API Enhancement

Future Work

- API Enhancement
- Integration with NanoBLASTer

Future Work

- API Enhancement
- Integration with NanoBLASTer
- Testing

Future Work

- API Enhancement
- Integration with NanoBLASTer
- Testing
- Developing New Aligner

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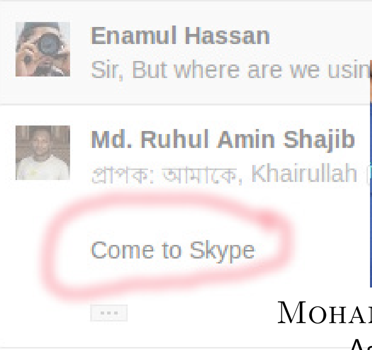
Special Thanks



Enamul Hassan

Sir, But where are we usin n of vectors? Are not we taking t...

Md. Ruhul Amin Shajib

প্রাপক: আমাকে, Khairullah

Come to Skype

...

Mohammad Ruhul Amin

Assistant Professor,

Department of Computer Science and Engineering

Shahjalal University of Science and Technology

Thank You