ANGLE

Antibody-free Microfluidics-based Circulating Tumor Cell Enrichment by Parsortix™ and Downstream Molecular Characterization by QuantiGene® Branched DNA Technology

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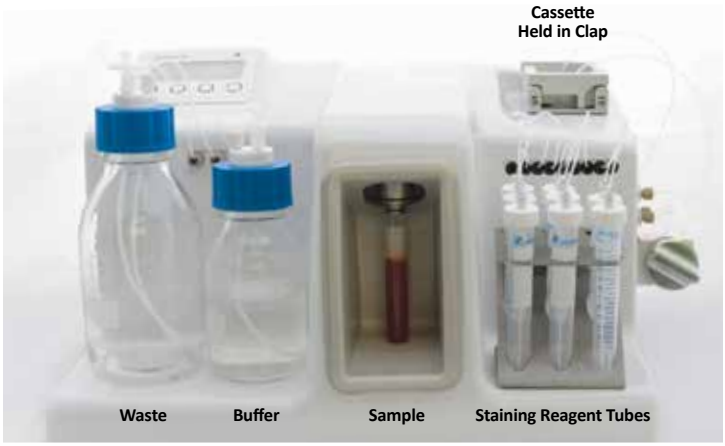
Introduction

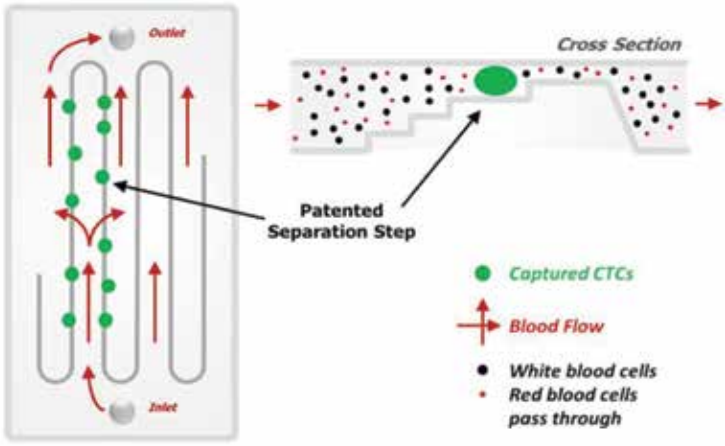
Enumeration of circulating tumor cells (CTCs) in blood is a prognostic and predictive marker in metastatic breast cancer. However, enumeration of CTCs by current approved methodology is of limited clinical utility and could be enhanced by molecular characterization. The unique feature of the Angle plc. Parsortix™ system that sets it apart from many other existing and nascent technologies is that it captures CTCs without antibodies. It relies on the size and deformability of CTCs with the advantage of easy harvesting for subsequent downstream molecular characterization. The prime objective of this study is to validate the isolation of spiked breast cancer cell lines in healthy donor blood (HDB) with Parsortix followed by molecular characterization using Affymetrix® QuantiGene® Plex, a sensitive assay exploiting branch DNA technology.

Methods

Four breast cancer cell lines (hormone receptor positive MCF-7, HER2 positive MDA-MB-453, mesenchymal MDA-MB-231 and inflammatory breast cancer SUM190) were separately spiked into 7.5 ml of HDB with EDTA anticoagulant and processed through Parsortix 10µm microfluidic cassettes for tumor cell enrichment. The captured tumor cells were harvested and then suspended in 300µl of lysis buffer before analysis by QuantiGene to detect the transcripts of 5 epithelial genes (CDH1, EGFR, ERBB2, KRT18, and MUC1) in addition to 20 CTCs and/or breast cancer-related genes. A gene was considered detectable if the transcript level was 2.5 standard deviations above the mean transcript level of the gene in four unspiked HDB samples. Individual cell lines were similarly analyzed to determine the linearity and sensitivity of QuantiGene. Human Universal RNA was included as a technical control for QuantiGene. In pursuit of higher sensitivity, the analysis was also performed by real time PCR.

Parsortix™ Cell Separation System





Signal Amplification (specificity)	Target Amplification (sensitivity)
Branched DNA assay	Real-Time QRT-PCR
(QuantiGene Assay-*Affymetrix*)	(PrimePCR™ Assay-*Bio-Rad)*
22 Target genes + 3 Housekeeping genes	16 Target genes + 3 Housekeeping genes

Balancing Specificity and Sensitivity

Genes Tested:

Branched DNA		
STAT3	FAS	GUSB
FOXO3	CTNNB1	ERBB2
HPRT1	ALDH1A1	IGF1R
GATA3	FN1	CDH2
KRT18	MUC1	EGFR
TBP	CD44	FASLG
PDGFRB	TGFB1	ESR2
CDH1	AR	
ERS1	VEGFA	

Real-Time QRT-PCR		
ESR1	KRT18	KRT5
EGFR	KRT8	PDL1
EPCAM	CD45	VIM
CDH1	HER2	CDH2
MUC1	SRC	
SCGB2A2	ZEB2	

Results

Specificity

For genes known to be expressed by these cells, good correlation was seen between expected and observed gene expression using branched DNA:

- 4 of 5 genes were detected in SUM190 and MCF-7 cells
- 2 of 4 genes were detected in MDA-MB-453 cells
- 3 of 3 genes were detected by MDA-MB-231

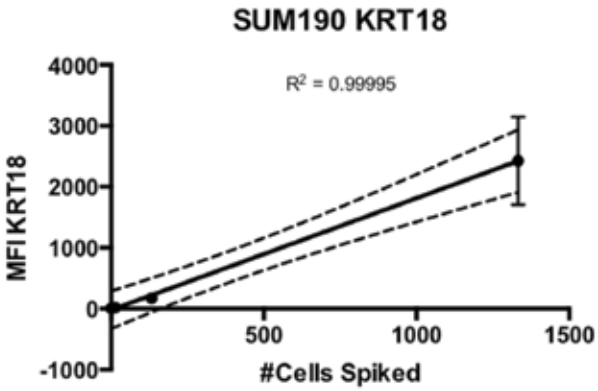
		SUM190	MCF-7	MDA-453	MDA-231
Transcripts	CDH1	+	+	–	N/A
	KRT18	+	+	+	+
	ERBB2	+	+	+	+
	EGFR	–	+	–	+
	MUC1	+	–	N/A	N/A

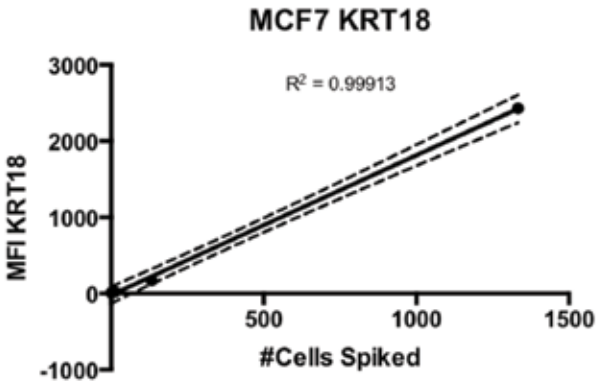
\+ Detected
N/A Not typical for this cell line
– Expected but not detected

Linearity of Detection

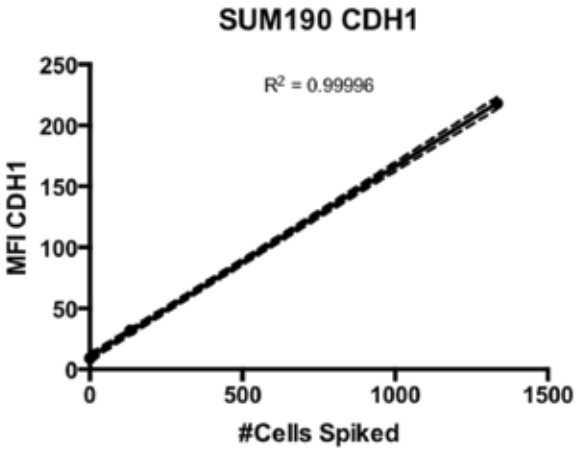
In *linearity* studies, expression levels correlated well with the number of cells spiked into normal donor blood and such a correlation was maintained (R2 > 0.9) for most of the 25 genes tested. Representative data from tests of 2 genes (**LOW** and **HIGH** expression) in 2 cell lines are shown.

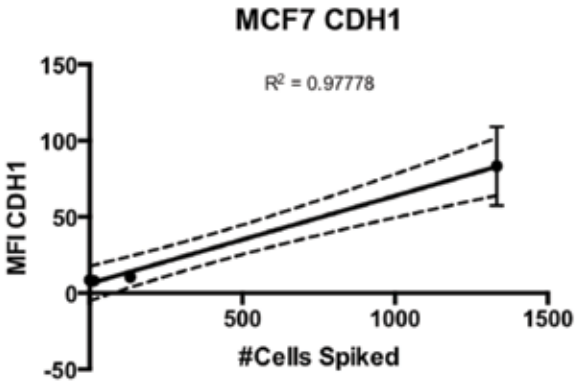
HIGH





LOW





Sensitivity as measured by branched DNA

KRT18 gene transcripts were detected in HDB spiked with as few as 50 SUM190 cells or MCF-7 cells. Several gene transcripts were detected when >50 cells were spiked. MDA-MB-453 gene transcripts were detected only in cell spikes of 500 cells or higher. Gene transcripts were detected in the highly mesenchymal cell line MDA-MB-231 only when several thousand cells were spiked.

Gene transcripts detected at **50** spiked cells by

	SUM190	MCF-7	MDA-MB-453	MDA-MB-231
Epithelial	KRT18	KRT18		
Mesenchymal				
Stem	CD44			
Other	GATA3			

Gene transcripts detected at **500** spiked cells by (above plus)

	SUM190	MCF-7	MDA-MB-453	MDA-MB-231
Epithelial	CDH1		KRT18	
Mesenchymal	CTNNB1, FN1			
Stem				

Gene transcripts detected at **500** spiked cells by (above plus)

	SUM190	MCF-7	MDA-MB-453	MDA-MB-231
Other	ERBB2, IGFR1, HPRT1	ERBB2, GATA3	ERBB2	

Gene transcripts detected at **5000** spiked cells by (above plus)

	SUM190	MCF-7	MDA-MB-453	MDA-MB-231
Epithelial	MUC1	CDH1		KRT18
Mesenchymal		CTNNB1		CTNB1
Stem		CD44		CD44
Other	AR, STAT3, VEGFA, GUSB, TBP	EGFR1, FOXO3, IGFR1, GUSB, HPRT1	GATA3	ERBB2, EGFR, HPRT1

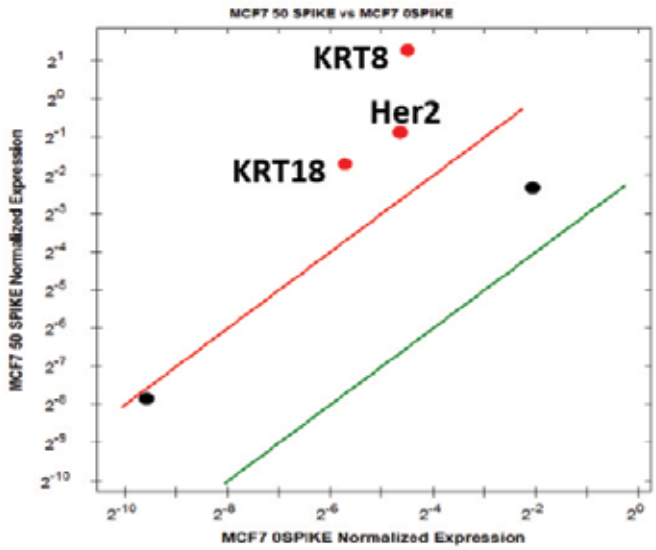
(N.B. Numbers of cells spiked are specified here: the numbers of cells captured and therefore analyzed will be lower)

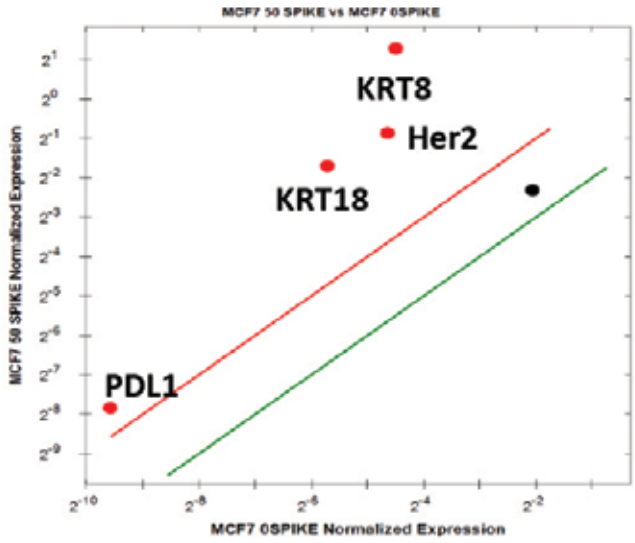
Sensitivity as measured by qRT-PCR

Amplification enables detection of a larger number of transcripts in smaller numbers of spiked cells. Additional gene transcripts were detected with increasing numbers of cells spiked. Genes above the red line are expressed at least 4 times (left plots) or two times (right plots) higher in spiked samples compared to unspiked samples.

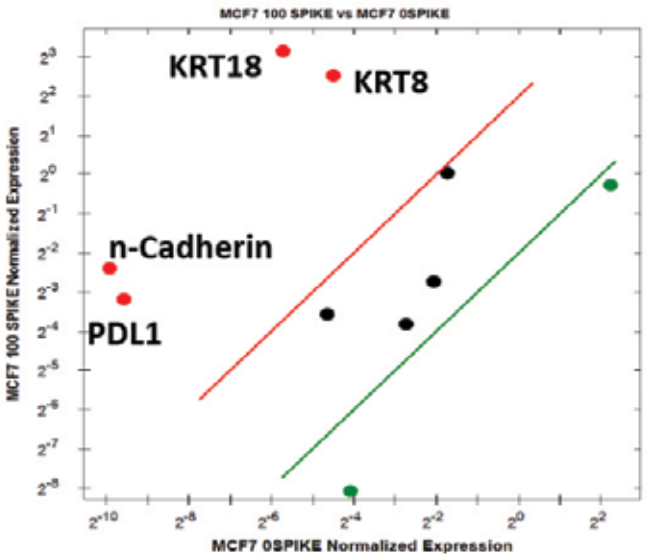
MCF-7 CELLS (Hormone Receptor Positive)

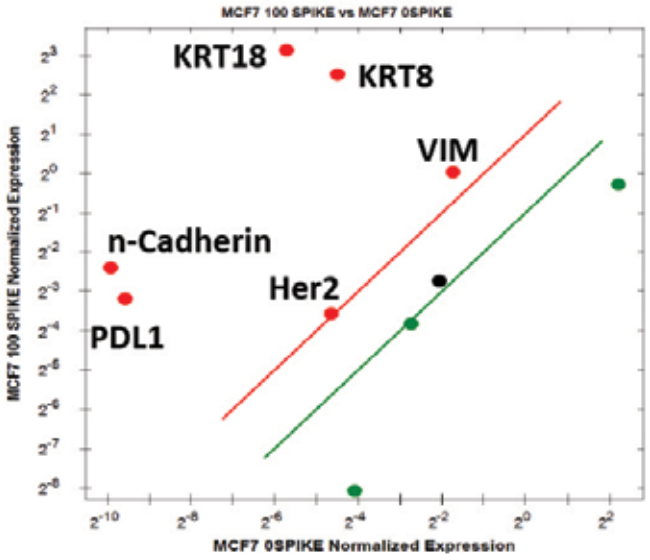
50 cells





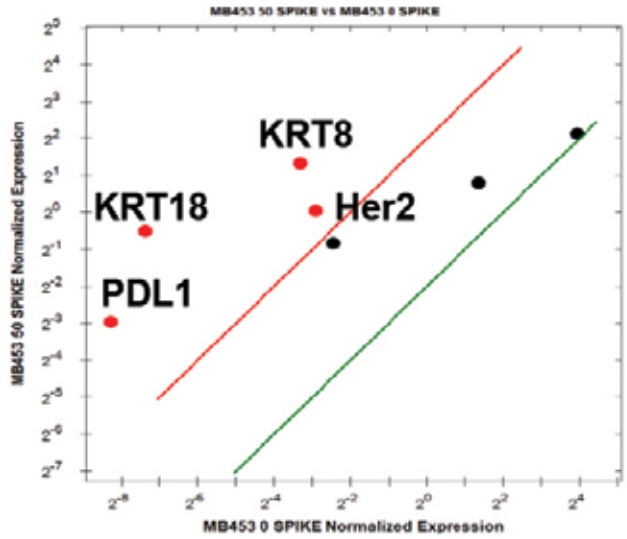
100 cells

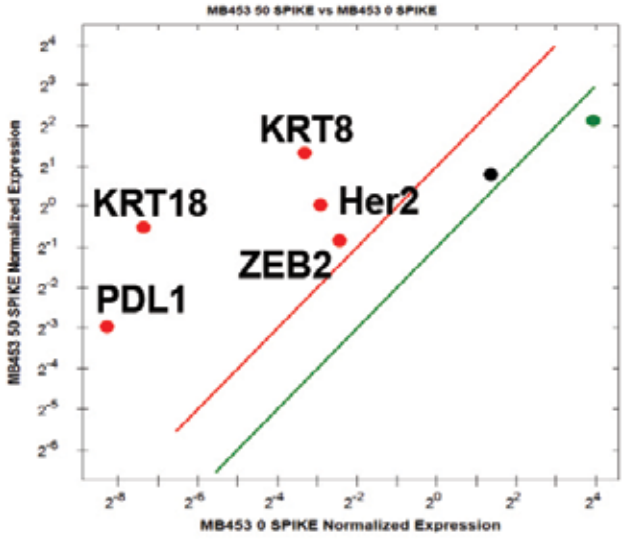




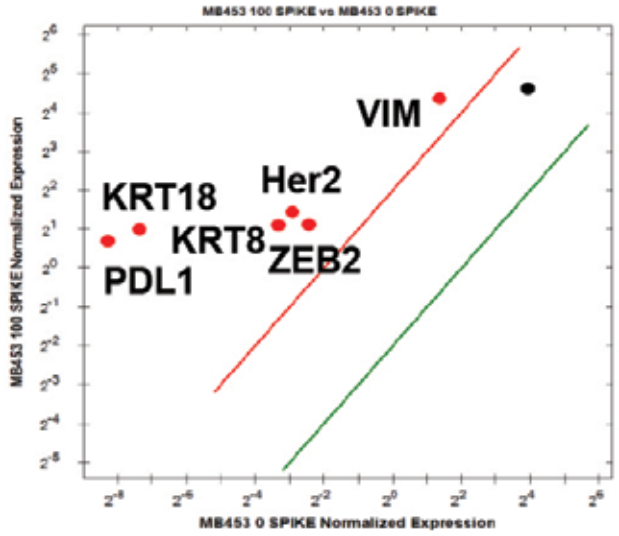
MB-453 CELLS (Her2)

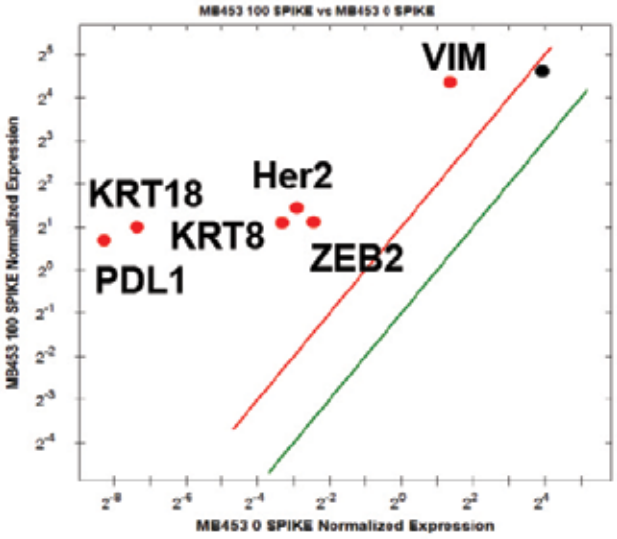
50 cells





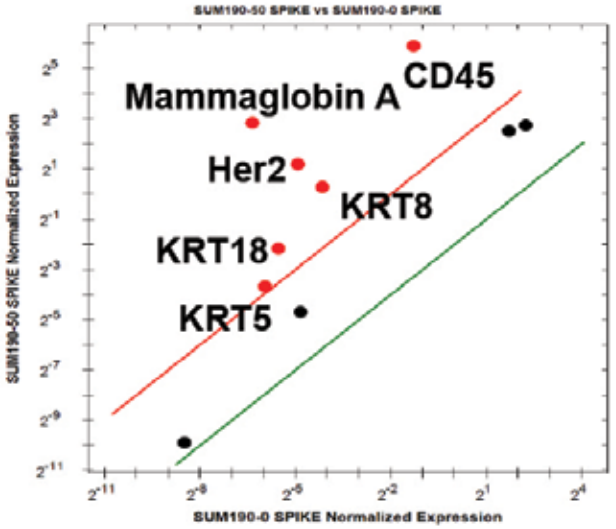
100 cells

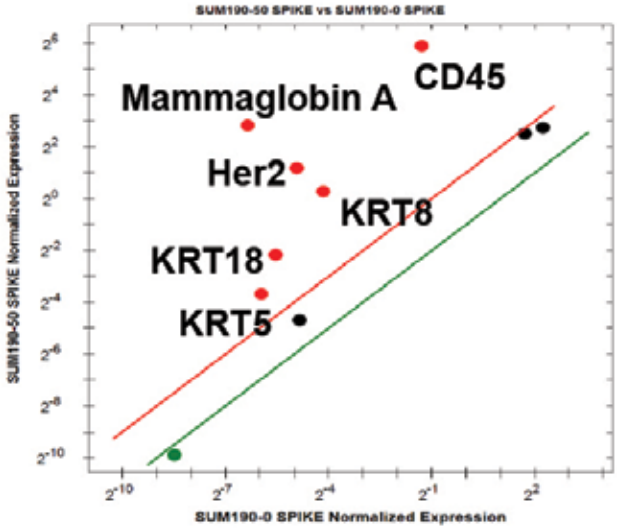




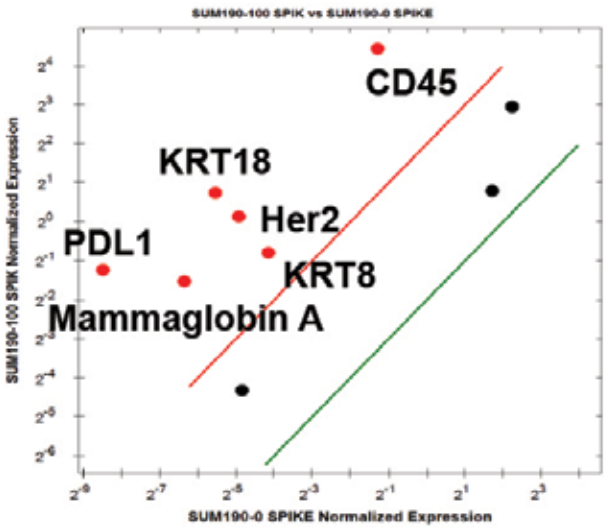
SUM-190 CELLS (Inflammatory Breast Cancer)

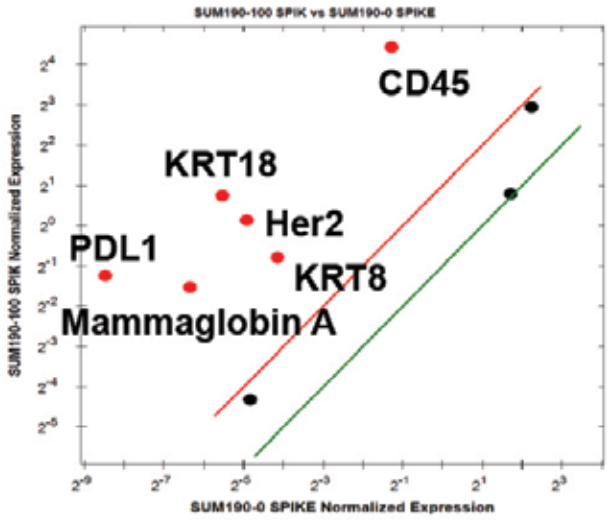
50 cells





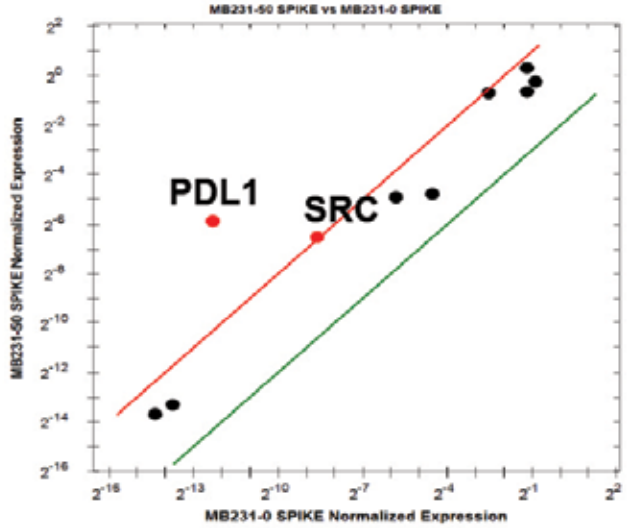
100 cells

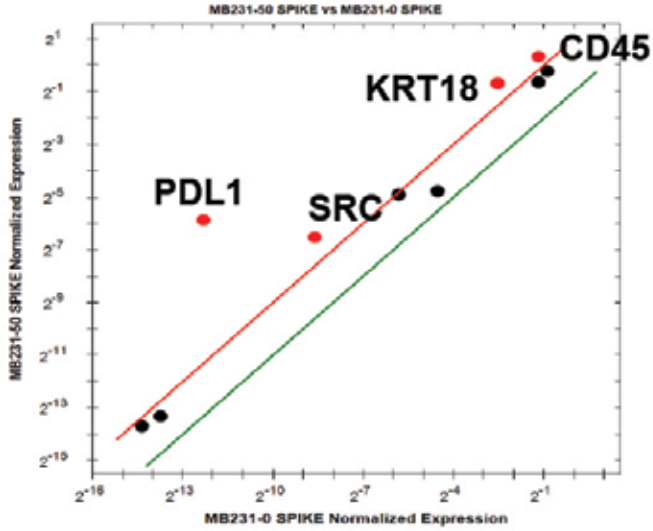




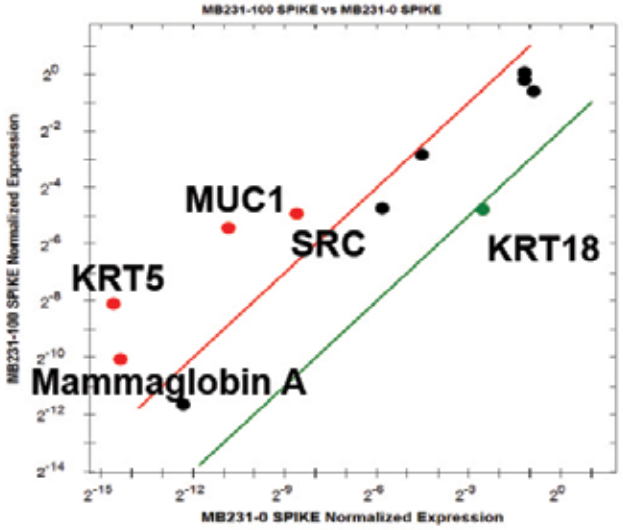
MB-231 CELLS (Triple Negative)

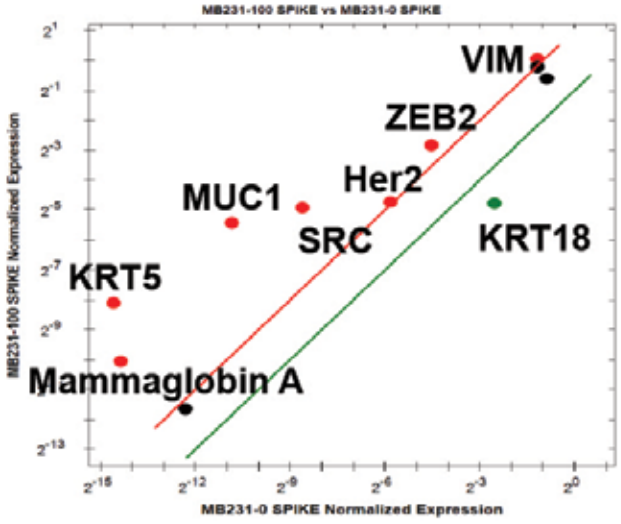
50 cells





100 cells





Summary and Conclusions

These data show that cultured cells harvested from the Parsortix™ system are in a condition enabling molecular characterization. Using two different gene expression analysis methods, it was possible to detect several gene transcripts at very high levels of sensitivity; in some cases the detection limit being 50 cells or less. In addition, we observed a linear correlation between quantities of transcript of RNA detected and the number of cells being processed. Gene expression analysis is of increasing importance in the development of new clinical diagnostics, and these observations have positive potential use in liquid biopsy.

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