GENE ON BIOTECH

GENE ON

Human Protein Microarray

Experiment Analysis Report

User Name	○○○
Protein chip	21,000 인간단백질칩
Sample name	○○○

2024. 1. 11

1-2 Introduction

Introduction

Large-scale protein microarrays are a versatile and sensitive platform for discovery of autoantibody specificity, screening of molecular interactions with proteins and for evaluation of protein-binding reagents which are employed in research and in clinical applications. The HuProt™ v4.0 Human Proteome Microarray provides the largest number of unique, full-length and individually purified human proteins on single microarray slide, allowing thousands of interactions to be profiled in a high-throughput manner. It contains over 21,000 full-length human recombinant proteins that cover more than 80% of the annotated human genome. These full-length recombinant proteins are expressed in the yeast S. cerevisiae, purified, and printed on glass slides in duplicate, along with a control set of proteins like GST, BSA, Histones, etc.

Human Protein Expression

Human clones in a yeast high-copy expression vector are expressed as N-terminal GST-His6 fusion proteins under the control of the galactose-inducible GAL1 promotor. Using a high-throughput yeast expression and purification system, proteins are purified via the GST affinity tag under conditions that retain their activities. A subset of purified protein samples is assayed to ensure that the proteins have the expected molecular weight. The purified proteins are transferred to 384-well plates and stored at -80℃. The proteins include all major classes: secreted, nuclear, membrane, metabolic, transcription factors, kinases, signal transduction, cell death, cell communication, etc.

HuProt™ v4.0 Microarray Manufacture

A non-contact microarray printer with 48 quill-tip pins is used to produce the HuProt arrays. Each of the purified proteins along with a set of control proteins are printed in duplicate on 75mm x 25mm SuperEpoxy 3 glass slides manufactured by ArrayIt Corp. The printing of these arrays is carried out in a cold room under dust-free conditions in order to preserve the integrity of both samples and printed microarrays. The HuProt™ v4.0 is designed to accommodate 50,000 spots. Before releasing protein microarrays for use, each lot of slides is subjected to a rigorous quality control procedure, including (i)a visual inspection of all the printed slides to check for scratches, fibres and merging and (ii) immunostaining against GST-tags to confirm the print quality.

2-1 Experiment Protocol

Blocking

Add 3.0 mL of blocking solution (2% BSA in PBST) to each compartment of a 4-well plate. Carefully use finenosed tweezers to remove one microarray from the plastic slide holder resting on dry ice. Immediately submerge the protein microarray, active side up, in a compartment containing blocking solution. Incubate with gentle shaking for 5 min. Carefully pour off the blocking buffer or remove it by aspiration. Add 3.0 mL fresh blocking buffer to the well of the plate and incubate the microarray with gentle shaking for 2 hrs at 4°C.

Assay:...

Add 9 µg ...

Assay: ...

Add 1 µg of ...

2-2 Experiment Method

Identification of ... HuProt™ microarrays.

To identify ...

Name	Brand	Cat#	Final Concentration

3-1 Sample Optimization

TEST

- CL01 에 대한 SDS-PAGE 및 Dot blot 진행

Dot Blot

Materials

- Nitrocellulose membrane, ...

Method

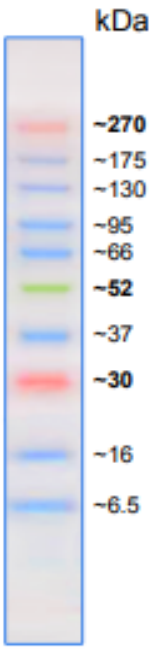
- Load 1 μL ...

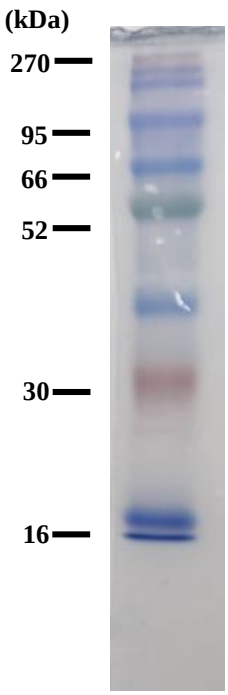
3-1 Sample Optimization

SDS-PAGE result

- ...
- ...

SDS-PAGE Results image





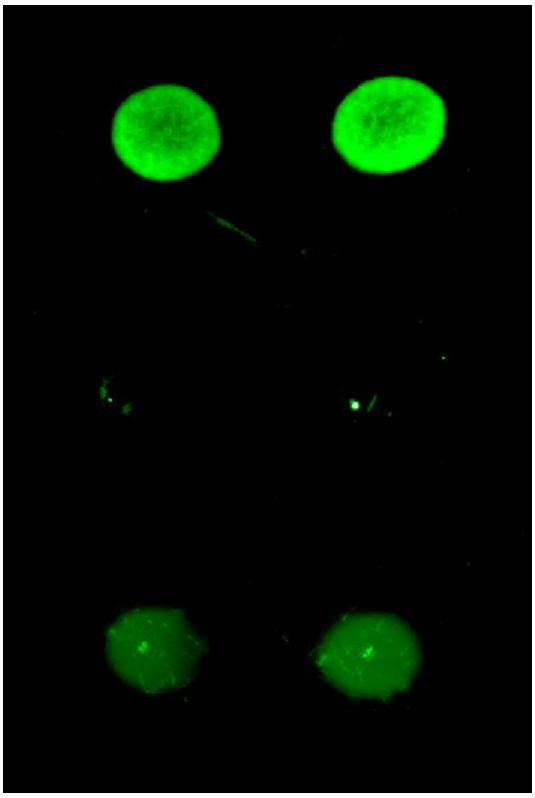
Sample 5 ug loading
Reducing condition
12% SDS-PAGE

3-1 Sample Optimization

Dot blot result

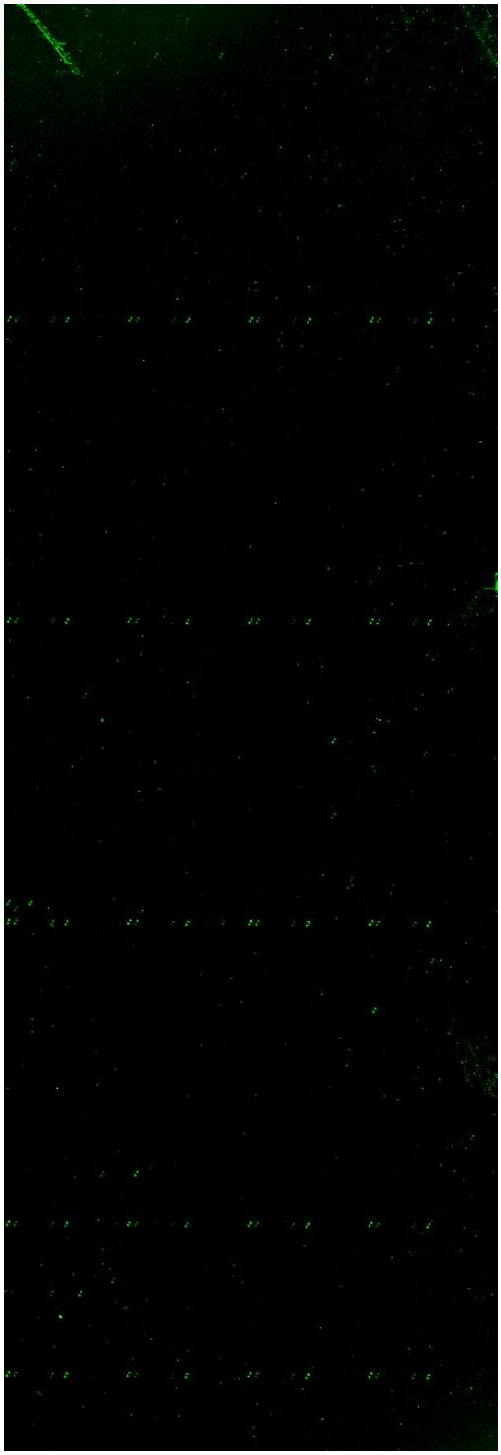
- ...

Dot-Blot Results image



4-1 Experiment: 결과 개요

Protein Microarray
Experiment Result image



Protein microarray

- …

Result

- **분석 기준**

1. 전체 21,000여개의 단백질 …

2. 이후 노이즈 등을 제거하고, A-score …
   (A=Affinity, S=Specificity)

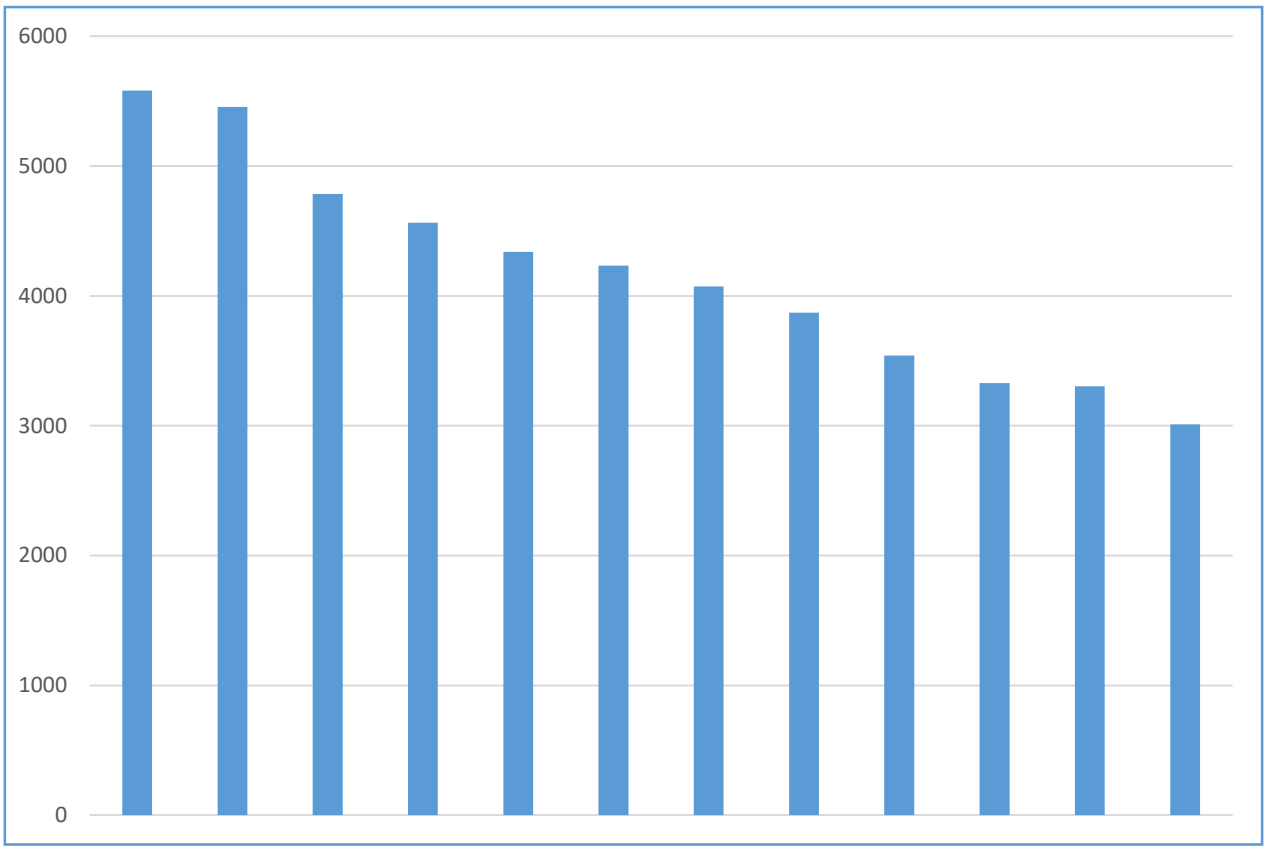
- **분석 결과**

1. A-score 상 총 …
2. 그 중 1개의 결합은 …

4-2 Experiment: 결합 단백질 정보

- ...
- ...

Signal intensity 그래프



4-2 Experiment: 결합 단백질 정보

Signal intensity, A / S-score

Name	Signal intensity	A-score	S-score
	1182.5		8.4
	11376.5		5.3
			0.6
			2.0
			2.3
			0.0
			0.2
			0.1
			0.0
			0.7
			0.4
			0.4
			0.4
			0.2
			0.3
			0.4
			0.6
	3541		0.4
			0.0
			0.5
	3010		0.3

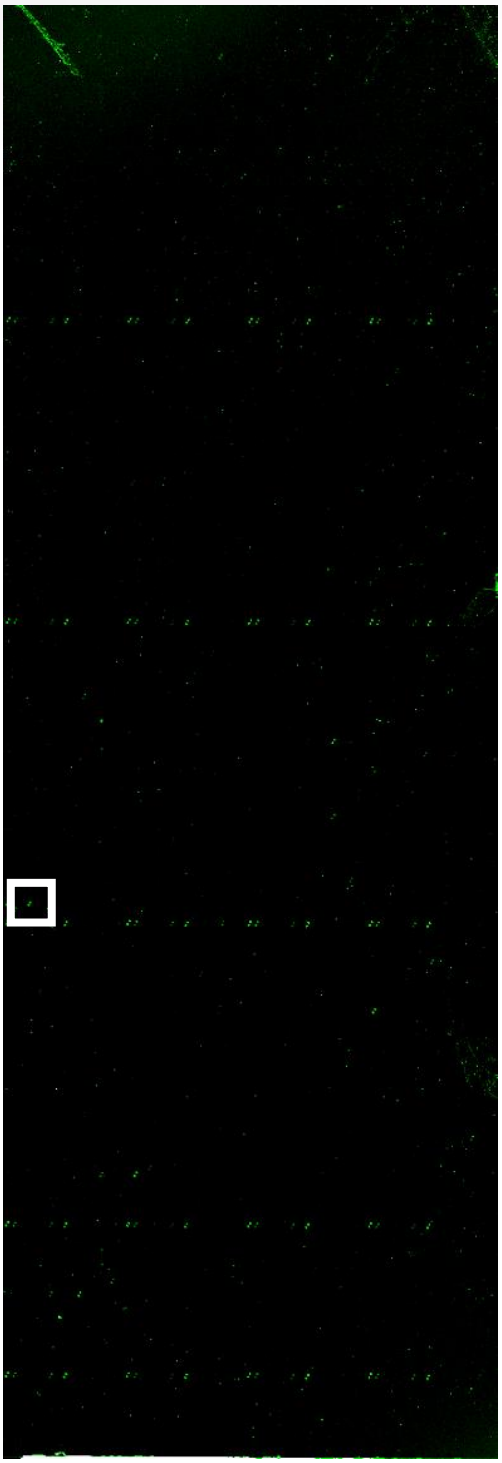
A-score: 결합 점수.

A-score 는 …

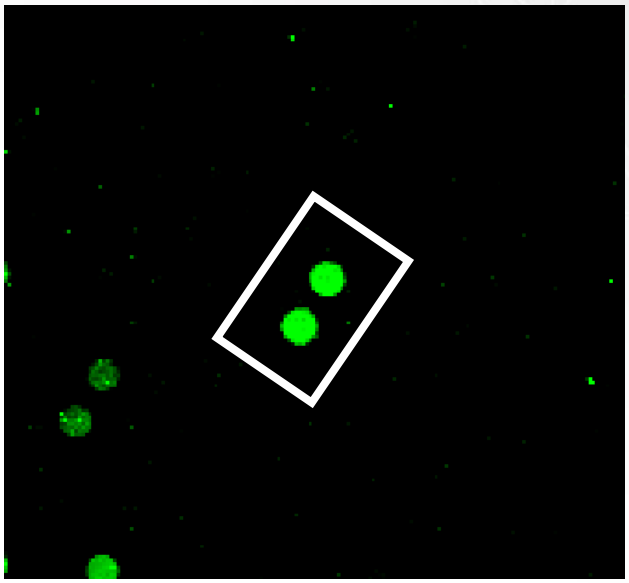
S-score: 특이성 점수.

S-score는 …

4-3 Experiment: 결합 단백질 상세정보



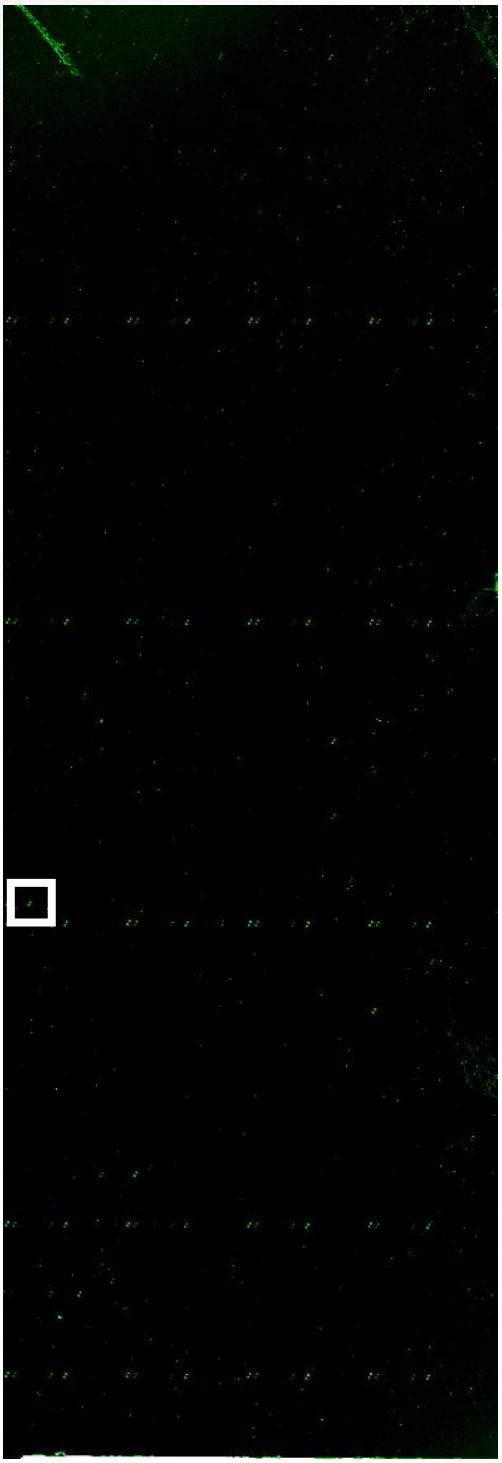
Signal image



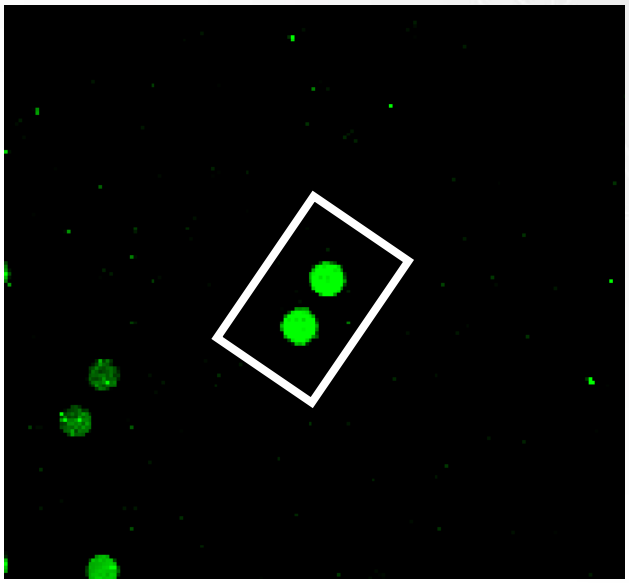
Protein information

Gene Title	...
Protein	...
Molecular function	
Biological process	
Cellular location	
Sequence	
Additional information	

4-3 Experiment: 결합 단백질 상세정보



Signal image



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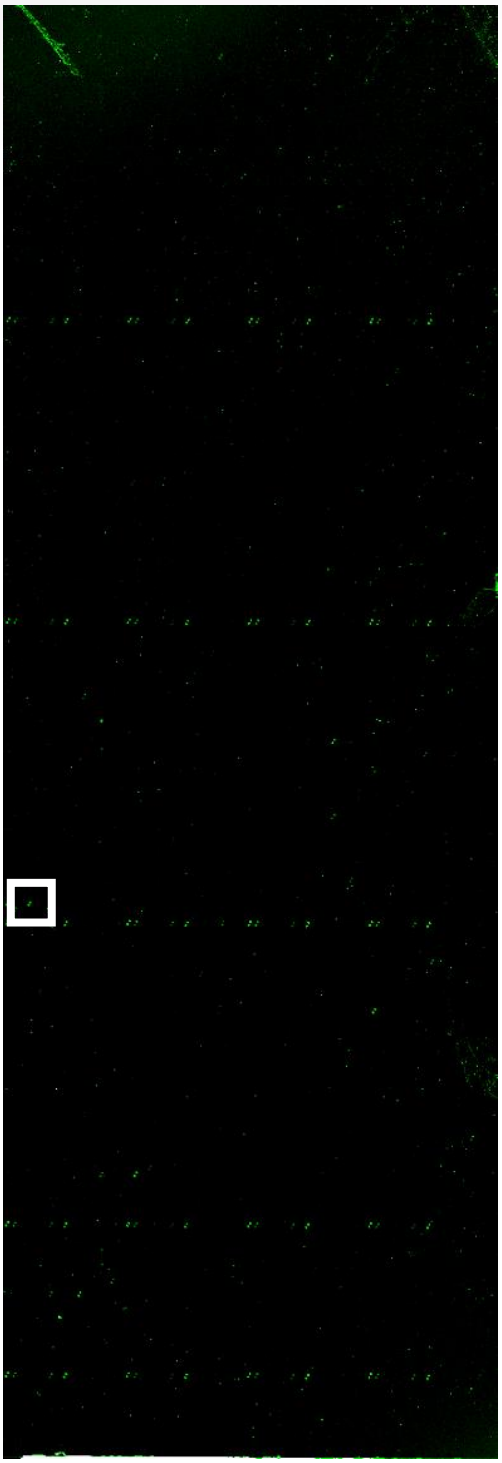

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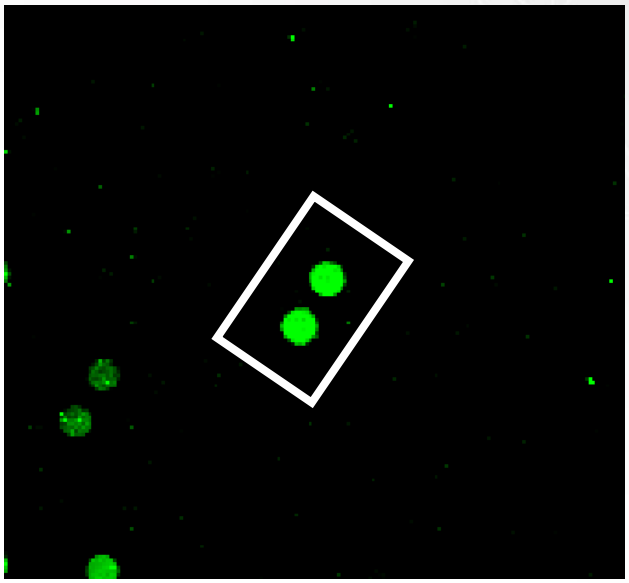

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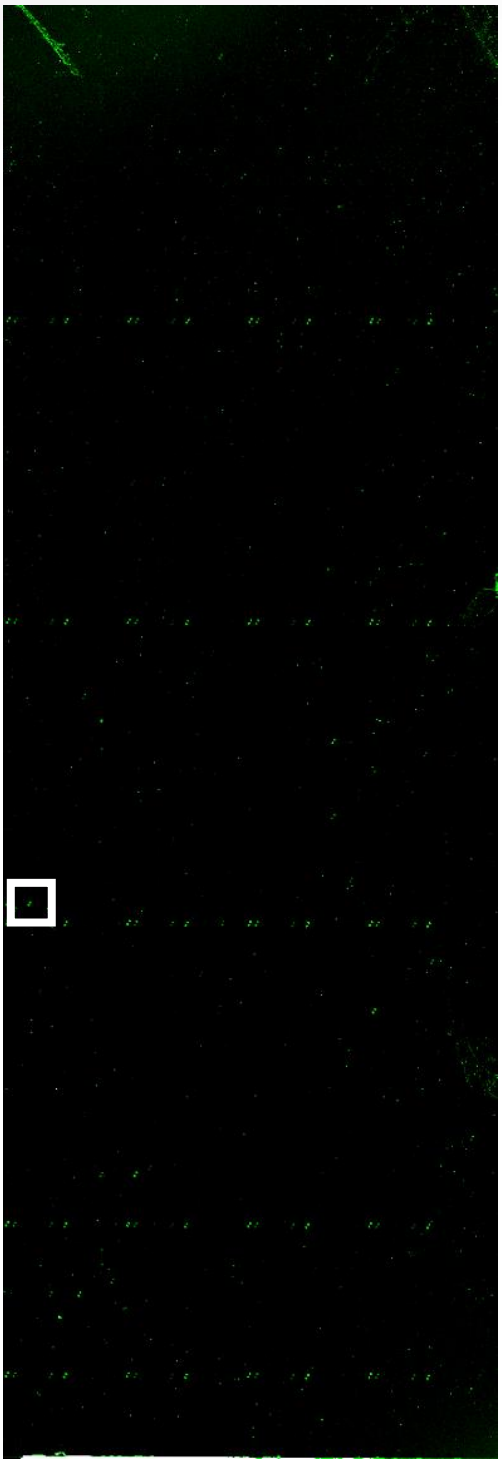
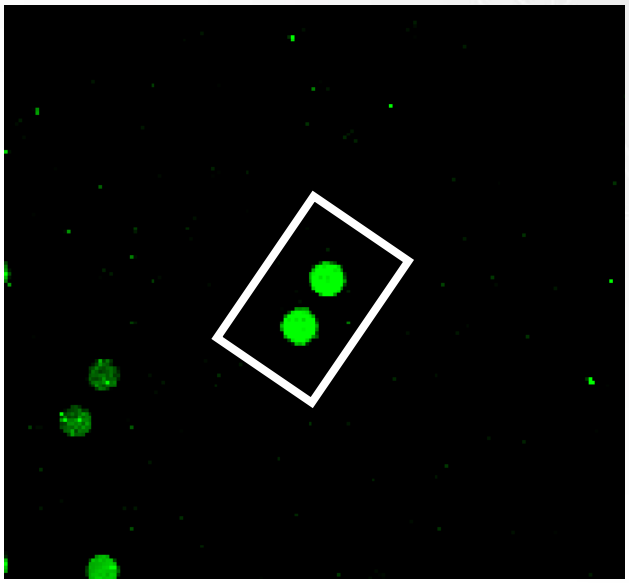


Signal image



Protein information

Gene Title	...
Protein	...
Molecular function	
Biological process	
Cellular location	
Sequence	
Additional information	

4-4 Experiment: 결합 단백질 정보

Nodes:

Network nodes represent proteins

splice isoforms or post-translational modifications are collapsed, i.e. each node represents all the proteins produced by a single, protein-coding gene locus.

Node Color

colored nodes:
query proteins and first shell of interactors

white nodes:
second shell of interactors

Node Content

empty nodes:
proteins of unknown 3D structure

filled nodes:
some 3D structure is known or predicted

Edges:

Edges represent protein-protein associations

associations are meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding to each other.

Known Interactions

from curated databases

experimentally determined

Predicted Interactions

gene neighborhood

gene fusions

gene co-occurrence

Others

textmining

co-expression

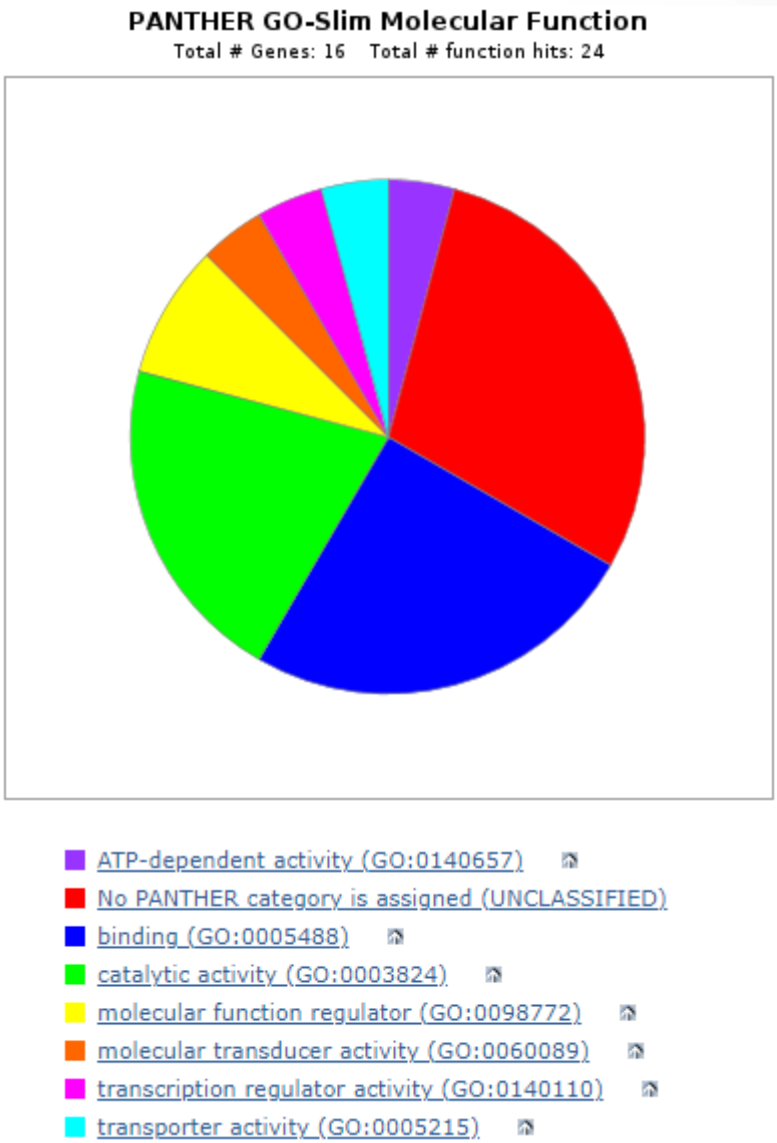
protein homology

Link: https://string- db.org/

4-4 Experiment: 결합 단백질 정보

결합 단백질 분석

- PANTHER 분석은 샘플과 결합한 단백질들의 어떤 특징을 가지는지 확인하는 것임.
- 결합단백질들의 Molecular function / Biological process / Cellular component 등의 Ontology 분석이 포함됨.



4-4 Experiment: 결합 단백질 정보

결합 단백질 분석 (PANTHER classification system)

- PANTHER 분석은 샘플과 결합한 단백질들의 어떤 특징을 가지는지 확인하는 것임.
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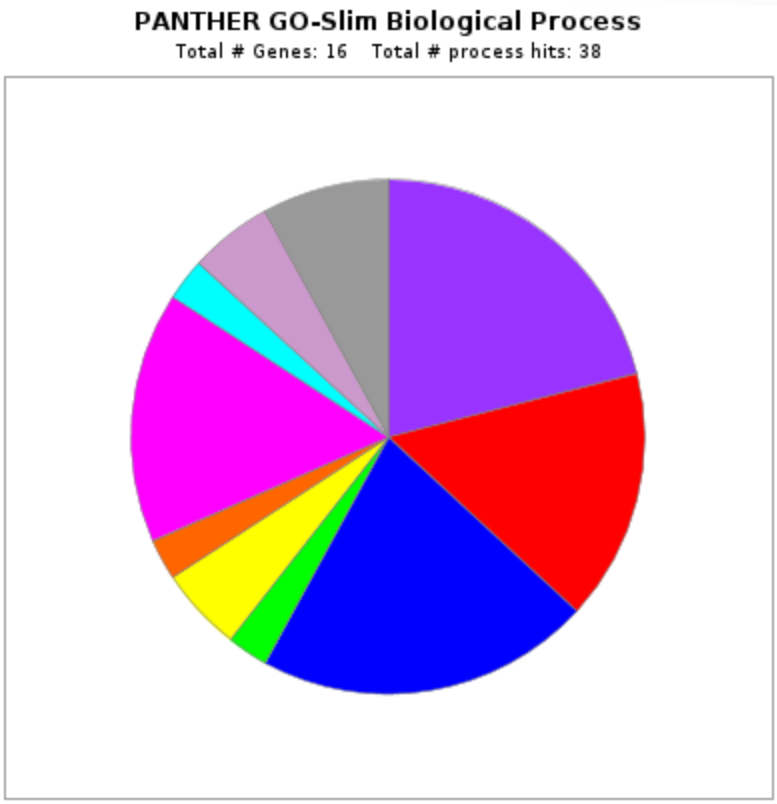

4-4 Experiment: 결합 단백질 정보

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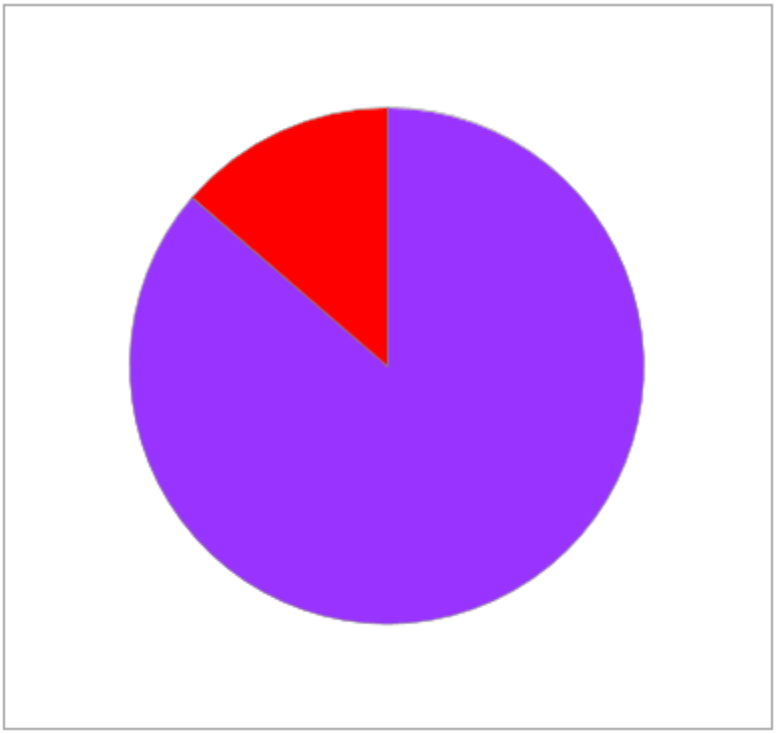

4-4 Experiment: 결합 단백질 정보

전체 결합 단백질 리스트

...

Disease related

	SPARC related modular calcium binding 1(SMOC1)
	Microphthalmia with limb anomalies,
	cytochrome b561(CYB561)
	Orthostatic hypotension 2,
	laminin subunit beta 2(LAMB2)
	Pierson syndrome, Nephrotic syndrome, type 5, with or without ocular abnormalities,
	prolyl endopeptidase like(PREPL)
	Myasthenic syndrome, congenital, 22,
	propionyl-CoA carboxylase subunit alpha(PCCA)
	Propionicacidemia,

4-4 Experiment: 결합 단백질 정보

전체 결합 단백질 리스트

...

Pathways

	fibroblast growth factor 2(FGF2)
	EGFR tyrosine kinase inhibitor resistance, MAPK signaling pathway, Ras signaling pathway, Rap1 signaling pathway, Calcium signaling pathway, PI3K-Akt signaling pathway, Signaling pathways regulating pluripotency of stem cells, Regulation of actin cytoskeleton, Kaposi sarcoma-associated herpesvirus infection, Pathways in cancer, Proteoglycans in cancer, Chemical carcinogenesis - receptor activation, Melanoma, Breast cancer, Gastric cancer,
	laminin subunit beta 2(LAMB2)
	PI3K-Akt signaling pathway, Focal adhesion, ECM-receptor interaction, Toxoplasmosis, Amoebiasis, Human papillomavirus infection, Pathways in cancer, Small cell lung cancer,
	laminin subunit gamma 1(LAMC1)
	PI3K-Akt signaling pathway, Focal adhesion, ECM-receptor interaction, Prion disease, Toxoplasmosis, Amoebiasis, Human papillomavirus infection, Pathways in cancer, Small cell lung cancer,
	olfactory receptor family 2 subfamily D member 3(OR2D3)
	Olfactory transduction,
	propionyl-CoA carboxylase subunit alpha(PCCA)
	Valine, leucine and isoleucine degradation, Glyoxylate and dicarboxylate metabolism, Propanoate metabolism, Metabolic pathways, Carbon metabolism,
	serine/threonine kinase 3(STK3)
	MAPK signaling pathway, Hippo signaling pathway, Hippo signaling pathway - multiple species,
	solute carrier family 27 member 2(SLC27A2)
	PPAR signaling pathway, Peroxisome, Insulin resistance,

4-5 Discussion

· 000샘플을 21,000가지 단백질이 심겨진
인간단백질칩을 이용하여 테스트했습니다.

· 실험 결과, …

· …

· …

· …

· …

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