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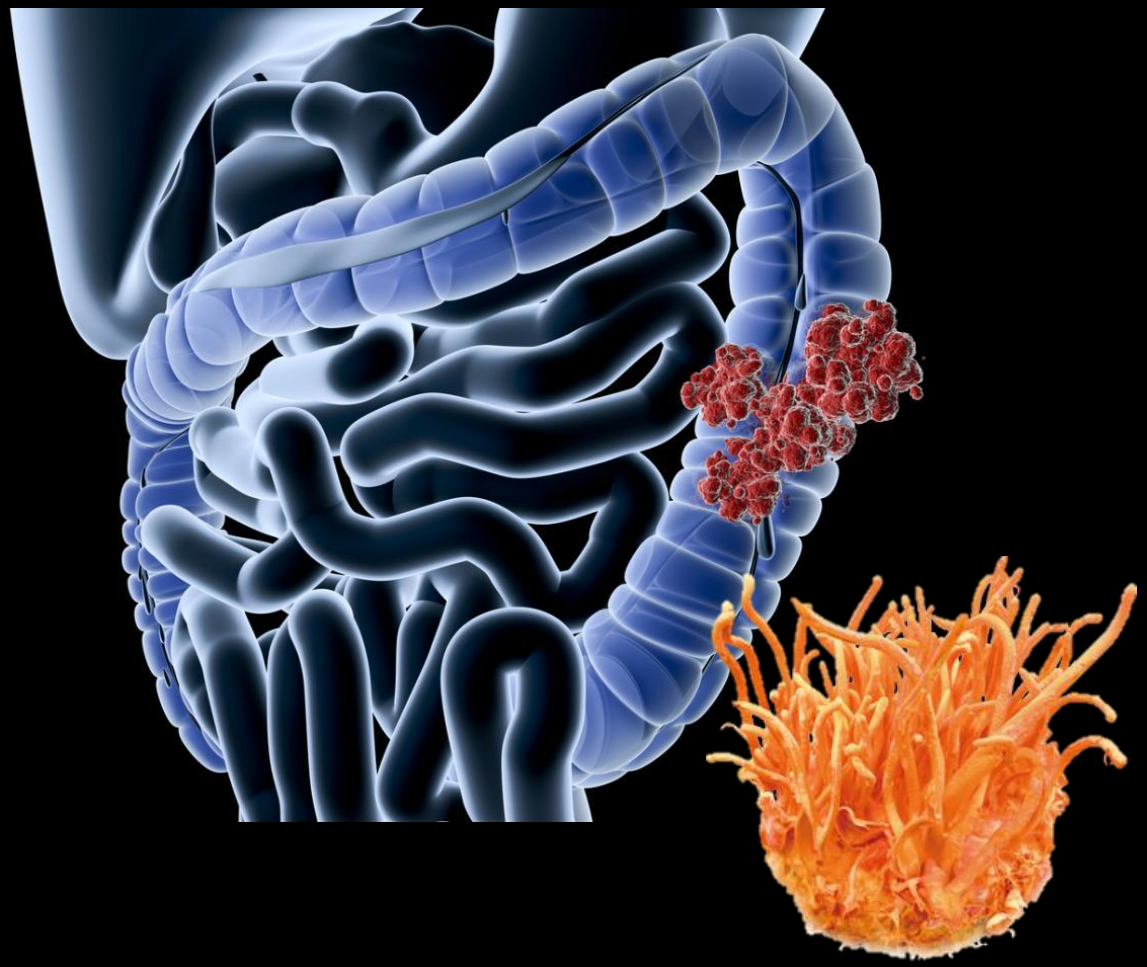

Prediction and Selection of anticancer peptide from *Cordyceps militaris* peptidome using bioinformatics

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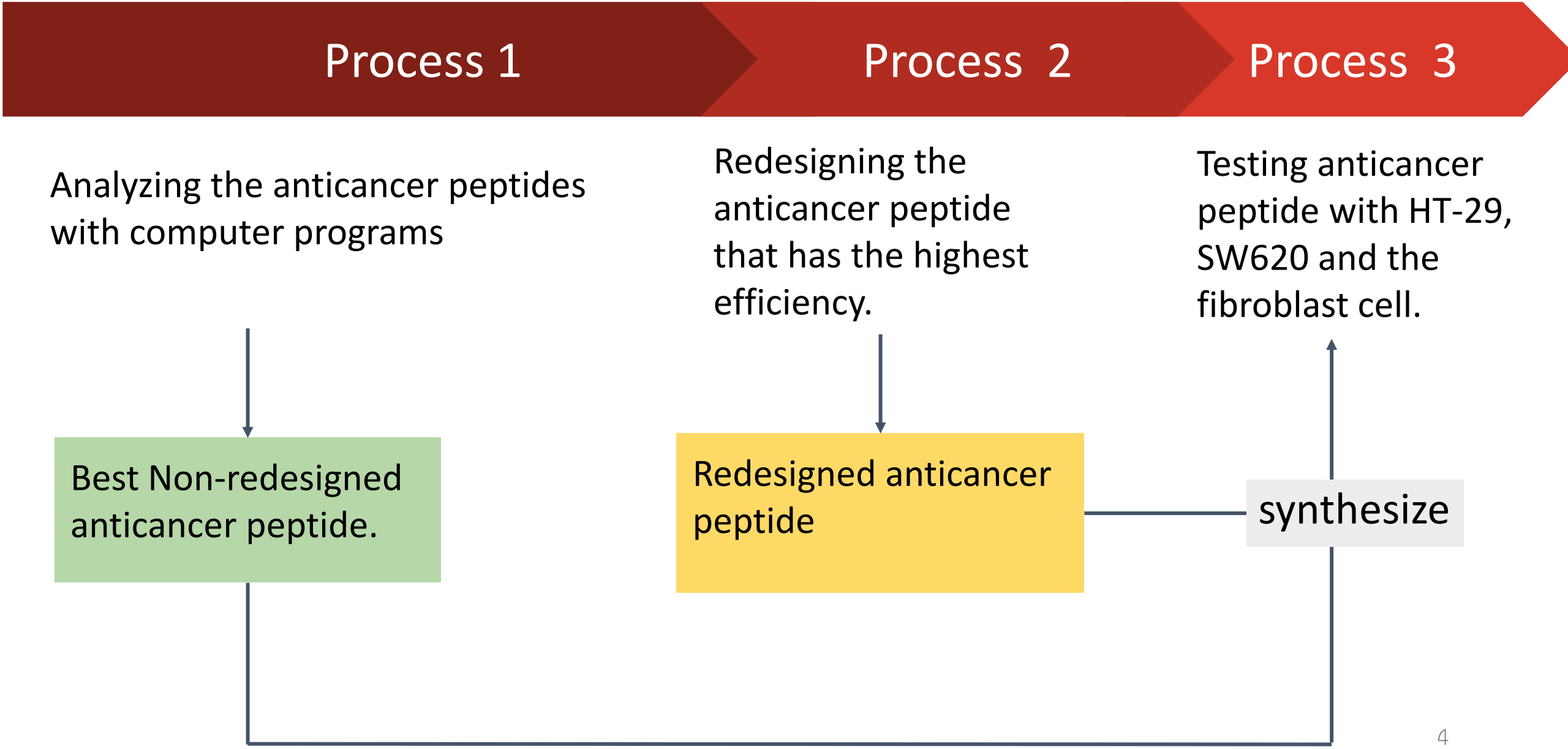
INTRODUCTION

Colorectal cancer is 2nd in Thailand

Cordyceps militaris can cure breast, lung, and bladder cancer

Rare and expensive

Method outline

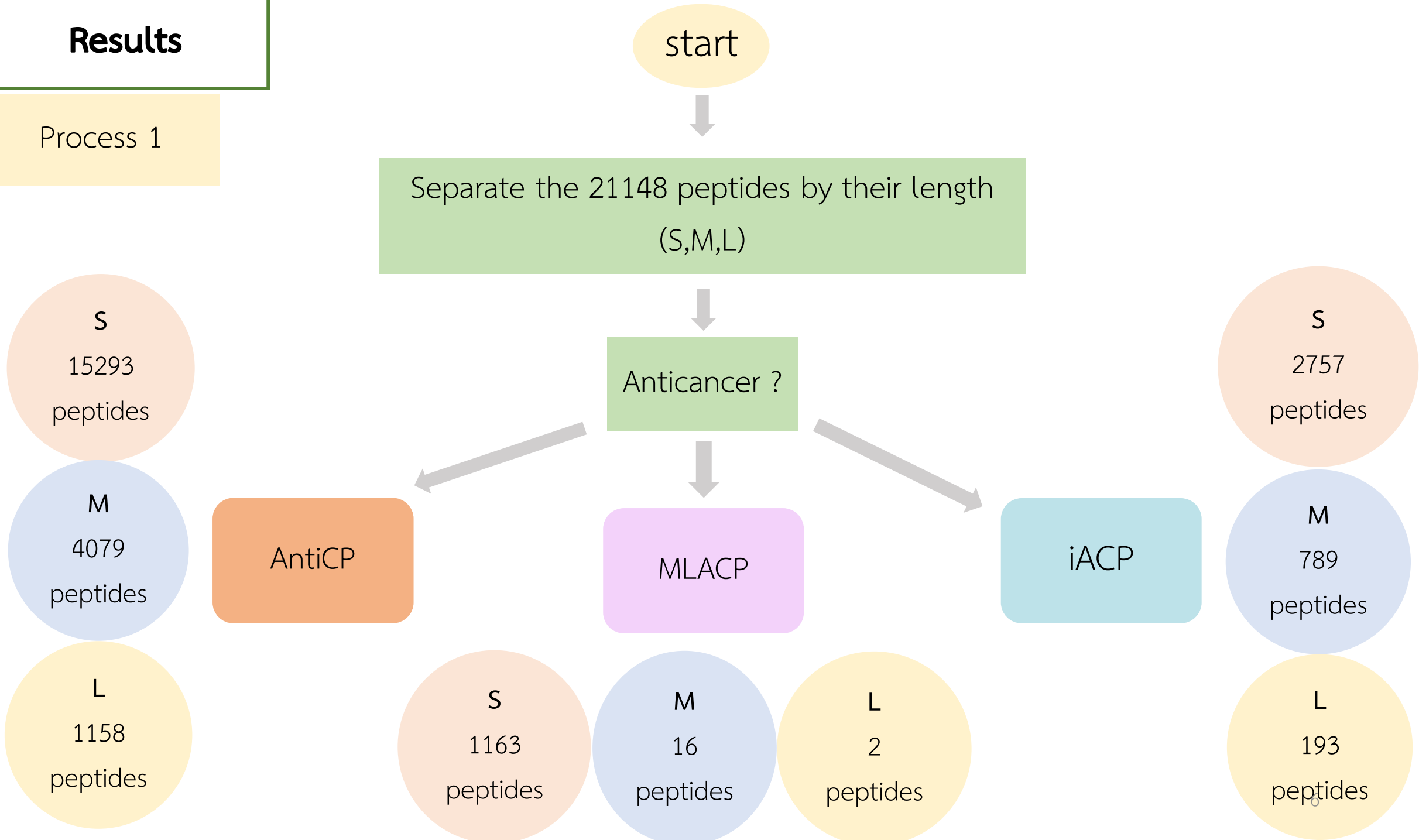


Process 1

Analyzing the peptides

Results

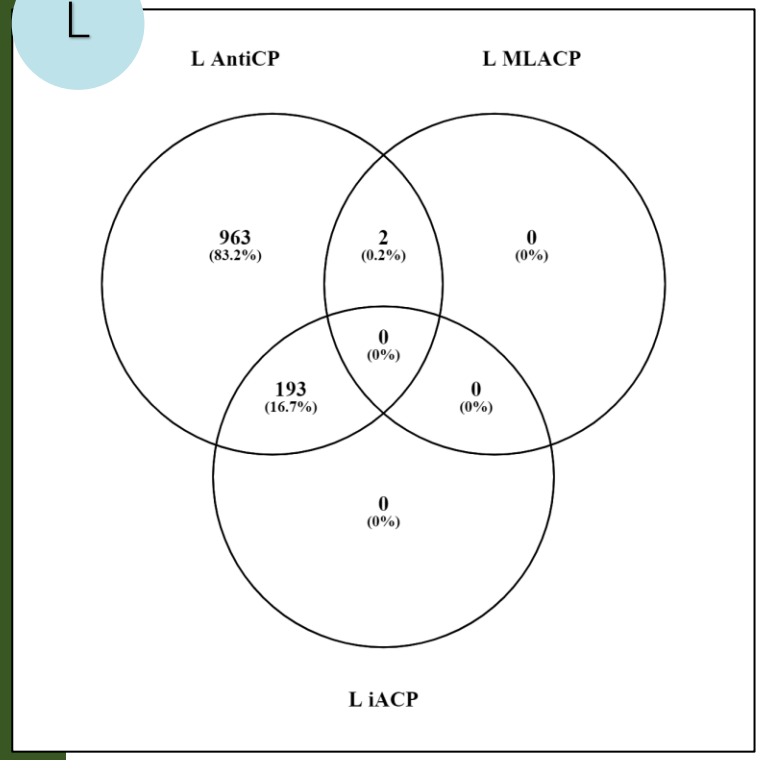
Process 1



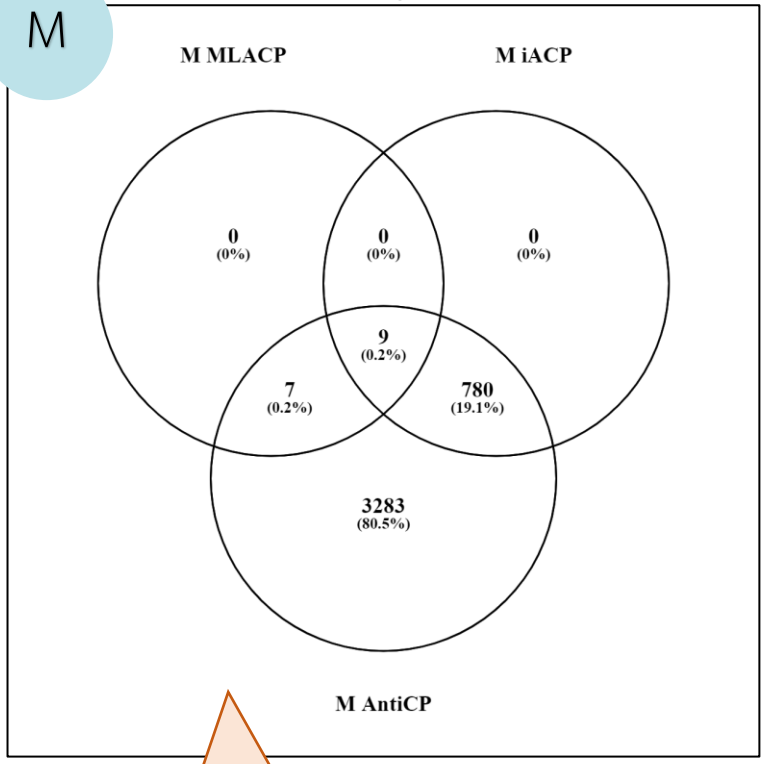
Results

Use **Venny** to find the peptides that pass every computer programs

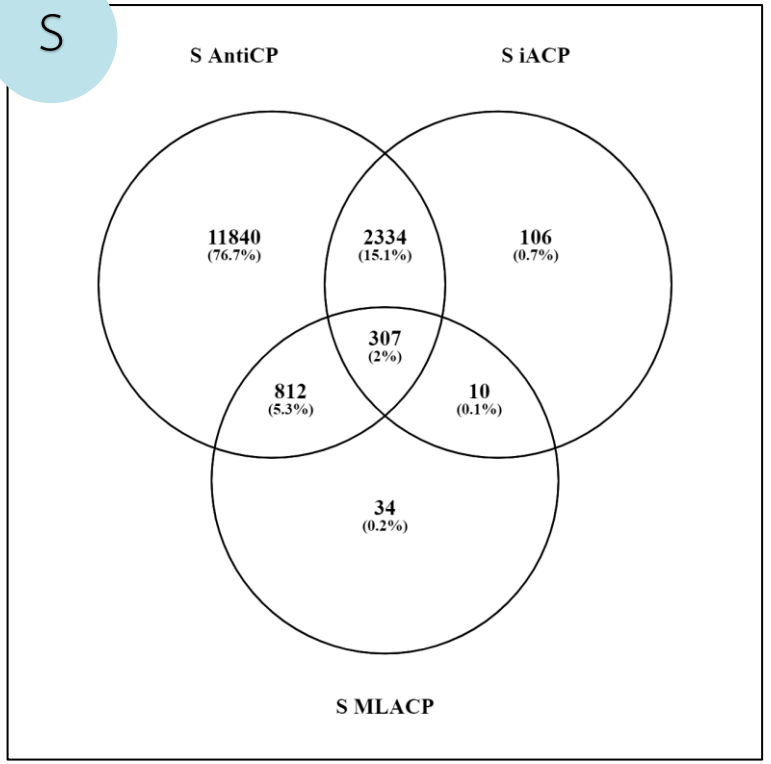
L



M



S



L 0 peptides

M 9 peptides

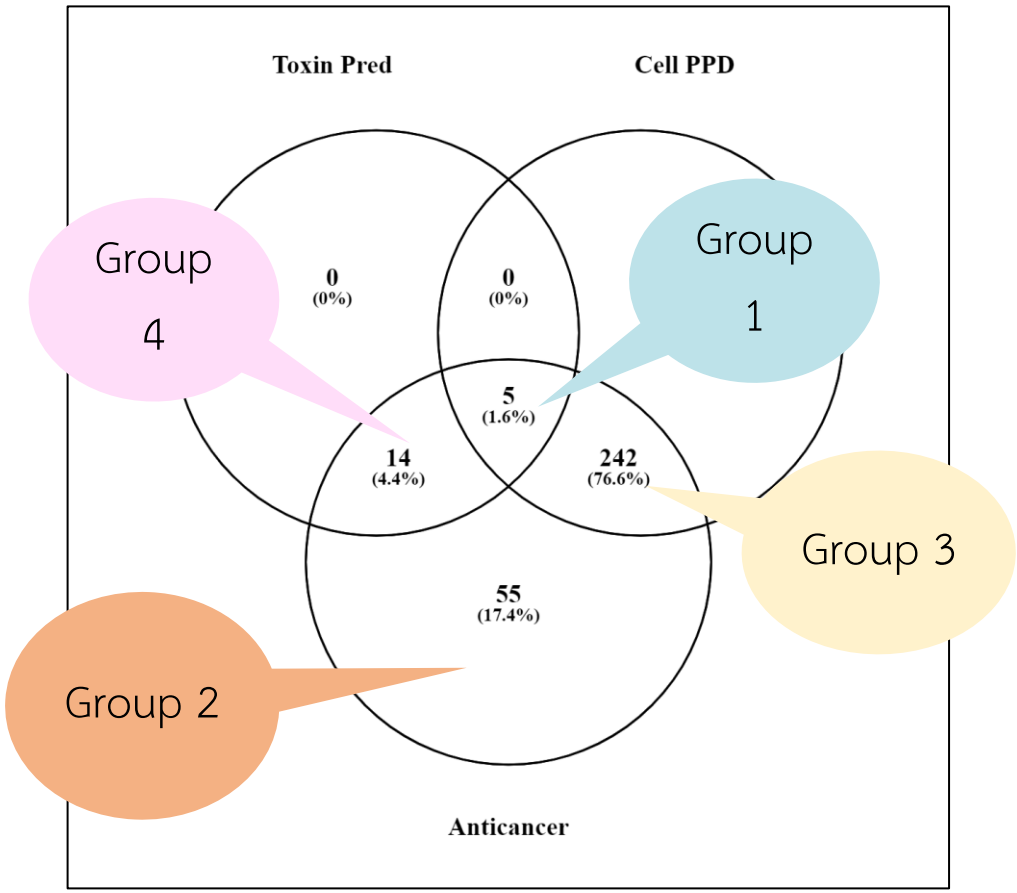
S 307 peptides

316 peptides in total

316 peptides

MLCPP

Toxin-Pred



Group 1 anticancer+toxin+cell penetrate

= 5 peptides

Group 2 anticancer

= 55 peptides

Group 3 anticancer+cell penetrate

=242 peptides

Group 4 anticancer+toxin

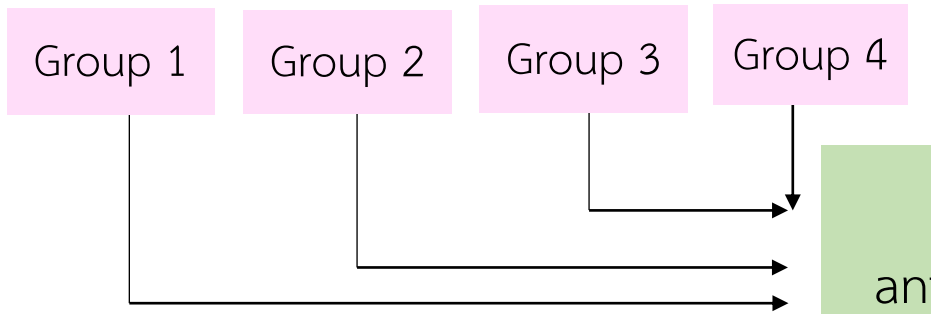
=14 peptides

Process 2

Redesigning the peptides

Results

Process 2



Best original anticancer peptides

Non-redesigned anticancer peptide

Group 1 TTMICLTCAR
Group 2 VTFVLIAAK
Group 3 FGSWEHR
Group 4 CDDCIDK

Redesign the peptide by changing some amino acids for better anticancer efficiency using **AntiCP**

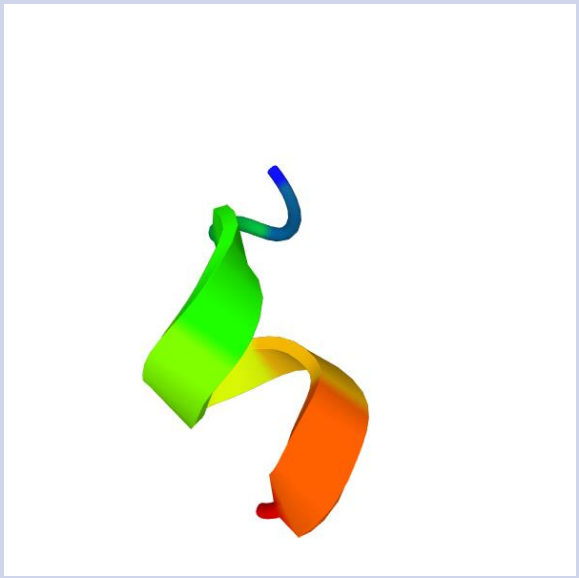
Group 1 TTGICLTCCR
Group 2 FTFVLLAAK
Group 3 FGSWEEE
Group 4 CDDCIEE

Redesigned anticancer peptide

Analyze the molecular structure with PEP-FOLD

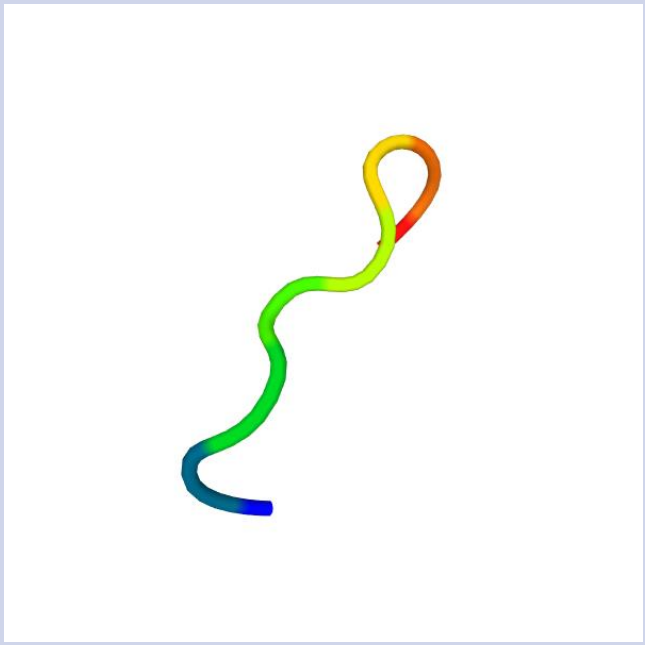
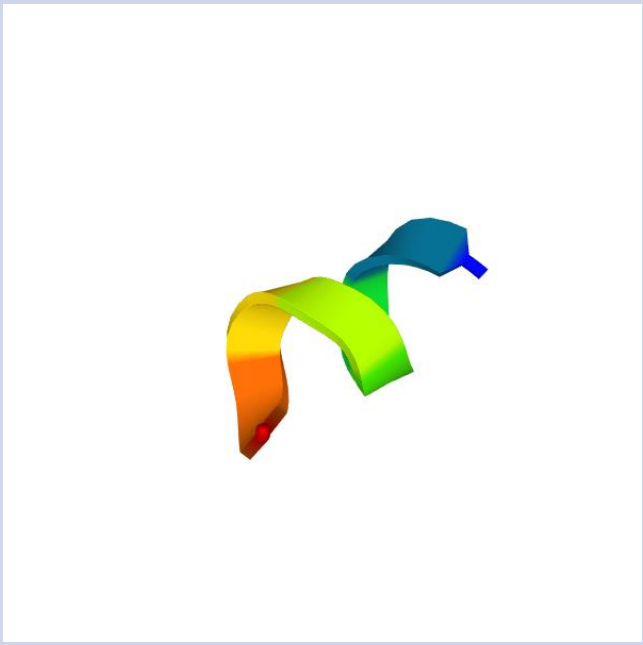
PEP-FOLD

anticancer+toxin+cell penetrate

Group	Non-Redesigned anticancer peptide	Redesigned anticancer peptide
1	 TTMICLTCAR (single helix)	 TTGICLTCCR (single helix)

PEP-FOLD

anticancer

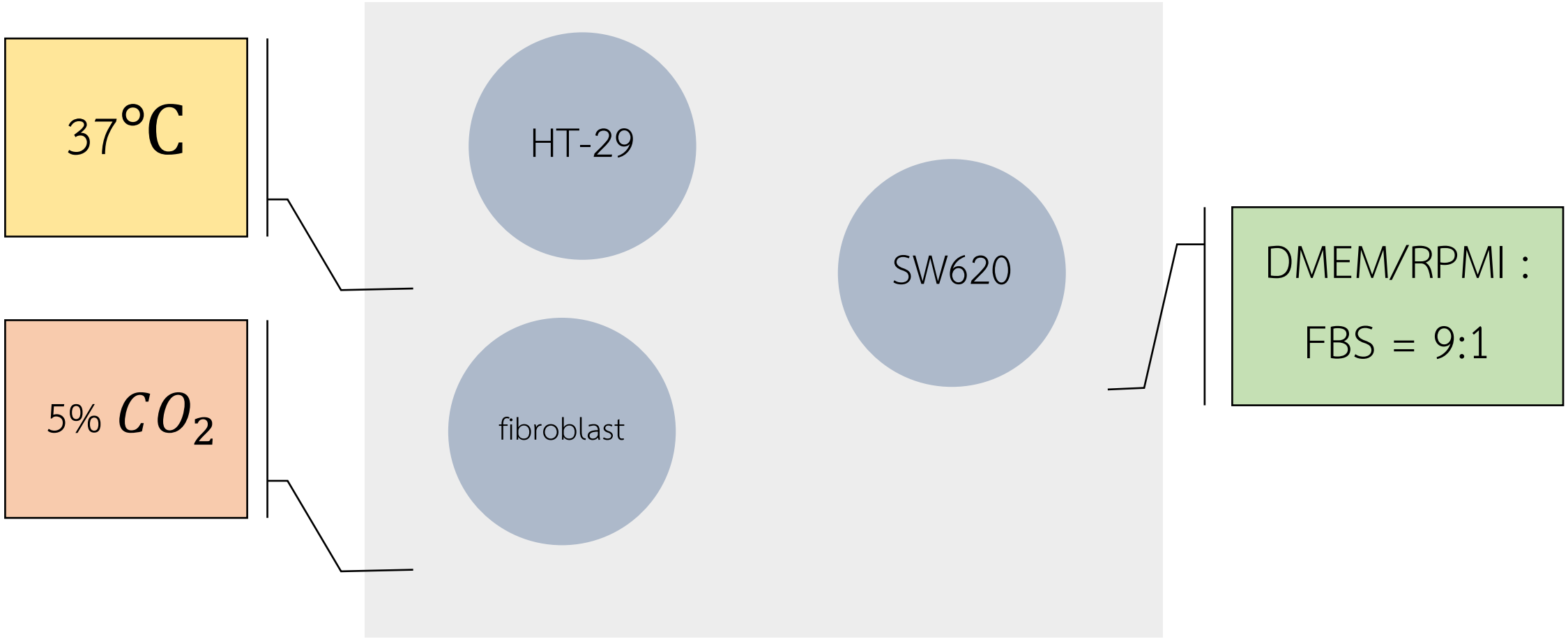
Group	Non-Redesigned anticancer peptide	Redesigned anticancer peptide
2	 VTFVLIAAK (random coiled)	 FTFVLLAAK (single helix)

Process 3

Testing the peptides

Cell Culture

3. Examining the properties



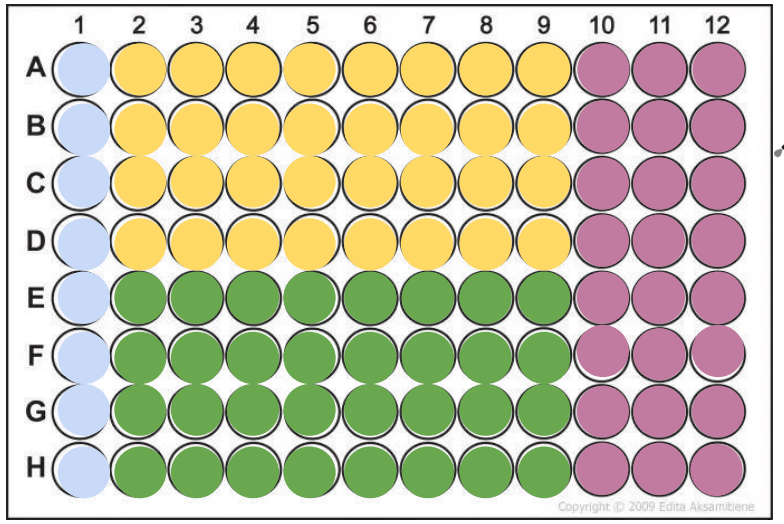
Peptide x4

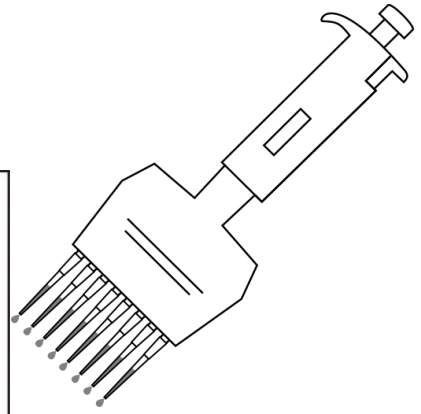
Dissolve with DMSO

peptide 100 mM

Dissolve with media

peptide + media 200μM





Two fold dilution

Keep in the incubator for

24, 48, 72 hours

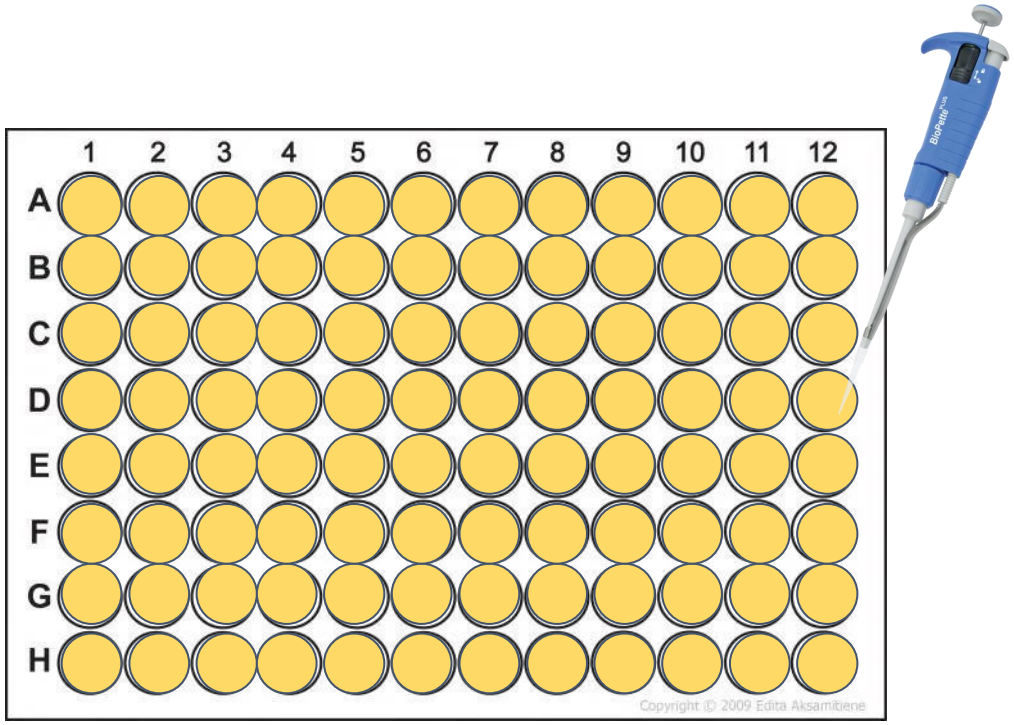
Peptide 1

Peptide 2

DMSO solution

control

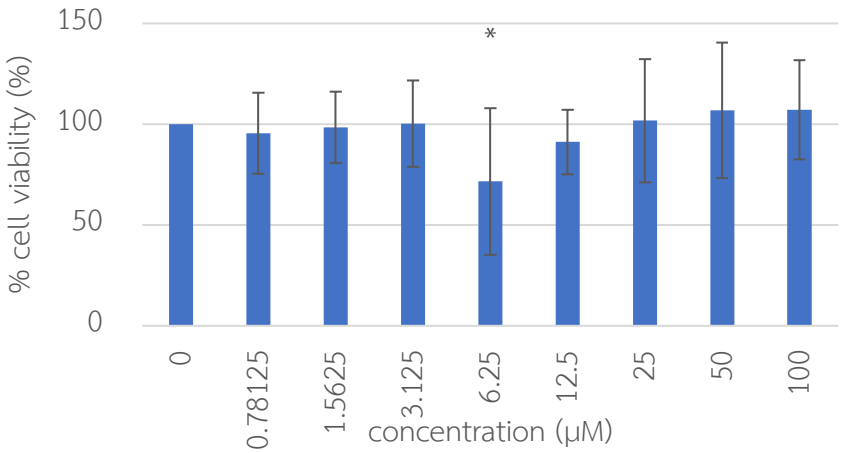
MTT Assay

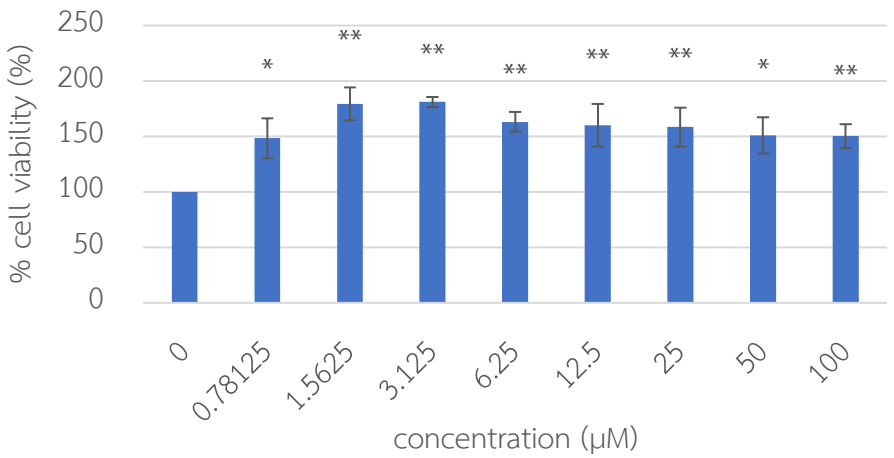


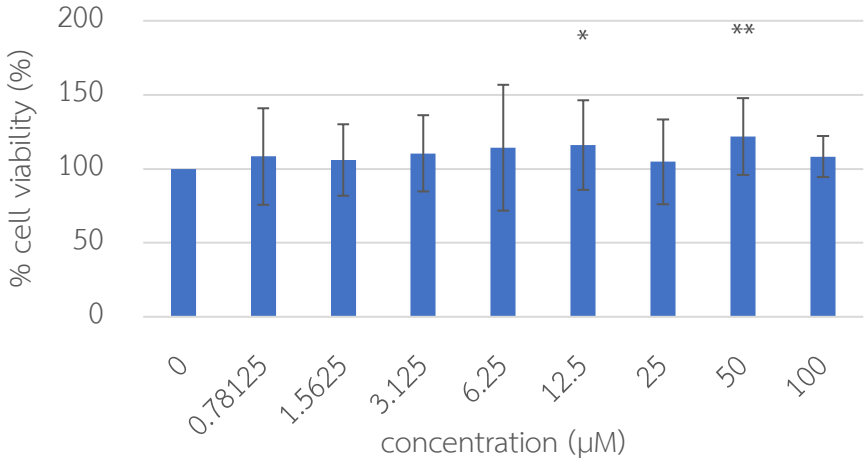
Drop 10μL MTT solution to each well

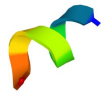
Measure the absorbance by using microplate reader at 570 nm

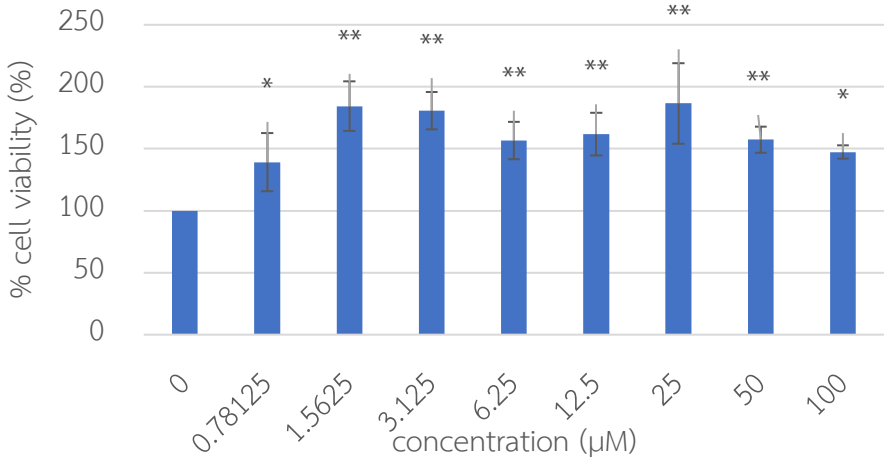
SW620


SW620 TTMICLTCAR
% cell viability (%)
150
100
50
0
*
0
0.78125
1.5625
3.125
6.25
12.5
25
50
100
concentration (μM)

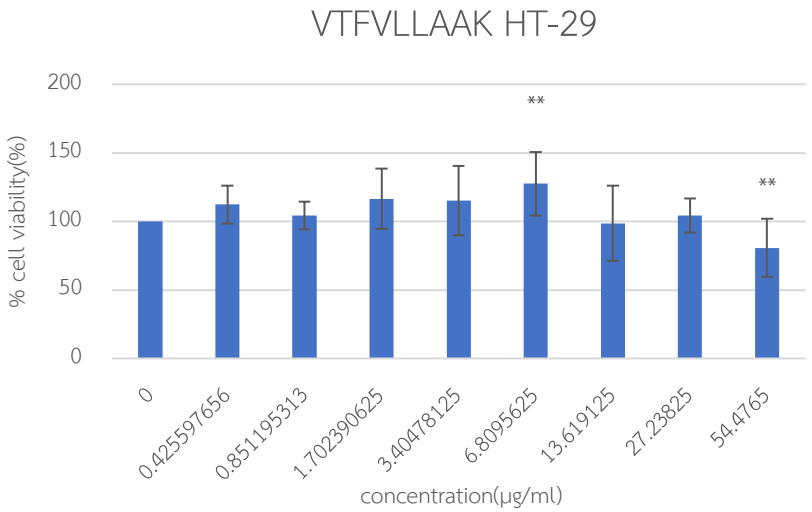

SW620 TTGICLTCCR
% cell viability (%)
250
200
150
100
50
0
*
**
**
**
**
**
*
**
0
0.78125
1.5625
3.125
6.25
12.5
25
50
100
concentration (μM)

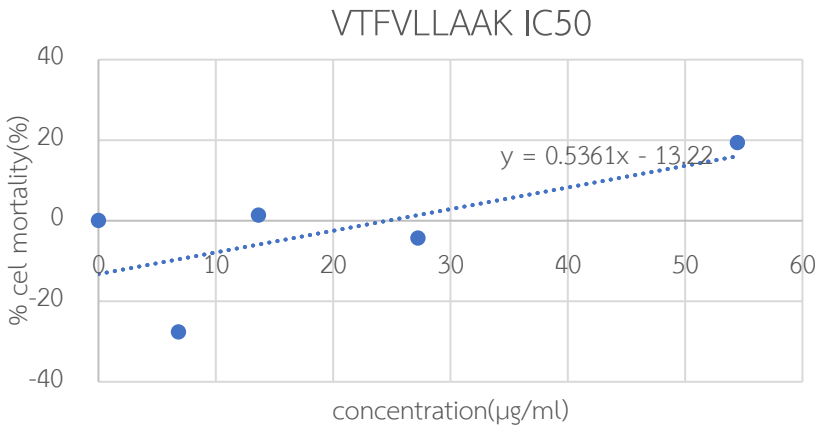

SW620 VTFVLIAAK
% cell viability (%)
200
150
100
50
0
*
**
0
0.78125
1.5625
3.125
6.25
12.5
25
50
100
concentration (μM)



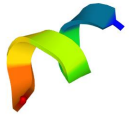

SW620 FTFVLLAAK
% cell viability (%)
250
200
150
100
50
0
*
**
**
**
**
**
**
*
0
0.78125
1.5625
3.125
6.25
12.5
25
50
100
concentration (μM)

VTFVLIAAK
(original)

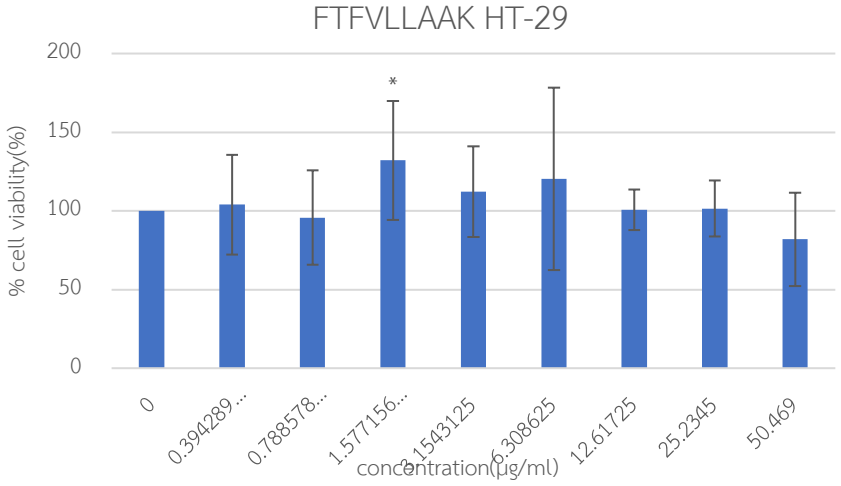


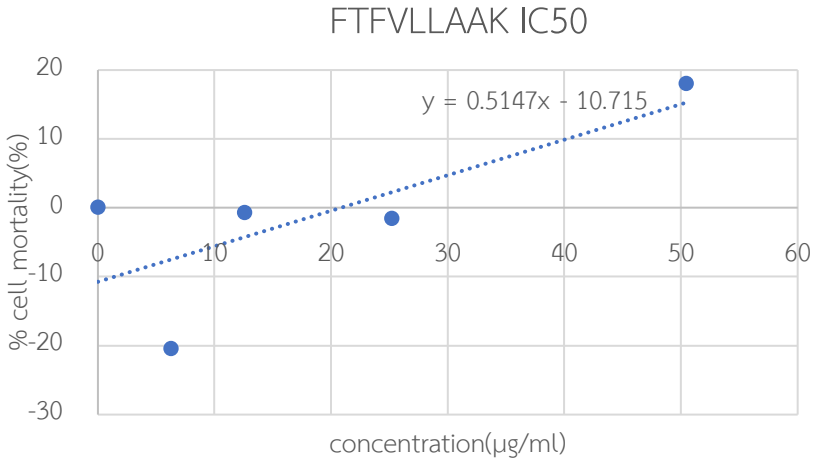


117.93
µg/ml



FTFVLLAAK
(redesigned)





117.96
µg/ml

CONCLUSIONS

- The study of prediction and selection of anticancer peptide from *Cordyceps militaris* by using bioinformatics predicted TTMICLTCAR, TTGICLTCCR, VTFVLIAAK and FTFVLLAAK as effective peptides.
- All of the peptides cannot inhibit the growth of SW620
- The VTFVLIAAK and FTFVLLAAK (redesigned peptides) can inhibit the growth of HT-29, and the IC_{50} of 108.23 and 116.87 μM, (117.93, and 117.96 μg/ml) respectively.
- The demonstrated data could be used as the database for further research on molecular mechanism, and experiment on other cancer cell types.

Acknowledgement

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