An Approach To Measure Quality among Multiple Alignments and Align Oxford Nanopore Single Molecule Sequence Reads

By

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What is Sequence Alignment

Sequence alignment is a way to arrange several sequences of DNA or RNA to identify similar regions that may have consequences on relationships among the sequences.

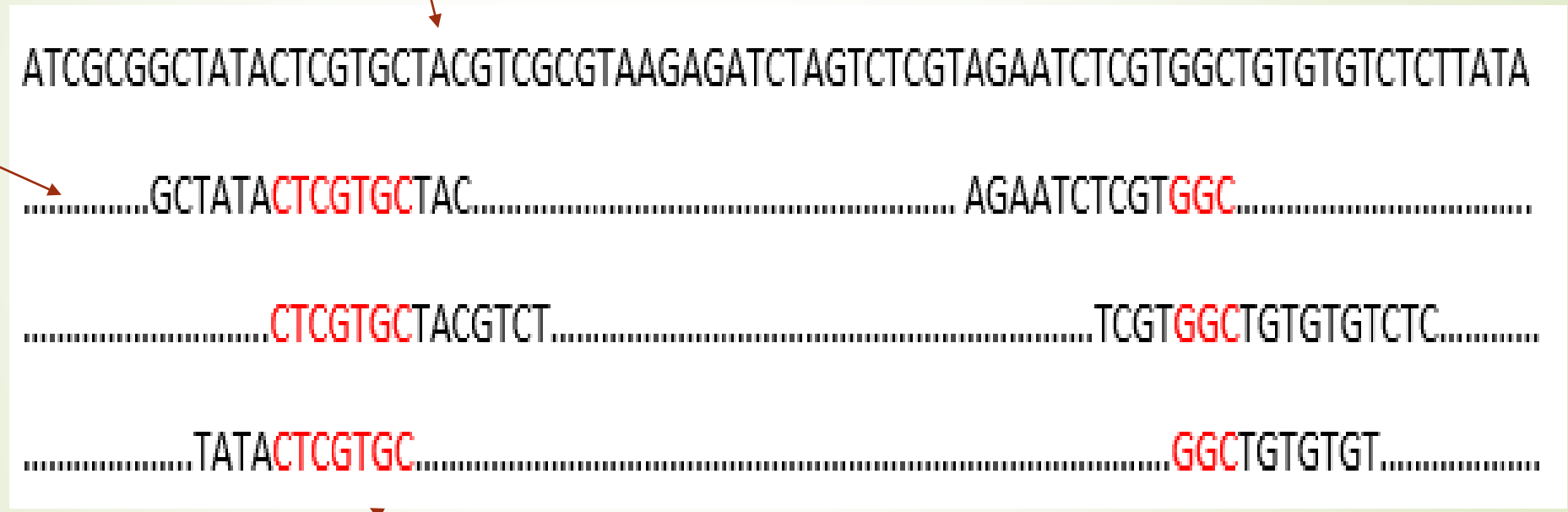
Sequence Alignment

- Global Alignment
- Local Alignment

Our task on Sequence Alignment

- Here we are working on finding similarity between a reference genome and some reads.

Reference Genome



Short Reads

Common Area
Coverages

Sequence Alignment Mapping

- Sequence Alignment Mapping (SAM) is a standard format to show how a tool makes alignment on several reads with any other genome reference.

Sequence Alignment Mapping File Format Specifications

Col	Field	Type	Regexp/Range	Brief description
1	QNAME	String	`[!-?A-~]{1,254}`	Query template NAME
2	FLAG	Int	$[0,2^{16}-1]$	bitwise FLAG
3	RNAME	String	`\*|[!-()+-<>-~][!-~]*`	Reference sequence NAME
4	POS	Int	$[0,2^{31}-1]$	1-based leftmost mapping POSition
5	MAPQ	Int	$[0,2^{8}-1]$	MAPping Quality
6	CIGAR	String	`\*|([0-9]+[MIDNSHPX=])+`	CIGAR string
7	RNEXT	String	`\*|=|[!-()+-<>-~][!-~]*`	Ref. name of the mate/next read
8	PNEXT	Int	$[0,2^{31}-1]$	Position of the mate/next read
9	TLEN	Int	$[-2^{31}+1,2^{31}-1]$	observed Template LENgth
10	SEQ	String	`\*|[A-Za-z=.]+`	segment SEQuence
11	QUAL	String	`[!-~]+`	ASCII of Phred-scaled base QUALity+33

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255
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Tools To Evaluate SAM Files

- Qualimap
- Picard tools
- RNA-SeQC
- RSeQC

	Picard tools	RNA-SeQC	RSeQC	**Qualimap**
Scope	Various NGS applications	RNA-seq	RNA-seq	Various NGS applications
User interface	Command-line	GUI + Command-line	Command-line	GUI + Command-line
Input formats	Alignment: BAM; annotations: RefSeq; reference: FASTA	Alignment: BAM; annotations: GTF; reference: FASTA	Alignment: BAM; annotations: BED.	Alignment: BAM; annotations: GFF/GTF or BED
Output	Raw data and plots. Different for each tool	Summary report including both html reports and raw data	Raw data and plots. Different for each tool	Summary report (HTML or PDF) and raw data
Alignment statistics	Yes	Partially	Partially	Yes
Overall Summary	No	Yes	No	Yes
Coverage analysis	Genome-wide or genes	Genes and transcripts	Genes and transcripts	Genome-wide or arbitrary regions
Average gene 5'-3' coverage plot	No	Yes	Yes	No
Counts computation	No	Yes	Yes	Yes
Insert size estimation	Yes	Yes	Yes	Yes
Sequence quality	No	Yes	Yes	Yes
Multiple samples support	No	Yes	No	2 samples

Analysis On NanoBLASTer

- Evaluation of corresponding SAM file

Analysis On NanoBLASTer

- Evaluation of corresponding SAM file
- Analysis on results

Analysis On NanoBLASTer

- Evaluation of corresponding SAM file
- Analysis on results
- Experimenting new method on tool's used algorithm

Analysis On NanoBLASTer

- Evaluation of corresponding SAM file
- Analysis on results
- Experimenting variations on tool's used algorithm
- Analysis results on experimental changes

Data Set

- We took 1000 reads from Oxford Nanopore Single Molecule Reads with minimum 100 length and maximum of 1000 length.

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Scale Measurement Definition

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- POI = Total number of matches/ (Total Matches + Total Penalties)

Scale Measurement Definition

- Counted-
- Total Matches
- Total Mismatches
- Total Insertions
- Total Deletions

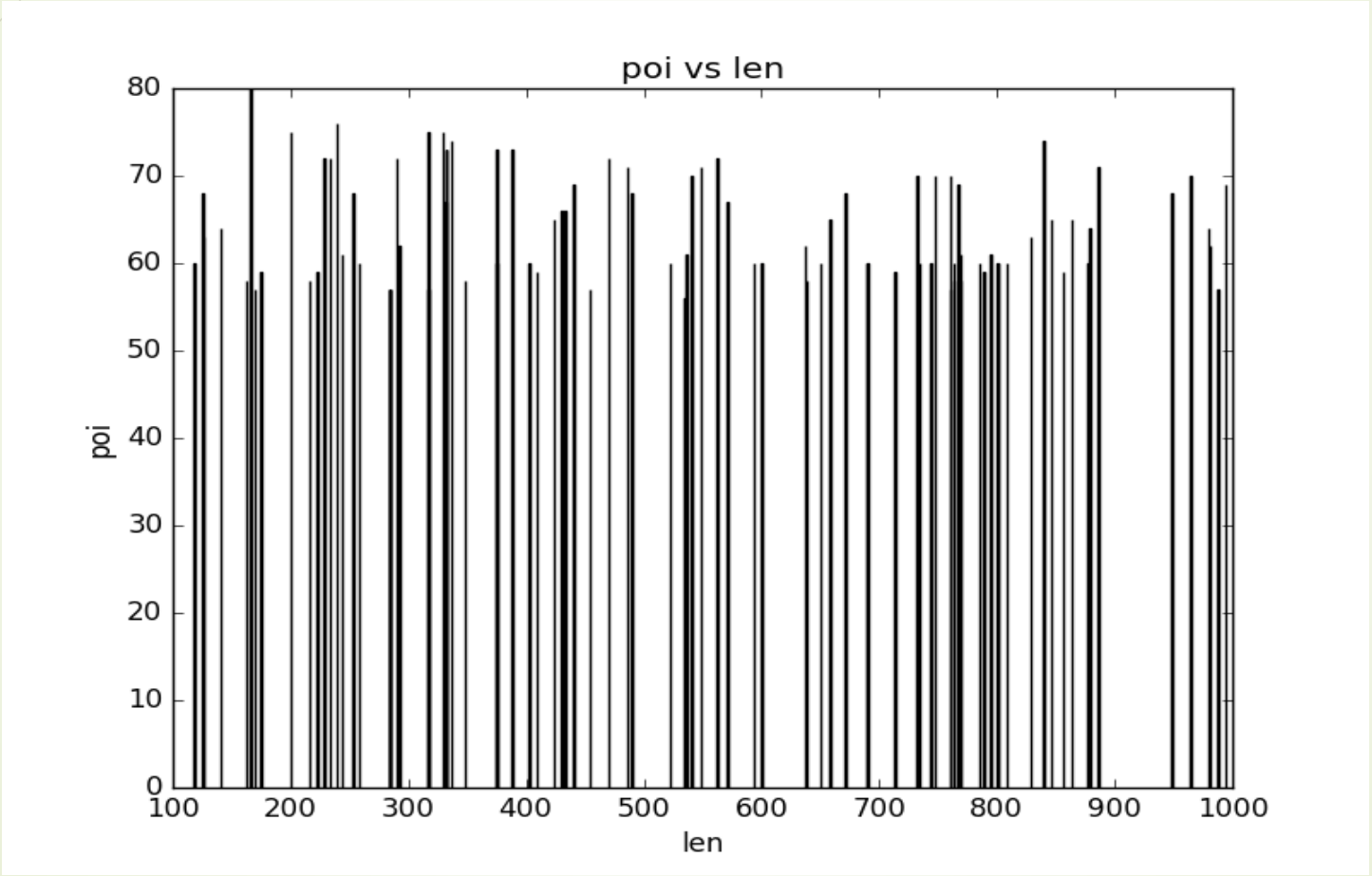
Measuring Quality

- Measured POI of sample data and compared with implemented alignment POI
- If POI (from implemented alignment) > POI (from sample data) then quality falls otherwise it will be called better in this scale

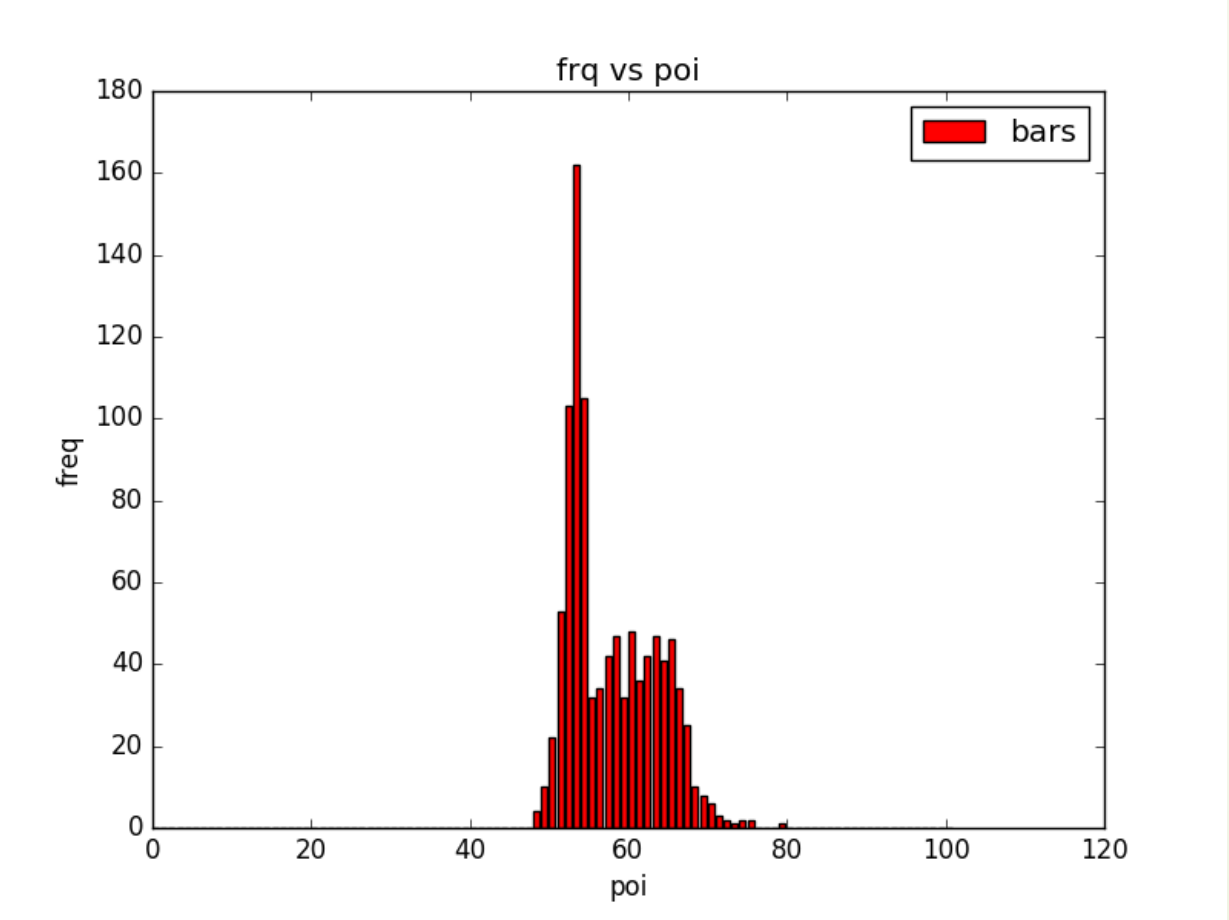
Graph Plotting

- X - axis is POI from 1 to 100
- Y - axis is frequency of POIs'

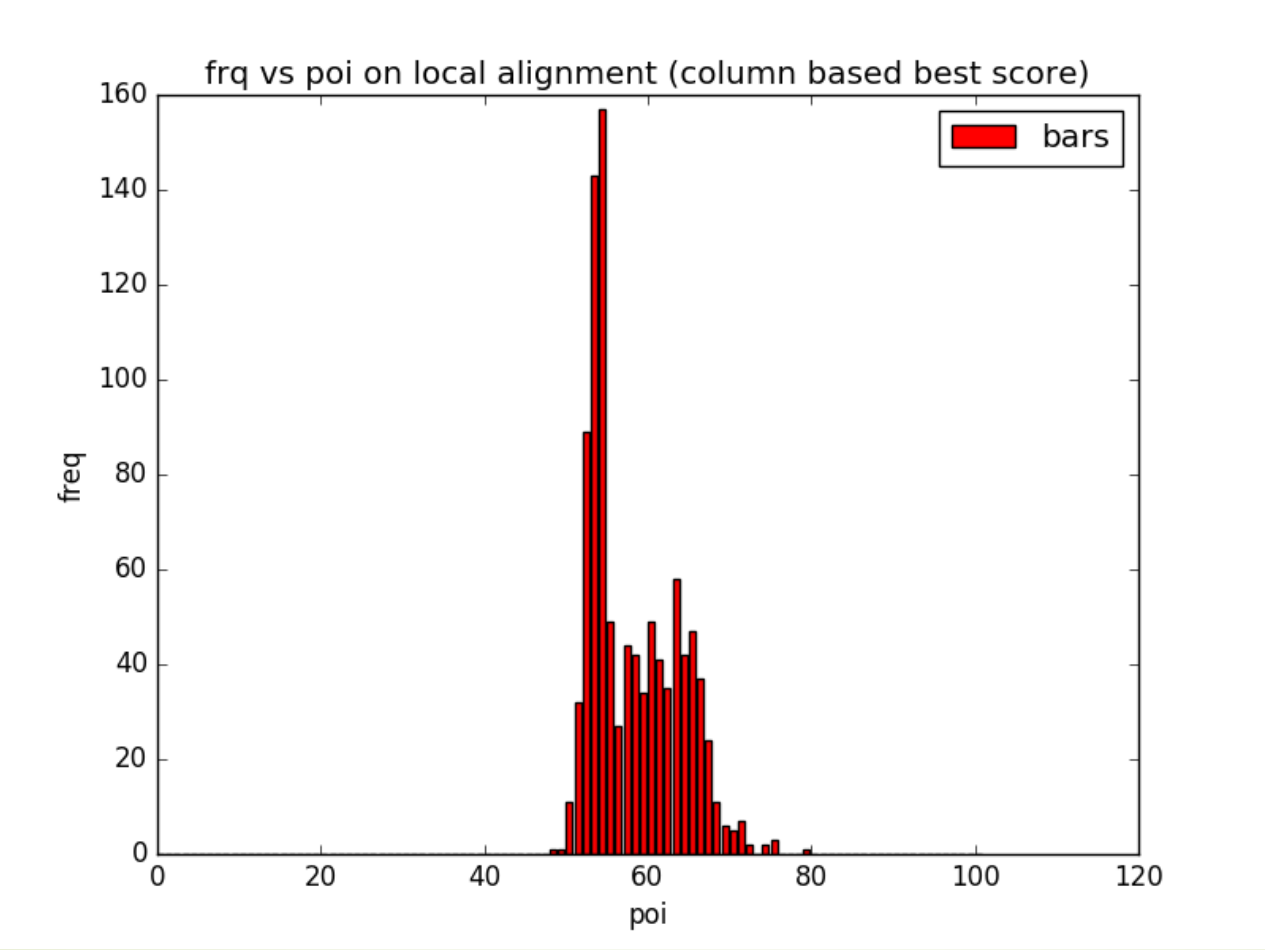
Max POI vs. Length



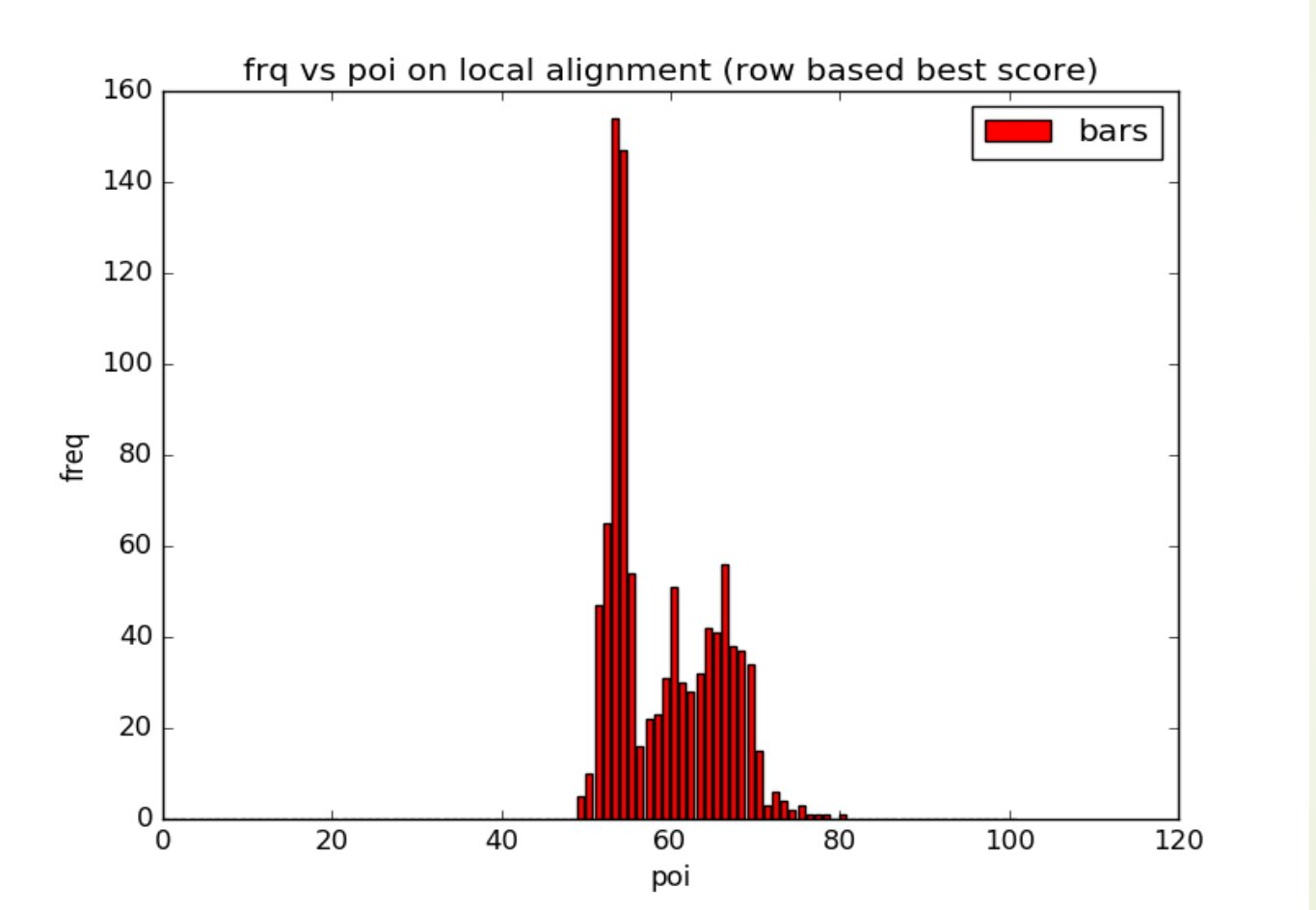
Frequency vs. POI (Global Alignment)



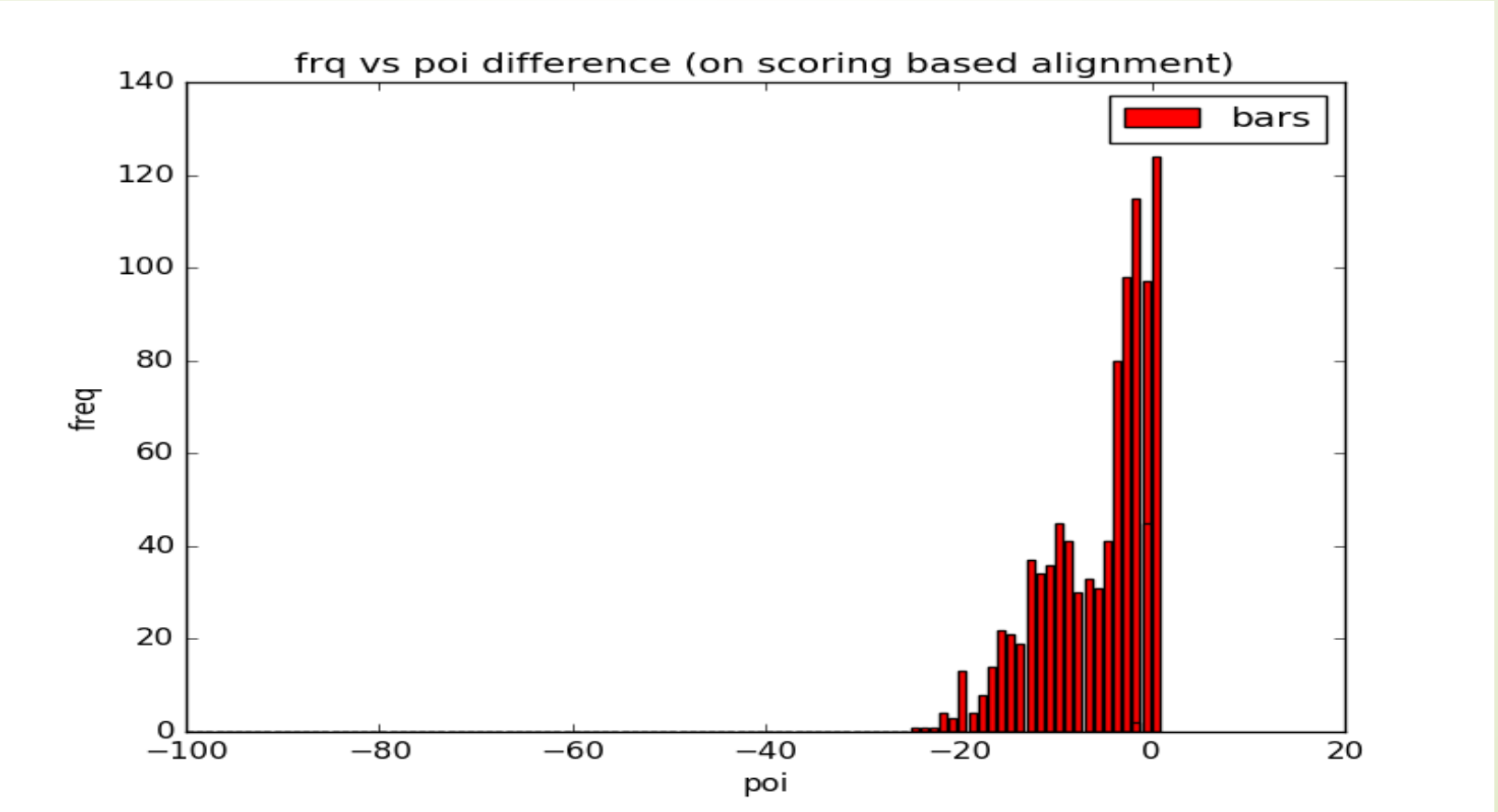
Local Alignment (Considering Column)



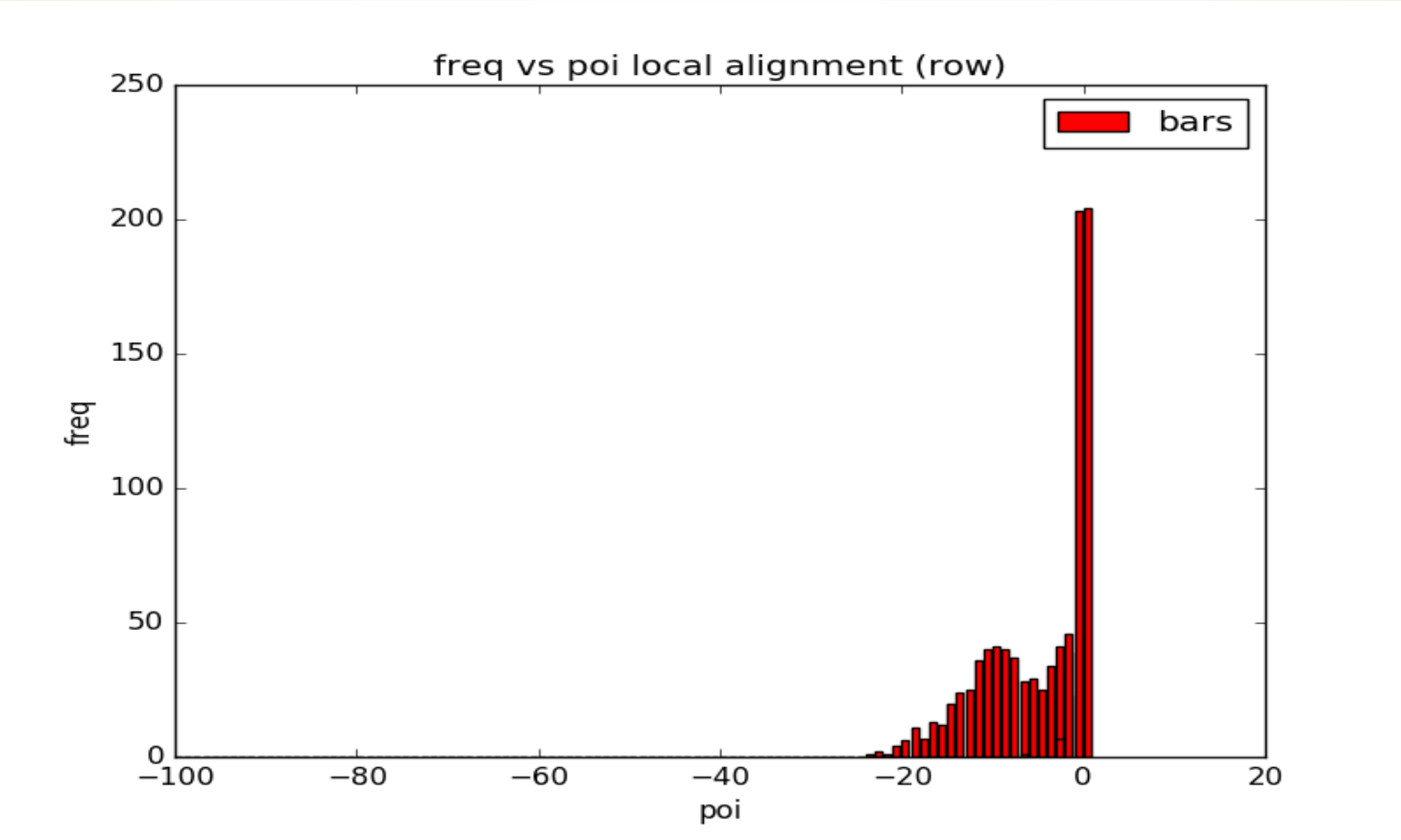
Local Alignment (Considering Row)



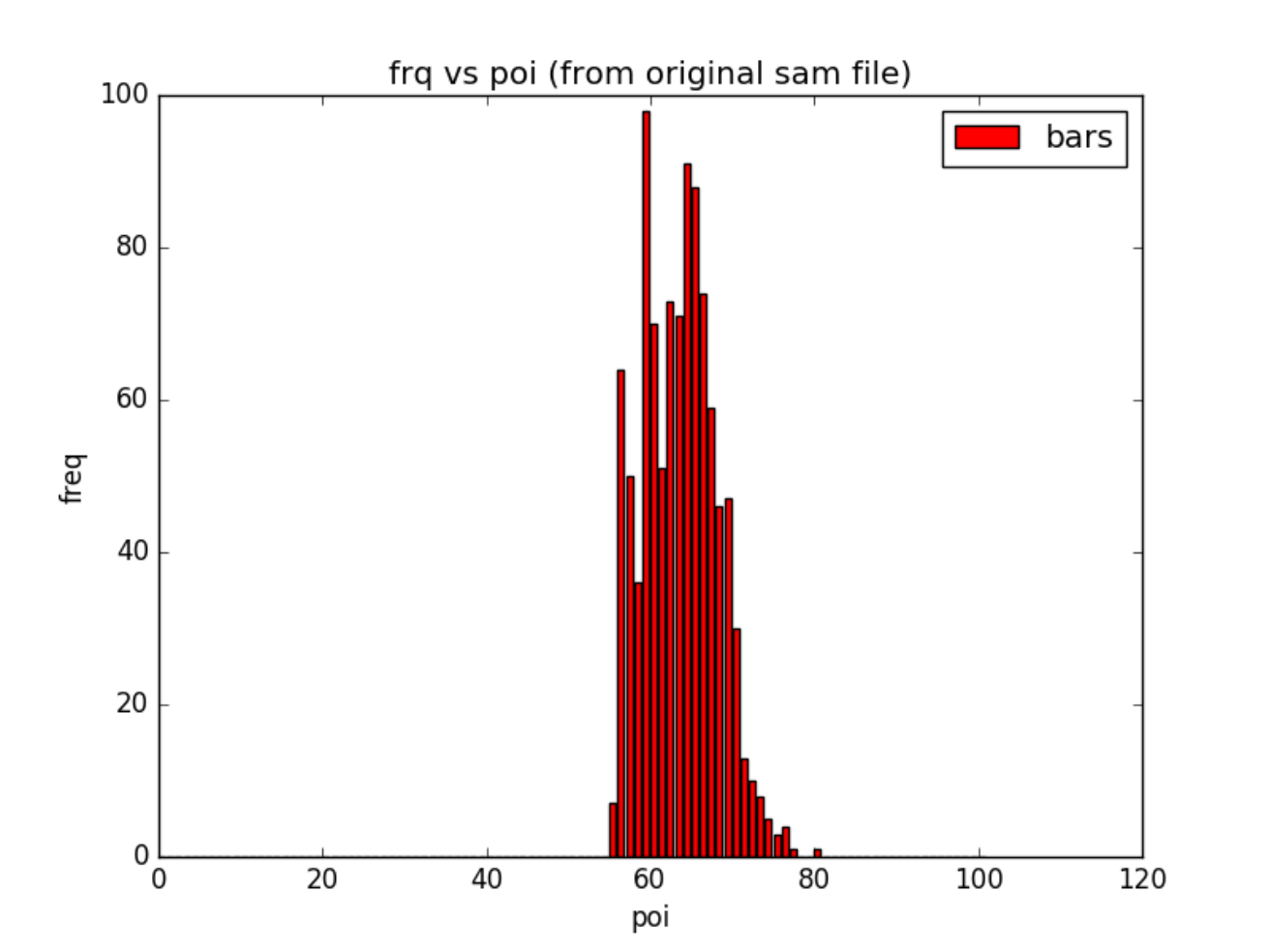
Difference with Implemented Global Alignment



Difference with Implemented Local Alignment



Freq. vs. POI (from original Input Data)



Experimented Method

- Mapping reference genome into several K-mers

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- Mapping reads into several K-mers

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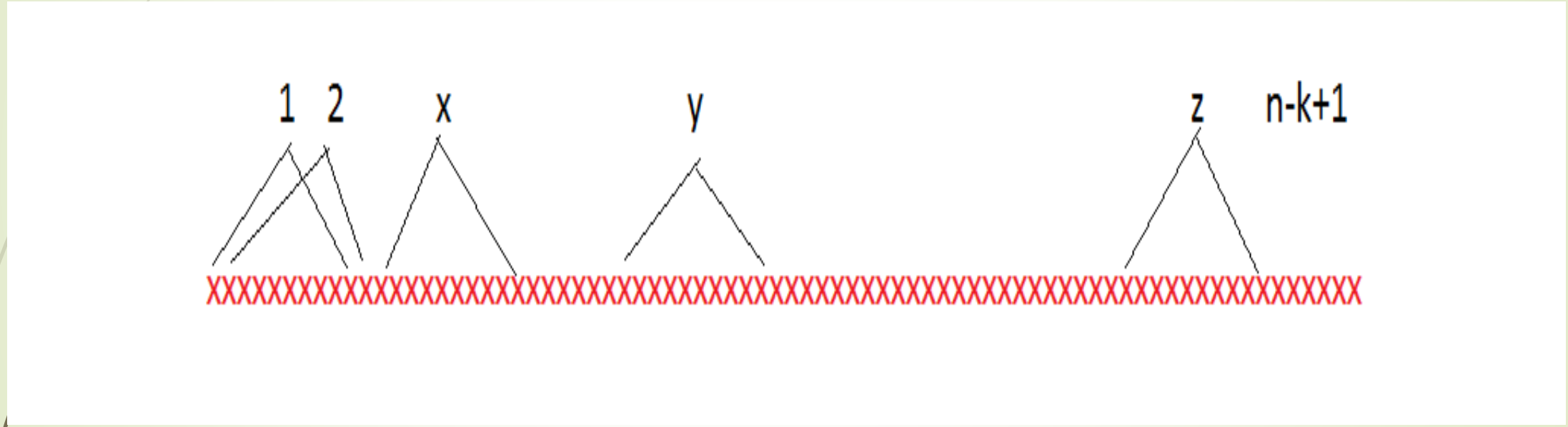
- Mapping reference genome into several K-mers
- Mapping reads into several K-mers
- Preparing 2-D vector with the mapped values with matching considering threshold values.

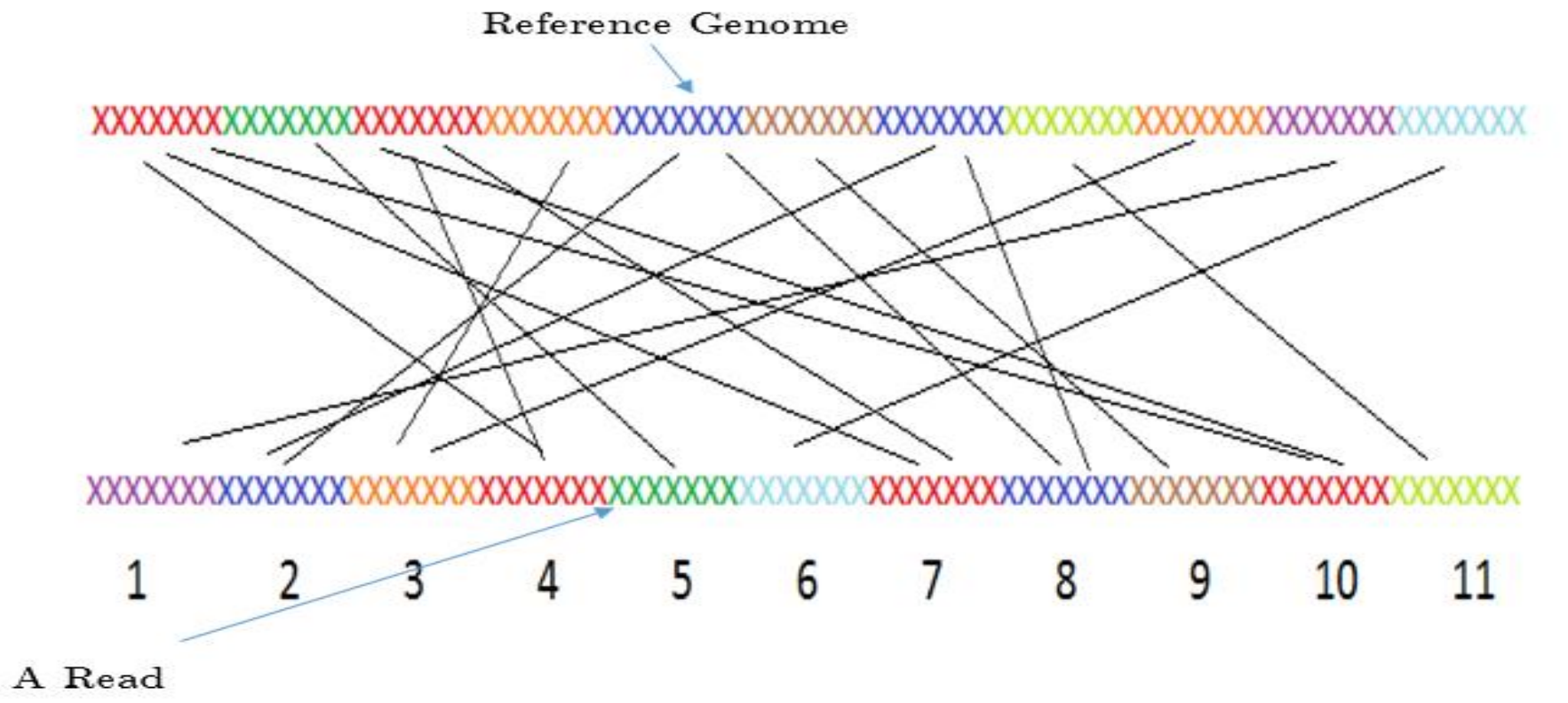
Mapping Reference Genome



Mapping Reference Genome

1	xxxxxxx
2	xxxxxxx
3	xxxxxxx
4	xxxxxxx
5	xxxxxxx
n-k-1	xxxxxxx
n-k	xxxxxxx
n-k+1	xxxxxxx

Preparing 2-D Vector

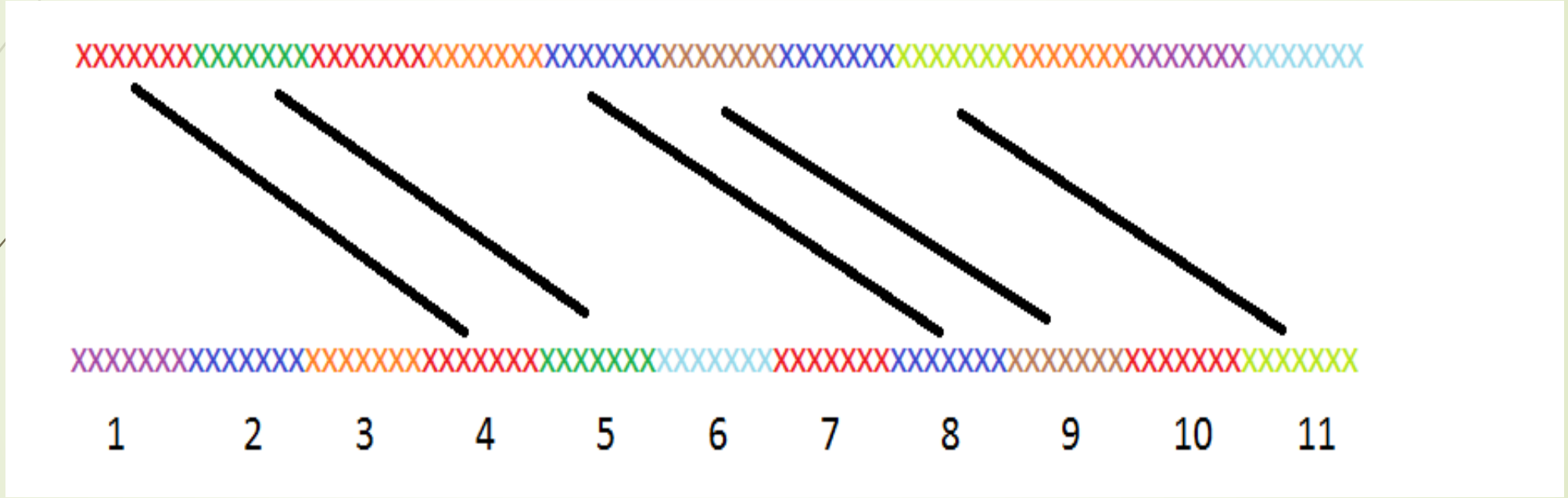


K-Mer starting positon from Reference Genome	K-Mer starting position from a Read
1	4
1	7
1	10
2	5
3	4
3	7
3	10
4	3
5	2
5	8
6	9
7	2
7	8
8	11
9	3
10	1
11	6

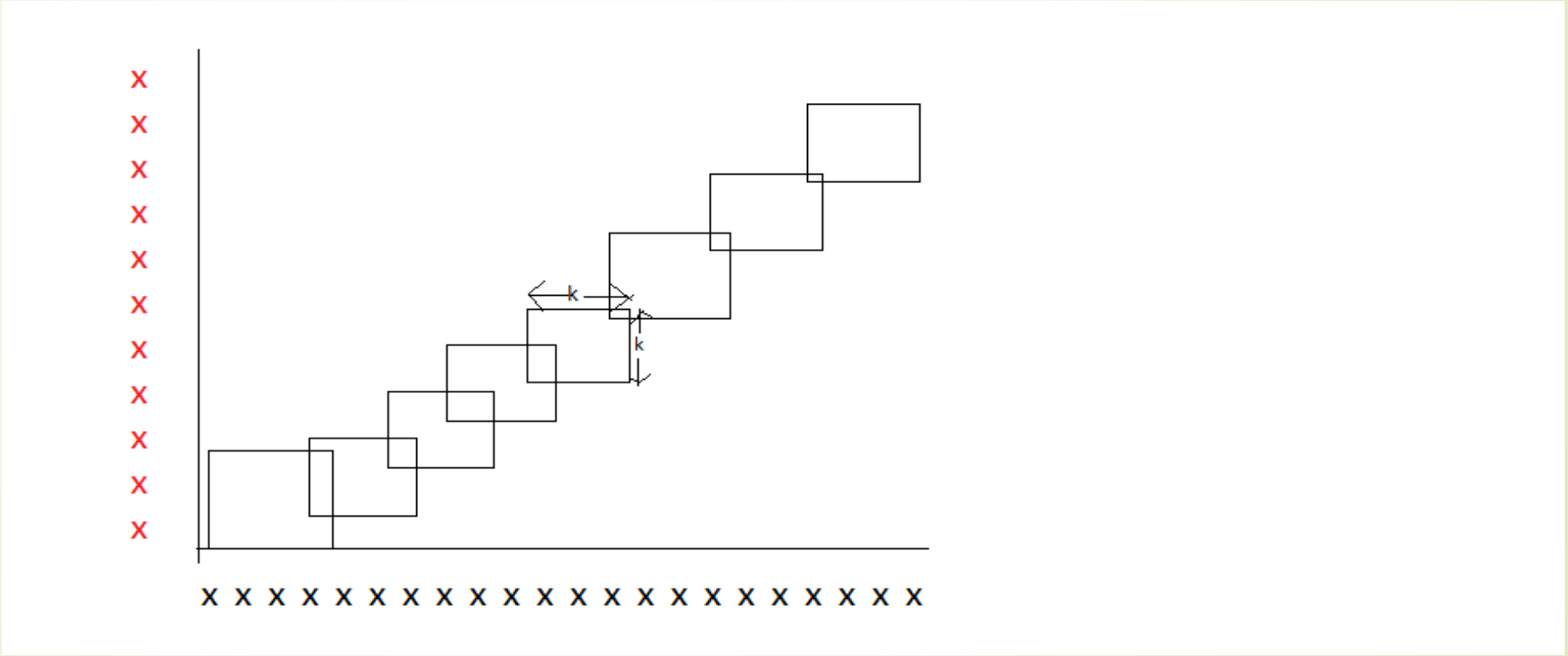
Result After Running Longest Increasing Subsequence Algorithm on the Vector

K-Mer starting positon from Reference Genome	K-Mer starting position from a Read
1	4
2	5
5	8
6	9
8	11

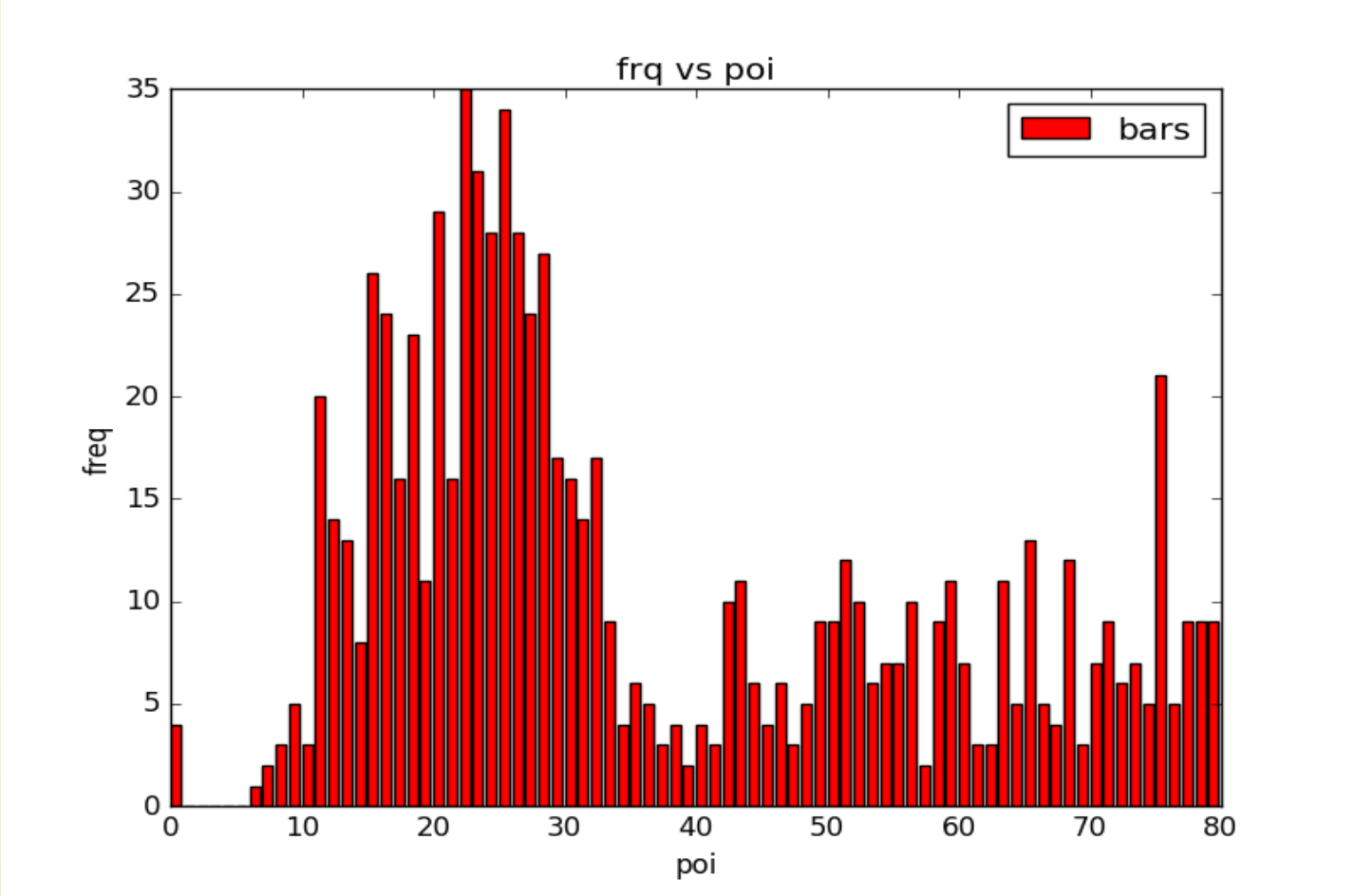
Visualizing LIS result



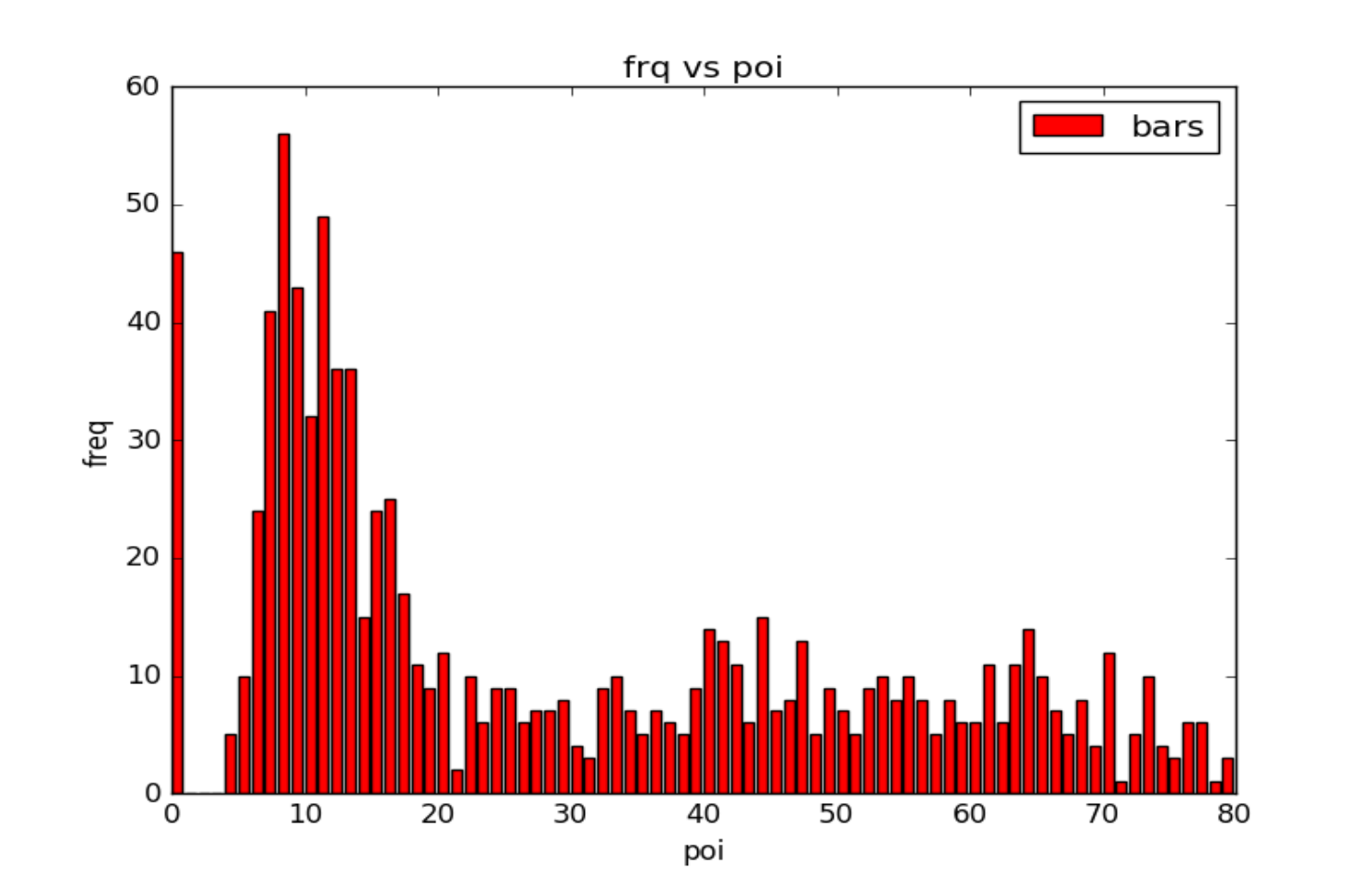
Block Alignment On Unaligned Areas



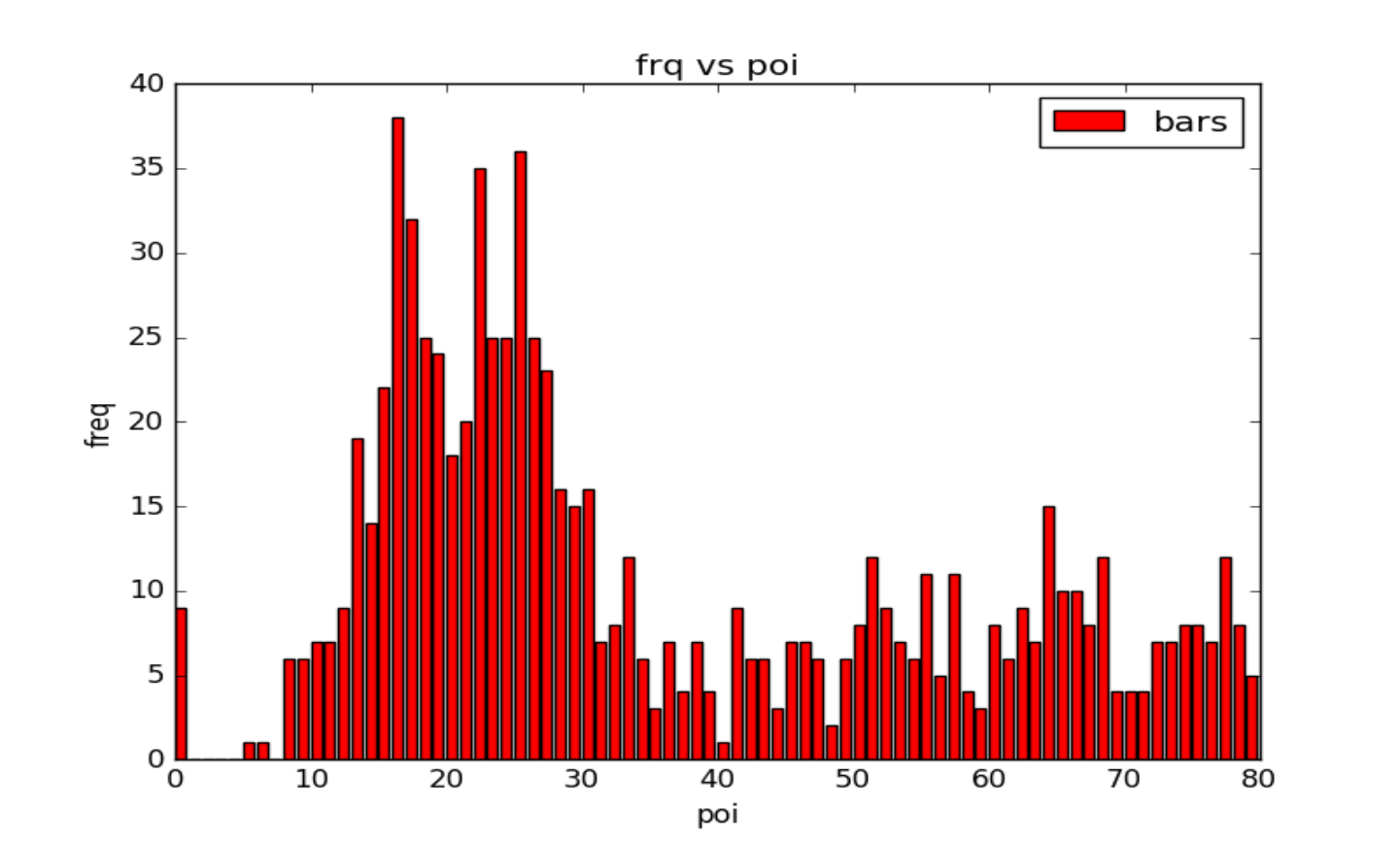
For k=11


frq vs poi
bars
freq
poi
0
5
10
15
20
25
30
35
0
10
20
30
40
50
60
70
80

For k=12



For k=13


frq vs poi
bars
freq
poi
0
5
10
15
20
25
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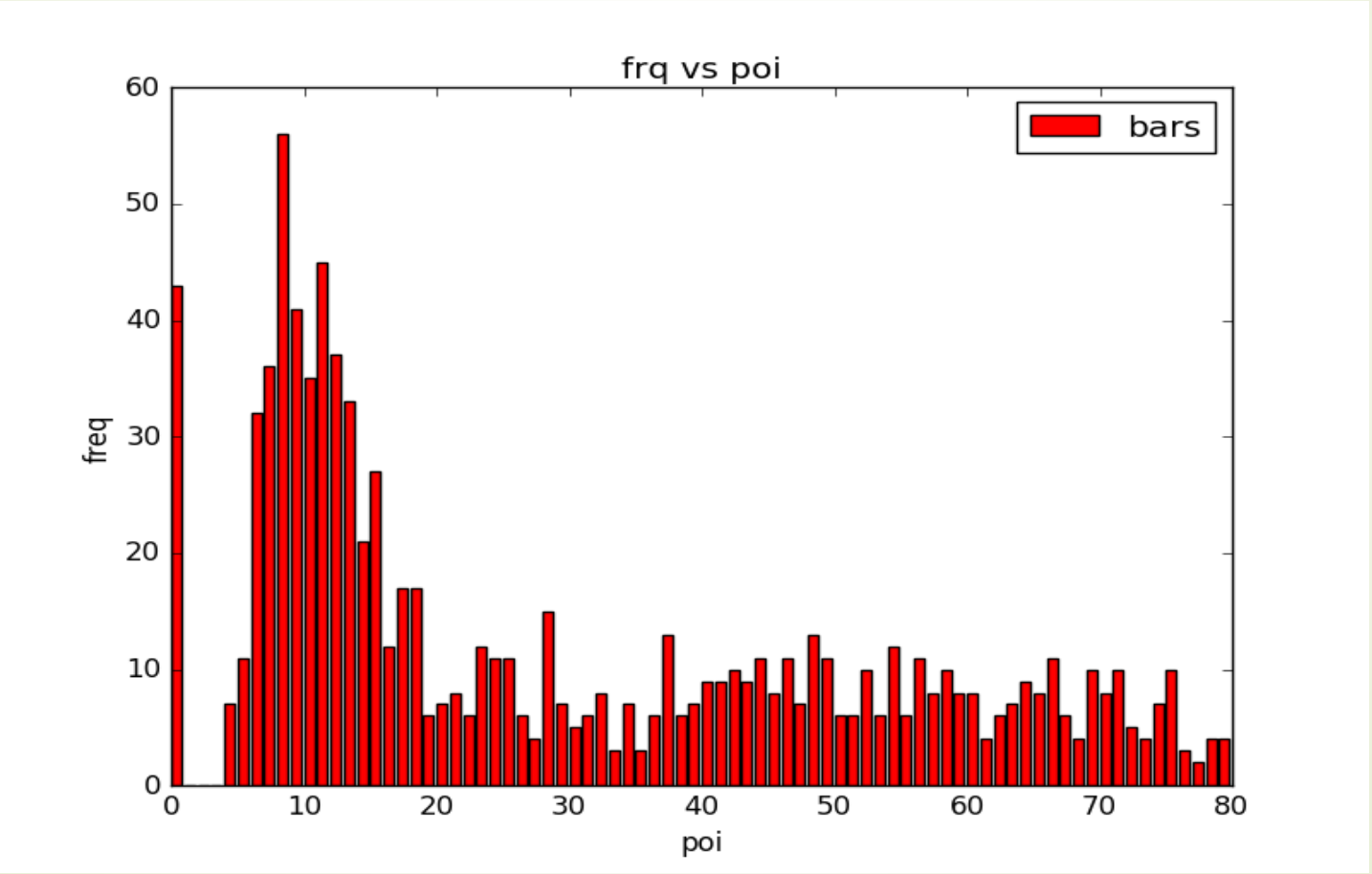
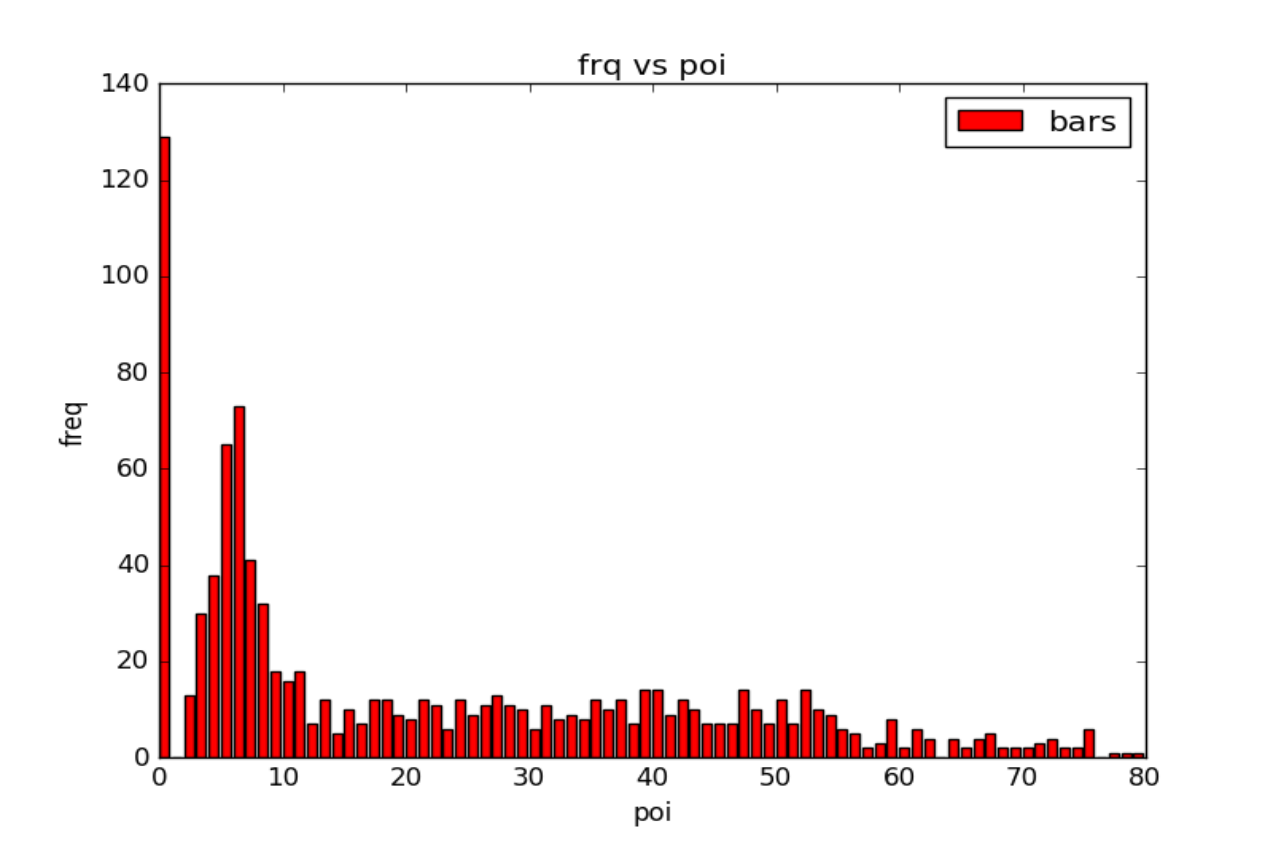
35
40
0
10
20
30
40
50
60
70
80

For k=14



For k = 15


frq vs poi
bars
freq
poi
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40
60
80
100
120
140
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20
30
40
50
60
70
80

Observation

- Result has been getting worse with incrementing values of k

Observation

- Result has been getting worse with incrementing values of k
- Best result got with k=11 and k=13

Questions…..