楓城新聞與評論
Maple News and Commentary

美國加州 City of Hope 暑期實習

－旅行，給自己多一些機會－

醫技系三年級 陳俐芸

It was really a great journey! During the summer of 2017, I attended to the summer program in City of Hope, which is a research center and also a hospital for patients with cancer and diabetes in Los Angeles. I learned in DR. Ann's laboratory and had a research project with one of the research assistance. In addition to working in the laboratory, I also experienced the life in LA, which broaden my horizon.

The first day I stepped into the laboratory, I was amazed by what I saw. The equipment, the people working extremely hard, and the atmosphere. They work with passion and with all those incredible ideas to their research. Although I was just a senior in college, I still wanted to learn something new from them and eventually be like them. The best thing about the summer program was that I can really 'think' in my own way. Whenever I encounter difficulties, I pulled out all knowledge in my brain and read papers to look for solutions. My mentor urged me to think. She kept asking me, "how would you design the experiment?" or "what do you think about the results?" This is the best way to learn, especially for doing research. Sometimes I would be suspicious of myself, but she always told me that I would never know how it will go until l tried. I did fail in the experiments; however, these were the accesses to new knowledge. I treasured all these experiences.

During the time without works, I went on a lot of sightseeing. I loved it but what I loved the most was to pretend myself an American and lived a life just like American, which helped me really fit in the culture. In the first week, I was an observer. Observing what people bought in the supermarkets or how people talked to each other was my favorite. Wherever I went, I would just stand beside and watch first. Watching beside at what the locals were doing and listening to how they speak. Though I learned English in Taiwan for many years, when communicating with the local Americans, there were still a lot of phrases I've never heard of. After few weeks, I was able to understand and use these special phrases. Although I still can't speak like a native, I have confidence that I've improved in this language.

I really enjoyed the journey in LA. This was an amazing life experience. Before this summer, I couldn't really understand why people keep saying that traveling abroad is one of the best ways to broaden one's horizons. Now I know! I will bring all I've learned with me at all times and will take advantage of these new knowledge in the future.

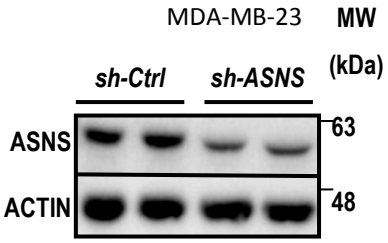
Expression Human Asparagine Synthase in *Escherichia coli.*

Introduction

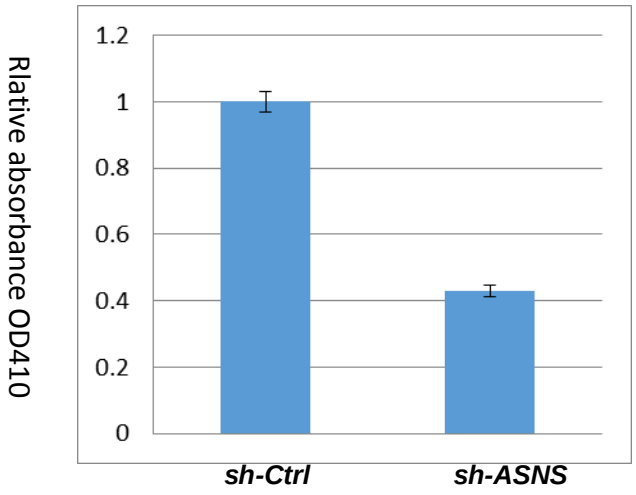
The previous study shows that Asparagine plays an important role in cancer cells' proliferation and motility. Asparagine regulates uptake of amino acids, mTORC1 activity, protein synthesis and nucleotide synthesis, which influences the growth of cancer cells. Asparagine synthetase(ASNS) is an enzyme which converts aspartate into asparagine. When knocking down ASNS in breast cancer cell(figure1A), we can see the reduction of cell proliferation(figure1B) and motility(figure1C). Furthermore, by analyzing The Cancer Genome Altas(TCGA) Pan-Cancer RNA-seq dataset(Figure2), we can conclude that ASNS is overexpressed in multiple cancer cells. Thus, we target ASNS to look for the inhibitor of ASNS.

Figure1

A.



B. Acid Phosphatase (Cell viability) assay



C. Wound healing assay

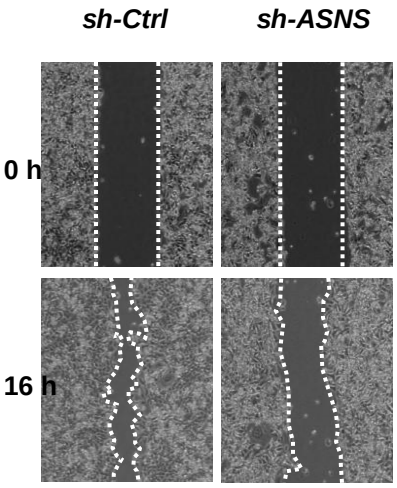
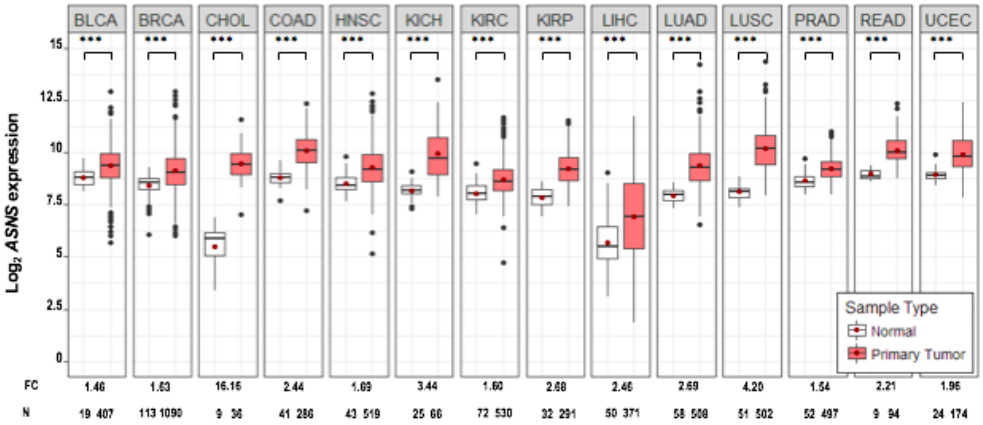
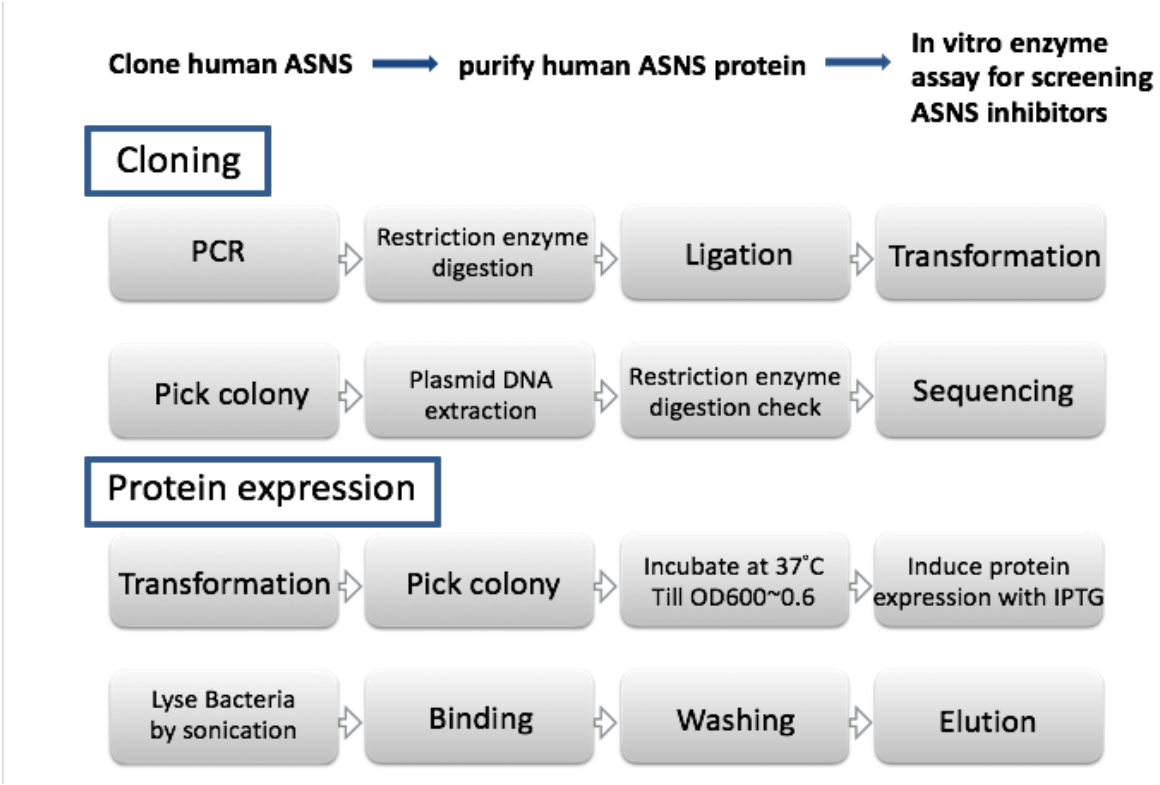


Figure2



Based on The Cancer Genome Atlas (TCGA) Pan-Cancer RNA-seq dataset

Procedure and method



Cloning

I. PCR

For PCR, we first designed primer for the reaction of amplifying human ASNS strain. We put the sequence of the cutting site of the restriction enzymes we used for restriction enzyme digestion to the forward and reverse primer respectively.

II. Restriction enzyme digestion

PCR products and vectors were cut with same restriction enzymes. In the experiment, we used two vectors, pGEX-4T-1(with GST-Tag) and pET-28a(+)(with HIS-Tag). For pGEX-4T-1, we used SmaI and NotI to cut the strain and plasmid. For pET-28a(+), we used XhoI and SacI to cut the strain and plasmid.

III. Ligation

For pGEX-4T-1

insert:vector=3:1(mole)/ 16°C overnight

For pET-28a(+)

Insert:vector=5:1(mole)/RT 1 hr

IV. Transformation

Competent cell

For pGEX-4T-1:DH5a

For pET-28a(+):Stable 3

For each, we added 5 μl of ligation products to competent cell and transfer the plasmid into the cell by heat shock method. Incubated in the 37 °C shaker for 1hr. Pour the competent cell with our plasmid in the plate(For pGEX-4T-1: Ampicillin resistant, for pET-28a(+): Kantamycin resistant). Cultured O/N

at 37°C

V. Pick colony

We picked 4 colonies for pGEX-4T-1 and 8 colonies for pET-28a(+).

VI. Plasmid DNA extraction

Use Miniprep KIT to do the plasmid DNA extraction.

VII. Restriction enzyme digestion check

In order to confirm whether we got the correct plasmid with hASNS sequence, we cut both of the plasmids with BamHI.

VIII. Sequencing

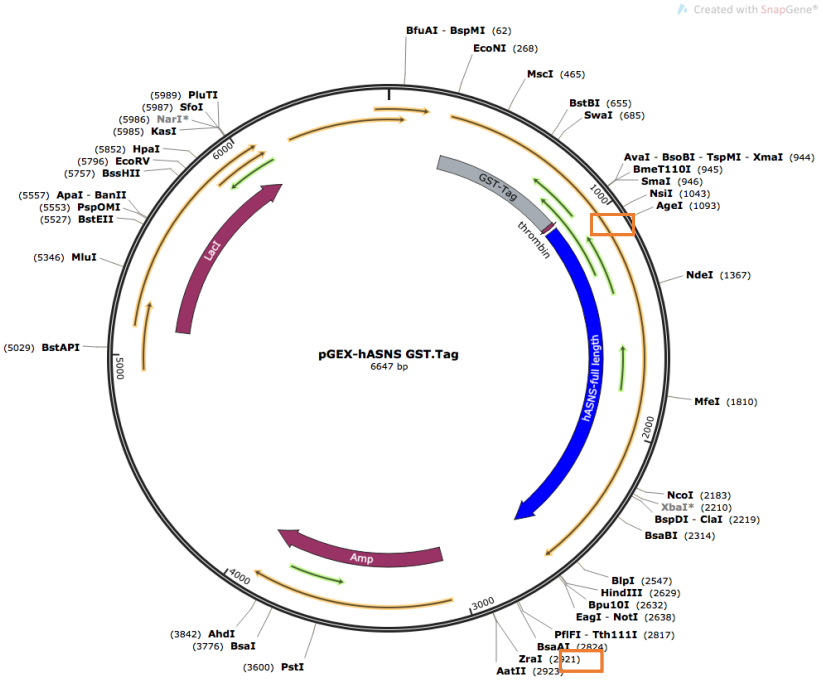
Plasmid DNA sequences were verified by sequencing.

Protein expression

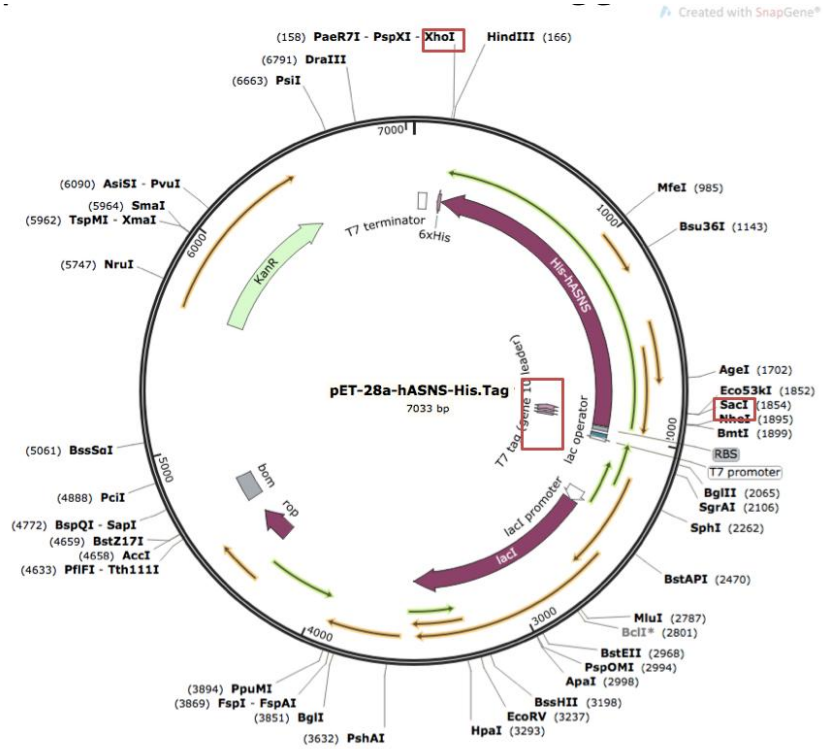
After we got the correct plasmids, we did the transformation(using competent cell BL21). Pick colony and incubate the bacteria at 37°C till OD600 reached 0.5 -0.6. Then, induce protein expression by IPTG and tested different concentrations of IPTG and temperatures to find the best conditions for induction. Finally, we purified protein and got purified hASNS.

Result

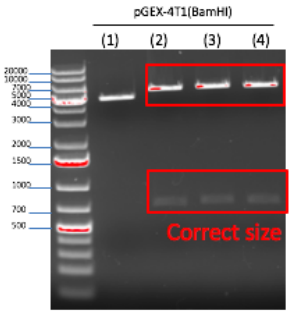
I. Expression plasmid for GST-Tagged hASNS



Expression plasmid for HIS-Tagged hASNS

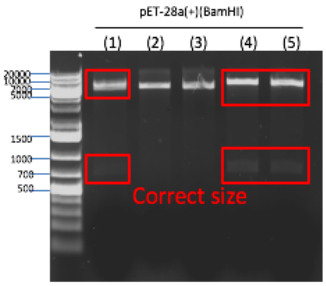


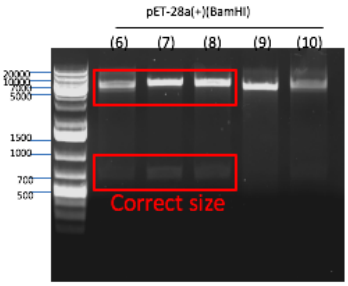
II. Restriction enzyme digestion check



For pGEX-4T-1
Restriction enzyme check with BamHI
Product size:727bp and 5920bp

For pET-28a(+)
Restriction enzyme check with BamHI
Product size:724bp and 6309bp



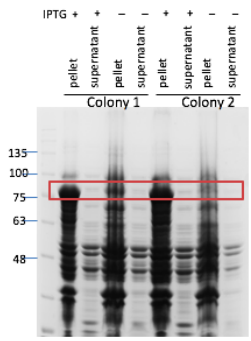


III. Protein expression

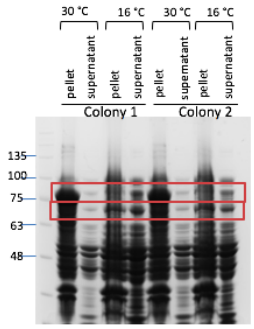
For GST-Tagged protein expression

SDS-PAGE stained with Coomassie blue forGST-Tagged hASNS protein expression(cell lysate)

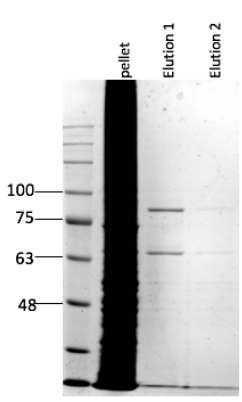
Product size:90kDa



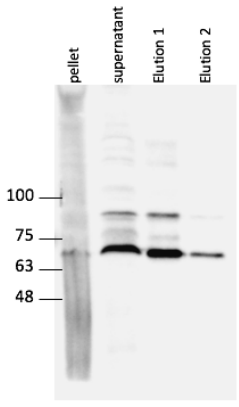
hASNS was induced at 30°C with or without IPTG. Expression of hASNS has been induced by IPTG.



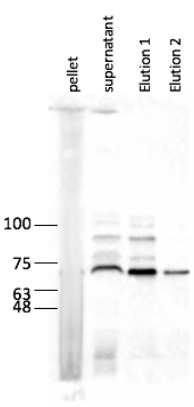
hASNS was induced at 30°C and 16°C with IPTG.
There is more hASNS in pellet when induced at 30°C . However, there is more soluble hASNS when induced at 16°C.



SDS-PAGE stained with Coomassie blue for GST-Tagged hASNS



Western blot for GST-Tagged hASNS probed with anti-GST-Tag antibody

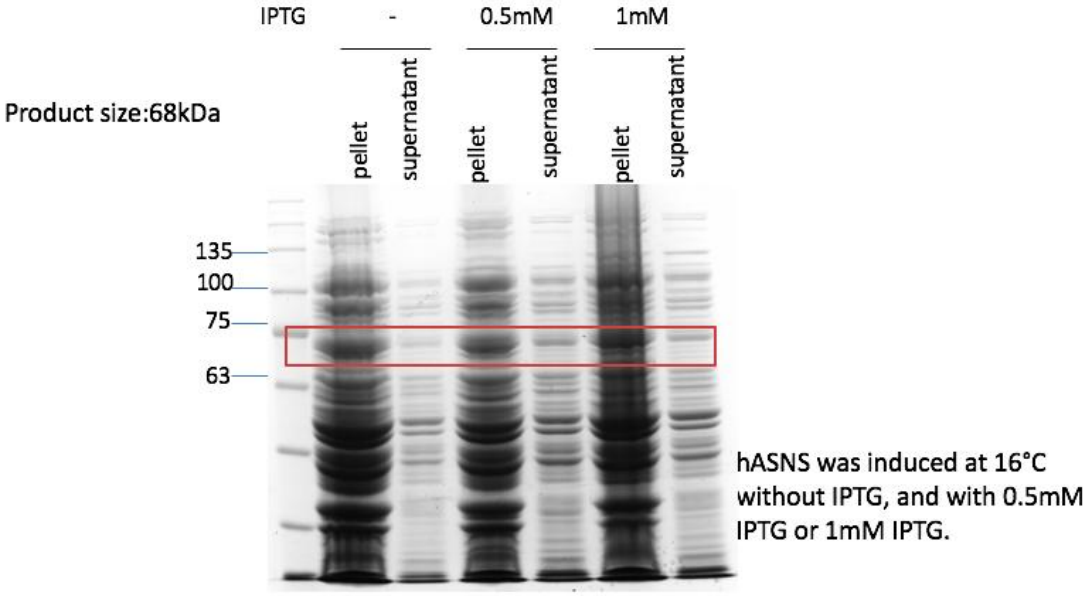


Western blot for GST-Tagged hASNS probed with anti-ASNS antibody

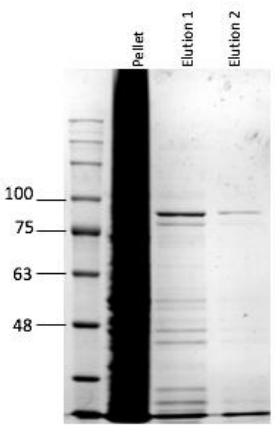
For HIS-Tagged hASNS expression

As SDS-PAGE stained with Commassie blue and Western blot showed, we haven't successfully purified HIS-Tagged hASNS.

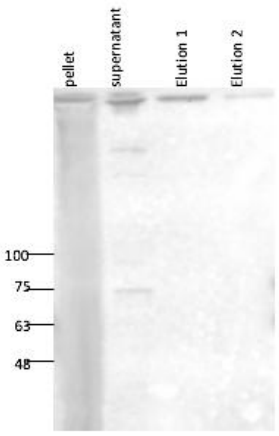
SDS-PAGE stained with Coomassie blue for HIS-Tagged hASNS protein expression(cell lysate)



hASNS was induced at 16°C without IPTG, and with 0.5mM IPTG or 1mM IPTG.



SDS-PAGE stained with Coomassie blue for HIS-Tagged hASNS



Western blot for HIS-Tagged hASNS probed with anti-ASNS antibody

Future direction

I. optimize induction and purification for Hig-tagged hASNS

II. test enzyme activity

To test the activity of hASNS:

$$PP_i + \text{F-6-P} \xrightarrow{PP_i\text{-PFK}} \text{F-1,6-DP} + P_i$$

$$\text{F-1,6-DP} \xrightarrow{\text{Adolase}} \text{GAP} + \text{DHAP}$$

$$\text{GAP} \xrightarrow{\text{TPI}} \text{DHAP}$$

$$2\ \text{DHAP} + 2\ \beta\text{-NADH} + 2\ H^+ \xrightarrow{\text{GDH}} 2\ \text{glycerol-3-phosphate} + 2\ \beta\text{-NAD}^+$$

The reaction is monitored spectrophotometrically at 340 nm for the amount of NAD^+. If these reactions work successfully, we can confirm that hASNS works well.

III. small molecule screening by using in vitro enzyme assay

前排右一 Dr. Ann 右二 Evelyn, Chung (指導老師)


JEEP TRADING
COMPANY
FACE MASK