Whole Plasmid Sequencing

By Nanopore Technology

Nanopore DNA sequencing is a unique, scalable technology that enables direct, real-time analysis of long DNA or RNA fragments. It works by monitoring changes to an electrical current as nucleic acids are passed through a protein nanopore. The resulting signal is decoded (basecalling) to provide the specific DNA or RNA sequence (see next page for work-flow details).

The **Quintara-Poochon DNA Sequencing Center** offers rapid and cost-effective whole plasmid sequencing services using Oxford Nanopore Technologies. Submit your plasmid DNAs and receive results the next business day.

Sample Preparation and Submission

- **Sample Type:** Plasmid DNA up to 30kb or PCR products ≥2kb
- **Submission of Samples**: Please consider to use 96-well plates for ≥ 36 samples, tubes or 8-well strips for < 36 samples. Plasmid DNA up to 30 kb, or purified PCR product ≥ 2 kb, at 100-200 ng/µl with **an OD260/OD280** reading ≥ 1.80 in 5-10 µl is preferred. Send to Poochon at ambient temperature using our drop-box service or any overnight shipping service. To submit sample forms online, please visit https://www.quintarabio.com/corder/plsexpress
- **Turnaround Time:** Sequencing data is available for download the following day
- **Unit Price:** $15 per sample

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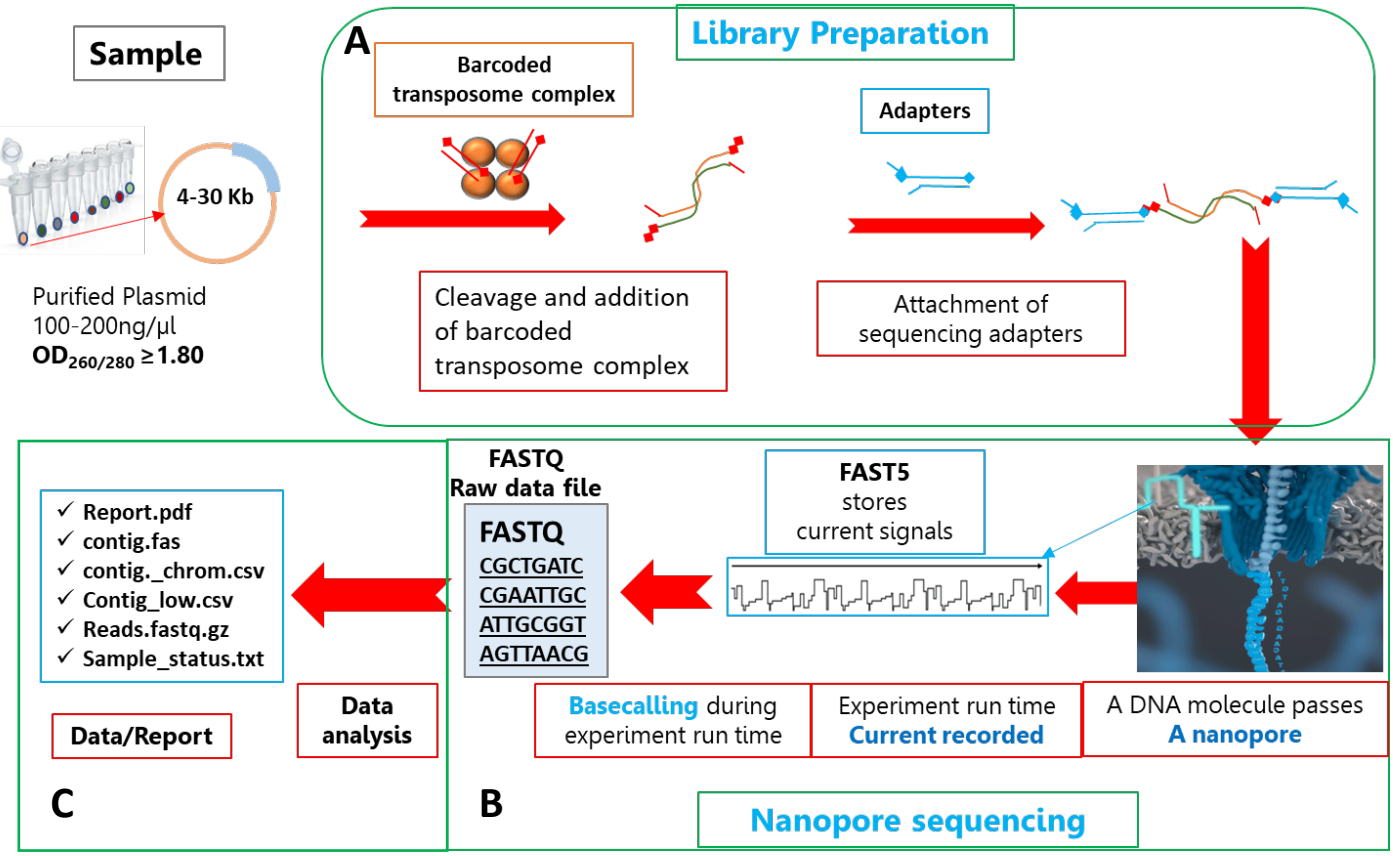
Whole Plasmid Sequencing Workflow



(A) **Creation of the library:** High purity plasmid samples (100-200 ng/µl with **OD260/OD280** ≥ 1.80) were processed for linearization and barcoding followed by the attachment of adaptors to the barcoded linear DNA molecules.

(B) **Nanopore Sequencing and Basecalling:** The library preparation is loaded onto a nanopore sequencer (flow-cell). Changes in current (signal) caused by the strand of DNA or RNA as it passes through the pore were recorded as FAST5 files. The signal stored within FAST5 files was then processed by Basecalling Algorithms to decode the sequence of bases into FASTQ files.

(C) **Data Analysis (Bioinformatics):** The FASTQ files (sequence data) were processed by bioinformatics tools to filter the data for quality based on length and scores. As a result, a report in PDF file format and five other data files per sample were generated.

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Whole Plasmid Analysis Example: MGIN Plasmid

Sample: Purified MGIN plasmid DNA size at 7262 bp, 100 ng/µl, OD260/OD280 = 1.81.

→ 5 µl was used for the analysis

→ A Report in PDF file format and five other data files were generated for this sample

1. **Report.pdf – Next page**
2. **contig.fas – *Open this file using SnapGene viewer (free download: https://www.snapgene.com/snapgene-viewer), or other software to visualize the annotation and analyze the sequence (example of MGIN circle map opened using Snapgene Viewer on a following page).***
3. **contig._chrom.csv – The list of whole sequence determined; open this file using Microsoft Excel**
4. **Contig_low.csv – The list of nt sites with low score/confidence; open this file using Microsoft Excel**
5. **Reads.fastq.gz – Raw data file**
6. **Sample_status.txt – Data statistics and barcode number**

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PlasmidExpress Report: MIGN

QuintaraBio 2023-03-17
PlasmidExpress

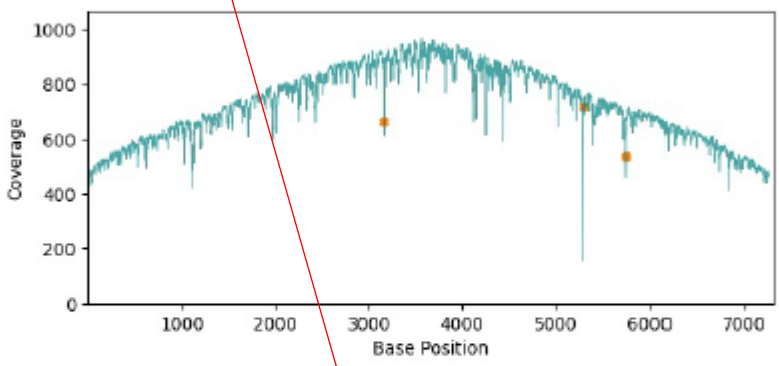
Name	Reads	Bases
Total	1304	7257754
Host Genomic DNA	0.23%	0.14%

Assembly Status: form 1 contig

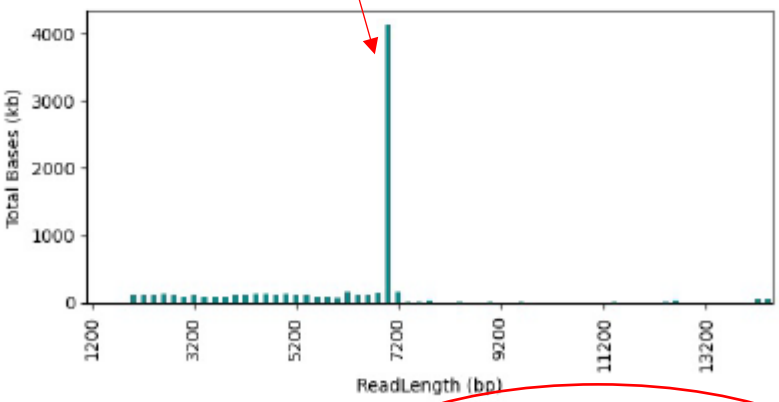
Contig	Length (bp)	Read Count	Bases Mapped
MIGN_contig	7262	1291	7197951 (99.18%)

MIGN_contig Coverage Map

low confidence base positions are markered with x



Read Length Distribution



Files Included

- read fastq file
- contig fasta
- base information for each contig position
- low confidence bases for each contig

Good Data Indicators

- number of reads >= 500
- read length peaks at expected plasmid size
- all reads form 1 contig only
- more than 90% bases mapped back to contig sequence

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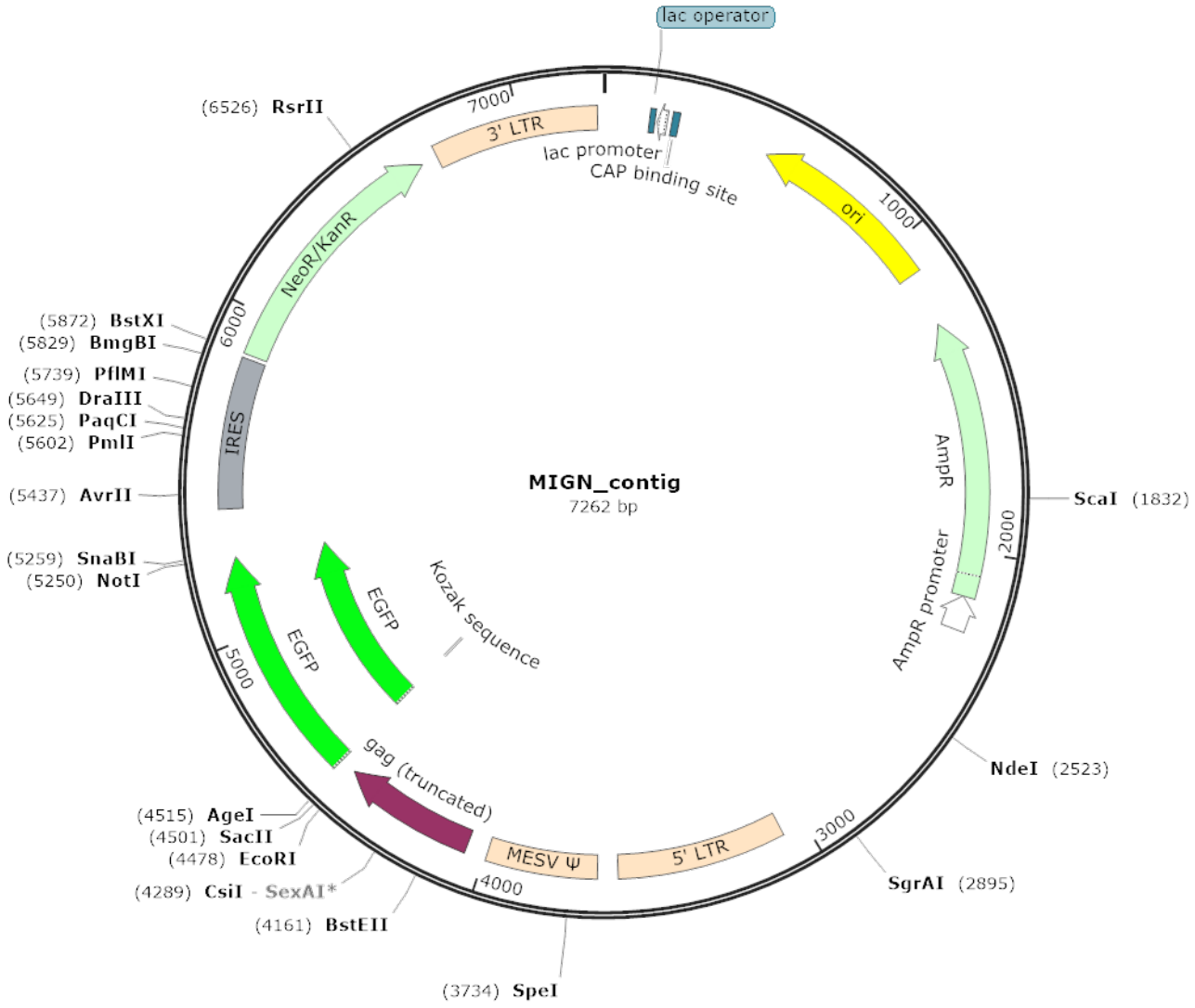
MGIN_contig.fas



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MGIN_contig.fas - 7262 bp exported from SnapGene viewer

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AGT

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