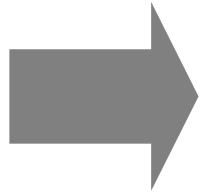


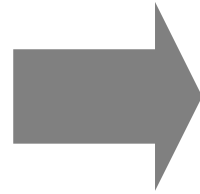
奈良先端科学技術大学院大学
遺伝子教育研究センター
生体情報学

森 浩禎

細胞
生命体



分子



細胞
生命体

細胞の概念 → 遺伝子の概念 → 分子機構 → 個々の機構から全体の構築

形態観察

遺伝学
生化学

分子生物学

ゲノム生物学
バイオインフォマティクス
シミュレーション
システムズバイオロジー

育種

遺伝子工学

生命体の設計

Functional classification of ORFs

Total number of ORFs = **4,388 orfs / 4,641,433 bp**



		2088	49	(%)
1	<u>Uncharacterized</u>			
2	Transport/Binding proteins	410	10	
3	Energy metabolism	388	9	
4	Cell envelope	212	5	
5	Outer membrane	157	4	
6	Translation	145	3	
7	Central intermediary metabolism	138	3	
8	Biosynthesis of cofactors, prosthetic group, carriers	127	3	
9	Regulatory functions	119	3	
10	Cellular processes	112	3	
11	Amino acid biosynthesis	107	3	
12	Purines, pyrimidines, nucleosides and nucleotides	107	3	
13	Replication	93	2	
14	Fatty acid/Phospholipid metabolism	45	1	
15	Transcription	38	1	

Escherichia coli

Clones



Molecular mechanism

Gene disruption



Gene Function

**DNA microarray
Protein array etc.**



**Gene network
Protein-protein interaction**

Bioinformatics

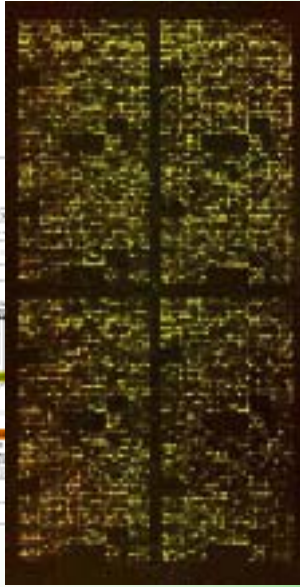


Analysis, Prediction

**Complete understandings of a cell
Cell simulation**

Transcriptome

DNA micro array

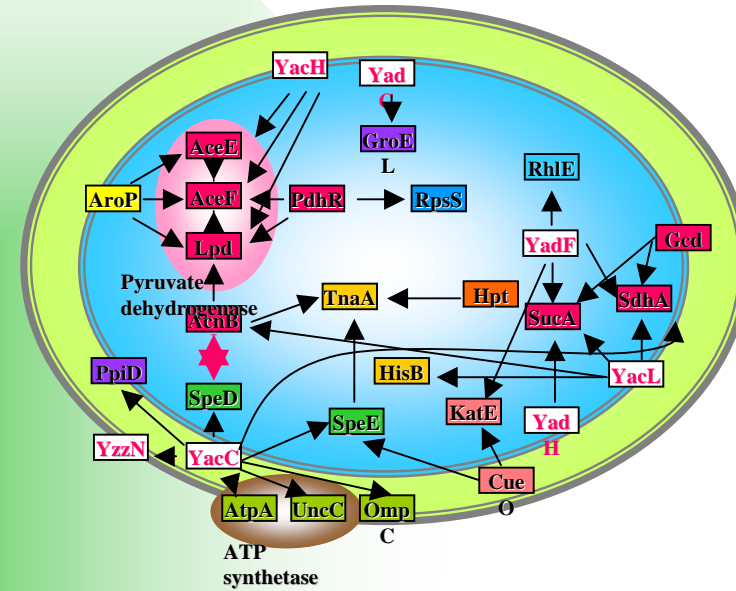


-ome analyses

Life
Life

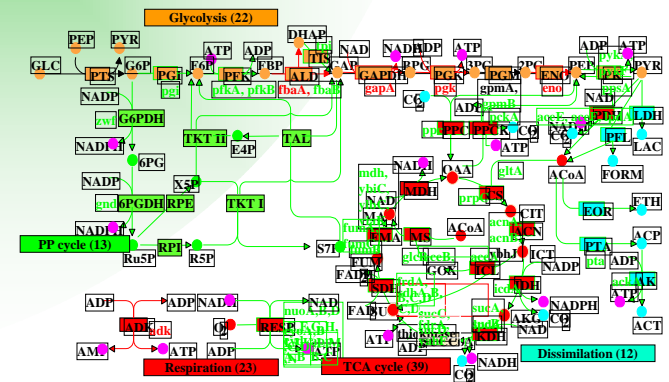
Interactome

Protein-protein interaction



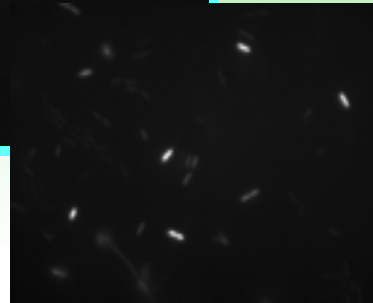
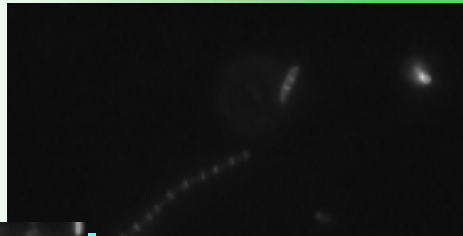
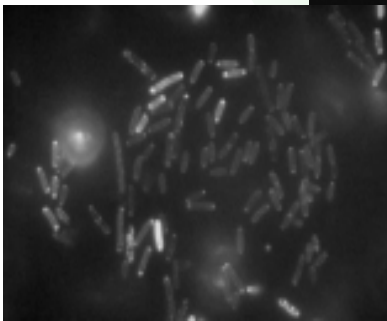
Metabolome

E. coli energy metabolic pathway



Physiome

Protein localization
Functional network

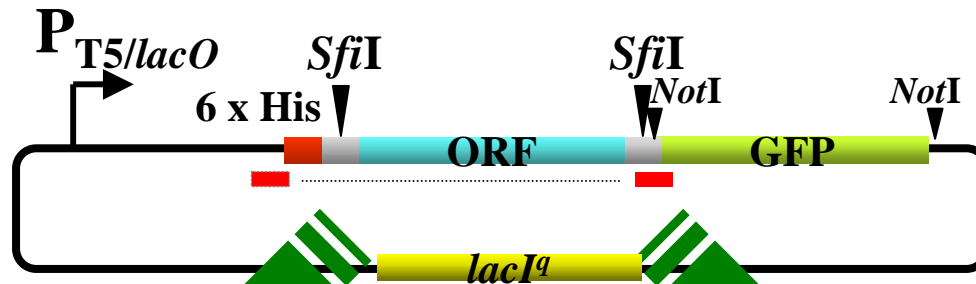




PCR amplification



Archive vector



DNA

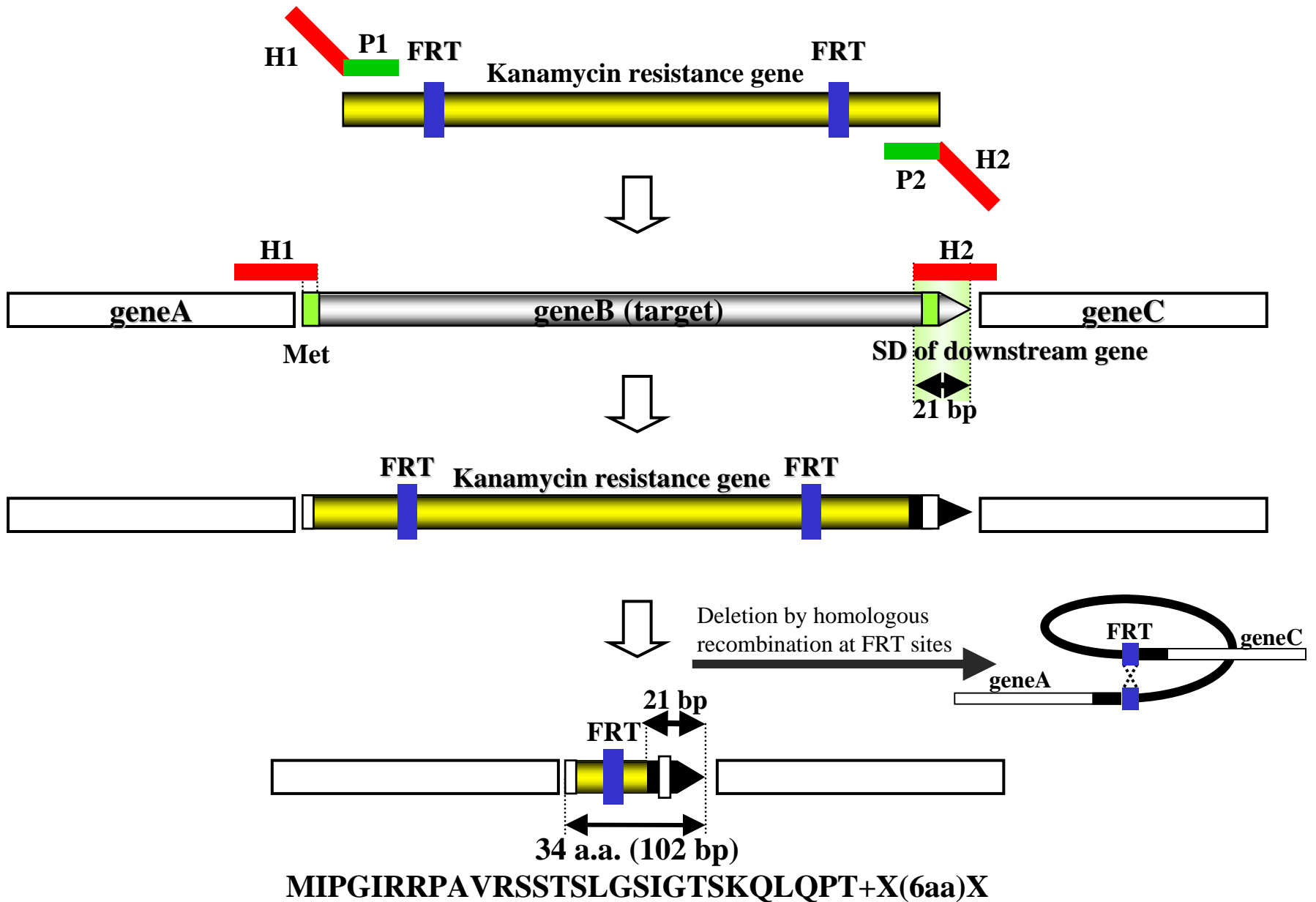
DNA fragment
for DNA microarray

Proteins

3D structure
Protein-protein interaction

Functional analysis
Molecular genetics

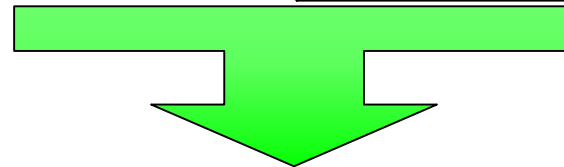
Design of single gene deletion mutants



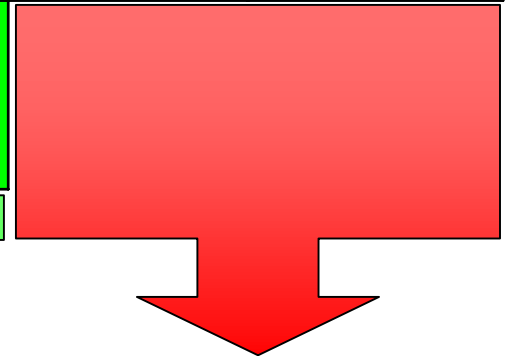
Progress of “KO (Knock Ot) Collection”

gene	<i>E. coli</i> K-12	Tested	Number of candidates obtained			
			more than 2 out of 8	just 1	0	
Chromosomal structure checking by PCR						
Known-gene	2366	1512	179	1185	19	129
y-gene	2024	1678	166	1450	22	40
Total	4390	3190	345	2635	41	169
Evaluation			Quasi-non-essential	Non-essential	Quasi-essential	Essential

KO
Collection

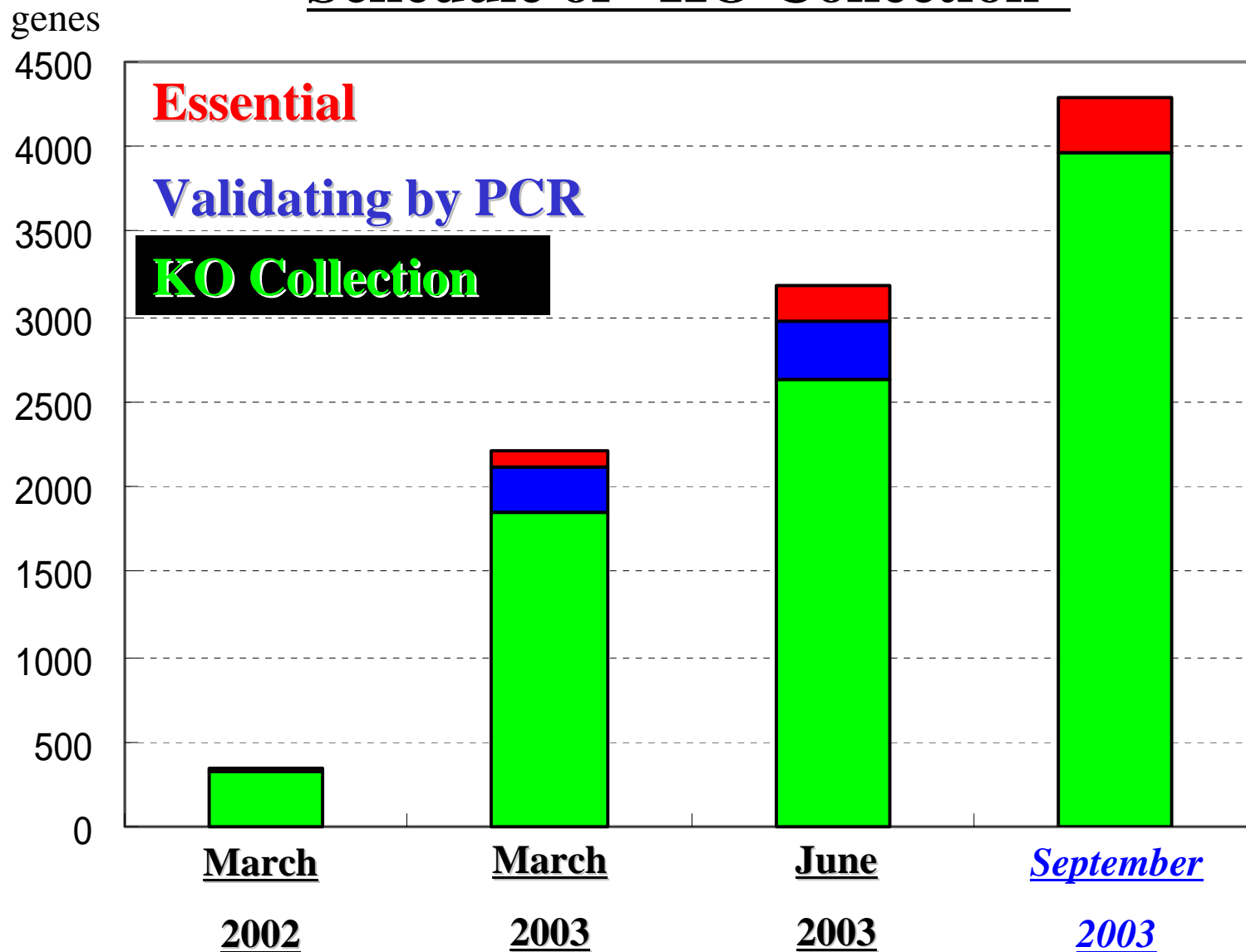


non-essential



essential

Schedule of “KO Collection”



“KO Collection” will be open to IECA members via the database, “GenoBase” (<http://ecoli.aist-nara.ac.jp/>).



ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-A 0010

ANB-C 0010

ANB-A 0010

ANB-A 0010

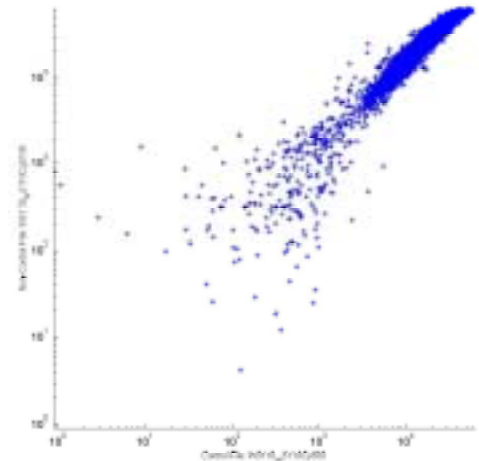
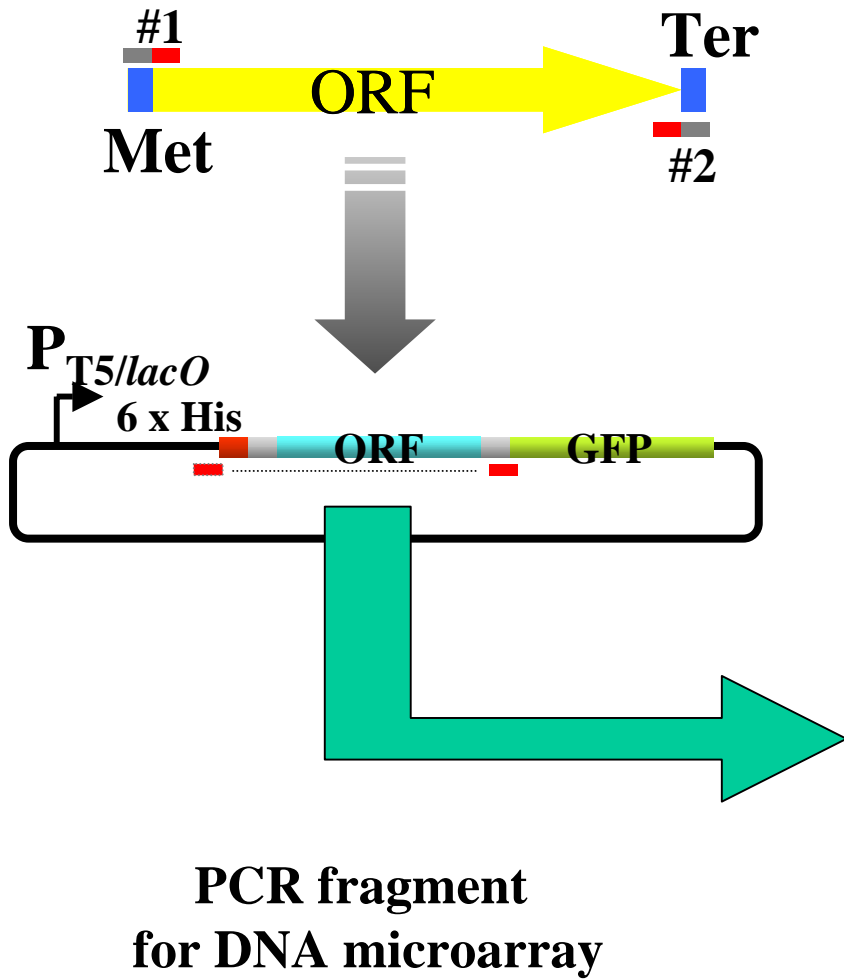
ANB-A 0010

ANB-A 0010

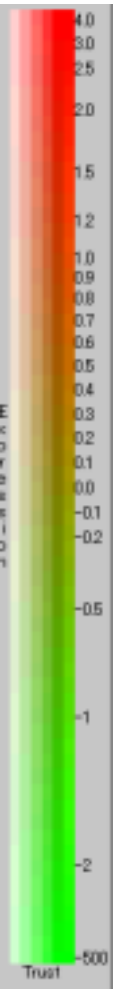
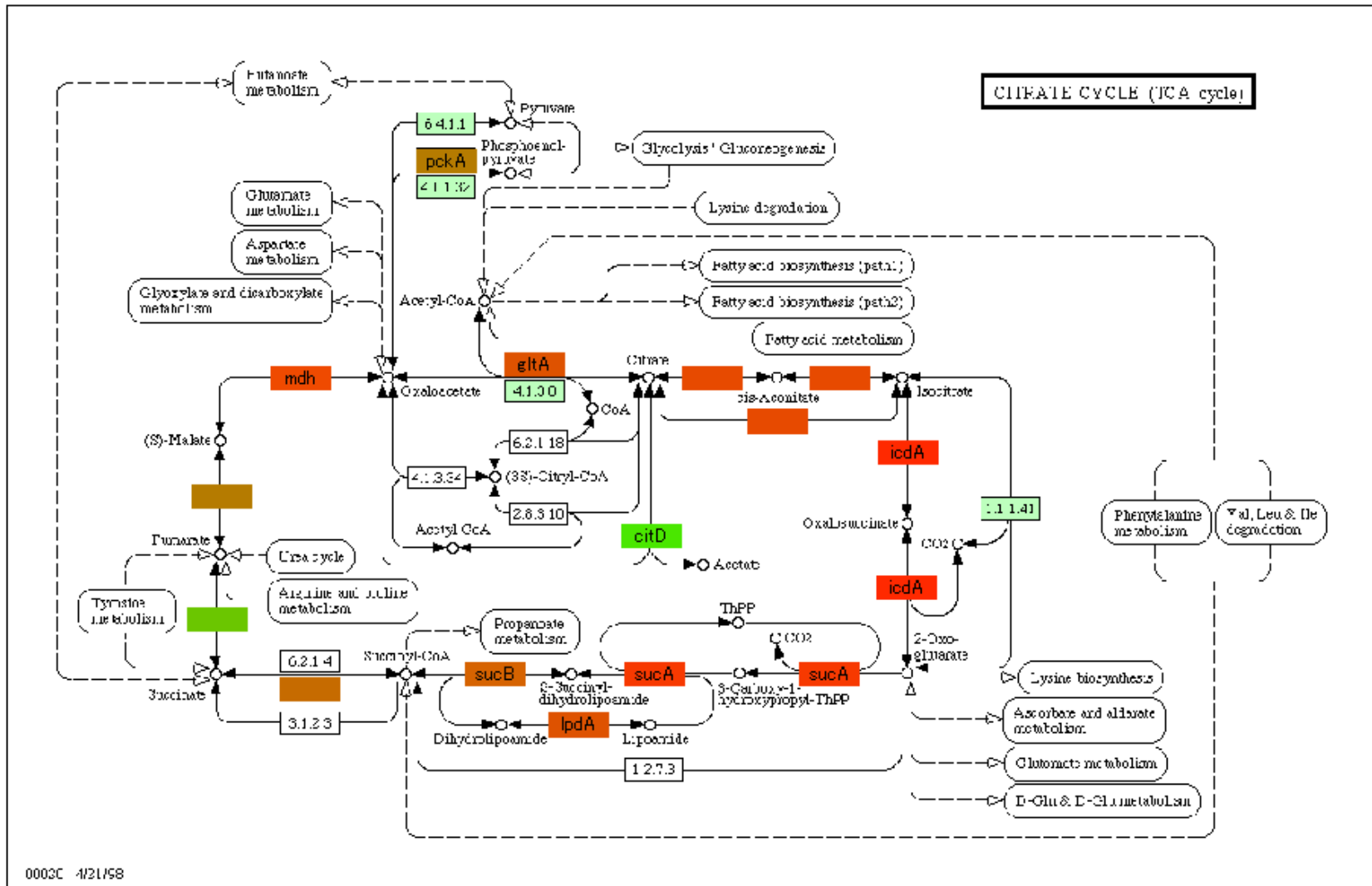
ANB-A 0010

ANB-A 0010

Transcriptome



TCA cycle



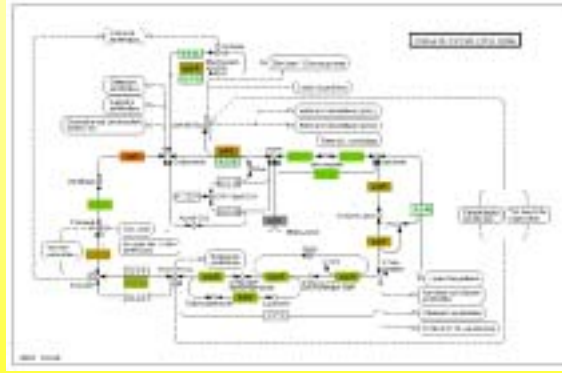
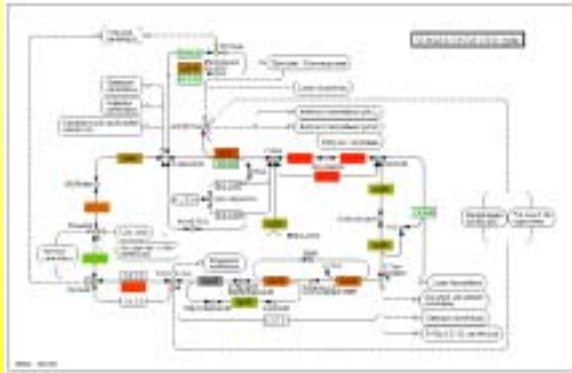
Time course of dynamic change in a cell

TCA cycle as an example



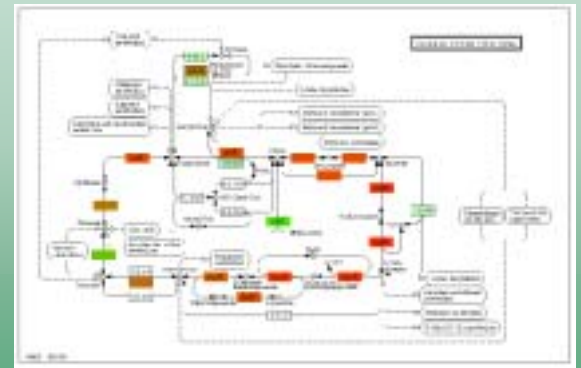
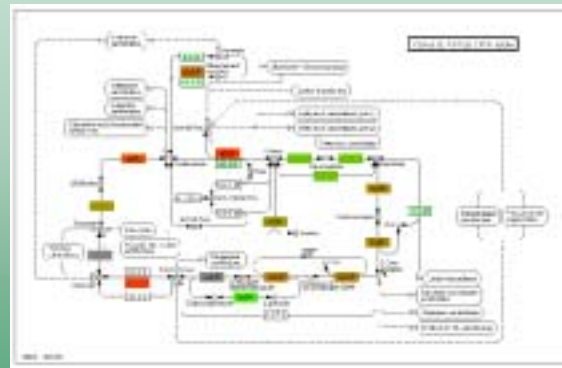
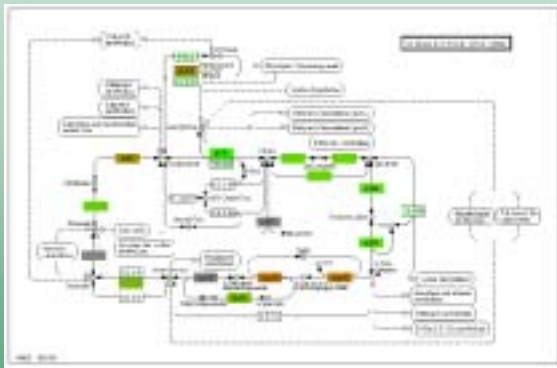
Taku Oshima

Condition A

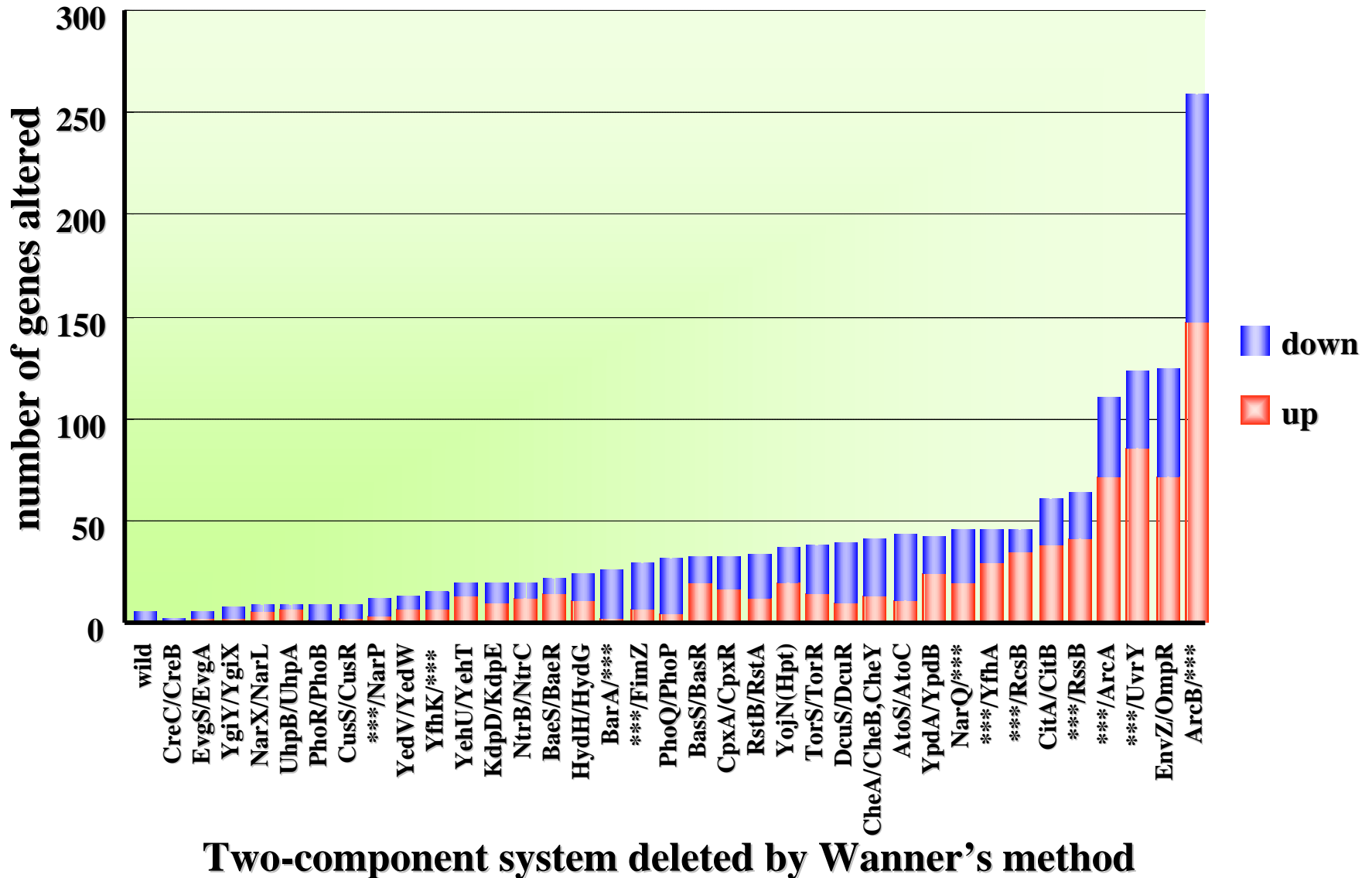


Time course

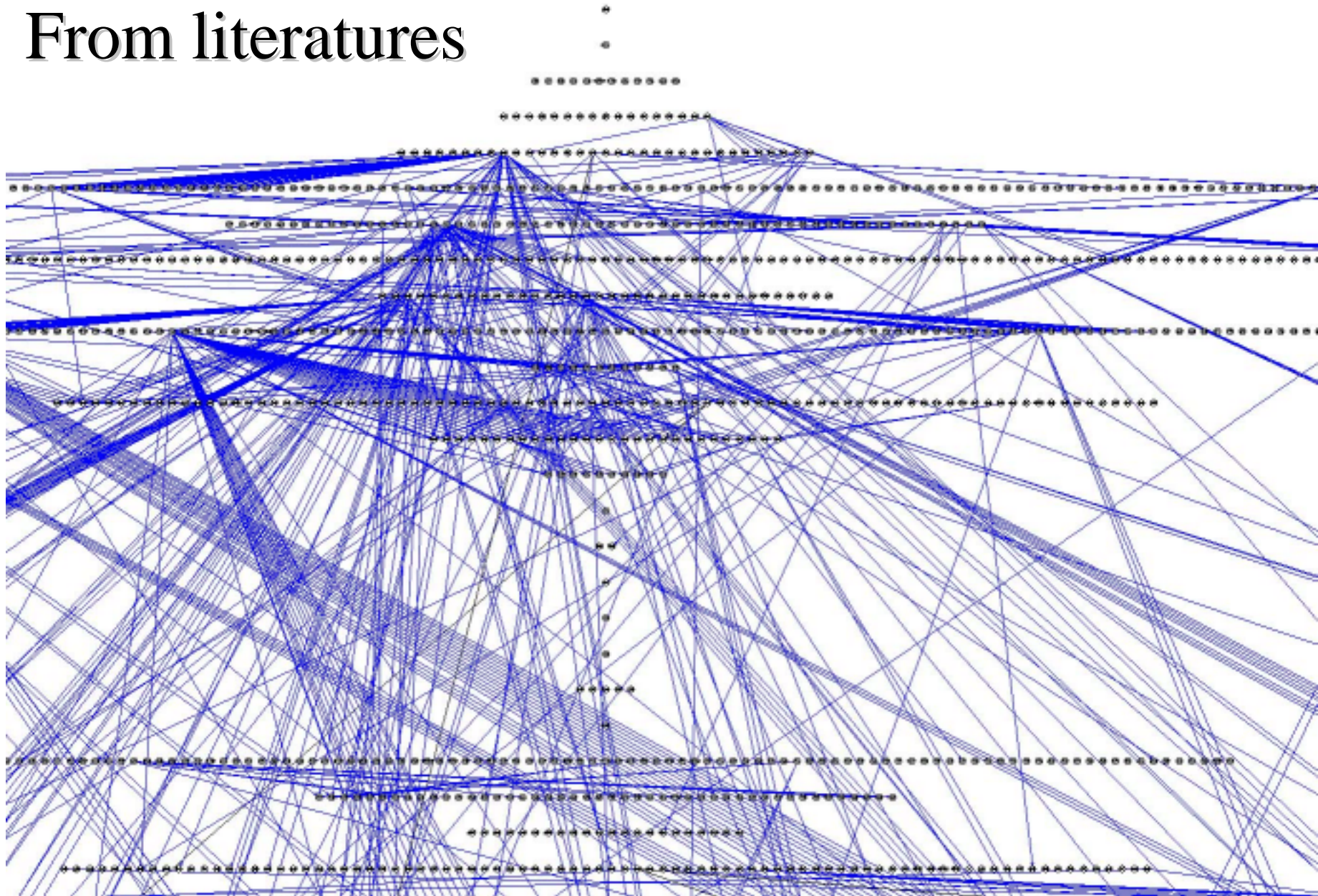
Condition B



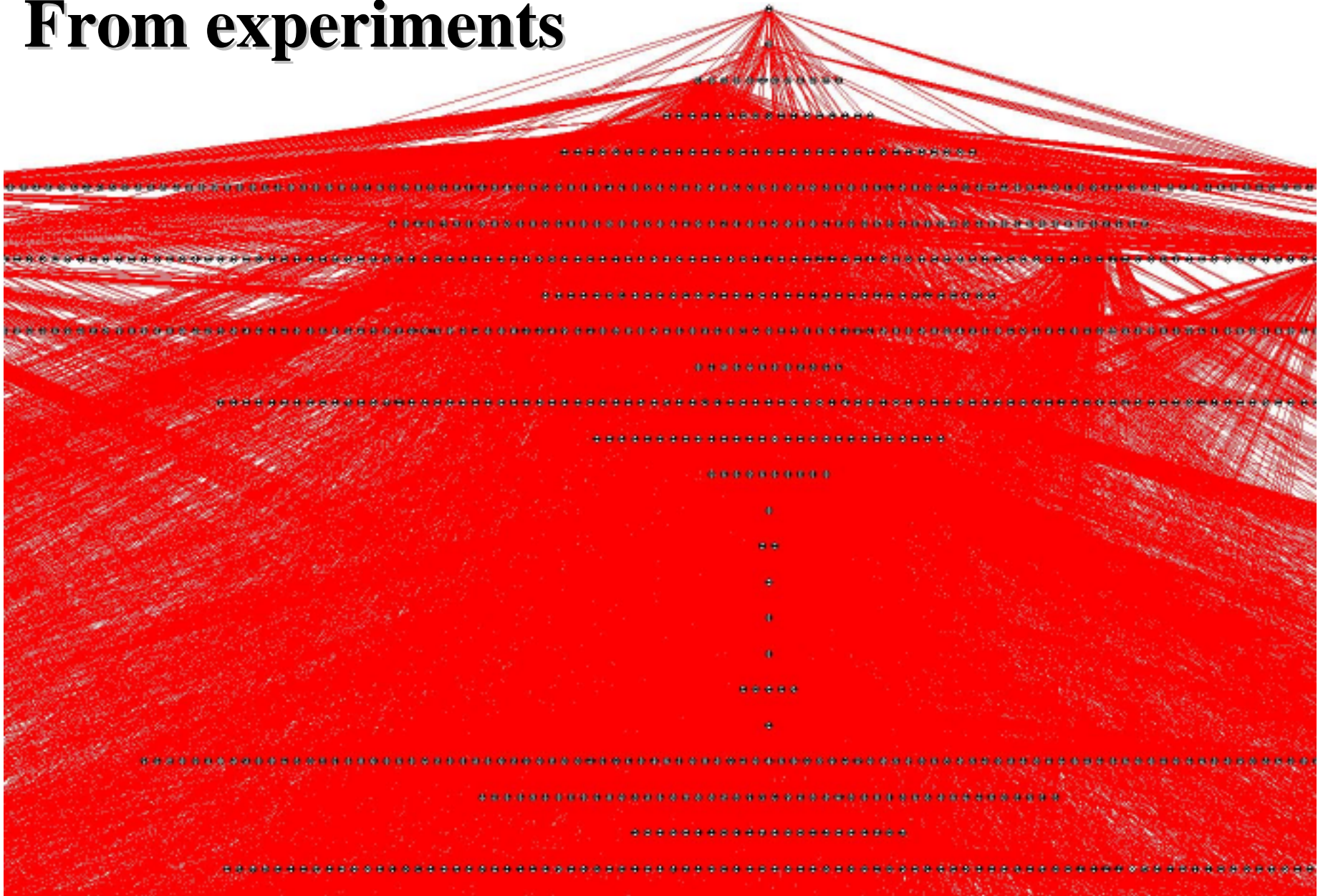
Deletion of two-component systems



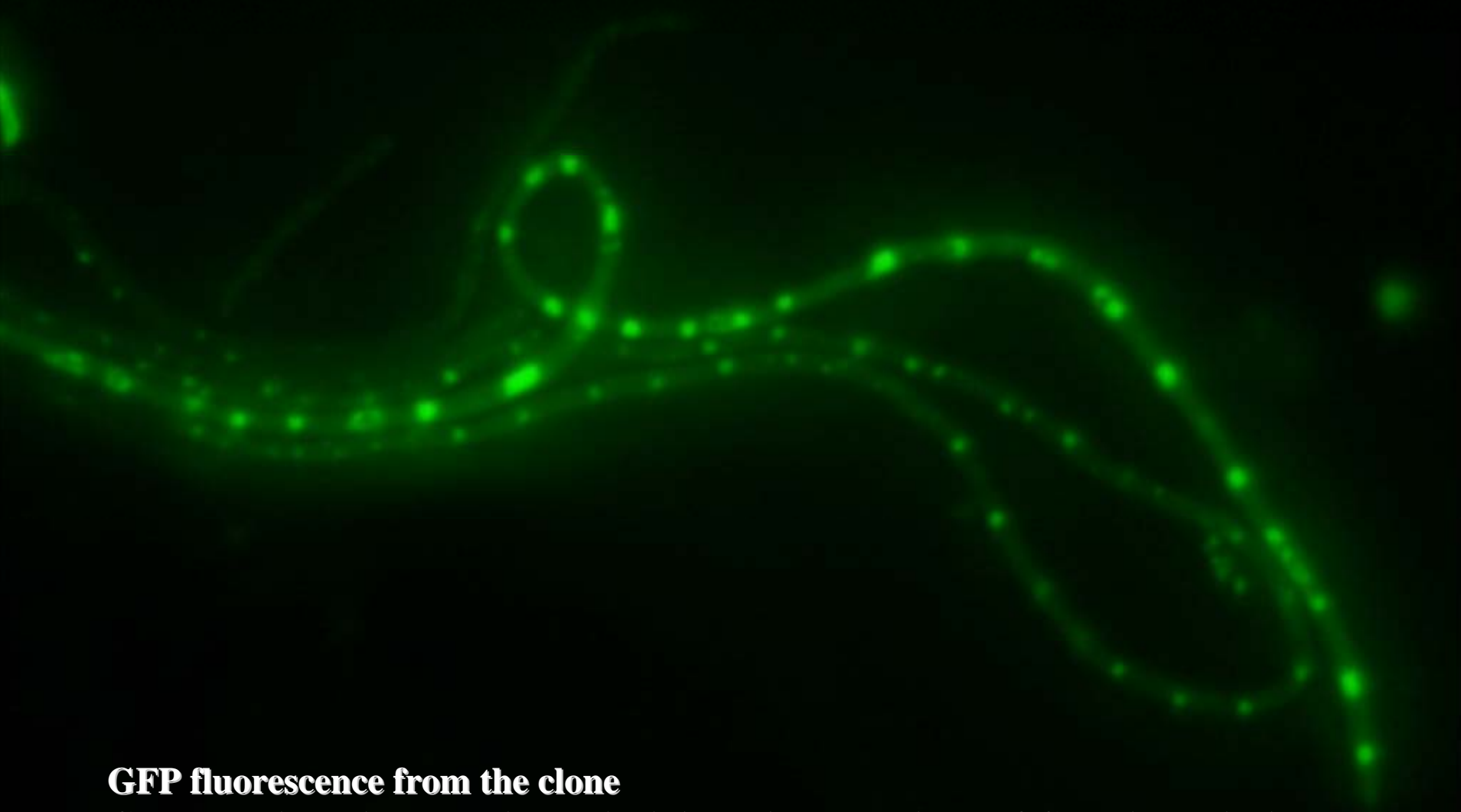
From literatures



From experiments



Protein localization



GFP fluorescence from the clone

Collaboration with Dr. Hironori Niki, National Institute of Genetics, Mishima

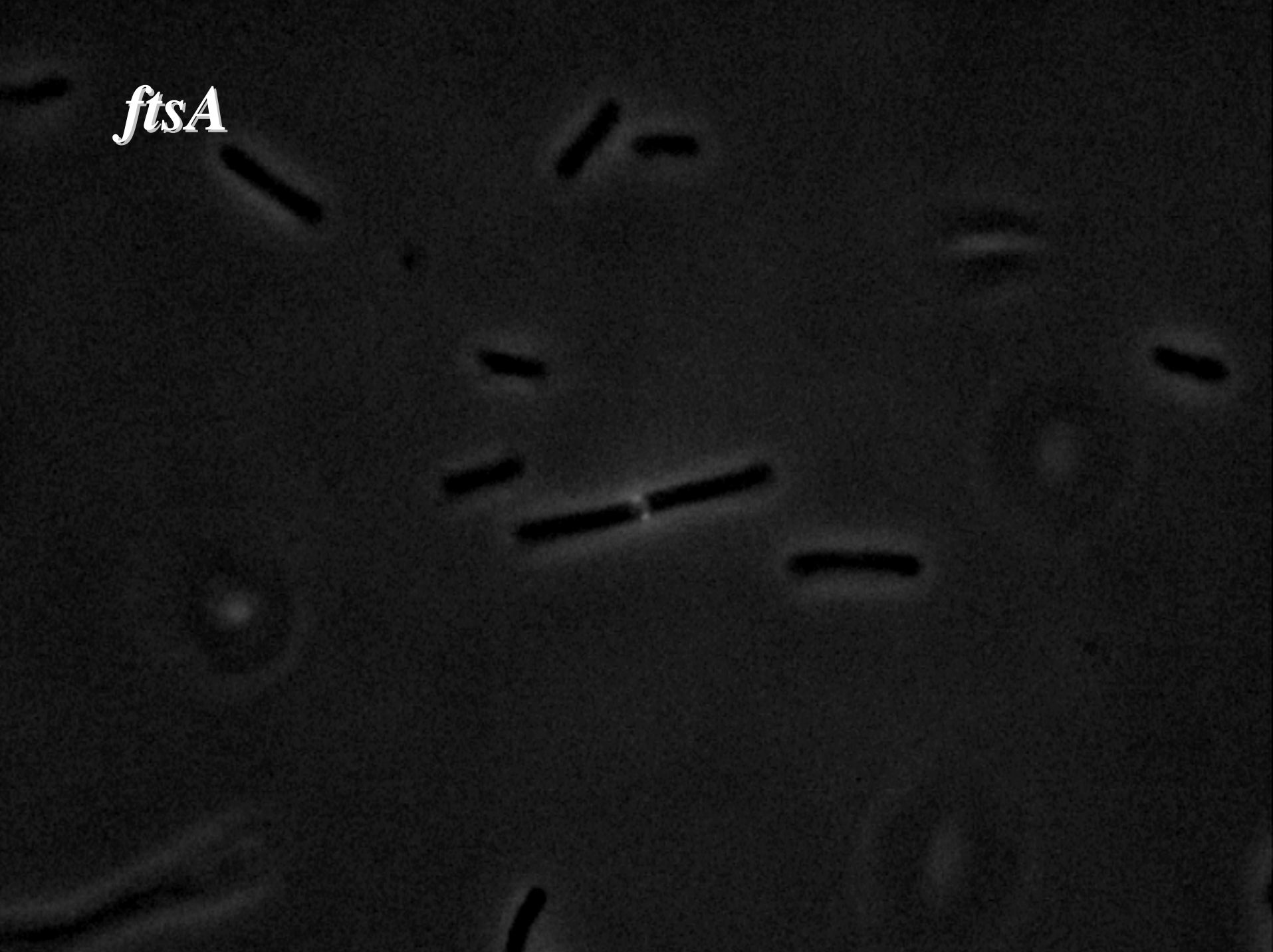
Summary of GFP-fusion library

Total screened cells	4351
----------------------	------

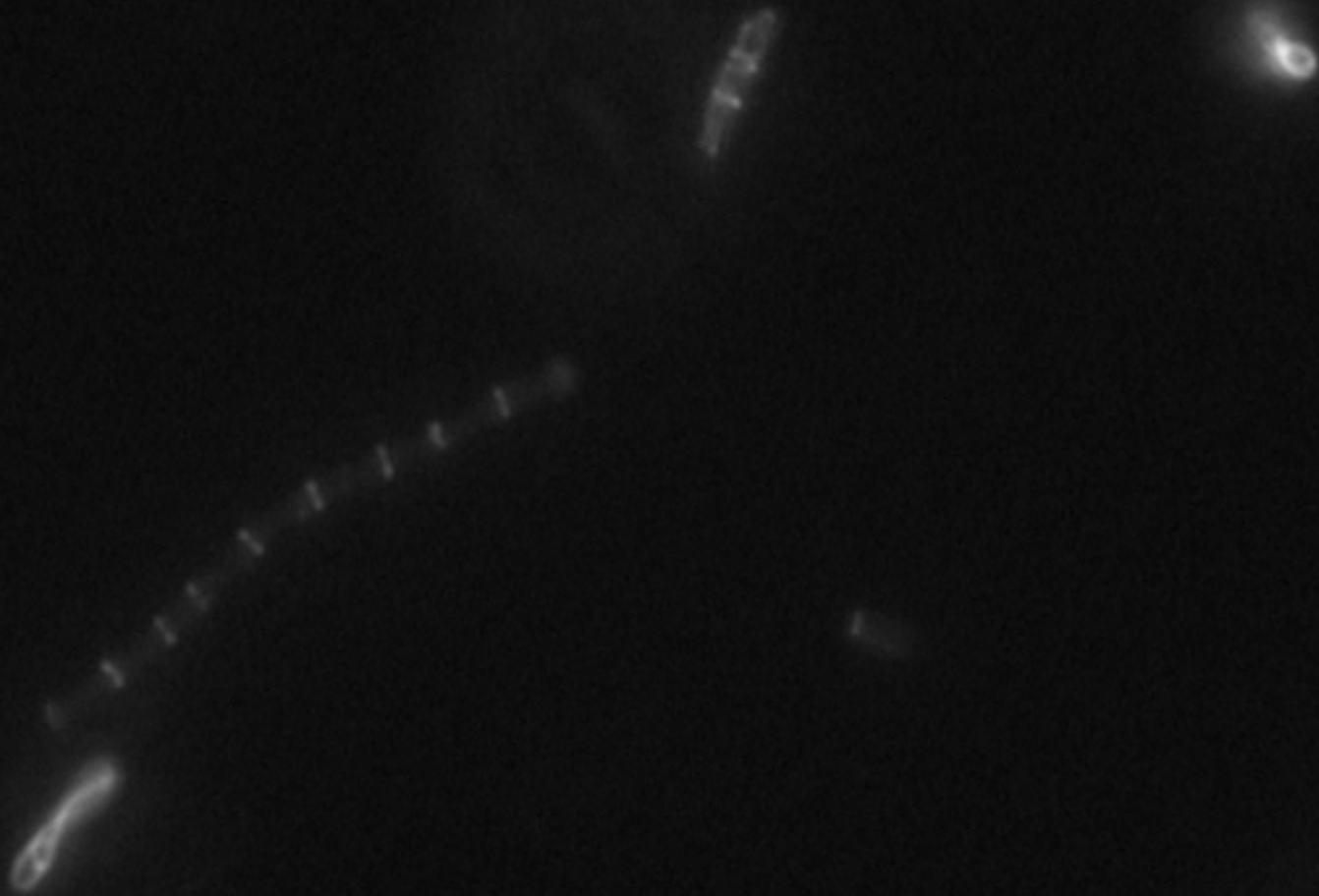
Cells with detected GFP signal	3996
--------------------------------	------

Cells without GFP signal	355
--------------------------	-----

ftsA

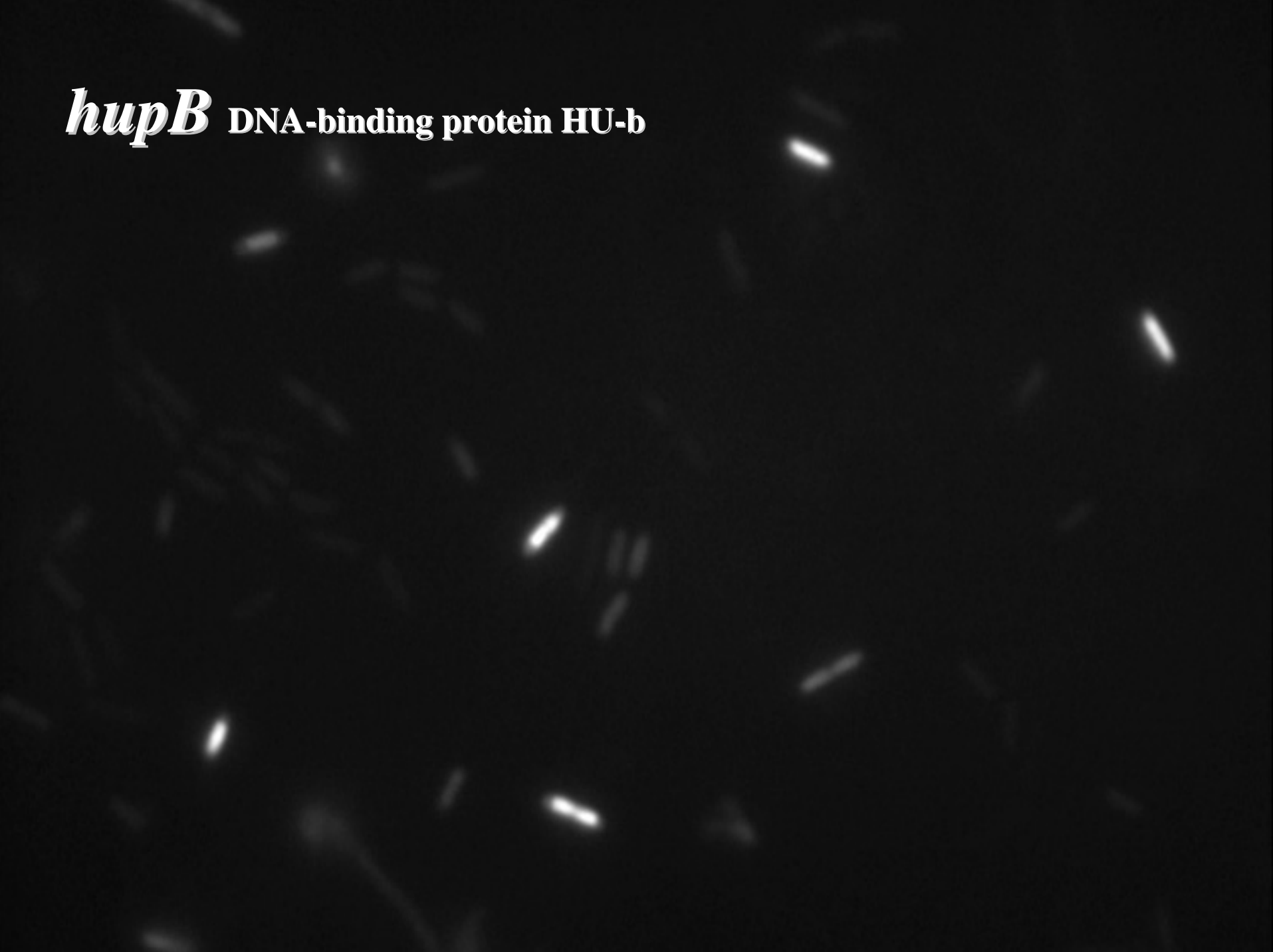


ftsZ



gsK Nucleotide metabolism; **INOSINE-GUANOSINE KINASE (EC 2.7.1.73)**

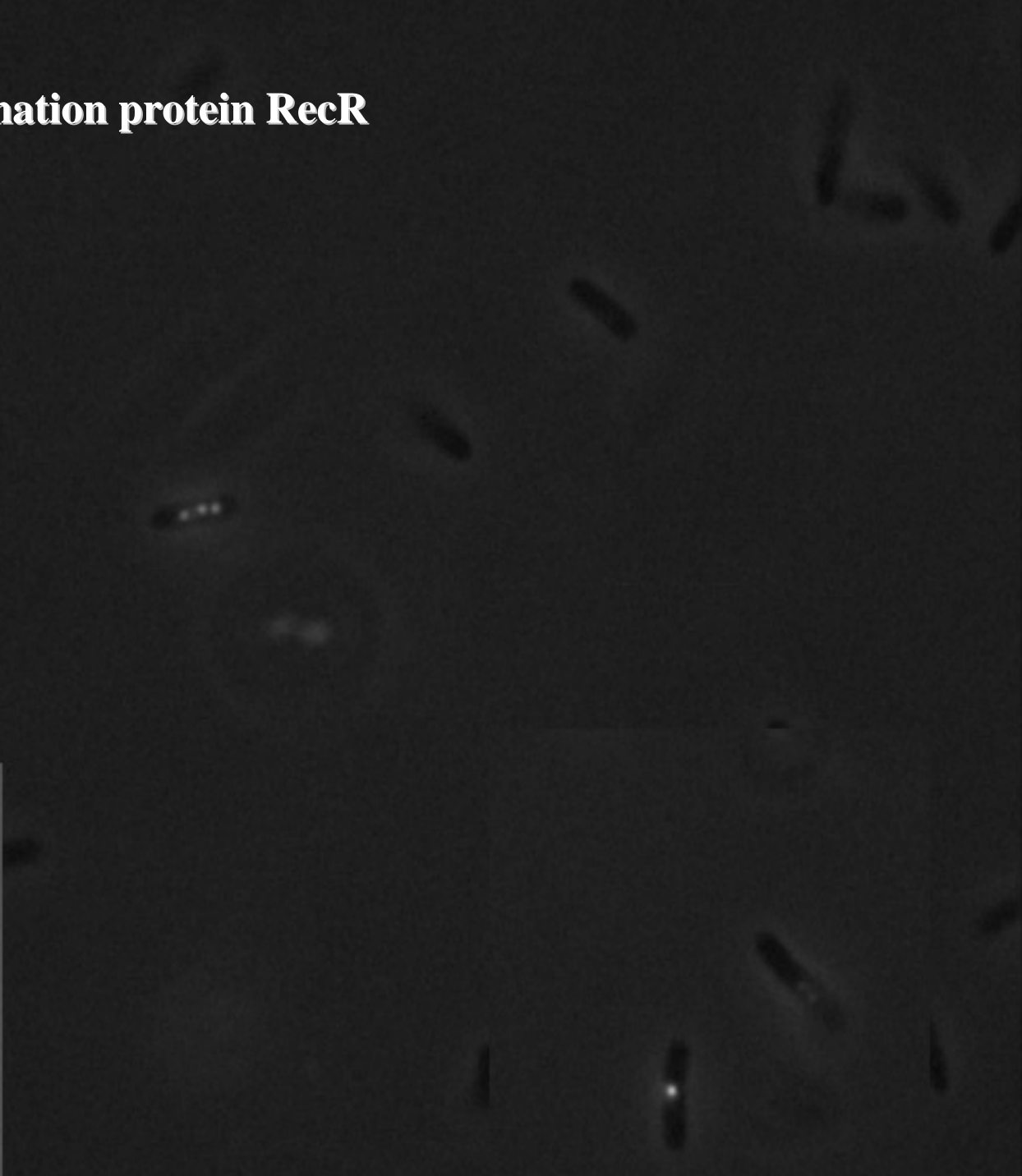
hupB DNA-binding protein HU-b



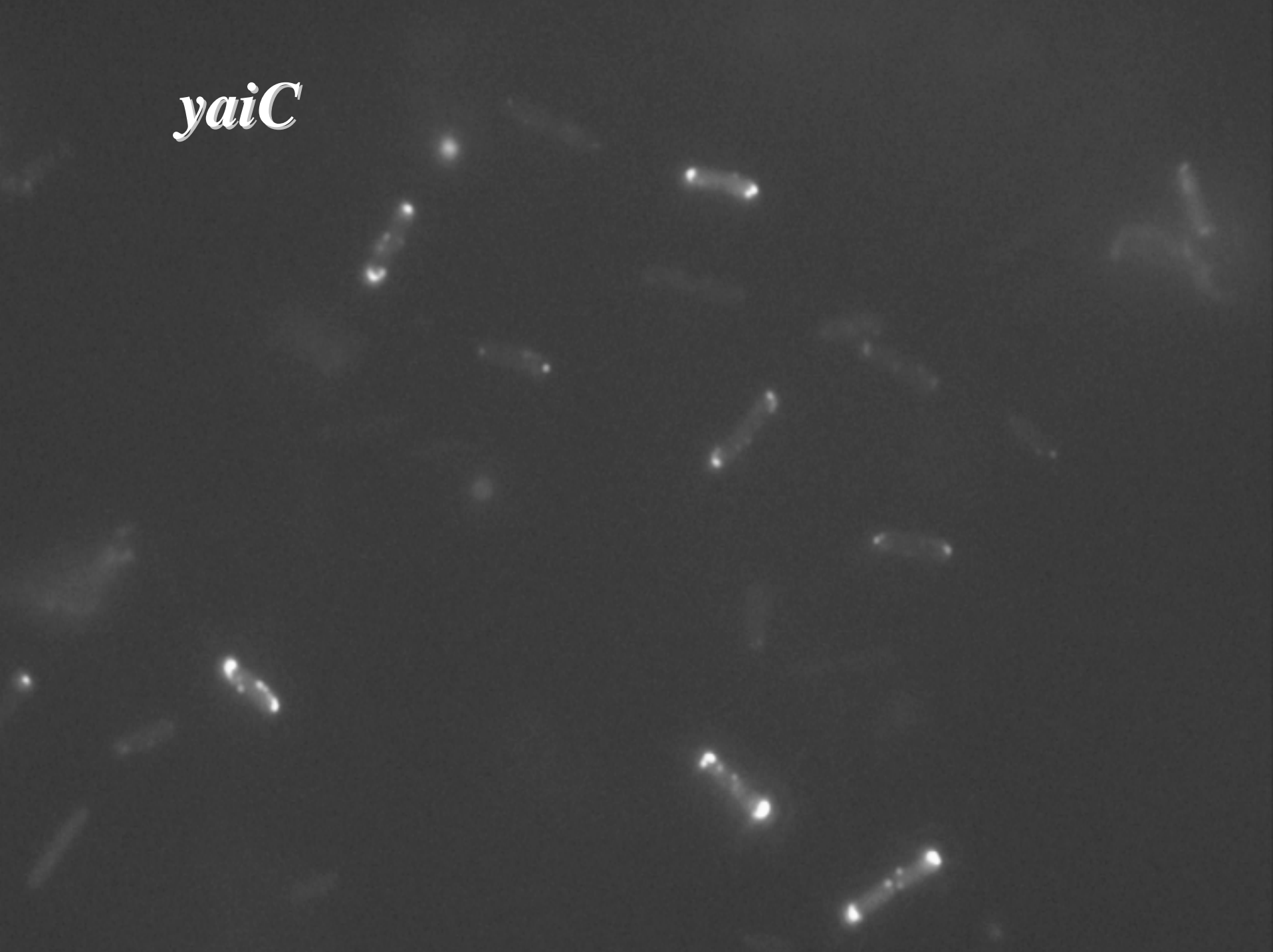
yajK



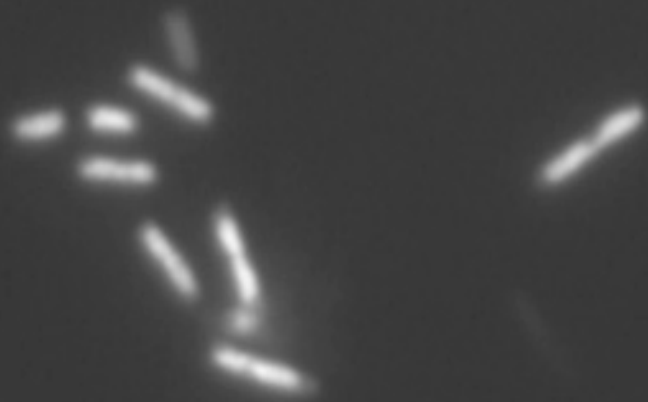
recR Recombination protein RecR



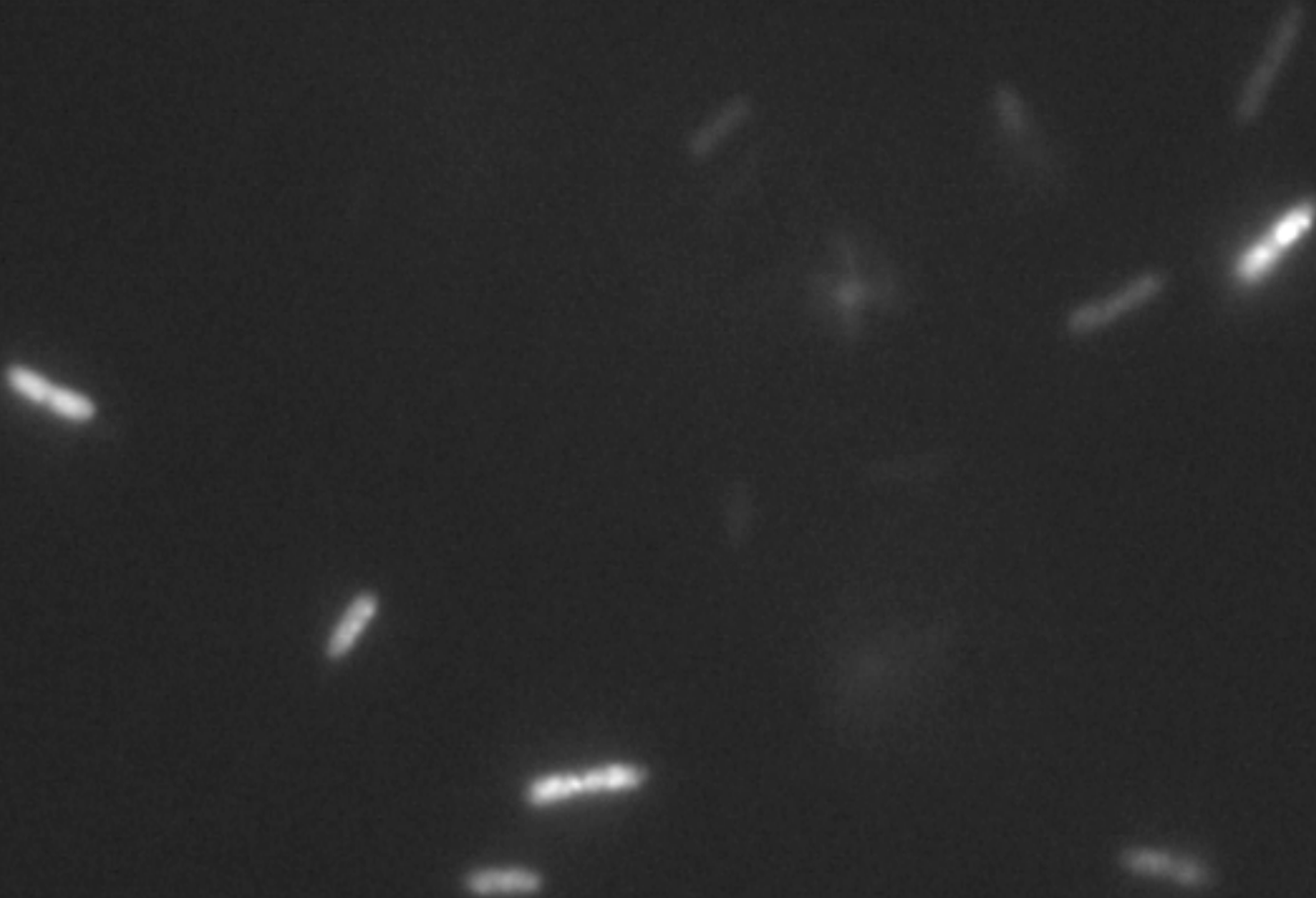
yaiC



yagQ

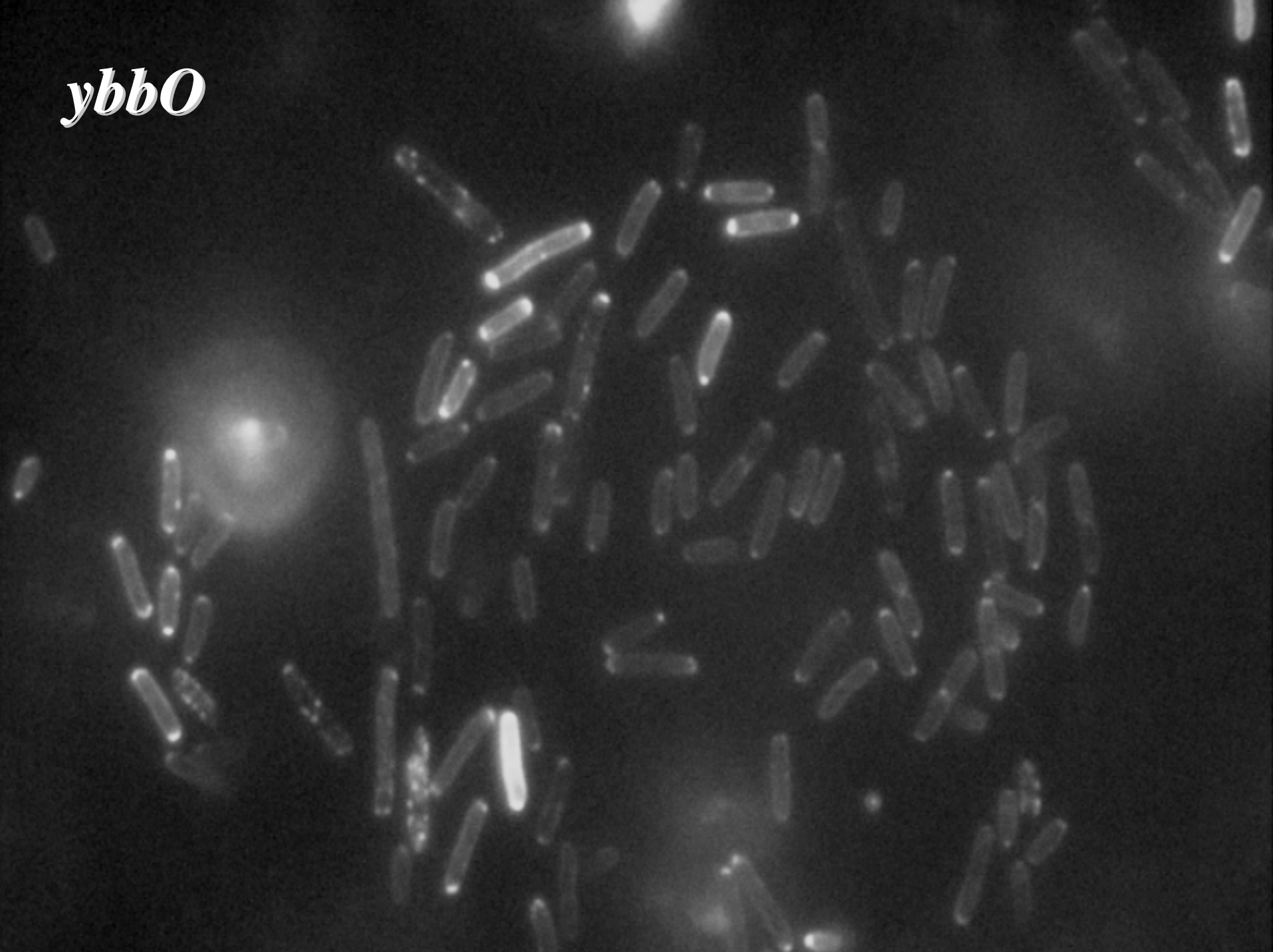


prpR Propionate catabolism operon regulatory protein

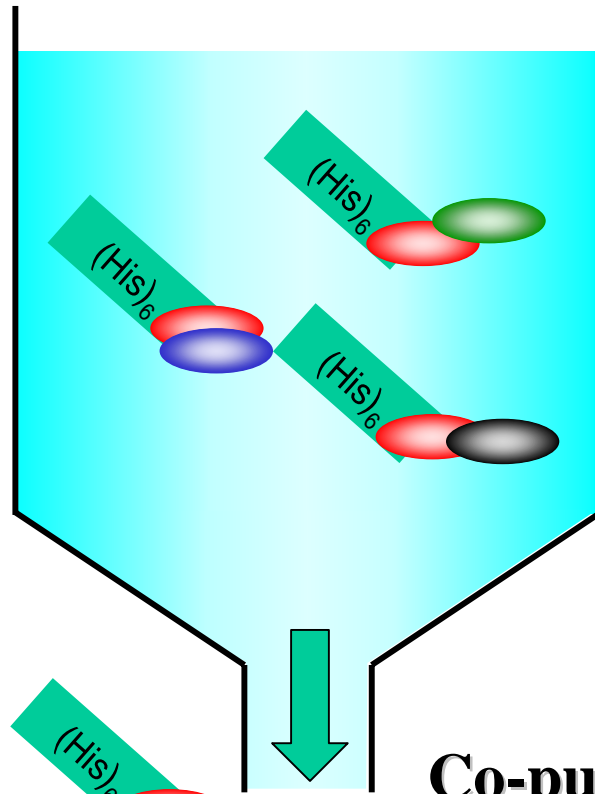


allA utilization of allntoin; ureidoglycolate hydrolase

ybbO

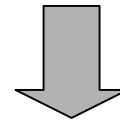


Identification of interacting proteins

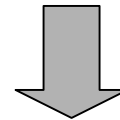


Ni-NTA column

Co-purified proteins with His tag protein

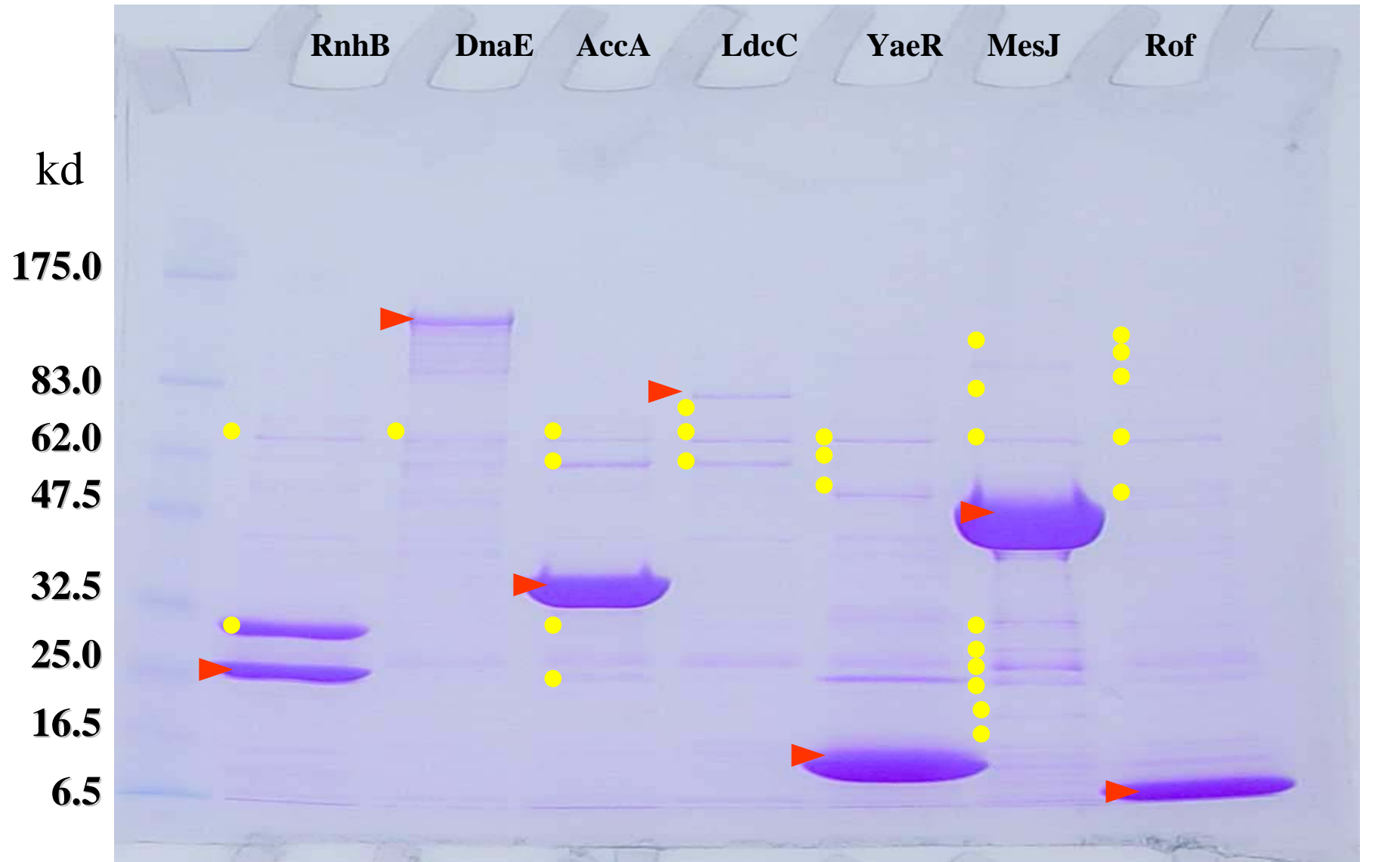


Excised the copurified protein bands from SDS-Gel



Identification by Mass spectrometry

Identification of interacting proteins by Mass Spectrometry



7.5 ~ 15% SDS PAGE Stained by CBB

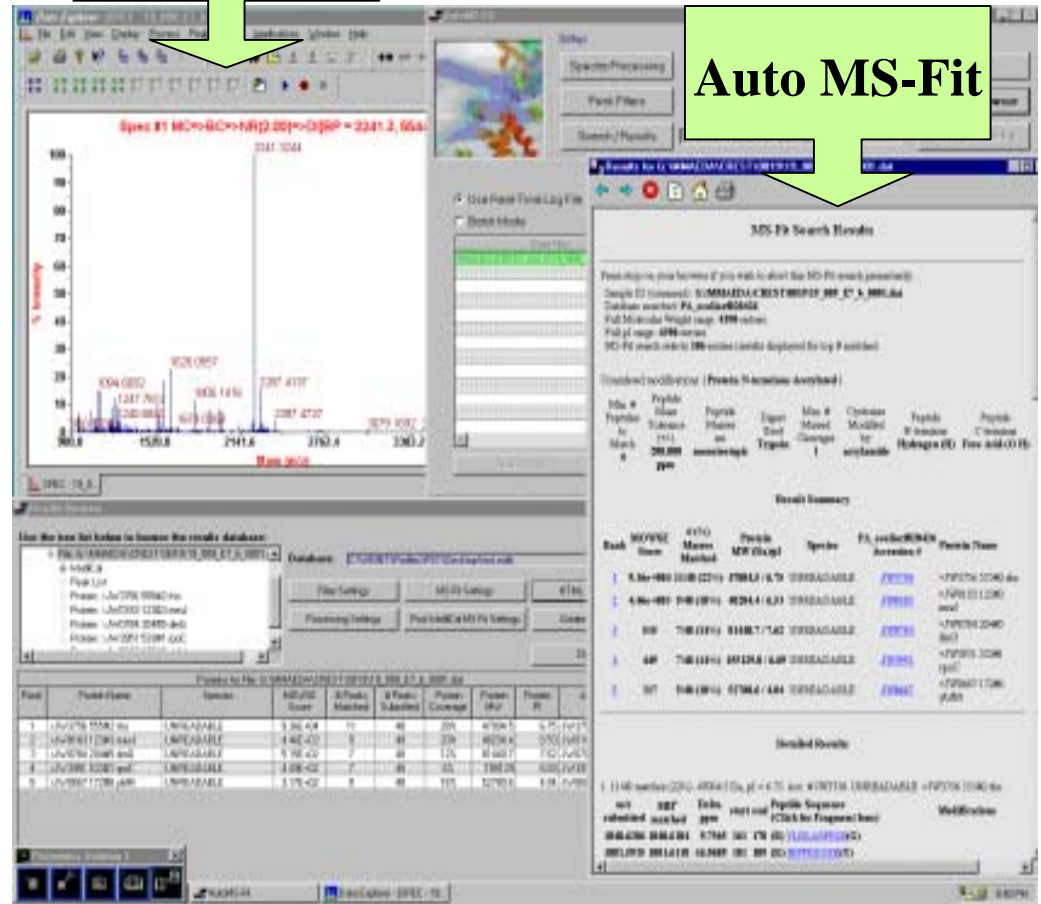
▶ His tagged Protein
● Identified

Protein identification by Mass spectrometry

MALDI-TOFMS (Applied Biosystems Voyager DE-PRO)



PS1 program



Auto MS-Fit



Database

E.coli database Genobase
(<http://ecoli.aist-nara.ac.jp>)

Identification of interacting proteins by Mass Spectrometry

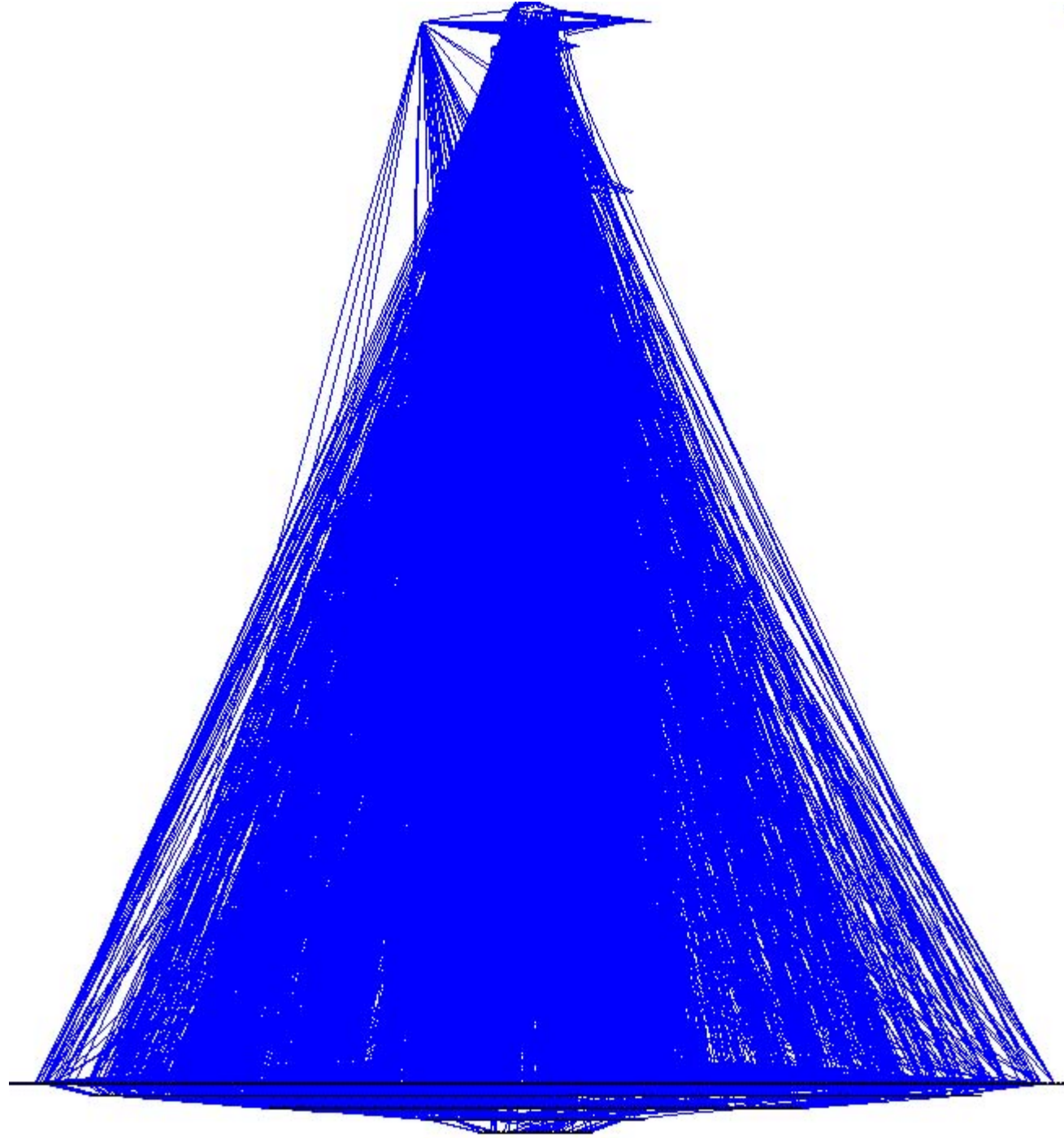


7.5 ~ 15% SDS PAGE Stained by CBB

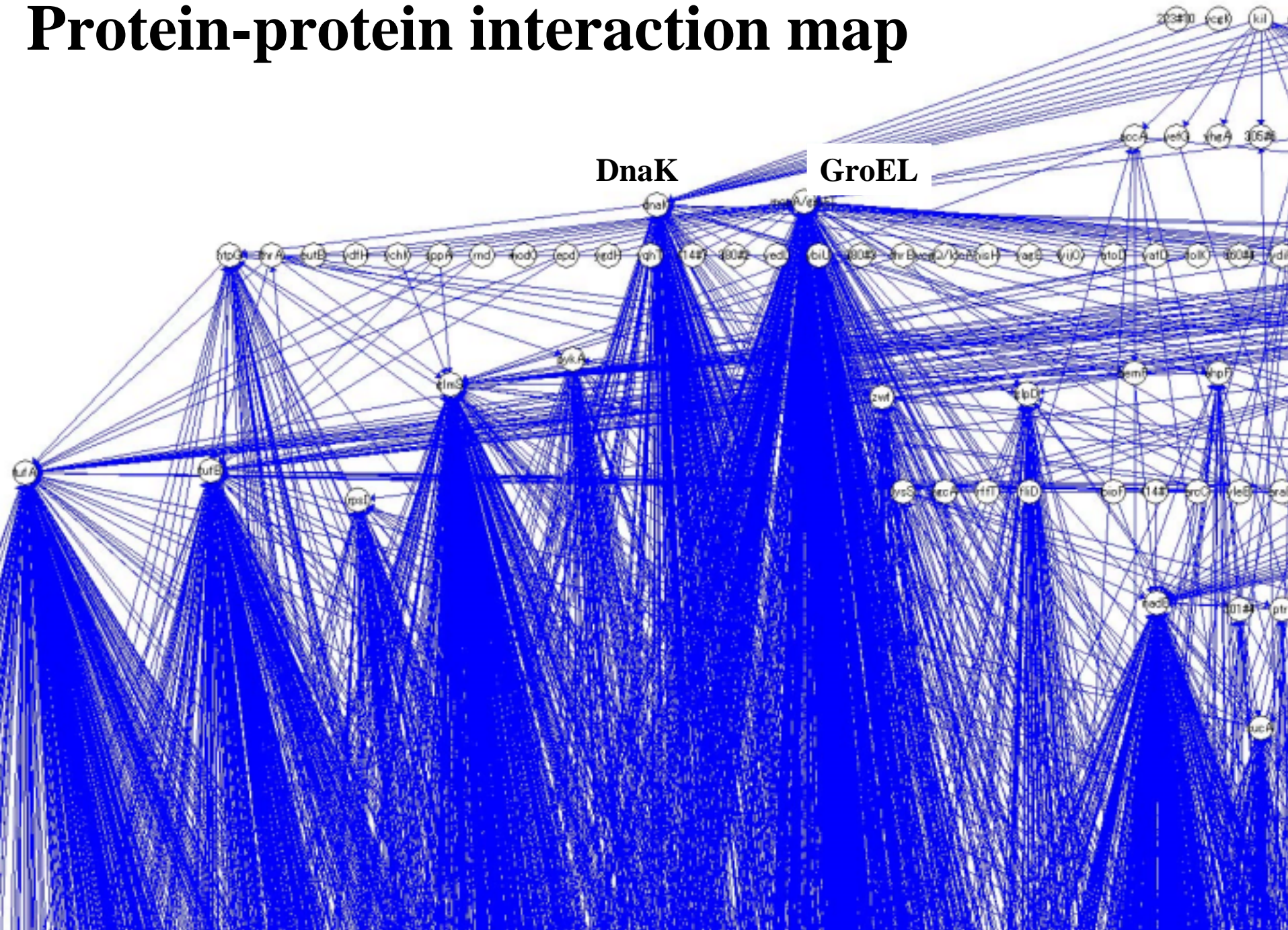
▶ His tagged Protein
● Identified

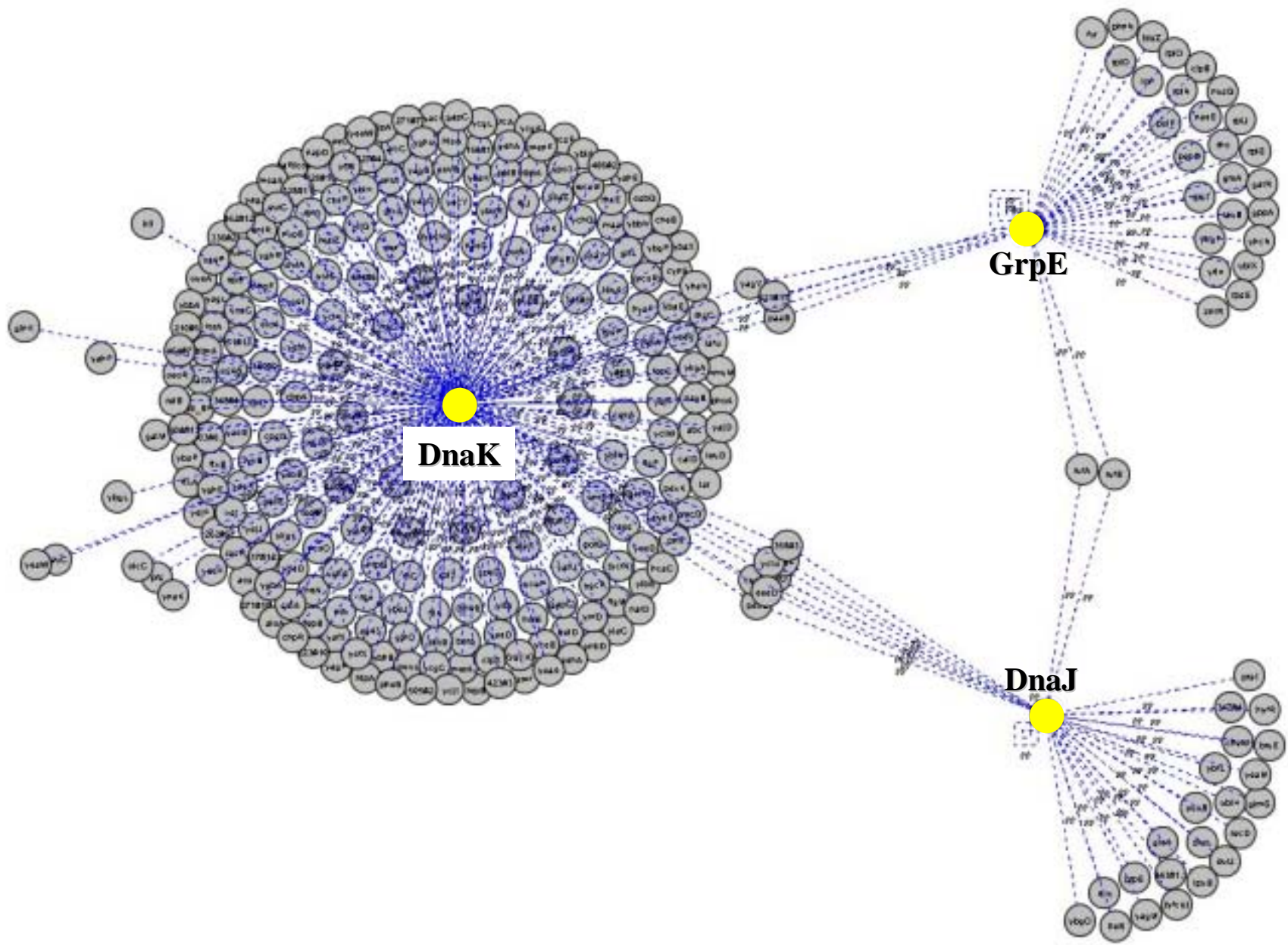
home

Gel. No	His-tagged	Gene	MW	Protein	Sample ID	Coverage (%)	JW NO.	Gene	MW (MW/pI)	pI	Comments01	Comments02
NA053	JW0392	proY	52919.547	Proline-specific permease	ProY	ND						
	JW0393	malZ	71592.557	Maltodextrin glucosidase (EC 3.2.1.20)	53_1	27%	JW0393 > JW0393 144#8	malZ	69172.60	5.93		
						22%	JW0013 > JW0013 102#5	dnaK	69115.30	4.83		
					53_2	34%	JW0393 > JW0393 144#8	malZ	69172.60	5.93		
					53_3	21%	JW4103 > JW4103 649#9	mopA/groEL	57329.20	4.85		
	JW0394	yajH	23660.572	Hypothetical protein	53_4	31%	JW3707 > JW3707 560#3	glmS	66894.70	5.56		
					53_5	36%	JW4103 > JW4103 649#9	mopA/groEL	57329.20	4.85		
					53_6	28%	JW3301 > JW3301 626#1	tufA	43251.70	5.30		
						28%	JW3943 > JW3943 534#6	tufB	43313.80	5.30		
					53_7	40%	JW2004 > JW2004 350#12	hisB	40278.20	5.76		
					53_8	35%	JW0394 > JW0394 145#2	yajH	22961.20	5.88		
						22%	JW2833 > JW2833 465#2	(npD)	26546.70	10.11		?
	JW0395	queA	41142.788	S-adenosylmethionine:tRNA ribosyltransferase-isomerase (EC 5.-.-.-)	53_9	22%	JW0013 > JW0013 102#5	dnaK	69115.30	4.83		
						14%	JW1431 > JW1431 272#6	prtC	72670.10	6.71		
					53_10	21%	JW3707 > JW3707 560#3	glmS	66894.70	5.56		
					53_11	13%	JW4198 > JW4198 657#25	treC	63838.00	5.51		
						9%	JW1691 > JW1691 321#13	ydiD	62759.70	6.13		
					53_12	27%	JW0395 > JW0395 145#3	queA	39431.0	5.09	manual	
						19%	JW4333 > JW4333 673#6	yjiT/rsmG	37624.9	6.00	manual	?
					53_13	12%	JW1729 > JW1729 327#3	nadE	30637.00	5.41		
	JW0396	tgt	44188.557	Queuine tRNA-ribosyltransferase (EC 2.4.2.29)	53_14	29%	JW3707 > JW3707 560#3	glmS	66894.70	5.56		
					53_15	13%	JW4103 > JW4103 649#9	mopA/groEL	57329.2	4.85	manual	
						13%	JW3974 > JW3974 629B#3	aceB	60273.9	5.39	manual	
					53_16	33%	JW0396 > JW0396 145#4	tgt	42593.90	5.98		
						18%	JW1126 > JW1126 240#9	intE	42799.20	10.05		



Protein-protein interaction map





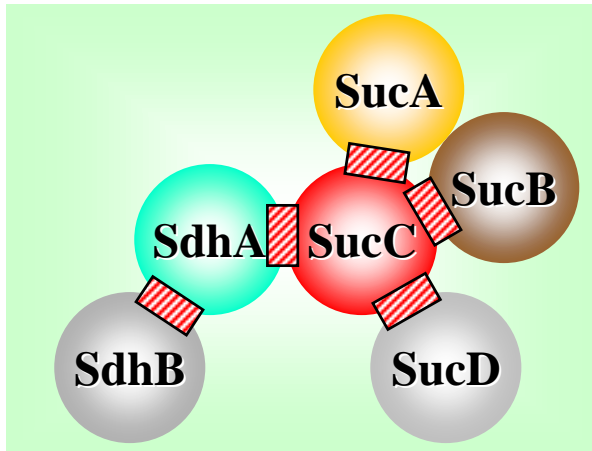
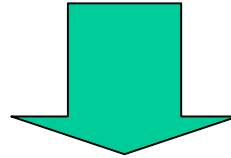
Complexes Detected in TCA Cycle



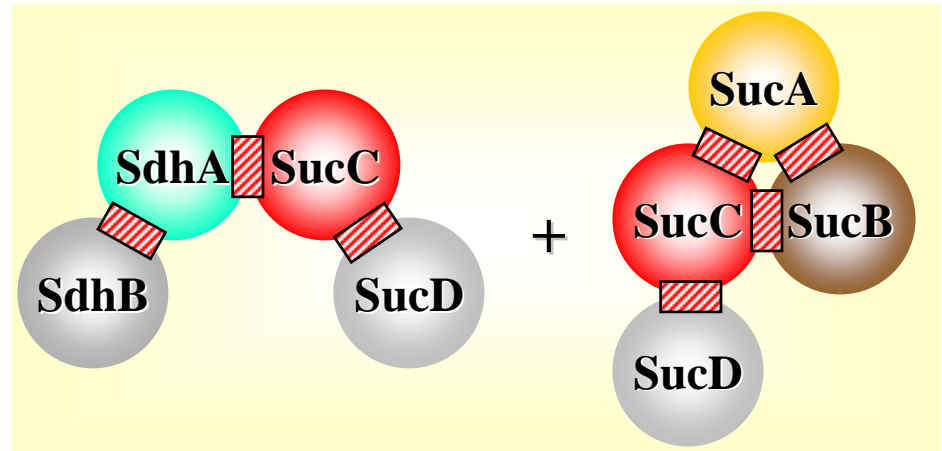
From the literatures



Our experiments

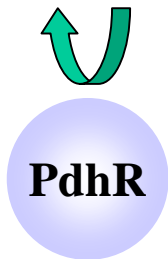
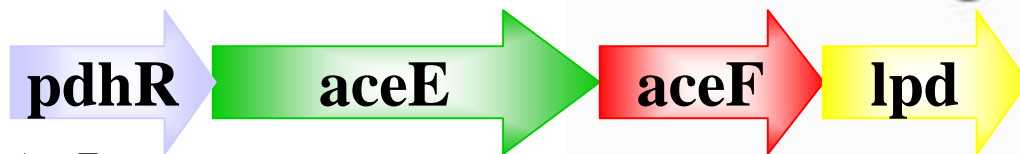
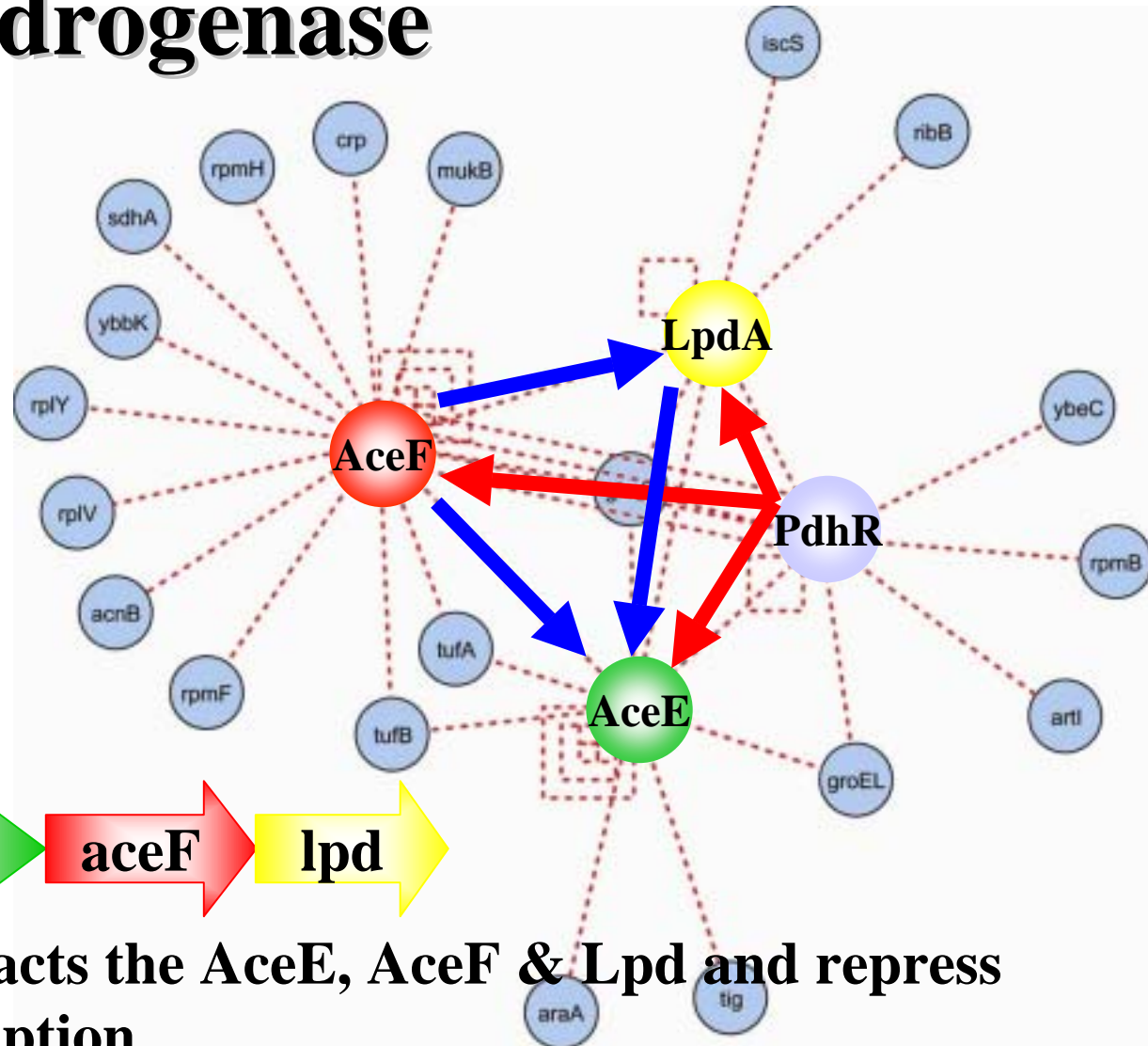
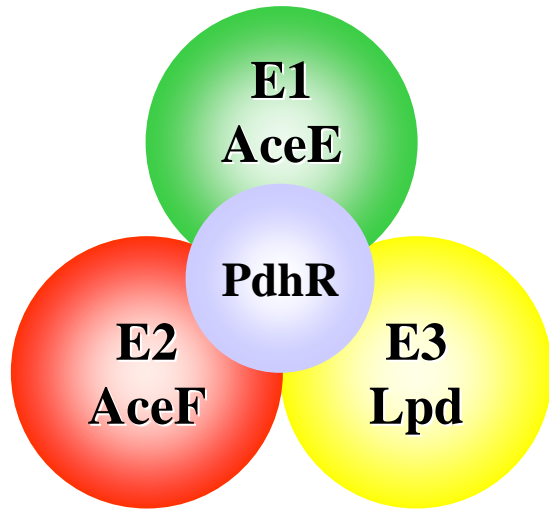


or



- SucA, B, C & D form a large complexes.
- Proteins belong to the same operon are potentially prone to form complexes (especially Enzyme complexes).

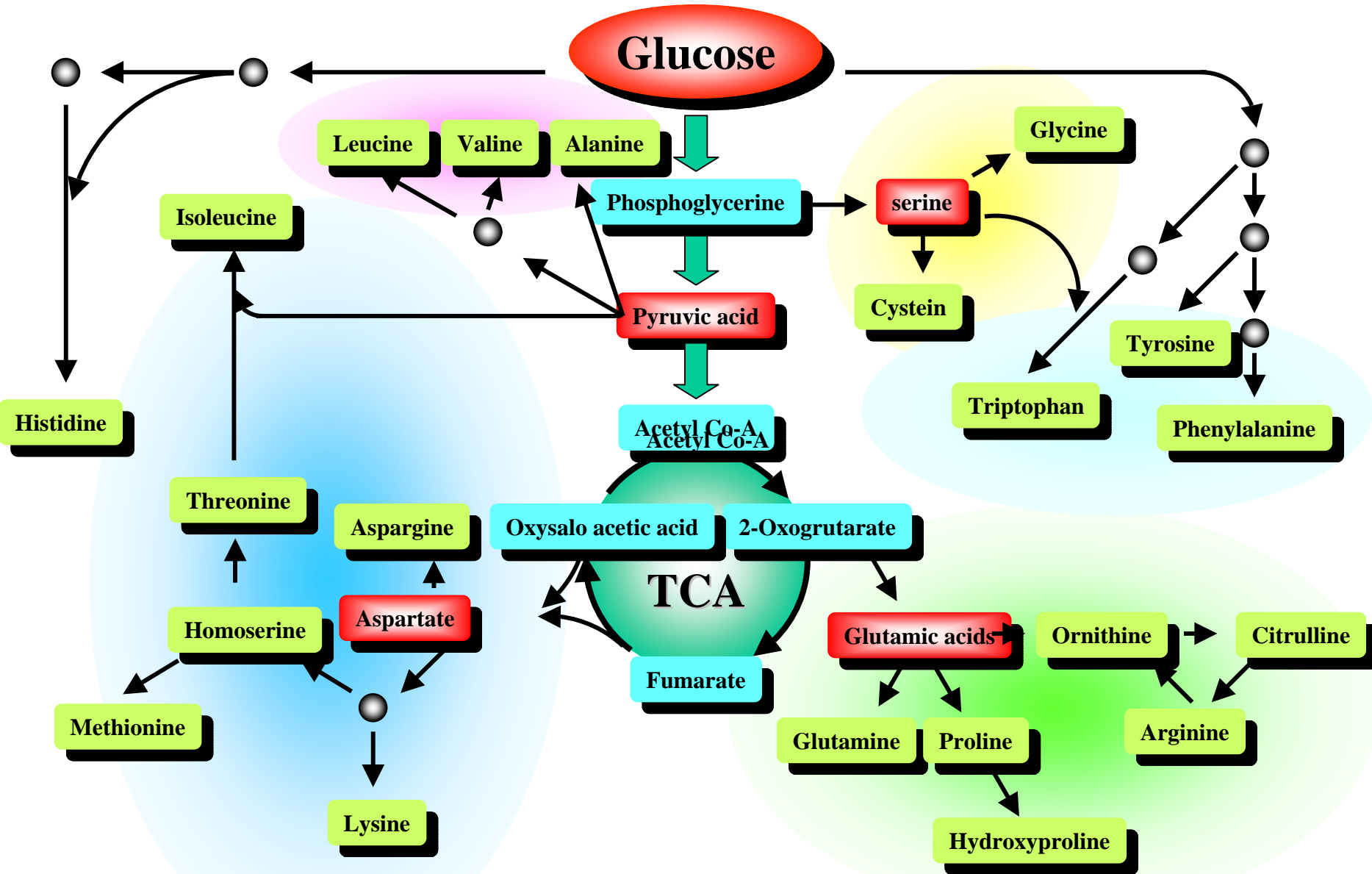
Pyruvate dehydrogenase



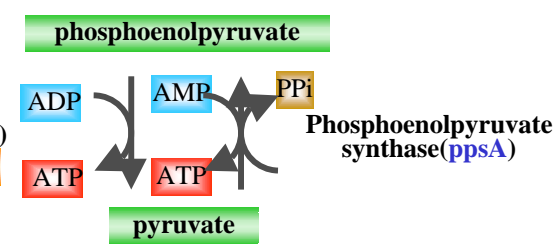
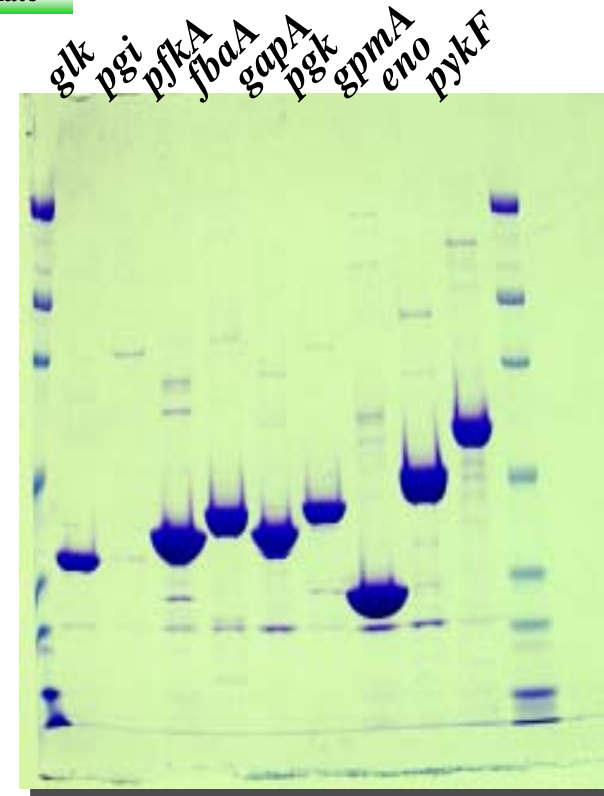
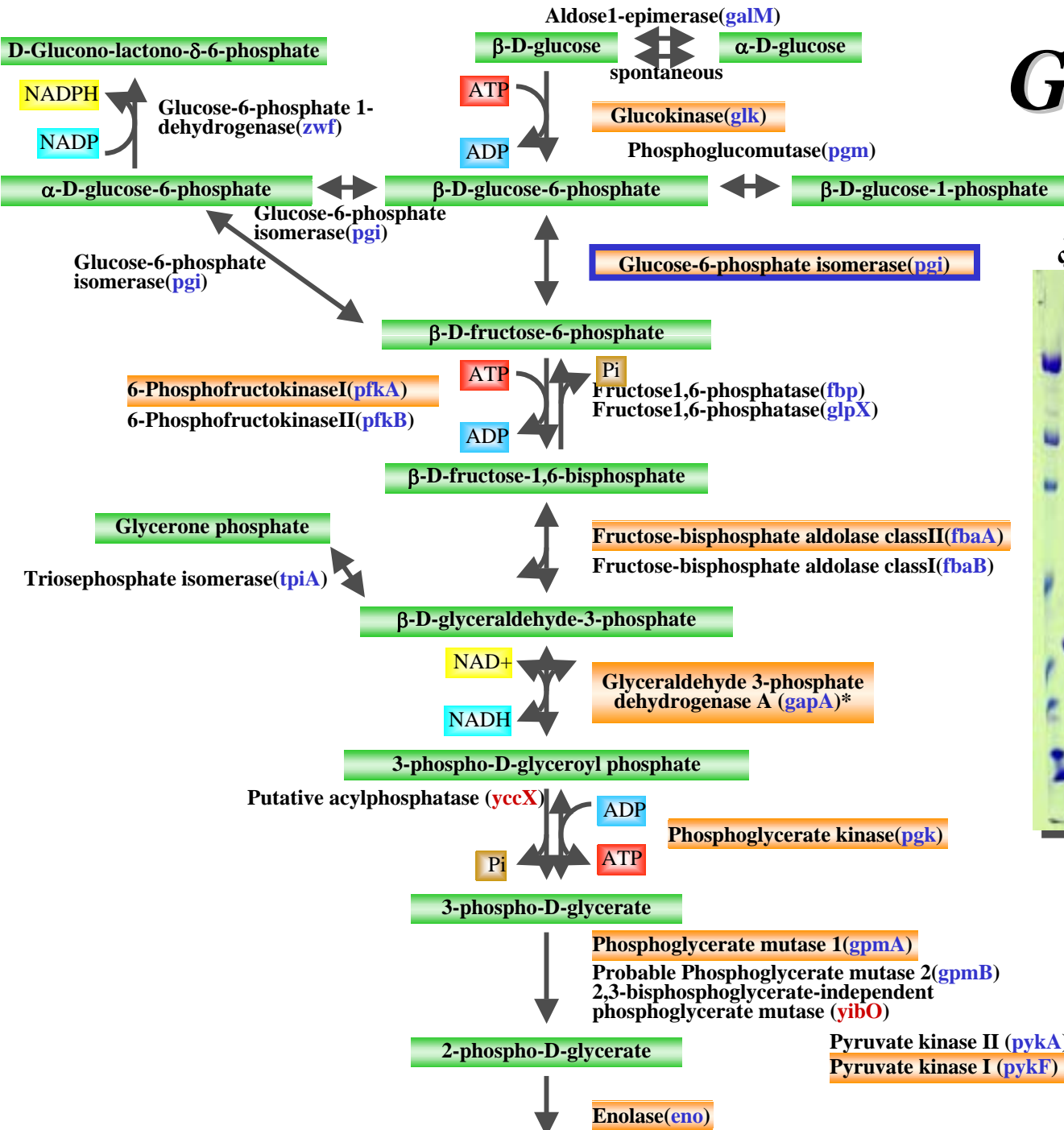
PdhR interacts the AceE, AceF & Lpd and repress the transcription.

Proteins belong to same operon are form complexes (especially Enzymes).

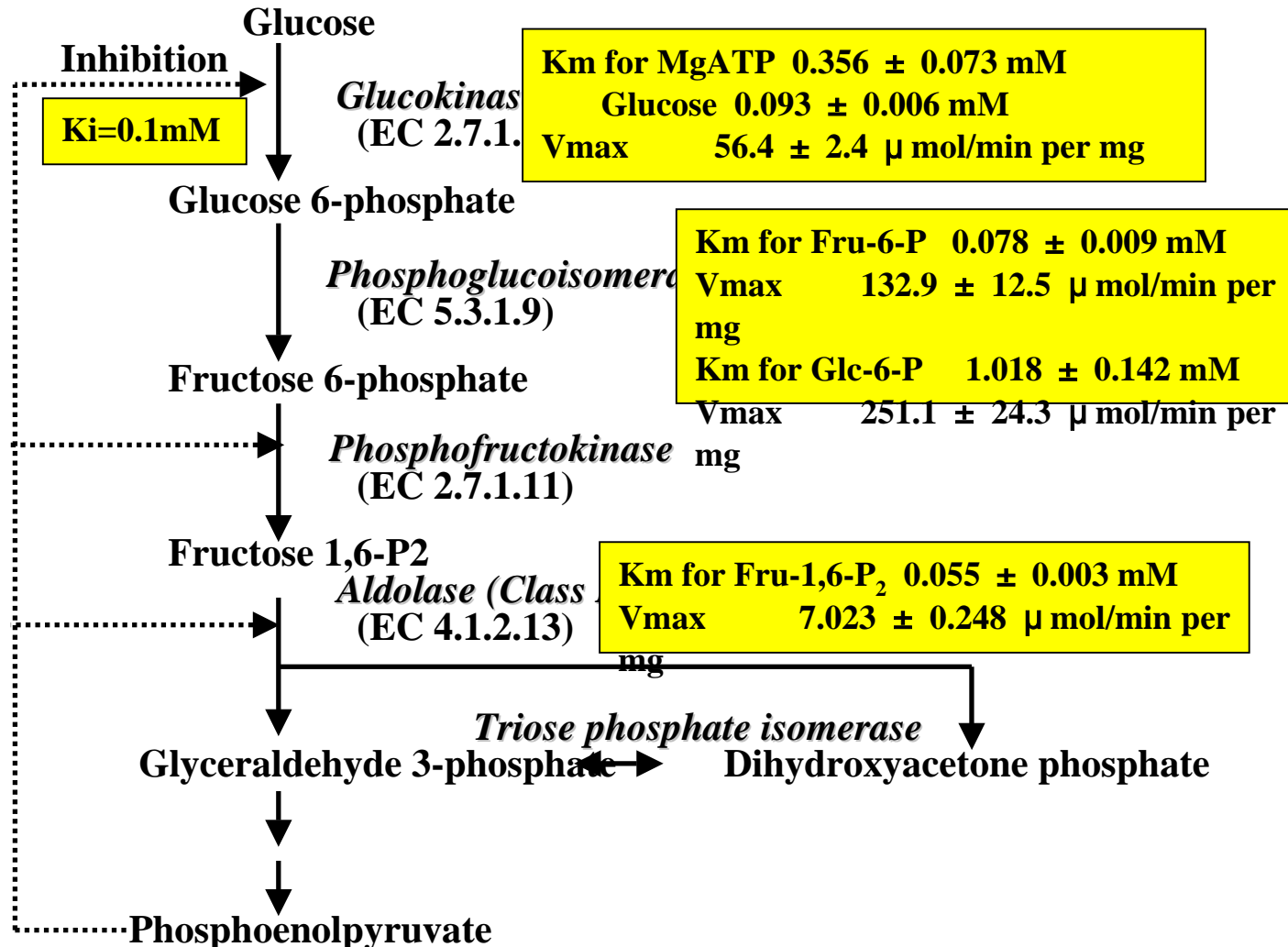
Protein number with interaction observed	1,844
Total number of interaction	13,376
Average number of interaction	7.3
Protein with no interaction observed	1,384

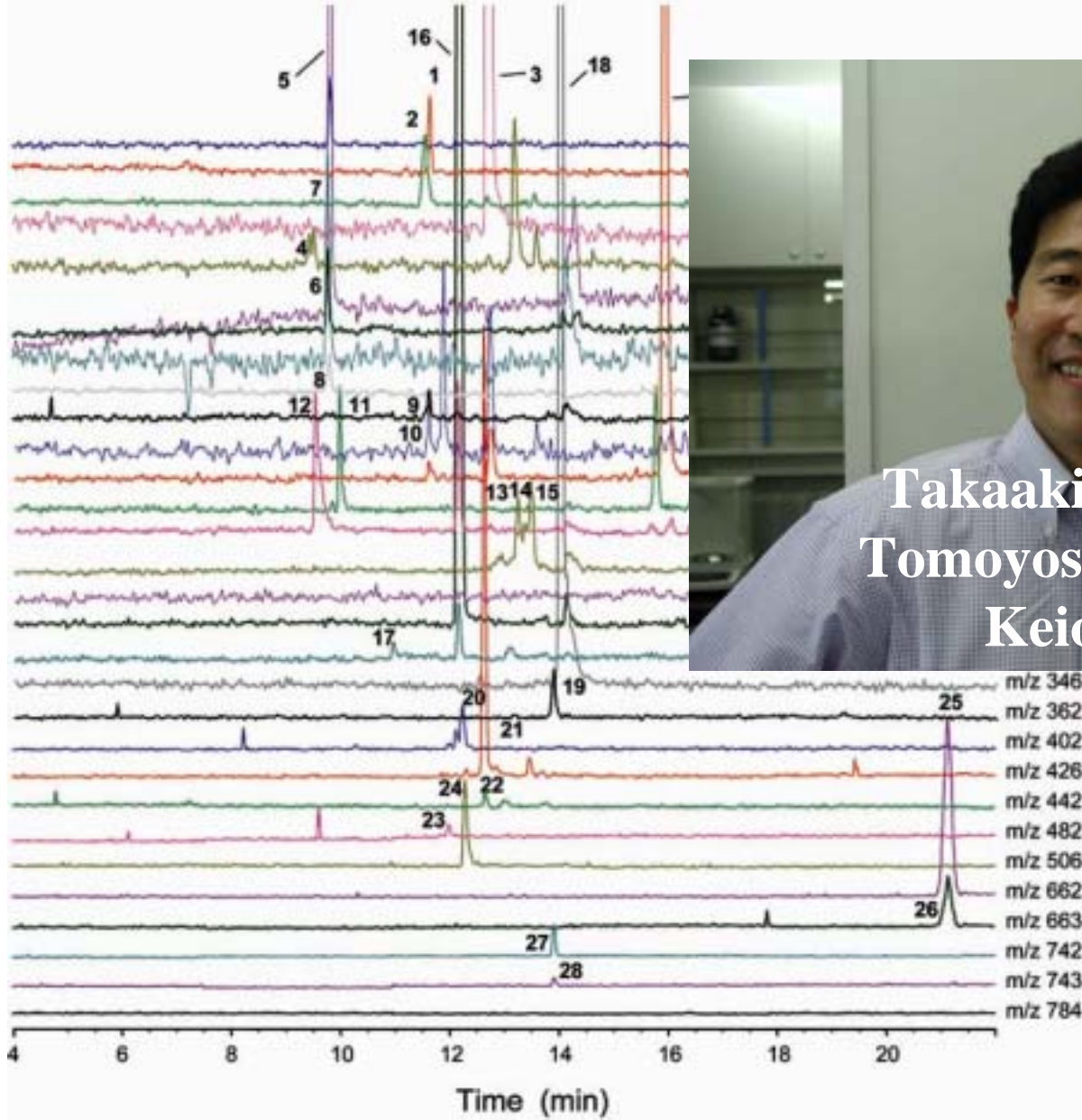


Glycolysis



Kinetic Parameters of Glycolytic Enzymes





1	Glycolate
---	-----------

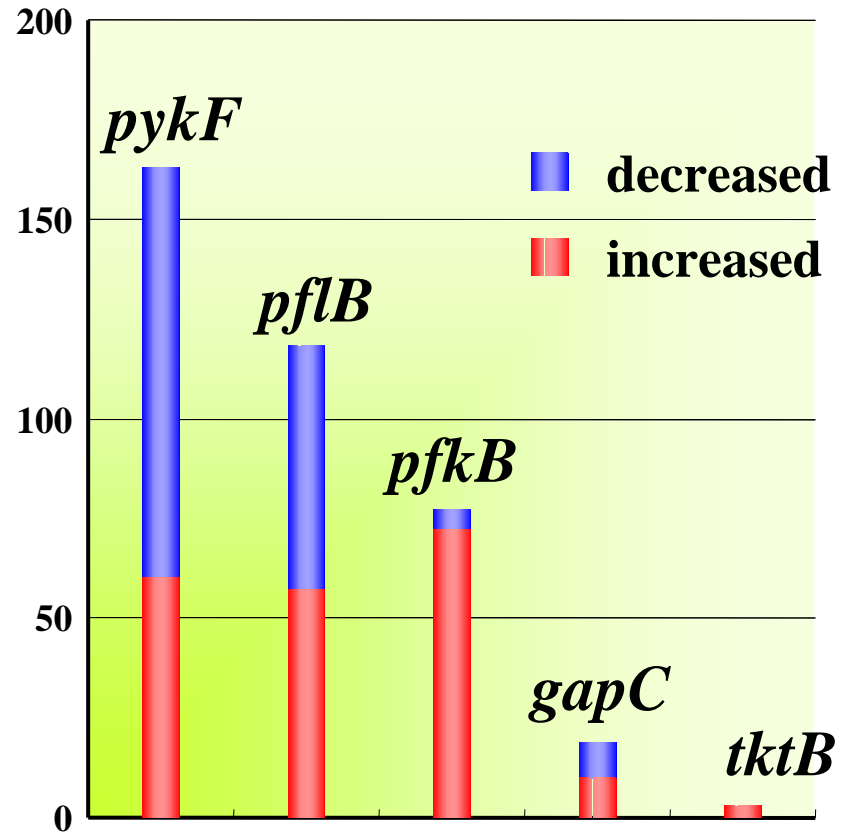
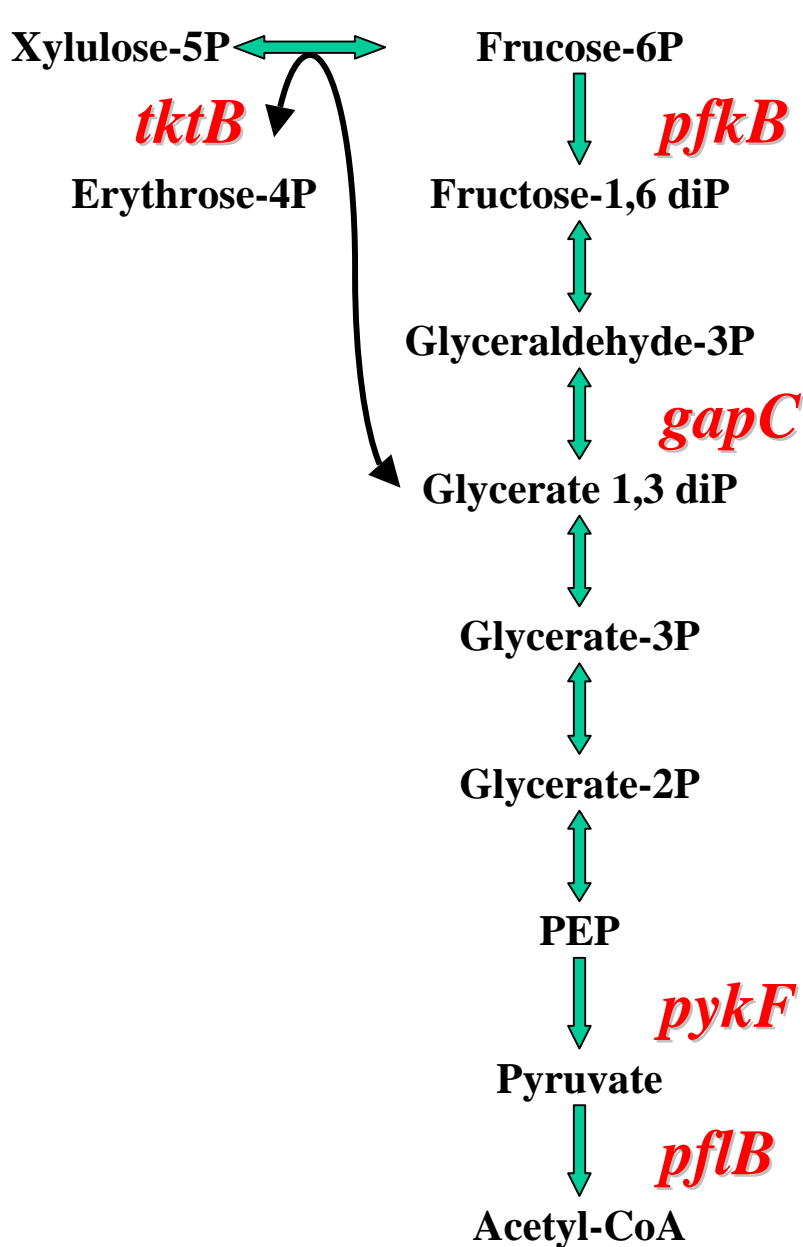


Takaaki Nishioka Prof.
Tomoyoshi Soga As. Prof.
Keio University

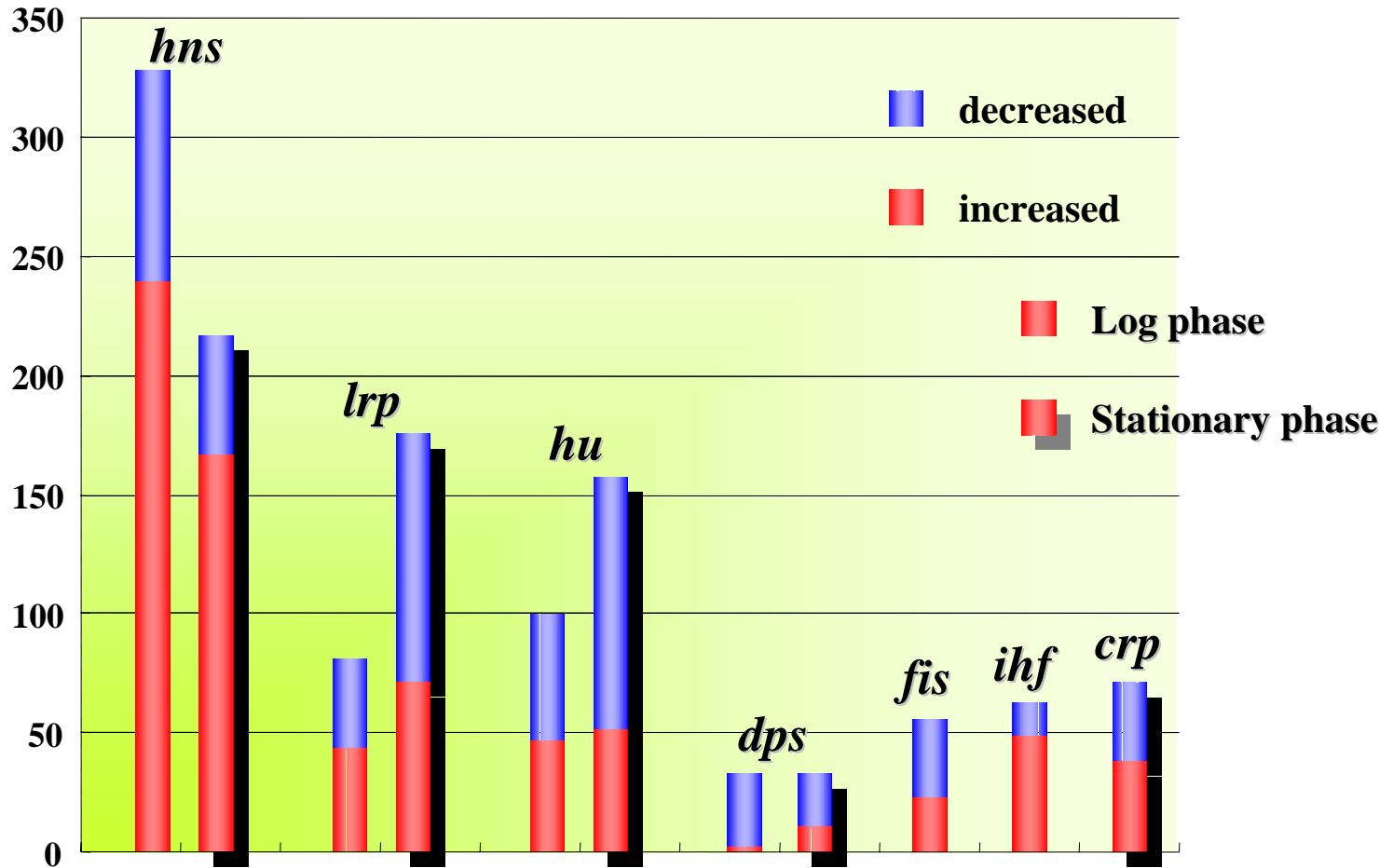
16	PIPES(is)
17	F1,6P
18	AMP
19	GMP
20	CDP
21	ADP
22	GDP
23	CTP
24	ATP
25	NAD
26	NADH
27	NADP
28	NADPH
29	unknown

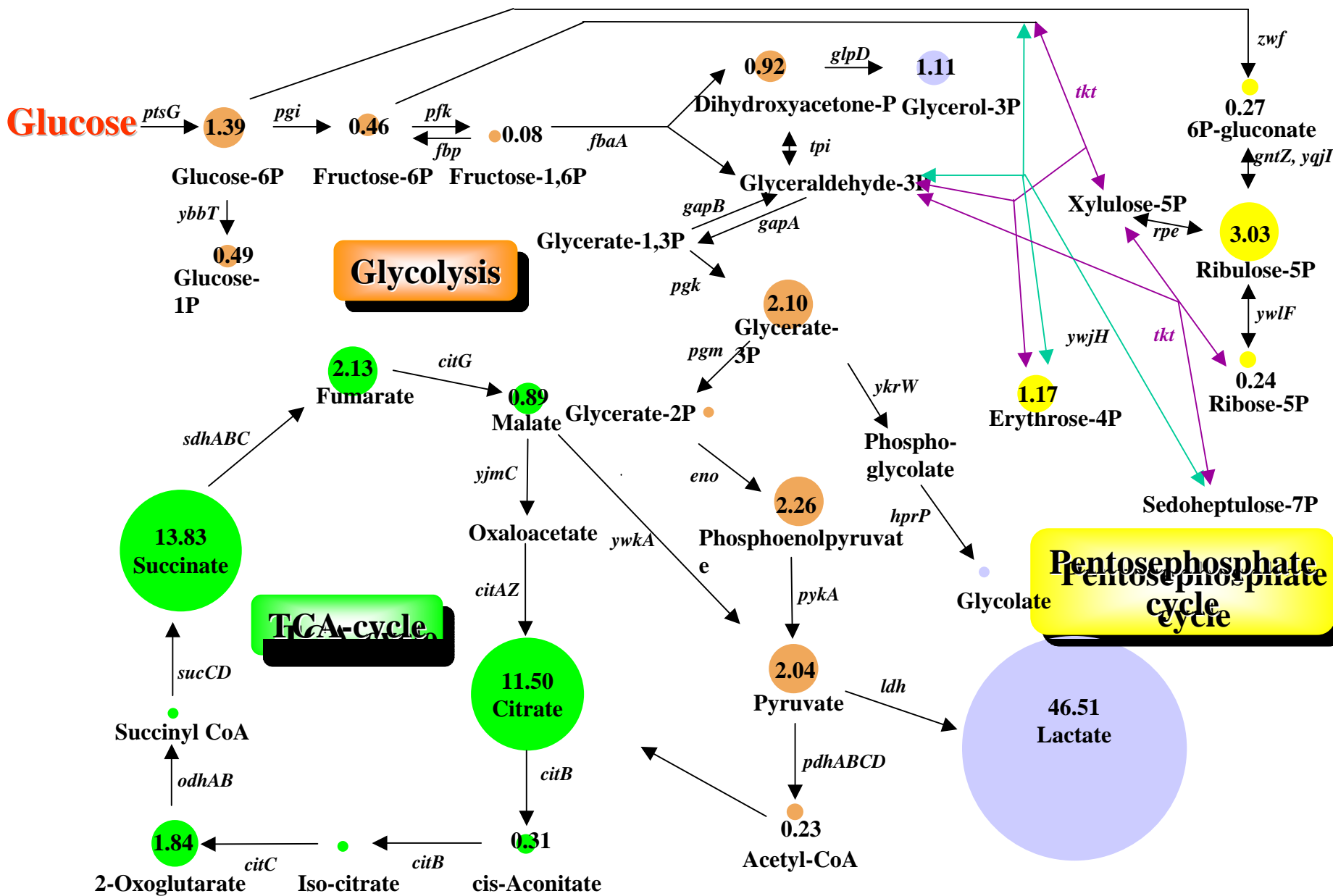
Metabolic intermediates

Deletion of metabolic enzymes



Deletion of nucleoid proteins





(Intracellular concentration in mM)

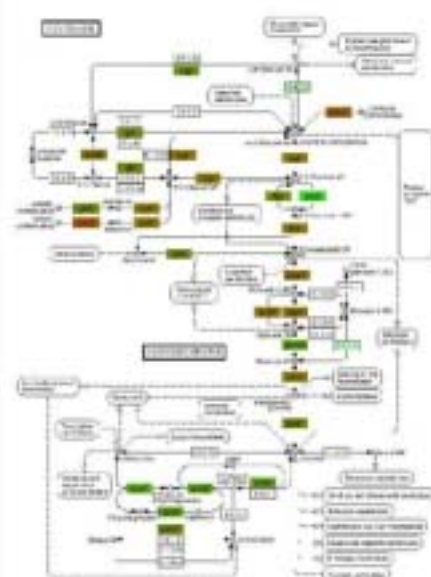
Kindly provided by T. Nishioka, Keio Univ & Kyoto Univ.



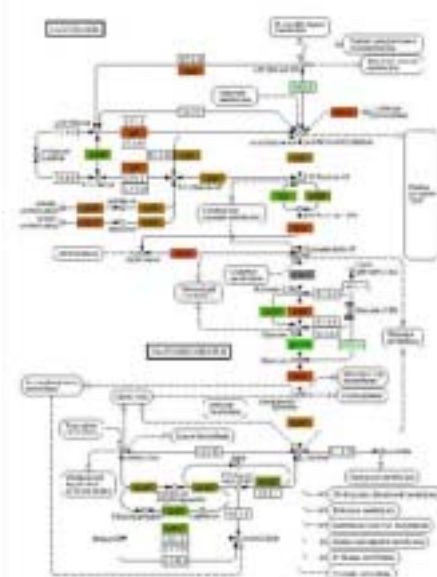
gapC



gndC



pfkB



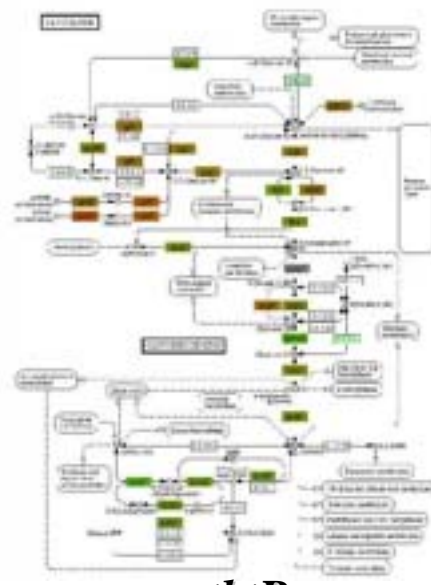
pflA



pflB

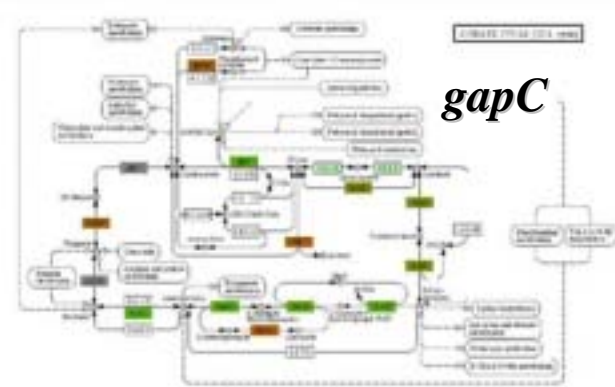


pykF

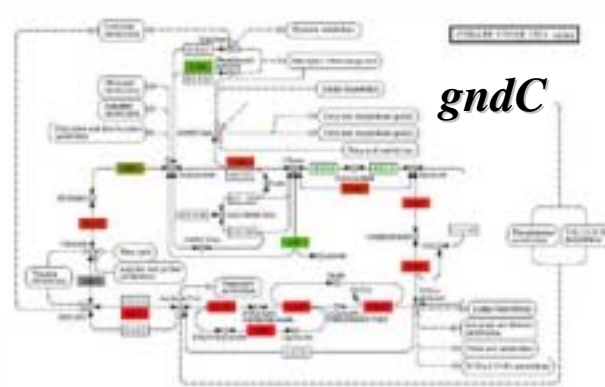


tktB

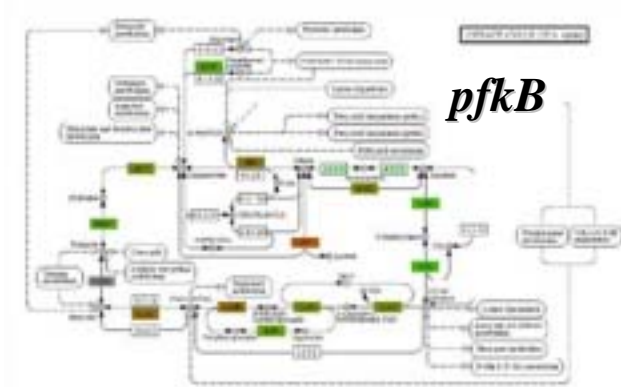
Glycolysis



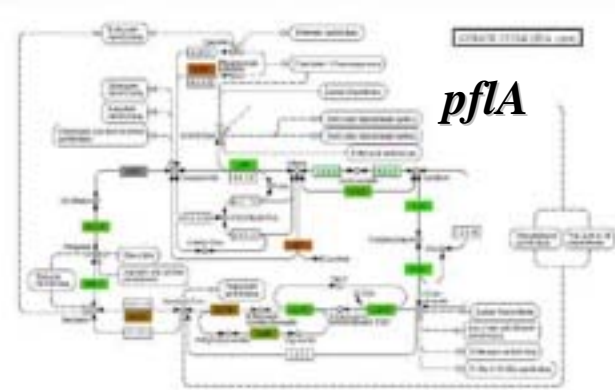
gapC



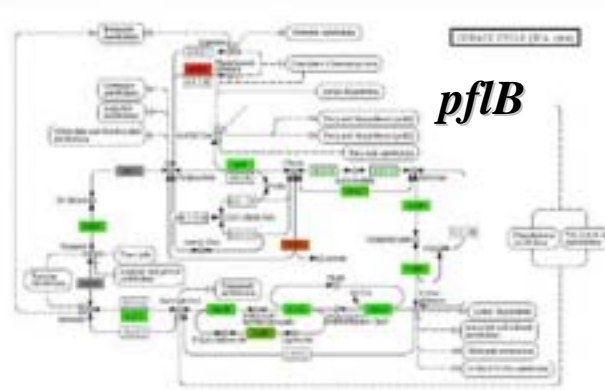
gndC



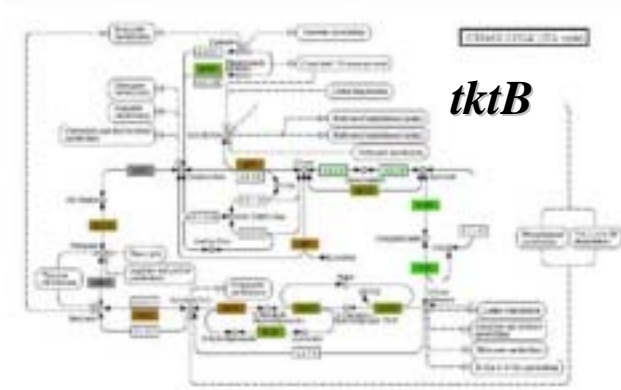
pfkB



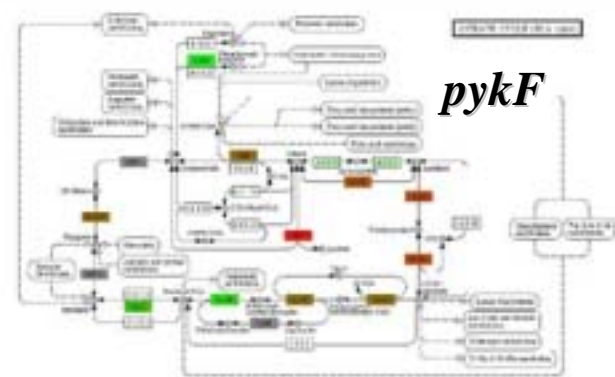
pflA



pflB

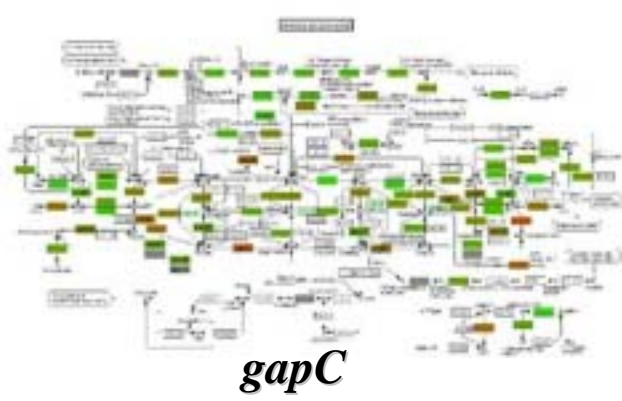


tktB

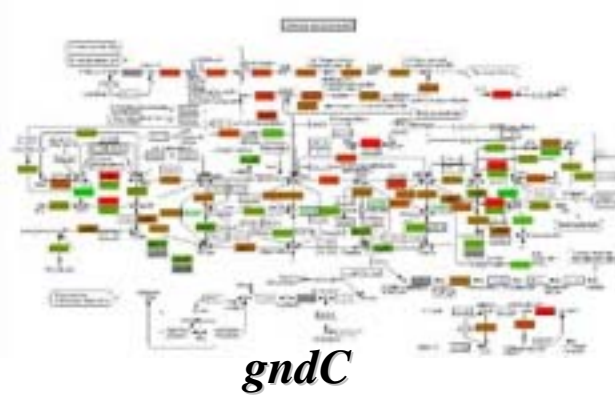


pykF

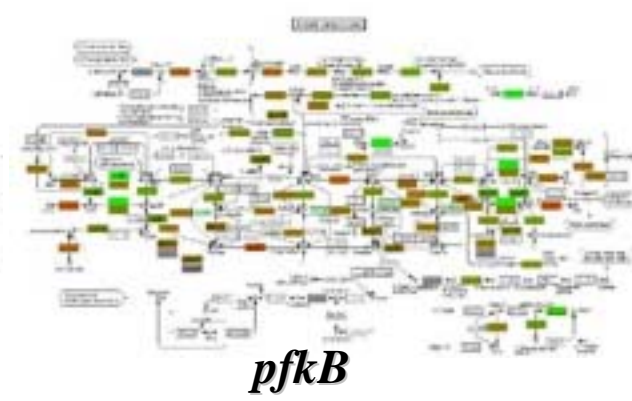
TCA cycle



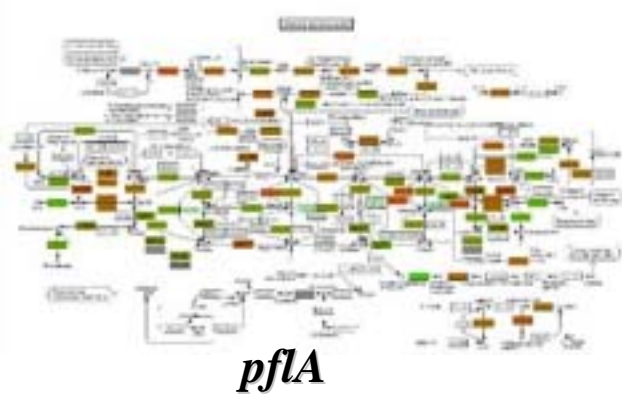
gapC



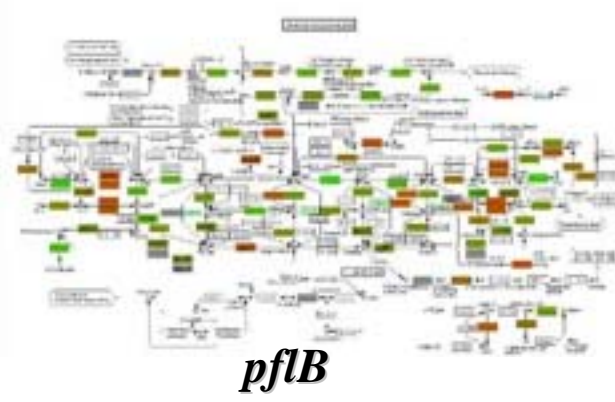
gndC



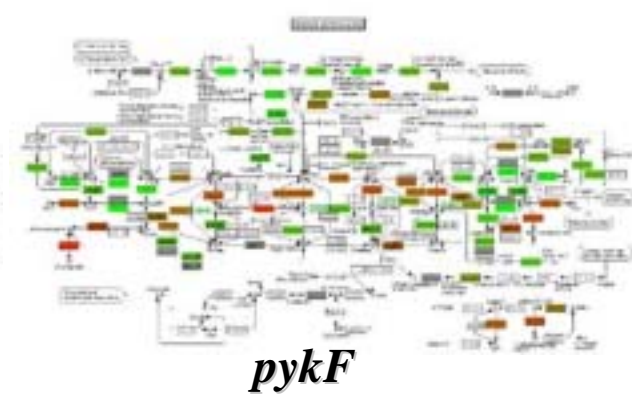
pfkB



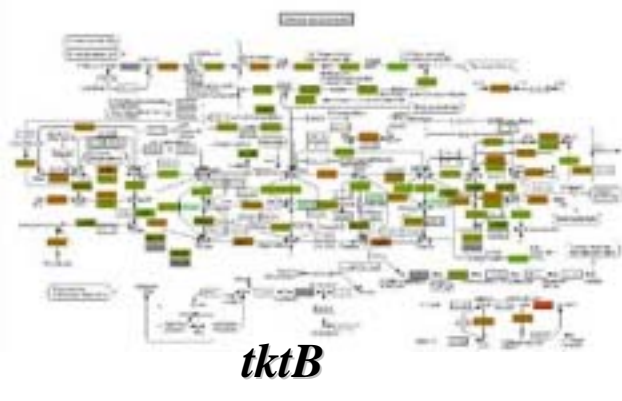
pflA



pflB



pykF



tktB

Purine biosynthesis

Toward modeling & simulation



→ 発現変化

→ mRNA量

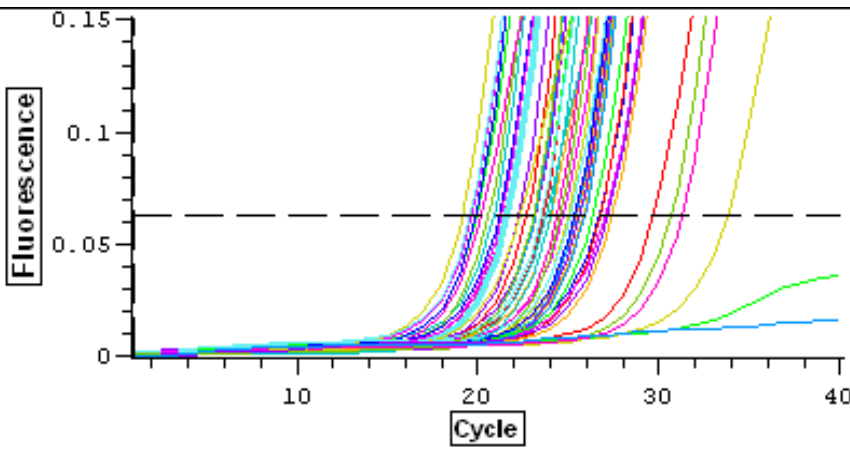
→ タンパク質濃度

→ 酵素パラメーター

→ 基質濃度

→ 反応経路

Quantification of mRNA level energy metabolism related genes Towards the modeling of transcription



<i>gene</i>	CT	<i>gene</i>	CT	<i>gene</i>	CT
<i>aceA</i>	22.7	<i>frdD</i>	30.7	<i>rpiA</i>	22.3
<i>aceE</i>	20.0	<i>fumA</i>	25.9	<i>rpiB</i>	31.3
<i>aceF</i>	19.9	<i>fumB</i>	29.7	<i>sdhA</i>	27.1
<i>aceK</i>	None	<i>fumC</i>	25.6	<i>sdhB</i>	23.8
<i>acnA</i>	25.6	<i>gapA</i>	19.2	<i>sdhC</i>	21.2
<i>acnB</i>	33.8	<i>glcB</i>	26.3	<i>sdhD</i>	24.1
<i>bfkA</i>	26.7	<i>glk</i>	23.6	<i>sucA</i>	21.6
<i>bfkB</i>	25.5	<i>gltA</i>	24.7	<i>sucB</i>	24.6
<i>bgi</i>	23.5	<i>gnd</i>	21.3	<i>sucC</i>	22.2
<i>bykA</i>	25.4	<i>gpmA</i>	20.6	<i>sucD</i>	23.1
<i>eno</i>	23.2	<i>icdA</i>	20.2	<i>talB</i>	23.5
<i>epd</i>	21.4	<i>mdh</i>	25.4	<i>tktA</i>	23.1
<i>fba</i>	19.7	<i>pdhR</i>	26.8	<i>tktB</i>	27.2
<i>frdA</i>	25.1	<i>pgk</i>	19.6	<i>tpiA</i>	20.9
<i>frdB</i>	24.4	<i>ppsA</i>	25.8	<i>zwf</i>	27.4
<i>frdC</i>	25.1	<i>rpe</i>	24.0		



What is GenoBase

- » getting started
- » user's manual
- » update news
- » publications
- » environments
- » outline

SEARCH

- » simple search
- » individual-table-search
- » homology search
- » transcriptome
- » 2D proteome
- » transcription/regulation
- » references(mutation)
- » map position search
- » sequence
- » SQL execution

TOOLS

- » complement/translate

FTP Service

- » FTP service list

Resources

- databases
- experimental resource
- analysis tools

Links

- » *E.coli*-related & bacteriology

Old version *E.coli* Database

- » GenoBase 3.0 / 2.0

Research activities

- » CREST(Japanese)

Comments & Requests

- » *E.coli* mailing list
- » request

Genome Analysis Project Japan

Escherichia coli K-12 W3110



Notice!

Java applet This mark stands for Java Applet by Java2 SDK (JDK1.3).

We are checking of normal operation on Windows2000/NT ,IE5 & Netscape4.7.

2002.10.10 GenoBase4.1 release.

What's new?



Gene List GENE_NAME , ORF_ID TABLE

Search

simple

individual table

homology

transcriptome

2D proteome

transcription

references

map position

sequence

SQL execution

» by keyword (exact match)

- Enter a word for JW id or gene name -

Search

» by keyword (fuzzy search)

- Enter a word for gene id, gene name or biological keywords -

protease Search

» by Kohara clone

- Enter Kohara-clone NO.(101 - 676) -

Search

» Function category

View