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Instructions for bacterial genome sequencing

Our Oxford Nanopore sequencing library prep does not include DNA amplification. Therefore, **your DNA quality & purity will directly affect the quality & quantity of sequencing data you receive**.

DNA input requirements

- ***Quality:*** We recommend **>50% of the DNA be >15kb in length,** as measured via Fragment Analyzer, Bioanalyzer, or gel (ideally pulsed-field).

- ***Purity:*** We recommend **260/280 >1.8 and 260/230 2.0.-2.2**, as measured by Nanodrop.

- ***Quantity:*** We require **5 µg of HMW gDNA,** as measured only by fluorometric method (e.g. Qubit or plate reader). ***Spectrophotometry (Nanodrop) is NOT reliable for quantification!*** We recommend drying down your sample(s) in a speed-vac concentrator with no heat; if that is not possible, please normalize to **100 ng/µL in >50 µL of elution buffer.**
    - If you'd like to send extra DNA, please increase the **volume** of the gDNA that you send, rather than the concentration; dilute as necessary to achieve 100 ng/µL. Sending samples below 100 ng/µL above may impact our ability to obtain sufficient genome coverage to produce an assembly.

Submitting a sequencing request

1. **Register for an account.** When you log in, you will be directed to your dashboard and will see options on the screen to submit a bacterial genome sequencing request.
    1. For genomes <7 Mb: Submit a "Bacterial Genome - Standard" request
    2. For genomes 7-12 Mb: Submit a "Bacterial Genome - Big" request

2. Prepare your sample(s) in a **PCR strip tube** and label your strip with your **assigned 3-digit order label** according to our instructions.

3. **Carefully package your samples** with parafilm and a falcon tube according to our instructions.

4. For free overnight shipping, place your samples along with a copy of your plasmidsaurus order confirmation into a **plasmidsaurus dropbox.** Otherwise, you can ship your samples at **room temperature** (no cold packs or dry ice required) via overnight express courier (UPS, FedEx, DHL, etc.) to the following address:

    Plasmidsaurus
    1850 Millrace Drive, Suite 200
    Eugene, OR 97403
    Phone#: (541) 514-0871

5. Turnaround time is **3-5 business days** from sample receipt to data delivery. We deliver the raw sequencing data in .fastq format, a genome assembly in .fasta format, and folder of genome annotations in various file formats.

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Recommended DNA extraction kits

For bacterial samples, we pre-treat gram positive samples with lysozyme and use the Zymo® Quick-DNA Miniprep Plus Kit for extraction. This kit is also available in a 96 well plate format.

Other extraction methods we and others have had success with:

- Wizard® Genomic DNA Purification kit
- Qiagen® MagAttract HMW kit
- Qiagen® DNeasy kit

Tips for preparing high quality, high purity, high molecular weight gDNA that is free of nicks, gaps, breaks, and contaminants

Sample handling:

- Avoid **vortexing** and fast or unnecessary pipetting; pipet with **wide-bore tips** only
- Elute in **elution buffer or TE**, not water
- Do not expose to **high temperatures** (>37°C) for >1 hour, **pH extremes** (<6 or >9), intercalating **fluorescent dyes**, or **UV radiation**
- Avoid **freeze-thaw cycles;** store gDNA at 4°C for 1-2 months
- Avoid **over-drying** of gDNA; do not use heat when drying in a speed-vac
- Must not contain **RNA**; we strongly recommend RNase treatment during extraction
- Must not contain **denaturants** (guanidinium salts, phenol, etc.) **or detergents** (SDS, Triton-X100, etc.)
- Must not contain **residual contaminants** from the organism/tissue (heme, humic acid, polyphenols, etc.)
- Must not contain **insoluble material** or be colored or cloudy
- DNA must be **double stranded**

Sample quality control (QC):

- **Evaluate quality (gDNA length/integrity)** via Fragment Analyzer, Bioanalyzer, or gel (ideally pulsed-field). We recommend >50% of the DNA be >15kb in length. If the sample looks degraded, either re-extract the sample or clean up the sample to remove the small fragments using a Qiagen kit or AMPure XP beads.

- **Evaluate purity (lack of contaminants)** via Nanodrop or other spectrophotometer. We recommend 260/280 >1.8 and 260/230 2.0.-2.2.

- **Evaluate quantity (total DNA yield)** using only fluorometric methods (e.g. Qubit or plate reader) for DNA quantification. We require at least **5 µg** of HMW gDNA. ***Spectrophotometry is NOT reliable for quantification!***

Note that HMW gDNA typically requires extra effort in **homogenization** (longer incubation times, increased incubation temperatures, very extensive gentle mixing, etc.) in order to obtain accurate quantification. If separate measurements from the top and bottom of the sample are within 15% of each other, this is usually a good indication of adequate DNA homogeneity.