



Whole exome sequencing

with Celero™ EZ DNA-Seq.

Application Note

**EXOME SEQUENCING WORKFLOWS USING CELERO LIBRARIES
PROVIDE HIGHER YIELDS, EXCEPTIONAL UNIFORMITY OF COVERAGE AND
TUNABLE FRAGMENTATION**



INTRODUCTION

Whole exome sequencing only covers protein-coding genes, accounting for approximately 3 percent of the whole genome. However, it is these protein-coding genes that harbor the majority of genetic variations associated with human diseases. Exome sequencing therefore provides many of the benefits of whole genome sequencing, while greatly reducing sequencing costs and turnaround times.

Combining Celero EZ DNA-Seq library preparation with exome enrichment using Twist Bioscience's Human Core Exome Kit provides several key advantages – including a single bead clean-up step – simplifying the workflow and saving cost and time.

Traditional whole genome workflows tend to be laborious, lengthy and costly. As shown in Figure 1, the Celero EZ DNA-Seq library preparation kit shortens and simplifies the NGS library prep process, removing an additional bead purification step between adapter ligation and PCR amplification to significantly improve final library yields.

The Celero EZ DNA-Seq library preparation kit also incorporates a number of other proprietary technologies to reduce cost, simplify the workflow, and increase the quality of final sequence data. This includes NuQuant®, a proprietary library quantification method for efficient and accurate quantitation of NGS libraries. NuQuant eliminates the need for time-consuming or inaccurate library quantification methods such as qPCR, allowing libraries to be constructed and quantified in a single day. The resulting higher quality output from the Celero EZ DNA-Seq kit provides a perfect starting point for high quality exome sequencing with Twist Bioscience's exome enrichment kits.

This application note describes a comparison of the Celero EZ DNA-Seq library preparation kit with a kit from Competitor K in combination with Twist Bioscience's Universal Blockers and the Human Core Exome Kit.

Traditional workflow



Celero EZ DNA-Seq workflow



Figure 1: Comparison between traditional and Celero EZ DNA-Seq workflows demonstrates the ease of use of Tecan's library preparation kit.

MATERIALS AND METHODS

Materials

1. Starting material: 400 ng human NA12878 genome reference standard
2. Celero EZ DNA-Seq kit (Tecan, Part No. 0569-24),
3. Human Core Exome Kit (Twist Bioscience, Part No. 100254)
4. Universal Blockers (Twist Bioscience, Part No. 100865/ Part No. 100578)
5. Kit from Competitor K

Methods

The experiment was designed as a direct comparison between the workflow using the Celero EZ DNA-Seq library preparation kit and kit from Competitor K, in combination with Twist Bioscience's Universal Blockers and the Human Core Exome Kit.



Protocol

1. Library preparation using Celero EZ DNA-Seq kit with 300 bp fragmentation
2. 8 PCR cycles (pre-hybridization)
3. Addition of 3.5 µl of Twist Universal Blockers to each sample
4. Triplicate libraries pooled (200 ng of each library) and hybridization performed
5. 8 PCR cycles (post-hybridization) using Twist hybridization workflow

Results and analysis

The Celero EZ DNA-Seq library preparation kit showed significant improvements in exome sequencing performance across a range of critical parameters, including:

- Increased yield (Table 1)
- Advantageous fragment sizing (Table 1)
- Greater uniformity of target coverage (Figure 2)
- Increased density of target coverage (Figure 3)
- Consistent depth of coverage for target sequence (Figures 4A and 4B)

	Sample	Molarity qPCR (nM)	Library size
Competitor K	1.1	55.88	average ~348 bp
	1.2	59.94	
	1.3	75.88	
Celero EZ DNA-Seq	2.1	255.89	average ~282 bp
	2.2	344.80	
	2.3	245.05	

Table 1: Pre-hybridization QC. Yield comparison between kit from Competitor K and Celero EZ DNA-Seq.

As shown in Table 1, the Celero EZ DNA-Seq library preparation kit provides significantly higher yields, due to the reduced number of clean-up steps required. Each clean-up step in the competitor kits reduces the yield, with multiple clean-ups having a compounding negative effect on the final yield. The Celero EZ kit also enables robust, tunable fragmentation sizing providing the option to generate smaller fragments, known to be beneficial for exome sequencing and other target enrichment applications.

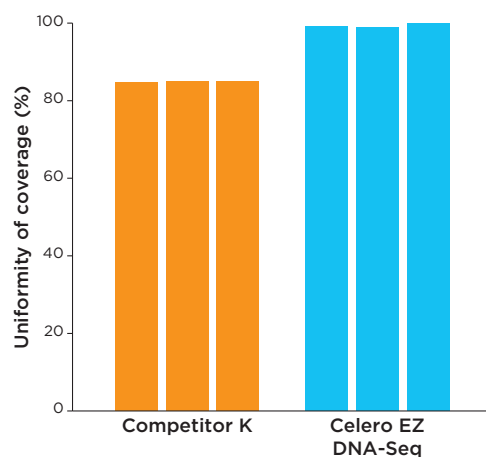


Figure 2: Comparison of sequencing results between kit from Competitor K and Celero EZ DNA-Seq, showing an improved uniformity of coverage (>99 %) for the Celero kit.

The Celero EZ DNA-Seq library preparation kit provides greater uniformity of coverage compared to Competitor K, with a comparable on-target rate. As seen in Figure 2, Celero provides an even uniformity of coverage across all targets (~98 %), meaning less sequencing is required and resulting in an appreciable savings of time and sequencing costs.

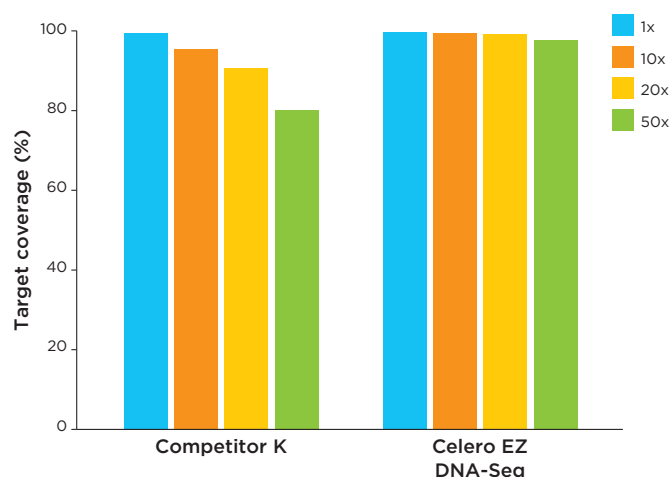


Figure 3: Comparison of target coverage between kit from Competitor K (1) and Celero EZ DNA-Seq (2).

In Figure 3, the percentage of target coverage can be seen for the two kits tested. For the kit from Competitor K, target coverage starts to drop off becoming apparent at 10x, increasing at 20x, and dropping to <80 % at 50x. The Celero EZ DNA-Seq kit consistently maintains greater than 98 % target coverage, which enables more accurate variant calling.



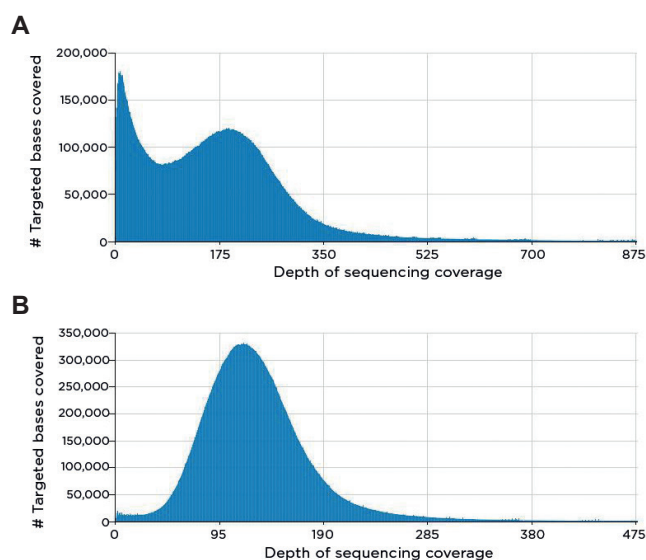


Figure 4: Depth of coverage in target regions between the kit from Competitor K (A) and Celero EZ DNA-Seq kit (B).

Figure 4 shows a critical advantage of the Celero EZ DNA-Seq library preparation kit – the even coverage of target sequences. The kit from Competitor K offered insufficient coverage for large numbers of targets, while Celero libraries provided an even depth of coverage across all target sites.

SUMMARY

The results in this application note demonstrate the suitability of the Celero EZ DNA-Seq library preparation kit for whole exome sequencing applications. This kit offered improvements for almost all critical performance parameters across the experiments performed.

When used as part of an exome sequencing workflow, the Celero EZ DNA-Seq library preparation kit offers several key benefits:

- **Single bead clean-up step** – simplifying the workflow to save time and costs
- **Greater uniformity of coverage** – reducing the number of reads required, and enabling further cost savings
- **Even depth of coverage across target sites** – for more accurate variant calling
- **Robust and tunable fragmentation size** – ideal for target enrichment applications

The robust and easy-to-use Celero EZ DNA-Seq library preparation kit ensures high quality libraries, reduces costs and increases the quality of final sequencing data. Using proprietary technologies, such as NuQuant, it simplifies the workflow and offers automation-friendly approach for high throughput target enrichment applications.

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