SarcomaFusion Detection

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CE IVD

Introduction

Sarcomas are a form of cancer that develops in the body's connective tissues, such as bone, muscle, cartilage, fat, blood vessels, or lymphatic tissue. Unlike carcinomas, which develop in epithelial cells, sarcomas tend to be rarer and can be more difficult to diagnose and treat. Diagnosis of sarcoma often requires a combination of medical imaging, biopsy and histological analysis to determine the type of cancer cells present. Treatment may include surgery, radiotherapy, chemotherapy, immunotherapy or a combination of these options, depending on the type and stage of the sarcoma. Close follow-up is usually required to monitor response to treatment and detect any early recurrence.

In this complex context, our brochure is positioned as an innovative solution for sarcoma detection.

The SarcomaFusion test delivers fast, accurate results, even from small quantities of tumor samples. By providing detailed characterization of sarcomas at the molecular level, this test paves the way for targeted, individualized treatments, improving treatment prospects for patients affected by these rare and complex cancers.

SarcomaFusion

For the detection of fusion transcripts

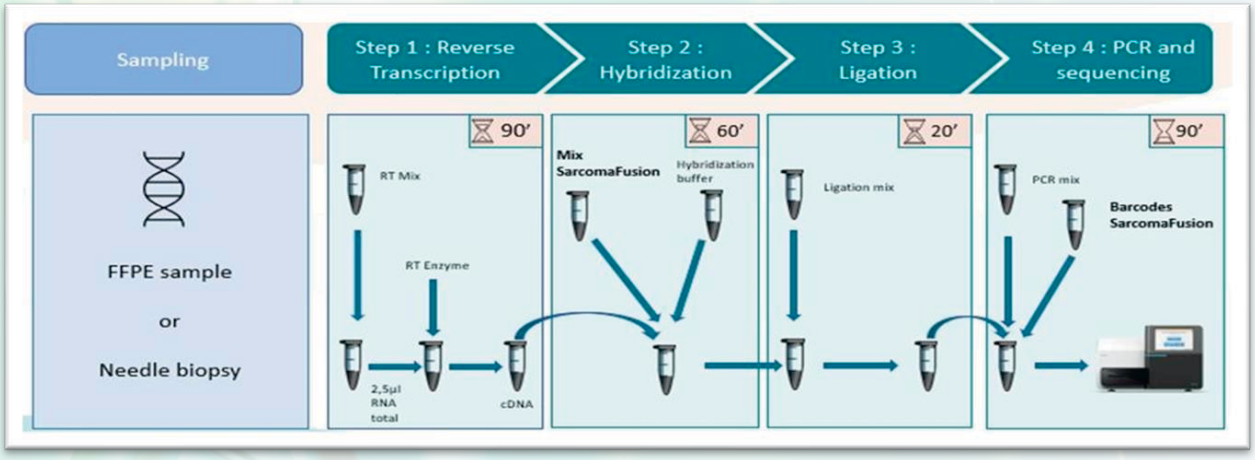
A unique test, based on patented ligation-dependent PCR technology, allows the pathologist or molecular biologist to detect fusion transcripts, from a fresh, frozen, or paraffin-embedded sample, among 140 fusion transcripts associated with sarcomas in only one reaction.

The simplicity of the test protocol makes it possible to obtain the results of the analysis from the tumor RNA in 48 hours.
Since the amount of tumoral RNA required is very small, a needle biopsy is sufficient to obtain results. The SarcomaFusion test is extremely robust and sensitive.

The detection and quantification of these fusion transcripts are made possible by combining molecular biology and **high-throughput sequencing**. The data obtained is analyzed using our **RT-MIS platform**.

RT-MLPSeq - a simple and fast technique

The **SarcomaFusion** test uses the **RT-MLPSeq** method.
The multi-step *in vitro* test simultaneously evaluates a large number of **genetic markers** (chromosomal translocations) using pairs of specific oligo-nucleotide probes for each of them.

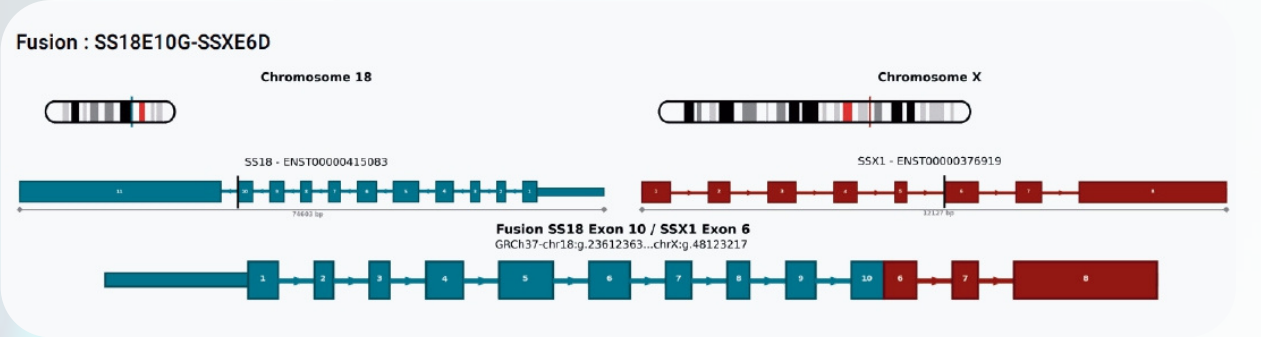


- The *In vitro* consists of 4 steps with a total duration of approximately 1/2 day including 2h to 2h30 hands-on
- NGS sequencing of the SarcomaFusion test requires only 100,000 reads per sample
- SarcomaFusion can be sequenced with other libraries and the barcodes are provided with the kit

Post sequencing analysis using dedicated software

After sequencing, the **FASTQ** file is loaded onto the **RT-MIS** platform which carries out demultiplexing, identification and quantification of any fusion transcripts.

RT-MIS delivers in a few minutes a **complete analysis** of the sequencing results, from the raw data (number of reads and Unique Molecular Identifiers (UMI)) to the **bibliography associated** to the transcript.



Characteristics

- **1/2 day of manipulation**
- **Low RNA quantity needed**
- **Suitable for FFPE samples**
- **Sensitive thanks to short probes**
- **Sequencing with other libraries possible**
- **Increased specificity thanks to UMI**
- **100,000 reads are sufficient**
- **Bioinformatic analysis included**
- **Access to complete raw data**
- **Needle biopsie possible**

Application domain	Fusion transcript detection		
Handling duration	5h30	Actual working time	1h-1h30
Type of samples	Fresh, frozen or fixed and paraffin-embedded tissue biopsies		
Input quantity	Between 50 and 500ng of RNA in a volume of 2,5µL		
Contents of the reagent kit	Probes targeting 140 fusion transcripts, barcodes, sequence primers		
Material compatibility	Sequencer Illumina®		

SarcomaFusion KIT	Cond.
GEP-SF08	8 reactions
GEP-SF16	16 reactions
GEP-SF24	24 reactions
GEP-SF48	48 reactions

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Please read the instructions carefully.
For in vitro diagnostic use.
For professional use only.

Experience our products firsthand by watching our informative videos to learn how they work. Click now to discover more.

Frequently Asked Questions :SarcomaFusion test

1. Which genes are targeted in the test?
2. What regions are targeted with the kit probes?
3. What method can I use to quantify my starting RNA?
4. What is the starting RNA recommendation? For an FFPE sample?
5. What is the effect of residual DNA contamination?
6. What control must we use?
7. What are the available kits and how many analyses can you get?
8. How many barcodes can you use in each kit?
9. What is the breakpoint in this protocol?
10. How long does it take to obtain libraries before the purification step?
11. What is the recommended Library Read size?
12. Which sequencer can I use for the obtained libraries with the *in vitro* test?
13. What workflow is needed to create a sample sheet using Illumina Experiment Manager?
14. What workflow is required to create a template with Local Run Manager?
15. What sequencing primers do you need?
16. Should we always use Phi? If so, what percentage?
17. What is the loading concentration of the libraries?
18. How many reads does the sample require for precise detection?
19. What software can analyze the SarcomaFusion test result?
20. What is the Mitelman database?
21. How many reads associated with a potential fusion are necessary for the fusion to be determined as such?
22. Why do some detected fusions not have any associated references?
23. Where can I find details on the sample results?
24. Do I need to target the 2 genes to detect a gene fusion?

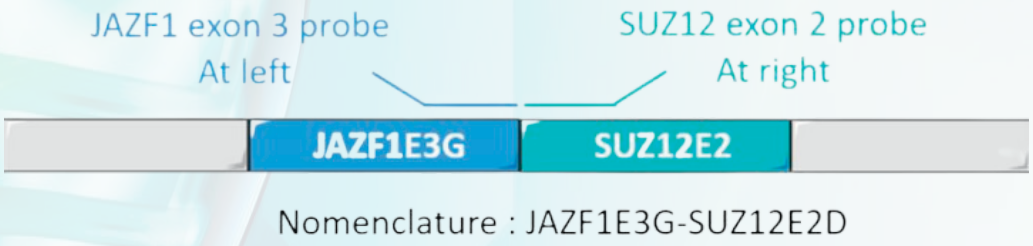
1. Which genes are targeted in the test?

The targeted genes are:

A2M	BRD8	COL3A1	DDIT3	ETV1	FOXO1	KIF5B
ACTB	CAMTA1	COL6A3	DVL2	ETV4	FUS	KLF17
AFF3	CARS	CREB1	EEF1G	ETV6	HAS2	LIFR
ALK	CCBL1	CREB3L1	EGF	EWSR1	HEY1	LMNA
ARL1	CCNB3	CREB3L2	EMILIN2	FEV	HMGA2	MAML2
ASPSCR1	CDX1	CREBBP	EML4	FGF1	HSPA8	MAML3
ATF1	CITED2	CREM	EP400	FGFR1	IRF2BP2	MEAF6
ATIC	CLTC	CRTC1	EPC1	FLI1	JAZF1	MEIS1
BCOR	COL1A1	CTNNB1	EPC2	FN1	KHDRBS1	MET
BCORL1	COL1A2	DCTN1	ERG	FOSB	KIAA2026	MKL2
MSN	NTRK1	PBX3	RBPMS	SRF	TFCP2	WWTR1
MXD4	NTRK3	PDGFB	RELA	SS18	TFE3	YAP1
MYH9	NUDT11	PDGFD	ROS1	SS18L1	TFG	YWHAE
NAB2	NUTM1	PHF1	RREB1	SSX1	THBS1	ZC3H7B
NCOA1	NUTM2A	PLAG1	RUNX2	STAT6	TPM3	ZFP36
NCOA2	OPHN1	POU5F1	SFPQ	STRN	TPR	ZNF444
NFATC1	PAFAH1B1	PPFIBP1	SMAD3	SUZ12	USP6	
NFATC2	PATZ1	PRDM10	SMARCA5	TAF15	VCL	
NONO	PAX3	RAD51B	SP3	TCF12	VGLL2	
NR4A3	PBX1	RANBP2	SQSTM1	TEAD1	WT1	

2. What regions are targeted with the kit probes?

Probes target the end or beginning of an exon of interest



3. What method can I use to quantify my starting RNA?

The recommended method for quantifying RNA is fluorimetry. Other techniques can be used, such as spectrometry or the determination of the RING.

4. What is the starting RNA recommendation? For an FFPE sample?

The starting amount should be between 50 and 500 ng of RNA, even FFPE. It is recommended to use the Promega Maxwell® RSC RNA FFPE kit (Promega, ref AS1440 and AS4500).

5. What is the effect of residual DNA contamination?

A DNA contamination can induce an underestimated quantity of starting RNA. But contamination will have no impact on the technique since it is based on fixing the probes at the exon-exon junctions, except for mutation points.

6. What control must we use?

The SarcomaFusion test contains an internal control with the GAPDH gene (exon 3-exon 4) by performing the corresponding PCR in parallel with your samples.

Remember to use the same GAPDH barcode number as the classic barcode of your analysis.

During computer analysis, RT-MIS combines the raw data from both barcodes to generate a single report.

The sample with the control barcode should be sequenced like a normal sample with 100,000 reads.

However, its use has no impact on the scan count.

7. What are the available kits and how many analyses can you get?

The kit can contain:

	Reagent kit - U = number of analyses			
	8U	**16U**	**24U**	**48U**
GEP-SFPM probe mix	30 µL	48 µl	54 µl	108 µL
Barcodes **GEP-BC-xxx (from 001 to 032 depending on number of analyses purchased)** BC=barcode	8 BC N°017 to 024 5µL/BC	8 BC N°001 to 008 8µL/BC	12 BC N°021 to 032 6µL/BC	24 BC N°001 to 024 6µL/BC
GEP-SP-001 sequencing primers	96 µL	144 µL	180 µL	360 µL
For the internal control				
GAPDH barcodes **GEP-BCC-xxx (from 001 to 032 depending on number of analyses purchased)** BCC=control barcode	8 BCC N°017 to 024 5µL/BCC	8 BCC N°001 to 008 8µl/BCC	12 BCC N°021 to 032 6µL/BCC	24 BCC N°001 to 024 6µL/BCC
GAPDH sequencing primers **GEP-SP-002**	96 µL	144 µL	180 µL	360 µL

8. How many barcodes can you use in each kit?

For a kit of 8 analyses, 8 different barcodes are provided. For each kit of more than 8 analyses, each barcode will be used twice (for 2 different analyses).

9. What are the breakpoints in this protocol?

Breakpoints can be issued at the end of the following steps:

- Reverse transcription
- Ligation
- PCR
- Purification

They are indicated by the following symbols:
During breakpoint, samples must be stored between -30°C et -15°C.

10. How long does it take to obtain libraries before the purification step?

Libraries' obtention will go through different steps:

- Reverse transcription.
- Probes hybridization.
- Probes ligation and PCR amplification.

All these steps will last around 4 hours.

11. What is the recommended Library Read size?

The smallest recommended size is 120 pb.

12. Which sequencer can I use for the obtained libraries with the *in vitro* test?

The tests are optimized for Illumina MiSeq et NextSeq 500/550 sequencer.
During the PCR step, the Illumina technology-specific adapters are added to the ligation products.

13. What workflow is needed to create a sample sheet using Illumina Experiment Manager?

NextSeq sequencer	NextSeq sequencer
• Category: Other • Application: FASTQ only • Library Prep Workflow: Illumina DNA Prep • Index Read: 0 (None) • Read Type: Single Read • Cycles Read 1: 120 • Workflow-Specific Setting: • Select: Custom Primer for Read 1 • Uncheck: Use Adapter Trimming	• Library Prep Workflow: Illumina, DNA Prep • Index Read: 0 (None) • Read Type: Single Read • Cycles Read 1: 120 • Workflow-Specific Setting: Uncheck Use Adapter Trimming

14. What workflow is required to create a template with Local Run Manager?

After downloading the template, Fill in the file with the following information:

- IndexStrategy: NoIndex
- ReadType: Single
- DefaultReadLenght1: 120

15. What sequencing primers do you need?
The sequencing primer is included in the SarcomaFusion kit (GEP-SP-001 for the probes mix and GEPSP-002 for the GAPDH probes).

16. Should we always use Phi? If so, what percentage?
Illumina recommends phiX use for internal control in order to check the run functioning.
We
recommend at least the use given by Illumina (1% for most of the libraries).

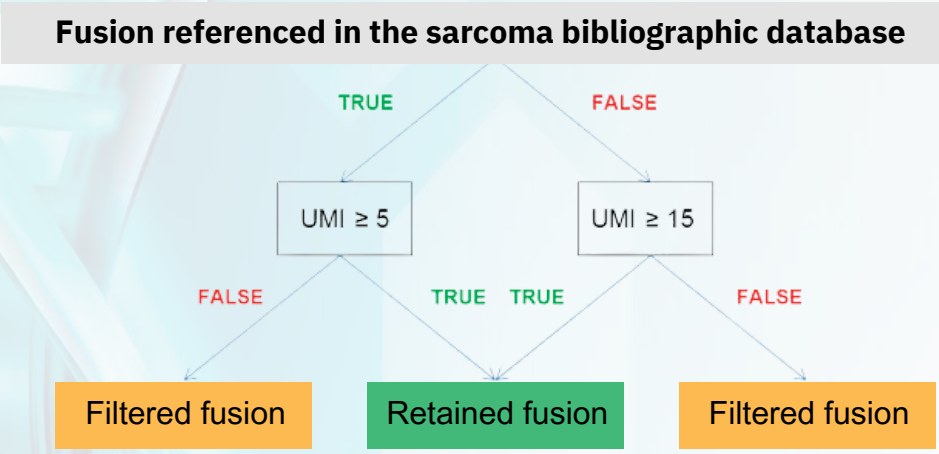
17. What is the loading concentration of the libraries?
- Miseq: 8 to 10 pM
- NextSeq 500/550: 0.8-1 nM

18. How many reads does the sample require for precise detection?
Only 100 000 reads per sample are necessary.

19. What software can analyze the SarcomaFusion test result?
The NGS results from the SarcomaFusion test can be quickly and completely analyzed using the RT-MIS software. It establishes the fusion(s) found in the sample connected to the sarcoma and the literature related to it.
No other software enables results analysis.

20. What is the Mitelman database?
This is the reference database for common mergers. It was used to create our bibliography.

21. How many reads associated with a potential fusion are necessary for the fusion to be determined as such?
The pairs of probes detected correspond to potential genetic anomalies. They are compared to the bibliographic database. The objective is to determine whether the fusion detected has already been described in the literature in the context of sarcomas or not. Depending on this referencing, the merge is kept or filtered according to the algorithm below:



22. Why do some detected fusions not have any associated references?

These fusions are either:

- A fusion associated with another pathology
- A fusion not referenced in the associated bibliography
- Background noise

23. Where can I find details on the sample results?

At the time of uploading all the documents in the analysis detail, the uploaded documents are:

- The summary PDF generated
- The Excel file with the number of counts by reads and by UMI

24. Do I need to target the 2 genes to detect a gene fusion?

Yes, the test contains a defined number of probes specific to target genes. The destination of the test is the detection of fusion transcripts previously known in the literature. It is not intended to discover new fusions.

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Version of 19/02/2024