## Built To Adapt

Comprehensive next-generation sequencing promotes efficiencies in rare disease analysis

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illumina®

### Move

TACTTGTCTAGCTTAACTGATC CTAGCTACTTA ATCTGGGAG GGAGAGCA GCTACTTAG GCTACTTGT ACTGATCTT CTAGCTTA CTTAGCTAC TTAGCTACT CTACTTGTCTAGCTTAACTGATCTT TCTTAGCTACTTAGCTACTTGTC TGTCTAGCTAGCTACTTAGCTAC TTAGCTACTI TGATCTTAA ATTACTTAG ACTTGTCG TTGATCT GTCTAGC CTACTTAGC FAACTGATCC TAACTGATGCTA TCTAGCATGA AGCTAGCTACTTAGCTACTTGTCT TAGCTACTTGTCGCTTGAT

GCTACTTGTCTA TAACTGATC ACTGATCTT CTTAGCTAC AACTGATCT TTGTCTAG TGTCTAGCT TAGCTTTTGATCTGGGAGAGCAGC GTCTAGCTTAACTGATCTTAACTG TACTTAGCTA ATCTTCTTA ACTTAGCTA TAGCTTAAC GAGAGCAG GCTACTTAG GGGAGCAT ATGATGCTT TGCTTGATCTGGGAGAGCAGCTACT AGCTTAACTGATAGCTACTTGTCTA CTGGGAGAGCAGCTACTTAGCTACT

TACTTGTCT	
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TTCTTAGCT	ACT
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ATGCT	TGATCTO
TAGC	TACTTGI
GTC	TAGCTTA
CTA	CTTAGCI
TT	AACTGAT
G	TCTAGC
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TGATCTO CTACTTA CTACTTG

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ACTGATCTTACTTA CTAGCTTCT TTGTCTAGO CTTGTCTA TAGCTAGC AGAGCAGC TGATCTTA AGCTTAAC TGATCTTAA AGCTTAA TCTACTTAGCTACTTGT TAACTATCTTACTT

GGGAGAGCAGCTACTTAGCT ACTGATCT AGCTAGCT TTGTCTAG CTAGCTTA CTGATCTTA CTGATCTT# ACTGATCT TAGCTTAA GGAGAGCA TTGATCTG GCTACTTAG GATCTCTA GCAGCTACI TTAGCTACT TAGCTACTT CTGGGAG. ГАССТАС СТАСТТАС GCTTAAC TGATCTTA CTTAGCTAC AACTGATCI GCTTAACTO TACTTAGCT ATCTCTACT TAGCTAC TTGTCTTI CTTAG<u>CTACTTAG</u> GTCTAGCTTCTTAG TTAACTGATCTI ACTTAGCTA CTTGTCTTTAACTGATCTTACT

### WGS and WES offer higher diagnostic utility than CMA

40

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The pace of genomic discovery in rare disease is breathtaking. Two hundred and fifty new gene-disease associations are identified annually. Over nine thousand new variant-disease associations are reported per year.<sup>1</sup>

Deeper understanding of the genome is being uncovered with each new day.

traditional methods

AGCTACTTGTC

GCTACTTGTCTA

AACTGATCTCTAC

CTTGTCTA

GATCTTA

TGTCTAGO AGCTACTI

CTAGCTAG

CTTCTAGC TACTTAG ACTTAGCT ACTTGT

ATGATGCC ATGATGC

Chromosomal microarrays (CMA) are a traditional testing method used by investigators in individuals with unexplained developmental disabilities.<sup>2</sup>

It enables profiling of chromosomal abnormalities, such as duplications and microdeletions, down to 5-10kb in size.<sup>3</sup> While highly effective, CMA accesses only a portion of the genome and does not enable interrogation of sequence variants.

### utility 20 Diagnostic

Figure 1 95% CI: 4.7-14.9.P < 0.0001.4



Evidence-based guidelines issued by the American College of Genetics and Genomics (ACMG) have recognized the value of whole-genome sequencing (WGS) or whole-exome sequencing (WES) in first or second tier use. Improved management, higher diagnostic yield, and improved costs were cited as support to using early in a genomic evaluation.<sup>5</sup>

Thirty-seven studies comprising 20,068 children published between January 2011 and August 2017 were reviewed for the diagnostic utility of CMA, WES, and WGS

TAGCTACTTGT	TAGC
TTGTCTTTAACT	GATCTTAC
TAGCTTAACTAT	CTTACTTAGC
AGCTACT	TGTCTAGCT
TAGCTAC	TTAGCTAC
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CTTGTCTA	GCTTAAC
CTAGCTT	AACTGAT
ATGCTTGATCTG	G G A G A G C A
CTAGCTTAACTG	ATCTCTACT
TCTTAACT	GATCTTCTT
GCTTAAC	TGATCTTA
GTCTAGC	TAGCTACT
TAGCTAC	TTGTCTAG
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CTACTTGT	CTAGCTTA
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G G G A G A G C A G C	TACTTAGCTAC
CTACTTAGCTAC	TTGTCTAGC
A G A G C A G C T A C	TTAG

TTGTCGCT TAGCTTAA TGATCTGG CTGATCTC CTTAACTG ATCTTAAC TCTAGCTA ACTTAGCT GATGCTTG ACTTGTCT ATCTGGG GCTACTTG GCTACTTG TGTCTAGC AGCTACTT ACTTAGCT TTAACTGA CTTTCTAC GAGAGCA TCATGATG GCTACTTA CTTGATCT GCTACTTG ACTGATCT CTACTTAG TCTTAACT TGTCTAGCTTAACTGATCTTAC

TAGCTACTTAGCTAC AGCTACTT TTAGCTAC GAGCATGA CTTAGCTA CTACTTG1 TGATCTTA CTACTTAG TTACTTAG TGTCTAGC CTTAGCTA AGCTTCTT CAGCCAT AACTGATC CTACTTAG GATGCCAT TTACTTAG ACTTAGCTACTTGTCTAGCTAG TCATGATGCTTGATCTGGG

CTACTTGT TTAACTGA CTTGTCTA

G A G A G C A TACTTAGC

TGATGCTT AGCTTAAC AGAGCAG

AACTGATG

AACTGATC TAGCTACT TCTAGCTA

G G G A G A G T C T A G C T T

CTAGCTAG

ATCTTACTTAGCTACTTGTCT

for the future

#### Create "virtual panels" with a genome or exome foundation

Use of whole-genome or whole-exome sequencing as an assay foundation enables dynamic creation and modification of "virtual panels" as more is understood about the genome.

#### The burden of multigene panels

TTAGCTAC TACTTGTC

TAACTGAT

G C T A C T T G A G C A G C T

CTTACTTA

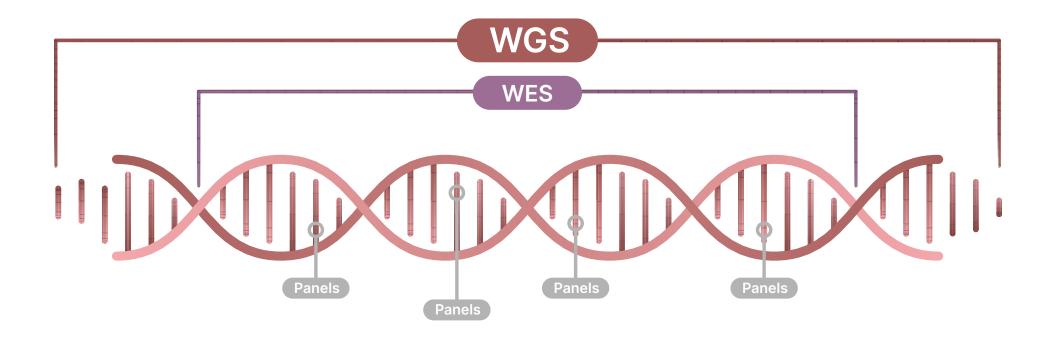
TAGCTACI AGCTACII

The velocity of change brings challenges for the modern molecular genomics laboratory to stay current. One lab found 23% of positive WES findings were in genes described within the last two years, while 7% of positive variants were in novel gene discoveries.<sup>6</sup>

Labs face a continuous cycle of new panel design and validation with every new gene or variant association with rare disease, requiring significant expenditure of time and resources, all while being unable to engage in gene discovery themselves.

In contrast, with WGS and WES labs can create a comprehensive assay, amenable to the latest genomic discoveries. New findings can be incorporated into existing workflows and "future-proof" the test menu.

Re-analysis of existing data sets can identify novel associations without the need to re-sequence samples or re-validate an assay. "Virtual panels" can be created out of a genome or exome output, providing ordering health care providers a bespoke panel of their choosing (Figure 2).



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TTAACTG	ATCTCTAC	TTAGCTAC	TTGTCTAG	CTAGCTAC	TTAGCTAC	TTGTCTT	TAACTGA	TCTTACTT	AGCTACTT	GTCGCTT	GATCTGG	GAGAGCAGCTAC
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### your analysis with WES

Scale variant interpretation and benefit from Next-Generation Sequencing (NGS)

For labs that want to increase capabilities and gain proficiency in comprehensive NGS analysis, WES is a targeted sequencing approach that enables them to focus resources on genes likely to affect the phenotype.

WES targets protein-coding regions, which comprise less than 2% of the genome but contain ~90-95% of known disease-related variants.6 It produces a manageable data set for focused analysis that can help build competencies.

### WES can:

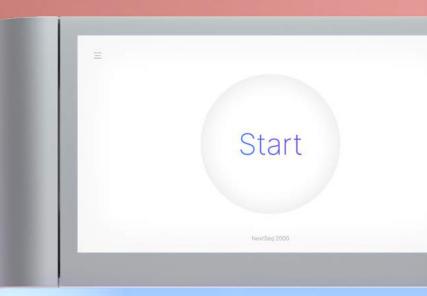
CTTGTCTA TACTTGTC GCAGCTAC TGTCTAGC





# With WES

find a molecular



Provide the laboratory professional a broad view of coding variants.

Enhance laboratory proficiencies associated with data management and interpretation at scale.

Offer greater opportunity for re-analysis or discovery potential than CMA or gene panels.



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### No single test is more

T A G C T G A G C A G C T A		TTG GCTAC	T C T A G C TT G T C T A	TTAACT GCTTAA	GATCTCT		CTTAGC GCTACT		CTTGTCTAGCTA AGCTTAACTGAT	G C T A C T C T	C T T A A C T T	GCTACTTGTCTT AGCTACTTGTCT	TAACT AGCTA	G A T C G C T A	TTACT CTTAGCTAC	T A G C T T G T	TACTT CTAG	G T C G C T T A	CTTGATCTGGGA ACTGATCTTAAC
TGAT CTT	C TTAG	CTAC	TTAGCTA	CTTGTCT	AGCT	TCTA	GCTA	CTTA	GCTA	CTTG	TCTA	GCTT	AACTG	а тстт	AACT GATC	TTCT	TAGC	TACT	TAGC
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AGCT	ACTT	GTCT	AGCT TAA	CTG ATCT	TACT	TAGC	TACT	TGTC	TAGC	TTAA	CTGA	T C C A	TGAT G	CT TGAT	CTGG	G A G A	G C A G	CTAC	TTAG
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TTAAC TGAT	CTTAC	TTAGC	TACT TGT	CT AGCT	TAAC		TGAT	СТСТ	ACTTAGCTACTT	GTCT	AGCT	AGCTACTTAGCT	ACTT	GTCTA	GACC TTAA	CTGA	TCT	TAA	CTGATCTTCTTA
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#### WGS offers unparalleled analysis

For labs that want to streamline operational efficiency, WGS provides the most comprehensive view of the human genome.

WGS enables simultaneous analysis of thousands of genes with known or suspected associations with rare disease, as well as discovery of novel causative variants. Enabling uniform coverage of coding regions, WGS provides advantages evaluating exons compared to WES. As a single assay, there is no other test that can detect as many diverse variant types (Table 1).

Comprehensive variant calling can result in greater opportunities to interrogate the genome, as 13% of WGS diagnoses in a large national pilot study were not expected to be discovered with exome sequencing.<sup>7</sup> WGS also provides a foundational assay for other emerging applications including pharmacogenomics, human leukocyte antigen (HLA) typing, and polygenic risk scores.

### WGS provides the most comprehensive analysis of geneomic variants among all clinical genomic testing methods<sup>9</sup>

Single-Nucl

Insertions 8

Copy Numb

Repeat Exp

Structural V

Mitochandr

Paralogs

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& Deletions (Indels)	~	~	~	~	~	~
ber Variants (CNVs)		~	~	~	~	~
oansions	· · ·		~		1	~
Variants (SVs)				~	~	~
rial	~	~			~	~
	~		~			~

Table 1: Comparaison of WGS to Standard Testing

 Limited capabilities Capable

It is clear whole-genome sequencing is contributing significantly to end diagnostic odysseys in rare disease. With guidelines advocating use as a first-tier test<sup>5</sup>, inclusion in national health care systems<sup>7</sup>, and increasing evidence of economic value when used as a first-tier test<sup>8</sup>, genome sequencing appears to be on the path toward standard of care.

\*Variant detection may vary depending on laboratory and test offering

CMA = chromosomal microarray, CNV = copy number variant, FISH = fluorescence in-situ hybridization, indel = small insertion deletion, NGS = next-generation sequencing, PCR = polymerase chain reaction, SNV=single nucleotide variant, WES = whole-exome sequencing, WGS = whole-genome sequencing

TAGCTA	CTTGTCT AGC	TT AACTGATCTCTACTTAGCTA	CTTGTCTAGCTAGCTAC	TTAGCTACT	TGTCTTTAACTGA	TCTTACTT	AGCTACTTGTCGCTTGATCT	G G G A G A G C A G C T A C T T	AGCTACTTGTCT
AGCTTA	ACTATCT TAC	TT AGCTACTTGTCTAGCTTAAC	TGATCTCTACTTAGCTA	CTTGTCTAGCT	AGCTACTTAGCTACT	TGTCTAGC	TTAACTGATCTTAACTGATC	TTCTTAGCTACTTAGCT	ACTTGTCTAGCTTC
TAGCTA	CTTAGCTA CTT	GT CTAGCTTAACTGATCTTAAC	TGATCTTCTTAGCTACT	TAGCTACTTGTCTAG	CTTCTTAGCTACTTGTC	TAGCTAGCT	ACTCATGATGCTTGATCTGG	GAGCATGATGCTTGAT	CTGGGAGAGCAGCTAC
TTAGCT	ACTTGTCT AGC	TT AACTGA	TCTTAC	TTAGCT ACTTGT	CTAGCT TAACTGA	TCCATGATG	CTTGAT	CTGGGA	GAGCAG CTACTTAG
CTACTT	GTCTAGCTT AAC	TG ATCTTA	CTTAGC	TACTTG TCTAGC	TTAACT GATGCT	ACTTG TCTAG	CATGAT	GCTTGA	TCTGGG AGAGCA
GCTACT	TAGCTACTT GTC	TA GCTTAA	CTGATC	TTACTT AGCTAC	TTGTCT AGCTTA	ACTGA TCTCT	ACTTAG	CTACTT	GTCTAG CTAGCT
ACTTAG	CTACTTGTC TAG	CT TAACTG	ATCTTA	ACTGAT	CTTCTT AGCTAC	TTAGC TACTT	GTCTAG	CTTCTA	GCTACT TAGCTA
CTTGTC	TAGCT TAAC TGA	TC TTAACT	GATCTT	CTTAGC	TACTTA GCTACT	TGTCT AGCTT	CTTAGC	TACTTG	TCTAGC TAGCTA
CTTAGC	TACTT GTCT AGC	TT AACTGA	TCTTAA	CTGATC	TTCTTA GCTAC	TTAGC TACTT	GTCTAG	СТТТСТ	ACTCAT GATGCT
TGATCT	GGGAG AGCA GCC		ATGATGCTTGATCTGG	GAGAGC	AGCTAC TTAGCT	ACTTG TCTAG	CTTAAC	TGATCTTACTTAGCTA	CTTGTC TAGCTT
AACTGA	TCTCT ACTTA GCT	AC TTGTCT	AGCTAGCTACTTAGCT	ACTTGT CTAGACCTTA	ACTGATCTTAACTGA	TCTTC TTAGC	TACTTA	GCTACTTGTCTAGCTT	TTGATC TGGGAG
AGCAGC	TACTT AGCT ACT	GT CTAGCT	TAACTGATCTTACTTA	GCTACT TGTCTAGCTT	AACTGATCTCTACTTA	GCTAC TTGTC	TAGCTA	GCTACTTAGCTACTTG	TCTAGC TTAACT
GATCTT	AACTG ATCTT CTT	AG CTACTT	AGCTAC	TTGTCT AGCTTC	ATGATG CTTGAT	CTGGG AGAGC	AGCTAC	TTAGCT	ACTTGT CTAGCT
TAACTG	ATCTT ACTTA GCT		AGCTTA	ACTGAT CTCTAC	TTAGCT ACTTGT	CTAGCTAGCTACTTAGC		TCTAGC	TTAACT GATCTT
AACTGA	TCTTC TTAGCTA		CTTGTC	TAGCTT TACTTA	GCTACT TGTCTA	GCTTAACTGATCTTACT		TTGTCC	TCTATT ACTTAG
CTACTT	GTCTA GCTTAAC	TG ATCTCT	ACTTAG	CTACTT GTCTAG	CTAGCT ACTTAG	CTACTTGTCTTTAACTG	A TCTTAC	TTAGCT	ACTTGT CGCTTG
ATCTGG	GAGAG CAGCTAC	TT AGCTAC	TTGTCT	AGCTTA ACTGAT	CTTACT TAGCTA	CTTGT CTAG	CT TAACTG	ATCTCT	ACTTAG CTACTT
GTCTAG	CTAGC TACTTA	GC TACTTG	TCTAGC	TTAACT GATCTTA	ACTGAT CTTCTT	AGCTA CTTA	GC TACTTG	TCTAGC	TTCTAG CTACTT
AGCTAC	TTGTC TAGCTT		TTAACTGATCTTCTTAG	CTACTTA GCTACTTG	TCTAGC TTCTTA	GCTAC TTGT		CTACTCATGATGCTTGAT	CTGGGA GCATGATG
CTTGAT	CTGGG AGAGC	AG CTACTT	AGCTACTTGTCTAGCTT	AACTGATCTTACTTAGC	TACTTG TCTAGC	TTAACT GAT	CA TGATGC	T T G A T C T G G G A G A G C A G	CTACTTAGCTACTTGT
CTAGCT	TAACT GATCI	TA CTTAGC	TACTTGTCTAGCTTAAC	TGATGCTAC TTGTC	TAGCAT GATGC	TTGATC TGG	GAG AGCAGC	TACTTAGCTACTTGTCT	AGCTTAACTGATC

### workflows for NGS methods

Illumina offers investigators integrated, streamlined workflows for WES and WGS research that follow the same three steps labs may be familiar with and already use for targeted sequencing (Figure 4). Regardless of the method, prepared libraries are loaded onto an Illumina platform for sequencing.

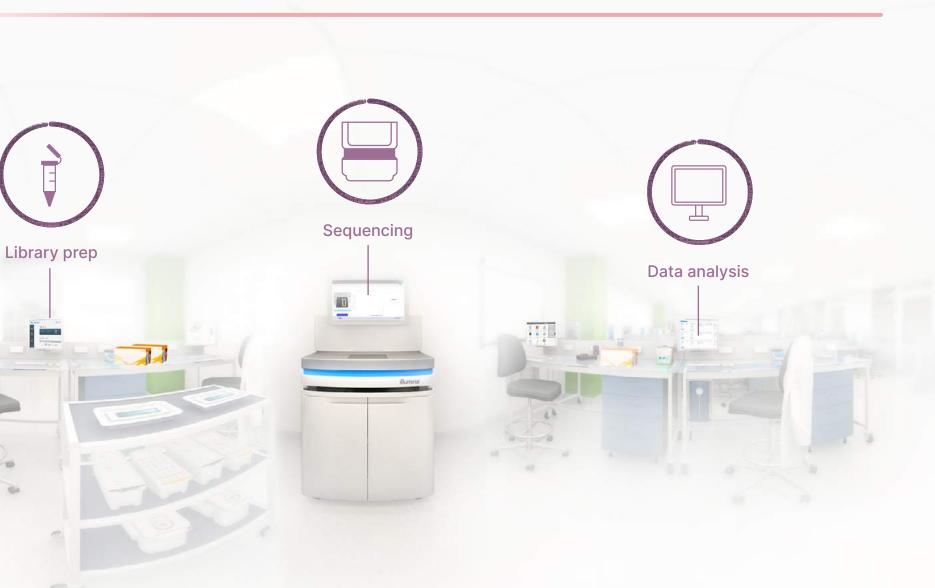
WES research can be performed on a range of Illumina systems from the benchtop MiSeq™ System to the NovaSeq<sup>™</sup> 6000 System. The output capabilities and scalability of the NextSeq<sup>™</sup> 1000, NextSeq 2000, and NovaSeq 6000 Systems make them ideal for WGS investigations.

Illumina sequencing systems are powered by the same sequencing by synthesis (SBS) chemistry, so data generated across systems can be compared and integrated, enabling labs to transition to new methods with confidence.

#### The Illumina NGS workflow

Regardless of the specific method used, all Illumina NGS workflows consist of three basic steps: library prep, sequencing, and data analysis.

Figure 4: The Illumina NGS workflow



TCTAGCTT		ACTT	GTCT					
CTTAGCTACTT TGTCTAGCTTCTAGCTA		CTACTT	GTCTA	GCTTAA	C T G A	TCTTA	ACTGATC	TTCTT
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	TACTTGT	CTAGCTT	CTTAGCTA	CTTGTCTA	GCTAG	CTACTC	ATGATGC	TTGAT
	TTGATCT	GGGAGA	G C A G C T	ACTTAGC	TACTT	GTCTAG	CTTAACT	GATCT
	CTTAGCT	ACTTGTC	TAGCTTA	ACTGATC	CATGA	TGCTTG	ATCTGGG	AGAG
	GCTACTT		AGCTACT		TGTCTA	GCTTAA	CTGATCT	TACTT
	CTACTTG		TCTAGCT		TAACTG	ATGCTA	CTTGTCT	AGCA
	ATGCTTGAT		C T G G G A G		AGCAG	CTACTT	AGCTACT	TGTCT
CTTAACTGATCT TCTAGCTAGCTA		TACTTAG		CTACTT	GTCTAG	CTTAACT	GATCT	
		CTTAGCT		ACTTGT	CTAGCT	TAACTGA	TCTTA	
		ACTTAGCTAC	TTGTCTA		GCTTCT	AGCTAC	TTAGCTA	CTTGT
		AGCTTAACT	GATCTTA		A C T G A T	CTTCTTA	GCTACTT	AGCTA
		TGTCTAG	CTTCTTA		GCTACTTGTC	TAGCTAGCTAC	TTAGCTA	CTTGT
		AGCTTAA	CTGATCT		TAACTGATCTT	CTTAGCTACTT	AGCTACT	TGTCT
	CTTTCTA	CTCATGA	TGCTTGA	TCTGGG	A G A G C A	GCCATG	ATGCCAT	GATGC
	GATCTGG	GAGAGC	AGCTACT	TAGCTAC	TTGTCT	AGCTTAA	CTGATCT	TACTT
	CTACTTG	TCTAGCT	T A A C T G A	TCTCTAC	T T A G C T	ACTTGTC	TAGCTAG	CTACT
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	GCTACTG	TCTAGCTT	AACTGAT	CTTACTT	AGCTAC	TTGTCTA	GCTTAACTGATCTCTACTTA	GCTAC
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### with automated interpretation and XAI

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TACTTAGCTACTTG CTGATCTTCTTAGC

G A G A G C A G C T A C T T A

TCTAGCTTCATGAT

The cornerstone of rare disease analysis is interpretation. With variability in the method, the genes interrogated, and the output generated by an application, a software solution to provide an investigator a complete view of the data is crucial.

Illumina's Emedgene tertiary analysis platform has been designed to translate the vast amounts of data produced by WGS, WES and virtual panels into meaningful insights, enabling rapid analysis. Illumina's Emedgene intuitive genomic analysis platform enables 2-5x improvement in efficiency:

- Streamline interpretation and automate evidence curation with explainable artificial intelligence (XAI) and machine-learning
- Integrate with the cloud-based DRAGEN™
  Bio-IT Platform to enable comprehensive, streamlined secondary and tertiary analysis workflows and ultrarapid variant calling

Illumina offers users an ecosystem of end-to-end high-throughput products, designed for diverse researcher needs. Whether it is including automation to increase efficiency, ensuring quality of a run, or providing a seamless experience with scalable software for sample-to-report generation, laboratories can have confidence knowing they have the very latest to equip them in their search for answers.

#### Learn more

→ Whole-genome sequencing
 → Whole-exome sequencing

#### References

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