

Supplemental Table S1. Breakdown of type of sample used for NanoString sarcoma panel testing per sarcoma fusion.

Tumour entities	Translocation	Sarcoma Fusion	Tested Samples	RT-PCR	NanoString Frozen	NanoString FFPE	NanoString Rapid Extraction
Alveolar rhabdomyosarcoma	t(1;13)(p36;q14)	PAX7(Exon7)-FOXO1(Exon2)	Patient 1	●	●		●
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14)	PAX3(Exon7)-FOXO1(Exon2)	Patient 2	●		●	
			Patient 3	●	●		
			Patient 4	●		●	
			Patient 5	●	●		
Ewing sarcoma	t(2;22)(q33;q12)	EWSR1(Exon7)-FEV(Exon2)	Patient 6	●	●		
Ewing sarcoma	t(7;22)(p22;q12)	EWSR1(Exon7)-ETV1(Exon12)	Not tested				
Ewing sarcoma	t(11;22)(q24;q12)	EWSR1(Exon7)-FLI1(Exon6)	Patient 7	●		●	
			Patient 8	●		●	
		EWSR1(Exon7)-FLI1(Exon5)	Patient 9	●	●		
			Patient 10	●	●		
			Patient 11	●		●	
			Patient 12	●	●		●
		Patient 13	●		●		
EWSR1(Exon10)-FLI1(Exon5)	Not tested						
EWSR1(Exon7)-FLI1(Exon8)	Patient 14	●		●			
Ewing sarcoma	t(17;22)(q12;q12)	EWSR1(Exon7)-ETV4(Exon9)	Not tested				
Ewing sarcoma	t(21;22)(q22;q12)	EWSR1(Exon7)-ERG(Exon8)	Patient 15	●	●		
			Patient 16	●			●
		EWSR1(Exon7)-ERG(Exon11)	Patient 17	●		●	
			Patient 18	●		●	
		EWSR1(Exon10)-ERG(Exon8)	Patient 19	●	●		
		EWSR1(Exon7)-ERG(Exon9)	Patient 20	●		●	
EWSR1(Exon7)-ERG(Exon10)	Not tested						
BCOR-CCNB3 (Ewing-like) Sarcoma	inv(X)(p11.2p11.4)	BCOR(Exon15)-CCNB3(Exon5)	Patient 20	●		●	
			Patient 21	●		●	
Primitive round cell sarcoma	t(4;19)(q13;p13)	CIC(Exon20)-DUX4(Exon1)-KSM2007-Ins	Not tested				
		CIC(Exon20)-DUX4(Exon1)-GC2012-Del	Not tested				
		CIC(Exon20)-DUX4(Exon1)-SK-Del	Not tested				
Synovial sarcoma	t(X;18)(p11;q11)	SS18(Exon10)-SSX1/2(Exon6)	Patient 22	●	●		●
			Patient 23	●	●		
			Patient 24	●		●	
			Patient 25	●		●	
			Patient 26	●	●		
Angiomatoid fibrous histiocytoma	t(2;22)(q33;q12)	EWSR1(Exon7)-CREB1(Exon7)	Patient 27	●	●	●	
Angiomatoid fibrous histiocytoma	t(12;16)(q13;p11)	FUS(Exon5)-ATF1(Exon5)	Not tested				
Angiomatoid fibrous histiocytoma	t(12;22)(q13;q12)	EWSR1(Exon7)-ATF1(Exon5)	Patient 28	●		●	
		EWSR1(Exon8)-ATF1(Exon4)	Patient 29	●	●		
Infantile/congenital fibrosarcoma	t(12;15)(p13;q25)	ETV6(Exon5)-NTRK3(Exon15)	Patient 30	●	●	●	
			Patient 31	●	●		
Desmoplastic small round cell tumour	t(11;22)(p13;q12)	EWSR1(Exon7)-WT1(Exon8)	Patient 32	●		●	
			Patient 33	●		●	
		EWSR1(Exon9)-WT1(Exon8)	Not tested				
Inflammatory myofibroblastic tumour	t(1;2)(q22;p23)	TPM3/4(Exon7)-ALK(Exon20)	Patient 34	●	●		
Inflammatory myofibroblastic tumour	t(2;19)(p23;p13)	TPM3/4(Exon7)-ALK(Exon20)	Patient 35	●	●	●	
Inflammatory myofibroblastic tumour	t(2;2)(p23;q13)	RANBP2(Exon18)-ALK(Exon20)	Not tested				
Lipoblastoma	t(7;8)	COL1A2(Exon1)-PLAG1(Exon3)	Not tested				
Lipoblastoma	der(8)t(8pter->8q12::8q24.1->8ater)	HAS2(Exon1)-PLAG1(Exon3)	Not tested				
Nodular fasciitis	t(17;22)(p13;q13.1)	MYH9(Exon1)-USP6(Exon1partial) or MYH9(Exon1)-USP6(Exon2)	Patient 36	●		●	
			Patient 37	●		●	
			Patient 38	●		●	

Supplemental Table S2. Comparison between RT-PCR, nanoString and the Illumina TruSight RNA PanCancer.

Method	pros	cons	Turn around time
RT-PCR	Ideal when testing one or two candidate fusions.	Test one fusion per run, low throughput.	2 days; includes extraction, RT-PCR, and interpretation.
	Necessitates a thermal cycler only, low capital cost.	Requires 500 to 1000 ng of total RNA.	
	Minimum hands-on time.	Possible reverse transcription and amplification bias.	
NanoString	Can test up to 216 fusions in a single reaction.	Not a discovery tool, probe design necessitates prior knowledge of the fusion.	3 days; includes extraction, hybridization, capture, scan and interpretation.
	Works well on fragmented nucleic acids extracted from FFPE tissues.	Not cost effective for rare fusions or fusions with many variants.	
	Reasonable reagent cost.	Substantial capital equipment.	
	Needs only 200ng of total RNA.		
	Based on target capture by hybridization, no reverse transcription, no amplification.		
	Minimum hands-on time.		
Simple interpretation of the results.			
NGS Illumina TruSight RNA PanCancer	No prior knowledge on the tumour or fusion type. Covers known fusions, rare variants, rare fusions and novel fusions.	Possible reverse transcription and amplification bias.	5 to 6 days, includes extraction, library preparation, sequencing, interpretation.
	Data analysis is pretty simple and straightforward for fusion transcripts.	Relatively expensive.	
	Requires only 20 to 100ng of total RNA extracted from FFPE tissue.	Substantial capital equipment.	
		Very laborious.	