

# Detection of microRNA genes in individual cancer stem cells from breast cancer cell lines using the Advalytix AmpliGrid™ single cell platform and a single cell quantitative RT-PCR method

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## Background

Breast cancer is one of the most common malignancies in women with estimated 182,460 new cases diagnosed in 2008 (Jemal, 2008). Breast cancer patients represent a heterogeneous group whose prognosis depends on different host and tumor related factors.

Cancer stem cells (CSC) are rare cells, responsible for disease recurrence and treatment failure (Al-Hajj, 2003). Cells able to efflux Hoechst 33342 dye, the side population (SP) of cells, are enriched for CSCs (Hagnagy, 2006). It is presumed that elimination of these cells is tightly linked to cancer cure. MicroRNAs are small non-coding RNAs able to regulate gene expression at the post-transcriptional level. They play an important role in different biological processes involved in the development of breast cancer (Iorio, 2008).

Traditionally, characterization of microRNA relied on the analysis of a population of cells, ignoring the inherent heterogeneity among individual cells that may obscure important prognostic differences. This is a particularly important consideration when studying putative metastatic stem cells, which are a small but poorly defined population of cells that can not be easily purified. Single cell PCR is a novel powerful tool that is particularly suitable for the analysis of rare cells with biological significance, including candidate cancer stem cells.

The aim of this study was to determine feasibility of microRNAs detection at the individual cell level in SP and non-SP cells of breast cancer cell lines.

## Methods

Three breast cancer cell lines, SUM-149, MDA-231, and KPL-4, and immortalized human mammary epithelial cells (HMEC) were interrogated for expression of 7 microRNA genes (miR-10b, miR-21, miR-27a, miR-125a, miR-145, miR-155 and let-7a; see box below) known to be deregulated in breast cancer (Iorio, 2008) and 2 endogenous control microRNA genes (RNU48, RNU44).

Cells from each of the cell line were stained with Hoechst 33342 dye and sorted based on efflux of the dye. Using a MoFlo™ XDP cell sorter (Beckman Coulter), SP and non-SP subsets were deposited into each well of a 48-well AmpliGrid slide (Olympus, Advalytix Products, Germany) at a frequency of one cell per well. As positive control, we used RNA isolated from the corresponding cell line (Figure 1).

Reverse transcription reaction of individual cells was performed on the AmpliGrid slide. The expression of microRNA genes was determined by quantitative RT-PCR using TaqMan microRNAs assays. Our current set of primers have been optimized for a PCR cycle that includes an initial 10-minute step at 95 °C; followed by 45 cycles of 95 °C for 15 sec, and 60 °C for 60 seconds; and held at 20 °C. Expression of each microRNA genes was determined on 6 to 11 cells of each cell line.

Fisher's Exact test was used to compare the expression of microRNA genes between different cell populations.

### Characteristics of cell lines

**MDA231** is a triple negative breast cancer cell line

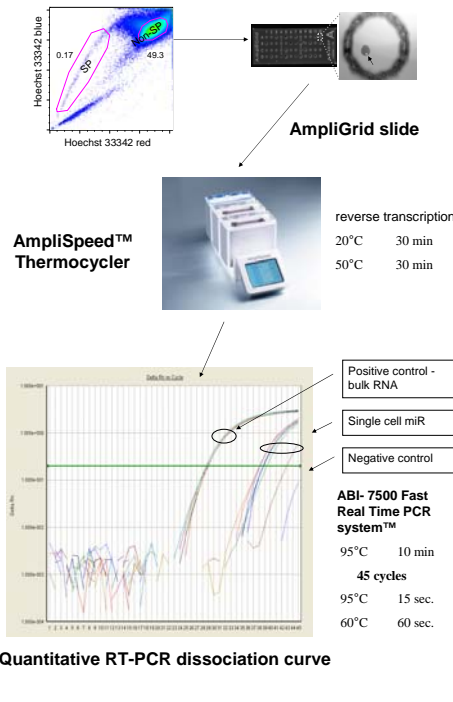
**SUM149** is a triple negative inflammatory breast cancer cell line

**KPL-4** is a Her2/neu positive inflammatory breast cancer cell line

**HMEC** is immortalized human mammary epithelial cell line

MicroRNA	Expression/role in breast cancer	Targets (ref.3)
MiR-21	oncogenic role	Bcl-2,TPM1, PDCD4
MiR-155	oncogenic role	
MiR-125a	oncosuppressor	ERBB2, ERBB3
MiR-145	down-modulated	
miR-10b	metastatic potential	Homeobox D10
MiR-27a	oncogenic role	ZBTB10
Let-7 a	down-modulated	RAS, HMGA2

**Figure 1. Representative side population (SP) and non-side population (non-SP) of cell lines**



## Results

A total of 628 individual cells were interrogated for expression of microRNA genes.

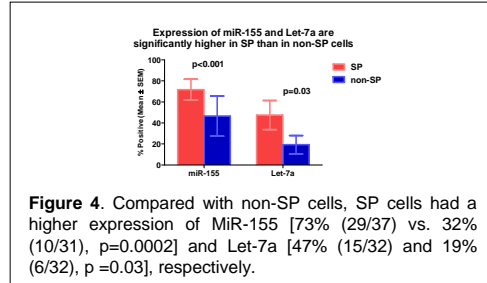
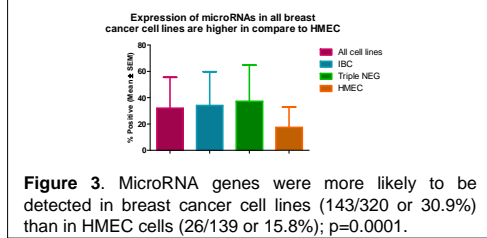
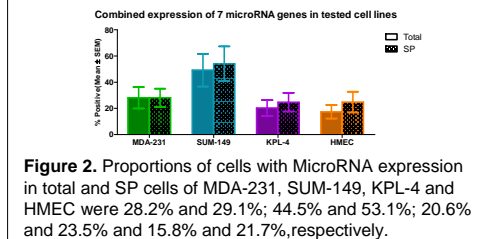
The median number of cycles (Ct number) at which amplification of microRNA genes for SP and non-SP cells was 38.9 cycles and 39.0 cycles, respectively (p = 0.99).

**Table 1. Proportion of cells expressing microRNA genes**

Gene	CELL LINES TESTED							
	MDA231		SUM149		KPL-4		HMEC	
	% SP	% non-SP	% SP	% non-SP	% SP	% non-SP	% SP	% non-SP
let-7a	30	10	88	29	29	0	43	38
miR-21	40	60	86	71	29	14	50	13
miR-10	13	0	0	0	11	25	0	0
miR-125a	25	36	11	0	0	0	9	11
miR-145	0	0	11	0	0	0	0	0
miR-27a	0	0	36	71	29	0	10	11
miR-155	56	30	100	100	71	45	60	11
RNU-48	56	89	88	86	29	50	43	11
RNU-44	33	30	67	43	25	20	9	0

Endogenous control microRNA genes, RNU48 and RNU44, were detected in 56.2% (36/64) and 26.6% (20/75) of examined cells, respectively without a significant difference between SP and non-SP cells.

MicroRNA genes were preferentially detected in SP (100/312) cells compared with non-SP (69/316) cells (32.1% vs. 21.8%; p=0.004).



## Conclusions

- Detection of microRNA genes is feasible at the single cell level.
- Expression of MicroRNA genes by individual cells of breast cancer cell lines is variable with higher expression of in the SP subset than in the non-SP cell subset.
- MicroRNA genes were more likely to be detected in breast cancer cell lines than in HMEC cells.
- Single cell quantitative RT-PCR is a novel method that is suitable for determining the biological characteristics of very rare cells such as cancer stem cells that may be responsible for disease recurrence or micrometastasis.

## References

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