

Detection of Leukemia–Associated Structural Variants Using Electronic Genome Technology



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Background

Short-read sequencing (SRS) often misses large SVs due to read-length constraints, while long-read sequencing (LRS) and traditional cytogenetic techniques struggle to balance resolution, throughput, and cost. Electronic genome mapping (EGM), a solid-state nanochannel-based platform, was used to detect *TP53* deletions, various translocations, gene fusions, and copy number variants associated with leukemia in 11 cell lines.

High Molecular Weight (HMW) DNA was extracted, processed with a dual-enzyme workflow, and analyzed on the OhmX™ Platform. SVs were detected in Nabsys Navigator™ SV-Discover™ and confirmed with Nabsys Navigator™ SV-Verify™, a targeted, alignment-based variant detection application. Copy number variants (CNVs) were assessed using the CNV analysis pipeline. Variants were confirmed with orthogonal techniques such as sequencing, optical genome mapping (OGM), and CMA.

EGM accurately detected *TP53* deletions ranging from 3.8–87 kb, a paracentric chromosome 3 rearrangement leading to a *MECOM* rearrangement, a 22.3 kb *KMT2A*-PTD, an 848 kb deletion forming *FIPIL1::PDGFRA*, a microRNA cluster deletion of miR-15a/16-1, as well as gene fusions such as *BCR::ABL1*, *PML::RARA*, and *RUNX1::RUNX1T1*, with accompanying CNV data. SV breakpoints, lengths, and zygosity were resolved with high concordance to published data and orthogonal techniques.

Data for three cancer cell lines were selected for this poster to highlight the range of structural variants that can be detected by EGM.

About Electronic Genome Mapping (EGM)

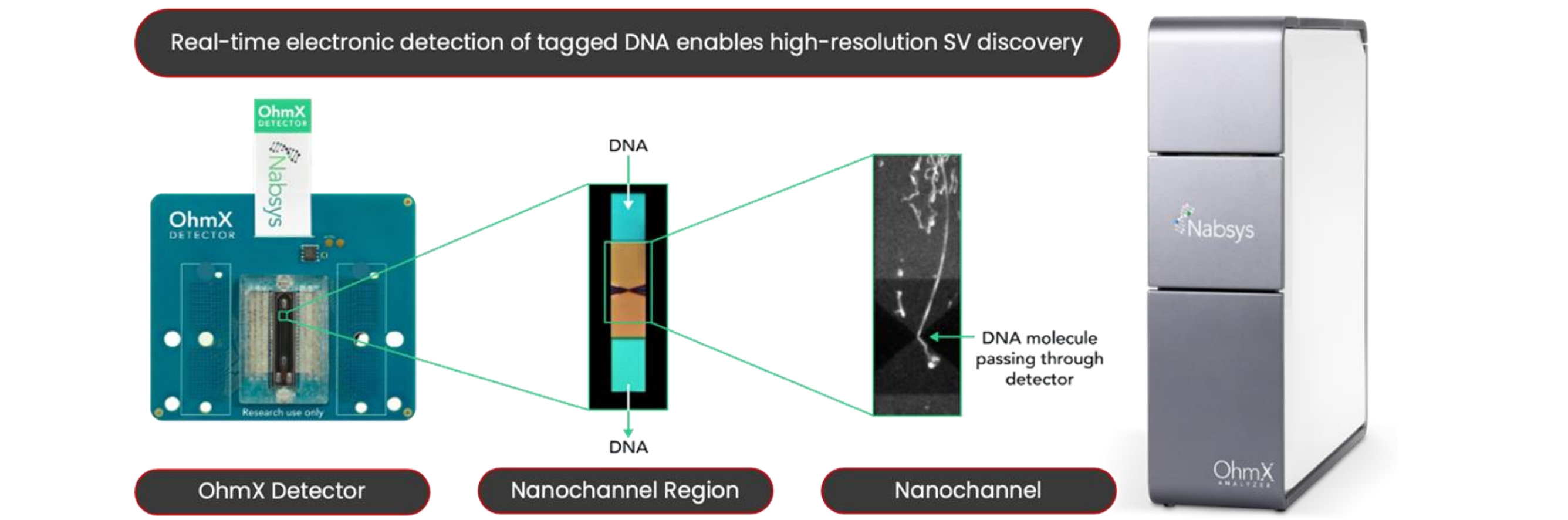


Figure 1. EGM Detector and Analyzer. EGM is enabled by the novel, solid-state nano-detectors developed by Nabsys. The OhmX Detector houses 256 parallel nanochannels, each with its own electronic sensor. Nabsys has developed DNA tagging chemistry that provides a high signal-to-noise ratio. The high signal and spatial resolution allows tags to be closely spaced on the high molecular weight DNA molecules. This yields optimal tag density such that structural variations as small as 300 bp can be routinely identified and mapped genome-wide.

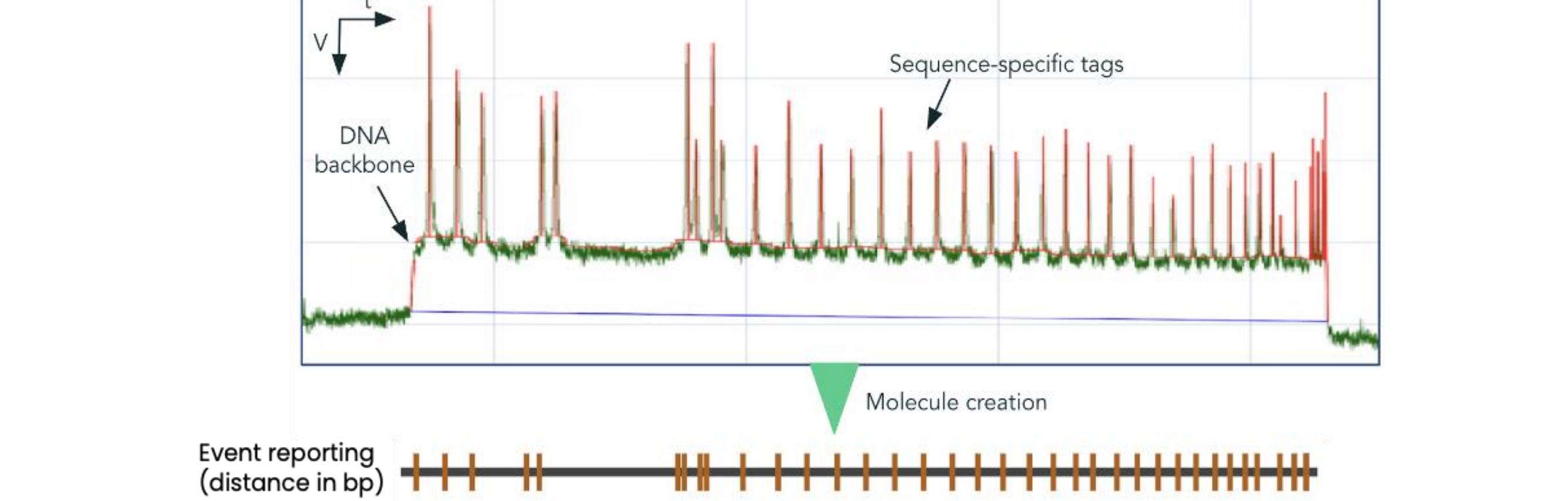


Figure 2. Electronic profile for tagged DNA molecules over 150 kb. The baseline voltage is shown on the left. When the DNA molecules enter the nanochannel, a change in voltage is observed. Whenever a site-specific tag on the DNA is also present, the voltage changes again. The time between tags is converted to distance in base pairs.

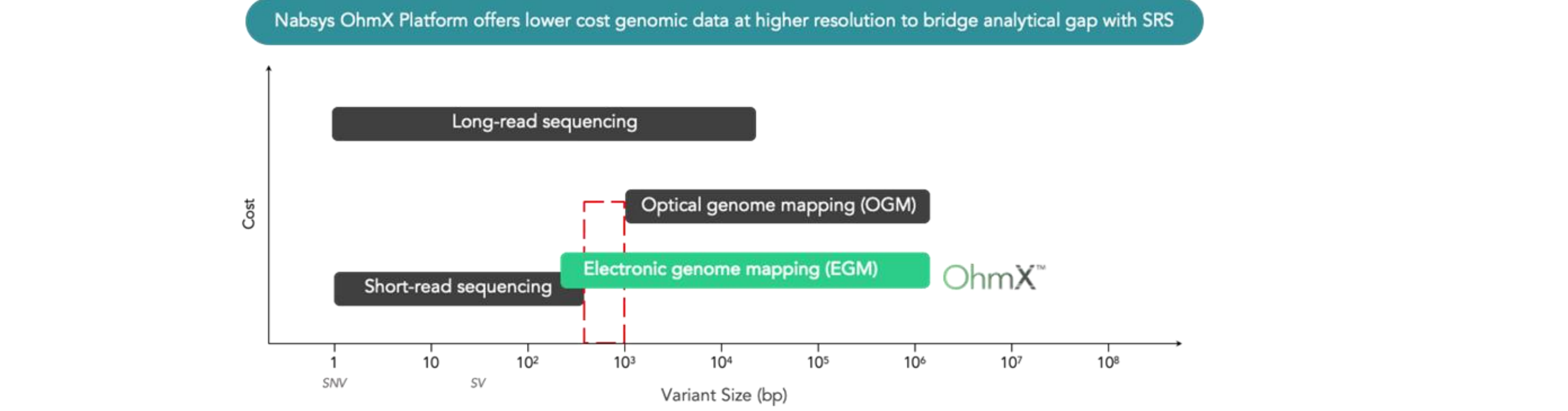


Figure 3. Genomic alteration size scale across cytogenetic and molecular methods. Cytogenetic, array-based, sequencing, and genome mapping approaches provide different views of genomic variation across alteration sizes. EGM bridges the gap between SNV and large SV detection and has the potential to be a complementary technique to sequencing and OGM.

TP53 Deletions in H1299 and Hep3B

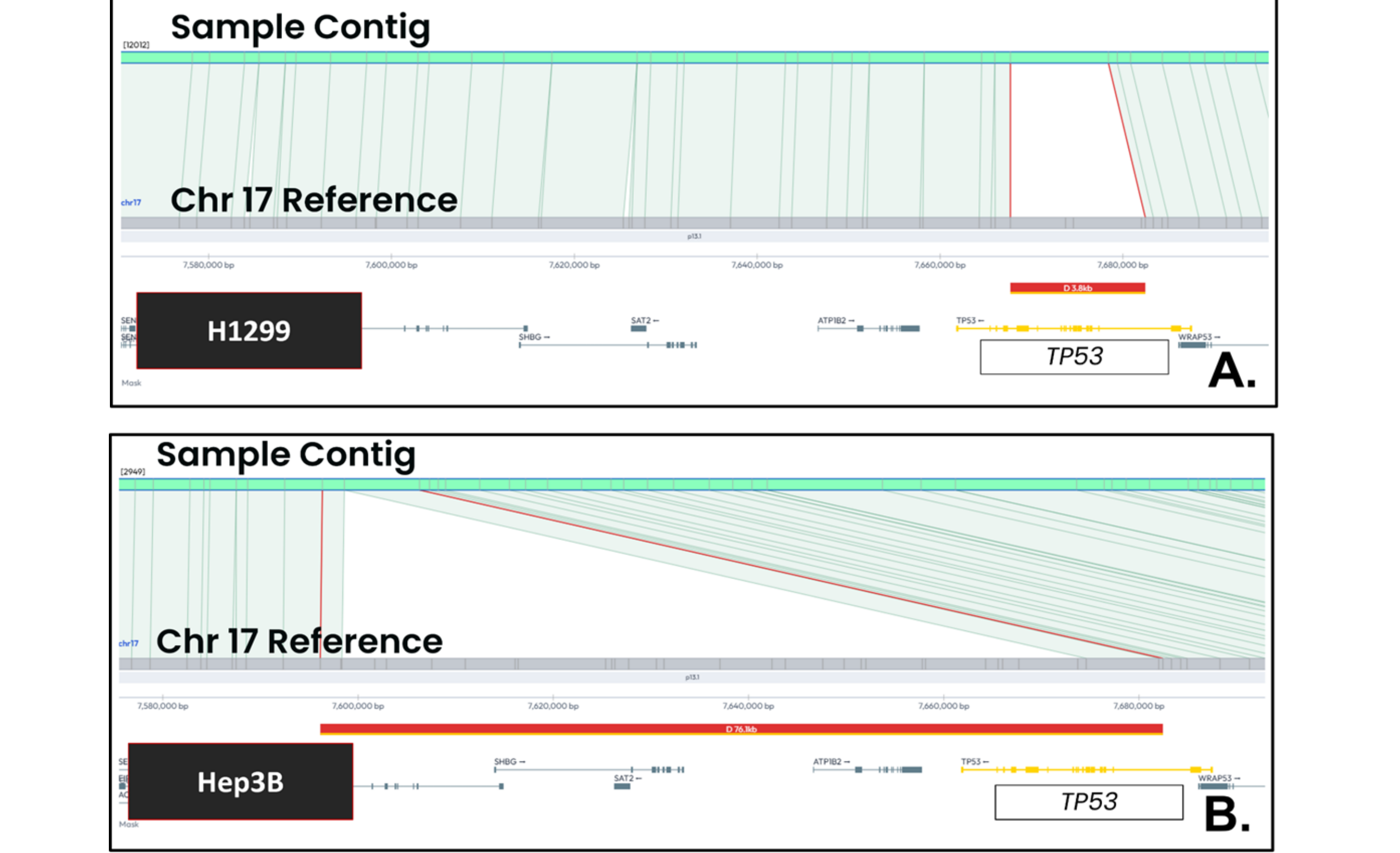


Figure 4. *TP53* deletions in H1299 and Hep3B, visualized in Nabsys Navigator SV-Discover. *TP53* gene is shown in yellow. A deletion is represented as a red rectangle below the reference genome (grey). A) Small ~3.8 kb *TP53* deletion in H1299 cell line. B) Large ~76 kb *TP53* deletion in Hep3B cell line.

Sample	Chromosome	Start	End	Length (bp)	SV Type	Zygosity
H1299	17	7,667,694	7,682,447	3,828	Deletion	Homozygous
Hep3B	17	7,596,133	7,682,447	76,165	Deletion	Homozygous

Table 1. Nabsys Navigator *TP53* SV calls. *TP53* deletions were called in H1299 and Hep3B. Start and end coordinates are breakpoints of the deletion.

Sample	Chromosome	Start	Length (bp)	SV Type	Molecules Aligned to WT	Molecules Aligned to Construct	Total Molecule	SV Frequency
H1299	17	7,667,694	3,828	Deletion	25.5	34	59.5	0.57
Hep3B	17	7,596,133	76,165	Deletion	8	90	98	0.92

Table 2. SV-Verify summary statistics for *TP53* SV calls. *TP53* deletions were called in H1299 and Hep3B.

FIPIL1::PDGFRA Fusion in EOL-1

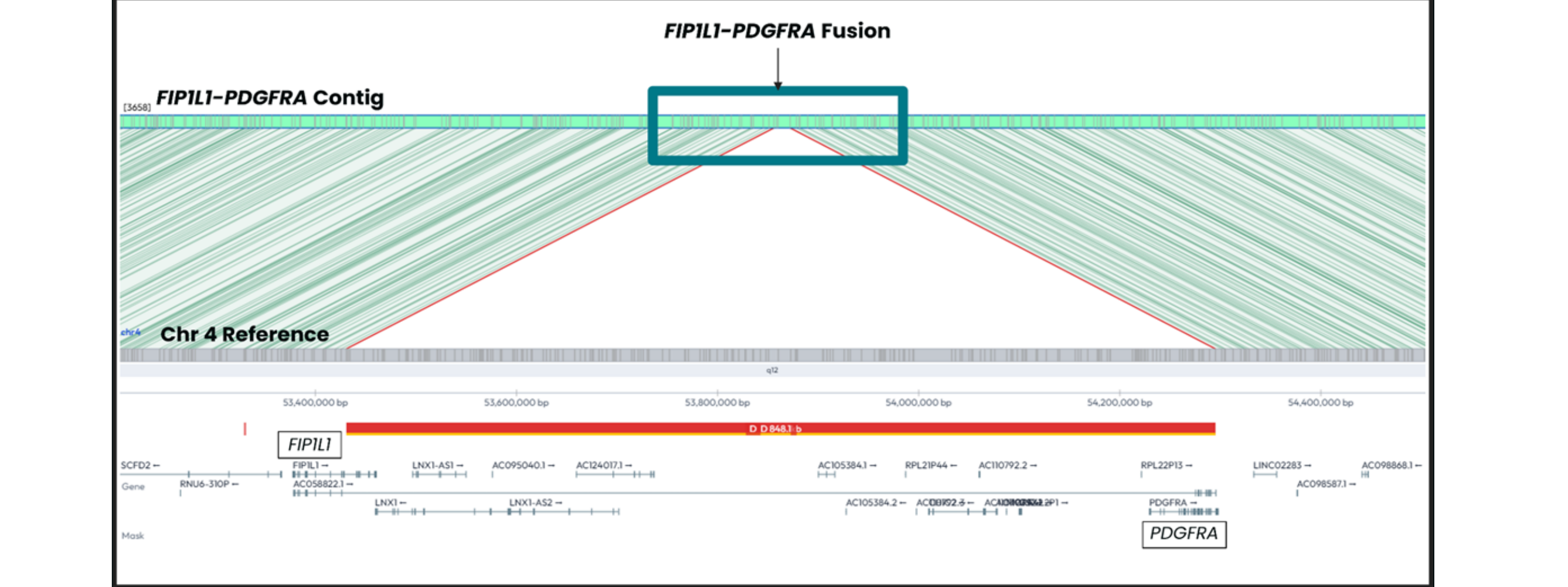


Figure 5. Deletion involving the *FIPIL1* and *PDGFRA* genes in EOL-1 cell line, resulting in the *FIPIL1::PDGFRA* fusion, visualized in Nabsys Navigator SV-Discover. The red inverted triangle marks the deletion spanning the start and end breakpoints on the reference. Exons are shown as vertical dashes along the gene transcript in the gene track. The green box highlights the contig region forming the *FIPIL1::PDGFRA* fusion resulting from an ~850 kb deletion involving the *FIPIL1* and *PDGFRA* genes.

Sample	Chromosome	Start	End	Length (bp)	SV Type	Zygosity
EOL-1	4	53,430,845	54,295,110	848,084	Deletion	Heterozygous

Table 3. Nabsys Navigator *FIPIL1::PDGFRA* SV call. Deletion involving *FIPIL1* and *PDGFRA* genes were called in EOL-1. Start and end coordinates are breakpoints of the deletion.

Sample	Chromosome	Start	Length (bp)	SV Type	Molecules Aligned to WT	Molecules Aligned to Construct	Total Molecule	SV Frequency
EOL-1	4	53,430,845	848,046	Deletion	54	81	135	0.6

Table 4. SV-Verify summary statistics for *FIPIL1::PDGFRA* SV calls. Deletion involving *FIPIL1* and *PDGFRA* genes were called in EOL-1.

KMT2A Partial Tandem Duplication in EOL-1

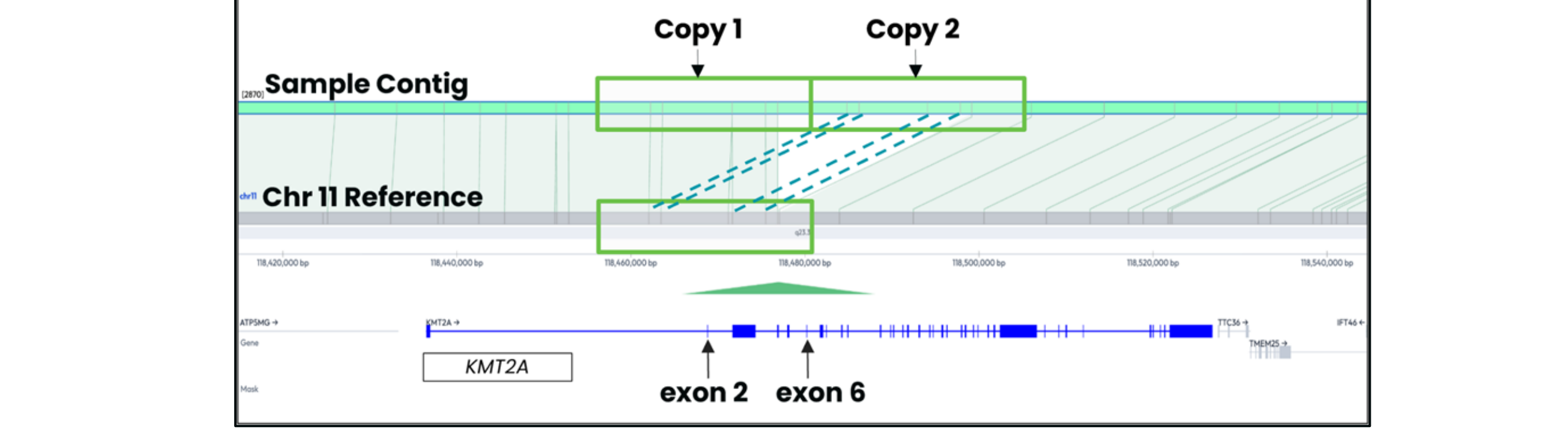


Figure 6. *KMT2A* partial tandem duplication (PTD) in EOL-1, visualized in Nabsys Navigator SV-Discover. *KMT2A*-PTD is visualized in SV-Discover as a ~22 kb insertion. The green triangle below the reference genome (grey) denotes an insertion call. *KMT2A* gene is shown in blue, and blue vertical lines or boxes in the *KMT2A* gene indicate exons along the *KMT2A* transcript. Green dashed lines and green boxes were added manually to illustrate duplication of *KMT2A* exons 2–6, consistent with prior reports.

Sample	Chromosome	Start	End	Length (bp)	SV Type	Zygosity
EOL-1	11	118,476,863	118,477,066	22,305	Insertion*	Heterozygous

Table 5. Nabsys Navigator *KMT2A*-PTD SV call. *KMT2A*-PTD was called in EOL-1 as a ~22 kb insertion. Start and end coordinates are breakpoints of the insertion.

Copy Number Detection in Hep3B

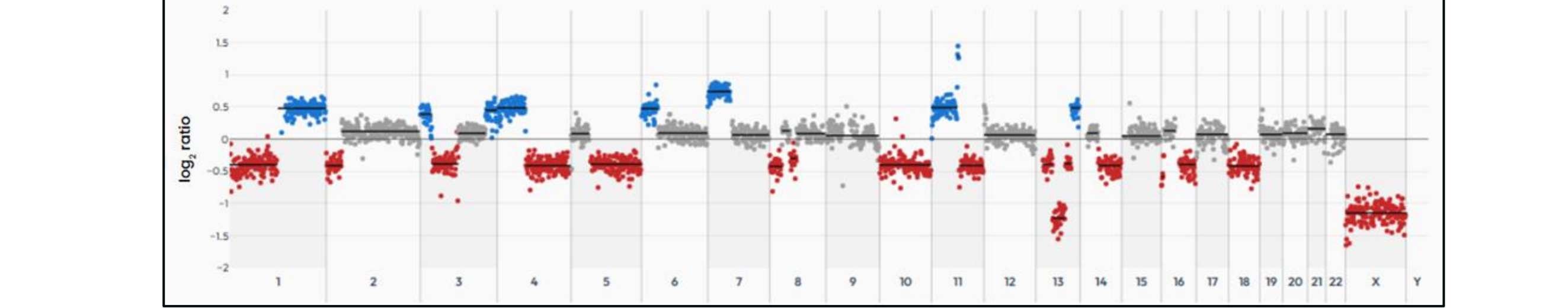


Figure 7. Detection of copy number alterations in the Hep3B cell line. Genome-wide CNV analysis of EGM data showing copy number gains (blue) and losses (red) across multiple chromosomes, consistent with known aberrations in Hep3B cell line.

Expected CN Aberrations in Hep3B	Detected via CNV Pipeline	Expected CN Aberrations in Hep3B	Detected via CNV Pipeline
gain(1q)	✓	del(1)(p13p35)	✓
gain(3)(q25q29)	✓	del(3)(p12p21)	✓
gain(4p)	✓	del(4)(q22q35)	✓
gain(4)(q12q13)	✓	del(5)(q14q35)	✓
gain(7p)	✓	del(8p)	✓
gain(11p)	✓	-10	✓
gain(11)(q12q13)	✓	del(13)(q12q31)	✓
gain(13)(q32q34)	✓	del(14)(q21q32)	✓
gain(21)(q22)	✓	del(16q)	✓
		-18	✓
		-X	✓

Table 6. CNV detection results in Hep3B cell line. A summary of copy number aberrations detected by the CNV pipeline, showing concordance with reported CN events in Hep3B cell line.
Legend:
✓ detected
– not detected

Conclusions

- EGM accurately detected various *TP53* deletions, fusions, and CNVs in human cancer cell lines with high concordance to published data and orthogonal techniques.
- EGM is a robust, high-resolution tool for detecting SVs and CNVs associated with hematological malignancies, complementing traditional cytogenetic and molecular technologies.
- Additional human blood samples are being evaluated using enhanced analytics software to further refine performance.
- The cost-effectiveness, precision, and throughput of EGM using the OhmX Platform make it a strong candidate for integration into hematopathology workflows.

References

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