Nanopore Sequencing FAQ

Q: What is the service name for whole plasmid sequencing?
A: PlasmidExpress - this service is for sequencing analysis of full-length plasmids, up to 30kb, by Nanopore long-read sequencing technology, the 3rd Generation Sequencing Technology.

Q: I'm a new customer, how do I create an account?
A: https://www.poochonscientific.com/services/dna-sequencing/nanopore-sequencing/
The link will direct you to the resources you need regarding the PlasmidExpress service. Click "Place an order" to place your order online. Poochon Scientific and Quintarabio partner to offer this service. Please use Quintarabio's website to place orders and check results. Poochon is responsible for sample pick-up, analysis, billing, and customer service.

Q: Where are the pickup locations and what is the drop off deadline?
A: Please use the Poochon-Quintara sample drop boxes (link to the dropbox location list). Pickup times are the same as Sanger sequencing. The cut off time for NIH is 5:00 PM and the cut off time for JHU is 4:00 PM. Please email support@poochonscientific.com if you have any questions.

Q: What is the turnaround time?
A: The turnaround time is 24 hours for standard plasmid samples.

Q: What is Nanopore Technology?
A: Nanopore long-read sequencing technology is used to provide PlasmidExpress, whole plasmid sequencing service. No primers are required. Using Nanopore technology, it is possible to sequence through ITRs, LTRs, large repeats, and hairpins. However, there may be difficulties sequencing homopolymer regions such as a Poly-T (TTTTTTTTTTT) region, for which we recommend confirming potential artifacts by Sanger sequencing.

Q: What are the template/sample submission requirements?
A: OD260/280 = 1.80 – 1.90; Concentration: 30-100 ng/µl, **10 µl**

- For custom service sequencing of very large circular plasmids, ≥100 ng (30-150 kb) /µl, **10 µl,** or ≥200 ng (150-300 kb) /µl, **10 µl**.
- For double-stranded DNA, normalize to the specific concentrations listed above.
- Please refer to published literature for plasmid extraction protocols. Submit the final purified plasmids in elution buffer (10 mM Tris, pH 8.5) or **nuclease-free water**; avoid buffers containing EDTA (e.g. TE or AE buffer) whenever possible.
- Plasmid samples are sequenced WITHOUT primers, so please DO NOT ship any primers with your samples or mixed into your samples.
- Our low sequencing prices and fast turnaround times do not include DNA extraction or quality control (QC) services, so please verify with full QC that your samples meet the following requirements prior to shipping.
- For best results, samples should NOT contain any of the following:
- RNA (RNase treatment is recommended during extraction)
- Denaturants (guanidinium salts, phenol, etc.) or detergents (SDS, Triton-X100, etc.)
- Residual contaminants from the organism (heme, humic acid, polyphenols, etc.)
- Insoluble material, colors, or cloudiness
- Samples should also be "pure" in the sense that they should only contain copies of a single clonal plasmid molecule.
- Sending mixtures of molecular species will give mixed results and is at your own risk!

Q: What is the data output format and how do I read results?
A: Please check out the link below for results reading instructions: https://www.poochonscientific.com/wp-content/uploads/2023/06/Poochon-Whole-Plasmid-Sequencing_results-reading.pdf

Q: How do I receive results?
A: Data is automatically emailed once the sequencing is complete and run through our rigorous QC procedure. You can directly download your data in a secure attachment, and you will receive a custom sequence summary from a member of our QC team.

Q: What is the price and how does the payment work?
A: The price per sample is $15. You may use the same standing PO number with us. We accept credit cards, standing purchase orders, and checks for payment. Feel free to contact us for a quote, if needed.

Q: I cannot find out the target sequence?
A: Please use the NCBI **BLAST** tool to blast the target sequence against the PlasmidExpress resulted sequence.

Q: What can I do if I see a gap in the result sequence?
A: Please email us at support@poochonscientific.com and provide the order ID and sample name as well as the predicted missed sequence (gap) and predicted full-length sequence of the plasmid tested. Our technical team will answer your inquiry within 24 hours.

Q: Why should I sequence the entire plasmid instead of just my region of interest?
A:
(1) Scientific rigor and peace of mind.
(2) Identify any possible mutation sites outside of the gene analyzed by Sanger sequencing which could be occurred during cloning.
(3) For target DNA insertion longer than 1 kb, instead of multiple Sanger runs or synthesizing a sequencing primer or doing primer walking, sequence the whole plasmid not only save time but also save cost.

Q: Can you sequence my mixture of plasmids?
A: Our service is intended for purified plasmids prepared from a clonal population of molecules. You can send mixtures of molecular species, but we can't predict the analysis outcome, so it's at your own risk. There two most common outcomes:

1) If your species are very similar (e.g. differ by only a few nucleotides), the pipeline will most likely create a single SampleName_contig.fas file (one contig) with low confidence positions at SNP/in/del locations. You can view those locations in your provided sampleName_contig_chrom.csv and sampleName_contig_lowc.csv.
2) If your species are sufficiently distinct (e.g. vastly different in size or sequence), the pipeline will most likely create more than one contigs, SampleName_contig001.fas, SampleName_contig002.fas etc. Ultimately, which species end up producing a consensus will vary depending on overall sample quality, coverage, and relative abundance/degradation of each species.
3) Sequencing is considered successful if the pipeline is able to generate any consensus contigs, even if it is not your target. Re-sequencing mixtures won't change the relative proportions of the species, but you can submit multiple aliquots if you need higher total coverage. If the pipeline does not produce a consensus for your target, you can analyze the raw reads from your provided SampleName_reads.fastq.gz by your own tools.

Q: What about these errors or low confidence positions I found in a homopolymer region?
A: The most common error modes for Oxford Nanopore are deletions in homopolymer stretches and errors at the Dam methylation site GATC. This limitation is expected to improve with future updates to their sequencing chemistry and basecalling software.

Q: How much sequencing coverage will I get for plasmids?
A: We do not guarantee any specific level of coverage. The number of raw reads generated can vary substantially depending on sample quality. Successful samples sent at the required concentration typically yield in the high dozens to hundreds (or thousands!) of raw sequencing reads. Final coverage of the consensus depends on how many of the raw sequencing reads are full-length plasmids and whether any degraded plasmid reads can be aligned to the full-length consensus.

Q: What data will I receive for plasmid sequencing?
A: Six data files will be generated for each plasmid sample.

1) A Summary as ".pdf" – A report with the summary of results and data
2) One or more than one contigs as "contig.fas" – A continuous sequence with annotations resulting from the reassembly of the passed reads generated by the Nanopore sequencing analysis [Note: *You may use SnapGene viewer (free download: https://www.snapgene.com/snapgene-viewer), or other software to visualize the annotation as a plasmid circle map or linear map to analyze the sequence*]
3) One excel file as "contig._chrom.csv" – List of the whole sequence determined (open via Microsoft Excel)
4) Another excel file as "contig_low.csv" –List of nucleotides with low score/confidence (open via Microsoft Excel)
5) "Reads.fastq.gz" – fastq raw data file
6) "Sample_status.txt" – Data statistics and barcode number.

Q: Why did my plasmid sample fail?
A: For plasmids, "failure" refers to the failure of your sample to produce a consensus sequence with at least 10x coverage. Our low sequencing prices and fast turnaround times do not include extensive QC to determine why plasmid samples fail. Although we do not provide definitive reasons for failure, by far the most common reasons are:

1) Samples are not prepared to meet with the required DNA concentration and quality, concentration ≥ 30 ng/µl, OD260/280 = 1.80 -1.90.
2) Samples contain a mixture of plasmid species and/or fragmented genomic DNA or fragmented plasmids. You may see evidence of this failure mode in a wide range of read lengths reported in the raw read length histogram.

Q: What is your policy when plasmid samples fail?
A: It is relatively rare that we cannot return a plasmid sequence, but some rate of failure is unavoidable. We may attempt to re-sequence failed samples if your sample quality and quantity permits (with follow-up results delivered in 2-3 business days). If the sample fails a second time, we will conclude that something about the sample makes it un-sequenceable. Unfortunately, we must still charge for failed samples, since we spend more time and resources on them than we do on successes.