Alissa Interpret | The next evolution of Cartagenia Bench

White Paper:
Challenges in Molecular Pathology: NGS variant assessment and reporting on actionable findings

At a Glance
In this case study, you will learn:

- How pathology labs adopting NGS technology can significantly improve turnaround times through automation of their interpretation and reporting protocols.

- How Alissa Interpret allows labs to automate variant assessment SOPs and draft reports on actionable findings.

Introduction

Clinical genetics and molecular pathology labs alike are facing new challenges with the advent of Next Generation Sequencing (NGS). This brief white paper illustrates how the user-friendly features of the Agilent Alissa Interpret, the variant assessment module on the Agilent Alissa Clinical Informatics platform, can efficiently address the needs in sample analysis automation and lab reporting. As an illustration of these automation features, we illustrate these features in the context of molecular pathology.

Case

As an example, we take the outcome of a gene panel capture kit, representing about 50 well known and characterized cancer genes. After wet-lab processing, and raw data analysis to call SNP and InDel variants from the resulting sequencing output, the resulting identified variants (available as a VCF file) are uploaded to the Alissa Interpret, either as a single sample, or as a batch of tests. The resulting variants are available as Lab Results to the patient record at hand, as shown in **Figure 1**.

Figure 1. Vcf files with SNPs and indels are linked to the oncology patient sample record. A total of 169 variants are available for analysis.

Conclusion Summary

Alissa Interpret allows very significant speedup as well as workflow standardization in the context of clinical oncology.

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The Analysis function will now allow to automatically execute a Standard Operating Procedure on the identified variants. The analysis and interpretation steps are represented as a decision tree, that was previously constructed and validated on a number of cancer samples with known results. Variants flow through the decision tree and are classified according to the protocol.

Labs typically wish to perform filter steps as shown in **Figure 2**:

- Presence in public databases that represent frequent variants. This particular protocol will label variants as "benign" as soon as 2 out of 3 databases (Exome Variant Server, 1000 Genomes and dbSNP) have this variant with a minor allele frequency over 1%;
- A transcription effect other than "synonymous." Only variants with a likely effect on protein are considered;
- A check against a number of custom databases. In this case, the following databases were configured:
    - The COSMIC cancer variants database,
    - A selection of variants this lab has curated from online resources such as PubMed,
    - Known and curated TP53 and BRCA1 variants from Locus Specific Databases as chosen and validated by the lab,
    - A selection of ClinVar variants relevant to oncology,
    - A lab-specific database of genetic markers with known drugs.

This classification tree represents only an illustration — many more filters, databases, algorithms and strategies are available out of the box on the Alissa clinical informatics platform. Examples include tumor-healthy sample comparison, inheritance analysis for germline markers, various prediction algorithms, frequency statistics calculation and use for large sets of samples, and much more. Moreover, the platform can be customized and tailored towards the needs of the lab.
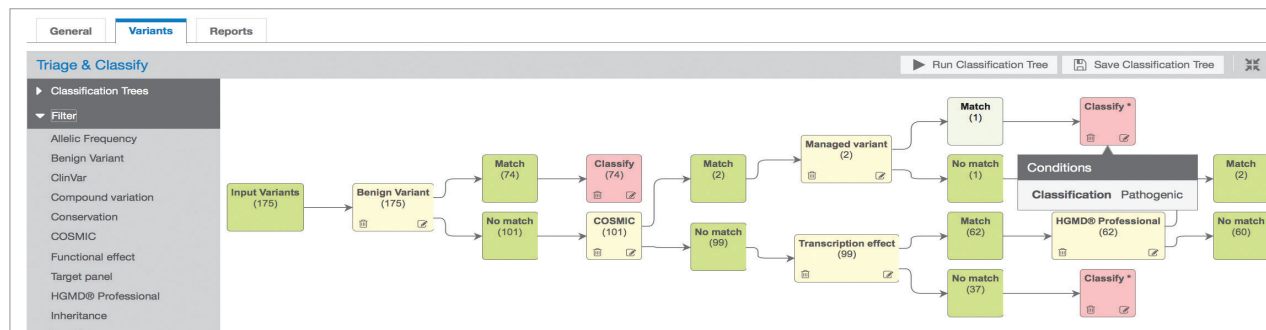

Figure 2. A basic filtration strategy represented as a validated and stored 'decision tree'. Variants percolate through the tree and receive a classification. They can then be reviewed, and included in a report

Immediately, the protocol identified one pathogenic variant that is flagged for review and reporting. **Figure 3** shows the short list — in this case, a well known variant with known drug the lab has annotated in its database.

Summary

Effect	Benign	Likely benign	VOUS	Likely pathogenic	Pathogenic	Not Classified	Total
frameshift	0	0	0	0	0	41	41
nonsynonymous	31	0	0	1	1	19	52
stopgain	0	0	0	0	0	2	2
synonymous	31	18	0	0	0	0	49
Not specified	12	19	0	0	0	0	31
Total	74	37	0	1	1	62	175

Variant List: 175 variants (total) ▶ 1 variant (1 classification tree bin)   Export   Summary   Filter

Gene	Position	Ref	Patient	Read Depth	Type	Transcript	cDNA	Location	Exon	Effect	Protein	Classification	Confirmed Classification	References	Biom	BRCA	Canc	Clin	Cosm	Other	Similarities	Info	Report	Actions
BRAF	7:140,453,136	A	A T	105	snp	NM_004333.4	c.1799T>A	exonic	15	nonsynonymous	p.Val600Glu	Pathogenic	Pathogenic											

Figure 3. The resulting variants – in this case, one single variant is identified and flagged for reporting. Many additional tools are available: (a) check wether this was seen before in the lab, (b) links out to many web resources, (c) all variant information at a glance, (d) c. and p. notations, (e) bar graphs showing occurrences on other samples on the database, as well as on curated databases called "managed variant lists".

As a final step in the work-flow, the Alissa Interpret allows creation of lab reports through a convenient templating system. Default templates are available, but typically, templates are tailored towards lab reporting needs and layout requirements.

The power of this templating system lies in its automation capabilities:

- Automatically insert 'blurbs' of text based on the content of the analysis. For example, when this particular BRAF p.Val600Glu variant is present, the report template can be configured to automatically insert information on the BRAF gene, on this particular variant, on drugs known for this variant, or on clinical trials linked to an internal database.
- Automatically generate paragraphs with findings.
- Automatically generate overview tables of variants deemed relevant.
- Automatically include protocols, disclaimers, follow-up suggestions.
- Automatically include information on database versions, protocol versions, ...
  to provide full traceability in a clinical context.

An automatically drafted report customized to the needs of a laboratory, ready for review, processing, download or export as PDF or Word file, is shown in **Figure 4** on the last page of this case study.

Alissa Interpret NGS - Conclusion

Alissa Interpret allows very significant speedup as well as workflow standardization in the context of clinical oncology – helping pathology and genetics labs, but also providing informative and customized reports to oncologists.

If you want to learn more, ask for a demonstration of the system via www.agilent.com/lifesciences/alissa.

MyHospital
Department for Clinical Genetics

Patient

Name: **P_HP01**

Gender: Male

Creation date: 16.09.2014

Analysis

Reference number: A_HP01

Domain: Oncology

Target panel: 50 gene target panel used

Date : 16.09.2014

Clinical details

HP:0012056 Cutaneous melanoma - The presence of a `melanoma` (MPATH:359) of the `skin` (FMA:7163).

Reported variants

Gene	Transcript	Zyg	cNomen	protein	Read	Label
BRAF	NM_004333.4	0/1	c.1799T>A	p.Val600Glu	105.0	Pathogenic

Affected gene information

BRAF This gene encodes a protein belonging to the raf/mil family of serine/threonine protein kinases. This protein plays a role in re the MAP kinase/ERKs signaling pathway which affects cell division, differentiation, and secretion.

Mutations in this gene are associated with **cardiofaciocutaneous syndrome**, a disease characterized by **heart defects**, **mental retardation** and a **distinctive facial appearance**. Mutations in this gene have also been associated with various cancers, including **non-Hodgkin lymphoma**, **colorectal cancer**, **malignant melanoma**, **thyroid carcinoma**, **non-small cell lung carcinoma**, and **adenocarcinoma of lung**. A pseudogene, which is located on chromosome X, has been identified for this gene. An important paralo gene is FPGT-TNNI3K.

Interpretation

Tafinlar, a BRAF inhibitor, is approved to treat patients with melanoma whose tumors express the BRAF V600E gene mutation. M a MEK inhibitor, is approved to treat patients whose tumors express the BRAF V600E or V600K gene mutations. Approximately ha

Figure 4. An example of an automatically drafted report based on a report template.

Note: Reports can be fully customized towards lab needs, both in content as well as layout.

(a) all information on the sample, findings, public information, databases, literature annotations... can be included into a template;

(b) easy-to-read overviews of variants, with their various characteristics (read depth, nomenclature, transcripts, gene definitions, classification, etc.) can be included in table listings;

(c) automatically generated text can be injected in a context-sensitive way: if variant x or gene Y occurs in the reportable findings, then preconfigured text will be included accordingly. In this case, the "affected gene information" heading automatically includes a section on the BRAF gene, because that variant is reported.

LEARN MORE:

www.agilent.com/lifesciences/alissa

Email us at **cad.info@agilent.com**

Trusted Answers. Together.

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

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