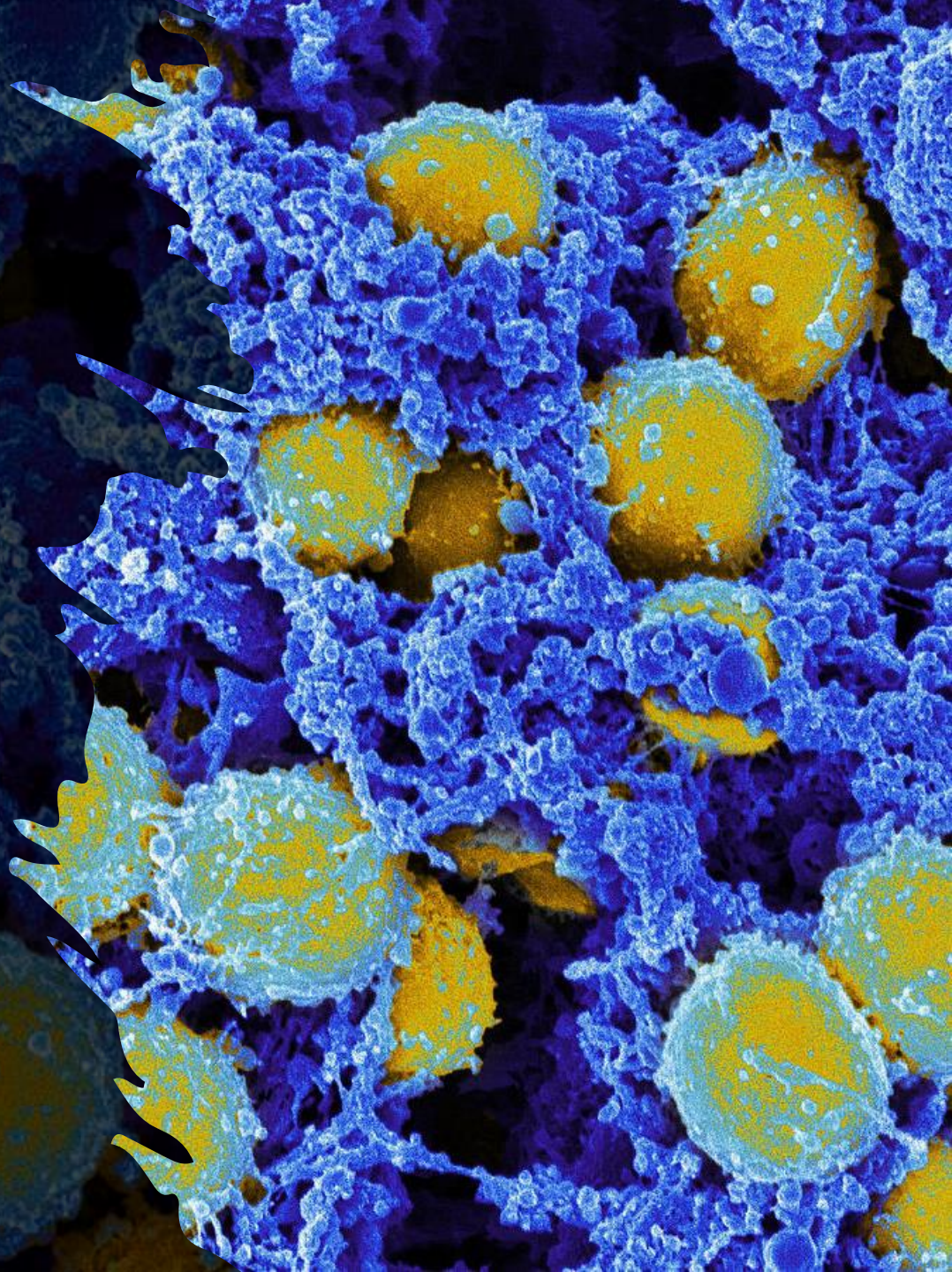
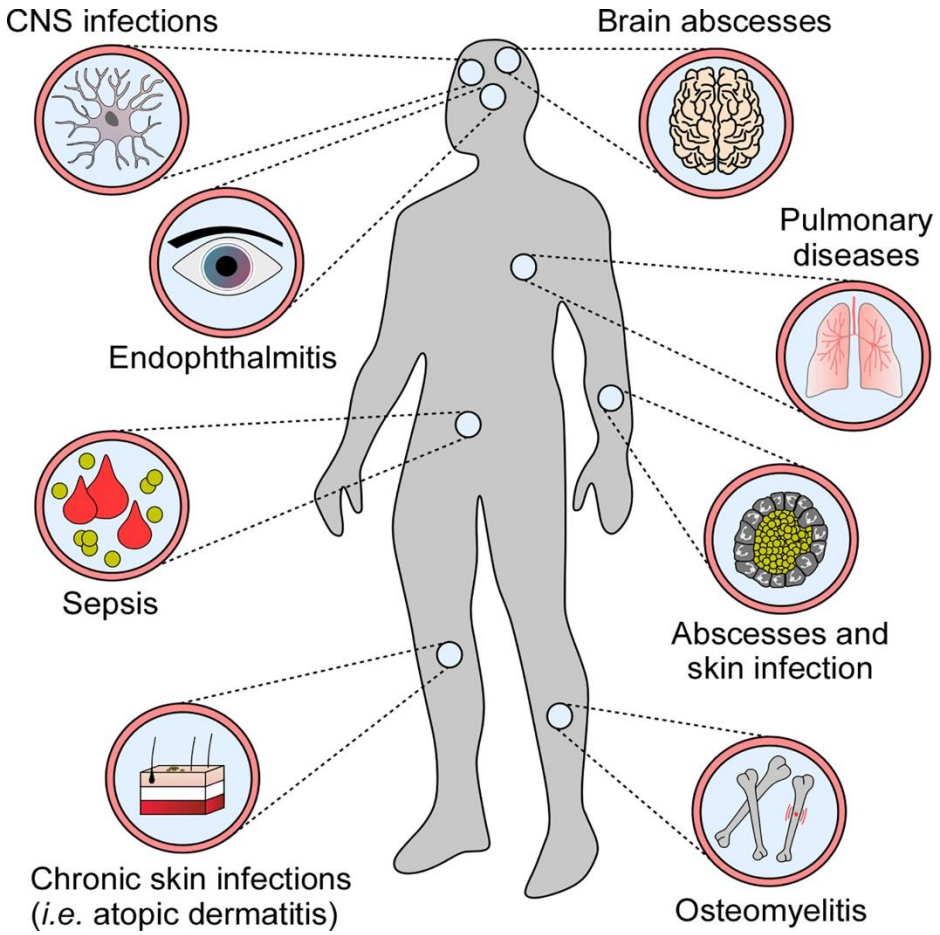
Deciphering the role of **endoribonuclease RNase III** in regulating **Methicillin-resistant *Staphylococcus aureus* (MRSA) gene expression** using **Nanopore sequencing**



Linqing Hu
2023 BSc (Hons) Biological Science (Biotechnology)
Sander Granneman's Lab

Background 1: Why studying **MRSA gene expression**



Diseases caused by S. aureus on human body

https://www.frontiersin.org/articles/10.3389/fimmu.2020.621733/full

Staphylococcus aureus

Opportunistic pathogen

Serious infections !

Methicillin-resistant *Staphylococcus aureus* (MRSA)

Strong antibiotics resistance

Global prevalent

Lack of treatment

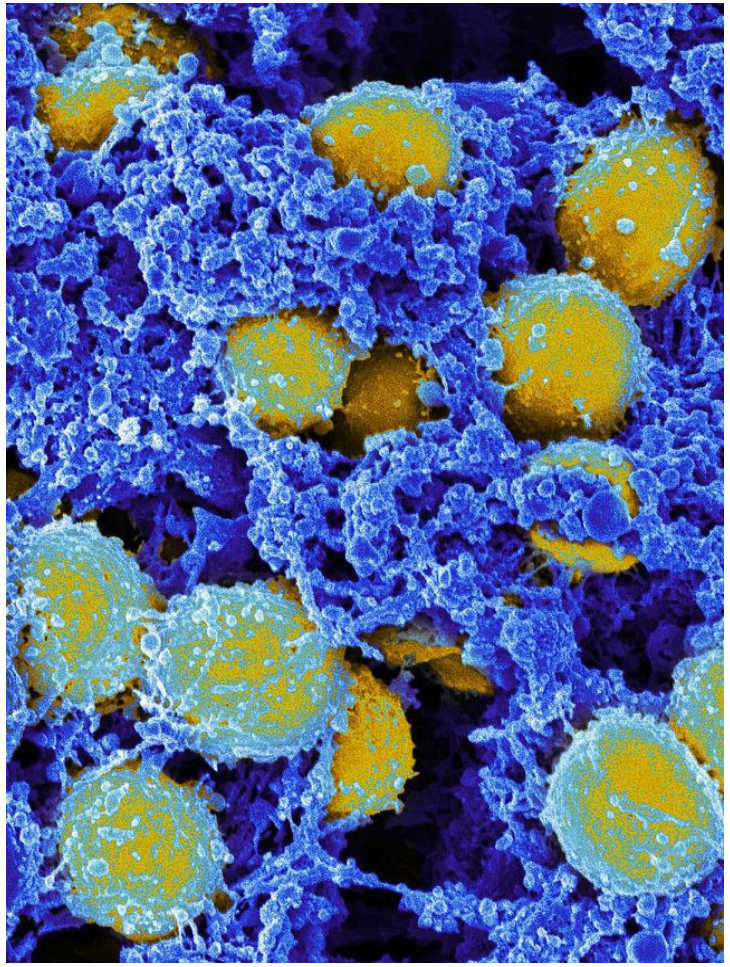
New drug targets !

New treatment strategies !

MRSA USA300

Many virulence factors

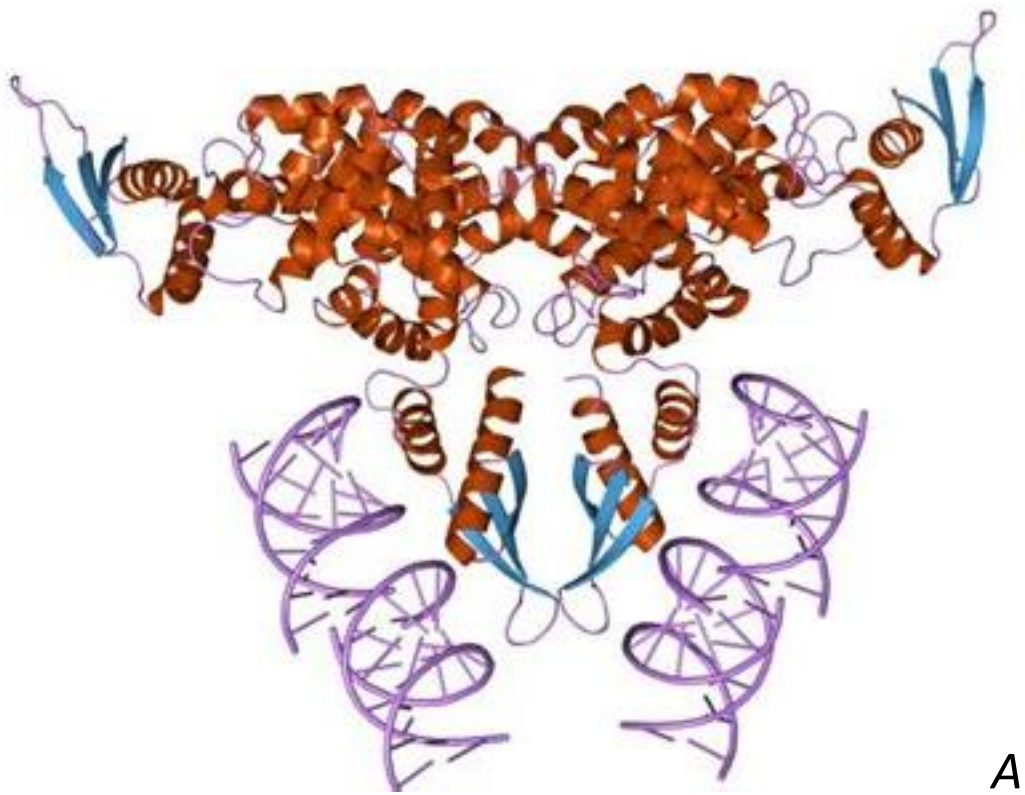
A good repository to decipher gene expression mechanisms !



S. aureus EM scan

https://fineartamerica.com/featured/5-staphylococcus-aureus-bacteria-sem-science-source.html

Background 2: Why studying **RNase III**



RNase III homodimer
protein *structure*
https://www.ecosia.org/images?q=RNase%20III%20protein%20structure#id=0873E3D3743233F1BACAFA786C20FFEBB27F349C

Features:

- **Mg^{2+}** dependent **endoribonuclease**
- Cleaves **RNA double strands**

Functions:

- **RNA processing**---cleave **sRNA-mRNA duplex**, release **mature mRNA**
- **RNA maturation**---cleave **rRNA** and **tRNA precursor** release **mature rRNA** and **tRNA**
- **RNA degradation**---degraded unwanted transcript
  ---Transcriptional level control
  ---nucleotide recycling

Achieving functions in help with:

- **small RNAs (sRNAs)**
- **RNA binding proteins (RDBs)**

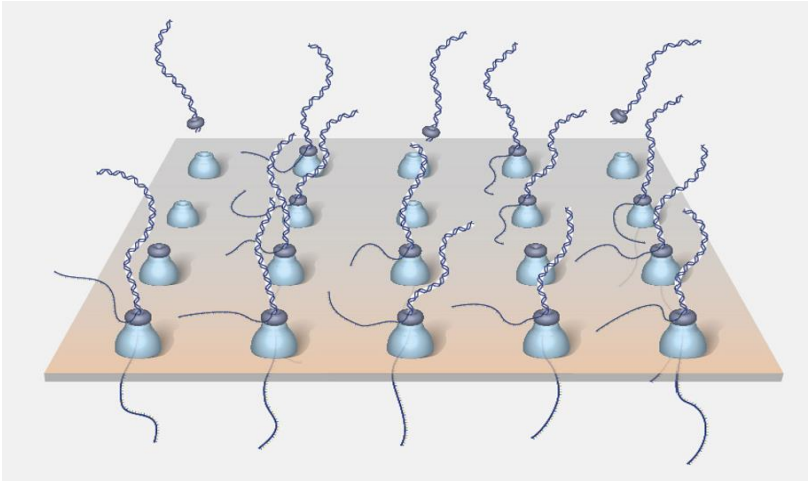
Functions** and **targets** are **underestimated !

Aim:** discover more **RNase III targets !

Background 3: Why using **Nanopore sequencing**

Oxford Nanopore sequencing machine PromethION

https://nanoporetech.com/products/promethion

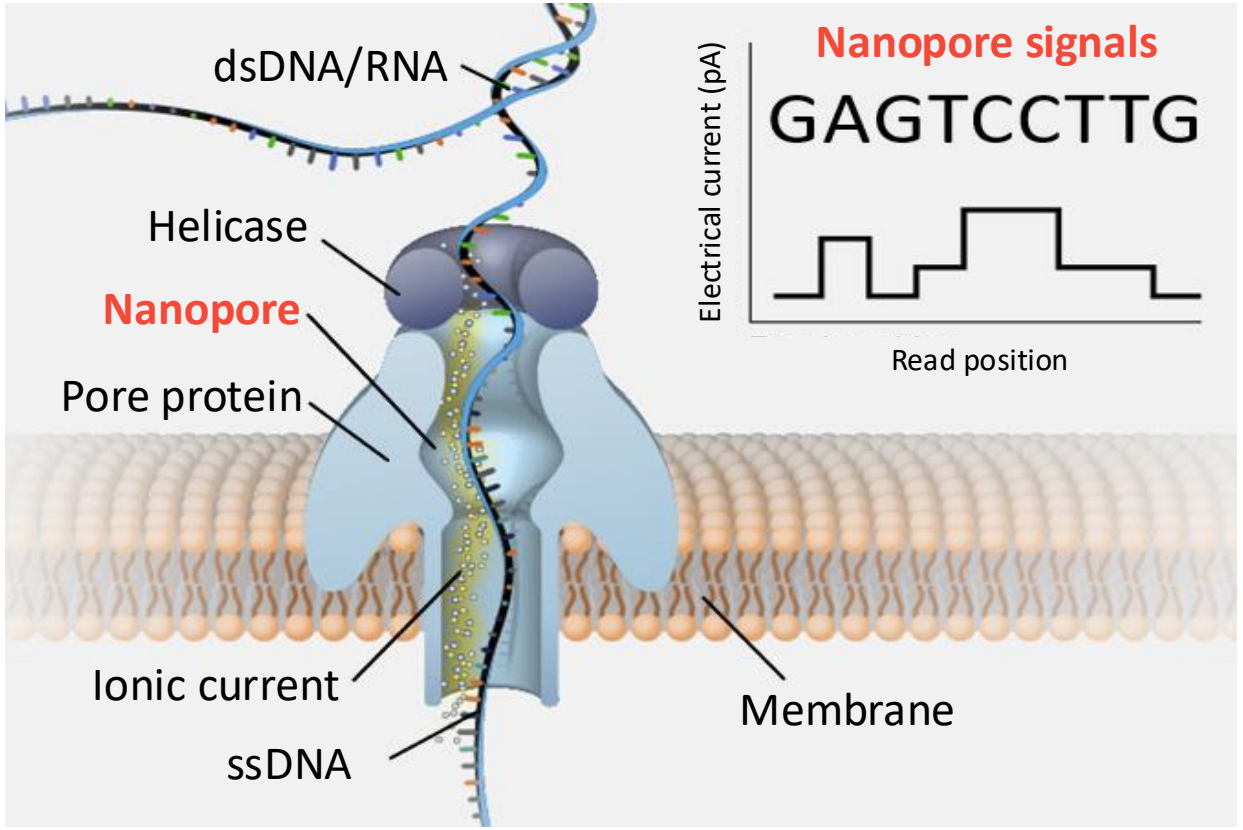


Many sequencing happen together

https://www.genome.gov/genetics-glossary/Nanopore-DNA-Sequencing?fbclid=IwAR31BY-R_LVsBCL4VsBLwihRt3Xm47uVK7dy9QcRHYTkZJbsMeGxcylw20Q

Illumina sequencing---Short reads, lack 3' UTR information

Oxford Nanopore sequencing---Long reads



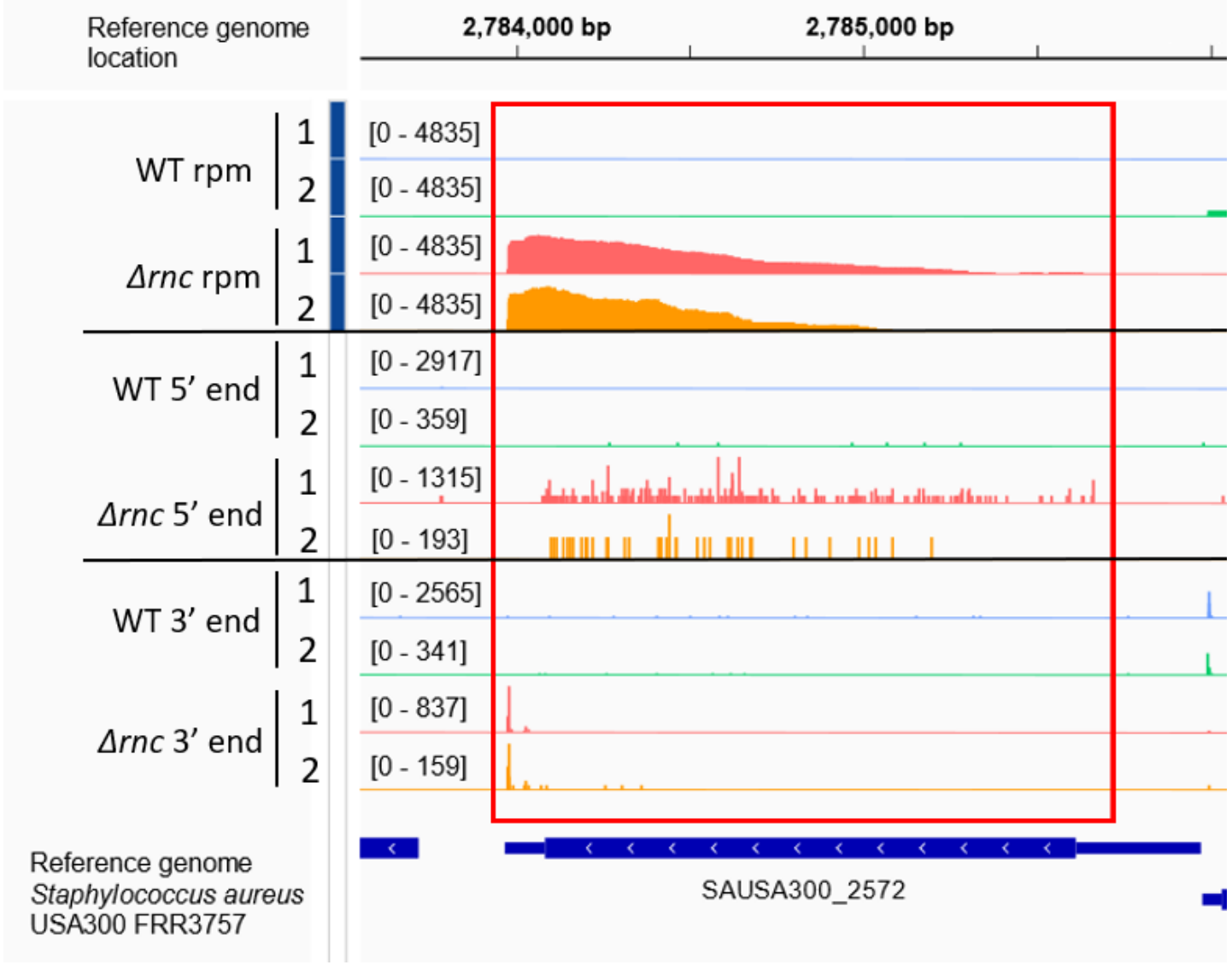
Hope: Read transcripts in full length
---get complete information of:
transcripts boundaries and **RNase III cleavage sites**

Experiment 1: Discover **Differential Gene Expression (DGE)** in Integrated Genome Brower (IGV)

DGE

(1) Variation in coverage

mRNA aur
SAUSA300_2572



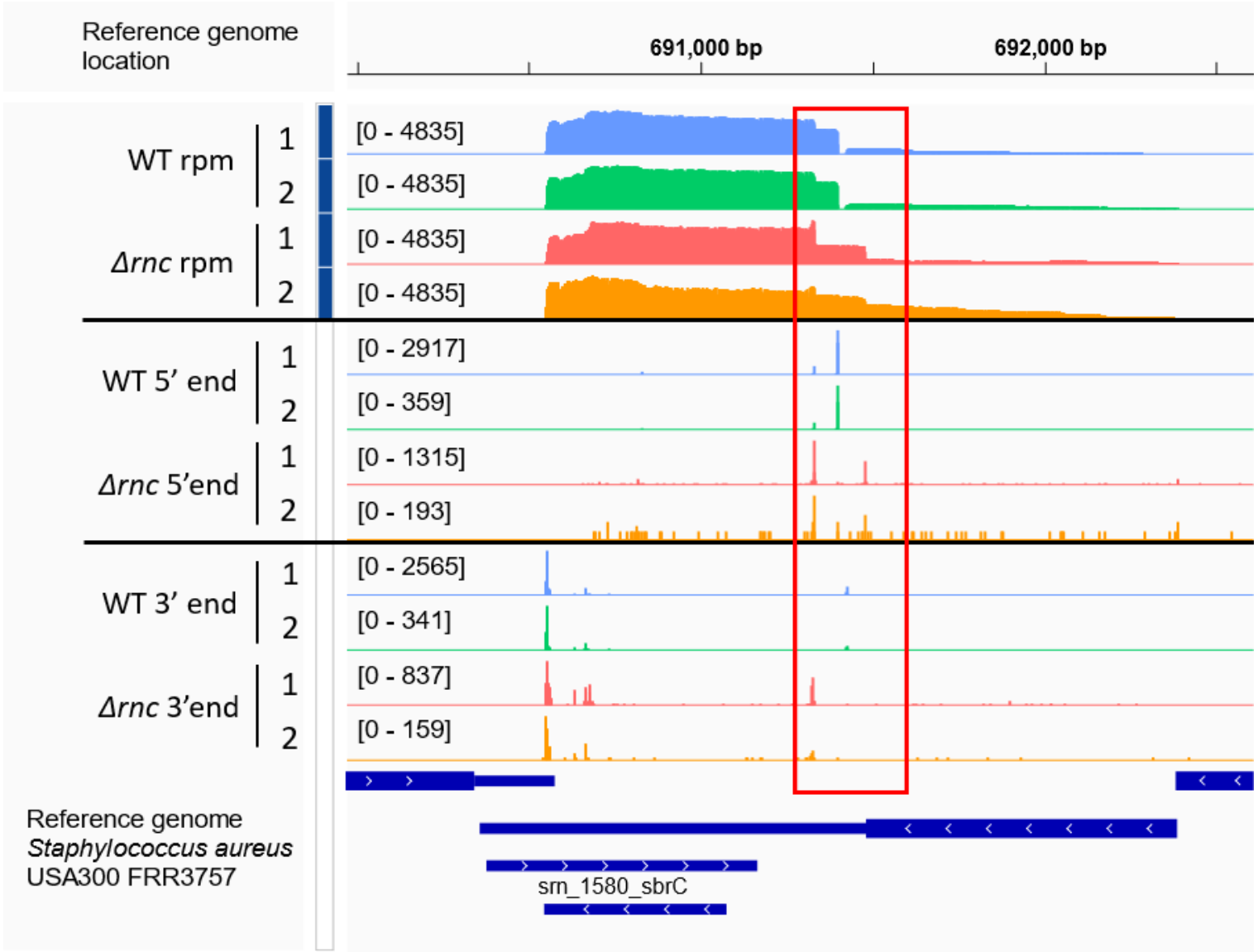
Experiment 1: Discover **Differential Gene Expression (DGE)** in Integrated Genome Brower (IGV)

DGE

(2) New cleavage site

sRNA

srn_1580_sbrC



Experiment 2: Performing DEseq2 to filter DEGs

Step 1: **Remove genes has ≤ 10 counts**

Method 1: 'Single sample'--- ≤ 10 counts in any single replicate

Method 2: 'All samples' --- ≤ 10 counts in the sum of 4 replicates

Step 2: **Prepare two input tables**

(1) Counts table

gene	ssrA	PSMa1	PSMa2	PSMa3	PSMa4
WT1	694607.0	779.0	1365.0	4612.0	6294.0
WT2	191721.0	393.0	618.0	1361.0	1788.0
drnc1	193327.0	344.0	637.0	2234.0	3411.0
drnc2	36197.0	157.0	285.0	777.0	1118.0

...

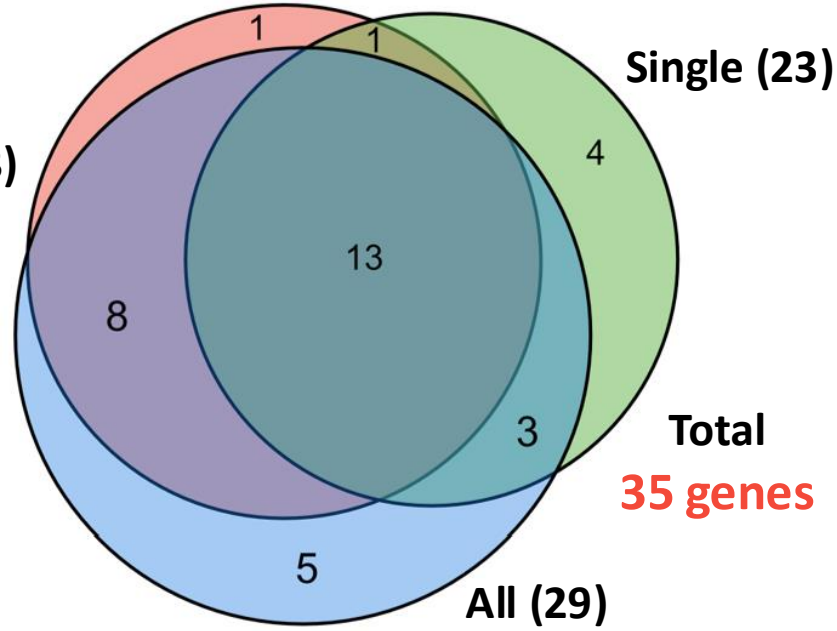
(2) Clinical table

gene	Condition
WT1	Wild Type
WT2	Wild Type
drnc1	RNase III Knock Out
drnc2	RNase III Knock Out

Step 3: **Run Deseq2 analysis, using adjusted P-value**

gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
ssrA	175653.769888	0.328625	0.463209	0.709452	0.478044	0.954306
PSMa1	408.012614	-0.634902	0.826624	-0.768066	0.442448	0.954306
PSMa2	713.146031	-0.801779	0.816327	-0.982178	0.326012	0.871300
PSMa3	2000.145241	-0.975179	0.675426	-1.443799	0.148796	0.653505
PSMa4	2844.013245	-1.109305	0.651677	-1.702231	0.088712	0.531057

Datasets too small !



Step 4: Run Deseq2 analysis, using **P-value**

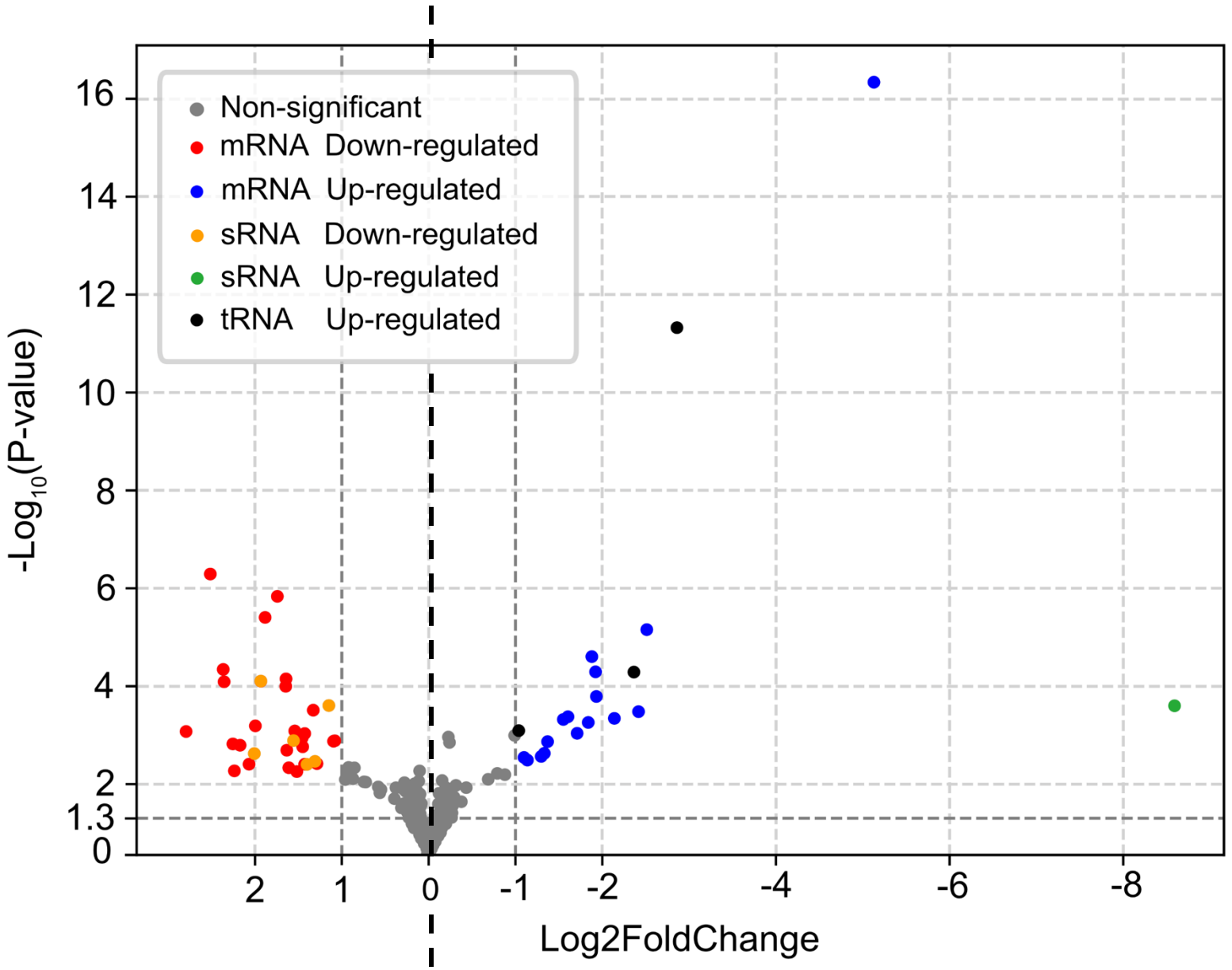
P-value ≤ 0.05, log2FC ≤ |1|

114 genes

significant differential expression

Experiment 2: Visualising DGE result in volcano plot

Down regulation in *Δrnc*



Up regulation in *Δrnc*

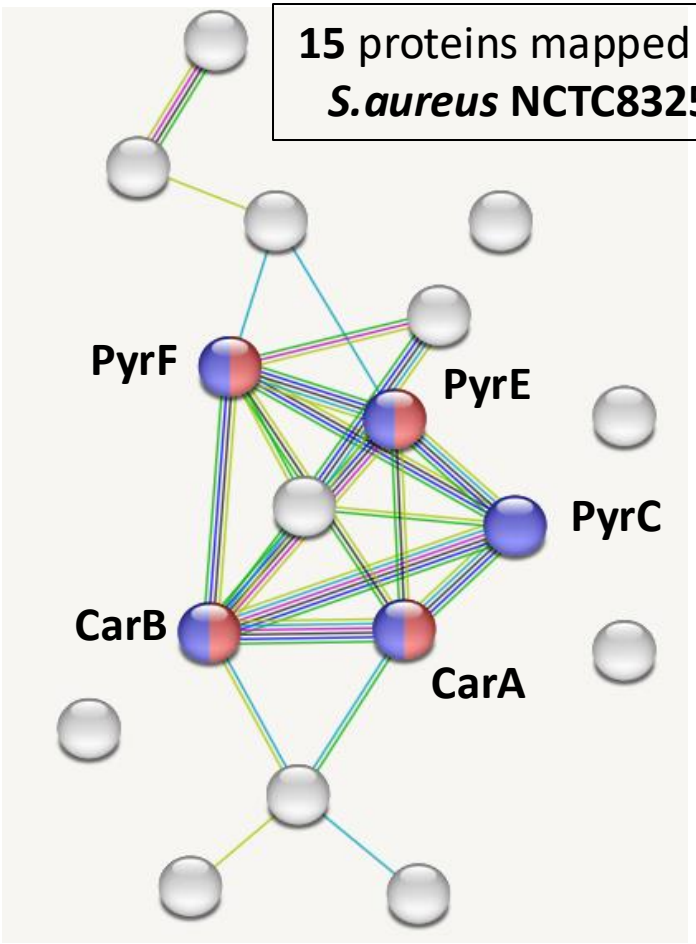
Experiment 3: GO term and KEGG enrichment analysis in STRING

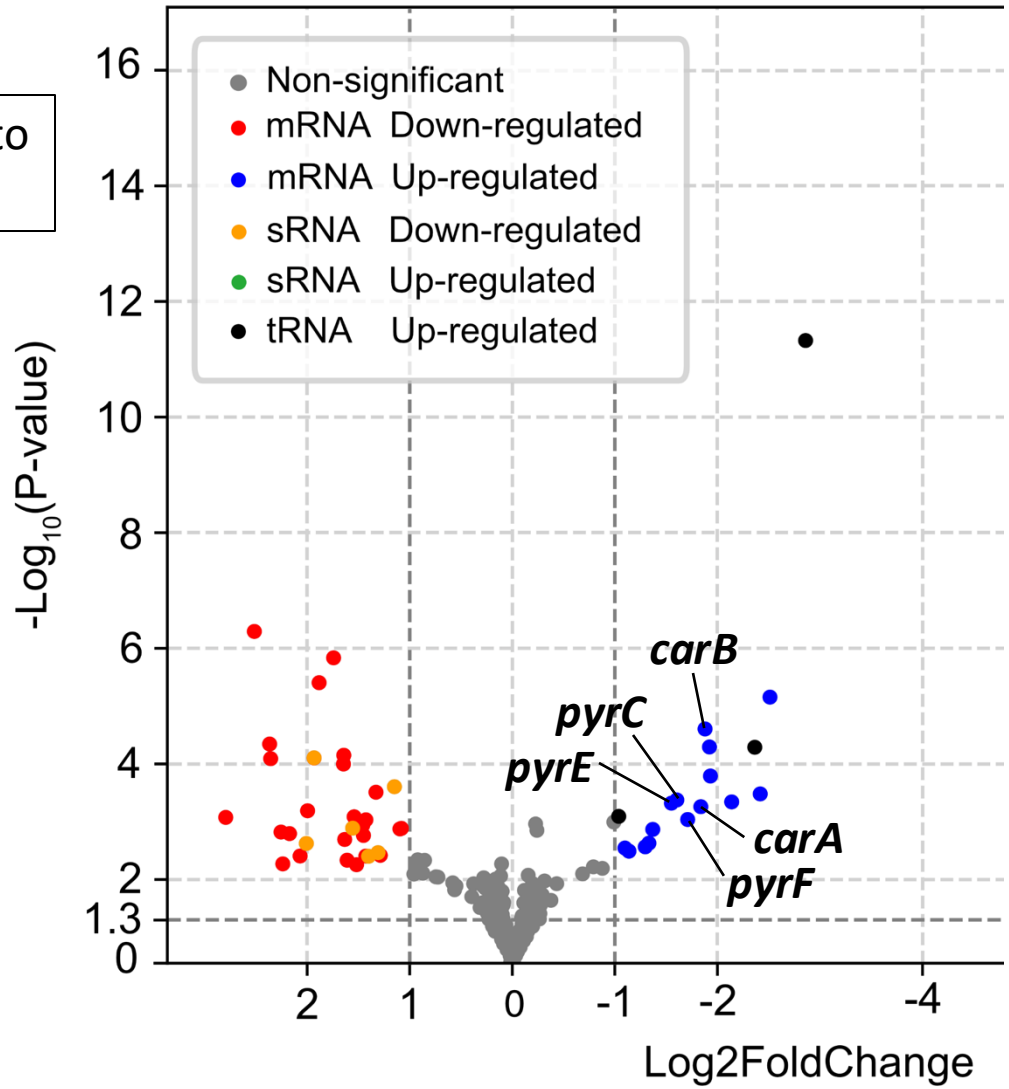
Gene Ontology (GO)

KEGG (Kuydo)

De novo Uridine monophosphate (UMP) biosynthetic process

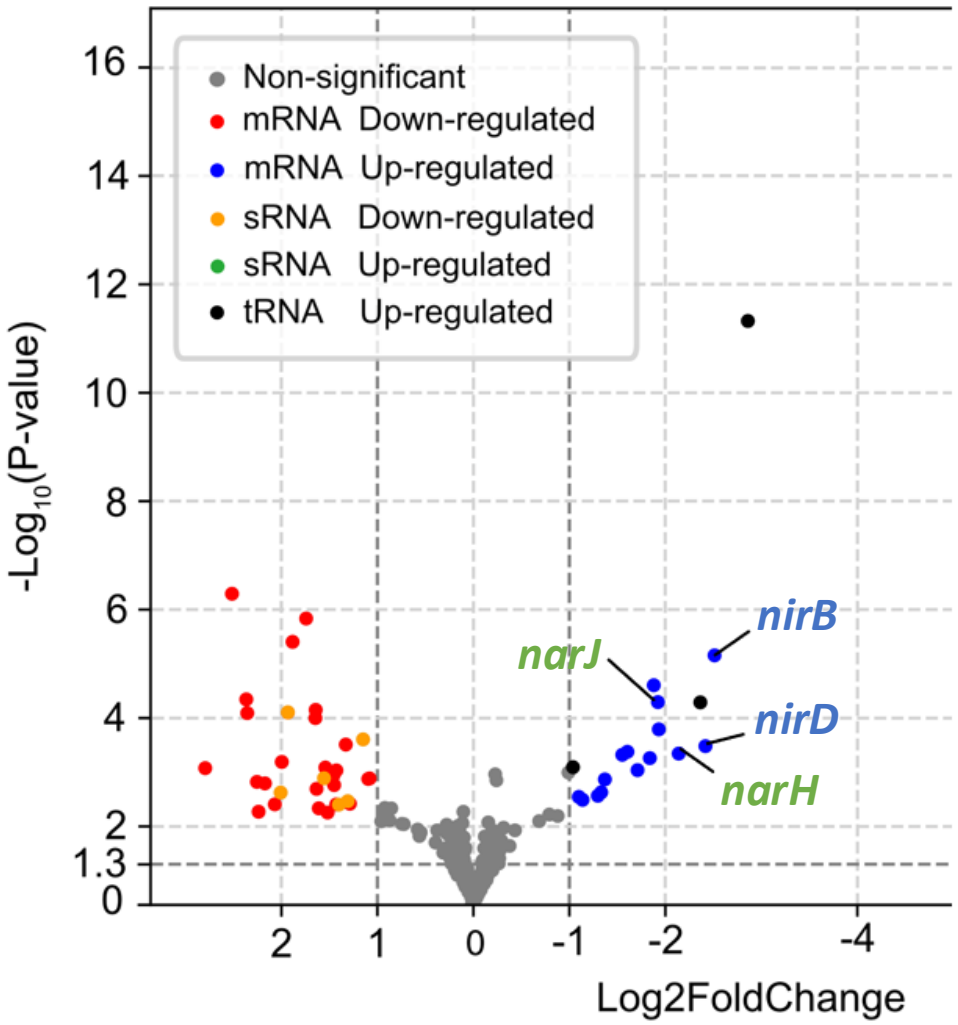
Pyrimidine metabolism





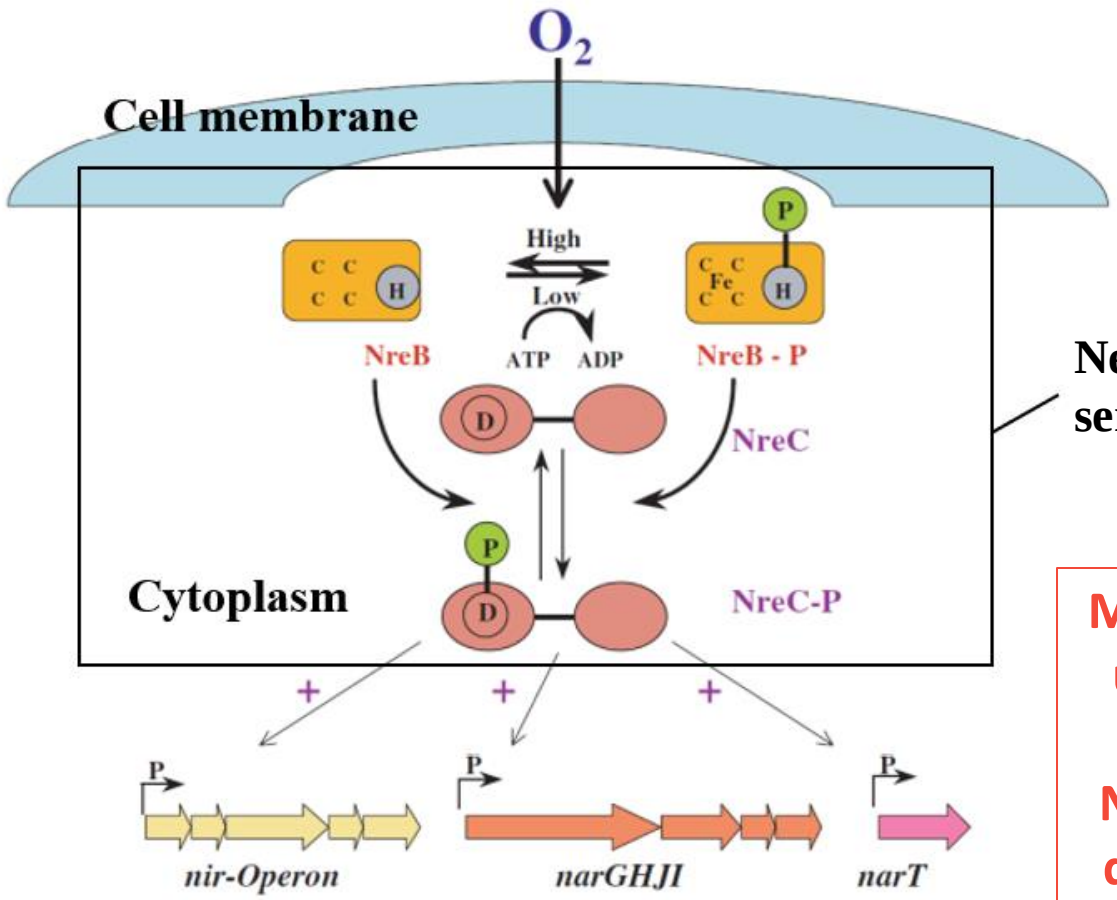
Analysis result 1: Nir and Nar families are up regulated in *Δrnc*

Nir family---Nitrite reductase
Nar family---Nitrate reductase



In Native condition:
On---low O2/iron
Off---with O2/iron

In *Δrnc*---up regulated
RNase III---suppress gene expression
---same effect as when with O2/iron

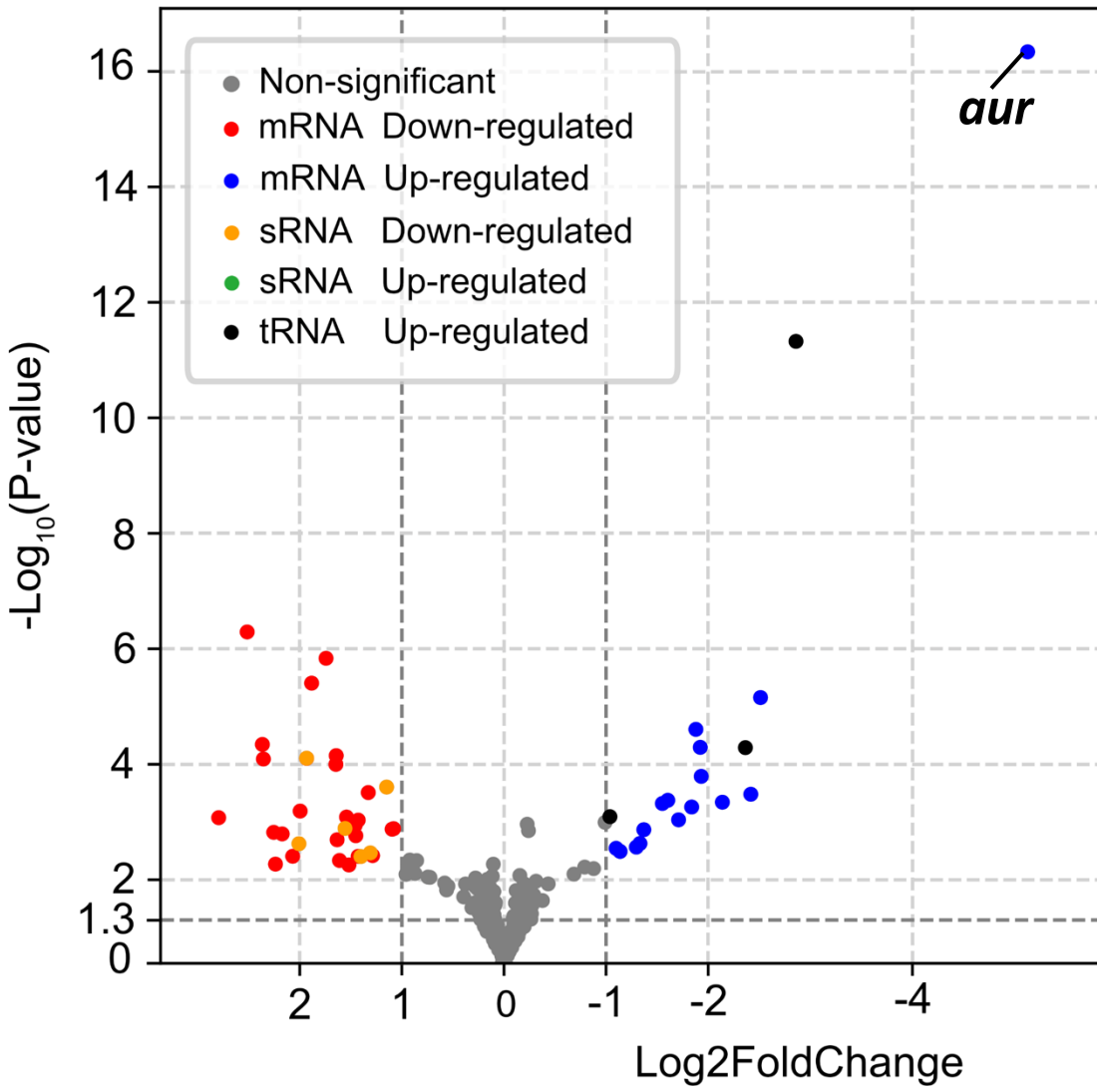


Mechanism unknown

New study direction !

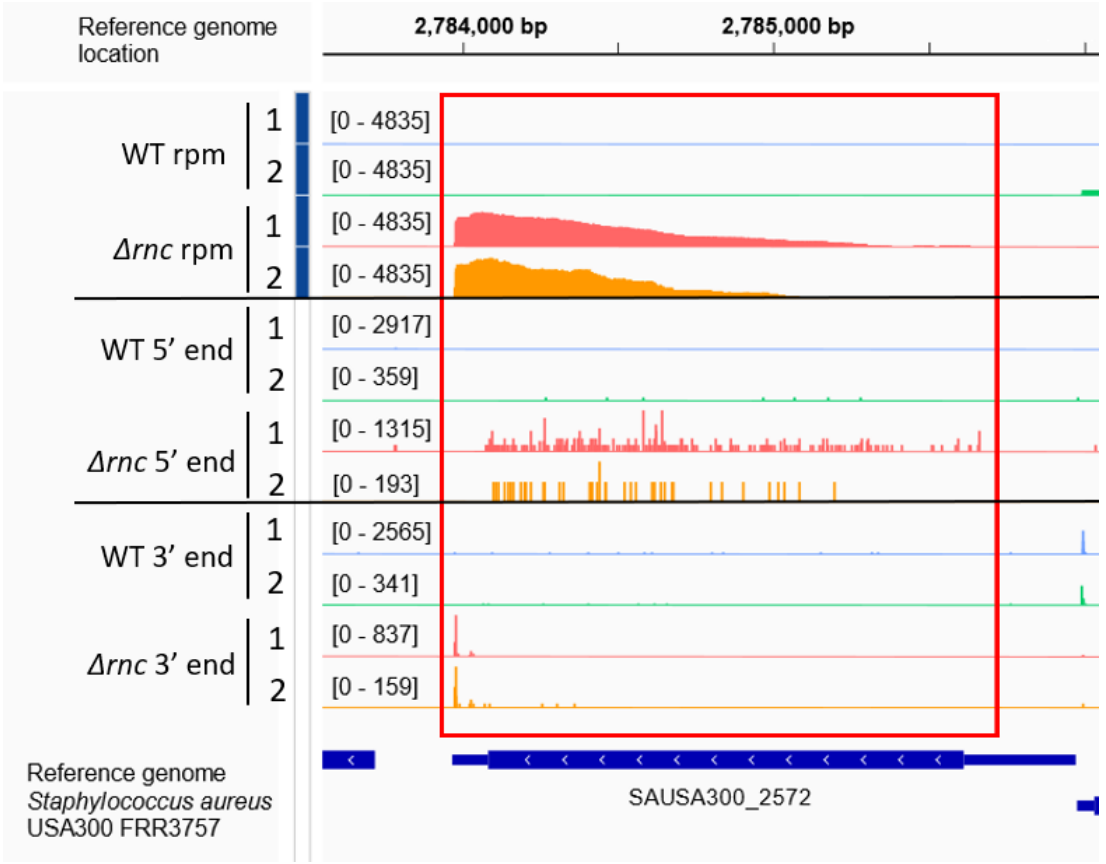
https://theses.hal.science/tel-01424170/preview/VD2_LELAM_THAONGUYEN_24092015.pdf

Analysis result 2: genes ***aur*/sspABC/scpA** are **up regulated** in ***Δrnc***



aur **log2FC**: 5.24 --- **38** fold up regulated
p-value: 3.70E-13 --- significant

	WT1	WT2	*Δrnc1*	*Δrnc2*
aur raw counts	14	8	285	37

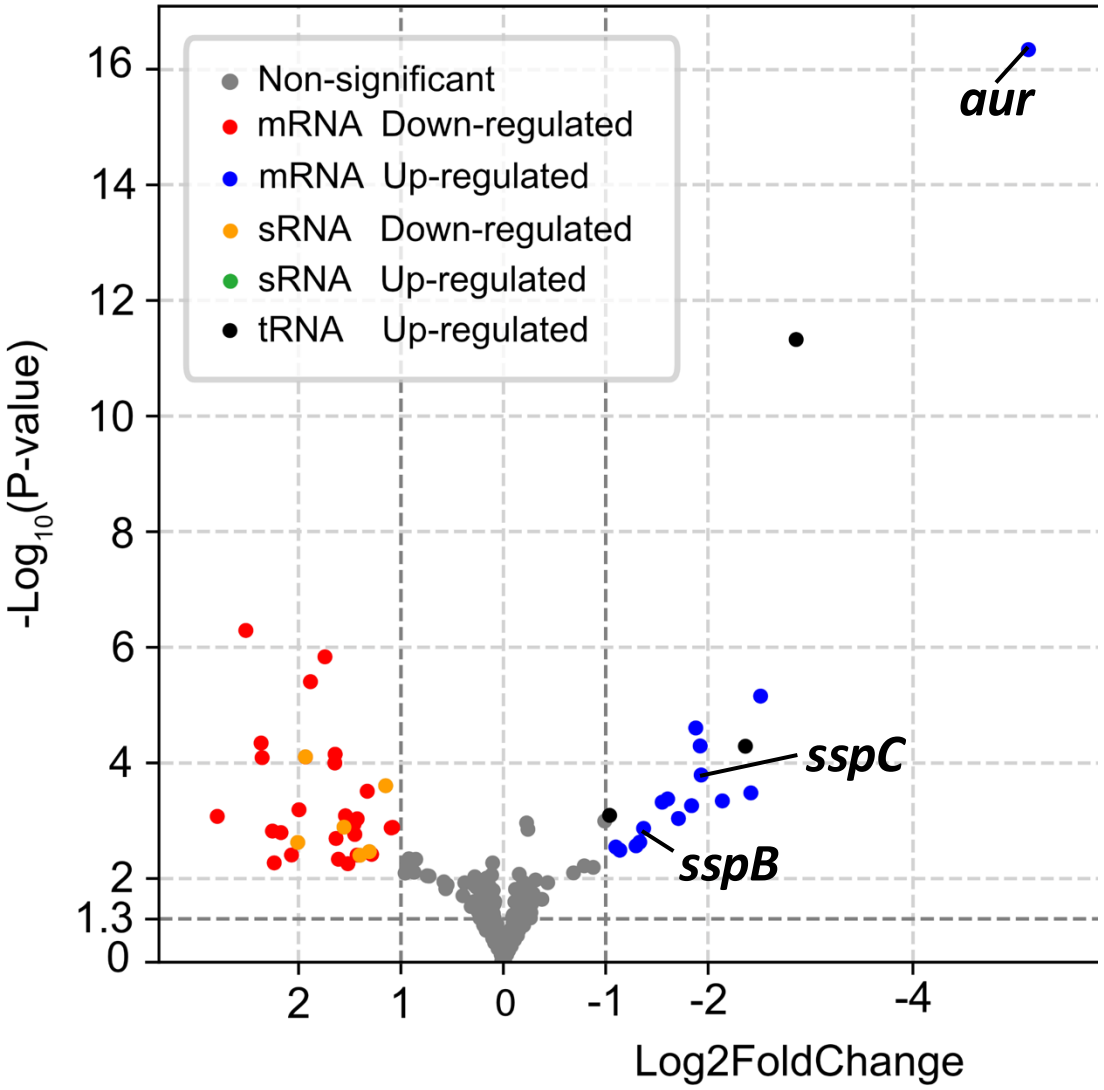


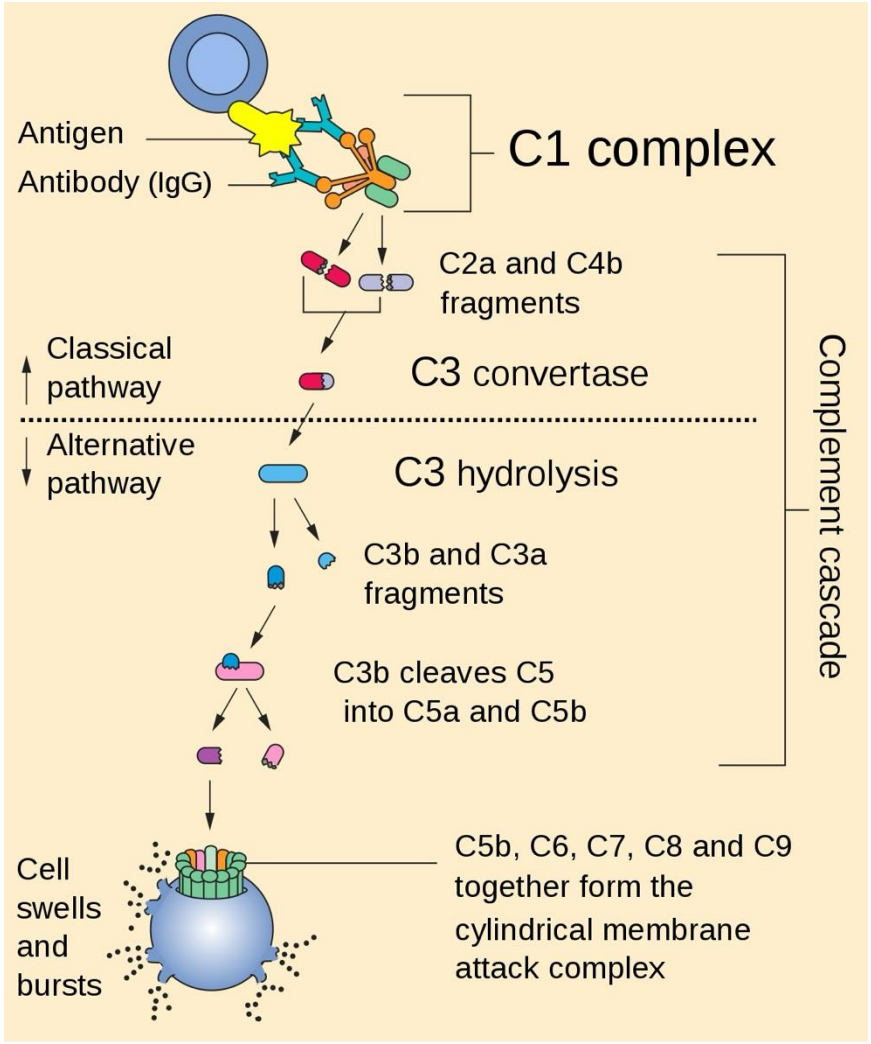
Analysis result 2: genes ***aur*/sspABC/scpA** are **up regulated** in ***Δrnc***

aur :

- **zinc dependent metalloproteinase**
- **509 aa, secretary enzyme**

Type	Protein	Gene	Regulation	Significance
Cystine protease	Stapthopin A	*scpA*	Little up	No
	Stapthopin B	*sspB*	Very up	Yes
		sspC	Very up	Yes
Serine protease	V8	*sspA*	Very up	No
Metallo-proteinase	**Aur**	*aur*	Very up	Yes





Human complement immune pathway

https://www.ecosia.org/images?q=human%20complement%20system#id=E77DDA1C45D1FB2874E194DC0CD72AE4AAD320FD

Aur function

1. Cleave host complement protein **C3** to **C3B**
   ---C3b rapidly degraded in serum by other proteases
   ---Host cell has poor C3b opsonising
   ---attenuates **neutrophils phagocytosis** and **killing**
2. Cleave host **LL-37,** a **cathelicidin**
   --- prevent host cell membranes puncturing in reacting with pathogens
3. Activates host **prothrombin**
   --- increase pathogenicity in causing septic infection

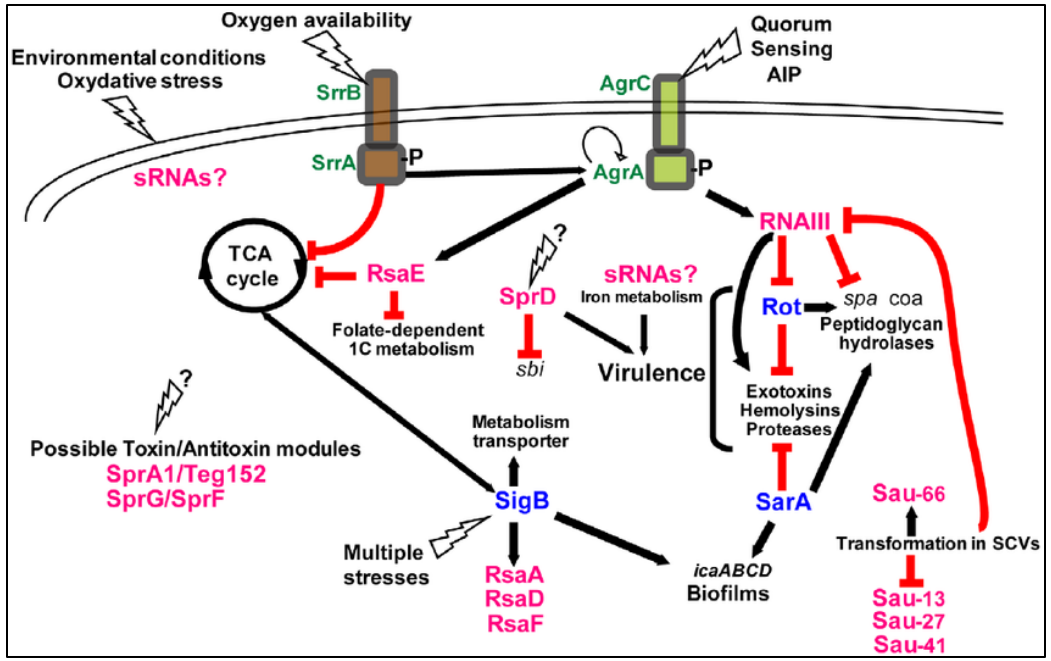
Other evidence

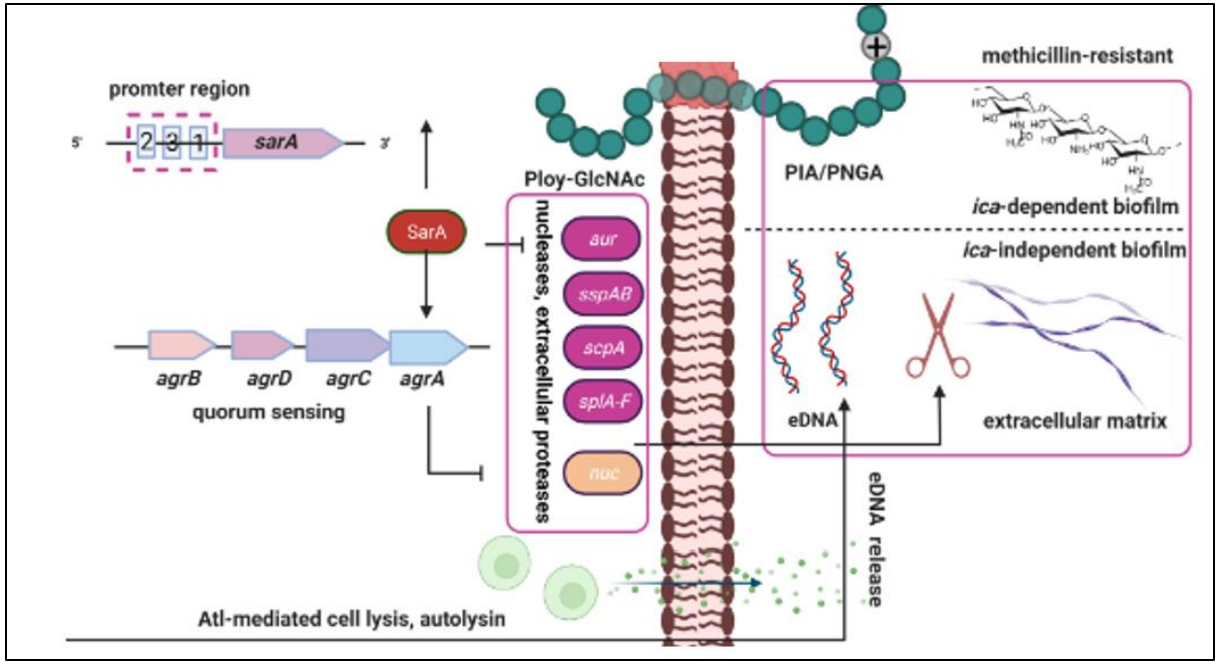
Δaur strain---*more efficiently killed in human blood*

Conclusion

1. Gene ***aur*** can help *S.aureus* to **escape host cell immune system**
2. This is **linked with RNase III activity**

RNase III function---suppress Aur/SspAB/ScpA---biofilm formation↑





Arg↑ — SarA↑ — Aur↓ / SspA/B↓ / ScpA↓ — Biofilm↑ — RNase III

Mechanism unknown

New study direction !

Further experiments…

- WT vs *Δrnc* Biofilm Assay
- rt-qPCR, Northern blotting

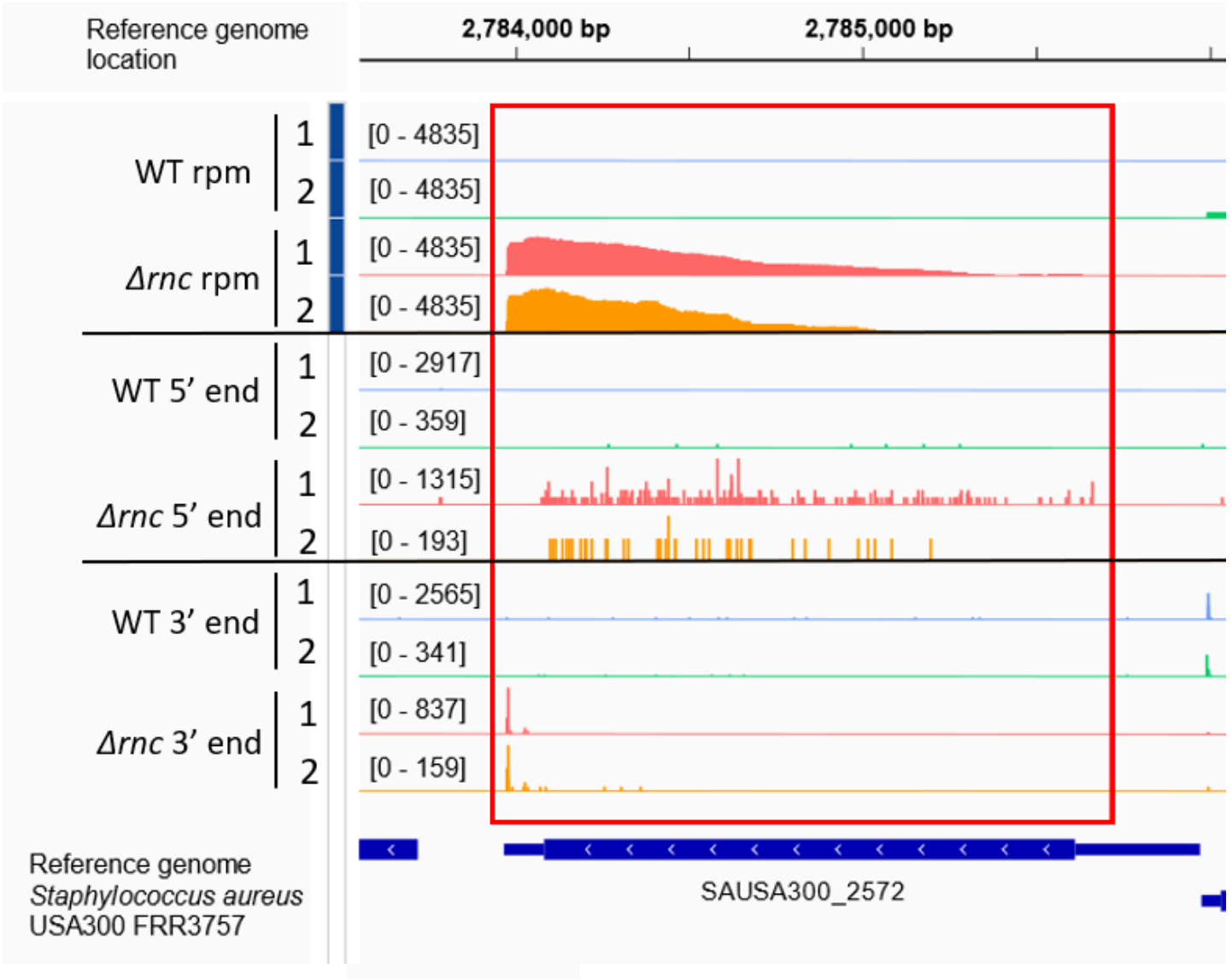
Discussion: Problem with this experiment…

1. Inaccurate mapping

DGE

(1) Variation in coverage

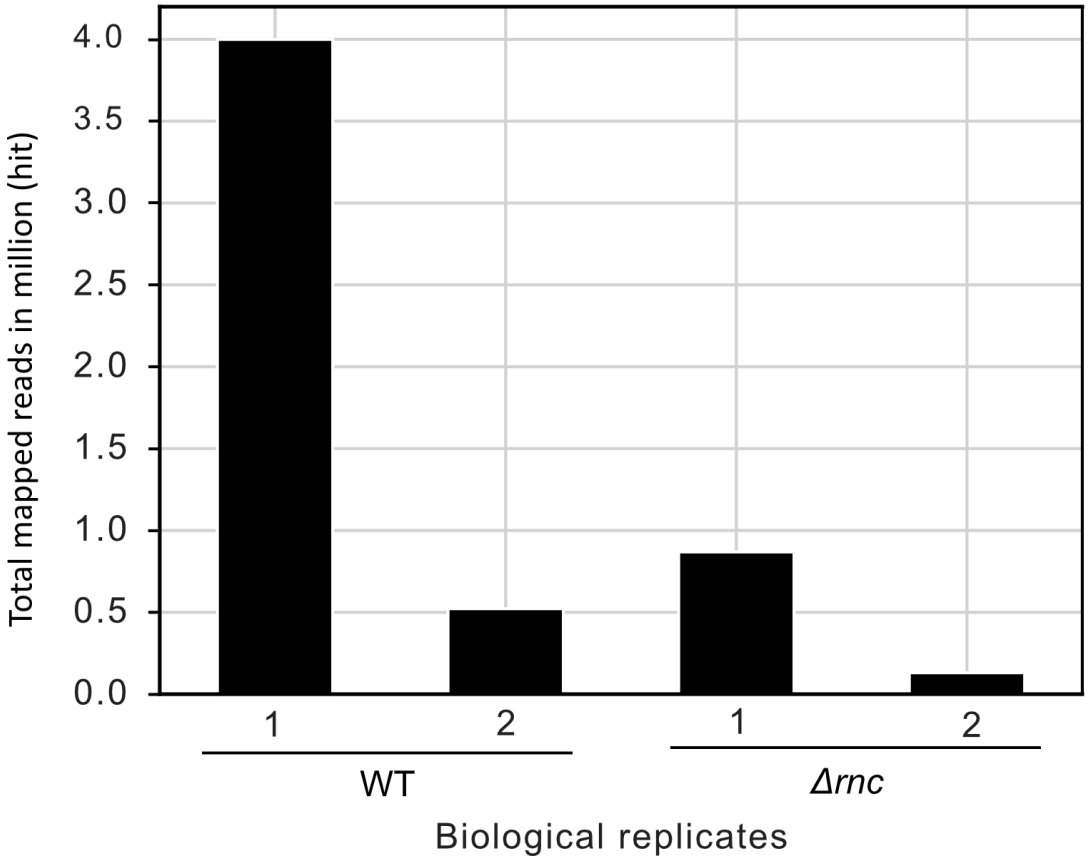
mRNA aur
SAUSA300_2572



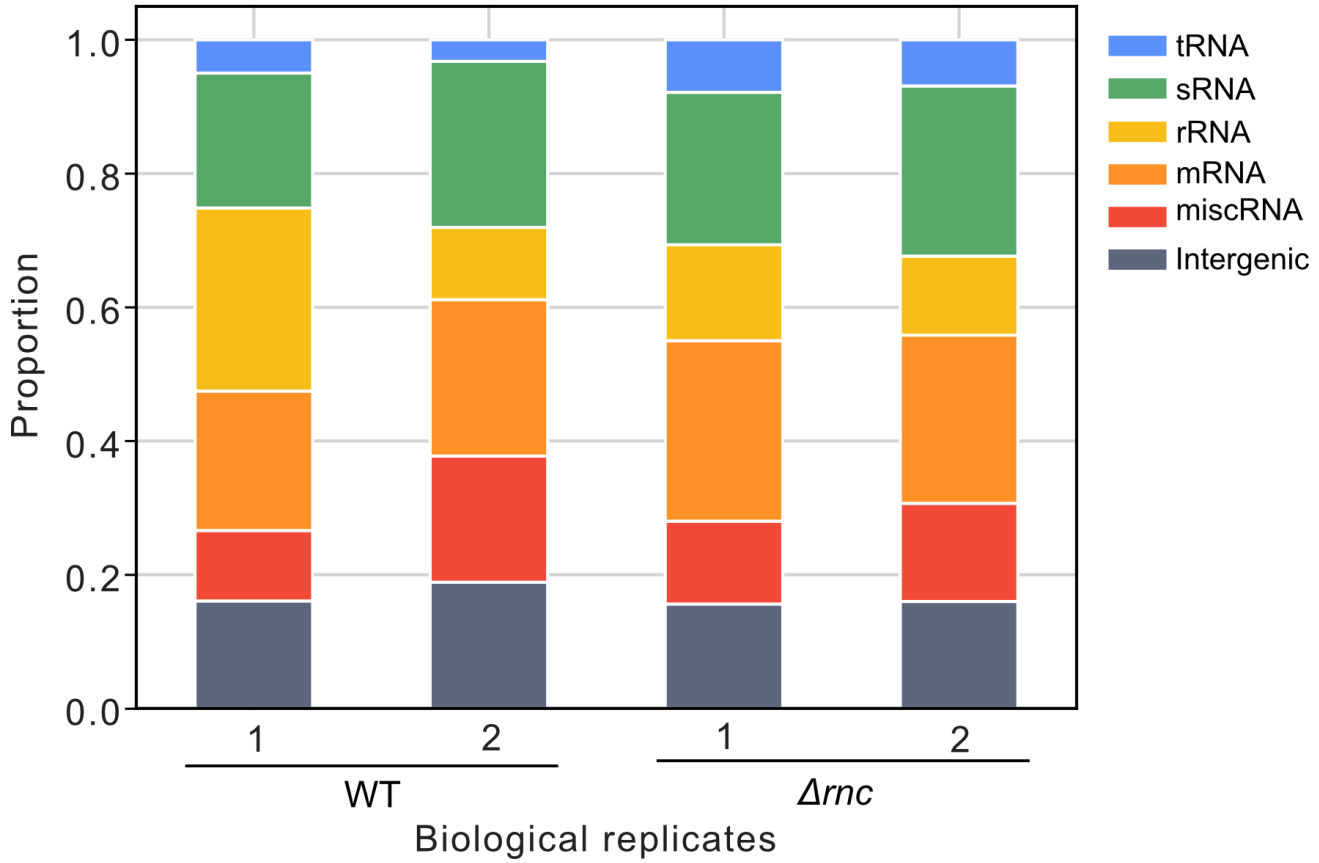
Discussion: Problem with this experiment…

2. Large variation between replicates

Total mapped read counts in different replicate

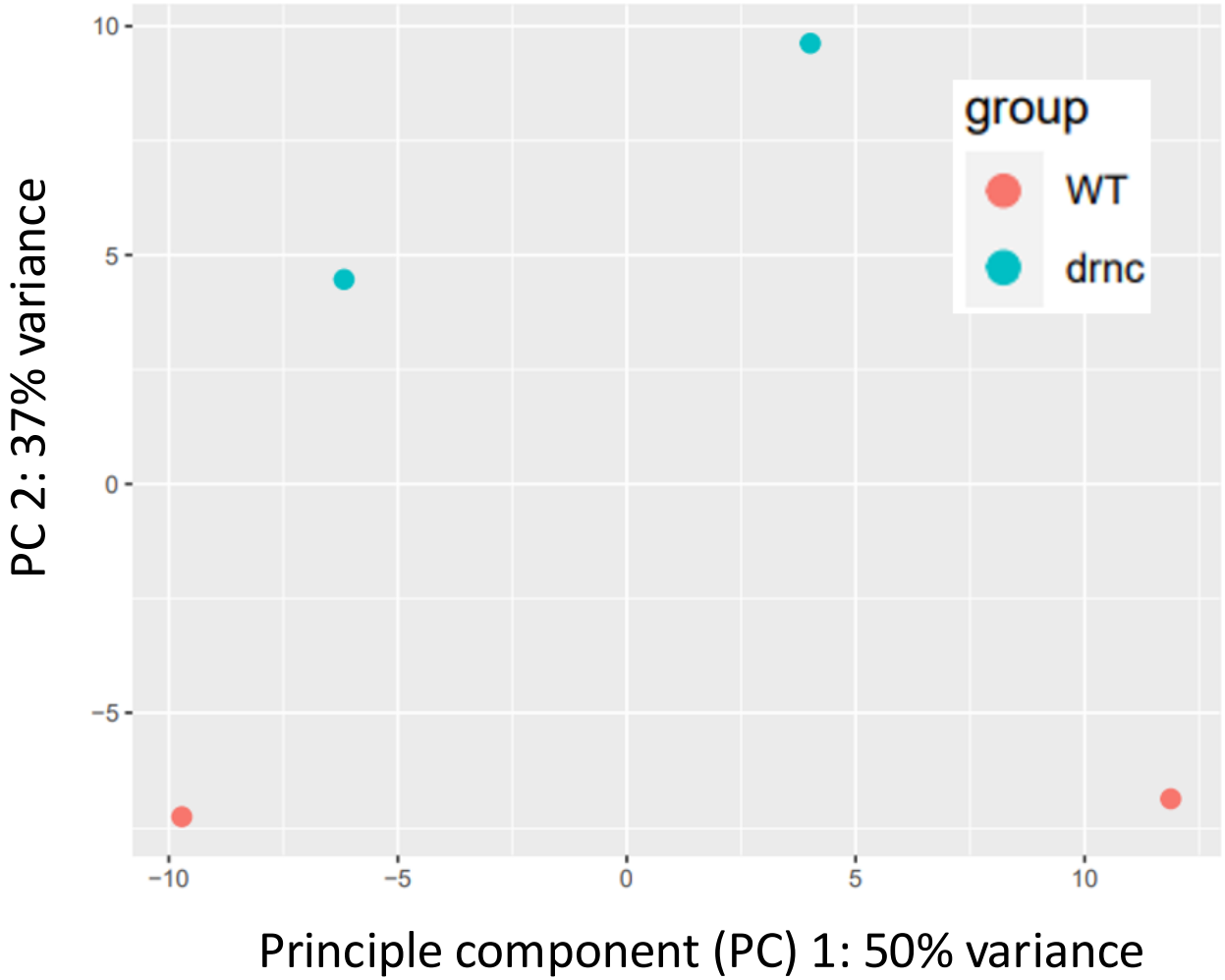


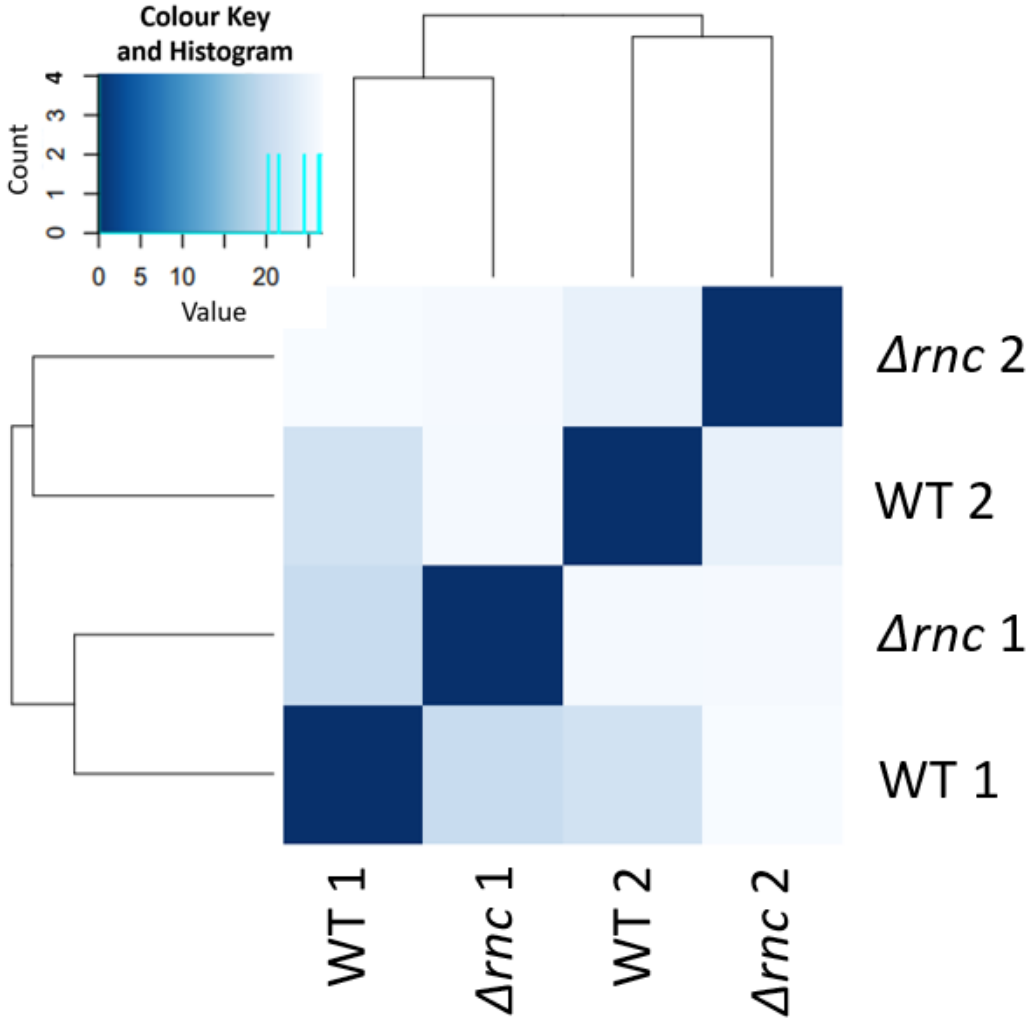
RNA proportion in different replicate



Discussion: Problem with this experiment…

3. The variation is larger **within** treatments than **between**





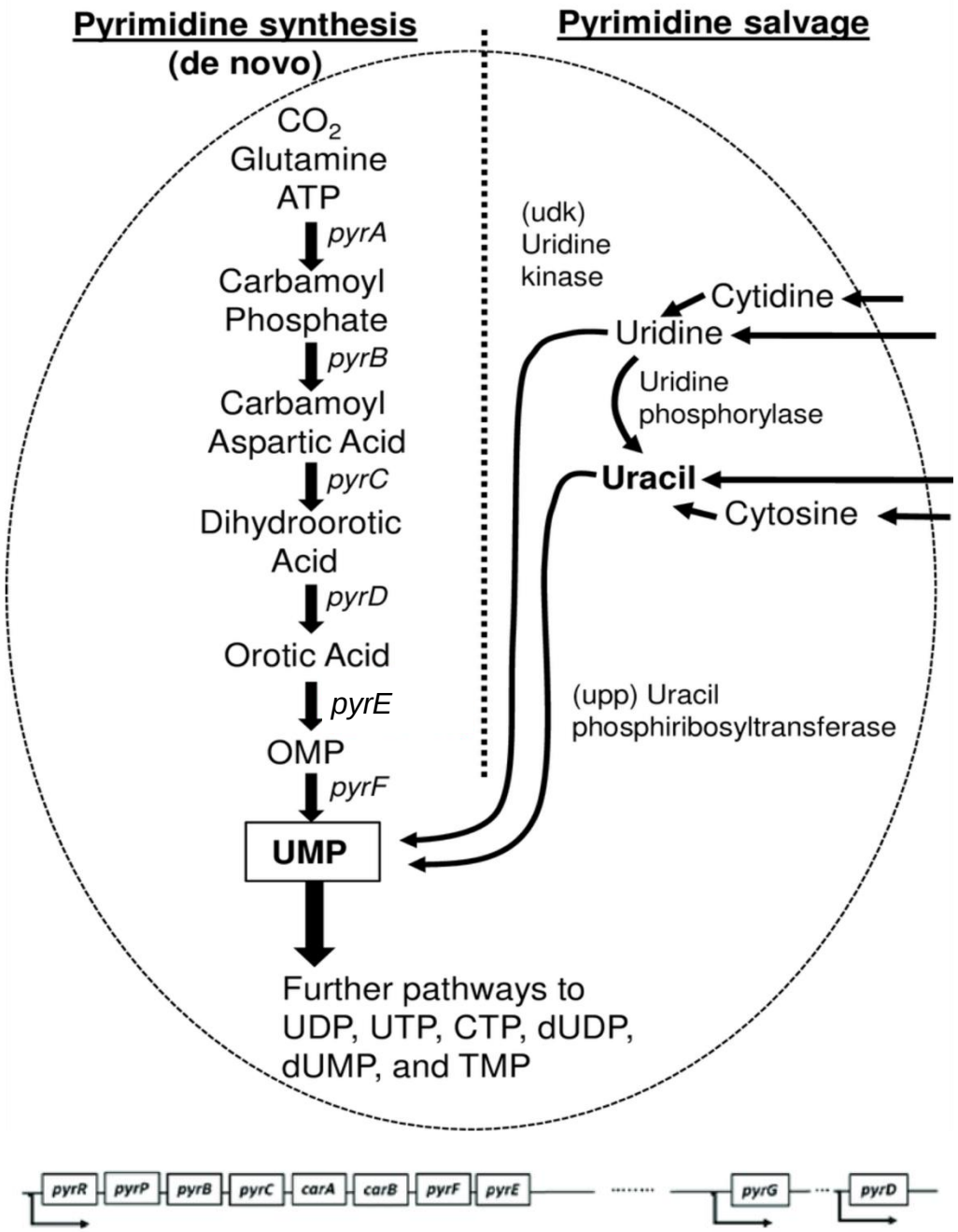
Acknowledgement

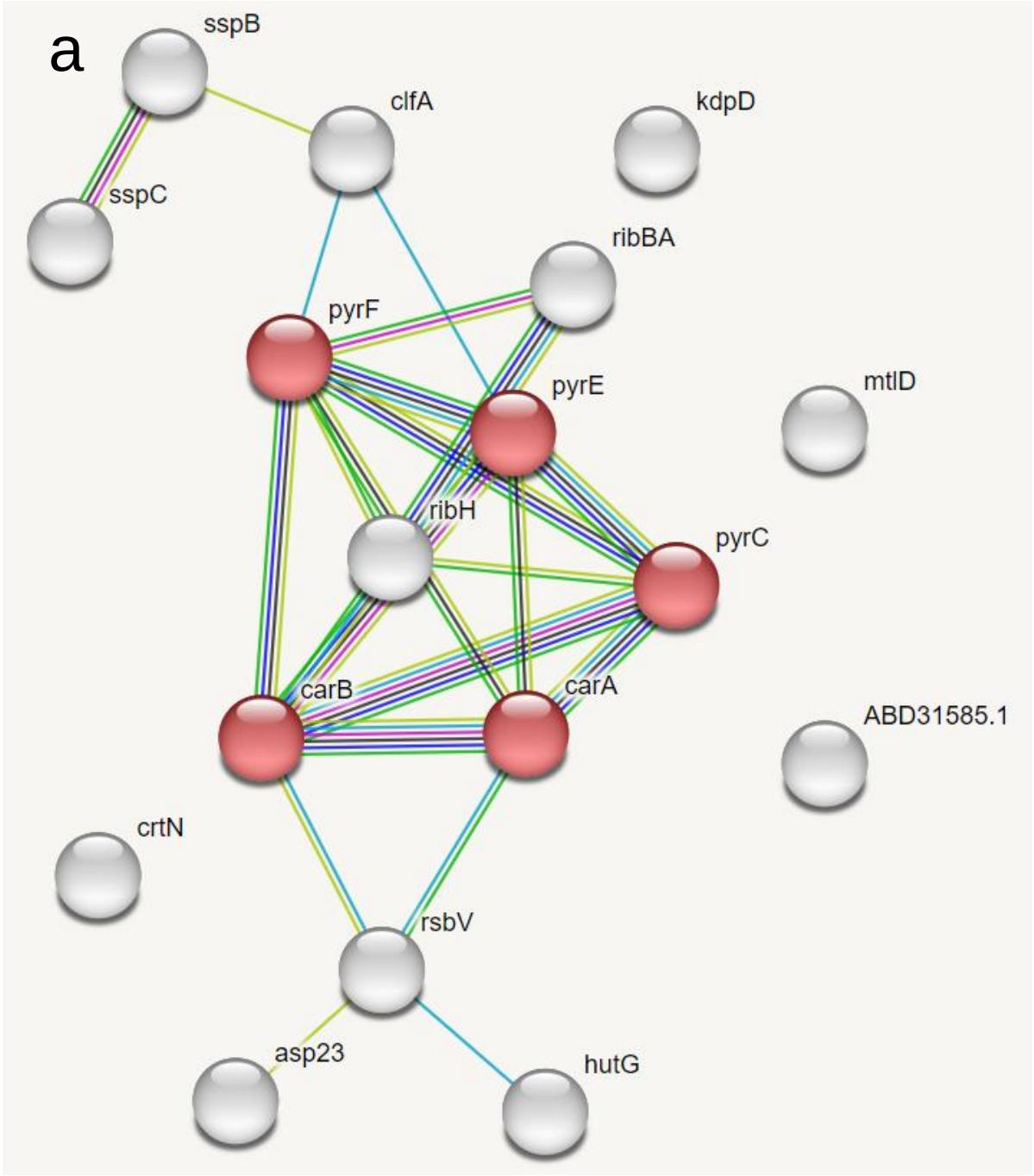
I would like to thank my supervisor Dr. Sander Granneman for his generous guidance and enthusiastic support in providing knowledge and working flow throughout my project.

I would like to thank my fellow honours student Saulė Zubytė for her generous helps, encouragements and inspiration while working on parallel projects at the same lab.

I would like to thank the other members in Sander's group for their instructions and accompany. Although I didn't get chance to talk with many people, but I appreciate all the helps in the past.

Finally, I would like to thank for the generous financial support from my family and enormous emotional support from my friends to make me complete my study at this University of Edinburgh possible and to make my career pathway this far.


Pyrimidine synthesis
(de novo)
Pyrimidine salvage
CO_2
Glutamine
ATP
pyrA
Carbamoyl
Phosphate
pyrB
Carbamoyl
Aspartic Acid
pyrC
Dihydroorotic
Acid
pyrD
Orotic Acid
pyrE
OMP
pyrF
UMP
(udk)
Uridine
kinase
Cytidine
Uridine
Uridine
phosphorylase
Uracil
Cytosine
(upp) Uracil
phosphiribosyltransferase
Further pathways to
UDP, UTP, CTP, dUDP,
dUMP, and TMP
pyrR
pyrP
pyrB
pyrC
carA
carB
pyrF
pyrE
pyrG
pyrD


a
sspB
clfA
kdpD
sspC
ribBA
pyrF
pyrE
mtlD
ribH
pyrC
carB
carA
ABD31585.1
crtN
rsbV
asp23
hutG

b

Functional enrichments in your network

explain columns

Biological Process (Gene Ontology)

GO-term	*description*	*count in network*	*strength*	*false discovery rate*
GO:0006525	Arginine metabolic process	3 of 18	1.45	0.0112
GO:0044271	Cellular nitrogen compound biosynthetic process	8 of 327	0.62	0.0138
GO:0006207	De novo pyrimidine nucleobase biosynthetic process	2 of 5	1.83	0.0303
GO:0044205	De novo UMP biosynthetic process	5 of 7	2.08	3.67e-06
GO:0018130	Heterocycle biosynthetic process	7 of 229	0.72	0.0112

(more ...)

Local network cluster (STRING)

cluster	*description*	*count in network*	*strength*	*false discovery rate*
CL:331	De novo UMP biosynthetic process	4 of 5	2.13	5.87e-05
CL:291	Ribonucleoside monophosphate biosynthetic process	5 of 27	1.5	0.00019

KEGG Pathways

pathway	*description*	*count in network*	*strength*	*false discovery rate*
sao00240	Pyrimidine metabolism	5 of 45	1.28	0.00071

Annotated Keywords (UniProt)

keyword	*description*	*count in network*	*strength*	*false discovery rate*
KW-0464	Manganese	3 of 25	1.31	0.0366
KW-0665	Pyrimidine biosynthesis	5 of 9	1.98	1.53e-06
KW-0686	Riboflavin biosynthesis	2 of 3	2.05	0.0352
KW-0843	Virulence	4 of 67	1.01	0.0366