LCD-array for molecular diagnosis of Synovial Sarcoma

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Aims: Synovial sarcoma (SS) is an aggressive, highly malignant tumors and account for 7 to 10% of all human soft-tissue sarcomas. SS is mainly located in the extremities in the vicinity of large joints, and occurs most frequently in adolescents and young adults. It is characterized by the chromosomal translocation t(X;18) (p11.2;q11.2), which results in the fusion of the SYT gene on chromosome 18 with a SSX gene on chromosome X. In the majority of cases, SYT is fused to exon 5 of SSX1 (64%), SSX2 (36%), or, rarely, SSX4. These translocations have been reported in 89–96% of cases, are apparently specific for SS and have proved to be a valuable tool for confirming the diagnosis of SS in cases where the microscopic features are not typical.

We used new LCD-array (Sarcoma 1.5 and SYT-SSX 2.0) to improve the molecular analysis of SS and to evaluate a low cost density Array (LCD-Array; Chipron, Berlin / Germany) for molecular analysis of different types of sarcomas.

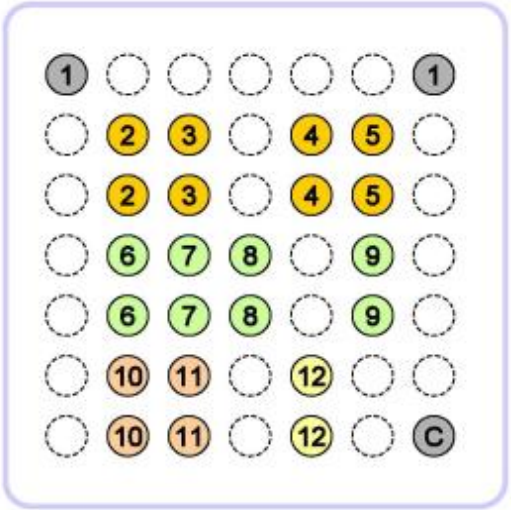
Methods: In this study, we conducted a reverse transcription-polymerase chain reaction-based (RT-PCR) assay to detect the primary fusion transcript type (SYT-SSX) using formalin-fixed, paraffin embedded (FFPE) tissues and compared the results by using LCD-array. Because it is the first attempt to analyse SS by using LCD-array, we chose 21 samples which gave positive results for FISH and RT-PCR.

One LCD-Array contains eight identical microarrays, separated in pre-structured reaction chambers. Each chamber is designed to detect different mutations of sarcomas.

Sarcoma 1.5: There are 11 capture probes for the most frequent mutations in sarcomas. Two probes represent SYT-SSX fusion point and one probe harbour the sequence for the SSX-1 (3'-fusion part). Other probes represent different mutations for different sarcomas.

Array-Pattern

Sarcoma 1.5
CSNr.: 0228



Nr.	ID	Spezifität
2	EWS	5'-Fusion Part
3	TLS/FUS	5'-Fusion Part
4	PAX-3	5'-Fusion Part
5	PAX-7	5'-Fusion Part
6	FLI-1 - B	3'-Fusion Part
7	ERG	3'-Fusion Part
8	CHOP	3'-Fusion Part
9	FKHR	3'-Fusion Part
10	SYT-SSX	Fusion Point
11	SYT-SSX	Fusion Point
12	SSX-1	3'-Fusion Part
1	Hyb-C	Hyb-Control

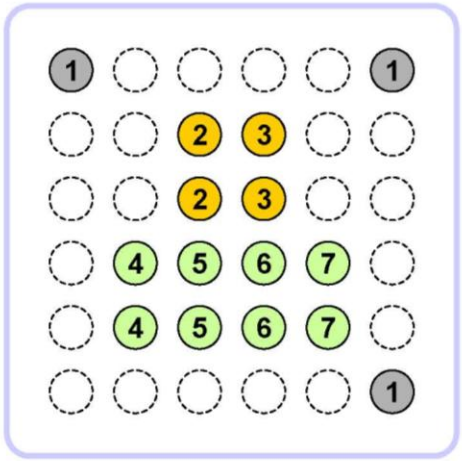
SYT-SSX 2.0: There are 6 capture probes for type of fusion in SS. Two probes represent SYT-SSX fusion point (A and B) and four probes harbour the sequence for the SSX-1, SSX-2 A, SSX-2 B and SSX-4.

Array-Pattern

chipron

SYT-SSX 2.0

CSNr.: 0286



Nr.	ID	Spezifität
1	Hyb-C	Hyb-Control
2	SYT-SSX FUS-A	Fusionssonde
3	SYT-SSX FUS-B	Fusionssonde
4	SSX-1	Dist. SSX-1
5	SSX-2 A	Dist. SSX-2
6	SSX-2 B	Dist. SSX-2
7	SSX-4	Dist. SSX-4

*** PCR amplification for fusion gene SYT-SSX was performed with 3 - 5 µl of DNA in 25 µl reaction volume. Hybridizations were performed according to the manufacturer's instructions.

Results: In total, 21 synovial sarcomas with a SYT-SSX fusion gene were correctly identified by the LCD-Arrays. Eleven of 21 were monophasic SS and 10 were biphasic SS.

LCD-ARRAY REPORT Sarcoma 1.5 2010-0030 Field 3 chipron

General Information

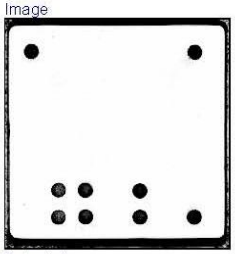
User:	default
Date:	16.03.2010
CS-Nr.:	00228
Chip-Lot:	002
Chip-ID:	008

Sample: T 983 / 07

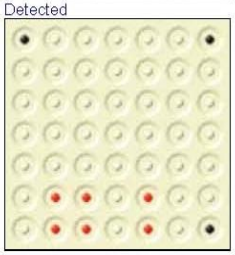
Name 1:
Name 2:
Name 3:
Name 4:
Name 5:

Image path: SYT-SSX _ 16 März 2010.tif

Image



Detected



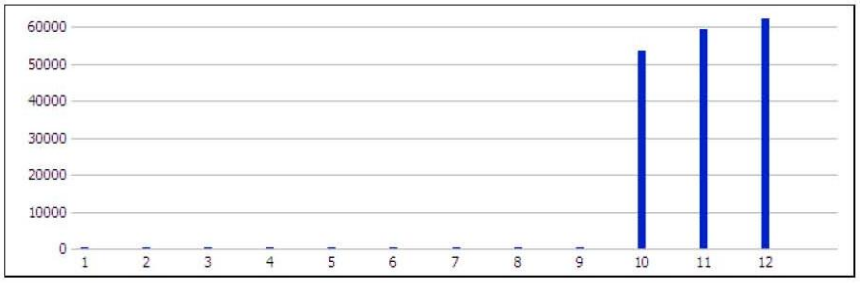
Results

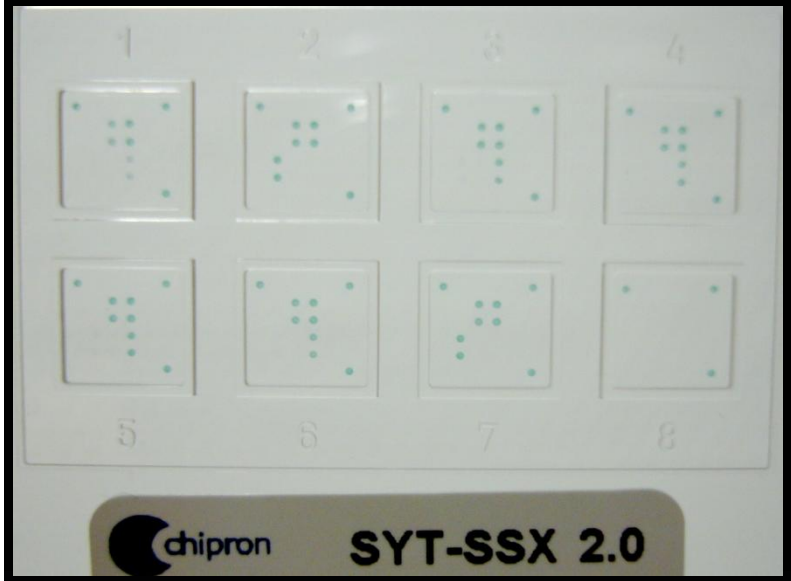
Number	Identity	Value
12	SSX-1 3´	62002
11	SYT-SSX B	59093
10	SYT-SSX A	53327
Empty	Empty	0

Treshholds

User Defined Features

Action	Features Effected
Set Positive	
Set Negative	





LCD-ARRAY REPORT **SYT-SSX 2.0** **2010-0032** **Field 2** chipron

General Information

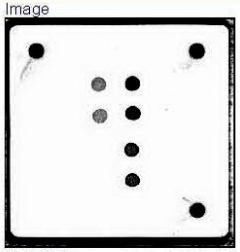
User:	default
Date:	18.03.2010
CS-Nr.:	00286
Chip-Lot:	001
Chip-ID:	001

Sample: T 2131 / 08

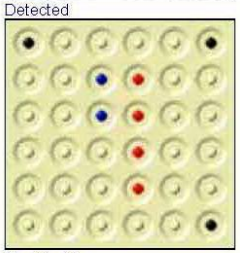
Name 1:	
Name 2:	
Name 3:	
Name 4:	
Name 5:	

Image path: SYT-SSX 2.0 - 18 März 2010.tif

Image



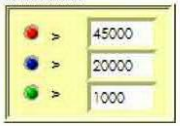
Detected



Results

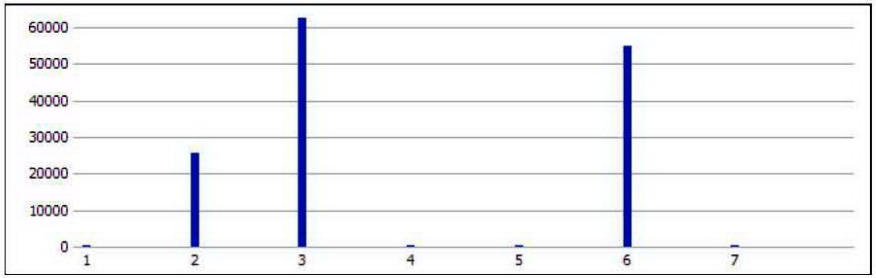
Number	Identity	Value
3	SYT-SSX FUS-B	62154
6	Dist. SSX-2 B	54318
2	SYT-SSX FUS-A	25090
Empty	Empty	0

Treshholds



User Defined Features

Action	Features Effected
Set Positive	
Set Negative	



Conclusions: As we gain information regarding the clinical and a treatment implication of the presence of particular chromosomal translocations and chimeric genes, LCD-Array is an easy, rapid and convenient method to characterise and diagnosis SYT-SSX mutation in SS and it will become ever more valuable and necessary for the diagnosis and classification of sarcomas.