# 2024 SMaHT SNV/indel Detection Challenge
# A Summary of Results

(prepared by DAC; last update on 7/8/24)

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## Data Release - Version 1.0

Version 1.0 (June 26, 2024) is an **unofficial internal release.**
*** **The V1.0 data are to be shared within the SMaHT Network only.** ***
The indel truth set will be made available in the near future.
The official public release of the final truth sets may be made after the consortium-wide approval.

**What is released in V1.0:**
1. Truth set for SNVs in COLO829-BLT50.
2. Negative controls for SNVs: (i) germline variant sites based on COLO829-BL and (ii) homozygous reference / non-variant sites.
3. Genome stratification: (i) easy, (ii) difficult, and (iii) extreme regions.
4. [*For the final call sets OK to be published*] Submitted VCFs from GCCs and accuracy metrics (precision, sensitivity, F1 score) from the SNV/Indel Challenge.

The original calls submitted by centers are normalized (i.e., split multiallelic variants and left align indels), and, where applicable, files have been re-formatted in the standard VCF format for proper comparisons. The README files describing the pipelines and additional notes provided by submitters are included in the same directory as the VCFs.

All data are immediately available via Globus, a secure data transfer tool that can move large amounts of data between the source and destination storages directly. The data are organized into subfolders. The top folder, denoted as the **SMaHT-DAC SNV/Indel Challenge Release V1** collection on Globus, is the endpoint to which you will be added to access all subfolders. To access the data via Globus, please contact DAC [`smhelp@hms-dbmi.atlassian.net`] with your Globus ID.

### Directory structure at DAC's Globus endpoint

```
.
└── snvIndelChallenge_Release_V1
    ├── callsets_from_centers
    │   ├── GCC_Broad
    │   │   ├── accuracy_scores
    │   │   └── submitted_vcf_normalized
    │   ├── GCC_NYGC
    │   │   ├── accuracy_scores
    │   │   └── submitted_vcf_normalized
    ├── genomeTiers
    ├── negControl_snv_COLO829
    │   ├── germline
    │   └── homozygous_ref
    └── truthset_snv_COLO829
    └── SMaHT_Benchmark_SNVIndel_Challenge-DataRelease_V1.pdf (this document)
```

Under the `accuracy_scores` directory for each center are accuracy matrices that contain precision, sensitivity, and F1 scores across VAF bins for *in silico* mixture and cell-admixture BLT50 samples.

The file name prefix of an accuracy matrix is the following:

[SUBMITTER]_[SAMPLE DESCRIPTION]_[DATA GENERATOR]

E.g. File names of the accuracy matrices of the Broad's pipelines applied on 100X *in silico* mixture BLT50 and the cell-admixture BLT50 from BCM:

`BROAD_insilico-BLT50-100X.accuracy.tsv`
`BROAD_BLT50_BCM.accuracy.tsv`

### Other Files in Version 1 Release:

**Table 1.** The SNV truth set used for the COLO829 SNV/Indel Detection Challenge.

| Data description | File name | File size |
|---|---|---|
| COLO829 SNV truth set - Version 1.0 | `SMaHT_COLO829_SNV_truth_set_v1.0.vcf` | 3.3 MB |

**Table 2.** The negative control sets for SNVs in COLO829-BLT50.

| Data description | File name | Total file size |
|---|---|---|
| Germline variant sites | `colo829bl_germline.vcf.gz` | 280 MB |
| Homozygous reference sites | (Grouped by chromosomes)<br>`/byChr/chr*.merged.bed`<br>(Grouped by genomic tiers and chromosomes)<br>`/byGenomeTiers/[easy\|difficult\|extreme]/chr*.merged.bed` | 2.2 GB |

**Table 3.** Genome stratification used for the COLO829 SNV/Indel Detection Challenge.

| Category | Tier(s) | File name | File size (MB) |
|---|---|---|---|
| Easy | Tier 0 | `SMaHT_easy_regions_GRCh38_v1.0.bed` | 111 |
| Difficult | Tiers 1-4 merged | `SMaHT_difficult_regions_all_GRCh38_v1.0.bed` | 116 |
| | Tier 1 | `SMaHT_difficult_regions_tier1_GRCh38_v1.0.bed` | 141 |
| | Tier 2 | `SMaHT_difficult_regions_tier2_GRCh38_v1.0.bed` | 76 |
| | Tier 3 | `SMaHT_difficult_regions_tier3_GRCh38_v1.0.bed` | 43 |
| | Tier 4 | `SMaHT_difficult_regions_tier4_GRCh38_v1.0.bed` | 23 |
| Extreme | Tier 5 | `SMaHT_extreme_regions_GRCh38_v1.0.bed` | 5.7 |

# COLO829 truth set

The truth set in the Version 1 release includes SNVs with VAF ≥ 25% in the pure COLO829-T cancer cell line (corresponding to VAF ≥ 0.5%, expected in the BLT50 mixture samples) to analyze variants considered for the SNV/Indel Detection Challenge.

## Overview of the truth set generation

To achieve high sensitivity, the truth set of the COLO829-T cancer cell line was derived by taking the union of somatic variants identified by four callers (Mutect2, Strelka2, VarNet, and RUFUS) using the Illumina WGS data. To achieve high precision, the truth set was validated by PacBio HiFi reads using `bcftools mpileup` with BQ and MQ ≥ 30 (**Figure 1**). We considered SNVs in three different genomic regions where Illumina short-reads are mapped with different levels of confidence.

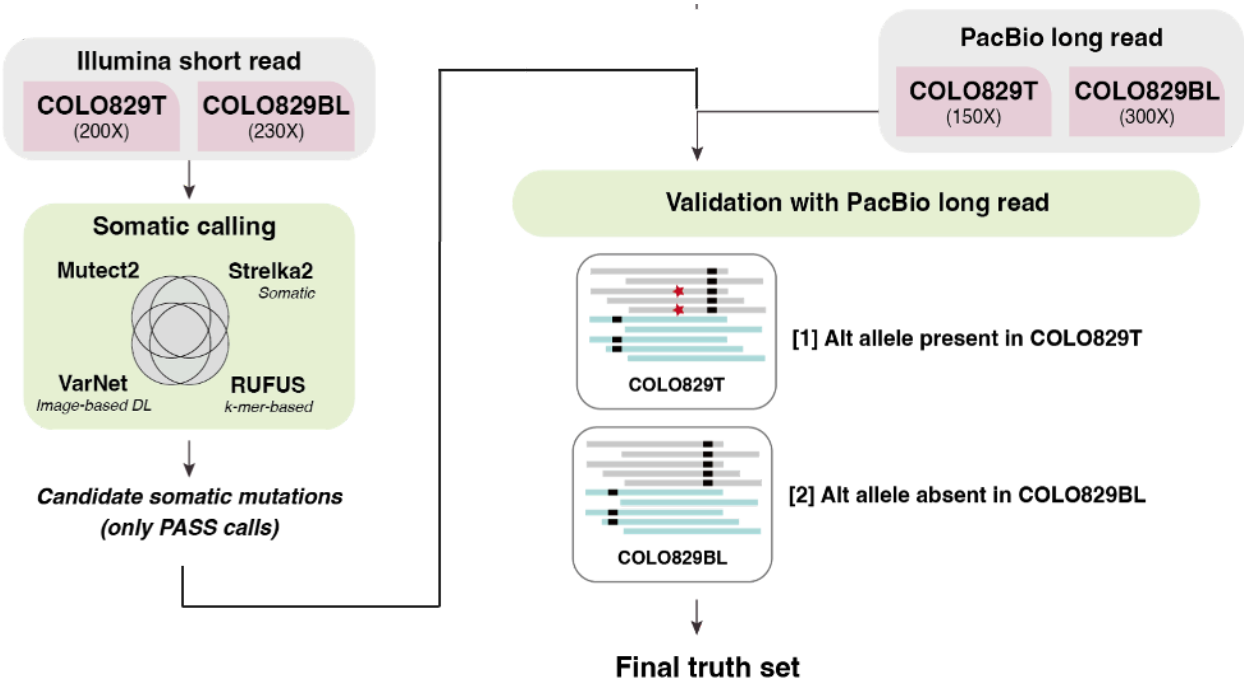



**Figure 1.** Overview of the COLO829-T truth set generation method.

## Distribution of the truth set variants across VAF ranges

The true variants in COLO829-BLT50 are expected to have VAF under 2% based on the mixing ratio of 49-parts COLO829-BL to 1-part COLO829-T. As expected, the distribution of VAF of the truth set variants shows the majority under 2%, as shown in **Figure 2**.

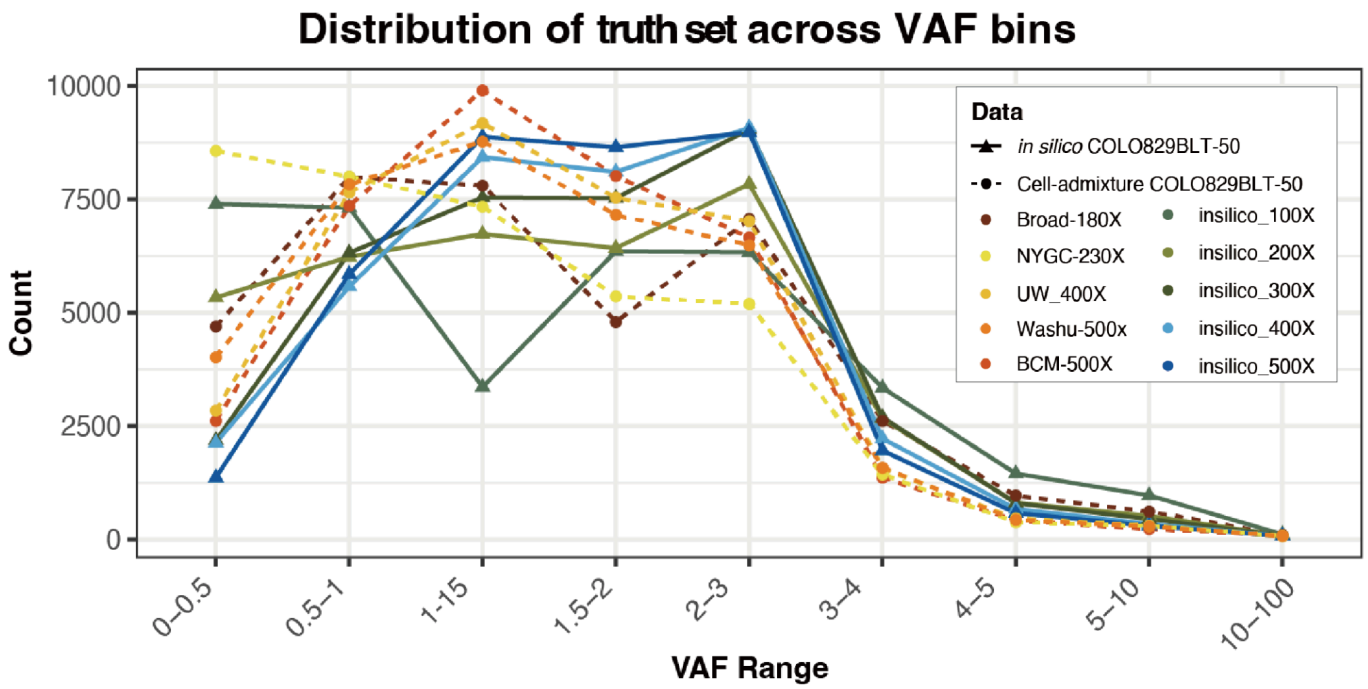



**Figure 2.** VAF distribution of SNVs in the truth set across different COLO829-BLT50 samples.

## Defining the negative controls for the COLO829-T truth set

Sometimes an algorithm makes a call using Illumina data, which is not cross-validated by an orthogonal dataset (e.g., PacBio). We cannot be sure that such a call is false-positive, e.g., due to PacBio data having insufficient coverage at this position. Thus, we derived a “negative control” set consisting of positions that are (almost certainly) not true mosaic variant sites. If the negative control set is not defined, true mosaic variants can be miscategorized as false positive calls (e.g. true variants among the non-PASS Mutect2 calls), potentially leading to the underestimation of precision.

We determined the negative controls as the following:

- Non-variant, homozygous reference positions
    - Two types of candidate sites were identified across the genome, based on raw pileup counts compared between the number of supporting reads for the alt allele in COLO829-T and -BL:
      (1) Homozygous reference sites
        - Positions that were annotated as 0/0 in GT (genotype), not 1/0 nor 1/1, by Sentieon Haplotyper (which implements GATK HaplotypeCaller) using the `GVCF` mode.
      (2) Positions that failed during the COLO829-T truth set generation, consisting of:
        a. Variants that were filtered out during the short-read variant calling process (e.g., those annotated as ‘weak_evidence’ in the FILTER field instead of ‘PASS’)
        b. Variants that were not validated by PacBio reads because there was no supporting alt allele in COLO829-T.

- Germline variant positions

- COLO829-BL germline variants were identified by Sentieon Haplotyper using PacBio data.

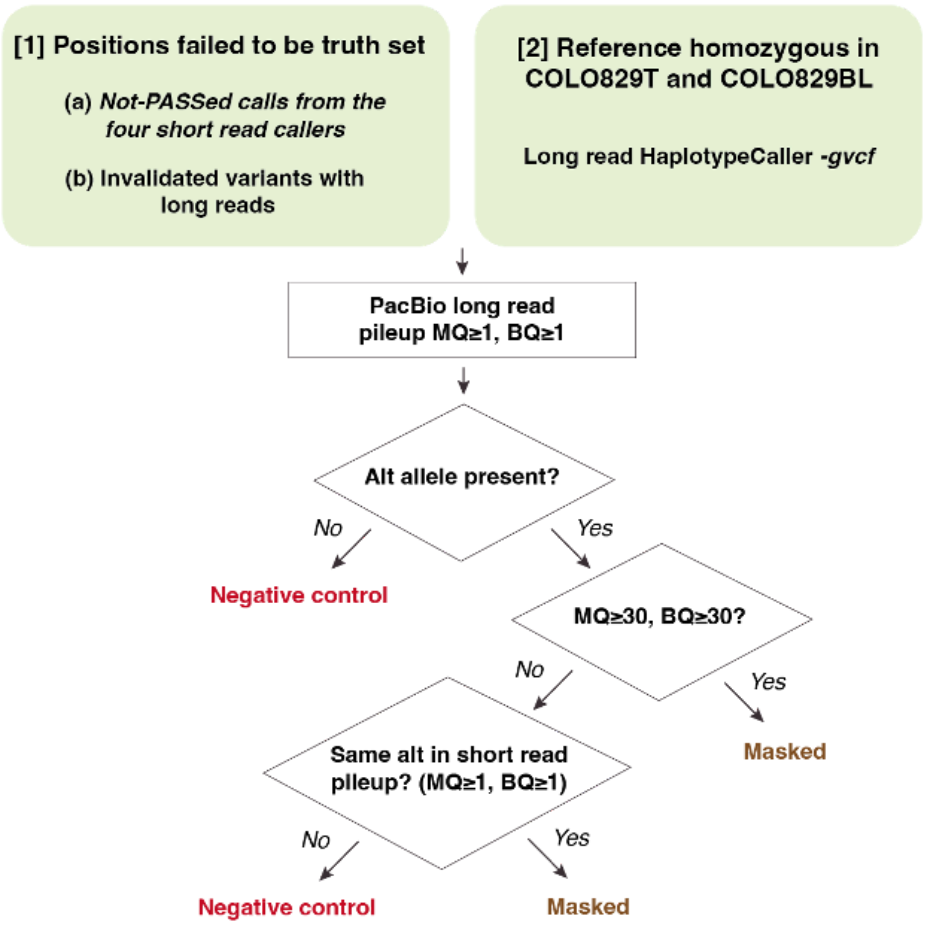



**Figure 3.** Overview of the negative control design and generation.

## Pipelines Used by GCCs/TTDs

**Table 4.** Pipelines and filtering methods used by GCCs, TTD, and DAC. Column names in the accuracy matrix contain abbreviations of the pipelines and filtering methods, as shown in blue.

| Center | Main algorithm | Filtering method & Note |
|---|---|---|
| BCM-GCC | DRAGEN (v4.3) | VAF 10%; variant calling with mosaic detection on |
| Broad-GCC | BROAD_MUTECT2: Mutect2 using the tumor-only mode | Filtered against the publicly available panel of normal and gnomAD; Ran with the GATK best practice.<br>Post-calling filter: Exact binomial test to remove germline calls; Removed calls with Depth <50X or >1,000X; Removed calls in pseudoautosomal regions of chr X and Y; FilterMutectCalls |
| | BROAD_DRAGEN: DRAGEN (v4.3 - beta) | Used parameters to avoid the RG issue ("--use-single-read-group-for-bam-list) and VAF < 20% ("--vc-mosaic-af-filter-threshold = 0.2")<br>Same post-calling filter as above; Variants with the "PASS" flag |

| | | |
|---|---|---|
| | BROAD_DRAGEN_Direct: DRAGEN (v4.3 - beta) | The same DRAGEN pipeline described above was run directly on their original CRAM file. |
| NYGC-GCC | NYGC_Lancet: Lancet2 - single sample mode | Filtered against germline calls using HaplotypeCaller + GenotypeGVCFs workflow; removed calls in gnomAD (>0.00001% AF) and 1KGenome (high-coverage panel), and in UCSC simpleRepeats and segDup regions; Depth = 150 - 250, whole coverage = 200 - 300, allele depth > 4, VAF < 20%, strand, allele quality and mapping quality considered; Removed indels of 1 bp. |
| WashU-GCC | WASHU_Vaf: Mutect2 (GATK 4.4.0.0) - VAF | FilterMutectCalls; VAF < 2 % |
| | WASHU_Mutect2dbSNP: Mutect2 (GATK 4.4.0.0) - dbSNP | FilterMutectCalls; Remove calls in dbSNP v151 |
| Utah-TTD | Utah_Lenient: RUFUS - Lenient | 3 filter levels to reduce the noise levels in the call set: (1) Lenient; (2) moderate, and (3) strict.<br><br>(* RUFUS only works with a normal control sample - 230X COLO829-BL was used) |
| | Utah_Moderate: RUFUS - Moderate | |
| | Utah_Strict: RUFUS - Strict | |
| UW-GCC | UW_MUTECT2: Mutect2 using the tumor-only mode | FilterMutectCalls; VAF > 5% with Depth > 20 |
| | UW_DRAGEN: DRAGEN Somatic (v3.9.5) | Variants with the “PASS” flag: VAF > 5% |
| DAC | DAC_MF: MosaicForecast | Removed calls in UCSC SimpleRepeats, SegmentalDuplication, MosaicForecast clustered regions. |

## Comparisons of mosaic SNV methods across *in silico* COLO829-BLT50 samples

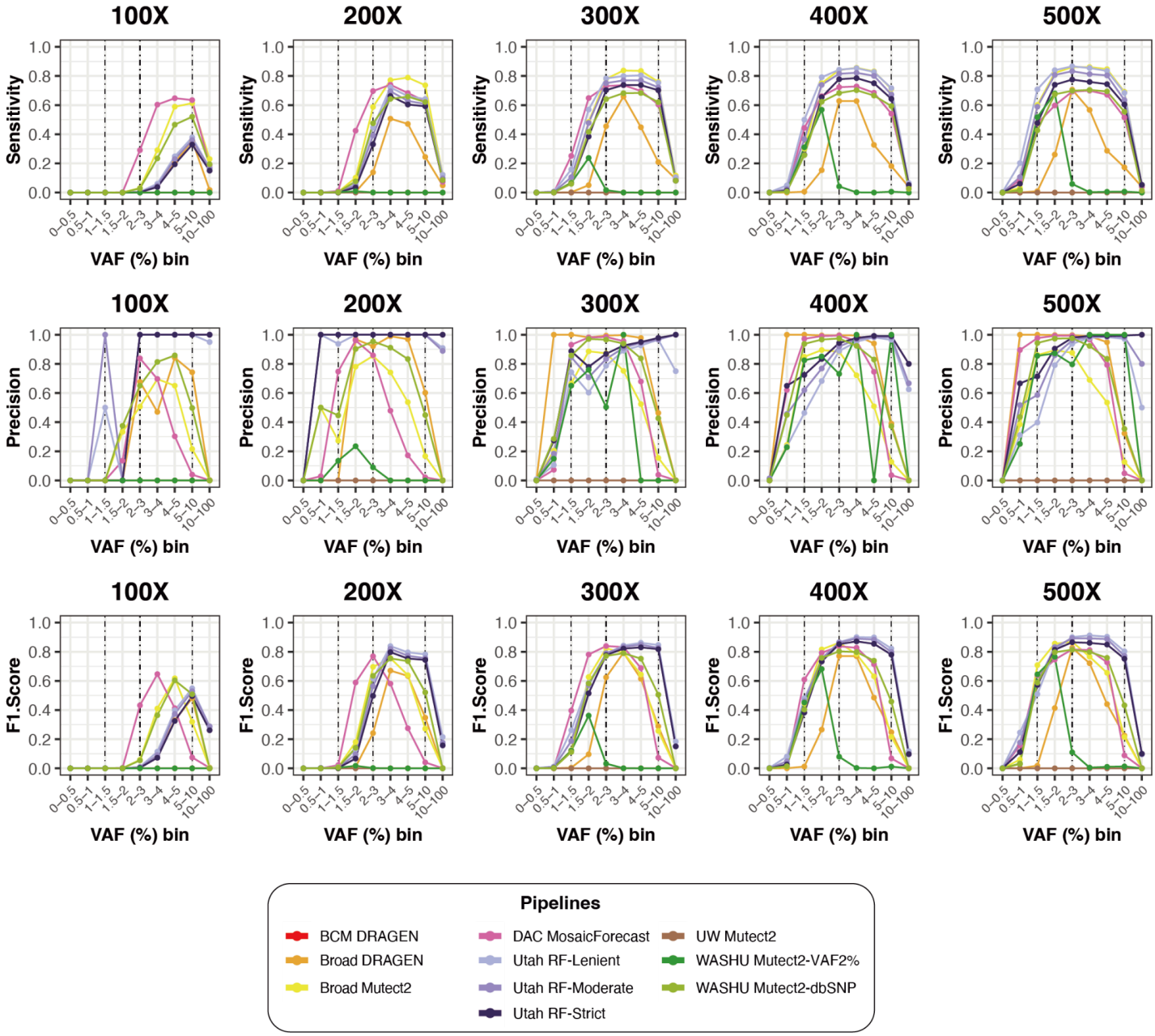



**Figure 4.** Pipeline performance comparisons across five *in silico* COLO829-BLT50 samples.

## Comparisons of mosaic SNV methods across cell-admixture COLO829-BLT50 samples

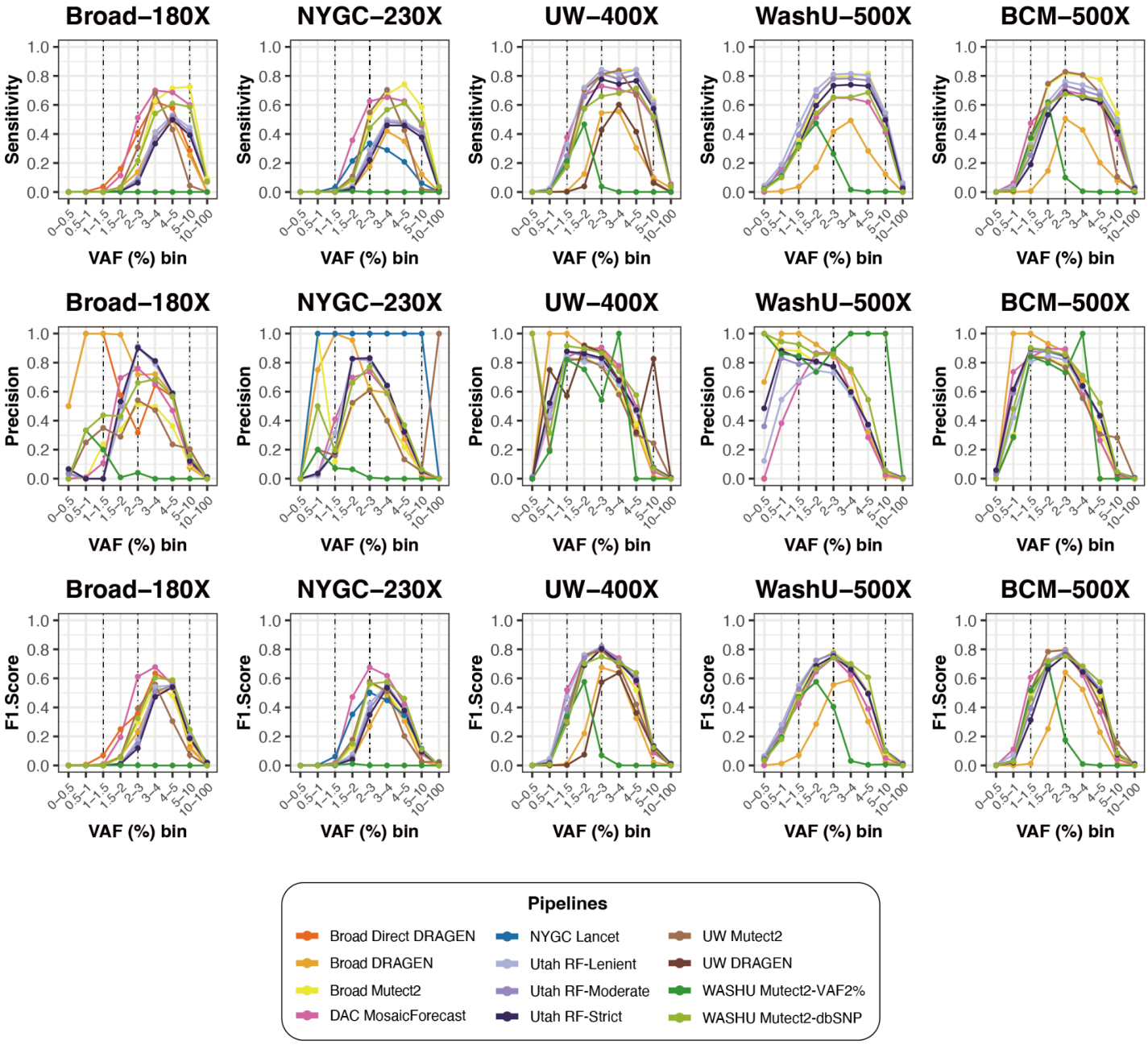



**Figure 5.** Pipeline performance comparisons across five cell-admixture COLO829-BLT50 samples.

## Genome stratification for SNV benchmarking

### Expanding the truth set to include "difficult" genomic regions

Mosaic variant detection in genomic regions with low mappability can be challenging. If the variants in the truth set were considered only in the regions with high mappability (i.e. "high-confidence" regions), it becomes an "easy" benchmark study with inflated performance measures.

Thus, we took the GIAB ("Genome in a Bottle") easy regions as the baseline (in the "Easy" category) and expanded the genome categorization based on k-mer-based read mappability scores using the UMAP software (Karimzadeh M *et al.* (2018) NAR).

**Table 5.** Genome stratification was applied to categorize the variants in the truth set. The "Easy" regions were determined based on the GIAB regional stratification, which excludes the GIAB difficult region defined as (1) Low Complexity; (2) Functional Technically Difficult; (3) Genome Specific (4) Functional Regions; (5) GC content; (6) mappability; (7) Other Difficult; (8) Segmental Duplications; (9) Union; (10) Ancestry, or (11) XY.

| Category | Tier | Description | Bp | % of genome | |
|---|---|---|---|---|---|
| **Easy** | **Tier 0** | GIAB easy | 2,310,050,737 | 74.80 | |
| **Difficult** | **Tier 1** | Additional UMAP24 regions | 371,562,536 | 16.68 | 12.03 |
| | **Tier 2** | Additional UMAP36 regions | 53,704,601 | | 1.74 |
| | **Tier 3** | Additional UMAP50 regions | 35,085,249 | | 1.14 |
| | **Tier 4** | Additional UMAP100 regions | 54,908,647 | | 1.78 |
| **Extreme** | **Tier 5** | Rest of the hg38 genome | 262,958,062 | 8.51 | |
| **Total** | | **Entire hg38 genome** | **3,088,269,832** | **100** | |

## Overview of the truth set across the stratified genomic regions

The truth set contains 44K high-quality SNVs (plus 644 putative indels; to be distributed in the next release after more refined validation) across the genomic regions described above.

We also categorized the negative control sets into the genomic regions as follows:

- Negative controls for non-variant, homozygous reference positions
    - Total number of sites = 2,772,580,858 (90% of the entire genome)
        - Easy region: 2,229,413,903 (72% of the genome)
        - Difficult region: 475,890,667 (15% of the genome)
        - Extreme region: 67,276,288 (2% of the genome)
- Negative controls for germline variants (COLO829-BL)
    - Total number of sites = 6,208,223
        - Easy: 3,205,125 (52% of the germline variants)
        - Difficult: 2,803,519 (45% of the germline variants)
        - Extreme: 203,189 (3% of the germline variants)

**Table 6.** True SNV and indel calls across the easy, difficult, and extreme categories of the genomic region. The "All" variant set includes all somatic calls; The "Challenge" set includes only the variants with VAF ≥ 25% in COLO829-T, to consider the variants with VAF ≥ 0.5% expected in the BLT50 mixture samples for the Challenge.

| Category | Tier | SNVs (All) | | SNVs (Challenge) | | Indels (All) | | Indels (Challenge) | |
|---|---|---|---|---|---|---|---|---|---|
| **Easy** | **Tier 0** | 35,128 | | **29,155** | | 252 | | **166** | |
| **Difficult** | **Tier 1** | 8,989 | 6465 | **7149** | **5,107** | 389 | 278 | **230** | **176** |
| | **Tier 2** | | 999 | | **784** | | 78 | | **39** |
| | **Tier 3** | | 620 | | **505** | | 22 | | **8** |
| | **Tier 4** | | 905 | | **753** | | 11 | | **7** |
| **Extreme** | **Tier 5** | 433 | | **345** | | 3 | | **2** | |
| **Total** | | **44,550** | | **36,649** | | **644** | | **398** | |

## Comparisons of mosaic SNV detection across genomic regions

We evaluated the performance of the variant-calling pipelines across different genomic regions. As representative datasets, below are the accuracy of variant calls made from the cell-admixture BLT50 samples with the lowest (180X from Broad-GCC) and the highest (510X from BCM-GCC) sequencing depths, shown in **Figures 6** and **7**, respectively.

## Broad-GCC: 180X (COLO829-BLT50)

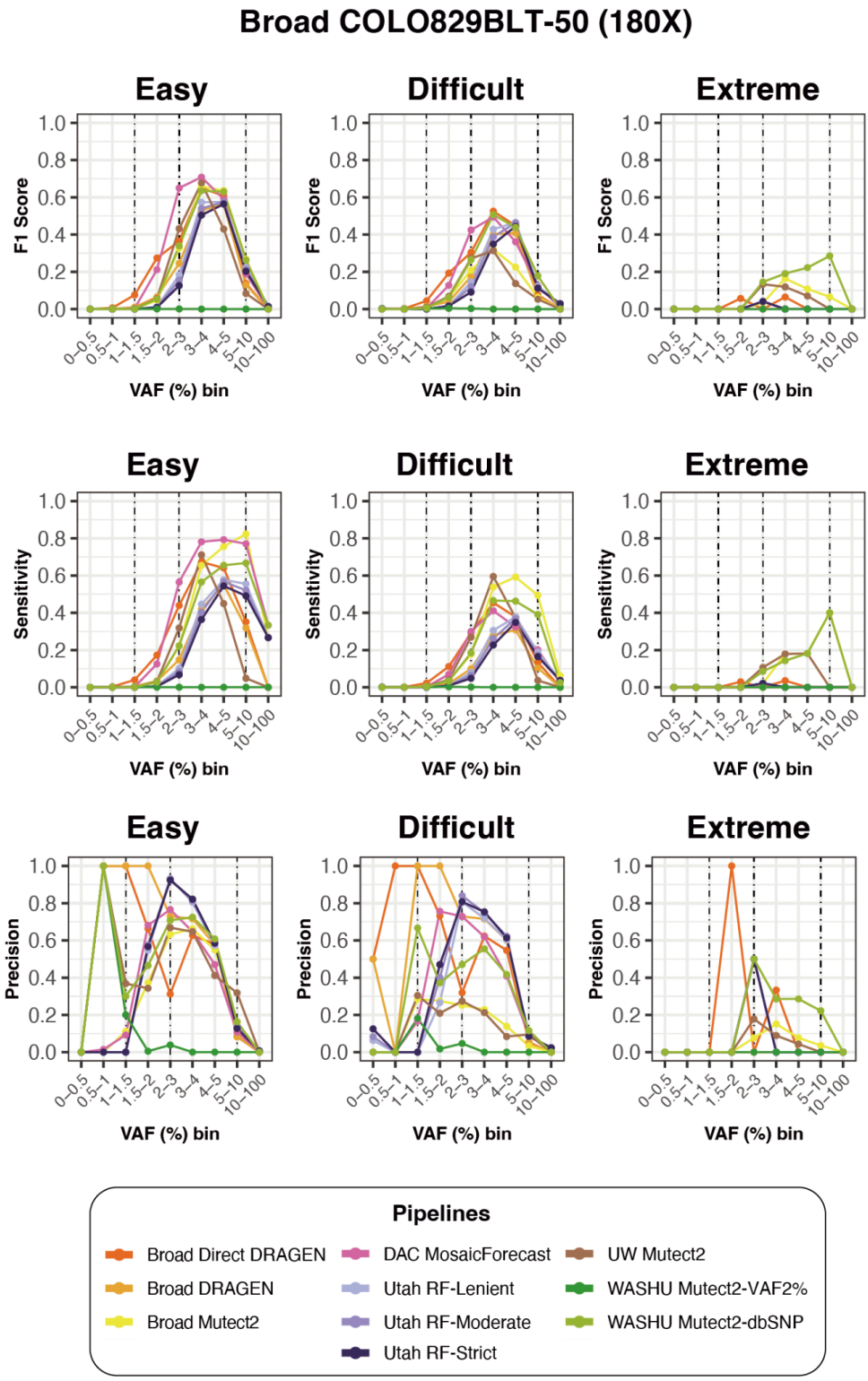



**Figure 6.** Performance comparisons across different genomic regions in 180X cell-admixture COLO829-BLT50.

## BCM-GCC: 510X (COLO829-BLT50)

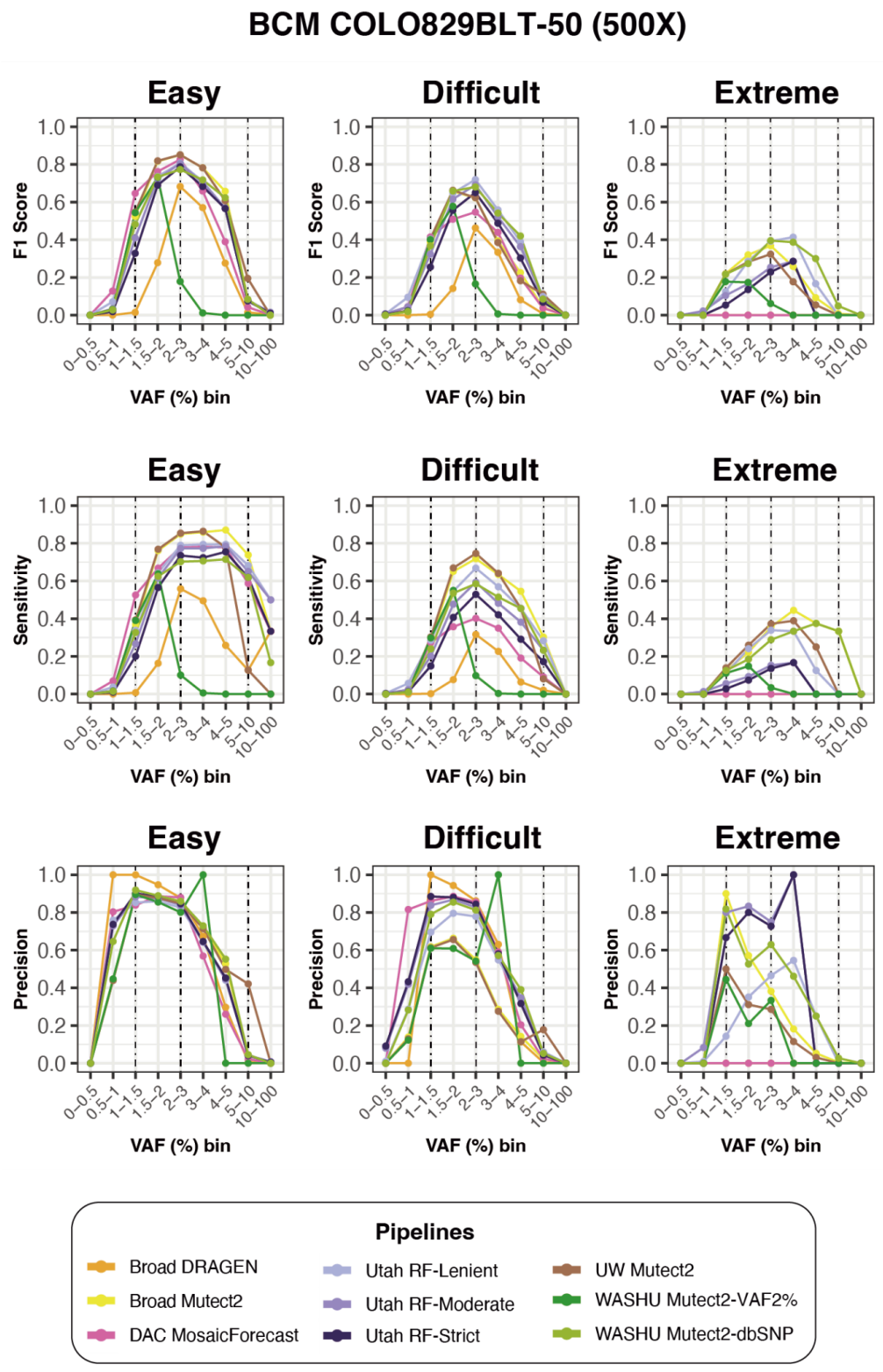



**Figure 7.** Performance comparisons across different genomic regions in 500X cell-admixture COLO829-BLT50.

# Supplementary Information

## Genome Region Stratifications for SMaHT benchmarking truth call sets of SNV/Indels

**Version**: 1.0
**Last updated:** July 1, 2024

The Data Analysis Center (DAC) of the NIH Somatic Mosaicism across Human Tissues (SMaHT) consortium generates BED files containing genomic regions with different levels of confidence for mosaic variant detection, stratified into three groups, i.e. “Easy”, “Difficult”, and “Extreme”. The “Difficult” region is subclassified into four tiers based on the Umap mappability score. The regions are based on the GRCh38 reference human genome.

Please refer to **Table 3** for the full list of BED files for all genomic regions released in Version 1.

Easy regions
“Easy” regions are high-confidence regions that exclude the difficult regions defined by Genome in a Bottle (GIAB v3.3) in autosomes and sex chromosomes, i.e. Chr1 - 22, X, Y.

We used the BED file for the GIAB v3.3 difficult regions, `GRCh38_alldifficultregions.bed.gz` under
https://ftp-trace.ncbi.nlm.nih.gov/ReferenceSamples/giab/release/genome-stratifications/v3.3/GRCh38@all/Union/

Difficult regions
Difficult regions are tiered based on the read mappability scores determined by Umap (Karimzadeh M *et al.* (2018) NAR; https://doi.org/10.1093/nar/gky677), which identifies genomic regions with uniquely mappable k-mers. A subset of the GIAB difficult regions is included and tiered based on the mappability of reads of different lengths:

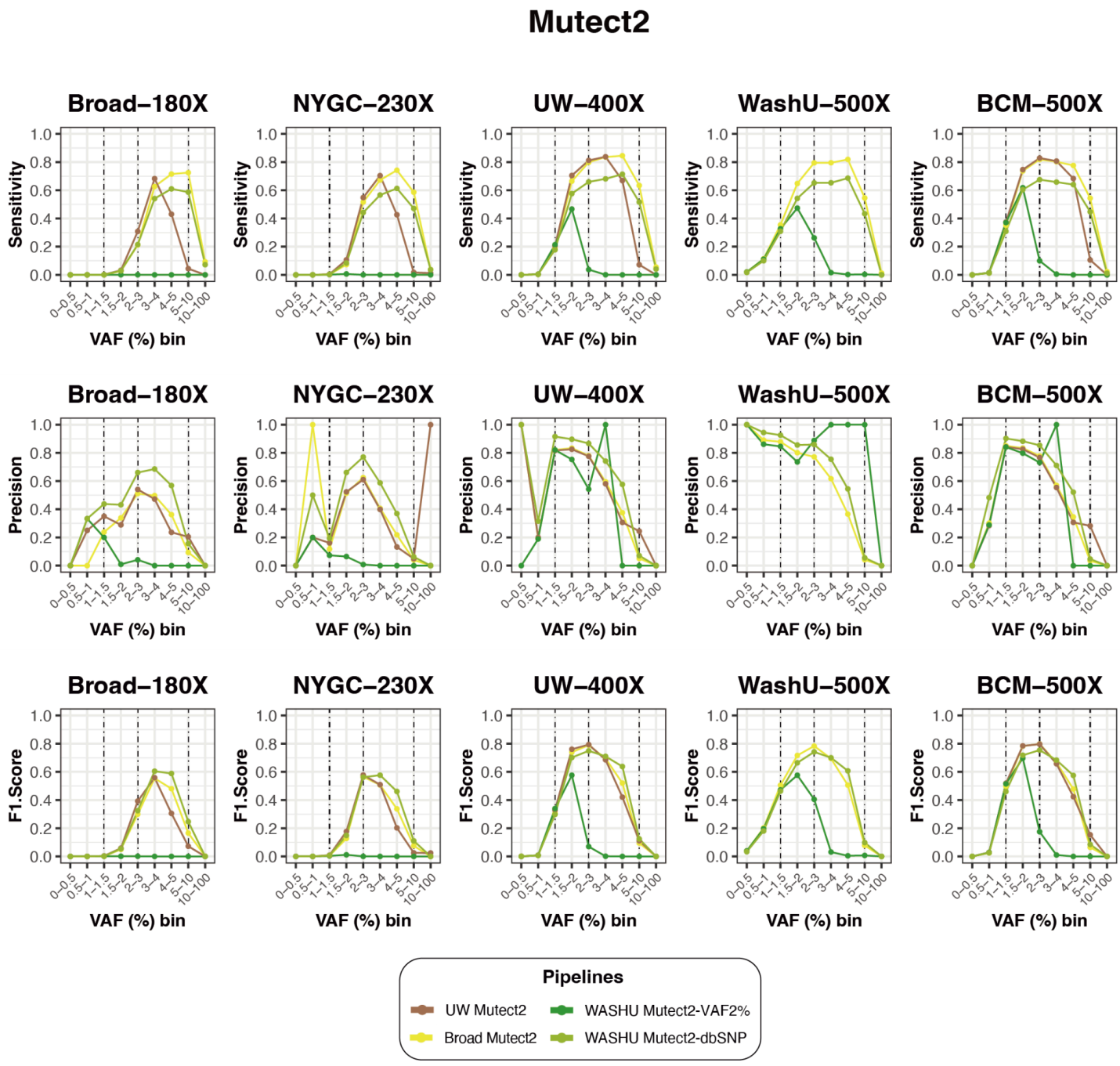
- Tier 1 [*most stringent, good mappability*]: Regions with uniquely mappable reads of 24 bp in length
- Tier 2: Regions with uniquely mappable reads of 36 bp in length
- Tier 3: Regions with uniquely mappable reads of 50 bp in length
- Tier 4 [*most lenient*]: Regions with uniquely mappable reads of 100 bp in length

*Note: The tiers of the “Difficult” regions do not overlap.

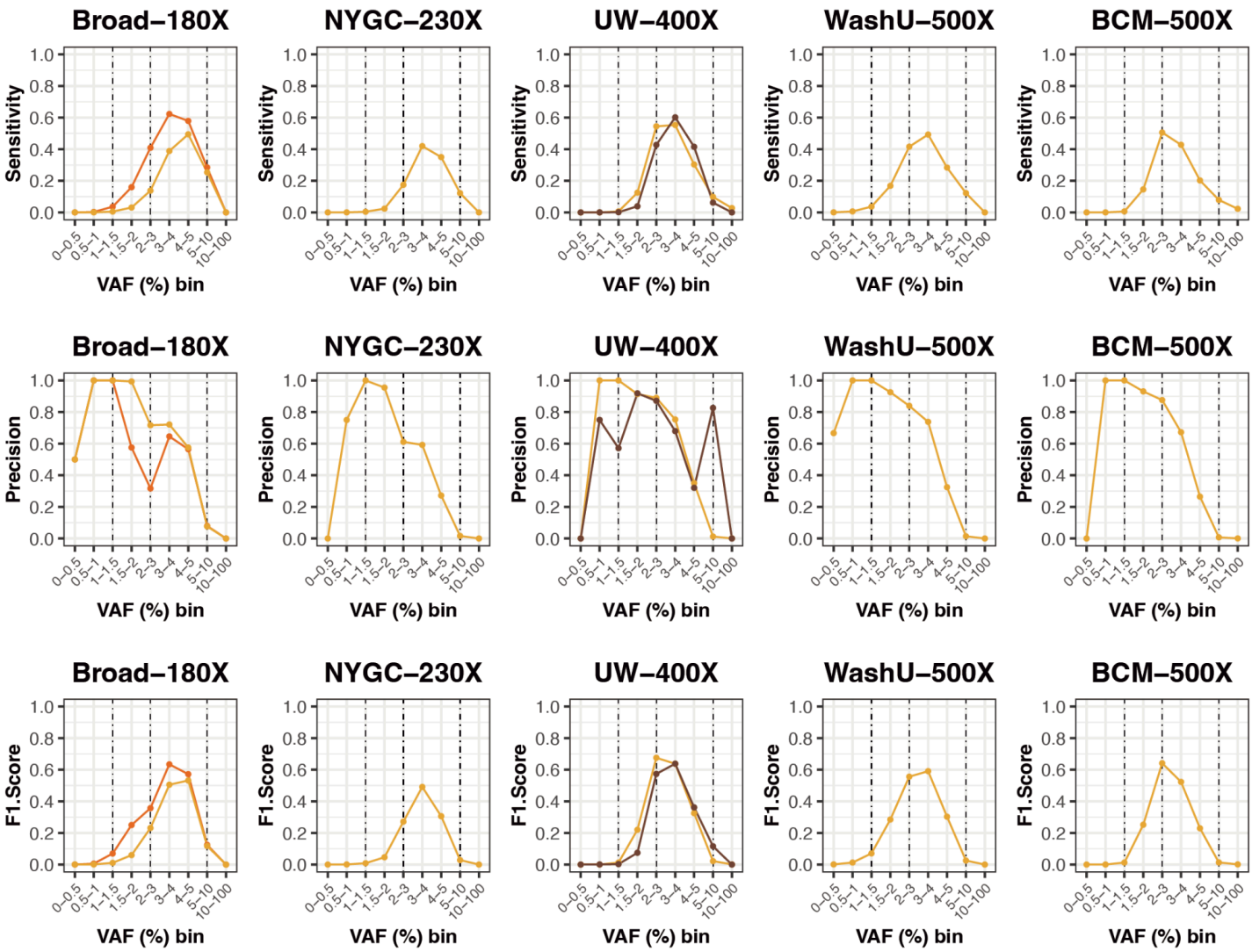
Extreme regions
Genomic regions that are not in the “Easy” and “Difficult” regions are defined as “Extreme” where mosaic variant detection is most challenging due to poor mappability.

## Pipeline evaluation for the SNV/Indel Detection Challenge - Additional Plots

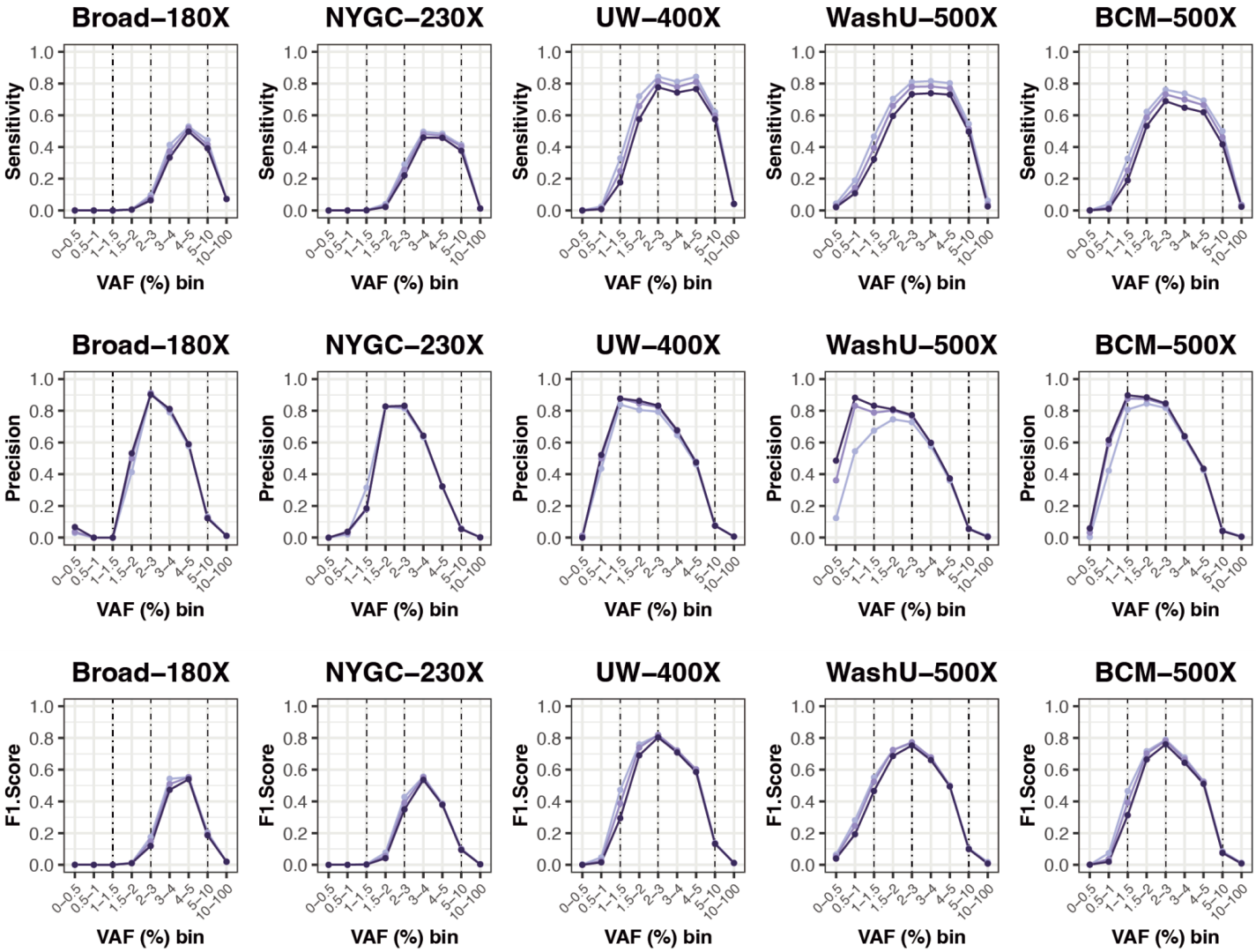
The following plots show the performance of the pipelines grouped by common variant callers used by the centers.



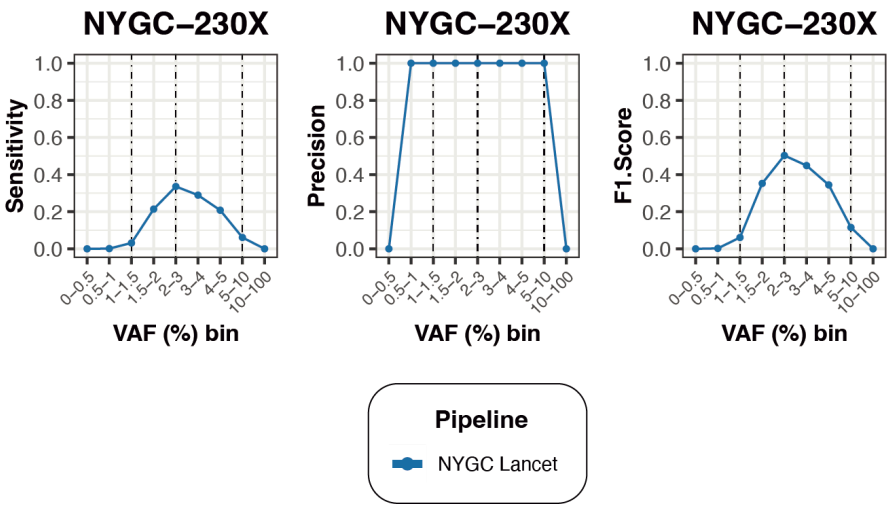
# DRAGEN

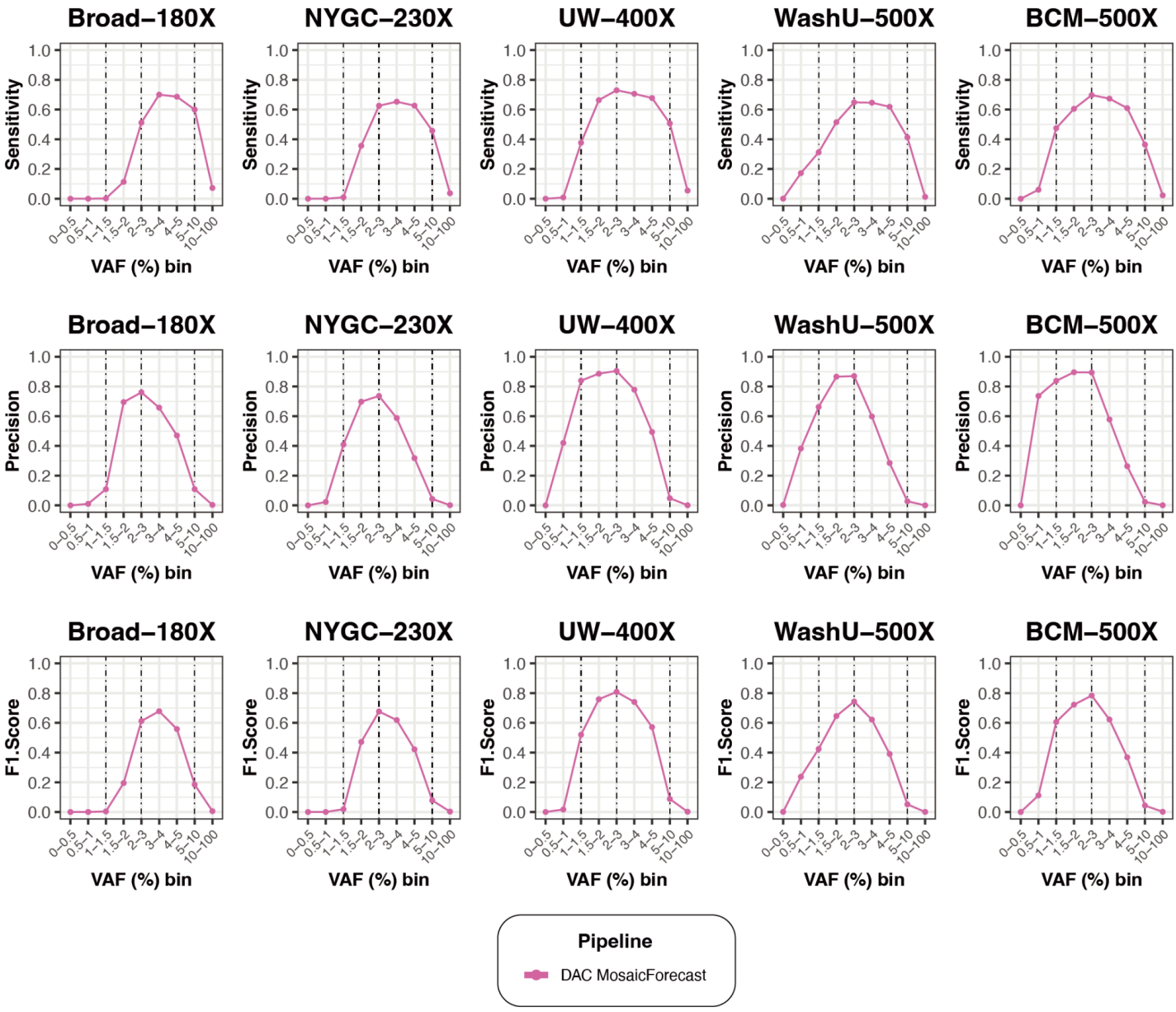

Broad–180X
NYGC–230X
UW–400X
WashU–500X
BCM–500X
Sensitivity
Precision
F1.Score
VAF (%) bin
0–0.5
0.5–1
1–1.5
1.5–2
2–3
3–4
4–5
5–10
10–100
Pipelines
Broad Direct DRAGEN
Broad DRAGEN
UW DRAGEN

# RUFUS


Broad–180X
NYGC–230X
UW–400X
WashU–500X
BCM–500X
Sensitivity
Precision
F1.Score
VAF (%) bin
0–0.5
0.5–1
1–1.5
1.5–2
2–3
3–4
4–5
5–10
10–100
Pipelines
Utah RF-Lenient
Utah RF-Moderate
Utah RF-Strict

# Lancet


NYGC–230X
Sensitivity
Precision
F1.Score
1.0
0.8
0.6
0.4
0.2
0.0
0–0.5
0.5–1
1–1.5
1.5–2
2–3
3–4
4–5
5–10
10–100
VAF (%) bin
Pipeline
NYGC Lancet




**Supplementary Figure 1.** Pipeline performance comparisons across five cell-admixture COLO829-BLT50 samples. The plots are grouped by the pipelines that use the same variant callers by the centers.