Georgia Tech

Team 1 Final Presentation

Assembly, Gene Prediction, Functional Annotation, Comparative Genomics, Predictive Webserver

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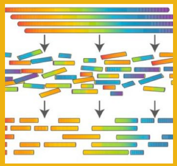
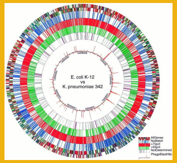
Outline

1 Overall Project Goals

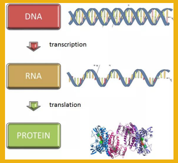
2 Genome Assembly



3 Gene Prediction



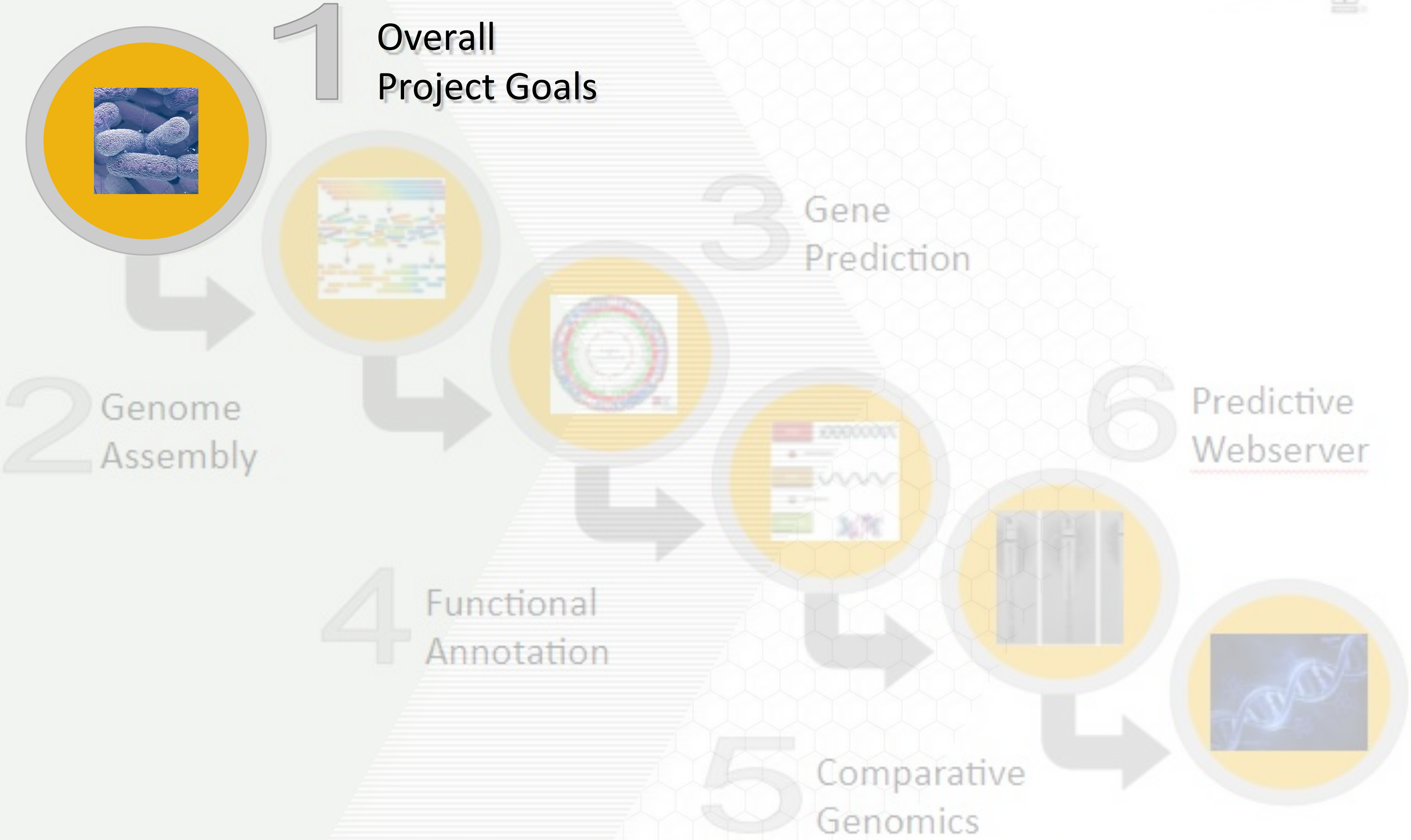
4 Functional Annotation



5 Comparative Genomics

6 Predictive Webserver

Outline



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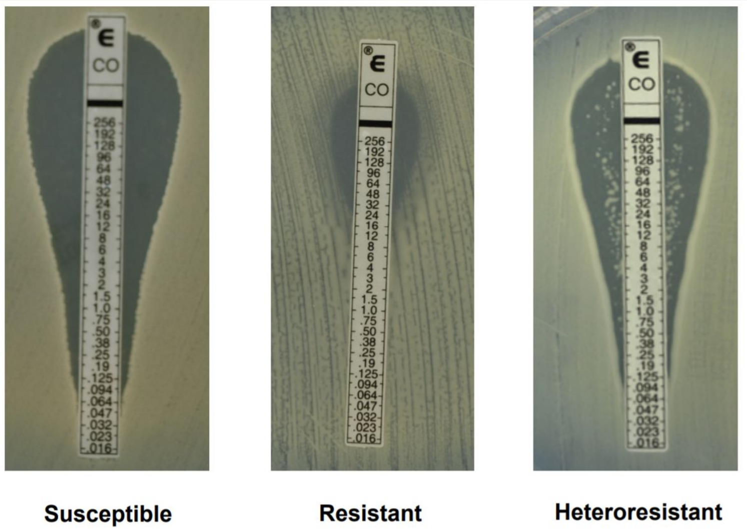
Overall Project Goals



Susceptible Resistant Heteroresistant

1 Vial
Colistimethate Sodium for Injection IP 1 Million IU
Colistin
कोलिस्टिन
1 MIU
Single Use Only
For Intravenous / Inhalation use only
cbc

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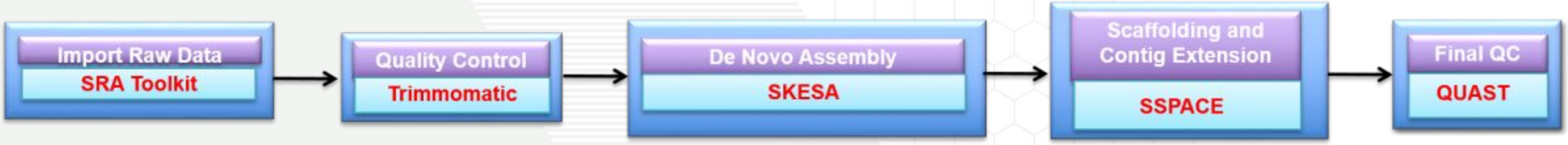
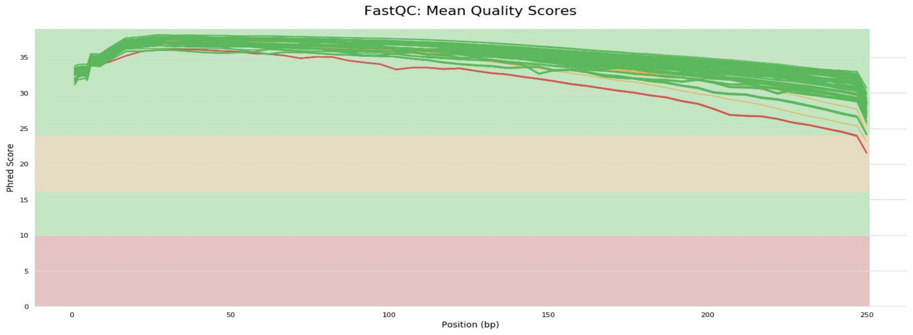
Genome Assembly

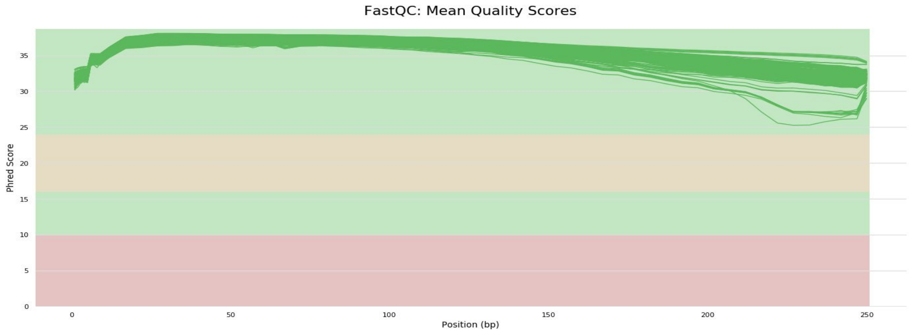
Assembly Pipeline



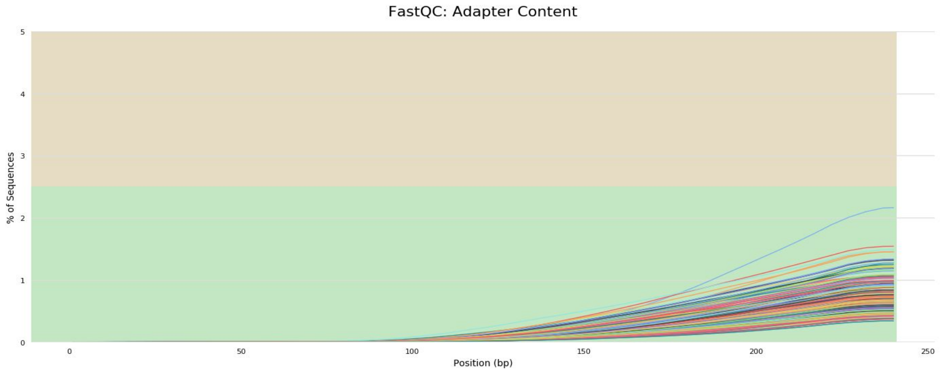
Trimming

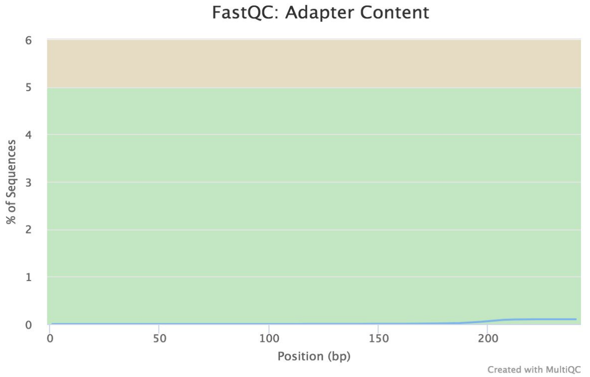


Before Trimming



After Trimming





De Novo Assembly

SKESA -

- The binary for Skesa was provided by CDC
- It is an assembler that works based on DeBruijn graphs
- Creates breaks at repeat regions in genomes
- It works for haploid genome
- Multi-threaded application - so good for scaling

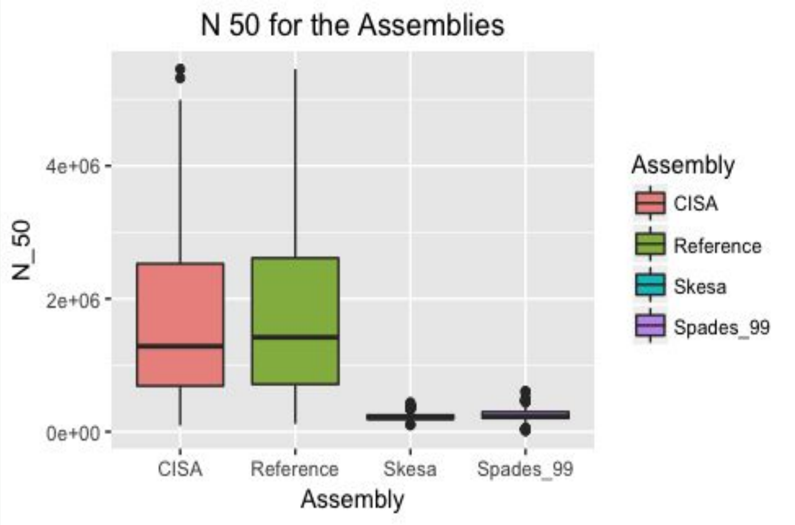
Scaffolding -

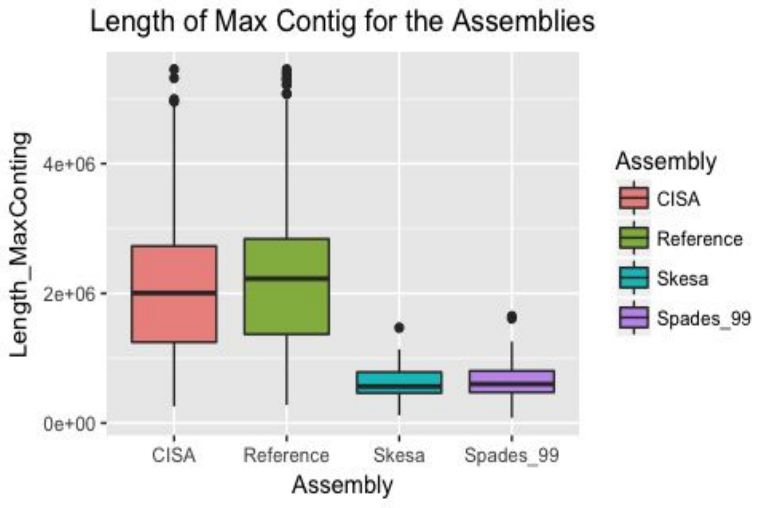
- Scaffolding was performed using SSPACE
- Extends and scaffolds pre-assembled contigs

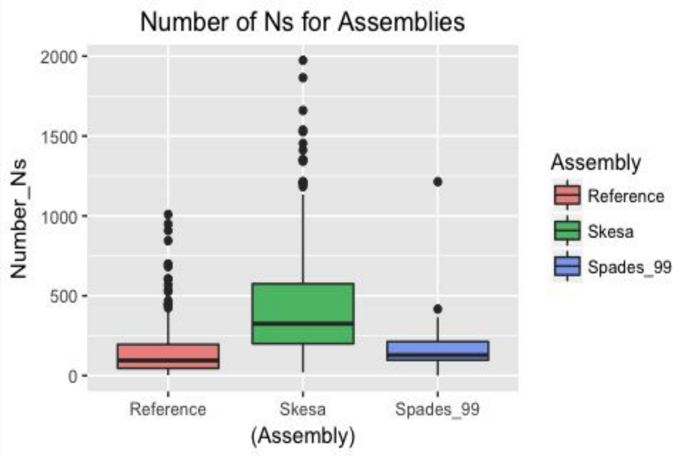
The output contains final scaffolds in fasta format, scaffolds with initial numbered contigs, a log file and a summary file

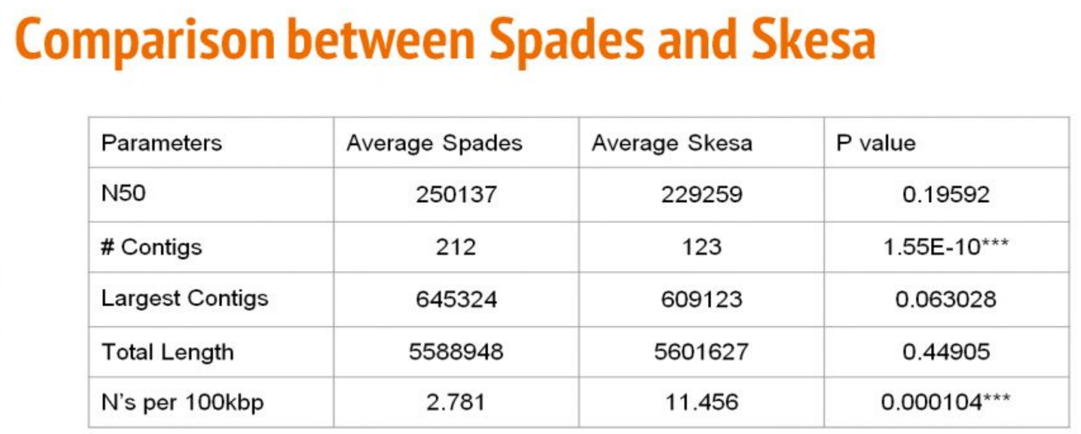
De Novo Assembly

Quality of assemblies





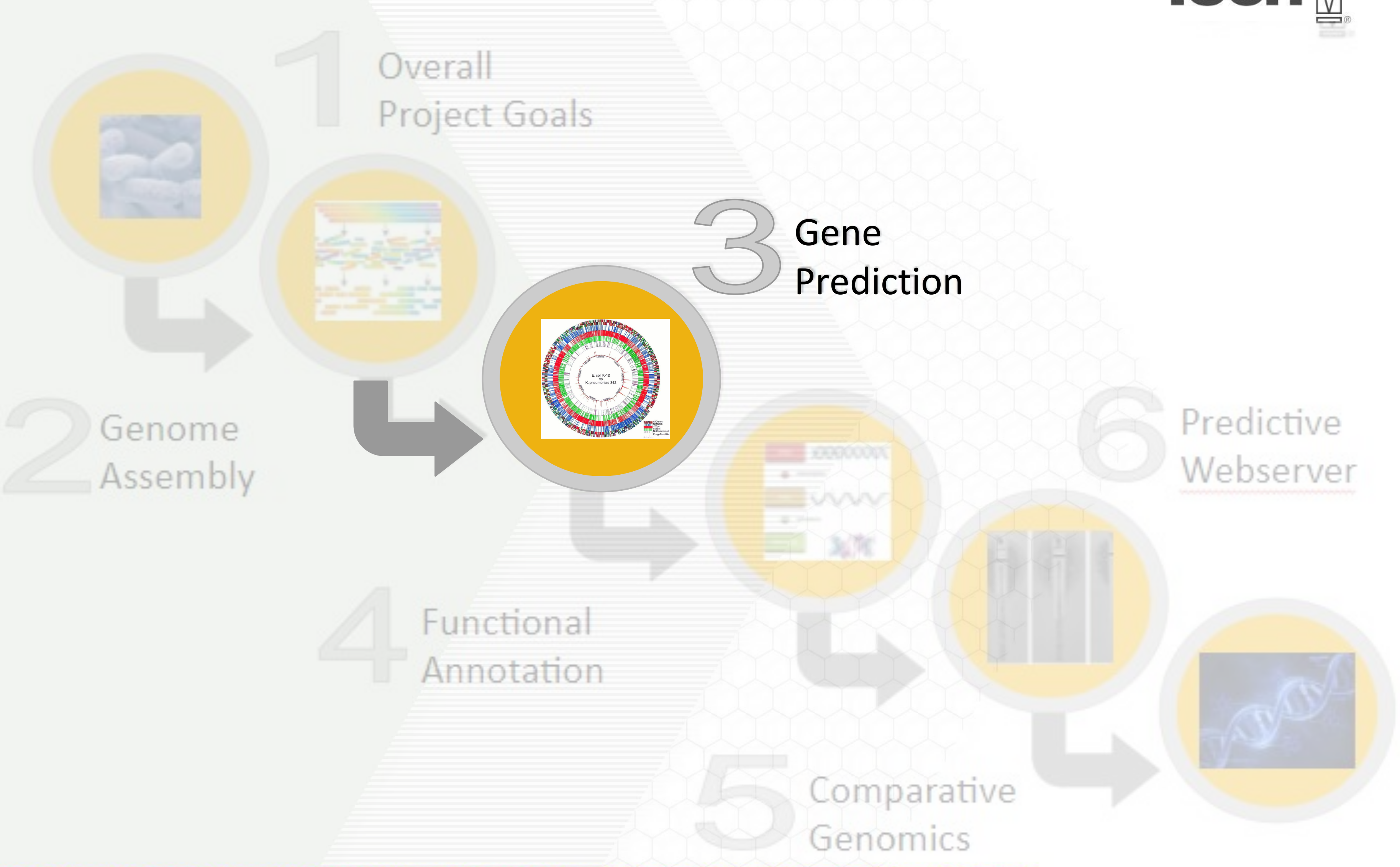




Comparison between Spades and Skesa

Parameters	Average Spades	Average Skesa	P value
N50	250137	229259	0.19592
# Contigs	212	123	1.55E-10***
Largest Contigs	645324	609123	0.063028
Total Length	5588948	5601627	0.44905
N's per 100kbp	2.781	11.456	0.000104***

Outline



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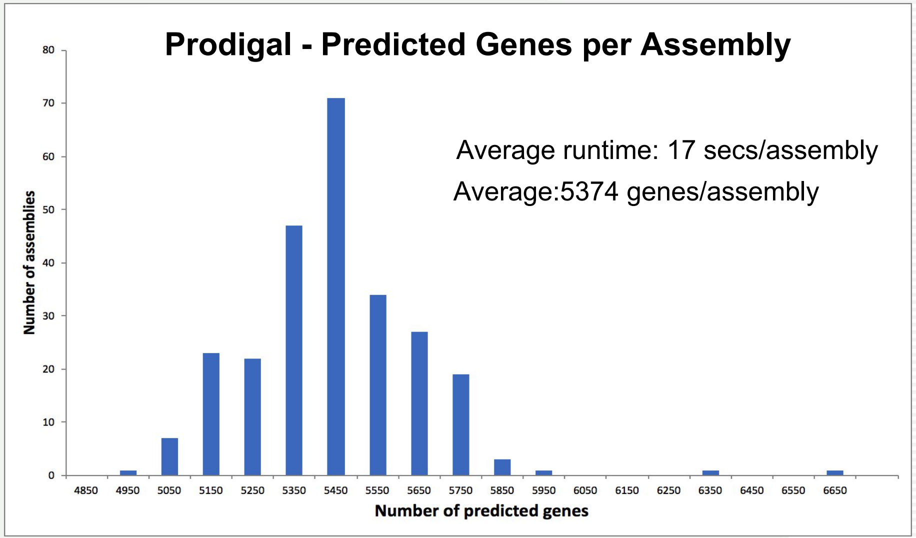
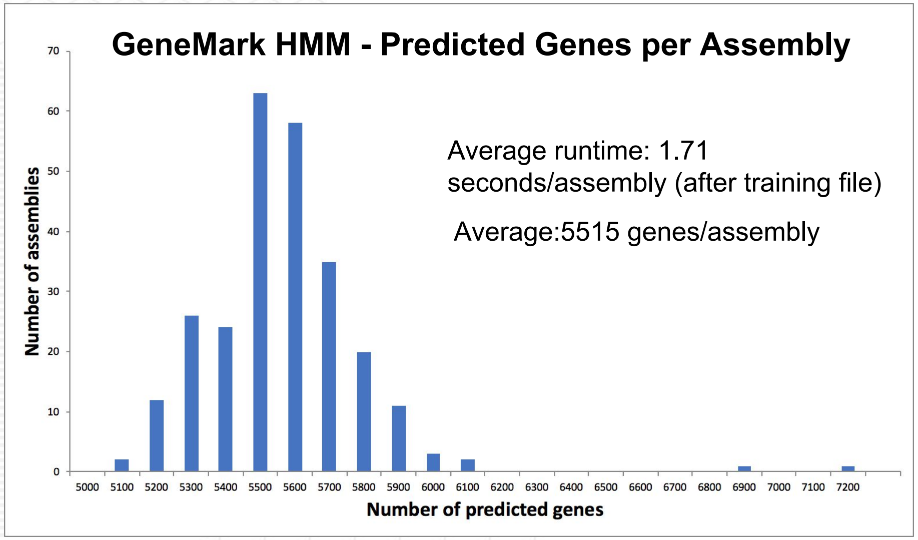
Gene Prediction Methods



Prodigal - Predicted Genes per Assembly

Average runtime: 17 secs/assembly

Average:5374 genes/assembly

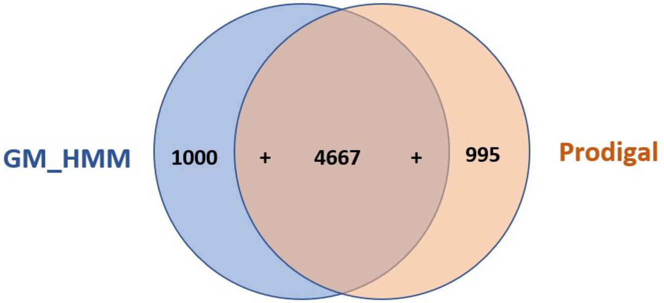


GeneMark HMM - Predicted Genes per Assembly

Average runtime: 1.71 seconds/assembly (after training file)

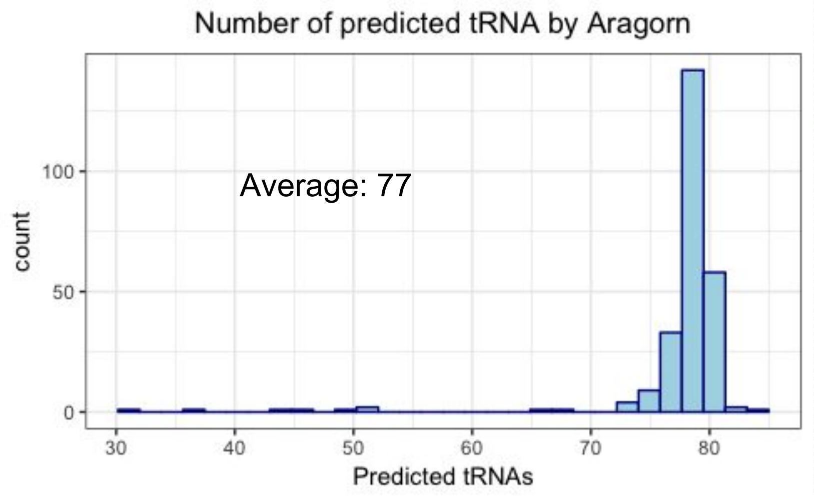
Average:5515 genes/assembly

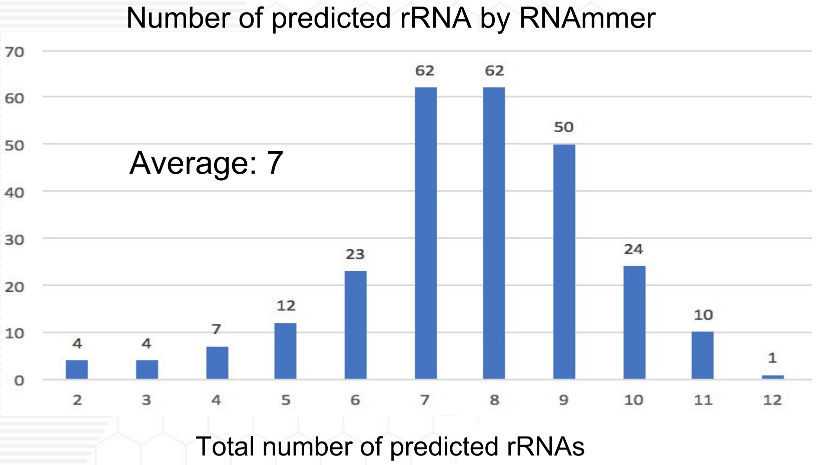
Method	True Positives	False Positives	False negatives	Sensitivity	PPV
Prodigal	5015.8	437.7	480.6	91.2	92.0
GeneMark HMM	5061.5	507.1	456.4	91.7	91.1
Intersect	4383.4	323.6	1096.4	80.0	93.1
Union	5693.9	618.5	423.1	93.1	90.25



Gene Prediction Methods

	Aragon	RNAmmer	Infernal
Method	Homology Base	ab initio	ab initio
Run Time	<10 sec/assembly	~3min/assembly	<10 min/assembly
Type of RNA	tRNA	rRna	istR





Gene Prediction Methods

	Aragon	RNAmmer	Infernal
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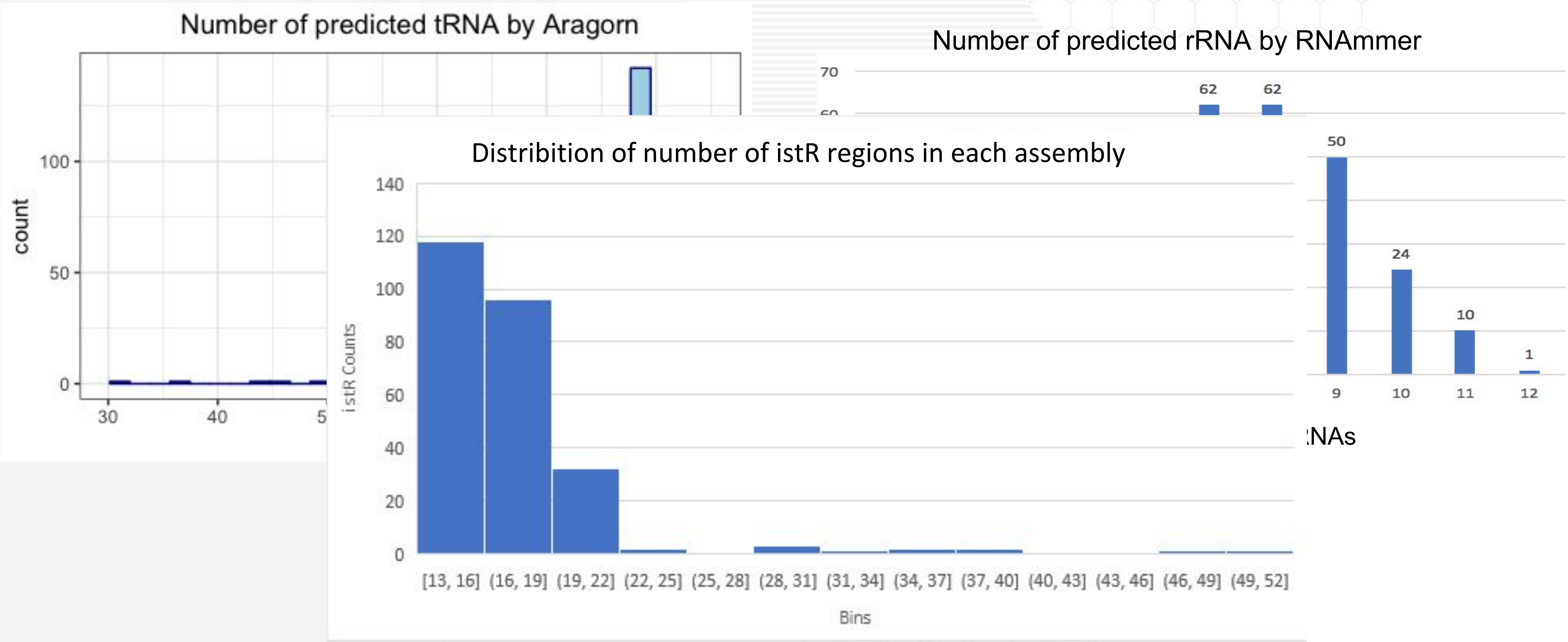
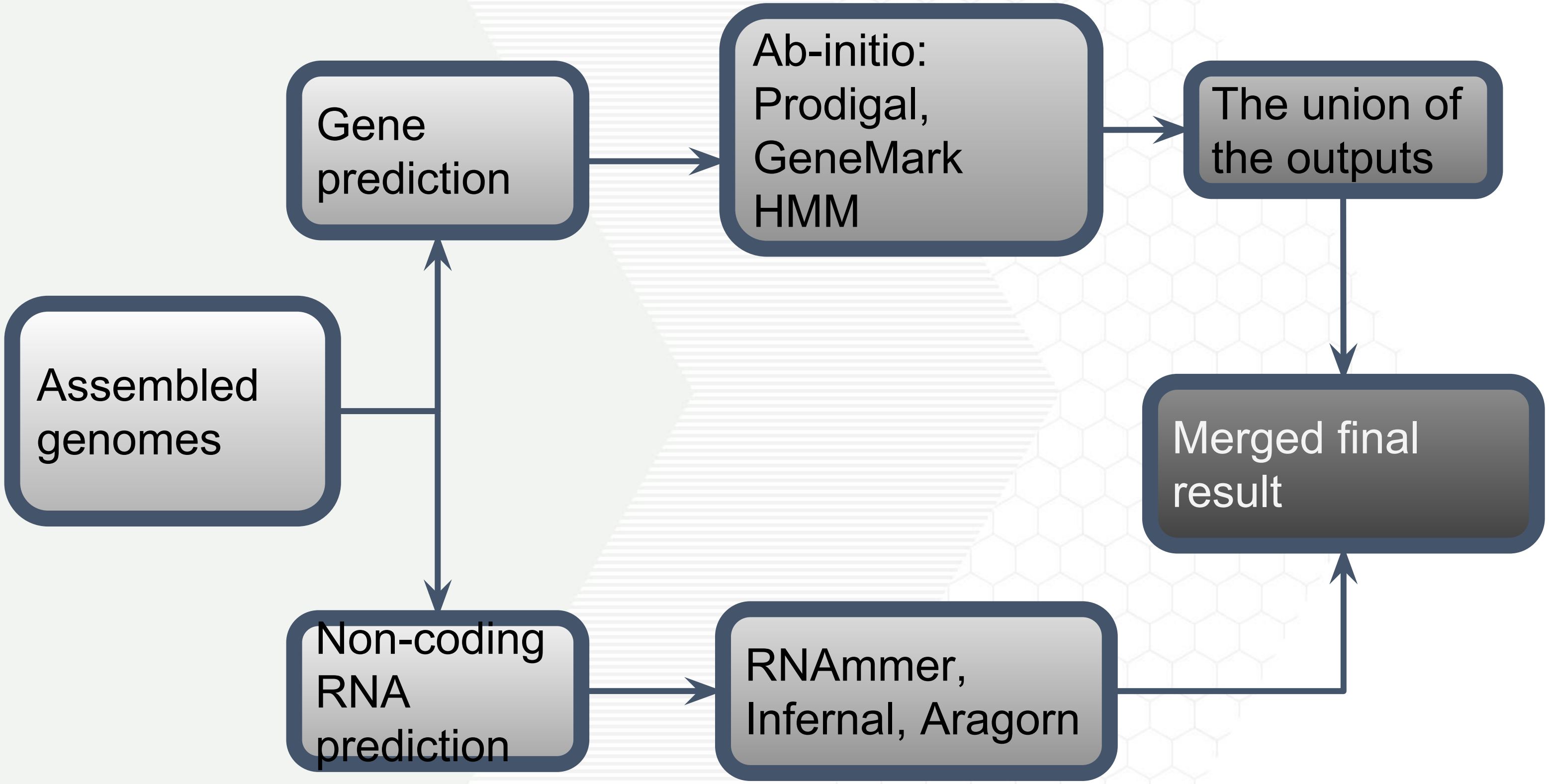

Number of predicted tRNA by Aragorn

Number of predicted rRNA by RNAmmer

Distribition of number of istR regions in each assembly

Gene Prediction Final Pipeline


Assembled genomes

Gene prediction

Ab-initio: Prodigal, GeneMark HMM

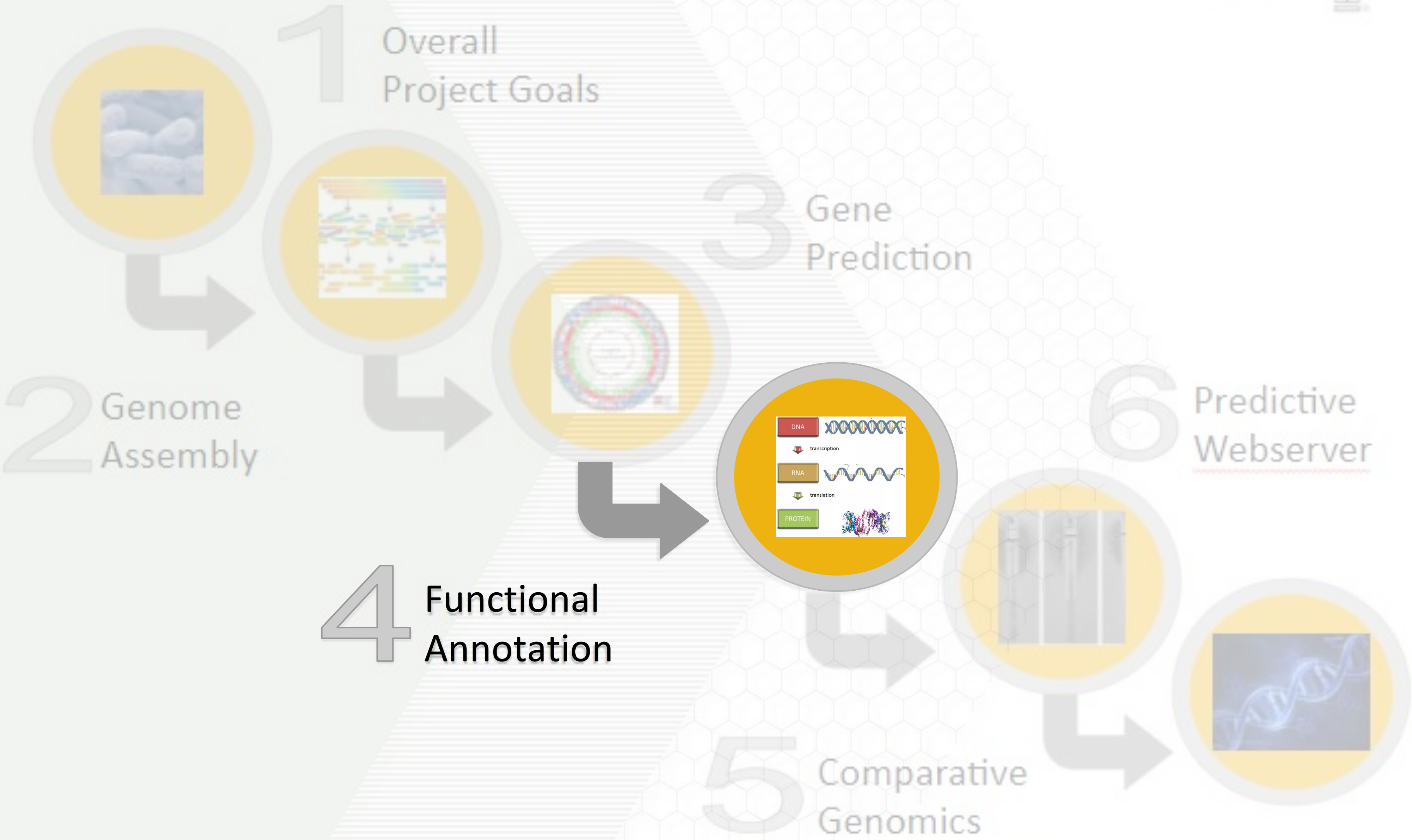
The union of the outputs

Non-coding RNA prediction

RNAmmer, Infernal, Aragorn

Merged final result

Outline



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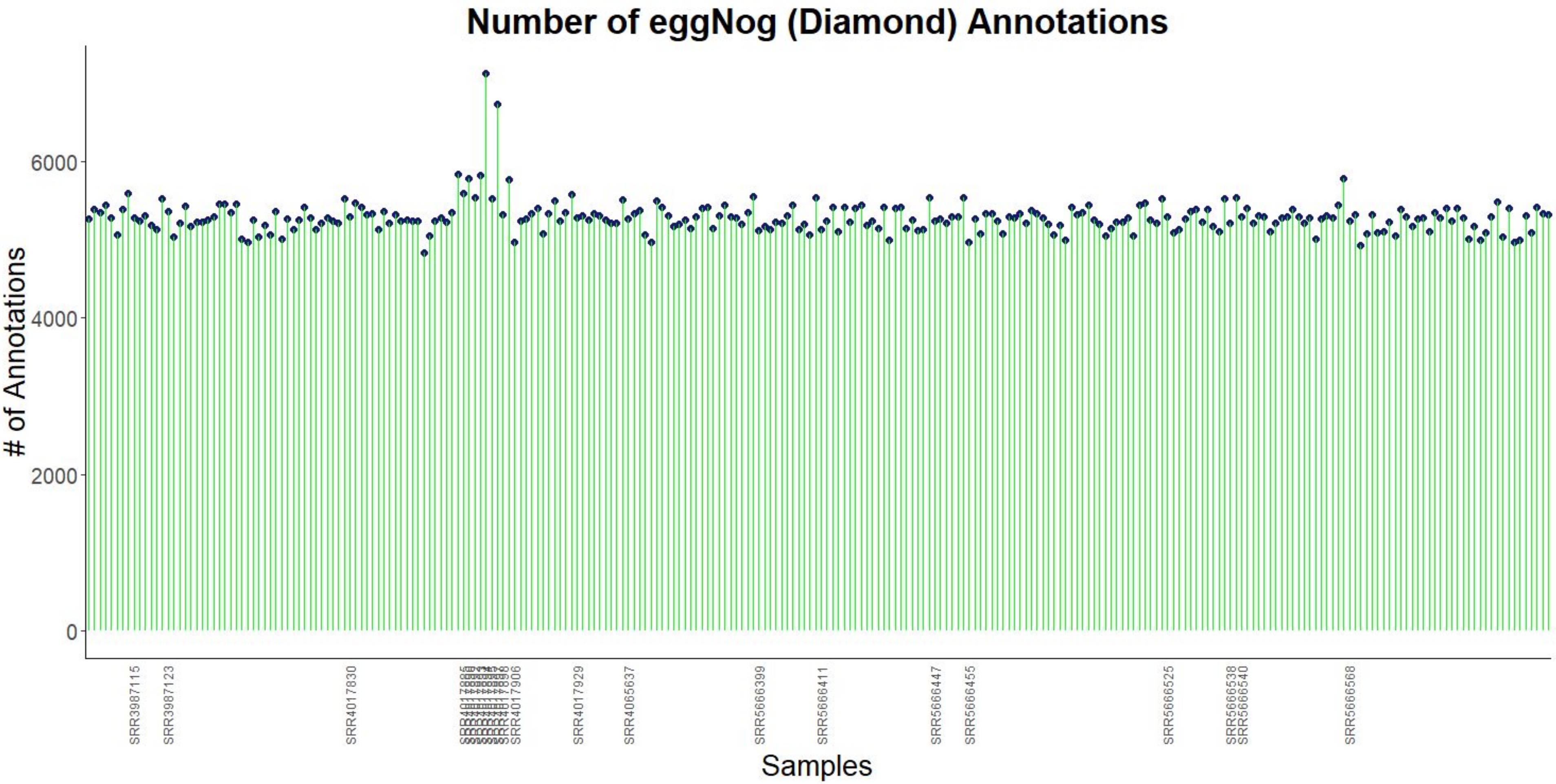
Functional Annotation Methods



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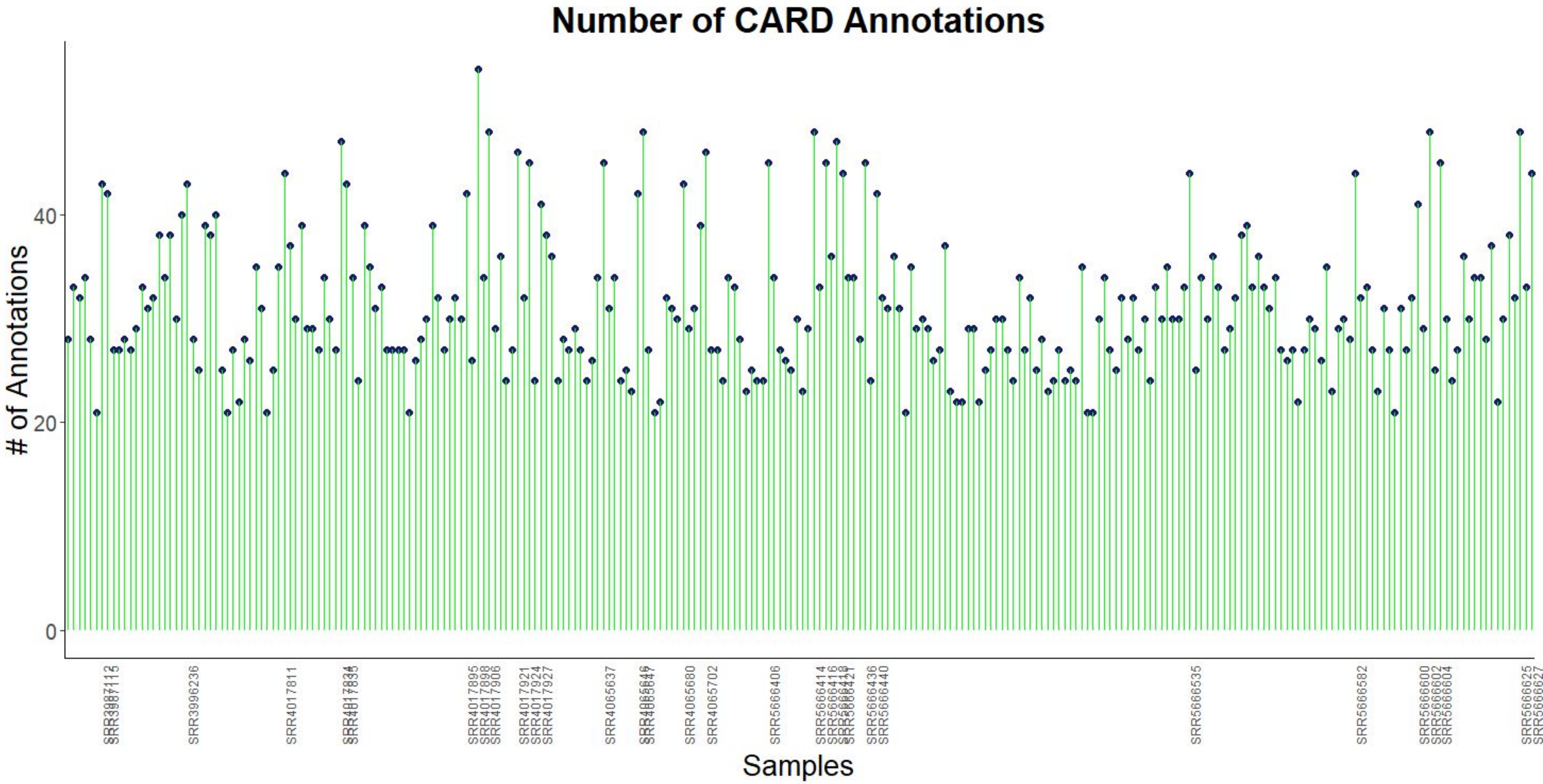
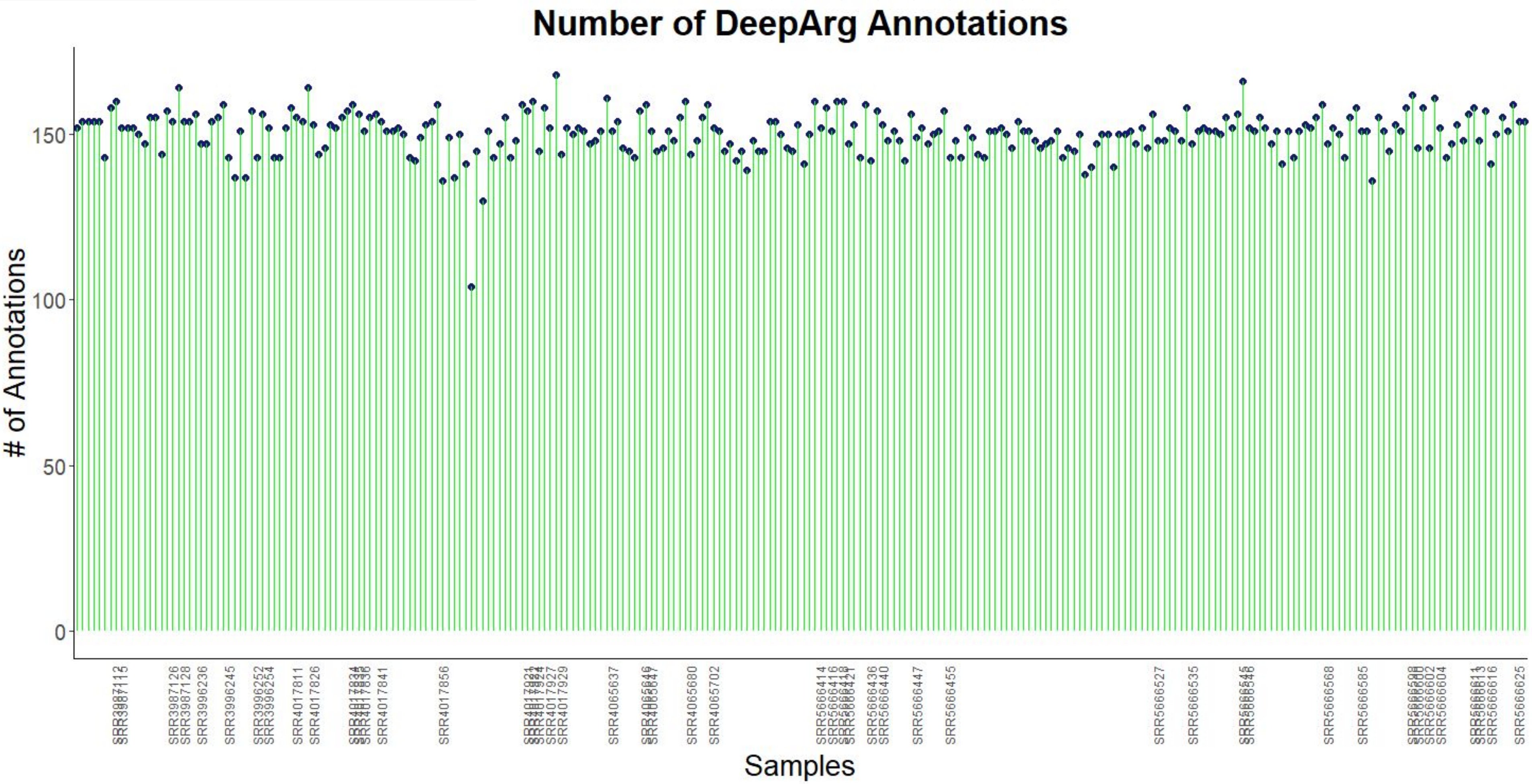
Functional Annotation Methods

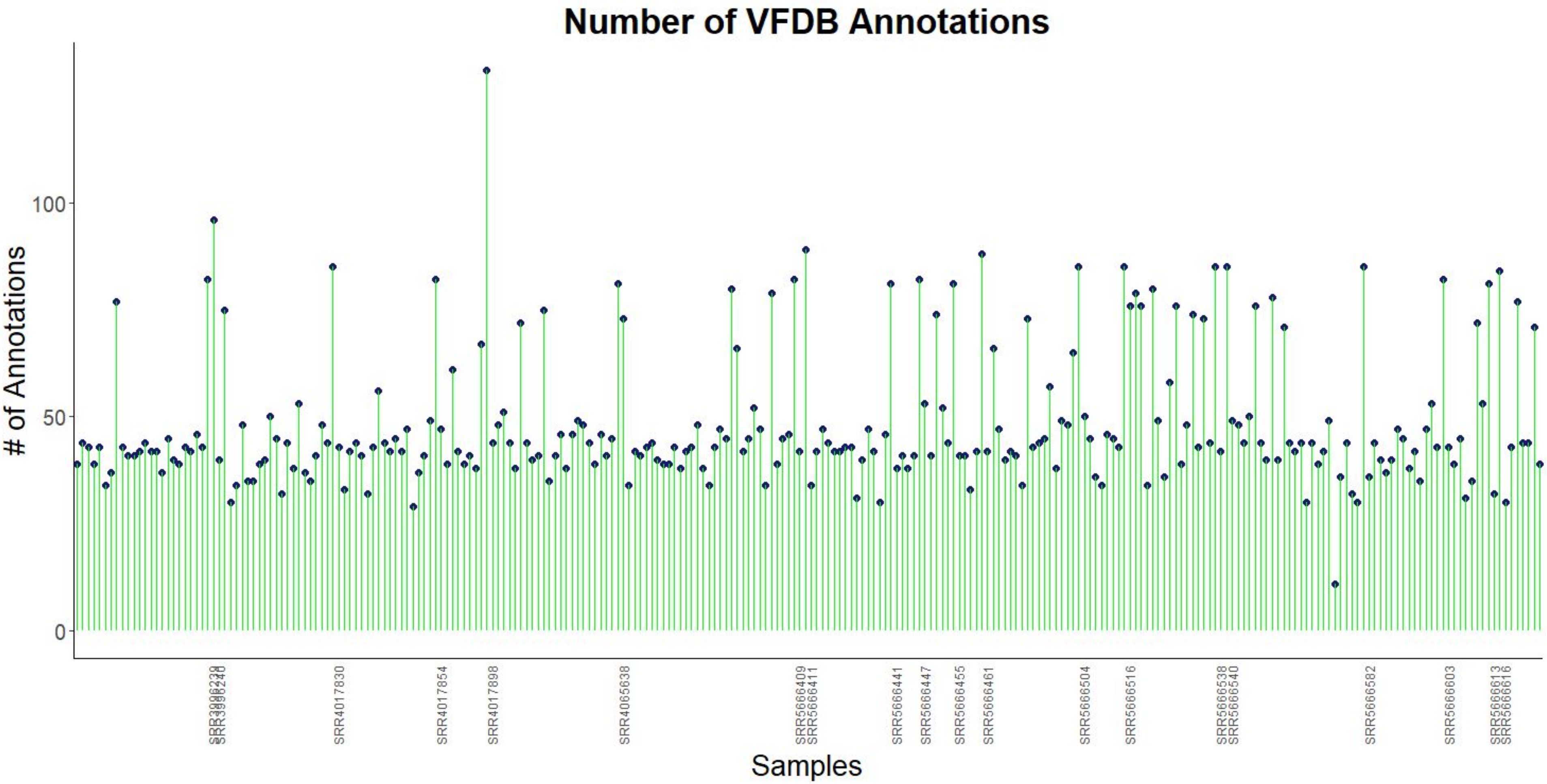


Number of CARD Annotations

Functional Annotation Methods



Functional Annotation Methods

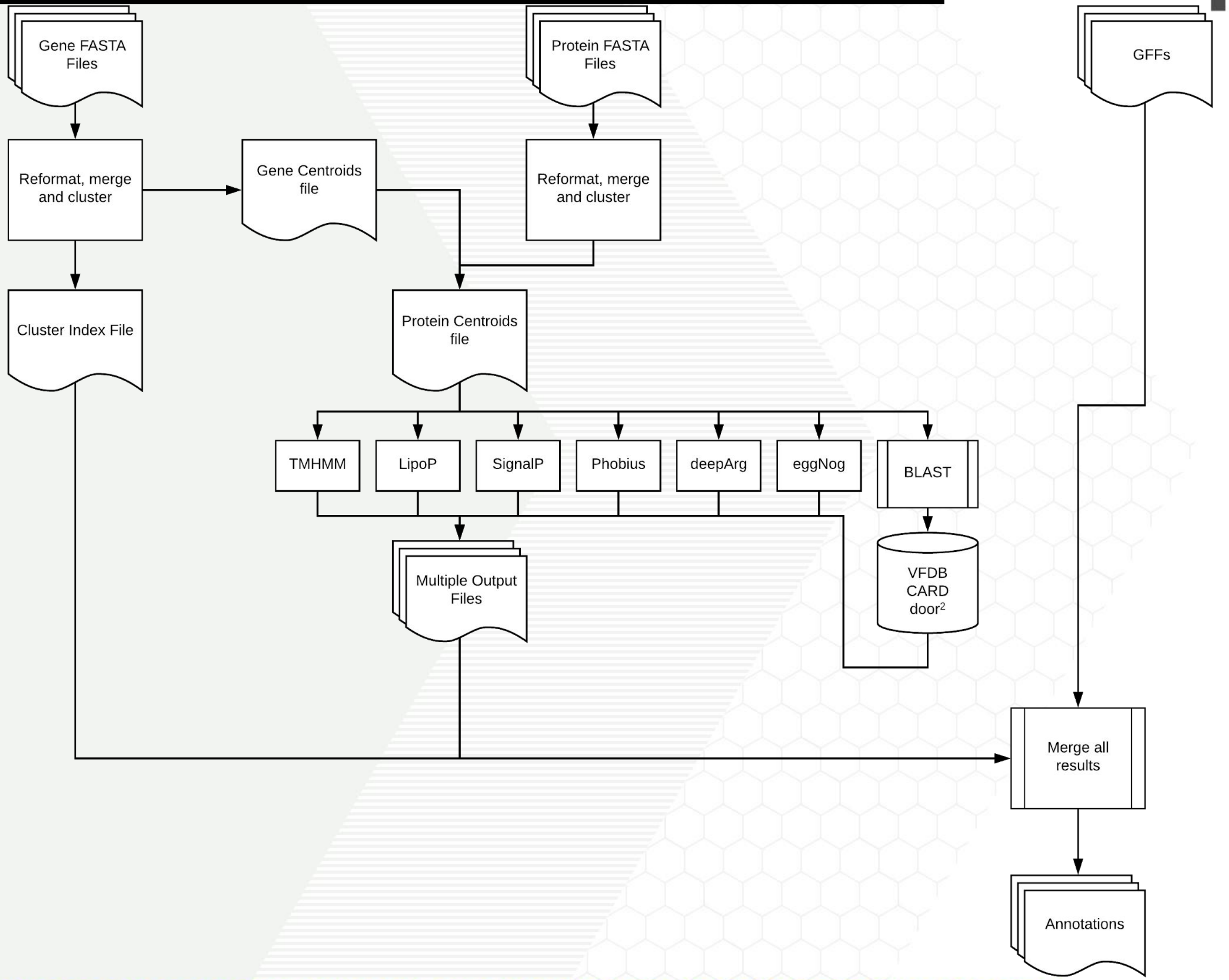


Functional Annotation Methods

Overall statistics

Tools / Database	Total # of annotations	Average # of annotations
CARD	8,051	31
DeepARG	38,799	150
Door2	1,330,879	5158
Eggnog (diamond)	1,364,546	5289
GeneMark.hmm	110,235	427
LipoP	1,502,024	5822
Phobius	1,501,560	5820
Prodigal	1,391,789	5395
SignalP	1,501,569	5820
TMHMM	1,501,577	5820
VFDB	12,517	49

Functional Annotation Final Pipeline



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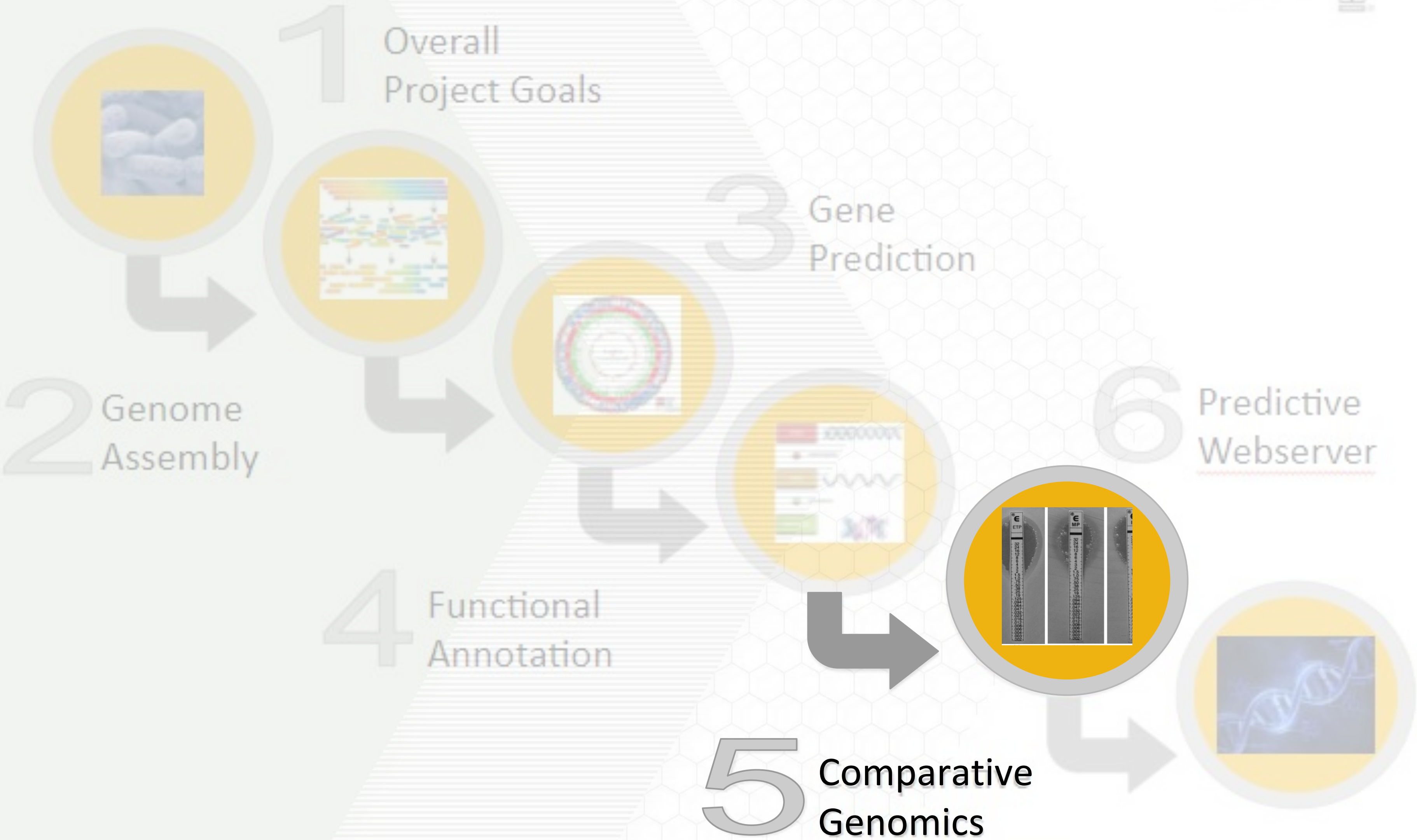

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Comparative Genomics Final Pipeline

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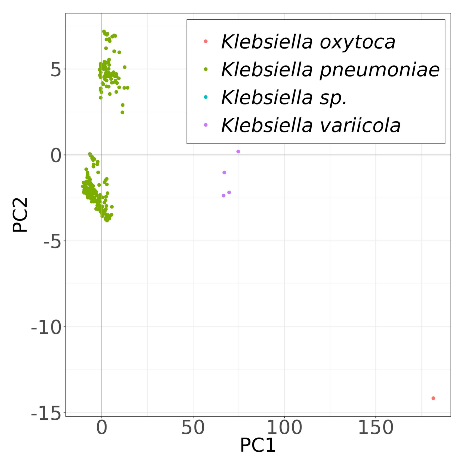
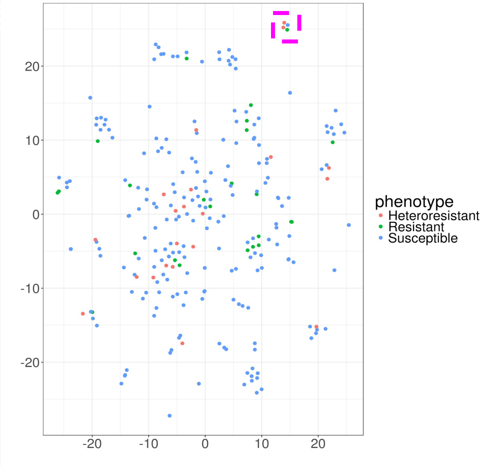
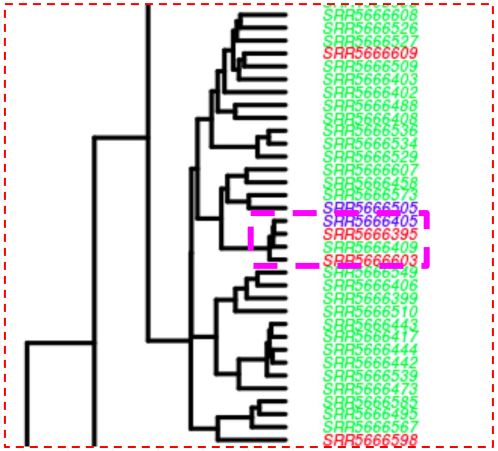
PCA analysis



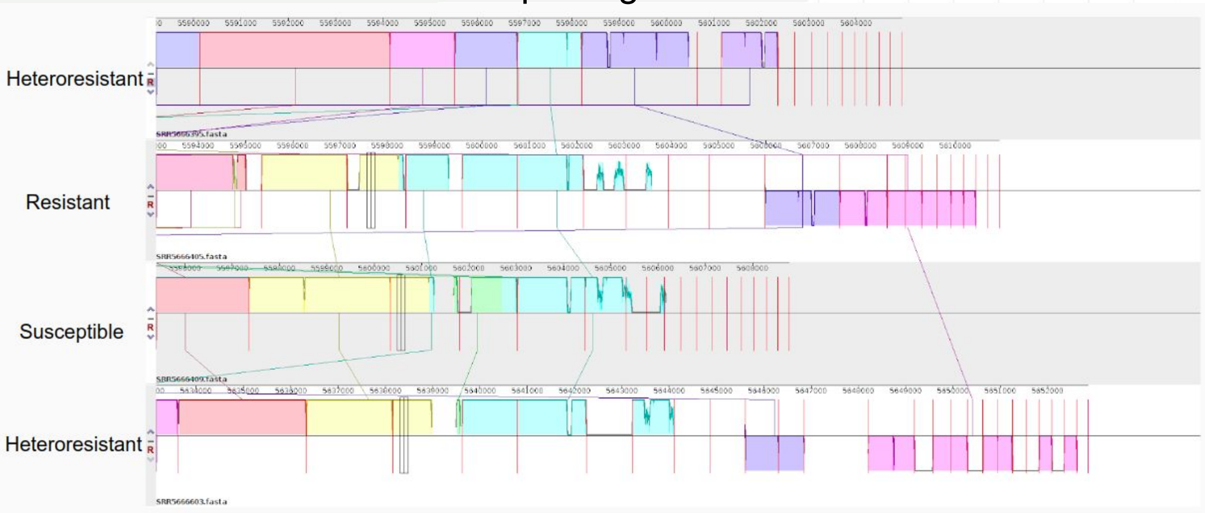
tSNE



Hierarchical clustering

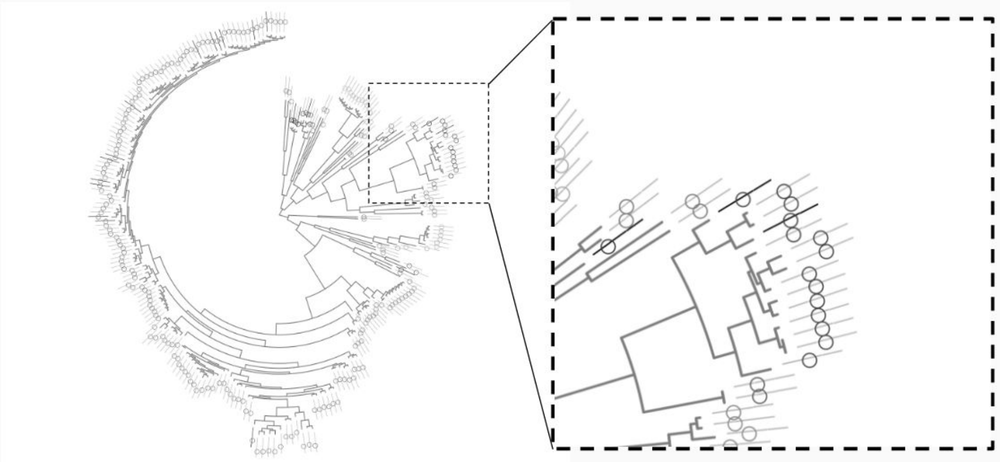


Multiple alignment

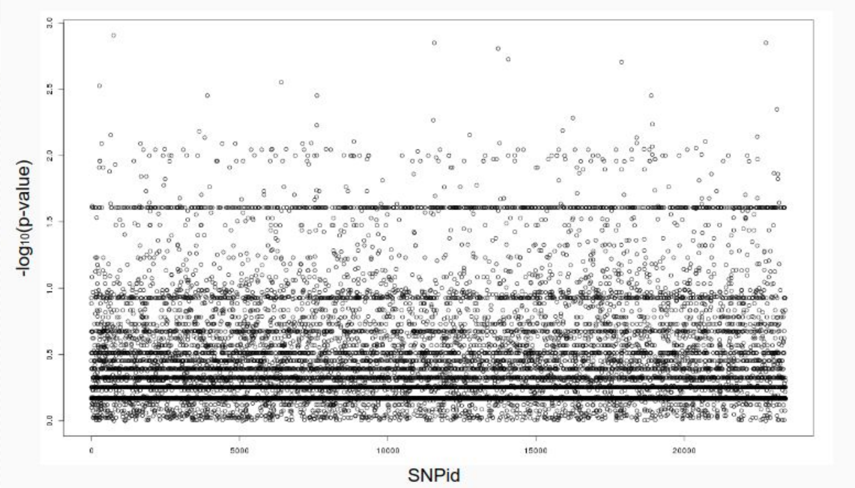


Comparative Genomics Methods

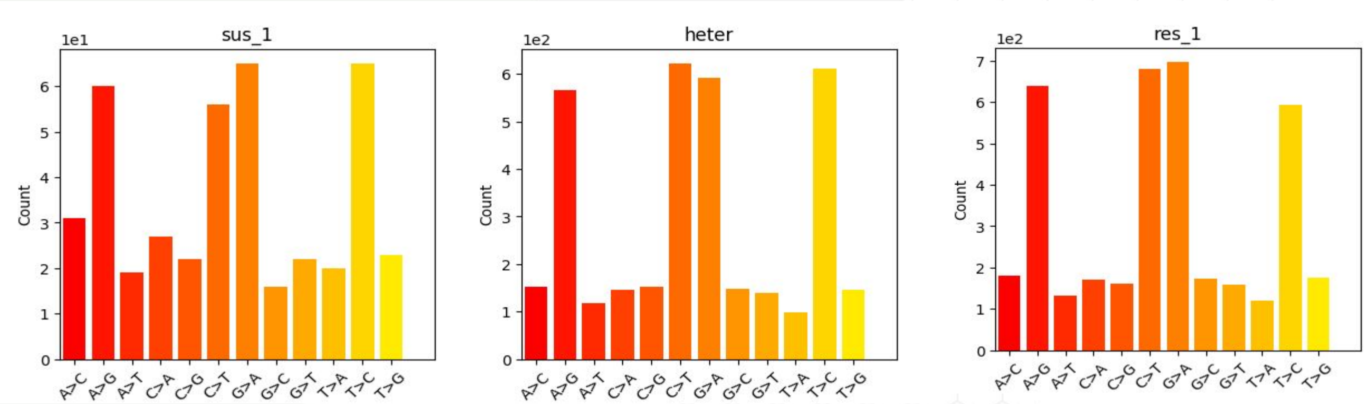
buGWAS



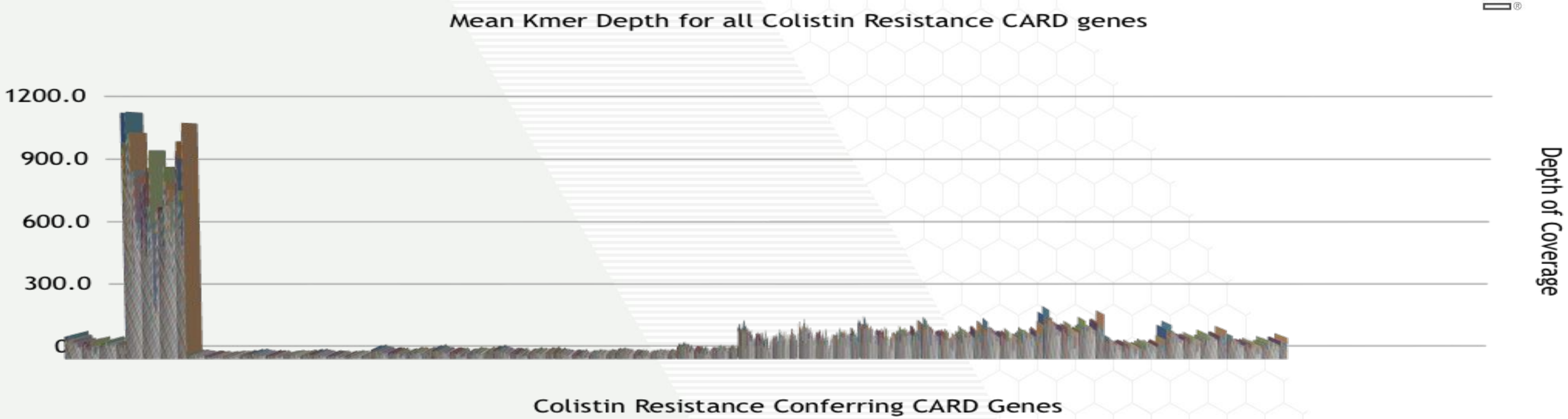
bacterialGWAS

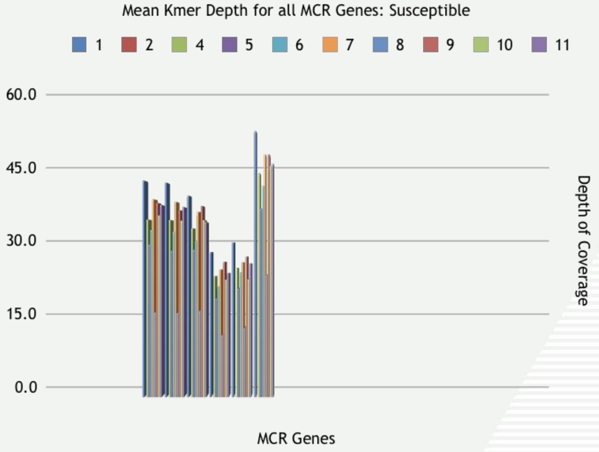


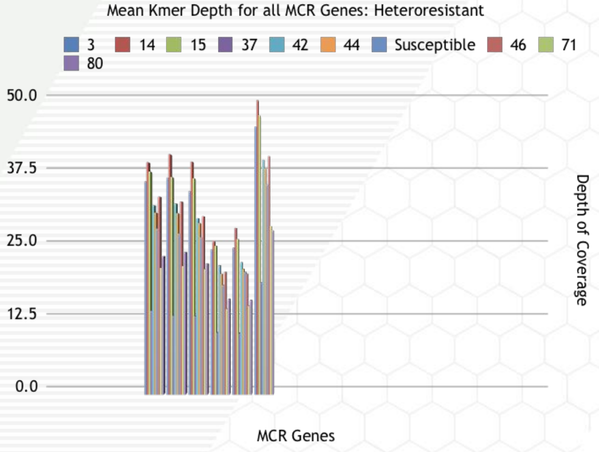
Substitution rates

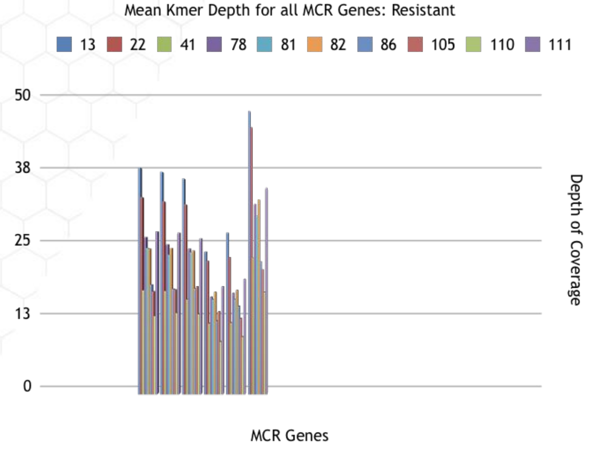


Comparative Genomics Methods


Mean Kmer Depth for all Colistin Resistance CARD genes


Mean Kmer Depth for all MCR Genes: Susceptible


Mean Kmer Depth for all MCR Genes: Heteroresistant


Mean Kmer Depth for all MCR Genes: Resistant

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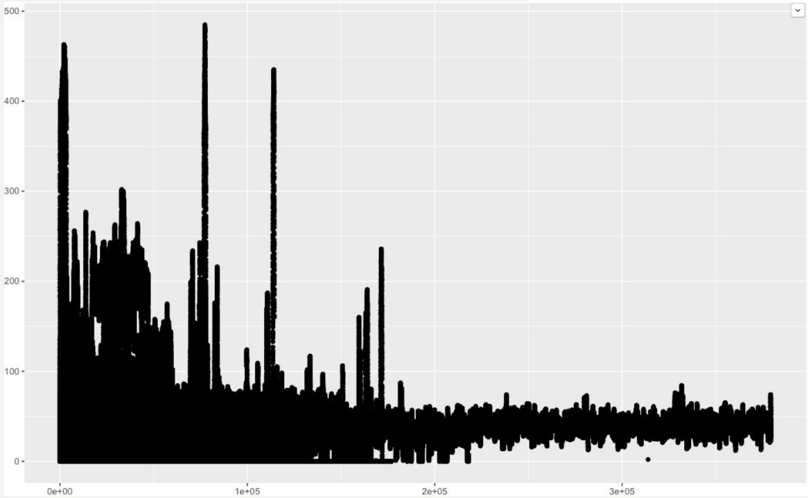
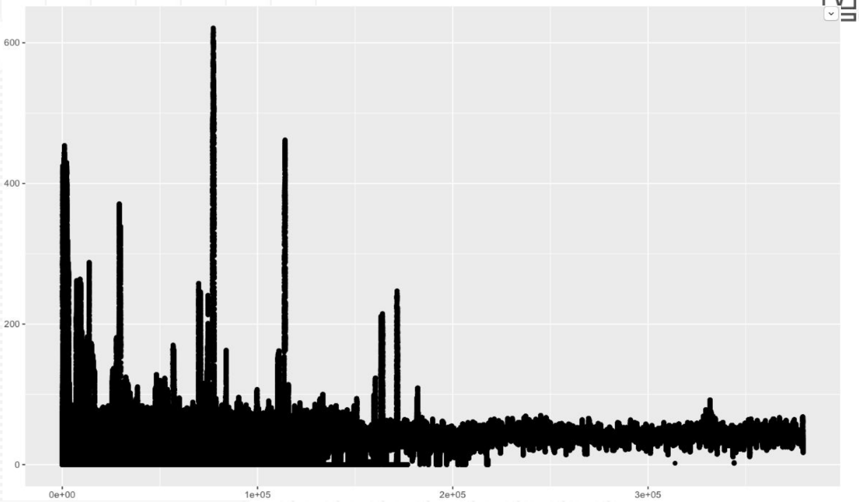
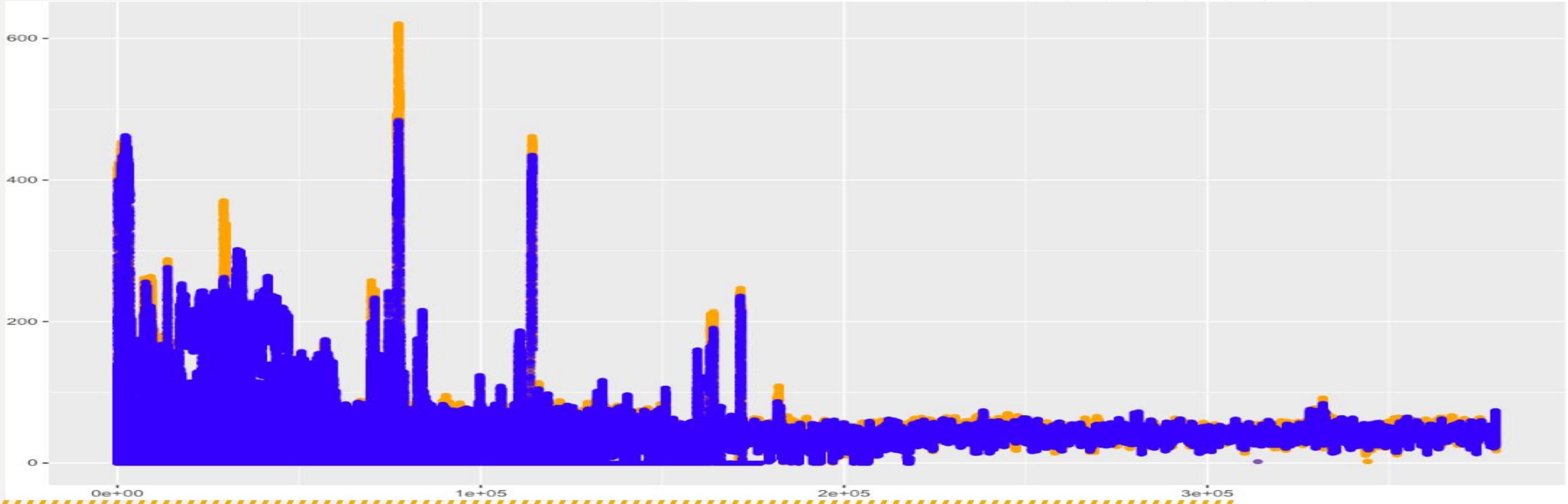
Comparative Genomics Methods



Susceptible



Heteroresistant



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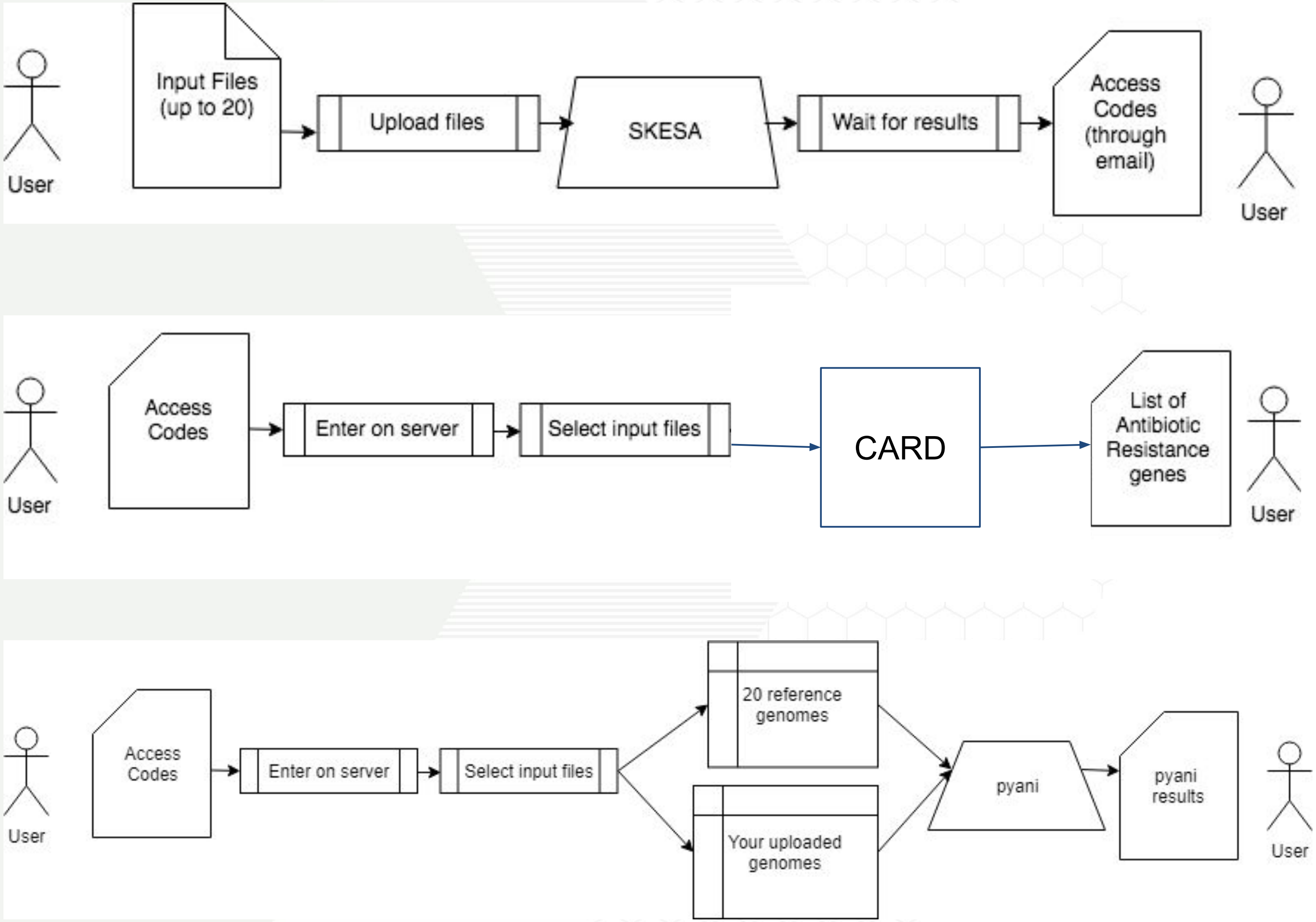
Predictive Webserver Goals

Goal - to build a tool that can provide high quality assemblies and relevant information about genomes

- Computational phenotyping
- Assembly
- Average Nucleotide Identity
- Strain Seeker

Predictive Webserver Methods



Predictive Webserver Demo

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Thank you

Questions?