SOPHiA GENETICS™

RNAtarget TECHNOLOGY

Expect more from your RNA analyses

RNAtarget Technology by SOPHiA GENETICS is a novel approach that maximizes the insights from small RNA samples. As RNA sequencing is being recognized as the method of choice to maximize fusion detection, notably in the case of *NTRK* fusion in lung cancer samples[1], we have aimed to expand RNA-based sequencing beyond fusion detection. Our technology offers novel (partner-agnostic) fusion detection combined with the ability to detect SNV/Indels (in selected genes) and unlocking the capacity to assess expression changes[2].

We have packaged this technology in a single workflow that supports all stages of the analysis; then, researchers can access the answers they seek in record time.

BENEFITS

Design

- Customizable technology
- Set up to work with as little as 10ng RNA/TNA
- Streamlined capture-based 1.5-day workflow
- Automation friendly

Analytical performance

High-performance fusion detection
- Novel fusions
- High analytical sensitivity

Ability to detect SNV/Indels
- Detection in expressed genes/regions
- Confident calling when >200 molecules are sequenced

Expression level assessment
- Supports detection of promoter fusions
- Provides additional insights for CNV calls

Supportive platform

- Intuitive and user-friendly interface
- Dedicated features for accelerated data visualization and interpretation
- Customizable reporting
- Secure storage of anonymized data
- Comprehensive QA report

Customization | Laboratory Workflow | Data Analysis | Variant Interpretation

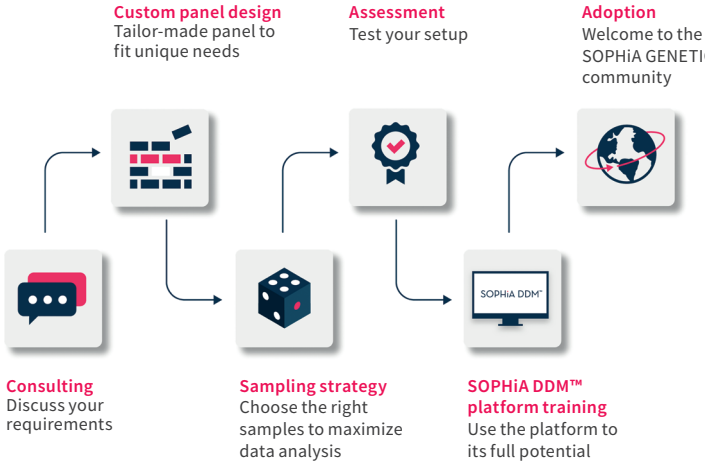

Figure 1: Overview of customization process.

Designed with customization in mind

Based on a robust probe design process and backed by numerous quality controls, we can scale and tailor our technology to your needs.

From consultative support to deployment, our trusted customization process adapts to your requirements while guaranteeing optimized chemistry and high coverage uniformity. At each step, our team of experts supports the delivery of your solution, ensuring reliable and swift adoption for the solution you envisioned (*Figure 1*).

Laboratory workflow overview:

RNAtarget Technology requires FFPE or fresh frozen tissue as starting material, and works with as low as 10ng of RNA/TNA input regardless of sample type.

RNAtarget Technology can be deployed via 1.5 days streamlined workflow that can optionally be automated using a liquid handling robot (*Table 1*).

Sample type
FFPE: No fragmentation is required; the sample is processed as is.
Fresh frozen tissue: Short and simple fragmentation (7' at 94°C in the thermocycler just before the RT step).

Starting material
RNA or TNA

Input amount
Minimum: 10ng
Recommended: 50ng
Maximum: 200ng

Quality control
DV200 (% of RNA fragments above 200bp) > 20%

Step	Hands-on time	Total time
Reverse Transcription (RT): Double Stranded cDNA Synthesis	3h30	Full day
End Repair + Adapters Ligation		
PCR: Library Amplification + QC (Concentration + Profile)		
Overnight Capture		
Capture + Amplification + QC	1h30	1/2 day
Total	**5h**	**1.5 days**

Table 1: Overview of the RNAtarget Technology laboratory workflow.

RNA sequencing is being recognized as a technology of choice for fusion detection, while its ability to detect SNVs is gaining traction over the last decade[3]. We designed RNAtarget Technology to combine those two detection capacities, keeping focus on optimal analytical performance. The RNAtarget Technology performance was evaluated in a series of analyses conducted on a base panel of 47 guideline-recommended genes *(Figure 2)*.

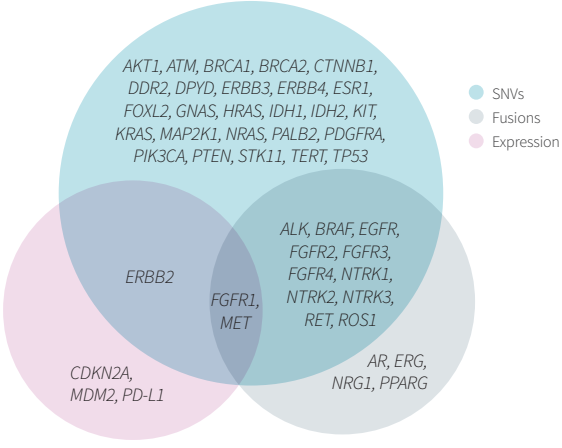

Figure 2: Base gene panel used in the RNAtarget Technology design.

Fusion detection

Unique fusion calling model for good accuracy
With RNAtarget Technology, reads are aligned to the full genome, so the fusion partner does not need to be known. Chimeric reads that align far apart or to different chromosomes are used for fusion calling. The output is then refined using a probabilistic model, which uses multiple features to reduce false positives and provide good accuracy of fusion calling[2].

More fusions detected in minimal sample input
RNAtarget Technology detects more fusions in minimal input material samples (20ng) compared to standard amplicon-based solution, with novel fusion detection capabilities. Based on analysis of SeraCare and Horizon Discovery reference standards, RNAtarget Technology detects additional fusions (CD74-ROS1, EGFR-SEPT14, METex14 and SLC34A2-ROS1) that are not detected by an amplicon solution. Please note that both panels are designed to detect all fusions shown due to partner-agnostic panel design (*Figure 3*).

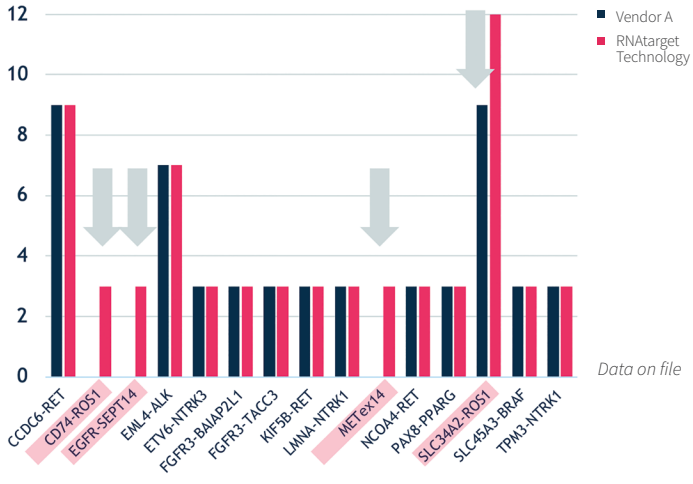

Figure 3: Comparison of RNAtarget Technology detection capacity.

Two methods for identifying exon skipping
Detection of *MET* exon 14, *EGFR* exons 2-8, and *EGFR* exons 2-7 (vIII) exon skipping:

1. Use alignment file and pull out reads that overlap the breakpoint and match the sequence on either side of the breakpoint
2. Use alignment position to find reads that support the exon-skipping for when there is a deletion or mutation near the junction

Expression imbalance assesment for additional confidence in fusion calling

Our detection model identifies expression imbalance on either side of the breakpoint *(Figure 4)*. The output is provided in a downloadable format.

Replicate	5' gene	3' gene	Molecules	Imbalance
1	*FGFR1*	*TACC1*	2	No
	EGFR	*VOPP1*	41	Yes
2	*EGFR*	*VOPP1*	24	Yes

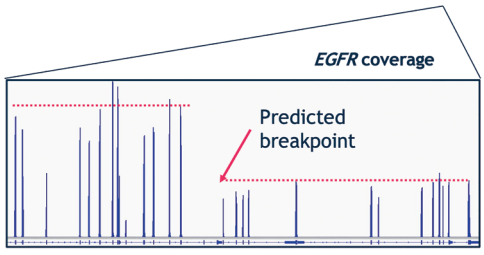

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Figure 4: Illustration of expression imbalance assessment.

SNV/Indel detection

Sophisticated SNV/Indel calling algorithm

Figure 5 illustrates how, in addition to the usual steps present in any standard variant calling algorithm (read trimming, adapter removal, alignment), the RNAtarget Technology removes:

- Deamination artefacts frequent in FFPE samples
- Background noise due to hard-to-align regions and sequencing artefacts

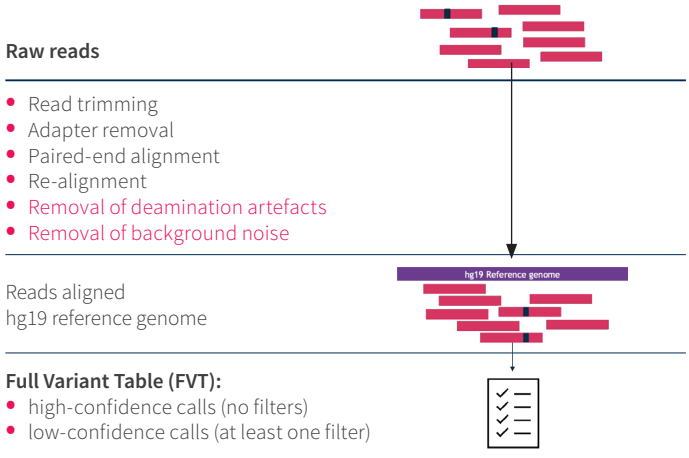

Figure 5: Illustration of the RNAtarget Technology SNV calling algorithm.

SNV/Indel calling parameters

RNAtarget Technology variant caller identifies PCR duplicates to enhance results accuracy. The analytical requirements were optimized to balance the sensitivity and specificity of our technology. The analytical parameters were carefully chosen to balance the need to remove deamination artifacts and background noise.

SNV/Indel detection confidence

SNV/Indel calling in RNA is strongly dependent on the biological level of expression of the gene of interest in the sample and/or tissue. Our SNV calling algorithm accounts for these biases. During our assesment, SNVs were able to be called with high confidence in lung cancer ESMO (Tier I and II) and NCCN guideline-recommended genes, while providing additional insights in other potentially relevant genes (*Table 2*).

SNV detection confidence	% of samples with at least 200 molecules coverage of targeted gene	Gene examples
High	>90%	*HER2, KRAS, PIK3CA, EGFR, IDH1/2, AKT1, ATM, BRAF, CTNNB1, GNAS, HRAS, MAP2K1, NRAS, PTEN, STK11, TP53*
Medium	50% - 90%	*MET, BRCA1, PALB2, DPYD, DDR2*
Low	<50%	*TERT, ESR1, BRCA2, KIT*

Table 2: Overview of SNV detection confidence for RNAtarget Technology.

SNV/Indel detection compared to other DNA and RNA-based assays

The analytical performance of our technology was assessed head-to-head with alternative DNA and RNA-based assays in analysis of confirmed variants with VAF% of at least 5% in 9 commercial characterized reference standards. Comparisons were made only considering overlapping target regions. For the RNA-based assay, 20 ng of RNA was the minimum requirement of the comparator's protocol. When comparing SNV calling to a DNA-based assay, 10ng of RNA input for RNAtarget Technology and 10ng of DNA input for comparator assay (minimal requirement) were needed. The results showed a higher sensitivity of the RNAtarget Technology compared to an alternative RNA-based assay (Figure 6). Moreover, the comparison with a DNA-based assay resulted in the detection of the majority of confirmed variants with RNAtarget Technology (Figure 7).

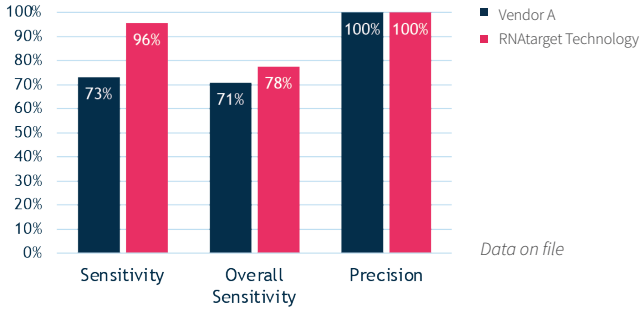

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Figure 6: Comparison of RNAtarget Technology with alternative RNA-based assay.

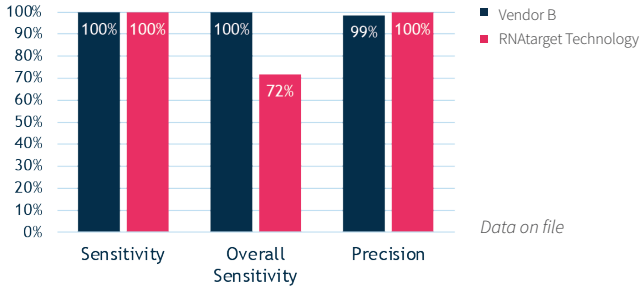

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Figure 7: Comparison of RNAtarget Technology with alternative DNA-based assay.

Beyond Analytics

Results are displayed in SOPHiA DDM™ (*Figure 8*). The platform allows immediate focus on relevant genomic alterations with several features facilitating the interpretation process:

- Fusion display and flagging.
- SNV/Indel display and flagging.
- Enhanced downloadable quality report with important indicators like control gene molecule coverage, group size (based on PASS variants), and on-target and on-flank bases.

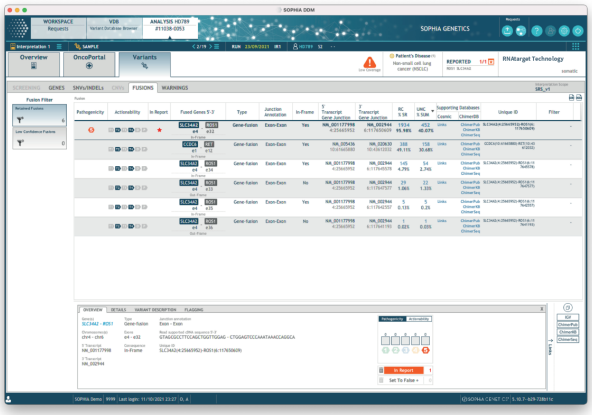

Figure 8: Fusions visualisation in SOPHiA DDM™.

About SOPHiA GENETICS

SOPHiA GENETICS (Nasdaq: SOPH) is a healthcare technology company dedicated to establishing the practice of data-driven medicine as the standard of care and for life sciences research. It is the creator of the SOPHiA DDM™ platform, a cloud-based SaaS platform capable of analyzing data and generating insights from complex multimodal data sets and different diagnostic modalities. The SOPHiA DDM™ platform and related solutions, products and services are currently used by more than 780 hospital, laboratory, and biopharma institutions globally.

Want to know more?

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References:
1. Harada G, Santini FC, Wilhelm C, Drilon A. NTRK fusions in lung cancer: From biology to therapy. Lung Cancer. 2021 Nov;161:108-113.
2. Data on file - SOPHiA GENETICS 2021.
3. Heyer EE et al. Diagnosis of fusion genes using targeted RNA sequencing. Nat Commun. 2019 Mar 27;10(1):1388.