Cartagenia Bench Lab NGS, now Alissa Interpret, case study

An Automated Pipeline for NGS Testing and Reporting in a Commercial Molecular Pathology Lab: the Genoptix Case

Molecular pathology labs are increasingly automating their pipelines for somatic variant assessment. Read how Genoptix Medical Laboratory pioneered a pipeline in their lab and in the field of pathology using Cartagenia Bench Lab NGS, which is now Alissa Interpret, the next evolution of Cartagenia Bench on the Alissa Clinical Informatics platform.

This case study will show you how Genoptix

- Implemented an automated pipeline for somatic variant assessment.
- Built a variant knowledge base, integrated it into routine workflow, and reduced variant assessment time significantly.
- Established a traceable and reproducible workflow to help the lab scale to higher test volumes.

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 demonstrate the development, validation and implementation of the Cartagenia Bench Lab NGS 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.

"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.

Authors

Matthew J. McGinniss[1], PhD FACMG, Executive Director, Molecular Genetics

Shareef A. Nahas[1], PhD FACMG, Associate Director, Molecular Genetics

Dianne Keen-Kim[1] PhD FACMG, Executive Director, Cytogenetics

Peter Bui[1], PhD FACMG, Associate Director, Cytogenetics

Steven W. Van Vooren[2], PhD, Product Marketing Manager

1. *Molecular Genetics, Genoptix Medical Laboratory, Genoptix, Inc. (a Novartis company), Carlsbad, CA*
2. *Cartagenia, Inc. (an Agilent company), Boston, MA*

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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.

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 Cartagenia Bench Lab NGS 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 classiication scheme allows us to ilter variants based on their population frequency, annotations in COSMIC (Forbes et al. 2008), dbSNP and Clinvar. For example, any variant with a population frequency of >2% is filtered out automatically as a benign polymorphism.

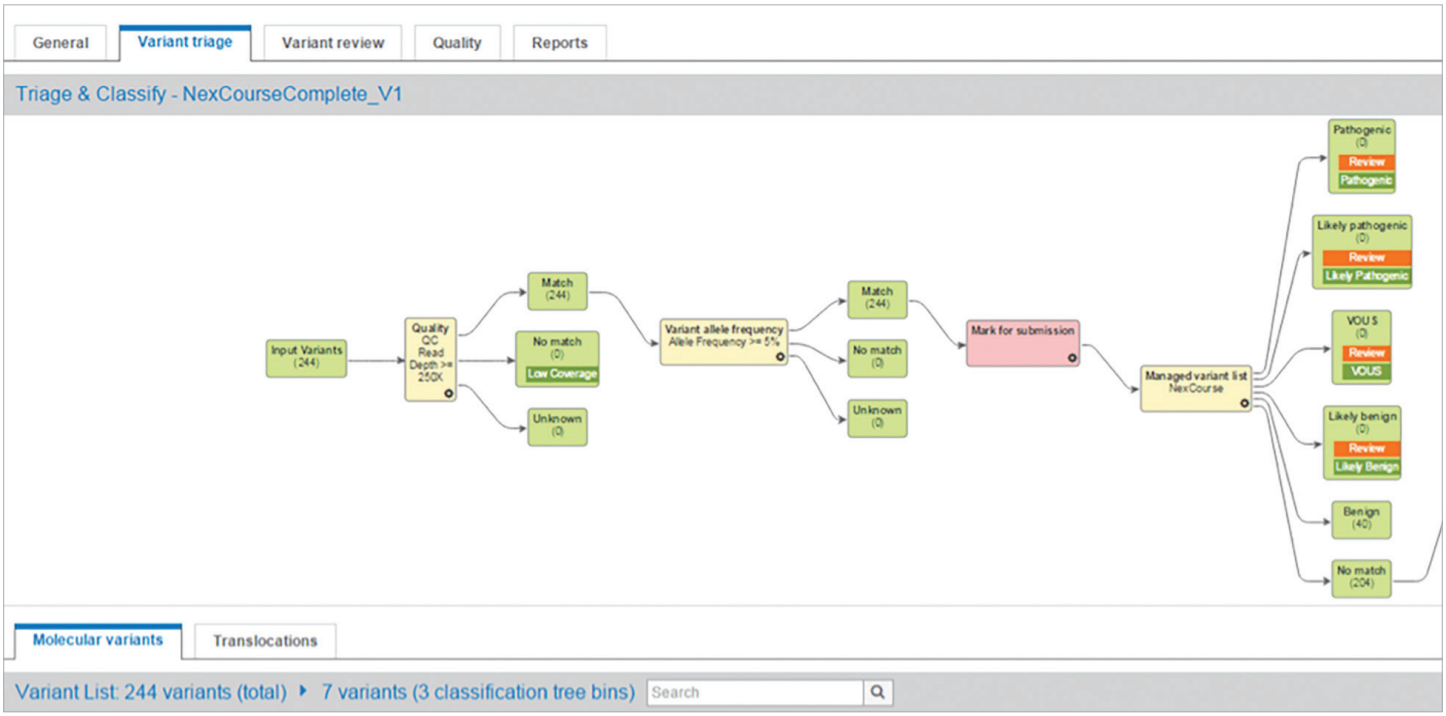

Figure 1a. An example classification tree. This tree shows our filtration scheme that was validated and stored. The number of variants in the input VCF file are checked against common population databases, and based on a specific Minor Allele Frequency are labeled as "benign" with ACMG/Genoptix guidelines.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

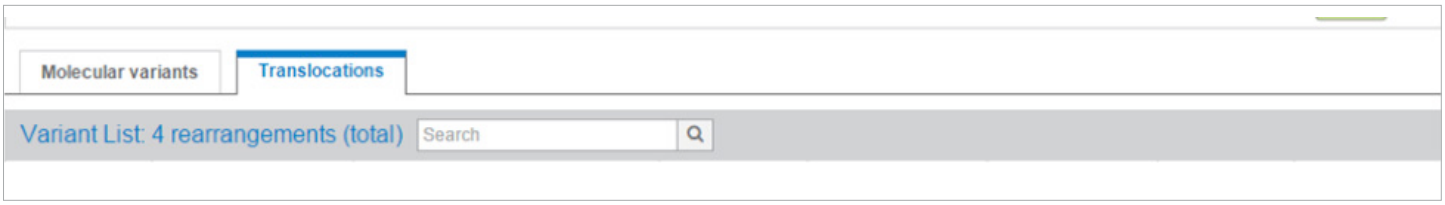

Figure 1b. Translocations are visualized separately. Four genomic alterations were identified in the input VCF file and displayed in the translocation tab.

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.

Variant review

Shown below is an example of a translocation variant.

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

Table 1. Typical Number of Reportable Variants from Different Reportable Proilessequencing VCF files are then filtered down to 7 variants and marked for review by a clinical molecular geneticist.

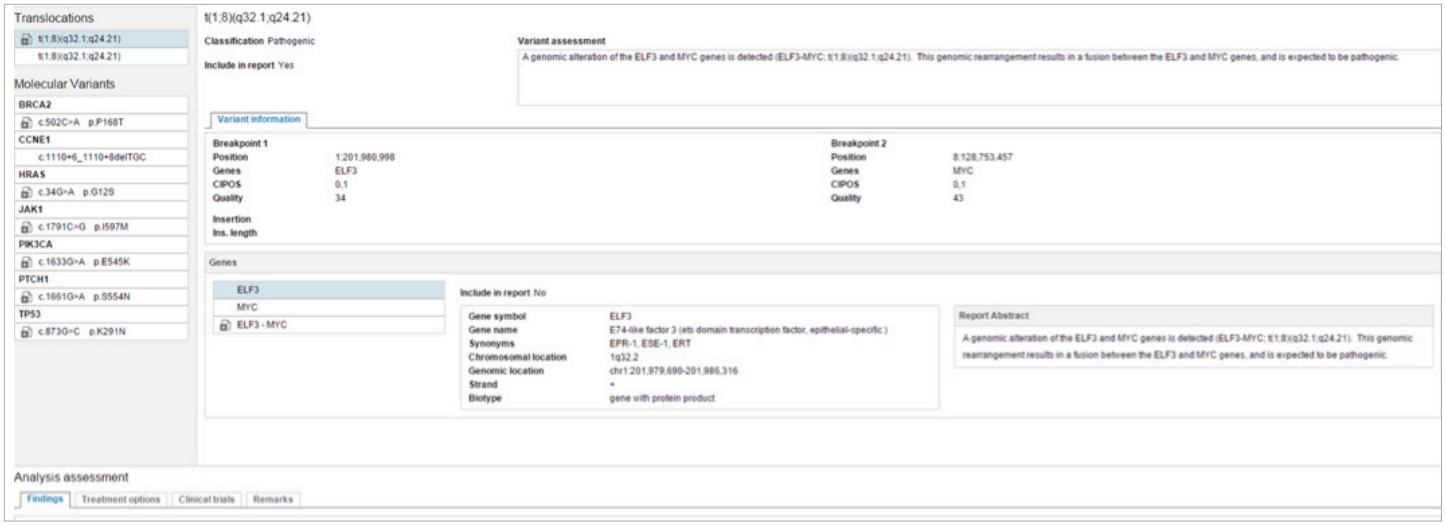

Figure 2. Variant Review Tab.

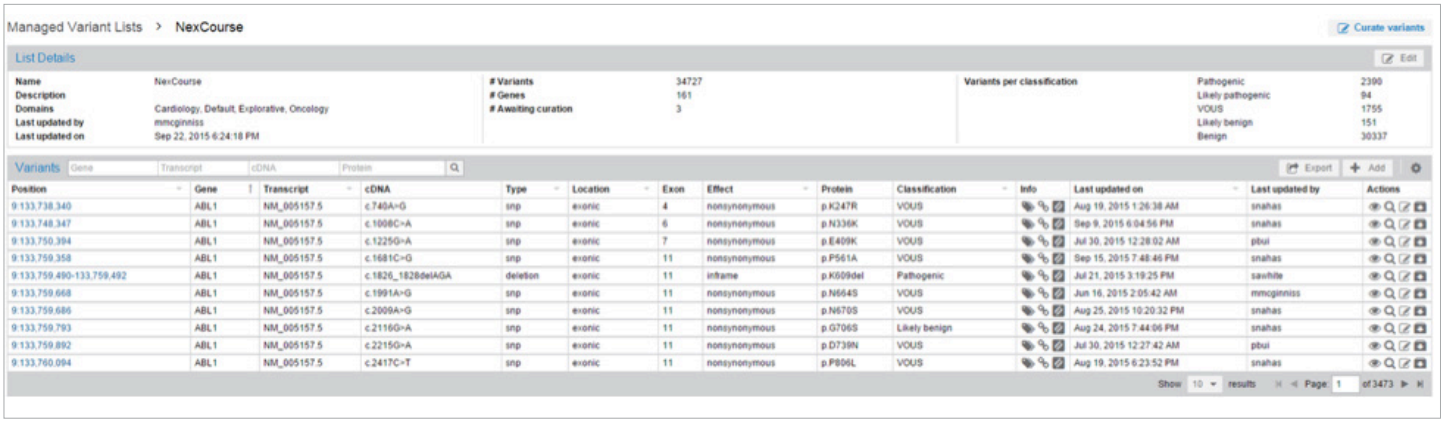

Figure 3. Managed Variant List.

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 Proile. (Source: Bench Lab 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.

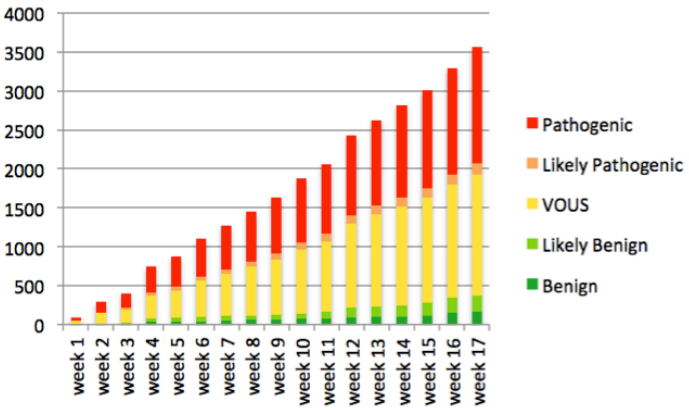

Figure 4. Linear growth of internal variant database over time, binned by week and colored by variant classification.

A workflow ecosystem: integration with IT

The Cartagenia Bench solution is fully integrated with our IT systems to automatically update and build our client-facing reports. The Cartagenia Bench system is used by board certiied clinical molecular geneticists, with user roles ranging from clinical user, to curator, to administrator. The Bench 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 Bench 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 worklow. This insight into internal knowledge, with our previous history already loaded when launching a test, makes a very powerful expert system.

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