Assembly of Foc isolates from TNAU

By Jamie Pike

Contents

Data download and QC checks

Isolate raw data supplied on 24/2/23, by Dr Raveendran Muthurajan and downloaded directly from ONEMOMICS via sftp.

> Dear Sir,
>
> Greetings from ONEOMICS PRIVATE LIMITED!
>
> The raw data for whole genome sequencing (>5GB per sample) of three fungal samples are available for download from our FTP server. Also, we have attached the Data QC report for your reference.
>
> Please use the below credentials to access the data:
>
> Host : sftp://117.247.108.171
> Username : Dr_RVN
> Password : pRlJAFrl
> Path: /home/Dr_RVN/OPL_WGS_3_172/Rawdata
>
> The following files are available for download:
>
> 1. Rawdata

Isolates sequenced using ONEOMICS using Illumina 1.9 PE (PLATFORM?)

Following download, I assessed the quality of each isolate using FastQC, although a QC html is provided by ONEOMCIS and attached to email from Dr Raveendran Muthurajan.

FastQC checks

Performed using FastQC (version 0.11.8).

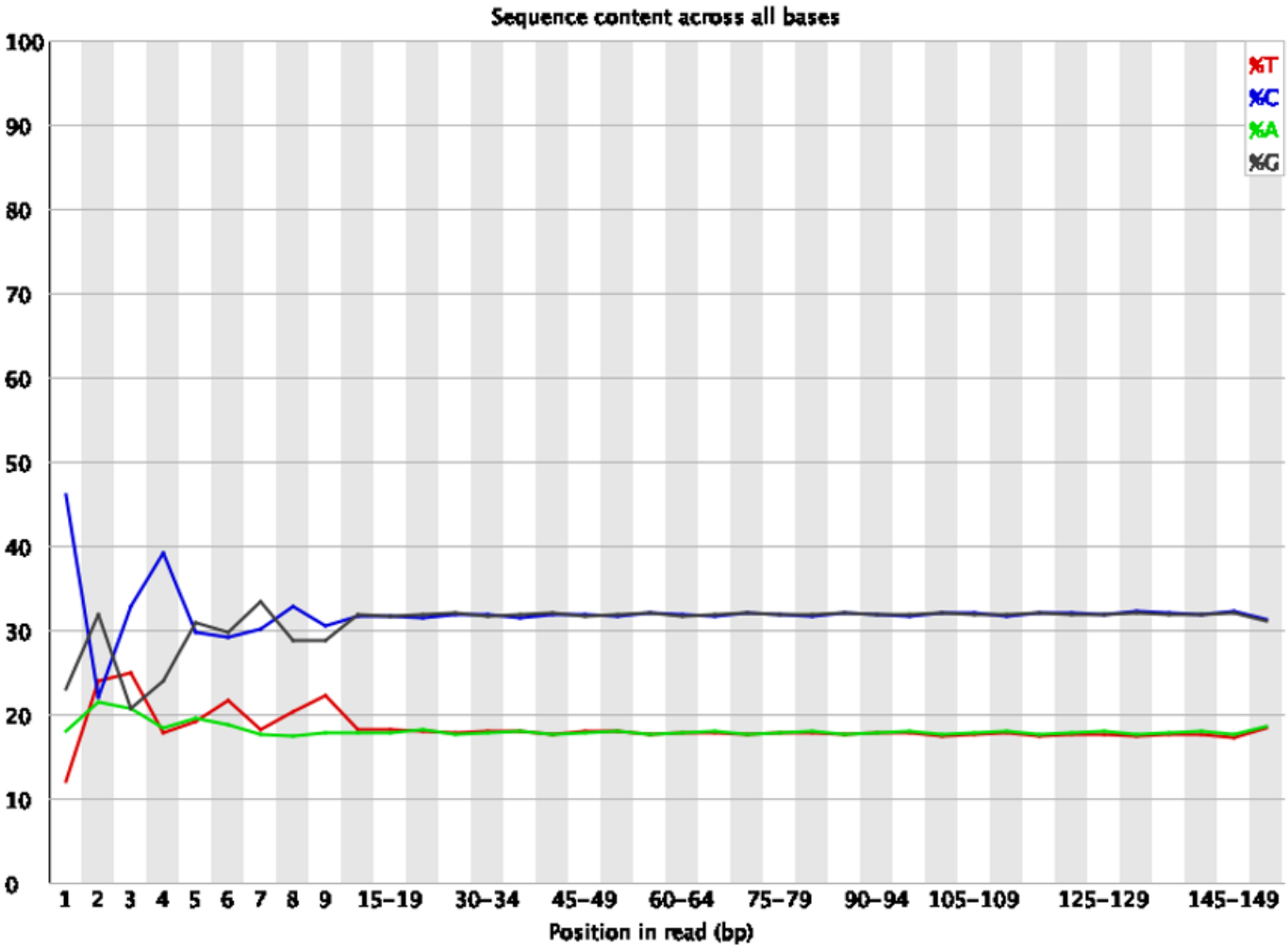
FASTQ: S6-1.fq.gz

GRADE	TEST
PASS	Basic Statistics
PASS	Per base sequence quality
PASS	Per tile sequence quality
PASS	Per sequence quality scores
FAIL	Per base sequence content

GRADE	TEST
FAIL	Per sequence GC content
PASS	Per base N content
PASS	Sequence Length Distribution
FAIL	Sequence Duplication Levels
PASS	Overrepresented sequences
PASS	Adapter Content

S6-1.fq.gz failed for Per base sequence content, Per sequence GC content and Sequence duplication levels.

Per base sequence content:



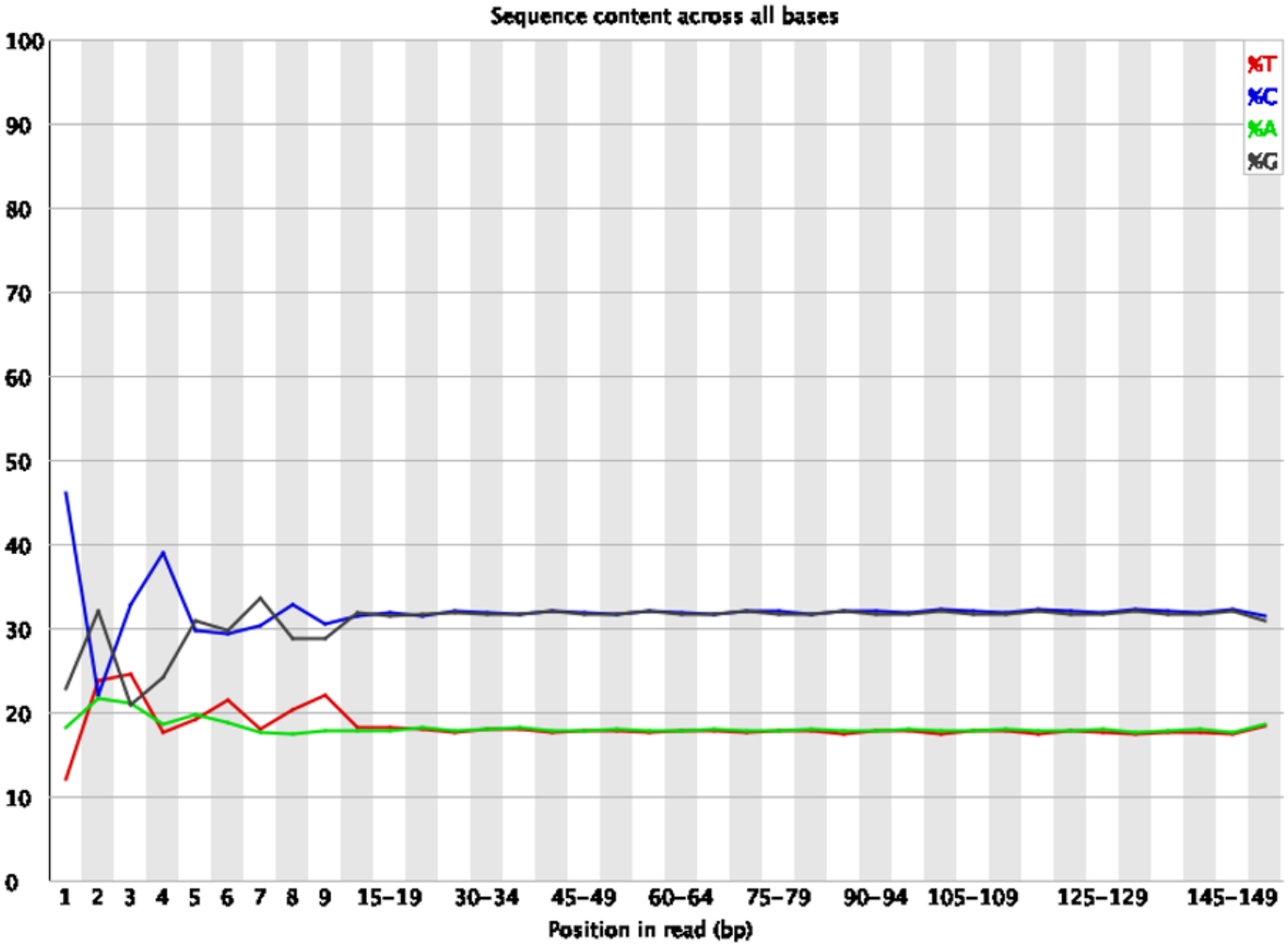
S6-1.fq has a biased sequence in the first ~12 bases. This is commonly observed in RNAseq analysis, as explained here.

FASTQ: S6-2.fq.gz

GRADE	TEST
PASS	Basic Statistics
PASS	Per base sequence quality
PASS	Per tile sequence quality
PASS	Per sequence quality scores
FAIL	Per base sequence content

GRADE	TEST
FAIL	Per sequence GC content
PASS	Per base N content
PASS	Sequence Length Distribution
WARN	Sequence Duplication Levels
PASS	Overrepresented sequences
PASS	Adapter Content

Per base sequence content:



Similarly, S6-2.fq failed for Per base sequence content, Per sequence GC content and Sequence duplication levels and has a biased sequence in the first ~12 bases.

The same was observed for S16 and S32. See the tables below and the HTML outputs:

`/Users/u1983390/Library/CloudStorage/OneDrive-UniversityofWarwick/Research/Genomics/NewToolsProject/Indian-Foc-Genome/Indian Foc Genomes-Feb23/QC-Analysis`

FASTQ: S16-1.fq.gz

GRADE	TEST
PASS	Basic Statistics
PASS	Per base sequence quality

GRADE	TEST
PASS	Per tile sequence quality
PASS	Per sequence quality scores
WARN	Per base sequence content
FAIL	Per sequence GC content
PASS	Per base N content
PASS	Sequence Length Distribution
PASS	Sequence Duplication Levels
PASS	Overrepresented sequences
PASS	Adapter Content

FASTQ: S16-2.fq.gz

GRADE	TEST
PASS	Basic Statistics
PASS	Per base sequence quality
PASS	Per tile sequence quality
PASS	Per sequence quality scores
WARN	Per base sequence content
FAIL	Per sequence GC content
PASS	Per base N content
PASS	Sequence Length Distribution
PASS	Sequence Duplication Levels
PASS	Overrepresented sequences
PASS	Adapter Content

FASTQ: S32-1.fq.gz

GRADE	TEST
PASS	Basic Statistics
PASS	Per base sequence quality
PASS	Per tile sequence quality
PASS	Per sequence quality scores
WARN	Per base sequence content
FAIL	Per sequence GC content
PASS	Per base N content
PASS	Sequence Length Distribution
FAIL	Sequence Duplication Levels
PASS	Overrepresented sequences
PASS	Adapter Content

FASTQ: S32-2.fq.gz

GRADE	TEST
PASS	Basic Statistics
PASS	Per base sequence quality
WARN	Per tile sequence quality
PASS	Per sequence quality scores
WARN	Per base sequence content
FAIL	Per sequence GC content
PASS	Per base N content
PASS	Sequence Length Distribution
FAIL	Sequence Duplication Levels
PASS	Overrepresented sequences
PASS	Adapter Content

After speaking to Dr Laura Baxter about the Per base sequence content, she advised not trimming and continuing with assembly. Additionally, she warned me to be careful of the Per sequence GC content, as most do not follow a completely normal distribution and have a very narrow peak in the centre.

From: Baxter, Laura <[Laura.Baxter@warwick.ac.uk>(mailto:Laura.Baxter@warwick.ac.uk)> **Date:** Friday, 24 February 2023 at 15:31 **To:** PIKE, JAMIE (PGR) <Jamie.Pike@warwick.ac.uk> **Subject:** Re: FastQC Per base sequence content advice

Hi Jamie,

I agree with what you've found - I often see this not-so-random sequence bias at the start of RNA seq data and don't consider it a problem. It's less common in WGS data but I think essentially it will be the same/similar issue, probably down to the library preparation steps. It would be interesting/useful to know what method/kit they used.

I wouldn't trim, as they aren't sequencing errors as such, and if you hard trim the first 10 bases you'll lose ~7% your data.

The per sequence GC content is a bit odd, in that it has a very narrow peak centred around 67% (however, this might be normal for your genome's GC content), but also there's a small 'shoulder' around 50% (again, this might be a biological feature of your genome, if there's say one chromosome with a very different GC content to the rest, or it might be an indicator of reads from different contaminant species being present).

I say proceed with the untrimmed data as the quality is high and there's no adapter etc, keeping an eye out for any contaminants.

Is this fusarium data, and are you doing a de novo assembly, or reference-guided?

Cheers,

Laura

I continued without trimming, as recommended, and aligned the isolates to the reference quality *Foc* TR4 assembly from David Studholme's group. I will use the % mapping and blast searching of unmapped reads to gauge the level of contamination and subsequent assembly approach.

Bowtie2 Mapping of raw reads

In the `Bowtie2Mapping` directory:

Vettel: `/home/u1983390/Projects/NewTools-Proj/IndianGenomeAssemblies/Bowtie2Mapping`

Bowtie2 version: 2.4.5

An index directory was created for the *Foc* TR4 reference assembly GCA-007994515.1-UK0001: `UK0001-Index.`

And then the reference assembly was indexed for Bowtie2 using the following command in the conda AlignEnv:

```
bowtie2-build F._oxysporum_f._sp._cubense_UK0001.fna UK0001_bowtie2
```

A directory was created for each isolate within the Bowtie2Mapping directory for the SAM output. The following command was then run for each isolate in the corresponding directory:

```
nohup ../Bowtie2Command.sh [Path/to/fastq_1] [Path/to/fastq_2] 1>Bowtie_S[X].log &
```

The `Bowtie2Command.sh` file contains:

```
#!/bin/bash

One=${1} #Where One is the first fastq.

Two=${2} #Where Two is the second fastq.

python -c "print('=' * 75)"

echo "Bowtie2 Mapping"

echo "---------------"

echo $(date)

python -c "print('=' * 75)"

#Perform Bowtie2 Mapping using UK0001 TR4 as the reference.

bowtie2 --local -x /home/u1983390/Projects/NewTools-
Proj/IndianGenomeAssemblies/Bowtie2Mapping/UK0001_Index/UK0001_bowtie2 -1 $One -2 $Two -S
./S16_MappedTo_UK0001.sam

python -c "print('=' * 75)"

#Send email notification of job completion.

SendEmail.py "Bowtie2 Complete" "Bowtie2 Mapping complete. Check for results."

python -c "print('=' * 75)"
```

The Results of BOWTIE2 mapping of raw reads:

S6

Command:

```
nohup ../Bowtie2Command.sh /home/u1983390/Projects/NewTools-
Proj/IndianGenomeAssemblies/rawdata/S6/S6_1.fq.gz /home/u1983390/Projects/NewTools-
Proj/IndianGenomeAssemblies/rawdata/S6/S6_2.fq.gz 1>Bowtie2_S6.log &
```

Bowtie2_S6.log:

```
===========================================================================

Bowtie2 Mapping

---------------
```

```
Wed 1 Mar 16:43:38 GMT 2023

================================================================

26213258 reads; of these:

26213258 (100.00%) were paired; of these:

24042619 (91.72%) aligned concordantly 0 times

1935086 (7.38%) aligned concordantly exactly 1 time

235553 (0.90%) aligned concordantly >1 times

----

24042619 pairs aligned concordantly 0 times; of these:

52956 (0.22%) aligned discordantly 1 time

----

23989663 pairs aligned 0 times concordantly or discordantly; of these:

47979326 mates make up the pairs; of these:

47857196 (99.75%) aligned 0 times

37425 (0.08%) aligned exactly 1 time

84705 (0.18%) aligned >1 times

8.72% overall alignment rate

================================================================

Sending job status email...

================================================================
```

S16

Command:

```
nohup ../Bowtie2Command.sh /home/u1983390/Projects/NewTools-
Proj/IndianGenomeAssemblies/rawdata/S16/S16_1.fq.gz /home/u1983390/Projects/NewTools-
Proj/IndianGenomeAssemblies/rawdata/S16/S16_2.fq.gz 1>Bowtie_S16.log &
```

Bowtie2_S16.log:

```
================================================================

Bowtie2 Mapping

---------------

Wed 1 Mar 09:36:59 GMT 2023

================================================================

22473220 reads; of these:
```

```
22473220 (100.00%) were paired; of these:

11044640 (49.15%) aligned concordantly 0 times

10676063 (47.51%) aligned concordantly exactly 1 time

752517 (3.35%) aligned concordantly >1 times

----

11044640 pairs aligned concordantly 0 times; of these:

272737 (2.47%) aligned discordantly 1 time

----

10771903 pairs aligned 0 times concordantly or discordantly; of these:

21543806 mates make up the pairs; of these:

20761361 (96.37%) aligned 0 times

552679 (2.57%) aligned exactly 1 time

229766 (1.07%) aligned >1 times

53.81% overall alignment rate

=================================================================

Sending job status email...

=================================================================
```

S32

Command:

```
nohup ../Bowtie2Command.sh /home/u1983390/Projects/NewTools-
Proj/IndianGenomeAssemblies/rawdata/S32/S32_1.fq.gz /home/u1983390/Projects/NewTools-
Proj/IndianGenomeAssemblies/rawdata/S32/S32_2.fq.gz 1>Bowtie_S32.log &
```

Bowtie2_S32.log:

```
=================================================================

Bowtie2 Mapping

---------------

Wed 1 Mar 14:04:56 GMT 2023

=================================================================

34526189 reads; of these:

34526189 (100.00%) were paired; of these:

29377154 (85.09%) aligned concordantly 0 times

4873959 (14.12%) aligned concordantly exactly 1 time

275076 (0.80%) aligned concordantly >1 times
```

```
----

29377154 pairs aligned concordantly 0 times; of these:

124174 (0.42%) aligned discordantly 1 time

----

29252980 pairs aligned 0 times concordantly or discordantly; of these:

58505960 mates make up the pairs; of these:

58215294 (99.50%) aligned 0 times

170055 (0.29%) aligned exactly 1 time

120611 (0.21%) aligned >1 times

15.69% overall alignment rate

=================================================================

Sending job status email...

=================================================================
```

Searching Unmapped Reads

SAM files were downloaded onto my local machine and converted from SAM to BAM. Unmapped reads were then extracted and searched using the BLAST web server.

Local Path:

```
/Volumes/Jamie_EXT/Research/Genomics/NewToolsProject/FocGenomes/Indian_Genomes/Bowtie2Mapping/S*
```

Command example:

```
samtools view -S -b S16_MappedTo_UK0001.sam > S16_MappedTo_UK0001.bam

samtools fasta -f 4 S16_MappedTo_UK0001.bam > S16_MappedTo_UK0001_UnmappedReads.fasta

#The fasta file was chuncked using the following command for BLAST searching.

awk 'BEGIN {n=0;} /^>/ {if(n%500==0){file=sprintf("S6_UNmappedReads_UK0001-chunk%d.fa",n);}
print >> file; n++; next;} { print >> file; }' < ../S16_MappedTo_UK0001_UnmappedReads.fasta
```

For isolates S6 and S32, the majority of hits were for *Stenotrophomonas* species, particularly *Stenotrophomonas maltophilia*. A reference assembly for *Stenotrophomonas maltophilia* was therefore downloaded from GenBank.

Map to *Stenotrophomonas maltophilia*

Reference Assembly: GCA_900475405.1

The reference assembly was indexed for Bowtie2 mapping in the following Vettle directory:

```
/home/u1983390/Projects/NewTools-
Proj/IndianGenomeAssemblies/Bowtie2Mapping/S.maltophilia_44087_C01_Index
```

```
bowtie2-build Stenotrophomonas_maltophilia_44087_C01_reference.fna S_maltophilia__ref-bowtie2
```

S6, S16 and S32 were then mapped to the *Stenotrophomonas maltophilia* reference using the Bowtie2Command.sh file:

```
#!/bin/bash

One=${1} #Where One is the first fastq.

Two=${2} #Where two is the second fastq.

python -c "print('=' * 75)"

echo "Bowtie2 Mapping"

echo "---------------"

echo $(date)

python -c "print('=' * 75)"

#Perform Bowtie2 Mapping using S. maltophilia as the reference.

bowtie2 --local -x /home/u1983390/Projects/NewTools-
Proj/IndianGenomeAssemblies/Bowtie2Mapping/S.maltophilia_44087_C01_Index/S_maltophilia__ref-
bowtie2 -1 $One -2 $Two -S ./S6_MappedTo_S_maltophilia__ref.sam

python -c "print('=' * 75)"

#Send email notifciation of job completion.

SendEmail.py "Bowtie2 Complete" "Bowtie2 Mapping complete. Check for results."

python -c "print('=' * 75)"

S6, S16 and S32 were then mapped to the _Fusarium sacchari_ FS66 reference using the
Bowtie2Command.sh file:

#!/bin/bash

One=${1} #Where One is the first fastq.

Two=${2} #Where two is the second fastq.

python -c "print('=' * 75)"

echo "Bowtie2 Mapping"

echo "---------------"

echo $(date)

python -c "print('=' * 75)"

#Perform Bowtie2 Mapping using S. maltophilia as the reference.

bowtie2 --local -x /home/u1983390/Projects/NewTools-
Proj/IndianGenomeAssemblies/Bowtie2Mapping/F._fujikuori_FS66_Index/F.fujikuroi-bowtie2 -1 $One
-2 $Two | samtools view -Shu | samtools sort -o S32_mappedto_FS66.bam

python -c "print('=' * 75)"

#Send email notifciation of job completion.

SendEmail.py "Bowtie2 Complete" "Bowtie2 Mapping complete. Check for results."
```

```
python -c "print('=' * 75)"
```

The following mapping rates are recorded in the corresponding log files.

Species	S6 Bowtie2 AlignmentRate	S16 Bowtie2 AlignmentRate	S32 Bowtie2 AlignmentRate	Strain	GenBank Accession	No. of Contigs	Contig N50 (Mb)
F. oxysporum f. sp. cubense (TR4)	8.72%	53.81%	15.69%	UK0001	GCA_007994515.1	15	4.49
F. sacchari	5.24%	68.65%	22.49%	FS66	GCA_017165645.1	48	1.97
Stenotrophomonas maltophilia	49.32%	0.01%	53.93%	NCTC10258	GCA_900475405.1	1	4.5

As S6 had a 49.32% mapping rate to the *Stenotrophomonas maltophilia* reference, unmapped reads were extracted into a fasta file and searched using web BLAST against the NCBI database using the same approach as pg 9 (original BLAST search of unmapped reads).

`/Volumes/Jamie_EXT/Research/Genomics/NewToolsProject/FocGenomes/Indian_Genomes/Bowtie2Mapping/S6/S6_UNmappedReads_S_maltophilia-ChunkedFastas/`

Hits are mixed, with some for *Fusarium* species, and others for *Stenotrophomonas* species and *Achromobacter* species.

The S16 reads which did not map to *FS66* were extracted and searched against the NCBI database using web BLAST. A lot of the searches had no significant similarity found, which is curious – many of the sequences which did have hits were for *Fusarium* species. I will therefore generate a *de novo* assembly for S16 and will then extract the TEF1-a region as well as perform a Blobltools analysis.

De novo assemblies

Isolate S16

SPAdes was used to generate the S16 *de novo* assembly, as it was for the SY-2 assembly prepared in 2022. The SPAdes assembly was generated in the following directory:

`/home/u1983390/Projects/NewTools-Proj/IndianGenomeAssemblies/S16`

Using the following command:

```
nohup ./SPAdesAssembly-S16.sh 1>S16_SpadesAssembly.log &
```

Where `SPAdesAssembly-S16.sh` contains:

```
#!/bin/bash

#Command used to generate the S16 SPAdes Assembly

python -c "print('=' * 75)"

echo "S16 Spades Assembly"

echo "--------------------"

echo $(date)

python -c "print('=' * 75)"

#Run the Spades command.

spades.py -1 /home/u1983390/Projects/NewTools-
```

```
Proj/IndianGenomeAssemblies/rawdata/S16/S16_1.fq.gz -2 /home/u1983390/Projects/NewTools-
Proj/IndianGenomeAssemblies/rawdata/S16/S16_2.fq.gz --careful -isolate --cov-cutoff auto -o
/home/u1983390/Projects/NewTools-Proj/IndianGenomeAssemblies/S16/

python -c "print('=' * 75)"

#Send an email notification of job completion.

SendEmail.py "S16 SPAdes Assembly Complete" "Spades assembly for S16 is now complete. Please
check: /home/u1983390/Projects/NewTools-Proj/IndianGenomeAssemblies/S16/ for the results."

python -c "print('=' * 75)"
```

Quality of the S16 assembly was assessed using the following commands:

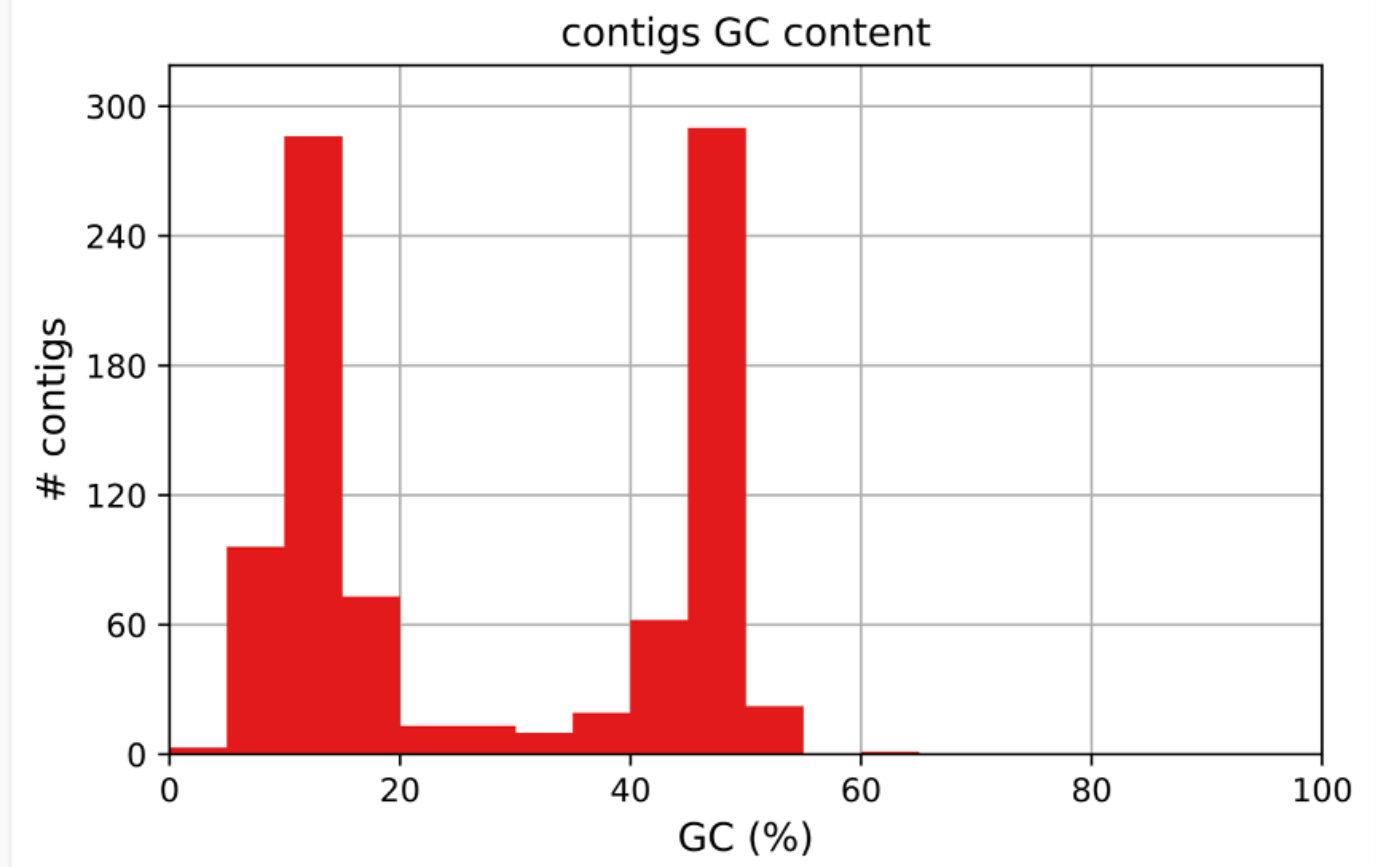
```
nohup ../AssemblyQualityCheck.sh contigs.fasta 1>AssemblyQualityCheck.log &
```

Output can be found in the following directories:

`/home/u1983390/Projects/NewTools-Proj/IndianGenomeAssemblies/S16/QuastResult`

Like the SY-2 assembly, there is not a normal distribution of GC content contigs. Overall GC content is 46.28%. Some contigs have a low GC content. Therefore, alike the SY-2 isolate, low GC contigs were removed using the `gcTrimmer.py` script with a 25% GC threshold.

BUSCO is 97%.



After running `gcTrimmer.py`, the gc trimmed fasta was assessed using Quast and BUSCO.

`gcTrimmer.py` command:

```
gcTrimmer.py contigs.fasta 25
```

Assembly Quality Check:

```
nohup ../AssemblyQualityCheck.sh contigs.fasta_GCcontentAbove25perc.fasta
1>AssemblyQualityCheck_GCTrimmed-25perc.log &
```

In:

/home/u1983390/Projects/NewTools-Proj/IndianGenomeAssemblies/S16

The GC trimmed assembly has reduced the number of contigs from 1666 to 768. BUSCO remains at 97.5% and the low GC hip has been removed from the GC% plot. Further, GC% has increased to 47.53.

Next, the raw reads were aligned to the SPAdes GC-Trimmed assembly using Bowtie2.

First, a Bowtie2 index was generated for the SPAdes GC-Trimmed assembly:

```
bowtie2-build S16_GC-Trimmed.fasta S16_GC-Trimmed.bowtie2
```

Then the following script and commands used to run Bowtie2:

```
nohup ./Bowtie2Command.sh /home/u1983390/Projects/NewTools-
Proj/IndianGenomeAssemblies/rawdata/S16/S16_1.fq.gz /home/u1983390/Projects/NewTools-
Proj/IndianGenomeAssemblies/rawdata/S16/S16_2.fq.gz 1>Bowtie2_S16RawReads_MappedTo_S16GC-
TrimmedAssemb.log &
```

```
#!/bin/bash

One=${1} #Where One is the first fastq.

Two=${2} #Where two is the second fastq.

python -c "print('=' * 75)"

echo "Bowtie2 Mapping"

echo "---------------"

echo $(date)

python -c "print('=' * 75)"

#Perform Bowtie2 Mapping using S. maltophilia as the reference.

bowtie2 --local -x /home/u1983390/Projects/NewTools-
Proj/IndianGenomeAssemblies/S16/Mapping/Bowtie2Index/S16_GC-Trimmed.bowtie2 -1 $One -2 $Two |
samtools view -Shu | samtools sort -o S16RawReads_Mappedto_S16GC-TrimmedAssemb.bam

python -c "print('=' * 75)"

#Send email notifciation of job completion.

SendEmail.py "Bowtie2 Complete" "Bowtie2 Mapping complete. Check for results."

python -c "print('=' * 75)"
```

I then ran Qualimap bamQC to determine coverage etc using the following commands and scripts in the directory below:

/home/u1983390/Projects/NewTools-Proj/IndianGenomeAssemblies/S16/Mapping/BamQC

Mean Coverage is 148.8038x - other data can be found:

`/Users/jamiepike/Library/CloudStorage/OneDrive-UniversityofWarwick/Research/Genomics/NewToolsProject/Indian_Foc_Genome/Indian Foc Genomes_Feb23/Assemblies/S16/Mapping`

BlobTools Analysis

Directory:

`/home/u1983390/Projects/NewTools-Proj/IndianGenomeAssemblies/S16/Blobtools`

The BlobTools analysis was conducted on the *de novo* S16 assembly to look for contamination, as was performed for the SY-2 isolate.

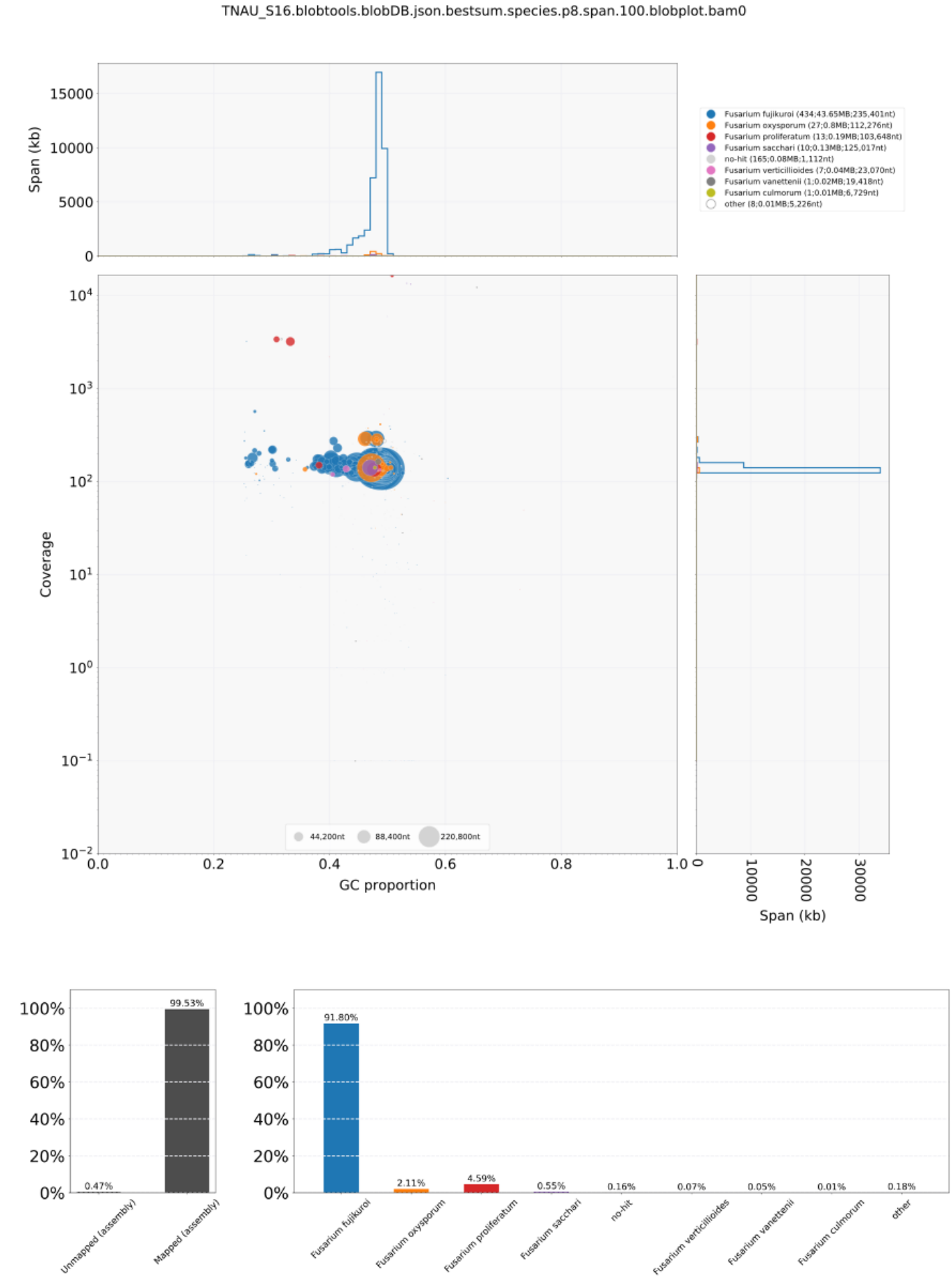
The BAM input required for BlobTools (raw S16 reads mapped back to assembly) has already been generated, as has the S16 *de novo* assembly. The preliminary BLAST search required for BlobTools was then conducted using the recommended BLAST search parameters from BlobTools and the following command:

```
nohup ./BlobtoolsCommand.sh /home/u1983390/Projects/NewTools-Proj/IndianGenomeAssemblies/S16/S16_GC-Trimmed.fasta /home/u1983390/Projects/NewTools-Proj/IndianGenomeAssemblies/S16/Mapping/S16RawReads_Mappedto_S16GC-TrimmedAssemb.bam TNAU_S16 1>BlobtoolsS16.log &
```

Script:

```
#!/bin/bash

Assemb=${1?Please provide the assembly input fasta.} #Input Assembly.

Bam=${2?Please provide a bam file of raw reads mapped back to the assembly} #Input BAM.

prefix=${3?Please provide a prefix for you outputs.}

python -c "print('=' * 75)"

echo "Blobtools BLAST"

echo "---------------"

echo $(date)

python -c "print('=' * 75)"

echo "Blasting..."

#Perform BLASTN for Blobtools.

blastn -task megablast -query ${Assemb} -db /shared/reference/NCBI_NT/BLASTDB/nt -outfmt '6 qseqid staxids bitscore std' -max_target_seqs 1 -max_hsps 1 -num_threads 16 -evalue 1e-25 -out ${prefix}.vs.nt.mts1.hsp1.1e25.megablast.out

python -c "print('=' * 75)"

echo "Running Blobtools..."

#Perform the Blobtools Analysis

/home/u1983390/apps/blobtools/blobtools create -i ${Assemb} -b ${Bam} -t ${prefix}.vs.nt.mts1.hsp1.1e25.megablast.out -o ${prefix}.blobtools

echo "Generating Blobtools figures..."
```

```
#To view results.

/home/u1983390/apps/blobtools/blobtools view -i ${prefix}.blobtools.blobDB.json -r species

/home/u1983390/apps/blobtools/blobtools view -i ${prefix}.blobtools.blobDB.json -r phylum

#To generate graphs.

/home/u1983390/apps/blobtools/blobtools plot -i ${prefix}.blobtools.blobDB.json -r species

/home/u1983390/apps/blobtools/blobtools plot -i ${prefix}.blobtools.blobDB.json -r phylum

#To generate PDF versions.

/home/u1983390/apps/blobtools/blobtools plot -i ${prefix}.blobtools.blobDB.json -r species --
format pdf

/home/u1983390/apps/blobtools/blobtools plot -i ${prefix}.blobtools.blobDB.json -r phylum --
format pdf

python -c "print('=' * 75)"

#Send email notifciation of job completion.

SendEmail.py "Blobtools Complete" "Blobtools analysis now complete. Check for results."

echo $(date)

python -c "print('=' * 75)"
```



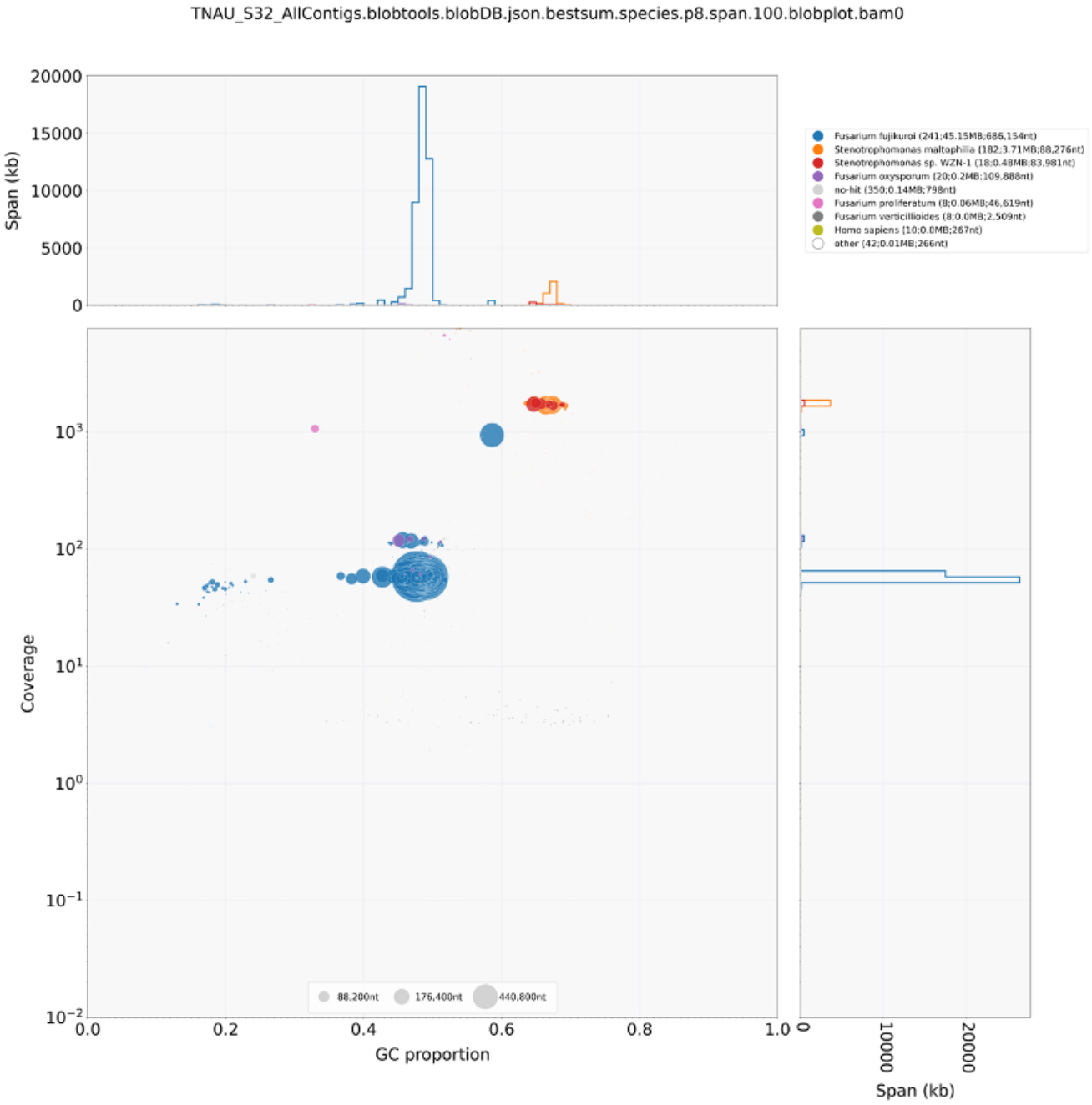
As all the contigs are from *Fusarium* using BlobTools, I am wondering if the >25% GC removed contigs are from any contaminants or other phylum_._ I therefore ran BlobTools on the Untrimmed SPAdes assembly.

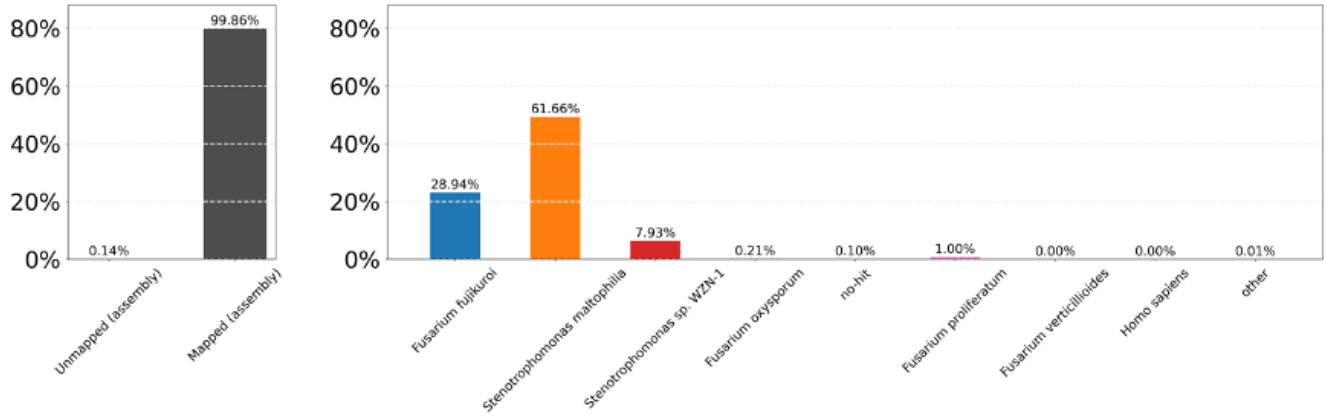
The output for the second BlobTools search using all contigs matches the first BlobTools. Most of the sequences are from *F. fujikuroi*. Interestingly, only 0.55% of the reads are from *F. saccahri*. I will conduct a *TEF-1a* and *RPBII* phylogeny and Fusariod DB search to try and work out what species this may be. I think the high *F. fujikuroi* hits is because of bias in the BLAST nt DB om Vettel.

For the S32 and S6 assemblies

I am going to try to create a *de novo* assembly for S32 using the same SPAdes command and approach to see what the BlobTools results are. If I get a lot of hits for the higher GC contigs looking like the bacterial species, I can try to filter those out using the `gcTrimmer.py` script.

The BlobTools results indicate a fair amount of contamination, but the majority of assembled hits are for *F. fujikori* – as 20% of the reads mapped to FS66, I am going to extract these into a fastq and create a *de novo* SPAdes assembly from that. I'll then search and extract the *TEF1-a* and *RBPII* regions to see if it looks like *F. saccahri*.





This whole process (*de novo* assembly from all reads, blobtools, *de novo* assembly from *F. saccahari* mapped reads) will also be performed for S6.