

Linear Amplicon Sequencing Analysis Report

Research Use Only (RUO)
25 January, 2024

Eurofins Order ID: **12345**
 Sample Name: **sample1**
 Amplicon Length: **10 Kbp**
 Sample Received on: **15 January, 2024**
 Sample Analyzed on: **25 January, 2024**
 Technology Used: **Oxford Nanopore Technology (ONT) Sequencing**
 Pipeline: **Amplicon Analysis Pipeline v1.0**
 Report Version: **v1.0**

Eurofins proprietary Nanopore data analysis pipeline is used to prepare and sequence samples with Oxford Nanopore Technologies sequencers, which utilize a third-generation sequencing technology capable of real-time long-read sequencing of DNA. The technology involves feeding a single-stranded DNA molecule through a protein nanopore and measuring changes in electrical current as the DNA passes through. The resulting reads are then subjected to quality filtering, assembly, and annotation using the Nanopore data analysis pipeline developed by Eurofins.

Results

Amplicon Assembly

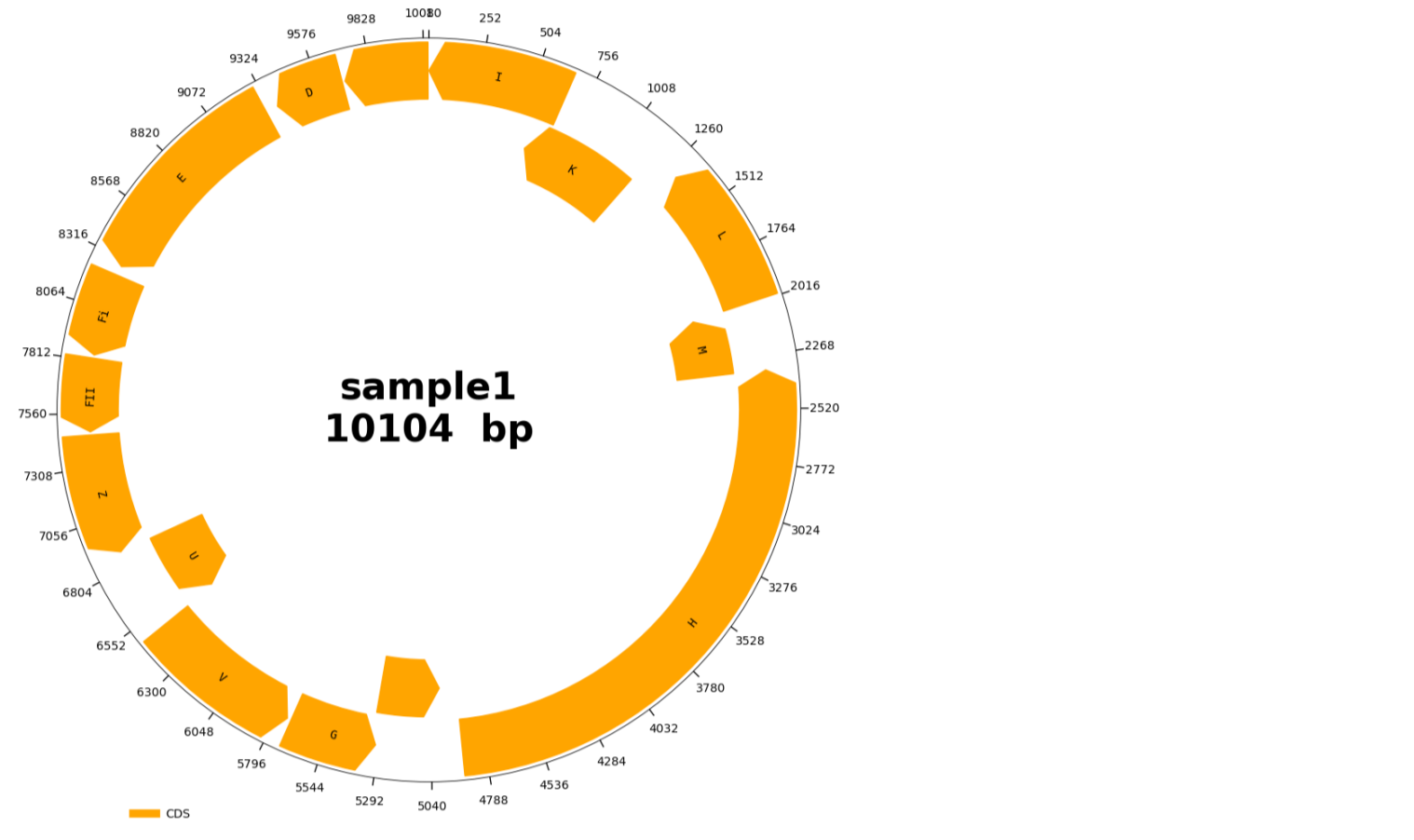
The assembly statistics of the assembled amplicon is shown in the following table -

SAMPLE NAME	AMPLICON LENGTH	GC %	TOTAL READS	COVERAGE DEPTH
sample1	10104	57.59	1645	377

- SAMPLE NAME column represents the name of sample processed.
- AMPLICON LENGTH column represents the size of the assembled amplicon in basepairs (bp).
- GC % column represents percentage GC content of the assembled amplicon.
- TOTAL READS column represents the number of reads in amplicon assembly.
- COVERAGE DEPTH column represents the average coverage depth of the assembled amplicon.

Amplicon Map

The assembled amplicon is represented graphically showing the locations and types of genetic features such as genes, promoters, restriction enzyme sites, and other functional elements as a amplicon map. Typically, a circular amplicon map is shown with the DNA sequence depicted as a circle, with the start and end points of the sequence joined together. amplicon maps may also include labels indicating the names or functions of the genetic features and their relative positions on the amplicon.



Amplicon Features Table

The features annotated and displayed in the amplicon map are shown in the table below. The full annotations, including nucleotide sequence and descriptions of each feature are found in the deliverables.

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Show entries Search:

Feature	Type	Start Location	End Location	Percent Identity
H	CDS	2336	4895	100
E	CDS	8279	9302	100
V	CDS	5744	6482	100
L	CDS	1312	2008	100
G	CDS	5306	5726	100
Fi	CDS	7839	8235	100
U	CDS	6492	6885	100
D	CDS	9360	9690	100
K	CDS	563	1160	100
Z	CDS	6884	7460	100

Showing 1 to 10 of 15 entries Previous 2 Next

Amplicon Clonal Purity Check

To check the assembled amplicon purity, the sequenced reads are mapped against the assembled amplicon and the variants (SNPs, insertions & deletions) are determined. Variants detected with at least 0.3 minor allele frequency(MAF) and >30x read support are shown below

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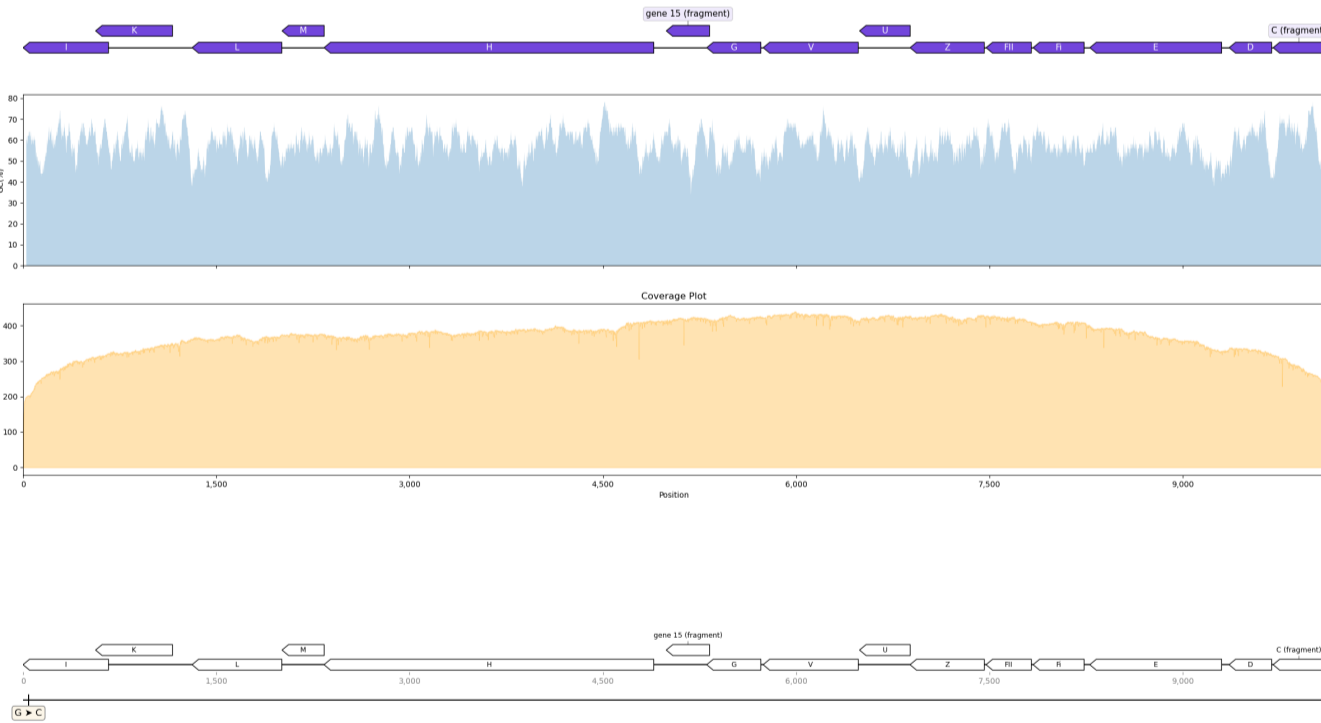
Show entries Search:

Sample	Position	Main Allele	Minor Allele	MAF	Total Depth
sample1	43	G	C	0.3	203

Showing 1 to 1 of 1 entries Previous Next

Amplicon Coverage Plot

The coverage and GC content of the assembled amplicon is shown below. If variants are observed, they are also plotted below along with the annotated features.



Deliverables

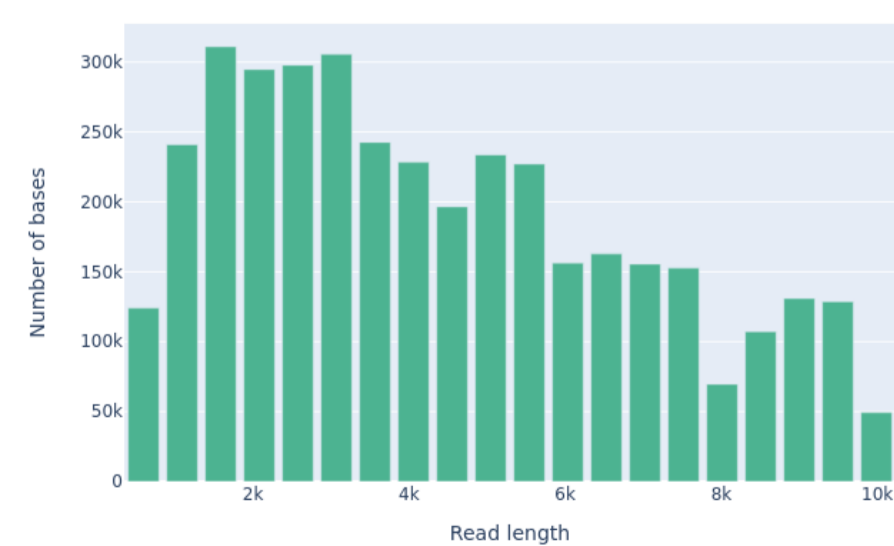
The **ORDERID.SAMPLE.amplicon_analysis.zip** archive contains the following files:

- SAMPLE.amplicon_analysis_report.html**: This is the analysis report.
 - SAMPLE.amplicon_assembly.fasta**: Assembled amplicon FASTA sequence.
 - SAMPLE.amplicon_assembly.fastq**: Assembled amplicon FASTQ sequence.
 - SAMPLE.amplicon_assembly_per_base_qualities.csv**: Per base Quality with confidence score.
 - SAMPLE.amplicon_annotations.gbk**: Annotated amplicon sequence in GENBANK* format.
 - SAMPLE.amplicon_annotations.csv**: Annotated feature table, including the nucleotide sequence of each feature.
- *Use this file to import the amplicon sequence and features in software like SnapGene, Geneious Prime, Benchling, SeqBuilder Pro etc.)

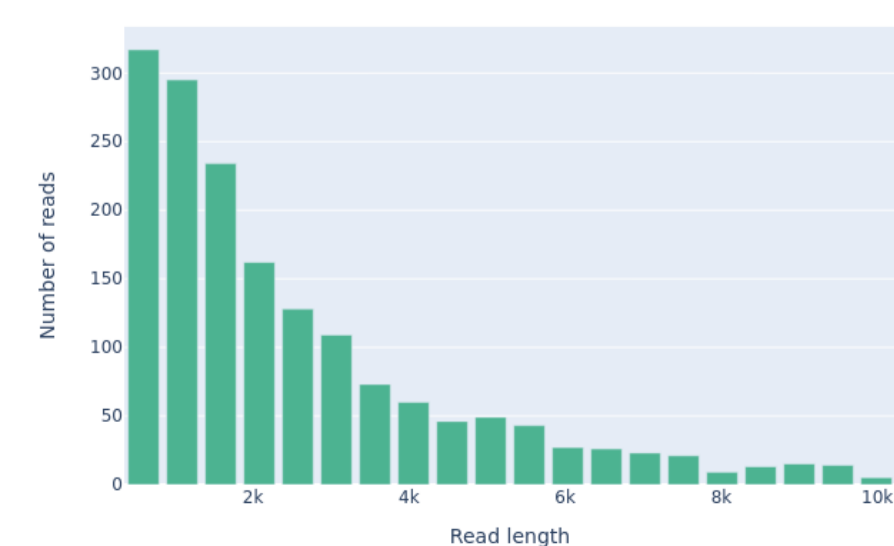
Sequencing Reads QC

Distribution of read lengths from sequenced data is shown in the following histogram. The histogram displays the number of sequenced bases (bp) on the y-axis and the read length on the x-axis. Each bar in the histogram represents a range of read lengths, and the height of the bar indicates the total number of bases (bp) falling within that range. This results in a weighted plot by the number of nucleotides per bin, as longer reads carry more weight in the histogram. Read length histograms can be used to assess the quality of sequencing data, as the distribution of read lengths can indicate the presence of contaminants or biases in the sequencing process. They can also be used to determine the size of the amplicon being sequenced.

Weighted histogram of read lengths



Non weighted histogram of read lengths



REMARKS

Amplicon sequencing using nanopore technology has some limitations. One limitation is the relatively high error rate associated with long-read nanopore sequencing in comparison to short read sequencing technologies, which can lead to errors in the assembled sequence. Additionally, nanopore sequencing can be sensitive to sequencing errors, particularly in homopolymer regions, which can affect the accuracy of the sequencing data. The quality of input DNA is a very important factor that can influence the accuracy of generated sequence data. Any impurities in the DNA sample can significantly affect the accuracy of the sequencing data, which may result in failure of amplicon assembly reconstruction.

DISCLAIMER

The results presented and delivered are generated by following best practices available for nanopore sequencing of amplicons. Before interpretation of the results, customers are advised to inspect the results thoroughly and consider the technological and bioinformatical limitations carefully. This report and the provided deliverables are for research use only (RUO). Please see the interpretation guide <https://eurofinsgenomics.eu/en/custom-dna-sequencing/eurofins-services/whole-plasmid-sequencing/wps-data-interpretation/>.