Final Report

Whole Plasmid Sequencing

Project Id: SNP25XXXX

Submitted To:

Dr. XX
Address
Email: xx@x.com

1. Project Details

Type of Service	Plasmid Sequencing
Platform	Oxford Nanopore
Sample Source	Plasmid DNA
Number of Samples	1

2. Methods

2.1 Qualitative and Quantitative Analysis of Plasmid DNA Sample

The quality and quantity of the received plasmid DNA sample was checked using Qubit Fluorometer followed by agarose gel electrophoresis.

2.2 Preparation of Nanopore library

The Nanopore library was prepared from the QC passed plasmid DNA samples using Rapid Barcoding Kit V14 (SQK-RBK114.24). Briefly, 50-100ng of DNA sample was processed for Rapid barcoding as per the kit recommendation followed by AMPure XP bead purification. The barcoded sample was processed for sequencing adaptor ligation and bead purification. The purified library pool was quantified using Qubit Fluorometer. The final library pool was loaded onto Oxford Nanopore Platform and processed for sequencing 6-8hours.

3. Results

3.1 DNA Quality Control Using Qubit

Table 1: Quantification of received DNA sample on Qubit

Sr. No.	Sample Id	Qubit Readings (ng/µl)	Total Yield (ng)	Status
1	Sample-1	50.0	500	Pass

3.2 DNA Quality Control by Agarose Gel Electrophoresis

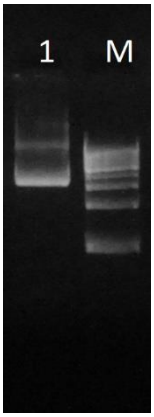


Figure 1: QC of received DNA sample on 0.8% agarose gel. W1: received plasmid DNA; W2: 1Kb DNA ladder.

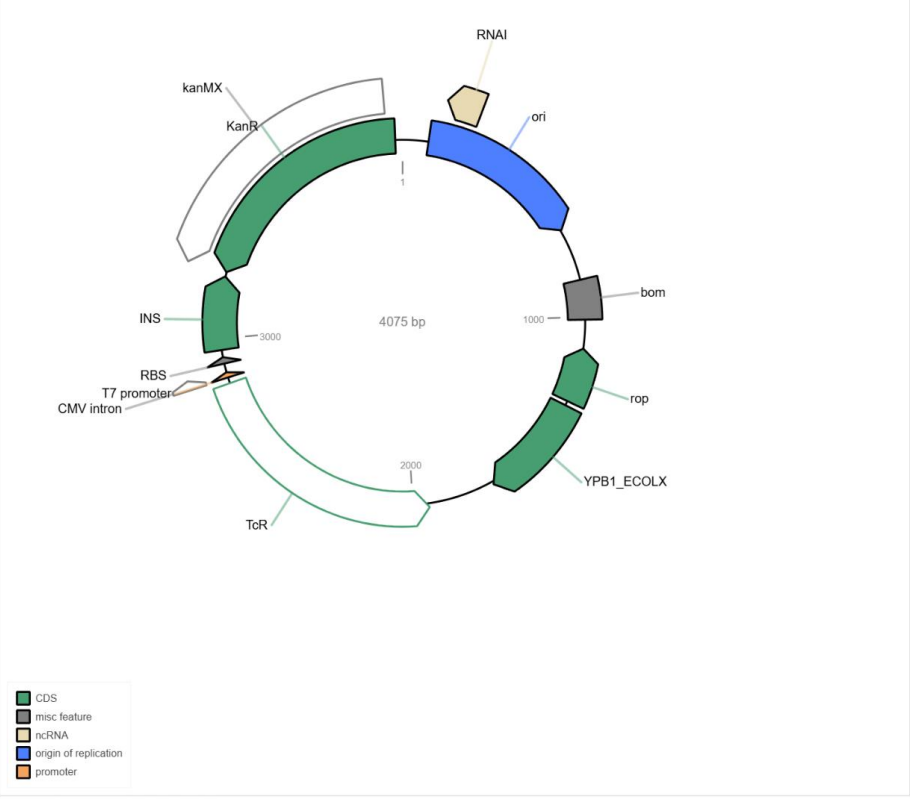
4. Wet lab inference

The quality and quantity of the received plasmid DNA sample was checked using Qubit Fluorometer followed by agarose gel electrophoresis. The QC passed DNA sample was processed for Nanopore library using Rapid Barcoding Kit. The plasmid DNA library was loaded onto Oxford Nanopore platform and processed for sequencing.

5. Bioinformatics Analysis

The Plasmid annotation plot and feature table are produced using pLannotate

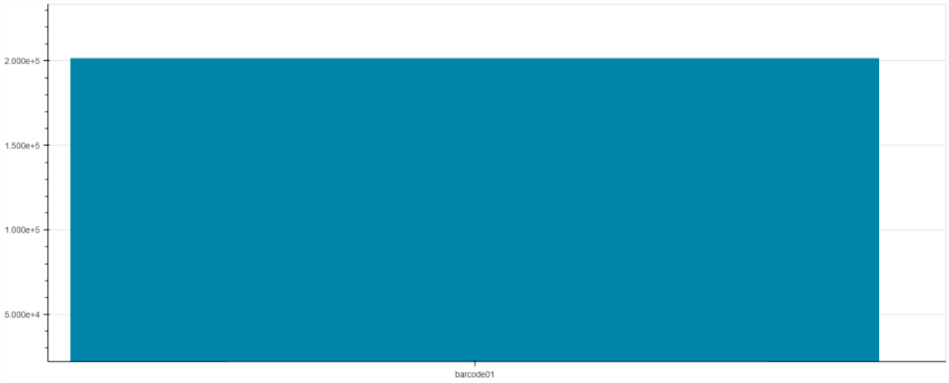
A pLannotate plot is shown for each assembly. A feature table provides descriptions of the annotated sequence. Unfilled features on the pLannotate plots are incomplete features; the sequence match in the plasmid covers less than 95% of the full length of the feature in the database. These elements may be leftover fragments from earlier cloning steps used to create a plasmid. If they include only a small fraction of the feature, they likely do not still have the annotated function. However, even small feature fragments may affect plasmid function if they result in cryptic gene expression or are inadvertently combined with other elements during later cloning steps. The plannotate plot may have overlapping annotation labels, use the zoom and hover tools to decipher the labels.



Feature	Database	Identity	Match Length	Description	Start Location	End Location	Length	Strand
KanR	snapgene	100.0%	100.0%	aminoglycoside phosphotransferase; aph(3')-Ia; confers resistance to kanamycin in bacteria or G418 (Geneticin®) in eukaryotes	3233	4049	816	-
ori	snapgene	100.0%	100.0%	high-copy-number ColE1/pMB1/pBR322/pUC origin of replication	95	684	589	+
rop	snapgene	100.0%	100.0%	Rop protein, which maintains plasmids at low copy number; rop	1110	1302	192	-
TcR	snapgene	100.0%	75.7%	tetracycline efflux protein; tet; confers resistance to tetracycline	1940	2841	901	-
kanMX	snapgene	99.9%	57.9%	yeast selectable marker conferring kanamycin resistance (Wach et al., 1994)	3233	4019	786	-
RBS	snapgene	100.0%	100.0%	efficient ribosome binding site from bacteriophage T7 gene 10 (Olins and Rangwala, 1989)	2912	2935	23	+
T7 promoter	snapgene	100.0%	100.0%	promoter for bacteriophage T7 RNA polymerase	2862	2881	19	+
bom	snapgene	99.3%	100.0%	basis of mobility region from pBR322	869	1009	141	-
INS	swissprot	83.7%	100.0%	INS_HORSE - Experimental evidence at protein level: Swiss-Prot protein existence level 1. Insulin decreases blood glucose concentration. It	2960	3218	258	+

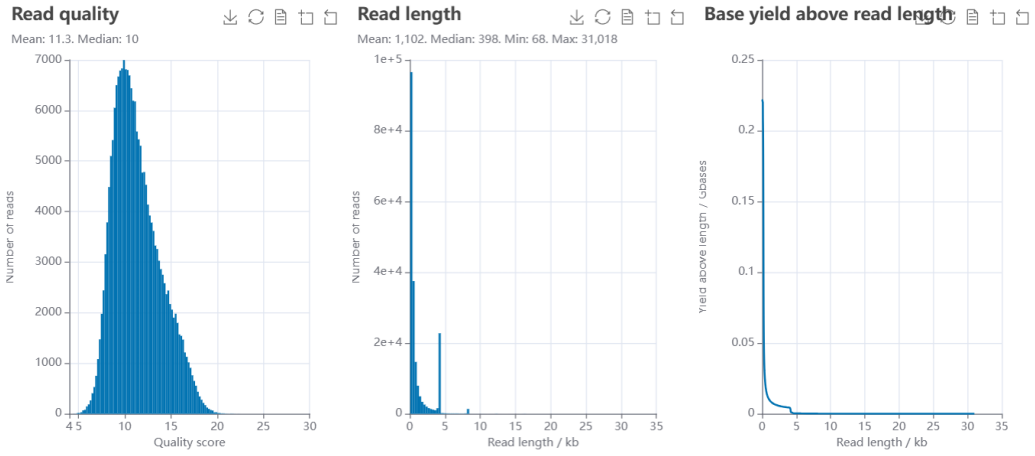
Feature	Database	Identity	Match Length	Description	Start Location	End Location	Length	Strand
				increases cell permeability to monosaccharides, amino acids and fatty acids. It accelerates glycolysis, the pentose phosphate cycle, and glycogen synthesis in liver. From Equus caballus (Horse).				
RNAI	Rfam	100.0%	97.1%	Accession: RF00106 - RNAI	134	239	105	-

5.1 Read Counts



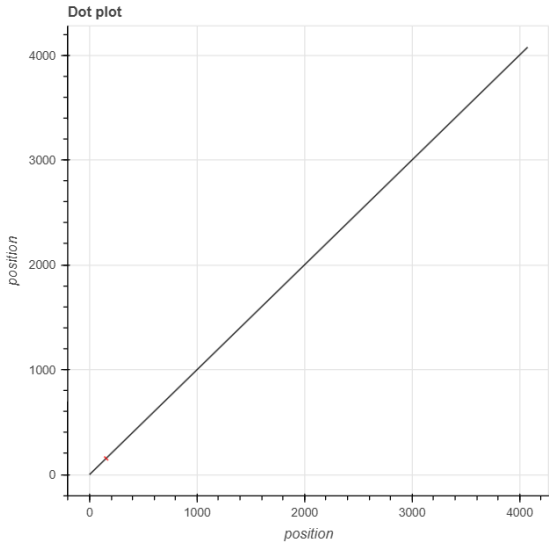
Total Reads: **201411**

5.2 Read stats



5.3 Dot plots

These dot plots have been created by aligning the assembly to itself to reveal any repeats or repetitive regions. Black is for repeats found in the forward strand and red for repeats found in the reverse complement.



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