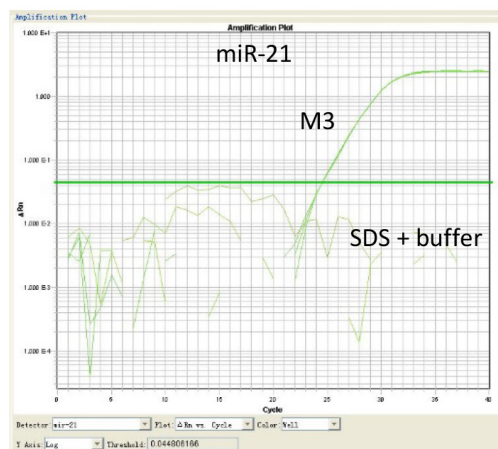
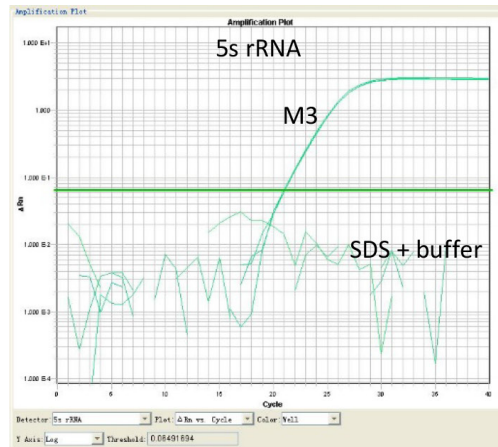
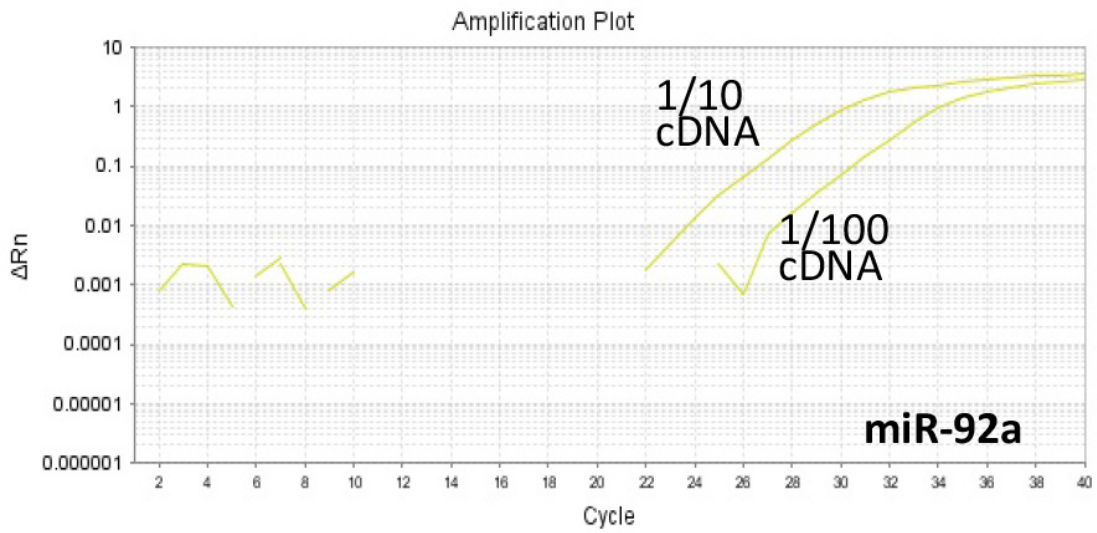


# A direct quantification method for measuring plasma MicroRNAs identified potential biomarkers for detecting metastatic breast cancer

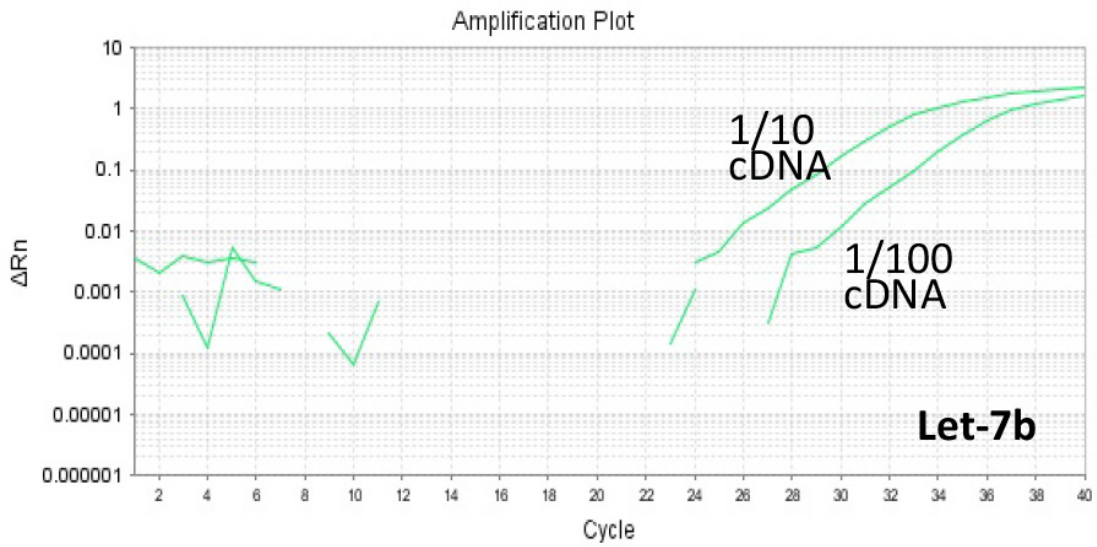
## Supplementary Materials



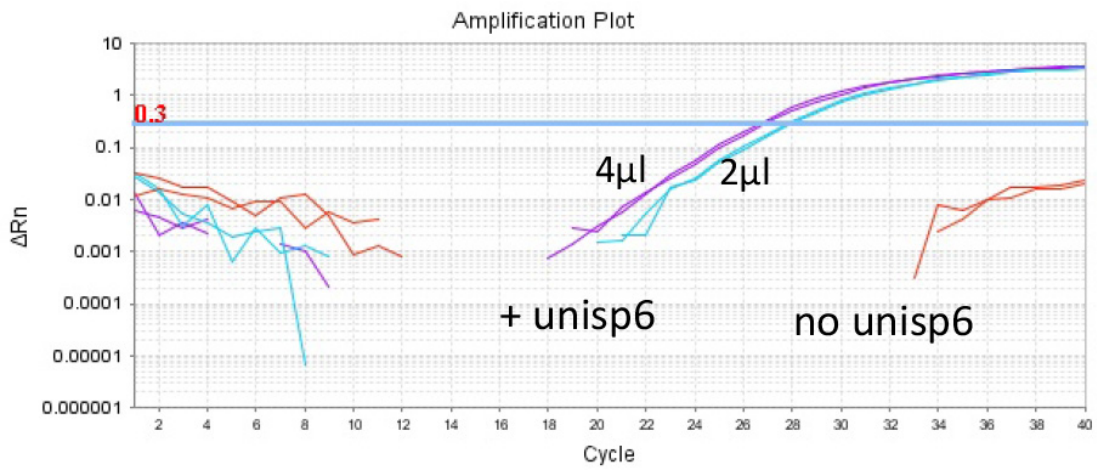
Supplementary Figure S1: Quantitative analysis of 5s rRNA, miR-16 and miR-21 in plasma sample treated with 10% SDS or method M3.



Supplementary Figure S2: Quantitative analysis of miR-92a in plasma sample with 1:10 and 1:100 cDNA dilutions using the method M3.



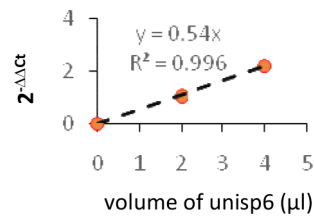
Supplementary Figure S3: Quantitative analysis of let-7b in plasma sample with 1:10 and 1:100 cDNA dilutions using the method M3.



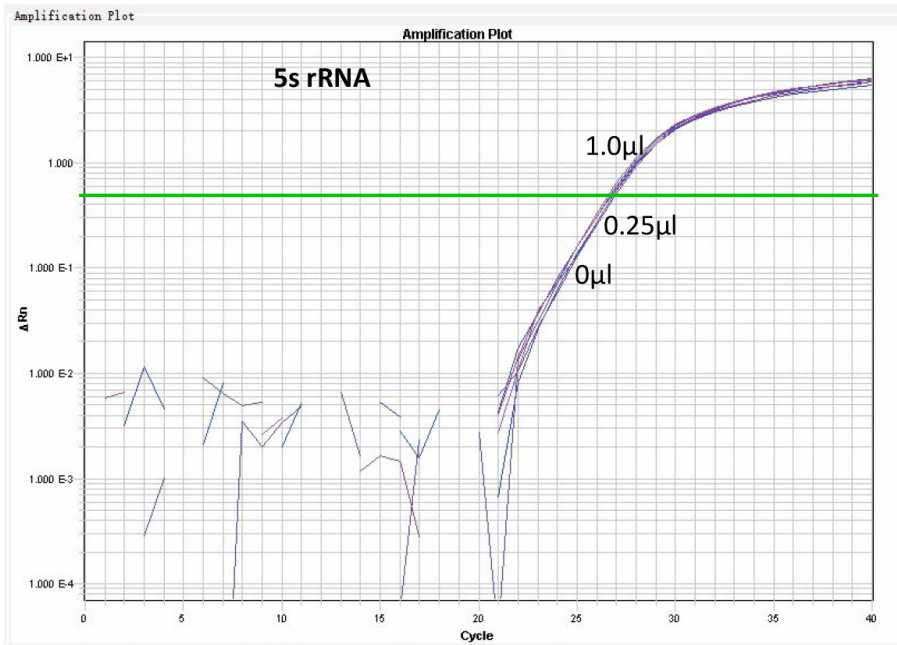
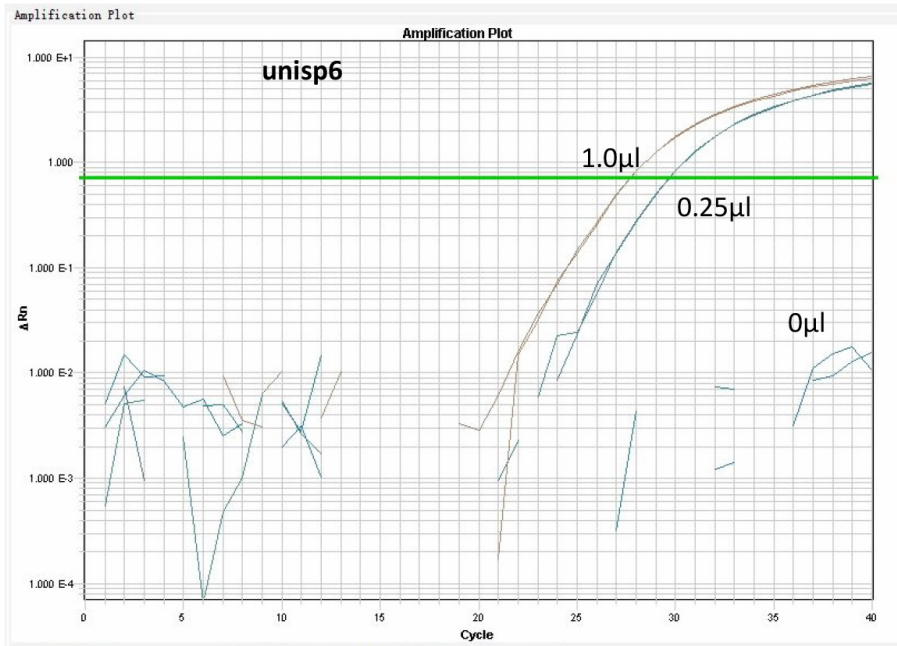
Supplementary Figure S4: Quantitative analysis of exogenous small RNA unisp6 which was added into plasma with volume 0, 2 and 4  $\mu\text{l}$ .

unisp6 (spike-in)	$Ct_{\text{sp6}}$
0	Undetermined
2 $\mu\text{l}$	$27.83 \pm 0.13$
4 $\mu\text{l}$	$26.69 \pm 0.15$

Supplementary Figure S5:  $Ct$  values for unisp6 amplification in S4.



Supplementary Figure S6: Linear positive correlation in S4 between the relative abundance of unisp6 and the unisp6 concentration in plasma with  $R^2 = 0.997$ .



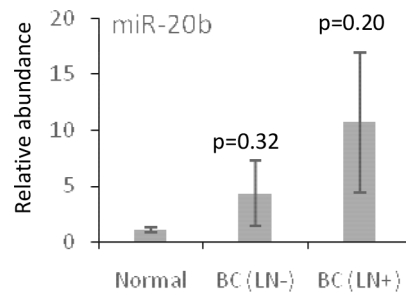
**Supplementary Figure S7: Quantitative analysis of exogenous small RNA unisp6 which was added into plasma with volume 0, 0.25 and 1  $\mu$ l. 5s rRNA was analyzed as well.**

Date	$\Delta$ Ct (miR-16 vs 5s rRNA) (mean $\pm$ SEM)
day 1	4.15 $\pm$ 0.04
day 5	4.12 $\pm$ 0.23

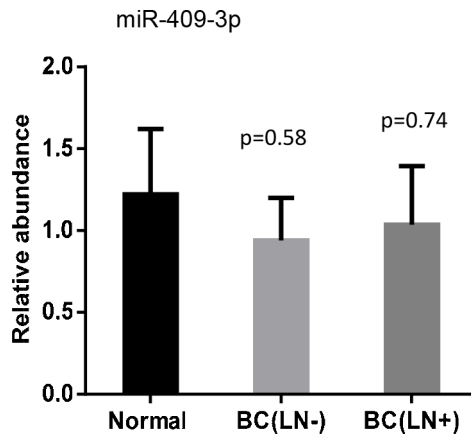
**Supplementary Figure S8: Ct values for miR-16 amplification in a plasma sample at day 1 and day 5 using the method M3.**

Date	$\Delta Ct$ (let-7b vs 5s rRNA) (mean $\pm$ SEM)
day 1	4.53 $\pm$ 0.41
day 5	4.30 $\pm$ 0.06

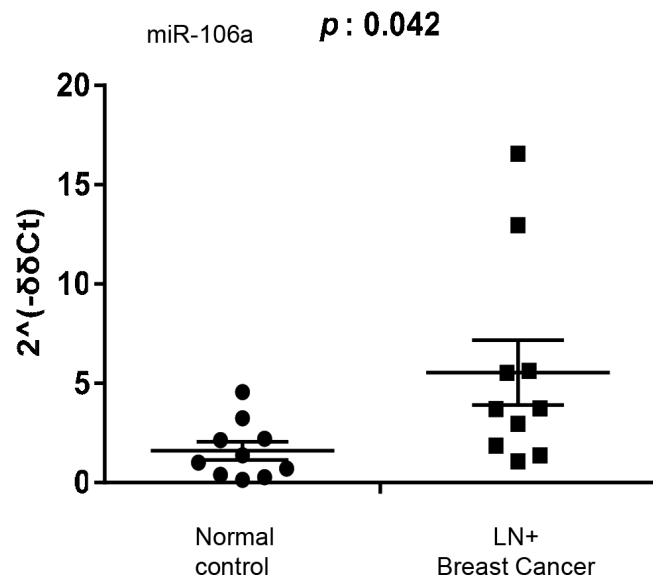
Supplementary Figure S9: Ct values for let-7b amplification in a plasma sample at day 1 and day 5 using the method M3.



Supplementary Figure S10: The relative abundance of miR-20b in the plasma samples from three LN+ breast cancer patients, three LN- breast cancer patients and three normal controls.



Supplementary Figure S11: The relative abundance of miR-409-3p in the plasma samples from three LN+ breast cancer patients, three LN- breast cancer patients and three normal controls.



Supplementary Figure S12: The relative abundance of miR-106a in the plasma samples from additional 10 LN+ breast cancer patients and 10 normal controls.