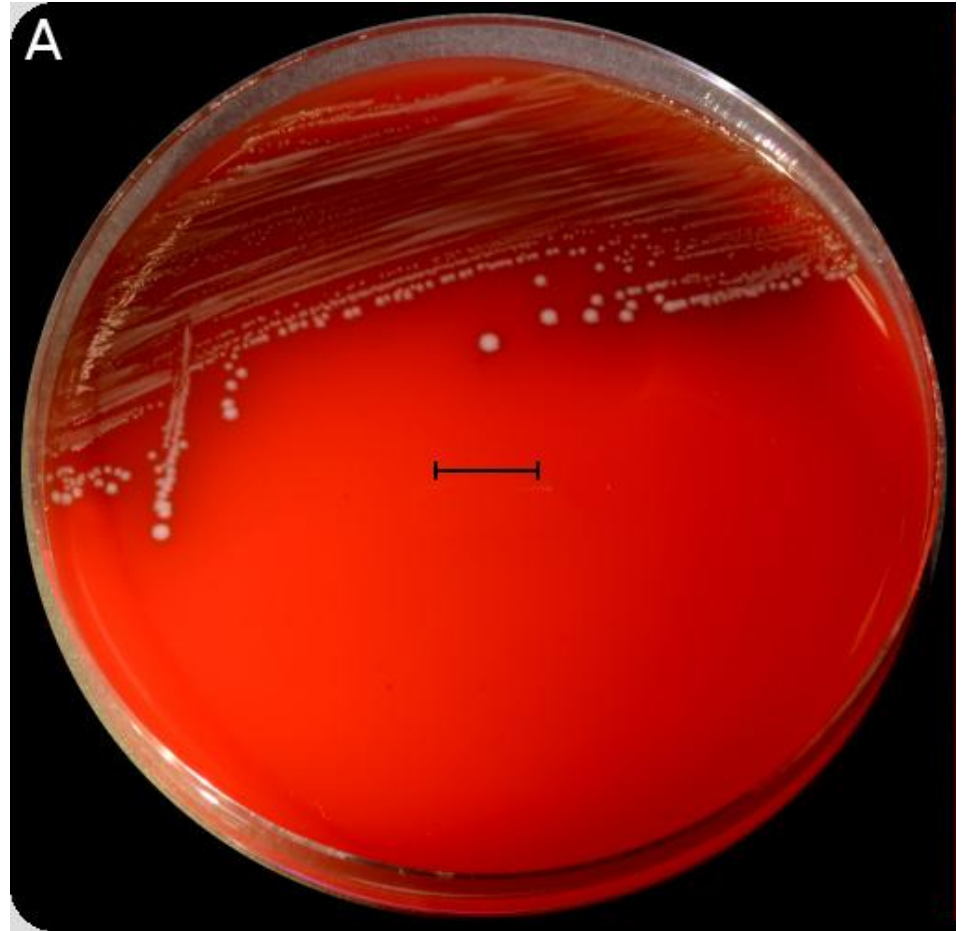


**Paper: 1;** “RNA-seq and Tn-seq reveal fitness determinants of vancomycin-resistant *Enterococcus faecium* during growth in human serum” (Zhang et al., 2017)

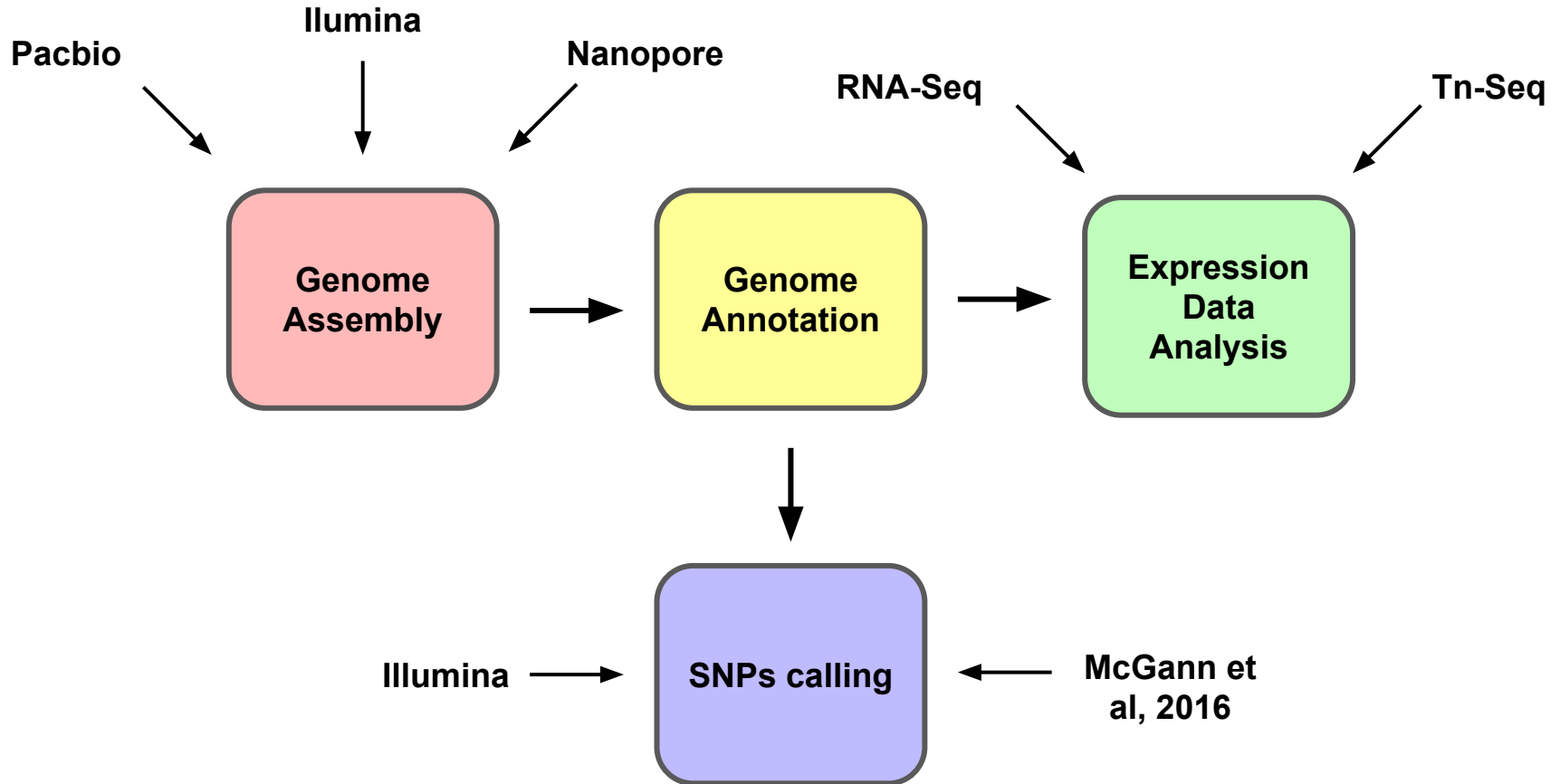
**Erik Zhivkoplías**

Genome Analysis 2019

29/05/2019



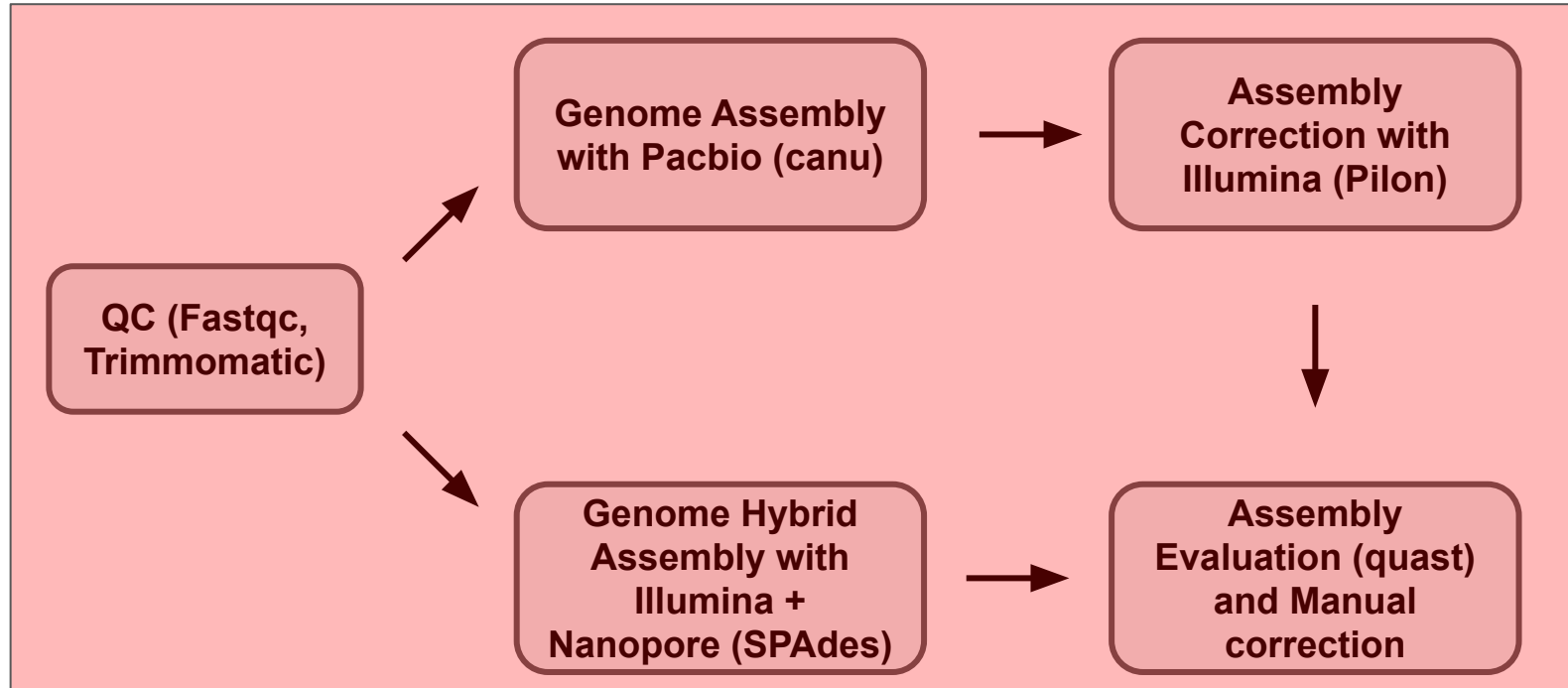
# Analysis overview



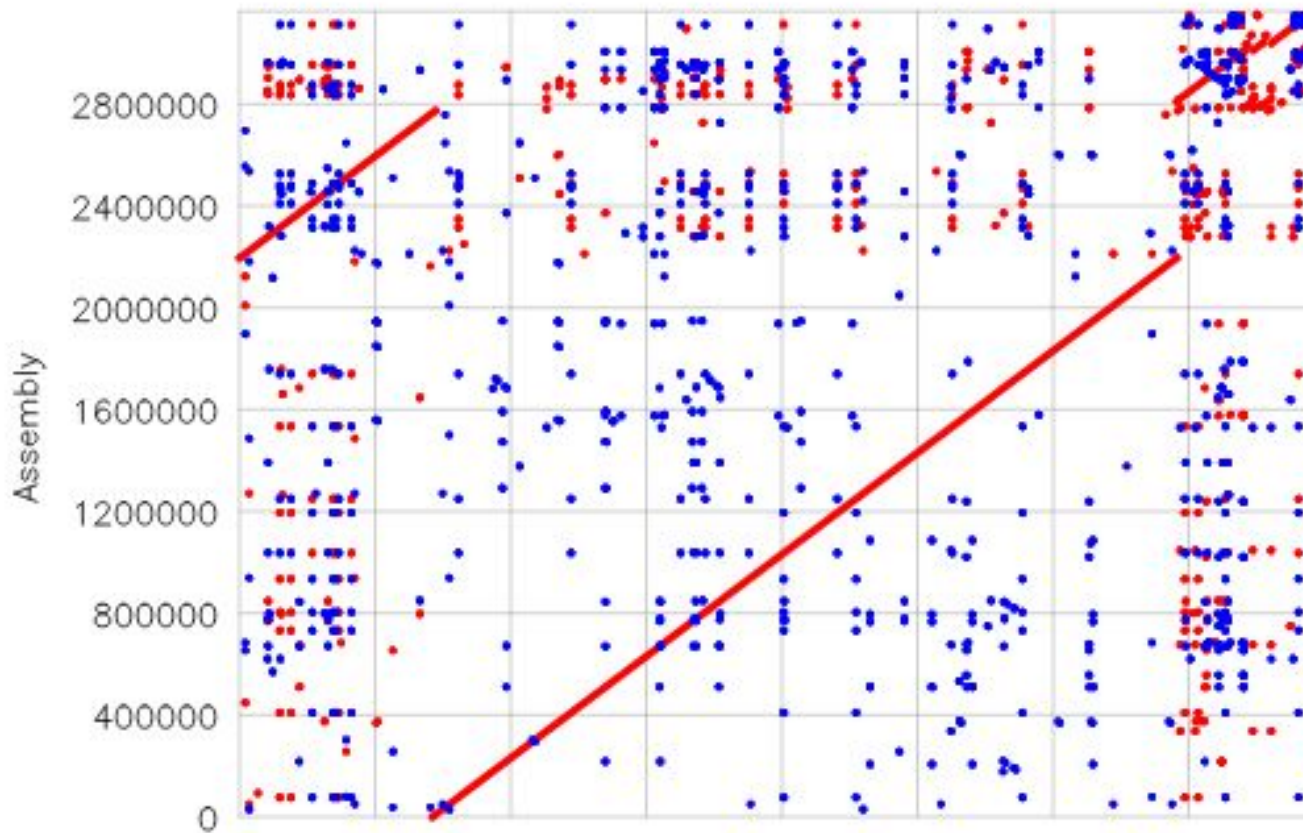
# Goals

- Reproduce the results in the chosen paper (Paper 1: “RNA-seq and Tn-seq reveal fitness determinants of vancomycin-resistant *Enterococcus faecium* during growth in human serum”)
- Learn how to assemble and annotate prokaryotic genomes, analyze RNA-Seq data, and make biological conclusions out of NGS data
- Find any (novel?) candidate genes to target *E. faecium* in bloodstream

# Genome Assembly: pipeline



# Genome Assembly: result



**Number of contigs:**  
9

**Largest contig:**  
2773702

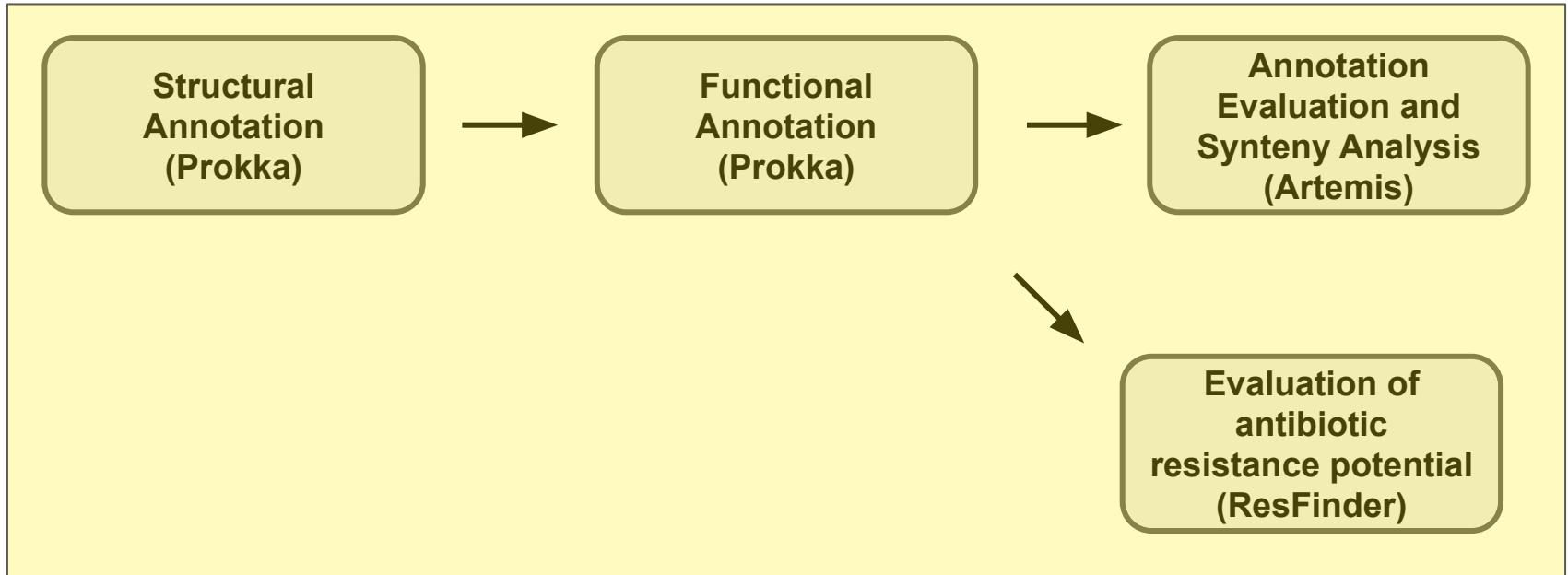
**Total length:**  
3 164 587 bp

**Reference size:**  
3 168 410 bp

**Genome fraction (%):**  
98.935

FWD   
REV 

# Genome Annotation pipeline



# Genome Annotation: results

**Canu Assembly:** contigs: 9, bases: 3126531, tmRNA: 1, tRNA: 70, **CDS: 3036**, rRNA: 18

**SPAdes assembly:** contigs: 43, bases: 3151876, tmRNA: 1, tRNA: 70, rRNA: 18, **CDS: 3060**

**Hybrid (final) assembly:** contigs: 9, bases: 3164587, **tRNA: 70**, **CDS: 3083**, rRNA: 18, tmRNA: 1

**Reference:** <..>the chromosome and plasmids have **3095 predicted coding sequences**<..>

# Genome Annotation: antibiotic resistance potential (ResFinder)

## Aminoglycoside

Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
<b>aac(6')-li</b>	99.64	549/549	tig00000001_pilon	2471910..2472458	L12710	

## Glycopeptide

Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
<b>VanH</b>	91.85	969/969	tig00000025_pilon	37402..38370	1_Y15705	
<b>VanA</b>	93.51	1032/1032	tig00000025_pilon	38363..39394	<b>Vancomycin resistance</b> (Glycopeptid resistance)	1_Y15704
<b>VanX</b>	96.79	249/249	tig00000025_pilon	39400..39648	1_Y15708	
<b>VanHAX</b>	100.00	2607/2607	tig00000025_pilon	4980..7586	M97297	

## Macrolide, Lincosamide and Streptogramin B

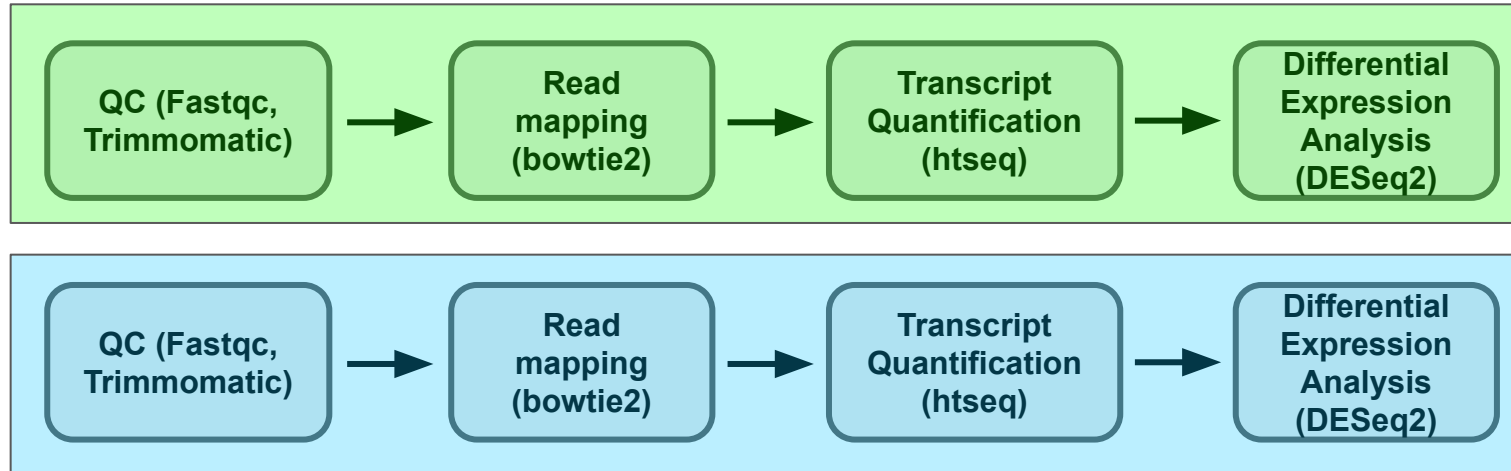
Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
<b>msr(C)</b>	98.99	1479/1479	tig00000001_pilon	743512..744990	Macrolide, Lincosamide and Streptogramin B resistance	AY004350

## Trimethoprim

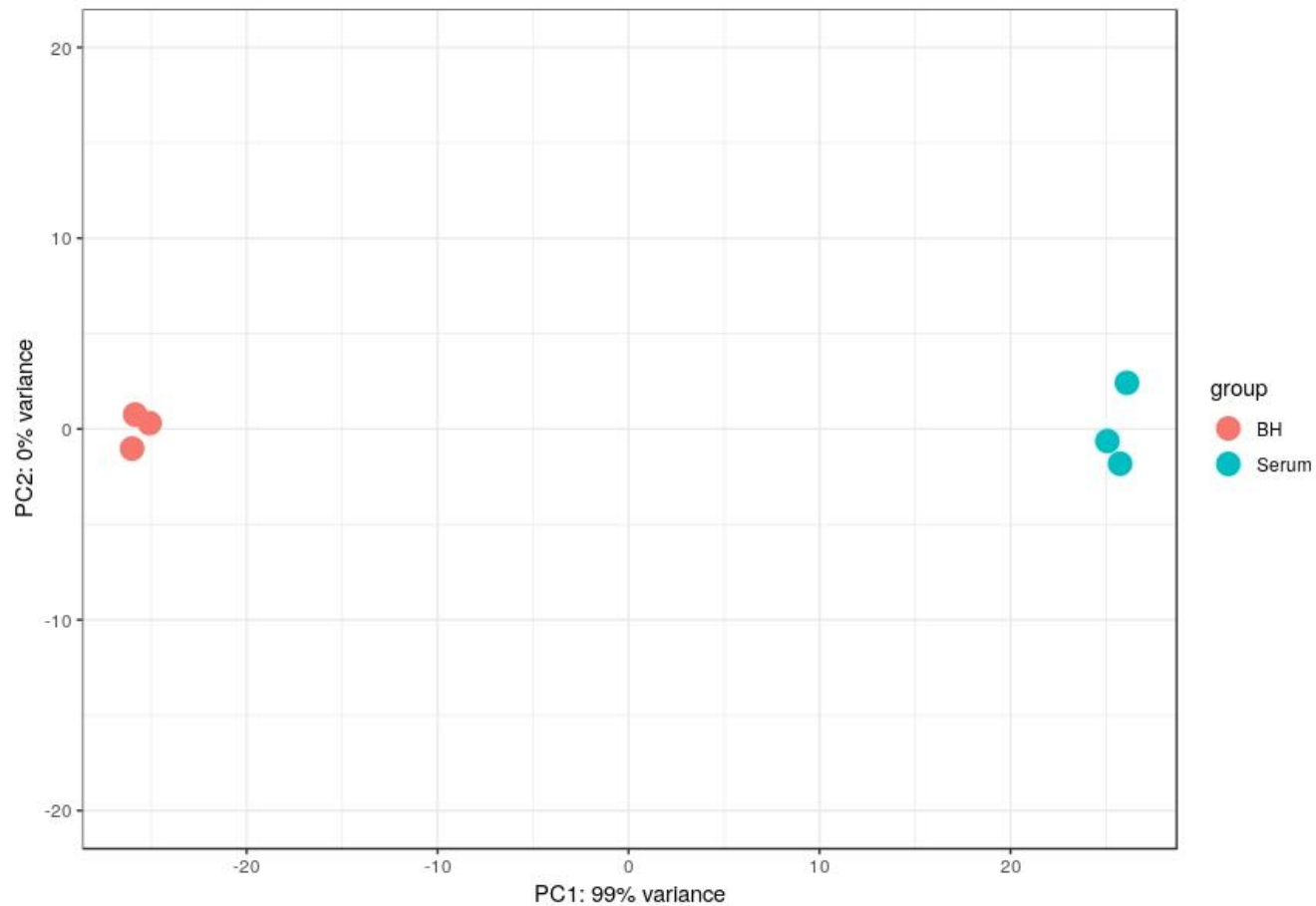
Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
<b>dfrG</b>	100.00	498/498	NODE_3_length_64343_cov_75.9457	2210..2707	Trimethoprim resistance	AB205645



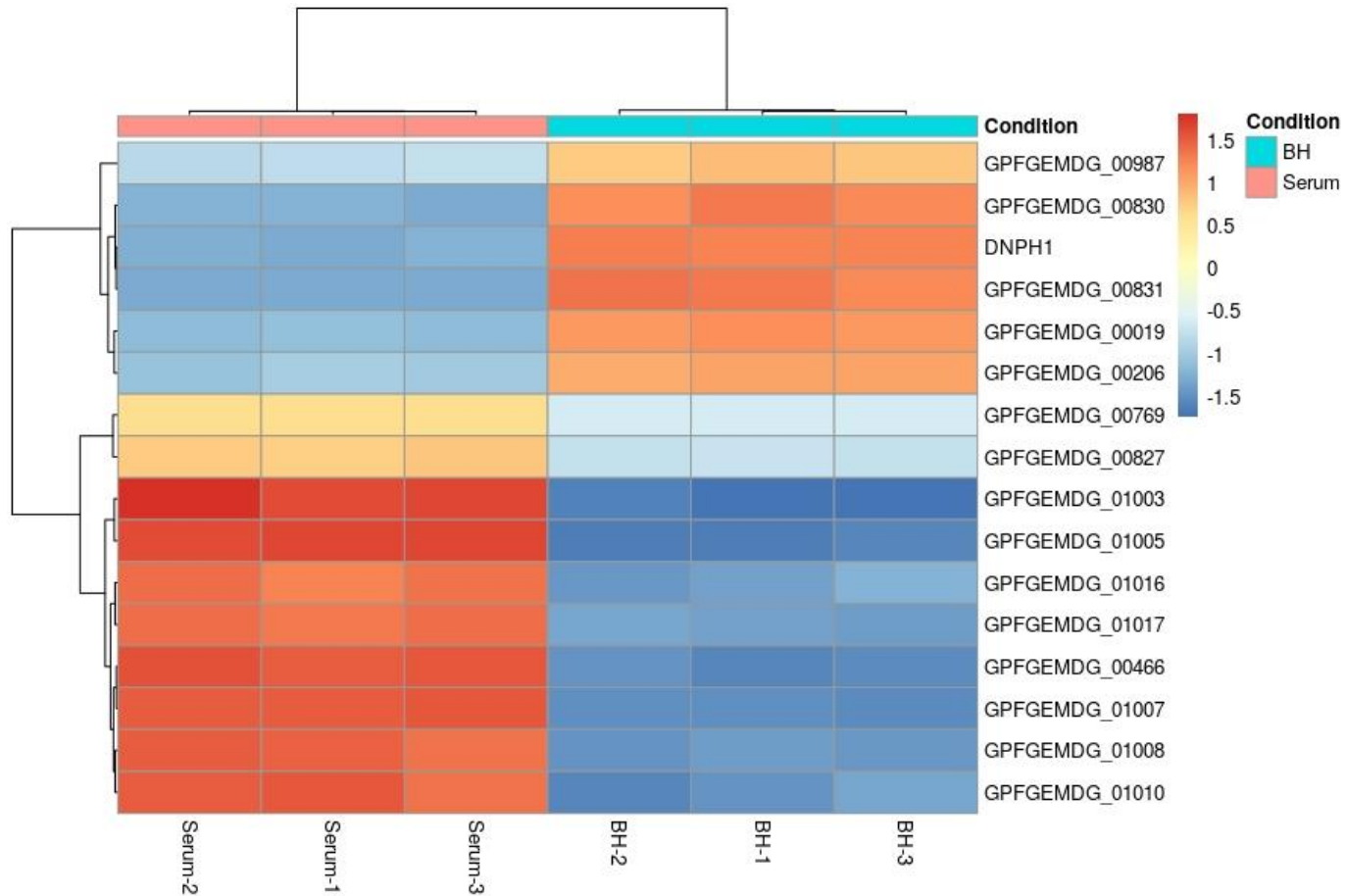
# RNA Seq and Tn-Seq pipeline



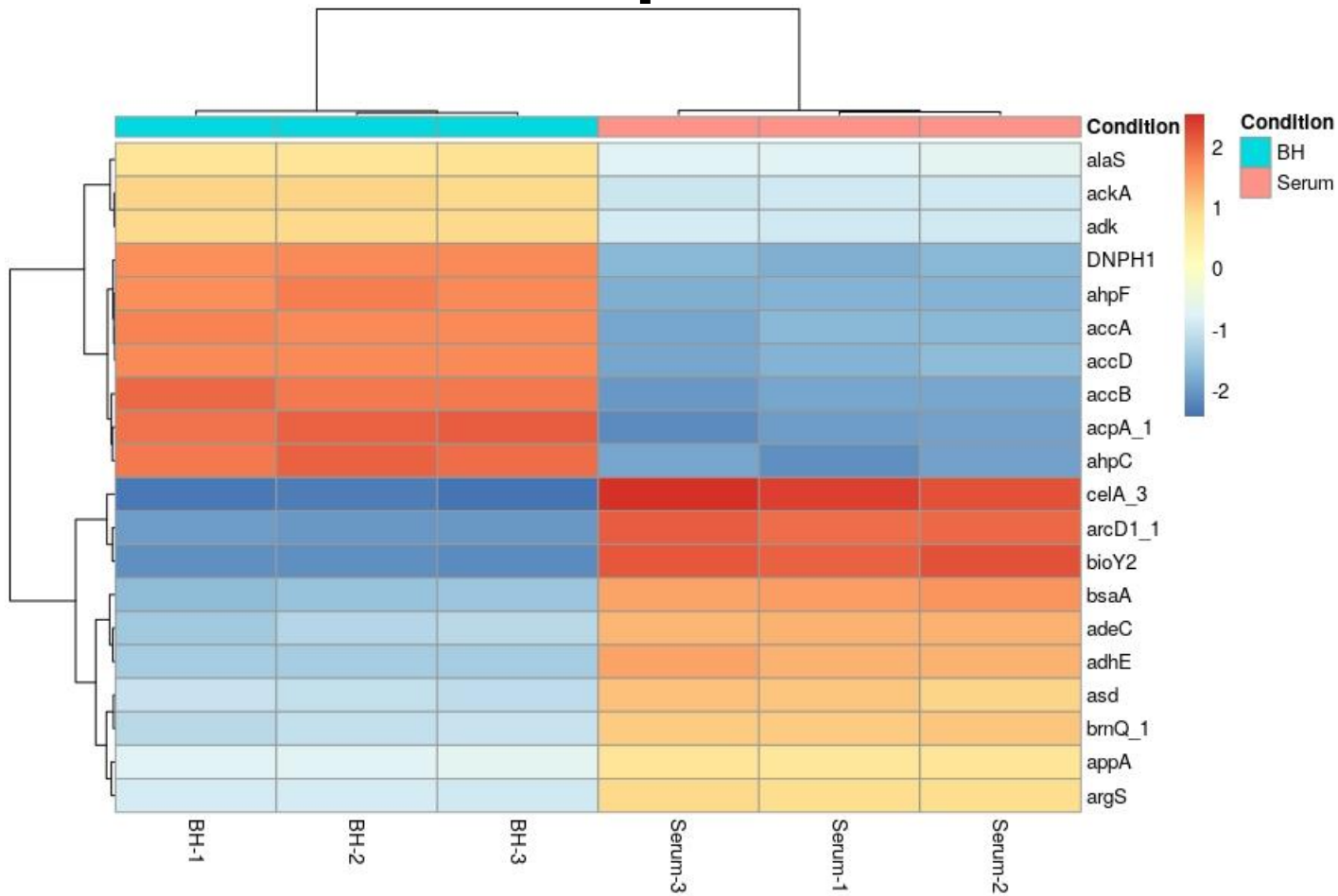
# RNA Seq: results



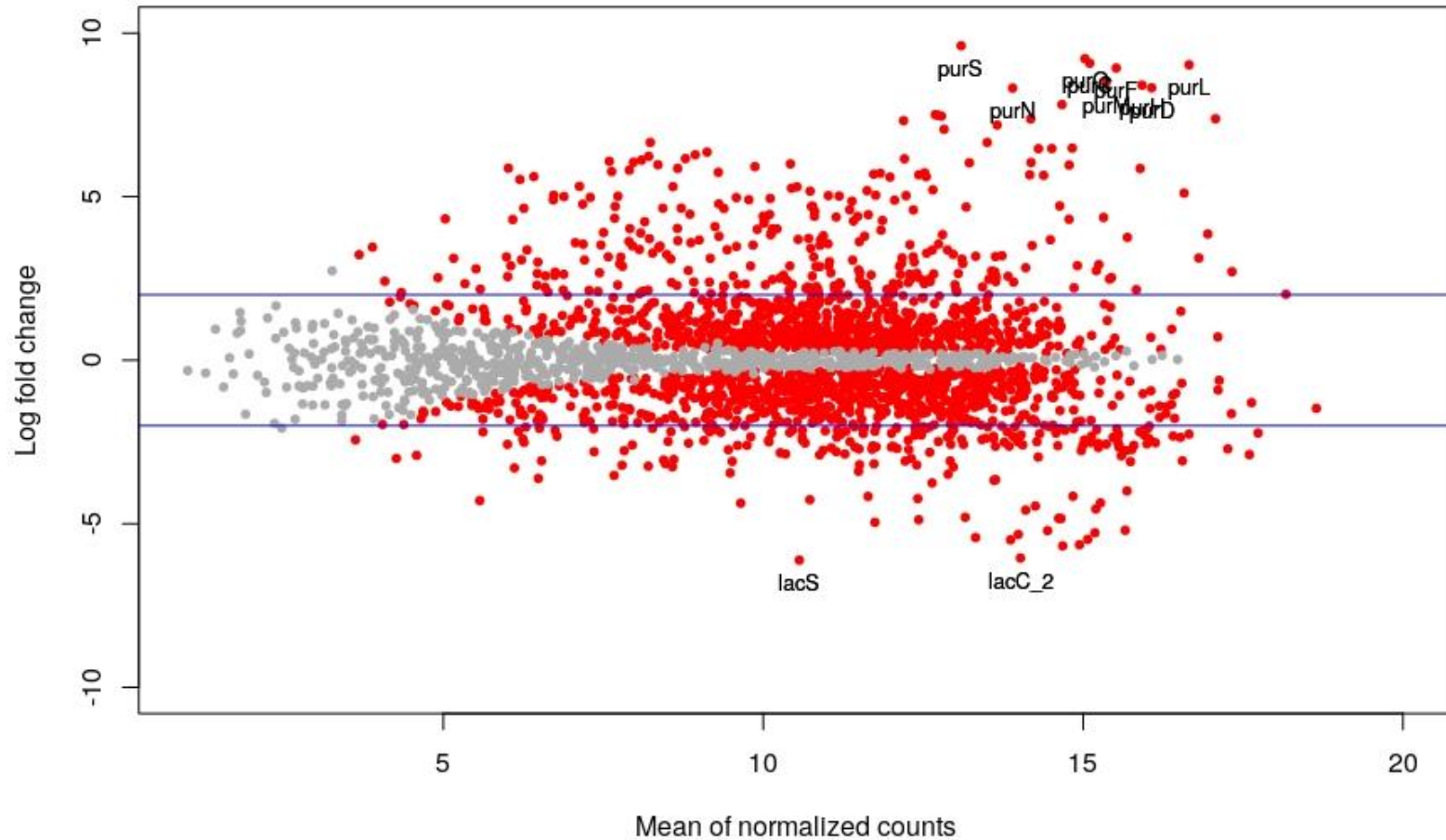
# RNA Seq: results



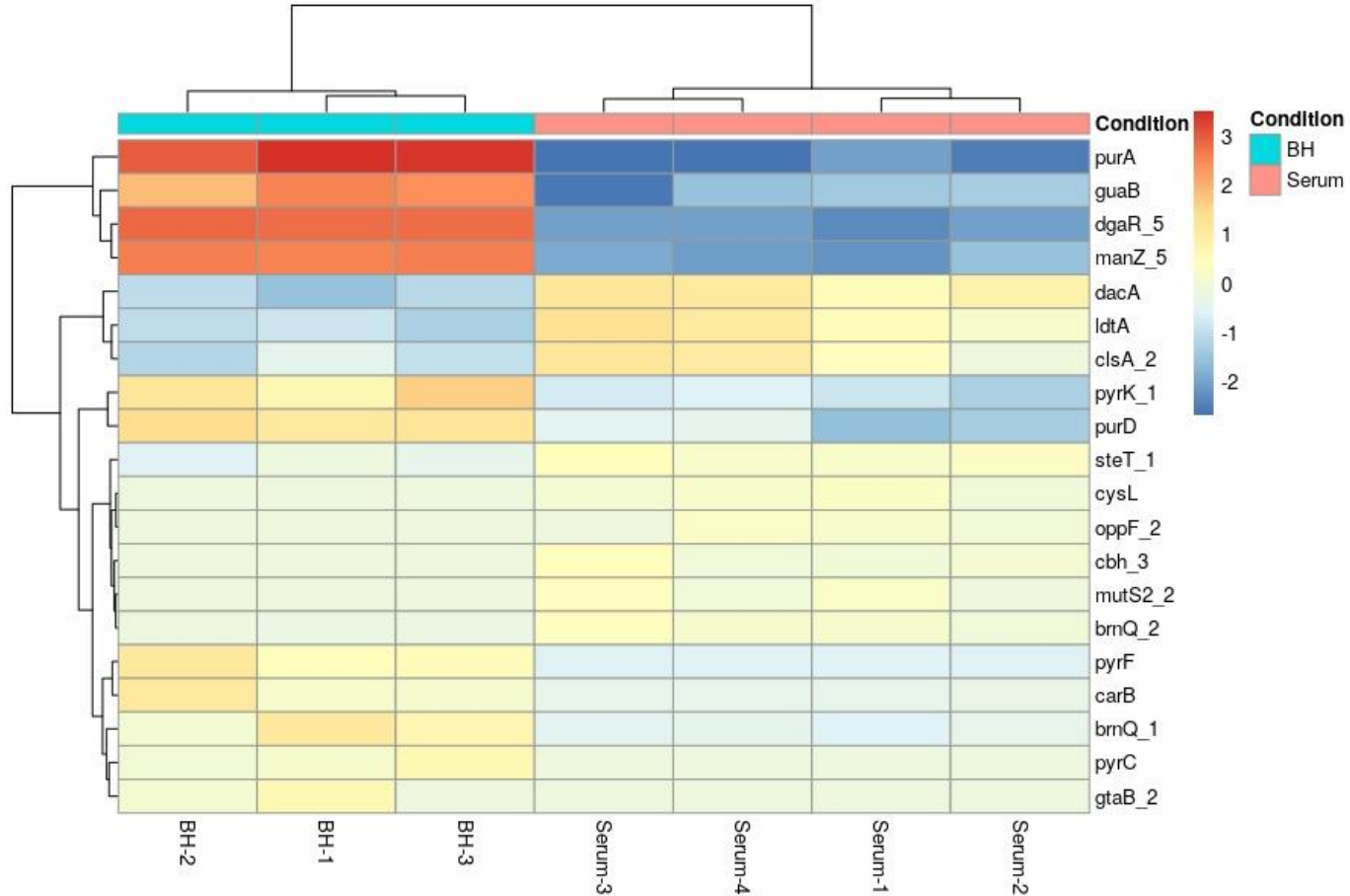
# RNA Seq: results



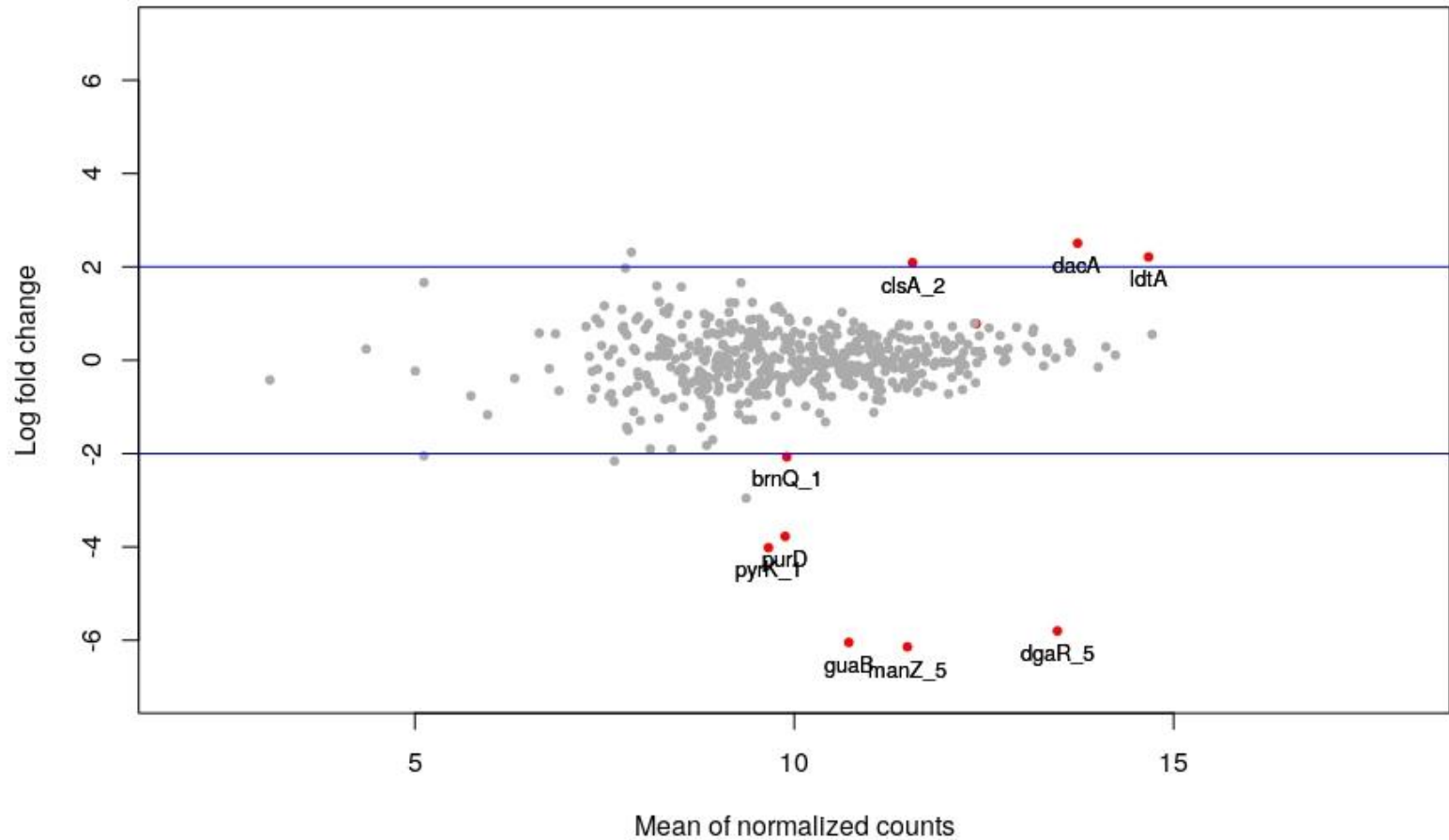
MA plot (padj<0.001)



# Tn-Seq: results



MA plot (padj<0.05)



# Conclusion

- Found 31 candidate genes (37 in paper) of which 20 are hypothetical proteins and 11 have known gene names
- Most genes are related to purine metabolism genes (the same finding as in paper), however, there are a few genes that participate in carbohydrate metabolism
- One gene that is involved in carbohydrate uptake (manZ\_5: part of hosphoenolpyruvate-dependent sugar phosphotransferase system) might be a good candidate for antibiotic targeting, as it is a membrane-associated protein, and a quite distant homolog in primates (huntingtin: 40% homology).



**Thank you for your attention.**