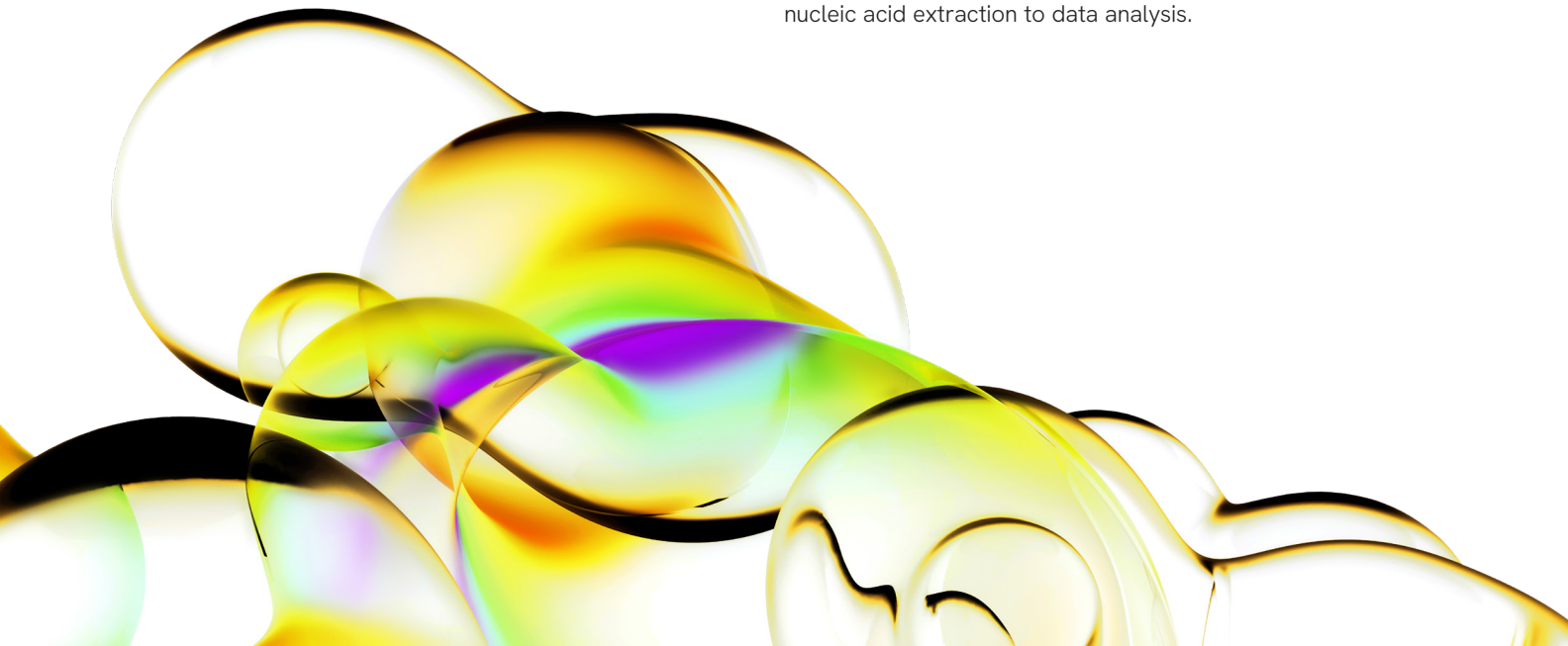
Enhancing NGS precision in oncology with Mimix Geni somatic cancer reference standards.

Introduction and Background

In 2011, the U.S. Centers for Disease Control and Prevention (CDC) brought together experts to determine how to support the integration of NGS into clinical workflows. The absence of a universal truth for comparison against clinical samples and kits led to the 2012 publication, outlining guidelines for standardisation of NGS in clinical laboratory practice[1].

This publication highlighted the need for highly characterized reference materials, prompting the launch of the Genone in a Bottle (GIAB) consortium in 2012. Under this consortium, benchmark human genome cell lines, like Han Chinese and Ashkenazi Jewish, were comprehensively characterized and then introduced as gold-standard reference material for NGS clinical workflows. However, the GIAB cell lines lacked oncology-specific reference material. To genetically edit these cells lines proved challenging, leading the Medical Device Innovation Consortium (MDIC) to convene the Somatic Reference Samples (SRS) Initiative (https://mdic.org/our-work/somatic-reference-samples/) and select Horizon Discovery (now Revvity) in 2020 for its gene editing capabilities as a development collaborator.

Reference standards are essential for validating the accuracy, sensitivity, reproducibility, and end-to-end performance assessment of NGS-based assays, especially in clinical oncology[2,3]. In particular, cell line-based formalin-fixed paraffin-embedded (FFPE) reference material play a critical role by closely mimicking the biological complexity of clinical tumour samples. With mutations embedded in their native genomic background, cell line-based FFPE standards support the comprehensive evaluation of the entire NGS workflow from nucleic acid extraction to data analysis.



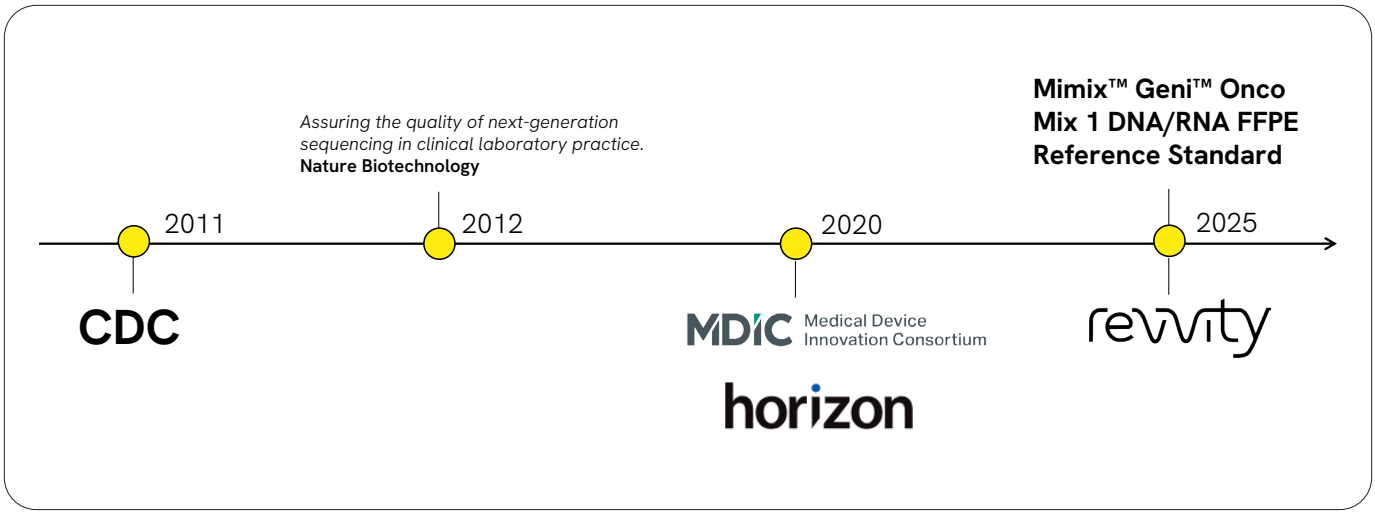


Figure 1: Timeline of key events driving the development of the Mimix Geni Reference Standard.

In 2025, the MDIC and Revvity collaboration resulted in the development of the first somatic reference standard (SRS) based on the GIAB cell line Ashkenazi Jewish Son HG002/GM24385. The Mimix™ Geni™ Onco Mix 1 DNA/RNA FFPE Reference Standard is an industry-first pan-cancer dual DNA/RNA reference material with seven mutations clinically relevant in oncology testing, as selected by the SRS Steering Committee of industry experts. The mutations were engineered by Revvity into the genome of GM24385 GIAB Consortium cell line, providing a highly characterized reference material which can be trusted to verify and monitor oncology detection assays and workflows.

Methods

Engineering Oncogenic Mutations in GM24385

Seven variants with the highest correlation to disease, representing different cancer types, mutation categories, prevalence levels, and technical difficulty, were selected by the SRS Steering Committee. Table 1 lists the selected variants. The GM24385 cell line was engineered by Revvity to independently integrate each of the seven oncogenic mutations into the genome, creating 7 cell line clones. The standards were then rigorously tested and validated by MDIC and Revvity. All seven clones were tested for the presence of edited mutations using whole genome sequencing (WGS) by MDIC. Sequencing was performed on two platforms: Illumina and PacBio. Illumina sequencing achieved an average coverage of 30X, while PacBio sequencing was performed at 40X coverage. NGS data was processed using DRAGEN™ software and PacBio HiFi somatic pipeline.

Table 1: List of Selected Variants for Engineering in GM24385

Gene	Variant	Location	Variant type	Relevant cancer
ERBB2	V659E/V659E	Chr17	MNV	NSCLC, Breast cancer
BRAF	V600E/+	Chr7	SNV	Most cancers[4]
EGFR	A763_Y764ins FQEA/+	Chr7	INDEL	NSCLC
PDGFRA	I843del/ I843_D846del	Chr4	INDEL	GIST
FGFR3	S249C/S249C	Chr4	SNV	Bladder cancer, all solid tumours
NTRK1	TPM3e7:: NTRK1e10	Chr1	FUSION	All solid tumours
RET	CCDC6e1:: RETe12	Chr10	FUSION	NSCLC, thyroid cancer

GIST: gastrointestinal stromal tumour; INDEL: insertion/deletion; MNV: multi-nucleotide variant; NSCLC: non-small cell lung cancer; SNV: single-nucleotide variant.

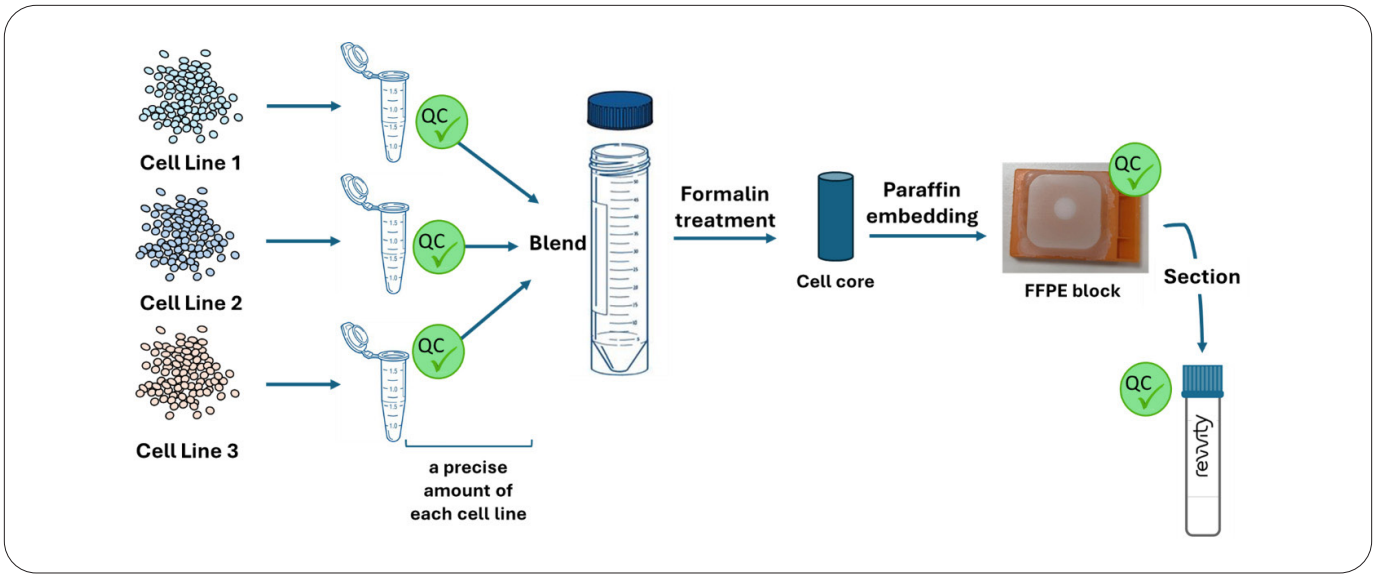


Figure 2: Workflow for creating Mimix FFPE Reference Standards.

Mimix Geni Onco Mix 1 DNA/RNA FFPE reference standard development

The Mimix Geni Onco Mix 1 DNA/RNA FFPE standard is a curl, designed to mimic the patient tissue sections. It is prepared by sectioning an FFPE block derived from the seven independently engineered GM24385 cell lines. These cell lines were combined at a fixed blending ratio after pelleting of cells from culture, followed by fixation in 4% paraformaldehyde (PFA), cell core production and paraffin embedding (See Figure 2).

Each FFPE block contains approximately $3x10^8$ cells, corresponding to 3.5 $x10^5$ cells per section. Either DNA or RNA can be extracted from individual curls of this product. Variant allelic frequency (VAF) for the seven variants and RNA expression for two fusions was validated using Illumina 600X WES and RNA-seq, respectively, by MDIC. Minimum yields for gDNA and RNA per FFPE section extracted using Promega Maxwell® and Revvity Chemagic™ 360 extraction platforms, respectively, were confirmed by Quantifluor® (Promega) and Qubit™ (Thermo Fischer Scientific) instruments. VAF and fusion RNA expression levels were further assessed using ddPCR and RT-ddPCR.

Results

Verification of engineered clones and Mimix Geni reference standard by MDIC

It is critical to confirm that the engineered variants are present and at the correct allelic frequency for validating the performance of this reference material. The presence of engineered variants in the GM24385 cell line clones was assessed using NGS by MDIC. Two sequencing platforms - Illumina 30X WGS and PacBio HiFi 40X WGS - were used for variant detection in the engineered clones. Both platforms confirmed the presence of the intended variants in their respective clones (see Table 2).

Additionally, VAF of all seven variants was assessed using Illumina 600X WES high-depth sequencing on DNA extracted from Mimix Geni Onco Mix 1 DNA/RNA FFPE sections. To evaluate the impact of cell cycle passage number on the genetic stability of these cell lines, VAF were compared between FFPE blocks prepared from cell line clones at passage 5 (P5) and passage 15 (P15). The observed VAFs for all seven variants were comparable across passages, ranging from 8% to 14%, indicating no evidence of genetic drift (see Table 2).

Table 2: NGS data confirming presence of edits and VAF in engineered clones and Mimix Geni Onco Mix 1 DNA/RNA FFPE sections.

Engineered clone	Variant type	Expected genotype	VAF in clone		VAF in Mimix Geni Onco Mix 1 (Illumina 600X WES data)		Edit confirmation
			Illumina 30X WGS	PacBio 40X WGS	P5 FFPE	P15 FFPE	
ERBB2 V659E	MNV	Homozygous	100%	100%	7.9%	10.5%	√
BRAF V600E	SNV	Heterozygous	45.7%	52.6%	9.6%	8.1%	√
EGFR A763_Y764ins FQEA	INDEL	Heterozygous	43.1%	37.5%	9.4%	8.3%	√
PDGFRA I843del	INDEL	Homozygous	100%	96.3%	9.9%	8%	√
FGFR3 S249C	SNV	Homozygous	100%	100%	10.1%	10.6%	√
TPM3-NTRK1	FUSION	Homozygous	100%	100%	11%	10.3%	√
CCDC6-RET	FUSION	Homozygous	100%	93.3%	13.8%	12.5%	√

RNA sequencing (RNASeq) was performed to evaluate fusion transcript detection and expression levels. Analysis of engineered clones and FFPE samples confirmed the presence of fusion transcripts through split, soft-clipped, and paired-end reads. Both TPM3-NTRK1 and CCDC6-RET fusions were identified, with TPM3-NTRK1 showing substantially higher read counts. Fusion transcripts were consistently detected in FFPE material with measurable transcript per million (TPM) values. TPM3-NTRK1 showed strong expression whereas CCDC6-RET showed lower expression levels.

Analytical validation data of Mimix Geni reference standard by Revvity

The Mimix Geni Onco Mix 1 DNA/RNA FFPE Reference Standard underwent extensive validation to ensure its reliability for oncology specific end-to-end NGS workflow. The team at Revvity conducted a series of analytical evaluations, including measurements of DNA and RNA yield per section, VAF determination, and fusion RNA expression analysis. These assessments were performed using Maxwell and Chemagic extraction platforms and detection technologies such as ddPCR and RT-ddPCR. The average gDNA and RNA yield obtained per section from three validation batches is shown in Figure 3.

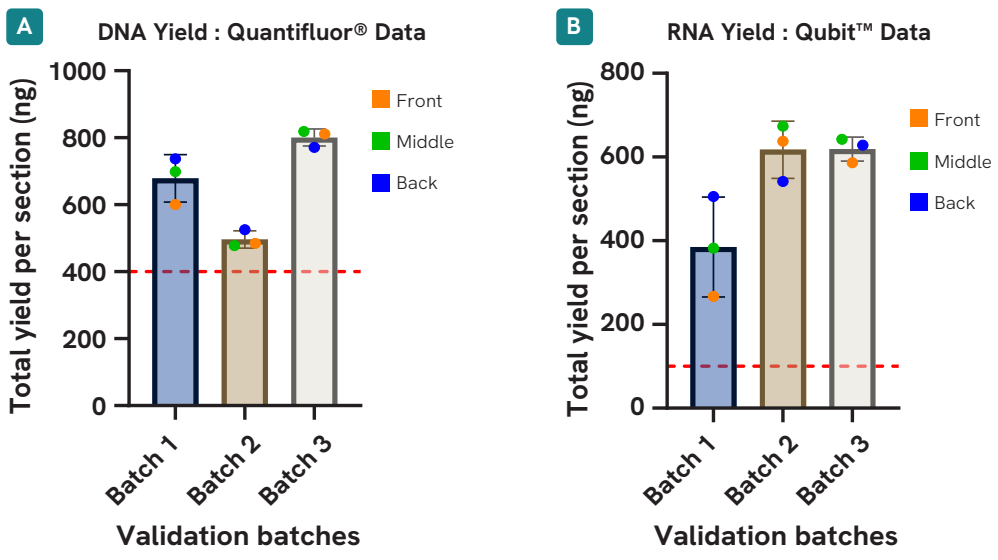


Figure 3: Average yields of nucleic acids extracted from sections taken from the front, middle, and back of FFPE blocks: **(a)** gDNA and **(b)** RNA. The red dotted line indicates the acceptance criteria of 400 ng for DNA and 100 ng for RNA. Quantification of gDNA and RNA was performed using the Quantifluor® and Qubit™ systems, respectively.

Table 3: ddPCR data confirming VAF in Mimix Geni Onco Mix 1 DNA/RNA FFPE sections for three validation batches.

Gene	Mutation	Acceptance Criteria (%AF)		Batch 1 (%VAF)	Batch 2 (%VAF)	Batch 3 (%VAF)
		Lower	Upper			
ERBB2	V659E	8.65	12.98	11.49	9.25	11.70
TPM3-NTRK1	Fusion	10.78	16.17	12.79	15.00	12.64
CCDC6-RET	Fusion	8.98	13.46	11.02	11.58	11.06
BRAF	V600E	7.80	11.70	9.48	9.86	9.91
EGFR	A763_Y764insFQEA	7.53	11.30	9.37	9.70	9.16
PDGFRA	I843del	9.21	13.81	12.40	10.61	11.52
FGFR3	S249C	8.12	12.17	10.15	9.88	10.41

The %VAF of seven DNA variants in the Mimix Geni Onco Mix 1 DNA/RNA FFPE Reference Standard was quantified using ddPCR across validation batches. For all batches, the observed %VAF values fell within the defined acceptance range, as shown in Table 3 and Figure 4. VAF in Mimix Geni Onco Mix 1 DNA/RNA FFPE Reference Standard ranged from 9% to 15%

RNA expression levels of the TPM3-NTRK1 and CCDC6-RET fusions in the Mimix Geni Onco Mix 1 DNA/RNA FFPE Reference Standard were quantified using RT-ddPCR across validation batches. As shown in Table 4, all expression data were within the acceptance criteria. The RT-ddPCR results were consistent with RNAseq data, confirming moderate expression of TPM3-NTRK1 and low expression of CCDC6-RET.

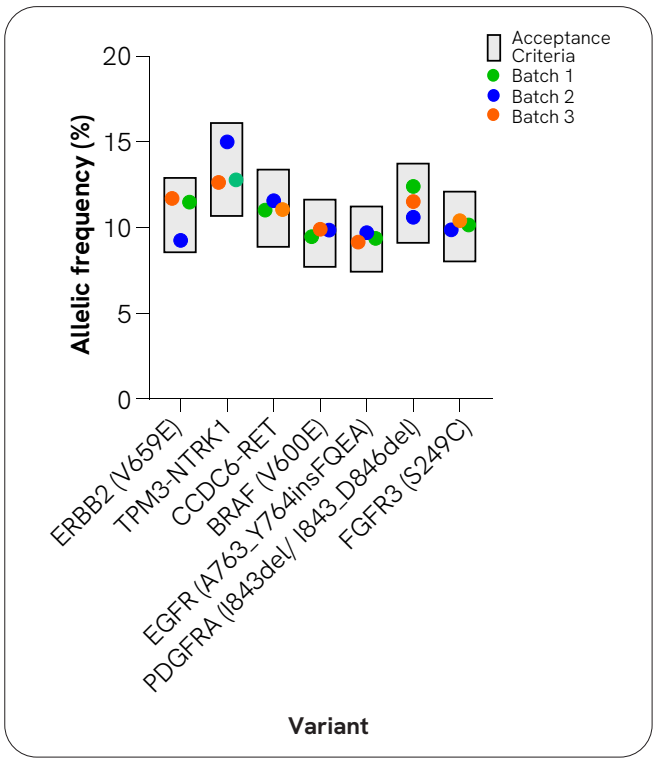


Figure 4: Graph illustrating the % AF of seven DNA Variants in the Mimix Geni Onco Mix 1 DNA/RNA FFPE Reference Standard across validation batches. % AFs were measured using ddPCR.

Table 4: RNA expression levels of fusions in Mimix Geni Onco Mix 1 DNA/RNA FFPE Reference Standard for Validation batches.

Fusion	Acceptance criteria (copies/ng)	Expression category	RNA Expression level (copies/ng)		
			Batch 1	Batch 2	Batch 3
TPM3-NTRK1	≥40	Medium	45.17	76.81	48.69
CCDC6-RET	≥4	Low	6.18	8.32	6.87

Discussion and conclusion

The collaboration between MDIC and Revvity has led to a significant development in the field of oncology reference standards. By leveraging Revvity's expertise in gene editing and reference standard development, alongside the regulatory and scientific capabilities of MDIC, the team has successfully developed the first GIAB Consortium cell line engineered to contain seven clinically relevant oncology mutations. The Mimix Geni Onco Mix 1 DNA/RNA FFPE reference standard sets a new benchmark for cancer diagnostics and NGS validation.

This reference standard highlights the need for precise editing of clinically relevant cancer variants into the well-characterised GIAB (HG002/ GM24385) cell line, followed by their transformation into FFPE DNA/RNA reference standards. As the first of its kind, this reference standard not only helps to enhance the accuracy and reliability of NGS-based oncology diagnostic tests but also fills a critical gap in the NGS validation landscape, by providing a cancer variant reference material within a deeply studied human genome background.

Beyond the technical innovations involved, this initiative exemplifies how cross-sector collaboration can drive meaningful progress in precision medicine. The resulting reference standard is poised to strengthen confidence in oncology testing and will contribute to improved outcomes.

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