illumına[®]

Informing analysis and empowering interpretation of variants in rare disease

A use case with the TruSight[™] Software Suite.

Introduction

Whole-genome sequencing (WGS) using next-generation sequencing (NGS) technology is a powerful tool for investigating variants linked to genetic disease. It provides a high-resolution, unbiased view across the entire genome to discover causative variants of inherited disorders. However, the vast amounts of data produced by WGS requires comprehensive data analysis tools that can efficiently translate the raw sequencing data into meaningful, interpretable results. To address these challenges, Illumina offers the TruSight Software Suite.

This hypothetical use case describes the workflow a laboratory analyst would follow using the TruSight Software Suite to identify variants associated with a genetic disease (Figure 1).

Case receipt and request for variant analysis

A laboratory analyst receives a new request for WGS variant analysis. The case is an example trio data set (Family 1) that consists of a male proband and unaffected parents (Table 1). After libraries are prepared from DNA samples from the proband and each parent, they are sequenced on the NovaSeq[™] 6000 System. The analyst then begins working in the TruSight Software Suite to find disease-associated variants that correspond with the phenotypic features of the proband.

Table 1: Example data set case description

		Proband		
Case	Gender	Phenotype	Mother	Father
Family 1	Male	night blindnessoptic disc pallor	Unaffected	Unaffected

Case management

Using the Case Management Portal in the TruSight Software Suite, the lab analyst creates a new case, imports data files, and associates sequencing data files with the case. Alternatively, this information can be easily imported via an application programming interface (API). Next, the lab analyst enters pedigree information about the case (up to five individuals), including family structure, proband gender, proband phenotypic features (optional), and affected status of family members (Table 1). Real-time updates of case status are displayed in the TruSight Software Suite, allowing the analyst to monitor progress through the analysis workflow.

After sequencing is complete, the DRAGEN[™] Bio-IT Platform automatically performs sequence alignment and variant calling for the proband and associated family members. Comprehensive variant calling includes small variants, copy number variants (CNVs), structural variants, mitochondrial variants, repeat expansions, and more. After reviewing information in the Quality Control (QC) Metrics tab for the proband and each family member, the lab analyst proceeds with variant triage, visualization, and interpretation (Figure 1).

Variant triage

The lab analyst begins variant triage by opening the Interpretation tab. Variant filtering can follow a custom plan or a prebuilt filter plan. Family-based filtering in the TruSight Software Suite enables comparison of the proband with other family members. Additional filter options include filtering on population frequencies from sources such as the Genome Aggregation Database (gnomAD), variant consequences, sample metrics, ClinVar pathogenicity, and more



Figure 1: Variant analysis in the TruSight Software Suite—After the DRAGEN platform performs alignment and variant calling, users proceed with triaging, visualizing, and interpreting variants.

•) TI	EST-0	CAS	E6 SUBJE	CT INFO INTERPRETAT	TION REPORT	ГS						PO
S	ubject	PROBA	ND V								⊟ Case Visualizat	ion	Columns 🗸
А ВЕ	TA_PRO	TOCOL_FI	LTERS	GENERAL	- DENOVO	RECES	SIVE -	SHORT +	NEGATIVE	SECONDARY FINDINGS	× MOST LI	KELY -	+ NEW VIEW
≢	Show Fil	ters	View va	ariants by flag 🗸 🖸	isplaying 28 of 5004596 \	/ariants.							(1) Legend
FLAG	STATUS	LINKED	IGV	CATEGORY	POSITION	GENE	OVERLAP	CONSEQUENCE	HGVS	ZYGOSITY	POP FREQ	LENGTH	CLINVAR
٦×	-	сн ~	Ξ	Small Variant	6:65622398	EYS	5	Stop Gained	c.2620C>T p.(Gl	Heterozygous (Father)	0.000079 NFE gno	1	P
٦×	-	сн ~	=	Copy Number Variant	6:65985254-66085786	EYS (+1)	5	Transcript Truncation Co		Heterozygous (Mother)		100533	
٦×	-		=	Run of Homozygosity	8:17453812-22542961	PDGFRL (+47)	5			Hemizygous		5089150	
٦v	-		Ξ	Run of Homozygosity	13 : 9506311017984	DCT (+57)	5			Hemizygous		6735353	
□ ~	-	сн ~	=	Small Variant	10 : 126103126103	OAT		Intron Variant	c29-288829-2	Heterozygous		14	
□~	-		Ξ	Small Variant	20:5092253-5092254	TMEM230 (+1)	4	Splice Region Variant Intr	c.69-5_69-3dup	Homozygous (Conflict)		3	
٦×	-	сн ~	Ξ	Small Variant	2:210640622	UNC80		Stop Gained	c.151C>T p.(Arg	Heterozygous		1	P
٦v	-	сн ~	=	Small Variant	10:14139140-14139141	FRMD4A	.1	Intron Variant	c.45+232913_4	Heterozygous		4	
٦×	-	сн ~	Ξ	Short Tandem Repe	18 : 53253387-53253458	TCF4		Short Tandem Repeat Exp		Heterozygous		57	
٦v	-	сн ~	=	Small Variant	1:39879371	MACF1 (+1)		Intron Variant	c.9631-8675T>C	Heterozygous	0.000149 EAS gno	1	
ب ک	-	сн ~	=	Small Variant	2:26022394	ASXL2		Missense Variant	c.263C>T p.(Pro	Heterozygous	0.000027 NFE gno	1	

Figure 2: Variant filtering and prioritization—The lab analyst can perform family-based variant filtering for identification of inherited and *de novo* variants. As shown, the variant grid shows information on category of variant, chromosomal position, gene affected, overlap (number of overlapped phenotypes for the variant), consequence of the variant, population frequency of the variant (if known), and more.



Figure 3: Variant calling confirmation—The lab analyst can access an embedded IGV for detailed variant inspection and comparison to other cases and external databases.

BETA_PROTOCOL_F Show Filters	FILTERS	GENERAL	DEN	ovo 🔍											
	View varia	the building of the			RECESS	SIVE	SHORT		NEGATIVE	SECONDARY FINDINGS	× MOST LI	KELY	+ NEW VIEW		
		nts by nag 🔹	Displaying 28 of 50	04596 Varian	nts.								(i) I	Leg	
STATUS LINKED	IGV C	CATEGORY	POSITION	GEN	E	OVERLAP	CONSEQUENCE		HGVS	ZYGOSITY	POP FREQ	LENGTH	CLINVAR		
· — сн ·	≡ \$	Small Variant	6 : 65622398	EYS		5	Stop Gained		c.2620C>T p.(Gl	Heterozygous (Father)	0.000079 NFE gno	1	P		
- сн ×	= 0	Copy Number Varian	6:65985254-660	85786 EYS	(+1)	5	Transcript Tru	uncation Co	r.	Heterozygous (Mother)		100533			
riant Details	Transcript	Gene Deta	ile					1		_	Knowledge Ne	etwork	Send for Curation	1	
SNV chr6:65622398 REF: G		208	Zygosity & Metrics					Population I All Population (SpliceAI (EYS)		
LT: A			PROBAND	MOTHER	FATHER			GnomAD	í		Acceptor Gain		onor Gain		
ote Ø	9	Affected	True	False	False		ALL	0.00004			Score:		ore:		
		Genotype	0/1	0/0	0/1		AFR	0.00000			Distance:	Di	stance:		
dd a note		VRF	0.475	0	0.4		AMR	0.00004			Acceptor Loss	De	onor Loss		
		Total Dept	40	33	45		ASJ	0.00000			Score:	Sc	ore: 0.1		
		Allele Dept	ns 19	0	18		EAS	0.00000			Distance:	Di	stance:-21		
		GT Quality	48	84	46		EUR								
		Filter	PASS	PASS	PASS		FIN	0.00000							
		Quality Sco	re 125	125	125		NFE	0.00008							

Figure 4: Variant interpretation and curation—Users can search multiple external databases within the software to aid in variant interpretation.



Figure 5: CaseLog - The lab analyst can visualize aggregate data for both variants and genes of interest.



Figure 6: Variants of interest in Family 1—Using the tools in the TruSight Software Suite, the lab analyst has identified variants of interest in the proband: a compound heterozygous variant in the EYS gene consisting of a small stop-gain variant and a CNV. This image depicts the normalized coverage plot with a clear copy number loss in both the proband and the mother, highlighted in the blue box.

(Figure 2). Also, the analyst can access a growing list of tools powered by artificial intelligence (AI)–based machine learning, such as SpliceAI and PrimateAI to further prioritize variants of interest. These deep learning networks provide unbiased, highly accurate classification of mRNA splice sites and missense variants, respectively.^{1,2} After triage, the lab analyst has filtered out millions of irrelevant variants and is able to focus on the top variants of interest for the case for visualization and interpretation.

Variant visualizations

The analyst uses embedded visualization tools such as the Integrative Genomics Viewer (IGV) to further inspect genomic data such as readlevel alignments and pair-end reads, b-allele frequency and normalized coverage tracks for all subjects within a case (Figure 3). In addition to variant level visualizations, the analyst can zoom out to view either an entire chromosome or whole genome to look for large anomalies. After variant calls have been confirmed, the lab analyst proceeds with interpretation for the case.

Variant interpretation and curation

Finally, the lab analyst works to determine which prioritized variants are relevant to the current case. To do this, the lab analyst interrogates gene and variant disease associations with overlapping phenotypic features similar to those in the proband. TruSight Software helps by aggregating and integrating preferred external databases such as the Online Mendelian Inheritance in Man (OMIM) catalog, ClinVar, and others into the Variant Details tab (Figure 4). The lab analyst can view the aggregated data to gain valuable insights into a variant, eliminating the need for repeated online searches in separate databases. Additionally, the TruSight Software Suite integrates standard terminology for variant classification developed by the American College of Medical Genetics (ACMG), which helps with recording variant details and associations. This enables customers to access and store gene-level information (eg, tolerance to loss-of-function variation, etc) and characteristics of gene-disease relationships (via preferred external databases).

The lab analyst can also use features such as the Note field to add case-specific notes pertaining to a specific variant, or the Comments field to record case-independent information about the variant or gene, which may be valuable if observed in future cases. CaseLog is used to view and aggregate gene, variant, and phenotypic information for each case across both private and public data sets (Figure 5). This customer-specific database of variant information enables reanalysis of historical data based on new discoveries in the scientific community.

Case results and reporting

After interpretation, the lab analyst has identified and curated variants of interest in the proband with known disease associations. A compound heterozygous variant was found in the *EYS* gene that includes a small stop-gain variant and a CNV that results in deletion of multiple exons (Table 2, Figure 6). Mutations in the *EYS* gene have known associations with retinal pigmentosa (RP), a genetic disease that results in an incomplete loss of vision that includes night and peripheral vision.

The lab analyst creates a new case report and adds gene and variant associations relevant to the case. The lab analyst can send the report for additional review and approval within the software. For ease of data sharing, the report can be downloaded as a PDF or JSON format. The Case status is now updated to Reported in the Case Management Portal.

Table 2: Family 1 proband variant interpretation

Variant of interest	Interpretation
EYS Small Variant 6:65622398 (c.2620C>T; p.Gln874Ter) Heterozygous EYS CNV 6:65985254-66085786 Heterozygous	The c.2620C>T; p.Gln874Ter variant introduces a premature stop codon that results in early truncation of the EYS protein. The CNV results in a loss of ~100 Kb of sequence that includes multiple exons of the EYS gene.

Summary

As demonstrated in this use case, a laboratory analyst can use the intuitive and comprehensive features and tools of the TruSight Software Suite to visualize, triage, and interpret variants associated with genetic disease.

Learn more

Learn more about the TruSight Software Suite at www.illumina.com/ trusight-software-suite.html

References

- Jaganathan K, Kyriazopoulou Panagiotopoulou S, McRae JF, et al. Predicting splicing from primary sequence with deep learning. *Cell*. 2019;176(3):535–548.
- Sundaram L, Gao H, Padigepati SR, et al. Predicting the clinical impact of human mutation with deep neural networks. *Nat Genet*. 2018;50(8):1161– 1170.

About TruSight Software Suite

The TruSight Software Suite is a software as a service (SaaS) analytics solution that integrates with the NovaSeq 6000 Sequencing System and the DRAGEN Bio-IT Platform to enable comprehensive and streamlined secondary and tertiary analysis workflows for NGS. The TruSight Software Suite offers:

- Simplified, customizable case management—Manage cases from sequencing data to report, assign cases to users, configure pipeline settings, and set quality control (QC) thresholds
- Comprehensive, ultra-rapid variant calling—Use DRAGEN secondary analysis to call small variants, CNVs, and structural variants, mitochondrial variants, repeat expansions, and more
- Intuitive, high-powered interpretation—Filter variants via gene lists, inheritance modes, custom annotations, and complex logic; flag, sort, and prioritize important variants

For a full list of current and future functionality, please contact an Illumina sales associate for additional information.

Illumina • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com © 2020 Illumina, Inc. All rights reserved. All trademarks are the property of Illumina, Inc. or their respective owners. For specific trademark information, see www.illumina.com/company/legal.html. 970-2019-024-A QB9184

