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Agilent Technologies

Release Notes

Agilent SureCall 4.1.2

Product Name and Version Number

Agilent SureCall v4.1.2.11

Product Number

G4980AA – SureCall Client 6-month named license supports installation of one client and server (to host the SureCall database) on one machine. For additional client only installations that connect to the same database on the central server, additional copies of this license are needed. There is no limit on the number of free-of-charge licenses provided.

The software comes with 2 installers: (1) SureCall installer (2) GenAligners (contains BWA, BWA-MEM) v4.1.1.

*Note :- The GenAligners installer has been updated for SureCall MAC version 4.1.1. The installer was updated to make it compatible with macOS Mojave 10.14.6 and High Sierra 10.13.6.

Overview

SureCall is a research desktop application combining both novel and widely accepted open-source algorithms for end-to-end NGS data analysis from alignment to categorization and annotation of mutations. SureCall addresses the critical need for an easy-to-use analysis tool that incorporates the most widely accepted open source libraries and algorithms, augments them with tools specific to Agilent assays and deploys them in a convenient and user-friendly manner. SureCall provides five different types of analysis: Single, Pair, Trio, SureSelect All-In-One (AIO), and OneSeq CNV and Mutation analysis. Analysis in SureCall begins with raw reads from Illumina HiSeq/MiSeq sequencing of genomic DNA enriched with HaloPlex or SureSelect target enrichment reagents. After removal of the adapter sequences and lower-quality bases from the end of each of each read, the reads are aligned to the reference genome using BWA-MEM or BWA. Subsequently, the appropriate variant caller is selected to detect variants in a sample. The SNPPET SNP caller is an Agilent algorithm, which is optimal for detecting low-frequency single nucleotide variants with high sensitivity and specificity. SNP filtering, mutation classification and annotations are applied to the

called variant list as part of the analysis workflow. VCF export is also available for any further downstream data interpretation needs.

Single Sample analysis

Run a single sample analysis when you want to find mutations, insertions or deletions (indels), and translocations in individual samples. SNPPET, an in-house algorithm developed specifically for the detection of low allele frequency variants, is used to call mutations. For samples that were target-enriched using Agilent's HaloPlex^{HS} or SureSelect$^{XT\ HS}$, duplicate reads will be flagged and merged, allowing for an even more accurate detection of alleles at low frequencies. Several tools are then used to provide input for the mutation classification. Each mutation is evaluated based on its location, amino acid change, and effect on protein function (SIFT). Further information regarding the mutation is then aggregated from various public sources, including NCBI, COSMIC (Catalog of Somatic Mutations in Cancer), PubMed, and Locus-Specific Databases. In addition, SureCall also supports variant annotation with NCBI ClinVar files with a local database source. After collecting the various inputs for classification, the proprietary mutation classifier evaluates the significance of the mutation following default or customized guidelines. Each mutation is then categorized with the user triaging each mutation and reviewing supporting evidence in the built-in viewer, including raw data and confidence measures, as well as links to external databases such as OMIM, dbVar, dbSNP, etc.

Pair analysis

A pair analysis can have two different applications: 1) to determine copy number changes in a test sample relative to a reference that does not have a copy number change in your region of interest, or 2) to find somatic mutations in a tumor sample by comparing it to a normal sample.

Trio analysis

Select trio analysis to find mutations and indels in a trio of samples, typically mother, father and child. The analysis focuses on de novo mutations, i.e., mutations that are only found in the child and mutations that are homozygous in the child but not in either parent.

SureSelect AIO analysis

Select AIO analysis to identify copy number variants (CNVs), point mutations, indels, and translocations in samples that were target-enriched using one of Agilent's SureSelect AIO panels. You need to provide a reference sample (either matched or unmatched) to which SureCall compares the experimental sample.

OneSeq CNV and Mutation analysis

A OneSeq analysis simultaneously finds CNVs, copy-neutral LOH, point mutations, and indels in a single sample. The OneSeq workflow type is only suitable for samples that were target-enriched using Agilent's OneSeq kits. Copy number changes are detected by comparing an experimental sample to a known reference sample. The in-house developed SNP calling algorithm SNPPET is used to call point mutations and indels. The high-frequency, minor allele SNPs covered by the OneSeq backbone design are used to determine copy-neutral LOH.

New Key Features of SureCall 4.1.2

- **Detect SNVs outside exon track boundaries**
  Custom AIO analysis methods can be configured with an 'Advanced Filter' that enables SureCall to report/detect SNV's outside the exon track boundaries. The user-specified regions are defined in the Advanced Filter parameter of the SNP Filter settings.

System Requirements

SureCall 4.1.2 is only supported on Windows operating systems (64-bit Windows 7 Enterprise, Windows 10 Enterprise and Professional, or Windows Server 2016) and MAC OS X Mojave and OS X High Sierra. Additionally, only the English language versions of these operating systems are supported. If using a non-English version of Windows, switch the language to English before installing SureCall.

See the SureCall Installation Guide (publication G4890-90006) or the SureCall web site (https://www.agilent.com/en/download-software-surecall) for a complete list of minimum and recommended system requirements and installation instructions.

Workflow analysis memory settings: In SureCall, the default memory allocated to workflow analysis is 8 GB. This memory settings may need to be increased for analysis of larger data sets (e.g., Exome, HaloPlex^{HS} and SureSelect$^{XT\ HS}$). If needed, first install additional RAM in your computer. Then, increase the memory allocation in SureCall from the Admin > Memory Management screen of the software.

Installation Instructions

New installation
Refer to instructions in http://www.agilent.com/cs/library/usermanuals/public/G4890-90006.pdf.

Points to note for upgrade from older version of SureCall to SureCall 4.1.2:

- Upgrade to SureCall 4.1.2 is only supported from released versions of SureCall 4.0 or SureCall 4.1.1.
- **For MAC upgrade is only supported from version 3.5 and 4.1.1.9.*
- SureCall versions prior to 4.0 (i.e., 2.1, 2.0, 3.0) cannot be directly upgraded to 4.1.2. You must first upgrade from version 1.0/1.1/2.0/3.0 to version 3.5 using the SureCall 3.5 installer. Then, you can upgrade to either version 4.0 or 4.1.1, followed by upgrading to 4.1.2 with the SureCall 4.1.2 installer.
- During upgrade to SureCall 4.1.2, the earlier version of the PostgreSQL server is upgraded, but there is not a new server installation. Hence, after upgrade, server and client installation

folders are saved to different locations on disk. If you need to uninstall SureCall, you must uninstall the client and server separately.

Upgrade instructions from earlier (v4.0 or later) SureCall version

1. Double-click the Agilent SureCall 4.1.2.x.exe file to start the installation wizard. You will be prompted that a version of SureCall client already exists on the local machine.
2. Click OK to proceed with uninstalling the existing Agilent SureCall client. The Uninstaller of the existing SureCall installation is launched.
3. Click Next to proceed.
4. Select "Uninstall specific features", and click Next.
5. In the top panel, check the Client checkbox, and click Uninstall. ***Note***: Do not remove server as doing so will remove all previously analyzed samples from the database.
6. After client uninstallation of earlier version is complete, you are automatically returned to the v4.1.2 installation wizard.
7. Select "Both Client and Server" option and click Next. The installer notifies you that SureCall server already exists and will be upgraded to latest version.
8. Click OK and proceed with the installation.
9. The installer installs SureCall 4.1.2 client application and upgrades existing SureCall server to 4.1.2.
10. ***Optional:*** Install GenAligners v3.0/v4.1(for MAC) if you plan to use unaligned FASTQ files in your analysis workflows.

Upgrade instructions from SureCall v4.1.1.x version via cloud

1. Open SureCall 4.1.1 and ensure you have an internet connection. You will see a notification that version 4.1.2 is now available.
2. Click the notification and follow the prompts to upgrade to 4.1.2.

Issues Fixed in SureCall 4.1.2

1. The deduplication algorithm encounters memory limitations during analysis of large data sets. (TT#282627, TT#284436)
2. The translocation detection algorithm encounters memory limitations during analysis of some FFPE sample data. (TT#288170)
3. VCF does not properly report translocation calls with multiple mates. (TT#286877)
4. In PDF Variant Report the variant position is off by 1 bp. (TT#287800)
5. File names assigned to the output BAM files are sometimes too long to allow for proper import/export with 3rd party software tools. (TT#273039)
6. Triage View for Pair-CNV analyses should display the counts for losses and gains at the bottom of the screen. (TT#284198)
7. In the Job Summary report for SureSelect AIO analyses, include the user-defined settings from the Describe Samples step of the analysis workflow setup. (TT#284456)

8. Users are not notified of error when they import a SureSelect AIO reference sample with special characters in the file name. (TT#284485)
9. The Add Call feature available in Triage View is problematic for Trio and Pair analyses and should be disabled. (TT#286155)
10. In Pair and Trio analyses in which the Add Call feature was used to add a call (in versions of SureCall prior to 4.1.2) the exported VCF does not always include the added calls. (TT#286156)
11. In the Mutations table in Triage View, the 'Number of variant alleles' is not consistently populated and should be disabled. (TT#286250)
12. In QC report for OneSeq analyses, null values are incorrectly displayed for some of the reference sample metrics. (TT#286256)
13. In analyses that include translocation calling, if no translocations are detected in the sample, then the VCF file is not valid and cannot be exported. (TT#286266)
14. After upgrading SureCall, previously analyzed samples that included translocation calling display a 'VCF does not exist' error message. (TT#287110, TT#287477)
15. When configuring analysis methods, the SNP Filter option needs to be mandatory in order to avoid issues in which removal of the filter causes fewer variants to be called. (TT#259762)
16. In Pair-CNV analyses in which regions of chrM are covered in the design the analysis job fails or stalls. (TT#287847)
17. In some cases, duplicate analysis job with the same sample can produce different translocation results. (TT#289511)
18. For some sample BAM files, some translocations are incorrectly assigned a mapping quality of zero during the analysis job. (TT# 289924)

Known/Open issues

1. The GT, GQ and PL values reported in the VCF file may not be reliable. Alissa Interpret users are advised not to set up variant filter based on the GT, GQ and PL values until the issues are fixed in SureCall (TT#258991, TT#247517)
2. When upgrading from 4.1.1 to 4.1.2, the hg38 and hg19 annotations are properly downloaded but the installer screen freezes until the download process is complete. (TT#290148)
3. For analyses that include multiple tabs in Triage View (i.e. multiple variant types are called), signing off on the sample without first checking it in may cause the application to discard any changes made to the results while the sample was last checked out. (TT #290235)
4. For analyses that include a known variants file, the contents of the Comments column of that file are inadvertently deleted for the insertion and deletion type variants. (TT#289131)
5. Following a SureCall upgrade, previously analyzed samples that included a known variants file now show duplicate entries in the Known Variants tab of the Triage View. In some cases, the VCF file for the known variants may be inadvertently removed from the output folder, causing an error when a user attempts to launch the Triage View for that sample. (TT#287785, TT#290152)

6. For AIO, Pair, and Trio analyses, the values in the "Filtered read depth (per sample)" column in Triage View are higher than those found in the BAM file in the Genome Viewer. (TT#289988)

PR7000-2059