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vcfdist: accurately benchmarking phased small variant calls

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Introduction: VCF Benchmarking

- With many new emerging sequencing technologies and methods, it's important to accurately assess the relative performance of each option
- This is done by comparing the set of variants called to a known ground truth set of variant calls in VCF format

Technology #1
Reference: ACCGTTGAAG
Query #1: ACAGTAGAAG

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

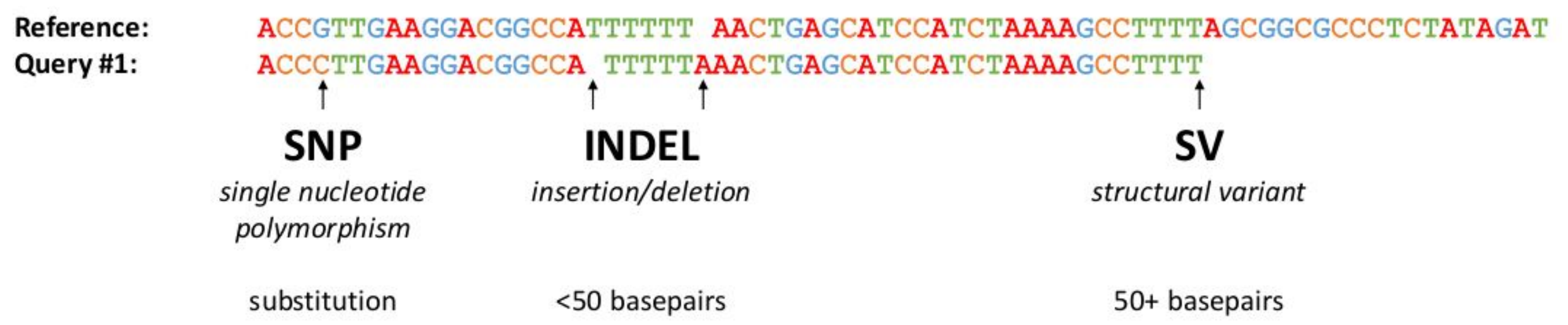
Technology #2
Reference: ACCGTTGAAG
Query #2: ACCGTAGAGG

	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

- Performance metrics such as precision and recall are reported independently for each category of variant


Reference: ACCGTTGAAGGACGGCCATTTTTT AACTGAGCATCCATCTAAAAGCCTTTTAGCGGCGCCCTCTATAGAT
Query #1: ACCCTTGAAGGACGGCCA TTTTTAAACTGAGCATCCATCTAAAAGCCTTTT

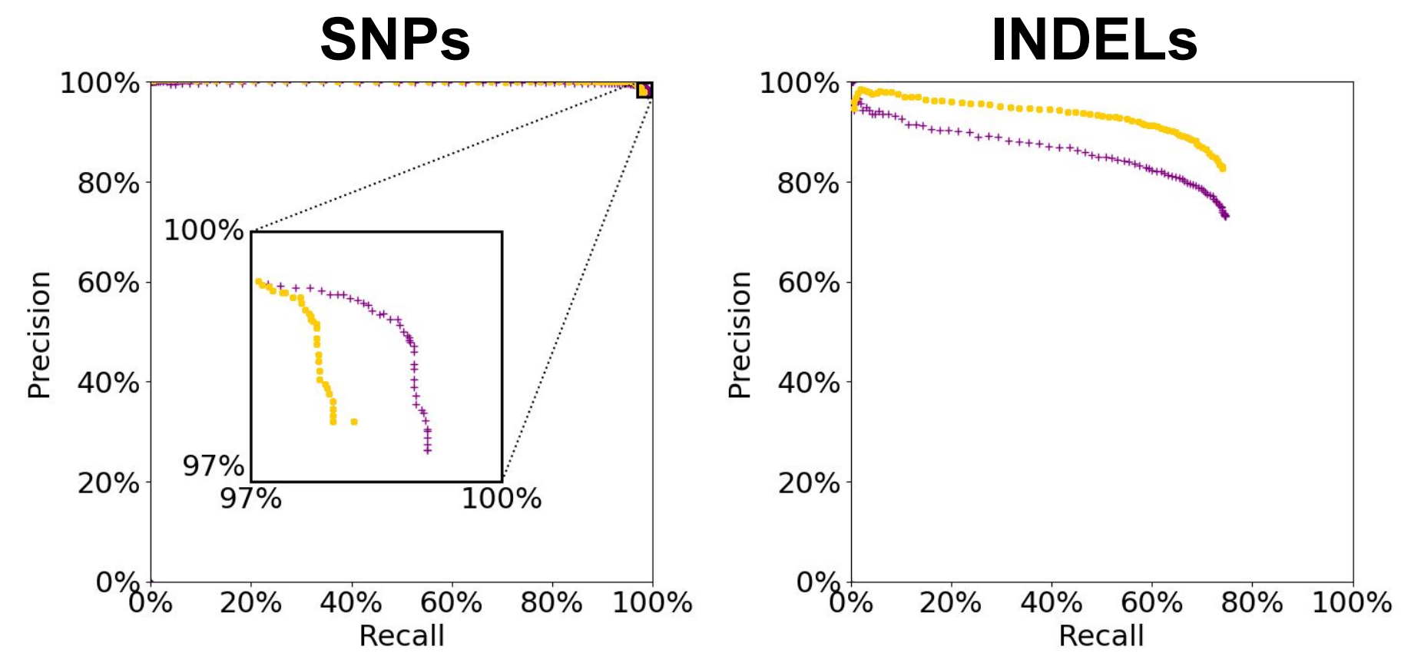
SNP
single nucleotide polymorphism
substitution

INDEL
insertion/deletion
<50 basepairs

SV
structural variant
50+ basepairs

Motivation

- Generating SNP/INDEL precision-recall curves for phased VCFs using vcfeval[1] does not result in stable evaluations
- The following figure shows benchmarking results for two VCFs which both encode the exact same underlying query sequence, but prefer different variant representations

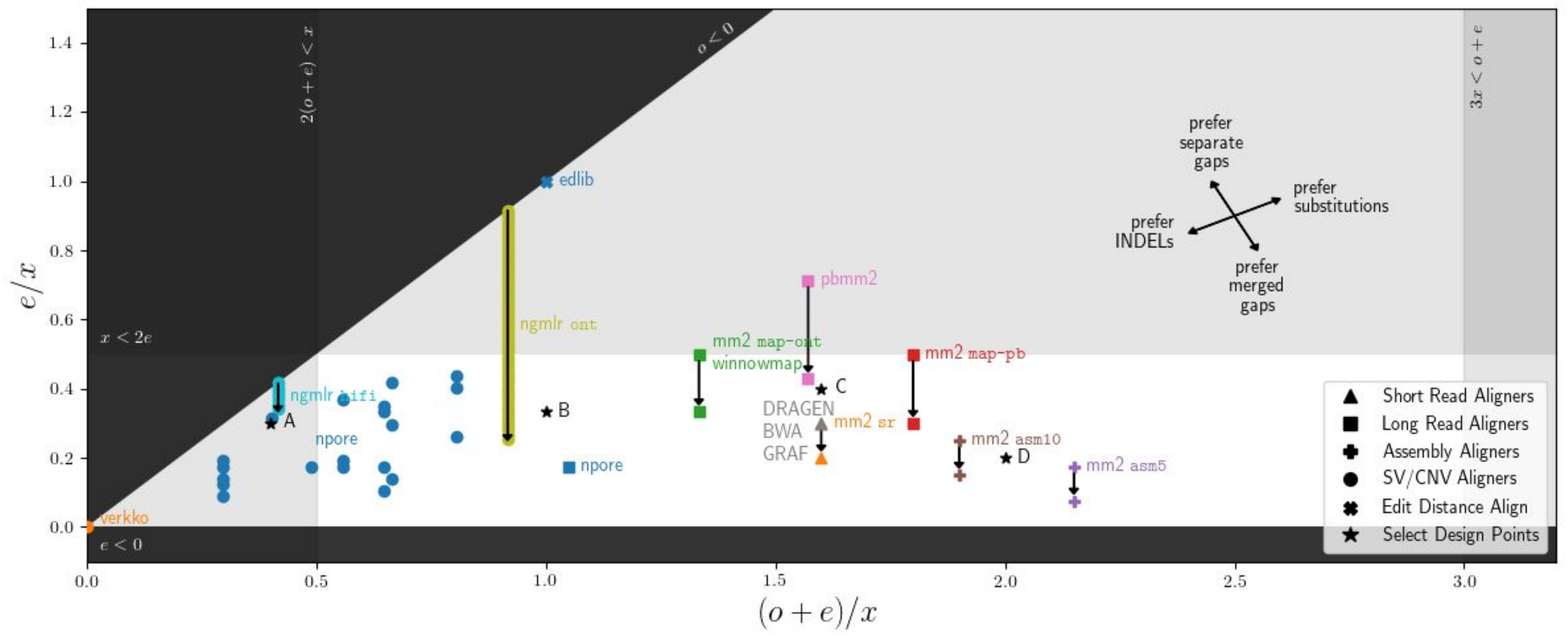


1. Variant Clustering

- The human genome is prohibitively large for exact alignment of entire chromosomes, and must be partitioned into independent subproblems for efficient analysis
- We present an alignment-based algorithm for identifying potential variant dependencies and grouping variants into independent clusters

2. Variant Normalization

- We present a thorough exploration of "Best Alignment Normalization" [2] affine-gap parameters



Reference AGGCGACA   Query ATACCGAGCTTA

Point A
$m, x, o, e = 0, 10, 1, 3$

Alignment

```
AGG---CGA-C--A
.     ... .  .
A--TACCGAGCTTA
```

VCF

POS	REF	ALT
1	AGG	A
3	G	GTAC
6	A	AG
7	C	CTT

Point B
$m, x, o, e = 0, 3, 2, 1$

```
A-GGCGA-C--A
.   ... .  .
ATACCGAGCTTA
```

POS	REF	ALT
1	A	AT
2	G	A
3	G	C
6	A	AG
7	C	CTT

Point C
$m, x, o, e = 0, 5, 6, 2$

```
A-GGCGA---CA
.   ...    .
ATACCGAGCTTA
```

POS	REF	ALT
1	A	AT
2	G	A
3	G	C
6	A	AGCT
7	C	T

Point D
$m, x, o, e = 0, 5, 9, 1$

```
AGGCGAC-------A
.    ..       .
A---TACCGAGCTTA
```

POS	REF	ALT
1	AGGC	A
5	G	T
7	C	CCGAGCTT

3. Enforce Local Variant Phasing

- Previous work vcfeval[1] was designed for short read variant calls and assumes all variant calls are unphased
- For vcfdist, local phasing is enforced within each cluster of variants and arbitrary phase swaps are allowed to occur between clusters

4. Partial Credit for Variant Calls

- Using a novel alignment algorithm, we can assign partial credit to mostly-correct calls
- This allows inexact matches for long or complex variants

Ref. ACCCTTTTTTG   **Query** ACCTTTG   **Truth** ACCCTTTG

Query VCF Representation 1

POS	REF	ALT
3	CCTTT	C

Query VCF Representation 2

POS	REF	ALT
1	AC	A
4	CTTT	C

Truth VCF

POS	REF	ALT
4	CTTT	C

vcfeval Summary Statistics

	TP	FP	FN	PP	Prec.	Recall	F1
Query Repr. 1	0	1	1	0	0.00	0.00	0.00
Query Repr. 2	1	1	0	0	0.50	1.00	0.67

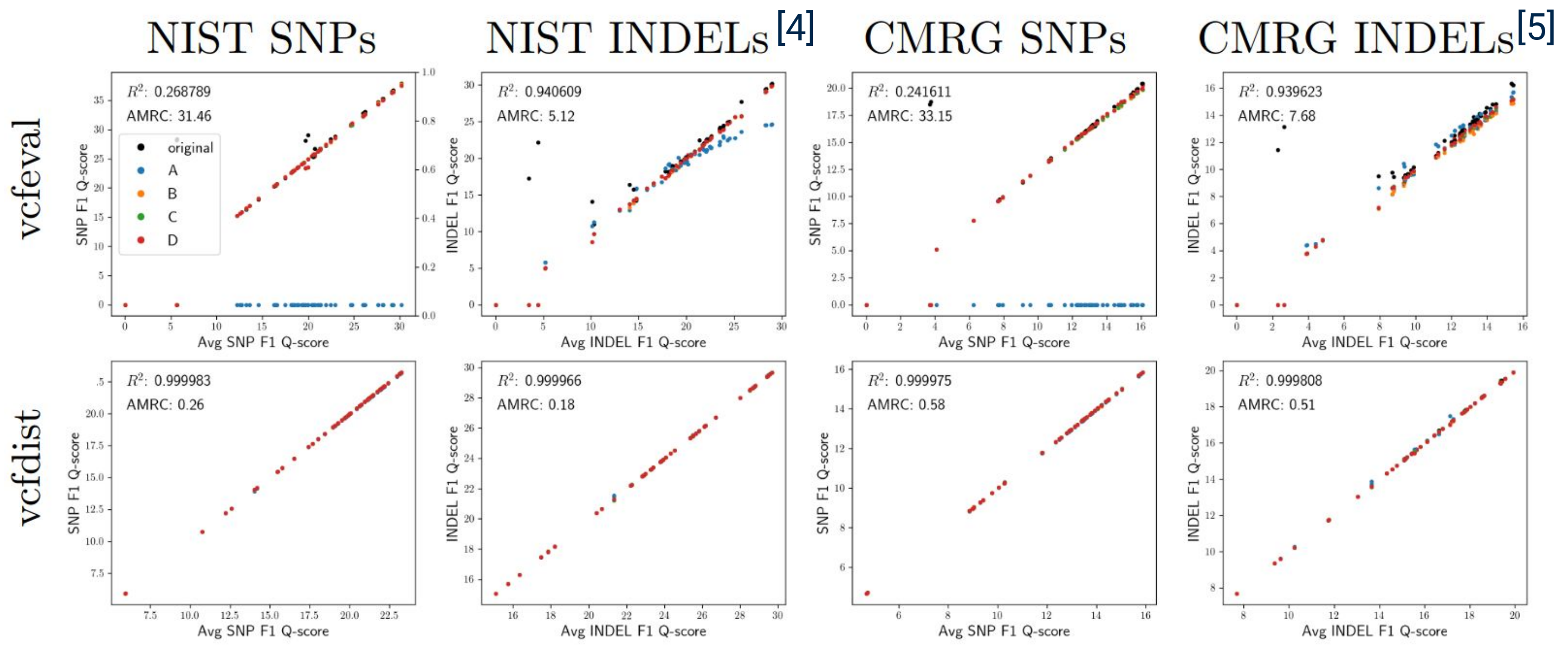
vcfdist Summary Statistics

	TP	FP	FN	PP	Prec.	Recall	F1
Query Repr. 1	0	0	0	1	0.67	0.67	0.67
Query Repr. 2	1	1	0	0	0.50	1.00	0.67

5. Alignment Distance Metrics

- Since SNP and INDEL variants are ill-defined in the case of complex variants, we can avoid classifying variants and simply align the query and truth sequences
- High-level summary information regarding the alignment can then be used to evaluate performance
  - **Edit Distance:** the total number of bases which differ
  - **Distinct Edits:** the total number of variants
  - **Alignment Distance:** the affine-gap alignment score

Results[3]



References

[1] Cleary et al. *"Comparing variant call files for performance benchmarking of next-generation sequencing variant calling pipelines"*. bioRxiv, 2015.
[2] Bayat et al. *"Improved VCF normalization for accurate VCF comparison"*. Bioinformatics, 2017.
[3] Olson et al. *"PrecisionFDA Truth Challenge V2: Calling variants from short and long reads in difficult-to-map regions"*. Cell Genomics, 2022.
[4] Wagner et al. *"Benchmarking challenging small variants with linked and long reads"*. Cell Genomics, 2022.
[5] Wagner et al. *"Curated variation benchmarks for challenging medically relevant autosomal genes"*. Nature Biotechnology, 2022.

Additional Information

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Code: github.com/TimD1/vcfdist