vcfdist: accurately benchmarking phased variant calls

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Outline

1. **Context**
2. **Problem**
3. **Discussion**
4. **Solution**
5. **Implementation**
6. **Results**
7. **Next Steps**

Outline

1. **Context:** whole genome sequencing evaluation
2. **Problem**
3. **Discussion**
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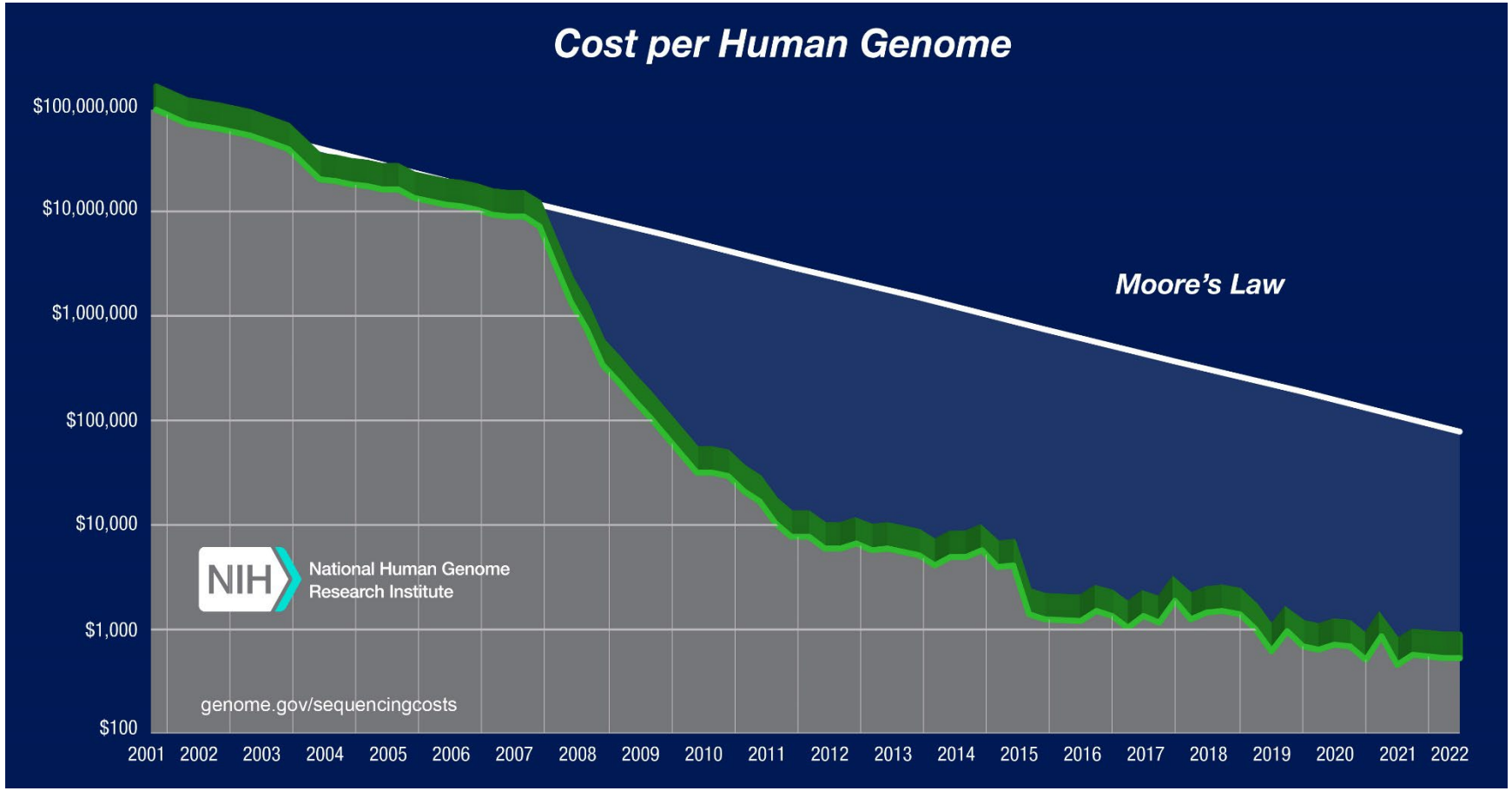
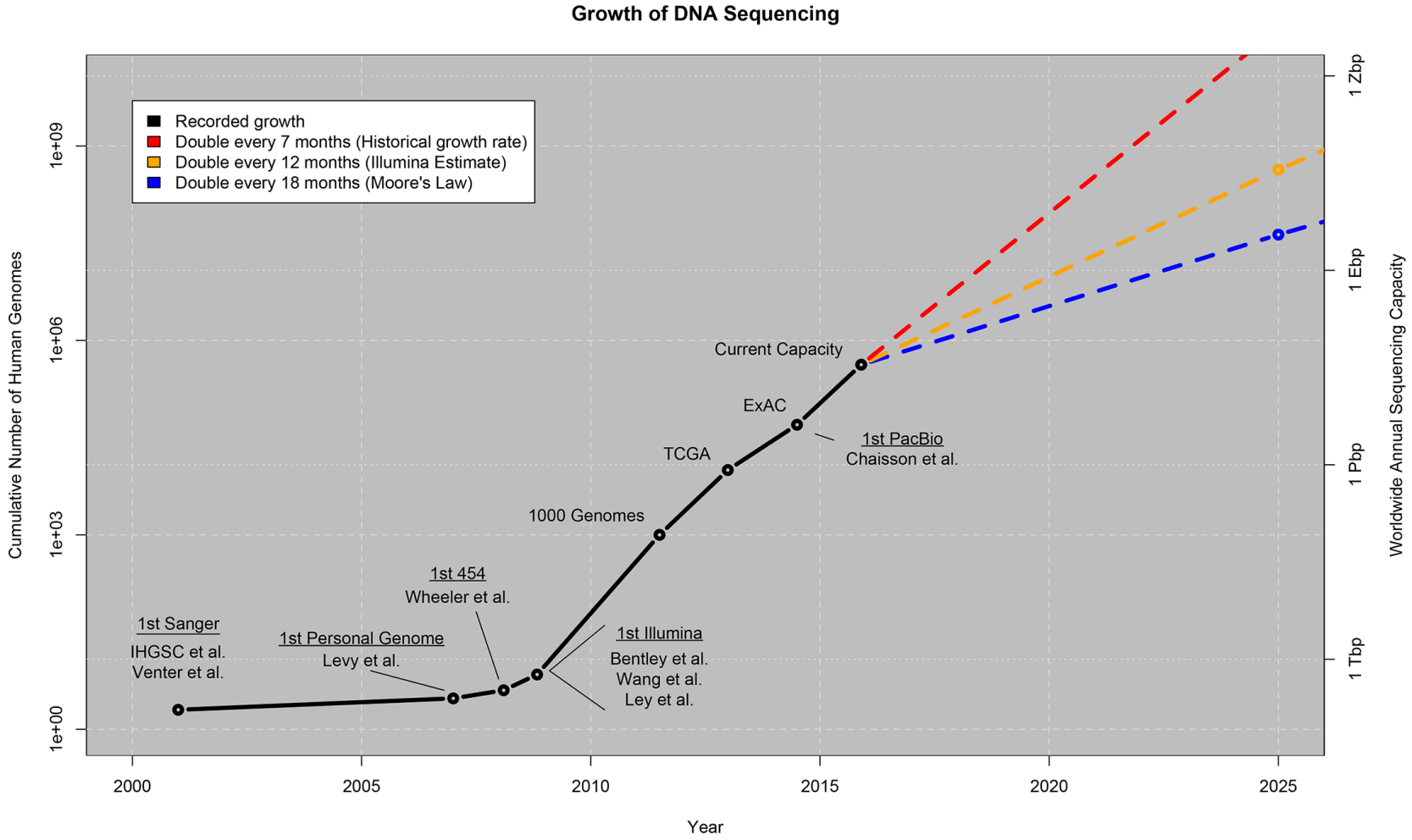
Sequencing: *cost is rapidly declining*



NHGRI. "DNA Sequencing Costs: Data". https://www.genome.gov/about-genomics/fact-sheets/DNA-Sequencing-Costs-Data, 2023.

Sequencing: *exponential growth in genomes*



Stephens et al. “Big Data: Astronomical or Genomical?”.PLOS Biology, 2015.

Applications

- Genome wide association studies
- Pharmacogenomics
- Clinical diagnostics
- Benchmarking new methods and tech

Applications: *genome comparison required*

- Genome wide association studies
- Pharmacogenomics
- Clinical diagnostics
- Benchmarking new methods and tech

Comparison: *genomes are mostly identical*

Reference: ACCGTTGAAGGACGGCCATTTTTT AACTGAGCATCCATCTAAAAGCCTTTTAGCGGCGCCCTCTATAGAT

Query #1: ACCCTTGAAGGACGGCCA TTTTTAAACTGAGCATCCATCTAAAAGCCTTTT

Variant Call Format: *difference-based*

POSITION	REFERENCE	ALTERNATE
4	G	C
18	AT	A
25	T	TA
53	TAGCGGCGCCC…	T

Applications: *benchmarking*

- Genome wide association studies
- Pharmacogenomics
- Clinical diagnostics
- **Benchmarking new methods and tech**

Applications: *benchmarking*

- Genome wide association studies
- Pharmacogenomics
- Clinical diagnostics
- **Benchmarking new methods and tech**

Belton et al, “Hi-C: a comprehensive technique to capture the conformation of genomes”. Nature Methods, 2012.
DelveInsight Business Research. “Global DNA Sequencing Market Set to Reach USD 28.85 billion by 2027”. Web, 2022.

Benchmarking: *a simple example*

Technology #1

Reference: ACCGTTGAAG
Query #1: ACAGTAGAAG

CHROM	POS	REF	ALT
chr14	3	C	A
chr14	6	T	A

Technology #2

Reference: ACCGTTGAAG
Query #2: ACCGTAGAGG

CHROM	POS	REF	ALT
chr14	6	T	A
chr14	9	A	G

Benchmarking: *a simple example*

Technology #1

Reference: ACCGTTGAAG
Query #1: ACAGTAGAAG

CHROM	POS	REF	ALT
chr14	3	C	A
chr14	6	T	A

Technology #2

Reference: ACCGTTGAAG
Query #2: ACCGTAGAGG

CHROM	POS	REF	ALT
chr14	6	T	A
chr14	9	A	G

Ground Truth

Reference: ACCGTTGAAG
Query: ACCGTAGAGG

CHROM	POS	REF	ALT
chr14	6	T	A
chr14	9	A	G

Benchmarking: *a simple example*

Technology #1

Reference: ACCGTTGAAG
Query #1: ACAGTAGAAG

	CHROM	POS	REF	ALT
✗	chr14	3	C	A
✓	chr14	6	T	A

Technology #2

Reference: ACCGTTGAAG
Query #2: ACCGTAGAGG

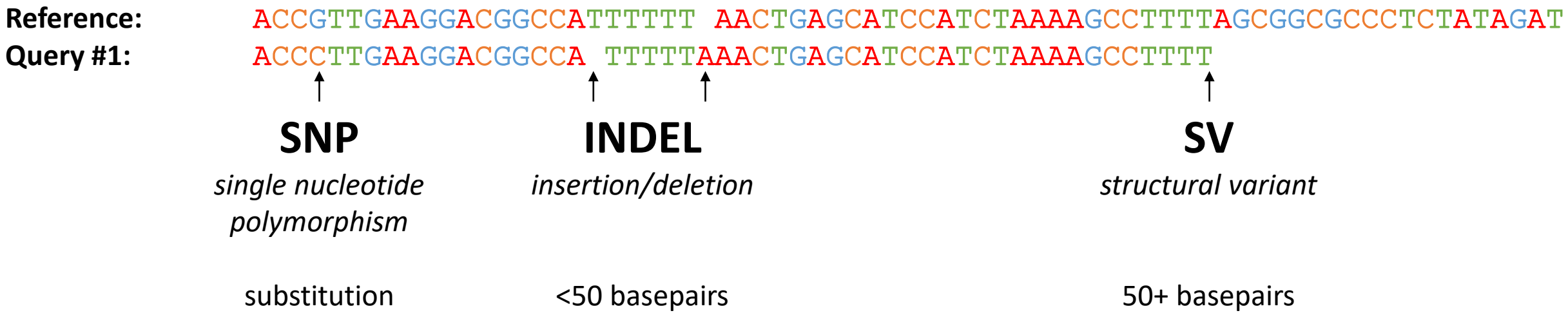
	CHROM	POS	REF	ALT
✓	chr14	6	T	A
✓	chr14	9	A	G

Ground Truth

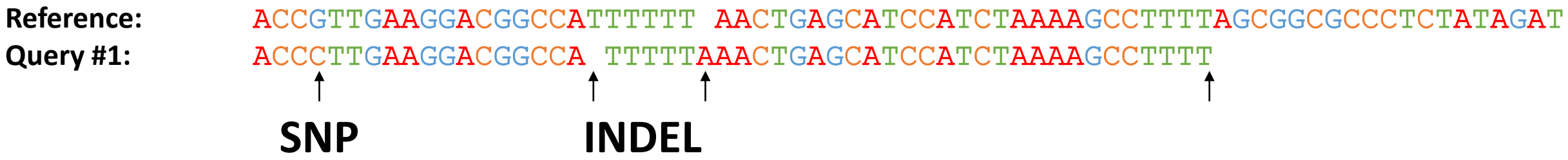
Reference: ACCGTTGAAG
Query: ACCGTAGAGG

CHROM	POS	REF	ALT
chr14	6	T	A
chr14	9	A	G

Benchmarking: *stratification by variant type*



Benchmarking: *precision-recall curves*



$$Precision = \frac{TP}{TP + FP}$$

$$Recall = \frac{TP}{TP + FN}$$

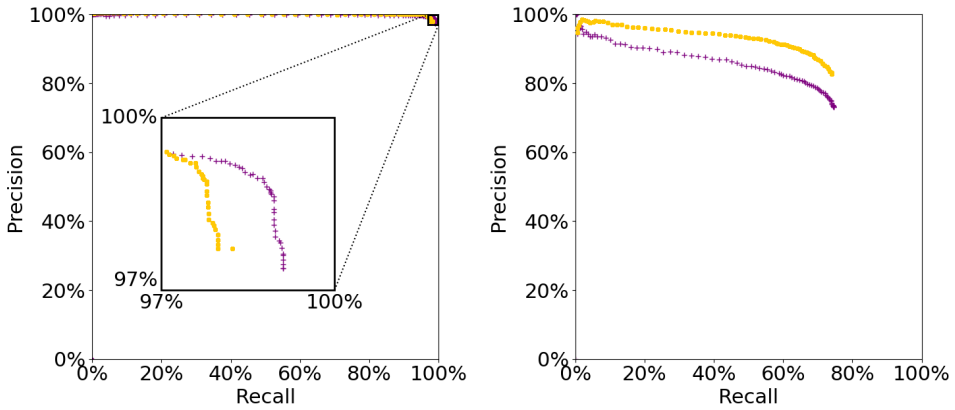
$$F1 = 2 \cdot \frac{precision \cdot recall}{precision + recall}$$

Benchmarking: *precision-recall curves*

Reference: ACCGTTGAAGGACGGCCATTTTTT AACTGAGCATCCATCTAAAAGCCTTTTAGCGGCGCCCTCTATAGAT

Query #1: ACCCTTGAAGGACGGCCA TTTTTAAACTGAGCATCCATCTAAAAGCCTTTT

SNP **INDEL**



$$Precision = \frac{TP}{TP + FP}$$

$$Recall = \frac{TP}{TP + FN}$$

$$F1 = 2 \cdot \frac{precision \cdot recall}{precision + recall}$$

Outline

1. **Context**
2. **Problem:** complex variants
3. **Discussion**
4. **Solution**
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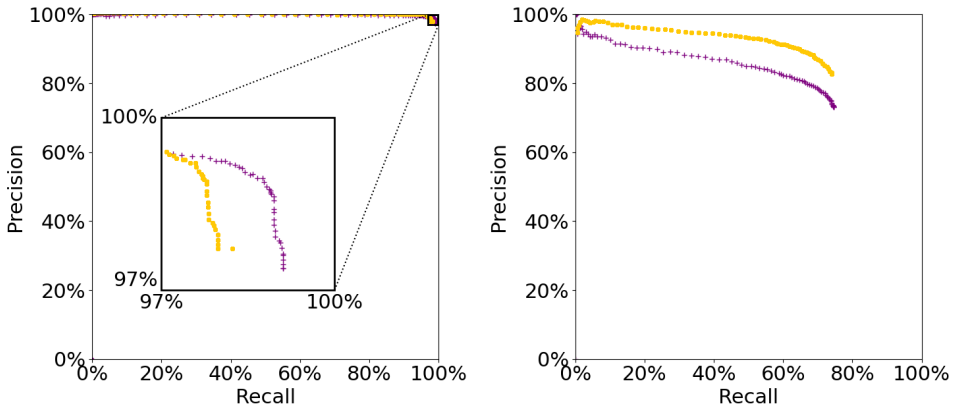
Problem: *evaluation consistency*

Reference: ACCGTTGAAGGACGGCCATTTTTT AACTGAGCATCCATCTAAAAGCCTTTTAGCGGCGCCCTCTATAGAT

Query #1: ACCCTTGAAGGACGGCCA TTTTTAAACTGAGCATCCATCTAAAAGCCTTTT

Query #2: ACCCTTGAAGGACGGCCATTTTTA AACTGAGCATCCATCTAAAAGCCTTTT

SNP **INDEL**



$$Precision = \frac{TP}{TP + FP}$$

$$Recall = \frac{TP}{TP + FN}$$

$$F1 = 2 \cdot \frac{precision \cdot recall}{precision + recall}$$

Challenge: *comparing complex variants*

Ground Truth

Representation #1

Reference: AAGG AAATC
Truth: ATCGAAAATC

CHROM	POS	REF	ALT
chr14	2	A	T
chr14	3	G	C
chr14	4	G	GA

Representation #2

Reference: AAGG AAATC
Truth: A TCGAAAATC

CHROM	POS	REF	ALT
chr14	1	AAGG	A
chr14	1	A	ATCGA

Challenge: *comparing complex variants*

Ground Truth

Representation #1

Reference:	AAGG AAATC
Truth:	ATCGAAAATC

CHROM	POS	REF	ALT
chr14	2	A	T
chr14	3	G	C
chr14	4	G	GA

Representation #2

Reference:	AAGG AAATC
Truth:	A TCGAAAATC

CHROM	POS	REF	ALT
chr14	1	AAGG	A
chr14	1	A	ATCGA

Challenge: *comparing complex variants*

Technology #1

Reference: AAGGAAATC
Query #1: ATCGAAATC

CHROM	POS	REF	ALT
chr14	2	A	T
chr14	3	G	C

Technology #2

Reference: AAGGAAATC
Query #2: A AAATC

CHROM	POS	REF	ALT
chr14	1	AAGG	A

Ground Truth

Representation #1

Reference: AAGG AAATC
Truth: ATCGAAAATC

CHROM	POS	REF	ALT
chr14	2	A	T
chr14	3	G	C
chr14	4	G	GA

Representation #2

Reference: AAGG AAATC
Truth: A TCGAAAATC

CHROM	POS	REF	ALT
chr14	1	AAGG	A
chr14	1	A	ATCGA

Challenge: *comparing complex variants*

Technology #1

Reference: AAGGAAATC
Query #1: ATCGAAATC

	CHROM	POS	REF	ALT
✓	chr14	2	A	T
✓	chr14	3	G	C

SNP Precision: 100%
SNP Recall: 100%
INDEL Precision: NA
INDEL Recall: 0%

Technology #2

Reference: AAGGAAATC
Query #2: A AAATC

	CHROM	POS	REF	ALT
✗	chr14	1	AAGG	A

SNP Precision: NA
SNP Recall: 0%
INDEL Precision: 0%
INDEL Recall: 0%

Ground Truth

Representation #1

Reference: AAGG AAATC
Truth: ATCGAAAATC

CHROM	POS	REF	ALT
chr14	2	A	T
chr14	3	G	C
chr14	4	G	GA

Representation #2

Reference: AAGG AAATC
Truth: A TCGAAAATC

CHROM	POS	REF	ALT
chr14	1	AAGG	A
chr14	1	A	ATCGA

Challenge: *comparing complex variants*

Technology #1

Reference: AAGGAAATC
Query #1: ATCGAAATC

	CHROM	POS	REF	ALT
✗	chr14	2	A	T
✗	chr14	3	G	C

SNP Precision: 100%
SNP Recall: 100%
INDEL Precision: NA
INDEL Recall: 0%

SNP Precision: 0%
SNP Recall: NA
INDEL Precision: NA
INDEL Recall: 0%

Technology #2

Reference: AAGGAAATC
Query #2: A AAATC

	CHROM	POS	REF	ALT
✓	chr14	1	AAGG	A

SNP Precision: NA
SNP Recall: 0%
INDEL Precision: 0%
INDEL Recall: 0%

SNP Precision: NA
SNP Recall: NA
INDEL Precision: 100%
INDEL Recall: 50%

Ground Truth

Representation #1

Reference: AAGG AAATC
Truth: ATCGAAAATC

CHROM	POS	REF	ALT
chr14	2	A	T
chr14	3	G	C
chr14	4	G	GA

Representation #2

Reference: AAGG AAATC
Truth: A TCGAAAATC

CHROM	POS	REF	ALT
chr14	1	AAGG	A
chr14	1	A	ATCGA

Outline

1. **Context**
2. **Problem**
3. **Discussion:** complex variant representation
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Complex variants: *standard VCF normalization*

Reference AAGGAAATC **Query** ATCGAAAATC

Heng Li. “Toward better understanding of artifacts in variant calling from high-coverage samples.” Bioinformatics, 2014.
Tan et al. “Unified representation of genetic variants.” Bioinformatics, 2015.

Complex variants: *standard VCF normalization*

Reference AAGGAAATC **Query** ATCGAAAATC

Alignment

```
AAGGAAA-TC
·  ···· ··
ATCGAAAATC
```

VCF

POS	REF	ALT
2	AG	TC
6	AATC	AAATC

Original

Heng Li. “Toward better understanding of artifacts in variant calling from high-coverage samples.” Bioinformatics, 2014.
Tan et al. “Unified representation of genetic variants.” Bioinformatics, 2015.

Complex variants: *standard VCF normalization*

Reference AAGGAAATC **Query** ATCGAAAATC

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VCF

POS	REF	ALT
2	AG	TC
6	AATC	AAATC

Original

```
AAGGAAA-TC
·   ···· ··
ATCGAAAATC
```

POS	REF	ALT
2	A	T
3	G	C
6	AATC	AAATC

Decomposed

Heng Li. “Toward better understanding of artifacts in variant calling from high-coverage samples.” Bioinformatics, 2014.
Tan et al. “Unified representation of genetic variants.” Bioinformatics, 2015.

Complex variants: *standard VCF normalization*

Reference AAGGAAATC **Query** ATCGAAAATC

Alignment

```
AAGGAAA-TC
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ATCGAAAATC
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VCF

POS	REF	ALT
2	AG	TC
6	AATC	AAATC

Original

```
AAGGAAA-TC
·   ···· ··
ATCGAAAATC
```

POS	REF	ALT
2	A	T
3	G	C
6	AATC	AAATC

Decomposed

```
AAGGAAA-TC
·   ···· ··
ATCGAAAATC
```

POS	REF	ALT
2	A	T
3	G	C
7	A	AA

Trimmed

Heng Li. “Toward better understanding of artifacts in variant calling from high-coverage samples.” Bioinformatics, 2014.
Tan et al. “Unified representation of genetic variants.” Bioinformatics, 2015.

Complex variants: *standard VCF normalization*

Reference AAGGAAATC **Query** ATCGAAAATC

Alignment

Original	Decomposed	Trimmed	Left shifted
AAGGAAA-TC	AAGGAAA-TC	AAGGAAA-TC	AAGG-AAATC
· · ···· ··	· · ···· ··	· · ···· ··	· · · ·····
ATCGAAAATC	ATCGAAAATC	ATCGAAAATC	ATCGAAAATC

VCF

POS	REF	ALT
2	AG	TC
6	AATC	AAATC

POS	REF	ALT
2	A	T
3	G	C
6	AATC	AAATC

POS	REF	ALT
2	A	T
3	G	C
7	A	AA

POS	REF	ALT
2	A	T
3	G	C
4	G	GA

Heng Li. “Toward better understanding of artifacts in variant calling from high-coverage samples.” Bioinformatics, 2014.
Tan et al. “Unified representation of genetic variants.” Bioinformatics, 2015.

Complex variants: *standard VCF normalization*

Reference AAGGAAATC **Query** ATCGAAAATC

Alignment

Original	Decomposed	Trimmed	Left shifted	Alternate
AAGGAAA-TC	AAGGAAA-TC	AAGGAAA-TC	AAGG-AAATC	AAGG----AAATC
ATCGAAAATC	ATCGAAAATC	ATCGAAAATC	ATCGAAAATC	A---TCGAAAATC

VCF

Original			Decomposed			Trimmed			Left shifted			Alternate		
POS	REF	ALT	POS	REF	ALT	POS	REF	ALT	POS	REF	ALT	POS	REF	ALT
2	AG	TC	2	A	T	2	A	T	2	A	T	1	AAGG	A
6	AATC	AAATC	3	G	C	3	G	C	3	G	C	1	A	ATCGA
			6	AATC	AAATC	7	A	AA	4	G	GA			

Heng Li. “Toward better understanding of artifacts in variant calling from high-coverage samples.” Bioinformatics, 2014.
Tan et al. “Unified representation of genetic variants.” Bioinformatics, 2015.

Complex variants: *standard VCF normalization*

Reference AAGGAAATC **Query** ATCGAAAATC

Alignment

```
AAGGAAA-TC
·  ···· ··
ATCGAAAATC
```

VCF

POS	REF	ALT
2	AG	TC
6	AATC	AAATC

Original

```
AAGGAAA-TC
·  ···· ··
ATCGAAAATC
```

POS	REF	ALT
2	A	T
3	G	C
6	AATC	AAATC

Decomposed

```
AAGGAAA-TC
·  ···· ··
ATCGAAAATC
```

POS	REF	ALT
2	A	T
3	G	C
7	A	AA

Trimmed

```
AAGG-AAATC
·  · ·····
ATCGAAAATC
```

POS	REF	ALT
2	A	T
3	G	C
4	G	GA

Left shifted

```
AAGG----AAATC
·       ·····
A---TCGAAAATC
```

POS	REF	ALT
1	AAGG	A
1	A	ATCGA

Alternate

Heng Li. “Toward better understanding of artifacts in variant calling from high-coverage samples.” Bioinformatics, 2014.
Tan et al. “Unified representation of genetic variants.” Bioinformatics, 2015.

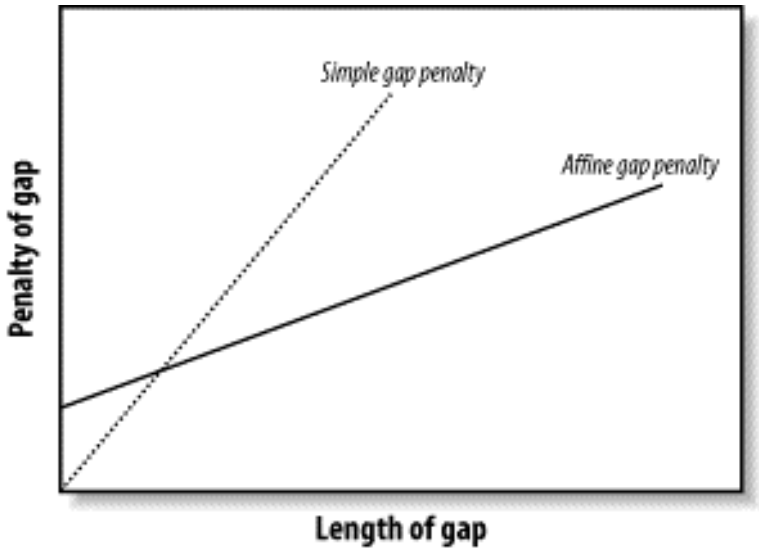
Choosing representations: *best-alignment normalization*

m = match

x = mis-match

o = gap opening

e = gap extension



Bayat et al. “Improved VCF normalization for accurate VCF comparison”. Oxford Bioinformatics, 2017.

Choosing representations: *best-alignment normalization*

m = match

x = mis-match

o = gap opening

e = gap extension

Option #1

```
AAGG-AAATC
.  .  .....
ATCGAAAATC
```

POS	REF	ALT
2	A	T
3	G	C
4	G	GA

x + x + (o+e)

Option #2

```
AAGG----AAATC
.       .....
A---TCGAAAATC
```

POS	REF	ALT
1	AAGG	A
1	A	ATCGA

(o+3e) + (o+4e)

Bayat et al. “Improved VCF normalization for accurate VCF comparison”. Oxford Bioinformatics, 2017.

Choosing representations: *best-alignment normalization*

$m = 0$ = match

$x = 5$ = mis-match

$o = 6$ = gap opening

$e = 2$ = gap extension

Option #1

```
AAGG-AAATC
.  .  .....
ATCGAAAATC
```

POS	REF	ALT
2	A	T
3	G	C
4	G	GA

x + x + (o+e)

18

Option #2

```
AAGG----AAATC
.       .....
A---TCGAAAATC
```

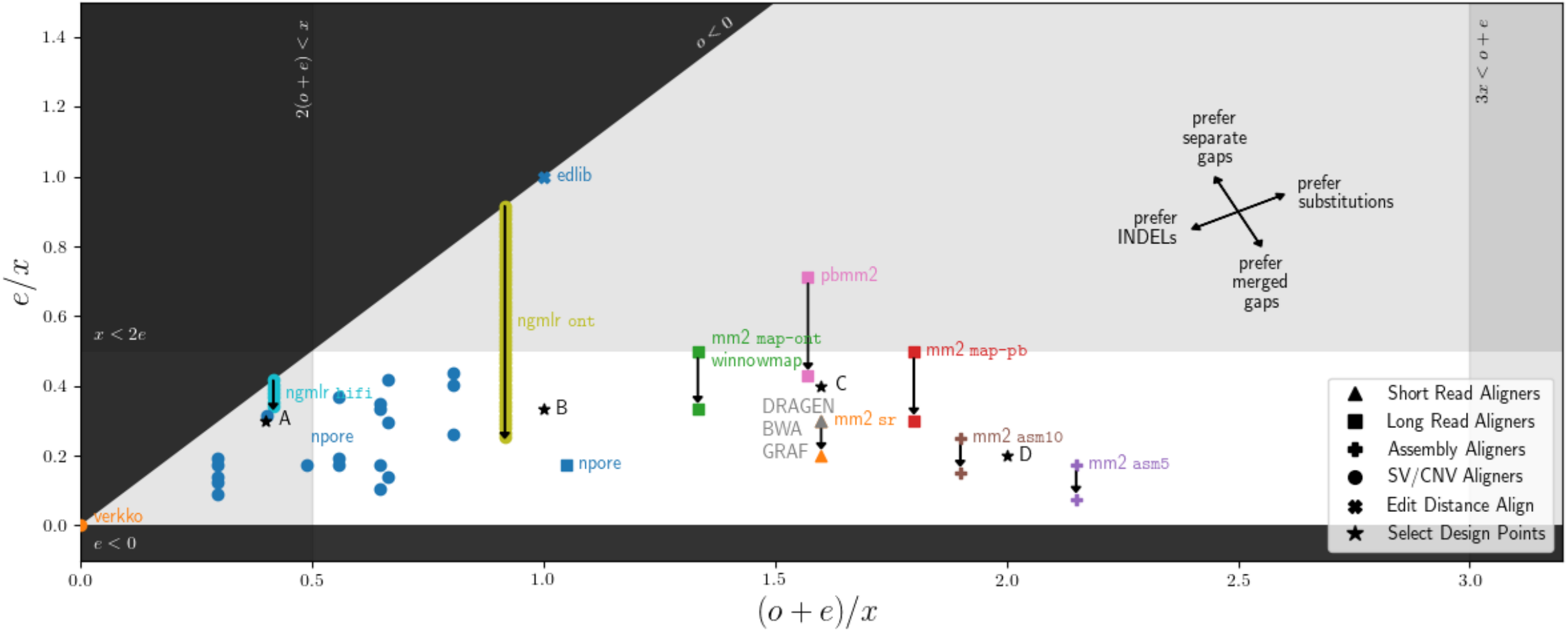
POS	REF	ALT
1	AAGG	A
1	A	ATCGA

(o+3e) + (o+4e)

26

Bayat et al. “Improved VCF normalization for accurate VCF comparison”. Oxford Bioinformatics, 2017.

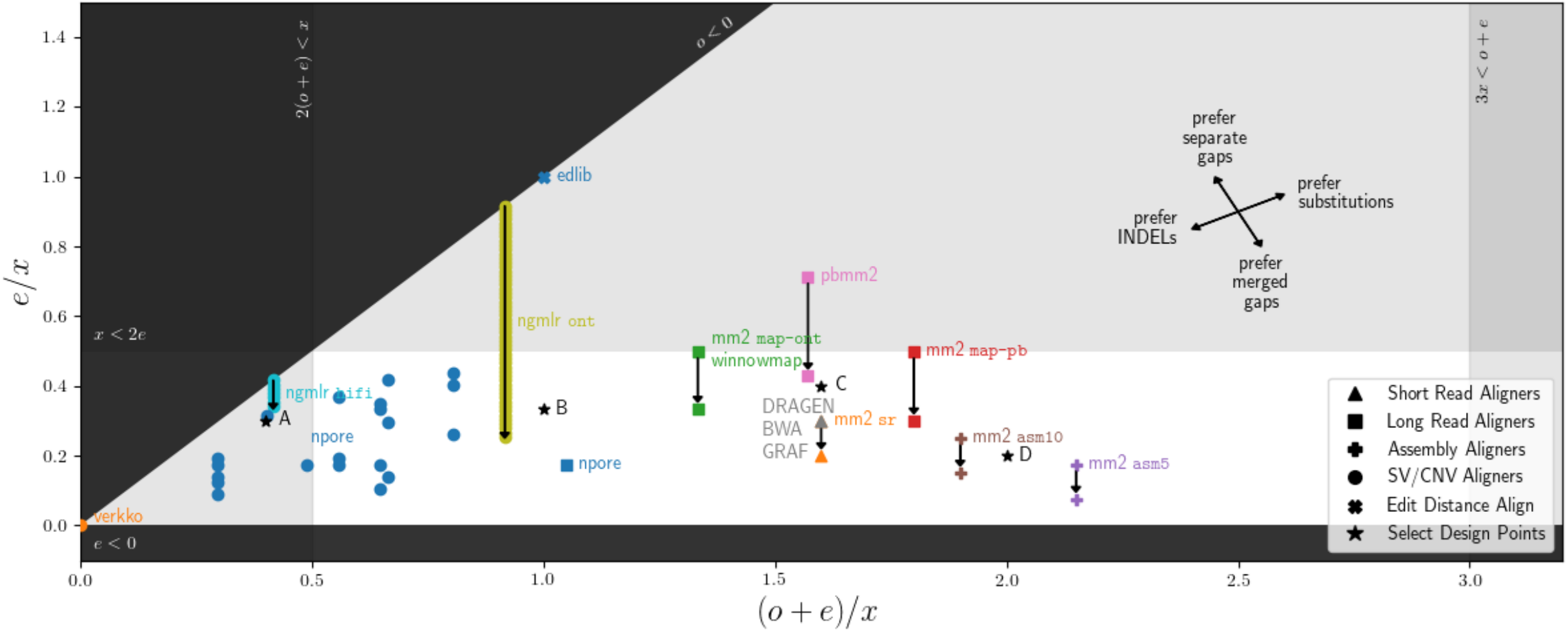
Alignment-based normalization design space



To what extent do parameters matter?

Representation	SNPs	INDELs
Original	3,367,320	548,602
A	0	7,185,103
B	3,366,095	547,654
C	3,369,257	545,077
D	3,369,279	544,664

Design point A: *structural and copy number variants*



SV / CNV Analysis: *only recently enabled by long reads*

- **2014**: NIST/GIAB initial small variant benchmark (77% of GRCh38)
- **2019**: NIST/GIAB small variant benchmark expansion (84% of GRCh38)
- **2020**: NIST/GIAB structural variant benchmarks
- **2022**: NIST/GIAB challenging small variants (92% of GRCh38)
- **2023**: NIST/GIAB tandem repeat benchmarks

- **2022**: T2T Consortium “The first complete human genome”

Motivation: *GIAB tandem repeat benchmark*

Dataset	SNPs	INDELS
Original GIAB TR	917,255	431,545
Normalized GIAB TR	502,076	461,258

Outline

1. **Context**
2. **Problem**
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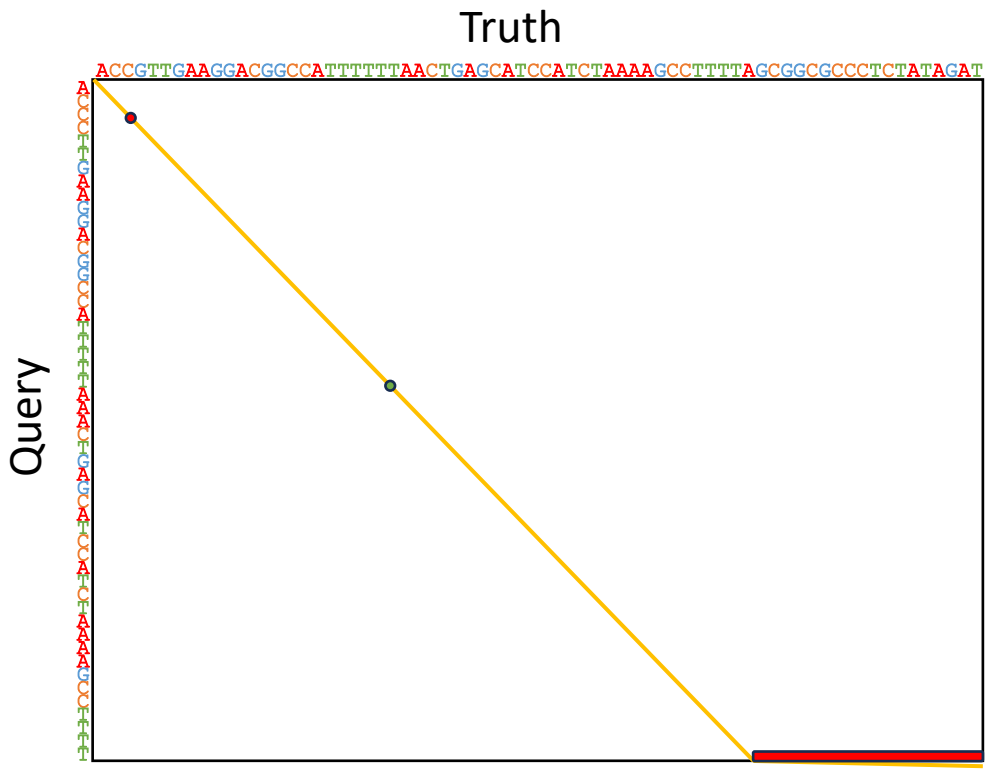
4. **Solution:** new comparison methods
5. **Implementation**
6. **Results**
7. **Next Steps**

Idea #1: *sequence-based evaluation metrics*

Reference: ACCGTTGAAGGACGGCCATTTTTT AACTGAGCATCCATCTAAAAGCCTTTTAGCGGCGCCCTCTATAGAT
Query #1: ACCCTTGAAGGACGGCCA TTTTTAAACTGAGCATCCATCTAAAAGCCTTTT
Truth: ACCGTTGAAGGACGGCCA TTTTTAAACTGAGCATCCATCTAAAAGCCTTTTAGCGGCGCCCTCTATAGAT

- Edit Distance
- Distinct Edits
- Alignment Distance



Idea #2: *standardize complex variant representation*

Reference: ACCGTTGAAGGACGGCCATTTTTT AACTGAGCATCCATCTAAAAGCCTTTTAGCGGCGCCCTCTATAGAT

Query #1: ACCCTTGAAGGACGGCCA TTTTTAAACTGAGCATCCATCTAAAAGCCTTTT

Query #2: ACCCTTGAAGGACGGCCATTTTTA AACTGAGCATCCATCTAAAAGCCTTTT

Query

POS	REFERENCE	ALTERNATE
4	G	C
18	AT	A
25	T	TA
53	TAGCGGCG…	T

POS	REFERENCE	ALTERNATE
4	G	C
24	T	A
53	TAGCGGCG…	T

Idea #3: *allow partial credit for variant calls*

Reference: ACCGTTGAAGGACGGCCATTTTTTAACTGAGCATCCATCTAAAAGCCTTTTAGCGGCGCCCTCTATAGAT
Query #1: ACCCTTGAAGGACGGCCATTTTTAAACTGAGCATCCATCTAAAAGCCTTTT 20 bases
Truth: ACCCTTGAAGGACGGCCATTTTTAAACTGAGCATCCATCTAAAAGCCTTTTAG 18 bases

Query

POS	REFERENCE	ALTERNATE
4	G	C
24	T	A
53	TAGCGGCG…	T

Truth

POS	REFERENCE	ALTERNATE
4	G	C
24	T	A
55	GCGGCG…	G

Idea #3: *allow partial credit for variant calls*

Reference: ACCGTTGAAGGACGGCCATTTTTTAACTGAGCATCCATCTAAAAGCCTTTTAGCGGCGCCCTCTATAGAT
Query #1: ACCCTTGAAGGACGGCCATTTTTAAACTGAGCATCCATCTAAAAGCCTTTT 20 bases
Truth: ACCCTTGAAGGACGGCCATTTTTAAACTGAGCATCCATCTAAAAGCCTTTTAG 18 bases

Query

	POS	REFERENCE	ALTERNATE
✓	4	G	C
✓	24	T	A
✗	53	TAGCGGCG…	T

Truth

	POS	REFERENCE	ALTERNATE
✓	4	G	C
✓	24	T	A
✗	55	GCGGCG…	G

Idea #3: *allow partial credit for variant calls*

Reference: ACCGTTGAAGGACGGCCATTTTTTAACTGAGCATCCATCTAAAAGCCTTTTAGCGGCGCCCTCTATAGAT
Query #1: ACCCTTGAAGGACGGCCATTTTTAAACTGAGCATCCATCTAAAAGCCTTTT (20 bases)
Truth: ACCCTTGAAGGACGGCCATTTTTAAACTGAGCATCCATCTAAAAGCCTTTTAG (18 bases)

Query

	POS	REFERENCE	ALTERNATE
✓	4	G	C
✓	24	T	A
.9 ✓ + .1 ✗	53	TAGCGGCG…	T

Truth

	POS	REFERENCE	ALTERNATE
✓	4	G	C
✓	24	T	A
.9 ✓ + .1 ✗	55	GCGGCG…	G

Idea #4: *enforce local variant phasing*

Reference: GAGCC

Query #1:
1 GACCC
2 GTGAC

Phased Query

POS	REF	ALT	GENOTYPE
2	A	T	0|1
3	G	C	1|0
4	C	A	0|1

"Correct" Query haps:
1 GACCC
2 GTGAC

Idea #4: *enforce local variant phasing*

Reference: GAGCC

Query #1:

1 GACCC

2 GTGAC

Phased Query

POS	REF	ALT	GENOTYPE
2	A	T	0|1
3	G	C	1|0
4	C	A	0|1

“Correct” Query haps:

1 GACCC

2 GTGAC

Idea #4: *enforce local variant phasing*

Reference: GAGCC

Query #1:

1 GACCC

2 GTGAC

Phased Query

POS	REF	ALT	GENOTYPE
2	A	T	0|1
3	G	C	1|0
4	C	A	0|1

"Correct" Query haps:

1 GACCC

2 GTGAC

Unphased Query

POS	REF	ALT	GENOTYPE
2	A	T	0/1
3	G	C	0/1
4	C	A	0/1

Idea #4: *enforce local variant phasing*

Reference: GAGCC

Query #1:

1 GACCC
2 GTGAC

Phased Query

POS	REF	ALT	GENOTYPE
2	A	T	0|1
3	G	C	1|0
4	C	A	0|1

"Correct" Query haps:

1 GACCC
2 GTGAC

Unphased Query

POS	REF	ALT	GENOTYPE
2	A	T	0/1
3	G	C	0/1
4	C	A	0/1

"Correct" Query haps:

1 GAGCC GAGAC GACCC GTGCC
2 GTCAC GTCCC GTGAC GACAC

1 GTCAC GTCCC GTGAC GACAC
2 GAGCC GAGAC GACCC GTGCC

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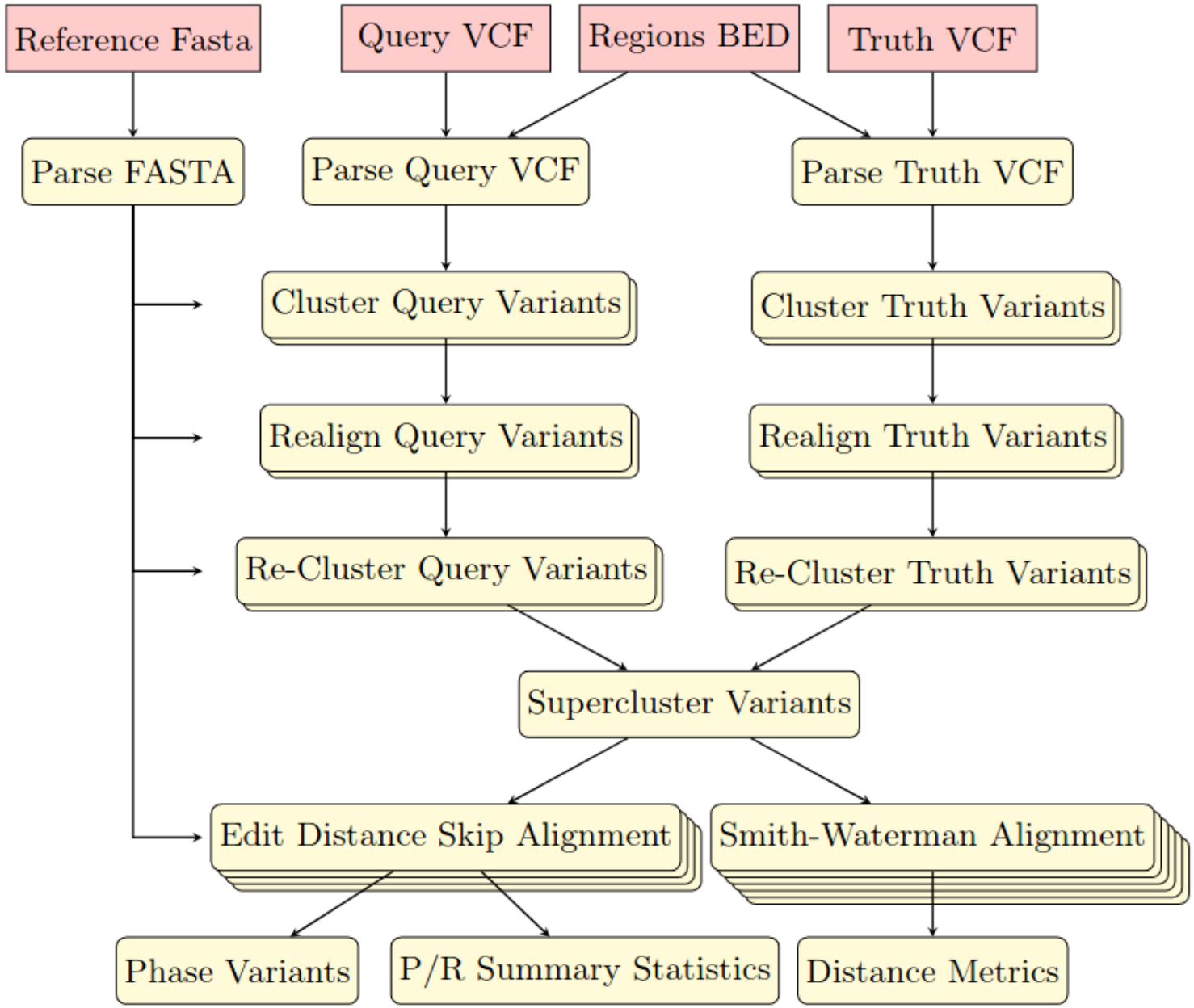
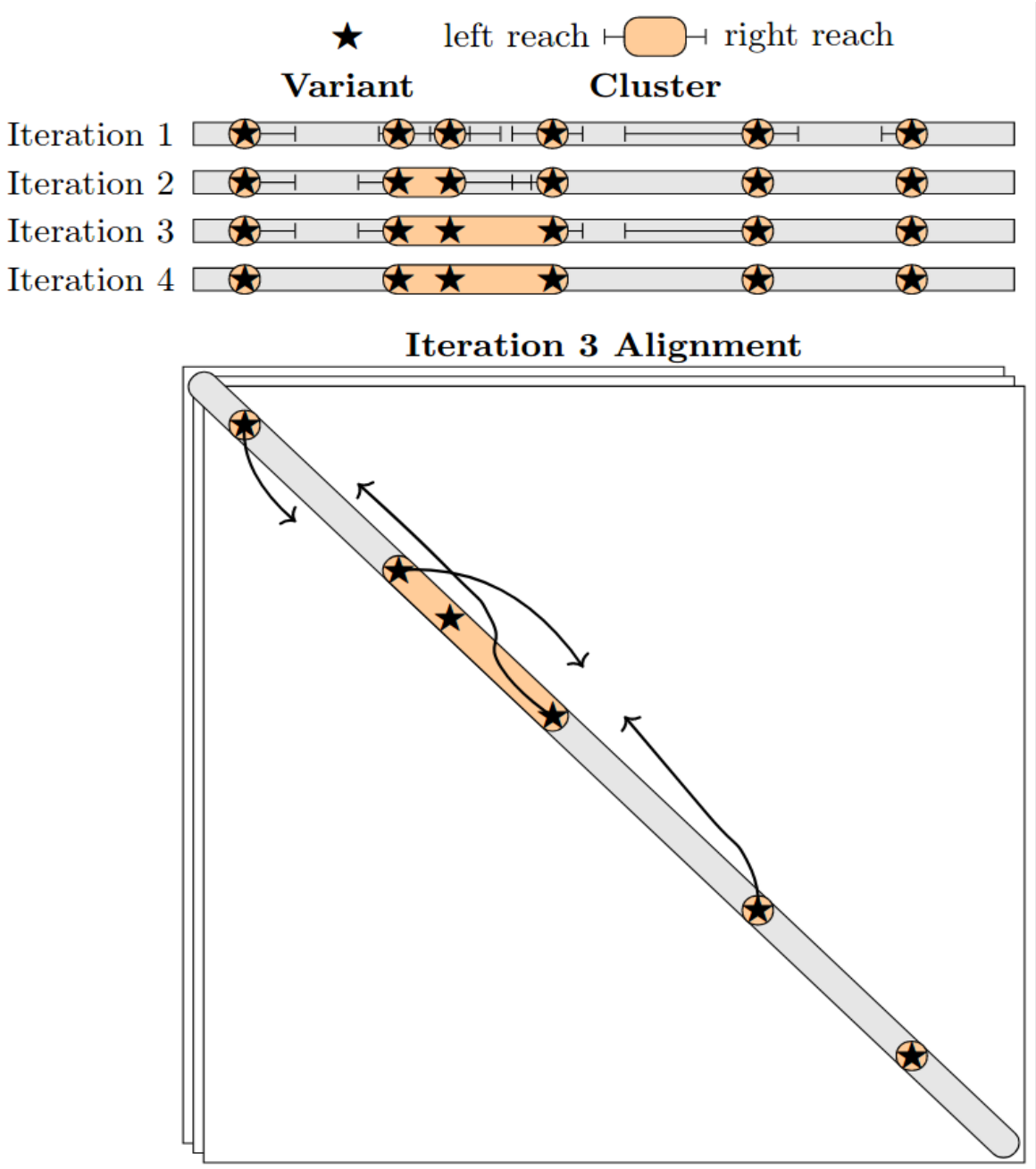
2. **Problem**
3. **Discussion**
4. **Solution**
5. **Implementation:** dynamic programming / alignment
6. **Results**
7. **Next Steps**

Overview


Reference Fasta
Query VCF
Regions BED
Truth VCF
Parse FASTA
Parse Query VCF
Parse Truth VCF
Cluster Query Variants
Cluster Truth Variants
Realign Query Variants
Realign Truth Variants
Re-Cluster Query Variants
Re-Cluster Truth Variants
Supercluster Variants
Edit Distance Skip Alignment
Smith-Waterman Alignment
Phase Variants
P/R Summary Statistics
Distance Metrics

Clustering:
iterative bi-directional wavefront alignment



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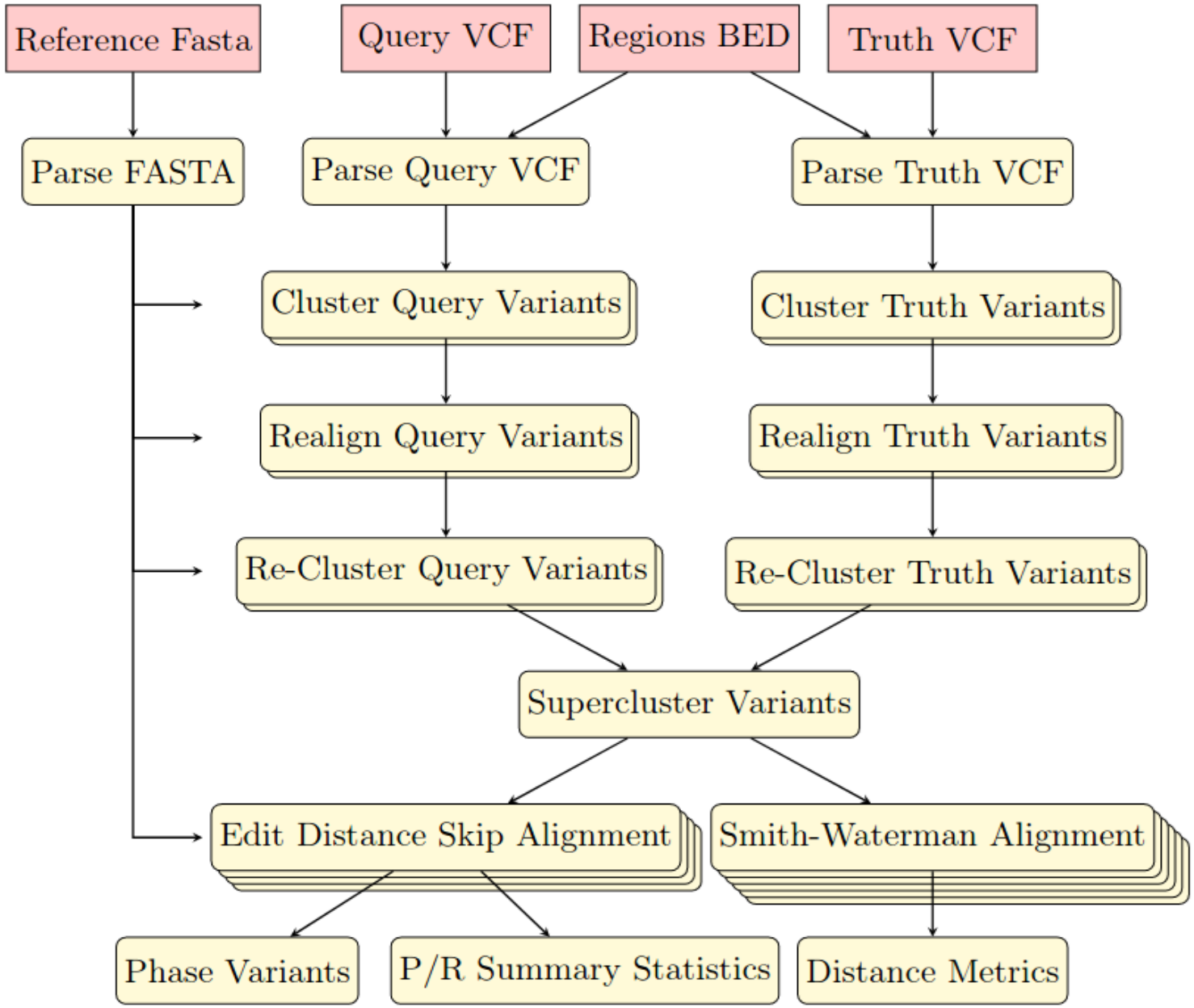
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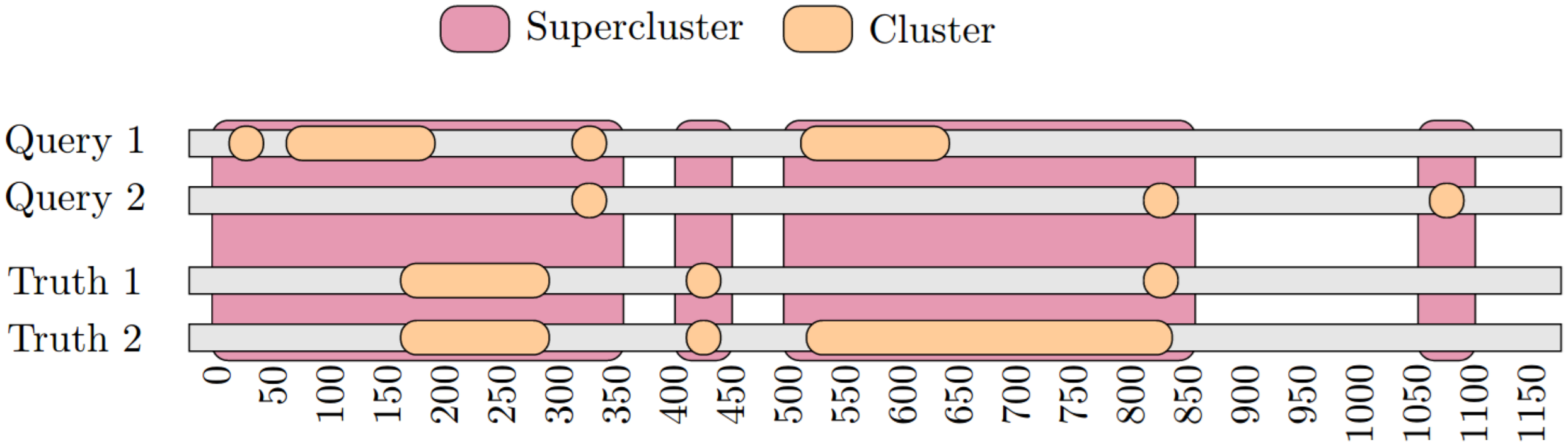
Superclustering: *simple reference distance heuristic*



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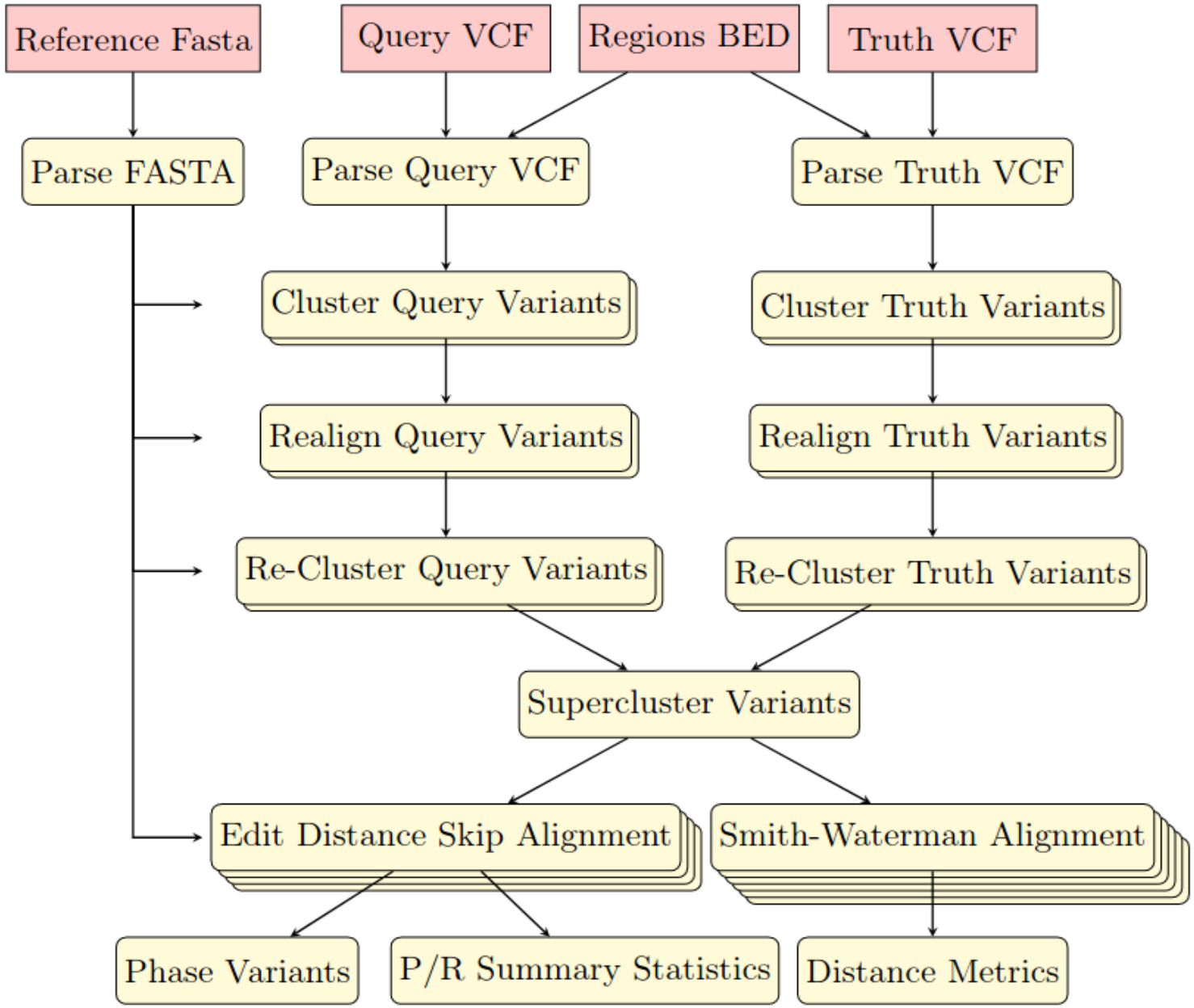
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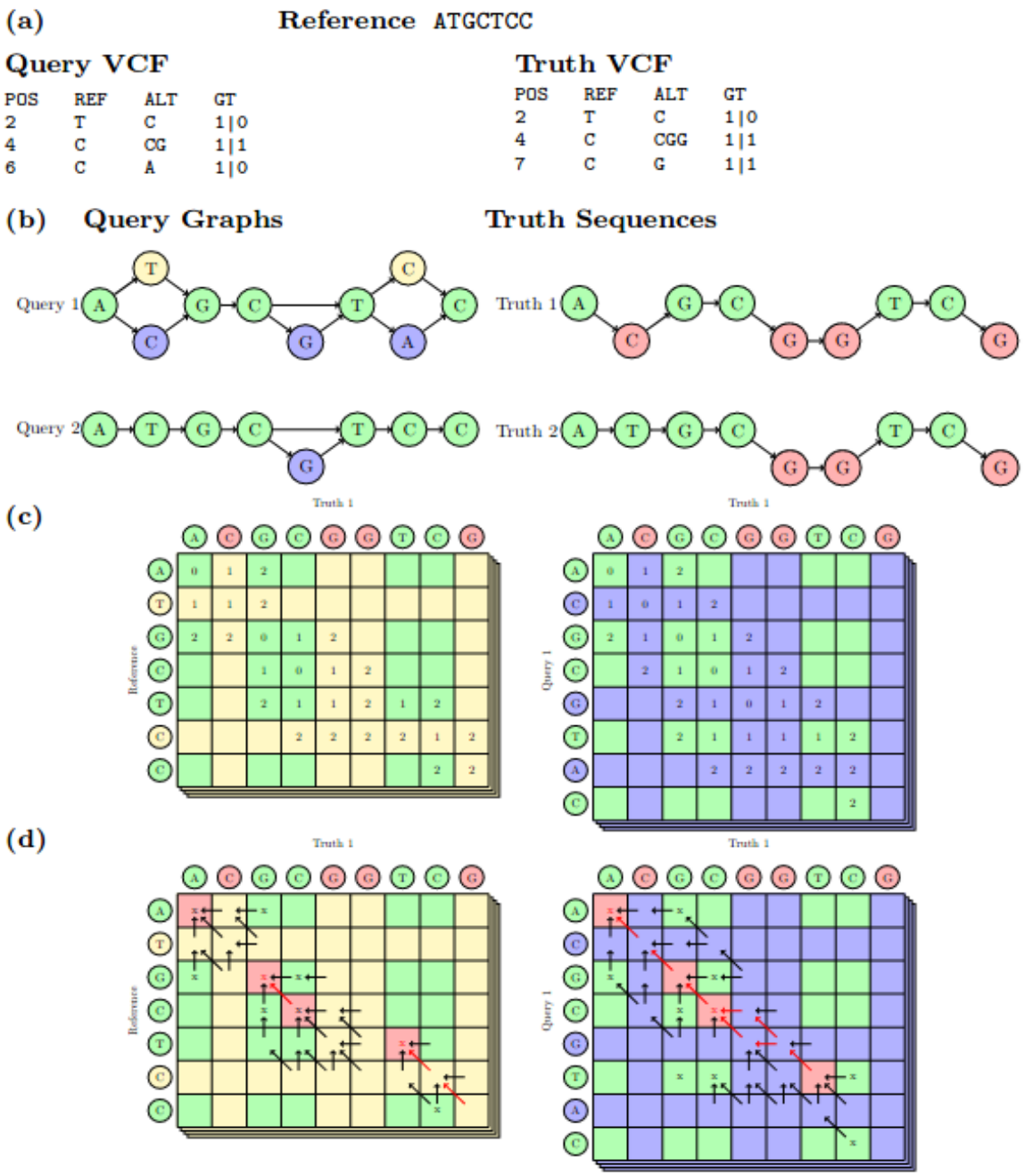
Precision/Recall:

edit distance, allows skipping FP query variants, backtracking



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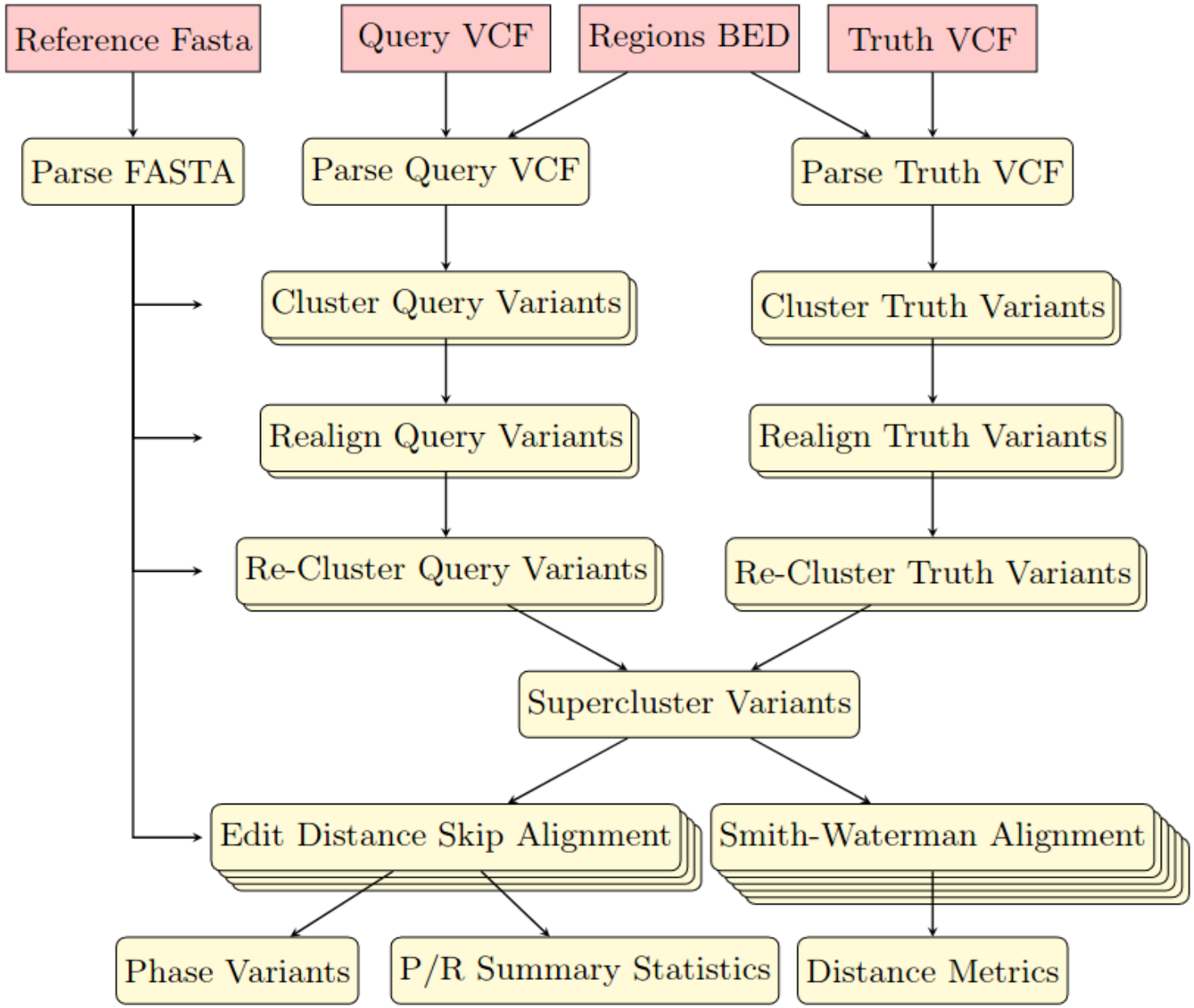
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Outline

1. **Context**
2. **Problem**
3. **Discussion**
4. **Solution**
5. **Implementation**
6. **Results:** improved evaluation stability
7. **Next Steps**

Example #1: *complex variant normalization*

Original VCF: *GIAB Tandem Repeats*

chr20 278985 A C
chr20 278986 C G
chr20 278990 G C
chr20 278993 C A
chr20 278994 G GGGAGGGAGGGCGGGACGGAGGGA
GGGAGGGAGGGACGGAGGGCGGGACGGCGGGAGGGCGGGAC
GGAGGGACGGAGGGAGGGCGGGACGGAGGGCGGGAGGGCGG
GACGGAGGGAGGGAGGGAGGGAGGGCGGGACGGAGGGAGGG
AGGGCGGGACGGAGGGAGGGAGGGAGGGACGGAGGGCGGGA
CGGCGGGAGGGCGGGACGGAGGGACGGAGGGAGGGCGGGAC
GGAGGGCGGGAGGGCGGGACGGAGGGAGGGAGGGCGGGACG
GAGGGACGGAGGGAGGGAGGGCGGGACGGAGGGAGGGAGGG
CGGGACGGAGGGACGGAGGGAGGGAGGGCGGGACGGAGGGA
GGGAGGGAGGGACGGAGGGCGGGACGGCGGGAGGGCGGGAC
GGAGGGACGGAGGGAGGGCGGGACGGAGGGCGGGAGGGAGG
GAGGGCGGGACGGAGGGAGGGAGGGAGGGAGGGCGGGACGG
AGGGAGGGAGGGAGGGAGGGACGGAGGGACGGAGGGAGGGA
GGGAGGGAGGGACGGAGGGCGGGACGGAGGGAGGGAGGGCG
GAGGGAGGGAGGGCGGGACGGAGGGAGGGAGGGAGGGACGG
AGGGCGGGACGGAGGGAGGGAGGGC
chr20 278998 C G
chr20 279001 C A
chr20 279022 C G
chr20 279029 A C
chr20 279033 C A
chr20 279038 C T
chr20 279045 C A
chr20 279069 A C

12 SNPs
1 INS (622bp)

Normalized VCF: *vcfdist design point C*

chr20 278984 G GCGGGACGGAGGGAGGGAGGGCG
GGACGGAGGGAGGGAGGGAGGGACGGAGGGCGGGA
CGGCGGGAGGGCGGGACGGAGGGACGGAGGGAGGG
CGGGACGGAGGGCGGGAGGGCGGGACGGAGGGAGG
GAGGGAGGGAGGGCGGGACGGAGGGAGGGAGGGCG
GGACGGAGGGAGGGAGGGAGGG
chr20 279069 A AGGGCGGGACGGAGGGACGGAGG
GAGGGAGGGCGGGACGGAGGGAGGGAGGGCGGGAC
GGAGGGACGGAGGGAGGGAGGGCGGGACGGAGGGA
GGGAGGGAGGGACGGAGGGCGGGACGGCGGGAGGG
CGGGACGGAGGGACGGAGGGAGGGCGGGACGGAGG
GCGGGAGGGAGGGAGGGCGGGACGGAGGGAGGGAG
GGAGGGAGGGCGGGACGGAGGGAGGGAGGGAGGGA
GGGACGGAGGGACGGAGGGAGGGAGGGAGGGAGGG
ACGGAGGGCGGGACGGAGGGAGGGAGGGCGGAGGG
AGGGAGGGCGGGACGGAGGGAGGGAGGGACG
GAGGGCGGGACGGAGGGAGGGAGGGCGGAGGGAGG
GAGGGCGGGACGGAGGGAGGGAGGGCGGGAGGGAT
GGAGGGAGGGAGGGCGGGACGGAGGGAGGGC

2 INS (438bp, 184bp)

Example #2: *complex variant near-equivalence*

Query:

CHROM	POS	REF	ALT	CALL	CREDIT
chr1	976722	C	CAGGAACCGCCTCCCACTCCCCCCACAACCCCGGGAACCGCCTCCCACTC		
CCCCCGCAACCCCGGGAACCGCCTCCCACTCCCCCCGCAACCCC				INS PP	0.979167
chr1	976745	G	A	SNP PP	0.979167

Truth:

CHROM	POS	REF	ALT	CALL	CREDIT
chr1	976715	A	ACAACCCCAGGAACCGCCTCCCACTCCCCCCA	INS PP	0.979167
chr1	976747	C	CAACCCCGGGAACCGCCTCCCACTCCCCCCG	INS PP	0.979167
chr1	976777	G	A	SNP PP	0.979167
chr1	976811	C	CAACCCCGGGAACCGCCTCCCACTCCCCCCG	INS PP	0.979167
chr1	976840	C	G	SNP PP	0.979167
chr1	976841	G	A	SNP PP	0.979167

Dataset: *PrecisionFDA Truth Challenge V2*

- 64 whole genome sequencing submissions
- Illumina, PacBio, ONT, and Multi

Olson et al. “PrecisionFDA Truth Challenge V2: Calling variants from short and long reads in difficult-to-map regions.” Cell, 2022.

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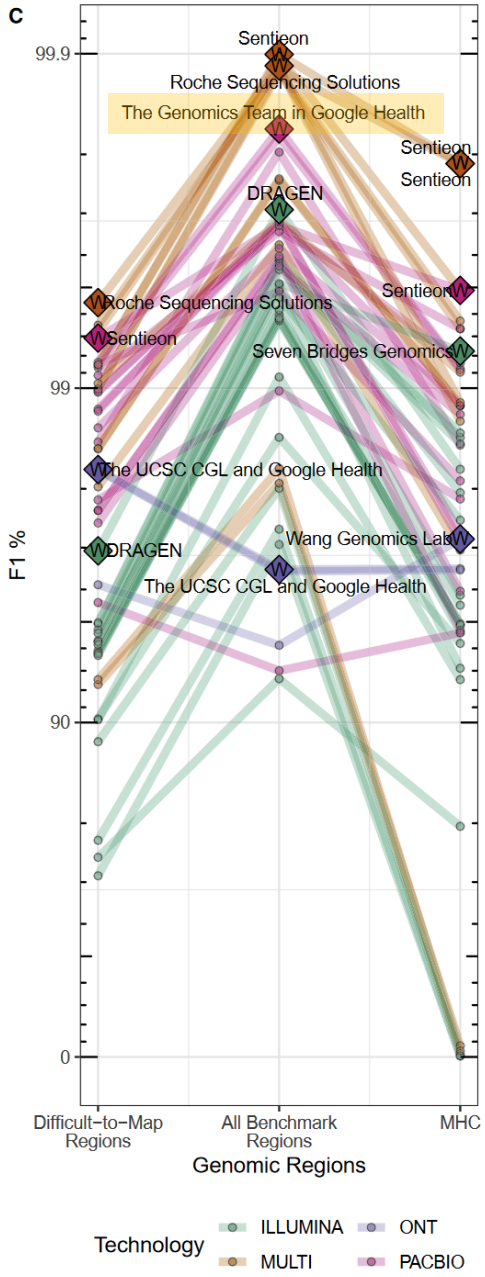
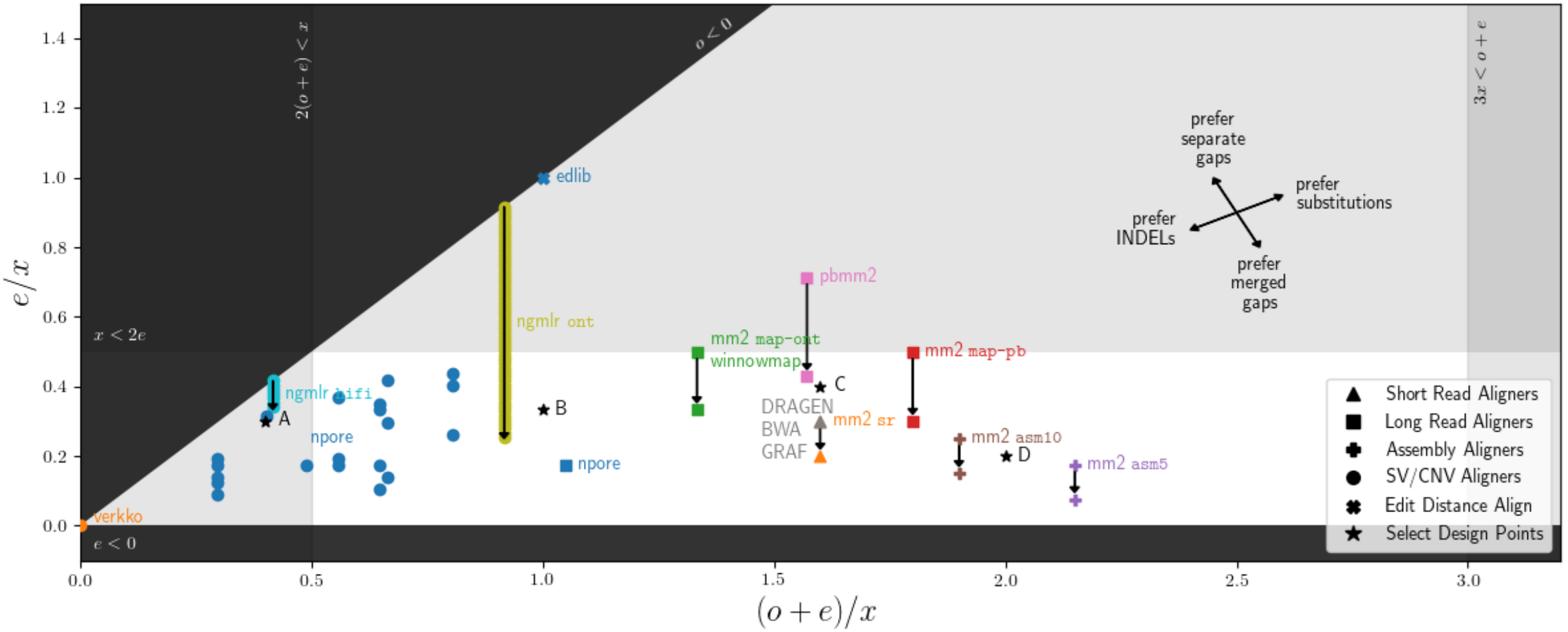


Olson et al. “PrecisionFDA Truth Challenge V2: Calling variants from short and long reads in difficult-to-map regions.” Cell, 2022.

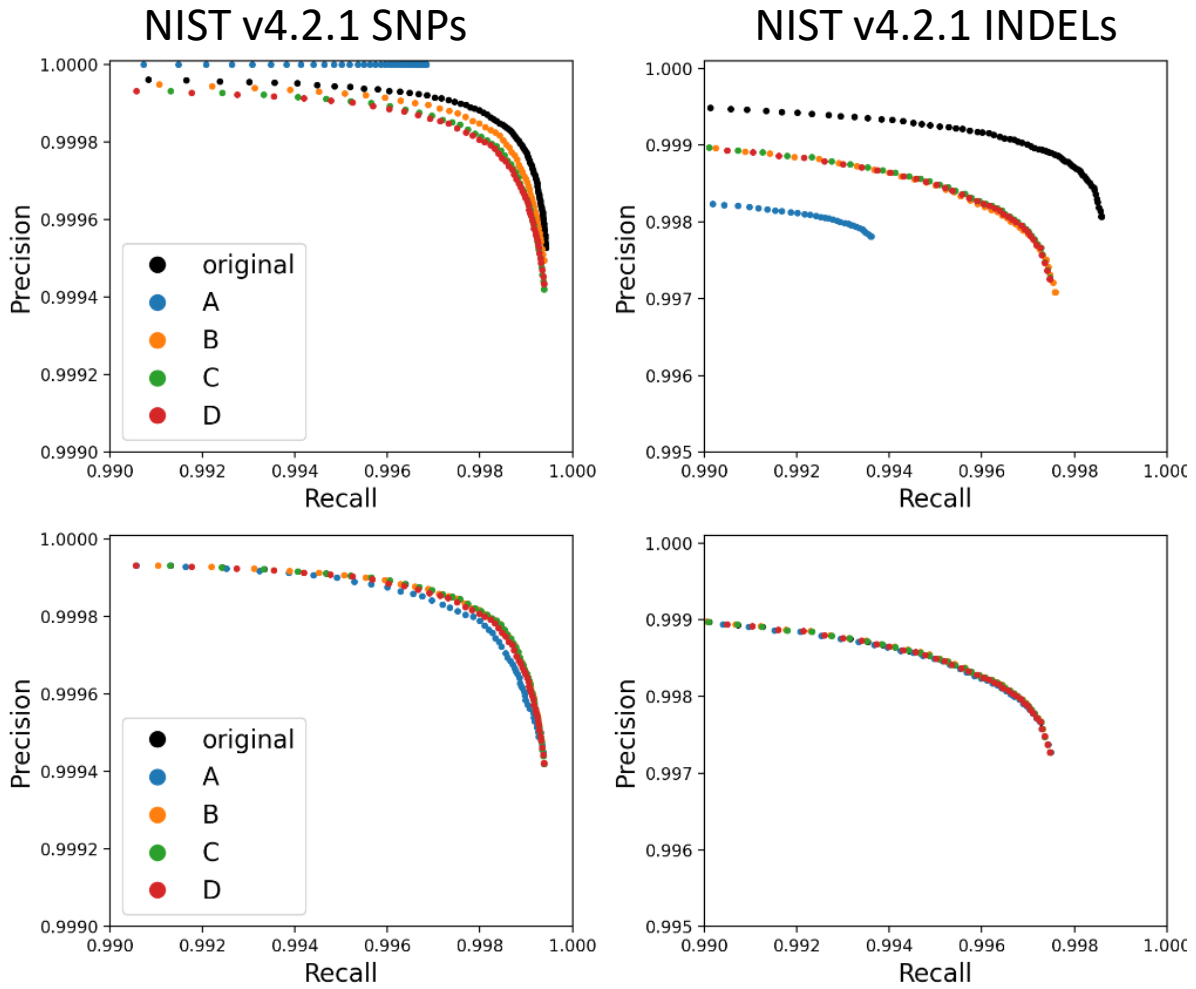
Analysis: *select design points*



Results: *normalization fixes representation bias*

Before

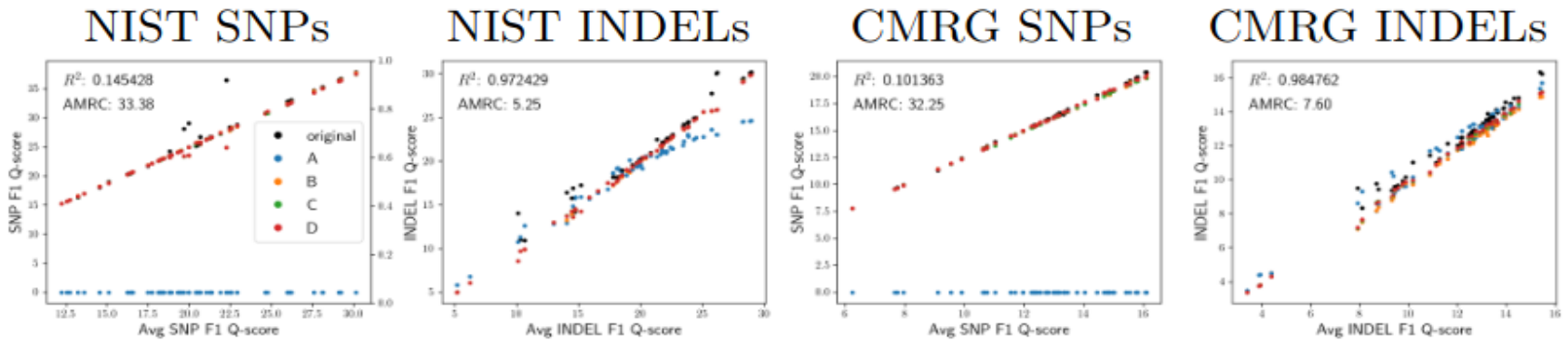
After



Prior Work
vcfeval

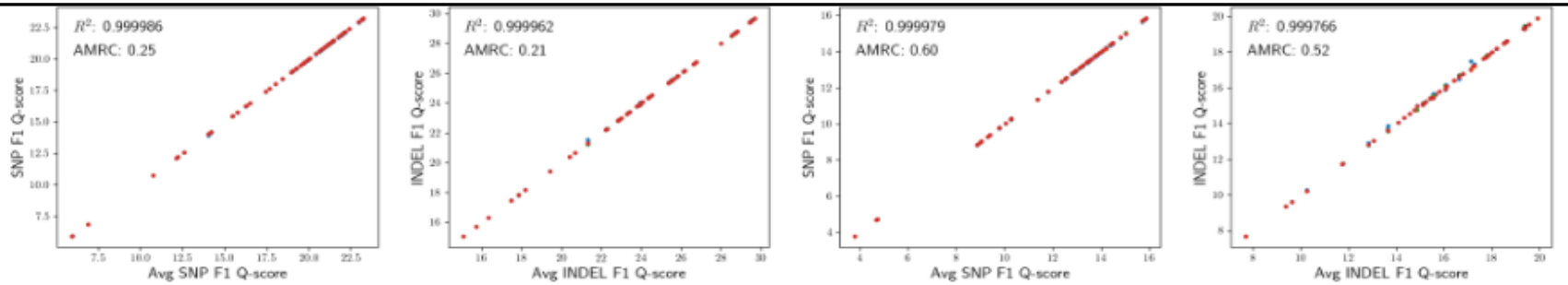
Results: *stable performance across representations*

vcfeval precision/recall metrics



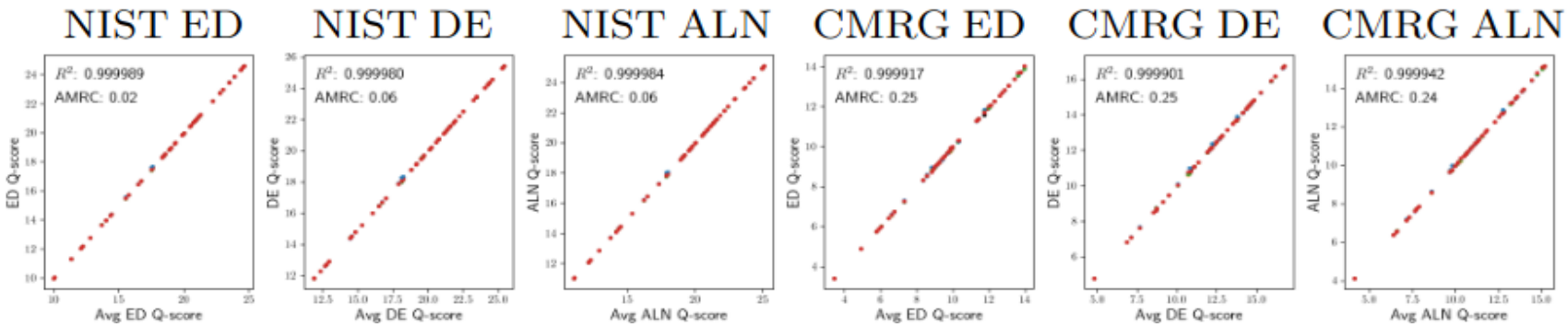
Prior Work

vcfdist precision/recall metrics



This Work

vcfdist distance metrics



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7. **Next Steps:** structural and unphased variants

Next Steps: *structural and unphased variants*

NIST Collaboration

- vcfdist now works with structural variants up to 10,000bp
- Comprehensive evaluation of recent Verkko assemblies
- Simultaneous benchmarking of SNPs/INDELs/TRs/SVs

Next Steps: *structural and unphased variants*

NIST Collaboration

- vcfdist now works with structural variants up to 10,000bp
- Comprehensive evaluation of recent Verkko assemblies
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Planned Research

- Extend vcfdist's alignment algorithm to more general graphs
- This allows vcfdist to evaluate unphased query variant call sets

Conclusion

`github.com/`**`TimD1/vcfdist`**

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Expected Graduation: **Summer 2024**