Introduction

Next Generation Sequencing (NGS) utilizing large scale tumor profiles is becoming increasingly common in the molecular pathology setting. The adoption of this technology brings challenges in data management and clinical interpretation, and requires bioinformatics tools to analyze, interpret, and database the large number of variants originating from NGS assays. In a high-throughput context, the delivery of actionable results from NGS data needs to be clinically robust (informed, traceable and reproducible). Moreover, in a cancer diagnostics setting, fast turnaround times are essential for patient care.

Development of an automated system to support variant assessment, classification and reporting requires maintaining standards established by the American College of Medical Genetics and Genomics (ACMG) for the interpretation of sequencing variants (Richards et al. 2015) and those developed for the classification and reporting of cancer susceptibility genes (Plon et al. 2008).

In this case study, we will:

• Describe Genoptix’s approach to somatic genetic testing in routine setting, covering the routine workflow: upstream VCF calling integration, filtration standardization, working with preconfigured profiles, and report automation.
• Describe how the approach was validated, supported by numbers.
• Discuss example cases to illustrate workflow and validation.

Conclusion Summary

Using Alissa Interpret to automate our routine variant assessment workflow and reporting pipeline resulted in achieving three key measures of success for scaling to higher test volumes: (1) regardless of which operator, our lab provides the same answer; (2) we have scaled efficiency through automation, and (3) our variant knowledge base is now accessible and integrated it into our workflow as a very powerful internal expert system.

“On average we have reduced the time to perform variant assessments for myeloid cases from 12 minutes per patient result to less than 5 minutes!” Matthew J. McGinniss PhD FACMG, Executive Director Molecular Genetics, Genoptix, Inc.
In this case study, we demonstrate the development, validation and implementation of the Alissa Interpret platform in routine clinical diagnostic use for support of somatic variant and genomic alteration assessment and lab reporting. Also, we were able to supplement ACMG guidelines with our own Genoptix-specific rules and guidelines that we developed and incorporated into our internal standard work instructions. While clinical laboratory geneticist’s professional judgment cannot be replaced with an automated platform, automation of the criteria a lab adopts for variant assessment is essential to scaling efficiently.

Approach

We developed our own internal variant classification scheme to filter raw variants and genomic alterations coming off our sequencing instruments to improve throughput and consistency of the assessment process. We consecutively implemented our variant filtration and classification approach on the Alissa Interpret platform.

We also build internal databases of variants and curated information. This content is integrated into our assessment protocol through the use of filtration trees and database checks as well as in our variant review workflow and reporting workup.

Illustrations of classification scheme

**Figure 1a** shows an example of a solid tumor case where our own classification scheme filtered 244 raw variants down to 7 variants and 4 genomic rearrangements that then needed to be reviewed and classified by the molecular geneticist (**Figures 1a and 1b**).

This classification scheme allows us to filter variants based on their population frequency, annotations in:

- COSMIC (Forbes et al. 2008)
- dbSNP
- ClinVar

For example, any variant with a population frequency of >2% is filtered out automatically as a benign~ polymorphism.

**Figure 1b.** Translocations are visualized separately. Four genomic alterations were identified in the input VCF file and displayed in the translocation tab.
Figure 1a. An example classification tree. This tree shows our filtration scheme that was validated and stored. The number of variants in the input VCF file are checked against common population databases, and based on a specific Minor Allele Frequency are labeled as “benign” with ACMG/Genoptix guidelines. The 244 variants embedded within sequencing VCF files are then filtered down to 7 variants and marked for review by a clinical molecular geneticist.

Variant filtration by the numbers

Table 1 shows the various profiles available with numbers of genes. This table illustrates the ratio between typical lower and upper bounds of numbers of raw variants identified on a single sample and relates this to the typical number of reportable variants. On average only 4-11% of variants are reportable.

<table>
<thead>
<tr>
<th>Profile</th>
<th>No of Genes</th>
<th>Typical No of Raw Variants</th>
<th>Typical No of Reportable Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS Profile</td>
<td>5</td>
<td>40-50</td>
<td>0-6</td>
</tr>
<tr>
<td>Melanoma Profile</td>
<td>15</td>
<td>10-12</td>
<td>1-3</td>
</tr>
<tr>
<td>AML Profile</td>
<td>21</td>
<td>15-20</td>
<td>1-5</td>
</tr>
<tr>
<td>Lung Profile</td>
<td>25</td>
<td>50-100</td>
<td>2-5</td>
</tr>
<tr>
<td>Myeloid Profile</td>
<td>41</td>
<td>50-90</td>
<td>0-3</td>
</tr>
<tr>
<td>Lymphoid Profile</td>
<td>75</td>
<td>120-140</td>
<td>0-10</td>
</tr>
<tr>
<td>NexCourse Solid</td>
<td>110</td>
<td>240-?</td>
<td>2-24</td>
</tr>
<tr>
<td>NexCourse Complete</td>
<td>173</td>
<td>300-540</td>
<td>4-24+</td>
</tr>
</tbody>
</table>

Table 1. Typical Number of Reportable Variants from Different Reportable Profiles.
Variant review

Shown below is an example of a translocation variant.

**Figure 2.** Variant Review Tab.

**Figure 3.** Managed Variant List.

Variant review

Storing variants and the annotations made by experts in a traceable, versioned, and accessible way is a key requirement to assess samples efficiently in a high sample throughput environment. We store variant information in so-called “Managed Variant Lists.” Our managed variant list (Table 2) so far comprises over 30,000 variants. This list has been developed over the last few years and recently expanded as we just clinically validated and launched our 173-gene pan-cancer gene profile.
Classification | No. of Variants
--- | ---
Benign | 30,324
Likely Benign | 139
VOUS | 1,713
Likely Pathogenic | 94
Pathogenic | 2,370
Totals | 34,640

Table 2. Managed Variant Listing for the NexCourse Pan-Cancer Profile. (Source: Alissa Interpret NGS Managed Variant List, 161 genes, 21 Sept 2015)

To assess the growth and the growth rate of our internal variant knowledge base, we tracked variant additions to our database over a period of 17 weeks post-test launch. As shown in Figure 4 below, the growth remains linear over time, which points to the importance of populating a variant database systematically and incorporating its content in variant assessment on new samples in an automated fashion.

![Figure 4](image)

**Figure 4.** Linear growth of internal variant database over time, binned by week and colored by variant classification.

A workflow ecosystem: integration with IT

The Alissa Interpret platform is fully integrated with our IT systems to automatically update and build our client-facing reports. The Alissa Interpret solution is used by board-certified clinical molecular geneticists, with user roles ranging from clinical user, to curator, to administrator. The Alissa Interpret system supports enterprise-wide single sign-on (SSO) and other features required for security and access controls. We also partnered with CollabRx, another third party software solutions company, to assist with annotations of solid tumor related genes for any therapies or clinical trials that may be associated with a given variant. CollabRx has published an actionability framework (Vidwans *et al.* 2014) and this expert system is used to help inform therapeutic decision making by the ordering physician.
Conclusion

In setting up our routine variant assessment and reporting pipeline, three elements have proven key.

1) Regardless which operator, our lab provides the same answer. For this, standardization is essential: Genoptix has a defined, tested, and validated classification strategy, a well-defined process and a systematic method, and can register assessment by person. With the Alissa Interpret software platform, a lab can set up the tools and procedures necessary for this.

2) We have scaled efficiency through automation. Scale should not impact turnaround times, and standardization has allowed us to reach high throughput while minimizing the need to add/hire more directors, which is an important cost avoidance.

3) Variant assessment is the key challenge when adopting NGS. We have not only built a variant knowledge base, but also made it accessible and integrated it into our workflow. This insight into internal knowledge, with our previous history already loaded when launching a test, makes a very powerful expert system.

Publications


Intended Use Statement

Alissa Interpret software is intended for variant storage, visualization, and annotation using public, commercial and customer internal data sources. It allows end users to set up pipelines to perform or automate the triage and classification of genetic variants. It provides features for recording variant assessments and the drafting of variant analysis reports. The integration capabilities allow for the automated exchange of variant and report information with external software systems.

Alissa Interpret software is intended to be used by trained lab professionals, clinical geneticists and molecular pathologists as a decision-support software platform for the analysis and interpretation of genetic variants identified in human samples in the context of clinical information recorded for a sample.
An Automated Pipeline for NGS Testing and Reporting

Trusted Answers. Together.

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PR7000-0690
© Agilent Technologies, Inc. 2017 Printed in USA.
Original publication date November 01, 2015
Revision Date June 30, 2017
5991-8529EN

Agilent Technologies