Pillar® Biosciences RUO PiVAT® Software Release Notes Version 2023.1.0

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Pillar Biosciences PiVAT RUO, Version 2023.1.0

Introduction

Pillar Variant Analysis Toolkit, or PiVAT, is a software product intended for use as a data processing tool that will accept input from a genetic sequencer.

Scope

The software provides optimized secondary analysis including alignments and variant calling. It is for research use only.

Features

- Detects the target variants in the proposed intended use.
- Reports qualitative results of the target variants for each sample.
- Reports Quality Metrics for each analysis run.
- Assigns uniquely mapped reads to corresponding targeted amplicons. Non-uniquely mapped reads or reads that do not have proper amplicon structure are removed. Based on each amplicon group, variants are determined.

Changes and upgrades

Changes in 2023.1.0

New Features

- cfDNA CNV Caller:
  - New CNV algorithm with an advanced Bayesian non-parametric method for improved sensitivity and specificity in cell-free DNA copy number amplification calling.
    - Enabled on oncoReveal™ Core LBx panel (P-LBX-01).
- Annotation/Filtering:
  - New filtering strategy (custom variant filter queries) for flexible filtering on any PiVAT Customer Results (xlsx) Variant Report columns. Not webapp accessible.
    - Enabled on oncoReveal™ Core LBx (P-LBX-01).
  - New PiVAT denoising algorithm for greatly improved sensitivity and specificity in small variant (SNV/indel) calling.
    - Enabled on oncoReveal™ Core LBx panel (P-LBX-01).
- Fusion:
  - New **InFrame** column in fusion customer report that is True when the fusion frame caller detects an *in-frame* fusion and False if *out of frame*.
    - We consider a fusion out of frame if:
      - Open Reading Frame (ORF) is not divisible by 3 or has an early stop.
      - Coding region start or end are missing.
      - Left and/or right transcript is non-coding, unless one of the transcripts is coding, and the fusion transcript contains the coding transcript's full open reading frame.
- Customer Results Variant Report:
  - New columns:
    - **Transcript**: The VEP annotated transcript name (e.g., NM_001127500.3).
    - **c_dot**: VEP HGVSc annotation with transcript name removed (e.g., `c.3912C>T`).
    - **p_dot**: VEP HGVSp annotation with transcript removed (e.g., `p.Asp1304=`).
    - **Max_Impact**: The maximum VEP **Impact** for a single transcript. For a given transcript, VEP Impact is based on the first VEP Consequence, whereas **Max_Impact** uses the most disruptive change according to VEP criteria

(https://useast.ensembl.org/info/genome/variation/prediction/predicted_data.html).

- Webapp
  - **Tumor Content %** column added to sample **Edit Definition** page. Can be left blank, but if tumor content is known, should be specified for improved performance.
    - This column is used only by the new **cfDNA CNV (CNV2)** algorithm for the oncoReveal™ Core LBx panel.
- Installation
  - SSL/TLS certificate renewal script is provided for semi-automated renewal. The script must be manually configured by the user. Contact Pillar Tech Support if you want to implement this feature.

Improvements

- Significantly improved SNV, Indel, CNV, Fusion variant caller performance in cell-free DNA:
  - These changes impact the oncoReveal™ Core LBx (P-LBX-01) panel and a subset of custom LBx panels.
- MSI:
  - Improved support for matched and unmatched MSI calling.
- Reporting:
  - The SNV/Indel section of PDF reports has been improved, now has the following columns:

Gene	Transcript	Amino Acid Change	Coding	VAF(%)	Exon

- Annotation/Filtering:
  - VEP upgraded to version 106.
- Webapp:
  - Upgraded IGV to improve user experience.
  - Updated upload and download functionality for improved experience and handling of large files.
  - Post Analysis Filtering Filters now always have an SA Status Match True Positive filters added by default. A brief help section has also been added to this section to guide usage.
  - General UI improvements for a better user experience.
  - Added customizable file grouping parameter in the administration section to allow alternative fastq input file naming conventions.

Bugfixes

- Fusion:
  - Corrects logic for handling intronic breakpoints in fusion frame calling. When intronic breakpoints are present, the intronic fragments retained will be considered as part of the open reading frame when they are between the coding sequence start and coding sequence end.
  - Fix NormalizedCount calculation: was previously dividing fusion variant read count by total read count, rather than by total unfiltered read count.
- Customer Report:
  - Fixed COSMIC Linking so links will now be populated using the cosmic IDs.
  - Removed multiple values in the gnome_ad and 1000 Genomes population allele frequencies.

Changes in 2022.1.3

Bugfixes

- Resolve post-filter crashes in CNV runs.
- Corrects logic error in fusion filtering which could potentially cause false positives and false negatives.

Changes in 2022.1.2

Bugfixes

- Resolves crash caused by error in softclip activated by edge-case data when writing bam file.

Changes in 2022.1.1

Bugfixes

- Resolves fatal error caused by fusion breakpoint in intron.

Changes in 2022.1.0

New Features

- PiVAT will only display in results fusions specified in the Product Sheet for that panel
- CNV output now contains all unfiltered cluster-level CNV calls.

Improvements

- Improved accuracy of SA Status bar displayed in PiVAT web application.
- Improved performance for large, multi-sample jobs.
- PiVAT API retuns Task ID upon task instantiation.
- Improved detection of failed jobs.
- Reduced potential for calling false positive fusions based on low quality data by reducing the threshold for removing mispriming reads.
- Implemented performance improvement that reduces time required for alignment step.
- Updated parameter BALANCE_MIN_MATCH threshold to 35 to improve the accuracy of the balance read count.
- boolean parameter "POSITIVE_FUSIONCOUNT" exposed within the panel json. For fusion panels with this parameter set to TRUE, strong evidence fusion reads will be included in the control reads count. Setting to True could allow fusion samples previously failing QC due to low control read counts to be 'rescued' by the addition of strong evidence fusion reads to the control read count.
- Accuracy of fusion calling improved by addition of driver-gene-specific imbalance ratio thresholds.
- Improved Calculation for NormalizedCount. Calculation for NormalizedCount was previously (FusionCount / Total Raw Reads) * 10^5. Calculation for NormalizedCount was updated to (FusionCount / Total Reads After Filtering) * 10^5. This prevents low quality reads (potentially present in Total Raw Reads, but absent from Total Reads After Filtering) from contributing to NormalizedCount, standardizing the NormalizedCount across samples of varying qualities.
- Panel-level filtering on "% Total Unfiltered Reads set lower for cfTNA assay relative to SFv2 because of the expected presence of a large number of aligned DNA reads in total nucleic acid assays relative to a more limited number of expected fusion RNA reads. This is a sample-level QC, if the sample falls below this threshold, nothing is called (the sample is failed. cfTNA: Panel-level filtering on "% Total Unfiltered Reads threshold = 0.1 %. SFv2 Panel-level filtering on "% Total Unfiltered Reads threshold = 3%.
- MAPQ parameter threshold set to 30. If the MAPQ score of the primary alignment of a read output by BWA is < 30, that read will not be used in fusion calling.

Bugfixes

- Resolved bug which had caused long values to be truncated in pdf output.
- Resolved bug that caused crash in bwa in edge cases in which all reads were filtered out.
- Fixed User Guide Link in GUI of local installations.
- Resolved bug which could cause crash during analysis of SMA/Thal panel.
- Resolved bug which caused crash in CNV analysis when control amplicons were designated to be (expression) outliers.

Changes in 2020.2.2

New Features

- Implemented the new Spinal Muscle Atrophy (SMA) caller on the inheritReveal™ Thalassemia plus SMN Panel. The copy number ratios of Exon-07 and Exon-08 in the SMN1 and SMN2 genes are calculated. The SMA caller requires at least 2 normal samples to be defined before running the task.
- Implemented the new SMA caller's output in the form of a report table as well as an interactive plot. The report table contains the copy number ratios of Exon-07 and Exon-08 in the SMN1 and SMN2 genes, as well as those of the control regions. The plot generated shows the copy number ratios of the testing sample overlaid with the normal samples' copy number ratios. The plot contains various interfaces, including zoom in/out, toggle info on mouse-hover, and download.
- Implemented new CNV per gene filters.
    - Default values were set for oncoReveal™ Multi-Cancer with CNV v4 Panel (HS341) to report only copy gains >= 1.4x with at least 5 amplicon count on the gene.
    - Default values were set for oncoReveal™ BRCA1 & BRCA2 plus CNV Panel (BR283) to report copy gain/loss on the BRCA1 and BRCA2 genes only.
    - The default values can be updated in the Post Filtering section of a custom Parameter, and these filters can be toggled on or off in the CNV results page.
- Added a "Download" button for the CNV plots, which enables downloading the CNV plot as a PNG file. Any changes to the plot like updating the upper or lower threshold guidelines are reflected in the downloaded PNG image.
- Implemented new table to report UID performance summary as a table containing minimum, maximum, and mean values in the existing UIDBAM Stats Summary worksheet of the UID customer report excel file.

- Unique software keys are displayed in the footer of any page and the Help page under Versions. Identifying each local installation with a unique key will aid in customer support, particularly for uniquely configured local installations. If a customer needed to reset their software, the unique installation key and the records associated with it would allow a quick and identical PiVAT re-installation.

Improvements

- Updated to Striped Smith-Waterman (SSW) library to reduce runtime of all panels.
- Made improvements in the logic of CNV sample QC by making targeted correlation check on positive samples.
- Implemented a new filter for fusion detection within oncoReveal™ PillarHS TNA Panel (cfTNA). This filter will detect and remove DNA reads that might be mistaken for fusions to reduce the false positive rate in cfTNA fusion detection.
- Added fusion specific run parameters to the parameter sheet of the customer report. The fusion parameters used can now be viewed to verify what settings were used to run a fusion task.
- Resolved an issue with creating excels that have more rows than allowed by excel (1,048,576 rows). PiVAT will now separate extremely large datasets into separate sheets within a single Excel file, allowing large data sets to be downloaded and viewed without error.
- Fixed the logic of determining the start and end positions of a CNV call to ensure the accuracy of location information on CNV calls.
- Improved accuracy of fusion alignments in PBAM, namely to include any final adjustments to overlaps or gaps between the primary and secondary alignments to better reflect the breakpoints.
- Added a filter to the CNV call to remove false positive calls on amplicons without a gene name (e.g., control amplicons on intergenic regions).
- Corrected a typo in fusion control files, from "TranscriptomMapping" to "TranscriptomeMapping".
- Corrected a defect in UID in which unreliable reads were incorporated into variant calling.
- Corrected a defect in fusion analysis that inaccurately inflated the imbalance counts of control samples.
- Corrected Fusion Imbalance ratios.
- Added a revision number to control files, which is visible both in the parameters sheet of customer results and the log files.
- Updated the CFTNA mispriming threshold to avoid falsely flagging valid amplicons.

Bugfixes

- Fixed an edge case in CNV in which a run with a single noisy sample was mistakenly allowed to be used to call CNV if all other samples were flagged as outliers.
- Fixed an edge case in which fastq files were not paired correctly if an empty Fastq file was included.
- Fixed a rare failure in the fusion caller which could occur if no clusters needed realignment.
- Fixed a bug that would cause the software to crash if control amplicons were flagged as outliers.

Limitations

- If CNV per-gene filtering is desired for CNV results from previous PiVAT version, it is necessary to re-run the data for the CNV filtering to take proper effect.
- The "Re-run" button does not support old tasks from previous version.

Changes in 2020.2.1

New Features

- New "idReveal COVID-19 Panel (COVID218)" + pipeline to detect SNPs and InDels within the COVID genome.
- Added "MSI_Start" column to the report of oncoReveal MSI Panel. "MSI_Start" represents the genomic coordinate of the start of the MSI site.
- oncoReveal Multi-Cancer with CNV v4 Panel (HS341) is now applicable to both FFPE and cell line samples.

Improvements

- Reduced memory overhead required to run Fusion.
- Removed the following invalid stats from CNV stats report: "Total_Number_Of_Reads", "Total_Valid_Reads", "On_Target_Ratio" (Real values still available on Overall Stats page)
- Reduced memory overhead when running very large numbers of samples.
- Added PPL gene to Fusion calling.
- Aesthetic improvements to "Task Number Per Panel" display on PiVAT landing page.
- Updated footnote on variant output report to note that Clinical significance is determined using "ClinVar" instead of the less specific "Medical science databases".
- Updated HGVS notation for called fusions to truncate inserted bases for all inserts longer than 10 bases.
- Updates to Panel Settings:

  - oncoReveal BRCA1 & BRCA2 plus CNV Panel– ROIs trimmed to exclude noisy regions, preferred transcripts selected for annotation.
  - oncoReveal Myeloid panel, – ROIs trimmed to exclude noisy regions, custom filters added, quality threshold reduced to 25.
  - oncoReveal cfTNA panel –Removed unnecessary gene transcripts.
  - oncoReveal Multi-Cancer RNA Fusion v2 Panel –Removed unnecessary gene transcripts. Added missing gene transcripts for PPL.
  - oncoReveal MSI Panel – custom filters updated – will now no longer call unnecessary SNVs in MSI regions.
  - oncoReveal Multi-Cancer with CNV v4 Panel – removed cell vs ffpe, no changes except name.
  - oncoReveal Lynch Panel – removed amplicon that was not working from control file.
- Updated wording on "Download All files" button on "Analysis/Analysis Results/Analysis Task/" and "Analysis/Analysis Results/Analysis Task/Download Result Files" pages. Buttons now read "Download All Zip Files" and "Download Result Zip Files" (respectively).
- Attempting to use a file which lacks a file extension as PiVAT input now provides a "File format not supported" error, instead of a 404 error.
- If no zip files are found for the corresponding run, error message now correctly specifies "No Zip Files Found" instead of the previous, inaccurate "No Results Files Found" message.
- Updated vcf output to include "Read Depth".

Bugfixes

- Fixed rare CNV bug in which a negative reference would be included in the test samples.
- Fusion bugfixes:
  - Fixed bugs in the mispriming filter that was leading to some over-aggressive filtering.
  - Fixed bugs in code to generate Fusion PBAM.
- Fixed non-responsive create/rename objects projects.
- Fixed timeout at login error triggered by large number of files stored under user's PiVAT folder.
- Fixed long delay that occurred when creating or deleting files on the Web App via sftp.
- Resolves issue that prevented admin users from using the "Search" function on Audit Logs after a warning had been issued.
- Resolved rare and intermittent defect resulting from incomplete run abort command that caused runs to remain stuck in queue indefinitely.

Changes in 2020.2.0

- Enhancements to the user interface for an improved user experience.
- Potential system halts that occurred when analyzing targets too close to the edges of a chromosome have been corrected.
- Potential for aborted runs to continue in the background has been removed.
- Handling of overlapping regions has been improved.
- Updated VCF output format to improve compatibility with third party applications.
- Improved extraction of equipment information.
- Run reports are now available in PDF format.
- Improved filtering interface for SNP/Indel call to enable immediate updates to filtering.
- Current process display has been improved.
- MSI analysis added for tumor normal panel.
- Improved visualizations and reporting for CNV and Thalasssemia.
- CNV and Thalasssemia results updates:
    - Results now included in the final Report.
    - Caller performance improved.
- Fusion report output enhanced, including Fusion stat reporting.
- Fusion caller performance enhancements.
- The version of VEP used has been upgraded to VEP 100.
- Panel support has been added to include:
    - ID/Reveal COVID-19 Panel.
    - ONCO/Reveal MSI Panel.
    - onco Reveal PillarHS TNA Panel.

Known issues and limitations

Limitations

- Samples cannot be named Log, Common, or Report (upper or lower-case).
- Directories used with PiVAT cannot contain spaces.
- Sample file names cannot contain the underscore character.
- Sorting of input fields in PiVAT Admin pages does not display correctly. Users are advised not to utilize the sort function for input fields within the PiVAT Admin page.

Known potential issues

- In the case of a power failure or other system shutdown, there may be occurrences where a task is shown as running incorrectly.

  - The task incorrectly listed as running will not affect any tasks started after the system restarts. The task incorrectly listed as running will remain in the task list.
  - This is only an issue for local installations of PiVAT.
- It is possible to lock out the default Admin account through multiple unsuccessful login attempts.
  - It is recommended to create an additional account with admin privileges as a backup.

Legal notices

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