plasmids NG

PLASMID SEQ

Service Description

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Background

Plasmids are extrachromosomal genetic elements that can encode a variety of functions. Plasmids are used to express proteins, act as selection markers, and as vectors for gene transfer.

Working with plasmids can be challenging - they don't always do what you expect! That's why it's important to know what you are working with. But sequence verification can be costly, it requires time, resources, and expertise in bioinformatics to handle the data outputs.

We want to solve these problems, which is why we are excited to launch a rapid turnaround plasmid sequencing service that makes characterisation and verification of plasmids quick, simple and cost effective.

Service Overview

We charge £10 (excluding VAT) per plasmid, up to 30 kb in length.

Our target turnaround time is 1-5 business days, from sample receipt to data delivery.

Send us

• Pure plasmid DNA that has been extracted from its host organism (mini-prep is perfect) and has little or no host DNA contamination

  - A260:A280 ratio of between 1.8-2.0
  - A230:A260 ratio >2

• One plasmid per sample

  - We recommend you perform agarose gel electrophoresis, to ensure that you have a single plasmid at the approximate size you are expecting

- Normalise each plasmid to 25 ng/µL in nuclease-free water or EB (no EDTA!)
    - If using a spectrophotometric platform (e.g. NanoDrop) to quantify your DNA, the minimum concentration is 100 ng/µL
- Minimum volume of 10 µL - though the more, the better

We will perform long read sequencing using the latest R10.4.1 chemistry from Oxford Nanopore Technologies. We have carried out extensive testing to optimise this process and all samples that meet our submission requirements produce good quality data, with sufficient yield to produce complete and accurate assemblies.

Service Outputs

The outputs will include:

- Raw R10.4.1 ONT long reads in .fastq format
    - Allows you to perform your own analysis and upload to public repositories
- A histogram showing read length distribution, a virtual gel image, and a list of identified peaks
    - Allows you to ensure your plasmid is the right size, and that your prep did not contain non target DNA (e.g. host DNA, plasmid dimers etc.)
- A high quality consensus assembly of your plasmid
    - Allows you to query your plasmid for key information, and where possible will be rotated to an appropriate start gene
- Annotations in genbank and csv format, as well as interactive plasmid maps
    - Allows you to look at the CDS identified in your plasmid
- Custom sequencing guide + QC report
    - Allows you to quickly check your results, and find details of our methodology

We will not repeat or provide refunds on any sequencing of samples that fail, unless we identify a technical issue on our side.

Any samples that fail will require a resubmission under a new order.

Samples can fail if you provide an inadequate concentration or sample volume, or if your plasmid preparation has non-target contamination (e.g. additional plasmids, host-contamination, plasmid quasispecies). In all cases, we will still provide you with the sequence data that we are able to generate, but it might not be sufficient in quantity or quality to produce an assembly we'd be confident in.

We run a number of internal quality control checks to ensure that each sequencing run is robust to a variety of inputs, and to validate our end-to-end sequencing workflow. Provided your DNA is at the required standards for quantity, quality and purity, it is very unlikely that your sample will fail.

Good applications

This service is particularly useful for verifying plasmid constructs and vectors, and can provide a much richer source of information than typical validation methods. It's also way easier; you don't have the hassle of designing primers and targeting specific regions, with a simple mini-prep, you'll get the sequence of the whole plasmid back.

We would also recommend checking your plasmid stocks before embarking on any long lab experiments. We bought a commercial plasmid to use as a positive control, and noticed that there were 3 errors in the commercial grade reference assembly provided by the supplier! Now imagine what's lurking in that plasmid you received from a collaborator all those years ago...

Bad applications

Samples with multiple plasmids, significant host contamination, or plasmids with multiple large repetitive elements will produce a lower quality output. It can be hard to obtain pure plasmid extractions with larger (>30 Kb) plasmids at low copy numbers.

In these circumstances you can use our Enhanced Genome Service. This service will provide sequence data for the host chromosome as well as any plasmids present. By using both Illumina short reads and Oxford Nanopore long reads, we can better resolve multiple complex plasmids, and provide a high confidence, reference quality genome.

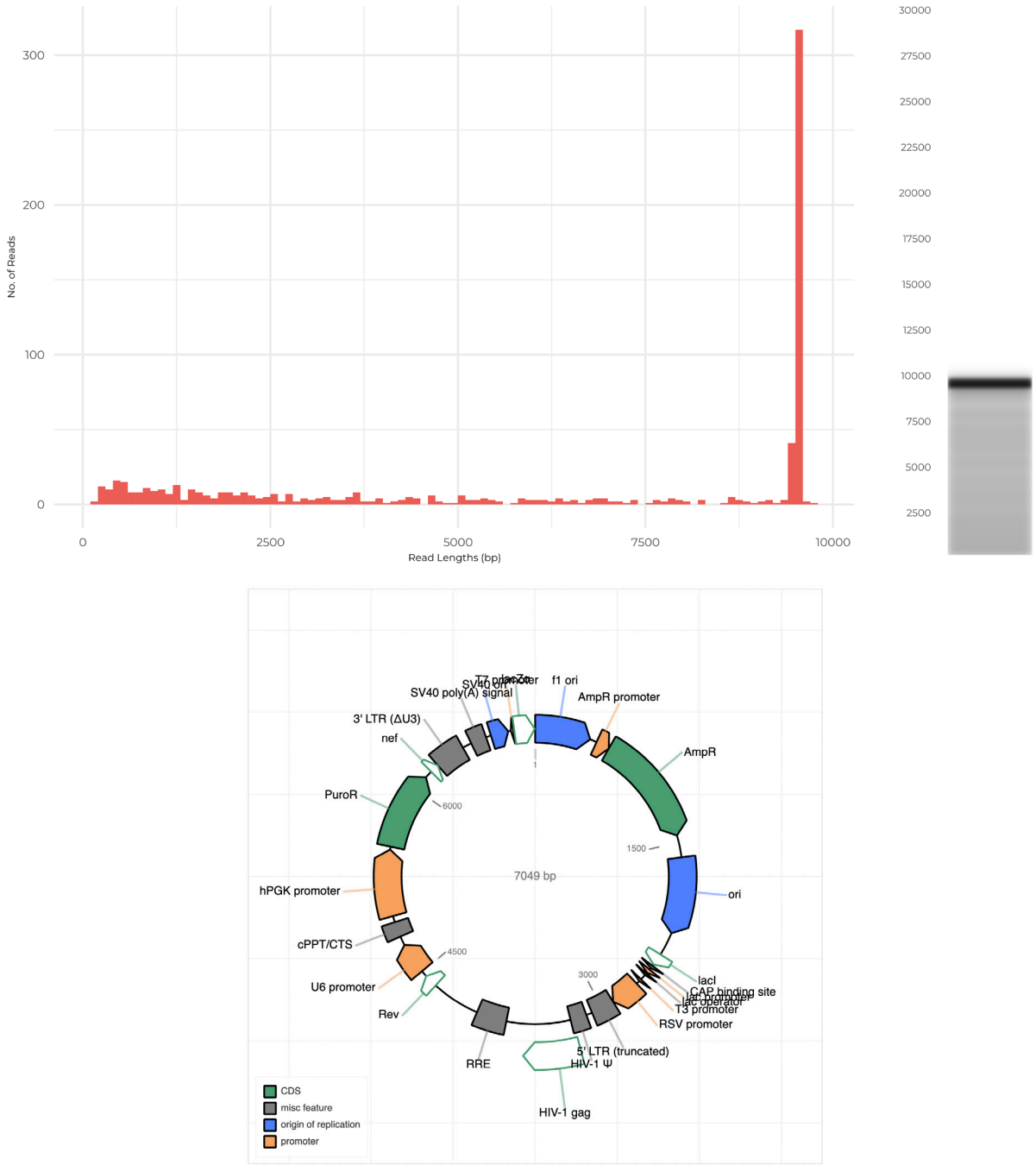

Figure 1 - Examples of service outputs, including a read length histogram, interactive plasmid map and virtual gel image.