Agilent
Trusted Answers

Alissa Case Study Compendium–2nd Edition

Alissa Clinical Informatics Platform

Alissa Align & Call
Alissa Interpret

These case studies and application notes show how our customers, with the help of our team of field application scientists (FAS), have established their laboratories' routine testing on the Alissa Clinical Informatics Platform.

You'll learn how labs use Alissa to:

– Improve and align with their standard operating procedures (SOP) for greater efficiency.

– Improve their turnaround times from pipeline analysis to variant assessment.

– Seamlessly implement their NGS and CGH workflows, panels and exomes.

– Address molecular diagnostic challenges: from clinical genetics to molecular pathology.

Alissa Align & Call

Alissa Align & Call ushers in the next generation of genomic data analysis to unlock NGS complex data and accelerate time-to-results. Optimized for Agilent SureSelect, HaloPlex and OneSeq libraries and reagents for an integrated NGS workflow of detection, annotation, and visualization of called and aligned variants.

Alissa Interpret

Using any VCF file, Alissa Interpret automates variant assessment for NGS and CGH workflows, performing triage and curation for routine diagnostic testing. With ease, Alissa Interpret provides access to a wealth of annotation sources and peer reviewed databases, builds the lab's internal knowledge base, and drafts clinical-grade lab reports with ease. On the leading edge, Alissa Interpret supports end-to-end workflows for genomic testing across multiple independent testing laboratories and hospitals.

Contents

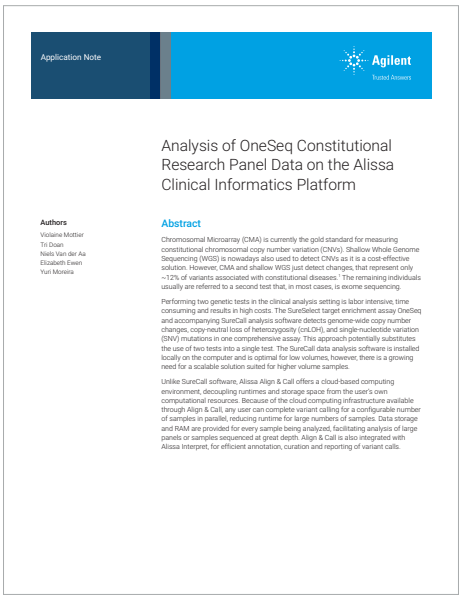
To download visit **www.agilent.com** and enter document number in search window

Alissa Clinical Informatics Platform

Analysis of OneSeq Constitutional Research Panel Data on the Alissa Clinical Informatics Platform

Chromosomal Microarray (CMA) is currently the gold standard for measuring constitutional chromosomal copy number variations (CNVs). Shallow Whole Genome Sequencing (WGS) is nowadays also used to detect CNVs as it is a cost-effective solution. However, CMA and shallow WGS just detect changes, that represent only ~12% of variants associated with constitutional diseases.[1] The remaining individuals usually are referred to a second test that, in most cases, is exome sequencing.

1. Villela, D.; Costa, S.S.; Vianna-Morgante, A.M.; Krepischi, A.C.V.; Rosenberg, C., *et al.* Efficient detection of chromosome imbalances and single nucleotide variants using targeted sequencing in the clinical setting. European Journal of Medical Genetics **2017,** 60(2017) 667-674 doi:10.1016/j.ejmg.2017.08.020.


Application Note

Agilent
Trusted Answers

Analysis of OneSeq Constitutional Research Panel Data on the Alissa Clinical Informatics Platform

Authors
Violaine Mottier
Tri Doan
Niels Van der Aa
Elizabeth Ewen
Yuri Moreira

Abstract

Chromosomal Microarray (CMA) is currently the gold standard for measuring constitutional chromosomal copy number variation (CNVs). Shallow Whole Genome Sequencing (WGS) is nowadays also used to detect CNVs as it is a cost-effective solution. However, CMA and shallow WGS just detect changes, that represent only ~12% of variants associated with constitutional diseases.[1] The remaining individuals usually are referred to a second test that, in most cases, is exome sequencing.

Performing two genetic tests in the clinical analysis setting is labor intensive, time consuming and results in high costs. The SureSelect target enrichment assay OneSeq and accompanying SureCall analysis software detects genome-wide copy number changes, copy-neutral loss of heterozygosity (cnLOH), and single-nucleotide variation (SNV) mutations in one comprehensive assay. This approach potentially substitutes the use of two tests into a single test. The SureCall data analysis software is installed locally on the computer and is optimal for low volumes, however, there is a growing need for a scalable solution suited for higher volume samples.

Unlike SureCall software, Alissa Align & Call offers a cloud-based computing environment, decoupling runtimes and storage space from the user's own computational resources. Because of the cloud computing infrastructure available through Align & Call, any user can complete variant calling for a configurable number of samples in parallel, reducing runtime for large numbers of samples. Data storage and RAM are provided for every sample being analyzed, facilitating analysis of large panels or samples sequenced at great depth. Align & Call is also integrated with Alissa Interpret, for efficient annotation, curation and reporting of variant calls.

This application note illustrates how:

– The SureSelect target enrichment assay OneSeq and accompanying Align & Call analysis module on the Alissa Clinical Informatics Platform detect genome-wide copy number variations (CNVs), copy-neutral loss of heterozygosity (cnLOH), and single-nucleotide variations (SNVs) in one comprehensive assay.

– This approach potentially substitutes the use of two tests into a single test.

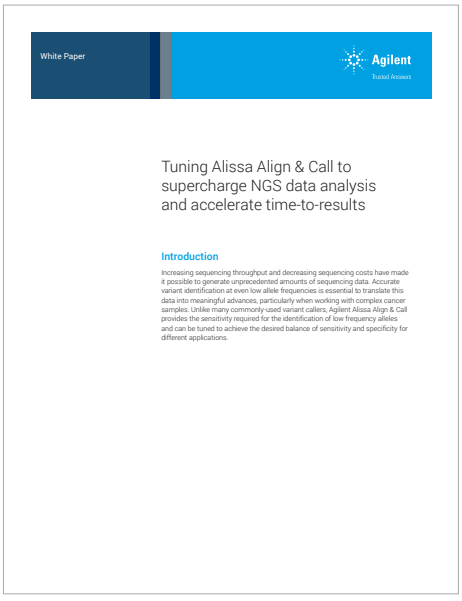
5994-1090EN

Alissa Align & Call

Tuning Alissa Align & Call to Supercharge NGS Data Analysis and Accelerate Time-to-Results

Increasing sequencing throughput and decreasing sequencing costs have made it possible to generate unprecedented amounts of sequencing data. Accurate variant identification at even low allele frequencies is essential to translate this data into meaningful advances, particularly when working with complex cancer samples. Unlike many commonly-used variant callers, Alissa Align & Call provides the sensitivity required for the identification of low frequency alleles and can be tuned to achieve the desired balance of sensitivity and specificity for different applications.

To assess the performance of Alissa Align & Call and four other common variant callers (GATK4 Haplotype Caller, SAMtools, VarDict, and platypus) on SNPs in a normal diploid sample, we used the Genome in a Bottle (GIAB) sample NA12878 (HG001).


White Paper

Agilent
Trusted Answers

Tuning Alissa Align & Call to supercharge NGS data analysis and accelerate time-to-results

Introduction

Increasing sequencing throughput and decreasing sequencing costs have made it possible to generate unprecedented amounts of sequencing data. Accurate variant identification at even low allele frequencies is essential to translate this data into meaningful advances, particularly when working with complex cancer samples. Unlike many commonly-used variant callers, Agilent Alissa Align & Call provides the sensitivity required for the identification of low frequency alleles and can be tuned to achieve the desired balance of sensitivity and specificity for different applications.

This white paper finds that:

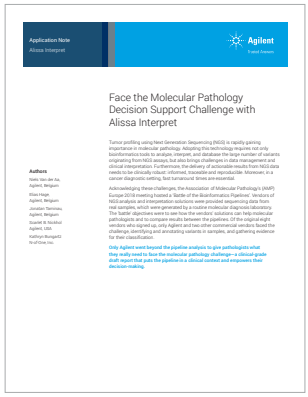
- Align & Call produces high sensitivity and specificity variant calls even for variants at low allele frequencies.
- Align & Call provides the traceability of Agilent SureCall, combined with improved ease-of-use and increased analysis speed of many samples via parallelization.
- Integration with Alissa Interpret enables downstream interpretation and annotation of variant calls.
- The other variant callers tested do not offer this combination of performance, accessibility, and integration with downstream interpretation.

5994-0169EN

Alissa Interpret: Molecular Pathology

Face the Molecular Pathology Decision Support Challenge with Alissa Interpret

This application note shows you how to:

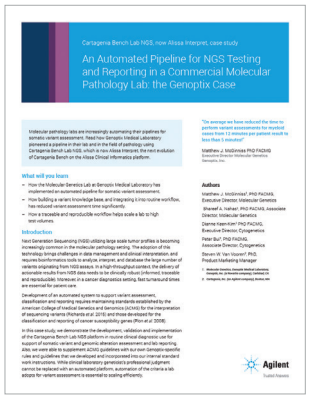


- Implement an automated pipeline for somatic variant assessment.
- Support efficient triage and confident variant classification in context of the tumor type and by public and premium third-party knowledge databases.
- Build a curated variant knowledgebase tailored to collect diagnostic evidence for somatic variants.

5994-0349EN

An Automated Pipeline for NGS Testing and Reporting in a Commercial Molecular Pathology Lab

This case study will show you how:



- The Molecular Genetics Lab at Genoptix Medical Laboratory has implemented an automated pipeline for somatic variant assessment.
- Building a variant knowledge base, and integrating it into routine workflow, has reduced variant assessment time significantly.
- A traceable and reproducible workflow helps scale a lab to higher test volumes.

5991-9205EN

Challenges in Molecular Pathology: NGS Variant Assessment and Reporting on Actionable Findings

This white paper illustrates how:

- Pathology labs adopting NGS technology can significantly improve turnaround times through automation of their interpretation and reporting protocols.
- Alissa Interpret allows labs to automate variant assessment SOPs and draft reports on actionable findings.

5991-8534EN

Identifying Somatic Tumor-Only Variants with Intelligent Variant Filtration Strategies in Alissa Interpret

This case study will show how University Health Network has:

- Developed a custom variant filtration strategy in Alissa Interpret to improve the identification of somatic tumor-specific variants in tumor-only testing, removing the need to sequence a reference sample.
- Built an internal knowledgebase in Alissa Interpret.

5991-8531EN

Alissa Interpret: Clinical Genetics

University Medical Center Utrecht Genetics–One Plus One Equals Three: Using Arrays + NGS

In many genomic diagnostics laboratories both array-testing and Whole Exome Sequencing (WES) are currently offered in case of prenatal multiple congenital anomalies and/or intellectual disability (MCA/ID). Since 2005, the University Medical Center (UMC) Utrecht Genetics lab has offered comparative genomic hybridization (CGH) and single-nucleotide polymorphism (SNP) array-based testing, detecting copy-number variations (CNVs) and regions of homozygosity (ROH). Annually, approximately 1,500 arrays on prenatal MCA/ID patients are performed. WES, detecting single-nucleotide variants (SNVs), small deletions and duplications and indels, was introduced at the UMC Utrecht diagnostic department in 2014.

The University Medical Center Utrecht Genetics is currently transitioning towards Whole Genome Sequencing (WGS), which allows for a combined CNV/SNV analysis on the same data. While this is in the research phase, performing both WES and array-based testing continues to be needed for combined CNV/SNV analysis in case of MCA/ID. Depending on the severity of the phenotype and/or the urgency, both tests are initiated either simultaneously or subsequently.


Alissa Interpret case study: UMC Utrecht Genetics

One plus one equals three

Combined analysis of CNVs and SNVs in genomic diagnostics

What will you learn

This case study shows how the University Medical Center (UMC) Utrecht Genetics Department built a foundation to efficiently transition to whole genome sequencing with combined CNV and SNV analysis on the same data set and is:

- evolving to a single software platform for its assessment of genetic abnormalities detected by two tests — one array test and another for whole exome sequencing – previously analyzed using Cartagenia Bench Lab CNV and Cartagenia Bench Lab NGS, respectively.
- deploying Alissa Interpret, the next evolution of Cartagenia Bench, for analysis of CNVs and SNVs in a single workflow and achieved better detection of recessive disorders, and quicker response time to urgencies and severe phenotypes.

Introduction

In many genomic diagnostics laboratories both array-testing and whole exome sequencing (WES) are currently offered in case of (prenatal) congenital anomalies and or intellectual disability (MCA/ID). Since 2005, the University Medical Center (UMC) Utrecht Genetics lab has offered comprehensive genomics hybridization (CGH) and single-nucleotide polymorphism (SNP) array-testing, detecting copy-number variation (CNV) and regions of homozygosity (ROH). Annually, approximately 1,500 arrays on (prenatal) MCA/ID patients are performed. Whole Exome Sequencing, detecting single-nucleotide variants (SNVs), small deletions and duplications and indels, was introduced at the UMC Utrecht diagnostic department in 2014.

Array-CGH/SNP-array tests detect pathogenic CNV's in on average 10-15% of MCA/ID cases and the diagnostic yield of WES analysis is estimated to be 30-40%. Hence, a combined analysis of CNVs and SNVs is an effective approach to increase the diagnostic yield of genomic testing for diagnostic applications in MCA/ID and beyond. This is corroborated by several cases in literature in which a combination of a CNV and a SNV gave rise to detection of a recessive disorder.

Dr. Ellen van Binsbergen

Clinical Genetics Laboratory Specialist, University Medical Center (UMC) Utrecht Genetics, The Netherlands

Agilent

Trusted Answers

This case study will show you how UMC is:

- Evolving to a single software platform for its assessment of genetic abnormalities detected by two tests — one array test and another for Whole Exome Sequencing – previously analyzed using Cartagenia Bench Lab CNV and Cartagenia Bench Lab NGS, respectively.
- Deploying Alissa Interpret, the next evolution of Cartagenia Bench, for analysis of CNVs and SNVs in a single workflow.
- Achieving better detection of recessive disorders, and quicker response time to urgencies and severe phenotypes.

5991-9111EN

Comprehensive Genomic Analysis of Complementary Microarray and Next Generation Sequencing Data for Clinical Diagnostics

This case study shows you how:

- Greenwood Genetic Center brings together NGS Microarray results in routine genetic testing for improved diagnostic yield.
- Alissa Interpret allows for a seamless integration of copy number and molecular variants.
- Greenwood Genetic Center used this feature to address a diagnostic case of Brittle Cornea.

5991-8528EN

An Efficient Clinical Pipeline for Microcephaly, RASopathy and Leukodystrophy Gene Panels Using Alissa Interpret's Flexible Classification Functionality: The Hôpital Robert-Debré Experience

This case study shows you how:

- The molecular geneticists at the Robert-Debré hospital save time by using Alissa Interpret to set up a variant classification strategy that helps diagnose patients suffering from rare developmental pathologies.
- Alissa Interpret can be used in a flexible way, through the extensive labeling and variant review functionality.

5991-8530EN

Implementing ACMG Guidelines on Sequence Variant Interpretation: Software-Assisted Variant Curation and Filtering

This case study shows you how:

- Uppsala University Hospital's Molecular Genetics lab has implemented the ACMG guidelines and standards on sequence variant interpretation.
- Alissa Interpret facilitates automation of variant classification pipelines.
- The ACMG classification functionality enables evaluation of all ACMG criteria on a single variant level providing a complete evidence overview.

5991-8532EN

Whole Exome Sequencing Diagnostics for Patients with Intellectual Disability at University Medical Center Utrecht: A Tiered and Automated Approach Using Alissa Interpret

This case study illustrates how:

- The UMC Utrecht has implemented an automated and tiered approach for WES diagnostics using Alissa Interpret.
- UMC Utrecht's tiered analysis workflow has been used for a specific clinical case.
- The tiered approach maximizes clinical utility and time efficiency, while minimizing uncertain and unsolicited findings.

5991-8533EN

www.agilent.com/lifesciences/alissa

OneSeq and Alissa Align & Call are for Research use Only. Not for use in diagnostic procedures.

Alissa Interpret is a USA Class I Exempt Medical Device, Europe CE IVD, Canada and Australia Class I IVD Device.

This information is subject to change without notice.

PR7000-2047

Revised July 30, 2019
Printed in the USA
5991-8535EN

Agilent
Trusted Answers