**Agilent**

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Release Notes

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# Agilent SureCall 4.2.2

## Product Name and Version Number

Agilent SureCall v4.2.2.3

## 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).

## 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.

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## What's new in SureCall 4.2.2

The SureCall application was updated to the latest secure version of  Apache's Log4j software library to address log4j2 vulnerability risk.  Log4Shell is a critical vulnerability affecting many versions of the Apache Log4j application.  The vulnerability allows unauthenticated remote code execution.

## Special analysis method setting needed for certain SureSelect DNA designs

If any of your SureSelect DNA designs are an Agilent catalog panel, or were created in SureDesign using the "Select Optimized Probes" method, then when running a SureCall analysis with that design, make sure to use a custom analysis method in which the Region Padding parameter is enabled and set to at least 50 bp. This padding ensures that all variants that fall in an intended target region are reported by SureCall. See the SureCall help system for instructions on creating and customizing analysis methods.

## System Requirements

SureCall 4.2.2 is only supported on Windows operating systems (64-bit  Windows 10 Enterprise and Professional, or Windows Server 2019) and macOS Catalina . The regional setting for the operating system must be set to English. In Windows 10, you can find the regional format setting in **Windows Settings > Time & Language > Region**.

See the SureCall 4.2 Installation Guide (publication G4890-90007) 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.

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# Installation Instructions

**New installation**
For new installations, the process for installing SureCall 4.2.2 is the same as the process for installing SureCall 4.2.1. Refer to instructions in:
https://www.agilent.com/cs/library/usermanuals/public/GSD-MAN-G4890-90007.pdf.

**Points to note for upgrade from older version of SureCall to SureCall 4.2.2:**

- Upgrade to SureCall 4.2.2 is only supported from released versions of SureCall 4.2.0/4.2.1 or SureCall 4.1.2.11.
- SureCall versions prior to 4.1.2.11 (i.e., 2.1, 2.0, 3.0, 4.0) cannot be directly upgraded to 4.2.2. If you have an older version of SureCall and would like to upgrade to this new version, please email informatics_support@agilent.com for advice.
- Versions of SureCall prior to version 4.2.1 are not compatible with the macOS Catalina operating system. Please email informatics_support@agilent.com for advice on upgrading from a previous version of SureCall to SureCall 4.2.2 on a Macintosh computer.

**Upgrade instructions from earlier (v4.1.2 or later) SureCall version using the new installer**

1. Double-click the Agilent SureCall 4.2.2.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 for the existing version of SureCall is launched.
3. Click Next to proceed with the uninstallation.
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 v4.2.2 installation wizard.
7. Select the "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 to proceed with the installation.
9. The installer installs SureCall 4.2.2 client application and upgrades existing SureCall server to v4.2.2.
10. **Optional:**  Install GenAligners application of Windows/Mac available on Agilent Genomics download page, in order to perform analysis for unaligned FASTQ files input (if GenAligners already not installed on machine).

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## Issues Fixed in SureCall 4.2.1

1. Indel variants are not called in Tumor/Normal Pair analysis workflow. (TT#291011)
2. Tumor/Normal Pair analysis workflow, some variants in the tumor sample are mistakenly called as somatic when that same variant in the normal sample fails the read depth threshold filter. (TT#291012)
3. For Pair analysis, the analysis method parameter "somatic variant genotype quality filter" is applied to both somatic and non-somatic variants. (TT#258820)
4. In some Pair analyses, the somatic likelihood calculation was erroneously high. (TT#258883)
5. OneSeq analyses miss homozygous deletions when no reads from the deleted region are present. (TT#291610)
6. The "minimum read depth" SNPPET parameter is not being applied to the indel caller. (TT#292382)
7. Errors when setting up distributed common storage on external drive. (TT#293374)
8. For TL variants, exported VCF file shows GENE name in one mate's INFO field while not showing GENE names in other mate INFO field. (TT#293663)
9. In VCF file, zygosity value in INFO field changes after reannotation for some variants. (TT#293776)
10. In some cases, importing a design can fail if the design name includes certain words. (TT#293816)
11. In certain cases, during setup of a SureSelect AIO analysis, the interface may display the wrong drop-down selections if samples are added and then removed. (TT#293866)
12. Sometimes submitted analysis jobs are not shown in the application even though the samples are properly processed. (TT#293987)
13. In the Triage View Mutation table, when the Allele Frequency column is reordered, the format used to display the frequecny values changes. (TT#294322)
14. 1000 genome Allele Frequency annotations are not working for hg38 analysis. (TT#295915)
15. MBC deduplication is not performed properly for HaloPlex HS workflows. (TT#295329)
16. During analysis set, toggling the Library Prep selection multiple times can interfere with default selections for use of the molecular barcode file. (TT#295316)
17. For Pair analyses, known somatic calls are not detected in some cases. (TT#267872)
18. For indels variants in Pair analyses, Reverse REF/ALT values in DP4 set incorrectly as 0. (TT#295290)

## Issues Fixed in SureCall 4.2.2

1. The SureCall application was updated to use the latest version of Apache's Log4j2 software library.

## Known/Open issues

1. CoveragInfo stat is not calculated when intervals in design starts 0. (TT#294677)
2. For some variant calls, the "MAF dbSNP" annotation is not available when rsID is present. (TT#295186)
3. Mismatch in annotations for some variants are observed. In some cases, the Reference SNP cluster ID (rsID) of a variant might be shown incorrectly in the software when there are multiple dbSNP entries at the same position. (TT#295191)
4. Variants in ChrM are not annotated. (TT#295272)

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