WES All-in-One Solution: Market-leading performance of Celemics' Whole Exome Sequencing Panel and seamless integration to robust VarSome Clinical reporting platform

ABSTRACT

Celemics WES panel offers the all-in-one solution that enables highly efficient and affordable NGS-based genetic analysis application of the human whole exome with superior performance in respect of coverage and uniformity. The objective of this study was to demonstrate and compare the sequencing result of Celemics' WES panel with other major competitors' using reference standard material, not only to address the market-leading performance of the panel but also to present the proprietary probe design and assay optimization technologies that ensures robust sequencing results. In addition to exceptional sequencing result, VarSome Clinical enabled flawless integration of bioinformatics analysis and support efficient and accurate variant detection and interpretation. Through this collaboration, the most comprehensive human whole exome regions were sequenced and established accurate data analysis with higher than 99% of the variants detected among tens of thousands of acknowledged variants in exome regions. The results showed that Celemics' proprietary probe design and panel synthesis technology along with VarSome clinical's convenient and effective data analysis tool can ensure incomparable quality sequencing results regardless of sample type or target region of interest.

INTRODUCTION

The significance and demand for Whole Exome Sequencing (WES) is increasing as WES through targeted sequencing technology suggests better and efficient performance with higher throughput and incomparable cost-effectiveness for the exome represents only about 2% of the whole human genome but contains most of the known disease-related variants. Considering these advantages of WES, Celemics WES panel offers the all-in-one solution that enables highly efficient and affordable genetic analysis application of the human whole exome with superior performance in respect of coverage and uniformity. With a panel target size of target size of 37.1 Mb, in addition to incorporation of customizable gene add-on options, Celemics' WES panel ensures the most comprehensive set of target regions of major WES panels available in the market. Through Celemics' proprietary probe design and panel synthesis technology, the Celemics WES panel, is able to successfully cover even the challenging 'hard-to-capture' regions, such as high and low GC ratio regions; along with the integration to VarSome Clinical reporting platform, can demonstrate outstanding performance for comprehensive client-specific data interpretation for genomic variant discovery.

MATERIALS AND METHODS

Panel Design

Celemics Inc. (Korea) has designed and developed WES panel as an effort to cover the most comprehensive human whole exome regions by covering beyond all target regions of four major WES panels in the market, including each WES panel from company A, company I, and two different WES panels from company T (Figure 1). However, the WES panels in the market vary in their target regions, and some compromise with coverage, even deleting 'hard-to-capture' areas to enhance performance. With a target size of 37.1 Mb, Celemics WES panel does not compromise performance in terms of coverage and uniformity, enabling time-saving and cost-effective sequencing of the human whole exome that spans exon regions from RefSeq, CCDS, and GENCODE. Through Celemics' highly optimized probe design and panel synthesis technology, Celemics WES panel provides the most comprehensive coverage even in intrinsically challenging regions, providing the optimal balance of high coverage with high uniformity for deeper profiling of both rare and inherited genetic variation.

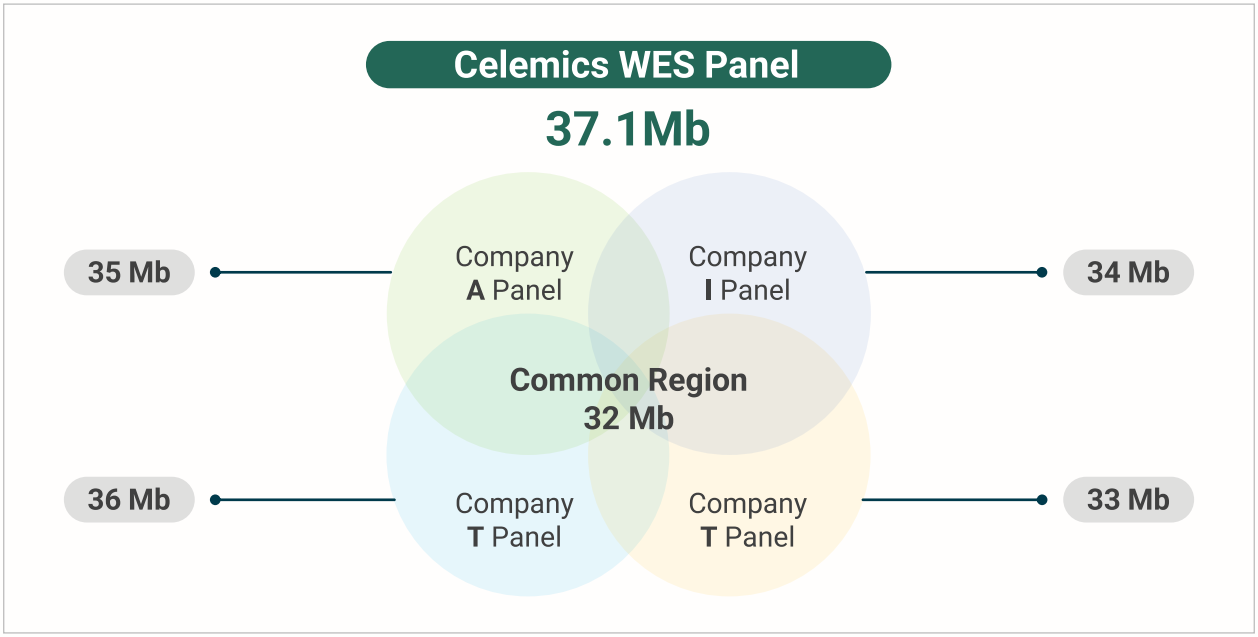


FIGURE 1 : The Celemics WES panel covers the target regions of all major whole exome panels in the market, which include WES panels from company A, company I and company T

Among the regions that Company A and T failed to cover from their own target regions, Celemics covers an additional 60 Kb and 306 Kb of Company A and T respectively (data not shown). Most of these regions are challenging to capture due to GC-rich and repeated sequences. Through Celemics' optimized probe design and panel synthesis technology, the Celemics WES panel is able to successfully cover the challenging regions that other company products fail to cover.

Library Preparation

Celemics designed and produced capture probes to cover 37.1 Mb of the human whole exome region. 200 ng of genomic DNA was processed for an Illumina sequencing run. The process included the following steps; fragmentation, end-repair, dA-tailing, adapter ligation, and pre-PCR for indexed NGS library. NGS-prepared DNA library and capture probes were hybridized in buffer to capture target regions of interest through the use of a Celemics target enrichment kit. After the capture and washing process were finished, the captured library was amplified by the post-PCR process. The PCR products were sequenced by the Illumina platform.

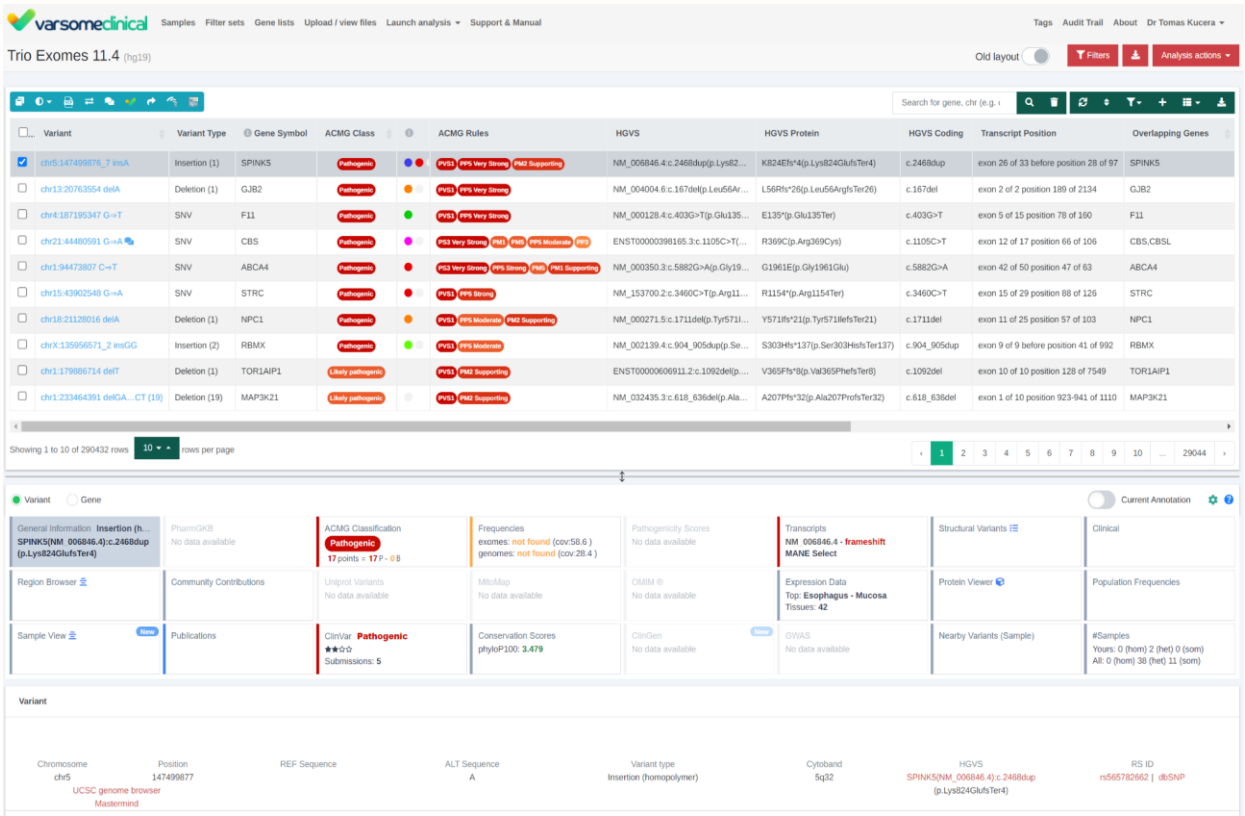
Samples

For the panel comparison studies, 200 ng of three Hapmap standard reference material samples, NA12878, NA12891 and NA12892 were used on Illumina NextSeq 500 (2x150 cycles). The sequencing of competitor products was conducted by third party laboratories (certified service providers). For VarSome Clinical platform studies, 200 ng of three NIST Genome In A Bottle (GIAB) standard reference material samples HG001 - HG003 were used on Illumina NextSeq 500 (2x150 cycles).

Analysis in VarSome Clinical

VarSome Clinical is a CE-IVD certified and HIPAA-compliant platform allowing fast and accurate variant discovery, annotation, interpretation, and reporting for NGS data for whole genomes, exomes, and gene panels. It allows for variant prioritization based on patient phenotypes using HPO and OMIM terms. VarSome Clinical leverages the open knowledgebase of VarSome.com with aggregated clinical evidence consisting of 130+ cross referenced public data resources and contributions from its community of more than 350,000 users worldwide. For variant calling, VarSome's clinical platform uses Sentieon, the state-of-the-art, award-winning variant caller. Sentieon offers several applications tailored to germline and somatic sample analysis and has repeatedly demonstrated its superiority in accurate variant detection.

[https://www.sentieon.com/products/]



RESULTS

The most comprehensive exome coverage achieved with market-leading performance of on-target ratio and uniformity even against 'hard-to-capture' regions.

Celemics WES panel is the most comprehensive panel in the market. Not only covering the target regions of the competitor products, but the panel also includes additional 890,675 bp regions that other companies failed to cover due to technical issues. The issues usually occur against 'hard-to-capture' regions such as GC rich, repeats, and/or homologous regions. But through Celemics' optimized probe design and panel synthesis technology, even the coverage of those challenging regions is secured (Figure 2).

Figure 2 : Exhaustive coverage for each gene
The Celemics WES Panel covers each gene with thorough coverage in comparison to competitor products. The bar graphs indicate the percentage of genes that are covered at (A) 20X depth and (B) 30X depth. The data from the three panels were downsampled to 5.4 Gb. (C) The IGV figure demonstrates the superior coverage performance of the Celemics WES panel against the TGID1 gene compared to other competitor products.

Celemics provides market-leading target capture performance due to probe design and reagent optimization technology. Despite some companies who resort to masking the 'hard-to-capture' regions (such as GC- or AT-rich regions and homologous regions) or completely omit the regions from their target in order to enhance the result quality, Celemics provides both high coverage and on-target ratio without reducing the number of target regions. With Celemics' proprietary technology, the WES panel captures regions that no other companies could capture with quality coverage (Figure 2C), on-target ratio (Figure 3A) and uniformity (Figure 3B, 3C). The all-around performance of Celemics' WES panel allows for highly sensitive, cost-effective and time-saving sequencing of the whole exome.

Figure 3 : Superior performance in the market
Celemics WES panel shows exceptional performance compared to other competitor products when measured by (A) on-target read ratio, (B) 0.2x mean depth coverage uniformity (higher the better), and (C) Fold-80 base penalty (lower the better). Third-party laboratories (certified service providers) conducted a comparison study between the Celemics WES panel, Company A and Company T panels. Reference materials NA12878, NA12891, and NA12892 were used with same amount. Illumina instruments were used for the sequencing. The data from the three panels were downsampled to 5.4 Gb

If the regions of the customer's interest are either in the GC- or AT-rich regions, for Company A product, the sequencing has to be repeated since not enough sequencing reads are generated. Also, unnecessarily high sequencing depth around 70-80% GC ratio regions of Company T would result in unproductive sequencing, causing large amount of needless data. (Figure 4B). In contrast to other competitors' products, Celemics' panel shows no lack of sequencing reads either in GC- or AT-rich regions and maintains better uniformity (Figure 4).

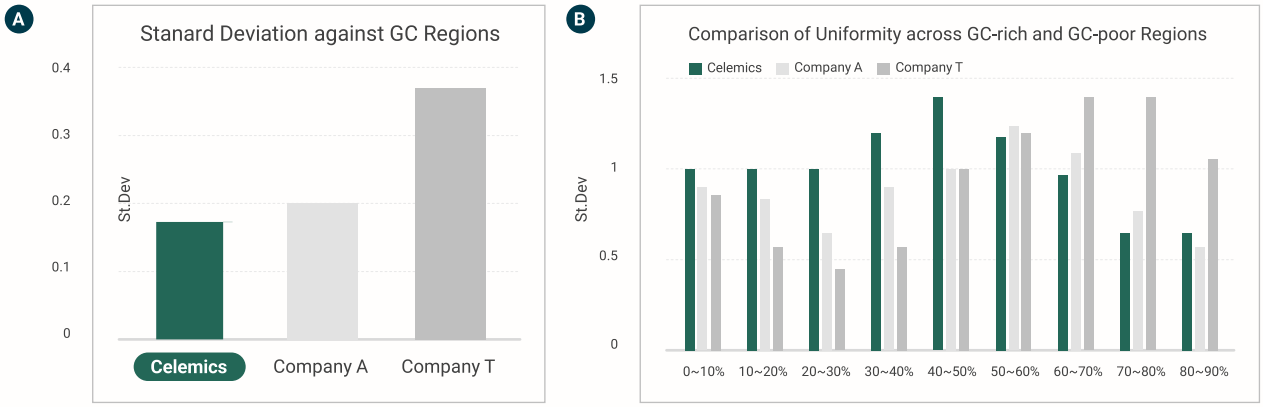


Figure 4. Exceptional uniformity across low and high GC regions
Figure A. The Celemics WES panel yields a 0.166 standard deviation (lower the better), while the other two competitor panels yield a 0.199 and 0.356 standard deviation respectively. Figure B. demonstrates relatively consistent uniformity across GC-rich and AT-rich regions in comparison to competitor products.

Flexible customization and integration of Celemics WES panel afforded by outstanding proprietary hybrid capture probe design.

In order to perform library preparation prior to target enrichment and sequencing, it is often required to have heavy instruments (such as a vacuum concentrator or sonicator, or others), which are barriers against complete automation set-up. Even with an automation protocol, using these heavy instruments is inevitable and is often burdensome for the users. Celemics has successfully eliminated the need for heavy instruments by substituting them with a more convenient solution of enzymes and beads. After rigorous validation that consistently showed reliable performance, we have optimized this workflow to enable the benefit of a complete walkaway solution. The Celemics WES panel is also seamlessly integrated with all NGS instruments from Illumina, MGI, and Ion Torrent. Since there are no heavy instruments required, the experiment can be carried out with complete automation.

Streamlined same-day workflow

Although hybridization capture has great advantages including minimized bias, stable and reliable data results from a variety of sample types, the complexity of the workflow and the long prep time have been obstacles to the users. Celemics has developed a new workflow and have incorporated it into the WES package to significantly simplify the process and reduce the experiment time (Figure 5). Compared to the conventional method took 2-3 days to complete one sequencing experiment, Celemics' newly developed method allows the whole experiment and the NGS run can be started on the same day.

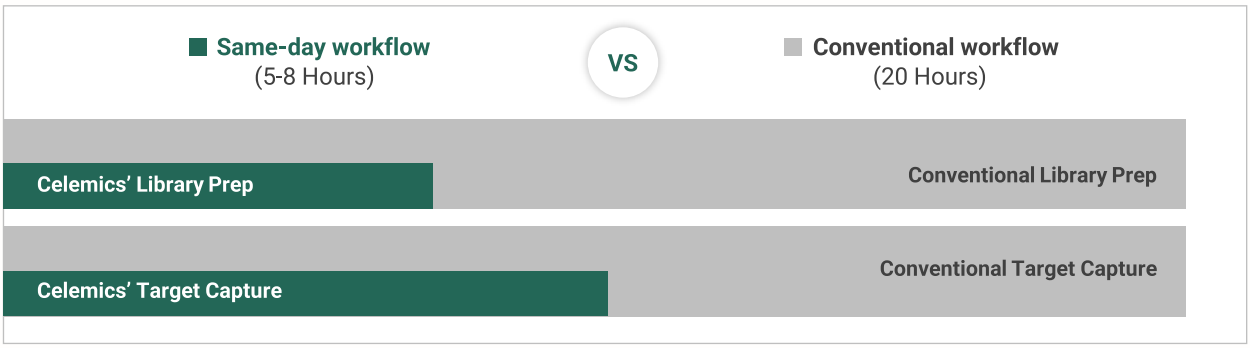


Figure 5 : Newly developed same-day workflow
The figure demonstrates that Celemics has significantly reduced the time for performing WES from the conventional 20 hours to a now 5-hour minimum workflow.

Variant calling performance

Using GIAB samples [https://www.nist.gov/programs-projects/genome-bottle], the standard reference for determining germline variants, the VarSome Clinical pipeline proves capable of detecting virtually all of the tens of thousands of variants these samples contain. Less than 1% of variants are missed, and results turn out to be incorrect less than 1% of the time. In the precision competition sponsored by the U.S. Food and Drug Administration, these numbers were established as the de facto limit for NGS technologies [https://precision.fda.gov/challenges/truth/results].

VarSome variant calling pipeline validation					
Reference data set	**variants in the data set**	**Variants found**	**Percentage variants found**	**Variants missed**	**wrong calls (not a variant)**
NA12878	23926	23849	99.68%	77	215
NA24149	22631	22425	99.09%	206	207
NA24385	26043	25917	99.52%	126	303

CONCLUSION

Despite its small composition in human genome, WES can provide substantial information in regards to disease-causing variants or disease-related mutations. In order for efficient, accurate detection of variants in exon regions, Celemics has designed and developed exclusive WES panel. With the most comprehensive target region commercially available, Celemics also utilizes proprietary probe design and assay optimization technology to maximize the sequencing efficiency, guaranteeing the market-leading capture performance even in GC-rich or homologous regions. Along with Celemics' exceptional WES panel, VarSome Clinical introduces the state-of-the-art variant caller and data interpretation platform for effective and accurate data analysis for clinical diagnostics. VarSome's clinical interpretation follows renowned ACMG and AMP guidelines for classifying pathogenicity, ensuring the precision and competence for disease-related variant discoveries in human exome.

About

CELEMICS

Celemics has developed and manufactured over a thousand different panels to our customers, including hospitals, clinical labs, research institutes, and biopharma companies. The outstanding performance of the panel is enabled by Celemics' proprietary probe design technology and its RNA-based biotinylated probes, providing an even greater binding capacity than DNA-based probes. Celemics has been providing services to the top CROs and clinical labs in Korea and other countries since the company foundation, also collaborating with major hospitals in Korea including Asan Medical Center, Samsung Medical Center, and Seoul National University Hospital. Celemics is certified to ISO 13485, GMP, ISO 9001, and CE-IVD.

About

varsomeclinical

VarSome suite of tools for clinical analysis of NGS data is created by Saphetor SA, a Swiss-based precision medicine company operating on the global market since with 2018

VarSome Clinical is a CE IVD-certified and HIPAA-compliant platform that brings the power of VarSome.com to your Illumina and BGI FASTQs and any VCFs, consisting of 130+ cross referenced public data resources and contributions from its community of more than 350,000 users worldwide.

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