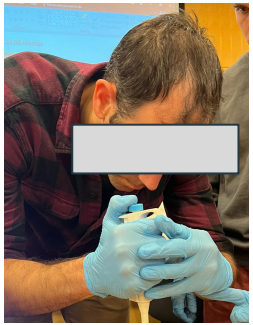
DNA Sequencing and Data Analysis

Final Project - 2023

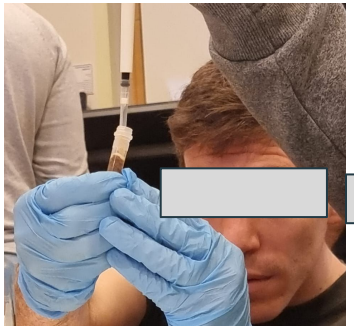
Tomer Lev, Elad Solomon, Shir Mousseri, Omer Wachman, Rotem Lapid, Gil Geva

The Team

Gil

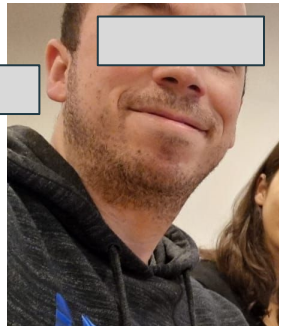


Rotem



Omer

Tomer



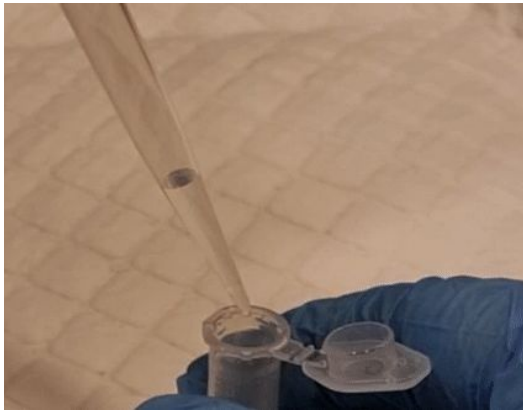
Elad

Shir

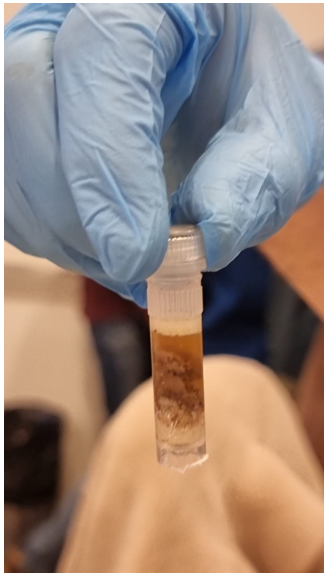
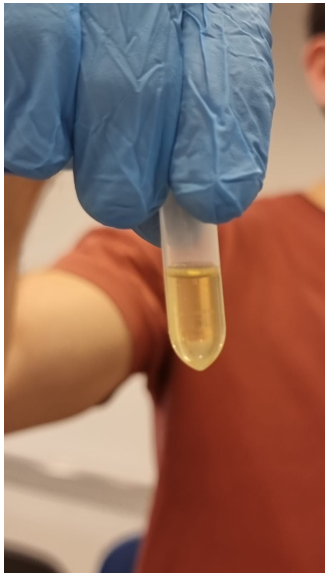
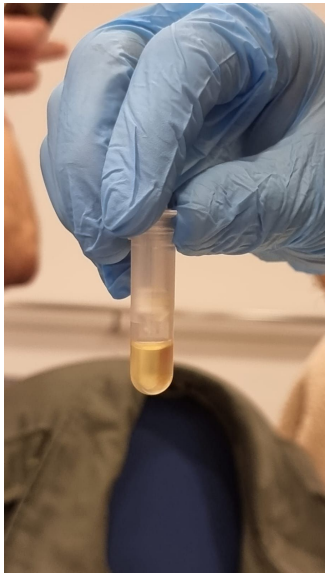
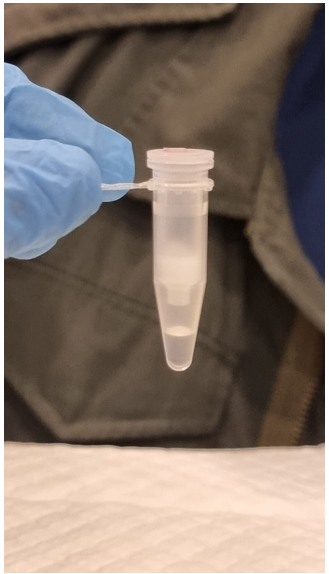
Agenda

- From poo to DNA
- From DNA to Reads
- From Reads to a DNA Sequences
- From Sequences to -
    - Animal detection
    - Gene Analysis
    - Microbiome analysis

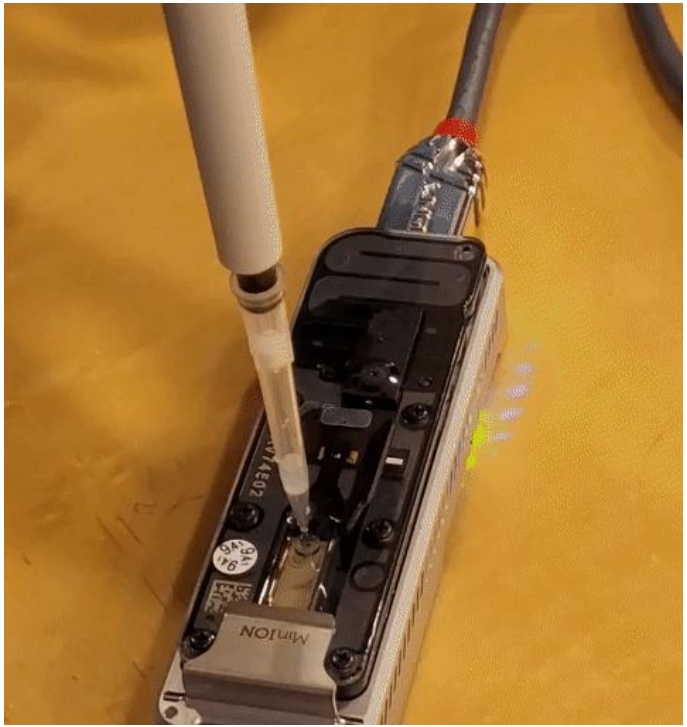
Extracting DNA - O



Extracting DNA - O

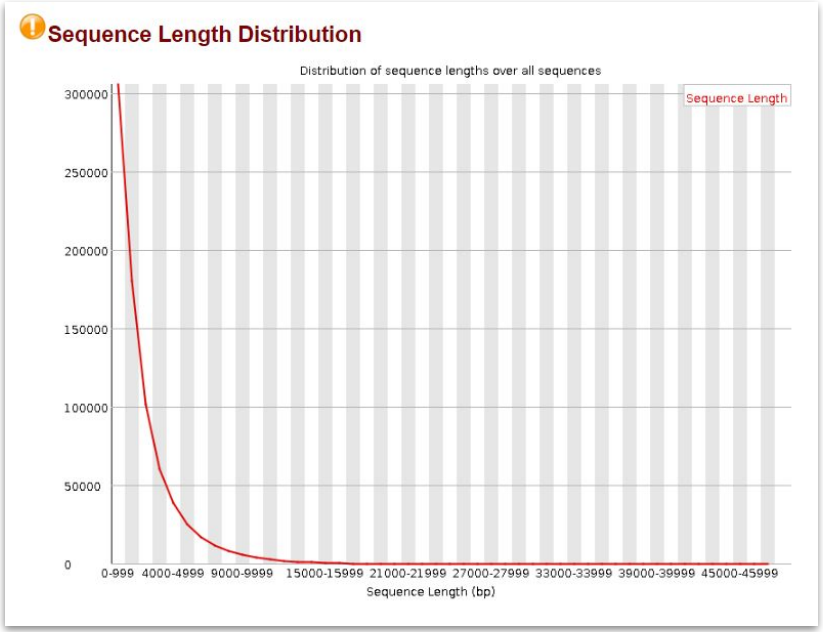






Sequencing - A

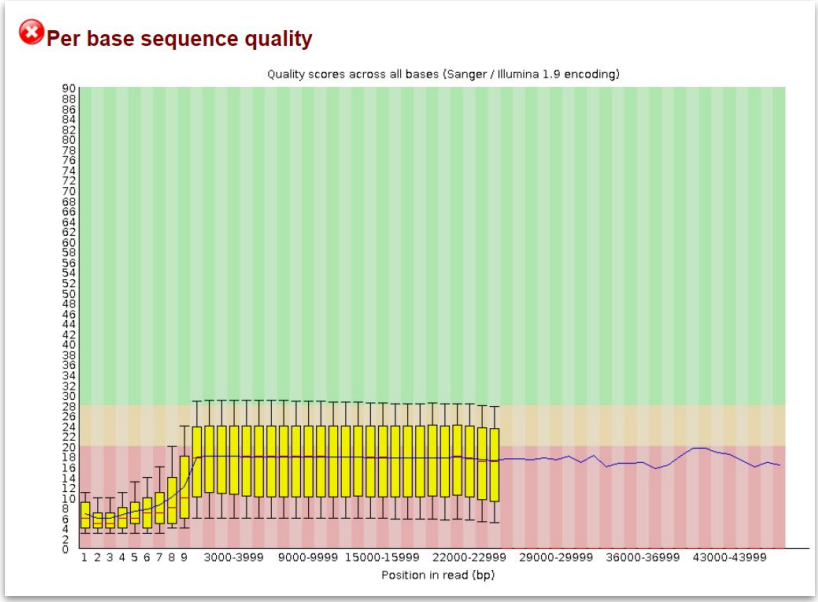


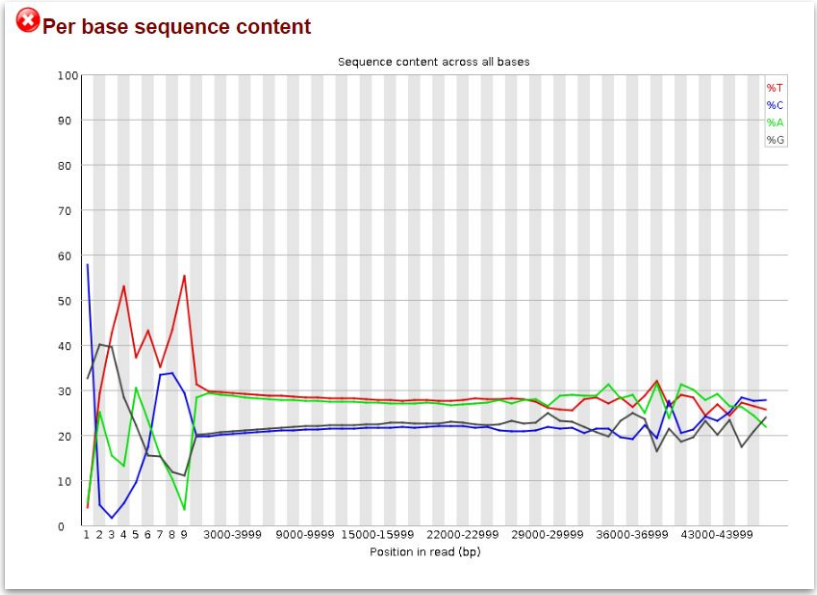
Analyzing the Reads File - M2

- 773,977 reads
- Length - 50-47,639



Analyzing the Reads File





Processing

- Trimming and filtering bad reads - NanoFilt
- Alignement - Minimap2
- We aligned the FASTA vs all reference genomes

```
fastas=("GCF_015227675.2_mRatBN7.2_genomic.fna" \
  "GCF_000001635.27_GRCm39_genomic.fna" \
  "GCA_019924945.1_FelChav1.0_genomic.fa" \
  "GCF_003160815.1_VulVul2.2_genomic.fna" \
  "GCF_014441545.1_ROS_Cfam_1.0_genomic.fna")

function align() {
  for fasta in ${fastas[@]}; do
[redacted]
bam
    echo "---- Finished ---"
  done
}

function count_alignments() {
[redacted]
    done
}
```

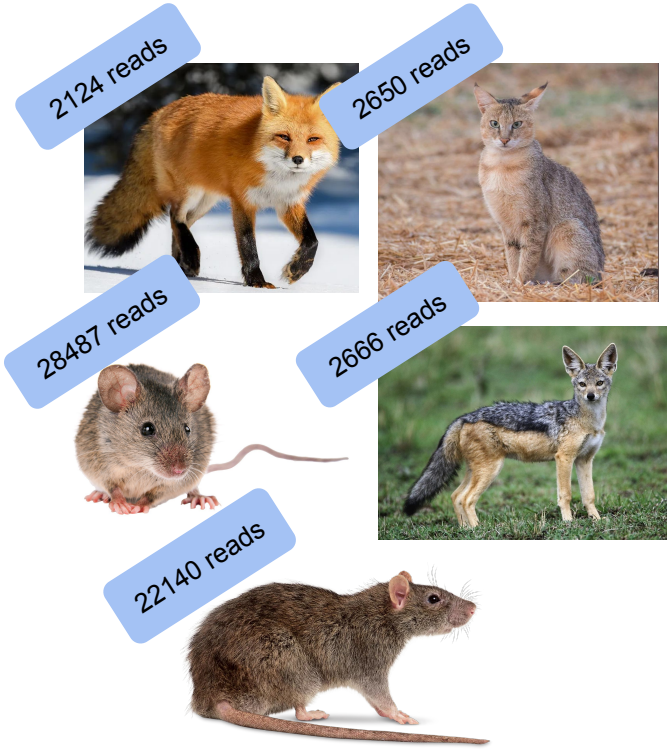
Determining the Animal - Strategy

- We compared the number of aligned reads in each SAM file
- The maximal number of aligned reads - this will be the animal

First Try

- We filtered out reads with less than 500 bases
- Got very similar numbers between rat and mouse

```
gunzip -c ./M2.raw.fastq.gz | \
                                  --logfile $fasta.nanofilt.log| \
```



First Try

Rat (mRatBN7.2)		
Count	Sequence	Chromosome
2134	NC_051336.1	chr1
1881	NC_051337.1	chr2
1245	NC_051338.1	chr3
1633	NC_051339.1	chr4
1270	NC_051340.1	chr5
1377	NC_051341.1	chr6
2204	NC_051342.1	chr7
979	NC_051343.1	chr8
1006	NC_051344.1	chr9
666	NC_051345.1	chr10
662	NC_051346.1	chr11
301	NC_051347.1	chr12
749	NC_051348.1	chr13
852	NC_051349.1	chr14
812	NC_051350.1	chr15
610	NC_051351.1	chr16
861	NC_051352.1	chr17
682	NC_051353.1	chr18
359	NC_051354.1	chr19
416	NC_051355.1	chr20
1321	NC_051356.1	X
30	NC_051357.1	Y

Mouse (GRCm39)		
Count	Sequence	Chromosome
1160	NC_000067.7	chr1
2789	NC_000068.8	chr2
1129	NC_000069.7	chr3
1006	NC_000070.7	chr4
1165	NC_000071.7	chr5
1245	NC_000072.7	chr6
986	NC_000073.7	chr7
802	NC_000074.7	chr8
5997	NC_000075.7	chr9
817	NC_000076.7	chr10
637	NC_000077.7	chr11
930	NC_000078.7	chr12
776	NC_000079.7	chr13
914	NC_000080.7	chr14
563	NC_000081.7	chr15
585	NC_000082.7	chr16
616	NC_000083.7	chr17
508	NC_000084.7	chr18
335	NC_000085.7	chr19
1565	NC_000086.8	X
367	NC_000087.8	Y

First Try

Rat (mRatBN7.2)		
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1245	NC_051338.1	chr3
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1270	NC_051340.1	chr5
1377	NC_051341.1	chr6
2204	NC_051342.1	chr7
979	NC_051343.1	chr8
1006	NC_051344.1	chr9
666	NC_051345.1	chr10
662	NC_051346.1	chr11
301	NC_051347.1	chr12
749	NC_051348.1	chr13
852	NC_051349.1	chr14
812	NC_051350.1	chr15
610	NC_051351.1	chr16
861	NC_051352.1	chr17
682	NC_051353.1	chr18
359	NC_051354.1	chr19
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Mouse (GRCm39)		
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1006	NC_000070.7	chr4
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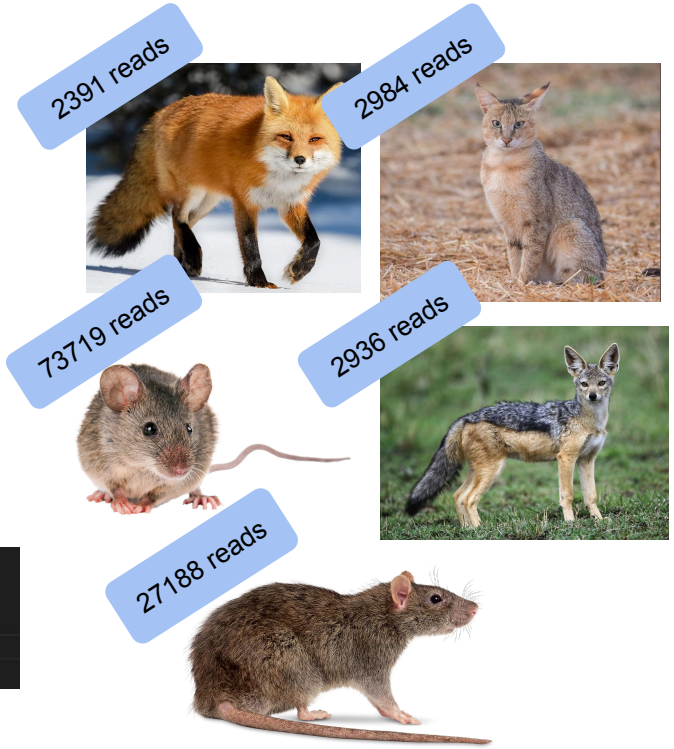
1245	NC_000072.7	chr6
986	NC_000073.7	chr7
802	NC_000074.7	chr8
5997	NC_000075.7	chr9
817	NC_000076.7	chr10
637	NC_000077.7	chr11
930	NC_000078.7	chr12
776	NC_000079.7	chr13
914	NC_000080.7	chr14
563	NC_000081.7	chr15
585	NC_000082.7	chr16
616	NC_000083.7	chr17
508	NC_000084.7	chr18
335	NC_000085.7	chr19
1565	NC_000086.8	X
367	NC_000087.8	Y

Second Try

- We decided to align again with different parameters
- This time we didn’t filter short reads
- Now the mouse was significantly better




```
gunzip -c ./M2.raw.fastq.gz | \
```

Second Try

Rat (mRatBN7.2)				Mouse (GRCm39)		
Count	Sequence	Chromosome		Count	Sequence	Chromosome
2697	NC_051336.1	chr1		2742	NC_000067.7	chr1
2353	NC_051337.1	chr2		8237	NC_000068.8	chr2
1585	NC_051338.1	chr3		2581	NC_000069.7	chr3
1985	NC_051339.1	chr4		2456	NC_000070.7	chr4
1546	NC_051340.1	chr5		2529	NC_000071.7	chr5
1642	NC_051341.1	chr6		2758	NC_000072.7	chr6
2472	NC_051342.1	chr7		2405	NC_000073.7	chr7
1228	NC_051343.1	chr8		1859	NC_000074.7	chr8
1236	NC_051344.1	chr9		17155	NC_000075.7	chr9
873	NC_051345.1	chr10		1904	NC_000076.7	chr10
836	NC_051346.1	chr11		1533	NC_000077.7	chr11
341	NC_051347.1	chr12		2206	NC_000078.7	chr12
926	NC_051348.1	chr13		1728	NC_000079.7	chr13
1067	NC_051349.1	chr14		2811	NC_000080.7	chr14
1006	NC_051350.1	chr15		1385	NC_000081.7	chr15
758	NC_051351.1	chr16		1362	NC_000082.7	chr16
1037	NC_051352.1	chr17		1357	NC_000083.7	chr17
859	NC_051353.1	chr18		1221	NC_000084.7	chr18
446	NC_051354.1	chr19		809	NC_000085.7	chr19
513	NC_051355.1	chr20				
1640	NC_051356.1	X		3815	NC_000086.8	X
37	NC_051357.1	Y		603	NC_000087.8	Y
14	NC_001665.2	?		34	NC_005089.1	?
27097				**63490**		

A Male Mouse

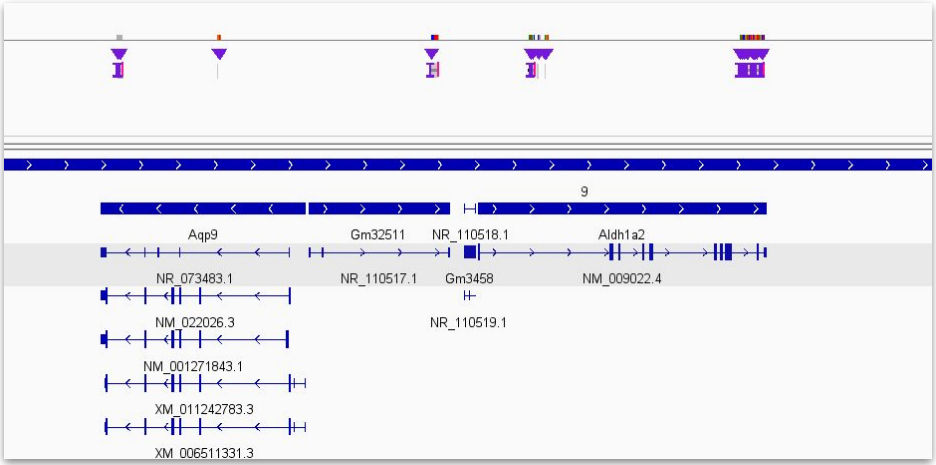
Let’s just verify with the MAPQ values…

Rat (mRatBN7.2)			
Average MAPQ	Count	Sequence	Chromosome
15.20	2697	NC_051336.1	chr1
16.33	2353	NC_051337.1	chr2
18.78	1585	NC_051338.1	chr3
15.51	1985	NC_051339.1	chr4
16.34	1546	NC_051340.1	chr5
14.42	1642	NC_051341.1	chr6
8.36	2472	NC_051342.1	chr7
17.49	1228	NC_051343.1	chr8
15.58	1236	NC_051344.1	chr9
19.83	873	NC_051345.1	chr10
18.14	836	NC_051346.1	chr11
16.54	341	NC_051347.1	chr12
16.37	926	NC_051348.1	chr13
17.29	1067	NC_051349.1	chr14
16.06	1006	NC_051350.1	chr15
18.90	758	NC_051351.1	chr16
13.31	1037	NC_051352.1	chr17
16.26	859	NC_051353.1	chr18
18.60	446	NC_051354.1	chr19
14.93	513	NC_051355.1	chr20
15.70	1640	NC_051356.1	X
0.35	37	NC_051357.1	Y
40.67	14	NC_001665.2	?
	27097		

Mouse (GRCm39)			
Average MAPQ	Count	Sequence	Chromosome
50.06	2742	NC_000067.7	chr1
22.45	8237	NC_000068.8	chr2
45.91	2581	NC_000069.7	chr3
40.82	2456	NC_000070.7	chr4
40.72	2529	NC_000071.7	chr5
40.53	2758	NC_000072.7	chr6
36.54	2405	NC_000073.7	chr7
45.45	1859	NC_000074.7	chr8
5.51	17155	NC_000075.7	chr9
50.79	1904	NC_000076.7	chr10
51.36	1533	NC_000077.7	chr11
36.34	2206	NC_000078.7	chr12
46.51	1728	NC_000079.7	chr13
29.64	2811	NC_000080.7	chr14
50.15	1385	NC_000081.7	chr15
51.96	1362	NC_000082.7	chr16
46.24	1357	NC_000083.7	chr17
50.93	1221	NC_000084.7	chr18
48.96	809	NC_000085.7	chr19
29.82	3815	NC_000086.8	X
1.36	603	NC_000087.8	Y
39.06	34	NC_005089.1	?
	63490		

A Female Mouse!

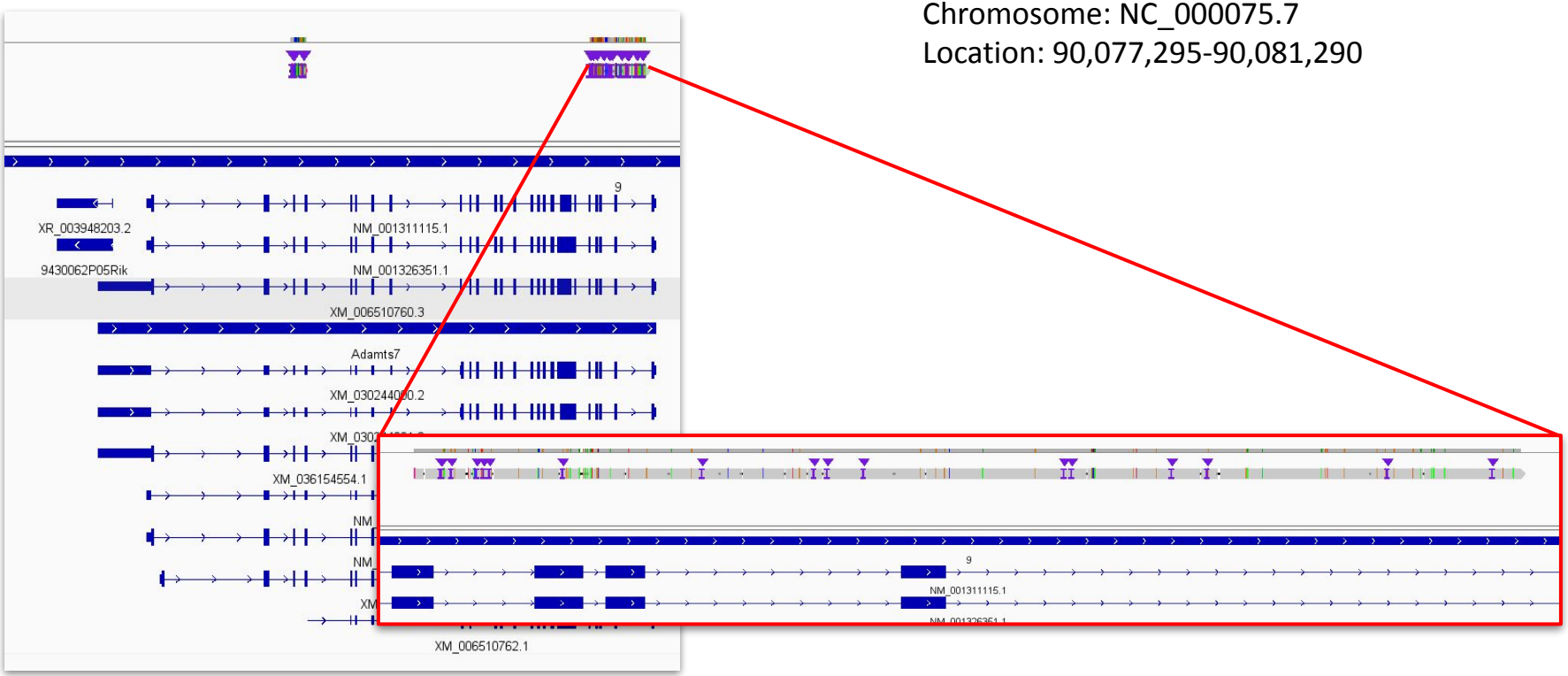
Exploring the results on IGV



Genes Analysis

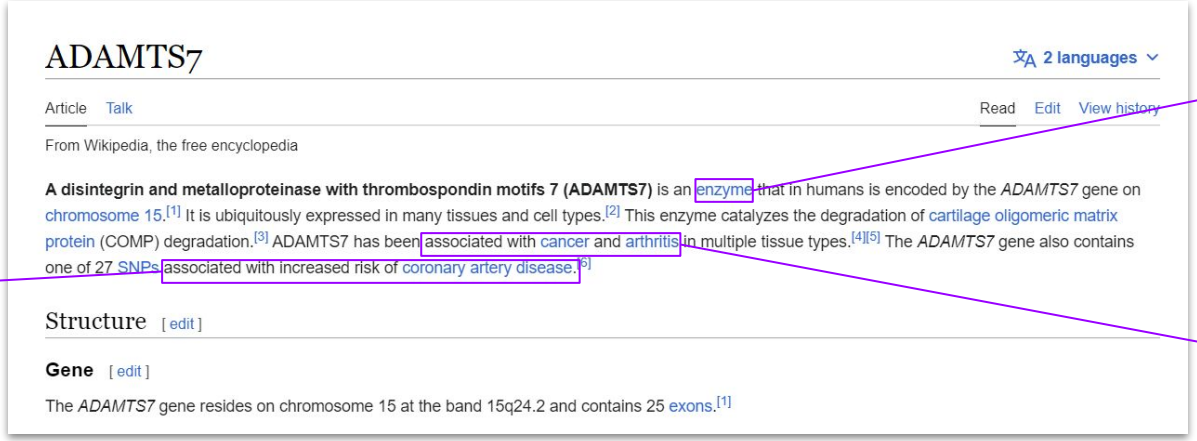
- We found interesting alignments in the IGV and looked for their source in the DB
- We used the famous website for Mouse Genome

Genes Analysis



Chromosome: NC_000075.7
Location: 90,077,295-90,081,290

Genes Analysis - ADAMTS7



ADAMTS7

2 languages

Article Talk Read Edit View history

From Wikipedia, the free encyclopedia

A disintegrin and metalloproteinase with thrombospondin motifs 7 (ADAMTS7) is an enzyme that in humans is encoded by the *ADAMTS7* gene on chromosome 15.[1] It is ubiquitously expressed in many tissues and cell types.[2] This enzyme catalyzes the degradation of cartilage oligomeric matrix protein (COMP) degradation.[3] ADAMTS7 has been associated with cancer and arthritis in multiple tissue types.[4][5] The *ADAMTS7* gene also contains one of 27 SNPs associated with increased risk of coronary artery disease.[6]

Structure [edit]

Gene [edit]

The *ADAMTS7* gene resides on chromosome 15 at the band 15q24.2 and contains 25 exons.[1]

Enzymes are proteins that act as biological catalysts by accelerating chemical reactions

Coronary Artery Disease - מחלת לב כלילית

Arthritis = דלקת מפרקים

Genes Analysis - ADAMTS7

They turned off the gene in mice (probably using CRISPR)

Knockout of Adamts7, a novel coronary artery disease locus in humans, reduces atherosclerosis in mice

Robert C Bauer [1 2], Junichiro Tohyama [1 2], Jian Cui [1 3], Lan Cheng [1 3], Jifu Yang [1 3], Xuan Zhang [1 3], Kristy Ou [1 3], Georgios K Paschos [1 4], X Long Zheng [5], Michael S Parmacek [1 3], Daniel J Rader [# 1 2 3 4], Muredach P Reilly [# 1 3]

Affiliations + expand

PMID: 25712206 PMCID: PMC4382454 DOI: 10.1161/CIRCULATIONAHA.114.012669

Free PMC article

Abstract

Background: Genome-wide association studies have established ADAMTS7 as a locus for coronary artery disease in humans. However, these studies fail to provide directionality for the association between ADAMTS7 and coronary artery disease. Previous reports have implicated ADAMTS7 in the regulation of vascular smooth muscle cell migration, but a role for and the direction of impact of this gene in atherogenesis have not been shown in relevant model systems.

Methods and results: We bred an Adamts7 whole-body knockout mouse onto both the Ldlr and Apoe knockout hyperlipidemic mouse models. Adamts7(-/-)/Ldlr(-/-) and Adamts7(-/-)/Apoe(-/-) mice displayed significant reductions in lesion formation in aortas and aortic roots compared with controls. Adamts7 knockout mice also showed reduced neointimal formation after femoral wire injury. Adamts7 expression was induced in response to injury and hyperlipidemia but was absent at later time points, and primary Adamts7 knockout vascular smooth muscle cells showed reduced migration in the setting of tumor necrosis factor-α stimulation. ADAMTS7 localized to cells positive for smooth muscle cell markers in human coronary artery disease lesions, and subcellular localization studies in cultured vascular smooth muscle cells placed ADAMTS7 at the cytoplasm and cell membrane, where it colocalized with markers of podosomes.

Conclusions: These data represent the first in vivo experimental validation of the association of Adamts7 with atherogenesis, likely through modulation of vascular cell migration and matrix in atherosclerotic lesions. These results demonstrate that Adamts7 is proatherogenic, lending directionality to the original genetic association and supporting the concept that pharmacological inhibition of ADAMTS7 should be atheroprotective in humans, making it an attractive target for novel therapeutic interventions.

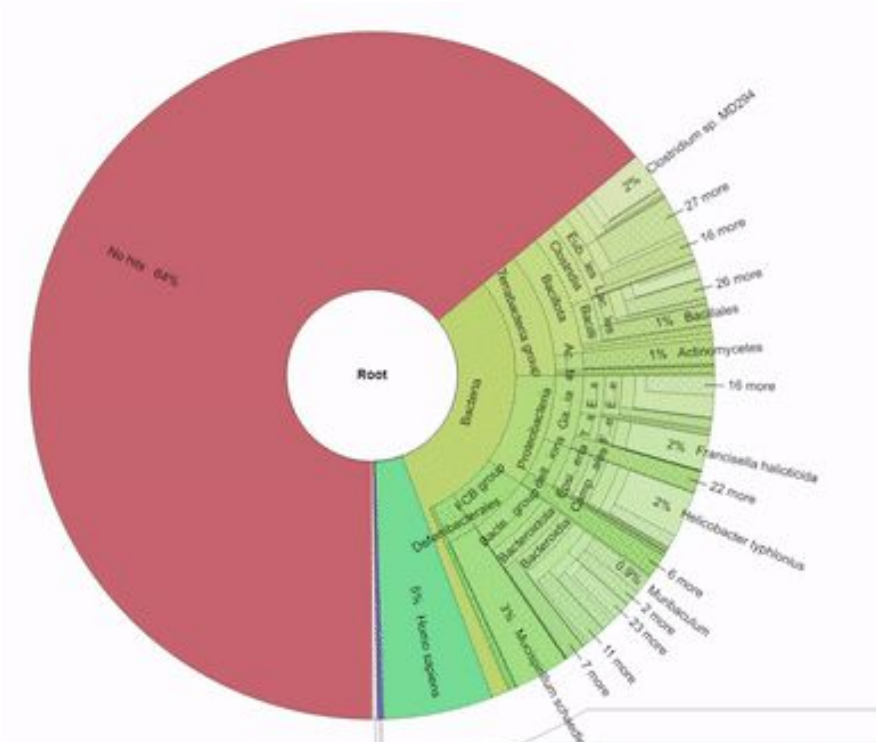
And reduced atherosclerosis (טרשת עורקים)

Microbiome Analysis

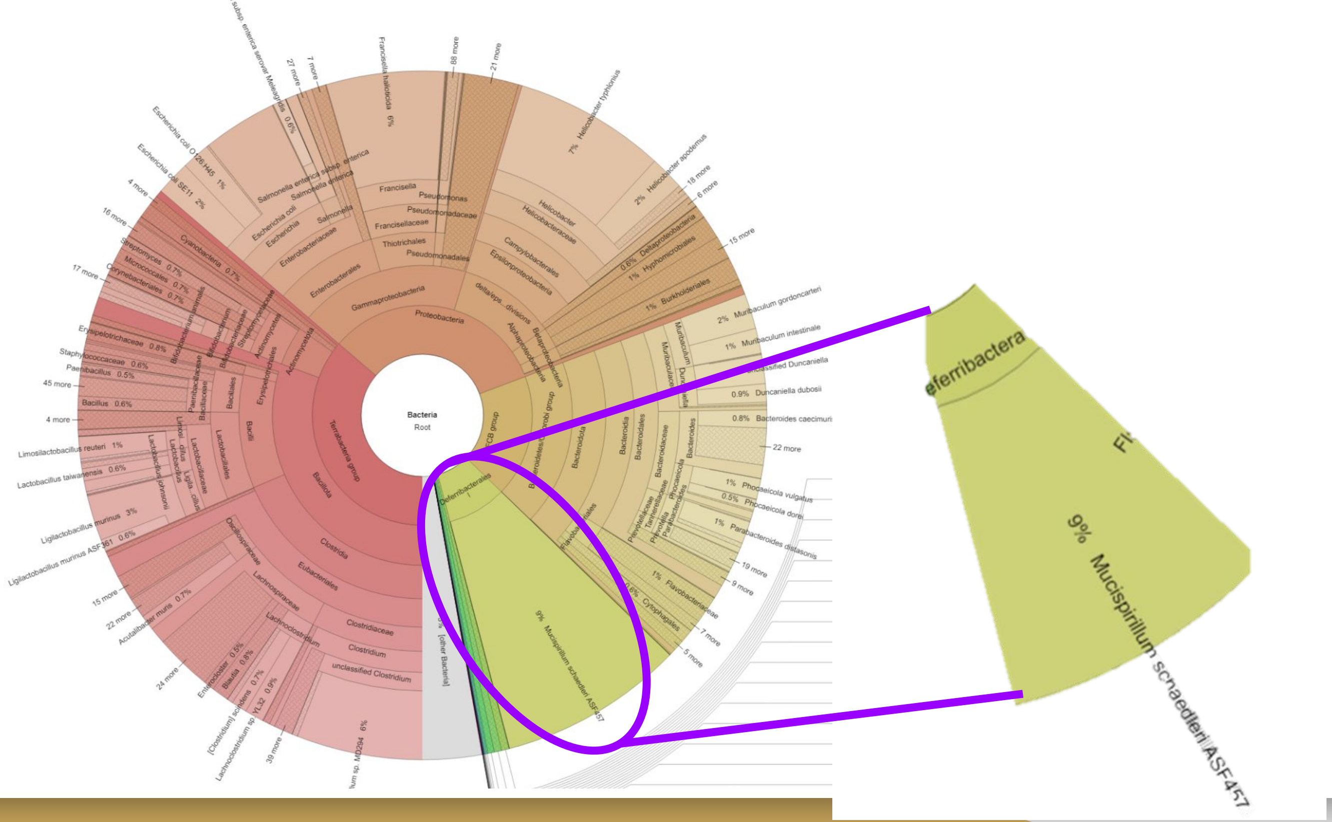
We mapped the the unaligned reads in the sequenced DNA using Kraken2, and got a mapping of the other DNA sources in the sample.

```
# Download standard 16Gb DB
mkdir db-16 && cd db-16
                                                                    gb_20221209.tar.gz

# Invoke Kraken
c
s                                                                   ed.bam > grc.fasta
k                                                                   t.txt --output out.txt

# Invoke Krona
ktImportTaxonomy -t 5 -m 3 report.txt
```

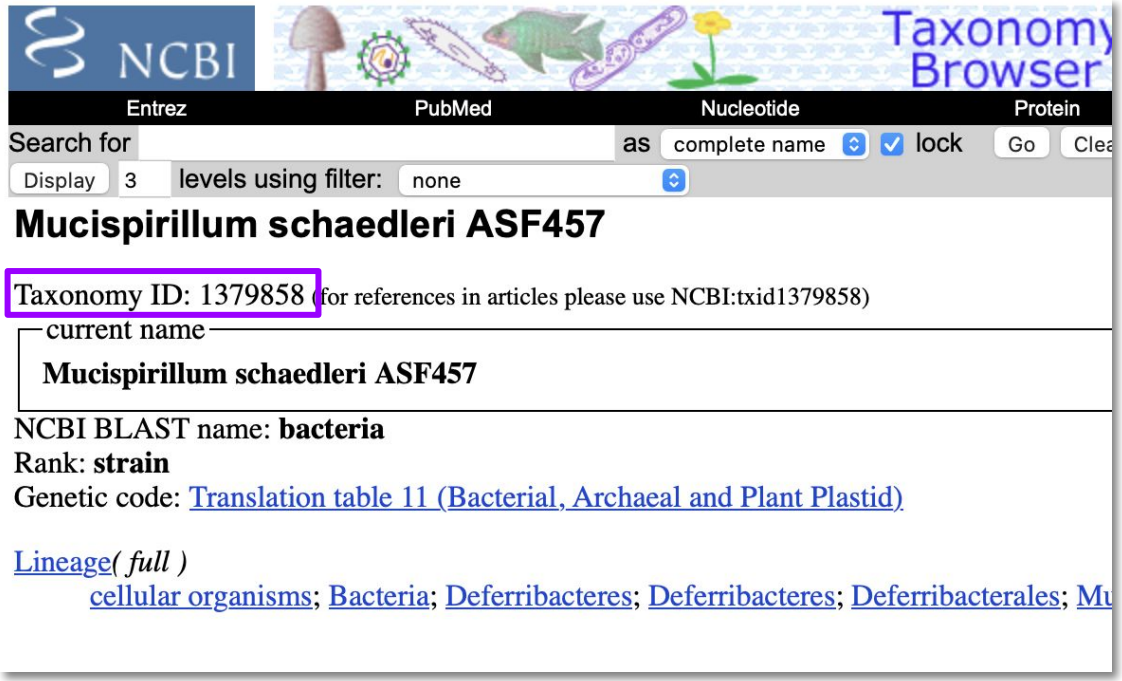
Microbiome Analysis



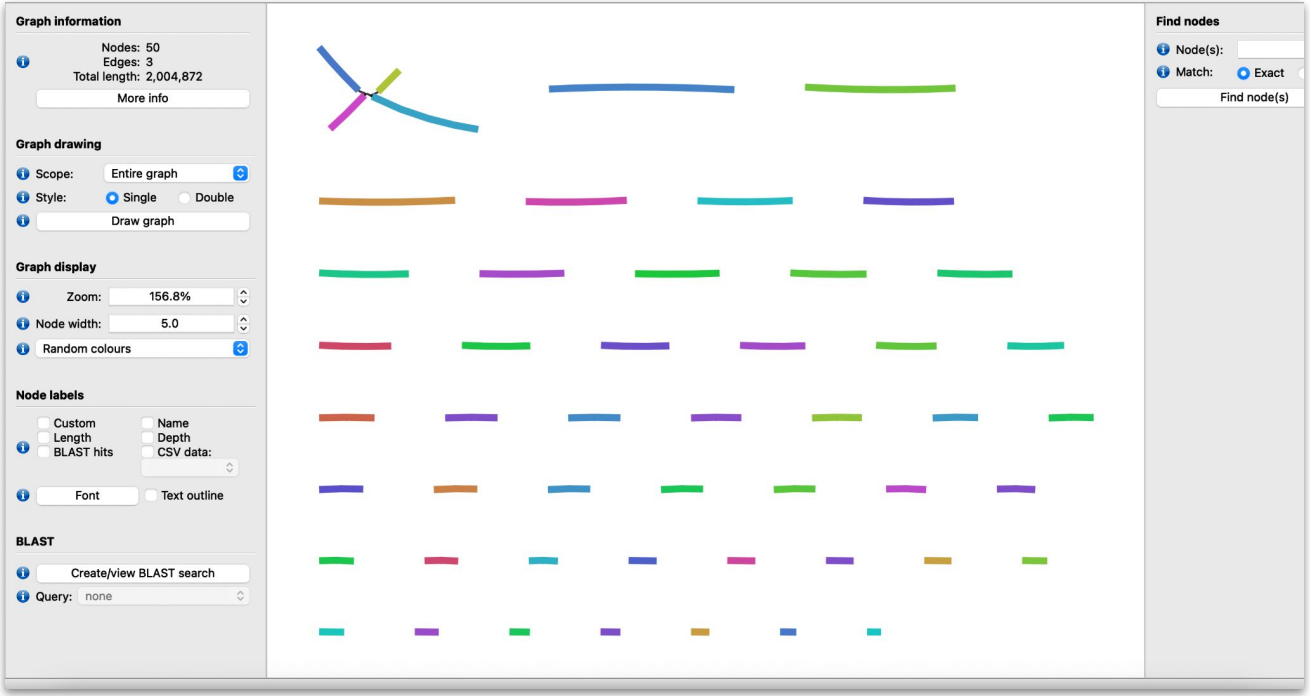
Microbiome Analysis - Bacteria



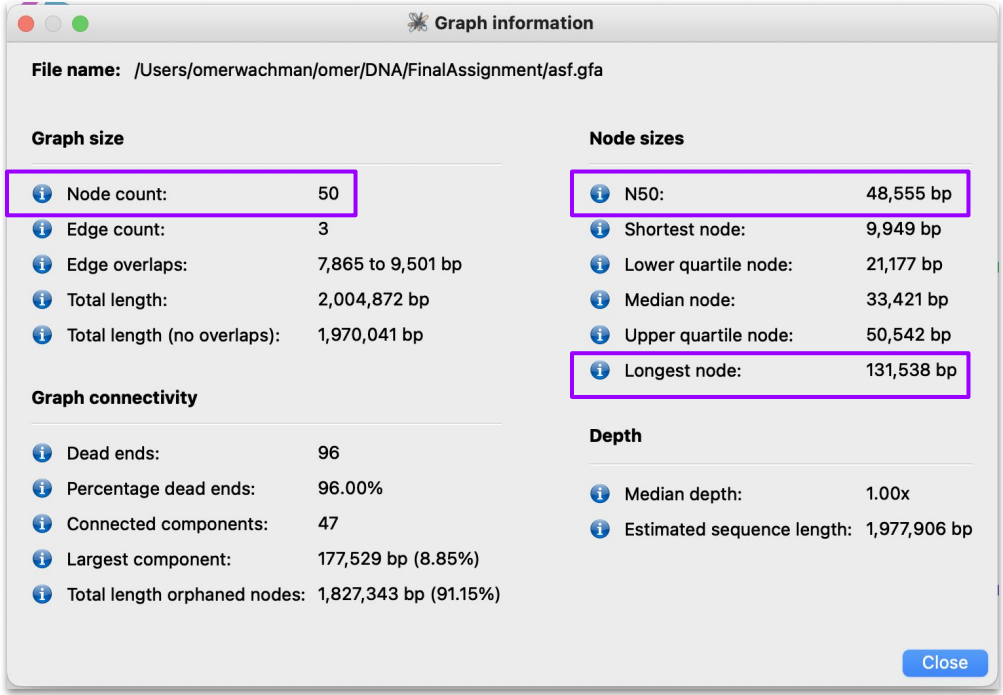
Mucispirillum Schaedleri ASF457



Mucispirillum Schaedleri ASF457 - Assembly



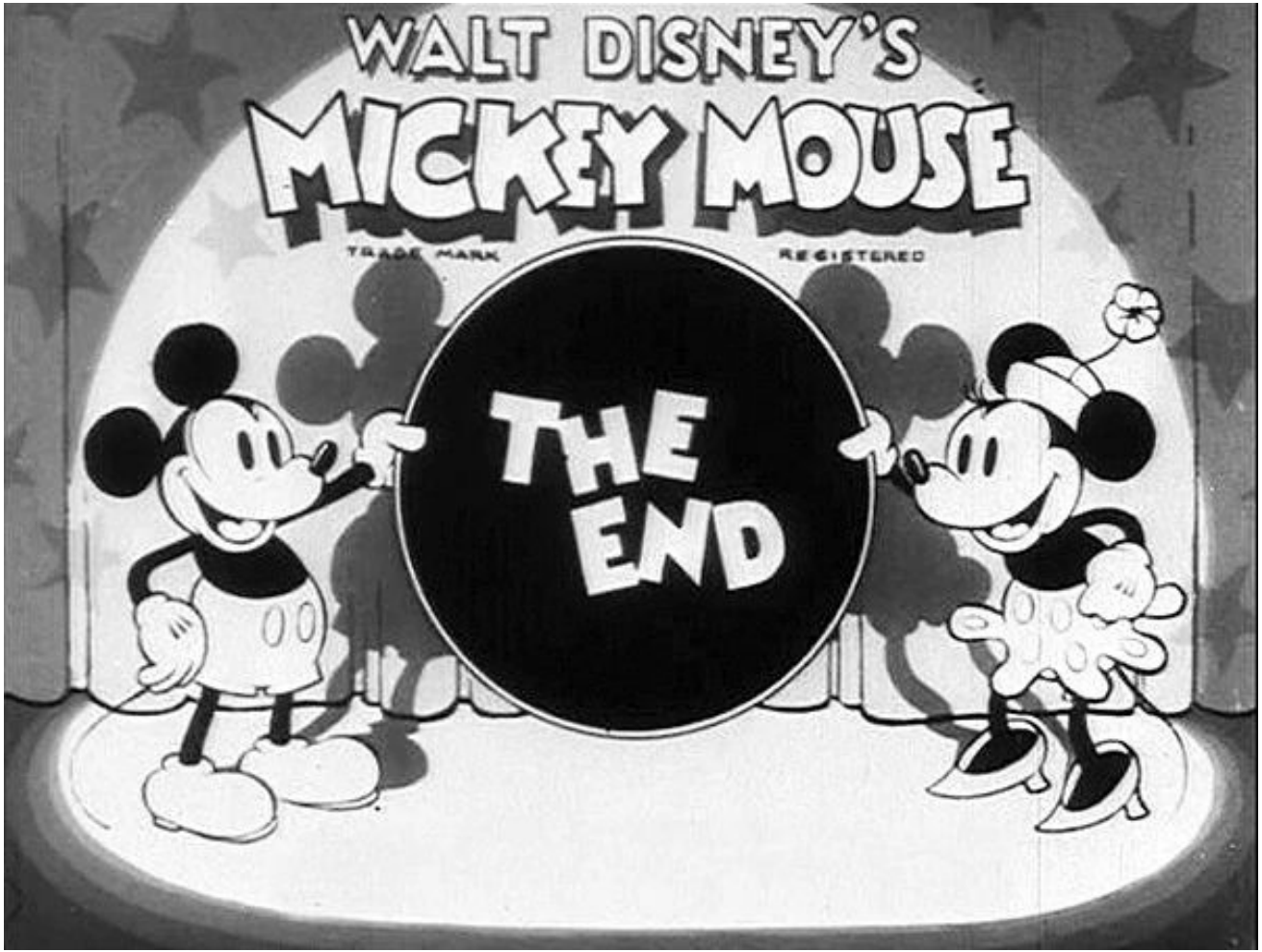
Assembly Assessment



	[REF]	[QRY]
[Sequences]		
TotalSeqs	1	50
AlignedSeqs	1(100.00%)	50(100.00%)
UnalignedSeqs	0(0.00%)	0(0.00%)
[Bases]		
TotalBases	2347083	2004872
AlignedBases	1975841(84.18%)	1901458(94.84%)
UnalignedBases	371242(15.82%)	103414(5.16%)

N50	48,555 bp
L50	14 contigs

Mucispirillum schaedleri ASF457


WALT DISNEY'S
MICKEY MOUSE
TRADE MARK
REGISTERED
THE END