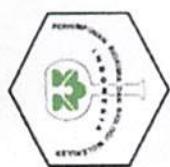




UNIVERSITAS AIRLANGGA



AnMicro  
ASEAN Network  
on Microbial Utilization



## CERTIFICATE FOR ATTENDANCE

This certificate is awarded to

**Dessy Arisanty**

Who has participated in the 23<sup>rd</sup> Seminar of Indonesian Society for Biochemistry and Molecular Biology as

Oral Presenter

Held at Universitas Airlangga, Campus C Surabaya, Indonesia, October 11<sup>th</sup> – 12<sup>th</sup> October 2018

Surabaya, October 12<sup>th</sup> 2018

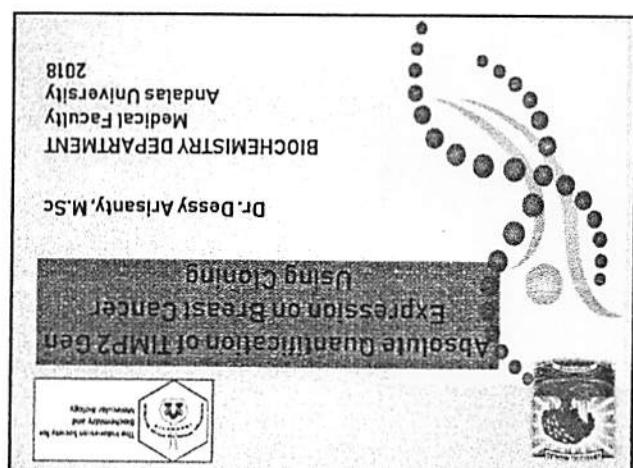
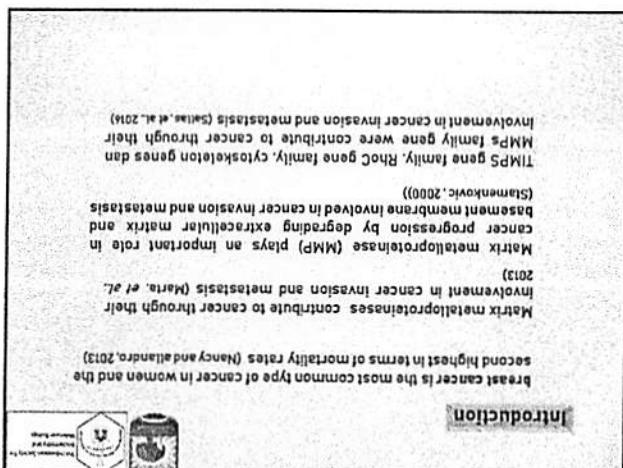
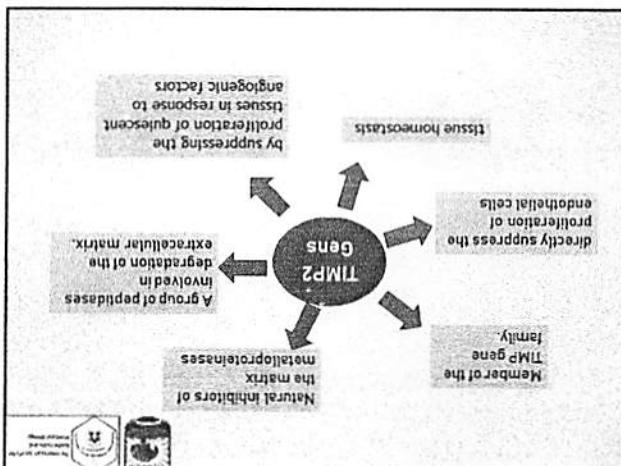
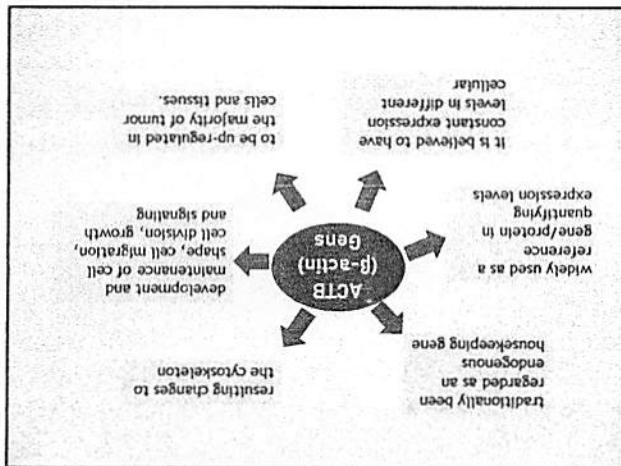
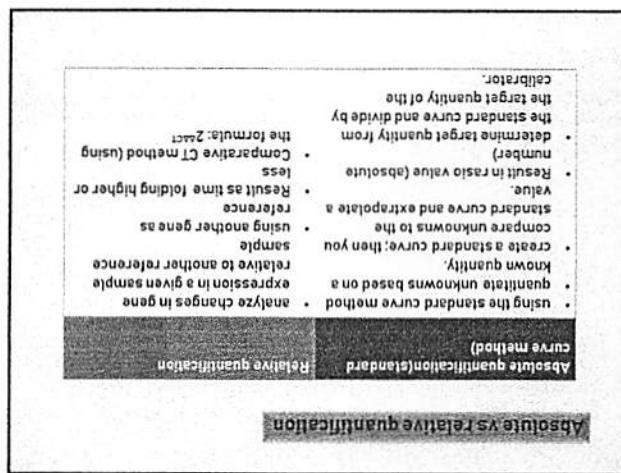
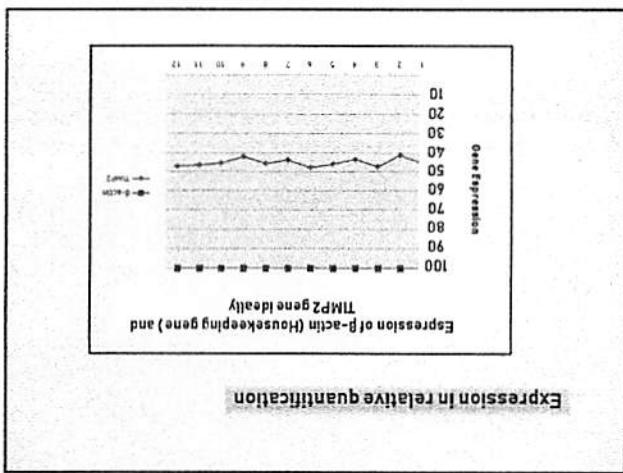
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Dr. Rahmawati Ridwan, Apt., MS.

President of ISBMB

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Prof. Dr. Nyoman Tri Puspaningsih, M.Si.  
Chairman of 12<sup>th</sup> Congress and 23<sup>rd</sup> Seminar of ISBMB  
AnMicro and Marine Workshop



## MATERIAL AND METHOD

### 2. cDNA synthesis

1 µg total RNA was used for cDNA conversion using iScript cDNA synthesis kit (Bio-Rad, catalog number 1708890). The total volume reaction 20 µl was incubated according to the manufacturer's instruction. The converted cDNA was stored at -20°C.

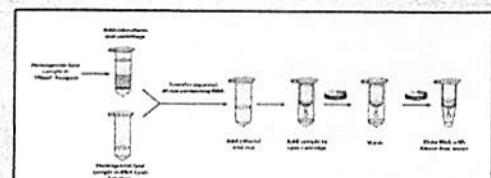
### 3. Primer Design

Quantitative reverse-transcription PCR primers were designed to span an exon-exon junction to reduce the risk of false positives

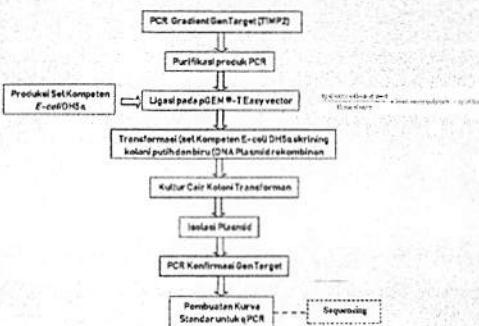


### 1. RNA extraction

Total RNA was extracted from 30 samples of frozen breast cancer tissues and 10 samples of frozen FAM tissues, using TRIzol Reagent with the PureLink® RNA Mini Kit (Ambion, Life Technologies) according to the manufacturer's instructions. Total RNA concentration and purity was quantified in duplicate using NanoDrop spectrophotometer. RNA samples were stored at -80°C.



### 4. Construction of standard for absolute quantitative PCR



### Pembuatan Kurva Standar qPCR (Wheland et al., 2003)

Sebelum membuat kurva standar, terlebih dahulu dihitung massa molekul plasmid tunggal dengan rumus di bawah ini

$$m = \left( n \right) \left[ 1.656 \times 10^{-21} \text{ g} \right] \text{ bp}$$

where: n = plasmid size (bp)  
m = mass  
 $\times 21 \times 10^{-11}$

Note: Use the size of the entire plasmid (plasmid + insert) in the calculation above instead of the size of the insert alone

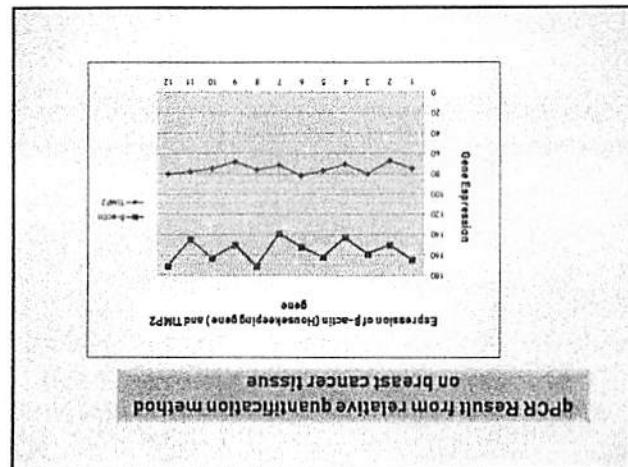
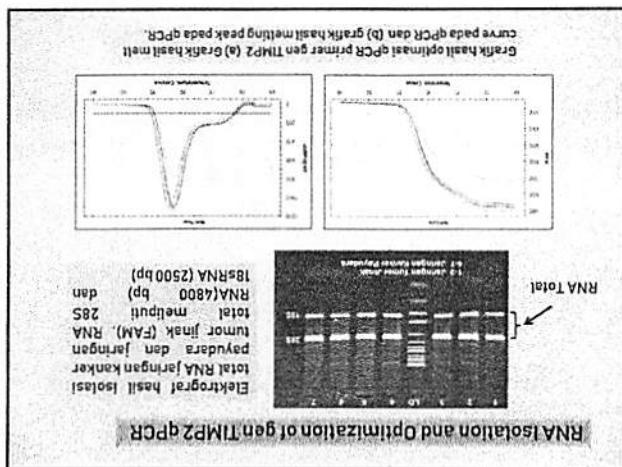
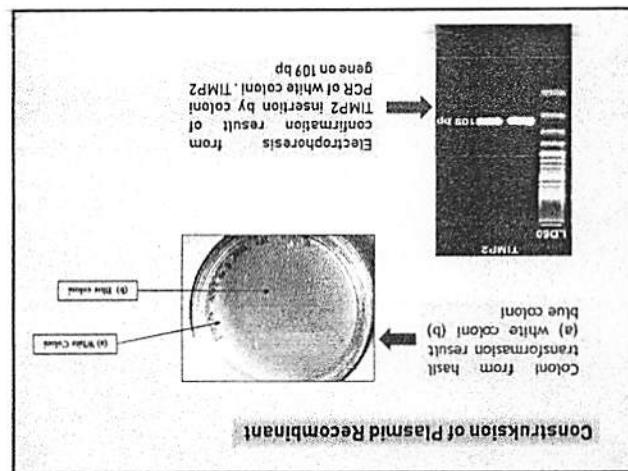
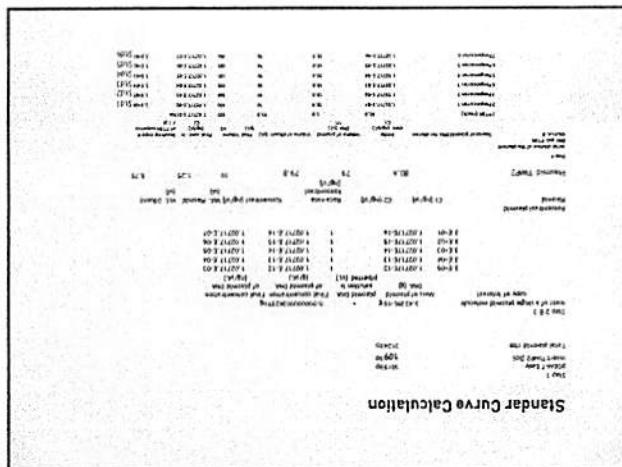
Hitung massa plasmid yang mengandung *copy's of interest*, misalnya 300.000 copy number sampai dengan 30 copy number dengan rumus berikut

$$\text{Copy's of interest} \times \text{mass of single plasmid} = \text{mass of plasmid DNA needed}$$

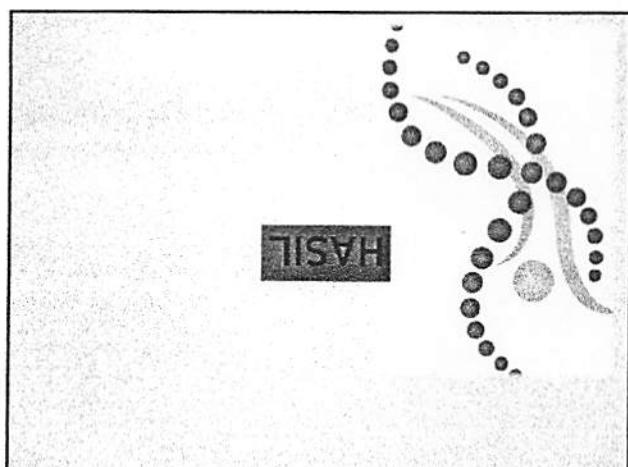
### 5. Realtime absolute quantitative PCR

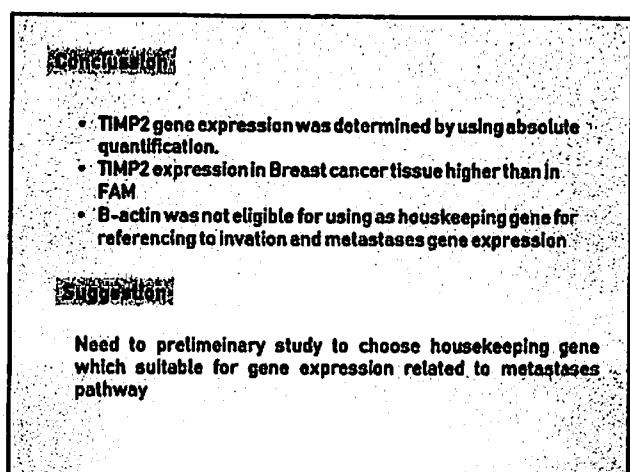
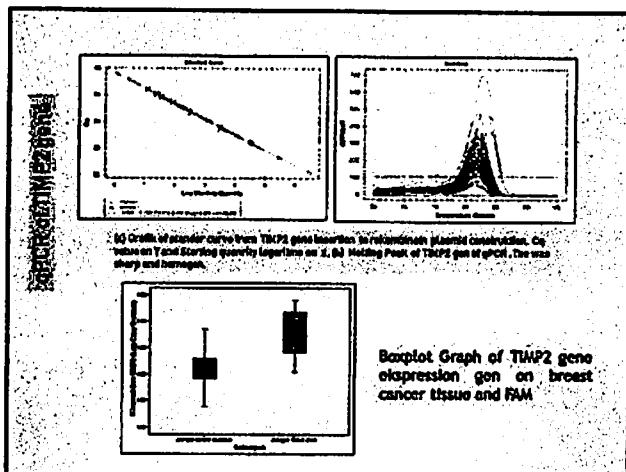
Real time PCR were performed in optical strip PCR tubes in duplicate which contained 500 nM TIMP2 -Forward and 500 nM TIMP2-Reverse (synthesized by Integrated DNA Technologies, Singapore), 1x Sso EvaGreen Supermix (Bio-Rad, catalog number 172-5200), and 2 µl template (cDNA or TIMP2 plasmid standard) in a final volume 20 µl. The cycling conditions were enzyme activation at 95°C for 30 seconds followed by 40 cycles of denaturation

Konstruksi Plasmid Rekombinan



- The expression of TIMP2 gene was relatively stable.
- Due to the real concentration of these TIMP2 genes expression on breast cancer tissue than FAM.
- expression of housekeeping gene  $\beta$ -actin in relative quantity technique was relatively unstable and fluctuate results.
- This may be due to  $\beta$ -actin was also a gene in the cellular matrix.
- $\beta$ -actin revealed to be associated with the invasiveness and metastasis of cancer.
- provides suggestions for future studies of the roles of  $\beta$ -actin in tumors.
- level of gene expression when using the absolute quantification technique of qPCR by using TIMP2 gene cloning insertion into pGEM-T-easy cloning vector.





Terima  
Kasih

Stamenkovic I (2000) Matrix metalloproteinases in tumor invasion and metastasis. Seminars in cancer biology 10: 415–433.